Design, Synthesis and Applications of an Imidazole-Phosphonate-Based Mimic of 3-Phosphohistidine

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Dissertation

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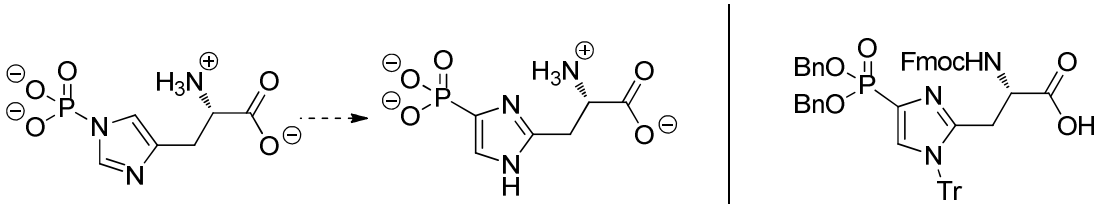
Dedicated to my father

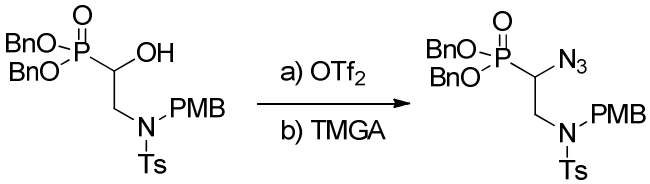
1 Abstract

Protein phosphorylation is a post-translational modification that plays a significant role in many cell processes and regulatory pathways. In eukaryotic cells, six percent of total protein phosphorylation occurs on the nitrogen atoms of the imidazole moiety of histidine. While phosphate esters (P-O bond) are relatively stable to hydrolysis, phosphoramidates (P-N bond) hydrolyze easily. Hydrolysis of 3-phosphohistidine **7** (pHis) is even faster under acidic conditions, since the protonated imidazole behaves as an excellent leaving group. Therefore, research focused on phophorylated histidine containing proteins is complicated by the lack of analytical techniques that are compatible with pHis's inherent instability. Following the success of phosphotyrosine antibodies, attempts have been made to raise antibodies specific for pHis containing peptides. However, pHis itself cannot be used for immunisation as the phosphoramidate is hydrolyzed before a strong immune response can be realized. Therefore, a new pHis analogue **63** was designed and synthesized. This mimic closely resembles pHis's electronic and structural properties under physiological conditions, due to the conserved imidazole ring, while the P-N bond is exchanged for a stable P-C bond. In order to raise antibodies the pHis mimic should be incorporated into a peptide. Therefore, the mimic was decorated with protective groups suitable for Fmoc-based SPPS (**65**).

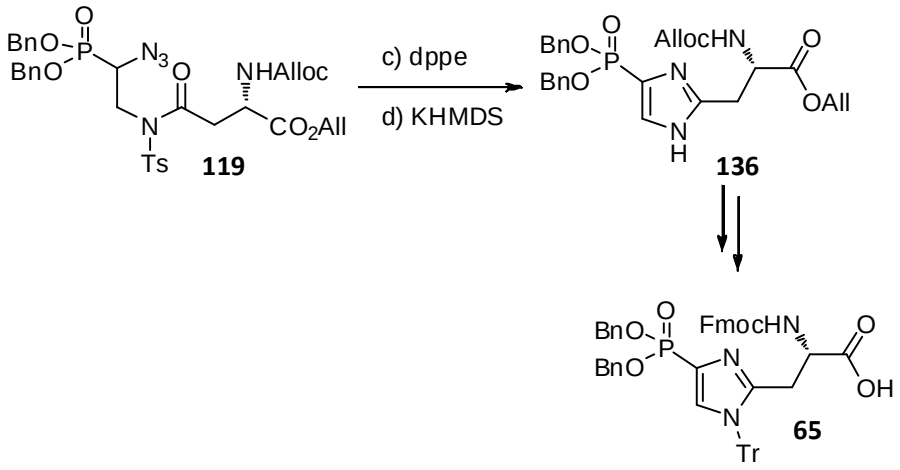


7 **63** **65**

Various routes have been investigated to synthesize **65**. The successful retrosynthetic strategy involved coupling of compound **66** with an Alloc/allyl protected aspartic acid to yield amide **119**. Compound **66** was made via a nine step sequence, which included the activation of compound **113** as a triflate followed by substitution with TMGA. A dppe promoted aza-wittig ring closing reaction of the amide was used to form the imidazoline. Aromatization of the imidazoline was realized by base induced elimination of the tosylate to yield **136**. Several protecting group manipulations gave final compound **65** in 22.7 percent yield over 15 steps.



113 **66**



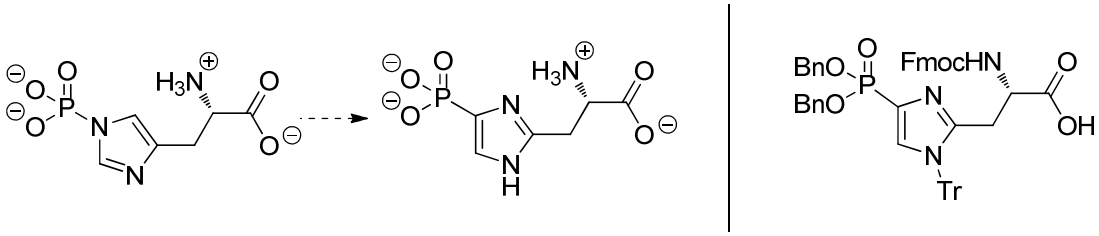
The resulting compound **65** was used to synthesize several peptides, of which two have been employed to raise antibodies. These antibodies successfully recognized natural pHis, both in dot blot tests with pHis containing peptides, as well as in cell lysates. These antibodies can be used in the near future to study the biological functions of pHis.

In order to study the related enzyme PHPT1, responsible for phophohistidine dephosphorylation, a library of 5-phosphonic acid imidazoles with various substituents on C-2 has been synthesized to act as a starting point for the development of a PHPT1 inhibitor. The synthetic strategy described for the synthesis of mimic **65** was applied to generate this compound set.

The antibodies, inhibitors and other tool compounds described in this thesis provide an invaluable toolset to increase the understanding of phosphorylation.

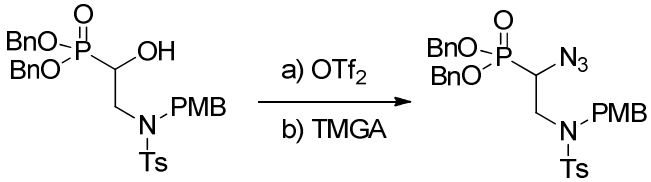
2 Zusammenfassung

Die Proteinphosphorylierung ist eine posttranslationale Modifikation, die eine entscheidende Funktion in vielen zellulären Prozessen und regulatorischen Signalwegen erfüllt. Sechs Prozent der gesamten Proteinphosphorylierungen in eukaryotischen Zellen geschieht an den Stickstoffatomen der Imidazolgruppe von Histidin. Während Phosphatester (P-O Bindung) relativ hydrolysestabil sind, werdenPhosphoramidite (P-N Bindung) leicht hydrolysiert. Die Hydrolyse von 3-Phosphohistidin **7** (pHis) geschieht unter sauren Bedingungen noch schneller, da protoniertes Imidazol eine exzellente Abgangsgruppe darstellt. Aus diesem Grund wird die Untersuchung von phosphoryliertenHistidinen innerhalb von Proteinen durch das Fehlen von analytischen Methoden, die mit der inhärenten Instabilität des pHis kompatibel sind, erschwert. Dem Beispiel der erfolgreichen Phosphotyrosin-Antikörper folgend, wurden Versuche unternommen Antikörper zu generieren, die spezifisch pHis innerhalb von Peptiden erkennen. pHis selber kann jedoch nicht für Immunisierungen verwendet werden, weil das Phosphoramidit hydrolysiert wird, bevor eine starke Immunantwort realisiert werden kann. Deshalb wurde ein neues pHis Analogon **63** entworfen und synthetisiert. Das Analogon imitiert die elektronischen und strukturellen Eigenschaften von pHis unter physiologischen Bedingungen aufgrund des konservierten Imidazolringes, während die P-N Bindung durch eine stabile P-C Bindung ersetzt wurde. Um Antikörper zu generieren, sollte das pHis Analogon in einen Peptid integriert werden. Daher wurde das Analogon mit Schutzgruppen versehen, die den Einsatz in Fmoc SPPS erlauben (**65**).

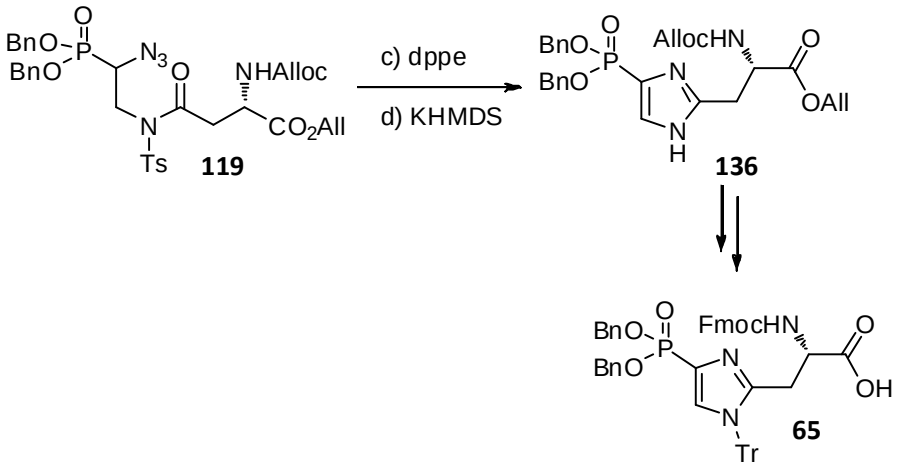


7 **63** **65**

Zur Synthese von **65** wurden verschiedene Syntheserouten untersucht. Die erfolgreiche retrosynthetische Strategie beinhaltet die Kupplung von Verbindung **66** mit Alloc/Allyl geschützter Asparaginsäure, um das Amid **119** zu erhalten. Verbindung **66** wurde in einer Neun-Stufen-Synthese hergestellt, die eine Aktivierung von Verbindung **113** als Triflat, gefolgt von einer Substitution mit TMGA, beinhaltete. Ein dppe-vermitteltеAza-Wittig Ringschlussreaktion des Amids wurde zur Bildung des Imidazolins eingesetzt. Aromatisierung des Imidazolinsdurch Eliminierung des Tosylates unter basischen Bedingungen ergab **136**. Verschiedene Manipulationen der Schutzgruppen ergaben die finale Verbindung **65** in 22.7 Prozent Ausbeute über 15 Stufen.



113 **66**



Verbindung **65** wurde zur Synthese verschiedener Peptide eingesetzt, von denen zwei zur Antikörpergenerierung verwendet wurden. Diese Antikörper erkennen erfolgreich natürliches pHis, sowohl in Dot-Blot Tests mit pHisinnerhalb von Peptiden, als auch in Zelllysat. In naher Zukunft werden diese Antikörper für die Untersuchung der biologischen Funktionen von pHis eingesetzt werden.

Um das in Beziehung stehende Enzym PHPT1, das für die Dephosphorylierung von Phosphohistidin verantwortlich ist, zu untersuchen, wurde eine Bibliothek von 5-Phosphonsäure Imidazolen mit verschiedenen Substituenten an C2-Position synthetisiert, die als Ausgangspunkt für die Entwicklung von PHPT1 Inhibitoren dient. Die beschriebene Synthesestrategie für Verbindung **65** wurde zur Generierung dieser Bibliothek angewendet.

Die Antikörper, Inhibitoren und andere Hilfsmittel, die in dieser Dissertation beschrieben werden, gewährleisten einen wertvollen Ansatz, um das Verständnis von Proteinphosphorylierung zu erweitern.

3 List of Abbreviations

AA	amino acid
Ac	acetyl
ad (NMR)	apparent doublet
All	allyl
Alloc	allyloxycarbonyl
aq.	aqueous
ATP	adenosine triphosphate
Bn	benzyl
Boc	*tert*-butoxycarbonyl
br	broad
Bu	butyl
Bz	benzoyl
°C	degrees Celcius (centigrade)
calc.	calculated
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CDI	carbonyldiimidazole
m-CPBA	*meta*-chloroperoxybenzoic acid
CTAN	cerric tetrabutylammonium nitrate
d (NMR)	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
dba	dibenzylidene acetone
DBU	1,8-diazabicyclo [5.4.0]undec-7-ene
dd (NMR)	doublet of doublets
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DCC	*N*,*N*-dicyclohexylcarbodiimide
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIB	(diacetoxyiodo)benzene
DIC	diisopropyl carbodiimide
DIPEA	diisopropylethylamine
DMAD	dimethyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMB	2,4-dimethoxybenzyl
DMBA	2,4-dimethoxybenzaldehyde
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DMTSF	dimethyl(methylthio)sulfonium tetrafluoroborate
DBP	dibenzylphosphite
dppa	diphenylphosphoryl azide
dppe	ethane-1,2-diylbis(diphenylphosphane)
dppf	1,1'-bis(diphenylphosphino)ferrocene
dt (NMR)	doublet of triplets
e.e.	enantiomeric excess
EDC	3-(ethyliminomethyleneamino)-N,N-dimethyl-propan-1-amine

EDT	ethane dithiol
equiv	equivalents
ESI	electrospray ionization
Et	ethyl
et al.	*et alia* (and others)
Fmoc	9-fluorenylmethoxycarbonyl
h	hours
HATU	2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium
HBTU	O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate
HOAt	3-Hydroxytriazolo[4,5-*b*]pyridine
HOBt	*N*-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
iPr	isopropyl
IR	infrared
HMDS	bis(trimethylsilyl)amine
KLH	keyhole limpet hemocyanin
LCMS	high performance liquid chromatography mass spectrometry
m (NMR)	multiplet
Me	methyl
MeCN	acetonitrile
min	minutes
mp	melting point
MS	mass spectrometry
Ms	mesyl
m/*z*	mass to charge ratio
NDPK	nucleoside diphosphate kinases
NBS	*N*-bromosuccinimide
NMP	1-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
Ns	4-nitrobenzenesulfonyl
o	ortho
OSu	hydroxysuccinimide
p	phospho
p	para
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PCC	pyridinium chlorochromate
PEG	polyethyleneglycol
PEPPSI	pyridine-enhanced precatalyst preparation stabilization and initiation
Ph	phenyl
PHP	phosphohistidine phosphatase
PMB	4-methoxybenzyl
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
PTM	posttranslational modification
PyBrOP	bromo-tris-pyrrolidino phosphoniumhexafluorophosphate
q	quartet

quant.	quantitatively
R_f	retention factor
rt	room temperature
s	singlet (NMR)
sat.	saturated
S-Phos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
SPPS	solid phase peptide synthesis
t (NMR)	triplet
TBS	*tert*-butyldimethylsilyl
tBu	*tert*-butyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography
TMGA	N,N,N',N'-tetramethylguanidinium azide
TMP	2,2,6,6-tetramethylpiperidine
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
TPS	2,4,6-triisopropylbenzenesulfonyl
Tr	trityl (triphenylmethyl)
Ts	*p*-toluenesulfonyl
$V_{S,max}$	local surface maximum
$V_S(\mathbf{r})$	surface electrostatic potential
XantPhos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene
X-Phos	2-dicyclohexylphosphino-2',4',6',-triisopropylbiphenyl

Amino acids

Ala	alanine (A)
Arg	arginine (R)
Asn	asparagine (N)
Asp	aspartate (D)
Cys	cysteine (C)
Gly	glycine (G)
Glu	glutamate (E)
Gln	glutamine (Q)
His	histidine (H)
Ile	isoleucine (I)
Leu	leucine (L)
Lys	lysine (K)
Met	methionine (M)
Phe	phenylalanine (F)
Pro	proline (P)
Ser	serine (S)
Thr	threonine (T)
Trp	tryptophane (W)
Tyr	tyrosine (Y)
Val	valine (V)

4 Contents

5 Introduction

Diseases are often related to aberrant function of the biological machinery. In order to understand these diseases knowledge of the particular malfunction and the underlying mechanisms is of crucial importance. Using this knowledge, eventually ways can be found to alter the malfunction or the specific pathway, usually by a small molecule. In this regard, post-translational modifications (PTMs) are of particular interest, since PTMs are responsible for all structural changes proteins undergo after ribosomal synthesis.

Posttranslational modifications are essential for protein function, and about five percent of the eukaryotic genome is dedicated to enzymes effecting PTMs. Several types of PTMs are known, the most well-studied are: acylation, alkylation, glycosylation, oxidation and phosphorylation.[1] Of these five, phosphorylation is arguably the most widespread and investigated post-translational modifications in eukaryotic organisms. In the human proteome alone, more than 500 kinases responsible for phosphorylation are known, divided over several sub-groups.[2]

PTMs in general, and phosphorylation in particular, induce conformational changes in proteins, thereby changing their function. In the case of phosphorylation the conformational change is induced by the introduction of an anionic phosphate, which changes the local hydrogen bonding pattern. Additionally, the presence of an anionic phosphonate changes the electrostatic properties of the protein. Often, phosphorylation acts as an on/off switch, changing the protein from an inactive to an active conformation. Phosphorylation has been shown to be involved in numerous diseases, most prominently various forms of cancer, by constitutively activation of its target protein. Therefore, kinase inhibition has become a common strategy in the pharmaceutical industry, spearheaded by the success of Gleevec, an inhibitor of tyrosine kinase that is used in the treatment of leukemia and other types of cancer.[3] Understanding the effects and causes of protein phosphorylation is of critical importance and many techniques have been developed to aid in their study. Whereas O-phosphorylation has been widely studied, the effect of N-phosphorylation remains unclear. The short life-time of such N-phosphorylated enzymes hampers the studies. In order to understand N-phosphorylation, a chemical biology approach could be pursued.

Chemical biology is the science of understanding biological processes through the use of synthetic chemistry.[4] Organic chemistry is employed to synthesize tool compounds that can be used to investigate and manipulate biological systems. A recent example of this strategy by the group of Hedberg involves the synthesis of a solid phase peptide synthesis (SPPS) ready adenylylated tyrosine building block. This building block was used to synthesize peptides that were used to raise antibodies specific for adenylylated tyrosine. These antibodies can be used to investigate the functions of this PTM.[5]

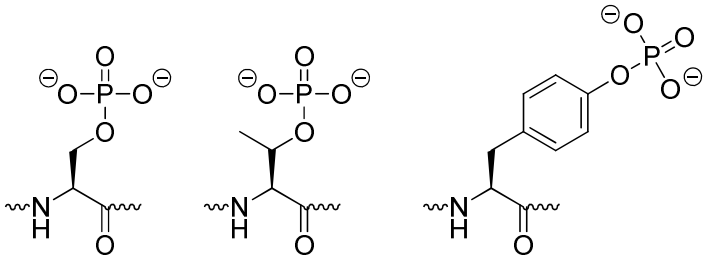
In this thesis, efforts towards the understanding of N-phosphorylation of histidine will be described. For this reason, a stable phosphorylated histidine analog has to be designed and synthesized. Before the design and synthesis of this analog will be described, a short overview of the current state of research will be given, divided in a biological background of phosphorylation and synthetic strategies to obtain imidazoles.

5.1 Phosphorylation

The first phosphorylated protein was discovered in 1906 by Levene and coworkers. It took many years of work before the phosphorylated amino acid was identified to be serine.[6] Since then, it has been discovered that about 30 percent of the proteins in mammalian cells are phosphorylated at any time.[7] 80 percent of all phosphorylation occurs on the hydroxy functionalities of three amino acids: serine, threonine and tyrosine.[8] While O-phosphorylation is the best studies type of phosphorylation it is not the only type. To date six more amino acids are known to be phosphorylated: arginine, aspartate, cysteine, glutamate, histidine and lysine.[9] Since phosphohistidine (pHis) is the focus of this work it will be discussed in more detail.

5.1.1 O-phosphorylation

The most common form of phosphorylation is O-phosphorylation, which occurs on the residues of serine, threonine and tyrosine. Serine is by far the most commonly phosphorylated amino acid followed by threonine. Tyrosine phosphorylation is quite rare; however, since anti-phosphotyrosine antibodies can detect phosphotyrosine very well, it has been extensively studied.



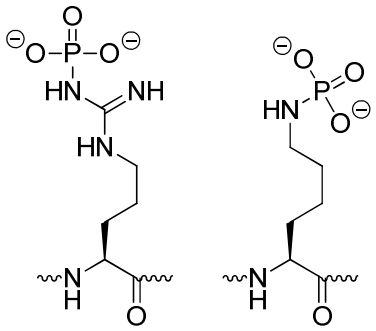
1 **2** **3**

Figure 1: O-phosphorylated amino acids pSer (**1**), pThr (**2**) and pTyr (**3**).

There is a wide range of kinases and phosphatases linked to these amino acids and their phosphorylation has numerous biological functions. For an overview of this field one can turn to several excellent literature sources.[7, 8b, 10]

5.1.2 N-phosphorylation

As mentioned before, N-phosphorylation represents a significant amount of all protein phosphorylation in organisms. Besides histidine, there are two other basic amino acids that get phosphorylated: Arginine and Lysine (figure 2). Little is known about these phospho-amino acids due to the instability of the phosphoramidate bond. Several of the kinases and phosphatases that have these amino acids as substrates have been found and some reports exist on proteins containing pArg and pLys.[11]



4 **5**

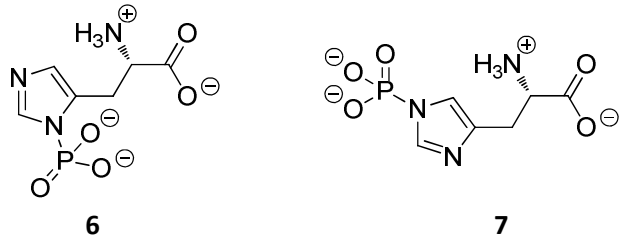
Figure 2: N-phosphorylated amino acids pArg (**4**), and pLys (**5**).

Not many proteins that contain Arginine or Lysine residues that are phosphorylated are known. The few that are known include: Histones H1 and H3,[12] Myelin basic protein[13] and VP12, a basic internal core protein of the virus *Plodia interpunctella*.[14]

Regrettably, little is known about the biological function of both these phosphorylated amino acid. While their abundance does not rival that of phosphohistidine, they might still be involved in important processes and should be investigated further.

5.2 Phosphohistidine

Compared to the O-phosphorylated amino acids, pHis was discovered fairly recently in 1947, when Severin and coworkers synthesized pHis for the first time.[15] It was identified for the first time in a biological setting in 1963 by Boyer, who discovered pHis in rat liver mitochondria.[16] Subsequent research revealed that pHis is of crucial importance in prokaryotes, which have two-component histidine kinase signaling pathways that enable these organisms to react to different kinds of stimuli (paragraph 5.2.2). In eukaryotes, pHis has been found in high concentration, in the slime mold *Physarum polycephalum* pHis makes up six percent of all phosphoamino acids of its basic nuclear proteins.[17] The importance of pHis in higher eukaryotes has not been sufficiently studied to date as pHis's chemical properties (its instability) make it difficult to study. There are two biologically relevant phosphohistidine isomers, 1- and 3-pHis (figure 3, **6** and **7**), of which 3-pHis is the more stable one.



6 **7**

Figure 3: 1- and 3-pHis at pH 8.[18]

Recently, pHis has been discovered in several proteins of higher eukaryotes. Among these proteins are: Rat ATP-citrate lyase,[19] KCa3.1 ion channel,[20] β-subunit of G proteins[21] and the N-terminus of histone H4.[22] These instances of histidine phosphorylation were discovered by studying histidine phosphatases and kinases and mutational studies. Additionally, there was a recent publication describing a possible

connection between a phosphohistidine phosphatase and certain lung cancer cell migration and invasion properties.[23]

5.2.1 Phosphatases and kinases

In vertebrates only one type of histidine phosphatase and one type of histidine kinase are known. These two enzyme types are the Nucleoside diphosphate kinases (NDPK's), which phosphorylate histidine and Phosphohistidine phosphatase (PHP), which removes the phosphate from pHis.[24] NDPK's are found in almost all organisms, while PHP is restricted to higher eukaryotes, it is one of the smallest enzymes known with a weight of 14 kD.

5.2.1.1 NDPK's

Of the nine known isoforms of NDPK only two, NDPK-A and NDPK-B, have been known to phosphorylate histidine. NDPK's influence various processes in the cell, such as nucleotide metabolism, regulation of tumor metastasis, cell differentiation and cell motility. They are not exclusively histidine kinases but also phosphorylate aspartate, serine and threonine in some proteins. As histidine kinases they target the proteins mentioned in the previous paragraph, except for the histone: Rat ATP-citrate lyase, KCa3.1 ion channel and β-subunit of G proteins. It is not known which protein phosphorylates the histone H4 histidine. NDPK's mode of action involves autophosphorylation on a histidine, using ATP, followed by transfer of the phosphate to the target protein.[25]

5.2.1.2 PHP

PHP has been first identified by Zetterqvist in 2002,[26] and the first structure was obtained by Hallberg and coworkers in 2006.[27] These studies showed that the protein is not similar to any known phosphatase. As mentioned before, it is unusually small (14 kD) and it is not completely clear whether its active form is a monomer or a trimer.[24] Mutational studies have shown that Arg^{45}, His^{53} and His^{102} are important for the phosphatase function and may contribute to the mechanism of action.[27-28] The substrates of this enzyme are the same as for the NDPK's.

On the other hand, several other phosphatases and kinases are known to occur in prokaryotes.[29]

5.2.2 Two component system

The phosphohistidine kinase two component signaling systems are ubiquitous in prokaryotes (bacteria) and to an extent in lower eukaryotes (fungi and plants). These systems play a large role in the interaction between an organism and its environment. The systems are used as sensors that can sense a variety of external variables including: temperature, pH, osmotic pressure and availability of nutrients.[30] A well-known example is ethylene induced fruit ripening in plants, which is regulated by histidine kinase activity of ethylene receptors.[31]

The two component signaling system comprises five active domains, divided over two proteins. These two proteins are: (1) a histidine kinase, containing an extracellular sensing domain, a histidine substrate domain and a kinase domain. (2) the response regulator, containing the aspartate substrate domain and the effector (figure 4). The substrate domain on the histidine kinase also doubles as a dimerization domain; the kinase proteins are always present as dimers when active.

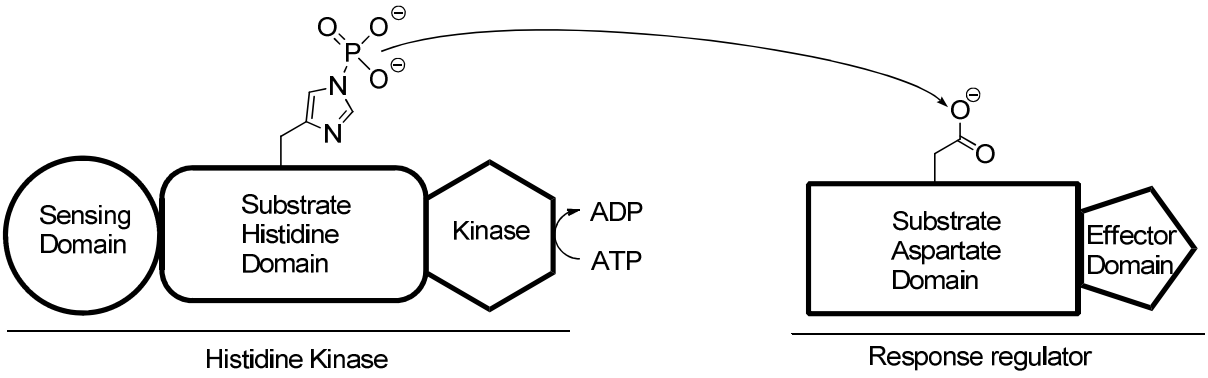


Figure 4: schematic representation of the two component signaling system.

When the sensing domain senses an event, the two monomers phosphorylate each other's histidine residue, using ATP. The resulting unstable phosphoryl group is then transferred to an aspartate on the response regulator protein. This in turn activates the effector, resulting in a cell response, such as change in flagella motion or a change in transcription.[32]

There is a variety of two component systems, including those with more components, but each is dependent on the easily transferred phosphoryl group on histidine. For a more in depth look on phosphohistidine kinase two component signaling systems there are excellent reviews by Stock,[33] Dhillon[34] and Grefen[31].

5.3 Properties of Phosphohistidine

As mentioned before, the biological function of phosphohistidine is dependent on the ease with which it can transfer its phosphate group. While this property is useful in its biology it has made pHis difficult to study. This section will discuss the reasons behind the pHis instability and some attempts made to circumvent it and analyze the biological functions of pHis.

5.3.1 Phosphohistidine Nomenclature

The numbering of the phosphohistidine isomers is somewhat confusing. In the literature isomer **6** is designated as the 1-isomer while compound **7** is the 3-isomer. However, according to the IUPAC rules the naming should be reversed as the substituent on the imidazole should always have the lowest possible number when the nitrogen substituent is the same (figure 5: **6b**). To avoid confusion when comparing to the literature the conventional naming will be used for phosphohistidine (making **6a** 1-pHis), all other imidazoles will be named according to IUPAC rules.

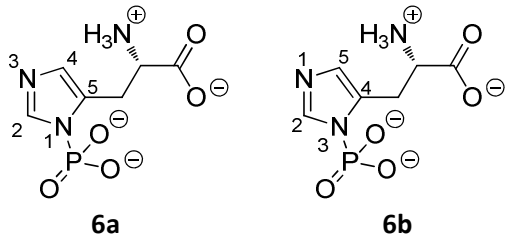


Figure 5: 1-pHis using conventional (literature) numbering (**6a**) and using IUPAC numbering (**6b**).

5.3.2 Phosphohistidine stability

The lifetime of phosphohistidine is dependent on its environment; in outside of a small pH range (pH 8 – 9.5), it is highly unstable. This makes it difficult to measure or analyze phosphohistidine, unlike the O-phosphorylated amino acids mentioned before. There is a stark difference in stability between phosphate esters and phosphoramidates. The ΔG° of hydrolysis of a phosphoester is between -6.5 and -9.5 kcal mol^{-1}, while for a phosphoramidate the ΔG° of hydrolysis is between -12 and -14 kcal mol^{-1}.[33] This is due to the fact that phosphoramidate nitrogens are basic. Therefore they are protonated at acidic-, and even at neutral pH, greatly improving their leaving group ability. When considering the reasons behind this one might expect enhanced stability from donation of the nitrogen lone pair into the P=O π*-orbital, thereby giving some double bond characteristics to the P-N bond. However, the donation of the nitrogen lone pair, is not a major contributor to the phosphoramidate electronic structure. Since the phosphorus d-orbital is involved in the P=O π-bond and overlap between this d-orbital and the lone pair orbital of the nitrogen is very poor. Therefore, the lone pair is not donated to the phosphorus and there is little or no double bond characteristic in the phosphoramidate bond (figure 6).

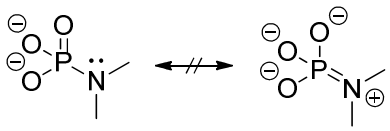


Figure 6: canonical resonance structures of a phosphoramidate.

In the case of pHis the effect is even more pronounced, as donation from the nitrogen lone pair to the phosphorus would break the aromaticity of the imidazole (figure 7: **A**). Additionally, the acid sensitivity of pHis is even greater than for other phosphoramidates, though the phosphoramidate nitrogen in pHis is not basic, due to the aromaticity loss upon protonation at that nitrogen. The other nitrogen on the imidazole however, is basic. Its protonation not only leads to a reduction of the electron density on the phosphoramidate, making it more electrophilic. It also makes the imidazole an excellent leaving group as the ejected imidazole is a neutral species (figure 7: **B**).[32c] Therefore, pHis's reactivity is closer to that of an acyl transfer reagent, like CDI or even an acyl chloride, than a phosphate ester or an amide.[35]

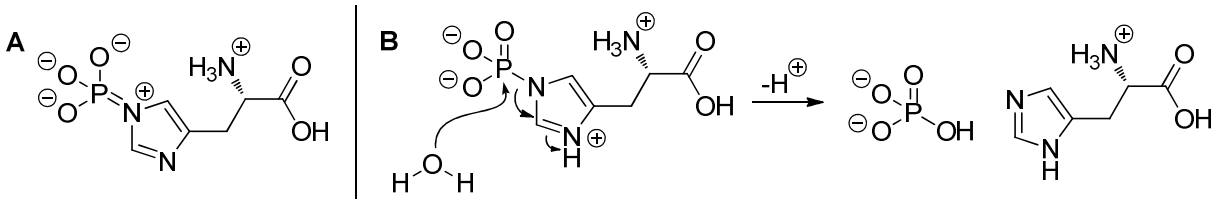


Figure 7: donation of the nitrogen lone pair leads to loss of aromaticity (**A**). Protonated imidazole is an excellent leaving group (**B**).

Due to their general instability and their acid sensitivity 1- and 3-pHis have very short half-lives under normal acidic phospho-protein partial digestion conditions. In 1M HCl at 49°C 1- and 3-pHis have a half-live of 18 and 25 seconds respectively,[36] compared to 18 hours for phosphoserine and phosphothreonine and 5 hours for phosphotyrosine.[37] Both pHis isomers are stable under basic conditions, because the imidazole is not protonated under those conditions.

The inherent instability of phosphohistidine makes it an excellent phosphoryl transfer residue, a function for which it is extensively used in biological systems.

5.4 pHis analysis

A myriad of methods is used to detect phosphorylated proteins, including: partial acid hydrolysis, mass spectrometry, sample fractionation, ^{31}P NMR, gel separation, affinity purification and immuno-based techniques.[11, 32b] However, most of these methods use acidic conditions, and are therefore not suitable to be used for the detection of acid labile phosphohistidine. Not only the detection of pHis complicated, its instability also requires special measures during sample preparation and work-up. For example a study on the tryptic digests of CheA, a part of the bacterial two component signaling system, used a combination of HPLC-ESI-MS and element mass spectroscopy under acidic conditions. Initially 40 percent phosphorylation was measured, but after one hour the fraction of phosphorylated peptide had fallen to 10 percent.[38]

It is possible to perform partial alkali hydrolysis of a peptide to preserve pHis, but pSer and pThr are not stable under those conditions and are lost. Trypsination also produces protein fragments containing intact pHis, however most methods to analyze the fragments require acidic conditions. Moreover, these methods cannot differentiate between 1- and 3-pHis. Additionally, due to pHis's instability one cannot be completely sure no sample degradation has taken place before measuring, leading to a problem with underestimation of the amount of pHis present.

These problems could be solved with the availability of a pHis specific antibody, as was the case when a phosphotyrosine-specific antibody was raised.[39] However, raising antibodies specific for pHis is very difficult, for the same reasons that make its detection difficult, its instability. If pHis is introduced in an animal for the purpose of antibody generation (see chapter 5.5), it decomposes before a strong immune response can be realized. A non-hydrolysable mimic of pHis would help to generate these antibodies and some compounds have been tested, with limited success.

Several non-hydrolysable mimics have been synthesized; among them are a pyrrole analog **8**,[32c] a furan analog **9**,[40] a thiophosphoryl analog **10**[41] and a triazole analog **11**.[42] Of these four, only two have been reported to be used in attempts to raise antibodies specific for pHis (**8** and **11**). Antibodies raised using pyrrole analog **8** were selective for the analog but did not show any affinity for pHis containing peptides. During the course of the thesis the triazole analog **11**, designed and synthesized in the group of Muir, was incorporated into a peptide using SPPS. The resulting peptides were immunized in rabbits and antibodies were obtained that were specific for pHis containing peptides. However, they did not report on the affinity of their antibodies for pHis. Therefore, it is not sure if their antibodies are qualitatively good enough to study histidine phosphorylation.

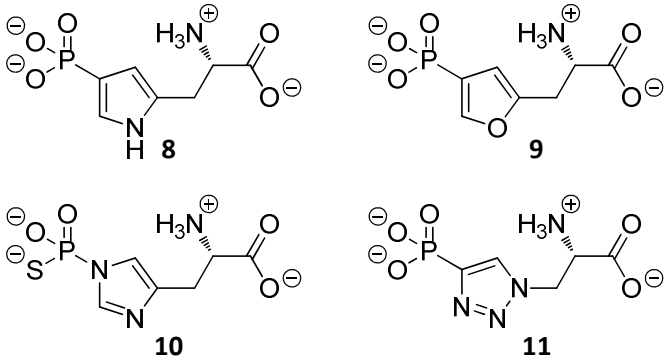


Figure 8: non-hydrolyzable phosphohistidine analogs.

The reason why the analogs mentioned above are not successful in raising high affinity antbodies for pHis is that although they are structurally similar, their electronic properties are widely different. A more in depth look at these analogs will be given in chapter 7.

This thesis will describe the design and synthesis of a pHis analog that resembles histidine both electronically and structurally. This will be achieved by conserving the imidazole of pHis and synthesizing an imidazole containing mimic. The design of this analog will be discussed in chapter 7.

5.5 Antibody generation

Antibodies are powerful tools in biochemistry and biology. Their high specificity and binding affinity ensures their use as reagents in protein recognition. Antibodies are generally raised using native or recombinant proteins, however, it is also possible to use peptides, including phosphorylated peptides, as haptens in antibody generation. Peptides themselves are too small to generate a significant immune response. Therefore, they are usually attached to a carrier protein, most commonly keyhole limpet hemocyanin (KLH). The peptides can be attached to the carrier protein by coupling with an exposed amino acid on the protein surface. Several bond types can be used for the conjugation of peptides to their carrier proteins, coupling using a thiol on a cysteine that is incorporated on one end of the peptide is ideal. Using other residues (amino, hydroxyl, carbonyl) has the disadvantage that they present a much larger fraction of the amino acids in proteins, encouraging multipoint attachment.

Designing the right peptide to generate antibodies that can recognize the target protein is of critical importance in antibody generation. A greater chance of obtaining antibodies with a good titer can be realized if the peptides are from a part of the protein sequence that is on the surface, hydrophilic and flexible. Furthermore, it is important to choose sequences at the N- or C- terminus of the protein because these regions are usually on the surface of the protein. Ideal peptide length has been shown to be between eight and 20 amino acids. Longer peptides might lose specificity while antibodies raised with shorter peptides might not recognize the protein. Acylation of the N-terminus and and a C-terminal amide are also important to better mimic the protein. Other variables to consider are peptide stability, ease of peptide synthesis and conjugation and immunogenicity of the host animal.[43]

After the peptides have been designed, synthesized and attached to the carrier protein, they are injected into the host animal. A wide variety of animals are used for this purpose, such as mice, rabbits, chickens, pigs, cows and horses. Animal selection is mainly based upon the amount of antibody required and the cost. The peptides are injected together with an adjuvant, which is a mixture of chemicals designed to illicit a strong immune response. A commonly used adjuvant is Freund's complete adjuvant, which contains cell membranes of mycobacteria tuberculosis, paraffin oil and mannide monooleate. Since it is highly toxic (immunogenic shock) to the host animal, other adjuvants have been used as replacement recently.[44] After the initial injection, several booster injections can be administered to maintain a strong immune response. The time it takes to raise antibodies depends on the animal used; usually there are several points of serum collection finalized by the terminal bleed at the end.

The collected serum is purified by affinity purification. In the case of phospho-peptides two affinity columns are used; first a column functionalized with the phospho-peptide is used to obtain only antibody that recognize the correct sequence. Second a depletion step is performed over the native, un-phosphorylated peptide to remove all antibodies that bind the non-phosphorylated peptide.

This workflow typically results in high titer, polyclonal antibodies that can be used in biochemical experiments to detect the presence of their respective antigen.

5.6 Solid Phase Peptide synthesis

Solid phase peptide synthesis (SPPS) is the default method for the synthesis of peptides. The method was pioneered by Merrifield in 1963.[45] The method involves the immobilization of an amino acid on a resin, followed by a stepwise build-up of the peptide chain. The advantage of having the growing peptide chain attached to a solid resin is that after each coupling step all reagents can simply be washed away. Since many peptides are quite long (between 10 and 50 amino acids) and require many coupling steps, purification by any other method becomes impossible. To prevent side reactions the amino acids are protected using a range of protecting groups. The correct choice of these groups is of critical importance; the right protecting groups should be removed at the right time. There are two main methods of peptide chain protection, the Fmoc- and the boc-method. The Fmoc method is the most widely used method at the moment and was used for all peptides described in this thesis; thus it will be the focus of this paragraph.

The Fmoc method refers to the protecting group that protects the N-terminus of the peptide. The first amino acid is connected to the resin at the C-terminus, this can be done using a variety of linkers, which can impart different functionality to the C-terminus upon cleavage. The peptide synthesis starts with deprotection of the Fmoc group on the terminal amino acid using a base (usually a piperidine solution). The resin is washed, a second Fmoc protected amino acid is added together with a coupling reagent, which extends the peptide by one amino acid. Finally, the peptide is washed and sequence can be started again (figure 9). When the desired peptide is synthesized it can be cleaved from the resin, in the case of the Fmoc method this is usually done with an acidic cleavage step. If the protecting groups on the amino acids are well chosen, they will also be cleaved during this step. The group on the C-terminus of the peptide after deprotection is determined by the linker – resin combination that is used. Since the peptides in this thesis were needed for antibody generation a tentagel resin with an S-RAM linker was used, yielding an amide at the C-terminus after peptide cleavage.[46]

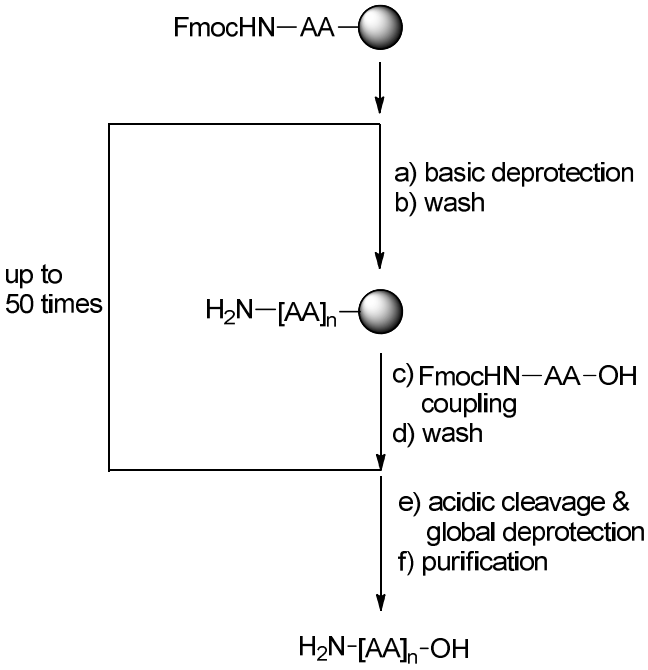


Figure 9: Fmoc SPPS flowchart.

After cleavage and global deprotection the peptide is first roughly purified by ether precipitation, which removes most of the organic impurities. Then the peptide is purified by preparative HPLC. The overall yield of a peptide synthesis sequence is very much dependent on the amino acid sequence and length.

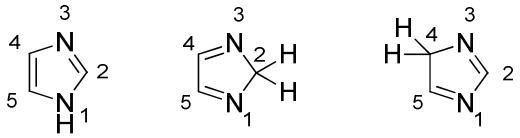
5.7 Imidazole review

Compared to other five membered heterocycles 1*H*-imidazoles have some unique properties due to the fact that it contains two nitrogens. The properties include its basicity, ability to form hydrogen bonds and leaving group ability. 1*H*-Imidazoles are present in a variety of natural products but is most well known as the defining structural feature of the amino acid histidine.[47] They are used, among others, as pharmaceuticals,[48] ionic liquids,[49] and organometallic chemistry (mostly as N-heterocyclic carbenes).[50] In this chapter, first the properties of 1*H*-imidazoles will be discussed, followed by some synthesis strategies used to make them.

5.7.1 Imidazole properties

Imidazoles are five membered aromatic heterocycles containing two nitrogen atoms in a 1-3 relationship. There are three isomers that are called imidazoles, 1*H*-imidazoles (**12**), 2*H*-imidazoles (**13**) and 4*H*-imidazoles (**14**) (figure 10). There are large differences between these three compounds, most notably, 2*H*- and 4*H*-imidazoles are not aromatic, while 1*H*-imidazoles are. This imparts stability to 1*H*-imidazoles, in fact unsubstituted 2*H*- and 4*H*-imidazoles have not been synthesized as they rearrange to

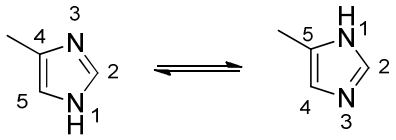
the 1*H*-imidazole isomer immediately. 2*H*- and 4*H*-imidazoles that have large substituents on C-2 and C-4 respectively, or are part of ring systems can be synthesized and are used in dyes, semiconductor chemistry and as reactive intermediates.[51] However, they are not as important and widely studied as their 1*H*-imidazole counterpart.



12 **13** **14**

Figure 10: Imidazole numbering 1*H*-imidazole (**12**), 2*H*-imidazole (**13**) and 4*H*-imidazole (**14**).

The atom count of imidazoles start at the substituted nitrogen, proceeds to the other nitrogen via the shortest path and continues to the final carbon. Imidazoles that have a hydrogen substituent at the nitrogens are present as a mixture of tautomers. If these imidazoles have a substituent on the four or five position they are designated as 4(5)-substituted imidazoles (figure 11).



15a **15b**

Figure 11: 4(5)-methyl imidazole.

Compared to other five membered heterocycles imidazoles have a high boiling point of 256°C. This is due to the formation of hydrogen bonds between the individual imidazole molecules (figure 12: **A**). The imine nitrogen of imidazole is basic (pK_a 7.0) and can easily donate to an N-hydrogen of another imidazole (figure 12: **B**). Imidazole is such a strong base because the positive charge of the protonated imidazole can be shared over both nitrogens (figure 12: **C**).[52]

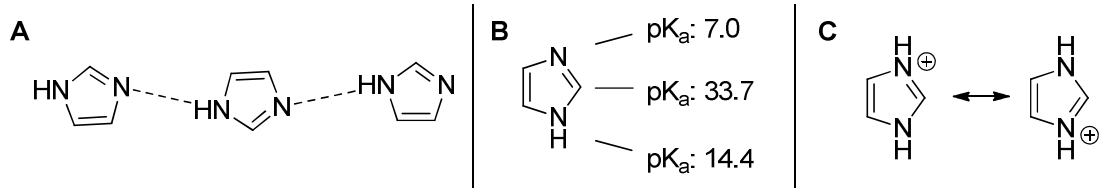


Figure 12: **A**: pK_a of several positions on the imidazole. **B**: hydrogen bonding in imidazoles. **C**: Charge sharing in protonated imidazoles.

This ability of imidazole, to accept a proton and then release a proton, is exploited in numerous enzymes where histidine is a part of the active site. One of these enzymes is serine protease, an enzyme that breaks peptide bonds in proteins.[53] In figure 13 it is shown how, during the cleavage of the peptide, the imidazole acts as both a proton acceptor and a donor.

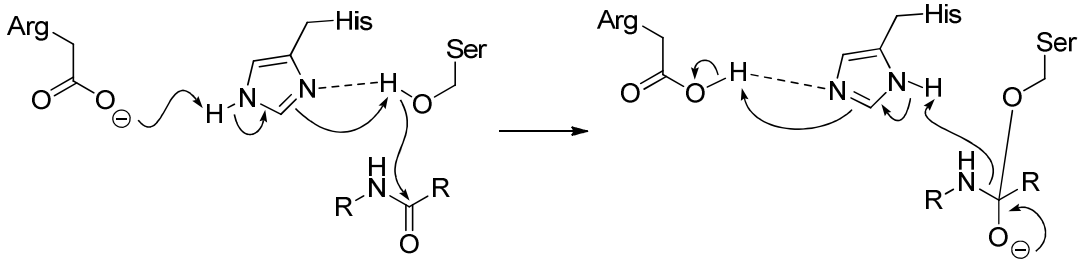


Figure 13: Breaking of a peptide bond in the active site of serine protease.

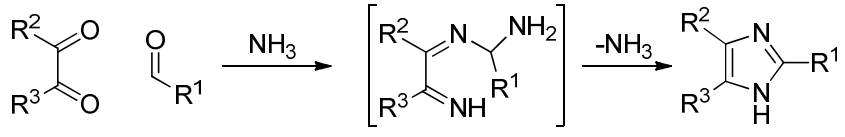
Imidazoles are also nucleophilic at the nitrogen and will quickly methylate on both nitrogens in the presence of an excess of methyl iodide. Imidazoles can be easily deprotonated at the nitrogen by sodium hydride or similar bases, as the pK_a of that proton is only 14.4. N-protected imidazoles can be metalated using lithium or magnesium reagents, preferentially at C-2, if that position is not eligible for deprotonation, C-5 is preferred. Imidazoles are electron rich and are thus easily halogenated and nitrated. Halogenated imidazoles have been extensively used in halogen-metal exchange reactions and metal catalyzed couplings.[52, 54]

5.7.2 Imidazole synthesis

While there are many synthetic methods to make imidazoles, there is not a condensed set of methods that allows access to most imidazoles, like there are for, indoles or pyridines, for instance. Therefore, there are many different routes and every imidazole synthesis requires its own synthetic plan. In this chapter various methods for imidazole synthesis will be discussed that are of historical significance or important to this thesis. The initial discovery of the method will be mentioned along with selected examples of their use in modern organic chemistry.

5.7.2.1 Debus-Radziszewski synthesis

Debus discovered the reaction of a 1,2-diketone **16** and an aldehyde **17**, in the presence of ammonia to synthesize 2,4,5-substituted imidazoles **19**, the method was later elaborated by Radziszewsk (figure 14).[55] The mechanism likely involves attack of ammonia on all the carbonyls, followed by attack of the diimine on the imine to yield **18**. This intermediate can then close by elimination of one equivalent of ammonia and tautomerization yields the imidazole **19**. This method suffers from mediocre yields (50 – 60%), however a wide variety of building blocks can be use, yielding many differently substituted imidazoles.[56] The yields and reaction times of this reaction have been be improved by the use of microwave irradiation[57] or catalysts[58], where yields between 85 – 99 percent can be obtained for selected examples.

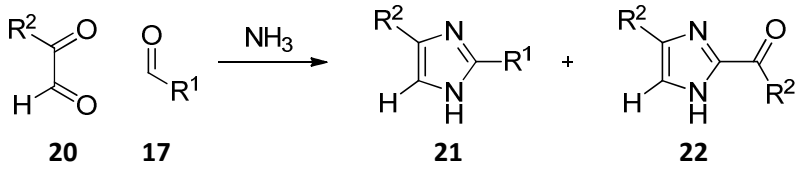


16 **17** **18** **19**

Figure 14: Debus- Radziszewsk synthesis.

Recently, a variation of this reaction was reported using α-keto aldehydes **20** instead of 1,2-diketones **16** (figure 15). The obvious problem of self-condensation of the α-keto aldehydes was solved by careful

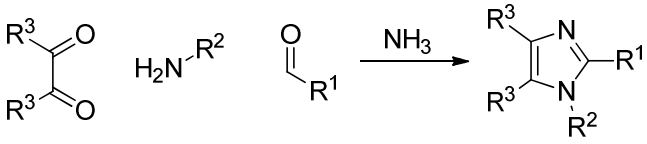
optimization of the reaction conditions. In acetic acid, DCM and DMF compound **22** was the major product, but using methanol at room temperature yielded a mixture of **21** and **22** in an 8:1 ratio and reasonable to good yields.[59]



20 **17** **21** **22**

Figure 15: Debus type synthesis using α-keto aldehydes.

Another useful variation is the Gridnev modification of the Debus synthesis, which uses an amine to take the place of one of the ammonia equivalents (figure 16). In this way also 1,2,4,5-substituted imidazoles are accessible.[60] This method has been used recently to synthesize imidazoles with optically active substituents on the N-1 position, without loss of enantiopurity during the reaction.[61]

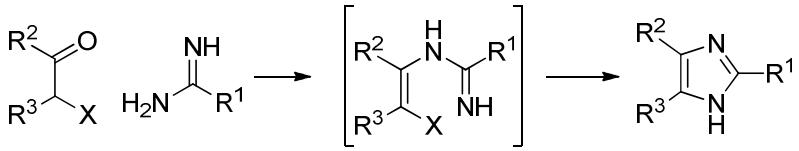


23 **24** **17** **25**

Figure 16: Gridnev modification.

5.7.2.2 Kunckell synthesis

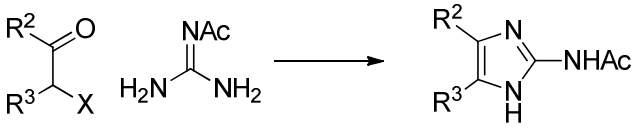
The Kunckell synthesis, first reported in 1901,[62] involves the reaction between an α-haloketone **26** and an amidine (figure 17). In its original form it provides access to the same substitution pattern as the Debus synthesis.



26 **27** **28** **19**

Figure 17: Kunckell imidazole synthesis.

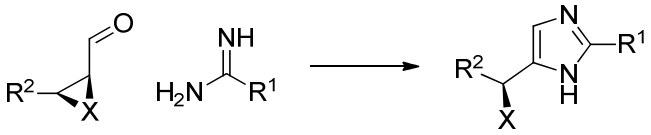
Useful variations include the Webber synthesis, which exchanges the amidine for acetyl guanidine **29** (figure 18). This method gives access to 2-amino imidazoles after deprotection of the amine.[63] The acetyl group has also been changed for a boc, leading to higher yields in some examples.[64]



26 **29** **30**

Figure 18: Webber's modification.

The α-haloketone **26** can also be changed for compounds with a similar reactivity pattern. For instance, recently 2,3-epoxy- and 2,3-aziridine aldehydes (**31** and **32**) were reported to work well in this reaction (figure 19). Using optically pure aldehydes yielded products **33** with good ee's in average to good yield. The 2,3-epoxy- and 2,3-aziridine aldehydes were made in situ from α,β-unsaturated aldehydes and hydrogen peroxide or TsNHOTs in the presence of a pyrrolidine organo-catalyst.[65]

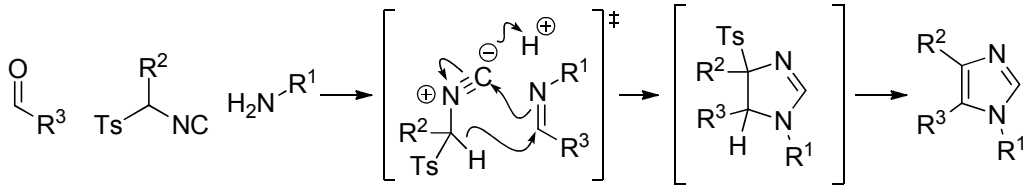


X = O **31**, NTs **32** **27** **33**

Figure 19: use of 2,3-epoxy- and 2,3-aziridine aldehydes.

5.7.2.3 Van Leusen synthesis

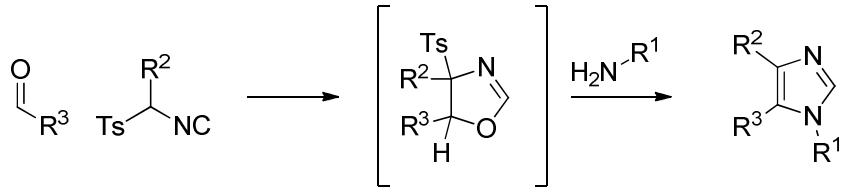
The van Leusen synthesis is a multi-component reaction between a toluenesulfonylmethyl isocyanide **34** (usually TosMIC, R_2 = H), an amine **24** and an aldehyde **17** (figure 20). In this reaction initially the amine and aldehyde react to form a Schiff base. The isocyanide is then added, which attacks the Schiff base leading to ring closure to the imidazoline. The imidazoline is then aromatized by the elimination of the tosylate, yielding a 1,5- and some 1,4,5-substituted imidazole.[66] This method is used extensively in the pharmaceutical industry for a synthesis of pharmaceutically active imidazoles,[67] and has also been used in solid phase synthesis.[68]



17 **34** **24** **35** **36** **37**

Figure 20: van Leusen imidazole synthesis.

One modification of the van Leusen synthesis involves the reaction between a toluenesulfonylmethyl isocyanide **34** and an aldehyde **17** to form a 4-tosyloxazoline. Then the amine is added to yield the imidazole (figure 21).[69] This method gives regioselective access to 4-, 1,4- and 4,5-imidazoles, 1,4,5-imidazoles are difficult to make using this method as a mixture of regio-isomers is usually obtained.

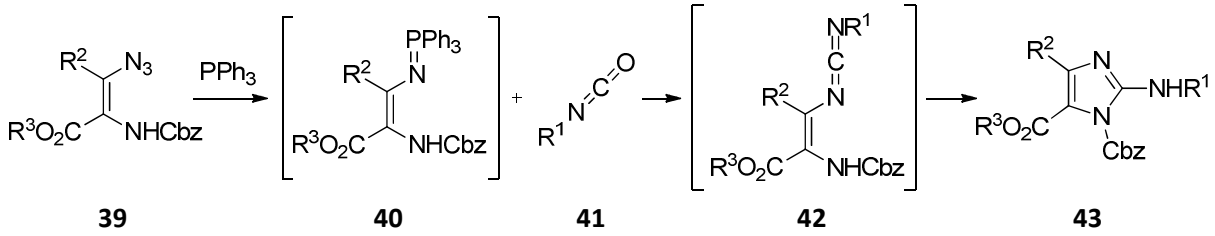


17 **34** **38** **37**

Figure 21: Modified van Leusen imidazole synthesis.

5.7.2.4 Aza-wittig strategy and imidazoline aromatization

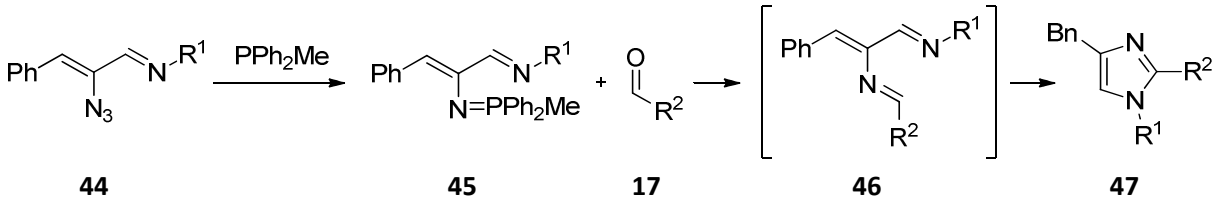
There are several synthetic strategies for imidazoles based on the Aza-wittig reaction, the reaction of an iminophosphorane and a carbonyl group. Molina et al. published the use of an aza-wittig reaction to produce 2-aminoimidazoles **43** (figure 22). Triphenylphosphine (PPh_3) and the azide **39** are mixed at 0°C to form the iminophosphorane **40** by the expulsion of molecular nitrogen. The iminophosphorane reacts with isocyanate **41** to yield a carbodiimide **42**, which ring closes at room temperature to form the target 2-aminoimidazole **43**. The reaction works with both aryl and alkyl isocyanates but the other substituents are restricted to a rather small set. The iminophosphorane was isolated in one case but in all other cases the reaction was performed in one pot.[70]



39 **40** **41** **42** **43**

Figure 22: Aza-wittig reaction with isocyanates.

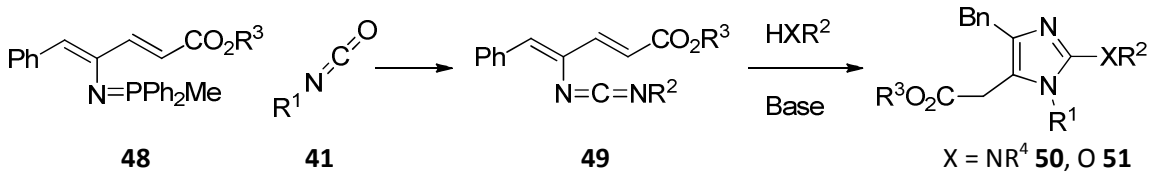
Very recently, Nie et al. reported the use of an aza-wittig reaction between azides (**44**) and aldehydes (**17**) to yield 1,2,4-substituted imidazoles (**47**) (figure 23).[71] Mixing the iminophosphorane **45** and the aldehyde **17** at room temperature did not result in any product, but upon heating to 80°C the reaction proceeded smoothly to yield a range of substituted imidazoles, presumably going through intermediate **46**. The substituent on the imine moiety of **44** (R^1) is limited to phenyl or 4-chlorobenzene; however aldehydes with a wide range of aromatic groups were successfully used. In all cases the intermediate iminophosphorane was isolated as a crystalline solid.



44 **45** **17** **46** **47**

Figure 23: Aza-wittig reaction for the synthesis of 1,2,4-substituted imidazoles.

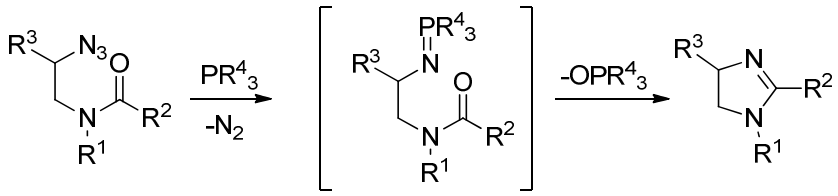
In line with the work of Molina et al. similar iminophosphoranes were allowed to react with isocyanates to give carbodiimides, which could be continued to imidazoles with either amines or ethers at C-2 using amines or alcohol respectively (figure 24). The 2-oxo-imidazoles **51** were not stable and all oxidized at the benzylic position to yield a final benzoyl group a C-4.[72]



48 **41** **49** X = NR^4 **50**, O **51**

Figure 24: Aza-wittig reaction with isocyanides to synthesize 2-amino- or 2-oxo-imidazoles.

The aza-wittig reaction has been widely used in the synthesis of five membered dihydro-heterocycles like oxazolines and thiazolines by reaction of iminophosphoranes with esters or thioesters, respectively. These dihydro-heterocycles can then be aromatized to their unsaturated analogs by various methods. The synthesis of imidazolines by this method is less investigated, mostly due to the low reactivity of the amide bond in this reaction (figure 25).

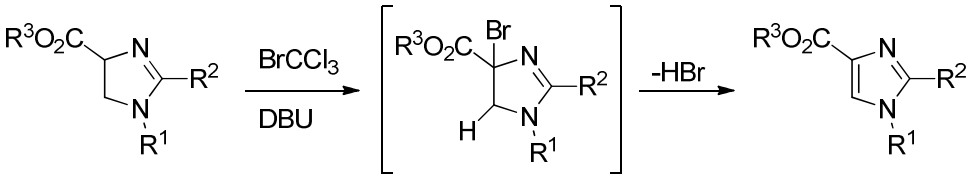


52 **53** **54**

Figure 25: Aza-wittig imidazoline synthesis.

The first time an aza-wittig reaction was used to synthesize an imidazoline was in 1985, in this instance the low reactivity was overcome by heating the reaction to 180-250°C.[73] Unfortunately, these conditions are not suitable for many functional groups. To enhance the reaction rate, Loos et al. used a sulfonamide to activate the amide group of compound **52** (R_1 = Ts). The electron withdrawing character of the tosyl group increased the electrophilicity of the amide, allowing the reaction to proceed at a more agreeable 80°C.[74] The reaction is heavily influenced by the substituents on the phosphine, where triphenylphosphine showed the best reactivity.[75]

Once an imidazoline-ring is created, using an aza-wittig reaction or other reactions, it needs to be dehydrogenated to make an imidazole. One popular method for the aromatization of all kinds of 1,3-azolines is dehydrogenation using bromo trichloromethane ($BrCCl_3$) and DBU (figure 26). This method, first discovered by Williams et al.[76] likely involves deprotonation of the imidazoline C-4, followed by bromination. The imidazoline then eliminates hydrogen bromide to become aromatic. This method is extensively used for the aromatization of sensitive imidazolines due to its mildness and functional group tolerance.[74, 77] The only requirement is that the imidazoline has a strong electron withdrawing group at C-4, ideally an ester. If such an electron withdrawing group is absent, the C-4 hydrogen is not acidic enough and deprotonation does not occur, retarding the reaction.



55 **56** **57**

Figure 26: Dehydrogenation of imidazolines to imidazoles using bromo trichloromethane and DBU.

Recently, a similar reaction, using trichloroisocyanuric acid, instead of bromo trichloromethane, showed good results in aromatizing 2-imidazolines, without an ester at C-4. However, no substituents other than at C-2 are tolerated in this case.[78]

Many other reagents have been used to dehydrogenate imidazolines including MnO_2,[79] $BaMnO_4$,[80] Pd/C,[81] Zn-Al_2O_3,[82] DMSO/$(COCl)_2$,[83] $KMnO_4$,[84] IBX,[85] (Diacetoxyiodo)benzene (DIB),[86] O_2[87] and Magtrieve.[88]

5.7.3 Additional Imidazole Syntheses

There are an enormous amount of other imidazole syntheses, however their detailed discussion is beyond the scope of this thesis. Some examples from the recent literature (2002 – present) include (1) by rhodium catalyzed transannulation of 1,2,3-triazoles with nitriles,[89] (2) by treatment of imidazolones with phosphorus oxybromide or -oxychloride,[90] (3) by 1,5-electrocyclization of azavinyl azomethine ylides,[91] (4) by gold catalyzed hydroamination of fluorinated N'-aryl-N-propargyl amidines,[92] (5) by the Wallach reaction,[93] (6) from β-ketoamides,[94] (7) from two different isocyanides or formamides,[95] (8) from nitriles and amines,[96] (9) by palladium catalyzed cyclization of O-pentafluorobenzoylamidoximes,[97] (10) from thioamides,[98] (11) from thioiminoether hydrobromides,[99] (12) by the Bredereck synthesis,[100] (13) by nickel catalyzed nitrile cyclization,[101] (14) by SeO_2 mediated oxidation of 1,3-diazabuta-1,3-dienes,[102] (15) from 2-azido acrylates and nitrones,[103] (16) from (N-acylated)α-aminonitriles,[104] (17) from allenes, imines and nitriles,[105] (18) by cross-coupling of aldimines,[106] (19) from keto-oximes, aldehydes and ammonia,[107] (20) by the Lantos-Eggelston synthesis (hetero-Cope rearrangement),[108](21) from 3-N,N-(diemthylamino)-2-isocyanoacrylates[109] and (22) from 2-bromo-isocyanoacrylates and amines.[110]

For older imidazole syntheses (pre-2002) there are some excellent reviews available.[51d, 111]

6 Aim of the Project

Selective antibodies that recognize 3-phosphohistidine would be valuable tools to understand the epigenetic importance of 3-phosphohistidine. The aim of the main project described in this thesis is to provide a tool set to raise such antibodies. To this end several steps were undertaken:

1. The design of a stable 3-phosphohistidine mimic.
2. The synthesis of the 3-phosphohistidine mimic.
3. Incorporation of the 3-phosphohistidine mimic in peptide that can serve as a antigen.

The design of the 3-phosphohistidine mimic had to take into consideration both the electronic and the structural aspects of phosphohistidine. The mimic has to be stable under physiological conditions to enable the raising of antibodies. In order to incorporate the 3-phosphohistidine mimic in a peptide, the synthetic building block needed to be suitable for SPPS. The synthesis of the mimics should minimize synthetic steps and maximize yields, especially since multi gram quantities of the final compound are required for SPPS. Finally, the peptides needed to be well designed so antibodies could be raised successfully.

After coupling of the peptide to a carrier, antibodies can be raised to investigate phosphohistidine function. The raising of the antibodies as well as some of the subsequent studies are being performed in the group of Robert Schneider.

6.1 Side projects

Several side projects were also pursued in parallel with the work described above:

Toward potential phosphohistidine phosphatase inhibitors: The aim of this project was to use chemistry developed in the 3-pHis analog project to synthesize a small library of 4-phosphonic acid imidazoles. This library might serve as a starting point for the development of an inhibitor for PHP.

1-phosphohistidine project: This project is analogues to the 3-pHis project, where the aim is to generate antibodies for 1-pHis. It involves the same key steps as discussed above.

Adenylylated histidine project: This project is also analogous to the pHis projects but the aim was to generate a mimic for adenylylated histidine in order to raise the relevant antibodies.

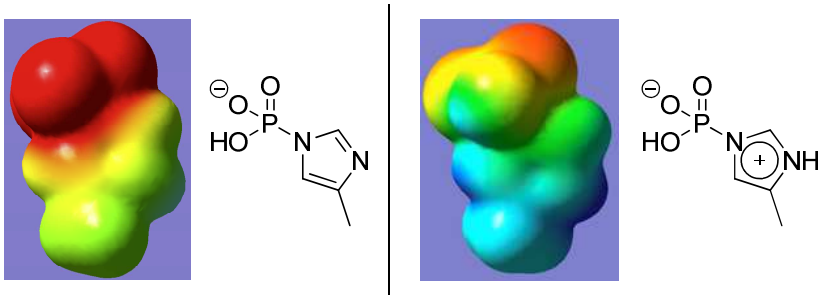
N^{δ}-Methylarginine project: The aim of this project was to synthesize an SPPS building block that enabled the synthesis of peptides containing an arginine methylated at the N^{2} position.

Piperazine probe project: The aim of this project was to investigate the feasibility of a proposed synthesis for a probe that was to be used for exploring the relation between phosphorylation and lipidation in proteins.

7 Design and synthetic consideration of pHis mimic

To achieve the goal of raising antibodies specific for phosphohistidine it would be necessary to design a pHis mimic that is stable under most conditions and that can be incorporated into a peptide, preferably using SPPS.

To design an analog of pHis that not only had similar structural but also electronic features it was clear that the imidazole ring should be conserved. The properties of the imidazole are unique among five membered heterocycles and cannot be easily mimicked. Importantly, imidazole is a base with a pKa around 7.0. Thus the imidazole is protonated at physiological pH (7.2-7.4).Protonation of the imidazole has a profound impact on its electronic structure. To compare unprotonated vs. protonated phosphohistidine the surface electrostatic potentials (V_S(**r**))of their respective heterocyclic cores were calculated at the PCM-B3LYP/6-31+G(d) level by electron density contour using Gaussian 03 and the HS95v09 program (figure 27) in collaboration with prof Tore Brink at the Royal institute of technology, Stockholm, Sweden.

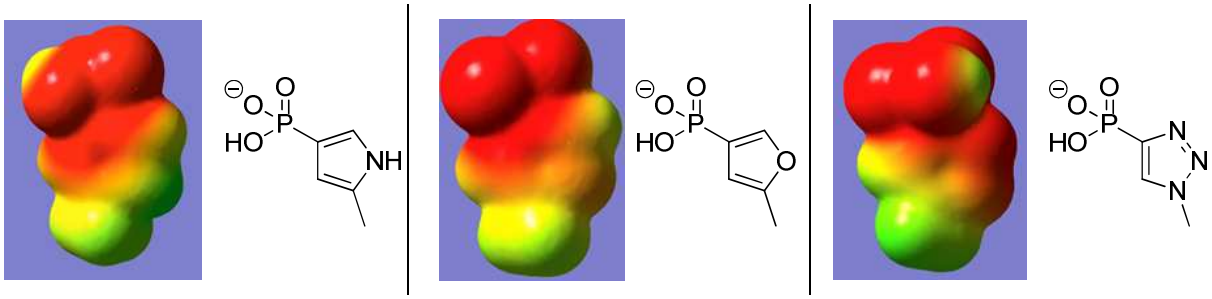


58 **59**

Figure 27: calculated surface electrostatic potentials of the pHis heterocyclic core unprotonated (**58**) and at pH 7.2 (**59**).

Figure 27 shows pHis with an unprotonated (**58**) and protonated imidazole moiety (**59**). It is quite striking how different their V_S(**r**)'s are. The phosphate has a strong negative potential in both cases; however, the phosphate in **59** also shows a surprisingly strong local surface maximum ($V_{S,max}$) located at the hydrogen of the OH group. Thus the OH group of pHis under physiological conditions is a good hydrogen bond donor, even though the phosphate overall is negatively charged. Furthermore, the V_S(**r**) over the imidazole ring of **59** has two positions with a high $V_{S,max}$ at the N-1 and C-4 positions, whilst **58** does not. Overall the imidazole ring is more positive in **59** than in **58**.

Taking these differences into consideration the two pHis homologs were compared to the cores of the pHis mimics that were synthesized by other groups (figure 28).



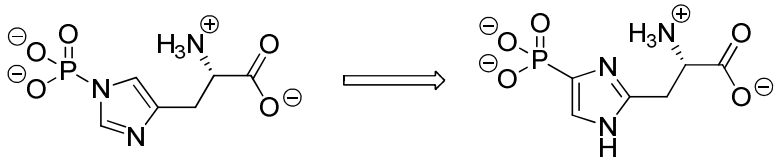
60 **61** **62**

Figure 28: calculated surface electrostatic potentials of several non-hydrolysable analogs at physiological pH.

At first glance it is clear that these mimics have similar electronic properties to unprotonated imidazole **58**. They also have a fully negative phosphonate; the OH group on the phosphonate is no hydrogen bond donor due to its high electron density. Also the V_S(**r**) over the heterocyclic rings are highly negative, they are more negative than **58** and certainly than **59**. Therefore, it is not surprising that compound **60** failed to raise antibodies specific for pHis, since it does not resemble the electronic structure of pHis at physiological pH at all. It is also surprising that the triazole mimic **62** did produce antibodies that recognized pHis. However, using the same logic it can be deduced that they should not have a high affinity.

The antibodies produced using a peptide containing a mimic with heterocyclic core **62**, showed some cross reactivity with pTyr and, to a lesser extent pThr and pSer.[112] However, the cross reactivity was only seen in the antibodies obtained from one of three rabbits. Antibodies generated using compound **63** might also show some cross-reactivity with other phosphorylated amino acids. To which extent this would be an issue has to be determined using affinity experiments using non-pHis phospho-amino acids.

Thus, it is clear that the imidazole ring needs to be conserved in the analog to mimic pHis correctly under physiological conditions. Additionally, to ensure a stable compound, the labile phosphoramidate P-N bond has to be exchanged for a stable phosphonate P-C bond. Both these goals can be achieved by simply turning the imidazole ring relative to its substituents (figure 29).

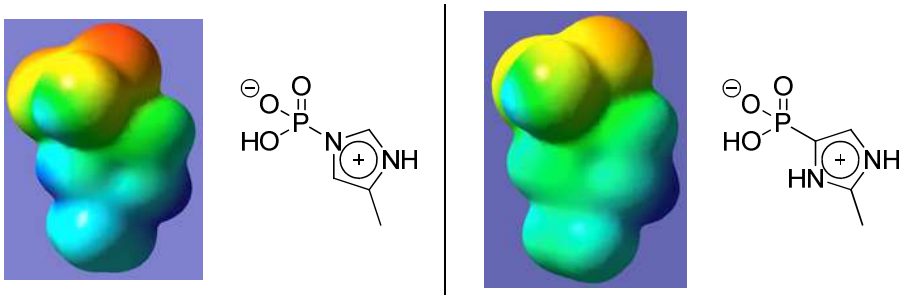


7 **63**

Figure 29: Design of a better pHis analog by turning the imidazole ring.

Surface electrostatic potential calculations were performed on this proposed mimic **63**. The results are compared with protonated pHis (figure 30). It can be clearly seen from the pictures that the two compounds are very similar, not only structural but also in an electronic sense. In contrast to the other analogs, the OH group on the phosphonate has a similar $V_{S,max}$ for pHis (63 kcalmol^{-1}) as for the imidazole analog (58 kcalmol^{-1}). This means that the phosphonate OH can be a hydrogen bond donor even though the phosphonate is negatively charged overall. Additionally, the V_S(**r**) over the imidazole ring is very

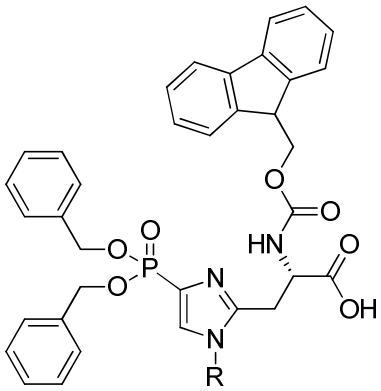
similar. Both structures have a positive potential at the protonated nitrogen. The only difference between **63** and pHis is seen at C-4. pHis has an additional high local $V_{S,max}$ at C-4, while the mimic has a much less pronounced positive potential at that point. Nevertheless, it is clear that the pHis analogue **63** should make an excellent mimic of pHis that should give antibodies selective for pHis.



59 **64**

Figure 30: calculated surface electrostatic potentials of the pHis core (**59**) and the non-hydrolysable analog **64** core at physiological pH.

With the basic design of the pHis established, the next step was the incorporation of these design principles in a SPPS building block. Therefore, **63** had to be synthesized with protective groups amenable for solid phase peptide synthesis. Firstly, it should have an Fmoc protecting group on the amino acid amine moiety, to allow its use in Fmoc based SPPS. Secondly, the other protecting groups on the building block should preferably be acid labile, so they can be cleaved during the global deprotection and cleavage step, when peptide synthesis is finished. Taking these constraints into consideration compound **65** was envisioned as a good building block.



65

This compound has two benzyl groups as protecting groups for the phosphonate. More common groups on phosphonates are methyl or ethyl groups, however these are much harder to cleave, requiring TMS bromide or even stronger reagents. The benzyl groups, on the other hand, are easily cleavable using TFA and a scavenger. A disadvantage of benzyl protecting groups is that they can also be removed by strong nucleophiles and (very) strong bases; however, these conditions can be avoided during the synthesis of compound **65**.

Several options could be considered as protecting group 'R' on the imidazole. Trityl is used in SPPS for histidine and would be a good choice as a protective group for analog **63**, since it is stable and easily cleaved with acid. Another possibility could be a boc group, even though they are less stable on imidazoles, they should remain stable as long as the pH stays basic. 4-Methoxybenzyl and 2,4-dimethoxybenzyl are two other protecting groups that have been used during these studies. Finally, a tosyl protecting group could be used. However, cleavage of the tosyl group would require different conditions than the acidolytic cleave of the peptide.[113]

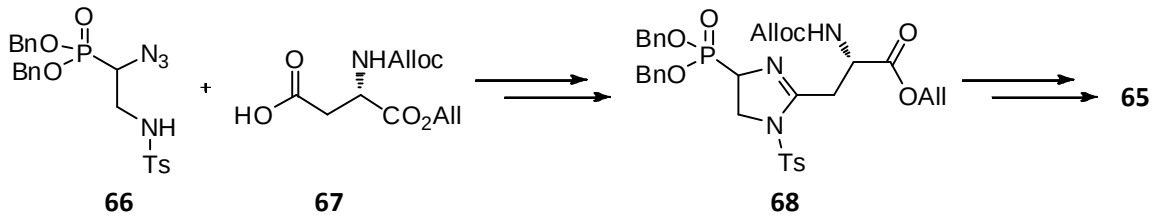
The Fmoc group, however, cannot be used for the entire synthesis as basic conditions are expected to be employed. Additionally, the carboxylic acid moiety also needs to be protected to avoid side reactions. Several protecting groups were considered but finally the Alloc and allyl ester groups were chosen for the amine and acid respectively. The main advantage of these two protecting groups is that they are stable under most acidic and basic conditions. They are both cleaved in the presence of palladium(0) and a scavenger, usually in high yield. After their removal an Fmoc group can be used to make the building block SPPS ready.

8 Research toward a non-hydrolysable 3-phosphohistidine analog

In order to obtain the non-hydrolysable 3-pHis mimic, a versatile route toward the synthesis of **65** needed to be developed. Even though there are many syntheses towards imidazoles (chapter 5.7), only a few publications exist about phosphonic acid imidazoles.[114] Therefore the synthesis of the 3-phosphohistidine mimic is not as straightforward as one might expect. Many different synthetic strategies can be considered to synthesize compound **65** and many different routes have been pursued during the studies reported in this thesis. Below is a graphical abstract of the different routes toward the pHis mimic that are reported in this work. They can be roughly grouped by those that started with a fully formed imidazole that was to be substituted and those routes that formed the imidazole during synthesis. The routes are briefly described below.

8.1.1 Routes that included imidazole synthesis

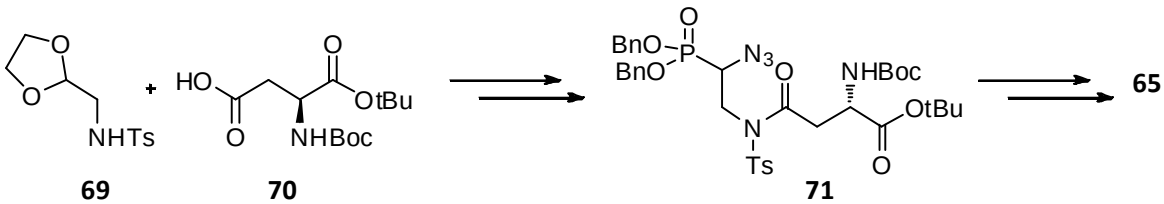
8.1.1.1 Aza-wittig route



66 **67** **68**

The Aza-wittig route was the route that eventually provided **65** in good overall yield, amenable to scale up into preparative amounts. It started with dibenzylphosphite and 4-methoxybenzyl amine and involved an aza-wittig ring closing reaction-aromatization as central, imidazole forming step. The major challenges were the oxidation/aromatization of the imidazoline and selection of a suitable protecting group for the imidazole (chapter 8.2).

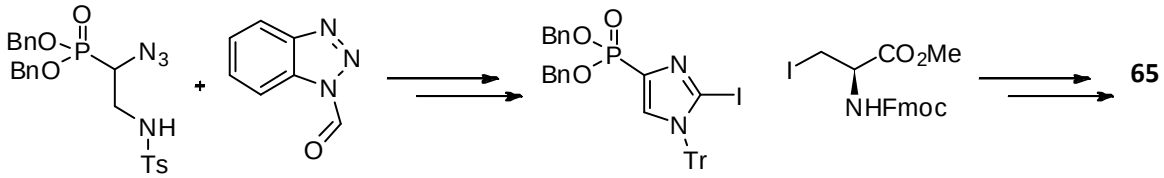
8.1.1.2 Alternative Aza-wittig route



69 **70** **71**

The alternative Aza-wittig route would also close the imidazole using an aza-wittig reaction, but introduced the amino acid part much earlier in the synthesis. This route was one of the first attempts and failed due to an unstable intermediate (chapter 8.3.4).

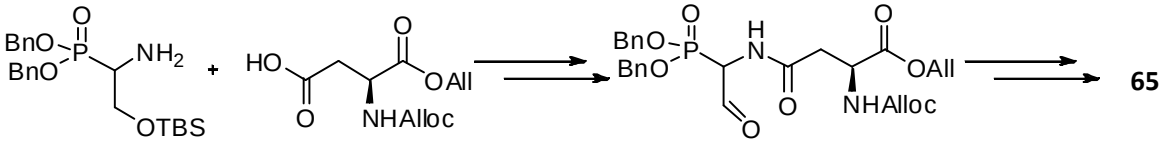
8.1.1.3 Negishi route I



66 **72** **73** **74**

The first part of the Negishi route involved the synthesis of the imidazole by Aza-wittig after a formylation of the tosylamide. Then the imidazole would be iodinated and a Negishi coupling would be performed to obtain the target compound. Regrettably, the yield of the imidazole obtained using the Aza-wittig strategy was to low too make this strategy feasible (chapter 8.3.1). Later on, a different route to iodo-imidazole was devised, which allowed the exploration of the Negishi reaction itself (chapter 8.4.2).

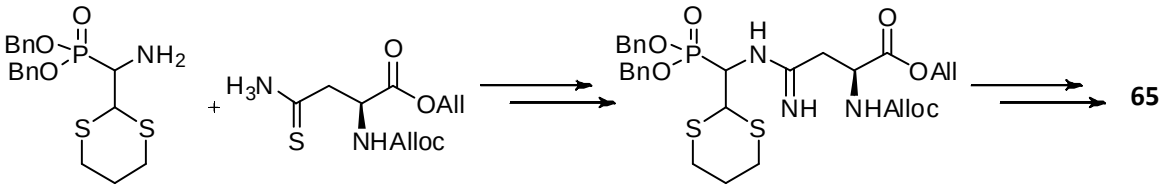
8.1.1.4 Aldehyde route



75 **67** **76**

This route was pursued in an attempt to circumvent the oxidation of the imidazole by having all atoms at the desired oxidation state before ring closure. The route started with dibenzylphosphite and (tert-butyldimethylsilyloxy)acetaldehyde. This route failed due to an unstable intermediate (chapter 8.3.2).

8.1.1.5 1,3-Dithiane route

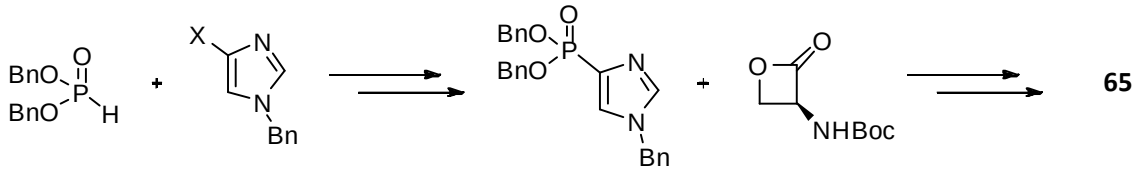


77 **78** **79**

The 1,3-dithiane route was analogues to the aldehyde route except that it did not have an unstable aldehyde intermediate but a dithiane instead. This route failed due to a failure of the synthesis of intermediate **77** (chapter 8.3.3).

8.1.2 Routes starting with an imidazole

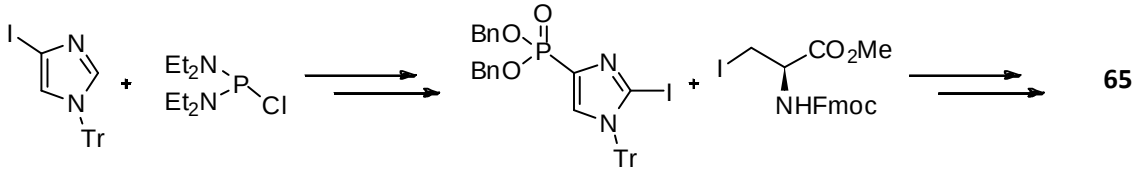
8.1.2.1 Metal catalyzed & Halogen-metal exchange



80 X = Br (**81**), I(**82**) **83** **84**

This imidazole substitution was chronologically the first strategy that was pursued. Several attempts were made to phosphorylate imidazoles at the C-4 position, including halogen metal exchange and metal catalyzed couplings. Regrettably all these reactions did not work (chapter 8.4.1).

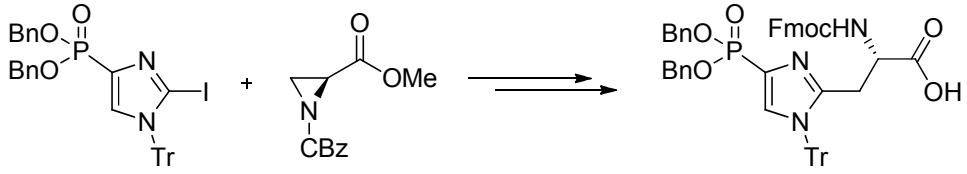
8.1.2.2 Negishi route II



85 **86** **73** **74**

This second part of the Negishi route started after a successful route toward the imidazole was developed. Many conditions were investigated to couple these two iodo compounds but none worked (chapter 8.4.2).

8.1.2.3 Aziridine opening route



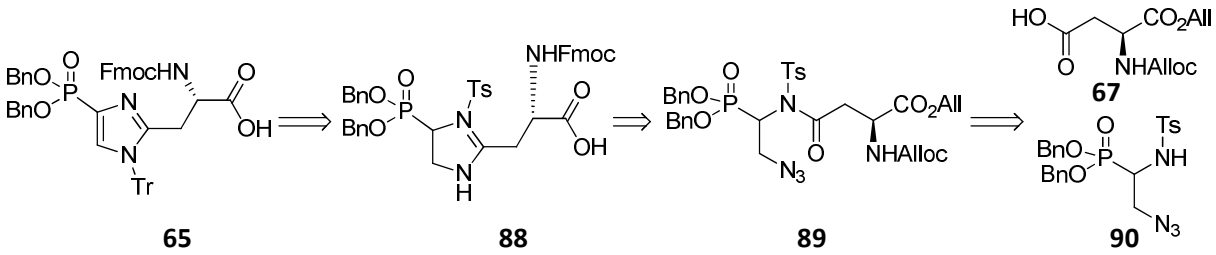
73 **87** **65**

Finally, it was attempted to use halogen metal exchange to synthesize the C-2 anion of the imidazole, followed by nucleophilic ring opening of an aziridine (chapter 8.4.3).

Although the routes depicted above are reported separately in this thesis, many were pursued in parallel, sometimes coming to a halt and later being picked up again. Chemistry discovered in one route was often used in other routes. Therefore, the different paragraphs will refer to each other frequently. Now the routes will be discussed separately, starting with the synthesis that was finally successful to yield **65**, using the aza-wittig reaction to assemble the imidazole.

8.2 Aza-wittig strategy

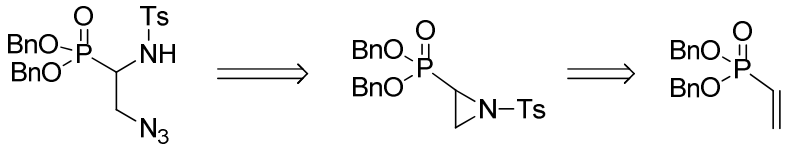
As mentioned above, the strategy presented in this chapter led to the successful synthesis of 3-pHis analogue **65** (figure 31). The retrosynthetic analysis involved the formation of the imidazoline **88** via an aza-wittig reaction, followed by the adjustment of the heterocycle's oxidation state. The required precursor for the aza-wittig reaction **89** was expected to be available by an amide coupling reaction of compounds **67** and **90**. Key challenges for this route were expected to be the synthesis of compound **90** and oxidation of the imidazoline.



65 **88** **89** **90**

Figure 31: Retrosynthetic analysis of compound **65**.

Compound **67** could be easily synthesized via a literature procedure.[115] Compound **90** should be available by nucleophilic ring opening of aziridine **91** with an azide.[116] The aziridine should be accessible via a reaction with chloramine-T (figure 32).[43]

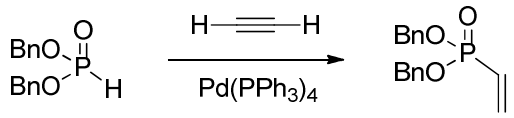


90 **91** **92**

Figure 32: Retrosynthetic analysis of compound **90**.

8.2.1 Synthesis toward compound 90

The synthesis toward **90** started with dibenzyl vinylphosphonate **92**, which was easily obtained by bubbling acetylene through a solution of dibenzylphosphite **80** and tetrakis(triphenylphosphine)palladium(0) in THF (scheme 1) until all of the dibenzylphosphite was consumed.[116]

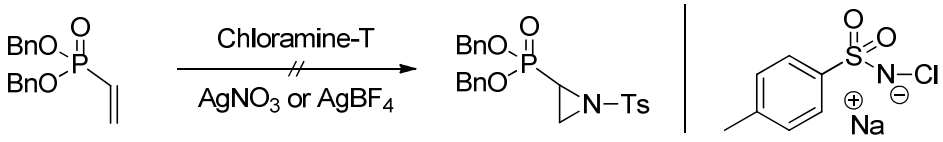


80 **92**

Scheme 1: Synthesis of dibenzyl vinylphosphonate **92**.Reagents and conditions: acetylene, tetrakis(triphenylphosphine)palladium(0) (0.05 equiv), THF, rt, 7h, 72%.

Next, attempts were made to react **92** with chloramine-T **93** and silver nitrate to obtain aziridine **91**. Kumar et al. reported that chloramine-T and silver nitrate react to form a nitrene, which should react with alkenes.[117] Unfortunately, these reaction conditions were not successful in providing intermediate

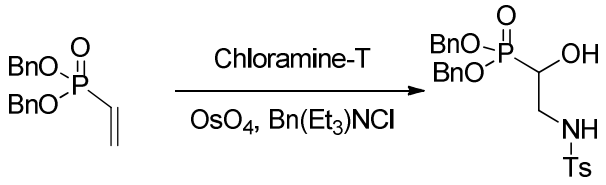
91. Using rigorously dry reaction conditions or silver tetrafluoroborate, as a more soluble silver source, also did not produce the target compound (scheme 2).



92 **91** **93**

Scheme 2: Unsuccessful aziridation strategy. Reagents and conditions: chloramine-T (1 equiv), silver nitrate or silver tetrafluoroborate (1 equiv), THF, rt.

Since the nitrene conditions did not yield the desired product, it was decided to synthesize the aziridine via a multi-step synthesis starting with aminohydroxy compound **94** (scheme 3). This compound might then be submitted to ring closing conditions to yield the aziridine **91**. Sharpless et al. had shown that vinyl phosphonates could be aminohydroxylated using chloramine-T and osmium tetroxide,[118] in line with the more well-known Sharpless dihydroxylation.[119] However, in their case only aryl substituted olefins could successfully be oxyaminated. It was gratifying to see that our vinyl phosphonate performed well using this reaction conditions, giving up to 50 percent yield. During acidic work-up and solvent evaporation of the reaction it was noticed that the aminohydroxide **94** was decomposed. A final basic wash during the work-up and low temperature (30°C) evaporation of the solvent were essential to prevent any decomposition of the product.

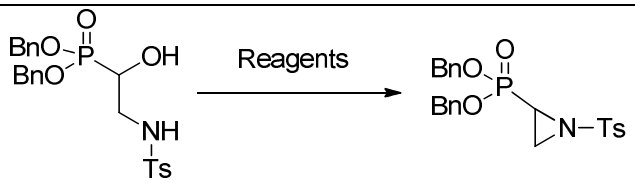


92 **94**

Scheme 3: Aminohydroxylation of dibenzyl vinylphosphonate **92**. Reagents and conditions: chloramine-T (1.25 equiv), osmium tetroxide (0.01 equiv), benzyl triethylammonium chloride (0.05 equiv), $CHCl_3$, water, 60°C, 16h, 50%.

To facilitate ring closure to the aziridine, activation of the hydroxyl moiety was attempted in two ways; via the mesylate and via a mitsunobu reaction. The mesylate formed quickly and was isolated in high purity. However, the ring closing reaction using potassium carbonate did not yield any product. Conversely, the Mitsunobu reaction gave the aziridine in a single step and high yield. The product of the Mitsunobu reaction was contaminated with triphenylphosphine oxide (PPh_3O) and diisopropyl hydrazine-1,2-dicarboxylate. The PPh_3O was easily removed by column chromatography but the hydrazine eluted in the same fractions as the product. This problem was solved by using dimethylazodicarboxylate (DMAD) instead of diisopropylazodicarboxylate (DIAD) for the activation of the triphenylphosphine. The hydrazine product from DMAD had a different R_f and was thus easily separated on column together with PPh_3O. The reaction conditions used to obtain the aziridine are summarized in table 1.

Table 1: Synthesis of aziridine **91**.

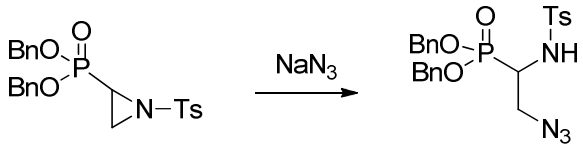


94 **91**

#	Reagents	Result
1	MsCl,[a] K_2CO_3[b]	96% mesylate of **94**
2	PPh_3, DIAD[c]	100% conversion
3	PPh_3, DMAD[d]	93%

[a]: methyl sulfonylchloride (2 equiv), triethylamine (3 equiv), DCM, -10°C to rt, 75 min, 96%. [b]: potassium carbonate (5 equiv), DMF, rt, 16h, trace. [c]: triphenylphosphine (1 equiv), diisopropylazodicarboxylate (1 equiv), THF, 0°C to rt, 2h, not isolated.[d]: triphenylphosphine (1 equiv), dimethylazodicarboxylate (1 equiv), THF, 0°C to rt, 2h, 93%.

