Synthesis of Mannose Extended *N*- and *O*- Glycopeptides for Antibody Generation and Studies of Protein-Carbohydrate Interactions

Dissertation zur Erlangung des Grades

“Doktor der Naturwissenschaften” (Dr. rer. nat.)

in der Fakultät Chemie und Chemische Biologie

an der Technischen Universität Dortmund

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Die vorliegende Arbeit wurde in der Zeit

von Januar 2010 bis Oktober 2015

am Leibniz-Institut für Analytische

Wissenschaften – ISAS in Dortmund

unter Anleitung von Frau. Dr. Ulrika Westerlind ausgeführt.

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Abbreviations

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
Ac	Acetyl
Ala	Alanine
All	Allyl
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
BSA	Bovine serum albumin
Bu	Butyl
Bz	Benzoyl
CAN	Cerium ammonium nitrate
CHO	Chinese hamster ovary
CIP	Contact ion pair
ConA	Concanavalin A
conc.	concentrated
COSY	Correlation spectroscopy
CSA	Camphorsulfonic acid

Cys	Cysteine
DBU	1,8-Diazabicyclo[5.4.0] undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	Distortionless Enhancement by Polarization Transfer.
DIPEA	N,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl(methylthio)-sulfonium triflate
DSG	Dystroglycan
DTBMP	2,6-Di-*tert*-butyl-4-methylpyridine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDT	1,2-Ethanedithiol
ELISA	Enzyme-linked immunosorbent assay
ENGases	endo-β-*N*-acetylglucosaminidases
EPO	Erythropoietin
ER	Endoplasmic reticulum
Et	Ethyl

EtSH	Ethanethiol
Fmoc	9-Fluorenylmethoxycarbonyl
Fmoc-OSu	N-(9-Fluorenylmethoxycarbonyloxy) succinimide
Fuc	Fucose
Gal	Galactose
Glc	Glucose
GlcN	Glucosamine
GlcNAc	*N*-Acetylglucosamine
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
HBTU	*O*-(Benzotriazol-1-yl)- *N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
HCD	Higher energy dissociation
His	Histidine
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple-bond correlation
HOAt	1-Hydroxy-7-azabenzotriazole

HOBt	1-Hydroxybenzotriazole
HSQC	Heteronuclear Single Quantum Coherence
IgG	Immunoglobulin G
Ile	Isoleucine
KLH	Keyhole limpet hemocyanin
Leu	Leucine
LLO	Lipid-linked oligosaccharide
Lys	Lysine
MALDI	Matrix-assisted laser desorption/ionization
Man	Mannose
Me	Methyl
MeCN	Acetonitrile
MS	Mass spectrometry
NCL	Native chemical ligation
Neu5Ac	*N*-Acetylneuraminic acid
NIS	*N*-Iodosuccinimide
NMP	*N*-Methylpyrrolidone
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
OST	Oligosaccharyl transferase

PgIB	Undecaprenyl-diphosphooligosaccharide-protein glycotransferase
Ph	Phenyl
Phe	Phenylalanine
PhSH	Thiophenol
Phth	Phthalimide
PMB	*p*-Methoxybenzyl
Pro	Proline
PTM	Posttranslational modification
PTSA	*p*-Toluenesulfonic acid
py	Pyridine
r.t.	room temperature
Ser	Serine
SPPS	Solid-phase peptide synthesis
SSIP	Solvent separated ion pair
Su	Succinimide
TBAHS	Tetrabutylammonium hydrogen sulfate
TBS	*tert*-Butyldimethylsilyl
t-Bu	*tert*-Butyl
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid

THF	Tetrahydrofuran
Thr	Threonine
TIPS	Triisopropylsilane
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Tn antigen	GalNAcα1-*O*-Ser/Thr
TOCSY	Total Correlation Spectroscopy
Tol	Toluene
Troc	2,2,2-Trichloroethoxycarbonyl
Trp	Tryptophan
Trt	Trityl
Tyr	Tyrosine
Val	Valine

1 Introduction

1.1 Biological roles of *N*- and *O*-glycosylation

Protein glycosylation is regarded as one of the most complex post-translational modifications.[1-2] Glycans are found on diverse protein classes and are often located on the extracellular side of the cell-surface. Toxins, lectins, antibodies, hormones, viruses and bacteria are interacting with membrane bound glycoproteins and are thereby participating in various biological processes.[3-5] Glycoproteins are also involved in intracellular interactions, for instance *O*-GlcNAcylation play a major role in signal transduction. Protein glycosylation may further influence protein folding and controlling resistance to proteases.[6-7]

Two major types of protein glycosylation exist, *N*-glycosylation and *O*-glycosylation. In *N*-glycosylation the glycans are attached to asparagine (Asn) through an amide bond and in *O*-glycosylation the glycan structure is linked through an *O*-glycosidic bond to serine (Ser), threonine (Thr) and sometimes to tyrosine (Tyr), hydroxylysine or hydroxyproline. The biosynthetic pathways of these modifications are significantly different. In *N*-glycan biosynthesis[8] a presynthesized triantennary tetradecasaccharide ($Glc_3Man_9GlcNAc_2$) is transferred from dolichol pyrophosphate to an Asn residue of the newly produced protein employing the enzyme oligosaccharyl transferase (OST).[9] The terminal glucose residues are then enzymatically removed in a sophisticated process involving specific chaperones and are forming the common pentasaccharide core ($Man_3GlcNAc_2$). The pentasaccharide are then further extended to obtain high mannose-, hybrid- or complex type *N*-glycans (**Figure 1** A). A consensus peptide sequence consisting of Asn-Xaa-Ser/Thr are usually found for recognition of sites that are modified with *N*-glycans, Xaa can be any of the 20 natural amino acids except proline. In *O*-glycoprotein biosynthesis single monosaccharides are directly attached to the Ser/Thr or Tyr, which usually are further extended by sequential enzymatic addition of up to 3-9 sugar residues. The mucin-type *O*-glycosylation is the most common modification, which is initiated with the attachment of an α-linked *N*-acetylgalactosaminyl (GalNAc) residue to Ser or Thr (Tn-antigen). The GalNAc residue is usually extended with GlcNAc, Gal or GalNAc in 3- or 6-position forming core 1-8 structures.[10] These core structures can be further extended and often carry terminal sialylation or fucosylation (**Figure 1** B). In the recently discovered *O*-GlcNAcylation,[11] found on intracellular glycoproteins, a

N-acetylglucosamine monosaccharide is usually β-linked to Ser/Thr without further extension (**Figure 1** C).

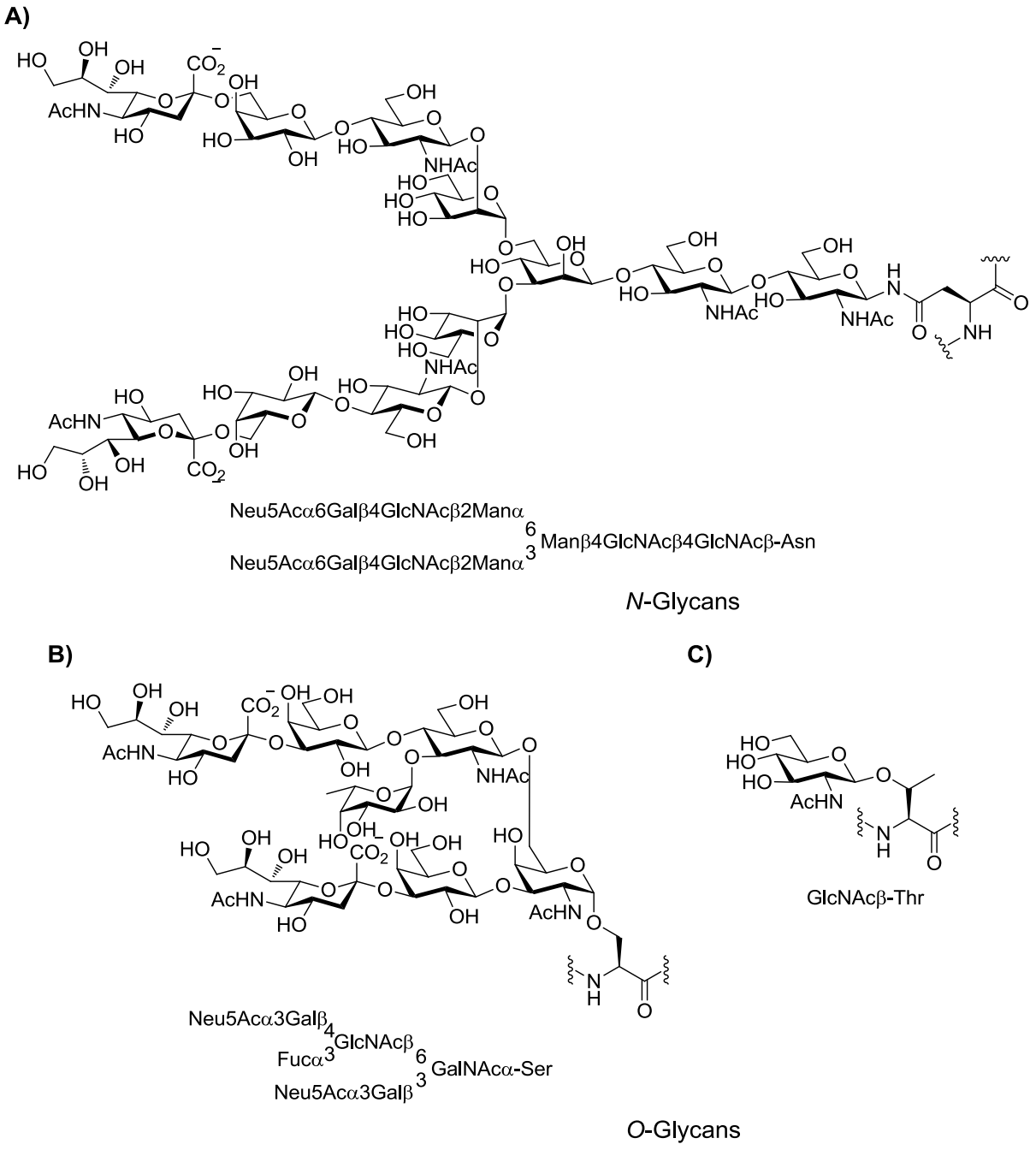


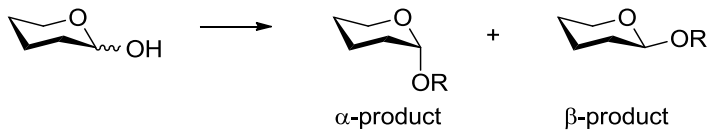
Figure 1. Examples of typical *N*- and *O*-linked glycans.

1.2 General introduction to carbohydrate synthesis

1.2.1 Common protecting groups

An optimal protecting group strategy is essential during synthesis of complex oligosaccharides. By modulation of the protecting groups under different protection/ deprotection conditions, highly functionalized monosaccharide units could be designed and applied in coupling reactions forming larger oligosaccharides. [12]

Starting from unprotected D-glucose, D-mannose or D-galactose monosaccharides, the first step often involves the protection of the anomeric center, since the cyclic hemiacetal can otherwise be ring opened, changing anomeric configuration or switch from hexose to furanose rings in solution. The conformation of the coupling product can be controlled by different methods (**Scheme 1**). In general, the α-anomer is thermodynamically more stable, due to the anomeric effect,[13-15] while the β-anomer is more reactive and typically generate kinetic products.



Scheme 1. α/β anomers of the pyranose ring.

An example is the synthesis of β-acetylated glucose under kinetic control, which is preformed by heating and refluxing glucose together with sodium acetate in acetic anhydride. The sodium acetate causes a rapid anomerization of the free sugar at high temperature resulting in acetylation of the more reactive β-anomer. Selective α-acetylation can perform under thermodynamic control by treatment of D-galactose with acetic anhydride and Lewis acid catalysis. Acetylation with acetic anhydride in pyridine usually gives the peracetylated product that retains the anomeric configuration found in the parent free sugar.

The selective protection of remaining monosaccharide hydroxyl groups is then proceeding. The choice of protecting groups applied on the saccharide would influence the

regioselectivity and reactivity in the next coming coupling reactions. In this thesis, several classes of common protecting groups are used for sugar ring protection.

Ester protections: *acetyl*, *benzoyl*;
Ether protections: *benzyl*, *p-methoxybenzyl*, *trityl*, *allyl*, *tert-butyldimethylsilyl*;
Acetal protections: *benzylidene acetal*, and *butane diacetal*.

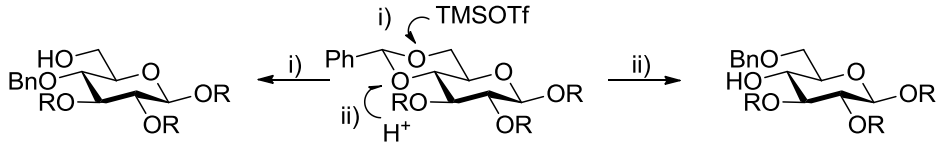
The acetyl group is the most widely applied protection group in carbohydrate synthesis, which is easy to form[16] and remove[17] by mild acid or base hydrolysis. Acetylation or deacetylation reactions are usually not performed with a particular selectively within the sugar ring, with exception of the anomeric acetyl group that selectively react forming a desired donor or stable protection of the position. The acetyl groups may then be removed followed by more selective protection of the ring or the acetylated donor is directly applied in coupling reactions. When acetyl protection is combined with other protecting group functionalization, the order of protection and deprotection needs to be considered, acyl migration may otherwise be a problem since the acetyl protecting group have a tendency of moving towards the more reactive positions within the ring.[18] The benzoyl protecting group behaves rather similar to acetyl protection, but is more stable.

Ether protection, is more stable than esters and in the case of benzyl (Bn) protection, which is the most common ether protection, it even survive strong acid/base conditions. The generation of benzyl ether protection often involves treatment with base (e.g. NaH) and halogenated reagents such as benzyl bromide.[19] By using dibutyltin oxide or tributyltin oxide regents, the benzyl ether protecting groups can be introduced with high selectively.[20]

For removal of the benzyl group protection, catalytic hydrogenation with 10% palladium on carbon (Pd(C)) is commonly used.[21] In order to introduce a protecting group that can be removed under milder conditions *p*-methoxybenzyl protection might be an alternative, which can be cleaved by CAN/DDQ treatment or diluted TFA.[21] The trityl protecting group, is even less stable and can be removed using 80% acetic acid. The allyl group is another option, which is selectively removed using a Rh(I) or Ir(II) catalyst, e.g. Wilkinson's/Baudry catalyst for double bond isomerization and followed by acid hydrolysis.[22] Further, *tert*-butyldimethylsilyl (TMS)[23] or similar reagents,[24] are commonly used in selective 6-OH

protection of the primary hydroxyl and is removed by acid hydrolysis[25-26] or by using a fluoride source.[27-28]

To selectively protect two hydroxyl groups simultaneously, acetal protection can be introduced, for instance by treatment with ketones or aldehydes in weak acidic conditions. The Benzylidene acetal is often used for 4,6-*O*-protection and by selective ring opening the formation of 4-OH or 6-OH products can be controlled. The cleavage of the benzylidene acetal by using borane and TMSOTf would favor formation of a free hydroxyl on the less sterically hindered 6-position, alternatively $LiAlH_4$, $AlCl_3$ in ether can be used.[29] On the other hand treatment with sodium cyanoborohydride in 2M HCl result in a free hydroxyl in 4-position (**Scheme 2**).[30]



Scheme 2. Selective benzylidene acetal ring opening. i) 1 M BH_3 in THF, TMSOTf, DCM, 0 °C 30 min, r.t. 16 h; ii) sodium cyanoborohydride, 2N HCl in Et_2O, THF, 0 °C 30 min.

The choice of protecting group strategy on the donor and acceptor saccharide residues may further be important to increase or decrease the reactivity or influence the stereoselectivity during coupling of the saccharide building blocks. For instance glycan donors protected with acetate groups (in particular in C2), which are electron withdrawing groups, disfavor the build-up of positive charge in the anomeric center (C1) and stabilize the saccharide. In contrast benzyl groups are electronically passive and do not hinder the build-up of positive charge in the pyranose ring. Differences in stability of in particular the glycan donors can in this way be used to modulate the reactivity in coupling reactions. These differences in reactivity influenced by protecting groups was initially studied by Paulsen[31] and later the armed/disarmed concept was described by Fraiser-Reid. [32]

In certain occasions it is better to skip protection of hydroxyl groups and instead make use of the individual OH group nucleophilicity within the pyranose ring. Thereby avoiding unnecessary protection and deprotection steps. The differences in hydroxyl group reactivity

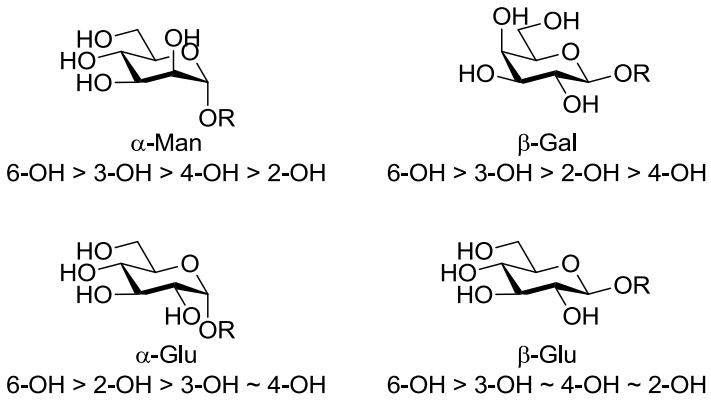
may further be used to selectively introduce protecting groups within the ring. Some general rules describing OH group reactivity within the pyranose ring is summarized below (**Scheme 3**).[33]

Reactivity of the hydroxyl groups in the pyranose ring:

i) 1° OH > 2° OH;

ii) Equatorial OH > axial OH;

iii) Equatorial OH with vicinal axial OH (or OR) > Equatorial OH without vicinal axial OH (or OR).



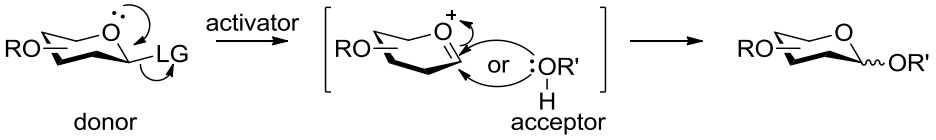
Scheme 3. Expected nucleophilicity of different monosaccharide residues.

In conclusions, by combining different protecting group methods, selective protected monosaccharide units are obtained. The protecting group strategy should be well planned and reducing the number of protection and deprotection steps. In particular, a unified protection pattern, requiring less deprotection steps in the end of a complex synthesis route is preferable. Alternation of the protecting groups results in variation of reactivity and selectivity of the saccharide building blocks. Sometimes reactions without complete hydroxyl protection[34-35] should be considered, since shorter synthesis routes usually result in higher overall yields.

1.2.2 Different glycosylation coupling methods and stereoselective control

Glycosylation coupling steps are other key aspect to control during the synthesis of large oligosaccharides. The glycosylation coupling procedure typically involves activation of a

leaving group on the donor saccharide to form an oxacarbenium ion intermediate. A nucleophile, the hydroxyl group on the acceptor, then attack the oxacarbenium ion in the anomeric center to form a glycosidic linkage (**Scheme 4**).

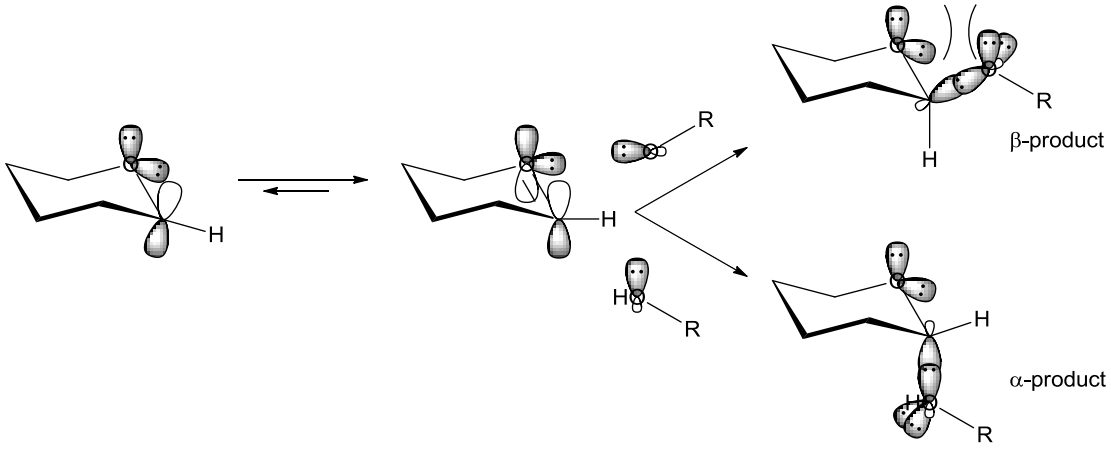


Scheme 4. General procedure of chemical glycosylation.

Several factors influence the glycosylation process,[36] which will be discussed below.

1.2.2.1 The anomeric effect

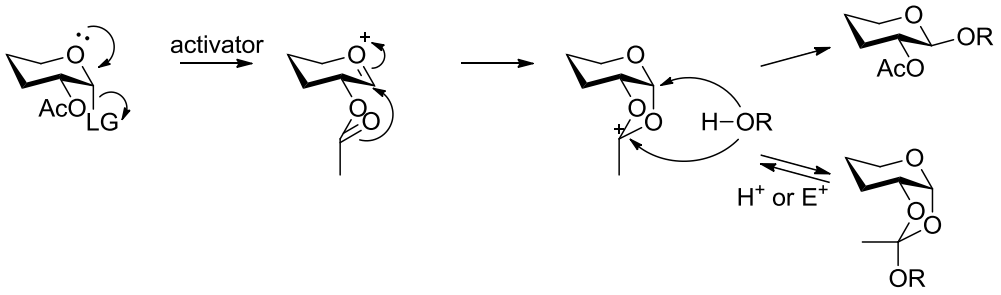
The propensity to favor axial configuration at the anomeric center (C1) with increased electronegativity of the substituent is called the anomeric effect.[15] To stabilize the formation of an oxacarbenium ion intermediate, an ion electron pair from the ring oxygen is preferably shared with the anomeric carbon. Since the electron density from the upper face is higher, nucleophile attack is favored from below, to form an α-glycosidic bond. Further, the two oxygen of the β-product will have unfavorable parallel dipole-dipole interactions, repelling each other, while the axial α-product have favorable dipole-dipole interactions resulting in a lower energy state. Another explanation of the anomeric effect is that the ion pair of electrons in an oxygen molecular orbital overlap with an anti-bonding molecular orbital of the α-glycosidic bond, which stabilize the α-anomer (**Scheme 5**).



Scheme 5. Anomeric effect on α/β glycosylation.

1.2.2.2 Neighboring group participation

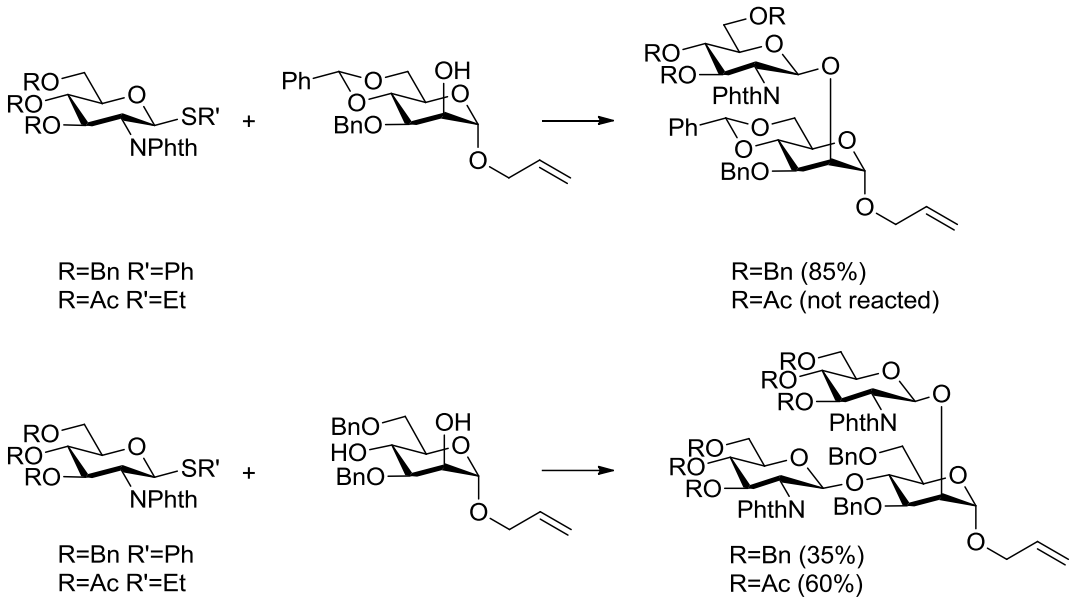
During synthesis of β-glycosylated products, neighboring group participation is often involved, for instance in presence of 2-*O*/*N* acetyl, benzoyl, 2,2,2-trichloroethoxycarbonyl (Troc) or phthalimido (Phth) protecting groups. Participation with acetyl and benzoyl groups involves formation of an orthoester ring intermediate, giving the opportunity of nucleophile attack from the equatorial site and thereby obtaining a β-glycosylated product (**Scheme 6**).[37-39]



Scheme 6. Neighboring group participation during glycosylation.

1.2.2.3 Protecting group effects

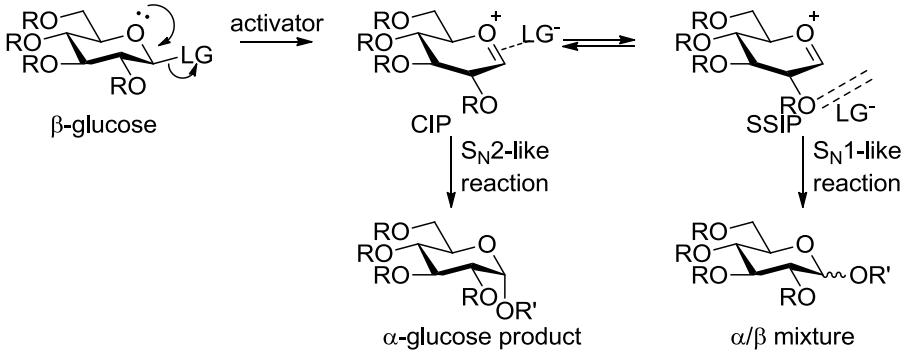
As briefly described in section 1.2.1., protecting groups influence the reactivity of glycosyl donor/acceptor pairs. In general, the electron withdrawing acyl protecting groups, disfavor the build-up of positive charge in the anomeric center and will deactivate the glycan donor/acceptor. Alkyl groups or deoxy modifications will on the other hand increase the reactivity. To perform a specific glycosylation reaction, a donor with suitable reactivity should be used. For example two glycosyl donors were here evaluated for the synthesis of the GlcNAc-Man di- and trisaccharide building blocks. The reactions involved thioglycoside donor couplings to 2-OH or 2,4-OH mannose acceptors activated by NIS-TfOH in DCM. The benzylated donor, was far more reactive during coupling to 2-OH mannose, while the acetylated donor was not reacting at all. During coupling to a more reactive acceptor, a 3,6-benzylated 2,4-OH mannose, the benzyl donor was too active resulting in multiple by-product formation and low yields. Instead, the less active acetylated donor, gave high yields of the desired product 2,4-glycosylated product (**Scheme 7**).



Scheme 7. Benzylated/acetylated donor compared in coupling reactions.

1.2.2.4 Ion-pairs and solvent effects

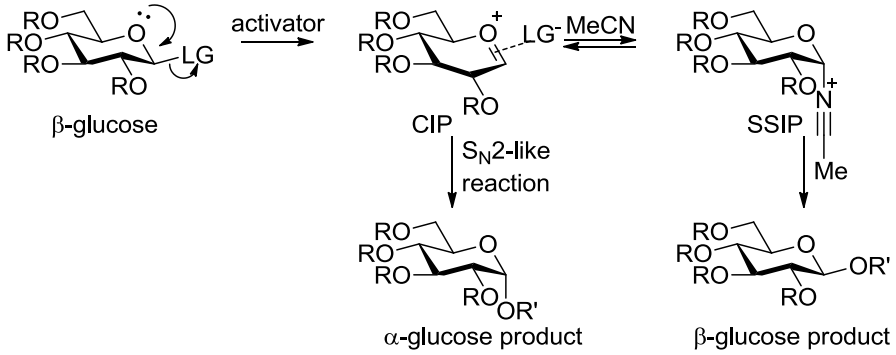
After glycosyl donor activation, the reactivity of the oxacarbenium-leaving group ion pair is influenced by the surrounding solvent molecules. In less polar solvents the cation and anion will still be in contact, which is described as a CIP: contact ion-pair or tight ion-pair. Similar to a S_N2 mechanism, the nucleophile attack will be directed by the leaving group and cause inversion of the anomeric conformation.[40-41] Solvents with a high dielectric constant can stabilize positive charge very well and the oxacarbenium ion will be completely solvated and shielded from the counterion, described as a SSIP: solvent separated ion pair (**Scheme 8**).



Scheme 8. An example of solvent effects on glycosylation.

The S_N1 resembled oxacarbenium cation can then be attacked by a nucleophile both from the upper and bottom side resulting in α/β mixtures.[42] In addition to the solvent, the kind of glycan residue, the neighboring protecting groups, conformation, acceptor and reagents are thus further influencing the ratio of the obtained anomeric mixture. For mannose, the 2-OR axial bond would for instance influence and often favor the formation of α-glycosylated products.

When acetonitrile is used as solvent, the glycosylation mechanism will be slightly different. Under S_N1-like conditions, the acetonitrile may coordinate to the anomeric center of the oxacarbenium ion, forming a nitrilium-nitrile on the α-face.[43] The acetonitrile SSIP effect will then result in a nucleophile attack from above leading to β-glycoside product formation.



Scheme 9. Solvent effect on glycosylation performed in MeCN

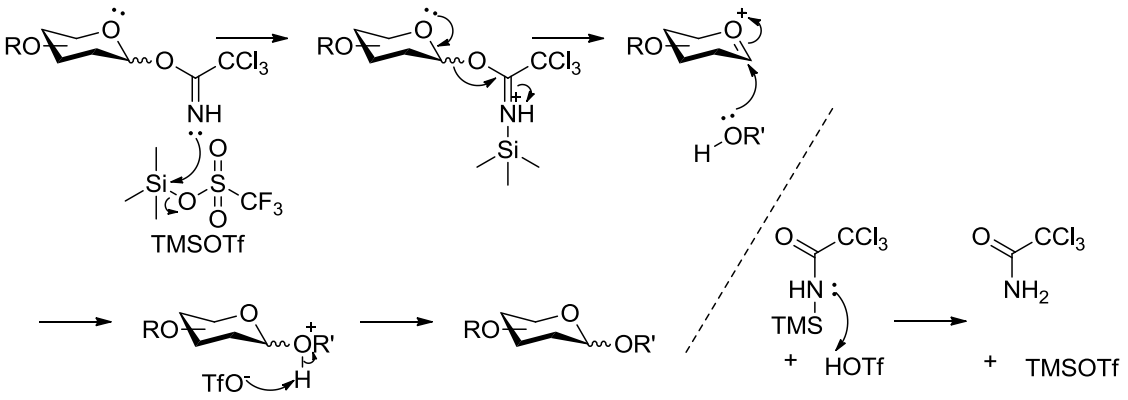
1.2.3 Common glycosylation reactions

In coupling reactions, activation of the donor leaving group results in the formation of a oxacarbenium ion intermediate[44] followed by nucleophilic attack by an acceptor.[45-46] Dependent on the desired structure of the final product, stereochemistry in glycosidic bonds and applied protecting groups, different leaving group/activation reagent combinations can be applied in the synthesis strategy.[47-49] Glycosyl halides was employed in the very first glycosylation synthesis[50] and a few years later the Königs-Knorr method, was developed, which involved glycosyl halide donors reacting with alcohols in the presence of silver (I) carbonate.[51] The Königs-Knorr method had an enormus impact in the method development of carbohydrate synthesis and is still commonly used in a modern form. Lemieux devloped the halide ion catalyzed *In situ anomerization* method for selective formation of 1,2-cis

glycosidic bonds.[52] This method is based on that the α-glycosylbromide donor rapidly equilibrate with the β-bromide under catalysis of tetraalkylammonium halides. The more reactive β-bromide is constantly consumed in the glycosylation reaction forming the desired α-glycoside coupling product. The trichloroacetimidate method developed by Schmidt, are commonly used in coupling reactions and are described in detail below. Stable benzyl or allyl ethers or acetyl groups in the anomeric center of the glycan are here typically converted to trichloroacetimidate donors in two simple steps. The couplings proceed generally using Lewis acid promotors like borontrifluoride (BF_3) etherate or catalytic amounts of trimethylsilyl triflate (TMSOTf). A great number of coupling reactions involving thioglycoside donors have further been developed. The thioglycosides are stable for storage and reactions involving protecting group modification steps or orthogonal glycosylations with other donor-activation systems. A few thioglycoside promoter systems employed in this work are described below. Other glycosyl donors employed in couplings are; n-pentenyl glycosides,[53-54] phenylselenoglycosides[55] and glycal donors.[56]

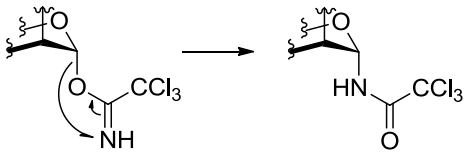
1.2.3.1 Trichloroacetimidate method

The trichloroacetimidate group is first activated by addition of a TMS group, the double bound is then transferred from a carboximidate into a carbonyl and is leaving meanwhile the glycan forms an oxacarbenium ion.[57-59] After nucleophilic attack by the acceptor, the TfO^- group abstracts a hydrogen atom from the acceptor to form triflic acid. The Triflic acid is regenerated into TMSOTf and only catalytically amounts of TMSOTf[60] were required in this reaction (**Scheme 10**).



Scheme 10. Trichloroacetimidate coupling mechanism.

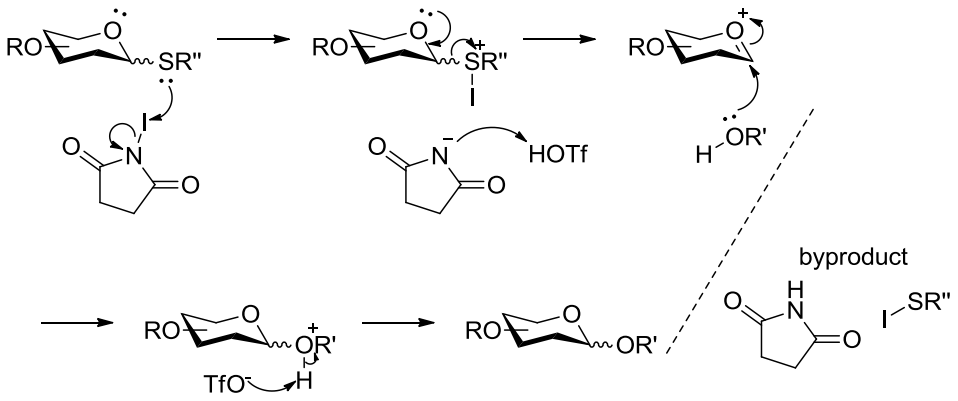
A major side-reaction upon activation of the glycosyl trichloroacetimidate is the Chapman rearrangement.[61-62] During trichloroacetimidate activation, a rearrangement into an inactive form can take place. This occurs particularly in slow coupling reactions, for instance by using large oligosaccharide donors (**Scheme 11**).



Scheme 11. The trichloroacetimidate rearrangement reaction.

1.2.3.2 Thioglycoside with NIS-TfOH coupling

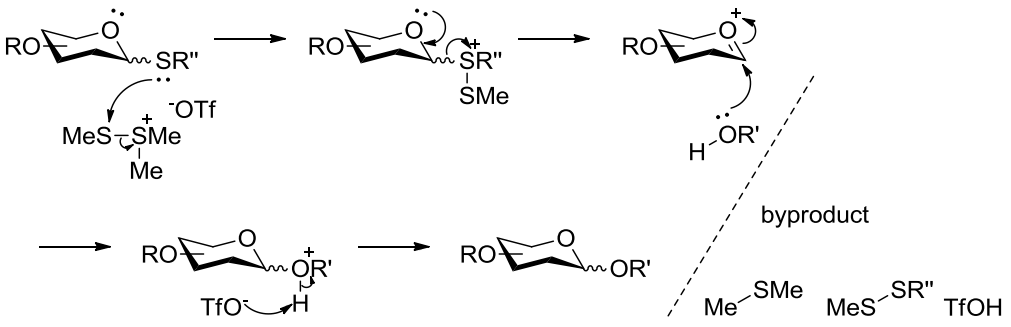
The thiol ethers (SEt/SPh) are good leaving groups[63] when activated with a bromine or iodine source e.g. NBS[64] or NIS.[65-66] Catalytic amounts of triflic acid are used, while equal amounts of the donor sugar and NIS would be required (**Scheme 12**).



Scheme 12. NIS-TfOH assisted thioglycoside activation.

1.2.3.3 DMTST coupling

DMTST should be synthesized freshly prior to synthesis.[67] Upon activation with DMTST the thioglycoside donor form a positively charged methyl disulfide, which serves as a good leaving group generating a oxacarbenium ion available for coupling with the acceptor (**Scheme 13**).



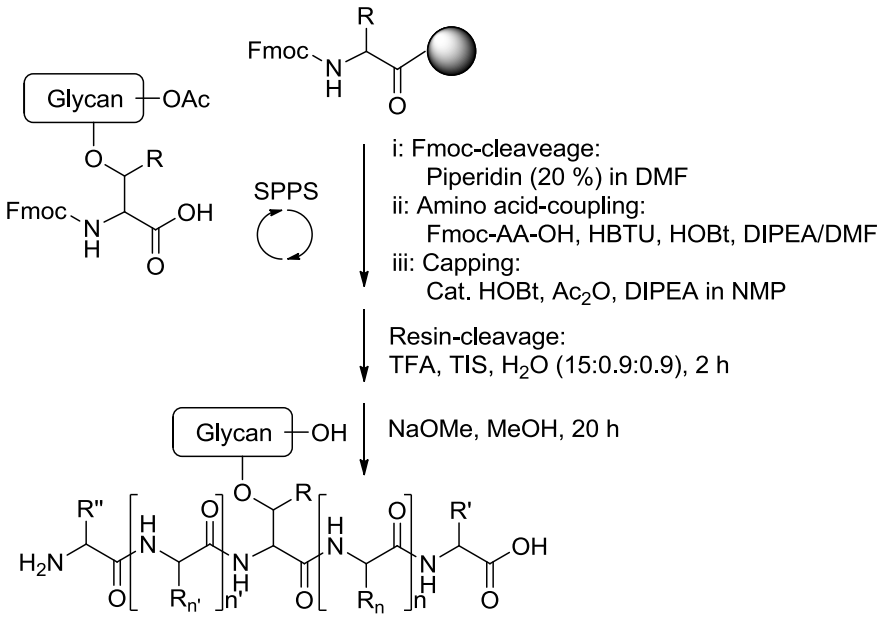
Scheme 13. DMTST thioglycoside coupling mechanism.

1.3 General introduction to glycopeptide synthesis[10, 68-70]

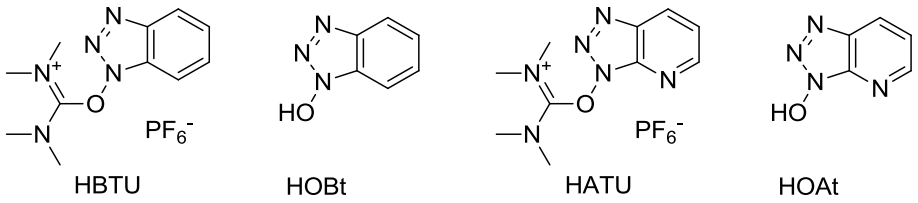
1.3.1 Fmoc-Solid-Phase glycopeptide synthesis

Fmoc-solid-phase synthesis (Fmoc-SPPS) is the most common method applied in peptide and glycopeptide synthesis. The Fmoc protecting group, can be removed under mild base treatment and is compatible with acid labile side-chain protecting groups such as the *tert*-butyl and trityl groups. In glycopeptide synthesis the use of mild conditions are in particular critical, since the glycosidic bond on the glycosylated amino acids are labile to strong acid such as HF, the other common peptide synthesis method, Boc-SPPS, is therefore not an option in glycopeptide synthesis. Harsh base treatment of *O*-glycopeptides may result in β-elimination, however the use of *O*-acetyl glycan protecting groups and the use of mild organic bases in the Fmoc peptide synthesis strategy, avoid that such undesired side reactions takes place. By using an automatic peptide synthesizer, the peptide assembly can perform in a stepwise fashion starting from a resin preloaded with the first *C*-terminal Fmoc-amino acid. The peptide assembly proceeds in coupling cycles, starting with Fmoc deprotection with 20% piperidine in DMF followed by amino acid (4-10 eq) coupling activated by HBTU/HOBt and DIPEA. For introducing a glycosylated amino acid (1.5-2 eq), the more reactive coupling reagent HATU/HOAt together with DIPEA are used instead (**Scheme 15**).[71-72] To compensate for the lower excess of the valuable glycosylated amino acids, the couplings perform more concentrated and during longer reaction times. After the completion of the desired peptide sequence, a resin cleavage step follows by treatment with a mixture of TFA/TIPS/H_2O (standard conditions, other TFA cleavage reagents are used if peptide contains oxidation sensitive amino acids). Simultaneously the acid sensitive amino acid side chain protecting groups are cleaved. The cleaved peptides are desalted on a C-18

cartridge or purified by HPLC. For glycan deprotection, deacetylation is performed under mild basic conditions with NaOMe/MeOH at pH 9.5-10 to avoid β-elimination or for larger saccharides NaOH in MeOH/H_2O is used. The final product is then typically purified by HPLC.



Scheme 14. Procedure for solid phase peptide synthesis.

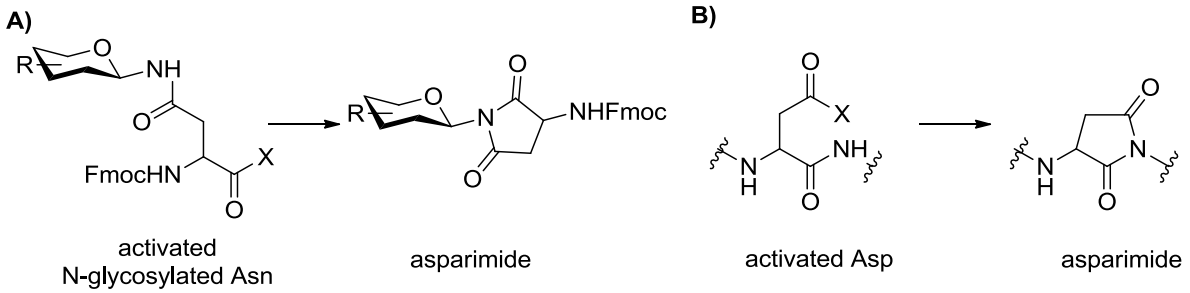


Scheme 15. Reagents used for peptide synthesis coupling reactions.

1.3.2 Glycopeptide synthesis by direct glycan coupling to the peptide backbone

The use of peracetylated Fmoc glycosylated amino acids in stepwise peptide assembly, as described above, is the most common approach for glycopeptide synthesis. The formation of an *O*-glycosidic bond connecting the glycan to the amino acid side-chain (Thr/Ser) is preferably introduced in an early stage of the glycosylated amino acid synthesis using optimal protecting groups for desired reactivity, stereochemistry and regioselectivity in the coupling step. Direct coupling of a protected *O*-glycan donor to a fully assembled peptide forming an

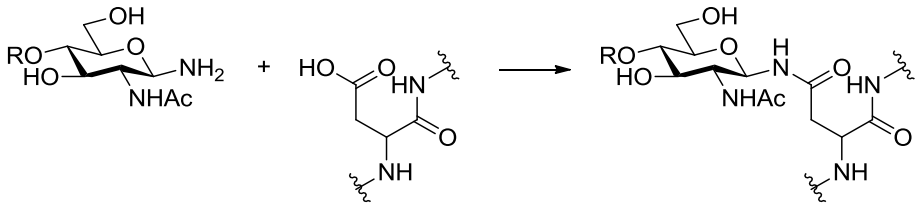
O-glycosidic bond is not desirable, since it often results in low yields and lack of stereoselectivity in the reaction. In contrast to *O*-glycopeptides, *N*-glycopeptides are connected through an amide bond to the peptide backbone and *N*-glycan attachment to a fully assembled peptide through a standard amide coupling is here an option. By using synthetic *N*-glyco-amino acid building blocks in stepwise Fmoc-SPPS, the yields for the glycosylated amino acid coupling step often drop as the size of the glycan structure increases. Due to sterical hindrance and slow amino acid reactivity, it might result in aspartimide formation on the *N*-glycan Asn (**Scheme 16** A). Since the glycan acetyl protecting groups dramatically reduce the reactivity, unprotected *N*-glycosylated amino acids and modified coupling conditions, might be an option to achieve *N*-glycopeptides in acceptable yields.[73-75]



Scheme 16. Formation of asparimide byproduct during peptide synthesis. A) asparimide formed on glycosylated amino acid during Fmoc-SPPS; B) asparimide formed on peptide backbone during Lansbury glycopeptides synthesis.

In the alternative approach, a protected peptide backbone is synthesized with an unprotected aspartate side-chain, which later is employed in an amide coupling with a 1-NH_2 reactive unprotected *N*-glycan (the Lansbury method, **Scheme 17**).[76] Also in this method asparimide byproduct formation can take place during coupling of large and slowly reacting *N*-glycans (**Scheme 16** B). Danishefsky and coworkers synthesized hybrid type *N*-glycopeptide gp120 fragments using the Lansbury method.[77-78] A synthetic $Man_7GlcNAc_2$ 1-NH_2 oligosaccharide was conjugated to a pentapeptide in 30% yield and a 20 amino acid gp120 fragment peptide in 20% yield. In another example by Danishefsky a homogeneous Erythropoietin *N*-glycopeptide fragment 1-28 was synthesized.[79] The 1-NH_2 disaccharide, hexasaccharide, and dodecasaccharide, were conjugated to a 28 residue peptide by the Lansbury reaction, in yields of 70%, 30% and 0%, showing the influence of the *N*-glycan size.

As it was not possible to join the biantenary dodecasaccharide to the long peptide directly, the glycan donor was first conjugated to a shorter 22-28 peptide fragment in 65% yield, which was then elongated by native chemical ligation[80-82] to give the desired *N*-glycopeptide.[83-84]



Scheme 17. Principle of Lansbury method by amide coupling of glycan donors to the Asp side chain.

1.3.3 Chemoenzymatic glycopeptide synthesis

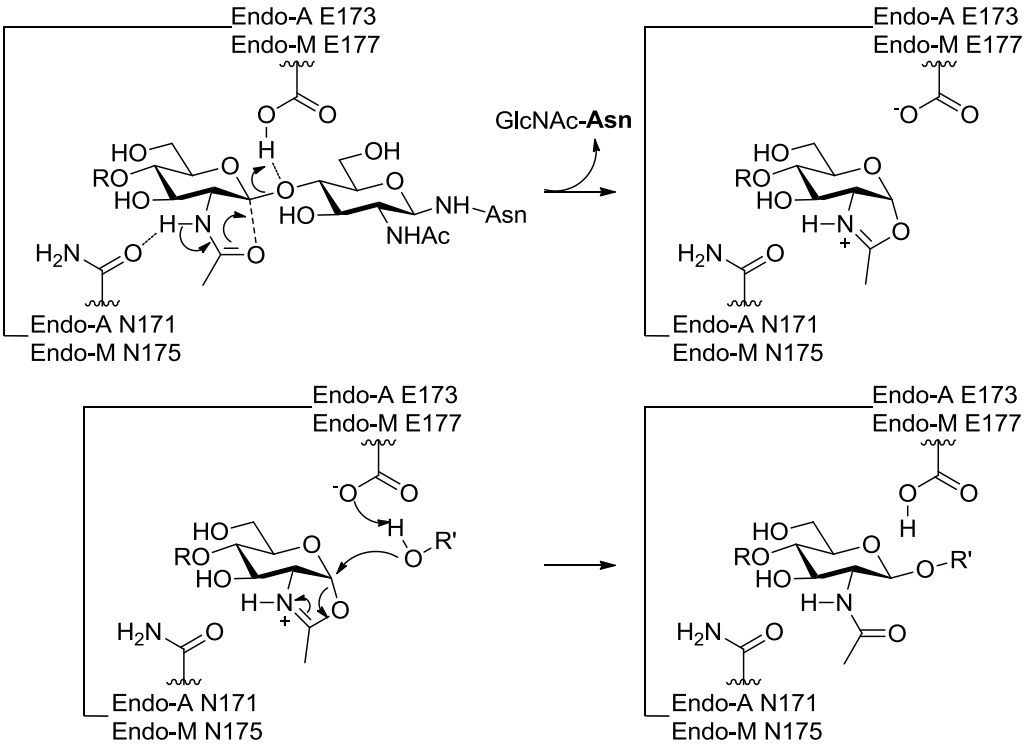
Another approach to synthesize *N*- and *O*-glycopeptides is to use chemoenzymatic methods.[85-86] Initially the peptide backbone is synthesized with one or a few glycans attached to the peptide backbone, and is then further glycosylated through enzyme catalysis.[87-88]

Different glycosyl transferases are employed together with UDP, GDP or CMP activated monosaccharide donors to sequentially extend the *O*-glycopeptide acceptors. However, if the enzymatic couplings do not go to completion, separation of the glycopeptide product mixture might be challenging. For *N*-glycopeptides, endoglycosidases can in one step be employed to transfer large oligosaccharide donors to a monoglycosylated GlcNAc-Asn side chain of peptides/proteins. In this thesis *N*-glycopeptides were prepared by the latter method, which is described in more detail below.

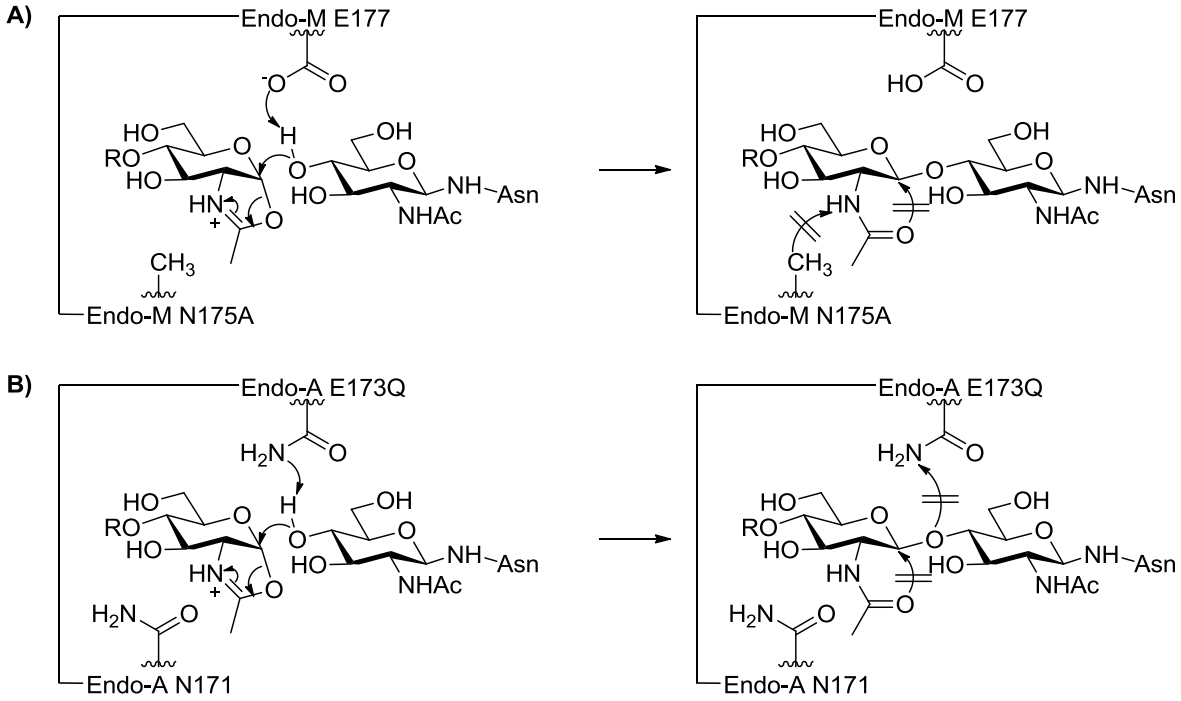
1.3.3.1 Endoglycosidase catalyzed oligosaccharide transglycosylation

For chemoenzymatic *N*-glycopeptide synthesis, *endo*-β-*N*-acetylglucosaminidases (ENGases) is an important enzyme class, which targets the chitobiose bond, a β-(1→4)-bond between two GlcNAc residues in the *N*-glycan pentasaccharide core structures.[89-90] Endo-H isolated from *Streptomyces plicatus* or *Streptomyces griseus*, was specified to cleave this bond,[91] two other enzymes; Endo-A from the bacteria *Arthrobactor protophormiae*[92] and Endo-M from the fungi *Mucor hiemalis*,[93] were able to exchange glycans through the chitobiose bond in a

transglycosylation reaction.[94] Endo-A is specific for high-mannose type *N*-glycans and Endo-M for complex type *N*-glycans. Using this approach, defined oligosaccharides could in one step be coupled[95-96] to a specific site on glycopeptides[97-99] or glycoproteins.[100]



Scheme 18. Enzymatic reaction mechanism for glycopeptide hydrolysis and coupling employing native Endo-A or Endo-M.



Scheme 19. Transglycosylation mechanism of mutant Endo-M-N175A and Endo-A-E173Q, which hydrolytic activity were suppressed.

However, until recently the yields employing this method were limited by hydrolytic activities of the native enzymes and further required large excess of complex oligosaccharide donors, which usually not are available in large amounts. To solve these problems, the method was improved by using endoglycosidase mutants less prone to hydrolyze the formed glycopeptide products and by using synthetic oxazoline donors, which function as more reactive transition state analogs.

Several studies were done to identify the endoglycosidase crystal structure[101-102] using thiol substituted oxazoline[103] transition state inhibitors. Similar to chitinases[104-106] or *N*-acetyl-β-hexosaminidases,[107-108] the mechanism of the enzymatic coupling was suggested to involve a Glu E173 on Endo-A or E177 on Endo-M[109] and an Asn N171 on Endo-A or N175 on Endo-M, resulting in oxazoline formation and hydrolysis of the β-(1→4) Chitin glycosidic bond. Catalyzed by E173/E177 the 4-OH from a GlcNAc glycopeptide acceptor then initiate an attack to the oxazoline intermediate, resulting in the formation of a new *N*-glycopeptide glycosidic bond. By using an oxazoline donor the glycan coupling is independent on the glycan chitin bond hydrolysis step and by mutation[110] of the Asn into Ala on Endo-M 175 or Glu into Gln on Endo-A 173, the hydrolysis properties of the ENGases are dramatically reduced, which hinder hydrolysis of the formed glycopeptide product.

In previous work, different types of oxazoline donors have been evaluated using both the endo-A and Endo-M ENGases.[111-113] For Endo-A, the shortest donor tolerated was a Manβ-(1→4)GlcNAc oxazoline,[114] additional protecting group modifications or oxazoline donors further extended with mannoses generating pentasaccharide core or high-mannose structures were also accepted. Extensions of the pentasaccharide core by using glycan donors containing terminal Gal or GlcNAc were not accepted by the enzyme. This means that complex-type *N*-glycan oxazoline donors are not accepted by Endo-A.

The ENGase enzymatic glycosylation could be performed on peptide level as well as on protein level, with multiple glycosylation sites modified in one step.[115] The GlcNAc monoglycosylated protein must first be obtained, various methods are available,[116-117] followed by ENGase glycan extension. In one example from Wang and co-workers,[118] a protein was expressed in *E*. coli,[119-120] a tailored lipid-linked oligosaccharide (LLO) containing a GlcNAc at the reducing end was attached to the Asn on the protein by the oligosaccharyl

transferase (OST), undecaprenyl-diphosphooligosaccharide-protein glycotransferase (PglB), and the *N*-glycan was trimmed by α-*N*-acetylgalactosaminidase to give a GlcNAc monoglycosylated protein. Then a $MaN_3GlcNAc$ oxazoline donor was transferred onto the protein by the Endo-A enzyme.

1.4 Analytical methods

1.4.1 NMR structural analysis of synthetically prepared carbohydrates

1.4.1.1 NMR chemical shifts of carbohydrate ring protons and common protection groups

A general overview of chemical shifts of ^{1}H- and ^{13}C NMR signals from common protecting groups and carbohydrate ring structures are illustrated below (**Figure 2**). Chemical shifts of the carbohydrate ring protons and carbons are highly influenced by the protecting groups employed, glycosidic linkages, configuration and conformation of the stereogenic centers.

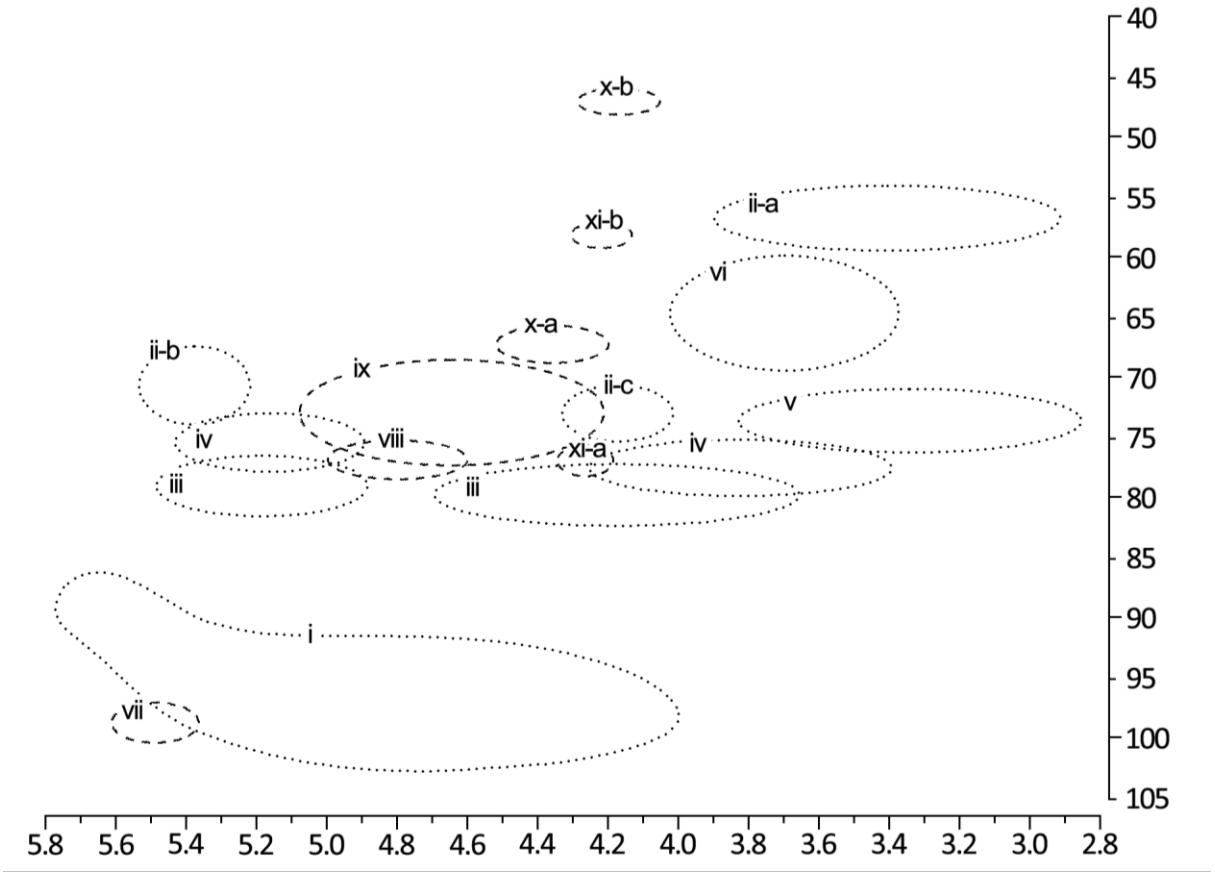


Figure 2. Summary of NMR chemical shifts from sugar structures as well as common protection groups. i) H1; ii) H2, N-linked(a)/acetyl(b)/glycosylated(c); iii) H3; iv) H4; v) H5; vi) H6; vii) benzylidene acetal CH; viii) Troc-CH_2; ix) benzyl CH_2; x) Fmoc CH_2(a)/CH(b); xi) Thr/Ser-C*H*α(a), Thr-CHβ(b).

Anomeric proton and carbon NMR signals are characterized by the extreme down-field shifts at 4.5-5.5 ppm for H-1 and at 90-105 ppm for C-1 (i). Additionally the α/β linkage configurations show differences in chemical shifts. Usually the α-anomeric H-1 signal is found around 5 ppm and β H-1 signal around 4.5 ppm. The α-anomeric C-1 signal is found 5 ppm up-field shifted compared to β C-1 signal. The H-2 signals are found in several areas, depending on if the 2-position is linked to a nitrogen (ii-a); to an acetyl or benzoyl (ii-b); or as a glycosylation site (ii-c). H-3 and H-4 signals often have similar chemical shifts, around 3.6-4.5 ppm (iii, iv). H-5 (v) signals are found with proton chemical shifts slightly up-field from the H-3 and H-4. The H-6 proton pair is found around 3.5-4.4 ppm and has a characteristic carbon shift around 60-65 and if glycosylated in this position around 70 ppm (vi). In general the substitution of the hydroxyl groups in the pyranose ring has a great impact on the ring proton and carbon chemical shifts. For instance acetylation and other electron withdrawing substituents have deshielding effects usually resulting in a down-field shift of the proton signal with around 1 ppm.

CH or CH_2 signals from common protecting groups or amino acids found in the carbohydrate NMR shift region are also listed: benzylidene acetal CH signal (vii), Troc-CH_2 signal (viii), benzyl CH_2 (ix), Fmoc-CH_2 signal (x-a) and CH signal (x-b), Thr/Ser-C*H*α (xi-a) and Thr-CHβ (xi-b).

1.4.1.2 Coupling constants and 2D-NMR experiments in carbohydrate structure elucidation

The α/β linkage in the anomeric center and other protons in the pyranose ring are distinguished by measurements of the ^3J coupling constants, which is dependent on the dihedral angel of vicinal hydrogen atoms. According to Karplus, trans-diaxial hydrogens with a dihedral angel of 180° have a large coupling constant, axial-equatorial and equatorial-equatorial orientations with a dihedral angel of 60° have a small coupling constant. The measurements of ^3J coupling constants are of outmost importance for carbohydrate structural elucidation. Besides ^1H and ^{13}C spectra, 2-dimentional (2D) NMR experiments give additional information about proton and carbon connectivities.

TOCSY and COSY NMR spectra give information about couplings of adjacent protons, COSY links ^3J couplings to the nearby protons and TOCSY gives additional information about long

distance couplings connecting signals from the same spin system, in this case, from the same pyranose ring.

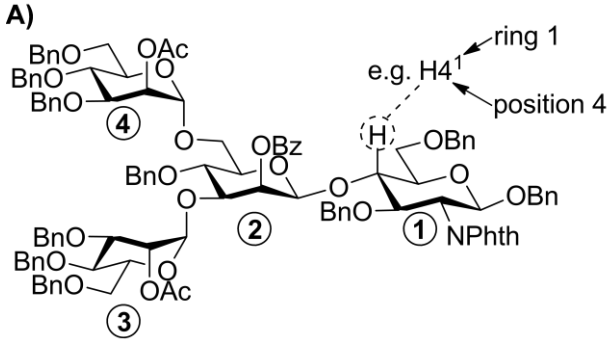
HSQC and HMBC experiments give information about linkages between carbon and protons. In HSQC spectra the direct H-C connectivities are outlined, which is highly useful to get an overview of the CH pairs and their corresponding chemical shifts. Additionally, signals with overlapping proton shifts in the 1D spectra are here separated from each other by the differences in carbon shift. The HSQC spectra are usually measured as phase-sensitive experiments, CH/CH_3 vs. CH_2 with positive/negative signals are labeled in different colors. HMBC spectra which measures C,H bond connectivities at 2-3 bond distance, are highly useful for structural assignment of the connectivities between different CH pairs in the ring and to elucidate for instance connections between glycosidic bonds.

1.4.1.3 NMR structural elucidation of a tetrasaccharide N-glycan building block

One tetrasaccharide synthesized in this thesis work, compound **87** (**Scheme 20** A) was selected as example to illustrate the process of interpreting complex glycan structures.

To assign each proton and carbon pair and to assign characteristic signals to the specific structure, the HSQC spectrum was initially inspected. By empirical knowledge of chemical shifts (**Figure 2**), certain signals could be distinguished (**Figure 3**), four anomeric CH pairs signals were observed (ii), proving the compound to be a tetrasaccharide. Acylated signals are found in area (iii) should be $H2^2$, $H2^3$ and $H2^4$ with high proton shifts, while the NPhth linked $C2^1$ should have a low carbon shift, identified in area (vii).

A)



B)

Ring Nr.	Positions 1	2	3	4	5	6
1	Bn	N	Bn			Bn
2		Bz		Bn		
3		Ac	Bn	Bn		Bn
4		Ac	Bn	Bn		Bn

Scheme 20. NMR interpretation of tetrasaccharide **87**. **A)** The chemical structure with numbering of the rings; **B)** Analysis of the substitution on each position, Ac = acylated, Bn = benzylated, Bz = benzoylated, N = NPhth.

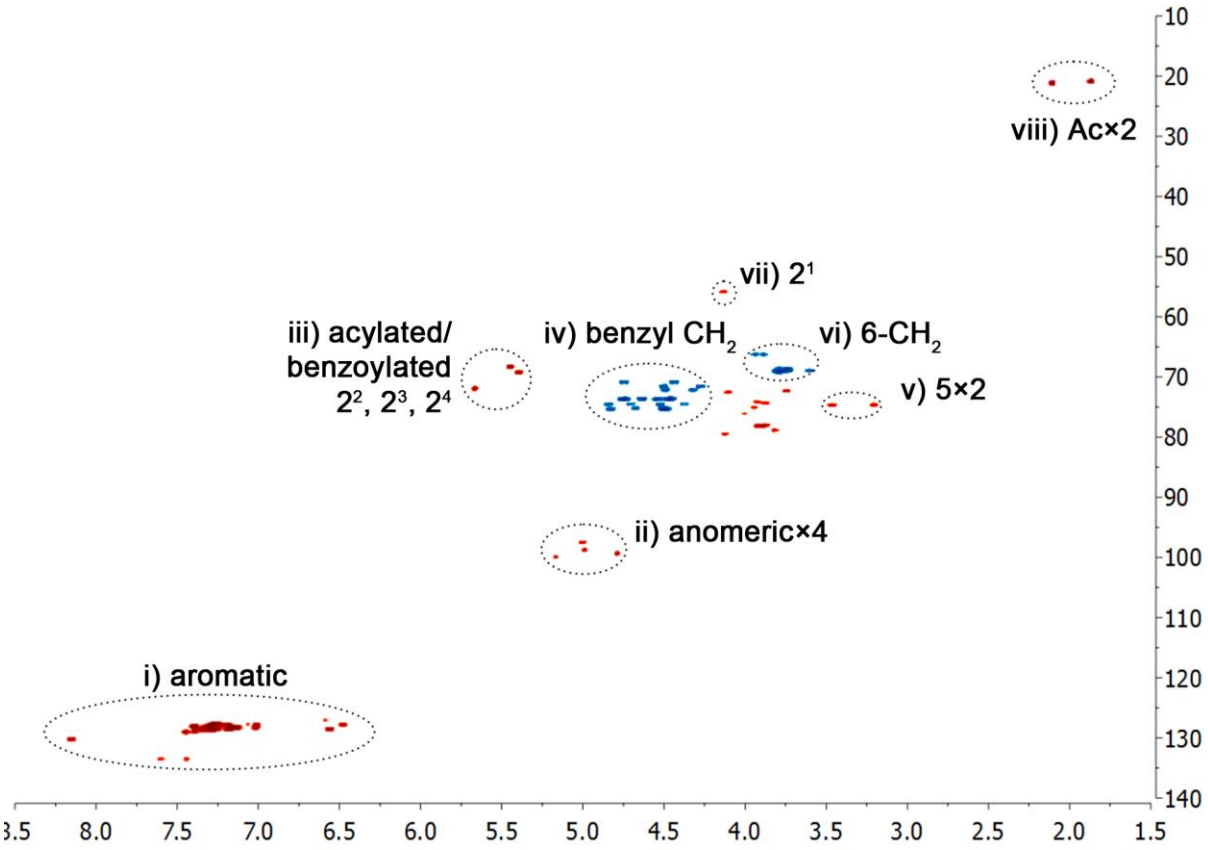


Figure 3. Characteristic signals in the HSQC spectrum.

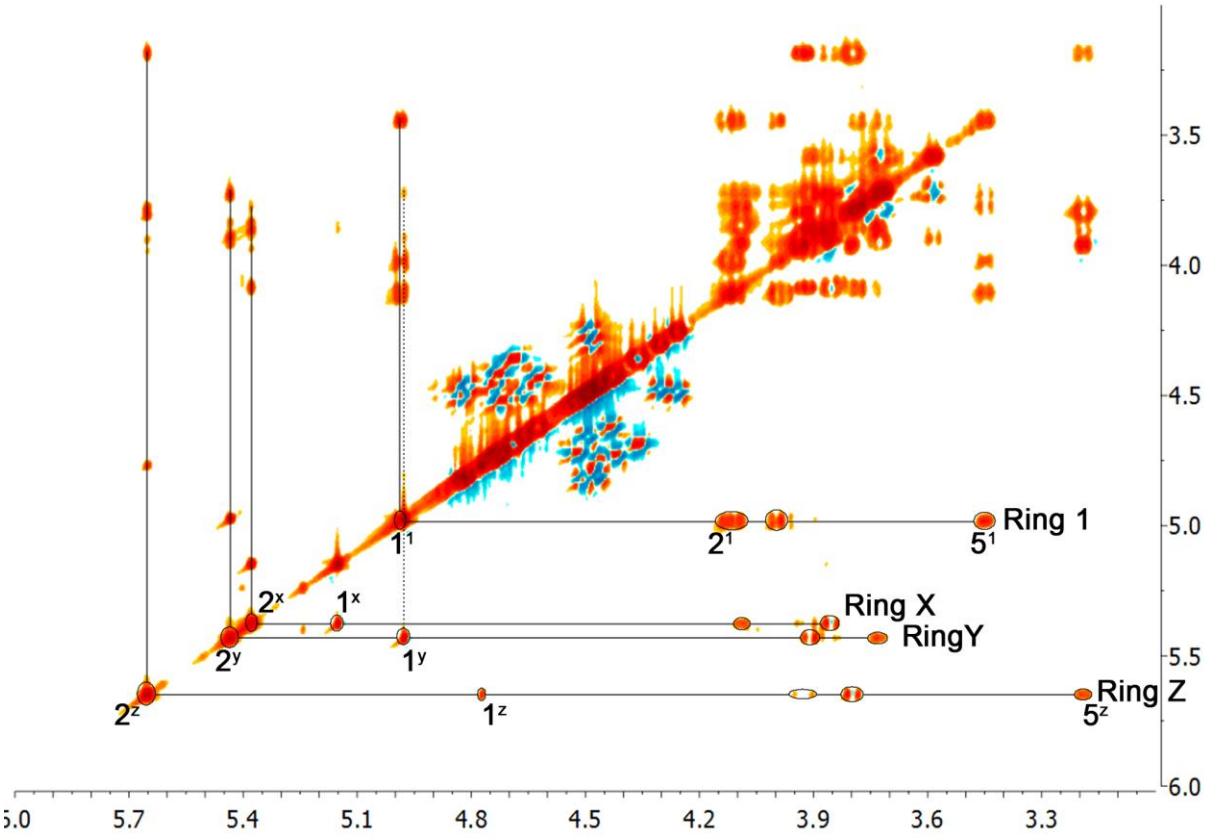


Figure 4. TOCSY spectrum identifying each ring system.

COSY and TOCSY spectra give information of couplings between protons, the COSY show H,H-couplings at three bond distances and TOCSY additionally provide information about long distance couplings and connectivities within the pyranose ring spin systems. Using TOCSY the four pyranose rings systems were identified **Figure 4.**

By comparing HSQC and TOCSY signals, the GlcNAc ring 1 could be distinguished since the $H2^1$ has a charcteristic proton shift determined by HSQC. While the other three mannose ring X, Y and Z containing Ac or Bz linked H-2 signals were found at down field shifts.

By employment of HMBC, the three mannose rings were further distinguished since differences between benzoyl and acetyl connectivities were observed (**Figure 5**). The tertiary carbon from the acetyl group coupled to the CH_3 around 2.0 ppm (**Figure 5** v) and the benzoyl carbonyl carbons coupled to the protons in the aromatic region (**Figure 5** i). The different carbonyl signals additionally coupled to the corresponding H-2 signal in the different rings and thereby the Z-ring was identified as pyranose ring 2, while ring X and Y remained as the two terminal mannose residues. The cross-coupling of the phthalimide carbonyl to $H2^1$ (**Figure 5** iv) was further observed in this area.

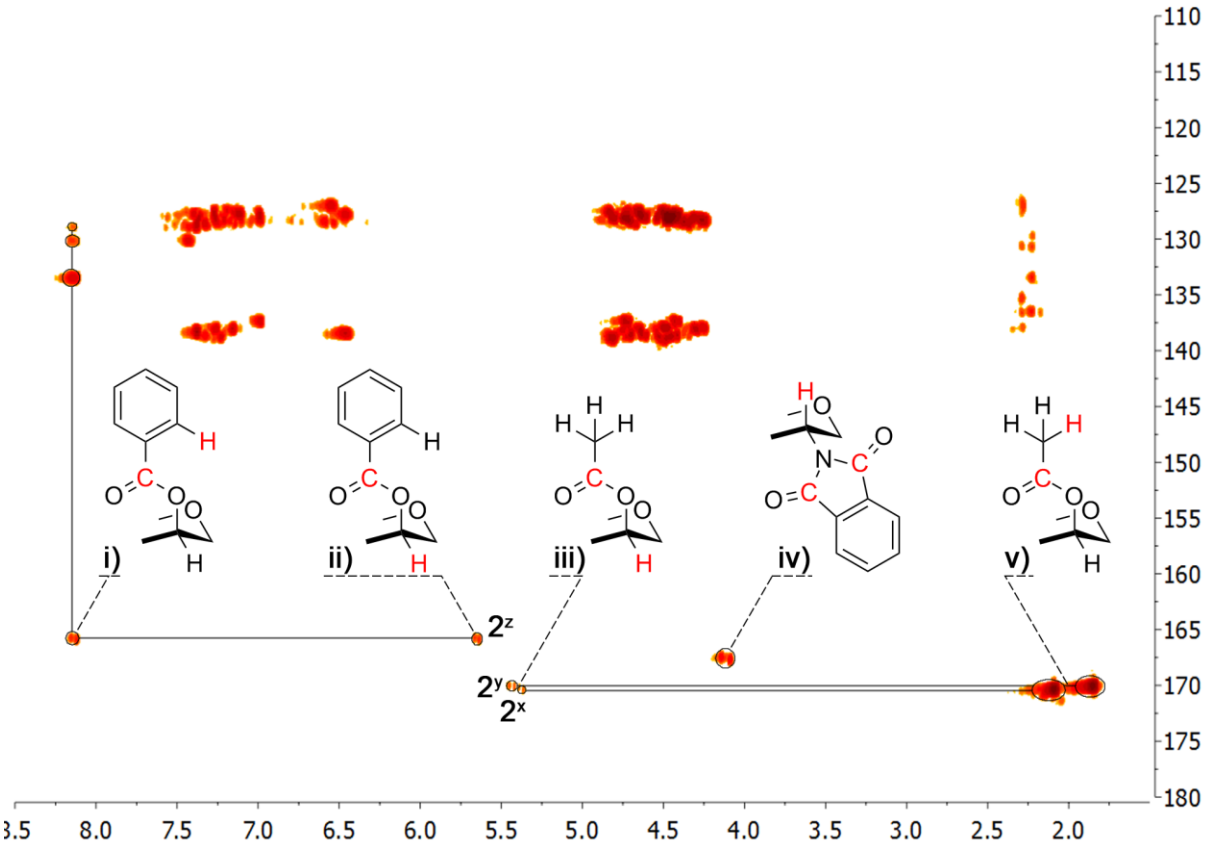


Figure 5. HMBC couplings of carbonyl carbons to protons at two or three bond distance.

To distinguish the remaining mannose residues extending the H-3 and H-6 position of the inner mannose (the assigned ring-2 structure), the anomeric HMBC cross couplings were investigated. The coupling signals were identified in pairs diagonally in HMBC (**Figure 6**) , the CH siganals involved in the HMBC cross-couplings are further marked, the marked × corresponds to the interacting signal in the HSQC spectrum (**Figure 7**). The two dashed lined rectangles represent the anomeric cross coupling of ring X and Y to the center mannose. The anomeric 1^{y} carbon was found to have a coupling to the 6^{2}-CH_2 (v and vi) and the anomeric 1^{x} carbon coupled to the 3^{2} proton(vii and viii), ring X was identified as 3-extended mannose and ring Y as the 6-extended.

After solving the key feature of the structure, a detailed interpretation was achieved by matching the assignments from the HSQC and TOCSY spectra (**Figure 8**).

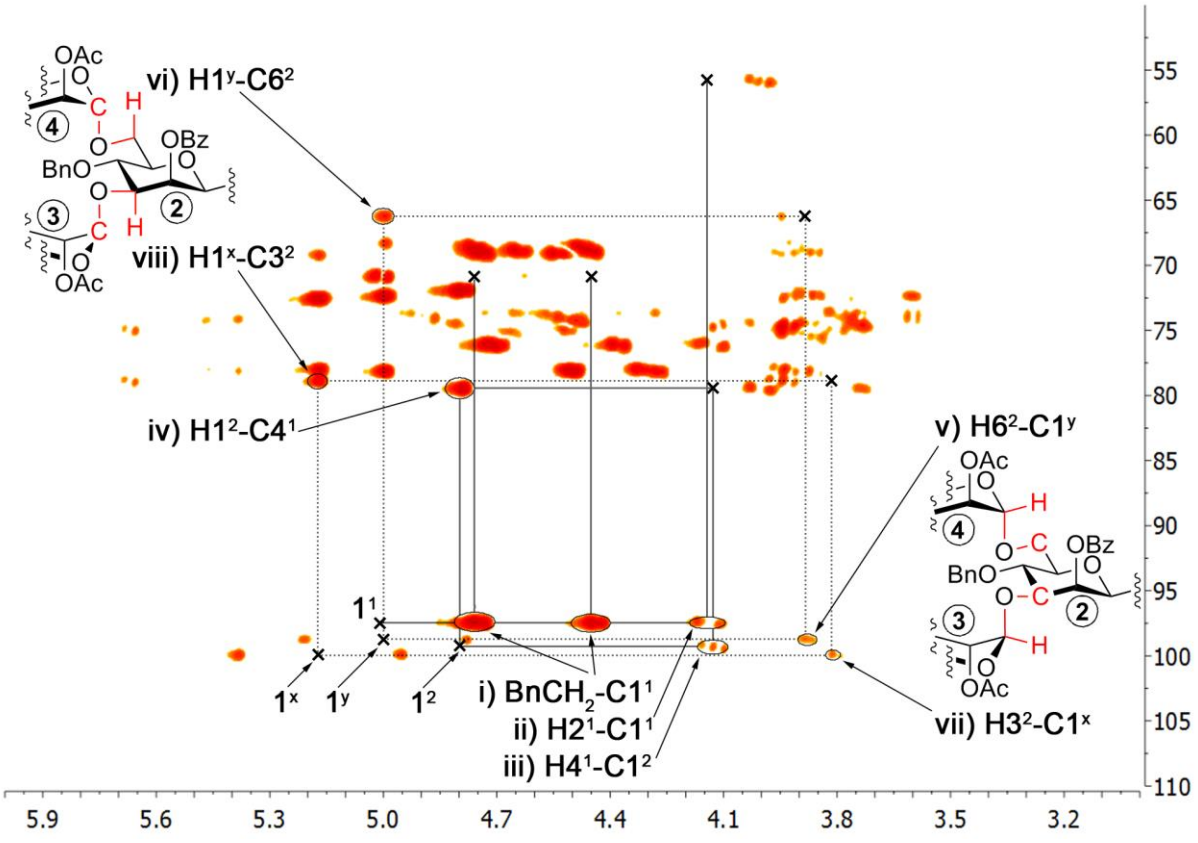


Figure 6. HMBC signals of the anomeric cross couplings

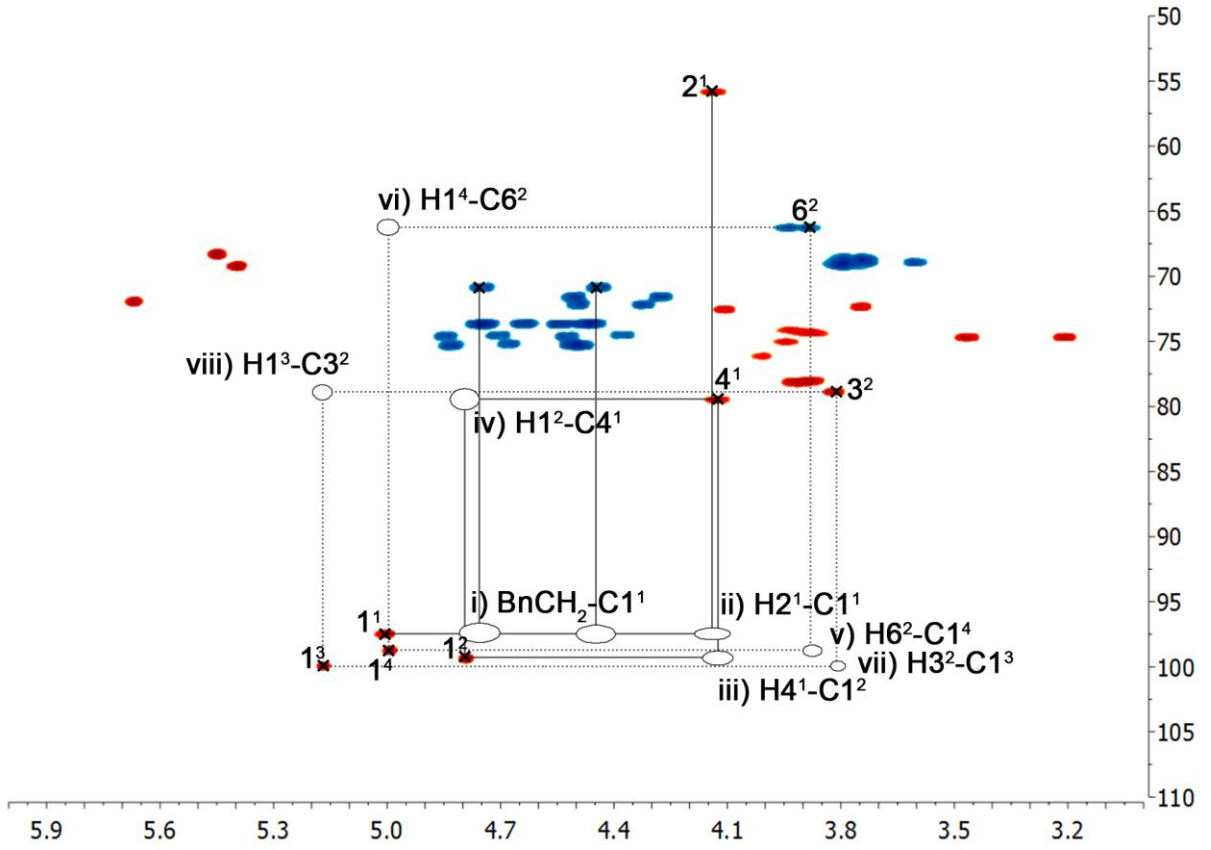


Figure 7. The corresponding HQSC signals identified from HMBC couplings.

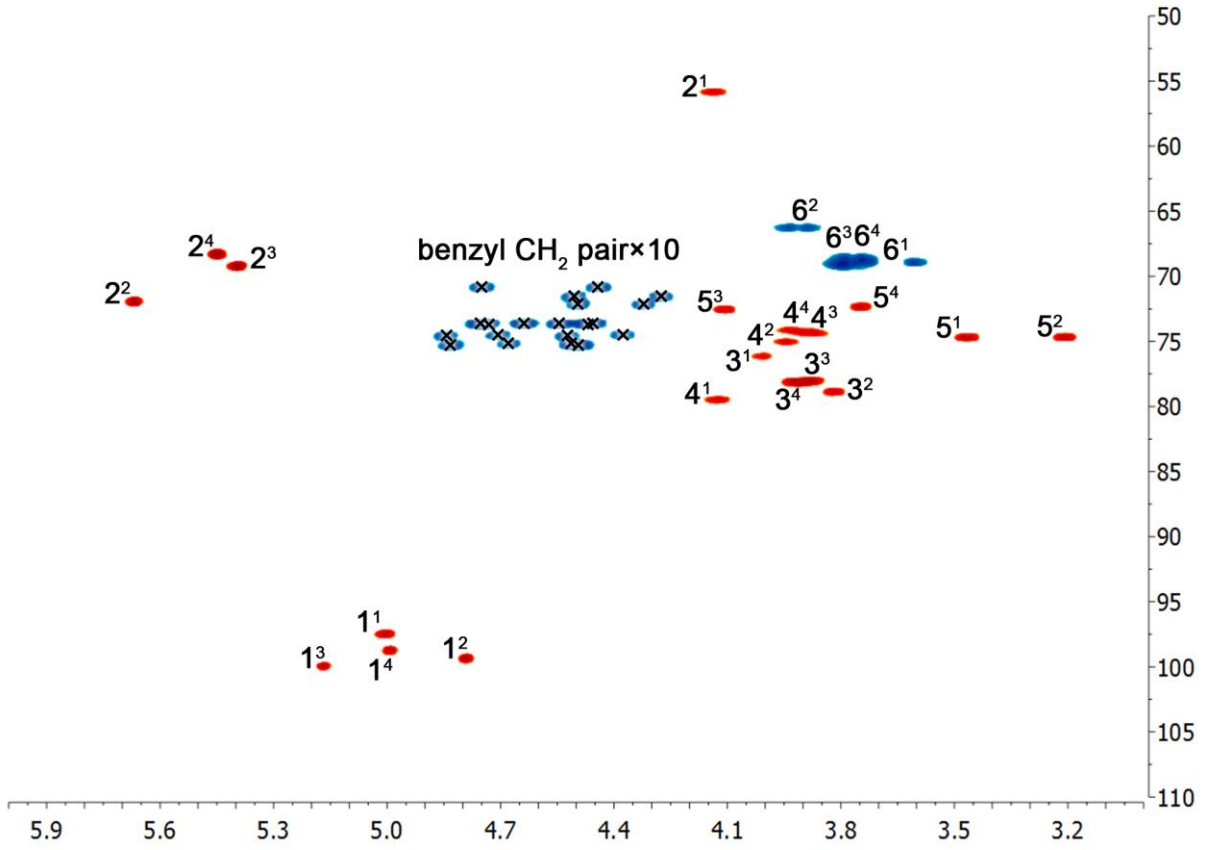


Figure 8. Fully interpreted HSQC spectrum.

1.4.2 MS structural analysis

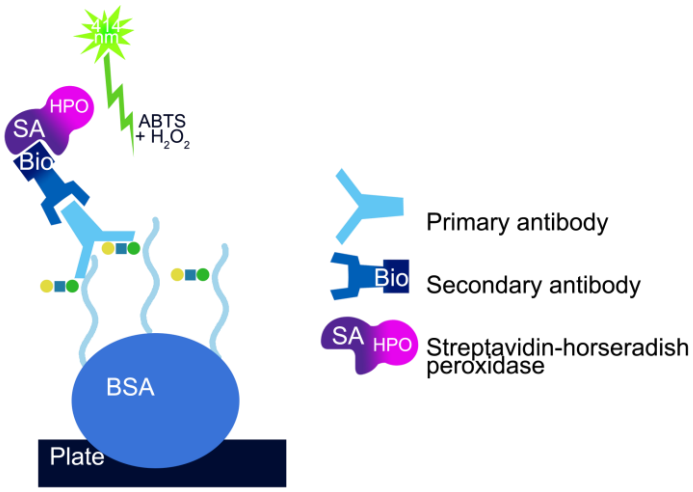
NMR enables identification and detailed characterization of glycans and glycopeptides. Mass spectrometry is a complement method that further support the identity of the synthesized or isolated molecules. For efficient analysis of more complex molecules, mass spectrometry have become the most powerful methodolgy since it enables fast analysis with low amounts of material. Further, technical and bioinformatic developments have resulted in that mass spectrometry have revolutionized the field of proteomics. In glycoproteomic analysis, glycoproteins are typically trypsin-digested into (glyco-)peptides before proceeding with MS detection. By direct analysis of intact glycopeptides, knowledge about the glycan structure, glycosylation site and protein identity may be obtained from the same molecule. Enriched fractions of glycopeptides originating from complex samples can be analysed via the use of proteomic dedicated protocols such as nano-flow liquid chromatography – electrospray ionisation/tandem mass spectrometry (LC-ESI-MS/MS). By the use of collision induced dissociation (CID), the identity of saccharide units can be designated based on stepwise glycosidic fragmentation patterns. By electron capture/transfer dissociation (ECD/ETD) the entire glycan mass can be pinpointed to its attachment site, but further glycan structure information is here not provided. By collisional activation, using positive mode higher energy dissociation (HCD), information about both the peptide backbone and glycan structure can simultaneously be obtained. Matrix-assisted laser desorption/ionization (MALDI) spectrometry are commonly used for analysis of intact proteins in ranges of higher molecular weights. In this thesis MALDI was employed to characterize glycopeptide-protein conjugates (common molecular weights of 50-100 kDa), to determine the loading of glycopeptide antigen attached on the protein immune carrier.

1.4.3 ELISA experiment for antibody evaluation

The enzyme-linked immunosorbent assay (ELISA) is one of the most common methods used to estimate the amount of induced serum antibodies (antibody titers) that recognize the antigen structure. The ELISA may further be used to briefly evaluate the binding specificity of the induced antibodies. The principle of this experiment is described as follows (**Scheme 21**). The antigen structure alone or conjugated BSA or other protein carriers are immobilized on a microtiter plate, the surface is blocked with a blocking solution. Then incubated with a

primary antibody, in our study immunized rabbit serum was applied in dilution series. After washing a secondary antibody was applied, here an anti-rabbit antibody labeled with biotin, followed by incubation with streptavidin-horseradish peroxidase, which binds to the biotin group of the secondary antibody. A mixture of ABTS and H_2O_2 was then added in each well resulting in oxidation of ABTS by H_2O_2 and horseradish peroxidase catalysis. A green color is obtained and the absorption measured at 414 nm.

ELISA neutralization experiments are further employed to elucidate the specificity of the antibody sera. In a neutralization experiment different peptides or antigen related structures, competing with the surface-coated antigen BSA conjugate, are added to the wells and incubated simultaneously with the primary antibody. If the (glyco-)peptide structures are able to interact with the primary antibody sera, a reduction of the detected ELISA signal “a neutralization” to the antigen structure will be observed. ELISA neutralization experiments consume relatively large amount of precious synthetic glycopeptides, microarray experiment were here considered to be a material- and time-saving alternative.

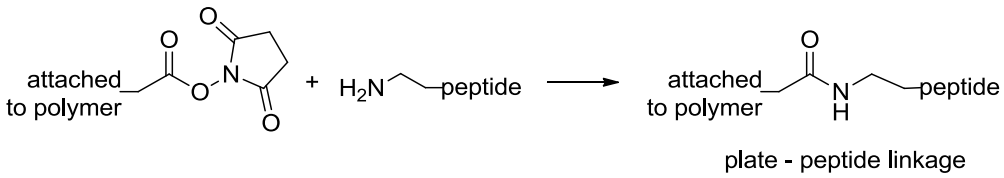


Scheme 21. Basic principle of ELISA experiment to evaluate the binding efficiency of a primary antibody to antigen structure. Primary antibody Secondary antibody Streptavidin-horseradish peroxidase

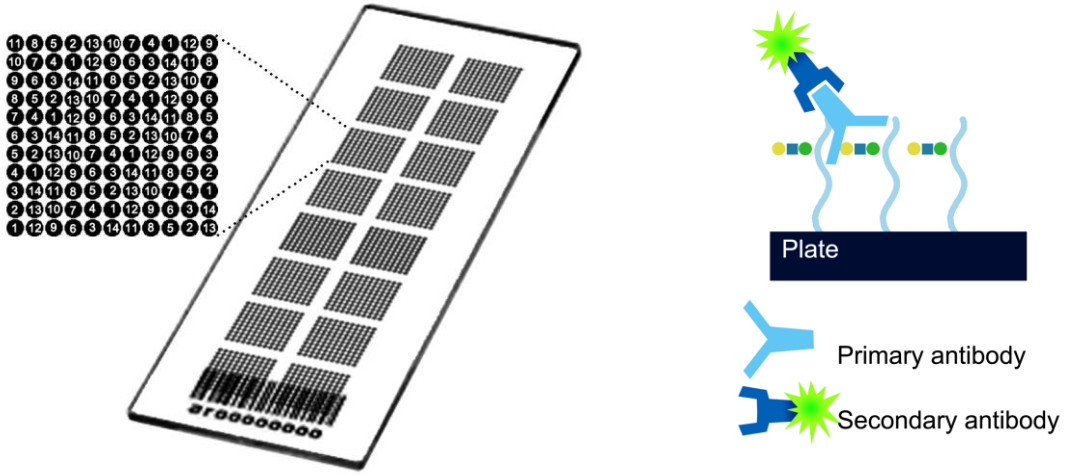
1.4.4 Microarray experiments for antibody and lectin binding evaluation

Microarray experiments were here used as an alternative approach for evaluation of binding specificity of induced antibody sera as well as to elucidate binding recognition of glycan binding proteins, lectins. A library of glycopeptides was spotted in a defined pattern on a

microarray glass slide (Shott® slide H) coated with *N*-Hydroxysuccinimide esters on the surface. The free amine groups on a N- or C-terminal spacer of the peptides, could react with the surface forming a native amide bond and thereby covalently immobilize the peptides (**Scheme 22**).



Scheme 22. The amide linkage from plate to peptides.



Scheme 23. Microarray printing and antibody recognition.

Each microarray slide could be divided into different wells, a common 8×2 format was for instance selected in order to spot a library of peptides with several spot duplications spread evenly over the well (**Scheme 23**). The spotted slide was kept in a 75% humidity chamber for immobilization followed by blocking with ethanolamine in sodium borate buffer to cap remaining reactive groups. The antibody incubation steps on the microarray slide are then rather similar to the ELISA experiments. A primary antibody, for instance immunized rabbit serum or a glycan binding protein are incubated with the immobilized peptide array, then a secondary antibody with a fluorophore are reacting to the primary antibody or lectin. The fluorescence are quantitatively detected by using a scanner. Sometimes the lectin or primary antibody can be directly labeled with biotin or a fluorophore for efficient read out. By comparing the relative fluorescence intensities of the different spots, the antibody or lectin

binding recognition to the peptide library are determined. In addition to evaluation of binding specificities of glycopeptides and peptides, the microarray technology has a great impact in glycomics to study interactions with different glycan binding partners and in DNA arrays to evaluate gene transcription.[121-122]

Project 1: *Chemoenzymatic strategies for synthesis and application of N-glycopeptides*

2.1 Introduction protein *N*-glycosylation

Glycosylation is one of the most complex post-translational modifications in eukaryotes. In *N*-glycoside biosynthesis all *N*-glycoproteins share a common pentasaccharide core structure, Man(α1-3)[Man(α1-6)]Man(β1-4)GlcNAc(β1-4)GlcNAc. Based on different extensions from the core, the *N*-glycosylated oligosaccharides are divided into three types.

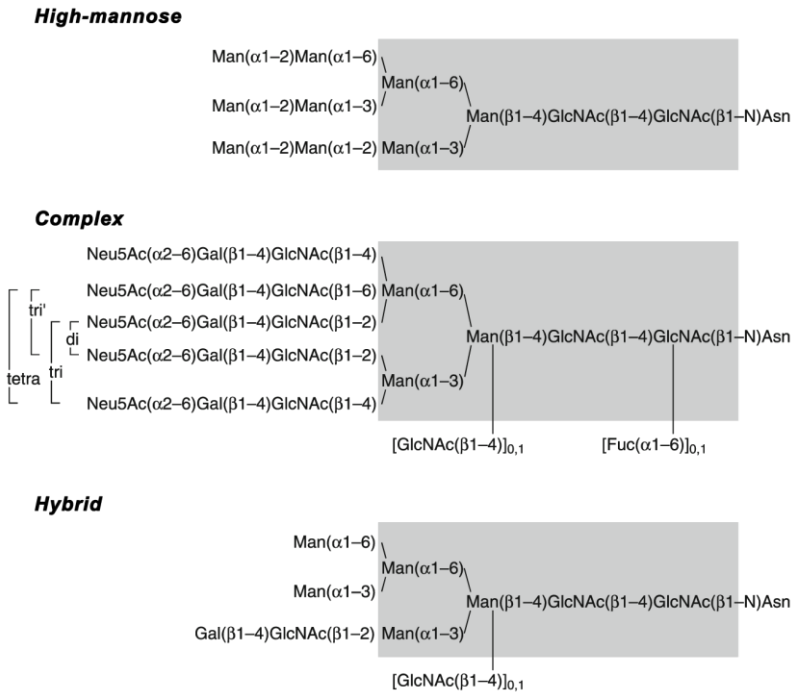


Figure 9. Homogeneous native *N*-glycan structures.[123]

In the high mannose form, the pentasaccharide core structure is extended with mannose residues, linear in the 2-position or 3,6-branched. In complex type *N*-glycosylation, the pentasaccharide core is extended with Gal-GlcNAc units (LacNAc, β1-4 or β1-3) in the core mannose 2-position, in a linear fashion or 2,6/2,4-branched. The hybrid form, consists of a combination of the high-mannose and complex type glycosylation. These *N*-glycans have been shown to have a wide range of specific biological functions and are essential in intra- and intercellular interactions. For instance, *N*-glycosylation is involved in protein folding processes, controlling of resistance to proteases and interactions to cellular receptors.[124-125]

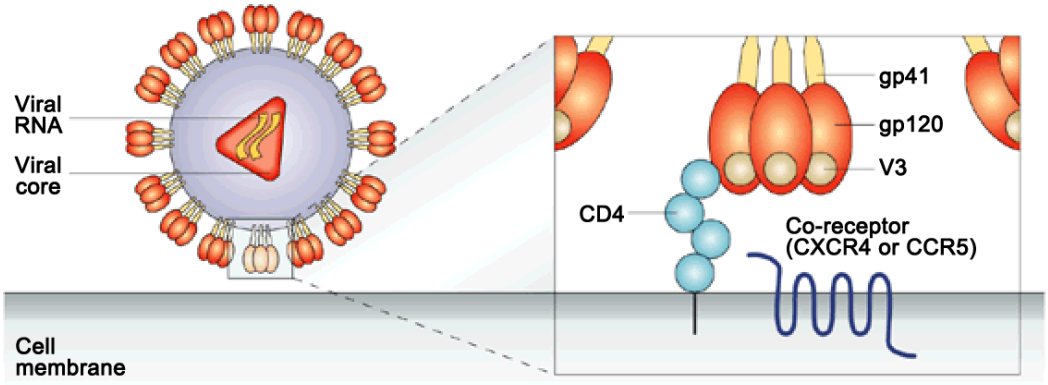


Figure 10. Extracellular docking glycoprotein gp120 and transmembrane glycoprotein gp41 on HIV virus.[126]

Availability of homogenous glycoprotein or glycopeptide material is important to generate tools to study these processes. Intensive work has been made to synthetically prepare homogeneous complex *N*-glycopeptides[127-128] and glycoproteins.[129-130] One well studied example is the glycoprotein gp120[131-133] and gp41[134-136] on HIV, which interacts with host cell surface receptors resulting in infection. The gp120 and gp41 glycoproteins are heavily *N*-glycosylated. By analysis of the glycoprotein gp120, it was found to contain 13 complex type *N*-glycans and 11 high-mannose *N*-glycan structures.[137-138] These glycans shield immune recognition and proteolysis by the host immune system and are also involved in the HIV infection and transmission processes.[139-140] As a result, the *N*-glycan is considered as the primary target for vaccination[141-142] against HIV infection.[143] Another example is the cell-surface *N*-glycosylation on tumor cells, where *N*-glycans are involved in many important recognition processes such as cell-adhesion, cell-differentiation and tumor metastasis. Here the *N*-glycosylation is dramatically changed, for instance the structural changes involve increased core fucosylation, attachment of a fucose connected α-1,6 to the innermost core GlcNAc residue. Increased complex type *N*-glycan branching due to changed expression of the β 1-6 GlcNAc transferase GnT-V also occurs. The changed glycosylation on tumor glycoproteins are potential targets in the discovery of novel biomarkers for diagnosis. Glycomimetics may further be developed to hinder tumor cell-surface adhesion events involving tumor associated glycans.[144-145]

2.2 Project aim

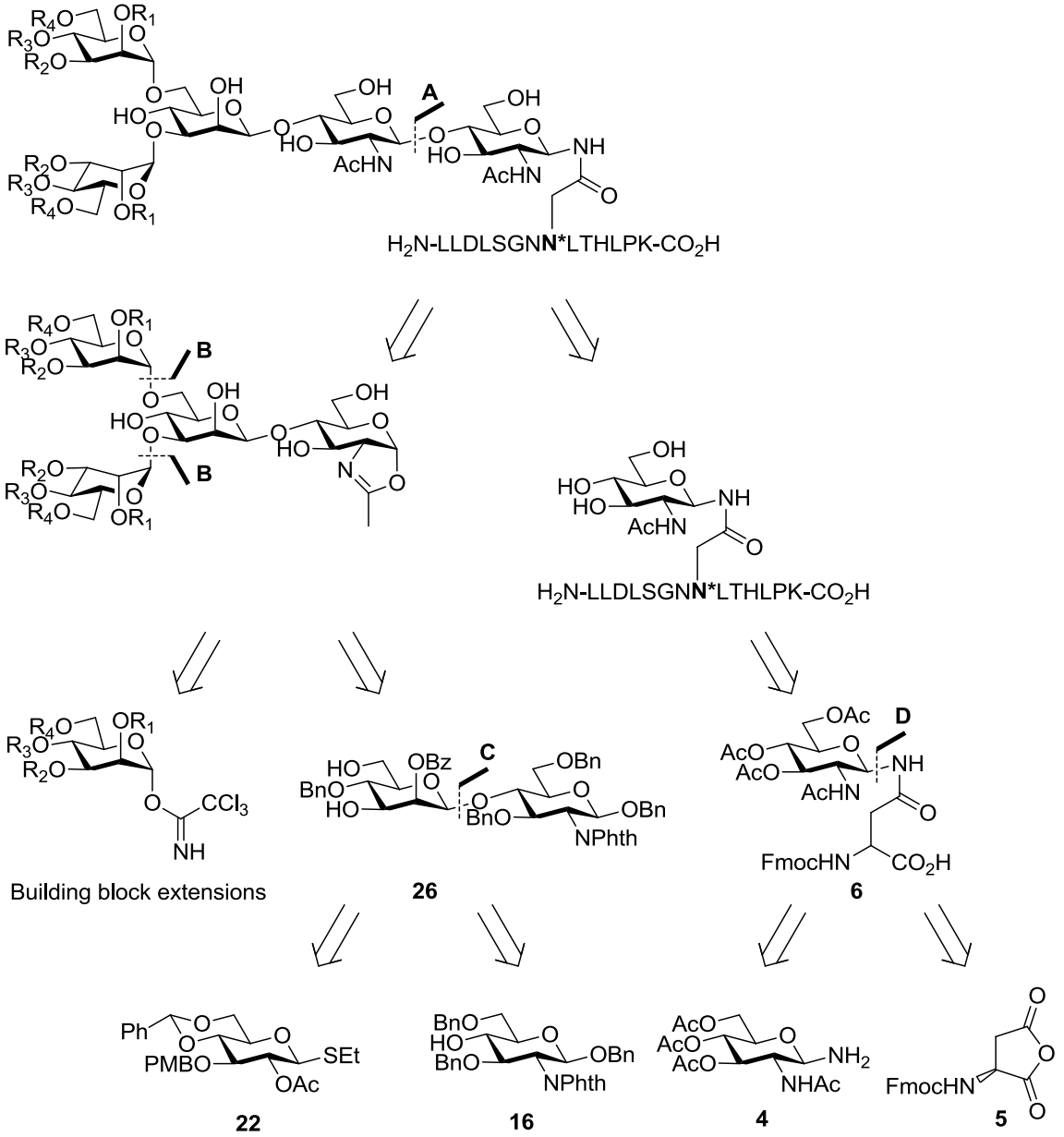
This PhD project aimed at making *N*-glycopeptides available for glycomic and glycoproteomic studies. By using a chemoenzymatic approach, combining synthetic *N*-glycan oxazoline donors with GlcNAc-Asn glycopeptides, an efficient strategy was available to synthesize high mannose and complex type *N*-glycopeptides carrying well-defined oligosaccharide structures. In this way glycopeptide libraries were going to be constructed and applied in microarray analysis to study binding events between glycopeptides and proteins. Synthetic *N*-glycopeptides were further aimed to be used to study MS-CID/HCD fragmentation, which would be helpful in order to predict the identity of *N*-glycopeptide structures, oxonium ion traces may here be useful by searching for glycopeptides employing bioinformatics tools. Additionally, availability of *N*-glycopeptide standards was of interest for evaluation of glycopeptide purification/enrichment protocols, and also of interest to spike-in as MS standards in glycoproteomic quantification analysis.

The synthesis of the oxazoline glycan donors and evaluation of the endoglycosidase chemoenzymatic coupling were considered to be a major challenge in this project. An *N*-glycopeptide with complex type N-glycosylation are often isolated from egg yolk and converted to a unique *N*-glycan oxazoline donor building block, however in this way only low amounts are obtained and it would not enable to make a library of different chemically defined *N*-glycans and *N*-glycopeptide structures.[146-147] Therefore, total synthesis of complex type and high mannose type oligosaccharides was considered as the best approach to generate *N*-glycopeptide structures. Since all *N*-glycans share a Man_3-$GlcNAc_2$ pentasaccharide core, the oligosaccharides synthesis was planned in a systematic fashion using common precursor building blocks responsible for glycan extension. After oxazoline donor conversion, the *N*-glycans were coupled to *N*-GlcNAc peptides using endoglycosidase.

2.3 Synthetic strategy

An optimized synthetic route was designed dividing the large *N*-glycopeptide structure into two parts, which could be connected by a chemoenzymatic approach using Endoglycosidase A or M (**Scheme 24** A).[148] The parts consisted of a peptide backbone glycosylated with a GlcNAc residue on the Asn amino acid and an oligosaccharide oxazoline donor responsible

for glycan extension. By using different oxazoline donors that could be coupled to a large number of peptide sequences in parallel employing an endoglycosidase enzyme, *N*-glycopeptides could be efficiently generated. Such an approach would be suitable for building up peptide libraries for microarray or MS analysis.

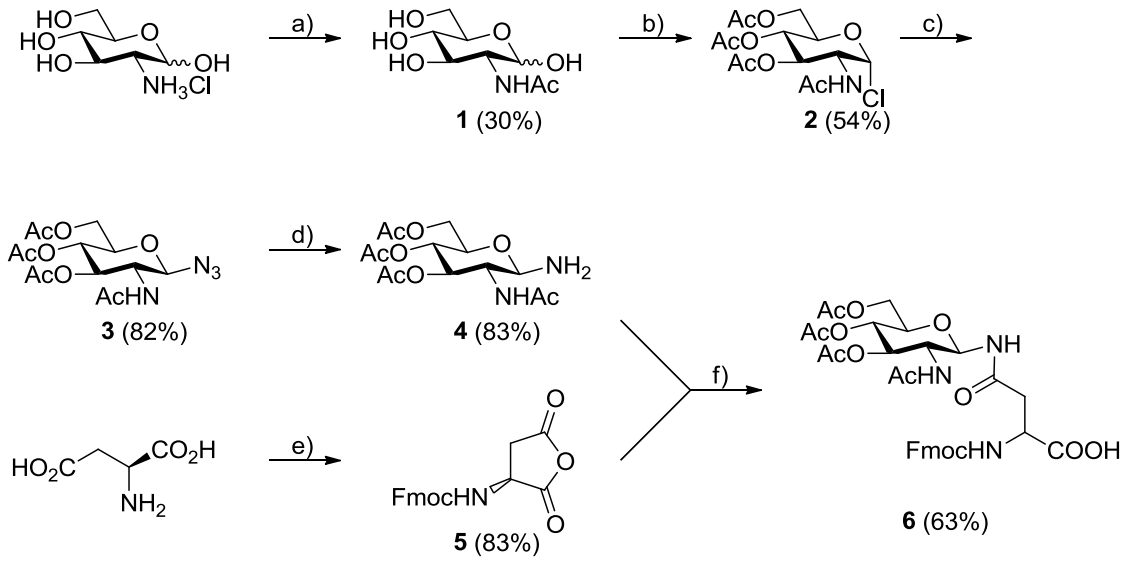


Scheme 24. Retrosynthetic analysis for *N*-glycopeptides synthesis by oxazoline conjugation to peptide.

Different oxazoline donors were synthesized, however since all *N*-glycans contain the same pentasaccharide core structure, a common disaccharide building block could be prepared Manβ1-4GlcNAc **26** and extended at the terminal Man 3- and 6-positions using different Man trichloroacetimidate donors (**Scheme 24** B). This functional disaccharide unit could be derived from the glucose donor **22** and a 4-OH glucosamine acceptor **16** (**Scheme 24** C). The neighboring equatorial acetyl group on the glucose donor would here ensure the formation of a β-glycosidic-linkage, which after coupling could be converted to an axial benzoyl group forming a β-mannoside. Removal of a *p*-methoxybenzyl and selective benzylidene acetal ring opening would generate free hydroxyl groups in the 3,6 positions, which then is available for additional glycan elongation. The GlcNAc glycopeptide part could be generated by Fmoc-solid-phase peptide synthesis (Fmoc-SPPS) using a *N*-glycosyl asparagine Fmoc-amino acid **6**, which according to a recent publication,[149] could be efficiently synthesized in an one-step coupling from the *N*-acetylglucosamine **4** and aspartic anhydride (**Scheme 24** D). The traditional synthesis[150-151] would involve a multi-step synthesis with the formation of glycosylamines[152-153] and coupling[154-155] to selectively protected aspartic acid.[156]

2.4 Synthesis of the *N*-GlcNAc-aspartate Fmoc-amino acid

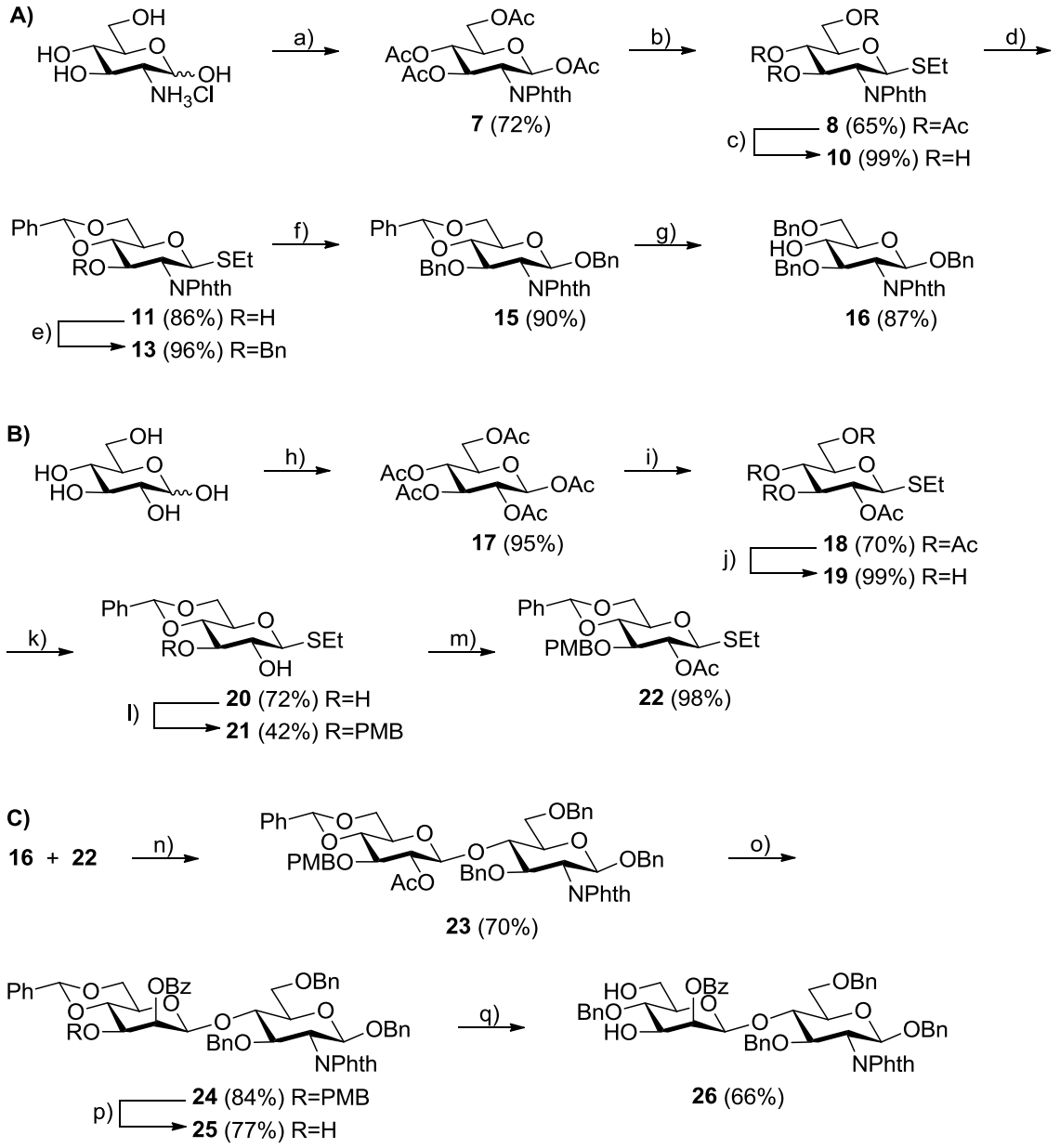
Starting from the *N*-glucosamine hydrochloride (**Scheme 25**), the free amine was protected with an acetyl group, followed by treatment with acetyl chloride to produce the peracetylated GlcNAc chloride **2**. The axial chloride was then replaced with an equatorial azide using TBAHS and NaN_3. The azide in the anomeric center was reduced by catalytic hydrogenation using Pd(C) to obtain the amine **4**.[157] The *N*-Fmoc-aspartic anhydride was prepared in two steps in high yield, and was then employed in the glycan coupling reaction. The choice of solvent was here critical for the coupling, in less polar solvents, an isoasparagine derivative is formed as main product, while more polar solvents promote the desired glycosylation. The reaction therefore performed in DMSO with consumption of the amino-glycoside followed by a ninhydrin test, indicating the reaction was finished in 2 h. The obtained glycosylated Fmoc-amino acid **6** was then employed in Fmoc-SPPS to generate the desired glycopeptides.



Scheme 25. a) glucosamine hydrochloride, MeOH, NaOMe, r.t. 2 h; AcOH, Ac_2O, r.t. 24 h; b) AcCl, 40 °C 24 h; c) TBAHS, NaN_3, Sat. $NaHCO_3$, r.t. 2 h; d) H_2, Pd(C), MeOH, r.t. 2 h; e) Asn, Na_2CO_3 in H_2O, Fmoc-OSu in THF, r.t. 20 h; Ac_2O, 120 °C, 5 min; f) DMSO, r.t. 2 h.

2.5 Synthesis of the Manβ1-4GlcNAc core

The formation of a β-mannose glycosidic bond is difficult, since both the anomeric effect and the use of neighboring group participation would favor the formation of an α-mannosyl product. To generate the β-mannosyl product in the synthesis of the Manβ1-4GlcNAc disaccharide core, a strategy was selected converting a β-glucoside into a mannose.[158-159] By neighboring group participation, a β-glucosidic bond would easily be obtained by coupling a 2-*O*-acetyl glucose donor to the GlcNAc acceptor. After removing the-+ acetyl group under basic condition and conversion to an equatorial triflate, a good leaving group is generated. According to a S_N2 mechanism, nucleophile attack from axial direction by a benzoate ion, would generate an axial 2-benzoyl product and thereby convert a glucose into the desired mannose. Therefore, the synthesis route was designed to prepare a glucose donor and glucosamine acceptor, to ensure the β-linkage first, and then convert the glucose to mannose.



Scheme 26. a) glucosamine hydrochloride in MeOH, NaOH in H_2O, 0 °C, 30 min; phthalic anhydride in acetone, r.t. 24 h; NaOAc, Ac_2O, 160 °C, 2 h; b) EtSH, $BF_3{\cdot}Et_2O$, DCM, r.t. 3 h; c) 0.08 M NaOMe in MeOH, r.t. 4 h; d) α,α-dimethoxytoluene, *p*-toluenesulfonic acid, DMF, 50 °C 1 h; e) NaH, BnBr, THF, reflux 2 h; f) BnOH, NIS, TfOH, molecular sieves, 0 °C 1h; g) sodium cyanoborohydride, 2N HCl in Et_2O, THF, 0 °C 30 min; h) NaOAc, Ac_2O, 160 °C, 1 h; i) EtSH, $BF_3{\cdot}Et_2O$, DCM, 0 °C 1 h; j) 0.05 M NaOMe in MeOH, r.t. 2 h; k) α,α-dimethoxytoluene, *p*-toluenesulfonic acid, DMF, 50 °C 1 h; l) dibutyltin oxide, MeCN, refluxed 24 h; CsF, 4-methoxybenzyl bromide, r.t. 48 h; m) Py/Ac_2O, r.t. 4 h; n) **22**, NIS, TfOH, molecular sieves, DCM, 0 °C 1h; o) 0.05 M NaOMe in MeOH/DCM 2:1, r.t. 72 h; Py, Tf_2O, DCM, 0 °C 15 min; tetrabutylammonium benzoate, Tol, 120 °C 2 h; p) CAN, MeCN/H_2O 9:1, r.t. 30 min; q) 1 M BH_3 in THF, TMSOTf, DCM, 0 °C 30 min, r.t. 16 h.

Other alternative routes for the synthesis of β-mannoside products were developed by Gorin and Perl,[160] in principle employing Königs-Knorr conditions, using an α-bromo mannosyl donor to generate β-products via a S_N2 mechanism. However, the reaction yields are usually moderate, and the method is not widely applied anymore. Instead of a bromo donor, an α-triflate donor intermediate was developed by Crich and coworkers.[161-163] The reaction was performed in a similar S_N2 style[164] and a 4,6-benzylidene acetal ring and a relatively large Bn or PMB protecting group at O3 here disfavor oxacarbenium cation formation.[165]

N-glucosamine hydrochloride was selected as starting material for the acceptor part (**Scheme 26** A), the hydrochloride was removed first and the free amine was protected with phthalic anhydride,[166] since phthalimide groups are quite stable during the whole synthesis process and favor β-linkage formation on the anomeric position by neighboring group participation. The glycan was then treated with sodium acetate / acetic anhydride at reflux to obtain the β-acetate product **7**. The acetyl group in the anomeric position then reacted with ethanethiol (or thiophenol) promoted by boron trifluoride diethyl etherate[167-168] to give the β-SEt product **8**. The acetyl groups on position 3, 4 and 6 were then removed by sodium methoxide in methanol to give **10**. The benzylidene acetal protection was then performed at pH 3.5 - 4 in DMF under reduced pressure, 50 mbar, at 50 °C, thereby methanol produced from the reaction was continuously removed as the reaction proceed to give **11**.[169] Benzylation of the remaining hydroxyl in the 3-position was unsuccessful in DMF, by altering the solvent to THF[170] and after refluxing for 2 hours, the reaction proceeded in excellent yield to give **13**. To replace the SEt in the anomeric position with an *O*-benzyl protecting group, a NIS/triflic acid approach was applied and the reaction was performed smoothly to give **15**. For benzylidene acetal ring opening to give a free hydroxyl group at the 4-position,[171] compound **15** was treated with sodium cyanoborohydride and HCl at optimized conditions, to give the *N*-glucosamine acceptor **16**.

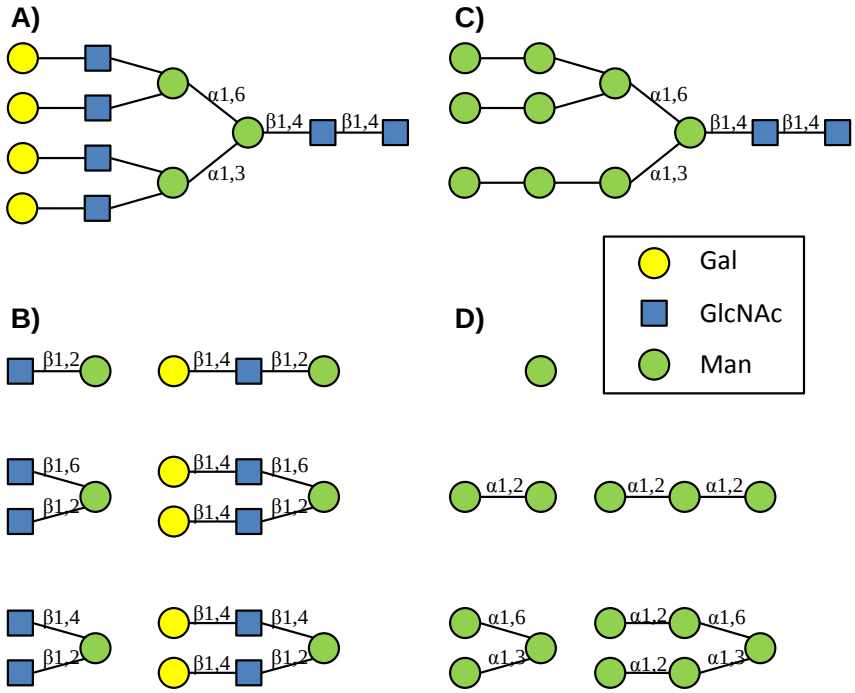
The synthesis of the glucose donor **22** started with acetylation of D-glucose by treatment with sodium acetate and acetic anhydride under reflux to give the β-acetylated product **17** (**Scheme 26** B). By reacting with ethanethiol and boron trifluoride diethyl etherate, the acetate was converted to the thioglycoside donor **18**. The remaining acetyl groups were then removed using sodium methoxide in methanol followed by benzylidene acetal formation

according to the standard conditions described above. A few procedures[172-173] were available for selective benzylation on the glucose 3-position in the presence of a free hydroxyl group in 2-position, the employed dibutyltin oxide promoted benzylation suffered from byproduct formation and only moderate yields were achieved to produce **21**. The remaining free 2-hydroxyl group was acetylated to give the SEt glucose donor **22**.

The NIS/TfOH promoted coupling reaction of donor **22** and acceptor **16** was finished after 1 h at 0 °C and the coupling product **23** was obtained in a good yield (**Scheme 26** C). A deacetylation step followed[174] and treatment with triflic anhydride then generated a triflate intermediate, which immediately was reacted with tetrabutylammonium benzoate to give the benzoyl group in axial position, converting glucose into mannose obtaining **24**. To prepare the disaccharide building block for further extension, the *p*-methoxybenzyl protecting group in the mannose 3-position was removed using cerium ammonium nitrate to give **25**.[175] A free hydroxyl group in the mannose 6-position was obtained by benzylidene acetal ring opening using borane, catalyzed by TMSOTf,[29] to give the 3,6-hydroxyl Manβ1-4GlcNAc acceptor **26**.

2.6 Design of building block elongations

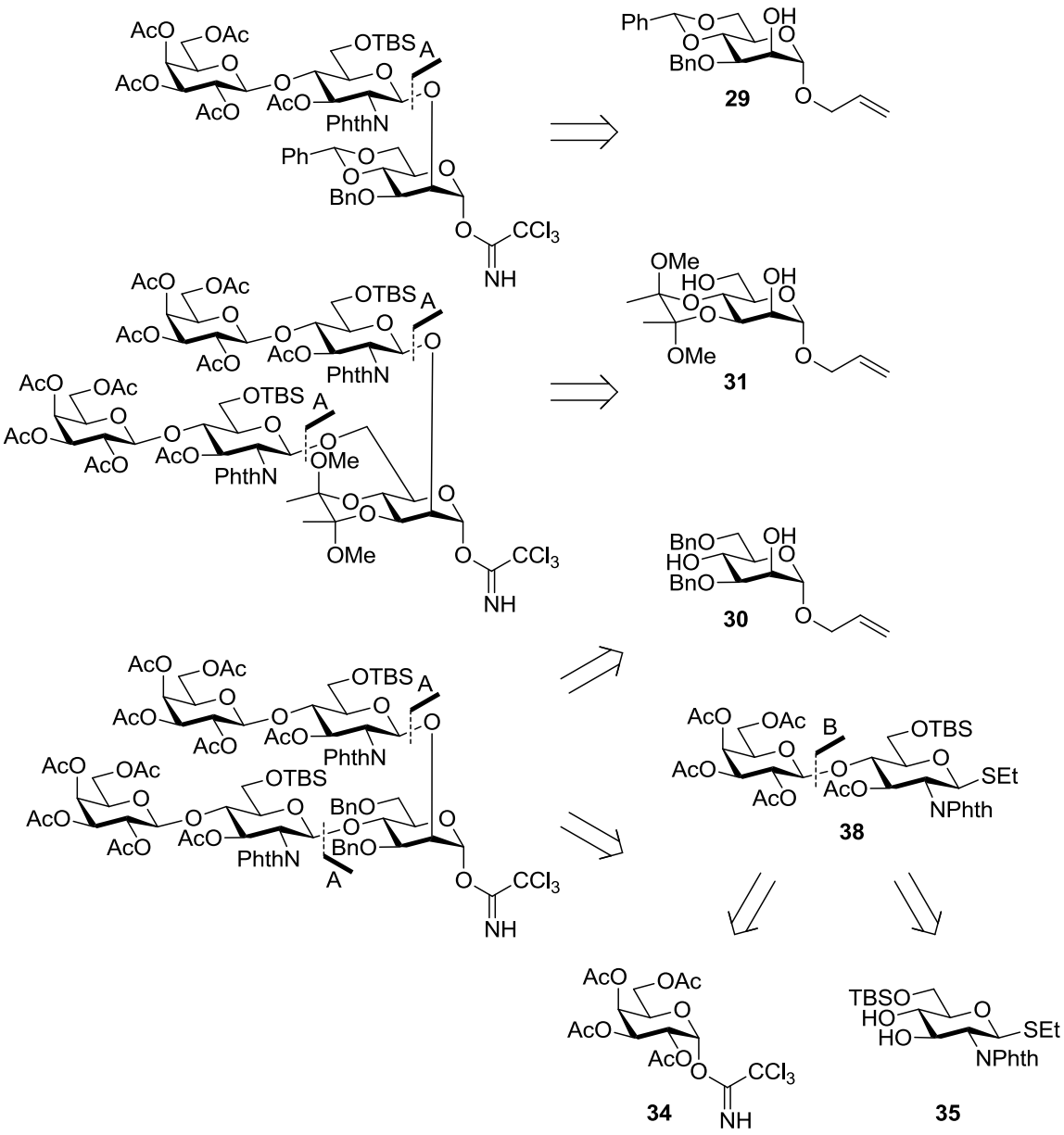
In order to prepare more complex oligosaccharide oxazoline donor building blocks for later use in couplings to monoglycosylated GlcNAc-Asn *N*-glycopeptides, the synthetic Manβ1-4GlcNAc disaccharide core structure **26** was extended with different trichloroacetimidate donors at the 3,6 position on mannose. The extension building blocks were divided into two types, resulting in complex type (**Scheme 27** A) or a high mannose type (**Scheme 27** C) *N*-glycosylation. In the complex type, the mannose was extended with GlcNAc monosaccharide or Glaβ1-4GlcNAc disaccharide units (**Scheme 27** B) forming a linear β1-2 linkage product or β1-2, β1-4 and β1-2, β1-6 branched products. For high mannose formation (**Scheme 27** C), a building block was repeatingly extending the mannose residue in 2-position for linear elongation or in 3,6-position for branched elongation(**Scheme 27** D).



Scheme 27. **A)** Complex type glycosylation; **B)** GlcNAc-Man and Gal-GlcNAc-Man linear and 2,4 / 2,6 branched elongation for generation of complex type building blocks; **C)** High mannose type glycosylation; **D)** Linear and 3,6 branched elongation for generation of high mannose type building blocks.

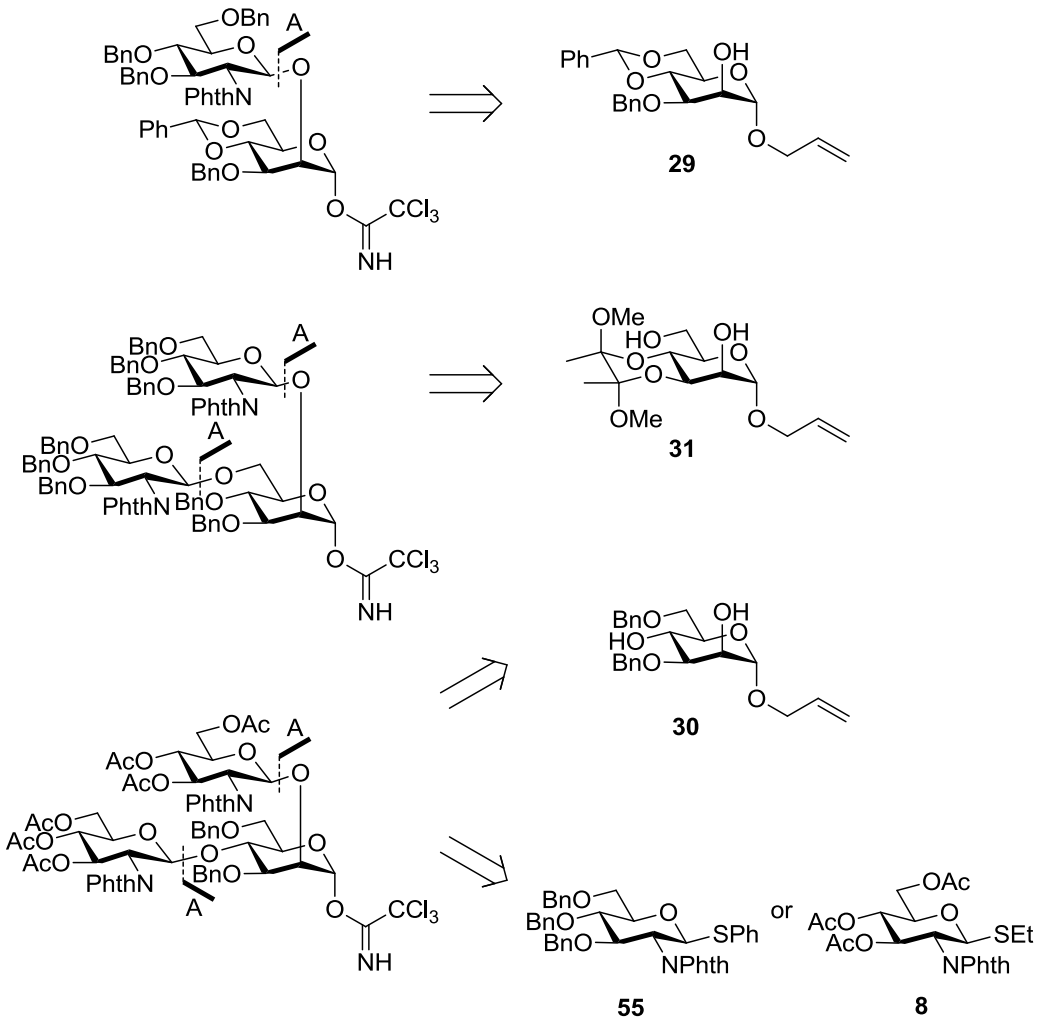
2.7 Synthesis of complex type building block extensions

A unified synthesis strategy was applied generating the different complex type *N*-glycan building blocks. A Galβ1-4GlcNAc disaccharide or a GlcNAc monosaccharide donor extended three kinds of mannose acceptors, a mannose with a free 2-hydroxyl **29** or a mannose with two free hydroxyls in 2,6- or 2,4-position compound **31** resp. compound **30** (**Scheme 28**, **Scheme 29**). An Allyl protecting group was used to protect the anomeric center of the mannose acceptors, which after glycan extension could be selectively removed by Wilkinson's or Baudry catalyst, converting the building block into a trichloroacetimidate donor.



Scheme 28. Retrosynthetic analysis for complex type building blocks with Gal-GlcNAc elongation.

The GlcNAc monosaccharide donor was available from the previous Manβ1-4GlcNAc disaccharide core synthesis. With simple protecting group manipulations, the reactivity of the GlcNAc donor could be modulated, and further Galβ1-4GlcNAc disaccharide donors with different protecting groups were also prepared to modulate the reactivity in the coupling reactions.



Scheme 29. Retrosynthetic analysis for complex type building blocks with GlcNAc elongation.

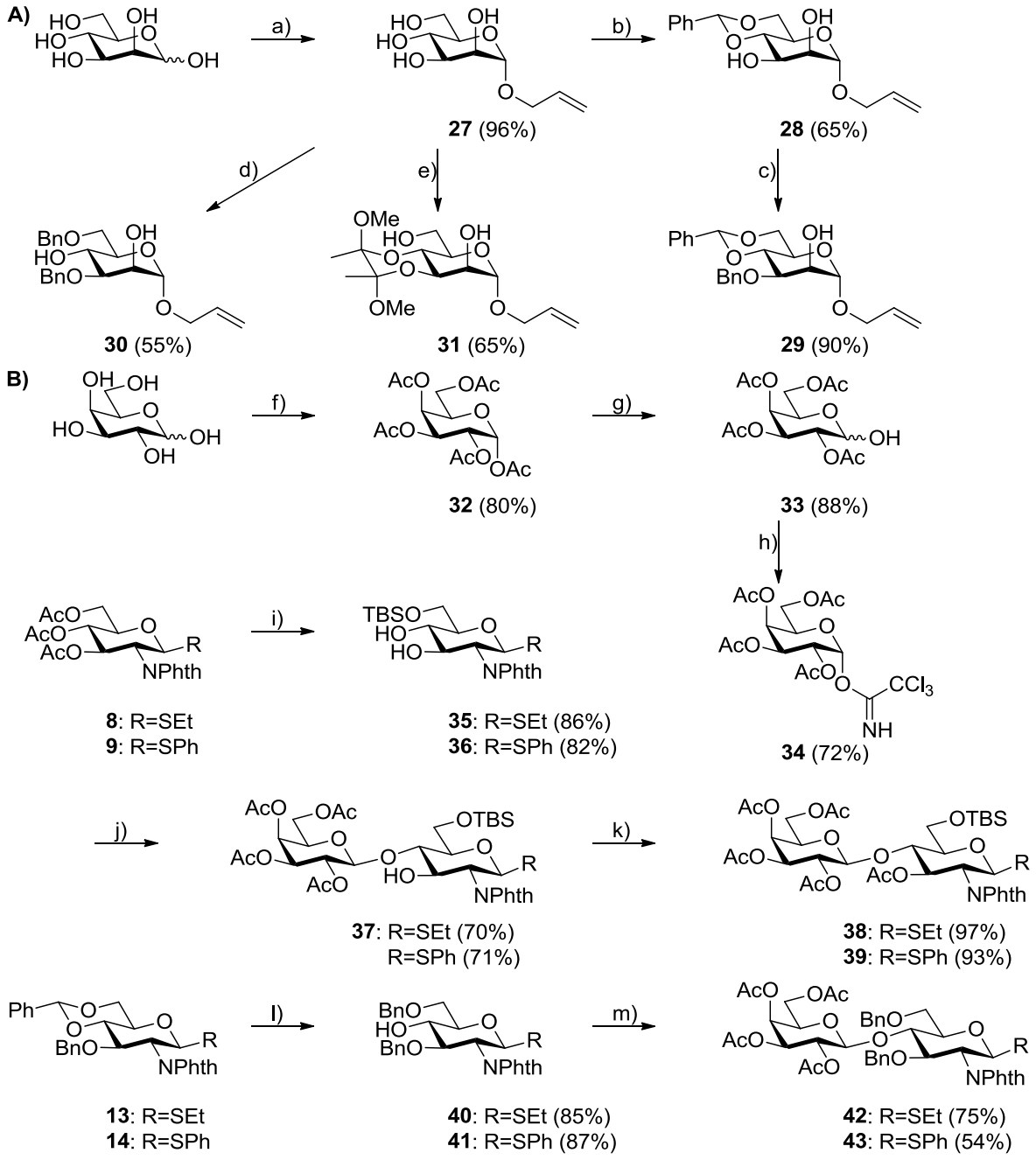
The synthesis of all mannose acceptor building blocks started with allylation of D-mannose in the anomeric center, generating **27** as a common intermediate (**Scheme 30** A). α,α-Dimethoxytoluene was used for 4,6-benzylidene acetal formation[176] to give **28** and for selective benzylation in the mannose 3-position, dibutyltin oxide was used[177] resulting in the 2-hydroxyl acceptor **29** in a high yield. A few methods were tested for selective benzylidene acetal ring opening[178-180] to generate a 2,4 or 2,6-hydroxyl acceptor, but the ring opening conditions were not compatible with the allyl group and therefore none of the attempts were successful. Instead, another procedure was adopted using tributyltin oxide that selectively benzylated the mannose 3- and 6-position in a moderate yield to give **30** as a 2,4- hydroxyl acceptor. By forming a 3,4-acetal ring with butanedione[181-182] **31** was formed as a 2,6- hydroxyl acceptor.

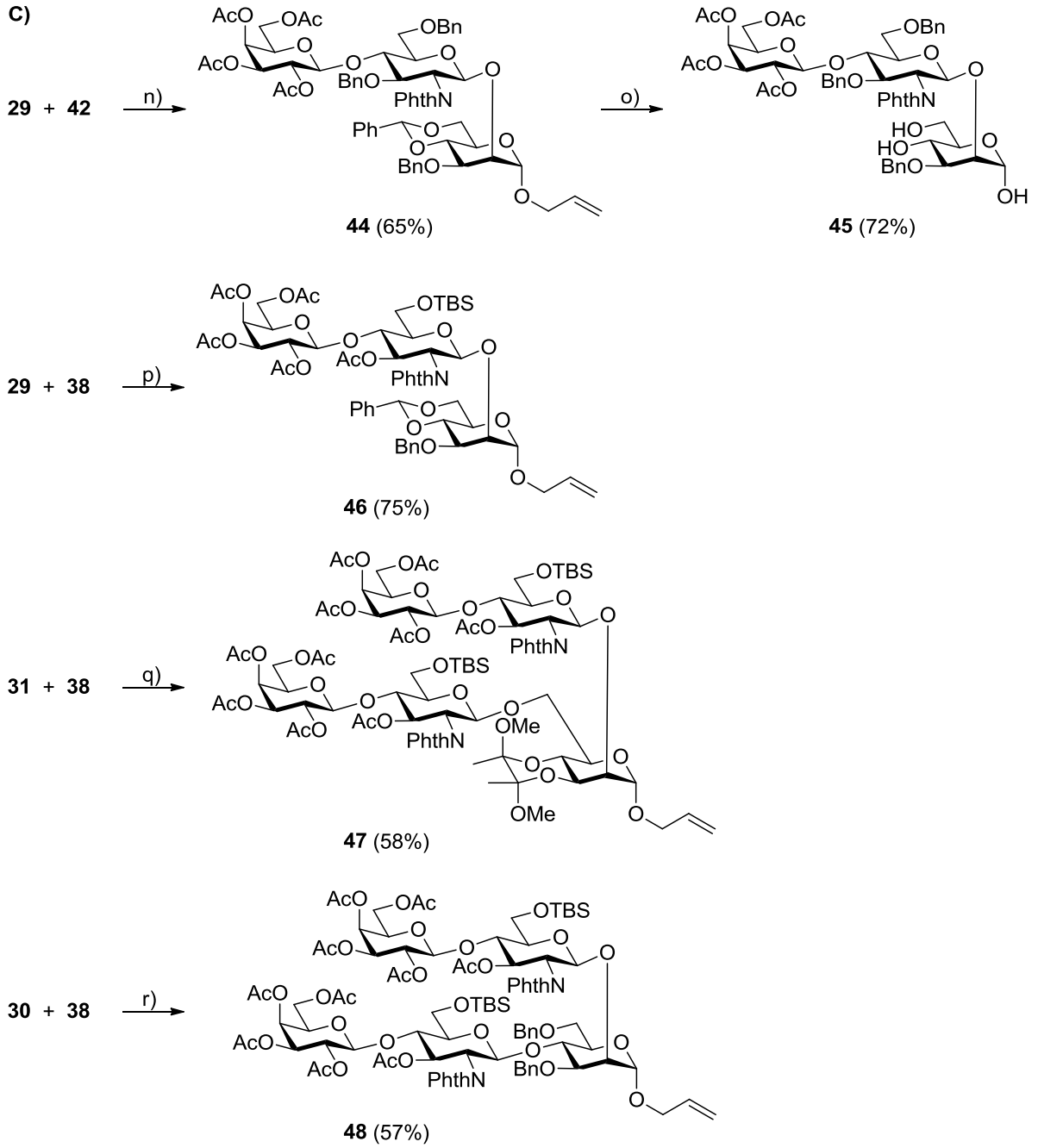
In order to tune the reactivity during coupling to the mannose acceptors **29** - **31,** lactosamine (Galβ1-4GlcNAc) disaccharide donors were prepared with different protecting groups and leaving groups in the anomeric center e.g. the SEt or SPh group (**Scheme 30** B). The disaccharide donor synthesis started with acetylation of D-galactose. A catalytical amount of perchloric acid was used resulting in the formation of the α-acetate **32**. Deacetylation of the anomeric center using hydrazine acetate was followed and the free hydroxyl was treated with trichloroacetonitrile and DBU to give the galactose trichloroacetimidate donor **34**. Two GlcNPhth acceptors were prepared starting from intermediates described in the Manβ1-4GlcNAc disaccharide core synthesis above, compound **8** and **9** (**Scheme 26** A). After deacetylation of **8** and **9**, protection with a TBS group in the primary hydroxyl 6-position was followed to give **35** and **36**. Reaction of the acceptors **35** and **36** with the Gal trichloroacetimidate **34** selectively gave the 4-coupling products, since the hydroxyl 4-position is more reactive then the 3 position in glucose. After coupling the remaining free hydroxyl was acetylated forming **38** and **39.** Two benzylated lactosamine disaccharide donors were prepared according to another route. A GlcNPhth acceptor was obtained by performing a reductive sodium cyanoborohydride benzylidene acetal ring opening[183] on compound **13** and **14** to give the 3,6-benzylated 4-hydroxyl acceptor **40** and **41**. Coupling of the acceptors **40** and **41** with the Gal trichloroacetimidate donor **34** generated the disaccharides **42** and **43**.

