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Synthesis and Biological Evaluation of a Compound Collection Inspired by Withanolides

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The present thesis was developed between September 2011 and August 2015 under the supervision of Prof. Dr. Dr. h.c. Herbert Waldmann at the Department of Chemistry and Chemical Biology of the Technical University of Dortmund and the Max Planck Institute of Molecular Physiology, Dortmund.

Teile dieser Arbeit wurden bereits in folgender Publikation veröffentlicht:

The present work was partly published in the following paper:

Biology-Oriented Synthesis of a Withanolide-Inspired Compound Collection Reveals Novel Modulators of Hedgehog Signaling

Jakub Švenda, Michael Sheremet, Lea Kremer, Lukáš Maier**,** Jonathan O. Bauer, Carsten Strohmann, Slava Ziegler, Kamal Kumar, Herbert Waldmann

Angew. Chem. Int. Ed. **2015**, *54*, 5596–5602; *Angew. Chem.* **2015**, *127*, 5688–5694

"So eine Arbeit wird eigentlich nie fertig, man muß sie für fertig erklären, wenn man nach Zeit und Umständen das Mögliche getan hat."

„A work as this is never finished; one must simply declare it finished when one has, within limits of time and circumstances, done what is possible."

Johann Wolfgang von Goethe

Dedicated to my wife and my love, Kristina

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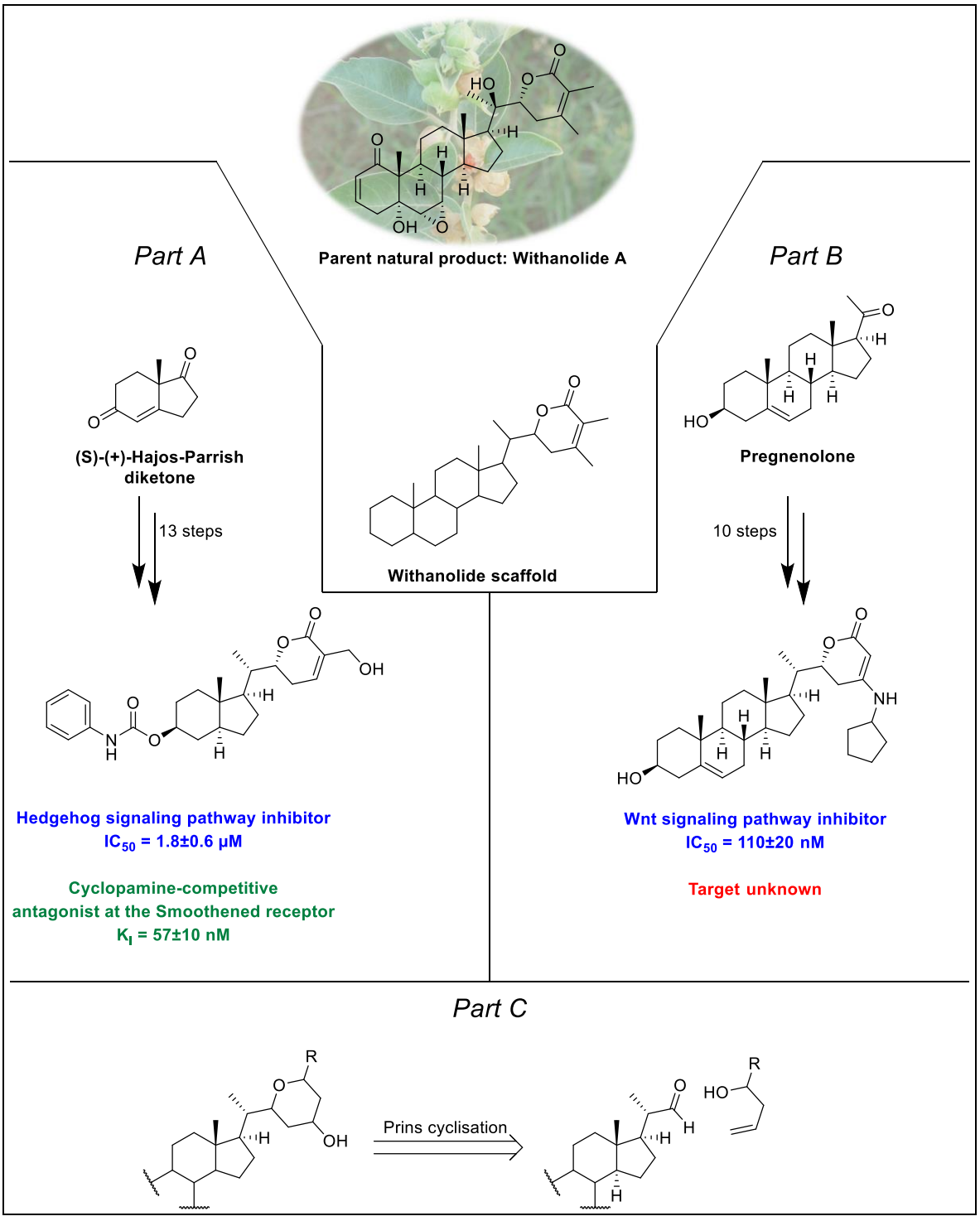
Abstract

In biology-oriented synthesis (BIOS) the scaffolds of natural products are chosen as the starting points for the synthesis of potentially bioactive compounds. Natural products have evolved to carry out diverse functions in the biological sphere. Therefore, their scaffolds represent a privileged section of the chemical space, enriched with bioactivity. Synthesis of compound collections with a common natural product scaffold provides a pool of structures from which the bioactive compounds are identified by various biological assays. Identified biologically active compounds can serve as probes in chemical biology research.

In the present work a compound collection inspired by the withanolide class of natural products was synthesized. The withanolides comprise a family of natural products embodying a steroid core, which share a γ- or δ-lactone/lactol as the common structural feature. Two complementary libraries were prepared in order to cover a possibly broad chemical space. Both synthetic pathways start from commercially available and enantiomerically pure compounds. In part A, a library of full steroidal analogues was prepared. The synthesis starts from commercially available pregnenolone and leads to withanolide analogues in 10–11 steps. In Part B, a compound collection based on the *trans*-hydrindane dehydro-δ-lactone scaffold was prepared. The synthesis starts from (S)-(+)-Hajos-Parrish diketone and takes 12–13 steps to withanolide analogues.

All synthesized compounds were submitted to cell-based assays for the modulation of cellular signaling pathways. One compound derived from the collection of full steroidal analogues is an inhibitor of the Wnt signaling pathway with an IC_{50} of 110±20 nM. It only modestly affects the enzymatic activity of TNKS1/2, a known target of Wnt inhibitors, and has most likely another target. One compound derived from collection B is an inhibitor of the Hedgehog signaling pathway with an IC_{50} of 1.8±0.6 µM. It acts a cyclopamine-competitive antagonist at the Smoothened receptor with a K_i of 57±10 nM.

Finally, in part C an alternative approach to the synthesis of withanolide analogues was discussed. Hereby, no lactone is assembled in multiple steps, but instead a Prins cyclization used for the quick and stereoselective synthesis of a six-membered ring.


Parent natural product: Withanolide A
Part A
(S)-(+)-Hajos-Parrish diketone
13 steps
Withanolide scaffold
Part B
Pregnenolone
10 steps
Hedgehog signaling pathway inhibitor
IC50 = 1.8±0.6 μM
Cyclopamine-competitive antagonist at the Smoothened receptor
KI = 57±10 nM
Wnt signaling pathway inhibitor
IC50 = 110±20 nM
Target unknown
Part C
Prins cyclisation

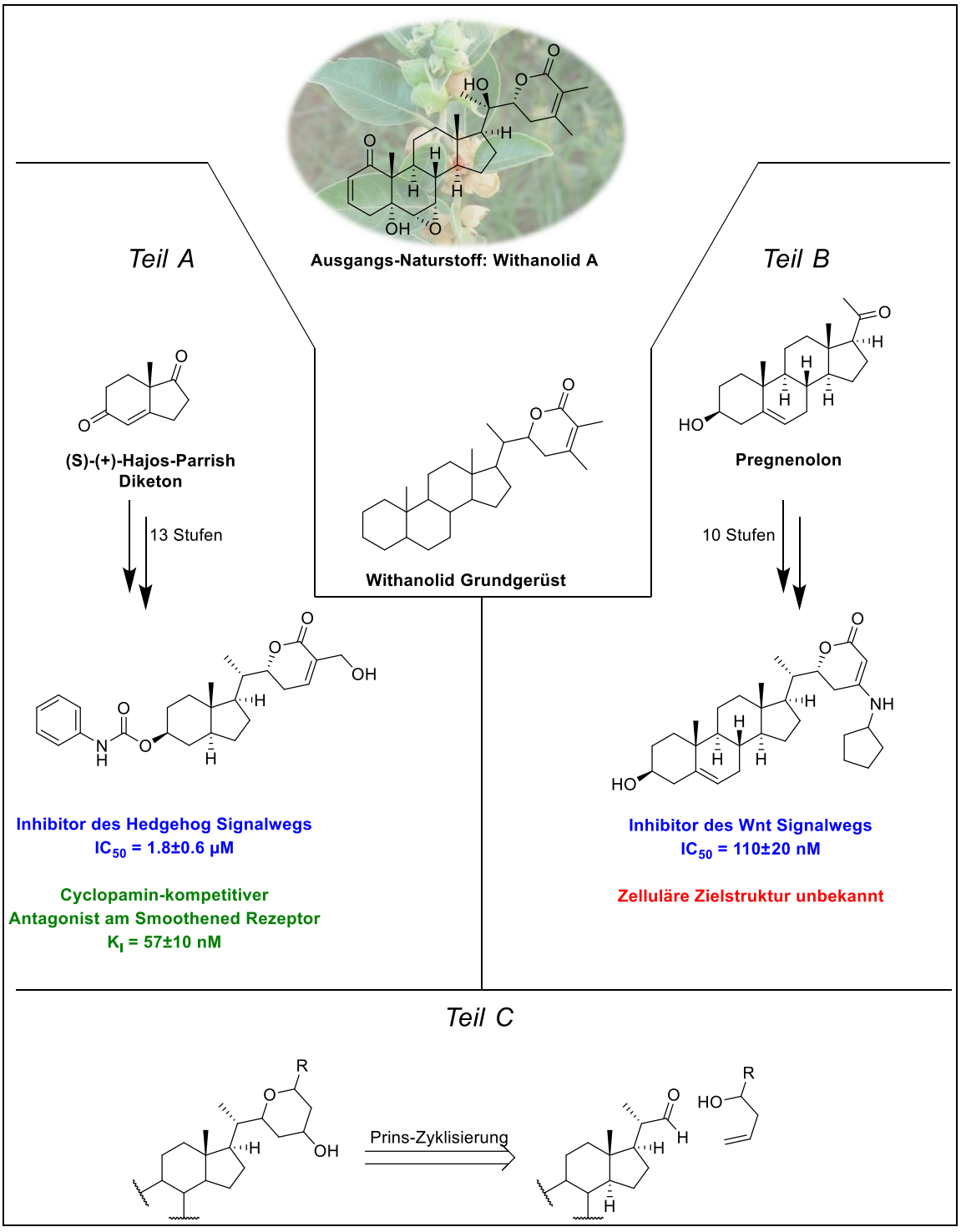
Kurzfassung

In der Biologie-orientieten Synthese (BIOS) werden die Gerüststrukturen von Naturstoffen als Startpunkte in der Suche nach potentiell biologisch aktiven Verbindungen gewählt. Naturstoffe sind evolviert um vielfältige Funktionen in der Biosphäre auszuüben. Ihre Gerüststrukturen repräsentieren daher einen priveligierten Teil des chemischen Strukturraums, angereichert mit biologischer Aktivität. Die Synthese von Substanzbibliotheken mit einer gemeinsamen naturstoffbasierten Gerüststruktur stellt eine Sammlung von Strukturen bereit, aus der die biologisch aktiven Verbindungen mittels biologischer Assays identifiziert werden. Die identifizierten biologisch aktiven Verbindungen können als Sonden in der chemischen Biologie eingesetzt werden.

In der vorliegenden Arbeit wurde eine Substanzbibliothek inspiriert von Withanoliden synthetisiert. Withanolide sind eine Familie von Naturstoffen mit einem Steroid-Grundgerüst, die ein γ- oder δ-Lacton/Lactol als gemeinsames Strukturmerkmal teilen. Um einen möglichst großen Strukturraum abzudecken, wurden zwei komplementäre Substanzbibliothekten hergestellt. Beide Synthesewege beginnen bei kommerziell erhältlichen und enantiomerenreinen Verbindungen. In Teil A wurde eine Substanzbibliothek von steroidhaltigen Analoga hergestellt. Die Synthese startet von kommerziell erhältlichem Pregnenolon und führt in 10-11 Schritten zu Withanlid-Analoga. In Teil B wurde eine Substanzbibliothek basierend auf dem *trans*-Hydrindan-Dehydro-δ-Lacton hergestellt. Die Synthese beginnt bei (S)-(+)-Hajos-Parrish Diketon und führt in 12-13 Schritten zu Withanlid-Analoga.

Alle synthetisierten Verbindungen wurden zell-basierten Untersuchungen bezüglich der Modulierung von zellulären Signalwegen unterzogen. Eine Verbindung aus der Gruppe der steroidhaltigen Analoga ist ein Inhibitor des Wnt-Signalwegs mit einem IC_{50} von 110±20 nM. Die Verbindung beeinflusst nur mäßig die enzymatische Aktivität von TNKS1/2, einem bekannten Zielprotein von gängigen Inhibitoren des Wnt-Signalwegs, und besitzt aller Wahrscheinlichkeit nach eine andere zelluläre Zielstruktur. Eine weitere Verbindung aus Teil B ist ein Inhibitor des Hedgehog-Signalweg mit einem IC_{50} von 1.8±0.6 µM. Sie ist ein Cyclopamin-kompetitiver Antagonist am membranständigen Smoothened-Rezeptorprotein mit einem K_i von 57±10 nM.

Zuletzt wurde in Teil C ein alternativer Zugang zu Withanolid-Analoga diskutiert. Hierbei wurde das Lakton nicht in einem mehrstufigen Prozess aufgebaut, sonderen eine Prins-Zyklisierung für die schnelle und stereoselektive Synthese eines sechsgliedrigen Rings genutzt.


Teil A
Ausgangs-Naturstoff: Withanolid A
Teil B
(S)-(+)-Hajos-Parrish Diketon
13 Stufen
Withanolid Grundgerüst
Pregnenolon
10 Stufen
Inhibitor des Hedgehog Signalwegs
IC50 = 1.8±0.6 µM
Cyclopamin-kompetitiver Antagonist am Smoothened Rezeptor
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Inhibitor des Wnt Signalwegs
IC50 = 110±20 nM
Zelluläre Zielstruktur unbekannt
Teil C
Prins-Zyklisierung

I Introduction

1.1 Natural products

1.1.1 Natural Products and their Role in Drug Discovery

In the broadest sense, a natural product is any compound produced by an organism. The three groups of polymeric structures, that is, proteins, nucleic acids and polysaccharides are generally excluded from this definition. Within the fields of chemical biology and medicinal chemistry the definition is further narrowed to secondary metabolites only.[1] Primary metabolites are those compounds who perform inevitable physiological functions in the organism. This covers all compounds of ubiquitous metabolic pathways like glycolysis, citric acid cycle or urea cycle, as well as compounds with structural functions like lipids. Primary metabolites are identical or similar among all organisms. Contrary to this, secondary metabolites are not essential for survival, but provide an evolutionary advantage to the producer. Secondary metabolites (SM) are found in bacteria, sponges, plants, lower animals like amphibians and others, they do not occur in higher animals like humans. The two main purposes of SM are protection against herbivores in case of plants and predators in case of amphibian etc., as well as protection against invasion by microbes and parasites. Plants are sessile and in contrast to most animals cannot escape in case of danger. Many species have therefore evolved to produce SM as a chemical defense mechanism. Alternatively, plants can forego the synthesis of SM if they possess mechanical and morphological barriers like spikes, thorns, impenetrable bark and so on. As a defense mechanism against microbes and parasites, secondary metabolites are a feature of organisms that lack an immune system, whereas they are absent in organisms which possess an immune system. Hence, sessile and slow-moving organisms, as well as defenseless plants use SM as a chemical defense mechanism against all kinds of threats in their ecological environment.[2]

Secondary metabolites with a defense function achieve this by acting as toxins. In evolution, several strategies have developed how the plant can protect itself from its own SM.[3] Oftentimes, plant natural products are stored as glycosides, that is, they have one or more sugar molecules attached to a hydroxyl group. In this form, the compound is stored in the vacuole, a compartment common to plant

cells. In case the cell is damaged by an herbivore, the compartment boundaries are destroyed and the vacuole content comes into contact with the cytosol. The cytosol contains glycosidases which cleave the glycoside bonds and release the biologically active aglycon.

Secondary metabolites can be grouped according to structural features or biosynthetic origin (Figure 1). According to the latter, many SM can be classified into three major categories: alkaloids, terpenoids and polyketides.[4] Two prominent alkaloids are the penicillins (**1**) and quinine (**2**)[5]. Because alkaloids are biosynthesized from amino acids they always contain at least one nitrogen atom. Often, the individual amino acid constituents can be distinguished in the structure, as for example in the penicillins (**1**)[6]. Terpenoids are derived from five-carbon isoprene units, assembled in thousands of ways. Prominent examples are the various isomers of pinene (**5**)[7], withanolide A (**6**)[8] and tetrahydrocannabinol (**7**)[9]. Due to their biosynthetic origin, the number of carbon atoms is oftentimes an integral multiple of five. Polyketides are biosynthesized from acyl-CoA units in a process similar to fatty acid synthesis. This polymerization-like biosynthetic origin is sometimes reflected in highly ordered degree of the the product structures, as for example in brevetoxin B (**8**).[10] Tetracycine (**9**) is another polyketide and a very important antibiotic.[11]

As mentioned above, a key feature of natural products is their organization in compound families. That means that organisms do not biosynthesize a certain natural product in a target-oriented metabolic pathway, but rather a large number of similar compounds sharing a common scaffold but varying in substitution. The diversity in the oxygenation patterns of secondary metabolites is for the most part achieved through the oxidase reactions catalysed by cytochrome P450 enzymes (CYPs). CYPs catalyze aliphatic and aromatic bond hydroxylations, epoxidations and many more reactions. The reactions generally occur with high chemo-, regio-, and stereoselectivity.[12]

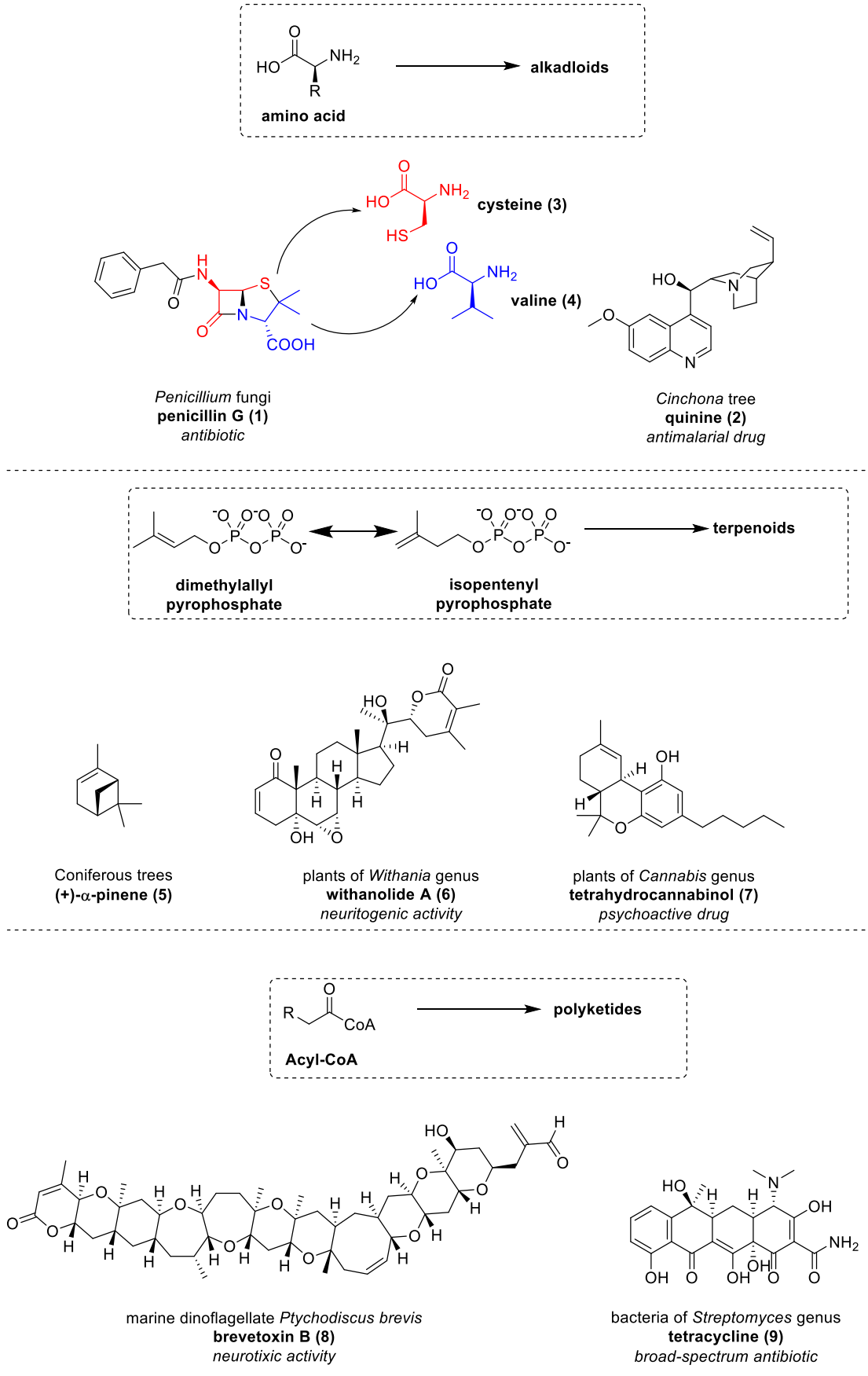


Figure 1: The three main categories of secondary metabolites, their biosynthetic origin and examples.