With the aziridine in hand a nucleophilic ring opening with sodium azide in DMF was performed.[120] The reaction proceeded uneventful and in good yield, providing key intermediate **90** as a single regio-isomer (scheme 4).



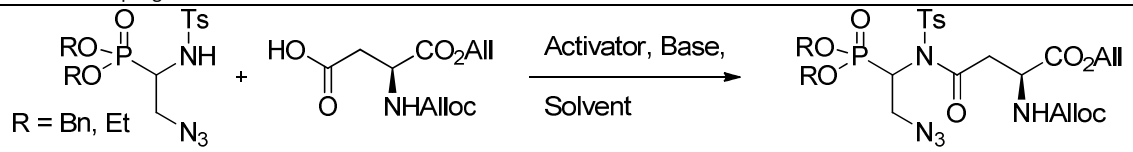
91 **90**

Scheme 4: Aziridine ring opening. Reagents and conditions: a) sodium azide (1.25 equiv), DMF, rt, 16h, 78%.

8.2.2 Amide coupling I

Next, sulfonamide **90** needed to be coupled to Alloc/allyl protected aspartic acid **67**. Due to the electron withdrawing character of the tosyl group, the amine was expected to be a weak nucleophile. Therefore, several strong activators were tested to activate acid **67** (table 2). The initial trial used the preformed HOAt-ester of **67** and DBU, conditions that were successful for other tosylamides (see chapter 8.3.4).[121] However, amide **89** was not detected in the complex mixture of products that resulted from the reaction. Changing amino acid protecting groups from allyl ester/ Alloc to tert-butyl ester/ boc to mimic more closely the conditions from chapter 8.3.4, did not yield any different result. These results, coupled with the discovery that **90** slowly decomposed in the presence of DBU, led us to consider other activation strategies (table 2).

Table 2: Amide coupling conditions.



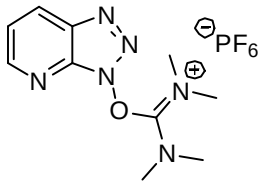
95 R = Et, **90** R = Bn **67** **96** R = Et, **89** R = Bn

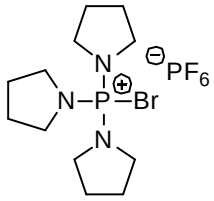
Reagents and conditions: **67** (1.1equiv), Activator (1.1 equiv), base, rt, 16h.

#	Activator	Base (equiv)	DMAP (equiv)	Solvents	Tosylamide	Result
1	HOAt	DBU (1.5)	—	DCM	**90**	Decomposition
2	HATU	DBU (2)	—	DCM, DMF	**90**	Decomposition
3	HATU	Cs_2CO_3 (4)	—	DCM, DMF	**90**	No reaction
4	HATU	—	2	DCM	**90**	Trace[a]
5	DIC	Cs_2CO_3 (1.1)	1.1	DMF	**90**	No reaction
6	PyBrOP	DIPEA (2.2)	1.1	DCM	**95**	Trace[a]
7	PyBrOP	DIPEA (3)	1.25	DCM	**95**	0 – 42%

[a]: The mass was detected on the LCMS but the compound was not isolated.

HATU is used as coupling reagent for a wide range of amide coupling reactions including SPPS strategies; therefore it might be successful in promoting this coupling. In these reactions the ester was activated in situ by the addition of base and coupling reagents for five minutes, after which the tosylamide was added. Using DBU as a base did not yield any product before the starting material decomposed (table 2: entry 2). Since the tosylamide was not stable to DBU a replacement was considered. Cesium carbonate was expected to perform better, as an inorganic base it might not decompose starting material **90**. While the tosylamide was indeed stable under these conditions, no product was obtained (table 2: entry 3). HATU with 2 equivalents of DMAP seemed to yield traces of product. Unfortunately, these conditions could not be optimized to obtain a satisfying amount of product (table 2: entry 4). DIC with DMAP did also not yield any product (table 2: entry 5). At this time supplies of dibenzyl vinylphosphonate ran out, as well as the starting material to make it, therefore, diethyl vinylphosphonate was temporarily utilized while exploring the chemistry of these compounds. The final coupling reagent that was tested in this reaction was PyBrOP. PyBrOP is a phosphonium based coupling reagent that does not require pre-activation of the acid. Under these conditions a moderate yield was obtained (table 2: entry 7) but the reaction was poorly reproducible and yielded inconsistent amounts of product. Since large amounts (multi-gram) of this compound were needed, another, more reliable strategy was considered.

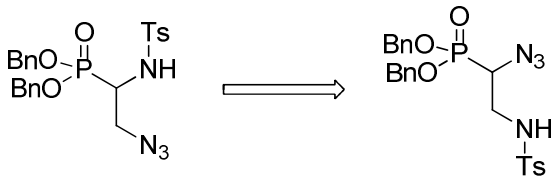

HATU


PyBrOP

8.2.3 New strategy

Looking closely at **90**, one can notice that, not only is the amine deactivated by the tosyl group, there is also considering steric hindrance from the bulky benzyl protecting groups on the phosphonate. Additionally, the phosphonate serves as an electron withdrawing group, which might have an additional deactivating effect on the amine. To test these hypotheses, and to successfully perform the amide

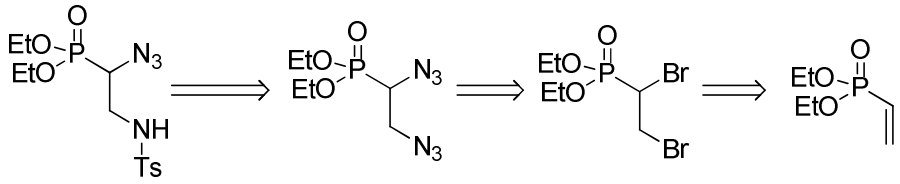
coupling, it was decided to switch the tosylamide and the azide moieties (figure 33). In doing so any negative influences from the phosphonate would be minimized, leading to a facile amide coupling and, after deprotection of the imidazole ring, the same final product would still be obtained.



90 **66**

Figure 33: Switching azide and tosylamide groups.

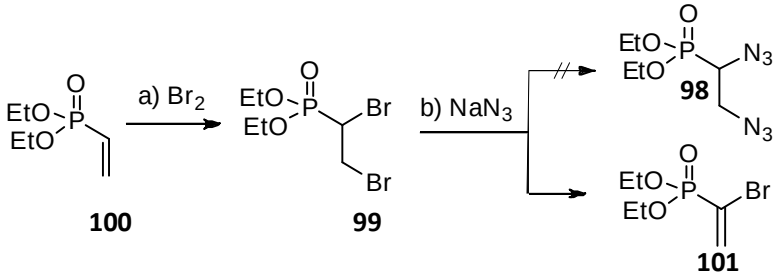
As the aziridine **91** had high selectivity to open at C-3 it was not suitable as an intermediate for the synthesis of **66**. Therefore, a new synthesis was designed starting from diethyl vinylphosphonate **100** (figure 34). After dibromination of diethylvinylphosphonate **100**, both bromines would be substituted with sodium azide.[41, 44] The azide beta to the phosphonate, would then be selectively reduced with one equivalent of triphenylphosphine, followed by tosylation.[26, 115]



97 **98** **99** **100**

Figure 34: Retrosynthetic analysis of **97**.

The bromination of diethyl vinylphosphonate proceeded smoothly.[122]The substitution reaction however, did not produce desired product **98**. Instead, the beta bromine eliminated to form diethyl (1-bromovinyl)phosphonate **101**. This reaction would be expected under basic conditions. However, it was surprising that the elimination took place with only sodium azide present. At this point it was decided to focus on a different route that used conditions previously successful in this project.



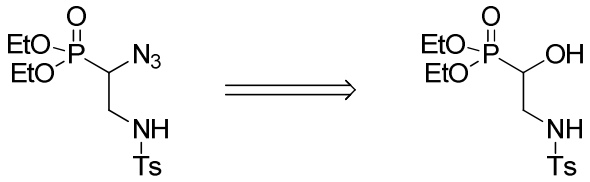
100 **99** **98** **101**

Scheme 5: Synthesis toward **97**. Reagents and conditions: a) bromine (1.02 equiv), $CHCl_3$, rt, 3h, quant. b) sodium azide (8 equiv), DMF, rt, 16h, quantitative.

8.2.4 Azide introduction

The new strategy relied on the previously described product of the Sharpless aminohydroxylation, **94** (paragraph 8.2.1). The alcohol could be activated and then substituted by an azide nucleophile. The key

transformation of this route was the activation of the hydroxyl function followed by its substitution without interference from intramolecular aziridine formation.

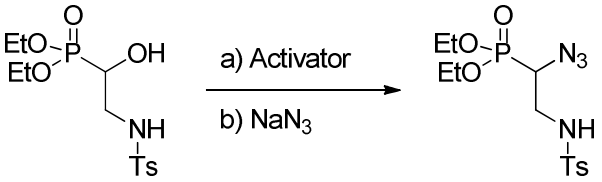


97 **94**

Figure 35: New retrosynthesis of **97.**

Initially, some sulfonyl chlorides were used to activate the alcohol followed by substitution with sodium azide (table 3). Activation with the sulfonyl chlorides went smoothly in all cases. However, problems became immediately apparent when the methyl sulfonyl activated alcohol did not react with sodium azide (entry 1). The tosyl activated alcohol yielded an unidentified product (entry 2) and the o-nitrobenzene sulfonyl activated alcohol mostly formed the aziridine along with some unidentified byproducts (entry 3).

Table 3: Azide formation I.

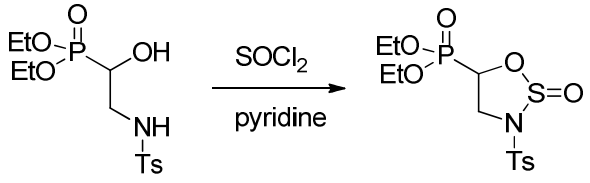


102 **97**

Reagents and conditions: a) Sulfonyl chloride (2 equiv), triethylamine (3 equiv), rt, 2h. b) Sodium azide (4 equiv), DMF, rt, 16h.

#	Activator	result
1	Mesyl	No reaction
2	Tosyl	Unidentified products
3	2-Nosyl	Aziridine formation

To prevent aziridine formation it was decided to protect the hydroxylamine **102** as compound **103** (scheme 6). This compound contains an oxathiazolidine that simultaneously activates the hydroxyl moiety and protects the tosylamide.[10a, 123] After substitution of the alcohol, the protecting group should leave the reaction as gaseous sulfur dioxide.[124]

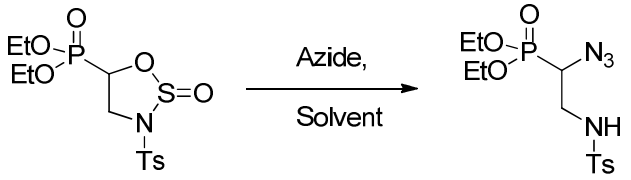


102 **103**

Scheme 6: Synthesis toward **103**. Reagents and conditions: a) thionyl chloride (1.4 equiv), pyridine (2.5 equiv), THF, -78°C to rt, 6h, 93% (NMR yield).

Synthesis of the oxathiazolidine was fairly straightforward. Reaction with thionyl chloride and pyridine in THF yielded the product in **93** percent yield. Interestingly, substituting pyridine for 2,6-lutidine yielded no product. This result emphasizes that an acyl transfer reagent (in this case pyridine) is required to drive the reaction. Purification of the oxathiazolidine proved to be problematic; the compound decomposed during silica flash column chromatography. Therefore, it was used as the crude mixture in all subsequent reactions. The oxathiazolidine was submitted to various reaction conditions in order to obtain azide **97**, summarized in table 4.

Table 4: Azide formation II.



103 **97**

Reagents and conditions: azide (5 equiv), solvent, rt, 16h.

#	Azide	Solvent	Result
1[a]	NaN_3	DMF	**102**
2	$TMSN_3$	THF	**102** and an unidentified by product
3[b]	$TMSN_3$, TBAF	THF	TMS adduct of **102**
4[c]	Bu_4NN_3	THF	**102** and an unidentified by product

[a]: reaction was done at 60°C. [b]: 0.25 equiv TBAF was added. [c]: 2.5 equiv azide were used, reaction was stirred for four hours.

As can be derived from the table, sodium azide in DMF did not yield desired product **97** but instead returned alcohol **102** (entry 1). Presumably, the azide was not reactive enough and subsequent aqueous work-up decomposed **103** to its precursor, **102**. Therefore, different azide sources that were more soluble in organic solvents were considered. TMS-azide (entry 2) resulted in **102** along with an unidentified byproduct. Adding a catalytic amount of TBAF was expected to promote the substitution reaction by scavenging the TMS cations, thereby creating a more soluble tert-butyl ammonium azide in situ. However, added TBAF (1M in THF) yielded a surprising quantitative amount of TMS-ether adduct of **102**. It seems that TBAF catalyzed the decomposition of the oxathiazolidine to **102**, which then reacted to the silyl ether with TMS-azide.

A possible mechanism for the formation of **104** is outlined in figure 36. In this proposed mechanism the fluorine anion attack the electrophilic sulfoxide that is further activated by the electron withdrawing effect of the tosylamide. The alkoxide attacks a TMS group, freeing an azide to attack the sulfoxide and free the tosylamide. The sulfurazidous fluoride can be attacked by a second equivalent of azide, freeing the fluorine to start the catalytic cycle again. Finally, an aqueous work-up with sodium bicarbonate removes the labile TMS group on the tosylamide, while leaving the TMS group on the alcohol. The diazidosulfoxide reacts further to form hydrazoic acid and sulfur dioxide.

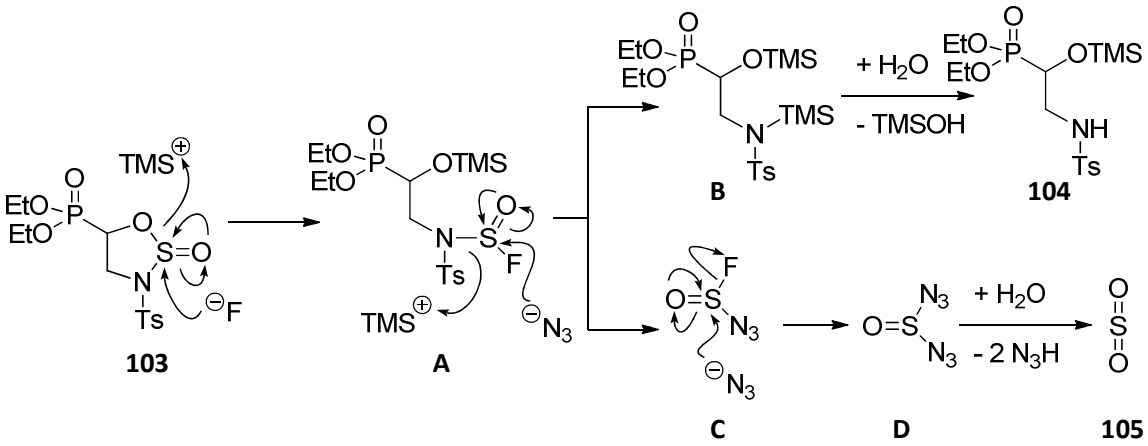
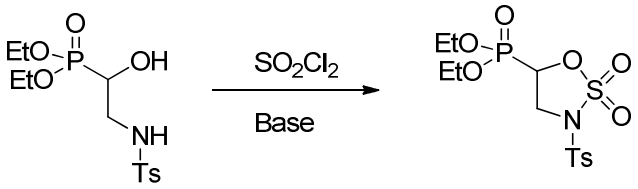


Figure 36: Possible mechanism for the decomposition of the oxathiazolidine by catalytic fluorine.

In order to increase the reactivity of the oxathiazolidine so it would react with one of the azide sources. Attempts were made to synthesize oxathiazolidine **106** with a sulfonyl group instead of a sulfoxide. However, attempts to synthesize such a compound, through direct synthesis from alcohol **102** with sulfuryl chloride or 1,1-sulfonyldiimidazole failed (table 5). Also oxidation of the sulfoxide **103** with $RuCl_3.3H_2O/NaIO_4$ did not produce any **106** (not shown).[125] A likely explanation is that the compound was not stable under the reaction conditions due to its high reactivity.[126]

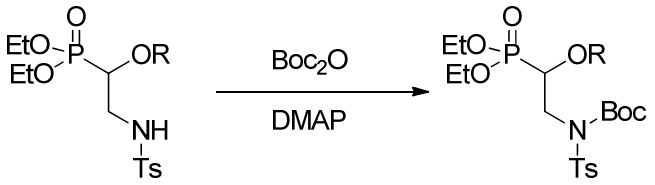
Table 5: Protection of **102** with a sulfonyl group.



102 **106**

#	Sulfonyl source (equiv)	Base (equiv)	Solvent	Temp. (°C)	Result
1	Sulfuryl chloride (1.4)	Pyridine (2.5)	THF	-78 to -41	Decomposition
2	1,1-sulfonyldiimidazole (1.5)	NaH (3)	DMF	0 to -41	Decomposition

Since the oxathiazolidine strategy was unsuccessful, a more conventional protecting group was needed. The Boc group seemed appropriate since it is generally easy to introduce. More importantly, it is also easy to remove under acidic conditions and stable to the basic conditions used in the synthesis. The Boc group was introduced reacting mesylate **107** or tosylate **108** (table 3) with Boc anhydride in the presence of a catalytic amount of DMAP, yielding **109** and **110** respectively (scheme 7).

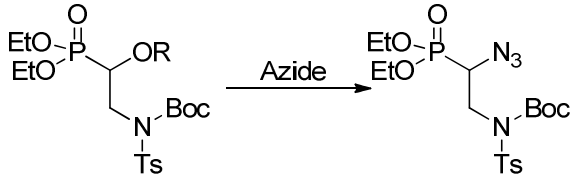


R = Ms **107**, Ts **108** R = Ms **109**, Ts **110**

Scheme 7: Boc protection of tosylamides. Reagents and conditions: Boc anhydride (1.2 equiv), DMAP (0.1 equiv), DCM, rt, 3 h, **109**: 77%, **110**: 49%.

The Boc protected mesylate was allowed to react with sodium azide in DMF, however only starting material was recovered (table 6: entry 1). When sodium azide was substituted for N,N,N',N'-tetramethylguanidinium azide (TMGA) in MeCN, an azide source that has been reported as successful in similar systems,[114b, 123, 127] also no reaction took place (entry 2). The tosylate did not do better, at room temperature and at 40°C no reaction took place in the presence of TMGA. At even higher temperature (80°C) decomposition of the starting material into an unidentified mixture of compounds occurred, possibly due to attack of TMGA on the benzyl protecting groups (entries 3-5).

Table 6: Azide formation III.



R = Ms **109**, Ts **110** **111**

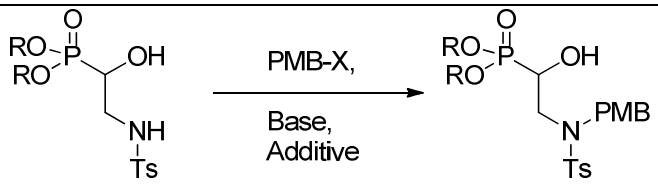
Reagents and conditions: azide (1.5 equiv), MeCN, 16 h.

#	R	Azide	Temp. (°C)	Result
1[a]	Ms	NaN_3	rt	No reaction
2	Ms	TMGA	rt	No reaction
3	Ts	TMGA	rt	No reaction
4	Ts	TMGA	40	No reaction
5	Ts	TMGA	80	Decomposition

[a]:3 equiv of sodium azide were used, DMF was used as the solvent.

The results summarized in table 6 indicate that the mesylate and the tosylate do not possess sufficient leaving group ability to allow substitution by the azide anion. Additionally, the Boc protecting group might retard the reaction through steric hindrance. Therefore, it was decided to change the Boc group to a PMB protecting group, which is less bulky than the Boc-group, and to change the activating group on the alcohol to a triflate, which is a better leaving group.[128] Optimization of the PMB introduction is summarized in table 7.

Table 7: PMB protection of tosylamides.



102 R = Et, **94** R = Bn **112** R = Et, **113** R = Bn

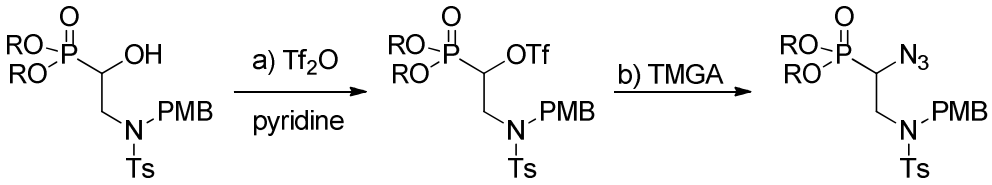
Reagents and conditions: p-methoxybenzyl halide, base, additive, solvent, rt, 16h.

#	PMB-X (equiv)	Base (equiv)	Additive (equiv)	Solvent	Tosylamide	Yield (%)
1	Cl (1.1)	Cs_2CO_3(1.1)	—	MeCN	**102**	63
2	Cl (1.5)	Cs_2CO_3(1.1)	—	DMF	**94**	55[a]
3	Cl (1.1)	K_2CO_3(1.1)	KI (0.1)	Acetone	**94**	No reaction
4	Cl (2.5)	K_2CO_3(1.1)	—	Acetone	**94**	No reaction
5	Cl (2)	K_2CO_3(2.5)	Bu_4NI (0.1)	Acetone	**94**	53[a]
6	Br (2)	K_2CO_3(2.5)	—	DMF	**94**	75[a]
7	Br (2)	Cs_2CO_3(2.1)	—	DMF	**94**	80[a]
8	Br (2)	Cs_2CO_3(2)	—	DMF	**94**	91

[a]: NMR yield.

The PMB group was installed by stirring **102** in acetonitrile in the presence of p-methoxybenzyl chloride (PMBCl) and cesium carbonate, although in modest yield (entry 1). In an attempt to increase the yield of the reaction a small optimization study was carried out. Switching solvent to DMF did not improve the yield of the reaction (entry 2). Finkelstein conditions initially showed no conversion; however, when tetrabutylammonium iodide was used instead of potassium iodide, a modest yield was seen (entries 3-5). Switching from PMB-Cl to PMB-Br and back to DMF as a solvent gave an immediate rise in yield (entry 6). Finally, returning to cesium carbonate as a base gave the product in 91% yield on a 0.5 mmol scale (entry 8).

Introduction of the triflate was uneventful; stirring **112** in DCM in the presence of triflic anhydride and pyridine gave triflate **114** quantitatively. Submitting the triflate to the TMGA conditions provided the azide in good yield (scheme 8). The lower yield for **117** compared to **116** is most likely due to slow decomposition of **115** by nucleophilic attack of the guanidine on the benzyl protecting group. Therefore, it was essential to monitor the reaction closely and quench it as soon as all starting material was consumed.

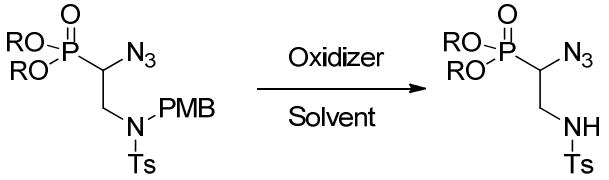


R = Et **112**, Bn **113** R = Et **114**, Bn **115** R = Et **116**, Bn **117**

Scheme 8: Synthesis of **116**. Reagents and conditions: a) triflic anhydride (1.5 equiv), pyridine (2 equiv), DCM, -15°C, 10 min, quantitative. b) TMGA (1.1 equiv), MeCN, 0°C to rt, 2-16h, **116** 90%, **117** 70%.

The deprotection of the tosylamide **117** was performed by oxidation of the PMB group (table 8). DDQ was initially investigated to remove the PMB group but no reaction occurred (entry 1). CAN was considerably more successful, giving 71 percent isolated yield after a few hours an acetonitrile: water mixture (entry 2). Upon switching back to the benzyl protecting groups on the phosphonate, a complex mixture was obtained that gave only a modest yield of product (entry 3), presumably due to the slight sensitivity of the benzyl groups to oxidation. A solvent switch to 1,4-dioxane containing 10 percent water yielded a much cleaner mixture and gave the compound in 85 percent yield (entry 4).

Table 8: PMB deprotection of tosylamides.



R = Et **116**, Bn **117** R = Et **97**, Bn **66**

Reagents and conditions: oxidizer (3 equiv), solvent, rt, 8h.

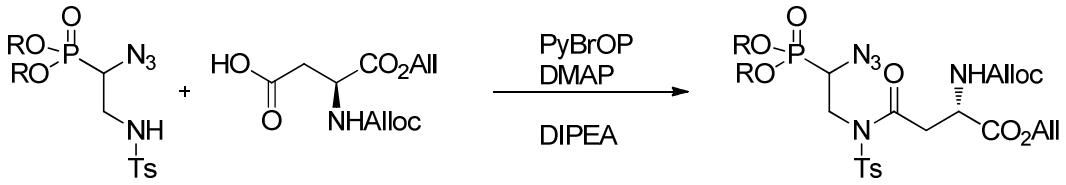
#	Oxidizer	Solvent (ratio)	Tosylamide	Yield (%)
1[a]	DDQ	DCM: H_2O (10: 1)	**116**	No Reaction
2	CAN	MeCN: H_2O (5: 1)	**116**	71%
3	CAN	MeCN: H_2O (5: 1)	**117**	56%
4	CAN	1,4-dioxane: H_2O (9: 1)	**117**	85%

[a]: 1.2 equiv DDQ was used and the mixture was stirred 20 hours.

8.2.5 Amide coupling II

With compounds **66** and **97** in hand the amide coupling was reinvestigated. The PyBrOP conditions proved to be the most successful when using **90** as a coupling partner. Therefore, the same conditions were used for this amide coupling, using both **66** and **97**. After minor optimization, coupling occurred fast and in a reproducible 79 percent yield of **119** (table 9: entry 2).

Table 9: Amide coupling.



R = Et **97**, Bn **66** **67** R = Et **118**, Bn **119**

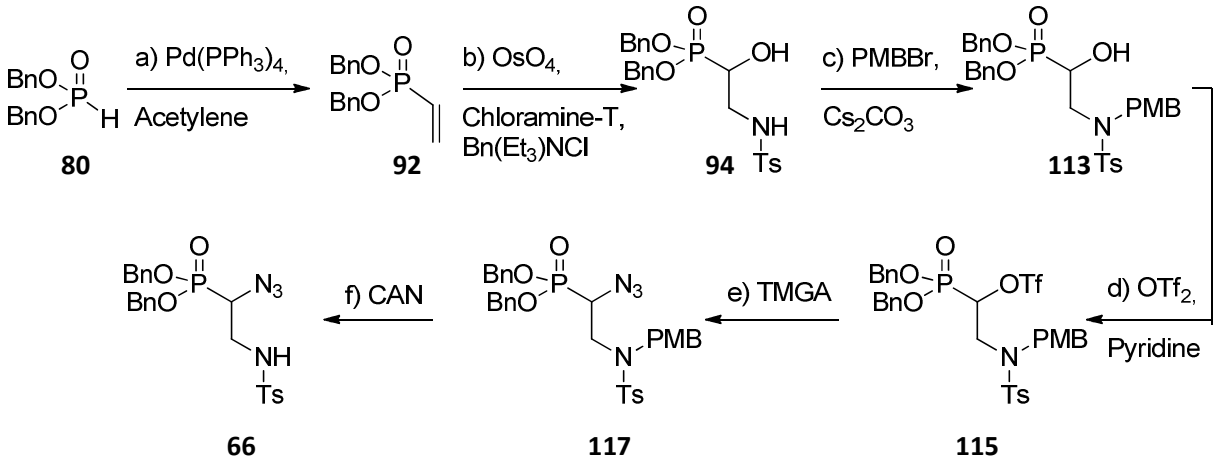
Reagents and conditions: **67**, PyBrOP, DMAP, DIPEA, DCM, rt, 4h.

#	**67** equiv	PyBrOP equiv	DMAP equiv	DIPEA equiv	Tosylamide	Yield (%)
1	1.1	1.1	1.1	2.2	**97**	73[a]
2	1.2	1.2	1.2	2.4	**66**	79

[a]: NMR yield.

A summary of the route described so far is given in scheme 9. Although feasible on a small scale (up to a few hundred milligram), some of the transformations require undesirable reagents (explosive acetylene,

non-commercially available, unstable 4-methoxybenzyl bromide), or were low yielding. Additionally, flash column chromatography was required after every step. To facilitate scale up, different routes were explored to allow multi-gram synthesis of **66** in a higher yield, preferably using purification by crystallization.



Scheme 9: Synthesis of **66**. Reagents and conditions: a) acetylene, tetrakis(triphenylphosphine)palladium(0) (0.05 equiv), THF, rt, 7h, 72%.b) chloramine-T (1.25 equiv), osmium tetroxide (0.01 equiv), benzyl triethylammonium chloride (0.05 equiv), $CHCl_3$, water, 60°C, 16 h, 50%.c) 4-methoxybenzylbromide (2 equiv), cesium carbonate (2 equiv), DMF, rt, 3 h, 91%. d) triflic anhydride (1.5 equiv), pyridine (2 equiv), DCM, -15°C, 10 min, 98%. h) TMGA (1.1 equiv), MeCN, rt, 2h, 70%. f) CAN (3 equiv), 1,4-dioxane: water 9:1, rt, 6.5 h, 82%.

8.2.6 Alternative routes to intermediate 66

Some constraints existed in designing different routes to **66**, the introduction of the azide via triflation and substitution seemed to be the only chemistry that worked well for this transformation and thus could not be changed. The azide introduction also only worked with a PMB group on the tosylamide and, while its introduction could be done at a different point in the synthesis, its removal could not. Therefore, the last three steps in the previous route toward **66** (scheme 9) were not changed. Thus, compound **113** became the focus of a new synthesis strategy. Two additional potential routes towards compound **113** were undertaken going via intermediate **122**. The retrosynthetic analysis is given in figure 37.

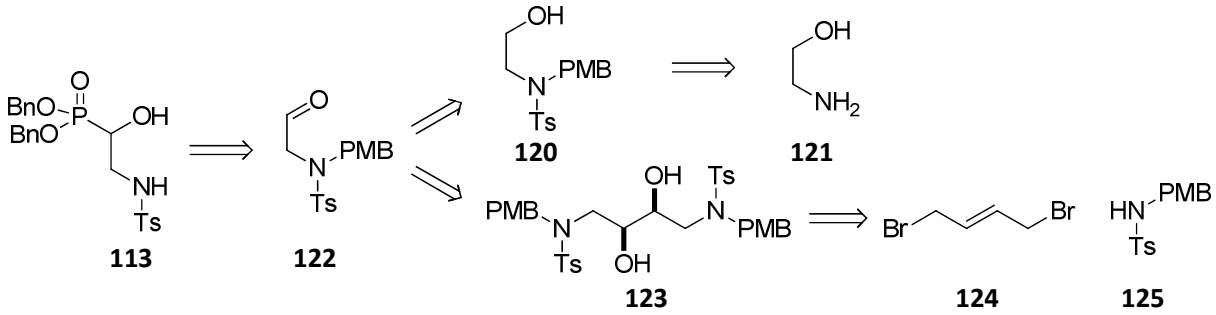
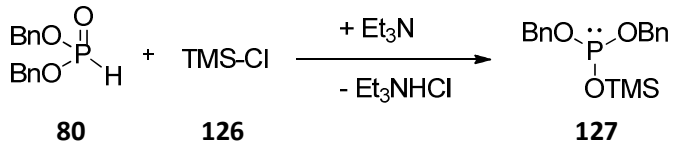


Figure 37: Retrosynthetic analysis of compound **113**.

Synthesis of **113** via **122** could be realized in line with a literature procedure. Bendikov et al. reported on the synthesis of alpha hydroxyl phosphonates by nucleophilic attack of a TMS activated dibenzylphosphite on an aldehyde.[129] Dibenzylphosphite occurs as the phosphonate (phosphorus V)

tautomer, however the phosphite (phosphorus III) tautomer is a better nucleophile due to its free electron pair. To obtain the phosphite tautomer, the phosphonate can be deprotonated and trapped using triethylamine and TMS chloride to provide **127** (figure 38). **127** can then be reacted with aldehyde **122** to provide **113**. This reaction then results in the trimethylsilyl ether adduct of **113**, which should be easy to deprotect. Thus, the target of the strategy became aldehyde **122**.

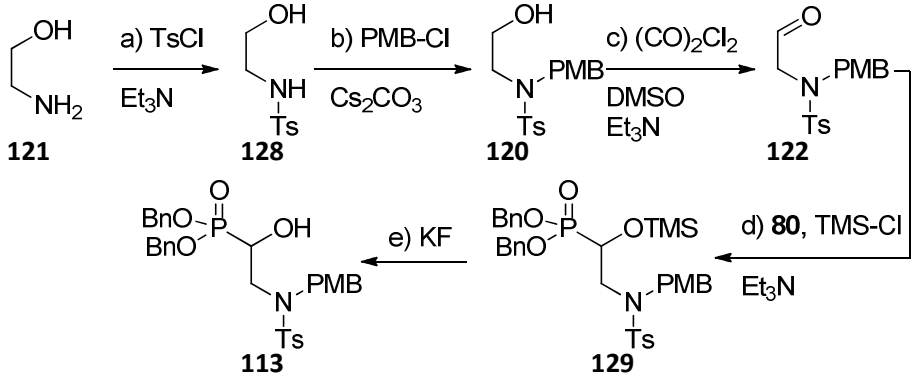


80 **126** **127**

Figure 38: Activation of dibenzylphosphite with TMS chloride.

Two alternative strategies toward aldehyde **122** were evaluated, one route starting with ethanolamine and one starting with p-methoxybenzyl amine (figure 37). Both routes use cheap commercially available starting materials. The ethanolamine route began by tosyl and PMB protection of the amine. Oxidation would then yield the aldehyde **122**. The p-methoxybenzyl amine route started by tosylation of the amine yielding **125**. A reaction with 1,4-dibromobut-2-ene (**124**) should afford an alkene that could be dihydroxylated using Sharpless conditions and then oxidatively cleaved affording the aldehyde **122**. Of the two new routes the first, ethanolamine route was the shortest; however, it was expected that most intermediates in the second p-methoxybenzyl amine route were solids and might be suitable for purification by crystallization.

For the new first route, ethanol amine was tosylated and subsequently PMB protected using PMB chloride, in good yield. A swern oxidation yielded aldehyde **122** in excellent yield. Although **122** is unstable at room temperature, it could be stored for several months at -20°C under argon before significant decomposition was observed. Nevertheless, the aldehyde was always used in the next reaction as soon as possible. The reaction of **122** with activated dibenzylphosphite proceeded smoothly to give phosphonate **129** in 88 percent yield, containing a silyl ether (scheme 10).

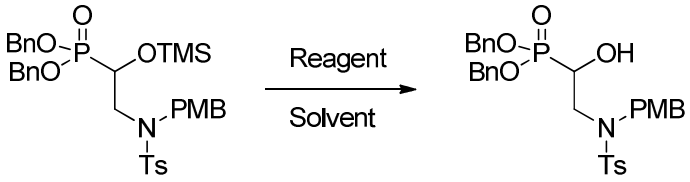


121 **128** **120** **122** **129** **113**

Scheme 10: Synthesis of **113** starting with ethanolamine. Reagents and conditions: a) ethanolamine (1.1 equiv), 4-toluenesulfonyl chloride (1 equiv), triethylamine (1.9 equiv), DCM, 0°C to rt, 16h, 83%. b) p-methoxybenzyl chloride (1.05 equiv), cesium carbonate (1.1 equiv), DMF, rt, 2h, 83%. c) oxalyl chloride (1.1 equiv), DMSO (2.4 equiv), triethyl amine (5 equiv), DCM, -78°C to rt, 2h, 95%.d) dibenzylphosphite (1.2 equiv), chlorotrimethylsilane (1.44 equiv), triethyl amine (1.44 equiv), DCM, -20°C to -78°C to rt, 17h, 88%. e) potassium fluoride (2 equiv), methanol, rt, 30 min, 94%.

Next the removal of the silyl ether was explored (table 10). Removal of the silyl ether was first attempted using citric acid in methanol, a method that worked well on similar compounds (see chapter 8.3.2), but not in this case (entry 1). Since acidic removal did not seem to work, basic removal with potassium carbonate was attempted but again there was no conversion to the free alcohol (entry 2). It is well known that silicon has a strong affinity for fluorine thus two fluorine reagents were tested in the removal of the silyl ether. HF.pyridine yielded mostly starting material and an unidentified byproduct. Similar results were obtained using potassium fluoride in THF: water 9:1 (entry 3 and 4). However, potassium fluoride in methanol gave the target alcohol **113** quickly, in high yield and purity (entry 5).

Table 10: TMS deprotection of phosphonate **129**.

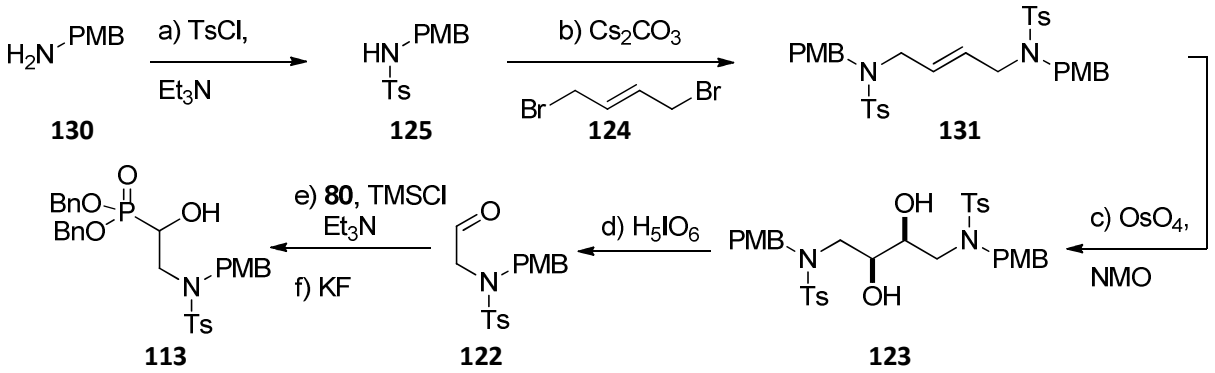


129 **113**

#	Reagent (equiv)	Solvent	Result
1	Citric acid (2.5)	MeOH	No reaction
2	K_2CO_3(3)	MeOH	No reaction
3	HF.Pyridine (0.2)	THF: water 9:1	**129** and unidentified byproduct
4	KF (3)	THF: water 9:1	**129** and unidentified byproduct
5	KF (3)	MeOH	94%

This sequence, although two steps longer than the original vinylphosphonate route, led to target compound **113** in 54 percent overall yield. It also circumvented many of the undesirable reagents and conditions of the vinylphosphonate route. However, still almost every step in the sequence required chromatographic purification. Therefore, yet another route was investigated.

The second new route started with p-methoxybenzyl amine, which was tosylated using tosyl chloride and triethyl amine.[129] Tosylamide **125** was allowed to react with half an equivalent of 1,4-dibromobut-2-ene (**124**, scheme 11), yielding alkene **131** in excellent yield. Sharpless dihydroxylation of the alkene, using osmium tetroxide and NMO, gave the 2,3-dihydroxyl compound **123**, which was oxidatively cleaved using periodic acid to yield aldehyde **122**. The aldehyde was then allowed to react with the activated phosphonate in the same manner as before (scheme 11).



Scheme 11: Synthesis of **113** starting with p-methoxybenzyl amine. Reagents and conditions: a) 4-toluenesulfonyl chloride (1 equiv), triethylamine (2 equiv), toluene, 0C to rt, 2 h, 94%. b) **125** (2 equiv), 1,4-dibromobut-2-ene (**124**) (1 equiv), cesium carbonate (1.2 equiv), DMF, rt, 2h, 91%. c) osmium tetroxide 5 wt% in water (0.05 equiv), N-methylmorpholine-N-oxide (3 equiv), chloroform: water: tert-butanol 20:2.5:1, 50C, 16h, 97%. d) periodic acid (1.1 equiv), 1,4-dioxane, 0C to rt, 1h, 84%. e) dibenzylphosphite (1.2 equiv), chlorotrimethylsilane (1.44 equiv), triethyl amine (1.44 equiv), DCM, -20°C to -78°C to rt, 17h, 88%. e) potassium fluoride (2 equiv), methanol, rt, 30 min, 94%.

This final sequence, although one step longer than the ethanolamine route and three steps longer than the vinylphosphonate route, gave a total of 58 percent yield of **113** over six steps (table 11). More importantly, until the introduction of the phosphonate all compounds were solids that were easily precipitated or crystallized in high purity, eliminating the need for column chromatography.

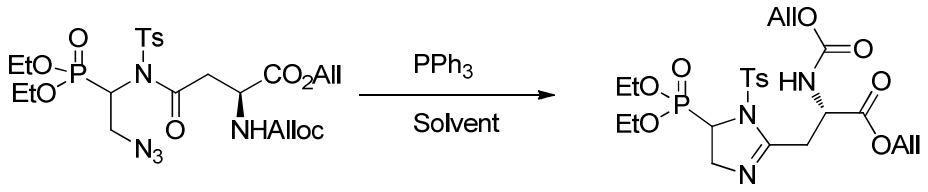
Obviously, the p-methoxybenzyl amine route was superior to the other two as it had a higher overall yield, more robust chemistry and easier purification protocols. This method was used to synthesize compound **113** for the entirety of the project; more than 125 grams were produced in total. **113** could then be transformed to azide **66** via the triflate/TMGA method described before (scheme 9).

Table 11: Numerical comparison of the three strategies for the synthesis of **113**.

#	Strategy	Steps	Yield **113** (%)
1	Vinylphosphonate route	3	32
2	Ethanolamine route	5	54
3	p-methoxybenzylamine route	6	58

8.2.7 Aza-wittig ring closure and aromatization

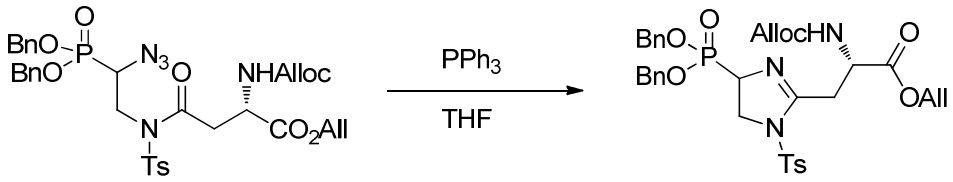
With a consistently high yielding route to **66** available and an amide coupling that was performing well, attention was focused on the aza-wittig ring closing reaction. Initial exploration of this reaction was performed using **96**, although only a small amount was available. Two solvents, THF and 2,6-lutidine, were reviewed, of which the latter should perform better according to literature.[74] The reaction was executed, by dissolving **96** and triphenylphoshpine (PPh_3) in the solvent at 0.045 M and -20°C. The high dilution was used to prevent intermolecular reactions. After addition of PPh_3 the iminophosphorane forms almost instantaneously and, after the mixture stopped bubbling, the Schlenk was closed and heated to 80°C for six hours to bring about the ring closure (scheme 12).



96 **132**

Scheme 12: Initial aza-wittig tests. Reagents and conditions: triphenylphosphine (1.5 equiv), solvent, -20°C to 80°C, 6 h.

Surprisingly, THF showed a much cleaner reaction profile (^{1}H- &^{31}P-NMR) than 2,6-lutidine, thus THF was chosen for all subsequent reactions. A problem became clear during the purification of the reaction; both the triphenylphosphine (PPh_3) and triphenylphosphine oxide (PPh_3O) byproducts were inseparable from **132** by flash column chromatography. It was hoped that, upon switching to **119** as a starting material, with benzyl protecting groups on the phosphonate, this problem might be solved.



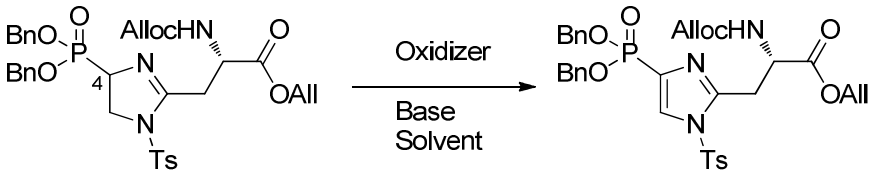
119 **68**

Scheme 13: Aza-wittig using **119**. Reagents and conditions: triphenylphosphine (1.5 equiv), THF, -20°C to 80°C, 6h.

The aza-wittig using **119** as starting material was performed under identical conditions as for **96**. No side products were observed except PPh_3O and some residual PPh_3. Purification was again a problem however; both PPh_3O and PPh_3 remained in the material, in essentially unchanged amounts after column chromatography. Several different chromatography conditions, as well as precipitation conditions, were investigated, but removing the side products completely proved impossible.

8.2.8 Oxidation

The next step in the synthesis was the oxidation of **68** to **133** (scheme 14). It was decided to continue with crude **68** as its oxidation should change its behavior on column substantially, allowing separation. It was expected that PPh_3O would not interfere in the oxidation reactions as it is not a reductor. PPh_3 is a reductor and therefore might quench the oxidator used to oxidize **68**, however the PPh_3 was only present in small amounts (5-10%) and should be easily compensated for by adding a super stiochiometric amount of oxidizer. Many different oxidizing agents are known in literature. In order to oxidize the imidazoline to the imidazole, at first oxidizers that were reported for heterocyclic systems were investigated (chapter 5.7.2.4). All oxidation trials were performed on small scale (10-20 mg) and monitored by TLC, if the TLC showed conversion LCMS and NMR analysis was also performed. Therefore, several entries in the result sections of the following tables are marked as 'conversion' and further explained in the text.



68 **133**

Scheme 14: Oxidation of imidazoline **68** to imidazole **133**.

8.2.8.1 Oxidation with electrophilic halogens

A common way to oxidize imidazolines to imidazoles uses $BrCCl_3$ and DBU (chapter 5.7.2.4). According to the literature, an imidazoline with an ester at C-4 is deprotonated at that position by DBU. The resulting negative charge attacks the electrophilic bromide, followed by elimination of hydrogen bromide to yield the imidazole. Since phosphonates are also electron withdrawing it was expected that this method would also work in this case. Initial tests with $BrCCl_3$ and DBU gave no reaction, even upon heating. Other sources of electrophilic halogens were also reported in literature as useful reagents in this reaction. Therefore, several were investigated in this reaction (table 12).

Table 12: Oxidation I, electrophilic halogens.

#	Lewis acid (equiv)	Base (equiv)	Solvent	Temp. (°C)[a]	Result
1	$BrCCl_3$ (1.5)	DBU (3)	DCM	0 to 70	No reaction
2	CCl_4 (9.5)	DBU (5)	MeCN	0 to 70	Decomposition
3	NBS (1.5)	DBU (3)	DCM	rt	No reaction

[a]: All reactions were performed in a closed vial.

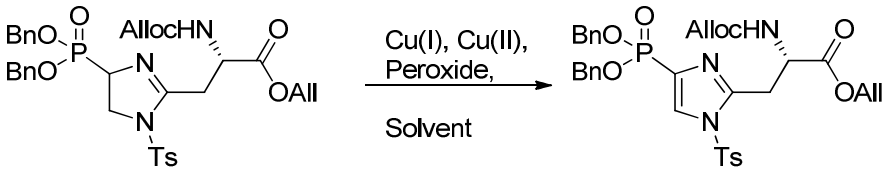
Reactions with carbon tetrachloride did not show any conversion until the mixture was heated to 70 degrees for several hours, after which all **68** had decomposed (entry 2). Also no conversion of the starting material was seen when N-bromosuccinimide was used as the bromine cation source (entry 3).

A possible explanation for these results could be the substituent pattern of the imidazoline. The phosphonate might not be as electron withdrawing as the ester groups that are usually on that position in literature examples, which could lead to a less activated proton at C-4. Also steric shielding by the benzyl groups might be a problem, as the large protecting groups might block DBU's access to the C-4 proton.

8.2.8.2 Oxidation with the Kharash-Sosnovsky reaction

The Kharash-Sosnovsky reaction has been used to oxidize imidazolines, oxazolines and thiazolines and various other heterocycles with electron withdrawing groups, to their respective aromatic heterocycle.[130] Therefore, it was thought that the method might be adapted to imidazolines with a phosphonate at C-4. To this end imidazoline **68** was dissolved in benzene and copper(I)bromide, copper(II)acetate and tert-butyl peroxybenzoate were added, after stirring four hours at 80°C a trace of product **133** was detected by LCMS. Thus, attempts were made to optimize the conditions and obtain a useful yield of **133** (table 13).

Table 13: Oxidation II, Kharasch-Sosnovsky.



68 **133**

#	Cu(I) (equiv)	Cu(II) (equiv)	Peroxide (equiv)	Solvent	Temp (°C)	Result
1	Cu(I)Br (1.1)	$Cu(II)(OAc)_2$ (1.1)	tBuOOCOPh (1.5)	PhH	rt to 80	Trace **133**[a]
2	Cu(I)Br (1.1)	$Cu(II)(OAc)_2$ (1.1)	tBuOOCOPh (3)	PhCl	90	Decomposition
3	Cu(I)Br (1.1)	$Cu(II)(OAc)_2$ (1.1)	tBuOOCOPh (3)	DCE	84	Decomposition
4	Cu(I)Br (1.1)	$Cu(II)(OAc)_2$ (1.1)	tBuOOCOPh (2)	PhH	80	Decomposition
5	Cu(I)OAc (1.1)	—	$(BzO)_2$ (2)	PhH	80	Trace **136**[a]

[a]: detected by LCMS.

However, changing the solvent, temperature or the amount of peroxide did not result in a higher yield of **133**. Only when copper(I)acetate was used as copper source and benzoyl peroxide as peroxide a small amount of tosyl eliminated product **136** was detected by LCMS. Due to its lack of success this method was not further investigated.

8.2.8.3 Oxidation with Manganese based oxidizers

Manganese dioxide has been reported for the oxidation of heterocycles including imidazoles, and was investigated next (table 14). The manganese dioxide was activated before the reaction by co-evaporation with toluene to remove residual water. [79]

Table 14: Oxidation III, manganese oxidizers.

#	Manganese oxidizer (equiv)	Solvent	Temp (°C)[a]	Result
1	MnO_2 (10)	THF	70	No reaction[b]
2	MnO_2(10)	$CHCl_3$	70	No reaction[b]
3	MnO_2 (10)	toluene	70	No reaction[b]
4	MnO_2 (10)	DCM	70	No reaction[b]
5	n-Bu_4NMnO_4 (1.1)	MeCN	rt	Decomposition
6	n-Bu_4NMnO_4 (1.1)	$CHCl_3$	rt	Decomposition
7	n-Bu_4NMnO_4 (1.1)	DCM	rt	Decomposition
8	$Mn(OAc)_3$. 2 H_2O (4)	AcOH	rt to 60	Decomposition
9	$Mn(OAc)_3$. 2 H_2O (4)	benzene	100	No reaction[c]

[a]: All reactions were performed in a closed vial. [b]: Stirred for 16 hours. [c]: Stirred for 2 days

Manganese dioxide in various solvents gave no reaction (entries 1-4). Since it is a relatively mild oxidizer some manganese compounds with a higher oxidizing potential were investigated. Tetrabutylammonium permanganate was selected since it dissolves in organic solvents due to its highly soluble counter-ion.[128a] This permanganate, however, was found to be too harsh leading to complete decomposition at

room temperature, using only 1.1 equivalents (entries 5-7). Manganese(III) acetate also did not yield any product (entries 8-9).[131]

8.2.8.4 Oxidation with selenium dioxide

Selenium dioxide is an oxidizer known for the oxidation of allylic alcohols and it has been reported as an oxidizer of 1,4-dihydropyridines.[132] Thus imidazoline **68** was subjected to these conditions (table 15).

Table 15: Oxidation IV, selenium dioxide.

#	Equivalents SeO_2	Solvent	Temp. (°C)	Result
1	1.5	1,4-dioxane	60 to 40	Trace, many side products
2	1.5	Pyridine	rt	No reaction
3	1	1,4-dioxane:pyridine 10:1	40	Trace, did not go to completion
4[a]	3	1,4-dioxane	60	12%
5	1.5	1,4-dioxane	40	20%

[a]: 3 A molsieve was added.

Selenium dioxide in 1,4-dioxane gave a surprising result, on LC-MS the correct mass for compound **134** could be identified, among other side products. The ^{31}P-NMR (entry 1) showed a single diastereomer, suggesting that a stereocenter had been destroyed, as one would expect when the imidazoline was aromatized. However, only an impure, small amount of product could be obtained, which was insufficient for a full characterization.

In order to obtain enough isolated compound, optimization studies were performed. Selenium dioxide has also been used with pyridine as a solvent.[133] However, changing the solvent to pyridine did not yield any product (entry 2). Mixing pyridine and 1,4-dioxane produced mixture containing some of the target compound but mostly unreacted starting material was recovered (entry 3). Performing the reaction in 1,4-dioxane with addition of 3A molsieve led to a 12% isolated yield after purification. It was very gratifying to see the PPh_3O byproduct from the previous, aza-wittig reaction, was easily removed from the product (entry 4). Lowering the temperature from 60 to 40 degrees improved the yield even more (entry 5). Unfortunately, the analytical data showed that the stating material **68** was oxidized at the amino acid side chain instead of the imidazoline, yielding compound **135** (figure 39).

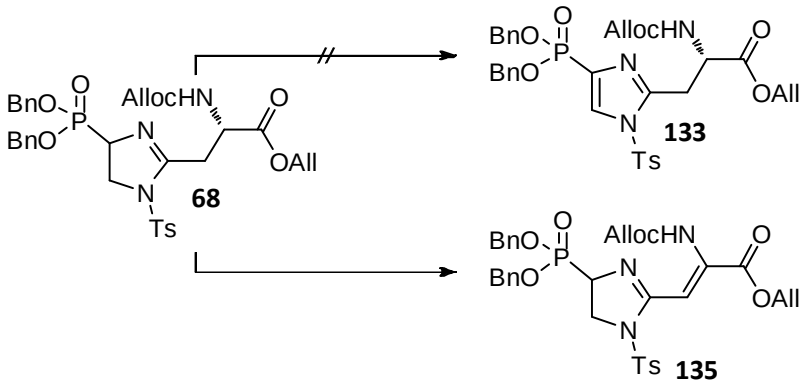


Figure 39: alternative product for the reaction of **68** with selenium dioxide.

A possible mechanism for this reaction (analogous for the selenium dioxide allylic oxidation mechanism),[134] is shown is figure 40. Initially, selenium dioxide and compound **68** undergo an ene-reaction, to form intermediate **A**. This intermediate then undergoes a [2+3] sigmatropic rearrangement, followed by a tautomerisation and a second ene-reaction, to yield compound **135** and selanediol. After elucidating the structure of **135** further investigations into this reaction were ceased.

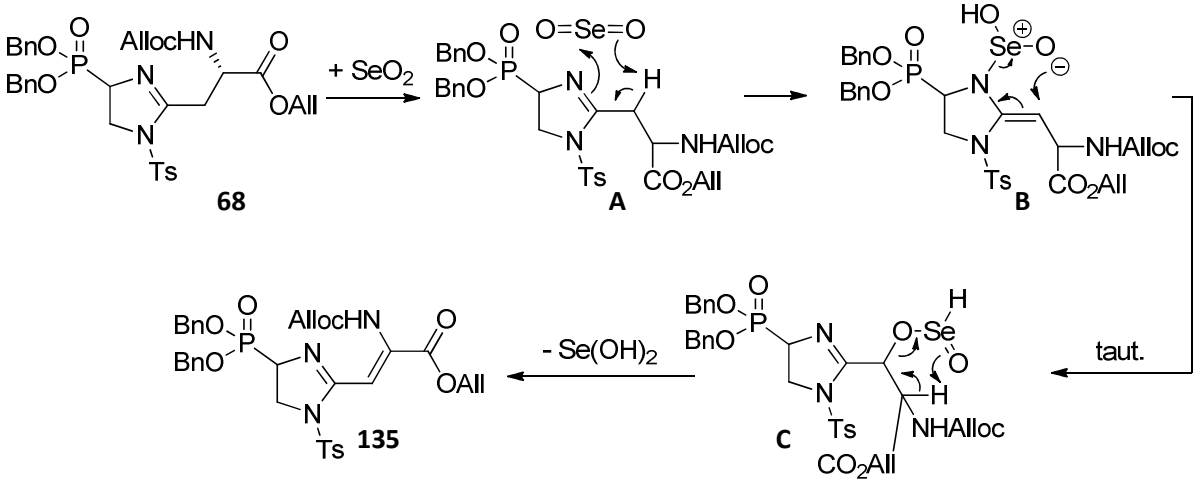


Figure 40: Selenium dioxide oxidation, possible mechanism.

8.2.8.5 Oxidation with hypervalent iodine reagents

Since its re-introduction by Nicolaou IBX has found many uses as a mild oxidizing agent, among which the oxidation of imidazolines to imidazoles.[85] The initial trials with IBX were encouraging (table 16); conversion was noticed after 16 hours at 45°C, however much starting material remained (TLC). Increasing the temperature to 60°C for three hours yielded a mixture showing three new signals in the ^{31}P-NMR in addition to some unreacted starting material (entry 1). Increasing the amount of IBX to two equivalents ensured the reaction went to complete conversion (entry 2). Using more than two equivalents resulted in decomposition of the products (entry 3). Attempts to select for one of the three products were not successful, and the products were inseparable. Therefore, it was impossible to identify these products. Using Dess-Martin periodinane (DMP) instead of IBX in either DCM of $CHCl_3$, yielded no product (entries 4 and 5). Further investigation into this reaction was not done as at this time a more promising method was found (vide infra).

Table 16: Oxidation V, hypervalent iodine reagents.[a]

#	Hypervalent iodine reagent (equiv)	Temp. (°C)	Result
1	IBX (1.5)	45 to 60	Conversion
2	IBX (2)	60	100% Conversion
3	IBX (10)	60	Decomposition
4[b]	DMP (1.1)	rt	No reaction
5[c]	DMP (1.1)	rt	No reaction

[a]: DMSO was used as the solvent. [b]: DCM was used as the solvent. [c]: $CHCl_3$ was used as the solvent.

8.2.8.6 Base induced aromatization

Since the oxidation seemed a bigger challenge than expected, another strategy was envisioned. Instead of oxidizing the imidazoline, it might be possible to aromatize it by base induced aromatization. To achieve this; a base would abstract a proton from C-4, which would lead to elimination of the tosyl protecting group, the imidazole would then be obtained after tautomerisation (figure 41).

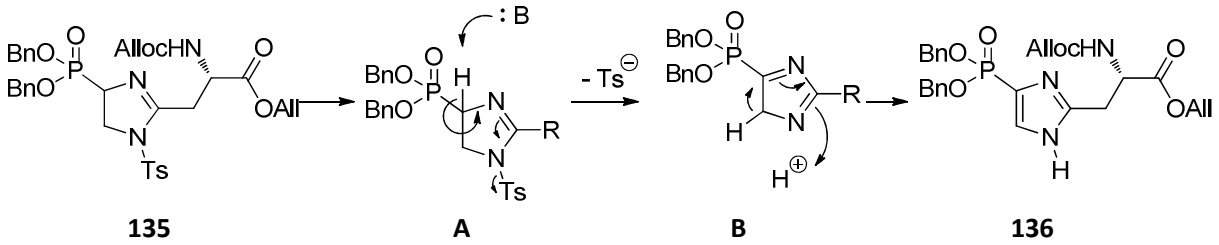


Figure 41: Base induced aromatization mechanism.

Thus, several bases were screened (table 17); DBU was not strong enough a base to deprotonate the imidazole. DBN also did not show any conversion until the mixture was heated; then the reaction mixture contained only decomposed starting material (entry 3). The absence of a reaction at room temperature indicated that there was a reactivity problem. This could be solved in two ways: increase the acidity of the proton at C-4 or use a stronger base. First it was attempted to increase the acidity of the C-4 proton by adding lewis acids; these could coordinate to the imidazole and the phosphonate, thereby decreasing the electron density on C-4 (figure 42). Silver was initially investigated as a chelating agent. Silver carbonate, which could both act as base and chelator, did not show any reaction (entry 4). Silver tetrafluoroborane and trimethylsilyl triflate, a strong lewis acid, also did not result in any reaction (entries 5 and 6). Therefore a look at the second option, using a stronger base, was required.

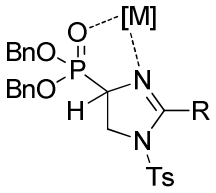
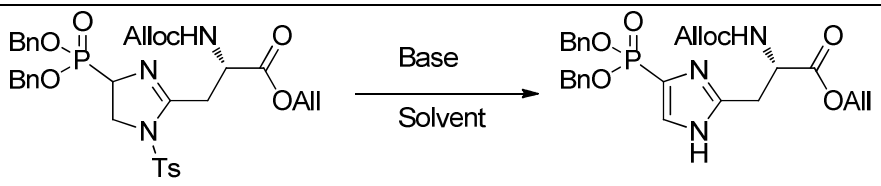


Figure 42: lewis acid coordination

Using strong base has the disadvantage that it might induce racemization of the amino acid by abstracting the alpha proton. However, it was expected that the proton on the Alloc carbamate was more acidic, and its removal would strongly deactivate the alpha proton on the amino acid. Later this was found to be the case (vide infra).

Table 17: Base induced aromatization.

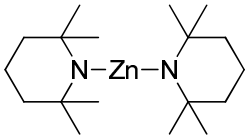


135 **136**

#	Base (equiv)	Solvent	Temp. (°C)	Result
1	DBU (15)	DMF	rt	No reaction
2	DBU (33)	—	rt	No reaction
3	DBN (41)	—	rt to 60	Decomposition
4	$AgCO_3$(6)	DCM	rt	No reaction
5	DBU (3) $AgBF_4$ (1.5)	DCM	rt	No reaction
6	DBU (3) TMSOTf (1.5)	DCM	0	No reaction
7	NaH (1.1)	DMF	rt	No reaction
8	KHMDS (2)	THF	-78 to 0	0 - 72%[a]
9	$Zn(TMP)_2$ (2.2)	THF	-78	Trace

[a]: NMR yield.

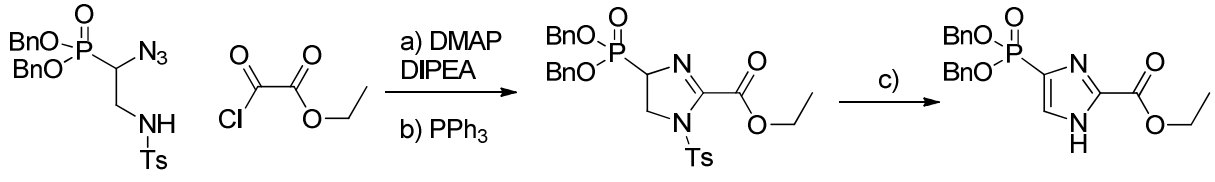
The first strong base that was used was sodium hydride, however no reaction occurred (entry 7).Upon addition of KHMDS to compound **135** in THF some product was formed. Regrettably, there was also a lot of side product formation and the reaction was not reproducible. Sometimes the reaction gave good yield (up to 72 percent), while other times no product was recovered. $Zn(TMP)_2$ was tested as a 'softer' alternative for KHMDS, but only traces of product were detected by NMR.



$Zn(TMP)_2$

8.2.9 Aromatization of an electron deficient imidazoline

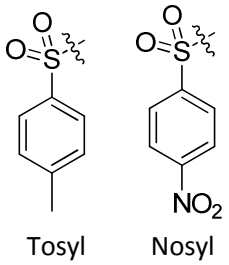
Convinced that the base induced elimination strategy could work, attempts were made to test if the reaction went better with a more electron deficient imidazoline. To that end compound **138** was synthesized, this imidazoline has an ethyl ester on the 2-position and therefore is much more electron deficient (scheme 15). Its synthesis was straightforward, **66** was dissolved in DCM with chloroethyl oxoacetate in the presence of DMAP and DIPEA to furnish the amide. The amide proved to be unstable to basic conditions and was thus quickly cyclized to the imidazoline with PPh_3 yielding **138** as a mixture with PPh_3 and $OPPh_3$.



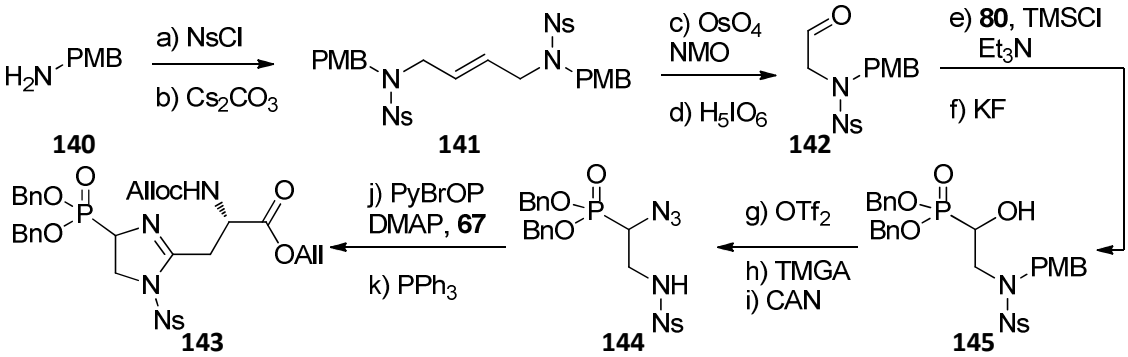
66 **137** **138** **139**

Scheme 15: Synthesis of **138**. Reagents and conditions: a) chloroethyl oxoacetate (2 equiv), DMP (1 equiv), DIPEA (2 equiv), DCM, rt, 1 h, 45%. b) triphenylphosphine (1.5 equiv), THF, -18°C to 80°C, 6h, assumed quant. c) DBU (15 equiv), DMF, 0°C, 5 min, **139** quantitative

Imidazoline **138** was submitted to base induced aromatization conditions (DBU in DMF), which led to immediate quantitative formation of **139**. It seemed that the deprotonation reaction was very fast and working quite well using electron deficient imidazolines.



It was clear that reducing the electron density of the imidazoline was very effective in promoting the base induced aromatization. Therefore, a way was sought to decrease the electron density on the imidazoline, while still allowing the other chemistries to work. A possible solution was found in exchanging the tosyl group for a nosyl. Due to the nitro group on the 4 position a nosyl is significantly more electron withdrawing than a tosyl. This should allow the use of weaker bases, like DBU, and circumvent the danger of racemization. A disadvantage was that, due to our synthetic strategy, the nosyl imidazole had to be synthesized starting with p-methoxybenzyl amine instead of simply swapping the tosyl for a nosyl using imidazoline **68**.

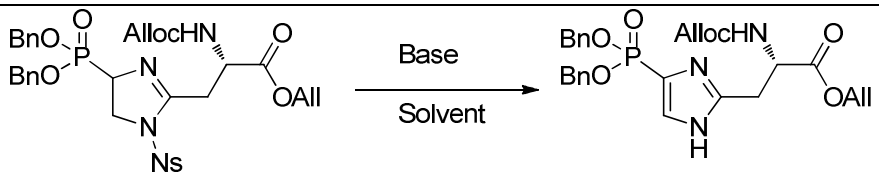


Scheme 16: Synthesis of **146**. Reagents and conditions: a) 4-toluenesulfonyl chloride (1 equiv), triethylamine (2 equiv), toluene, 0°C to rt, 2 h, 90%. b) 1,4-dibromobut-2-ene (**124**) (0.5 equiv), cesium carbonate (1.2 equiv), DMF, rt, 2h, 98%. c) osmium tetroxide 5 wt% in water (0.05 equiv), N-methylmorpholine-N-oxide (3 equiv), chloroform: water: tert-butanol 20:2.5:1, 50°C, 16h, 95%. d) periodic acid (1.1 equiv), 1,4-dioxane, 0°C to rt, 1h, 92%. e) dibenzylphosphite (1.2 equiv), chlorotrimethylsilane (1.44 equiv), triethyl amine (1.44 equiv), DCM, -20°C to -78°C to rt, 17h, 57%. f) potassium fluoride (2 equiv), methanol, rt, 30 min, 93%. g) triflic anhydride (1.5 equiv), pyridine (2 equiv), DCM, -15°C, 10 min. h) TMGA (1.1 equiv), MeCN, rt, 2h, 76% over two steps. i) CAN (3 equiv), 1,4-dioxane: water 9:1, rt, 6.5 h, 85%. j) **67**, PyBrOP (1.2 equiv), DMAP (1.2 equiv), DIPEA (2.4 equiv), DCM,0°C to rt, 3.5 h, 47%. k) PPh_3 (1.5 equiv), THF, 80°C, 6 h, 88%.

The synthesis of the nosyl imidazoline was similar to the synthesis of **68** (scheme 16). However, several reactions gave lower yield using a nosyl group than with the tosyl group. The phosphonate introduction gave the TMS-ether adduct of compound **145** in only 57 percent and the amide coupling between **144** and **67** provided a modest yield of 47 percent. The latter low yield is most likely due to increased deactivation of the sulfonamide compared to the tosylamide, due to the stronger electron withdrawing properties of the nitro group. Additionally, the nosyl compounds had a lower solubility in most solvents. This lengthened some reaction times due to the higher dilution required. The overall yield to **143** was 23 percent.

143 was submitted to similar reaction conditions as imidazolines **135** and **138**, the results are summarized in table 18.

Table 18: Base induced aromatization.



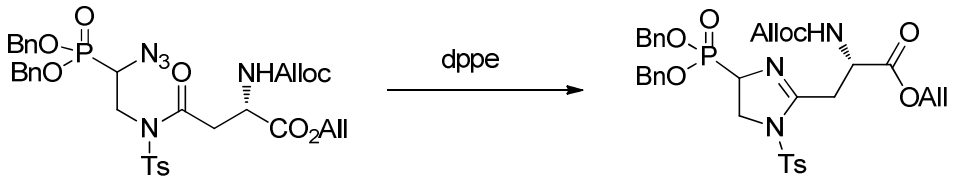
143 **136**

#	Base (equiv)	Solvent	Temp. (°C)	Result
1	DBU (7.5)	DMF	0	No reaction
2	Cs_2CO_3 (2)	MeCN	rt	No reaction
3	DBU (5) CCl_4 (4)	MeCN	rt	Decomposition
4	DBU (1)	HMDS	rt	No reaction
5	DBU (3) TMSOTf (1.5)	DCM	rt	No reaction
6	$Zn(TMP)_2$ (6.6)	THF	-78	Decomposition

As clear from table 18, no satisfying results were obtained for the nosyl-imidazoline employing weak base. Thus, no noticeable difference was seen in ease of deprotonation between the tosyl and the nosyl imidazoline. DBU failed to deprotonate **143** (entry 1), as did other methods investigated for the tosyl imidazoline (entries 2-5). Also using the strong base $Zn(TMP)_2$ no product was obtained (entry 6). It seems that the nosyl group cannot reduce the electron density of the imidazoline as sufficiently as a C-2 ester. Thus, since the yield and handleability of the tosyl imidazoline and its precursors were better than for the nosyl analogues, the tosyl compounds were used in all follow up chemistry.

8.2.10 Successful base induced elimination

Some promising, but irreproducible results were obtained with the base induced aromatization strategy, using KHMDS (vide supra). To solve the reproducibility issues it seemed necessary to perform the reaction on pure **135** without any residual PPh_3 and $OPPh_3$. Therefore, another phosphine that did not have the same retention time as **68** was required. In the literature several phosphines have been tested in the aza-wittig reaction, but they display inferior performance compared to PPh_3. It was thought that bis(diphenylphosphino)ethane (dppe) could be a good replacement for PPh_3 from a purification standpoint as a di-oxidized dppe would be quite polar and thus easily removable by column chromatography. dppe has not been described in literature as a phosphine source in the aza-wittig reaction. However, upon testing dppe in the aza-wittig reaction it was found to perform similar to PPh_3 (scheme 17).



119 **135**

Scheme 17: Synthesis of **147**. Reagents and conditions: dppe (1.5 equiv), THF, rt to 80°C, 8h, 98%.

Using similar conditions as described for PPh_3, dppe gave full conversion in six to eight hours at 80 degrees and, after oxidizing residual dppe with hydrogen peroxide, all side products were easily removed by filtration through a silica plug. Since the formation of the iminophosphorane was slower with dppe compared to PPh_3, it was no longer necessary to cool the reaction mixture during phosphine addition. With pure **68** in hand the aromatization reaction was further optimized.

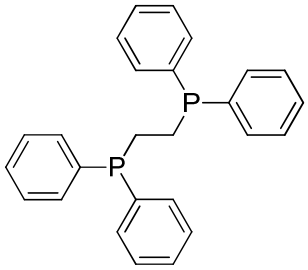


Figure 43: dppe

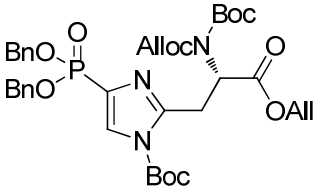
Table 19: Base induced aromatization of pure **68**.

#	Equivalents KHMDS	Quencher (equiv)	Temp. (°C)	Result
1	2	water	-78	60% conversion[a]
2	2.5	water	-78 to -15	100% conversion
3	3	TrCl (1)	-78 to -15	**136** recovered
4	3	TsCl (2.1)	-78 to rt	**136** recovered
5	3	TsCl (1)	-78	**136** recovered
6	3	Boc_2O (2)	-78	Double addition
7	3	AcOH / water	-78	100% conversion

[a]: determined by NMR.

Using 2 equivalents of KHMDS the reaction only proceeded to 60 percent conversion (table 19: entry 1). Increasing the amount of KHMDS to 2.5 equivalents, in combination with a slow increase of temperature during the reaction to -15 degrees, led to full conversion (entry 2). Due to the polarity of the unprotected imidazole it was difficult to purify using column chromatography, the imidazole seemed to have a high affinity for the silica and much product was lost during purification. Therefore, in situ quenching of the deprotonated imidazole with various protecting groups was also investigated.

Unfortunately, in situ quenching with a protecting group was not successful. The reagent was added after complete consumption of **68**. Reactions with trityl- or tosyl chloride only yielded **136** (entries 3 to 5). However, when boc anhydride was used to quench the reaction double addition of Boc was observed (entry 6). NMR and LCMS suggested the compound was **148**, the compound was not isolated but this result did suggest that in situ protection was not a good strategy.



148

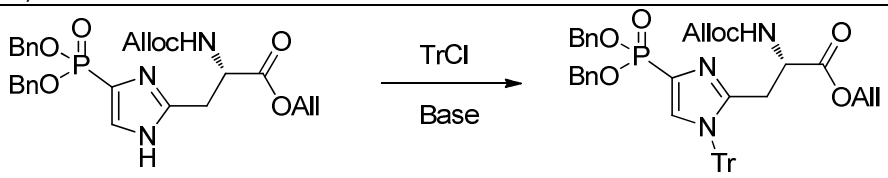
Thus, a quench with water, followed by a conventional protection seemed the only way to go forward. Since **136** behaved so poorly during column chromatography it was decided to submit it to the next reaction after only an aqueous work-up. The optimized conditions involved the slow addition of three equivalents of

KHMDS, driving the reaction to completion immediately. To prevent decomposition during the quench the mixture was transferred, by canula, to a stirring, ice cold solution of six equivalents acetic acid in water. This ensured that all KHMDS was quenched immediately. These optimizations provided **136** in a high yield and purity so it could be submitted to the next reaction without further purification.

8.2.11 Protecting the imidazole: trityl

As discussed before (chapter 7) a trityl group would be preferred for SPPS. Installing a trityl group on an imidazole is normally done by reaction with trityl chloride and an amine base. Attempts to synthesize **149** are summarized in table 20.

Table 20: Attempted synthesis of **149**.



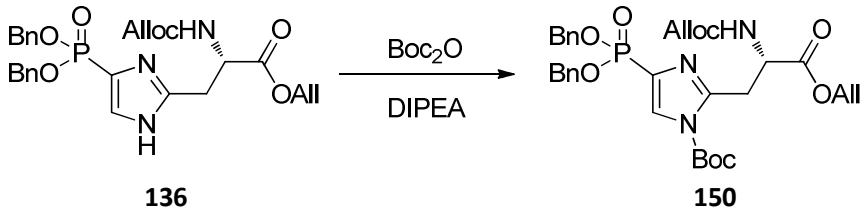
136 **149**

#	Equivalents Trityl chloride	Base (equiv)	Solvent	Result
1	1.5	Pyridine (10)	DCM	No reaction
2	1.5	Pyridine (10)	THF	No reaction
3	1.5	DIPEA (4)	THF	No reaction
4	2.2	KOtBu	DMF	No reaction

Protecting the imidazole using trityl chloride and 10 equivalents of pyridine did not lead to any conversion of the starting material, using either DCM or THF as a solvent (entries 1 and 2). Changing the base to DIPEA was also not successful (entry 3). It was thought that the bases were not sufficiently strong to deprotonate the imidazole. Therefore potassium tert-butoxide was used, however again no reaction was observed. Steric effects from the amino acid part and the benzyl group could be preventing the big trityl group from access to the imidazole, thus retarding the reaction.

8.2.12 Protecting the imidazole: Boc

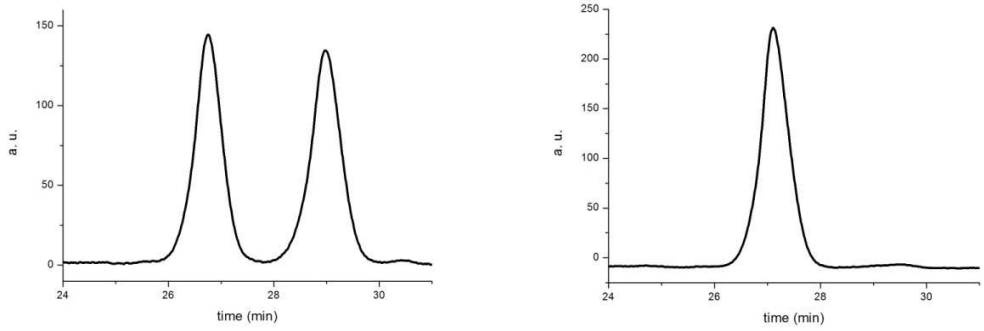
Therefore, an alternative protecting group was required. The Boc group is known as an easily removable protecting group on imidazoles, due to its propensity to fall off if the imidazole becomes protonated. Since all the following reactions were under basic conditions Boc was deemed a suitable protection group. Introduction of the Boc group was achieved by stirring crude **136** with Boc anhydride in the presence of DIPEA. In contrast to in situ protection, no reaction of Boc anhydride with the carbamate was observed under these reaction conditions. Purification of the protected imidazole using column chromatography proceeded without problems.



136 **150**

Scheme 18: Synthesis of **150**. Reagents and conditions: boc anhydride (1 equiv), DIPEA (1 equiv), 1,4-dioxane: water 1:1, 0°C to rt, 66%.

At this time the enantiomeric purity of **150** was investigated, in order to analyze if the base induced aromatization compromised the stereocenter (vide supra). To this end a racemic version of **150** was synthesized, by using di-protected DL-aspartic acid as the acid partner for the amide coupling. Both L-**150** and DL-**150** were injected on a chiral HPLC. Gratifyingly it showed that L-**150** had an ee of 96%, proving that the KHMDS does not induce racemization by abstracting the alpha proton from the amino acid (figure 44).

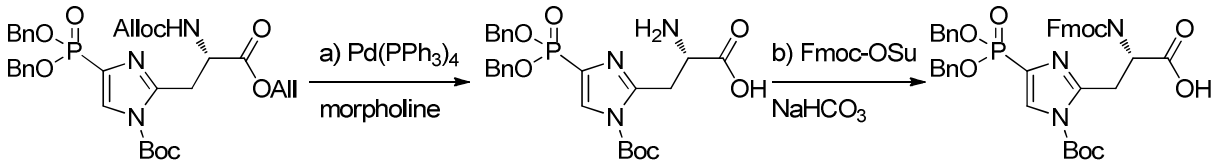


DL-**150** L-**150**

150	S-enantiomer		R-enantiomer	
	t_R(min)	Area %	t_R(min)	Area %
DL	26.75	49.5	28.98	50.5
L	27.11	98.0	29.57	2.0

Figure 44: Chiral HPLC data for compound **150.**

Next the Alloc and allyl protecting groups were removed using tetrakis(triphenylphoshino)palladium(0) in THF and morpholine as allyl scavenger. The reaction went to completion and showed the right mass in LCMS. The mixture was not purified beyond evaporation of all volatiles, due to the polarity of the free amino acid. Fmoc protection was performed on the crude mixture using Fmoc-OSu and sodium bicarbonate in dioxane: water 9:1 (scheme 19).



150 **151** **152**

Scheme 19: Attempted synthesis of **152**. Reagents and conditions: a) tetrakis(triphenylphosphine)palladium(0) (0.1 equiv), morpholine (20 equiv), THF, rt, 15 min. b) Fmoc-OSu (1.1 equiv), Sodium bicarbonate (1.1 equiv), 1,4-dioxane: water 9:1, rt, 16h.

Unexpectedly, the reaction did not yield any of the target product, only starting material **151** was recovered. It was hypothesized that residual morpholine might be the cause of the failure by reacting with all the Fmoc-OSu. Therefore, the alloc and allyl deprotection was performed again but this time using phenyl silane as allyl scavenger. The crude reaction mixture was suspended in mesitylene and evaporated to dryness three times to ensure no more volatiles were present. However, once again the Fmoc protection did not work.

Taking a closer look at the LCMS data from both the de-allylations and Fmoc protection something peculiar was noticeable. After the deallylation using phenylsilane there are two peaks visible in the LCMS trace, at 9.0 min (**A**) and 9.5 min (**B**) (figure 45, **I**). Both show the correct product mass of 516 $[M+H]^+$. However, when looking at the LCMS spectra of the same mixture after exposure to base only one peak of that mass is visible (figure 45, **II**).

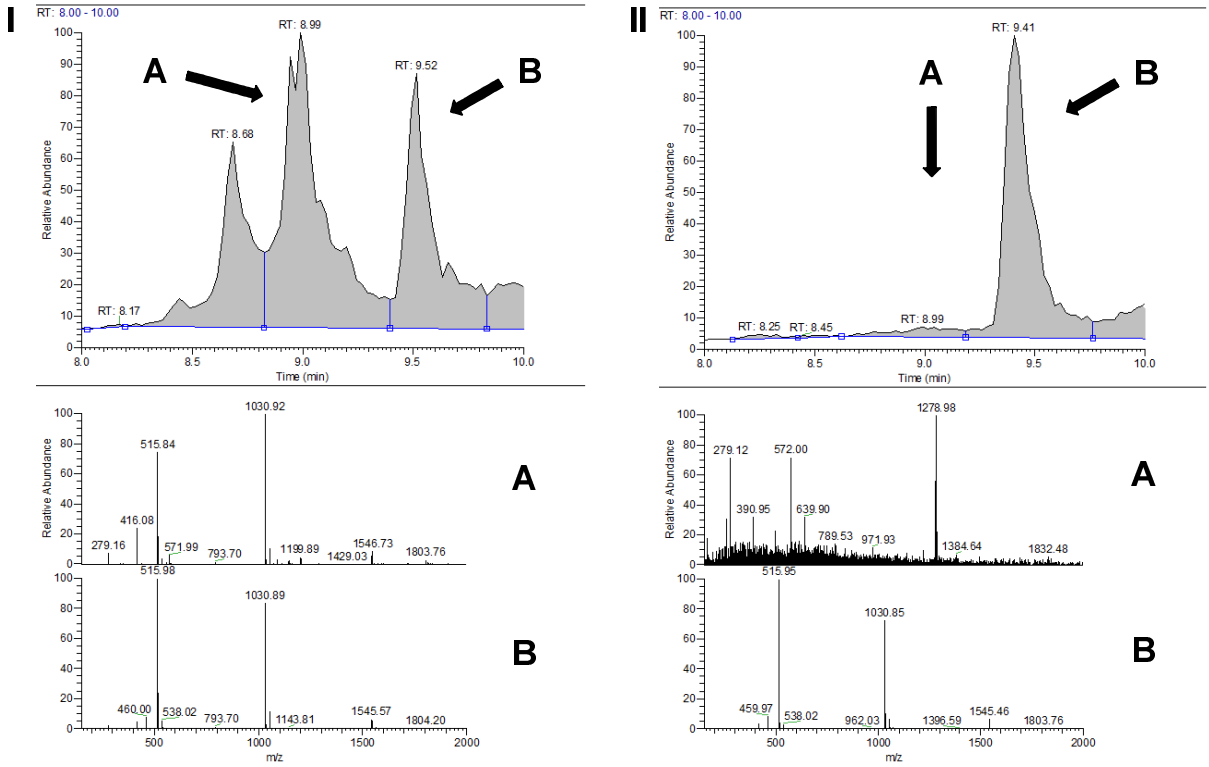


Figure 45: LCMS spectra of the product of the deallylation before (**I**) and after (**II**) the exposure to base. **A** = **153**, **B** = **154**.

It seems that upon exposure to base a reaction occurs that yields a product with the same mass but that is unreactive toward Fmoc-OSu. Most likely exposure of compound **153** to base induces an attack of the free amine of the amino acid on the carbamate and abstracts it from the imidazole through a six membered transition state to yield **154** (figure 46). While **154** was isolated, it proved impossible to obtain a good HMBC NMR spectrum to conclusively prove its structure, most likely due to its zwitterionic nature. However, the ^{31}P-NMR shows a singlet at 6 ppm, this is a chemical shift that would be expected for the unprotected imidazole (between 5-15 ppm). This problem precluded the use of Boc as a protecting group. Even if the problem could be solved at this stage by careful pH control, the Boc scramble would retard peptide synthesis during removal of the Fmoc under basic conditions, due to capping of the peptide sequence with the Boc group.

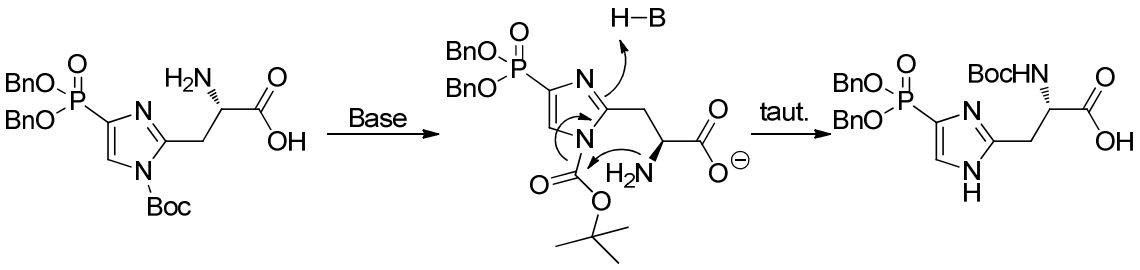
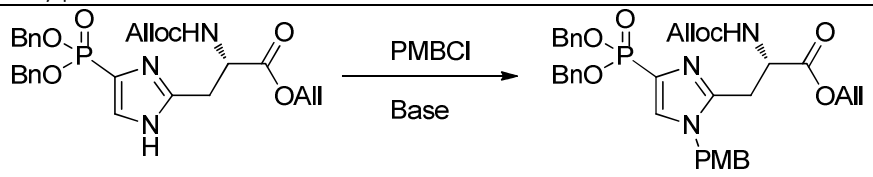


Figure 46: Boc rearrangement mechanism.

8.2.13 Protecting the imidazole: p-Methoxybenzyl

Since Boc protection was no longer a possibility, another protecting group was needed and the PMB group seemed to have the main qualities that were needed; stability under peptide coupling conditions and acid lability. It also did not have an electrophilic functionality that could be attacked by the amine after allyl deprotection. To install the PMB group p-methoxybenzyl chloride was used in conjunction with a base (table 21). The first base tested was DIPEA but no product was obtained after stirring for three days at room temperature (entry 1). Potassium carbonate in acetonitrile led to decomposition (entry 2), the same base in DMF gave quick formation of **155** but also led to decomposition if the mixture was allowed to age at room temperature (entry 3). Finally, **155** was obtained in 84 percent yield after three hours (entry 4).

Table 21: p-methoxybenzyl protection of **136**.

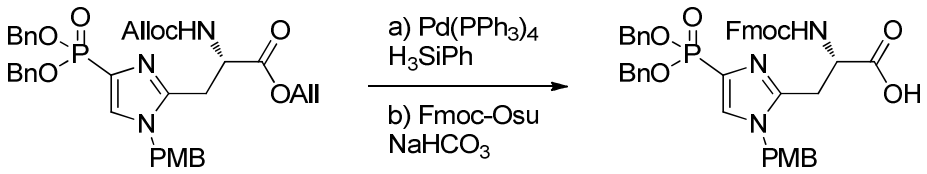


136 **155**

Reagents and conditions: p-methoxybenzyl chloride (1.5 equiv), base, solvent, rt, time.

#	Base (equiv)	Solvent	Time	Result
1	DIPEA (1.6)	THF	3 days	No reaction
2	K_2CO_3 (5)	MeCN	7 hours	Decomposition
3	K_2CO_3 (5)	DMF	7 hours	Decomposition
4	K_2CO_3 (5)	DMF	3 hours	84%

Removal of the allyl and alloc protecting groups was done without incident using tetrakis(triphenylphoshino)palladium(0) in THF and phenylsilane. The reaction mixture was again co-evaporated with mesitylene and used in the Fmoc protection without further purification. Fmoc-Osu and sodium bicarbonate in 1,4-dioxane: water 10:1 was successful in Fmoc protecting the amine yielding **156** in 51 percent over two steps (scheme 20).



155 **156**

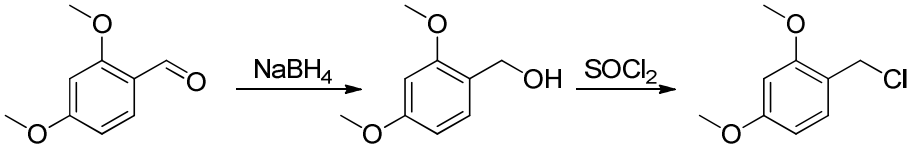
Scheme 20: Synthesis of **156**. Reagents and conditions: a) tetrakis(triphenylphoshino)palladium (0.1 equiv), phenylsilane (5 equiv), THF, rt, 1h. b) Fmoc-OSu (1.5 equiv), sodium bicarbonate (1.5 equiv), dioxane: water 10:1, rt, 16h, 51% over two steps.

Having obtained a suitable protected Fmoc SPPS building block a cleavage test was performed to make sure all acid labile protecting groups could be removed by acidolytic cleavage. To this end a small amount of **156** was dissolved in TFA:TIPS:water 95:2.5:2.5 and stirred for 16 hours. Periodic LCMS samples showed that first one of the benzyl protecting groups was removed and then slowly the second benzyl group was removed. However, no matter how long the reaction mixture was allowed to age, the PMB group could not be removed using this method. This made it very unlikely that the PMB group could be removed after peptide synthesis; therefore a new protective group was again needed.

8.2.14 Protecting the imidazole: 2,4-dimethoxybenzyl

2,4-Dimethoxybenzyl (DMB) is structurally similar to PMB, only the extra methoxy group makes it more labile toward acidic- and oxidative cleavage.[128b] Oxidative cleavage provided a good alternative method for removal of the protecting group in case acidolytic cleavage did not work.[ref greene] To attach the DMB group to the imidazole, DMB chloride was synthesized in two easy steps from 2,4-dimethoxybenzaldehyde (scheme 21).[135] Sodium borohydride was used to reduce the aldehyde to the alcohol, which was then transformed into the chloride by thionyl chloride. DMB chloride is a very

reactive compound, sensitive to nucleophiles and prone to polymerization, therefore the reaction was quickly worked up with ice cold solvents and immediately used in the next reaction.

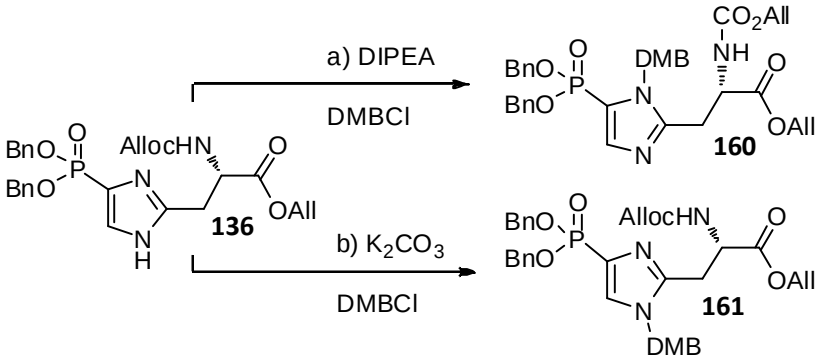


157 **158** **159**

Scheme 21: Synthesis of **159**. Reagents and conditions: a) sodium borohydride (1 equiv) EtOH, rt, 30 min, 98%. b) thionyl chloride (2.5 equiv), 2,6-lutidine (2.5 equiv), chloroform, -15°C, assumed quantitative.

The protection of **136** with DMBCl was optimized (table 22). First DIPEA in DCM was investigated but this led to a rather complex mixture of mostly **160**. Switching to potassium carbonate in DMF and 1.2 equivalents of **159** yielded the desired product in 62 percent isolated yield. The product still contained 6 percent **160** that was inseparable using flash column chromatography or preparative HPLC. Increasing the amount of DMB chloride to 1.4 equivalents lowered the yield but no **160** was present in the final product. However, since both **160** and **161** yield the same product after deprotection 1.2 equivalents of DMBCl were used in subsequent syntheses of the compound.

Table 22: 2,4-dimethoxybenzyl protection of **136**.

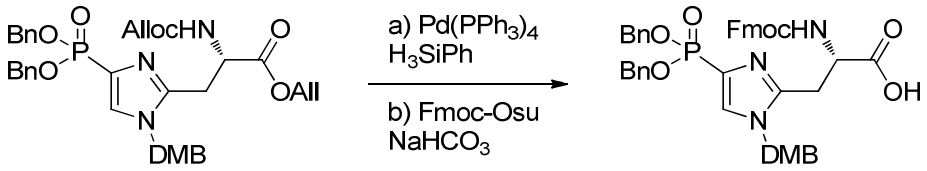


Reagents and conditions: 2,4-dimethoxybenzyl chloride, base, solvent, rt, 16h.

#	Equivalents DMBCl	Base (equiv)	Solvent	Result[a]
1	1.5	DIPEA (1.6)	DCM	**160**:**161** (10:1)[b]
2	1.2	K_2CO_3 (5)	DMF	**161** (62%)[c]
3	1.4	K_2CO_3 (5)	DMF	50%

[a]: yield over two steps. [b]: yield not determined, ratio determined by ^{31}P-NMR. [c]: containing 6% **160**

Having obtained a suitably protected imidazole the next step was concomitant deprotection of the alloc and allyl groups, followed by Fmoc protection of the amine. Deprotection of the allyl groups and Fmoc protection were done similarly to the PMB imidazole, yielding 50 percent **162** over two steps (scheme 22).



161 **162**

Scheme 22: Synthesis of**162**. Reagents and conditions: a) tetrakis(triphenylphoshino)palladium(0) (0.1 equiv), phenylsilane (5 equiv), THF, rt, 1h. b) Fmoc-OSu (1.5 equiv), sodium bicarbonate (1.5 equiv), dioxane: water 10:1, rt, 16h, 50% over two steps.