The lactosamine disaccharide donors **38**, **39**, **42** and **43** were employed in coupling reactions with the 2-OH, 2,4-OH and 2,6-OH mannose acceptors **29**-**31**, the successful reactions are shown in **Scheme 30** C. Certain differences among the donors were found, the SEt leaving group were considered more reactive, but if the reactivity was too high more byproducts could be formed compared with using SPh groups. However, the suitable reactivity required for a coupling reaction also depends on the acceptor and the mannose acceptors **29**-**31** reacted considerably slower to the donors with SPh groups **43** and **39** compared with the SEt donors **42** and **38**, which resulted lower yields.

The reactivity of the Galβ1-4GlcNAc disaccharide donors were also affected by the protecting groups on the glucosamine, the benzyl and TBS groups were considered to have an electron donating effect, increasing the reactivity of the glycan donor, while the acetyl groups are electron withdrawing, decreasing the donor reactivity. In practice, the 3,6 benzylated

disaccharide donor **42** or 3-acetyl 6-TBS donor **38** containing the same leaving group (SEt), did not show much differences, but **38** showed a cleaner reaction on TLC with 10% higher yield. Additionally, the synthesis of the disaccharide donor **38** was two steps shorter and therefore the donor of choice.





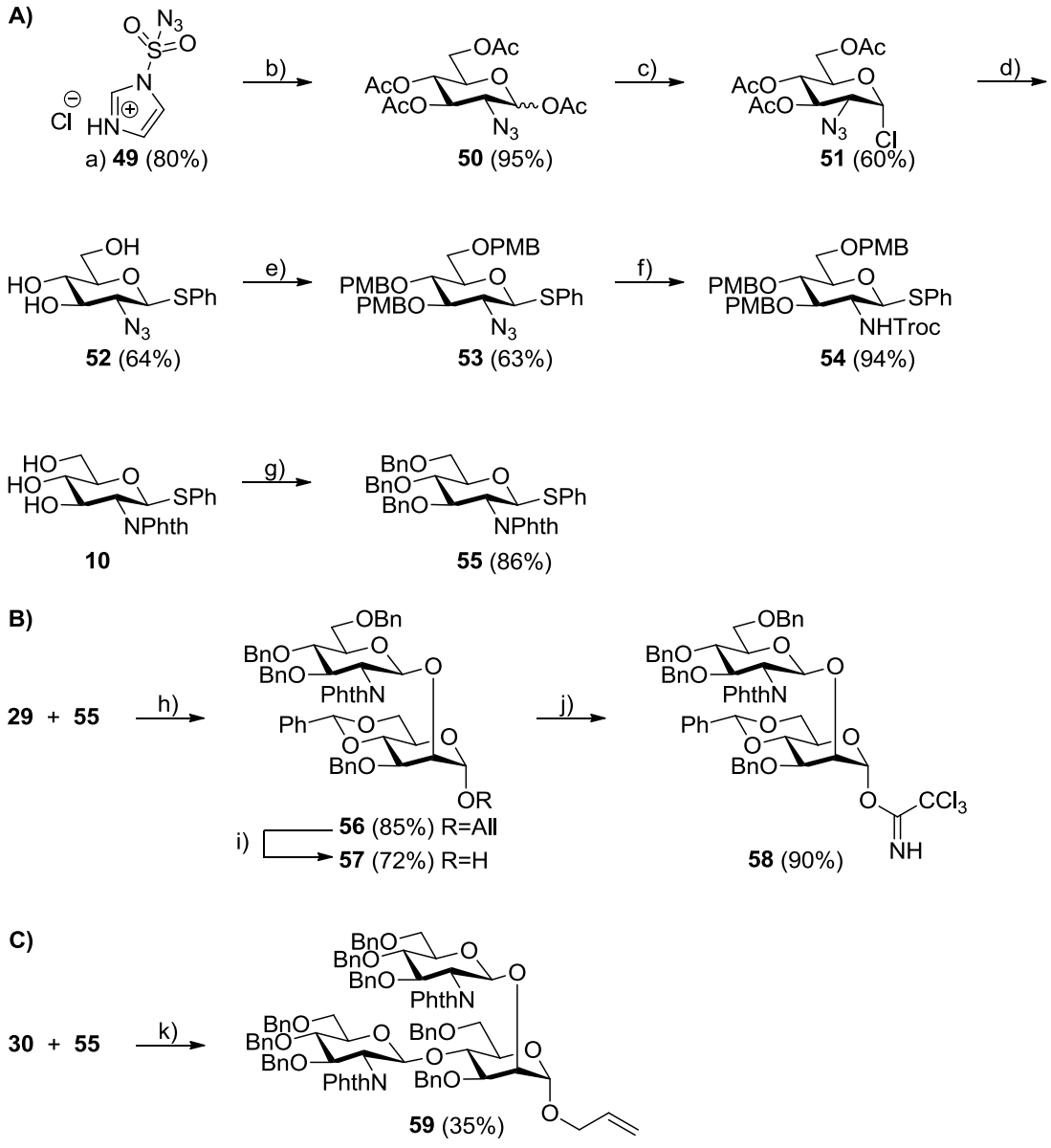
Scheme 30. a) D-mannose, allyl alcohol, $BF_3{\cdot}Et_2O$, 120 °C 30 min; b) α,α-dimethoxytoluene, *p*-toluenesulfonic acid, DMF, 50 °C 1 h; c) dibutyltin oxide, Tol, refluxed 24 h; tetra-*n*-butylammonium bromide, CsF, BnBr, r.t. 24 h, 80 °C 24 h, 120 °C 1 h; d) tributyltin oxide, Tol, refluxed 24 h; BnBr, r.t. 72 h; e) trimethyl orthoformate, CSA, butanedione, MeOH, 90 °C 24 h; f) D-galactose, Ac_2O, $HClO_4$, 25 - 30 °C 30 min; g) hydrazine acetate, DMF, 50 °C 3 h; h) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; i) 0.08 M NaOMe in MeOH, r.t. 4 h; TBSCl, imidazole, DMF, r.t. 2 h; j) **34**, TMSOTf, molecular sieves, DCM, -60 °C 1 h; k) Py/Ac_2O, r.t. 24 h; l) sodium cyanoborohydride, 2N HCl in Et_2O, THF, 0 °C 30 min; m) **34**, TMSOTf, molecular sieves, Et_2O, -50 °C 4 h; n) NIS, TfOH, molecular sieves, DCM, -50 °C 1h; o) Wilkinson's catalyst, $Tol/EtOAc/H_2O$ 20:10:1, reflux 5.5 h; I_2, THF/H_2O 4:1, r.t. 30 min; p) NIS, TfOH, molecular sieves, DCM, 0 °C 4h; q) NIS, TfOH, molecular sieves, DCM, 0 °C 3h; r) NIS, TfOH, molecular sieves, DCM, 0 °C 4h.

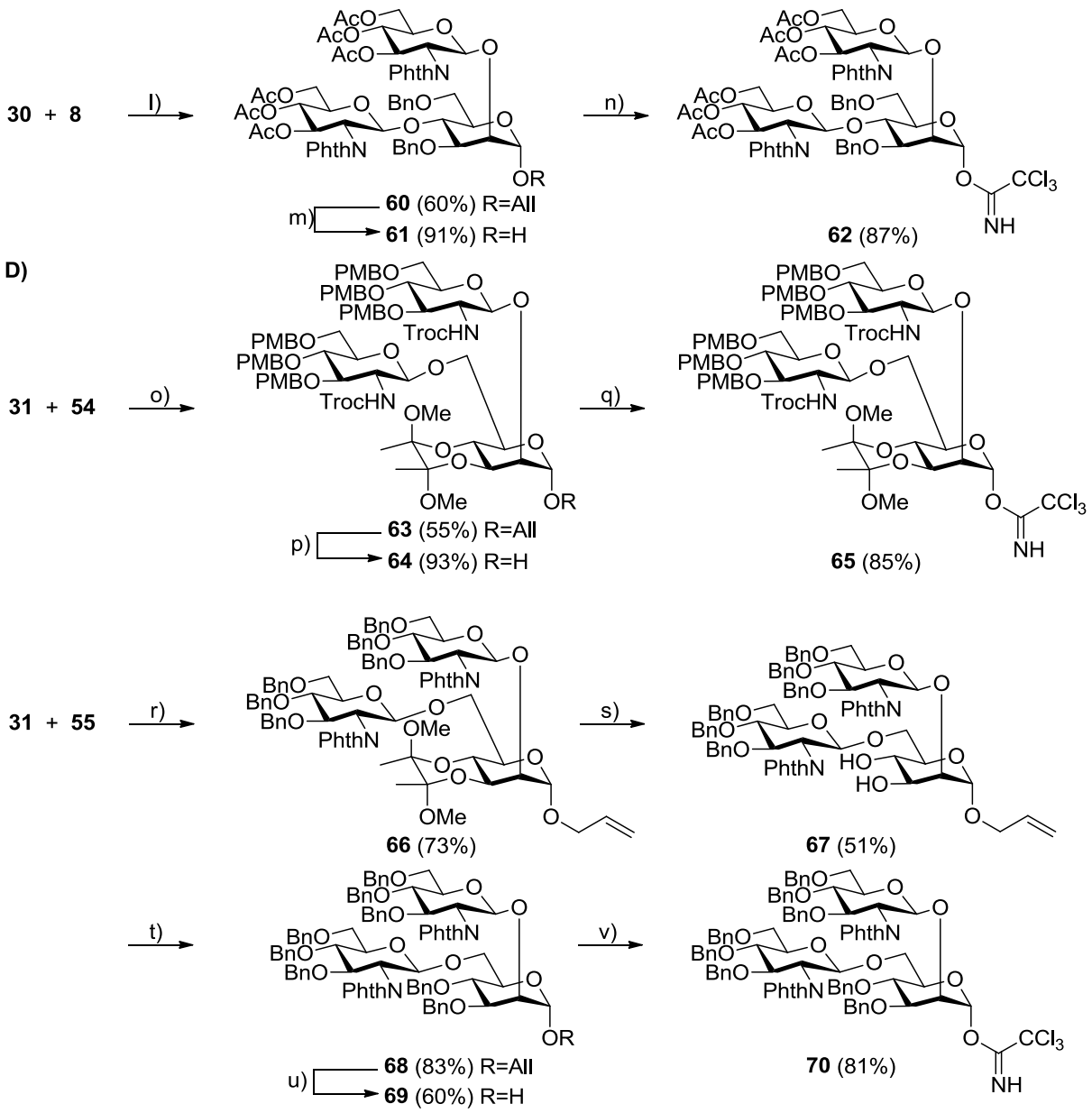
Deallylation was performed on the simplest coupling product **44** by Wilkinson's catalyst,[184-185] which required heating to reflux in a Tol/EtOAc/H_2O 20:10:1 mixture. A single product was obtained, verified by NMR the benzylidene acetal group on the mannose residue was also removed during the reflux. The results indicated that the glycan allyl groups could be efficiently removed by Wilkinson's catalyst, but additional optimization would be required depending on the applied glycan protecting groups.

The mannose acceptors **29** - **31,** were also extended with glucosamine monosaccharide donors. The glucosamine donors were prepared with different protecting groups, acetylated or benzylated GlcNPhth or *p*-methoxybenzyl GlcNTroc donors (**Scheme 31** A). Glycan donors containing NHTroc protection were considered to be slightly more reactive than GlcNPhth donors. However, in contrast to the phthalimido group, the Troc group is quite sensitive to base and would be removed by treatment with sodium hydride during the benzylation step. On the other hand the Troc group could be deprotected under mild conditions by zinc reduction, while the deprotection of the phthalimide require rather harsh base treatment by heating with hydrazine monohydrate. To synthesize the *N*-Troc glucosamine glycan donor with benzyl or *p*-methoxybenzyl protection, the glucosamine was first converted to the corresponding azide and acetylated to give **50**.[186] Treatment with titanium tetrachloride generated the α-chloride **51**, which reacted with thiophenol to give the SPh-β-thioglycoside **52**. Deacetylation by sodium methoxide and *p*-methoxybenzylation followed to obtain **53**.[187] The azide was then reduced and the amine protected with a Troc group forming the GlcNTroc donor **54**. The benzylated phthalimide donor was synthesized in one step since the intermediate **10** was available from the previous synthesis described above (**Scheme 26** A). Compound **10** was benzylated in high yield to give the GlcNPhth donor **55**. The peracetylated GlcNPhth donor **8** was available from previous synthesis as well.

The GlcN donors **8**, **54** and **55** were employed in couplings with the mannose acceptors **29** - **31** (**Scheme 31**). The GlcNPhth donor **55** was applied in a NIS-triflic acid promoted with the mannose acceptor **29** to give the 2-extended coupling product **56** in high yield (**Scheme 31** B). A deallylation step was followed by using the Baudry catalyst and the deallylated product was then converted into a trichloroacetimidate donor **58**.

The GlcNPhth donor **55** was also used in the coupling with the 2,4 hydroxyl acceptor **30** (**Scheme 31** C), in this case, both donor and acceptor were completely protected with benzyl groups, the reaction was instant and a lot of side-products were formed even by reducing the reaction temperature to - 60 °C and gave the desired product **59** only in a low yield. The benzylated acceptor-donor pair was probably too reactive and the less active donor **8** with acetyl protection was instead used in the coupling, which gave **60** in a significantly increased yield. Deallylation and trichloroacetimidate formation performed in high yield to give **62**.



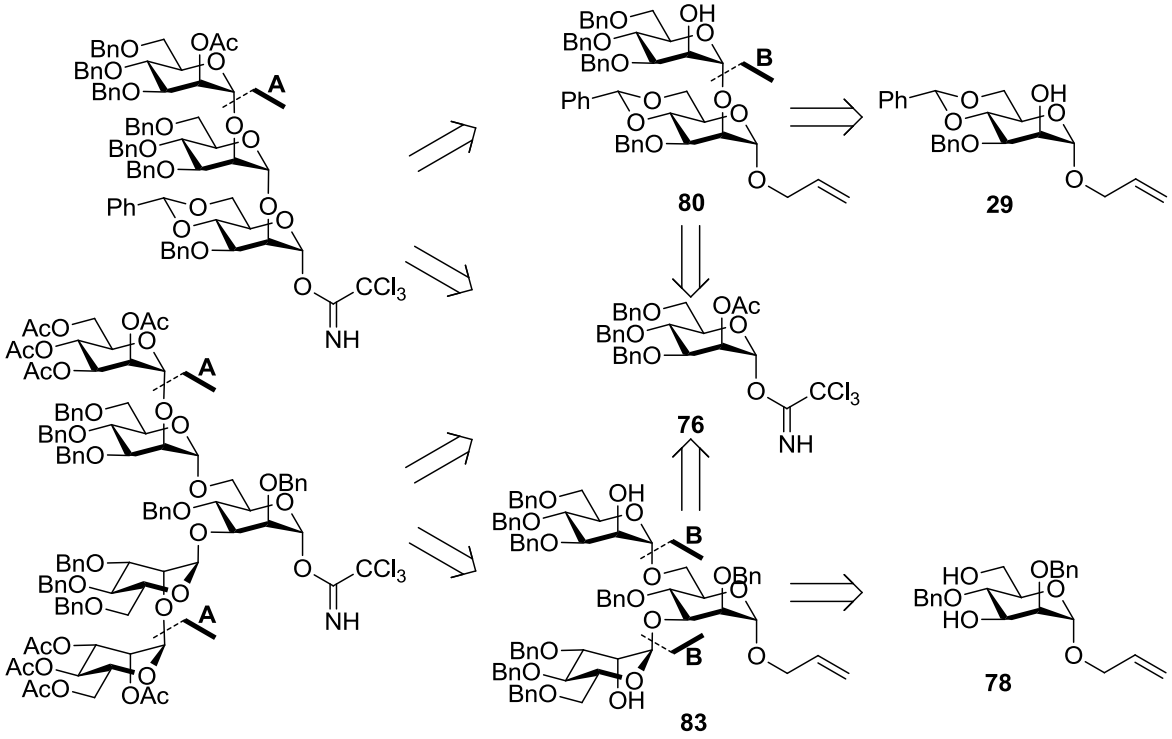


Scheme 31. a) sodium azide, sulfuryl chloride, MeCN, r.t. 18 h; imidazole, r.t. 4 h; b) glucosamine hydrochloride, K_2CO_3, $CuSO_4 \cdot 5H_2O$, MeOH, r.t. 4 h; Py/Ac_2O, r.t. 24 h; c) titanium (IV) chloride, $CHCl_3$, reflux 4 h; d) thiophenol, KOH, EtOH, $CHCl_3$, r.t. 4 h; 0.25 M NaOMe in MeOH, r.t. 4 h; e) NaH, 4-methoxybenzyl chloride, DMF, r.t. 2 h; f) Zn, dioxane/AcOH 10:1, r.t. 24 h; g) NaH, BnBr, DMF, r.t. 24 h; h) NIS, TfOH, molecular sieves, DCM, 0 °C 1h; i) Baudry catalyst, THF, r.t. 2 h; I_2, $NaHCO_3$, H_2O, r.t. 0.5 h; j) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; k) NIS, TfOH, molecular sieves, DCM, -18 °C 0.5 h, 0 °C 1h; l) NIS, TfOH, molecular sieves, DCM, 0 °C 15 min; m) Baudry catalyst, THF, r.t. 2 h; I_2, $NaHCO_3$, H_2O, r.t. 0.5 h; n) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; o) NIS, TfOH, molecular sieves, DCM, 0 °C 15 min; p) Baudry catalyst, THF, r.t. 2 h; I_2, $NaHCO_3$, H_2O, r.t. 0.5 h; q) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; r) NIS, TfOH, molecular sieves, DCM, 0 °C 2 h; s) TFA/TIPS/H_2O 15:0.9:0.9, r.t. 2 h; t) NaH, BnBr, DMF, r.t. 24 h; u) Baudry catalyst, THF, r.t. 2 h; I_2, $NaHCO_3$, H_2O, r.t. 0.5 h; v) trichloroacetonitrile, DBU, DCM, 0 °C 3 h.

Both the *p*-methoxybenzyl GlcNHTroc **54** and the benzyl GlcNPhth **55** donors were used in the coupling with **31** to generate 2,6 branched mannose products **63** and **66** (**Scheme 31** D). Since the dimethoxymethyl 3,4-acetal might influence the reactivity in later coupling steps, the 3,4-acetal group was removed by acid treatment on compound **66**. In this case the acetal was easily removed by TFA and replaced by benzyl groups and then converted to a trichloroacetimidate donor. For compound **63** the *p*-methoxybenzyl groups, which are acid sensitive, would not allow cleavage of the acetal in this stage. As a result, **63** was directly deallylated and converted to trichloroacetimidate **65**. Compared with Wilkinson's catalyst, the reaction conditions are much milder using the Baudry catalyst in the deallylation step. The Baudry catalyst is therefore more suitable for deallylation of larger and more complex molecules, while Wilkinson's catalyst usually works perfect for monosaccharide or disaccharides.

2.8 Synthesis of high mannose building block extension units

High mannose building blocks were prepared by repeated extensions with the 2-acetyl 3,4,6-benzyl mannose trichloroacetimidate donor **76** in the 2-position of the mannose acceptor **29** forming a linear oligosaccharide or in the 3,6-position of acceptor **78** forming a branched oligosaccharide (**Scheme 32**).

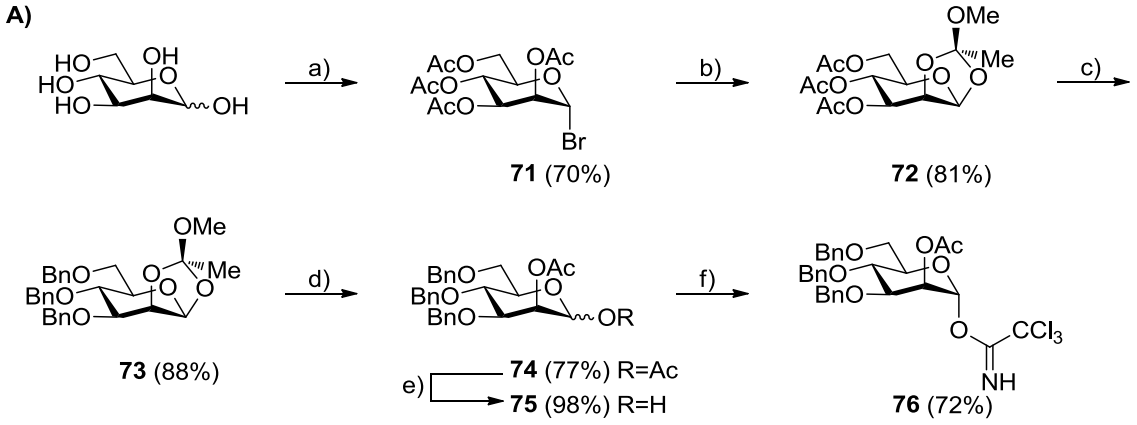


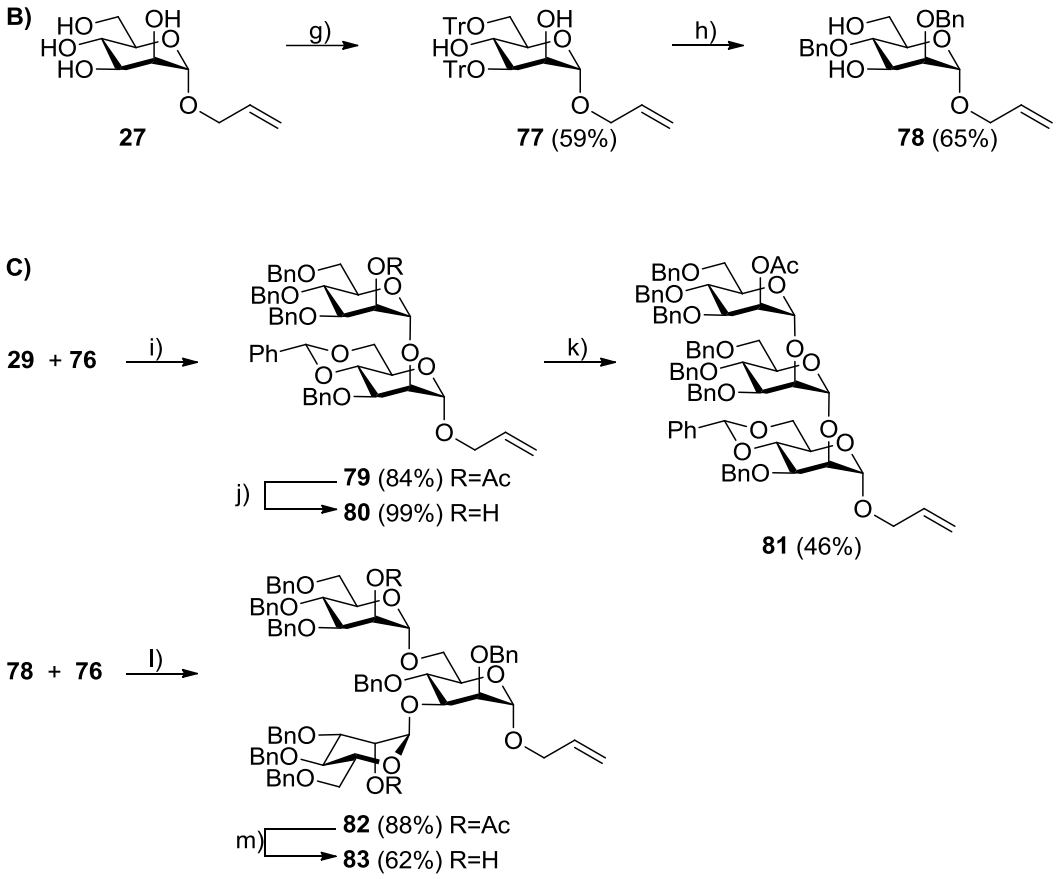
Scheme 32. Retrosynthetic analysis for high mannose building blocks elongated with a mannose monosaccharide donor and employing different acceptors.

The 2-acetyl 3,4,6-benzyl mannose trichloroacetimidate donor **76** was prepared within a few steps of synthesis (**Scheme 33** A). Catalyzed by Iodine, the starting material D-mannose was acetylated in acetic anhydride,[188-189] and brominated with 30% HBr in AcOH to give the α-bromo-mannoside **71**.[190] The bromide was considered to be a good leaving group when treated with 2,4,6-collidine to form the 1,2-ortho ester **72**.[191] The acetyl groups were removed by treatment with K_2CO_3 in MeOH and replaced with benzyl groups using standard conditions to give **73**.[192-193] The orthoester was converted into acetates by treatment with acetic acid, and gave the desired 1,2-di-acetyl **74** as main product and 10% of the 1-hydroxyl 2-acetyl product **75**,[194] which is the desired product in the next step. Compound **74** was converted to **75** by removing the anomeric acetate group using hydrazine acetate and then converted to the trichloroacetimidate donor **76**.

The synthesis of the 3,6-hydroxyl acceptor **78** started with selective tributyl tin oxide assisted trityl protection in the 3,6-positions of the allyl-mannoside **27**.[195] Then the 2,4 positions were benzylated and the trityl groups were removed to give the 3,6-hydroxyl mannose acceptor **78** (**Scheme 33** B). The mannose acceptor **29** responsible for linear glycan extension in the 2-position, were the same as described above in the synthesis complex type *N*-glycan donors.

By stepwise coupling of the mannose donor **76** with the linear acceptor **29** or the branched acceptor **78**,[196] the high mannose type building blocks **79** and **82** were obtained (**Scheme 33** C). By deacetylation of the coupling product **79** and coupling again with donor **76**, the mannose elongation product **81** was formed.



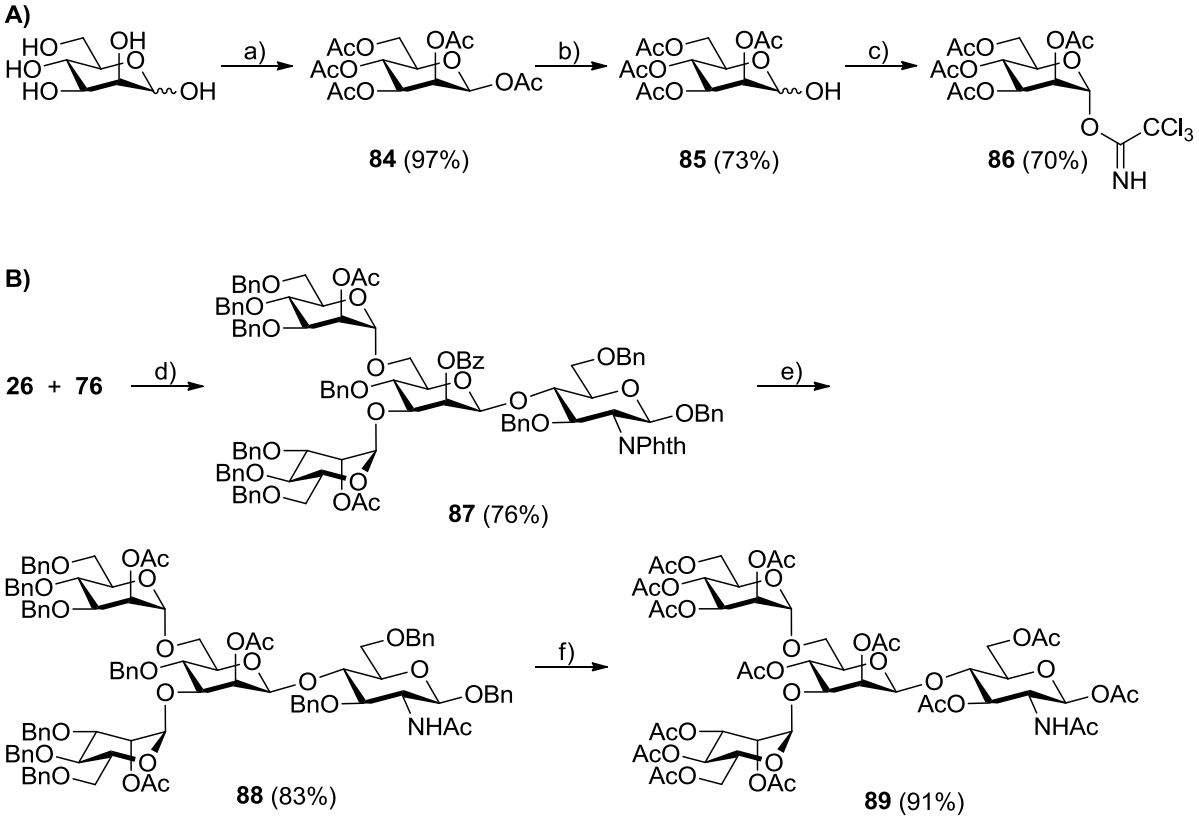


Scheme 33. a) D-mannose, I_2, Ac_2O, 90 °C 0.5 h; 30% HBr in AcOH, AcOH, r.t. 24 h; b) 2,4,6-collidine, MeOH/$CHCl_3$ 1:4, r.t. 24 h; c) K_2CO_3, MeOH, r.t. 0.5 h; NaH, BnBr, DMF, r.t. 2 h; d) AcOH/H_2O 1.54:1, r. t. 24 h; e) hydrazine acetate, DMF, 50 °C 3 h; f) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; g) tributyltin oxide, Tol, refluxed 24 h; trityl chloride, r.t. 24 h; h) NaH, BnBr, DMF, r.t. 2 h; AcOH, r.t. 2 h; i) TMSOTf, molecular sieves, DCM, r.t. 0.5 h; j) 0.07 M NaOMe in MeOH/DCM 4:1, r.t. 24 h; k) TMSOTf, molecular sieves, DCM, r.t. 2 h; l) TMSOTf, molecular sieves, DCM, r.t. 0.5 h; m) 0.08 M NaOMe in MeOH/DCM 4:1, r.t. 24 h.

2.9 Extension of the Manβ1-4GlcNAc disaccharide core with complex type building blocks

The synthetic Manβ1-4GlcNAc disaccharide core acceptor **26** was used for glycan extension at the 3,6-positions of the terminal mannose, employing the above described trichloroacetimidate donor building blocks. The simple peracetylated mannose donor **86** was first synthesized and used in the coupling of **26**, forming a precursor to the *N*-glycan pentasaccharide core (**Scheme 34**). The peracetylated donor **86** was not fully converting **26** to product during the coupling reaction, suggesting that the acetylated donor was not active enough. The 3,4,6-benzylated donor **76** was instead employed (**Scheme 34** B), which was far

more reactive and the reaction was finished in 15 min in a good yield. A few protecting group manipulation steps followed. The Phthalimido groups and the benzoyl group were removed by hydrazine monohydrate. Then all benzyl groups were removed by catalytic Pd(C) hydrogenation, followed by global acetylation. The synthesized peracetylated tetrasaccharide **89** was stored at this stage, since the oxazoline product was considered less stable. Final conversion to the oxazoline proceeded short before use in the chemoenzymatic couplings to GlcN-Asn-*N*-glycopeptides.

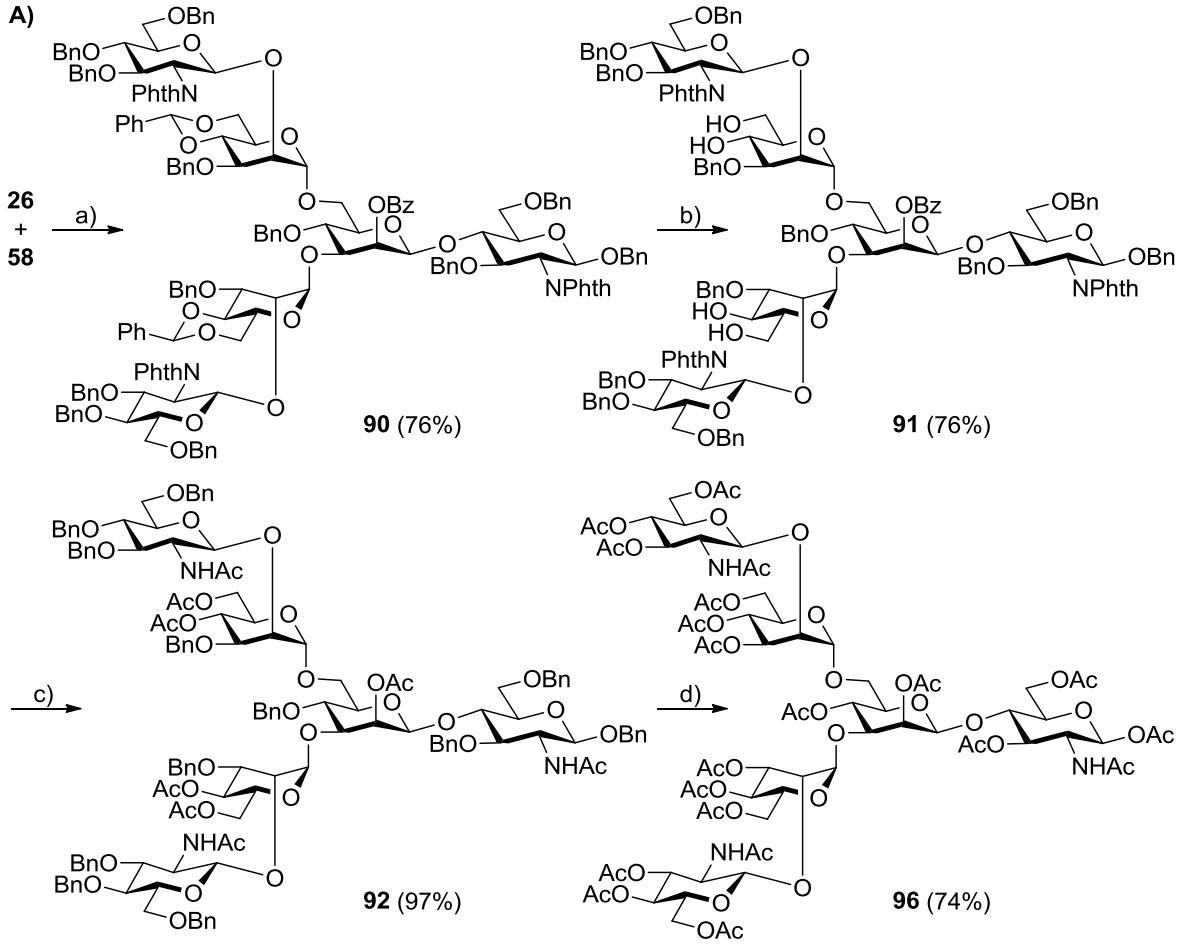


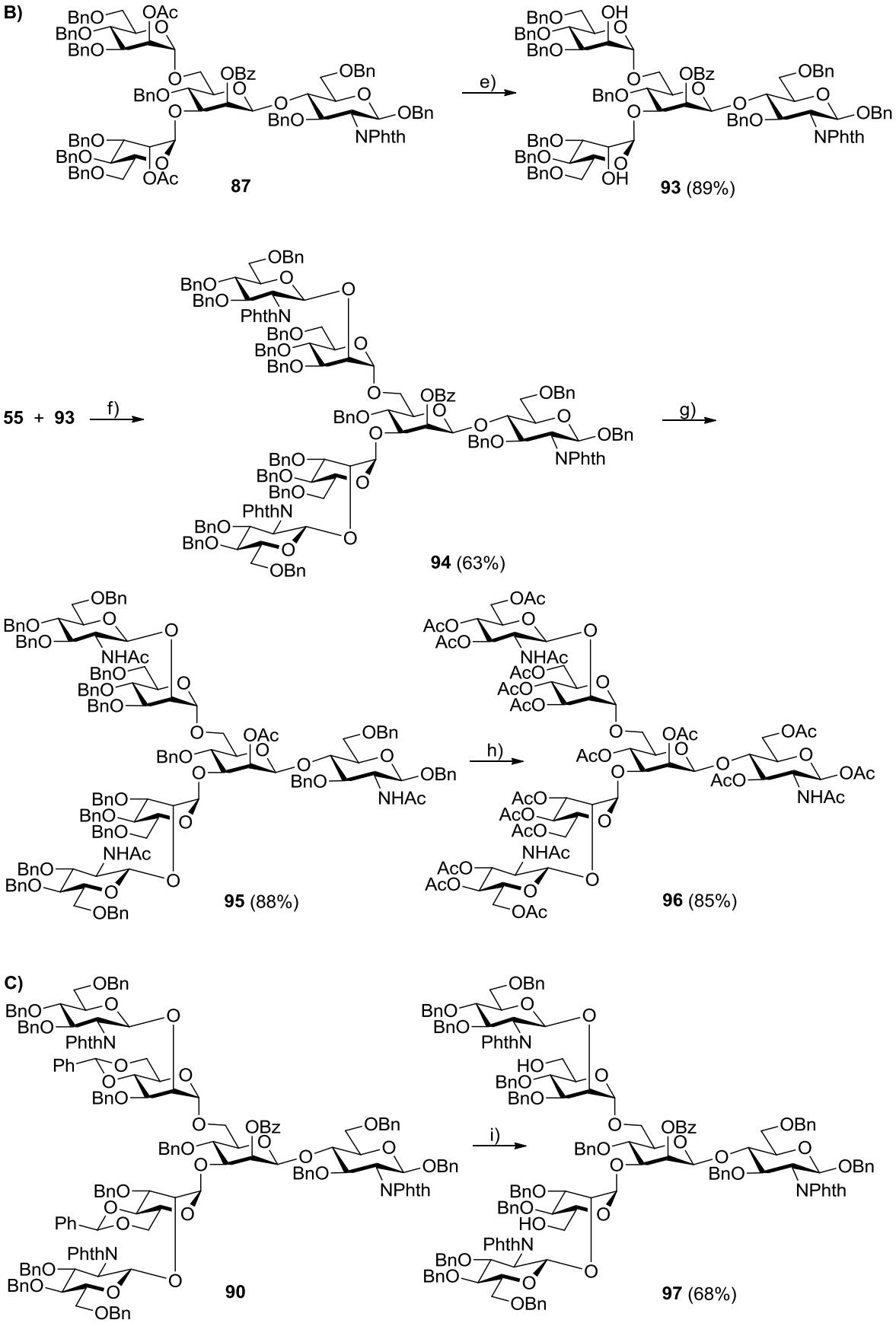
Scheme 34. a) Py/Ac_2O, r.t. 24 h; b) hydrazine acetate, DMF, 50 °C 3 h; c) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; d) TMSOTf, molecular sieves, DCM, 0 °C 15 min; e) hydrazine monohydrate, MeOH, 70 °C 24 h; Py/Ac_2O, r.t. 24 h; f) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 48 h; Py/Ac_2O, r.t. 24 h.

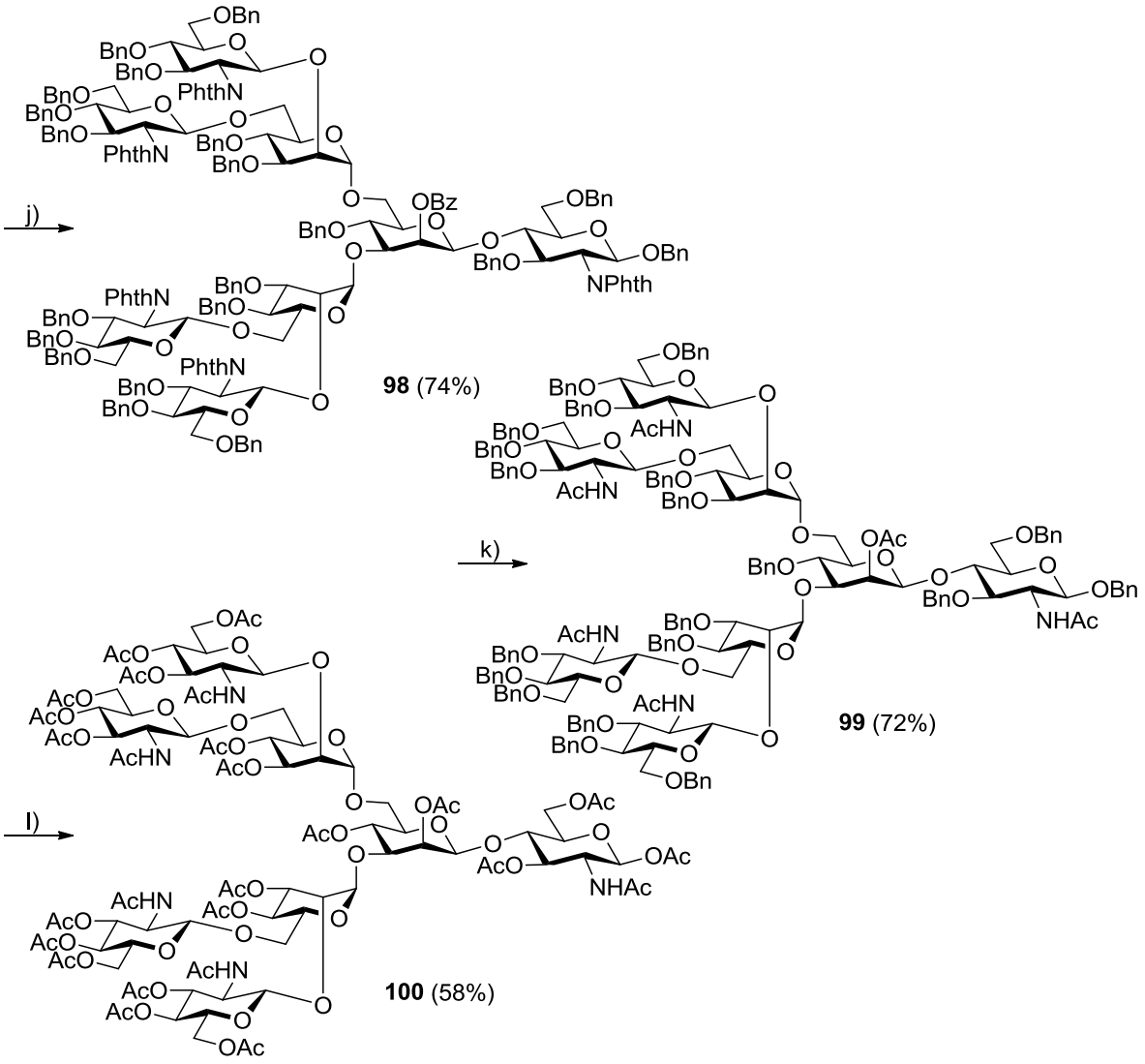
After successfully coupling of the monosaccharide donor **76** symmetrically on **26**, more complex donors were used, the 2,6 branched trisaccharide **70** and 2,4 branched trisaccharide **62** were tested according to similar coupling conditions.[197-198] The results showed that a mono glycosylated coupling product on the 3- or 6-position of the mannose on the Manβ1-4GlcNAc disaccharide acceptor was formed to a large extend. More equivalents of

donor could not increase the product formation. To avoid potential problems with steric hindrance, the synthesis strategy was therefore changed and the coupling reactions were instead performed stepwise, glycosylating the Man 6- and 3-position with the smaller linear GlcNAcβ1-2Man glycan donors that after coupling was branched in the 4 or 6 position of the terminal mannose residues.

By using the slightly smaller GlcNAcβ1-2Man disaccharide donor **58** in the coupling to acceptor **26**, the reaction gave the double glycosylated product **90** in a good yield, 76% (**Scheme 35** A). After final coupling, the benzylidene acetal was cleaved off under acidic conditions using *p*-toluenesulfonic acid in MeCN,[199] then the phthalimido groups were removed and replaced with acetyl groups. Deprotection of the benzyl groups and acetylation again followed forming the peracetylated (GlcNAcβ1-2Manα1)$_2$-3,6-Manβ1-4GlcNAc hexasaccharide **96**.




B)
87
e)
93 (89%)
55 + 93
f)
94 (63%)
g)
95 (88%)
h)
96 (85%)
C)
90
i)
97 (68%)



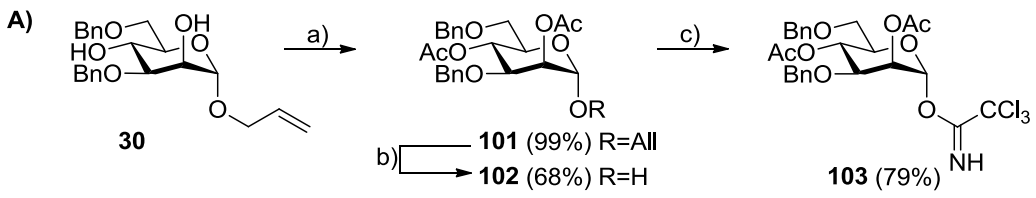
Scheme 35 a) TMSOTf, molecular sieves, DCM, -50 °C to 0 °C over 6 h; b) *p*-toluenesulfonic acid, MeCN, r.t. 30 min; c) hydrazine monohydrate, Tol/EtOH 1:1, 80 °C 72 h; Py/Ac_2O, r.t. 24 h; d) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 48 h; Py/Ac_2O, r.t. 24 h; e) NaOMe, MeOH/DCM 5:1, r.t. 3 days; f) **55**, NIS, TfOH, molecular sieves, DCM, 0 °C 30 min; g) hydrazine monohydrate, Tol/EtOH 1:1, 80 °C 5 days; Py/Ac_2O, r.t. 2 days; h) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 16 days; Py/Ac_2O, r.t. 3 days; i) 1 M BH_3 in THF, TMSOTf, DCM, 0 °C 30 min, r.t. 16 h; j) **55**, NIS, TfOH, molecular sieves, DCM, 0 °C 30 min; k) hydrazine monohydrate, Tol/EtOH 1:1, 80 °C 10 days; Py/Ac_2O, r.t. 2 days; l) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 15 days; Py/Ac_2O, r.t. 3 days.

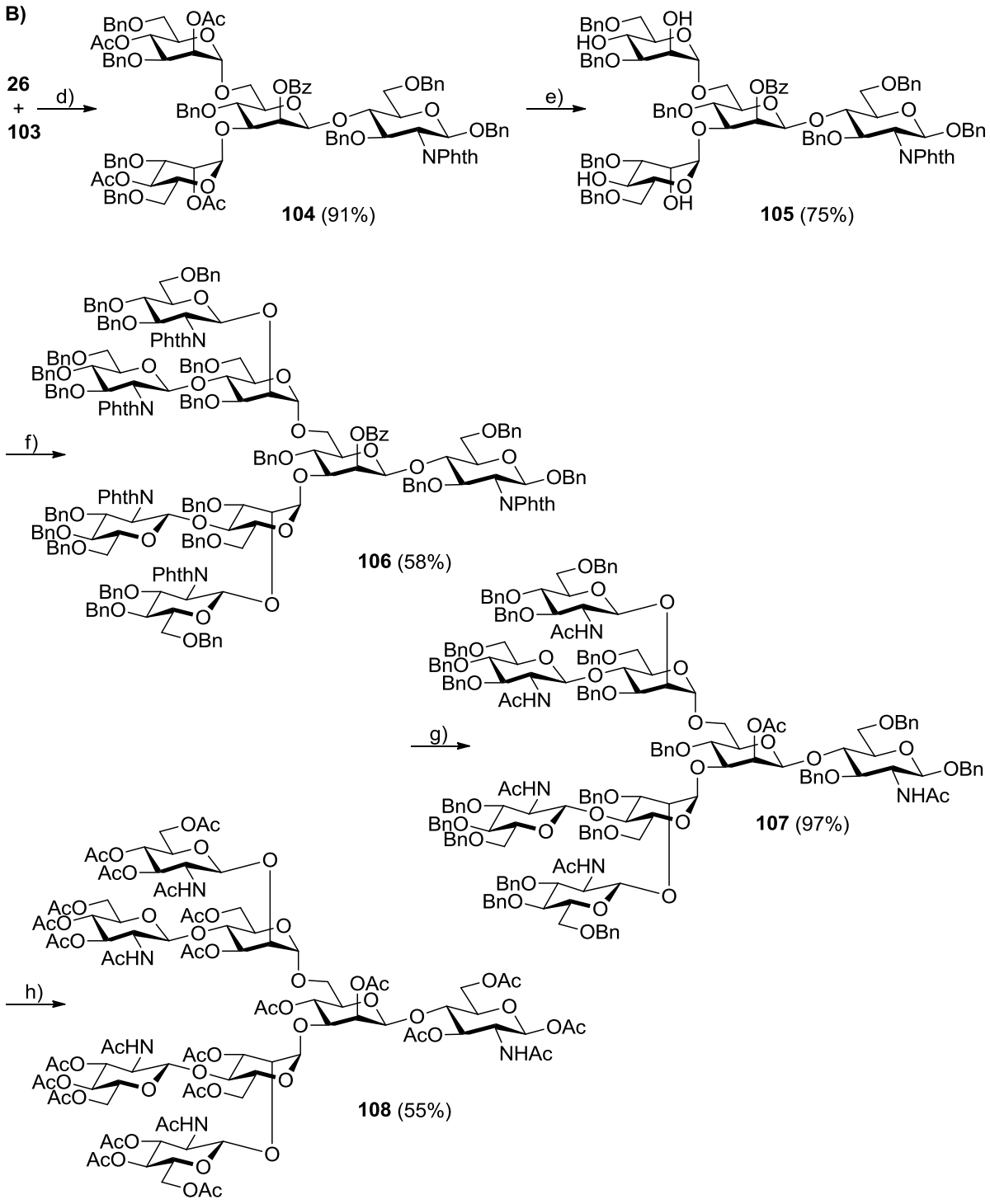
The 2-extended hexasaccharide **96** was also synthesized by another route (**Scheme 35** B). From previous synthesis (**Scheme 34** B), the tetrasaccharide core unit **87** was deacetylated to give **93**, which was extended in the terminal mannose 2-OH positions using the benzylated GlcNPhth donor **55** forming the hexasaccharide **94**. After a few protecting group modification steps, the acetylated hexasaccharide **96** was obtained.

The coupling product **90**, described above, was also used for generating more branched saccharides, the benzylidene acetal was selectively ring opened generating a free hydroxyl at the 6-position on the two mannose residues, with 1 M BH_3 in THF catalyzed by TMSOTf (**Scheme 35** C). The hexasaccharide was then elongated in the two 6-OH positions using the GlcNAc donor **55**. After similar protecting group modulations as described above, the peracetylated 2,6 branched octasaccharide **100** was obtained. Benzylidene acetal ring opening in the 4-position instead to generate a 2,4 branched extended octasaccharide was also tested on the intermediate **90** but suffered from formation of break down products.

To generate the 2,4 branched extended octasaccharide, the mannose residue **30** was in a few steps converted to the mannose donor **103** which could potentially extended the 2,4 positions (**Scheme 36** A). The trichloroacetimidate donor **103** was coupled to the Manβ1-4GlcNAc acceptor **26** in a high yield (**Scheme 36** B). Deacetylation of the coupling product **104** performed carefully at pH 9.5 to avoid simultaneous cleavage of the benzoyl group. A quadruple coupling was then performed with 6.0 eq of the benzylated GlcNPhth donor **55** to the symmetrical 2,4-hydroxyl acceptor **105** to give the coupling product **106** in a good yield. The protection groups were modified to give the final 2,4 branched extended octasaccharide **108**.

In conclusion, with generation of larger oligosaccharide structures, the difficulty of the elongation step increased with the size of the donor building blocks, probably due to sterical hindrance. Monosaccharide donors achieved 70-90% yield in a double glycosylation of the Manβ1-4GlcNAc acceptor **26** or 50-60% yield in a quadruple couplings of the tetrasaccharide acceptor **105**. Using disaccharide donors, the double coupling to acceptor **26** performed in 70-80% coupling yields, while using trisaccharide donors, the yields dropped to only 10-15% and in attempts to form double glycosylated products, mono glycosylated coupling products were dominating instead. By coupling monosaccharide donors stepwise with functional group orthogonally, large building blocks were synthesized efficiently.

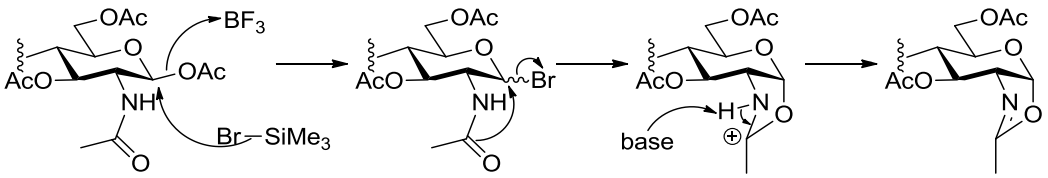




Scheme 36. a) Py/Ac_2O, r.t. 24 h; b) Wilkinson's catalyst, Tol/EtOAc/H_2O 20:10:1, reflux 24 h; I_2, THF/H_2O 4:1, r.t. 2 h; c) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; d) TMSOTf, molecular sieves, DCM, 0 °C 2 h; e) NaOMe, MeOH/DCM 5:1, r.t. 12 days; f) **55**, NIS, TfOH, molecular sieves, DCM, 0 °C 30 min; g) hydrazine monohydrate, Tol/EtOH 1:1, 80 °C 4 days; Py/Ac_2O, r.t. 2 days; h) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 12 days; Py/Ac_2O, r.t. 3 days.

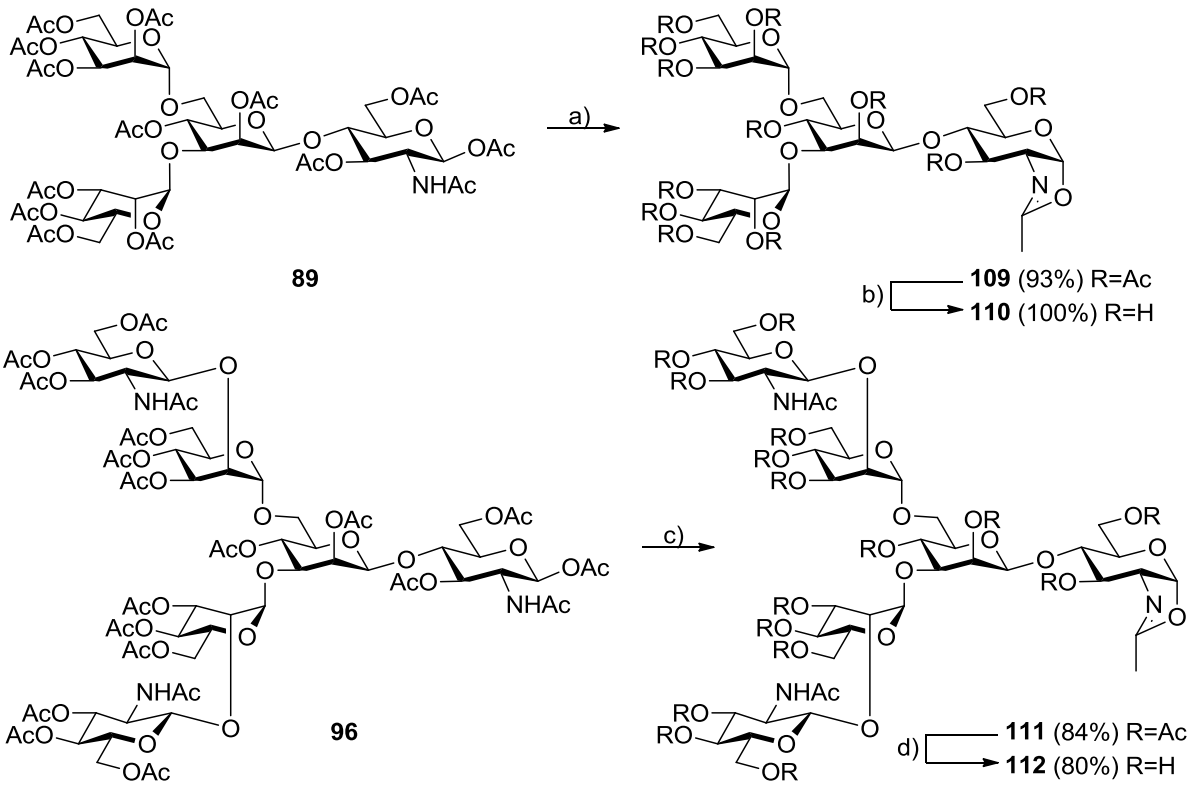
2.10 Producing oxazoline donors for chemoenzymatic coupling with *N*-glycopeptides

A few examples of converting *N*-glycans into oxazoline donors have been reported in recent years,[200-201] the structures reported vary from monosaccharides to hexasaccharide or decasaccharides.[202-203] The following conditions were used, treatment of the glycan acetate with BF_3 etherate, trimethylsilyl bromide in the presence of a base, 2,4,6-collidine or tri-*tert*-butyl pyrimidine in dichloroethane (DCE) at 40 °C. A proposed reaction mechanism involve that the acetyl group leave, promoted by the BF_3 etherate, and the trimethylsilyl bromide support the formation of a glycosyl bromide to create an intermediate that lower the energy barrier of the reaction. Then, the carbonyl oxygen of the neighboring NHAc group make a nucleophilic attack to form a five membered ring, which with the help of base abstract the hydrogen from the NH group to form the oxazoline (**Scheme 37**).



Scheme 37. Mechanism of oxazoline formation.

The oxazoline formation was performed on tetrasaccharide **89** and hexasaccharide **96** (**Scheme 38**) in high yields. For smaller molecules, the product showed a distinguished change of R_f value on TLC, but for the larger saccharide structures, the difference were not so pronounced. Instead the reaction needed to be followed by mass spectrometry or NMR. Since the oxazolines were acid sensitive, ammonium buffer was used in the MS measurements. During these conditions potential formation of hydrolysis product could be discriminated from the oxazoline peak in the high-resolution mass spectrum. Characterization by NMR showed a unique CH_3 peak from the oxazoline in the ^{13}C spectrum around 13 - 15 ppm and a quaternary carbon from the oxazoline ring at 165 ppm, which gave cross coupling signals to H1, H2 and the CH_3 group in the HMBC spectrum. Deacetylation performed similar to the deacetylation steps during glycopeptide synthesis, keeping the pH constant at 9.5-10 for 24 h.



Scheme 38. a) Trimethylsilyl bromide, $BF_3{\cdot}Et_2O$, tri-*tert*-butyl pyrimidine, 40 °C 4 h; b) NaOMe, MeOH, r.t. 24 h; c) Trimethylsilyl bromide, $BF_3{\cdot}Et_2O$, tri-*tert*-butyl pyrimidine, 40 °C 4 h; d) NaOMe, MeOH, r.t. 48 h.

2.11 *N*-GlcNAc glycopeptides synthesis and in solution chemoenzymatic coupling of a tetrasaccharide oxazoline donor

Two sets of *N*-glycopeptides were synthesized, with or without a spacer, the glycopeptides with a spacer were aimed for spotting on glycopeptide microarrays to study interactions with glycan binding proteins. The peptides without spacer aimed to be used as standards to study glycopeptide MS-fragmentation and to evaluate glycoproteomic methodology. Initially synthetic mono-GlcNAc *N*-glycopeptides were used to evaluate the enzymatic coupling of the oxazoline donor in solution and later also in on-slide glycosylations reacting directly on the microarray slide. Mono-GlcNAc *N*-glycopeptides were synthesized based on peptide sequences with *N*-glycosylation sites previously identified from human platelet proteins (**Table 2**).[204-205] A Fmoc-solid-phase peptide synthesis strategy was applied here, the glycosylated Fmoc-GlcNAc-Asn amino acid **6** was added manually, using less excess and longer reaction times.[206] After complete peptide assembly the peptides were released from resin using a mixture of TFA:TIPS:H_2O. A deacetylation step removing the glycan protecting

groups was followed, achieving the glycopeptides in good total yields after the final HPLC purification step. The synthetic glycopeptides are summarized in **Table 1** and **Table 2**.

Comp Nr	Peptides	Sequence	MS	Mass (mg)	Yield
136	ADAM10:	IN*TTADEKDPTNPFR	1922.02	17.5	73%
137	Integrin alpha-6:	AN*HSGAVVLLKR	1467.67	15.2	83%
138	Thrombospondin-1:	VVN*STTGPGEHLR	1569.68	16.0	82%
139	Antithrombin III:	WVSN*KTEGR	1279.36	14.0	88%
140	Integrin beta-3:	LRPDDSKN*FSIQVR	1878.05	20.1	86%
141	Integrin alpha-2:	GEYFVN*VTTR	1388.48	1.3	7.5%
142	P-Selectin:	AYSWN*ISR	1199.27	9.6	64%
143	Platelet glycoprotein 1b alpha:	N*LTALPPDLPK	1381.57	10.7	62%
144	Multimerin 1:	LQN*LTLPTNASIK	1615.83	11.5	57%
145	Coagulationfactor V:	SN*YSSPSNISK	1386.42	8.8	51%
146	Endoplasmin:	ELISN*ASDALDK	1478.56	6.6	34%
146	Platelet glycoprotein V:	LLDLSGNN*LTHLPK	1737.95	13.0	60%
148	Multimerin 1:	LQNLTLPTN*ASIK	1615.83	12.0	60%
149	Coagulationfactor V:	SNYSSPSN*ISK	1386.42	12.9	74%

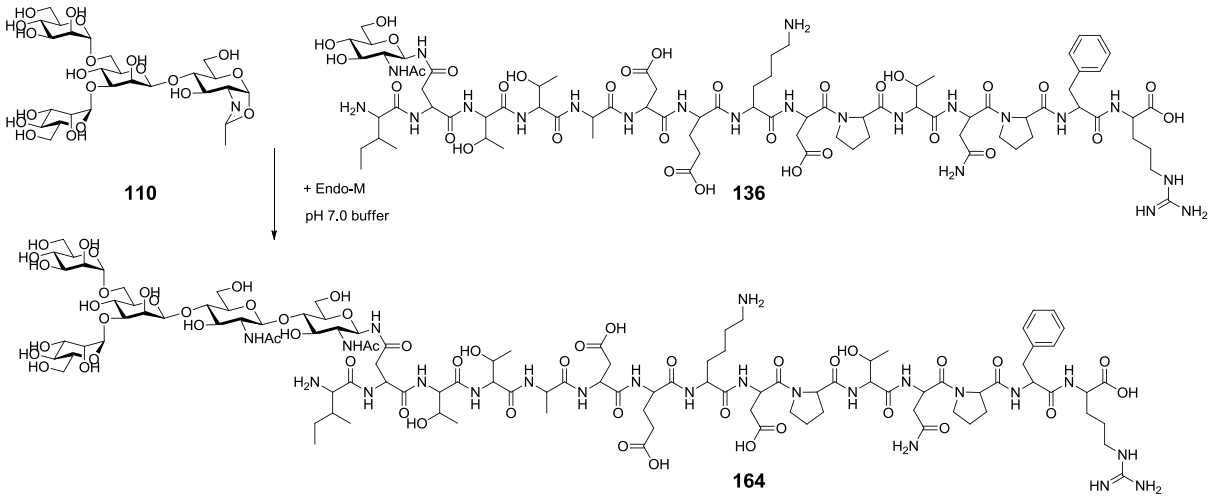
Table 1. Synthesis of tryptic *N*-glycopeptides, selected from previously identified human platelet proteins.

Comp Nr	*Peptide Nr on microarray plate*	Sequence with *N*-terminal spacer	MS	Mass (mg)	Yield
150	*1*	*Sp*IN*TTADEKDPTNPFR	2125.26	17.5	66%
151	*2*	*Sp*AN*HSGAVVLLKR	1670.91	17.8	85%
152	*3*	*Sp*VVN*STTGPGEHLR	1772.92	19.0	86%
153	*4*	*Sp*WVSN*KTEGR	1482.6	9.43	51%
154	*5*	*Sp*LRPDDSKN*FSIQVR	2081.29	10.7	41%
155	*6*	*Sp*GEYFVN*VTTR	1591.72	4.7	23%
156	*7*	*Sp*AYSWN*ISR	1402.51	10.2	58%
157	*8*	*Sp*N*LTALPPDLPK	1584.81	12.7	64%
158	*9*	*Sp*LQN*LTLPTNASIK	1819.07	14.7	64%
158	*10*	*Sp*SN*YSSPSNISK	1589.66	7.45	37%
160	*11*	*Sp*ELISN*ASDALDK	1681.8	13.4	64%
161	*12*	*Sp*LLDLSGNN*LTHLPK	1941.19	18.3	75%
162	*13*	*Sp*LQNLTLPTN*ASIK	1819.07	15.0	66%
163	*14*	*Sp*SNYSSPSN*ISK	1589.66	9.6	48%

Table 2. Second set of *N*-glycopeptides with a triethyleneglycol spacer, which was used in the microarray experiments.

After completing the synthesis of the mono-GlcNAc *N*-glycopeptides and the oxazoline donors, the endoglycosidase coupling was tested.[207-208] The following conditions were used, 6-8 eq of oxazoline donor was mixed with 1.0 eq target peptide using 12.5 mU Endo-M-N175Q in 50 mM phosphate buffer pH 7.1. The reaction was kept at 25 °C and followed by analytical HPLC. The mono-GlcNAc *N*-glycopeptide **136** was first tested under these conditions, reacting with the tetrasaccharide oxazoline **110** resulting in the formation of the pentasaccharide core glycopeptide **164**. The progress of the reaction was followed by HPLC from 0.5 h to 6 h and compared with the starting material (**Figure 11**).

Followed by analytical HPLC, the chromatogram showed the consumption of the two starting materials and formation of the enzyme coupling product. The fully deprotected oxazoline donor was very hydrophilic coming out with the front around 4.4 min, and peptide substrate **136** eluted at 23.3 min, while the enzymatically extended *N*-glycopeptide product **164** eluted slightly faster than the starting peptide, at 22.4 min. The enzymatic coupling was already finished after 30-40 min and by waiting longer, a clear glycan hydrolysis back to the starting peptide was observed after 2-3 hours and after 6 h around 24% of the product was hydrolyzed back to the starting peptide. Although the endoglycosidase is a N175Q mutant of Endo-M, in which the hydrolysis property should be suppressed, after certain time, the back hydrolysis will still take place.



Scheme 39. Enzymatic endoglycosidase coupling of an oxazoline donor to a *N*-GlcNAc peptide **136**, sequence: IN*TTADEKDPTNPFR from ADAM10.

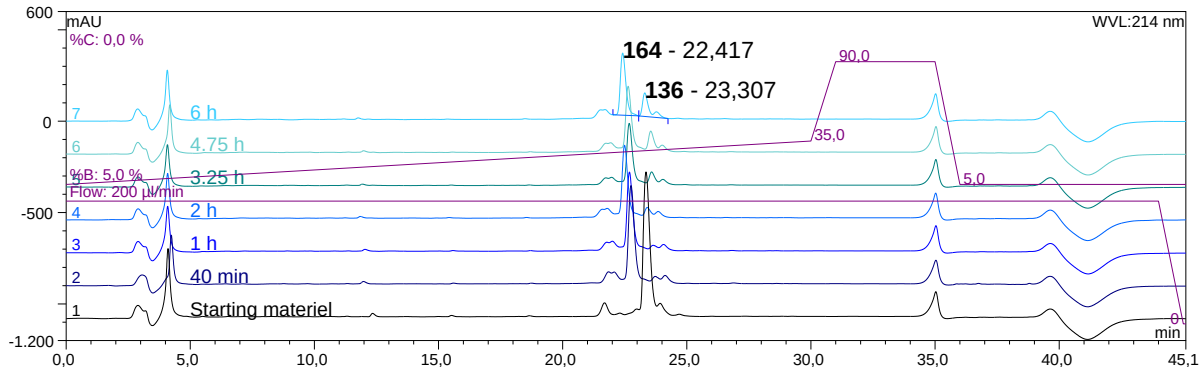


Figure 11. HPLC chromatogram following the enzymatic coupling, taken from 40 min, 1 h, 2 h, 3.25 h, 4.75 h up to 6 hours.

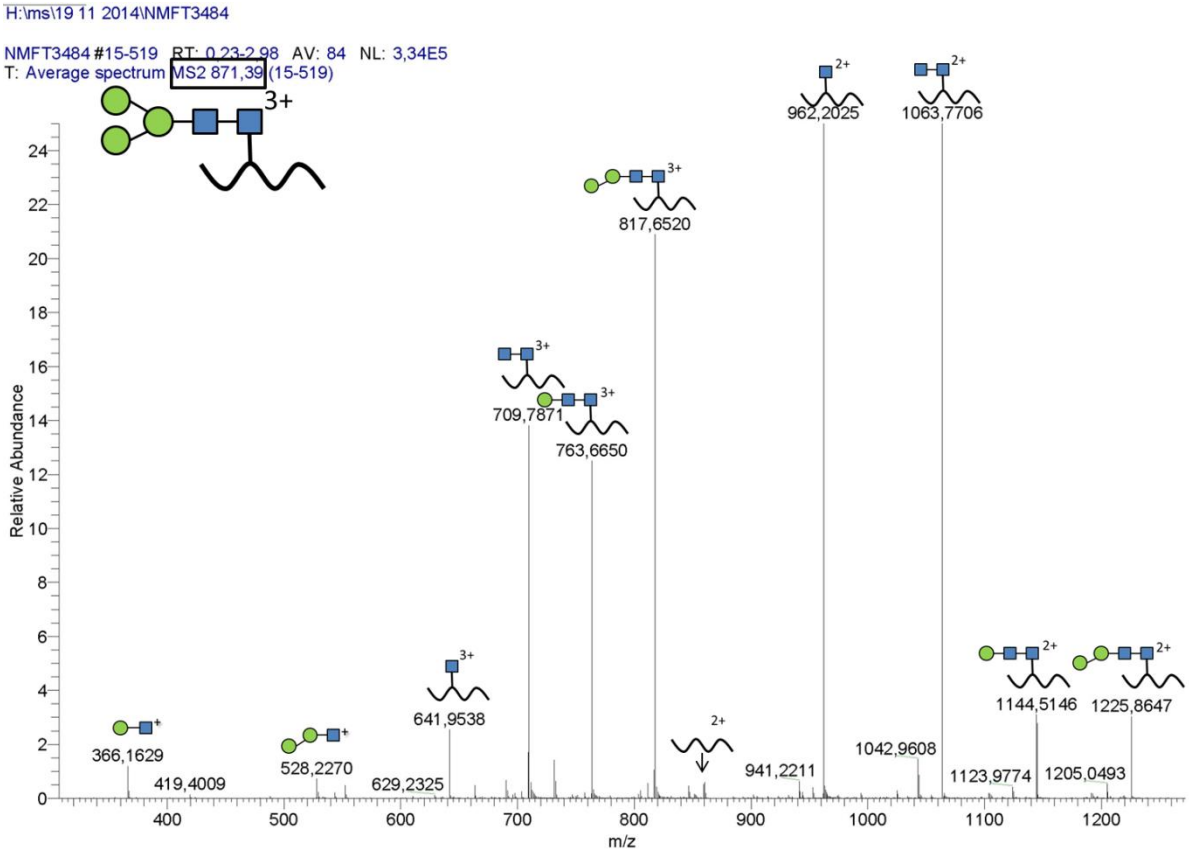


Figure 12. MS2 fragmentation of the enzyme coupling product, pentasaccharide core *N*-glycopeptide **164**.

The coupling product was verified by high resolution mass spectrometry, and a major 3^+ ion peak was identified and further fragmented by tandem MS/MS. Different MS2 peaks were identified showing that the sugar residue on the glycopeptide was cleaved sequentially in 2^+

and 3^+ ions until the peptide backbone was observed as well as the cleaved Man-GlcNAc and Man-Man-GlcNAc fragments.

Based on previous conditions, four more triethyleneglycol spacer peptides (**152**, **153**, **157** and **162**) were coupled with the tetrasaccharide oxazoline **110** under endoglycosidase catalysis. All these couplings were finished in 0.5 h with full conversion, confirmed by HPLC and MS. The obtained pentasaccharide core peptides were all used in the followed microarray studies.

2.12 Enzymatic on-slide coupling of the tetrasaccharide oxazoline to synthetic N-GlcNAc N-glycopeptides combined with microarray studies

To generate a *N*-glycopeptide library, by enzymatic coupling of the *N*-GlcNAc peptides to different oxazoline donors one by one in solution is time consuming. Another approach is that the *N*-GlcNAc peptide library first is printed on a microarray slide and then the enzymatic coupling with the oxazoline donor take place directly on the slide, on-slide glycosylation. In this way, multiple *N*-glycopeptides can in parallel be extended with the oligosaccharide oxazoline donor in one synthetic step. To evaluate this approach, several enzymatic couplings were first evaluated in solution, indicating that the coupling conditions are optimal and that no hydrolysis took place. Although the in-solution reactions behave slightly different from performing the enzymatic coupling on-slide, it could at least be indicative for the on-slide reaction progress. By enzymatic coupling in solution, testing the coupling conditions, four selected *N*-GlcNAc peptides **152**, **153**, **157** and **162** (peptide Nr *3*, *4*, *8* and *13* in **Table 2**) were converted into pentasaccharide glycopeptides (containing a triethyleneglycol spacer) **165** - **168** (referring to Nr *3p*, *4p*, *8p*, *13p* in **Figure 13** A). These glycopeptides were printed on the microarray slide as internal standards together with the mono glycosylated *N*-GlcNAc peptide acceptors **150** - **163,** which were going to be enzymatically extended with the oxazoline donor oligosaccharides (peptide Nr *1* - *14* in **Table 2**, referring to Nr *1* - *14* in **Figure 13** A).

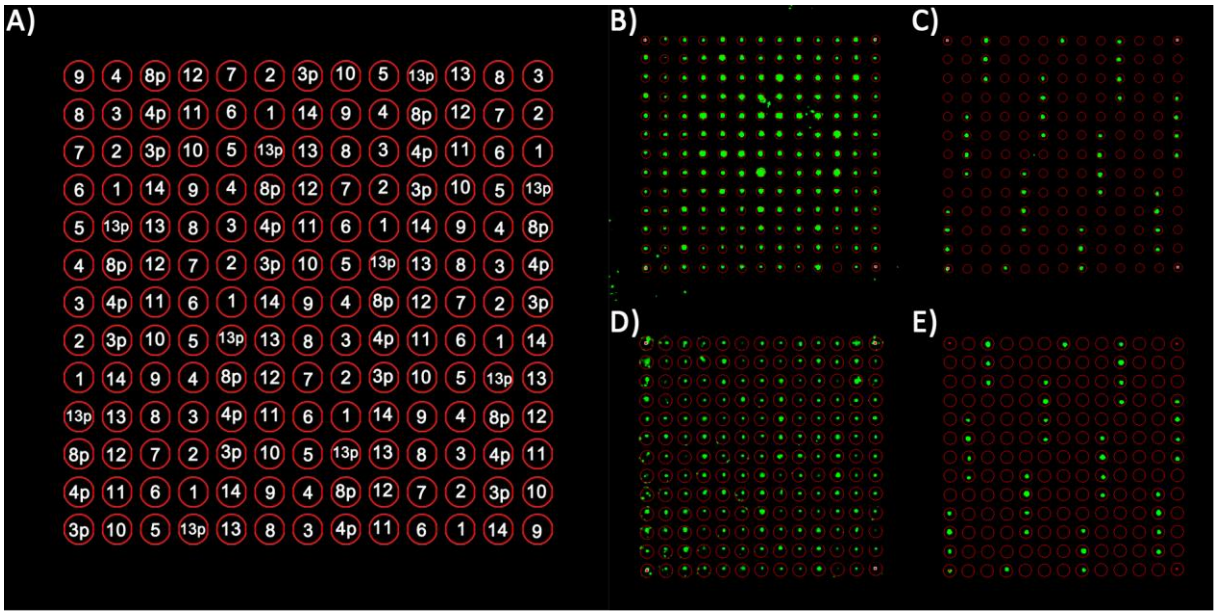
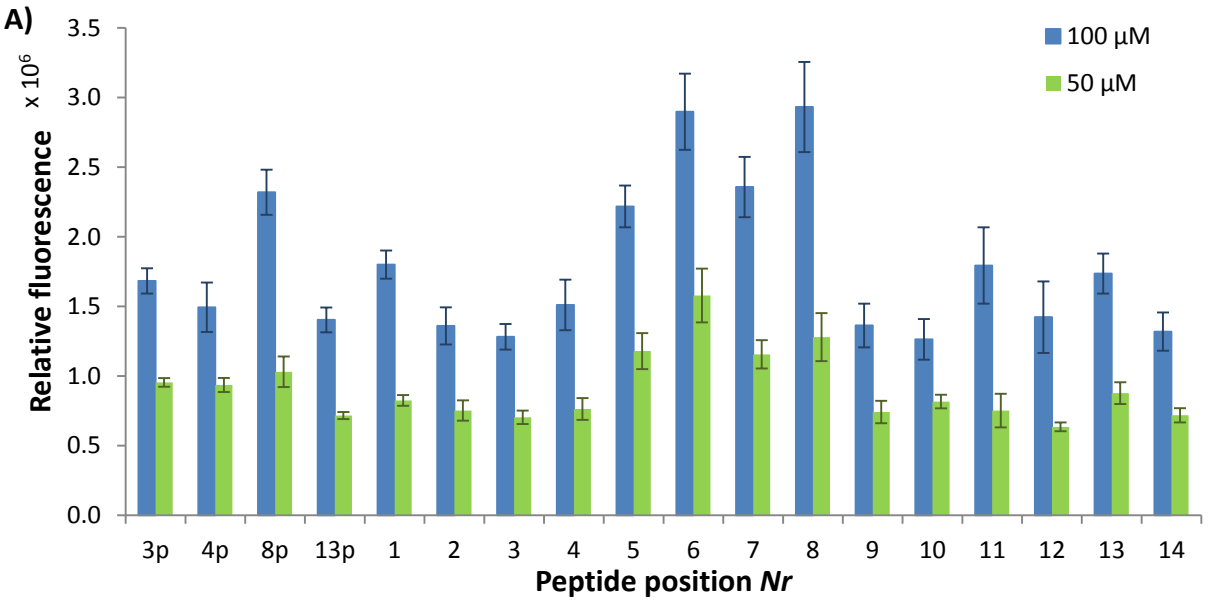


Figure 13. Enzymatic *N*-glycan extension performed on a microarray slide including a few in-solution extended reference peptides. **A)** Spotting pattern, Nr *1* - *14* corresponds to the *N*-GlcNAc peptides **150** - **163** in **Table 2**, and Nr *3p*, *4p*, *8p*, *13p* refer to the reference peptides **165** - **168**, already extended with pentasaccharide core *N*-glycosylation; **B)** Peptides printed at 50 µM, with enzymatic on-slide *N*-glycan extension and recognized by incubation with ConA; **C)** Peptides printed at 50 µM, directly recognized by incubation with ConA without enzymatic on-slide extension; **D)** Peptides printed at 100 µM, with enzymatic *N*-glycan on-slide extension and recognized by incubation with ConA; **E)** Peptides printed at 100 µM, directly recognized by incubation with ConA without enzymatic on-slide extension.



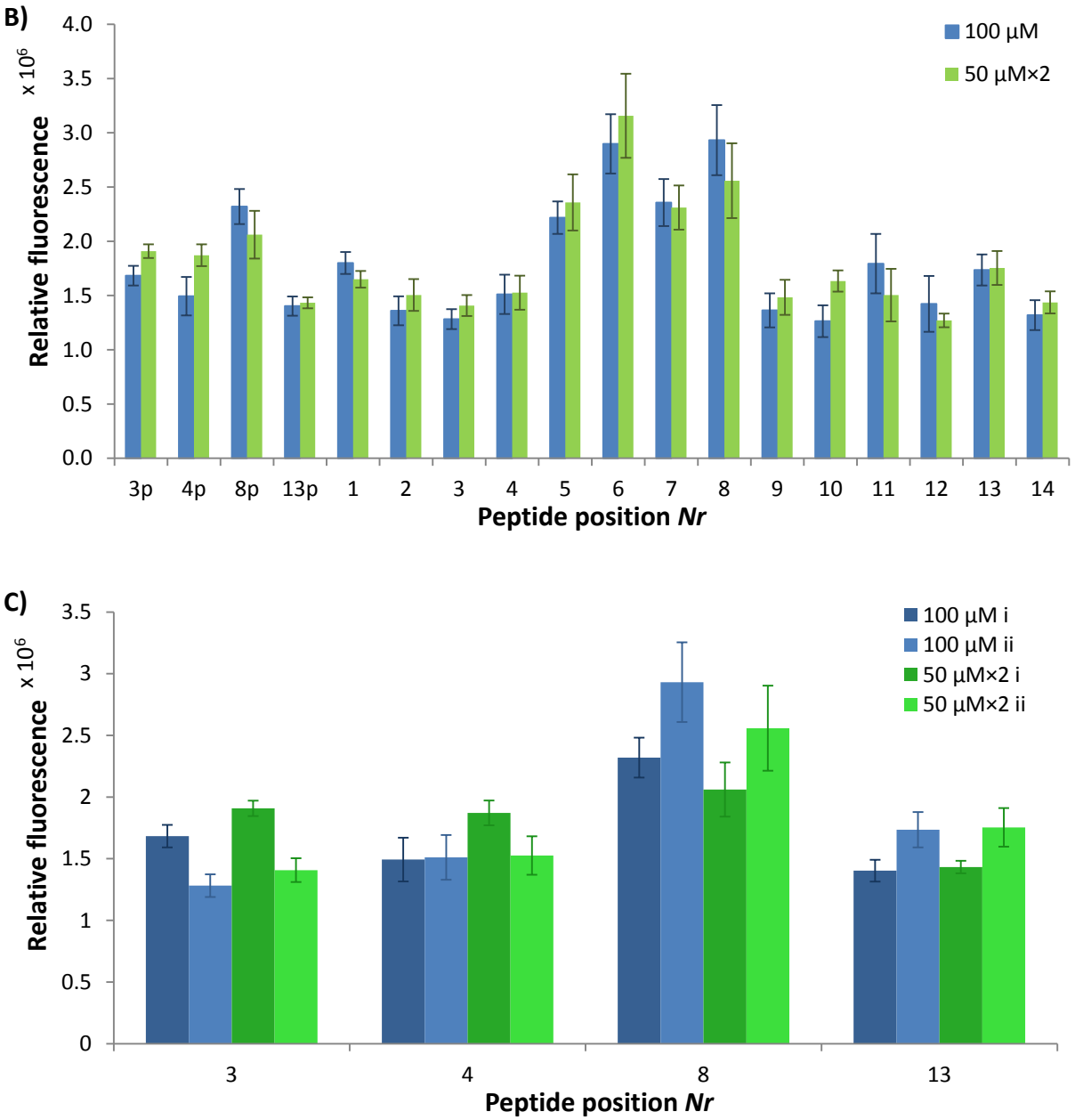


Figure 14. **A)** Direct data comparing well D and B in **Figure 13**, the peptide substrate was printed in two concentrations, 50 µM and 100 µM; **B)** Comparing the intensity of the 100 µM signals with the double intensity of the 50 µM signals; **C)** Comparing signal intensities of four peptides spotted at 100 µM concentration and the double signal intensities spotted at 50 µM concentration. i, internal standard, in solution *N*-glycan extended peptides from well D and B in **Figure 13**, ii, the same peptides synthesized by on-slide enzymatic *N*-glycan extension from well D and B in **Figure 13**.

Two concentrations, 50 µM and 100 µM, were selected for printing the *N*-glycopeptide library and after immobilization the library were treated with the tetrasaccharide oxazoline donor **110** and the N175Q Endo-M enzyme for 1 h. The on-slide enzymatically extended

glycopeptides (**Figure 13** B and D) and the blank control without enzymatic on-slide extension (**Figure 13** C and E) were incubated with the biotin-labeled glycan binding lectin ConA, which recognize α-mannoside residues. After treatment with Cy5 streptavidin, the relative fluorescence were detected and the binding data analyzed.

Shown in **Figure 14** A, in solution enzyme extended *N*-glycopeptides and the on-slide enzyme extended *N*-glycopeptides showed similar average signal intensity, indicating that all of the 14 *N*-GlcNAc peptides were converted to the pentasaccharide core *N*-glycopeptides. Shown in **Figure 14** B, comparing the signals of the peptides spotted at 100 µM with the double signal intensity of the peptides spotted at 50 µM, indicated that the binding affinity observed from two experiments are similar. Certain fluctuations were observed between the different peptides, which in part can be explained by differences in lectin recognition to the *N*-glycan residue presented on different peptide backbones. In **Figure 14** C, four glycopeptides pre-synthesized by in-solution enzymatic extension, were used as internal standards. The internal standard peptides were directly compared with the on-slide enzymatic reaction and showed similar signal intensities. In conclusion, it is feasible to generate an *N*-glycopeptide library with on-slide Endo-M enzymatic extension using an active oxazoline glycan donor and multiple *N*-GlcNAc peptide acceptors immobilized on the slide. Thus the procedure needs to be further optimized to reduce background problems and thereby achieving higher reproducibility.

2.13 Coupling of the hexasaccharide oxazoline to *N*-GlcNAc peptides

A second oxazoline donor, the hexasaccharide **112**, was also evaluated for enzymatic glycopeptide extension using the Endo-M mutant. The conditions were in accordance with the description above, using 0.07 - 0.1 µmol substrate, 8.0 eq donor and 12.5 µM enzyme. The reactions were kept at pH 7.1, 25 °C and followed by analytical HPLC.

Two different monoglycosylated peptide substrates **136** and **157** were tested in the in-solution coupling with hexasaccharide **112**, the coupling products were observed on analytical HPLC, which were slightly faster than the starting peptides. The conversion rate reached up to 20%, and afterwards back hydrolysis to the starting peptides were then observed, the same results were shown in both cases.

Scheme 40 Enzymatic coupling of hexasaccharide oxazoline donor **112** on *N*-GlcNAc peptide **136** sequence: IN*TTADEKDPTNPFR from ADAM10 and **157** sequence: *Sp*N*LTALPPDLPK from Platelet glycoprotein 1b alpha.

The reason for the slow product formation causing hydrolysis back to the starting peptides cannot be completely explained, it might be a substrate limitation by the particular Endo-M enzyme mutant, but other Endo-M mutants, have previously shown to catalyze complex type oxazoline donors in higher yields. Since the oxazoline donor was not considered to be very stable, a desalting step was avoided after deacetylation, however it might be that these salt impurities of the donor caused problems in the followed enzymatic coupling reaction.

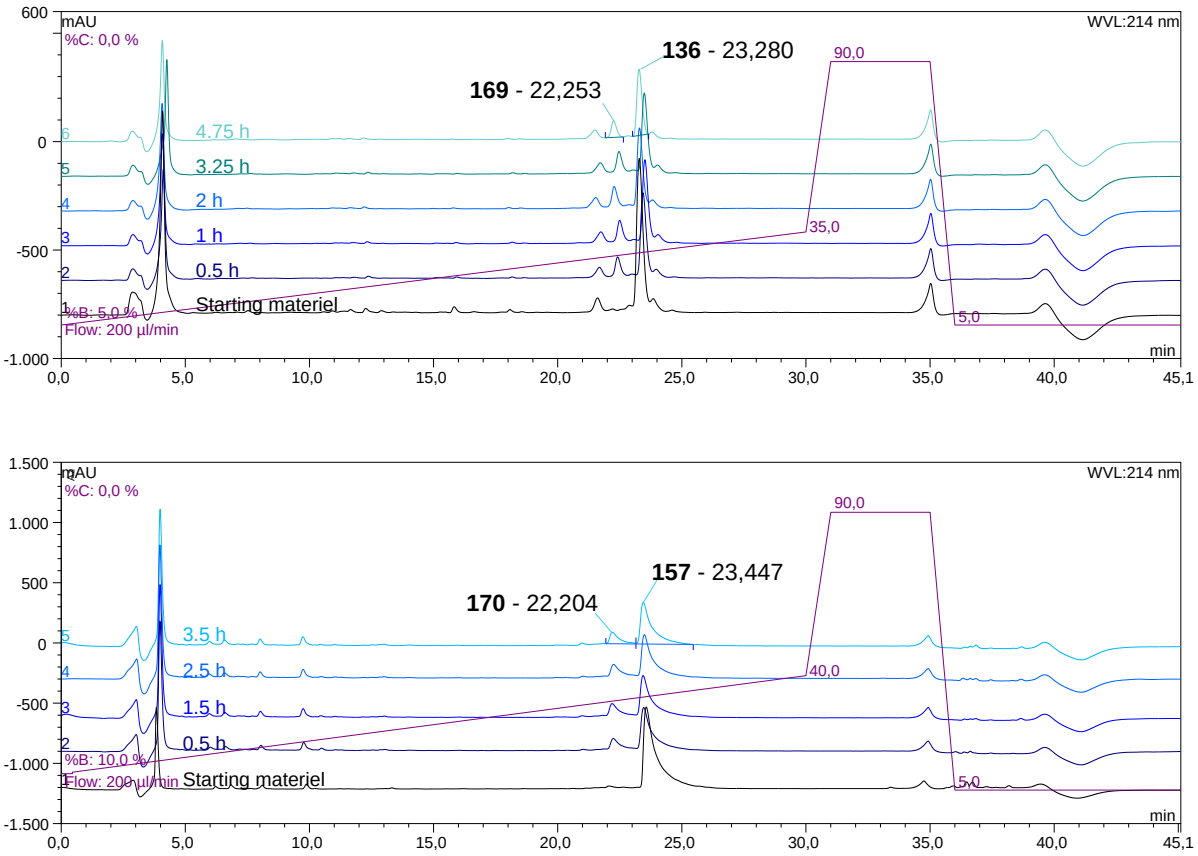


Figure 15. HPLC chromatogram following the hexasaccharide enzymatic coupling. Upper chromatogram figure: converting peptide **136** to the coupling product **169**; lower chromatogram figure: converting peptide **157** to **170**.

136 enzyme coupling with oxazoline 112 upper HPLC chromatograms			**157 enzyme coupling with oxazoline 112 lower HPLC chromatograms**		
Reaction time	Prod 169 (%)	Starting peptide (%)	Reaction time	Prod 170 (%)	Starting peptide (%)
0.5 h	19	81	0.5 h	17	83
1 h	21	79	1.5 h	20	80
2 h	21	79	2.5 h	19	81
3.25 h	21	79	3.5 h	18	82
4.75 h	20	80			

Table 3. Peak integration on HPLC chromatogram following the enzymatic coupling.

The coupling product **169** and **170** were purified and measured by high resolution ESI-MS. The major glycopeptide peak of the 3^+ ion was detected and further fragmented by MS2 for detailed structural analysis.

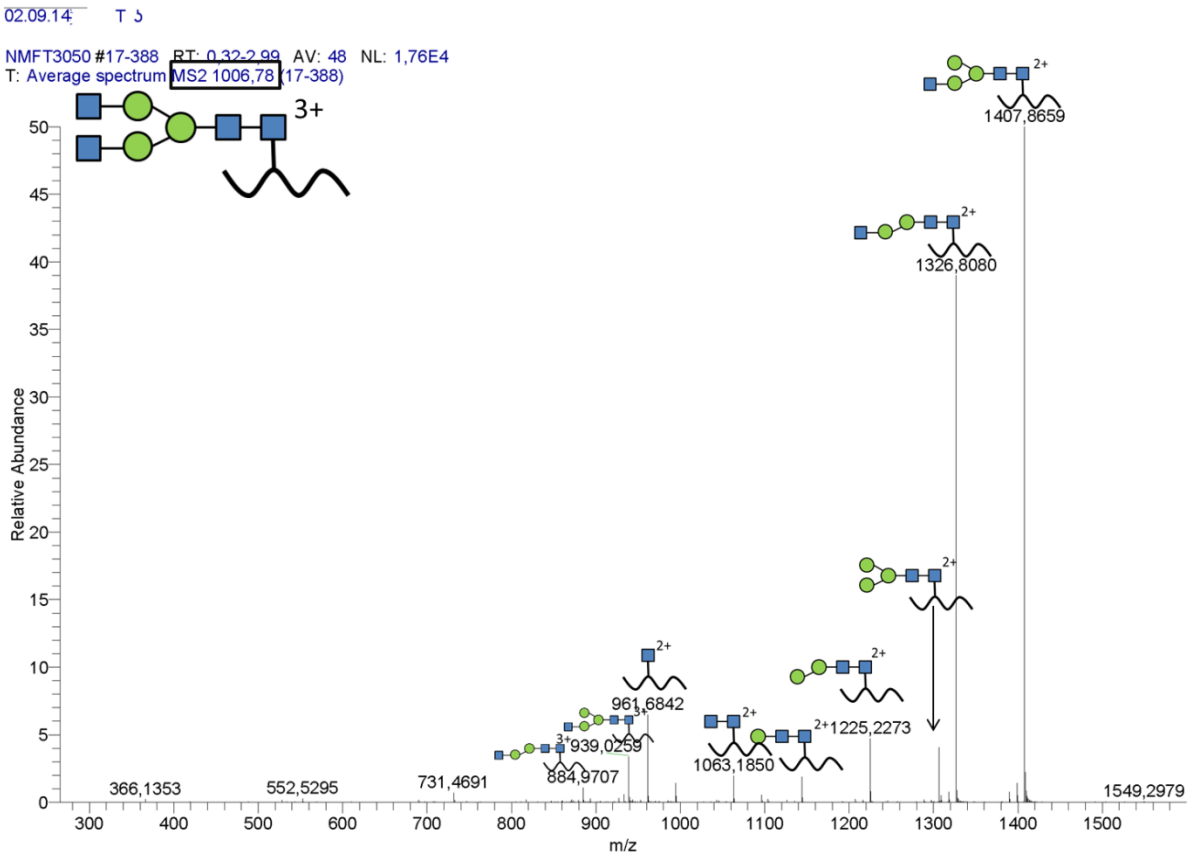


Figure 16. MS2 fragmentation of the heptasaccharide *N*-glycopeptide **169**.

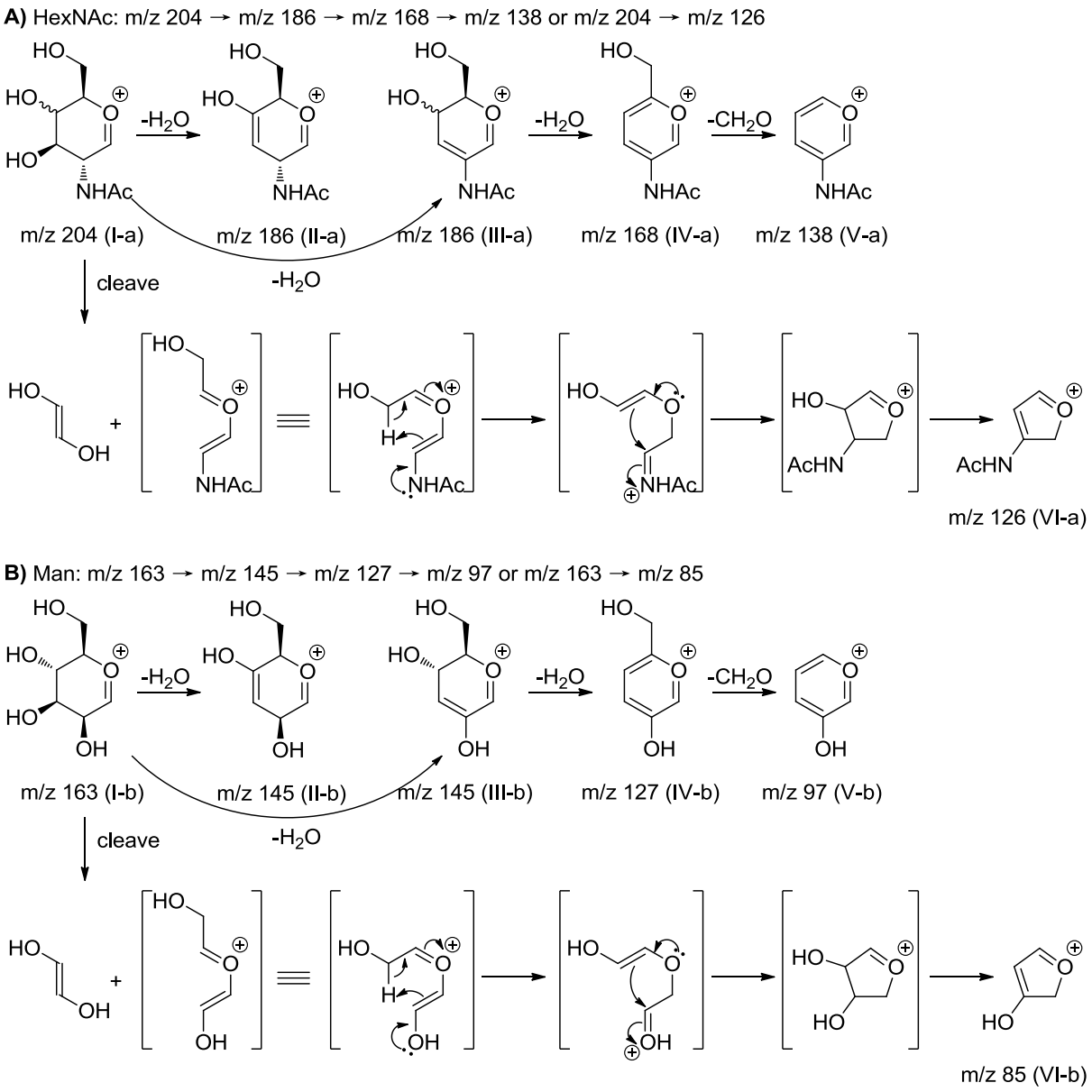
2.14 Distinctive MS/MS fragmentation pathways of *N*-glycopeptides for saccharide structure identification

Mass spectrometry (MS)-based proteomics has in the recent years developed into a powerful method to qualitatively and quantitatively characterize a substantial number of all the proteins expressed in a cell. The use of proteomic methods for the identification of post-translational modifications (PTMs) of proteins has also emerged as an important tool to investigate protein regulation. In order to increase our knowledge about protein function and interactions it is important to continue the development of glycoproteomic methods aimed at characterizing glycosylation sites, and the glycan structures occupying such sites. MS strategies have frequently been used to determine the structures of released glycans,[209-210] the utilization of intact glycopeptides represents a bioconjugate structure where the glycosylation site structure and protein identity may be obtained from the same molecule. In a collaboration with Dr. Jonas Nilsson, we recently showed that the intensity profiles of saccharide oxonium ion spectra generated from collisional activation, using

positive mode higher energy dissociation (HCD),[211] of glycopeptides differed depending whether the epimeric structure was GalNAc or GlcNAc. A GlcNAc/GalNAc ratio term was introduced, where the (138+168)/(126+144) intensity ratio was used to designate glycopeptides as GlcNAc- or GalNAc-like showing typical values of 2-50 and 0.2-1, respectively.

To better understand the observed differences of oxonium ion spectra profiles, the *N*-glycopeptides **165** and **166** with synthetically well-defined structures were additionally subjected to HCD and multistage CID-MS^n experiments. The use of glycopeptides consisting of known Hex and HexNAc, linkages, core structures and anomeric configurations, enables investigations of the interrelation between small differences in glycan structure and the corresponding oxonium ion profiles. Effects of oxonium ion decomposition pathways for HexNAc containing glycoconjugates have partly been addressed previously,[212-213] but the precise correlation between them, their structure and mechanism of formation have not been fully investigated. The unraveling of various oxonium ion decomposition pathways will be of help in the design of new and improved methods for qualitative and quantitative glycopeptide analysis.

In addition to the HexNAc oxonium ions, the terminal Man residues on the synthetic pentasaccharide core glycopeptides also gave rise to oxonium ions at *m/z* 163, 145, 127, 97 and 85, having uneven *m/z* values and thus lacking nitrogen (**Figure 17** A and B). The *m/z* 163 → *m/z* 145 → *m/z* 127 → *m/z* 97 sequence was observed, which is analogous to the *m/z* 204 / 366 → *m/z* 138 decomposition of the HexNAc oxonium ions, and show that this pathway is also present for Man-generated Hex oxonium ions. Further, the occurrence of the *m/z* 85 ion is analogous to the *m/z* 204 → *m/z* 126 ring-fragmentation pathway for HexNAc oxonium ions, indicating that the decomposition of $[Hex]^+$ also is mechanistically similar. These Hex-generated oxonium ions are generally not detectable in the HCD spectra of glycopeptides carrying terminal HexNAc or HexHexNAc structures, i.e. for complex type *N*-glycopeptides, an example is presented here in (**Figure 17** C). Thus, terminal HexNAc is substantially more prone to form oxonium ions normally suppressing the simultaneous presence of Hex oxonium ions.



Scheme 41. Proposed reaction mechanisms for HCD and CID induced saccharide decomposition of **A)** HexNAc and **B)** mannose; unit loss of H_2O, anomerization and ring-cleavage.

This prevalence may depend on the tendency of the HexNAc *N*-acetyl group to become protonated during the ESI facilitating the formation of charged fragments originating from the HexNAc. Similar results, with prominent Man-and GlcNAc-generated oxonium ions, were obtained by fragmentation of **166** demonstrating that the peptide part does not influence the oxonium ion fragmentation patterns (Section 10). The realisation of these Man-specific oxonium ions prompted the investigation whether they also were observable in glycoproteomic samples. In a trypsinised and strong anion exchange (SAX) prepared human cerebrospinal fluid sample subjected to LC-MS/MS,[214] indeed the presence of

Hex-generated oxonium ions was observed in a number of HCD spectra. By analysis of the peptide backbone fragmented b- and y-ions one of these was identified to be TVLTPATNHMGN*VTFTIPANR from Complement C3 carrying a $(Man)_5(GlcNAc)_2$ glycan at the underlined Asn (**Figure 17** B). These results show that Man-generated oxonium ions indeed regularly are formed in the HCD of high-mannose *N*-glycopeptides and could become significant already at the relatively simple $(Man)_3$-substitution level (**Figure 17** A and B).

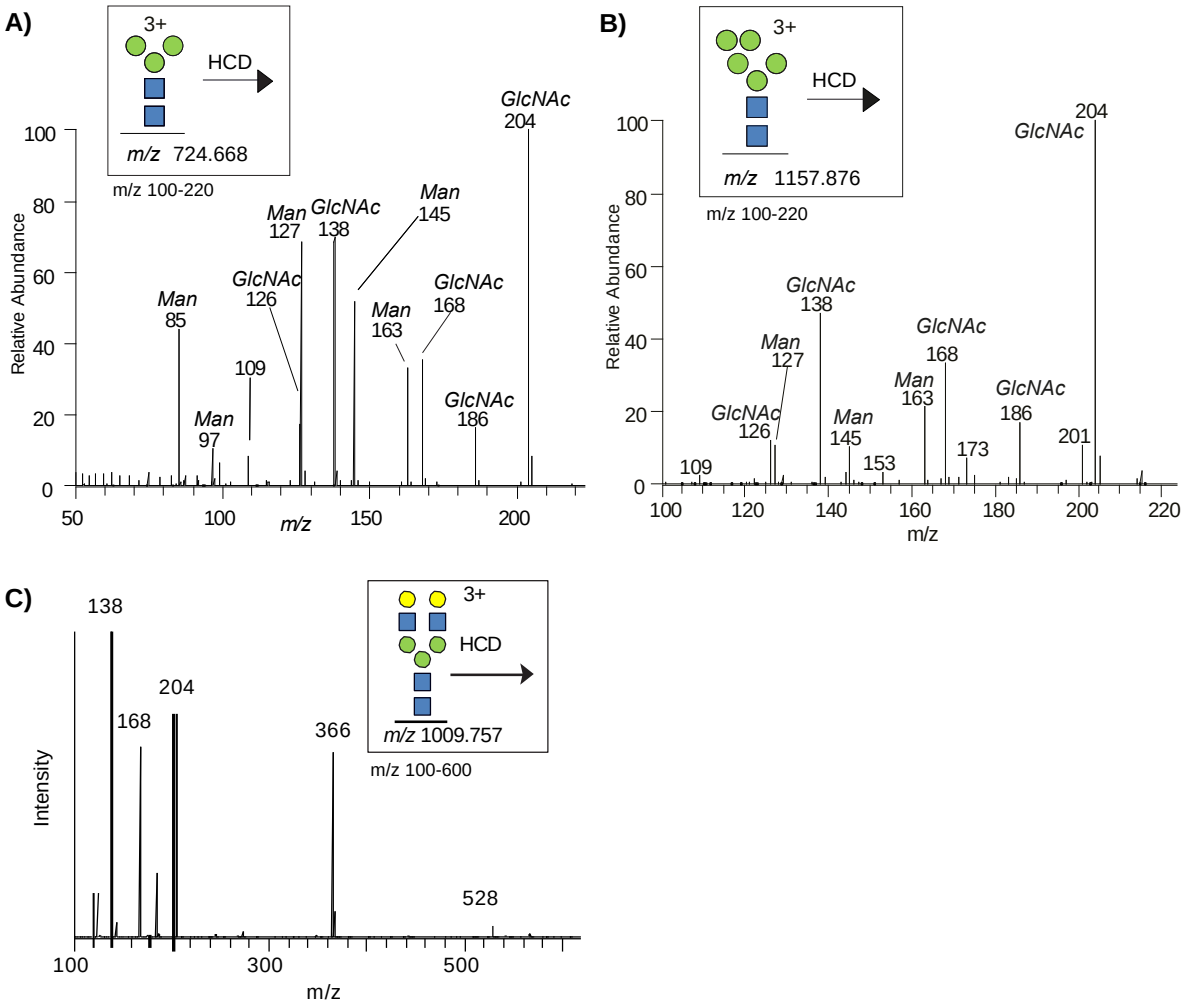
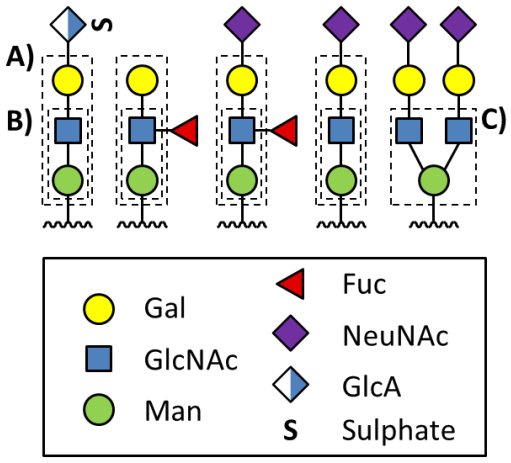


Figure 17. HCD of synthetic and native high-mannose *N*-glycopeptides. **A)** The HCD spectrum at the NCE 20% level shown for the *m/z* 50-220 region a synthetic $(Man)_3(GlcNAc)_2$ substituted Antithrombin III peptide **166**, sequence: NH_2-Spacer-WVSN*KTEGR. See also section 10. **B)** The HCD spectrum, including a *m/z* 100-220 expansion of a native $(Man)_5(GlcNAc)_2$ substituted peptide. The sequence is TVLTPATNHMGN*VTFTIPANR from complement C3 (UniprotKB id: CO3_HUMAN). **C)** The HCD spectrum of *m/z* 100-600 expansion of a native $(Gal\text{-}GlcNAc\text{-}Man)_2Man(GlcNAc)_2$ substituted SWPAVGN*CSSALR tryptic peptide fragment from hemopexin.

Project 2: *Synthesis of extended O-mannose glycopeptide constructs for generation of glycan and glycopeptide specific antibodies*

3.1 Introduction to *O*-Mannose modifications

Proteins modified with *O*-mannose glycans on backbone Ser and Thr residues, are involved in important functions in brain and muscle glycobiology.[215-216] For example in α-Dystroglycan (α-DSG),[217-219] the *O*-mannose glycosylation is essential for stabilization of the dystrophin-glycoprotein complex acting as a transmembrane linker between the extracellular matrix and intracellular cytoskeleton.[220] The *O*-mannose glycans on α-DSG are often extended with sialyl-*N*-acetyllactosamine[221] on the peptide tandem repeats resulting in the formation of NeuNAcα2-3Galβ1-4GlcNAcβ1-2Manα tetrasaccharide structures.[222-223] Other *O*-mannose glycan epitopes found on α-DSG are extension with fucose-*N*-acetyllactosamine, the HNK-1 epitope and branching on the 6-position of the mannose unit (**Scheme 42**).[224-225]



Scheme 42. *O*-Mannose glycans identified on mammalian proteins. **A)** Gal-GlcNAc-Man trisaccharide; **B)** GlcNAc-Man disaccharide and **C)** 2,6 di-GlcNAc-Man trisaccharide structures were employed as target for vaccine design.

α-DSG were long considered to be the only *O*-mannosylated protein in mammals, however a study on *O*-glycans from total rabbit brain gave a different picture concluding that about 30% of the released *O*-glycans[226] were identified as *O*-mannosylated.[227-229] The high abundance of protein *O*-mannosyl glycans in brain was also shown in a study of the mice brain

proteome.[230] Recently, a handful of additional mammalian glycoproteins were identified containing *O*-mannosyl modifications, the transmembrane protein CD24,[231] a monoclonal IgG2 light chain from CHO cells,[232] receptor protein-tyrosine phosphatase, known as RPTPβ,[233-234] phosphacan,[235] tenascin-R, neurofascin 186, neurocan,[236-237] brevican and versican.[238] These studies altogether raise a conviction that many more not yet identified proteins carry *O*-mannosyl glycans with critical functions in the mammalian brain.

3.2 Project aim

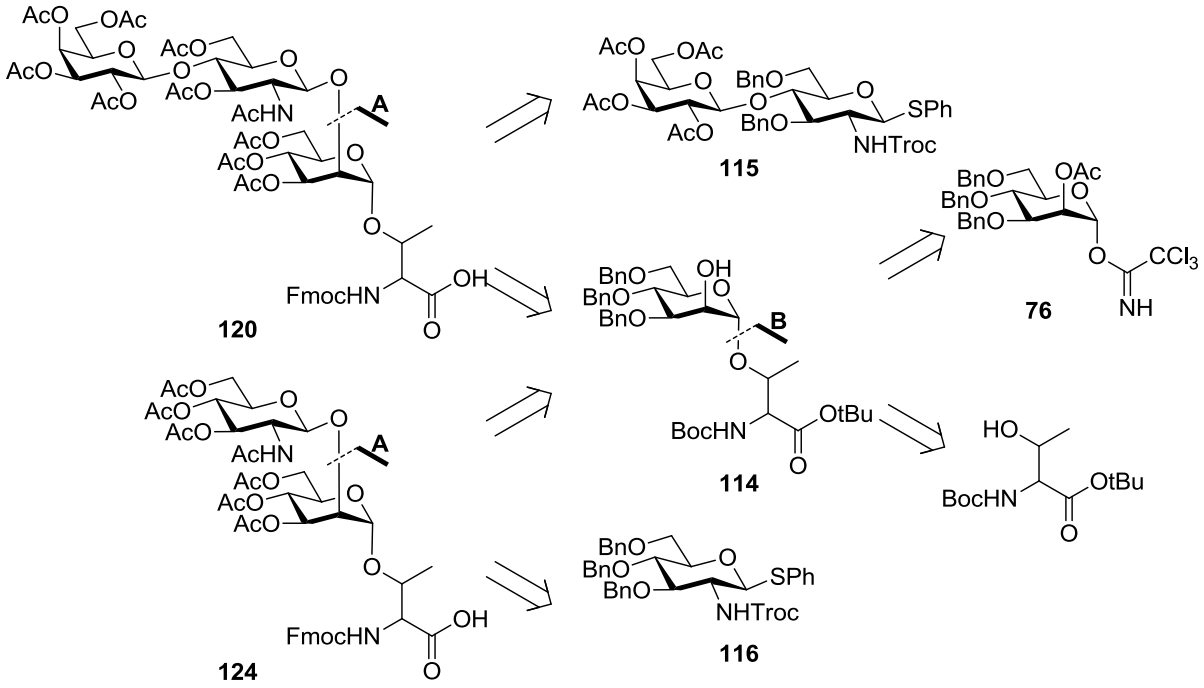
In spite of the clear importance of *O*-mannosyl glycosylation, no tools are to date available for specific detection, enrichment and identification of proteins containing these modifications. In order to study these processes, a project was initiated to generate glycan and glycopeptide specific antibodies, which could be useful tools to detect and enrich *O*-mannosylated proteins. Vaccine-constructs were prepared from glycopeptides containing these extended mannose moieties. Since the terminal *O*-glycans of the *O*-mannosyl modifications show structural similarities with the complex type *N*-glycans, a few common building blocks were used for construction of both *O*-mannosyl amino acids and *N*-glycans as described in the section above. The prepared Fmoc-*O*-mannosyl glycosylated amino acids were employed in glycopeptide synthesis. The glycopeptides were then incorporated into glycopeptide-immune carrier constructs for antibody induction in rabbits. For antibody evaluation additional glycopeptides were prepared and employed in ELISA neutralization experiments and on glycopeptide microarrays.

3.3 Synthetic strategy for synthesis of the linear Gal-GlcNAc-Man and GlcNAc-Man *O*-glycoamino acids

Protein *O*-mannosylation on mammals typically contain a common Galβ1-4GlcNAcβ1-2Manα trisaccharide core structure, which has been found further modified with branching or termination with the HNK epitope, sialic acid or fucose residues (**Scheme 42** A and B). A few chemical and chemoenzymatic synthesis strategies for preparation of simple *O*-mannosyl glycosylated amino acids and glycopeptides were previously reported.[239-243]

Glycosylated amino acids containing the linear core structures, the Galβ1-4GlcNAcβ1-2Manα trisaccharide and the GlcNAcβ1-2Manα disaccharide, were here chemically prepared

(**Scheme 43**). The disaccharide amino acid containing a terminal GlcNAc residue was considered to be more immunogenic, when included in a glycopeptide vaccine construct compared with the corresponding trisaccharide with a terminal Gal residue. The chemical strategy involved the synthesis of a protected α-mannosylated threonine amino acid building block, which could be extended through NIS activation with the Galβ1-4GlcNAc thioglycoside donor **115** or the GlcNAc thioglycoside donor **116** to obtain the di- or trisaccharide amino acids. The mannosyl threonine could in turn be prepared by coupling of a mannose trichloroacetimidate donor **76** with a protected threonine acceptor employing trichloroacetimidate chemistry.[244]

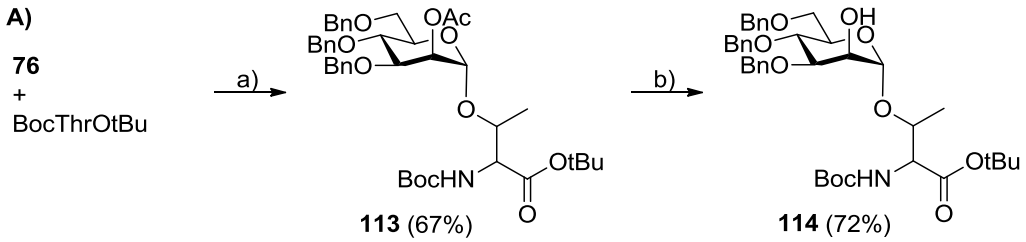


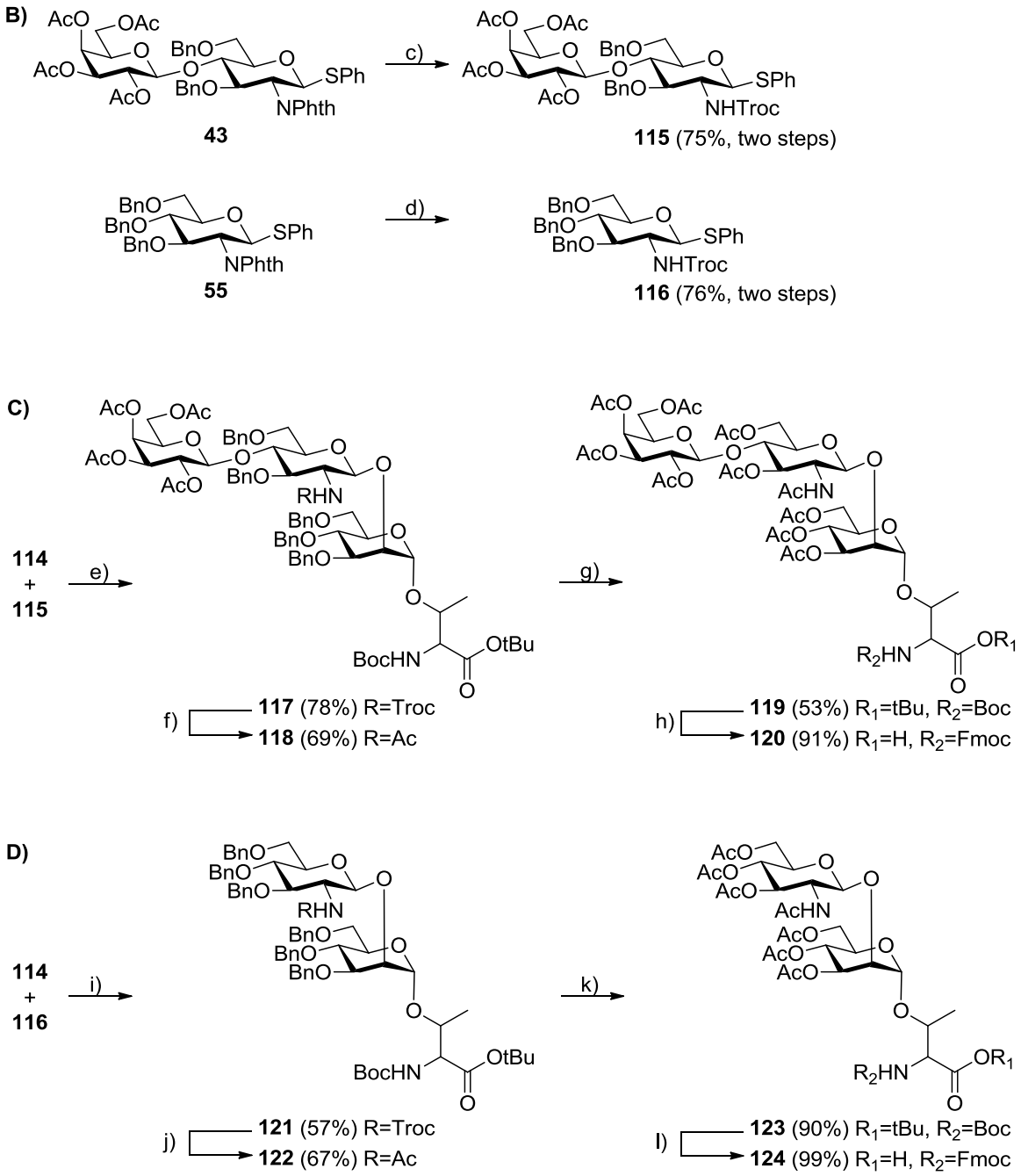
Scheme 43. Retrosynthetic analysis for synthesis of the glycosylated Fmoc amino acid building blocks.

3.4 Synthesis of the linear Gal-GlcNAc-Man and GlcNAc-Man *O*-glycoamino acids

The chemical synthesis commenced with the coupling of the mannose trichloroacetimidate donor **76,** described above in the *N*-glycan part (**Scheme 33** A), with the *N*-Boc and *O*-*t*Bu protected threonine acceptor[245] resulting in the α-Man-Thr coupling[246-247] product **113** (**Scheme 44** A). The mannose trichloroacetimidate donor **76** contained a single acetyl protecting group for selective deprotection and glycan extension in the 2-position, the remaining hydroxyls were protected with stable benzyl groups. By neighboring group

participation, the acetyl group in the mannose 2-position would further promote the formation of an α-glycosidic bond during coupling with the threonine acceptor. The threonine residue was Boc protected, since the later removal of the glycan benzyl groups by catalytic hydrogenation, would be problematic in the presence of Fmoc groups. Amino acid Fmoc-protection was necessary to proceed with the later peptide synthesis and the Fmoc group was therefore inserted after removal of the benzyl protecting groups.[245] For further extension of the α-Man-Thr coupling product **113**, the acetyl group in the Man 2-position was removed with sodium methoxide in methanol to obtain compound **114**. The glycan thioglycoside donors employed in α-Man-Thr extension, were converted from *N*-phthalimido protected thioglycoside donors, disaccharide **43** and monosaccharide **55** (**Scheme 44** B), to the *N*-Troc protected donors **115** and **116** using hydrazine hydrate followed by treatment with acetic anhydride in pyridine. The synthesis of the Phth saccharides **43** and **55** were described in the *N*-glycan section (**Scheme 30** B and **Scheme 31** A). The *N*-Troc protecting groups on the thioglycoside donors were considered to contribute with increased reactivity of the prepared donor building blocks. Additionally, removal of the phthalimido groups in later steps under strong base conditions may result in β-elimination and Phth protection was therefore not considered to be compatible with *O*-glycosyl amino acid synthesis.[248-249] The deacetylated α-Man-Thr **114** was then elongated with two *N*-Troc thioglycoside donors **115** and **116** under NIS, TfOH activation[250-251] to give compound **117** and **121** (**Scheme 44** C and D). The conversion of the obtained di- and trisaccharide amino acid coupling products into stable building blocks compatible with Fmoc solid-phase peptide synthesis (Fmoc-SPPS), was achieved by a few protecting group manipulation steps. Initially, the *N*-Troc groups of **117** and **121** were removed by zinc reduction followed by acetylation to give **118** and **122**. Catalytic hydrogenation with 10% Pd(C) and subsequent acetylation resulted in the formation of the compounds **119** and **123**. Simultaneous removal of the threonine *tert*-butyl ester (*t*Bu) and amine *tert*-butyloxycarbonyl (NHBoc) using trifluoroacetic acid (TFA) was followed by *in situ* reaction with Fmoc-*O*Su to give the desired glycosylated amino acid **120** and **124**, which were ready to use in Fmoc-SPPS.[252-253]



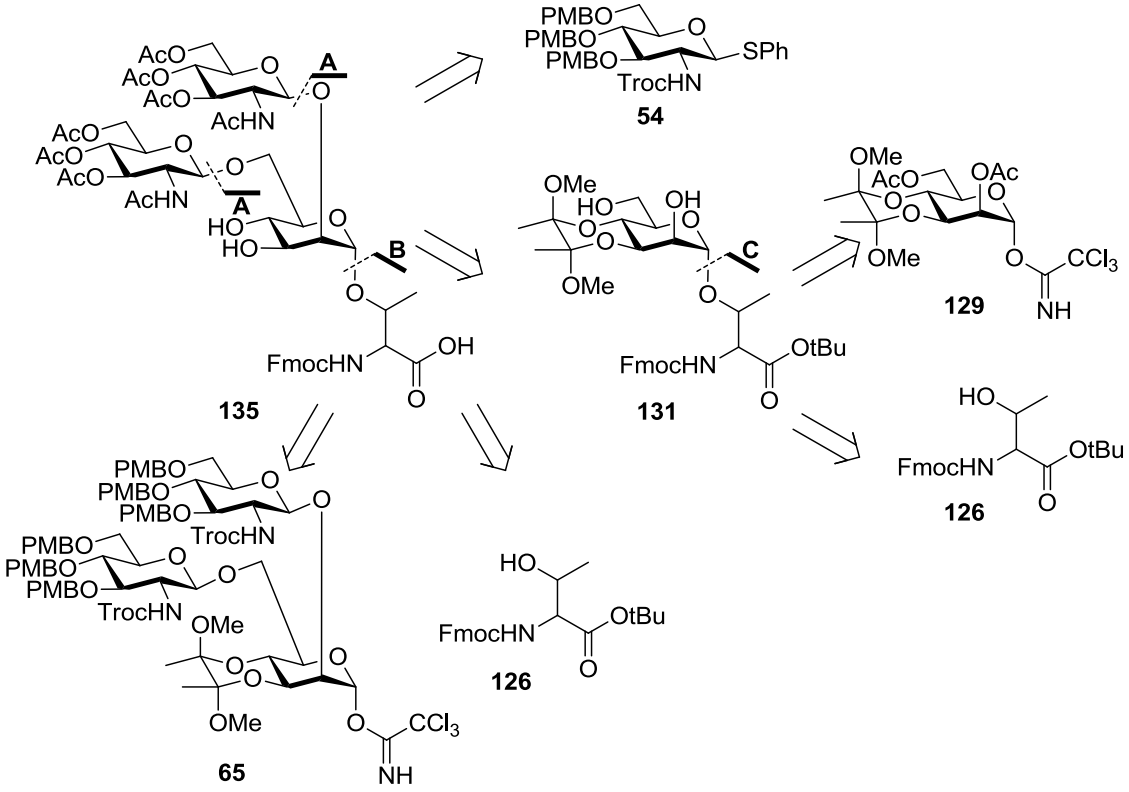


Scheme 44. a) TMSOTf, molecular sieves, DCM, -40 °C 2 h; b) 0.08 M NaOMe in MeOH, r.t. 2 h; c) hydrazine hydrate, MeOH, 70 °C, 2 h, 62%; TrocCl, $NaHCO_3$, dioxane/H_2O 2:1, r.t. 2 h; Py/Ac_2O, r.t. 24 h; d) hydrazine hydrate, Tol/EtOH 2:3, 105 °C 72 h; 1,4-dioxane, $NaHCO_3$ in H_2O, TrocCl, r.t. 2 h; e) NIS, TfOH, molecular sieves, DCM, -18 °C 2 h; f) Zn, AcOH, 40 °C 48 h; Py/Ac_2O, r.t. 24 h; g) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 48 h; Py/Ac_2O, r.t. 24 h; h) TFA/DCM/anisole 3:1:0.4, r.t. 24 h; Fmoc-OSu, $NaHCO_3$, dioxane, r.t. 4 h; i) NIS, TfOH, DCM, -40 °C, 30 min; j) Zn, AcOH, r.t. 36 h; k) H_2, Pd(C), MeOH/AcOH 30:2, r.t. 48 h; Py/Ac_2O, r.t. 24 h; l) TFA/DCM 3:1, r.t. 3 h; Fmoc-OSu, $NaHCO_3$, dioxane, r.t. 4 h.

3.5 Synthetic strategy for synthesis of a branched $GlcNAc_2$-Man-*O*-Thr amino acid building block

A 2,6 branched *O*-mannosyl glycosylated amino acid was also prepared for further binding specificity analysis of the induced antibodies directed to the linear core *O*-mannosyl structure, which was described above (**Scheme 42** C). Additionally it was considered interesting to study binding interactions of mannose specific lectins comparing the linear and branched *O*-mannosyl structures.

Two different synthetic strategies were evaluated for synthesis of the 2,6-branched *O*-mannosyl glycosylated amino acid **135**. Similar to the synthesis of the linear 2-elongated *O*-mannosyl glycosylated amino acid described above, a functional mannosyl trichloroacetimidate donor **129** was used in the coupling with a protected threonine **126.** The obtained α-mannosylated threonine amino acid building block was deacetylated on both the 2 and 6-positions, which thereafter were extended with the GlcN thioglycoside donor **54** on both hydroxyls simultaneously (**Scheme 45** A and C). In the second approach the trisaccharide donor **65,** was directly connected to the Fmoc threonine **126** (**Scheme 45** B).

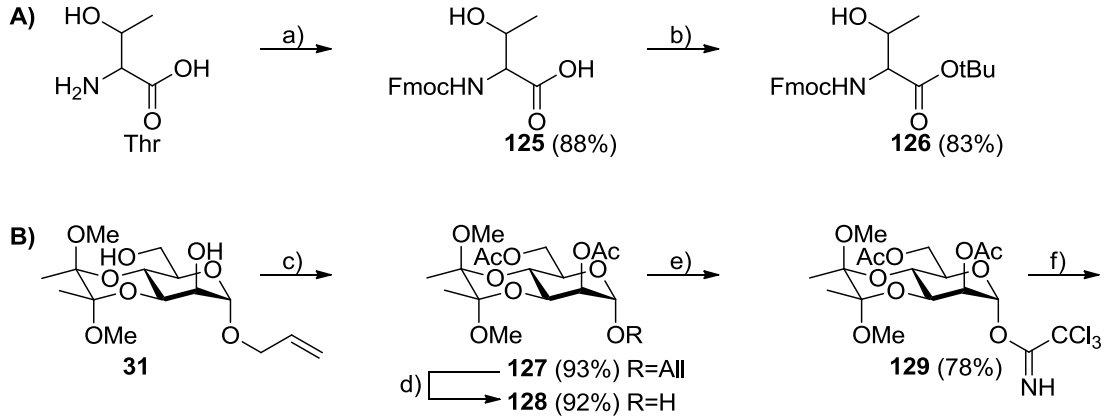


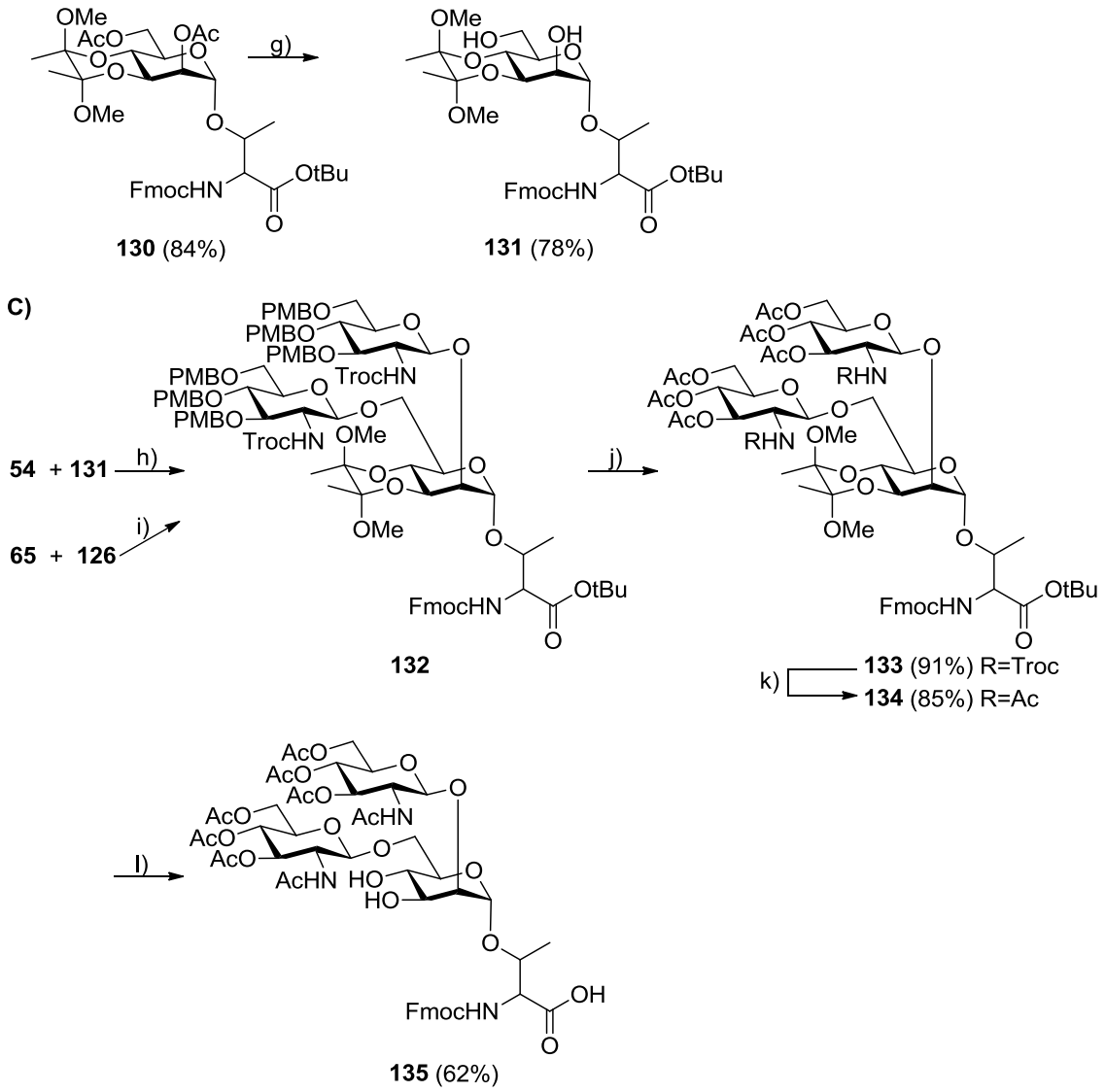
Scheme 45. Retrosynthetic analysis for two synthesis routs of the branched glycosylated Fmoc amino acid building blocks.

The trisaccharide donor **65** was in turn obtained by modification of one of the *N*-glycan complex type donor building blocks, which were further described in the *N*-glycan section. In both approaches for synthesis of the 2,6-branched *O*-mannosyl amino acids, *p*-methoxybenzyl protecting groups were used instead of benzyl groups, since a hydrogenation step, which would be problematic in the presence of Fmoc groups was then avoided. The *p*-methoxybenzyl groups were easily removed by CAN treatment. Additional steps for later replacement of amino acid Boc groups with Fmoc were thereby unnecessary.

3.6 Synthesis of a branched GlcNAc2-Man-O-Thr amino acid building block

The Fmoc protected threonine was directly prepared and employed in 2,6-branched *O*-mannosyl amino acid synthesis (**Scheme 46** A). In the first approach, building up a large glycan structure stepwise with small monosaccharide donors (**Scheme 46** B), the 2,6-hydroxyl mannose **31**, from the above described *N*-glycan synthesis (**Scheme 30** A), was converted into the trichloroacetimidate donor **129** in a few steps by acetylation, deallylation with Wilkinson's catalyst and reaction with trichloroacetonitrile in DBU. The coupling of trichloroacetimidate donor **129** with acceptor **126** was performed under TMSOTf activation to give the α-Man-Thr coupling product **130**. The reaction proceeded in Et_2O at r.t. to avoid orthoester formation, in DCM or at lower temperatures extensive formation of orthoester products was observed, confirmed by NMR analysis. Another coupling step then followed to elongate the α-Man-Thr **130** with the GlcNTroc thioglycoside donor **54** by NIS/TfOH activation to give the coupling product **132** in good yields. In the second synthesis strategy approach the trisaccharide donor **65**, synthesis described in the *N*-glycan section (**Scheme 31** D), was by TMSOTf activation connected to the threonine **126** directly and again obtained the coupling product **132** in a high yield.



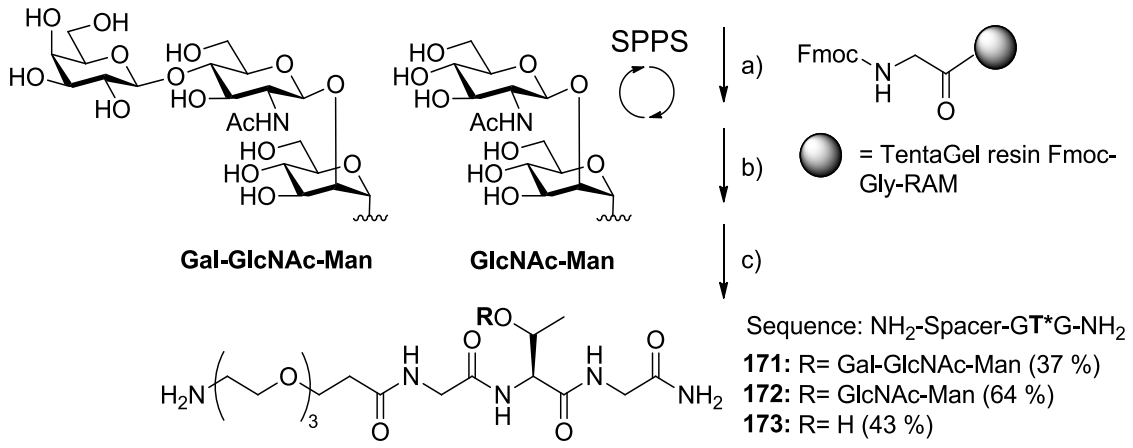


Scheme 46. a) $NaHCO_3$, Fmoc-OSu, acetone/H_2O 1:1, r.t. 24 h; b) *tert*-butanol, CuCl, DCC, r.t. 72 h under darkness; **125** in DCM added, r.t. 2 h; c) Py/Ac_2O, r.t. 24 h; d) Wilkinson's catalyst, Tol/EtOAc/H_2O 20:10:1, reflux 24 h; I_2, THF/H_2O 4:1, r.t. 2 h; e) trichloroacetonitrile, DBU, DCM, 0 °C 3 h; f) **126**, TMSOTf, molecular sieves, Et_2O, r.t. 30 min; g) NaOMe in MeOH, pH 9.5, r.t. 24 h; $NaHCO_3$, 1,4-dioxane/H_2O 1:1, Fmoc-OSu, r.t. 2 h; h) NIS, TfOH, DCM, -50 °C 4 h, 90%; i) TMSOTf, molecular sieves, DCM, r.t. 2 h, 82%; j) CAN, MeCN/H_2O 9:1, r.t. 30 min; Py/Ac_2O, r.t. 24 h; k) Zn, AcOH, r.t. 4 days; Py/Ac_2O, r.t. 24 h; l) TFA/DCM 3:1, r.t. 24 h.

The *p*-methoxybenzyl groups were then removed by CAN followed by acetylation. Subsequently the *N*-Troc groups were converted to NHAc groups by zinc reduction and acetylation. The dimethyldimethoxy acetal ring and amino *tert*-butyl protection groups were simultaneously removed[254] in the last step by treatment with TFA to obtain the 2,6-branched glycoamino acid **135** in a moderate yield, the slow removal of the acetal here seemed to be a problem (**Scheme 46**).

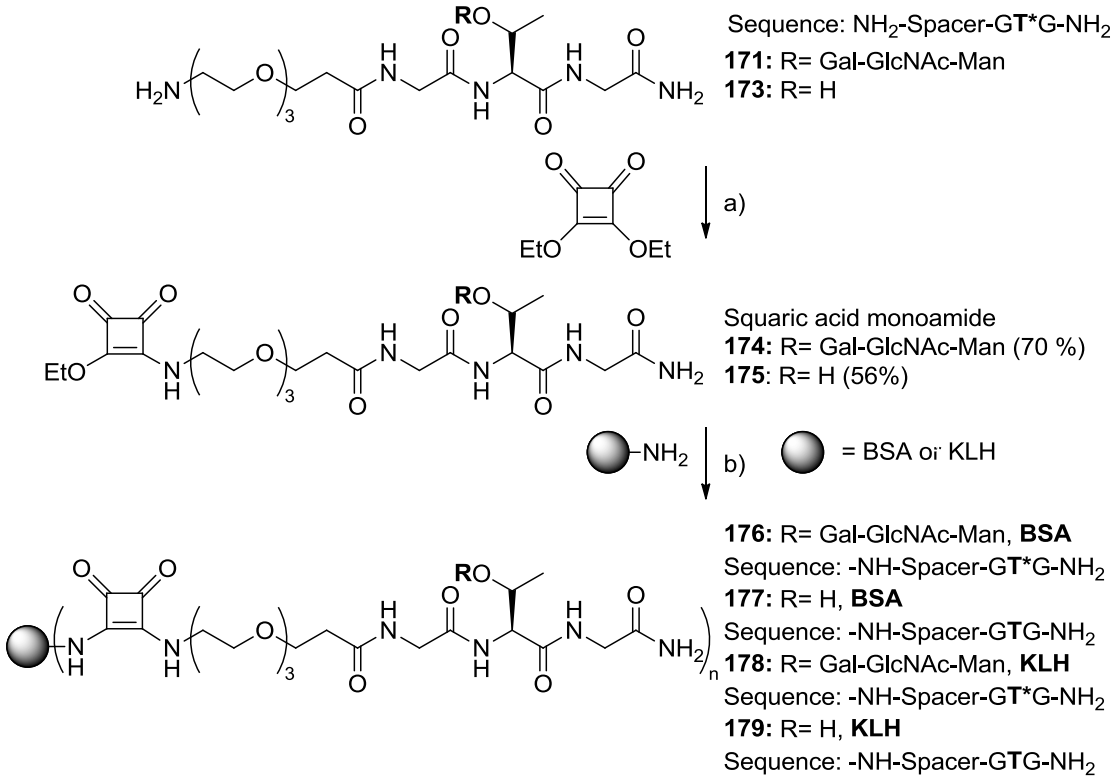
3.7 Synthesis of the Gal-GlcNAc-Man antigen glycopeptides and BSA- and KLH-conjugates

The obtained Galβ1-4GlcNAcβ1-2ManαThr **120** and GlcNAcβ1-2ManαThr **124** were employed in the synthesis of the glycopeptides **171** - **173** (**Scheme 47**).[255-256] Glycine residues were here incorporated on each side of the glycosylated amino acids in order to generate a vaccine construct inducing antibodies specific to the *O*-mannosyl glycan amino acid structure no matter of the underlying peptide backbone sequence. Starting from preloaded Fmoc-Gly Rink amide resin (12.5 µmol),[257-258] coupling of the standard Fmoc-amino acids (8 equivalents) was carried out using HBTU/HOBt and the glycosylated Fmoc-threonine building block **120** and **124** (2 equivalents) were coupled using HATU/HOAt.



Scheme 47. Synthesis of antigen peptide and similar peptides. a) i: Fmoc-cleavage: Piperidine (20%) in DMF, ii: Amino acid-coupling: Fmoc-AA-OH, HBTU, HOBt, DIPEA/DMF; b) Resin-cleavage: TFA/TIPS/H_2O (15:0.9:0.9), 2 h; c) NaOMe, MeOH, pH 9.5, 20 h.

The glycosylated amino acids were pre-activated in a smaller volume of solvent and added manually to the resin. Furthermore, the reaction time of the glycosylated amino acids were extended (to 6h instead of 40 min). After full assembly of the glycopeptides on the resin, the peptide was terminated with coupling of a triethylene glycol spacer and subsequent detachment from the resin by applying a mixture of trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/H_2O (90:5:5). After a desalting step on a C-18 cartridge, the *O*-acetyl groups were removed by treatment with catalytic amounts of NaOMe in methanol at pH 9.5.[259] The deprotected glycopeptides **171** - **173** were finally purified by preparative HPLC.



Scheme 48. Conjugation to BSA/KLH immure carrier. a) EtOH/H_2O 1:1, pH 8.0, r.t. 2 h; b) 50 mM Na_2HPO_4 buffer, pH 9.3, r.t. 48 h.

The glycopeptide **171** and non-glycosylated **173** was conjugated to BSA and KLH by an initial coupling at the *N*-terminal amine with diethyl squarate in EtOH/H_2O (1:1) at pH 8 to give the squaric acid monoamide **174** and **175**.[260-261] The coupling to BSA and KLH performed in aqueous Na_2HPO_4 buffer at pH 9.5 to give the glycopeptide BSA and KLH[262] conjugate **176** - **179** (**Scheme 48**). The vaccine construct **178** was used for immunization in Rabbit, while the other glycopeptide conjugates were used for ELISA antibody analysis.[263-264]

To determine the glycopeptide loading on the KLH-conjugate **178**, anthranilic acid was used to label[265-266] galactose/mannose residues cleaved from the synthesized KLH conjugate with 10% TFA. The released and labeled monosaccharides was then quantified by HPLC.[267] To determine the relationship of UV absorption at 214 nm on the HPLC chromatogram with the amount of sugar, a calibration curve was first made. A 1:1 mixture of galactose/mannose, was first treated with anthranilic acid labeling solution, then 1 µL of the reaction mixture was diluted with 100, 150, 200 and 600 times with 0.1% TFA-H_2O. The diluted samples were injected into the analytical HPLC, measuring the UV absorbance at 214 nm. The combined

galactose and mannose peak areas were detected and a calibration curve was plotted for the corresponding dilutions.