Since ancient times, people have attempted to cure diseases by producing remedies from natural materials. The traditional use of plants as the main delivery of pharmaceuticals has been coined ethnopharmacology. There are noteworthy examples of plant preparations with a real active ingredient. This way, people have been unwittingly using natural products as drugs. Prominent examples are aspirin and penicillin (Figure 2). The knowledge of the use of willow bark as a remedy for pains and fever dates back to the ancient Sumerians and Egyptians, as well as Hippocrates.[13] The active ingredient of the willow tree bark was discovered in 1763 by Edward Stone to be salicylic acid (**8**).[14] Since 1899 acetylsalicylic acid is marketed worldwide as aspirin. Many ancient cultures independently discovered the effectiveness of moulds to treat infections.[15] This could work because some moulds produce antibiotic substances. Today, the discovery of penicillin (**1**) is attributed to Alexander Fleming in 1928. After a long way of development, the mass production of penicillin started in the 1940s.[16] Penicillin was the first antibiotic substance to be discovered and has paved the way to many more substance classes, including other natural products.

Examples of natural products or natural product derived drugs approved in the last decades include paclitaxel (**11**), lovastatin (**12**) and amphotericin B (**13**).[1] In 1980, lovastatin (also called mevinolin) was reported as a metabolite of the fungus *Aspergillus terreus*.[17] The natural product can be used as a drug without any chemical modification. In 1987, lovastatin was the first statin approved by the U.S. Food and Drug Administration (FDA) as a drug for lowering cholesterol levels by inhibiting the enzyme HMG-CoA reductase.[18] Amphotericin B (**11**) is an antifungal drug originally isolated from *Streptomyces nodosus* in 1955. It was first approved by the FDA in 1966. Due to its severe and possibly lethal side effects, it is of limited use.[19] Paclitaxel is used in cancer chemotherapy. It was discovered in 1971 and received its first FDA approval in 1992. Paclitaxel was a major breakthrough in cancer therapy because it was not only a new active ingredient, but also added a completely new mechanism of action to the arsenal of cancer treatment. Paclitaxel stabilizes the microtubule polymer and protects it from disassembly. Microtubule dynamics is therefore impaired and mitosis haltet.[20] All above mentioned drugs or close synthetic derivatives thereof are on the 19th edition of the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system. One of the best examples for the successful truncation of a natural product is that of halichondrin B (**14**). A truncated synthetic derivative of this polyether macrolide, named eribulin (**15**), has been approved by the FDA in 2010 as a new treatment option for late-stage breast cancer.[21]

Many more examples of natural products as sources of drugs can be found in the literature.[22] Natural products are involved in ca. 50% of all newly approved small molecules drugs in the years

[1] The following FDA approval dates were retrieved from the official online database of the U.S. Food and Drug Administration.

2000–2010.[22c] The human usage of natural products as medications can be either according to their natural purpose, or not. An example for the former is penicillin. Members of the family *Penicillium* produce penicillin as a means of "chemical warfare" against bacteria. People have learnt to isolate the active ingredient and use it for the very same purpose. A contrary example is taxol. In fact, taxol did not evolve as a ligand for human tubulin and its activity in humans is a mere coincidence. Also, it is part of a comprehensive picture of natural products to note that they have been used by humans for many other purposes than medication as well. For example, indigo has been used as dye, vanillin and capsaicin as flavors, caffeine, nicotine as stimulants, et cetera.[2c]

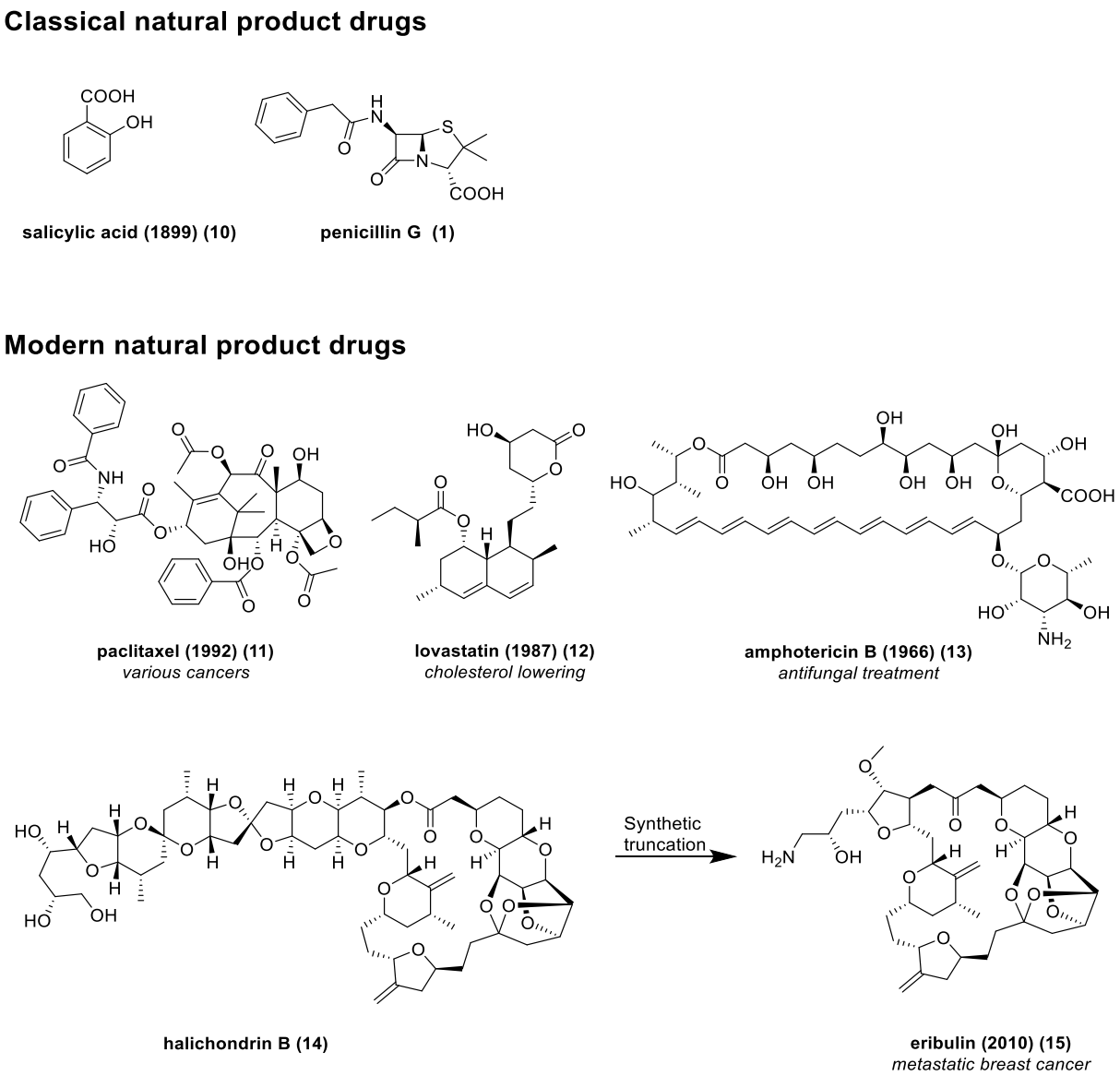
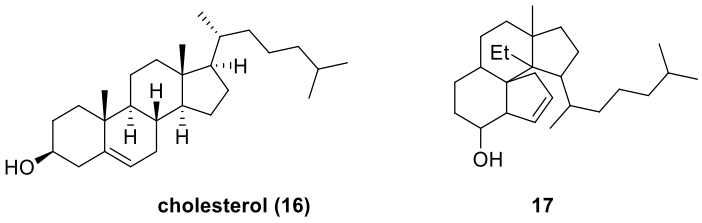


Figure 2: Prominent drugs based on natural products. The year of approval is given in brackets.

1.1.2 The Steroid Scaffold in Biology & Medicine

Steroidal compounds are a key feature of all eukaryotic organisms.[23] While absent in prokaryotes, the cellular membranes of plants, animals, and fungi all contain a type of steroid, where it influences the cell membrane's fluidity. The most common type of animal steroid is cholesterol (**16,** Figure 3), the most notable plant sterol (phytosterol) is stigmasterol. Besides its function in cell membranes, in vertebrates steroids have a second function as messenger molecules. In humans, the biosynthesis of all further steroid hormones starts from cholesterol and proceeds over many steps to five groups of compounds. These are glucocorticoids, mineralocorticoids, androgens, estrogens, and progestagens.[24] Given the ubiquitous distribution and countless bioactivities, the steroid scaffold is unique among natural products! At the beginning of the 20th century there was enormous interest in establishing the structure of steroids. Wieland and Windaus elucidated several of the key structural motifs, but unfortunately proposed the incorrect structure **17**. Among other things, this wrong structure was part of the reason for their separate receipt of the Nobel Prize in Chemistry in 1927 and 1928, respectively. The mistake was brought to light in 1932 when Bernal elucidated the first X-ray crystal structure of the steroid ergosterol.[25]



cholesterol (16) **17**

Figure 3: Correct cholesterol structure (16) and the cholesterol structure as proposed by Wieland and Windaus (17)

Steroidal hormones or their derivatives are widely used for therapeutic purposes (Figure 4). Most notable is the group of synthetic glucocorticoids, which are structural analogues of cortisone and bind as agonists to the glucocorticoid receptor. The activated GR complex up-regulates the expression of anti-inflammatory proteins which turn immune activity and inflammation down. Among the indications are allergies, asthma, autoimmune diseases, and sepsis. An important example of this compound class is dexamethasone (**18**). Another widely known group of steroidal drugs are oral contraceptives. They were first approved in the USA in 1960 for birth control and contain a combination of an estrogen and a progestogen. Further selected examples of synthetic steroid drugs are listed below.[26]

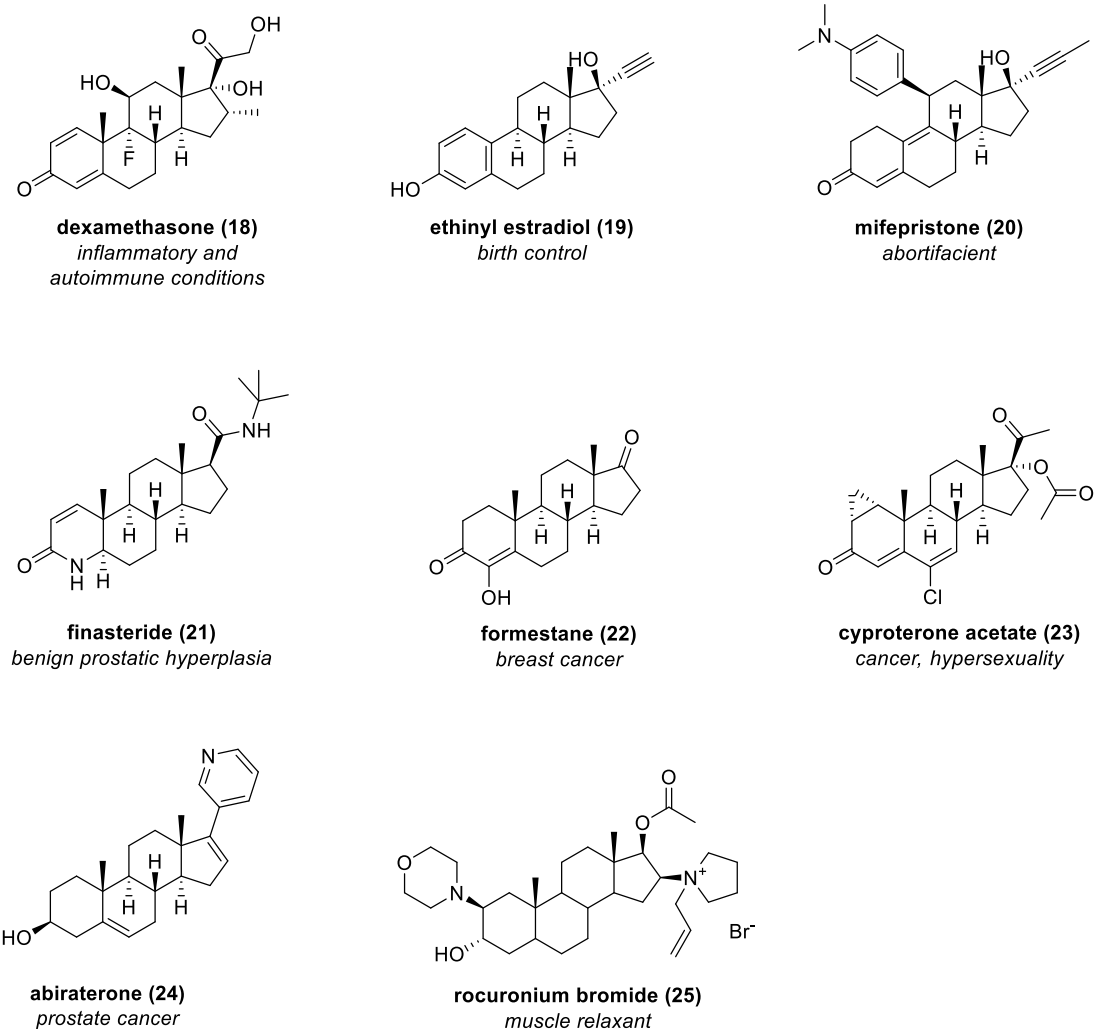


Figure 4: Steroidal drugs and their fields of application.

1.2 BIOS and the Synthesis of Natural Product inspired Libraries

The synthesis and biological evaluation of compound libraries is the main source of new medicines. Between 1999 and 2008, 45 of the 50 FDA approvals for first-in-class small molecules originated from a screen.[27] A new rational framework for the synthesis and biological evaluation of compound libraries based on natural products was introduced in the concept of "biology-oriented synthesis" (BIOS). BIOS is one of many guiding principles in the search for bioactive compounds in chemical biology research and drug discovery. The ultimate goal of chemical biology is on the one hand to completely chart and map the biologically relevant chemical space of drug-like small molecules, and on the other hand to be able to selectively manipulate all proteins (as well as other biological targets) encoded by the human genome.[28] However, this wish is hampered by the apparently endless number of chemical structures as well as proteins.

In reality, the sequence and shape variation among proteins is far lower than suggested by the seemingly endless pool of amino acid sequences, given by 20^n (n = number of amino acids in the protein). In fact, only a tiny portion of possible amino acid sequences can fold to a thermodynamically stable globular protein, and moreover, many proteins of unrelated amino acid sequence can have similar folds. Hence, the structure of the protein fold is conserved in nature on a far higher level than the amino acid sequence. The estimated total number of fold types in nature is in the range of a few 1000s and even lower if restricted to the structures of major protein families.[29]

Contrary to this, chemical space is enormous, even if limited to biologically relevant drug-like small molecules. It is estimated that there are potentially 10^{60} organic compounds with a molecular weight below 500 Da.[30] Obviously, given the natural limitations in time and matter it is impossible to synthesize all of them. Hence, at least in terms of numbers of compounds, even large compound libraries with millions of compounds used in drug discovery cover only a minute fraction of the complete chemical space. It is therefore clear that a rationale for the navigating and populating of biologically relevant chemical space must be found.

A possible solution to this problem is the use of natural products as starting points in the search for biologically active compounds. Firstly, in the course of their biosynthesis, the intermediates proceed through sequential binding to different enzymes. Secondly, many natural products display a variety of biological activities, either within one organism or across species. Taken together, natural products have evolved to interact with multiple proteins and therefore represent "privileged" chemical

structures. Besides the mere abundance of biological activity within natural products, the selectivity and specificity also plays a role. Due to the fact that natural products must also have evolved not to be toxic to their own producer, they are less likely to damage biological structures common to all organisms, such as membranes or DNA.[31] Last, chiral natural products are usually biosynthesized in enantiopure form. This is important, as the two enantiomers of a bioactive compound can have profoundly different effects. While one is active in the desired way, the other can be inactive or even possess undesired side effects.[32] However, in some cases both enantiomers of a natural product can be available.[33]

Several chemoinformatic analyses on the properties of drugs, natural products and compounds from combinatorial chemistry have been published in the past two decades.[34] It was found that natural products differ significantly from synthetic compounds, which are synthesized primarily on the basis of chemical accessibility. Natural products contain more stereogenic centers and fused rings, but fewer aromatic rings and rotatable bonds. Therefore, natural products represent more rigid, nonflat three-dimensional structures compared to synthetic compounds.[35] Indeed, it has been demonstrated that the fraction of sp^3-carbons increases in the transition of compounds from discovery, through clinical testing, to drugs.[36]

As well as proteins, natural products do not have random structures, but possess highly conserved scaffolds (Figure 5). Typically, natural products are organized in classes of compounds with one common scaffold and varying substituents around it (*vide infra*). The limited numbers of protein fold types with their conserved shapes of ligand binding sites parallels the limited number of natural product scaffolds classes with their conserved substituent orientation. The hypothesis of BIOS is that individual proteins of one conserved fold type can be addressed by one class of compounds sharing the same scaffold and substituted in different ways. In this approach, presented by Koch *et al*., a protein structure similarity cluster (PSSC) is identified, which contains proteins with similarly folded ligand binding cores, irrespective of sequence differences.[37] The scaffolds of ligands that bind to one member of this cluster can be used as starting points for the development of novel ligands for other members of the same cluster. To this end, Koch *et al*. used the scaffold of a natural product.

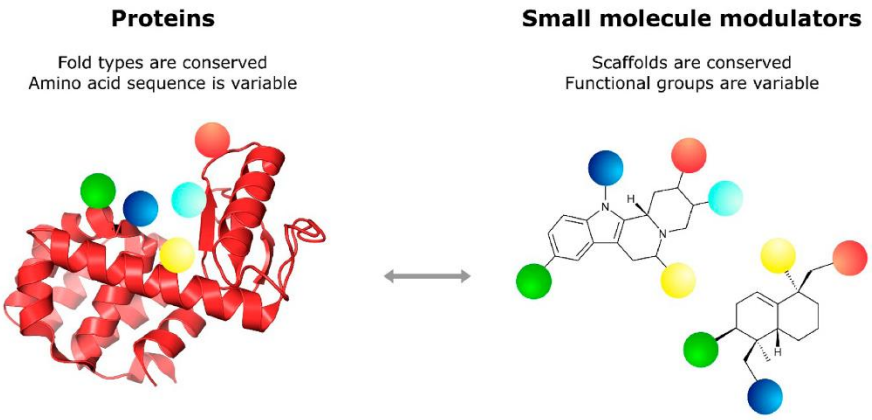


Figure 5: Individual proteins of one fold type can be addressed by one class of compounds sharing the same scaffold and substituted in different ways. Reprinted with permission from [29]. (Copyright (2014) American Chemical Society)

In addition to the clustering of proteins structures, natural product scaffolds can also be clustered in a systematic way. With the assistance of chemoinformatics, natural product scaffolds can be classified and arranged hierarchically, guided by a set of rules. Koch *et al*. have proposed the first Structural Classification of Natural Products (SCONP) (Figure 6) that was introduced by analysis of the *Dictionary of Natural Products* (DNP), the most comprehensive database resource of natural product structures.[38] For each scaffold, a branch is generated by iterative deconstruction of one ring at a time.

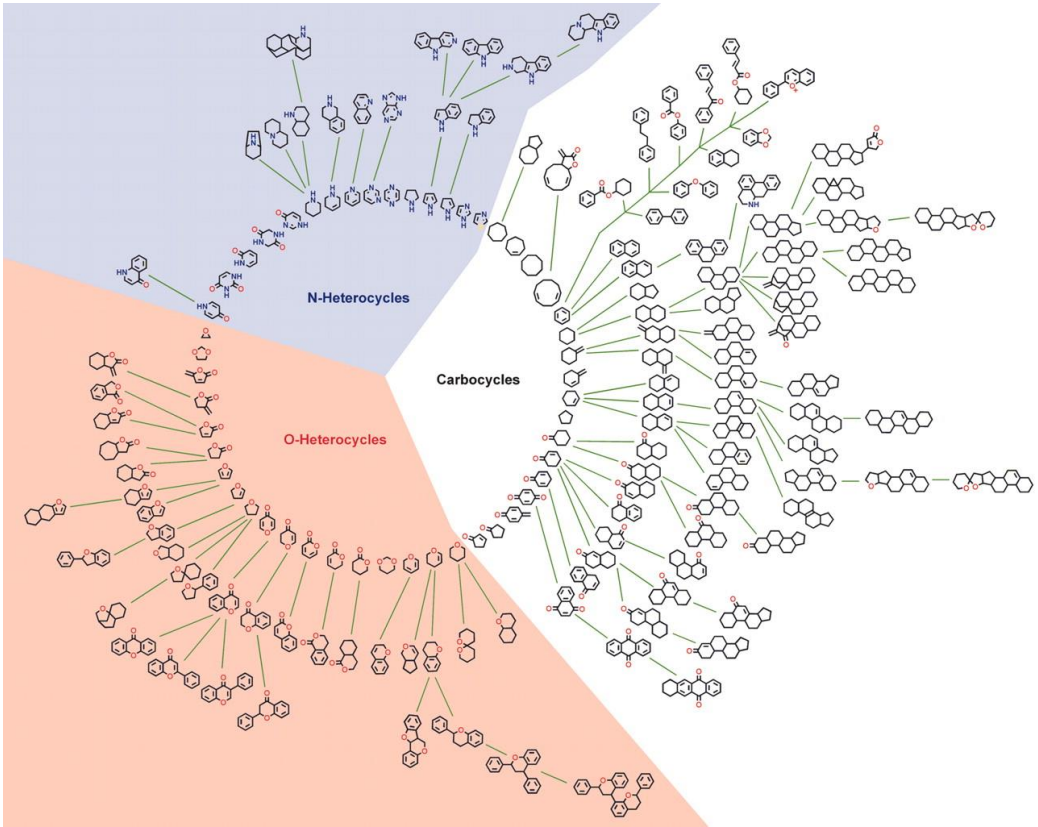


Figure 6: Graphical representation of the NP scaffold tree. For clarity, only scaffolds are shown that represent cumulatively at least 0.2% of the NP population in the DNP. Reprinted with permission from [38].