162 was exposed to several peptide cleavage mixtures (table 23). Standard TFA:TIPS:water 95:2.5:2.5 removed both benzyl groups but failed to remove the DMB group, heating the mixture to 80 degrees for several hours, after the benzyls were removed, did not result in any change (entry 1). In pure TFA the results were the same (entry 2). In TFA: EDT 1:1 very quick benzyl deprotection was seen. EDT (ethanedithiol) is a strong nucleophile that will quickly attack any electrophiles in the mixture, reducing side reactions during deprotection and improving its speed. However, these conditions also did not remove the DMB group (entry 3). Finally, the compound was dissolved in acetic acid with EDT present. No reaction occurred under these conditions until the reaction was heated to 60 degrees overnight, when slowly one of the benzyl groups was removed.

Table 23: Acidic 2,4-dimethoxybenzyl deprotection conditions.

#	Cleave mix (ratio)	Result
1	TFA: TIPS: water (95:2.5:2.5)	DMB on, two Bn removed
2	TFA	DMB on, two Bn removed
3	TFA: EDT (1:1)	DMB on, two Bn removed

Clearly, DMB removal did not proceed as hoped, to ensure it would also not work on a peptide, a small peptide, Ac-FKH*KG-NH_2 was synthesized. Standard cleavage mixture (TFA:TIPS:water 95:2.5:2.5) was enough to cleave the peptide from the resin and remove all protecting groups except the DMB group (table 24: entry 1). Changing the ratio of the cleavage mix (entry 2) or adding hydrobromic acid (entry 3) did not remove the DMB either.

Table 24: Acidic 2,4-dimethoxybenzyl deprotection conditions from a peptide.

#	Cleave mix (ratio)	DMB removed?
1	TFA: TIPS: water (95:2.5:2.5)	no
2	TFA: TIPS: water (90:5:5)	no
3	TFA: TIPS: HBr (48% in water): water (80:5:5:10)	no

A different peptide (Ac-GAKRH*RKVLRGC-NH_2) was exposed to TMSBr for several weeks, as well as to condensed HF, without any sign of DMB removal. At this point it was clear acidolytic cleavage was not possible for the DMB group, therefore oxidative cleavage was investigated.

Table 25: Oxidative 2,4-dimethoxybenzyl deprotection conditions.

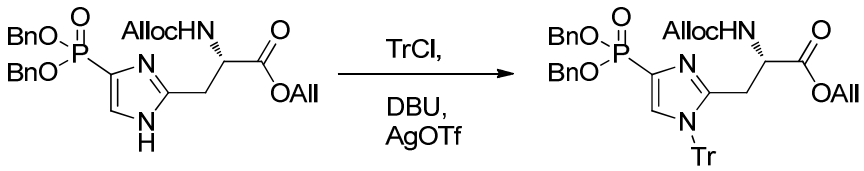
#	Substrate	Oxidizer (equiv)	Solvent	Result[a]
1	**162**	DDQ (3)	$CHCl_3$: water 9:1	No reaction
2	**162**	CTAN (3)	DCM	Decomposition
3	**162**	CAN (3)	DMF: water 9:1	DMB removal[b]
4	Ac-GAKRH*RKVLRGC-NH_2[c]	CAN (3)	DMF: water 9:1	No reaction
5	Ac-GAKRH*RKVLRGC-NH_2[c]	CAN (10)	DMF: water 9:1	No reaction
6	Ac-GAKRH*RKVLRGC-NH_2[c]	CAN (30)	DMF: water 9:1	No reaction

[a]: determined by LCMS. [b]: also some benzyl removal.[c]: on tentagel S-RAM resin.

First, oxidation of the DMB group was attempted on the SPPS building block **162** using DDQ, but no reaction was seen (table 25: entry 1). Cerric tetrabutylammonium nitrate, an analogue to CAN that is more soluble in organic solvents, decomposed **162** completely (entry 2). CAN in DMF: water 9:1 gave slow DMB removal (several days) with concomitant mono benzyl removal (entry 3). Encouraged by this result deprotection of the DMB group on a peptide was investigated. The peptide on resin, before global acidolytic deprotection, was subjected to 3, 10 and 30 equivalents of CAN in DMF: water 9:1. Regrettably, even after prolonged reaction times no reaction was observed.

8.2.15 Protecting the imidazole: Trityl revisited

Since the alternative protecting groups proved incompatible with SPPS, introduction of the trityl group was more thoroughly investigated. With this final failure to remove the DMB group it became clear that yet another protecting group was needed. Most likely, the lack of reactivity of trityl chloride (TrCl) was the limitation in the previous experiments. There are literature reports that TrCl can be activated by silver triflate (AgOTf).[136] The triflate can displace the chloride of TrCl, which precipitates as silver chloride; TrOTf, a strong electrophile was expected to be more reactive toward the imidazole. Thus, the imidazole was dissolved in DCM in the presence of TrCl and DBU and AgOTf were added. Immediately, a grey solid precipitated out of the solvent and compound **149** was obtained in 77 percent yield over two steps (from the imidazoline).

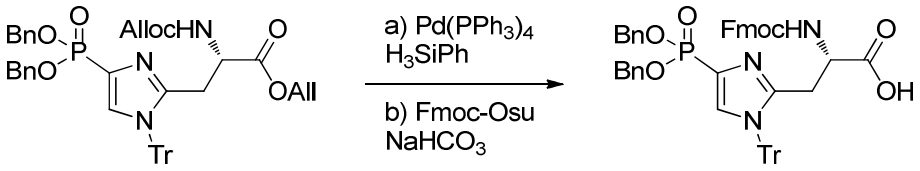


136 **149**

Scheme 23: Synthesis of. Reagents and conditions: Trityl chloride (2 equiv), DBU (3 equiv), silver triflate (2.2 equiv), DCM, rt, 2 h, 77% over two steps from **68**.

Alloc- and allyl deprotection and Fmoc protection were performed according to the previously used protocol. Flash column chromatography of **65** gave low isolated yield, most likely due to a high affinity of the compound to the silica. As an alternative the compound was purified over C18 sep-pak cartridges. The compound was dissolved in water and a minimal amount of acetonitrile and loaded on the cartridge. Elution was done by a gradient starting with 100 percent water to 100 percent acetonitrile in

steps of 10 percent. The compound elutes between 70 and 90 percent acetonitrile and was isolated in 96 percent yield. To date, more than 5.4 grams of **65** were synthesized using this method.



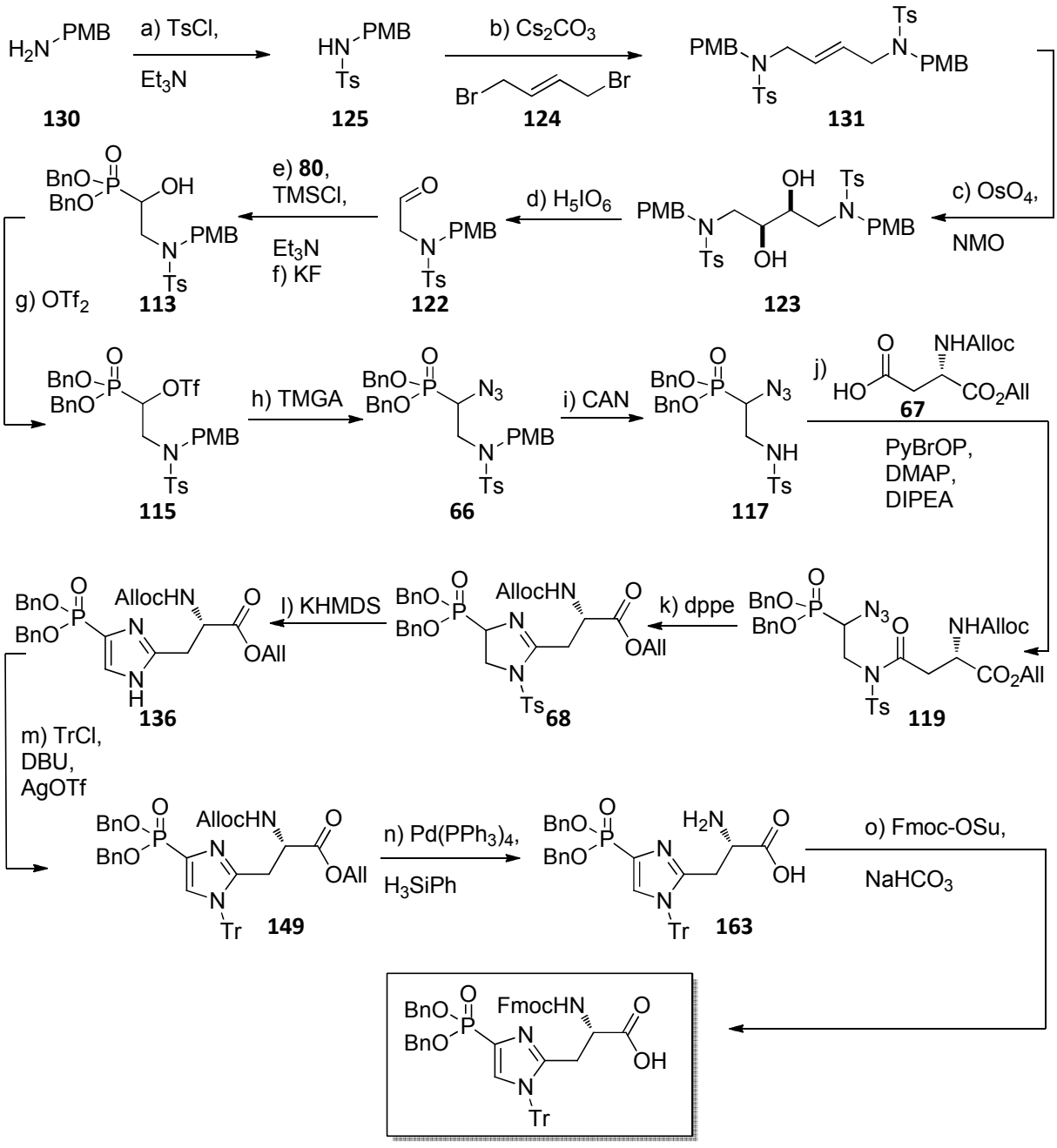
136 **65**

Scheme 24: Synthesis of **65**. Reagents and conditions: a) $Pd(PPh_3)_4$ (0.1 equiv), phenylsilane (5 equiv), THF, rt. b) Fmoc-OSu (1.5 equiv), $NaHCO_3$ (1.5 equiv), 1,4-dioxane: water 10:1, rt, 16h, 96% over two steps.

65 was used in SPPS to synthesize a range of peptides in moderate to good yield, these peptides were used to raise antibodies against pHis. This is further discussed in chapter 9.

8.2.16 Final synthetic route toward 65

In conclusion, a 15 step synthesis toward **65** was realized (scheme 25), beginning with the tosylation of p-methoxybenzyl amine. The resulting tosylamide was allowed to react with 1,4-dibromobut-2-ene, yielding alkene **124**, which was dihydroxylated with osmium tetroxide. The diol was oxidatively cleaved using periodic acid and the resulting aldehyde was subjected to TMS activated dibenzylphosphite, which after deprotection yielded the phosphonate **113**. An azide moiety was introduced by activating the hydroxyl as a triflate and substituting it with TMGA. Removal of the PMB group with CAN led to amide coupling partner **117** that was coupled to **67** using PyBrOP and DMAP. Aza-wittig ring closing of the resulting amide was promoted by dppe, followed immediately by aromatization by base induced elimination of the tosylate. Protecting the imidazole with a trityl group and removal of the Alloc and allyl protecting groups was followed by the final Fmoc protection yielding **65** in 22.7 percent over 15 steps.



Scheme 25: Synthesis of **65** starting with p-methoxybenzyl amine. Reagents and conditions: a) 4-toluenesulfonyl chloride (1 equiv), triethylamine (2 equiv), toluene, 0°C to rt, 2 h, 94%. b) **125** (2 equiv), 1,4-dibromobut-2-ene (**124**) (1 equiv), cesium carbonate (2.4 equiv), DMF, rt, 2h, 91%. c) osmium tetroxide 5 wt% in water (0.05 equiv), N-methylmorpholine-N-oxide (3 equiv), chloroform: water: tert-butanol 20:2.5:1, 50C, 16h, 97%. d) periodic acid (1.1 equiv), 1,4-dioxane, 0C to rt, 1h, 84%. e) dibenzylphosphite (1.2 equiv), chlorotrimethylsilane (1.44 equiv), triethyl amine (1.44 equiv), DCM, -20°C to -78°C to rt, 17h, 88%. f) potassium fluoride (2 equiv), methanol, rt, 30 min, 94%. g) triflic anhydride (1.5 equiv), pyridine (2 equiv), DCM, -15°C, 10 min, 98%. h) TMGA (1.1 equiv), MeCN, rt, 2h, 70%. i) CAN (3 equiv), 1,4-dioxane: water 9:1, rt, 6.5 h, 82%. j) XX, PyBrOP (1.2 equiv), DMAP (1.2 equiv), DIPEA (2.4 equiv), DCM,0°C to rt, 3.5 h, 81%. k) dppe (1.5 equiv), THF, rt to 80°C, 8h, 98%. l) KHMDS 0.5M in toluene (3 equiv), -78°C , 30 min. m) Trityl chloride (2 equiv), DBU (3 equiv), silver triflate (2.2 equiv), DCM, rt, 2 h, 77% over two steps. n) $Pd(PPh_3)_4$ (0.1 equiv), phenylsilane (5 equiv), THF, rt. o) Fmoc-OSu (1.5 equiv), $NaHCO_3$ (1.5 equiv), 1,4-dioxane: water 10:1, rt, 16 h, 96% over two steps.

8.3 Other routes toward a 3-phosphohistidine mimic – Imidazole synthesis

Besides the successful route described in the last paragraph, many different routes have been pursued. Here the other routes that were followed in an attempt to synthesize the 3-pHis mimic will be described. The routes are roughly divided into methods that relied on the construction of the imidazole and methods that started from the imidazole. First, the syntheses to construct the imidazole will be discussed, followed by the methods starting from the imidazole. All syntheses described here have failed at a certain point, which will be detailed in the text.

8.3.1 Negishi I

It was though that **65** could be prepared from the heteroaromatic phosphonate **73** and amino acid derivative **74**. In this case, a Negishi coupling should lead to the final product (figure 47).[54b] The choice of protecting groups of compound **74** was determined by success of **74** in reacting with systems analogous to **73**, containing a sulfonyl amide moiety at C-4 instead of the phosphonate.[137] The methyl ester can be easily removed using trimethyltin hydroxide.[138] Compound **74** should be easily available via a literature procedure,[139] whereas compound **73** had proved to be more challenging. Earlier attempts to make a phosphonic acid imidazole by addition of the phosphonate to the imidazole failed (chapter 8.4.1). Therefore intermediate **66**, described in chapter 8.2 was employed.

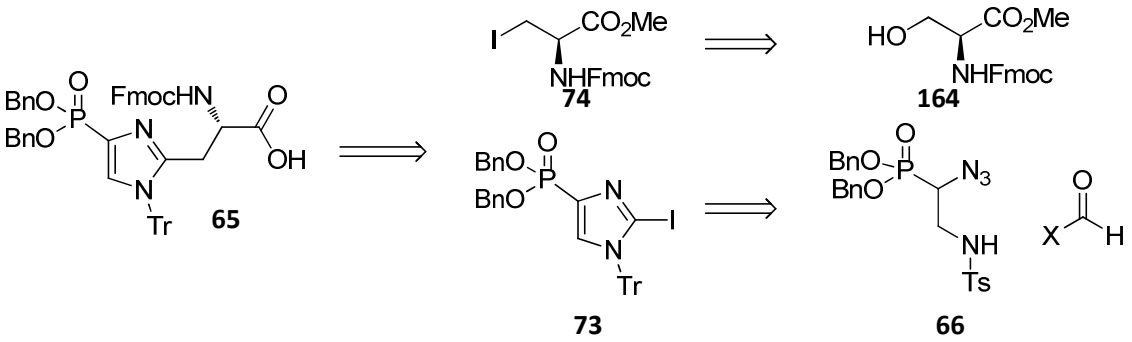
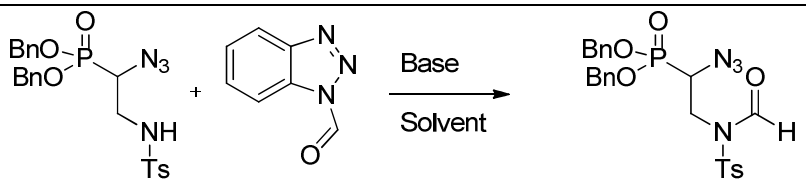


Figure 47: Negishi strategy.

To this end azide **66**, which is made via a nine step synthesis from p-methoxybenzyl amine (chapter 8.2.6), was allowed to react with formic acid using DCC and DMAP with formic acid. However, this reaction did not produce any product.[140] It was thought that 1H-benzo[d][1,2,3]triazole-1-carbaldehyde **72** could serve as a suitable formyl donor and was easily synthesized using benzyl triazole, formic acid and diisopropyl carbodiimide (DIC).[141] **72** was allowed to react with **66** in the presence of various bases and solvents, the results are summarized in table 26.

Table 26: Synthesis of amide **165**.

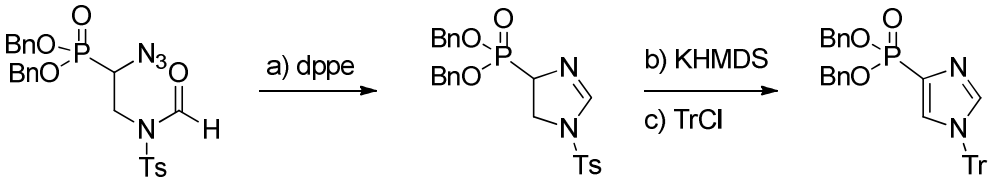


66 **72** **165**

#	Base (equiv)	Equivalents **72**	Solvent	Temp. (°C)	Result
1	Cs_2CO_3 (1.2)	1.2	DMF	rt	50% conversion
2	NaH (1.2)	2	DMF	0	Decomposition
3	NaH (1.2)	2	THF	0	76% yield

The amide coupling was first performed using cesium carbonate in DMF, however after 6 hours at room temperature there was only 50% conversion (entry 1). Therefore, sodium hydride was tested, being a stronger base. Surprisingly, sodium hydride in DMF yielded an unidentified mixture of products (entry 2). After a solvent switch to THF the reaction gave target compound **165** in good yield after only 30 minutes reaction time (entry 3).

Aza-wittig ring closing using dppe under standard conditions (see chapter 8.2.7) gave 87% yield of the imidazoline (scheme 26). Imidazoline **166** was then aromatized to the imidazole by base induced aromatization and quenching with trityl chloride. The in situ quench with trityl chloride gave about 50 percent conversion to **167** during test reactions; the rest was the unprotected imidazole.



165 **166** **167**

Scheme 26: Formyl synthesis of **167**. Reagents and Conditions: a) dppe (1.5 equiv), THF, 80°C, 6h, 87%. b) KHMDS 0.5M in toluene (2 equiv), THF, -78°C, 1 h. c) trityl chloride (1.5 equiv), THF, -78°C to rt, 2.5 h, 50 percent conversion.

The in situ quench of the imidazole failed completely in case of the pHis analogue described in chapter 8.2.11. The absence of a C-2 substituent on compound **166** most likely allows for in situ protection albeit in moderate yield.[1]

While this route toward **167** did yield the product, it was in quite low overall yield. Since parallel work on a different route toward **167** was successful at this point, the formyl route was abandoned (see chapter 8.4.2).

[1] At this time the introduction of the trityl chloride employing the DBU and silver triflate method was not yet developed.

8.3.2 Aldehyde route

The problematic oxidation of the imidazoline to the imidazole described in chapter 8.2.8, let to the consideration of a different strategy where this oxidation step could be discarded. Key intermediate **168** already possesses the right oxidation state and should therefore lead directly to **155** via attack of an amine on the carbonyl (Figure 2). The retrosynthetic analysis to obtain **155** is depicted in Figure 48.

The final compound **155** can be made by ring closing of imine **168**. The PMB was chosen as a protecting group because it should not deactivate the amine.[2] The imine should be obtained by reaction of **76** with 4-methoxybenzylamine. Aldehyde **76** should be available through the amide coupling of **67** and **75** followed by deptorection and oxidation. **67** can be easily synthesized according to a literature procedure.[115] It was thought that **75** could be obtained from the corresponding azide, which should be available from precursor **170** by activating the alcohol followed by substitution. Finally, **170** should be made accessible through a reaction between dibenzylphosphite **80** and aldehyde **169**. The TBS (tert-butyl dimethylsilyl) group was chosen as a protecting group on **169** due to its stability to a wide array of conditions and its ease of cleavage with fluorine sources.

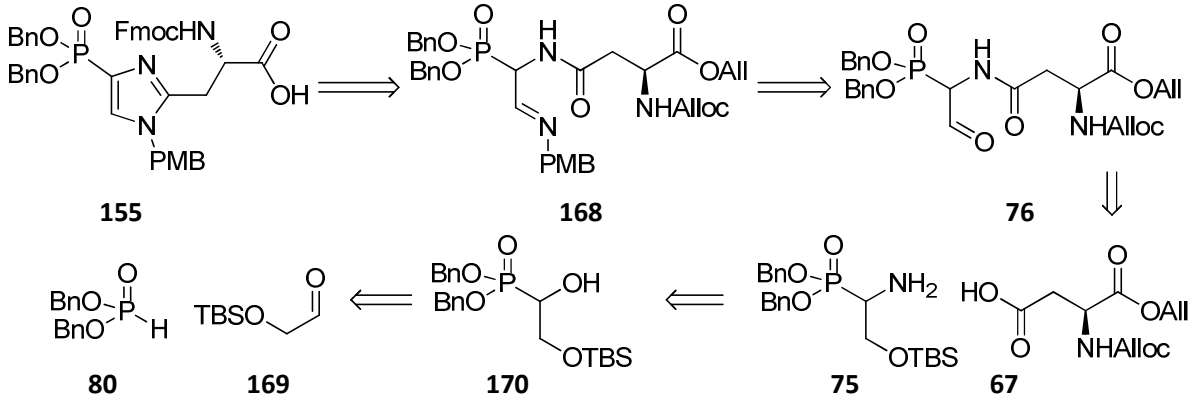
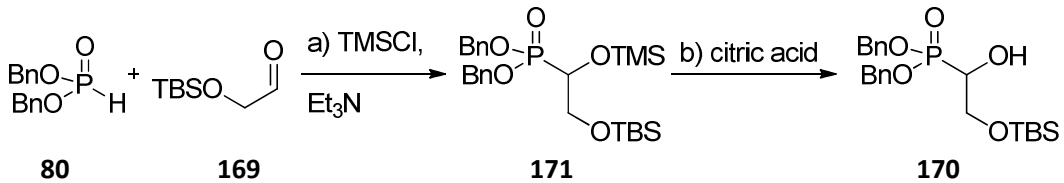


Figure 48: Retrosynthesis

Intermediate **170** was prepared in analogy to the strategy described in chapter 8.2.6. The phosphonate was activated by TMS chloride and then the aldehyde was added, yielding silyl ether **171**. The TMS group was easily removed using citric acid in methanol, leaving the TBS group untouched (scheme 27).



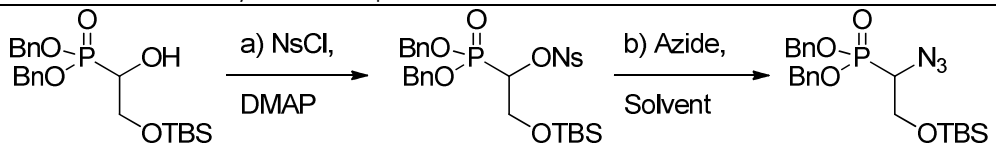
Scheme 27: Synthesis of . Reagents and conditions: a) TMS chloride (1.44 equiv), triethylamine (1.44 equiv), DCM, 0°C to -78°C to rt, 16 h, 69% b) citric acid (2.5 equiv), methanol, rt, 2 h, 98%.

Next, the alcohol needed to be activated so it could be displaced by an azide. Initially, the 4-nitrobenzenesulfonyl (4-nosyl) group was selected. Attempts that used 4-nosylchloride and pyridine did

[2]At this time the imidazoline was not yet oxidized to the imidazole using base-induced aromatization. Therefore, the problematic removal of the PMB protective group was not yet known.

not go to full conversion, even after extended reaction times. However, the 4-nosyl group was successfully introduced in high yield using DMAP. Attempts at aziridation of 4-nosyl compound **172** are summarized in table 27.

Table 27: synthesis of azide **173** via 4-nosyl activated compound **172**.



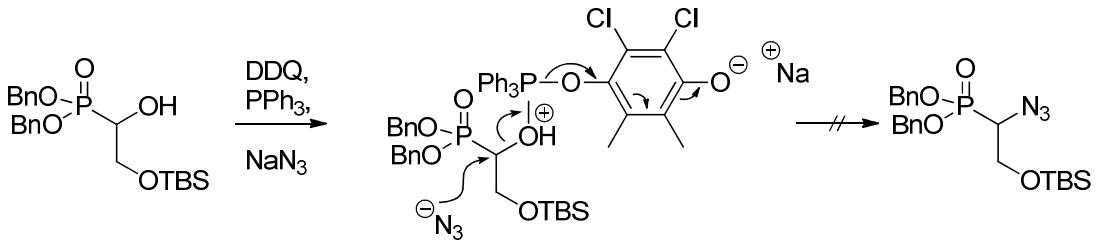
170 **172** **173**

Reagents and conditions: a) 4-nitrobenzenesulfonylchloride (1.1 equiv), DMAP (1.1 equiv), DCM rt, 3 h, 98%. b) azide, solvent, temperature, 16 h.

#	Azide Reagent (equiv)	Solvent	Temp (°C)	Result
1	NaN_3 (3)	DMF	rt	Unidentified byproducts
2	TMGA	MeCN	83	Decomposition

Exposing **172** to sodium azide in DMF resulted in a complex mixture containing several unidentified byproducts (entry 1). TMGA in acetonitrile at 83 degrees resulted in complete decomposition of the starting material. It is likely that, like in chapter 8.2.4, the TMGA removes the benzyl group as no phosphorus containing compounds were recovered. The 4-nosyl group did not seem to be a good activator for this kind of reaction, therefore other possibilities were investigated.

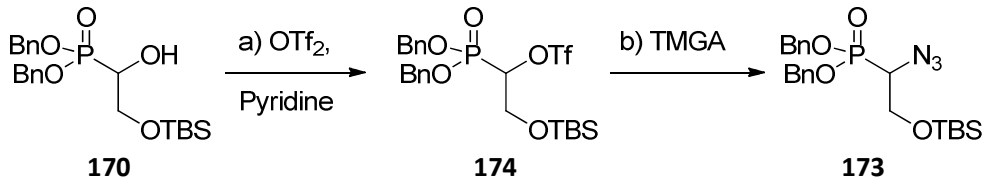
In the literature an example employing DDQ and PPh_3 to activate an alpha-hydroxyphosphonate (scheme 28: **A**), followed by successful substitution, was described.[142] In analogy to this report, 2 equivalents of sodium azide, DDQ and PPh_3 were mixed and compound **170** was added. After stirring for several hours, no conversion was observed.



170 **A** **173**

Scheme 28: Synthesis of azide **173** via PPh_3 and DDQ activation. Reagents and conditions: DDQ (2 equiv), PPh_3(2 equiv), NaN_3 (2 equiv), MeCN, 84°C, 3 h.

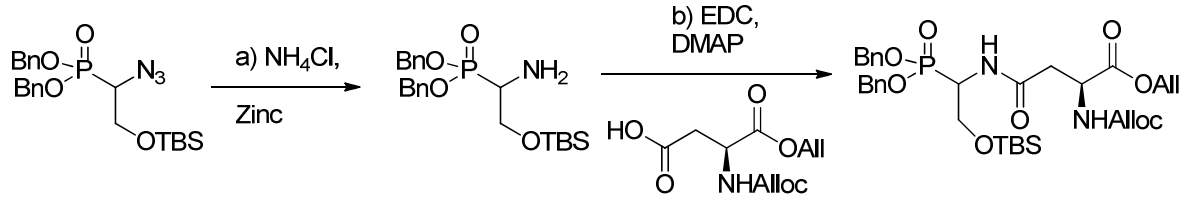
The triflate group was selected as a stronger activating group. The hydroxyl moiety was activated as a triflate using triflic anhydride and pyridine in DCM to obtain **174** quantitatively. Gratifyingly, dissolving **174** in acetonitrile in the presence of TMGA gave the azide **173** in high yield (Scheme 29).



170 **174** **173**

Scheme 29: synthesis of azide 173 via triflate activated compound **174**. Reagents and conditions: a) triflic anhydride (1.5 equiv), pyridine (2 equiv), DCM, -15C, 2 h, 96%. b) TMGA (1.2 equiv), MeCN, rt, 15 min, 83%.

With azide **173** in hand, the next goal was its reduction to the amine. 1.1 equiv PPh_3 in 1,4-dioxane: water 10:1 smoothly reduced the azide. However, the product was contaminated with a large amount of PPh_3O. Rather than spending time and effort in purifying the compound another method was used. Exposing azide **173** to a suspension of ammonium chloride and zinc powder in ethanol: water 3:1 gave the target amine **75** as a pure yellow oil after an aqueous work-up in 89 percent yield (scheme 30).

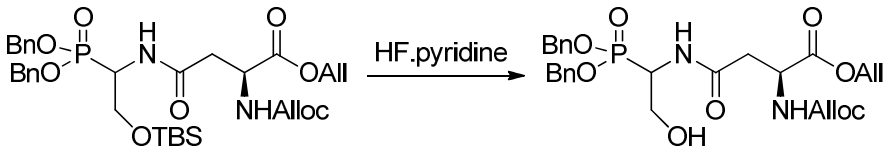


173 **75** **67** **175**

Scheme 30: synthesis of amide **175** via amine **75**. Reagents and conditions: a) ammonium chloride (2.5 equiv), zinc powder (3 equiv), ethanol: water 3:1, rt, 1 h, 87%. b) **67** (1.25 equiv), EDC (1.3 equiv), DMAP (0.1 equiv), DCM, rt, 1 h, 67%.

The amide coupling between **75** and **67** proceeded smoothly. Using previously established conditions (PyBrOP, DMAP, DIPEA, Chapter 8.2.5), gave **175** in 66 percent yield. The coupling was also performed using EDC as activator and a catalytic amount of DMAP. These conditions furnished compound **175** in comparable yields. Since EDC is cheaper than PyBrOP this method was used in subsequent reactions.

Subsequent removal of the TBS group using an excess of HF.pyridine in wet THF went in 99 percent yield (scheme 31).

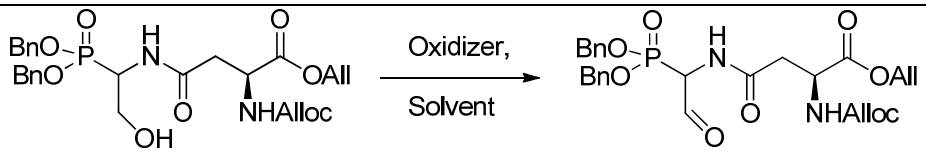


175 **176**

Scheme 31: Deprotection of **75**. Reagents and conditions: HF.pyridine (9 equiv), wet THF, rt, 1 h, 99%.

Next, the oxidation of **176** to the aldehyde was investigated (table 28). The swern oxidation (entry 1) was the first choice for the oxidation of **176** to **76**. Regrettably, all the starting material decomposed and no product was obtained. The Parikh-Doering oxidation, a close analogue to the swern oxidation provided similar results (entry 2). Various other oxidants and conditions were investigated, but all resulted in decomposition of the starting material (entries 3-6).

Table 28: Oxidation of **76**.



176 **76**

Reagents and conditions: oxidizer, solvent, 0°C.

#	Oxidizer (equiv)	Solvent	Result
1[a]	$(CO)_2Cl_2$ (1.2), DMSO (2.4), Et_3N (5)	DCM	Decomposition
2	SO_3.pyridine (3), Et_3N (6)	DMSO	Decomposition
3	DMP (2)	DCM	Decomposition
4[b]	TPAP (0.15), NMO (1.5)	DCM	Decomposition
5[b]	TPAP (0.15), NMO (2)	DCM: MeCN 8:1	Decomposition
6	PCC (1.5)	DCM	Decomposition

[a]: reaction was done at -78°C to rt. [b]: reaction was done at rt and 4A molsieve was used.

The reason why all oxidation methods failed is most likely due to the instability of the aldehyde that is formed. In an attempt to trap the aldehyde before it decomposed, PMB amine was added to a crude mixture of the swern oxidation, but no product was obtained. Since the intrinsic reactivity of the aldehyde seemed to exclude its isolation and the oxidation issues regarding the aza-wittig route were solved (chapter 8.2.10), this route was discontinued.

8.3.3 Dithiane route

To prevent the problems aromatizing the imidazoline described in chapter 8.2.8, an effort was made to circumvent this step by making use of a precursor already at the right oxidation state (**79**, figure 49). Because it was known from previous experiments that an aldehyde on the beta-position of the phosphonate is unstable (chapter 8.3.2), a 1,3-dithiane was used instead. 1,3-dithianes are used as protecting groups for aldehydes and ketones, in analogy to acetals and ketals and can be hydrolyzed back to their respective carbonyl. In this case however, the intention was to substitute the 1,3-dithiane directly without unmasking the unstable aldehyde (figure 49).[143]

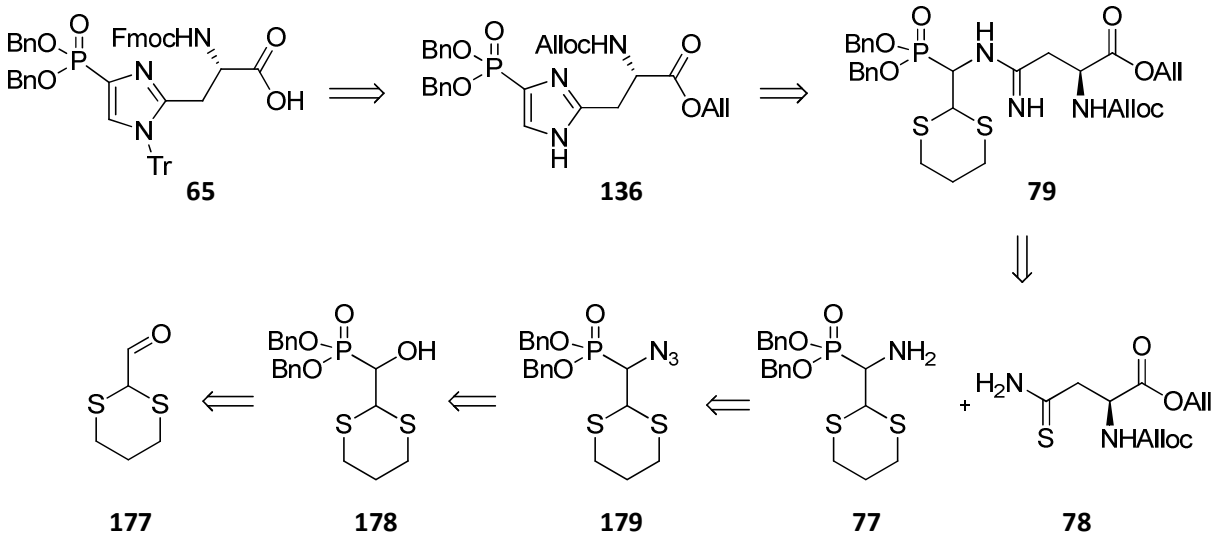
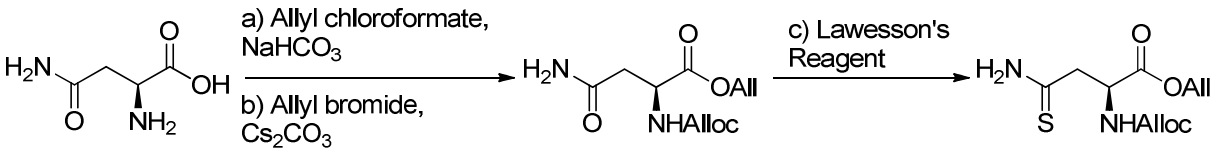


Figure 49: retrosynthetic strategy for the 1,3-dithiane route.

65 could be made from imidazole **136** via protecting group switches. **136** in turn should be available via amidine **79** by activation of the dithiane, using dimethyl(methylthio)sulfonium tetrafluoroborate (DMTSF)[144] or methylation, followed by substitution of the dithiane by the amidine nitrogen. Amidine **79** would be synthesized by a simple coupling of amine **77** and thioamide **78**.[145] Thioamide **78** can easily be prepared from L-asparagine using Lawesson's reagent. Amine **77** could be prepared from aldehyde **177** in three steps. Phosphorylation of **177** should provide alcohol **178**, which should then be activated, substituted for an azide (**179**) and reduced to give **77**.[146]

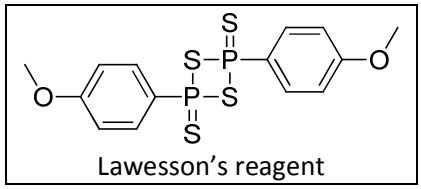
Thioamide **78** was synthesized by protecting the amine and acid moieties of L-asparagine **180** with an Alloc and allyl group respectively. The resulting amide **181** was transformed to the thioamide using Lawesson's reagent in excellent overall yield (scheme 32).

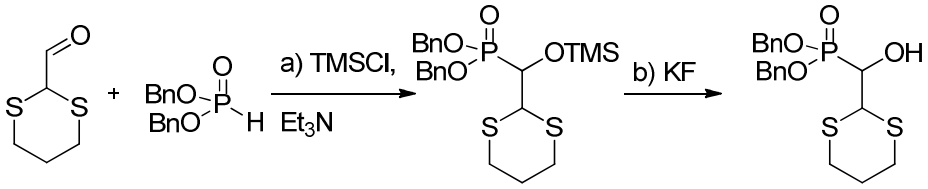


180 **181** **78**

Scheme 32: Synthesis of **78**. Reagents and conditions: a) Allyl chloroformate (1.05 equiv), water, rt, 7 h, quantitative. b) allyl bromide (1.2 equiv), cesium carbonate (1.5 equiv), DMF, rt, 8h, 92%. c) Lawesson's reagent (0.5 equiv), THF, rt, 4h, 88%.

Aldehyde **177** was synthesized according to a literature procedure.[147] Introduction of the phosphonate was done in a similar way as in chapter 8.2.6. Dibenzylphosphite was activated using TMS chloride and triethylamine, after which the aldehyde was added. The yield for the phosphorylation of this aldehyde was not as good as for other aldehydes, with a maximum yield of 50 percent (scheme 33). Removal of the TMS group with KF in methanol went in quantitative yield, setting the stage for the azide introduction.



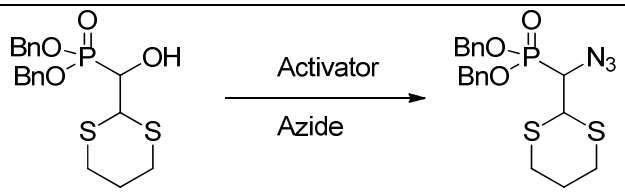


177 **80** **182** **178**

Scheme 33: Phosphorylation of **177**. Reagents and conditions: a) dibenzylphosphite (1.2 equiv), chlorotrimethylsilane (1.44 equiv), triethyl amine (1.44 equiv), DCM, -20°C to -78°C to rt, 17h, 50 %. b) potassium fluoride (2 equiv), methanol, rt, 30 min, 99%.

Attempts to introduce the azide were done by activation of the alcohol, followed by substitution with an azide source. Several condition were used, a summary is shown in table 29.

Table 29: Azide introduction.



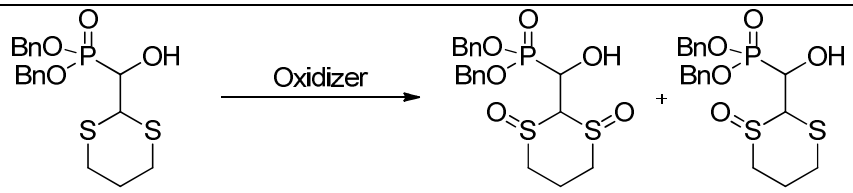
178 **179**

#	Activator (equiv)	Azide (equiv)	Solvent	Result
1	OTf_2 (1.5), pyridine (2)	—	DCM	Decomposition
2	OTf_2 (1.5), 2,6-lutidine (2)	TMGA	DCM	Decomposition
3	TsCl (1.2), Et_3N (1.2)	—	DCM	No reaction
4	dppa (1.2), DBU (1.1)	—	Toluene	No reaction
5	PPh_3 (1.05), DIAD (1.05)	TMGA (1.5)	THF	No reaction
6	PPh_3 (1.05), DIAD (1.05)	HN_3 (3)	THF	No reaction
7	PPh_3 (1.05), DIAD (1.05)	dppa (1.5)	THF	Decomposition

Initially, triflate activation was attempted, using triflic anhydride and pyridine; however, a mixture containing several unidentified products was obtained (entry 1). Changing pyridine for 2,6-lutidine, followed by addition of TMGA as soon as the starting material was consumed, did not improve the reaction (entry 2). It was hypothesized that the triflate of **178** was too reactive and therefore the tosyl group, a weaker activating group, was introduced. However, the reaction with tosyl chloride yielded a mixture containing mostly starting material (entry 3). Next diphenylphosphoryl azide (dppa) was tested as both activator and azide source;[148] but again no reaction was observed (entry 4). Mitsunobu reactions were explored as an alternative activation strategy, using several azides.[146b, 149] However, these reactions either gave back the starting material (entries 5 and 6) or decomposition (entry 7).

The reason for the failure of the azide introduction might be due to the low electrophilicity of the carbon alpha to the phosphonate. In the case of the triflate activation in chapter 8.2.4 it was assumed that the tosylamide lowered the electron density on that carbon promoting sufficient activation. This effect is absent in the case of the 1,3-dithiane. Therefore, it was thought that the activated alcohol might be successfully substituted if the dithiane was more electron withdrawing. To that end, attempts were made to oxidize both dithiane sulfurs to the sulfoxide (Table 30).

Table 30: 1,3-dithiane oxidation.

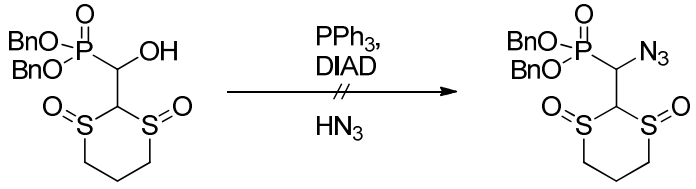


178 **183** **184**

#	Oxidizer (equiv)	Solvent	Result
1	*m*-CPBA (2.1)	$CHCl_3$	Decomposition
2	*m*-CPBA (2.2)	$CHCl_3$: EtOAc 2:1	Decomposition
3	$NaIO_4$ (2.1)	1,4-dioxane: water 2.3:1	**183**: **184** 2:1[a]
4	$NaIO_4$ (2.3)	1,4-dioxane: water 2.3:1	36% **183**[b]

[a]: yield not determined. [b]: still contained 6% **184**.

Initially, m-CPBA was investigated as oxidizer; however, a complex mixture of unidentified products was obtained (entry 1).[150] Changing the solvent to include ethyl acetate did not change the outcome of the reaction (entry 2). After switching oxidant to sodium periodate,[151] a mixture containing mostly **183** and **184**, in a 2:1 ratio, was obtained (entry 3). The two analogues were challenging to separate, finally **183** was obtained in 36 percent yield (entry 4), albeit contaminated with six percent **184**.



183 **185**

Scheme 34: Mitsunobu reaction with **183** and hydrazoic acid. Reagents and conditions: PPh_3 (1.05 equiv), DIAD (1.05 equiv), HN_3 2.5 M in benzene (3 equiv), THF, -40°C, 16 h.

A Mitsunobu reaction between **183** and hydrazoic acid was attempted (scheme 34). Hydrazoic acid was freshly prepared as a solution in benzene and **183** was added as a solution in DMF as it did not dissolve in THF. Regrettably, the reaction did not show any conversion.

Due to the failure of the initial azidation and the low yield of the 1,3-dithiane oxidation, combined with its low reactivity in the Mitsunobu reaction, it was decided to not pursue this route any further.

8.3.4 Alternative Aza-wittig route

This was chronologically the first strategy that required the synthesis of the imidazole, opposed to merely substituting an imidazole. The strategy is similar to the one presented in chapter 8.2. The main difference between this route and the one described in chapter 8.2 is the early introduction of the amino acid moiety and the aldehyde intermediate. The retrosynthetic analysis is shown in figure 50. Compound **65** can be accessed from imidazoline **186** by aromatization and protecting group changes. The imidazoline should be available from amide **71**, using an intramolecular aza-wittig reaction. The azide can be made from **189** by activation of the hydroxyl function and substitution. Alcohol **189** should be available by a nucleophilic attack of a TMS activated phosphonate on aldehyde **188** (chapter 8.2.6), followed by deprotection. Aldehyde **188** can be liberated by hydrolysis of acetal **187**, which in turn can be synthesized by an amide coupling between compounds **70** and **69**. The protected amino acid **70** can be made using a literature procedure.[152]

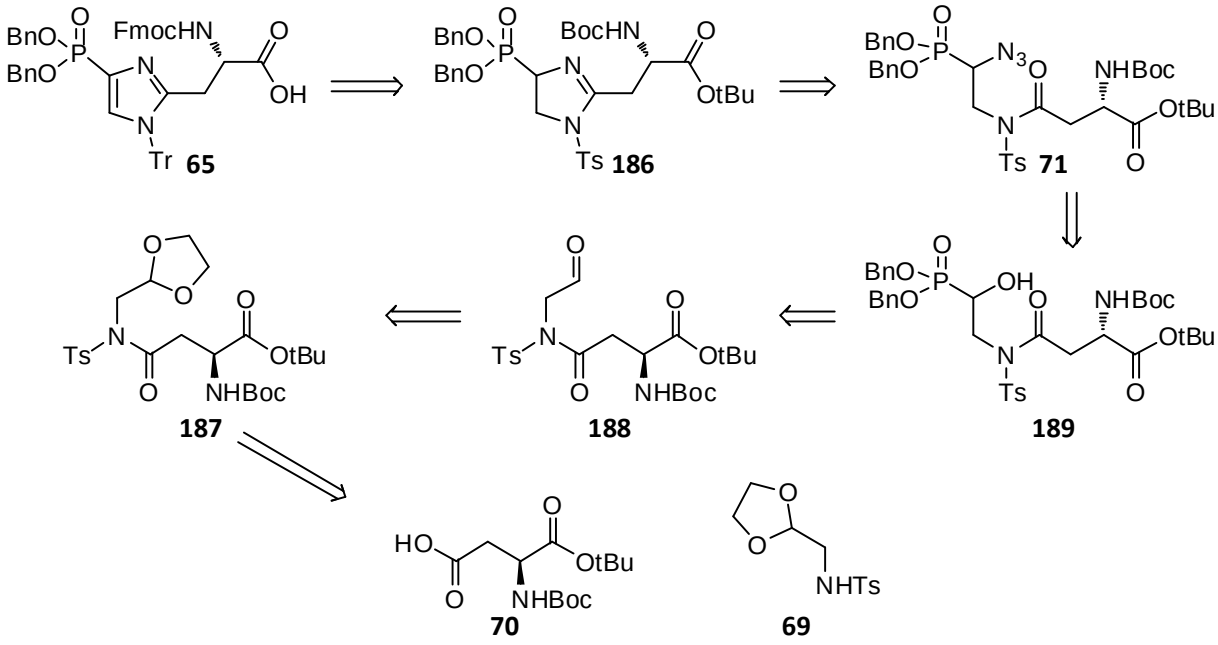
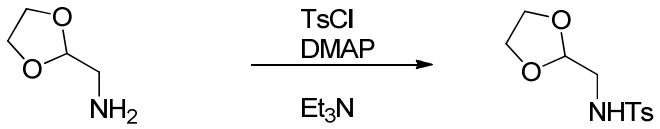


Figure 50: Retrosynthetic strategy toward **65** starting from acetal **69**.

This route features the Boc and tert-butyl ester as acid cleavable protecting groups. Acidolytic cleavage is not convenient, as it will also cleave the trityl group as well as the benzyl groups that should be in the final imidazole. Later, it was decided to use an Alloc and allyl group to protect the amine and the ester, respectively. These two protecting groups could be much more easily removed (chapter 7).

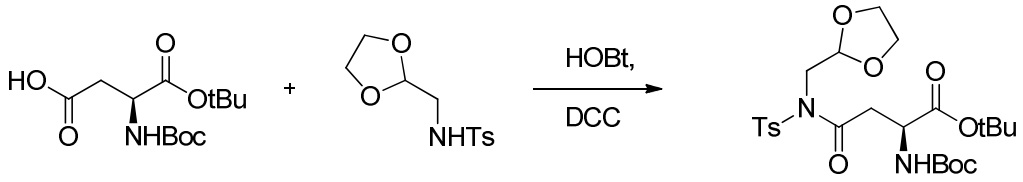
Synthesis of **65** started with the tosylation of **190** to make **69**. Simply combining **190** and tosyl chloride in DCM, in the presence of DMAP and triethylamine gave **69** in 93 percent yield (scheme 35).



190 **69**

Scheme 35: Tosylation of **190**. Reagents and conditions: Tosyl chloride (1 equiv), DMAP (0.01 equiv), triethylamine (1 equiv), DCM, 0°C to rt, 16 h, 93%.

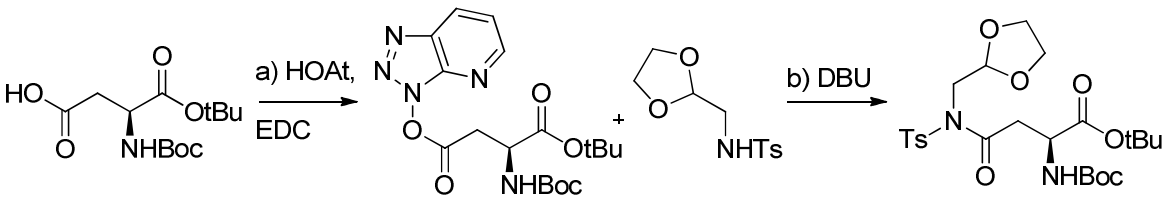
Next, **69** had to be coupled to **70**. The coupling between **70** and **69** was initially attempted with DCC and HOBt. Regrettably, no reaction occurred (scheme 36). There were two potential explanations for the failure of this reaction. Either the tosylamide was not a good enough nucleophile to attack the HOBt-ester of **70** or the HOBt-ester of **70** was not formed, due to unsuccessful activation by DCC.



70 **69** **187**

Scheme 36: Amide coupling of **70** and **69**. Reagents and conditions: HOBt (1 equiv), DCC (1 equiv), THF, 0°C to rt, 16 h.

A possible solution to both problems was found in the literature, where a premade HOAt-ester was successfully coupled with a sulfonyl amide.[121] In this way it is assured that the activated ester forms and, since HOAt is a better activating group than HOBt, a possible reactivity problem might also be solved. In line with this strategy, compound **70** was subjected to HOAt and EDC in DCM, yielding the HOAt-ester **191** in 93 percent isolated yield. Combining **69** and **191** in DCM in the presence of DBU gave the amide **187** in good yield.

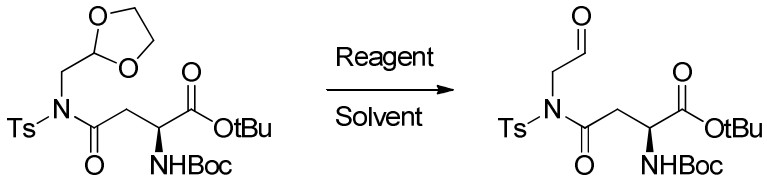


70 **191** **69** **187**

Scheme 37: Amide coupling of **70** and **69**, via HOAt activated ester **191**. Reagents and conditions: a) HOAt (1.3 equiv), EDC (1.5 equiv), DCM, 0°C to rt, 16 h, 93%. b) **191** (1.1 equiv), DBU (1.5 equiv), DCM, 0°C to rt, 1.5 h, 73%.

With the amide in hand, the next step was the hydrolysis of the acetal. This reaction appeared to be less straightforward than expected. The conditions that were tested are summarized in table 31.

Table 31: Acetal hydrolysis.



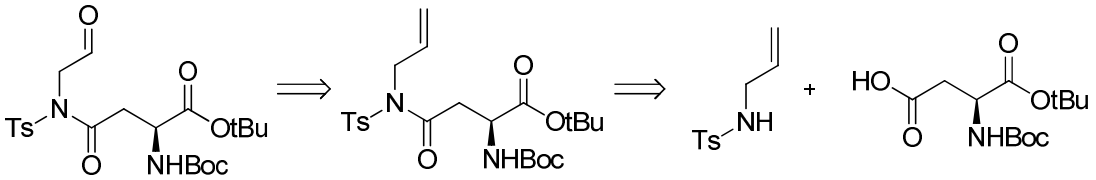
187 **188**

Reagents and conditions: reagent, solvent, temp, 16 h.

#	Reagents (equiv)	Solvent	Temp (°C)	Result
1	AcOH: water 9:1 (excess)	—	rt	No Reaction
2	AcOH: water 9:1 (excess)	—	65	No Reaction
3	AcOH: water 2:1 (excess)	—	90	deprotection of boc/tbu
4	1M HCl (excess)	THF	0 to rt	No Reaction
5	PPTS (1)	Acetone: water 9:1	rt	No Reaction
6	PPTS (1)	Acetone: water 9:1	57	No Reaction
7	$FeCl_3.6H_2O$ (3.5)	DCM	rt	Decomposition
8	$FeCl_3.SiO_2$ (1)	Acetone	rt	No Reaction
9	$FeCl_3.SiO_2$ (1)	$CHCl_3$	rt	No Reaction
10	H_5IO_6 (3.9)	1,4-dioxane: water 2.4:1	rt	No Reaction
11	DDQ (0.1)	MeCN: water 9:1	rt	No Reaction

Initially, acid catalyzed hydrolysis with acetic acid was attempted, but dissolving compound **188** in a large excess of acetic acid and water did not produce any conversion, even upon mild heating (entries 1 and 2). Upon heating to 90°C in acetic acid: water 2:1 both the Boc and tert-butyl group were lost, whereas the acetal remained (entry 3). The acetal was also stable to stronger acids (HCl[153] and PPTS[154]), even upon heating no reaction was observed (entries 4-6). Lewis acid ferric trichloride in DCM[155] led to complete decomposition of the starting material (entry 7). Silica gel with ferric chloride impregnated on the surface did not succeed in hydrolyzing the acetal, nor did periodic acid[156] or catalytic DDQ in acetonitrile: water 9:1.[157]

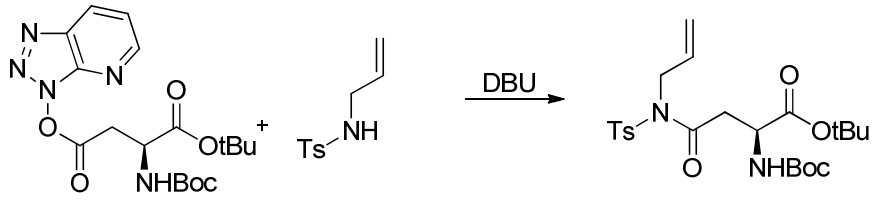
Since acetal **187** seemed to be to unreactive toward hydrolysis, an alternative route to the aldehyde was considered. Aldehydes can be easily made by oxidative cleavage of an alkene using osmium tetroxide and sodium periodate. Therefore, aldehyde **188** might be available from allyl tosylamide **192**, which in turn is easily made by amide coupling of **193** and **70** (figure 51).



188 **192** **193** **70**

Figure 51: New retrosynthetic strategy toward **188**.

Compound **193** was made according to a modified literature procedure.[158] Its coupling with **70** was performed in line with the previous amide coupling (scheme 38).

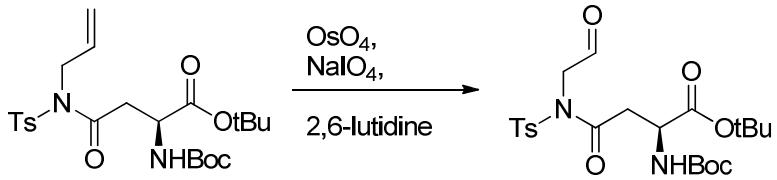


191 **193** **192**

Scheme 38: Amide coupling of **191** and **193**. Reagents and conditions: **193** (1.1 equiv), DBU (1.5 equiv), DCM, 0°C to rt, 1.5 h, 78%.

Unfortunately, amide **192** appeared to be difficult to purify. During silica column chromatography residual starting material **193** and amide **192** eluted in the same fractions and seemed inseparable. Eventually it was found that alumina was able to separate the two compounds and **192** could be obtained in 78% yield.

Next, oxidative cleavage of **192** was attempted with osmium tetroxide and sodium periodate in the presence of 2,6-lutidine (scheme 39).[159] During the reaction quick conversion to one new product was observed, after 30 minutes all starting material was consumed. However, during work-up and subsequent evaporation the mixture turned from a clear solution to a brown and then black oil. Analysis of this oil revealed an unidentifiable mixture of decomposition products. Attempts to do the work-up and evaporation at low temperature and under an argon atmosphere could not prevent the decomposition to occur.



192 **188**

Scheme 39: Oxidative cleavage of alkene **192**. Reagents and conditions: osmium tetroxide (0.2 equiv), sodium periodate (4 equiv), 2,6-lutidine (2 equiv), 1,4-dioxane: water 3:1, rt, 30 min.

It seemed that the aldehyde **188** did form during the oxidative cleavage reaction, but that it was too unstable to survive work-up. Such an unstable aldehyde could not be used as an intermediate in the synthesis of **65**. Therefore, this route was not pursued any further.

8.4 Other routes toward a 3-phosphohistidine mimic – Imidazole substitution

8.4.1 Metal catalyzed & Halogen-metal exchange

The initial retrosynthetic strategy toward a stable 3-pHis analogue was build around the substitution of an imidazole. Introduction of the amino acid part was envisioned through a nucleophilic attack of an imidazole cuprate onto a β-lactone, which was easily available from L-serine.[45-46, 160] The required phospho-imidazole should be available through either a metal mediated coupling or through halogen metal exchange and attack on an activated phosphite.[161] The 4-halo-imidazoles were easily made from 1-benzylimidazole according to literature procedure.[162]

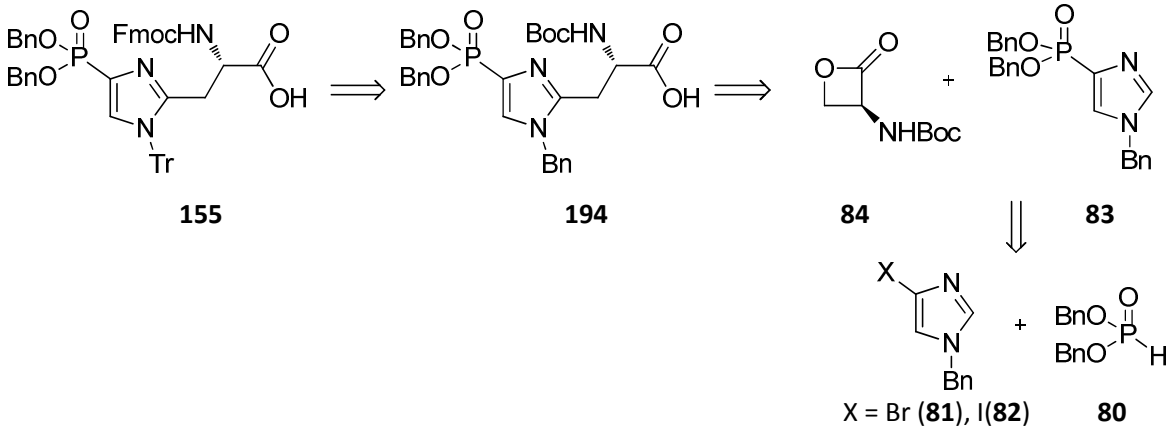
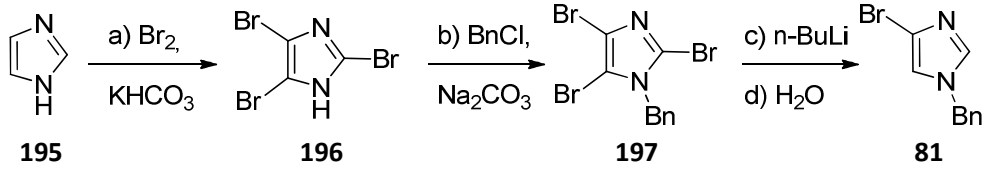


Figure 52: First retrosynthetic analysis toward non-hydrolysable pHis analog.

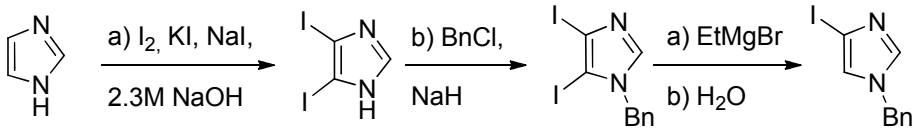
As protecting group on the imidazole the benzyl group was selected since there was literature precedent for the 1-benzyl-4-iodoimidazole as well as for the 1-benzyl-4-bromoimidazole that were targeted. In addition, this benzyl group could be removed together with the phosphonate protecting groups after SPPS using hydrogenation. Both the bromo- and iodoimidazole were investigated to account for possible differences in reactivity.

4-bromo-1-benzylimidazole **81** was synthesized according to a literature procedure.[163] The synthesis started with imidazole **195**, which was tribrominated using molecular bromine; albeit at low yield. Most probably, addition of extra equivalents of bromine would have driven the reaction to completion. Since enough material was isolated, this has not been further investigated. Benzyl protection was realized using benzyl chloride, setting the stage for halogen metal exchange of the two most reactive bromines, at the two- and four positions, followed by quenching with water to yield 4-bromo-1-benzylimidazole **81** in acceptable yield.



Scheme 40: Synthesis of 4-bromo-1-benzylimidazole.Reagents and Conditions: a) Bromine (1.5 equiv), $KHCO_3$ (3 equiv), DMF, 0-100°C, 5h, 32%; b) Benzylchloride (1 equiv), Na_2CO_3 (1 equiv), DMF, 130°C, 2 days, 82%; c) n-BuLi (2.4 equiv), Et_2O, -78°C, 2h, then; d) water, 64%.

4-iodo-1-benzylimidazole **82** was synthesized in an analogues way; imidazole **195** was first diiodinated using molecular iodine, sodium iodide and potassium iodide in aqueous sodium hydroxide.[164] 4,5-Diiodo-1H-imidazole **198** was benzyl protected using benzyl chloride and sodium hydride. Finally, the iodine on C-5 was selectively removed using a halogen metal exchange reaction with ethyl magnesium bromide and quenching with water, yielding 4-iodo-1-benzylimidazole**82** in acceptable yield. The next step was phosphorylation of these 4-haloimidazoles.



195 **198** **199** **82**

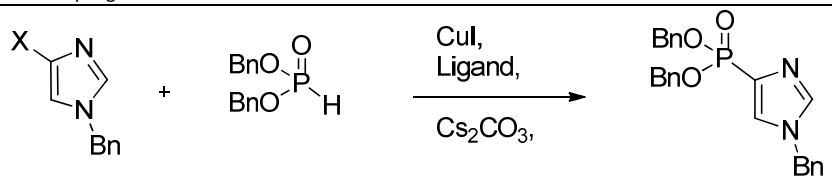
Scheme 41: Synthesis of 4-bromo-1-benzylimidazole. Reagents and conditions: a) iodine (1.0 equiv), potassium iodide (1.1 equiv), sodium iodide (2.3 equiv), 2.3M NaOH (aq), rt, 3h, 27%; b) benzyl chloride (1.1 equiv), NaH (1.1 equiv), THF, 0-65°C, 2.5 days, 94%; c) ethyl magnesium bromide 1M in MTBE (1.1 equiv), THF, rt, 3.5h, then; d) water (6.7 equiv), 78%.

8.4.1.1 Metal catalyzed

While literature concerning the phosphorylation of imidazoles is rather small, some precedent can be found. A halogen-metal exchange strategy had been successful in the past.[165] Metal mediated couplings of vinyl- and aryl-iodides and bromides might also be successfully adapted to our imidazoles.[166] A great advantage of metal-mediated formation of phosphorus – aryl bonds is that they use commercially available, stable phosphites as phosphorus source. They usually employ either a copper or a palladium catalyst, of which the former is especially attractive due to its low price and easy availability.[127b]

The synthesis of several phosphonates from aryl- and vinyl-iodides and bromides, using CuI and proline or N,N'-dimethylethylenediamine (DMEDA) as ligand have been reported.[167] Iodides were reported to show superior reactivity compared to bromides. Therefore, coupling reactions between 4-halo-1-benzylimidazole and dibenzylphosphite were investigated (table 32).

Table 32: Copper mediated coupling reactions.



X= Br (**81**), I (**82**) **80** **83**

Reagents and Conditions: **80** (1.5 equiv), CuI (0.1 equiv), Ligand (0.3 equiv), Cs_2CO_3 (2 equiv), toluene, 16 h.

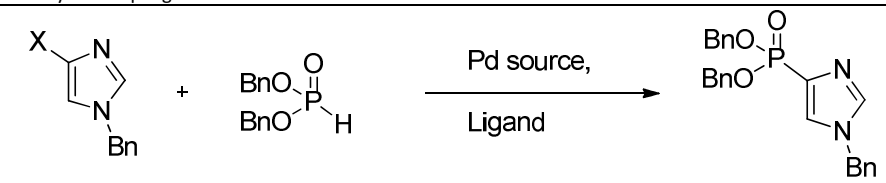
#	Imidazole	Ligand	Temp. (°C)	Result
1[a]	**82**	Proline	rt	No reaction
2[a]	**82**	DMEDA	rt	No reaction
3[b]	**82**	Proline	110	Decomposition
4[b]	**82**	DMEDA	110	Decomposition
5[b]	**81**	Proline	110	Decomposition
6[b]	**81**	DMEDA	110	Decomposition

[a]: 0.2 equiv CuI was used. [b]: the reaction was stirred for 3 days.

Initial trails with iodoimidazole **82** at room temperature did not show any conversion, regardless of the ligand used (entries 1 and 2). When the reactions were heated to reflux, starting material was consumed very slowly, over several days. Regrettably, no product **83** was recovered, as all starting material had decomposed.

The halo-imidazoles seemed to be inert under the copper catalyzed reaction conditions; therefore, palladium-mediated couplings were investigated. Stawinski *et al.* compared different palladium(0) sources in coupling reactions between diethyl phosphite and bromo- and iodo-benzene.[168] They showed that $Pd(OAc)_2$ and PPh_3 was the best catalyst – ligand combination and that iodides are superior to bromides. The attempts to apply the palladium chemistry to the 4-halo imidazoles are summarized in table 33.

Table 33: Palladium catalyzed coupling reactions.



X= Br (**81**), I (**82**) **80** **83**

Reagents and Conditions: **80**(1.5 equiv), Pd (0.1 equiv), L (0.3 equiv), NEt_3 (1.2 equiv), toluene, 90°C, 1 day.

#	Imidazole	Palladium source	Ligand	Result
1	**81**	$Pd(OAc)_2$	PPh_3	No reaction
2	**82**	$Pd(OAc)_2$	PPh_3	No reaction
3	**82**	$Pd(PPh_3)_4$[a]	—	Decomposition

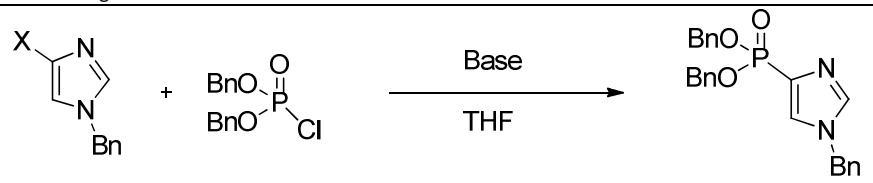
[a]: 0.3 equiv [Pd] was used, mixture was stirred for 2 days.

Tests with palladium(II)acetate and PPh_3 were unsuccessful, both for the bromo- and iodoimidazole no conversion was observed (entries 1 and 2). Switching catalyst system to tetrakis(triphenylphosphine)palladium(0) and stirring for several days led to decomposition (entry 3).

8.4.1.2 Halogen metal exchange

At this point it seemed that the metal catalyzed coupling conditions could not successfully be adapted to our system, thus it was decided to try a halogen-metal exchange strategy. Dibenzyl phosphorylchloride **200**, which was easily prepared from dibenzylphosphite and N-chlorosuccinimide,[169] was used as the electrophile. Results from the halogen-metal exchange trials are summarized in table 34.

Table 34: Halogen metal exchange reactions.



X=Br (**81**), I (**82**) **200** **83**

Reagents and Conditions:

#	Imidazole	Base (equiv)	Temp (°C)	Result[a]
1	**81**	n-BuLi (2)	-78 to rt	Benzyl imidazole
2	**82**	^{i}PrMgCl.LiCl (1.2)	0 to rt	No reaction
3	**82**	^{i}PrMgCl.LiCl (1.2)	rt to -78 to rt	Benzyl imidazole

[a]: reaction monitored by TLC.

Initially, bromo imidazole **81** and n-BuLi were mixed at low temperature, stirred for 20 minutes at low temperature followed by quenching with **200**. Regrettably, only benzyl imidazole and starting material were recovered (entry 1). Clearly the combination of **200** and the lithium salt of **81** was not reactive enough. A Grignard reagent might perform better due to the better coordinating ability of magnesium.[170] This not only yields a more stable metalate, but might also bring the two reactants closer together due to coordination of the phosphite. Therefore, **82** was subjected to ^{i}PrMgCl.LiCl at 0°C, followed by addition of **200** and slow warming to room temperature. Again, only starting material was recovered. Increasing the initial temperature to room temperature, to ensure complete halogen metal exchange, followed by low temperature, careful quenching with **200** led to a combination of benzyl imidazole and starting material. At this point it was realized that **200** was likely not a good electrophile in this reaction and an alternative pathway was needed.

8.4.2 Negishi II

Although the synthesis of compound **167** was successfully accomplished using the formyl method (chapter 8.3.1) the yield was disappointing. Therefore an alternative route was developed that gave the same product but in higher yield and fewer steps, employing a halogen-metal exchange and a more reactive phosphor source. Instead of the dibenzylphosphoryl chloride **200**, bis(diethylamino)phosphine chloride was used, a better electrophilic phosphor source. It was thought that the obtained intermediate **202** could be converted to **167** in situ. This indeed resulted in a one pot synthesis of compound **167** (scheme 42). This success allowed the investigation of the Negishi reaction (figure 53).

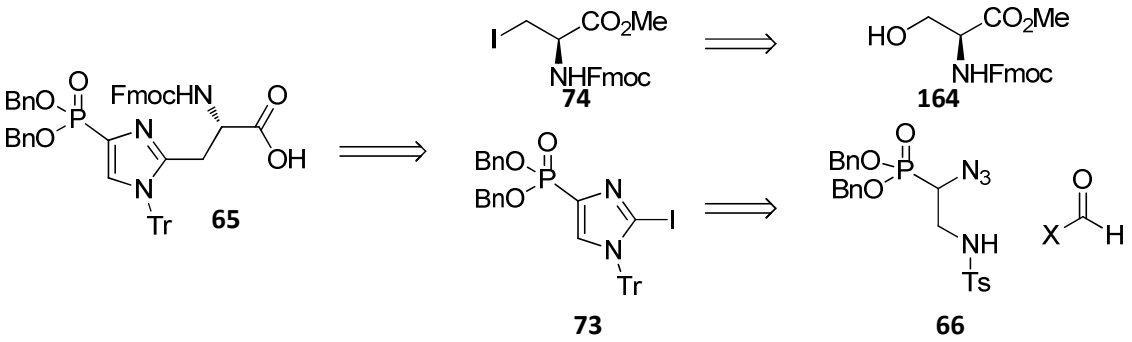
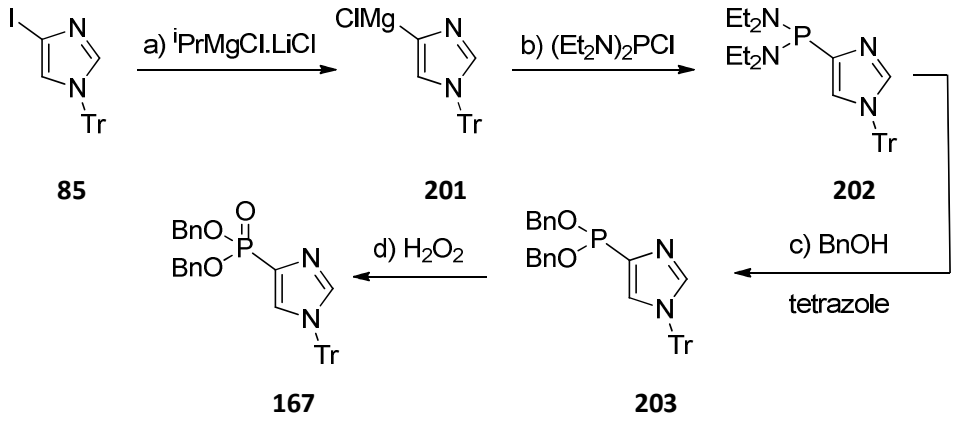


Figure 53: Updated Negishi strategy.

8.4.2.1 One pot synthesis of compound 167

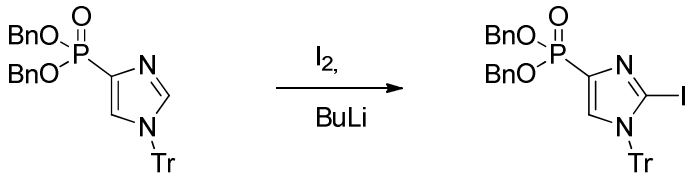
The alternative, one-pot route toward **167** started with 1-trityl-4-iodoimidazole **85**, thereby circumventing the nine steps of the aza wittig route to **66**. **85** was dissolved in THF, cooled to 0°C and isopropylmagnesium chloride lithium chloride complex in THF was added, resulting in halogen metal exchange with the iodine (scheme 42). The reaction was stirred at room temperature for 20 minutes to ensure full conversion during this step. Then the mixture was cooled to -78 degrees and bis(diethylamino)phosphine chloride was added. This P(III)-compound is a much better electrophile than dibenzyl phosphorylchloride **200** that was previously used in a similar reaction (chapter 8.4.1.2).[169b] After stirring the reaction for several hours the THF was removed in vacuo and benzyl alcohol was added. After adding acetonitrile as solvent, the reaction mixture was cooled down and a tetrazole solution was added to catalyze the substitution of the diethylamino groups to obtain the benzyl phosphoester groups. After stirring overnight at room temperature, the mixture was again cooled to 0°C and hydrogen peroxide in water was added to oxidize the phosphite to the phosphonate. In four steps, this one-pot method gave **167** in 59 percent yield.



Scheme 42: One-pot synthesis of **167**. Reagents and Conditions: a) iPrMgCl.LiCl 1.3M in THF (1.05 equiv), THF, 0°C to rt, 30 min. b) bis(diethylamino)phosphine chloride (1.1 equiv), -78 to rt, 3 h. c) benzyl alcohol (5.05 equiv), tetrazole 0.3M in MeCN (0.1 equiv), MeCN, 0°C to rt, 12h. d) hydrogen peroxide 30% in water (3.85 equiv), -20°C to 0°C, 2h, 59% over 4 steps.

The next challenge was to iodinate **167** on the 2 position of the imidazole. This was done by adding n-butyl lithium to a solution of **167** in THF at -30°C.[171] After stirring the solution for 20 minutes to ensure

full deprotonation of the imidazole, iodine in THF was slowly added until the iodine color persisted. After work-up and purification **73** was obtained in 53 percent yield (scheme 43).

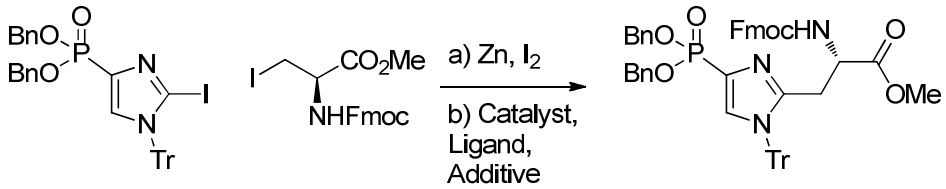


167 **73**

Scheme 43: Synthesis of trityl protected 2-iodoimidazole **73**. Reagents and conditions: Iodine (1.5 equiv), butyl lithium 1.6M in hexane (1.1 equiv), THF, -30°C to -60°C, 30 min, 53%.

The Negishi reaction itself requires an iodo-zinc species and an aryl- (or vinyl-)iodide. Zinc powder was activated using a catalytic amount of molecular iodine at about 300°C under vacuum. This activated zinc powder was added to compound **74** in DMF to prepare the iodo-zinc counterpart.[172] The mixture was then added to a solution of iodo-imidazole **73**. Finally, the catalyst and ligand were added and the mixture was heated to 40 degrees. The results of the first attempts at the Negishi reaction are summarized in table 35.

Table 35: Negishi reaction I.



73 **74** **204**

Reagents and Conditions: a) **74** (2.5 equiv), iodine (0.03 equiv), zinc (8.2 equiv), DMF, 300°C to rt. b) catalyst, ligand, additive, 40°C, 16h.

#	Catalyst (equiv)	Ligand (equiv)	Additive (equiv)	Result
1	$Pd_2(dba)_3$ (0.1)	X-Phos (0.2)	—	Beta elimination
2	$Pd_2(dba)_3$ (0.05)	S-Phos (0.1)	—	Beta elimination
3	$Pd_2(dba)_3$ (0.05)	X-Phos (0.1)	LiCl (2.5)	Beta elimination
4	$Pd_2(dba)_3$ (0.05)	S-Phos (0.1)	LiCl (2.5)	Beta elimination
5	$Pd(OAc)_2$ (0.1)	X-Phos (0.2)	—	Beta elimination
6	$Pd(OAc)_2$ (0.1)	S-Phos (0.2)	—	Beta elimination
7	$Pd(OAc)_2$ (0.1)	X-Phos (0.2)	LiCl (2.5)	Beta elimination
8	$Pd(OAc)_2$ (0.1)	S-Phos (0.2)	LiCl (2.5)	Beta elimination

[a]: reaction was performed at room temperature.

Initially $Pd_2(dba)_3$ was used as palladium source and X-Phos as ligand, this combination has been reported as particularly suitable for Negishi reaction by the group of Buchwald.[173] However, in this case no product was obtained (entry 1); switching to S-Phos[174] did not change the outcome of the reaction (entry 2). Addition of lithium chloride, reported to enable difficult Negishi reactions by activation of the

zinc reagent,[175] also did not produce any product (entries 3 and 4). Reactions using palladium(II)acetate as palladium source did not fare better (entries 5-8).

All reactions performed so far resulted in formation of dehydroamino acid derivative **205**, along with reduced starting material. The occurrence of this compound indicated that after the transmetalation step, beta elimination (path **I**) was preferred over reductive elimination (path **II**), normally a quick reaction (figure 54).[176] Therefore, conditions had to be found that favor the reductive elimination over beta elimination.

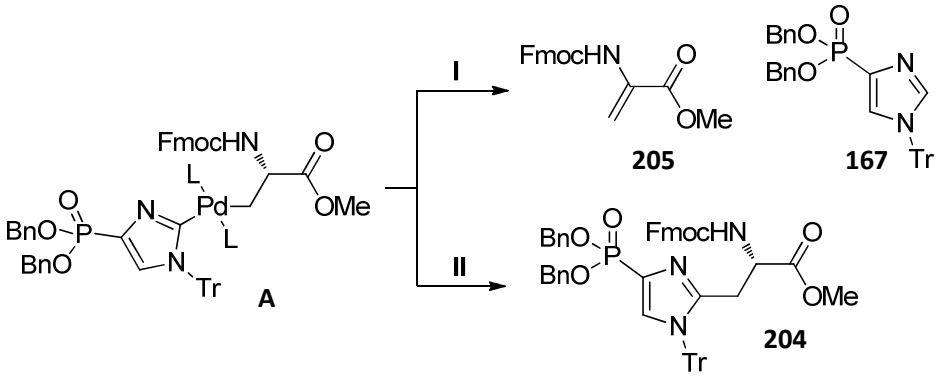
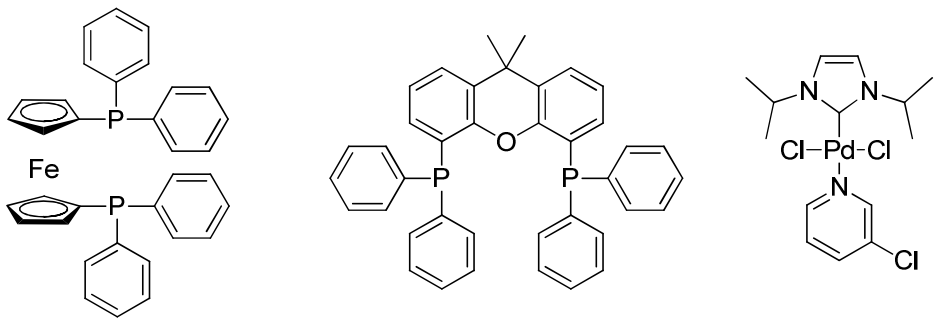


Figure 54: Negishi mechanism. Path I: beta elimination leads to alkene **205**. Path II reductive elimination leads to the desired product **204**.

It is known that ligands with a larger bite angle induce a bias for reductive elimination over beta elimination. Two ligands with large bite angles were examined; 1,1'-bis(diphenylphosphino)ferrocene (dppf) (**206**) and XantPhos (**207**).[175] Furthermore, an 'all-in-one' catalyst system called PEPPSI-iPr (**208**), is also reported to strongly favor reductive elimination over beta elimination.[177] Thus, these compounds were tested in the Negishi reaction (table 36).



206 **207** **208**

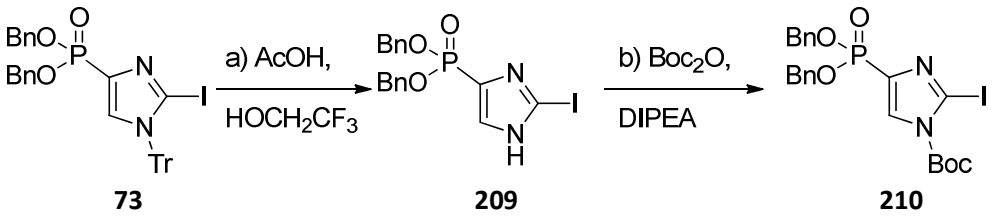
Table 36:Negishi reactions II.[a]

#	Catalyst (equiv)	Ligand (equiv)	Additive (equiv)	Solvent	Result
1[b]	$Pd_2(dba)_3$ (0.05)	dppf (0.1)	—	DMF	Beta elimination
2[b]	$Pd_2(dba)_3$ (0.05)	Xant-phos (0.1)	—	DMF	Beta elimination
3	$Pd_2(dba)_3$ (0.05)	dppf (0.1)	LiBr (5)	DMF	Beta elimination
4	$Pd_2(dba)_3$ (0.05)	Xant-phos (0.1)	LiBr (5)	DMF	Beta elimination
5[c]	PEPPSI (0.01)	—	LiBr (3.2)	THF: NMP 1:1	Beta elimination
6[d]	PEPPSI (0.1)	—	LiBr (5)	THF: NMP 1:1	Beta elimination

[a]:Reagents and Conditions: a)74 (2.5 equiv), iodine (0.03 equiv), zinc (8.2 equiv), DMF or NMP, 300°C to rt. b) catalyst, ligand, additive, 40°C, 16h. [b]: this reaction was performed at room temperature and at 40°C.[c]: this reaction was performed with 1.6 equiv 74 at room temperature. [d]: this reaction was performed at 60°C.

Regrettably, in all reactions only the beta elimination byproduct was recovered. To examine the reactivity of the organo-zinc compound, a Negishi reaction with iodobenzene as aryl halide was performed. This reaction went quickly to completion and no beta elimination byproduct was seen. It is known that Negishi reactions on imidazoles are sensitive to steric bulk at the N-1 position.[178] Therefore, imidazole **73** was thought to be too sterically hindered, to be a good substrate for the Negishi reaction. The trityl protecting group on **73** gives substantial steric shielding to C-2, thereby interfering with the reductive elimination. PMB and Boc[179] were investigated as different protecting groups of imidazole **73** to drive the equilibrium to reductive elimination product **204**.

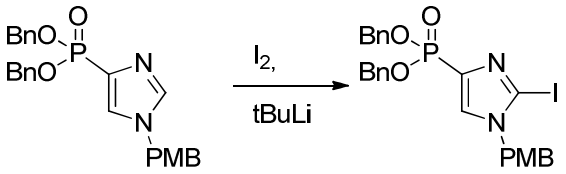
The Boc group was introduced by removing the trityl group from **73** using acetic acid and trifluoroethanol (scheme 44). The unprotected imidazole **209** was not isolated but immediately used in the next reaction. **209** was dissolved in DCM in the presence of DIPEA and Boc anhydride was added. **210** was obtained in 57 percent over two steps.



73 **209** **210**

Scheme 44: Synthesis of Boc protected 2-iodoimidazole **73**. Reagents and conditions: a) acetic acid (82 equiv), trifluoroethanol (3.1 equiv), rt, 5 h. b) Boc anhydride (1 equiv), DIPEA (1.1 equiv), DCM, 0°C to rt, 16 h, 57% over two steps.

The PMB protected 2-iodoimidazole was synthesized by deprotonation of **211** using tert-butyl lithium and quenching the resulting metalate with iodine. A large amount of 5-iodoimidazole and 2,5-iodoimidazole byproducts were recovered from the reaction, but could be separated from the desired product **212** by column chromatography. The large amount of 5-iodonation suggests a strong ortho-effect of the phosphonate, where it would stabilize the metalate on the C-5 position by coordination of the lithium cation. The large amount of side products did result in a low yield of 16 percent of **212**.

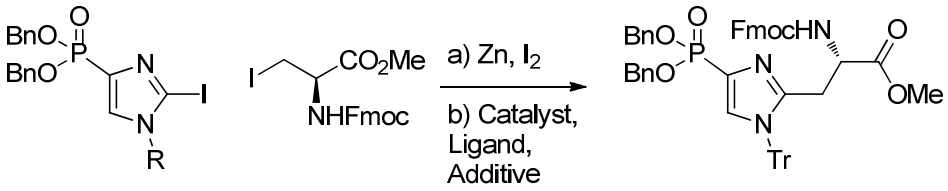


211 **212**

Scheme 45: Synthesis of PMB protected 2-iodoimidazole **212**. Reagents and conditions: Iodine (1.5 equiv), tert-butyl lithium 1.5M in pentane (1.2 equiv), THF, -78°C, 1 h, 16%.

Attempts to obtain the coupling products of these two imidazoles using the Negishi reaction are summarized in table 37.

Table 37: Negishi reactions III.



212 R = PMB, **210** R = Boc **74** **213** R = PMB, **214** R = Boc

Reagents and Conditions: a) **74** (2.5 equiv), iodine (0.03 equiv), zinc (8.2 equiv), DMF or NMP, 300°C to rt. b) catalyst, ligand, LiBr (5 equiv), 40°C, 16 h.

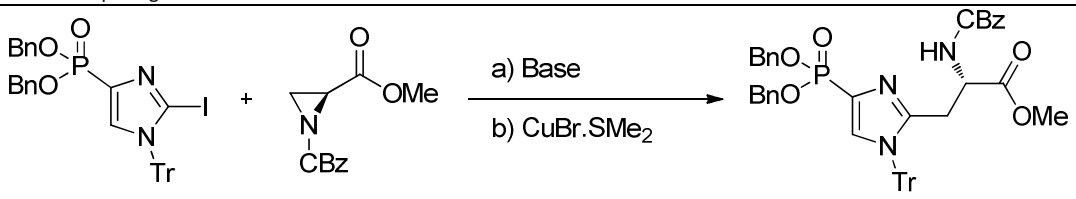
#	R	Catalyst (equiv)	Ligand (equiv)	Solvent	Result
1	PMB	$Pd(OAc)_2$ (0.1)	X-Phos (0.2)	DMF	Beta elimination
2	PMB	$Pd(OAc)_2$ (0.1)	S-Phos (0.2)	DMF	Beta elimination
3	PMB	$Pd_2(dba)_3$ (0.05)	dppf (0.1)	DMF	Beta elimination
4	PMB	$Pd_2(dba)_3$ (0.05)	Xant-phos (0.1)	DMF	Beta elimination
5	PMB	PEPPSI	—	THF: NMP 1:1	Beta elimination
6	Boc	$Pd(OAc)_2$ (0.1)	S-Phos (0.2)	DMF	Beta elimination

Unfortunately, all conditions yielded only beta eliminated products. Due to the continued failure of the Negishi this strategy was abandoned at this point.

8.4.3 Aziridine

As an alternative method to introduce the amino acid part to 2-iodoimidazole **73** a reaction with aziridine **87** was attempted. **87** was made according to literature procedure[180] and submitted to the metalate of **73** (table 37).

Table 38: Aziridine opening reactions.



73 **87** **215**

Reagents and conditions: a) base, THF, -30°C to -20°C, 1h. b) **87**, $CuBr.SMe_2$, -30°C to rt, 16 h.

#	Equivalents **73**	Base (equiv)	Equivalents $CuBr.SMe_2$	Result
1	3	iPrMgCl.LiCl (3.4)	0.3	Decomposition
2	1.2	iPrMgCl.LiCl (1.4)	1.4	Decomposition
3	1.2	n-BuLi (1.4)	1.4	Decomposition

Regrettably, all the attempts led only to decomposition of the starting material. Initially, isopropyl magnesium chloride lithium chloride complex was used as base, but no product was obtained (entries 1 and 2). Reactions with butyl lithium as base also led to complete decomposition (entry 3). At this point the route in chapter 8.2 was successful in delivering the final target compound **65**, thus this route was not pursued any further.

8.4.4 Follow-up studies

Later, Martijn Eerland, a colleague, discovered that the iodination of the 1-trityl-5-phosphonic acid imidazole was highly favored towards the C-5 position. The ortho-effect of the phosphonate was much stronger than expected. Even with a bulky trityl group on N-1, C-5 iodonation was highly favored. It could only be circumvented by performing the metalation with tert-butyl lithium at -100°C and immediate quenching of the metalate with an excess of iodine as soon as addition of base was complete. The 2- and 5-iodo-1-tritylimidazole isomers have extremely similar spectroscopic properties, as can be seen from the ^{1}H-NMR spectra in figure 55 (^{31}P-NMR shifts 11.43 and 11.75 ppm respectively). Another difference can be seen in the proton NMR spectrum, where the benzyl protons of the 5-iodo imidazole have a more complicated splitting pattern, due to the restricted rotation of the benzyls by the iodine.

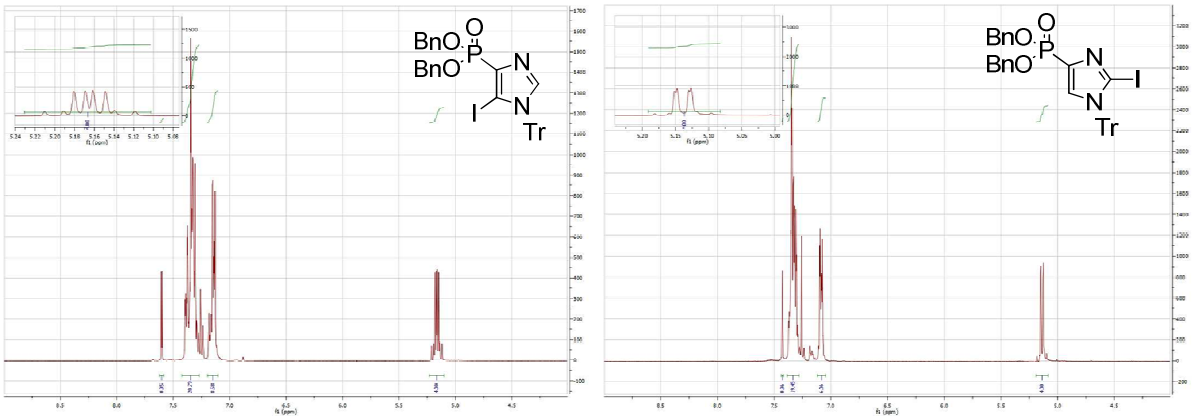


Figure 55: ^{1}H-NMR spectra of 5-iodo-**73** and 2-iodo-**73**.

This explained the failure of the Negishi- and halogen metal exchange reactions previously described. The Negishi reaction was tested again with the correct 2-iodoimidazole and the reaction went to completion, albeit delivering a low yield of the final product.[3]

[3] unpublished results, manuscript in preparation.

9 Peptides and antibodies

As the final target of the project is to obtain tools to deepen the understanding of the role of phosphohistidines, the 3-pHis analog building block will have to be incorporated in a peptide to raise antibodies.

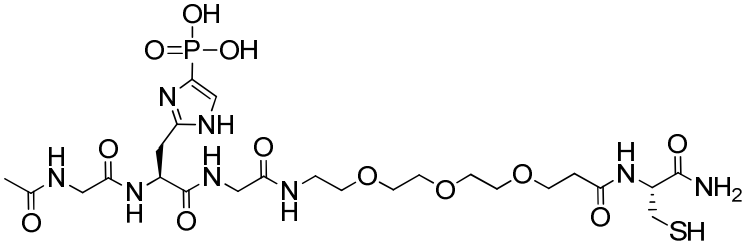
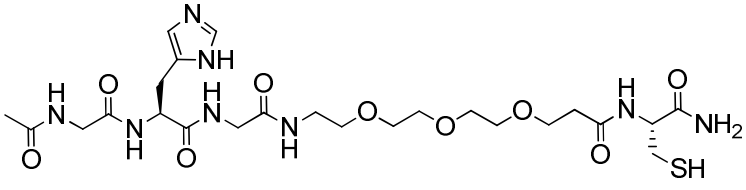
Using the building block described in chapter 7 several peptides were synthesized. These peptides were based on peptide sequences found in proteins that are known to phosphorylate on histidine (chapter 5.2). The peptides were to be used as antigens for antibody generation; therefore the peptides had to be prepared as C-terminal amides with an acetylated N-terminus. These end groups make sure that the peptide resembles the ionization state of a regular protein, increasing the chance for successful antibody generation (chapter 5.5). All peptides had a C-terminal cysteine for ligation purposes. The free thiol of the cysteine is an excellent handle for selective modification. It can be used for coupling to KLH with a bifunctional maleamide linker. In addition the thiol can be used to immobilize the peptides on sepharose, which provides the material to purify the respective antibodies.

The peptides were made by standard Fmoc based SPPS using HBTU as coupling reagent and HOBt as the activator (chapter 5.6). The peptides were synthesized on Tentagel resin functionalized with a RAM-anchored Fmoc-(Trt)-cysteine-amide. The initial amino acids were coupled on an automated peptide synthesizer using 10 equivalents of amino acid relative to the amount of peptide. The precious pHis building block was coupled manually, using HATU/HOAt as coupling reagents. Two equivalents of pHis mimic were used and the reaction was performed at high concentration, using just enough solvent to dissolve the reactants and wet the resin completely. The remaining parts of the sequences were completed on the automated peptide synthesizer.

Following the final Fmoc deprotection and acetylation of the N-terminus; the cleavage from the resin and concomitant global deprotection was achieved using a mixture of TFA, TIPS, EDT and water. After evaporation of the cleavage mixture the residue was triturated with diethyl ether, dissolved in water and lyophilized. The resulting fluffy white solid was further purified using prep-HPLC.