Dilution Gal-Man mix	Amount of injected monosaccharide (nmol)	SUM Gal+Man peak area at 214 nm
1:100	1.8	187.43
1:150	1.2	114.78
1:200	0.9	93.48
1:600	0.3	33.86

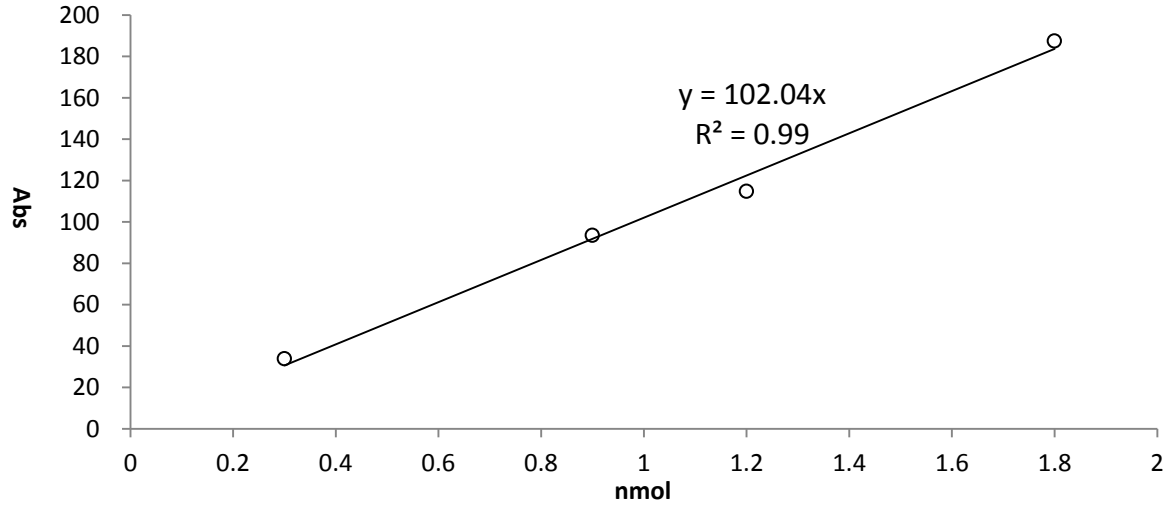


Figure 18. Calibration curve peak area absorbance at 214 nm versus dilution in nmol of the 1:1 galactose/mannose mixture.

The monosaccharides cleaved from KLH conjugate **178** were incubated with anthranilic acid labeling solution and the diluted reaction mixture was analyzed by HPLC, measuring the UV absorbance at 214 nm with a peak area of 83.549 mAu, which was corresponding to 0.818 nmol of galactose and mannose respectively. As the molecular weight of KLH (400-3000 kDa, given by Sigma-Aldrich), the glycopeptide to KLH ratio was determined to 65 (400 kDa KLH) - 491 (3000 kDa KLH) mol glycopeptide per mol KLH.

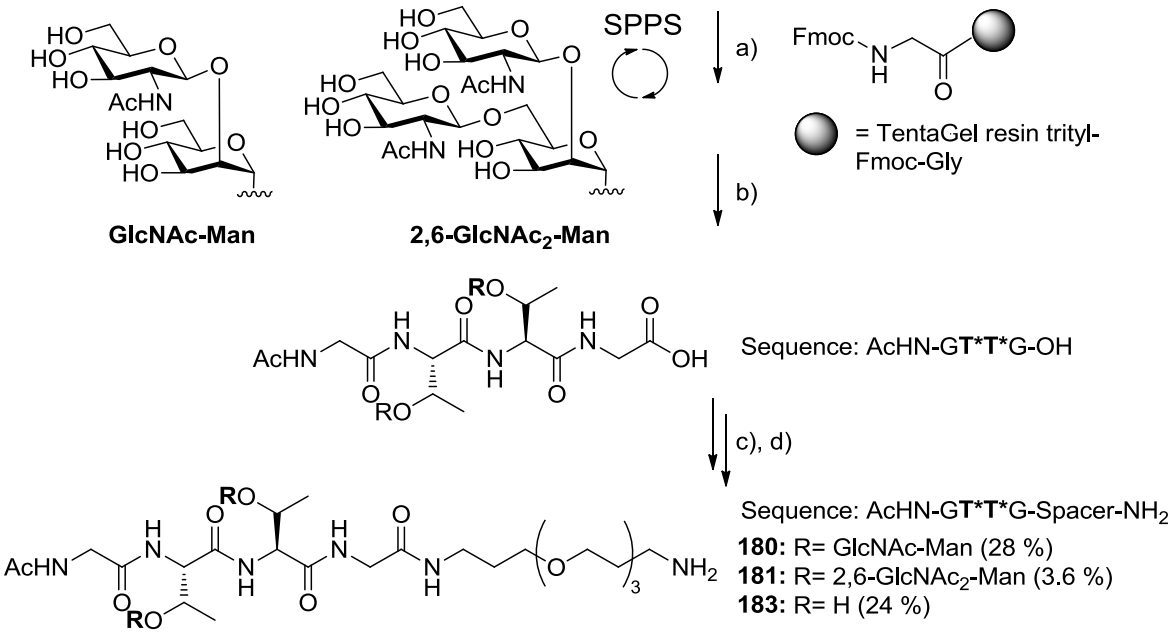
3.8 Synthesis of the GlcNAc-Man antigen and branched $GlcNAc_2$-Man antigen

In addition to the synthesis of the KLH vaccine construct **178** containing the Galβ1-4GlcNAcβ1-2Manα glycan structure, the GlcNAc-β1-2Manα and the branched GlcNAc-β1-2-(GlcNAc-β1-6)Manα glycopeptides were synthesized and conjugated to an immune carrier protein (**Scheme 49**). Starting from Fmoc-Gly trityl resin, the glycosylated

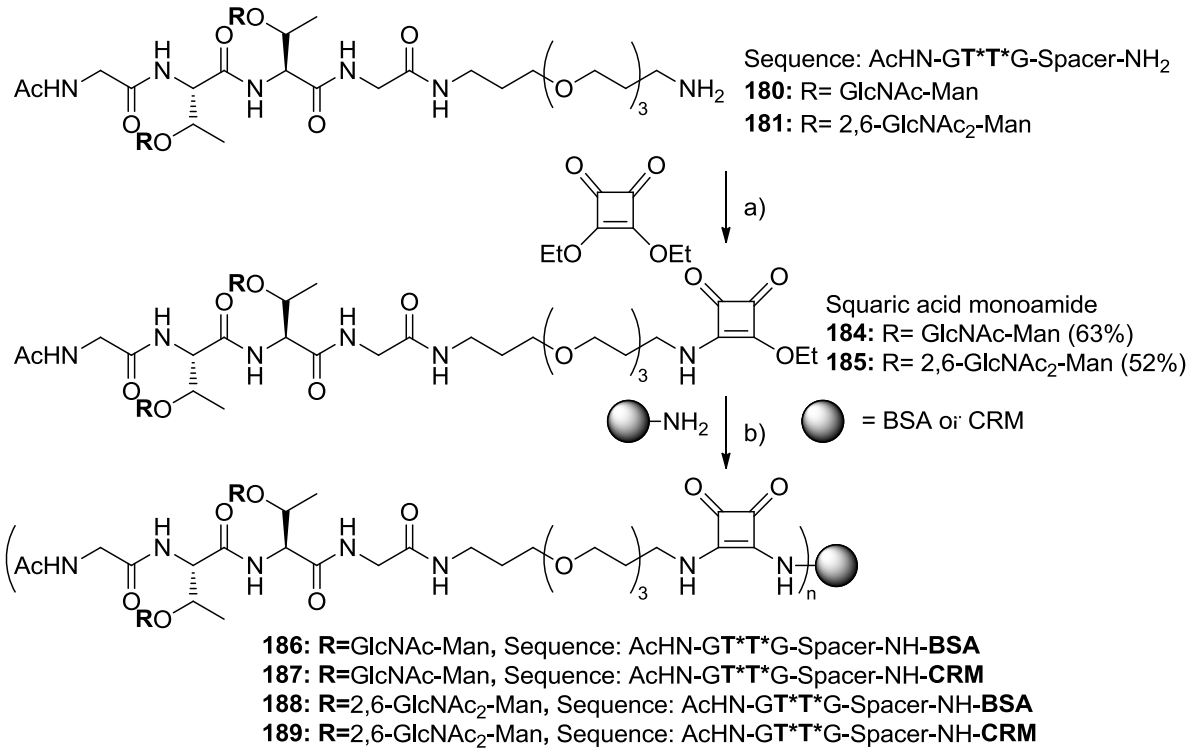
Fmoc-threonine building blocks **124** and **135** (2 equivalents) were coupled using HATU/HOAt, pre-activated in a smaller volume of solvent and reacting for 8 h. After capping the *N*-terminal with Ac_2O, HOBt and DIPEA, the peptides were cleaved form the resin by applying a mixture of TFA/TIPS/H_2O (90:5:5). A spacer was then connected to the *C*-terminal for later conjugation with an immune carrier or for immobilization on a microarray surface. The position of the linker in the *C*-terminal instead of the *N*-terminal aimed to avoid having a potential immunogenic *C*-terminal amide or free carboxylic acid close to the glycosylation site. After linkage coupling a deacetylation step was followed to obtain peptide **180** - **183**. The synthesis of the 2,6 branched glycopeptide **181** proceeded in very low yield compared to the other glycopeptides and it was believed that steric hindrance with two branched glycosylated amino acids next to each other caused a problem. Additionally, a large extend of the monoglycosylated peptide byproduct **182** was formed.

The obtained glycosylated antigen peptides **180** and **181** were extended with a squarate linker and conjugated to an immune carrier protein (**Scheme 50**). The CRM^{197} immune carrier mutant originating from Difteria toxin have successfully been used for immune stimulation of different glycoconjugates and was therefore selected for conjugation to our peptides.

The conjugation proceeded in phosphate buffer at pH 9.3 and after washing using spin filters, the glycopeptide loading to the protein carrier was measured be MALDI mass spectrometry. For the GlcNAc-Man glycosylated antigen **187**, 6.3 equivalents of peptides were loaded on each CRM molecule, reacting from 26.4 eq antigen peptide **184,** and 13.3 eq of peptides was conjugated on the BSA carrier **186** using 17.7 eq of the antigen peptide substrate. The BSA conjugates were prepared for ELISA determination of the antibody titers after immunization with the desired vaccine constructs. The conjugation of 2,6 branched $GlcNAc_2$-Man glycopeptide **185** to BSA/CRM resulted in a loading of around 2-3 eq of peptides, using 28-30 eq of peptide substrate in the coupling reaction.



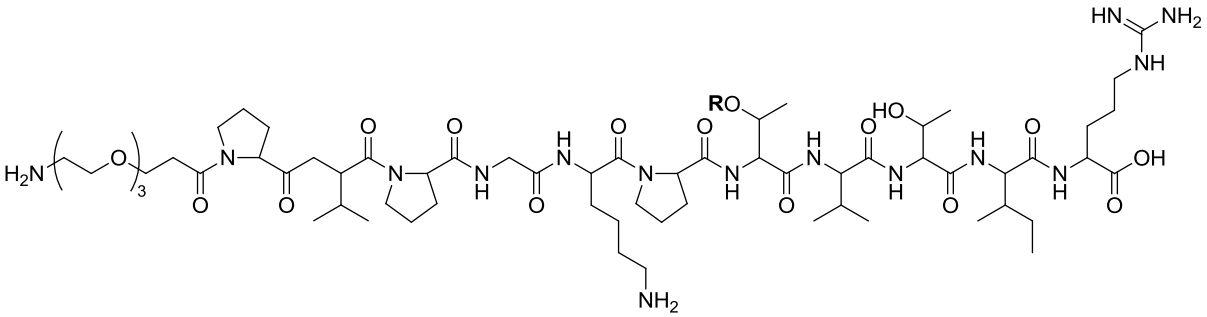
Scheme 49. Synthesis of antigen peptide and similar peptides with *C*-terminal spacer. a) i: Fmoc-cleavage: Piperidine (20%) in DMF, ii: Amino acid-coupling: Fmoc-AA-OH, HBTU, HOBt, DIPEA/DMF, iii: *N*-terminal capping: Ac_2O, HOBt, DIPEA/DMF; b) Resin-cleavage: TFA/TIPS/H_2O (15:0.9:0.9), 2.5 h; c) i: *N*-Boc-4,7,10-trioxa-1,13-tridecanediamine spacer, HATU, HOAt, DIPEA/DMF, 8 h, ii: TFA/TIPS/H_2O (15:0.9:0.9), 2.5 h; d) NaOMe, MeOH, pH 9.5, 20 h.



Scheme 50. Glycopeptide conjugations to BSA/CRM immure carriers. a) EtOH/H_2O 1:1, pH 8.0, r.t. 2 h; b) 50 mM Na_2HPO_4 buffer, pH 9.3, r.t. 72 h.

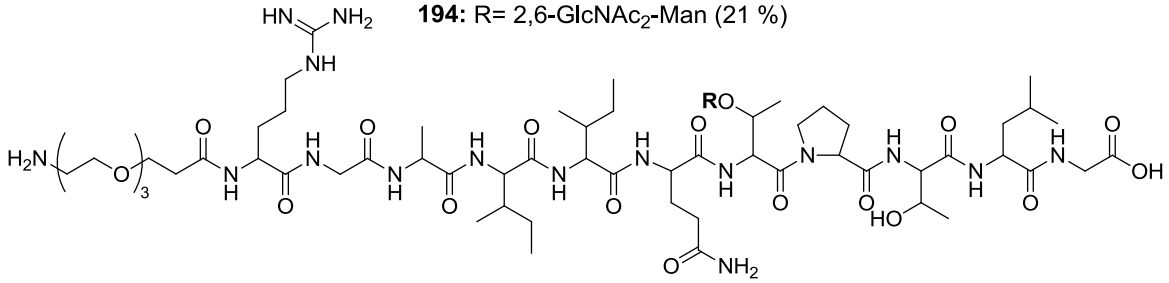
3.9 Synthesis of different linear and branched *O*-mannosyl glycopeptide sequences for antibody and lectin analysis

The glycopeptides **190** - **195**, which contained the three different linear and branched *O*-mannosyl-glycans, were synthesized (**Scheme 51**). Two glycopeptide sequences were selected from α-Dystroglycan (α-DSG), a glycoprotein that is known to be densely modified with *O*-mannosylation. Starting from Fmoc-Gly trityl resin or Fmoc-Arg trityl resin (12.5 µmol) and in accordance with the above described glycopeptide synthesis, the linear glycopeptides **190** - **193** were obtained in good yields, while the more bulky 2,6 branched *O*-mannosyl glycopeptides **194** and **195** were obtained in lower yields. The synthetic *O*-glycopeptides were used in ELISA and microarray studies to investigate the induced *O*-mannosyl antibodies and to evaluated binding interactions with glycan specific lectins.



Sequence: NH_2-Spacer-PVPGKPT*VTIR

190: R= Gal-GlcNAc-Man (70 %)
192: R= GlcNAc-Man (62 %)
194: R= 2,6-$GlcNAc_2$-Man (21 %)



Sequence: NH_2-Spacer-RGAIIQT*PTLG

191: R= Gal-GlcNAc-Man (21 %)
193: R= GlcNAc-Man (18 %)
195: R= 2,6-$GlcNAc_2$-Man (5 %)

Scheme 51. Synthesis of α-DSG *O*-mannosyl glycopeptides.

3.10 Immunization of *O*-man antigen and evaluation of the antibody serum

In order to induce antibodies with specific recognition of the Galβ1-4GlcNAcβ1-2Manα glycosylated structures on proteins and peptides, two rabbits **21390** and **21391** were immunized with the *O*-mannosyl-glycopeptide KLH conjugate vaccine **178,** together with Freunds complete adjuvant (CFA). Six booster immunizations were carried out over 8 weeks. The induced antibody titers were analyzed on ELISA plates coated with the Galβ1-4GlcNAcβ1-2Manα glycopeptide BSA conjugate **176**. Analysis of the sera from the pre-immune, first bleed and final bleed by ELISA showed that a strong immune response was directed against the antigen structure (**Figure 19** A). The binding specificity of the obtained antibody sera was further evaluated by incubations with glycopeptides **171**, **172**, **173**, **190** and **191** in a microarray[268] format (**Figure 20**) or by ELISA neutralization experiments (**Figure 19** B and C).

Both the ELISA neutralization experiments and the microarray analysis showed that the antibody sera were highly specific to the Galβ1-4GlcNAcβ1-2Manα glycopeptide antigen structure **171** and with some reactivity to the structurally similar GlcNAcβ1-2Manα glycopeptide **172**. The antibody sera recognized neither the non-glycosylated antigen peptide **173** nor the α-DSG peptide **191** glycosylated with the Galβ1-4GlcNAcβ1-2Manα trisaccharide. The α-DSG glycopeptide **190** containing the antigen trisaccharide showed a weak neutralizing effect by the ELISA neutralization experiments, but no binding interaction was observed on the glycopeptide microarray. The obtained results showed that both the glycan residues and the peptide backbone were important for the binding recognition of the induced antibodies. The induced antibodies were in this case not suitable for applications to generally detect *O*-mannosyl glycosylation on proteins. The results thus prove that antibodies could be produced with very high selectivity to chemically defined *O*-mannosyl glycopeptide structures.

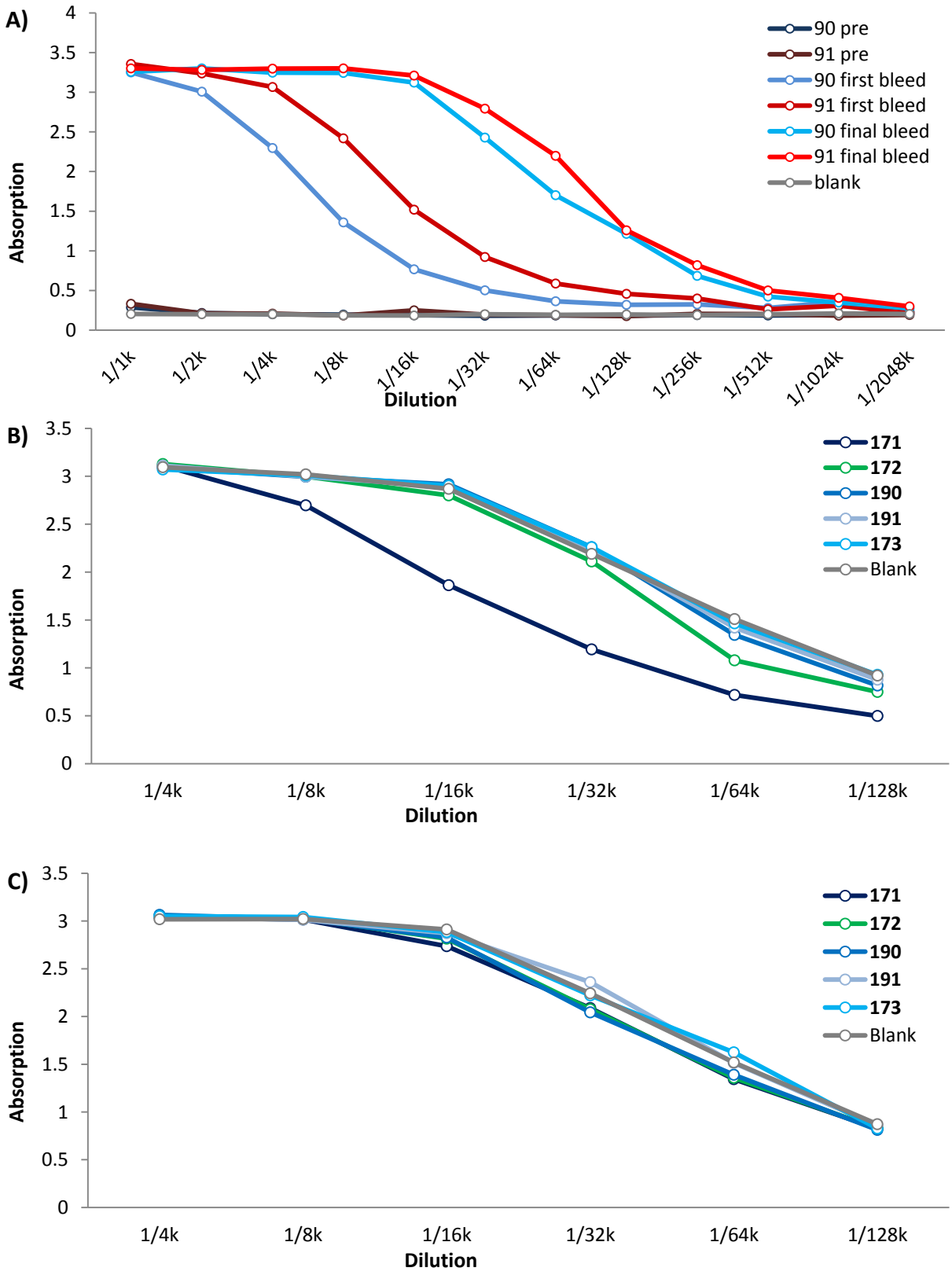
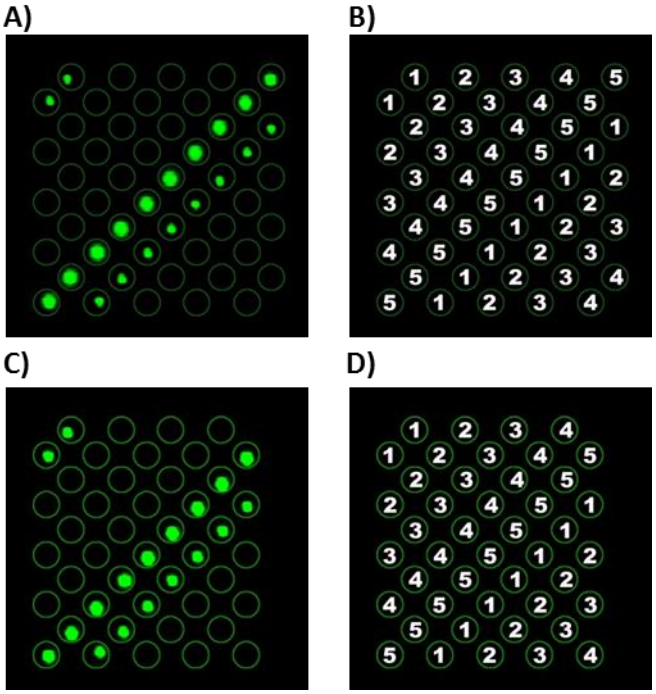


Figure 19. **A)** ELISA analysis of pre-immune, first bleed and final bleed of Rabbit **21390** and **21391**, immunized with Gal-GlcNAc-Man KLH **178**. The ELISA plate was coated with Gal-GlcNAc-Man BSA **176**; **B)** ELISA neutralization experiments with peptides **171**, **172**, **173**, **190**, **191** and Rabbit **21390** final bleed; **C)** ELISA neutralization experiments with peptides **171**, **172**, **173**, **190**, **191** and Rabbit **21391** final bleed.



Nr	Compound Nr	Sequence	Glycan structure
1	**172**	SpacerGT*G	GlcNAc-Man
2	**173**	SpacerGTG	NON
3	**190**	SpacerPVPGKPT*VTIR	
4	**191**	SpacerRGAIIQT*PTLG	Gal-GlcNAc-Man
5	**171**	SpacerGT*G	

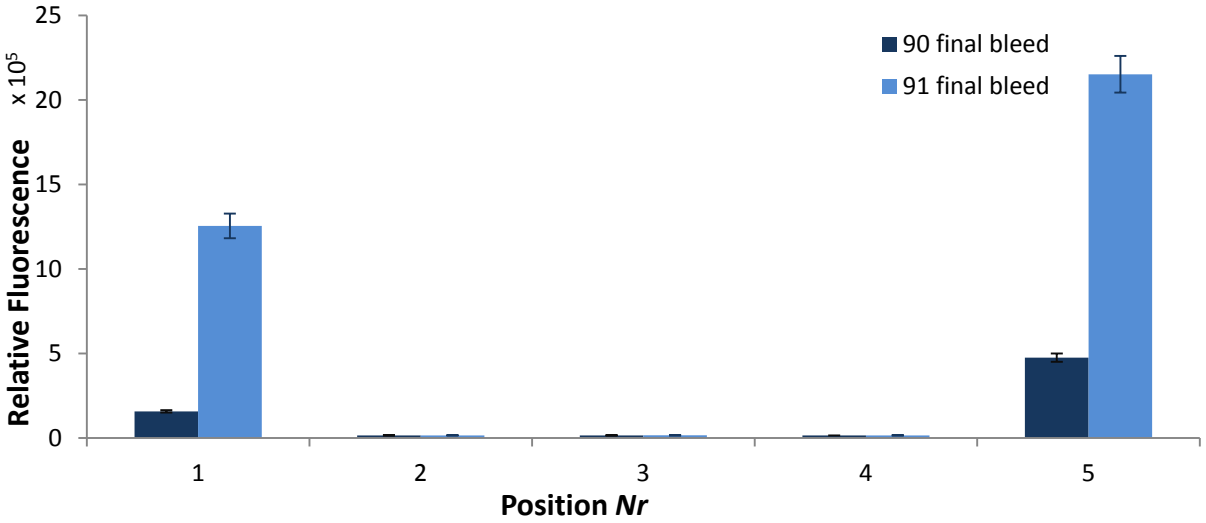


Figure 20. Microarray results of rabbit serum **23190** and **23191**. **A)** Serum **23190** final bleed 1:5000; **B)** Peptide spotting pattern designed for analysis of serum **23190**; **C)** Serum **23191** final bleed 1:100; **D)** Peptide spotting pattern designed for analysis of serum **23191**.

The CRM vaccine construct **187**, conjugated with the GlcNAcβ1-2Manα glycopeptide was immunized in rabbit **23803** and **23804** with Freunds complete adjuvant (CFA). Six booster immunizations were carried out over 8 weeks. Analysis of the antibody sera from the pre-immune, first bleed, second bleed and final bleed by ELISA, coated with the GlcNAcβ1-2Manα glycopeptide BSA conjugate **186**, showed that a strong immune response was directed against the antigen structure (**Figure 21**).

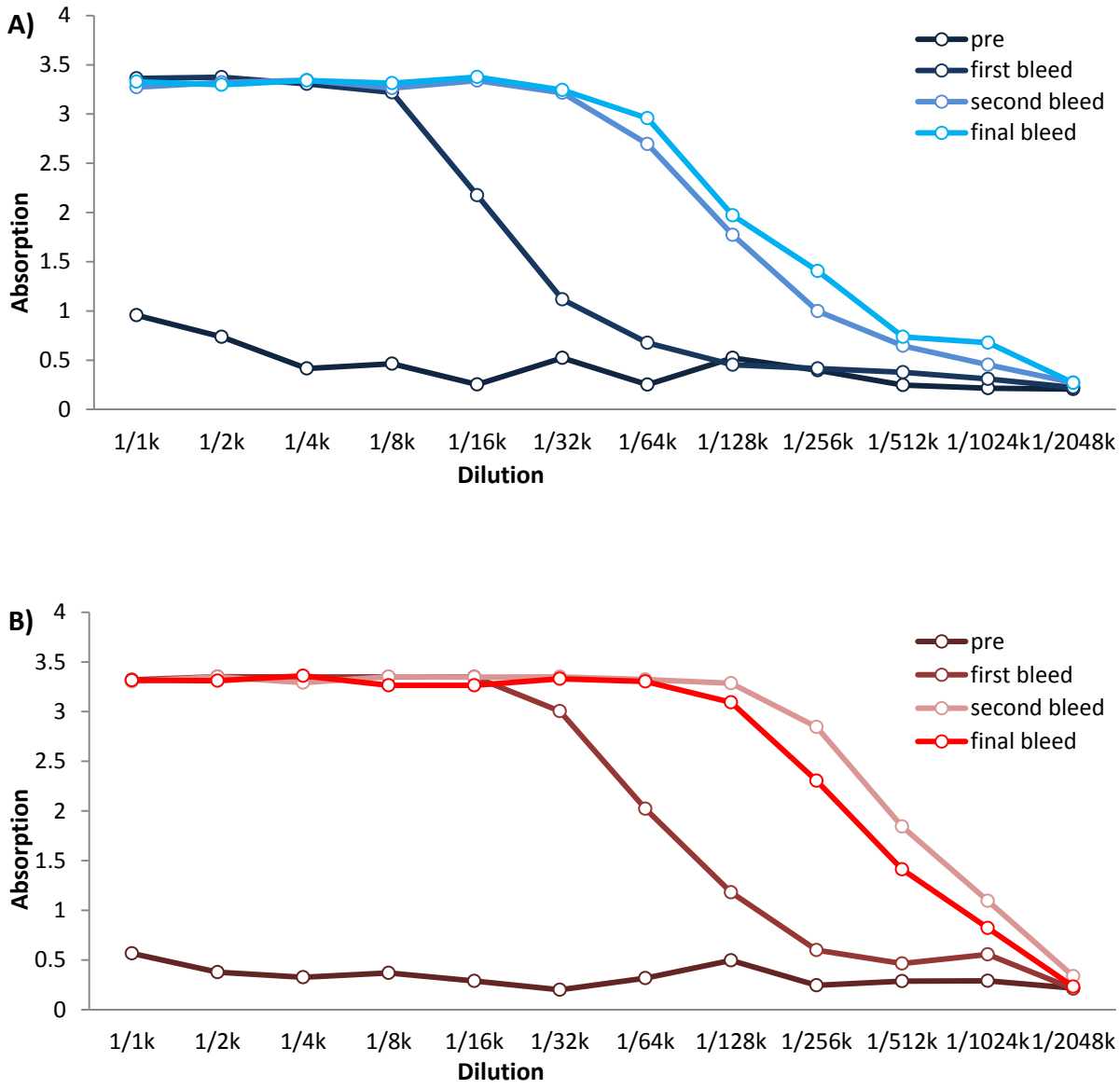


Figure 21. **A)** ELISA of pre-immune, first bleed, second bleed and final bleed of Rabbit **23803**, immunized with GlcNAc-Man CRM **187**, ELISA plate coated with GlcNAc-Man BSA **186**; **B)** ELISA of preimmunization, first bleed, second bleed and final bleed of Rabbit **23804**, immunized with GlcNAc-Man CRM **187**, ELISA plate coated with GlcNAc-Man BSA **188**.

The binding specificity of the induced antibody sera were analyzed by incubations with glycopeptides **171** - **173**, **180** - **183** and **190** - **195** in a microarray format (**Figure 22**). Additionally, the *O*-mannosyl glycopeptide microarray was employed to evaluate the glycan binding lectin Concanavalin A (ConA), which is known to recognize α-mannose residues on *N*-glycoproteins. This lectin might as well be useful for detection or enrichment of *O*-mannosyl proteins.

Nr	Compound Nr	Peptide sequences	Glycan structure
1	**190**	SpacerPVPGKPT*VTIR	Gal-GlcNAc-Man
2	**191**	SpacerRGAIIQT*PTLG	
3	**171**	SpacerGT*G	
4	**192**	SpacerPVPGKPT*VTIR	GlcNAc-Man
5	**193**	SpacerRGAIIQT*PTLG	
6	**172**	SpacerGT*G	
7	**194**	SpacerPVPGKPT*VTIR	2,6-GlcNAc$_2$-Man
8	**195**	SpacerRGAIIQT*PTLG	
9	**182**	T*GSpacer	
10	**183**	GTTGSpacer	Non
11	**173**	SpacerGTG	

Nr	Peptides	Conc
12	**180** GT*T*GSpacer	100 µM
13		50 µM
14		10 µM
15		2 µM
16		0.5 µM
17	**181** GT*T*GSpacer	100 µM
18		50 µM
19		10 µM
20		2 µM
21		0.5 µM

(Nr *1-11* applied at 250µM)

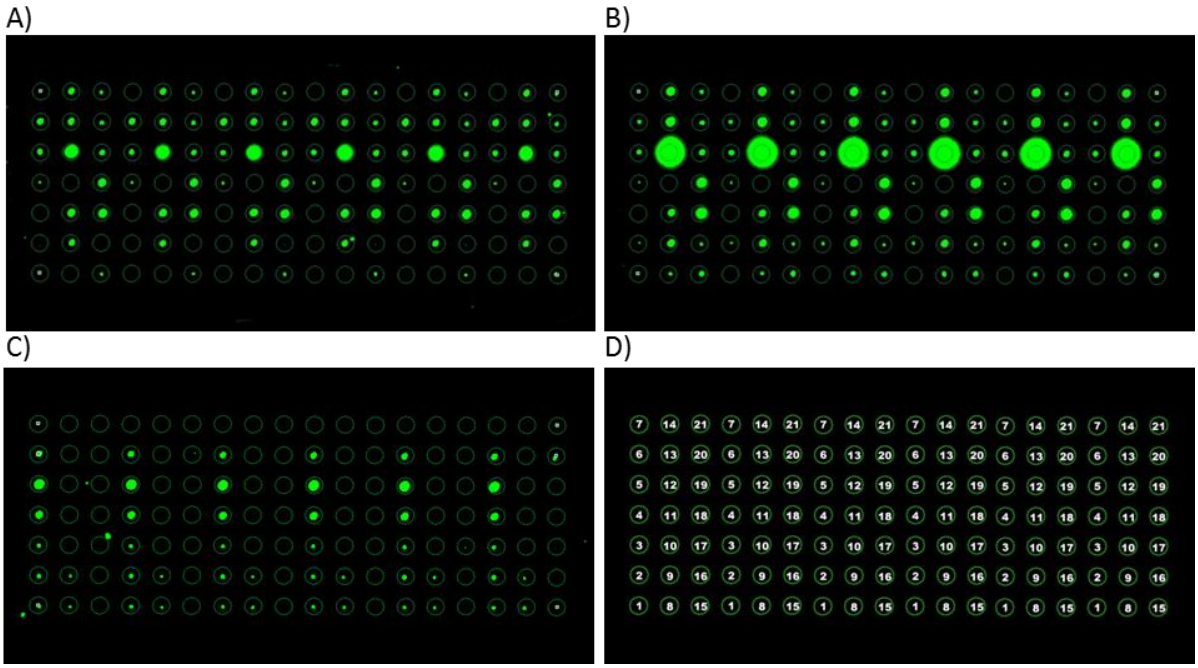


Figure 22. Microarray experiment designed for rabbit serum **23803** and **23804**. **A)** Serum **23803** final bleed 1:25 dilution; **B)** Serum **23804** final bleed 1:25 dilution; **C)** ConA 0.1 mg/mL; **D)** Peptide spotting pattern.

The antibody sera **23803** and **23804** recognized the double glycosylated GlcNAc-Man antigen peptide **180** exceptionally strong (position *12-16*, **Figure 22** A and B, **Figure 23**). Additionally, the double-glycosylated peptide 2,6-branched $GlcNAc_2$-Man peptide **181** were also partly recognized (position *17-21*, **Figure 22** A and B, **Figure 23**). The antigen structure was here assembled differently from the previous KLH vaccine construct **178** and, a dimer with higher glycan density aimed to increase induction of antibodies directed to the glycosylated amino acid structure. As a result, a major binding epitope recognizing the clustered GlcNAc-Man structure seems to be dominating in the antibody sera. Additionally the GlcNAc-Man monoglycosylated peptides **192, 193** and **172** (position *4-6*) and mono-glycosylated 2,6-branched $GlcNAc_2$-Man peptides **195** and **182** (position *8-9*), were recognized to some extent. The non-glycosylated backbone sequences were as well partly recognized by the immune sera (position *10-11*).

By microarray analysis of the lectin ConA the three mono-glycosylated GlcNAc-Man glycopeptides **192, 193** and **172** were in particular well recognized (position *4-6,* **Figure 22** C and **Figure 23**). The mono-glycosylated linear Gal-GlcNAc-Man glycopeptides **190, 191** and **171** (position *1-3*) and the 2,6-branched $GlcNAc_2$-Man peptides **195** and **182** (position *8-9*) were only weakly recognized by ConA. The double-glycosylated peptide **180** and **181** were not recognized at all (position *12-21*). This means that the ConA lectin recognition is both influenced by the glycan structure and the presentation in a clustered or monoglycosylated form. The observed discriminations, can be very useful for future *O*-mannosyl structural analysis work using the ConA lectin. Further, clustered or elongated *O*-mannosyl glycopeptide or glycoprotein structures will be discriminated by ConA lectin enrichment.

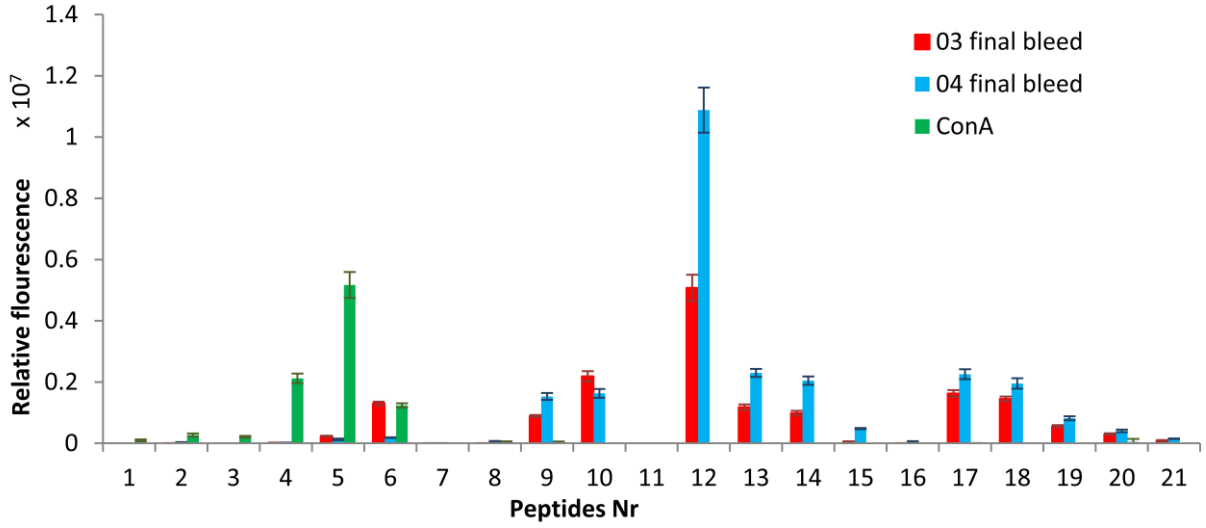


Figure 23. Microarray results of rabbit serum **23803** and **23804** compared with ConA.

In conclusion, efficient methodology was developed for the synthesis of the glycosylated amino acid building blocks, Galβ1-4GlcNAcβ1-2ManαThr **120**, GlcNAcβ1-2ManαThr **124** and GlcNAcβ1-2(GlcNAcβ1-6)ManαThr **135**. The glycosylated amino acids were employed in the construction of *O*-mannosyl glycopeptides and the corresponding BSA**,** KLH and CRM glycopeptide conjugates **171** - **195**. Immunizations of vaccine **178** in rabbit gave evidence that a strong immune response was directed to the glycopeptide antigen structure. Evaluation of the antibody sera binding specificity using glycopeptide microarrays and ELISA neutralization experiments showed that highly specific antibodies were obtained recognizing the *O*-mannosyl glycopeptide antigen structure. Immunizations of vaccine **187** in rabbit showed immune responses directed to the antigen glycan structure presented on different peptides evaluated by ELISA and microarrays. In particular a clustered glycan binding epitope also present in the glycopeptide antigen structure was highly recognized. Analysis of the glycan binding lectin Concanavalin A (ConA), which is known to recognize α-mannose residues, showed that unique glycan and glycopeptide structural binding epitopes were recognized. The obtained results will have an impact in the structural analysis work of *O*-mannosyl glycoproteins.

4 Summary

This PhD thesis work includes the synthesis of glycans and glycosylated amino acid building blocks to generate various glycopeptides to study protein *N*- and *O*-glycosylation binding events and to provide structural analysis tools. Diverse *N*-glycan structures and *O*-mannosyl glycosylation, were here of the main focus. These structures include certain structural resemblance. Complex type *N*-glycans and the elongated *O*-mannosyl glycosylated amino acids contain the same terminal structural units and were therefore prepared from common building blocks (**Figure 24**). The high mannose type *N*-glycosylation further resembles the α1-2 *O*-mannosyl extension commonly found in yeast,[269] while the complex type *N*-glycosylation resembles the elongated *O*-mannosylation, which extensively have been found modifying proteins in eukaryotic brain tissues.

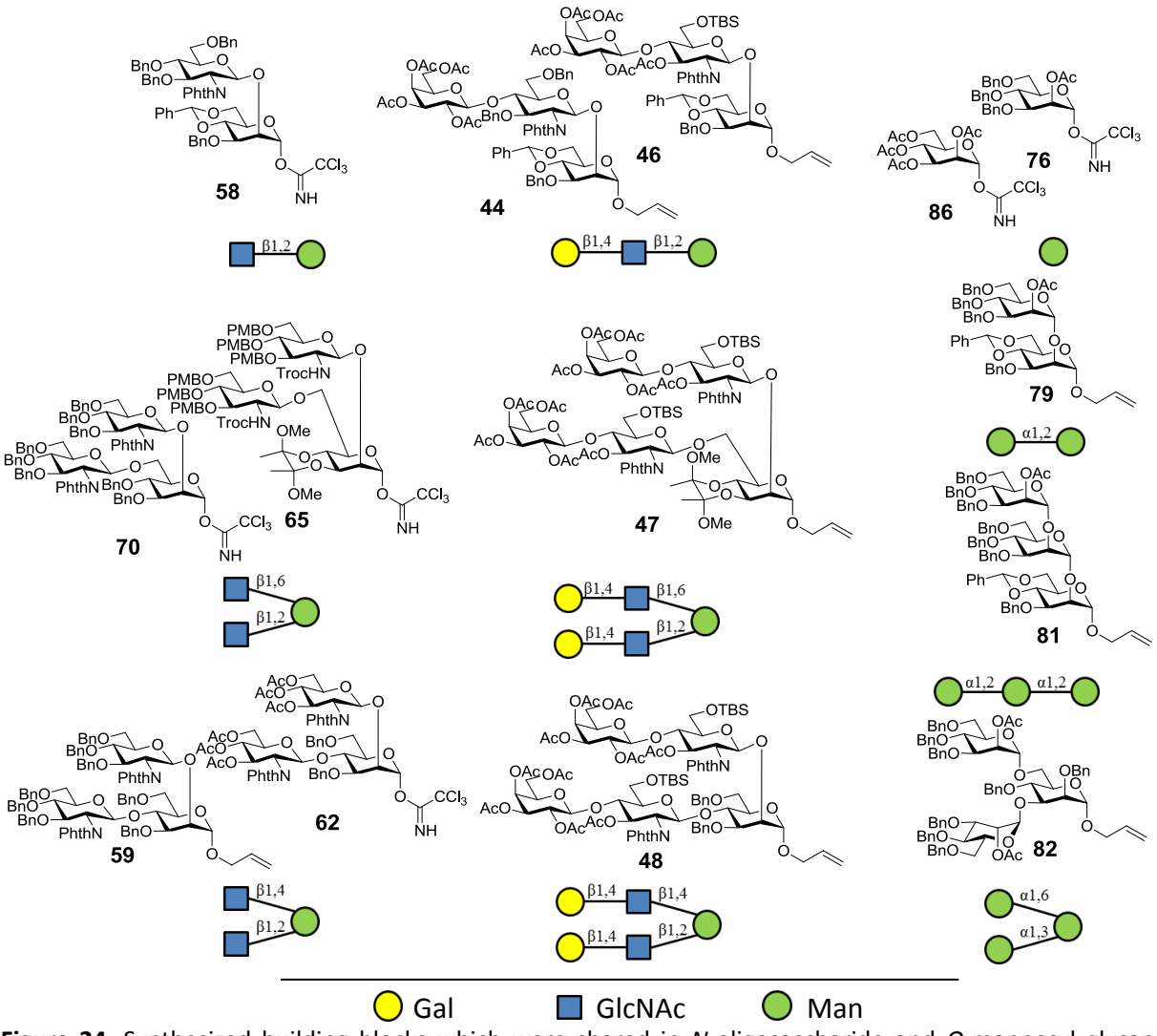
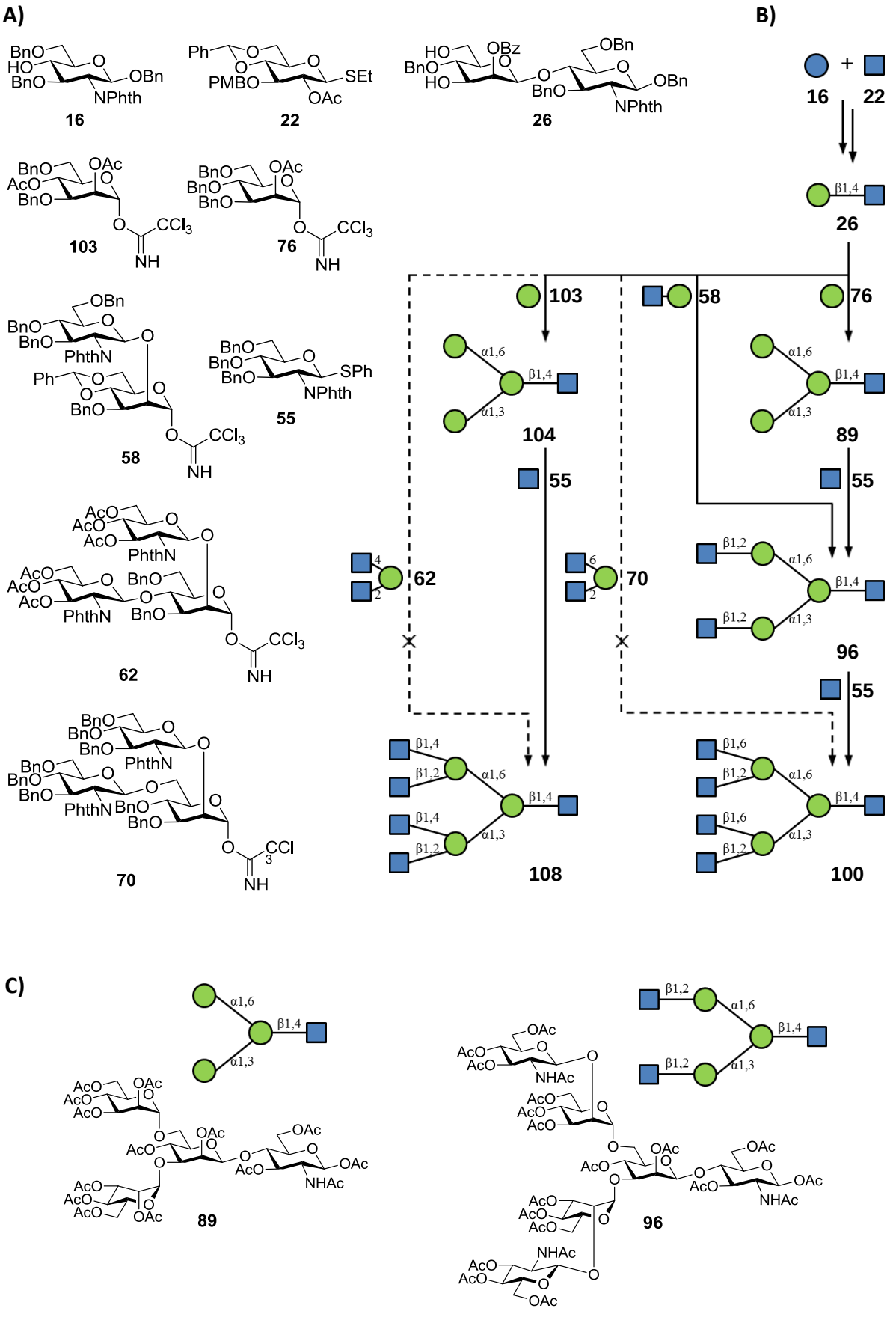


Figure 24. Synthesized building blocks which were shared in *N*-oligosaccharide and *O*-mannosyl glycan synthesis.

To prepare the *N*-glycan oligosaccharides, which contains a common pentasaccharide core unit linked to asparagine on proteins and peptides, a Manβ1-4GlcNAc disaccharide core **26** was initially synthesized. Due to difficulties to perform stereoselective couplings to generate the β-Man glycosidic bond, a Glc-GlcNAc disaccharide was initially formed. This disaccharide was obtained starting with the coupling of glucose donor **22** to the glucosamine acceptor **16**, to form a β1-4 linkage (**Scheme 26**). The disaccharide Glc unit was then converted into a Man residue. The symmetrical extensions with trichloroacetimidate donor building blocks on the 3,6 positions of the disaccharide core mannose structure **26**, were tested using the monosaccharide donors **76** and **86**, disaccharide donor **58**, and trisaccharide donors **62** and **70** (**Figure 24**). The results indicated that, i) The glycan donor must be active enough for high conversion to the desired product, comparing the acetylated donor **86** and benzylated donor **76**, the later donor as expected gave a much higher yield. ii) In case of coupling of monosaccharide and disaccharide donors **76** and **58**, the core disaccharide acceptor **26** was completely consumed in yields of 70-80% (**Scheme 34**, **Scheme 35** A), however when the donor size grow larger, for instance with the trisaccharide **62** and **70**, symmetrical couplings simultaneously extending the Man 3- and 6-position were problematic. Mixtures of mono- and double glycosylated products were obtained. Therefore, glycan donors larger than disaccharides were not used in these symmetrical coupling. iii) An alternative approach was to building up the large oligosaccharides in a stepwise fashion, employing smaller donor units. To obtain the 2,6 branched oligosaccharide **100**, the disaccharide donor **58** was first coupled to acceptor **26** forming hexasaccharide **96**. Then extended with the monosaccharide donor **55** to generate the desired octasaccharide (**Scheme 35** C). The 2,4 branched oligosaccharide **108** was generated in a good yield by quadruple mannose donor **103** extensions on the 2,4 positions of the two terminal glycan residues of acceptor **104** (**Scheme 36**). In summary four complex type *N*-glycan structures **89**, **96**, **100** and **108** were synthesized and two of them were converted into the oxazoline donors **110** and **112** and employed in endoglycosidase enzymatic couplings using a synthetic *N*-GlcNAc peptide library (**Figure 25**).


A)
B)
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16
22
26
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89
104
108
100
96

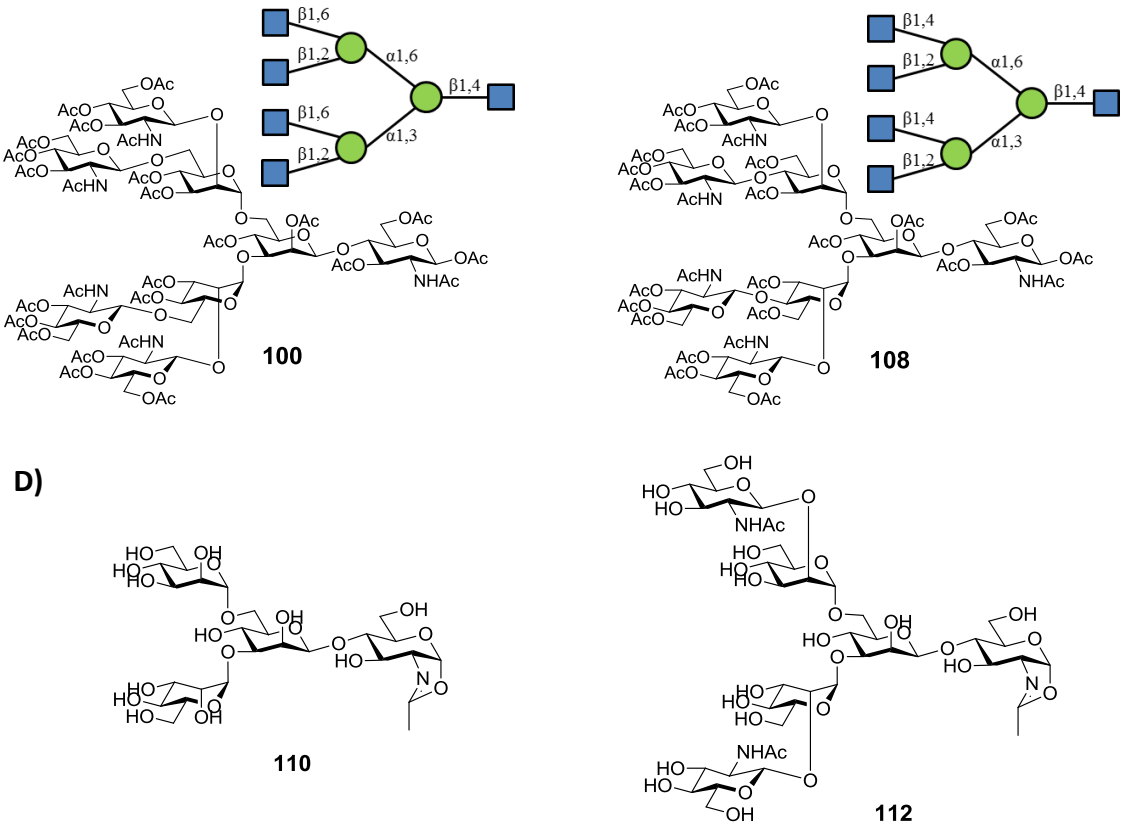

Figure 25. **A)** Building block units used in *N*-glycan synthesis. **B)** Stepwise synthesis routes to generate complex type *N*-glycans. **C)** Synthesized complex type *N*-glycan oligosaccharides. **D)** Converted oxazolines for enzymatic glycopeptide extension.

The couplings of the tetrasaccharide oxazoline **110** to different *N*-GlcNAc peptides were successful and all in-solution couplings were finished in 0.5 h with full conversion verified by HPLC and high resolution ESI-MS. The hexasaccharide oxazoline **112**, on the other hand, usually only reached 20% conversion to the desired *N*-glycopeptide products. The couplings with this more bulky donor were limited by back-hydrolysis to the starting material, which is a general problem by using endoglycosidases combined in *N*-glycan coupling reactions. Endoglycosidase mutants like the employed Endo M 175Q mutant should thus reduce back-hydrolysis reactions.

To efficiently generate a *N*-glycopeptide library with homogenous complex type glycosylation, instead of performing enzymatic couplings one by one in solution, different *N*-GlcNAc peptides were extended by on-slide enzymatic glycosylation. In principle, *N*-GlcNAc peptides were spotted and immobilized on a microarray slide followed by a one step on-slide

enzymatic *N*-glycan extension using oxazoline donor building blocks. To evaluate the on-slide enzymatic glycosylation, four *N*-GlcNAc reference peptides **165** - **168** were prepared in solution using a tetrasaccharide oxazoline, HPLC-purified and spotted on the microarray slide together with the *N*-GlcNAc peptide library **150** - **163**. Two experiments performed with different concentration of the spotted peptides. By comparing, the on-slide reaction with pre-synthesized internal standard peptides it was concluded that the endoglycosidase couplings were completed within 1 h. Further the results indicated that the location of the *N*-GlcNAc-Asn residue on the peptide backbone sequence had no dramatic influence on the on-slide enzymatic coupling, although the *N*-GlcNAc peptides were linked to the microarray slide through a *N*-terminal spacer. In summary, the experiments proved that it is possible to efficiently generate a library of *N*-glycopeptides with complex glycosylation directly on the microarray slide. The enzymatic extension using larger *N*-glycan donor building blocks need to be further optimized in future studies. The obtained hepta- and pentasaccharide *N*-glycopeptide library was in this work employed in initial microarray studies analyzing binding interactions with the lectin Concanavalin A (ConA). Additionally, selected peptides were employed in ESI-MS fragmentation studies to improve methodology for *N*-glycopeptide structural analysis.

Some of the building blocks from the *N*-glycan synthesis, were also employed in the synthesis of *O*-mannosylated glycosylated amino acids to generate the building blocks **120**, **124** and **135** (**Figure 26**). Proteins modified with *O*-mannose glycans on backbone Ser and Thr residues, are involved in important functions in brain and muscle glycobiology. In spite of the clear importance of *O*-mannosyl glycosylation, no tools are to date available for specific detection, enrichment and identification of proteins containing these modifications. In order to study these processes, a project was initiated to generate glycan and glycopeptide specific antibodies, which could be useful tools to detect and enrich *O*-mannosylated proteins. Vaccine-constructs were prepared from glycopeptides containing these extended mannose moieties, which would be essential for *O*-mannosylation studies. Different Fmoc-*O*-mannosyl glycosylated amino acids were prepared and employed in glycopeptide synthesis. The glycopeptides were then incorporated into glycopeptide-immune carrier constructs for antibody induction in rabbits. For antibody evaluation additional glycopeptides were prepared and employed in ELISA neutralization experiments and on glycopeptide microarrays.

The obtained *O*-mannosyl glycopeptide library was further employed to study interactions with glycan binding lectins. These lectins were evaluated as potential analytical tools for specific *O*-mannosyl glycoprotein or glycopeptide detection and enrichment.

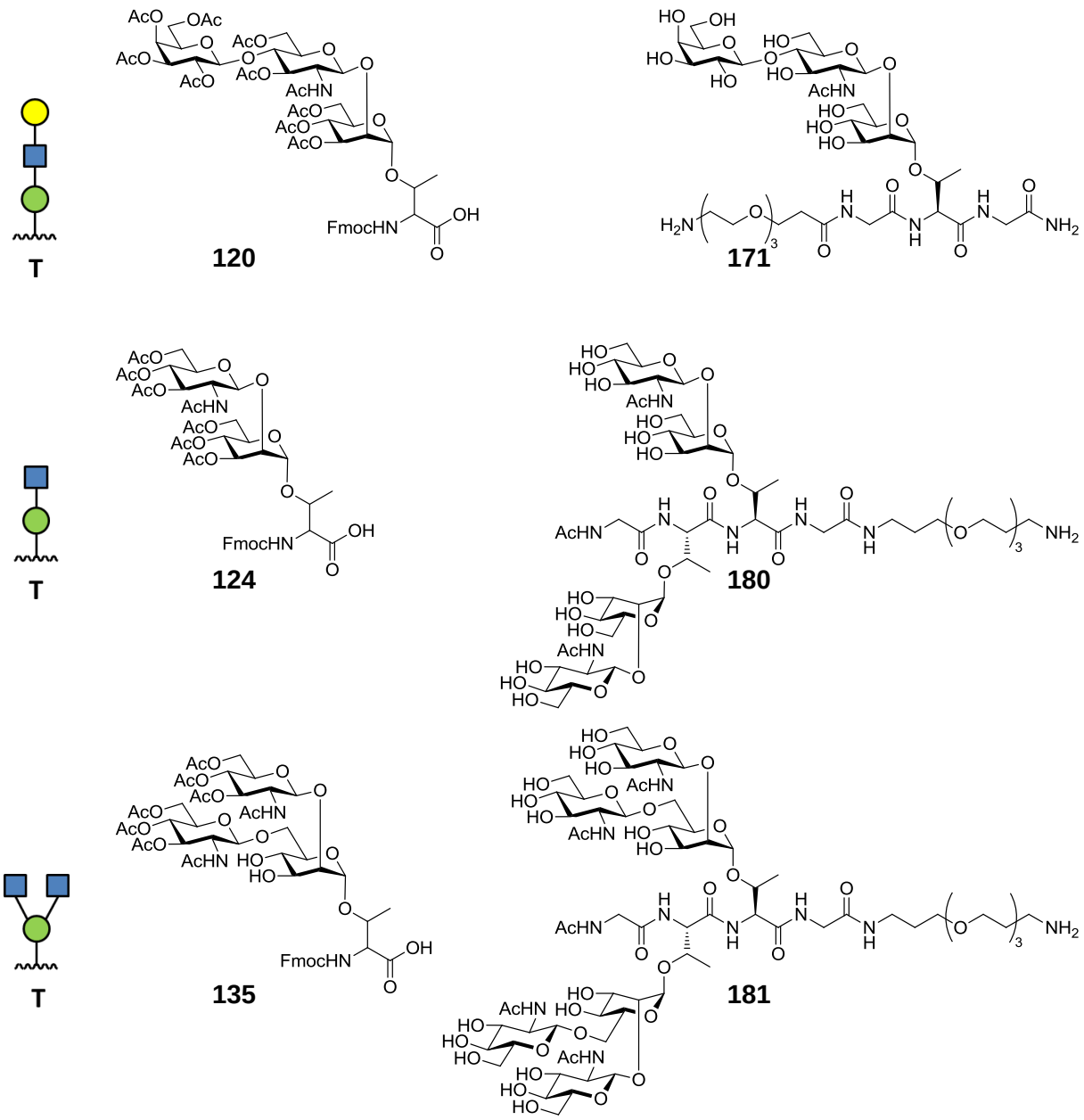

Figure 26. Synthesized *O*-mannosyl amino acids and the corresponding antigen peptides included in vaccine constructs for antibody induction.

Procedures were developed to synthesize two linear *O*-mannosyl amino acids, Galβ1-4GlcNAcβ1-2ManαThr **120**, GlcNAc-β1-2ManαThr **124** and one branched *O*-mannosyl amino acid, GlcNAc-β1-2-(GlcNAc-β1-6)ManαThr **135**. During the synthesis of the *O*-glycosylated amino acids, major considerations about the protecting group strategy were

taken. The *O*-glycosidic bond connected to serine and threonine residues is base labile and, protecting group manipulation steps involving strong basic conditions may cause β-elimination. Therefore, the *N*-phthalimide groups, employed in building blocks from the *N*-glycan synthesis, were replaced by *N*H-Troc groups, which could be removed in mild acidic conditions. Glycan donors protected with benzyl groups are usually more reactive than the corresponding acetyl group protected donors, which sometimes makes benzyl protection more attractive. On the other hand the later debenzylation step is not compatible with the desired *N*HFmoc-protection on the glycosylated amino acid building blocks. During synthesis of the linear *O*-mannosyl amino acid, Galβ1-4GlcNAcβ1-2ManαThr **120**, a Boc protected amino acid was initially used and converted to a Fmoc protecting group after the benzyl groups were removed. To avoid extra steps converting a Boc to a Fmoc group, *p*-methoxybenzyl (PMB) groups were used instead of benzyls in the later synthesis of the 2,6 branched *O*-mannosyl Fmoc amino acid GlcNAc-β1-2-(GlcNAc-β1-6)Manα Thr **135**. Before incorporation of the glycosylated Fmoc-amino acids into peptides, the glycan protection was unified and converted to acetyl groups, which could be removed under mild conditions and in one step after peptide synthesis.

To induce hapten-antibodies that generally recognize specific *O*-mannosyl glycan structures on peptides and proteins, short peptides containing glycine residues on each side, were synthesized and extended with a spacer to give **171**, **180** and **181**. The peptides were conjugated to KLH and CRM immure carrier proteins through a squarate linker. Accordingly BSA conjugates were prepared for ELISA antibody analysis. For further antibody analysis by glycopeptide microarrays, a small library of *O*-mannosyl glycopeptides were prepared. The antigen peptide **171** with a Gal-GlcNAc-Man trisaccharide was prepared with monovalent glycosylation on the peptide and the *N*-terminal was extended with a spacer for protein conjugation. The Gal-GlcNAc-Man antigen peptide **171** conjugated to the KLH immune carrier was administrated into rabbit. The obtained antibody sera was analyzed by normal ELISA, ELISA neutralization experiments and glycopeptide microarray analysis. A strong antibody titer recognizing the antigen peptide **171** was detected. The microarray analysis showed that peptide **172** containing the shorter disaccharide GlcNAc-Man, which was presented on the same peptide backbone as the antigen, was recognized to some extend by the antibodies. Further, the antibody specificity to the antigen structure was extremely high and when the

O-mannosyl glycans were presented on other peptide sequences like the glycopeptides **190** and **191**, antibody recognition was completly or partly lost.

To increase the specificity of the induced antibodies to the glycan structure and reducing the recognition of the underlying peptide sequence, a second antigen peptide **180** was prepared with the GlcNAc-Man disaccharide, di-valent glycosylated on the peptide backbone. The *N*-terminal was capped with an acetyl group and the *C*-terminal was extended with a spacer for protein conjugation. After immunization the obtained antibody sera was again analyzed by ELISA and glycopeptide microarray experiments. The immune response directed to the antigen glycopeptide was very strong. The raised antibodies additionally recognized other glycopeptide sequences containing the same GlcNAc-Man-Thr *O*-glycosylation like peptide **192**, **193** and **172**, and cluster glycan presentation on the peptide backbone dramatically favored antibody recognition. The 2,6 branched $GlcNAc_2$-Man-Thr presented with di-valent glycosylation on the antigen peptide sequence were also well recognized. Larger *O*-mannosyl glycans with monovalent glycosylation on the peptide backbone like the 2,6 branched $GlcNAc_2$-Man-Thr or the linear Gal-GlcNAc-Man-Thr structures were only weakly recognized. The non-glycosylated peptide backbone sequence of the GTTG-Spacer was to some extend recognized by the induced antibodies. In summary, antibodies were obtained with very high specificity to both the *O*-mannosyl glycan structure and the underlying peptide sequence. Presentation with higher glycan density on the peptide backbone, could increase the antibody specificity to the glycan structure.

The *O*-mannosyl glycopeptide library was also employed to analyze interactions with certain lectins. Analysis of the glycan binding lectin Concanavalin A (ConA), which is known to recognize α-mannose residues, showed that unique glycan and glycopeptide structural binding epitopes were recognized. Here, monoglycosylated peptides containing the simpler GlcNAc-Man disaccharide structure was highly recognized, while reactivity to the 2,6 branched $GlcNAc_2$-Man-Thr or the linear Gal-GlcNAc-Man-Thr structures were rather weak. Clustered glycan presentation resulted in no recognition by ConA. These results will have an impact in the structural analysis work of *O*-mannosyl glycoproteins. Additionally, selected *O*-mannosyl peptides were employed in ESI-MS fragmentation studies to improve methodology for *O*-glycopeptide structural analysis work.

5 Acknowledgements

I would like to express my sincere gratitude to my supervisor Dr. Ulrika Westerlind for her motivating supervision, helpful discussions, inspiring ideas and excellent guidance. This work was supported by ISAS - Leibniz institute for Analytical Sciences. I am grateful to Prof. Dr. Albert Sickmann for accepting my position to work in this excellent institute.

Special thanks to Prof. Dr. Thomas Norberg (Department of Chemistry-BMC, Uppsala University, Uppsala, Sweden) who introduced me into the carbohydrate synthesis field and Prof. Dr. Hebert Waldmann, Dr. Christian Hedberg, and Dr. Yaowen Wu from the Max-Planck Institute of Molecular Physiology, Dortmund, for their unconditional support.

Many thanks to Rita Fobbe, Helma Geltenpoth, Rolf Bandur from ISAS and Dr. Petra Janning, Chantale Martin from MPI and TU Dortmund, for the large amount of work involving high-resolution mass spectrometry measurements of the synthesized compounds. Dr. Jörg Lambert from ISAS-City Dortmund is acknowledged for his help of solving difficulties during NMR measurements of large glycan molecules.

Also, I would like to thank my lab-mates Christian Pett, Manuel Schorlemer, Vanessa Caixeta and Dr. Hui Cai, as good friends and for fruitful discussions. Many thanks to colleagues, in particular from the Sickmann, Zahedi and Waldmann group, for their support and for creating a nice working environment.

Finally, I would like to thank my wife for her long distance spiritual support, my parents for their endorsement of sending me for education oversea so that I could have the chance of pursuing further achievement in Science.

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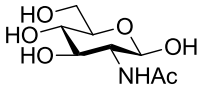
7 Experimental part

Reagents and solvents were purchased with high commercial quality and were used without further purification unless otherwise stated. Molecular sieves (powdered 4Å) were dried at 280°C /0.5 torr overnight. Dry dichloromethane and acetonitrile was prepared by distillation from CaH_2. Solvent concentrations were performed at a reduced pressure (bath temperature <50°C). TLC was performed on Silica Gel F_{254} (Merck, Darmstadt, Germany) with detection by UV-light and by staining with 5% sulfuric acid in ethanol. Column chromatography was performed on silica gel 60 Å (35-70 m, Acros). NMR spectra were recorded at 303 K with a Varian Mercury 400 Oxford NMR spectrometer or Bruker DRX 600 MHz spectrometer using $CDCl_3$ (internal $CHCl_3$, δ_H 7.26 ppm; δ_C 77.23 at 303 K) or DMSO-d_6 (internal DMSO, δ_H 2.50; δ_C 39.51 ppm at 303 K). Assignments were corroborated by appropriate 2-D experiments. The HR-ESI-MS spectra were recorded with a Thermo LTQ-FT ultra mass spectrometer. The MALDI-TOF MS were performed with a Bruker autoflex II LRF 50-CID. Specific rotations ($[\alpha]_D^{20}$) were recorded on a Polartronic HH8 from Schmidt and Haensch. HPLC measurements were performed using a Dionex ultimate 3000 LC system. The synthesis of compounds **1-195** is described herein.

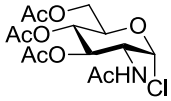
7.1 Synthesis of *N*-GlcNAc asparagine

N-acetyl glucosamine (1)[270]



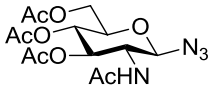
To a solution of glucosamine hydrochloride (25 g, 116 mmol, 1.0 eq) in MeOH (200 mL), sodium methoxide (6.45 g, 119 mmol, 1.03 eq) was added slowly. After intensive stirring at for 2 h, acetic acid was added until pH reached 6 - 7, followed by addition of acetic anhydride (9.05 g, 8.64 mL, 151 mmol, 1.3 eq). The mixture was mechanically stirred for 24 h. The organic layer turned yellow with a white solid that precipitated. The volume of MeOH was reduced to 100 mL by rotary evaporation, and the residue was kept in a -20 °C freezer for 4 h. After the product was fully precipitated, it was collected by filtration together with other inorganic salt from the reaction, NaCl, NaAc for instance. The crude product was then dissolved in hot MeOH (100 mL), filtered again the remove the undissolved inorganic salt, concentrated to dryness to give the GlcNAc **1** as a white powder, yield 30% (7.78 g, 35.2 mmol). R_f = 0.4 (EtOAc/MeOH/AcOH/H_2O 12:3:3:2)

2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosylchloride (2)[271]



GlcNAc **1** (7.78 g, 35.2 mmol) was dissolved in acetyl chloride (40 mL) in a 1 L round bottom flask with cooling under Ar, kept stirring at 40 °C for 24 h followed by TLC. The acetyl chloride was removed and diluted with DCM (100 mL). A solution of 10% of $NaHCO_3$ was added slowly under ice bath with strong stirring until pH reached 8-9. The aqueous layer was extracted with DCM (100 mL×3). The combined organic phase was dried with $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:2) gave **2** yield 54% (6.96 g, 19.0 mmol). R_f = 0.6 (^{C}Hex/EtOAc 1:3). ***^{1}H-NMR*** (400 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.04 (d, *J*=9.2, 1H, N*H*), 5.09 (t, *J*=9.9, 1H, H3), 4.88 (dd, *J*=10.5, 8.8, 1H, H4), 4.86 (d, *J*=7.3, 1H, H1), 4.16 (dd, *J*=12.4, 4.9, 1H, H6a), 4.06 (dd, *J*=12.3, 2.1, 1H, H6b), 4.00 (ddd, *J*=10.1, 4.8, 2.2, 1H, H5), 3.80 (dd, *J*=19.3, 9.8, 1H, H2), 2.01 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.78 (s, 3H, NHAc). ***^{13}C-NMR*** (100.6 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 170.68, 170.27, 170.18, 169.89 (170.3 - 169.9 CO_2CH_3), 88.16 (C1), 73.47 (C5), 72.94 (C3), 68.83 (C4), 62.34 (C6), 53.18 (C2), 23.22, 21.15, 21.03, 20.94 (23.2 - 20.9 CO_2CH_3).

2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylazide (3)[272]

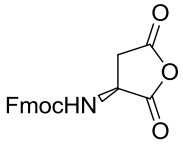


To a stirring mixture of **2** (6.96 g, 19.0 mmol, 1.0 eq) and TBAHS (6.51 g, 19.2 mmol, 1.01 eq) with NaN_3 (5.61 g, 86.3 mmol, 4.5 eq) in DCM (70 mL), Sat. $NaHCO_3$ (70 mL) was added. The two phase mixture was stirred at r.t. for 2 h and followed by TLC. Then the reaction was diluted with EtOAc (300 mL) and the organic phase was washed with Sat. $NaHCO_3$ (150 mL×2), H_2O (150 mL×2), brine (150 mL×1), dried over Na_2SO_4, the solvent was removed and the crude product was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:2) and recrystallized from cyclohexane-ethyl acetate to give **3**, yield 82% (5.83 g, 15.7 mmol). R_f = 0.6 (^{C}Hex/EtOAc 1:2). $[\alpha]_D^{20}$ = -38° (c = 1.2, $CHCl_3$). *ESI-MS (pos), m/z*: 372.73 ($[M+H]^+$, calc. 373.14). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.90 (d, *J*=8.9, 1H, N*H*), 5.25 (dd, *J*=10.5, 9.4, 1H, H3), 5.08 (t, *J*=9.7, 1H, H4), 4.77 (d, *J*=9.3, 1H, H1), 4.26 (dd, *J*=12.4, 4.9, 1H, H6a), 4.16 (dd, *J*=12.4, 2.3, 1H, H6b), 3.91 (dd, *J*=19.6, 9.1, 1H, H2), 3.80 (ddd, *J*=10.0, 4.9, 2.4, 1H, H5), 2.09 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.97 (s, 3H, NHAc). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.15, 170.88, 170.70, 169.48 (171.2 - 169.5 CO_2CH_3), 88.62 (C1), 74.17 (C5), 72.38 (C3), 68.35 (C4), 62.10 (C6), 54.35 (C2), 23.42 ($NHCO_2CH_3$), 20.92 (CO_2CH_3), 20.83 (CO_2CH_3), 20.78 (CO_2CH_3).

2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (4)[273-274]

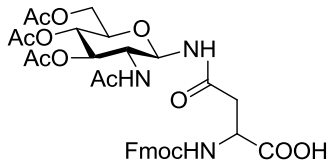
A dry flask containing Pd(C) (100 mg) was flushed with Ar several times. A solution of **3** (500 mg, 1.34 mmol) in MeOH (15 mL) was added via syringe under Ar. The reaction was then flushed with H_2 and kept under H_2 atmosphere for 2 h. After completion of the reaction, the flask was flushed again with Ar and the reaction mixture was filtered through a layer of Celite, followed by concentration and purification by column chromatography (DCM-MeOH 100:1 to 100:5) to give **4**, yield 83% (386 mg, 1.11 mmol). R_f = 0.5 (DCM/MeOH 10:1). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.75 (d, *J*=9.3, 1H, N*H*), 5.10 - 4.98 (m, 2H, H3, H4), 4.20 (dd, *J*=12.3, 4.9, 1H, H6a), 4.14 - 4.06 (m, 2H, H6b, H1), 4.04 - 3.93 (m, 1H, H2), 3.63 (ddd, *J*=9.4, 5.0, 2.5, 1H, H5), 2.08 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.96 (s, 3H, NHAc). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.76, 170.97, 169.53 (171.8 - 169.6 CO_2CH_3), 86.64 (C1), 73.66 (C3), 72.99 (C5), 68.76 (C4), 62.64 (C6), 55.11 (C2), 23.52 ($NHCO_2CH_3$), 21.00 (CO_2CH_3), 20.92 (CO_2CH_3), 20.83 (CO_2CH_3).

_N_-Fmoc-L-aspartic anhydride (5)[149]



To a stirring solution of Asn (2.0 g, 15 mmol, 1.0 eq) and Na_2CO_3 (3.5 g, 33 mmol, 2.2 eq) in H_2O (30 mL), a solution of Fmoc-OSu (5.1 g, 15 mmol, 1.0 eq) in THF (30 mL) was added. The reaction was stirred at r.t. and the progression was followed by TLC. After 20 h the reaction was completed and the THF was removed under reduced pressure. The residue was diluted with H_2O (30 mL), washed with Et_2O (20 mL×2). The aqueous layer was then acidified with conc. HCl to pH = 2, and extracted with EtOAc (30 mL×2). The organic layer was washed with brine (50 mL×1), dried over $MgSO_4$ and the solvent was evaporated to dryness. The crude product was then dissolved in Ac_2O (15 mL) with rapid heating and shaking at 120 °C for 5 min and then quickly cooled to r.t. The product precipitated and was filtered, washed with Et_2O (30 mL×2) and dried under high vacuum, yield 83% (4.02 g, 11.9 mmol). R_f = 0.9 (EtOAc/MeOH/AcOH/H_2O 12:3:3:2). *ESI-MS (pos), m/z*: 369.87 ($[M+H]^+$, calc. 684.00), 1366.80 ($[2M+H]^+$, calc. 1367.47), 1384.80 ($[2M+NH_4]^+$, calc. 1384.50).***^{1}H-NMR*** (400 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.16 (d, *J*=7.7, 1H, N*H*), 7.87 (d, *J*=7.5, 2H, Fmoc), 7.65 (d, *J*=7.5, 2H, Fmoc), 7.40 (t, *J*=7.4, 2H, Fmoc), 7.32 (t, *J*=7.4, 2H, Fmoc), 4.65 (dt, *J*=9.9, 6.9, 1H, Asp-C*H*α), 4.48 - 4.32 (m, 2H, FmocCH_2), 4.23 (t, *J*=6.4, 1H, FmocC*H*), 3.22 (dd, *J*=18.5, 10.0, 1H, Asp-CH_2β), 2.84 (dd, *J*=18.4, 6.2, 1H, Asp-CH_2β). ***^{13}C-NMR*** (100.6 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 172.79, 170.51, 156.53, 144.30, 144.26, 141.46, 128.36, 127.79, 127.75, 125.76, 125.68, 120.86 (128.4 - 120.9 Fmoc), 66.75 (FmocCH_2), 51.05 (Asp-CH_2α), 47.25 (Fmoc*C*H), 35.39 (Asp-CH_2β).

2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*N*-[*N*-Fmoc-L-aspart-4-oyl]-β-D-glucopyranosylamide (6)[149]

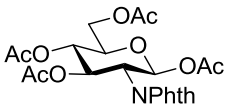


To a stirring solution of **4** (2.62 g, 7.55 mmol, 1.0 eq) in DMSO (4.5 mL), *N*-Fmoc-asp anhydride **5** (2.54 g, 7.53 mmol, 1.0 eq) was added. The reaction was stirred at r.t. for 2 h, full conversion indicated by TLC and then diluted with MeOH (50 mL). The precipitate was filtered and recrystallized from MeOH to give **6**, yield 63% (3.25 g, 4.75 mmol). R_f = 0.4 (^{C}Hex/EtOAc/MeOH/AcOH/H_2O 15:60:3:3:2). *ESI-MS (pos), m/z*: 684.24 ($[M+H]^+$, calc. 684.00), 1366.80 ($[2M+H]^+$, calc. 1367.47), 1384.80 ($[2M+NH_4]^+$, calc. 1384.50).***^{1}H-NMR*** (400 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.56 (d, *J*=9.2, 1H, GlcN*H*), 7.87 (d, *J*=7.8, 3H, Fmoc, N*H*Ac), 7.69 (d, *J*=7.4, 2H, Fmoc), 7.48 (d, *J*=8.4, 1H, AsnN*H*), 7.40 (t, *J*=7.4, 2H, Fmoc), 7.31 (t, *J*=7.4, 2H, Fmoc), 5.17 (t, *J*=9.6, 1H, H1),

5.09 (t, *J*=9.9, 1H, H3), 4.81 (t, *J*=9.8, 1H, H4), 4.37 (dd, *J*=13.6, 7.3, 1H, Asn-C*H*α), 4.31 - 4.14 (m, 4H, FmocC*H*$_2$, H6a, FmocC*H*), 3.93 (d, *J*=11.7, 1H, H6b), 3.87 (dd, *J*=19.8, 9.9, 1H, H2), 3.80 (d, *J*=9.4, 1H, H5), 2.65 (dd, *J*=16.2, 5.3, 1H, Asn-CH$_2$β), 2.54 - 2.45 (m, 1H, Asn-CH$_2$β), 1.98 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.71 (s, 3H, NHAc). ***^{13}C-NMR*** (100.6 MHz, DMSO-d$_6$, gHSQC, gHMBC), *δ (ppm)*: 173.62, 170.70, 170.48, 170.17, 169.98 (170.7 - 170.0 *C*O$_2$CH$_3$), 156.51, 144.46, 141.38, 128.31, 127.75, 125.92, 120.78 (128.3 - 120.8 Fmoc), 113.54, 78.78 (C1), 74.06 (C3), 72.98 (C5), 69.09 (C4), 66.39 (Fmoc*C*H$_2$), 62.83, 62.52 (C6), 52.81 (C2), 50.69 (Asn-*C*Hα), 47.29 (Fmoc*C*H), 37.55 (Asn-CH$_2$β), 23.27, 21.20, 21.08, 21.05 (23.3 - 21.1CO$_2$*C*H$_3$).

7.2 Synthesis of the 4-OH glucosamine acceptor

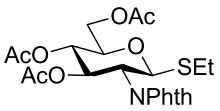
2-deoxy-2-phthalimido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (7)[166]



NaOH (36.2 g, 904 mmol, 1.3 eq) in H_2O (600 mL) was added to a stirring solution of glucosamine hydrochloride (150 g, 696 mmol, 1.0 eq) in MeOH (300 mL), at 0 °C. Then a solution of phthalic anhydride (149.3 g, 1007 mmol, 1.45 eq) in acetone (1200 mL) was slowly added over 30 min. The reaction was then kept at r.t. for 2 h and more phthalic anhydride (61.8 g, 417 mmol, 0.6 eq) and $NaHCO_3$ (149.6 g, 1781 mmol, 2.56 eq) was added. After stirring at r.t. for 24 h, the reaction mixture was brought to pH 1 by adding a small portion of conc. HCl. The acetone was removed under reduced pressure and the crude product was kept in the fridge for 24 h, filtered, washed with cold water, coevaporated with toluene, dried under vacuum to give a white powder, yield 88% (190 g, 614 mmol). R_f = 0.6 (EtOAc/MeOH/AcOH/H_2O 6:3:3:2)

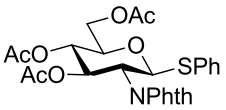
The intermediate product (87 g, 281 mmol, 1.0 eq) was added in portions into a refluxing mixture of NaOAc (25.4 g, 309 mmol, 1.1 eq) in acetic anhydride (265 mL, 2.81 mol, 10 eq) and kept refluxing at 160 °C for 2 h, then concentrated followed by dilution with EtOAc (800 mL), washed with H_2O (500 mL×3), Sat. $NaHCO_3$ (500 mL×3), brine (500 mL×1), dried over $MgSO_4$. The organic solvent was removed under reduced pressure, the crude product was purified by column chromatography (cyclohexane-ethyl acetate 1:1) and was then recrystallized from cyclohexane-ethyl acetate to give **7**, yield 72% (97 g, 203 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +76° (c = 1.1, $CHCl_3$). *ESI-MS (pos), m/z*: 494.87 ($[M+NH_4]^+$, calc. 495.16), 500.00 ($[M+Na]^+$, calc. 500.12). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.86 - 7.81 (m, 2H, Phth), 7.75 - 7.70 (m, 2H, Phth), 6.49 (d, *J*=8.9, 1H, H1), 5.87 (dd, *J*=10.6, 9.1, 1H, H3), 5.19 (dd, *J*=10.1, 9.2, 1H, H4), 4.45 (dd, *J*=10.6, 8.9, 1H, H2), 4.35 (dd, *J*=12.4, 4.4, 1H, H6a), 4.12 (ddd, *J*=12.2, 5.0, 2.1, 1H, H6b), 4.01 (ddd, *J*=10.2, 4.4, 2.1, 1H, H5), 2.09 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.84 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.87, 170.22, 169.68, 168.83 (170.9 - 169.8 CO_2CH_3), 134.69 (Phth), 134.67 (Phth), 124.00 (Phth), 89.99 (C1), 72.85 (C5), 70.73 (C3), 68.54 (C4), 61.76 (C6), 53.72 (C2), 20.96, 20.93, 20.84, 20.80, 20.59 (21.0 - 20.6 CO_2CH_3).

Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (8)[166]



Under cooling with an ice bath and under Ar atmosphere, BF_3 etherate (2.7 mL, 21.9 mmol, 1.3 eq) was added via syringe to a stirring solution of **7** (8.0 g, 16.8 mmol, 1.0 eq) and EtSH (1.58 mL, 21.9 mmol, 1.3 eq) in dry DCM (100 mL). The reaction mixture was stirred at r.t. for 3 h, and was then neutralized by addition of Et_3N (1 mL), concentrated to dryness to remove most of the EtSH, dissolved again in DCM (100 mL) washed with Sat. $NaHCO_3$ (50 mL×3), H_2O (50 mL×3), brine (50 mL×1), dried over $MgSO_4$, and the organic solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 4:1 to 1:1), and recrystallized from cyclohexane-ethyl acetate to give **8**, yield 65% (5.2 g, 10.8 mmol). R_f = 0.7 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +44° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 496.93 ($[M+NH_4]^+$, calc. 497.16), 502.07 ($[M+Na]^+$, calc. 502.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.83 (dd, *J*=5.3, 3.1, 2H, Phth), 7.72 (dd, *J*=5.5, 2.9, 2H, Phth), 5.80 (dd, *J*=10.2, 9.2, 1H, H3), 5.46 (d, *J*=10.6, 1H, H1), 5.15 (t, *J*=9.7, 1H, H4), 4.37 (t, *J*=10.4, 1H, H2), 4.28 (dd, *J*=12.3, 4.9, 1H, H6a), 4.15 (dd, *J*=12.3, 2.2, 1H, H6b), 3.87 (ddd, *J*=10.2, 4.9, 2.3, 1H, H5), 2.75 - 2.55 (m, 2H, SCH_2CH_3), 2.07 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.19 (t, *J*=7.4, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.85, 170.26, 169.65 (170.9 - 169.7 CO_2CH_3), 167.95 (Phth C=O), 167.34 (Phth C=O), 134.64, 134.50, 131.80, 131.38, 123.90 (134.6 - 123.9 Phth), 81.39 (C1), 76.13 (C5), 71.76 (C3), 69.11 (C4), 62.51 (C6), 53.89 (C2), 24.55 (SCH_2CH_3), 20.96, 20.82, 20.64 (21.0 - 20.7 CO_2CH_3), 15.10 (SCH_2CH_3).

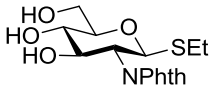
Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (9)[275]



Under cooling with an ice bath and under Ar atmosphere, BF_3 etherate (12.8 mL, 100.8 mmol, 1.5 eq) was added via syringe to a stirring solution of **7** (32.1 g, 67.2 mmol, 1.0 eq) and EtSPh (13.7 mL, 134.4 mmol, 2.0 eq) in dry DCM (150 mL). The reaction mixture was stirred at r.t. for 3 h, and was then neutralized by addition of Et_3N (7.5 mL), concentrated to dryness to remove most of the EtSPh, dissolved again in DCM (200 mL) washed with Sat. $NaHCO_3$ (100 mL×3), H_2O (100 mL×3), brine (100 mL×1), dried over $MgSO_4$, and the organic solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 4:1 to 1:1), and recrystallized from cyclohexane-ethyl acetate to give **9**, yield 58% (20.4 g, 38.7 mmol). R_f = 0.6 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +55° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 544.93 ($[M+NH_4]^+$, calc.

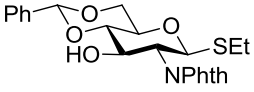
545.16), 550.00 ([M+Na]$^+$, calc. 550.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.86 (dd, *J*=5.5, 3.1, 2H, Phth), 7.74 (dd, *J*=5.4, 3.1, 2H, Phth), 7.42 - 7.37 (m, 2H, SPh), 7.31 - 7.22 (m, 3H, SPh), 5.79 (dd, *J*=10.2, 9.2, 1H, H3), 5.71 (d, *J*=10.6, 1H, H1), 5.13 (t, *J*=9.7, 1H, H4), 4.34 (t, *J*=10.4, 1H, H6a), 4.28 (dd, *J*=12.3, 5.1, 1H, H6b), 4.20 (dd, *J*=12.2, 2.3, 1H, H2), 3.89 (ddd, *J*=10.2, 5.1, 2.4, 1H, H5), 2.09 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.82 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.79 (CO_2CH_3), 170.28 (CO_2CH_3), 169.64 (CO_2CH_3), 168.01 (Phth C=O), 167.13 (Phth C=O), 134.67 (Phth), 134.55 (Phth), 133.49, 131.20, 129.11, 128.63 (133.5 - 128.6 SPh), 123.92 (Phth), 83.27 (C1), 76.13 (C5), 71.85 (C3), 68.96 (C4), 62.45 (C6), 53.81 (C2), 20.97 (CO_2CH_3), 20.82 (CO_2CH_3), 20.61 (CO_2CH_3).

Ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10)[166]



Compound **8** (10.2 g, 21.2 mmol) was dissolved in 0.08 M NaOMe in MeOH (100 mL), stirred at r.t. for 4 h. The deacetylated product was then neutralized with *Dowex* 50WX8 ion-exchanger (1 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL), dried under high vacuum to give **10**, yield 99% (7.4 g, 20.9 mmol). R_f = 0.4 (DCM/MeOH 9:1). $[\alpha]_D^{20}$ = +6° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 370.80 ([M+NH_4]$^+$, calc. 371.13), 375.93 ([M+Na]$^+$, calc. 376.08). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.78 (dd, *J*=5.1, 3.3, 2H, Phth), 7.67 (dd, *J*=5.4, 3.0, 2H, Phth), 5.28 (d, *J*=10.4, 1H, H1), 4.33 - 4.24 (m, 1H, H3), 4.09 (t, *J*=10.3, 1H, H2), 3.81 (d, *J*=2.7, 2H, H6a, H6b), 3.62 (t, *J*=9.2, 1H, H4), 3.41 (d, *J*=9.3, 1H, H5), 2.71 - 2.51 (m, 2H, SCH_2CH_3), 1.12 (t, *J*=7.4, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.50, 134.33 (Phth), 131.94, 131.80, 123.97 (Phth), 123.51 (Phth), 81.44 (C1), 79.95 (C5), 72.66 (C3), 71.26 (C6), 62.00 (C4), 56.12 (C2), 24.49 (SCH_2CH_3), 15.11 (SCH_2CH_3).

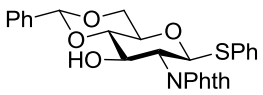
Ethyl 2-deoxy-2-phthalimido-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (11)[166]



Compound **10** (7.4 g, 20.9 mmol, 1.0 eq) and α,α-dimethoxytoluene (6.37 g, 41.8 mmol, 2.0 eq) were dissolved in DMF (100 mL). *p*-toluenesulfonic acid was added to the reaction mixture to reach pH 4 and was then kept at 50 mbar, 50 °C for 1 h. The reaction was neutralized with Et_3N to pH 7 and DMF was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 8:1 to 2:1), and recrystallized from cyclohexane-ethyl acetate to give **11**, yield 86% (7.95 g, 18.0 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +1° (c = 0.9, $CHCl_3$).

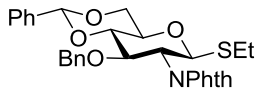
ESI-MS (pos), m/z: 899.60 ($[2M+NH_4]^+$, calc. 900.28), 1340.33 ($[3M+NH_4]^+$, calc. 1341.41). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.90 - 7.85 (m, 2H, Phth), 7.74 - 7.70 (m, 2H, Phth), 7.52 - 7.46 (m, 2H, Ph), 7.39 - 7.31 (m, 3H, Ph), 5.57 (s, 1H, C*H*PH), 5.40 (d, *J*=10.6, 1H, H1), 4.66 (dd, *J*=10.0, 8.9, 1H, H3), 4.39 (dd, *J*=10.3, 4.7, 1H, H6a), 4.32 (t, *J*=10.3, 1H, H2), 3.80 (t, *J*=10.1, 1H, H6b), 3.70 (td, *J*=9.7, 4.7, 1H, H5), 3.61 (t, *J*=9.1, 1H, H4), 2.75 - 2.59 (m, 2H, SCH_2CH_3), 1.19 (t, *J*=7.4, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 137.18, 134.42 (Phth), 134.38 (Phth), 129.97, 129.57, 129.21, 128.59 (benzylidene acetal), 126.54 (benzylidene acetal), 124.07 (Phth), 123.55 (Phth), 102.16 (*C*HPH), 82.35 (C4), 82.11 (C1), 70.61 (C5), 69.77 (C3), 68.86 (C6), 55.76 (C2), 24.42 (SCH_2CH_3), 15.10 (SCH_2CH_3).

Phenyl 2-deoxy-2-phthalimido-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (12)[276]



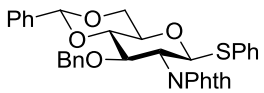
Compound **9** (20.4 g, 38.7 mmol) was dissolved in a solution of MeOH/DCM 3:1 (266 mL), NaOMe (1.61 g, 29.8 mmol, 0.11 M) was added. The reaction mixture was stirred at r.t. for 4 h, and was then neutralized with *Dowex* 50WX8 ion-exchanger (1.5 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL), dried under high vacuum to give the deacetylated product. The residue and α,α-dimethoxytoluene (8.81 g, 57.8 mmol, 1.5 eq) were dissolved in DMF (200 mL). *p*-toluenesulfonic acid was added to the reaction mixture to reach pH 4 and was then kept at 50 mbar, 50 °C for 1 h. The reaction was neutralized with Et_3N to pH 7 and DMF was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 8:1 to 2:1), and recrystallized from cyclohexane-ethyl acetate to give **12**, yield 82% (15.4 g, 31.5 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +41° (c = 1.1, $CHCl_3$). *ESI-MS (pos), m/z*: 995.73 ($[2M+NH_4]^+$, calc. 996.28), 1484.40 ($[3M+NH_4]^+$, calc. 1485.41). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.81 - 7.76 (m, 1H, Phth), 7.73 (s, 1H, Phth), 7.67 - 7.61 (m, 2H, Phth), 7.41 - 7.36 (m, 2H, Ph), 7.33 - 7.28 (m, 2H, S*Ph*), 7.28 - 7.23 (m, 3H, Ph), 7.19 - 7.15 (m, 3H, S*Ph*), 5.60 (d, *J*=10.6, 1H, H1), 5.47 (s, 1H, C*H*PH), 4.54 (t, *J*=9.5, 1H, H3), 4.30 (dd, *J*=10.4, 4.8, 1H, H6a), 4.23 (t, *J*=10.3, 1H, H2), 3.73 (t, *J*=10.2, 1H, H6b), 3.59 (td, *J*=9.7, 4.8, 1H, H5), 3.50 (t, *J*=9.1, 1H, H4). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 134.47 (Phth), 134.42 (Phth), 132.84, 132.41, 132.03, 129.58, 129.19, 128.59, 128.32, 126.56 (132.8 - 126.6 aromatic), 124.08 (Phth), 123.61 (Phth), 102.17 (*C*HPH), 84.52 (C1), 82.09 (C4), 70.56 (C5), 69.92 (C3), 68.80 (C6), 55.86 (C2).

Ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (13)[170]



Compound **11** (82.13 g, 186 mmol, 1.0 eq) was dissolved in dry THF (400 mL) under Ar and under cooling at 0 °C, NaH (60% suspension, 11.17 g, 279 mmol, 1.5 eq) was added in portions. The reaction was kept at 0 °C for 40 min before BnBr (33.18 mL, 279 mmol, 1.5 eq) was added dropwise via syringe. The mixture was heated to reflux for 2 h before the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (800 mL), filtered through a layer of Celite, washed with H_2O (500 mL×2), brine (500 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **13**, yield 96% (94.56 g, 178 mmol). R_f = 0.7 (Tol/EtOAc 3:1). $[\alpha]_D^{20}$ = +65° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 548.67 ($[M+NH_4]^+$, calc. 549.21), 1079.53 ($[2M+NH_4]^+$, calc. 1080.38). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.85 (d, *J*=7.1, 1H, Phth), 7.70 (d, *J*=4.9, 2H, Phth), 7.65 (d, *J*=6.6, 1H, Phth), 7.53 (dd, *J*=7.7, 1.8, 2H, CH*Ph*), 7.46 - 7.34 (m, 3H, CH*Ph*), 7.04 - 6.96 (m, 2H, CH_2*Ph*), 6.96 - 6.81 (m, 3H, CH_2*Ph*), 5.63 (s, 1H, C*H*Ph), 5.35 (d, *J*=10.6, 1H, H1), 4.80 (d, *J*=12.3, 1H, CH_2Ph), 4.51 (d, *J*=12.3, 1H, CH_2Ph), 4.46 (dd, *J*=9.8, 9.1, 1H, H3), 4.42 (dd, *J*=4.5, 10.9, 1H, H6a), 4.30 (t, *J*=10.3, 1H, H2), 3.87 - 3.79 (m, 2H, H6b, H4), 3.71 (td, J=9.8, 4.9, H5), 2.78 - 2.54 (m, 2H, SCH_2CH_3), 1.17 (t, *J*=7.4, 3H, SCH_2CH_3).***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.04 (*C*=O), 137.54 (*C*=O), 134.15 (Phth), 134.04 (Phth), 129.24, 128.51, 128.31, 128.26, 127.63, 126.27 (129.2-126.3, CH_2*Ph*, CH*Ph*), 123.77 (Phth), 123.53 (Phth), 101.55 (*C*HPh), 83.28 (C4), 82.03 (C1), 75.67 (C3), 74.43 (CH_2Ph), 70.69 (C5), 68.97 (C6), 54.91 (C2), 24.30 (SCH_2CH_3), 15.08 (SCH_2CH_3).

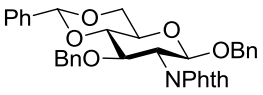
Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (14)[276]



Compound **12** (11.95 g, 24.4 mmol, 1.0 eq) was dissolved in dry THF (120 mL) under Ar and under cooling at 0 °C, NaH (60% suspension, 1.95 g, 48.8 mmol, 2.0 eq) was added in portions. The reaction was kept at 0 °C for 40 min before BnBr (5.26 mL, 44.2 mmol, 1.8 eq) was added dropwise via syringe. The mixture was heated to reflux for 2 h before the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL), filtered through a layer of Celite, washed with H_2O (100 mL×2), brine (100 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **14**, yield 96% (94.56 g, 178 mmol). R_f = 0.7 (Tol/EtOAc 3:1). $[\alpha]_D^{20}$ = +98° (c = 0.8,

$CHCl_3$). *ESI-MS (pos), m/z*: 579.67 ($[M+H]^+$, calc. 580.18), 596.80 ($[M+NH_4]^+$, calc. 597.21), 1175.60 ($[2M+NH_4]^+$, calc. 1176.38). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.87 (d, *J*=7.0, 1H, Phth), 7.77 - 7.68 (m, 2H, Phth), 7.65 (d, *J*=6.3, 1H, Phth), 7.53 (dd, *J*=7.7, 1.7, 2H, CH*Ph*), 7.44 - 7.32 (m, 5H, CH*Ph*, S*Ph*), 7.29 - 7.22 (m, 3H, S*Ph*), 7.02 - 6.96 (m, 2H, CH_2*Ph*), 6.94 - 6.84 (m, 3H, CH_2*Ph*), 5.64 (d, *J*=10.5, 1H, H1), 5.63 (s, 1H, C*H*Ph), 4.79 (d, *J*=12.3, 1H, CH_2Ph), 4.51 (d, *J*=12.3, 1H, CH_2Ph), 4.48 - 4.40 (m, 2H, H3, H6a), 4.32 (t, *J*=10.2, 1H, H2), 3.86 (t, *J*=8.5, 1H, H6b), 3.84 - 3.79 (m, 1H, H4), 3.74 (dd, *J*=9.7, 4.7, 1H, H5). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.00 (Phth C=O), 167.42 (Phth C=O), 137.94, 137.53, 134.23 (Phth), 134.09 (Phth), 132.99 (S*Ph*), 131.88, 131.82, 129.27, 129.14, 128.53, 128.38, 128.32, 128.28, 127.67 (131.9 - 127.7 aromatic), 126.30 (CH*Ph*), 123.77 (Phth), 123.60 (Phth), 101.57 (*C*HPh), 84.35 (C1), 83.06 (C4), 75.73 (C3), 74.46 (CH_2Ph), 70.64 (C5), 68.91 (C6), 55.00 (C2).

Benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (15)[277]



Compound **13** (24.23 g, 45.6 mmol, 1.0 eq), benzylalcohol (9.44 mL, 78.8 mmol, 1.73 eq), *N*-iodosuccinimide (15.35 g, 68.3 mmol, 1.5 eq) and 4 Å molecular sieves (48 g) in dry dichloromethane (240 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (606 μL, 6.8 mmol, 0.15 eq). After stirring for 1 h at 0 °C, the reaction was quenched by diluting the reaction mixture with more DCM (100 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (75 mL×3), brine (100 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (toluene-ethyl acetate 100:1 to 10:1) to give **15**. Yield: 90% (23.76 g, 41.1 mmol). R_f = 0.5 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +6° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 577.80 ($[M+H]^+$, calc. 578.22), 594.73 ($[M+NH_4]^+$, calc. 595.24), 1171.73 ($[2M+NH_4]^+$, calc. 1172.45). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.69 (s, 2H, Phth), 7.52 (dd, J=7.6, 1.8, 2H, Phth), 7.41 - 7.34 (m, 5H, CH*Ph*), 7.05 - 6.95 (m, 6H, CH*Ph*), 6.91 - 6.83 (m, 4H, CH*Ph*), 5.63 (s, 1H, C*H*Ph), 5.20 (d, J=8.5, 1H, H1), 4.80 (d, J=12.0, 1H, 1-CH_2Ph), 4.77 (d, J=11.8, 1H, 3-CH_2Ph), 4.48 (d, J=12.3, 2H, 3-CH_2Ph, 1-CH_2Ph), 4.45 - 4.37 (m, 2H, H6a, H3), 4.26 (dd, J=10.4, 8.5, 1H, H2), 3.88 (t, J=10.3, 1H, H6b), 3.82 (t, J=9.1, 1H, H4), 3.63 (td, J=9.8, 5.0, 1H, H5).***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.14, 137.58, 137.07, 133.90, 131.80, 129.23, 128.92, 128.76, 128.50, 128.39, 128.25, 128.22, 127.88, 127.85,

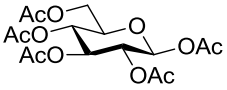
127.56, 127.19 (129.2 - 126.3, CH_2*Ph*), 126.29, 123.46, 101.56 (*C*HPh), 98.13 (C1), 83.24 (C4), 74.68 (C3), 74.26 (3-CH_2Ph), 71.39 (1-CH_2Ph), 69.03 (C6), 66.28 (C5), 56.06 (C2).

Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D- glucopyranoside (16)[278]

Compound **15** (19.49 g, 33.7 mmol, 1.0 eq) and sodium cyanoborohydride (8.48 g, 135 mmol, 4.0 eq) was dissolved in anhydrous THF (117 mL). Under Ar atmosphere, at 0 °C 2N HCl in Et_2O (59.0 mL, 118 mmol, 3.5 eq) was added dropwise via syringe. The reaction was followed by TLC. After 30 min stirring at 0 °C, the reaction was neutralized by Et_3N (10 mL) and the solvent was removed by evaporation under reduced pressure and without heating. The residue was purified by column chromatography (toluene-ethyl acetate 10:1 to 5:1) to give **16**. Yield: 87% (17.07 g, 29.4 mmol). R_f = 0.3 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +1° (c = 1.2, $CHCl_3$). *ESI-MS (pos), m/z*: 597.00 ($[M+NH_4]^+$, calc. 597.26), 1175.73 ($[2M+NH_4]^+$, calc. 1176.49). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.68 (d, *J*=2.4, 2H, Phth), 7.37 (m, 6H, Phth, CH_2*Ph*), 7.04 (m, 8H, CH_2*Ph*), 6.97 - 6.92 (m, 3H, CH_2*Ph*), 5.16 (d, *J*=8.2, 1H, H1), 4.79 (d, *J*=12.3, 1H, 1-CH_2Ph), 4.72 (d, *J*=12.2, 1H, 3-CH_2Ph), 4.67 (d, *J*=11.9, 1H, 6-CH_2Ph), 4.60 (d, *J*=11.9, 1H, 6-CH_2Ph), 4.52 (d, *J*=12.2, 1H, 3-CH_2Ph), 4.48 (d, *J*=12.3, 1H, 1-CH_2Ph), 4.26 - 4.21 (m, 2H, H2, H3), 3.84 (m, 3H, H6ab, H4), 3.64 (dt, *J*=9.8, 4.9, 1H, H5). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.41, 137.87, 137.35, 133.90, 128.75, 128.36, 128.35, 128.14, 128.08, 128.04, 127.82, 127.78, 127.63 (128.8 - 127.6, CH_2Ph), 97.65 (C1), 78.80 (C3), 74.55 (C4), 74.48 (3-CH_2Ph), 74.04 (6-CH_2Ph), 73.79 (C5), 71.06 (1-CH_2Ph), 70.93 (C6), 55.64 (C2).

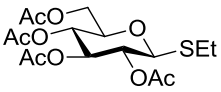
7.3 Synthesis of the glucose donor building block

1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (17)



A mixture of sodium acetate (25 g, 305 mmol, 1.1 eq) in acetic anhydride (170 mL, 1.80 mol, 6.5 eq) was heated to reflux in a three necked round bottom flask, the heating was removed. D-glucose (50 g, 278 mmol, 1.0 eq) was added in portions and the reaction started to boil intensively. When the boiling stopped, heating was applied again, and the reaction was refluxed for another 1 h. The reaction mixture was concentrated followed by dilution with EtOAc (500 mL), washed with H_2O (300 mL×3), Sat. $NaHCO_3$ (300 mL×3), brine (500 mL×1), dried over $MgSO_4$. The organic solvent was removed under reduced pressure, the crude product was purified by column chromatography (cyclohexane-ethyl acetate 1:1) and was then recrystallized from cyclohexane-ethyl acetate to give **17**, yield 95% (107.3 g, 264 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +22° (c = 1.2, $CHCl_3$). *ESI-MS (pos), m/z*: 407.80 ($[M+NH_4]^+$, calc. 408.15), 412.93 ($[M+Na]^+$, calc. 413.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.70 (d, *J*=8.3, 1H, H1), 5.24 (t, *J*=9.4, 1H, H3), 5.15 - 5.08 (m, 2H, H2, H4), 4.28 (dd, *J*=12.5, 4.5, 1H, H6a), 4.10 (dd, *J*=12.5, 2.2, 1H, H6b), 3.83 (ddd, *J*=10.0, 4.5, 2.2, 1H, H5), 2.10 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.02 (s, 6H, Ac×2), 2.00 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.77, 170.26, 169.56, 169.41, 169.12 (170.8 - 169.1, CO_2CH_3), 91.91 (C1), 73.00 (C3), 72.94 (C5), 70.45 (C2), 67.97 (C4), 61.66 (C6), 20.76 (Ac).

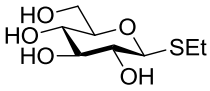
Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (18)[279]



Under cooling with an ice bath and under Ar atmosphere, BF_3 etherate (60 mL, 471 mmol, 1.2 eq) was added via syringe to a stirring solution of **17** (150 g, 384 mmol, 1.0 eq) and EtSH (87 mL, 1.15 mol, 3.0 eq) in dry DCM (500 mL). The reaction was stirred at r.t. and after 2h TLC indicated full conversion to product. The reaction mixture was then neutralized by addition of Et_3N (80 mL) under cooling with an ice bath. Then concentrated to dryness to remove most of the EtSH. The residue was dissolved again in DCM washed with Sat. $NaHCO_3$ (500 mL×3), H_2O (500 mL×3), brine (500 mL×1), dried over $MgSO_4$, and the organic solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 4:1 to 1:1), and recrystallized from cyclohexane-ethyl acetate to give **18**, yield 70% (106 g, 270 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1).

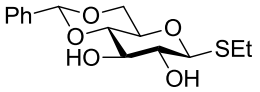
$[\alpha]_D^{20}$ = -26° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 409.87 ($[M+NH_4]^+$, calc. 410.15), 415.00 ($[M+Na]^+$, calc. 415.10). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.21 (t, *J*=9.4, 1H,H3), 5.12 - 4.98 (m, 2H, H4, H2), 4.49 (d, *J*=10.0, 1H, H1), 4.23 (dd, *J*=12.4, 5.0, 1H, H6a), 4.13 (dd, *J*=12.3, 2.4, 1H, H6b), 3.70 (ddd, *J*=10.0, 5.0, 2.4, 1H, H5), 2.79 - 2.60 (m, 2H, SCH_2CH_3), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.26 (t, *J*=7.5, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.82, 170.38, 169.60, 169.58 (170.8 - 169.6, *C*O_2CH_3), 83.73 (C1), 76.10 (C5), 74.14 (C3), 70.07 (C2), 68.58 (C4), 62.39 (C6), 24.38 (SCH_2CH_3), 20.93, 20.82, 20.79 (20.9 - 20.8, CO_2CH_3), 15.04 (SCH_2CH_3).

Ethyl 1-thio-β-D-glucopyranoside (19)[280]



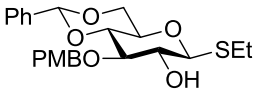
Compound **18** (12.1 g, 30.9 mmol) was dissolved in 0.05 M NaOMe in MeOH (40 mL), stirred at r.t. for 2 h. Then neutralized with Dowex 50 ion exchange beads (1 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL), dried under high vacuum to give **19**, yield 99% (6.92 g, 30.9 mmol), continued to the next step synthesis without further purification.

Ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (20)[280]



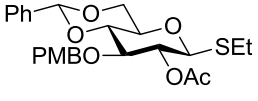
Compound **19** (7.16 g, 31.9 mmol, 1.0 eq) and α,α-dimethoxytoluene (9.72 g, 63.8 mmol, 2.0 eq) were dissolved in DMF (50 mL). *p*-toluenesulfonic acid was added to the reaction mixture to reach pH 4 and was then kept at 50 mbar, 50 °C for 1 h. The reaction was neutralized with Et_3N to pH 7 and DMF was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1), and recrystallized from cyclohexane-ethyl acetate to give **20**, yield 72% (7.22 g, 23.1 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +75° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 312.80 ($[M+H]^+$, calc. 313.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.52 - 7.46 (m, 2H, benzylidene acetal), 7.40 - 7.33 (m, 3H, benzylidene acetal), 5.53 (s, 1H, C*H*Ph), 4.44 (d, *J*=9.8, 1H, H1), 4.34 (dd, *J*=10.5, 4.8, 1H, H6a), 3.84 - 3.78 (m, 1H, H4), 3.78 - 3.72 (m, 1H, H6b), 3.55 (t, *J*=9.2, 1H, H3), 3.51 - 3.44 (m, 2H, H2, H5), 2.80 - 2.69 (m, 2H, SCH_2CH_3), 1.32 (t, *J*=7.4, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 137.13, 129.53, 128.57, 126.52 (129.5 - 126.5 benzylidene acetal aromatic), 102.14 (*C*HPh), 86.80 (C1), 80.60 (C3), 74.78 (C4), 73.45 (C2), 70.77 (C5), 68.82 (C6), 27.14, 24.97 (SCH_2CH_3), 15.51 (SCH_2CH_3).

Ethyl 4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (21)



Compound **20** (17.5 g, 56 mmol, 1.0 eq) and dibutyltin oxide (16.7 g, 67 mmol, 1.2 eq) was dissolved in MeCN (500 mL) and refluxed for 24 h. The reaction mixture was then cooled and diluted with more MeCN (1000 mL), cesium fluoride (9.37 g, 62 mmol, 1.1 eq) and 4-methoxybenzyl bromide (13.6 g, 67 mmol, 1.2 eq) were added. The reaction mixture was stirred at r.t. for 48 h. The mixture was filtered through a layer of Celite, and MeCN was removed under reduced pressure, without heating. The residue was diluted with DCM (400 mL), washed with 1 M potassium fluoride (150 mL×3), H_2O (200 mL×3), brine (300 mL×1), dried over $MgSO_4$, and the organic solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane-ethyl acetate 6:1 to 1:1), and recrystallized from cyclohexane-ethyl acetate to give **21**, yield 42% (10.1 g, 23.4 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -31° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 432.80 ($[M+H]^+$, calc. 433.17), 449.87 ($[M+NH_4]^+$, calc. 450.20), 881.60 ($[2M+NH_4]^+$, calc. 882.36), 886.67 ($[2M+Na]^+$, calc. 887.31). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.42 (dd, *J*=7.2, 2.0, 2H, benzylidene acetal), 7.35 - 7.27 (m, 3H, benzylidene acetal), 7.22 (d, *J*=8.4, 2H, PMB), 6.78 (d, *J*=8.5, 2H, PMB), 5.49 (s, 1H, C*H*Ph), 4.82 (d, *J*=11.3, 1H, $PMBCH_2$), 4.66 (d, *J*=11.3, 1H, $PMBCH_2$), 4.37 (d, *J*=9.7, 1H, H1), 4.27 (dd, *J*=10.5, 4.9, 1H, H6a), 3.73 - 3.65 (m, 4H, H6b, OCH_3), 3.65 - 3.53 (m, 2H, H3, H4), 3.47 (t, *J*=8.9, 1H, H2), 3.40 (td, *J*=9.3, 4.9, 1H, H5), 2.74 - 2.60 (m, 2H, SCH_2CH_3), 1.23 (t, *J*=7.4, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.60 (PMB *C*OCH_3), 137.49 (benzylidene acetal aromatic), 130.61 (PMB), 129.97 (PMB), 129.22, 128.47, 126.25 (129.2 - 126.3 benzylidene acetal aromatic), 114.12 (PMB), 101.49 (*C*HPh), 86.78 (C1), 81.48 (C3), 81.41 (C4), 74.60 ($PMBCH_2$), 73.20 (C2), 71.02 (C5), 68.89 (C6), 55.49 (OCH_3), 24.76 (SCH_2CH_3), 15.47 (SCH_2CH_3).

Ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (22)

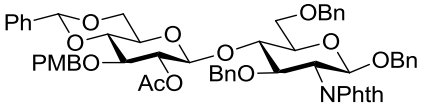


Compound **21** (10.1 g, 23.4 mmol) was dissolved pyridine / acetic anhydride 2:1 (90 mL) and stirred at r.t. for 4 h. The solvent was removed and coevaporated with toluene (100 mL×3) and the residue was purified by column chromatography (cyclohexane-ethyl acetate 6:1 to 1:1). Then recrystallized from cyclohexane-ethyl acetate to give **22**, yield 98% (10.9 g, 23.0 mmol). R_f = 0.6 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -24° (c = 1.2, $CHCl_3$). *ESI-MS (pos), m/z*: 491.80 ($[M+NH_4]^+$, calc. 492.21), 497.07

([M+Na]$^+$, calc. 497.16), 965.73 ([2M+NH_4]$^+$, calc. 966.38), 970.80 ([2M+Na]$^+$, calc. 971.33). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.47 - 7.39 (m, 2H, benzylidene acetal), 7.35 - 7.26 (m, 3H, benzylidene acetal), 7.13 (d, *J*=8.5, 2H, PMB), 6.77 (d, *J*=8.7, 2H, PMB), 5.50 (s, 1H, C*H*Ph), 5.01 - 4.92 (m, 1H, H2), 4.72 (d, *J*=11.6, 1H, PMBCH_2), 4.55 (d, *J*=11.6, 1H, PMBCH_2), 4.38 (d, *J*=10.1, 1H, H1), 4.30 (dd, *J*=10.5, 5.0, 1H, H6a), 3.74 - 3.69 (m, 4H, H6b, OCH_3), 3.69 - 3.63 (m, 2H, H3, H4), 3.46 - 3.36 (m, 1H, H5), 2.69 - 2.55 (m, 2H, SCH_2CH_3), 1.96 (s, 3H, Ac), 1.17 (t, *J*=7.5, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 169.63 (*C*O_2CH_3), 159.48 (PMB *C*OCH_3), 137.41 (benzylidene acetal aromatic), 130.47 (PMB), 129.76 (PMB), 129.24, 128.49, 126.22 (129.2 - 126.2 benzylidene acetal aromatic), 113.94 (PMB), 101.45 (*C*HPh), 84.44 (C1), 81.76 (C4), 79.40 (C3), 74.15 (PMBCH_2), 71.52 (C2), 70.92 (C5), 68.84 (C6), 55.49 (OCH_3), 24.12 (SCH_2CH_3), 21.17 (CO_2CH_3), 15.03 (SCH_2CH_3).

7.4 Synthesis of the Man-GlcNAc disaccharide core

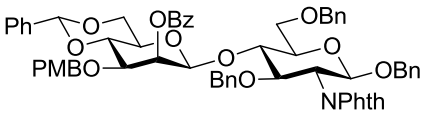
Benzyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (23)[148]



Compounds **22** (2.09 g, 4.40 mmol, 1.2 eq), **16** (2.13 g, 3.67 mmol, 1.0 eq), *N*-iodosuccinimide (1.63 g, 7.25 mmol, 2.0 eq) and 4 Å molecular sieves (5 g) were suspended in dry dichloromethane (50 mL) and were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (48.9 μL, 0.55 mmol, 0.15 eq). After stirring for 1 h at 0 °C, the reaction was quenched by dilution with more DCM (100 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (75 mL×3), brine (100 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (toluene-ethyl acetate 100:1 to 10:1) to give **23**. Yield: 70% (2.55 g, 2.57 mmol). R_f = 0.6 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +1° (c = 1.3, $CHCl_3$). *ESI-MS (pos), m/z*: 1009.00 ($[M+NH_4]^+$, calc. 1009.41). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.65 (s, 2H, Phth×2), 7.51 - 7.45 (m, 2H, benzylidene acetal aromatic×2), 7.44 - 7.29 (m, 10H, Phth×2, benzylidene acetal aromatic×3, benzyl aromatic×5), 7.23 - 7.17 (m, 2H, PMB aromatic×2), 7.12 - 7.03 (m, 5H, benzyl aromatic×5), 7.01 (dd, *J*=7.6, 1.7, 2H, benzyl aromatic×2), 6.93 - 6.82 (m, 5H, PMB aromatic×2, benzyl aromatic×3), 5.48 (s, 1H, C*H*Ph), 5.11 (d, *J*=8.3, 1H, H1), 5.02 - 4.93 (m, 1H, H2'), 4.83 - 4.77 (m, 3H, 1-BnCH_2, 6-BnCH_2, PMBCH_2), 4.75 (d, *J*=12.3, 1H, 3-BnCH_2), 4.62 (d, *J*=5.1, 1H, H1'), 4.59 (d, *J*=8.7, 1H, PMBCH_2), 4.51 (d, *J*=9.5, 1H, 6-BnCH_2), 4.48 (d, *J*=9.8, 1H, 1-BnCH_2), 4.42 (d, *J*=12.3, 1H, 3-BnCH_2), 4.26 (dd, *J*=10.6, 5.0, 1H, H6a'), 4.24 - 4.20 (m, 2H, H2, H3), 4.12 - 4.05 (m, 1H, H4), 3.81 (s, 3H, OCH_3), 3.80 - 3.75 (m, 2H, H6a, H6b), 3.65 (t, *J*=9.2, 1H, H4'), 3.56 (t, *J*=9.1, 1H, H3'), 3.53 - 3.48 (m, 1H, H5), 3.48 (t, *J*=10.3, 1H, H6b'), 3.22 (td, *J*=9.7, 4.9, 1H, H5'), 2.01 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 169.32, 167.91 (Phth *C*=O), 159.49, 138.74, 138.22, 137.54, 137.43 (138.7 - 137.4 aromatic *C*CH_2O), 133.82 (Phth), 132.56, 131.87, 130.63, 129.63, 129.60, 129.28, 129.25, 128.78, 128.50, 128.47, 128.37, 128.27, 128.18, 128.14, 128.04, 127.81, 127.79, 127.29 (131.9 - 127.3 aromatic), 126.28 (benzylidene acetal aromatic), 125.55, 123.42, 113.97 (PMB aromatic), 101.42 (*C*HPh), 101.01 (C1'), 97.64 (C1), 81.95 (C4'), 78.32 (C3'), 78.24 (C4), 76.86 (C3), 75.14 (C5), 74.76 (3-BnCH_2), 73.92

(6-BnCH_2), 73.64 (C2'), 71.03 (1-BnCH_2), 68.86 (C6'), 67.88 (C6), 66.21 (C5'), 55.94 (C2), 55.53 (OCH_3), 21.18 (Ac).

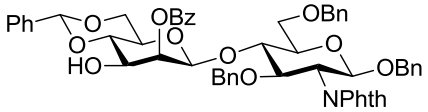
Benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (24) [148]



Compound **23** (15.6 g, 15.7 mmol) was dissolved in 0.05 M NaOMe (1.22 g, 22.5 mmol) in MeOH/DCM 2:1 (450 mL) and stirred at r.t. for 72 h. Then neutralized with Dowex 50WX8 ion-exchanger (2 g, prewashed with MeOH), concentrated and coevaporated with toluene (100 mL×3), dried under high vacuum to give the deacetylated intermediate with an equatorial hydroxyl group in the glucose 2-position, R_f = 0.6 (Tol/EtOAc 4:1). The residue was dissolved in anhydrous DCM (300 mL), treated with pyridine (27.2 mL, 337.6 mmol, 20 eq) at 0 °C before triflic anhydride (19.0 mL, 112.9 mmol, 7.2 eq) was added dropwise using a syringe. The reaction was finished in 15 min as a product with R_f = 0.8 (Tol/EtOAc 4:1) was formed, concentrated, dissolved again with DCM (300 mL) followed by washing with 0.1 M HCl (150 mL×2), Sat. $NaHCO_3$ (150 mL×2), H_2O (150 mL×2), brine (150 mL×1), dried over $MgSO_4$. The solvent was removed and the crude product was dried under high vacuum. The residue was dissolved in anhydrous toluene (300 mL) with tetrabutylammonium benzoate (12.3 g, 33.8 mmol 2.2 eq), refluxed for 2 h at 120 °C to form the axial 2-*O*-benzoyl β-mannose product with R_f = 0.7 (Tol/EtOAc 4:1). The reaction mixture was cooled to r.t. and then diluted with DCM (300 mL), washed with Sat. $NaHCO_3$ (200 mL×2), H_2O (200 mL×2), brine (300 mL×1), dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **24**, yield 84% (13.9 g, 13.1 mmol). R_f = 0.7 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = -28° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 1071.07 ($[M+NH_4]^+$, calc. 1071.43). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.10 (d, *J*=7.2, 2H, benzoyl aromatic×2), 7.62 (s, 2H, Phth×2), 7.53 - 7.45 (m, 4H, benzoyl aromatic×2, benzylidene acetal aromatic×2, benzyl aromatic×5), 7.44 - 7.31 (m, 11H, Phth×2, benzylidene acetal aromatic×3), 7.29 - 7.23 (m, 3H, PMB aromatic×2, benzoyl aromatic), 7.10 - 6.98 (m, 5H, benzyl aromatic×5), 6.85 - 6.80 (m, 2H, PMB aromatic×2), 6.79 - 6.70 (m, 5H, benzyl aromatic×5), 5.69 (d, *J*=3.4, 1H, H2'), 5.57 (s, 1H, C*H*Ph), 5.04 (d, *J*=8.5, 1H, H1), 4.83 - 4.70 (m, 4H, H1', 1-BnCH_2, 3-BnCH_2, 6-BnCH_2), 4.65 (d, *J*=11.9, 1H, PMBCH_2), 4.55 - 4.42 (m, 3H, 1-BnCH_2, 6-BnCH_2, PMBCH_2), 4.33 (dd, *J*=10.5, 4.8, 1H, H6a'), 4.28 (d, *J*=12.6, 1H,

3-BnCH$_2$), 4.20 - 4.13 (m, 2H, H2, H4), 4.06 (dd, *J*=10.6, 8.5, 1H, H3), 3.98 (t, *J*=9.6, 1H, H4'), 3.88 (dd, *J*=11.2, 2.9, 1H, H6a), 3.80 (dd, *J*=4.1, 3.0, 1H, H6b), 3.77 (s, 3H, OCH$_3$), 3.74 - 3.69 (m, 1H, H6b'), 3.57 (dd, *J*=9.8, 3.4, 1H, H3'), 3.53 (d, *J*=9.6, 1H, H5), 3.23 (td, *J*=9.7, 4.8, 1H, H5'). ***^{13}C-NMR*** (100.6 MHz, CDCl$_3$, gHSQC, gHMBC), *δ (ppm)*: 167.77 (Phth *C*=O), 165.98 (benzoyl Ph*C*O$_2$), 159.46 (PMB *C*OCH$_3$), 138.60 , 138.09, 137.70, 137.36 (138.6 - 137.4 aromatic *C*CH$_2$O), 133.68 (Phth), 133.34 (benzoyl), 131.82, 130.18, 130.12, 130.04, 129.41, 129.15, 128.84, 128.75, 128.62, 128.39, 128.32, 128.27, 128.25, 128.05, 127.88, 127.83, 127.74, 127.12 (131.8 - 127.1 aromatic), 126.36 (benzylidene acetal aromatic), 123.30, 114.00 (PMB aromatic), 101.77 (*C*HPh), 99.66 (C1'), 97.56 (C1), 79.30 (C4), 78.55 (C4'), 76.60 (C3), 75.76 (C3'), 74.70 (3-BnCH$_2$), 74.55 (C5), 73.87 (6-BnCH$_2$), 71.46 (3'-PMBCH$_2$), 70.99 (1-BnCH$_2$), 69.87 (C2'), 68.93 (C6'), 68.68 (C6), 67.27 (C5'), 55.89 (C2), 55.47 (OCH$_3$).

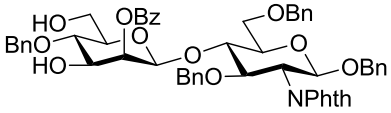
Benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (25)[148]



To a stirring solution of compound **24** (10.87 g, 10.3 mmol, 1.0 eq) in MeCN/H$_2$O 9:1 (200 mL), ceric ammonium nitrate (28.4 g, 51.8 mmol, 5.0 eq) was added. The reaction was stirred at r.t. for 30 min and then quenched with Sat. NaHCO$_3$ until the orange color was removed. The mixture was filtered through a layer of Celite, washed with EtOAc (40 mL ×2), H$_2$O (40 mL ×2) then MeOH (40 mL ×2) and DCM (40 mL ×2). The filtrate was combined and concentrated to remove most of the organic solvent, and the residue was extracted with DCM (20 mL ×3). The organic layer was then washed with brine (30 mL), concentrated to dryness. The crude product was acetylated with pyridine/acetic anhydride 2:1 (100 mL) for 24 h, concentrated and coevaporated with toluene (150 mL ×3), and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **25**. Yield 77% (7.38 g, 7.90 mmol). R$_f$ = 0.5 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = -14° (c = 1.0, CHCl$_3$). *ESI-MS (pos), m/z*: 933.80 ([M+H]$^+$, calc. 934.34), 950.87 ([M+NH$_4$]$^+$, calc. 951.37), 1884.73 ([2M+NH$_4$]$^+$, calc. 1885.71). ***^{1}H-NMR*** (400 MHz, CDCl$_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.10 (d, J=8.4, 2H, benzoyl aromatic×2), 7.62 (s, 2H, Phth×2), 7.55 - 7.34 (m, 15H, Phth×2, benzylidene acetal aromatic×5, benzyl aromatic×5, benzoyl aromatic×3), 7.10 - 6.96 (m, 5H, benzyl aromatic×5), 6.80 - 6.67 (m, 5H, benzyl aromatic×5), 5.54 (s, 2H, H2', C*H*Ph), 5.05 (d, J=8.5, 1H, H1), 4.89 - 4.80 (m, 2H, H1' , 6-BnCH$_2$), 4.76 (dd, J=14.5, 12.5, 2H, 1-BnCH$_2$, 3-BnCH$_2$), 4.53 (d, J=12.0, 1H, 6-BnCH$_2$), 4.46 (d, J=12.3, 1H, 1-BnCH$_2$), 4.33 (dd, J=10.5, 4.9,

1H, H6a'), 4.28 (d, J=12.5, 1H, 3-BnCH$_2$), 4.23 - 4.12 (m, 2H, H2, H4), 4.06 (dd, J=10.6, 8.6, 1H, H3), 3.91 (dd, J=11.3, 2.9, 1H, H6a), 3.89 - 3.83 (m, 1H, H4'), 3.83 - 3.74 (m, 2H, H6b, H3'), 3.70 (t, J=10.3, 1H, H6b'), 3.52 (d, J=9.8, 1H, H5), 3.26 (td, J=9.5, 5.0, 1H, H5'). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 167.78 (Phth *C*=O), 166.21 (benzoyl Ph*C*O$_2$), 138.61, 138.13, 137.35, 137.32 (138.6 - 137.3 aromatic *C*CH$_2$O), 133.70 (Phth), 133.49 (benzoyl), 131.81, 130.16, 129.90, 129.51, 129.26, 128.89, 128.70, 128.57, 128.45, 128.37, 128.33, 128.30, 128.23, 128.04, 127.93, 127.85, 127.81, 127.76, 127.17 (131.8 - 127.2 aromatic), 126.51 (benzylidene acetal aromatic), 123.33, 102.37 (*C*HPh), 99.67 (C1'), 97.56 (C1), 79.61 (C4), 79.25 (C4'), 76.60 (C3), 74.72 (3-BnCH$_2$), 74.59 (C5), 73.93 (6-BnCH$_2$), 72.01 (C2'), 71.00 (1-BnCH$_2$), 70.19 (C3'), 68.88 (C6'), 68.59 (C6), 66.95 (C5'), 55.90 (C2).

Benzyl 2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (26)[148]

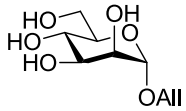


To a stirring solution of **25** (3.48 g, 3.72 mmol, 1.0 eq) in anhydrous DCM (37 mL, 10 mL/mmol) at 0 °C, a 1 M solution of BH_3 in THF (18 mL, 5 mL/mmol) was added slowly over 30 min, followed by addition of TMSOTf (101 μL, 0.56 mmol, 0.15 eq). The mixture was stirred at r.t. for 16 h and neutralized with Et_3N (0.2 mL), then MeOH (5 mL) was added until no more hydrogen gas was eluted. The organic solvent was evaporated under reduced pressure without heating, and the residue was dissolved in DCM (100 mL), washed with $NaHCO_3$ (50 mL ×2), brine (50 mL ×2), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **26**. Yield: 66% (2.31 g, 2.46 mmol). R_f = 0.6 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = -4° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 953.07 ($[M+NH_4]^+$, calc. 953.39), 1871.53 ($[2M+H]^+$, calc. 1872.71), 1888.73 ($[2M+NH_4]^+$, calc. 1889.74). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.10 - 8.03 (m, 2H, benzoyl aromatic×2), 7.60 (d, J=19.5, 2H, Phth×2), 7.54 - 7.25 (m, 15H, Phth×2, benzyl aromatic×10, benzoyl aromatic×3), 7.11 - 6.98 (m, 5H, benzyl aromatic×5), 6.85 - 6.67 (m, 5H, benzyl aromatic×5), 5.45 (d, J=2.3, 1H, H2'), 5.05 (d, J=8.4, 1H, H1), 4.84 - 4.72 (m, 5H, H1, 4'-BnCH$_2$, 3-BnCH$_2$, 6-BnCH$_2$, 1-BnCH$_2$), 4.66 (d, J=11.3, 1H, 4'-BnCH$_2$), 4.53 (d, J=12.1, 1H, 6-BnCH$_2$), 4.46 (d, J=12.3, 1H, 1-BnCH$_2$), 4.28 (d, J=12.5, 1H, 3-BnCH$_2$), 4.23 - 4.04 (m, 3H, H2, H4, H3), 3.88 - 3.82 (m, 2H, H6a, H6a'), 3.80 - 3.74 (m, 1H, H6b), 3.75 - 3.57 (m, 3H, H3', H4', H6b'), 3.54 (d, J=9.0, 1H, H5), 3.30 - 3.21 (m, 1H, H5'). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 167.82 (Phth C=O), 166.37 (benzoyl

Ph*C*O_2), 138.41, 138.27, 138.04, 137.37 (138.4 - 137.4 aromatic *C*CH_2O), 133.77 (Phth), 133.49 (benzoyl), 131.79, 130.13, 129.94, 128.86, 128.76, 128.71, 128.56, 128.34, 128.20, 128.09, 127.84, 127.76, 127.59, 127.28 (131.8 - 127.3 aromatic), 123.35, 98.73 (C1'), 97.55 (C1), 78.93 (C4), 76.63 (C3), 75.78 (C4', C5'), 75.07 (4'-BnCH_2), 74.63 (C5), 74.51 (3-BnCH_2), 73.92 (6-BnCH_2), 73.36 (C3'), 72.39 (C2'), 70.98 (1-BnCH_2), 68.51 (C6), 62.26 (C6'), 55.86 (C2).

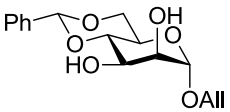
7.5 Synthesis of a mannosyl acceptor building block

Allyl α-D-mannopyranoside (27)[176, 281]



D-mannose (45.0 g, 250 mmol, 1.0 eq) was dissolved in allyl alcohol (300 mL) and BF_3 etherate (4.6 mL, 50 mmol, 0.2 eq) was then added. The mixture was refluxed at 120 °C. After 10 min the mannose was dissolved and the reaction was kept refluxing for another 30 min to full conversion, cooled and quenched by addition of Et_3N (10 mL). The solvent was removed and the residue was purified by column chromatography (DCM-MeOH 100:1 to 4:1) to give **27**, yield 96% (53.1 g, 241 mmol). R_f = 0.6 (EtOAc/MeOH/AcOH/H_2O 20:3:3:2). $[\alpha]_D^{20}$ = +83° (c = 2.0, H_2O). ***¹H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.87 (dtd, *J*=15.9, 10.7, 5.6, 1H, CH_2CH=CH_2), 5.27 (dd, *J*=17.2, 1.5, 1H, CH_2CH=CH_2), 5.18 (dd, *J*=10.5, 1.2, 1H, CH_2CH=CH_2), 4.86 (s, 1H, H1), 4.19 - 4.07 (m, 1H, CH_2CH=CH_2), 4.03 - 3.88 (m, 4H, CH_2CH=CH_2, H6a, H2, H4), 3.85 (dd, *J*=9.7, 2.9, 1H, H3), 3.77 (d, *J*=11.3, 1H, H6b), 3.53 (d, *J*=9.0, 1H, H5). ***¹³C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 133.86 (CH_2*C*H=CH_2), 117.75 (CH_2CH=*C*H_2), 99.51 (C1), 72.58 (C5), 71.78 (C3), 71.17 (C2), 68.34 (*C*H_2CH=CH_2), 66.47 (C4), 61.19 (C6).

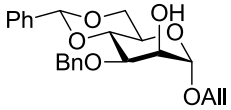
Allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (28)[176, 281]



The compound **27** (31.6 g, 143 mmol, 1.0 eq) and α,α-dimethoxytoluene (24.0 g, 158 mmol, 1.1 eq) were dissolved in DMF (150 mL). Then *p*-toluenesulfonic acid was added to the reaction mixture to reach pH 4 and was kept at 50 mbar, 50 °C for 1 h. The reaction was neutralized with Et_3N to pH 7 and DMF was removed under reduced pressure. The crude product was purified by column chromatography (toluene-ethyl acetate 6:1 to 2:1) to give **28**, yield 65% (28.7 g, 93 mmol). R_f = 0.3 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +53° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 308.87 ($[M+H]^+$, calc. 309.13), 638.93 ($[2M+Na]^+$, calc. 639.24). ***¹H-NMR*** (400 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.54 - 7.46 (m, 2H, aromatic), 7.40 - 7.29 (m, 3H, aromatic), 6.05 - 5.89 (m, 1H, CH_2CH=CH_2), 5.59 (s, 1H, C*H*Ph), 5.32 (dddd, *J*=17.2, 8.9, 3.4, 1.7, 1H, CH_2CH=CH_2), 5.24 - 5.15 (m, 1H, CH_2CH=CH_2), 4.82 (s, 1H, H1), 4.25 - 4.13 (m, 2H, CH_2CH=CH_2, H6a), 4.05 - 3.98 (m, 1H, CH_2CH=CH_2), 3.95 - 3.91 (m, 2H, H3, H4), 3.90 (s, 1H, H2), 3.87 - 3.71 (m, 2H, H5, H6b). ***¹³C-NMR*** (100.6 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 138.12, 134 (CH_2*C*H=CH_2).15, 128.69, 127.84, 126.33 (128.7 - 126.3

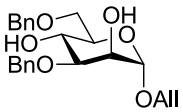
aromatic), 116.37 ($CH_2CH=\mathit{C}H_2$), 102.19 (*C*HPh), 100.61 (C1), 79.01 (C4), 71.48 (C2), 68.64 (C3), 68.41 (C6), 67.98 ($\mathit{C}H_2CH=CH_2$), 64.16 (C5).

Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (29)[176, 282]



Compound **28** (11.6 g, 37.6 mmol, 1.0 eq) and dibutyltin oxide (9.37 g, 37.6 mmol, 1.0 eq) were dissolved in toluene (200 mL) and refluxed for 24 h using a Dean-Stark apparatus. The reaction mixture was then cooled to r.t. followed by addition of tetra-*n*-butylammonium bromide (13.3 g, 41.4 mmol, 1.1 eq), cesium fluoride (5.86 g, 38.5 mmol, 1.025 eq) and benzyl bromide (4.72 mL, 39.5 mmol, 1.05 eq). The mixture was stirred at r.t. for 24 h, then heated to 80 °C for 24 h and refluxed at 120 °C for 1 h. The mixture was filtered through a layer of Celite and diluted with EtOAc (400 mL), washed with 1 M potassium fluoride (200 mL×3), H_2O (200 mL×3), brine (300 mL×1), dried over $MgSO_4$ and concentrated. Then purified by column chromatography (cyclohexane-ethyl acetate 8:1 to 1:1) to give **29**, yield 90% (13.5 g, 33.8 mmol). R_f = 0.6 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +46° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 398.87 ($[M+H]^+$, calc. 399.18), 415.93 ($[M+NH_4]^+$, calc. 416.21), 421.13 ($[M+Na]^+$, calc. 421.16). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.46 - 7.39 (m, 2H, benzylidene acetal aromatic), 7.35 - 7.19 (m, 7H, aromatic), 5.82 (dddd, *J*=17.2, 10.4, 6.2, 5.3, 1H, $CH_2C\mathit{H}=CH_2$), 5.54 (s, 1H, C*H*Ph), 5.21 (dq, *J*=17.2, 1.6, 1H, $CH_2CH=C\mathit{H}_2$), 5.14 (ddd, *J*=10.4, 2.8, 1.2, 1H, $CH_2CH=C\mathit{H}_2$), 4.84 (d, *J*=1.4, 1H, H1), 4.79 (d, *J*=11.9, 1H, $BnCH_2$), 4.64 (d, *J*=11.9, 1H, $BnCH_2$), 4.22 - 4.18 (m, 1H, H6a), 4.11 (ddt, *J*=12.9, 5.2, 1.5, 1H, $C\mathit{H}_2CH=CH_2$), 4.07 - 4.01 (m, 1H, H4), 4.00 (dd, *J*=3.5, 1.5, 1H, H2), 3.95 - 3.89 (m, 1H, $C\mathit{H}_2CH=CH_2$), 3.87 (dd, *J*=9.6, 3.5, 1H, H3), 3.80 - 3.76 (m, 2H, H5, H6b). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.26, 133.72 ($CH_2\mathit{C}H=CH_2$), 129.13, 128.69, 128.43, 128.12, 128.03, 126.27 (129.1 - 126.3 aromatic), 118.06 ($CH_2CH=\mathit{C}H_2$), 101.81 (*C*HPh), 99.40 (C1), 79.14 (C4), 75.93 (C3), 73.31 ($BnCH_2$), 70.26 (C2), 69.09 (C6), 68.41 ($\mathit{C}H_2CH=CH_2$), 63.62 (C5).

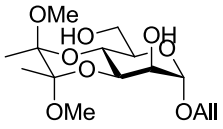
Allyl 3,6-di-*O*-benzyl-α-D-mannopyranoside (30)[283]



The compound **27** (16.1 g, 73.1 mmol, 1.0 eq) and tributyltin oxide (61.0 g, 102.3 mmol, 1.4 eq) was dissolved in toluene (250 mL) and refluxed for 24 h with a Dean-Stark apparatus. The reaction mixture was then cooled to r.t., benzyl bromide (19.1 mL, 160.8 mmol, 2.2 eq) was added and the mixture was stirred at r.t. for 72 h. Then the mixture

was shaken with 1 M potassium fluoride solution (120 mL) intensively, the precipitated salt was filtered off and the filtrate was separated. The organic layer was washed with H_2O (100 mL×3), brine (150 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **30**, yield 55% (16.1 g, 40.2 mmol). R_f = 0.3 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +31° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 418.00 ($[M+NH_4]^+$, calc. 418.22), 800.67 ($[2M+H]^+$, calc. 801.39), 817.73 ($[2M+NH_4]^+$, calc. 818.41), 822.80 ($[2M+Na]^+$, calc. 823.37). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.44 - 7.24 (m, 10H, aromatic), 5.90 (ddd, *J*=22.3, 10.8, 5.6, 1H, CH_2CH=CH_2), 5.28 (dd, *J*=17.2, 1.6, 1H, CH_2CH=CH_2), 5.20 (dd, *J*=10.4, 1.5, 1H, CH_2CH=CH_2), 4.92 (s, 1H, H1), 4.74 - 4.64 (m, 2H, $BnCH_2$), 4.63 - 4.55 (m, 2H, $BnCH_2$), 4.23 - 4.16 (m, 1H, CH_2CH=CH_2), 4.04 - 4.01 (m, 1H, H2), 4.01 - 3.95 (m, 1H, CH_2CH=CH_2), 3.92 (d, *J*=9.1, 1H, H4), 3.82 - 3.78 (m, 1H, H5), 3.76 (s, 2H, H6a, H6b), 3.72 (dd, *J*=9.2, 3.3, 1H, H3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.28, 138.17, 133.96 (CH_2*C*H=CH_2), 128.83, 128.61, 128.26, 128.15, 127.89 (128.8 - 127.9 aromatic), 117.72 (CH_2CH=*C*H_2), 98.87 (C1), 79.81 (C3), 73.83 ($BnCH_2$), 72.19 ($BnCH_2$), 70.92 (C5), 70.57 (C6), 68.24 (*C*H_2CH=CH_2), 68.13 (C2), 68.11 (C4).

Allyl 3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D- mannopyranoside (31)[182]

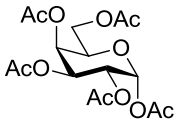


To a stirring solution of **27** (14.21 g, 64.5 mmol), trimethyl orthoformate (25.8 mL, 0.4 mL/mmol) and camphorsulfonic acid (1.10 g, 17 mg/mmol) in MeOH (250 mL) was added. Followed by addition of butanedione (6.45 mL, 0.1 mL/mmol). The mixture was refluxed for 24 h at 90 °C, then the reaction was cooled to r.t. and neutralized by addition of Et_3N (1.3 mL). Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 2:1 to 1:4) to give **31**, yield 65% (14.0 g, 41.9 mmol). R_f = 0.4 (Tol/EtOAc 1:3). $[\alpha]_D^{20}$ = +219° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 351.93 ($[M+NH_4]^+$, calc. 352.20), 357.13 ($[M+Na]^+$, calc. 357.15), 685.80 ($[2M+NH_4]^+$, calc. 686.36), 690.93 ($[2M+Na]^+$, calc. 691.32). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.97 - 5.78 (m, 1H, CH_2CH=CH_2), 5.24 (dq, *J*=17.2, 1.5, 1H, CH_2CH=CH_2), 5.16 (dd, *J*=10.4, 1.3, 1H, CH_2CH=CH_2), 4.86 (d, *J*=1.3, 1H, H1), 4.18 - 4.05 (m, 2H, CH_2CH=CH_2), 4.01 (dd, *J*=10.3, 3.0, 1H, H4), 3.95 (ddd, *J*=12.9, 6.2, 1.2, 1H, CH_2CH=CH_2), 3.92 (dd, *J*=2.9, 1.5, 1H, H2), 3.76 (dd, *J*=9.0, 3.5, 3H, H3, H6a, H6b), 3.24 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 1.28 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 133.89 (CH_2*C*H=CH_2), 117.81 (CH_2CH=*C*H_2), 100.51 (*C*$OCH_3(CH_3)$), 100.01 (*C*$OCH_3(CH_3)$), 99.52 (C1), 70.97 (C3),

69.87 (C2), 68.36 (C4), 68.33 (*C*H_2CH=CH_2), 63.15 (C5), 61.40 (C6), 48.26 (OCH_3), 48.05 (OCH_3), 17.97 (CH_3), 17.87 (CH_3).