BIOS defines a viable alternative to the concept of combinatorial chemistry. Combinatorial chemistry includes synthetic methods that enable the preparation of a large number (tens of thousands or even millions) of compounds in a single process. In spite of the large number of produced compounds, the success rate of the approach proved to be poor. This has been attributed to the molecular properties typical of molecules generated by combinatorial chemistry.[34a, 36] The synthetic methods available in combinatorial chemistry allowed only for the synthesis of achiral, aromatic compounds. This goes hand in hand with a lack of chirality as well as structure rigidity and is the exact opposite of natural products. These properties of compound collections which are subjected to high-throughput screening are at least part of the reason for the high attrition rate in drug discovery.

The typical approach in BIOS is the identification of a promising class of natural products or scaffold, respectively. A synthetic strategy is devised that should feature a compromise between a maximum of substitution variability and a minimum of steps.[39] In this regard, it has to be differentiated between natural product-derived and -inspired collections. The synthesis of natural product derived compound collections usually starts from the natural product itself. Derivatization occurs in only a few steps, determined by the reactivity of the natural product. Hence, the synthesis of stereoisomers is generally not possible. On the other hand, collections inspired by natural products are synthesized from smaller building blocks, in a way that the different substituents are introduced in the course of the synthesis. Substantial variations in substitution pattern and stereochemistry compared to the parent natural product are possible.

The goal of the synthesis of compound collections is to delineate a structure-activity relationship (SAR) for a bioactive compound. The detailed knowledge of the SAR allows the reduction of structural complexity towards a compound which is amenable to chemical synthesis and further optimization. In the past, reduction of complexity and even truncation of natural products has in many cases shown to be very effective.[21]

There are recent examples of natural product inspired compound libraries endowed with biologically active compounds. Dakas *et al*. have developed an enantioselective, catalytic synthesis strategy towards a class of neuritogenic iridoids.[40] The iridoid family of natural products has been known before to possess pronounced neuritogenic properties. In another approach, the synthesis of a iridoid-inspired compound collection led to the discovery of inhibitors of the Wnt and Hedgehog signaling pathways.[41] Voigt *et al*. synthesized a natural product Inspired tetrahydropyran collection and subjected the products to a phenotypic screen. This led to the identification mitosis inhibitors and the unravelling of their mechanism of action.[42]

1.3 Withanolides

1.3.1 Withanolide Structures and Bioactivities

The withanolides comprise a family of natural products embodying a steroid core, which share a γ- or δ-lactone/lactol as the common structural motif. The polyoxygenated character of withanolides is the basis for a variety of modifications of the steroid core, resulting in complex structural features. Most withanolides can be roughly grouped in two types of structures, which are designated A (Figure 7) and B (Figure 8). Type A withanolides bear a δ-lactone or δ-lactol, whereas type B withanolides are those with a γ-lactone or γ-lactol. In type A withanolides, the oxygenation is concentrated around the A- and B-rings, while the *trans*-hydrindane dehydro-δ-lactone part is largely conserved. Furthermore, there are natural products which are biosynthetically related to withanolides, but possess a modified steroid skeleton. Physalins, neophysalins, withajardins and others belong to that group. Like many natural products, withanolides also occur as glycosides, linked to one or more sugar residues at the A-ring. Withanolides can be mainly found in 19 genera of *Solanaceae,* which live in the temperate and tropical zones around the world. The most prominent source of withanolides is *Withania somnifera*, known commonly as ashwagandha or Indian ginseng. Herbal medicines containing withanolides as active ingredients have a wide range of ethnopharmacological applications. Specifically, *Withania somnifera* is well known for its use in Ayurvedic medicine[43] and its extract is commercially available.

Withaferin A (**26**) was the first withanolide-type natural product isolated from *Withania somnifera* in 1965.[44] The withanolides were classified for the first time in 1981 by Kirson & Glotter.[45] Since then, withanolides have been reviewed for several times.[46] The latest comprehensive review on the classification and bioactivities of withanolides appeared in 2011.[47]

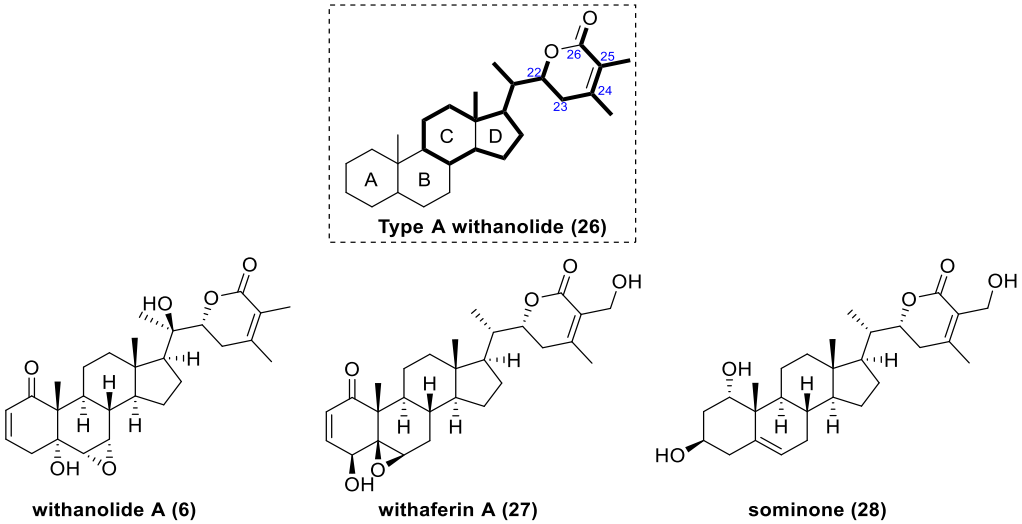


Figure 7: Type A withanolides. The common *trans*-hydrindane dehydro-δ-lactone scaffold is highlighted in bold.

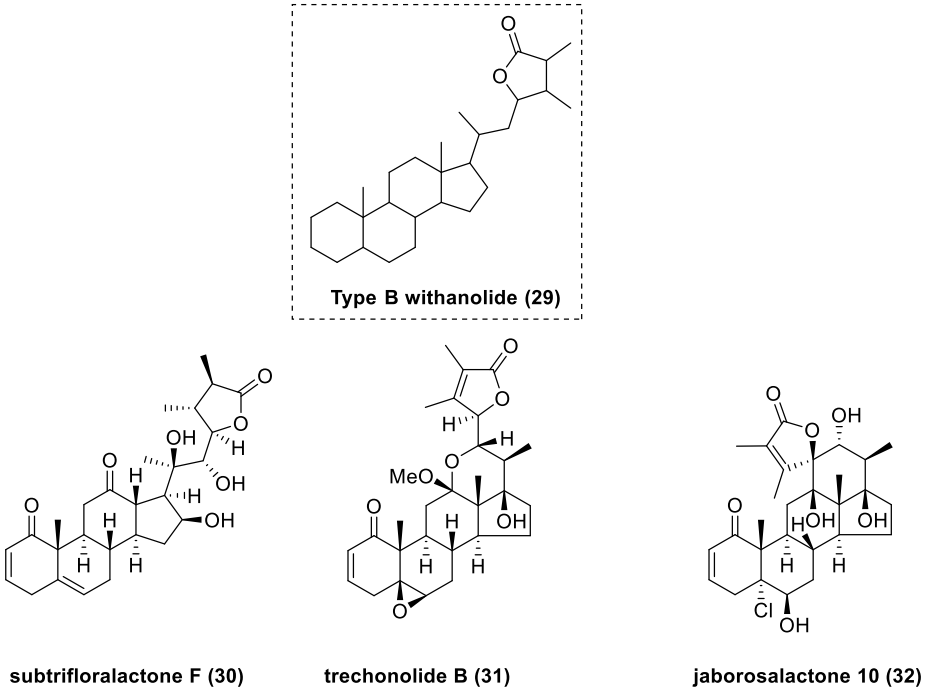


Figure 8: Type B withanolides.

Natural withanolides and withanolide analogues are reported to possess diverse bioactivities.[47] Among them are potent anti-inflammatory effects[48], as well as the modulation of the mTOR[49], the Wnt pathway[50] and the Hedgehog pathway[51]. A large portion of the literature about withanolides is concerned with their neuritogenic and neuroprotective activity. For example, withanolide A and derivatives were recently shown to promote neurite outgrowth[8b, 52] and the synthetic, simplified withanolide analogue denosomin exhibits neuroprotective activity, exceeding the original natural compound sominone.[53] Although no FDA-approved withanolide drug is on the market, Ashwagandha

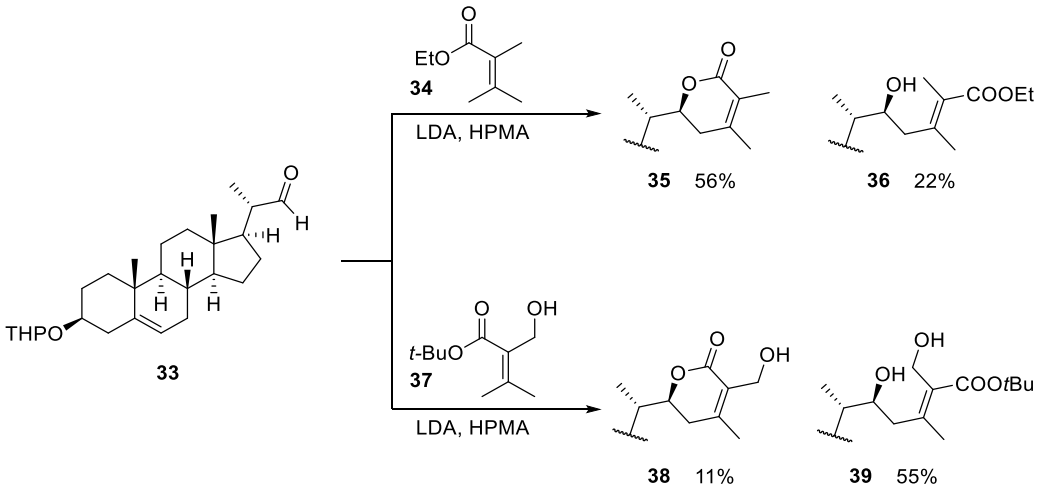
(*Withania somnifera*) extract containing withanolides is commercially available without prescription. It is advertised for a number of beneficial effects, including anti-aging, stress relief or blood sugar stabilization.

1.3.2 Synthetic Studies towards Withanolides

Numerous synthetic efforts have been reported in the literature towards generating withanolides.[46c] Some chosen examples are presented in the following section.

Synthetic works on the withanolides have always used naturally available steroids as starting materials. The challenge remained to elaborate the right oxygenation pattern in the steroid core and to build up the lactone side chain in a stereoselective manner. The only stereocenter in the lactone side chain is C-22 and has always the *R*-configuration in natural withanolides (Figure 7, page 13).

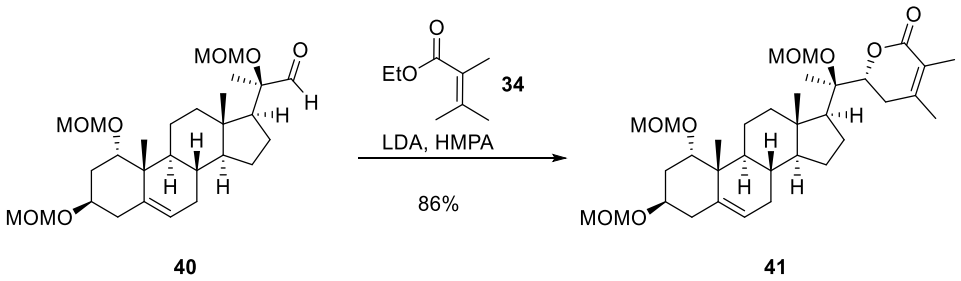
Ishiguro *et al*. were the first to accomplish the partial synthesis of a withanolide model compound, which had the A- and B-ring of withaferin A, but the side chain of cholesterol.[54] Only one year later the same group has accomplished the elaboration of the typical α,β-unsaturated δ-lactone on a steroidal aldehyde via an aldol reaction with an α,β-unsaturated ester. However, the proper stereochemistry at C-22 was not achieved (Scheme 1).[55]



Scheme 1: Lactone construction via γ-coupling of lithium dienolates with the steroidal 22-aldehydes. [55]

Later on this strategy was systematically investigated and it was discovered that a MOM-protected hydroxyl group at C-20 is key to the desired 22(*R*)-configuration.[56] The first application of this methodology in the synthesis of withanolides was achieved by Gamoh *et al.* in 1984.[57] In their stereocontrolled synthesis of withanolide D the side chain moiety was installed in one step with full

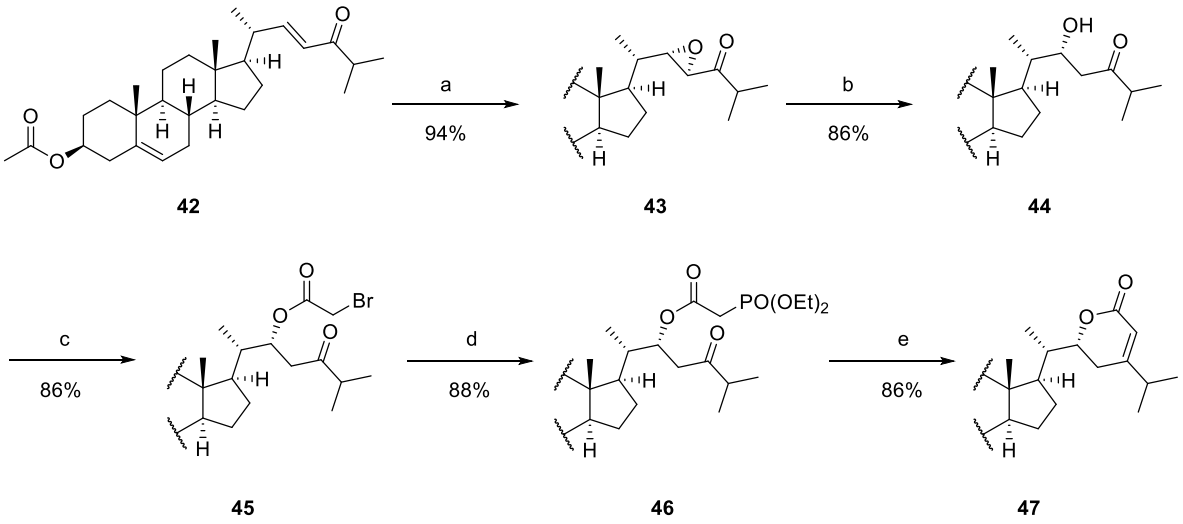
stereocontrol at C-22 (Scheme 2). Although this methodology is remarkable, it has been shown effective only for this particular dimethyl substitution pattern of the lactone.



40 **41**

Scheme 2: Side chain construction according to Gamoh *et al*. [57]

A general strategy for the establishment of the C-22(*R*)-configuration was developed by Weihe *et al*. in 1978 (Scheme 3).[58] It relies on the diastereoselective epoxidation of α,β-unsaturated ketone **42**. Treatment of **42** with 30% hydrogen peroxide and dilute sodium hydroxide furnishes after reacetylation the product **43** in 94% yield. The NMR spectrum indicated that it was a mixture of **43** and the isomeric epoxide in a ratio of approximately 95:5. The epoxy ketone **43** was transformed to the unsaturated lactone **47** in four further steps.

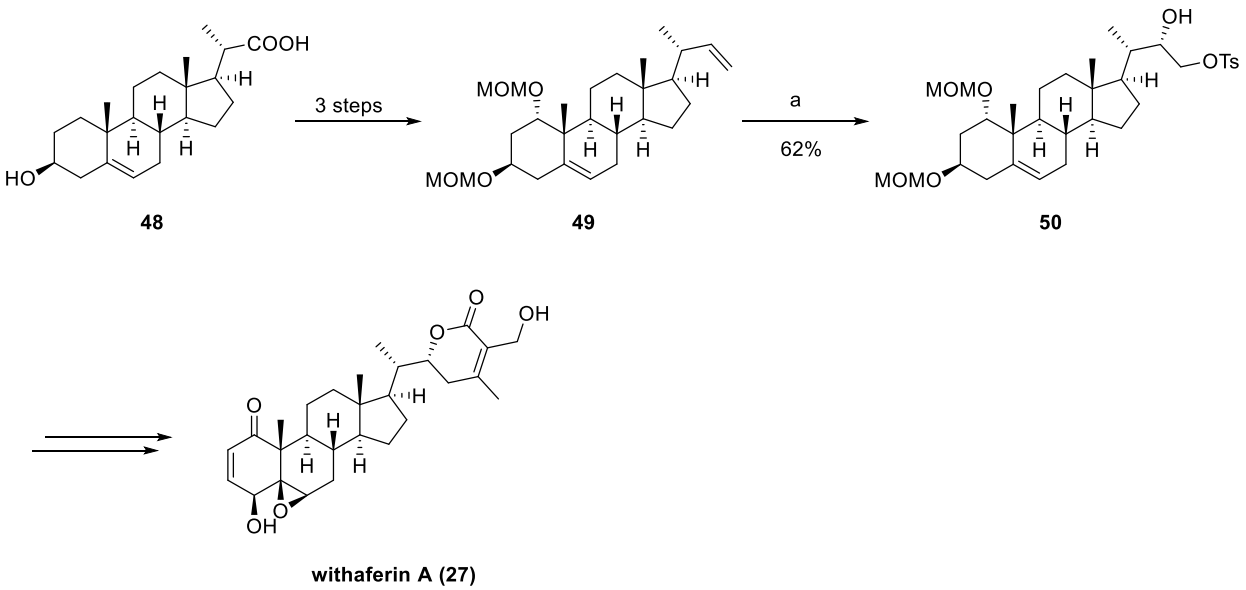


42 **43** **44**

45 **46** **47**

Scheme 3: Withanolide synthesis according to Weihe *et al*.[58] (a) H_2O_2, NaOH, ethanol; **(b)** Al(Hg), ether/ethanol; **(c)** bromoacetyl bromide, pyridine, ether; **(d)** triethyl phosphite; **(e)** NaH, THF, reflux.

One more strategy for the establishment of the right C-22-configuration was presented by Hirayama *et al*. in 1982 (Scheme 4). Starting from commercially available steroidal acid **48** several withanolides were prepared, including withaferin A (**27**).[59] The key stereodefining step is the diastereoselective dihydroxylation of **49** with osmium tetraoxide, followed by tosylation. This transformation furnishes a 5:1 mixture of epimers, with the major diastereomer **50**.[60]

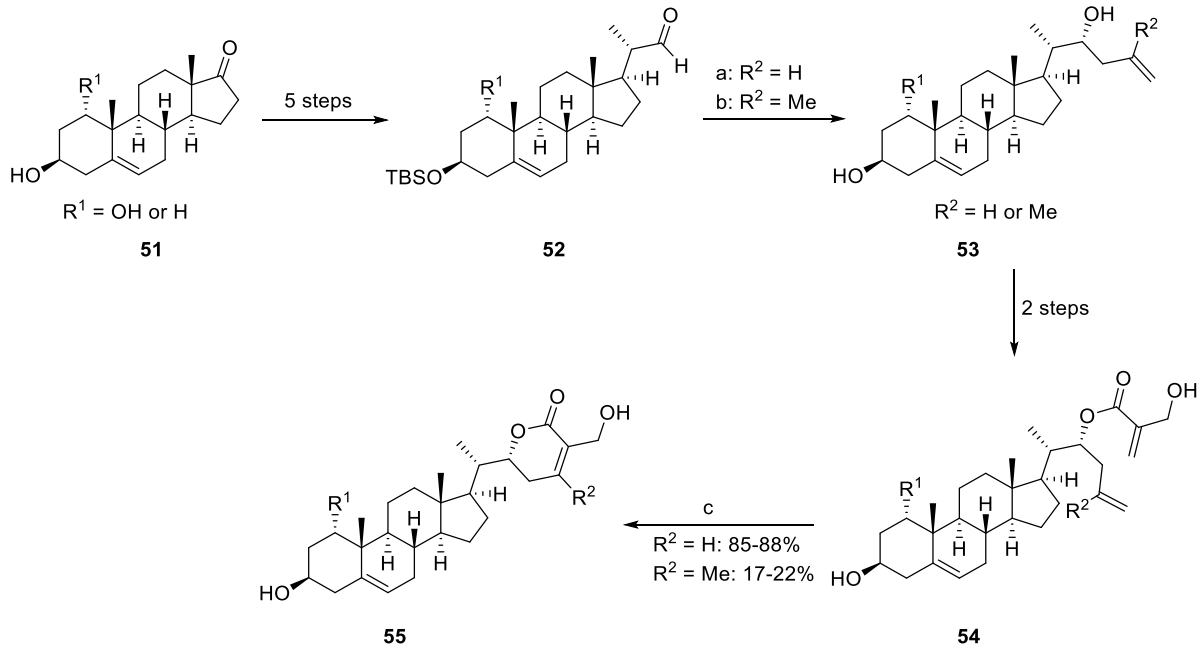


48 49 50

withaferin A (27)

Scheme 4: Withanolide synthesis according to Hirayama *et al*.[59a] (a) OsO_4, *N*-methylmopholine oxide, *t*-BuOH/THF/H_2O/*p*-TsCl·Py.