Table 39 includes the peptides that were synthesized. Each peptide was synthesized in two versions, either containing normal histidine (H) or the pHis mimic (H*). Entries 1-5 are the peptides containing the mimic, entries 6-10 are their equivalents that contain a normal histidine.

Table 39: peptides synthesized using the pHis mimic and normal histidine.

#	Peptide sequence	Yield (%)
1	Ac-GAKRH*RKVLRGC-NH_2 (**216**)	37
2	Ac-VQFGH*AGAGC-NH_2 (**217**)	53
3	Ac-ADQELMTYSH*DNIIC-NH_2 (**218**)	1.8
4	Ac-FRQVLKH*RKLREQGC-NH_2 (**219**)	50
5	 (**220**)	42
6	Ac-GAKRHRKVLRGC-NH_2 (**221**)	70
7	Ac-VQFGHAGAGC-NH_2 (**222**)	78
8	Ac-ADQELMTYSHDNIIC-NH_2 (**223**)	9.8
9	Ac-FRQVLKHRKLREQGC-NH_2 (**224**)	55
10	 (**225**)	34

As previously mentioned, the peptide sequences were based on proteins that are known to be phosphorylated on histidine. The sequence length varied between 10 and 15 amino acids, which is in the optimal range to raise antibodies. The sequences were based on histone H4 (entries 1 and 6), rat ATP-citrate lysase (entries 2 and 7), G-protein β-1 subunit sequence (entries 3 and 8), and KCa3.1 ion channel protein (entries 4 and 9). In addition, two probes were designed containing a His or pHis analog with a PEG linker (entries 5 and 10). It was thought that this peptide could be used to raise a more general antibody that would recognize diverse sequences containing pHis, opposed to the other peptide sequences, which were expected to be more selective towards specific proteins.

Isolated yields for most peptides were moderate to good. The only exceptions are both G-protein β-1 subunit sequences (entries 3 and 8), which yielded only 1.8 and 9.8 percent respectively. The reason for this low yield is the insolubility of this peptide. During sample preparation for prep-HPLC it was noticed that the peptide was poorly soluble in most combinations of acetonitrile and water. After purification by prep-HPLC and lyophilization, only a small amount could be recovered.

Peptides **216** and **220** were used to raise antibodies, by the group of Dr. Robert Schneider, Max Planck Institute of Immunobiology and Epigenetics, Freiburg. They also performed all antibody purifications and blot tests. The antigen peptides were conjugated to Keyhole Limper Hemocyanin (KLH) carrier protein using the bifunctional linker N-hydroxysuccinimidyl-3-maleimidobenzoate (MBS). The conjugate was injected into two rabbits with Freund's complete adjuvant. Over the next eight weeks, five booster immunizations were carried out. After final bleeding and serum isolation the antibodies were purified by selection over peptide **216** or **220** immobilized on sepharose, followed by depletion over peptide **221** or

225 respectively. This method resulted in about 6 mg of monoselective poly-clonal antibody from each animal.

The antibodies were then tested for their affinity for the natural pHis containing peptides (figure 56). Peptide **221** was phosphorylated using potassium phosphoramidate and dot blots were performed to assess the affinity of the antibodies for the natural pHis containing peptides. The dot blots for anti-**216** antibodies are shown in figure 56. The blots clearly show that there is no backbone recognition, as non-phosphorylated peptide **221** was not stained by the antibody. The phosphorylated peptide (**221**-P) on the other hand was stained even at very low concentration (0.19 pmol).

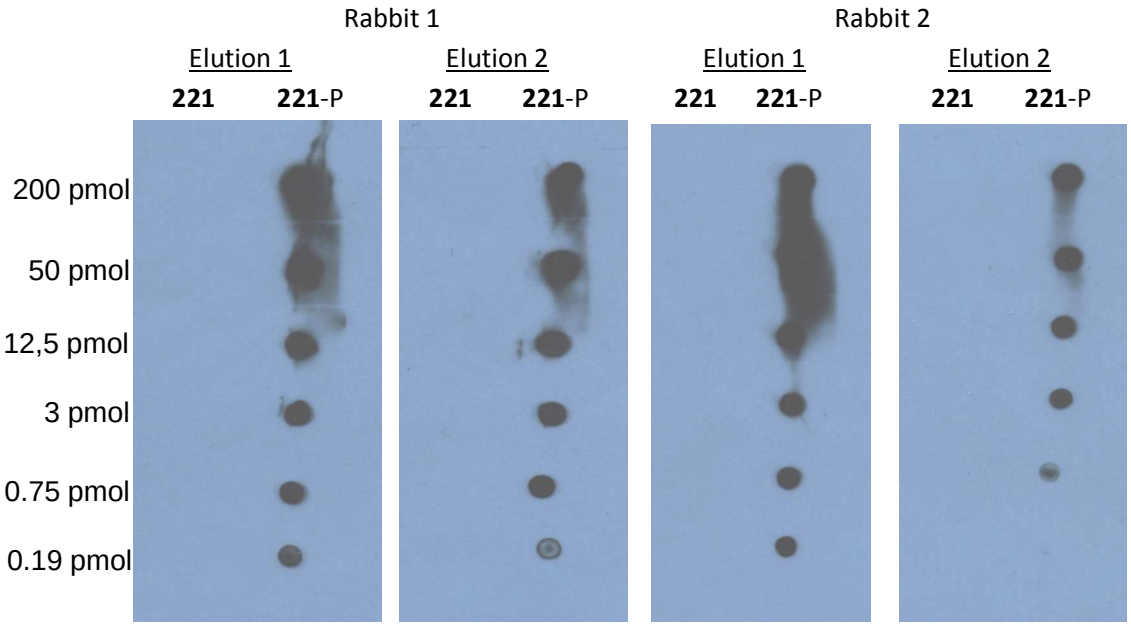


Figure 56: Dot blots of anti-**216** antibodies against peptide **221** and **221** containing a phosphorylated histidine (**221**-P), blots by the Schneider group.

Anti-**220** antibodies were tested in a similar manner; these antibodies should recognize only pHis and should require no other amino acids for recognition purposes. Thus, these antibodies were also tested against **221** and **221**-P. Additionally, they were also tested against peptide **216** containing the pHis mimic (figure 57).

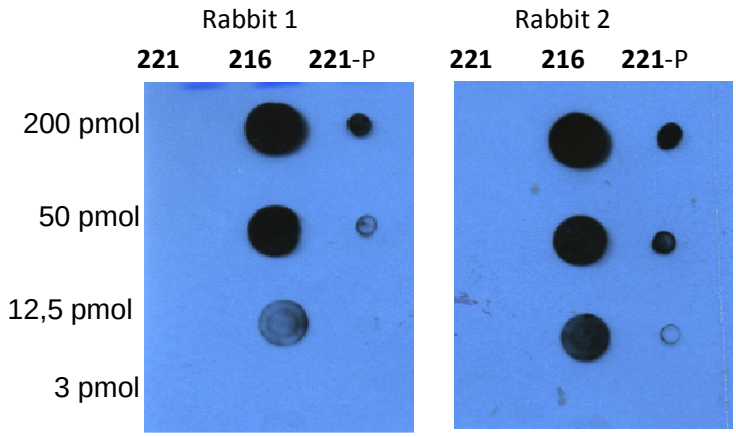


Figure 57: Dot blots of anti-**220** antibodies against peptide **221**, **221** containing a phosphorylated histidine (**221**-P) and **216,** blots by the Schneider group.

Again the blots clearly show that the peptide back bone is not recognized by the anti-**220** antibodies as peptide **221** is not stained at any concentration. Phosphorylated **221** is stained, however the detection limit seems to be around 3 pmol, much higher than for the anti-**216** antibodies. The anti-**220** antibodies also recognize peptide **216** containing the pHis mimic.

The antibodies were then used to investigate H4 histone histidine phosphorylation. To this end mouse hepatocytes were fractionated and analyzed by western blot using the anti-**216** antibodies. The results are shown in figure 58.

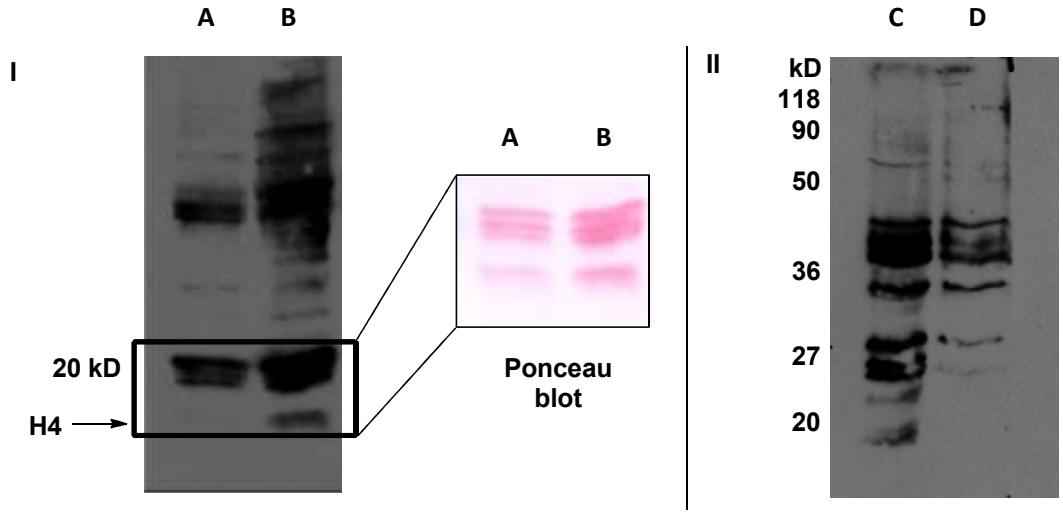


Figure 58: Western blots of (**I**) mouse hepatocyte nuclear protein fractions. **A**: Soluble chromatin fraction. **B**: Pellet/Insoluble chromatin fraction. (**II**) Fractionated mouse hepatocytes. **C**: Nuclear extract. **D**: Cytoplasm. Blots by the Schneider group.

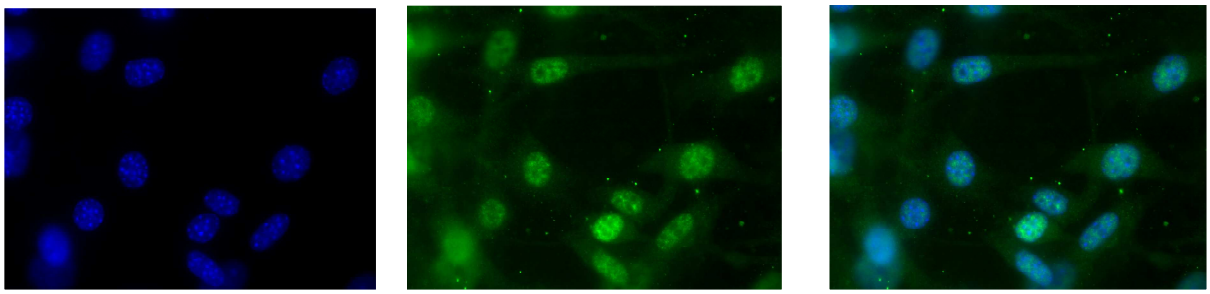
The first blot (figure 58: **I**) shows two mouse liver nuclear protein fractions: the soluble chromatin fraction (**A**) and the pellet or insoluble chromatin fraction (**B**). The Ponceau blot is used as a loading control. When comparing the loading control and the western blot one can see that the band for the H4 histone is detected in fraction **B** but not in fraction **A**. This indicates that the insoluble histone fraction contains H4 histone proteins that are phosphorylated on histidine, while the H4 proteins of the soluble

fraction are not. Since the insoluble chromatin fraction contains a high concentration of DNA that is tightly wound around the histones, this result suggests that phosphohistidine has a function in the packing of DNA.

A second striking result from both western blots (figure 58: **I** and **II**) is the detection of many proteins other than the H4 histone. This might be caused by cross-reactivity of the anti-**216** antibodies with other phosphoamino acids. As mentioned in chapter 7, the antibodies produced by the Muir group showed cross reactivity with phosphotyrosine and to a lesser extent phosphothreonine and phosphoserine. The anti-**216** antibodies might also recognize these phosphoamino acids to some extent. This question can be easily answered by experiments using versions of the histone H4 peptide **221** with the histidine exchanged for a phosphotyrosine, phosphothreonine or phosphoserine. Since the anti-**216** antibodies do not recognize the backbone of **221** any recognition of the modified peptide indicates cross-reactivity with that particular phosphoamino acid.

Other possibilty are the recognition of the phosphates of protein-DNA complexes or, more intriguingly, the detection of polybasic stretches. Preliminary experiments, performed in the groups of Prof. Bastiaens and Dr. Hedberg suggest that a large number of these polybasic stretches containing a histidine are encoded in the human genome. The occurrence of these polybasic stretches seems to be highest among membrane proteins. More experiments will be necessary to investigate this further.

To investigate in which location in the cell proteins that are recognized by the anti-**216** antibodies reside mouse hepatocytes were fixed and then stained (figure 59). The cells were first stained with DAPI, a dye that colors the cell nucleus (**A** – blue), the cells were then stained with the anti-**216** antibodies (**B** – green). As expected the overlay (**C**) makes clear that the anti-**216** antibodies stain the nucleus, with minor background in the rest of the cells.



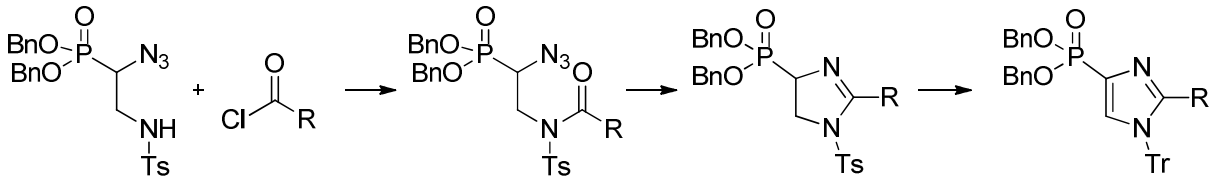
A **B** **C**

Figure 59: Immunofluorescent stained fixed mouse hepatocytes. **A**: DAPI stain. **B**: Anti-**216** antibody stain. **C**: Overlay.

Thus the pHis mimic that was designed for this project was successful in raising antibodies selective for phosphohistidine. The antibodies have a high affinity for peptides containing phosphohistidine. They have been successfully used to detect histone H4 in complex mixtures and in the future they can be used to investigate the function of histidine phosphorylation.

10 Toward potential PHPT1 inhibitors

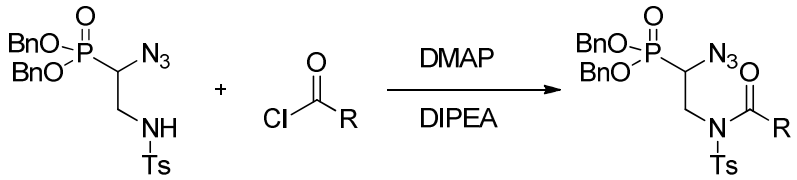
The aza-wittig approach was very successful in providing a large amount of the pHis analog that was needed for biological studies (chapter 8.2). To investigate the generality of the approach to synthesize a range of C-2 substituted 4-phophonic acid imidazoles a small library was synthesized, with the help of Momotaro Takeda. Such a library could provide a source of inhibitors for PHP (chapter 5.2.1.2). Inhibitors of this phosphatase could be used to study its function in vivo. To this end, it was planned to react azide **66** with a variety of acid chlorides, in the presence of DMAP, to yield the amide. The amide could then be cyclized to the imidazoline by aza-wittig reaction, followed by base induced aromatization. Finally, the imidazoles will be protected with a trityl group to aid in their purification and ensure stability during storage (figure 60).



66 **226** **227** **228** **229**

Figure 60: Strategy to obtain a small library of C-2 substituted 4-phophonic acid imidazoles

Practically, the coupling procedure is straightforward, the azide, DIPEA and DMAP were dissolved in DCM under air. Once the DMAP was completely dissolved the acyl chloride was added dropwise, which led to the immediate formation of a white precipitate. The mixture was stirred for an hour at room temperature to ensure the reaction went to completion and the reaction was submitted to an aqueous work-up. Final purification was done by flash column chromatography (scheme 46).



66 **226a-t** **227a-t**

Scheme 46: Reagents and conditions: **226a-t** (2 equiv), DMAP (1 equiv), DIPEA (2 equiv), DCM, rt, 1 h.

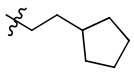
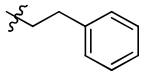
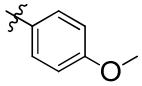
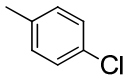
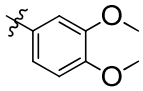
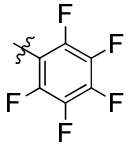
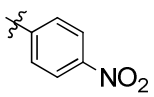
Most of the amide coupling reaction went well, with yields up to 94 percent (table 40). On average the benzoyl chlorides gave higher yields than the alkyl substituted acyl chlorides, likely due to the activating properties of the benzyl group (entries a-k vs. l-r). The alkyl substituted acyl chlorides seemed to be somewhat sensitive to steric bulk, as **227a** and **227b** formed in higher yield than **227c** and **227d**.

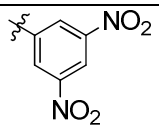
The low yield retrieved for compound **227e** (42 percent) was mainly related to purification issues. **227e** was inseparable by normal silica flash column chromatography from myristic acid, the hydrolysis product of the acyl chloride starting material. The similar behavior on column of these two compounds is most likely due to the long alkyl tail. Separation could be achieved when the crude mixture was dissolved in methanol and a large excess of sodium bicarbonate was added. After stirring this mixture

for two hours, some celite was added, the solvent was evaporated and the residue was loaded on a column. This ensured that the myristic acid was loaded on the column as a sodium salt, which had a much higher retention than **227e**.

Even though similarly long alkyl tails were present, purification of **227f** and **227g** was not as problematic. The yield of **227g** was only 65 percent, but 23 percent of unreacted starting material **66** was recovered. This was due to impurity of freshly prepared **226g**, still containing some of the corresponding alcohol. After optimizing the synthesis of the acyl chloride, **227f** was obtained in a good 86 percent yield.

Table 40: Synthesis of a small library of imidazoles.

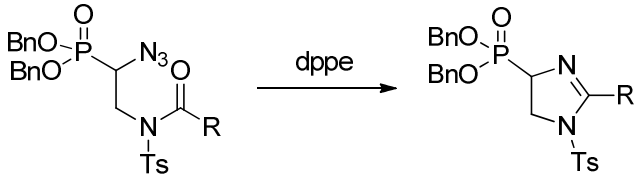
#	R	Yield **227** (%)	Yield **228** (%)	Yield **229** (%)[a]
a	Me	91[b]	91	54
b	Et	91	87	74
c		76	85	78
d		74	93	57
e	$CH_2(CH_2)_{11}CH_3$	42	41[c]	74
f	$CH_2(CH_2)_8C{\equiv}CH$	86[d]	100	72
g	$CH_2(CH_2)_{12}C{\equiv}CH$	65 (85)[e]	96	76
h		74	84	78
i		84	91	81
j		76	93	88
k		69	76	75
l	Ph	93	93	87
m		91	91	81
n		77	93	70
o		80	87	79
p		94	70	56
q		82	86	67

r		94	72	—[f]
s		—[g]	—	—
t		—[g]	—	—

[a]: over two steps.[b]: no DMAP was used.[c]: 25 percent **230** was recovered. [d]: 1.2 equivalent acyl chloride was used.[e]:>1 equivalents of acyl chloride was used. Yield based upon recovered starting material..[f]: total decomposition of the starting material.[g]: no reaction.

Finally, oxalyl chloride (**226s**) and diethylcarbamoyl chloride (**226t**) did not yield any of the corresponding amide. Oxalyl chloride was used in an attempt to make the double coupled product. Upon submission of **66** and **226s** the mono-coupling product likely did form but decomposed back to **66** with the release of carbon monoxide and carbon dioxide. Addition of the second equivalent of **66** was apparently to slow to yield the double coupled product even after longer reaction times. In the case of diethylcarbamoyl chloride it seems likely that no reaction ever occurred as the carbonyl was deactivated since it was part of an amine moiety.

Next, compounds **227a-r** were submitted to the aza-wittig reaction to obtain the imidazoline (scheme 47).



227a-r **228a-r**

Scheme 47: Reagents and conditions: dppe (1.5 equiv), THF, 80°C, 16 h.

The aza-wittig reaction was performed by dissolving compounds **227a-r** in THF, followed by addition of dppe. The mixture was then heated to 50°C until bubbling stopped, indicating that iminophosphorane formation was complete. The reaction vessel was then sealed and heated to 80°C for 16 hours to perform the aza-wittig cyclization. Afterwards, the reaction was cooled to around 50°C and a one-to-one mixture of 30% hydrogen peroxide in water and saturated aqueous sodium bicarbonate was added to ensure complete oxidation of the dppe. After 30 minutes the mixture was worked-up with chloroform and saturated aqueous sodium bicarbonate. Final purification was performed by filtering through a silica plug.

The aza-wittig reaction performed admirably, yielding over 80 percent **228a-r** in almost all cases (table 40). Notable is that **227p** and **227r**, bearing electron withdrawing substituents provided lower yields. Compound **228e** was recovered in only 41 percent yield. However, additionally to compound **228e**, compound **230** was recovered in 25 percent yield (figure 61). This compound likely formed during the work-up of the aza-wittig reaction. If the reaction had not gone to completion and there was still unreacted iminophosphorane left (figure 61: **A**), the iminophosphorane could have been quenched

during the aqueous work-up, yielding the amine. In this case, the tosylamide becomes an acyl donor as the amine attacks on the carbonyl (figure 61: **B**), resulting in compound **230**. Why there was still so much (25 percent) unreacted iminophosphorane after stirring for more than 16 hours at 80°C is unknown. None of the aza-wittig reaction using the other amides yielded side products like **230**. **228f** and **228g**, both containing long carbon chains, were recovered in excellent yields.

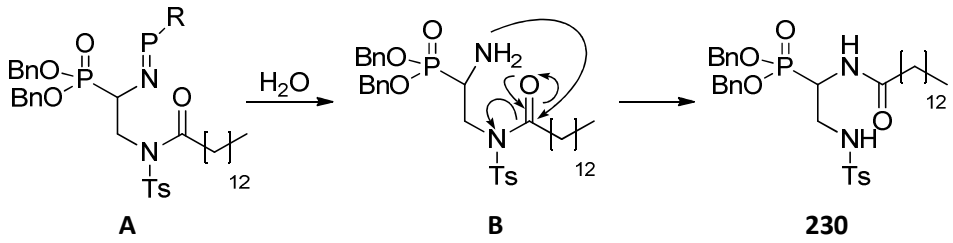
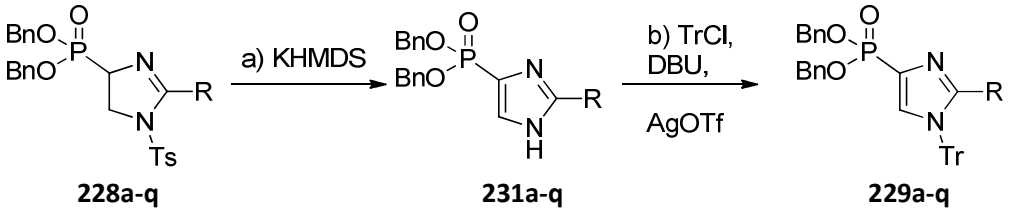


Figure 61: mechanism for the formation of compound **230**.

The base induced aromatization to provide the imidazole was performed using KHMDS (scheme 48, table 40). The imidazoline was dissolved in THF and cooled to -78°C, after which KHMDS was slowly added as a 0.5M solution in toluene. A color change from colorless or yellow to orange or red was observed during addition. Upon completion of addition, the reaction mixture was transferred by canula to a flask containing six equivalents of acetic acid in water to quench all reactive species (chapter 8.2.10). After aqueous work-up and evaporation, the unprotected imidazole was not isolated but immediately used in the next step. The described conditions smoothly converted all substrates, except for compound **228r**. Addition of KHMDS to **228r** made the reaction mixture turn black immediately and complete decomposition of the starting material was observed after work-up. Presumably, the KHMDS reduced one or more of the nitro groups, resulting in a complex mixture of products. With this in mind the aromatization of **228q**, bearing a single nitro-group, was performed with DBU in DMF instead of KHMDS. It was gratifying to see that the previously discovered fact, that the imidazolines with an electron withdrawing groups at C-2 were easy to deprotonate (compound **138**, chapter 8.2.9), held for compound **228n**.



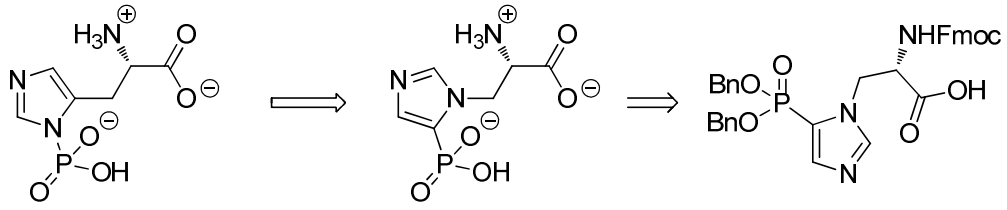
Scheme 48: Reagents and conditions: a) KHMDS (3 equiv), THF, -78°C, 15 min. b) TrCl (2 equiv), DBU (3 equiv), AgOTf (2.2 equiv), DCM, rt, 1 h.

Trityl protection, by stirring the crude unprotected imidazoles with trityl chloride in the presence of DBU and silver triflate, under air, was straightforward in all cases (scheme 48, table 40). All imidazoles were protected in reasonable to good yield, whereas imidazoles (**229p** and **229q**) were obtained in moderate yields. This was most likely due to their electron withdrawing properties, decreasing the nucleophilicity of the imidazole.

In conclusion, the presented synthetic strategy can be used to generate tritylprotected 4-phosphonic acid imidazoles with a wide variety of substituents on the C-2 position. Aromatization to the imidazoles has been realized in general with KHMDS. In addition, it has been shown that imidazolines with electron withdrawing groups at C-2 can be aromatized under mild conditions (DBU). Imidazoles **229f** and **229g** containing a long alkyl chain with a terminal alkyne could be used to fashion molecular probes, using 'click chemistry', also these two substituents performed well in the synthetic strategy presented here.[181] The potential inhibitors synthesized will be tested in a PHPT1 assay developed in the Hedberg group.[137]

11 Studies toward a 1-phosphohistidine analog

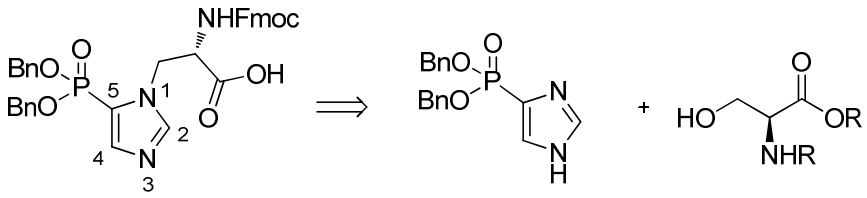
As described in the introduction, histidine can be phosphorylated at both nitrogens of the imidazole, providing either 3-pHis or 1-pHis (chapter 5.2). In chapter 7 the design and synthesis of the 3-pHis mimic have been described. In order to obtain a stable 1-pHis mimic a similar design was used. To preserve the electronic and steric properties of natural 1-pHis, the imidazole was conserved whereas the positions of the substituents were changed (figure 62).



6 **232** **233**

Figure 62: 1-pHis, the stable analogue **232** and the SPPS building block **233**.

Compound **232** was derived following these design principles. As shown in figure 62, this analog maintains the 1-2 relationship of the phosphate and amino acid in **6**, while the unstable phosphoramidate bond is exchanged for a stable phosphonate bond. In order to raise antibodies the stable pHis analog needs to be incorporated in a peptide (chapter 5.5). Therefore, SPPS building block **233** needed to be prepared. The protecting groups of choice are the same as for the 3-pHis analog: benzyl groups on the phosphonate and an Fmoc group on the amine. The retrosynthetic analysis towards building block **233** is shown in figure 63.



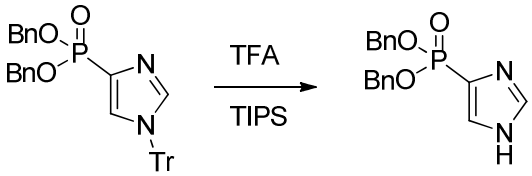
233 **234** **235**

Figure 63: Retrosynthetic analysis of compound **233**.

Compound **233** should be available by the coupling of compound **234** and an activated serine **235**. **234** can be prepared by deprotection of its trityl protected analog **167**, which has been successfully prepared before in order to obtain the 3-pHis analog (see chapter 8.4.2.1). The critical step of this synthesis would be the regio-selectivity of the serine addition. In general, a bulky group at C-5, the phosphonate in this case, leads to introduction of the alkyl on the opposite side of the ring, N-3. Thus, the steric bulk of the phosphonate is expected to interfere with the addition, favoring a 4-phosphonc acid imidazole instead of the desired 5-phosphonic acid imidazole. Several strategies, such as making use of directing groups or particular protective groups could be explored to obtain the desired regioisomers. The synthesis of **234** and the coupling to **235** are detailed below.

11.1 Unprotected imidazole

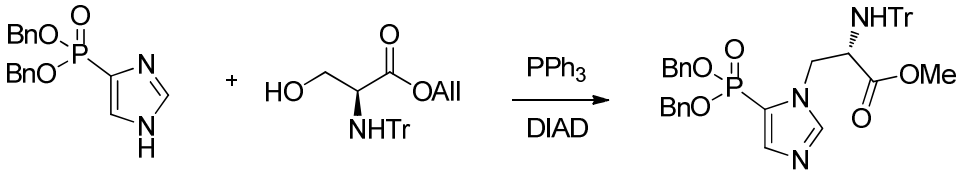
The synthesis of **234** involved the deprotection of imidazole **167** using TFA and TIPS. Compound **167** was synthesized as described in chapter 8.4.2.1. Compound **234** was submitted to an acid-base work-up and not purified further, as it was very polar and did not behave well during column chromatography.



167 **234**

Scheme 49: Synthesis of compound **234**. Reagents and conditions: TFA (1 equiv), TIPS (2 equiv), AcOH, rt, 20 h, 65%.

Introduction of the amino acid part was initially attempted by a Mitsunobu reaction between imidazole **234** and protected serine **236** (scheme 50).



234 **236** **237**

Scheme 50: Synthesis of compound **237**. Reagents and conditions: PPh_3 (1.18 equiv), DIAD (1.2 equiv), THF, 0°C to rt, 16 h.

It was hypothesized that the phosphonate group could serve as a directing group to obtain the desired regio-isomer. The Mitsunobu intermediate would coordinate to the phosphonate oxygen, thereby selecting for the right product (figure 64: **A**). The main problem with this strategy is the propensity of the activated serine to ring close to the aziridine under Mitsunobu conditions (figure 64: **B**). In that case selectivity for the unwanted regio-isomer of **233** would be expected during aziridine ring opening due to the steric bulk of the phosphonate.[182]

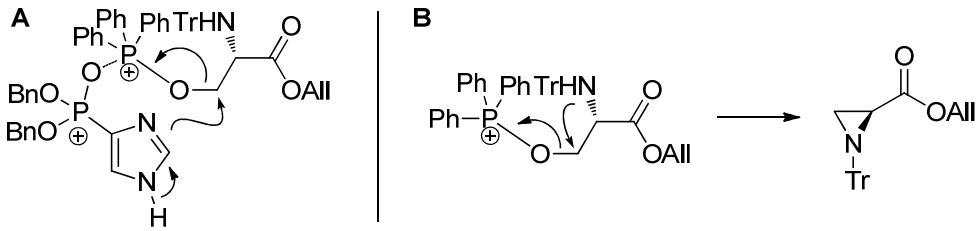
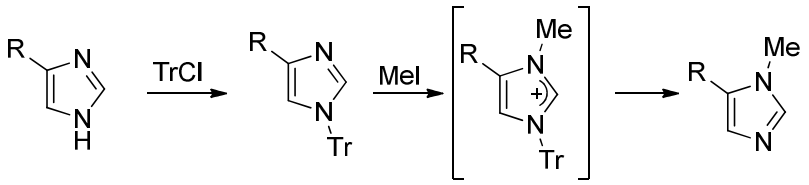


Figure 64: Mitsunobu considerations. **A**: Induction of selectivity due to coordination to the phosphonate. **B**: formation of the aziridine.

Compound **234** and **236** were submitted to Mitsunobu conditions (scheme 50). Only a 1:1 mixture of products was obtained and attempts to isolate these products using column chromatography failed. A 1:1 mixture of products is a much better ratio than one would expect if the reaction was governed by sterics alone. Therefore, it seemed that the induction of selectivity did work, but was insufficient to push the reaction to the desired regio isomer completely. Attempts to optimize the reaction did not change the ratio significantly, thus other strategies had to be considered.

11.2 Protected imidazole

In the literature there are examples of enforcing regioselectivity during imidazole substitution by smart use of leaving groups.[183] A well-known example is the introduction of a methyl group on N-3 of 4-substituted imidazoles. The natural selectivity of this reaction, like in our case, is for N-1. Therefore, N-1 is first trityl protected, after which the compound is subjected to methyliodide or another methylation reagent to introduce a methyl group on N-3. Because the trityl group is good at stabilizing a positive charge it will fall off during the reaction, sometimes with the help of some acid and/or silver salt, yielding the imidazole with the desired substitution pattern (figure 65).[184]

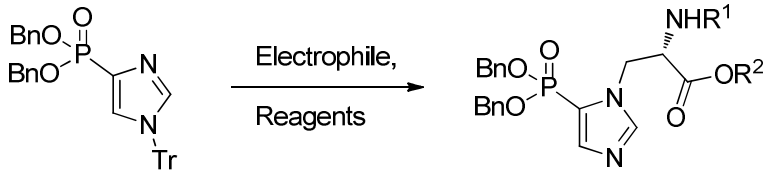


238 **239** **240** **241**

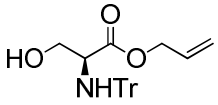
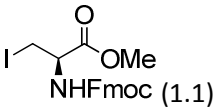
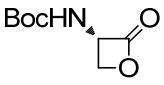
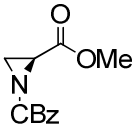
Figure 65: 5-substituted N-methyl imidazole synthesis.

In analogy to this strategy, trityl protected imidazole **167** was submitted to various reaction conditions in order to obtain compound **242**, as summarized in table 41.

Table 41: Substitution of **167**.



167 **242**

#	Electrophile (equiv)	Reagents (equiv)	Solvent	Result
1	 (1) **236**	PPh_3 (1.18), DIAD (1.2)	THF	No reaction
2	 (1.1) **243**	—	MeCN	No reaction
3	 (1.1) **244**	—	MeCN	No reaction
4	 (1.1) **245**	—	MeCN	No reaction

Unfortunately, in all cases only unreacted starting material was recovered. Neither a Mitsunobu reaction nor an iodide was successful in activating the hydroxy function (entries 1 and 2). Using β-lactone **244** or aziridine **245** also did not result in the formation of any product (entries 3 and 4).

A possible reason for the failure of these reactions might be the electron withdrawing properties of the phosphonate, which deactivate the imidazole. Additionally, there might also be a steric factor, the two benzyl protecting groups might shield the side of the molecule that should be substituted. Therefore, attempts were made to use 1-trityl-4-iodoimidazole **85** as a starting material. The rationale being that the iodine is weakly activating and much smaller than the phosphonate, allowing for substitution to the desired compound **249**. This compound might then be subjected to the same conditions as discussed in (chapter 8.4.2.1, scheme 42) to introduce the phosphonate. Thus, compound **85** was allowed to react with various serine derivatives, the results are summarized in table 42.

Table 42: Attempted substitution of **85**.

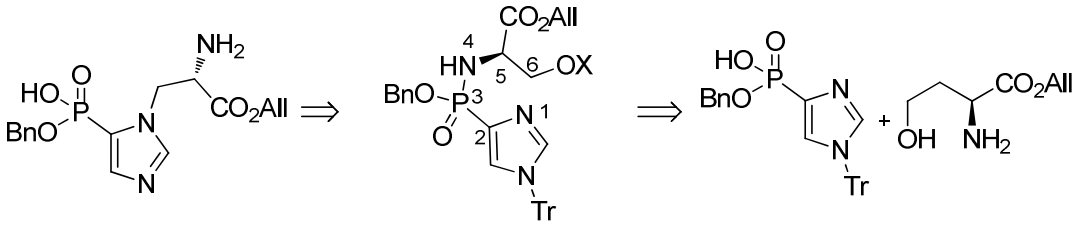
85 X = OH **246**, Br **247**, I **248** **249**

#	Serine derivative (equiv)	Reagents (equiv)	Solvent	Result
1	**246** (1)	PPh_3 (1.2), DIAD (1.2)	THF	Decomposition
2	**247** (1.2)	—	MeCN	No reaction
3	**248** (1.2)	—	MeCN	No reaction
4	**247** (1.1)	AgOTf (1.5)	MeCN	No reaction
5	**248** (1.1)	AgOTf (1.5)	MeCN	Decomposition

The 4-iodoimidazole was first submitted to Mitsunobu conditions using protected serine **246**. However, these conditions resulted in recovery of **85** and decomposition of **246** (entry 1). Attempts to react compound **85** with halo-serines **247** or **248** failed to show any conversion (entries 2 and 3). Adding silver triflate in order to exchange the bromine or iodine for a triflate did not result in any conversion in the case of **247** (entry 4). In the case of **248** the serine derivative decomposed and most of **85** was recovered (entry 5).

11.3 Intramolecular strategy

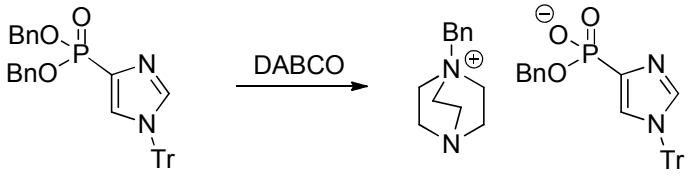
As a solution to the apparent reactivity problem as well as the regio selectivity issues, a modification to the above strategy was developed. It was hypothesized that the amino acid part could be connected to the phosphorus via a phosphoramidate bond (figure 66).This would position the amino acid side chain in such a way that reaction with the correct imidazole nitrogen would go over a six-membered transition state, which is favored over the seven-membered transition state for the other nitrogen. More importantly, the intramolecular nature of the reaction should increase the effective reactivity of the system, solving the reactivity problem of the previous attempts.



250 **251** **252** **253**

Figure 66: Strategy using the phosphoramidate tether.

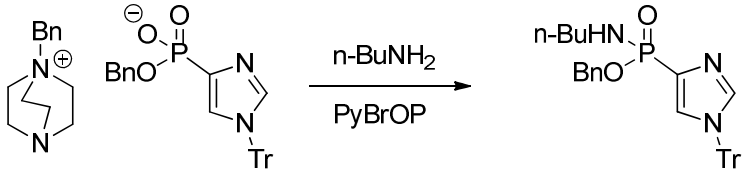
To obtain intermediate **251**, one benzyl has to be removed from **167**, while leaving all other protecting groups unaffected. This was accomplished by submitting **167** to DABCO in toluene at 80°C for 20 hours, yielding the mono-benzyl DABCO salt (scheme 51) in analogy to a procedure reported by Saady et al.[185]



167 **254**

Scheme 51: Synthesis of compound **254**. Reagents and conditions: DABCO (2 equiv), toluene, 80°C, 20 h, quantitative.

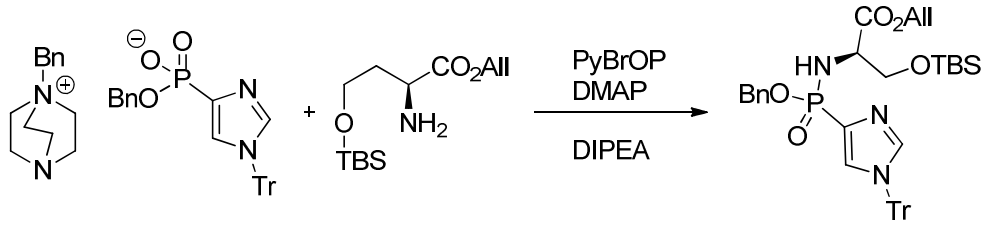
In order to investigate the initial P-N coupling, n-butyl amine was used instead of the amino acid (scheme 52). The mono-benzyl phosphonate was activated using PyBrOP and n-butyl amine was added. NMR and LCMS data clearly showed that there was conversion and suggested that the target molecule **255** was indeed the product. However, attempts to isolate the compound by column chromatography failed. Nevertheless, the reaction seemed to work, thus it was decided to proceed with the amino acid.



254 **255**

Scheme 52: Synthesis of compound **255**. Reagents and conditions: n-butyl amine (1.2 equiv), PyBrOP (1.2 equiv), DIPEA (2.4 equiv), DCM-d^2, rt, 20 h.

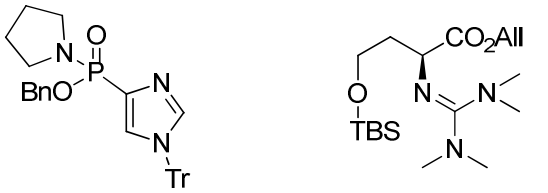
The side chain of the protected serine was protected as a tert-butyl dimethyl silyl (TBS) ether, to prevent cross reactivity. Even though similar conditions as for the reaction with n-butyl amine were used, no product **257** was observed (scheme 53). Fortunately, addition of a catalytic amount of DMAP pushed the reaction to completion. It proved impossible to purify the compound by conventional column chromatography. Fortunately, by using a reverse-phase C18 sep-pak 14 milligrams of **257** could be isolated, corresponding to a 48% yield.



254 **256** **257**

Scheme 53: Synthesis of compound **257**. Reagents and conditions: **256** (1.2 equiv), PyBrOP (1.2 equiv), DMAP (0.1 equiv), DIPEA (2.4 equiv), DCM, rt, 2 days, 48%.

The low yield was a source of worry, as gram amounts of the final compound would be needed for SPPS. Therefore, the byproducts of the reaction were further investigated. It was discovered that compound **258** was a major side product. Pyrrolidine analogue **258** forms due to pyrrolidine exchange between **254** and PyBrOP. Since no conditions were discovered that suppressed this behavior, some other activators were tested. The results are summarized in table 43.



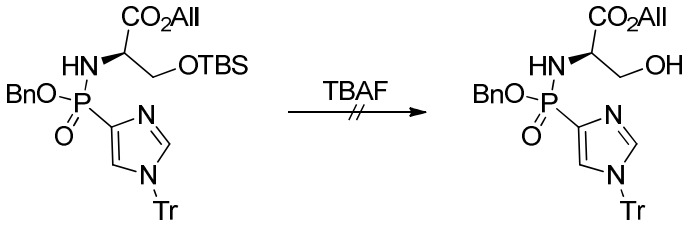
258 **259**

Initially, HATU and HOAt were investigated to activate the phosphonate, but **254** was not reactive under these conditions. Even though there was an initiation time of 5 minutes before the amino acid was added, compound **259** was the only new compound found in the reaction mixture (entry 1). Reducing the amount of HATU and HOAt suppressed the formation of **259** but did not yield any of the target product (entry 2). Attempts to make a chlorophosphonate using Vilsmeier conditions,[186] which could then be attacked by the amino acid **260** led to complete decomposition of the starting material (entry 3).

Table 43: different activating agents in the synthesis of compound **257**

#	Activator (equiv)	Base	Result
1	HATU (2.4), HOAt (2.4)	DIPEA (4.8)	No reaction
2	HATU (1.1), HOAt (1.1)	DIPEA (4.8)	No reaction
3	$(COCl)_2$ (1.4), DMF (0.2)	—	Decomposition

The small amount of **257** that was obtained using the PyBrOP method (Scheme 53) was used in an attempt to remove the TBS group. To this end **257** was dissolved in THF and TBAF was added and the mixture was stirred for two hours (Scheme 54). Unfortunately, a complex mixture of unidentified products was obtained, none of these products showed a mass consistent with compound **261** on LCMS.



257 **261**

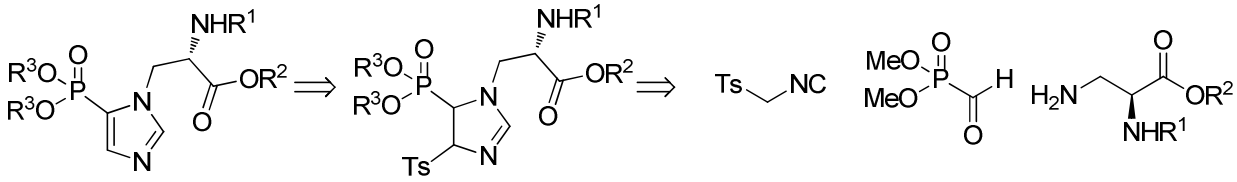
Scheme 54: Attempted synthesis of compound **261**. Reagents and conditions: TBAF (1.2 equiv), THF, rt, 2 h.

Since the synthesis of phosphoramidate **257** did not give the product in high yield and the subsequent deprotection did not work, it was decided to cease work on this route.

11.4 Outlook

Making use of the phosphonate as directing group resulted in the formation of the desired 1-pHis regioisomer as a 1:1 inseparable mixture with the undesired regioisomer. The protective group strategy, unfortunately, did not yield any 1-pHis. Therefore, other strategies will have to be considered. Many different strategies toward 1-pHis analogs could be envisioned, one is discussed below.

The van Leusen imidazole synthesis is a multicomponent reaction (MCR) that gives fast access to a variety of substituted imidazoles from simple starting materials (chapter 5.7.2.3). In this method the imidazoline is aromatized by elimination of the tosyl group, a method that also works without a electron withdrawing group on C-4 (in contrast to, for instance, $BrCCl_3$/DBU aromatization).[66] The imidazoline could be synthesized in one step from the multicompontent reaction between TosMIC (**263**), an aldehyde (**264**) and an amine (**265**), in the presence of base.

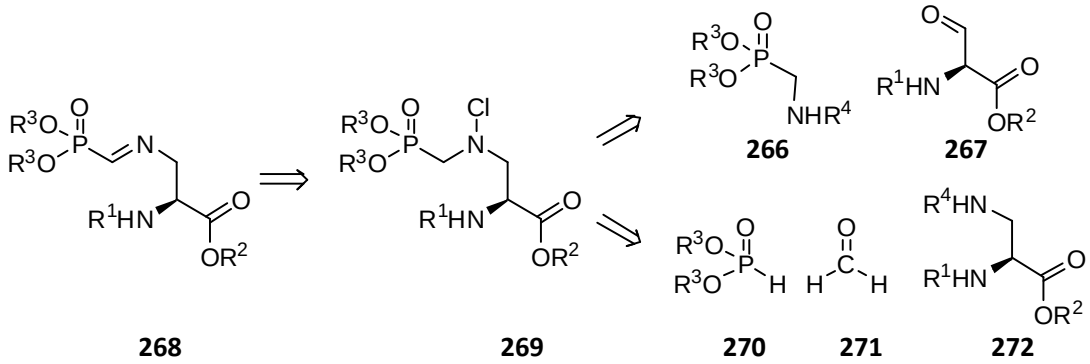


242 **262** **263** **264** **265**

Figure 67: Van Leusen strategy to synthesize compound **242**.

The protecting groups on the amino acid should likely be the Alloc and allyl ester groups, as they have served well in other chemistries discussed in this thesis. Unfortunately, the dibenzyl formylphosphonate is not a stable compound.[187] However, the dimethyl equivalent is stable and can be easily synthesized from dimethyl phosphonate and formic acetic anhydride.[188] Both methyl groups should be removable using TMS bromide,[189] after which they can be reprotected with benzyl groups. Unfortunately, initial experiments toward the synthesis of **264** and **265** did not show promising results. If these compounds cannot be made or if the reaction does not work due to issues with the imine formation, the imine **268** might be synthesized separately in advance.

This could be achieved by eliminating HCl from chloramine **269** using base. The chloramine itself should be available through either a reductive amination between an alpha amino phosphonate (**266**) and a protected 3-oxo-L-alanine (**267**).[190] Another possibility would be to use a Kabachnick-Fields reaction between a phosphite (**270**), formaldehyde (**271**) and a protected L-2,3-diaminopropionic acid (**272**).[191] The amine could then be chlorinated using tert-butyl hypochlorite.[192] This method also allows greater flexibility with the phosphonate protecting groups.



268 **269** **266** **267** **270** **271** **272**

Figure 68: Alternative access to the imine **268** for the van Leusen strategy.

12 Adenylylated histidine analog

Apart from phosphorylation, histidine can also undergo methylation or adenylylation.[193] As for the phosphohistidine, little is known about the biological function of adenylylated histidine. Since an intermediate in the 3-pHis synthesis could serve as a practical handle to prepare an adenylylated histidine analog, it was decided to look into the development of a histidine adenylylation toolset as well.

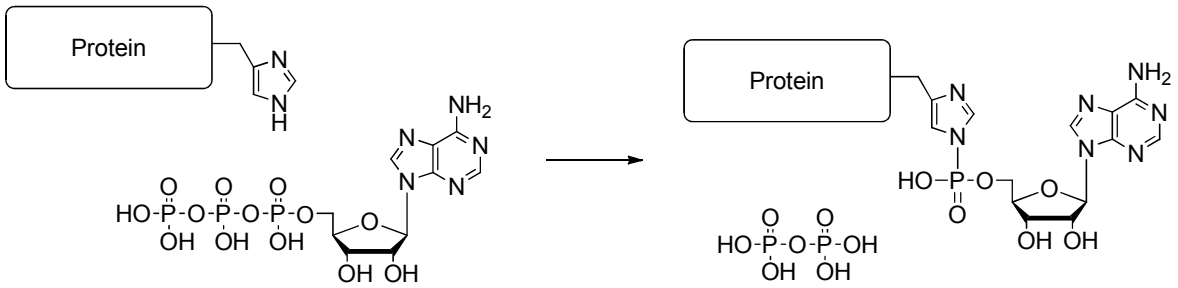


Figure 69: Adenylylation of a histidine residue on a protein using ATP.

It was hypothized that an intermediate in the 3-pHis analog synthesis could be modified to an adenylylated histidine analog in line with earlier work in our group.[5, 194] This compound would also be used in SPPS to yield antigen peptides for immunization. Therefore, it should contain, the now familiar, Fmoc group on the amine and preferably a benzyl on the phosphonate. The sugar moiety of the adenosine was protected with an acetal, as the more common ester protecting groups make the adenosine decompose under acidic conditions. The purine moiety was di-boc protected to deactivate the system further (figure 70).[5, 194]

It was thought that building block **274** could be obtained by a coupling of protected adenosine **275** (provided by Michael Albers)[194] and mono benzylphosphonate **273** (figure 70). **273** could be prepared by mono deprotection of compound **136**, an intermediate of the synthesis of the 3-pHis analog synthesis (chapter 8.2).

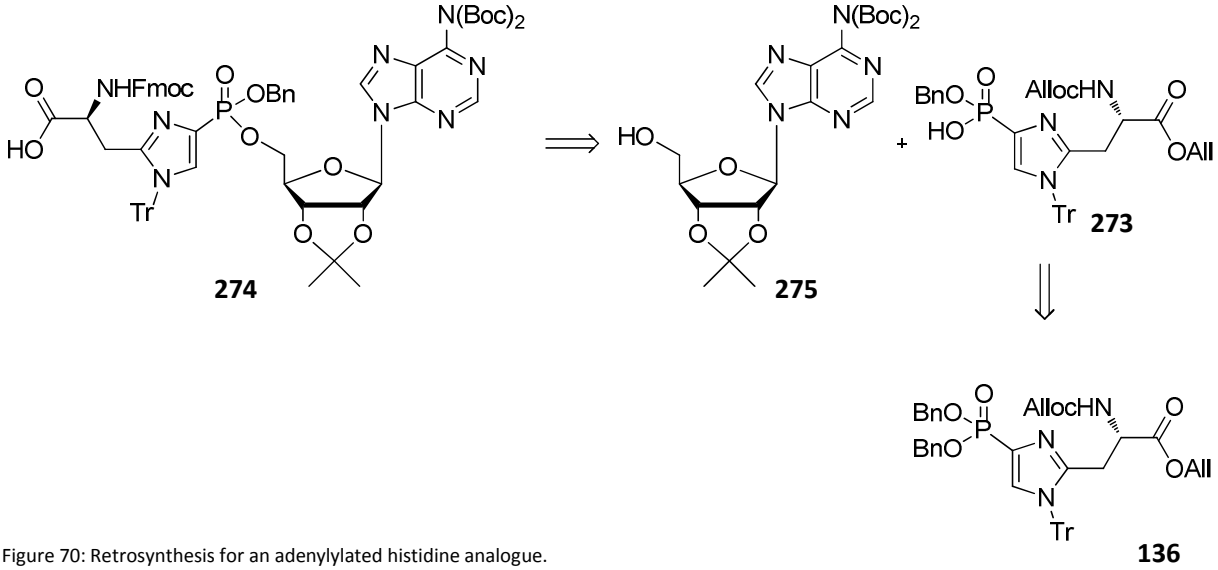
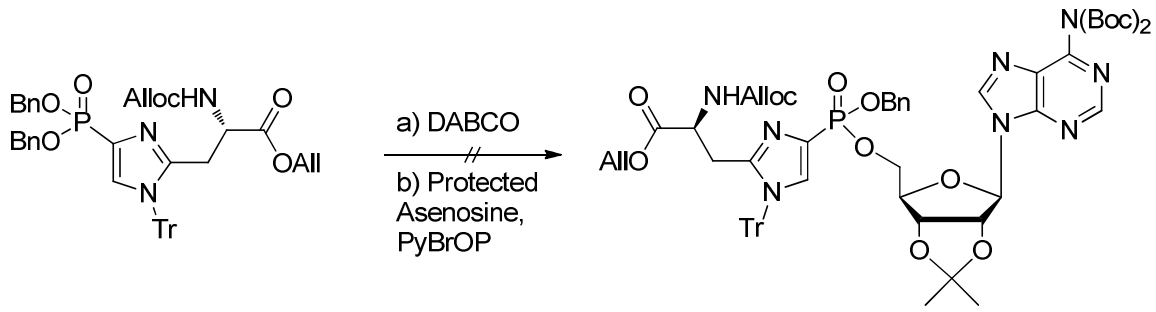


Figure 70: Retrosynthesis for an adenylylated histidine analogue.

Mono debenzylation of a phosphonate was already done with DABCO on compound **167** (chapter 11), and was also successful in this case. The intermediate mono-benzylphosphonate **273** was not isolated but adenosine **275** and PyBrOP were added to the crude reaction mixture. Conversion of the mono-benzylphosphonate **273** was observed, but no **276** was detected.

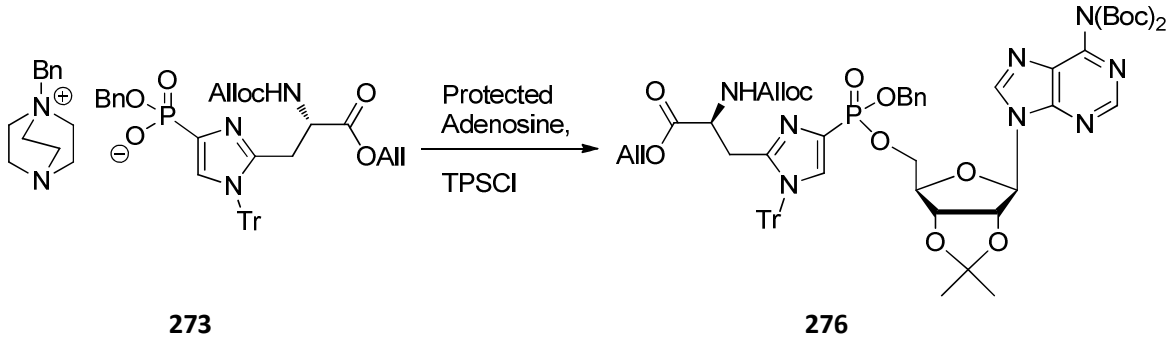


136 **276**

Scheme 55: Attempted synthesis of compound **276** using PyBrOP. Reagents and conditions: a) DABCO (2 equiv), toluene, 80°C, 16 h. b) Adenosine **275** (1.1 equiv), PyBrOP (1.1 equiv), rt, 6 h.

It was already known from studies on other mono-benzylphosphonates (chapter 11) that it was hard to substitute the phosphonate mono-ester. A particular problem for PyBrOP was the exchange of a pyrrolidine group from the PyBrOP phosphorus to the phosphonate. Therefore, a better activator was needed.

The combination of 2,4,6-Triisopropylbenzenesulfonyl chloride (TPSCl) and pyridine has been successful in coupling phosphonates and alcohols.[195] Thus, it was investigated for this coupling as well. Mono-benzyl phosphonate **273** and adenosine **275** were dissolved in pyridine and TPSCl was added. The mixture was stirred for five days, after which a compound of the correct mass for **276** was detected in the LCMS. Unfortunately, many side products were also present and attempts to isolate the target compound failed.



273 **276**

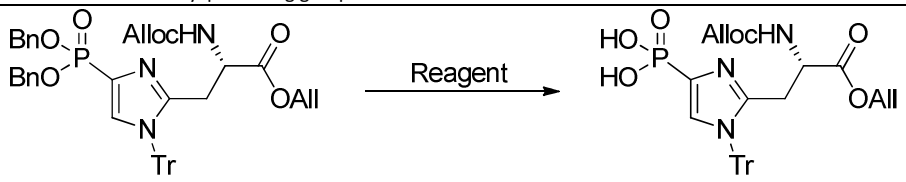
Scheme 56: Attempted synthesis of compound **276** using TPSCl. Reagents and conditions: a) Adenosine**275** (1.2 equiv), TPSCl (3 equiv), pyridine, rt, five days.

Since DABCO mono debenzylation worked quite well it was clear that the benzyl protecting groups were sensitive to nucleophiles. It was hypothesized that the pyridine solvent was responsible for the large amount of side products in the TPSCl promoted reaction. To test the hypothesis compound **136** was dissolved in pyridine and heated to 80°C for three days. LCMS clearly showed slow mono debenzylation

followed by decomposition of **273**. The reaction had not gone to completion after three days but it became clear that a reaction performed in pyridine was not an option.

As an alternative method to increase the reactivity of the phosphonate both benzyl groups might be removed. It was already known that one free hydroxy group on the phosphonate did not interfere with SPPS[194] and a doubly activated phosphonic acid should be more reactive than a mono activated one. Thus, it was attempted to remove both benzyl groups without removing any of the other protecting groups of **136**. Especially the trityl protecting group on the imidazole was a source of concern as it is quite acid labile. Several methods were tested, they are summarized in table 44.

Table 44: Attempts to remove both benzyl protecting groups.



136 **277**

#	Reagent (equiv)	Solvent	Temperature (°C)	Result
1	PhSH (3), KOtBu (3)	DMF	40	Deallylation
2	PhSH (6), KOtBu (6)	DMF	80	Deallylation
3	TMSBr (2.1)	DCM	rt	**273**:**277** 1:1[a]
4	TMSBr (3)	DCM	rt	**277**: **277**-Tr 1:1[a]

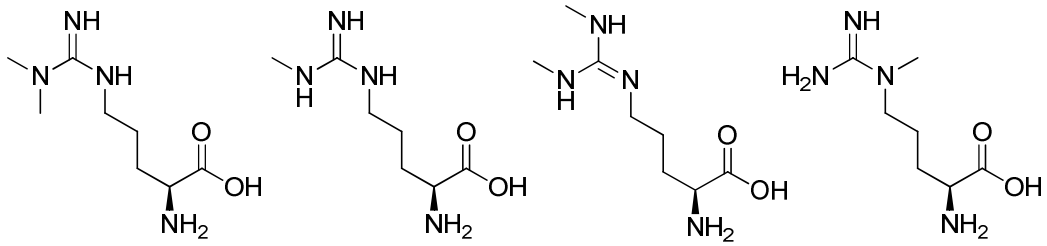
[a]: no yield was determined, ratios determined by ^{1}H and/or ^{31}P NMR.

Initially thiophenol and potassium tert-butoxide were tested.[196] At 40°C fast mono-benzyl removal was seen however the resulting compound **273** was slowly deallylated to the free acid over several hours (entry 1). Increasing the temperature to 80°C speeded up the reaction to yield fully deallylated product in one hour (entry 2). The second benzyl group was not removed during these tests. Secondly trimethylsilyl bromide (TMSBr) in DCM was investigated, after five hours a mixture of mono benzyl compound **273** and free phosphonic acid **277** were detected (entry 3). Repeating the reaction with slightly more TMSBr pushed the reaction to completion but also resulted in a significant amount of free phosphonic acid with the trityl group removed (entry 4). NMR analysis of the crude product also indicated that the phosphonic acid contained two TMS groups. Explorative reactions suggested that these TMS groups could easily be removed using KF in methanol.

At this point work on this project had to be halted due to a shift in priorities. Future work on this project might include fine-tuning of the TMSBr debenzylation reaction to yield the desired product **277** exclusively, perhaps through the use of a suitable buffer system. The free phosphonic acid could then be activated using TPSCl, using 2,6-lutidine instead of pyridine might stop the decomposition problems seen with that base. After coupling of the adenosine and **277** the protecting group manipulations to obtain the SPPS building block should be straightforward.

13 N^{δ}-methylarginine SPPS building block synthesis

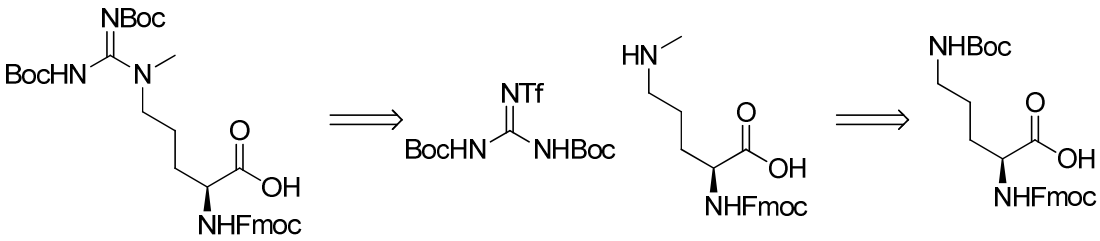
Arginine methylation is an essential posttranslational modification that plays a role in many physiological processes. There are several forms of methylated arginine, the most prevalent is asymmetric dimethyl arginine **278**, where both methyls are located on the same nitrogen. Less frequent are mono-methylated arginine **279** and symmetric dimethylated arginine **280**. There was interest to discover if monomethylated arginine **281** was also one of the methyl arginine homologues, to aid in the investigation antibodies specific for proteins containing **281** had to be raised.[197]



278 **279** **280** **281**

Figure 71: Different homologs of methyl arginine.

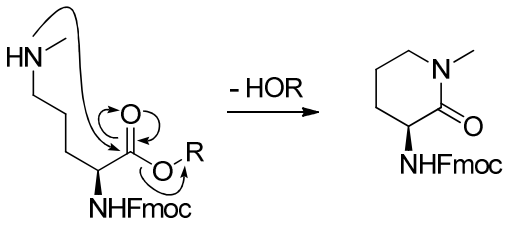
In order to raise these antibodies, peptides containing N^{δ}-methylarginine needed to be synthesized. Thus a SPPS building block had to be synthesized based on **281**. The SPPS building block should have the guanidine moiety protected, preferably with a Pbf or with two boc groups. Naturally, Fmoc protection is necessary for the amine so the building block can be used in Fmoc based strategies. A retrosynthetic analysis of the target building block is shown in Figure 72.



282 **283** **284** **285**

Figure 72: retrosynthesis of protected N^{δ}-methyl arginine **282.**

At first glance, synthesis of the N^{δ}-methylarginine building block **282** seems straightforward. However, there are some difficulties to be expected. Protection of the acid moiety as an ester could lead to formation of **287** via the attack of the side chain amine on the carbonyl, due to its activation by the methyl group (figure 73).



286 **287**

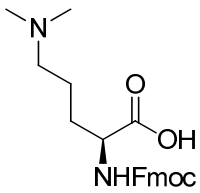
Figure 73: Cyclization of **286**.

Two possibilities to prevent the formation of **287** were envisioned: to leave the acid unprotected (1) or to reduce the acid to the alcohol first and re-oxidize to the acid as the last step (2). Option 1 was investigated first since less synthetic steps were required.

13.1 The free acid approach

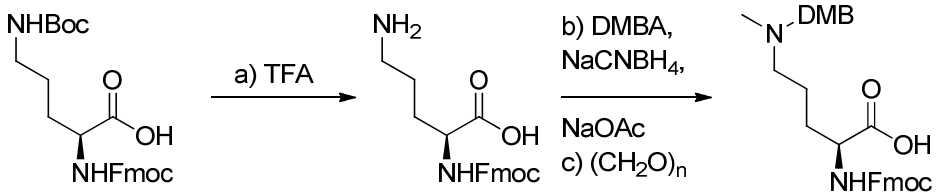
Thus, final compound **282** was envisioned to be synthesized by reaction of **283**[198] and Fmoc protected methyl ornithine **284**.[199] This compound should be available by two subsequent reductive aminations of FmocN-Orn-OH, first using 2,4-dimethoxybenzyl aldehyde (DMBA), then using formaldehyde. The initial DMB protection is to prevent di-addition of the formaldehyde; the DMB group will then be removed using acidic or oxidative conditions. FmocN-Orn-OH can easily be made from commercially available **285** in one step.

Thus, the Boc group was removed from FmocN-Orn(Boc)-OH (**285**) with TFA: DCM 1:1, giving the TFA salt in quantitative yield (scheme 2). The resulting amine was subjected to reductive amination conditions using sodium cyanoborohydride and 2,4-dimethoxybenzyl aldehyde in methanol. After one hour, para-formaldehyde was added with another portion of sodium cyanoborohydride and the mixture was aged overnight.



288

Initially, up to 50 percent dimethyl adduct **288** was recovered from the reductive amination reactions. Presumably, this was caused by the reduction of 2,4-dimethoxybenzyl aldehyde by sodium cyanoborohydride. The problem was solved by slow addition of reductor, giving **290** in 66 percent yield (scheme 57). Sodium triacetoxyborohydride and 30 percent formaldehyde in water were also tested but these reaction conditions were unsuccessful.

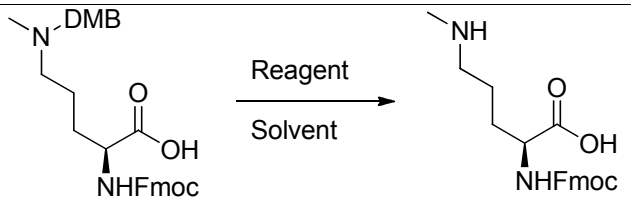


285 **289** **290**

Scheme 57: Synthesis of **290**. Reagents and Conditions: a) TFA: DCM 1:1, rt, 5 h, quant. b) 2,4-dimethoxybenzaldehyde (1.1 equiv), sodium cyanoborohydride (2 equiv), sodium acetate (2.1 equiv), 3A molsieve, MeOH, rt, 1h. c) para-formaldehyde (4 equiv), sodium cyanoborohydride (2 equiv), rt, 16 h, 66% over two steps.

Next, conditions were screened to remove the DMB protective group (Table 2). Initial DMB group removal with TFA did not result in any conversion. The DMB group proved to be quite stable to acidic conditions (entries 1 and 2). Therefore, oxidating conditions were investigated. DDQ was not a strong enough oxidizer to oxidize the DMB group (entry 3). However, CAN in 1,4-dioxane and water did produce **284** after a long reaction time and with a significant amount of unidentified byproducts (entry 4). A solvent switch to acetonitrile and water produced compound **284** at 81 percent yield in only an hour (entry 5).

Table 45: DMB deprotection.



290 **284**

Reagents and conditions: Reagent, solvent, rt, 16 h.

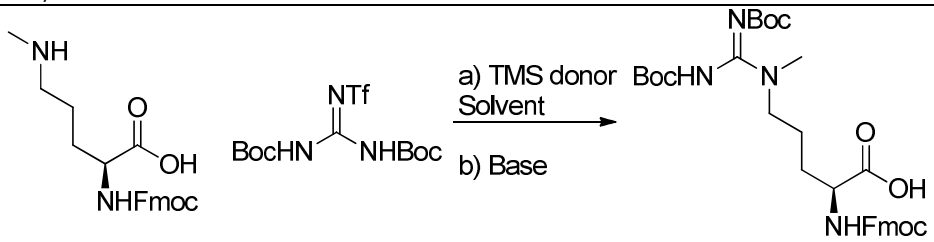
#	Reagent (equiv)	Solvent	Result
1	TFA (excess)	DCM	No reaction
2	TFA (excess)	Water	No reaction
3[a]	DDQ (1.5)	DCM	No reaction
4	CAN (3)	1,4-dioxane: water 9:1	Full conversion
5[b]	CAN (3)	MeCN: water 4:1	81%

[a]: reaction was performed at 80°C. [b]: reaction was done in 1 hour.

Pure **284** was obtained as a brown, sticky, extremely hydrophobic foam and was therefore very hard to handle. Due to its unprotected acid moiety it was also very insoluble in organic solvents. For a similar compound, Feichtinger et al. reported that a TMS adduct of the acid could be prepared in situ to enhance solubility, after which the guadinilation reagent can be added.[199]

Similar conditions were tested to prepare a TMS ester of **284** in situ (table 3). The initial test used bis(trimethylsilyl)acetamide (BSA) as TMS donor. A mixture of **284** suspended in DCM and BSA was heated to 45°C for 3 hours, after which no more suspended solid was visible. Then compound **283** and triethylamine were added at room temperature and the reaction mixture was stirred overnight. Regrettably, only complete decomposition of **284** was observed; a large amount of unreacted **283** was still present (entry 1). It might be that formation of the TMS adduct was too slow, allowing for decomposition of **284** before protection could be complete. Therefore, a better TMS donor, trimethylsyliltrifluoromethanesulfonate (TMSOTf), was used. The addition of TMSOTf to a suspension of **284** in DCM was done at 0°C to prevent decomposition of the starting material; the mixture was then allowed to warm to room temperature and stirred for an hour. After addition of the base and stirring for 16 hours the compound had again completely decomposed (entry 2). Finally, a reaction of **284** and **283** without the TMS protection was attempted. DCE was used as solvent to increase the solubility of **284**, however again no product was obtained (entry 3).[200]

Table 46: Guanidine synthesis I.



284 **283** **282**

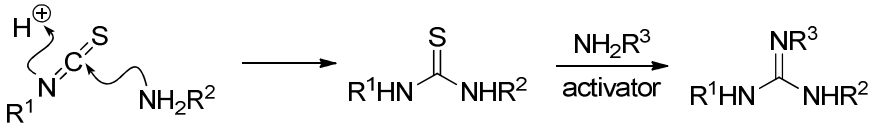
Reagents and conditions: a) TMS donor, solvent, rt, 16 h.b) **283** (1.2 equiv), base.

#	TMS donor (equiv)	Base (equiv)	Solvent	Result
1[a]	BSA (2.2)	Et_3N (1.2)	DCM	Decompostion
2[b]	TMSOTf (3)	DIPEA (3)	DCM	Decompostion
3	—	DIPEA (4)	DCE	Decompostion

[a]: reaction was done at 45°C then rt. [b]: reaction was done at 0°C to rt.

The reason for the decomposition of the starting material during these reactions is unclear. The Fmoc group should be stable to triethylamine and DIPEA. Since, a large amount of **283** was recovered it seems unlikely it had a role in the decomposition of **284**. Finally, entry 3 also had decomposition of **284** while there was no silylating agent present.

Reactions with compound **283** were not successful; therefore other guanidinylation reagents were considered. Guanidines are often made from thioureas by activating the thiourea and substituting the sulfur for a nitrogen nucleophile.[201] Thioureas can be synthesized by mixing an isothiocyanate and an appropriate amine (figure 74).

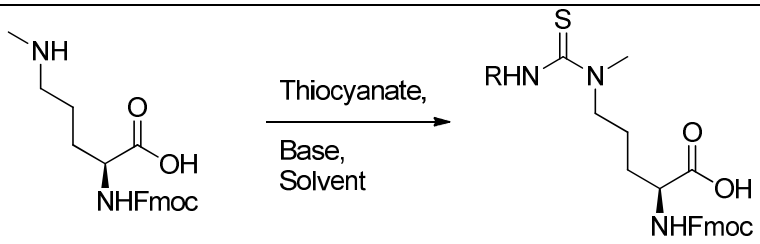


A **B** **C**

Figure 74: guanidine synthesis starting from an isothiocyanate.

Thus several isothiocyanates were employed in an effort to synthesize derivatives of thiourea **11** (Table 4).The first test, using potassium isothiocyanate in DMF, did not show any conversion (entry 1). Initially no base was added, and the mixture was stirred for an hour. When no conversion of **284** was observed by TLC three equivalents of DIPEA were added and the mixture was stirred for several hours. After addition of base, no turnover of the starting material was observed. This was somewhat surprising as conditions were similar to those in table 3, where complete decomposition was seen in all cases.

Table 47: Guanidine synthesis II.

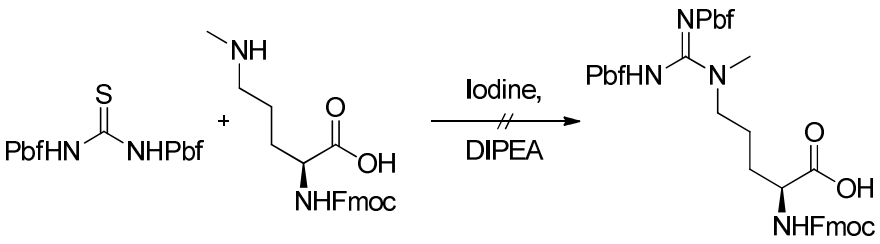


284 **291**

#	Thiocyanate (equiv)	Base (equiv)	R	Solvent	Result
1	KSCN (1.2)	DIPEA (3)	H	DMF	No reaction
2	PbfNCS (1.2)	DIPEA (2)	Pbf	DCM	Decomposition
3	PbfNCS (1.3)	DIPEA (3)	Pbf	DCM	Trace
4	PbfNCS (1.2)	K_2CO_3 (3)	Pbf	DCM	Decomposition

Pbf-isothiocyanate would be a suitable isothiocyanate to use as it would lead to a guanidine with the Pbf protecting group, which is very amiable to SPPS.[202] However, only a trace of compound with the right mass was observed in the LCMS (entry 3), which could not be isolated. The use of potassium carbonate as base led to complete decomposition, presumably due to Fmoc removal (entry 4).

Finally, an attempt was made to turn around the guanidine formation. Instead of preparing thiourea **291** from an isothiocyanate and **284**, followed by attack by a nitrogen nucleophile to obtain the guanidine. It was attempted to react compound **284** with a di-Pbf protected thiourea **292** (scheme 58). Following a procedure described by Qin et al, **292** was activated with DIPEA and Iodine, followed by addition of **284**.[203] However, this strategy also did not yield the desired product.

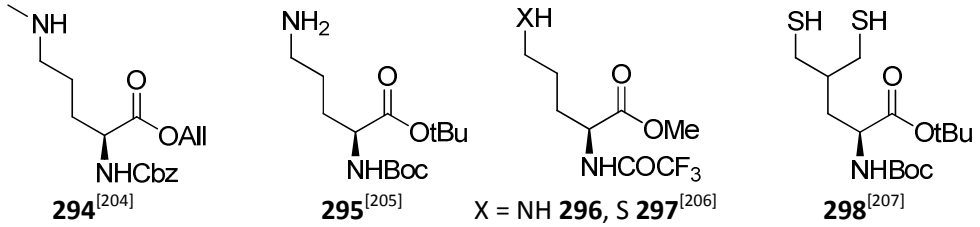


292 **284** **293**

Scheme 58: attempted synthesis of **293**. Reagents and Conditions: Iodine (2.9 equiv), DIPEA (2.9 equiv), THF, rt, 16 h.

13.2 Protected acid approach

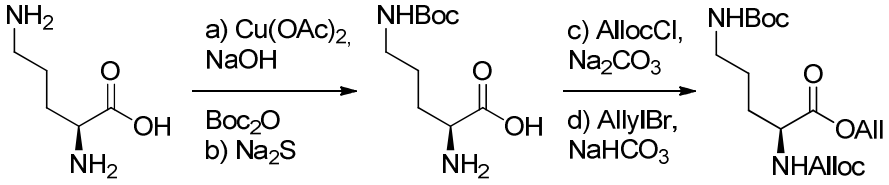
Due to the solubility problems of **284**, the synthetic strategy was revised. A literature search revealed that several compounds similar to **284** with an ester protecting group on the acid moiety are known. These compounds were reported to be stable and did not spontaneously ring close (figure 75).



294[204] **295**[205] X = NH **296**, S **297**[206] **298**[207]

Figure 75: Stable N-methyl ornithine ester and analogues.

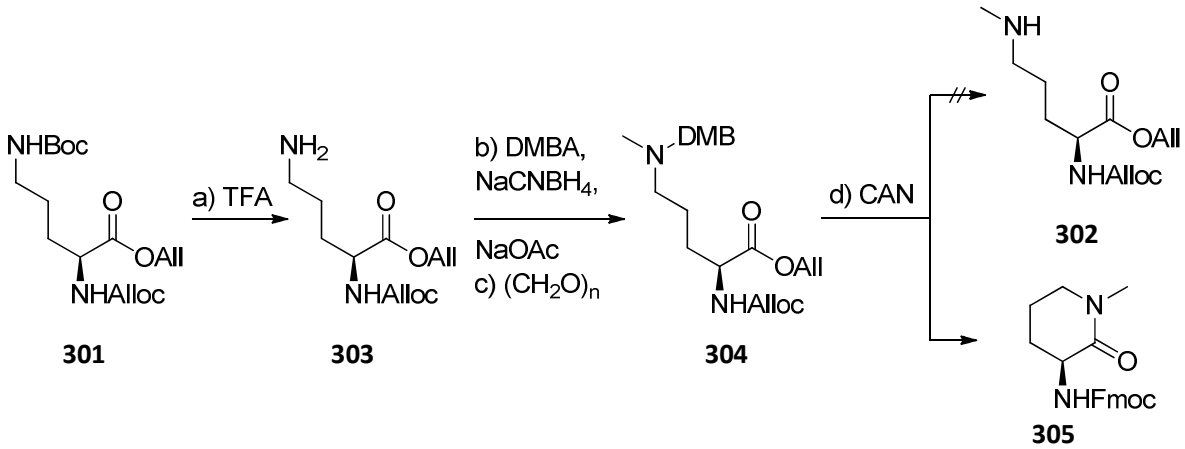
Therefore, it was decided to synthesize a diprotected analogue of **284**. Since the Alloc and allyl ester protecting group combination had served well in the past (chapter 8.2), it was used here as well. Synthesis started with ornithine, which could be selectively boc protected on the side chain after protection of the amino acid as a copper complex.[208] After reaction with Boc anhydride, the copper complex was removed with sodium sulfide.[209] The amine and carboxylic acid were protected with an Alloc and allyl group respectively, using standard methods (scheme 59).



299 **300** **301**

Scheme 59: Synthesis of **301**. Reagents and Conditions: a) Copper acetate (0.5 equiv), sodium hydroxide (2 equiv), Boc anhydride (1.3 equiv), acetone: water 4:3, rt, 2.5 days. b) sodium sulfide (0.75 equiv), water, rt, 2 h, quantitative over two steps. c) allyl chloroformate (1 equiv), sodium carbonate (1 equiv), acetonitrile: water 1:2, rt, 2 days. d) allyl bromide (1.1 equiv), sodium bicarbonate (1 equiv), DMF, rt, 2 days, 68% over two steps.

Removal of the Boc group with TFA and reductive amination, using the same procedure as before proceeded as expected. Regrettably, removal of the DMB group with CAN lead to the ring closed product. Several attempts were made to perform an in situ guanidinylation after the CAN deprotection but only traces of the guanidine were obtained.



301 **303** **304** **302** **305**

Scheme 60: attempted synthesis of **302**. Reagents and Conditions: a) TFA: DCM 1:1, rt, 5 h, quant. b) 2,4-dimethoxybenzaldehyde (1.1 equiv), sodium cyanoborohydride (2 equiv), sodium acetate (2.1 equiv), 3A molsieve, MeOH, rt, 1h. c) para-formaldehyde (4 equiv), sodium cyanoborohydride (2 equiv), rt, 16 h, 78% over two steps. d) CAN (3 equiv) acetonitrile: water 4:1, rt, 15 minutes.

Finally, for future attempts toward compound **282** it would be best to use a strategy similar to the method of Marletta et al. although the sequence is much longer than the routes pursued above.[210] They first introduced a tosyl group on the N^{δ} position using the copper protection strategy used above for the boc introduction. After adding a protecting group to the primary amine the methyl group was established using methyl iodide and all protecting groups were removed. Then they protected the amino acid as a boron complex followed by synthesis of the guanidine with N,N'-diboc thiourea. Removal of the borane complex can be achieved using weak acid, leaving the boc groups untouched. At this point an Fmoc protecting group could be introduced yielding the final product (figure 76).

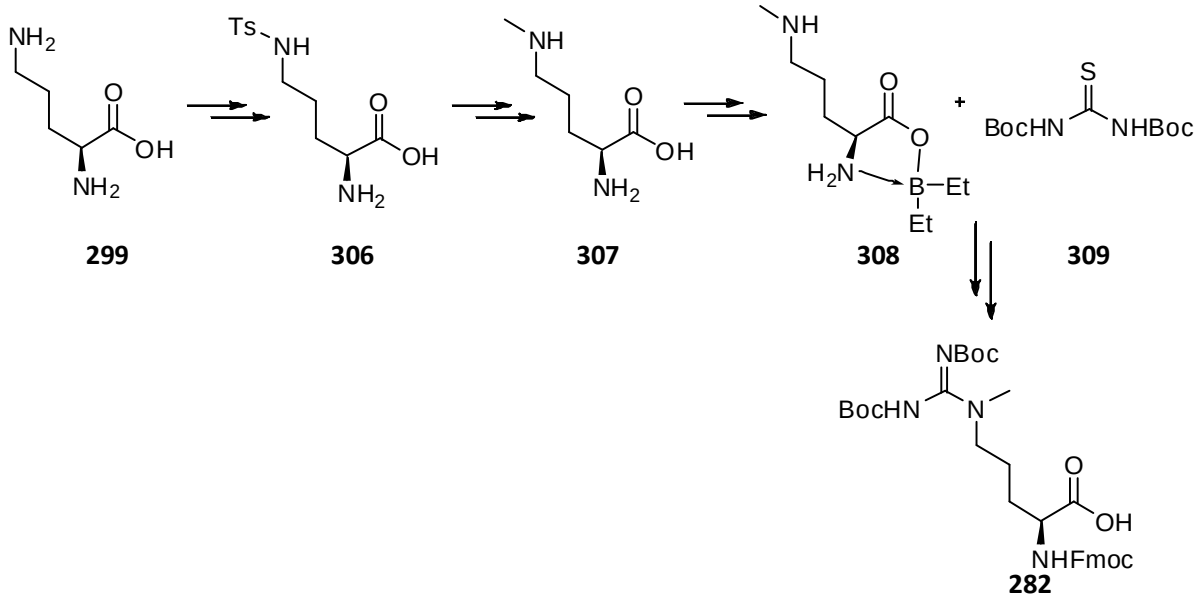


Figure 76: Alternative synthesis toward **282**.

Alternatively, a strategy centered around compound **310** (figure 77). The alcohol moiety should not give the problems related to the acid (bad solubility, hygroscopicity or ring closure) and can be protected as an easily handled silyl ether if needed. In contrast to the Fmoc group, the Alloc protecting group is stable to acidic and basic conditions, allowing for the introduction of the guanidine without decomposition. The Alloc can then be easily removed and an Fmoc installed. This route incorporates lessons learned during the previously discussed chemistry allowing quick access to target compound **282**.

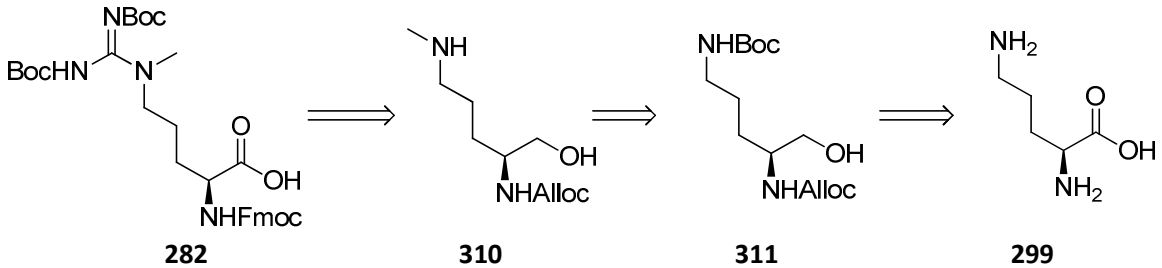


Figure 77: Second alternative retrosynthesis toward **282**.

To synthesize compound **310**, initial protection of **299** could be performed with the copper methodology used before, to introduce the Boc protection. Alloc protection would likewise be done using chemistry

mentioned previously. Reduction of the carboxylic acid can be done by first activating it with, for instance, PyBrOP or as the asymmetric anhydride, followed by sodium borohydride reduction.[211] Then removal of the Boc group followed by the reductive amination procedure detailed above would yield compound **310**. This could then be guadinylated by any of the methods mentioned above. The final compound could then be obtained by oxidation and a protecting group switch.

14 Probes

In this chapter, the design and partial synthesis of a probe to study the synergy between lipidation and phosphorylation will be described. In contrast to the other chapters, this probe is not meant to raise antibodies but will be used as a MS tag for proteomic studies. To study lipidation and phosphorylation of a single protein, a bifunctional probe was developed.

The probe consisted of three parts. The bottom part, a maleimide, was designed to be attacked by the surface cysteine residues of a protein (figure 78, **312**) (normal lipidation sites). The middle part, a piperazine, was designed to enable easy fragmentation in the mass spectrometer, together with the maleimide (**313**). The top part, a phosphonate, was designed to allow titanium oxide purification, together with other phosphate containing proteins. These functions allow the probe to be used in pull-down experiments where information about proteins that get lipidated and phosphorylated can be obtained.

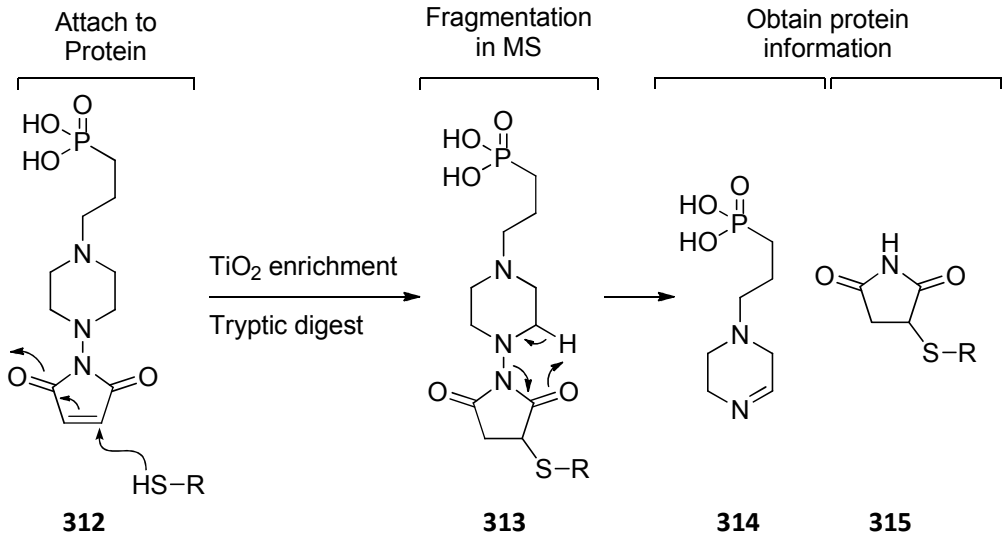


Figure 78: Flow chart to use probe **312**.

The retrosynthetic analysis of the probe **312** starts with a disconnection of the piperazine and the alkyl chain. The final product could be obtained using a reductive amination between aldehyde **316** and piperazine **318**. The aldehyde can be made by hydroboration of **317** and oxidation of the resulting alcohol. **317** can be made from an addition of di-tert-butylphosphonate to allyl bromide. Piperazine **318** should be available through coupling of piperazin-1-amine **319** and maleic anhydride **320**. The piperazine was protected with a boc protecting group to prevent cross reactivity (figure 79).

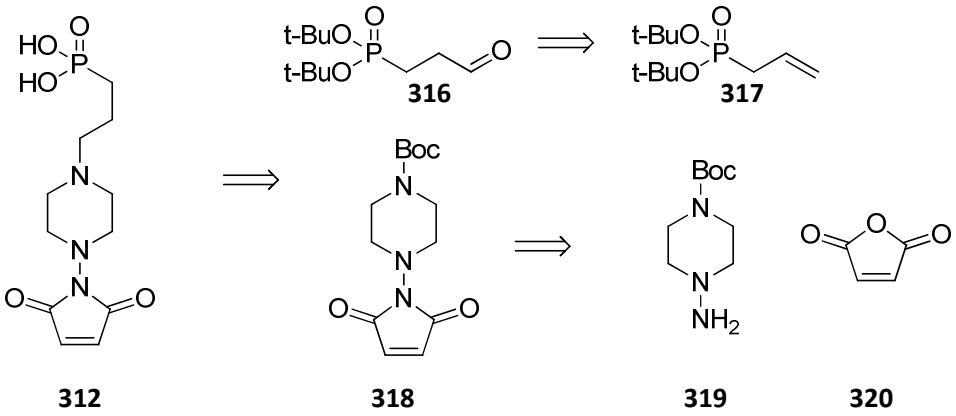
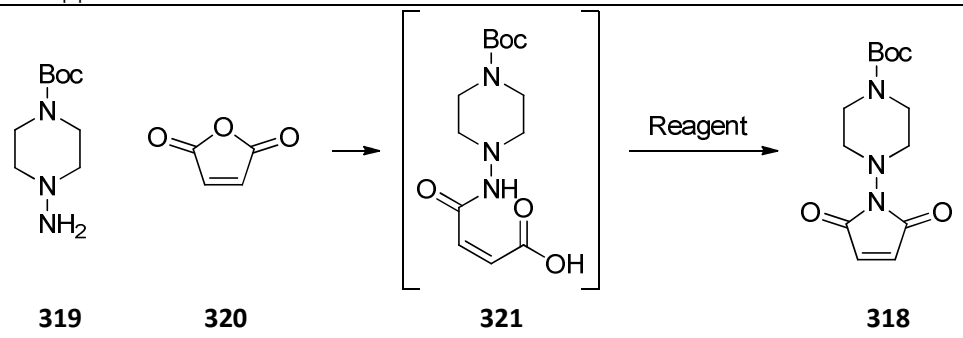


Figure 79: Retrosynthetic analysis of compound **312**.

14.1 Synthesis of the bottom part

Thus, synthesis of the piperazine moiety started with N-boc-piperazine, which was aminated using sodium nitrite, followed by reduction using zinc and acetic acid.[212] Synthesis of the urea was envisioned as a simple condensation between compound **319** and maleic anhydride **320**. However, after starting the reaction using DIPEA in DCM, the reaction stalled at the intermediate amide stage **321**. To make sure the urea ring closed an acetic anhydride promoted strategy was pursued, the results are summarized in table 48.

Table 48: synthesis of piperazine **318**.



Reagents and conditions: maleic anhydride **320** (1 equiv), DCM, rt, 2.5 h then: evaporate DCM, reagent, 3 h.

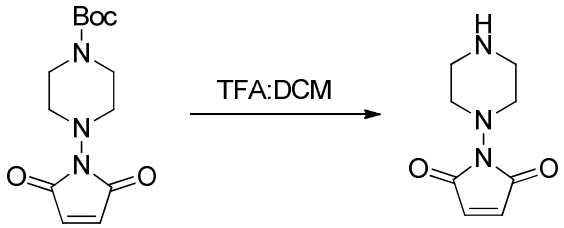
#	Reagent (equiv)	Temp (°C)[a]	Result[b]
1[c]	DIPEA (2)	50	**321**
2[c]	–	rt	**321**
3	Ac_2O (42)	120	**318**:byproduct 1:1
4	Ac_2O (42), NaOAc (3)	120	**318**:byproduct 3:2
5	Ac_2O (42), NaOAc (3)	60	**318**:byproduct 4:1
6	Ac_2O (42), NaOAc (3)	rt	**321**

[a]: temperature for the acetic anhydride promoted ring closing reaction. [b]: ratios determined by ^{1}H-NMR, no yields were determined. [c]: no acetic anhydride or sodium acetate was added.

Running the reaction without any acid or base (entry 2) gave the same result as the reaction with DIPEA (entry 1). Next the acetic anhydride promoted ring closure reaction was performed by first heating the

piperazine **319** and maleic anhydride **320** together to form intermediate **321**. Then acetic anhydride was added and the mixture was heated to 120°C for three hours. This led to a mixture of target molecule **318** and an unidentified byproduct, likely an addition product from boc deprotection (entry 3). Adding sodium acetate to decrease the acidity of the reaction led to a more favorable ratio of **318** and the byproduct (entry 4). Finally, decreasing the temperature to 60°C gave a ratio of 4:1 of **318**:byproduct (entry 5) as optimal conditions. After chromatographic purification this method delivered compound **318** in 63 percent yield. Lowering the temperature further to room temperature to prevent the formation of the byproduct, only resulted in intermediate **321** (entry 6). It should be noted that after introduction of the maleimide moiety, the piperazine ring was present as a mixture of conformations. This mixture made NMR interpretation more difficult. In one case one of the conformers was isolated successfully; the fact that this is possible shows that there is little exchange between conformers. Since these two conformers are the same compound no further attempts were made to separate them.

Compound **318** could be easily deprotected using a one-to-one mixture of TFA and DCM to yield the unprotected piperazine **322** (scheme 61), ready for coupling with the aldehyde **316**.

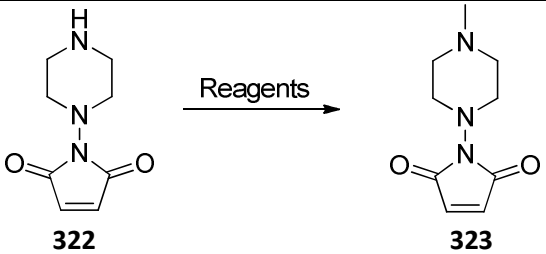


318 **322**

Scheme 61: Boc deprotection using TFA:DCM 1:1. Reagents and conditions: TFA:DCM 1:1, rt, 30 min, quantitative.

Before moving to the synthesis of the full probe a test probe was prepared to verify the behavior in the mass spectrometer. To this end compound **323** was synthesized, containing a methyl group on top of the piperazine. Initially, the attempts were made to methylate compound **323** using a reductive amination with formaldehyde or a substitution reaction with methyl iodide. The results are summarized in table 49.

Table 49: attempted synthesis of **323**.

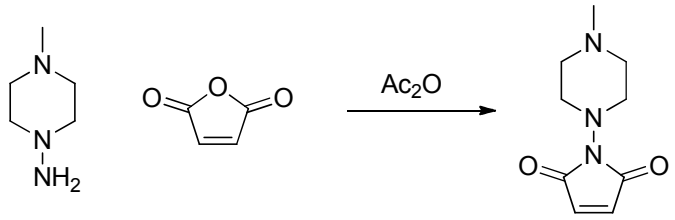


322 **323**

#	Reagents (equiv)	Solvent	Result
1	p-formaldehyde (5), $NaBH_3CN$ (5)	MeCN	Decomposition
2	p-formaldehyde (5), $NaBH_3CN$ (5)	EtOH:AcOH 1:1	Decomposition
3	37 wt% formaldehyde (aq) (2), $NaBH_3CN$ (1.2)	MeOH	Decomposition
4	MeI (1), DIPEA (1.2)	THF	Decomposition

The reductive amination strategy seemed attractive as it was planned to furnish the final probe **312** in the same manner. However, reductive amination using para-formaldehyde let to decomposition of the starting material (entries 1 and 2). Using an aqueous formaldehyde solution did not yield a different result (entry 3). It might be that the maleimide was susceptible to nucleophilic attack from the $NaBH_3CN$, leading to its decomposition. Therefore, methyl iodide and DIPEA were investigated as methylation reagents. Unfortunately, a mixture of unidentified products was again obtained (entry 4).