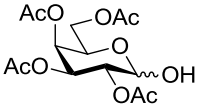
7.6 Synthesis of Gal-GlcNAc donors

1,2,3,4,6-penta-*O*-acetyl-α-D-glactopyranose (32)[284]



Acetic anhydride (330 mL, 3.5 mol, 7.0 eq) with $HClO_4$ (2.5 mL, 4.2 mmol, 0.08 eq) was stirred at r.t. in a three-necked round bottom flask with a thermometer inside the solution. D-galactose (90 g, 0.5 mol, 1.0 eq) was then added in portions, since the reaction temperature raised during addition, an ice bath was applied to keep the reaction at 25 - 30 °C. The reaction was instant, and was stirred for another 15 min after addition of the final portion of galactose. Then the mixture was concentrated and the residue diluted with EtOAc (1 L), washed with Sat. $NaHCO_3$ (300 mL×3), H_2O (300 mL×3), brine (500 mL×1) and dried over $MgSO_4$. The organic solvent was removed under reduced pressure, the crude product was recrystallized from Et_2O to give **32**, yield 80% (157 g, 402 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +101° (c = 1.1, $CHCl_3$). *ESI-MS (pos), m/z*: 407.87 ($[M+NH_4]^+$, calc. 408.15), 413.00 ($[M+Na]^+$, calc. 413.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 6.34 (d, *J*=1.9, 1H, H1), 5.49 - 5.45 (m, 1H, H4), 5.32 - 5.29 (m, 2H, H3, H2), 4.31 (t, *J*=6.7, 1H, H5), 4.12 - 4.01 (m, 2H, H6a, H6b), 2.13 (s, 3H), 2.12 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H, 2.13 - 1.97 Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.50, 170.29, 170.26, 170.02, 169.07 (170.5 - 169.1 CO_2CH_3), 89.90 (C1), 68.95 (C5), 67.62 (C4), 67.55 (C3), 66.64 (C2), 61.42 (C6), 21.05, 20.82, 20.80, 20.77, 20.70 (21.0 - 20.7 CO_2CH_3).

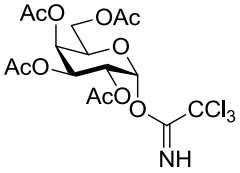
2,3,4,6-tetra-*O*-acetyl-α/β-D-glactopyranose (33)[285]



Compound **32** (43.97 g, 113 mmol, 1.0 eq) was dissolved in dry DMF (100 mL) and hydrazine acetate (10.70 g, 116 mmol, 1.03 eq) was added. The reaction was finished after heating at 50 °C for 3 h, concentrated and taken up in EtOAc (500 mL), washed with Sat. $NaHCO_3$ (300 mL×1), H_2O (200 mL×3), brine (300 mL×1) and dried over $MgSO_4$. The organic solvent was removed under reduced pressure to give **33**, as a 2:1 α:β mixture, yield 88% (33.57 g, 96 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +77° (c = 1.1, $CHCl_3$). *ESI-MS (pos), m/z*: 365.87 ($[M+NH_4]^+$, calc. 366.14), 371.00 ($[M+Na]^+$, calc. 371.10), 715.13 ($[2M+NH_4]^+$, calc. 714.25). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.50 (d, *J*=3.3, 1H, H1α), 5.46 (d, *J*=3.3, 1H, H2α), 5.41 (d, *J*=3.4, 1H, H3β), 5.39 (d, *J*=3.3, 1H, H3α), 5.14 (dd, *J*=10.8, 3.6, 1H, H4), 5.08 - 5.06 (m, 1H, H4β), 5.06 (d, J=1.1, 1H, H2β), 4.69 (d, *J*=5.7, 1H, H1β), 4.46 (t, *J*=6.6, 1H, H5α), 4.14 (d, *J*=6.6, 2H, H6β), 4.12 - 4.03 (m, 2H, H6α), 3.95 (t, *J*=6.7,

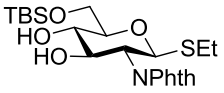
1H, H5β), 2.17 - 1.94 (Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.36, 170.78, 170.74, 170.61, 170.46, 170.39, 170.29, 170.24 (171.4 - 170.2 CO_2CH_3), 96.20 (C1β), 90.88 (C1α), 71.26 (C5β), 71.23 (C2β), 70.58 (C4β), 68.55 (C4α), 68.42 (C2α), 67.46 (C3α), 67.36 (C3β), 66.44 (C5α), 62.02 (C6α), 61.68 (C6β), 21.02, 20.92, 20.90, 20.86, 20.84, 20.81, 20.76 (21.0 - 20.8 CO_2CH_3).

2,3,4,6-tetra-*O*-acetyl-α-D-glactopyranosyl trichloroacetimidate (34)[286]



DBU (2.90 mL, 19.4 mmol, 0.2 eq) was added to a solution of **33** (33.57 g, 96 mmol, 1.0 eq) and trichloroacetonitrile (29.16 mL, 292 mmol, 3.0 eq) in anhydrous DCM (380 mL) and under Ar atmosphere. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:1) and recrystallized from cyclohexane-ethyl acetate to give **34**, yield 72% (34.5 g, 70 mmol). R_f = 0.6 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +115° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 513.80 ($[M+Na]^+$, calc. 514.01). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.66 (s, 1H, N*H*), 6.60 (d, *J*=3.3, 1H, H1), 5.58 - 5.52 (m, 1H, H4), 5.42 (dd, *J*=10.8, 3.0, 1H, H3), 5.36 (dd, *J*=10.8, 3.5, 1H, H2), 4.43 (t, *J*=6.6, 1H, H5), 4.16 (dd, *J*=11.3, 6.6, 1H, H6a), 4.08 (dd, *J*=11.3, 6.6, 1H, H6b), 2.16 (s, 3H, Ac), 2.03 - 2.00 (m, 9H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.47, 170.28, 170.26, 170.15 (170.5 - 170.2 CO_2CH_3), 161.16 (*C*=NH), 93.77 (C1), 69.22 (C5), 67.73 (C3), 67.60 (C4), 67.13 (C2), 61.47 (C6), 20.86, 20.83, 20.81, 20.75 (20.9 - 20.8 CO_2CH_3).

Ethyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (35)[287]



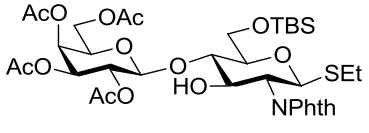
Compound **8** (16.8 g, 35.0 mmol, 1.0 eq) was dissolved in 0.08 M NaOMe in MeOH (150 mL), stirred at r.t. for 4 h and neutralized with *Dowex* 50WX8 ion-exchanger (1 g, prewashed with MeOH). Then concentrated and coevaporated with toluene (50 mL×3) under reduced pressure to give **10**. The crude product was dissolved in dry DMF (170 mL) and TBSCl (5.8 g, 38.5 mmol, 1.1 eq) and imidazole (5.48 g, 80.5 mmol, 2.3 eq) was added. The reaction mixture was stirred at r.t. for 2 h. The solvent was removed and the residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **35**. Yield: 86% (14.4 g, 30.1 mmol). R_f = 0.5 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = -15° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 484.87 ($[M+NH_4]^+$, calc. 485.21), 951.67 ($[2M+NH_4]^+$, calc. 952.39). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY,

gHSQC, gHMBC), *δ* (ppm): 7.83 (s, 2H, Phth), 7.74 - 7.67 (m, 2H, Phth), 5.31 (d, *J*=10.4, 1H, H1), 4.39 (dd, *J*=10.3, 8.5, 1H, H3), 4.19 (t, *J*=10.4, 1H, H2), 3.99 (dd, *J*=10.4, 4.7, 1H, H6a), 3.85 (dd, *J*=10.4, 6.3, 1H, H6b), 3.62 (t, *J*=8.9, 1H, H4), 3.58 - 3.52 (m, 1H, H5), 2.74 - 2.53 (m, 2H, SCH_2CH_3), 1.17 (t, *J*=7.4, 3H, SCH_2CH_3), 0.90 (s, 9H, *t*Bu), 0.11 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 134.23 (Phth), 123.67 (Phth), 81.10 (C1), 77.75 (C5), 75.20 (C4), 72.60 (C3), 65.20 (C6), 55.25 (C2), 25.95 (C(CH_3)$_3$), 23.98 (SCH_2CH_3), 18.32, 15.04 (SCH_2CH_3), -5.36 ($SiCH_3$).

Phenyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (36)[288]

Compound **9** (5.0 g, 9.48 mmol, 1.0 eq) was dissolved in 0.08 M NaOMe in MeOH (50 mL) and was stirred at r.t. for 4 h. Then neutralized with *Dowex* 50WX8 ion-exchanger (0.5 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL×3) to give a deacetylated intermediate. The crude product was dissolved in dry DMF (170 mL) and TBSCl (1.57 g, 10.4 mmol, 1.1 eq) and imidazole (1.48 g, 21.8 mmol, 2.3 eq) was added. The reaction mixture was stirred at r.t. for 2 h. The solvent was removed and the residue purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **36**. Yield: 82% (4.02 g, 7.80 mmol). R_f = 0.4 (Tol/EtOAc 1:1). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.72 (s, 2H, Phth), 7.65 - 7.58 (m, 2H, Phth), 7.30 - 7.23 (m, 2H, SPh), 7.16 - 7.09 (m, 3H, SPh), 5.50 (d, *J*=10.4, 1H, H1), 4.26 (dd, *J*=10.4, 8.2, 1H, H3), 4.08 (t, *J*=10.4, 1H, H2), 3.87 (dd, *J*=10.5, 4.7, 1H, H6a), 3.78 (dd, *J*=10.5, 5.4, 1H, H6b), 3.56 - 3.40 (m, 2H, H4, H5), 0.81 (s, 9H, *t*Bu), 0.02 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 134.28 (Phth), 132.65 (Ph), 132.37 (Ph), 131.85 (Ph), 128.99 (Ph), 127.87 (Ph), 123.84 (Phth), 83.78 (C1), 78.65 (C5), 74.10 (C4), 72.87 (C3), 64.62 (C6), 55.48 (C2), 26.04 (C(CH_3)$_3$), 18.41, -5.25 ($SiCH_3$).

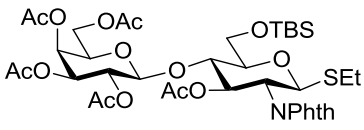
Ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (37)[289]



The trichloroacetimidate donor **34** (7.34 g, 14.9 mmol, 1.1 eq) and acceptor **35** (6.34 g, 13.6 mmol, 1.0 eq) was dissolved in dry DCM (60 mL) and 4 Å molecular sieves (12 g) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to -70 °C, then trimethylsilyl

-trifluoromethanesulfonate (TMSOTf) (369 μL, 2.02 mmol, 0.15 eq) was added. The reaction was kept stirring at -70 to -60 °C for 1 h, then quenched with triethylamine (0.7 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **37**. Yield 70% (7.60 g, 9.52 mmol). R_f = 0.7 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +18° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 814.87 ($[M+NH_4]^+$, calc. 815.31), 1594.40 ($[2M+H]^+$, calc. 1595.56), 1611.67 ($[2M+NH_4]^+$, calc. 1612.58). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.87 - 7.79 (m, 2H, Phth), 7.70 (dd, *J*=5.4, 3.1, 2H, Phth), 5.35 (d, *J*=3.4, 1H, H4'), 5.29 (d, *J*=10.5, 1H, H1), 5.22 (dd, *J*=10.4, 8.1, 1H, H2'), 4.96 (dd, *J*=10.4, 3.4, 1H, H3'), 4.61 (d, *J*=8.0, 1H, H1'), 4.43 (dd, *J*=10.0, 8.6, 1H, H3), 4.16 (t, *J*=10.4, 1H, H2), 4.06 (d, *J*=6.6, 2H, H6a', H6b'), 3.96 (t, *J*=6.6, 1H, H5'), 3.87 (d, *J*=11.4, 1H, H6a), 3.75 (dd, *J*=11.5, 3.5, 1H, H6b), 3.66 (t, *J*=9.0, 1H, H4), 3.51 (dd, *J*=9.6, 1.9, 1H, H5), 2.73 - 2.54 (m, 2H, SCH_2CH_3), 2.11 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.17 (t, *J*=7.4, 3H, SCH_2CH_3), 0.92 (s, 9H, *t*Bu), 0.12 (s, 3H, $Si(CH_3)_2$), 0.09 (s, 3H, $Si(CH_3)_2$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.49, 170.17, 170.08, 169.28, 168.20, 167.82, 134.14, 131.97, 131.81, 123.70, 123.33 (134.1 - 123.3 Phth), 101.80 (C1'), 81.82 (C4), 80.51 (C1), 78.84 (C5), 71.30 (C5'), 71.03 (C3'), 70.69 (C3), 68.86 (C2'), 66.90 (C4'), 61.79 (C6), 61.53 (C6'), 55.31 (C2), 26.00 ($C(CH_3)_3$), 23.44 (SCH_2CH_3), 20.81 (Ac), 20.67 (Ac), 20.61 (Ac), 20.40 (Ac), 18.40, 15.02 (SCH_2CH_3), -4.80 ($Si(CH_3)_2$), -5.05 ($Si(CH_3)_2$).

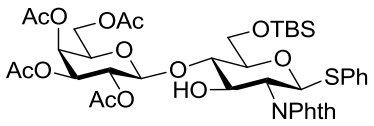
Ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (38)[289]



The compound **37** (3.73 g, 4.67 mmol) was dissolved in pyridine / acetic anhydride 2:1 (36 mL) and stirred at r.t. for 24 h. Then the reaction mixture was concentrated and coevaporated with toluene (50 ml×3). The residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) and gave **38** in 97% yield (3.36 g, 4.00 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +18° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 839.47 ($[M+H]^+$, calc. 840.29), 856.87 ($[M+NH_4]^+$, calc. 857.32), 1695.47 ($[2M+NH_4]^+$, calc. 1696.61). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.86 - 7.80 (m, 2H, Phth), 7.73 - 7.68 (m, 2H, Phth), 5.72 (t, *J*=9.7, 1H, H3), 5.40 (d, *J*=10.5, 1H, H1), 5.32 (d, *J*=3.5, 1H, H4'), 5.07 (dd, *J*=10.4, 8.0, 1H, H2'), 4.92 (dd, *J*=10.4, 3.5, 1H, H3'), 4.71 (d, *J*=8.0, 1H, H1'), 4.23 (t, *J*=10.4, 1H, H2'), 4.07 (dd, *J*=6.8, 3.1, 2H, H6a', H6b'),

4.00 - 3.90 (m, 2H, H4, H6a), 3.86 (dd, *J*=11.6, 2.8, 1H, H6b), 3.81 (t, *J*=6.7, 1H, H5'), 3.52 (d, *J*=9.8, 1H, H5), 2.72 - 2.52 (m, 2H, SCH_2CH_3), 2.10 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.87 (s, 3H, Ac), 1.18 (t, *J*=7.4, 3H, SCH_2CH_3), 0.93 (s, 9H, *t*Bu), 0.13 (s, 3H, $Si(CH_3)_2$), 0.11 (s, 3H, $Si(CH_3)_2$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.42, 170.29, 170.21, 170.11, 168.93, 167.80, 167.58, 134.42, 134.18, 131.84, 131.37, 123.77, 123.66 (134.2 - 123.7 Phth), 100.43 (C1'), 80.39 (C1), 79.58 (C5), 74.97 (C4), 71.84 (C3), 71.30 (C3'), 70.71 (C5'), 69.34 (C2'), 66.99 (C4'), 61.26 (C6), 61.13 (C6'), 54.12 (C2), 26.00 ($C(CH_3)_3$), 23.65 (SCH_2CH_3), 20.84 (Ac), 20.73 (Ac), 20.71 (Ac), 20.65 (Ac), 18.39, 15.10 (SCH_2CH_3), -4.85 ($Si(CH_3)_2$), -5.11 ($Si(CH_3)_2$).

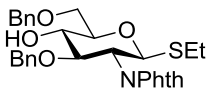
Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethyl silyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (39)



The trichloroacetimidate donor **34** (2.26 g, 4.74 mmol, 1.1 eq) and acceptor **36** (2.23 g, 4.32 mmol, 1.0 eq) was dissolved in dry DCM (22 mL) and 4 Å molecular sieves (2.2 g) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to -50 °C and then trimethylsilyl trifluoromethanesulfonate (TMSOTf) (157 μL, 0.86 mmol, 0.2 eq) was added. The reaction was kept stirring at -45 to -50 °C for 2 h, then quenched with triethylamine (0.3 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and the residue purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give the disaccharide product, Yield 71% (2.59 g, 3.06 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). The disaccharide (3.89 g, 4.60 mmol) was then dissolved in pyridine / acetic anhydride 2:1 (24 mL) and stirred at r.t. for 24 h. The reaction mixture was then concentrated, coevaporated with toluene (50 ml×3), followed by purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **39** in 93% yield (3.78 g, 4.26 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +23° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 887.27 ($[M+H]^+$, calc. 887.29), 904.93 ($[M+NH_4]^+$, calc. 905.32), 1791.47 ($[2M+NH_4]^+$, calc. 1792.61). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.71 (tt, *J*=7.9, 4.0, 2H, Phth), 7.64 - 7.56 (m, 2H, Phth), 7.29 - 7.24 (m, 2H, SPh), 7.16 - 7.07 (m, 3H, SPh), 5.60 - 5.53 (m, 2H, H3, H1), 5.20 (dd, *J*=3.4, 0.8, 1H, H4'), 4.95 (dd, *J*=10.4, 8.0, 1H, H2'), 4.79 (dd, *J*=10.4, 3.5, 1H, H3'), 4.58 (d, *J*=8.0, 1H, H1'), 4.07 (t, *J*=10.4, 1H, H2), 3.95 (dd, *J*=6.8, 2.3, 2H, H6a', H6b'), 3.87 - 3.78 (m, 2H, H4, H6a), 3.74 (dd, *J*=11.7, 2.9, 1H, H6b), 3.68 (td, *J*=6.7, 0.7, 1H, H5'),

3.40 (d, *J*=9.9, 1H, H5), 1.98 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.73 (s, 3H, Ac), 0.83 (s, 9H, C(CH_3)$_3$), 0.04 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.43, 170.31, 170.22, 170.17, 168.91 (170.4-168.9 *C*OCH_3), 167.91, 167.31, 134.46 (Phth), 134.24 (Phth), 133.43, 131.84, 131.49, 131.39, 129.14, 128.95, 128.33, 128.30 (131.8 - 128.3 aromatic), 125.41, 123.83 (Phth), 123.65 (Phth), 100.42 (C1'), 83.07 (C1), 79.68 (C5), 74.78 (C4), 71.95 (C3), 71.31 (C3'), 70.72 (C5'), 69.33 (C2'), 66.98 (C4'), 61.20 (C6), 61.10 (C6'), 54.12 (C2), 26.06 (C(CH_3)$_3$), 20.84, 20.75, 20.68, 20.67 (20.8-20.7 $COCH_3$), 18.43, -4.82 ($SiCH_3$), -5.11 ($SiCH_3$).

Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (40)[290]

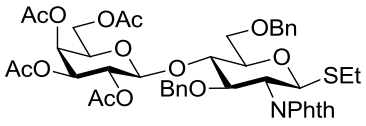


Compound **13** (23.65 g, 44.5 mmol, 1.0 eq) was dissolved in anhydrous THF (71 mL) and stirred at 0 °C under Ar atmosphere. Sodium cyanoborohydride (11.19 g, 178 mmol, 4.0 eq) was added followed by dropwise addition of 2N HCl in Et_2O (77.9 mL, 156 mL, 3.5 eq). After stirring for 30 min at 0 °C, the reaction was neutralized by addition of Et_3N (10 mL) and the solvent was removed under reduced pressure without heating. The residue was purified by column chromatography (toluene-ethyl acetate 10:1 to 5:1) to give **40**. Yield: 85% (20.18 g, 37.8 mmol). R_f = 0.2 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = +48° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 550.93 ($[M+NH_4]^+$, calc. 551.22), 1083.67 ($[2M+NH_4]^+$, calc. 1084.41). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.82 (d, *J*=5.6, 1H, Phth), 7.72 - 7.66 (m, 3H, Phth), 7.40 - 7.29 (m, 5H, CH_2*Ph*), 7.08 - 7.02 (m, 2H, CH_2*Ph*), 6.98 - 6.92 (m, 3H, CH_2*Ph*), 5.28 (d, *J*=10.0, 1H, H1), 4.75 (d, *J*=12.2, 1H, 3-CH_2Ph), 4.67 - 4.57 (m, 2H, 6-CH_2Ph), 4.54 (d, *J*=12.1, 1H, 3-CH_2Ph), 4.32 - 4.19 (m, 2H, H3, H2), 3.88 - 3.74 (m, 3H, H6ab, H4), 3.69 (dd, *J*=9.7, 4.9, 1H, H5), 2.72 - 2.54 (m, 2H, SCH_2CH_3), 1.16 (t, *J*=7.4, 3H, SCH_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.34, 137.79, 134.12 (Phth), 134.02 (Phth), 131.88, 128.73, 128.37, 128.13, 128.11, 128.02, 127.65 (128.7 - 127.7, CH_2*Ph*), 123.74 (Phth), 123.48 (Phth), 81.40 (C1), 79.81 (C3), 77.77 (C5), 74.80 (C4), 74.68 (3-CH_2Ph), 74.02 (6-CH_2Ph), 71.16 (C6), 54.62 (C2), 24.19 (SCH_2CH_3), 15.13 (SCH_2CH_3).

Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (41)[291]

Compound **14** (49.95 g, 86.2 mmol, 1.0 eq) was dissolved in anhydrous THF (290 mL) and stirred at 0 °C under Ar atmosphere. Sodium cyanoborohydride (21.66 g, 345 mmol, 4.0 eq) was added followed by dropwise addition of 2N HCl in Et_2O (150.5 mL, 302 mL, 3.5 eq). After stirring for 30 min at 0 °C, the reaction was neutralized by Et_3N (15 mL) and the solvent was removed under reduced pressure without heating. The residue was purified by column chromatography (toluene-ethyl acetate 10:1 to 5:1) to give **41**. Yield: 87% (43.66 g, 75.1 mmol). R_f = 0.3 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = +56° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 598.93 ($[M+NH_4]^+$, calc. 599.22), 1179.73 ($[2M+NH_4]^+$, calc. 1180.41). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.84 (d, *J*=6.6, 1H, 1H, Phth), 7.70 (dd, *J*=7.9, 4.4, 3H, Phth), 7.43 - 7.13 (m, 10H, CH_2*Ph*, S*Ph*), 7.04 (dd, *J*=6.3, 2.6, 2H, CH_2*Ph*), 6.99 - 6.88 (m, 3H, CH_2*Ph*), 5.59 - 5.55 (m, 1H, H1), 4.74 (d, *J*=12.2, 1H, CH_2Ph), 4.63 (d, *J*=11.9, 1H, CH_2Ph), 4.58 (d, *J*=11.8, 1H, CH_2Ph), 4.54 (d, *J*=12.2, 1H, CH_2Ph), 4.29 - 4.25 (m, 2H, H3, H2), 3.88 - 3.78 (m, 3H, H6ab, H4), 3.70 (dt, *J*=9.6, 4.8, 1H, H5). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.23, 137.93, 134.20 (Phth), 134.09 (Phth), 132.67, 129.05, 128.72, 128.40, 128.17, 128.09, 128.03, 128.01, 127.70 (129.1 - 127.7, aromatic), 123.76 (Phth), 123.56 (Phth), 83.89 (C1), 79.94 (C3), 78.12 (C5), 74.77 (CH_2Ph), 74.27 (C4), 74.00 (CH_2Ph), 70.90 (C6), 54.68 (C2), 27.16.

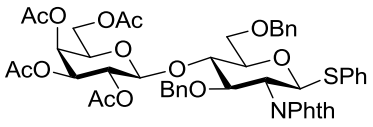
Ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (42)[292]



The trichloroacetimidate donor **34** (572 mg, 1.2 mmol, 1.2 eq) and acceptor **40** (534 g, 1.0 mmol, 1.0 eq) was dissolved in dry DCM (8 mL) and 4 Å molecular sieves (427 mg) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to -50 °C, trimethylsilyl -trifluoromethanesulfonate (TMSOTf) (469 μL, 2.58 mmol, 0.3 eq) was added. The reaction was kept stirring at -45 to -50 °C for 4 h, then quenched with triethylamine (0.5 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **42**. Yield 75% (650 mg, 0.75 mmol). R_f = 0.3 (Tol/EtOAc 5:1). $[\alpha]_D^{20}$ = 30° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 881.00 ($[M+NH_4]^+$, calc. 881.32), 1743.40 ($[2M+NH_4]^+$, calc. 1744.60). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.71 (d, *J*=5.9, 1H, Phth), 7.65 - 7.52 (m, 3H, Phth),

7.36 - 7.13 (m, 5H, aromatic), 6.99 - 6.92 (m, 2H, aromatic), 6.86 - 6.77 (m, 3H, aromatic), 5.20 (d, *J*=3.5, 1H, H4'), 5.14 (d, *J*=10.0, 1H, H1), 5.08 (dd, *J*=10.4, 8.0, 1H, H2'), 4.79 (dd, *J*=10.4, 3.5, 1H, H3'), 4.74 (d, *J*=3.6, 1H, 3-BnCH$_2$), 4.71 (d, *J*=3.4, 1H, 6-BnCH$_2$), 4.55 (d, *J*=8.0, 1H, H1'), 4.43 (d, *J*=12.0, 1H, 6-BnCH$_2$), 4.37 (d, *J*=12.2, 1H, 3-BnCH$_2$), 4.25 - 4.12 (m, 2H, H3, H2), 4.02 (dd, *J*=9.7, 8.3, 1H, H4), 3.95 - 3.83 (m, 2H, H6a', H6b'), 3.72 (d, *J*=2.3, 2H, H6a, H6b), 3.57 (t, *J*=6.9, 1H, H5'), 3.48 (dt, *J*=10.3, 2.5, 1H, H5), 2.66 - 2.46 (m, 2H, SCH$_2$CH$_3$), 1.99 (s, 3H, Ac), 1.95 (s, 6H, Ac), 1.90 (s, 3H, Ac), 1.10 (t, *J*=7.5, 3H, SCH$_2$CH$_3$). ***^{13}C-NMR*** (100.6 MHz, CDCl$_3$, gHSQC, gHMBC), *δ (ppm)*: 170.48, 170.39, 170.22, 169.38 (170.5 - 169.4 *C*O$_2$CH$_3$), 138.71, 138.11, 134.02 (Phth), 133.89 (Phth), 131.84, 128.80, 128.22, 128.10, 128.06, 127.30 (128.8 - 127.3 aromatic), 123.65 (Phth), 123.46 (Phth), 100.52 (C1'), 81.33 (C1), 79.32 (C5), 78.06 (C4), 77.86 (C3), 74.76 (3-BnCH$_2$), 73.82 (6-BnCH$_2$), 71.23 (C3'), 70.68 (C5'), 69.75 (C2'), 67.98 (C6), 67.14 (C4'), 60.92 (C6'), 54.92 (C2), 27.13, 24.08 (SCH$_2$CH$_3$), 21.02, 20.88, 20.81, 20.77 (CO$_2$*C*H$_3$), 15.12 (SCH$_2$CH$_3$).

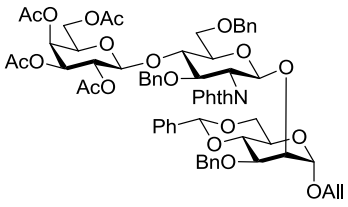
Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (43)[293]



The trichloroacetimidate donor **34** (5.32 g, 11.2 mmol, 1.3 eq) and acceptor **41** (5.00 g, 8.60 mmol, 1.0 eq) was dissolved in dry Et$_2$O (50 mL) and 4 Å molecular sieves (6.5 g) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to -50 °C, trimethylsilyl -trifluoromethanesulfonate (TMSOTf) (469 μL, 2.58 mmol, 0.3 eq) was added. The reaction was kept stirring at -45 to -50 °C for 4 h, then quenched with triethylamine (0.5 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **43**. Yield 54% (4.23 g, 4.64 mmol). R$_f$ = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = 48° (c = 1.5, CHCl$_3$). *ESI-MS (pos), m/z*: 929.00 ([M+NH$_4$]$^+$, calc. 929.32), 1839.87 ([2M+NH$_4$]$^+$, calc. 1840.40).

7.7 Synthesis of complex type building blocks with Gal-GlcNAc extension

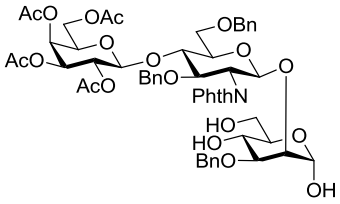
Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (44)



Compounds **42** (1.05 g, 1.22 mmol, 1.0 eq), **29** (578 mg, 1.45 mmol, 1.2 eq), *N*-iodosuccinimide (652 mg, 2.90 mmol, 2.4 eq) and 4 Å molecular sieves (1.0 g) was suspended in dry dichloromethane (10 mL). The mixture was cooled to -50 °C, stirred for 30 min under argon atmosphere, followed by slow addition of trifluoromethanesulfonic acid (30.7 μL, 0.35 mmol, 0.28 eq). After stirring for 2 h at -40 to -50 °C, the reaction was quenched by diluting with more DCM (40 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (15 mL×3), brine (30 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **44**. Yield: 65% (952 mg, 0.79 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +14° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1217.4732 ($[M+NH_4]^+$, calc. 1217.4706), 1222.4290 ($[M+Na]^+$, calc. 1222.4260). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.67 (s, 2H, Phth), 7.47 - 7.15 (m, 17H, aromatic), 7.10 - 6.98 (m, 2H, aromatic), 6.95 - 6.85 (m, 3H, aromatic), 5.72 (ddd, *J*=22.3, 11.1, 5.8, 1H, $CH_2CH=CH_2$), 5.42 (s, 1H, C*H*Ph), 5.28 (dd, *J*=7.9, 3.8, 1H, H4''), 5.21 - 5.06 (m, 4H, H2'', H1, $CH_2CH=CH_2$), 4.90 (dd, *J*=10.3, 3.6, 1H, H3''), 4.81 (d, *J*=12.3, 1H, $BnCH_2$), 4.76 - 4.71 (m, 2H, $BnCH_2$), 4.69 - 4.62 (m, 2H, H1'', $BnCH_2$), 4.51 - 4.43 (m, 3H, $BnCH_2$, H1'), 4.33 - 4.25 (m, 2H, H3', H2'), 4.10 - 4.05 (m, 2H, H4', H2), 4.03 - 3.97 (m, 2H, H6a'', H6b''), 3.96 - 3.83 (m, 3H, H4, H3, $CH_2CH=CH_2$), 3.83 - 3.76 (m, 2H, H6a', H6b'), 3.73 - 3.64 (m, 3H, $CH_2CH=CH_2$, H5'', H6a), 3.56 (d, *J*=10.0, 1H, H5'), 3.52 - 3.45 (m, 1H, H5), 3.08 (t, *J*=10.2, 1H, H6b), 2.07 (s, 3H, Ac), 2.02 (s, 6H, Ac), 1.98 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.52, 170.40, 170.22, 169.44 (170.5 - 169.4 CO_2CH_3), 138.85, 138.82, 138.16, 137.81, 134.66, 133.84 (Phth), 133.30 ($CH_2CH=CH_2$), 132.05, 129.95, 129.22, 128.95, 128.92, 128.84, 128.76, 128.62, 128.50, 128.46, 128.42, 128.34, 128.31, 128.28, 128.14, 128.12, 128.09, 127.93, 127.80, 127.61, 127.48, 127.41, 127.38, 127.32 (129.2 - 127.3 aromatic), 126.24, 123.21 (Phth), 118.06 ($CH_2CH=CH_2$), 101.61 (*C*HPh), 100.68 (C1''), 97.15 (C1, C1'), 78.39 (C4', C4), 76.79 (C3'), 75.48 (C5'), 75.31 (C2), 74.52 ($BnCH_2$), 74.03 ($BnCH_2$), 74.00 (C3), 71.58 ($BnCH_2$), 71.26 (C3''), 70.81 (C5''), 69.84 (C2''), 68.58 (C6),

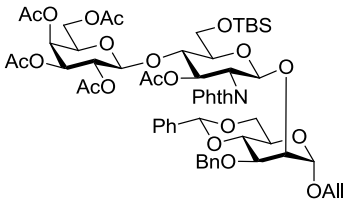
68.32 (C6', *C*H_2CH=CH_2), 67.21 (C4''), 64.07 (C5), 61.08 (C6''), 55.86 (C2'), 29.91, 21.01, 20.89, 20.82, 20.79 (21.0 - 20.8 CO_2CH_3).

2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-α-D-mannopyranose (45)



Compound **44** (60 mg, 0.05 mmol, 1.0 eq) was dissolved in Tol/EtOAc/H_2O 20:10:1 (930 μL) and Wilkinson's catalyst (6.9 mg, 7.5 μmol, 0.15 eq) was added. After refluxing for 5.5 h, the reaction mixture was cooled to r.t., filtered through a layer of Celite and concentrated. The residue was dissolved in THF/H_2O 4:1 (3 mL) followed by addition of I_2 (50.7 mg, 0.20 mmol, 4.0 eq). The reaction was kept stirring at r.t. for 30 min and neutralized with 1 M $Na_2S_2O_3$. The suspension was concentrated to remove most of the THF and was then diluted with DCM (10 mL), washed with 1 M $Na_2S_2O_3$ (5 mL×2), H_2O (5 mL×2), brine (5 mL×1) and dried over $MgSO_4$. The solvent was removed and the residue was purified by column chromatography (toluene-ethyl acetate 1:1 to 1:3) to give **45**, yield 72% (42 mg, 0.036 mmol). R_f = 0.2 (Tol/EtOAc 1:3). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.58 (dd, *J*=11.5, 7.7, 2H, Phth), 7.47 (t, *J*=7.3, 2H, Phth), 7.42 - 7.08 (m, 10H, aromatic), 6.97 (d, *J*=6.6, 2H, aromatic), 6.86 - 6.75 (m, 3H, aromatic), 5.22 - 5.18 (m, 1H, H4''), 5.13 - 5.06 (m, 2H, H1', H2''), 4.82 (s, 1H, H1), 4.78 (dd, *J*=11.6, 2.9, 1H, H3''), 4.75 - 4.70 (m, 2H, $BnCH_2$), 4.61 (d, *J*=13.0, 1H, $BnCH_2$), 4.56 (d, *J*=8.9, 1H, H1''), 4.39 (d, *J*=12.4, 1H, $BnCH_2$), 4.30 (dd, *J*=11.5, 4.2, 2H, $BnCH_2$), 4.25 (dd, *J*=10.9, 2.0, 1H, H3'), 4.16 (d, *J*=8.4, 1H, H2'), 4.06 (s, 1H, H2), 4.02 - 3.96 (m, 1H, H4'), 3.94 - 3.85 (m, 2H, H6a'', H6b''), 3.73 - 3.64 (m, 2H, H6a', H6b'), 3.62 - 3.56 (m, 2H, H3, H4), 3.51 (d, *J*=9.6, 1H, H5''), 3.47 (d, *J*=7.3, 2H, H5', H5), 3.26 (dd, *J*=11.0, 1.5, 1H, H6a), 2.85 (dd, *J*=11.1, 5.2, 1H, H6b), 2.00 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.90 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.52, 170.39, 170.23, 169.44 (170.5 - 169.4 *C*O_2CH_3), 138.78, 138.17, 138.01, 132.37 (Phth), 132.27 (Phth), 132.22, 128.81, 128.78, 128.69, 128.61, 128.47, 128.14, 128.03, 128.01 (128.8 - 128.0 aromatic), 123.35 (Phth), 100.71 (C1''), 96.65 (C1'), 91.76 (C1), 78.47 (C4'), 76.83 (C3'), 76.69 (C4), 75.26 (C5''), 74.54 ($BnCH_2$), 73.99 ($BnCH_2$), 72.57 (C2), 71.77 (C5'), 71.25 (C3''), 70.78 (C3), 70.22 ($BnCH_2$), 69.84 (C2''), 68.46 (C6'), 67.38 (C5), 67.17 (C4''), 63.27 (C6), 61.03 (C6''), 55.82 (C2'), 21.00, 20.88, 20.82, 20.78 (21.0 - 20.8 CO_2CH_3).

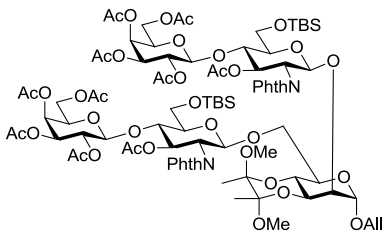
Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (46)



Compounds **38** (1.09 g, 1.30 mmol, 1.08 eq), **29** (479 mg, 1.20 mmol, 1.0 eq), *N*-iodosuccinimide (675 mg, 3.00 mmol, 2.5 eq) and 4 Å molecular sieves (1 g) in dry dichloromethane (10 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (21 μL, 0.24 mmol, 0.2 eq). After stirring for 4 h at 0 °C, the reaction was quenched by diluting with more DCM (50 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (30 mL×3), brine (30 mL×1) and dried over $MgSO_4$. Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **46**. Yield: 75% (1.05 g, 0.90 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +8° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1176.4486 ($[M+H]^+$, calc. 1176.4472), 1193.4742 ($[M+NH_4]^+$, calc. 1193.4737), 1198.4321 ($[M+Na]^+$, calc. 1198.4291), 1214.4051 ($[M+K]^+$, calc. 1214.4031). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.83 - 7.72 (m, 2H, Phth), 7.68 - 7.61 (m, 2H, Phth), 7.38 - 7.07 (m, 11H, aromatic), 5.73 - 5.59 (m, 2H, H3' , CH_2CH=CH_2), 5.35 - 5.23 (m, 3H, H1', C*H*Ph, H4''), 5.09 - 4.98 (m, 3H, H2'', CH_2CH=CH_2), 4.85 (dd, *J*=10.4, 3.5, 1H, H3''), 4.66 (d, *J*=7.9, 1H, H1''), 4.64 (d, *J*=10.7, 1H, $BnCH_2$), 4.57 (d, *J*=12.5, 1H, $BnCH_2$), 4.54 (d, *J*=1.2, 1H, H1), 4.19 (dd, *J*=10.8, 8.5, 1H, H2'), 4.06 - 3.97 (m, 3H, H2, H6a'', H6b''), 3.94 - 3.85 (m, 2H, H4' , CH_2CH=CH_2), 3.83 - 3.65 (m, 6H, H4, H3, H5'', H6a', H6b', CH_2CH=CH_2), 3.56 (dd, *J*=10.2, 4.7, 1H, H6a), 3.40 (d, *J*=9.8, 2H, H5, H5'), 2.86 (t, *J*=10.2, 1H, H6b), 2.05 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.84 (s, 3H, Ac), 0.84 (s, 9H, *t*Bu), 0.02 (s, 3H, $Si(CH_3)_2$), -0.00 (s, 3H, $Si(CH_3)_2$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.47, 170.31, 170.19, 168.94 (170.5 - 168.9 *C*O_2CH_3), 138.68, 137.65, 134.08 (Phth), 133.18 (CH_2*C*H=CH_2), 128.86, 128.23, 128.20, 127.73, 127.39, 126.09 (128.9 - 126.1 aromatic), 123.74 (Phth), 123.55 (Phth), 123.32 (Phth), 117.95 (CH_2CH=*C*H_2), 101.47 (*C*HPh), 100.39 (C1''), 96.65 (C1), 96.22 (C1'), 78.58 (C4), 75.84 (C5'), 75.13 (C2), 75.06 (C4'), 73.67 (C3), 71.89 ($BnCH_2$), 71.33 (C3''), 70.78 (C3', C5''), 69.40 (C2''), 68.44 (C6), 68.30 (*C*H_2CH=CH_2), 67.07 (C4''), 63.94 (C5), 61.25 (C6''), 61.14 (C6'), 55.16 (C2'),

27.04, 26.07 (C(*C*H_3)$_3$), 20.87, 20.81, 20.76, 20.67 (20.9 - 20.7 CO_2*C*H_3), 18.40, -4.69 (Si(CH_3)$_2$), -5.22 (Si(CH_3)$_2$).

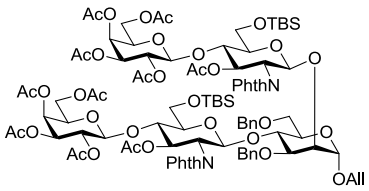
Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-(1→6)-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranoside (47)



Compounds **38** (1.48 g, 1.76 mmol, 2.2 eq), **31** (267 mg, 0.80 mmol, 1.0 eq), *N*-iodosuccinimide (538 mg, 2.39 mmol, 3.0 eq) and 4 Å molecular sieves (0.8 g) in dry dichloromethane (8 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (14 μL, 0.16 mmol, 0.2 eq). After stirring for 3 h at 0 °C, the reaction was quenched by diluting with more DCM (50 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (30 mL×3), brine (30 mL×1) and dried over $MgSO_4$. Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **47**. Yield: 58% (877 mg, 0.46 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +24° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1890.6982 ($[M+H]^+$, calc. 1889.7034), 1906.7319 ($[M+NH_4]^+$, calc. 1906.7300). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.90 - 7.59 (m, 8H, Phth), 5.64 - 5.55 (m, 2H, H3^2, H3^3), 5.47 - 5.34 (m, 1H, CH_2C*H*=CH_2), 5.28 (dd, *J*=14.6, 3.3, 2H, H4^4, H4^5), 5.23 (d, *J*=8.4, 1H, H1^2), 5.02 (ddd, *J*=12.9, 10.4, 7.9, 2H, H2^4, H2^5), 4.94 - 4.80 (m, 4H, CH_2CH=C*H_2*, H3^4, H3^5), 4.74 (d, *J*=8.3, 1H, H1^3), 4.61 (d, *J*=8.1, 2H, H1^4, H1^5), 4.25 (s, 1H, H1^1), 4.12 (dd, *J*=10.9, 8.4, 1H, H2^2), 4.00 (d, *J*=6.7, 4H, H6a^4, H6b^4, H6a^5, H6b^5), 3.92 (dd, *J*=9.4, 6.9, 1H, H2^3), 3.89 - 3.66 (m, 11H, H4^2, H4^3, H6a^2, H6b^2, H6a^3, H6b^3, H6a^1, H5^4, H5^5, H4^1, H2^1), 3.46 - 3.37 (m, 2H, H5^1, C*H_2*CH=CH_2), 3.31 (t, *J*=9.9, 2H, H5^2, H5^3), 3.24 - 3.17 (m, 1H, H3^1), 3.04 (s, 3H, OCH_3), 3.03 - 2.98 (m, 1H, C*H_2*CH=CH_2), 2.94 (s, 3H, OCH_3), 2.30 - 2.24 (m, 1H, H6b^1), 2.06 (s, 3H), 2.05 (s, 4H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H), 1.82 (s, 3H), 1.80 (s, 3H, 2.06 - 1.80 Ac), 1.04 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.85 (s, 9H, *t*Bu), 0.85 (s, 9H, *t*Bu), 0.05 (s, 3H, Si(CH_3)$_2$), 0.02 (s, 3H, Si(CH_3)$_2$), 0.01 (s, 3H, Si(CH_3)$_2$), 0.00 (s, 3H, Si(CH_3)$_2$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.46, 170.42, 170.29, 170.21, 170.06, 169.09,

168.97 (170.5 - 168.9 CO_2CH_3), 167.67, 167.36, 134.37, 133.98 (134.4 - 133.9 Phth), 133.10 ($CH_2CH=CH_2$), 131.72, 123.67 (Phth), 123.48 (Phth), 117.91 ($CH_2CH=CH_2$), 100.67 ($C1^4$), 100.51 ($C1^5$), 99.83 ($COCH_3(CH_3)$), 99.35 ($COCH_3(CH_3)$), 97.76 ($C1^3$), 95.72 ($C1^1$), 95.25 ($C1^2$), 75.62 ($C4^3$), 75.57 ($C5^2$), 75.21 ($C5^3$), 75.11 ($C4^2$), 73.19 ($C3^4$, $C3^5$), 71.27 ($C3^2$), 70.83 ($C3^3$), 70.74 ($C5^4$), 70.70 ($C5^5$), 70.25 ($C5^1$), 69.42 ($C2^4$), 69.31 ($C2^5$), 68.41 ($C6^1$), 67.19 ($CH_2CH=CH_2$), 67.04 ($C4^4$), 66.94 ($C4^5$), 66.58 ($C4^1$), 63.42 ($C3^1$), 61.24 ($C6^4$, $C6^5$), 61.09 ($C6^2$, $C6^3$), 55.85, 55.17 ($C2^3$), 54.50 ($C2^2$), 47.77 (OCH_3), 47.40 (OCH_3), 29.72, 27.01, 26.09 ($C(CH_3)_3$), 26.01 ($C(CH_3)_3$), 20.88, 20.81, 20.74, 20.71, 20.65 (20.9 - 20.7 CO_2CH_3), 18.41, 17.69 (CH_3), 17.56 (CH_3), -4.54, -4.86, -5.14, -5.18 (-4.5 - -5.2 $Si(CH_3)_2$).

Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-acetyl-β-D-glactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (48)

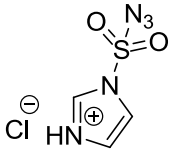


Compounds **38** (2.55 g, 3.04 mmol, 2.2 eq), **30** (553 mg, 1.38 mmol, 1.0 eq), *N*-iodosuccinimide (1.19 g, 5.30 mmol, 3.8 eq) and 4 Å molecular sieves (1 g) in dry dichloromethane (10 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (49 μL, 0.55 mmol, 0.4 eq). After stirring for 4 h at 0 °C, the reaction was quenched by diluting with more DCM (50 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (30 mL×3), brine (30 mL×1), dried over $MgSO_4$. Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **48**. Yield: 57% (1.55 g, 0.79 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -2° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1956.7187 ($[M+H]^+$, calc. 1956.7326), 1973.7480 ($[M+NH_4]^+$, calc. 1973.7591), 995.8998 ($[M+2NH_4]^{2+}$, calc. 995.8968), 997.8476 ($[M+Na+NH_4]^{2+}$, calc. 997.8728). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.88 - 7.61 (m, 8H, Phth), 7.36 - 7.11 (m, 10H, aromatic), 5.72 (ddd, *J*=9.9, 8.0, 4.4, 1H, $CH_2CH=CH_2$), 5.67 - 5.62 (m, 1H, $H3^2$), 5.56 (dd, *J*=10.7, 9.2, 1H, $H3^3$), 5.43 (d, *J*=8.4, 1H, $H1^2$), 5.38 (d, *J*=8.5, 1H, $H1^3$), 5.31 (dd, *J*=8.8, 3.5, 2H, $H4^4$, $H4^5$), 5.10 - 4.98 (m, 4H, $CH_2CH=CH_2$, $H2^4$, $H2^5$), 4.91 (dd, *J*=10.4, 3.5, 1H, $H3^4$), 4.86 (dd, *J*=10.4, 3.5, 1H, $H3^5$), 4.74 (d, *J*=8.1, 1H, $H1^4$), 4.71 (d, *J*=3.2, 1H, $BnCH_2$), 4.67 (d, *J*=8.0, 1H, $H1^5$), 4.58 (d, *J*=3.4, 1H, $H1^1$),

4.43 (d, *J*=12.5, 1H, $BnCH_2$), 4.18 (dd, *J*=10.8, 8.5, 1H, $H2^2$), 4.13 - 3.99 (m, 6H, $H2^1$, $H6a^4$, $H6b^4$, $H6a^5$, $H6b^5$, $H2^3$), 3.96 - 3.84 (m, 6H, $H4^2$, $H6a^3$, $CH_2CH=CH_2$, $BnCH_2$, $H4^3$), 3.84 - 3.75 (m, 5H, $H5^4$, $H6b^3$, $H4^1$, $H5^5$, $CH_2CH=CH_2$), 3.71 (dd, *J*=12.8, 6.2, 1H, $H3^1$), 3.53 - 3.39 (m, 3H, $H6a^2$, $H5^1$, $H5^2$), 3.35 (d, *J*=11.5, 1H, $H6b^2$), 3.14 (dd, *J*=10.9, 2.2, 1H, $H6a^1$), 3.00 (d, *J*=9.5, 1H, $H5^3$), 2.93 (dd, *J*=10.8, 5.9, 1H, $H6b^1$), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.03 (s, 6H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.87 (s, 3H), 1.83 (s, 3H, 2.10 - 1.83 Ac), 0.90 (s, 9H, *t*Bu), 0.89 (s, 9H, *t*Bu), 0.07 (s, 3H, $Si(CH_3)_2$), 0.06 (s, 3H, $Si(CH_3)_2$), 0.03 (s, 3H, $Si(CH_3)_2$), 0.01 (s, 3H, $Si(CH_3)_2$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.45, 170.42, 170.32, 170.20, 170.16, 169.23, 168.93, 168.78 (170.5 - 168.8 CO_2CH_3), 167.95, 167.68, 139.04, 138.48, 134.38 (Phth), 133.91 ($CH_2CH=CH_2$), 131.73, 131.56, 128.21, 128.12, 127.23, 127.03, 126.89 (128.2 - 126.9 aromatic), 123.68 (Phth), 123.38 (Phth), 117.30 ($CH_2CH=CH_2$), 100.31 ($C1^5$), 100.16 ($C1^4$), 97.73 ($C1^3$), 96.78 ($C1^1$), 96.06 ($C1^2$), 77.36 ($C1^3$), 75.64 ($C5^2$), 75.11 ($C4^2$), 75.04 ($C5^3$), 74.72 ($C4^1$), 74.50 ($C4^3$), 73.08 ($C1^2$), 72.44 ($BnCH_2$), 71.36 ($C3^4$), 71.30 ($C3^5$), 71.10 ($C3^1$, $C3^3$), 70.95 ($C5^1$), 70.83 ($BnCH_2$), 70.71 ($C5^4$), 70.63 ($C5^5$), 69.38 ($C2^4$), 69.31 ($C6^1$), 69.29 ($C2^5$), 68.32 ($CH_2CH=CH_2$), 67.02 ($C4^4$), 66.98 ($C4^5$), 61.19 ($C6^4$, $C6^5$), 61.04 ($C6^3$), 60.48 ($C6^2$), 55.58 ($C2^3$), 55.13 ($C2^2$), 29.79, 29.71, 26.09 ($C(CH_3)_3$), 26.01 ($C(CH_3)_3$), 20.84, 20.74, 20.66 (20.8 - 20.7 CO_2CH_3), 18.38, 18.30, -4.63, -4.85, -5.28, -5.33(-4.6 - -5.3 $Si(CH_3)_2$).

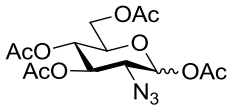
7.8 Synthesis of GlcNAc donor for building blocks extension

Imidazole-1-sulfonyl azide hydrochloride (49)[186]



To a suspension of sodium azide (35.2 g, 541 mmol, 1.0 eq) in MeCN (650 mL) at 0 °C, sulfuryl chloride (72.6 g, 537 mmol, 1.0 eq) was added dropwise. After stirring at r.t. for 18 h, imidazole (69.6 g, 1.02 mol, 1.9 eq) was added in portions and the reaction mixture was kept stirring for 4 h, diluted with 1.2 L EtOAc followed by washing with H_2O (400 mL×2) and Sat. $NaHCO_3$ (400 mL×2) and dried over $MgSO_4$. The organic solvent was concentrated and then the residue was cooled with an ice-bath. The mixture was acidified with HCl in MeOH solution (65 mL AcCl dropped into 225 mL ice-cooled anhydrous MeOH), stored at -20 °C freezer for 24 h. The precipitate was filtered off and washed with EtOAc to give **49**, yield 80% (89.5 g, 427 mmol).

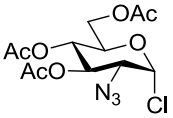
1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranose (50)[186]



To a suspension of K_2CO_3 (80.7 g, 583 mmol, 2.07 eq) and $CuSO_4 \cdot 5H_2O$ (694 mg, 2.8 mmol 0.01 eq) in MeOH (800 mL), glucosamine hydrochloride (60.9 g, 282 mmol, 1.0 eq) and compound **49** (64.2 g, 306 mmol, 1.09 eq) were added sequentially. After mechanical stirring at r.t. for 4 h, the solvent was removed by rotary evaporation and coevaporated with toluene (200 ml×3). The residue was then acetylated with pyridine / acetic anhydride 2:1 (900 mL) at r.t. for 24 h, concentrated and coevaporated with toluene (200 ml×3). The residue was then acetylated with pyridine / acetic anhydride 2:1 (900 mL) at r.t. for 24 h and concentrated, coevaporated with toluene (200 ml×3). The residue was diluted with EtOAc (800 mL), washed with H_2O (300 mL×2), Sat. $NaHCO_3$ (300 mL×2), brine (500 mL×1), dried over $MgSO_4$, the solvent was removed and crude product was purification by column chromatography (cyclohexane-ethyl acetate 8:1 to 2:1) gave **50** in 95% yield (99.6 g, 227 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +35° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 390.87 ($[M+NH_4]^+$, calc. 391.15). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.54 (d, *J*=8.6, 1H, H1), 5.13 - 4.97 (m, 2H, H3, H4), 4.27 (dd, *J*=12.5, 4.5, 1H, H6a), 4.06 (dd, *J*=12.5, 2.0, 1H, H6b), 3.79 (ddd, *J*=9.7, 4.4, 2.1, 1H, H5), 3.64 (t, *J*=8.9, 1H, H2), 2.16 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.00 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.72, 169.96, 169.80, 168.73 (170.7 - 168.7 CO_2CH_3), 92.75 (C1), 72.91

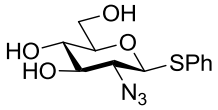
(C3), 72.89 (C5), 68.01 (C4), 62.78 (C2), 61.61 (C6), 21.04, 20.85, 20.79, 20.72 (21.0 - 20.7 CO_2CH_3).

3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosylchloride (51)[294]



To a stirring solution of **50** (99.3 g, 266 mmol, 1.0 eq) in anhydrous $CHCl_3$ (600 mL) at 0 °C, titanium (IV) chloride (58.3 mL, 532 mL, 2.0 eq) was added dropwise. The temperature was raised to r.t. and the mixture heated to reflux for 4 h. The reaction mixture was diluted with DCM (600 mL), washed with H_2O (500 mL×2), Sat. $NaHCO_3$ (500 mL×2), brine (800 mL×1), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **51** in 60% yield (56.3 g, 161 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +140° (c = 1.0, $CHCl_3$). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 6.10 (d, *J*=3.8, 1H, H1), 5.51 - 5.42 (m, 1H, H3), 5.08 (t, *J*=9.6, 1H, H4), 4.35 - 4.27 (m, 2H, H5, H6a), 4.09 (dt, *J*=4.6, 3.2, 1H, H6b), 3.84 (dd, *J*=10.3, 3.8, 1H, H2), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.02 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.59, 169.88, 169.79 (170.6 - 169.8 CO_2CH_3), 91.83 (C1), 70.92 (C5), 70.86 (C3), 67.79 (C4), 62.43 (C2), 61.31 (C6), 20.82, 20.79, 20.71 (20.8 - 20.7 CO_2CH_3).

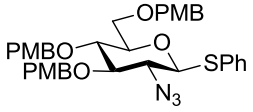
Phenyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (52)[295]



To a stirring solution of thiophenol (18.0 mL, 176 mmol, 1.1 eq) and KOH (9.89 g, 176 mmol, 1.1 eq) in EtOH (300 mL), a solution of **51** (56.0 g, 160 mmol, 1.0 eq) in $CHCl_3$ (300 mL) was added dropwise. After stirring at r.t. for 3 h. The reaction was diluted with DCM (600 mL), washed with H_2O (500 mL×2), Sat. $NaHCO_3$ (500 mL×2), brine (800 mL×1), dried over Na_2SO_4. The solvent was removed and the crude product was dissolved in 0.25 M NaOMe in MeOH (400 mL) solution. Then stirred at r.t. for 2 h, neutralized with *Dowex* 50WX8 ion-exchanger (3 g, prewashed with MeOH), concentrated and coevaporated with toluene (250 mL×3). The residue was purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:2) gave **52** in 64% yield (30.2 g, 102 mmol). R_f = 0.3 (EtOAc). $[\alpha]_D^{20}$ = -13° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 298.13 ($[M+H]^+$, calc. 298.09). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.50 (dd, *J*=8.3, 1.3, 2H, SPh), 7.37 - 7.30 (m, 2H, SPh), 7.30 - 7.25 (m, 1H, SPh), 4.69 (d, *J*=10.2, 1H, H1), 3.69 (dd, *J*=11.9, 1.7, 1H, H6a), 3.46 (dd, *J*=11.9, 5.7, 1H, H6b), 3.35 (t, *J*=9.0, 1H, H3), 3.27 (ddd, *J*=9.8, 5.6, 1.7,

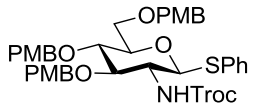
1H, H5), 3.19 - 3.10 (m, 2H, H2, h4). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 133.54, 131.62, 129.70, 127.98 (133.5 - 128.0 SPh), 84.96 (C1), 81.66 (C5), 77.22 (C3), 70.31 (C4), 66.03 (C2), 61.44 (C6).

Phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (53)



Compound **52** (20.82 g, 73.5 mmol, 1.0 eq) was dissolved in dry DMF (200 mL) under Ar and NaH (60% suspension, 10.29 g, 257 mmol, 3.5 eq) was added in portions under cooling with an ice-bath. The reaction was kept at 0 °C for 40 min before 4-methoxybenzyl chloride (31.0 mL, 237 mmol, 3.23 eq) was added dropwise using a syringe. After 2 h stirring at r.t., the organic solvent was removed under reduced pressure and the residue was dissolved in EtOAc (300 mL), washed with H_2O (150 mL×2), brine (150 mL×1), dried over $MgSO_4$. The solvent was removed, the residue purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) and then recrystallized from cyclohexane-ethyl acetate to give **53**, yield 63% (30.60 g, 46.5 mmol). R_f = 0.5 (^{C}Hex/EtOAc 3:1). $[\alpha]_D^{20}$ = -67° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 675.00 ($[M+NH_4]^+$, calc. 675.29), 1331.27 ($[2M+NH_4]^+$, calc. 1332.54). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.66 - 7.57 (m, 2H, PMB), 7.34 - 7.23 (m, 7H, PMB, SPh), 7.16 - 7.11 (m, 2H, PMB), 6.91 - 6.80 (m, 6H, PMB), 4.80 (s, 2H, $PMBCH_2$), 4.73 (d, *J*=10.5, 1H, $PMBCH_2$), 4.57 (d, *J*=11.6, 1H, $PMBCH_2$), 4.51 (d, *J*=8.0, 1H, $PMBCH_2$), 4.49 (d, *J*=9.6, 1H, $PMBCH_2$), 4.41 (d, *J*=10.1, 1H, H1), 3.83 - 3.79 (m, 9H, $PMBCH_3$), 3.75 (d, *J*=10.9, 1H, H6a), 3.70 (dd, *J*=10.9, 4.2, 1H, H6b), 3.56 (t, *J*=9.3, 1H, H3), 3.52 - 3.46 (m, 1H, H4), 3.46 - 3.40 (m, 1H, H5), 3.34 (t, *J*=9.6, 1H, H2). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.69 (PMB), 159.60 (PMB), 159.44 (PMB), 133.79, 131.55, 130.54, 130.30, 130.10, 129.74, 129.51, 129.20, 128.52 (133.8 - 128.5 aromatic), 114.17 (PMB), 114.10 (PMB), 114.01 (PMB), 86.17 (C1), 84.99 (C4), 79.66 (C5), 77.61 (C3), 75.72 ($PMBCH_2$), 74.89 ($PMBCH_2$), 73.33 ($PMBCH_2$), 68.64 (C6), 65.33 (C2), 55.51 ($PMBCH_3$).

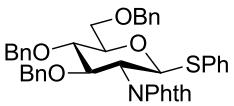
Phenyl 2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-1-thio-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (54)



To a stirring solution of **53** (32.14 g, 48.9 mmol, 1.0 eq) in dioxane/AcOH 10:1 (330 mL) at 0 °C, zinc powder (15.88 g, 244.3 mmol, 5.0 eq) was added. After stirring at r.t. for 24 h, the zinc was removed by filtering through a layer of Celite, the filtrate was concentrated, diluted with EtOAc (500 mL) and washed with H_2O (250 mL×3), Sat. $NaHCO_3$

(250 mL×2), brine (300 mL×1) and dried over $MgSO_4$. The organic solvent was removed under reduced pressure. The crude product was dissolved in dioxane/H_2O 2:1 (600 mL) and $NaHCO_3$ (9.46 g, 112.6 mmol, 2.3 eq) was then added. The mixture was stirred at room temperature for 15 min then cooled to 0 °C followed by slow addition of TrocCl (8.07 ml, 58.6 mmol, 1.2 eq). The reaction was kept stirring at r.t. for 24 h and concentrated under reduced pressure to remove most of the dioxane. The residue was extracted with EtOAc (200 mL×3), the organic layers were combined and washed with brine (300 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (toluene-ethyl acetate 20:1 to 4:1). Then recrystallized from cyclohexane-ethyl acetate to give **54**, yield 94% (37.04 g, 45.9 mmol). R_f = 0.4 (^{C}Hex/EtOAc 3:1). $[\alpha]_D^{20}$ = +12° (c = 1.0 $CHCl_3$). *ESI-MS (pos), m/z*: 822.80 ($[M+NH_4]^+$, calc. 823.20), 1629.20 ($[2M+NH_4]^+$, calc. 1630.36). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.56 - 7.47 (m, 2H, PMB), 7.30 - 7.19 (m, 7H, PMB, SPh), 7.14 (d, *J*=8.5, 2H, PMB), 6.92 - 6.80 (m, 6H, PMB), 5.09 (d, *J*=8.4, 1H, N*H*), 4.90 (d, *J*=10.2, 1H, H1), 4.80 - 4.69 (m, 4H, $PMBCH_2$, $TrocCH_2$), 4.64 (d, *J*=10.8, 1H, $PMBCH_2$), 4.56 (d, *J*=11.6, 1H, $PMBCH_2$), 4.52 (d, *J*=10.7, 1H, $PMBCH_2$), 4.48 (d, *J*=11.7, 1H, $PMBCH_2$), 3.83 - 3.77 (m, 10H, $PMBCH_3$, H3), 3.77 - 3.72 (m, 1H, H6a), 3.69 (dd, *J*=10.9, 4.6, 1H, H6b), 3.60 (t, *J*=9.1, 1H, H4), 3.52 (dd, *J*=12.1, 1.8, 1H, H5), 3.48 - 3.42 (m, 1H, H2). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.60 (PMB), 159.57 (PMB), 159.41 (PMB), 154.04 (Troc*C*=O), 132.52, 130.36, 129.99, 129.81, 129.60, 129.13, 127.92 (132.5 - 127.9 aromatic), 114.12 (PMB), 114.08 (PMB), 114.00 (PMB), 95.77 (CCl_3), 86.18 (C1), 82.01 (C3), 79.59 (C5), 78.46 (C4), 74.97 ($PMBCH_2$), 74.73 ($PMBCH_2$, $TrocCH_2$), 73.33 ($PMBCH_2$), 68.84 (C6), 56.85 (C2), 55.51 ($PMBCH_3$).

Phenyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (55)[296]

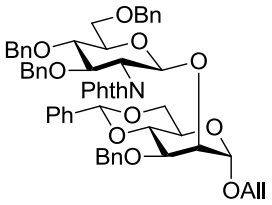


Compound **9** (50.1 g, 95.0 mmol, 1.0 eq) was dissolved in 0.08 M NaOMe in MeOH (500 mL) and was stirred at r.t. for 4 h. Then neutralized with *Dowex* 50WX8 ion-exchanger (0.5 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL×3) to give a crude product, yield 99% (37.6 g, 94.0 mmol). The deacetylated intermediate (34.8 g, 86.8 mmol, 1.0 eq) was dissolved in dry DMF (150 mL) under Ar, NaH (60% suspension, 9.89 g, 247 mmol, 2.8 eq) was added in portion and the reaction was kept at 0 °C for 40 min before BnBr (41.3 mL, 347 mmol, 4.0 eq) was added dropwise via syringe. The solvent was removed by high vacuum after 24 h stirring at r.t. and the residue was dissolved in EtOAc (800 mL), filtered through a layer of *Celite*, washed with

H_2O (500 mL×2), brine (500 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **55**, yield 80% (46.4 g, 69.1 mmol). R_f = 0.7 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +64° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 688.93 ($[M+NH_4]^+$, calc. 689.27), 1359.73 ($[2M+NH_4]^+$, calc. 1360.50). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.82 (d, *J*=6.8, 1H, Phth), 7.73 - 7.60 (m, 3H, Phth), 7.46 - 7.14 (m, 15H, CH_2*Ph*, S*Ph*), 7.00 (d, *J*=7.7, 2H, CH_2*Ph*), 6.93 - 6.82 (m, 3H, CH_2*Ph*), 5.57 (d, *J*=10.4, 1H, H1), 4.86 (d, *J*=11.0, 1H, CH_2Ph), 4.80 (d, *J*=12.0, 1H, CH_2Ph), 4.69 (d, *J*=8.3, 1H, CH_2Ph), 4.66 (d, *J*=9.4, 1H, CH_2Ph), 4.59 (d, *J*=12.0, 1H, CH_2Ph), 4.46 (d, *J*=12.0, 1H, CH_2Ph), 4.41 (dd, *J*=10.3, 8.5, 1H, H3), 4.29 (t, *J*=10.3, 1H, H2), 3.88 - 3.81 (m, 2H, H6a, H6b), 3.79 (d, *J*=8.3, 1H, H4), 3.76 - 3.70 (m, 1H, H5). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.25 (Phth C=O), 167.53 (Phth C=O), 138.52, 138.23, 138.06, 134.09 (Phth), 133.98 (Phth), 132.82, 132.40, 129.02, 128.70, 128.60, 128.30, 128.18, 128.17, 128.08, 128.01, 127.92, 127.80, 127.61 (129.0 - 127.6, aromatic), 123.67 (Phth), 123.55 (Phth), 83.53 (C1), 80.59 (C3), 79.70 (C4), 79.65 (C5), 75.24 (CH_2Ph), 75.21 (CH_2Ph), 73.70 (CH_2Ph), 69.13 (C6), 55.21 (C2).

7.9 Synthesis of complex type building blocks with GlcNAc extension

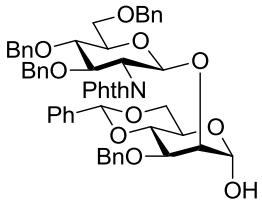
Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (56)



Compounds **55** (6.02 g, 8.96 mmol, 1.2 eq), **29** (2.96 g, 7.43 mmol, 1.0 eq), *N*-iodosuccinimide (2.08 g, 9.25 mmol, 1.25 eq) and 4 Å molecular sieves (3 g) in dry dichloromethane (30 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -18 °C followed by slow addition of trifluoromethanesulfonic acid (98.6 μL, 1.12 mmol, 0.15 eq). After stirring for 0.5 h at -18 °C, the temperature was raised to 0 °C continued for another 1h, then the reaction was quenched by diluting with more DCM (100 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (75 mL×3), brine (100 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 8:1) to give **56**. Yield: 85% (6.03 g, 6.28 mmol). R_f = 0.5 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = +25° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 977.4254 ($[M+NH_4]^+$, calc. 977.4225), 982.3814 ($[M+Na]^+$, calc. 982.3778). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.69 (s, 2H, Phth), 7.48 - 7.42 (m, 2H, Phth), 7.43 - 7.19 (m, 20H, benzyl aromatic), 7.08 (d, *J*=6.9, 2H, benzylidene acetal aromatic), 6.99 - 6.85 (m, 3H, benzylidene acetal aromatic), 5.77 (ddd, *J*=22.2, 10.9, 5.7, 1H, CH_2CH=CH_2), 5.47 (s, 1H, C*H*Ph), 5.25 (d, *J*=8.4, 1H, H1'), 5.16 (d, *J*=5.2, 1H, CH_2CH=CH_2), 5.12 (dd, *J*=13.3, 1.6, 1H, CH_2CH=CH_2), 4.91 (d, *J*=10.9, 1H, 4'-BnCH_2), 4.86 (d, *J*=12.1, 1H, 3'-BnCH_2), 4.79 (d, *J*=12.4, 1H, 3-BnCH_2), 4.71 (d, *J*=11.0, 1H, 4'-BnCH_2), 4.67 (d, *J*=12.4, 1H, 3-BnCH_2), 4.62 (q, *J*=12.0, 2H, 6'-BnCH_2), 4.55 - 4.45 (m, 3H, 3'-BnCH_2, H1, H3'), 4.38 (dd, *J*=10.8, 8.4, 1H, H2'), 4.18 - 4.14 (m, 1H, H2), 3.97 - 3.90 (m, 2H, CH_2CH=CH_2, H4), 3.87 (dd, *J*=10.0, 2.8, 1H, H3), 3.85 - 3.80 (m, 2H, H6a', H6b'), 3.79 - 3.72 (m, 3H, CH_2CH=CH_2, H5', H4'), 3.70 (dd, *J*=10.0, 4.5, 1H, H6a), 3.52 (td, *J*=9.7, 4.6, 1H, H5), 3.11 (t, *J*=10.2, 1H, H6b). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.68, 138.24, 138.14, 137.97, 137.70, 133.65 (Phth), 133.21 (CH_2*C*H=CH_2), 131.93, 128.76, 128.54, 128.42, 128.17, 128.14, 128.12, 127.97, 127.82, 127.77, 127.67, 127.63, 127.39, 127.27, 126.09 (128.8 - 126.1 aromatic), 123.01, 117.77 (CH_2CH=*C*H_2), 101.44 (*C*HPh), 97.12 (C1), 96.84 (C1'), 79.81 (C4'), 79.17 (C3'), 78.20 (C4), 75.52 (C5'), 75.16 (4'-BnCH_2), 74.85 (C2), 74.78 (3'-BnCH_2), 73.72

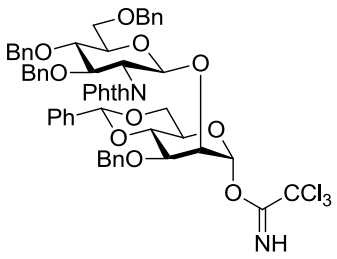
(6'-$BnCH_2$), 73.67 (C3), 71.20 (3-$BnCH_2$), 69.38 (C6'), 68.44 (C6), 68.21 (*C*H_2CH=CH_2), 63.89 (C5), 55.97 (C2').

3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranose (57)



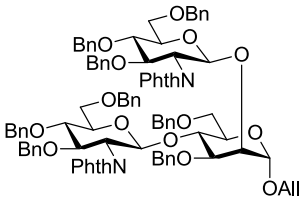
A suspension of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (228 mg, 0.27 mmol, 0.05 eq) in anhydrous THF (12 mL) was stirred under Ar atmosphere and H_2 gas was added until the orange color disappeared. The activated catalyst suspension was then added into a solution of **56** (5.17 g, 5.39 mmol, 1.0 eq) in anhydrous THF (60 mL), after 2 h stirring at r.t. a faster main product on TLC was observed, R_f = 0.6 (Tol/EtOAc 4:1). $NaHCO_3$ (1.72 g, 20.5 mmol, 3.8 eq) and H_2O (18 mL) was added to the previous reaction mixture followed with I_2 (3.45 g, 13.5 mmol, 2.5 eq), continued stirring at r.t. for 0.5 h as the deallylated product was formed. 1 M $Na_2S_2O_3$ was added until the purple color disappeared, and THF was then removed under reduced pressure. The residue was diluted with DCM (200 mL), washed with 1 M $Na_2S_2O_3$ (100 mL×2), H_2O (100 mL×2), brine (150 mL×1), dried over $MgSO_4$, the solvent was removed and crude product was purified by column chromatography (cyclohexane-ethyl acetate 8:1 to 1:1) to give **57**, yield 72% (3.57 g, 3.88 mmol). R_f = 0.1 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = -10° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 920.3353 ($[M+H]^+$, calc. 920.3646), 937.3927 ($[M+NH_4]^+$, calc. 937.3912), 942.3475 ($[M+Na]^+$, calc. 942.3465). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.67 (s, 2H, Phth), 7.46 - 7.12 (m, 22H, Phth, benzyl aromatic), 7.10 (d, *J*=7.5, 2H, benzylidene acetal aromatic), 6.90 (t, *J*=7.5, 3H, benzylidene acetal aromatic), 5.45 (s, 1H, C*H*Ph), 5.40 (d, *J*=8.5, 1H, H1'), 5.01 - 4.84 (m, 4H, H1, 4'-$BnCH_2$, 3'-$BnCH_2$, H3), 4.75 (d, *J*=14.1, 1H, 3-$BnCH_2$), 4.63 - 4.50 (m, 4H, 4'-$BnCH_2$, 3'-$BnCH_2$, 3-$BnCH_2$, 6'-$BnCH_2$), 4.43 - 4.30 (m, 3H, H2', 6'-$BnCH_2$, H2), 3.96 (t, *J*=8.7, 1H, H5'), 3.89 - 3.80 (m, 3H, H4, H3, H6a'), 3.68 - 3.51 (m, 4H, H5, H6b', H4', H6a), 3.05 (t, *J*=10.0, 1H, H6b). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.85, 138.29, 138.05, 137.25, 133.81 (Phth), 133.63, 132.12, 128.93, 128.83, 128.78, 128.76, 128.71, 128.42, 128.34, 128.19, 128.12, 128.05, 127.69, 127.46, 126.34 (128.9 - 126.3 aromatic), 101.65 (*C*HPh), 97.10 (C1'), 92.86 (C1), 80.83 (C4'), 79.55 (C3'), 78.47 (C4), 75.83 (4'-$BnCH_2$), 75.68 (C2), 75.17 (3'-$BnCH_2$), 75.06 (C5'), 73.46 (3-$BnCH_2$), 73.15 (C3), 70.89 (6'-$BnCH_2$), 69.83 (C6'), 68.88 (C6), 63.68 (C5), 56.28 (C2').

3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl trichloroacetimidate (58)



To a solution of **57** (3.47 g, 3.77 mmol, 1.0 eq) and trichloroacetonitrile (1.13 mL, 11.2 mmol, 3.0 eq) in anhydrous DCM (80 mL) under Ar at 0 °C, DBU (0.11 mL, 0.75 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:1) to give **58**, yield 90% (3.63 g, 3.41 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +49° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1080.3018 ($[M+NH_4]^+$, calc. 1080.3008), 1085.2570 ($[M+Na]^+$, calc. 1085.2562), 1101.2311 ($[M+K]^+$, calc. 1101.2301). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.49 (s, 1H, N*H*), 7.68 (d, *J*=2.3, 2H, Phth), 7.47 - 7.39 (m, 2H, Phth), 7.39 - 7.24 (m, 17H, benzyl aromatic), 7.24 - 7.16 (m, 3H, benzyl aromatic), 7.05 (d, *J*=6.7, 2H, benzylidene acetal aromatic), 6.95 - 6.81 (m, 3H, benzylidene acetal aromatic), 5.93 (d, *J*=1.5, 1H, H1), 5.46 (s, 1H, C*H*Ph), 5.37 (d, *J*=8.3, 1H, H1'), 4.89 (d, *J*=10.9, 1H, 4'-$BnCH_2$) , 4.83 (d, *J*=12.1, 1H, 3'-$BnCH_2$), 4.79 - 4.66 (m, 3H, 4'-$BnCH_2$, 3-$BnCH_2$), 4.60 (q, *J*=12.0, 2H, 6'-$BnCH_2$), 4.51 - 4.41 (m, 2H, 3'-$BnCH_2$, H3'), 4.36 (dd, *J*=10.8, 8.3, 1H, H2'), 4.20 (dd, *J*=2.9, 2.1, 1H, H2), 3.99 (t, *J*=9.8, 1H, H3), 3.89 - 3.74 (m, 5H, H4, H6a', H6a, H6b', H4'), 3.73 - 3.63 (m, 1H, H5), 3.08 (t, *J*=10.1, 1H, H6b). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 160.06, 138.39, 138.22, 138.15, 137.55, 133.98 (Phth), 131.97, 129.09, 128.74, 128.64, 128.49, 128.40, 128.34, 128.30, 128.24, 128.16, 127.99, 127.87, 127.76, 127.64, 126.24 (129.1 - 126.2 aromatic), 123.42, 101.69 (*C*HPh), 97.14 (C1'), 95.72 (C1), 90.77, 79.96 (C4'), 79.37 (C3'), 77.82 (C3), 75.82 (C5'), 75.35 (4'-$BnCH_2$), 75.07 (3'-$BnCH_2$), 73.89 (6'-$BnCH_2$), 73.45 (C2), 72.66 (C4), 71.75 (3-$BnCH_2$), 69.50 (C6'), 68.32 (C6), 66.70 (C5), 56.00 (C2').

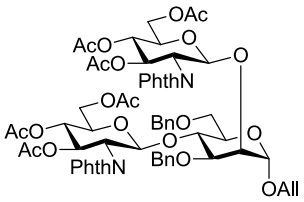
Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (59)



Compounds **55** (819 mg, 1.22 mmol, 2.2 eq), **30** (222 mg, 0.55 mmol, 1.0 eq), *N*-iodosuccinimide (312 mg, 1.39 mmol, 2.5 eq) and 4 Å molecular sieves (0.45 g) in dry dichloromethane (4.5 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -18 °C followed by

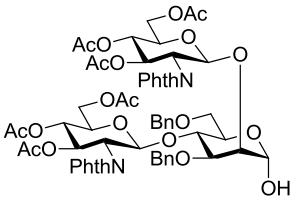
slow addition of trifluoromethanesulfonic acid (19.6 μL, 0.22 mmol, 0.4 eq). After stirring for 0.5 h at -18 °C, the temperature was raised to 0 °C continued for another 1h, then the reaction was quenched by diluting with more DCM (20 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (15 mL×3), brine (20 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **59**. Yield: 35% (293 mg, 0.19 mmol). R_f = 0.6 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +25° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1524.6299 ($[M+H]^+$, calc. 1524.6300), 1541.6448 ($[M+NH_4]^+$, calc. 1541.6566), 1546.6130 ($[M+Na]^+$, calc. 1546.6120), 1562.5881 ($[M+K]^+$, calc. 1562.5859). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.78 - 7.42 (m, 8H, Phth), 7.38 - 7.13 (m, 30H, aromatic), 6.98 (dd, *J*=11.6, 7.6, 4H, aromatic), 6.92 - 6.82 (m, 6H, aromatic), 5.71 (dq, *J*=10.4, 5.6, 1H, CH_2CH=CH_2), 5.28 (d, *J*=8.3, 1H, H1'), 5.25 (d, *J*=8.4, 1H, H1''), 5.08 - 4.98 (m, 2H, CH_2CH=CH_2), 4.85 - 4.68 (m, 5H, $BnCH_2$), 4.62 (dd, *J*=10.7, 3.8, 2H, $BnCH_2$), 4.56 - 4.36 (m, 7H, $BnCH_2$), 4.35 - 4.29 (m, 1H, H3'), 4.29 - 4.21 (m, 2H, H2', H3''), 4.12 (t, *J*=9.6, 1H, H2''), 4.07 (t, *J*=2.8, 1H, H2), 3.94 (dd, *J*=5.3, 2.4, 1H, H3), 3.86 (dd, *J*=12.8, 5.4, 1H, CH_2CH=CH_2), 3.81 - 3.57 (m, 9H, H4' , $BnCH_2$, CH_2CH=CH_2, H4'', H4, H6a', H6b', H5''), 3.54 (dd, *J*=11.2, 3.4, 1H, H6a''), 3.51 - 3.46 (m, 1H, H5), 3.40 (d, *J*=10.8, 1H, H6b''), 3.30 (d, *J*=9.8, 1H, H5'), 3.10 (d, *J*=10.9, 1H, H6a), 2.94 (dd, *J*=10.9, 6.3, 1H, H6b). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 139.31, 138.66, 138.56, 138.50, 138.43, 138.32, 138.30, 138.25, 134.14 (CH_2CH=CH_2), 133.68 (Phth), 133.61 (Phth), 131.82, 128.66, 128.61, 128.57, 128.49, 128.25, 128.22, 128.17, 128.13, 128.08, 128.06, 128.04, 128.02, 127.93, 127.73, 127.63, 127.47, 127.44, 127.40, 127.23, 127.10 (128.7 - 127.1 aromatic), 123.42 (Phth), 117.29 (CH_2CH=CH_2), 98.89 (C1''), 97.82 (C1), 96.99 (C1'), 79.93 (C4''), 79.61 (C4'), 79.50 (C3'), 79.29 (C3''), 77.10 (C3), 76.21 (C4), 75.38 (C5''), 75.19 ($BnCH_2$), 75.04 (C5'), 74.99 ($BnCH_2$), 74.92 ($BnCH_2$), 74.88 ($BnCH_2$), 74.21 (C2), 73.75 ($BnCH_2$), 73.56 ($BnCH_2$), 72.52 ($BnCH_2$), 71.41 ($BnCH_2$), 70.88 (C5), 69.68 (C6), 69.23 (C6'), 68.36 (CH_2CH=CH_2), 68.26 (C6''), 56.62 (C2''), 56.14 (C2').

Allyl 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-2-deoxy-2-phthalimido-(1→2)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (60)[297]



Compounds **8** (1269 mg, 2.65 mmol, 2.2 eq), **30** (482 mg, 1.20 mmol, 1.0 eq), *N*-iodosuccinimide (676 mg, 3.01 mmol, 2.5 eq) and 4 Å molecular sieves (1.0 g) in dry dichloromethane (10 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -0 °C followed by slow addition of trifluoromethanesulfonic acid (37 μL, 0.42 mmol, 0.35 eq). After stirring for 15 min at 0 °C, the reaction was quenched by diluting with more DCM (40 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (30 mL×3), brine (50 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **60**. Yield: 60% (970 mg, 0.72 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +13° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1235.4087 ($[M+H]^+$, calc. 1235.4084), 1252.4363 ($[M+NH_4]^+$, calc. 1252.4349), 1257.3928 ($[M+Na]^+$, calc. 1257.3903). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.72 (dd, J=4.6, 3.7, 2H, Phth), 7.60 (dd, J=5.4, 2.9, 2H, Phth), 7.49 (s, 2H, Phth), 7.38 (d, J=7.5, 2H, Phth), 7.32 (t, J=7.4, 3H, aromatic), 7.28 - 7.15 (m, 5H, aromatic), 6.86 - 6.77 (m, 2H, aromatic), 5.76 - 5.59 (m, 3H, H3', CH_2CH=CH_2, H3''), 5.50 (d, *J*=8.4, 1H, H1''), 5.45 (d, *J*=8.5, 1H, H1'), 5.19 - 4.98 (m, 4H, H4', CH_2CH=CH_2, H4''), 4.77 (d, *J*=11.7, 1H, $BnCH_2$), 4.51 (d, *J*=2.8, 1H, H1), 4.51 (d, *J*=11.8, 1H, $BnCH_2$), 4.38 (dd, *J*=10.7, 8.5, 1H, H2'), 4.17 (dd, *J*=10.7, 8.5, 1H, H2''), 4.13 - 3.99 (m, 4H, H6a', H6b', H2, H6a''), 3.89 (dd, *J*=7.3, 3.2, 1H, H3), 3.87 - 3.64 (m, 8H, H3, CH_2CH=CH_2, H4, H5', $BnCH_2$, H6b''), 3.51 - 3.42 (m, 1H, H5), 3.37 (d, *J*=10.0, 1H, H5''), 3.08 (dd, *J*=11.5, 1.4, 1H, H6a), 3.00 (dd, *J*=11.4, 5.2, 1H, H6b), 2.02 (s, 3H), 1.99 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.82 (s, 3H), 1.78 (s, 3H, 2.02 - 1.78, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 177.90, 170.93, 170.85, 170.41, 170.31, 169.67 (170.9 - 169.7 *C*O_2CH_3), 138.79, 138.34, 134.18 (Phth), 133.70 (CH_2*C*H=CH_2), 128.42, 128.18, 127.55, 127.51, 127.33, 127.29 (128.4 - 127.3 aromatic), 123.53 (Phth), 117.73 (CH_2CH=*C*H_2), 98.44 (C1''), 97.29 (C1'), 97.02 (C1), 76.69 (C3), 75.60 (C4), 74.57 (C2), 72.49 ($BnCH_2$), 72.12 (C5'), 71.69 (C5''), 71.04 (C5), 70.97 (C3', C3'', $BnCH_2$), 69.16 (C4'), 68.68 (C4''), 68.62 (C6), 68.35 (*C*H_2CH=CH_2), 62.28 (C6'), 61.65 (C6''), 60.62, 55.28 (C2''), 54.71 (C2'), 29.79, 21.25, 20.93, 20.81, 20.65, 20.60 (21.3 - 20.6 CO_2*C*H_3), 14.39.

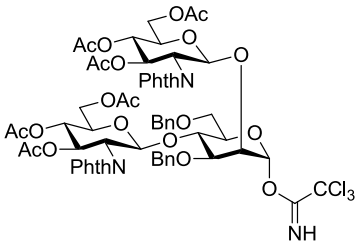
3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranose (61)



A suspension of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (140 mg, 0.17mmol, 0.25 eq) in fresh dry THF (7 mL) was stirred under Ar atmosphere and H_2 gas was added until the orange color disappeared. The activated catalyst suspension was then added into a solution of **60** (793 mg, 0.64 mmol, 1.0 eq) in anhydrous THF (8 mL) via syringe, after 2 h stirring at r.t. a faster main product was observed on TLC, R_f = 0.5 (^{C}Hex/EtOAc 1:1). $NaHCO_3$ (178 mg, 2.12 mmol, 3.3 eq) and H_2O (10 mL) was added to the previous reaction mixture followed with I_2 (356 mg, 1.39 mmol, 2.2 eq), continued stirring at r.t. for 0.5 h as the deallylated product was formed. 1 M $Na_2S_2O_3$ was added until the purple color disappeared, and THF was then removed under reduced pressure. The residue was diluted with DCM (20 mL), washed with 1 M $Na_2S_2O_3$ (10 mL×2), H_2O (10 mL×2), brine (15 mL×1), dried over $MgSO_4$, the solvent was removed and the residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **61**, yield 91% (693 mg, 0.58 mmol). R_f = 0.2 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +1° (c = 0.8, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1195.3774 ($[M+H]^+$, calc. 1195.3771), 1212.4049 ($[M+NH_4]^+$, calc. 1212.4036), 1217.3595 ($[M+Na]^+$, calc. 1217.3590). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.74 (dd, *J*=5.3, 3.2, 2H, Phth), 7.64 (dd, *J*=5.5, 3.1, 2H, Phth), 7.49 (s, 2H, Phth), 7.40 (d, *J*=7.2, 2H, Phth), 7.36 - 7.30 (m, 3H, aromatic), 7.29 - 7.16 (m, 5H, aromatic), 6.88 - 6.80 (m, 2H, aromatic), 5.77 (dd, *J*=10.8, 9.1, 1H, H3'), 5.67 (dd, *J*=10.7, 9.1, 1H, H3''), 5.59 (d, *J*=8.5, 1H, H1'), 5.45 (d, *J*=8.4, 1H, H1''), 5.18 - 5.12 (m, 1H, H4'), 5.10 - 5.03 (m, 1H, H4''), 4.90 (s, 1H, H1), 4.73 (d, *J*=11.9, 1H, $BnCH_2$), 4.55 (d, *J*=11.9, 1H, $BnCH_2$), 4.39 (dd, *J*=10.8, 8.5, 1H, H2'), 4.17 (dd, *J*=10.7, 8.4, 1H, H2''), 4.14 - 4.09 (m, 2H, H6a', H6b'), 4.08 - 4.01 (m, 2H, H2, H6a''), 3.93 - 3.78 (m, 3H, H5', H6b'', H3), 3.74 - 3.59 (m, 4H, $BnCH_2$, H4, H5), 3.41 (ddd, *J*=10.2, 3.6, 2.5, 1H, H5''), 3.13 - 3.03 (m, 1H, H6a), 2.65 (dd, *J*=10.4, 6.0, 1H, H6b), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.83 (s, 4H), 1.79 (s, 3H, 1.99 - 1.79, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.91, 170.86, 170.28, 170.25, 169.67, 169.62 (170.9 - 169.6 CO_2CH_3), 138.74, 137.97, 134.20 (Phth), 131.56, 128.60, 128.41, 128.36, 128.29, 127.70, 127.63, 127.57, 127.52, 127.26 (128.6 - 127.3 aromatic), 123.58 (Phth), 97.82 (C1''), 96.49 (C1'), 91.61 (C1), 75.43 (C4), 75.12 (C3), 74.13

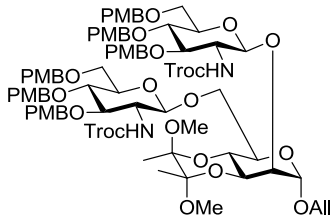
(C2), 72.73 ($BnCH_2$), 72.09 (C5'), 71.69 (C5''), 70.97 (C3''), 70.85 (C3', $BnCH_2$), 70.58 (C5), 69.38 (C6), 69.22 (C4'), 68.79 (C4''), 62.23 (C6'), 61.70 (C6''), 55.31 (C2''), 54.72 (C2'), 20.93, 20.82, 20.67, 20.61 (20.9 - 20.6 CO_2CH_3).

3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3,6-di-*O*-benzyl -α-D-mannopyranosyl trichloroacetimidate (62)



To a solution of **61** (645 mg, 0.54 mmol, 1.0 eq) and trichloroacetonitrile (161 μL, 1.61 mmol, 3.0 eq) in anhydrous DCM (6.5 mL) under Ar at 0 °C, DBU (16 μL, 0.11 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:1) to give **62**, yield 87% (630 mg, 0.47 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +29° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1355.3133 ($[M+NH_4]^+$, calc. 1355.3133), 1360.2717 ($[M+Na]^+$, calc. 1380.2687). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.42 (s, 1H, N*H*), 7.68 (s, 2H, Phth), 7.59 (s, 2H, Phth), 7.47 (s, 2H, Phth), 7.40 (d, *J*=7.8, 2H, Phth), 7.33 (t, *J*=7.6, 3H, aromatic), 7.30 - 7.16 (m, 5H, aromatic), 6.77 (d, *J*=3.4, 2H, aromatic), 5.99 (d, *J*=2.0, 1H, H1), 5.69 (dt, *J*=19.4, 9.9, 2H, H3', H3''), 5.58 (d, *J*=8.4, 1H, H1'), 5.53 (d, *J*=8.4, 1H, H1''), 5.16 (t, *J*=9.6, 1H, H4'), 5.08 (t, *J*=9.6, 1H, H4''), 4.80 (d, *J*=11.9, 1H, $BnCH_2$), 4.59 (d, *J*=11.9, 1H, $BnCH_2$), 4.43 (dd, *J*=10.5, 8.7, 1H, H2'), 4.25 - 4.14 (m, 3H, H2'', H2, H6a'), 4.14 - 4.06 (m, 2H, H6', H6a''), 4.04 - 3.97 (m, 1H, H3), 3.90 (dd, *J*=7.3, 2.6, 1H, H4), 3.83 (d, *J*=10.1, 1H, H5'), 3.77 - 3.69 (m, 3H, H6b'', $BnCH_2$), 3.68 - 3.63 (m, 1H, H5), 3.41 (d, *J*=10.1, 1H, H5''), 3.09 (s, 2H, H6a, H6b), 2.03 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.82 (s, 3H), 1.78 (s, 3H, 2.03 - 1.78, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.84, 170.75, 170.32, 170.23, 169.63, 169.60 (170.8 - 169.6 CO_2CH_3), 160.34 (*C*=NH), 138.36, 138.12, 134.17 (Phth), 131.40, 128.53, 128.20, 127.71, 127.63, 127.61, 127.38 (128.5 - 127.4 aromatic), 123.63 (Phth), 98.62 (C1''), 97.33 (C1'), 96.16 (C1), 90.92 (CCl_3), 75.86 (C4), 74.78 (C3), 73.96 (C5), 72.92 (C2), 72.55 ($BnCH_2$), 72.30 (C5'), 71.81 (C5''), 71.18 ($BnCH_2$), 71.01 (C3'), 70.98 (C3''), 69.05 (C4'), 68.64 (C4''), 68.01 (C6), 62.15 (C6'), 61.59 (C6''), 55.33 (C2''), 54.66 (C2'), 31.87, 21.25, 20.93, 20.82, 20.62, 20.59, 19.47 (21.3 - 19.5 CO_2CH_3), 14.41, 14.11.

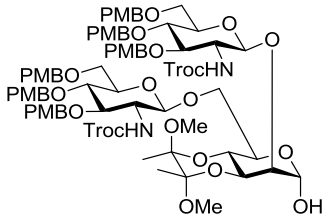
Allyl 2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-[2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→6)]-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranoside (63)



Compounds **54** (5.00 g, 6.19 mmol, 2.2 eq), **31** (941 mg, 2.81 mmol, 1.0 eq), *N*-iodosuccinimide (1.57 g, 7.04 mmol, 2.5 eq) and 4 Å molecular sieves (2.0 g) in dry dichloromethane (20 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -0 °C followed by slow addition of trifluoromethanesulfonic acid (49.8 μL, 0.65 mmol, 0.2 eq). After stirring for 15 min at 0 °C, the reaction was quenched by diluting with more DCM (100 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (50 mL×3), brine (50 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **63**. Yield: 55% (2.69 g, 1.56 mmol). R_f = 0.3 (Tol/EtOAc 3:1). $[\alpha]_D^{20}$ = +39° (c = 0.8, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1744.4860 ($[M+NH_4]^+$, calc. 1744.4853), 1749.4977 ($[M+Na]^+$, calc. 1749.4407), 881.2613 ($[M+2NH_4]^{2+}$, calc. 881.2599), 883.7098 ($[M+Na+NH_4]^{2+}$, calc. 883.7376). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.32 - 7.17 (m, 8H, aromatic), 7.11 (dd, *J*=10.2, 8.7, 4H, aromatic), 6.90 - 6.76 (m, 12H, aromatic), 6.35 (s, 1H, NH'), 5.84 (ddd, *J*=22.3, 10.8, 5.6, 1H, CH_2CH=CH_2), 5.24 (dd, *J*=17.2, 1.6, 1H, CH_2CH=CH_2), 5.15 (dd, *J*=10.4, 1.3, 1H, CH_2CH=CH_2), 5.03 (s, 1H, NH''), 5.02 (d, *J*=8.4, 1H, H1'), 4.93 (d, *J*=12.2, 1H, $PMBCH_2$), 4.83 (s, 1H, H1), 4.80 - 4.63 (m, 8H, $TrocCH_2$, $PMBCH_2$), 4.60 (d, *J*=9.4, 1H, H1''), 4.58 - 4.42 (m, 9H, H3' , H3'', $TrocCH_2$, $PMBCH_2$), 4.18 - 4.13 (m, 1H, CH_2CH=CH_2), 4.13 - 4.08 (m, 1H, H2), 4.08 - 4.04 (m, 1H, H3), 3.97 - 3.85 (m, 3H, H4, CH_2CH=CH_2, H5), 3.80 - 3.77 (m, 18H, $PMBOCH_3$), 3.74 - 3.64 (m, 6H, H6a, H6b, H6a', H6b', H6a'', H6b''), 3.64 - 3.57 (m, 1H, H4''), 3.57 - 3.49 (m, 3H, H2''. H5'', H4'), 3.48 - 3.44 (m, 1H, H5'), 3.26 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 1.36 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.54, 159.50, 159.45, 159.41, 159.39 (159.5 - 159.4 PMB), 154.49 (Troc), 154.27 (Troc), 134.02 (CH_2CH=CH_2), 131.10, 130.77, 130.60, 130.46, 130.38, 129.83, 129.79, 129.72, 129.69, 129.61, 129.55 (131.1 - 129.6 aromatic), 117.66 (CH_2CH=CH_2), 114.05 (PMB), 113.99 (PMB), 100.95 (C1''), 100.40 ($C(CH_3)OCH_3$), 99.76 ($C(CH_3)OCH_3$), 99.00 (C1), 97.37 (C1'), 96.17 (Troc), 95.76 (Troc), 80.66 (C3''), 79.18 (C3'), 78.64 (C4'), 78.35 (C4''), 75.71 (C5''),

75.27 (C5'), 74.71, 74.65, 74.56, 74.52, 74.36, 73.39, 73.31 (74.7 - 73.3 $TrocCH_2$, $PMBCH_2$), 70.91 (C2, C5), 68.68 (C6', C6''), 68.09 ($CH_2CH=CH_2$), 67.78 (C6), 67.43 (C3), 64.00 (C4), 57.74 (C2'), 57.62 (C2''), 55.49 ($PMBOCH_3$), 55.47 ($PMBOCH_3$), 48.51 (OCH_3), 48.23 (OCH_3), 17.98 (CH_3), 17.92 (CH_3).

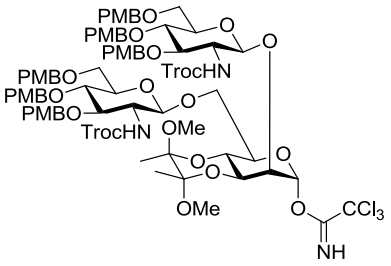
2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-[2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→6)]-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranose (64)



A suspension of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (258 mg, 0.31mmol, 0.15 eq) in anhydrous THF (12.5 mL) was stirred under Ar atmosphere and H_2 gas was added until the orange color disappeared. The activated catalyst suspension was then added into a solution of **63** (3.51 g, 2.03 mmol, 1.0 eq) in anhydrous THF (25 mL) via syringe, after 24 h stirring at r.t. a faster main product was observed. $NaHCO_3$ (0.64 g, 7.62 mmol, 3.8 eq) and H_2O (10 mL) was added to the reaction mixture followed with I_2 (1.29 g, 5.04 mmol, 2.5 eq) and continued stirring at r.t. for 0.5 h as the deallylated product was formed. 1 M $Na_2S_2O_3$ was added until the purple color disappeared, and THF was then removed under reduced pressure. The residue was diluted with DCM (100 mL), washed with 1 M $Na_2S_2O_3$ (50 mL×2), H_2O (50 mL×2), brine (75 mL×1), dried over $MgSO_4$, the solvent was removed and the residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **64**, yield 93% (3.20 g, 1.90 mmol). R_f = 0.3 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +42° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1687.4352 ($[M+H]^+$, calc. 1687.4275), 1704.4615 ($[M+NH_4]^+$, calc. 1704.4540), 861.2448 ($[M+2NH_4]^{2+}$, calc. 861.2442), 863.6932 ($[M+Na+NH_4]^{2+}$, calc. 863.7219). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.34 - 7.22 (m, 8H, aromatic), 7.20 - 7.03 (m, 4H, aromatic), 6.91 - 6.78 (m, 12H, aromatic), 6.29 (s, 1H, N*H*'), 5.22 (d, *J*=38.6, 1H, H1), 5.06 (d, *J*=4.2, 1H, H1'), 4.98 (d, *J*=11.7, 1H, $PMBCH_2$), 4.81 - 4.67 (m, 9H, H1'', $PMBCH_2$, $TrocCH_2$), 4.63 - 4.40 (m, 9H, H3', H3'', $PMBCH_2$), 4.25 - 4.14 (m, 3H, H2, H3, H5), 3.87 - 3.76 (m, 19H, H4, $PMBOCH_3$), 3.75 - 3.47 (m, 11H, H2'', H5', H5'', H4'', H4', H6a, H6b, H6a', H6b', H6a'', H6b''), 3.34 - 3.21 (m, 7H, H2', OCH_3), 1.41 (s, 3H, CH_3), 1.30 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.29, 159.25, 159.18 (159.3 - 159.2 PMB), 154.52 (Troc), 154.21 (Troc), 130.51, 130.31, 130.12,

129.76, 129.68, 129.57, 129.53, 129.45, 129.38 (130.5 - 129.4 aromatic), 113.87 (PMB), 113.80 (PMB), 113.78 (PMB), 100.16 ($C(CH_3)OCH_3$), 99.54 ($C(CH_3)OCH_3$), 97.30 (C1'), 95.21 (C1), 93.84 (C1''), 78.87 (C3'), 78.72 (C3''), 78.42 (C4'), 78.13 (C4''), 75.39 (C5'), 74.94 (C5''), 74.65, 74.46, 74.40, 74.28, 74.12, 73.19, 73.07, 72.99 (74.7 - 73.0 $TrocCH_2$, $PMBCH_2$), 72.06 (C5), 71.47 (C2), 68.42 (C6), 68.31 (C6'), 68.18 (C6''), 66.84 (C3), 64.72 (C4), 57.69 (C2''), 57.43 (C2'), 55.28 ($PMBOCH_3$), 55.24 ($PMBOCH_3$), 48.45 (OCH_3), 48.11 (OCH_3), 17.74 (CH_3), 17.69 (CH_3).

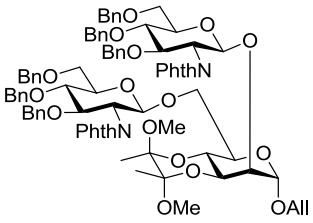
2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-[2-deoxy-3,4,6-tri-*O*-*p*-methoxybenzyl-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→6)]-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl trichloroacetimidate (65)



To a solution of **64** (3.09 g, 1.83 mmol, 1.0 eq) and trichloroacetonitrile (542 μL, 5.42 mmol, 3.0 eq) in anhydrous DCM (30 mL) under Ar at 0 °C, DBU (54.3 μL, 0.37 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **65**, yield 85% (2.87 g, 1.56 mmol). R_f = 0.6 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +42° (c = 1.4, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1849.3727 ($[M+NH_4]^+$, calc. 1849.3607), 1854.3125 ($[M+Na]^+$, calc. 1854.3161), 925.6590 ($[M+H+NH_4]^{2+}$, calc. 925.6860), 927.6561 ($[M+Na+H]^{2+}$, calc. 927.6620), 933.6576 ($[M+2NH_4]^{2+}$, calc. 933.6467), 936.1464 ($[M+Na+NH_4]^{2+}$, calc. 936.1753), 938.6557 ($[M+2Na]^{2+}$, calc. 938.6530). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.54 (s, 1H, C=N*H*), 7.32 - 7.17 (m, 8H, aromatic), 7.10 (dd, *J*=20.2, 8.6, 4H, aromatic), 6.94 - 6.74 (m, 2H, aromatic), 6.35 (s, 1H, N*H*'), 6.18 (s, 1H, H1), 5.07 (d, *J*=7.9, 2H, H1', N*H*''), 4.96 (d, *J*=11.9, 1H, $PMBCH_2$), 4.76 - 4.63 (m, 8H, $PMBCH_2$), 4.60 (d, *J*=5.7, 1H, H1''), 4.58 - 4.40 (m, 9H, H3', H3'', $PMBCH_2$), 4.23 (s, 1H, H2), 4.12 (s, 1H, H6a), 4.09 (s, 1H, H3), 4.06 - 3.98 (m, 2H, H4, H5), 3.81 - 3.76 (m, 18H, $PMBOCH_3$), 3.75 - 3.44 (m, 10H, H6b, H2'', H6a', H6b', H6a'', H6b'', H4'', H4', H5', H5''), 3.25 - 3.20 (m, 7H, H2', OCH_3), 1.35 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.44 (PMB), 159.40 (PMB), 131.02, 130.77, 130.41, 129.82, 129.74, 129.52 (131.0 - 129.5 aromatic), 113.98 (PMB), 100.82 (C1''), 100.42 ($C(CH_3)OCH_3$), 99.82 ($C(CH_3)OCH_3$), 97.54 (C1'), 97.46 (C1), 79.22 (C3'), 79.15 (C3''), 78.53 (C4''), 78.30 (C4'), 75.84 (C5'), 75.21

(C5''), 74.66, 74.57, 74.49, 74.43, 74.32 (C5), 73.44, 73.27 (74.7 - 73.3 $TrocCH_2$, $PMBCH_2$), 69.93 (C2), 68.68 (C6'), 68.59 (C6''), 67.02 (C3), 66.89 (C6), 63.13 (C4), 57.79 (C2), 57.43 (C2''), 55.49 ($PMBOCH_3$), 55.46 ($PMBOCH_3$), 48.28 (OCH_3), 17.97 (CH_3), 17.86 (CH_3).

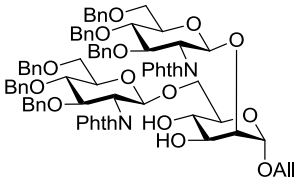
Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranoside (66)



Compounds **55** (871 mg, 1.30 mmol, 2.2 eq), **31** (222 mg, 0.59 mmol, 1.0 eq), *N*-iodosuccinimide (331 mg, 1.48 mmol, 2.5 eq) and 4 Å molecular sieves (0.4 g) in dry dichloromethane (4.0 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -0 °C followed by slow addition of trifluoromethanesulfonic acid (20.8 μL, 0.24 mmol, 0.4 eq). After stirring for 2 h at 0 °C, the reaction was quenched by diluting with more DCM (20 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (15 mL×3), brine (20 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **66**. Yield: 73% (629 mg, 0.43 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +77° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1474.6299 ($[M+H]^+$, calc. 1474.6274), 1479.5822 ($[M+NH_4]^+$, calc. 1479.5828), 1491.6283 ($[M+NH_4+NH_3]^+$, calc. 1491.6540), 748.7799 ($[M+Na+NH_4]^{2+}$, calc. 748.8086). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.64 (s, 8H, Phth), 7.40 - 7.21 (m, 20H, aromatic), 6.99 (d, *J*=7.6, 4H, aromatic), 6.93 - 6.84 (m, 6H, aromatic), 5.46 (qd, *J*=11.2, 5.7, 1H, CH_2CH=CH_2), 5.21 (d, *J*=8.3, 1H, H1'), 4.95 - 4.88 (m, 2H, CH_2CH=CH_2), 4.85 - 4.73 (m, 5H, H1'', $BnCH_2$), 4.68 - 4.61 (m, 4H, $BnCH_2$), 4.57 (dd, *J*=12.1, 5.9, 2H, $BnCH_2$), 4.42 (dd, *J*=12.1, 3.2, 2H, $BnCH_2$), 4.36 (s, 1H, H1), 4.36 (t, *J*=10.0, 1H, H3''), 4.32 - 4.25 (m, 1H, H3'), 4.27 - 4.20 (m, 1H, H2'), 4.08 (dd, *J*=10.9, 8.5, 1H, H2''), 3.85 - 3.68 (m, 9H, H3, H6a, H6a', H6b', H6a'', H6b'', H2, H4', H4''), 3.60 - 3.45 (m, 5H, CH_2CH=CH_2, H5, H5', H5''), 3.36 (t, *J*=10.5, 1H, H4), 3.15 (ddd, *J*=13.1, 6.1, 1.0, 1H, CH_2CH=CH_2), 3.10 (s, 3H, OCH_3), 2.96 (s, 3H, OCH_3), 2.61 (dd, *J*=10.3, 8.7, 1H, H6b), 1.10 (s, 3H, CH_3), 0.97 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.51, 138.48, 138.36, 138.33, 138.25, 133.89 (Phth), 133.81 (Phth), 133.45 (CH_2CH=CH_2), 132.05, 128.68, 128.61, 128.58, 128.57, 128.21, 128.16, 128.13, 128.07, 127.96, 127.94, 127.88, 127.81, 127.79, 127.46 (128.7 - 127.5 aromatic), 123.35 (Phth), 117.50 (CH_2CH=CH_2), 99.93 ($C(CH_3)OCH_3$), 99.44

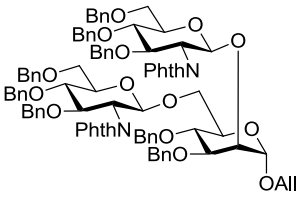
(*C*(CH_3)OCH_3), 98.71 (C1''), 96.35 (C1), 96.30 (C1'), 79.91 (C4''), 79.61 (C4'), 79.58 (C3''), 79.50 (C3'), 75.84 (C5'), 75.33 (C5''), 75.20 ($BnCH_2$), 75.08 ($BnCH_2$), 74.99 ($BnCH_2$), 74.78 ($BnCH_2$), 73.74 ($BnCH_2$), 73.62 (C2), 70.54 (C5), 68.93 (C6'), 68.84 (C6), 68.55 (C6''), 67.24 (*C*H_2CH=CH_2), 67.02 (C3), 63.83 (C4), 56.23 (C2''), 55.18 (C2'), 47.97 (OCH_3), 47.57 (OCH_3), 27.15, 17.86 (CH_3), 17.59 (CH_3).

Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-α-D-mannopyranoside (67)



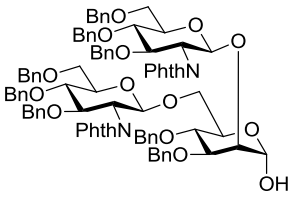
Compound **66** (663 mg, 0.45 mmol) was dissolved in TFA/TIPS/H_2O 15:0.9:0.9 (3.36 mL) and stirred at r.t. for 2 h. The solution was then concentrated and coevaporated three times with toluene, followed by purification by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:2) to give **67**. Yield: 51% (310 mg, 0.23 mmol). R_f = 0.2 (^{C}Hex/EtOAc 1:1). *MALDI-TOF-MS* (Bruker autoflex II MALDI spectrometer): 1367.494 ($[M+Na]^+$, calc. 1366.518), 1383.531 ($[M+K]^+$, calc. 1382. 492).

Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranoside (68)



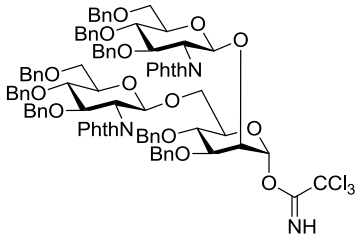
Compound **67** (310 mg, 0.23 mmol, 1.0 eq) was dissolved in dry DMF (2 mL) under Ar and NaH (60% suspension, 27.7 mg, 0.69 mmol, 3.0 eq) was added in portions. The reaction mixture was kept at 0 °C for 40 min before BnBr (82.3 μL, 0.69 mmol, 3.0 eq) was added dropwise via syringe. After 24 h stirring at r.t., the solvent was removed by evaporation under reduced pressure and the residue was dissolved in EtOAc (10 mL), filtered through a layer of Celite, washed with H_2O (10 mL×2), brine (10 mL×1), dried over $MgSO_4$. Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **68**, yield 83% (291 mg, 0.19 mmol). R_f = 0.7 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +12 (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1524.6307 ($[M+H]^+$, calc. 1524.6300), 1541.6596 ($[M+NH_4]^+$, calc. 1541.6566), 1545.6062 ($[M+Na]^+$, calc. 1545.6068).

3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranose (69)



A suspension of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (24 mg, 0.028 mmol, 0.15 eq) in anhydrous THF (1.2 mL) was stirred under Ar atmosphere and H_2 gas was added until the orange color disappeared. The activated catalyst suspension was then added into a solution of **68** (290 mg, 0.19 mmol, 1.0 eq) in anhydrous THF (1.2 mL) via syringe. After stirring for 24 h at r.t., a faster major product was observed on TLC. $NaHCO_3$ (60 mg, 0.71 mmol, 3.8 eq) and H_2O (0.6 mL) was added to the reaction mixture followed with I_2 (120 mg, 0.47 mmol, 2.5 eq), continued stirring at r.t. for 0.5 h as the deallylated product was formed. 1 M $Na_2S_2O_3$ was added until the purple color disappeared, and THF was then removed by evaporation under reduced pressure. The residue was diluted with DCM (10 mL), washed with 1 M $Na_2S_2O_3$ (5 mL×2), H_2O (5 mL×2), brine (7.5 mL×1), dried over $MgSO_4$. The solvent was removed and the residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **69**, yield 60% (170 mg, 0.11 mmol). R_f = 0.3 (^{C}Hex/EtOAc 1:1). *MALDI-TOF-MS* (Bruker autoflex II MALDI spectrometer): 1507.161 ($[M+Na]^+$, calc. 1506.581), 1523.207 ($[M+K]^+$, calc. 1522.555).

3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (70)

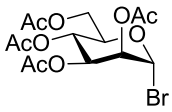


To a stirred solution of **69** (170 mg, 0.11 mmol, 1.0 eq) and trichloroacetonitrile (34.4 μL, 0.34 mmol, 3.0 eq) in anhydrous DCM (2 mL) under Ar at 0 °C, DBU (3.4 μL, 0.023 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:1) and recrystallized from cyclohexane-ethyl acetate to give **70**, yield 81% (145 mg, 0.089 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +12 (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 824.5727 ($[M+H+Na]^+$, calc. 824.7474), 831.4875 ($[M+2NH_4]^{2+}$, calc. 931.2847). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.98 (s, 1H, N*H*), 7.71 (s, 4H, Phth), 7.59 (s, 4H, Phth), 7.40 - 7.12 (m, 30H, aromatic), 7.04 (d, *J*=6.5, 2H, aromatic), 7.01 - 6.96 (m, 2H, aromatic), 6.95 - 6.85 (m, 6H, aromatic), 5.78 (d, *J*=1.8, 1H, H1), 5.33 (d, *J*=8.2, 1H, H1'), 4.91 -

4.73 (m, 4H, $BnCH_2$), 4.71 - 4.58 (m, , 5H, H1'', $BnCH_2$), 4.57 - 4.51 (m, 2H, $BnCH_2$), 4.47 (dd, *J*=11.9, 4.2, 2H, $BnCH_2$), 4.43 - 4.28 (m, 3H, H3', H2', $BnCH_2$), 4.26 - 4.19 (m, 2H, H3'', $BnCH_2$), 4.14 - 4.10 (m, 1H, H2), 3.97 (dd, *J*=10.7, 8.5, 1H, H2''), 3.81 - 3.64 (m, 9H, H4', H4, H4'', H3, H6a, H6a', H6b', H6a'', H6b''), 3.63 - 3.57 (m, 1H, H5), 3.40 (dt, *J*=10.1, 3.0, 1H, H5'), 3.29 (t, *J*=9.4, 1H, H5''), 2.66 (dd, *J*=10.7, 7.1, 1H, H6b). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.72 (*C*=NH), 138.28, 138.19, 138.17, 138.13, 138.11, 138.09, 138.07, 137.95, 133.81 (Phth), 133.53, 131.73, 128.57, 128.56, 128.51, 128.47, 128.40, 128.34, 128.31, 128.28, 128.17, 128.11, 128.09, 128.04, 127.95, 127.88, 127.80, 127.76, 127.74, 127.70, 127.61, 127.46, 127.38 (128.6 - 127.4 aromatic),, 123.37 (Phth), 98.89 (C1''), 96.62 (C1'), 94.99 (C1), 90.69 (*C*Cl_3), 79.78 (C4'), 79.65 (C4''), 79.52 (C3'), 79.41 (C3''), 76.69 (C3), 75.36 (C4), 75.09 (C5'), 75.01 ($BnCH_2$), 74.91 ($BnCH_2$), 74.83 ($BnCH_2$), 74.77 ($BnCH_2$), 74.31 (C5''), 73.79 (C5), 73.65 ($BnCH_2$), 73.60 ($BnCH_2$), 71.38 (C2), 70.86 ($BnCH_2$), 69.73 (C6), 69.41 (C6'), 68.78 (C6''), 55.74 (C2''), 55.72 (C2').

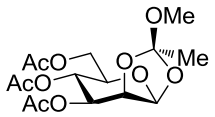
7.10 Synthesis of 2-OAc-3,4,6-OBn mannosyl donor

2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosylbromide (71)[193]



Acetic anhydride (330 mL, 3.5 mol, 7.0 eq) at was heated to 90 °C and I_2 (4.8 g, 19 mmol, 0.038 eq) was then added. After the heating was removed, mannose (90 g, 0.5 mol, 1.0 eq) was added in portions to the reaction mixture. After 30 min of stirring the reaction was completed. Sat. $Na_2S_2O_3$ (45 mL) was added to neutralize the remaining I_2 and the mixture was concentrated under reduced pressure. The residue was diluted with DCM (1 L), washed with Sat. $NaHCO_3$ (300 mL×3), H_2O (300 mL×3), brine (500 mL×1), dried over $MgSO_4$ and the solvent was removed to give penta-acetylated mannose. R_f = 0.3 (CHex/EtOAc 2:1). The acetylated product was dissolved in glacial AcOH (270 mL) and the stirred solution was cooled with an ice bath. 30% HBr in AcOH (112 mL, 0.65 mol, 1.3 eq) was added. After stirring at r.t. for 24 h, the reaction mixture was concentrated under reduced pressure to remove most of the AcOH and HBr at 10 – 15 °C. The residue was washed with H_2O (200 mL×3), and the aqueous layer was re-extracted with DCM (300 mL×3), the combined organic layers were washed with Sat. $NaHCO_3$ (300 mL×3), brine (500 mL×1), dried over $MgSO_4$. The solvent was removed at low temperature and to give **71**, yield 70% (144.7g, 352 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1).). $[\alpha]_D^{20}$ = +125° (c = 1.0, $CHCl_3$). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.39 (dd, *J*=10.1, 3.4, 1H, H2), 5.28 (dd, *J*=6.7, 3.2, 1H, H4), 5.26 - 5.22 (m, 1H, H3), 5.21 (s, 1H, H1), 4.26 - 4.18 (m, 2H, H5, H6a), 4.15 - 4.08 (m, 1H, H6b), 2.13 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.97 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.15, 170.49, 170.33, 170.08 (171.2 - 170.1 CO_2CH_3), 92.33 (C1), 70.33 (C3), 69.04 (C2), 68.61 (C5), 66.43 (C4), 62.82 (C6), 21.10, 20.95, 20.90, 20.89 (21.1 - 20.9 CO_2CH_3).

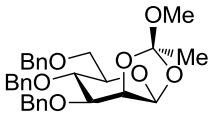
3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (72)[193]



Compound **71** (150.6 g, 365 mmol) and 2,4,6-collidine (88.5 g, 97.2 mL, 730 mmol, 2.0 eq) was dissolved in a methanol/chloroform 1:4 mixture (500 mL), stirred at r.t. for 24 h. The reaction was diluted with DCM (400mL), washed with H_2O (300 mL×3), brine (500 mL×1), dried over $MgSO_4$, the solvent was removed and the crude product was dried at reduced pressure to remove the remaining collidine. Then the residue was recrystallized from cyclohexane-ethyl acetate to give **72**, yield 81% (103.3 g, 285 mmol). R_f = 0.4 (Tol/EtOAc

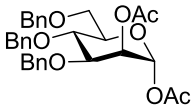
2:1). $[\alpha]_D^{20}$ = -20° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 379.67 ($[M+NH_4]^+$, calc. 380.16), 385.00 ($[M+Na]^+$, calc. 385.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.48 (d, *J*=2.6, 1H, H1), 5.28 (t, *J*=9.7, 1H, H4), 5.13 (dd, *J*=9.9, 4.0, 1H, H3), 4.60 (dd, *J*=3.9, 2.6, 1H, H2), 4.22 (dd, *J*=12.1, 4.9, 1H, H6a), 4.13 (dd, *J*=12.1, 2.7, 1H, H6b), 3.67 (ddd, *J*=9.5, 4.9, 2.7, 1H, H5), 3.26 (s, 3H, OCH_3), 2.10 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.72 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.82, 170.55, 169.61 (170.8 - 169.6 CO_2CH_3), 124.69 ($COCH_3(CH_3)$), 97.59 (C1), 76.78 (C2), 71.55 (C5), 70.82 (C3), 65.73 (C4), 62.53 (C6), 50.11 (OCH_3), 24.55 (CH_3), 20.94, 20.90, 20.86 (20.9 - 20.8 CO_2CH_3).

3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (73)[193]



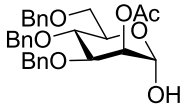
To a stirring mixture of **72** (18.9 g, 52.2 mmol, 1.0 eq) in MeOH (80 mL), anhydrous K_2CO_3 (629 mg, 4.6 mmol, 0.087 eq) was added. The reaction mixture was stirred at r.t. for 30 min, then concentrated and coevaporated with toluene (80 mL×3) and dried under reduced pressure. The residue was dissolved in dry DMF (150 mL), stirred under Ar and NaH (60% suspension, 8.33 g, 208 mmol, 4.0 eq) was then added in portions. The reaction was kept at 0 °C for 40 min, before BnBr (30.9 mL, 260 mmol, 5.0 eq) was added dropwise. After stirring at r.t. for 2 h, the organic solvent was removed under reduced pressure and the residue was dissolved in EtOAc (400 mL), filtered through a layer of Celite, washed with H_2O (150 mL×2), brine (150 mL×1), dried over $MgSO_4$. Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **73**, yield 88% (23.7 g, 46.2 mmol). R_f = 0.7 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +33° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 528.73 ($[M+Na]^+$, calc. 529.22). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.44 - 7.23 (m, 15H, aromatic), 5.35 (d, *J*=2.5, 1H, H1), 4.90 (d, *J*=10.8, 1H, $BnCH_2$), 4.79 (s, 2H, $BnCH_2$), 4.64 - 4.53 (m, 3H, $BnCH_2$), 4.41 (dd, *J*=3.9, 2.6, 1H, H2), 3.93 (t, *J*=9.3, 1H, H4), 3.79 - 3.68 (m, 3H, H3, H6a, H6b), 3.46 - 3.40 (m, 1H, H5), 3.29 (s, 3H, OCH_3), 1.75 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.47, 138.08, 128.78, 128.75, 128.71, 128.62, 128.53, 128.26, 128.22, 127.99, 127.86, 127.75, 127.73, 127.57, 127.19 (128.8 - 127.2 aromatic), 124.22, 97.80 (C1), 79.27 (C3), 77.36 (C2), 75.46 ($BnCH_2$), 74.46 (C4), 74.42 (C5), 73.61 ($BnCH_2$), 72.60 ($BnCH_2$), 69.27 (C6), 49.99 (OCH_3), 24.62 (CH_3).

1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranose (74)[194]



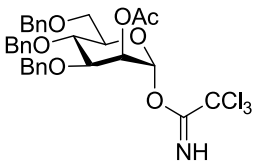
Compound **73** (74 g, 146 mmol) was dissolved in AcOH/H_2O 1.54:1 (740 mL : 480 mL) and stirred at r. t. for 24 h. The reaction was concentrated and diluted with DCM (500 mL), washed with Sat. $NaHCO_3$ (200 mL×3), H_2O (250 mL×2), brine (300 mL×1), dried over $MgSO_4$. The solvent was removed and crude product was identified as a mixture of **74** and **75**, which were separated by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1). to give **75**, yield 11% (7.8 g, 15.8 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1) and a major product of **74**, yield 77% (60.2 g, 113 mmol), R_f = 0.6 (^{C}Hex/EtOAc 2:1). *ESI-MS (pos), m/z*: 557.22 ($[M+Na]^+$, calc. 557.22). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.40 - 7.15 (m, 15H, aromatic), 6.13 (d, J=2.0, 1H, H1), 5.37 (t, J=2.4, 1H, H2), 4.87 (d, J=10.6, 1H, $BnCH_2$), 4.74 (d, J=11.2, 1H, $BnCH_2$), 4.69 (d, J=12.1, 1H, $BnCH_2$), 4.60 - 4.49 (m, 3H, $BnCH_2$), 4.01 - 3.94 (m, 2H, H4, H3), 3.89 - 3.79 (m, 2H, H5, H6a), 3.70 (dd, J=10.9, 1.6, 1H, H6b), 2.17 (s, 3H, Ac), 2.08 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.31 (CO_2CH_3), 168.57 (CO_2CH_3), 138.38, 137.89, 128.67, 128.60, 128.56, 128.33, 128.18, 128.11, 128.04, 127.98, 127.85 (128.7 - 127.9 aromatic), 91.53 (C1), 77.92 (C3), 75.59 ($BnCH_2$), 74.09 (C5), 73.96 (C4), 73.78 ($BnCH_2$), 72.19 ($BnCH_2$), 68.74 (C6), 67.78 (C2), 27.15, 21.21 (CO_2CH_3), 21.13 (CO_2CH_3).

2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranose (75)[194]



Compound **74** (60.2 g, 113 mmol, 1.0 eq) was dissolved in dry DMF (120 mL) and hydrazine acetate (11.4 g, 124 mmol, 1.1 eq) was added. The reaction was heated at 50 °C for 3 h, then concentrated and coevaporated with toluene (200 mL×3) and dried under reduced pressure to give **75**, yield 98% (54.8 g, 111 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +28° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 509.93 ($[M+NH_4]^+$, calc. 510.25), 515.13 ($[M+Na]^+$, calc. 515.20). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.40 - 7.16 (m, 15H, aromatic), 5.38 (dd, *J*=3.2, 1.9, 1H, H2), 5.21 (s, 1H, H1), 4.89 (d, *J*=10.9, 1H, $BnCH_2$), 4.72 (d, *J*=11.2, 1H, $BnCH_2$), 4.62 (d, *J*=12.1, 1H, $BnCH_2$), 4.58 - 4.47 (m, 3H, $BnCH_2$), 4.13 - 4.02 (m, 2H, H4, H3), 3.79 - 3.68 (m, 3H, H5, H6a, H6b), 2.16 (s, 3H, CO_2CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.77 (CO_2CH_3), 138.55, 138.20, 138.05, 128.70, 128.64, 128.62, 128.56, 128.34, 128.30, 128.12, 127.98, 127.88 (128.7 - 127.9 aromatic), 92.64 (C1), 77.94 (C3), 75.31 ($BnCH_2$), 74.90 (C5), 73.63 ($BnCH_2$), 72.02 ($BnCH_2$), 71.24 (C4), 69.57 (C2), 69.47 (C6), 27.18, 21.39 (CO_2CH_3).

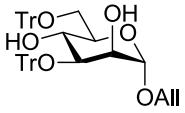
2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (76)[194]



To a stirred solution of **75** (62.6 g, 127 mmol, 1.0 eq) and trichloroacetonitrile (25.5 mL, 254 mmol, 2.0 eq) in anhydrous DCM (380 mL) under Ar at 0 °C, DBU (3.8 mL, 25.4 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 4:1) to give **76**, yield 72% (58.1 g, 91 mmol). R_f = 0.6 (Tol/EtOAc 6:1). *ESI-MS (pos), m/z*: 635.87 ($[M+H]^+$, calc. 636.13). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.70 (s, 1H, N*H*), 7.41 - 7.18 (m, 15H, aromatic), 6.32 (d, *J*=1.8, 1H, H1), 5.52 (t, *J*=2.1, 1H, H2), 4.89 (d, *J*=10.6, 1H, 4-BnCH_2), 4.76 (d, *J*=11.3, 1H, 3-BnCH_2), 4.70 (d, *J*=12.0, 1H, 6-BnCH_2), 4.60 (d, *J*=11.2, 1H, 3-BnCH_2), 4.58 - 4.50 (m, 2H, 4-BnCH_2, 6-BnCH_2), 4.10 - 3.97 (m, 3H, H4, H3, H5), 3.86 (dd, *J*=11.2, 3.6, 1H, H6a), 3.74 (dd, *J*=11.1, 1.5, 1H, H6b), 2.20 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.32, 160.19, 138.40, 138.35, 137.74, 128.76, 128.69, 128.61, 128.56, 128.53, 128.31, 128.22, 128.16, 128.02, 127.94, 127.85 (128.8 - 127.9 aromatic), 95.61 (C1), 77.57 (C3), 75.68 (4-BnCH_2), 74.63 (C5), 73.93 (C4), 73.68 (6-BnCH_2), 72.32 (2-BnCH_2), 68.64 (C6), 67.59 (C2), 27.16, 21.25 (CO_2CH_3).

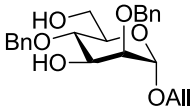
7.11 Synthesis of 3,6-OH mannosyl acceptor

Allyl 3,6-di-*O*-triphrnylmethyl-α-D-mannopyranoside (77)[195]



Compound **27** (26.9 g, 122 mmol, 1.0 eq) and tributyltin oxide (87.2 mL, 171 mmol, 1.4 eq) was dissolved in toluene (400 mL) and was refluxed for 24 h using a Dean-Stark apparatus. The reaction mixture was then cooled to 50 – 60 °C and trityl chloride (88.0 g, 316 mmol, 2.6 eq) was added. The reaction mixture was stirred at r.t. for 24 h, was then concentrated and the residue was diluted with EtOAc (1200 mL) followed by intensive extraction with 1 M potassium fluoride solution (200 mL). The precipitated salt was filtered off and the filtrate was separated. The EtOAc layer was reduced to 900 mL under reduced pressure, washed with H_2O (400 mL×3), brine (500 mL×1) and dried over $MgSO_4$. The concentrated residue was dissolved in cyclohexane (400 mL), the excess of trityl chloride was precipitated and removed by filtration. The crude product was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **77**, yield 59% (50.7 g, 72 mmol). R_f = 0.6 (Tol/EtOAc 2:1). $[\alpha]_D^{20}$ = +34 (c = 0.8, $CHCl_3$). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.63 - 7.18 (m, 30H, aromatic), 5.80 (ddt, *J*=17.2, 10.3, 5.1, 1H, CH_2CH=CH_2), 5.19 - 5.10 (m, 2H, CH_2CH=CH_2), 4.63 (d, *J*=1.8, 1H, H1), 4.11 (ddt, *J*=13.5, 4.7, 1.6, 1H, CH_2CH=CH_2), 3.95 (td, *J*=9.3, 2.9, 1H, H4), 3.90 - 3.82 (m, 2H, H3, CH_2CH=CH_2), 3.62 (dt, *J*=9.5, 4.7, 1H, H5), 3.40 - 3.30 (m, 2H, H6a, H6b), 2.80 (dd, *J*=5.2, 3.4, 1H, H2). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 144.69, 144.14, 134.08 (CH_2CH=CH_2), 129.23, 128.96, 128.21, 128.03, 127.67, 127.22 (129.2 - 127.2 aromatic), 116.53 (CH_2CH=CH_2), 98.65 (C1), 87.63 (CPh_3), 87.12 (CPh_3), 75.06 (C3), 71.67 (C5), 69.41 (C2), 68.32 (C4), 67.64 (CH_2CH=CH_2), 64.90 (C6).

Allyl 2,4-di-*O*-benzyl-α-D-mannopyranoside (78)[298]

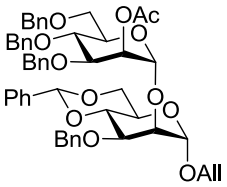


Compound **77** (47.2 g, 67.0 mmol, 1.0 eq) was dissolved in dry DMF (200 mL) and was stirred under Ar atmosphere. The solution was cooled to 0°C and NaH (60% suspension, 8.03 g, 201 mmol, 3.0 eq) was added in portions. The reaction was kept at 0 °C for 20 min and BnBr (31.8 mL, 268 mmol, 4.0 eq) was then added dropwise. After stirring for 2 h at r.t., the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (400 mL), filtered through a layer of Celite, washed with H_2O (150 mL×2), brine (150 mL×1), dried over $MgSO_4$. Then concentrated, dried and the residue (R_f = 0.5 (Tol/EtOAc

10:1)) was dissolved in AcOH (400 mL) followed by addition of H_2O (100 mL). After stirring at r.t. for 2 h, the mixture was concentrated and diluted with EtOAc (400 mL), washed with H_2O (150 mL×3), Sat. $NaHCO_3$ (150 mL×2), brine (300 mL×1) and dried over $MgSO_4$. The concentrated residue was dissolved in cyclohexane (400 mL) and the precipitate was removed by filtration. The crude product was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **78**, yield 65% (17.4 g, 43 mmol). R_f = 0.5 (Tol/EtOAc 3:1). $[\alpha]_D^{20}$ = +31° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 418.2230 ($[M+NH_4]^+$, calc. 418.2230), 423.1780 ($[M+Na]^+$, calc. 423.1784). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.57 - 7.13 (m, 10H, aromatic), 5.91 - 5.79 (m, 1H, $CH_2CH=CH_2$), 5.25 (dq, *J*=17.2, 1.6, 1H, $CH_2CH=CH_2$), 5.18 (ddd, *J*=10.4, 2.8, 1.3, 1H, $CH_2CH=CH_2$), 4.91 (dd, *J*=6.2, 4.9, 2H, H1, $BnCH_2$), 4.76 - 4.58 (m, 3H, $BnCH_2$), 4.18 - 4.11 (m, 1H, $CH_2CH=CH_2$), 4.07 - 4.01 (m, 1H, H3), 3.94 (ddt, *J*=13.0, 6.0, 1.3, 1H, $CH_2CH=CH_2$), 3.89 - 3.78 (m, 2H, H6a, H6b), 3.77 - 3.60 (m, 3H, H2, H4, H5). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.56, 137.89, 133.75 ($CH_2CH=CH_2$), 128.94, 128.83, 128.70, 128.30, 128.26, 128.10, 128.07, 128.05 (128.9 - 128.0 aromatic), 117.68 ($CH_2CH=CH_2$), 96.53 (C1), 78.70 (C2), 76.72 (C4), 75.18 ($BnCH_2$), 73.37 ($BnCH_2$), 71.97 (C3), 71.66 (C5), 68.19 ($CH_2CH=CH_2$), 62.51 (C6).

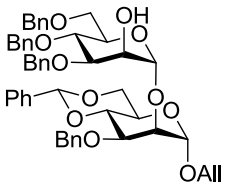
7.12 Synthesis of high mannose building blocks

Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (79)



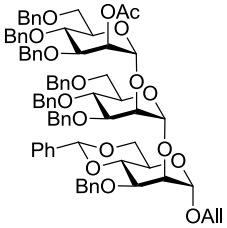
The trichloroacetimidate donor **76** (11.90 g, 18.7 mmol, 1.2 eq) and **29** (6.21 g, 15.6 mmol, 1.0 eq) was dissolved in dry DCM (90 ml) and 4 Å molecular sieves (9 g) was added. The suspension was stirred under argon at room temperature for 30 min and then cooled to 0°C followed by dropwise addition of a solution of trimethylsilyl-trifluoromethanesulfonate (TMSOTf) (0.14 mL, 0.77 mmol, 0.05 eq) in DCM (7 mL). The reaction was almost instant and kept stirring at r.t. for 30 min, then quenched with triethylamine (2.8 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **79**. Yield 84% (11.45 g, 13.1 mmol). R_f = 0.7 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +22° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 890.07 ($[M+NH_4]^+$, calc. 890.41). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.65 - 7.10 (m, 25H, aromatic), 5.83 (ddd, *J*=15.8, 10.8, 5.5, 1H, $CH_2CH=CH_2$), 5.66 (s, 1H, C*H*Ph), 5.64 - 5.59 (m, 1H, H2'), 5.28 - 5.17 (m, 2H, $CH_2CH=CH_2$), 5.15 (s, 1H, H1'), 4.92 (s, 1H, H1), 4.88 (dd, *J*=11.4, 5.2, 2H, 3-$BnCH_2$, 4'-$BnCH_2$), 4.80 (d, *J*=11.0, 1H, 3'-$BnCH_2$), 4.72 - 4.66 (m, 2H, 3-$BnCH_2$, 6'-$BnCH_2$), 4.62 - 4.47 (m, 3H, 3'-$BnCH_2$, 6'-$BnCH_2$, 4'-$BnCH_2$), 4.27 - 4.22 (m, 1H, H6a), 4.12 - 4.03 (m, 4H, $CH_2CH=CH_2$, H2, H3', H4), 4.01 (dd, *J*=9.8, 2.9, 1H, H3), 3.96 - 3.90 (m, 1H, H5'), 3.90 - 3.69 (m, 5H, H6, H4', $CH_2CH=CH_2$, H6b, H6a', H6b'), 2.16 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.23 (CO_2CH_3), 138.77, 138.47, 138.39, 138.24, 137.84, 133.76 ($CH_2CH=CH_2$), 129.08, 128.74, 128.62, 128.56, 128.54, 128.51, 128.47, 128.42, 128.41, 128.34, 128.28, 128.07, 127.99, 127.95, 127.88, 127.78, 127.70, 126.33 (129.1 - 126.3 aromatic), 117.68 ($CH_2CH=CH_2$), 101.72 (*C*HPh), 100.40 (C1'), 99.19 (C1), 79.30 (C4), 78.26 (C3'), 77.01 (C2), 75.92 (C3), 75.58 (4'-$BnCH_2$), 74.68 (C4'), 73.71 (6'-$BnCH_2$), 73.22 (3-$BnCH_2$), 72.18 (C5'), 72.10 (3'-$BnCH_2$), 69.28 (C6'), 69.06 (C6), 68.74 (C2'), 68.29 ($CH_2CH=CH_2$), 64.20 (C5), 21.33 (CO_2CH_3).

Allyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (80)



Compound **79** (5.90 g, 6.76 mmol) was dissolved in MeOH/DCM 4:1 (75 mL) and NaOMe (280 mg, 0.07 M) was added. The reaction mixture was stirred for 24 h at r.t. and then neutralized by a small amount of *Dowex* 50WX8 ion-exchanger (1.4 g, prewashed with MeOH), filtered and concentrated. The residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **80**. Yield: 99% (5.61 g, 6.76 mmol). R_f = 0.4 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +32° (c = 1.0, $CHCl_3$). *ESI-MS (pos), m/z*: 848.00 ($[M+NH_4]^+$, calc. 848.40). ***¹H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.58 - 7.17 (m, 25H, aromatic), 5.83 (ddd, *J*=21.8, 10.6, 5.4, 1H, CH_2CH=CH_2), 5.64 (s, 1H, C*H*Ph), 5.25 - 5.11 (m, 3H, H1' , CH_2CH=CH_2), 4.96 (s, 1H, H1), 4.89 - 4.81 (m, 2H, 3-$BnCH_2$, 4'-$BnCH_2$), 4.77 - 4.68 (m, 3H, 3'-$BnCH_2$, 3-$BnCH_2$), 4.67 - 4.62 (m, 1H, 6'-$BnCH_2$), 4.60 - 4.51 (m, 2H, 6'-$BnCH_2$, 4'-$BnCH_2$), 4.27 - 4.22 (m, 2H, H6a, H2'), 4.12 - 4.00 (m, 4H, CH_2CH=CH_2, H2, H3, H4), 3.98 - 3.90 (m, 2H, H5', H3'), 3.88 - 3.78 (m, 3H, CH_2CH=CH_2,H5, H6b, H4'), 3.76 - 3.70 (m, 2H, H6a', H6b'). ***¹³C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.65, 138.44, 138.38, 138.18, 137.86, 133.84 (CH_2CH=CH_2), 129.10, 128.77, 128.67, 128.64, 128.61, 128.58, 128.53, 128.52, 128.41, 128.31, 128.16, 128.13, 128.11, 128.05, 127.87, 127.86, 127.80, 127.77, 126.32 (129.1 - 126.3 aromatic), 117.60 (CH_2CH=CH_2), 102.10 (C1'), 101.72 (*C*HPh), 99.34 (C1), 80.09 (C3'), 79.22 (C4), 77.38 (C2), 76.00 (C3), 75.51 (4'-$BnCH_2$), 74.75 (C4'), 73.72 (6'-$BnCH_2$), 73.25 (3-$BnCH_2$), 72.28 (3'-$BnCH_2$), 71.91 (C5'), 69.41 (C6'), 69.10 (C6), 68.54 (C2'), 68.34 (CH_2CH=CH_2), 64.20 (C5).

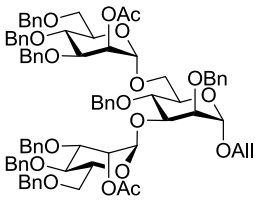
Allyl 3,4,6-tri-*O*-benzyl-2-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (81)



The trichloroacetimidate donor **76** (322 mg, 0.51 mmol, 1.2 eq) and the disaccharide **80** (350 g, 0.42 mmol, 1.0 eq) was dissolved in dry DCM (5 ml) and 4 Å molecular sieves (500 mg) was added. The suspension was stirred under argon at room temperature for 30 min and then cooled to 0°C followed by addition of trimethylsilyl-trifluoromethanesulfonate (TMSOTf) (3.8 μL, 0.021 mmol, 0.05 eq). The suspension was kept stirring at r.t. for 2 h and more donor **76** was added, after another 2 h, the reaction was quenched with triethylamine (76 μL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and

purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **81**. Yield 46% (250 mg, 0.19 mmol). R_f = 0.8 (Tol/EtOAc 3:1). $[\alpha]_D^{20}$ = +21° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1305.5743 ($[M+H]^+$, calc. 1305.5787), 1322.6072 ($[M+NH_4]^+$, calc. 1322.6052), 1327.5422 ($[M+Na]^+$, calc. 1327.5606). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.49 - 7.00 (m, 40H, aromatic), 5.71 (ddd, *J*=22.3, 10.7, 5.6, 1H, CH_2CH=CH_2), 5.52 (s, 1H, C*H*Ph), 5.44 (s, 1H, H2''), 5.13 (d, *J*=5.5, 1H, H1'), 5.13 - 5.02 (m, 2H, CH_2CH=CH_2), 5.01 (s, 1H, H1''), 4.83 (s, 1H, H1), 4.75 (dt, *J*=22.6, 11.3, 2H, $BnCH_2$), 4.65 - 4.50 (m, 6H, $BnCH_2$), 4.49 - 4.41 (m, 3H, $BnCH_2$), 4.37 (t, *J*=10.7, 2H, $BnCH_2$), 4.26 (d, *J*=12.0, 1H, $BnCH_2$), 4.15 - 4.08 (m, 2H, H6a, H2'), 3.99 - 3.92 (m, 2H, H2, CH_2CH=CH_2), 3.92 - 3.85 (m, 2H, H3'', H4), 3.85 - 3.77 (m, 5H, H3, H4', H4'', H5', H5''), 3.77 - 3.66 (m, 3H, H3', CH_2CH=CH_2, H5), 3.66 - 3.57 (m, 4H, H6a', H6b', H6a'', H6b''), 3.48 (d, *J*=11.1, 1H, H6b), 2.05 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.26 (CO_2CH_3), 138.67, 138.48, 138.41, 138.35, 138.29, 138.15, 137.76, 133.71 (CH_2CH=CH_2), 128.94, 128.50, 128.43, 128.38, 128.34, 128.32, 128.26, 128.19, 128.03, 127.92, 127.88, 127.84, 127.79, 127.75, 127.72, 127.68, 127.60, 127.53, 127.45, 126.19 (128.9 - 126.2 aromatic), 117.45 (CH_2CH=CH_2), 101.50 (*C*HPh), 101.36 (C1'), 99.39 (C1''), 99.21 (C1), 79.17 (C4), 78.18 (C3'), 77.16 (C2), 75.64 (C3), 75.34 ($BnCH_2$), 75.20 ($BnCH_2$), 74.93 (C3'), 74.73 (C2'), 74.39 (C4', C4''), 73.43 ($BnCH_2$), 72.72 ($BnCH_2$), 72.41 (C5'), 72.07 (C5''), 71.99 ($BnCH_2$), 69.57 (C6'), 68.94 (C6'', C2''), 68.89 (C6), 68.21 (CH_2CH=CH_2), 64.01 (C5), 21.27 (CO_2CH_3).