The most recent synthetic strategy towards withanolides uses a ring-closing metathesis (RCM) for the buildup of the unsaturated lactone (Scheme 5).[53] Matsuya *et al*. synthesized 8 withanolide derivatives and evaluated their neuritogenic and neuroprotective properties. The disadvantage of this strategy is the low-yielding RCM in the last step for R^2 = Me. The formation of a tetrasubstituted olefin is a big challenge and diminishes the yield as illustrated by the fact that R^2 is limited to H or Me.



R1 = OH or H

51 52 53

R2 = H or Me

54 55

Scheme 5: Withanolide synthesis according to Matsuya *et al*.[53] (a) (+)-Ipc$_2$B(allyl)borane; (b) MeC(=CH$_2$)CH$_2$Br, Mg; (c) 10 mol% Stewart-Grubbs catalyst, toluene, 80 °C.

The research on withanolides in the context of their neuritogenic and neuroprotective activity was continued by Gademann *et al*. In 2011 they published the total synthesis of the neuritogenic compound withanolide A[8b], followed in 2013 by semisynthetic derivatives.[52] Gademann *et al*. relied on the method shown in Scheme 2.

II Aim of the Project

The various bioactivities of withanolides, in particular their modulation of signaling pathways, inspired us to design a compound collection based on the withanolide class of natural products. It was anticipated that library synthesis based on these natural products would cover a promising section of the biologically relevant chemical space and may yield interesting and novel small molecule modulators of signaling pathways. The compound collection should be subjected to a series of cell-based screens for various biological activities. Subsequent synthesis of analogues and derivatives based on a possible hit structure should enable us to delineate a structure–activity relationship and identify a lead structure for further research into medicinal chemistry and chemical biology. If possible, the mechanism of action of hit compounds should be investigated through cell-based and biochemical assays.

As mentioned above, the structures of many bioactive type A withanolides significantly vary in the oxygenation pattern of the A- and B-rings, while the *trans*-hydrindane dehydro-δ-lactone part is conserved. In order to cover a greatest possible chemical space, the library should include both systematic variations of the A- and B-rings on the one hand, as well as in the lactone part on the other hand (Figure 9). The idea was to use a commercially available steroid or a segment of the steroid scaffold as starting material.

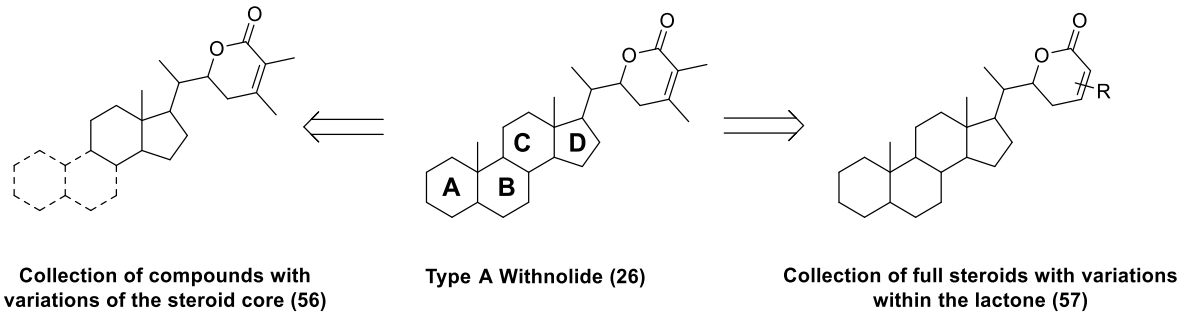


Figure 9: The two possible synthetic precursors to a withanolide inspired compound collection.

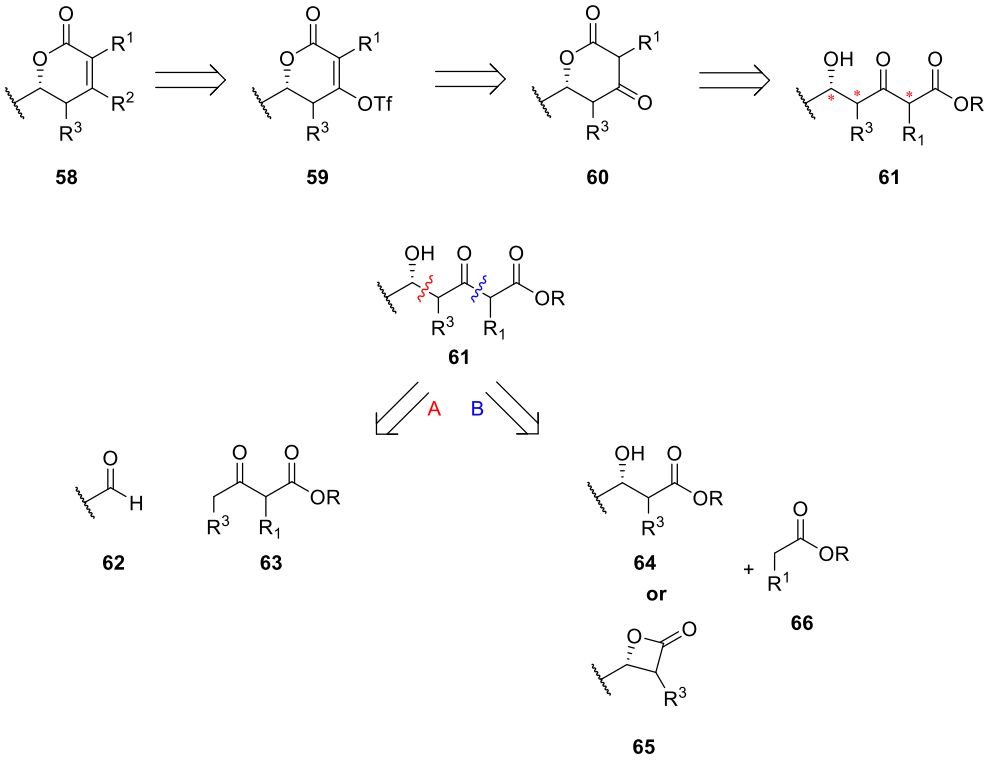
III Results and Discussion

3.1 Part A: Full Steroid Analogues

3.1.1 Synthesis Planning

The synthetic pathway to the compound collection should feature the possibility to introduce a braod array of substituents in the variable positions R^1 and R^2, if possible also in R^3 (Scheme 6). As discussed above, RCM of the double bond in the lactone is not a promising retrosynthetic step. Instead, it was planned to introduce R^2 via a cross coupling reaction of enol triflate **59**. The enol triflate functional group can be accessed from keto ester **60**, which in turn leads to the open-chain compound **61**. **61** possesses three stereogenic centers, only one of which needs to be established with the indicated configuration in order to match with natural withanolides. The stereocenter at R^3 can have either configuration as it is unknown which effect any substituent R^3 would have, the stereocenter at R^1 is inconsequential. There are tho ways for the synthesis of **61**, depicted by routes A and B. Disconnection A corresponds to a vinylogous aldol reaction in the forward direction and reveals an aldehyde **62** and keto ester **63**. Disconnection B corresponds to a Claisen condensation between aldol adduct **64** and substituted ester **66** in the forward direction. An alternative to an aldol adduct is β-lactone **65**, which is in fact a ring-closed aldol adduct. Enantioselective, catalytic methods for vinylogous aldol reactions (Route A) are reported and do usually employ a synthetic equivalent of an acetoacetate ester as nucleophile.[61] However, it was anticipated that this would be a troublesome route, given that substrates **63** would have to be prepared and a narrow substate scope for R^1 and R^3 was expected according to literature reports. The more promising strategy seemed to be disconnection B to a chiral

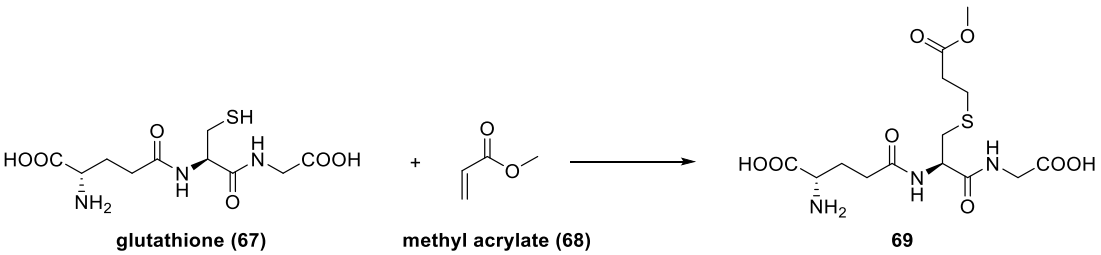
β-lactone and a substituted ester. A methodology for the stereoselective synthesis of β-lactones **65** is known.[62] The desired configuration at C-22 would be established using this method. Herein a new strategy towards the establishment of the right C-22(*R*)-confuguration in the construction of withanolides was proposed. In the synthesis of other natural products with an α,β-unsaturated δ-lactone the asymmetic [2+2]-cyclocondensation was already successfully used.[63]



Scheme 6: Retrosynthesis of the unsaturated lactone

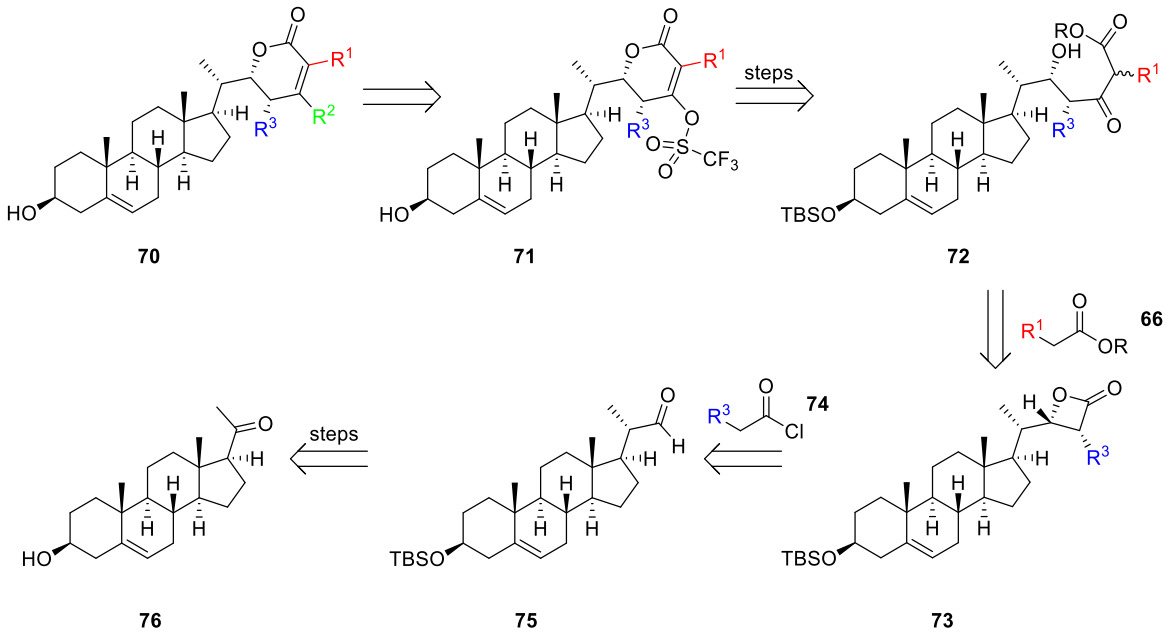
During the synthesis planning attention was focused to the presence of substituents at the unsaturated lactone because any additional substituent decreases the rate of Michael additions.[64] Table 1 shows kinetic data for the reaction of several α,β-unsaturated esters with glutathione (**67**). As electrophilic functional groups, Michael acceptors may form covalent bonds to nucleophilic sites of proteins and the DNA. Therefore, Michael acceptor reactivity of compounds is known to be a major reason for toxicity and other biological activity.[65] Because withanolides are always at least α-substituted and oftentimes also β-substituted at the lactone, their Michael acceptor reactivity should be weak. Accordingly, electrophilicity of the lactone is not invoked as the basis for biological activities of withanolides.

Table 1: α,β-unsaturated carbonyls with information on their Michael acceptor reactivity. Depicted are experimental second-order rate constants of their reaction with GSH. Data copied from [64]



	methyl acrylate	methyl crotonate	methyl methacrylate	methyl tiglate
Experimental log kGSH (L mol-1 min-1)	1.06	-0.79	-1.14	-2.15
Relative experimental kGSH (L mol-1 min-1)	100	1.41	0.63	0.062

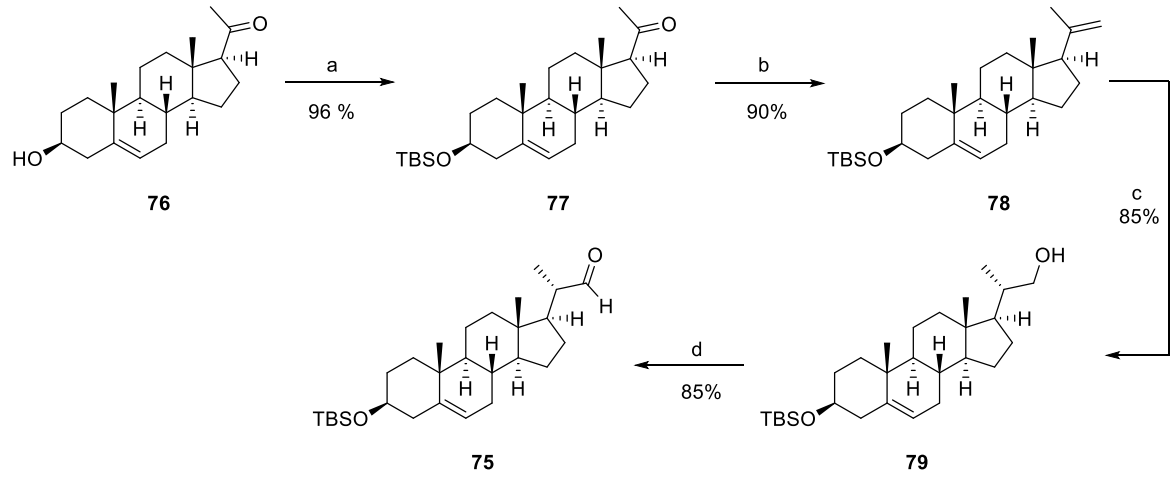
The preparation of the compound collection was planned to start from a commercially available steroidal compound. Although numerous steroids are commercially available, for several reasons the steroid of choice was the endogenous steroid hormone pregnenolone. First of all, it is inexpensive, so that many grams can be used as starting material. Second, after manipulation of the ketone, it has only one secondary hydroxyl group as single functional group. This means a lower molecular weight compared to other, higher oxygenated steroids, while at the same time the steroid core is unreactive and compatible to all kinds of reagents and conditions. Withanolide analogues were planned to be synthesized via the pathway shown in Scheme 7. The pathway from **70** to **73** equals to Scheme 6. The β-lactone was planned to be opened by ester enolates, introducing the second variable group R^1. Δ-lactone closure and triflation would transform **72** to vinyl triflate **71**. The triflate functionality allows for the introduction of the last variable group R^2. The key stereo-defining step is the asymmetric cinchona alkaloid-catalyzed formal cycloaddition between **75** and acid chlorides **74** to β-lactone **73**. This transformation was supposed to create two stereocenters and introduce a variable substituent R^3 in the configuration shown below. Aldehyde **75** can be prepared from commercially available and inexpensive pregnenolone (**76**).



Scheme 7: Outline of the synthetic pathway of part A

3.1.2 Synthesis

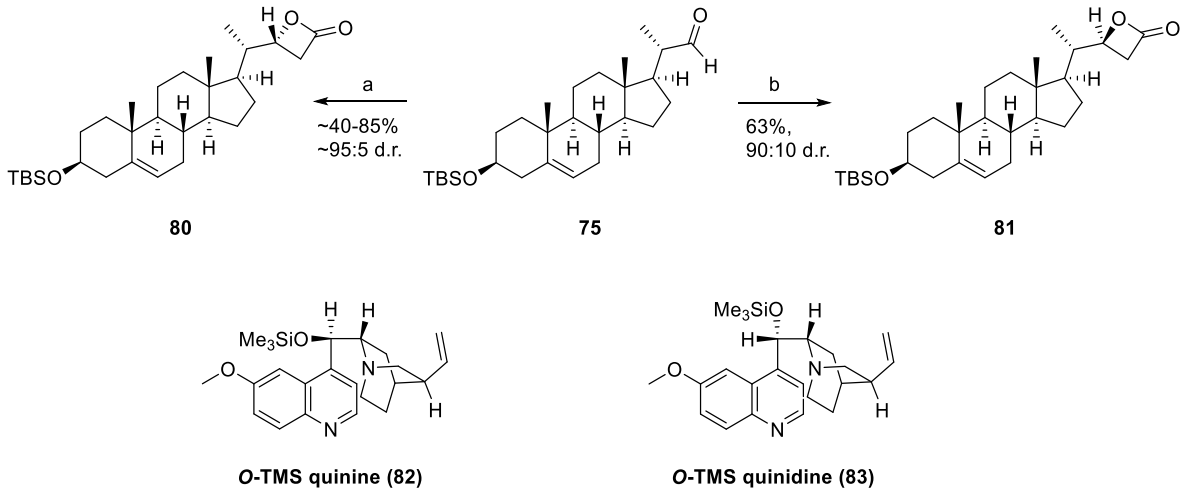
The synthesis of aldehyde **75** is effected in 4 steps as shown in Scheme 8. Pregnenolone (**76**) is TBS-protected and the keto group subjected to a Wittig reaction using methyltriphenylphosphonium bromide. The resulting alkene is hydroborated with 9-BBN, followed by the oxidation of primary alcohol **79** to known aldehyde **75**. These four steps were performed starting from 5 g pregnenolone with slightly different conditions than described in the literature.[66] Hydroboration of olefin **78** yields an epimeric mixture of alcohols with **79** being the major isomer. The ratio of epimers is 14:1 according to literature and was not determined in this work.[67] The major diastereomer was purified by flash column chromatography (FCC).



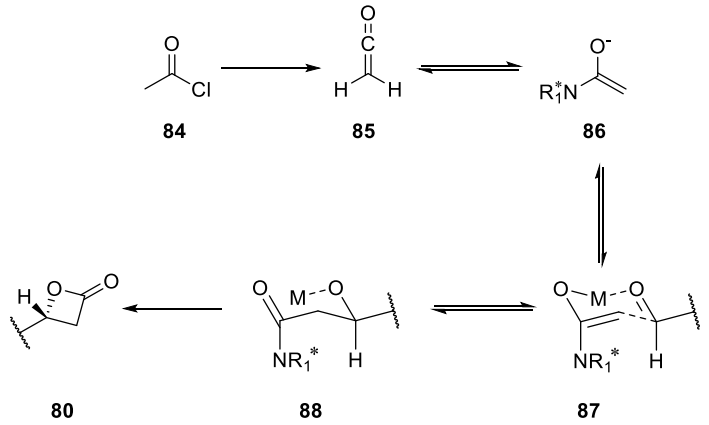
76 **77** **78** **79** **75**

Scheme 8: Synthesis of aldehyde 75. (a) TBS-Cl, *i*-Pr_2EtN, CH_2Cl_2, RT; **(b)** methyltriphenylphosphonium bromide, potassium *tert*-butoxide, toluene, RT; **(c)** 9-BBN, H_2O_2, NaOH, THF; **(d)** DMSO, oxalyl chloride, Et_3N, THF.

The next step is the asymmetric cinchona alkaloid-catalyzed cyclocondensation between **75** and acetyl chloride to β-lactone **80** (Scheme 9).[62] It is important to note that this reaction is a cyclocondensation and not a cycloaddition. While cycloadditions progress in a concerted fashion without ionic intermediates, cyclocondensations are classical reactions between nucleophile and electrophile, involving ionic intermediates, as shown in Scheme 10. Readily available and inexpensive *O*-trimethylsilyl quinine **82** (TMSQ), prepared by silylation of commercially available quinine[68], catalyzes this transformation. TMSQ catalyzes the ketene-aldehyde addition through nucleophilic addition to the ketene, generating the acylammonium enolate. The stereochemical induction is proposed to proceed via a six-membered transition state. Compared to the original publication, 30 mol% instead of 10 mol% catalyst were added due to the hindered aldehyde **75**. The product was isolated as an epimeric mixture in a ratio of ~95:5. The reaction proceeds cleanly but did never run to completion. Typically 40–50% of the product was isolated, together with most of the unreacted starting material. The total recovery of material is >95% and the reisolated starting material can be subjected to the same conditions again. The best yield achieved was 85%. Unfortunately, no conversion was achieved with propionyl chloride instead of acetyl chloride. Most likely steric hindrance in the transition state is the reason for this, probably caused by the stereocenter in α-position to the aldehyde. Therefore, no substituent R^3 (Scheme 6 and Scheme 7) could be introduced. When epimeric catalyst *O*-trimethylsilyl quinidine (**83**) instead of *O*-trimethylsilyl quinine was used, the epimeric β-lactone **81** was isolated as the major product in 63% yield and with a stereochemical ratio of 90:10.