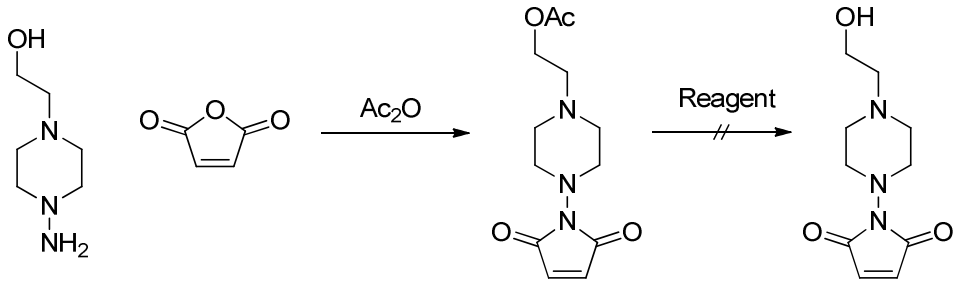
To obtain target molecule **323** a different strategy was used, instead of functionalizing compound **322**. It was decided to synthesize **323** starting from commercially available 1-amino-4-methylpiperazine **324** and maleic anhydride **320** (scheme 62).



324 **320** **323**

Scheme 62: synthesis of compound **323**. Reagents and conditions: maleic anhydride **320** (1 equiv), DCM, rt, 1 h then: evaporate DCM, acetic anhydride (42 equiv), sodium acetate (3 equiv), 50°C, 13 h, 94%.

Using conditions similar to those for the synthesis of **318** an excellent yield of the target compound **323** was achieved. In a similar way compound **326** was synthesized from commercial **325**, to provide a way to the phosphate through **327**.



325 **320** **326** **327**

Scheme 63: synthesis of compound **326**. Reagents and conditions: maleic anhydride **320** (1 equiv), DCM, rt, 3.5 h then: evaporate DCM, acetic anhydride (42 equiv), sodium acetate (3 equiv), 50°C, 2.5 h, 33%.

While the initial reaction to yield **326** proceeded albeit in a poor yield, the following deprotection to obtain **327** did not work. Several conditions were screened. The results are summarized in table 50.

Table 50: attempted synthesis of **327**.

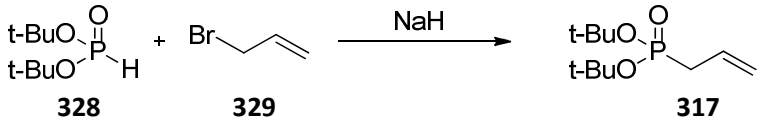
#	Reagent (equiv)	Solvent	Result
1	Cs_2CO_3 (0.1)	MeOH	Addition of methoxide
2	MgOMe (0.1)	MeOH	Addition of methoxide
3	H_2SO_4 (2.2)	MeOH	Decomposition

First the bases cesium carbonate and magnesium methoxide in methanol were investigated but both resulted in fast addition of methoxide to the maleimide (entries 1 and 2). Sulfuric acid in methanol led to complete decomposition of the starting material.

Despite a failure to deliver **327** due to deprotection issues, compounds **322** and **323** were successfully synthesized to ensure continuation of the project. The next step was the synthesis of the phosphonate 'top' part, required to furnish final probe **312**.

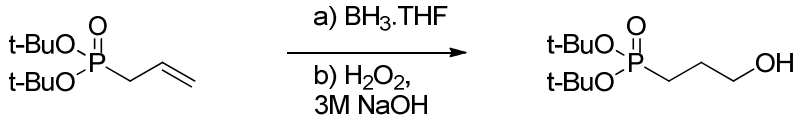
14.2 Synthesis of the top part

Synthesis of the aldehyde **316** started with the literature synthesis of ditert-butylphosphonate **328** from phosphorus trichloride and tert-butanol.[213] Ditert-butylphosphonate **328** was then deprotonated with sodium hydride and allowed to react with allyl bromide **329**, yielding allyl phosphonate **317** (scheme 64).



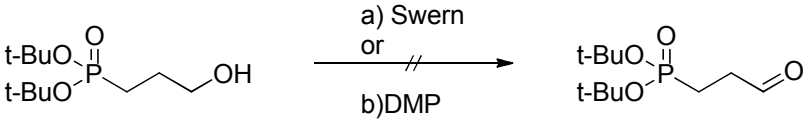
Scheme 64: synthesis of allyl ditert-butylphosphonate **317**. Reagents and conditions: allyl bromide (1.2 equiv), sodium hydride (1.1 equiv), THF, 0°C to rt, 5 h, 70%.

Allyl phosphonate was then subjected to hydroboration conditions followed by hydrogen peroxide oxidation to obtain the alcohol (scheme 65). Borane in THF was found to be a successful reagent, whereas 9-BBN did not result in any product. After oxidative work-up the target alcohol **330** was recovered in only 31 percent yield. While this yield is quite low it was decided to test the oxidation to the aldehyde before attempting to optimize the yield.



Scheme 65: Hydroboration of **317**. Reagents and conditions: a) BH_3.THF (2.5 equiv), THF, rt, 1 h. b) MeOH (excess), 3M NaOH (aq) (7.5 equiv), 30% H_2O_2 (aq) (22 equiv), 0°C to rt, 2 h, 31%.

The oxidation of **330** was attempted by swern reaction and with Dess-Martin periodinane. In both cases there was full conversion of the starting alcohol and some of the target aldehyde was seen in NMR. However, none of the product could be isolated, instead a brown mixture of unidentified products was obtained. It seems likely that the target aldehyde **316** is not stable under the reaction conditions.



Scheme 66: Attempted oxidation of **330**. Reagents and conditions: a) oxalyl chloride (1.2 equiv), DMSO (2.4 equiv), Et_3N (5 equiv), DCM, -78°C to rt, 2 h. b) DMP (1.2 equiv), DCM, rt, 4 h.

This project was not pursued further due to a shift in priorities. Future research might include the coupling of the alcohol **330** with **322** using a Mitsunobu reaction. Alternatively, oxidative cleavage of a longer alkene derivative osmium tetroxide and sodium periodate could be used to make related aldehydes that might be stable. If no stable aldehyde homolog can be found and other coupling methods coupling prove to be unsuccessful, the required molecule could still be made using a different strategy (figure 80).

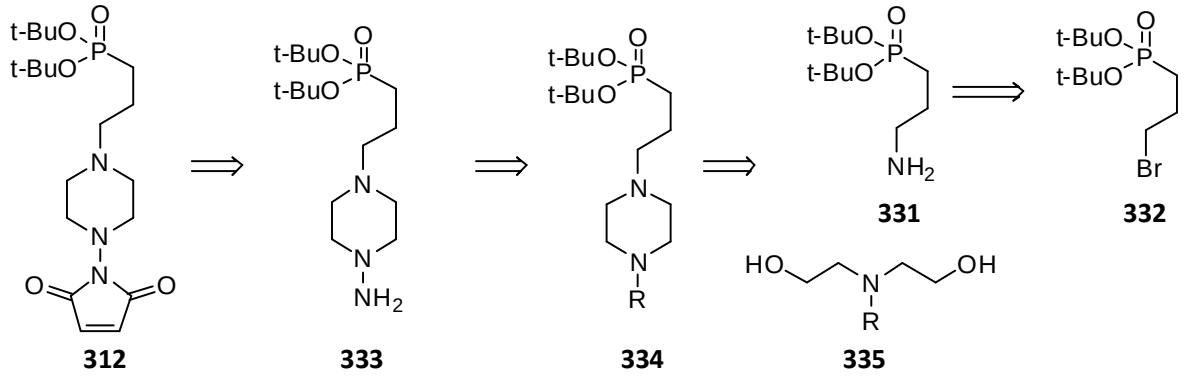


Figure 80: Alternative retrosynthetic analysis of compound **312**.

In this strategy the maleimide is introduced at the end of the sequence after converting protected **334** into hydrazine **333**, using similar conditions as described before (table 48). **334** could be made by metal catalyzed coupling of **331** and protected **335**,[38] thereby bypassing unstable aldehyde **316**. **331** should be easily available by reducing the corresponding azide, which can be made from bromide **332**. Bromide **332** can be synthesized by refluxing 1,3-dibromopropane and tri-tert-butylphosphite.[39] Alternatively, the bromide might also be accessed by a radical reaction between hydrogen bromide and allyl phosphonate **317**.

15 Concluding remarks

Post-translational modifications such as phosphorylation, methylation and adenylylation can alter protein function in a dynamic way. Phosporylation and dephosphorylation for example, have been shown to function as an on/off switch for proteins such as kinases. A good understanding of these processes can eventually lead to disease modifying drugs such as illustrated in the anti-cancer drug Gleevec. The short life-time of some post-translation modified residues, such as phosphorylated histidine, makes it impossible to study these processes using conventional biological methods.

Synthetic chemistry can be a powerful method to generate tool compounds to better understand biological processes as illustrated in the projects described in this thesis. In this thesis, the design and synthesis of a stable 3-pHis analog and its incorporation into a peptide has been described. This peptide in turn was succsefully used to raise antibodies, which can be used to study phosphorylation of histidine in more detail. In addition, major advances have been made in the synthesis of a 1-pHis analog, an adenylyated histidine analog and a methylated arginine, which all can be used to raise their respective antibodies in the near future. With the completion of these syntheses even more antibodies to understand post-translational modifications can be added to the biological toolbox.

Synthetically, the development of the tool compounds was challenging. Many different synthetic routes towards the 3-phis mimic were designed and pursued. The final synthetic strategy toward this mimic proved suitable to deliver multigram amounts of the mimic for future peptide synthesis campaigns. The synthesis started with cheap starting materials and used several innovative steps to get to the final product. Many difficulties have been overcome during the synthesis. The crucial transformations are highlighted in figure 81. Azide introduction to yield **66** was succesfully achieved by substitution of a triflate using TMGA. The difficulties to remove byproducts from the aza-wittig product, related to reagent PPh_3, could successfully be circumvented by the use of dppe. Aromatization of the imidazoline was achieved using base induced elimination of the tosylate. The imidazole was successfully trityl protected using trityl chloride, silver triflate and DBU. Finally, facile protecting group manipulations led to the final product.

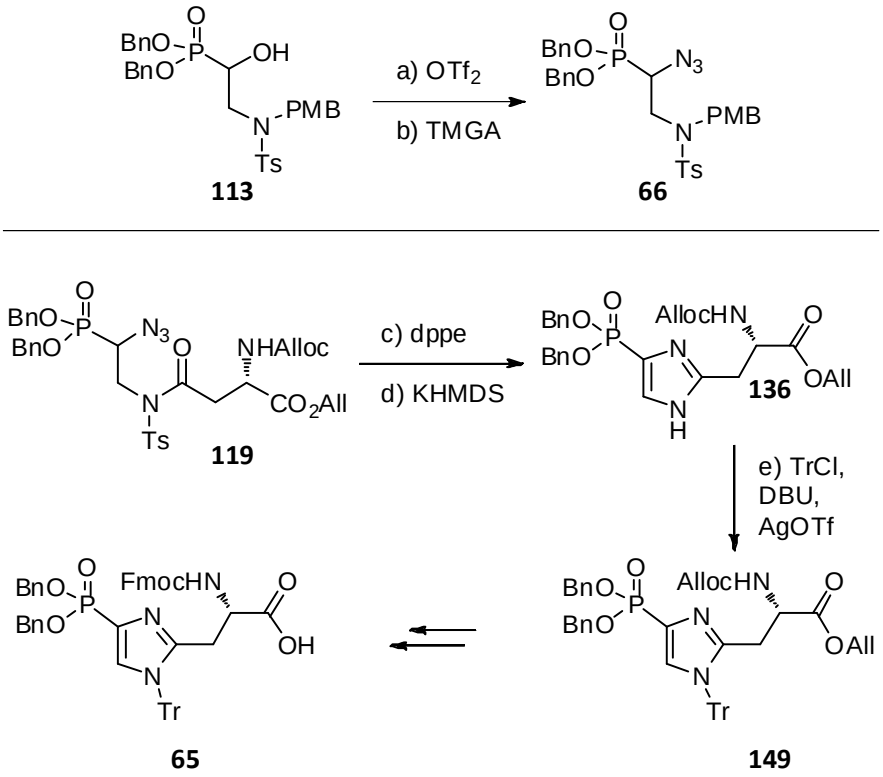


Figure 81: Highlights of the synthesis of 3-pHis mimic **65**.

With this synthetic route available, enough **65** has been synthesized to make a wide range of peptides available for antibody generation. The antibodies raised during the course of this project showed very good affinity. The high affinity likely reflects the electrostatic similarities of pHis and its stable analog, emphasizing the importance of a well thought out design of the mimic. These antibodies can now be used to detect the presence of pHis in various biological systems. This will aid in the study of the biology of phosphohistidine.

The synthetic methodology used to prepare the 3-pHis method was further explored to generate a small library of 4-phosphonic acid imidazoles. It is expected that such a library will provide a valuable starting point for the discovery of inhibitors of phosphohistidine phosphatase.

As mentioned above, the side projects have all generated important data on which future research can be build. Initial studies were performed towards the synthesis of a 1-phosphohistidine analog. It is expected that successful completion of this project will provide antibodies that will allow researchers to distinguish 1-pHis from 3-pHis containing proteins, thereby discovering the potentially different effects they have on biological systems.

The synthesis of an adenylylated histidine mimic was also investigated. It is expected that only minimal efforts will be required to complete the synthesis of this compound. This adenylylated histidine mimic in turn can be used to raise antibodies for proteins that are adenylylated on histidine.

The work done in the N^{δ}-methylarginine project provided a first view into the chemistry of this unexpectedly hard to synthesize compound. While the synthesis did not result in the target compound, the suggested follow-up studies should enable its synthesis with relative ease.

A version of the piperazine probe was successfully synthesized, allowing for proof of concept studies. The complete probe should be available following the suggestions at the end of the chapter and will aid proteomic studies focusing on palmitoylation and phosphorylation.

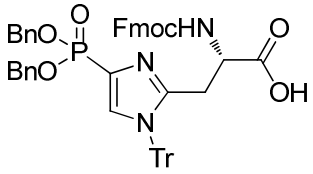
In conclusion, this thesis provides a clear example of the power of chemical biology. Using rational design several tool compounds were designed and synthesized. This led to antibodies that can shed new light on important biological processes. The work in thesis also emphasizes that tool compounds needed for biological investigation can be difficult to synthesize. Therefore, a good knowledge of chemistry is essential for the success of chemical biological studies.

16 Experimental Section

16.1 General remarks

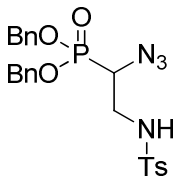
All chemicals were obtained from Acros, Aldrich, Alfa Aesar, Merck or Rapp Polymere and were used without further purification except when noted. Solvents were used as received; except dichloromethane, which was distilled from CaH_2. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on Varian Mercury 400- or a Bruker DRX 500 NMR spectrometer. Chemical shifts are reported in δ values relative to residual solvent peaks (Abbreviations: s: singlet, bs: broad singlet, d: doublet, dd: doublet of doublets, ad: apparent doublet, t: triplet, q: quartet, m: multiplet). Specific optical rotations were measured on a Schmidt + Haensch Polartronic HH8 Polarimeter. IR spectra were measured on a Bruker Tensor 27 with compounds deposited as a neat film onto the crystal. Column chromatography was performed using silica gel for chromatography (0.035-0.070 mm, 60A) purchased from Acros Organic. Compounds on TLC were visualized by UV and/or basic $KMnO_4$ staining. High resolution mass spectra (HR-MS, 70 eV) were measured on a Thermo Orbitrap, coupled to a Thermo Accela nano-HPLC machine, using electron spray ionization (ESI). Analytical HPLC-MS data were recorded on an Agilent HPLC (1100 series) coupled to a Finnigan LCQ ESI spectrometer, using a Nucleodur C18 gravity column, ID 4 mm, length 125 mm, particle size 3 µm from Macherey-Nagel. A gradient was applied beginning at 10% acetonitrile and ending at 90% after 15 min, followed by 3 min at 90% acetonitrile; finally, the column was washed for 5 min with 100% acetonitrile. The column was equilibrated for 3 min with 10% acetonitrile. All RP-HPLC solvents contained TFA (0.1% v/v). Residence time (t_R) was taken from the UV detector, unless otherwise noted. Chiral HPLC was performed on an Agilent 1100 system using a Diacel Chiralpak IA column, as mobile phase an isocratic mixture of 15% dichloromethane: ethanol (100:2) in hexanes was used, at a flow of 0.5 mL/minute over 60 minutes.

16.2 Compound information



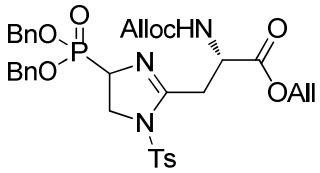
(*S*)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-trityl-1H-imidazol-2-yl)propanoic acid (65): Under argon, in a 50 mL schlenk flask, equipped with an egg-shaped stirring bar, compound **136** (1.2 g, 1.52 mmol, 1 equiv) was dissolved in anhydrous THF (10 mL) and phenylsilane (822 mg, 0.94 mL, 7.6 mmol, 5 equiv) was added, followed by palladium tetrakis(triphenylphosphine) (173 mg, 0.15 mmol, 0.1 equiv). Immediately after addition was complete, the mixture turns yellow and bubbles are formed. As the mixture ages, it slowly turned black. After 1 hour the reaction mixture was transferred to a 25 mL round bottom flask and the solvent was evaporated *in vacuo*. The residue was suspended in 4 mL mesitylene and evaporated to dryness on a rotary evaporator equipped with a dry ice cooler and an oil pump, this procedure was repeated twice to remove excess phenylsilane. The resulting black solid was not purified further and used 'as is' in the next step. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.91. R_f: 0.08 (chloroform:methanol 9:1). LC-MS (ESI): t_R 7.71 min. HRMS (ESI): for $[M+H]^+$ calc.: 658.24653, found: 658.24633. The crude reaction mixture (1.52 mmol) was

dissolved in dioxane (9 mL), upon complete dissolution, water (1 mL) was added followed by Fmoc-OSu (769 mg, 2.28 mmol 1.5 equiv) and sodium hydrogen carbonate (192 mg, 2.28 mmol, 1.5 equiv). The mixture was stirred at room temperature for 16 hours, when a TLC sample (Ethyl acetate or $CHCl_3$ containing 10% MeOH) indicated complete consumption of the starting material. Water (10 mL) and chloroform (10 mL) were added and the mixture was acidified to pH 4 with 0.5 M aqueous potassium hydrogen sulfate solution. The layers were separated and the aqueous layer was washed with 3 additional portions of chloroform (10 mL), the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a black foam (2.16 g) that was further purified by C_{18} sep-pak cartridge (loading 100 mg compound per gram C_{18}-material, 100% water to 100% acetonitrile in steps of 10%, each step 7 mL solvent per gram C_{18}, compound eluted at 70- to 90% acetonitrile), removal of the solvent by lyophilization yielded 1.1 g (1.24 mmol, 82%) of a brown amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.50 (d, *J* = 7.2 Hz, 2H), 7.46 – 7.17 (m, 22H), 7.07 – 6.94 (m, 6H), 5.92 (bs, 1H), 5.14 (dd, *J* = 16.9, 9.0 Hz, 4H), 4.59 – 4.48 (m, 1H), 4.32 – 4.18 (m, 2H), 4.13 (t, *J* = 6.9 Hz, 1H), 2.57 (d, *J* = 17.0 Hz, 1H), 1.80 (dd, *J* = 17.3, 9.4 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.18(C=O), 155.28(C=O), 150.38 (d, *J* = 19.1, C), 143.86 (C), 141.34, 141.33, 140.50, 135.96 (d, *J* = 5.5, C), 135.92 (d, *J* = 5.8, 1xC), 134.34, 134.27, 131.36 (d, *J* = 35.8, CH), 129.81, [128.68, 128.64, 128.59, 128.45, 128.11, 128.07, 127.80, 127.15] this area is unrevolvable, 125.24 (CH), 125.15 (CH), 120.07 (CH), 76.77 (C), 68.36 (d, *J* = 23.7, CH_2), (d, *J* = 23.7, CH_2), 67.16(CH_2), 51.65 (CH), 47.12 (CH), 34.01 (CH_2). ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.61. R_f: 0.16 (chloroform:methanol 95:5). LC-MS (ESI): t_R = 10.99 min. HRMS (ESI): for $[M+H]^+$ calc.: 880.31461, found: 880.31426. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ +219.3° (*c*=0.0023, $CHCl_3$).

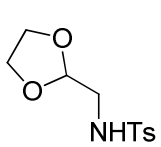


Dibenzyl 1-azido-2-(4-methylphenylsulfonamido)ethylphosphonate (66): In a 250 mL three-necked, round bottom flask containing an egg-shaped stirrer bar, azide **117** (18.3 g, 29.4 mmol, 1 equiv) was dissolved in 1,4-dioxane (130 mL). After complete solution of the starting material, water (15 mL) and ceric ammonium nitrate (48.4 g, 88.2 mmol, 3 equiv) were added, turning the mixture bright orange. After 6.5 hours TLC showed a small amount of starting material remaining, in order to drive the reaction to completion, additional portions of ceric ammonium nitrate (2 g,) and water (5 mL) were added. After stirring for an additional 15 minutes, TLC indicated complete conversion of the starting material. The reaction mixture was partitioned between water (200 mL) and ethyl acetate (200 mL), the aqueous layer was washed with additional ethyl acetate (2 x 100 mL), the organic layers were combined, washed with brine (100 mL) and dried over anhydrous magnesium sulfate. Evaporation yielded an orange oil that was purified by flash column chromatography (toluene:ethyl acetate 24:1 in increasing gradient to 4:1) evaporation of the solvent yielded a yellow oil (12 g, 24 mmol, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (ad, *J* = 8.3, 2H), 7.41 – 7.30 (m, 10H), 7.22 (ad, *J* = 8.2, 2H), 6.40 (t, *J* = 6.2, 1H), 5.16 – 5.00 (m, 4H), 3.95

– 3.82 (m, 1H), 3.47 – 3.35 (m, 1H), 3.22 – 3.09 (m, 1H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.4, 136.7, 135.3 (d, *J* = 5.4, 1C), 135.3 (d, *J* = 5.5, 1C), 129.6, 128.6 (br, 2x2C), 128.5 (br, 2x2C), 128.0 (br, 2x2C), 126.9, 68.6 (d, *J* = 6.9, 2C), 57.5 (d, *J* = 154.1, 1C), 42.3 (d, *J* = 5.1, 1C), 21.3. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.9. IR ν = 3151 (bw), 3066 (w), 3035 (w), 2894 (bw), 2107 (s), 1598 (w), 1497 (w), 1455 (m), 1331 (s), 1238 (s), 1157 (s), 1092 (w), 990 (s), 873 (m), 813 (s), 732 (s), 696 (s), 660 (s). R_f: 0.34 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 9.39 min. HRMS (ESI): for $[M+H]^+$ calc. 501.13560, found 501.13500.

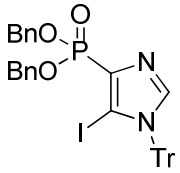


(2*S*)-Allyl 2-(allyloxycarbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-tosyl-4,5-dihydro-1H-imidazol-2-yl)propanoate (68): Under argon, in a 250 mL, flame dried schlenk flask azide **119** (2.7g, 3.6 mmol, 1 equiv) was dissolved in anhydrous THF (80 mL), 1,2-Bis(diphenylphosphino)ethane (dppe) (2.2 g, 5.4 mmol, 1.5 equiv) was added in one portion. The schlenk flask was closed and immersed in an 80^{o}C oil bath for 6 hours, after which the reaction mixture was allowed to cool just below boiling. A solution of hydrogen peroxide (30% in water) (15 mL) and saturated aqueous sodium hydrogen carbonate (15 mL) was added and the mixture was stirred for 10 minutes. When TLC indicated that no trace of un-oxidized DPPE remained, the mixture was transferred to a separatory funnel, chloroform (100 mL) and saturated aqueous sodium hydrogen carbonate (100 mL) were added, layers separated and the aqueous layer was washed with additional portions of chloroform (2 x 75 mL). Organic layers were combined, washed with brine (100 mL) and dried over sodium sulfate, evaporation yielded an off-white sticky solid that was further purified by filtration through a 4 cm high plug of silica eluting with chloroform:ethyl acetate 3:1, yielding **68** as a yellow oil as a mixture of diastereoisomers (1:1.24 by ^{31}P-NMR) (2.5 g, 3.5 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.66 (m, *J* = 8.3, 2H), 7.44 – 7.18 (m, 12H), 6.22 – 5.71 (m, 3H), 5.38 – 5.06 (m, 4H), 5.05 – 4.87 (m, 4H), 4.83 – 4.70 (m, *J* = 4.3, 9.0, 1H), 4.64 – 4.39 (m, 4H), 4.24 – 4.08 (m, 1H), 4.05 – 3.74 (m, 2H), 3.55 – 3.34 (m, 1H), 3.14 – 2.93 (m, 1H), 2.45 – 2.35 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 170.3, 159.3, 159.2, 156.2, 156.1, 145.19, 145.15, 136.17, 136.11, 136.06, 136.00, 134.96, 134.91, 132.8, 131.9, 131.8, 130.4, 128.73, 128.68, 128.65, 128.61, 128.03, 128.01, 127.99, 127.3, 118.4, 118.3, 118.0, 117.8, 68.78, 68.75, 68.71, 68.69, 68.40, 68.33, 68.26, 68.19, 66.30, 66.12, 66.05, 65.96, 62.0, 61.9, 60.4, 60.3, 51.1, 50.8, 48.96, 48.92, 48.89, 48.83, 32.5, 32.1, 21.74, 21.73. ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.7, 22.2. R_f: 0.36 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.61 min. HRMS (ESI): for $[M+H]^+$ calc.: 696.21391, found: 696.21318.

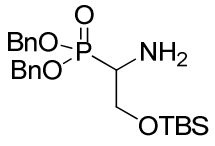


***N*-((1,3-Dioxolan-2-yl)methyl)-4-methylbenzenesulfonamide (69)**: In a 250 ml round bottom schlenk flask, equipped with a large egg-shaped stirring bar, under argon, (1,3-dioxolan-2-yl)methanamine (2.5

g, 2.3 mL, 24 mmol, 1 equiv) was dissolved in anhydrous dichloromethane (25 mL), the mixture was cooled to 0°C with an ice bath and triethylamine (2.4 g, 3.4 mL, 24 mmol, 1 equiv) was added followed by 4-dimethylaminopyridine (30 mg, 0.24 mmol, 0.01 equiv). Tosyl chloride (4.6g, 24 mmol, 1 equiv) was dissolved in 25 mL anhydrous dichloromethane containing 1 mL anhydrous THF to aid solvation. The tosyl chloride solution was added to the reaction mixture at 0°C, dropwise, over 5 minutes. After addition was complete the mixture was allowed to warm to room temperature with the ice bath in 16 hours. The mixture was added to 50 mL dichloromethane and was washed 3 times with 10% aqueous citric acid (100 mL), once with water (100 mL), 3 times with 5% aqueous sodium carbonate (100 mL) and once with brine (100 mL). The mixture was dried over anhydrous magnesium sulfate and the solvent was evaporated yielding 5.7 g (22 mmol, 93 %) of a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.70 (m, 2H), 7.00 – 6.90 (m, 2H), 4.96 (t, *J* = 6.3, 1H), 4.89 (t, *J* = 3.8, 1H), 3.92 – 3.73 (m, 7H), 3.11 (dd, *J* = 3.8, 6.4, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.94, 131.70, 129.27 (2xCH), 114.27 (2xCH), 101.53, 65.34 ($2xCH_2$), 55.70, 45.51. R_f: 0.23 (toluene:ethyl acetate 3:1). Melting point: 106.9°C. LC-MS (ESI): t_R = 6.87 min. The compound is poorly visible in the MS.

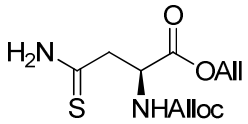


Dibenzyl 5-iodo-1-trityl-1H-imidazol-4-ylphosphonate (73): Under argon, in a 50 mL Schlenk imidazole **167** (500 mg, 0.88 mmol, 1 equiv) was dissolved in 5 mL THF. The mixture was cooled to -30°C and 1.6M butyl lithium in hexanes (0.6 mL, 1.0 mmol, 1.1 equiv) was added dropwise. The mixture was stirred for 30 minutes, followed by the addition of Iodine (330 mg, 1.3 mmol, 1.5 equiv) dissolved in 1 mL THF until the iodine color persisted. The mixture was poured into sat. aqueous sodium thiosulfate solution (15 mL and ethyl acetate (15 mL). The aqueous layer was washed with three additional 10 mL portions of ethyl acetate, the organic layers were combined, washed with brine (30 mL), dried over magnesium sulfate and the solvent was removed in vacuo. The resulting white foam was further purified by flash column chromatography (toluene:ethyl acetate 2:1) yielding 0.326 g (0.47 mmol, 53%) of a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.6 Hz, 1H), 7.41 – 7.28 (m, 20H), 7.16 – 7.08 (m, 6H), 5.22 – 5.09 (m, 4H). ^{31}P NMR (162 MHz, $cdcl_3$) δ 11.75. LC-MS (ESI): t_R = 10.72 min. HRMS (ESI): for $[M+H]^+$ calc.: 571.21451, found: 571.21491.

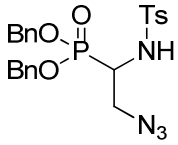


Dibenzyl 1-amino-2-(tert-butyldimethylsilyloxy)ethylphosphonate (75): Azide **173** (3.7 g, 8.02 mmol, 1 equiv) was dissolved in 22 mL ethanol and 7 mL water. Ammonium chloride (0.98 g, 18.4 mmol, 2.3 equiv) was added followed by zinc powder (1.6 g, 24.1 mmol, 3 equiv). After stirring for 30 minutes the

reaction was poured into saturated ammonia (50 mL) and ethyl acetate (50 mL). The layers were separated and the aqueous phase was washed with 3 additional portion of ethyl acetate (30 mL), the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded **75** as a yellow oil (3.1 g, 7.1 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36 – 7.23 (m, 10H), 5.13 – 4.90 (m, 4H), 3.90 – 3.78 (m, 1H), 3.71 (ddd, *J*=10.2, 7.5, 6.1, 1H), 3.18 (ddd, *J*=13.1, 7.5, 3.7, 1H), 1.58 (s, 2H), 0.84 (s, 9H), 0.00 (s, 3H), -0.00 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 28.34. LC-MS (ESI): t_R = 8.47 min. HRMS (ESI): for $[M+H]^+$ calc.: 436.20675, found: 436.20671.

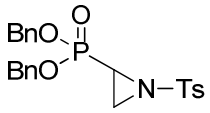


(*S*)-Allyl 2-(allyloxycarbonylamino)-4-amino-4-thioxobutanoate (78): under argon, in a 50 mL round bottom flask, (*S*)-allyl 2-(((allyloxy)carbonyl)amino)-4-amino-4-oxobutanoate (494 mg, 1.9 mmol, 1 equiv) was dissolved in THF (7.5 mL). Lawesson's reagent (389 mg, 0.96 mmol, 0.5 equiv) was added and the mixture was stirred for four hours. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate solution (20 mL) and ethyl acetate (20 mL). The layers were separated and the aqueous phase was washed with 3 additional portion of ethyl acetate (20 mL), the organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was further purified by flash column chromatography (chloroform:ethyl acetate 3:1) yielding 453 mg (1.7 mmol, 88%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.47 (bs, 1H), 7.32 (bs, 1H), 6.00 (bd, *J*=6.5, 1H), 5.91 – 5.75 (m, 2H), 5.35 – 5.10 (m, 4H), 4.70 – 4.62 (m, 1H), 4.60 (ddd, *J*=5.8, 2.8, 1.4, 2H), 4.51 (dt, *J*=5.6, 1.4, 2H), 3.30 – 3.06 (m, 2H). LC-MS (ESI): t_R = 7.30 min. HRMS (ESI): for $[M+H]^+$ calc.: 273.09035, found: 273.09052.

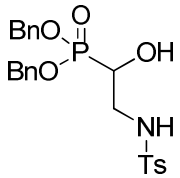


Dibenzyl 2-azido-1-(4-methylphenylsulfonamido)ethylphosphonate (90): In a 25 mL round bottom flask, containing an egg-shaped stirring bar, aziridine **91** (595 mg, 1.3 mmol, 1 equiv) was dissolved in 8.6 mL DMF. Sodium azide (106 mg, 1.6 mmol, 1.25 equiv) was added in one batch, changing the clear solution to a white suspension. After stirring overnight, a TLC sample indicated complete consumption of the starting materials. The DMF was evaporated in vacuo and the residue partitioned between water (10 mL) and ethyl acetate (10 mL). The aqueous layer was washed once with 10 mL of ethyl acetate, the organic layers were combined, washed with brine (10 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded a grey oil that was further purified by filtering through a silica plug (3 cm high, 25% ethyl acetate in toluene) yielding a grey oil (509 mg, 1 mmol, 78%). R_f: 0.24 (toluene:ethyl acetate 3:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 – 7.61 (m, 2H), 7.33 – 7.05 (m, 12H), 6.06 (dd, *J* = 3.9, 9.4, 1H), 5.03 – 4.73 (m, 4H), 3.91 – 3.73 (m, 1H), 3.54 – 3.31 (m, 2H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.79 (C), 137.90 (C), 135.70 (d, *J* = 30.3, CH), 135.64 (d, *J* = 30.6, CH), 129.76 (CH), 128.86 (C), 128.78 (CH), 128.77 (CH), 128.34 (CH), 128.31 (CH), 127.18 (CH), 68.94 (d, *J* = 78.3, CH_2), 68.87 (d, *J* = 78.4, CH_2),

51.32 (CH_2), 50.56 (d, *J* = 163.9, CH), 21.65 (CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.94. IR: 3114 (w), 2922 (w), 2103 (s), 1332 (s), 1235 (s), 1160 (s), 995 (s), 814 (m), 735 (s). LC-MS (ESI): t_R = 9.30 min. HRMS (ESI): for $[M+H]^+$ calc.: 501.13560, found: 501.13514.



Dibenzyl 1-tosylaziridin-2-ylphosphonate (91): Under argon, a schlenk tube containing a magnetic stirring bar was charged with sulphonamide (**94**) (710 mg, 1.5 mmol, 1 equiv), triphenylphosphine (394 mg, 1.5 mmol, 1 equiv) and 3 mL anhydrous THF. The solution was cooled to 0°C and dimethylazodicarboxylate (219 mg, 1.5 mmol, 1 equiv) was added dropwise, upon complete addition the mixture is stirred at 0°C for 30 minutes followed by warming to room temperature over 1 hour. Water (5 mL) and diethyl ether (5 mL) were added, the layers were separated and the organic layer washed with 2 additional 5 mL portions of water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. The resulting oil was purified by column chromatography (toluene 25% ethyl acetate) yielding 639 mg (1.4 mmol, 93%) of a clear oil. R_f: 0.54 (toluene:ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.77 (m, 2H), 7.40 – 7.20 (m, 12H), 5.04 – 4.83 (m, 4H), 2.87 (ddd, *J* = 4.6, 7.6, 16.4, 1H), 2.67 (dd, *J* = 7.6, 9.3, 1H), 2.44 (dd, *J* = 4.6, 9.4, 1H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.31, 135.73 (d, *J* = 13.4, 1C), 135.67 (d, *J* = 13.0, 1C), 134.01, 129.88 (2xCH), 128.79, 128.72 (2xCH), 128.64 (2xCH), 128.62, 128.37 (2xCH), 128.19 (2xCH), 128.04 (2xCH), 68.64 (d, *J* = 31.7, 1C), 68.58 (d, *J* = 31.7, 1C), 31.83 (d, *J* = 207.5, 1C), 30.26 (d, *J* = 4.4, 1C), 21.75. ^{31}P NMR (162 MHz, $CDCl_3$) δ 18.84. LC-MS (ESI): t_R = 10.01 min. HRMS (ESI): for $[M+H]^+$ calc.: 458.11856, found: 458.11846.

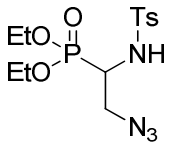


Dibenzyl 1-hydroxy-2-(4-methylphenylsulfonamido)ethylphosphonate (336):

Method A: In a 100 mL three-necked round bottom flask, vinyl dibenzylphosphonate (1 g, 3.47 mmol, 1 equiv) was dissolved in chloroform (17.5 mL). To this mixture chloramine T trihydrate (223 mg, 4.34 mmol, 1.25 equiv), benzyl triethylammonium chloride (40 mg, 0.17 mmol, 0.05 equiv), water (17.5 mL) and osmium tetroxide as a 2.5% solution in tert-butanol (0.44 mL, 0.035 mmol, 0.01 equiv) were sequentially added. On addition of the osmium tetroxide the mixture changes from a pink suspension to a green clear solution. The mixture was heated to 60°C over 16 hours, after which the mixture turned had brown. After confirming complete conversion with a TLC sample, sodium bisulfite (361 mg, 3.47 mmol, 1 equiv) was added and the mixture stirred for one hour, turning the reaction black. The mixture was poured into a separatory funnel, the layers were separated, the organic layer was washed with brine (10 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded 1.5 g of a

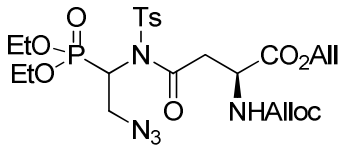
brown oil that was purified further by flash column chromatography (toluene : ethyl acetate 1 : 1) yielding 0.82 g (1.7 mmol, 50%) of a yellow oil that solidified on standing.

Method B: In a 100 mL round bottom flask, containing an egg-shaped stirring bar, hydroxysulfonamide **117** (2 g, 3.36 mmol, 1 equiv) was dissolved in 15.3 mL 1,4-dioxane, water (1.7 mL) was added followed by ceric ammonium nitrate (5.5 g, 10.1 mmol, 3 equiv) and the orange mixture was stirred for 8 hours. After confirming the complete conversion of the starting material with a TLC sample, the reaction mixture was poured into ethyl acetate (30 mL) and water (30 mL). The layers were separated, the aqueous layer was washed with two additional 15 mL portions of ethyl acetate, the organic layers were combined and washed with brine (50 mL). After drying over anhydrous magnesium sulfate, the solvent was evaporated yielding a yellow oil that was purified further by flash column chromatography (toluene:ethyl acetate 1:1) yielding 1.28 g (2.7 mmol, 80%) of an orange oil that solidified on standing. R_f: 0.28 (toluene:ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (d, *J*=8.2, 2H), 7.37 – 7.25 (m, 10H), 7.20 (d, *J*=8.5, 2H), 5.99 (dd, *J*=7.1, 5.1, 1H), 5.10 – 4.97 (m, 4H), 4.86 (bs, 1H), 4.09 (bs, 1H), 3.42 – 3.29 (m, 1H), 3.28 – 3.14 (m, 1H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 143.65, 136.86, 136.01 (d, *J*=5.7), 129.94, 128.86, 128.84, 128.81, 128.79, 128.27, 127.33, 68.82 (d, *J*=7.1), 67.39 (d, *J*=161.8), 44.71 (d, *J*=6.5), 21.72. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.72. LC-MS (ESI): t_R = 8.82 min. HRMS (ESI): for $[M+H]^+$ calc.: 476.12912, found: 476.12885.

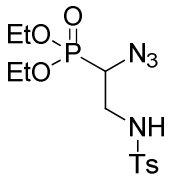


Diethyl 2-azido-1-(4-methylphenylsulfonamido)ethylphosphonate (337): In a 25 mL round bottom flask, containing an egg-shaped stirring bar, 595 mg (1.3 mmol, 1 equiv) of aziridine **91** was dissolved in 8.6 mL DMF. Sodium azide (106 mg, 1.6 mmol, 1.25 equiv) was added at room temperature and the resulting mixture was stirred for 16 hours at room temperature during which the mixture changed from a clear solution into a white suspension. A TLC sample confirmed complete consumption of the starting material. The DMF was evaporated on a rotary evaporator with a dry ice condenser and an oil pump. The residue was partitioned between water (20 mL) and ethyl acetate (20 mL), the aqueous layer was washed with two additional 20 mL portions of ethyl acetate, the organic layers were combined, washed with brine, dried over anhydrous magnesium sulfate and the solvent was evaporated yielding a yellow oil. This was further purified by passing through a short (3 cm) silica column (toluene 25% ethyl acetate) yielding a clear oil (509 mg, 1 mmol, 78%). ^{1}H NMR (400 MHz, MeOH-d^4) δ 7.82 – 7.75 (m, 2H), 7.44 – 7.30 (m, 2H), 4.24 – 3.99 (m, 4H), 3.96 – 3.83 (m, 1H), 3.55 – 3.33 (m, 2H), 2.42 (s, 3H), 1.30 (t, *J* = 7.1, 3H), 1.28 (t, *J* = 7.1, 3H). ^{13}C NMR (101 MHz, MeOH-d^4) δ 144.97, 140.25, 130.80 (2xCH), 128.23 (2xCH), 64.90 (d, *J* = 50.1, 1H), 64.83 (d, *J* = 50.0, 1H), 52.43 (d, *J* = 5.9, 1H), 52.36 (d, *J* = 6.7, 1H), 50.87 (the second peak of the doublet is obfuscated by the methanol peak), 21.60, 16.83 (d, *J* = 10.6, 1H), 16.77 (d, *J* = 10.8, 1H). ^{31}P NMR (162 MHz, MeOH-d^4) δ 25.78. IR: 3103 (bw), 2992 (w), 2885 (w), 2099 (s), 1753 (w), 1666 (w), 1596 (w), 1468 (w), 1441 (w), 1394 (w), 1332 (s), 1304 (w), 1279 (w), 1243 (w), 1225 (s), 1191 (m), 1157 (s), 1109 (m), 1090 (m), 1025 (s), 984 (s), 953 (s), 921 (s), 846 (w), 823 (s), 798 (m), 761

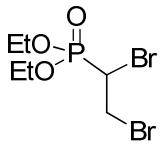
(w), 705 (w), 663 (s). R_f: 0.24 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 8.26 min. HRMS (ESI): for $[M+H]^+$ calc.: 377.10430, found: 377,10395.



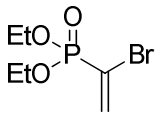
Compound 96: Under argon, in a 5 mL, flame dried, schlenk flask containing an egg-shaped stirring bar, Azide **338** (30 mg, 0.08 mmol, 1 equiv) and amino acid **67** (33 mg, 0.09 mol, 1.1 equiv) were dissolved in 0.4 mL anhydrous dichloromethane. After cooling to 0°C, PyBrOP (42 mg, 0.09 mmol, 1.1 equiv), DIPEA (31 mg, 0.24 mmol, 3 equiv) and DMAP (12 mg, 0.1 mmol, 1.25 equiv) were added sequentially. After stirring the resulting mixture for 3.5 hours, a TLC confirmed complete conversion of the azide. The mixture was poured into 10% citric acid (5 mL), the layers were separated and the aqueous layer was washed twice with dichloromethane (5 mL). The organic layers were combined, washed with saturated aqueous sodium hydrogen carbonate solution (5 mL), water (5 mL) and brine (5 mL). Drying over anhydrous magnesium sulfate, followed by evaporation of the solvent, yielded a yellow oil that was purified further by flash column chromatography (Petroleum ether:ethyl acetate 1:1) yielding 13.6 mg (0.02 mmol, 28%) of a yellow oil as a mixture of diastereoisomers (1:1 by ^{31}P-NMR). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.76 (m, 2H), 7.36 – 7.28 (m, 2H), 5.99 – 5.56 (m, 3H), 5.27 – 5.07 (m, 4H), 4.55 – 4.31 (m, 5H), 4.25 – 3.86 (m, 4H), 3.86 – 3.61 (m, 2H), 3.55 – 3.12 (m, 2H), 3.08 – 3.00 (m, 1H), 2.40 – 2.37 (m, 3H), 1.34 – 1.12 (m, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.19, 20.64.



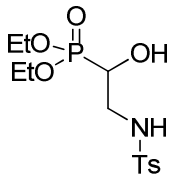
Diethyl (1-azido-2-(4-methylphenylsulfonamido)ethyl)phosphonate (97): in a 5 mL round bottom flask, azide **116** (123 mg, 0.25 mmol, 1 equiv) was dissolved in acetonitrile:water 5:1 (1.25 mL). CAN (411 mg, 0.75 mmol, 3 equiv) was added and the mixture was stirred for 6 hours and poured into ethyl acetate (10 mL). The organic phase was washed six times with saturated sodium bisulfite (10 ml) to remove the aldehyde. The organic layer was washed with brine (10 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated yielding an orange oil (67 mg, 0.18 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.77 (m, 2H), 7.77 – 7.69 (m, 2H), 5.25 – 5.12 (m, 1H), 4.24 – 4.04 (m, 4H), 3.66 (ddd, *J* = 11.5, 8.8, 4.9 Hz, 1H), 3.44 – 3.29 (m, 1H), 3.19 – 3.01 (m, 1H), 2.49 – 2.34 (m, 3H), 1.41 – 1.28 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.03, 136.92, 130.02, 127.21, 77.48, 77.37, 77.16, 76.84, 63.67 (d, *J* = 6.7 Hz), 63.60 (d, *J* = 6.5 Hz), 57.03 (d, *J* = 153.6 Hz), 42.52 (d, *J* = 3.7 Hz), 21.68, 16.60 (d, *J* = 5.5 Hz), 16.57 (d, *J* = 5.5 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.07.



Diethyl 1,2-dibromoethylphosphonate (99): In a 100 mL three-necked flask, vinyl diethylphosphonate (1 g, 6.1 mmol, 1 equiv) was dissolved in chloroform (7.7 mL). Bromine (0.99 g, 6.2 mmol, 1.02 equiv) was added dropwise over 5 minutes. The resulting brown solution was stirred for 3 hours, after which it was poured into 5% aqueous sodium thiosulfate (20 mL), chloroform (10 mL) was added, the layers were separated and the aqueous layer was washed once with an additional 20 mL portion of chloroform, the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded 2.1 g (quantitative yield) of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.37 – 4.16 (m, 4H), 4.16 – 3.92 (m, 2H), 3.69 – 3.59 (m, 1H), 1.37 (tt, *J*=7.1, 0.6, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 64.19 (d, *J*=29.8), 64.13 (d, *J*=29.8), 41.96 (d, *J*=151.1), 31.94 (d, *J*=2.5), 16.35 (d, *J*=5.7). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 16.91. LC-MS (ESI): t_R = 8.01 min. HRMS (ESI): for $[M+H]^+$ (Br_2) calc.: 322.90418, found: 322.90514. (^{81}BrBr) calc.: 324.90214, found: 324.90256. ($^{81}Br_2$) calc.: 326.90009, found: 326.90024.

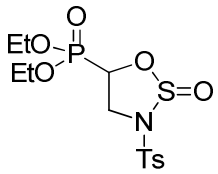


Diethyl (1-bromovinyl)phosphonate (101): In a 5 mL round bottom flask, dibromo compound **99** (30 mg, 0.09 mmol, 1 equiv) was dissolved in DMF (0.45 mL). Sodium azide (48 mg, 0.74 mmol, 8 equiv) was added and the mixture was stirred at room temperature for three hours. The solvent was removed in vacuo and the residue was dissolved in water (5 mL) and ethyl acetate (5 mL). The layers were separated and the organic layer was washed with brine (5 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo yielding 32 mg (full conversion) of a yellow oil that was not purified further. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (dd, *J* = 14.4, 1.8 Hz, 1H), 6.45 (dd, *J* = 37.2, 1.8 Hz, 1H), 4.26 – 4.03 (m, 4H), 1.37 (td, *J* = 7.1, 0.7 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.12.

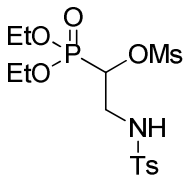


Diethyl 1-hydroxy-2-(4-methylphenylsulfonamido)ethylphosphonate (102): In a 250 mL, three necked, round bottom flask containing an egg-shaped stirring bar, diethyl vinylphosphonate (5 g, 4.7 mL, 30.5 mmol, 1equiv) was dissolved in 115 mL tert-butanol and 35 mL water was added. Chloramine-T (10.7 g, 38.1 mmol, 1.25 equiv) was added in one batch, followed by osmium tetroxide as a 2.5% solution in tert-butanol (3.8 mL, 0.3 mmol). Resulting mixture is stirred overnight, after which a TLC sample indicated complete consumption of the starting material. A solution of sodium hydrogensulfate (4g) in 50 mL water was added to reduce the osmium catalyst, after stirring for 2 hours the mixture was transferred to

a separatory funnel and extracted with four 100 mL portions of chloroform. The organic layers were combined and washed with saturated sodium hydrogencarbonate (100 mL), water (100 mL) and brine (100 mL), followed by drying over anhydrous magnesium sulfate and evaporation of the solvent yielding a dirty solid. Final purification by flash column chromatography (chloroform 50% ethyl acetate) and evaporation of the fractions yielded a white solid (5.1 g, 14.4 mmol, 47%). R_f: 0.38 (chloroform:methanol 9:1). Melting point: 70.9°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (ad, *J* = 8.3, 2H), 7.24 (ad, *J* = 7.9, 2H), 6.05 (dd, *J* = 4.9, 7.2, 1H), 5.02 – 4.92 (m, 1H), 4.16 – 4.01 (m, 4H), 4.01 – 3.89 (m, 1H), 3.36 – 3.18 (m, 1H), 3.18 – 3.00 (m, 1H), 2.36 (s, 3H), 1.25 (dd, *J* = 7.2, 14.9, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.57, 137.00, 129.88 (2xCH), 127.28 (2xCH), 67.00 (d, *J* = 162.9, 1H), 63.48 (d, J = 7.9, CH_2), 63.40 (d, J = 7.5, CH_2), 44.72 (d, *J* = 7.1, 1H), 21.67, 16.58 (d, *J* = 5.6, CH_3), 16.57 (d, *J* = 5.5, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.93. LC-MS (ESI): t_R = 6.87 min. HRMS (ESI): for $[M+H]^+$ calc.: 352.09782, found: 352.09828.

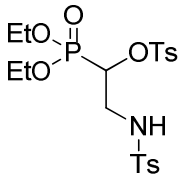


Diethyl (2-oxido-3-tosyl-1,2,3-oxathiazolidin-5-yl)phosphonate (103): Under argon, in a 50 mL, flame dried schlenk flask, alcohol **102** (575 mg, 1.64 mmol, 1 equiv) was dissolved in 3.7 mL anhydrous THF. The mixture was cooled to -45C and thionyl chloride (166 µL, 273 mg, 2.29 mmol, 1.4 equiv) was added dropwise over 2 minutes. Pyridine (330 µL, 324 mg, 4.1 mmol, 2.5 equiv) was added dropwise over 10 minutes, after addition was complete the mixture was stirred at the same temperature for one hour, before being allowed to warm to rt, the solution changed into a thick white suspension during this time. After stirring for an additional hour saturated aqueous sodium bicarbonate (10 mL) was added followed by 10 mL ethyl acetate. The layers were separated and the aqueous phase was washed with one additional portion of ethyl acetate (10 mL), the organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue (633 mg) was used in the next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.80 – 7.71 (m, 2H), 7.36 – 7.27 (m, 2H), 5.24 (ddd, *J*=10.7, 6.8, 4.0, 1H), 4.19 – 4.08 (m, 4H), 3.63 (dddd, *J*=19.7, 13.0, 8.8, 7.2, 2H), 2.39 (s, 3H), 1.26 (t, *J*=7.1, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.75. LC-MS (ESI): t_R = 8.57 min. HRMS (ESI): for $[M+H]^+$ calc.: 398.04916, found: 398.04894.

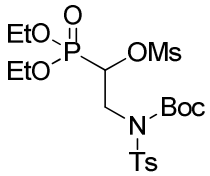


1-(Diethoxyphosphoryl)-2-(4-methylphenylsulfonamido)ethyl methanesulfonate (107): At -10°C, in a round bottom flask alcohol **102** (100 mg, 0.29 mmol, 1 equiv) was dissolved in 1.1 mL dichloromethane. Triethylamine (87 mg, 120 µL, 0.86 mmol, 3 equiv) and mesyl chloride (65 mg, 44 µL, 0.57 mmol, 2 equiv) were sequentially added. The mixture was stirred at the same temperature for 15 minutes and then allowed to warm to room temperature over one hour. The mixture was poured into water (5 mL)

and dichloromethane (5 mL) was added. The phases were separated and the aqueous phase was washed with two additional 5 mL portions of dichloromethane. The organic layers were combined and washed with 10% aqueous sodium bicarbonate (10 mL), brine (10 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated yielding 115 mg (0.25 mmol, 85% (corrected for 10 percent residual starting material by NMR)) of a clear oil that was used in the next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.71 (m, 2H), 7.31 – 7.27 (m, 2H), 5.75 (t, *J* = 6.3 Hz, 1H), 4.86 (ddd, *J* = 10.7, 7.9, 3.8 Hz, 1H), 4.28 – 4.06 (m, 4H), 3.51 – 3.27 (m, 2H), 3.17 (s, 3H), 2.40 (s, 3H), 1.31 (qd, *J* = 7.1, 0.4 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 16.13.

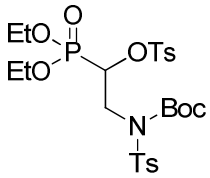


1-(Diethoxyphosphoryl)-2-(4-methylphenylsulfonamido)ethyl 4-methylbenzenesulfonate (108): tosyl amide **102** (200 mg, 0,57 mmol, 1 equiv) was dissolved in dichloromethane (2.5 mL). Triethylamine (0.42 mL, 303 mg, 1.71 mmol, 3 equiv) was added, followed by tosyl chloride (217 mg, 1.14 mmol, 2 equiv). The mixture was stirred for two hours and poured into water (20 mL). Dichloromethane (20 mL) was added and the layers were separated and the aqueous phase was washed with 3 additional portion of dichloromethane (10 mL), the organic layers were combined, washed with saturated aqueous sodium bicarbonate solution (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. The compound was obtained as a 1:1 mixture with p-toluenesulfonic acid (303 mg, 0.6 mmol, 96% (corrected)). No further purification was performed due to the compounds instability. R_f: . ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.82 – 7.77 (m, 2H), 7.75 – 7.70 (m, 2H), 7.38 – 7.34 (m, 2H), 7.33 – 7.29 (m, 2H), 4.76 – 4.65 (m, 1H), 4.22 – 3.95 (m, 5H), 3.51 – 3.18 (m, 2H), 2.46 (s, 3H), 2.43 (s, 3H), 1.27 (dtd, *J*=11.0, 7.1, 0.5, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = . ^{31}P NMR (162 MHz, $CDCl_3$) δ = 16.13. LC-MS (ESI): t_R = 8.93 min. HRMS (ESI): for $[M+H]^+$ calc.: 506.10667, found: 506.10611.

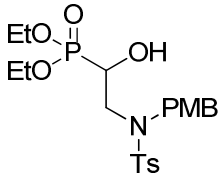


2-(*N*-(Tert-butoxycarbonyl)-4-methylphenylsulfonamido)-1-(diethoxyphosphoryl)ethyl methanesulfonate (109): Under argon, in a 5 mL round bottom schlenk, sulfonyl amide **107** (134 mg, 0.31 mmol, 1 equiv) was dissolved in 1.24 mL dichloromethane. Boc-anhydride (82 mg, 0.37 mmol, 1.2 equiv) was added followed by triethylamine (47 µL, 34 mg, 0.34 mmol, 1 equiv) and DMAP (4 mg, 0.031 mmol, 0.1 equiv). The mixture was stirred until disappearance of the starting material on TLC, diluted with 10 mL dichloromethane and poured into 10% aqueous citric acid (20 mL). The layers were

separated and the aqueous phase was washed with 3 additional portion of dichloromethane (10 mL), the organic layers were combined, washed with saturated aqueous sodium bicarbonate (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was further purified by flash column chromatography (toluene:ethyl acetate 1:1) yielding 127 mg (0.24 mmol, 77%) of a clear oil that solidifies on standing. R_f: 0.31 (toluene:ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.87 – 7.77 (m, 2H), 7.37 – 7.29 (m, 2H), 5.32 (ddd, *J*=10.6, 9.7, 2.5, 1H), 4.50 (ddd, *J*=15.3, 10.7, 5.6, 1H), 4.33 – 4.22 (m, 4H), 4.10 (ddd, *J*=15.3, 2.5, 1.5, 1H), 3.23 (s, 3H), 2.44 (s, 3H), 1.40 (t, *J*=7.1, 6H), 1.36 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = . ^{31}P NMR (162 MHz, $CDCl_3$) δ = 16.20. LC-MS (ESI): t_R = 9.29 min. HRMS (ESI): for $[M+H]^+$ calc.: 530.12780, found: 30.12760.

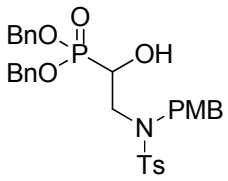


2-(*N*-(Tert-butoxycarbonyl)-4-methylphenylsulfonamido)-1-(diethoxyphosphoryl)ethyl 4-methylbenzenesulfonate (110): Under argon, in a 5 mL round bottom schlenk, sulfonyl amide **108** (348 mg, 0.48 mmol, 1 equiv) was dissolved in 2 mL dichloromethane. Boc-anhydride (126 mg, 0.58 mmol, 1.2 equiv) was added followed by triethylamine (67 µL, 49 mg, 0.48 mmol, 1 equiv) and DMAP (6 mg, 0.048 mmol, 0.1 equiv). The mixture was stirred until disappearance of the starting material on TLC, diluted with 10 mL dichloromethane and poured into water. The layers were separated and the aqueous phase was washed with 3 additional portion of dichloromethane (10 mL), the organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was further purified by flash column chromatography (toluene:ethyl acetate 3:1) yielding 143 mg (0.24 mmol, 49%) of a clear oil that solidifies on standing. R_f: . ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.91 – 7.82 (m, 4H), 7.35 – 7.28 (m, 4H), 5.43 – 5.32 (m, 1H), 4.40 (ddd, *J*=15.3, 10.1, 7.9, 1H), 4.23 – 4.03 (m, 5H), 2.43 (t, *J*=6.9, 6H), 1.39 – 1.24 (m, 15H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = . ^{31}P NMR (162 MHz, $CDCl_3$) δ = 16.10. LC-MS (ESI): t_R = 10.13 min. HRMS (ESI): for $[M+H]^+$ calc.: 606.15910, found: 606.15970.



Diethyl 1-hydroxy-2-(*N*-(4-methoxybenzyl)-4-methylphenylsulfonamido)ethylphosphonate (112): In a 25 mL round bottom flask, tosyl amide **102** (256 mg, 0.54 mmol, 1 equiv) was dissolved in 3 mL DMF. Cesium carbonate (352 mg, 1.08 mmol, 2 equiv) was added followed by 4-methoxybenzyl bromide (217 mg, 1.08 mmol, 2 equiv). The resulting mixture was stirred for three hours and then poured into saturated aqueous ammonium chloride (10 mL) and ethyl acetate (10 mL). The layers were separated and the aqueous phase was washed with 3 additional portion of ethyl acetate (10 mL), the organic layers

were combined, washed with water (10 mL), brine (2x 10 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was further purified by flash column chromatography (chloroform:ethyl acetate 4:1 in increasing gradient to 1:1) yielding 292 mg (0.49 mmol, 91%) of a yellow oil that solidifies on standing. R_f: . ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (bs, 1H), 7.74 – 7.69 (m, *J*=6.6, 1.1, 2H), 7.34 – 7.30 (m, 2H), 7.19 – 7.14 (m, 2H), 6.84 – 6.80 (m, 2H), 4.50 (d, *J*=14.4, 1H), 4.19 (d, *J*=14.5, 1H), 4.14 – 4.00 (m, 4H), 3.77 (s, 3H), 3.77 – 3.76 (m, 1H), 3.46 (ddd, *J*=12.9, 10.3, 5.2, 1H), 3.40 – 3.30 (m, 1H), 2.43 (s, 3H), 1.31 – 1.20 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.71, 143.97, 136.37, 130.19, 130.07, 128.81, 127.54, 114.37, 114.17, 67.17 (d, *J*=160.9), 63.12 (d, *J*=35.0), 63.05 (d, *J*=34.9), 55.48, 54.96, 53.13, 49.67 (d, *J*=9.1), 36.67, 21.75, 16.64 (d, *J*=5.5), 16.60 (d, *J*=5.5). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 22.20. LC-MS (ESI): t_R = 8.61 min. HRMS (ESI): for $[M+H]^+$ calc.: 472.15534, found: 472.15527.

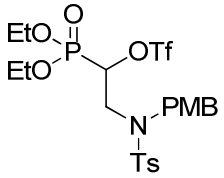


Dibenzyl 1-hydroxy-2-(*N*-(4-methoxybenzyl)-4-methylphenylsulfonamido)ethylphosphonate (113):

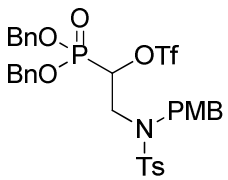
Method A: In a 10 mL round bottom flask alcohol **94** (256 mg, 0.54 mmol, 1 equiv) was dissolved in DMF (2.7 mL). Cesium carbonate (352 mg, 1.08 mmol, 2 equiv) was added followed by 4-methoxybenzyl bromide (217 mg, 1.08 mmol, 2 equiv) and the mixture was vigorously stirred for three hours. It was then poured into saturated aqueous ammonium chloride (10 mL) and ethyl acetate (10 mL). The layers were separated and the aqueous layer was extracted twice with ethyl acetate (10 mL). The organic layers were combined, washed with water (20 mL) and twice with brine (20 mL). The resulting solution was dried over anhydrous magnesium sulfate and the solvent was removed in vacuo. The resulting yellow oil was purified by column chromatography (chloroform:ethyl acetate 4:1 in increasing gradient to 1:1) yielding 292 mg (0.49 mmol, 91%) of a yellow oil that solidified on standing.

Method B: In a 250 mL three-necked, round bottom flask containing an egg-shaped stirring bar, TMS-alcohol **129** (36 g, 54 mmol, 1 equiv) was dissolved in methanol (100 mL). Potassium fluoride (6.3 g, 108 mmol, 2 equiv) was added in one portion, which dissolves completely. After 15 minutes a white solid precipitated out of the reaction mixture and an additional 100 mL of methanol was added to ensure efficient stirring. After an additional 15 minutes, TLC indicated complete consumption of the starting material. The reaction mixture was poured into a separatory funnel containing saturated aqueous ammonium chloride (200 mL), ethyl acetate (200 mL) was added and upon shaking all white solid dissolved. The layers were split and the aqueous layer was washed with two additional portions of ethyl acetate (200 mL), organic layers were combined, washed with saturated aqueous sodium hydrogen carbonate (300 mL), water (300 mL) and brine (300 mL), the mixture was dried over anhydrous sodium sulfate and evaporation of the solvent yielded a white solid (30.3 g, 50.9 mmol, 94%) that required no further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (ad, *J* = 8.3, 2H), 7.39 – 7.28 (m, 10H), 7.26 (ad, *J* = 8.0, 2H), 7.14 (ad, *J* = 8.6, 2H), 6.77 (ad, *J* = 8.6, 2H), 5.10 – 4.93 (m, 4H), 4.49 (d, *J* = 14.5, 1H), 4.21 (d, *J* =

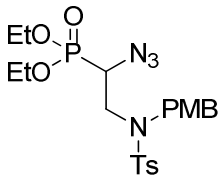
14.5, 1H), 3.98 (ddd, *J* = 2.3, 7.9, 10.2, 1H), 3.76 (s, 3H), 3.51 (ddd, *J* = 5.3, 10.3, 15.5, 1H), 3.45 – 3.37 (m, 1H), 2.43 (s, 3H), OH is not visible. ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.6, 143.8, 136.4, 136.2 (d, *J* = 6.5 Hz, 1C), 136.2 (d, *J* = 6.7 Hz, 1C), 130.1, 129.9, 128.7 (broad, 2x2C), 128.6, 128.6, 128.1, 128.1, 127.7, 127.5, 114.3, 68.4 (d, *J* = 21.6 Hz, 1C), 68.4 (d, *J* = 21.7, 1C), 67.5 (d, *J* = 160.1, 1C), 55.4, 52.9, 49.4 (d, *J* = 9.6, 1C), 21.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.9. Melting point: 96.3-97.2°C. R_f: 0.40 (toluene:ethyl acetate 1:1). LC-MS (ESI): t_R = 10.59 min. HRMS (ESI): for $[M+H]^+$ calc.: 596.18664, found: 596.18603. R_f: 0.40 (toluene:ethyl acetate 1:1).



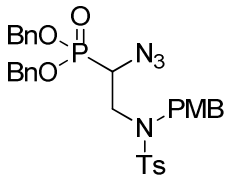
1-(Diethoxyphosphoryl)-4-(4-methoxyphenyl)-3-tosylbutyl trifluoromethanesulfonate (114): Under argon in a flame dried 25 mL schlenk flask equipped with an egg-shaped stirrer bar, alcohol **112** (157 mg, 0.36 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (3.5 mL). Pyridine (57 mg, 58 µL, 0.72 mmol, 2 equiv) was added in one batch, followed by cooling to -15°C with an ice-salt bath. Dropwise addition of triflic anhydride (152 mg, 91 µL, 0.54 mmol, 1.5 equiv) over 5 minutes yielded a yellow solution that turned to bright pink within minutes. After stirring at the same temperature for 1.5 hours, TLC confirmed complete consumption of the starting material. The mixture was poured into an ice cold solution of 5% aqueous citric acid (5 mL), turning the reaction mixture yellow, a few shakes of the separatory funnel turned the organic layer bright pink again. The layers were split and the aqueous layer was washed with three portions of CH_2Cl_2 (5 mL). Organic layers are combined, washed with water (10 mL) and brine (10 mL), dried over anhydrous magnesium sulfate and the solvent was evaporated, yielding a pink oil (assumed quantitative). The oil is co-evaporated with toluene twice to remove any traces of CH_2Cl_2, and used in the next reaction without further purification due to its potential instability. The yield was assumed quantitative. R_f: 0.21 (toluene:ethyl acetate 3:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.68 (m, 2H), 7.34 – 7.30 (m, 2H), 7.06 – 7.01 (m, 2H), 6.82 – 6.77 (m, 2H), 5.07 (td, *J* = 3.2, 9.4, 1H), 4.42 (d, *J* = 14.5, 1H), 4.22 (d, *J* = 14.5, 1H), 4.19 – 4.06 (m, 4H), 3.77 (s, 3H), 3.70 – 3.57 (m, 1H), 3.51 (dt, *J* = 3.2, 15.9, 1H), 2.44 (s, 3H), 1.36 – 1.27 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.83 (C), 144.13 (C), 135.71 (C), 130.72 (CH), 130.02 (CH), 127.74 (CH), 126.31 (C), 116.85 (C), 114.36 (CH), 79.33 (d, *J* = 159.8, CH), 64.30 (d, *J* = 11.8, CH_2), 64.23 (d, *J* = 10.9, CH_2), 55.42 CH_3), 53.30 (CH_2), 47.12 (d, *J* = 7.6, CH_2), 21.68 (CH_3), 16.39 (d, *J* = 10.2, CH_3), 16.33 (d, *J* = 10.3, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.84. ^{19}F NMR (377 MHz, $CDCl_3$) δ -74.41. LC-MS (ESI): t_R = 10.13 min. HRMS (ESI): for $[M+H]^+$ calc.:604.10462, found: 604.10431.



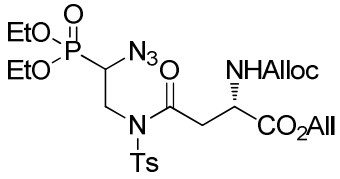
1-(Bis(benzyloxy)phosphoryl)-2-(*N*-(4-methoxybenzyl)-4-methylphenylsulfonamido)ethyl trifluoromethanesulfonate (115): Under argon in a flame dried 250 mL schlenk flask equipped with an egg-shaped stirrer bar, alcohol **113** (11.2 g, 18.8 mmol, 1 equiv) was dissolved in anhydrous dichloromethane (130 mL). Pyridine (3.0 g, 3.1 mL, 37.6 mmol, 2 equiv) was added in one batch, followed by cooling to -15°C with an ice-salt bath. Dropwise addition of triflic anhydride (8.0 g, 4.8 mL, 28.2 mmol, 1.5 equiv) over 5 minutes yielded a yellow solution that turned to bright pink after a few minutes. After stirring at -15°C for 1.5 hours, TLC confirmed complete consumption of the starting material. The mixture was poured into an ice cold solution of 5% aqueous citric acid (150 mL). The layers were split and the aqueous layer was washed with three portions of dichloromethane (100 mL). Organic layers were combined, washed with water (200 mL) and brine (200 mL), dried over anhydrous magnesium sulfate and the solvent was evaporated, yielding a pink oil (13.4g, 18.4 mmol, 98%). The oil was co-evaporated with toluene twice to remove any traces of dichloromethane, and used in the next reaction without further purification due to its instability. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65 – 7.58 (m, 2H), 7.38 – 7.31 (m, 6H), 7.31 – 7.23 (m, 6H), 6.99 – 6.93 (m, 2H), 6.76 – 6.70 (m, 2H), 5.20 – 5.11 (m, 1H), 5.04 – 4.92 (m, 4H), 4.35 (d, *J* = 14.5, 1H), 4.17 (d, *J* = 14.5, 1H), 3.75 (s, 3H), 3.64 – 3.54 (m, 1H), 3.52 – 3.43 (m, 1H), 2.43 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ 159.8, 144.1, 135.7, 135.3 (d, *J* = 8.2 Hz, 1C), 135.2 (d, *J* = 8.5 Hz, 1C), 130.7, 130.0, 129.1 (br, 2x2CH), 128.9 (br, 2x2CH), 128.6, 128.6, 127.8, 126.3, 114.4, 79.5 (d, *J* = 160.9, 1C), 69.6 (d, *J* = 11.6 Hz, 1C), 69.5 (d, *J* = 11.2 Hz, 1C), 55.5, 53.4, 47.2 (d, *J* = 7.6, CH_2)., 21.8. ^{31}P NMR (162 MHz, $CDCl_3$): δ 14.9. ^{19}F NMR (377 MHz, $CDCl_3$): δ -74.3. R_f: 0.64 (toluene:ethyl acetate 3:1). HRMS (ESI): for $[M+H]^+$ calc.: 728.13592, found: 728.13607.



Diethyl (1-azido-2-(*N*-(4-methoxybenzyl)-4-methylphenylsulfonamido)ethyl)phosphonate (116): Under argon in a 5 mL round bottom Schlenk, triflate **114** (173 mg, 0.29 mmol, 1 equiv) was dissolved in 1.2 mL acetonitrile. TMGA (34 mg, 0.33 mmol, 1.1 equiv) was added and the mixture was stirred for 16 hours. The solvent was evaporated, the residue dissolved in water (5 ml) and ethyl acetate (5 mL) and the layers were separated. The aqueous layer was washed with two additional portions of ethyl acetate (5 mL) and the organic layers were combined, washed with saturated aqueous sodium bicarbonate (10 mL), water (10 mL) and brine (10 mL). After removal of the solvent in vacuo a yellow oil remained that was not purified further (123 mg, 0.25 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.62 (m, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.16 – 7.06 (m, 2H), 6.81 – 6.72 (m, 2H), 4.33 (d, *J* = 14.6 Hz, 1H), 4.23 (d, *J* = 14.6 Hz, 1H), 4.13 – 3.94 (m, 4H), 3.72 (s, 3H), 3.63 (ddd, *J* = 13.7, 8.6, 3.1 Hz, 1H), 3.37 (dt, *J* = 15.2, 2.7 Hz, 1H), 3.27 – 3.15 (m, 1H), 2.38 (s, 3H), 1.97 (s, 2H), 1.22 (ddd, *J* = 20.8, 10.8, 6.4 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.99. LC-MS (ESI): t_R = 9.46 min.

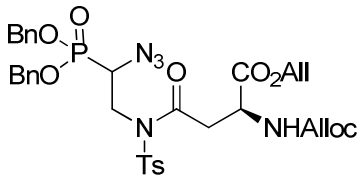


Dibenzyl 1-azido-2-(*N*-(4-methoxybenzyl)-4-methylphenylsulfonamido)ethylphosphonate (117): Under an argon atmosphere, in a 250 mL, flame dried schlenk flask, triflate **115** (136.7 g, 50.4 mmol, 1 equiv) was dissolved in anhydrous acetonitrile (130 mL). N,N,N',N'-tetramethylguanidinium azide (8.8 g, 55.4 mmol, 1.1 equiv) was rapidly weighed (hygroscopic!) and added to the pink solution in one portion. The resulting mixture was allowed to stir for 2 hours, after which a TLC confirmed complete consumption of the starting material (If the reaction was left to age after completion, yield was diminished). Reaction mixture was poured into saturated aqueous sodium hydrogen carbonate (250 mL), layers were separated and the aqueous layer was washed with ethyl acetate (3 x 150 mL). The organic layers were combined, washed with 10% citric acid (250 mL), water (250 mL), brine (250 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded a thick orange oil that was purified by flash column chromatography (toluene:ethyl acetate 24:1 in increasing gradient to 9:1) yielding a clear oil that solidified on standing (21.9 g, 35.2 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.3, 2H), 7.45 – 7.30 (m, 10H), 7.27 (d, *J* = 8.1, 2H), 7.15 (d, *J* = 8.6, 2H), 6.82 (d, *J* = 8.7, 2H), 5.12 – 4.92 (m, 4H), 4.34 (d, *J* = 14.6, 1H), 4.28 (d, *J* = 14.6, 1H), 3.82 (ddd, *J* = 2.6, 10.8, 13.6, 1H), 3.77 (s, 3H), 3.48 – 3.38 (m, 1H), 3.35 – 3.26 (m, 1H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.5, 143.7, 136.1, 135.6 (d, *J* = 5.8 Hz, 1C), 135.5 (d, *J* = 5.7 Hz, 1C), 130.0, 129.8, 128.7, 128.7, 128.7 (br, 2x2C), 128.1, 128.1, 127.4, 127.3, 114.1, 68.6 (d, *J* = 12.9 Hz, 1C), 68.6 (d, *J* = 13.0 Hz, 1C), 57.8 (d, *J* = 151.8, 1C), 55.2, 53.0, 47.1 (d, *J* = 7.8, 1C), 21.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.7. IR ν = 3064 (w), 3032 (w), 2950 (bw), 2836 (w), 2134 (s), 1611 (m), 1511 (s), 1456 (m), 1371 (w), 1333 (s), 1287 (m), 1246 (s), 1217 (m), 1174 (m), 1155 (s), 1119 (m), 1101 (m), 1087 (m), 1032 (m), 992 (s), 951 (s), 920 (s), 878 (m), 815 (m), 801 (s), 786 (w) 764 (w), 741 (s), 697 (s), 653 (s), 636 (w). Melting point: 88.6-89.8°C. R_f: 0.50 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.40 min. HRMS (ESI): for $[M+H]^+$ calc.: 621.19312, found: 621.19268.

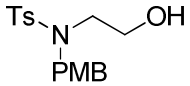


(2*S*)-Allyl 2-(((allyloxy)carbonyl)amino)-4-(*N*-(2-azido-2-(diethoxyphosphoryl)ethyl)-4-methylphenylsulfonamido)-4-oxobutanoate (118): Under argon, in a 5 mL, flame dried, schlenk flask containing an egg-shaped stirring bar, Azide **97** (50 mg, 0.13 mmol, 1 equiv) and amino acid **67** (56 g, 0.15 mol, 1.1 equiv) were dissolved in 0.5 mL anhydrous dichloromethane. After cooling to 0°C, PyBrOP (70 g, 0.15 mmol, 1.1 equiv), DIPEA (38 g, 51 µL, 0.15 mmol, 2.2 equiv) and DMAP (18 g, 0.15 mmol, 1.1 equiv) were added sequentially. After stirring the resulting mixture for 3.5 hours, a TLC confirmed complete conversion of the azide. The mixture was poured into 10% citric acid (5 mL), the layers were separated and the aqueous layer was washed twice with dichloromethane (5 mL). The organic layers were combined, washed with saturated aqueous sodium hydrogen carbonate solution (5 mL), water (5

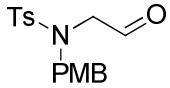
mL) and brine (5 mL). Drying over anhydrous magnesium sulfate, followed by evaporation of the solvent, yielded a yellow oil that was purified further by flash column chromatography (petroleum ether:ethyl acetate 1:1) yielding 72 mg of a yellow oil that still contained some impurities (73% corrected by NMR) and was a mixture of diastereoisomers (1:1 by ^{31}P-NMR). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.69 (m, 2H), 7.32 – 7.28 (m, 1H), 7.24 – 7.20 (m, 1H), 5.91 – 5.61 (m, 2H), 5.29 – 5.07 (m, 5H), 4.61 – 4.40 (m, 5H), 4.27 – 3.90 (m, 7H), 3.37 – 3.21 (m, 2H), 2.39 (s, 2H), 2.35 (s, 1H), 1.32 (td, *J* = 7.1, 1.9 Hz, 4H), 1.30 – 1.21 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.55, 171.41, 170.38, 156.00, 145.77, 143.53, 137.37, 135.93, 135.89, 132.89, 132.68, 132.01, 131.61, 131.58, 130.26, 129.83, 127.91, 127.87, 127.22, 118.78, 118.67, 118.30, 117.91, 117.60, 66.41, 66.22, 66.02, 63.74, 63.67, 63.50, 63.43, 58.17, 57.65, 57.50, 56.65, 56.15, 56.01, 53.65, 50.74, 50.55, 46.67, 45.74, 45.35, 45.26, 42.68, 42.63, 39.54, 39.46, 36.91, 26.14, 24.49, 21.83, 21.65, 16.66, 16.61, 16.57. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.30, 19.28.



(2*S*)-Allyl 2-(allyloxycarbonylamino)-4-(*N*-(2-azido-2-(bis(benzyloxy)phosphoryl)ethyl)-4-methylphenylsulfonamido)-4-oxobutanoate (119): Under argon, in a 250 mL, flame dried, round bottom schlenk flask containing an egg-shaped stirring bar, azide **66** (12 g, 24 mmol, 1 equiv) and amino acid **67** (7.4 g, 28.8 mol, 1.2 equiv) were dissolved in 120 mL anhydrous dichloromethane. After cooling to 0°C, PyBroP (13.4 g, 28.8 mmol, 1.2 equiv), DIPEA (7.4 g, 19 mL, 57.6 mmol, 2.4 equiv) and DMAP (3.5 g, 28.8 mmol, 1.2 equiv) were added sequentially. After stirring the resulting mixture for 3.5 hours, a TLC confirmed complete conversion of the azide. The mixture was poured into 10% citric acid (100 mL), the layers were separated and the aqueous layer was washed twice with dichloromethane (100 mL). The organic layers were combined, washed with saturated aqueous sodium hydrogen carbonate solution (150 mL), water (150 mL) and brine (100 mL). Drying over anhydrous magnesium sulfate, followed by evaporation of the solvent, yielded a yellow oil that was purified further by flash column chromatography (toluene:ethyl acetate 50:1 in increasing gradient to 12:1) yielding 14.1 g (19 mmol, 79%) of a yellow oil as a mixture of diastereoisomers (1:1.14 by ^{31}P-NMR). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (ad, *J* = 8.5, 2H), 7.41 – 7.33 (m, 10H), 7.33 – 7.28 (m, 2H), 5.97 – 5.66 (m, 2H), 5.33 – 4.99 (m, 8H), 4.62 – 4.46 (m, 5H), 4.14 – 3.92 (m, 3H), 3.54 – 3.01 (m, 1H), 2.43 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 171.3, 170.3, 155.9, 145.6, 135.7, 135.6, 135.49, 135.48, 135.47, 135.44, 135.43, 132.6, 131.5, 131.5, 130.11, 130.10, 128.96, 128.95, 128.93, 128.90, 128.83, 128.82, 128.81, 128.45, 128.41, 128.38, 127.88, 127.82, 118.7, 118.6, 117.9, 69.16, 69.10, 69.05, 69.04, 69.02, 68.98, 68.95, 66.3, 66.0, 57.9, 57.7, 56.4, 56.2, 50.4, 45.15, 45.11, 45.06, 45.02, 39.4, 39.3, 21.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.3, 20.2. IR v: 3293 (b w), 3034 (w), 2955 (w), 2118 (s), 1719 (s), 1649 (w), 1596 (w), 1500 (m), 1455 (m), 1362 (s), 1332 (m), 1258 (s), 1209 (s), 1164 (s), 1119 (w), 1087 (m), 989 (s), 813 (m), 777 (w), 733 (s), 697 (s), 668 (s). R_f: 0.76 (chloroform:ethyl acetate 1:1). LC-MS (ESI): t_R = 10:15 min. HRMS (ESI): for $[M+H]^+$ calc.: 740.21498, found: 740.21490.



***N*-(2-Hydroxyethyl)-*N*-(4-methoxybenzyl)-4-methylbenzenesulfonamide (120)**: In a 25 mL round bottom flask, equipped with a small, egg-shaped stirring bar, N-tosyl-ethanolamine (200 mg, 0.93 mmol, 1 equiv) was dissolved in three mL DMF and cesium carbonate (333 mg, 1.02 mmol, 1.1 equiv) was added. p-methoxybenzyl chloride (153 mg, 0.133 mL, 0.98 mmol, 1.05 eq.) was added dropwise over 1 minute. The resulting reaction mixture was stirred for two hours after which a TLC sample indicated complete conversion. The solvent was evaporated on a rotary evaporator equipped with a dry ice condenser and an oil pump, the residue was dissolved in 20 mL ethyl acetate and 20 mL water. The layers were separated, the aqueous layer was washed once with 20 mL ethyl acetate, the organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated yielding a clear oil that was further purified by flash column chromatography (hexanes:ethyl acetate 3:1 in increasing gradient to 2:1) yielding 259 mg (0.77 mmol, 83%) of a clear oil that slowly solidifies on standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.67 (m, 2H), 7.32 – 7.26 (m, 2H), 7.19 – 7.14 (m, 2H), 6.82 – 6.77 (m, 2H), 4.25 (s, 2H), 3.73 (s, 3H), 3.42 (t, *J* = 5.8, 2H), 3.17 (t, *J* = 5.8, 2H), 2.45 (bs, 1H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.27 (C), 143.50 (C), 136.15 (C), 129.75 (2xCH), 129.65 (2xCH), 128.10 (C), 127.15 (2xCH), 114.03 (2xCH), 60.78 (CH_2), 55.16 (CH_3), 52.69 (CH_2), 50.12 (CH_2), 21.41 (CH_3). R_f: 0.40 (toluene:ethyl acetate 1:1). LC-MS (ESI): t_R = 9.01 min. HRMS (ESI): for $[M+H]^+$ calc.: 336.12641, found: 336.12671.

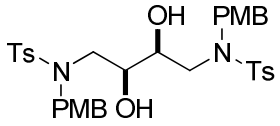


***N*-(4-methoxybenzyl)-4-methyl-*N*-(2-oxoethyl)benzenesulfonamide (122):**

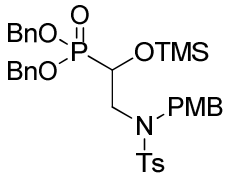
Method A: Under argon, in a 10 mL round bottom schlenk flask, oxalyl chloride (42 mg, 28 µL, 0.33 mmol, 1.1 equiv) was dissolved in anhydrous dichloromethane (1 mL) and cooled to -78°C in an acetone-dry ice bath. Dimethyl sulfoxide (56 mg, 51 µL, 0.72 mmol, 2.4 equiv) was added dropwise over one minute and the mixture was stirred at the same temperature for 15 minutes. Alcohol **120** (100mg, 0.3 mmol, 1 equiv), dissolved in 0.5 mL dichloromethane, was added dropwise over one minute and the resulting mixture was stirred 30 minutes before dropwise addition of triethyl amine (152 mg, 209 µL, 1.5 mmol, 5 equiv) over one minute. After addition was complete the mixture was stirred at -78°C for 30 minutes followed by removal of the cooling bath and warming to room temperature over 45 minutes. Water (5 mL) was added to the resulting white milky solution, turning it clear, the aqueous layer was extracted with three 5 mL portions of DCM, organic layers were combined and washed with water (5 mL), brine (5 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded 95 mg (0.28 mmol, 95%) of a white solid that requires no further purification. Can be recrystallized from ethyl acetate:petroleum ether.

Method B: In a 1 liter round bottom flask, equipped with a large egg-shaped stirring bar, diol **123** (20 g, 30 mmol, 1 equiv) was dissolved in 400 mL dioxane by sonicating the suspension one hour. The mixture was cooled in an ice bath and periodic acid (7.5 g, 33 mmol, 1.1 equiv) was slowly added, a slight

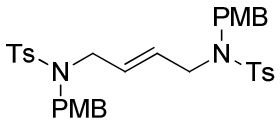
exotherm was noticed. Upon complete addition the cooling bath was removed and the mixture was stirred for one hour. TLC indicated complete conversion of the starting material. 400 mL of DCM were added, followed by about 15 g of anhydrous magnesium sulfate. After stirring for five minutes about 10 grams of celite were added and, after stirring for an additional 5 minutes, the mixture was filtered through a celite plug. The filter cake was washed with two 100 mL portions of DCM, after which the filtrate was evaporated to dryness. The residue was redissolved in 250 mL DCM and the volume of the solution reduced to about 100 mL, upon addition of 50 mL petroleum ether 40-60 and cooling white crystals crystallize out of the solution. The crystals were obtained by filtration and the mother liquor was evaporated to dryness and resubmitted to the crystallization conditions for a total of three crops (15.9 g, 0.9 g, 0.7 g) for a total of 17.5 g of white crystals (50.1 mmol, 83.5%). The aldehyde is sensitive and decomposes slowly, even under argon at -18°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (t, *J* = 1.4, 1H), 7.71 (ad, *J* = 8.3, 2H), 7.33 (ad, *J* = 8.0, 2H), 7.15 (ad, *J* = 8.7, 2H), 6.82 (ad, *J* = 8.7, 2H), 4.24 (s, 2H), 3.76 (s, 3H), 3.65 (d, *J* = 1.4, 2H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.03, 159.99, 144.21, 135.63, 130.48, 130.13, 127.61, 126.57, 114.53, 56.21, 55.45, 52.89, 21.74. R_f : 0.51 (toluene:ethyl acetate 3:1). Melting point: 117.9-118.4°C. LC-MS (ESI): t_R = 7.82 min. HRMS (ESI): for $[M+H]^+$ calc.:334.11076, found:.334.11103



***N*,*N*'-((Z)-2,3-Dihydroxybutane-1,4-diyl)bis(*N*-(4-methoxybenzyl)-4-methylbenzenesulfonamide) (123):** In a 3-necked 1L round bottom flask, fitted with a condenser and containing a large egg-shaped stirring bar, alkene **131** (73.4 g, 116 mmol, 1 equiv) was dissolved in chloroform (500 mL). Water (24 mL) and tert-butanol (57 mL) were added and the mixture was stirred vigorously. Osmium tetroxide as a 5% solution in water (5.9 mL, 1.16 mmol, 0.01 equiv) (*Osmium tetroxide is very toxic and should be handled with care*) was added via syringe, followed by spoon wise addition of N-methyl morpholine-N-oxide (41 g, 347 mmol, 3 equiv). After addition was complete the mixture was heated to 50°C in an oil-bath and stirred 16 hours, after which a TLC sample indicated complete conversion of the starting material. Sodium hydrogen sulphite (6 g, 57.6 mmol, 0.49 equiv) in 40 mL water was added and stirred for an hour to reduce excess osmium tetroxide, turning the water layer of the reaction black. The reaction mixture was transferred to a separatory funnel, the layers were separated and the aqueous layer was washed with three 200 mL portions of chloroform. The organic layers were combined, washed with water (300 mL, brine (300 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the resulting yellow foam was dissolved in a minimal amount of methanol and petroleum ether 40-60 was added, precipitating a white solid, which was filtered and the residue dried *in vacuo*, yielding 75.1 g (112.3 mmol, 97%) of a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.2, 4H), 7.31 (d, *J* = 8.0, 4H), 7.12 (d, *J* = 8.6, 4H), 6.82 (d, *J* = 8.6, 4H), 4.29 (d, *J* = 14.3, 2H), 4.11 (d, *J* = 14.3, 2H), 3.78 (s, 6H), 3.31 (s, 2H), 3.10 – 2.94 (m, 4H), 2.43 (s, 8H). ^{13}C NMR (126 MHz, CDCl3) δ 159.60 (2xC), 143.69 (2xC), 136.29 (2xC), 130.10 (2x2 CH), 129.93 (2x2 CH), 127.90 (2xC), 127.35 (2x2 CH), 114.32 (2x2 CH), 68.39 (2xCH), 55.39 ($2xOCH_3$), 53.26 ($2xCH_2$), 50.54 ($2xCH_2$), 21.63 ($2xCH_3$). R_f: 0.40 (toluene:ethyl acetate 1:1). Melting point: 157.6°C. LC-MS (ESI): t_R = 10.63 min. HRMS (ESI): for $[M+H]^+$ calc.: 669.22988, found: 669.22976.

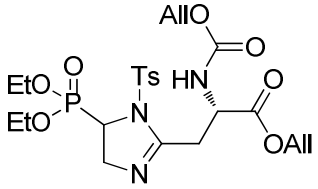


Dibenzyl 2-(*N*-(4-methoxybenzyl)-4-methylphenylsulfonamido)-1-(trimethylsilyloxy)ethylphosphonate (129): Under an argon atmosphere, in a 500 mL flame dried schlenk flask, dibenzylphosphite (11 g, 9.2 mL, 41.8 mmol, 1.2 equiv) and triethylamine (5 g, 7.0 mL, 50 mmol, 1.44 equiv) were dissolved in 150 mL of anhydrous dichloromethane. The resulting mixture was cooled to -20°C with an ice-salt bath, and chlorotrimethylsilane (5.4 g, 6.1 mL, 50 mmol, 1.44 equiv) was added dropwise over 5 minutes. After addition was complete, the mixture was stirred for one hour at -20°C, and a white precipitate formed. The reaction mixture was cooled to -78°C with acetone-dry ice and aldehyde **122**, dissolved in 100 mL dichloromethane, was added via cannula. Upon addition, the white precipitate dissolved and the mixture changes color from clear to slightly yellow. After stirring for 16 hours, complete consumption of the aldehyde was confirmed by TLC. The reaction mixture was poured into saturated aqueous NH_4Cl (250 mL), a white precipitate formed that was dissolved by addition of a small amount of water (15 mL). The layers were split and the aqueous layer was washed with three 100 mL portions of dichloromethane. The organic layers were combined and sequentially washed with aqueous saturated sodium hydrogen carbonate (200 mL), water (200 mL) and brine (200 mL); dried over anhydrous magnesium sulfate and the solvent was evaporated. The resulting crude oil was purified by flash column chromatography (toluene:ethyl acetate 49:1 in increasing gradient to 9:1) yielding **129** (17g, 26 mmol, 73%) as colorless oil. *Important: the product is unstable and slowly decomposes to the alcohol in solution, particularly under acidic conditions*. ^{1}H NMR (400 MHz, C_6D_6) δ 7.67 – 7.62 (m, 2H), 7.24 – 7.00 (m, 12H), 6.75 – 6.69 (m, 2H), 6.65 – 6.59 (m, 2H), 5.04 – 4.87 (m, 4H), 4.75 (ddd, *J* = 2.9, 7.0, 9.7, 1H), 4.54 (d, *J* = 14.7, 1H), 4.43 (d, *J* = 14.7, 1H), 3.89 – 3.81 (m, 1H), 3.63 (ddd, *J* = 8.0, 9.5, 15.1, 1H), 3.26 (s, 3H), 1.89 (s, 3H), 0.32 (s, 9H). ^{13}C NMR (101 MHz, C_6D_6) δ 159.8, 142.8, 138.1, 137.1 (d, *J* = 8.1, C), 137.0 (d, *J* = 8.1, C), 130.8, 129.7, 128.8, 128.7, 128.6, 128.56, 128.48, 128.46, 128.4, 128.4, 114.3, 70.4 (d, *J* = 161.3, C), 68.2 (d, *J* = 6.8, C), 67.9 (d, *J* = 7.0, C), 54.8, 53.5, 50.2 (d, *J* = 10.2, C)., 21.1, 0.5. ^{31}P NMR (162 MHz, C_6D_6) δ 23.4. R_f: 0.44 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.32 min. HRMS (ESI): for $[M+H]^+$ calc.: 668.22616, found: 668.22590.

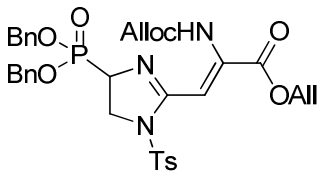


(E)-*N*,*N*'-(But-2-ene-1,4-diyl)bis(*N*-(4-methoxybenzyl)-4-methylbenzenesulfonamide) (131): In a 500 mL round bottom flask charged with a large egg-shaped stirring bar, sulphonamide **125** (73.8 g, 254 mmol, 2 equiv) was dissolved in DMF (300 mL) and 1,4-dibromo but-2-ene (27.2 g, 127 mmol, 1 equiv) was added to the stirring solution. Cesium carbonate (99.4 g, 305 mmol, 2.4 equiv) was added and the reaction mixture was stirred at room temperature for 2 hours, after which a TLC sample indicated

complete consumption of the starting material. The solvent was evaporated on a rotary evaporator equipped with a dry ice condenser and an oil pump, the residue was partitioned between chloroform (400 mL) and water (400 mL), the layers were separated and the aqueous layer was washed with five 100 mL portions of chloroform. The combined organic layers were washed 3 times with brine (100 mL), dried over magnesium sulfate and the solvent was evaporated *in vacuo*. The residue was triturated with diethyl ether (100 mL), precipitating white crystals. The solid was filtered and the residue was dried *in vacuo* overnight. The filtrate was evaporated to dryness and the brown residue was recrystallized from chloroform – diethyl ether yielding a total of 73.5 g (116 mmol, 91%) of white crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 – 7.61 (m, 4H), 7.34 – 7.27 (m, 4H), 7.09 – 7.00 (m, 4H), 6.83 – 6.74 (m, 4H), 5.14 (t, *J* = 3.3, 2H), 4.11 (s, 4H), 3.77 (s, 6H), 3.54 (d, *J* = 4.5, 4H), 2.42 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.33 (2xC), 143.43 (2xC), 137.39 (2xC), 129.85 (2x2 CH), 129.82 (2x2 CH), 128.99 (2xC), 127.85 (2xC), 127.26 (2x2 CH), 114.04 (2x2 CH), 55.34 (2xOCH_3), 50.16 (2xCH_2), 48.03 (2xCH_2), 21.59 (2xCH_3). R_f: 0.50 (toluene:ethyl acetate 9:1). Melting point: 134.9°C. LC-MS (ESI): t_R = 10.99 min. HRMS (ESI): for $[M+H]^+$ calc.: 635.22440, found: 635.22429.