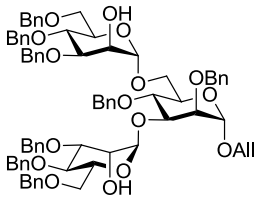
Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzyl-α-D-mannopyranoside (82)



The trichloroacetimidate donor **76** (14.01 g, 22.0 mmol, 2.2 eq) and **78** (4.00 g, 10.0 mmol, 1.0 eq) was dissolved in dry DCM (100 ml) and 4 Å molecular sieves (10 g) was added. The suspension was stirred under argon at room temperature for 30 min and then cooled to 0°C followed by dropwise addition of a solution of trimethylsilyl-trifluoromethanesulfonate (TMSOTf) (0.15 mL, 0.8 mmol, 0.08 eq) in DCM (5 mL). The reaction was instant and kept stirring at r.t. for 30 min, then quenched with triethylamine (3.0 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and recrystallized from cyclohexane-ethyl acetate to remove solid impurities. The mother liquid was then

concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **82**. Yield 88% (11.87 g, 8.80 mmol). R_f = 0.5 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +39° (c = 0.8, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1349.6056 ($[M+H]^+$, calc. 1349.6049), 1366.6333 ($[M+NH_4]^+$, calc. 1366.6314), 1371.5882 ($[M+Na]^+$, calc. 1371.5868). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.43 - 7.12 (m, 40H, aromatic), 5.80 (ddd, *J*=15.6, 10.7, 5.6, 1H, CH_2CH=CH_2), 5.56 - 5.52 (m, 1H, H2''), 5.51 - 5.47 (m, 1H, H2'), 5.25 - 5.17 (m, 2H, H1'', CH_2CH=CH_2), 5.13 (dd, *J*=10.5, 1.2, 1H, CH_2CH=CH_2), 4.99 (d, *J*=1.3, 1H, H1'), 4.92 - 4.82 (m, 3H, H1, $BnCH_2$), 4.78 (d, *J*=11.1, 1H, $BnCH_2$), 4.72 - 4.60 (m, 6H, $BnCH_2$), 4.54 - 4.43 (m, 7H, $BnCH_2$), 4.20 (dd, *J*=9.4, 3.0, 1H, H3), 4.14 - 4.03 (m, 2H, CH_2CH=CH_2, H3''), 4.01 - 3.85 (m, 7H, CH_2CH=CH_2, H3', H4', H4'', H2, H4, H6a), 3.84 - 3.80 (m, 1H, H5'), 3.78 - 3.58 (m, 6H, H5'', H6b, H6a', H6a'', H6b'', H6b'), 2.17 (s, 3H, Ac), 2.11 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.49 (CO_2CH_3), 170.32 (CO_2CH_3), 138.89, 138.79, 138.50, 138.41, 138.16, 138.11, 138.08, 133.91 (CH_2CH=CH_2), 128.85, 128.65, 128.63, 128.62, 128.58, 128.55, 128.51, 128.49, 128.46, 128.45, 128.38, 128.35, 128.30, 128.23, 128.18, 128.02, 127.97, 127.94, 127.89, 127.87, 127.86, 127.82, 127.76, 127.74, 127.69 (128.9 - 127.7 aromatic), 117.53 (CH_2CH=CH_2), 99.95 (C1''), 98.27 (C1'), 96.38 (C1), 78.97 (C3), 78.36 (C3''), 77.88 (C3'), 77.86 (C2), 75.31 ($BnCH_2$), 75.26 ($BnCH_2$), 75.14 ($BnCH_2$), 74.65 (C4''), 74.44 (C4'), 73.71 ($BnCH_2$), 73.58 ($BnCH_2$), 72.48 ($BnCH_2$), 72.07 ($BnCH_2$), 71.73 ($BnCH_2$, C5'), 71.60 (C5''), 71.47 (C5'), 69.40 (C6''), 69.06 (C6'), 68.99 (C2''), 68.69 (C2'), 68.13 (CH_2CH=CH_2), 66.69 (C6), 21.38 (CO_2CH_3), 21.24 (CO_2CH_3).

Allyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzyl-α-D-mannopyranoside (83)



Compound **82** (8.90 g, 6.82 mmol) was dissolved in a solution of NaOMe (540 mg, 0.08 M) in MeOH/DCM 4:1 (125 mL). The reaction mixture was stirred for 24 h at r.t. and then neutralized by a small amount of *Dowex* 50WX8 ion-exchanger (2.7 g, prewashed with MeOH), filtered and concentrated. The residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **83**. Yield: 62% (5.75 g, 4.26 mmol). R_f = 0.5 (Tol/EtOAc 3:1). $[\alpha]_D^{20}$ = +53° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1265.5870 ($[M+H]^+$, calc. 1265.5838), 1282.6148 ($[M+NH_4]^+$, calc. 1282.6103), 1288.5751 ($[M+Na]^+$, calc. 1288.5691), 1303.5456 ($[M+K]^+$, calc. 1303.5396). ***^{1}H-NMR*** (400 MHz, $CDCl_3$,

gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.43 - 7.16 (m, 40H, aromatic), 5.88 - 5.77 (m, 1H, CH_2CH=CH_2), 5.28 - 5.21 (m, 2H, H1' , CH_2CH=CH_2), 5.16 (dd, *J*=10.4, 1.6, 1H, CH_2CH=CH_2), 5.12 (d, *J*=1.4, 1H, H1''), 4.91 - 4.84 (m, 3H, $BnCH_2$, H1), 4.73 (d, *J*=11.2, 1H, $BnCH_2$), 4.69 (d, *J*=8.0, 1H, $BnCH_2$), 4.68 - 4.59 (m, 7H, $BnCH_2$), 4.58 - 4.49 (m, 5H, $BnCH_2$), 4.21 (dd, *J*=9.4, 3.0, 1H, H3), 4.16 - 4.13 (m, 1H, H2''), 4.13 - 4.08 (m, 1H, CH_2CH=CH_2), 4.07 (dd, *J*=3.1, 1.8, 1H, H2'), 4.04 - 4.00 (m, 1H, H5'), 3.98 - 3.86 (m, 8H, H3', H4, H3'', H2, H4'', H4' , CH_2CH=CH_2, H6a), 3.85 - 3.80 (m, 1H, H5''), 3.78 - 3.69 (m, 5H, H5, H6a', H6b', H6b, H6a''), 3.68 - 3.64 (m, 1H, H6b''). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 138.81, 138.73, 138.57, 138.56, 138.53, 138.34, 138.15, 138.14, 133.97 (CH_2CH=CH_2), 128.84, 128.73, 128.63, 128.55, 128.53, 128.51, 128.34, 128.21, 128.14, 128.13, 128.07, 128.02, 128.00, 127.97, 127.94, 127.83, 127.78, 127.75 (128.8 - 127.8 aromatic), 117.66 (CH_2CH=CH_2), 101.74 (C1'), 99.94 (C1''), 96.45 (C1), 80.36 (C3'), 79.91 (C3''), 79.44 (C3), 78.14 (C4), 75.31 ($BnCH_2$), 75.20 ($BnCH_2$), 75.12 (C2), 74.73 (C4'), 74.53 (C4''), 73.79 ($BnCH_2$), 73.64 ($BnCH_2$), 72.52 ($BnCH_2$), 72.27 ($BnCH_2$), 72.24 (C5'), 71.95 (C5), 71.77 (C5''), 71.41 ($BnCH_2$), 69.55 (C6'), 69.11 (C6''), 68.98 (C2'), 68.31 (C2''), 68.24 (CH_2CH=CH_2), 66.32 (C6).

7.13 Synthesis of a tetra acetylated mannosyl donor building block

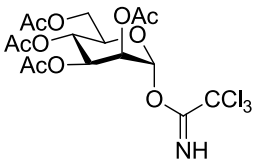
1,2,3,4,6-penta-*O*-acetyl-β-D-mannopyranose (84)[299]

A mixture of sodium acetate (25.9 g, 315 mmol, 1.05 eq) in acetic anhydride (198 mL, 2.10 mol, 7.0 eq) was heated to reflux at 140 - 160 °C. The heating was removed and D-mannose (54 g, 300 mmol, 1.0 eq) was added in portions. The reaction started to boil intensively, when the boiling stopped, heating was applied again, and the reaction refluxed for another 4 h. The reaction mixture was concentrated followed by dilution with EtOAc (500 mL), washed with H_2O (300 mL×3), Sat. $NaHCO_3$ (300 mL×3), brine (500 mL×1) and dried over $MgSO_4$. The organic solvent was removed under reduced pressure, the crude product was purified by column chromatography (cyclohexane-ethyl acetate 1:1) to give **84**, yield 97% (114 g, 292 mmol). R_f = 0.6 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +36° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 407.87 ($[M+NH_4]^+$, calc. 408.15), 412.93 ($[M+Na]^+$, calc. 413.11). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 6.06 (d, *J*=1.6, 1H, H1), 5.32 (dt, *J*=3.1, 1.9, 2H, H3, H4), 5.24 - 5.21 (m, 1H, H2), 4.28 - 4.21 (m, 1H, H6a), 4.11 - 4.05 (m, 1H, H6b), 4.05 - 3.99 (m, 1H, H5), 2.15 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.98 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.77, 170.13, 169.88, 169.68, 168.21 (170.8 - 168.2 CO_2CH_3), 90.80 (C1), 70.81 (C5), 68.93 (C3), 68.53 (C2), 65.75 (C4), 62.30 (C6), 21.03, 20.94, 20.88, 20.83, 20.81 (21.0 - 20.8 CO_2CH_3).

2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose (85)[299]

Compound **84** (21.4 g, 55.0 mmol, 1.0 eq) was dissolved in dry DMF (50 mL) and hydrazine acetate (5.57 g, 59.7 mmol, 1.1 eq) was added. The reaction was heated at 50 °C for 3 h, then concentrated and coevaporated with toluene (100 mL×3) and dried under reduced pressure to give **85**, yield 73% (14.0 g, 40.4 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +20° (c = 0.8, $CHCl_3$). *ESI-MS (pos), m/z*: 365.87 ($[M+NH_4]^+$, calc. 366.14), 370.93 ($[M+Na]^+$, calc. 371.10). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.40 (dd, *J*=10.1, 3.4, 1H, H3), 5.32 - 5.26 (m, 1H, H4), 5.26 - 5.23 (m, 1H, H2), 5.21 (d, *J*=1.6, 1H, H1), 4.27 - 4.19 (m, 2H, H5, H6a), 4.15 - 4.07 (m, 1H, H6b), 2.14 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.98 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.08, 170.44, 170.26, 170.04 (171.1 - 170.0 CO_2CH_3), 92.35 (C1), 70.36 (C2), 69.04 (C3), 68.59 (C5), 66.44 (C4), 62.81 (C6), 21.10, 20.96, 20.91, 20.89 (21.1 - 20.9 CO_2CH_3).

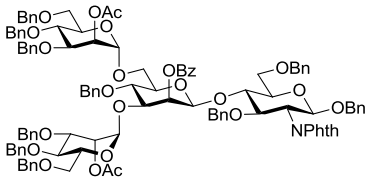
2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (86)[299]



Compound **85** (18.2 g, 52.6 mmol, 1.0 eq) and trichloroacetonitrile (10.6 mL, 105.1 mmol, 2.0 eq) was dissolved in anhydrous DCM (300 mL) and stirred under Ar at 0 °C, DBU (1.58 mL, 10.5 mmol, 0.2 eq) was added and the reaction mixture was stirred at 0 °C for 3 h, then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 4:1) to give **86**, yield 70% (18.1g, 36.8 mmol). R_f = 0.6 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +53° (c = 0.9, $CHCl_3$). *ESI-MS (pos), m/z*: 513.80 ($[M+Na]^+$, calc. 514.01). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.78 (s, 1H, N*H*), 6.26 (d, J=1.8, 1H, H1), 5.45 (dd, J=3.0, 2.0, 1H, H2), 5.40 - 5.36 (m, 2H, H3, H4), 4.26 (dd, J=11.9, 4.7, 1H, H6a), 4.21 - 4.11 (m, 2H, H5, H6b), 2.18 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.99 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.66, 169.89, 169.81, 169.71 (170.7 - 169.7 *C*O_2CH_3), 159.86 (*C*=NH), 94.63 (C1), 90.62 (*C*Cl_3), 71.32 (C5), 68.91 (C3), 67.98 (C2), 65.52 (C4), 62.16 (C6), 20.87, 20.79, 20.71 (20.9 - 20.7 CO_2*C*H_3).

7.14 Synthesis of tetrasaccharide for oxazoline conversion

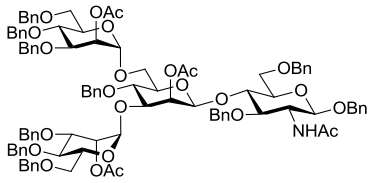
Benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (87)



The trichloroacetimidate donor **76** (1539 mg, 2.42 mmol, 3.0 eq) and acceptor **26** (754 mg, 0.81 mmol, 1.0 eq) was dissolved in dry DCM (15 mL) and 4 Å molecular sieves (1.5 g) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to 0 °C, trimethylsilyl -trifluoromethanesulfonate (TMSOTf) (29.3 μL, 0.16 mmol, 0.2 eq) was added. The reaction was kept stirring at 0 °C for 15 min, then quenched with triethylamine (0.1 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **87**. Yield 76% (1151 mg, 0.61 mmol). R_f = 0.4 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = +9° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 960.4149 ($[M+2NH_4]^{2+}$, calc. 960.4162), 962.3620 ($[M+Na+NH_4]^{2+}$, calc. 962.3922). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.97 (dd, *J*=6.5, 3.0, 2H, benzoyl), 7.42 (s, 2H, Phth), 7.30 - 6.78 (m, 50H, aromatic), 6.43 - 6.35 (m, 3H, aromatic), 6.29 (t, *J*=7.4, 2H, aromatic), 5.48 (d, *J*=3.1, 1H, $H2^2$), 5.28 - 5.25 (m, 1H, $H2^4$), 5.23 - 5.20 (m, 1H, $H2^3$), 4.98 (d, *J*=0.9, 1H, $H1^3$), 4.82 (d, *J*=8.5, 1H, $H1^1$), 4.81 (s, 1H, $H1^4$), 4.69 - 4.43 (m, 9H, $H1^2$, $BnCH_2$), 4.38 - 4.23 (m, 9H, $BnCH_2$), 4.19 (d, *J*=13.1, 1H, $BnCH_2$), 4.13 (d, *J*=11.0, 1H, $BnCH_2$), 4.09 (d, *J*=11.0, 1H, $BnCH_2$), 3.99 - 3.88 (m, 3H, $H2^1$, $H5^3$, $H4^1$), 3.82 (dd, *J*=10.5, 8.5, 1H, $H3^1$), 3.79 - 3.72 (m, 4H, $H6a^2$, $H4^4$, $H4^2$, $H3^4$), 3.71 - 3.66 (m, 3H, $H6b^2$, $H4^3$, $H3^3$), 3.65 - 3.59 (m, 3H, $H6a^4$, $H6a^3$, $H3^2$), 3.58 - 3.52 (m, 4H, $H6b^4$, $h6b^3$, $H6a^1$, $H5^4$), 3.42 (d, *J*=9.3, 1H, $H6b^1$), 3.28 (d, *J*=9.8, 1H, $H5^1$), 3.02 (d, *J*=9.6, 1H, $H5^2$), 1.93 (s, 3H, Ac), 1.69 (s, 3H, Ac). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.35 (CO_2CH_3), 170.07 (CO_2CH_3), 167.60, 165.83 (benzoyl), 138.98, 138.74, 138.60, 138.56, 138.43, 138.31, 138.08, 137.97, 137.42 (139.0 - 137.4 benzyl), 133.49 (Phth), 131.87, 130.18 (benzoyl), 130.15 (benzoyl), 128.98, 128.87, 128.62, 128.59, 128.55, 128.49, 128.46, 128.44, 128.42, 128.32, 128.30, 128.18, 128.13, 128.09, 128.03, 128.00, 127.88, 127.83, 127.80, 127.79, 127.70, 127.58, 126.99 (129.0 - 127.0 aromatic), 123.23, 99.93 ($C1^3$), 99.34 ($C1^2$), 98.76 ($C1^4$), 97.47 ($C1^1$), 79.49 ($C4^1$), 78.88 ($C3^2$), 78.16 ($C3^4$), 78.05 ($C3^3$), 76.14 ($C3^1$), 75.36 ($BnCH_2$), 75.22 ($BnCH_2$), 75.03 ($C4^2$), 74.71 ($C5^2$, $C5^1$), 74.60

($BnCH_2$), 74.53 ($BnCH_2$), 74.35 ($C4^3$), 74.18 ($C4^4$), 73.69 ($BnCH_2$), 73.66 ($BnCH_2$), 72.58 ($C5^3$), 72.39 ($C5^4$), 72.22 ($BnCH_2$), 71.96 ($C2^2$), 71.59 ($BnCH_2$), 70.88 ($BnCH_2$), 69.25 ($C2^3$), 69.13 ($C6^3$), 68.95 ($C6^1$), 68.59 ($C6^4$), 68.32 ($C2^4$), 66.28 ($C6^2$), 55.87 ($C2^1$), 21.25 (CO_2CH_3), 20.94 (CO_2CH_3).

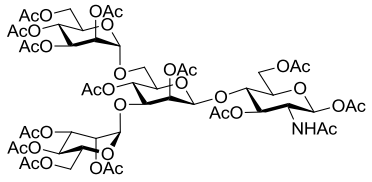
Benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (88)



To a heated solution of **87** (1.83 g, 0.97 mmol) in MeOH (60 mL) at 70 °C, hydrazine monohydrate (12 mL) was added. The reaction was kept at 70 °C for 24 h, the solvent was removed and coevaporated with toluene (50 mL×3). The residue was taken up in pyridine / acetic anhydride 2:1 (30 mL) and stirred at room temperature overnight. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:2) gave **88**. Yield: 83% (1.40 g, 0.81 mmol). R_f = 0.5 (Tol/EtOAc 1:1). $[\alpha]_D^{20}$ = +14° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1735.7661 ($[M+H]^+$, calc. 1735.7608), 1752.7704 ($[M+NH_4]^+$, calc. 1752.7873), 887.3584 ($[M+Na+NH_4]^{2+}$, calc. 887.3869). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.44 - 7.12 (m, 50H, aromatic), 5.50 (dd, *J*=2.9, 1.8, 1H, $H2^3$), 5.47 - 5.44 (m, 1H, $H2^4$), 5.43 (d, *J*=2.7, 1H, $H2^2$), 5.19 (d, *J*=1.6, 1H, $H1^3$), 5.14 (d, *J*=7.5, 1H, N*H*), 4.96 (d, *J*=1.5, 1H, $H1^4$), 4.95 - 4.77 (m, 6H, $H1^1$, $BnCH_2$), 4.75 - 4.65 (m, 4H, $H1^2$, $BnCH_2$), 4.62 (d, *J*=12.1, 1H, $BnCH_2$), 4.59 - 4.41 (m, 10H, $BnCH_2$), 4.37 (d, *J*=12.1, 1H, $BnCH_2$), 4.11 (t, *J*=8.1, 1H, $H4^1$), 4.03 (t, *J*=8.3, 1H, $H3^1$), 4.00 - 3.90 (m, 5H, $H5^3$, $H4^3$, $H4^4$, $H3^4$, $H3^3$), 3.86 - 3.80 (m, 3H, $H6a^1$, $H5^4$, $H3^2$), 3.77 - 3.73 (m, 5H, $H4^2$, $H6a^2$, $H6b^1$, $H6a^3$, $H6b^3$), 3.72 - 3.69 (m, 1H, $H6b^2$), 3.62 - 3.54 (m, 2H, $H6a^4$, $H5^1$), 3.39 (dd, *J*=16.0, 7.8, 1H, $H2^1$), 3.33 - 3.27 (m, 1H, $H5^2$), 3.23 (dd, *J*=10.8, 1.2, 1H, $H6b^4$), 2.14 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.63 (s, 3H, NHAc). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.56, 170.42, 170.26 (CO_2CH_3), 139.36, 138.96, 138.85, 138.69, 138.56, 138.35, 138.26, 138.17, 138.13, 137.81, 128.83, 128.75, 128.64, 128.61, 128.51, 128.48, 128.44, 128.38, 128.19, 128.07, 128.01, 127.99, 127.96, 127.91, 127.88, 127.85, 127.82, 127.80, 127.74, 127.72 (128.8 - 127.7 aromatic), 100.19 ($C1^3$), 99.63 ($C1^1$), 98.10 ($C1^4$), 97.68 ($C1^2$), 78.91 ($C3^2$), 78.59 ($C3^4$), 77.95 ($C3^1$), 77.91 ($C3^3$), 76.23 ($C4^1$), 75.67 ($BnCH_2$), 75.50 ($C4^2$), 75.31 ($BnCH_2$),

74.94 ($C5^2$, $BnCH_2$), 74.66 ($C4^4$), 74.39 ($C5^1$), 74.27 ($C4^3$), 74.17 ($BnCH_2$), 73.70 ($BnCH_2$), 73.68 ($BnCH_2$), 73.59 ($BnCH_2$), 72.64 ($C5^3$), 72.13 ($BnCH_2$), 72.11 ($BnCH_2$), 71.81 ($C5^4$), 71.42 ($C2^2$), 71.21 ($BnCH_2$), 69.17 ($C2^4$, $C2^3$), 69.12 ($C6^3$), 69.06 ($C6^4$), 68.81 ($C6^1$), 66.08 ($C6^2$), 56.42 ($C2^1$), 23.56 ($NHCO_2CH_3$), 21.27 (CO_2CH_3), 21.25 (CO_2CH_3), 21.19 (CO_2CH_3).

2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-1,3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranose (89)[148]

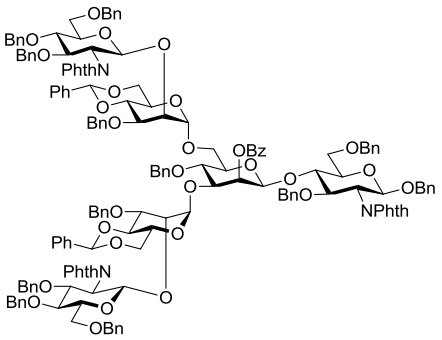


Compound **88** (1.33 g, 0.77 mmol) was dissolved in MeOH / acetic acid 30:2 (31 mL). The solution was added to a suspension of 10% Pd(C) (950 mg) in MeOH (30 mL), stirred under argon. The reaction mixture was then stirred under hydrogen atmosphere for 48 h. After flushing the mixture with argon, the suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (30 mL) and stirred at room temperature overnight. The solvent was removed and co-evaporation three times with toluene was followed by column chromatography (cyclohexane-ethyl acetate 1:1 to 1:5) to give **89**. Yield: 91% (0.88 g, 0.70 mmol). R_f = 0.2 (^{C}Hex/EtOAc 1:3). $[\alpha]_D^{20}$ = +32° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1254.3946 ($[M+H]^+$, calc. 1254.3936), 1271.4204 ($[M+NH_4]^+$, calc. 1271.4201), 1278.3788 ($[M+Na]^+$, calc. 1276.3755), 647.1755 ($[M+Na+NH_4]^{2+}$, calc. 647.2050). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 6.05 (d, *J*=3.5, 1H, $H1^1$), 5.90 (s, 1H, N*H*), 5.36 - 5.23 (m, 6H, $H4^3$, $H4^4$, $H2^3$, $H2^2$, $H3^3$, $H3^1$), 5.09 (dd, *J*=10.2, 3.3, 1H, $H3^4$), 5.05 (t, *J*=7.5, 1H, $H4^2$), 4.98 (dd, *J*=3.2, 1.8, 1H, $H2^4$), 4.97 (d, *J*=1.6, 1H, $H1^3$), 4.82 (d, *J*=1.4, 1H, $H1^2$), 4.77 (s, 1H, $H1^4$), 4.37 - 4.30 (m, 2H, $H2^1$, $H6a^1$), 4.29 - 4.24 (m, 3H, $H6b^1$, $H6a^4$, $H6b^4$), 4.23 - 4.18 (m, 1H, $H6a^3$), 4.13 - 4.06 (m, 2H, $H6b^3$, $H5^4$), 4.00 - 3.88 (m, 5H, $H5^3$, $H6a^2$, $H5^1$, $H4^1$, $H3^2$), 3.67 (dd, *J*=10.4, 4.0, 1H, $H6b^2$), 3.64 - 3.58 (m, 1H, $H5^2$), 2.17 (s, 3H), 2.13 (s, 6H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 9H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H) (2.17 - 1.90 Ac). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.35, 170.83, 170.75, 170.67, 170.42, 170.13, 170.12, 170.10, 170.07, 169.94, 169.89, 169.80, 169.21 (171.4 - 169.2 CO_2CH_3), 98.04 ($C1^3$), 97.56 ($C1^4$), 97.17 ($C1^2$), 90.78 ($C1^1$), 74.54 ($C3^2$), 72.74 ($C5^2$), 71.15 ($C3^1$), 70.28 ($C4^1$, $C5^1$), 69.44 ($C2^4$), 69.39 ($C2^2$, $C5^4$), 69.14 ($C2^3$), 68.91 ($C3^3$), 68.73 ($C5^3$), 68.49 ($C3^4$), 68.38 ($C4^2$),

66.96 (C6^2), 66.01 (C4^3), 65.52 (C44), 62.24 (C6^3, C6^4), 61.94 (C6^1), 50.79 (C2^1), 22.97, 21.10, 20.96, 20.91, 20.88, 20.87, 20.83, 20.79, 20.72, 20.66 (23.0 - 20.7 CO_2CH_3).

7.15 Synthesis of complex type hexasaccharide for oxazoline conversion

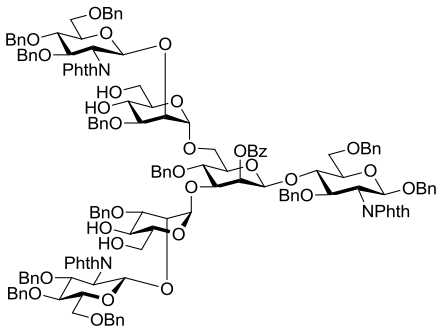
Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (90)



The trichloroacetimidate donor **58** (2.37 mg, 2.23 mmol, 2.4 eq) and acceptor **26** (866 mg, 0.93 mmol, 1.0 eq) was dissolved in dry DCM (30 mL) and 4 Å molecular sieves (3 g) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to -50 °C, trimethylsilyl -trifluoromethanesulfonate (TMSOTf) (25.4 µL, 0.14 mmol, 0.15 eq) was added. The reaction was kept stirring at -50 °C for 1 h, -45 °C for 1 h, -40 °C for 1 h, -30 °C for 1 h and 0 °C for 2 h, then quenched with triethylamine (0.1 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **90**. Yield 76% (1.92 g, 0.71 mmol). R_f = 0.4 (^{C}Hex /EtOAc 2:1). $[\alpha]_D^{20}$ = -20° (c = 1.2, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1387.5600 ($[M+2NH_4]^{2+}$, calc. 1387.5581), 1390.5403 ($[M+Na+NH_4]^{2+}$, calc. 1390.5375). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.32 (d, *J*=7.5, 2H, benzoyl), 7.74 - 6.57 (m, 85H, aromatic), 5.65 (s, 1H, $PhCH_3$), 5.59 (s, 1H, Ph*CH*^{4}), 5.48 (d, *J*=8.5, 1H, H1^5), 5.37 (d, *J*=3.5, 1H, H2^2), 5.22 (d, *J*=13.3, 1H, $BnCH_2$), 5.07 (d, *J*=8.3, 1H, H1^1), 5.04 (s, 1H, $BnCH_2$), 5.02 (d, *J*=5.2, 1H, H1^6), 4.93 (d, *J*=13.5, 1H, $BnCH_2$), 4.91 - 4.86 (m, 3H, H3^5, $BnCH_2$), 4.85 - 4.78 (m, 3H, $BnCH_2$), 4.77 - 4.70 (m, 5H, H1^2, $BnCH_2$), 4.67 (d, *J*=9.3, 1H, $BnCH_2$), 4.65 (d, *J*=11.5, 1H, $BnCH_2$), 4.61 (d, *J*=11.7, 1H, $BnCH_2$), 4.55 - 4.44 (m, 6H, $BnCH_2$), 4.42 (d, *J*=10.3, 2H, H2^5, $BnCH_2$), 4.38 (d, *J*=2.6, 1H, H2^3), 4.35 (s, 1H, H1^3), 4.32 (d, *J*=2.4, 1H, H2^4), 4.25 - 4.19 (m, 4H, H2^6, $BnCH_2$), 4.17 - 4.12 (m, 2H, H2^1, H3^1), 4.11 - 4.05 (m, 4H, H4^1, H6a^1, H4^5, H6a^3), 3.98 - 3.87 (m, 6H, H6b^1, H5^5, H4^3, H4^4, H4^6, H6b^3), 3.79 - 3.77 (m, 2H, H6a^5, H6b^5), 3.75 - 3.70 (m, 2H, H3^2, H1^4), 3.62 - 3.57 (m, 4H, H6a^2, H5^4, H5^1, H5^3), 3.55 - 3.51 (m, 2H, H6a^4, H6a^2), 3.45 - 3.38 (m, 2H, H5^3, H6b^4), 3.37 - 3.32 (m, 3H, H5^2, H6a^6, H6b^6), 3.06 (t, *J*=9.9, 1H, H6b^2), 2.92 (td, *J*=9.6, 4.9, 1H, H5^4), 2.14 (d, *J*=9.6, 1H, H5^6). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 167.88 (Phth C=O), 167.08 (Phth C=O), 166.28 (benzoyl

C=O), 139.63, 139.20, 139.16, 138.82, 138.78, 138.73, 138.58, 138.49, 138.12, 138.02, 138.01, 137.94, 137.61, 137.17 (139.6 - 137.2 benzyl), 133.85 (Phth), 133.49 (Phth), 131.64, 130.51, 129.74, 129.51, 128.90, 128.78, 128.75, 128.72, 128.65, 128.49, 128.44, 128.36, 128.33, 128.27, 128.26, 128.23, 128.17, 128.09, 128.04, 127.98, 127.96, 127.95, 127.88, 127.86, 127.82, 127.79, 127.73, 127.71, 127.67, 127.63, 127.61, 127.60, 127.54, 127.53, 127.43, 127.40, 127.34, 127.31, 127.25, 127.05, 127.00, 126.86, 126.26, 126.22, 126.16 (129.7 - 126.2 aromatic), 123.37, 123.13, 102.38 ($C1^4$), 101.27 (PhC^3H), 101.24 (PhC^4H), 100.80 ($C1^3$), 99.11 ($C1^5$), 97.83 ($C1^6$), 97.25 ($C1^1$), 96.99 ($C1^2$), 82.06 ($C3^2$), 79.34 ($C4^5$), 79.10 ($C5^3$), 78.71 ($C3^5$), 78.23 ($C4^6$), 77.92 ($C4^1$), 77.12 ($C4^3$), 76.81 ($C4^4$), 76.30 ($C3^1$), 76.10 ($C5^3$), 75.72 ($C5^1$), 74.91 ($C5^5$), 74.48 ($BnCH_2$), 74.34 ($BnCH_2$), 74.28 ($BnCH_2$), 74.24 ($C5^2$), 73.92 ($BnCH_2$), 73.89 ($BnCH_2$), 73.82 ($C5^4$), 73.62 ($BnCH_2$), 73.47 ($BnCH_2$), 73.38 ($C5^6$), 73.33 ($BnCH_2$), 73.27 ($BnCH_2$), 72.57 ($C2^4$), 71.72 ($C2^3$), 71.01 ($C6^2$), 70.60 ($BnCH_2$), 69.70 ($BnCH_2$), 69.61 ($C6^1$), 69.58 ($BnCH_2$), 69.48 ($C2^2$), 68.81 ($C6^6$), 68.42 ($C6^3$), 68.01 ($C6^4$), 67.01 ($C5^4$), 56.54 ($C2^5$), 56.22 ($C2^6$), 55.93 ($C2^1$).

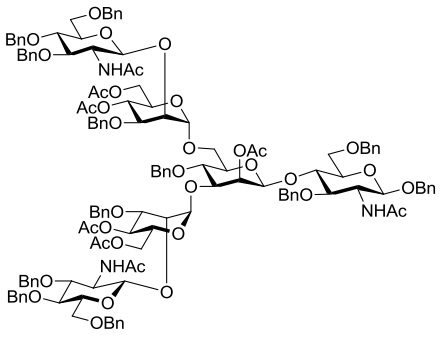
Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (91)



To a stirring solution of **90** (540 mg, 0.197 mmol) in MeCN (4 mL), a solution of *p*-toluenesulfonic acid (100 mg) in MeCN (4 mL) was added. Et_3N (75 μL) was used to neutralize the reaction after stirring at r.t. for 30 min. The reaction was concentrated and coevaporated with toluene and the crude product was purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:2) to give **91**. Yield 76% (384 mg, 0.150 mmol). R_f = 0.2 (Tol/EtOAc 1:1). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1291.0153 ($[M+H+NH_4]^{2+}$, calc. 1291.0136), 1299.5268 ($[M+2NH_4]^{2+}$, calc. 1299.5273), 1301.9730 ($[M+Na+NH_4]^{2+}$, calc. 1302.0045). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.24 (d, *J*=5.3, 2H, benzoyl), 7.89 - 6.44 (m, 75H, aromatic), 5.49 (d, *J*=7.7, 1H, $H1^5$), 5.39 (s, 1H, $H2^2$), 5.21 (d, *J*=10.6, 1H, $BnCH_2$), 5.07 (d, *J*=7.5, 1H, $H1^1$), 5.06 (d, *J*=7.0, 1H, $H1^6$), 5.01 - 4.94 (m, 2H, $BnCH_2$), 4.93 - 4.82 (m, 3H, $H3^5$, $BnCH_2$), 4.79 - 4.71 (m, 2H,

$BnCH_2$), 4.70 - 4.59 (m, 4H, $H1^2$, $BnCH_2$), 4.54 (dd, *J*=29.1, 11.3, 3H, $BnCH_2$), 4.49 - 4.22 (m, 14H, $H2^5$, $H2^4$, $H2^3$, $H1^3$, $H1^4$, $BnCH_2$), 4.14 (d, *J*=13.8, 3H, $H2^6$, $H2^2$, $BnCH_2$), 4.09 - 3.99 (m, 3H, $H4^1$, $H3^1$, $H6a^1$), 3.99 - 3.86 (m, 5H, $H4^5$, $H3^6$, $H5^5$, $BnCH_2$, $H6b^1$), 3.83 - 3.75 (m, 3H, $H6a^5$, $H6b^5$, $H4^3$), 3.72 (d, *J*=11.3, 2H, $H6a^3$, $H6a^4$), 3.68 - 3.56 (m, 6H, $H3^2$, $H4^6$, $H5^2$, $H5^1$, $H6a^2$, $H4^4$), 3.55 - 3.49 (m, 1H, $H6b^3$), 3.42 (d, *J*=8.8, 1H, $H3^3$), 3.39 - 3.32 (m, 2H, $H4^2$, $H6b^4$), 3.28 (d, *J*=10.7, 3H, $H6a^6$, $H5^4$, $H3^4$), 3.24 (s, 1H, $H6b^6$), 3.14 (t, *J*=9.5, 1H, $H6b^2$), 3.04 (s, 1H, $H5^3$), 2.12 (d, *J*=9.1, 1H, $H5^6$). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.61, 168.33, 167.62 (Phth C=O), 167.50 (Phth C=O), 166.19 (benzoyl C=O), 138.85, 138.81, 138.75, 138.51, 138.28, 138.06, 138.00, 137.95, 137.76, 137.05 (138.9 - 137.1 benzyl), 133.89 (Phth), 133.83 (Phth), 133.62, 133.29, 132.99, 132.29, 132.24, 131.54, 131.43, 130.37 (benzoyl), 129.60, 129.23, 128.87, 128.78, 128.69, 128.49, 128.46, 128.39, 128.34, 128.31, 128.28, 128.27, 128.21, 128.18, 128.09, 128.05, 127.98, 127.87, 127.82, 127.79, 127.73, 127.69, 127.66, 127.64, 127.60, 127.56, 127.49, 127.46, 127.38, 127.34, 127.31, 127.19, 127.09, 126.99 (129.6 - 127.0 aromatic), 123.38 (Phth), 123.12 (Phth), 122.71, 122.66, 101.34 ($C1^6$), 100.46 ($C1^3$), 99.20 ($C1^5$), 97.49 ($C1^4$), 97.12 ($C1^1$), 96.80 ($C1^2$), 81.42 ($C3^2$), 79.69 ($C3^3$), 79.44 ($C4^5$), 79.26 ($C3^4$), 79.14 ($C4^6$), 78.75 ($C3^5$), 78.27 ($C3^6$), 77.55 ($C1^4$), 76.11 ($C3^1$, $C5^4$), 75.33 ($C5^3$), 74.84 ($C5^5$), 74.70 ($C5^2$), 74.55 ($BnCH_2$), 74.43 ($BnCH_2$), 74.33 ($C4^2$), 74.04 ($BnCH_2$), 73.96 ($C5^1$), 73.86 ($BnCH_2$), 73.82 ($BnCH_2$), 73.60 ($BnCH_2$), 73.41 ($C5^6$), 73.34 ($BnCH_2$), 73.24 ($BnCH_2$), 72.63 ($C2^3$), 70.65 ($BnCH_2$), 69.91 ($BnCH_2$), 69.66 ($BnCH_2$), 69.57 ($C6^1$), 69.50 ($C2^4$), 69.44 ($C2^2$), 69.40 ($C6^2$), 68.63 ($C6^6$), 67.84 ($C6^5$), 67.29 ($C4^3$), 66.88 ($C4^4$), 63.45 ($C6^3$), 63.22 ($C6^4$), 56.57 ($C2^5$), 56.20 ($C2^6$), 55.97 ($C2^1$).

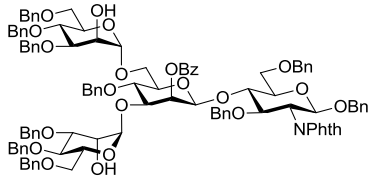
Benzyl 2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (92)



To a heated solution of **91** (284 mg, 0.111 mmol) in Tol / EtOH 1:1 (10 mL) at 80 °C, hydrazine monohydrate (2 mL) was added. The reaction was kept at 80 °C for 72 h, the solvent was removed and coevaporated with toluene (20 mL×3). The residue was taken up in pyridine / acetic anhydride 2:1 (10 mL) and stirred at r.t.

for 24 h. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:3) gave **92**. Yield: 97% (260 mg, 0.108 mmol). R_f = 0.2 (EtOAc). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1203.5214 ($[M+2H]^{2+}$, calc. 1203.5212), 1212.0370 ($[M+H+NH_4]^{2+}$, calc. 1212.0345), 1220.5514($[M+2NH_4]^{2+}$, calc. 1220.5478), 1222.9987 ($[M+Na+NH_4]^{2+}$, calc. 1223.0255). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.48 - 7.05 (m, 60H, aromatic), 6.01 (s, 1H, N*H*^{5}), 5.93 (d, *J*=5.6, 1H, N*H*^{1}), 5.81 (s, 1H, N*H*^{6}), 5.25 - 5.10 (m, 5H, H2^2, H1^1, H4^3, H4^4, $BnCH_2$), 5.00 (dd, *J*=14.2, 8.0, 2H, H1^5, $BnCH_2$), 4.92 - 4.59 (m, 11H, H1^6, $BnCH_2$), 4.59 - 4.50 (m, 5H, H1^2, $BnCH_2$), 4.49 - 4.38 (m, 6H, H1^3, $BnCH_2$), 4.37 - 4.24 (m, 4H, H2^3, H3^1, $BnCH_2$), 4.21 - 4.07 (m, 4H, H2^4, H6a^3, H6b^3, $BnCH_2$), 4.07 - 4.00 (m, 3H, H6a^4, H3^5, H1^4), 3.97 - 3.91 (m, 2H, H6b^4, H4^1), 3.90 - 3.75 (m, 6H, H2^5, H2^6, H6a^1, H4^2, H3^2, H3^6), 3.76 - 3.63 (m, 9H, H6a^5, H6b^5, H6a^4, H6b^4, H6b^1, H6a^2, H5^5, H5^6, H4^5), 3.63 - 3.53 (m, 3H, H6b^2, H5^5, H4^6), 3.52 - 3.44 (m, 3H, H5^1, H3^3, H5^2), 3.40 (d, *J*=10.1, 1H, H3^4), 3.08 (d, *J*=8.1, 1H, H2^1), 2.60 (d, *J*=8.3, 1H, H5^4), 2.10 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.06 (s, 6H, OAc), 1.97 (s, 3H, OAc), 1.85 (s, 2H, NHAc), 1.60 (s, 3H, NHAc), 1.58 (s, 3H, NHAc). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 174.48, 171.27, 171.24, 170.89, 170.78, 170.60, 169.65, 169.45 (171.3 - 169.5 *C*O_2CH_3), 138.99, 138.72, 138.66, 138.61, 138.46, 138.40, 138.34, 138.23, 137.97, 137.89, 137.85, 137.70 (138.9 - 137.9 benzyl), 129.37, 128.82, 128.70, 128.59, 128.52, 128.48, 128.42, 128.40, 128.32, 128.28, 128.23, 128.20, 128.05, 128.00, 127.98, 127.93, 127.87, 127.86, 127.78, 127.75, 127.66, 127.61, 127.54, 127.46, 127.44, 127.38, 127.35, 127.32 (129.4 - 127.3 aromatic), 102.48 (C1^4), 101.94 (C1^6), 100.07 (C1^5), 99.30 (C1^1), 98.73 (C1^3), 97.69 (C1^2), 82.71 (C3^6), 79.49 (C3^2), 79.05 (C3^5), 77.95 (C4^1), 77.88 (C3^1), 77.72 (C4^2), 77.37 (C4^5) 77.24 (C5^2), 76.14 (C3^4), 75.25 (C2^4), 74.92 (C5^2), 74.80 (C3^4), 74.06 (C5^5), 74.02 (C5^6), 73.82 (C5^1), 73.77 ($BnCH_2$), 73.64 ($BnCH_2$), 73.50 ($BnCH_2$), 73.36, ($BnCH_2$) 73.31 ($BnCH_2$), 73.21 ($BnCH_2$), 73.05 (C5^5), 72.17 (C2^3), 72.05 (C5^4), 71.64 ($BnCH_2$), 70.58 (C6^2), 70.26 ($BnCH_2$), 70.22 ($BnCH_2$), 70.05 ($BnCH_2$), 69.39 (C6^6), 68.87 (C6^1), 68.38 (C6^5), 68.21 (C2^2), 67.40 (C4^3), 66.10 (C4^4), 63.68 (C6^3), 62.39 (C6^4), 58.27 (C2^1), 56.12 (C2^5, C2^6), 21.11, 21.08, 21.00, 20.97, 20.94, 20.89, 20.83 (21.1 - 20.8 CO_2CH_3).

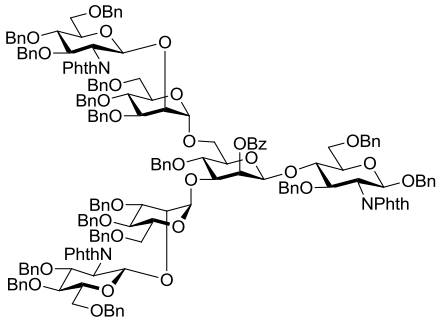
Benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (93)



To a solution of **87** (1115 mg, 0.592 mmol) in MeOH / DCM 5:1 (60 mL), NaOMe (65 mg) was added to reach pH 9.5. The reaction was kept at r.t. for 3 days and neutralized with *Dowex* 50WX8 ion-exchanger (1 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL×3). Then purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:2) and recrystallized from cyclohexane-ethyl acetate to give **93**, yield 89% (947 mg, 0.526 mmol). R_f = 0.6 (Tol/EtOAc 1:1). $[\alpha]_D^{20}$ = +7° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1818.7658 ($[M+NH_4]^+$, calc. 1818.7768), 918.4052 ($[M+ NH_4+NH_4]^{2+}$, calc. 918.4056), 920.8824 ($[M+ Na+NH_4]^{2+}$, calc. 920.8833). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.13 (d, *J*=7.3, 2H, benzoyl), 7.65 (s, 1H, Phth), 7.62 - 7.57 (m, 1H, Phth), 7.46 - 7.14 (m, 47H, aromatic), 7.12 - 7.01 (m, 4H, aromatic), 6.67 - 6.47 (m, 4H, aromatic), 5.66 (d, *J*=2.9, 1H, $H2^2$), 5.20 (s, 1H, $H1^3$), 5.13 (s, 1H, $H1^4$), 5.02 (d, *J*=8.5, 1H, $H1^1$), 4.83 - 4.80 (m, 2H, $BnCH_2$), 4.80 - 4.76 (m, 4H, $H1^2$, $BnCH_2$), 4.75 (d, *J*=12.4, 1H, $BnCH_2$), 4.64 (d, *J*=11.1, 1H, $BnCH_2$), 4.61 (d, *J*=12.3, 1H, $BnCH_2$), 4.59 - 4.56 (m, 2H, $BnCH_2$), 4.56 - 4.52 (m, 3H, $BnCH_2$), 4.52 - 4.49 (m, 2H, $BnCH_2$), 4.48 - 4.43 (m, 3H, $BnCH_2$), 4.42 (d, *J*=11.4, 1H, $BnCH_2$), 4.33 (d, *J*=13.2, 1H, $BnCH_2$), 4.18 - 4.13 (m, 2H, $H2^1$, $H4^1$), 4.08 - 4.06 (m, 1H, $H2^4$), 4.06 - 4.03 (m, 1H, $H5^3$), 3.99 (dd, *J*=10.5, 8.7, 1H, $H3^1$), 3.97 - 3.92 (m, 2H, $H6a^2$, $H4^4$), 3.90 (t, *J*=9.7, 4H, $H6b^2$, $H2^3$, $H4^3$, $H4^2$), 3.86 (dd, *J*=9.2, 3.1, 1H, $H3^4$), 3.83 - 3.78 (m, 4H, $H6a^3$, $H6a^4$, $H6b^4$, $H5^4$), 3.78 - 3.72 (m, 3H, $H6b^3$, $H3^3$, $H3^2$), 3.70 (dd, *J*=10.9, 4.2, 1H, $H6a^1$), 3.60 (d, *J*=9.6, 1H, $H6b^1$), 3.48 (d, *J*=9.8, 1H, $H5^1$), 3.26 (d, *J*=9.5, 1H, $H5^2$). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 165.40 (benzoyl), 138.61, 138.39, 138.38, 138.36, 138.22, 138.02, 137.87, 137.83, 137.18 (138.6 - 137.2 benzyl), 133.17 (Phth), 130.06, (benzoyl) 129.99, 128.71, 128.57, 128.43, 128.36, 128.32, 128.31, 128.29, 128.16, 128.13, 128.11, 127.98, 127.95, 127.94, 127.88, 127.85, 127.80, 127.73, 127.72, 127.66, 127.62, 127.55, 127.53, 127.50 (128.7 - 127.5 aromatic), 126.84, 101.70 ($C1^3$), 99.80 ($C1^4$), 98.97 ($C1^2$), 97.17 ($C1^1$), 80.10 ($C3^4$), 79.66 ($C3^2$), 79.37 ($C3^3$), 79.03 ($C4^1$), 75.92 ($C3^1$), 75.24 ($BnCH_2$), 75.05 ($BnCH_2$), 74.83 ($C4^2$), 74.49 ($BnCH_2$), 74.44 ($C5^1$), 74.38 ($C5^2$), 74.30 ($BnCH_2$), 74.18 ($C4^3$), 73.89 ($C4^4$), 73.46 ($BnCH_2$), 73.44 ($BnCH_2$), 72.21 ($BnCH_2$), 72.02 ($C2^2$), 71.86

($C5^3$), 71.68 ($C5^4$), 71.56 ($BnCH_2$), 70.65 ($BnCH_2$), 68.91 ($C2^3$), 68.89 ($C6^3$), 68.67 ($C6^1$), 68.27 ($C6^4$), 68.18 ($C2^4$), 66.13 ($C6^2$), 55.61 ($C1^2$).

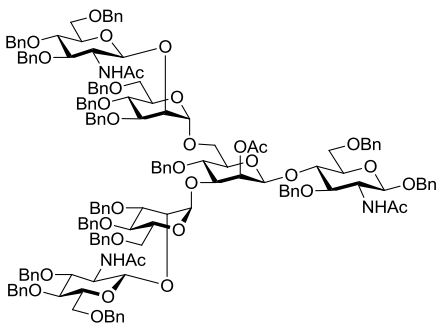
Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (94)



Compounds **55** (1291 mg, 1.92 mmol, 4.0 eq), **93** (865 mg, 0.48 mmol, 1.0 eq), *N*-iodosuccinimide (432 mg, 1.92 mmol, 4.0 eq) and 4 Å molecular sieves (1 g) in dry dichloromethane (20 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (16.4 μL, 0.12 mmol, 0.25 eq). After stirring for 0.5 h at 0 °C, the reaction was quenched by diluting with more DCM (20 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (15 mL×3), brine (30 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 8:1) to give **94**. Yield: 63% (883 mg, 0.30 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -5° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1471.1086 ($[M+H+NH_4]^{2+}$, calc. 1471.1075), 1479.6320 ($[M+NH_4+NH_4]^{2+}$, calc. 1479.6207), 1482.0734 ($[M+ Na+NH_4]^{2+}$, calc. 1482.0984). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.15 (d, *J*=7.5, 2H, benzoyl), 7.71 (s, 2H, Phth), 7.64 - 7.56 (m, 4H, Phth), 7.54 - 6.86 (m, 67H, aromatic), 6.71 (d, *J*=7.3, 2H, aromatic), 6.49 (d, *J*=6.9, 3H, aromatic), 5.39 (d, *J*=2.2, 1H, $H2^2$), 5.01 (s, 1H, $H1^3$), 4.99 (d, *J*=8.6, 1H, $H1^1$), 4.95 - 4.91 (m, 2H, $H1^5$, $H1^6$), 4.89 (d, *J*=10.9, 1H, $BnCH_2$), 4.87 - 4.64 (m, 10H, $BnCH_2$), 4.64 - 4.59 (m, 2H, $H1^4$, $BnCH_2$), 4.59 - 4.44 (m, 7H, $BnCH_2$), 4.44 - 4.38 (m, 5H, $H1^2$, $BnCH_2$), 4.38 - 4.31 (m, 2H, $BnCH_2$), 4.29 - 4.20 (m, 4H, $H2^6$, $H2^5$, $BnCH_2$), 4.16 - 4.08 (m, 6H, $H2^1$, $H2^4$, $H3^5$, $BnCH_2$), 4.08 - 4.00 (m, 3H, $H4^1$, $H3^6$, $H3^3$), 3.99 - 3.94 (m, 1H, $H3^1$), 3.88 - 3.81 (m, 4H, $H2^3$, $H3^4$, $BnCH_2$), 3.74 - 3.64 (m, 5H, $H6a^1$, $H4^2$, $H4^3$, $H4^5$, $H4^6$), 3.61 - 3.52 (m, 4H, $H6a^3$, $H6b^3$, $H4^4$, $H3^2$), 3.52 - 3.44 (m, 4H, $H6b^1$, $H6a^6$, $H6a^5$, $H5^3$), 3.43 - 3.34 (m, 4H, $H6b^6$, $H6a^2$, $H5^1$, $H5^4$), 3.28 (d, *J*=10.5, 1H, $H6b^5$), 3.23 (d, *J*=10.4, 1H, $H6a^4$), 2.91 (dd, *J*=11.0, 5.9, 1H, $H6b^4$), 2.78 (d, *J*=9.6, 1H, $H5^2$), 2.74 (dd, *J*=10.6, 6.6, 1H, $H6b^2$), 2.52 (d, *J*=9.2, 1H, $H5^6$), 2.20 (d, *J*=8.7, 1H, $H5^5$). ***^{13}C-NMR***

(150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 167.55 (Phth C=O), 165.06 (benzoyl C=O), 139.07, 138.85, 138.72, 138.66, 138.51, 138.38, 138.32, 138.28, 138.26, 138.19, 138.09, 138.01, 137.21 (138.9 - 137.2 benzyl), 133.49 (Phth), 133.08 (Phth), 131.97, 131.82, 130.36, 130.02, 129.13, 128.97, 128.53, 128.47, 128.44, 128.41, 128.36, 128.34, 128.30, 128.26, 128.20, 128.15, 128.12, 128.08, 128.04, 128.00, 127.95, 127.90, 127.80, 127.75, 127.72, 127.66, 127.56, 127.51, 127.47, 127.39, 127.34, 127.30, 127.26, 127.13 (129.6 - 127.1 aromatic), 125.98, 123.09 (Phth), 98.89 ($C1^2$), 98.35 ($C1^3$), 97.63 ($C1^4$), 97.17 ($C1^1$), 97.12 ($C1^6$), 95.69 ($C1^5$), 79.38 ($C4^5$), 79.30 ($C4^6$), 79.20 ($C3^3$), 78.94 ($C3^6$), 78.88 ($C3^5$), 77.68 ($C3^2$), 77.44 ($C3^4$), 77.21 ($C4^3$), 75.38 ($C3^1$), 75.29 ($BnCH_2$), 74.67 ($BnCH_2$), 74.62 ($C5^6$), 74.57 ($BnCH_2$), 74.34 ($C4^4$, $C5^1$, $C5^5$), 74.14 ($BnCH_2$), 74.07 ($C4^2$), 73.97 ($BnCH_2$), 73.81 ($BnCH_2$), 73.71 ($C5^3$), 73.46 ($BnCH_2$), 73.43 ($BnCH_2$), 73.15 ($C5^2$), 73.10 ($BnCH_2$), 72.80 ($C2^3$), 72.68 ($BnCH_2$), 72.57 ($C2^4$), 72.19 ($C2^2$), 72.10 ($C5^4$), 72.06 ($C4^1$), 71.20 ($BnCH_2$), 70.78 ($BnCH_2$), 70.67 ($C6^2$), 69.75 ($C6^4$), 69.60 ($BnCH_2$), 68.79 ($C6^6$), 68.76 ($C6^5$), 68.22 ($C6^3$), 66.67 ($C6^1$), 55.73 ($C2^1$), 55.67 ($C2^5$), 55.65 ($C2^6$).

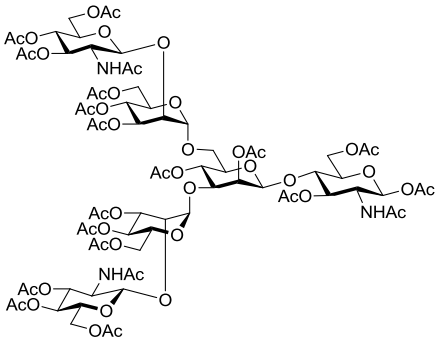
Benzyl 2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (95)



To a heated solution of **94** (750 mg, 256 μmol) in Tol / EtOH 1:1 (20 mL) at 80 °C, hydrazine monohydrate (4 mL) was added. The reaction was kept at 80 °C for 5 days, the solvent was removed and coevaporated with toluene (20 mL×3). The residue was taken up in pyridine / acetic anhydride 2:1 (20 mL) and stirred at r.t. for 48 h. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:3) gave **95**. Yield: 88% (590 mg, 227 μmol). R_f = 0.8 (Tol/EtOAc 1:2). $[\alpha]_D^{20}$ = +19° (c = 1.2, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1299.5947 ($[M+2H]^{2+}$, calc. 1299.5940), 1308.1093 ($[M+H+NH_4]^{2+}$, calc. 1308.1073), 1319.0694 ($[M+ Na+NH_4]^{2+}$, calc. 1319.0982). ***¹H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.47 - 7.06 (m, 80H, aromatic), 5.92 - 5.82 (m, 2H, NH^6, NH^1), 5.42 (d, *J*=2.9, 2H, $H2^2$, NH^5), 5.05 (s, 1H, $H1^3$), 5.03 (d, *J*=8.1, 1H, $H1^6$), 4.97 (d,

J=11.3, 1H, $BnCH_2$), 4.92 (d, *J*=10.8, 1H, $BnCH_2$), 4.88 - 4.85 (m, 2H, $H1^1$, $BnCH_2$), 4.84 - 4.74 (m, 9H, $H1^4$, $H1^2$, $H1^5$, $BnCH_2$), 4.73 - 4.70 (m, 2H, $BnCH_2$), 4.69 - 4.58 (m, 7H, $BnCH_2$), 4.58 - 4.53 (m, 5H, $H3^6$, $BnCH_2$), 4.53 - 4.40 (m, 9H, $BnCH_2$), 4.32 (d, *J*=11.7, 1H, $BnCH_2$), 4.25 (s, 1H, $H2^4$), 4.18 (t, *J*=9.5, 1H, $H3^5$), 4.13 (t, *J*=8.3, 1H, $H3^1$), 4.11 - 4.05 (m, 4H, $H2^3$, $H4^3$, $H4^1$, $H4^4$), 3.92 (dd, *J*=9.4, 2.7, 1H, $H3^4$), 3.85 - 3.78 (m, 3H, $H4^2$, $H3^2$, $H3^3$), 3.78 - 3.71 (m, 3H, $H6a^4$, $H6b^4$, $H6a^3$), 3.70 - 3.63 (m, 2H, $H6a^1$, $H6b^3$), 3.63 - 3.55 (m, 7H, $H6a^5$, $H6b^5$, $H5^1$, $H5^3$, $H5^4$, $H2^1$, $H4^6$), 3.55 - 3.46 (m, 4H, $H6b^1$, $H6a^2$, $H5^6$, $H4^5$), 3.19 (d, *J*=10.5, 1H, $H6b^2$), 3.18 - 3.12 (m, 2H, $H2^5$, $H5^2$), 3.08 (d, *J*=4.9, 1H, $H2^6$), 3.03 (dd, *J*=9.6, 2.9, 1H, $H5^5$), 1.95 (s, 3H, Ac), 1.73 (s, 3H, Ac), 1.69 (s, 3H, Ac), 1.65 (s, 3H, Ac). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.98, 171.67, 170.31, 169.87 (171.9 - 169.8 *C*=O), 139.17, 139.02, 138.97, 138.85, 138.77, 138.52, 138.45, 138.31, 138.29, 138.25, 138.19, 138.01, 128.74, 128.67, 128.65, 128.60, 128.56, 128.53, 128.51, 128.45, 128.42, 128.41, 128.37, 128.36, 128.30, 128.15, 128.10, 128.07, 127.96, 127.93, 127.90, 127.87, 127.82, 127.78, 127.75, 127.73, 127.69, 127.66, 127.64, 127.63, 127.60, 127.58, 127.51, 127.49, 126.72 (139.2 - 126.7 aromatics), 99.99 ($C1^1$), 99.71 ($C1^3$), 98.01 ($C1^4$), 97.97 ($C1^2$), 97.57 ($C1^5$), 97.50 ($C1^6$), 80.04 ($C3^5$), 79.94 ($C3^6$), 79.55 ($C3^3$), 78.89 ($C3^1$, $C4^6$), 78.82 ($C4^5$), 78.02 ($C3^4$), 77.58 ($C3^2$), 76.39 ($C4^4$), 75.43 ($BnCH_2$), 74.89 ($BnCH_2$), 74.80 ($C5^6$, $BnCH_2$), 74.70 ($BnCH_2$), 74.60 ($C5^5$), 74.56 ($BnCH_2$), 74.52 ()$C5^3$, 74.43, ($C5^4$) 74.34, ($C4^3$) 74.19, ($C5^2$) 74.12 ($BnCH_2$), 73.91 ($C4^1$), 73.58 ($BnCH_2$), 73.48 ($BnCH_2$), 73.43 ($BnCH_2$), 73.39 ($BnCH_2$), 73.21 ($BnCH_2$), 73.18 ($BnCH_2$), 72.84 ($C2^4$), 72.42 ($C4^2$), 71.62 ($C5^1$), 71.47 ($C2^2$), 71.05 ($BnCH_2$), 70.81 ($BnCH_2$), 69.36 ($C6^6$), 69.30 ($C6^5$), 69.21 ($C6^3$), 68.99 ($C6^4$), 68.94 ($C6^2$), 66.24 ($C6^1$), 58.67 ($C2^6$), 58.16 ($C2^5$), 56.05 ($C2^1$), 23.47 (Ac), 23.44 (Ac), 23.33 (Ac), 21.08 (Ac).

2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-1,3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranose (96)



Compound **92** (200 mg, 83 μmol) and 10% Pd(C) (111 mg) was suspended in MeOH / acetic acid 30:2 (6.82 mL). The suspension was stirred under argon and then flushed with H_2 gas. The reaction was stirred under hydrogen

atmosphere for 5 days and after completion flushed with argon again. The suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (10 mL) and stirred at room temperature overnight. The solvent was removed and co-evaporated three times with toluene, purified by column chromatography (cyclohexane-ethyl acetate 1:1 to MeOH-ethyl acetate 5%) to give **96**. Yield: 74% (112 mg, 61 μmol). R_f = 0.2 (MeOH/EtOAc 5%).

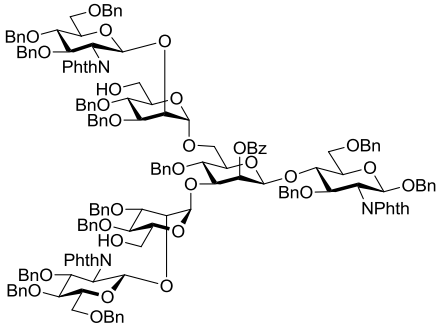
Compound **95** (518 mg, 199 μmol) and 10% Pd(C) (300 mg) was suspended in MeOH / acetic acid 30:2 (16 mL). The suspension was stirred under argon and flushed with H_2 gas. The reaction was stirred under hydrogen atmosphere for 16 days and after completion flushed with argon again. The suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (15 mL) and stirred at room temperature for 3 days. The solvent was removed and co-evaporated three times with toluene, purified by column chromatography (cyclohexane-ethyl acetate 1:1 to MeOH-ethyl acetate 5%) to give **96**. Yield: 85% (310 mg, 170 μmol). R_f = 0.4 (EtOAc/MeOH/AcOH/H_2O 60:3:3:2).

$[\alpha]_D^{20}$ = -37° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1828.6148 ($[M+H]^+$, calc. 1828.5946), 1845.6204 ($[M+NH_4]^+$, calc. 1845.6211), 1850.5700 ($[M+Na]^+$, calc. 1850.5765), 914.8005 ($[M+2H]^{2+}$, calc. 914.8012), 923.2163 ($[M+H+NH_4]^{2+}$, calc. 923.3145), 934.2767 ($[M+Na+NH_4]^{2+}$, calc. 934.3055).***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 6.33 (d, *J*=9.1, 1H, N*H*^{5}), 6.10 (d, *J*=3.6, 1H, H1^1), 5.97 (s, 1H, N*H*^{6}), 5.83 (d, *J*=9.1, 1H, N*H*^{1}), 5.51 - 5.42 (m, 2H, H2^2, H3^6), 5.31 - 5.12 (m, 3H, H3^4, H3^3, H3^1), 5.11 - 5.06 (m, 1H, H3^5), 5.06 - 5.00 (m, 1H, H4^6), 4.99 - 4.93 (m, 1H, H4^5), 4.93 - 4.86 (m, 2H, H1^6, H4^2), 4.80 - 4.74 (m, 2H, H1^2, H4^3), 4.72 (dd, *J*=10.1, 3.0, 1H, H4^4), 4.61 - 4.55 (m, 2H, H1^4, H1^3), 4.52 (d, *J*=8.3, 1H, H1^5), 4.43 - 4.38 (m, 3H, H2^1, H2^4, H6a^1), 4.33 - 4.10 (m, 7H, H2^5, H6a^4, H6a^6, H6b^1, H6a^3, H2^3, H6a^5), 4.07 - 3.92 (m, 4H, H6b^6, H6b^4, H6b^3, H6b^5), 3.92 - 3.74 (m, 5H, H2^6, H6a^2, H4^1, H5^1, H3^2), 3.72 - 3.64 (m, 3H, H6b^2, H5^2, H5^6), 3.59 - 3.53 (m, 3H, H5^4, H5^3, H5^5), 2.18 (s, 3H), 2.17 (s, 3H), 2.14 (s, 6H), 2.12 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 - 1.99 (m, 15H), 1.99 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.89 (s, 3H). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.20, 171.16, 171.08, 171.04, 170.94, 170.91, 170.83, 170.78, 170.43, 170.36, 170.28, 170.10, 169.66,

169.57, 169.49, 169.26, 168.88 (171.2 - 168.9 CO_2CH_3), 100.66 ($C1^6$), 100.18 ($C1^4$), 99.89 ($C1^5$), 99.23 ($C1^3$), 98.53 ($C1^2$), 90.47 ($C1^1$), 78.58 ($C3^2$), 75.63 ($C1^4$), 73.83 ($C3^5$), 73.37 ($C5^6$), 73.28 ($C2^4$), 72.89 ($C5^3$), 72.80 ($C5^4$), 72.23 ($C4^4$), 72.06 ($C3^6$), 71.95 ($C3^1$), 71.76 ($C5^5$), 71.66 ($C5^2$), 71.47 ($C4^3$), 71.05 ($C2^3$), 70.61 ($C5^1$), 69.11 ($C4^5$), 68.90 ($C4^6$), 68.67 ($C2^2$), 68.23 ($C6^2$), 66.84 ($C4^2$), 65.63 ($C3^4$), 65.31 ($C3^3$), 62.44, 62.38, 62.02 (62.4 - 62.0 $C6^1$, $C6^3$, $C6^4$, $C6^5$), 55.23 ($C2^6$), 53.95 ($C2^5$), 50.85 ($C2^1$), 23.45, 23.29, 23.05 (23.3 - 23.1 NHAc), 21.29, 21.19, 21.14, 21.08, 21.01, 20.98, 20.89, 20.84, 20.82, 20.80, 20.79, 20.74, 20.10 (21.3 - 20.1 OAc).

7.16 Synthesis of complex type 2,6 branched octasaccharide

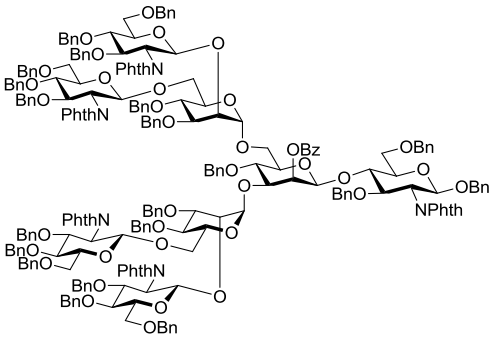
Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (97)



To a stirring solution of **90** (868 mg, 0.32 mmol) in anhydrous DCM (6.4 mL, 20 mL/mmol) and at 0 °C, a solution of 1 M BH_3 in THF (3.17 mL, 10 mL/mmol) was slowly added over 30 min. Addition of TMSOTf (17.1 µL, 0. 96 mmol, 0.3 eq) followed and the mixture was stirred at r.t. for 16 h. Neutralization with first Et_3N (0.1 mL) was followed and then MeOH was added until no more hydrogen gas was formed. The organic solvent was evaporated under reduced pressure without heating, and the residue was dissolved in DCM (20 mL), washed with $NaHCO_3$ (20 mL ×2), brine (20 mL ×2) and dried over $MgSO_4$. Then concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **97**. Yield: 68% (595 g, 0.22 mmol). R_f = 0.2 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -10° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1381.0651 ($[M+H+NH_4]^{2+}$, calc. 1381.0605), 1389.5671 ($[M+2NH_4]^{2+}$, calc. 1389.5738), 1392.0249 ($[M+Na+NH_4]^{2+}$, calc. 1392.0515). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.18 - 8.08 (m, 2H, benzoyl), 7.57 - 6.41 (m, 85H, aromatic), 5.34 (d, *J*=8.5, 1H, $H1^5$), 5.24 (d, *J*=3.3, 1H, $H2^2$), 5.13 (d, *J*=12.0, 1H, $BnCH_2$), 5.01 - 4.92 (m, 3H, $BnCH_2$, $H1^1$, $H1^6$), 4.92 - 4.80 (m, 3H, $BnCH_2$), 4.80 - 4.73 (m, 2H, $BnCH_2$, $H3^5$), 4.68 (dd, *J*=11.6, 3.3, 2H, $BnCH_2$), 4.64 - 4.45 (m, 9H, $H1^2$, $BnCH_2$), 4.45 - 4.16 (m, 13H, $H2^5$, $H2^4$, $H2^3$, $BnCH_2$, $H1^3$), 4.13 (dd, *J*=10.2, 1.9, 2H, $H2^6$, $BnCH_2$), 4.11 - 4.01 (m, 2H, $H2^1$, $H6a^1$), 4.01 - 3.83 (m, 6H, $BnCH_2$, $H6b^1$, $H3^6$, $H3^1$, $H4^1$, $H4^5$), 3.83 - 3.71 (m, 3H, $H5^5$, $H6a^5$, $BnCH_2$), 3.71 - 3.57 (m, 4H, $H6b^5$, $H5^2$, $H4^3$, $H1^4$), 3.56 - 3.26 (m, 10H, $H6a^4$, $H6a^3$, $H4^4$, $H5^1$, $H4^6$, $H3^2$, $H3^4$, $H6a^2$, $H6b^3$, $H3^3$), 3.25 - 3.09 (m, 5H, $H6b^4$, $H6a^6$, $H6b^6$, $H4^2$, $H5^4$), 2.86 (d, *J*=10.1, 2H, $H6b^2$, $H5^3$), 2.10 (d, *J*=6.7, 1H, $H5^6$). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.96 (Phth C=O), 168.04 (Phth C=O), 167.13, 166.32 (benzoyl C=O), 139.63, 139.37, 139.32, 139.23, 139.03, 138.85, 138.80, 138.61, 138.40, 138.32, 138.26, 138.11, 137.36 (139.6 - 137.4 benzyl), 133.93 (Phth), 133.65 (Phth), 133.37 (Phth), 132.21, 131.83, 131.68, 130.51, 129.50, 128.92, 128.87, 128.76,

128.71, 128.63, 128.59, 128.50, 128.42, 128.39, 128.37, 128.30, 128.23, 128.14, 127.94, 127.92, 127.78, 127.73, 127.67, 127.64, 127.58, 127.55, 127.48, 127.28, 127.20 (129.5 - 127.2 aromatic), 123.19, 102.08 ($C1^4$), 100.69 ($C1^3$), 99.58 ($C1^5$), 97.74 ($C1^1$), 97.46 ($C1^6$), 97.26 ($C1^2$), 82.19 ($C3^2$), 80.51 ($C3^3$), 80.31 ($C3^4$), 79.48 ($C4^5$), 79.31 ($C4^6$), 78.98 ($C3^5$), 78.61 ($C3^6$), 78.09 ($C4^1$), 76.76 ($C3^1$), 76.06 ($C5^4$), 75.45 ($BnCH_2$), 75.38 ($BnCH_2$), 75.34 ($C4^2$), 75.17 ($C5^3$), 74.99 ($C5^5$), 74.63 ($BnCH_2$), 74.49 ($C4^3$, $BnCH_2$), 74.37 ($BnCH_2$), 74.28 ($BnCH_2$), 74.15 ($C4^4$, $C5^1$), 74.07 ($BnCH_2$), 73.77 ($C5^6$, $C5^2$), 73.68 ($BnCH_2$), 73.58 ($BnCH_2$), 72.56 ($C2^3$), 70.85 ($C6^2$), 70.77 ($BnCH_2$), 70.30 ($C2^4$), 69.93 ($C2^2$, $BnCH_2$), 69.79 ($C6^1$), 69.68 ($BnCH_2$), 68.91 ($C6^6$), 68.37 ($C6^5$), 63.24 ($C6^3$), 62.92 ($C6^4$), 56.88 ($C2^5$), 56.57 ($C2^6$), 56.13 ($C2^1$).

Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-{3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)}-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (98)

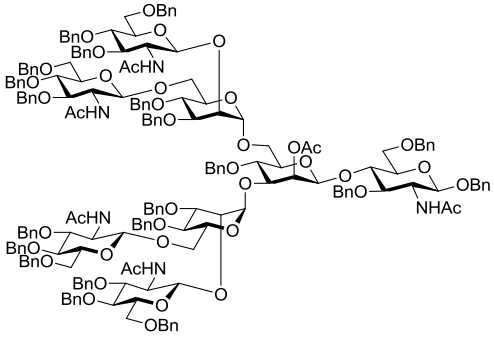


Compounds **55** (485 mg, 0.72 mmol, 4.0 eq), **97** (495 mg, 0.18 mmol, 1.0 eq), *N*-iodosuccinimide (162 mg, 0.72 mmol, 4.0 eq) and 4 Å molecular sieves (0.5 g) in dry dichloromethane (10 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (16.4 μL, 0.12 mmol, 0.25 eq). After stirring for 0.5 h at 0 °C, the reaction was quenched by dilution with more DCM (20 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (15 mL×3), brine (30 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (toluene-ethyl acetate 5% to 11%) to give **98**. Yield: 74% (545 mg, 0.13 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -2° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1951.2720 ($[M+2NH_4]^{2+}$, calc. 1951.2906), 1953.7541 ($[M+Na+NH_4]^{2+}$, calc. 1953.7683), 1962.2458 ($[M+Na+NH_3+NH_4]^{2+}$, calc. 1962.2816). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.21 (d, *J*=7.9, 2H, benzoyl), 7.78 (d, *J*=6.4, 1H, Phth), 7.69 (d, *J*=6.5, 1H, Phth), 7.65 - 6.82 (m, 110H, aromatic), 6.82 - 6.74 (m, 5H, aromatic), 6.71 (d,

J=6.8, 2H, aromatic), 6.63 (t, *J*=7.3, 1H, aromatic), 6.41 (t, *J*=7.4, 2H, aromatic), 6.34 (d, *J*=7.3, 3H, aromatic), 5.49 (d, *J*=8.5, 1H, $H1^5$), 5.24 - 5.20 (m, 2H, $H1^6$, $BnCH_2$), 5.19 (d, *J*=3.2, 1H, $H2^2$), 5.10 - 5.05 (m, 2H, $H1^7$, $BnCH_2$), 4.98 (d, *J*=8.4, 1H, $H1^1$), 4.97 - 4.90 (m, 5H, $H1^8$, $H3^5$, $BnCH_2$), 4.88 (d, *J*=9.1, 1H, $BnCH_2$), 4.86 - 4.77 (m, 6H, $BnCH_2$), 4.74 - 4.65 (m, 6H, $BnCH_2$), 4.63 - 4.54 (m, 4H, $BnCH_2$), 4.53 - 4.48 (m, 4H, $BnCH_2$), 4.48 - 4.40 (m, 8H, $H1^2$, $H3^6$, $BnCH_2$), 4.40 - 4.36 (m, 3H, $H2^5$, $BnCH_2$), 4.33 (d, *J*=12.2, 1H, $BnCH_2$), 4.30 - 4.24 (m, 6H, $H1^3$, $H3^8$, $H2^3$, $H2^4$, $H2^6$, $BnCH_2$), 4.24 - 4.16 (m, 3H, $H2^8$, $H6a^1$, $BnCH_2$), 4.15 - 4.06 (m, 5H, $H4^5$, $H2^1$, $H6b^1$, $BnCH_2$), 4.06 - 3.99 (m, 4H, $H3^7$, $H4^1$, $H3^1$, $BnCH_2$), 3.96 (d, *J*=9.7, 1H, $H5^5$), 3.89 - 3.79 (m, 5H, $H4^8$, $H4^6$, $H4^2$, $H6a^4$, $H6a^6$), 3.79 - 3.72 (m, 3H, $H6a^3$, $H6b^6$, $H6a^5$), 3.72 - 3.66 (m, 4H, $H3^2$, $H1^4$, $H5^4$, $H6a^8$), 3.65 - 3.59 (m, 4H, $H4^7$, $H6a^2$, $H6b^5$, $H6b^8$), 3.50 - 3.43 (m, 3H, $H3^4$, $H5^6$, $H5^1$), 3.42 - 3.37 (m, 2H, $H5^2$, $H5^8$), 3.35 - 3.32 (m, 3H, $H4^4$, $H6b^4$, $H6b^3$), 3.25 (t, *J*=9.4, 1H, $H4^3$), 3.17 (dd, *J*=9.2, 2.4, 1H, $H3^3$), 3.14 (dd, *J*=10.5, 2.8, 1H, $H6a^7$), 3.10 (d, *J*=10.2, 1H, $H6b^2$), 3.02 (d, *J*=10.0, 1H, $H6b^7$), 2.99 - 2.96 (m, 1H, $H5^3$), 2.14 (d, *J*=9.3, 1H, $H5^7$). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.64, 168.34, 168.28, 167.96, 167.66, 167.40, 166.91, 166.66 (168.6 - 166.7 Phth C=O), 166.08 (benzoyl C=O), 139.70, 139.43, 139.25, 139.19, 138.87, 138.80, 138.78, 138.64, 138.47, 138.31, 138.24, 138.13, 138.07, 138.04, 138.00, 137.84, 137.24, 136.78, 133.77, 133.54, 133.47, 133.43, 133.22 (133.8 - 133.2 Phth), 132.45, 131.89, 131.84, 131.53, 131.48, 131.18, 130.22, 130.05, 129.80, 129.18, 129.04, 128.72, 128.64, 128.48, 128.40, 128.35, 128.28, 128.24, 128.21, 128.15, 128.12, 128.10, 128.07, 128.05, 128.00, 127.98, 127.95, 127.93, 127.88, 127.83, 127.76, 127.70, 127.64, 127.62, 127.56, 127.51, 127.49, 127.39, 127.36, 127.30, 127.25, 127.17, 127.12, 126.95, 126.91, 126.88, 126.83, 126.76, 126.62 (129.2 - 126.6 aromatic), 123.59, 123.46, 123.31, 123.16, 122.93, 122.84, 102.21 ($C1^4$), 99.97 ($C1^6$), 99.46 ($C1^5$), 99.30 ($C1^8$), 99.18 ($C1^3$), 97.54 ($C1^7$), 97.24 ($C1^1$), 96.63 ($C1^2$), 80.93 ($C3^2$), 80.76 ($C3^3$), 80.52 ($C3^4$), 79.52 ($C4^6$), 79.39 ($C3^6$), 79.37 ($C3^8$), 79.16 ($C4^8$), 79.04 ($C4^5$), 78.74 ($C4^7$), 78.55 ($C3^5$), 78.49 ($C3^7$), 77.04 ($C4^1$), 76.90 ($C5^4$), 76.36 ($C5^3$), 76.13 ($C3^1$), 75.12 ($BnCH_2$), 75.07 ($BnCH_2$), 74.96 ($BnCH_2$), 74.89 ($C5^6$), 74.83 ($C4^4$), 74.81 ($C5^8$), 74.72 ($C5^2$), 74.68 ($C5^5$), 74.60 ($C4^3$), 74.46 ($C5^1$), 74.21 ($BnCH_2$), 74.11 ($BnCH_2$), 74.03 ($BnCH_2$), 73.91 ($BnCH_2$), 73.81 ($C5^7$), 73.71 ($BnCH_2$), 73.68 ($BnCH_2$), 73.51 ($C4^2$), 73.29 ($BnCH_2$), 73.24 ($BnCH_2$), 73.18 ($BnCH_2$), 72.45 ($C6^2$), 72.20 ($C2^3$), 72.14 ($C6^1$), 70.61 ($C6^3$), 70.48 ($BnCH_2$), 70.23 ($C2^4$, $BnCH_2$), 69.75 ($BnCH_2$), 69.62 ($BnCH_2$), 69.59 ($C6^1$), 69.54 ($C2^2$),

68.58 (C6^6), 68.48 (C6^5), 68.33 (C6^7), 66.78 (C6^8), 56.21 (C2^5), 56.17 (C2^7), 55.90 (C2^8), 55.85 (C2^1), 55.78 (C2^6).

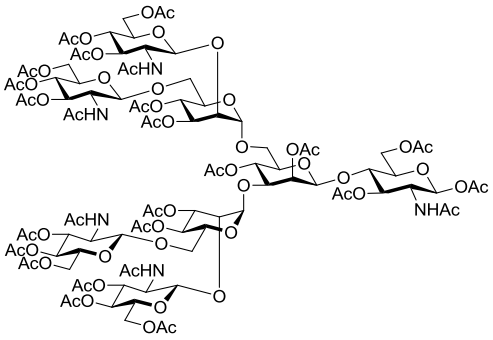
Benzyl 2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-{2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)}-2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (99)



To a heated solution of **94** (515 mg, 133 μmol) in Tol / EtOH 1:1 (20 mL) at 80 °C, hydrazine monohydrate (4 mL) was added. The reaction was kept at 80 °C for 10 days, the solvent was then removed and coevaporated with toluene (20 mL×3). The residue was taken up in pyridine / acetic anhydride 2:1 (20 mL) and stirred at r.t. for 2 days. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:3) gave **99**. Yield: 72% (322 mg, 96 μmol). R_f = 0.7 (Tol/EtOAc 1:1). *MALDI-TOF-MS* (Bruker autoflex II MALDI spectrometer): 3388.168 ([M+Na]$^+$, calc. 3387.512), 3404.361 ([M+K]$^+$, calc. 3403.486). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.48 – 7.15 (benzyl aromatics), 5.42 (H2^2), 5.04 (H1^6), 5.02 (H1^8), 4.88 (H3^5), 4.85 (H1^1), 4.78 (H1^5), 4.75 (H1^2), 4.55 (H1^7), 4.52 (H1^3), 4.39 (H2^3), 4.24 (H3^7), 4.21 (H2^7), 4.20 (H2^8), 5.06 – 4.15 (benzyl CH_2), 4.07 (H1^4), 4.01 (H2^4), 3.98 (H4^1), 3.91 (H4^2), 3.90 (H4^3), 3.75 (H4^4), 3.59 (H3^2), 3.55 (H6^3), 3.46 (H6^4), 3.97 – 3.66 (H6s), 3.29 (H5^3), 3.11 (H2^6), 3.10 (H2^5), 2.95 (H5^4), 2.38, 2.17, 2.12, 2.07, 2.04, 1.79 (Acetyl CH_3). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 173.85, 172.51, 172.10, 170.54, 169.67, 168.55 (acetyl *C*=O), 139.22, 138.72, 138.66, 138.54, 138.38, 138.22, 138.16, 138.12, 138.10, 137.88, 137.81, 137.79, 137.75, 135.27, 134.05, 133.97, 133.49, 132.62, 131.87, 129.04, 128.76, 128.57, 128.56, 128.51, 128.48, 128.45, 128.44, 128.40, 128.37, 128.34, 128.25, 128.20, 128.14, 128.06, 128.04, 127.94, 127.90, 127.86, 127.83, 127.68, 127.66, 127.61, 127.55, 127.48, 127.44, 127.39 (139.22 – 127.39 benzyl aromatics), 102.28 (C1^7), 101.27 (C1^4), 101.08 (C1^5), 100.68 (C1^6), 100.54 (C1^8), 99.34 (C1^1), 98.87 (C1^2), 98.24 (C1^3), 83.00, 80.59, 80.28 (C3^2), 79.81, 79.62 (C3^5), 79.26, 79.20, 78.60, 78.34 (C3^7), 77.92 (C4^1),

76.53 ($C4^2$), 75.34 ($C5^3$), 75.21, 75.10, 75.01, 74.77, 74.67, 74.64, 74.47 ($C5^4$), 74.42, 74.26, 74.10, 73.60, 73.53 ($C2^4$), 73.46, 73.41, 72.31 ($C4^4$), 72.24 ($C4^3$), 71.26 ($C2^3$), 70.98, 69.93, 69.69, 69.51 ($C6^3$), 69.42 ($C6^4$), 69.12, 69.08, 68.60, 68.11 ($C2^2$), 67.17, 66.57, 59.57 ($C2^5$), 59.19 ($C2^6$), 55.26 ($C2^8$), 55.65 ($C2^7$), 25.04, 24.06, 23.89, 23.11, 21.39, 20.83 (25.04- 20.83 acetyl CH_3).

2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→3)-{2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→6)}-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-1,3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranose (100)

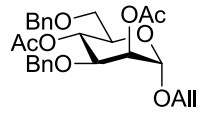


Compound **99** (447 mg, 133 μmol) and 10% Pd(C) (300 mg) was suspended in MeOH / acetic acid 30:2 (22.4 mL). The suspension was stirred under argon and flushed with H_2 gas. The reaction was stirred under hydrogen atmosphere for 15 days and after completion flushed with argon again. The suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (15 mL) and stirred at room temperature for 3 days. The solvent was removed and co-evaporated three times with toluene, purified by column chromatography (cyclohexane-ethyl acetate 1:1 to MeOH-ethyl acetate 5%) to give **100**. Yield: 58% (186 mg, 77 μmol). R_f = 0.3 (EtOAc/MeOH/AcOH/H_2O 60:3:3:2). $[\alpha]_D^{20}$ = -42° (c = 0.8, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1202.4050 ($[M+2H]^{2+}$, calc. 1202.4034), 1210.9251 ($[M+H+NH_4]^{2+}$, calc. 1210.9167), 1221.8867 ($[M+Na+NH_4]^{2+}$, calc. 1221.9077). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 6.86 (s, 1H, N*H*^{7}), 6.57 (d, *J*=5.3, 1H, N*H*^{8}), 6.40 (d, *J*=8.1, 1H, N*H*^{6}), 6.16 (d, *J*=3.1, 1H, $H1^1$), 6.04 (t, *J*=9.8, 1H, $H3^5$), 5.66 (d, *J*=8.3, 1H, N*H*^{1}), 5.44 - 5.34 (m, 4H, $H1^5$, $H3^7$, $H3^8$, $H2^2$), 5.31 (t, *J*=9.9, 1H, $H3^3$), 5.22 (t, *J*=9.9, 1H, $H3^4$), 5.18 - 5.10 (m, 2H, $H3^6$, $H3^1$), 5.04 (t, *J*=9.5, 1H, $H4^6$), 4.99 - 4.89 (m, 3H, $H1^2$, $H4^8$, $H4^7$), 4.86 (t, *J*=9.5, 1H, $H4^5$), 4.79 (dd, *J*=10.3, 2.7, 1H, $H4^4$), 4.73 - 4.66 (m, 4H, $H1^7$, $H4^3$, $H4^2$, $H6a^1$), 4.55 (s, 1H, $H1^3$), 4.49 - 4.46 (m, 4H, $H1^8$, $H1^4$, $H6a^6$, $H6a^7$), 4.36 (d, *J*=2.6, 1H, $H2^3$), 4.34 - 4.30 (m, 3H, $H6b^6$, $H6b^7$, $H2^1$), 4.29 - 4.20 (m, 5H, $H2^4$,

H6a^5, H6b^1, H6a^8, H2^6), 4.19 - 4.11 (m, 3H, H1^6, H6a^3, H6a^4), 4.10 - 4.04 (m, 2H, H4^1, H6b^8), 3.98 - 3.91 (m, 3H, H5^2, H3^2, H6b^5), 3.89 - 3.79 (m, 3H, H5^1, H5^6, H6a^2), 3.79 - 3.74 (m, 1H, H2^7), 3.73 - 3.63 (m, 3H, H5^6, H5^8, H2^8), 3.60 - 3.56 (m, 1H, H5^7), 3.53 - 3.45 (m, 2H, H5^4, H6b^2), 3.39 (d, *J*=9.5, 1H, H5^3), 3.27 (d, *J*=11.0, 1H, H6b^4), 3.19 (d, *J*=11.3, 1H, H6b^3), 3.07 (d, *J*=6.0, 1H, H2^5), 2.20 (s, 3H), 2.19 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.07 (s, 6H), 2.06 - 2.03 (m, 15H), 2.00 (t, *J*=8.1, 24H), 1.94 (s, 6H), 1.85 (s, 3H) (2.20 - 1.85 Ac). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.94, 171.70, 171.57, 171.20, 170.84, 170.75, 170.68, 170.54, 170.48, 170.45, 170.36, 170.18, 170.03, 170.00, 169.68, 169.59, 169.57, 169.38, 168.78 (171.94 - 168.78 *C*=O), 103.03 (C1^6), 101.24 (C1^8), 99.54 (C1^4), 99.34 (C1^7), 99.21 (C1^3), 98.45 (C1^5), 97.75 (C1^2), 90.19 (C1^1), 77.88 (C3^2), 75.04 (C4^1), 72.91 (C5^4), 72.74 (C5^3), 72.55 (C3^8, C3^7), 72.51 (C5^2), 72.23 (C3^6), 72.17 (C5^8), 72.02 (C4^3), 71.73 (C5^7), 71.71 (C4^4), 71.62 (C5^6), 71.35 (C3^1), 71.28 (C2^3), 71.13 (C5^5), 71.07 (C5^1), 70.90 (C2^4), 70.72 (C3^5), 70.08 (C4^5), 69.33 (C4^7), 69.17 (C4^8), 68.99 (C2^2), 68.74 (C4^6), 68.36 (C6^2), 68.08 (C4^2), 67.77 (C6^3), 67.07 (C6^4), 65.39 (C3^3), 65.27 (C3^4), 63.05 (C6^5), 62.37 (C6^1), 62.15 (C6^6), 62.12 (C6^7), 62.02 (C6^8), 56.91 (C2^5), 55.01 (C2^7), 54.82 (C2^8), 53.56 (C2^6), 51.29 (C21), 23.48, 23.41, 23.16, 22.81, 22.68, 21.49, 21.16, 21.05, 21.02, 20.95, 20.91, 20.89, 20.85, 20.73, 20.69, 20.63, 20.59, 20.52 (23.48 - 20.52 CH_3).

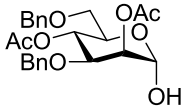
7.17 Synthesis of a 2,4-OAc-3,6-OBn mannosyl donor building block

Allyl 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (101)[283]



Compound **30** (4.69 g, 11.7 mmol) was dissolved in pyridine / acetic anhydride 2:1 (45 mL) and stirred at r.t. for 24 h. Then concentrated, coevaporated with toluene (50 ml×3) and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **101** in 99% yield (5.65 g, 11.7 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +12° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 485.3472 ($[M+H]^+$, calc. 485.2175), 502.2441 ($[M+NH_4]^+$, calc. 502.2435), 507.1988 ($[M+Na]^+$, calc. 507.1995). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.36 - 7.23 (m, 10H, aromatic), 5.90 (dddd, *J*=21.8, 10.4, 6.1, 5.2, 1H, CH_2CH=CH_2), 5.37 (dd, *J*=3.3, 1.8, 1H, H2), 5.32 - 5.18 (m, 3H, CH_2CH=CH_2, H4), 4.89 (d, *J*=1.6, 1H, H1), 4.65 (d, *J*=12.1, 1H, 3-$BnCH_2$), 4.58 - 4.50 (m, 2H, 6-$BnCH_2$), 4.42 (d, *J*=12.1, 1H, 3-$BnCH_2$), 4.19 (ddt, *J*=12.9, 5.2, 1.4, 1H, CH_2CH=CH_2), 4.01 (ddt, *J*=12.9, 6.2, 1.3, 1H, CH_2CH=CH_2), 3.92 - 3.84 (m, 2H, H3, H5), 3.61 - 3.52 (m, 2H, H6a, H6b), 2.13 (s, 3H, Ac), 1.91 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.63 (CO_2CH_3), 170.00 (CO_2CH_3), 138.21, 138.11, 133.54 (CH_2CH=CH_2), 128.53, 128.50, 127.95, 127.91, 127.80 (128.5 - 127.8 aromatic), 118.10 (CH_2CH=CH_2), 97.02 (C1), 74.97 (C3), 73.73 (6-$BnCH_2$), 71.56 (3-$BnCH_2$), 70.25 (C5), 69.69 (C6), 68.59 (C2), 68.52 (C4, CH_2CH=CH_2), 21.26 (CO_2CH_3), 21.08 (CO_2CH_3).

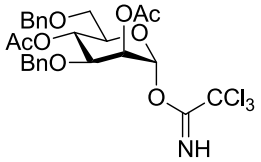
2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranose (102)[283]



Compound **101** (4.82 g, 9.95 mmol, 1.0 eq) was dissolved in Tol/EtOAc/H_2O 20:10:1 (62 mL) and Wilkinson's catalyst (920 mg, 0.99 mmol, 0.1 eq) was added. After refluxing for 24 h, the reaction mixture was cooled to r.t., filtered through a layer of Celite and concentrated. The residue was dissolved in THF/H_2O 4:1 (50 mL) followed by addition of I_2 (10.2 g, 39.8 mmol, 4.0 eq). The reaction was kept stirring at r.t. for 2 h and neutralized with 1 M $Na_2S_2O_3$. The suspension was concentrated to removed most of the THF and then diluted with DCM (100 mL), washed with 1 M $Na_2S_2O_3$ (50 mL×2), H_2O (50 mL×2), brine (50 mL×1), dried over $MgSO_4$, the solvent was removed and the crude product was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **102**, yield 68% (3.02 g, 6.79 mmol). R_f = 0.3 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -13° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 462.2124 ($[M+NH_4]^+$, calc. 462.2128),

467.1676 ([M+Na]$^+$, calc. 467.1682). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.37 - 7.21 (m, 10H, aromatic), 5.32 (dd, *J*=3.2, 1.9, 1H, H2), 5.17 (d, *J*=1.6, 1H, H1), 5.10 (t, *J*=9.9, 1H, H4), 4.64 (d, *J*=12.2, 1H, 3-BnCH_2), 4.50 (s, 2H, 6-BnCH_2), 4.40 (d, *J*=12.2, 1H, 3-BnCH_2), 4.09 (ddd, *J*=10.0, 7.2, 2.5, 1H, H5), 3.90 (dd, *J*=9.7, 3.3, 1H, H3), 3.56 (dd, *J*=10.5, 7.2, 1H, H6a), 3.45 (dd, *J*=10.5, 2.6, 1H, H6b), 2.12 (s, 3H, Ac), 1.91 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.71 (CO_2CH_3), 170.20 (CO_2CH_3), 138.08, 137.67, 128.61, 128.58, 128.55, 128.34, 128.31, 128.03, 127.96, 127.94 (128.6 - 127.9 aromatic), 92.52 (C1), 74.35 (C3), 73.73 (6-BnCH_2), 71.53 (3-BnCH_2), 69.87 (C6), 69.82 (C5), 68.97 (C2), 68.54 (C4), 21.29 (CO_2CH_3), 21.07 (CO_2CH_3).

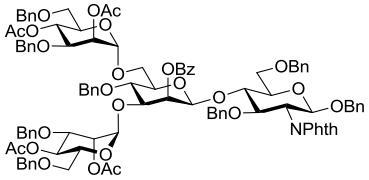
2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (103)[300]



A solution of **102** (3.0 g, 6.75 mmol, 1.0 eq) and trichloroacetonitrile (1.35 mL, 13.5 mmol, 2.0 eq) in anhydrous DCM (30 mL) was stirred under Ar at 0 °C, DBU (0.20 mL, 1.35 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:1) to give **103**, yield 79% (3.16 g, 5.37 mmol). R_f = 0.5 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +25° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 605.4454 ([M+NH_4]$^+$, calc. 605.1224), 610.1842 ([M+Na]$^+$, calc. 610.0778). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.73 (s, 1H, N*H*), 7.38 - 7.21 (m, 10H, aromatic), 6.30 (d, *J*=1.5, 1H, H1), 5.52 - 5.48 (m, 1H, H2), 5.38 (t, *J*=10.0, 1H, H4), 4.67 (d, *J*=12.2, 1H, 3-BnCH_2), 4.59 - 4.43 (m, 3H, 3-BnCH_2, 6-BnCH_2), 4.04 (dt, *J*=8.5, 4.0, 1H, H5), 3.93 (dd, *J*=9.8, 3.3, 1H, H3), 3.58 (d, *J*=4.0, 2H, H6a, H6b), 2.17 (s, 3H, Ac), 1.94 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.26 (CO_2CH_3), 169.85 (CO_2CH_3), 159.92 (*C*=NH), 138.07, 137.51, 128.64, 128.51, 128.32, 128.19, 128.13, 128.06, 127.84 (128.6 - 127.8 aromatic), 95.25 (C1), 73.99 (C3), 73.70 (6-BnCH_2), 73.03 (C5), 71.75 (3-BnCH_2), 69.27 (C6), 67.77 (C4), 66.98 (C2), 21.15 (CO_2CH_3), 21.07 (CO_2CH_3).

7.18 Synthesis of a complex type 2,4 branched octasaccharide

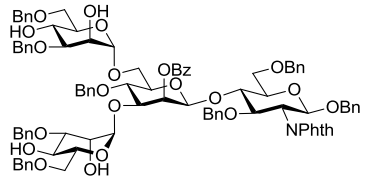
Benzyl 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside(104)



The trichloroacetimidate donor **103** (2.49 mg, 4.23 mmol, 3.0 eq) and acceptor **26** (1.33 g, 1.42 mmol, 1.0 eq) was dissolved in dry DCM (30 mL) and 4 Å molecular sieves (3 g) was added. The suspension was stirred under argon at r.t. for 30 min and cooled to 0 °C, trimethylsilyl trifluoromethanesulfonate (TMSOTf) (77.3 µL, 0.43 mmol, 0.3 eq) was added. The reaction was kept stirring at 0 °C for 2 h, then quenched with triethylamine (0.1 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) to give **104**. Yield 91% (2.32 g, 1.30 mmol). R_f = 0.2 (^{C}Hex /EtOAc 2:1). $[\alpha]_D^{20}$ = +9° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1789.6967 ($[M+H]^+$, calc. 1789.6986), 1806.7173 ($[M+NH_4]^+$, calc. 1806.7251), 906.3388 ($[M+Na+H]^{2+}$, calc. 906.3442), 914.8286 ($[M+Na+NH_4]^{2+}$, calc. 914.8575). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.23 - 8.14 (m, 2H, benzoyl), 7.63 (s, 2H, Phth), 7.57 - 6.98 (m, 35H, aromatic), 6.70 - 6.51 (m, 5H, aromatic), 5.69 (d, *J*=2.9, 1H, $H2^2$), 5.48 (dd, *J*=3.0, 1.8, 1H, $H2^4$), 5.38 (dd, *J*=2.7, 1.4, 1H, $H2^3$), 5.32 (dd, *J*=10.0, 4.7, 1H, $H4^4$), 5.29 (dd, *J*=9.9, 4.7, 1H, $H4^3$), 5.21 (s, 1H, $H1^3$), 5.01 (d, *J*=8.5, 1H, $H1^1$), 5.00 (d, *J*=1.4, 1H, $H1^4$), 4.80 (d, *J*=11.5, 1H, $H1^2$), 4.77 - 4.62 (m, 4H, $BnCH_2$), 4.61 - 4.50 (m, 5H, $BnCH_2$), 4.49 - 4.38 (m, 3H, $BnCH_2$), 4.38 - 4.27 (m, 4H, $H5^3$, $BnCH_2$), 4.18 - 4.11 (m, 2H, $H2^1$), 4.05 (dd, *J*=10.3, 8.5, 1H, $H4^1$), 3.95 - 3.77 (m, 7H, $H4^2$, $H3^2$, $H3^4$, $H3^3$, $H5^4$, $H6a^2$, $H6b^2$), 3.73 - 3.68 (m, 2H, $H6a^4$, $H6b^4$), 3.68 - 3.62 (m, 2H, $H6a^3$, $H6b^3$), 3.55 (dd, *J*=11.0, 4.7, 1H, $H6a^1$), 3.49 - 3.41 (m, 2H, $H6b^1$, $H5^1$), 3.22 (dd, *J*=9.1, 3.2, 1H, $H5^2$), 2.09 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.88 (s, 3H, Ac). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.31, 170.20, 170.15, 170.12 (CO_2CH_3), 167.61 (Phth C=O), 165.89 (benzoyl C=O), 138.51, 138.43, 138.35, 138.27, 137.98, 137.91, 137.83, 137.38 (138.5 - 137.4 benzyl), 133.62 (Phth), 130.12 (benzoyl), 129.26, 129.01, 128.87, 128.63, 128.50, 128.44, 128.42, 128.38, 128.30, 128.13, 128.05, 128.00, 127.98, 127.88, 127.84, 127.76, 127.72, 127.68, 127.63, 127.05 (129.3 - 127.1 aromatic), 123.23, 99.48 ($C1^3$), 98.63 ($C1^2$), 98.41 ($C1^4$), 97.53 ($C1^1$), 78.62 ($C4^1$), 77.39 ($C3^2$), 76.32 ($C3^1$), 75.57

($C4^2$), 75.21 ($C3^3$, $BnCH_2$), 74.88 ($C5^2$), 74.72 ($C5^1$), 74.66 ($C3^4$), 74.57 ($BnCH_2$), 73.78 ($BnCH_2$), 73.68 ($BnCH_2$), 73.62 ($BnCH_2$), 72.04 ($BnCH_2$), 71.14 ($C2^2$), 70.93 ($C5^3$), 70.64 ($BnCH_2$), 69.57 ($C5^4$), 69.40 ($C6^3$), 68.86 ($C6^1$), 68.63 ($C2^3$), 68.54 ($C6^2$), 68.05 ($C4^3$, $C4^4$), 67.88 ($C2^4$), 66.28 ($C6^2$), 55.90 ($C2^1$), 21.18 (CO_2CH_3), 21.15 (CO_2CH_3), 20.90 (CO_2CH_3).

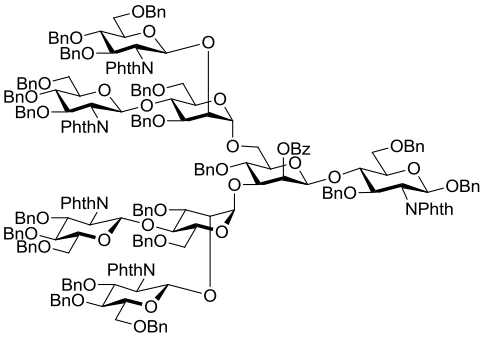
Benzyl 3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (105)



To a solution of **104** (2.15 g, 1.20 mmol) in MeOH / DCM 5:1 (60 mL), NaOMe (100 mg) was added to reach pH 9.5. The reaction was kept at r.t. for 12 days and quenched with *Dowex* 50WX8 ion-exchanger (1 g, prewashed with MeOH), concentrated and coevaporated with toluene (50 mL×3). Then purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:2) and recrystallized from cyclohexane - EtOAc to give **105**, yield 75% (1.46 g, 0.90 mmol). R_f = 0.6 (Tol/EtOAc 1:1). $[\alpha]_D^{20}$ = +17° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1621.6588 ($[M+H]^+$, calc. 1621.6563), 1638.6880 ($[M+NH_4]^+$, calc. 1638.6829), 1643.6425 ($[M+Na]^+$, calc. 1643.6383), 828.3586 ($[M+2NH_4]^{2+}$, calc. 828.3586), 830.3058 ($[M+Na+NH_4]^{2+}$, calc. 830.3347). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.11 (d, *J*=7.3, 2H, benzoyl), 7.60 (s, 2H, Phth), 7.46 - 7.16 (m, 36H, aromatic), 7.09 - 6.96 (m, 5H, aromatic), 6.61 (dq, *J*=8.1, 4.0, 1H, aromatic), 6.47 (d, *J*=4.4, 4H, aromatic), 5.65 (d, *J*=2.9, 1H, $H2^2$), 5.17 (s, 1H, $H1^3$), 5.06 (s, 1H, $H1^4$), 5.01 (d, *J*=8.5, 1H, $H1^1$), 4.78 (s, 1H, $H1^2$), 4.75 - 4.66 (m, 5H, $BnCH_2$), 4.65 - 4.53 (m, 5H, $BnCH_2$), 4.52 - 4.47 (m, 3H, $BnCH_2$), 4.43 (dd, *J*=12.2, 6.7, 2H, $BnCH_2$), 4.28 (d, *J*=13.1, 1H, $BnCH_2$), 4.20 - 4.11 (m, 3H, $H4^1$, $H2^1$), 4.09 - 4.03 (m, 2H, $H5^4$, $H3^2$), 4.03 - 3.99 (m, 1H, $H3^1$), 3.98 - 3.88 (m, 4H, $H6a^2$, $H4^3$, $H4^4$, $H3^4$), 3.85 - 3.65 (m, 12H, $H6b^2$, $H5^3$, $H6a^1$, $H6b^1$, $H6a^3$, $H6b^3$, $H6a^4$, $H6b^4$, $H3^2$, $H4^2$, $H3^4$), 3.51 (dd, *J*=9.1, 3.2, 1H, $H3^3$), 3.46 (d, *J*=10.0, 1H, $H5^1$), 3.39 - 3.33 (m, 1H, $H5^2$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 167.75 (Phth C=O), 165.70 (benzoyl C=O), 138.48, 138.45, 138.36, 138.18, 138.06, 137.78, 137.42 (138.5 - 137.4 benzyl), 133.56 (Phth), 133.44, 131.83, 130.15 (benzoyl), 130.13 (benzoyl), 128.90, 128.83, 128.78, 128.70, 128.68, 128.61, 128.49, 128.42, 128.30, 128.26, 128.18, 128.17, 128.11, 128.07, 128.06, 127.91, 127.90, 127.87, 127.78, 127.76, 127.68, 127.66, 127.04 (128.9 - 127.0 aromatic), 123.31, 101.78 ($C1^3$), 99.42 ($C1^4$), 98.39 ($C1^2$), 97.47 ($C1^1$), 79.84 ($C3^4$), 79.37 ($C3^3$), 78.98 ($C3^2$), 78.31 ($C4^1$), 76.21 ($C3^1$), 76.13

($C4^2$), 75.48 ($BnCH_2$), 74.78 ($C5^2$), 74.68 ($C5^1$), 74.46 ($BnCH_2$), 73.80 ($BnCH_2$), 73.77 ($BnCH_2$), 72.65 ($BnCH_2$), 72.55 ($BnCH_2$), 72.17 ($C2^2$), 71.66 ($C3^3$), 71.57 ($C3^4$), 70.79 ($C6^3$, $BnCH_2$), 70.64 ($C6^4$), 68.65 (C53), 68.50 ($C5^4$, $C6^1$), 68.31 ($C4^3$, $C4^4$), 66.01 ($C6^2$), 55.90 ($C2^1$).

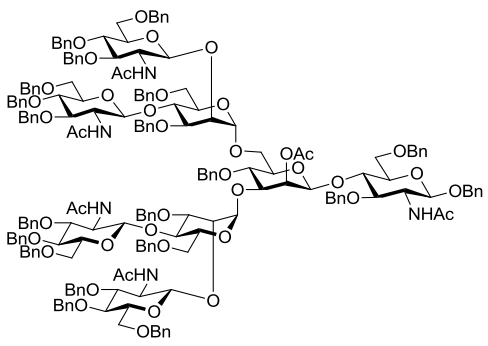
Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-{3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)}-2-*O*-benzoyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (106)



Compounds **55** (2.92 g, 4.35 mmol, 6.0 eq), **105** (1.18 g, 0.73 mmol, 1.0 eq), *N*-iodosuccinimide (1.31 g, 5.83 mmol, 8.0 eq) and 4 Å molecular sieves (1.5 g) in dry dichloromethane (15 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to 0 °C followed by slow addition of trifluoromethanesulfonic acid (49.8 μL, 0.36 mmol, 0.5 eq). After stirring for 0.5 h at 0 °C, the reaction was quenched by diluting with more DCM (50 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (30 mL×3), brine (50 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (toluene-ethyl acetate 5% to 11%) to give **106**. Yield: 58% (1.62 g, 0.42 mmol). R_f = 0.3 (Tol/EtOAc 6:1). $[\alpha]_D^{20}$ = +10° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1942.7640 ($[M+H+NH_4]^{2+}$, calc. 1942.7773), 1951.2735 ($[M+2NH_4]^{2+}$, calc. 1951.2906). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.16 (d, *J*=7.3, 2H, benzoyl), 7.77 (d, *J*=7.0, 2H, Phth), 7.68 - 6.85 (m, 114H, aromatic), 6.68 (d, *J*=6.8, 3H, aromatic), 6.58 - 6.44 (m, 5H, aromatic), 5.38 (d, *J*=8.4, 1H, $H1^6$), 5.32 (d, *J*=8.3, 1H, $H1^8$), 5.15 (s, 1H, $H2^2$), 4.94 (d, *J*=8.5, 1H, $H1^1$), 4.89 - 4.82 (m, 3H, $H1^4$, $H1^7$, $BnCH_2$), 4.82 - 4.77 (m, 3H, $H1^5$, $BnCH_2$), 4.77 - 4.64 (m, 10H, $BnCH_2$), 4.59 - 4.52 (m, 4H, $BnCH_2$), 4.52 - 4.45 (m, 5H, $H1^3$, $BnCH_2$), 4.45 - 4.38 (m, 5H, $BnCH_2$), 4.38 - 4.31 (m, 4H, $H3^8$, $BnCH_2$), 4.28 - 4.22 (m, 7H, $H3^6$, $H2^8$, $H1^2$, $BnCH_2$), 4.21 - 4.13 (m, 3H, $H2^6$, $H2^7$, $BnCH_2$), 4.13 - 4.11 (m, 2H, $H2^5$, $BnCH_2$), 4.11 - 4.05 (m, 2H, $H3^7$, $H2^1$), 3.99 (d, *J*=12.2, 2H, $BnCH_2$), 3.92 - 3.84 (m, 6H, $H3^5$, $H3^1$, $H4^1$, $H4^8$, $BnCH_2$), 3.82 - 3.70 (m, 7H, $H4^6$, $H4^2$, $H3^3$, $H3^4$, $H2^3$, $H2^4$, $BnCH_2$), 3.69 (s, 2H, $H6a^1$, $H6b^1$),

3.63 - 3.54 (m, 5H, H4^{7}, H4^{5}, H3^{2}, H4^{4}, H4^{3}), 3.54 - 3.44 (m, 3H, H5^{8}, H5^{3}, H6a^{3}), 3.42 - 3.29 (m, 5H, H6a^{8}, H6b^{8}, H6a^{7}, H6b^{7}, H6b^{3}), 3.28 - 3.20 (m, 5H, H5^{1}, H5^{4}, H6a^{2}, H6a^{5}, H6a^{4}), 3.15 - 3.06 (m, 2H, H5^{6}, H6b^{5}), 3.00 (d, *J*=10.5, 1H, H6b^{4}), 2.80 (d, *J*=11.4, 1H, H6a^{6}), 2.78 - 2.70 (m, 2H, H6b^{2}, H6b^{6}), 2.70 - 2.66 (m, 1H, H5^{2}), 2.33 (s, 1H, H5^{5}), 2.16 (d, *J*=6.6, 1H, H5^{7}). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 168.26, 167.98, 167.82, 167.51, 167.18 (168.3 - 167.2 Phth C=O), 164.74 (Bz*C*=O), 139.23, 138.88, 138.84, 138.73, 138.71, 138.64, 138.53, 138.41, 138.37, 138.35, 138.25, 138.21, 138.18, 138.08, 138.03, 137.95, 137.91, 137.18 (139.2 - 137.2 aromatics), 133.37, 133.26, 133.08, 132.08 (133.4 - 132.1 Phth), 131.93, 131.75, 131.62, 131.48, 131.38, 130.44, 130.32, 130.19 (Benzoyl), 129.80, 129.09, 129.06, 129.02, 128.95, 128.87, 128.73, 128.59, 128.41, 128.36, 128.35, 128.30, 128.28, 128.27, 128.24, 128.20, 128.16, 128.11, 128.03, 128.01, 127.99, 127.94, 127.91, 127.87, 127.83, 127.82, 127.79, 127.77, 127.72, 127.67, 127.65, 127.61, 127.53, 127.47, 127.42, 127.40, 127.36, 127.33, 127.31, 127.27, 127.22, 127.13, 126.94, 126.88, 126.79, 126.73, 126.07, 125.96, 125.35 (129.8 - 125.4 aromatics), 123.24, 123.07, 122.88 (123.2 - 122.9 Phth), 99.47 (C1^{6}), 98.60 (C1^{4}, C1^{2}), 98.06 (C1^{3}), 97.91 (C1^{8}), 97.39 (C1^{5}), 97.02 (C1^{1}), 95.69 (C1^{7}), 79.61 (C4^{8}), 79.36 (C4^{6}), 79.16 (C3^{8}), 79.09 (C3^{6}, C4^{7}, C3^{2}), 78.93 (C4^{7}), 78.77 (C3^{7}), 78.68 (C3^{5}), 77.33 (C4^{2}), 74.99 (C3^{1}), 74.77 (C5^{8}, C5^{3}, $BnCH_2$), 74.69 (C3^{3}, C5^{6}, $BnCH_2$), 74.53 ($BnCH_2$), 74.41 (C5^{5}, $BnCH_2$), 74.32 ($BnCH_2$), 74.23 (C5^{7}), 74.14 (C5^{1}), 73.95 (C4^{3}), 73.83 (C4^{4}), 73.58 (C3^{4}), 73.40 ($BnCH_2$), 73.34 ($BnCH_2$), 73.19 ($BnCH_2$), 73.14 ($BnCH_2$), 73.11 ($BnCH_2$), 73.00 ($BnCH_2$), 72.91 (C5^{2}), 72.82 ($BnCH_2$), 72.70 ($BnCH_2$), 72.26 ($BnCH_2$), 71.82 (C2^{2}, C6^{8}), 71.72 (C2^{3}), 71.57 (C2^{4}), 71.40 (C4^{1}), 71.19 (C5^{4}), 70.72 ($BnCH_2$), 70.64 ($BnCH_2$), 70.19 (C6^{2}), 69.64 ($BnCH_2$), 68.55 (C6^{5}), 68.13 (C6^{1}), 67.93 (C6^{7}), 67.65 (C6^{6}), 67.13 (C6^{4}), 66.69 (C6^{3}), 56.95 (C2^{6}), 56.67 (C2^{8}), 55.60 (C2^{1}), 55.50 (C2^{7}, C2^{5}).

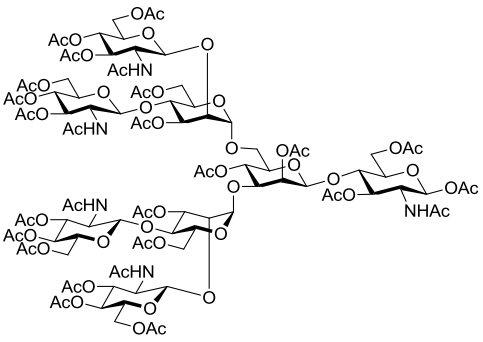
Benzyl 2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-{2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)]-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)}-2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-3,6-di-*O*-b



enzyl-2-deoxy-β-D-glucopyranoside (107)

To a heated solution of **106** (1.54 g, 398 μmol) in Tol / EtOH 1:1 (40 mL) at 80 °C, hydrazine monohydrate (8 mL) was added. The reaction was kept at 80 °C for 4 days, the solvent was removed and coevaporated with toluene (40 mL×3). The residue was taken up in pyridine / acetic anhydride 2:1 (30 mL) and stirred at r.t. for 2 days. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 5:1 to 1:3) gave **107**. Yield: 97% (1.31 g, 388 μmol). R_f = 0.4 (Tol/EtOAc 1:1). $[\alpha]_D^{20}$ = +17° (c = 1.2, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1683.2711 ($[M+2H]^{2+}$, calc. 1683.2689), 1691.7825 ($[M+H+NH_4]^{2+}$, calc. 1691.7822), 1122.1886 ($[M+3H]^{3+}$, calc. 1122.1808), 1128.2035 ($[M+2H+NH_4]^{3+}$, calc. 1128.1907). ***^{1}H-NMR*** (600 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.45 -7.07 (benzyl aromatics), 5.37 ($H2^2$), 5.04 ($H1^1$), 4.97 ($H1^3$), 4.87, 4.81 ($H1^4$), 4.72, 4.69, 4.67, 4.60 (4.87 – 4.60, H1s), 4.35 (H3), 4.31 (H2-NHAc), 4.23 (H3), 4.92 – 4.22 (benzyl CH_2), 3.86 (H2-NHAc), 3.82 ($C3^2$), 3.72 (H2-NHAc), 3.53 (H2-NHAc), 3.77- 3.35 (H6s), 3.17 (H2-NHAc), 2.94 ($H6b^2$), 1.95, 1.91, 1.79, 1.77, 1.62, 1.61 (1.95 – 1.61 acetyl CH_3). ***^{13}C-NMR*** (150.9 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.72, 171.43, 170.73, 170.54, 169.95 (171.72 – 169.95 acetyl *C*=O), 139.31, 139.03, 138.68, 138.63, 138.49, 138.44, 138.39, 138.29, 138.24, 138.13, 138.08, 138.02, 137.92, 137.82, 128.80, 128.73, 128.69, 128.60, 128.55, 128.52, 128.46, 128.44, 128.40, 128.36, 128.34, 128.26, 128.19, 128.17, 128.15, 128.10, 128.00, 127.90, 127.88, 127.84, 127.83, 127.81, 127.71, 127.63, 127.60, 127.57, 127.45, 127.42, 127.40, 127.38, 127.33, 127.23, 127.09 (139.31 – 127.09 benzyl aromatics), 101.09, 100.30, 99.91 ($C1^3$), 99.71 ($C1^1$), 97.52, 97.40, 97.38 (101.09 – 97.38 H1s), 97.28 ($C1^4$), 82.35, 82.14, 80.08 (C3), 79.85 ($C3^2$), 78.82 (C3), 78.63, 78.37, 78.15, 76.41, 75.14, 74.96, 74.85, 74.79, 74.72, 74.66, 74.55, 74.38, 74.21, 74.10, 73.51, 73.39, 73.36, 73.32, 73.28, 73.17, 72.95, 72.03, 71.84, 71.58, 71.28 ($C2^2$), 71.01, 70.62, 69.37 ($C6^2$), 69.20, 69.03, 68.88, 67.16 (70.62 – 67.16 C6s), 57.46, 57.03, 56.29, 55.75, 53.82 (57.46 – 53.82 C2-NHAc), 23.51, 23.45, 23.27, 23.19, 20.96, 20.83 (23.51 – 20.86 acetyl *C*H_3).

2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→3)-{2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→6)}-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-*N*-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α/β-D-glucopyranose (108)

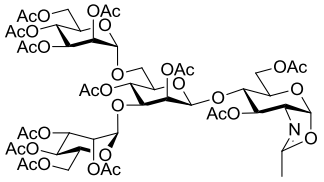


Compound **107** (1.23 g, 364 μmol) and 10% Pd(C) (700 mg) was suspended in MeOH / acetic acid 30:2 (44.8 mL). The suspension was stirred under argon and flushed with H_2 gas. The reaction was stirred under hydrogen atmosphere for 12 days and after completion flushed with argon again. The suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (30 mL) and stirred at room temperature for 3 days. The solvent was removed and co-evaporated three times with toluene, purified by column chromatography (cyclohexane-ethyl acetate 1:1 to MeOH-ethyl acetate 5%) to give a α/β (2:1) mixture of **108**. Yield: 55% (480 mg, 200 μmol). R_f = 0.3 (EtOAc/MeOH/AcOH/H_2O 60:3:3:2). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1202.4022 ($[M+2H]^{2+}$, calc. 1202.4034), 1210.9191 ($[M+H+NH_4]^{2+}$, calc. 1210.9167), 1213.3974 ($[M+H+Na]^{2+}$, calc. 1213.3944), 801.6074 ($[M+3H]^{3+}$, calc. 801.6037), 807.6151 ($[M+2H+NH_4]^{3+}$, calc. 807.6137), 809.2631 ($[M+2H+Na]^{3+}$, calc. 809.2670), 601.4722 ($[M+4H]^{4+}$, calc. 601.4548), 605.6893 ($[M+3H+NH_4]^{4+}$, calc. 605.7114). ***^{1}H-NMR*** (600 MHz, DMSO -d_3, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.11 – 7.76 (N*H*s), 5.86 (d, *J*=3.2, H1^{1}α), 5.74 (d, *J*=8.6, 1H, H1^{1}β), 5.31 (d, *J*=6.4, H2^2), 5.20 (H3^{1}β), 5.08 (H3^{1}α), 4.97 (H4^2), 4.96 (H1^3), 4.88 (H1^2), 4.84 (H1^4), 4.80, 4.76, 4.66, 4.43 (4.80 – 4.43 H1s from terminal GlcNAc), 5.18 – 4.64 (H33, H34 and H3s, H4s from terminal GlcNAc), 4.13 (H2^{1}α), 4.07 (H3^2), 3.91 (H5^{1}α), 4.40 – 3.83 (H6s), 3.82 (H4^1), 3.79 (H5^{1}β), 3.76 (H6a^2), 3.70 (H2^{1}β), 3.87 – 3.63 (H2s from terminal GlcNAc) 3.58 (H5^2), 3.92 – 3.57 (H2^3, H4^3, H2^4, H4^4, H5^3, H5^4 and H5s from terminal GlcNAc), 3.48 (H6b^2), 2.16 – 1.68 (acetyl C*H*$_3$). ***^{13}C-NMR*** (150.9 MHz, DMSO -d_3, gHSQC, gHMBC), *δ (ppm)*: 170.75, 170.66, 170.63, 170.56, 170.48, 170.27, 170.11, 170.02, 169.91, 169.85, 169.81, 169.73 (170.75 – 169.73), 100.87, 100.73, 100.56, 100.47 (100.87 – 100.47 H1s from terminal GlcNAc), 99.61 (C1^3), 97.90 (C1^4), 97.72 (C1^2), 92.02 (C1^{1}β), 90.07 (C1^{1}α), 77.56

($C3^2\alpha/\beta$), 77.42 ($C3^2\alpha/\beta$), 74.56, 74.45, 74.29, 74.20, 73.76 ($C4^1$), 73.14, 72.81 ($C3^1\alpha$), 72.73, 72.55, 72.39 ($C3^1\beta$), 72.12, 71.88, 71.60 ($C5^2$), 71.40 ($C5^1\beta$), 71.33, 71.09, 70.82 ($C5^1\alpha$), 70.73, 70.45 ($C4^2$), 70.46 ($C2^2$), 70.19, 69.24 ($C6^2$), 69.06, 69.02, 68.96, 68.88, 68.82, 68.55, 68.40, 62.86, 62.59, 62.54, 62.40, 61.94 (62.86 – 61.94 C6s), 54.02, 53.80 ($C2^1\beta$), 53.03, 52.88, 52.70 (54.02 – 52.70 C2s from terminal GlcNAc), 50.35 ($C2^1\alpha$), 23.16, 23.03, 22.97, 22.92, 22.71, 21.36, 21.21, 21.16, 21.13, 21.06, 21.00, 20.95, 20.89, 20.86, 20.82, 20.80, 20.77, 20.67 (23.16 – 20.67 acetyl CH_3).

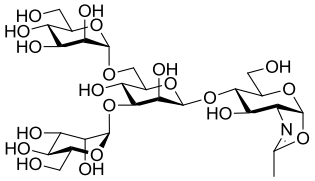
7.19 Synthesis of oxazoline donor

2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-acetyl-1,2-dideoxyl-α-D-glucopyrano-[2,1-*d*]-2-oxazoline (109)[200]



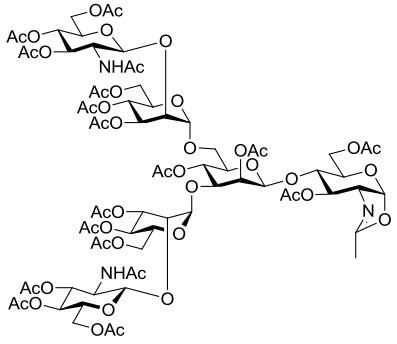
A solution of **89** (350 mg, 279.1 µmol, 1.0 eq) and tri-*tert*-butyl pyrimidine (972 mg, 3.91 mmol, 14 eq) in freshly distilled DCE (5 mL) was stirred under Ar atmosphere, then bromotrimethylsilane (441 µL, 3.34 mmol, 12 eq) and BF_3 etherate (414 µL, 3.34 mmol, 12 eq) was added. The reaction was kept at 40 °C for 4 – 6 h, until TLC indicated that a faster product was formed, then the mixture was neutralized by addition of a few drops of Et_3N. The crude mixture was diluted with DCM (30) and washed with Sat. $NaHCO_3$ (15 mL×2), brine (15 mL×1) and dried over $MgSO_4$. The solvent was removed under reduced pressure without heating and the residue was purified by column chromatography (cyclohexane-ethyl acetate 1:1 to ethyl acetate-MeOH 100:10) to give **109**, yield 93% (310 mg, 269.6 µmol). R_f = 0.6 (Pure EtOAc). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1194.3733 ($[M+H]^+$, calc. 1194.3724). ***^{1}H-NMR*** (600 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.97 (d, *J*=7.3, 1H, $H1^1$), 5.33 (d, *J*=3.3, 1H, $H2^2$), 5.33 - 5.31 (m, 1H, $H3^1$), 5.19 - 5.11 (m, 4H, $H2^3$, $H2^4$, $H4^3$, $H4^4$), 5.04 - 4.96 (m, 3H, $H1^3$, $H4^2$, $H3^4$), 4.96 - 4.92 (m, 2H, $H1^2$, $H3^3$), 4.87 (s, 1H, $H1^4$), 4.22 - 4.02 (m, 8H, $H3^2$, $H2^1$, $H6a^1$, Hb^1, $H6a^3$, $H6b^3$, $H6a^4$, $H6b^4$), 4.00 - 3.94 (m, 2H, $H5^3$, $H5^4$), 3.76 - 3.67 (m, 3H, $H4^1$, $H5^2$, $H6a^2$), 3.54 (dd, *J*=10.6, 3.4, 1H, $H6b^2$), 3.39 (ddd, *J*=8.9, 6.1, 2.7, 1H, $H5^1$), 2.13 (s, 3H), 2.12 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.95 (s, 6H), 1.94 (s, 3H) (2.13 - 1.94 Ac, CH_3). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 170.64, 170.52, 170.47, 170.36, 170.26, 170.20, 170.02, 170.01, 169.97, 169.94, 169.91, 169.56 (170.6- 169.6 CO_2CH_3), 165.23 (*C*=N), 99.21 ($C1^1$), 98.84 ($C1^2$), 98.45 ($C1^3$), 97.26 ($C1^4$), 75.91 ($C3^2$), 75.77 ($C4^1$), 72.03 ($C5^2$), 70.61 ($C2^2$), 70.46 ($C3^1$), 69.36 ($C3^3$), 69.13 ($C2^3$), 69.10 ($C2^4$), 68.94 ($C5^3$), 68.64 ($C4^2$), 68.44 ($C3^4$), 68.34 ($C5^4$), 68.17 ($C5^1$), 66.98 ($C6^2$), 65.70 ($C4^4$), 65.40 ($C4^3$), 64.79 ($C2^1$), 63.78 ($C6^1$), 62.28 ($C6^3$), 62.01 ($C6^4$), 21.25, 21.08, 21.05, 20.99, 20.97, 20.94, 20.89, 20.87 (21.3 - 20.9 CO_2CH_3), 13.81 (N=CCH_3).

α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-1,2-dideoxyl-α-D-glucopyrano-[2,1-*d*]-2-oxazoline (110)[200]



Compound **109** (28 mg, 23.4 µmol) was dissolved in anhydrous MeOH (10 mL) and stirred under Ar atmosphere. NaOMe (8 mg, 148.1 µmol) was added in portions to keep the reaction pH around 9.5 - 10. After 24 h, the reaction was checked by MALDI MS, indicating that all acetyl groups were removed. The reaction was neutralized by addition of 3-5 µL 10% AcOH solution in MeOH to reach a pH of 7.5. The solvent was removed and the residue freeze dried to give the deacetylated product **110** (28 mg, containing 12 mg NaOAc, 146.3µmol, 16 mg product, 23.2 µmol) in 99% yield. *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 690.2446 ($[M+H]^+$, calc. 690.2457), 712.2279 ($[M+Na]^+$, calc. 712.2276).***^{1}H-NMR*** (600 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.91 (d, *J*=7.3, 1H, $H1^1$), 4.84 (s, 2H, $H1^3$), 4.65 (d, *J*=1.2, 1H, $H1^4$), 4.50 (s, 1H, $H1^2$), 4.12 - 4.09 (m, 1H, $H3^1$), 3.92 (d, *J*=7.2, 1H, $H2^1$), 3.89 (d, *J*=2.3, 1H, $H2^2$), 3.76 - 3.74 (m, 1H, $H2^3$), 3.68 - 3.58 (m, 7H, $H4^3$, $H4^4$, $H2^4$, $H6a^2$, $H6b^2$, $H6a^3$, $H6b^3$), 3.58 - 3.47 (m, 4H, $H4^1$, $H3^3$, $H6a^4$, $H6b^4$), 3.47 - 3.40 (m, 5H, $H3^4$, $H5^2$, $H5^3$, $H6a^1$, $H6b^1$), 3.39 - 3.34 (m, 2H, $H3^2$, $H5^4$), 3.30 - 3.25 (m, 1H, $H4^2$), 3.18 - 3.15 (m, 1H, $H5^1$), 1.92 (s, 3H, CH_3). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 164.21 (*C*=N), 102.44 ($C1^3$), 101.18 ($C1^2$), 99.57 ($C1^1$, $C1^4$), 81.15 ($C3^2$), 78.86 ($C4^1$), 75.27 ($C4^2$), 73.91 ($C4^3$), 73.62 ($C3^4$), 70.88 ($C5^1$), 70.82 ($C3^3$), 70.61 ($C4^4$), 70.24 ($C2^3$), 70.19 ($C2^4$), 69.74 ($C2^2$, $C3^1$), 67.47 ($C2^1$), 67.25 ($C5^4$), 66.97 ($C5^3$), 65.95 ($C6^2$), 65.57 ($C5^2$), 61.31 ($C6^1$), 61.20 ($C6^4$), 61.09 ($C6^3$), 13.62 (N=C*C*H_3).

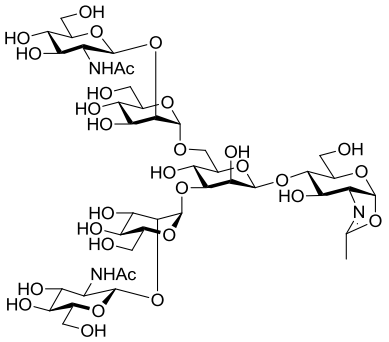
2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2-*N*-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)]-3,6-di-*O*-acetyl-1,2-dideoxyl-α-D-glucopyrano-[2,1-*d*]-2-oxazoline (111)[203]



A solution of **96** (52 mg, 28.7 µmol, 1.0 eq) with tri-*tert*-butyl pyrimidine (61.4 mg, 247.2 µmol, 8.6 eq) in freshly distilled DCE (1 mL), was stirred under Ar atmosphere, then bromotrimethylsilane (28 µL, 211.9 µmol, 7.4 eq) and BF_3 etherate (26 µL, 211.9 µmol, 7.4 eq) was added. The reaction was kept at 40 °C for 4 - 6 h and then neutralized by addition of a few drops of Et_3N. The

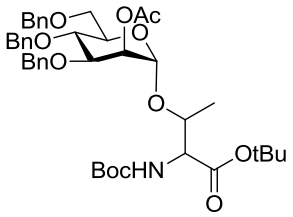
crude mixture was diluted with DCM (10 mL) and washed with Sat. $NaHCO_3$ (10 mL×2), brine (10 mL×1), dried over $MgSO_4$. The solvent was removed by evaporation under reduced pressure without heating and the residue was purified by column chromatography (cyclohexane-ethyl acetate 1:3 to ethyl acetate-MeOH 100:10) to give **111**, yield 93% (47 mg, 26.8 µmol). R_f = 0.3 (EtOAc/MeOH 100:5). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 884.7939 ($[M+2H]^{2+}$, calc. 884.7907), 893.3070 ($[M+NH_4+H]^{2+}$, calc. 893.3039), 895.7838 ($[M+Na+H]^{2+}$, calc. 895.7816), 903.7695 ($[M+K+H]^{2+}$, calc. 903.7686), 906.7763 ($[M+2Na]^{2+}$, calc. 906.7726). ***^{1}H-NMR*** (600 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.87 (d, *J*=9.0, 1H, NH^5), 7.81 (d, *J*=9.5, 1H, NH^6), 5.97 (d, *J*=7.2, 1H, $H1^1$), 5.36 - 5.34 (m, 1H, $H3^1$), 5.32 (d, *J*=3.0, 1H, $H2^2$), 5.12 - 5.07 (m, 1H, $H3^5$), 5.07 - 5.01 (m, 4H, $H4^3$, $H4^4$, $H4^2$, $H3^6$), 5.01 - 4.96 (m, 2H, $H1^2$, $H1^3$), 4.88 (s, 1H, $H1^4$), 4.87 - 4.81 (m, 3H, $H4^5$, $H4^3$, $H4^6$), 4.72 (dd, *J*=10.3, 3.3, 1H, $H3^4$), 4.59 (d, *J*=8.5, 1H, $H1^6$), 4.57 (d, *J*=8.4, 1H, $H1^5$), 4.21 - 4.11 (m, 3H, $H6a^6$, $H6a^5$, $H6a^1$), 4.09 - 3.96 (m, 8H, $H61^3$, $H6b^3$, $H6b^6$, $H6b^5$, $H6b^1$, $H2^1$, $H2^4$, $H3^2$), 3.93 (d, *J*=10.4, 2H, $H6a^4$, $H6b^4$), 3.90 - 3.87 (m, 1H, $H2^3$), 3.86 - 3.81 (m, 3H, $H2^6$, $H5^3$, $H5^6$), 3.79 (dd, *J*=9.8, 2.7, 1H, $H5^4$), 3.76 - 3.72 (m, 1H, $H6a^2$), 3.72 - 3.62 (m, 4H, $H2^5$, $H5^2$, $H5^5$, $H4^1$), 3.44 (dd, *J*=10.0, 4.0, 1H, $H6b^2$), 3.36 (s, 1H, $H5^1$), 2.18 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.01 (s, 3H), 2.00 (s, 6H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H, $N=CCH_3$), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H) (2.18 - 1.92 OAc), 1.76 (s, 3H, NHAc), 1.75 (s, 3H, NHAc). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 170.20, 170.14, 170.12, 169.81, 169.80, 169.68, 169.65, 169.61, 169.58, 169.30, 169.26, 169.24 (170.2 - 169.3 *C*=O), 164.89 (*C*=N), 99.82 ($C1^6$), 99.45 ($C1^5$), 98.86 ($C1^2$), 98.72 ($C1^3$), 98.66 ($C1^1$), 97.38 ($C1^4$), 76.10 ($C3^2$), 75.49 ($C4^1$), 73.81 ($C2^4$, $C2^3$), 72.12 ($C3^6$), 71.83 ($C3^5$), 71.31 ($C5^2$), 71.06 ($C5^5$), 70.81 ($C5^6$), 70.24 ($C2^2$), 70.18 ($C3^1$), 69.82 ($C4^6$), 69.28 ($C3^4$), 68.81 ($C3^3$), 68.62 ($C4^2$, $C4^5$, $C5^3$), 67.90 ($C5^4$), 67.56 ($C5^1$), 67.41 ($C6^2$), 64.80 ($C4^4$), 64.59 ($C4^3$), 64.21 ($C2^1$), 63.40 ($C6^1$), 62.16 ($C6^5$), 61.99 ($C6^4$), 61.75 ($C6^6$), 61.52 ($C6^3$), 53.07 ($C2^5$), 52.46 ($C2^6$), 22.62, 22.54, 20.85, 20.84, 20.78, 20.62, 20.57, 20.55, 20.53, 20.49, 20.45, 20.43, 20.41, 20.39, 20.37, 20.34 (22.6 - 20.3 Ac), 13.35 ($N=CCH_3$).

2-*N*-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-*N*-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-1,2-dideoxyl-α-D-glucopyrano-[2,1-*d*]-2-oxazoline (112)[203]



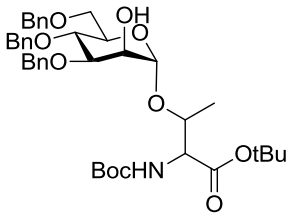
Compound **111** (47 mg, 26.6 µmol) was dissolved in anhydrous MeOH (10 mL) and stirred under Ar atmosphere. NaOMe (18 mg, 0.33 mmol) was added in portions to keep the reaction pH around 9.5 - 10. After 24 h, the completion of the reaction was checked by MALDI MS, which indicated that all acetyl groups were removed. The reaction was neutralized by addition of 3-5 µL 10% AcOH solution in MeOH to reach pH 7.5. The solvent was removed and the residue freeze dried to give the deacetylated product **112** (46 mg, containing 23 mg NaOMe, 0.26 mmol and 23.4 mg product, 21.3 µmol) in 80% yield. *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1096.4073 ($[M+H]^+$, calc. 1096.4044), 1118.3892 ($[M+Na]^+$, calc. 1118.3864), 1136.4008 ($[M+NH_3+Na]^{2+}$, calc. 1136.4163). ***^{1}H-NMR*** (600 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.99 (d, *J*=9.0, 1H, NH^5), 7.87 (d, *J*=9.0, 1H, NH^6), 5.93 (d, *J*=7.3, 1H, $H1^1$), 4.98 (s, 1H, $H1^3$), 4.78 (s, 1H, $H1^4$), 4.53 (s, 1H, $H1^2$), 4.39 (d, *J*=8.4, 1H, $H1^6$), 4.37 (d, *J*=8.0, 1H, $H1^5$), 4.14 (s, 1H, $H3^1$), 3.93 (d, *J*=6.7, 1H, $H2^1$), 3.90 (s, 1H, $H2^2$), 3.86 (s, 1H, $H2^3$), 3.79 - 3.74 (m, 1H, $H2^4$), 3.69 - 3.62 (m, 10H, $H6a^5$, $H6b^5$, $H6a^4$, $H6b^4$, $H6a^2$, $H6b^2$, $H4^5$, $H4^6$, $H3^3$, $H4^1$), 3.50 - 3.40 (m, 9H, $H2^5$, $H2^6$, $H6a^6$, $H6b^6$,$H6a^1$, $H6b^1$, $H5^2$, $H3^4$, $H3^2$), 3.38 - 3.25 (m, 5H, $H5^3$, $H5^4$, $H3^6$, $H3^5$, $H4^2$), 3.19 - 3.12 (m, 2H, $H4^3$, $H5^4$), 3.12 - 3.05 (m, 3H, $H4^4$, $H5^1$, $H5^5$), 1.93 (d, *J*=1.5, 3H, $N=CCH_3$), 1.83 (s, 3H, NHAc), 1.81 (s, 3H, NHAc). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 169.88 (*C*=O), 169.75 (*C*=O), 164.40 (*C*=N), 101.49 ($C1^5$), 101.41 ($C1^6$), 101.22 ($C1^2$), 99.75 ($C1^3$), 99.57 ($C1^1$), 97.26 ($C1^4$), 81.31 ($C3^2$), 79.06 ($C2^3$), 78.92 ($C4^1$), 78.72 ($C1^4$), 77.11 ($C5^5$), 77.03 ($C5^6$), 75.24 ($C4^2$), 74.13 ($C3^3$), 73.97 ($C3^5$), 73.87 ($C3^4$), 73.79 ($C3^6$), 70.83 ($C4^3$), 70.53 ($C4^4$, $C5^1$), 69.87 ($C4^6$), 69.72 ($C3^1$), 69.63 ($C3^2$), 69.55 ($C4^5$), 67.49 ($C5^4$), 67.44 ($C2^1$), 66.98 ($C5^3$), 66.31 ($C6^2$), 65.45 ($C5^2$), 61.37, 61.19, 60.95 (61.4 - 61.0 $C6^1$, $C6^3$, $C6^4$, $C6^5$, $C6^6$), 55.59 ($C2^6$), 55.44 ($C2^5$), 23.25 (NHAc), 23.24 (NHAc), 13.60 ($N=C\mathit{C}H_3$).

7.20 Synthesis of Gal-GlcNAc-Man/GlcNAc-Man glycosylated amino acid



_N_-_tert_-butyloxycarbonyl-_O_-(2-_O_-acetyl-3,4,6-_O_-benzyl-α-D-mannopyranosyl)-L-threonine-_tert_-butylester (113)[301]

The trichloroacetimidate donor **76** (11.31 g, 17.8 mmol, 1.0 eq) and Boc-Thr-O*t*Bu (4.97 g, 18.1 mmol, 1.02 eq) was dissolved in dry DCM (150 mL) and 4 Å molecular sieves (15 g) was added. The suspension was stirred under argon at room temperature for 30 min and then cooled to -40 °C followed by dropwise addition of trimethylsilyl-trifluoromethanesulfonate (TMSOTf) (0.807 ml, 4.4 mmol, 0.25 eq). The reaction was stirred at -40 °C for 2 h, then quenched with triethylamine (2 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **113**. Yield 67% (8.90 g, 11.9 mmol). R_f = 0.6 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +23° (c = 0.7, $CHCl_3$). *HR-ESI-MS (pos), m/z*: 750.3847 ($[M+H]^+$, calc. 750.3853), 767.4113 ($[M+NH_4]^+$, calc. 767.4119), 772.3665 ($[M+Na]^+$, calc. 772.3673). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.30 - 7.03 (m, 15H, CH_2*Ph*), 5.15 (s, 1H, H2), 4.95 (d, *J*=9.7, 1H, NH), 4.83 (d, *J*=1.5, 1H, H1), 4.77 (d, *J*=10.6, 1H, 4-CH_2Ph), 4.62 (d, *J*=2.6, 1H, 3-CH_2Ph), 4.59 (d, *J*=3.6, 1H, 6-CH_2Ph), 4.45 (d, *J*=11.3, 1H, 3-CH_2Ph), 4.42 (d, *J*=12.5, 1H, 6-CH_2Ph), 4.38 (d, *J*=10.6, 1H, 4-CH_2Ph), 4.20 (ddd, *J*=12.4, 6.0, 1.8, 1H, Thr-Hβ), 4.11 (dd, *J*=9.8, 2.0, 1H, Thr-Hα), 3.81 (d, *J*=2.8, 1H, H3), 3.79 (s, 1H, H4), 3.77 (s, 1H, H5), 3.74 - 3.67 (m, 1H, H6a), 3.63 - 3.57 (m, 1H, H6b), 2.05 (s, 3H, CH_3CO), 1.41 - 1.39 (m, 9H, $CO_2C(CH_3)_3$), 1.35 (s, 9H, $CO_2C(CH_3)_3$), 1.21 (d, *J*=6.3, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.3 ($COCH_3$), 169.7 ($CHCO_2$tBu), 156.2 ($NHCO_2$tBu), 138.4, 138.0, 128.7, 128.6, 128.5, 128.3, 128.1, 127.9, 127.8 (138.4-127.9, CH_2*Ph*), 99.7 (C1), 82.6 (CCH_3), 80.1 (CCH_3), 78.0 (Thr-Cβ), 77.8 (C3), 75.6 (4-CH_2Ph), 74.5 (C4), 73.7 (6-CH_2Ph), 72.1 (C5), 71.9 (3-CH_2Ph), 69.0 (C6), 68.8 (C2), 59.0 (Thr-Cα), 28.6, 28.2, 28.1, 27.1 (28.6-27.1, $C(CH_3)_3$), 21.2 ($COCH_3$), 18.5 (Thr-CH_3).

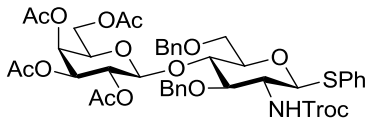


_N_-_tert_-butyloxycarbonyl-_O_-(3,4,6-_O_-benzyl-α-D-mannopyranosyl)-L-threonine-_tert_-butylester (114)[301]

Compound **113** (6.48 g, 8.6 mmol) was dissolved in MeOH (68 ml) followed by addition of NaOMe (294 mg, 5.4 mmol, 0.08 M) in small portions over 2 h to reach pH 9.0-9.5. The reaction mixture was stirred overnight at room

temperature and then neutralized by a small amount of *Dowex* 50WX8 ion-exchanger, filtered and concentrated. The residue was purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 5:1) to give **114**. Yield: 72% (4.42 g, 6.3 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = +55° (c = 0.8, $CHCl_3$). *HR-ESI-MS (pos), m/z*: 708.3736 ($[M+H]^+$, calc. 708.3748), 725.4005 ($[M+NH_4]^+$, calc. 725.4013), 730.3560 ($[M+Na]^+$, calc. 730.3567). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.38 - 7.26 (m, 13H, CH_2*Ph*), 7.19-7.16 (m, 2H, CH_2*Ph*), 5.05 (d, *J*=9.8, 1H, NH), 4.95 (s, 1H, H1), 4.82 (d, *J*=10.7, 1H, CH_2Ph), 4.70 (s, 2H, CH_2Ph), 4.65 (d, *J*=12.2, 1H, CH_2Ph), 4.54 (d, *J*=12.23, 1H, CH_2Ph), 4.49 (d, *J*=10.72, 1H, CH_2Ph), 4.31 (dd, *J*=6.3, 2.1, 1H, Thr-C*H*β), 4.18 (dd, *J*=9.9, 2.1, 1H, Thr-C*H*α), 3.89 (s, 1H, H2), 3.83 (m, 3H, H5, H4, H3), 3.78 - 3.73 (m, 1H, H6a), 3.68 (d, *J*=10.1, 1H, H6b), 1.49-1.47 (s, 18H, $CO_2C(CH_3)_3$), 1.29 (d, *J*=6.2, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 169.9 (CH*C*O_2tBu), 156.1 (NH*C*O_2tBu), 138.3, 137.9, 128.7, 128.5, 128.4, 128.1, 128.0, 127.9, 127.9, 127.7 (128.7-127.7, CH_2*Ph*), 101.3 (C1), 82.3 (CCH_3), 80.0 (CCH_3), 79.8 (C3), 77.6 (Thr-Cβ), 75.4 (CH_2Ph), 74.4 (C4), 73.6 (CH_2Ph), 72.1 (CH_2Ph), 71.6 (C5), 69.0 (C6), 68.5 (C2), 59.0 (Thr-Cα), 28.5, 28.2, 27.0 (28.5-27.0, $C(CH_3)_3$), 18.7 (Thr-CH_3).