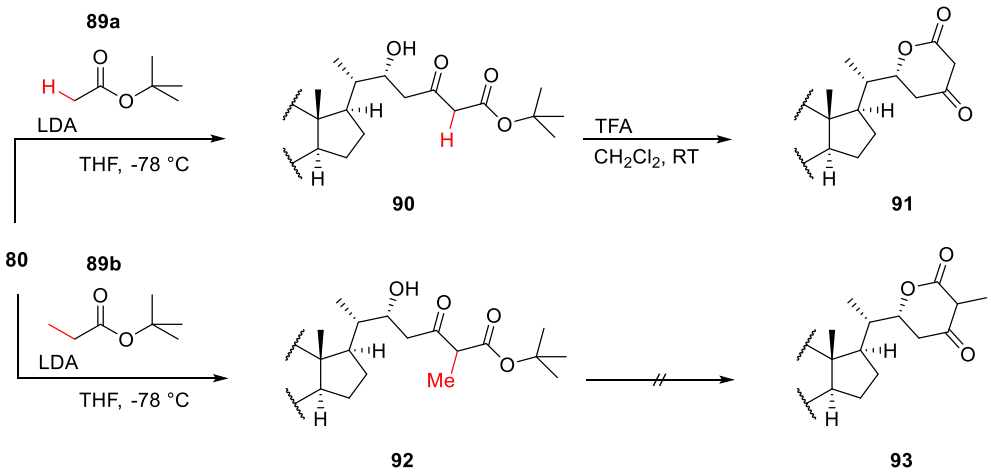


Scheme 9: Both epimers of the β-lactone are accessible. (a) acetyl chloride, *O*-TMS quinine, $LiClO_4$, *i*-Pr_2EtN, CH_2Cl_2/Et_2O, −78 °C; **(b)** acetyl chloride, *O*-TMS quinidine, $LiClO_4$, *i*-Pr_2EtN, CH_2Cl_2/Et_2O, −78 °C.



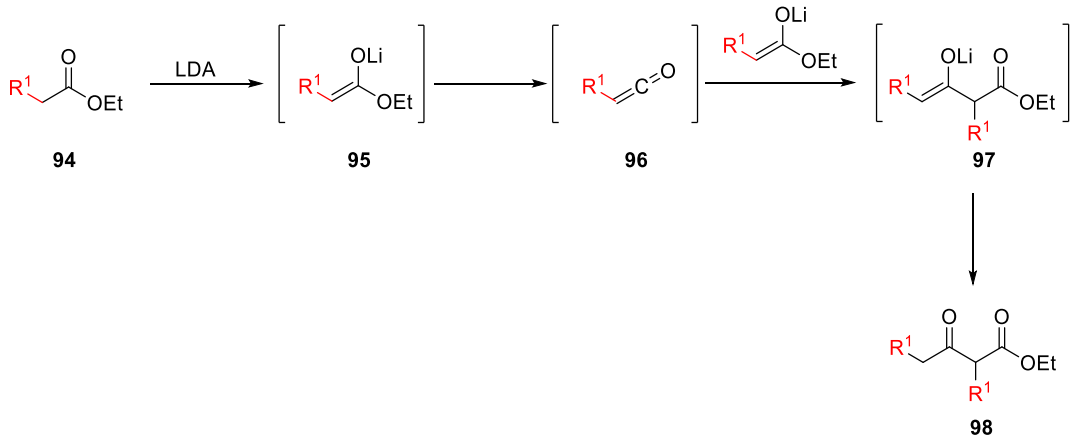
Scheme 10: Model for stereoselectivity in the assymetric cyclocondensation between 75 and acetyl chloride.[62]

Initially, **80** could be opened by the lithium-enolates of *tert*-butyl acetate (**89a**) and *tert*-butyl propionate (**89b**) to furnish δ-hydroxy-β-keto-esters **90** and **92** (Scheme 11). After β-lactone opening with *tert*-butyl acetate, the product **90** could be easily ring-closed with TFA in dichloromethane. However, the product of β-lactone opening with *tert*-Butyl propionate (**92**) could not be effectively ring-closed under acidic (TFA, formic acid, HCl in CH_2Cl_2) and basic (K_2CO_3 in methanol) conditions.



Scheme 11: Initial observations in β-lactone openings and δ-lactone closures.

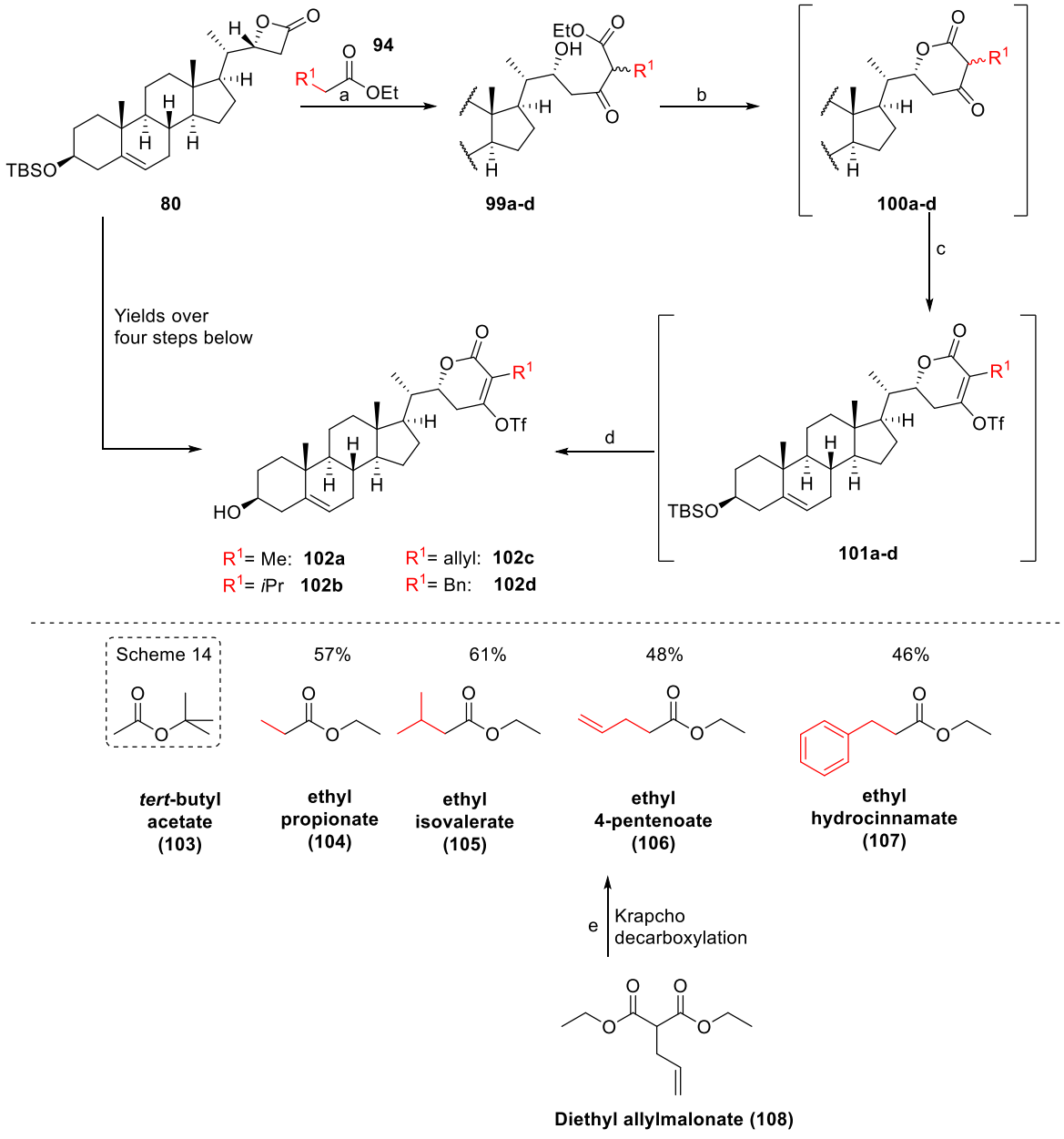
Therefore, ethyl esters instead of *tert*-butyl esters were used in order to introduce a substituent $R^1 \neq H$ (Scheme 13). For $R^1 \neq H$, a new stereocenter is generated in the β-lactone opening, but it is inconsequential. *Tert*-butyl acetate, ethyl propionate, ethyl isovalerate and ethyl hydrocinnamate are commercially available and inexpensive. Ethyl 4-pentenoate is commercially available as well, but very costly. Therefore, it was prepared from diethyl allylmalonate by Krapcho decarboxylation. The short lifetime of lithium enolates generated from ethyl esters upon treatment with LDA, especially from ethyl hydrocinnamate and ethyl isovalerate (next page), was problematic. The lithium enolates decomposed in solution at −78 °C within minutes, probably by ketene formation and following side reactions (Scheme 12). Inclusion of 1 equivalent hexamethylphosphoramide (HMPA) did not solve the problem. Therefore, a large excess of the lithium enolates was used for the β-lactone openings.



Scheme 12: Side reactions of ester enolates.

Compounds **99a-d** readily underwent cyclization under basis conditions (K_2CO_3 in methanol) to keto lactones **100a-d**. Crude products **100a-d** were converted to the vinyl triflates **101a-d** with

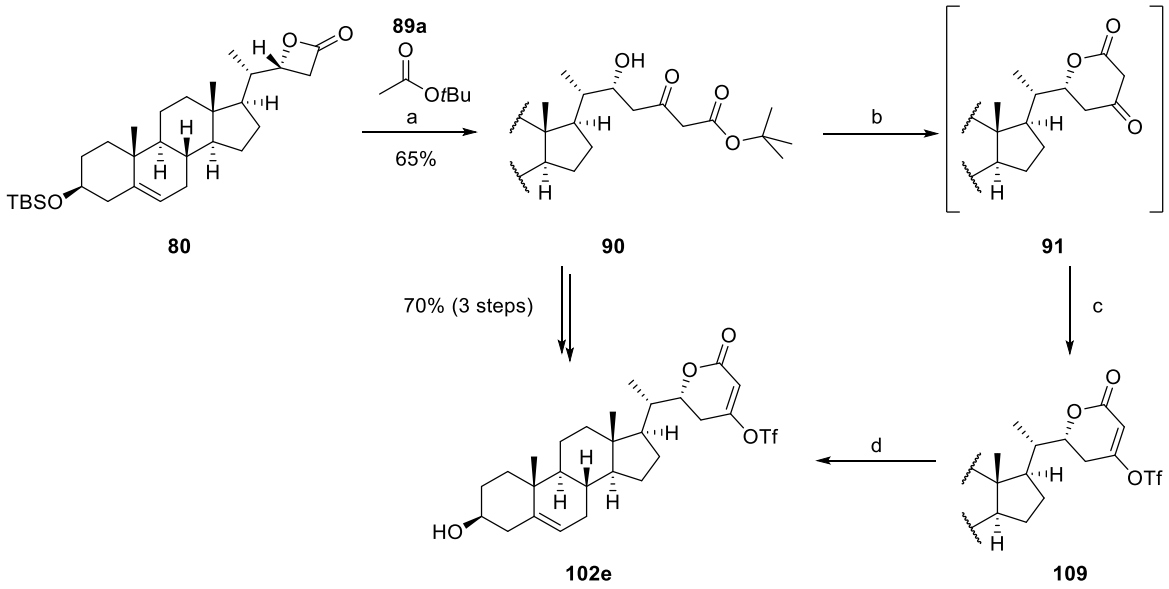
trifluoromethanesulfonic anhydride and triethylamine at −78 °C. The products were again not purified and used as the crude for the next transformation. Finally, the vinyl triflates **101a-d** were TBS-deprotected to furnish a series of compounds amenable for various late-stage functionalizations. Because of the difficulty to purify and characterize a diastereomeric mixture, the yield was calculated over four steps from **80** to **102a-d**. The vinyl triflates **102a-d** were benchstable and when stored at low temperature (4 °C) for many months did not show any sign of decomposition.



Scheme 13: Synthesis of enol triflates 101. (a) LDA, THF, −78 °C; **(b)** K_2CO_3, MeOH, RT; **(c)** trifluoromethanesulfonic anhydride, Et_3N, CH_2Cl_2, −78 °C; **(d)** triethylamine trihydrofluoride, CH_2Cl_2, RT; **(e)** NaCl, DMSO/H_2O.

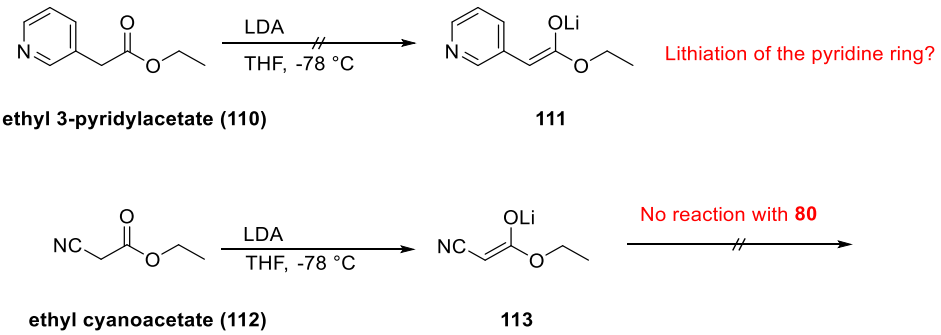
As written above, for R^1=H (*tert*-butyl ester), TFA in dichloromethane was most effective for the ring closure of **90** to **91** (Scheme 14). Column chromatographic purification of **91** did always lead to a

diminished yield of only ~50% and therefore the compound was used as the crude for the next transformation. Probably the slightly acidic conditions on silica gel effect lactone opening to the very polar carboxylic acid. The yield over four steps from **80** to **102e** was 46%.



Scheme 14: Sequence for R^1 = H. (a) LDA, THF, -78 °C; **(b)** TFA, CH_2Cl_2, RT; **(c)** trifluoromethanesulfonic anhydride, Et_3N, CH_2Cl_2, −78 °C; **(d)** triethylamine trihydrofluoride, CH_2Cl_2, RT.

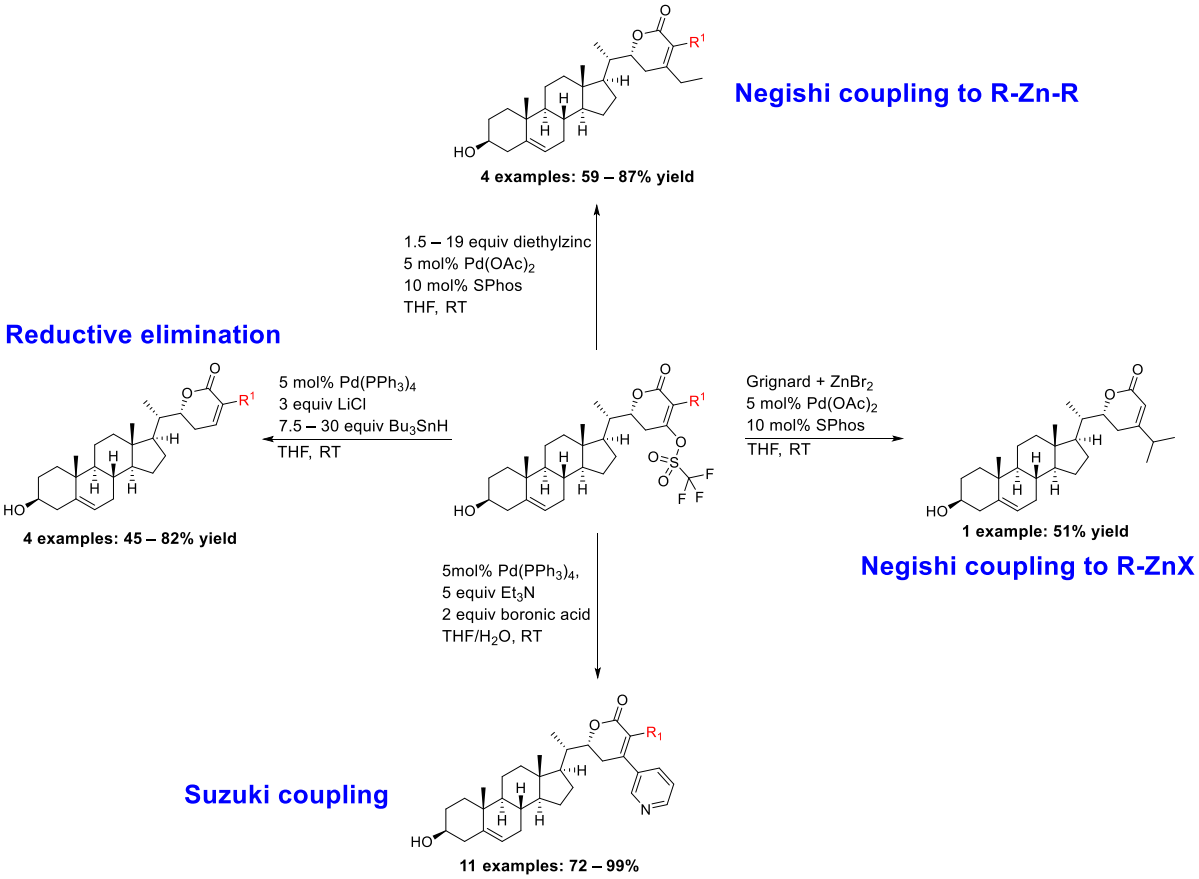
The presented ring-opening of the β-lactone posed limitations regarding the nucleophiles that can be used (Scheme 15). The lithium enolate of ethyl 3-pyridylacetate could not be generated, probably due to lithiation of the pyridine ring. Ethyl cyanoacetate on the other hand could be transformed to the lithium enolate, but did not react with the β-lactone under the conditions used.



Scheme 15: Failed attempts of β-lactone opening.

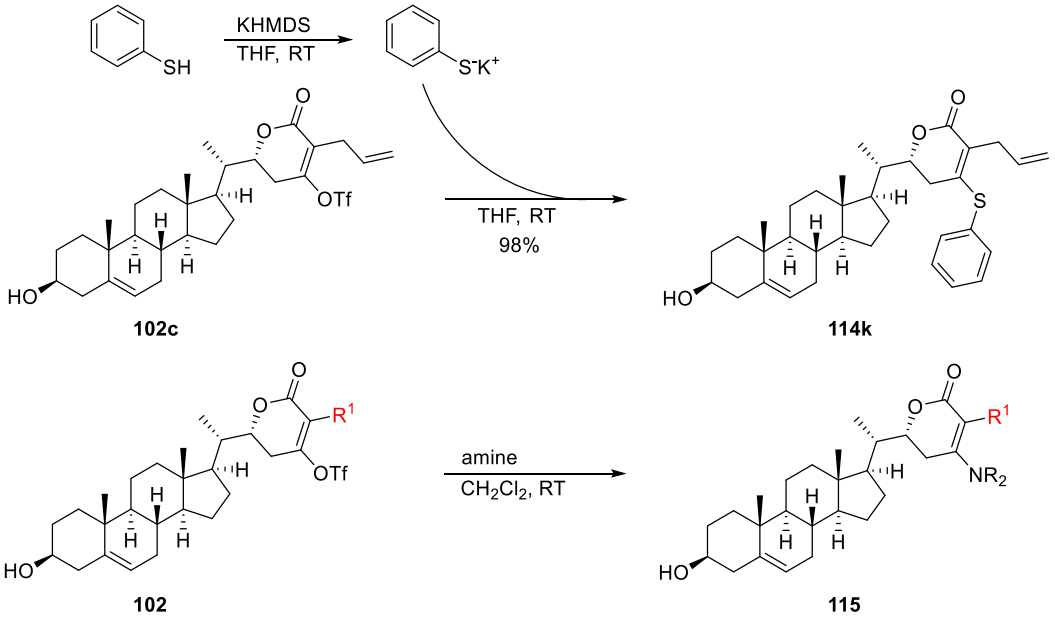
The vinyl triflates **102a-e** served as the starting materials for the following diversifying reactions (Scheme 16). Suzuki-couplings, Negishi-couplings[69] and reductive elimination of the triflate were the performed cross coupling reactions. Suzuki couplings were performed with a variety of aryl and heteroaryl boronic acids under the same conditions. All reactions were reliable and clean, with yields

ranging from 72% to 99%. Negishi couplings were performed with diethyl zinc, isopropyl zinc halide derived from transmetalation of isopropyl Grignard, and an alkyl zinc halide derived from zinc insertion into the alkyl halide. Zinc insertion was performed using a procedure from Knochel *et al*.[70] Reductinve elimination of the enol triflates was achieved with tributyltin hydride and catalytic amounts of tetrakis(triphenylphosphine)palladium(0).[71]



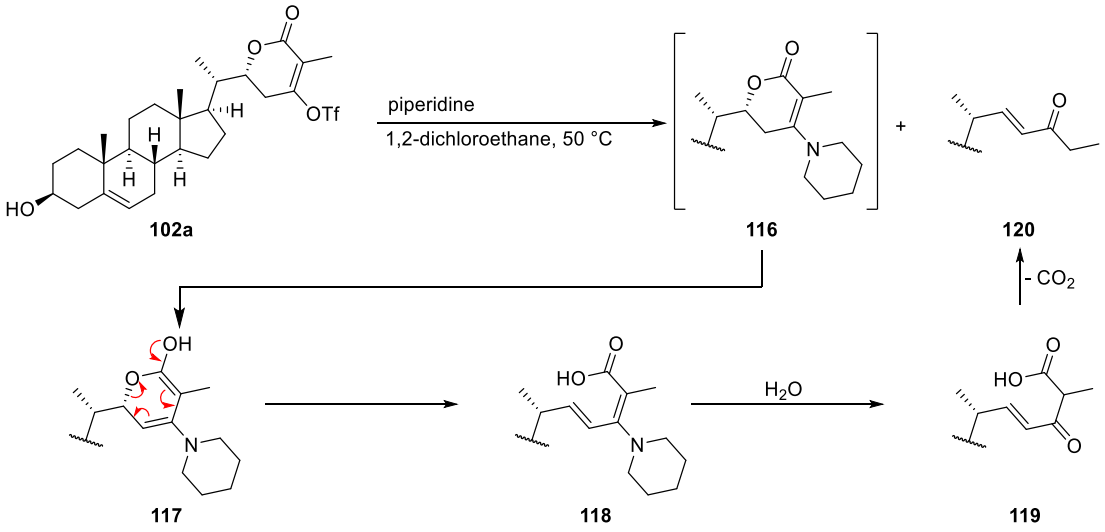
Scheme 16: Cross coupling reactions and reductive elimination. Only the yields for individual reactions are given, yields over two steps are excluded.