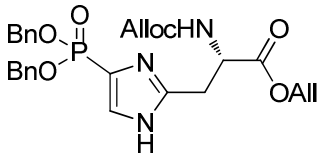


(2*S*)-Allyl 2-(allyloxycarbonylamino)-3-(5-(diethoxyphosphoryl)-1-tosyl-4,5-dihydro-1H-imidazol-2-yl)propanoate (132): In a 5 mL, flame dried pear shaped schlenk, under argon, azide **90** (20 mg, 0.03 mmol, 1 equiv) was dissolved in anhydrous THF. The reaction mixture was cooled to -41C and triphenyl phosphine (13 mg, 0.05 mmol, 1.5 equiv) was added. 5 minutes after addition the schlenk was closed and the content heated to 80C and stirred at that temperature for six hours. The solvent was evaporated and the residue loaded on a silica column, elution with dichloromethane with 3% methanol yielded 30 mg of a yellow oil that still contained a lot of TPP and TPPO (66% product according to ^{31}P-NMR) in addition to other impurities, a yield was not determined. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 30.13 ($OPPh_3$), 21.48, 21.32, 15.64 (PPh_3). LC-MS (ESI): t_R = 8.93 min. HRMS (ESI): for $[M+H]^+$ calc.: 572.18261, found: 572.18286.

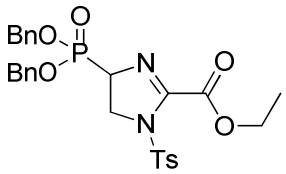


(*E*)-Allyl 2-(((allyloxy)carbonyl)amino)-3-(4-(bis(benzyloxy)phosphoryl)-1-tosyl-4,5-dihydro-1H-imidazol-2-yl)acrylate (135): Under an argon atmosphere, in a 10 mL round bottom Schlenk, imidazoline **68** (176 mg, 0.25 mmol, 1 equiv) was dissolved in 1,4-dioxane (1.3 mL). Selenium dioxide (42 mg, 0.38 mmol, 1.5 equiv) was added and the mixture was heated to 40°C. After stirring for 16 hours the mixture was poured into saturated aqueous sodium bicarbonate (5 mL). The aqueous layer was washed three times with ethyl acetate (5 mL), the organic layers were combined, washed with brine (10 mL) twice and

dried over anhydrous sodium sulfate. The resulting solution was evaporated to dryness and the residue was purified by column chromatography (chloroform:ethyl acetate 10:1) yielding a yellow oil (35 mg, 0.05 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.4 Hz, 2H), 7.33 – 7.11 (m, 12H), 6.38 (s, 1H), 6.00 – 5.86 (m, 1H), 5.69 (ddd, *J* = 16.3, 11.1, 5.2 Hz, 1H), 5.44 – 5.02 (m, 4H), 4.99 – 4.82 (m, 4H), 4.73 (dd, *J* = 5.8, 1.3 Hz, 2H), 4.51 – 4.39 (m, 2H), 4.23 (ddd, *J* = 15.2, 11.5, 8.4 Hz, 1H), 4.00 – 3.73 (m, 2H), 2.30 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.85. LC-MS (ESI): t_R = 10.60 min. HRMS (ESI): for $[M+H]^+$ calc.: 694.19826, found: 694.19812.

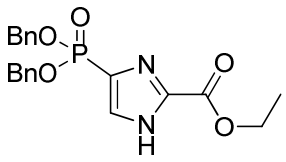


(*S*)-Allyl 2-(allyloxycarbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1H-imidazol-2-yl)propanoate (136): Under argon, in a flame dried, 500 mL schlenk flask imidazoline **68** (7 g, 10.1 mmol, 1 equiv) was dissolved in 120 mL anhydrous THF. The mixture was cooled to -78°C with acetone-dry ice and KHMDS (0.5M in toluene, 60.4 mL, 30.2 mmol, 3 equiv) was added dropwise over 20 minutes, during addition the mixture turned orange followed by red after addition of the first equivalent. After addition was complete the mixture was stirred for 15 minutes, after which a TLC indicated a small amount of starting material remained. To push the reaction to completion an additional 5 mL KHMDS solution (2.5 mmol, 0.25 equiv) was added. A second TLC taken immediately after addition was complete, showed complete conversion of the starting material. The reaction mixture was transferred by cannula to a 500 mL round bottom flask, containing an egg-shaped stirring bar and 200 mL of water with acetic acid (3.6 g, 3.5 mL, 60.4 mmol, 6 equiv) at 0°C, while vigorously stirring. After addition was complete, solid sodium hydrogen carbonate was added until the pH was 8. Chloroform (200 mL) was added and the layers were separated. The aqueous layer was washed with additional chloroform (2 x 200 mL), the organic layers were combined, washed with brine (200 mL) and dried over sodium sulfate. Evaporation yielded **136** as colorless oil that was not purified further and used 'as is' in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.11 (br s, 0.34H), 11.78 (br s, 0.62H), 11.95 (m, 1H), 7.45 (s, 1H), 7.36 – 7.20 (m, 10H), 6.51 (s, 1H), 5.78 (m, 2H), 5.30 – 4.95 (m, 8H), , 4.76 – 4.67 (m, 1H), 4.60 – 4.37 (m, 4H), 3.27 (d, *J* = 5.4, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 156.3, 136.0, 132.6, 131.5, 128.6, 128.5, 128.0, 127.9, 118.7, 117.9, 68.3, 66.3, 66.0, 52.9, 30.6. ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.4, 10.2 very broad. R_f: 0.49 (chloroform:methanol 9:1). LC-MS (ESI): t_R = 9.64 min. HRMS (ESI): for $[M+H]^+$ calc.: 540.18941, found: 540.18833.



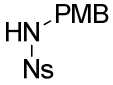
Ethyl 4-(bis(benzyloxy)phosphoryl)-1-tosyl-4,5-dihydro-1H-imidazole-2-carboxylate (138): In a 25 mL round bottom flask, containing an egg-shaped stirring bar, azide **66** (500 mg, 1 mmol; 1 equiv) was dissolved in 2 mL anhydrous THF. While stirring, DIPEA (259 mg, 0.348 mL, 2 mmol, 2 equiv) and DMAP (122 mg, 1 mmol, 1 equiv) were added, followed by chloroethyl oxoacetate (273 mg, 0.223 mL, 2 mmol,

2 equiv). Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After stirring for 1 hour at room temperature a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was removed by evaporation in vacuo resulting in a yellow oil. The oil was further purified by column chromatography (toluene:ethyl acetate 10:1) resulting in 268 mg of a yellow oil (0.45 mmol, 45%) that was unstable and was used in the next reaction as soon as possible. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.85 – 7.80 (m, 2H), 7.42 – 7.32 (m, 10H), 7.32 – 7.27 (m, 2H), 5.19 – 4.97 (m, 4H), 4.47 – 4.39 (m, 2H), 4.06 – 3.94 (m, 2H), 3.83 – 3.69 (m, 1H), 2.44 (s, 3H), 1.42 (t, *J*=7.2, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.77, 161.34, 146.46, 135.61 (d, *J*=5.8), 133.98, 130.34, 129.07, 129.06, 128.97, 128.71, 128.50, 128.48, 69.16 (d, *J*=11.4), 69.09 (d, *J*=11.5), 63.40, 56.39 (d, *J*=152.2), 43.84 (d, *J*=9.6), 21.97, 14.01. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 19.81. IR: 2983 (w), 2118 (s), 1745 (m), 1694 (s), 1596 (w), 14.96 (w), 1455 (s), 1373 (m), 1318 (m), 1295 (s), 1261 (s), 1198 (s), 1090 (m), 988 (s), 861 (m), 813 (m), 786 (m), 734 (s), 697 (s), 666 (s), 593 (s), 546 (s), 486 (s), 436 (s). R_f: 0.65 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.41 min. HRMS (ESI): for $[M+H]^+$ calc.: 601.15165, found: 601.15201. Under argon a flame dried, 100 mL schlenk flask was charged with the azide from the previous reaction (243mg, 0.40 mmol, 1 equiv) and 10 mL anhydrous THF. dppe (242 mg, 0.61 mmol, 1.5 equiv) was added and the reaction was heated to 50°C in an oil bath, steady bubble formation was noticed. Once bubble formation stopped the oil bath was set to 80°C and the schlenk flask was closed. After heating for 16 hours the flask was allowed to cool to about 50°C and 7 mL saturated aqueous sodium bicarbonate solution: 30% hydrogen peroxide 1:1 was added. The mixture was stirred until a TLC sample confirmed complete oxidation of DPPE. Chloroform (20 mL) was added, the layers were separated and the aqueous layer was extracted twice with 5 mL portions of chloroform. Organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated and the resulting oil was further purified by flash column chromatography (chloroform:ethyl acetate 3:1) yielding 166 mg (0.30 mmol, 74%) of a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.85 – 7.75 (m, 2H), 7.37 – 7.22 (m, 12H), 5.09 – 4.90 (m, 4H), 4.51 – 4.38 (m, 3H), 3.92 (dd, *J*=22.1, 10.0, 2H), 2.39 (s, 3H), 1.40 (t, *J*=7.2, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.84, 153.11 (d, *J*=13.1), 145.41, 135.97 (d, *J*=5.9), 135.95 (d, *J*=5.4), 134.29, 130.20, 128.81, 128.80, 128.74, 128.33, 128.07, 127.97, 68.87 (d, *J*=35.6), 68.80 (d, *J*=35.7), 64.22 (d, *J*=164.3), 63.48, 48.04, 21.86, 14.04. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.98. R_f: 0.15 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 9.88 min. HRMS (ESI): for $[M+H]^+$ calc.: 557.15058, found: 557.15055.

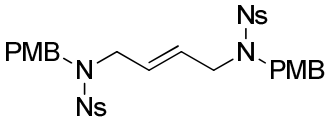


Ethyl 4-(bis(benzyloxy)phosphoryl)-1H-imidazole-2-carboxylate (139): In a flame dried 10 mL schlenk flask, containing an egg-shaped stirring bar, imidazoline **138** (20 mg, 0.036 mmol, 1 equiv) was dissolved

in 0.18 mL of anhydrous DMF. The mixture was cooled to 0°C and DBU (82 mg, 81 µL, 0.54 mmol, 15 equiv) was added. Upon complete addition a TLC was taken to confirm complete conversion of the imidazoline, and the reaction mixture was partitioned between chloroform (5 mL) and saturated aqueous sodium bicarbonate solution (5 mL). The layers were separated and the aqueous layer was washed with two additional 5 mL portions of chloroform, the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a yellow oil (quantitative) that was used as is in the next reaction. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 11.49, 7.82. R_f: 0.44 (ethyl acetate). LC-MS (ESI): t_R = 8.81 min. HRMS (ESI): for $[M+H]^+$ calc.: 401.12608, found: 401.12531.

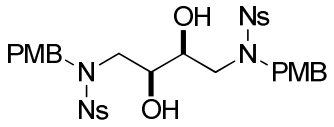


***N*-(4-Methoxybenzyl)-4-nitrobenzenesulfonamide (141-S1):** In a 250 mL round bottom flask, containing a large, egg-shaped stirring bar, 4-nitrobenzene sulfonylchloride (5.87 g, 26.5 mmol, 1 equiv) was dissolved in 80 mL of toluene. The yellow mixture was cooled to 0°C and triethylamine (5.35 g, 7.4 mL, 53 mmol, 2 equiv) was added, followed by stirring for 30 minutes. 4-Methoxybenzyl amine (3.64 g, 26.5 mmol, 1 equiv) was added dropwise over 5 minutes, forming a white precipitate. The mixture was stirred at 0°C for 30 minutes followed by stirring at room temperature for 4 hours, after which about half the toluene was removed in vacuo, followed by filtration. The residue was washed with two 20 mL portions of ice cold toluene, dissolved in boiling chloroform (200 mL), transferred to a separatory funnel and extracted twice with 100 mL hot water, once with hot brine (50 mL), dried over anhydrous sodium sulfate and the solvent was removed in vacuo yielding 7.66 g of an off-white solid (23.8 mmol, 90%). ^{1}H NMR (500 MHz, $CDCl_3$:MeOD 1:1) δ 8.38 – 8.19 (m, 2H), 8.04 – 7.87 (m, 2H), 7.06 (ad, *J* = 8.6, 2H), 6.79 – 6.65 (m, 2H), 4.29 (s, 1H), 4.08 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$:MeOD 1:1) δ 159.53, 150.07, 147.19, 129.58 (2xCH), 128.77, 128.50 (2xCH), 124.37 (2xCH), 114.16 (2xCH), 55.44, 46.83. R_f: 0.40 (toluene:ethyl acetate 8:1). Melting point: 137.4°C. LC-MS (ESI): t_R = 9.41 min. HRMS (ESI): for $[M-H]^-$ calc.: 321.05507, found: 321.05450.

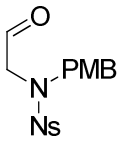


(E)-*N*,*N*′-(But-2-ene-1,4-diyl)bis(*N*-(4-methoxybenzyl)-4-nitrobenzenesulfonamide) (141): In a 250 mL round bottom flask, containing an egg-shaped stirring bar, the nosyl amine **141-S1** (7.66 g, 23.8 mmol, 2 equiv) and 1,4-dibromo-2-butene (2.54 g, 11.9 mmol, 1 equiv) were dissolved in 30 mL DMF, resulting in an orange solution. Cesium carbonate (9.3 g, 28.6 mmol, 2.4 equiv) was added turning the mixture into a dark grey-green suspension that was stirred for four hours, after which a TLC sample showed complete conversion of the starting material. 250 mL water was added precipitating a white solid. The mixture was filtered and the residue was washed with 100 mL water followed by two 50 mL portions of ethanol and two 50 mL portions of diethylether. The solid was dried in vacuo yielding 8.1 g of an off-white solid (11.6 mmol, 98%). The product is very insoluble, hot chloroform seems to work best. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 – 8.28 (m, 2H), 8.01 – 7.89 (m, 2H), 7.08 – 6.98 (m, 2H), 6.86 – 6.76 (m, 2H), 5.25 – 5.18 (m, 1H), 4.20 (s, 2H), 3.78 (s, 3H), 3.70 – 3.58 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.70, 150.12, 146.14,

129.96, 128.93, 128.37, 126.80, 124.61, 114.27, 55.46, 50.74, 48.33. R_f: 0.63 (toluene:ethyl acetate 3:1). Mp: 199.4°C (decomp.). LC-MS (ESI): t_R = 10.31 min. HRMS (ESI): for $[M+Na]^+$ calc.: 719.14521, found: 719.14451.

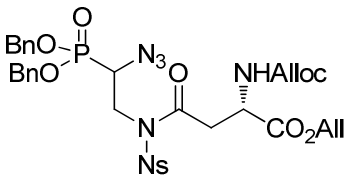


N,N'-((2*S*,3*S*)-2,3-Dihydroxybutane-1,4-diyl)bis(*N*-(4-methoxybenzyl)-4-nitrobenzenesulfonamide) (142-S1): In a 250 mL three-necked, round bottom flask, fitted with a reflux condenser and an egg-shaped stirring bar, nosyl amide **141** (8.1 g, 11.6 mmol, 1 equiv) was suspended in 100 mL chloroform, water (4.8 mL) and tert-butanol (11.4 mL). Osmium tetroxide as a 4 wt% solution in water (0.73 mL, 0.76 g, 0.12 mmol 0.01 equiv) was added followed by 4-methylmorphonline N-oxide (4.1 g, 34.8 mmol, 3 equiv), the resulting mixture was vigorously stirred and heated to 50°C. After stirring for 3 days the yellow suspension had turned into an orange solution, a TLC sample confirmed complete conversion of the starting material. Sodium thiosulfate (5.5 g, 34.8 mmol, 3 equiv) in 60 mL water was added and stirred for an hour to reduce excess osmium tetroxide and 4-methylmorphonline N-oxide, turning the water layer of the reaction black. The reaction mixture was transferred to a separatory funnel, the layers were separated and the aqueous layer was washed with three 40 mL portions of chloroform. The organic layers were combined, washed with water (70 mL), brine (50 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the resulting off-white solid was re-dissolved in a minimal amount of hot chloroform (around 80 mL) and petroleum ether 40-60 was added, precipitating a white solid, which was filtered and the residue was washed with two 100 mL portions of petroleum ether, dried *in vacuo*, yielding 7.36 g of a white solid, a second crop yielded 0.73 g for a total of 8.09 g (11.1 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, *J* = 8.5, 4H), 7.96 (d, *J* = 8.6, 4H), 7.12 (d, *J* = 8.3, 4H), 6.82 (d, *J* = 8.3, 4H), 4.43 (d, *J* = 14.2, 2H), 4.13 (d, *J* = 14.4, 2H), 3.78 (s, 6H), 3.34 – 3.20 (m, 2H), 3.08 (d, *J* = 3.3, 4H), 2.42 (d, *J* = 4.9, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.93, 150.20, 145.20, 130.10, 128.48, 126.96, 124.59, 114.53, 68.02, 55.46, 53.26, 50.47. R_f: 0.31 (toluene:ethyl acetate 3:1). Melting point: 96.6-97.8°C. LC-MS (ESI): t_R = 9.55 min. HRMS (ESI): for $[M+Na]^+$ calc.: 753.15068, found: 753.15054.

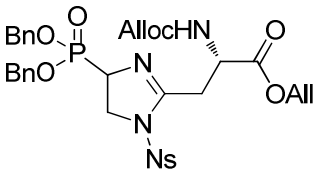


***N*-(4-Methoxybenzyl)-4-nitro-*N*-(2-oxoethyl)benzenesulfonamide (142):** In a 50 mL round bottom flask, equipped with an egg-shaped stirring bar, the diol **142-S1** (800 mg, 1.1 mmol, 1 equiv) was dissolved in 15 mL 1,4-dioxane, slight heating was required to achieve full dissolution of the diol. Periodic acid (275 mg, 1.2 mmol, 1.1 equiv) was added, turning the clear solution to a white suspension. The mixture was stirred for 90 minutes, after which a TLC sample indicated complete conversion of the starting material. Eight milliliters of DCM were added followed by about 2 grams of anhydrous magnesium sulfate were added, mixture was stirred for 5 minutes and about 2 grams of celite were added. After an additional 5

minutes of stirring the mixture was filtered through celite. The filter cake was washed with two additional portions of DCM (8 mL) and the filtrate was evaporated to dryness, yielding a yellow solid that was purified by crystallization from chloroform-petroleum ether 40-60, white crystals 734 mg (2 mmol, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.39 (d, *J* = 8.8, 2H), 8.03 (d, *J* = 8.8, 2H), 7.16 (d, *J* = 8.6, 2H), 6.86 (d, *J* = 8.6, 2H), 4.37 (s, 2H), 3.92 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.62, 160.42, 150.62, 145.40, 130.64, 128.97, 125.95, 124.83, 114.89, 77.66, 77.56, 77.41, 77.15, 55.93, 55.72, 52.54. R_f: 0.71 (toluene:ethyl acetate 1:1). Melting point: 149.7-150.4°C. The compound doesn't show up in mass detectors.

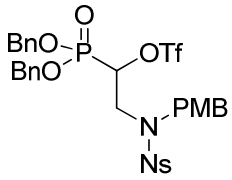


(2*S*)-Allyl 2-(((allyloxy)carbonyl)amino)-4-(*N*-(2-azido-2-(bis(benzyloxy)phosphoryl)ethyl)-4-nitrophenylsulfonamido)-4-oxobutanoate (143-S1): Under argon, in a 25 mL, flame dried, round bottom schlenk flask containing an egg-shaped stirring bar, azide **144** (1 g, 1.9 mmol, 1 equiv) and amino acid **67** (0.58 g, 2.26 mol, 1.2 equiv) were dissolved in 10 mL anhydrous dichloromethane. After cooling to 0°C, PyBroP (1.1 g, 2.26 mmol, 1.2 equiv), DIPEA (0.58 g, 0.79 mL, 4.5 mmol, 2.4 equiv) and DMAP (276 mg, 2.26 mmol, 1.2 equiv) were added sequentially. After stirring the resulting mixture for 3.5 hours, a TLC confirmed complete conversion of the azide. The mixture was poured into 10% citric acid (10 mL), the layers were separated and the aqueous layer was washed twice with dichloromethane (10 mL). The organic layers were combined, washed with saturated aqueous sodium hydrogen carbonate solution (15 mL), water (15 mL) and brine (10 mL). Drying over anhydrous magnesium sulfate, followed by evaporation of the solvent, yielded a yellow oil that was purified further by flash column chromatography (chloroform:ethyl acetate 8:1 in increasing gradient to 3:1) yielding 658 mg (0.89 mmol, 47%) of a yellow oil as a mixture of diastereoisomers (1:1.14 by ^{31}P-NMR). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.39 – 8.28 (m, 2H), 8.13 – 7.97 (m, 2H), 7.49 – 7.29 (m, 10H), 5.92 – 5.58 (m, 3H), 5.33 – 5.00 (m, 8H), 4.69 – 4.40 (m, 5H), 4.16 – 3.97 (m, 2H), 3.97 – 3.79 (m, 1H), 3.49 – 2.83 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.44, 170.24, 170.16, 155.96, 150.97, 144.13, 135.56, 135.49, 132.57, 131.45, 129.77, 129.26, 129.23, 129.21, 129.06, 128.73, 128.65, 128.63, 124.54, 119.02, 118.90, 118.20, 69.42, 69.39, 69.36, 69.25, 69.18, 66.67, 66.63, 66.20, 58.17, 56.67, 50.51, 45.66, 45.57, 39.26, 39.11. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 19.73, 19.71. R_f: 0.43 (chloroform:ethyl acetate 3:1). IR: 2119 (N_3). LC-MS (ESI): t_R = 10.04 min. HRMS (ESI): for $[M+H]^+$ calc.: 771.18440, found: 771.18515.



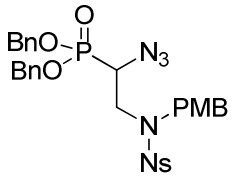
(2*S*)-Allyl 2-(allyloxycarbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-(4-nitrophenylsulfonyl)-4,5-dihydro-1H-imidazol-2-yl)propanoate (143): Under argon, in a 50 mL, flame dried schlenk flask the

amide **143-S1** (0.77 g, 1 mmol, 1 equiv) was dissolved in anhydrous THF (25 mL), 1,2-Bis(diphenylphosphino)ethane (DPPE) (0.60 g, 1.5 mmol, 1.5 equiv) was added in one batch. The schlenk flask was closed and immersed in an 80°C oil bath for 8 hours, after which the reaction mixture was allowed to cool just below boiling. A one-to-one solution of hydrogen peroxide (30% in water) (15 mL) and saturated aqueous sodium hydrogen carbonate (15 mL) was added and the mixture was stirred for 10 minutes. Once TLC indicated no more un-oxidized DPPE remained, the mixture was transferred to a separatory funnel, chloroform (50 mL) and saturated aqueous sodium hydrogen carbonate (50 mL) were added, layers separated and the aqueous layer was washed with additional portions of chloroform (2 x 30 mL). Organic layers were combined, washed with brine (50 mL) and dried over sodium sulfate, evaporation yielded an off-white sticky solid that was further purified by filtration over a 4 cm high plug of silica and elution with chloroform containing 25% ethyl acetate, yielding **143** as a yellow oil as a mixture of diastereoisomers (1:1.24 by ^{31}P-NMR) (0.596 g, 0.82 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.33 – 8.21 (m, 1H), 8.03 – 7.94 (m, 1H), 7.42 – 7.16 (m, 5H), 6.18 – 5.73 (m, 1H), 5.38 – 5.10 (m, 2H), 5.06 – 4.89 (m, 2H), 4.87 – 4.69 (m, 1H), 4.68 – 4.45 (m, 2H), 4.26 – 4.15 (m, 1H), 4.03 – 3.77 (m, 1H), 3.50 – 2.93 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.28, 158.55, 150.79, 143.57, 135.97, 135.78, 132.76, 131.85, 129.03, 128.99, 128.92, 128.61, 128.38, 128.17, 128.14, 125.01, 124.99, 124.60, 118.77, 118.63, 118.28, 118.07, 90.89, 87.85, 77.43, 73.48, 69.02, 68.96, 68.54, 68.45, 68.38, 66.57, 66.42, 66.25, 62.23, 60.61, 53.58, 51.24, 50.90, 49.17, 34.30, 32.67, 32.25. ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.26, 21.84. R_f: 0.31 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 9.91 min. HRMS (ESI): for $[M+H]^+$ calc.: 727.18334, found: 727.18373.

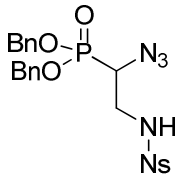


1-(Bis(benzyloxy)phosphoryl)-2-(*N*-(4-methoxybenzyl)-4-nitrophenylsulfonamido)ethyl trifluoromethanesulfonate (144-S1): Under argon in a flame dried 50 mL schlenk flask equipped with an egg-shaped stirrer bar, alcohol **145** (860 mg, 1.37 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (13 mL). Pyridine (217 mg, 221 µL, 2.74 mmol, 2 equiv) was added in one batch, followed by cooling to -15°C with an ice-salt bath. Triflic anhydride (465 mg, 277 µL, 1.65 mmol, 1.5 equiv) was added dropwise over 5 minutes. After stirring at the same temperature for 3 hours, TLC confirmed complete consumption of the starting material. The mixture was poured into an ice cold solution of 5% aqueous citric acid (20 mL). The layers were split and the aqueous layer was washed with three portions of CH_2Cl_2 (20 mL). Organic layers are combined, washed with water (50 mL) and brine (50 mL), dried over anhydrous magnesium sulfate and the solvent was evaporated, yielding a yellow oil (949 mg, 1.25 mmol, 91%).The oil is co-evaporated with toluene twice to remove any traces of CH_2Cl_2, and used in the next reaction without further purification due to its instability. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 – 8.14 (m, 2H), 7.81 – 7.71 (m, 2H), 7.45 – 7.23 (m, 10H), 6.94 (ad, *J* = 8.6, 2H), 6.76 – 6.64 (m, 2H), 5.12 (td, *J* = 3.3, 9.1, 1H), 5.06 – 4.92 (m, 4H), 4.50 – 4.39 (m, 1H), 4.33 – 4.20 (m, 1H), 3.74 (s, 3H), 3.70 – 3.46 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.00, 150.17, 144.74, 135.01 (d, J = 7.1, C), 134.94 (d, J = 7.4, C), 130.51 (2xCH), 129.28, 128.96 (2x2CH), 128.75 (2xCH), 128.67 (2xCH), 128.60 (2x2CH), 125.52, 124.42 (2xCH), 114.44 (2xCH),

78.95 (d, *J* = 161.7, CH), 69.70 (d, J = 7.1, CH_2), 69.60 (d, J = 6.7, CH_2), 55.44, 53.17, 47.48 (d, *J* = 7.8, CH_2). ^{19}F NMR (282 MHz, $CDCl_3$) δ -73.00. ^{31}P NMR (121 MHz, $CDCl_3$) δ 14.20. R_f 0.62 (toluene:ethyl acetate 2:1). HRMS (ESI): for $[M+H]^+$ calc.: 759.10535, found: 759.10565.

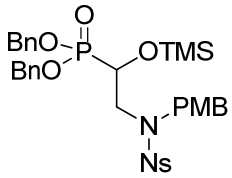


Dibenzyl (1-azido-2-(*N*-(4-methoxybenzyl)-4-nitrophenylsulfonamido)ethyl)phosphonate (144-S2): Under an argon atmosphere, in a 25 mL, flame dried schlenk flask, the triflate **144-S1** (949 mg, 1.25 mmol, 1 equiv) was dissolved in anhydrous acetonitrile (5 mL), after care was taken there was no more CH_2Cl_2 present in the triflate by co-evaporation with toluene. After cooling to 0°C in an ice bath, N,N,N',N'-tetramethylguanidinium azide (TMGA) (153 mg, 1.5 mmol, 1.2 equiv) is rapidly weighed and added to the pink solution in one batch (*Caution: very hygroscopic, forms explosive, toxic gas (HN_3) upon contact with acid*). The resulting mixture is allowed to warm up to 5°C in the ice bath over 16 hours, after which a TLC sample still showed starting material present, an additional 30 mg (0.29 mmol, 0.24 equiv) of TMGA was added followed two hours later by another 18 mg (0.18 mmol, 0.14 equiv). Thirty minutes after the final addition the starting material was consumed. Reaction mixture was poured into saturated aqueous sodium hydrogen carbonate (15 mL), layers were separated and the aqueous layer was washed with ethyl acetate (3 x 15 mL). The organic layers were combined, washed with 10% citric acid (30 mL), water (30 mL), brine (30 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded a thick orange oil that was purified by flash column chromatography (toluene containing 20% ethyl acetate) yielding a clear oil that solidified on standing (0.62 g, 0.96 mmol, 76%). ^1H NMR (400 MHz, $CDCl_3$) δ = 8.28 – 8.17 (m, 2H), 7.88 – 7.77 (m, 2H), 7.40 – 7.34 (m, 6H), 7.33 – 7.28 (m, 4H), 7.12 – 7.02 (m, 2H), 6.84 – 6.70 (m, 2H), 5.10 – 4.91 (m, 4H), 4.35 (s, 2H), 3.83 – 3.67 (m, 4H), 3.45 – 3.27 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.95, 150.19, 145.38, 135.59 (d, *J*=3.9), 130.14, 129.14, 129.13, 128.98, 128.62, 128.53, 128.49, 126.60, 124.50, 114.49, 69.02 (d, *J*=12.0), 68.95 (d, *J*=12.3), 57.76 (d, *J*=151.7), 55.52, 52.99, 47.27 (d, *J*=7.8). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.32. Ir: 2917 (w), 2122 (s), 1609 (m), 1526 (s), 1511 (s), 1456 (m), 1346 (s), 1310 (m), 1214 (s), 1178 (m), 1157 (m), 1104 (s), 1036 (s), 994 (s), 917 (s), 854 (s), 817 (m), 799 (m), 766 (w), 736 (s), 698 (s), 600 (s), 580 (m), 558 (m), 518 (m), 501 (m), 460 (m). R_f: 0.63 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 10.31 min. HRMS (ESI): for $[M+H]^+$ calc.: 652.16255, found: 652.16263.



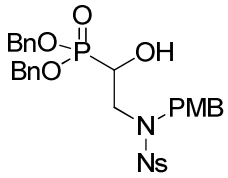
Dibenzyl 1-azido-2-(4-nitrophenylsulfonamido)ethylphosphonate (144):In a 25 mL three-necked, round bottom flask containing an egg-shaped stirrer bar, the azide **144-S2** (1.4 g, 2.2 mmol, 1 equiv) was dissolved in 1,4-dioxane (9 mL). After complete solution of the starting material water (1 mL) and Ceric

ammonium nitrate (3.6 g, 6.6 mmol, 3 equiv) were added, turning the mixture bright orange. After 6.5 hours TLC showed complete conversion of the starting material. The reaction mixture was partitioned between water (25 mL) and ethyl acetate (25 mL), the aqueous layer was washed with additional ethyl acetate (2 x 25 mL), the organic layers were combined, washed with brine (50 mL) and dried over anhydrous magnesium sulfate. Evaporation yielded an orange oil that was purified by flash column chromatography (toluene with 4% ethyl acetate gradient 20% ethyl acetate at which point compound was eluted) evaporation of the solvent yielded a white solid (941 mg, 1.87 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 – 8.16 (m, 2H), 8.02 – 7.89 (m, 2H), 7.44 – 7.28 (m, 10H), 6.51 – 6.41 (m, 1H), 5.16 – 4.95 (m, 4H), 3.88 – 3.72 (m, 1H), 3.45 – 3.31 (m, 1H), 3.24 – 3.05 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.24, 145.81, 135.43 (d, *J* = 5.8, 1C), 135.37 (d, *J* = 5.9, 1C), 129.27, 129.25, 129.05, 128.51, 128.50, 128.46, 124.61, 69.33 (d, *J* = 7.2, 1C), 69.30 (d, *J* = 7.0, 1C), 57.72 (d, *J* = 154.4, 1C), 42.69 (d, *J* = 4.7, 1C). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.60. Ir: 3105 (w), 2870 (w), 2102 (s), 1528 (s), 1455 (m), 1342 (s), 1307 (m), 1238 (s), 1213 (m), 1164 (s), 996 (s), 852 (s), 729 (s). R_f: 0.19 (toluene:ethyl acetate 4:1). Melting point: 82.5°C. LC-MS (ESI): t_R = 9.28 min. HRMS (ESI): for $[M+H]^+$ calc.: 532.10503, found: 532.10471.

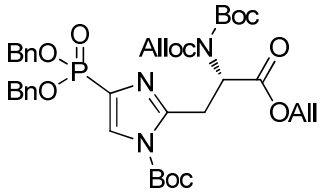


Dibenzyl (2-(*N*-(4-methoxybenzyl)-4-nitrophenylsulfonamido)-1-((trimethylsilyl)oxy)ethyl)phosphonate (145-S1): Under an argon atmosphere, in a 500 mL flame dried schlenk flask, dibenzylphosphite (12 g, 10 mL, 45.6 mmol, 1.2 equiv) and triethylamine (5.5 g, 7.6 mL, 55 mmol, 1.44 equiv) were dissolved in 150 mL of anhydrous dichloromethane. The resulting mixture was cooled to 0°C with an ice bath, and chlorotrimethylsilane (6.0 g, 6.8 mL, 55 mmol, 1.44 equiv) was added dropwise over 5 minutes. After addition was complete, the mixture was stirred for 1 hour at the same temperature. The reaction mixture was cooled to -78°C with acetone-dry ice and **142** (13.8 g, 38 mmol, 1 equiv), dissolved in 110 mL dichloromethane, was added via cannula. Upon addition the mixture's changes color from clear to slightly yellow. After stirring for 16 hours the complete consumption of the reaction is confirmed by TLC. The reaction mixture is poured into saturated aqueous NH_4Cl (250 mL), a white precipitate forms that was dissolved by addition of a small amount of water (15 mL). The layers are split and the aqueous layer was washed with three 100 mL portions of dichloromethane. The organic layers are combined and sequentially washed with aqueous saturated sodium hydrogen carbonate (200 mL), water (200 mL) and brine (200 mL); dried over anhydrous magnesium sulfate and the solvent is evaporated. The resulting crude oil was purified by filtration through a short 4 cm silica plug (toluene:ethyl acetate 9:1) followed by crystallization from hot methanol yielding a yellow solid (15.3 g, 29.1 mmol, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 – 8.01 (m, 2H), 7.73 – 7.61 (m, 2H), 7.40 – 7.27 (m, 10H), 6.98 – 6.88 (m, 2H), 6.75 – 6.61 (m, 2H), 5.08 – 4.89 (m, 4H), 4.53 (d, *J* = 15.1, 1H), 4.40 – 4.29 (m, 2H), 3.73 (s, 3H), 3.56 – 3.26 (m, 2H), 0.18 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.65, 149.79, 145.82, 136.17 (d, *J* = 5.9), 136.13 (d, *J* = 5.8), 130.32, 129.17 (toluene), 128.82, 128.77, 128.52, 128.37, 128.34, 126.99, 125.43 (toluene), 124.11, 114.13, 69.67 (d, *J* = 163.3), 68.37 (d, *J* = 16.3), 68.27 (d, *J* = 16.6),

55.42, 53.44, 50.01 (d, *J* = 10.6), 21.59 (toluene), 0.34. ^{31}P NMR (121 MHz, $CDCl_3$) δ 22.31. R_f: 0.15 (toluene:ethyl acetate 4:1). Mp: 110.9°C.

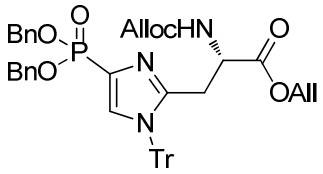


Dibenzyl 1-hydroxy-2-(*N*-(4-methoxybenzyl)-4-nitrophenylsulfonamido)ethylphosphonate (145): In a 250 mL round bottom flask, containing a larger egg-shaped stirring bar, the TMS protected nosyl amine **145-S1** (15.3 g, 22 mmol, 1 equiv) was suspended in 140 mL methanol. Potassium fluoride (2.6 g, 44 mmol, 2 equiv) was added in one batch, after stirring for a few minutes the suspension becomes a clear solution. Stirring was continued for an hour, after which a TLC sample indicated complete conversion of the starting material. The reaction mixture was poured into a separatory funnel containing saturated aqueous ammonium chloride (200 mL); ethyl acetate (200 mL) was added and the layers were separated. The aqueous layer was washed with two additional portions of ethyl acetate (200 mL), organic layers were combined, washed with saturated aqueous sodium hydrogen carbonate (300 mL), water (300 mL) and brine (300 mL), the mixture was dried over anhydrous sodium sulfate and evaporation of the solvent yielded a white amorphous solid (12.8 g, 20.4 mmol, 93%) that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.21 (d, *J*=8.6, 2H), 7.86 (d, *J*=8.7, 2H), 7.37 – 7.32 (m, 6H), 7.32 – 7.27 (m, 4H), 7.08 (d, *J*=8.5, 2H), 6.74 (d, *J*=8.6, 2H), 5.09 – 4.90 (m, 4H), 4.44 – 4.33 (m, 2H), 3.98 (t, *J*=7.9, 1H), 3.53 (ddd, *J*=15.6, 10.2, 5.4, 1H), 3.47 – 3.33 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.84, 150.10, 145.81, 135.97 (d, *J*=5.8), 130.20, 128.91, 128.90, 128.67, 128.30, 128.26, 126.84, 124.41, 114.42, 68.69 (d, *J*=7.0), 68.64 (d, *J*=7.1), 67.20 (d, *J*=158.7), 55.48, 52.4548.96 (d, *J*=10.0). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 22.76. R_f: 0.25 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 9.65 min. HRMS (ESI): for $[M+H]^+$ calc.: 627.15606, found: 627.15621.

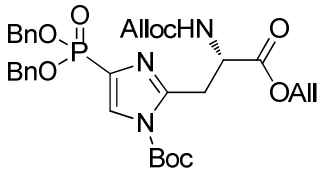


Imidazole 148: In a flame dried 10 mL Schlenk flask, under argon, imidazoline **68** (60 mg, 0.086 mmol, 1 equiv) was dissolved in 1 mL THF. The mixture was cooled to -78°C and KHMDS 0.5 M in toluene (0.52 mL, 0.26 mmol, 3 equiv) was slowly added. The mixture was stirred for eight hours and halve (0.75 mL) of it was removed and worked-up. To the remainder Boc_2O (38 mg, 0.17 mmol, 2 equiv) was added in 0.3 mL THF and the mixture was stirred for three hours while warming to room temperature. The mixture was poured into saturated aqueous sodium bicarbonate (5 mL) and the aqueous layer was extracted three times with ethyl acetate (5 mL). The organic layers were combined, washed with brine (10 mL), dried over anhydrous sodium sulfate and the solvent was removed in vacuo. The residual brown oil was not purified further. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 1.5 Hz, 1H), 7.26 – 7.09 (m, 11H), 5.82 – 5.55 (m, 2H), 5.52 (dd, *J* = 9.9, 4.8 Hz, 1H), 5.21 – 4.82 (m, 8H), 4.55 – 4.30 (m, 4H), 3.82 (dd,

J = 15.2, 4.9 Hz, 1H), 3.49 (dd, *J* = 15.2, 9.9 Hz, 1H), 1.46 (s, 9H), 1.25 (s, 9H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.18. LC-MS (ESI): t_R = 10.84 min.

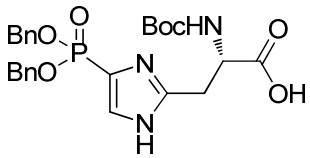


(*S*)-Allyl 2-(allyloxycarbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-trityl-1H-imidazol-2-yl)propanoate (149): Under an argon atmosphere, crude imidazole **136** (10.1 mmol, assumed) was added to a schlenk flask containing a magnetic stirring bar. Anhydrous dichloromethane (55 mL) was added, followed by trityl chloride (5.6 g, 20.2 mmol, 2 equiv) and DBU (4.6 g, 4.5 ml, 30.3 mmol, 3 equiv). To the brown mixture silver triflate (5.7 g, 22.2 mmol, 2.2 equiv) was added in one batch, forming a suspension. After stirring for 2 hours a TLC sample indicated complete consumption of the starting material, the reaction mixture was filtered through a plug of celite to remove the silver precipitates. The resulting filtrate was added to 100 mL saturated aqueous sodium bicarbonate solution. The layers were split and the aqueous layer was washed with three 50 mL portions of dichloromethane. The organic layers were combined, washed with brine (150 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo yielded a brown oil that was purified by flash column chromatography (toluene:ethyl acetate 3:1), yielding 6.11 g (7.8 mmol, 77% over two steps) of a brown oil.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.40 – 7.20 (m, 19H), 7.11 – 6.96 (m, *J* = 3.0, 6.5, 6H), 5.95 – 5.79 (m, 1H), 5.79 – 5.62 (m, 2H), 5.33 – 5.02 (m, 8H), 4.57 – 4.32 (m, 5H), 2.52 (dd, *J* = 4.8, 17.1, 1H), 2.04 (dd, *J* = 4.0, 17.2, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 155.9, 149.0 (d, *J* = 21.6, C), 140.9, 136.4 (d, *J* = 6.9, 2C), 132.74, 131.74, 131.5 (d, *J* = 38.2, CH), 129.8, 128.38, 128.36, 128.31, 127.63, 127.57, 126.0 (d, *J* = 247.8, C), 117.9, 117.6, 75.8, 67.9 (d, *J* = 5.6, 2CH), 65.7, 65.7, 51.4, 32.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.6. R_f: 0.19 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.81 min. HRMS (ESI): for $[M+H]^+$ calc.: 782.29896, found: 782.29946. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ +17.6° (*c*=0.0095, $CHCl_3$).



(*S*)-Tert-butyl 2-(3-(allyloxy)-2-(allyloxycarbonylamino)-3-oxopropyl)-4-(bis(benzyloxy)phosphoryl)-1H-imidazole-1-carboxylate (150): In a 25 mL round bottom flask, containing an egg-shaped stirring bar, crude imidazole **136** (556 mg, 1.03 mmol, 1 equiv) was dissolved in 4.44 mL 1,4-dioxane. DIPEA (133 mg, 179 µL, 1.03 mmol, 1 equiv) was added followed by 0.56 mL water. The resulting mixture was cooled to 0°C and boc anhydride (225 mg, 1.03 mmol, 1 equiv) was added. The mixture was stirred for 16 hours while warming to room temperature in the ice bath. A TLC sample indicated complete conversion of the starting material; the reaction mixture was poured into a saturated aqueous sodium bicarbonate solution (10 mL) and ethyl acetate (10 mL). The layers were separated and the aqueous layer was

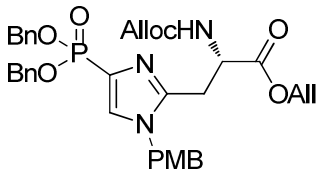
washed with three additional portion of ethyl acetate (10 mL). The organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated, yielding an oil that was purified by flash column chromatography (chloroform:ethyl acetate:triethyl amine 82:17:1) yielded a clear oil (433 mg, 0.68 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.52 – 7.15 (m, 11H), 5.97 (d, *J* = 9.1, 1H), 5.85 – 5.65 (m, 2H), 5.28 – 4.97 (m, 9H), 4.88 – 4.77 (m, 1H), 4.58 – 4.34 (m, 4H), 3.60 (dd, *J* = 5.7, 16.9, 1H), 3.41 (dd, *J* = 4.3, 16.8, 1H), 1.54 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 155.9, 149.0 (d, *J* = 20.2, C), 146.8, 136.04 (d, *J* = 7.4, C), 136.03 (d, *J* = 6.8, C), 132.6, 131.6, 130.0, 128.7 (d, *J* = 36.1, C), 128.47, 128.45, 128.3, 127.8, 127.8, 118.40, 118.37, 117.8, 87.1, 77.3, 77.0, 76.8, 68.10 (d, *J* = 5.5, C), 68.08 (d, *J* = 5.4, C), 66.1, 65.9, 51.6, 32.3, 27.9. ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.7. R_f: 0.24 (chloroform:ethyl acetate:triethylamine 5:1:0.01). LC-MS (ESI): t_R = 10.61 min. HRMS (ESI): for $[2M+Na]^+$ calc.: 1301.45835, found: 1301.45884. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ +21.1° (*c*=0.01, $CHCl_3$).



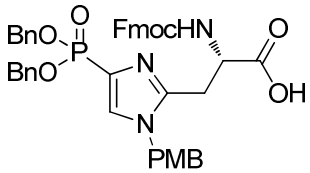
(*S*)-3-(4-(Bis(benzyloxy)phosphoryl)-1H-imidazol-2-yl)-2-(tert-butoxycarbonylamino)propanoic acid (339): Boc-protected imidazole **150** (54 mg, 0.08 mmol, 1 equiv) was dissolved in THF (1 mL), phenyl silane (46 mg, 52 µL, 0.42 mmol, 5 equiv) and tetrakis(triphenylphosphine)palladium (9.2 mg, 0.008 mmol, 0.1 equiv) were added and the resulting mixture was stirred for 2 hours, slowly turning black. Mesitylene was added and the mixture was evaporated to dryness, after repeating this twice, the residue was dissolved in water and acetonitrile and purified by prep-HPLC obtaining a white amorphous solid 27 mg (0.05 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.95 (bs, 3H), 7.49 (s, 1H), 7.29 (s, 10H), 6.36 (s, 1H), 5.18 – 5.00 (m, 4H), 4.62 (s, 1H), 3.59 (d, *J*=13.6, 1H), 3.45 – 3.23 (m, 1H), 1.29 (d, *J*=9.4, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.38, 162.53 (d, *J*=35.5), 156.18, 149.15 (d, *J*=14.5), 135.26 (d, *J*=6.3), 128.98, 128.84, 128.52, 128.42, 128.39, 116.79 (d, *J*=292.1), 80.47, 69.37 (d, *J*=5.6), 69.36 (d, *J*=5.4), 52.73, 30.08, 28.32. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 6.02. R_f: 0.10 (ethyl acetate). LC-MS (ESI): t_R = 8.27 min. HRMS (ESI): for $[M+H]^+$ calc.: 516.18941, found: 516.18878.

Prep-HPLC conditions solvent A was water with 0.1 % TFA, solvent B was acetonitrile:

Time (min)	B (%)
0	10
1	10
10	60
12	100
14	100
14.1	10
17	10

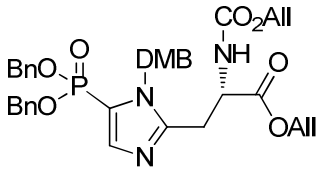


(*S*)-Allyl 2-(allyloxycarbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-(4-methoxybenzyl)-1H-imidazol-2-yl)propanoate (155): Under an argon atmosphere crude imidazole **136** (2.8 mmol, assumed) was added to a schlenk flask containing a magnetic stirring bar. Anhydrous DMF (15 mL) was added, followed by 4-methoxybenzyl chloride (657 mg, 570 µL, 4.2 mmol, 1.5 equiv) and potassium carbonate (1.93 g, 14 mmol, 5 equiv). After stirring for 3 hours a TLC sample indicated complete consumption of the starting material, the reaction mixture was added to 50 mL ethyl acetate and 50 mL saturated aqueous sodium bicarbonate solution. The layers were split and the aqueous layer was washed with three 50 mL portions of ethyl acetate. The organic layers were combined, washed with brine (2 x 150 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo yielded a yellow oil that was purified by flash column chromatography (chloroform:ethyl acetate 2:1), yielding **155** as a yellow oil that consisted of a mixture of regio-isomers (N-1: N-3 protection 5.7:1 by ^{31}P-NMR) 1.56 g (2.4 mmol, 84% over two steps). Only the major isomer is reported. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 – 7.21 (m, 11H), 7.02 (ad, *J*=8.5, 2H), 6.92 – 6.81 (m, 2H), 6.43 (d, *J*=8.7, 1H), 5.96 – 5.63 (m, 2H), 5.28 – 4.99 (m, 8H), 4.94 (s, 2H), 4.83 – 4.72 (m, 1H), 4.59 – 4.50 (m, 2H), 4.50 – 4.41 (m, 2H), 3.78 (s, 3H), 3.34 (dd, *J*=15.9, 5.0, 1H), 3.07 (dd, *J*=15.9, 4.7, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.50, 159.82, 156.03, 146.99 (d, *J*=22.1), 136.44 (d, *J*=7.4, 2C), 132.66, 131.52, 130.21 (d, *J*=36.8), 128.94, 128.43, 128.12, 128.06 (d, *J*=247.6), 127.78, 126.49, 118.48, 117.80, 114.65, 7.85 (d, *J*=5.5), 67.84 (d, *J*=5.4), 66.16, 65.89, 55.38, 51.99, 49.73, 28.93. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 12.65 (N-1), 9.32 (N-3). R_f: 0.71 (chloroform:methanol 9:1). LC-MS (ESI): t_R = 9.37 min. HRMS (ESI): for $[M+H]^+$ calc.: 660.24693, found: 660.24661.

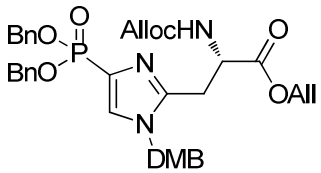


(*S*)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-(4-methoxybenzyl)-1H-imidazol-2-yl)propanoic acid (156): Under argon, in a 50 mL schlenk flask, equipped with an egg-shaped stirring bar, imidazole **155** (1.54 g, 2.3 mmol, 1 equiv) was dissolved in anhydrous THF (15 mL) and phenylsilane (1.26 g, 1.4 mL, 11.6 mmol, 5 equiv) was added, followed by palladium tetrakis(triphenylphosphine) (266 mg, 0.23 mmol, 0.1 equiv). Immediately after addition was complete, the mixture turned yellow and bubbles formed. As the mixture aged, it slowly turned black. After 1 hour the reaction mixture was transferred to a 100 mL round bottom flask and the solvent was evaporated *in vacuo*. The residue was suspended in 20 mL mesitylene and evaporated to dryness on a rotary evaporator equipped with a dry ice cooler and connected to an oil pump; this procedure was repeated twice to remove excess phenylsilane. The resulting black solid was not purified further and used 'as is' immediately in the next step. ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.8 (broad). R_f: 0.04 (chloroform:methanol 9:1). LC-MS (ESI): t_R = 6.75 min. HRMS (ESI): for $[M+H]^+$ calc.: 536.19450, found: 536.19387. The crude

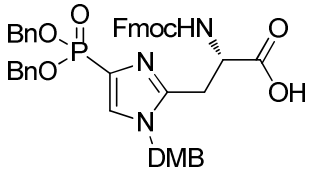
mixture (2.3 mmol, assumed) was dissolved in dioxane (12 mL), upon complete dissolution, water (1.2 mL) was added followed by Fmoc-OSu (1164 mg, 3.45 mmol 1.5 equiv) and sodium hydrogen carbonate (290 mg, 3.45 mmol, 1.5 equiv). The mixture was stirred at room temperature for 16 hours, when a TLC sample (Ethyl acetate or $CHCl_3$ containing 10% MeOH) indicated complete consumption of the starting material. Water (10 mL) and chloroform (10 mL) were added and the mixture was acidified to pH 4 with 0.5 M aqueous potassium hydrogen sulfate solution. The layers were separated and the aqueous layer was washed with 3 additional portions of chloroform (10 mL), the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a black foam that was further purified by flash column chromatography (chloroform:methanol 99:1 in increasing gradient to 49:1) yielding 0.893 g (1.18 mmol, 51% over two steps) of a dirty brown foam solid.^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.85 (s, 1H), 7.74 (d, *J*=7.5, 2H), 7.57 (t, *J*=6.9, 2H), 7.41 – 7.34 (m, 2H), 7.33 – 7.17 (m, 13H), 7.05 (d, *J*=8.3, 2H), 6.85 (d, *J*=8.5, 2H), 6.54 (d, *J*=5.8, 1H), 5.14 – 4.96 (m, 6H), 4.62 – 4.53 (m, 1H), 4.39 – 4.21 (m, 2H), 4.14 (t, *J*=7.3, 1H), 3.77 (s, 3H), 3.48 (dd, *J*=15.7, 4.3, 1H), 3.23 (dd, *J*=15.6, 8.0, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.73, 160.31, 156.09, 147.77 (d, *J*=15.5), 143.93, 143.69, 141.38, 135.67 (d, *J*=6.1), 135.61 (d, *J*=6.0), 134.66, 129.76 (d, *J*=31.4), 129.68, 128.61, 128.59, 128.23, 127.88, 127.24, 125.31 (d, *J*=8.0), 125.02, 120.11, 114.97, 68.75, 68.70, 67.40, 55.48, 52.30, 50.59, 47.15, 28.94. ^{31}P NMR (162 MHz, CD_3CN) δ = 11.93. R_f: 0.12 (chloroform:methanol 9:1). LC-MS (ESI): t_R = 9.63 min. HRMS (ESI): for $[M+H]^+$ calc.: 758.26258, found: 758.26231. $[\alpha]_D^{20}$ +18.7° (*c*=0.015 g/mL, $CHCl_3$).



(*S*)-Allyl 2-(((allyloxy)carbonyl)amino)-3-(5-(bis(benzyloxy)phosphoryl)-1-(2,4-dimethoxybenzyl)-1H-imidazol-2-yl)propanoate (**160**): in a 10 mL round bottom flask imidazole **136** (60 mg, 0.11 mmol, 1 equiv) was dissolved in 3.5 mL dichloromethane. To the mixture DIPEA (23 mg, 31 µL, 0.18 mmol, 1.6 equiv) and freshly prepared 1,4-dimethoxybenzyl chloride (31 mg 0.17 mmol, 1.5 equiv) were added. After stirring for 16 hours the reaction had still not gone to full conversion but work-up was performed anyway. The mixture was poured into 5 mL chloroform and 5 mL saturated aqueous sodium bicarbonate solution. The layers were split and the aqueous layer was washed with three 5 mL portions of chloroform. The organic layers were combined, washed with brine (2 x 150 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo yielded a yellow oil that was not purified further. ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.22 (**160**). LC-MS (ESI): t_R = 9.39 min (**160**).

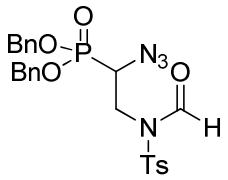


(*S*)-Allyl 2-(allyloxycarbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-(2,4-dimethoxybenzyl)-1H-imidazol-2-yl)propanoate (161): Under argon, in a flame dried, 50 mL schlenk flask imidazole **136** (2.9 mmol, 1 equiv) was dissolved in anhydrous DMF (13 mL) and potassium carbonate (2 g, 14.5 mmol, 5 equiv) was added. To the suspension, freshly prepared 1,4-dimethoxybenzyl chloride (0.65 g, 3.5 mmol, 1.2 equiv) in 2 mL DMF, was added by syringe. Resulting mixture was stirred for 2 hours, a TLC sample indicated there was still starting material left. A second portion of 1,4-dimethoxybenzyl chloride (0.22 g, 1.16 mmol, 0.4 equiv) in 1 mL DMF was added and the reaction mixture was stirred for 16 hours, after which the starting material was consumed. The reaction mixture was transferred to a 100 mL round bottom flask and the DMF was evaporated *in vacuo* on a rotary evaporator equipped with a dry ice condenser and an oil pump. The residue was partitioned between ethyl acetate (50 mL) and saturated sodium hydrogen carbonate (50 mL), the layers were separated and the aqueous layer was washed with additional portions of ethyl acetate (2 x 50 mL). The organic layers were combined and washed with brine (100 mL), dried over sodium sulfate and the solvent was evaporated, yielding a yellow oil. Further purification was done via flash column chromatography (chloroform with 33% ethyl acetate) yielding a yellow oil (1.25 g, 1.8 mmol, 62%), containing 5.6% of the **160**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.37 – 7.22 (m, 10H), 6.94 (d, *J* = 8.7, 1H), 6.50 – 6.41 (m, 2H), 5.95 – 5.80 (m, 1H), 5.80 – 5.66 (m, 1H), 5.32 – 5.01 (m, 8H), 4.93 – 4.89 (m, 2H), 4.87 – 4.77 (m, 1H), 4.54 (m, 2H), 4.49 (ad, *J* = 5.6, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 3.45 (dd, *J* = 5.0, 16.0, 1H), 3.16 (dd, *J* = 4.6, 16.1, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.80, 161.81, 158.45, 156.27, 147.22, 147.01, 136.72, 136.64, 134.92, 132.88, 131.77, 130.76, 130.65, 130.39, 128.68, 128.59, 128.25, 128.12, 128.08, 127.93, 118.56, 117.94, 115.57, 104.72, 99.09, 68.02, 67.99, 67.96, 67.94, 66.28, 66.05, 55.69, 55.62, 52.13, 45.42, 29.02. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.94 (**161**), 9.22 (**160**). R_f: 0.70 (chloroform:methanol 9:1). LC-MS (ESI): t_R = 9.51 min (**161**), 9.39 min (**160**). HRMS (ESI): for $[M+H]^+$ calc. 690.25749, found: 690.25666.

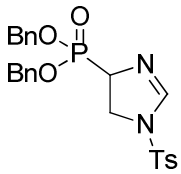


(*S*)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-(bis(benzyloxy)phosphoryl)-1-(2,4-dimethoxybenzyl)-1H-imidazol-2-yl)propanoic acid (162): Under argon, in a 50 mL schlenk flask, equipped with an egg-shaped stirring bar, imidazole **161** (1 g, 1.45 mmol, 1 equiv) was dissolved in anhydrous THF (8 mL) and phenylsilane (785 mg, 0.89 mL, 7.25 mmol, 5 equiv) was added, followed by palladium tetrakis(triphenylphosphine) (168 mg, 0.15 mmol, 0.1 equiv). Immediately after addition is complete, the mixture turns yellow and bubbles are formed. As the mixture ages, it slowly turns black. After 1 hour the reaction mixture is transferred to a 25 mL round bottom flask and the solvent in evaporated. The residue is suspended in 4 mL mesitylene and evaporated to dryness on a rotary evaporator equipped with a dry ice cooler and an oil pump, this procedure is repeated twice to remove excess phenylsilane. The resulting black solid is not purified further and used 'as is' in the next step. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.08. R_f: 0.08 (chloroform:methanol 9:1). LC-MS (ESI): t_R 6.93 min. HRMS (ESI): for $[M+H]^+$ calc.: 279.09333, found: 279.09346. In a 25 mL round bottom flask containing an egg-shaped stirring bar, the black oil (2.3 mmol, 1 equiv) was dissolved in dioxane (10.8 mL) followed by addition of

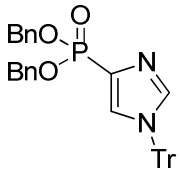
sodium hydrogen carbonate (290 mg, 3.45 mmol, 1.5 equiv), water (1.2 mL) and Fmoc-OSu (1.16 g, 3.45 mmol, 1.5 equiv). The resulting mixture was stirred for 16 hours after which a TLC sample indicated full consumption of the starting material. The reaction mixture was poured into water (20 mL) and chloroform (20 mL) was added. The pH of the water layer was adjusted to pH 4 with 0.5M aqueous potassium hydrogen sulfate (approximately 1 mL was added), the layers were separated and the aqueous layer was washed with additional chloroform (2 x 15 mL). The organic layers were combined, washed with brine (20 mL), dried over anhydrous sodium sulfate and the solvent was removed yielding an brown oil; this was further purified by flash column chromatography (chloroform : methanol : (methanol : water : acetic acid : ethyl acetate 3:3:2:3) 6:0.24:0.1) yielding 915 mg (1.16 mmol, 50.4% over two steps) of a brown oil. ^{1}H NMR (400 MHz, CD3CN) δ 7.76 (d, *J* = 7.5, 2H), 7.55 (d, *J* = 4.7, 2H), 7.43 (s, 1H), 7.35 (t, *J* = 6.9, 3H), 7.30 – 7.19 (m, 11H), 7.18 – 7.06 (m, 2H), 6.92 (d, *J* = 7.8, 1H), 6.54 – 6.38 (m, 2H), 5.09 – 4.92 (m, 6H), 4.73 – 4.60 (m, 1H), 4.30 – 4.01 (m, 3H), 3.73 (s, 3H), 3.69 (s, 3H), 3.54 – 3.37 (m, 2H). ^{13}C NMR (101 MHz, CD3CN) δ 172.57 (C), 162.99 (C), 159.64 (C), 157.13 (C), [144.95, 144.86, 142.06, 136.84, 132.32, 129.49, 129.48, 129.08, 129.05, 128.71, 128.13, 126.20] This area is unresolvable, 120.96 (2xCH), 115.40 (C), 105.95 (CH), 99.71 (CH), 69.46 (2xCH_2), 67.66 (CH_2), 56.32 (CH_3), 56.15 (CH_3), 53.09 (CH), 47.87 (CH), 47.17 (CH_2), 29.03 (CH_2). No phosphorus couplings were observed. ^{31}P NMR (162 MHz, CD3CN) δ 7.97. R_f: 0.15 (chloroform 10% methanol). LC-MS (ESI): t_R = 10.08 min. HRMS (ESI): for $[M+H]^+$ calc. 788.27314, found: 788.27264.



Dibenzyl (1-azido-2-(*N*-tosylformamido)ethyl)phosphonate (165): In a flame dried schlenk flask, under argon, azide **66** (1.5 g, 3.0 mmol, 1 equiv) was dissolved in THF (15 mL) and cooled to 0°C. 1H-benzo[d][1,2,3]triazole-1-carbaldehyde **72** (0.88 g, 6 mmol, 2 equiv) was added followed by sodium hydride (0.14 g, 3.6 mmol, 1.2 equiv). The mixture was stirred for 30 minutes after which it was poured into saturated aqueous sodium bicarbonate (50 mL) and ethyl acetate (50 mL). The layers were separated and the aqueous layer was washed with two additional portions of ethyl acetate (50 mL). The organic layers were combined, washed with water (100 mL), brine (100 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated and the residual oil was purified by column chromatography (toluene:ethyl acetate 24:1 in increasing gradient to 4:1) yielding a yellow oil (0.89 g, 1.7 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.42 – 7.22 (m, 12H), 5.16 – 4.96 (m, 4H), 4.09 – 3.84 (m, 2H), 3.66 – 3.51 (m, *J* = 14.4 Hz, 1H), 2.44 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.00. R_f: 0.29 (toluene:ethyl acetate 4:1)



Dibenzyl (1-tosyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (166): In a flame dried, 250 mL round bottom Schlenk, containing an argon atmosphere, formamide **165** (887 mg, 1.7 mmol, 1 equiv) was dissolved in 40 mL THF. dppe (1016 mg, 2.55 mmol, 1.5 equiv) was added, the mixture was warmed to 50°C and stirred until no more nitrogen bubble formation was seen. The Schlenk was closed and heated to 80°C for eight hours. The mixture was allowed to cool to about 50°C and a 1:1 mixture of 30% hydrogen peroxide (aq) and saturated sodium bicarbonate (aq) was added. The mixture was stirred for one hour, after which a TLC sample indicated all dppe had been completely oxidized. The mixture was poured into water (100 mL) and chloroform (150 mL), the layers were separated and the aqueous layer was washed with two additional 50 mL portions of chloroform. The organic layers were combined and washed with brine (200 mL). Evaporation of the solvent yielded a sticky white solid that was purified by passing through a short silica column (chloroform:ethyl acetate 3:1) yielding a clear oil (720 mg, 1.5 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.62 (m, 2H), 7.40 (dd, *J* = 3.6, 2.3 Hz, 1H), 7.37 – 7.20 (m, 12H), 4.97 (dd, *J* = 11.4, 8.5 Hz, 4H), 4.43 (dddd, *J* = 15.7, 11.1, 8.8, 2.3 Hz, 1H), 3.75 – 3.55 (m, 2H), 2.40 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.99.

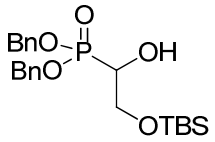


Dibenzyl 1-trityl-1*H*-imidazol-4-ylphosphonate (167):

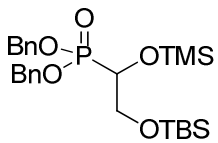
Method A: In a flame dried, argon filled, 10 mL schlenk imidazoline **166** (46 mg, 0.095 mmol, 1 equiv) was dissolved in 0.5 mL THF. The mixture was cooled to -78°C and KHMDS 0.5M in toluene (0.38 mL, 0.19 mmol, 2 equiv) was added drop wise. The mixture was stirred for two hours until all starting material was consumed (TLC chloroform:ethyl acetate 3:1). Trityl chloride (40 mg, 0.143 mmol, 1.5 equiv) was added and the mixture was allowed to warm to room temperature. It was poured into saturated aqueous sodium bicarbonate (10 mL) and the aqueous phase was washed with three 10 mL portions of chloroform. The organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. Removal of the solvent yielded a clear oil that contained 50 percent **167** and 50 percent the unprotected imidazole, no yield was determined.

Method B: To a flame-dried, argon filled 500 ml Schlenk flask equipped with a large egg-shaped stirring bar was added 4-iodo-1-trityl-1*H*-imidazole (8.00 g, 18.33 mmol), followed by anhydrous THF (100 mL). The suspension was briefly heated to 50°C to complete dissolution. The clear solution was cooled to 0°C and *i*-PrMgCl:LiCl (14.8 mL, 1.3 M in THF, 1.05 equiv 19.25 mmol) was added drop-wise over 10 min. The resulting clear reaction was stirred for 20 min at 0°C, followed by another 20 min at room temperature. The reaction mixture was cooled to -78°C in a acetone-dry ice bath and bis(diethylamino)phosphine chloride (4.76 g, 20.16 mmol, 1.10 equiv) was added drop-wise via a syringe over 10 min. The resulting clear reaction mixture was aged for 1 hour at -78°C, then allowed to reach ambient temperature over 2 hours. The Schlenk-flask was connected at the side arm to a large-bore cold trap and the THF was removed via vacuum transfer. After complete removal of THF, benzyl alcohol (10.0 g, 82 mmol, 5.05 equiv) was added, followed by anhydrous acetonitrile (60 mL) and the resulting pink reaction mixture was cooled to 0°C. A solution of tetrazole in acetonitrile (6 mL, 0.3 M, 1.80 mmol, 0.10 equiv) was added

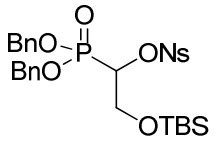
in one portion and the reaction mixture was allowed to reach ambient temperature and stirred over night (12 hours). Upon aging, the reaction mixture turns pale yellow and a thick precipitate is formed. The suspension was cooled to -20°C in an ice-salt bath and ice-cold 30% aq. hydrogen peroxide (8.00 mL, 70.58 mmol, 3.85 equiv) was added drop wise over 5 min. The reaction mixture was allowed to rise to 0°C over an hour and stirred at 0°C for another hour, when 5% aq Na_2CO_3 (100 mL) was added, followed by Ethyl acetate (100 mL). The resulting white suspension was stirred in the schlenk tube for 10 min, and subsequently transferred to a separatory funnel. The mixture was diluted with another 100 mL of water and extracted with additional Ethyl acetate (2x100 mL). The combined organic extracts were washed with 5% aq sodium thiosulfate (100 mL), followed by water (200 mL) and brine (2x200 mL), dried over Na_2SO_4 and evaporated to dryness. Traces of MeCN and some BnOH were removed by drying on oil-pump vacuum for 3 hours. The resulting yellow residue (20 g) was dissolved in a small amount of Ethyl acetate (10 mL) and cyclohexane (30 mL) was added. The solution was added to a silica gel pad (diam. 80 mm, 200 mm length, 20% Ethyl acetate in cyclohexane), the excess of BnOH was eluted at 20:80 ethyl acetate: cyclohexane, followed by the product when increasing the gradient stepwise from 20:80 to 80:20 ethyl acetate: cyclohexane. Evaporation of combined fractions yielded 6.2 g of clear oil, 10.86 mmol, 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.53 (m, 1H), 7.53 – 7.50 (m, 1H), 7.36 – 7.29 (m, 4H), 7.29 – 7.15 (m, 15H), 7.10 – 6.97 (m, 6H), 5.20 – 5.04 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.42 (3C), 141.17 (d, J = 22.0, 1C), 135.99 (d, J = 6.6, 2C), 130.67 (d, J = 38.4, 1C), 129.25 (3x2C), 128.62 (d, J = 247.9, 1C), 128.05 (3x1C & 2x2C), 127.95 (3x2C), 127.81 (2C), 127.48(2x2C), 75.64, 67.46 (d, J = 5.5, 2C). ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.18. R_f: 0.44 (c-hexane: ethyl acetate 1:4). LC-MS (ESI): t_R = 10.99 min. HRMS (ESI): for $[M+H]^+$ calc.: 571.21451, found: 571.21389.



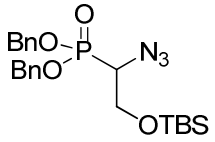
Dibenzyl (2-((tert-butyldimethylsilyl)oxy)-1-hydroxyethyl)phosphonate (170): In a 250 mL round bottom flask TMS-ether **171** (5.34 g, 10.5 mmol, 1 equiv) was dissolved in 100 mL methanol. Citric acid (5.04 g, 26.2 mmol, 2.5 equiv) was added and the mixture was stirred until complete consumption of the starting material. It was then added to 2.77 g sodium carbonate in 100 mL water. Ethyl acetate (200 mL) was added, the layers were separated and the aqueous layer was washed with two additional 100 mL portions of ethyl acetate. The organic layers were combined, washed with brine (200 mL), dried over anhydrous sodium sulfate and the solvent was evaporated yielding a yellow oil (4.5 g, 10.3 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.22 (m, 10H), 5.12 – 4.92 (m, 4H), 4.07 (q, *J* = 7.1 Hz, 1H), 4.03 – 3.93 (m, 1H), 3.91 – 3.77 (m, 2H), 2.77 (dd, *J* = 14.1, 5.8 Hz, 1H), 0.82 (s, 9H), -0.00 (s, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.97.



Dibenzyl (2,2,7,7,8,8-hexamethyl-3,6-dioxa-2,7-disilanonan-4-yl)phosphonate (171): Under an argon atmosphere, in a 250 mL flame dried schlenk flask, dibenzylphosphite (4 g, 3.4 mL, 15.3 mmol, 1.2 equiv) and triethylamine (1.9 g, 2.56 mL, 18.4 mmol, 1.44 equiv) were dissolved in 50 mL of anhydrous dichloromethane. The resulting mixture was cooled to -20°C with an ice-salt bath, and chlorotrimethylsilane (2.0 g, 2.27 mL, 18.4 mmol, 1.44 equiv) was added dropwise over 5 minutes. After addition was complete, the mixture was stirred for one hour at -20°C, and a white precipitate formed. The reaction mixture was cooled to -78°C with acetone-dry ice and (TBS-oxy)acetaldehyde **169**, dissolved in 50 mL dichloromethane, was added via cannula. Upon addition, the white precipitate dissolved. After stirring for 16 hours, complete consumption of the aldehyde was confirmed by TLC. The reaction mixture was poured into saturated aqueous NH_4Cl (100 mL). The layers were split and the aqueous layer was washed with three 50 mL portions of dichloromethane. The organic layers were combined and sequentially washed with aqueous saturated sodium hydrogen carbonate (100 mL), water (100 mL) and brine (100 mL); dried over anhydrous magnesium sulfate and the solvent was evaporated. The resulting crude oil was purified by flash column chromatography (toluene:ethyl acetate 9:1) yielding **171** (5.34 g, 10.5 mmol, 69%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.24 (m, 10H), 5.13 – 4.91 (m, 4H), 4.06 (ddd, *J* = 9.5, 8.1, 2.9 Hz, 1H), 3.90 (ddd, *J* = 10.9, 5.8, 2.9 Hz, 1H), 3.72 (ddd, *J* = 10.9, 8.2, 6.1 Hz, 1H), 0.84 (s, 9H), 0.11 (s, 9H), 0.00 (s, 3H), -0.00 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.62.

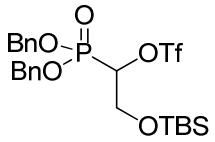


TBS-protected nosyl phosphonate (172): in a 25 mL round bottom flask, under air, alcohol **170** (100 mg, 0.23 mmol, 1 equiv) was dissolved in dichloromethane (2.3 mL). 4-nitrobenzene sulphonylchloride (56 mg, 0.25 mmol, 1.1 equiv) and DMAP (31 mg, 0.25 mmol, 1.1 equiv) were added and the mixture was stirred two hours. 10 mL dichloromethane was added and the mixture was poured into 10 % citric acid (20 mL). The aqueous layer was extracted once with dichloromethane (15 mL), the organic layers were combined, washed with saturated aqueous sodium bicarbonate (20 mL), brine (20 mL) and dried over magnesium sulfate. Evaporation of the solvent in vacuo yielded a colorless oil (140 mg, 0.23 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.15 – 8.09 (m, 2H), 8.01 – 7.96 (m, 2H), 7.35 – 7.17 (m, 10H), 5.09 – 4.82 (m, 5H), 4.04 (ddd, *J*=12.8, 9.8, 3.1, 1H), 3.94 (ddd, *J*=12.1, 7.3, 4.8, 1H), 0.84 – 0.82 (m, 9H), 0.01 (s, 3H), -0.00 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 17.21. LC-MS (ESI): t_R = 9.03 min. HRMS (ESI): for $[M+H]^+$ calc.: 525.10911, found: 525.10873.

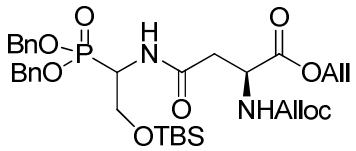


Dibenzyl 1-azido-2-(tert-butyldimethylsilyloxy)ethylphosphonate (173): Under an argon atmosphere, in a 100 mL, flame dried schlenk flask, triflate **174** (5.8 g, 10.3 mmol, 1 equiv) was dissolved in anhydrous acetonitrile (34 mL), after taken care there was no more CH_2Cl_2 present in the triflate by co-evaporation with toluene. N,N,N',N'-tetramethylguanidinium azide (1.26 g, 12.3 mmol, 1.2 equiv) is rapidly weighed

and added to the pink solution in one batch (*Caution: very hygroscopic, forms explosive, toxic gas upon contact with acid*). The resulting mixture is stirred at room temperature for 3 hours, after which a TLC showed remaining starting material. Another batch of N,N,N',N'-tetramethylguanidinium azide (0.13 g, 1.23 mmol, 0.12 equiv) was added and 15 minutes later the starting material was completely consumed. Reaction mixture is poured into saturated aqueous sodium hydrogen carbonate (50 mL), layers are separated and the aqueous layer is washed with ethyl acetate (3 x 30 mL). The organic layers are combined, washed with 10% citric acid (50 mL), water (50 mL), brine (50 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded a thick pink oil that was purified by filtration through a silica plug (toluene:ethyl acetate 6:1) yielding a pink oil (3.93 g, 8.5 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.29 (m, 10H), 5.22 – 4.98 (m, 4H), 4.03 (ddd, *J* = 3.2, 8.9, 11.0, 1H), 3.87 (ddd, *J* = 4.2, 8.4, 10.9, 1H), 3.64 (ddd, *J* = 3.2, 8.4, 15.1, 1H), 0.90 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.82 (d, *J* = 9.4, C), 135.77 (d, *J* = 9.3, C), 128.65 (br, 2x2CH&2xC), 128.14 (2x2CH), 68.43 (d, *J* = 17.8, CH_2), 68.35 (d, *J* = 17.8, CH_2), 62.44 (d, *J* = 6.3, CH_2), 60.28 (d, *J* = 155.5, CH), 25.75 ($3xCH_3$), 18.21, -5.52 ($2xCH_3$). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.26. R_f: 0.58 (toluene:ethyl acetate 3:1) IR = 3035 (w), 2954 (w), 2929 (w), 2885 (w), 2857 (w), 2093 (m), 1498 (w), 1457 (w), 1380 (w), 1314 (w), 1252 (s), 1213 (m), 1112 (m), 1080 (m), 992 (s), 918 (w), 833 (s), 778 (s), 732 (s), 695 (s), 665 (m), 618 (w). LC-MS (ESI): t_R = 11.56 min. HRMS (ESI): for $[M+H]^+$ calc.: 462.19725, found: 462.19718.

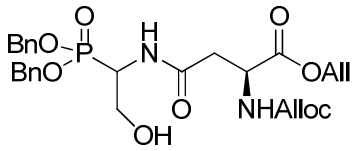


1-(Bis(benzyloxy)phosphoryl)-2-((tert-butyldimethylsilyl)oxy)ethyl trifluoromethanesulfonate (174): In an argon filled, flame dried 250 mL Schlenk, alcohol **170** (4.5 g, 10.3 mmol, 1 equiv) was dissolved in 100 mL dichloromethane and cooled to -15°C. Pyridine (1.6 g, 1.65 mL, 20.5 mmol, 2 equiv) and triflic anhydride (4.34 g, 2.6 mL, 15.4 mmol, 1.5 equiv) were added and stirred for two hours. The mixture was poured into 200 mL ice cold five percent citric acid (aq) and the aqueous layer was washed three times with 100 mL dichloromethane. The organic layers were combined, washed with brine (200 mL) and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo gave **174** as a yellow oil (5.6 g, 9.9 mmol, 96%) that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.27 (m, 10H), 5.05 (ddd, *J* = 9.5, 9.0, 6.1 Hz, 5H), 4.02 (ddd, *J* = 12.3, 5.9, 2.8 Hz, 1H), 3.92 (ddd, *J* = 12.3, 8.4, 3.8 Hz, 1H), 0.94 – 0.80 (m, 9H), 0.04 (d, *J* = 1.3 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.80. ^{19}F NMR (377 MHz, $CDCl_3$) δ -74.79.

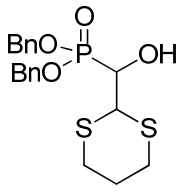


(2*S*)-Allyl 2-(((allyloxy)carbonyl)amino)-4-((1-(bis(benzyloxy)phosphoryl)-2-((tert-butyldimethylsilyl)oxy)ethyl)amino)-4-oxobutanoate (175): In a 100 mL round bottom flask, amino acid **67** (1.6 g, 6.3 mmol, 1.25 equiv) was dissolved in 26 mL dichloromethane. EDC (1.26 g, 6.6 mmol, 1.3

equiv) and DMAP (0.062 g, 0.51 mmol, 0.1 equiv) were added and the mixture was stirred for five minutes. Amine **75** (2.2 g, 5.1 mmol, 1 equiv) was added and the mixture was stirred for five hours. The mixture was poured into five percent aqueous citric acid (30 mL) and the aqueous layer was washed three times with dichloromethane (20 mL). The organic layers were combined, washed with saturated aqueous sodium bicarbonate (50 mL), water (50 mL) and brine (50 mL). The mixture was dried over anhydrous sodium sulfate and the solvent was removed. The residual oil was purified by column chromatography (toluene:ethyl acetate 4:1) resulting in a yellow oil (2.3 g, 3.4 mmol, 67%) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.17 (m, 10H), 6.00 (dd, *J* = 21.5, 9.0 Hz, 1H), 5.92 – 5.74 (m, 3H), 5.37 – 5.06 (m, 4H), 5.06 – 4.87 (m, 4H), 4.67 – 4.37 (m, 6H), 4.02 – 3.86 (m, 1H), 3.86 – 3.67 (m, 1H), 3.02 – 2.79 (m, 1H), 2.64 (ddd, *J* = 24.0, 16.1, 4.4 Hz, 1H), 0.84 (d, *J* = 1.6 Hz, 9H), 0.01 (dd, *J* = 3.9, 1.6 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.58, 24.43. LC-MS (ESI): t_R = 8.40 min. HRMS (ESI): for $[M+H]^+$ calc.: 675.28667, found: 675.28701.

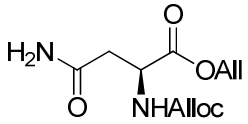


(2*S*)-Allyl 2-(((allyloxy)carbonyl)amino)-4-((1-(bis(benzyloxy)phosphoryl)-2-hydroxyethyl)amino)-4-oxobutanoate (176): In a 24 mL round bottom flask, TBS protected alcohol **175** (555 mg, 0.82 mmol, 1 equiv) was dissolved in 4.1 mL wet THF. HF.pyridine (212 mg, 233 µL, 7.35 mmol, 9 equiv) was added and the mixture was stirred for four hours. The mixture was poured into saturated aqueous sodium bicarbonate (20 mL) and the aqueous layer was extracted three times with ethyl acetate (15 mL). The organic layers were combined, washed with brine (20 mL), dried over anhydrous sodium sulfate and the solvent was removed in vacuo. The resulting yellow oil required no further purification (454 mg, 0.81 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 10H), 6.20 – 6.07 (m, 1H), 5.94 – 5.74 (m, 2H), 5.36 – 4.89 (m, 8H), 4.66 – 4.35 (m, 6H), 4.03 – 3.90 (m, 1H), 3.87 – 3.67 (m, 2H), 2.95 – 2.60 (m, 2H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.23, 25.08. LC-MS (ESI): t_R = 8.44 min. HRMS (ESI): for $[M+H]^+$ calc.: 561.200196, found: 561.19988.

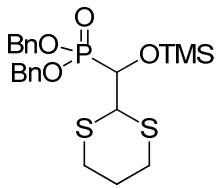


Dibenzyl (1,3-dithian-2-yl)(hydroxy)methylphosphonate (178): In a 100 mL three-necked, round bottom flask containing an egg-shaped stirring bar, TMS protected alcohol **182** (3.12 g, 6.5 mmol, 1 equiv) was dissolved in methanol (30 mL). Potassium fluoride (0.76 g, 13 mmol, 2 equiv) was added in one batch, dissolving completely. After 30 minutes, TLC indicated complete consumption of the starting material. The reaction mixture was poured into a separatory funnel containing saturated aqueous ammonium chloride (50 mL) and ethyl acetate (50 mL). The layers were split and the aqueous layer was washed with two additional portions of ethyl acetate (30 mL), organic layers were combined, washed

with saturated aqueous sodium hydrogen carbonate (30 mL), water (30 mL) and brine (30 mL), the mixture was dried over anhydrous sodium sulfate and evaporation of the solvent yielded a white solid (2.5 g, 6.09 mmol, 94%) that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 – 7.28 (m, 10H), 5.22 – 4.97 (m, 4H), 4.34 – 4.20 (m, 2H), 2.96 – 2.85 (m, 2H), 2.77 – 2.65 (m, 2H), 2.10 – 1.86 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 134.32 (d, *J* = 11.4 Hz), 134.26 (d, *J* = 11.6 Hz), 126.67, 126.66, 126.20, 126.15, 125.09, 68.30 (d, *J* = 164.4 Hz), 66.82 (d, *J* = 6.7 Hz), 66.29 (d, *J* = 7.0 Hz), 44.77 (d, *J* = 4.8 Hz), 26.24, 25.64, 23.09. ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.61. LC-MS (ESI): t_R = 8.68 min. HRMS (ESI): for $[M+H]^+$ calc.: 411.08481, found: 411.08504.

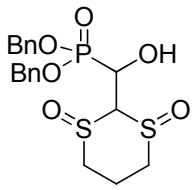


(*S*)-Allyl 2-(((allyloxy)carbonyl)amino)-4-amino-4-oxobutanoate (181): In a 250 mL round bottom flask, under air, asparagines monohydrate (5 g, 33.3 mmol, 1 equiv) was dissolved in 100 mL water. Sodium bicarbonate (5.6 g, 66.6 g, 2 equiv) was added followed by slow addition of allyl chloroformate (3.7 mL, 4.2 g, 35 mmol, 1.05 equiv) over six hours while ensuring the mixture is basic the whole time. After addition was complete the mixture was stirred for 30 minutes. The mixture was frozen and the solvent removed via lyophilization. The white solid residue was suspended in ethanol and filtered; the residue was washed three times with ethanol. The combined filtrates were evaporated to dryness in vacuo yielding a white foam (quantitative yield). ^{1}H NMR (400 MHz, CD_3OD) δ = 6.01 – 5.84 (m, 1H), 5.30 (ddd, *J*=17.3, 3.1, 1.6, 1H), 5.17 (ddd, *J*=10.5, 2.8, 1.4, 1H), 4.93 (bs, 4H), 4.60 – 4.43 (m, 3H), 2.75 (dd, *J*=6.0, 4.1, 2H). LC-MS (ESI): t_R = 4.61 min. HRMS (ESI): for $[M+H]^+$ calc.: 217.08190, found: 217.08203. In a 50 mL round bottom flask, the mono protected amino acid (500 mg, 2.3 mmol, 1 equiv) was dissolved in 9 mL DMF. Allyl bromide (339 mg, 242 mL, 2.8 mmol, 1.2 equiv) and cesium carbonate (1.12 g, 3.45 mmol, 1.5 equiv) were added and the reaction was stirred for eight hours. The mixture was added to 20 mL 1N HCl (aq) and the aqueous layer was extracted three ties with ethyl acetate (20 mL). The organic layers were combined, washed with brine (50 mL) twice, dried over anhydrous magnesium sulfate and evaporated to dryness yielding a clear oil that solidifies on standing to a white solid (514 mg, 2.11 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 – 5.82 (m, 3H), 5.62 (bs, 1H), 5.52 b(s, 1H), 5.38 – 5.16 (m, 4H), 4.70 – 4.52 (m, 5H), 3.03 – 2.95 (m, 1H), 2.78 (dd, *J* = 16.2, 4.1 Hz, 1H). LC-MS (ESI): t_R = 4.50 min.

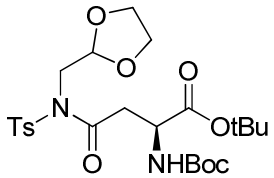


Dibenzyl (1,3-dithian-2-yl)(trimethylsilyloxy)methylphosphonate (182): Under an argon atmosphere, in a 250 mL flame dried schlenk flask, dibenzylphosphite (4.1 g, 3.45 mL, 15.6 mmol, 1.2 equiv) and triethylamine (1.9 g, 2.6 mL, 18.7 mmol, 1.44 equiv) were dissolved in 50 mL of anhydrous dichloromethane. The resulting mixture was cooled to -20^{o}C with an ice-salt bath, and chlorotrimethylsilane (2.0 g, 2.73 mL, 18.7 mmol, 1.44 equiv) was added dropwise over 5 minutes. After addition was complete, the mixture was stirred for one hour at the same temperature. The reaction mixture was cooled to -78^{o}C with acetone-dry ice and 1,3-dithiane-2-carbaldehyde (1.93 g, 13 mmol, 1

equiv), dissolved in 20 mL dichloromethane, was added via cannula. After stirring for 16 hours the complete consumption of the aldehyde was confirmed by TLC. The reaction mixture was poured into saturated aqueous NH_4Cl (100 mL), the layers were split and the aqueous layer was washed with three 50 mL portions of dichloromethane. The organic layers were combined and sequentially washed with aqueous saturated sodium hydrogen carbonate (100 mL), water (100 mL) and brine (100 mL); dried over anhydrous magnesium sulfate and the solvent was evaporated. The resulting crude oil was purified by flash column chromatography (toluene:ethyl acetate 9:1) yielding **182** (3.17g, 6.6 mmol, 50%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.24 – 7.10 (m, 10H), 4.99 – 4.71 (m, *J* = 25.9, 11.8, 7.5 Hz, 4H), 4.28 (dd, *J* = 7.5, 4.4 Hz, 1H), 4.12 (dd, *J* = 9.2, 4.4 Hz, 1H), 2.78 – 2.58 (m, 4H), 1.94 – 1.80 (m, 1H), 1.78 – 1.58 (m, 1H), -0.00 (s, 9H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 22.13. LC-MS (ESI): t_R = 8.59 min. HRMS (ESI): for $[M+H]^+$ calc.: 483.12434, found: 483.12411.

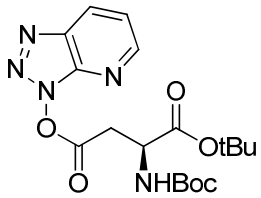


Phosphonate (183): At 40°C, sodium periodate (120 mg, 0.56 mmol, 2.3 equiv) was added to a stirring solution of dithiane **178** (100 mg, 0.24 mmol, 1 equiv) in dioxane (1 mL) and water (0.6 mL) and stirred for 16 hours. The reaction was poured into saturated aqueous sodium bicarbonate solution (10 mL) and ethyl acetate (10 mL). The layers were separated and the aqueous phase was washed with 3 additional portion of ethyl acetate (10 mL), the organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was further purified by flash column chromatography (chloroform:methanol 15:1) yielding 38 mg (0.086 mmol, 36%) of a yellow oil still containing about six percent **184**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 – 7.25 (m, 11H), 5.25 – 5.01 (m, 6H), 3.87 (t, *J* = 2.9 Hz, 1H), 3.63 – 3.49 (m, 1H), 3.01 (td, *J* = 12.2, 2.6 Hz, 2H), 2.88 – 2.65 (m, 2H), 2.34 – 2.24 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 135.98 (d, *J* = 5.6 Hz), 135.95 (d, *J* = 5.6 Hz), 128.88, 128.85, 128.45, 128.44, 128.39, 74.83 (d, *J* = 5.8 Hz), 69.54 (d, *J* = 7.4 Hz), 69.18 (d, *J* = 6.9 Hz), 64.88 (d, *J* = 167.5 Hz), 51.16, 46.85, 15.20. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.71. LC-MS (ESI): t_R = 6.95 min. HRMS (ESI): for $[M+H]^+$ calc.: 443.07464, found: 443.07454.

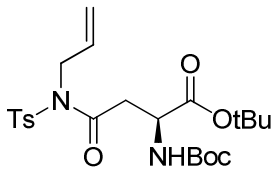


(*S*)-Tert-butyl 4-(N-((1,3-dioxolan-2-yl)methyl)-4-methylphenylsulfonamido)-2-(tert-butoxycarbonylamino)-4-oxobutanoate (187): Under an argon atmosphere, a 100 mL round bottom schlenk flask containing an egg-shaped stirring bar, was charged with HOAt ester **191** (2.57 g, 6.31 mmol, 1 equiv) and anhydrous dichloromethane (50 mL). The reaction was cooled to 0°C with an ice bath and N-allyl-4-methylbenzenesulfonamide (1.79 g, 6.94 mmol, 1.1 equiv) was added, followed by addition of 1,8-diazabicyclo-undec-7-ene (1.44 g, 1.42 mL, 9.46 mmol, 1.5 equiv), which turned the

reaction mixture yellow. After stirring for 15 minutes the ice bath was removed and the solution was allowed to stand at room temperature for an hour. The mixture was poured into 10 % aqueous citric acid (50 mL), the layers were separated and the organic layer was washed with an additional portion of 10 % aqueous citric acid (50 mL), two 50 mL portions of saturated aqueous sodium hydrogencarbonate and 50 mL brine. Drying over anhydrous magnesium sulfate and evaporation of the solvent yielded a yellow oil that was purified further by flash column chromatography over silica (Petroleum ether (40-60) with 33% ethyl acetate) yielding a white foam (2.34 g, 4.43 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (ad, *J* = 8.4, 2H), 7.28 (d, *J* = 8.4, 2H), 5.43 (d, *J* = 8.6, 1H), 5.17 (t, *J* = 4.0, 1H), 4.38 – 4.28 (m, 1H), 4.06 – 3.90 (m, 4H), 3.90 – 3.79 (m, 2H), 3.25 (dd, *J* = 4.1, 17.7, 1H), 3.06 (dd, *J* = 3.8, 17.8, 1H), 2.39 (s, 3H), 1.38 (s, 9H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.69, 169.89, 155.74, 145.02, 136.62, 129.76 (2xCH), 128.58 (2xCH), 101.74, 82.07, 79.82, 65.29, 65.25, 50.43, 47.96, 39.11, 28.50 (3xCH_3), 27.80 (3xCH_3), 21.77. R_f: 0.35 (petroleum ether:ethyl acetate 2:1). Melting point: 87.2-89.1°C. LC-MS (ESI): t_R = 10.51 min. HRMS (ESI): for $[M+H]^+$ calc.: 529.22143, found: 529.22119.

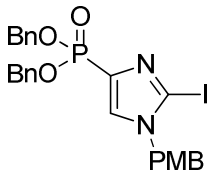


(*S*)-4-(3*H*-[1,2,3]Triazolo[4,5-b]pyridin-3-yl) 1-tert-butyl 2-(tert-butoxycarbonylamino)succinate (191): In a 50 mL round bottom flask, containing an egg-shaped stirring bar, L-Aspartic acid, N-[(1,1-dimethylethoxy)carbonyl]-, 1-(1,1-dimethylethyl) ester (1 g, 3.46 mmol, 1 equiv) was dissolved in 20 mL dichloromethane. The mixture was cooled to 0°C and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1 g, 5.2 mmol, 1.5 equiv) was added, followed by spoon wise addition of 1-hydroxyazabenzotriazole (612 mg, 4.5 mmol, 1.3 equiv) turning the mixture orange. The mixture was allowed to warm up in the ice bath over 16 hours, changing the color to yellow, after which a TLC confirmed complete conversion of the starting material. The mixture was poured into 10 % aqueous citric acid (20 mL), the layers were separated and the organic layer was washed with two additional portions of 10 % aqueous citric acid (20 mL), once with brine (20 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded a white foam (1.2 g, 2.9 mmol, 85%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (dd, *J* = 4.5, 1.4 Hz, 1H), 8.41 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.44 (dd, *J* = 8.4, 4.5 Hz, 1H), 5.62 (d, *J* = 7.4 Hz, 1H), 4.66 (d, *J* = 6.5 Hz, 1H), 3.49 (dd, *J* = 17.1, 4.3 Hz, 1H), 3.39 (dd, *J* = 17.1, 5.2 Hz, 1H), 1.48 (s, 9H), 1.46 (s, 9H).



(*R*)-Tert-butyl 4-(*N*-allyl-4-methylphenylsulfonamido)-2-(tert-butoxycarbonylamino)-4-oxobutanoate (192): Under an argon atmosphere, a round bottom schlenk flask containing an egg-shaped stirring bar, was charged with HOAt ester **191** (1 g, 2.45 mmol, 1 equiv) and anhydrous dichloromethane (20 mL). The reaction was cooled to 0°C with an ice bath and N-allyl-4-methylbenzenesulfonamide (570 mg, 2.7

mmol, 1.1 equiv) was added, followed by addition of 1,8-diazabicyclo-undec-7-ene (560 mg, 0.55 mL, 3.68 mmol, 1.5 equiv), which turned the reaction mixture yellow. After stirring for 15 minutes the ice bath was removed and the solution was allowed to stand at room temperature for an hour. The mixture was poured into 10 % aqueous citric acid (20 mL), the layers were separated and the organic layer was washed with an additional portion of 10 % aqueous citric acid (20 mL), two 20 mL portions of saturated aqueous sodium hydrogencarbonate and 20 mL brine. Drying over anhydrous magnesium sulfate and evaporation of the solvent yielded a yellow oil that was purified further by flash column chromatography over alumina (toluene 5% ethyl acetate gradient, compound eluted at 10% ethyl acetate) yielding a white solid (924 mg, 1.9 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (ad, *J* = 8.3, 2H), 7.32 (ad, *J* = 8.2, 2H), 5.84 (ddd, *J* = 5.4, 10.4, 15.8, 1H), 5.47 (d, *J* = 9.0, 1H), 5.32 – 5.17 (m, 2H), 4.52 – 4.31 (m, 3H), 3.31 (dd, *J* = 4.3, 17.8, 1H), 3.02 (dd, *J* = 3.6, 17.9, 1H), 2.42 (s, 3H), 1.41 (s, 9H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.02, 169.90, 155.70, 145.12, 136.42, 132.48, 129.85, 128.31, 118.55, 82.14, 79.87, 50.42, 48.66, 39.23, 28.44, 27.82, 21.74. R_f: 0.55 (toluene:ethyl acetate 3:1). Melting point: 64.1°C. LC-MS (ESI): t_R = 10.35 min. HRMS (ESI): for $[M+H]^+$ calc.: 483.21595, found: 483.21584.