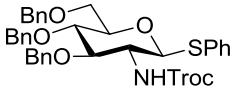
Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-1-thio-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (115)[301]



Compound **43** (14.35 g, 15.74 mmol) was dissolved in MeOH (215 ml) followed by addition of hydrazine hydrate (86.10 mL, 1.76 mmol). The reaction mixture was stirred at 70°C for 2 h, and then the solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (150 ml) and filtered over *Celite*. The filtrate was washed with saturated $NaHCO_3$ (50 ml×3), brine (100 ml×1) and dried over $MgSO_4$. The organic solvent was evaporated to give the deprotected disaccharide in 62% yield (6.00 g, 9.78 mmol). The crude material was taken up in 1,4-dioxane (60 ml) and a solution of $NaHCO_3$ (1.89 g, 22.5 mmol) in H_2O (20 ml) was added. The mixture was stirred at room temperature for 15 min followed by slow addition of TrocCl (1.97 ml, 14.7 mmol). After 2 h TLC (DCM/MeOH 9:1, R_f = 0.5) indicated full conversion to the desired product. The reaction mixture was concentrated and coevaporated with toluene (50 ml×3) and was then dissolved pyridine / acetic anhydride 2:1 (18 mL). After 24 h the solvent was removed and again coevaporated with toluene (50 ml×3). Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) gave **115** in

75% yield (6.88 g, 7.20 mmol). R_f = 0.4 (^{C}Hex/EtOAc 2:1). $[\alpha]_D^{20}$ = -7 (c = 0.7, $CHCl_3$). *HR-ESI-MS (pos), m/z*: 956.1891 ($[M+H]^+$, calc. 956.1888). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.54 - 7.44 (m, 2H, Ph), 7.41 - 7.18 (m, 13H, Ph), 5.32 (d, *J*=8.0, 1H, NH), 5.26 (d, *J*=3.4, 1H, H4'), 5.12 (dd, *J*=10.4, 8.0, 1H, H2'), 4.95 (d, *J*=10.0, 1H, H1), 4.91 - 4.82 (m, 2H, H3', $PhCH_2$), 4.81 - 4.64 (m, 3H, $TrocCH_2$, $PhCH_2$), 4.60 (m, 2H, H1', $PhCH_2$), 4.50 (d, *J*=11.9, 1H, $PhCH_2$), 3.99 (m, 2H, H4, H6a'), 3.88 (m, *J*=11.0, 6.0, 1H, H6b'), 3.83 (m, 1H, H3), 3.78 - 3.70 (m, 2H, H6ab), 3.60 (t, *J*=6.9, 1H, H5'), 3.47 (m, 1H, H5), 3.41 (m, 1H, H2), 2.07 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H) (2.07-1.95 $COCH_3$). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.3, 170.4, 170.4, 170.2, 169.5 (171.3-169.5 *C*OCH_3), 154.0, 138.4, 138.1, 132.8 (138.4 - 132.8 CH_2*Ph*), 100.3 (C1'), 95.7 (Troc-*C*Cl_3), 85.9 (C1), 79.7 (C3), 79.3 (C5), 76.5 (C4), 74.7 (CH_2Ph), 73.8 (CH_2Ph), 71.1 (C3'), 70.8 (C5'), 69.7 (C2'), 68.2 (C6), 67.0 (C4'), 60.9 (C6'), 56.3 (C2), 23.2, 21.2, 20.9, 20.8 (21.2-20.8 $COCH_3$).

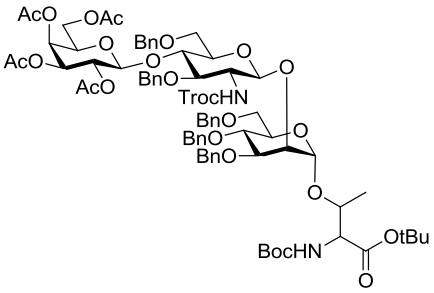
Phenyl 3,4,6-tri-*O*-benzyl-2-deoxy-1-thio-2-N-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (116)[301]



Compound **55** (8.9 g, 13.2 mmol) was dissolved in Tol/EtOH 2:3 (300 mL) followed by addition of hydrazine hydrate (10 mL, 204 mmol). The reaction mixture was refluxed at 105 °C for 72 h, and then the solvent was removed under reduced pressure. The residue was diluted with DCM (150 ml) and washed with saturated $NaHCO_3$ (50 mL×3), brine (100 mL×1) and dried over $MgSO_4$. The organic solvent was evaporated and the crude material was taken up in 1,4-dioxane (90 ml) and a solution of $NaHCO_3$ (2.56 g, 30.5 mmol, 2.3 eq) in H_2O (30 mL) was added. The mixture was stirred at r.t. for 15 min followed by slow addition of TrocCl (2.74 mL, 19.9 mmol, 1.5 eq). After 2 h TLC indicated full conversion to the desired product. The reaction mixture was concentrated and coevaporated with toluene (50 ml×3) and purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) gave **116** in 76% yield (7.2 g, 10.0 mmol). R_f = 0.7 (Tol/EtOAc 4:1). $[\alpha]_D^{20}$ = +11 (c = 1.2, $CHCl_3$). *ESI-MS (pos), m/z*: 732.73 ($[M+NH_4]^+$, calc. 733.17), 1451.07 ($[2M+NH_4]^+$, calc. 1450.30). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ* (ppm): 7.57 - 7.53 (m, 2H), 7.39 - 7.20 (m, 18H), 5.13 (d, *J*=8.1, 1H, N*H*), 4.95 (d, *J*=10.1, 1H, H1), 4.86 - 4.79 (m, 2H, $BnCH_2$), 4.78 - 4.70 (m, 3H, $BnCH_2$), 4.65 - 4.52 (m, 3H, $TrocCH_2$, $BnCH_2$), 3.89 (t, *J*=8.9, 1H, H3), 3.81 (d, *J*=10.8, 1H, H6a), 3.76 (dd, *J*=10.8, 4.4, 1H, H6b), 3.67 (t, *J*=9.0, 1H, H4), 3.59 (d, *J*=7.2, 1H, H5), 3.50 (dd, *J*=18.7, 9.5, 1H, H2). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*:

154.05 (Troc*C*=O), 138.47, 138.17, 138.10, 132.69, 129.16, 128.72, 128.70, 128.60, 128.30, 128.14, 128.12, 128.10, 128.03, 127.91, 127.83 (129.2 - 127.8, aromatic), 95.74 (*C*Cl_3), 86.02 (C1), 82.53 (C3), 79.53 (C5), 78.70 (C4), 75.46 (CH_2Ph), 75.1 (CH_2Ph)1, 74.73 (CH_2Ph), 73.70 (TrocCH_2), 69.22 (C6), 56.90 (C2).

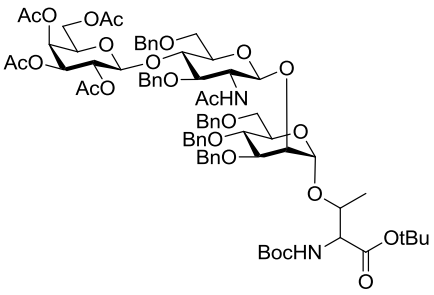
***N*-*tert*-butoxycarbonyl-*O*-{3,4,6-*O*-benzyl-2-*O*-[3,6-*O*-benzyl-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine-*tert*-butylester (117)[301]**



Compounds **114** (2.37 g, 3.35 mmol, 1.0 eq), **115** (3.85 g, 4.02 mmol, 1.2 eq) and 4 Å molecular sieves (3 g) in dry dichloromethane (35 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -18 °C followed by slow addition of *N*-iodosuccinimide (1.51 g, 6.71 mmol, 2.0 eq) and trifluoromethanesulfonic acid (0.059 mL, 0.67 mmol, 0.2 eq). After stirring for 2 h at -18 °C, the reaction was quenched by addition of Et_3N (0.5 mL). The molecular sieves were removed by filtration and the filtrate was concentrated. Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 5:1) gave **117**. Yield: 78% (4.08 g, 2.63 mmol). R_f = 0.4 (^{C}Hex/EtOAc 3:2). $[\alpha]_D^{20}$ = +12° (c = 0.9, $CHCl_3$). *HR-ESI-MS (pos), m/z*: 1553.3979 ($[M+H]^+$, calc. 1553.5368). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.42 - 7.15 (m, 25H, CH_2*Ph*), 5.36 (d, *J*=6.0, 1H, GlcN*H*), 5.24 (d, *J*=3.4, 1H, H4''), 5.12 (dd, *J*=10.3, 8.0, 1H, H2''), 4.99 (d, *J*=8.0, 1H, ThrN*H*), 4.93 - 4.86 (m, 2H, H1', $PhCH_2$×1), 4.86 - 4.77 (m, 4H, H3'', H1, $PhCH_2$×2), 4.71 - 4.56 (m, 6H, TrocCH_2, H1'', $PhCH_2$×3), 4.56 - 4.41 (m, 3H, $PhCH_2$×3), 4.36 (d, *J*=12.0, 1H, $PhCH_2$×1), 4.21 - 4.07 (m, 2H, Thr-C*H*β, Thr-C*H*α), 4.05 - 3.92 (m, 4H, H2, H3', H6a'', H3), 3.92 - 3.80 (m, 3H, H6b'', H4', H4), 3.77 - 3.65 (m, 4H, H5, H6ab, H6a'), 3.62 (d, *J*=8.8, 1H, H6b'), 3.55 (t, *J*=7.0, 1H, H5''), 3.43 - 3.33 (m, 1H, H5'), 3.28 - 3.12 (m, 1H, H2'), 2.09 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.97 (s, 6H, Ac), 1.47 (s, 18H, Boc, *t*Bu), 1.25 (d, *J*=6.9, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.4, 170.4, 170.3, 170.1, (170.4-170.1 Ac), 169.4 (CH*C*O_2tBu), 156.1 (NH*C*O_2tBu), 154.2 (NH*C*OTroc), 138.9, 138.7, 138.5, 138.2, 138.0, 137.6, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6 (138.9-127.6, CH_2*Ph*), 100.4 (C1''), 99.3 (C1), 98.7 (C1'), 95.9 (*C*Cl_3), 82.2 (*C*CH_3), 80.2 (*C*CH_3), 78.2 (C3'), 77.5 (Thr-Cβ), 77.4 (C4), 77.1 (C3), 75.2 (CH_2Ph), 74.9 (C4'), 74.9 (C5'), 74.5 (CH_2Ph), 74.4

(TrocCH$_2$), 73.9 (C2), 73.9 (CH$_2$Ph), 73.5 (CH$_2$Ph), 72.5 (C5), 71.3 (C3''), 71.2 (CH$_2$Ph), 70.8 (C5''), 69.9 (C2''), 69.6 (C6'), 68.5 (C6), 67.1 (C4''), 61.1 (C6''), 58.8 (Thr-Cα), 57.9 (C2'), 29.9, 28.6, 28.3, 27.1 (29.9-27.1, C(*C*H$_3$)$_3$), 20.9, 20.9, 20.8, 20.8 (20.9-20.8 Ac), 18.5 (Thr-CH$_3$).

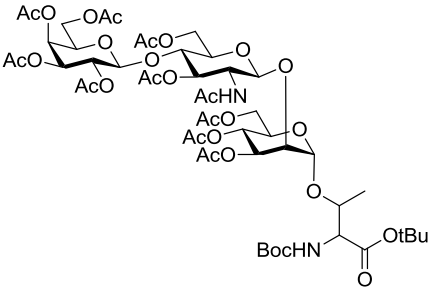
_N_-_tert_-butoxycarbonyl-_O_-{3,4,6-_O_-benzyl-2-_O_-[2-_N_-acetamido-3,6-_O_-benzyl-2-deoxy-4-_O_-(2, 3,4,6-tetra-_O_-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine-_tert_-butylester (118)[301]



Zinc powder was activated by treatment with 1 M HCl aq. for 20 min, washed with H$_2$O, MeOH, Et$_2$O and dried under reduced pressure for 30 min. Compound **117** (4.30 g, 2.77 mmol) was dissolved in glacial acetic acid (60 mL) followed by addition of the activated Zn powder (5.50 g, 84.6 mmol, 30 eq). The reaction mixture was stirred at room temperature for 36 h and was then filtered through a thin layer of *Celite*. The filtrate was concentrated and coevaporated 3 times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (15 mL) and stirred at room temperature overnight. Then the reaction mixture was concentrated and coevaporated three times with toluene. Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) gave **118**. Yield: 69% (2.70 g, 1.91 mmol). R_f = 0.3 (^{C}Hex/EtOAc 3:2). $[\alpha]_D^{20}$ = +8° (c = 0.7, CHCl$_3$). *HR-ESI-MS (pos), m/z*: 1421.6392 ([M+H]$^+$, calc. 1421.6431). ***^{1}H-NMR*** (400 MHz, CDCl$_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.42 - 7.17 (m, 25H, CH$_2$*Ph*), 5.68 (d, *J*=5.4, 1H, GlcN*H*), 5.27 (d, *J*= 3.4, 1H, H4''), 5.12 (dd, *J*=10.4, 8.0, 1H, H2''), 5.03 (d, *J*=7.3, 1H, H1'), 4.99 (d, *J*=9.8, 1H, ThrN*H*), 4.89 - 4.79 (m, 4H, H3'', H1, PhCH$_2$×2), 4.78 - 4.75 (m, 1H, PhCH$_2$), 4.67 - 4.55 (m, 4H, H1'', PhCH$_2$×3), 4.55 - 4.48 (m, 2H, PhCH$_2$×2), 4.47 - 4.39 (m, 2H, PhCH$_2$×2), 4.36 (d, *J*=11.9, H, PhCH$_2$), 4.27 (t, *J*=8.5, 1H, H3'), 4.20 (d, *J*=6.2, 1H, Thr-C*H*β), 4.17 - 4.14 (m, 1H, Thr-C*H*α), 4.04 - 3.96 (m, 2H, H2, H6a''), 3.96 - 3.86 (m, 3H, H6b'', H3, H4'), 3.85 - 3.79 (m, 1H, H4), 3.76 - 3.68 (m, 3H, H6ab, H5), 3.68 - 3.63 (m, 1H, H6a'), 3.61 - 3.54 (m, 2H, H6b', H5''), 3.50 - 3.42 (m, 1H, H5'), 3.25 - 3.16 (m, 1H, H2'), 2.09 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.46 (s, 18H, Boc, *t*Bu), 1.26 (d, *J*=7.1, 3H, Thr-CH$_3$). ***^{13}C-NMR*** (100.6 MHz, CDCl$_3$, gHSQC, gHMBC), *δ (ppm)*: 171.5, 170.4, 170.3, 170.0, (171.5-170.0 Ac), 169.5 (CH*C*O$_2$tBu), 139.3, 138.6, 138.5, 138.2, 128.7, 128.6, 128.4, 128.1, 128.0, 127.9, 127.7, 127.6, 127.6 (139.3-127.6 CH$_2$*Ph*), 100.4 (C1''), 99.4 (C1), 97.8 (C1'), 82.1 (*C*CH$_3$), 80.1 (*C*CH$_3$), 77.9 (Thr-Cβ), 77.4 (C3'), 77.4 (C4), 77.1 (C3), 75.4 (CH$_2$Ph), 74.8 (C5'), 74.6 (C4'), 74.1 (CH$_2$Ph),

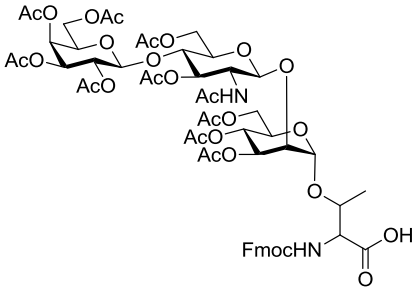
73.8 (C2), 73.8 (CH_2Ph), 73.5 (CH_2Ph), 72.2 (C5), 71.2 (C3''), 71.0 (CH_2Ph), 70.8 (C5''), 69.8 (C2''), 69.5 (C6'), 69.0 (C6), 67.1 (C4''), 61.1 (C6''), 59.0 (Thr-Cα), 57.1 (C2'), 28.6, 28.3, 27.1 (28.6-27.1, C(CH_3)$_3$), 23.5, 21.3, 21.0, 20.9, 20.8, 20.8 (21.3-20.8 Ac), 18.6 (Thr-CH_3).

_N_-_tert_-butoxycarbonyl-_O_-{3,4,6-_O_-acetyl-2-_O_-[2-_N_-acetamido-3,6-_O_-acetyl-2-deoxy-4-_O_-(2, 3,4,6-tetra-_O_-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-thre onine-_tert_-butylester (119)[301]



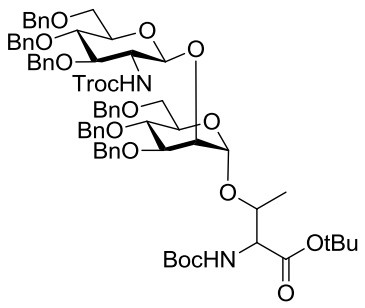
Compound **118** (2.50 g, 1.76 mmol) was dissolved in MeOH / acetic acid 30:2 (85 mL). The solution was added to a suspension of 10% Pd(C) (2.67 g) in MeOH (80 mL), stirred under argon. The reaction mixture was then stirred under hydrogen atmosphere for 48 h. After flushing the mixture with argon, the suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (25 mL) and stirred at room temperature overnight. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 1:1 to 1:5) finally gave **119**. Yield: 53% (1.10 g, 0.93 mmol). R_f = 0.3 (Tol/EtOAc 1:5). $[\alpha]_D^{20}$ = +3° (c = 0.7, $CHCl_3$). *HR-ESI-MS (pos), m/z*: 1181.4589 ($[M+H]^+$, calc. 1811.4612), 1203.4422 ($[M+Na]^+$, calc. 1203.4431). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.83 (d, *J*=9.2, 1H, NH), 5.32 (d, *J*=3.4, 1H, H4''), 5.20-5.05 (m, 3H, H4, H3', H2''), 4.99 (dd, *J*=9.7, 3.4, 1H, H3), 4.94 (dd, *J*=10.5, 3.4, 1H, H3''), 4.84 (s, 1H, H1), 4.46-4.48 (m, 2H, H1', H1''), 4.35 (dd, *J*=11.8, 2.6, 1H, H6a'), 4.26 - 4.17 (m, 2H, Thr-C*H*β, Thr-C*H*α), 4.13 - 4.02 (m, 5H, H6b', H6ab'', H6ab), 3.98 - 3.95 (m, 1H, H2), 3.91 (m, 1H, H2'), 3.87 (m, 1H, H5), 3.85 (m, 1H, H5''), 3.71 (t, *J*=8.6, 1H, H4'), 3.51 (m, 1H, H5'), 2.12 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.01 (m, 6H, Ac), 1.96 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.92 (s, 3H, NHAc), 1.47 (s, 9H, *t*Bu), 1.44 (s, 9H, *t*Bu), 1.27 - 1.24 (m, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.8, 170.5, 170.5, 170.4, 170.4, 170.3, 170.2, 170.1, 169.9, 169.4 (170.8-169.4 Ac), 169.3 (CH*C*O_2tBu), 156.0 (NH*C*O_2tBu), 101.1 (C1''), 99.7 (C1'), 98.8 (C1), 82.5 (*C*CH_3), 80.3 (*C*CH_3), 77.4 (Thr-Cβ), 76.1 (C4'), 74.6 (C2), 72.7 (C5'), 72.1 (C3'), 70.9 (C3''), 70.9 (C5''), 70.1 (C3), 69.2 (C2''), 69.2 (C5), 66.8 (C4''), 66.2 (C4), 62.8 (C6), 62.5 (C6'), 60.9 (C6''), 58.5 (Thr-Cα), 53.6 (C2'), 29.7, 28.8, 28.4, 28.1 (29.7-28.1, C(CH_3)$_3$), 23.2, 21.1, 20.9, 20.8, 20.8, 20.7, 20.7, 20.7, 20.6 (21.1-20.6 Ac), 17.9 (Thr-CH_3).

_N_-9-Fluorenylmethoxycarbonyl-_O_-{3,4,6-_O_-acetyl-2-_O_-[2-_N_-acetamido-3,6-_O_-acetyl-2-deoxy-4-_O_-(2,3,4,6-tetra-_O_-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine (120)[301]



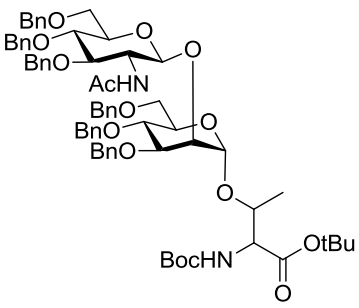
Compound **119** (1.04 g, 0.88 mmol) was dissolved in TFA/DCM/anisole 3:1:0.4 (34 mL) and was stirred at room temperature overnight. The solution was then concentrated and coevaporated three times with toluene. The residue was dissolved in a mixture of Dioxane / H_2O 1:1 (40 mL) followed by addition of Na_2CO_3 (224 mg, 2.67 mmol 3.0 eq). The mixture was cooled to 0°C and Fmoc-OSu (357 mg, 1.06 mmol, 1.2 eq) was slowly added. After stirring the reaction mixture for 4 h at room temperature, dioxane was removed by evaporation followed by addition of 5 M HCl until pH reached 1.5. The residue was diluted with H_2O /DCM (100 mL each) and after extraction, the organic layer was dried, filtered and concentrated. Purification by silica column chromatography (DCM-MeOH 100:1 to 10:1) gave **120**. Yield: 91% (1.00 g, 0.8 mmol). R_f = 0.1 (EtOAc/MeOH/AcOH/H_2O 60:3:3:2). $[\alpha]_D^{20}$ = +11° (c = 0.8, $CHCl_3$). *HR-ESI-MS (pos), m/z*: 1247.4121 ($[M+H]^+$, calc. 1247.4142). ***^{1}H-NMR*** (600 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.87 (d, *J*=7.5, 2H, Fmoc), 7.81 - 7.66 (m, 4H, Fmoc, NH×2), 7.39 (q, *J*=7.2, 2H, Fmoc), 7.36 - 7.27 (m, 2H, Fmoc), 5.21 (d, *J*=3.6, 1H, H4''), 5.16 (dd, *J*=10.1, 3.6, 1H, H3''), 5.02 (m, 2H, H1, H3), 4.94 (m, 1H, H4), 4.92 - 4.79 (m, 2H, H3', H2''), 4.75 (d, *J*=8.0 1H, H1''), 4.39 (d, *J*=8.5, 1H, H1'), 4.33 - 4.26 (m, 2H, FmocC*H*$_2$), 4.24 (m, 2H, FmocC*H*, H6a'), 4.20 (m, 3H, Thr-C*H*β, Thr-C*H*α, H5''), 4.08 - 3.91 (m, 6H, H5, H6b', H6ab, H6ab''), 3.83 (s, 1H, H2), 3.79 - 3.68 (m, 2H, H2', H4'), 3.62 - 3.49 (m, 1H, H5'), 2.08 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.01 - 1.94 (m, 15H, Ac), 1.88 (s, 6H, Ac), 1.73 (s, 3H, Ac), 1.23 (d, *J*=5.8, 3H, Thr-CH_3). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 171.5, 170.3, 170.1, 169.8, 169.5, 169.5, 169.4, 169.3, 169.1 (171.5-169.1 Ac), 156.58 (NH*C*OFmoc), 143.8, 143.7, 140.7, 137.3, 128.9, 128.2, 127.6, 127.0, 125.3, 125.3, 120.1 (128.9-120.1 Fmoc aromatic), 100.1 (C1'), 100.0 (C1''), 98.2 (C1), 76.3 (C4'), 76.2 (Thr-Cβ), 75.0 (C2), 72.7 (C3'), 72.0 (C5'), 70.3 (C3''), 69.6 (C3), 69.6 (C5'), 69.0 (C2''), 67.9 (C5), 67.0 (C4''), 65.9 (Fmoc*C*H_2), 65.7 (C4), 62.3 (C6'), 62.1 (C6), 60.8 (C6''), 58.6 (Thr-Cα), 52.6 (C2'), 46.7 (Fmoc*C*H), 22.5, 21.0, 20.5, 20.5, 20.4, 20.3, 20.3 (22.5-20.3 Ac), 17.5 (Thr-CH_3).

_N_-_tert_-butoxycarbonyl-_O_-{3,4,6-_O_-benzyl-2-_O_-[3,4,6-_O_-benzyl-2-deoxy-2-_N_-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine-_tert_-butylester (121)[301]



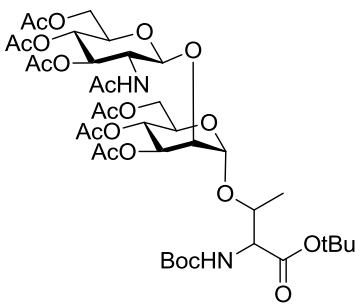
Compounds **114** (3.11 g, 4.40 mmol, 1.0 eq), **116** (5.04 g, 7.03 mmol, 1.6 eq), *N*-iodosuccinimide (2.37 g, 10.5 mmol, 2.4 eq) and 4 Å molecular sieves (5 g) in dry dichloromethane (50 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -40 °C followed by slow addition of trifluoromethanesulfonic acid (0.078 mL, 0.87 mmol, 0.2 eq). After stirring for 30 min at -40 °C, the reaction was quenched by addition of Et_3N (0.5 mL). The molecular sieves were removed by filtration and the filtrate was concentrated. Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 4:1) gave **121**. Yield: 57% (3.28 g, 2.50 mmol). R_f = 0.7 (Tol/EtOAc 4:1). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.29 (m, 30H, CH_2*Ph*), 5.29 (bs, 1H, GlcN*H*), 4.95 (d, *J*=8.7, 1H, ThrN*H*), 4.92 (d, *J*=8.1, 1H, H1'), 4.86 - 4.76 (m, 5H, H1', $PhCH_2$×4), 4.73 - 4.63 (m, 3H, TrocCH_2, $PhCH_2$×2), 4.61 - 4.55 (m, 3H, TrocCH_2, $PhCH_2$×2), 4.55 - 4.42 (m, 4H, $PhCH_2$×4), 4.18 (d, *J*=5.9, 1H, Thr-C*H*β), 4.15 (m, 2H, Thr-C*H*α, H3'), 4.04 (t, *J*=2.4, 1H, H2), 3.89 (d, *J*=7.8, 1H, H4), 3.83 (dd, *J*=8.0, 2.5, 1H, H3), 3.72 (m, 4H, H5, H6a, H6b, H6a'), 3.61 (m, 2H, H6b', H4'), 3.50 (dt, *J*=9.7, 3.3, 1H, H5'), 3.25 (d, *J*=7.6, 1H, H2'), 1.47 (s, 9H, *t*Bu), 1.47 (s, 9H, Boc), 1.25 (d, *J*=6.2, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.2 (CH*C*O_2tBu), 156.1 (CH*C*O_2tBu), 154.3 (NH*C*OTroc), 138.9, 138.5, 138.5, 138.2, 138.2, 128.6, 128.6, 128.5, 128.5, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.5 (138.8 - 127.6, CH_2*Ph*), 99.6 (C1), 98.4 (C1'), 95.9 (*C*Cl_3), 82.2 (*C*CH_3), 80.6 (C3'), 80.1 (*C*CH_3), 78.9 (C4'), 77.4 (Thr-Cβ), 77.0 (C3), 75.2 (C5', CH_2Ph), 75.1 (CH_2Ph), 75.1 (C4, CH_2Ph), 74.4 (TrocCH_2), 73.7 (C2, CH_2Ph), 73.4 (CH_2Ph), 72.4 (C5), 71.2 (CH_2Ph), 69.7 (C6'), 69.4 (C6), 58.8 (Thr-Cα), 58.4 (C2'), 28.5 (C(*C*H_3)$_3$), 28.3 (C(*C*H_3)$_3$), 18.6 (Thr-CH_3). *HR-ESI-MS (pos), m/z*: 1313.4928 ($[M+H]^+$, calc. 1313.4886), 1330.5157 ($[M+NH_4]^+$, calc. 1330.5155).

_N_-_tert_-butoxycarbonyl-_O_-{3,4,6-_O_-benzyl-2-_O_-[2-_N_-acetamido-3,4,6-_O_-benzyl-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine-_tert_-butylester (122)[301]



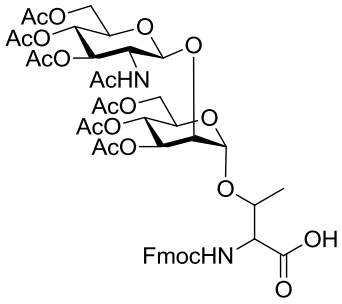
Zinc powder was activated by treatment with 1 M HCl aq. for 20 min, washed with H_2O, MeOH, Et_2O and dried under reduced pressure for 30 min. Compound **121** (2.87 g, 2.18 mmol) was dissolved in glacial acetic acid (50 mL) followed by addition of the activated Zn powder (2.84 g, 43.7 mmol, 20 eq). The reaction mixture was stirred at room temperature for 36 h and was then filtered through a thin layer of *Celite*. The filtrate was concentrated and coevaporated 3 times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (15 mL) and stirred at room temperature overnight. Then the reaction mixture was concentrated and coevaporated three times with toluene. Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) gave **122**. Yield: 67% (1.74 g, 1.47 mmol). R_f = 0.2 (^{C}Hex/EtOAc 3:2). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.44 - 7.16 (m, 30H, CH_2*Ph*), 5.57 (bs, 1H, GlcN*H*), 5.07 (d, *J*=8.3, 1H, H1'), 4.92 (t, *J*=11.1, $PhCH_2$×1), 4.87 - 4.75 (m, 4H, H1, $PhCH_2$×3), 4.67 - 4.46 (m, 7H, $PhCH_2$×7), 4.43 (m, 2H, H 3', $PhCH_2$×1), 4.21 (d, *J*=6.4, 1H, Thr-C*H*β), 4.15 (d, *J*=7.2, 1H, Thr-C*H*α), 4.03 (t, *J*=2.3, 1H, H2), 3.99 (d, *J*=9.1, 1H, H4), 3.83 (dd, *J*=8.9, 2.6, 1H, H3), 3.76 - 3.68 (m, 4H, H5, H6a, H6b, H6a'), 3.60 (m, 1H, H6b'), 3.55 (m, 2H, H4', H5'), 3.10 (dd, *J*=16.6, 8.2, 1H, H2'), 1.72 (s, 3H, NHAc), 1.48 (s, 9H, *t*Bu), 1.47 (s, 9H, Boc), 1.24 (d, *J*=6.2, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.8 (NHAc), 169.9 (CH*C*O_2tBu), 156.1 (NH*C*O_2tBu), 138.9, 138.6, 138.4, 138.3, 138.1, 128.9, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6 (138.9 - 127.6, CH_2*Ph*), 99.5 (C1), 97.4 (C1'), 82.1 (*C*CH_3), 80.3 (C3'), 80.0 (*C*CH_3), 79.2 (C4'), 78.1 (Thr-Cβ), 77.0 (C3), 75.3 (CH_2Ph), 75.2 (C5'), 75.0 (CH_2Ph), 75.0 (CH_2Ph), 74.4 (C4), 73.7 (CH_2Ph), 73.4 (CH_2Ph), 73.1 (C2), 72.1 (C5), 70.7 (CH_2Ph), 69.7 (C6), 69.5 (C6'), 59.0 (Thr-Cα), 58.9 (C2'), 28.5 (C(*C*H_3)$_3$), 28.3 (C(*C*H_3)$_3$), 23.6 (Ac), 18.6 (Thr-CH_3). *HR-ESI-MS (pos), m/z*: 1181.5978 ($[M+H]^+$, calc. 1811.5950), 1198.6211 ($[M+NH_4]^+$, calc. 1198.6215).

***N*-*tert*-butoxycarbonyl-*O*-{3,4,6-*O*-acetyl-2-*O*-[2-*N*-acetamido-3,4,6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine-*tert*-butylester (123)[301]**



Compound **122** (1.70 g, 1.40 mmol) was dissolved in MeOH / acetic acid 30:2 (64 mL). The solution was added to a suspension of 10% Pd(C) (1.88 g) in MeOH (60 mL), stirred under argon. The reaction mixture was then stirred under hydrogen atmosphere for 48 h. After flushing the mixture with argon, the suspension was filtered through *Celite* and the filtrate concentrated and co-concentrated three times with toluene. The residue was dissolved in pyridine / acetic anhydride 2:1 (30 mL) and stirred at room temperature overnight. Evaporation and co-evaporation three times with toluene was followed. Purification by column chromatography (cyclohexane-ethyl acetate 1:1 to 1:5) finally gave **123**. Yield: 90% (1.15 g, 1.29 mmol). R_f = 0.2 (Tol/EtOAc 1:5). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.74 (d, *J*=7.9, 1H, GlcN*H*), 5.37 (t, *J*=10.0, 1H, H3'), 5.16 (t, *J*=9.8, 1H, H4), 5.11 (d, *J*=9.5, 1H, BocN*H*), 4.97 (m, 2H, H3, H4'), 4.81 (s, 1H, H1), 4.78 (d, *J*=8.1, 1H, H1'), 4.22 (m, 3H, H6a', Thr-C*H*β, Thr-C*H*α), 4.14 (d, *J*=12.4, 6.1, 1H, H6a), 4.06 (d, *J*=2.5, 1H, H6b), 4.06 - 3.99 (m, 1H, H2), 3.67 - 3.63 (m,1H, H2), 3.58 - 3.54 (m, 1H, H5'), 2.05 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.98 (s, 6H, Ac×2), 1.92 (s, 3H, NHAc), 1.48 (s, 9H, *t*Bu), 1.45 (s, 9H, Boc), 1.26 (d, *J*=6.2, 3H, Thr-CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.8, 170.8, 170.7, 170.4, 169.9, 169.6, 169.5 (170.8 - 169.5 Ac, CH*C*O_2tBu), 156.1 (NH*C*O_2tBu), 99.2 (C1'), 99.1 (C1), 82.5 (*C*CH_3), 80.3 (*C*CH_3), 77.4 (Thr-Cβ), 74.4 (C2), 72.0 (C5'), 71.9 (C3'), 70.1 (C3), 69.3 (C5), 68.9 (C4'), 66.4 (C4), 63.0 (C6), 62.2 (C6'), 58.6 (Thr-Cα), 55.3 (C2'), 28.5 (C(*C*H_3)$_3$), 28.2 (C(*C*H_3)$_3$), 23.4 (NHAc), 21.2, 20.9, 20.9, 20.8, 20.8, 20.8 (21.2 - 20.8 Ac), 18.0 (Thr-CH_3). *HR-ESI-MS (pos), m/z*: 893.3779 ($[M+H]^+$, calc. 893.3767), 910.4025 ($[M+NH_4]^+$, calc. 910.4032), 915.3567 ($[M+Na]^+$, calc. 915.3586).

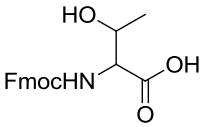
***N*-9-Fluorenylmethoxycarbonyl-*O*-{3,4,6-*O*-acetyl-2-*O*-[2-*N*-acetamido-3,6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranosyl}-L-threonine (124)[301]**



Compound **123** (1.11 g, 1.24 mmol) was dissolved in TFA/DCM 3:1 (40 mL) and was stirred at room temperature for 3 hours. The solution was then concentrated and coevaporated three times with toluene. The residue was dissolved in a mixture of Dioxane / H_2O 1:1 (60 mL) followed by addition of Na_2CO_3 (316 mg, 2.98 mmol, 2.4 eq). The mixture was cooled to 0°C and Fmoc-OSu (503 mg, 1.49 mmol, 1.2 eq) was slowly added. After stirring the reaction mixture for 4 h at room temperature, dioxane was removed by evaporation followed by addition of 5 M HCl until pH reached 1.5. The residue was diluted with H_2O /DCM (100 mL each) and after extraction, the organic layer was dried, filtered and concentrated. Purification by silica column chromatography (DCM-MeOH 100:1 to 10:1) gave **124**. Yield: 99% (1.20 g, 1.24 mmol). R_f = 0.5 (EtOAc/MeOH/AcOH/H_2O 60:3:3:2). ***^{1}H-NMR*** (400 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.88 (d, *J*=7.5, 2H, Fmoc), 7.84 - 7.70 (m, 4H, Fmoc, NH×2), 7.40 (t, *J*=7.4, 2H, Fmoc), 7.32 (dd, *J*=7.4, 6.3, 2H, Fmoc), 5.09 - 4.90 (m, 4H, H4, H3', H1, H3), 4.84 (t, *J*=9.7, 1H, H4'), 4.48 (d, *J*=8.5, 1H, H1'), 4.32 - 4.26 (m, 2H, FmocC*H*$_2$), 4.26 - 4.16 (m, 3H, FmocC*H*, Thr-C*H*β, Thr-C*H*α), 4.13 (dd, *J*=12.3, 4.8, 1H, H6a'), 4.06 - 3.86 (m, 5H, H6a, H2, H6b, H5, H6b'), 3.80 (dd, *J*=19.4, 9.4, 1H, H2'), 3.75 - 3.66 (m, 1H, H5'), 1.99 (s, 3H, Ac), 1.97 (d, *J*=3.9, 6H, Ac×2), 1.96 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.75 (s, 3H, NHAc), 1.24 (d, *J*=5.8, 3H, Thr-CH_3). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 172.2, 170.7, 170.7, 170.4, 170.3, 170.1, 170.0, 169.8 (172.2 - 169.8 Ac), 157.2 (NH*C*OFmoc), 144.4 (Fmoc-quaternary carbon), 141.3 (Fmoc-quaternary carbon), 128.3, 127.7, 126.0, 120.7 (128.3 - 120.7 Fmoc aromatic), 100.9 (C1'), 98.8 (C1), 76.9 (Thr-Cβ), 75.7 (C5), 72.7 (C3'), 71.5 (C5'), 70.2 (C4), 69.1 (C4'), 68.6 (C2), 67.0 (Fmoc*C*H$_2$), 66.6 (C3), 62.8 (C6), 62.5 (C6'), 59.2 (Thr-Cα), 53.2 (C2'), 47.3 (Fmoc*C*H), 23.2 (NHAc), 21.1, 21.1, 21.0 (21.18 - 21.05 Ac), 18.2 (Thr-CH_3). *HR-ESI-MS (pos), m/z*: 959.3311 ($[M+H]^+$, calc. 959.3297), 959.3297 ($[M+NH_4]^+$, calc. 976.3563), 981.3098 ($[M+Na]^+$, calc. 981.3117).

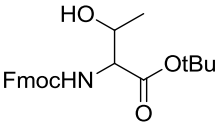
7.21 Synthesis of Fmoc protected threonine *tert*-butylester

_N_-9-Fluorenylmethoxycarbonyl-L-threonine (125)[302]



To a stirring mixture of Thr (17.7 g, 148.5 mmol, 1.0 eq) and $NaHCO_3$ (12.5 g, 148.6 mmol, 1.0 eq) in acetone/H_2O 1:1 (800 mL), Fmoc-OSu (50 g, 148.2 mmol, 1.0 eq) was added in portion. The reaction was stirred at r.t. for 24 h. Then the mixture was acidified by conc. HCl until pH reach 2.0, acetone was removed under reduced pressure and the aqueous layer was then extracted with DCM (200 mL×3). The DCM layer was washed with 1 M HCl (100 mL×2), H_2O (100 mL×2), brine (150 mL×1), dried over $MgSO_4$, and the solvent was removed to give **125**, yield 88% (44.6 g, 131 mmol). ***^{1}H-NMR*** (400 MHz, $CDCl_3$), *δ (ppm)*: 7.62 (d, *J*=7.6, 2H, Fmoc), 7.47 (m, 2H, Fmoc), 7.23 (t, *J*=7.2, 2H, Fmoc), 7.16 (dd, *J*=7.3, 6.2, 2H, Fmoc), 5.99 (d, 1H, *J*=7.2, N*H*), 4.26 - 4.07 (m, 5H, FmocCH_2, FmocC*H*, Thr-C*H*β, Thr-C*H*α), 1.13 (d, *J*=5.6, 3H, Thr-CH_3).

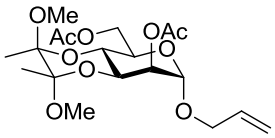
_N_-9-Fluorenylmethoxycarbonyl-L-threonine-*tert*-butylester (126)[303]



A mixture of *tert*-butanol (11.3 g, 14.5 mL, 152 mmol, 2.3 eq) with CuCl (0.6 g, 6 mmol, 0.09 eq) in DCC (55.35 g, 41.8 mL, 268 mmol, 4.0 eq) was stirred in the darkness under Ar for 72 h, the deep green solution was diluted with DCM (20 mL) and a solution of **125** (22.5 g, 66 mmol, 1.0 eq) in DCM (50 mL) was added dropwise over 1 h. The mixture was then stirred at r.t. for 2 h, filtered through a layer of Celite, washed with sat. $NaHCO_3$ (100 mL×2), H_2O (100 mL×2), brine (150 mL×1), dried over $MgSO_4$ and concentrated. The residue was dissolved in EtOAc (200 mL), stored in freezer at -20 °C for 24 h, and filtered through a layer of Celite. The EtOAc solution was concentrated and the residue was purified by column chromatography (cyclohexane-ethyl acetate 4:1 to 3:1) to give **126** yield 83% (21.7 g, 54.6 mmol). R_f = 0.4 (^{C}Hex/EtOAc 3: 1). ***^{1}H-NMR*** (400 MHz, $CDCl_3$), *δ (ppm)*: 7.68 (d, *J*=7.7, 2H, Fmoc), 7.53 (d, *J*=7.5 2H, Fmoc), 7.31 (t, *J*=7.2, 2H, Fmoc), 7.22 (dd, *J*=7.3, 6.2, 2H, Fmoc), 5.53 (d, 1H, *J*=7.6, N*H*), 4.33 (d, 2H, *J*=7.2, FmocCH_2), 4.21 - 4.15 (m, 3H, FmocC*H*, Thr-C*H*β, Thr-C*H*α), 1.41 (s, 9H, C(CH_3)$_3$), 1.16 (d, *J*=5.8, 3H, Thr-CH_3).

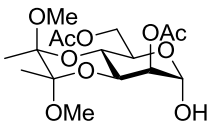
7.22 Synthesis of 2,6-OAc mannosyl donor for further elongation

Allyl 2,6-di-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranoside (127)



Compound **31** (6.93 g, 20.7 mmol) was dissolved in pyridine/acetic anhydride 2:1 (85 ml) and stirred at r.t. for 24 h. The solvent was then removed and the residue was coevaporated three times with toluene. Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 6:1) gave **127** yield 93% (8.10 g, 19.4 mmol). R_f = 0.8 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +137° (c = 1.0, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 436.2179 ($[M+NH_4]^+$, calc. 436.2183), 441.1729 ($[M+Na]^+$, calc. 441.1737). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.87 (dddd, *J*=15.7, 10.4, 6.2, 5.3, 1H, CH_2CH=CH_2), 5.25 (ddd, *J*=17.2, 3.1, 1.5, 1H, CH_2CH=CH_2), 5.18 (ddd, *J*=10.4, 2.7, 1.2, 1H, CH_2CH=CH_2), 5.04 (dd, *J*=3.3, 1.5, 1H, H2), 4.82 (d, *J*=1.4, 1H, H1), 4.32 (dd, *J*=12.0, 1.4, 1H, H6a), 4.27 - 4.20 (m, 1H, H6b), 4.18 - 4.10 (m, 2H, H3, CH_2CH=CH_2), 3.99 (dt, *J*=6.5, 1.3, 1H, CH_2CH=CH_2), 3.98 - 3.93 (m, 2H, H4, H5), 3.24 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 2.12 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.25 (s, 3H, CH_3), 1.25 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.96 (*C*O_2CH_3), 170.68 (*C*O_2CH_3), 133.52 (CH_2*C*H=CH_2), 118.13 (CH_2CH=*C*H_2), 100.44 (*C*$OCH_3(CH_3)$), 100.09 (*C*$OCH_3(CH_3)$), 97.51 (C1), 70.67 (C2), 68.89 (C4), 68.67 (*C*H_2CH=CH_2), 66.16 (C3), 63.81 (C5), 62.90 (C6), 48.27 (OCH_3), 48.02 (OCH_3), 21.34 (CO_2*C*H_3), 20.99 (CO_2*C*H_3), 17.95 (CH_3), 17.83 (CH_3).

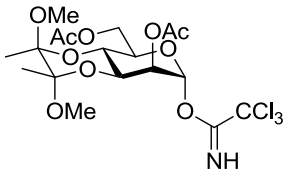
2,6-di-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranoside (128)



Compound **127** (8.02 g, 19.2 mmol, 1.0 eq) was dissolved in Tol/EtOAc/H_2O 20:10:1 (248 mL) and Wilkinson's catalyst (2.66 mg, 2.9 mmol, 0.15 eq) was added. After refluxing for 24 h, the reaction mixture was cooled to r.t., filtered through a layer of Celite and concentrated. The residue was dissolved in THF/H_2O 4:1 (375 mL) followed by addition of I_2 (19.4 g, 76.8 mmol, 4.0 eq). The reaction was kept stirring at r.t. for 2 h and neutralized with 1 M $Na_2S_2O_3$. The suspension was concentrated to remove the THF and then diluted with DCM (100 mL), washed with 1 M $Na_2S_2O_3$ (50 mL×2), H_2O (50 mL×2), brine (50 mL×1) and dried over $MgSO_4$. The solvent was removed and the crude product was purified by column chromatography (cyclohexane-ethyl acetate 6:1 to 1:1) to give **128**, yield 92% (6.67 g, 17.6 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +94° (c = 1.0,

$CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 396.1865 ($[M+NH_4]^+$, calc. 396.1870), 401.1416 ($[M+Na]^+$, calc. 401.1424). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 5.19 (s, 1H, H1), 5.06 - 5.03 (m, 1H, H2), 4.31 (d, *J*=11.5, 1H, H6a), 4.23 - 4.17 (m, 3H, H6b, H3), 4.15 (t, *J*=4.5, 1H, H5), 3.97 (t, *J*=10.0, 1H, H4), 3.24 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 2.12 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.25 (s, 6H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 171.17 (CO_2CH_3), 170.87 (CO_2CH_3), 100.43 ($COCH_3(CH_3)$), 100.09 ($COCH_3(CH_3)$), 93.02 (C1), 71.21 (C2), 68.69 (C3), 65.69 (C5), 63.85 (C4), 63.08 (C6), 48.26 (OCH_3), 48.01 (OCH_3), 21.39 (CO_2CH_3), 21.02 (CO_2CH_3), 17.97 (CH_3), 17.85 (CH_3).

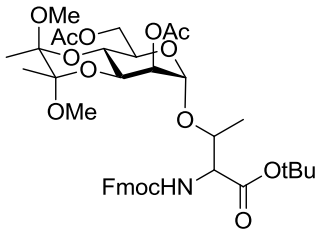
2,6-di-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl trichloroacetimidate (129)



A solution of **128** (6.67 g, 17.6 mmol, 1.0 eq) and trichloroacetonitrile (5.29 mL, 53.0 mmol, 3.0 eq) in anhydrous DCM (80 mL) was stirred under Ar at 0 °C and DBU (0.53 mL, 3.53 mmol, 0.2 eq) was added. After stirring at 0 °C for 3 h, the reaction mixture was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 2:1) to give **129**, yield 78% (7.2 g, 13.8 mmol). R_f = 0.8 (^{C}Hex/EtOAc 1:1). $[\alpha]_D^{20}$ = +98° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 539.1441 ($[M+NH_4]^+$, calc. 539.0966), 544.0520 ($[M+Na]^+$, calc. 544.0520). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 8.72 (s, 1H, N*H*), 6.25 (s, 1H, H1), 5.32 - 5.19 (m, 1H, H2), 4.37 (dd, *J*=12.1, 1.8, 1H, H6a), 4.23 (dd, *J*=12.0, 5.2, 1H, H6b), 4.19 (dd, *J*=9.9, 3.2, 1H, H3), 4.14 (ddd, *J*=10.3, 5.1, 1.4, 1H, H5), 4.13 - 4.02 (m, 1H, H4), 3.24 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 2.16 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.27 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.88 (CO_2CH_3), 170.23 (CO_2CH_3), 160.09 (*C*=NH), 100.44 ($COCH_3(CH_3)$), 100.17 ($COCH_3(CH_3)$), 95.49 (C1), 71.79 (C5), 68.86 (C2), 66.17 (C3), 63.19 (C4), 62.45 (C6), 48.17 (OCH_3), 48.11 (OCH_3), 21.18 (CO_2CH_3), 20.96 (CO_2CH_3), 17.94 (CH_3), 17.79 (CH_3).

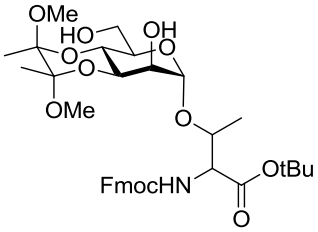
7.23 Synthesis of a 2,6-branched GlcNAc$_2$-Man glycosylated amino acid

***N*-9-Fluorenylmethoxycarbonyl-*O*-[2,6-di-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl]-L-threonine-*tert*-butylester (130)**



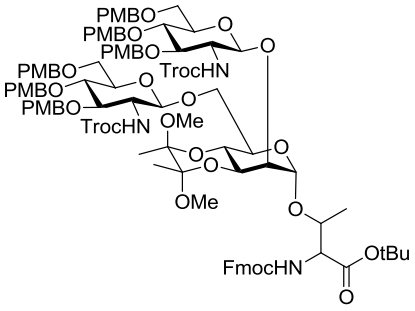
The trichloroacetimidate donor **129** (3.96 g, 7.58 mmol, 1.2 eq) and the protected amino acid **126** (2.51 g, 6.32 mmol, 1.0 eq) was dissolved in dry Et_2O (50 ml) and 4 Å molecular sieves (2.5 g) was added. The suspension was stirred under argon at r.t. for 30 min and trimethylsilyl -trifluoromethanesulfonate (TMSOTf) (0.23 mL, 1.27 mmol, 0.2 eq) was added. The reaction was kept stirring at r.t. for 30 min, then quenched with triethylamine (0.23 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:1) to give **130**. Yield 84% (4.02 g, 5.30 mmol). R_f = 0.8 (DCM/MeOH 95:5). $[\alpha]_D^{20}$ = +91° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 758.3380 ($[M+H]^+$, calc. 758.3388), 775.3658 ($[M+NH_4]^+$, calc. 775.3653), 780.3216 ($[M+Na]^+$, calc. 780.3207), 712.2744 ($[M+K]^+$, calc. 712.2735). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.77 (d, *J*=7.4, 2H, Fmoc), 7.68 - 7.61 (m, 2H, Fmoc), 7.40 (t, *J*=7.2, 2H, Fmoc), 7.33 (dd, *J*=12.9, 7.1, 2H, Fmoc), 5.40 (d, *J*=9.4, 1H, N*H*), 4.96 (s, 1H, H2), 4.88 (s, 1H, H1), 4.45 - 4.37 (m, 2H, FmocCH_2), 4.35 - 4.18 (m, 5H, Thr-C*H*β, H6a, H6b, Thr-C*H*α, FmocC*H*), 4.09 - 4.02 (m, 2H, H3, H5), 4.01 - 3.93 (m, 1H, H4), 3.30 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 2.13 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.49 (s, 9H, *t*Bu), 1.33 (d, *J*=6.3, 3H, Thr-CH_3), 1.30 (s, 3H, CH_3), 1.28 (s, 3H, CH_3).***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.91, 170.30, 169.23, 156.88 (NH*C*OFmoc), 144.12 (Fmoc quaternary), 144.03 (Fmoc quaternary), 141.51 (Fmoc quaternary), 127.93, 127.30, 125.40, 120.19 (127.9-120.2 Fmoc aromatic), 100.50 (O*C*(CH_3)OCH_3), 100.18 (O*C*(CH_3)OCH_3), 99.80 (C1), 82.99 (*C*CH_3), 77.98 (Thr-Cβ), 70.55 (C2), 69.37 (C5), 67.60 (Fmoc*C*H_2), 66.14 (C3), 63.90 (C4), 62.94 (C6), 59.35 (Thr-Cα), 48.45 (OCH_3), 48.11 (OCH_3), 47.40 (Fmoc*C*H), 28.20 (CCH_3), 27.13, 21.25 (Ac), 20.95 (Ac), 18.36 (Thr-CH_3), 17.99 (CH_3), 17.85 (CH_3).

_N_-9-Fluorenylmethoxycarbonyl-_O_-[3,4-_O_-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl]-L-threonine-_tert_-butylester (131)



Compound **130** (10.3 g, 13.6 mmol, 1.0 eq) was dissolved in MeOH (270 ml) followed by dropwise addition of a 1% solution of NaOMe in MeOH until pH 9.0-9.5. The reaction mixture was stirred 24 h at r.t. and then neutralized by a small amount of *Dowex* 50WX8 ion-exchanger, filtered and concentrated. The residue was dissolved in 1,4-dioxane/H_2O 1:1 (100 mL) with $NaHCO_3$ (1.49 g, 17.7 mmol, 1.3 eq), and Fmoc-OSu (4.59 g, 13.6 mmol, 1.0 eq) was added in portions. The reaction was stirred at r.t. for 2 h. Then the dioxane was removed under reduced pressure and the residue was extracted with DCM (100 mL×3). The DCM layer was washed with brine (200 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:3) to give **131**. Yield: 78% (7.2 g, 10.7 mmol). R_f = 0.4 (^{C}Hex/EtOAc 1:2). $[\alpha]_D^{20}$ = +114° (c = 1.1, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 674.3180 ($[M+H]^+$, calc. 674.3177), 691.3443 ($[M+NH_4]^+$, calc. 691.3442), 696.3003 ($[M+Na]^+$, calc. 696.2996), 1347.6301 ($[2M+H]^+$, calc. 1347.6275), 1364.6555 ($[2M+NH_4]^+$, calc. 1364.6540), 1369.6130 ($[2M+Na]^+$, calc. 1369.6094). ***^{1}H-NMR*** (400 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.87 (d, *J*=7.5, 2H, Fmoc), 7.74 (d, *J*=7.5, 2H, Fmoc), 7.64 (d, *J*=8.4, 1H, N*H*), 7.40 (t, *J*=7.4, 2H, Fmoc), 7.31 (td, *J*=7.5, 1.0, 2H, Fmoc), 5.05 (d, *J*=5.2, 1H, O*H*), 4.73 (s, 1H, H1), 4.54 (dt, *J*=11.7, 6.1, 1H, O*H*), 4.32 - 4.25 (m, 2H, FmocCH_2), 4.25 - 4.20 (m, 1H, FmocC*H*), 4.09 (d, *J*=6.8, 2H, Thr-C*H*β, Thr-C*H*α), 3.80 - 3.72 (m, 2H, H4, H3), 3.67 - 3.57 (m, 3H, H6a, H5, H2), 3.49 - 3.42 (m, 1H, H6b), 3.14 (s, 3H, OCH_3), 3.11 (s, 3H, OCH_3), 1.41 (d, *J*=10.1, 9H, *t*Bu), 1.19 (s, 3H, Thr-CH_3), 1.15 (m, 6H, CH_3×2). ***^{13}C-NMR*** (100.6 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 170.98, 169.89, 157.18, 144.45 (Fmoc quaternary), 144.43(Fmoc quaternary), 141.38 (Fmoc quaternary), 128.34, 127.73, 126.04, 120.77 (128.3-120.8 Fmoc aromatic), 102.59 (C1), 100.03 (O*C*(CH_3)OCH_3), 99.57 (O*C*(CH_3)OCH_3), 81.61 (*C*CH_3), 75.43 (Thr-Cβ), 72.64 (C2), 68.85 (C5), 68.38 (C3), 66.65 (Fmoc*C*H_2), 63.66 (C4), 60.92 (C6), 59.73 (Thr-Cα), 47.97 (OCH_3), 47.87, (OCH_3) 47.34 (Fmoc*C*H), 31.99, 28.32 (CCH_3), 21.42, 19.51, 18.61 (Thr-CH_3), 18.48 (CH_3), 18.35, (CH_3) 14.76, 14.44.

_N_-9-Fluorenylmethoxycarbonyl-_O_-{2-deoxy-3,4,6-tri-_O_-_p_-methoxybenzyl-2-_N_-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-[2-deoxy-3,4,6-tri-_O_-_p_-methoxybenzyl-2-_N_-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1→6)-3,4-_O_-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl}-L-threonine-_tert_-butylester (132)

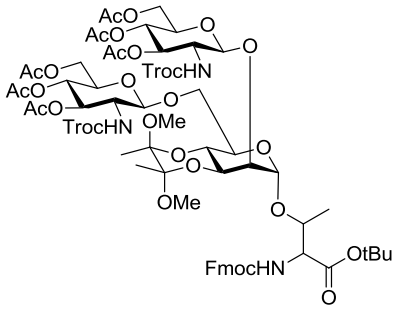


Compounds **54** (3.60 g, 4.46 mmol, 3.0 eq), **131** (1.00 g, 1.48 mmol, 1.0 eq), *N*-iodosuccinimide (1.00 g, 4.46 mmol, 3.0 eq) and 4 Å molecular sieves (1.5 g) in dry dichloromethane (15 mL) were stirred at room temperature for 30 min under argon atmosphere. Then the suspension was cooled to -50 °C followed by slow addition of trifluoromethanesulfonic acid (6.6 μL, 0.074 mmol, 0.05 eq). After stirring for 4 h at -40 to -50 °C, the reaction was quenched by diluting with more DCM (60 mL). The molecular sieves were removed by filtration and the filtrate was washed with 1 M $Na_2S_2O_3$ (50 mL×3), brine (100 mL×1), dried over $MgSO_4$, concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:3) to give **132**. Yield: 90% (2.78 g, 1.34 mmol). R_f = 0.6 (^{C}Hex/EtOAc 2:1).

The trichloroacetimidate donor **62** (731 mg, 0.40 mmol, 1.0 eq) and the protected amino acid **126** (317 mg, 0.80 mmol, 2.0 eq) was dissolved in dry DCM (10 ml) and 4 Å molecular sieves (1 g) was added. The suspension was stirred under argon at r.t. for 30 min and trimethylsilyl -trifluoromethanesulfonate (TMSOTf) (14.5 μL, 0.08 mmol, 0.2 eq) was added under cooling with an ice bath. The reaction was kept stirring at 0 °C for 2 h, then neutralized with triethylamine (0.14 mL). The molecular sieves were removed by filtering over a layer of *Celite*. The filtrate was concentrated and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:2) to give **132**. Yield 82% (672 mg, 0.33 mmol). R_f = 0.3 (DCM/MeOH 95:5). $[\alpha]_D^{20}$ = +32° (c = 1.3, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1044.7902 ($[M+H+Na]^{2+}$, calc. 1044.7978), 1050.8342 ($[M+2NH_4]^{2+}$, calc. 1050.8334), 1053.2823 ($[M+Na+NH_4]^{2+}$, calc. 1053.3111). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.76 (d, *J*=7.5, 2H, Fmoc), 7.63 - 7.56 (m, 2H, Fmoc), 7.39 (t, *J*=7.4, 2H, Fmoc), 7.34 - 7.15 (m, 10H, Fmoc, Ph), 7.08 (d, *J*=8.2, 4H, Ph), 6.84 (m, 12H, Ph), 6.39 (s, 1H, N*H*'), 5.44 (s, 1H, N*H*), 5.31 (s, 1H, N*H*''), 5.01 (d, *J*=8.0, 1H, H1'), 4.97 (d, *J*=12.7, 1H, CH_2Ph), 4.91 (s, 1H, H1), 4.81 - 4.63 (m, 8H, TrocCH_2×2, CH_2Ph×6), 4.61 - 4.52 (m, 3H,

CH_2Ph×3), 4.51 - 4.42 (m, 4H, TrocCH_2×2, CH_2Ph×2), 4.39 (d, *J*=7.2, 3H, FmocC*H*$_2$, H3'), 4.33 - 4.21 (m, 3H, Thr-C*H*β, Thr-C*H*α, FmocC*H*), 4.17 - 4.09 (m, 1H, H6a), 4.04 - 3.96 (m, 2H, H2, H3), 3.93 (d, *J*=4.4, 2H, H4, H5), 3.81 - 3.74 (m, 18H, PMBCH_3×6), 3.72 - 3.66 (m, 2H, H6a', H6a''), 6.66 - 3.54 (m, 5H, H6b', H6b'', H6b, H2'', H4''), 3.54 - 3.44 (m, H4', H5', H5''), 3.28 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 1.46 (s, 9H, *t*Bu), 1.33 (s, 3H, CH_3), 1.30 (d, *J*=6.1, 3H, Thr-CH_3), 1.28 (s, 3H, CH_3). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 159.44 (Ph*C*OCH_3), 159.40 (Ph*C*OCH_3), 144.08 (Fmoc quaternary), 141.50 (Fmoc quaternary), 130.73, 130.54, 130.48, 130.41, 129.80, 129.72, 129.66, 129.56, 127.92 (130.7-127.9 aromatic), 127.33, 125.44, 125.39, 120.18 (127.3-120.2 Fmoc aromatic), 114.03, 114.01, 113.98, 113.96 (114.0-113.9 aromatic), 100.78 (C1''), 100.37 (O*C*(CH_3)OCH_3), 99.84 (O*C*(CH_3)OCH_3), 99.56 (C1), 97.30 (C1'), 82.57 (*C*CH_3), 79.53 (C3'), 78.52 (C4'), 78.37 (C4''), 75.95 (C3'), 75.69 (C5'), 75.25 (C5''), 74.77 ($PMBCH_2$), 74.69 ($PMBCH_2$), 74.62 ($PMBCH_2$), 74.54 (TrocCH_2), 74.48 (TrocCH_2), 74.26 ($PMBCH_2$), 73.34 ($PMBCH_2$), 73.27 ($PMBCH_2$), 71.87 (C2), 71.25 (C5), 68.76 (C6'), 68.56 (C6''), 67.55 (Fmoc*C*H_2), 67.08 (C6, C3), 63.71 (C4), 58.82 (Thr-Cα), 58.00 (C2'), 57.78 (C2''), 55.49, 55.47, 55.42 (55.5-55.4 PMBCH_3), 48.44 (OCH_3), 48.20 (OCH_3), 47.38 (Fmoc*C*H), 28.35 (CCH_3), 18.36 (Thr-CH_3), 18.05 (CH_3), 17.97 (CH_3).

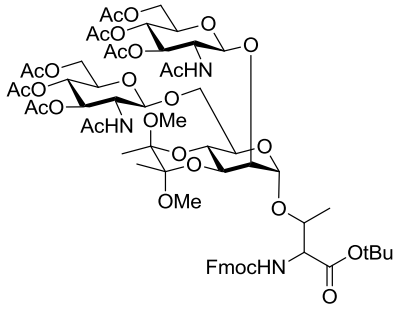
_N_-9-Fluorenylmethoxycarbonyl-_O_-{3,4,6-tri-_O_-acetyl-2-deoxy-2-_N_-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-_O_-acetyl-2-deoxy-2-_N_-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1→6)-3,4-_O_-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl}-L-threonine-_tert_-butylester (133)



To a stirring solution of **132** (1.59 g, 0.77 mmol, 1.0 eq) in MeCN/H_2O 9:1 (30 mL), cerium ammonium nitrate (5.04 g, 9.20 mmol, 12.0 eq) was added. The reaction was stirred at r.t. for 30 min and quenched with Sat. $NaHCO_3$ until the orange color was removed. The mixture was filtered through a layer of Celite, washed with EtOAc (20 mL ×2), H_2O (20 mL ×2), MeOH (20 mL ×2) and DCM (20 mL ×2). The filtrate was combined and concentrated to remove the organic solvent, and the residue was extracted with DCM (20 mL ×3). The organic layer was then washed with brine (30 mL) and concentrated to dryness. The crude product was acetylated with pyridine/acetic anhydride 2:1 (15 mL) for 24 h, concentrated and coevaporated with toluene (15 mL ×3), and purified by column chromatography (cyclohexane-ethyl acetate 10:1 to 3:2)

to give **133**. Yield 91% (1.12 g, 0.70 mmol). R_f = 0.5 (Tol/EtOAc 1:1). $[\alpha]_D^{20}$ = +19° (c = 1.2, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1600.3180 ($[M+H]^+$, calc. 1600.3212), 1634.3535 ($[M+NH_3+NH_4]^+$, calc. 1634.37743). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.75 (d, *J*=7.5, 2H, Fmoc), 7.61 (d, *J*=7.3, 2H, Fmoc), 7.38 (t, *J*=7.4, 2H, Fmoc), 7.29 (dd, *J*=13.2, 5.9, 2H, Fmoc), 6.30 (d, *J*=3.3, 1H, N*H*'), 5.83 (t, *J*=9.3, 1H, H3'), 5.67 (d, *J*=8.6, 1H, N*H*''), 5.61 (d, *J*=7.3, 1H, N*H*), 5.32 - 5.22 (m, 1H, H3''), 5.18 (d, *J*=8.0, 1H, H1'), 5.03 (dd, *J*=19.8, 10.0, 2H, H4'', H4'), 4.84 (d, *J*=10.9, 3H, H1, $TrocCH_2$), 4.70 (d, *J*=7.8, 1H, H1''), 4.68 - 4.56 (m, 2H, $TrocCH_2$), 4.45 (d, *J*=7.2, 1H, FmocCH_2), 4.36 (d, *J*=7.2, 1H, FmocCH_2), 4.34 - 4.27 (m, 3H, Thr-C*H*β, H6a', Thr-C*H*α), 4.26 - 4.20 (m, 2H, H6a'', FmocC*H*), 4.14 - 4.08 (m, 2H, H6b', H6b''), 4.06 (d, *J*=12.1, 1H, H6a), 3.98 (d, *J*=8.2, 2H, H2, H3), 3.88 (d, *J*=7.0, 2H, H4, H5), 3.78 - 3.71 (m, 2H, H5' H2''), 3.71 - 3.63 (m, 2H, H5'', H6b), 3.45 (dd, *J*=7.9, 5.6, 1H, H2'), 3.30 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.99 (s, 6H, Ac×2), 1.94 (s, 3H, Ac), 1.48 (s, 9H, *t*Bu), 1.31 (d, *J*=5.6, 3H, Thr-CH_3), 1.27 (s, 6H, CH_3×2). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 170.88, 169.99, 169.63 (170.9-169.6 Ac), 156.72 (NH*C*OFmoc), 154.29, 144.10, 144.00, 141.51, 141.49 (144.1 - 141.5 Fmoc quaternary), 127.92, 127.28, 125.34, 120.19 (127.9-120.2 Fmoc aromatic), 100.95 (C1''), 100.29 (O*C*(CH_3)OCH_3), 100.00 (O*C*(CH_3)OCH_3), 99.42 (C1), 97.02 (C1'), 82.69 (*C*CH_3), 75.48 (Thr-Cβ), 74.63 ($TrocCH_2$), 74.40 ($TrocCH_2$), 72.46 (C5'), 72.33 (C3''), 71.94 (C2, C5''), 71.29 (C5), 70.85 (C3'), 69.34 (C4'), 68.95 (C4''), 67.41 (C6, Fmoc*C*H_2), 66.99 (C3), 63.56 (C4), 62.29 (C6'), 62.18 (C6''), 58.63 (Thr-Cα), 56.48 (C2'', C2'), 48.52 (OCH_3), 48.26 (OCH_3), 47.39 (Fmoc*C*H), 28.27 (CCH_3), 20.95, 20.92, 20.87, 20.84, 20.82, 20.77 (21.0 - 20.8 Ac), 18.44 (Thr-CH_3), 17.96 (CH_3), 17.82 (CH_3), 14.40, 14.11.

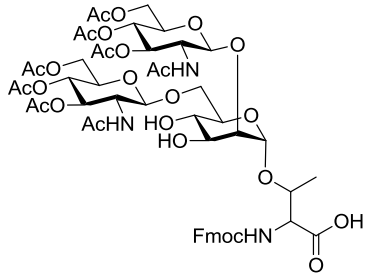
_N_-9-Fluorenylmethoxycarbonyl-_O_-{2-_N_-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-_N_-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-β-D-glucopyranosyl]-(1→6)-3,4-_O_-(2',3'-dimethoxybutane-2',3'-diyl)-α-D-mannopyranosyl}-L-threonine-_tert_-butylester (134)



Zinc powder was activated by treatment with 1 M HCl aq. for 20 min, washed with H_2O, MeOH, Et_2O and dried under reduced pressure for 30 min. Compound **133** (1.22 g, 0.76 mmol, 1.0 eq) was dissolved in glacial acetic acid (12 mL) followed by addition of the activated Zn powder (1 g, 15.4

mmol, 20 eq). The reaction mixture was stirred at room temperature for 96 h and was then filtered through a thin layer of *Celite*. The filtrate was concentrated and coevaporated with toluene (15 mL×3). The residue was dissolved in pyridine / acetic anhydride 2:1 (15 mL) and stirred at r.t. overnight. Then the reaction mixture was concentrated and coevaporated three times with toluene. Purification by column chromatography (cyclohexane-ethyl acetate 10:1 to 1:1) gave **134**. Yield: 85% (0.87 g, 0.65 mmol). R_f = 0.2 (EtOAc). $[\alpha]_D^{20}$ = +22° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1332.5401 ($[M+H]^+$, calc. 1332.5398), 1349.5706 ($[M+NH_4]^+$, calc. 1349.5663), 1354.5251 ($[M+Na]^+$, calc. 1354.5217). ***^{1}H-NMR*** (400 MHz, $CDCl_3$, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.69 (d, *J*=7.5, 2H, Fmoc), 7.59 - 7.50 (m, 2H, Fmoc), 7.32 (t, *J*=7.4, 2H, Fmoc), 7.28 - 7.19 (m, 2H, Fmoc), 6.91 (d, *J*=7.0, 1H, N*H*'), 6.11 (d, *J*=9.1, 1H, N*H*''), 5.77 (t, *J*=9.8, 1H, H3'), 5.50 (d, *J*=9.2, 1H, N*H*), 5.23 - 5.14 (m, 2H, H3'', H1'), 5.01 - 4.91 (m, 2H, H4'', H4'), 4.85 (s, 1H, H1), 4.54 (d, *J*=8.2, 1H, H1''), 4.33 (m, 2H, FmocCH_2), 4.26 - 4.10 (m, 5H, Thr-C*H*β, H6a', H6a'', Thr-C*H*α, FmocC*H*), 4.04 (d, *J*=12.1, 2H, H6b', H6b''), 3.99 - 3.82 (m, 4H, H6a, H3, H4, H2''), 3.81 - 3.67 (m, 2H, H2, H5), 3.65 - 3.57 (m, 2H, H5', H5''), 3.57 - 3.50 (m, 1H, H6b), 3.21 (m, 4H, H2', OCH_3), 3.14 (s, 3H, OCH_3), 1.98 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.93 (s, 6H, Ac×2), 1.91 (s, 3H, NHAc), 1.90 (s, 3H, NHAc), 1.43 (s, 9H, *t*Bu), 1.25 (d, *J*=6.3, 3H, Thr-CH_3), 1.23 - 1.16 (m, 6H, CH_3×2). ***^{13}C-NMR*** (100.6 MHz, $CDCl_3$, gHSQC, gHMBC), *δ (ppm)*: 172.11, 171.13, 170.89, 170.83, 170.77, 170.30, 169.95, 169.57 (172.1-169.6 Ac), 156.72 (NH*C*OFmoc), 144.09 (Fmoc quaternary), 143.96 (Fmoc quaternary), 141.48 (Fmoc quaternary), 127.93, 127.30, 125.34, 120.20 (127.9-120.2 Fmoc aromatic), 101.22 (C1''), 100.40 (O*C*(CH_3)OCH_3), 99.94 (O*C*(CH_3)OCH_3), 99.44 (C1), 97.42 (C1'), 82.57 (*C*CH_3), 76.58 (Thr-Cβ), 74.04 (C2), 72.56 (C3''), 72.17 (C5'), 71.93 (C5''), 71.26 (C3'), 70.56 (C5), 69.49 (C4'), 68.91 (C4''), 67.45 (Fmoc*C*H_2), 67.02 (C3), 66.60 (C6), 62.90 (C4), 62.42 (C6''), 62.28 (C6'), 59.07 (Thr-Cα), 56.28 (C2'), 54.70 (C2''), 48.36 (OCH_3), 48.28 (OCH_3), 47.38 (Fmoc*C*H), 28.29 (CCH_3), 23.69 (NHAc), 21.06, 20.98, 20.93, 20.89, 20.85, 20.81 (21.1 - 20.8 Ac), 18.45 (Thr-CH_3), 18.15 (CH_3), 18.01 (CH_3).

_N_-9-Fluorenylmethoxycarbonyl-_O_-{2-_N_-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-_N_-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-β-D-glucopyranosyl]-(1→6)-α-D-mannopyranosyl}-L-threonine (135)



Compound **134** (766 mg, 0.57 mmol) was dissolved in TFA/DCM 3:1 (10 mL) and was stirred at r.t. for 24 h. The solution was then concentrated and coevaporated with toluene (10 mL×3), purification by silica column chromatography (DCM-MeOH 100:1 to 10:1) gave **135**. Yield: 62% (415 g, 0.33 mmol). R_f = 0.2 (EtOAc/MeOH/AcOH/H_2O 60:3:3:2). $[\alpha]_D^{20}$ = +79° (c = 0.9, $CHCl_3$). *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1162.4094 ($[M+H]^+$, calc. 1162.4091), 1179.4368 ($[M+NH_4]^+$, calc. 1179.4357), 1184.3928 ($[M+Na]^+$, calc. 1184.3910). ***^{1}H-NMR*** (600 MHz, DMSO-d_6, gCOSY, gHSQC, gHMBC), *δ (ppm)*: 7.88 (dd, *J*=20.1, 8.3, 2H, Fmoc), 7.77 (dd, *J*=6.7, 4.4, 2H, Fmoc), 7.43 (t, *J*=7.4, 2H, Fmoc), 7.35 (t, *J*=7.2, 2H, Fmoc), 5.19 (t, *J*=9.9, 1H, H3'), 5.12 (t, *J*=9.9, 1H, H3''), 4.85 (m, 4H, NH, H1, H4'', H4'), 4.67 (d, *J*=8.2, 1H, H1'), 4.62 (d, *J*=8.3, 1H, H1''), 4.34 - 4.22 (m, 4H, NH, FmocC*H*$_2$, FmocC*H*), 4.20 (m, 2H, 6a', 6a''), 4.14 (m, 2H, Thr-C*H*β, Thr-C*H*α), 4.00 (t, *J*=9.8, 2H, 6a, 6b''), 3.96 (d, *J*=10.7, 1H, 6b'), 3.85 - 3.80 (m, 1H, H5''), 3.80 - 3.76 (m, 1H, H5'), 3.76 - 3.56 (m, 5H, H2, H2'', H2', H3, H4), 3.41 (m, 1H, 6b), 3.19 (dd, *J*=14.8, 9.4, 1H, H5), 2.02 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.97 (s, 6H, Ac), 1.95 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.81 (s, 3H, NHAc), 1.78 (d, *J*=11.3, 3H, NHAc), 1.19 (d, *J*=5.8, 3H, Thr-CH_3). ***^{13}C-NMR*** (150.9 MHz, DMSO-d_6, gHSQC, gHMBC), *δ (ppm)*: 172.38, 170.57, 170.54, 170.37, 170.15, 170.12, 169.77, 169.74 (172.4-169.7 Ac), 157.03 (NH*C*OFmoc), 144.27 (Fmoc quaternary), 141.16 (Fmoc quaternary), 129.36, 128.67, 128.15, 127.58, 125.90, 125.84, 121.84, 120.57, 120.49 (129.4-120.6 Fmoc aromatic), 101.45 (C1''), 99.96 (C1'), 99.00 (C1), 78.49 (C2), 76.04 (Thr-Cβ), 73.06 (C4), 72.96 (C3''), 72.63(C3'), 71.39 (C5'), 71.06 (C5''), 70.57 (C6), 69.64 (C3), 69.13 (C4',C4''), 67.58 (C5), 66.46 (Fmoc*C*H_2), 62.27 (C6', C6''), 59.38 (Thr-Cα), 53.89 (C2''), 53.78 (C2'), 47.13 (Fmoc*C*H), 46.13, 23.41, 23.18, 20.97, 20.94, 20.87, 20.82 (23.41-20.82 Ac), 18.62 (Thr-CH_3).

7.24 Synthesis of *N*-GlcNAc peptides

The *N*-glycopeptides **136** - **163** were synthesized by stepwise solid-phase peptide synthesis using the Fmoc strategy and starting with preloaded PHB Fmoc-Arg (Pbf) resin or tentagel PHB Fmoc-Lys (Boc) resin (12.5 µmol scale). The glycopeptide synthesis performed using 1.5 eq of the protected *N*-glycosylated building block **6,** which was pre-activated manually using 1.5 eq HATU/HOAt and 3.0 eq of DIPEA (8 h coupling time). The standard Fmoc amino acids were coupled automatically on a Multisyntech peptide synthesizer using 8 eq of the amino acid, 8 eq of the HBTU/HOBt reagents and 16 eq of DIPEA (40 min). Fmoc deprotection was done according to standard conditions, 20% piperidine in DMF. For peptides **150** - **163**, assembly of the peptide backbone was followed by coupling of a triethyleneglycol amino acid spacer to the *N*-terminus and then Fmoc deprotection. The obtained peptides were then released from the resin, and all acid sensitive side-chain protecting groups were simultaneously removed using TFA/TIPS/H_2O 15:0.9:0.9 for 3 h, followed by solvent concentration, lyophilization and purification using a C-18 cartridge (1 g of C-18 material, Waters). For saccharide deprotection, the *O*-acetyl groups were removed by transesterification in methanol using catalytic amounts of NaOMe at pH 9-9.5 for 24 h (the deprotection was followed by analytical HPLC) to yield glycopeptides **136** - **163** which were purified by preparative HPLC.

Sequence: NH_2-IN*TTADEKDPTNPFR, *=GlcNAc **(136)**

Yield: 73% (17.5 mg, 9.11 µmol). Analytical HPLC Rt= 23.01 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 12.92 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(75:25), 35 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 961.4615 ($[M+2H]^{2+}$, calc. 961.4611), 972.4557 ($[M+Na+H]^{2+}$, calc. 972.4521), 641.3091 ($[M+3H]^{3+}$, calc. 641.3100), 648.9727 ($[M+Na+2H]^{3+}$, calc. 648.9718), 654.2944 ($[M+Na+NH_4+H]^{3+}$, calc. 654.3128).

Sequence: NH_2-AN*HSGAVVLLKR, *=GlcNAc **(137)**

Yield: 83% (15.2 mg, 10.36 µmol). Analytical HPLC Rt= 22.25 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 12.58 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(75:25), 35 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1467.8326 ($[M+H]^+$, calc. 1467.8283), 734.4180 ($[M+2H]^{2+}$, calc. 734.4181), 745.4097 ($[M+Na+H]^{2+}$, calc. 745.4091), 489.9470 ($[M+3H]^{3+}$, calc. 489.9480)，502.9314 ($[M+Na+NH_4+H]^{3+}$, calc. 502.9508), 367.7123 ($[M+4H]^{4+}$, calc. 367.7129).

Sequence: NH_2-VVN*STTGPGEHLR, *=GlcNAc **(138)**

Yield: 82% (16.0 mg, 10.19 µmol). Analytical HPLC Rt= 17.34 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 10.23 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 785.3972 ($[M+2H]^{2+}$, calc. 785.3975), 796.3912 ($[M+Na+H]^{2+}$, calc. 796.3885), 523.9336 ($[M+3H]^{3+}$, calc. 523.9343), 536.9182 ($[M+Na+NH_4+H]^{3+}$, calc. 536.9371).

Sequence: NH_2-WVSN*KTEGR, *=GlcNAc **(139)**

Yield: 88% (14.0 mg, 10.94 µmol). Analytical HPLC Rt= 14.65 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 9.27 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 640.3170 ($[M+2H]^{2+}$, calc. 640.3180), 651.3103 ($[M+Na+H]^{2+}$, calc. 651.3090), 427.2140 ($[M+3H]^{3+}$, calc. 427.2146), 440.1982 ($[M+Na+NH_4+H]^{3+}$, calc. 440.2175).

Sequence: NH_2-LRPDDSKN*FSIQVR, *=GlcNAc **(140)**

Yield: 86% (20.1 mg, 10.70 µmol). Analytical HPLC Rt= 24.09 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 13.22 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(75:25), 35 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 939.4904 ($[M+2H]^{2+}$, calc. 939.4899), 950.4831 ($[M+Na+H]^{2+}$, calc. 950.4809), 626.6616 ($[M+3H]^{3+}$, calc. 626.6625), 639.6468 ($[M+Na+NH_4+H]^{3+}$, calc. 639.6654), 470.2486 ($[M+4H]^{4+}$, calc. 470.2489).

Sequence: NH_2-GEYFVN*VTTR, *=GlcNAc **(141)**

Yield: 7.5% (1.3 mg, 0.94 µmol). Analytical HPLC Rt= 24.82 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.09 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1388.6814 ($[M+H]^{+}$, calc. 1388.6697), 694.8384 ($[M+2H]^{2+}$, calc. 694.8388), 705.8313 ($[M+Na+H]^{2+}$, calc. 705.8298), 476.5459 ($[M+Na+NH_4+H]^{3+}$, calc. 476.5646).

Sequence: NH_2-AYSWN*ISR, *=GlcNAc **(142)**

Yield: 64% (9.6 mg, 8.00 µmol). Analytical HPLC Rt= 25.14 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.19 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 600.2878 ($[M+2H]^{2+}$, calc. 600.2887), 611.2804 ($[M+Na+H]^{2+}$, calc. 611.2797), 413.5121 ($[M+Na+NH_4+H]^{3+}$, calc. 413.5313).

Sequence: NH_2-N*LTALPPDLPK, *=GlcNAc **(143)**

Yield: 62% (10.7 mg, 7.74 µmol). Analytical HPLC Rt= 27.37 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 15.08 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad:

MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1381.7710 ($[M+H]^+$, calc. 1381.7578), 691.3824 ($[M+2H]^{2+}$, calc. 691.3828), 702.3757 ($[M+Na+H]^{2+}$, calc. 702.3738), 474.2422 ($[M+Na+NH_4+H]^{3+}$, calc. 474.2607).

Sequence: NH_2-LQN*LTLPTNASIK, *=GlcNAc **(144)**

Yield: 57% (11.5 mg, 7.12 µmol). Analytical HPLC Rt= 26.71 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.51 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 808.4493 ($[M+2H]^{2+}$, calc. 808.4492), 819.4442 ($[M+Na+H]^{2+}$, calc. 819.4402), 539.3034 ($[M+3H]^{3+}$, calc. 539.3021), 552.2873 ($[M+Na+NH_4+H]^{3+}$, calc. 552.3049).

Sequence: NH_2-SN*YSSPSNISK, *=GlcNAc **(145)**

Yield: 51% (8.8 mg, 6.35 µmol). Analytical HPLC Rt= 15.58 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 9.38 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1386.6535 ($[M+H]^+$, calc. 1386.6388), 693.8231 ($[M+2H]^{2+}$, calc. 693.8233), 704.8163 ($[M+Na+H]^{2+}$, calc. 704.8143), 475.8693 ($[M+Na+NH_4+H]^{3+}$, calc. 475.8877).

Sequence: NH_2-ELISN*ASDALDK, *=GlcNAc **(146)**

Yield: 34% (6.6 mg, 4.46 µmol). Analytical HPLC Rt= 23.12 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 12.99 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 739.8649 ($[M+2H]^{2+}$, calc. 739.8652), 750.8583 ($[M+Na+H]^{2+}$, calc. 750.8562), 506.5637 ($[M+Na+NH_4+H]^{3+}$, calc. 506.5822).

Sequence: NH_2-LLDLSGNN*LTHLPK, *=GlcNAc **(147)**

Yield: 60% (13.0 mg, 7.48 µmol). Analytical HPLC Rt= 30.21 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 16.00 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 869.4743 ($[M+2H]^{2+}$, calc. 869.4732), 880.3617 ($[M+Na+H]^{2+}$, calc. 880.4642), 579.9839 ($[M+3H]^{3+}$, calc. 579.9847).

Sequence: NH_2-LQNLTLPTN*ASIK, *=GlcNAc **(148)**

Yield: 60% (12.0 mg, 7.43 µmol). Analytical HPLC Rt= 26.99 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.66 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 808.4482 ($[M+2H]^{2+}$, calc. 808.4492), 819.4434 ($[M+Na+H]^{2+}$, calc. 819.4402), 539.3030 ($[M+3H]^{3+}$, calc. 539.3021).

Sequence: NH_2-SNYSSPSN*ISK, *=GlcNAc **(149)**

Yield: 74% (12.9 mg, 9.30 µmol). Analytical HPLC Rt= 15.40 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 9.16 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1386.6539 ($[M+H]^{+}$, calc. 1386.6388), 693.8233 ($[M+2H]^{2+}$, calc. 692.8233), 704.8167 ($[M+Na+H]^{2+}$, calc. 704.8143), 475.8694 ($[M+Na+NH_4+H]^{3+}$, calc. 475.).

Sequence: NH_2-Spacer-IN*TTADEKDPTNPFR, *=GlcNAc **(150)**

Yield: 66% (17.5 mg, 8.23 µmol). Analytical HPLC Rt= 23.56 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(35:65), 30 min, wavelength=214 nm). Preparative HPLC Rt= 13.72 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS*

(Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1063.0251 ($[M+2H]^{2+}$, calc. 1063.0190), 1074.0178 ($[M+Na+H]^{2+}$, calc. 1074.0100), 1082.5016 ($[M+Na+NH_4]^{2+}$, calc. 1082.5233), 709.0148 ($[M+3H]^{3+}$, calc. 709.0152), 716.6787 ($[M+Na+2H]^{3+}$, calc. 716.6770), 722.0003 ($[M+Na+NH_4+H]^{3+}$, calc. 722.0181).

Sequence: NH_2-Spacer-AN*HSGAVVLLKR, *=GlcNAc **(151)**

Yield: 85% (17.8 mg, 10.63 µmol). Analytical HPLC Rt= 23.56 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 12.79 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 835.9766 ($[M+2H]^{2+}$, calc. 835.9760), 846.9685 ($[M+Na+H]^{2+}$, calc. 846.9670), 557.6522 ($[M+3H]^{3+}$, calc. 557.6532), 716.6787 ($[M+Na+2H]^{3+}$, calc. 716.6770), 570.6370 ($[M+Na+NH_4+H]^{3+}$, calc. 570.6561).

Sequence: NH_2-Spacer-VVN*STTGPGEHLR, *=GlcNAc **(152)**

Yield: 86% (19.0 mg, 10.70 µmol). Analytical HPLC Rt= 21.13 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 11.71 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 886.9564 ($[M+2H]^{2+}$, calc. 886.9554), 897.9493 ($[M+Na+H]^{2+}$, calc. 897.9564), 591.6386 ($[M+3H]^{3+}$, calc. 591.6395), 599.3019 ($[M+Na+2H]^{3+}$, calc. 599.3013), 604.6232 ($[M+Na+NH_4+H]^{3+}$, calc. 604.6424).

Sequence: NH_2-Spacer-WVSN*KTEGR, *=GlcNAc **(153)**

Yield: 51% (9.43 mg, 6.36 µmol). Analytical HPLC Rt= 20.62 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 11.80 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1482.7529 ($[M+H]^{+}$, calc. 1482.7440), 741.8753 ($[M+2H]^{2+}$, calc. 741.8759), 752.8684 ($[M+Na+H]^{2+}$, calc. 752.8669), 494.9190 ($[M+3H]^{3+}$, calc. 494.9199), 507.9036 ($[M+Na+NH_4+H]^{3+}$, calc. 507.9227).

Sequence: NH_2-Spacer-LRPDDSKN*FSIQVR, *=GlcNAc **(154)**

Yield: 41% (10.7 mg, 5.14 µmol). Analytical HPLC Rt= 36.37 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 13.91 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1041.0495 ($[M+2H]^{2+}$, calc. 1041.0478), 1052.0402 ($[M+Na+H]^{2+}$, calc. 1052.0388), 694.3674 ($[M+3H]^{3+}$, calc. 694.3678), 702.0306 ($[M+Na+2H]^{3+}$, calc. 702.0296), 707.3524 ($[M+Na+NH_4+H]^{3+}$, calc. 707.3707), 521.0270 ($[M+4H]^{4+}$, calc. 521.0278).

Sequence: NH_2-Spacer-GEYFVN*VTTR, *=GlcNAc **(155)**

Yield: 23% (4.65 mg, 2.92 µmol). Analytical HPLC Rt= 27.09 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.81 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 796.3961 ($[M+2H]^{2+}$, calc. 796.3967), 807.3904 ($[M+Na+H]^{2+}$, calc. 807.3877), 544.2515 ($[M+Na+NH_4+H]^{3+}$, calc. 544.2699).

Sequence: NH_2-Spacer-AYSWN*ISR, *=GlcNAc **(156)**

Yield: 58% (10.2 mg, 7.24 µmol). Analytical HPLC Rt= 26.99 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.74 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 701.8464 ($[M+2H]^{2+}$, calc. 701.8466), 712.8390 ($[M+Na+H]^{2+}$, calc. 712.8376), 481.2177 ($[M+Na+NH_4+H]^{3+}$, calc. 481.2365).

Sequence: NH_2-Spacer-N*LTALPPDLPK, *=GlcNAc **(157)**

Yield: 64% (12.7 mg, 8.02 µmol). Analytical HPLC Rt= 28.87 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 15.35 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad:

MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1584.8936 ($[M+H]^+$, calc. 1584.8736), 792.9407 ($[M+2H]^{2+}$, calc. 792.9407), 803.9349 ($[M+Na+H]^{2+}$, calc. 803.9317), 528.9644 ($[M+3H]^{3+}$, calc. 528.9631), 541.9481 ($[M+Na+NH_4+H]^{3+}$, calc. 541.9659), 396.9659 ($[M+4H]^{4+}$, calc. 396.9743).

Sequence: NH_2-Spacer-LQN*LTLPTNASIK, *=GlcNAc **(158)**

Yield: 64% (14.7 mg, 8.06 µmol). Analytical HPLC Rt= 30.26 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 15.89 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 910.0074 ($[M+2H]^{2+}$, calc. 910.0071), 921.0021 ($[M+Na+H]^{2+}$, calc. 920.9981), 607.0094 ($[M+3H]^{3+}$, calc. 607.0073), 614.6717 ($[M+Na+2H]^{3+}$, calc. 614.6691), 619.9931 ($[M+Na+NH_4+H]^{3+}$, calc. 620.0102), 455.5122 ($[M+4H]^{4+}$, calc. 455.5075).

Sequence: NH_2-Spacer-SN*YSSPSNISK, *=GlcNAc **(159)**

Yield: 37% (7.45 mg, 4.69 µmol). Analytical HPLC Rt= 17.54 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 9.98 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); /*HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 795.3810 ($[M+2H]^{2+}$, calc. 795.3812), 806.3755 ($[M+Na+H]^{2+}$, calc. 806.3722), 543.5750 ($[M+Na+NH_4+H]^{3+}$, calc. 543.5929).

Sequence: NH_2-Spacer-ELISN*ASDALDK, *=GlcNAc **(160)**

Yield: 64% (13.35 mg, 7.94 µmol). Analytical HPLC Rt= 26.57 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.34 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1681.8656 ($[M+H]^+$, calc. 1681.8383), 841.4237 ($[M+2H]^{2+}$, calc. 841.4237), 852.4162 ($[M+Na+H]^{2+}$, calc. 852.4242), 568.9490 ($[M+Na+2H]^{3+}$, calc. 568.9464), 574.2704 ($[M+Na+NH_4+H]^{3+}$, calc. 574.2875), 421.2193 ($[M+4H]^{4+}$, calc. 421.2154).

Sequence: NH_2-Spacer-LLDLSGNN*LTHLPK, *=GlcNAc **(161)**

Yield: 75% (18.3 mg, 9.43 µmol). Analytical HPLC Rt= 33.32min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 17.23 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 971.0352 ($[M+2H]^{2+}$, calc. 971.0311), 982.0268 ($[M+Na+H]^{2+}$, calc. 982.0221), 647.6894 ($[M+3H]^{3+}$, calc. 647.6900), 655.3526 ($[M+Na+2H]^{3+}$, calc. 655.3518), 660.6743 ($[M+Na+NH_4+H]^{3+}$, calc. 660.6929).

Sequence: NH_2-Spacer-LQNLTLPTN*ASIK, *=GlcNAc **(162)**

Yield: 66% (15.0 mg, 9.43 µmol). Analytical HPLC Rt= 30.57 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 16.05 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1819.0355 ($[M+H]^+$, calc. 1819.0064), 910.0078 ($[M+2H]^{2+}$, calc. 910.0071), 921.0024 ($[M+Na+H]^{2+}$, calc. 920.9981), 607.0099 ($[M+3H]^{3+}$, calc. 607.0073), 614.6719 ($[M+Na+2H]^{3+}$, calc. 614.6691), 619.9933 ($[M+Na+NH_4+H]^{3+}$, calc. 620.0102).

Sequence: NH_2-Spacer-SNYSSPSN*ISK, *=GlcNAc **(163)**

Yield: 48% (9.6 mg, 6.04 µmol). Analytical HPLC Rt= 17.06 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 9.93 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 795.3813 ($[M+2H]^{2+}$, calc. 795.3912), 806.3755 ($[M+Na+H]^{2+}$, calc. 806.3722), 530.5915 ($[M+3H]^{3+}$, calc. 530.5901), 538.24483 ($[M+Na+2H]^{3+}$, calc. 538.2519), 543.5752 ($[M+Na+NH_4+H]^{3+}$, calc. 534.5929).

7.25 Enzymatic coupling of oxazoline to *N*-glycopeptides

Sequence: NH_2-IN*TTADEKDPTNPFR, *= $Man_3GlcNAc_2$ **(164)**

The tetrasaccharide oxazoline **110** (0.39 mg, 0.56 µmol 8.0 eq) and the GlcNAc-peptide **136** (0.13 mg, 0.07 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 6.25 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC. The reaction finished in 1 h and a faster product was observed on HPLC with full conversion. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **164**, yield 54% (0.10 mg, 0.038 µmol). Analytical HPLC Rt= 22.42 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(35:65), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 22.33 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1306.6200 ($[M+2H]^{2+}$, calc. 1306.5817), 871.3962 ($[M+3H]^{3+}$, calc. 871.3904), 663.2904 ($[M+K+3H]^{4+}$, calc. 663.2837).

Sequence: NH_2-Spacer-VVN*STTGPGEHLR, *= $Man_3GlcNAc_2$ **(165)**

The tetrasaccharide oxazoline **110** (1.03 mg, 1.49 µmol, 6.3 eq) and the GlcNAc-peptide **152** (0.42 mg, 0.24 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 7.75 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC. The reaction finished in 1 h and a faster product was observed on HPLC with full conversion. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **165**, yield 55% (0.32 mg, 0.13 µmol). Analytical HPLC Rt= 15.36 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 15.50 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1232.0969 ($[M+2H]^{2+}$, calc. 1232.0761), 821.7212 ($[M+3H]^{3+}$, calc. 821.7200), 616.5465 ($[M+4H]^{4+}$, calc. 616.5419), 501.0277 ($[M+K+4H]^{5+}$, calc. 501.0263).

Sequence: NH_2-Spacer-WVSN*KTEGR, *= $Man_3GlcNAc_2$ **(166)**

The tetrasaccharide oxazoline **110** (1.03 mg, 1.49 µmol, 5.3 eq) and the GlcNAc-peptide **153** (0.42 mg, 0.28 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 7.75 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC. The reaction finished in 1 h and a faster product was observed on HPLC with full conversion. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **166**, yield 57% (0.35 mg, 0.16 µmol). Analytical HPLC Rt= 14.15 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 14.63 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1086.5079 ($[M+2H]^{2+}$, calc. 1086.4949), 724.6675 ($[M+3H]^{3+}$, calc. 724.6658), 553.2415 ($[M+K+3H]^{4+}$, calc. 553.2403).

Sequence: NH_2-Spacer-N*LTALPPDLPK, *= $Man_3GlcNAc_2$ **(167)**

The tetrasaccharide oxazoline **110** (1.19 mg, 1.73 µmol, 8.2 eq) and the GlcNAc-peptide **157** (0.42 mg, 0.21 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 7.75 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC. The reaction finished in 1 h and a faster product was observed on HPLC with full conversion. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **167**, yield 89% (0.42 mg, 0.18 µmol). Analytical HPLC Rt= 21.99 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 22.41 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1138.0721 ($[M+2H]^{2+}$, calc. 1138.0614), 759.0502 ($[M+3H]^{3+}$, calc. 759.0435), 579.0296 ($[M+K+3H]^{4+}$, calc. 579.0236).

Sequence: NH_2-Spacer-LQNLTLPTN*ASIK, *= $Man_3GlcNAc_2$ **(168)**

The tetrasaccharide oxazoline **110** (1.03 mg, 1.49 µmol, 6.8 eq) and the GlcNAc-peptide **162** (0.40 mg, 0.22 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 7.75 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC. The reaction finished in 1 h and a faster product was observed on HPLC with full conversion. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **168**, yield 70% (0.35 mg, 0.15 µmol). Analytical HPLC Rt= 23.50 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 23.57 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1255.1527 ($[M+2H]^{2+}$, calc. 1255.1278), 837.0907 ($[M+3H]^{3+}$, calc. 837.0878), 637.5616 ($[M+K+3H]^{4+}$, calc. 637.5568).

Sequence: NH_2-IN*TTADEKDPTNPFR, *= $Man_3GlcNAc_4$ **(169)**

The hexasaccharide oxazoline **112** (0.61 mg, 0.56 µmol 8.0 eq) and the GlcNAc-peptide **136** (0.13 mg, 0.07 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 6.25 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC for a few hours. The reaction was stopped after 4 h and a faster product was observed on HPLC with 20% conversion and longer reaction times only resulted back-hydrolysis of the obtained product. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **169**. Analytical HPLC Rt= 22.42 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(35:65), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 21.72 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1509.6645 ($[M+2H]^{2+}$, calc. 1509.6611), 1006.7768 ($[M+3H]^{3+}$, calc. 1006.7766), 764.8211 ($[M+K+3H]^{4+}$, calc. 764.8234).

Sequence: NH_2-Spacer-N*LTALPPDLPK, *= $Man_3GlcNAc_4$ **(170)**

The hexasaccharide oxazoline **112** (0.92 mg, 0.84 µmol 8.0 eq) and the GlcNAc-peptide **157** (0.17 mg, 0.10 µmol 1.0 eq) were dissolved in a phosphate buffer (50 mM, pH 7.1, 6.25 µL) and mixed with the enzyme solution, Endo-M-N175Q (TCI chemistry, 12.5 milli-units in 6.25 µL buffer), incubated at 25 °C and followed by analytical HPLC for a few hours. The reaction was stopped after 3.5 h and a faster product was observed on HPLC with 21% conversion and longer reaction times only resulted back-hydrolysis of the obtained product. The crude product was diluted with H_2O and purified by semi-prep HPLC directly to give product glycopeptide **170**. Analytical HPLC Rt= 22.20 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm). Semi-preparative HPLC Rt= 23.08 min (Thermo Hypersil GOLD, 5 µm, 4.6x250mm, Grad: MeCN (84%)/water + 0.1% TFA (10:90)->(40:60), 30 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1341.1621 ($[M+2H]^{2+}$, calc. 1371.1407), 894.4315 ($[M+3H]^{3+}$, calc. 894.4297), 680.5668 ($[M+K+3H]^{4+}$, calc. 680.5632).

7.26 Mass fragmentation analysis of selected *N*-glycopeptides

Multistage CID-MSn

For the CID-MSn experiments (n=2-4), a HR-ESI-FTICR-MS (Thermo LTQ-FT Ultra) operated in positive ion mode and coupled to a nano-LC system was used. Precursor ion scans were acquired over the *m/z* range 300-2000 at a resolution setting of 50,000. CID-MS2 spectra were recorded for the most intense precursor ion in each full scan, at a normalised collision energy (NCE) level of 30% and an isolation width of 5 *m/z* units. An ion product list containing the *m/z* 366.14 and *m/z* 204.08 ions were used for the MS3 selection, and the seven most intense ions from each MS3 scan were selected for consecutive MS4 events.

HCD experiments

HCD fragmentation at the levels of 10 to 50% NCE in steps of 5% were performed on an Orbitrap Fusion Tribrid mass spectrometer interfaced to an Easy-nLC II (Thermo Fisher Scientific). Glycopeptides were separated using an analytical column (300x0.075 mm I.D.) packed with 3 µm Reprosil-Pur C18-AQ particles (Dr. Maisch, Germany), using a mobile phase of 0.2% formic acid in water and a gradient of 0.2% formic acid in acetonitrile for 30 min. Positive ions were injected into the mass spectrometer under a spray voltage of 1.6 kV. MS scans was performed at 120,000 resolution, *m/z* range 400-1,200 in the Orbitrap, followed by nine scan events of MS/MS analysis during a top speed cycle of 3s and precursor ion isolation in the quadrupole with a 1.6 *m/z* window. The nine individual consecutive MS/MS HCD fragmentation scans used 10, 15, 20, 25, 30, 35, 40, 45 and 50% NCE with detection in the Orbitrap at 60,000 resolution and *m/z* starting at 50.

7.27 Synthesis of *O*-mannosyl peptides

General procedure glycopeptide synthesis

The *O*-Mannosylated peptides **171** - **173**, **180** - **183** and **190** - **195** were synthesized by stepwise solid-phase peptide synthesis using the Fmoc strategy and starting with preloaded tentagel S RAM Fmoc-Gly resin or tentagel R Trt Fmoc-Gly or Fmoc-Arg resin (12.5 µmol scale). The glycopeptide synthesis performed using 2.0 eq of the protected *O*-Mannosyl amino acid building blocks **120, 124 and 135,** which were pre-activated manually using 2.0 eq HATU/HOAt and 4.0 eq of DIPEA (8 h coupling time). The standard Fmoc amino acids were coupled automatically on a Multisyntech peptide synthesizer using 8 eq of the amino acid and the HBTU/HOBt reagents (40 min). Fmoc deprotection was done according to standard conditions, 20% piperidine in DMF. After assembly of the peptide backbone:

i) peptide **171** - **173** and **190** - **195** were coupled with a triethyleneglycol amino acid spacer to the *N*-terminus followed by Fmoc deprotection. The obtained peptides were then released from the resin, and all acid sensitive side-chain protecting groups were simultaneously removed using TFA/TIPS/H_2O 15:0.9:0.9 for 2.5 h followed by solvent concentration, lyophilization and purification using a C-18 cartridge (1g of C-18 material, Waters). For saccharide deprotection, the *O*-acetyl groups were cleaved by transesterification in methanol using catalytic amounts of NaOMe at pH 9-9.5 for 24 h (deprotection followed by analytical HPLC) to yield glycopeptides **171** - **173** and **190** - **195** which were purified by preparative HPLC.

ii) peptide **180** - **183** were capped with an *N*-terminal acetyl group using a solution of 0.015 M HOBt, 0.5 M Ac_2O, 0.125 M DIPEA in NMP on *N*-terminus for 45 min. The obtained peptide was then released from the resin, and all acid sensitive side-chain protecting groups were simultaneously removed using TFA/TIPS/H_2O 15:0.9:0.9 for 2.5 h followed by solvent concentration, lyophilization and purification using a C-18 cartridge (1g of C-18 material, Waters). The *C*-terminal was then extended with *N*-Boc-4,7,10-trioxa-1,13-tridecanediamine spacer (1.5 eq), catalyzed by HATU/HOAt (1.25 eq) and DIPEA (2.5 eq) in DMF (200 µL per 12.5 mmol batch) for 8 h. The coupling product was diluted by H_2O and the solvent was removed by lyophilization. The Boc protection group was then removed by using DCM/TFA

3:1 solution (1.6 mL/10 mg peptide) in 3 h followed by solvent concentration, lyophilization and purification using a C-18 cartridge (500 mg of C-18 material, Waters). For saccharide deprotection, the *O*-acetyl groups were cleaved by transesterification in methanol (10 mL/10 mg peptide) using catalytic amounts of NaOMe at pH 9-9.5 for 24 h (deprotection followed by analytical HPLC) to yield glycopeptides **180** - **183** which were purified by preparative HPLC. The GlcNAc-Man and 2,6-GlcNac$_2$-Man glycopeptides loading on BSA and CRM-conjugates **186** - **189** were measured by MALDI. The spectra were listed in section 10 and the loading equivalence were calculated.

Sequence: NH_2-Spacer-GT*G, *= GalGlcNAcMan: **(171)**

Yield: 37% (4.8 mg, 4.63 µmol). Analytical HPLC Rt= 8.20 min (Phenomenex Luna C18 (2), 3µm, 2.0 x 150 mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(50:50), 45 min, wavelength = 214 nm). Preparative HPLC Rt= 5.99 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+H]^+$: 963.4258, found: 963.4247.

Sequence: NH_2-Spacer-GT*G, *= GlcNAcMan: **(172)**

Yield: 64% (5.2 mg, 6.49 µmol). Analytical HPLC Rt= 8.17 min (Phenomenex Luna C18(2), 3µm, 2.0 x 150 mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(35:65), 30 min, wavelength=214 nm). Preparative HPLC Rt= 6.07 min (Phenomenex Luna C18 (2), 21.2x250mm, 10 µm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm). HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+H]^+$: 801.3729, found: 801.3721.

Sequence: NH_2-Spacer-GTG: **(173)**

Yield: 43% (3.6 mg, 5.38 µmol). Analytical HPLC Rt= 7.07 min (Phenomenex Luna C18 (2), 2.00x150mm, 3µm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(35:65), 30 min, wavelength=214 nm). Preparative HPLC Rt= 5.88 min (Phenomenex Luna C18 (2), 21.2x250mm, 10 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(55:45), 25 min,

wavelength=214 nm). HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+H]^+$: 436.2407, found: 436.2398.

Sequence: Squarate-NH-Spacer-GT*G, *= GalGlcNAcMan: **(174)**

Peptide **171** (6.1 mg, 6.33 µmol) was dissolved in a 1:1 solution of EtOH / H_2O (6 mL) and 3,4-Diethoxy-3-cyclobuten-1,2-dione (1.03 µL, 6.96 µmol) was added. Then small portions of Sat. Na_2CO_3 solution (2.5 µL) was added every 5 min until pH reached 8.0. The reaction mixture was stirred at room temperature and after 2h HPLC showed complete conversion to the desired product. To quench the reaction AcOH (5 µL) was added, the solvent was then removed under reduced pressure and the residue was purified by HPLC to give **174**. Yield: 70% (4.4 mg, 4.43 µmol). Analytical HPLC Rt= 17.52 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(75:25), 70 min, wavelength=214 nm). Preparative HPLC Rt= 17.58 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+Na]^+$: 1109.4232, found: 1109.4253. $[M+2Na]^{2+}$: 556.2068, found: 556.2070

Sequence: Squarate-NH-Spacer-GTG: **(175)**

Peptide **173** (6.8 mg, 15.6 µmol, 1.0 eq) was dissolved in a 1:1 solution of EtOH / H_2O (16 mL) and 3,4-Diethoxy-3-cyclobuten-1,2-dione (2.54 µL, 17.2 µmol, 1.1 eq) was added. Then small portions of Sat. Na_2CO_3 solution (2.5 µL) was added every 5 min until pH reached 8.0. The reaction mixture was stirred at room temperature and after 2h HPLC showed complete conversion to the desired product. To quench the reaction AcOH (5 µL) was added, the solvent was then removed under reduced pressure and the residue was purified by HPLC to give **175**. Yield: 56% (4.9 mg, 8.73 µmol).

BSA-conjugate of peptide sequence: -Spacer-GT*G, *= GalGlcNAcMan: **(176)**

The squarate monoamide **174** (2.18 mg, 2.0 µmol) and BSA (6.67 mg, 0.1 µmol, 1/20 eq) was dissolved in a 60 mM Na_2HPO_4 buffer (0.5 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through an amicon filter (10 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide

material was removed from the obtained BSA conjugate product **176**. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **176** (5.0 mg, 5 mol/ mol BSA determined by MALDI).

BSA-conjugate of peptide sequence: -Spacer-GTG: **(177)**

The squarate monoamide **175** (1.12 mg, 2.0 µmol) and BSA (6.67 mg, 0.1 µmol, 1/20 eq) was dissolved in a 60 mM Na_2HPO_4 buffer (0.5 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through a amicon filter (10 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide material was removed from the obtained BSA conjugate product **177**. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **177** (5.0 mg).

KLH-conjugate of peptide sequence: -Spacer-GT*G, *= GalGlcNAcMan: **(178)**

The squarate monoamide **174** (2.18 mg, 2.0 µmol) and KLH (5 mg, 1.67-12.5 nmol, 1/160-1200 eq, average mass 400-3000 kDa given by Sigma Aldrich) was dissolved in a 60 mM Na_2HPO_4 buffer (0.5 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through a amicon filter (30 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide material was removed from the obtained KLH conjugate product **178**. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **178** (3.07 mg).

KLH-conjugate of peptide sequence: -Spacer-GTG: **(179)**

The squarate monoamide **175** (1.12 mg, 2.0 µmol) and KLH (0.6 mg, 0.20-1.5 nmol, 1/1333-10000 eq, average mass 400-3000 kDa given by Sigma Aldrich) was dissolved in a 60 mM Na_2HPO_4 buffer (0.5 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through a amicon filter (30 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide material was removed from the obtained KLH conjugate product **179**. The protein conjugate in the amicon filter was then

washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **179** (0.5 mg).

Sequence: AcHN-GT*T*G-Spacer-NH_2, *= GlcNAcMan **(180)**

Yield: 28% (9.0mg, 6.95 µmol). Analytical HPLC Rt= 8.97 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 5.12 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+2H]^{2+}$: 655.3038, found: 655.3064.

Sequence: AcHN-GT*T*G-Spacer-NH_2, *= $GlcNAc_2Man$ **(181)**

Yield: 3.6% (2.3 mg, 1.34 µmol). Analytical HPLC Rt= 9.07 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 6.28 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 869.3735 ($[M+Na+H]^{2+}$, calc. 869.3742), 880.3651 ($[M+2Na]^{2+}$, calc. 880.3653).

Sequence: AcHN-T*G-Spacer-NH_2, *= $GlcNAc_2Man$ **(182)**

Yield: 1.9% (0.7 mg, 0.71 µmol). Analytical HPLC Rt= 10.43 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 7.15 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 989.4817 ($[M+H]^{+}$, calc. 989.4778), 1011.4631 ($[M+Na]^{+}$, calc. 1011.4597), 506.2328 ($[M+Na+H]^{2+}$, calc. 506.2338), 517.2244 ($[M+2Na]^{2+}$, calc. 517.2248).

Sequence: AcHN-GTTG-Spacer-NH_2: **(183)**

Yield: 24% (3.4 mg, 5.88 µmol). Analytical HPLC Rt= 12.20 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214

nm). Preparative HPLC Rt= 6.92 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 579.3340 ($[M+H]^+$, calc. 579.3354), 601.3174 ($[M+Na]^+$, calc. 601.3173), 290.1737 ($[M+2H]^{2+}$, calc. 290.1716).

Sequence: AcHN-GT*T*G-Spacer-NH-Squarate, *= GlcNAcMan **(184)**

Peptide **180** (8.2 mg, 6.33 µmol, 1.0 eq) was dissolved in a 1:1 solution of EtOH / H_2O (11 mL) and 3,4-Diethoxy-3-cyclobuten-1,2-dione (1.03 µL, 6.96 µmol, 1.1 eq) was added. Then small portions of Sat. Na_2CO_3 solution (1.0 µL) was added every 5 min until pH reached 8.0. The reaction mixture was stirred at room temperature and after 2h HPLC showed complete conversion to the desired product. To quench the reaction AcOH (1 µL) was added, the solvent was then removed under reduced pressure and the residue was purified by HPLC to give **184**. Yield: 63% (5.5 mg, 3.96 µmol). Analytical HPLC Rt= 19.74 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(75:25), 70 min, wavelength=205nm). Preparative HPLC Rt= 11.99 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1433.6269 ($[M+H]^+$, calc. 1433.6158), 1455.6099 ($[M+Na]^+$, calc. 1455.5977), 717.3111 ($[M+2H]^{2+}$, calc. 717.3118), 728.3042 ($[M+Na+H]^{2+}$, calc. 728.3028), 736.7886 ($[M+Na+NH_4]^{2+}$, calc. 736.8161), 748.2696 ($[M+2Na+NH_3]^{2+}$, calc. 748.3087).

Sequence: AcHN-GT*T*G-Spacer-NH-Squarate, *= $GlcNAc_2Man$ **(185)**

Peptide **181** (1.50 mg, 0.87 µmol, 1.0 eq) was dissolved in a 1:1 solution of EtOH / H_2O (1 mL) and 3,4-Diethoxy-3-cyclobuten-1,2-dione (0.14 µL, 0.96 µmol, 1.1 eq) was added. Then small portions of Sat. Na_2CO_3 solution (1.0 µL) was added every 5 min until pH reached 8.0. The reaction mixture was stirred at room temperature and after 2h HPLC showed complete conversion to the desired product. To quench the reaction AcOH (1 µL) was added, the solvent was then removed under reduced pressure and the residue was purified by HPLC to give **185**. Yield: 52% (0.85 mg, 0.46 µmol). Analytical HPLC Rt= 17.93 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 10.87 min (Phenomenex Luna C18 (2), 10 µm,

21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 942.3747 ($[M+2Na]^{2+}$, calc. 943.3732).

BSA-conjugate of peptide sequence: AcHN-GT*T*G-Spacer-, *= GlcNAcMan **(186)**

The squarate monoamide **184** (2.00 mg, 1.40 µmol) and BSA (5.25 mg, 0.079 µmol, 1/17.7 eq) was dissolved in a 200 mM Na_2HPO_4 buffer (0.25 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through an amicon filter (10 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide material was removed from the obtained BSA conjugate product **186**. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **186** (6.5 mg). The average mass was measured around 85.5k as pure BSA for 66.4k. The loading of glycopeptide **184** (MS 1433.4) was calculated for 13.3 eq. See section 10.

CRM-conjugate of peptide sequence: AcHN-GT*T*G-Spacer-, *= GlcNAcMan **(187)**

The squarate monoamide **184** (0.6 mg, 0.419 µmol) and CRM (1.0 mg, 0.016 µmol, 1/26.4 eq) was dissolved in a 200 mM Na_2HPO_4 buffer (0.25 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through an amicon filter (10 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide material was removed from the obtained BSA conjugate product **187**. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **187** (1.2 mg). The average mass was measured around 72k as pure CRM for 63k. The loading of glycopeptide **184** (MS 1433.4) was calculated for 6.3 eq. See section 10.

BSA-conjugate of peptide sequence: AcHN-GT*T*G-Spacer-, *= $GlcNAc_2Man$ **(188)**

The squarate monoamide **185** (0.4 mg, 0.22 µmol) and BSA (0.5 mg, 0.0075 µmol, 1/30 eq) was dissolved in a 100 mM Na_2HPO_4 buffer (0.10 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through an amicon filter (10 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted

glycopeptide material was removed from the obtained BSA conjugate product. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **188** (0.6 mg). The BSA-conjugate was compared with pure BSA, see section 10. The zoom in spectrum showed clearly only 1-3 eq glycopeptide **185** (MS 1839.8) were loaded on the carrier protein.

CRM-conjugate of peptide sequence: AcHN-GT*T*G-Spacer-, *= $GlcNAc_2Man$ **(189)**

The squarate monoamide **185** (0.80 mg, 0.44 µmol) and CRM (1.0 mg, 0.016 µmol, 1/28 eq) was dissolved in a 75 mM Na_2HPO_4 buffer (0.10 mL, pH 9.5). The solution was stirred at room temperature and at pH 9.5 for 3 days. By passing the reaction mixture through an amicon filter (10 kDa cut-off, centrifugation at 14000 g for 15 min) the unreacted glycopeptide material was removed from the obtained CRM conjugate product. The protein conjugate in the amicon filter was then washed 5 times with H_2O (0.4 mL, centrifugation at 1000 g for 2 min each time) followed by lyophilization to give **189** (1.1 mg). The CRM-conjugate was compared with pure CRM, see section 10. The zoom in spectrum showed clearly only 2-3 eq glycopeptide **185** (MS 1839.8) were loaded on the carrier protein.

Sequence: NH_2-Spacer-PVPGKPT*VTIR, *= GalGlcNAcMan **(190)**

Yield: 70% (16.5 mg, 8.75 µmol). Analytical HPLC Rt= 21.01 min (Phenomenex Luna C18 (2), 3µm, 2.0 x 150 mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(35:65), 30 min, wavelength=214 nm). Preparative HPLC Rt= 11.95 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+2H]^{2+}$: 948.0096, found: 948.0098.

Sequence: NH_2-Spacer-RGAIIQT*PTLG, *= GalGlcNAcMan **(191)**

Yield: 21% (4.9 mg, 2.56 µmol). Analytical HPLC Rt= 23.84 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(50:50), 45 min, wavelength=214 nm). Semi-preparative HPLC Rt= 13.82 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm);

HR-ESI-TOF-MS (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: calc. for $[M+2H]^{2+}$: 928.9835, found: 928.9837.

Sequence: NH_2-Spacer-PVPGKPT*VTIR, *= GlcNAcMan **(192)**

Yield: 62% (15.5 mg, 7.8 µmol). Analytical HPLC Rt= 22.25 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 12.53 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 866.9857 ($[M+2H]^{2+}$, calc. 866.9872), 877.9768 ($[M+H+Na]^{2+}$, calc. 877.9782), 578.3239 ($[M+3H]^{3+}$, calc. 578.3274).

Sequence: NH_2-Spacer-RGAIIQT*PTLG, *= GlcNAcMan **(193)**

Yield: 18% (4.3 mg, 2.2 µmol). Analytical HPLC Rt= 25.35 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 14.45 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 1694.9332 ($[M+H]^{+}$, calc. 1694.9064), 847.9578 ($[M+2H]^{2+}$, calc. 847.9571), 578.6265 ($[M+H+Na+NH_4]^{3+}$, calc. 578.6435).

Sequence: NH_2-Spacer-PVPGKPT*VTIR, *= $GlcNAc_2Man$ **(194)**

Yield: 21% (5.2 mg, 2.69 µmol). Analytical HPLC Rt= 23.29 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 12.12 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 968.5279 ($[M+2H]^{2+}$, calc. 968.5269), 979.5183 ($[M+H+Na]^{2+}$, calc. 979.5179), 987.5007 ($[M+Na+NH_4]^{2+}$, calc. 987.5295), 646.0179 ($[M+3H]^{3+}$, calc. 646.0205).

Sequence: NH_2-Spacer-RGAIIQT*PTLG, *= $GlcNAc_2Man$ **(195)**

Yield: 5% (1.2 mg, 0.63 µmol). Analytical HPLC Rt= 22.09 min (Phenomenex Luna C18 (2), 2.0 x 150 mm, 3 µm, Grad: MeCN/water + 0.1% TFA (5:95)->(45:55), 40 min, wavelength=214 nm). Preparative HPLC Rt= 13.01 min (Phenomenex Luna C18 (2), 10 µm, 21.2x250mm, Grad: MeCN (84%)/water + 0.1% TFA (5:95)->(55:45), 25 min, wavelength=214 nm); *HR-ESI-TOF-MS* (Thermo LTQ-Orbitrap mass spectrometer), *m/z*: 949.4967 ($[M+2H]^{2+}$, calc. 949.4968), 960.4914 ($[M+H+Na]^{2+}$, calc. 960.4878), 633.3360 ($[M+3H]^{3+}$, calc. 633.3338), 640.6636 ($[M+2H+Na]^{3+}$, calc. 640.6611), 646.3197 ($[M+H+Na+NH_4]^{3+}$, calc. 646.3366).

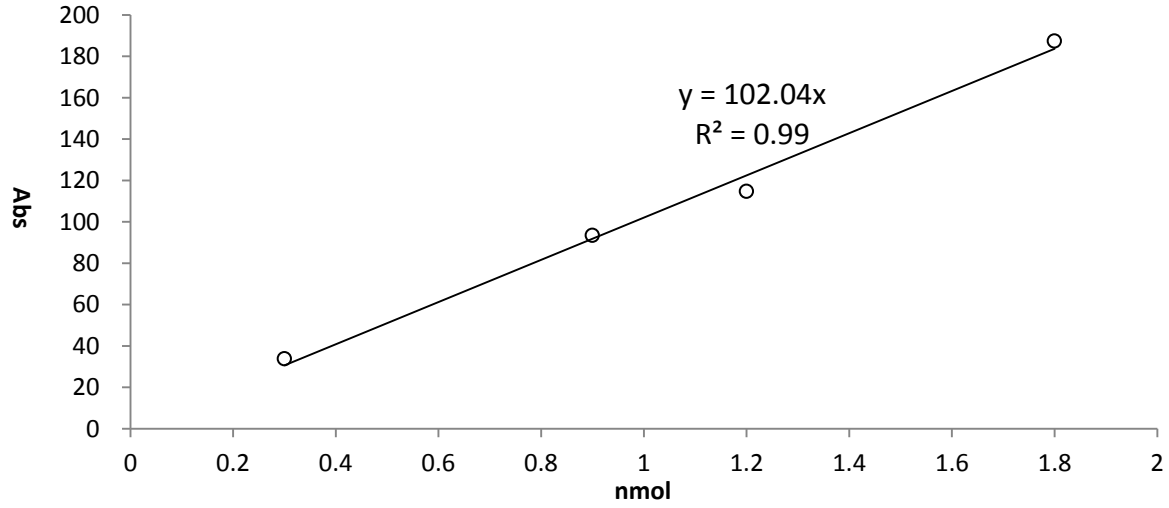
7.28 Determination glycopeptide loading on KLH-conjugate

To determine the glycopeptide loading on the glycopeptide-KLH conjugate **178,** monosaccharides were released from the peptide backbone by acid hydrolysis, derivatized and analyzed by HPLC in accordance with a previously published procedure. The KLH conjugate **178** (0.18 mg) was dissolved in 4 M trifluoroacetic acid (300 µL) and hydrolyzed for 3 h at 100 °C. The solvent was then removed using a speedvac, and the residue was dissolved in H_2O (10 µL) and mixed with Anthranilic acid (AA) reagent solution (20 µL) (AA regent solution = 3 mg of AA and 30 mg of sodium cyanoborohydride in 1 mL of 2% (w/v) boric acid in methanol) and incubated for 1 h at 80 °C. Then an aliquot of the reaction mixture (1 µL) was diluted with 0.1% TFA-H_2O (36 µL) and of the diluted sample 30µL was injected into the analytical HPLC. Measuring the UV absorbance at 214 nm a peak area of 83.549 mAu was detected for the combined galactose and mannose peaks (the combined area was used due to AA-derived mannose/galactose peak overlap). Using the AA-derived galactose/mannose calibration curve described below, it was concluded that the measured peak area was corresponding to 0.818 nmol of galactose and mannose respectively. Based on the obtained amount of released monosaccharides, the dilution of the KLH conjugate **178**, and the molecular weight of KLH (400-3000 kDa, given by Sigma-Aldrich), the glycopeptide to KLH ratio was determined to 65 (400 kDa KLH) - 491 (3000 kDa KLH) mol glycopeptide / mol KLH.

HPLC calibration curve of an AA derivatized 1:1 galactose-mannose mixture

Galactose (3.24 mg, 0.018 mmol) and mannose (3.24 mg, 0.018 mmol) was dissolved in H_2O (1 mL) and an aliquot (10 µL, 0.18 µmol) of the galactose/mannose 1:1 mixture was treated with 20 µL Anthranilic acid (AA) labeling solution at 80 °C for 1 h. Then an aliquot (1 µL) of the reaction mixture was diluted with 100 µL, 150 µL, 200 µL and 600 µL 0.1% TFA-H_2O and of the diluted sample 30µL was injected into the analytical HPLC. Measuring the UV absorbance at 214 nm the combined galactose and mannose peak area was detected and a calibration curve was plotted for the corresponding dilutions.

Dilution Gal-Man mix	Amount of injected monosaccharide (nmol)	SUM Gal+Man peak area at 214 nm
1:100	1.8	187.43
1:150	1.2	114.78
1:200	0.9	93.48
1:600	0.3	33.86



Calibration curve peak area absorbance at 214 nm versus dilution in nmol of the 1:1 galactose/mannose mixture.

7.29 Immunization of rabbit sera

For antibody induction against the Galβ1-4GlcNAcβ1-2Manα glycopeptide, two rabbits **21390 and 21391** were immunized with the KLH vaccine **178** together with Freunds complete adjuvant (CFA). Six booster immunizations were carried out over 8 weeks. Immunizations were carried out by Biogenes GmbH, Berlin, Germany.

For antibody induction against the GlcNAcβ1-2Manα glycopeptide, two rabbits **23803 and 23804** were immunized with the CRM vaccine **187** together with Freunds complete adjuvant (CFA). Six booster immunizations were carried out over 8 weeks. Immunizations were carried out by Biogenes GmbH, Berlin, Germany.

7.30 ELISA experiments

ELISA of Rabbit sera 21390 and 21391 in Figure 19 **A**

ELISA experiments performed according to a standard protocol. BSA conjugate **176** was coated on the ELISA plate (96-well, Maxisorb, nunc), 50 µL (10 µg/mL) substrate per well and incubation at 4 °C overnight. Coating buffer: 0.1 M Na_2HPO_4, pH 9.4. The coated plate was washed 3 times with PBST buffer followed by blocking of the surface using PBST buffer with 1% BSA (100 µL/well). The blocking buffer was removed and titration with the rabbit sera followed. 50 µL blocking buffer was added to each well and in the first row 50 µL pre-diluted serum was added followed by 1:2 dilution in each well. Incubation performed for 60 min at 37 °C. Rabbit sera **21390** and **21391**, pre-immune, first-bleed and final bleed were incubated in 12 dilutions starting from 1:1000. After incubation three PBST washing steps followed. Incubation with secondary antibody (anti-rabbit IgG biotin, Invitrogen, 1:200 dilution, 50 µL/well) in blocking buffer were done for 60 min at 37 °C. Thereafter washing and incubation with 50 µL streptavidin-horseradish peroxidase (1:2000, SA-HPO, Sigma-Aldrich), 37 °C for 30 min. Additional washing and incubation with 50 µL/well ABTS (1 mg/mL, Sigma-Aldrich) and H_2O_2 (30%, 1:4000 dilution) in citrate buffer (40 mM citric acid and 60 mM Na_2PO_4) was followed. After 5 min the UV absorbance at 410 nm was detected (Tecan infinity 700 scanner).

Glycopeptide ELISA neutralization experiments for analysis of antibody binding specificity of Rabbit serum 21390 in Figure 19 **B and C**

The ELISA neutralization experiments performed in accordance to the normal ELISA protocol with minor differences in the incubation step with the primary antibody. The ELISA plate was coated with BSA conjugate **176** followed by BSA blocking. 40 µL blocking buffer was added to each well and in the first row 40 µL pre-diluted serum (Rabbit sera 21390 final bleed, 1:4000, 6 dilutions) was added followed by 1:2 dilution in each well. Then 10 µL neutralization substance was added to each well (neg. contrl. and peptides **171** - **173**, **190**, **191**) and the mix incubation performed for 60 min at 37 °C. Incubation with secondary antibody, SA-HPO and ABTS-H_2O_2 and detection followed according to the ELISA protocol described above.

ELISA of Rabbit sera 23803 and 23804 in Figure 21

ELISA experiments performed according to a standard protocol. BSA conjugate **186** was coated on the ELISA plate (96-well, Maxisorb, nunc), 50 µL (10 µg/mL) substrate per well and incubation at 37 °C 1 h. Coating buffer: 0.1 M Na_2HPO_4, pH 9.4. The coated plate was washed 3 times with PBST buffer followed by blocking of the surface using PBST buffer with 1% BSA (100 µL/well). The blocking buffer was removed and titration with the rabbit sera followed. 50 µL blocking buffer was added to each well and in the first row 50 µL pre-diluted serum was added followed by 1:2 dilution in each well. Incubation performed for 60 min at 37 °C. Rabbit sera **23803** and **23804**, pre-immune, first bleed, second bleed and final bleed were incubated in 12 dilutions starting from 1:1000. After incubation three PBST washing steps followed. Incubation with secondary antibody (anti-rabbit IgG biotin, Invitrogen, 1:200 dilution, 50 µL/well) in blocking buffer were done for 60 min at 37 °C. Thereafter washing and incubation with 50 µL streptavidin-horseradish peroxidase (1:2000, SA-HPO, Sigma-Aldrich), 37 °C for 30 min. Additional washing and incubation with 50 µL/well ABTS (1 mg/mL, Sigma-Aldrich) and H_2O_2 (30%, 1:4000 dilution) in citrate buffer (40 mM citric acid and 60 mM Na_2PO_4) was followed. After 5 min the UV absorbance at 410 nm was detected (Tecan infinity 700 scanner).

7.31 Microarray experiments

On-slide enzymatic coupling of tetrasaccharide oxazoline 110 to *N*-GlcNAc peptides and examining binding efficiency using the lectin ConA in Figure 13

Two microarray slides (Slide H, Schott-Nexterion, Jena, Germany) were immobilized with *N*-GlcNAc peptides **150** - **163** as well as pre-synthesized pentasaccharide *N*-glycopeptides **165** - **168** with 50 µM/100 µM concentration (in 150 mM phosphate buffer pH 8.5, 0.005% Tween-20, 85 picoL spots) using a piezo-driven non-contact spotter (iTWO200, M2-automation, Berlin, Germany). The spotting were performed in a 16 array (well) format with 9 spot replicates for each peptides. The printed peptides were allowed to react in a humidity chamber (75% humidity) for 24 h and unreacted NHS-esters were capped with 1 mM ethanolamine at pH 9 for 1 h. The slides were washed with phosphate buffered saline (PBS), 0.05% Tween-20 buffer (PBST) and water and then dried with a stream of argon for storage. A solution of 1.5 mg oxazoline donor **110** with 12.5 mU Endo-M-N175Q (TCI chemistry) in 30 µL phosphate buffer (50 mM, pH 7.1) was added in one well and incubated at 25 °C for 1 h followed by washing 3 times for 10 min with PBST buffer. For recognition, another well without enzyme treatment was also included as blank control. The two wells were incubated with biotin labeled ConA (0.1 mg/mL in PBST buffer) for 1 h followed by washing 3×10 min with PBST buffer. For readout the slides were incubated with Cy5 streptavidin (Invitrogen, diluted 1/1000 in PBST buffer), washed 3 times for 10 min with PBST buffer, water, and dried. Fluorescence was detected on a Typhoon Trio^{+} scanner (General Electrics) and the signals were analyzed and quantified by means of the Image quant software.

***O*-Mannosyl glycopeptide microarrays for analysis of antibody binding specificity of Rabbit sera 21390 and 21391 in** Figure 20

The synthesized peptides **171** - **173**, **190** and **191** (0.25 mM of peptide in 150 mM phosphate buffer pH 8.5, 0.005% Tween-20, 85 picoL spots) were immobilized on amino reactive and biocompatible hydrogel slides with a surface functionalized with carboxylic acids activated as *N*-hydroxysuccinimide esters (Slide H, Schott-Nexterion, Jena, Germany). Using a piezo-driven non-contact spotter (iTWO200, M2-automation, Berlin, Germany) the spotting performed in a 16 array (well) format. Every well contained a spotting field divided into four blocks. In

every block glycopeptides **171** - **173**, **190** and **191** were printed with 10 spot replicates. The printed peptides were allowed to react in a humidity chamber (75% humidity) for 2.5 h and unreacted NHS-esters were trapped with 1 mM ethanolamine at pH 9 for 30 min. The slides were washed with phosphate buffered saline (PBS), 0.05% Tween-20 buffer (PBST) and water and then dried with a stream of argon for storage or directly incubated with primary antibodies. The slides were incubated with rabbit sera 21390 and 21391 (diluted 1/ 100 or 1/1000 in PBST buffer) for 45 min followed by washing 3 times for 10 min with PBST buffer. For readout the slides were subsequently incubated with a 488 fluorescent labeled secondary anti-rabbit antibody (Invitrogen, diluted 1/5000 in PBST buffer), washed 3 times for 10 min with PBST buffer, washed with water and dried. Fluorescence was detected on a Typhoon Trio^{+} scanner (General Electrics) and the signals were analyzed and quantified by means of the Image quant software.

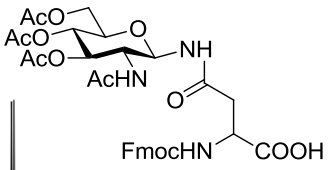
***O*-Mannosyl glycopeptide microarrays for analysis of antibody binding specificity of Rabbit sera 23803 and 23804** Figure 22

The synthesized peptides **171**- **173**, **182**, **183** and **190** - **195** (0.25 mM of peptide in 150 mM phosphate buffer pH 8.5, 0.005% Tween-20, 85 picoL spots), **180** and **181** at different concentration (0.1 mM, 0.05 mM, 0.01 mM, 2 µM and 0.5 µM of peptide in 150 mM phosphate buffer pH 8.5, 0.005% Tween-20, 85 picoL spots), were spotted and immobilized on amino reactive and biocompatible hydrogel slides with a surface functionalized with carboxylic acids activated as *N*-hydroxysuccinimide esters (Slide H, Schott-Nexterion, Jena, Germany). Using a piezo-driven non-contact spotter (iTWO200, M2-automation, Berlin, Germany) the spotting performed in a 8 array (well) format with 6 replicates for each glycopeptides in every block. The printed peptides were allowed to react in a humidity chamber (75% humidity) for 24 h and unreacted NHS-esters were trapped with 1 mM ethanolamine at pH 9 for 1 h. The slides were washed with phosphate buffered saline (PBS), 0.05% Tween-20 buffer (PBST) and water and then dried with a stream of argon for storage or directly incubated with primary antibodies. The slides were incubated with rabbit sera **23803, 23804** (diluted 1/25 in PBST buffer) and ConA (0.1 mg/mL in PBST buffer) for 45 min followed by washing 3 times for 10 min with PBST buffer. For readout the slides were subsequently incubated with a 488 fluorescent labeled secondary anti-rabbit antibody (Invitrogen, diluted 1/5000 in PBST buffer) or Cy5 streptavidin (Invitrogen, diluted 1/1000 in

PBST buffer), washed 3 times for 10 min with PBST buffer, washed with water and dried. Fluorescence was detected on a Typhoon Trio^{+} scanner (General Electrics) and the signals were analyzed and quantified by means of the Image quant software.