Addition-elimination reactions were performed with thiophenol and a series of amines (Scheme 17). Reaction with thiophenol was effected by treating **102c** with potassium thiophenolate, generated by deprotonating thiophenol with KHMDS in a separate flask. The addition-elimination with amines was an exceptionally clean reaction. The reactions were performed in dichloromethane at ambient temperature. Depending on the reactivity of the amine, the number of equivalents and the reaction time were varied. Reaction with secondary amines was significantly faster than with primary amines.



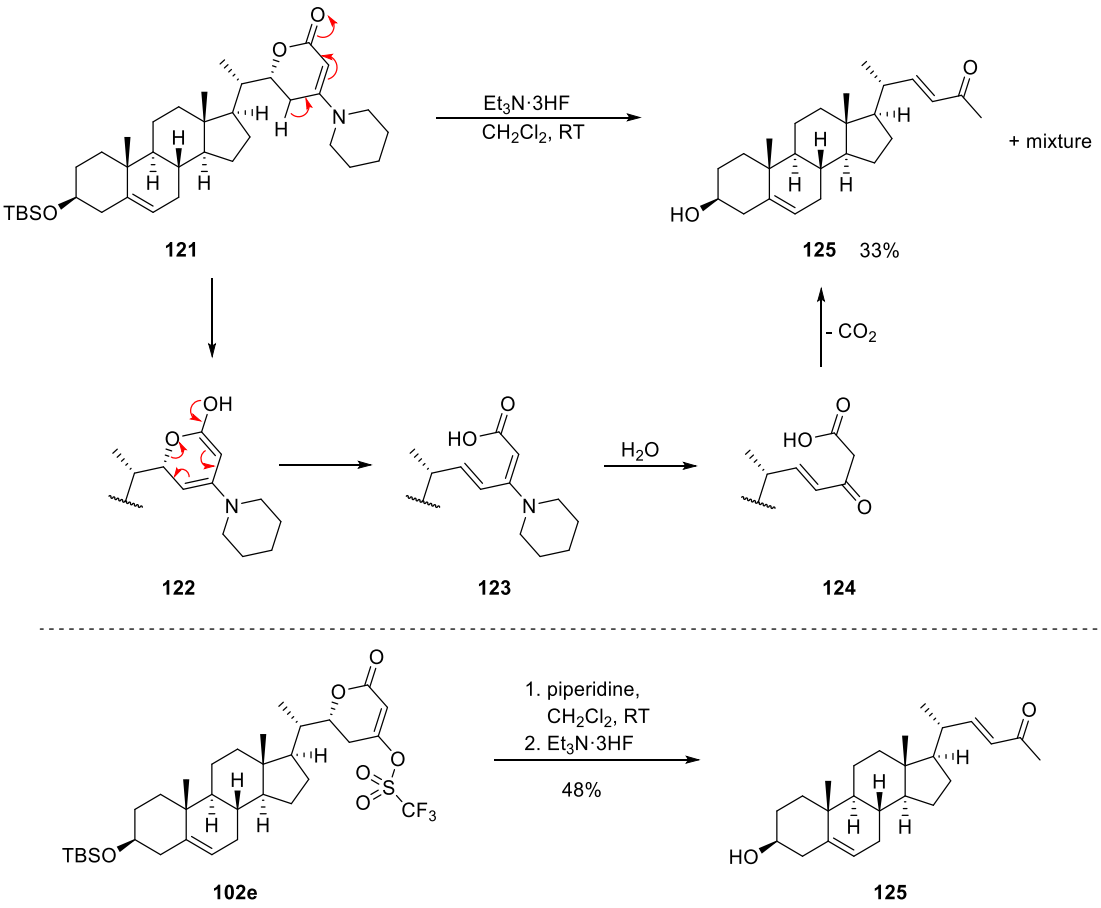
Scheme 17: Addition-elimination with amines and thiophenol.

While the α-unsubstituted enol triflate **102e** neatly underwent addition-elimination with primary and secondary amines, the reaction did not yield the desired product in the presence of an α-substituent R^1 other than H (Scheme 18). Reaction of enol triflate **102a** (R^1 = Me) with piperidine in dichloromethane at ambient temperature was very sluggish. In 1,2-dichloroethane at 50 °C a polar product was forming that could be observed by thin layer chromatography (TLC). However, this product could not be isolated because it decomposed to a less polar compound on silica. From the mixture of both products the desired product **116** was detected by HRMS. The decomposition product was isolated in 72% yield and determined to be α,β-unsaturated ketone **120**. The proposed mechanism is depicted below.[72].



Scheme 18: Failed addition elimination reaction.

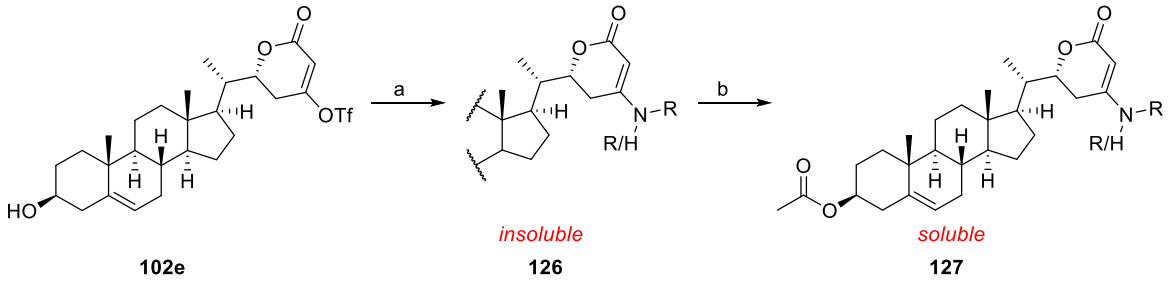
It was observed that the isolated amine adducts were of limited stability in organic solvents, though stable enough for characterization and biological evaluation (Scheme 19). Experiments revealed that the amine adducts are stable in DMSO-d_6 over one week at ambient temperature. In $CHCl_3$ however, the above mentioned fragmentation occured. Instability to slightly acidic conditions is further demonstrated by the fact that attempted TBS-deprotection of **121** with triethylamine trihydrofluoride led to decomposition. In order to be isolated and fully characterized, the fragmentation product **125** was later made on purpose. First, enol triflate **102e** was treated with piperidine to affect the addition-elimination reaction. After complete transformation, triethylamine trihydrofluoride was added and the mixture was stirred for one week at ambient temperature. The decomposition was still not complete, but the fragmentation product was isolated in 48% yield.



Scheme 19: Fragmentation of the lactone.

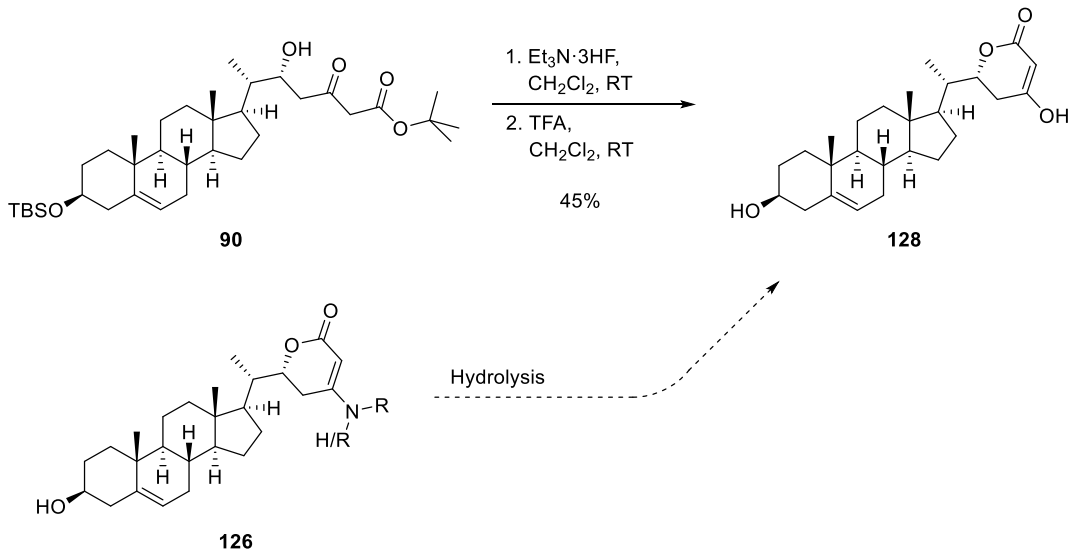
During the synthesis of amine adducts, the poor solubility in organic solvents, especially in DMSO, was noted. For biological assays it was necessary to prepare 10 mM solutions of the compounds in DMSO, which was in some cases far above the solubility limit. Several compounds could be only dissolved in a solvent mixture like CD_2Cl_2/CD_3OD or $CDCl_3/CD_3OD$ for NMR measurements. Interestingly, acetylation

of the secondary alcohol enhanced the solubility (Scheme 20). Acetylation is possible in the presence of the vinylogous urethane.



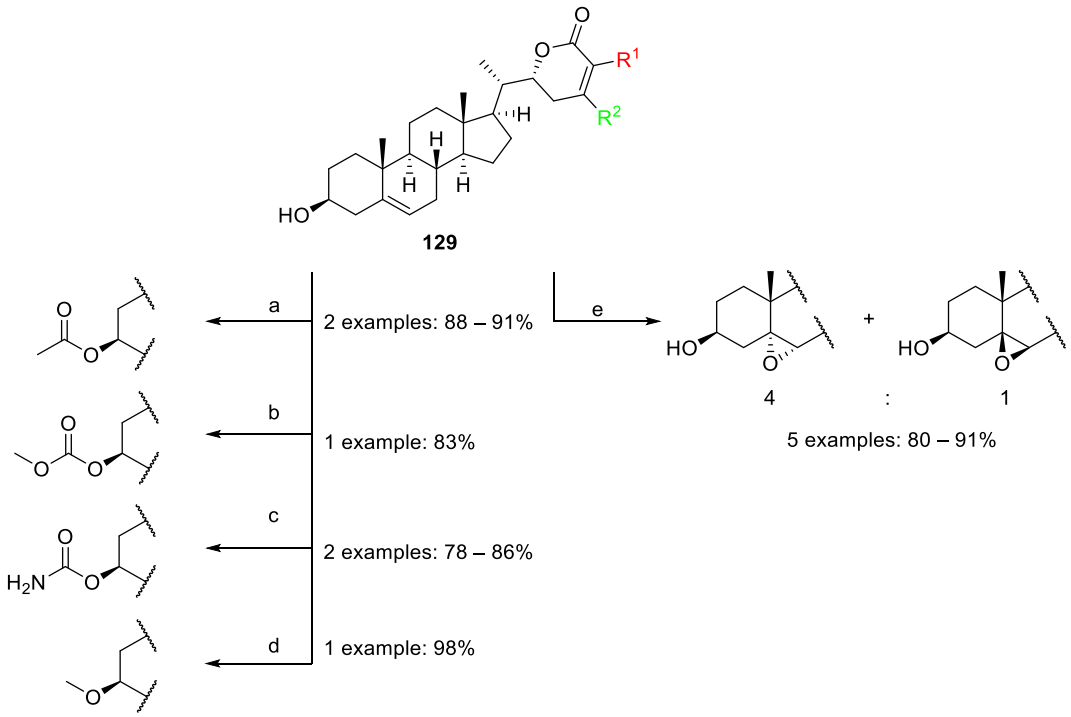
Scheme 20: Synthesis of amines. (a) amine, CH_2Cl_2, RT; **(b)** acetic anhydride, DMAP, CH_2Cl_2, RT.

Besides a possible fragmentation of the lactone, hydrolysis of the vinylogous urethane in aqueous solution is also a possible, yet never observed side reaction (Scheme 21). The hydrolysis product was prepared from **90** in two steps and also submitted for biological assays as a control.



Scheme 21: Synthesis of the putative enamine hydrolysis product 127.

In order to further increase the diversity of the library, one-step modifications of the cross-coupling and addition-elimination products were performed (Scheme 22). The easiest way to achieve this was the installation of appendages on the secondary alcohol in the A-ring. As written above, acetylation had the additional benefit to increase the solubility of the products in organic solvents and DMSO. In addition, carbonate formation, carbamoylation and methylation were also performed. Epoxidation of the C5-C6 olefin with *m*CPBA leads to an inseparable 4:1 mixture of diastereomers, with the α-epoxide being the major isomer.[73]



Scheme 22: Steroid core modifications. Only the yields for individual reactions are given, yields over two steps are excluded. **(a)** acetic anhydride, DMAP, CH_2Cl_2, RT; **(b)** methyl chloroformate, pyridine, CH_2Cl_2, RT; **(c)** chlorosulfonyl isocyanate, CH_2Cl_2; then H_2O/THF; **(d)** methyl iodide, NaH, DMF, RT; **(e)** *m*CPBA, CH_2Cl_2, RT.

The complete library is presented in Figure 10 and Table 2. It contains 50 compounds.

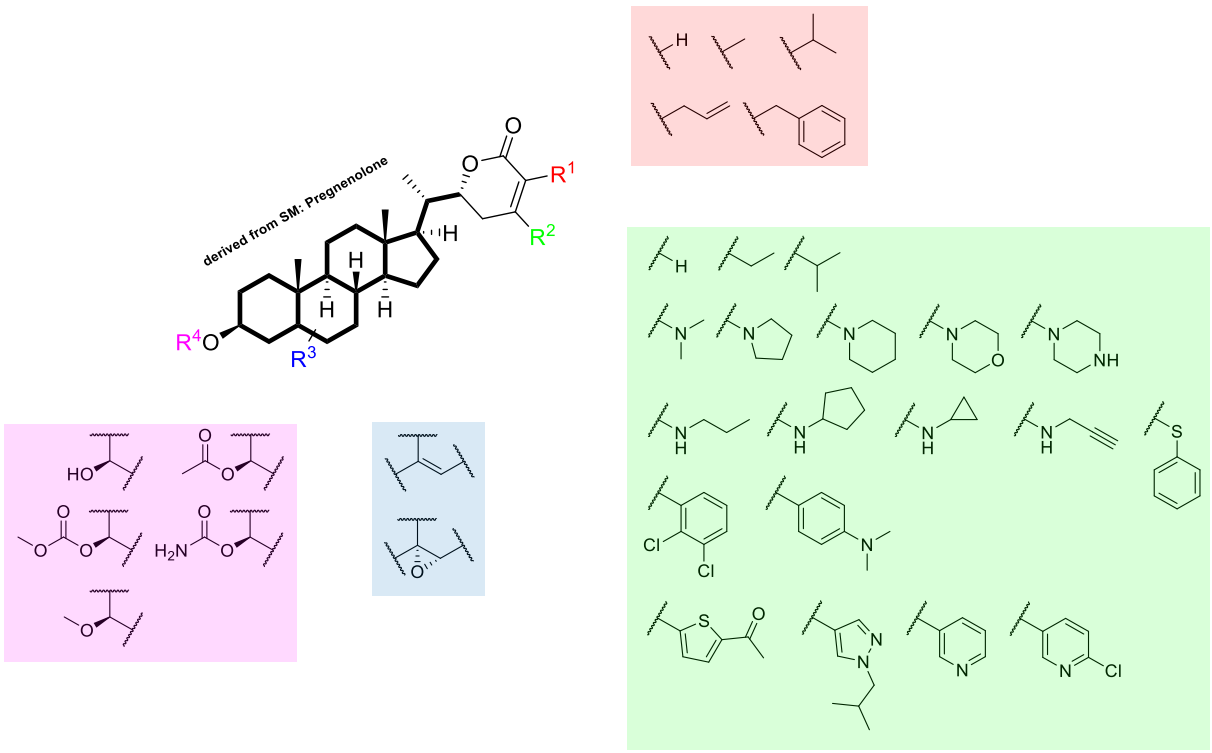
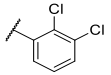
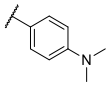
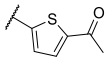
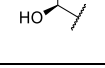
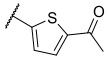
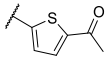
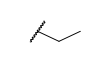
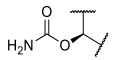
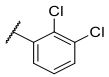
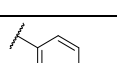
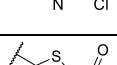


Figure 10: Summary of the compound collection synthesized in Part A.

Table 2: Full library

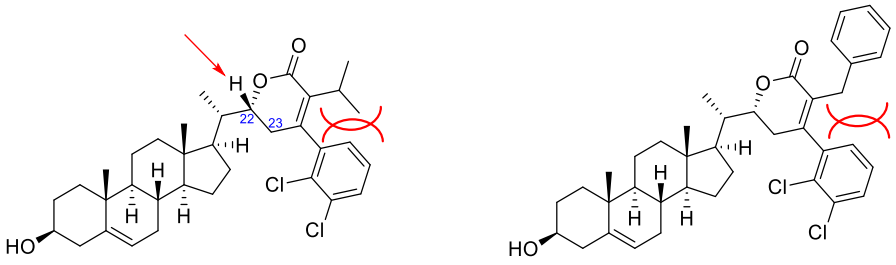
Entry	Cp. Nr	R_1	R_2	R_3	R_4
1	**130a**				
2	**130b**				
3	**130c**				
4	**130d**				
5	**130e**				
6	**130f**				
7	**130g**				
8	**130h**				
9	**130i**				
10	**130j**				
11	**130k**				
12	**130l**				
13	**130m**				
14	**130n**				
15	**130o**				
16	**130p**				
17	**130q**				
18	**130r**				
19	**130s**				
20	**130t**				
21	**130u**				
22	**130v**				
23	**130w**				
24	**131a**				

25	**131b**				
26	**131c**				
27	**131d**				
28	**131e**				
29	**131f**				
30	**132a**				
31	**132b**				
32	**132c**				
33	**132d**				
34	**132e**				
35	**132f**				
36	**132g**				

37	**132h**				
38	**114a**				
39	**114b**				
40	**114c**				
41	**114d**				
42	**114e**				
43	**114f**				
44	**114g**				
45	**114h**				
46	**114i**				
47	**114j**				
48	**114k**				

49	**133a**				
50	**133b**				

One problem was the occurance of conformers in NMR spectra. The reason is hindered rotation of the substituents at the double bond in the lactone (Figure 11). The ratio of conformers was determined in the ^{1}H NMR spectra by integration of the marked proton signal at C-22. The signals of the hydrogens at C-23 were also split. **132c** is a mixture of conformers of 85:15 in $CDCl_3$ at 27 °C. In C_6D_6 at 70 °C the ratio slightly changes to 81:19. **133a** is a mixture of conformers of 80:20 in $CDCl_3$ at 27 °C. In C_6D_6 at 70 °C the ratio slightly changes to 75:25.



132c **133a**

Figure 11: Compounds appearing as conformers in NMR spectra.

3.1.3 Biological Results

The two general ways of discovering drug candidates or, more generally speaking, bioactive compounds are the target-based approaches (target-first, forward chemical biology) and the phenotypic approaches (function-first, reverse chemical biology). Both are successfully used in drug discovery. The advantage of the phenotypic approach is that a prior knowledge of the molecular mechanism of action (MMOA) of a compound is not required. On the other hand, this makes it more difficult to optimize a hit compound.[27, 74] Biological screenings presented in this work use the phenotypic approach and were performed at the Compound Management and Screening Center (COMAS), Dortmund. Among others, COMAS screens compounds for the identification of modulators of the Wnt and Hedgehog signaling pathways. Both signaling pathways play roles in growth control and embryonic development. In embryonic development as well as tissue renewal in adults the growth of cells is limited by the optimal size and functions of organs and tissues. Cancer cells circumvent these growth limiting mechanisms and return to a style of growth which is limited by the availability of nutrients. This way, embryonic development, tissue renewal and cancer are interconnected. Hence, the Wnt and Hedgehog signaling pathways are relevant to drug discovery in cancer.[75]

Wnt signaling covers three different cellular signal transduction pathways, initiated by the binding of a Wnt protein to a membrane receptor on a cell surface, called Frizzled (Fz). Wnt proteins are a group of 19 secreted glycoproteins, which act as ligands in autocrine and paracrine signaling. The main function of Wnt signaling is the control of certain aspects of embryonic development and tissue renewal in adults. Aberrant activity of Wnt signaling in adults is associated with carcinogenesis. The mechanism of Wnt signaling and its role in diseases have been reviewed several times in the past years.[76] The three known Wnt signaling pathways are the canonical Wnt pathway, the noncanonical planar cell polarity pathway, and the noncanonical Wnt/calcium pathway. In this work, the focus lies on the canonical Wnt pathway.