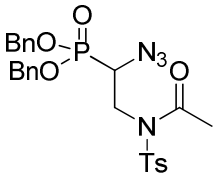


Dibenzyl 2-iodo-1-(4-methoxybenzyl)-1*H*-imidazol-4-ylphosphonate (212): At -78°C imidazole **211** (2.23 g, 5.0 mmol, 1 equiv) was dissolved in 35 mL anhydrous THF. 1.5 M t-butyl lithium in pentane (4.0 mL, 6 mmol, 1.2 equiv) was slowly added over 5 minutes. The mixture was stirred for 20 minutes, after which iodine (1.9 g, 7.5 mmol, 1.5 equiv) in 7 mL THF was slowly added until the iodine color persisted (about 5.5 mL was added). The reaction was poured into saturated aqueous sodium thiosulfate solution (50 mL) and ethyl acetate (50 mL). The layers were separated and the aqueous phase was washed with 3 additional portion of ethyl acetate (30 mL), the organic layers were combined, washed with brine (50 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue consisted of three main products, 2-iodoimidazole **212**, 5-iodoimidazole and 2,5 diiodoimidazole. The mixture was further purified by flash column chromatography (chloroform:methanol 49:1) yielding 487 mg (0.85 mmol, 17%) of a clear colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.63 (d, *J*=2.3, 1H), 7.33 – 7.27 (m, 4H), 7.26 – 7.17 (m, 6H), 7.02 (t, *J*=5.9, 2H), 6.87 – 6.77 (m, 2H), 5.16 – 5.01 (m, 4H), 4.99 (s, 2H), 3.74 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 11.29. LC-MS (ESI): t_R = 8.42 min. HRMS (ESI): for $[M+H]^+$ calc.: 575.05911, found: 575.05901.

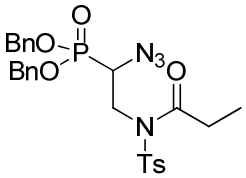
16.2.1 General procedure for the synthesis of amides 227a-t

This procedure was followed in all cases unless stated otherwise. In a 25 mL round bottom flask, containing an egg-shaped stirring bar, azide **66** (1 equiv) was dissolved in 2 mL anhydrous THF. While stirring, DIPEA (2 equiv) and DMAP (1 equiv) were added, followed by the acid chloride (2 equiv). Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After

stirring for 1 hour at room temperature, a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was removed by evaporation in vacuo and the residue was further purified by column chromatography.

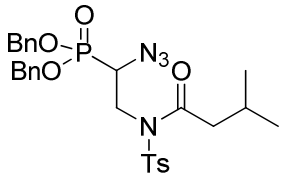


2-Azido-2-bis(benzyloxy)phosphoryl-*N*-acetyl-*N*-(4-toluenesulfonyl)-ethanamine (227a): In a 10 mL pear shaped flask, acetyl chloride (0.109 mL, 1.54 mmol, 2.0 equiv) was added to a solution of 2-azido-2-bis(benzyloxy)phospholyl-*N*-bis(4-toluenesulfonyl)-ethanamine **66** (385 mg, 0.77 mmol, 1.0 equiv), DIPEA (0.268 mL, 1.54 mmol, 2.0 equiv) in THF (1.5 mL) at 0 °C. Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After stirring for 1 hour at room temperature, a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo the residual yellow oil was purified with flash column chromatography on silica gel (toluene:ethyl acetate 8:1 in increasing gradient to 5:1) to afford **227a** as a colorless oil (380 mg, 0.70 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.5 Hz, 2H), 7.42 – 7.33 (m, 10H), 7.31 (d, *J* = 8.6 Hz, 2H), 5.22 – 5.01 (m, 4H), 4.20 – 4.01 (m, 3H), 2.43 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.48, 145.41, 136.20, 135.60 (d, *J* = 5.5 Hz), 130.02, 128.87, 128.85, 128.80 (d, *J* = 1.0 Hz), 128.38, 128.36, 128.03, 127.83, 68.92 (d, *J* = 7.0 Hz), 68.85 (d, *J* = 7.0 Hz), 57.29 (d, *J* = 150.7 Hz), 45.31 (d, *J* = 9.2 Hz), 24.94, 21.73. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.47. IR =3034 (w), 2116 (s), 1702 (s), 1596 (w), 1497 (w), 1456 (m), 1359 (s), 1242 (m), 1187 (m), 1165 (s), 1087 (m), 990 (s), 909 (s), 813 (m), 729 9s), 696 (s), 652 (s), 619 (w). R_f: 0.35 (chloroform:ethyl acetate 2:1). LC-MS (ESI): t_R = 9.75 min. HRMS (ESI): for $[M+H]^+$ calc.: 543.14617, found: 543.14586.

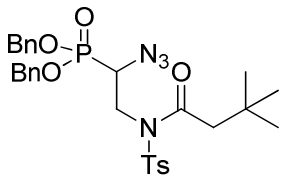


2-Azido-2-bis(benzyloxy)phosphoryl-*N*-propioyl-*N*-(4-toluenesulfonyl)-ethanamine (227b): In a 10 mL pear shaped flask, propionyl chloride (0.152 mL, 1.81 mmol, 2.0 equiv) was added to a solution of 2-azido-2-bis(benzyloxy)phospholyl-*N*-bis(4-toluenesulfonyl)-ethanamine **66** (452 mg, 0.90 mmol, 1.0

equiv), DIPEA (0.297 mL, 1.81 mmol, 2.0 equiv), and DMAP (110 mg, 0.91 mmol, 1.0 equiv) in THF (1.5 mL) at 0 °C. Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After stirring for 1 hour at room temperature, a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo the residual yellow oil was purified with flash column chromatography on silica gel (toluene:ethyl acetate 15:1 in increasing gradient to 8:1) and washed with 1 N NaOH *aq.* to afford **227b** as a colorless oil (459 mg, 0.82 mmol: 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.3 Hz, 2H), 7.43 – 7.33 (m, 10H), 7.31 (d, *J* = 8.4 Hz, 2H), 5.19 – 5.04 (m, 4H), 4.21 – 4.02 (m, 3H), 2.63 (dq, *J* = 17.5, 7.2 Hz, 1H), 2.55 – 2.43 (m, 1H), 2.43 (s, 3H), 1.01 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.12, 145.25, 136.40, 135.61 (d, *J* = 5.6 Hz), 129.96, 128.87 (d, *J* = 1.8 Hz), 128.81, 128.40 (d, *J* = 2.6 Hz), 127.89, 68.94 (d, *J* = 6.9 Hz), 68.85 (d, *J* = 7.0 Hz), 57.48 (d, *J* = 150.7 Hz), 45.13 (d, *J* = 9.0 Hz), 29.80, 21.73, 8.77. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.52. IR = 2944 (bw), 2116 (s), 1706 (s), 1596 (w), 1497 (w), 1456 (w), 1356 (s), 1260 (w), 1215 (w), 1163 (s), 1121 (w), 1087 (w), 989 (s), 910 (m), 813 (m), 729 (s), 696 (s), 665 (m). R_f: 0.20 (chloroform:ethyl acetate 5:1). LC-MS (ESI): t_R = 10.15 min. HRMS (ESI): for $[M+H]^+$ calc.: 557.16182., found: 557.16153.

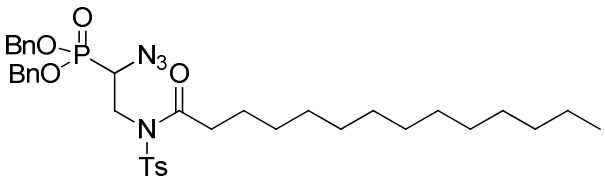


Aibenzyl 1-azido-2-(3-methyl-*N*-tosylbutanamido)ethylphosphonate (227c): 500 mg, 1 mmol of the azide was used. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 443 mg of a yellow oil (0.76 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.79 – 7.68 (m, 2H), 7.40 – 7.33 (m, 10H), 7.32 – 7.28 (m, 2H), 5.22 – 5.01 (m, 4H), 4.24 – 4.00 (m, 3H), 2.55 – 2.35 (m, 5H), 2.09 (dt, *J*=13.5, 6.7, 1H), 0.82 (d, *J*=6.6, 2H), 0.82 (d, *J*=6.7, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 172.79, 145.24, 136.62, 135.64 (d, *J*=5.7), 129.94, 128.90, 128.88, 128.84, 128.83, 128.41, 127.85, 68.94 (d, *J*=7.6), 68.86 (d, *J*=7.1), 57.61 (d, *J*=150.9), 45.04, 45.03 (d, *J*=9.0), 25.41, 22.43, 22.28, 21.76. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.48. IR: 2959 (w), 2116 (s), 1703 (m), 1596 (w), 1497 (w), 1456 (w), 1355 (s), 1260 (m), 1215 (m), 1162 (s), 1087 (m), 988 (s), 813 (m), 732 (s), 696 (s), 668 (s). R_f: 0.43 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.74 min. HRMS (ESI): for $[M+H]^+$ calc.: 585.19312, found: 585.19355.

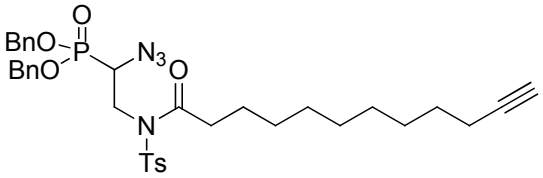


Dibenzyl 1-azido-2-(3,3-dimethyl-*N*-tosylbutanamido)ethylphosphonate (227d): 500 mg, 1 mmol of the azide was used. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 442 mg of a

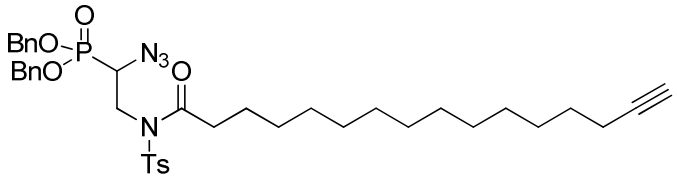
yellow oil (0.74 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.72 (d, *J*=8.3, 1H), 7.40 – 7.33 (m, 5H), 7.32 – 7.28 (m, 1H), 5.20 – 5.03 (m, 2H), 4.25 – 4.00 (m, 2H), 2.50 (s, 1H), 2.44 (s, 2H), 0.92 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 172.04, 145.17, 136.83, 135.67 (d, *J*=5.6), 129.93, 128.91, 128.89, 128.85, 128.44, 127.87, 68.94 (d, *J*=8.1), 68.87 (d, *J*=8.2), 57.65 (d, *J*=150.6), 47.85, 45.05 (d, *J*=9.2), 31.77, 29.59, 21.77. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.55. IR: 2955 (w), 2116 (s), 1703 (m), 1597 (w), 1497 (w), 1456 (w), 1354 (s), 1260 (m), 1216 (m), 1164 (s), 1087 (m), 989 (s), 813 (m), 732 (s), 696 (s), 669 (s). R_f: 0.48 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.00 min. HRMS (ESI): for $[M+H]^+$ calc.: 599.20877, found: 599.20931.



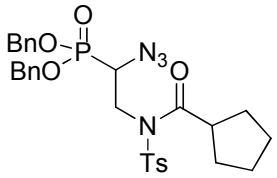
Dibenzyl 1-azido-2-(*N*-tosyltetradecanamido)ethylphosphonate (227e): In a 25 mL round bottom flask, containing an egg-shaped stirring bar, azide **66** (500 mg, 1 mmol; 1 equiv) was dissolved in 2 mL anhydrous THF. While stirring, DIPEA (259 mg, 0.348 mL, 2 mmol, 2 equiv) and DMAP (122 mg, 1 mmol, 1 equiv) were added, followed by myristoyl chloride (246 mg, 0.271 mL, 1 mmol, 1 equiv). Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After stirring for 1 hour at room temperature a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was removed by evaporation in vacuo resulting in a yellow oil. To get rid of any myristoleic acid the oil was dissolved in methanol (10 mL) and a large excess of sodium bicarbonate was added. After stirring for an hour, enough isolute was added to absorb all solvent. After evaporating the isolute to dryness it was loaded on a silica column and eluted (toluene:ethyl acetate 10:1) resulting in a yellow oil (301 mg, 0.42 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.83 – 7.70 (m, 2H), 7.45 – 7.28 (m, 12H), 5.20 – 4.99 (m, 4H), 4.27 – 3.99 (m, 3H), 2.68 – 2.36 (m, 5H), 1.56 – 1.43 (m, 2H), 1.37 – 1.13 (m, 20H), 0.88 (t, *J*=6.9, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.61, 145.33, 136.67, 135.74 (d, *J*=5.6), 130.06, 128.99, 128.97, 128.93, 128.51, 128.50, 127.98, 77.55, 77.24, 76.92, 69.04 (d, *J*=7.7), 68.97 (d, *J*=7.1), 57.62 (d, *J*=150.8), 45.21 (d, *J*=9.0), 36.47, 32.13, 29.89, 29.86, 29.81, 29.64, 29.57, 29.49, 29.08, 24.73, 22.90, 21.86, 14.33. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.51. IR: 2917 (s), 2850 (m), 2115 (s), 1705 (s), 1347 (s), 1292 (m), 1247 (s), 1167 (s), 1118 (s), 1090 (m), 996 (s), 870 (s), 813 (s), 725 (s), 696 (s), 573 (m), 562 (s), 533 (s), 512 (s), 476 (s), 456 (s). R_f: 0.53 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 14.38 min (AD card). HRMS (ESI): for $[M+Na]^+$ calc.: 711.33397, found: 711.33418.



Dibenzyl 1-azido-2-(*N*-tosyldodec-11-ynamido)ethylphosphonate (227f): 500 mg, 1 mmol of the azide was used, dodec-11-ynoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 586 mg of a yellow oil (0.86 mmol, 86%).^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73 (ad, *J*=8.4, 2H), 7.40 – 7.33 (m, 10H), 7.31 (d, *J*=8.5, 2H), 5.24 – 4.96 (m, 4H), 4.21 – 3.97 (m, 3H), 2.67 – 2.55 (m, 1H), 2.53 – 2.41 (m, 4H), 2.17 (td, *J*=7.1, 2.6, 2H), 1.93 (t, *J*=2.7, 1H), 1.58 – 1.44 (m, 4H), 1.42 – 1.30 (m, 4H), 1.30 – 1.05 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.57, 145.34, 136.65, 135.74 (d, *J*=5.6), 130.07, 128.99, 128.98, 128.93, 128.50, 127.98, 84.94, 69.04 (d, *J*=7.2), 68.97 (d, *J*=7.2), 68.32, 57.62 (d, *J*=150.7), 45.20 (d, *J*=8.9), 36.45, 29.44, 29.40, 29.21, 29.03, 28.90, 28.66, 24.71, 21.86, 18.60. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.50. IR: 3295 (w), 2924 (m), 2854 (w), 2117 (s), 1703 (m), 1596 (w), 1496 (w), 1404 (w), 1351 (s), 1255 (m), 1216 (m), 1164 (s), 1119 (m), 1088 (m), 991 (s), 813 (m), 734 (s), 734 (s), 697 (s), 666 (s), 641 (m), 585 (s), 560 (s), 543 (s), 527 (s), 484 (m). R_f: 0.52 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.51 min. HRMS (ESI): for $[M+H]^+$ calc.: 679.27137, found: 679.27271.

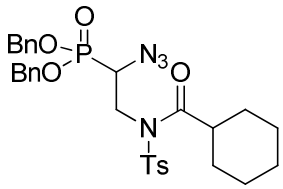


Dibenzyl 1-azido-2-(*N*-tosylhexadec-15-ynamido)ethylphosphonate (227g): 500 mg, 1 mmol of the azide was used, hexadec-15-ynoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 480 mg of a yellow oil (0.65 mmol, 65%) and 117 mg of the starting material recovered (0.23 mmol, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73 (ad, *J*=8.3, 2H), 7.40 – 7.33 (m, 10H), 7.30 (d, *J*=8.1, 2H), 5.23 – 5.02 (m, 4H), 4.22 – 4.03 (m, 3H), 2.79 – 2.38 (m, 5H), 2.17 (td, *J*=7.1, 2.6, 2H), 1.97 – 1.87 (m, 1H), 1.51 (dd, *J*=14.6, 7.1, 4H), 1.45 – 1.33 (m, 2H), 1.33 – 1.09 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.60, 145.33, 136.67, 135.75 (d, *J*=5.6), 130.07, 128.99, 128.98, 128.93, 128.51, 127.98, 85.02, 77.57, 77.26, 76.94, 69.04 (d, *J*=7.4), 68.97 (d, *J*=7.4), 68.27, 57.63 (d, *J*=150.7), 45.21 (d, *J*=9.1), 36.47, 29.82, 29.79, 29.79, 29.71, 29.63, 29.48, 29.32, 29.08, 28.98, 28.72, 24.73, 21.86, 18.62. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.51. IR: 3300 (w), 2924 (s), 2853 (m), 2116 (s), 1703 (m), 1596 (w), 1497 (w), 1456 (m), 1356 (s), 1260 (s), 1186 (s), 1163 (m), 1120 (m), 1087 (m), 989 (s), 814 (m), 734 (s), 696 (s), 665 (s), 583 (s), 543 (s), 484 (m). R_f: 0.54 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 12.52 min. HRMS (ESI): for $[M+NH_4]^+$ calc.: 752.36052, found: 752.36071.

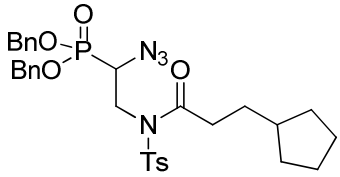


Dibenzyl 1-azido-2-(*N*-tosylcyclopentanecarboxamido)ethylphosphonate (227h): 500 mg, 1 mmol of the azide was used, cyclopentanecarbonyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 443 mg of a yellow oil (0.74 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73 (ad, *J*=8.3, 2H), 7.40 – 7.33 (m, 10H), 7.32 – 7.28 (m, 2H), 5.10 (m, 4H), 4.25 – 3.98 (m, 3H), 3.31 – 3.14 (m, 1H), 2.43 (s, 3H), 1.83 – 1.38 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 177.54, 145.08, 136.80, 135.65 (d, *J*=5.9), 129.93, 128.92, 128.90, 128.85, 128.45, 128.43, 127.83, 68.97 (d, *J*=9.9), 68.90 (d, *J*=10.1), 57.75 (d, *J*=151.0), 45.04 (d, *J*=9.2), 44.39, 31.18, 30.85, 26.24, 21.78. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.46. IR: 2955 (w), 2116 (s), 1699 (m), 1596 (w), 1497 (w), 1454 (m), 1354 (s),

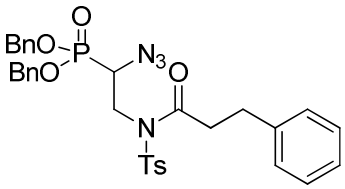
1260 (s), 1215 (m), 1162 (s), 1119 (m), 1087 (m), 988 (s), 813 (m), 733 (s), 696 (s), 666 (s). R_f: 0.46 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.81 min. HRMS (ESI): for $[M+H]^+$ calc.: 597.19312, found: 597.19341.



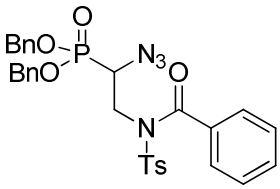
Dibenzyl 1-azido-2-(*N*-tosylcyclohexanecarboxamido)ethylphosphonate (227i): 500 mg, 1 mmol of the azide was used, cyclohexanecarbonyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 516 mg of a yellow oil (0.84 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.83 – 7.67 (m, 2H), 7.40 – 7.33 (m, 10H), 7.31 (m, 2H), 5.19 – 5.02 (m, 4H), 4.27 – 3.98 (m, 3H), 2.93 (m, 1H), 2.43 (s, 3H), 1.60 (m, 5H), 1.46 – 1.23 (m, 2H), 1.23 – 0.99 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 176.99, 145.17, 136.94, 135.67 (d, *J*=5.7), 129.98, 128.89, 128.88, 128.85, 128.84, 128.43, 128.42, 127.72, 68.94 (d, *J*=9.3), 68.82 (s, *J*=9.5), 57.61 (d, *J*=151.1), 44.90 (d, *J*=9.3), 44.40, 29.51, 29.34, 25.61, 25.58, 25.48, 21.78. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.45. IR: 2930 (w), 2856 (w), 2117 (s), 1698 (m), 1596 (w), 1496 (w), 1453 (m), 1355 (s), 1260 (s), 1215 (m), 1160 (s), 1087 (m), 986 (s), 813 (m), 731 (s), 696 (s), 667 (s). R_f: 0.54 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.07 min. HRMS (ESI): for $[M+Na]^+$ calc.: 633.19071, found: 633.19072.



Dibenzyl 1-azido-2-(3-cyclopentyl-*N*-tosylpropanamido)ethylphosphonate (227j): 500 mg, 1 mmol of the azide was used, cyclopentanepropionyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 489 mg of a yellow oil (0.76 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74 (d, *J*=8.3, 2H), 7.40 – 7.33 (m, 10H), 7.31 (d, *J*=8.5, 2H), 5.21 – 5.00 (m, 4H), 4.23 – 4.01 (m, 3H), 2.55 (m, 2H), 2.44 (s, 3H), 1.71 – 1.38 (m, 9H), 1.08 – 0.91 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 173.62, 145.27, 136.58, 135.66 (d, *J*=5.6), 129.97, 128.90, 128.89, 128.85, 128.42, 127.93, 68.95 (d, *J*=8.0), 68.88 (d, *J*=8.0), 57.54 (d, *J*=150.6), 45.17 (d, *J*=9.1), 39.49, 35.72, 32.50, 32.44, 30.74, 25.19, 21.77. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.51. IR: 2947 (w), 2116 (s), 1702 (m), 1596 (w), 1497 (w), 1455 (w), 1355 (s), 1259 (m), 1215 (m), 1163 (s), 1118 (w), 1087 (m), 988 (s), 813 (m), 732 (s), 696 (s), 666 (s). R_f: 0.48 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.36 min. HRMS (ESI): for $[M+H]^+$ calc.: 625.22442, found: 625.22460.

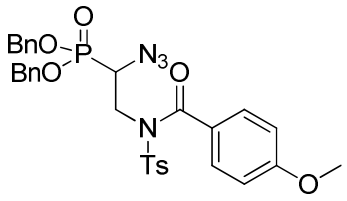


Dibenzyl 1-azido-2-(3-phenyl-*N*-tosylpropanamido)ethylphosphonate (227k): 500 mg, 1 mmol of the azide was used, hydrocinnamoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 438 mg of a yellow oil (0.69 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.69 (d, *J*=8.3, 2H), 7.39 – 7.33 (m, 10H), 7.28 (d, *J*=8.0, 2H), 7.25 – 7.15 (m, 3H), 7.10 – 7.01 (m, 2H), 5.19 – 5.02 (m, 4H), 4.17 – 3.98 (m, 3H), 3.05 – 2.74 (m, 4H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 172.62, 145.30, 140.23, 136.36, 135.62 (d, *J*=5.8), 130.00, 128.90, 128.88, 128.83, 128.58, 128.41, 128.40, 127.86, 126.37, 68.95 (d, *J*=9.9), 68.88 (d, *J*=10.0), 57.47 (d, *J*=150.6), 45.13 (d, *J*=9.1), 38.00, 30.70, 21.78. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.44. IR: 3031 (w), 2116 (s), 1702 (m), 1596 (w), 1496 (w), 1454 (w), 1355 (s), 1260 (m), 1215 (m), 1163 (s), 1118 (w), 1087 (w), 987 (s), 909 (m), 812 (m), 768 (m), 730 (s), 696 (s), 666 (s). R_f: 0.48 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.77 min. HRMS (ESI): for $[M+H]^+$ calc.: 633.19312, found: 633.19344.

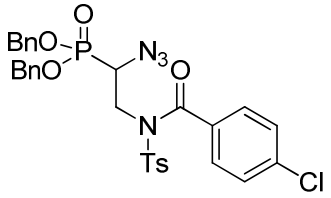


2-Azido-2-bis(benzyloxy)phosphoryl-*N*-benzoyl-*N*-(4-toluenesulfonyl)-ethanamine (227l): In a 10 mL pear shaped flask, benzoyl chloride (0.200 mL, 1.86 mmol, 2.0 equiv) was added to a solution of 2-azido-2-bis(benzyloxy)phospholyl-*N*-bis(4-toluenesulfonyl)-ethanamine (465 mg, 0.93 mmol, 1.0 equiv), DIPEA (0.323 mL, 1.86 mmol, 2.0 equiv), and DMAP (118 mg, 0.93 mmol, 1.0 equiv) in THF (1.5 mL) at 0 °C. Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After stirring for 1 hour at room temperature, a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo the residual yellow oil was purified with flash column chromatography on silica gel (toluene:ethyl acetate 15:1 in increasing gradient to 8:1) and washed with 1 N NaOH *aq.* to afford **227l** as a colorless oil (521 mg, 0.86 mmol: 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.3 Hz, 2H), 7.53 (d, *J* = 8.3 Hz, 2H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.42 – 7.26 (m, 14H), 7.25 (d, *J* = 8.2 Hz, 2H), 5.19 – 4.91 (m, 4H), 4.27 – 3.90 (m, 3H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.48, 145.30, 135.48 (d, *J* = 5.7 Hz, 2C), 135.35, 134.67, 132.08, 129.80, 128.86, 128.84, 128.79, 128.78, 128.75, 128.44, 128.30, 128.28, 77.48, 77.16, 76.84, 68.91 (d, *J* = 6.8 Hz), 68.79 (d, *J* = 7.0 Hz), 57.58 (d, *J* = 151.9 Hz), 46.30 (d, *J* = 8.4 Hz), 21.73. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.95. IR = 3065 (w), 3034 (w), 2959 (w), 2350 (bw), 2114 (s), 1689 (s), 1597 (w), 1496 (w), 1454

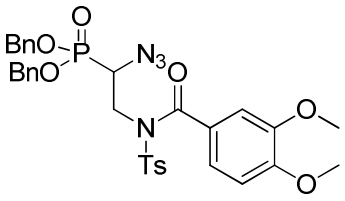
(m), 1359 (s), 1264 (s), 1214 (m), 1187 (s), 1165 (w), 1121 (m), 1088 (s), 989 (s), 964 (w), 924 (w), 845 (w), 814 (m), 773 (w), 732 (s), 695 (s), 661 (s). R_f: 0.55 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.41 min. HRMS (ESI): for $[M+H]^+$ calc.: 605.16182, found: 605.16205.



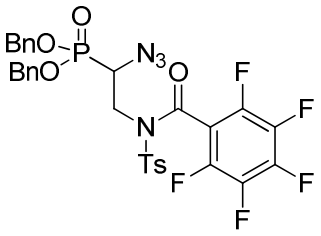
Dibenzyl (1-azido-2-(4-methoxy-*N*-tosylbenzamido)ethyl)phosphonate (227m): 500 mg, 1 mmol of the azide was used, 4-methoxybenzoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 in increasing gradient to 8:1) resulting in 577 mg of a colorless oil (0.91 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.65 (m, 4H), 7.39 – 7.26 (m, 10H), 7.24 (d, *J* = 8.0 Hz, 2H), 6.93 – 6.82 (m, 2H), 5.14 – 4.94 (m, 4H), 4.11 – 3.95 (m, 3H), 3.85 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.31, 163.50, 145.23, 135.66 (d, *J* = 5.7 Hz), 135.64 (d, *J* = 5.9 Hz), 132.06, 129.99, 128.96, 128.91, 128.90, 128.42, 128.40, 128.39, 126.96, 113.80, 69.00 (d, *J* = 11.2 Hz), 68.93 (d, *J* = 11.3 Hz), 57.83 (d, *J* = 152.2 Hz), 55.68, 46.54 (d, *J* = 8.2 Hz), 21.86. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.07. IR: 2957 (w), 2113 (s), 1683 (m), 1601 (s), 1509 (m), 1456 (m), 1359 (m), 1251 (s), 1163 (s), 1088 (s), 989 (s), 912 (m), 840 (m), 813 (m), 731 (s), 695 (s), 672 (s). R_f: 0.55 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.39 min. HRMS (ESI): for $[M+Na]^+$ calc.: 657.15433 found: 657.15468.



Dibenzyl (1-azido-2-(4-chloro-*N*-tosylbenzamido)ethyl)phosphonate (227n): 500 mg, 1 mmol of the azide was used, 4-chlorobenzoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 gradient to 8:1) resulting in 491 mg of a yellow oil (0.77 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.57 (m, 2H), 7.55 – 7.44 (m, 2H), 7.39 – 7.27 (m, 12H), 7.27 – 7.23 (m, 2H), 5.13 – 4.91 (m, 4H), 4.12 – 3.90 (m, 3H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.75, 145.64, 138.75, 135.58 (d, *J* = 5.7 Hz), 135.55 (d, *J* = 5.6 Hz), 135.21, 133.26, 130.53, 130.05, 129.05, 128.95, 128.74, 128.48, 128.45, 69.08 (d, *J* = 11.9 Hz), 69.01 (d, *J* = 12.1 Hz), 57.58 (d, *J* = 152.2 Hz), 46.29 (d, *J* = 8.5 Hz), 21.89. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.88. IR: 3034 (w), 2958 (w), 2113 (s), 1692 (m), 1688 (m), 1593 (m), 1456 (w), 1360 (s), 1261 (s), 1215 (m), 1187 (s), 1087 (s), 989 (s), 910 (m), 838 (s), 814 (s), 730 (s), 694 (s), 667 (s). R_f: 0.53 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.80 min. HRMS (ESI): for $[M+H]^+$ calc.: 639.12285, found: 639.12255.

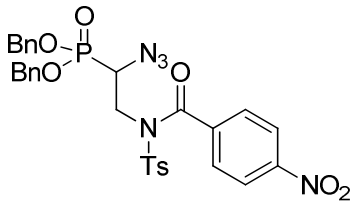


Dibenzyl 1-azido-2-(3,4-dimethoxy-*N*-tosylbenzamido)ethylphosphonate (227o): 500 mg, 1 mmol of the azide was used, 3,4-dimethoxybenzoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 530 mg of a yellow oil (0.80 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.63 (d, *J*=8.1, 2H), 7.39 – 7.27 (m, 11H), 7.24 (d, *J*=8.5, 2H), 7.17 (d, *J*=1.4, 1H), 6.82 (d, *J*=8.4, 1H), 5.13 – 4.96 (m, 4H), 4.10 – 3.99 (m, 3H), 3.91 (s, 3H), 3.85 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.23, 153.10, 148.79, 145.14, 135.53 (d, *J*=5.7), 135.50 (d, *J*=5.6), 135.24, 129.86, 128.87, 128.81, 128.79, 128.35, 128.30, 128.28, 126.83, 124.10, 112.24, 110.14, 68.90 (d, *J*=10.5), 68.84 (d, *J*=10.6), 57.74 (d, *J*=152.6), 56.16, 56.09, 46.56 (d, *J*=8.2), 21.74. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.02. IR: 2960 (w), 2117 (s), 1682 (m), 1596 (m), 1513 (s), 1415 (m), 1358 (m), 1265 (s), 1216 (s), 1163 (s), 11.39 (s), 1087 (s), 989 (s), 911 (s), 866 (m), 813 (m), 768 (m), 728 (s), 696 (s), 674 (s). R_f: 0.25 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.13 min. HRMS (ESI): for $[M+Na]^+$ calc.: 687.16489, found: 687.16529.

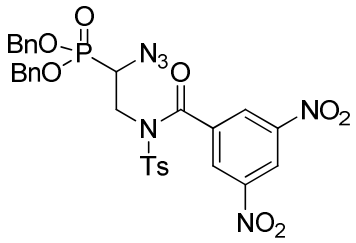


2-Azido-2-bis(benzyloxy)phosphoryl-*N*-pentafluorobenzoyl-*N*-(4-toluenesulfonyl)-ethanamine (227p): In a 10 mL pear shaped flask, pentafluorobenzoyl chloride (0.246 mL, 1.78 mmol, 2.0 equiv) was added to a solution of 2-azido-2-bis(benzyloxy)phospholyl-*N*-bis(4-toluenesulfonyl)-ethanamine (446 mg, 0.89 mmol, 1.0 equiv), DIPEA (0.323 mL, 1.86 mmol, 2.0 equiv) in THF (2 mL) at 0 °C. Upon addition of the acid chloride a white precipitate formed, turning the reaction to a thick suspension. After stirring for 1 hour at room temperature, a TLC sample indicated complete conversion of the starting azide and the reaction was quenched by addition of 5 mL saturated aqueous sodium bicarbonate, this dissolved the white precipitate. The resulting mixture was poured into 10 mL of ethyl acetate, the layers were separated and the aqueous layer was washed with 2 additional 10 mL portions of ethyl acetate. The organic layers were combined, washed with 10% aqueous citric acid (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo the residual yellow oil was purified with flash column chromatography on silica gel (toluene:ethyl acetate 15:1 gradient 10:1) to afford **227p** as a yellow oil (584 mg, 0.84 mmol: 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.4 Hz, 2H), 7.43 – 7.32 (m, 8H), 7.28 (d, *J* = 8.5 Hz, 2H), 5.15 (ddd, *J* = 11.5, 8.8, 2.9 Hz, 2H), 5.07 (ddd, *J* = 11.5, 8.8, 2.9 Hz, 2H), 4.26 – 3.96 (m, 3H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.67, 146.49, 145.0 – 143.9 (br), 142.5 – 141.2 (br), 139.1 – 138.4 (bs), 136.5 – 135.7 (bs), 135.41 (d, *J* = 5.8 Hz), 134.25, 130.09, 128.96 (d, *J* = 1.5 Hz), 128.82, 128.38, 128.17, 111.6 – 110.9, 69.11 (d, *J* = 6.9 Hz), 69.00 (d, *J* = 7.0 Hz), 57.27 (d, *J*

= 152.5 Hz). 21.76. ^{19}F NMR (377 MHz, $CDCl_3$) δ -140.16 (d, *J* = 844.6 Hz), -149.92 (s), -160.36 (d, *J* = 113.6 Hz).^{31}P NMR (162 MHz, $CDCl_3$) δ 19.68. IR = 3035 (w), 2122 (s), 1696 (m), 1655 (w), 1595 (w), 1521 (m), 1505 (s), 1456 (w), 1432 (w), 1374 (s), 1329 (m), 1238 (m), 1215 (m), 188 (w), 1170 (s), 1142 (w), 1089 (w), 992 (s), 907 (s), 840 (w), 813 (m), 728 (s), 697 (s), 666 (s), 648 (s). R_f: 0.55 (chloroform:ethyl acetate 5:1). LC-MS (ESI): t_R = 10.69 min. HRMS (ESI): for $[M+H]^+$ calc.: 695.11471, found: 605.11507.



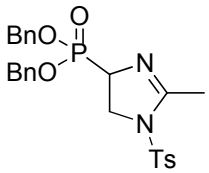
Dibenzyl 1-azido-2-(4-nitro-*N*-tosylbenzamido)ethylphosphonate (227p): 500 mg, 1 mmol of the azide was used, 3,3-dimethylbutyryl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 0.1% Et_3N) resulting in 533 mg of a yellow oil (0.82 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.16 (m, 2H), 7.60 (m, 4H), 7.43 – 7.19 (m, 12H), 5.17 – 4.95 (m, 4H), 4.17 – 3.89 (m, 3H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.76, 149.52, 146.12, 140.74, 135.51 (d, *J*=5.6), 135.45 (d, *J*=5.6), 134.94, 130.21, 129.62, 129.13, 128.98, 128.96, 128.53, 128.50, 128.43, 123.48, 77.57, 77.25, 76.94, 69.14 (d, *J*=6.8), 69.07 (d, *J*=6.9), 57.23 (d, *J*=152.1), 45.94 (d, *J*=7.6), 21.93. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 19.75. IR: 3034 (w), 2957 (w), 2113 (s), 1690 (m), 1597 (w), 1524 (s), 1496 (w), 1455 (w), 1346 (s), 1260 (s), 1215 (m), 1188 (w), 1166 (s), 1087 (m), 987 (s), 851 (s), 814 (m), 732 (s), 711 (s), 695 (s), 665 (s), 593 (s), 571 (s), 544 (m), 517 (m), 485 (m), 459 (m). R_f: 0.46 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.30 min. HRMS (ESI): for $[M+H]^+$ calc.: 650.14690, found:650.14698.



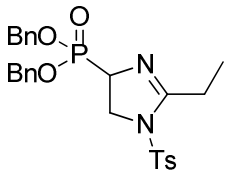
Dibenzyl (1-azido-2-(3,5-dinitro-*N*-tosylbenzamido)ethyl)phosphonate (227q): 500 mg, 1 mmol of the azide was used, 3,5-dinitrobenzoyl chloride was used as acid chloride. Column chromatography (toluene:ethyl acetate 10:1 gradient to 8:1) resulting in 653 mg of a yellow oil (0.94 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (t, *J* = 2.1 Hz, 1H), 8.59 (d, *J* = 2.1 Hz, 2H), 7.65 – 7.43 (m, 2H), 7.45 – 7.30 (m, 10H), 7.30 – 7.21 (m, 2H), 5.20 – 4.96 (m, 4H), 4.21 – 3.96 (m, 3H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.29, 147.87, 146.55, 138.31, 135.31 (d, *J* = 7.5 Hz), 135.27 (d, *J* = 7.4 Hz), 134.19, 130.32, 129.02, 128.95, 128.91, 128.76, 128.75, 128.42, 128.33, 128.32, 128.21, 127.99, 120.98, 6968.98 (d, *J* = 6.8 Hz), 68.95 (d, *J* = 7.0 Hz), 56.57 (d, *J* = 152.0 Hz), 45.47 (d, *J* = 7.0 Hz), 21.69. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.74. IR: 3092 (w), 2116 (s), 1692 (m), 1626 (w), 1595 (w), 1542 (s), 1496 (w), 1456 (m), 1343 (s), 1260 (s), 1165 (s), 1084 (m), 989 (s), 912 (s), 814 (s), 728 (s), 697 (s), 665 (s). R_f: 0.48 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.44 min. HRMS (ESI): for $[M+NH_4]^+$ calc.: 712.15852, found: 712.15921.

16.2.2 General procedure for the synthesis of amides 228a-r

This procedure was followed in all cases unless stated otherwise. Under argon a flame dried, 100 mL schlenk flask was charged with the azide (1 equiv) and 20 mL anhydrous THF. dppe (1.5 equiv) was added and the reaction was heated to 50°C in an oil bath, steady bubble formation was noticed. Once bubble formation stopped the oil bath was set to 80°C and the schlenk flask was closed. After heating for 16 hours the flask was allowed to cool to about 50°C and 7 mL saturated aqueous sodium bicarbonate solution: 30% hydrogen peroxide 1:1 was added. The mixture was stirred until a TLC sample confirmed complete oxidation of dppe. Chloroform (20 mL) was added, the layers were separated and the aqueous layer was extracted twice with 5 mL portions of chloroform. Organic layers were combined, washed with brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated and the resulting sticky solid was further purified by flash column chromatography.

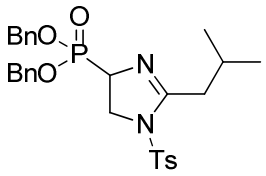


4-Bis(benzyloxy)phosphoryl-4,5-dihydro-2-methyl-1-(4-toluenesulfonyl)-1*H*-imidazole (228a): Azide **227a** (340 mg, 0.63 mmol, 1.0 equiv) was used. Column chromatography (toluene:ethyl acetate 2:1 gradient 1:1) to afford **228a** as a colorless oil (233 mg, 0.47 mmol; 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.3 Hz, 2H), 7.44 – 7.19 (m, 10H), 5.09 – 4.86 (m, 4H), 4.27 – 4.15 (m, 1H), 4.06 – 3.86 (m, 2H), 2.40 (s, 3H), 2.22 (dd, *J* = 3.3, 1.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.79 (d, *J* = 13.4 Hz), 144.90, 136.08 (d, *J* = 5.7 Hz), 136.01 (d, *J* = 5.4 Hz), 135.38, 130.19, 128.66, 128.60, 128.57, 128.09, 127.97, 127.17, 77.48, 77.16, 76.84, 68.34 (d, *J* = 6.7 Hz), 61.07 (d, *J* = 166.3 Hz), 49.14, 21.67, 16.72 (d, *J* = 1.9 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.01. R_f: 0.40 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 9.15 min. HRMS (ESI): for $[M+H]^+$ calc.: 499.145511, found: 499.11458.

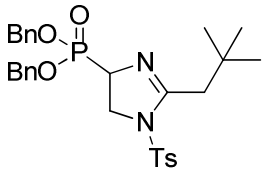


4-Bis(benzyloxy)phosphoryl-4,5-dihydro-2-ethyl-1-(4-toluenesulfonyl)-1*H*-imidazole (228b): Azide **227b** (384 mg, 0.69 mmol, 1 equiv) was used. Column chromatography (toluene:ethyl acetate 8:1 gradient 5:1) to afford **228b** as a colorless oil (306 mg, 0.60 mmol; 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.2 Hz, 2H), 7.39 – 7.20 (m, 12H), 5.14 – 4.87 (m, 4H), 4.32 – 4.13 (m, 1H), 4.10 – 3.82 (m, 2H), 2.75 – 2.59 (m, 1H), 2.59 – 2.45 (m, 1H), 2.39 (s, 3H), 1.17 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.06 (d, *J* = 13.2 Hz), 144.78, 136.13 (d, *J* = 6.0 Hz), 136.06 (d, *J* = 5.5 Hz), 135.49, 130.14, 128.53 (d, *J* = 2.1 Hz), 127.97, 127.93, 127.14, 68.40 (d, *J* = 6.8 Hz), 68.26 (d, *J* = 6.9 Hz), 61.20 (d, *J* = 165.7 Hz), 49.20, 23.37 (d, *J* = 1.6 Hz), 21.65, 10.81. ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.00. R_f: 0.20

(chloroform:ethyl acetate 5:1). LC-MS (ESI): t_R = 9.91 min. HRMS (ESI): for $[M+H]^+$ calc.: 513.16076, found: 513.16039.

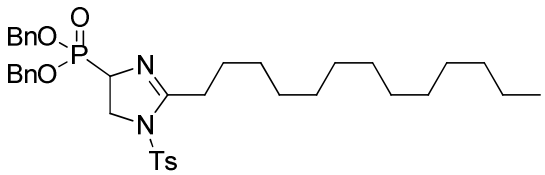


Dibenzyl 2-isobutyl-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228c): Azide **227c** (406 mg, 0.69 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 318 mg (0.59 mmol, 85%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.69 (ad, *J*=8.3, 2H), 7.42 – 7.20 (m, 12H), 5.08 – 4.89 (m, 4H), 4.30 – 4.11 (m, 1H), 2.54 – 2.45 (m, 2H), 2.40 (s, 3H), 2.16 (dp, *J*=13.4, 6.7, 1H), 0.93 (t, *J*=7.1, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.54 (d, *J*=13.4), 144.94, 136.26 (d, *J*=5.8), 136.22 (d, *J*=5.3), 135.59, 130.28, 128.78, 128.72, 128.70, 128.21, 127.36, 77.57, 77.26, 76.94, 68.57 (d, *J*=6.7), 68.36 (d, *J*=7.0), 61.42 (d, *J*=166.2), 49.20, 38.54, 26.82, 22.70, 22.62, 21.81. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.15. R_f: 0.30 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.31 min. HRMS (ESI): for $[M+H]^+$ calc.: 541.19206, found: 541.19171.

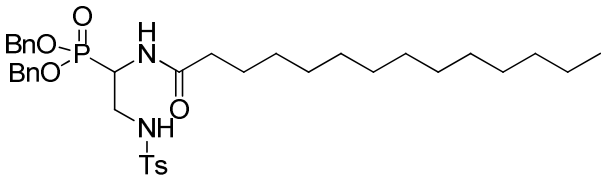


Dibenzyl 2-neopentyl-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228d): Azide **227d** (408 mg, 0.68 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielding 349 mg (0.63 mmol, 93%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.68 (m, 2H), 7.39 – 7.22 (m, 12H), 5.10 – 4.88 (m, 4H), 4.25 – 4.09 (m, 1H), 3.92 (m, 2H), 2.82 – 2.69 (m, 1H), 2.57 – 2.46 (m, 1H), 2.40 (s, 3H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.55 (d, *J*=14.0), 144.90, 136.24 (d, *J*=5.7), 136.22 (d, *J*=5.4), 135.52, 130.27, 128.78, 128.71, 128.27, 128.19, 127.41, 77.57, 77.25, 76.93, 68.59 (d, *J*=6.7), 68.27 (d, *J*=7.0), 61.51 (d, *J*=167.4), 48.79, 41.36, 41.34, 32.30, 32.28, 29.84, 21.81. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.32. R_f: 0.40 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.65 min. HRMS (ESI): for $[M+H]^+$ calc.: 555.20771, found: 555.20736.

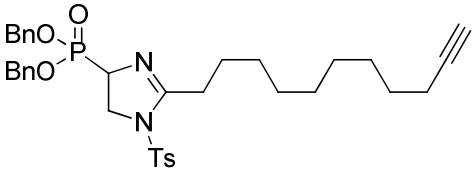
Dibenzyl 1-tosyl-2-tridecyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228e) and Dibenzyl 2-(4-methylphenylsulfonamido)-1-tetradecanamidoethylphosphonate (230): Azide **227e** (301 mg, 0.42 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 116 mg (0.17 mmol, 41%) of a clear oil (**228e**) and 73 mg (0.11 mmol, 25%) of a clear oil that solidified on standing (**230**).



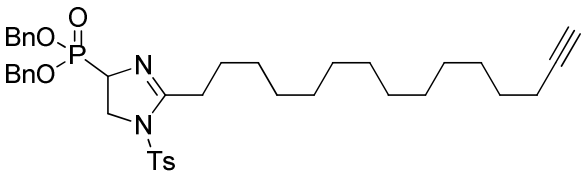
228e: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.75 – 7.64 (m, 2H), 7.34 – 7.22 (m, 12H), 5.08 – 4.92 (m, 4H), 4.30 – 4.15 (m, 1H), 4.09 – 3.80 (m, 2H), 2.67 – 2.45 (m, 2H), 2.40 (s, 3H), 1.72 – 1.56 (m, 2H), 1.36 – 1.16 (m, 20H), 0.88 (t, *J*=6.9, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.40 (d, *J*=13.3), 144.92, 136.29 (d, *J*=5.7), 136.23 (d, *J*=5.6), 130.28, 128.77, 128.70, 128.68, 128.15, 128.11, 127.34, 68.51 (d, *J*=12.1), 68.44 (d, *J*=12.4), 61.38 (d, *J*=165.6), 49.37, 32.14, 29.92, 29.90, 29.88, 29.76, 29.57, 29.56, 29.49, 26.75, 22.91, 21.81, 14.34. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.05. R_f: 0.69 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 14.24 min. HRMS (ESI): for $[M+H]^+$ calc.: 667.33291, found: 667.33275.



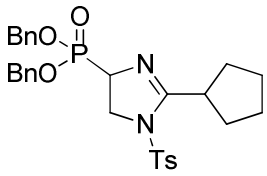
230: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74 (d, *J*=8.2, 2H), 7.38 – 7.15 (m, 12H), 7.01 (d, *J*=9.5, 1H), 6.55 (t, *J*=6.6, 1H), 5.11 – 4.88 (m, 4H), 4.72 (ddt, *J*=18.4, 9.5, 4.6, 1H), 3.38 – 3.17 (m, 2H), 2.38 (s, 3H), 2.23 – 2.04 (m, 2H), 1.61 – 1.43 (m, 2H), 1.38 – 1.11 (m, 20H), 0.88 (t, *J*=6.9, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.82 (d, *J*=6.1), 143.49, 137.23, 135.93 (d, *J*=16.5), 135.87 (d, *J*=16.5)129.89, 128.86, 128.84, 128.76, 128.28, 128.08, 127.35, 68.71 (d, *J*=44.8), 68.64 (d, *J*=44.8), 45.04 (d, *J*=157.9), 43.33 (d, *J*=1.5), 36.48, 32.15, 29.92, 29.90, 29.89, 29.75, 29.63, 29.58, 29.54, 25.73, 22.91, 21.71, 14.34. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 24.17. R_f: 0.26 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 12.44 min. HRMS (ESI): for $[M+H]^+$ calc.: 685.34347, found: 685.34435.



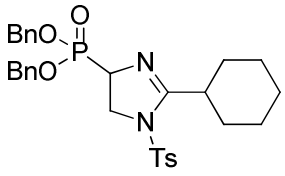
Dibenzyl 1-tosyl-2-(undec-10-ynyl)-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228f): Azide **227f** (523 mg, 0.77 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 489 mg (0.77 mmol, 100%) of a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.75 – 7.62 (m, 2H), 7.42 – 7.22 (m, 12H), 5.10 – 4.91 (m, 4H), 4.32 – 4.15 (m, 1H), 4.08 – 3.83 (m, 2H), 2.67 – 2.44 (m, 2H), 2.40 (s, 3H), 2.17 (td, *J*=6.9, 2.6, 2H), 1.93 (t, *J*=2.7, 1H), 1.73 – 1.56 (m, 2H), 1.56 – 1.44 (m, 2H), 1.44 – 1.16 (m, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.29 (d, *J*=13.3), 144.85136.19 (d, *J*=6.1), 136.13 (d, *J*=5.5), 130.19, 128.69, 128.61, 128.60, 128.06, 128.02, 127.25, 84.87, 68.42 (d, *J*=12.9), 68.35 (d, *J*=13.1), 68.23, 61.28 (d, *J*=165.8), 49.28, 29.84, 29.46, 29.37, 29.34, 29.19, 28.84, 28.60, 26.63, 21.73, 18.52. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.06. R_f: 0.46 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.31 min. HRMS (ESI): for $[M+H]^+$ calc.: 635.27031, found: 635.27046.



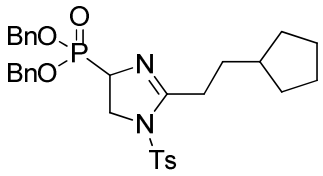
Dibenzyl 2-(pentadec-14-ynyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228g): Azide **227g** (410 mg, 0.56 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 372 mg (0.54 mmol, 96%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (ad, *J*=8.0, 2H), 7.40 – 7.21 (m, 12H), 5.14 – 4.91 (m, 4H), 4.32 – 4.15 (m, 1H), 4.11 – 3.82 (m, 2H), 2.67 – 2.44 (m, 2H), 2.40 (s, 3H), 2.21 – 2.11 (m, 2H), 1.93 (td, *J*=2.6, 0.8, 1H), 1.69 – 1.56 (m, 2H), 1.52 (dt, *J*=14.5, 7.0, 2H), 1.48 – 1.10 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.33 (d, *J*=13.3), 144.84136.20 (d, *J*=6.0), 136.15 (d, *J*=5.7), 130.20, 128.69, 128.62, 128.60, 128.07, 128.03, 127.26, 84.94, 68.43 (d, *J*=12.4), 68.36 (d, *J*=12.7), 68.17, 61.28 (d, *J*=165.7), 49.28, 29.86, 29.77, 29.74, 29.66, 29.65, 29.46, 29.40, 29.25, 28.90, 28.64, 26.66, 21.73, 18.54. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.06. R_f: 0.45 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 12.49 min. HRMS (ESI): for $[M+H]^+$ calc.: 691.33291, found: 691.33322.



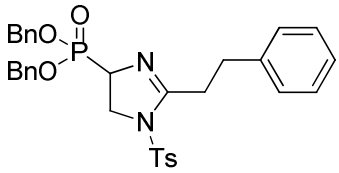
Dibenzyl 2-cyclopentyl-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228h): Azide **227h** (443 mg, 0.74 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 343 mg (0.62 mmol, 84%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (ad, *J*=8.3, 2H), 7.39 – 7.20 (m, 12H), 5.12 – 4.89 (m, 4H), 4.21 (ddd, *J*=14.6, 11.4, 7.8, 1H), 4.08 – 3.84 (m, 2H), 3.39 – 3.23 (m, 1H), 2.40 (s, 3H), 2.01 – 1.45 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.29 (d, *J*=13.0), 144.73, 136.21 (d, *J*=5.9), 136.18 (d, *J*=5.5), 135.68, 130.15, 128.67, 128.58, 128.02, 127.23, 77.48, 77.16, 76.84, 68.41 (d, *J*=9.9), 68.35 (d, *J*=10.3), 61.22 (d, *J*=165.2), 49.52, 38.91, 38.89, 32.25, 32.23, 31.81, 25.82, 25.75, 21.72. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.04. R_f: 0.40 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.51 min. HRMS (ESI): for $[M+H]^+$ calc.: 553.19206, found: 553.19166.



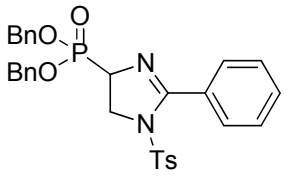
Dibenzyl 2-cyclohexyl-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228i): Azide **227i** (464 mg, 0.76 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 392 mg (0.69 mmol, 91%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.72 – 7.67 (m, 2H), 7.40 – 7.21 (m, 12H), 5.09 – 4.87 (m, 4H), 4.31 – 4.11 (m, 1H), 4.08 – 3.80 (m, 2H), 2.93 (t, *J*=10.7, 1H), 2.40 (s, 3H), 1.97 – 1.61 (m, 5H), 1.55 – 1.09 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.32 (d, *J*=13.2), 144.78, 136.20 (d, *J*=6.0), 136.19 (d, *J*=5.4), 135.65, 130.15, 128.66, 128.57, 128.08, 128.05, 127.20, 77.48, 77.16, 76.84, 68.39 (d, *J*=7.0), 68.38 (d, *J*=6.7), 61.38 (d, *J*=165.3), 49.28, 38.23, 31.58, 31.56, 31.23, 31.22, 26.05, 26.03, 25.91, 21.71. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.01. R_f: 0.37 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.69 min. HRMS (ESI): for $[M+H]^+$ calc.: 567.20771, found: 567.20739.



Dibenzyl 2-(2-cyclopentylethyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228j): Azide **227j** (455 mg, 0.73 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 396 mg (0.68 mmol, 93%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (m, 2H), 7.44 – 7.25 (m, 12H), 5.13 – 4.88 (m, 4H), 4.29 – 4.13 (m, 1H), 4.10 – 3.84 (m, 2H), 2.68 – 2.47 (m, 2H), 2.40 (s, 3H), 1.84 – 1.39 (m, 9H), 1.13 – 0.96 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.54 (d, *J*=13.3), 144.95, 136.28 (d, *J*=5.7), 136.23 (d, *J*=5.4), 135.67, 130.28, 128.78, 128.70, 128.69, 128.14, 128.10, 127.34, 77.60, 77.28, 76.96, 68.50 (d, *J*=9.1), 68.43 (d, *J*=9.3), 61.36 (d, *J*=165.7), 49.39, 39.91, 32.93, 32.59, 32.57, 29.25, 25.35, 21.81. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.07. R_f: 0.43 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.04 min. HRMS (ESI): for $[M+H]^+$ calc.: 581.22336, found: 581.22317.

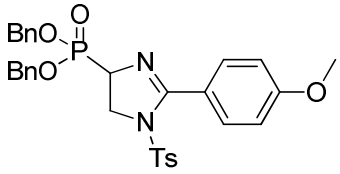


Dibenzyl 2-phenethyl-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228k): Azide **227k** (396 mg, 0.63 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 283 mg (0.48 mmol, 76%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73 – 7.62 (m, 2H), 7.44 – 7.23 (m, 14H), 7.23 – 7.15 (m, 3H), 5.09 – 4.89 (m, 4H), 4.32 – 4.18 (m, 1H), 4.11 – 3.82 (m, 2H), 3.06 – 2.77 (m, 4H), 2.40 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.44 (d, *J*=13.3), 144.93, 140.85, 136.16 (d, *J*=5.8), 136.11 (d, *J*=5.1), 135.37, 130.23, 128.71, 128.64, 128.62, 128.57, 128.56, 128.06, 128.02, 127.23, 126.30, 77.48, 77.16, 76.84, 68.43 (d, *J*=13.1), 68.36 (d, *J*=13.2), 61.34 (d, *J*=165.9), 49.33, 32.58, 31.62, 31.61, 21.72. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 22.89. R_f: 0.37 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.47 min. HRMS (ESI): for $[M+H]^+$ calc.: 589.19206, found: 589.19184.

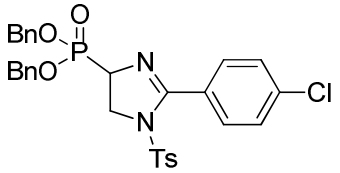


4-Bis(benzyloxy)phosphoryl-4,5-dihydro-2-phenyl-1-(4-toluenesulfonyl)-1*H*-imidazole (228l): Azide **227l** (450 mg, 0.74 mmol, 1 equiv) was used. Column chromatography (toluene:ethyl acetate 5:1) to afford **228l** as a colorless oil (386 mg, 0.69 mmol; 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.1 Hz, 2H), 7.48 (t, *J* = 7.5 Hz, 1H) 7.43 – 7.23 (m, 14H), 7.18 (d, *J* = 8.2 Hz, 1H), 5.22 – 4.85 (m, 4H), 4.36 – 3.96 (m, 3H), 2.38 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.90 (d, *J* = 15.4 Hz), 144.94, 136.09 (d, *J* = 5.7 Hz), 136.01 (d, *J* = 5.5 Hz), 134.82, 131.32, 129.93, 129.91, 129.75, 128.72, 128.69, 128.66, 128.19, 128.11, 122.75, 127.66. 68.58 (d, *J* = 6.8 Hz), 68.43 (d, *J* = 6.8 Hz), 62.65 (d, *J* = 168.6 Hz), 50.45, 21.73. ^{31}P

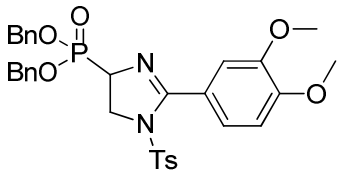
NMR (162 MHz, $CDCl_3$) δ 22.94. R_f: 0.25 (chloroform:ethyl acetate 5:1). LC-MS (ESI): t_R = 9.92 min. HRMS (ESI): for $[M+H]^+$ calc.: 560.16076, found: 560.16046.



Dibenzyl 2-(4-methoxyphenyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228m): Azide **227m** (541 mg, 0.85 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 456 mg (0.77 mmol, 91%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.60 (m, 2H), 7.39 (ad, *J*=8.2, 2H), 7.35 – 7.23 (m, 10H), 7.19 (ad, *J*=8.1, 2H), 6.88 (m, 2H), 5.00 (m, 4H), 4.27 – 4.04 (m, 2H), 3.97 (m, 1H), 3.86 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.34, 161.80 (d, *J*=15.9), 145.00, 136.21 (d, *J*=7.4), 136.15 (d, *J*=7.3), 134.89, 131.95, 130.05, 128.81, 128.77, 128.74, 128.72, 128.27, 128.19, 127.73, 122.10, 113.29, 77.59, 77.28, 76.96, 68.67 (d, *J*=6.8), 68.47 (d, *J*=6.8), 62.63 (d, *J*=169.7), 55.59, 50.66, 21.83. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.26. R_f: 0.23 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 9.94 min. HRMS (ESI): for $[M+H]^+$ calc.: 591.17132, found: 591.17104.

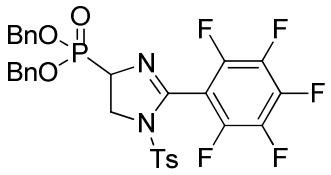


Dibenzyl 2-(4-chlorophenyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228n): Azide **227n** (455 mg, 0.73 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 396 mg (0.68 mmol, 93%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 (m, 2H), 7.44 – 7.24 (m, 14H), 7.21 (ad, *J*=8.3, 2H), 5.01 (m, 4H), 4.25 – 3.97 (m, 3H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.07 (d, *J*=15.5), 145.27, 137.71, 136.11 (d, *J*=7.7), 136.05 (d, *J*=7.4), 134.72, 131.38, 131.37, 130.16, 128.85, 128.82, 128.81, 128.28, 128.22, 127.69, 77.59, 77.27, 76.96, 68.67 (d, *J*=11.8), 68.60 (d, *J*=11.8), 62.84 (d, *J*=168.9), 50.58 (d, *J*=1.1), 21.86. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 22.80. R_f: 0.22 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 10.39 min. HRMS (ESI): for $[M+H]^+$ calc.: 595.12178, found: 595.12191.

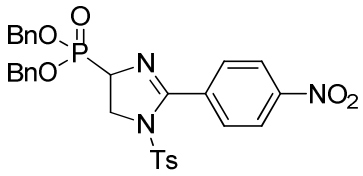


Dibenzyl 2-(3,4-dimethoxyphenyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228o): Azide **227o** (492 mg, 0.74 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 399 mg (0.64 mmol, 87%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42 – 7.36 (m, 2H), 7.35 – 7.24 (m, 11H), 7.21 – 7.16 (m, 2H), 7.13 (d, *J*=2.0, 1H), 6.85 (d, *J*=8.4, 1H), 5.12 – 4.94 (m, 4H), 5.09 – 4.97 (m, 2H), 4.06 – 3.88 (m, 4H), 3.84 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.82 (d, *J*=16.0), 151.91, 148.23, 145.00, 136.19 (d, *J*=8.2), 136.13 (d, *J*=8.2), 135.01, 130.00, 128.81, 128.77,

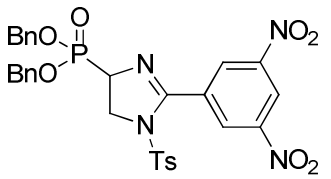
128.75, 128.73, 128.27, 128.19, 127.74, 123.91, 122.10, 113.11, 110.17, 77.57, 77.26, 76.94, 68.69 (d, *J*=6.8), 68.47 (d, *J*=6.8), 62.64 (d, *J*=169.7), 56.18, 50.82 (d, *J*=1.4), 21.82. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 23.29. R_f: 0.18 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 9.63 min. HRMS (ESI): for $[M+H]^+$ calc.: 621.18189, found: 621.18154.



4-Bis(benzyloxy)phosphoryl-4,5-dihydro-2-pentafluorophenyl-1-(4-toluenesulfonyl)-1*H*-imidazole (228p): Azide **227p** (451 mg, 0.65 mmol, 1 equiv) was used. Column chromatography (toluene:ethyl acetate 5:1) to afford **228p** as a colorless oil (297 mg, 0.46 mmol; 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.3 Hz, 2H), 7.39 – 7.23 (m, 12H), 5.11 – 4.88 (m, 5H), 4.49 (ddd, *J* = 16.7, 11.7, 8.6 Hz, 1H), 4.21 – 3.98 (m, 1H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7 - 148.4 (br), 146.6 – 146.2 (br), 145.82, 144.6 – 143.6 (br), 142.0 – 141.5 (br), 139.2 – 138.7 (br), 136.6 – 136.2, 135.97 (d, *J* = 2.3 Hz), 135.91 (d, *J* = 2.7 Hz), 134.03, 130.25, 128.75, 128.73, 128.71, 128.25, 128.08, 127.53, 106.7 – 106.2 (br), 77.36, 68.89 (d, *J* = 6.9 Hz), 68.53 (d, *J* = 6.9 Hz), 63.46 (d, *J* = 164.9 Hz). 48.79, 21.75. ^{19}F NMR (377 MHz, $CDCl_3$) δ -137.86 (dd, *J* = 79.6, 22.2 Hz), -150.16 (t, *J* = 20.8 Hz), -161.41 (dtd, *J* = 51.6, 21.5, 7.9 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.97. R_f: 0.40 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 10.31 min. HRMS (ESI): for $[M+H]^+$ calc.: 651.11365, found: 651.11360.



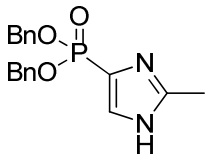
Dibenzyl 2-(4-nitrophenyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228q): Azide **227q** (464 mg, 0.71 mmol, 1 equiv) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded 371 mg (0.61 mmol, 86%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.21 (d, *J*=8.7, 2H), 7.69 (d, *J*=8.7, 2H), 7.45 – 7.17 (m, 14H), 5.10 – 4.93 (m, 4H), 4.26 – 4.04 (m, 3H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.06 (d, *J*=15.3), 149.42, 145.55, 135.91 (d, *J*=6.9), 135.89, 135.87 (d, *J*=9.3), 134.39, 130.87, 130.86, 130.24, 128.84, 128.80, 128.76, 128.22, 128.19, 127.55, 122.95, 68.65 (d, *J*=6.9), 68.63 (d, *J*=6.8), 63.17 (d, *J*=168.3), 50.40, 21.80. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 22.33. R_f: 0.31 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.13 min. HRMS (ESI): for $[M+H]^+$ calc.: 606.14583, found: 606.14580.



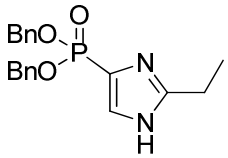
Dibenzyl 2-(3,5-dinitrophenyl)-1-tosyl-4,5-dihydro-1*H*-imidazol-4-ylphosphonate (228r): Azide **227r** (581 mg, 0.84 mmol, 1 equiv) was used. Column chromatography (toluene:ethyl acetate 3:1) yielded 396 mg (0.61 mmol, 72%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.11 (t, *J*=2.1, 1H), 8.66 (d, *J*=2.1, 2H), 7.46 – 7.40 (m, 2H), 7.39 – 7.27 (m, 12H), 5.03 (m, 4H), 4.25 – 4.00 (m, 3H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 158.06 (d, *J*=15.4), 147.99, 146.09, 135.76 (d, *J*=5.5), 135.74 (d, *J*=5.2), 134.20, 133.54 (d, *J*=2.2), 130.57, 129.59, 128.96, 128.86, 128.84, 128.77, 128.19, 127.38, 120.80, 68.79 (d, *J*=6.8), 68.72 (d, *J*=6.8), 63.47 (d, *J*=168.5), 50.35 (d, *J*=1.2), 21.81. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 21.94. R_f: 0.17 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.05 min. HRMS (ESI): for $[M+H]^+$ calc.: 651.13091, found:651.13096.

16.2.3 General procedure for the synthesis of amides 231a-q

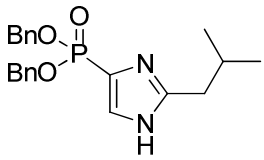
This procedure was followed in all cases unless stated otherwise. In a flame dried 50 mL schlenk flask, containing an egg-shaped stirring bar, the imidazoline (1 equiv) was dissolved in 3-8 mL anhydrous THF. The resulting mixture was cooled to -78°C with a dry-ice/acetone bath and 0.5 M KHMDS in toluene (3 equiv) was added dropwise over 5 minutes. At the start of addition color changes were usually seen. After addition of roughly two thirds of the KHMDS solution the reaction mixture stayed clear yellow during the addition of the remaining KHMDS. Upon complete addition a TLC was taken to confirm complete conversion of the imidazoline (if some imidazoline remained an additional 10% KHMDS solution was charged), and the reaction mixture was transferred by cannula to a 100 mL round bottom flask containing a vigorously stirred mixture of 20 mL of water containing acetic acid (6 equiv). Following the transfer saturated aqueous sodium bicarbonate solution was added until the mixture was pH 8, 50 mL of chloroform was added and the resulting mixture was vigorously stirred for five minutes. The layers were separated and the aqueous layer was washed with two additional 10 mL portions of chloroform, the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded an oil that was used 'as is' in the next reaction.



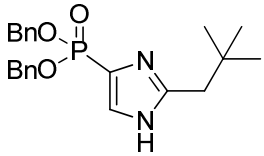
4-bBis(benzyloxy)phosphoryl-2-methyl-1*H*-imidazole (231a): Imidazoline **228a** (160 mg, 0.32 mmol, 1.0 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.64 (br). R_f: 0.20 (chloroform:methanol 10:1). LC-MS (ESI): t_R = 6.23 min. HRMS (ESI): for $[M+H]^+$ calc.: 343.12061, found: 343.12098.



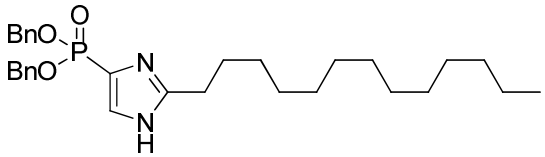
4-Bis(benzyloxy)phosphoryl-2-ethyl-1*H*-imidazole (231b): Imidazoline **228b** (200 mg, 0.39 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.32 (br), 11.01 (br). R_f: 0.55 (chloroform:methanol 10:1). LC-MS (ESI): t_R = 5.93 min. HRMS (ESI): for $[M+H]^+$ calc.: 357.13626, found: 357.13647.



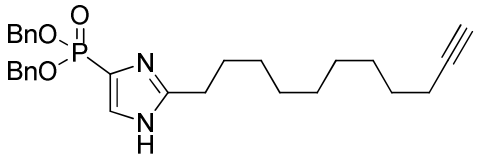
Dibenzyl 2-isobutyl-1*H*-imidazol-4-ylphosphonate (231c): Imidazoline **228c** (283 mg, 0.52 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 12.16. R_f: 0.22 (ethyl acetate). LC-MS (ESI): t_R = 6.91 min. HRMS (ESI): for $[M+H]^+$ calc.: 385.16756, found:385.16934.



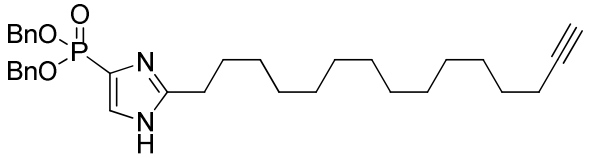
Dibenzyl 2-neopentyl-1*H*-imidazol-4-ylphosphonate (231d): Imidazoline **228d** (315 mg, 0.57 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 12.36. R_f: 0.09 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 7.33 min. HRMS (ESI): for $[M+H]^+$ calc.: 399.18321, found: 399.18214.



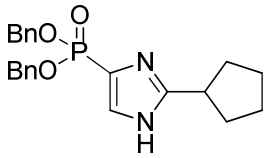
Dibenzyl 2-tridecyl-1*H*-imidazol-4-ylphosphonate (231e): Imidazoline **228e** (106 mg, 0.16 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.11. R_f: 0.56 (ethyl acetate). LC-MS (ESI): t_R = 11.01 min. HRMS (ESI): for $[M+H]^+$ calc.: 511.30841, found: 511.30832.



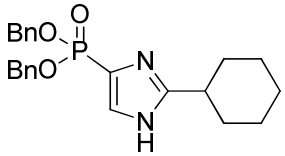
Dibenzyl 2-(undec-10-ynyl)-1*H*-imidazol-4-ylphosphonate (231f): Imidazoline **228f** (410 mg, 0.65 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.58, 11.96. R_f: 0.58 (ethyl acetate). LC-MS (ESI): t_R = 8.60 min. HRMS (ESI): for $[M+H]^+$ calc.: 479.24581, found: 479.24527.



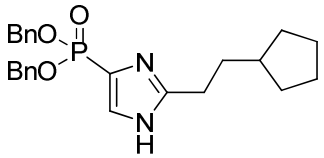
Dibenzyl 2-(pentadec-14-ynyl)-1*H*-imidazol-4-ylphosphonate (231g): Imidazoline **228g** (323 mg, 0.47 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 11.62. R_f: 0.5 (ethyl acetate). LC-MS (ESI): t_R = 9.94 min. HRMS (ESI): for $[M+H]^+$ calc.: 535.30841, found: 535.30770.



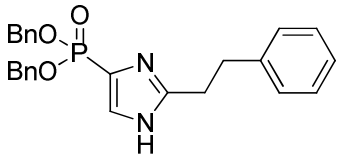
Dibenzyl 2-cyclopentyl-1*H*-imidazol-4-ylphosphonate (231h): Imidazoline **228h** (304 mg, 0.55 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 11.88. R_f: 0.29 (ethyl acetate). LC-MS (ESI): t_R = 7.09 min. HRMS (ESI): for $[M+H]^+$ calc.: 397.16756, found: 397.16640.



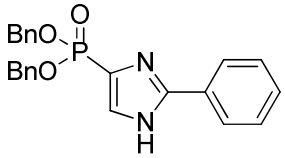
Dibenzyl 2-cyclohexyl-1*H*-imidazol-4-ylphosphonate (231i): Imidazoline **228i** (349 mg, 0.62 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 12.31. R_f: 0.24 (ethyl acetate). LC-MS (ESI): t_R = 7.27 min. HRMS (ESI): for $[M+H]^+$ calc.: 411.18321, found: 411.18324.



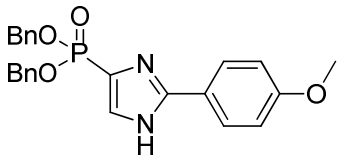
Dibenzyl 2-(2-cyclopentylethyl)-1*H*-imidazol-4-ylphosphonate (231j): Imidazoline **228j** (357 mg, 0.61 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 12.36. R_f: 0.29 (ethyl acetate). LC-MS (ESI): t_R = 7.73 min. HRMS (ESI): for $[M+H]^+$ calc.: 425.19886, found: 425.19746.



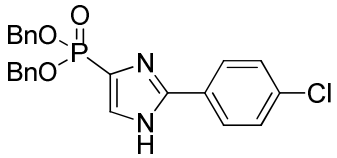
Dibenzyl 2-phenethyl-1*H*-imidazol-4-ylphosphonate (231k): Imidazoline **228k** (251 mg, 0.43 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 11.80. R_f: 0.25 (ethyl acetate). LC-MS (ESI): t_R = 7.59 min. HRMS (ESI): for $[M+H]^+$ calc.: 433.16756, found: 433.16632.



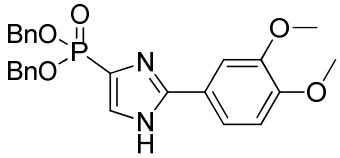
4-Bis(benzyloxy)phosphoryl-2-phenyl-1*H*-imidazole (231l): Imidazoline **228l** (302 mg, 0.54 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.50, 10.61. R_f: 0.30 (chloroform:methanol 20:1). LC-MS (ESI): t_R = 8.24 min. HRMS (ESI): for $[M+H]^+$ calc.: 405.13626, found: 405.13563.



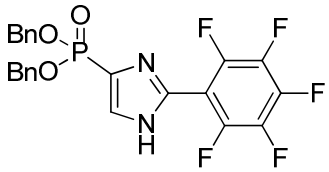
Dibenzyl 2-(4-methoxyphenyl)-1*H*-imidazol-4-ylphosphonate (231m): Imidazoline **228m** (423 mg, 0.72 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 11.59. R_f: 0.09 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 8.38 min. HRMS (ESI): for $[M+H]^+$ calc.: 435.14682, found: 435.114655.



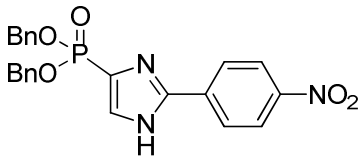
Dibenzyl 2-(4-chlorophenyl)-1*H*-imidazol-4-ylphosphonate (231n): Imidazoline **228n** (353 mg, 0.59 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.60, 10.43. R_f: 0.10 (toluene:ethyl acetate 1:1). LC-MS (ESI): t_R = 9.31 min. HRMS (ESI): for $[M+H]^+$ calc.: 439.09728, found: 439.09728.



Dibenzyl 2-(3,4-dimethoxyphenyl)-1*H*-imidazol-4-ylphosphonate (231o): Imidazoline **228o** (378 mg, 0.61 mmol, 1 equiv) was used. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 10.79. R_f: 0.19 (ethyl acetate). LC-MS (ESI): t_R = 7.93 min. HRMS (ESI): for $[M+H]^+$ calc.: 465.15738, found: 465.15651.



4-Bis(benzyloxy)phosphoryl-2-pentafluorophenyl-1*H*-imidazole (231p): Imidazoline **228p** (253 mg, 0.39 mmol, 1 equiv) was used. ^{19}F NMR (377 MHz, $CDCl_3$) δ 140.13, 152.33, 161.47. ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.55, 8.40. R_f: 0.30 (chloroform:methanol 10:1). LC-MS (ESI): t_R = 8.98 min. HRMS (ESI): for $[M+H]^+$ calc.: 495.08915, found: 495.08855.

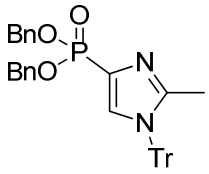


Dibenzyl 2-(4-nitrophenyl)-1*H*-imidazol-4-ylphosphonate (231q): In a flame dried 10 mL schlenk flask, containing an egg-shaped stirring bar, imidazoline **228q** (20 mg, 0.033 mmol, 1 equiv) was dissolved in

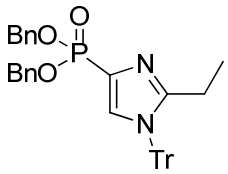
0.16 mL of anhydrous DMF. The mixture was cooled to 0°C and DBU (75 mg, 74 µL, 0.50 mmol, 15 equiv) was added turning the solution bright red. After stirring to 1 hour a TLC was taken to confirm complete conversion of the imidazoline, and the reaction mixture was partitioned between ethyl acetate (5 mL) and saturated aqueous sodium bicarbonate solution (5 mL). The layers were separated and the aqueous layer was washed with two additional 5 mL portions of ethyl acetate, the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a yellow oil that was used as is in the next reaction. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.58, 9.71. R_f: 0.44 (ethyl acetate). LC-MS (ESI): t_R = 9.61min. HRMS (ESI): for $[M+H]^+$ calc.: 450.12133, found: 450.12085.

16.2.4 General procedure for the synthesis of amides 229a-q

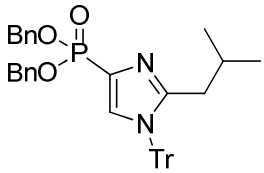
This procedure was followed in all cases unless stated otherwise. In a flame dried, 25 mL round bottom schlenk, containing an egg-shaped stirring bar and an argon atmosphere, the unprotected imidazole (1 equiv, assumed 100% yield in previous reaction) was dissolved in 2-3 mL DCM. Trityl chloride (2 equiv), DBU (3 equiv) and silver triflate (2.2 equiv) were sequentially added. After addition of the silver triflate a grey solid precipitated out of the solution. After one hour a TLC sample confirmed complete conversion and an additional 5 mL of DCM was added followed by one scoop of celite. The resulting mixture was filtered through celite, the filtrate was reduced in volume in vacuo and poured into saturated aqueous sodium bicarbonate solution (10 mL). The aqueous layer was washed with two additional portions of DCM (10 mL), the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated yielding a foam that was further purified by flash column chromatography.



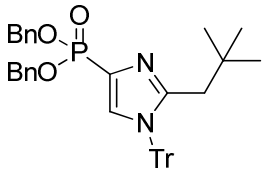
4-Bis(benzyloxy)phosphoryl-2-methyl-1-triphenylmethyl-1*H*-imidazole (229a): Imidazole **231a** (0.30 mmol, 1.0 equiv, assumed) was used. Column chromatography (toluene:ethyl acetate 2:1 gradient 1:1) afforded a yellow oil (77 mg, 0.13 mmol; 54% over two steps from **228a**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.27 (m, 19H), 7.18 – 6.97 (m, 6H), 5.21 – 5.04 (m, 4H), 1.65 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.01 (d, *J* = 22.4 Hz), 141.63, 136.61 (d, *J* = 6.5 Hz), 131.38 (d, *J* = 38.6 Hz), 129.99, 128.45, 128.31, 128.22, 128.18, 128.08, 127.33, 124.84, 75.83, 67.76 (d, *J* = 5.5 Hz),17.51. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.38. R_f: 0.40 (toluene:ethyl acetate 1:1). LC-MS (ESI): t_R = 10.52 min. HRMS (ESI): for $[M+H]^+$ calc.: 585.23016, found: 585.23032.



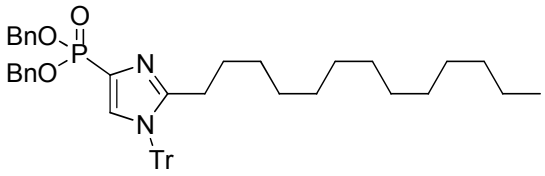
4-Bis(benzyloxy)phosphoryl-2-ethyl-1-triphenylmethyl-1*H*-imidazole (229b): Imidazole **231b** (0.28 mmol, 1.0 equiv, assumed) was used. Column chromatography (toluene:ethyl acetate 2:1) afforded a brown oil that solidified on standing (105 mg, 0.17 mmol; 74% over two steps from **228b**). ^{1}H NMR (400 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.23 (m, 19H), 7.14 – 6.98 (m, 5H), 5.31 – 5.02 (m, 4H), 1.94 (q, *J* = 7.4 Hz, 2H), 0.75 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.59 (d, *J* = 22.1 Hz), 141.94, 136.71 (d, *J* = 6.6 Hz), 130.80 (d, *J* = 38.8 Hz), 129.86, 128.43, 128.29, 128.14, 128.12, 128.07, 127.35, 124.86, 126.11 (d, *J* = 250.0 Hz), 75.54, 67.81 (d, *J* = 5.4 Hz), 24.23, 11.86. ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.60. R_f: 0.25 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 10.89 min. HRMS (ESI): for $[M+H]^+$ calc.: 599.24581, found: 599.24553.



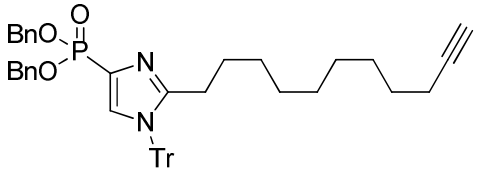
Dibenzyl 2-isobutyl-1-trityl-1*H*-imidazol-4-ylphosphonate (229c): Imidazole **231c** (0.52 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded a yellow foam (255 mg, 0.41 mmol, 78% over two steps from **228c**). *The compound is unstable and slowly reverts back to the unprotected imidazole.* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43 (s, 1H), 7.42 – 7.37 (m, 4H), 7.36 – 7.29 (m, 15H), 7.18 – 6.99 (m, *J*=6.4, 2.7, 6H), 5.23 – 5.13 (m, 4H), 2.02 – 1.90 (m, 1H), 1.77 (d, *J*=7.0, 2H), 0.59 (d, *J*=6.6, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 152.51 (d, *J*=22.0), 141.72, 136.62 (d, *J*=6.8), 131.02 (d, *J*=38.8), 129.92, 128.32, 128.13, 128.03, 128.00, 127.88, 125.66 (d, *J*=249.4), 75.51, 67.78 (d, *J*=5.4), 39.16, 26.25, 22.24. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.45. R_f: 0.57 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.58 min. HRMS (ESI): for $[M+H]^+$ calc.: 627.27711, found: 627.27788.



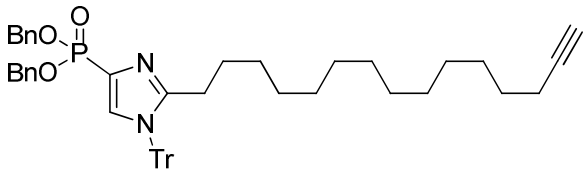
Dibenzyl 2-neopentyl-1-trityl-1*H*-imidazol-4-ylphosphonate (229d): Imidazole **231d** (0.57 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 18:1 gradient 9:1) yielded a yellow foam (209 mg, 0.33 mmol, 57% over two steps from **228d**). *The compound is unstable and slowly turns back into the unprotected imidazole.* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.50 (s, 1H), 7.39 (dd, *J*=7.8, 1.6, 4H), 7.36 – 7.29 (m, 16H), 7.15 – 7.09 (m, 5H), 5.29 – 5.16 (m, 4H), 1.85 (s, 2H), 0.77 (s, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = ^{13}C NMR (126 MHz, $CDCl_3$) δ = 151.47 (d, *J*=22.4), 141.79, 136.69 (d, *J*=7.1), 130.89 (d, *J*=38.6), 129.94, 128.33, 128.13, 127.98, 127.95, 127.69, 125.23 (d, *J*=248.6), 75.52, 67.88 (d, *J*=5.5), 42.79, 31.55, 29.61. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.99. R_f: 0.58 (chloroform:ethyl acetate 6:1). LC-MS (ESI): t_R = 11.97 min. HRMS (ESI): for $[M+H]^+$ calc.:641.29276, found: 641.29448.



Dibenzyl 2-tridecyl-1-trityl-1*H*-imidazol-4-ylphosphonate (229e): Imidazole **231e** (0.16 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 9:1) yielded a clear oil (89 mg, 0.12 mmol, 74% over two steps from **228e**).^{1}H NMR (400 MHz, $CDCl_3$) δ = ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43 – 7.23 (m, 20H), 7.14 – 6.98 (m, 6H), 5.21 – 5.08 (m, 4H), 2.01 – 1.87 (m, 2H), 1.42 – 1.01 (m, 20H), 1.01 – 0.76 (m, 7H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 153.88 (d, *J*=21.9), 142.05, 136.80 (d, *J*=6.6), 130.87 (d, *J*=38.7), 130.01, 128.54, 128.39, 128.26, 128.22, 128.18, 126.09 (d, *J*=249.6), 75.71, 67.94 (d, *J*=5.4), 32.15, 31.15, 29.91, 29.88, 29.85, 29.78, 29.63, 29.60, 29.58, 29.44, 27.82, 22.92, 14.35. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.52. R_f: 0.22 (chloroform:ethyl acetate 9:1). LC-MS (ESI): t_R = does not run. HRMS (ESI): for $[M+H]^+$ calc.: 753.41796, found: 753.41884.

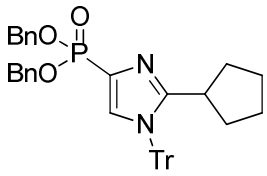


Dibenzyl 1-trityl-2-(undec-10-ynyl)-1*H*-imidazol-4-ylphosphonate (229f): Imidazole **231f** (0.65 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 9:1) yielded a yellow foam (336 mg, 0.47 mmol, 72% over two steps from **228f**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45 – 7.19 (m, 20H), 7.17 – 7.00 (m, 6H), 5.20 – 5.06 (m, 4H), 2.21 – 2.09 (m, 2H), 2.01 – 1.80 (m, 3H), 1.48 (dt, *J*=14.7, 7.1, 2H), 1.36 – 1.23 (m, 2H), 1.23 – 1.00 (m, 6H), 1.00 – 0.78 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 153.81 (d, *J*=22.0), 142.06, 136.81 (d, *J*=6.6), 130.87 (d, *J*=38.7), 130.01, 128.53, 128.39, 128.25, 128.21, 128.17, 126.13 (d, *J*=249.7), 84.97, 75.68, 68.29, 67.91 (d, *J*=5.4), 31.14, 29.53, 29.42, 29.34, 29.18, 28.88, 28.68, 27.78, 18.61. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.56. R_f: 0.34 (chloroform:ethyl acetate 6:1). LC-MS (ESI): t_R = 8.37 min. HRMS (ESI): for $[M+H]^+$ calc.: 721.35536, found: 721.35623.

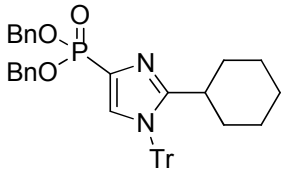


Dibenzyl 2-(pentadec-14-ynyl)-1-trityl-1*H*-imidazol-4-ylphosphonate (229g): Imidazole **231g** (0.47 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 9:1) yielded a yellow foam (277 mg, 0.37 mmol, 76% over two steps from **228g**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 – 7.20 (m, 20H), 7.14 – 7.00 (m, 6H), 5.21 – 5.07 (m, 4H), 2.18 (td, *J*=7.1, 2.6, 2H), 2.00 – 1.82 (m, 3H), 1.59 – 1.46 (m, 2H), 1.46 – 1.33 (m, *J*=14.3, 6.9, 2H), 1.33 – 1.00 (m, 14H), 1.00 – 0.76 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 153.84 (d, *J*=22.0), 142.08, 136.82 (d, *J*=6.6), 131.07, 130.87 (d, *J*=38.8), 128.53, 128.38, 128.24, 128.20, 128.17, 126.14 (d, *J*=249.7), 85.01, 75.68, 68.28, 67.91 (d, *J*=5.4), 31.16, 29.81, 29.81,

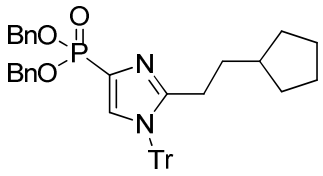
29.76, 29.73, 29.62, 29.59, 29.44, 29.33, 28.99, 28.72, 27.82, 18.63. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.59. R_f: 0.45 (chloroform:ethyl acetate 6:1). LC-MS (ESI): t_R = 10.47 min. HRMS (ESI): for $[M+H]^+$ calc.: 777.41796, found: 777.41885.



Dibenzyl 2-cyclopentyl-1-trityl-1*H*-imidazol-4-ylphosphonate (229h): Imidazole **231h** (0.55 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 6:1) yielded a yellow foam (274 mg, 0.43 mmol, 78% over two steps from **228h**). ^{1}H NMR (500 MHz, $CDCl_3$) δ = ^{1}H NMR 7.41 (d, *J*=2.0, 2H), 7.39 (d, *J*=1.5, 2H), 7.36 (s, 1H), 7.36 – 7.30 (m, 15H), 7.15 – 7.02 (m, 6H), 5.19 (d, *J*=8.1, 4H), 2.49 – 2.38 (m, 1H), 1.69 – 1.59 (m, 2H), 1.59 – 1.47 (m, 2H), 1.22 – 1.10 (m, 2H), 1.03 – 0.94 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = ^{13}C NMR (126 MHz, $CDCl_3$) δ = 158.32 (d, *J*=21.8), 142.15, 136.70 (d, *J*=6.7), 130.31 (d, *J*=38.9), 129.79, 128.35, 128.19, 128.05, 127.97, 127.96, 126.04 (d, *J*=248.2), 75.31, 67.78 (d, *J*=5.3), 40.81, 33.51, 25.73. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.70. R_f: 0.54 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.75 min. HRMS (ESI): for $[M+H]^+$ calc.: 639.27711, found: 639.27767.

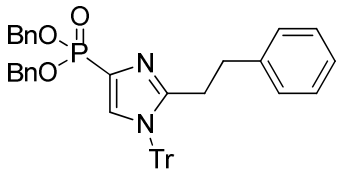


Dibenzyl 2-cyclohexyl-1-trityl-1*H*-imidazol-4-ylphosphonate (229i): Imidazole **231i** (0.62 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded a brown foam (326 mg, 0.50 mmol, 81% over two steps from **228i**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.46 (s, 1H), 7.45 – 7.37 (m, 4H), 7.37 – 7.24 (m, 15H), 7.23 – 7.06 (m, 6H), 5.26 – 5.08 (m, 4H), 2.09 (t, *J*=11.3, 1H), 1.52 – 1.32 (m, 5H), 1.11 (dt, *J*=13.0, 8.1, 1H), 0.79 (d, *J*=12.9, 2H), 0.74 – 0.57 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 157.52 (d, *J*=21.6), 141.97, 136.66 (d, *J*=6.6), 130.11 (d, *J*=38.9), 129.64, 128.32, 128.14, 128.03, 127.99, 127.86, 125.94 (d, *J*=248.1), 75.31, 67.74 (d, *J*=5.3), 39.35, 31.77, 26.42, 25.60. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.80. R_f: 0.50 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 7.17 min. HRMS (ESI): for $[M+H]^+$ calc.: 653.29276, found: 653.29320.

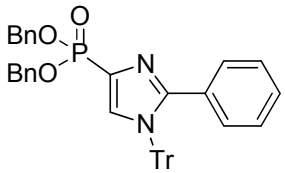


Dibenzyl 2-(2-cyclopentylethyl)-1-trityl-1*H*-imidazol-4-ylphosphonate (229j): Imidazole **231j** (0.61 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded a yellow foam (357 mg, 0.54 mmol, 88% over two steps from **228j**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43 – 7.23 (m, 20H), 7.16 – 7.00 (m, 6H), 5.14 (d, *J*=8.4, 4H), 1.98 – 1.88 (m, 2H), 1.51 – 1.25 (m, 7H), 1.15 (dt,

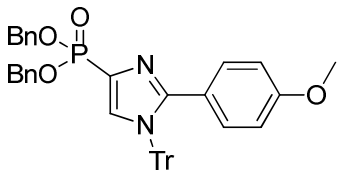
J=10.9, 7.6, 2H), 0.83 – 0.61 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = ^{13}C NMR (101 MHz, $CDCl_3$) δ = 153.88 (d, *J*=22.0), 142.02, 136.74 (d, *J*=6.5), 130.81 (d, *J*=38.7), 129.92, 128.44, 128.30, 128.16, 128.10, 126.02 (d, *J*=249.8), 75.6167.81 (d, *J*=5.4), 40.03, 33.84, 32.24, 30.32, 25.09. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.69. R_f: 0.62 (chloroform:ethyl acetate). LC-MS (ESI): t_R = 12.23 min. HRMS (ESI): for $[M+H]^+$ calc.: 667.30841, found: 667.30935.



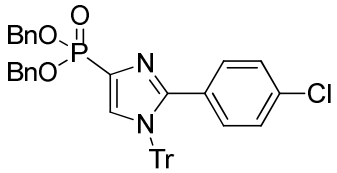
Dibenzyl 2-phenethyl-1-trityl-1*H*-imidazol-4-ylphosphonate (229k): Imidazole **231k** (0.43 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded a brown foam (217 mg, 0.32 mmol, 75% over two steps from **228k**). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43 (d, *J*=7.0, 4H), 7.39 (s, 1H), 7.38 – 7.29 (m, 15H), 7.19 – 7.10 (m, 3H), 7.08 (dd, *J*=8.1, 1.0, 6H), 6.85 (d, *J*=7.0, 2H), 5.22 (d, *J*=8.3, 4H), 2.59 – 2.48 (m, 2H), 2.34 – 2.23 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 152.54 (d, *J*=22.1), 141.82, 141.22, 136.58 (d, *J*=6.5), 130.89 (d, *J*=38.8), 129.77, 128.53, 128.39, 128.30, 128.12, 127.97, 127.91, 126.16 (d, *J*=249.9), 125.16, 75.57, 67.79 (d, *J*=5.4), 33.70, 32.76 (there is some overlap in the aromatic region). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 14.47. R_f: 0.15 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.41 min. HRMS (ESI): for $[M+H]^+$ calc.: 675.27711, found: 675.27897.



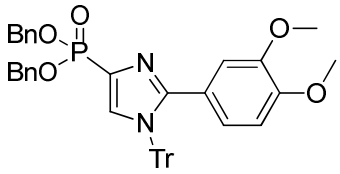
4-Bis(benzyloxy)phosphoryl-2-phenyl-1-triphenylmethyl-1*H*-imidazole (229l): Imidazole **231l** (0.47 mmol, 1 equiv, assumed) was used. Column chromatography with (toluene:ethyl acetate 5:1 gradient 2:1) afforded a brown oil that solidified on standing (224 mg, 0.35 mmol, 87% over two steps from **228l**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.44 – 7.35 (m, 4H), 7.35 – 7.13 (m, 15H), 7.04 (d, *J* = 6.9 Hz, 6H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.79 (t, *J* = 7.5 Hz, 2H), 6.67 (d, *J* = 7.8 Hz, 2H), 5.32 – 5.06 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.98 (d, *J*=22.4), 141.81, 136.76 (d, *J*=6.4), 132.59, 132.31 (d, *J*=38.2), 130.63, 130.57, 128.58, 128.29, 128.19, 128.16, 128.10, 127.88, 126.99, 125.95 (P(O)C) the other peak of this doublet is obscured by the other aromatic peaks), 76.85, 68.02 (d, *J*=5.5). ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.75. R_f: 0.25 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 11.17 min. HRMS (ESI): for $[M+H]^+$ calc.: 647.24581, found: 647.24584.