8 Appendix 1: NMR Spectra

Compound **6**



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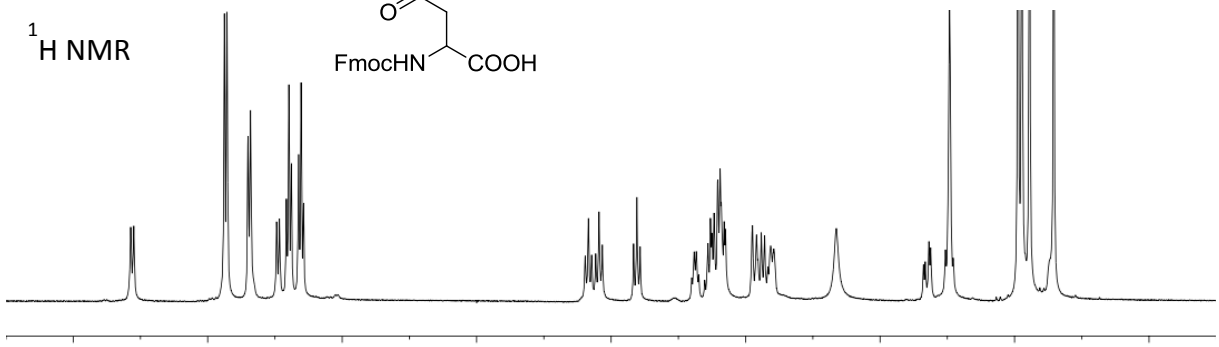
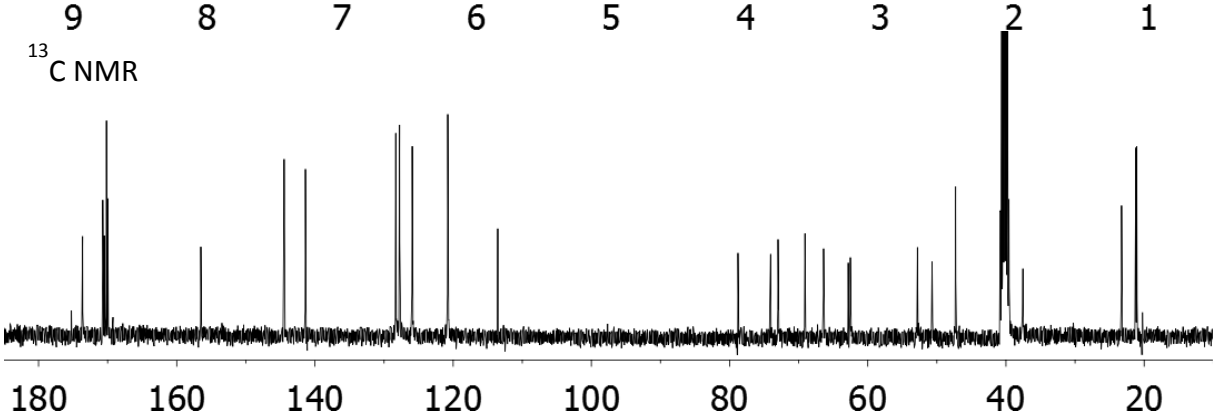


^{13}C NMR



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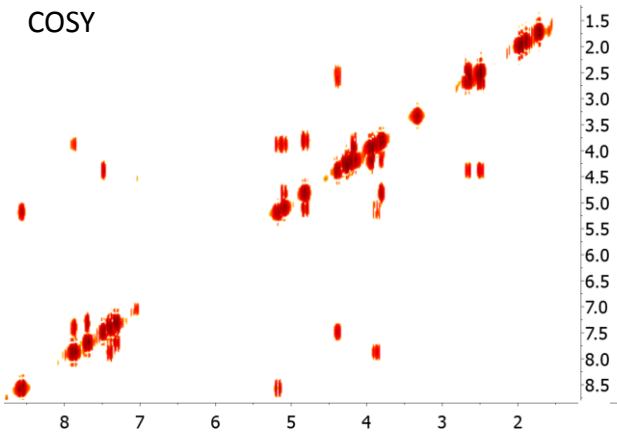

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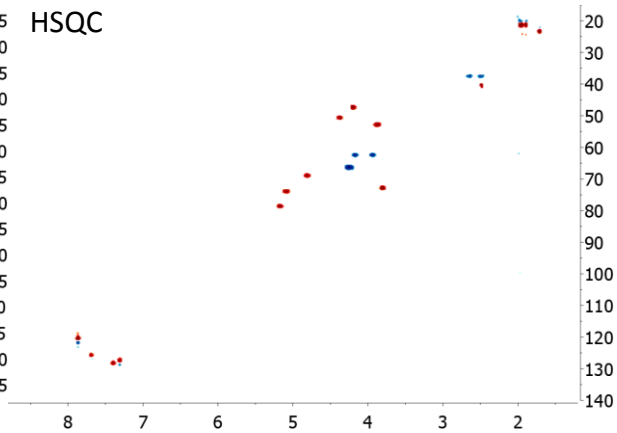

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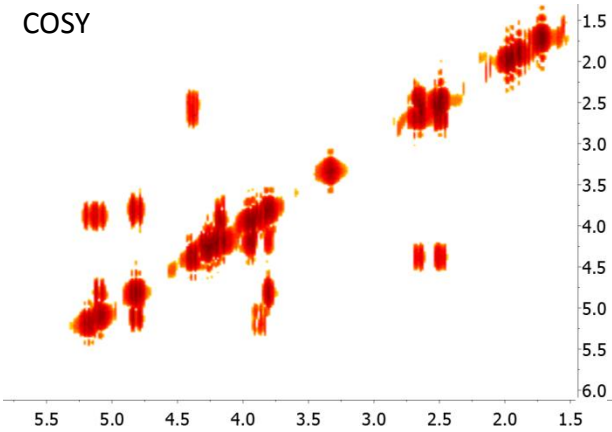

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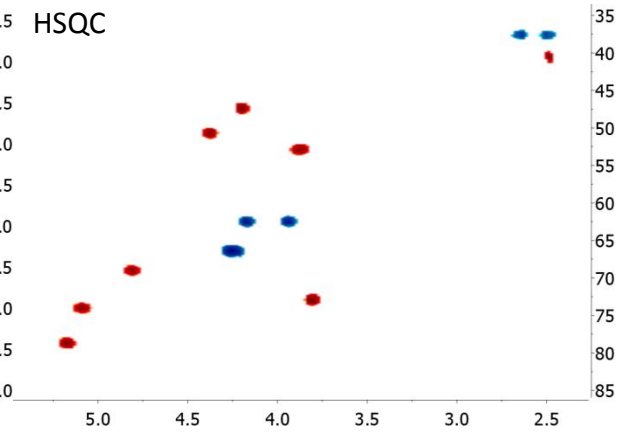
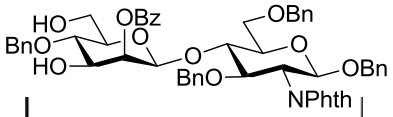
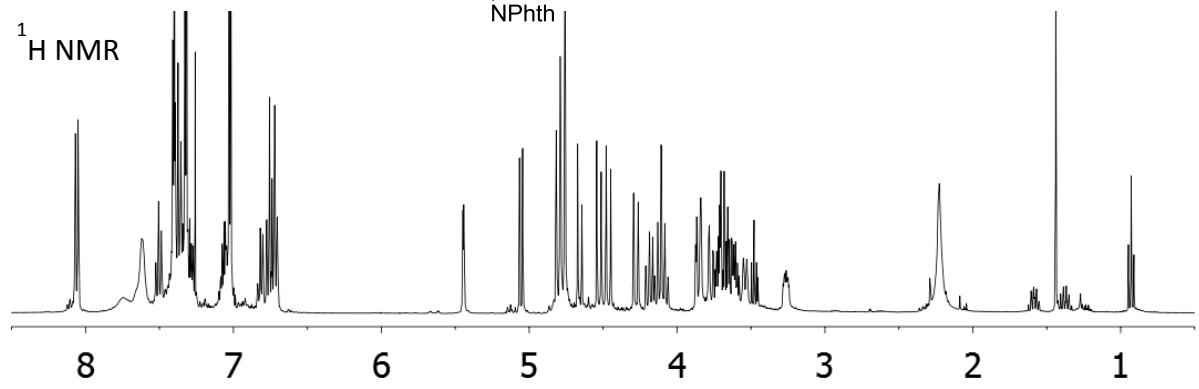


Compound **26**



^{1}H NMR



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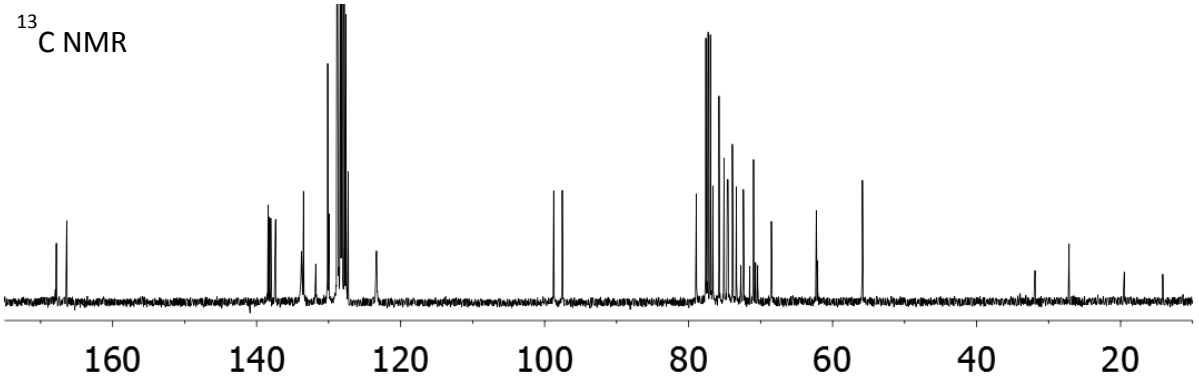

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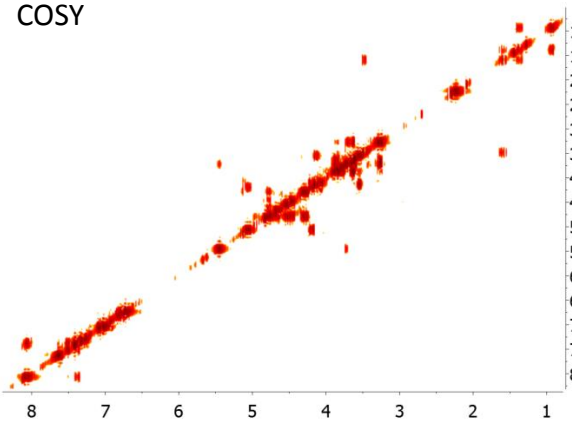

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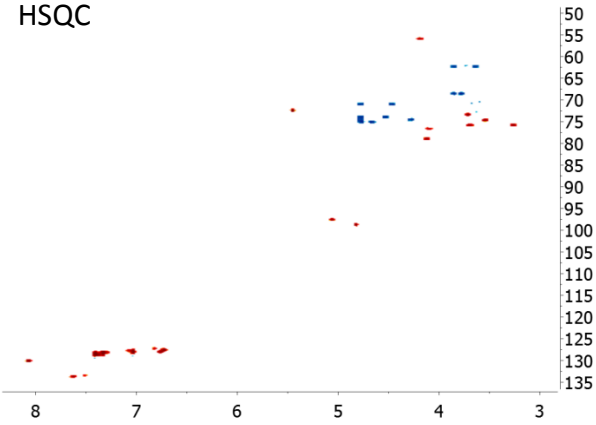
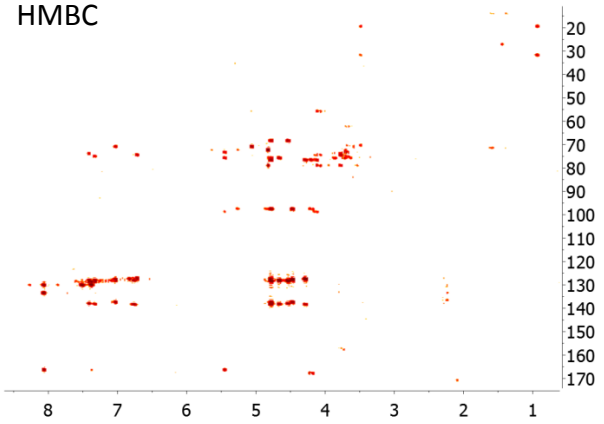
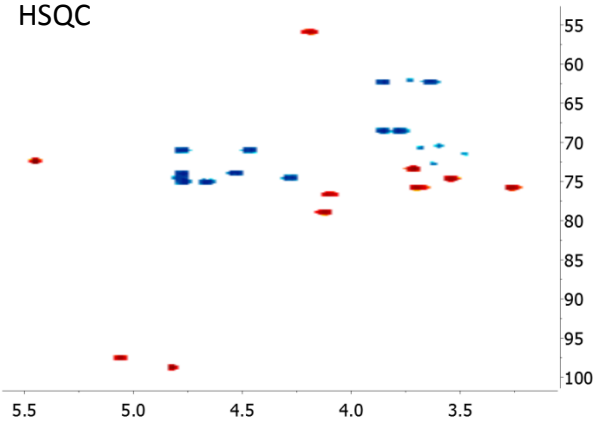


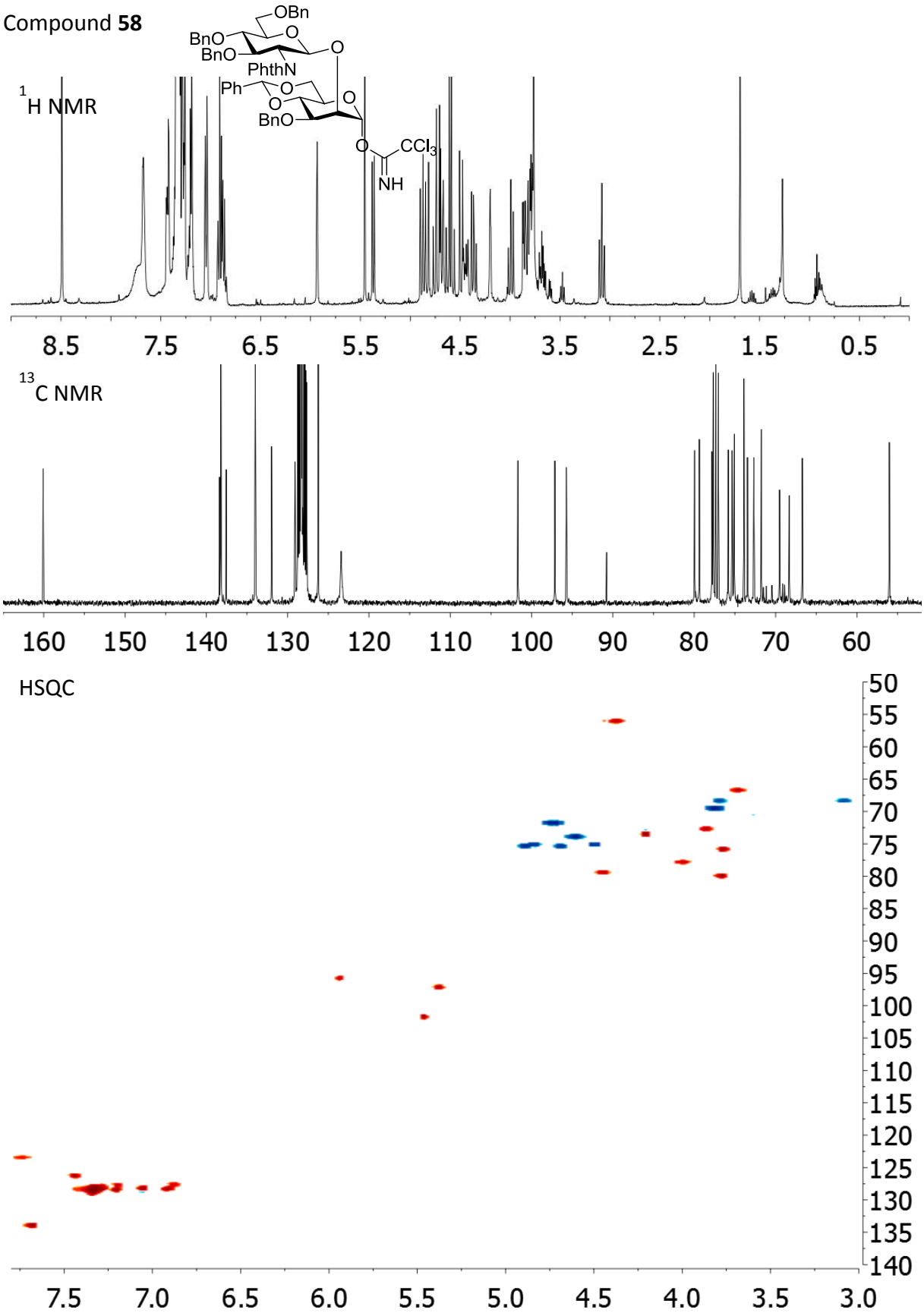
HMBC



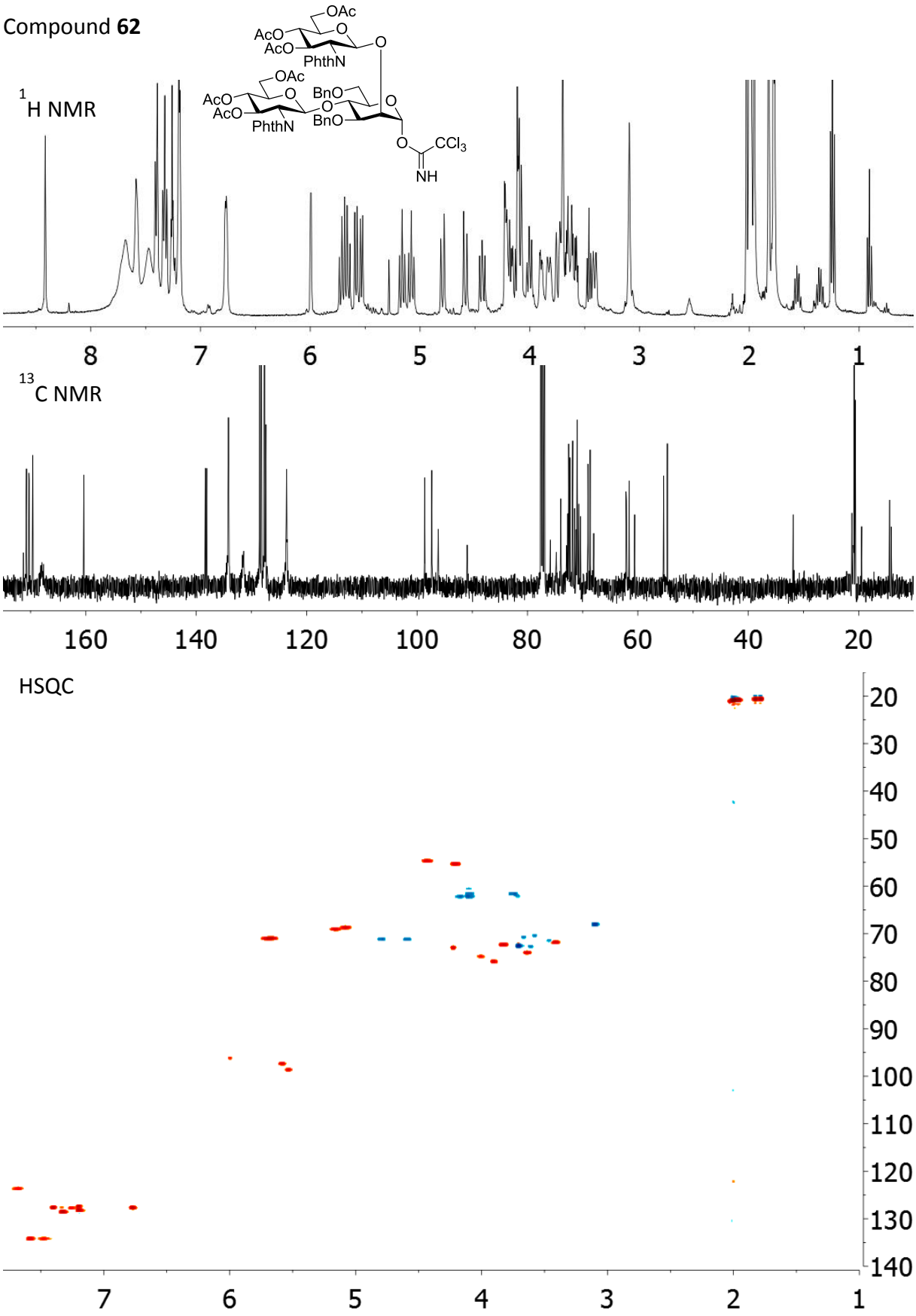
HSQC



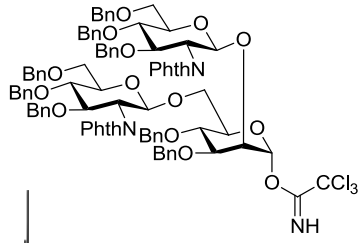
Compound **58**


OBn
BnO
BnO
PhthN
Ph
BnO
O
CCl3
NH
1H NMR
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
13C NMR
160
150
140
130
120
110
100
90
80
70
60
HSQC
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0

Compound **62**

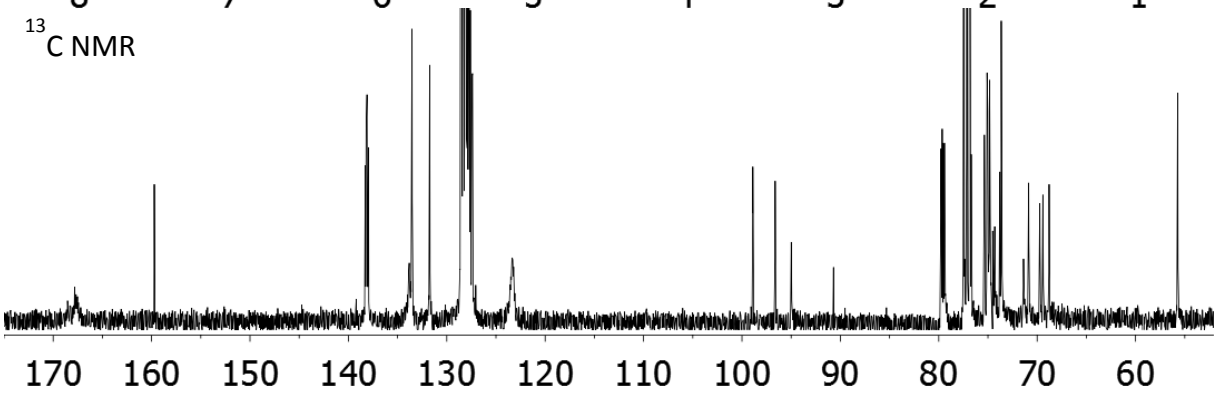


Compound **70**

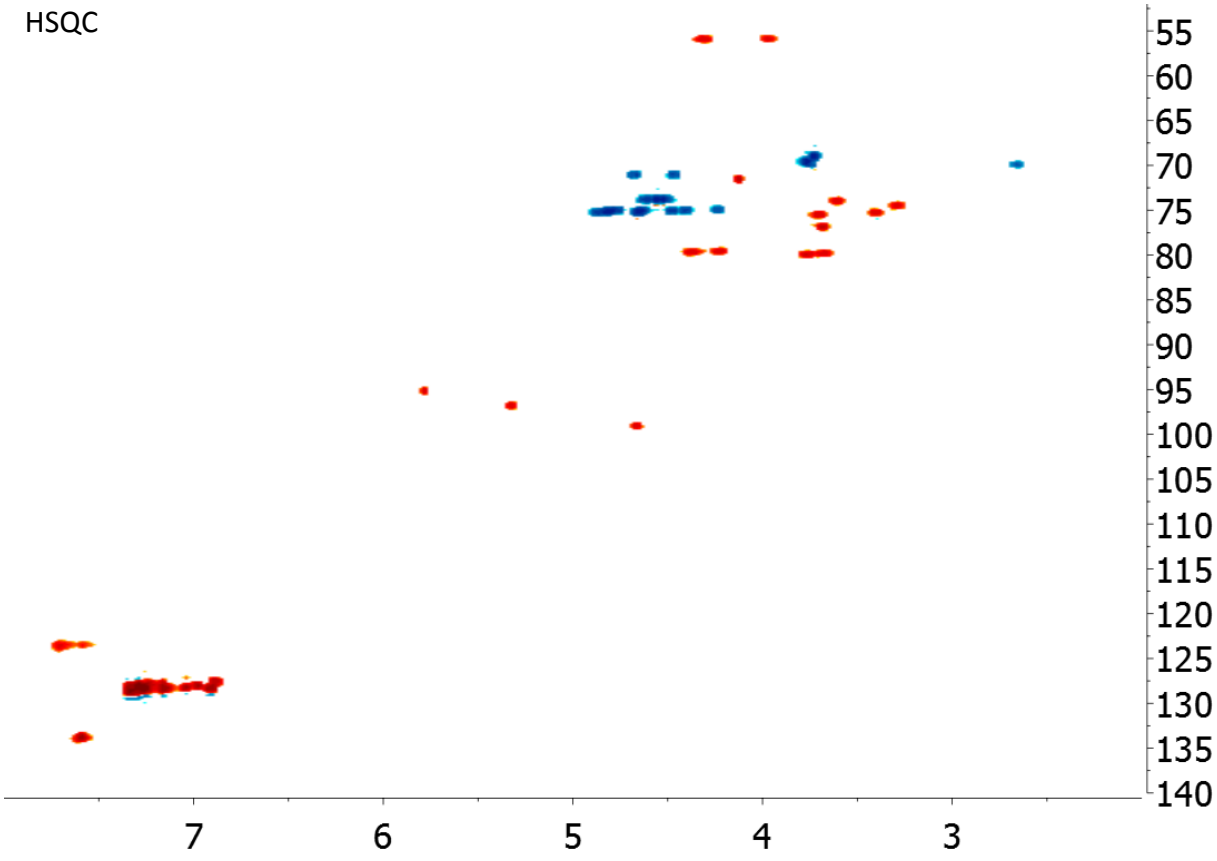


^{1}H NMR

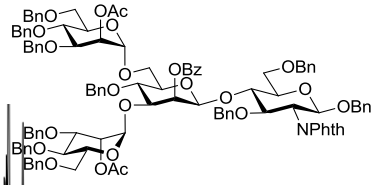
^{13}C NMR



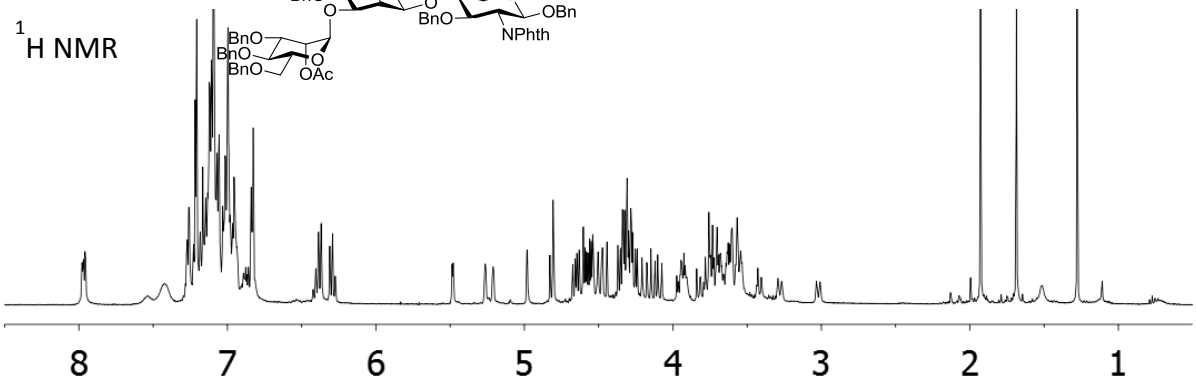
HSQC



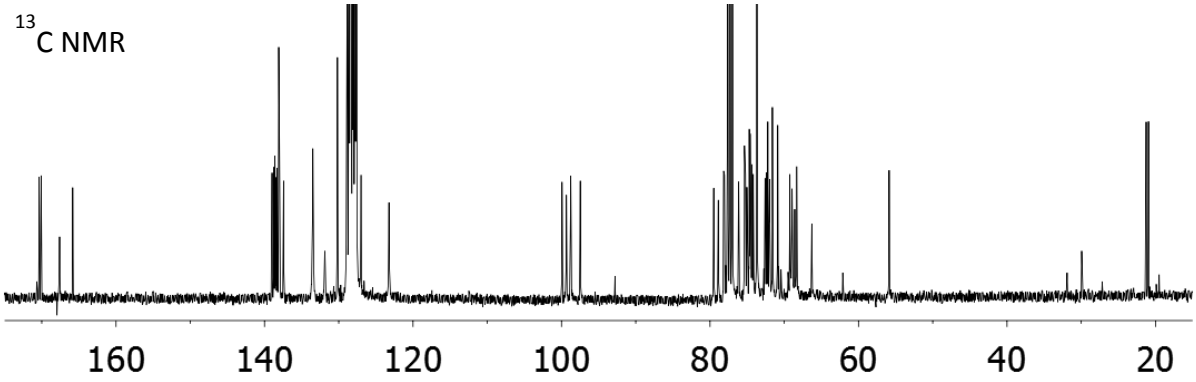
Compound **87**



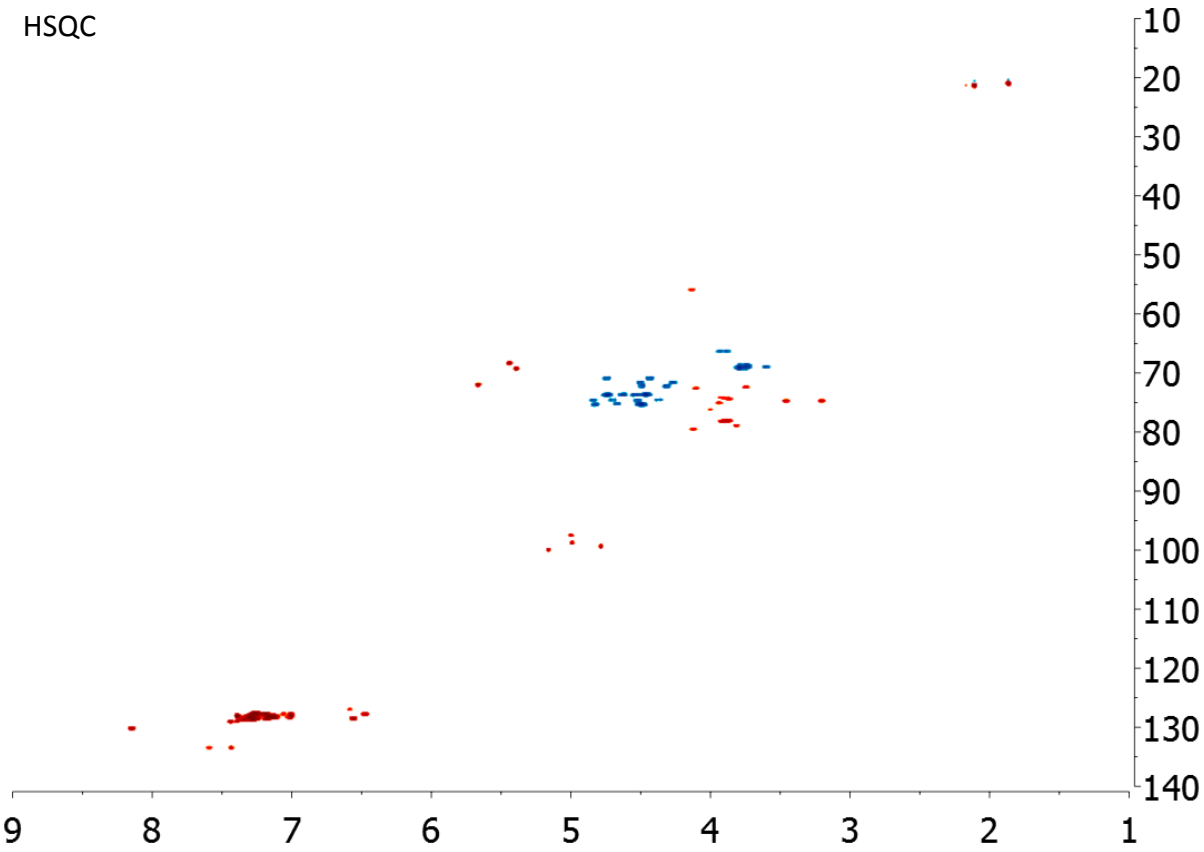
^{1}H NMR

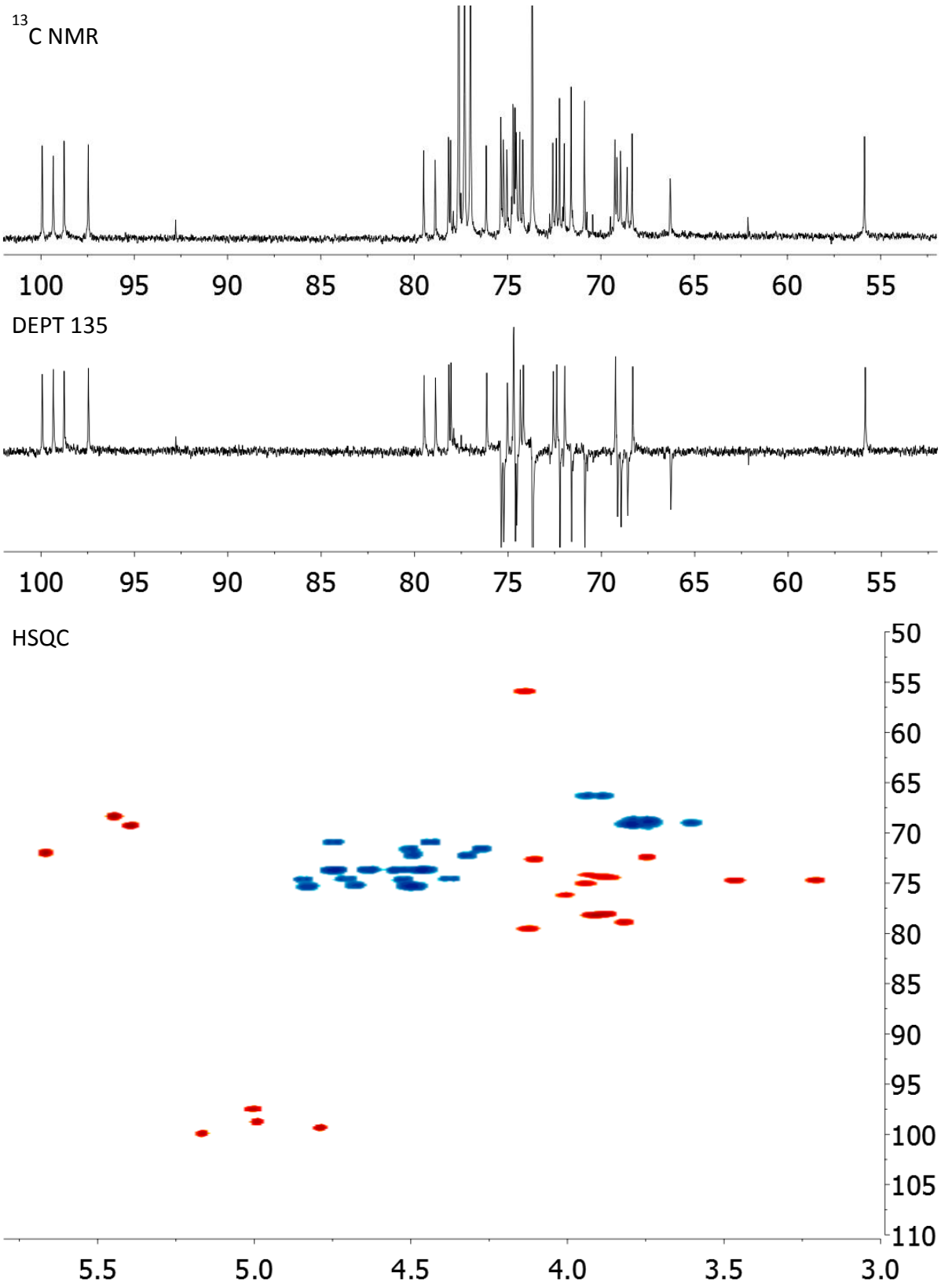


^{13}C NMR

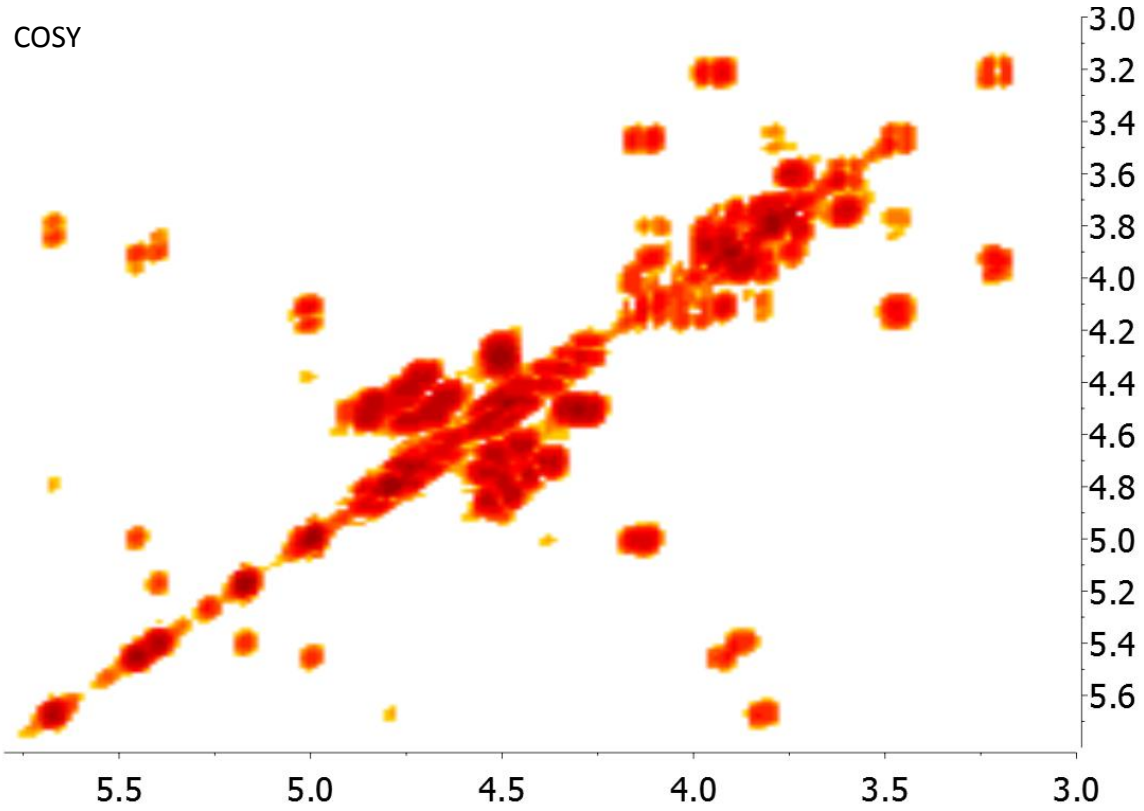


HSQC

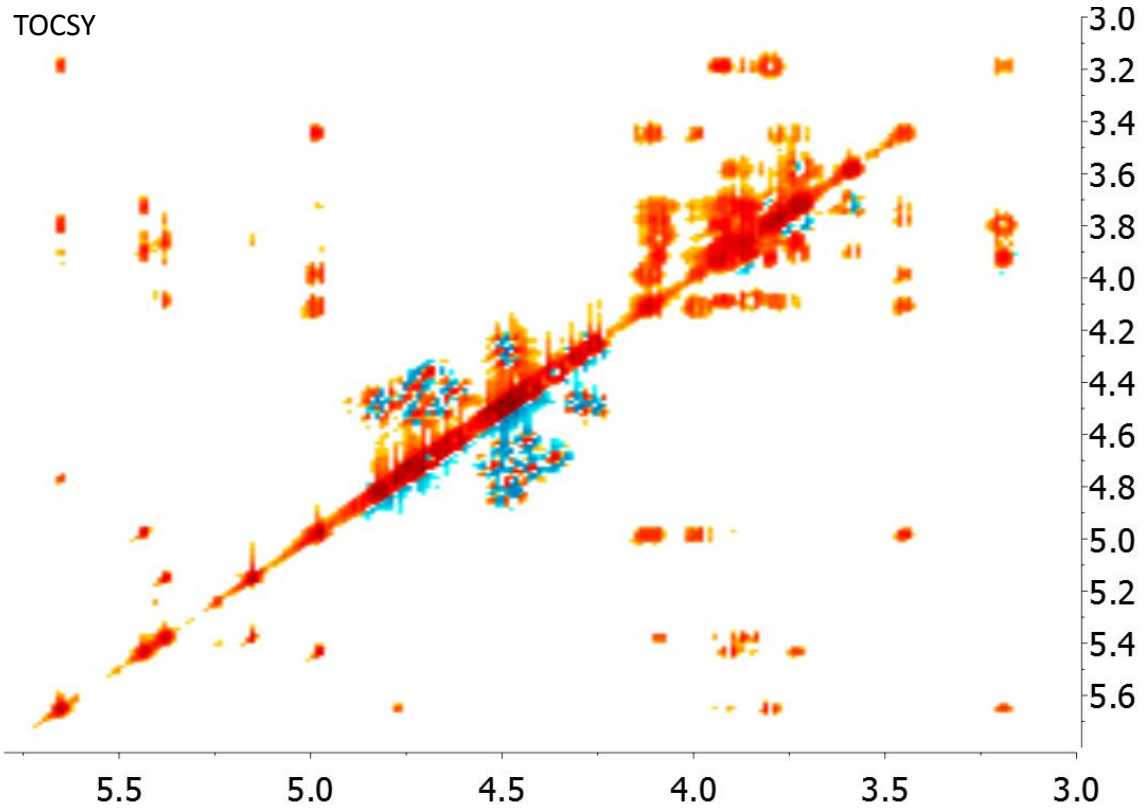



13C NMR
100 95 90 85 80 75 70 65 60 55
DEPT 135
100 95 90 85 80 75 70 65 60 55
HSQC
50 55 60 65 70 75 80 85 90 95 100 105 110
5.5 5.0 4.5 4.0 3.5 3.0

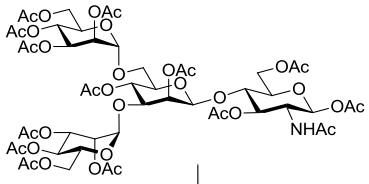
COSY


3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.5
5.0
4.5
4.0
3.5
3.0

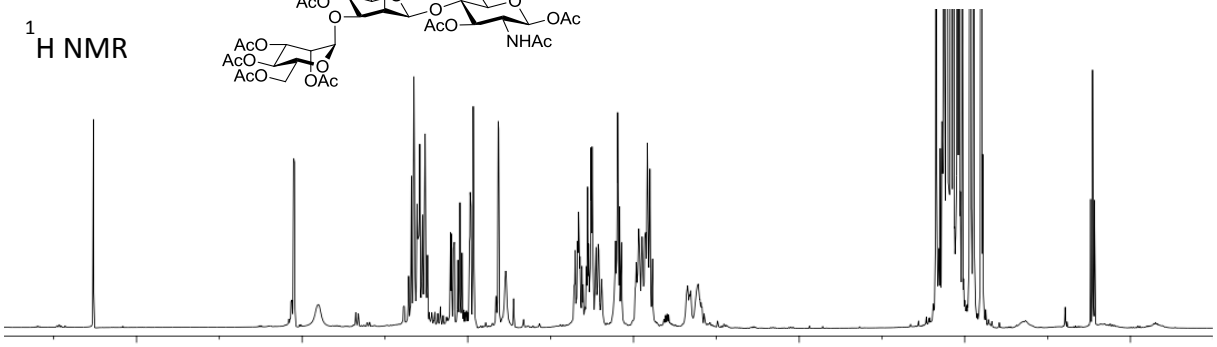
TOCSY


3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.5
5.0
4.5
4.0
3.5
3.0

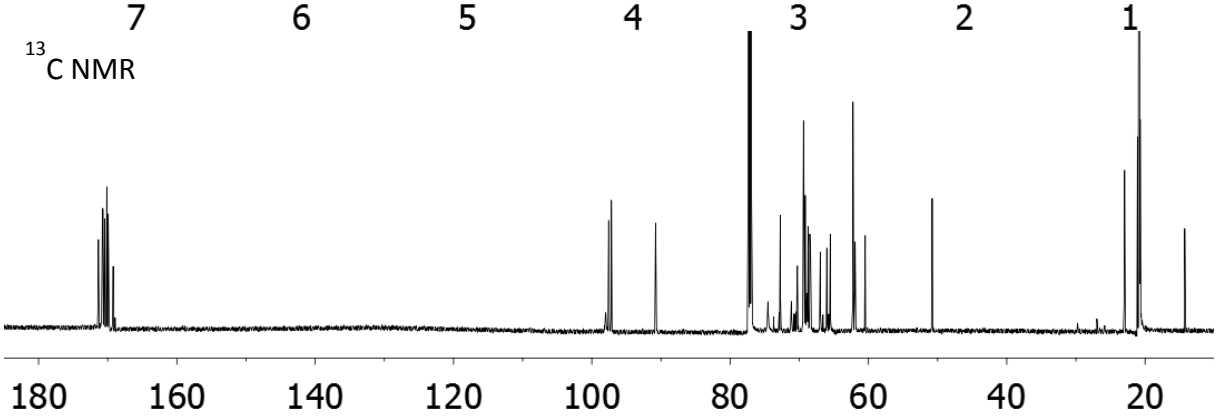
Compound **89**



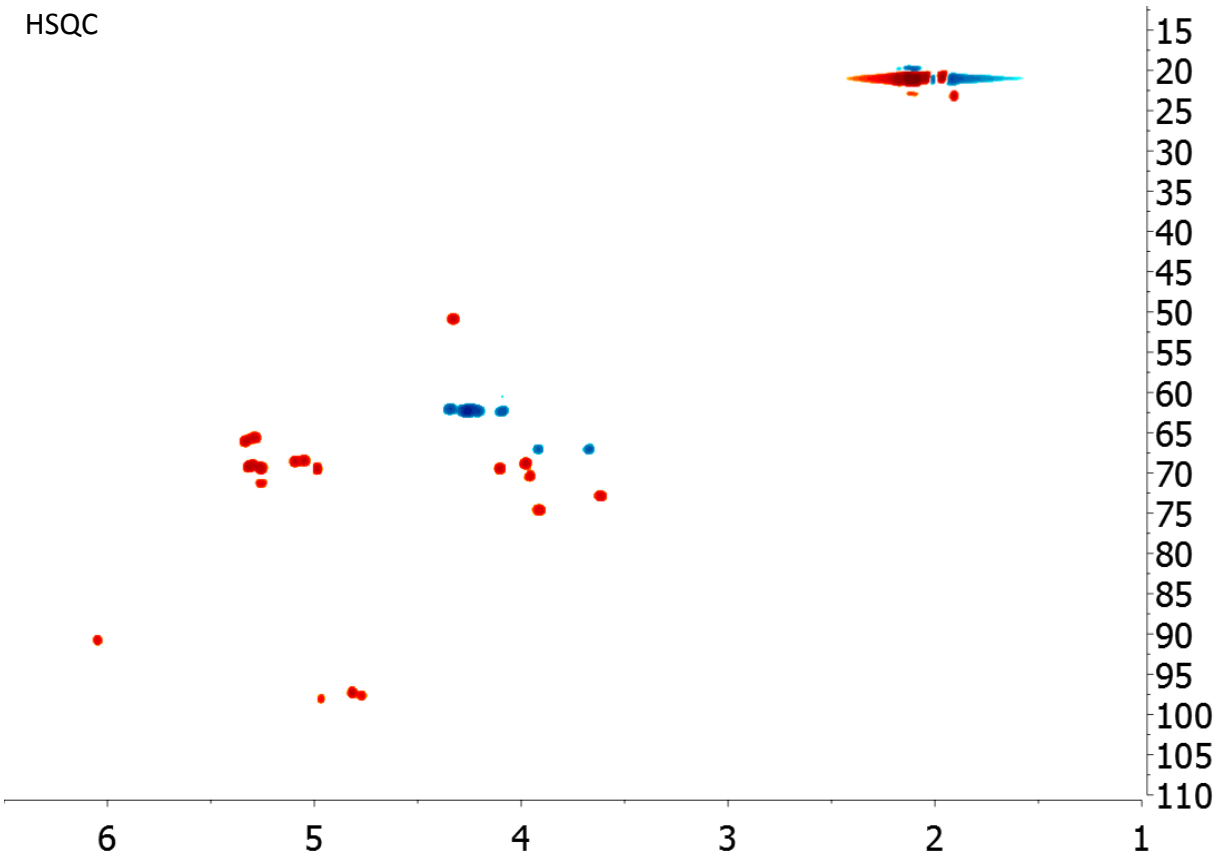
^{1}H NMR

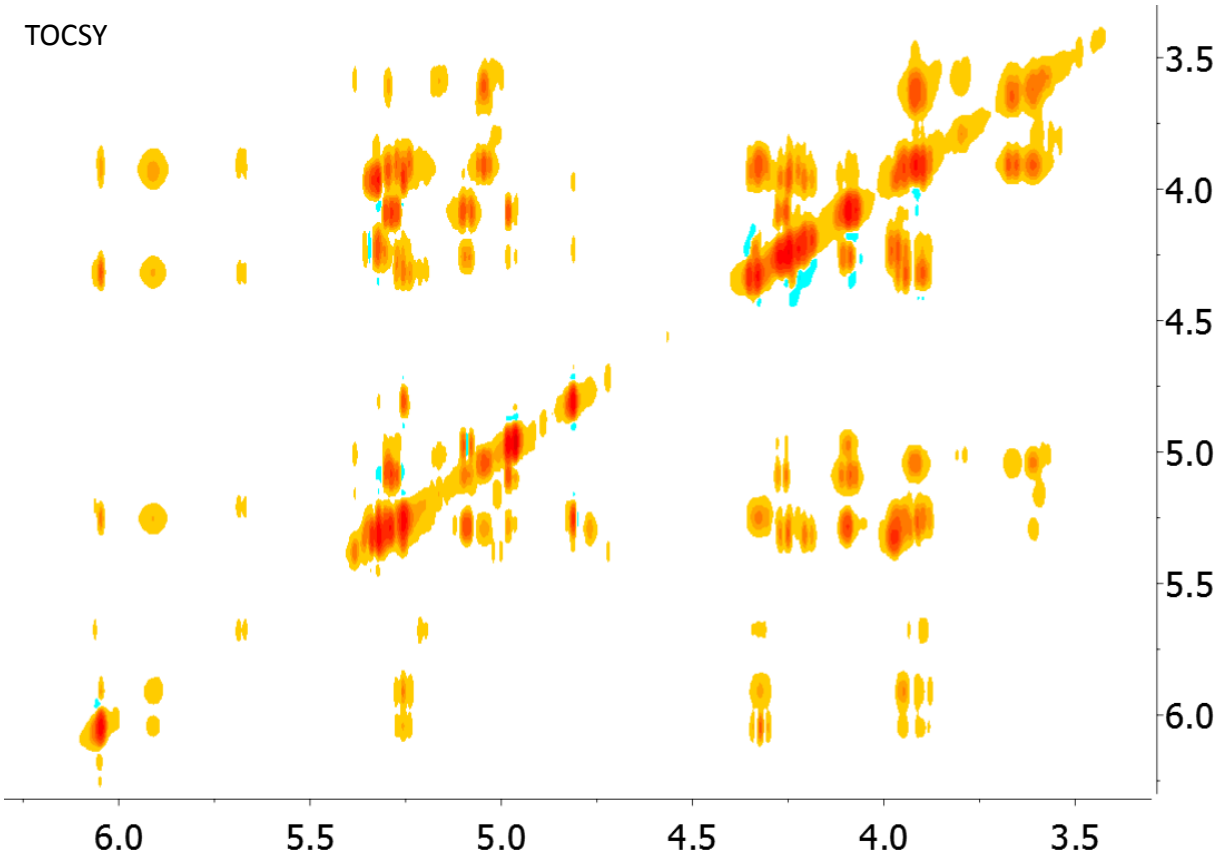


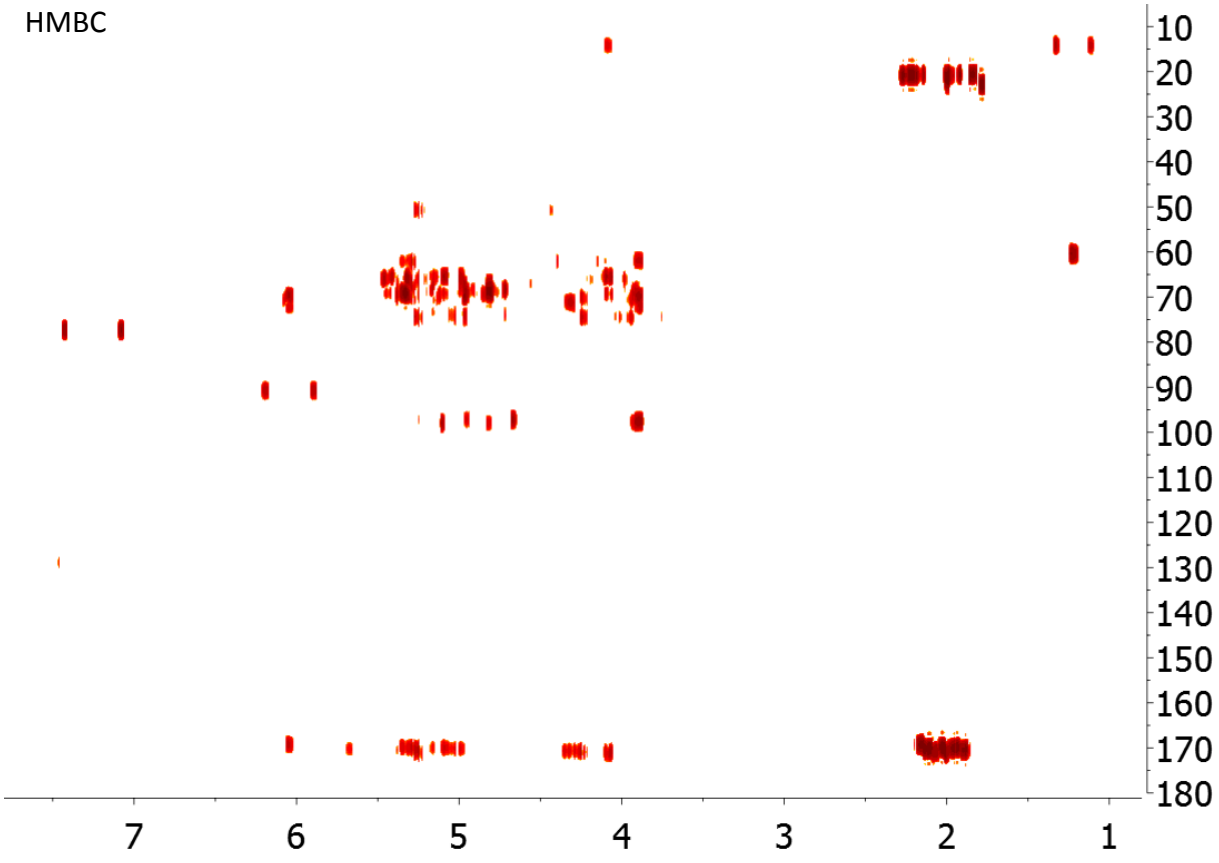
^{13}C NMR



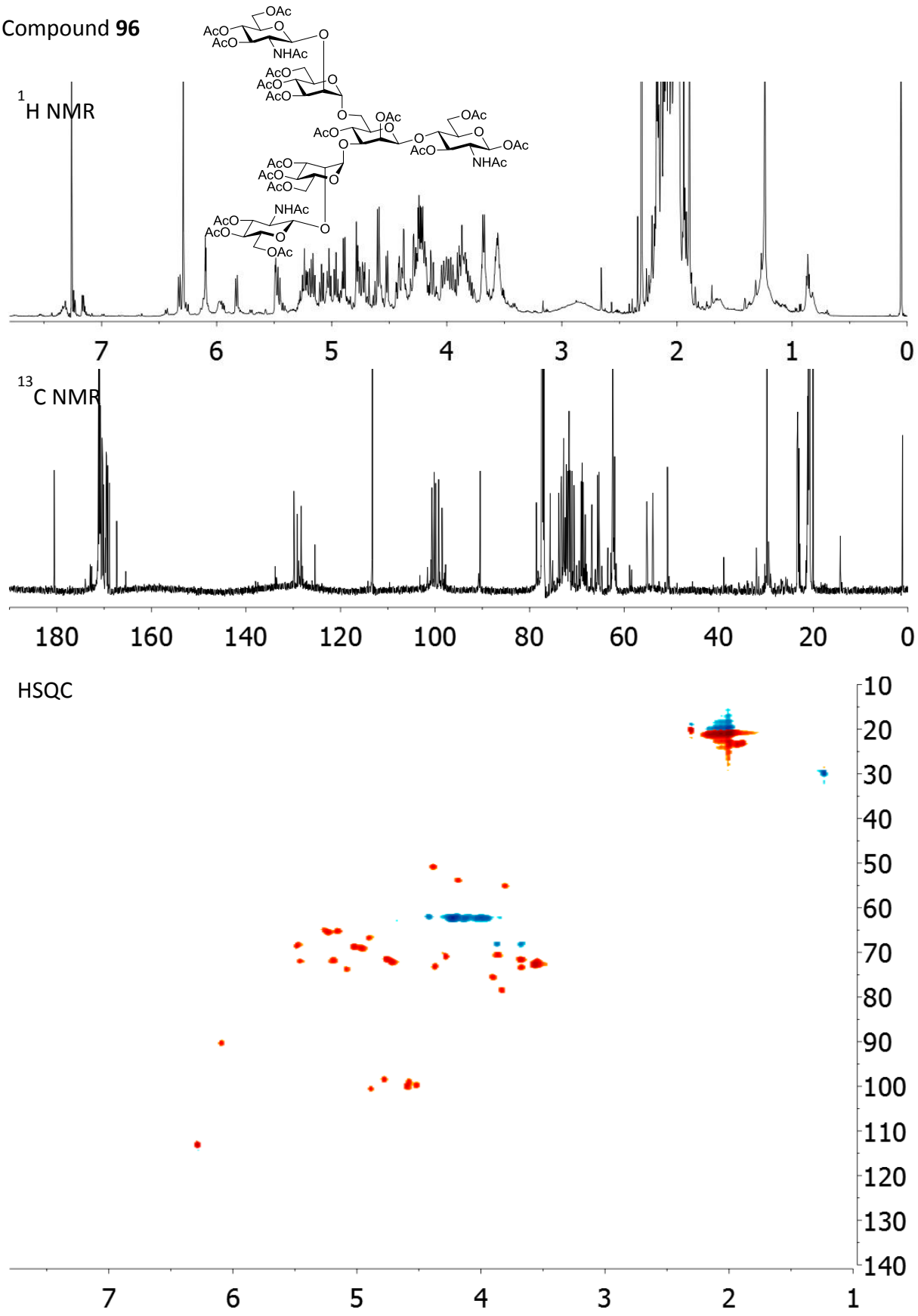
HSQC

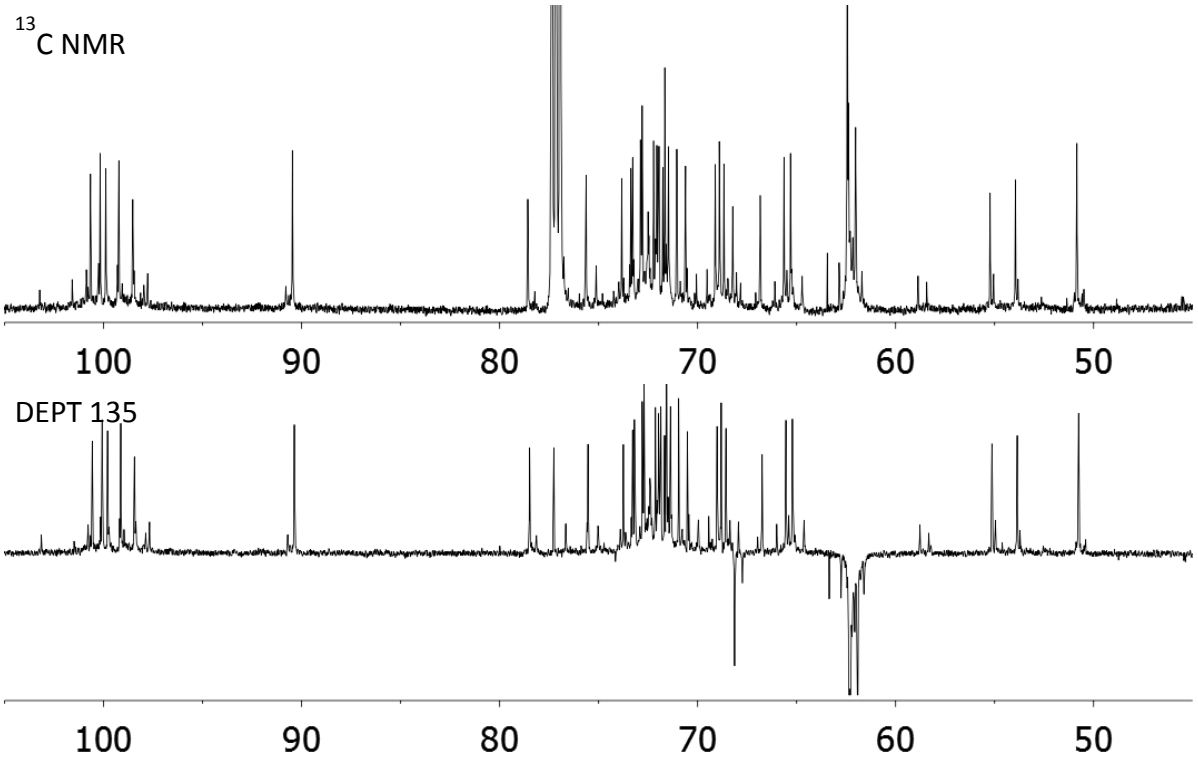


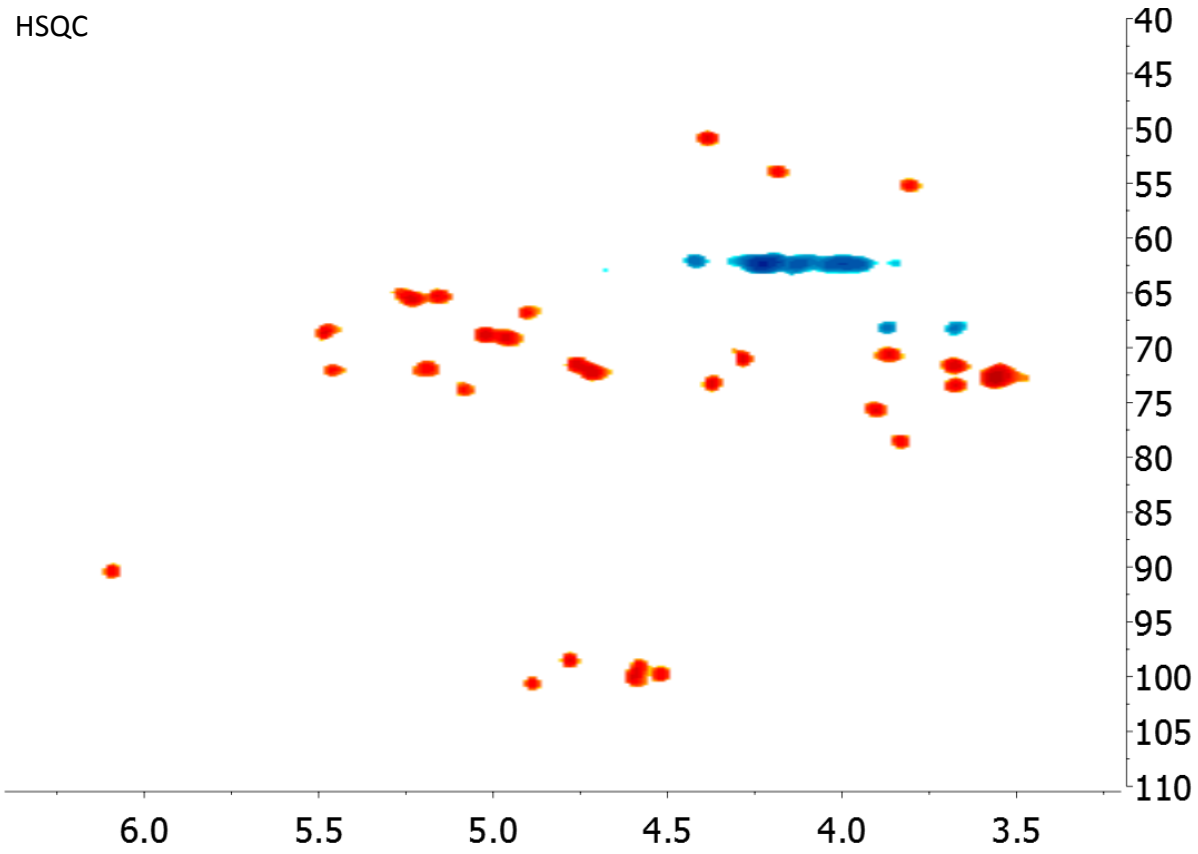

TOCSY
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5


HMBC
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
7
6
5
4
3
2
1

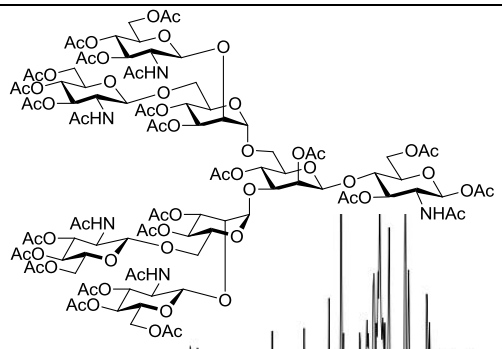
Compound **96**



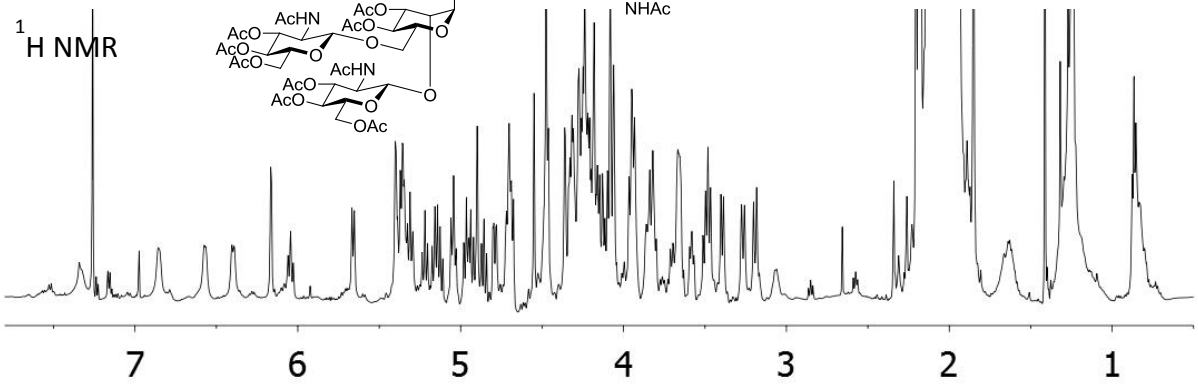

13C NMR
100
90
80
70
60
50
DEPT 135
100
90
80
70
60
50


HSQC
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
6.0
5.5
5.0
4.5
4.0
3.5

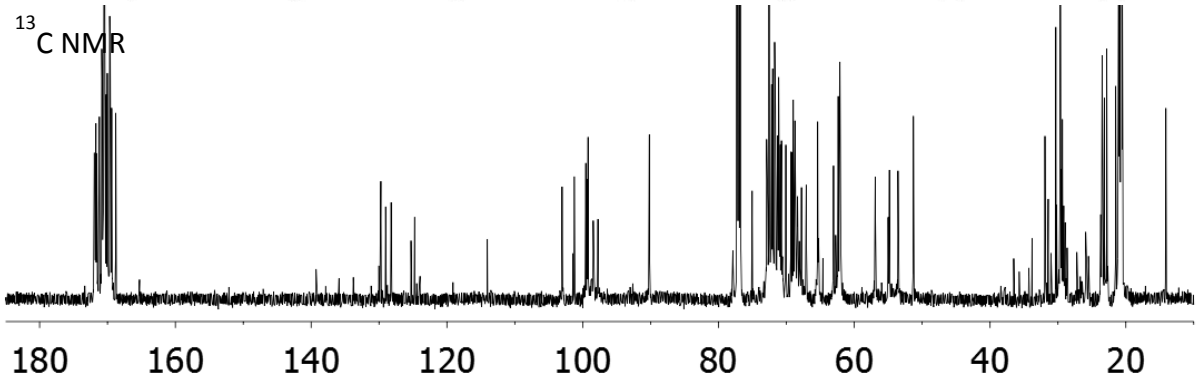
Compound **100**



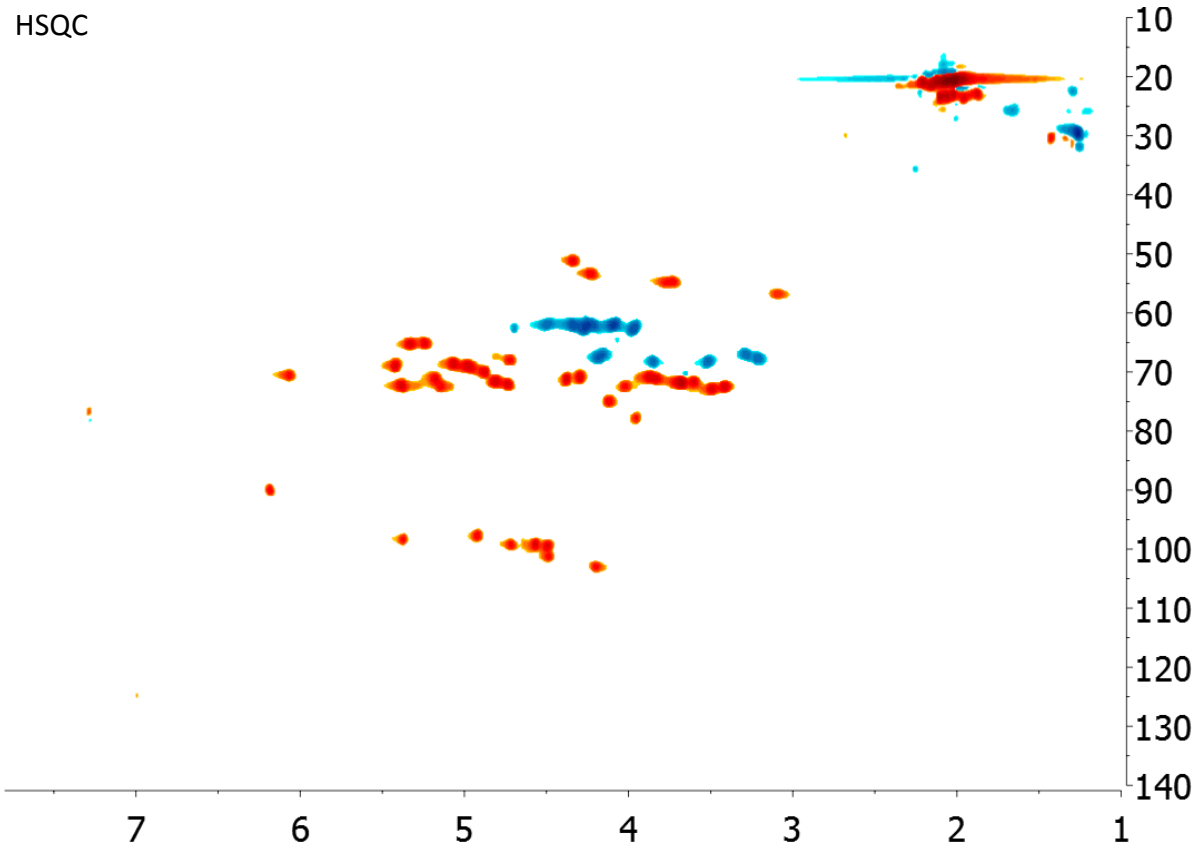
^{1}H NMR

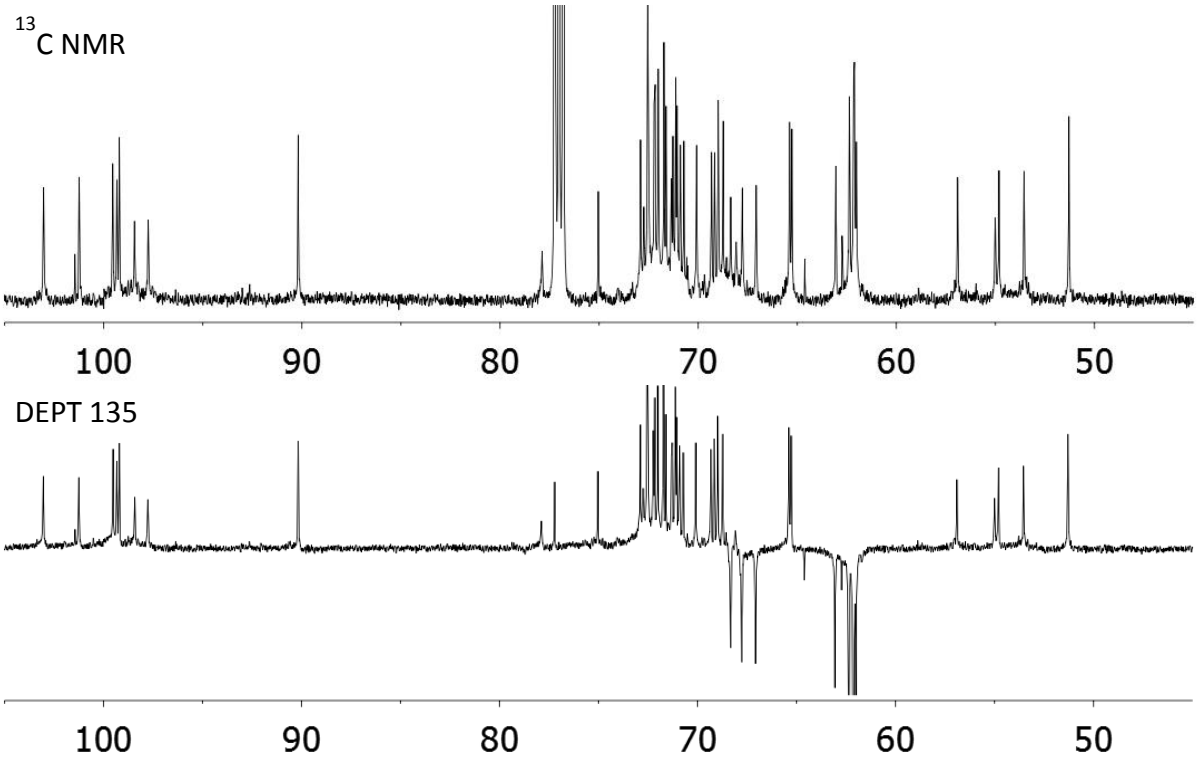


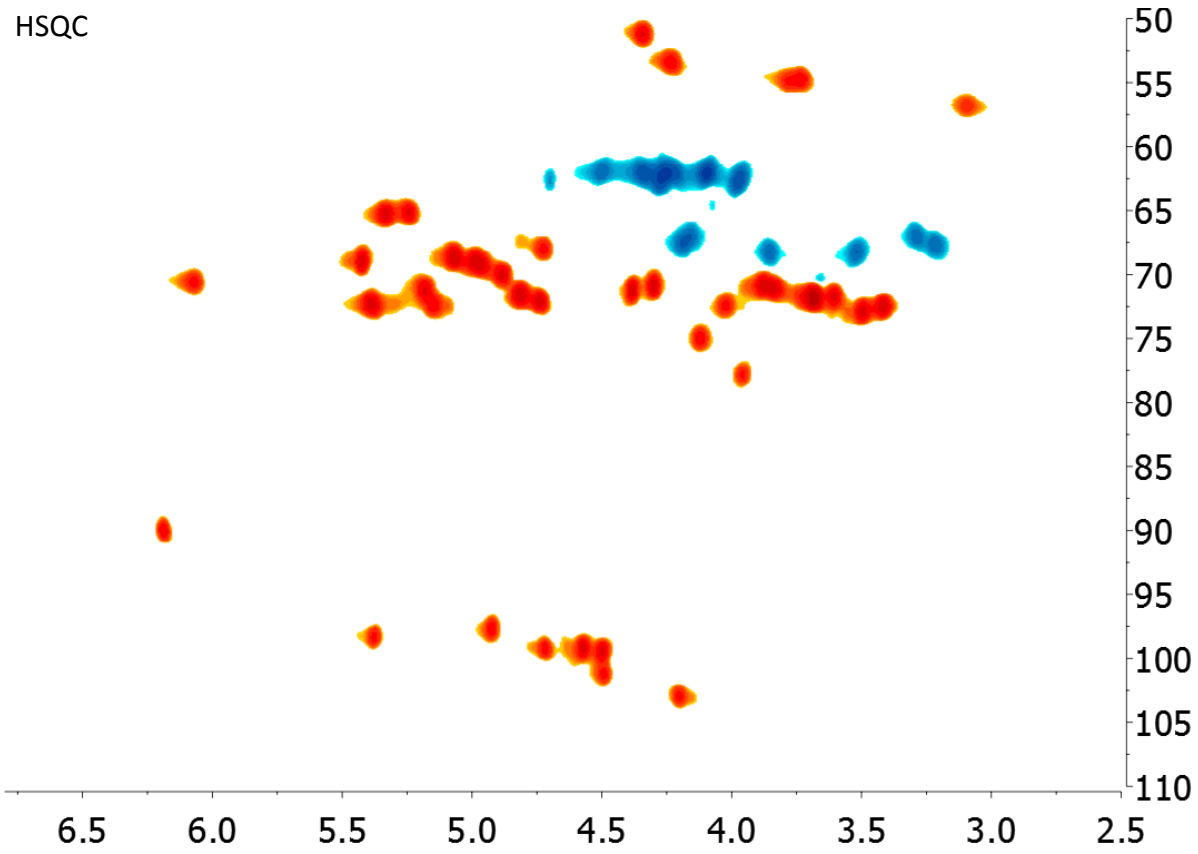
^{13}C NMR



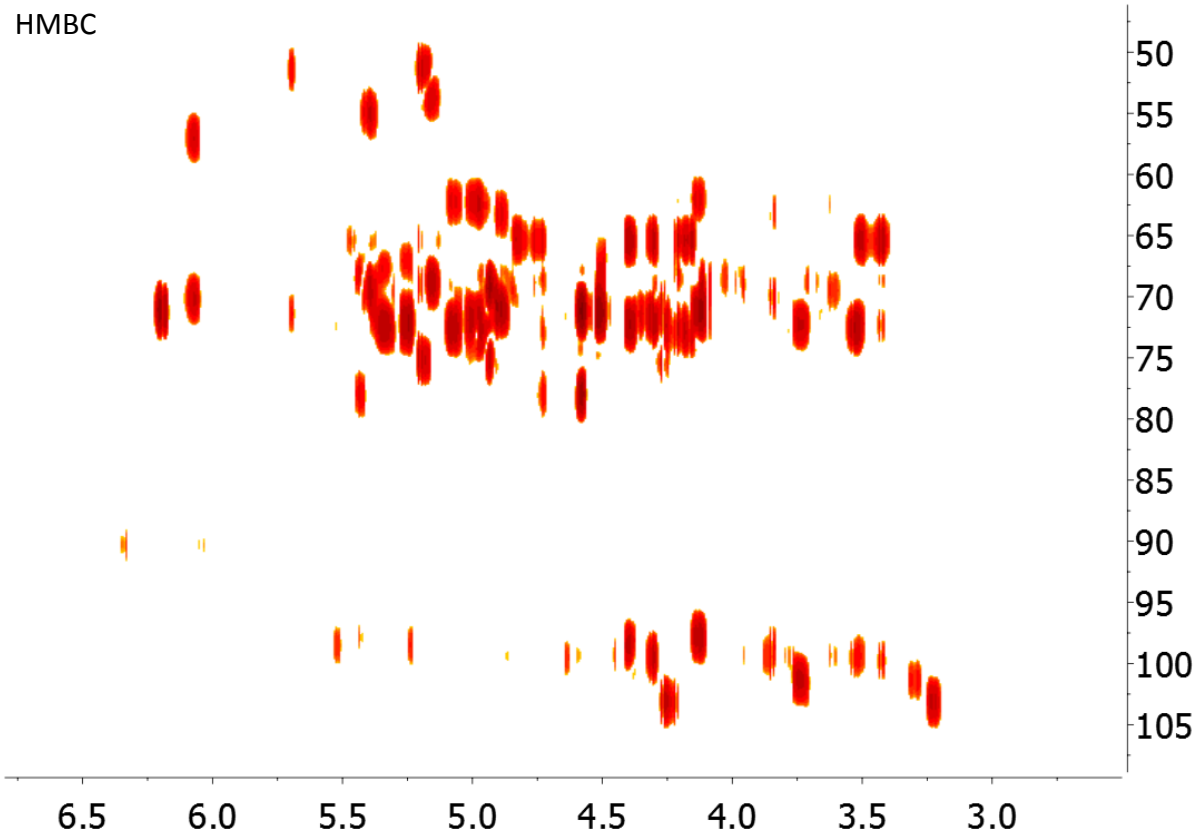
HSQC



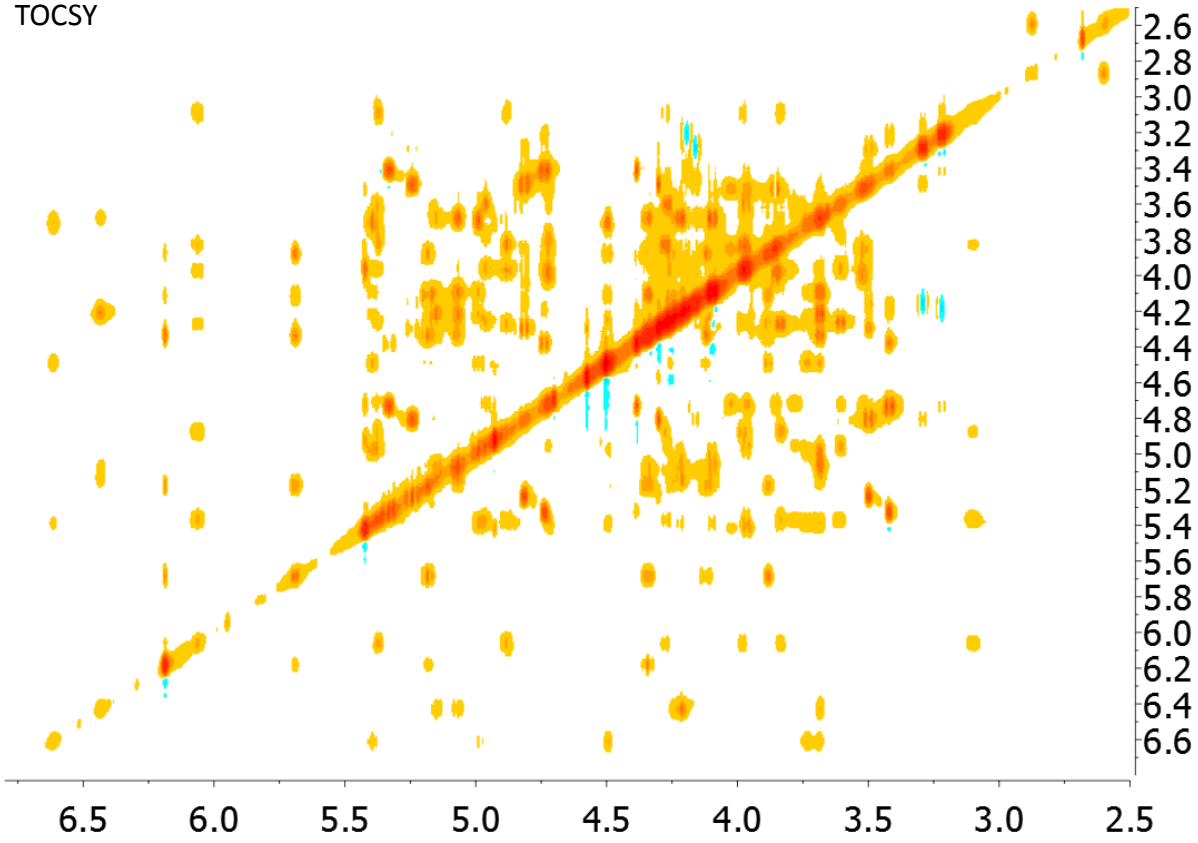

13C NMR
100
90
80
70
60
50
DEPT 135
100
90
80
70
60
50


HSQC
50
55
60
65
70
75
80
85
90
95
100
105
110
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5

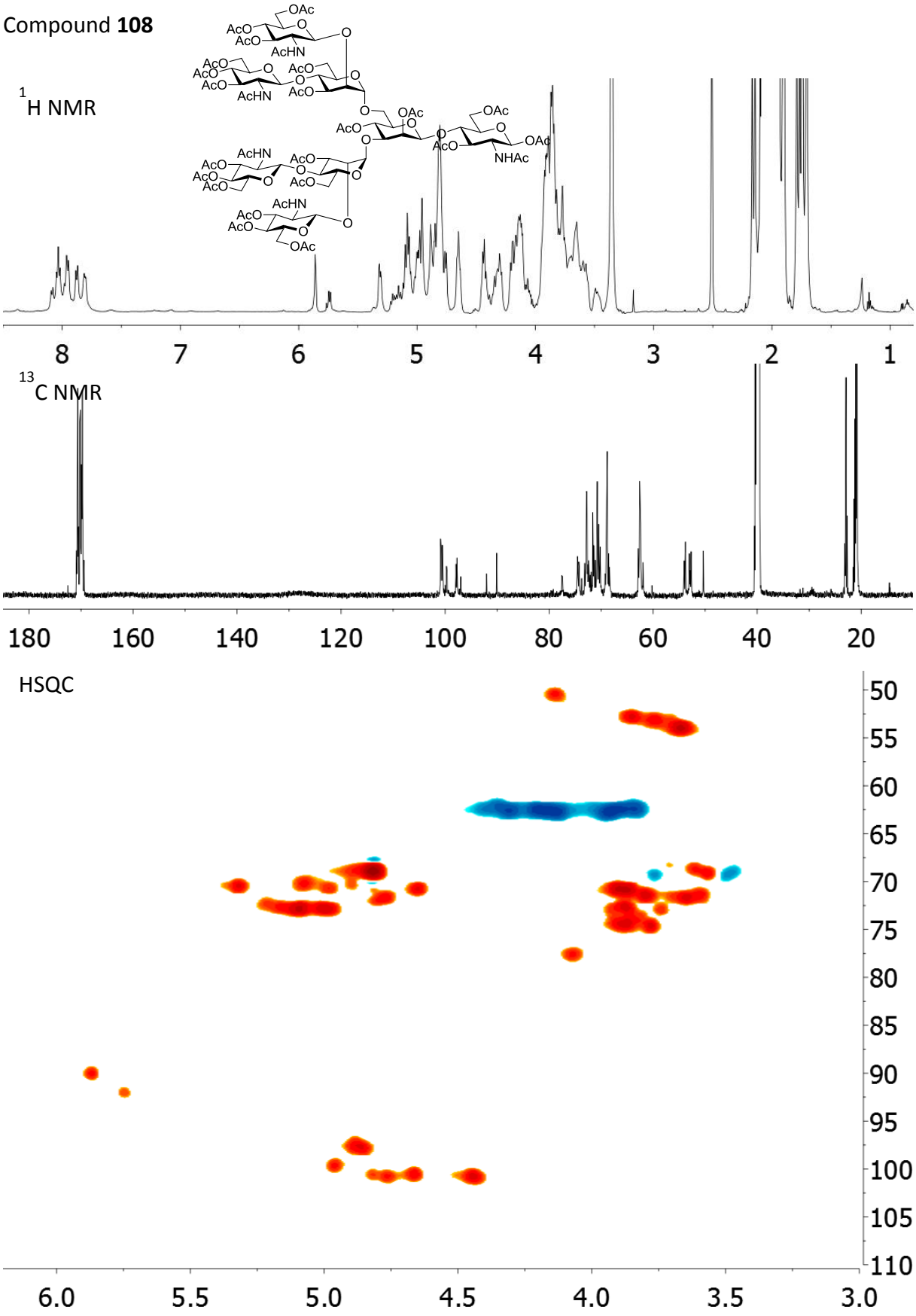
HMBC

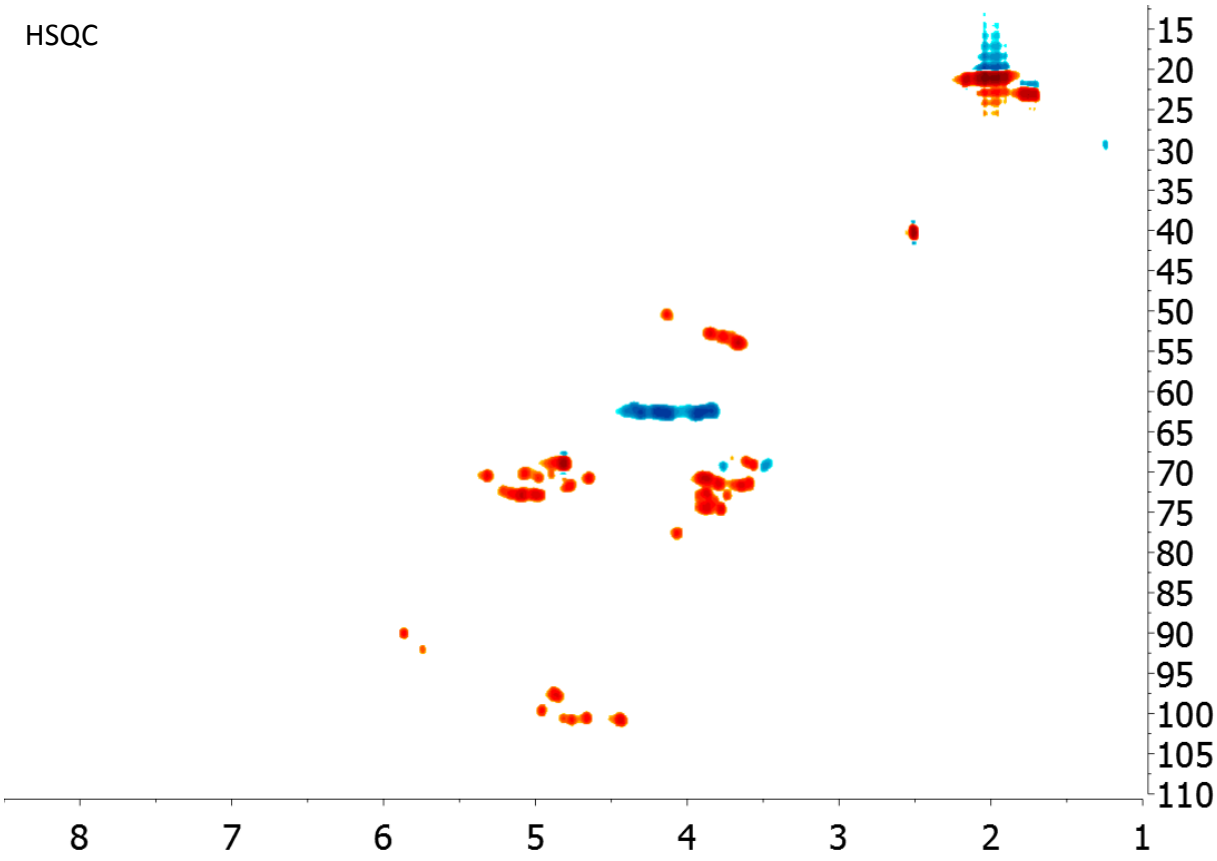

50
55
60
65
70
75
80
85
90
95
100
105
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0

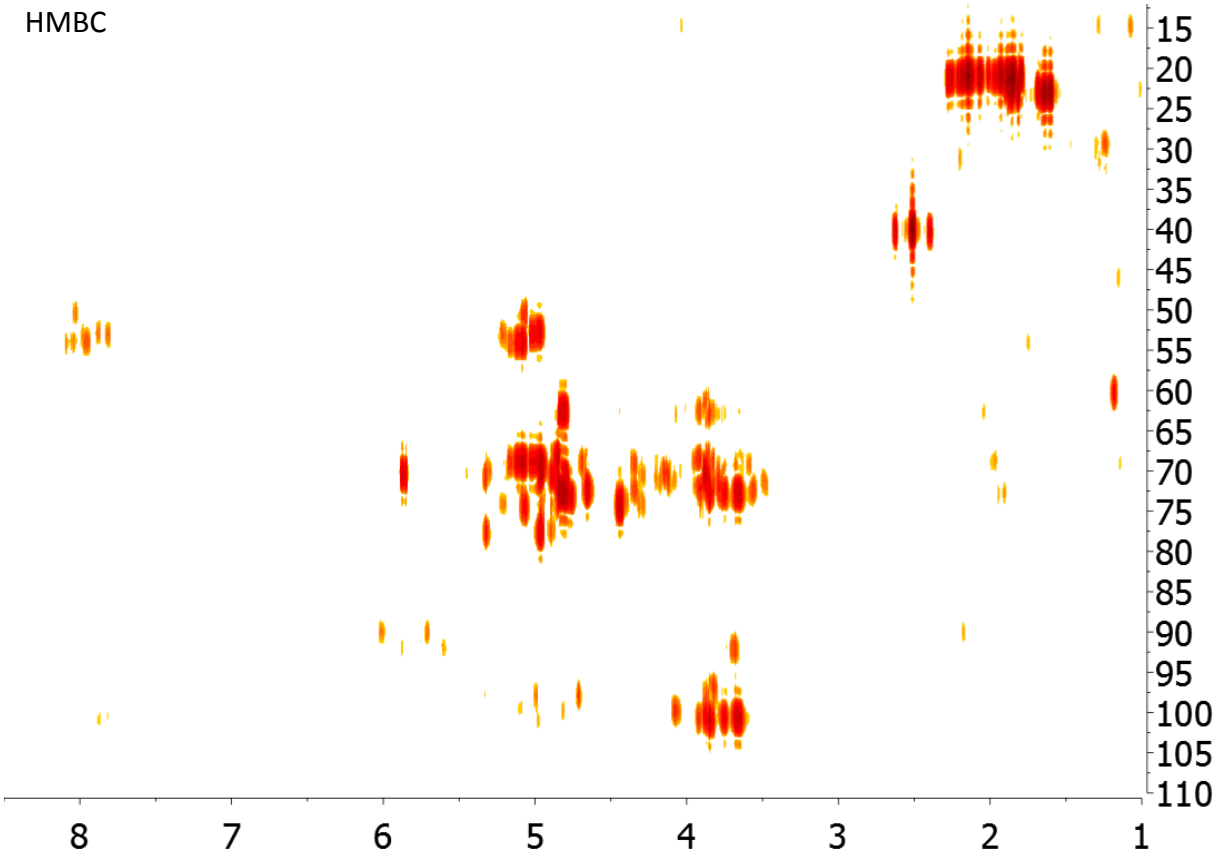
TOCSY


2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5

Compound **108**




HSQC
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
8
7
6
5
4
3
2
1


HMBC
15
20
25
30
35
40
45
50
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60
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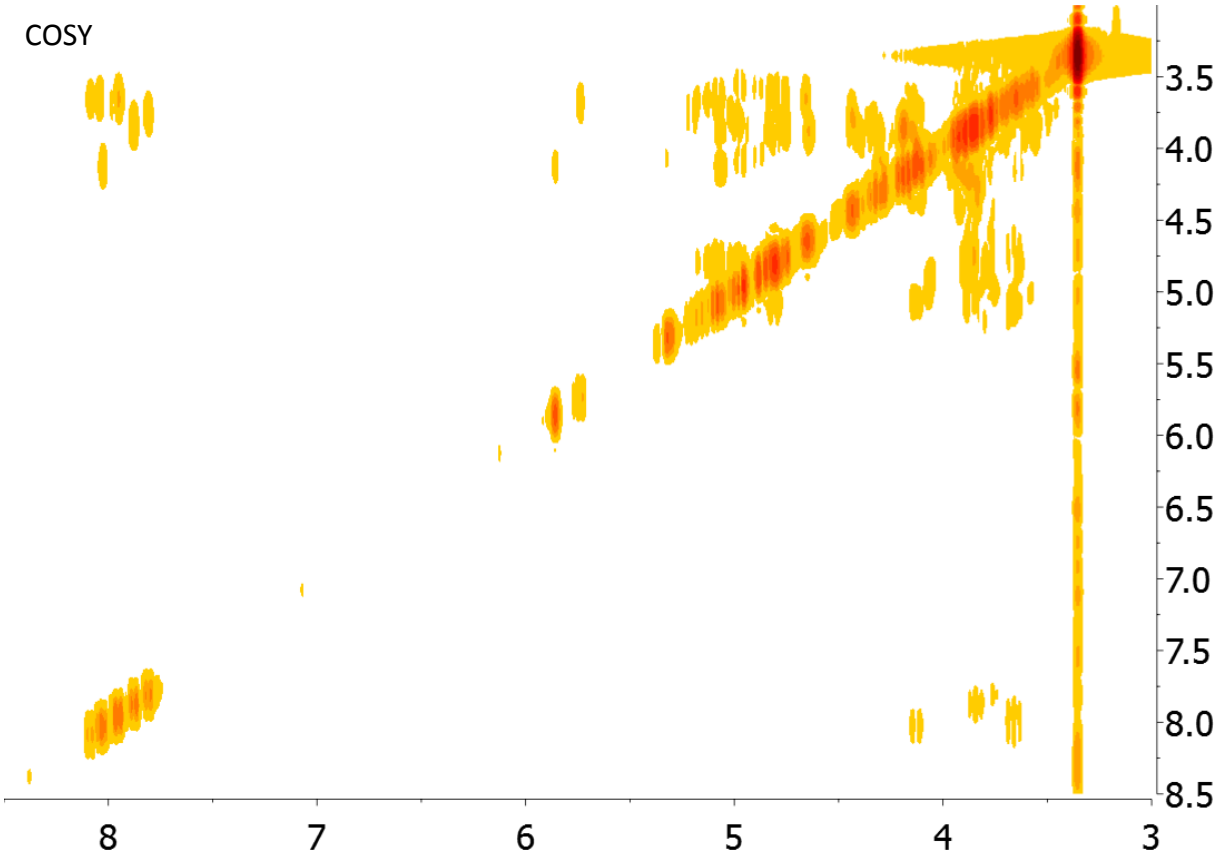
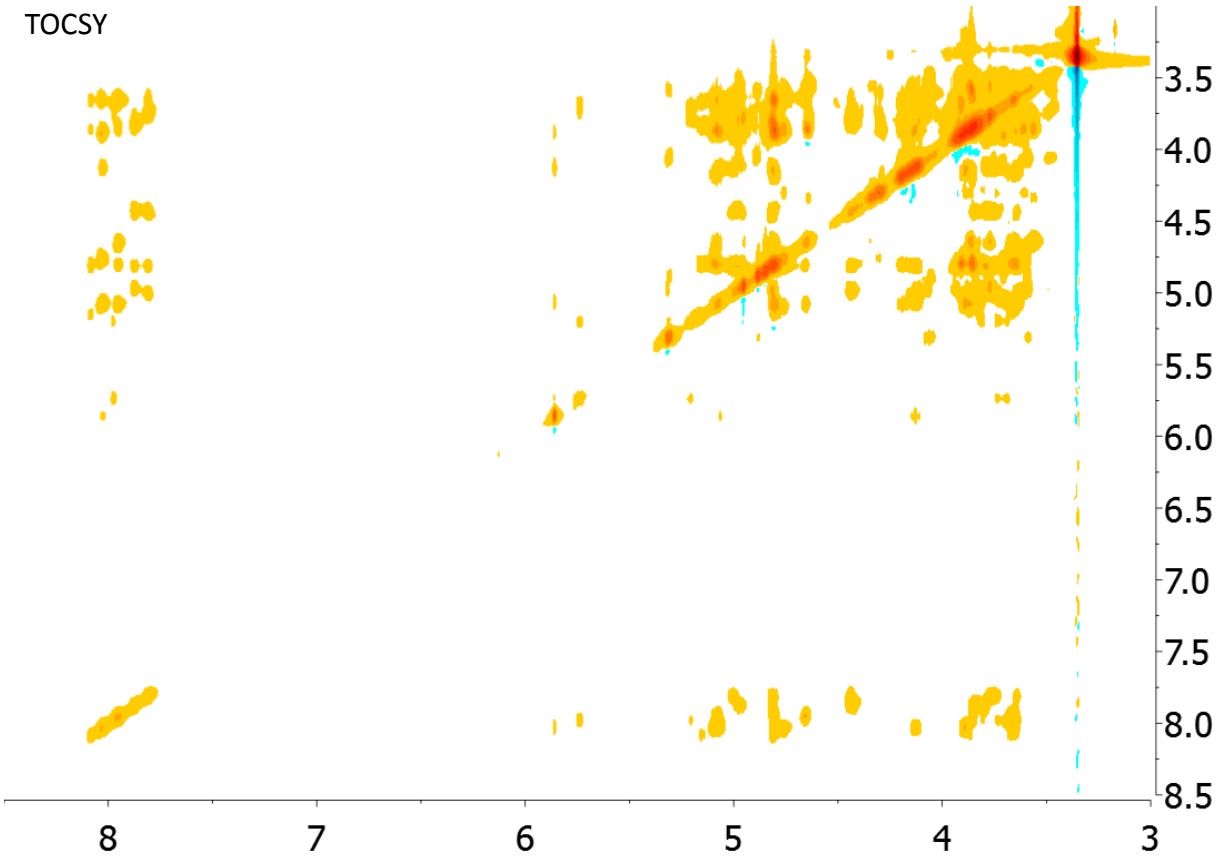
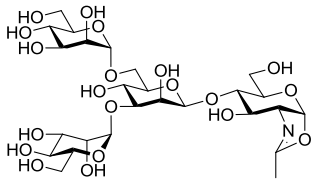
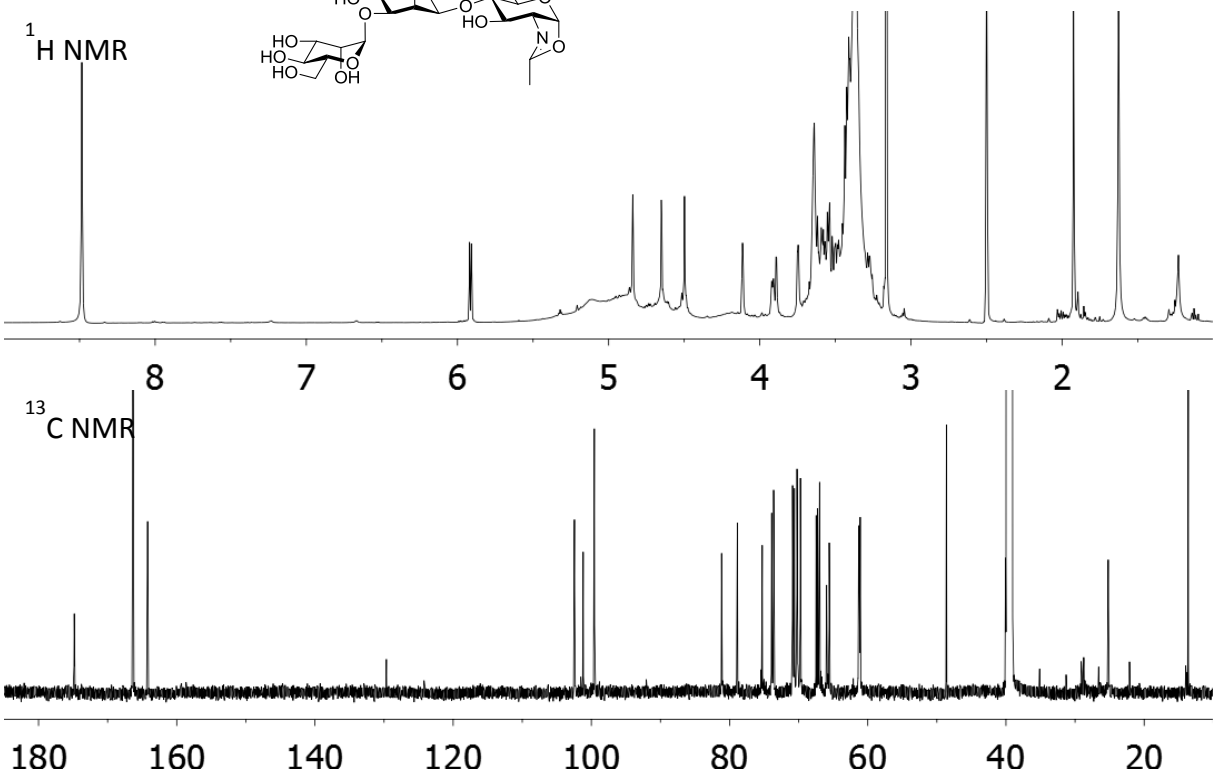
75
80
85
90
95
100
105
110
8
7
6
5
4
3
2
1

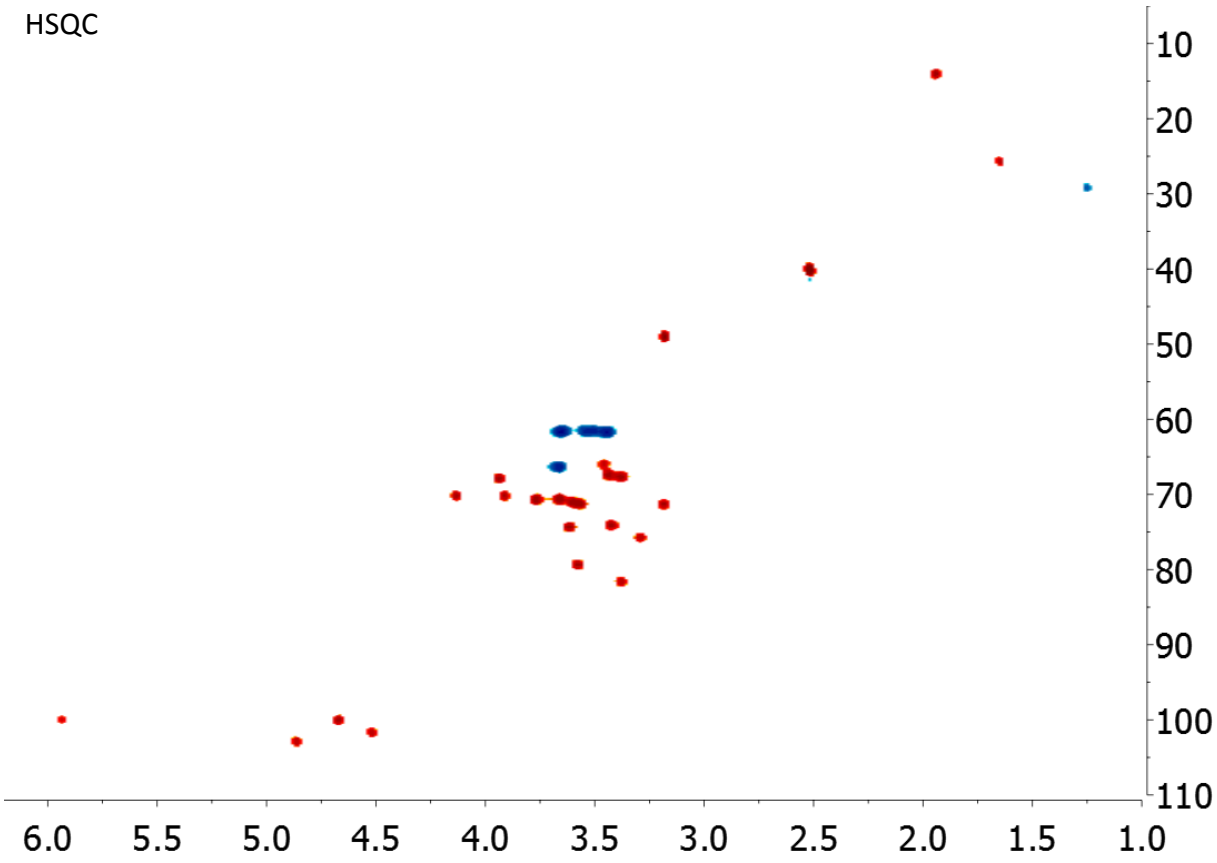

COSY
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8
7
6
5
4
3


TOCSY
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8
7
6
5
4
3

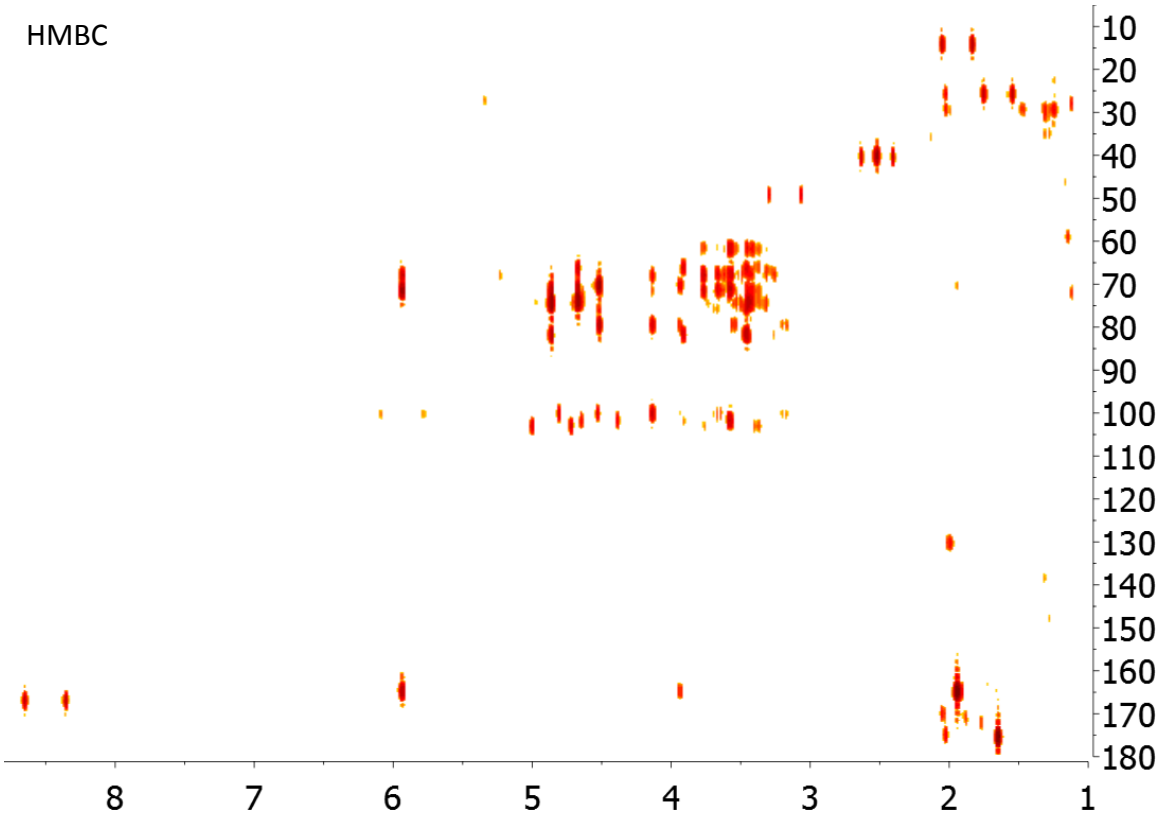
Compound **110**



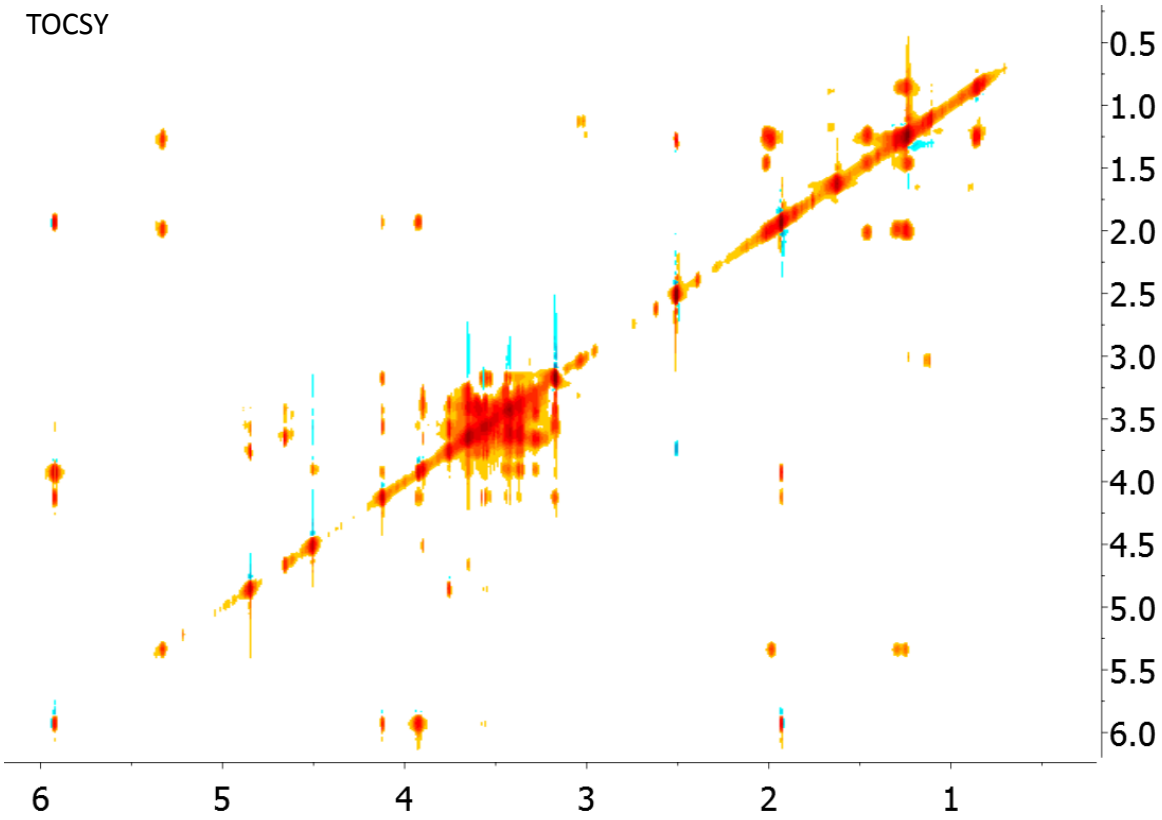




HMBC


10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
8
7
6
5
4
3
2
1

TOCSY


0.5
1.0
1.5
2.0
2.5
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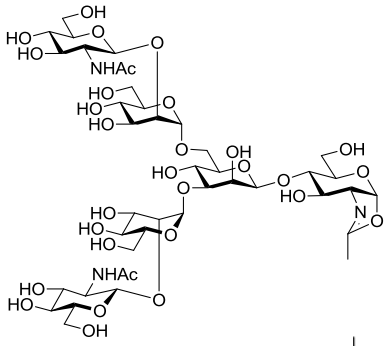
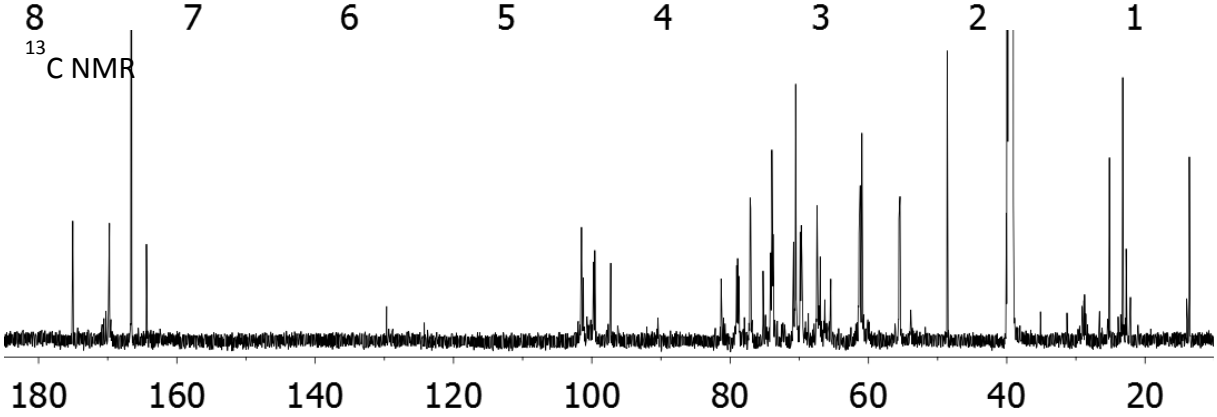
3.5
4.0
4.5
5.0
5.5
6.0
6
5
4
3
2
1

Compound **112**

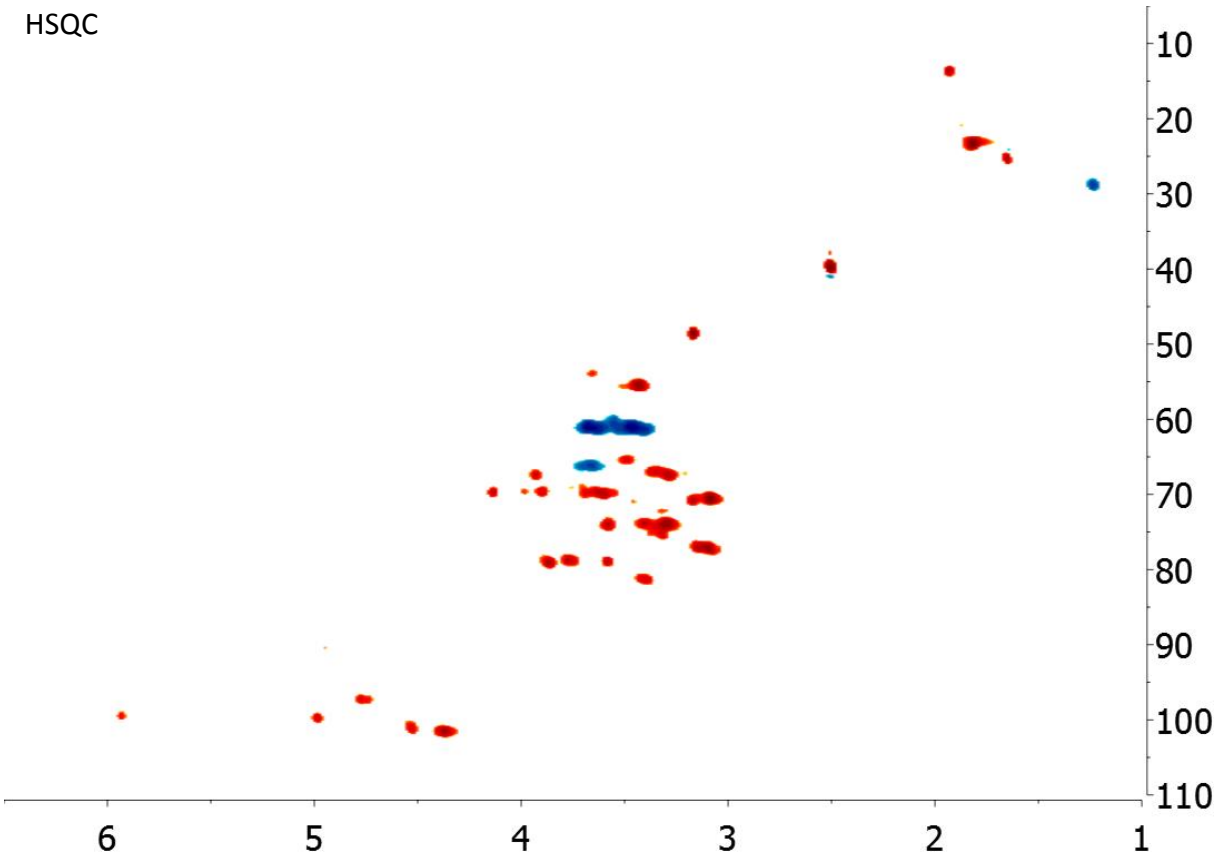


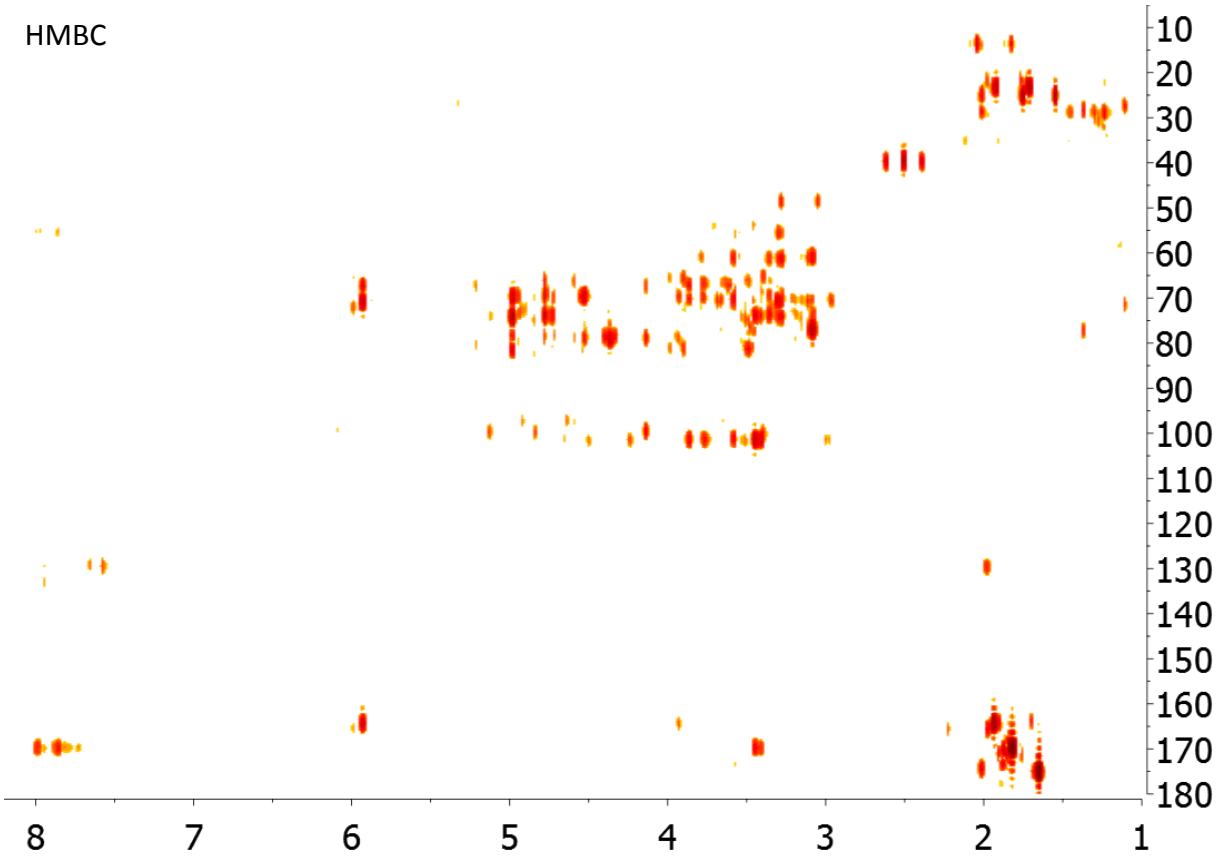
^{1}H NMR

^{13}C NMR



HSQC

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170
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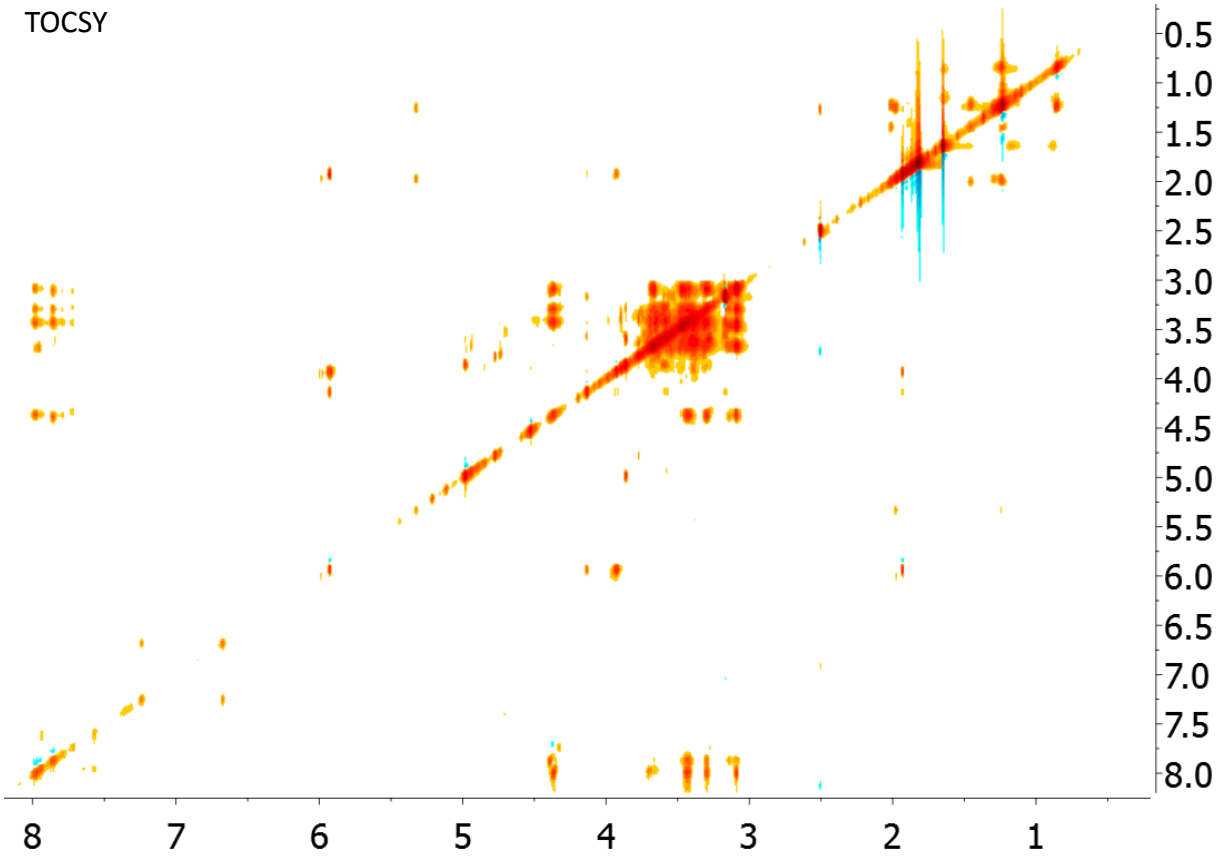
4
3
2
1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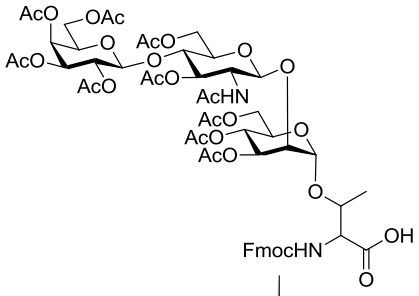
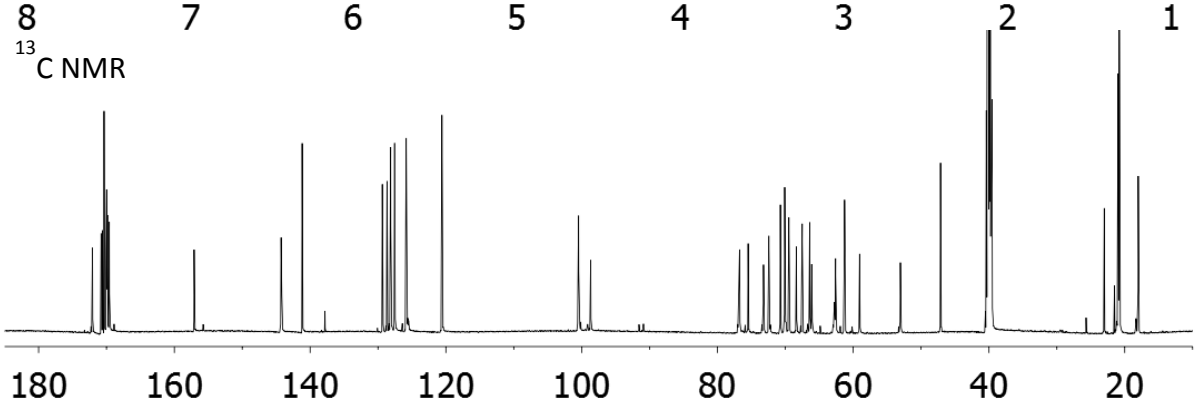
7.0
7.5
8.0
8
7
6
5
4
3
2
1

Compound **120**

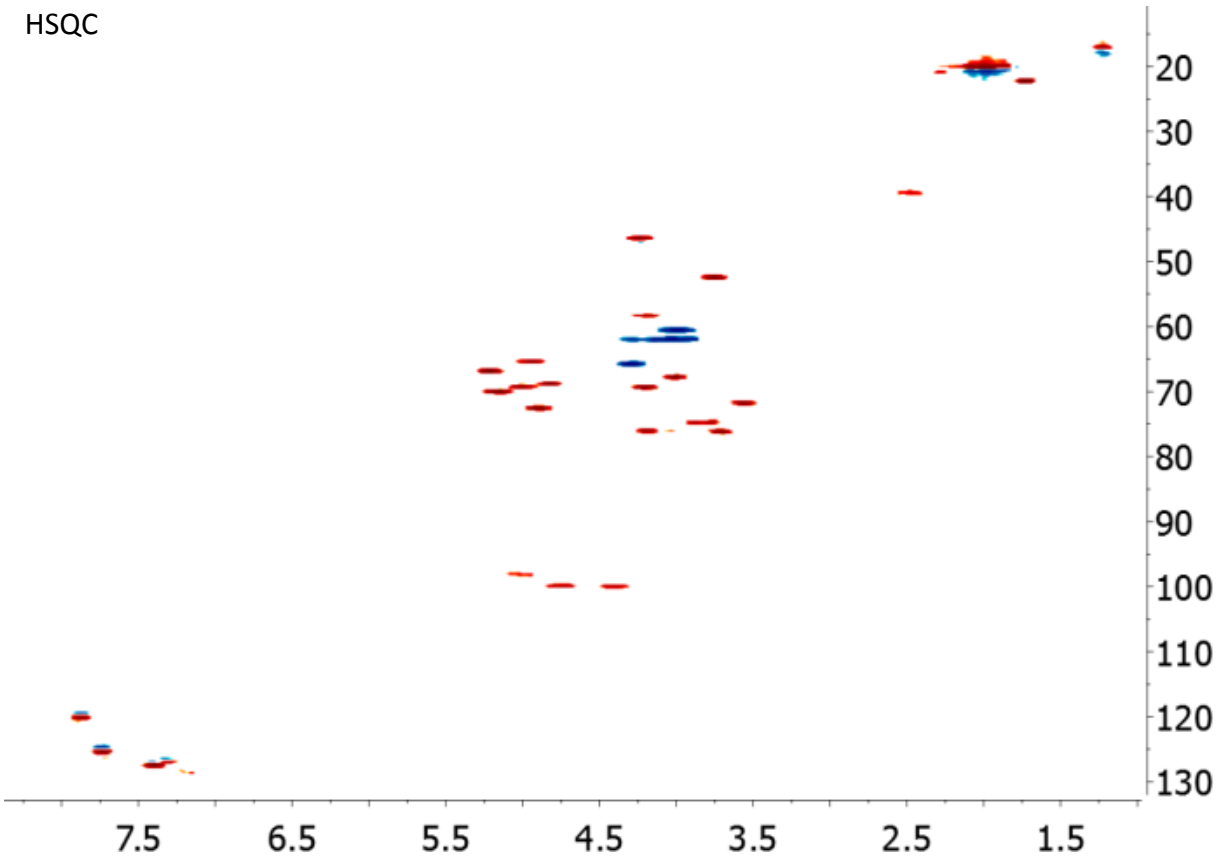


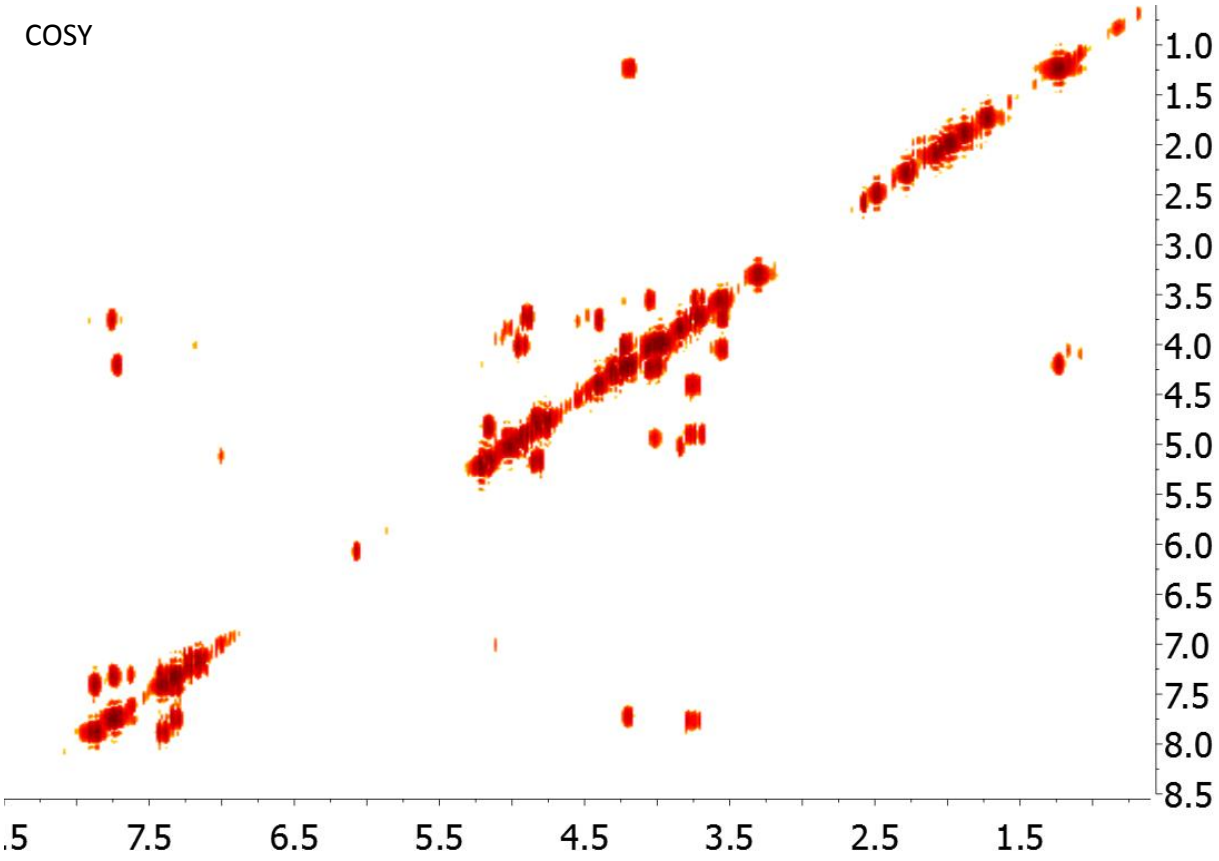
^{1}H NMR

^{13}C NMR



HSQC

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5.5
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6.5
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6.5
5.5
4.5
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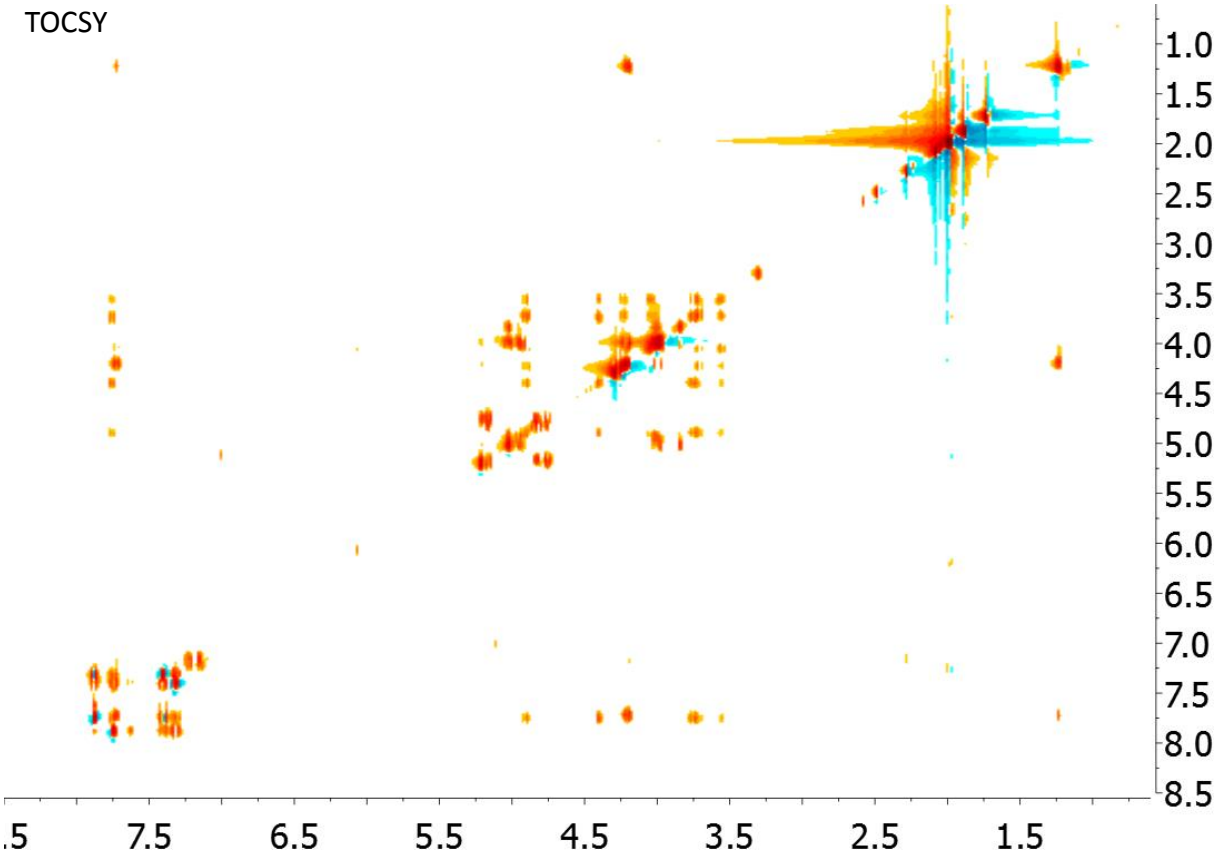
2.5
1.5


TOCSY
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3.0
3.5
4.0
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5.0
5.5
6.0
6.5
7.0
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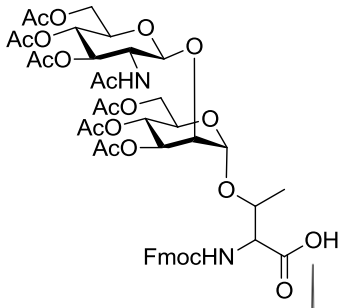
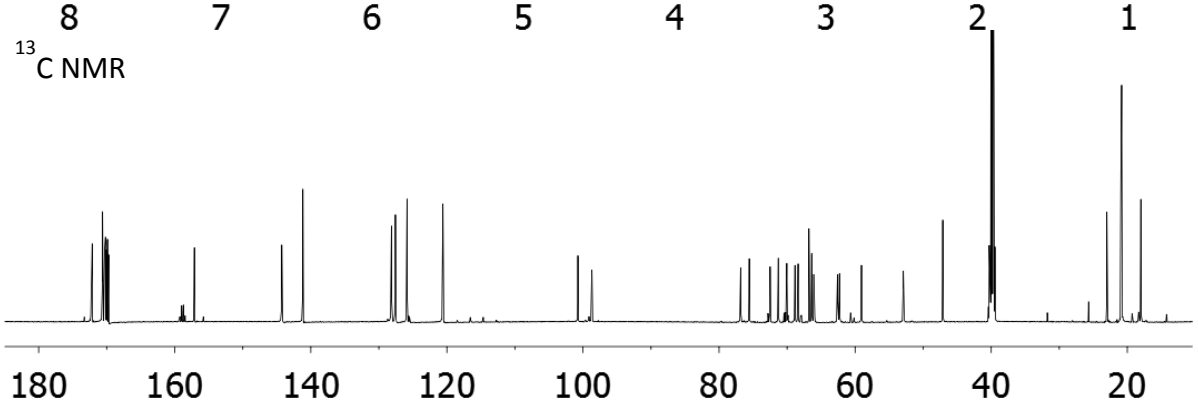
7.5
6.5
5.5
4.5
3.5
2.5
1.5

Compound **124**

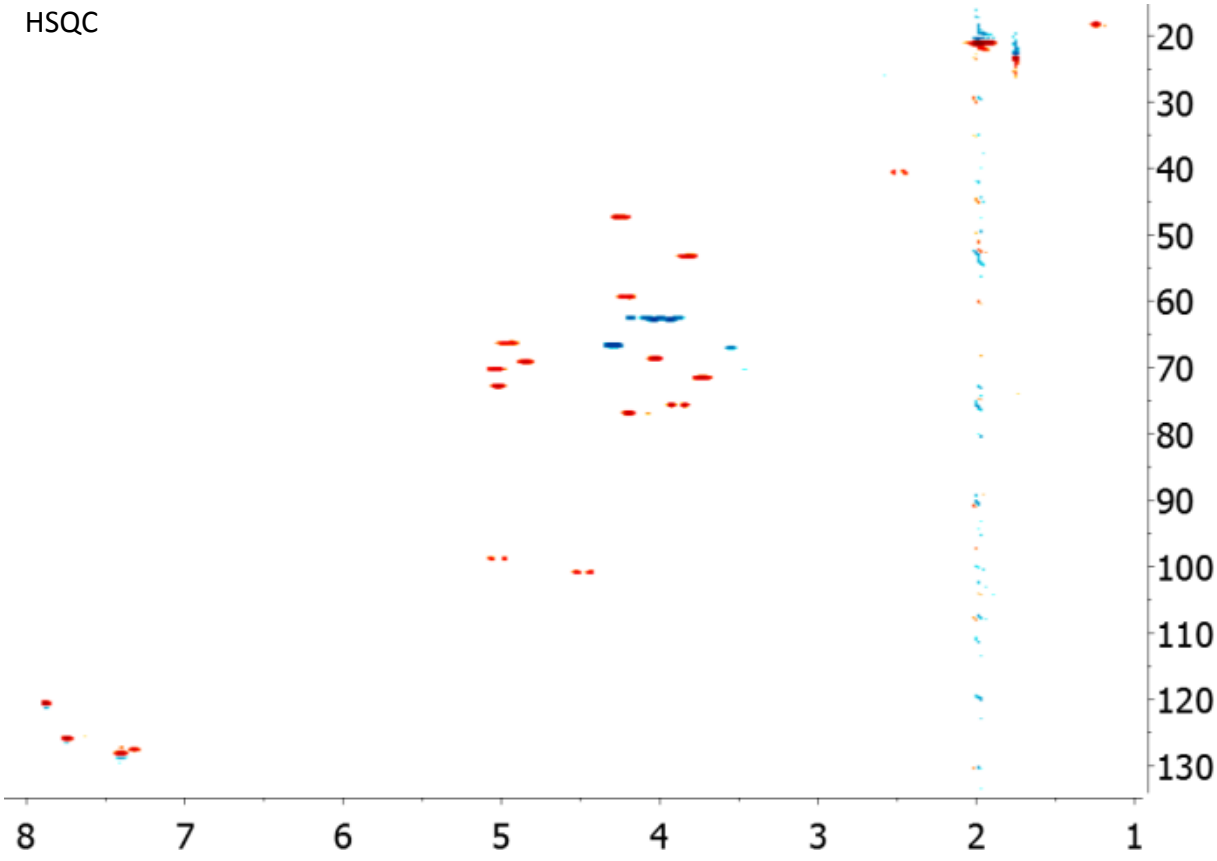


^{1}H NMR

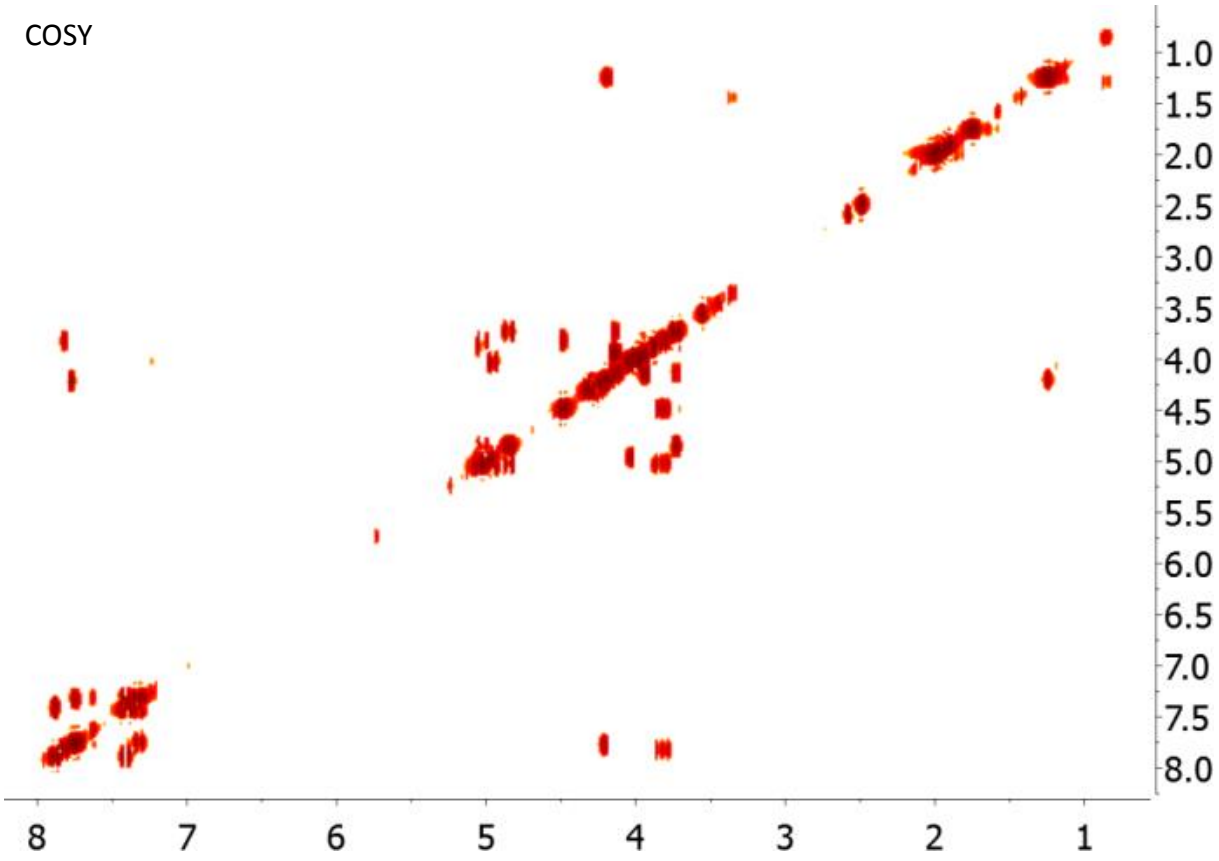
^{13}C NMR



HSQC



COSY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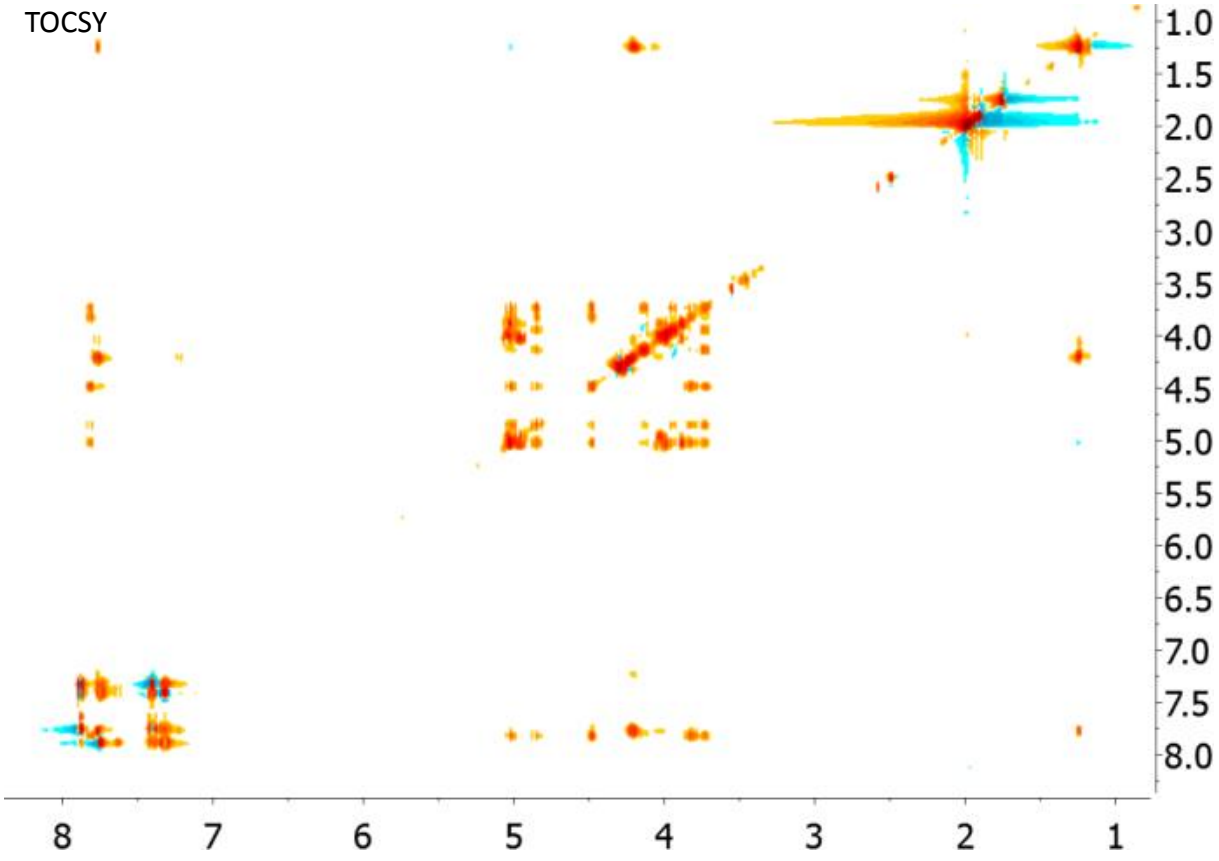
7
6
5
4
3
2
1

TOCSY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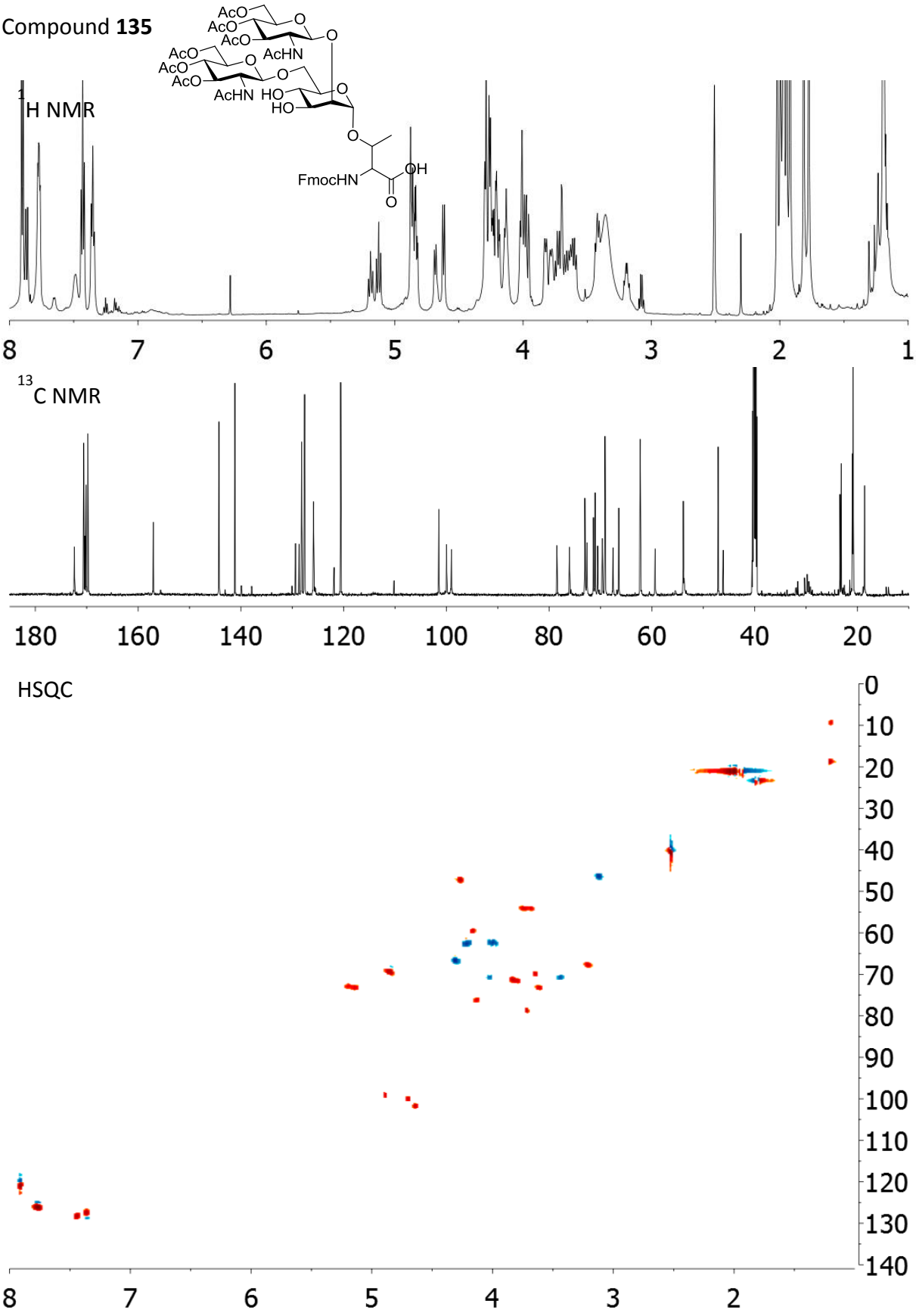
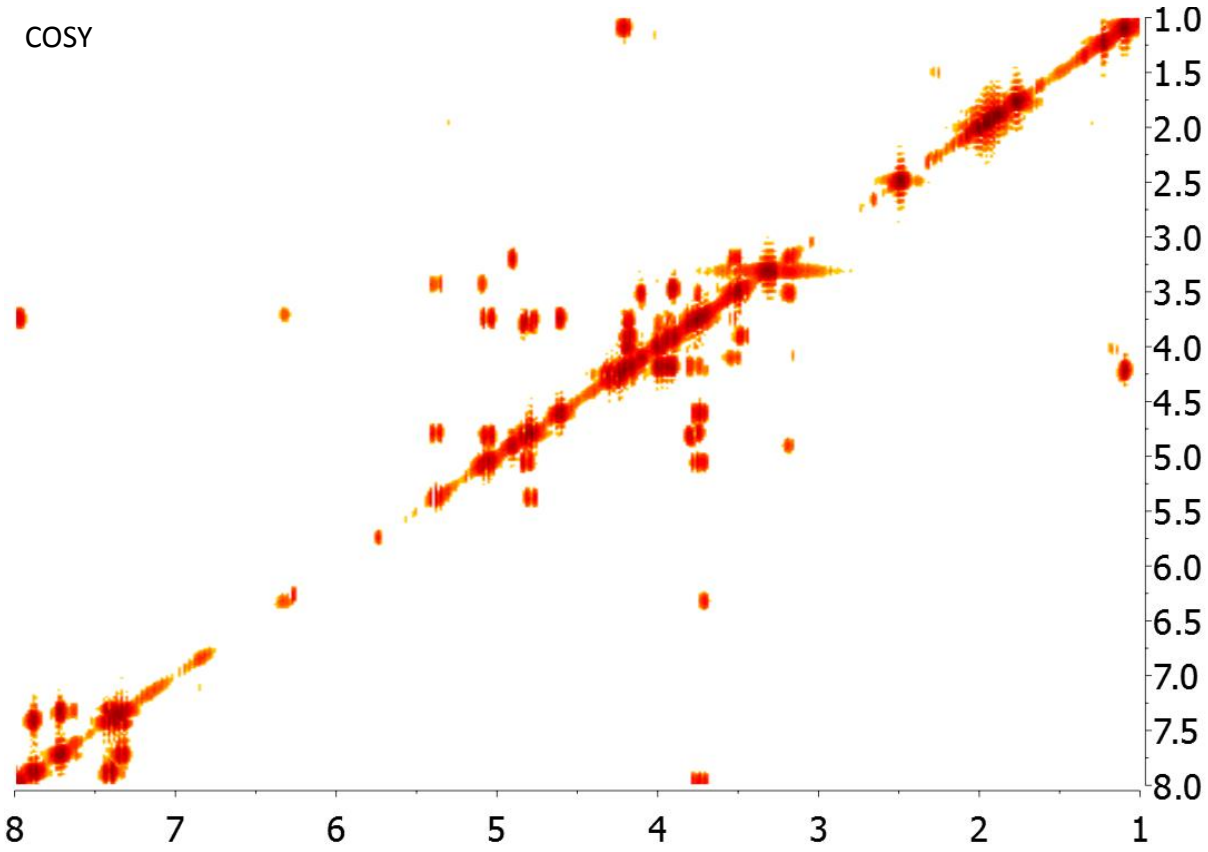
7.0
7.5
8.0
8
7
6
5
4
3
2
1

Compound **135**




COSY
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6.0
6.5
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8
7
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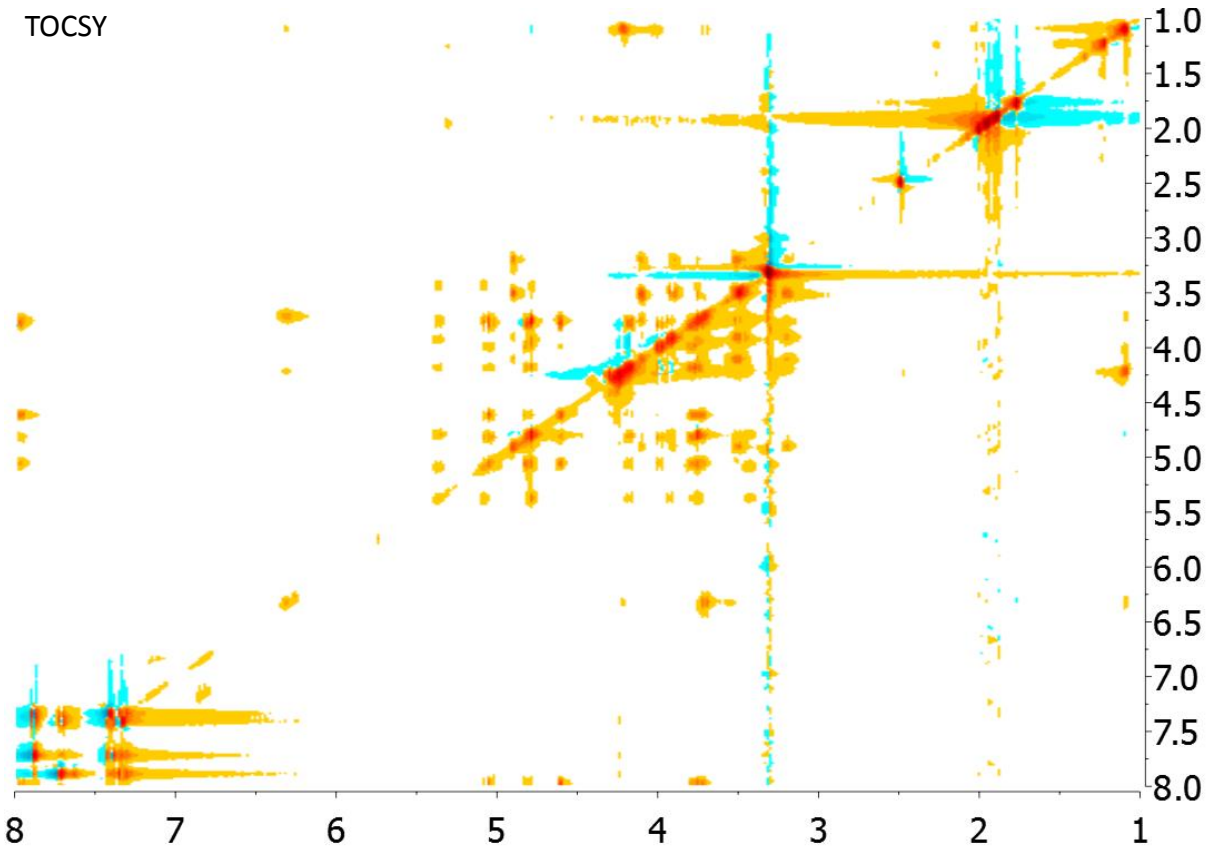
4
3
2
1


TOCSY
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2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8
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6
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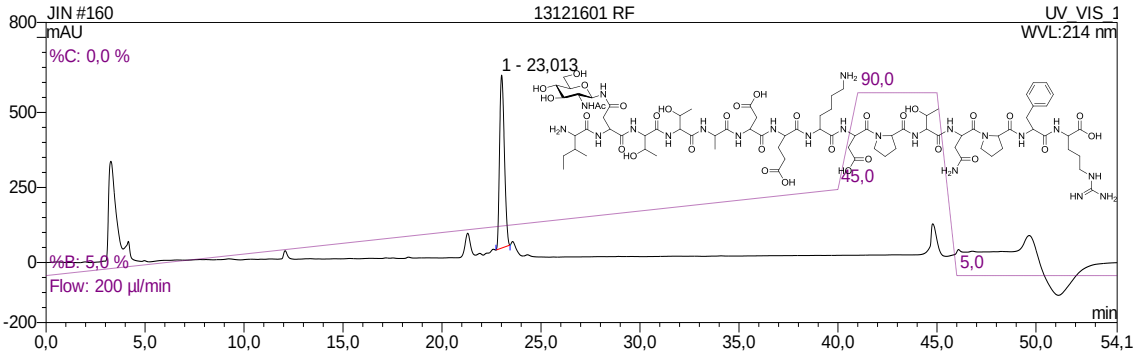
4
3
2
1

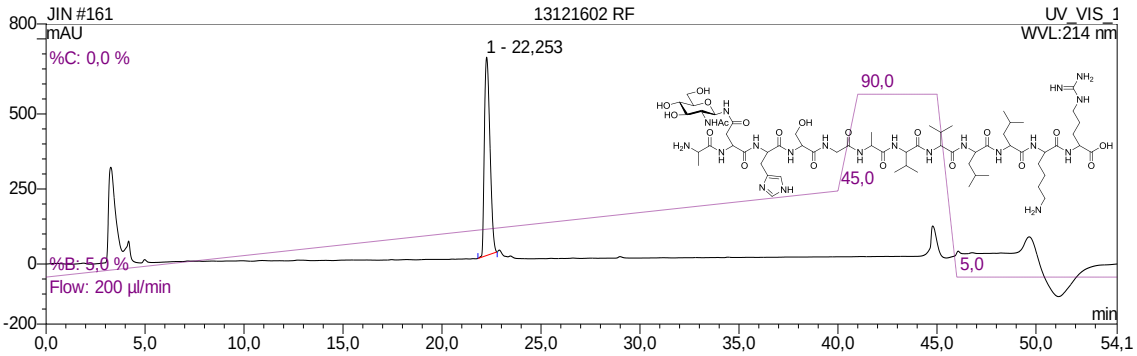
9 Appendix 2: HPLC chromatograms

9.1 HPLC Chromatogram *N*-glycosylated peptides

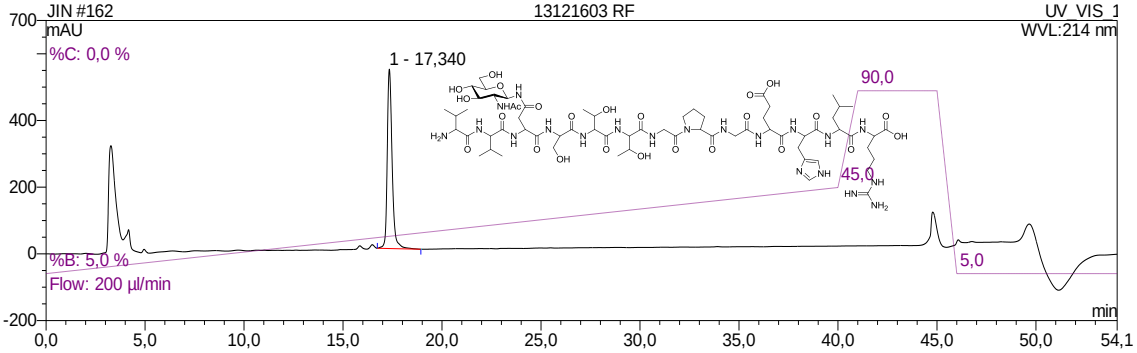
Compound **136**, ADAM10, Sequence: IN*TTADEKDPTNPFR.