The transcriptional coactivator β-catenin plays a central role in the canonical Wnt pathway (Figure 12). Its cytoplasmic level is regulated by the presence of Wnt proteins. In the absence of Wnt ligands, β-catenin is degraded by the destruction complex, which among others includes the following proteins: the scaffolding protein Axin, the tumor suppressor protein adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α). There are two Axin genes, which have different modes of expression. While Axin1 is constutively expressed, Axin2 is a Wnt target gene and at the same time part of the β-catenin destruction complex. It is therefore part of a negative feedback loop.[77] Following phosphorylation of β-catenin by the kinases GSK3β and CK1α, it is ubiquitinated and degraded by the proteasome. At low levels of β-catenin, Wnt target genes are transcriptionally repressed. Binding of Wnt proteins to Fz and the coreceptor LDL-related proteins (LRP-5/6) disrupts

the function of the destruction complex. β-catenin is no longer degraded and translocates to the nucleus. There it acts as a transcriptional coactivator of LEF/TCF and stimulates the transcription of Wnt target genes. Among the target genes are Axin2 and Cyclin D1. Other important components of the Wnt pathway are the poly-ADP-ribosylating enzymes Tankyrase 1 and Tankyrase 2 (TNKS1/2). The Tankyrases are poly(ADP-ribose) polymerases. Both Tankyrase isoforms directly PARsylate Axin and promote its degradation through the ubiquitin-proteasome pathway.[78] Tankrases are regarded as potential drug targets.[79]

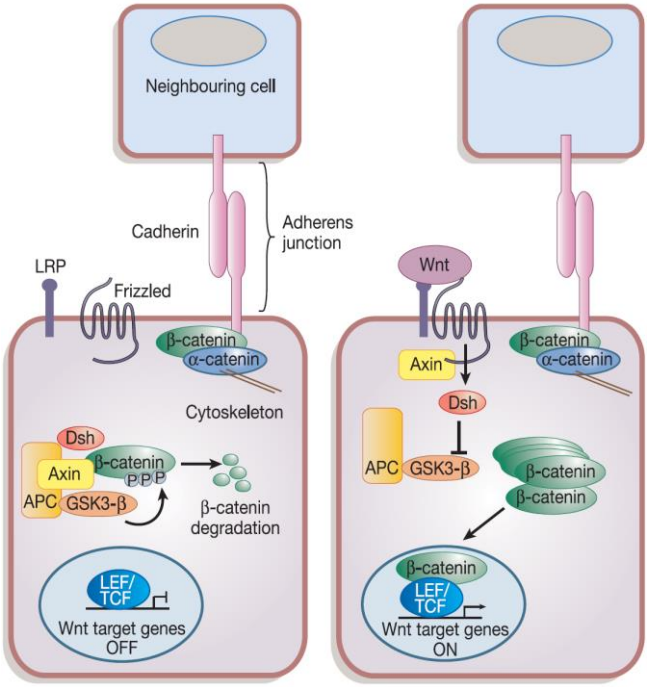


Figure 12: The canonical Wnt signalling pathway. Figure copied from [80] Reuse of this figure has been licensed by Nature Publishing Group.

Notably, colorectal cancers are strongly linked to mutations in the APC gene, underscoring the importance of Wnt/β-catenin pathway towards carcinogenesis. This includes the inherited disease familial adenomatous polyposis (FAP), as well as sporadic adenomas and cancers.[81] The colon contains a population of stem cells which give rise to all differentiated cell types. They are maintained in a proliferative state by continuous Wnt signaling from surrounding specialized cells. Cessation of exposure to Wnt signal proteins normally makes them stop dividing and leave the stem-cell niche. Loss-of-function mutations in the APC gene mimic the effect of continual exposure to Wnt signal and lead to an inappropriate expanding of colon epithelium, called an adenoma. Adenomas on their part have a tendency to become malignant and to lead to colon cancer.[80]

Currently, there is a high interest in developing a cancer treatment that specifically targets Wnt signaling. Agents targeting Wnt signaling can be grouped in small molecules, blocking antibodies and

peptides. As far as today, there is no FDA-approved drug which selectively targets Wnt signaling. However, a few small molecules with other cellular targets also modulate Wnt signaling *in vivo* (Figure 13).[82] For example, the FDA-approved anthelmintic drug pyrvinium (**134**) is a potent inhibitor of Wnt signaling with a half maximal effective concentration (EC_{50}) of ~10 nM, acting as an allosteric activator of CK1α.[83] In 2009, the small molecule XAV939 (**135**) was identified from a chemical genetic screen to inhibit β-catenin-dependent transcription.[78] XAV939 inhibits the poly-ADP-ribosylating enzymes Tankyrase 1 and Tankyrase 2, thereby stabilizing Axin and stimulating β-catenin degradation. Chen *et al*. screened a library of ~200.000 synthetic compounds and have identified two novel classes of small molecules that disrupt Wnt signaling. One class inhibits the activity of Porcupine, a membrane-bound enzyme that is essential to the secretion of Wnt proteins and the other abrogates the destruction of Axin proteins.[84] Interestingly, there are already reports on the inhibition of the Wnt pathway by withanolides! Coagulin-L (**136**) inhibits adipocyte differentiation by a mechanism which involves modulation of the Wnt pathway.[50a] Withanolide D (**137**) induced apoptosis in pancreatic ductal adenocarcinoma by abrogating β-catenin signaling.[50b]

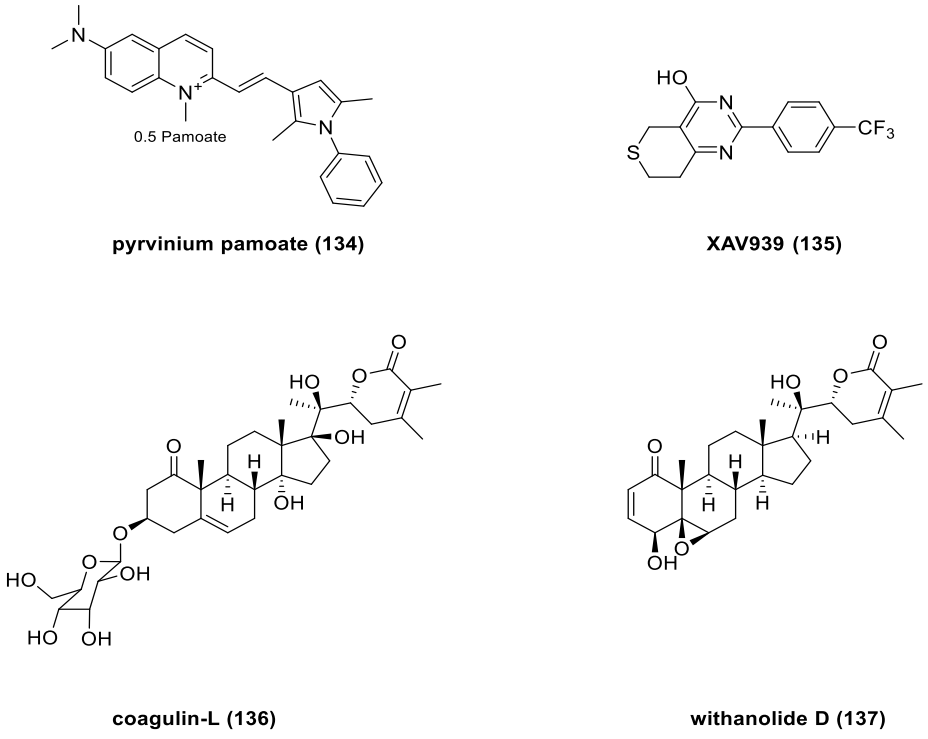


Figure 13: Wnt signaling modulators

All synthesized withanolide analogues were submitted to COMAS and underwent a screening for modulation of the Wnt and Hh signaling pathways. The activity in the Wnt pathway was investigated using a TOPFLASH-based Wnt reporter-gene assay. For this, a HEK293 reporter cell line was employed that contains the TOPFLASH reporter and is highly sensitive to stimulation by the protein Wnt3a, owing

to the presence of additional copies of the Frizzled receptor.[85] Stimulation with Wnt3a results in a 10–20-fold induction of a luciferase reporter. Compounds were screened at a concentration of 30 µM. In addition, the influence of the compounds on cell viability was determined, whereby only compounds that did not reduce viability by more than 20% were considered as hits. To rule out an inhibition of the luciferase protein or interference with transcription or translation, compounds were assayed in a HEK293 cell line that constitutively expresses firefly luciferase.

Initially, compound **130i,** the product of addition elimination reaction of **102e** with piperidine, was detected to inhibit the TOPFLASH reporter gene assay. However, it did interfere with the assay itself by increasing the luciferase activity. The initially synthesized compound library contained only **130i** as the single amine adduct. Therefore, a more focused library of amine adducts **130j**–**130w** was prepared (Figure 14, Table 3). All further biological experiments were performed by Shobhna Kapoor.

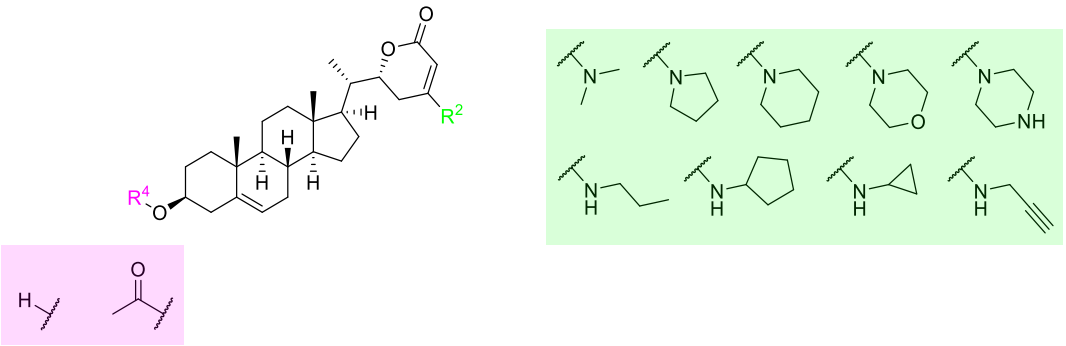


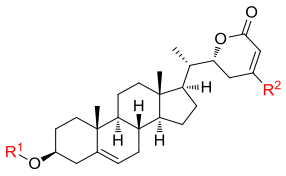
Figure 14: Focused library of amine adducts 130i–130w.

Experiments were performed in five different cell lines. Mouse L cells are fibroblasts and HEK 293 human embryonic kidney cells. For the reporter gene assay, a variant of the HEK 293 cell line was used, named HEK 293T. These cells contain the Simian Vacuolating Virus 40 (SV40) Large T-antigen, which enables them to replicate transfected plasmids containing the SV40 origin of replication. Besides that, the human colorectal cancer (CRC) cell lines HCT116 and SW480, both of which harbor mutations in key Wnt pathway components, were used. The cell line HCT116 has a Ser45 deletion in one β-catenin allele and one wild type allele.[86] This mutation of a GSK3β phosphorylation site prevents the degradation of β-catenin because the crucial phosphorylation at Ser45 cannot occur. In this cell line the canonical Wnt pathway is therefore constitutively active. The cell line SW480 expresses only truncated APC protein. The destruction complex remains intact and β-catenin can be phosphorylated, but however, ubiquitination of β-catenin is impaired.[87]

In the first step, a Wnt-responsive Super-Topflash (STF) luciferase reporter assay in HEK293T cells was performed. In this assay, the expression of firefly luciferase is under the control of the Wnt pathway.

Inhibition of the Wnt pathway results in a lower expression of the luciferase gene. As a control, constitutively expressed *Renilla* luciferase is used, which is not influenced by the Wnt pathway. If assayed compounds are toxic, the luminescence signals from both luciferase proteins would be diminished. The cells were transiently transfected with STF and control TK-*Renilla* plasmids. Canonical Wnt signaling was activated using Wnt3a conditioned medium (Wnt3a-CM), and the Wnt signaling pathway activity was determined at 10 and 30 µM of the respective compounds. A firefly luciferase inhibition of greater than 60% was regarded as a hit and the compound was tested for dose-response inhibition. The half maximal inhibitory concentration (IC_{50}) values of all assayed compounds are given in Table 3. **130k** and **130o** were insoluble in DMSO and could not be used.

Table 3: Chemical structures and Wnt pathway inhibition potency of withanolide analogues. Wnt reporter activity was measured in HEK293T cells transiently transfected with STF and *Renilla* control plasmid for 8 h, and luciferase activitiy was measured 22 h after compound addition in Wnt3a-CM. The luminescent signal for firefly luciferase was normalized to the signal of *Renilla* luciferase. Pathway activity set to 100 % for cells treated with DMSO. The IC_{50} values reported are mean value ± s.d. of three independent experiments each carried out in triplicate.



IC_{50} Wnt pathway inhibition [µM]

R^2 \ R^1		
	**130i** 2.61±0.67	**130j** 4.5±1.3
	**130k** **Insoluble in DMSO**	**130l** 3.21±0.84
	**130m** > 30	**130n** > 30
	**130o** **Insoluble in DMSO**	**130p** 2.40±1.0
	**130r** > 30	-
	**130s** 0.11±0.02	**130t** 0.35±0.06
	-	**130u** 0.77±0.1

	130v	130w
	> 30	1.14±0.42

The data do not allow any clear conclusion regarding the structure-activity relationship among amine adducts. However, **130s** was the most potent compound with an IC_{50} of 110±20 nM and much more active than the initial hit compound **130i**. At the same time, withanolide analogues are not toxic to HEK 293 cells for the duration of the experiment. Importantly, the decomposition product **125** as well as the putative hydrolysis product **128** (Figure 15) were totally inactive in the same assay.

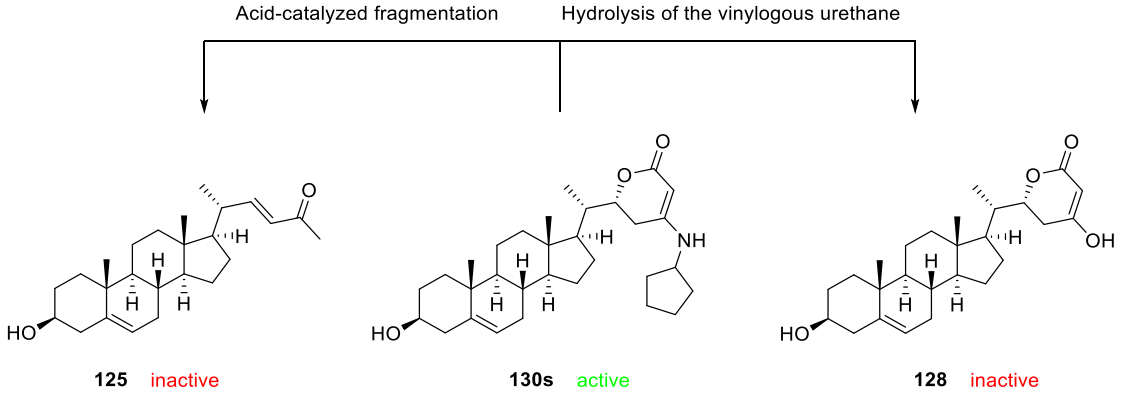


Figure 15: Possible decomposition products of Wnt inhibitors.

Deeper biological investigations were performed in order to elucidate the mechanism of action of Wnt pathway inhibition caused by withanolide analogues. For this, the most active analog **130s** and the less active compound **130i** were chosen.

130i and **130s** dit potently inhibit canonical Wnt signaling in HEK293T cells in a dose-dependent manner (**Figure 16**). Importantly, in this assay luciferase was not influenced by the compounds. The most active analog **130s** suppressed the STF-dependent reporter gene expression in HCT116 and SW480 cell lines (**Figure 17**). Further evidence for the inhibition of Wnt signaling comes from the analysis of Wnt target gene expression. Upon activation of the canonical Wnt pathway in HEK293 cells**,** **130i** and **130s** caused a dose-dependent decrease in the expression of the Wnt target genes Axin2 and Cyclin D1 on the mRNA level (**Figure 18**).

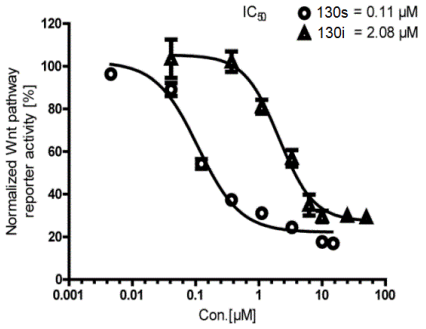


Figure 16: 130i and 130s potently reduce canonical Wnt signaling. Dose-response inhibition of Wnt signaling in HEK293T cells transiently transfected with STF and *Renilla* plasmids. Varying concentrations of the compounds or DMSO (0.3%) were added in Wnt3a-CM to the transfected cells and luciferase activities were measured 22 h later. The firefly luciferase activity was normalized to the activity of *Renilla* luciferase. Pathway activity was set to 100 % for cells treated with DMSO. Data represent mean values ± s.d of > 3 independent experiments each carried out in triplicate.

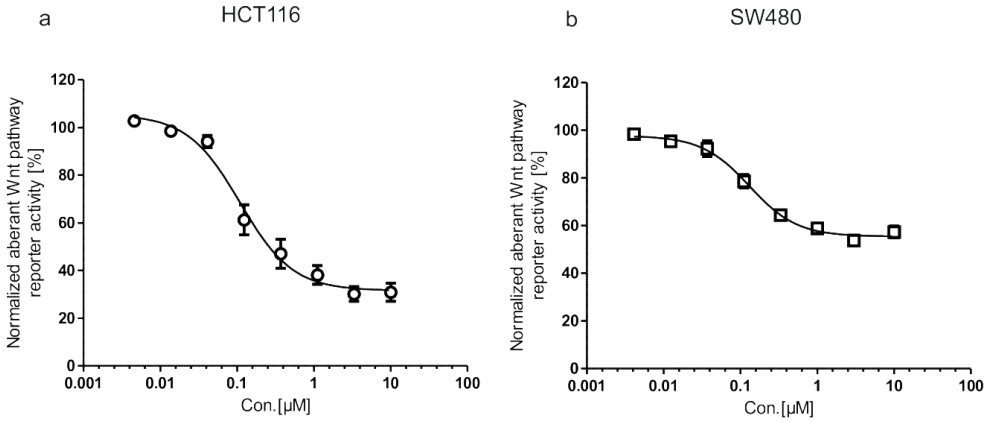


Figure 17: **130s inhibits aberrant Wnt signaling in colorectal cancer cells. 130s**-mediated reduction of reporter activity in β-catenin mutant HCT116 cells **(a)** and APC truncated mutant SW480 **(b)** cancer cells. Cells were transiently transfected with STF and *Renilla* plasmids and 8 h later **130s** was added in Wnt3a-CM for additional 22 h. Data are mean values ± s.d. of 3 independent experiments each carried out in triplicate.

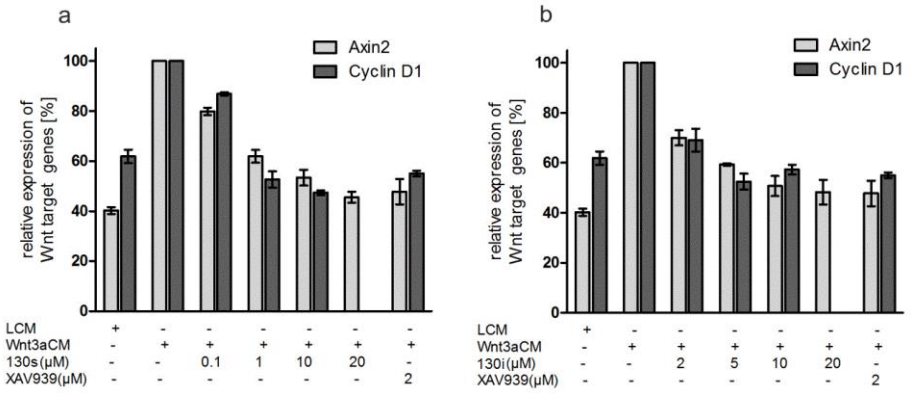


Figure 18: 130i and 130s reduce Wnt-specific target gene expression. Dose-dependent reduction in the relative expression of Wnt-target genes Axin2 and Cyclin D1 by **130s (a)** and **130i (b)**. HEK293 cells were treated with varying compound concentrations or DMSO in Wnt3a-CM for 24 h, followed by RNA isolation, reverse transcription and quantitative PCR with gene-specific oligonucleotides for Axin2, Cyclin D1 and GAPDH. The relative mRNA expression level for each gene was normalized to the level of GAPDH as an internal control. The gene expression level of cells treated with Wnt3a and DMSO was set to 100 %. Data represent mean values ± s.d. of three independent experiments each performed in triplicate.

The effect of **130s** and **130i** on the cellular levels of β-catenin was monitored via In-Cell-Western assay and re-confirmed for selected conditions by means of immunoblotting. **130s** and **130i** substantially reduced the β-catenin protein expression levels in Wnt3a-induced HEK293 and mouse L cells in a dose-dependent and temporal fashion (**Figure 19**). **130s** had no effect on the cytoplasmic β-catenin levels in mutant HCT116 cells and only modestly affected the β-catenin levels in SW480 cells (**Figure 20**), yet they potently inhibited the Wnt signaling in these cells (**Figure 17**). The fact that Wnt pathway inhibition and the β-catenin level do not necessarily correlate, suggests that the compounds target a pathway component that is omnipresent across all cell types upstream of β-catenin.