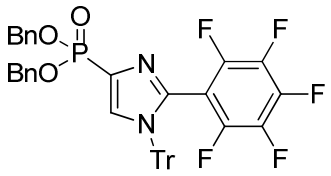
Dibenzyl 2-(4-methoxyphenyl)-1-trityl-1*H*-imidazol-4-ylphosphonate (229m): Imidazole **231m** (0.72 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 6:1 gradient 3:1) yielded a yellow foam (397 mg, 0.57 mmol, 81% over two steps from **228m**). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.72 (s, 1H), 7.41 (d, *J*=6.5, 4H), 7.37 – 7.14 (m, 13H), 7.14 – 7.00 (m, 6H), 6.62 (d, *J*=8.7, 2H), 6.35 (d, *J*=8.6, 2H), 5.31 – 5.14 (m, 4H), 3.64 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = ^{13}C NMR (126 MHz, $CDCl_3$) δ = 158.97, 151.69 (d, *J*=22.5), 141.70, 136.57 (d, *J*=6.4), 132.03 (d, *J*=38.2), 131.79, 130.32, 128.37, 128.08, 127.96, 127.94, 127.90, 126.78 (d, *J*=248.5), 124.82, 112.31, 76.54, 67.81 (d, *J*=5.5), 55.13. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.86. R_f: 0.36 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 8.17 min. HRMS (ESI): for $[M+H]^+$ calc.: 677.25637, found: 677.25705.



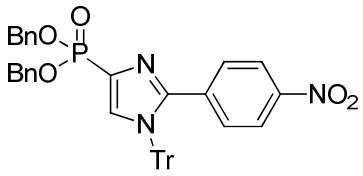
Dibenzyl (2-(4-chlorophenyl)-1-trityl-1*H*-imidazol-4-yl)phosphonate (229n): Imidazole **231n** (0.59 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 6:1 gradient 3:1) yielded a yellow foam (282 mg, 0.41 mmol, 70% over two steps from **228n**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71 (d, *J* = 0.9 Hz, 1H), 7.38 (dt, *J* = 4.7, 2.8 Hz, 4H), 7.35 – 7.16 (m, 15H), 7.07 – 6.97 (m, 6H), 6.82 – 6.71 (m, 2H), 6.66 – 6.56 (m, 2H), 5.20 (d, *J* = 8.6 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 150.54 (d, *J* = 22.5 Hz), 141.43, 136.42 (d, *J* = 6.3 Hz)9, 133.90, 132.12 (d, *J* = 38.1 Hz), 131.69, 130.77, 130.29, 128.39, 128.16, 128.00, 127.95, 126.95, 126.60 (d, *J* = 114.5 Hz), 76.68, 67.88 (d, *J* = 5.5 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.48. R_f: 0.21 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 11.50 min. HRMS (ESI): for $[M+H]^+$ calc.: 681.20683, found: 681.20700.



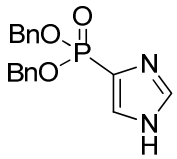
Dibenzyl 2-(3,4-dimethoxyphenyl)-1-trityl-1*H*-imidazol-4-ylphosphonate (229o): Imidazole **231o** (0.61 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 3:1) yielded a yellow foam (342 mg, 0.48 mmol, 79% over two steps from **228o**). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.75 (s, 1H), 7.41 (d, *J*=2.0, 3H), 7.40 (d, *J*=1.5, 2H), 7.36 – 7.21 (m, 16H), 7.13 – 7.02 (m, 6H), 6.35 – 6.22 (m, 3H), 5.28 – 5.14 (m, 4H), 3.73 (s, 3H), 3.40 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 151.54 (d, *J*=22.5), 148.47, 146.84, 141.68, 136.54 (d, *J*=6.4), 131.87 (d, *J*=38.1), 130.29, 128.35, 128.07, 127.94, 127.90, 127.87, 126.81 (d, *J*=249.4), 124.87, 123.62, 113.62, 109.72, 76.44, 67.79 (d, *J*=5.5), 55.77, 55.23. ^{31}P NMR (162 MHz, $CDCl_3$) δ = 13.82. R_f: 0.55 (ethyl acetate). LC-MS (ESI): t_R = 10.49 min. HRMS (ESI): for $[M+H]^+$ calc.: 707.26694, found: 707.26948.



4-Bis(benzyloxy)phosphoryl-2-pentafluorophenyl-1-triphenylmethyl-1*H*-imidazole (229p): Imidazole **231p** (0.29 mmol, 1 equiv, assumed) was used. Column chromatography (toluene:ethyl acetate 8:1 gradient 5:1) afforded a brown oil that solidified on standing (124 mg, 0.17 mmol, 56% over two steps from **228p**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.40 – 7.16 (m, 18H), 7.08 (d, *J* = 7.7 Hz, 6H), 5.32 – 4.94 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.8 – 145.5 (br), 143.3 – 142.9 (br), 140.93, 138.6 – 138.2 (br), 136.66 (d, *J* = 23.8 Hz), 136.23 (d, *J* = 6.4 Hz), 136.2 – 135.5 (br), 135.44 (d, *J* = 36.5 Hz), 135.26, 130.46, 130.34, 128.69, 128.54, 128.35, 127.97, 127.83, 109.10, 78.52, 68.13 (d, *J* = 5.4 Hz). ^{19}F NMR (377 MHz, $CDCl_3$) δ -135.12 (dd, *J* = 24.4, 6.3 Hz), -152.98 (t, *J* = 20.9 Hz), -163.40 (td, *J* = 21.8, 6.3 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.64. R_f: 0.30 (toluene:ethyl acetate 5:1). LC-MS (ESI): t_R = 9.17 min. HRMS (ESI): for $[M+H]^+$ calc.: 737.19870, found: 737.19920.

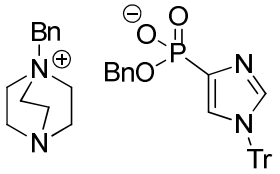


Dibenzyl 2-(4-nitrophenyl)-1-trityl-1*H*-imidazol-4-ylphosphonate (229q): Imidazole **231q** (0.19 mmol, 1 equiv, assumed) was used. Column chromatography (chloroform:ethyl acetate 18:1 gradient to 6:1) yielded a clear oil (88 mg, 0.13 mmol, 67% over two steps from **228q**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.69 (d, *J*=0.9, 1H), 7.67 – 7.62 (m, 2H), 7.41 – 7.19 (m, 19H), 7.06 – 7.00 (m, 6H), 6.94 – 6.88 (m, 2H), 5.18 (d, *J*=8.7, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 149.41 (d, *J*=22.6), 146.90, 141.39, 138.74, 136.52 (d, *J*=6.1), 132.71 (d, *J*=38.1), 131.42, 130.51, 128.68, 128.63, 128.43, 128.36, 128.36 (d, *J*=249.8), 128.19, 121.94, 77.17, 68.16 (d, *J*=5.5). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 12.93. R_f: 0.47 (chloroform:ethyl acetate 3:1). LC-MS (ESI): t_R = 10.86 min. HRMS (ESI): for $[M+H]^+$ calc.: 692.23088, found: 692.23166.

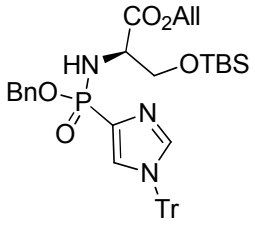


Dibenzyl 1*H*-imidazol-4-ylphosphonate (234): Trityl imidazole **167** (500 mg, 0.88 mmol, 1 equiv) was dissolved in acetic acid (5 mL), triisopropyl silane (278 mg, 359 µL, 1.75 mmol, 2 equiv) and trifluoroacetic acid (100 mg, 67 µL, 0.88 mmol, 1 equiv) were added and the mixture was stirred for 16 hours. TLC sample indicated conversion was not complete, a further 150 µL of TFA was added, after a further two hours stirring at room temperature the conversion was complete. The mixture was evaporated to dryness, partitioned between 0.5 M aqueous potassium hydrogen sulfate (10 mL) and methyl tert-butyl ether (10 mL). The organic phase was washed with 2 additional 5 mL portions of 0.5 M aqueous potassium hydrogen sulfate, the aqueous layers were combined, pH was adjusted to 8.5 with

sodium hydrogen carbonate and extracted with five 10 mL portions of chloroform. Evaporation of the solvent yielded an off-white oil (187 mg, 0.57 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.51 (s, 1H), 7.34 – 7.21 (m, 11H), 5.16 – 5.00 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.37 (CH), 138.86 (d, *J* = 22.5, CH), 136.04 (d, *J* = 6.9, 2xC), 129.01 (d, *J* = 162.9, C), 128.61 (2xCH), 128.47 (2xCH), 127.92 (2xCH), 68.11 (d, *J* = 5.4, 2xCH_2). ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.54 (br). R_f: 0.72 (chloroform : methanol : (ethyl acetate:methanol:acetic acid:water 3:3:3:2) 9:2:1). LC-MS (ESI): t_R = 6.65 min. HRMS (ESI): for $[M+H]^+$ calc.: 329.10496, found: 329.10507.

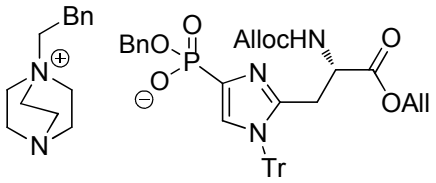


Benzyl hydrogen 1-trityl-1*H*-imidazol-4-ylphosphonate (254): A 25 mL round bottom flask, containing an egg-shaped stirring bar, was charged with imidazole **167** (426 mg, 0.75 mmol, 1 equiv) and toluene (4 mL). The mixture was stirred and DABCO (167 mg, 1.5 mmol, 2 equiv) was added in one batch. The mixture was heated to 80°C for 3 hours, after which a TLC sample (cyclohexane: ethyl acetate 1:4) showed complete consumption of the starting material. The solvent was evaporated in vacuo, the resulting solid was dissolved in 50 mM aqueous ammonium bicarbonate (ca. 3 mL) and a minimal amount of acetonitrile (ca. 1 mL) and purified over a C18 sep-pak cartridge (loading 100 mg per gram, 100% water to 100% acetonitrile in steps of 10%, each step 7 mL solvent per gram C_{18}, compound came at 30 - 50 % acetonitrile), removal of the solvent by lyophilization yielded 399 mg of a white fluffy solid (assumed quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.50 (m, 2H), 7.38 (s, 1H), 7.34 – 7.07 (m, 17H), 7.07 – 6.95 (m, 6H), 5.89 (s, 1H), 5.01 (s, 2H), 4.75 (d, *J* = 6.5 Hz, 2H), 3.66 – 3.54 (m, 6H), 2.94 (t, *J* = 7.3 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 5.57. LC-MS (ESI): t_R = 7.73 min. HRMS (ESI): for $[M+H]^+$ calc.: 481.16756, found: 481.16716.

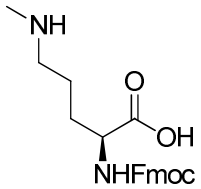


Imidazole (257): In a 5 ml, flame dried round bottom Schlenk, imidazole **254** (20 mg, 0.042 mmol, 1 equiv) was dissolved in 0.5 mL dichloromethane. NH_2-Ser(TBS)-OAll (16 mg, 0.063 mmol, 1.5 equiv) was added followed by PyBroP (23 mg, 0.05 mmol, 1.2 equiv) and DIPEA (22 µL, 16 mg, 0.126 mmol, 3 equiv). The mixture was stirred for 20 hours, the solvent was evaporated and the residue loaded on a C18 sep-pak cartridge (0.5 g). Elution was done with a gradient of 50 mM aqueous ammonium carbonate and acetonitrile (100% 50 mM aqueous ammonium carbonate to 100% acetonitrile in steps of 10%). Fraction nine and ten contained product, lyophilization of the solution yielded 14.2 mg (0.02 mmol, 48%) of a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.62 – 7.32 (m, 16H), 7.21 – 7.04 (m, 6H), 6.00 – 5.80 (m, 1H), 5.41 – 5.04 (m, 4H), 4.60 (ddd, *J*=10.4, 6.7, 4.3, 2H), 4.23 (bs, 1H), 4.15 – 3.64 (m, 4H), 3.24

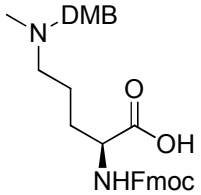
– 3.17 (m, 1H), 0.97 – 0.75 (m, 9H), 0.15 – -0.09 (m, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 16.06, 15.87. LC-MS (ESI): t_R = 11.84 min. HRMS: calc: 722.31736, found: 722.31722.



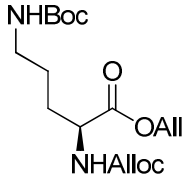
(*S*)-benzyl 2-(3-(allyloxy)-2-(allyloxycarbonylamino)-3-oxopropyl)-1-trityl-1*H*-imidazol-4-ylphosphonate 1-benzyl-4-aza-1-azoniabicyclo[2.2.2]octane salt (273): In a 5 mL, flame dried pear-shaped schlenk, containing a stirring bar and an argon atmosphere, imidazole **136** (39 mg, 0.05 mmol, 1 equiv) was dissolved in 0.5 mL of anhydrous toluene. DABCO (11.2 mg, 0.1 mmol, 2 equiv) was added and the mixture was stirred overnight. The solvent was evaporated yielding an off white, amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38 – 7.13 (m, 20H), 7.13 – 7.03 (m, 6H), 6.83 (d, *J*=8.8, 1H), 5.84 – 5.68 (m, 2H), 5.21 – 5.06 (m, 4H), 5.02 – 4.80 (m, 4H), 4.50 – 4.37 (m, 4H), 4.33 – 4.22 (m, 1H), 3.60 (t, *J*=7.3, 6H), 2.95 (t, *J*=7.2, 6H), 2.52 (dd, *J*=16.9, 5.8, 1H), 2.10 – 1.99 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 6.98. R_f: Doesn't run. LC-MS (ESI): t_R = 9.25 min. HRMS (ESI): for $[M+H]^+$ calc.: 692.25201, found: 692.25243.



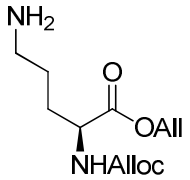
(S)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-5-(methylamino)pentanoic acid (284): In a 100 mL round bottom flask, containing an egg-shaped stirring bar, FmocNH-Orn(DMB, Me)-OH (30 mg, 0.06 mmol, 1 equiv) was dissolved in 0.8 mL acetonitrile and 0.2 mL water. CAN (132 mg, 0.24 mmol, 4 equiv) was added and the resulting orange mixture was stirred for one hour. The reaction mixture was evaporated; the residue was put on a silica column and eluted with chloroform: methanol: (acetic acid:ethyl acetate:methanol:water 3:3:3:2) 18:2:1 yielding 18 mg (0.05 mmol, 81%) of a brown, extremely hygroscopic foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.63 (d, *J* = 7.5 Hz, 2H), 7.50 (d, *J* = 5.7 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 2H), 7.24 – 7.11 (m, 2H), 7.11 – 6.99 (m, 1H), 4.20 – 3.98 (m, 4H), 2.91 (bs, 2H), 2.59 (s, 3H), 1.90 – 1.54 (m, 4H). LC-MS (ESI): t_R = 6.10 min. HRMS (ESI): for $[M+H]^+$ calc.: 369.18088, found: 369.18100.



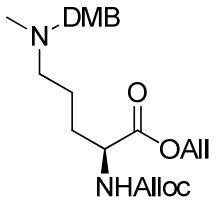
(S)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-5-((2,4-dimethoxybenzyl)(methyl)amino)pentanoic acid (290): In a 50 mL, flame dried schlenk flask, containing and argon atmosphere, FmocHN-Orn(TFA)-OH (1.1 mmol, 1 equiv) was dissolved in 30 mL anhydrous methanol. Molsieve (1 g) was added followed by 2,4- dimethoxybenzaldehyde (199 mg, 1.2 mmol, 1.1 equiv) and sodium acetate (189 mg, 2.3 mmol, 2.1 equiv). To the clear solution sodium cyanoborohydride (138 mg, 2.2 mmol, 2 equiv) was added batch wise, over one hour, turning it into a white suspension. After stirring for an additional hour paraformaldehyde (132 g, 4.4 mmol, 4 equiv) and a second portion of sodium cyanoborohydride (0138 mg, 2.2 mmol, 2 equiv) were added and the resulting mixture was stirred for 16 hours. The reaction mixture was acidified to pH 5 with acetic acid, evaporated to dryness and loaded on a silica column, the compound was eluted with chloroform:methanol:(acetic acid:ethyl acetate:methanol:water 3:3:3:2) 18:2:1 yielding 376 mg (0.73 mmol, 66%) of a clear oil that solidifies on standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.65 (d, *J*=7.5, 2H), 7.55 (dd, *J*=14.3, 7.3, 2H), 7.29 (t, *J*=7.4, 2H), 7.23 – 7.15 (m, 3H), 6.45 – 6.30 (m, 2H), 6.19 (bs, 1H), 4.32 – 3.96 (m, 6H), 3.68 (s, 6H), 3.15 – 2.66 (m, 2H), 2.50 (s, 3H), 2.01 – 1.63 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 175.74, 162.44, 159.52, 159.50, 155.95, 144.18, 143.95, 141.26, 141.23, 134.23, 127.62, 127.04, 125.31, 125.22, 119.90, 109.39, 105.05, 98.70, 77.32, 77.06, 76.81, 66.69, 55.45, 55.44, 55.27, 47.24, 38.98, 29.92, 20.26.(Some signals inferred from HSQC & HMBC). LC-MS (ESI): t_R = 6.69 min. HRMS (ESI): for $[M+H]^+$ calc.: 519.24896, found: 519.24844.



(*S*)-Allyl 2-(allyloxycarbonylamino)-5-(tert-butoxycarbonylamino)pentanoate (301): In a 250 mL round bottom flask NH2-Orn(Boc)-OH (59.3 mmol, assumed) was dissolved in 30 mL acetonitrile and 60 mL water. Sodium carbonate (6.3 g, 59.3 mmol, 1 equiv) was added followed by slow addition of 6.3 mL allyl chloroformate (7.1 g, 59.3 mmol, 1 equiv) over 5 hours. The resulting mixture was stirred for 16 hours after addition was complete, at which time a TLC sample showed complete conversion of the starting material. The acetonitrile was removed in vacuo followed by removal of the water by lyophilization. The resulting white solid was dissolved in 60 mL anhydrous DMF and sodium hydrogen carbonate (4.98 g, 59.3 mmol, 1 equiv) was added followed by allyl bromide (5.6 mL, 7.9 g, 65.2 mmol, 1.1 equiv). Resulting mixture was stirred for one day, the DMF was removed in vacuo and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layer was washed with three portions of water (30 mL), two portions of brine (30 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated yielding 14.39 g (40.36 mmol, 68%) of a yellow oil that solidifies on standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.05 – 5.80 (m, 2H), 5.40 – 5.13 (m, 4H), 4.72 – 4.48 (m, 4H), 4.37 (s, 1H), 3.13 (d, *J*=6.2, 2H), 1.95 – 1.83 (m, *J*=10.7, 6.0, 1H), 1.77 – 1.62 (m, 1H), 1.62 – 1.48 (m, 2H), 1.43 (s, 9H). LC-MS (ESI): t_R = 8.80 min. HRMS (ESI): for $[M+H]^+$ calc.: 357.20201, found: 357.20268.

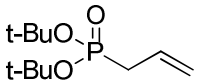


(*S*)-Allyl 2-(allyloxycarbonylamino)-5-aminopentanoate (303): In a 50 mL round bottom flask, containing an egg-shaped stirrer bar, Boc-protected Orthinine **301** (4 g, 11.2 mmol, 1 equiv) was dissolved in 6 mL anhydrous dichloromethane. 6 mL trifluoroacetic acid was added and the resulting mixture was stirred for 30 minutes. A TLC sample taken at this time confirmed complete conversion of the starting material. 24 mL toluene was added, followed by evaporating to dryness, another 24 mL toluene was added and the mixture was evaporated to dryness again to ensure all TFA was evaporated. After the final evaporation an orange oil remained 5.75 g (quant.) that was not purified further but used in the next reaction 'as is'. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (s, 3H), 5.95 – 5.76 (m, 3H), 5.42 – 5.11 (m, 4H), 4.73 – 4.39 (m, 4H), 4.39 – 4.08 (m, 1H), 2.98 (s, 2H), 2.09 – 1.64 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.20, 175.08, 172.02, 162.34 (q, *J*=34.7,TFA), 156.50, 132.65, 131.53, 119.28, 118.03, 66.47, 66.19, 53.63, 39.51, 29.34, 23.73. R_f: 0.10 (toluene:ethyl acetate 2:1). LC-MS (ESI): t_R = 5.36 min. HRMS (ESI): for $[M+H]^+$ calc.: 257.14958, found: 257.14964.

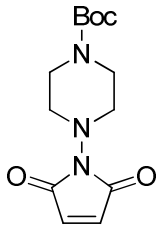


(*S*)-Allyl 2-(allyloxycarbonylamino)-5-((2,4-dimethoxybenzyl)(methyl)amino)pentanoate (304): In a 100 mL, flame dried round bottom schlenk, containing a magnetic stirring bar and an argon atmosphere, diprotected orthinine **303** (2.57 g, 5 mmol, 1 equiv) was dissolved in 37 mL anhydrous methanol. 5 g 3 A molsieve was added followed by sodium acetate (861 mg, 10.5 mmol, 2.1 equiv) and 2,4-dimethoxybenzaldehyde (831 mg, 5 mmol, 1 equiv). Sodium cyanoborohydride (620 mg, 10 mmol, 2 equiv) was added portion-wise over 2 hours, followed by an additional 2 hours of stirring. Paraformaldehyde (606 mg, 20 mmol, 4 equiv) was added followed by another portion of sodium cyanoborohydride (620 mg, 10 mmol, 2 equiv). The resulting mixture was stirred overnight, 5 g of celite was added and the suspension was filtered through celite. The filtrate was evaporated to dryness, and redissolved in chloroform (50 mL) and water (50 mL). The layers were separated and the aqueous phase was washed with 3 additional portion of chloroform (30 mL), the organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was further purified by flash column chromatography (chloroform:methanol 99:1 gradient 24:1) yielding 1.63 g (3.9 mmol, 78%) of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.16 (d, *J*=8.9, 1H), 6.63 (s, 1H), 6.51 – 6.37 (m, *J*=5.6, 2.3, 2H), 5.97 – 5.77 (m, 2H), 5.35 – 5.11 (m, 4H), 4.60 (d, *J*=5.7, 2H), 4.53 (d, *J*=4.6, 2H), 4.25 (d, *J*=4.7, 1H), 3.78 (d, *J*=1.1, 7H), 3.57 – 3.43 (m, 2H), 2.44 (t, *J*=6.5, 2H), 2.20 (s, 3H), 1.91 – 1.53 (m, 4H).^{13}C NMR (126 MHz, $CDCl_3$) δ = 172.29, 160.51, 159.00, 156.20, 132.91, 131.96, 131.78, 118.71,

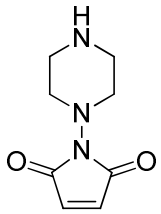
117.54, 117.40, 104.03, 98.47, 65.80, 65.57, 56.52, 55.37, 55.30, 53.97, 41.54, 30.17, 22.87. LC-MS (ESI): t_R = 6.05 min. HRMS (ESI): for $[M+H]^+$ calc.: 421.23331, found: 421.23262.



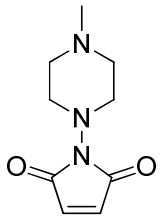
Di-tert-butyl allylphosphonate (317): In a 50 mL, flame dried, round bottom schlenk, containing an egg-shaped stirring bar and an argon atmosphere, di-tert-butyl phosphite (1 g, 5.15 mmol, 1 equiv) was dissolved in 20 mL anhydrous THF. The mixture was cooled to 0°C and sodium hydride 60% dispersion in mineral oil (227 mg, 5.7 mmol, 1.1 equiv) was added. The reaction mixture was stirred at 0°C for 3 minutes, after which allyl bromide (0.54 mL, 748 mg, 6.18 mmol, 1.2 equiv) was added and the cooling bath was removed. The resulting white suspension was stirred for 5 hours, after which an LCMS sample confirmed complete conversion of the starting material. Acetic acid (0.1 mL) was added to quench excess base, followed by 50 mL of water and 50 mL of ethyl acetate. The layers were separated, the aqueous layer was washed with 2 additional 25 mL portions of ethyl acetate, the organic layers were combined, washed with brine (40 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded 1.1 g of a clear liquid. The liquid was further purified over a silica column (chloroform:ethyl acetate 6:1) yielding 847 mg (3.6 mmol, 70%) of a clear liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.78 (dddd, *J*=24.7, 13.8, 9.5, 7.3, 1H), 5.17 – 5.04 (m, 2H), 2.50 (ddt, *J*=21.4, 7.3, 1.3, 2H), 1.49 (s, 18H). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 20.06. LC-MS (ESI): t_R = 8.67 min. HRMS (ESI): for $[M+H]^+$ calc.: 235.14576, found: 235.14601.



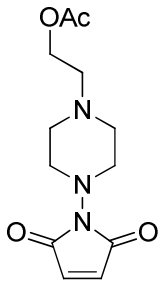
Tert-butyl 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)piperazine-1-carboxylate (318): In a 100 mL round bottom flask, hydrazine **319** (1.76 g, 8.78 mmol, 1 equiv) and maleic anhydride (861 mg, 8.78 mmol, 1 equiv) were dissolved dichloromethane (30 mL). The mixture was stirred for 1.5 hours at room temperature, while the reaction was monitored by ^{1}H-NMR. When the starting material was completely consumed, the solvent was evaporated and the residue was dissolved in acetic anhydride (40 mL) and sodium acetate (2.16 g, 26.3 mmol, 3 equiv) was added. The resulting mixture was stirred for 2.5 hours at 50°C, after which the solvent was evaporated. The residual brown solid was purified by column chromatography (toluene:ethyl acetate 20:1) to afford a yellow solid (1.55g, 5.52 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20 (d, *J*=5.4, 1H), 6.20 (d, *J*=5.4, 1H), 3.53 (dq, *J*=6.7, 3.7, 8H), 1.47 (s, 9H). R_f: 0.40 (toluene:ethyl acetate 3:1). LC-MS (ESI): t_R = 8.81 min. HRMS (ESI): for $[M+H]^+$ calc.: 282.14483, found: 282.14494.



1-(Piperazin-1-yl)-1*H*-pyrrole-2,5-dione (322): In a 50 mL round bottom flask a solution of N-Boc amine **318** (152.3 mg, 0.54 mmol, 1 equiv) in dichloromethane (3 mL) was exposed to TFA (3 mL). After stirring for 30 minutes at room temperature the starting material was consumed (TLC) and the solvent was evaporated yielding a yellow solid that required no further purification (quantitative as the TFA salt). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 2H), 7.24 (d, *J* = 5.5 Hz, 1H), 6.34 (d, *J* = 5.5 Hz, 1H), 3.82 – 3.70 (m, 4H), 3.40 (s, 4H).

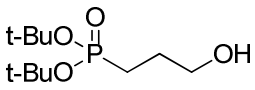


1-(4-Methylpiperazin-1-yl)-1*H*-pyrrole-2,5-dione (323): In a 500 mL round bottom flask, 4-methylpiperazin-1-amine (5.03 g, 43.7 mmol, 1 equiv) and maleic anhydride (4.3 g, 43.7 mmol, 1 equiv) were dissolved dichloromethane (150 mL). The mixture was stirred for one hours at room temperature, while the reaction was monitored by ^{1}H-NMR. When the starting material was completely consumed, the solvent was evaporated and the residue was dissolved in acetic anhydride (300 mL) and sodium acetate (10.8 g, 131 mmol, 3 equiv) was added. The resulting mixture was stirred for 12.5 hours at 50°C, after which the solvent was evaporated. The residual brown solid was purified by column chromatography (toluene:ethyl acetate 10:1) to afford a yellow oil (8.0 g, 41.1 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.17 (d, *J*=5.4, 1H), 6.14 (d, *J*=5.4, 1H), 3.61 – 3.54 (m, 4H), 2.55 – 2.50 (m, 4H), 2.30 (s, 3H). LC-MS (ESI): t_R = 1.31 min. HRMS (ESI): for $[M+H]^+$ calc.: 196.10805, found: 196.10811.



2-(4-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)piperazin-1-yl)ethyl acetate (326): In a 50 mL round bottom flask, maleic anhydride (338 mg, 3.44 mmol, 1 equiv) was added to a solution of 2-(4-aminopiperazin-1-yl)ethyl acetate (500 mg, 3.44 mmol, 1 equiv) in dichloromethane (20 mL). The mixture was stirred for 3.5 hours, after which the solvent was evaporated. The residue was dissolved in acetic anhydride (15

mL) and sodium acetate (847 mg, 10.3 mmol, 3 equiv) was added. The mixture was stirred at 50°C for 2.5 hours, and poured into saturated aqueous sodium bicarbonate (30 mL). The aqueous layer was extracted with three 30 mL portions of ethyl acetate, the organic layers were combined, washed with brine (50 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residual brown oil was purified by column chromatography (cyclohexane:ethyl acetate 2:1 gradient 1:1) to afford a yellow oil (303 mg, 1.13 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18 (d, *J* = 5.4 Hz, 1H), 6.16 (d, *J* = 5.4 Hz, 1H), 4.20 (t, *J* = 5.8 Hz, 2H), 3.62 – 3.52 (m, 4H), 2.71 – 2.58 (m, 7H), 2.07 (s, 3H). LC-MS (ESI): t_R = 1.80 min. HRMS (ESI): for $[M+H]^+$ calc.: 268.12918, found: 268.12913.



Di-tert-butyl 3-hydroxypropylphosphonate (330): In a flame dried, 100 mL round bottom schlenk, containing an egg-shaped stirring bar and an argon atmosphere, di-tert-butyl allylphosphonate (500 mg, 2.13 mmol, 1 equiv) was dissolved in THF (10 mL). Borane in THF (1M, 5.3 mL, 5.3 mmol, 2.5 equiv) was added dropwise over five minutes; after stirring for two hours a TLC sample showed complete conversion of the starting material. The reaction mixture was cooled to 0°C and methanol (10 mL was added) followed by addition of 3 M aqueous sodium hydroxide solution (5.75 mL, 17.3 mmol, 7.5 equiv) and 30% aqueous hydrogen peroxide (5.2 mL, 50.6 mmol, 22 equiv). The ice bath was removed and the reaction mixture was stirred at room temperature for two hours. The mixture was transferred to a separatory funnel containing 30 mL of saturated aqueous sodium bicarbonate solution and 30 mL of ethyl acetate, the layers were split and the aqueous layer was washed with two additional 10 mL portions of ethyl acetate. The organic layers were combined washed with brine, dried over anhydrous sodium sulfate and the solvent was removed in vacuo, yielding a clean oil that was further purified by flask column chromatography (toluene:ethyl acetate 1:1 gradient ethyl acetate: methanol 95:5) yielding 167 mg of a clear oil (0.66 mmol, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.69 (t, *J*=5.9, 2H), 3.05 (s, 1H), 1.91 – 1.66 (m, 4H), 1.49 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 82.08 (d, *J*=8.6), 62.96 (d, *J*=12.7), 30.64 (d, *J*=3.9), 28.33, 27.58 (d, *J*=151.9). ^{31}P NMR (162 MHz, $CDCl_3$) δ = 26.00. R_f: 0.14 (ethyl acetate). LC-MS (ESI): t_R = 6.85 min. HRMS (ESI): for $[M+H]^+$ calc.: 253.15632, found: 253.15638.

16.3 Fmoc Solid Phase Peptide Synthesis (Fmoc SPPS)

An Applied Biosystems automated peptide synthesizer was used for the automated portions of the syntheses of the peptides. Tentagel-RAM-Cys(Trt) was used as solid phase. Each coupling step employed 10 equiv amino acid and 10 equiv HBTU. The pHis analogue was coupled manually, using the following procedure:

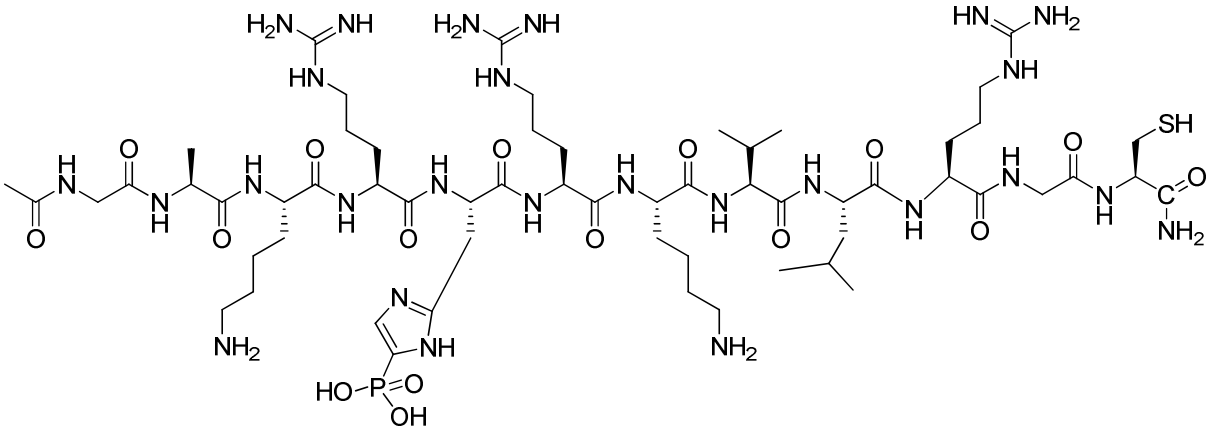
The resin was pre-swollen in DCM, washed five times with DMF and deprotected with 20 % piperidine in DMF (3 x 10 min). The resin was washed five times with DMF followed by addition of 2 equiv pHis analogue, 2 equiv HATU, 2 equiv HOAt and 6 equiv DIPEA in DMF. The mixture was shaken for 16 h at rt and subsequently washed 5 times with DMF. N-terminal acetylation was achieved by treatment of the unprotected N-terminus with a 1:1 mixture of 50 equiv DIPEA with 50 equiv acetic anhydride and DMF for 30 min at rt. After finishing the peptide sequence the resin was washed with DMF, DCM, methanol and diethyl ether (5 times each). Cleavage from the resin and parallel global deprotection was achieved with a cleavage mixture, consisting of 2.5 % water, 2.5 % TIPS-H and 2.5 % ethanedithiol in TFA. Cleavage was carried out by shaking the resin 1.5 hours with the cleavage mixture, followed by two times 30 minutes with a fresh amount of cleavage mixture, at room temperature. Afterwards, the cleavage solution was evaporated to dryness, the remaining solid was triturated with diethyl ether and subsequently dissolved in water containing as much acetonitrile as necessary for complete dissolution. After lyophilization the solid was re-dissolved in water/MeCN and purified by preparative HPLC.

16.3.1 Preparative HPLC purifications

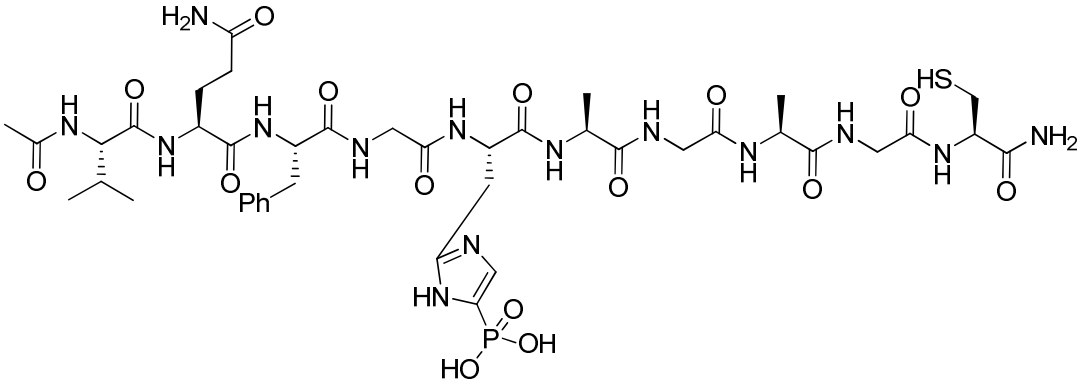
The peptides, both those containing the pHis analogue and the ones with normal histidine, were purified by preparative HPLC with an Agilent 1100 series instrument, connected to an Agilent MSD VL mass spectrometer and a fraction collector. As mobile phase water with 0.1 % TFA (solvent A) and acetonitrile (solvent B) were used. Peptides were eluted from a C_{18} column using the following program at a flow rate of 25 ml min^{-1}:

Time (min)	B (%)
0	5
1	5
20	30
25	100
27	100
26	5
29	5

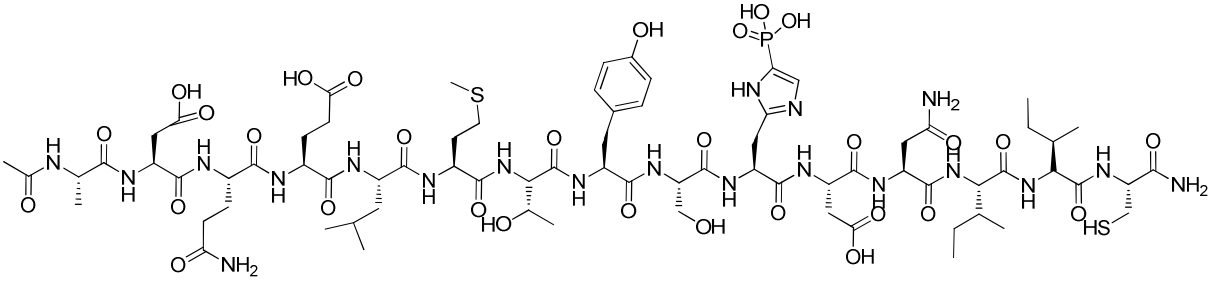
16.3.2 Peptide data



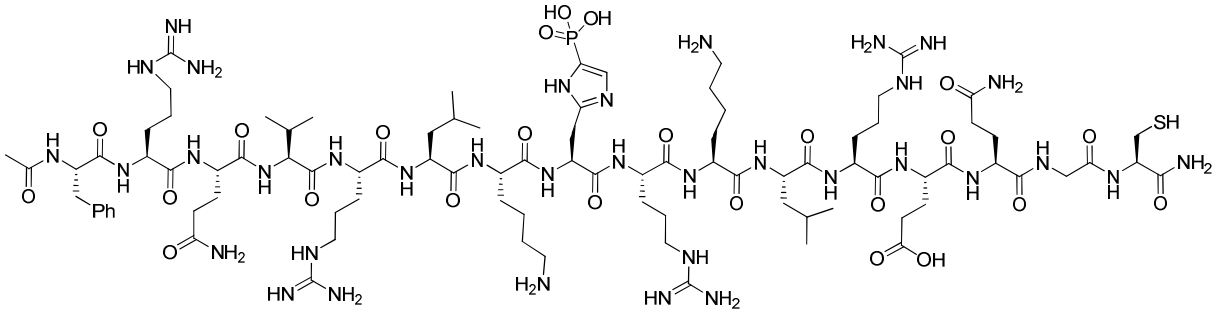
Ac-GAKRH*RKVLRGC-NH$_2$ (216): Synthesis by Fmoc-based SPPS automated and manual coupling at 0.09 mmol scale. Yield: 49.6 mg, 0.033 mmol, 37% (based on resin loading). ^{31}P NMR (162 MHz, D_2O) δ -2.9. LC-MS (ESI): t_R = 1.08 min (AD card). HRMS: for $[M+3H]^{3+}$ calc: 501.27305, found: 501.27285.



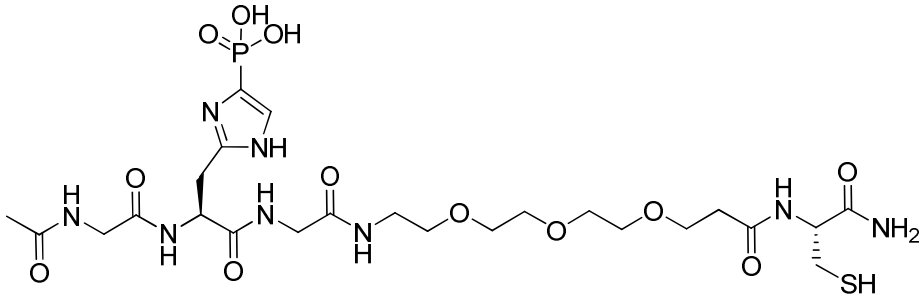
Ac-VQFGH*AGAGC-NH$_2$ (217): Synthesis by Fmoc-based SPPS automated and manual coupling at 0.05 mmol scale. Yield: 28.5 mg; 0.027 mmol; 53% (based on resin loading). ^{31}P NMR (162 MHz, D_2O) δ -3.1. HRMS: $[M+2H]^{2+}$: calculated: 534.21006, found: 534.21036.



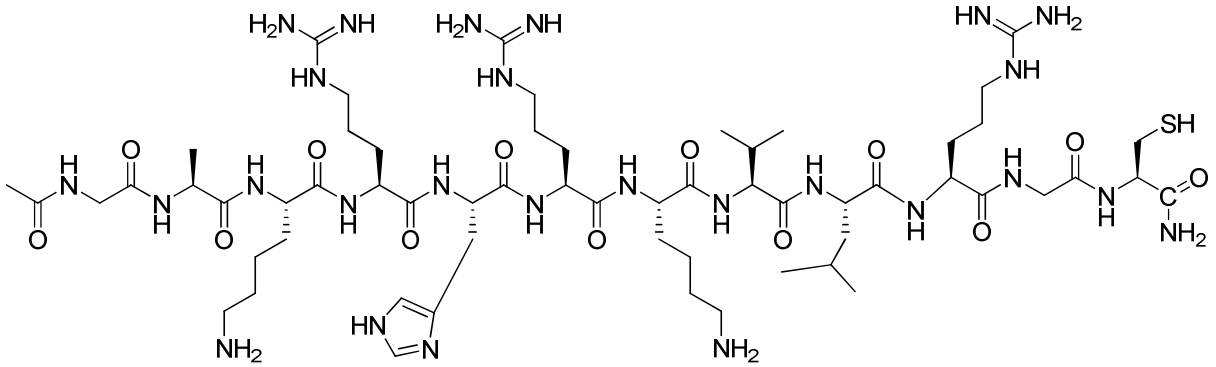
Ac-ADQELMTYSH*DNIIC-NH$_2$ (218): Synthesis by Fmoc-based SPPS automated and manual coupling at 0.05 mmol scale. Yield: 1.7 mg, 0.0009 mmol, 1.8% (based on resin loading). There was not enough compound available for ^{31}P NMR. LC-MS (ESI): t_R = 5.65 min. HRMS: $[M+2H]^{2+}$: calculated: 937.37845, found: 937.37879.



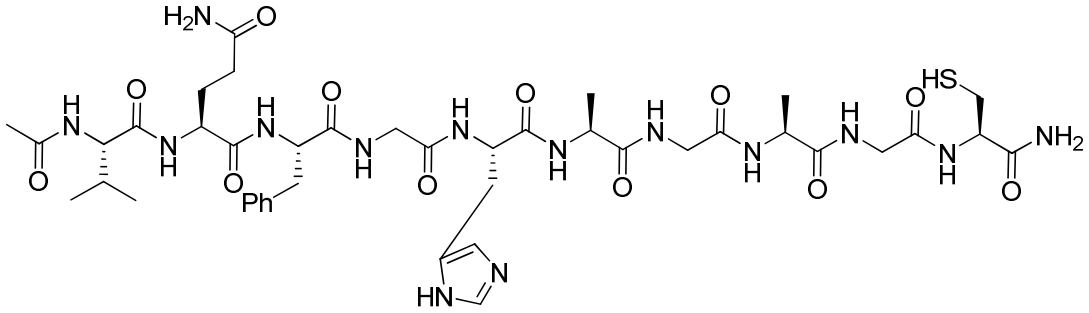
Ac-FRQVRLKH*RKLREQCG-NH$_2$ (219): Synthesis by Fmoc-based SPPS automated and manual coupling at 0.05 mmol scale. Yield: 54.5 mg; 0.025 mmol; 50% (based on resin loading). ^{31}P NMR (162 MHz, D_2O) δ -3.0. HRMS: $[M+3H]^{3+}$: calculated: 726.06058, found: 726.05984.



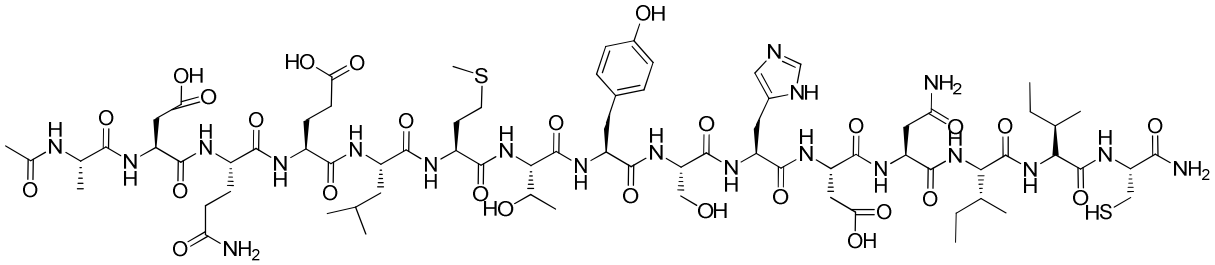
Ac-GH*G-PEG-C-NH$_2$ (220): Synthesis by Fmoc-based SPPS automated and manual coupling at 0.044 mmol scale. Yield: 12.9 mg; 0.019 mmol; 42% (based on resin loading). ^{31}P NMR (162 MHz, D_2O) δ -2.68. HRMS: $[M+H]^{+}$: calculated: 697.23750, found: 697.23692.



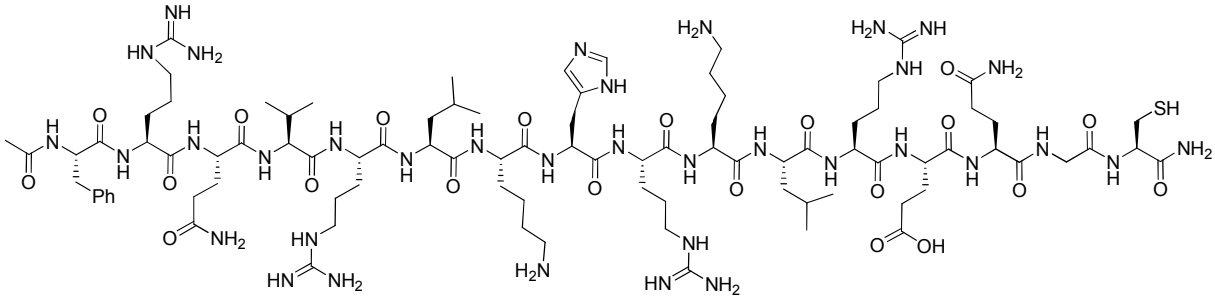
Ac-GAKRHRKVLRGC-NH$_2$ (221): Synthesis by Fmoc-based SPPS automated coupling at 0.1 mmol scale. Yield: 100 mg, 0.07 mmol, 70% (based on resin loading). LC-MS (ESI): t_R = 1.28 min (AD card). HRMS: for $[M+4H]^{4+}$ calc: 356.21502, found: 356.21527.



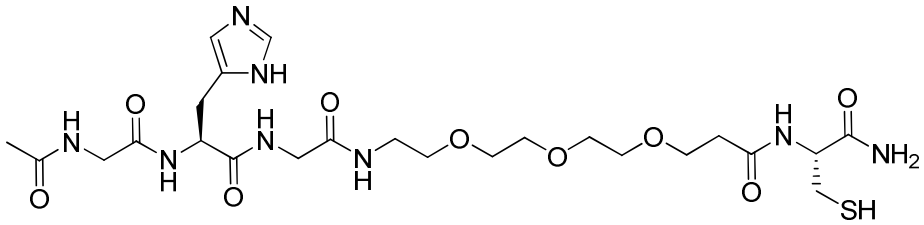
Ac-VQFGHAGAGC-NH_2 (222): Synthesis by Fmoc-based SPPS automated coupling at 0.05 mmol scale. Yield: 28.5 mg; 0.027 mmol; 53% (based on resin loading). HRMS: $[M+H]^+$: calculated: 987.44651, found: 987.44665.



Ac-ADQELMTYSHDNIIC-NH_2 (223): Synthesis by Fmoc-based SPPS automated and manual coupling at 0.05 mmol scale. Yield: 8.8 mg, 0.049 mmol, 9.8% (based on resin loading). LC-MS (ESI): t_R = 5.37 min. HRMS: $[M+2H]^{2+}$: calculated: 897.39529, found: 897.39536.



Ac-FRQVRLKHRKLREQCG-NH_2 (224): Synthesis by Fmoc-based SPPS automated coupling at 0.05 mmol scale. Yield: 57.2 mg; 0.027 mmol; 55% (based on resin loading). HRMS: $[M+3H]^{3+}$: calculated: 699.40513, found: 699.40339.



Ac-GHG-PEG-C-NH_2 (225): Synthesis by Fmoc-based SPPS automated at 0.138 mmol scale. Yield: 55 mg; 0.075 mmol; 55% (based on resin loading). LC-MS (ESI): t_R = 1.57 min. HRMS: for $[M+Na]^+$ calc: 639.25312, found: 639.25221.

17 References

[1] C. T. Walsh, S. Garneau-Tsodikova, G. J. Gatto, *Angew. Chem. Int. Ed.* **2005**, *44*, 7342-7372.

[2] G. Manning, D. B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, *Science* **2002**, *298*, 1912-1934.

[3] C. Harrison, *Nat Rev Drug Discov* **2012**, *11*, 21-21.

[4] K. Hubel, T. Lessmann, H. Waldmann, *Chem. Soc. Rev.* **2008**, *37*, 1361-1374.

[5] C. Smit, J. Bluemer, M. F. Eerland, M. F. Albers, M. P. Mueller, R. S. Goody, A. Itzen, C. Hedberg, *Angewandte Chemie-International Edition* **2011**, *50*, 9200-9204.

[6] a) P. A. Levene, C. L. Alsberg, *J. Biol. Chem.* **1906**, *2*, 127-133; b) F. A. Lipman, P. A. Levene, *J. Biol. Chem.* **1932**, *98*, 0109-0114.

[7] P. Cohen, *Trends Biochem. Sci* **2000**, *25*, 596-601.

[8] a) P. Cohen, *Annu. Rev. Biochem* **1989**, *58*, 453-508; b) T. Hunter, *Cell* **2000**, *100*, 113-127.

[9] A. W. Frank, *Crc Critical Reviews in Biochemistry* **1984**, *16*, 51-101.

[10] a) E. K. Dolence, G. Mayer, B. D. Kelly, *Tetrahedron: Asymmetry* **2005**, *16*, 1583-1594; b) M. K. Tarrant, P. A. Cole, in *Annu. Rev. Biochem, Vol. 78*, Annual Reviews, Palo Alto, **2009**, pp. 797-825; c) L. N. Johnson, *Biochem. Soc. Trans.* **2009**, *37*, 627-641.

[11] J. Ciesla, T. Fraczyk, W. Rode, *Acta Biochim. Pol.* **2011**, *58*, 137-147.

[12] a) C. C. Chen, B. B. Bruegger, C. W. Kern, Y. C. Lin, R. M. Halpern, R. A. Smith, *Biochemistry* **1977**, *16*, 4852-4855; b) C.-C. Chen, D. L. Smith, B. B. Bruegger, R. M. Halpern, R. A. Smith, *Biochemistry* **1974**, *13*, 3785-3789; c) B. T. Wakim, P. S. Grutkoski, A. T. M. Vaughan, G. L. Engelmann, *J. Biol. Chem.* **1995**, *270*, 23155-23158.

[13] L. S. Smith, C. W. Kern, R. M. Halpern, R. A. Smith, *Biochem. Biophys. Res. Commun.* **1976**, *71*, 459-465.

[14] M. E. Wilson, R. A. Consigli, *Virology* **1985**, *143*, 516-525.

[15] S. E. Severin, R. J. Judelovitch, *Biokhimiya* **1947**, *12*, 105-110.

[16] M. Deluca, P. D. Boyer, J. B. Peter, R. W. Moyer, K. E. Ebner, G. Kreil, D. E. Hultquist, *Biochemische Zeitschrift* **1963**, *338*, 512-&.

[17] H. R. Matthews, K. Pesis, Y. Wei, *Federation Proceedings* **1987**, *46*, 2161-2161.

[18] Hultquis.De, R. W. Moyer, P. D. Boyer, *Biochemistry* **1966**, *5*, 322-&.

[19] a) S. Klumpp, G. Bechmann, A. Mäurer, D. Selke, J. Krieglstein, *Biochem. Biophys. Res. Commun.* **2003**, *306*, 110-115; b) N. A. Elshourbagy, J. C. Near, P. J. Kmetz, G. M. Sathe, C. Southan, J. E. Strickler, M. Gross, J. F. Young, T. N. Wells, P. H. Groot, *J. Biol. Chem.* **1990**, *265*, 1430-1435.

[20] a) S. Srivastava, P. Choudhury, Z. Li, G. Liu, V. Nadkarni, K. Ko, W. A. Coetzee, E. Y. Skolnik, *Molecular Biology of the Cell* **2006**, *17*, 146-154; b) S. Srivastava, Z. Li, K. Ko, P. Choudhury, M. Albaqumi, A. K. Johnson, Y. Yan, J. M. Backer, D. Unutmaz, W. A. Coetzee, E. Y. Skolnik, *Molecular cell* **2006**, *24*, 665-675.

[21] a) F. Cuello, R. A. Schulze, F. Heemeyer, H. E. Meyer, S. Lutz, K. H. Jakobs, F. Niroomand, T. Wieland, *J. Biol. Chem.* **2003**, *278*, 7220-7226; b) A. Mäurer, T. Wieland, F. Meissl, F. Niroomand, R. Mehringer, J. Krieglstein, S. Klumpp, *Biochem. Biophys. Res. Commun.* **2005**, *334*, 1115-1120.

[22] J. A. Latham, S. Y. R. Dent, *Nat Struct Mol Biol* **2007**, *14*, 1017-1024.

[23] A. Xu, J. Hao, Z. Zhang, T. Tian, S. Jiang, J. Hao, C. Liu, L. Huang, X. Xiao, D. He, *Lung Cancer* **2010**, *67*, 48-56.

[24] S. Klumpp, J. Krieglstein, *Sci. Signal.* **2009**, *2*, 13.

[25] P. G. Besant, E. Tan, P. V. Attwood, *The International Journal of Biochemistry & Cell Biology* **2003**, *35*, 297-309.

[26] P. Ek, G. Pettersson, B. Ek, F. Gong, J. P. Li, O. Zetterqvist, *Eur. J. Biochem.* **2002**, *269*, 5016-5023.

[27] R. D. Busam, A.-G. Thorsell, A. Flores, M. Hammarström, C. Persson, B. M. Hallberg, *J. Biol. Chem.* **2006**, *281*, 33830-33834.

[28] R. Ma, E. Kanders, U. B. Sundh, M. Geng, P. Ek, Ö. Zetterqvist, J.-P. Li, *Biochem. Biophys. Res. Commun.* **2005**, *337*, 887-891.
[29] P. V. Attwood, *Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics*.
[30] J. A. Hoch, T. J. Silhavy, *Two-component signal transduction*, **1995**.
[31] C. Grefen, K. Harter, *Planta* **2004**, *219*, 733-742.
[32] a) V. L. Robinson, A. M. Stock, *Structure* **1999**, *7*, R47-R53; b) H. R. Matthews, *Pharmacology & Therapeutics* **1995**, *67*, 323-350; c) P. V. Attwood, M. J. Piggott, X. L. Zu, P. G. Besant, *Amino Acids* **2007**, *32*, 145-156.
[33] J. B. Stock, A. M. Stock, J. M. Mottonen, *Nature* **1990**, *344*, 395-400.
[34] N. K. Dhillon, S. Sharma, G. K. Khuller, *Critical Reviews in Microbiology* **2003**, *29*, 259-275.
[35] A. Kanavarioti, M. T. Rosenbach, *J. Org. Chem.* **1991**, *56*, 1513-1521.
[36] Hultquis.De, *Biochim. Biophys. Acta* **1968**, *153*, 329-&.
[37] B. Duclos, S. Marcandier, A. J. Cozzone, *Methods Enzymol.* **1991**, *201*, 10-21.
[38] Y. Tsuji, K. T. Huh, Y. Ohsugi, Y. Watanabe, *J. Org. Chem.* **1985**, *50*, 1365-1370.
[39] B. Basnar, M. Litschauer, S. Abermann, E. Bertagnolli, G. Strasser, M.-A. Neouze, *Chem. Commun.* **2011**, *47*, 361-363.
[40] C. Schenkels, B. Erni, J.-L. Reymond, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1443-1446.
[41] M.-a. Kakimoto, M. Kai, K. Kondo, *Chem. Lett.* **1982**, *11*, 525-526.
[42] J.-M. Kee, T. W. Muir, *ACS Chemical Biology* **2011**, *7*, 44-51.
[43] G. S. Singh, M. D'Hooghe, N. De Kimpe, *Chem. Rev.* **2007**, *107*, 2080-2135.
[44] M.-a. Kakimoto, M. Kai, K. Kondo, T. Hiyama, *Chem. Lett.* **1982**, *11*, 527-528.
[45] E. Marsault, K. Benakli, S. Beaubien, C. Saint-Louis, R. Déziel, G. Fraser, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4187-4190.
[46] T. Y. Zakharian, L. Di Costanzo, D. W. Christianson, *Organic & Biomolecular Chemistry* **2008**, *6*, 3240-3243.
[47] Z. Jin, *Natural Product Reports* **2011**, *28*, 1143-1191.
[48] L. D. Luca, *Current Medicinal Chemistry* **2006**, *13*, 1-23.
[49] a) P. Wasserscheid, W. Keim, *Angewandte Chemie-International Edition* **2000**, *39*, 3772-3789; b) R. Sheldon, *Chem. Commun.* **2001**, 2399-2407.
[50] a) W. A. Herrmann, *Angewandte Chemie-International Edition* **2002**, *41*, 1290-1309; b) V. Cesar, S. Bellemin-Laponnaz, L. H. Gade, *Chem. Soc. Rev.* **2004**, *33*, 619-636.
[51] a) M. P. Sammes, A. R. Katritzky, in *Adv. Heterocycl. Chem., Vol. Volume 35* (Ed.: R. K. Alan), Academic Press, **1984**, pp. 413-450; b) C. J. Moody, C. W. Rees, R. G. Young, *J. Chem. Soc., Perkin Trans. 1* **1991**, 335-339; c) M. Casey, C. J. Moody, C. W. Rees, *J. Chem. Soc., Perkin Trans. 1* **1987**, 1389-1393; d) M. R. Grimmett, in *Comprehensive Heterocyclic Chemistry II* (Eds.: R. K. Editors-in-Chief: Alan, W. R. Charles, C. W. R. Eric F.V. ScrivenA2 - Editors-in-Chief: Alan R. Katritzky, F. V. S. Eric), Pergamon, Oxford, **1996**, pp. 77-220.
[52] J. A. Joule, K. Mills, *Heterocyclic Chemistry*, fifth ed., Wiley, **2009**.
[53] a) A. Warshel, G. Naray-Szabo, F. Sussman, J. K. Hwang, *Biochemistry* **1989**, *28*, 3629-3637; b) A. Warshel, P. K. Sharma, M. Kato, Y. Xiang, H. Liu, M. H. M. Olsson, *Chem. Rev.* **2006**, *106*, 3210-3235.
[54] a) H. Du, Y. He, R. Sivappa, C. J. Lovely, *Synlett* **2006**, *2006*, 0965-0992; b) F. Bellina, R. Rossi, *Adv. Synth. Catal.* **2010**, *352*, 1223-1276.
[55] H. Debus, *Justus Liebigs Annalen der Chemie* **1858**, *107*, 199-208.
[56] a) X. Deng, N. S. Mani, *Org. Lett.* **2005**, *8*, 269-272; b) N. J. Liverton, J. W. Butcher, C. F. Claiborne, D. A. Claremon, B. E. Libby, K. T. Nguyen, S. M. Pitzenberger, H. G. Selnick, G. R. Smith, A. Tebben, J. P. Vacca, S. L. Varga, L. Agarwal, K. Dancheck, A. J. Forsyth, D. S. Fletcher, B. Frantz, W. A. Hanlon, C. F. Harper, S. J. Hofsess, M. Kostura, J. Lin, S. Luell, E. A. O'Neill, C. J.

Orevillo, M. Pang, J. Parsons, A. Rolando, Y. Sahly, D. M. Visco, S. J. O'Keefe, *J. Med. Chem.* **1999**, *42*, 2180-2190.

[57] a) S. E. Wolkenberg, D. D. Wisnoski, W. H. Leister, Y. Wang, Z. Zhao, C. W. Lindsley, *Org. Lett.* **2004**, *6*, 1453-1456; b) S. Balalaie, M. M. Hashemi, M. Akhbari, *Tetrahedron Lett.* **2003**, *44*, 1709-1711.

[58] a) N. D. Kokare, J. N. Sangshetti, D. B. Shinde, *Synthesis-Stuttgart* **2007**, *2007*, 2829-2834; b) G. V. M. Sharma, Y. Jyothi, P. S. Lakshmi, *Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry* **2006**, *36*, 2991 - 3000.

[59] V. Zuliani, G. Cocconcelli, M. Fantini, C. Ghiron, M. Rivara, *J. Org. Chem.* **2007**, *72*, 4551-4553.

[60] A. A. Gridnev, I. M. Mihaltseva, *Synth. Commun.* **1994**, *24*, 1547-1555.

[61] a) Y. Matsuoka, Y. Ishida, D. Sasaki, K. Saigo, *Tetrahedron* **2006**, *62*, 8199-8206; b) M. C. Perry, X. H. Cui, M. T. Powell, D. R. Hou, J. H. Reibenspies, K. Burgess, *J. Am. Chem. Soc.* **2003**, *125*, 113-123; c) W. L. Bao, Z. M. Wang, Y. X. Li, *J. Org. Chem.* **2003**, *68*, 591-593; d) E. Beccalli, G. Broggini, A. Contini, I. De Marchi, G. Zecchi, C. Zoni, *Tetrahedron-Asymmetry* **2004**, *15*, 3181-3187; e) A. R. Chianese, R. H. Crabtree, *Organometallics* **2005**, *24*, 4432-4436.

[62] F. Kunckell, *Berichte Der Deutschen Chemischen Gesellschaft* **1901**, *34*, 637-642.

[63] T. L. Little, S. E. Webber, *J. Org. Chem.* **1994**, *59*, 7299-7305.

[64] N. Ando, S. Terashima, *Tetrahedron* **2010**, *66*, 6224-6237.

[65] Ł. Albrecht, L. K. Ransborg, A. Albrecht, L. Lykke, K. A. Jørgensen, *Chemistry – A European Journal* **2011**, *17*, 13240-13246.

[66] A. M. Van Leusen, J. Wildeman, O. H. Oldenziel, *J. Org. Chem.* **1977**, *42*, 1153-1159.

[67] a) X. Beebe, V. Gracias, S. W. Djuric, *Tetrahedron Lett.* **2006**, *47*, 3225-3228; b) V. Gracias, D. Darczak, A. F. Gasiecki, S. W. Djuric, *Tetrahedron Lett.* **2005**, *46*, 9053-9056; c) V. Gracias, A. F. Gasiecki, S. W. Djuric, *Org. Lett.* **2005**, *7*, 3183-3186; d) V. Gracias, A. F. Gasiecki, T. G. Pagano, S. W. Djuric, *Tetrahedron Lett.* **2006**, *47*, 8873-8876; e) J. Sisko, A. J. Kassick, M. Mellinger, J. J. Filan, A. Allen, M. A. Olsen, *J. Org. Chem.* **2000**, *65*, 1516-1524; f) B.-C. Chen, R. Zhao, M. S. Bednarz, B. Wang, J. E. Sundeen, J. C. Barrish, *J. Org. Chem.* **2004**, *69*, 977-979; g) J. C. Boehm, J. M. Smietana, M. E. Sorenson, R. S. Garigipati, T. F. Gallagher, P. L. Sheldrake, J. Bradbeer, A. M. Badger, J. T. Laydon, J. C. Lee, L. M. Hillegass, D. E. Griswold, J. J. Breton, M. C. Chabot-Fletcher, J. L. Adams, *J. Med. Chem.* **1996**, *39*, 3929-3937; h) C. Almansa, J. Alfón, A. F. de Arriba, F. L. Cavalcanti, I. Escamilla, L. A. Gómez, A. Miralles, R. Soliva, J. Bartrolí, E. Carceller, M. Merlos, J. García-Rafanell, *J. Med. Chem.* **2003**, *46*, 3463-3475; i) L. Wang, K. W. Woods, Q. Li, K. J. Barr, R. W. McCroskey, S. M. Hannick, L. Gherke, R. B. Credo, Y.-H. Hui, K. Marsh, R. Warner, J. Y. Lee, N. Zielinski-Mozng, D. Frost, S. H. Rosenberg, H. L. Sham, *J. Med. Chem.* **2002**, *45*, 1697-1711.

[68] S. K. Samanta, I. Kylanlahti, J. Yli-Kauhaluoma, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3717-3719.

[69] a) D. A. Horne, K. Yakushijin, G. Buchi, *Heterocycles* **1994**, *39*, 139-153; b) D. A. Heerding, G. Chan, W. E. DeWolf Jr, A. P. Fosberry, C. A. Janson, D. D. Jaworski, E. McManus, W. H. Miller, T. D. Moore, D. J. Payne, X. Qiu, S. F. Rittenhouse, C. Slater-Radosti, W. Smith, D. T. Takata, K. S. Vaidya, C. C. K. Yuan, W. F. Huffman, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2061-2065.

[70] a) P. Molina, C. Conesa, M. Desamparados Velasco, *Synthesis-Stuttgart* **1996**, *1996*, 1459-1462; b) P. M. Fresneda, P. Molina, *Synlett* **2004**, *2004*, 1-17.

[71] Y.-B. Nie, L. Wang, M.-W. Ding, *J. Org. Chem.* **2011**, *77*, 696-700.

[72] Y.-B. Nie, Z. Duan, M.-W. Ding, *Tetrahedron* **2012**, *68*, 965-971.

[73] Y. G. Gololobov, N. I. Gusar, M. P. Chaus, *Tetrahedron* **1985**, *41*, 793-799.

[74] P. Loos, M. Riedrich, H.-D. Arndt, *Chem. Commun.* **2009**, 1900-1902.

[75] a) P. Molina, M. J. Vilaplana, *Synthesis-Stuttgart* **1994**, *1994*, 1197-1218; b) M. Riedrich, PhD Thesis, TU dortmund (Dortmund), **2009**.

[76] D. R. Williams, P. D. Lowder, Y.-G. Gu, D. A. Brooks, *Tetrahedron Lett.* **1997**, *38*, 331-334.

[77] a) H. Xie, J. Zhu, Z. Chen, S. Li, Y. Wu, *J. Org. Chem.* **2010**, *75*, 7468-7471; b) S.-L. You, J. W. Kelly, *Org. Lett.* **2004**, *6*, 1681-1683; c) S. Lauwagie, R. Millet, J. Pommery, P. Depreux, J.-P. Henichart, *Heterocycles* **2006**, *68*, 1149-+.
[78] I. Mohammadpoor-Baltork, M. A. Zolfigol, M. Abdollahi-Alibeik, *Synlett* **2004**, *2004*, 2803-2805.
[79] P. K. Martin, H. R. Matthews, H. Rapoport, Thyagara.G, *J. Org. Chem.* **1968**, *33*, 3758-&.
[80] J. L. Hughey, S. Knapp, H. Schugar, *Synthesis-Stuttgart* **1980**, 489-490.
[81] a) Y. Amemiya, D. D. Miller, F. L. Hsu, *Synth. Commun.* **1990**, *20*, 2483-2489; b) D. Gennet, S. Z. Zard, H. Zhang, *Chem. Commun.* **2003**, 1870-1871.
[82] T. Dockner, A. Frank, BASF A.-G., Fed. Rep. Ger. . **1979**, p. 12 pp.
[83] D. H. Huh, H. Ryu, Y. G. Kim, *Tetrahedron* **2004**, *60*, 9857-9862.
[84] a) M. E. Campos, R. Jimenez, F. Martinez, H. Salgado, *Heterocycles* **1995**, *40*, 841-849; b) M. Abdollahi-Alibeik, I. Mohammadpoor-Baltork, M. A. Zolfigol, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6079-6082; c) I. Mohammadpoor-Baltork, M. A. Zolfigol, M. Abdollahi-Alibeik, *Tetrahedron Lett.* **2004**, *45*, 8687-8690.
[85] K. C. Nicolaou, C. J. N. Mathison, T. Montagnon, *Angew. Chem. Int. Ed.* **2003**, *42*, 4077-4082.
[86] M. Ishihara, H. Togo, *Synlett* **2006**, *2006*, 227-230.
[87] a) Y. Huang, X. Zu, F. Wu, J. Xu, X. Wu, H. Yao, *Tetrahedron* **2012**, *68*, 3123-3128; b) S. Haneda, A. Okui, C. Ueba, M. Hayashi, *Tetrahedron* **2007**, *63*, 2414-2417.
[88] A. de la Hoz, Á. Díaz-Ortiz, M. del Carmen Mateo, M. Moral, A. Moreno, J. Elguero, C. Foces-Foces, M. L. Rodríguez, A. Sánchez-Migallón, *Tetrahedron* **2006**, *62*, 5868-5874.
[89] T. Horneff, S. Chuprakov, N. Chernyak, V. Gevorgyan, V. V. Fokin, *J. Am. Chem. Soc.* **2008**, *130*, 14972-14974.
[90] a) S.-H. Lee, K. Yoshida, H. Matsushita, B. Clapham, G. Koch, J. Zimmermann, K. D. Janda, *J. Org. Chem.* **2004**, *69*, 8829-8835; b) S. Laufer, G. Wagner, D. Kotschenreuther, *Angew. Chem. Int. Ed.* **2002**, *41*, 2290-2293.
[91] a) L. Preti, O. A. Attanasi, E. Caselli, G. Favi, C. Ori, P. Davoli, F. Felluga, F. Prati, *Eur. J. Org. Chem.* **2010**, *2010*, 4312-4320; b) O. A. Attanasi, E. Caselli, P. Davoli, G. Favi, F. Mantellini, C. Ori, F. Prati, *Org. Lett.* **2009**, *11*, 2840-2843; c) O. A. Attanasi, P. Davoli, G. Favi, P. Filippone, A. Forni, G. Moscatelli, F. Prati, *Org. Lett.* **2007**, *9*, 3461-3464.
[92] S. Li, Z. Li, Y. Yuan, D. Peng, Y. Li, L. Zhang, Y. Wu, *Org. Lett.* **2012**, *14*, 1130-1133.
[93] T. Benincori, E. Brenna, F. Sannicolo, *J. Chem. Soc., Perkin Trans. 1* **1993**, 675-679.
[94] a) M. A. Honey, R. Pasceri, W. Lewis, C. J. Moody, *J. Org. Chem.* **2012**, *77*, 1396-1405; b) W. Li, Y. Lam, *J. Comb. Chem.* **2005**, *7*, 644-647; c) D. E. Frantz, L. Morency, A. Soheili, J. A. Murry, E. J. J. Grabowski, R. D. Tillyer, *Org. Lett.* **2004**, *6*, 843-846.
[95] a) C. Kanazawa, S. Kamijo, Y. Yamamoto, *J. Am. Chem. Soc.* **2006**, *128*, 10662-10663; b) M.-A. Bonin, D. Giguère, R. Roy, *Tetrahedron* **2007**, *63*, 4912-4917.
[96] M. E. Voss, C. M. Beer, S. A. Mitchell, P. A. Blomgren, P. E. Zhichkin, *Tetrahedron* **2008**, *64*, 645-651.
[97] S. Zaman, K. Mitsuru, A. D. Abell, *Org. Lett.* **2005**, *7*, 609-611.
[98] S. Petit, C. Fruit, L. Bischoff, *Org. Lett.* **2010**, *12*, 4928-4931.
[99] C. H. Soh, W. K. Chui, Y. Lam, *J. Comb. Chem.* **2006**, *8*, 464-468.
[100] H. Bredereck, G. Theilig, *Chem. Ber. Recl.* **1953**, *86*, 88-96.
[101] J. J. Garcia, P. Zerecero-Silva, G. Reyes-Rios, M. G. Crestani, A. Arevalo, R. Barrios-Francisco, *Chem. Commun.* **2011**, *47*, 10121-10123.
[102] V. Kumar, A. Anand, M. P. Mahajan, *Synlett* **2006**, *2006*, 2199-2202.
[103] B. Hu, Z. Wang, N. Ai, J. Zheng, X.-H. Liu, S. Shan, Z. Wang, *Org. Lett.* **2011**, *13*, 6362-6365.
[104] a) M. McLaughlin, R. M. Mohareb, H. Rapoport, *J. Org. Chem.* **2002**, *68*, 50-54; b) Y.-L. Zhong, J. Lee, R. A. Reamer, D. Askin, *Org. Lett.* **2004**, *6*, 929-931.

[105] M. Gwiazda, H.-U. Reissig, *Synlett* **2006**, *2006*, 1683-1686.
[106] C. Kison, T. Opatz, *Chemistry – A European Journal* **2009**, *15*, 843-845.
[107] R. B. Sparks, A. P. Combs, *Org. Lett.* **2004**, *6*, 2473-2475.
[108] I. Lantos, W. Y. Zhang, X. Shui, D. S. Eggleston, *J. Org. Chem.* **1993**, *58*, 7092-7095.
[109] a) C. J. Helal, J. C. Lucas, *Org. Lett.* **2002**, *4*, 4133-4134; b) L. De Luca, G. Giacomelli, A. Porcheddu, *J. Comb. Chem.* **2005**, *7*, 905-908.
[110] K.-i. Nunami, M. Yamada, T. Fukui, K. Matsumoto, *J. Org. Chem.* **1994**, *59*, 7635-7642.
[111] a) M. R. Grimmett, in *Comprehensive Heterocyclic Chemistry* (Eds.: R. K. Editors-in-Chief: Alan, W. R. Charles), Pergamon, Oxford, **1984**, pp. 457-498; b) N. Xi, Q. Huang, L. Liu, in *Comprehensive Heterocyclic Chemistry III* (Eds.: R. K. Editors-in-Chief: Alan, A. R. Christopher, F. V. S. Eric, J. K. T. Richard), Elsevier, Oxford, **2008**, pp. 143-364.
[112] J.-M. Kee, B. Villani, L. R. Carpenter, T. W. Muir, *J. Am. Chem. Soc.* **2010**, *132*, 14327-14329.
[113] J. M. Van der Eijk, R. J. M. Nolte, J. W. Zwikker, *J. Org. Chem.* **1980**, *45*, 547-548.
[114] a) K. Moonen, I. Laureyn, C. V. Stevens, *Chem. Rev.* **2004**, *104*, 6177-6216; b) I. Billault, A. Vasella, *Helv. Chim. Acta* **1999**, *82*, 1137-1149.
[115] N. Shibata, J. E. Baldwin, A. Jacobs, M. E. Wood, *Synlett* **1996**, 519-520.
[116] J. E. Baldwin, A. C. Spivey, C. J. Schofield, J. B. Sweeney, *Tetrahedron* **1993**, *49*, 6309-6330.
[117] K. A. Kumar, K. M. L. Rai, K. B. Umesha, *Tetrahedron* **2001**, *57*, 6993-6996.
[118] a) E. Herranz, K. B. Sharpless, *J. Org. Chem.* **1978**, *43*, 2544-2548; b) K. B. Sharpless, A. O. Chong, K. Oshima, *J. Org. Chem.* **1976**, *41*, 177-179.
[119] A. A. Thomas, K. B. Sharpless, *J. Org. Chem.* **1999**, *64*, 8379-8385.
[120] a) E. K. Dolence, J. B. Roylance, *Tetrahedron-Asymmetry* **2004**, *15*, 3307-3322; b) A. Bisai, G. Pandey, M. K. Pandey, V. K. Singh, *Tetrahedron Lett.* **2003**, *44*, 5839-5841.
[121] D. Ellis, *Tetrahedron-Asymmetry* **2001**, *12*, 1589-1593.
[122] C. I. Sainz-Diaz, E. Galvez-Ruano, A. Hernandez-Laguna, J. Bellanato, *J. Org. Chem.* **1995**, *60*, 74-83.
[123] Z. Han, D. Krishnamurthy, P. Grover, Q. K. Fang, X. Su, H. S. Wilkinson, Z.-H. Lu, D. Magiera, C. H. Senanayake, *Tetrahedron* **2005**, *61*, 6386-6408.
[124] B. Aguilera, A. Fernandez-Mayoralas, *Chem. Commun.* **1996**, 127-128.
[125] G. C. G. Pais, R. A. Fernandes, P. Kumar, *Tetrahedron* **1999**, *55*, 13445-13450.
[126] K. K. Andersen, M. G. Kociolek, *J. Org. Chem.* **1995**, *60*, 2003-2007.
[127] a) A. J. Papa, *J. Org. Chem.* **1966**, *31*, 1426; b) I. P. Beletskaya, A. V. Cheprakov, *Coord. Chem. Rev.* **2004**, *248*, 2337-2364.
[128] a) Y. Génisson, N. L.-d. Viguerie, C. André, M. Baltas, L. Gorrichon, *Tetrahedron: Asymmetry* **2005**, *16*, 1017-1023; b) A. Isidro-Llobet, M. Alvarez, F. Albericio, *Chem. Rev.* **2009**, *109*, 2455-2504.
[129] M. Bendikov, H. M. Duong, E. Bolanos, F. Wudl, *Org. Lett.* **2005**, *7*, 783-786.
[130] a) A. I. Meyers, F. Tavares, *Tetrahedron Lett.* **1994**, *35*, 2481-2484; b) A. I. Meyers, F. X. Tavares, *J. Org. Chem.* **1996**, *61*, 8207-8215; c) K. Yamamoto, Y. G. Chen, F. G. Buono, *Org. Lett.* **2005**, *7*, 4673-4676; d) J. A. Mayoral, S. Rodríguez-Rodríguez, L. Salvatella, *Chemistry – A European Journal* **2008**, *14*, 9274-9285.
[131] a) T. K. M. Shing, Yeung, P. L. Su, *Org. Lett.* **2006**, *8*, 3149-3151; b) B. B. Snider, L. Han, C. Xie, *J. Org. Chem.* **1997**, *62*, 6978-6984.
[132] X.-h. Cai, H.-j. Yang, G.-l. Zhang, *Can. J. Chem.* **2005**, *83*, 273-275.
[133] F. Camps, J. Coll, A. Parente, *Synthesis-Stuttgart* **1978**, *1978*, 215-216.
[134] W.-D. Woggon, F. Ruther, H. Egli, *J. Chem. Soc., Chem. Commun.* **1980**, 706-708.
[135] T. M. Nicoletti, C. L. Raston, M. V. Sargent, *J. Chem. Soc., Perkin Trans. 1* **1990**, 133-138.
[136] Lundquist, A. D. Satterfield, J. C. Pelletier, *Org. Lett.* **2006**, *8*, 3915-3918.

[137] M. F. Eerland, C. Hedberg, *J. Org. Chem.* **2012**, *77*, 2047-2052.
[138] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chem. Int. Ed.* **2005**, *44*, 1378-1382.
[139] V. V. Sureshbabu, B. Vasantha, H. P. Hemantha, *Synthesis-Stuttgart* **2011**, 1447-1455.
[140] M. Mori, H. Wakamatsu, N. Saito, Y. Sato, R. Narita, Y. Sato, R. Fujita, *Tetrahedron* **2006**, *62*, 3872-3881.
[141] a) J. E. Mathieson, J. J. Crawford, M. Schmidtmann, R. Marquez, *Organic & Biomolecular Chemistry* **2009**, *7*, 2170-2175; b) D. Brückner, *Tetrahedron* **2006**, *62*, 3809-3814.
[142] H. Firouzabadi, N. Iranpoor, S. Sobhani, *Tetrahedron* **2004**, *60*, 203-210.
[143] Y. Wan, A. N. Kurchan, L. A. Barnhurst, A. G. Kutateladze, *Org. Lett.* **2000**, *2*, 1133-1135.
[144] a) A. Padwa, J. D. Ginn, *J. Org. Chem.* **2005**, *70*, 5197-5206; b) A. Padwa, A. G. Waterson, *J. Org. Chem.* **2000**, *65*, 235-244; c) D. Craig, N. P. King, A. N. Shaw, *Tetrahedron Lett.* **1997**, *38*, 8599-8602.
[145] N. R. Curtis, H. J. Diggle, J. J. Kulagowski, C. London, S. Grimwood, P. H. Hutson, F. Murray, P. Richards, A. Macaulay, K. A. Wafford, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 693-696.
[146] a) S. Cardani, C. Gennari, C. Scolastico, R. Villa, *Tetrahedron* **1989**, *45*, 7397-7404; b) C. A. M. Afonso, M. T. Barros, C. D. Maycock, *Tetrahedron* **1999**, *55*, 801-814.
[147] A. I. Meyers, R. C. Strickland, *J. Org. Chem.* **1972**, *37*, 2579-2583.
[148] A. S. Thompson, E. J. J. Grabowski, Merck and Co., Inc., USA . **1995**, p. 43 pp.
[149] K. C. K. Swamy, N. N. B. Kumar, E. Balaraman, K. V. P. P. Kumar, *Chem. Rev.* **2009**, *109*, 2551-2651.
[150] Z. Gultekin, H. Bayrak, W. Frey, *Asian J. Chem.* **2006**, *18*, 1474-1480.
[151] a) S. Yoshida, H. Yorimitsu, K. Oshima, *Org. Lett.* **2009**, *11*, 2185-2188; b) R. C. Cambie, G. R. Clark, S. R. Gallagher, P. S. Rutledge, M. J. Stone, P. D. Woodgate, *J. Organomet. Chem.* **1988**, *342*, 315-337.
[152] M. Ginisty, C. Gravier-Pelletier, Y. Le Merrer, *Tetrahedron: Asymmetry* **2006**, *17*, 142-150.
[153] P. A. Grieco, Y. Yokoyama, G. P. Withers, F. J. Okuniewicz, C. L. J. Wang, *J. Org. Chem.* **1978**, *43*, 4178-4182.
[154] a) M. Miyashita, A. Yoshikoshi, P. A. Grieco, *J. Org. Chem.* **1977**, *42*, 3772-3774; b) R. Sterzycki, *Synthesis-Stuttgart* **1979**, *1979*, 724-725.
[155] a) K. S. Kim, Y. H. Song, B. H. Lee, C. S. Hahn, *J. Org. Chem.* **1986**, *51*, 404-407; b) S. E. Sen, S. L. Roach, J. K. Boggs, G. J. Ewing, J. Magrath, *J. Org. Chem.* **1997**, *62*, 6684-6686.
[156] H. M. Walborsky, R. H. Davis, D. R. Howton, *J. Am. Chem. Soc.* **1951**, *73*, 2590-2594.
[157] K. Tanemura, T. Suzuki, T. Horaguchi, *J. Chem. Soc., Chem. Commun.* **1992**, 979-980.
[158] K. M. Brummond, H. Chen, B. Mitasev, A. D. Casarez, *Org. Lett.* **2004**, *6*, 2161-2163.
[159] W. Yu, Y. Mei, Y. Kang, Z. Hua, Z. Jin, *Org. Lett.* **2004**, *6*, 3217-3219.
[160] L. D. Arnold, J. C. G. Drover, J. C. Vederas, *J. Am. Chem. Soc.* **1987**, *109*, 4649-4659.
[161] S. Van der Jeught, C. V. Stevens, *Chem. Rev.* **2009**, *109*, 2672-2702.
[162] a) B. Iddon, N. Khan, *J. Chem. Soc., Perkin Trans. 1* **1987**, 1445-1451; b) B. Iddon, N. Khan, *J. Chem. Soc., Perkin Trans. 1* **1987**, 1453-1455; c) B. Iddon, N. Khan, B. L. Lim, *J. Chem. Soc., Perkin Trans. 1* **1987**, 1437-1443.
[163] M. Bahnous, C. Mouats, Y. Fort, P. C. Gros, *Tetrahedron Lett.* **2006**, *47*, 1949-1951.
[164] a) C. J. Lovely, H. Du, R. Sivappa, M. R. Bhandari, Y. He, H. V. R. Dias, *J. Org. Chem.* **2007**, *72*, 3741-3749; b) N. Matsunaga, T. Kaku, A. Ojida, A. Tasaka, *Tetrahedron-Asymmetry* **2004**, *15*, 2021-2028.
[165] T. Tschamber, F. Gessier, M. Neuburger, Sudagar S. Gurcha, Gurdyal S. Besra, J. Streith, *Eur. J. Org. Chem.* **2003**, *2003*, 2792-2798.
[166] T. Hirao, T. Masunaga, Y. Ohshiro, T. Agawa, *Tetrahedron Lett.* **1980**, *21*, 3595-3598.

[167] a) D. Gelman, L. Jiang, S. L. Buchwald, *Org. Lett.* **2003**, *5*, 2315-2318; b) C. Huang, X. Tang, H. Fu, Y. Jiang, Y. Zhao, *J. Org. Chem.* **2006**, *71*, 5020-5022; c) T. Hirao, T. Masunaga, N. Yamada, Y. Ohshiro, T. Agawa, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 909-913.
[168] M. Kalek, J. Stawinski, *Organometallics* **2007**, *26*, 5840-5847.
[169] a) S. Doussin, N. Birlirakis, D. Georgin, F. Taran, P. Berthault, *Chemistry-a European Journal* **2006**, *12*, 4170-4175; b) B. Punji, J. T. Mague, M. S. Balakrishna, *Inorg. Chem.* **2007**, *46*, 10268-10275.
[170] a) D. S. Carver, S. D. Lindell, E. A. SavilleStones, *Tetrahedron* **1997**, *53*, 14481-14496; b) M. Abarbri, J. Thibonnet, L. Berillon, F. Dehmel, M. Rottlander, P. Knochel, *J. Org. Chem.* **2000**, *65*, 4618-4634.
[171] R. M. de Figueiredo, S. Thoret, C. Huet, J. Dubois, *Synthesis-Stuttgart* **2007**, *2007*, 529-540.
[172] a) S. Tabanella, I. Valancogne, R. F. W. Jackson, *Organic & Biomolecular Chemistry* **2003**, *1*, 4254-4261; b) M. Kruppa, G. Imperato, B. König, *Tetrahedron* **2006**, *62*, 1360-1364.
[173] a) C. Han, S. L. Buchwald, *J. Am. Chem. Soc.* **2009**, *131*, 7532-7533; b) J. E. Milne, S. L. Buchwald, *J. Am. Chem. Soc.* **2004**, *126*, 13028-13032.
[174] A. J. Ross, H. L. Lang, R. F. W. Jackson, *J. Org. Chem.* **2009**, *75*, 245-248.
[175] V. F. Slagt, A. H. M. de Vries, J. G. de Vries, R. M. Kellogg, *Organic Process Research & Development* **2009**, *14*, 30-47.
[176] L. Jin, H. Zhang, P. Li, J. R. Sowa, A. Lei, *J. Am. Chem. Soc.* **2009**, *131*, 9892-9893.
[177] M. G. Organ, S. Avola, I. Dubovyk, N. Hadei, E. A. B. Kantchev, C. J. O'Brien, C. Valente, *Chemistry – A European Journal* **2006**, *12*, 4749-4755.
[178] J. E. Utas, B. Olofsson, B. Åkermark, *Synlett* **2006**, *2006*, 1965-1967.
[179] D. A. Evans, T. Bach, *Angew. Chem.* **1993**, *105*, 1414-1415.
[180] Y.-C. Wu, J. Zhu, *Org. Lett.* **2009**, *11*, 5558-5561.
[181] M. D. Smith, C. G. Sudhahar, D. Gong, R. V. Stahelin, M. D. Best, *Molecular BioSystems* **2009**, *5*, 962-972.
[182] B. H. Rotstein, V. Rai, R. Hili, A. K. Yudin, *Nat. Protocols* **2010**, *5*, 1813-1822.
[183] a) G. Basarab, J. Eyermann, M. Gowravaram, O. Green, L. MacPherson, M. Morningstar, T. Nguyen, Astrazeneca AB, Swed. . **2003**, p. 240 pp; b) D. Chen, C. L. Franklin, P. R. Guzzo, L. S. Lin, M. M. C. Lo, R. P. Nargund, I. K. Sebhat, Merck & Co., Inc., USA . **2008**, p. 165pp; c) S. Daninos-Zeghal, M. A. Al, A. Ahond, C. Poupat, P. Potier, *Tetrahedron* **1997**, *53*, 7605-7614; d) J. M. Kelly, A. Afonso, Schering Corporation, USA . **2007**, p. 247pp; e) L. Roumen, J. W. Peeters, J. M. A. Emmen, I. P. E. Beugels, E. M. G. Custers, G. M. de, R. Plate, K. Pieterse, P. A. J. Hilbers, J. F. M. Smits, J. A. J. Vekemans, D. Leysen, H. C. J. Ottenheijm, H. M. Janssen, J. J. R. Hermans, *J. Med. Chem.* **2010**, *53*, 1712-1725; f) P. K. Suryadevara, S. Olepu, J. W. Lockman, J. Ohkanda, M. Karimi, C. L. M. J. Verlinde, J. M. Kraus, J. Schoepe, V. W. C. Van, A. D. Hamilton, F. S. Buckner, M. H. Gelb, *J. Med. Chem.* **2009**, *52*, 3703-3715; g) A. G. Taveras, R. J. Doll, A. B. Cooper, J. A. Ferreira, T. Guzi, D. F. Rane, V. M. Girijavallabhan, C. J. Aki, J. Chao, C. Alvarez, J. M. Kelly, T. Lalwani, J. A. Desai, J. J. s. Wang, Schering Corporation, USA . **2002**, p. 215 pp.
[184] A. Malanda Kimbonguila, S. Boucida, F. Guibé, A. Loffet, *Tetrahedron* **1997**, *53*, 12525-12538.
[185] M. Saady, L. Lebeau, C. Mioskowski, *J. Org. Chem.* **1995**, *60*, 2946-2947.
[186] A. B. Smith, III, L. Ducry, R. M. Corbett, R. Hirschmann, *Org. Lett.* **2000**, *2*, 3887-3890.
[187] P. Savignac, B. Iorga, *Modern Phosphonate Chemistry*, first ed., CRC Press, **2003**.
[188] A. Vaseila, R. Voeffray, *Helv. Chim. Acta* **1982**, *65*, 1953-1964.
[189] a) S. Andreae, E. Schmitz, *J. Prakt. Chem./Chem.-Ztg.* **1994**, *336*, 366-368; b) J. S. Arora, N. Kaur, O. I. V. Phanstiel, *J. Org. Chem.* **2008**, *73*, 6182-6186; c) K. C. Calvo, *J. Org. Chem.* **1987**, *52*, 3654-3658; d) C. Sun, R. Bittman, *J. Org. Chem.* **2004**, *69*, 7694-7699.
[190] C. V. Stevens, E. V. Meenen, Y. Eeckhout, B. Vanderhoydonck, W. Hooghe, *Chem. Commun.* **2005**, 4827-4829.

[191] W. Pan, C. Ansiaux, S. P. Vincent, *Tetrahedron Lett.* **2007**, *48*, 4353-4356.
[192] M. Kawase, T. Kitamura, Y. Kikugawa, *J. Org. Chem.* **1989**, *54*, 3394-3403.
[193] T. W. Grebe, J. B. Stock, *Advances in Microbial Physiology, Vol 41* **1999**, *41*, 139-227.
[194] M. F. Albers, B. van Vliet, C. Hedberg, *Org. Lett.* **2011**, *13*, 6014-6017.
[195] C. D. Warren, I. Y. Liu, A. Herscovics, R. W. Jeanloz, *J. Biol. Chem.* **1975**, *250*, 8069-8078.
[196] G. W. Daub, E. E. Van Tamelen, *J. Am. Chem. Soc.* **1977**, *99*, 3526-3528.
[197] M. T. Bedford, S. G. Clarke, *Molecular cell* **2009**, *33*, 1-13.
[198] T. J. Baker, M. Tomioka, M. Goodman, *Org. Synth.* **2002**, *78*, No pp. given.
[199] K. Feichtinger, C. Zapf, H. L. Sings, M. Goodman, *J. Org. Chem.* **1998**, *63*, 3804-3805.
[200] R. Zhu, M. Xie, F. Wang, Q. Liu, T. Kang, *Chin. J. Chem .* **2010**, *28*, 1508-1509.
[201] a) J. Li, J. Kou, X. Luo, E. Fan, *Tetrahedron Lett.* **2008**, *49*, 2761-2763; b) R. G. S. Berlinck, A. C. B. Burtoloso, A. E. Trindade-Silva, S. Romminger, R. P. Morais, K. Bandeira, C. M. Mizuno, *Natural Product Reports* **2010**, *27*, 1871-1907; c) T. Suhs, B. Koenig, *Mini-Reviews in Organic Chemistry* **2006**, *3*, 315-331.
[202] S. Flemer, Jr., J. S. Madalengoitia, *Synthesis-Stuttgart* **2007**, 1848-1860.
[203] C. Qin, J. Li, E. Fan, *Synlett* **2009**, 2465-2468.
[204] a) D. Obrecht, P. Ermert, S. Oumouch, F. Lach, A. Luther, K. Marx, K. Moehle, Polyphor AG, Switz. **2011**, 528; b) D. Obrecht, P. Ermert, S. Oumouch, F. Lach, A. Luther, K. Marx, K. Moehle, Polyphor AG, Switz. . **2011**, 720.
[205] S. Bertrand, O. Duval, J.-J. Hélesbeux, G. Larcher, P. Richomme, *Tetrahedron Lett.* **2010**, *51*, 2119-2122.
[206] L. R. Reddy, B. V. S. Reddy, E. J. Corey, *Org. Lett.* **2006**, *8*, 2819-2821.
[207] E. Morera, F. Pinnen, G. Lucente, *Org. Lett.* **2002**, *4*, 1139-1142.
[208] S. Wiejak, E. Masiukiewicz, B. Rzeszotarska, *Chemical & Pharmaceutical Bulletin* **2001**, *49*, 1189-1191.
[209] S. Nowshuddin, A. R. Reddy, *Tetrahedron Lett.* **2006**, *47*, 5159-5161.
[210] S. D. Luzzi, M. A. Marletta, *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, 3934-3941.
[211] a) S. Rapireddy, L. Nhon, R. E. Meehan, J. Franks, D. B. Stolz, D. Tran, M. E. Selsted, D. H. Ly, *J. Am. Chem. Soc.* **2012**, *134*, 4041-4044; b) B. Nottelet, M. Patterer, B. François, M.-A. Schott, M. Domurado, X. Garric, D. Domurado, J. Coudane, *Biomacromolecules* **2012**, *13*, 1544-1553; c) M. J. Crawford, S. Rapireddy, R. Bahal, I. Sacui, D. H. Ly, *Journal of Nucleic Acids* **2011**, *2011*.
[212] E. Boman, R. Dahl, N. G. J. Delaet, J. Ernst, C. Lum, L. Sebo, J. Urban, Kemia, Inc., USA . **2006**, p. 235pp.
[213] V. Mark, J. R. V. Wazer, *J. Org. Chem.* **1964**, *29*, 1006-1008.

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19 Declaration/Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit selbständig und nur mit den angegeben Hilfsmittln angefertigt habe.

I hereby declare that I performed the work presented independently and did not use any other but the indicated aids.

Dortmund, Dezember 2012

Bart van Vliet