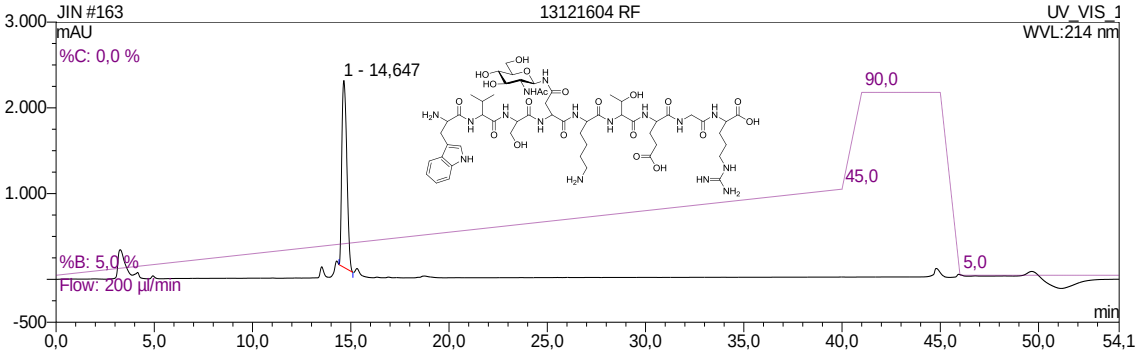
Compound **137**, Integrin alpha-6, Sequence: AN*HSGAVVLLKR.



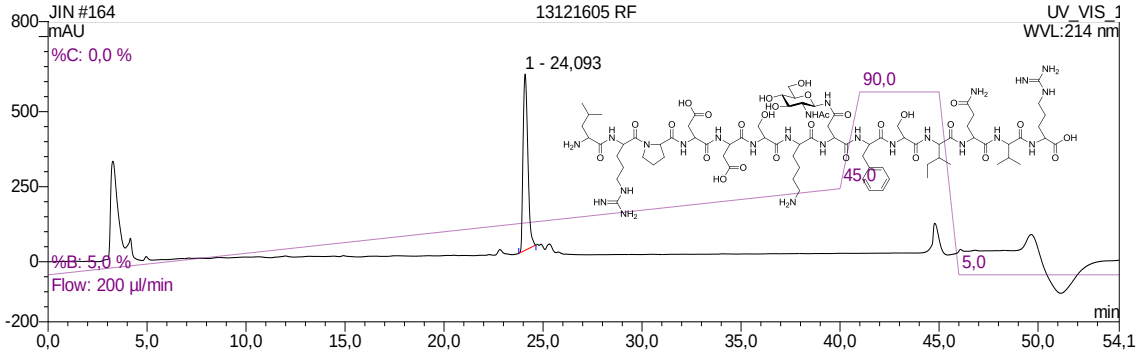
Compound **138**, Trombospondin-1, Sequence: VVN*STTGPGEHLR.



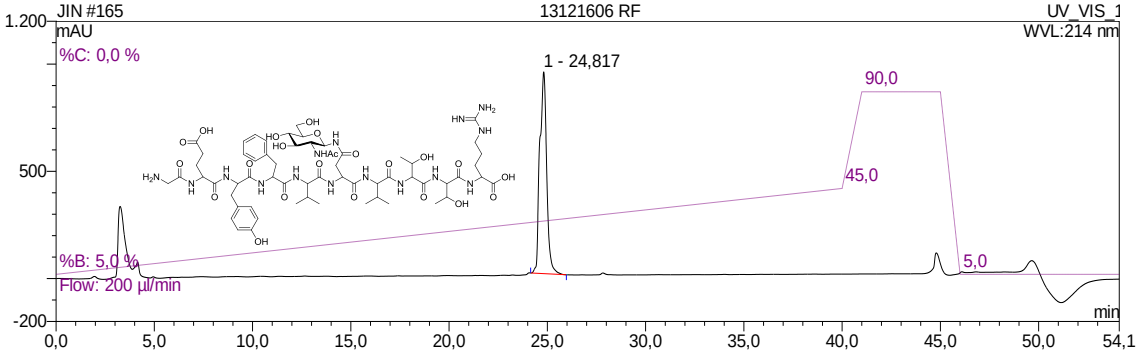
Compound **139**, Antithrombin III, Sequence: WVSN*KTEGR.



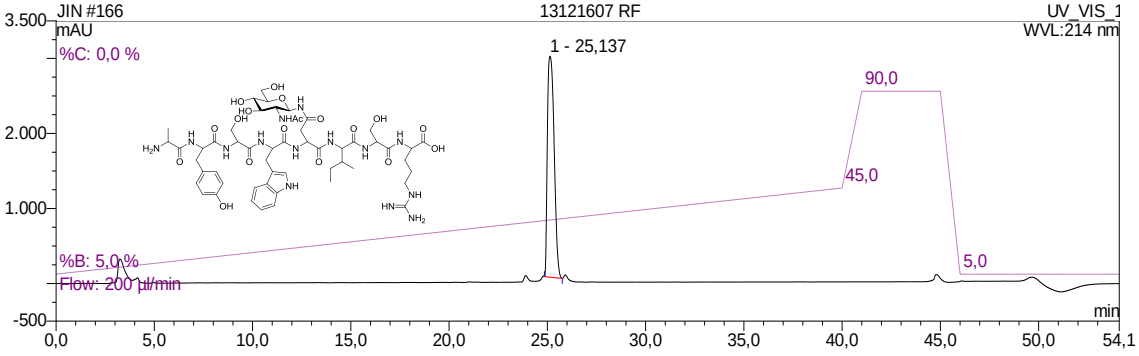
Compound **140**, Integrin beta-3, Sequence: LRPDDSKN*FSIQVR.



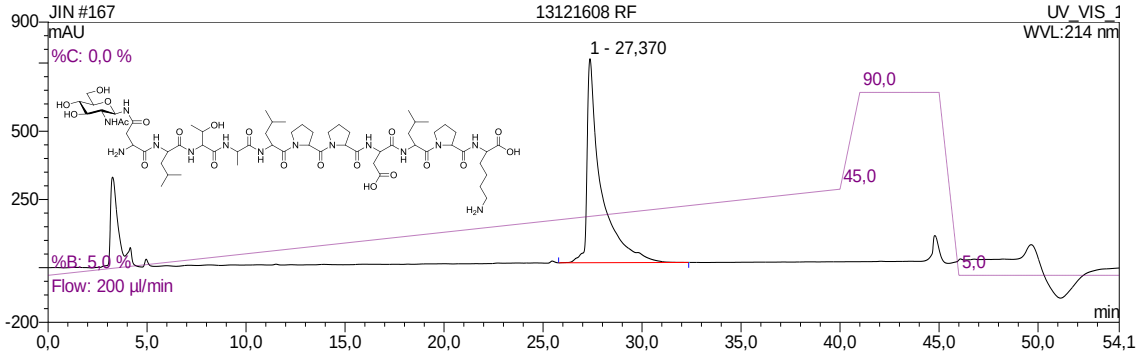
Compound **141**, Integrin alpha-2, Sequence: GEYFVN*VTTR.



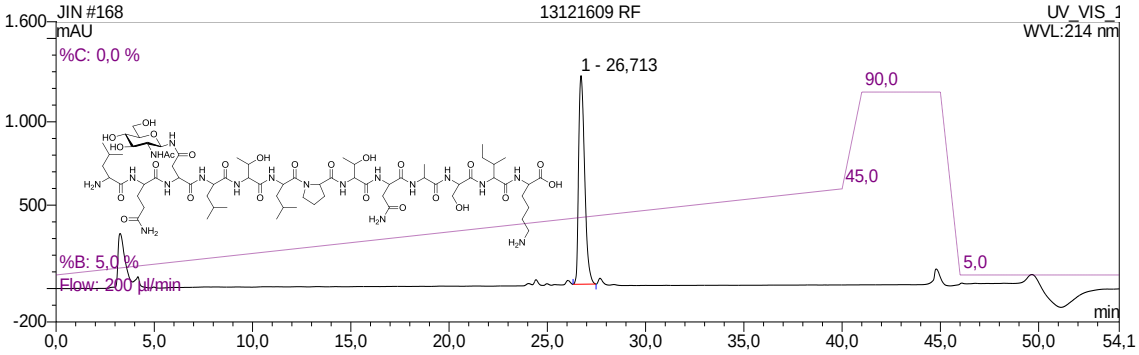
Compound **142**, P-Selectin, Sequence: AYSWN*ISR.



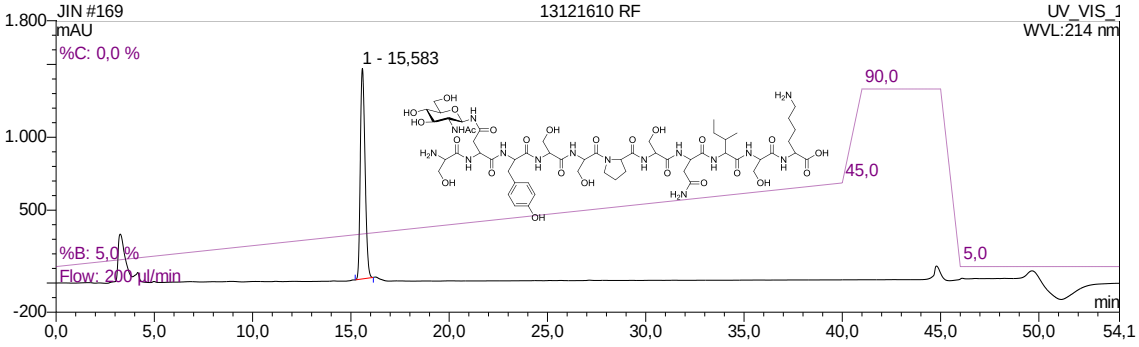
Compound **143**, Platelet glycoprotein 1b alpha, Sequence: N*LTALPPDLPK.



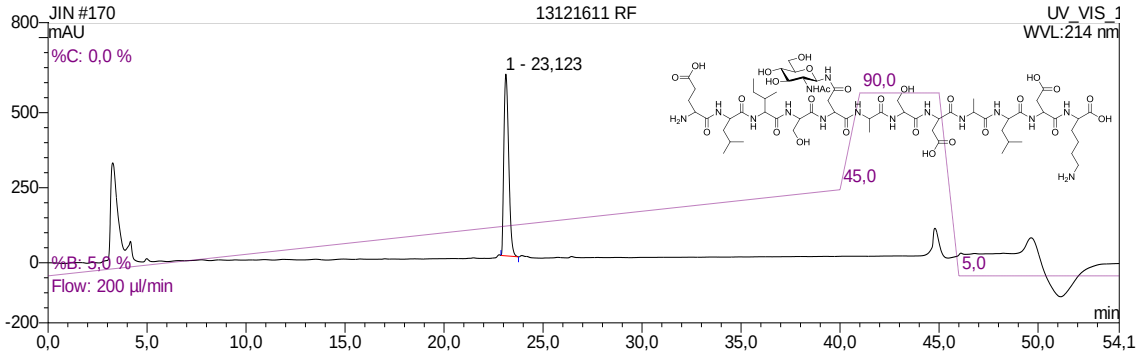
Compound **144**, Multimerin 1, Sequence: LQN*LTLPTNASIK.



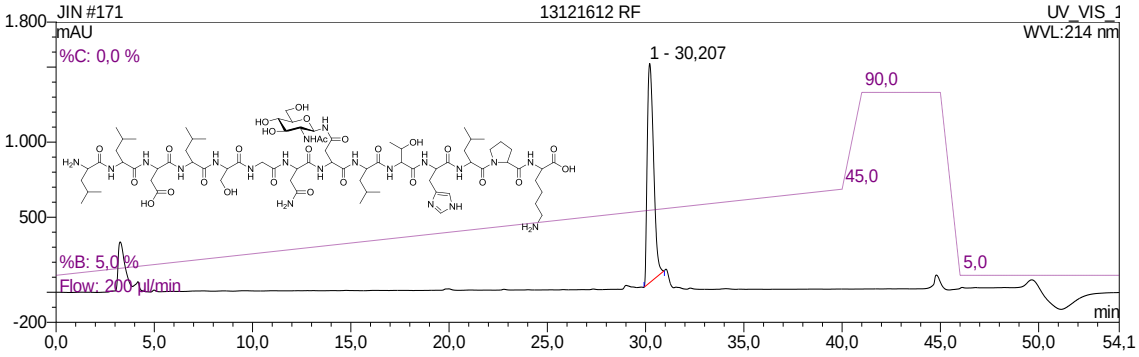
Compound **145**, Coagulationfactor V, Sequence: SN*YSSPSNISK.



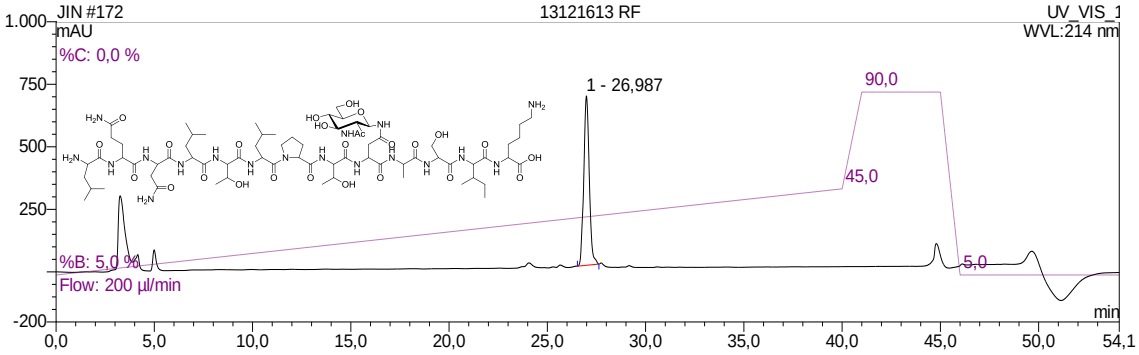
Compound **146**, Endoplasmin, Sequence: ELISN*ASDALDK.



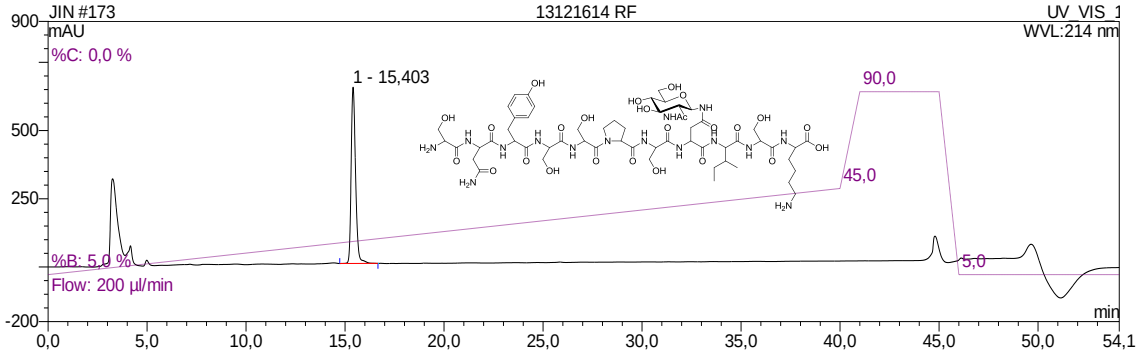
Compound **147**, Platelet glycoprotein V, Sequence: LLDLSGNN*LTHLPK.



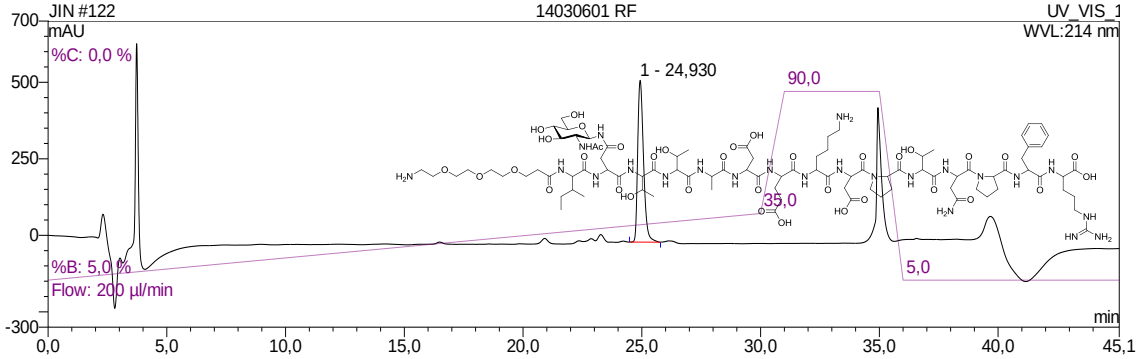
Compound **148**, Multimerin 1, Sequence: LQNLTLPTN*ASIK.



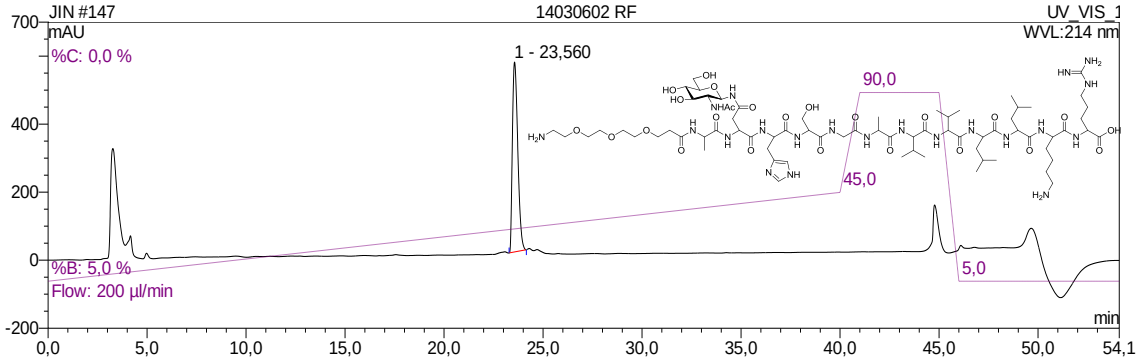
Compound **149**, Coagulationfactor V, Sequence: SNYSSPSN*ISK.



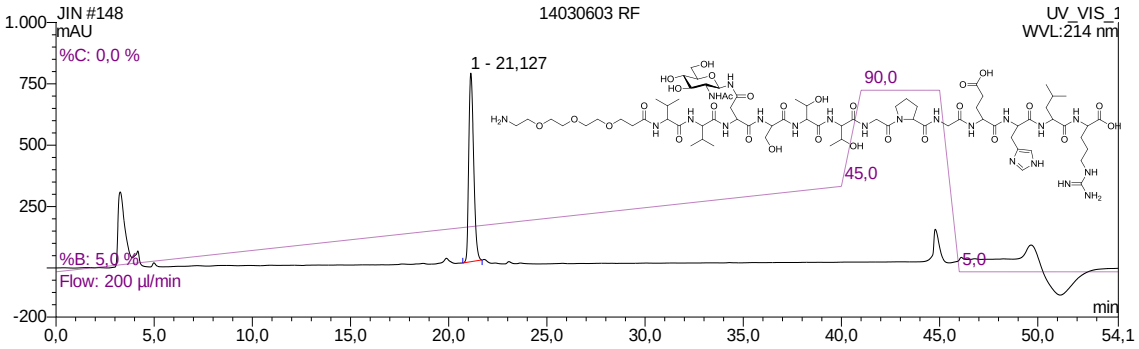
Compound **150**, Sequence: Spacer-IN*TTADEKDPTNPFR.



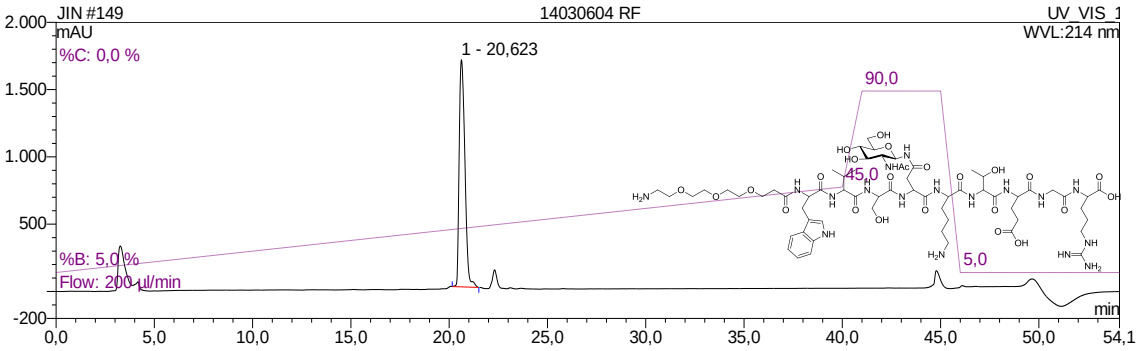
Compound **151**, Sequence: Spacer-AN*HSGAVVLLKR.



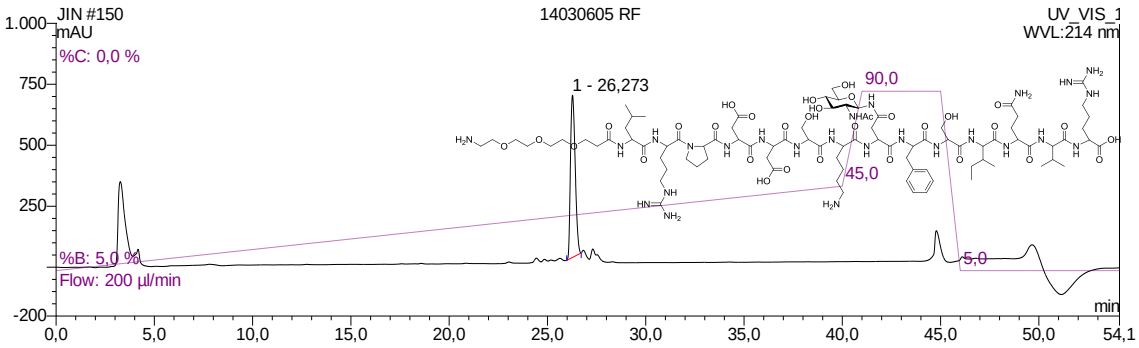
Compound **152**, Sequence: Spacer-VVN*STTGPGEHLR.



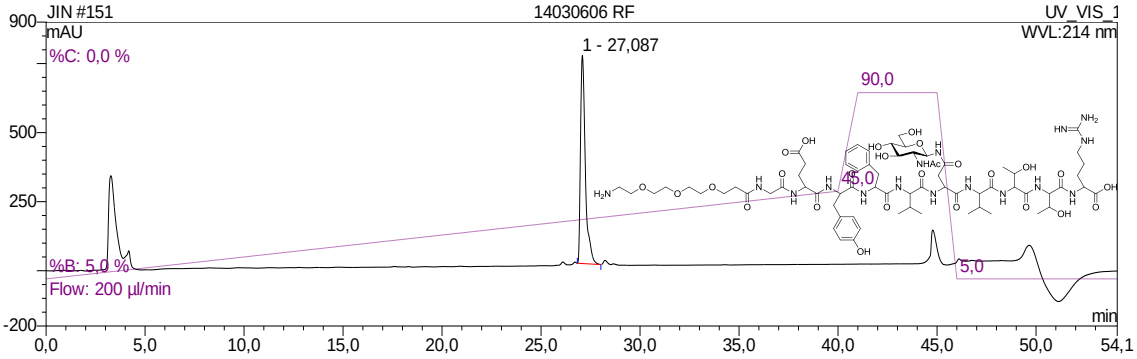
Compound **153**, Sequence: Spacer-WVSN*KTEGR.



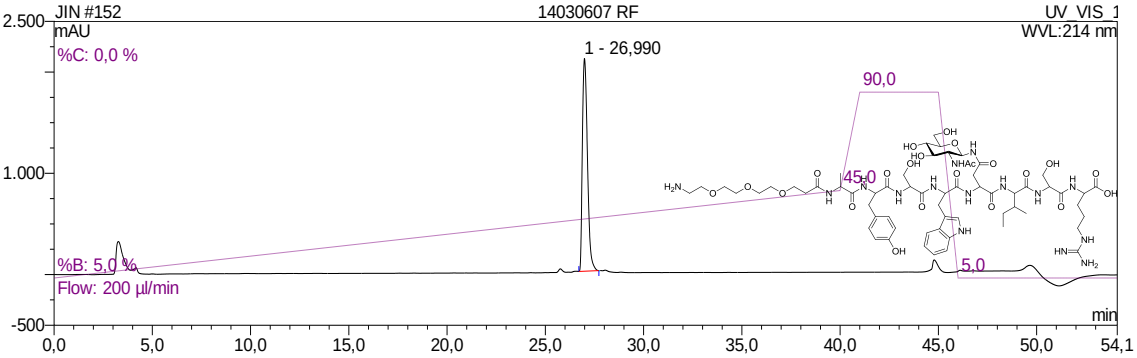
Compound **154**, Sequence: Spacer-LRPDDSKN*FSIQVR.



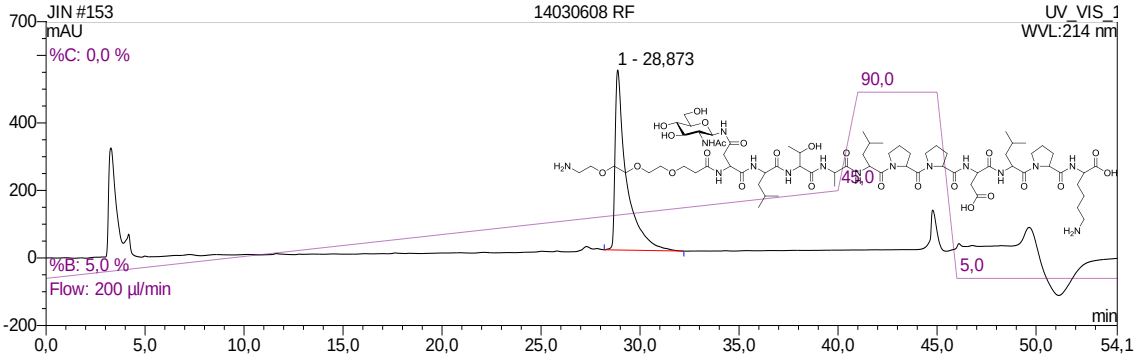
Compound **155**, Sequence: Spacer-GEYFVN*VTTR.



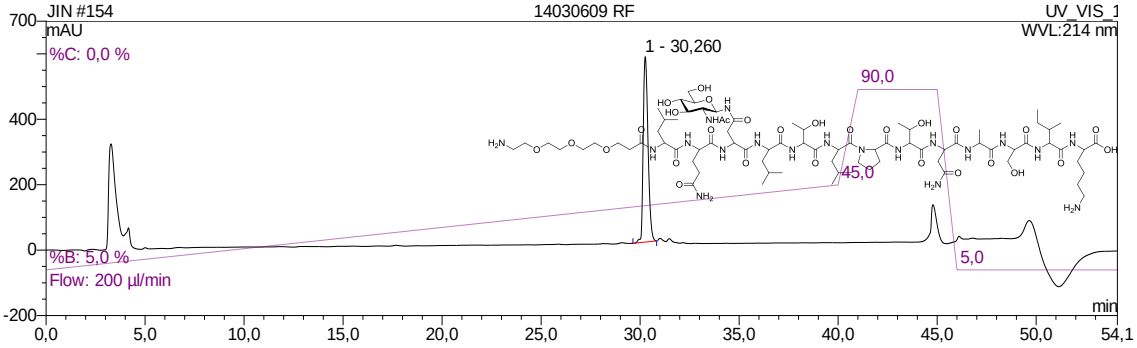
Compound **156**, Sequence: Spacer-AYSWN*ISR.



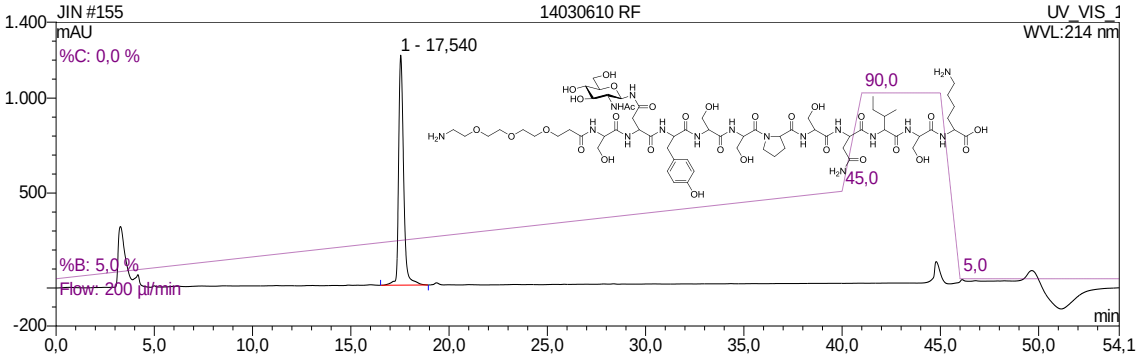
Compound **157**, Sequence: Spacer-N*LTALPPDLPK.



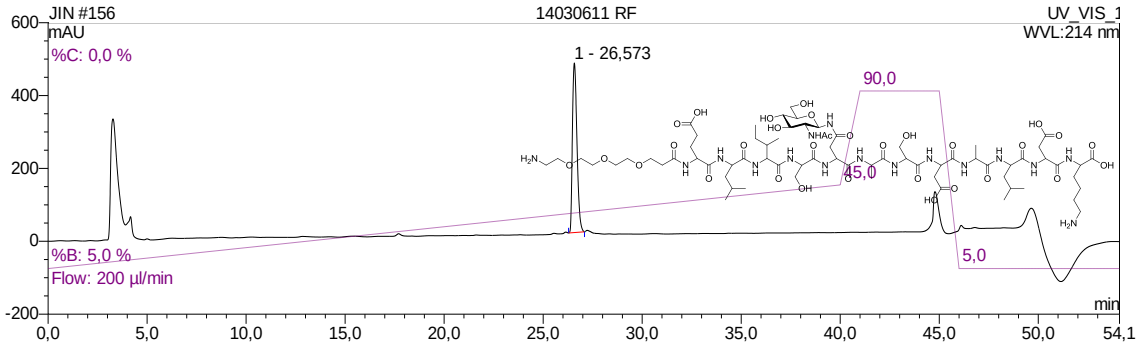
Compound **158**, Sequence: Spacer-LQN*LTLPTNASIK.



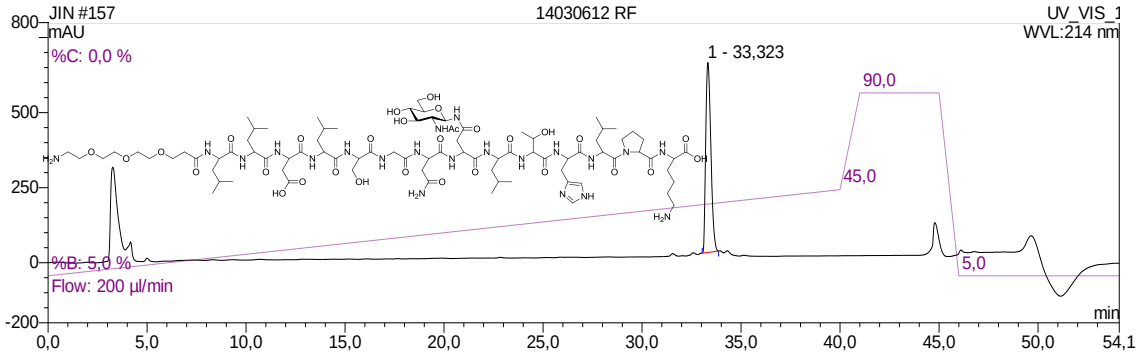
Compound **159**, Sequence: Spacer-SN*YSSPSNISK.



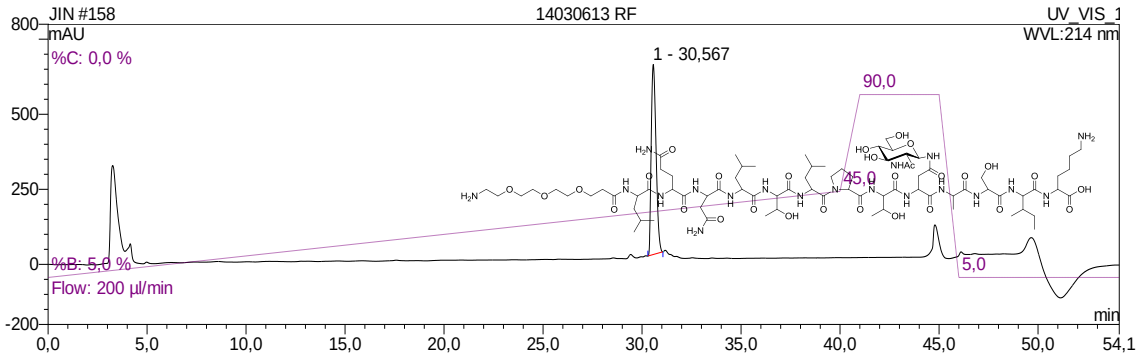
Compound **160**, Sequence: Spacer-ELISN*ASDALDK.



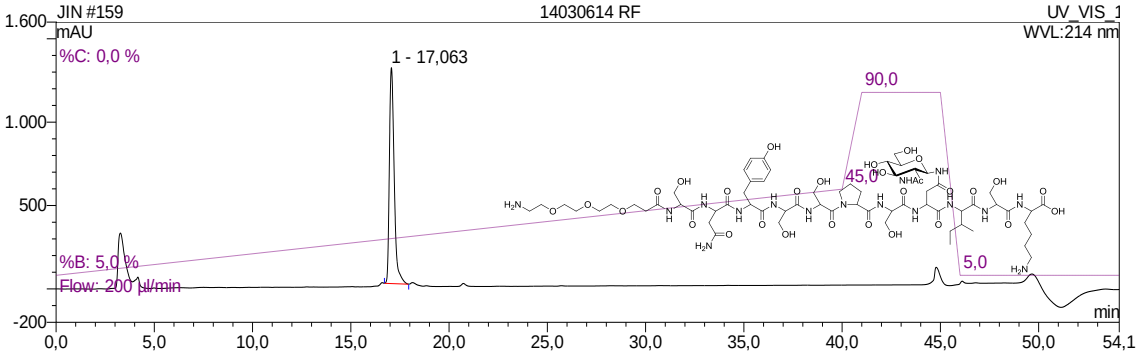
Compound **161**, Sequence: Spacer-LLDLSGNN*LTHLPK.



Compound **162**, Sequence: Spacer-LQNLTLPTN*ASIK.

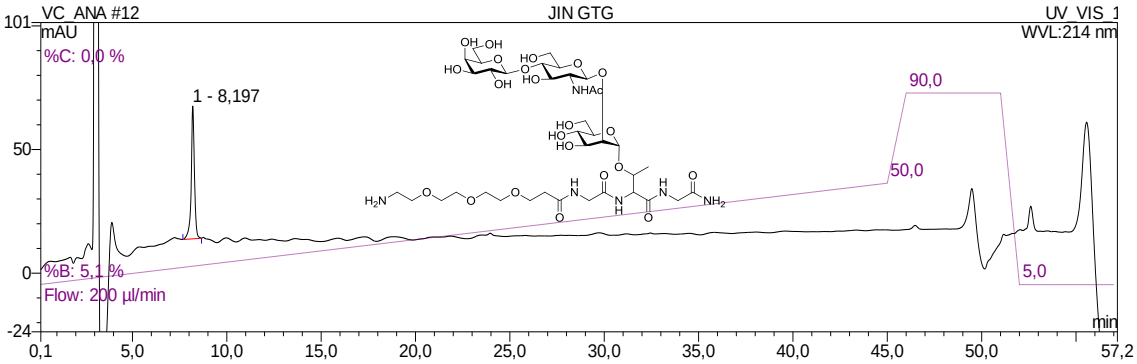


Compound **163**, Sequence: Spacer-SNYSSPSN*ISK.

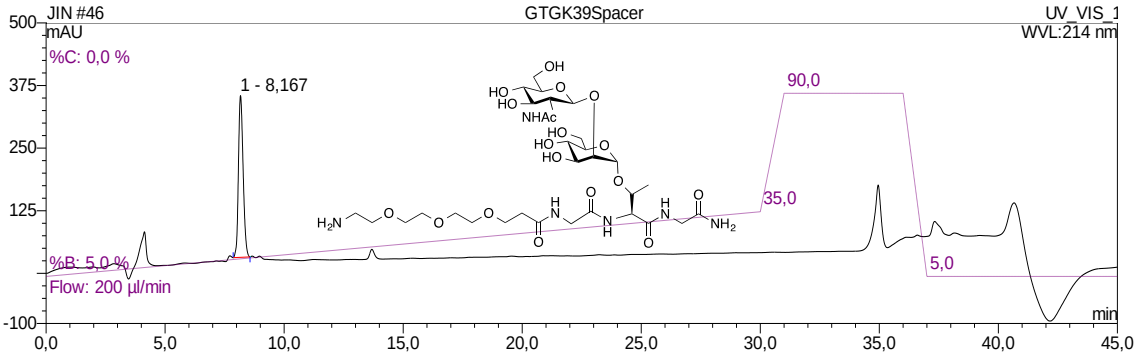


9.2 HPLC Chromatogram *O*-glycosylated peptides

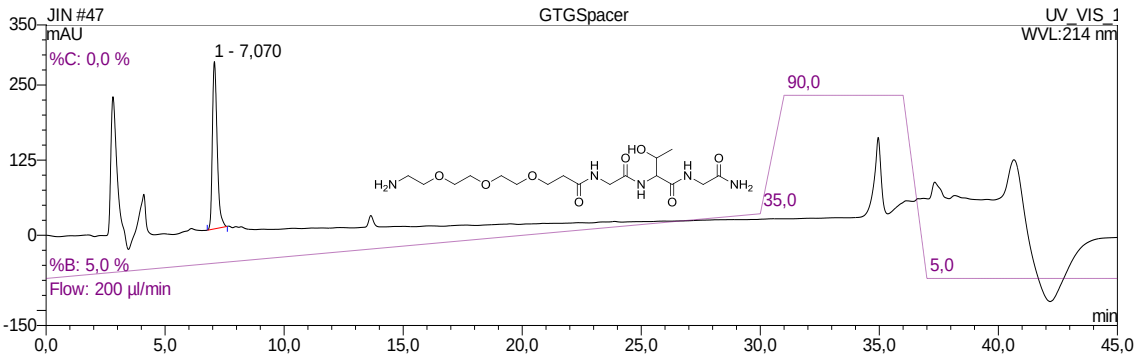
Compound **171**, Sequence: NH_2-Spacer-GT*G-NH_2, *=Gla-GlcNAc-Man.



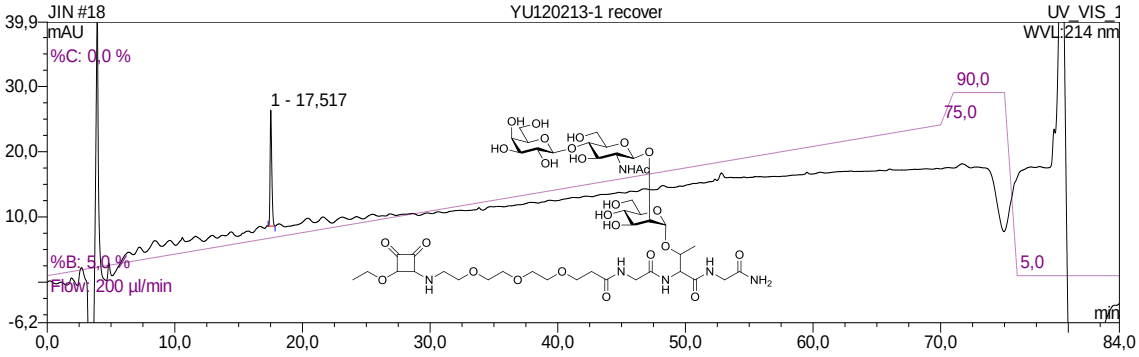
Compound **172**, Sequence: NH_2-Spacer-GT*G-NH_2, *=GlcNAc-Man.



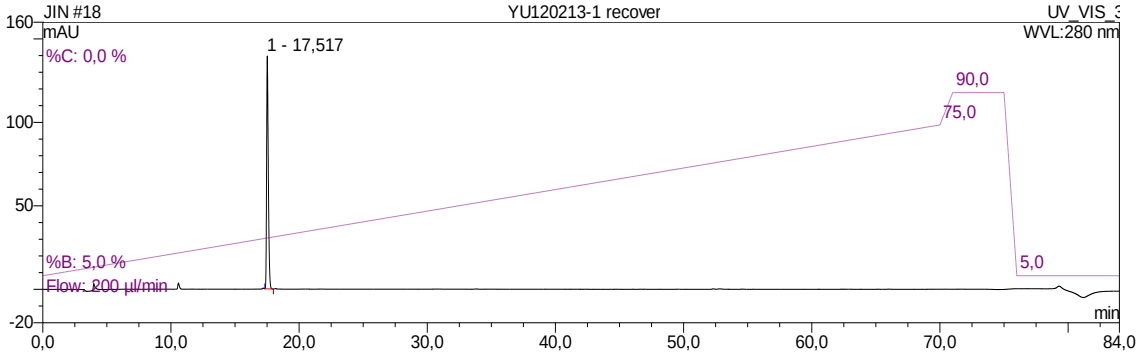
Compound **173**, Sequence: NH_2-Spacer-GTG-NH_2.



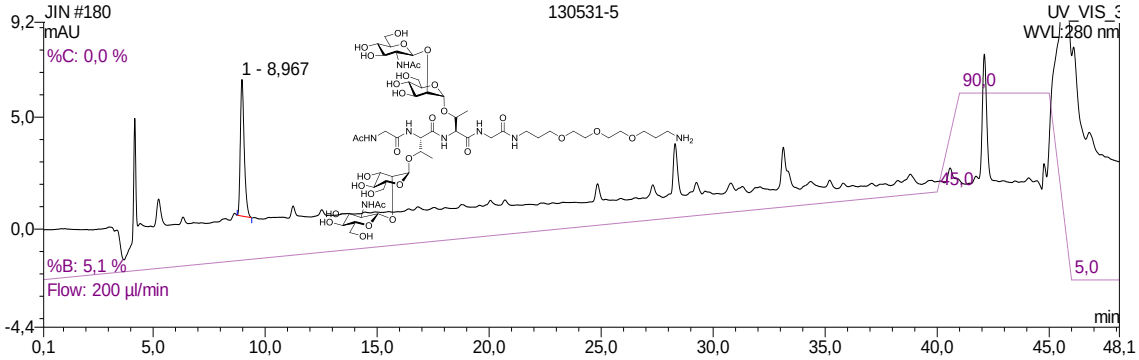
Compound **174**, Sequence: Linker-NH-Spacer-GT*G-NH_2, *=Gla-GlcNAc-Man.



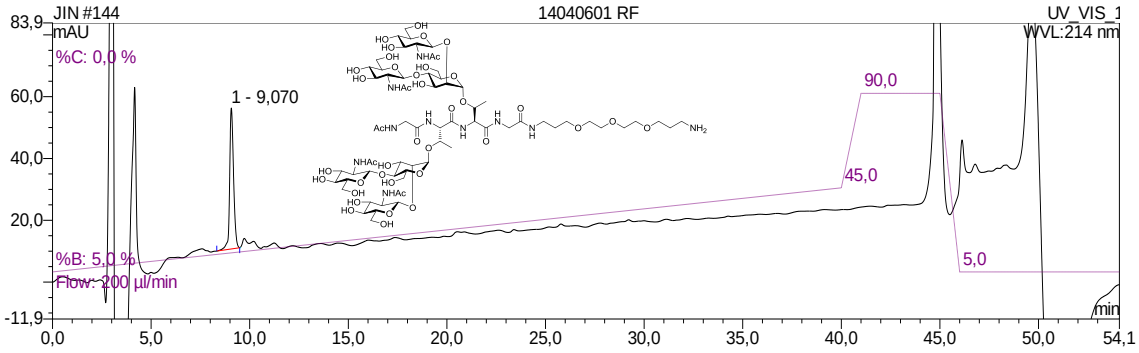
Compound **175**, UV channel 280 nm.



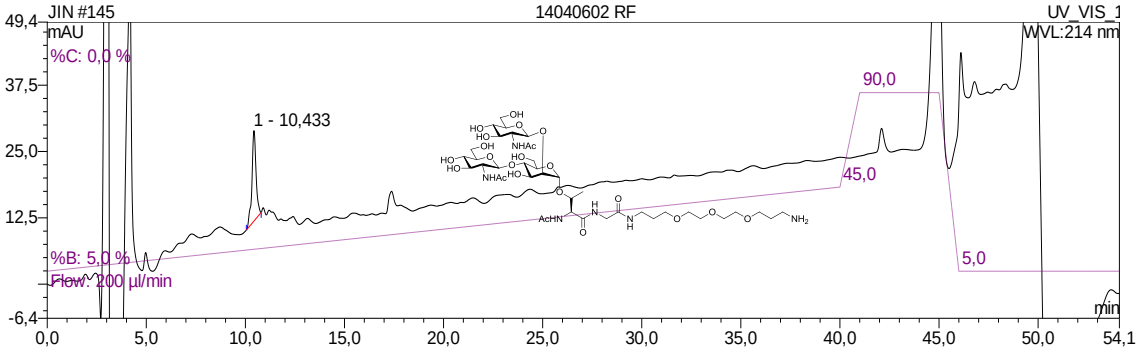
Compound **180**, Sequence: AcNH-GT*T*G-Spacer-NH_2, *=GlcNAc-Man.



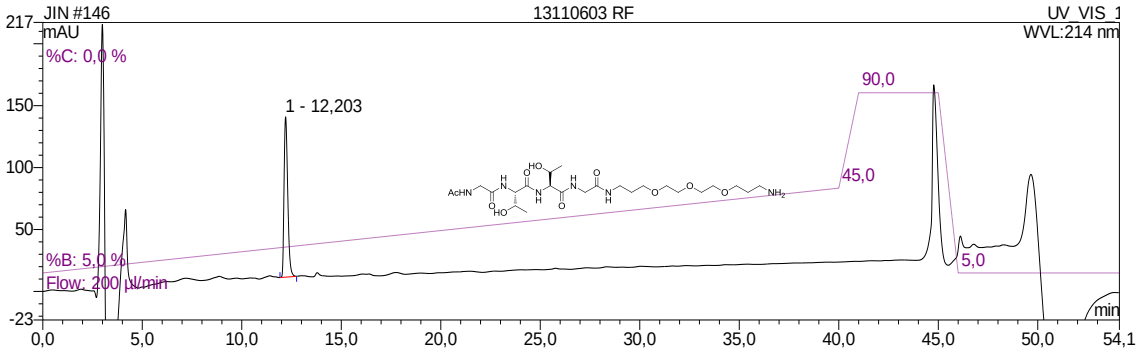
Compound **181**, Sequence: AcNH-GT*T*G-Spacer-NH_2, *=2,6 GlcNAc-Man.



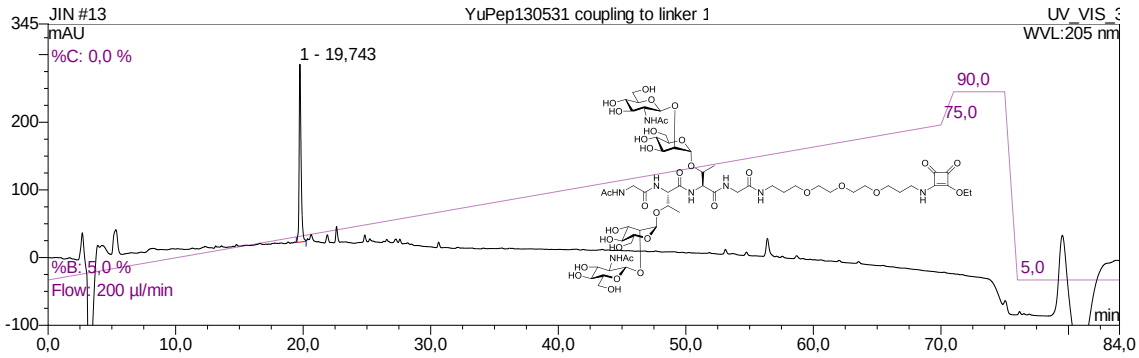
Compound **182**, Sequence: AcNH-T*G-Spacer-NH_2, *=2,6 GlcNAc-Man.



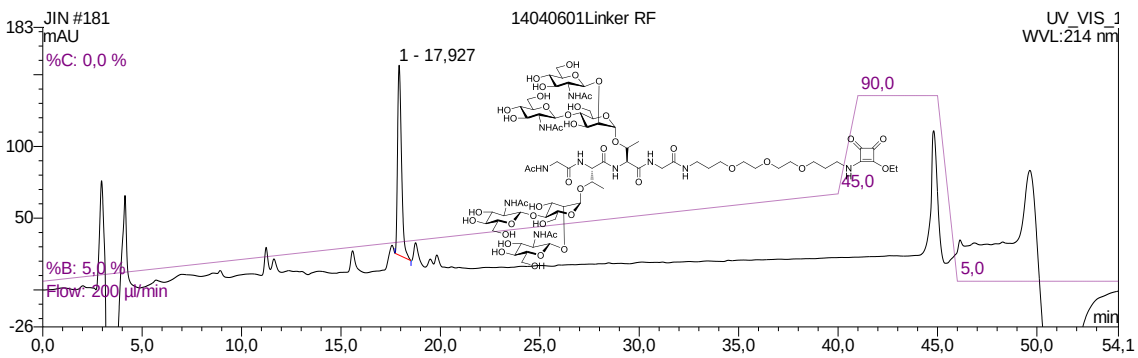
Compound **183**, Sequence: AcNH-GTTG-Spacer-NH_2.



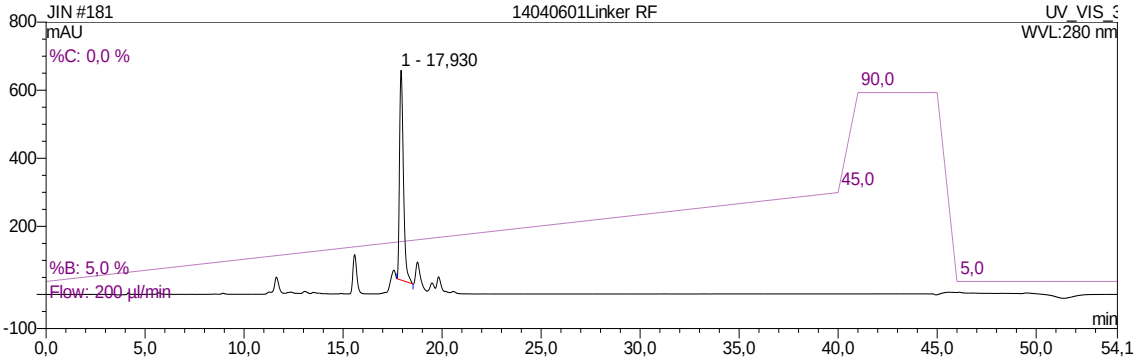
Compound **184**, Sequence: AcNH-GT*T*G-Spacer-NH-Linker, *=GlcNAc-Man.



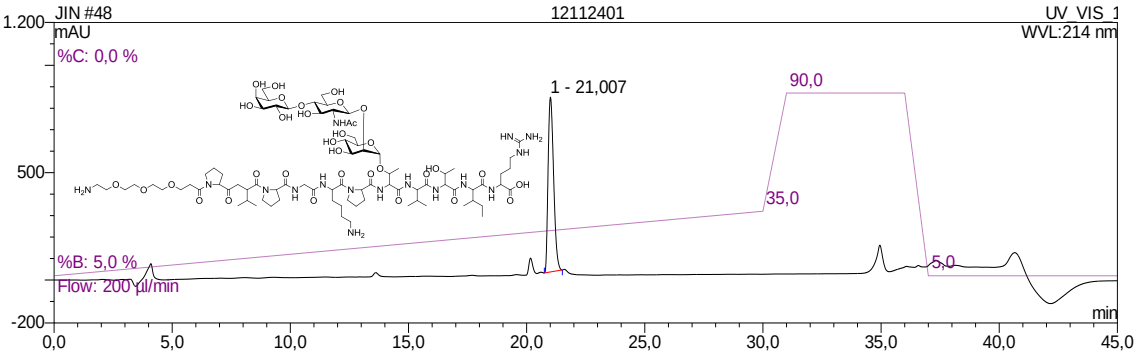
Compound **185**, Sequence: AcNH-GT*T*G-Spacer-NH-Linker, *=2,6 GlcNAc-Man.



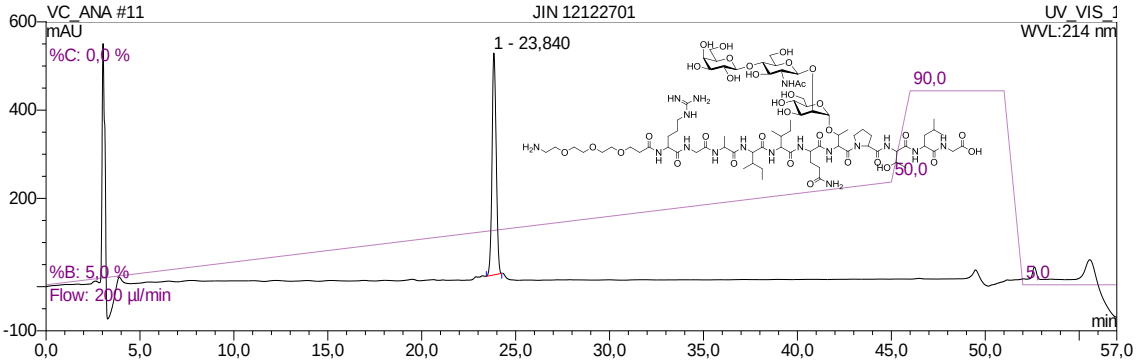
Compound **185**, UV channel 280 nm.



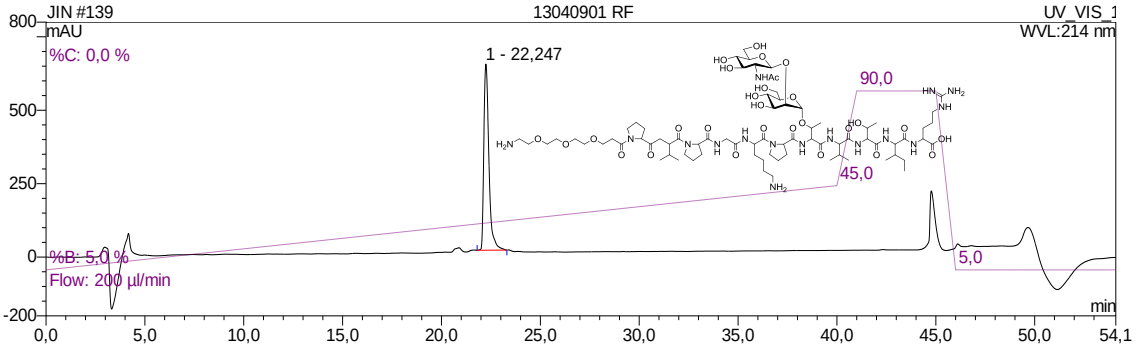
Compound **190**, Sequence: NH_2-Spacer-PVPGKPT*VTIR, *=Gla-GlcNAc-Man.



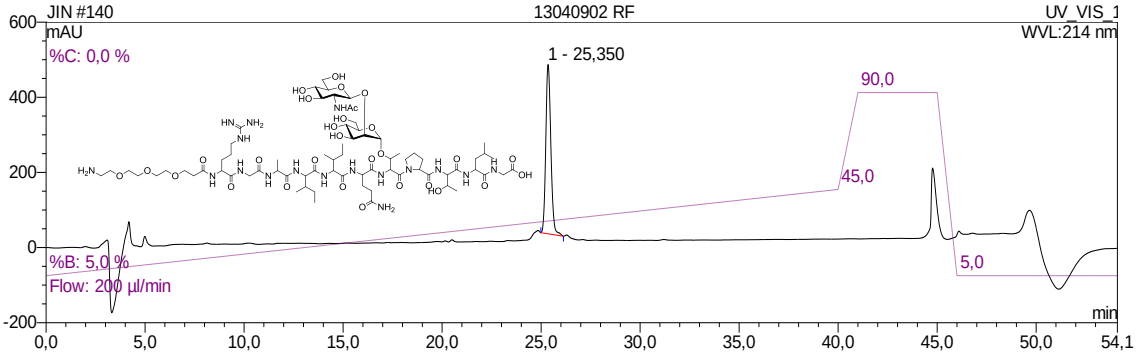
Compound **191**, Sequence: NH_2-Spacer-RGAIIQT*PTLG, *=Gla-GlcNAc-Man.



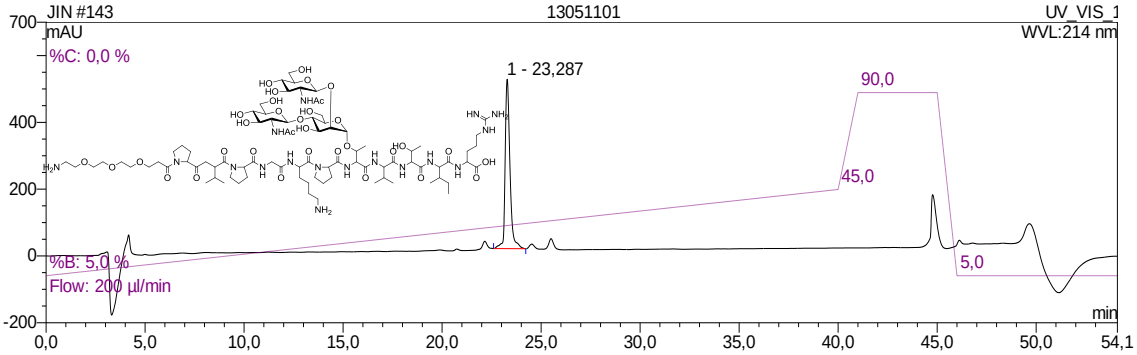
Compound **192**, Sequence: NH_2-Spacer-PVPGKPT*VTIR, *= GlcNAc-Man.



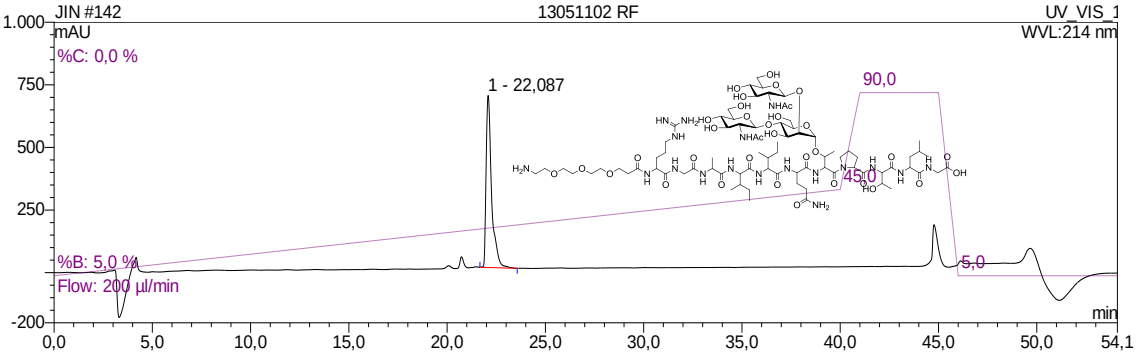
Compound **193**, Sequence: NH_2-Spacer-RGAIIQT*PTLG, *=GlcNAc-Man.



Compound **194**, Sequence: NH_2-Spacer-PVPGKPT*VTIR, *=2,6 GlcNAc-Man.



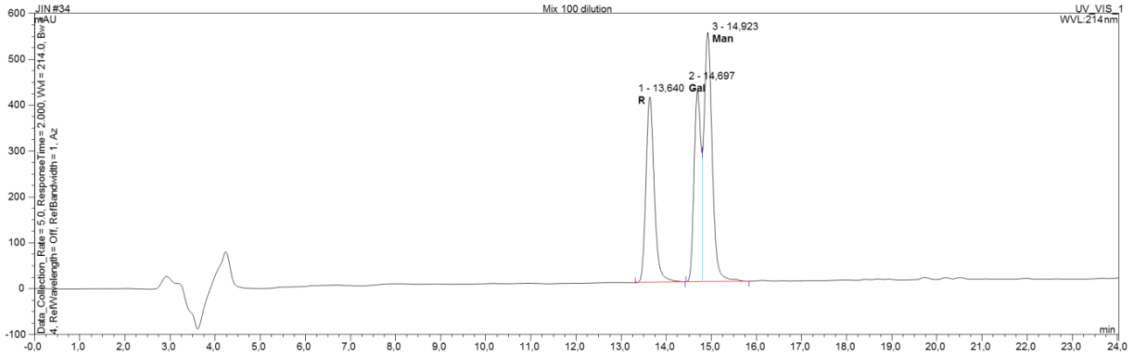
Compound **195**, Sequence: NH_2-Spacer-RGAIIQT*PTLG, *=2,6 GlcNAc-Man.

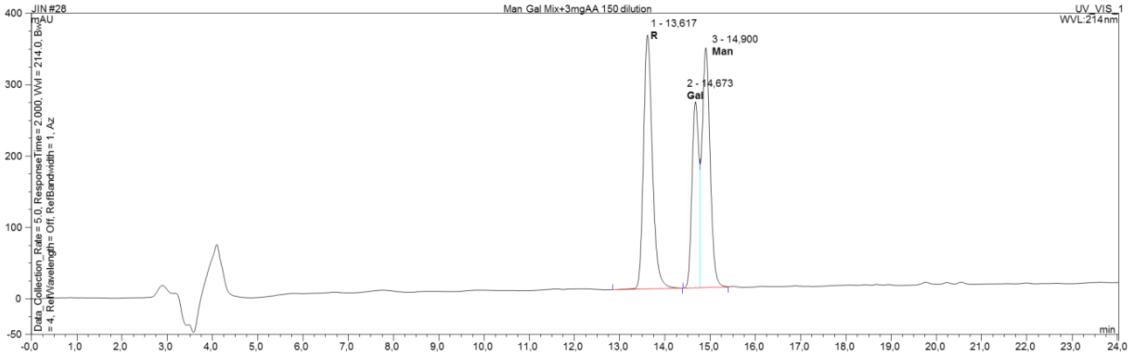


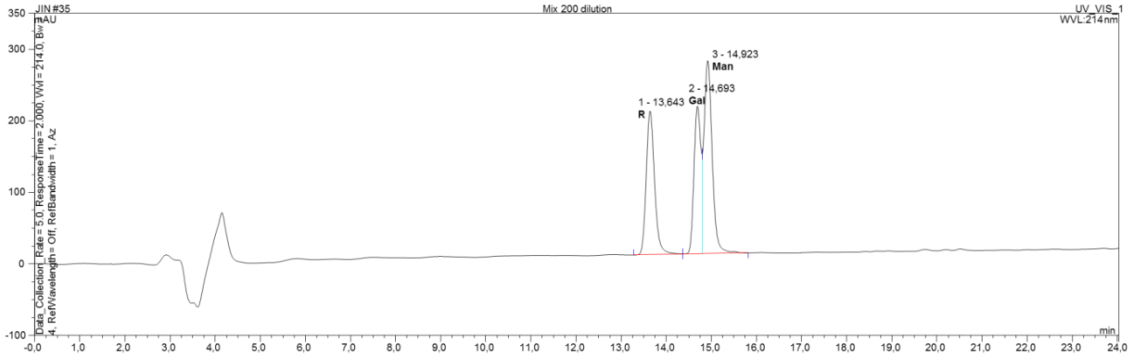
9.3 HPLC Chromatogram Monosaccharide Analysis (determination glycopeptide loading on KLH)

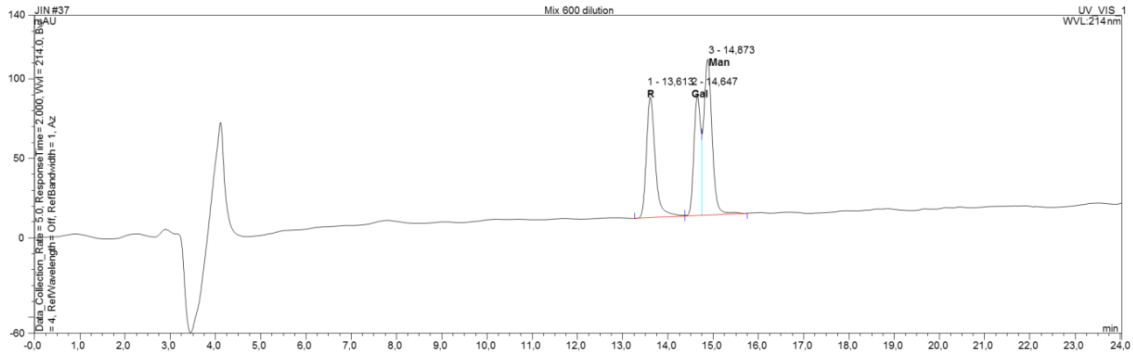
Chromatogram for calibration curve, integration of combined galactose/mannose peak after AA derivatization, R stands for reagent (Anthranilic acid, AA):

HPLC of dilution series of 1:1 Gal:Man mixture for calibration



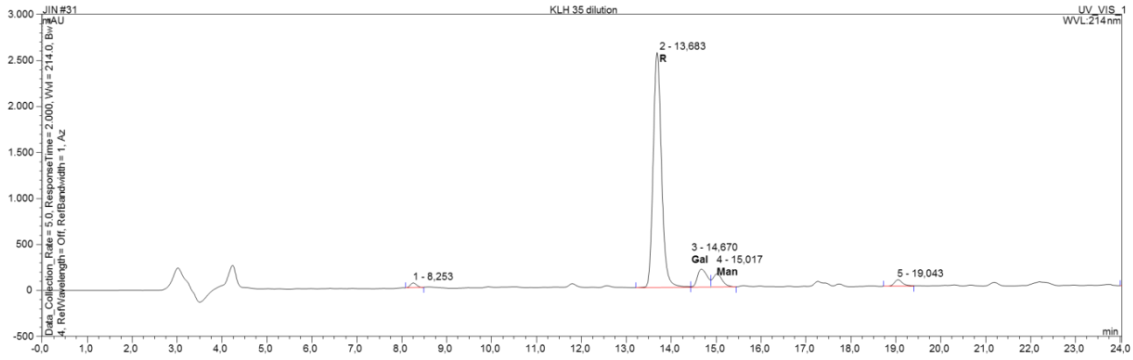






*Chromatogram after hydrolysis of glycopeptide-KLH conjugate **178** and monosaccharide AA derivatization, R stands for reagent (Anthranilic acid, AA):*

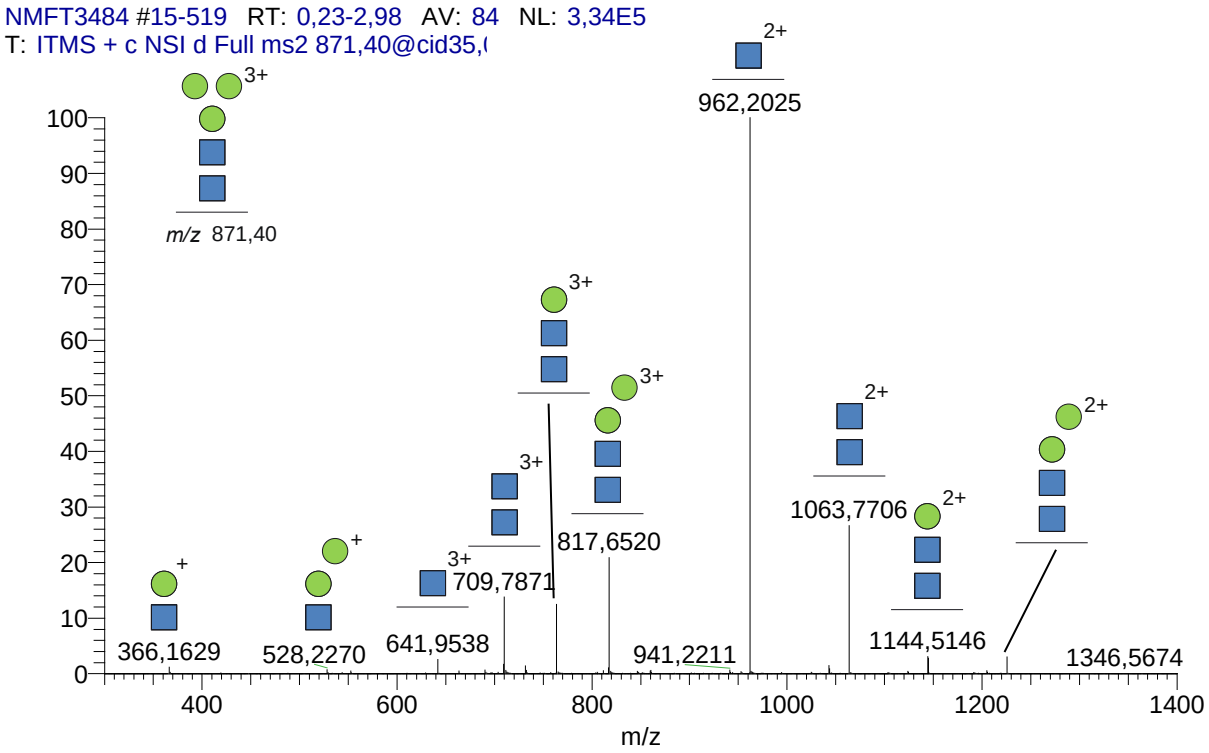
HPLC of labeled glycans cleaved from KLH conjugate



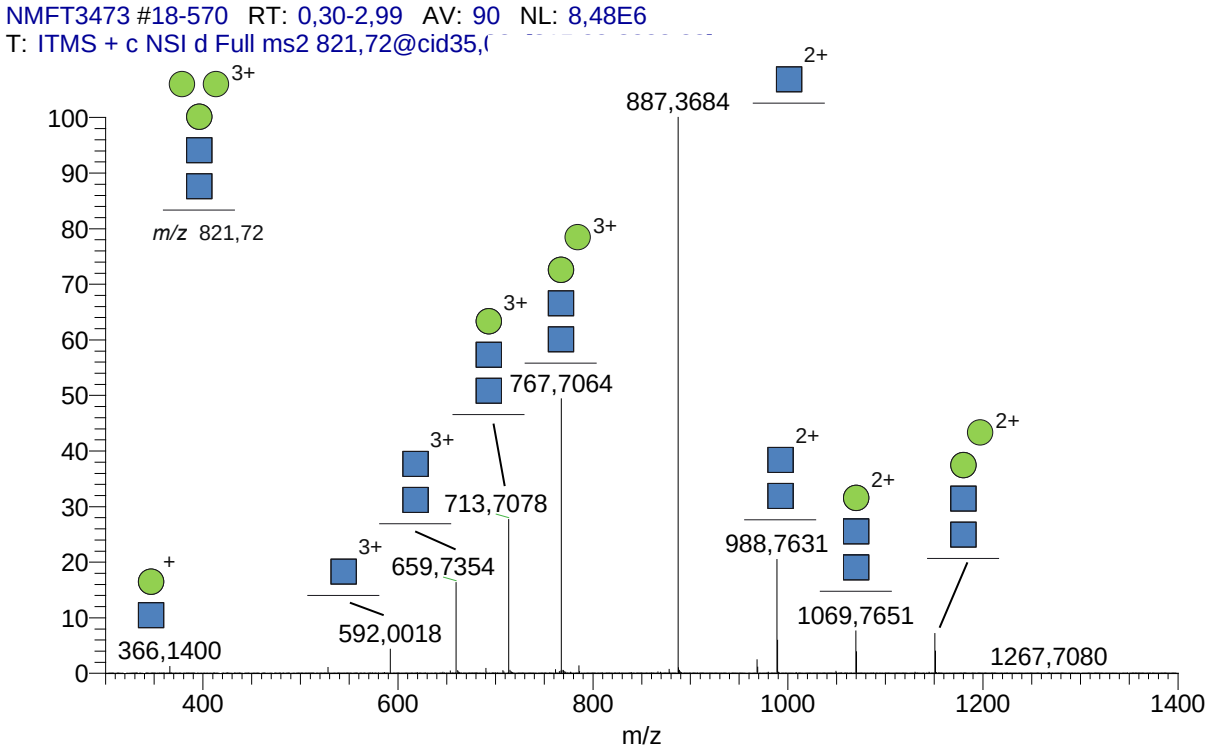
10 Appendix 3: MS spectra

10.1 MS2 fragmentation of enzymatically extended *N*-glycopeptides

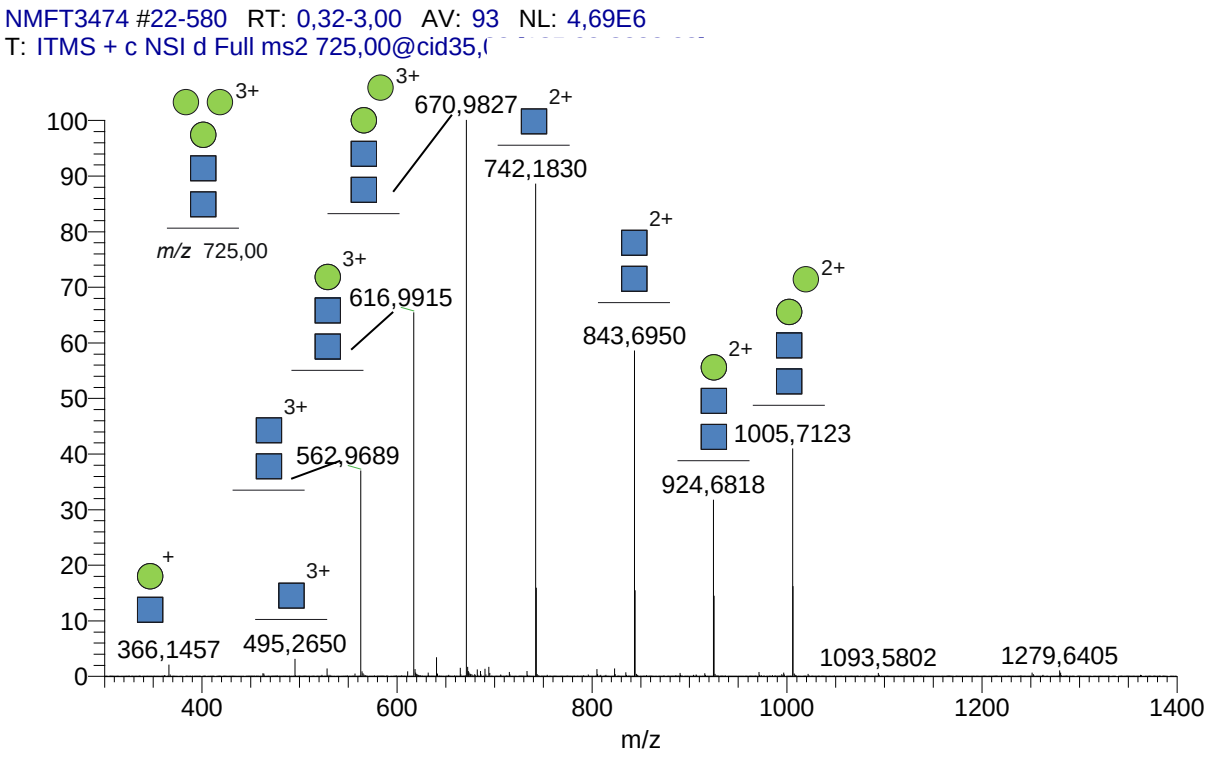
MS2 fragmentation of the pentasaccharide core glycopeptide **164**.



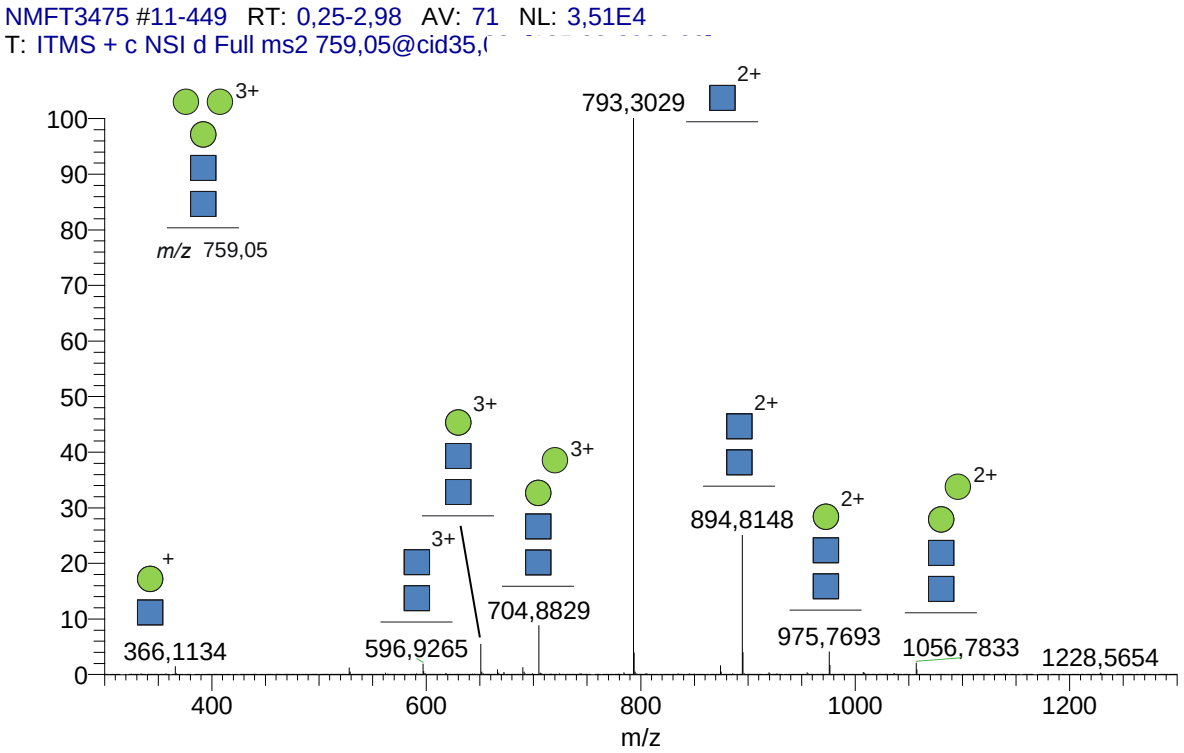
MS2 fragmentation of the pentasaccharide core glycopeptide **165**.



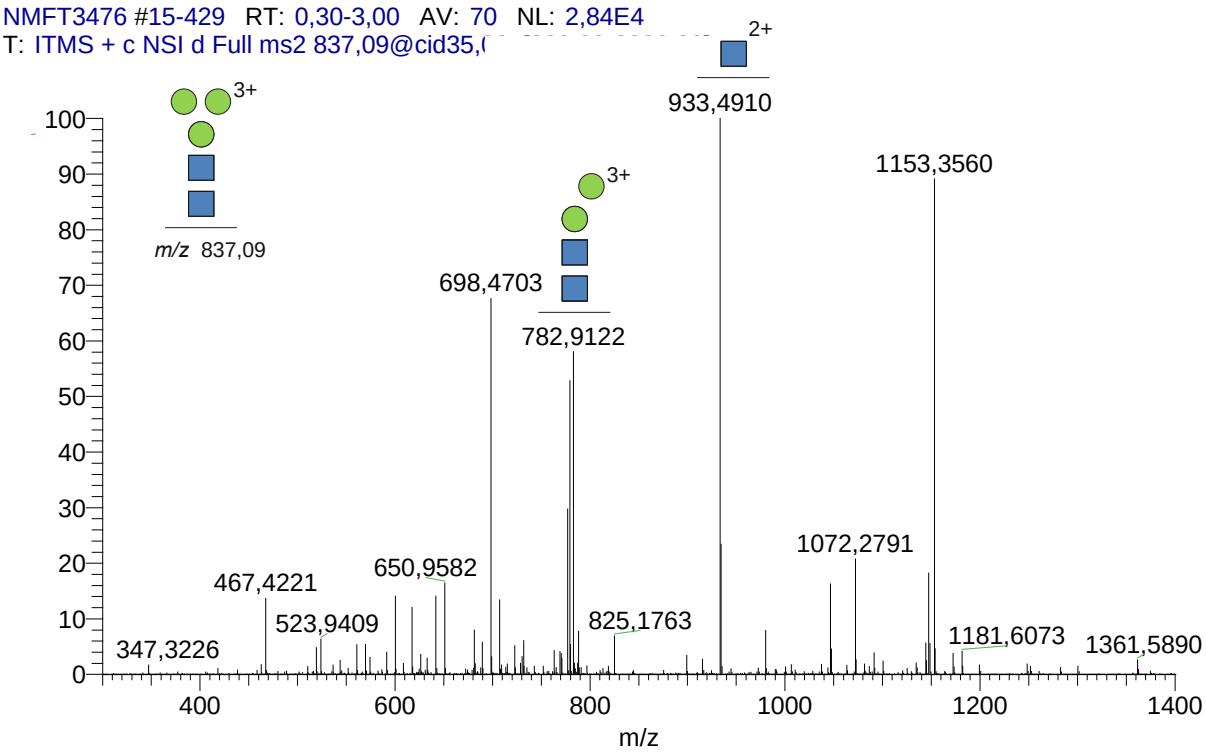
MS2 fragmentation of the pentasaccharide core glycopeptide **166**.



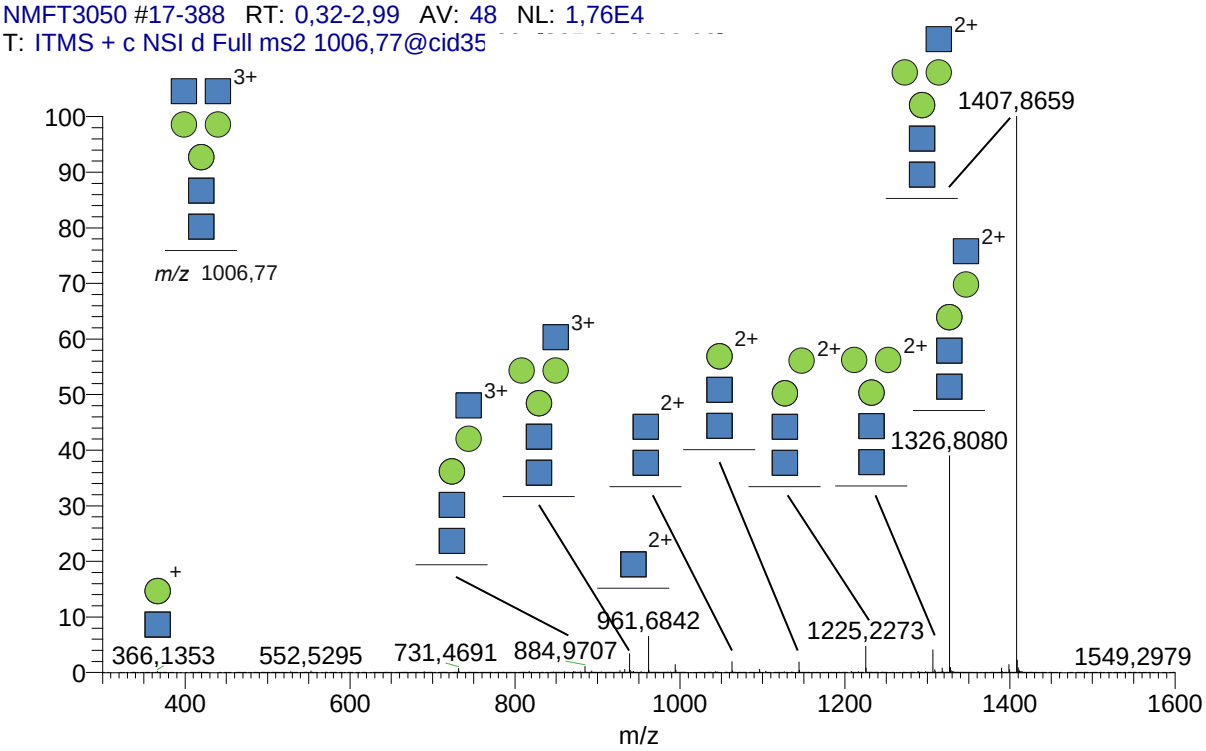
MS2 fragmentation of the pentasaccharide core glycopeptide **167**.



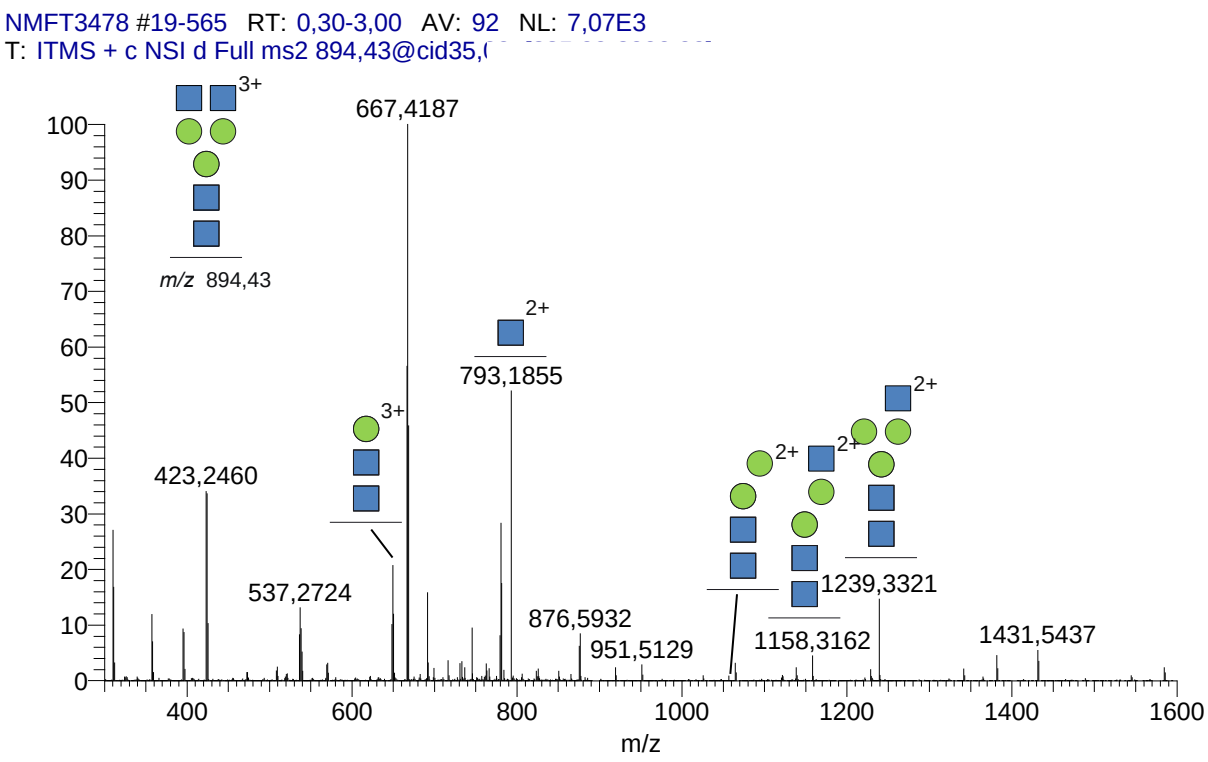
MS2 fragmentation of the pentasaccharide core glycopeptide **168**.



MS2 fragmentation of the heptasaccharide *N*-glycopeptide **169**.



MS2 fragmentation of the heptasaccharide *N*-glycopeptide **170**.



10.2 CID-MSn and HCD measurements of *N*- and *O*- mannosyl glycopeptides

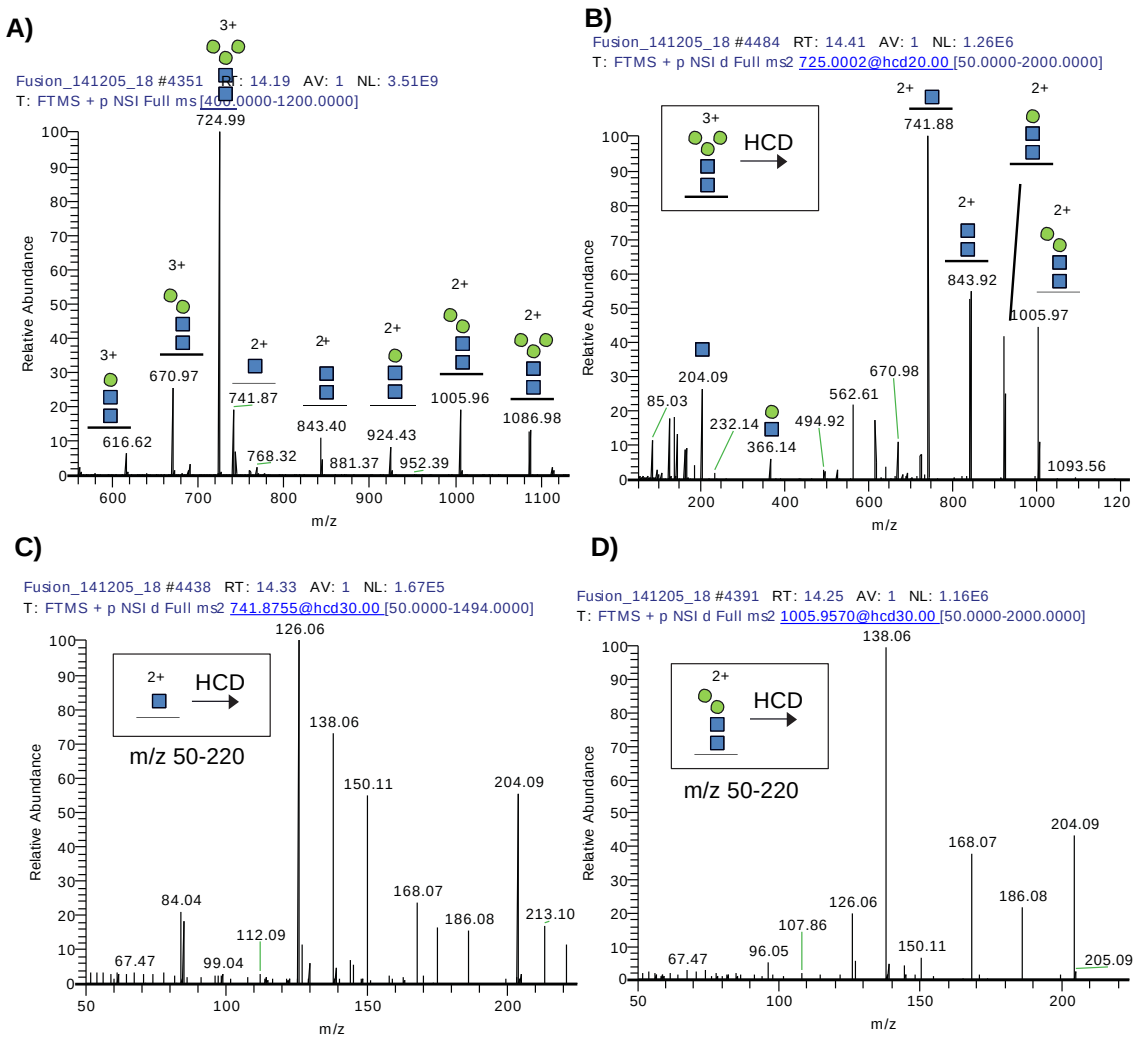


Figure 27. LC-MS/MS of the synthetic *N*-glycopeptide **165**. **A)** Precursor MS1 scan of **165** showing in source fragmentation. **B)** Full HCD spectrum of **165**. **C)** The oxonium ion m/z region of the HCD spectrum for the GlcNAc-*N*- substituted peptide.

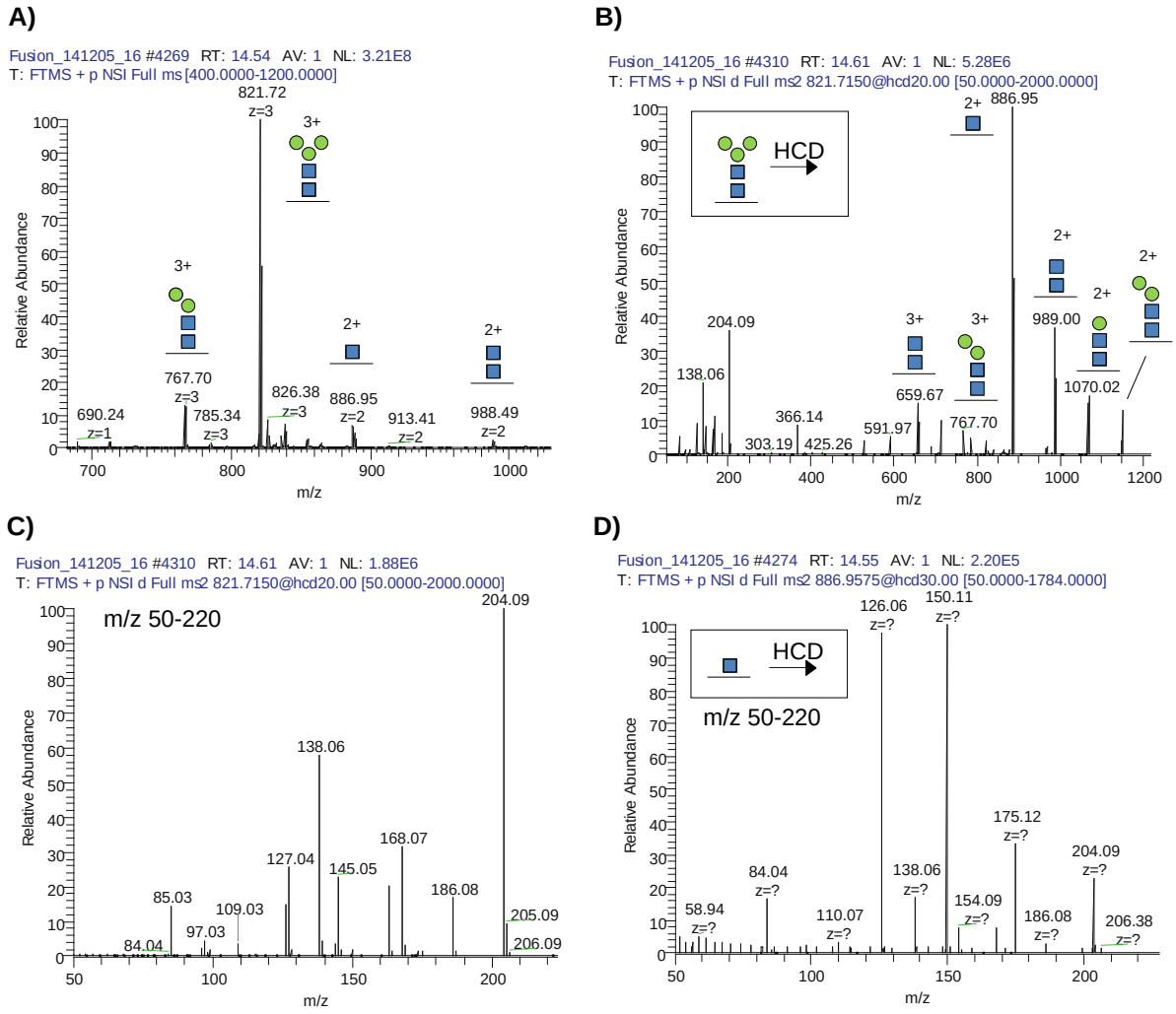


Figure 28. LC-MS/MS of the synthetic *N*-glycopeptide **166**. **A)** Precursor MS1 scan of **166** showing in source fragmentation. **B)** Full HCD spectrum of **166** and **C)** the oxonium ions of the *m/z* 50-220 region. **D)** The oxonium ion m/z region of the HCD spectrum for the GlcNAc-*N*- substituted peptide generated via the in source fragmentation.

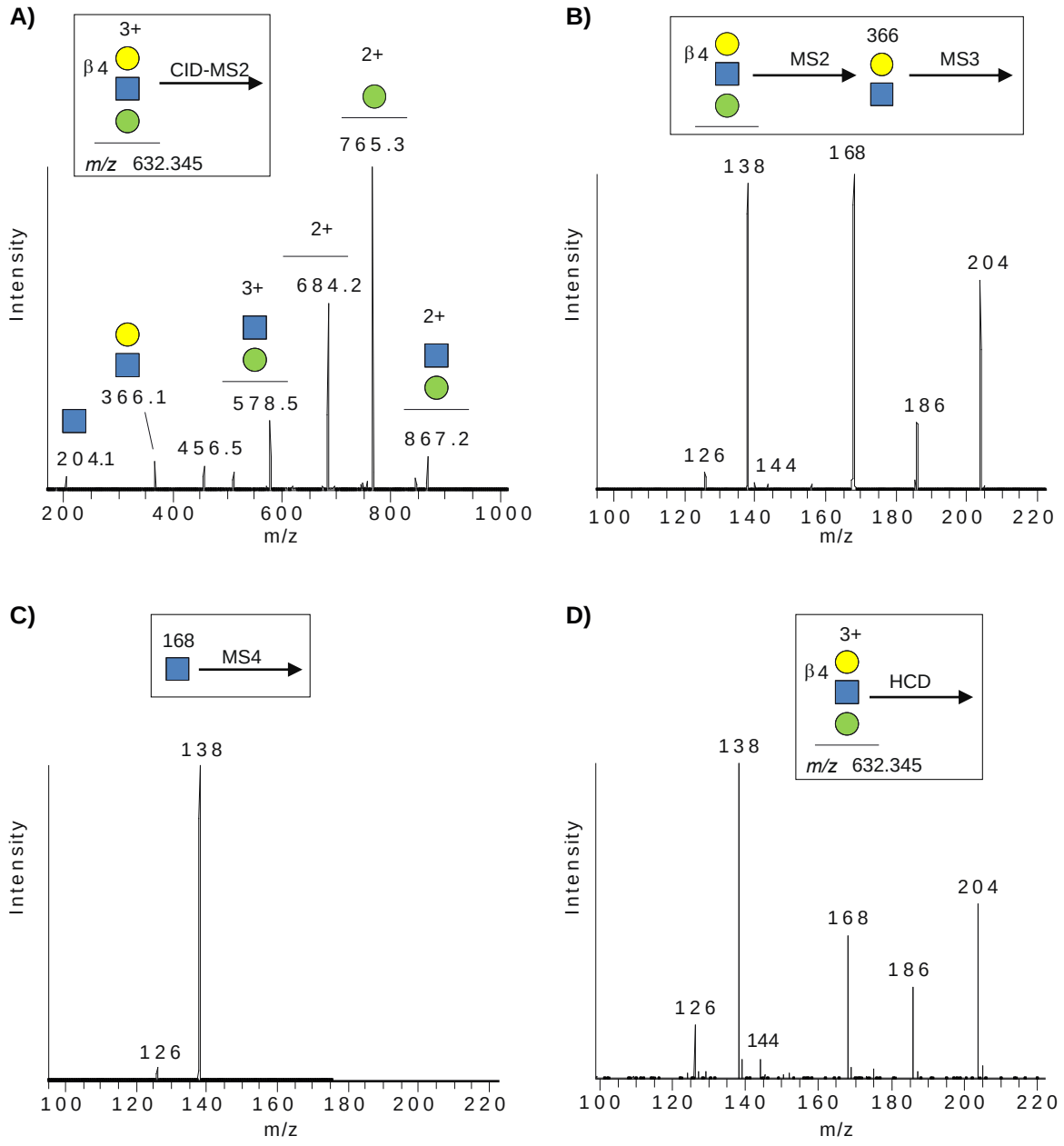


Figure 29. CID-MSn and HCD of Galβ4GlcNAc terminated glycopeptides **190**. **A)** The CID-MS2 spectrum of the Galβ4GlcNAcβ2Manα1-*O*- substituted peptide showed glycosidic fragmentation and formation of $[Gal\beta4GlcNAc]^+$ (*m/z* 366.1). **B)** CID-MS3 at *m/z* 366. **C)** Further CID-MS4 at *m/z* 168 to demonstrate the selective formation of the *m/z* 138 ion. **D)** The HCD spectrum at the 25% NCE level and the *m/z* 100-220 region. The peptide sequence is PVPGKPT*VTIR. Yellow circle is Gal (162 amu); green circle is Man (162 amu); blue square is GlcNAc (203 amu).

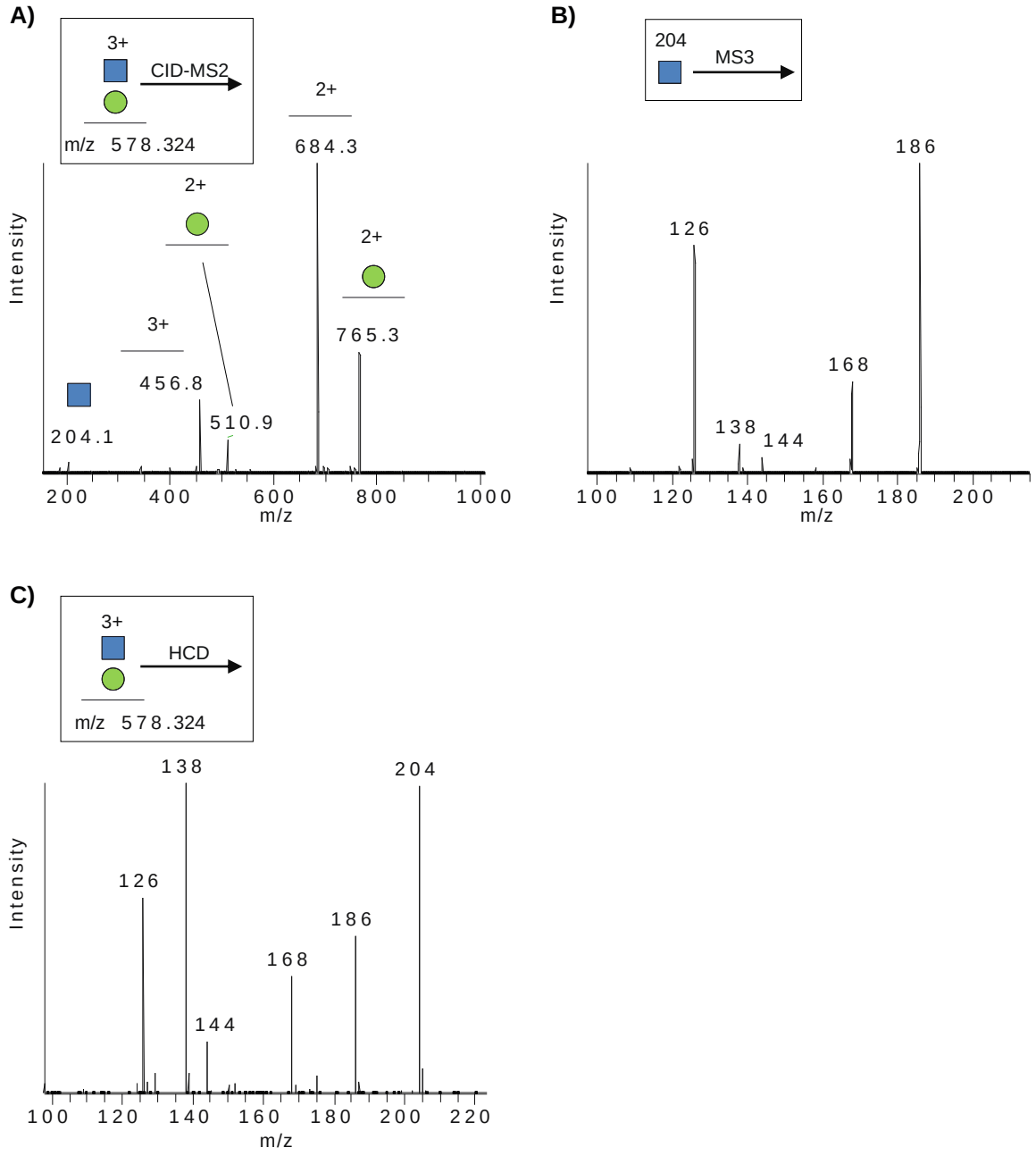
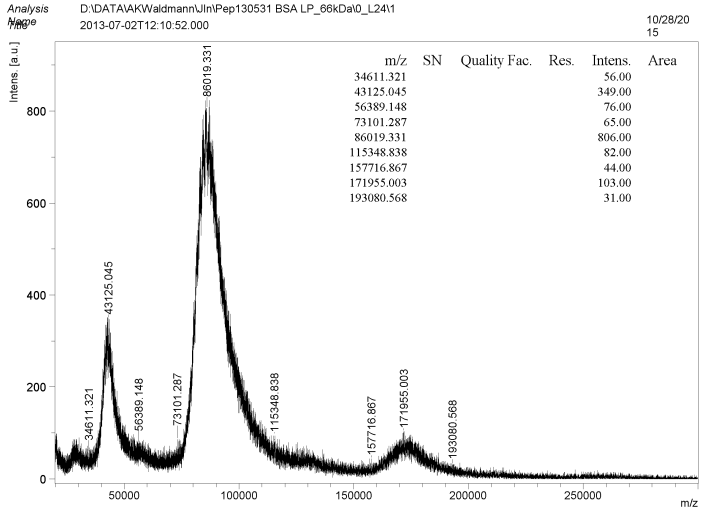
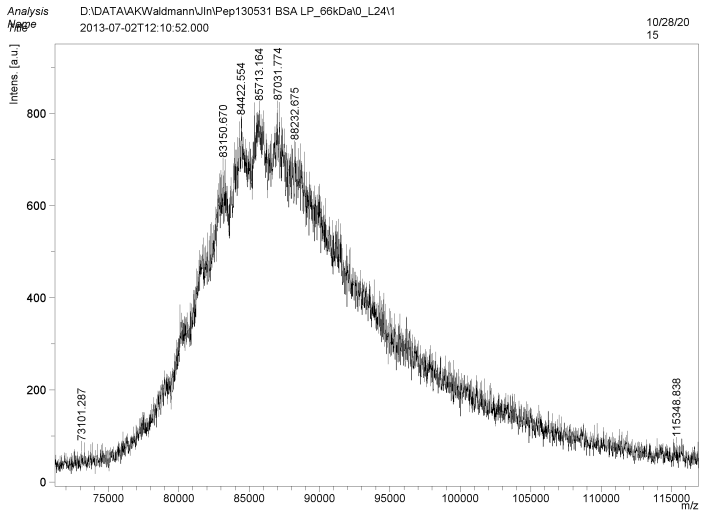


Figure 30. CID-MSn and HCD of GlcNAc terminated glycopeptides **192**. **A)** The CID-MS2 spectrum of the GlcNAcβ2Manα1-*O*- substituted peptide showed glycosidic fragmentation and formation of $[GlcNAc]^+$ (*m/z* 204.1). **B)** CID-MS3 at *m/z* 204. **C)** The HCD spectrum at the 25% NCE level and the *m/z* 100-220 region. The peptide sequence is PVPGKPT*VTIR. Green circle is Man (162 amu); blue square is GlcNAc (203 amu).

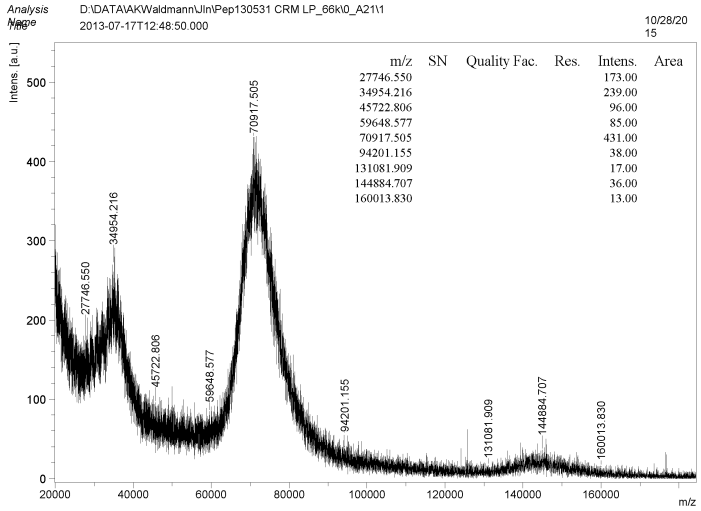
10.3 MALDI measurements of glycopeptide-protein conjugates

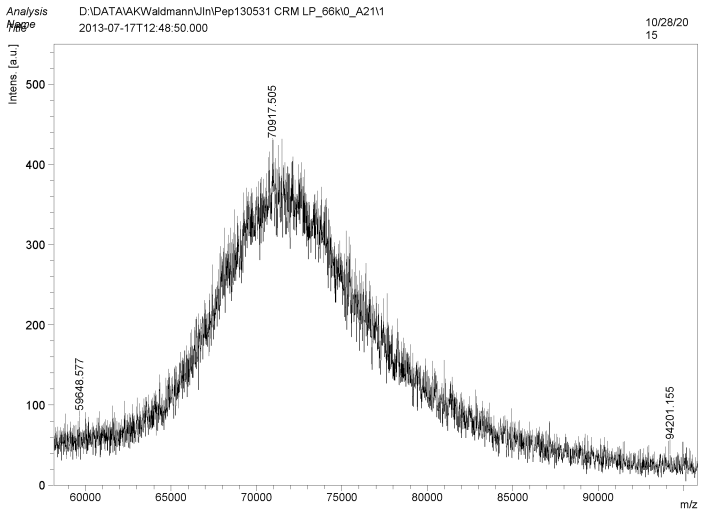
MALDI spectrum of BSA-conjugate **186**



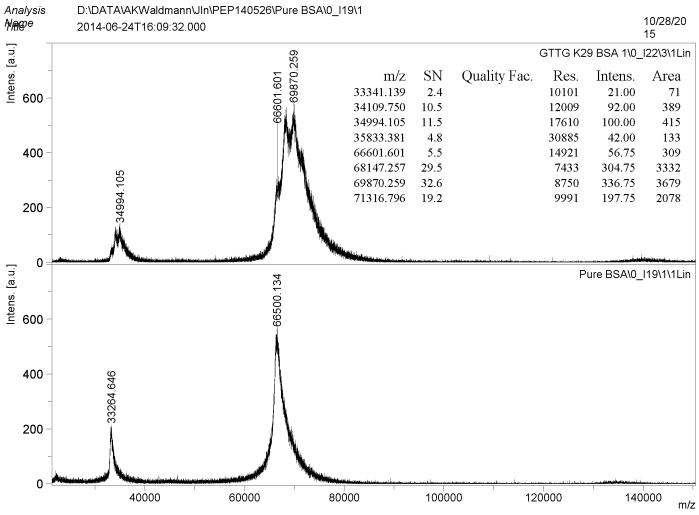


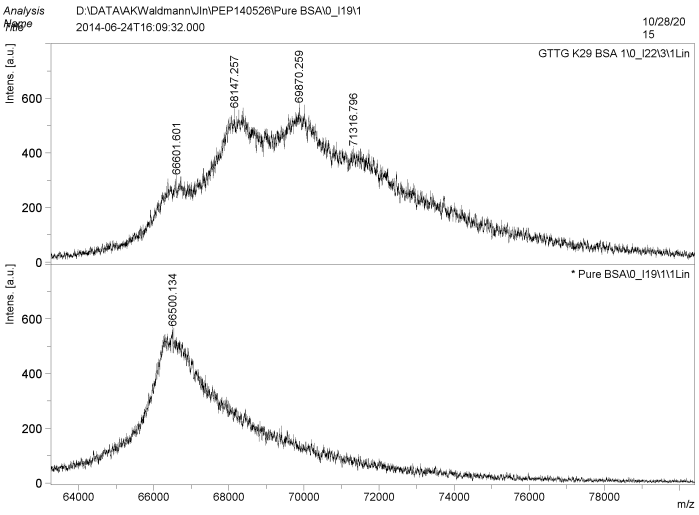
MALDI spectrum of CRM-conjugate **187**



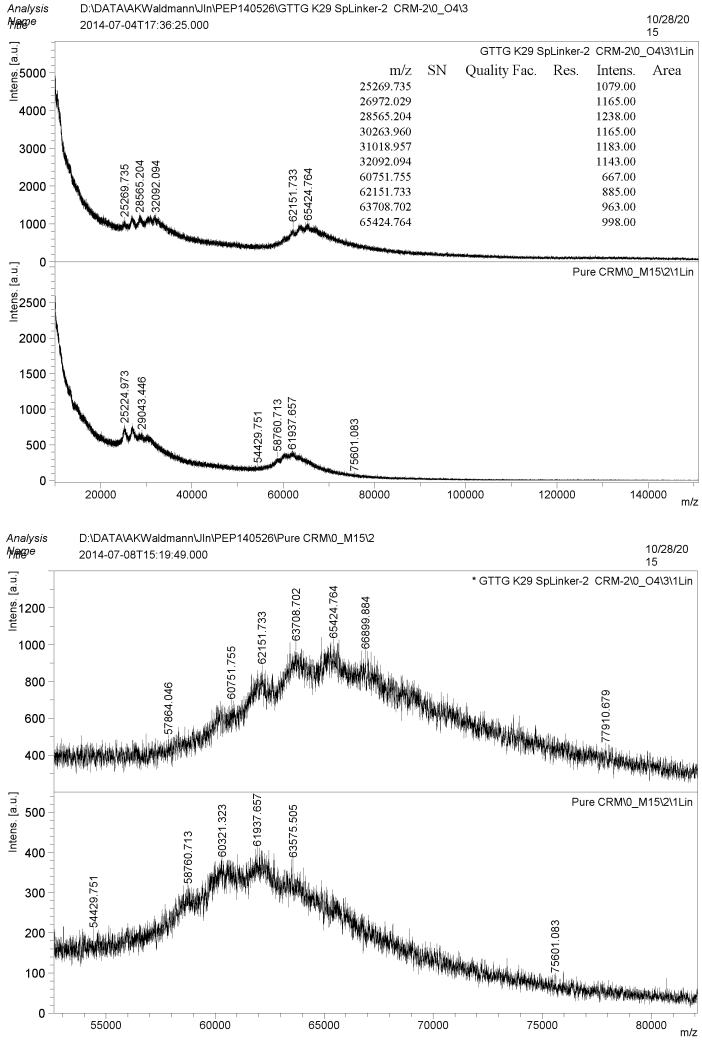


MALDI spectrum of BSA-conjugate **188** (top) compared with pure BSA (bottom)





MALDI spectrum of CRM-conjugate **189** (top) compared with pure CRM (bottom)



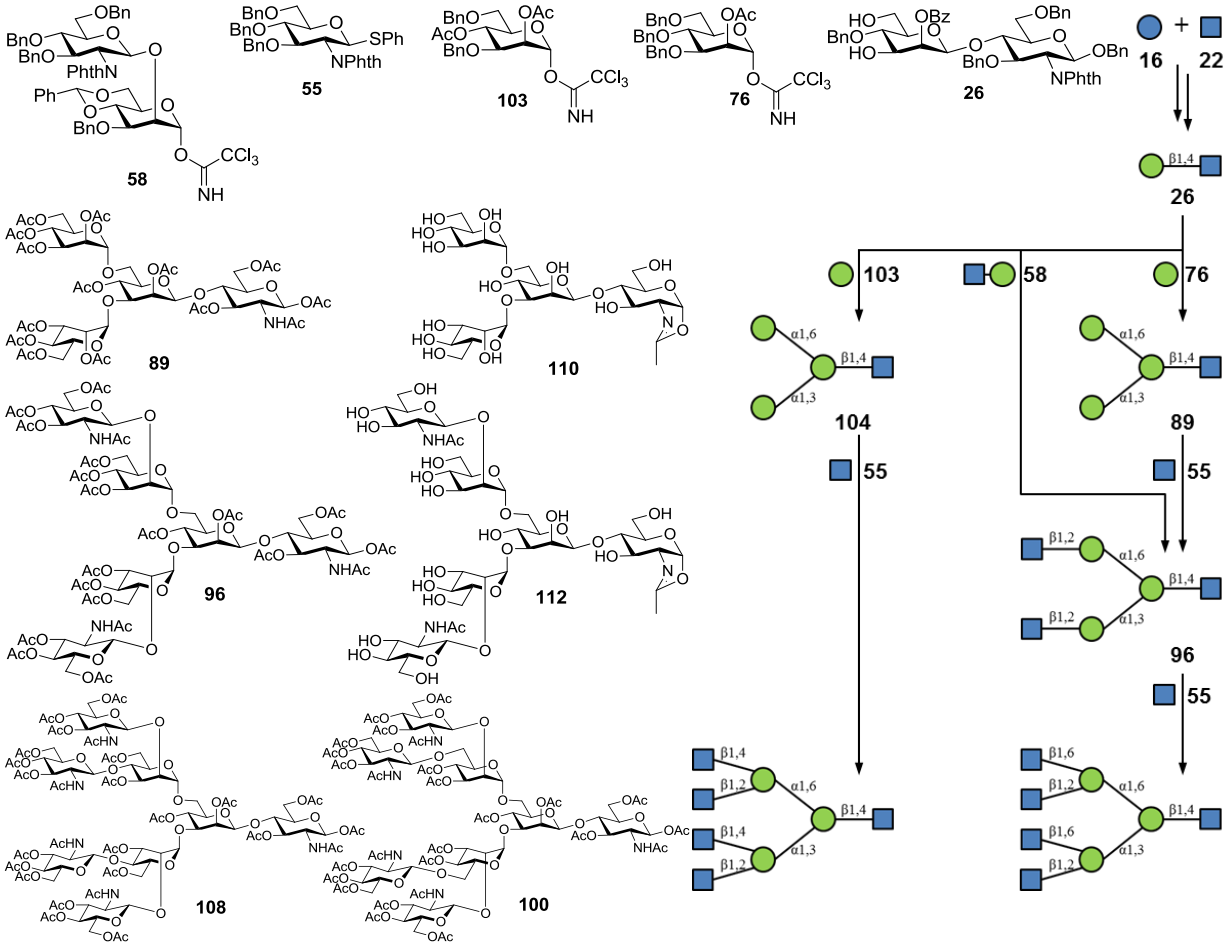
ZUSAMMENFASSUNG

Diese Dissertation beinhaltet die Synthese von Glykanen sowie glykosylierten Aminosäuren zu Herstellung verschiedener Glykopeptide. Anhand dieser wurden Interaktionen zwischen Proteinen und N- und O-Glykosylierung untersucht, sowie Tools zur Strukturanalyse entwickelt. Der Fokus lag dabei auf verschiedenen *N*-Glykanstrukturen sowie *O*-Mannosylierung. Diese Strukturen weisen eine strukturelle Ähnlichkeit auf. Komplexe *N*-Glykane und die verlängerten *O*-Mannosylierten Aminosäuren weisen gleiche terminale Struktureinheiten auf und wurden daher ausgehend von gemeinsamen Bausteinen hergestellt. Darüber hinaus ähnelt die mannosereiche *N*-Glykosylierung, der üblicherweise in Hefe vorkommenden O-Mannosylierung. Dagegen ähnelt die komplexe *N*-Glykosylierung der verlängerten *O*-Mannosylierung, welche Proteine in eukaryotischem Gehirngewebe modifiziert.

Zur Erstellung der *N*-Glykan-Oligosaccharide, welche einen gemeinsamen asparagingebundenen Pentassacharidkern aufweisen, wurde zunächst eine Manβ1-4GlcNAc Disaccharid Kerneinheit **26** synthetisiert. Verschiedene Untereinheiten **76**, **58**, **103** und **55** wurden symmetrisch und schrittweise an den Mannosekern gekuppelt. Vier komplexe *N*-Glykanstrukturen **89**, **96**, **100** und **108** wurden synthetisiert. Zwei davon wurden zu den Oxazolin-Donatoren **110** und **112** umgesetzt und in enzymatischen Endoglycosidse-Kupplungen mit einer synthetischen *N*-GlcNAc-Peptidbibliothek verwendet.

Die enzymatischen Endoglycosidasekupplungen des Tetrasaccharidoxazolins **110** an verschiedene *N*-GlcNAc-Peptide waren erfolgreich und alle Kupplungen in Lösung waren innerhalb von 0,5 h bei voller Umsetzung abgeschlossen. Dies wurde mittels HPLC und hochauflösendem ESI-MS verifiziert. Zur effizienten Erstellung einer *N*-Glykopeptidblibliothek mit homogener komplexer Glykosylierung wurden - anstelle von sequenziellen enzymatischen Kupplungen in Lösung – verschiedene *N*-GlcNAc-Peptide mittels *on-slide* enzymatischer Glykosylierung verlängert. Im Prinzip wurden *N*-GlcNAc-Peptide auf einen Microarrayslide gespottet und immobilisiert, gefolgt von einer enzymatischen einschritt-*on-slide*-*N*-Glykanverlängerung durch Nutzung von synthetischen Oxazolindonorbausteinen. Durch Vergleich der *on-slide*-Reaktionen mit vorsynthetisierten internen Peptidstandards wurde geschlussfolgert, dass die *on-slide* Kupplungen mit Endoglycosidase innerhalb einer Stunde abgeschlossen waren. Darüber hinaus wiesen die Ergebnisse darauf hin, dass die Position des *N*-GlcNAc-Asn-Restes im Peptidrückgrat keinen großen Einfluss auf die *on-slide*-Kupplung hat, obwohl die *N*-GlcNAc-Peptide durch einen *N*-terminalen Spacer an den Microarryslide gekoppelt waren. Zusammenfassend nachweisen die Experimente, dass es

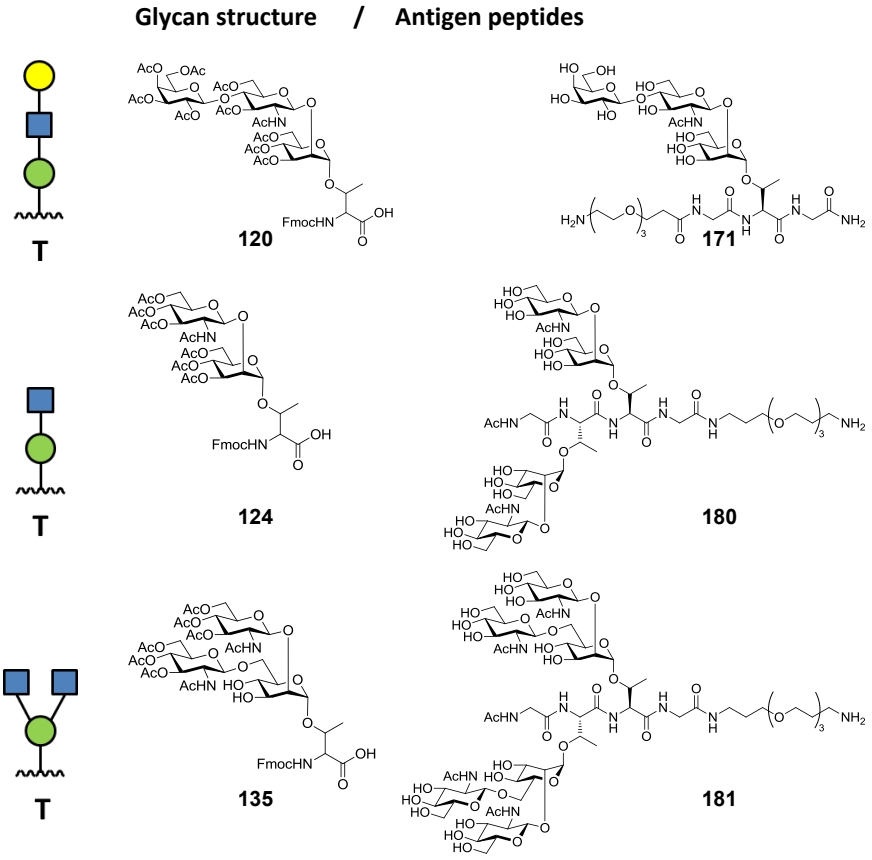
möglich ist eine *N*-Glykopeptidbibliothek mit komplexer Glykosylierung effizient auf einem Microarrayslide zu erzeugen. Die erhaltene Hepta- und Pentasaccharid-*N*-Glykopeptidbibliothek wurde im Rahmen dieser Arbeit in ersten Microarraystudien verwendet, um die Bindungsinteraktionen mit dem Lektin Concanavalin A (ConA) zu untersuchen. Zudem wurden ausgewählte Peptide zur ESI-MS-Fragmentierung verwendet, um die Methoden zur Strukturaufklärung von *N*-Glycopeptiden zu verbessern.



Einige der Bausteine aus der *N*-Glykansynthese wurden auch in der Synthese von *O*-mannosylierten Aminosäuren verwendet um die Bausteine **120**, **124** und **135** herzustellen. Proteine, welche an Ser- und Thr-Seitenketten *O*-mannosyliert sind, sind an wichtigen Funktionen in der Gehirn- und Muskelglykobiologie beteiligt. Trotz der klaren Bedeutung der *O*-Mannosylierung, existieren bislang keine Werkzeuge zur spezifischen Detektion, Anreicherung und Identifizierung von Proteinen, welche die Modifikation tragen. Um diese Prozesse zu studieren, wurde ein Projekt initiiert, um glykan- und

glykopeptidspezifische Antikörper, welche nützliche Werkzeuge zur Detektion und Anreicherung von *O*-mannosylierten Proteinen darstellen könnten, zu generieren.

Vakzinkonstrukte wurden ausgehend von Glykopeptiden, welche diese verlängerten Mannoseeinheiten enthielten, hergestellt. Diese stellen eine Voraussetzung für die Studien an *O*-Mannosylierung dar. Die Fmoc-*O*-mannosylierten Aminosäuren **120**, **124** und **135** wurden hergestellt und zur Glykopeptidsynthese genutzt, um **171**, **180** und **181** mit angekuppelten Spacern zu erhalten. Diese Glykopeptide wurden dann in Glykopeptid-Immuncarrier zur Antikörperinduktion in Kaninchen eingebaut. Zur Bewertung der Antikörper wurden zusätzliche Glykopeptide generiert und in ELISA-Neutralisationsexperimenten sowie in Glykopeptidmicroarrays verwendet. Weiter wurde die erhaltene *O*-Mannosylglykopeptidbibliothek verwendet, um Interaktionen mit glykanbindenden Lektinen zu untersuchen. Diese Lektine wurden hinsichtlich ihres Potentials als analytische Tools für die Detektion und Anreicherung spezifischer *O*-Mannosylglykoproteine oder -glykopeptide bewertet.



Das Antigenpeptid **171** mit einem Gal-GlcNAc-Man Trisaccharid wurde mit monovalenter Glykosylierung am Peptid und *N*-terminalem Spacer zur Proteinkonjugation synthetisiert. Das an den KLH-Immuncarrier

konjugierte Gal-GlcNAc-Man Antigenpeptid **171** wurde Kaninchen verabreicht. Das erhaltene Antikörperserum wurde mittels normalem ELISA, ELISA-Neutralisationsexperimenten sowie Glykopeptid-Microarrayanalyse analysiert. Es wurde ein starker Antikörpertiter, welcher das Antigenpeptid **171** erkannte detektiert. Die Microarrayanalyse zeigte, dass Peptid **171**, welches das kürzere, auf demselben Peptidrückgrat wie das Antigen präsentierte, GlcNAc-Man enthielt, zu einem gewissen Grad von den Antikörpern erkannt wurde. Des Weiteren war die Antikörperspezifität zur Antigenstruktur extrem hoch, sodass wenn die *O*-Mannosylglykane an andere Peptidsequenzen wie Glykopeptid **190** und **191** präsentiert wurden, die Antikörpererkennung vollständig oder teilweise verloren war.

Um die Spezifität der induzierten Antikörper zur Glykanstruktur zu erhöhen und die Erkennung der darunterliegenden Peptidsequenz zu reduzieren wurde ein zweites Antigenpeptid **180** mit dem GlcNAc-Man-Disaccharid hergestellt, welches divalent am Peptidrückgrat glykosyliert war. Der *N*-Terminus wurde mit einer Acetylkappe und der C-Terminus mit einem Spacer zur Proteinkonjugation versehen. Nach der Immunisierung wurde das erhaltene Antikörperserum erneut mittels ELISA und Glykopeptidmikroarryexperimenten analysiert. Die Antigenglycopeptid-gerichtete Immunantwort war sehr stark. Die erhaltenen Antikörper erkannten zudem andere Glykopeptidsequenzen, welche die gleiche GlcNAc-Man-Thr-*O*-Glykosylierung aufwiesen, wie Peptid **192**, **193** und **172**. Cluster-Glykanpräsentation am Peptidrückgrat begünstigte die Antikörpererkennung dramatisch. Zusammenfassend wurden Antikörper mit sehr hoher Spezifität gegenüber der *O*-Mannosylglykanstruktur sowie der darunterliegenden Peptidsequenz erhalten. Präsentation mit höherer Glykandichte am Peptidrückgrat würde die Antikörperspezifität gegenüber der Glykanstruktur erhöhen.

Die *O*-Mannosylglykopeptidbibliothek wurde zudem verwendet, um Interaktionen mit bestimmten Lektinen zu untersuchen. Eine Analyse des glykanbindenden Lektins Concanavalin A (ConA), welches bekanntlich α-Mannosereste erkennt zeigte, dass einzigartige strukturelle Glykan- und Glykopeptidbindungsepitope erkannt wurden. Während monoglykosylierte Peptide, welche die simplere GlcNAc-Man-Disaccharidstruktur enthielten, stark erkannt wurden, war die Reaktivität gegenüber der 2,6-verzweigten $GlcNAc_2$-Man-Thr- oder der linearen Gal-GlcNAc-Man-Thr-Struktur eher schwach. Die Präsentation von geclusterten Glykanen ergab keine Erkennung von ConA. Diese Ergebnisse werden einen Impact auf die Strukturanalyse von *O*-Mannosylglykoproteinen haben. Zusätzlich wurden ESI-MS-Fragmentierungsexperimente an ausgewählten *O*-Mannosylpeptiden durchgeführt, um die Methodik für die *O*-Glykopeptid-Strukturanalyse zu verbessern.

EIDESSTATTLICHE ERKLÄRUNG

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Die Arbeit wurde weder bisher im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt. Es haben bisher keine Promotionsverfahren stattgefunden.

Dortmund, den 26.01.2016

Jin Yu