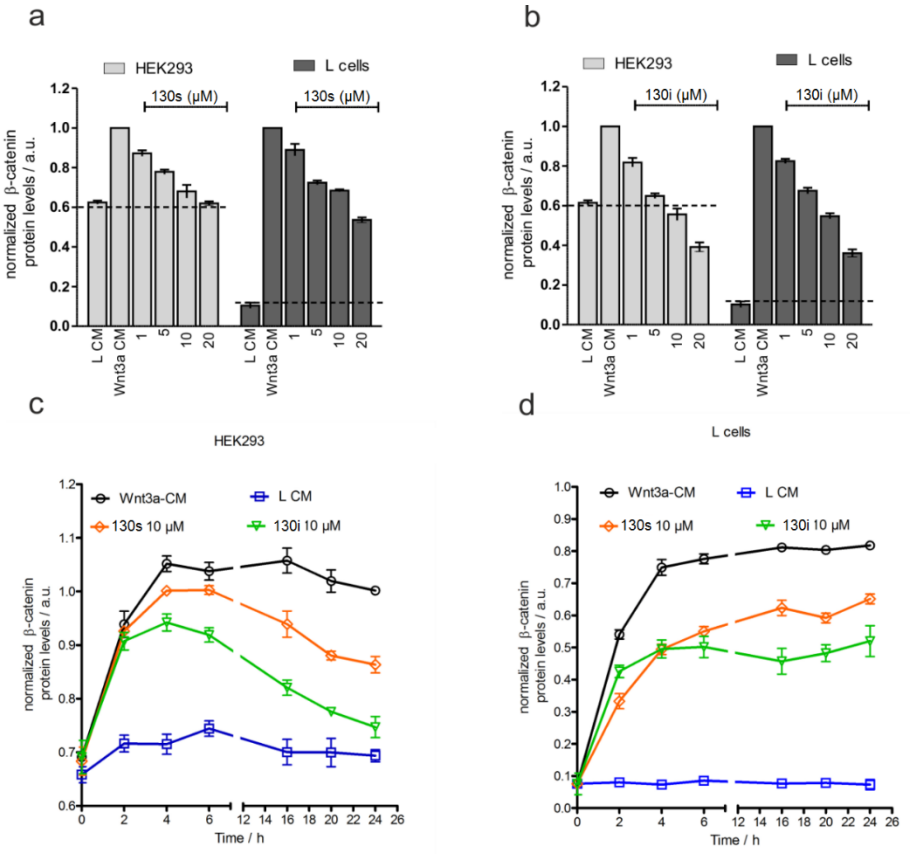


Figure 19: 130i and 130s inhibit Wnt-induced β-catenin stabilization in a time- and dose-dependent manner. Reduction in Wnt-induced cellular accumulation of β-catenin in HEK293 and L cells in response to **130s (a)** and **130i (b)**. Cells were treated with various concentrations of compounds or DMSO in Wnt3a-CM for 24 h, followed by quantification of β-catenin levels using In-cell Western. Dashed lines represent the basal levels of endogenous catenin levels in non-activated cells. Normalized β-catenin levels represented are mean values ± s.d. of 3 independent experiments, each in quadruplicates. Temporal changes in β-catenin abundance in HEK293 **(a)** and L cells **(b)** upon treatment with 10 μM of compounds in Wnt3a-CM. For **(c)** and **(d)**, representative curves are shown.

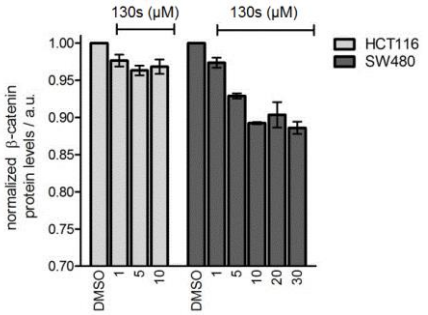


Figure 20: 130s-mediated inhibition of aberrant Wnt pathway activity in colorectal cancer cells. In-cell quantification of endogenous cellular β-catenin levels in HCT116 and SW480 upon treatment with indicated concentration of **130s** for 24 h. Normalized β-catenin levels represented are mean ± s.d. of three independent experiments, each performed in quadruplicates.

Treatment of HEK293 cells with **130i** and **130s,** as well as HCT116 and SW480 cells with **130s** led to a rise in the protein levels of Axin2 (**Figure 21** and **Figure 22**). In HEK293 and SW480 cells the rise of Axin2 was accompanied by an increase of the protein level of phosphorylated β-catenin ($(S^{33}/S^{37}/Thr^{41})$-pβ-catenin). As expected from the fact that β-catenin harbors a crucial point mutation in HCT116 cells, no phosphorylated β-catenin was observed here. In HCT116 and SW480 cells, the effect of **130s** was similar to that of IWR-1, a known compound which stabilizes Axin proteins by abrogating their turnover.[84]

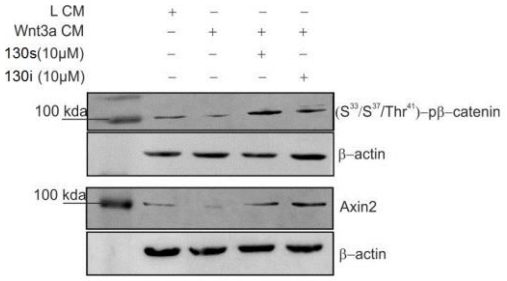


Figure 21: 130i and 130s induce stabilization of Axin2 proteins and increase β-catenin phosphorylation. HEK293 cells were incubated with 10 µM of indicated compounds in Wnt3a-CM for 24 h prior to cell lysis. Cell lysates were subjected to immunoblotting to determine the levels of Axin2, and β-catenin phosphorylation. β-actin was used as the loading control.

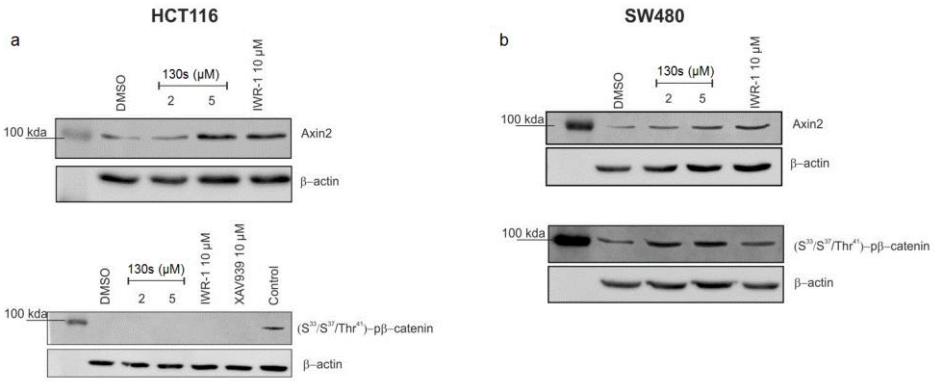


Figure 22: 130s induces stabilization of Axin2 protein in SW480 and HCT116 cells. Western blot analyses were performed in in SW480 (a) and HCT116 (b) at the indicated concentrations upon compound treatement for 24 h. The cells were incubated with 10 µM of indicated compounds in Wnt3a-CM for 24 h prior to cell lysis. Cell lysates were subjected to immunoblotting to determine the levels of Axin2 and β-catenin phosphorylation. β-actin was used as the loading control.

Taken together, the results suggest that in HEK293 and mouse L cells **130s** causes the degradation of β-catenin by increasing the the protein level of Axin2. Axin2 is the least abundant component and the rate-limiting factor of the destruction complex.[86] Notably, **130s** caused a reduction of Axin2 mRNA and at the same time an increase in the protein level. Therefore, **130s** seems to stabilize the existing pool of Axin2. In CRC cell lines, where the protein level of β-catenin was weakly influenced by **130s**, albeit observed inhibitory activity on Wnt signaling suggests that compound treatment elevates sequestration of β-catenin by the destruction complex. According to the results above, sequestered β-catenin is not degraded, but is prevented to transfer to the nucleus and drive Wnt-dependent transcription. The observed increase in Axin2 protein levels upon treatment with **130s** could be the consequence of Tankyrase (TNKS) inhibition or other reasons. However, **130i** and **130s** did only moderately inhibit the enzymatic activity of TNKS1/2 (**Figure 23**). Presumably another target is involved in the observed Wnt pathway inhibition in cellular assays. Hence, the molecular mechanism of action is currently unknown.

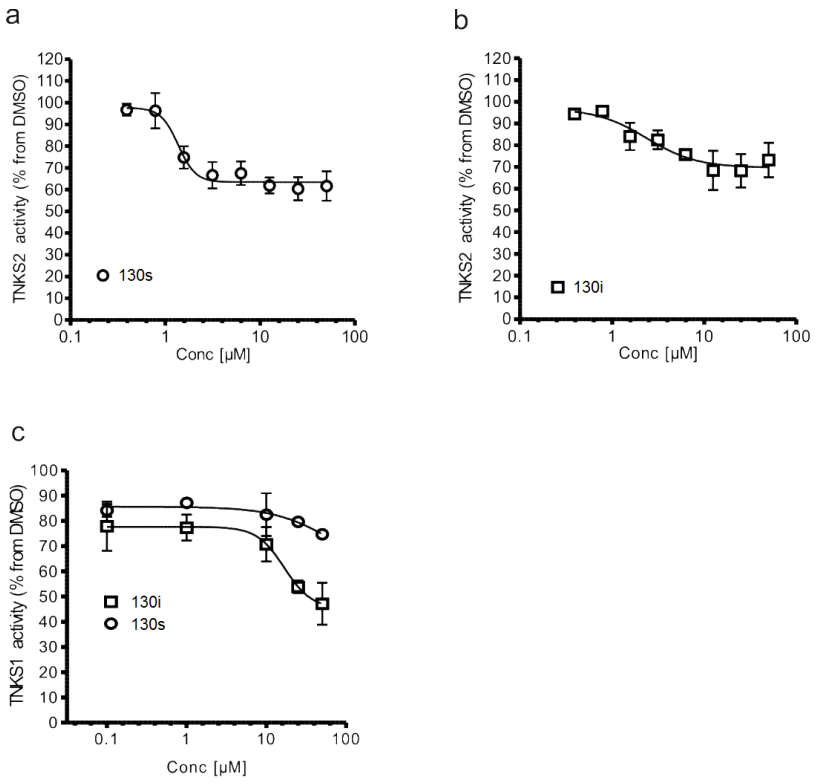


Figure 23: 130i and 130s only modestly affect the TNKS1/2 enzymatic activity. TNKS2 enzymatic activity in response to treatment with 130s **(a)** and 130i **(b)** was determined using TNKS2 histone ribosylation kit. **(c)** The effect of **130i** and **130s** on the enzymatic activity of TNKS1. Data shown are mean values ± s.d. of three independent measurements performed in duplicates.

3.1.4 Synthesis of Probes for Target Identification

Since the synthesized Wnt inhibitors only modestly affect Tankyrase activity, the question remains what their cellular target is. This question can be addressed with chemical proteomics techniques based on affinity isolation.[88] In order to perform pulldown experiments, positive and negative probes are required. Compound **130s** serves as the positive probe, while two negative probes were planned to be used (Figure 24). Negative probe **138** contains the lactone, but without any functionality. In the other negative probe **139,** the lactone side chain is completely removed. Decomposition product **125** was not used as a negative probe in order to avoid Michael acceptor reactivity. Vinylogous carboxylic acid **128** is also not suitable (page 42).

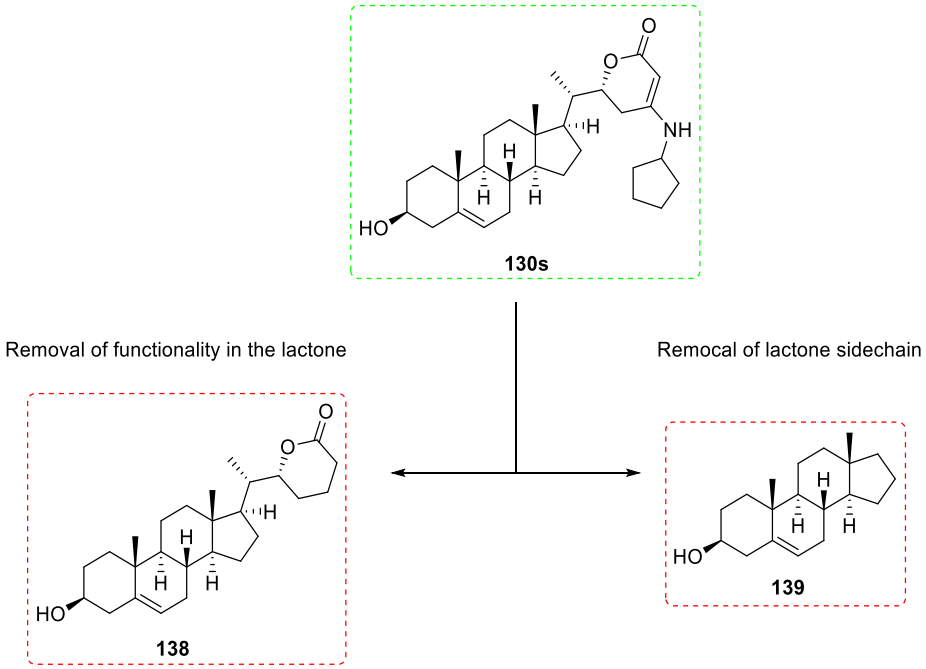
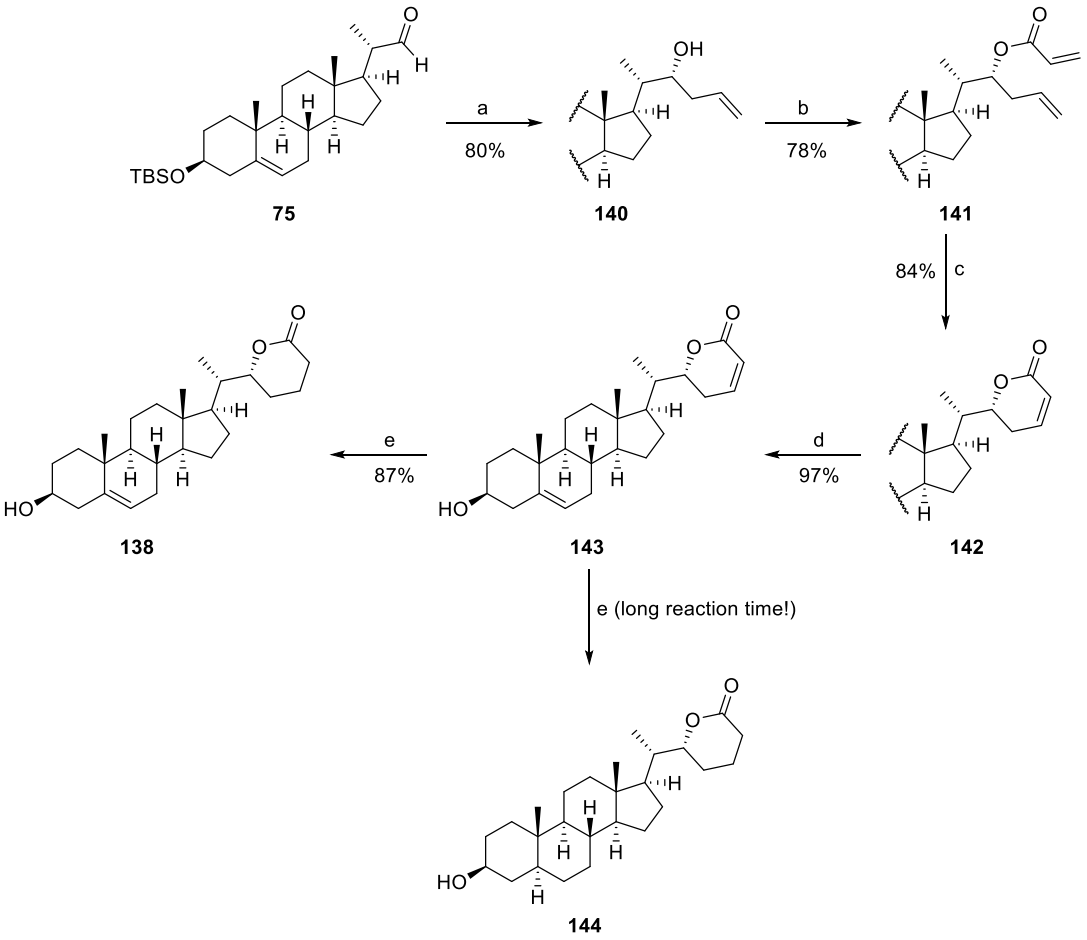


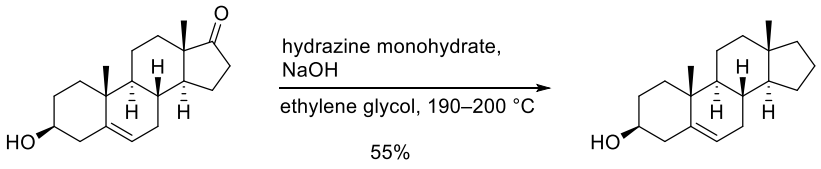
Figure 24: The planned positive and negative probes for pulldown experiments.

Compound **138** was prepared in 5 steps from aldehyde **75**, as shown in Scheme 23. The pathway from **75** to **143** is identical to a synthesis performed in part B (*vide infra*) and works well. The hydrogenation of **143** required careful control because undesired hydrogenation of the olefin in the B-ring occured with prolonged reaction time![89] Because the starting material **143**, the desired product **138** and the product of undesired overhydrogenation **144** all have the same chromatographic retention factor on TLC, the conversion was monitored by NMR.



Scheme 23: Synthesis of inactive compound 129. (a) (+)-Ipc$_2$B(allyl)borane, NaOH, H_2O_2, THF; **(b)** acryloyl chloride, Et_3N, CH_2Cl_2, RT; **(c)** Grubbs Catalyst, 2nd Generation, toluene, 80 °C; **(d)** triethylamine trihydrofluoride, CH_2Cl_2, RT; **(e)** Pd/C, H_2, THF, RT.

Compound **139** was prepared in one step by Wolff–Kishner reduction of commercially available (+)-dehydroisoandrosterone (**145**) (Scheme 24)[90]. It was observed that the hydrazone formation between the starting material and hydrazine is very fast, while the following evolution of nitrogen is the rate-limiting step. The yield is relatively low because full conversion from the hydrazone intermediate to the product was not achieved.

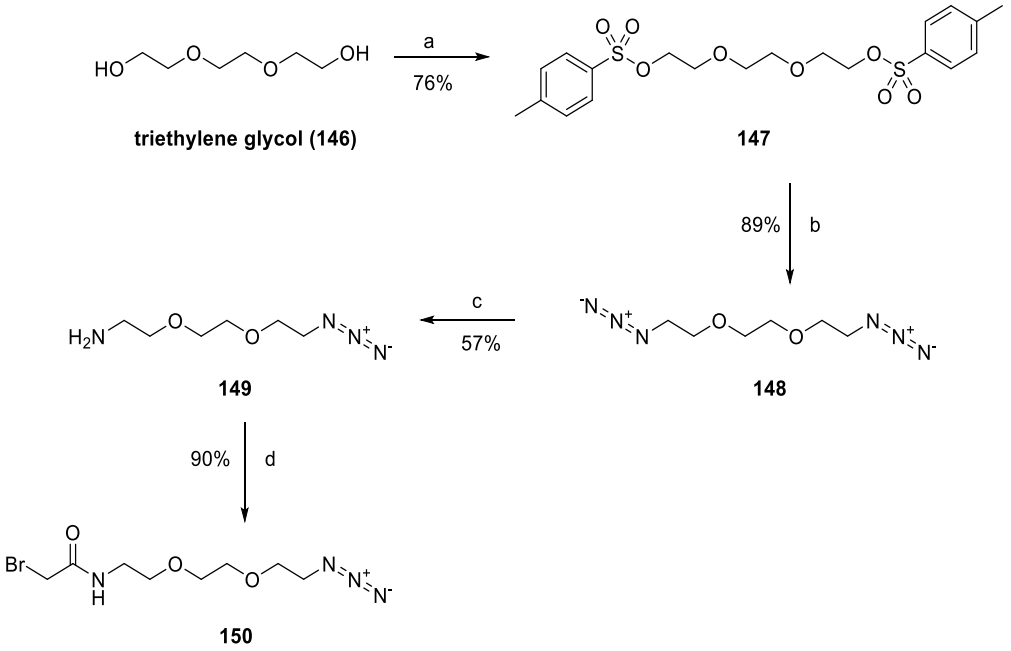


(+)-dehydroisoandrosterone (145) **139**

Scheme 24: Wolff–Kishner reduction of dehydroisoandrosterone.

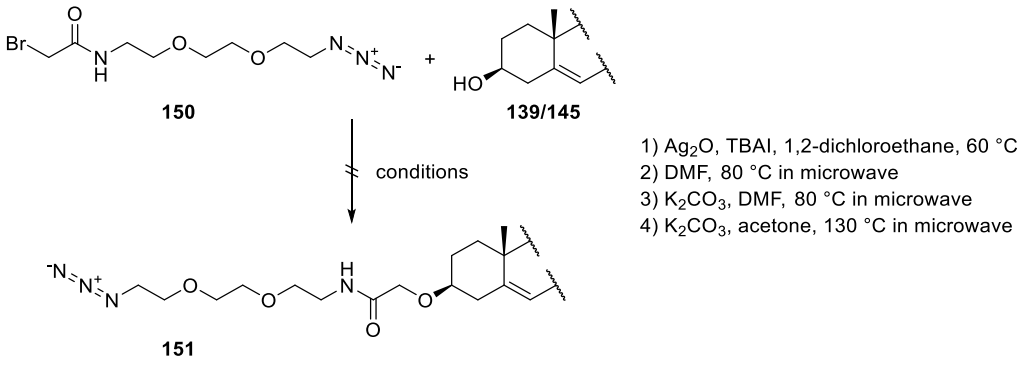
The linker for the probe molecules was prepared based on a known procedure with some minor changes (Scheme 25).[91] Triethylene glycol (**146)** was in four steps transformed into compound **150**

with an azide group at one end and a bromoacetyl group at the other. Most importantly, the Staudinger reaction of **148** was performed with tributylphosphine instead of triphenylphosphine. This was necessary because the crude product **149** does always contain traces of the respective phosphine oxide, generated in the Stauinger reaction. After the carbamate formation with **130s** (*vide infra*), triphenylphosphine oxide was inseparable from the product by column chromatography.



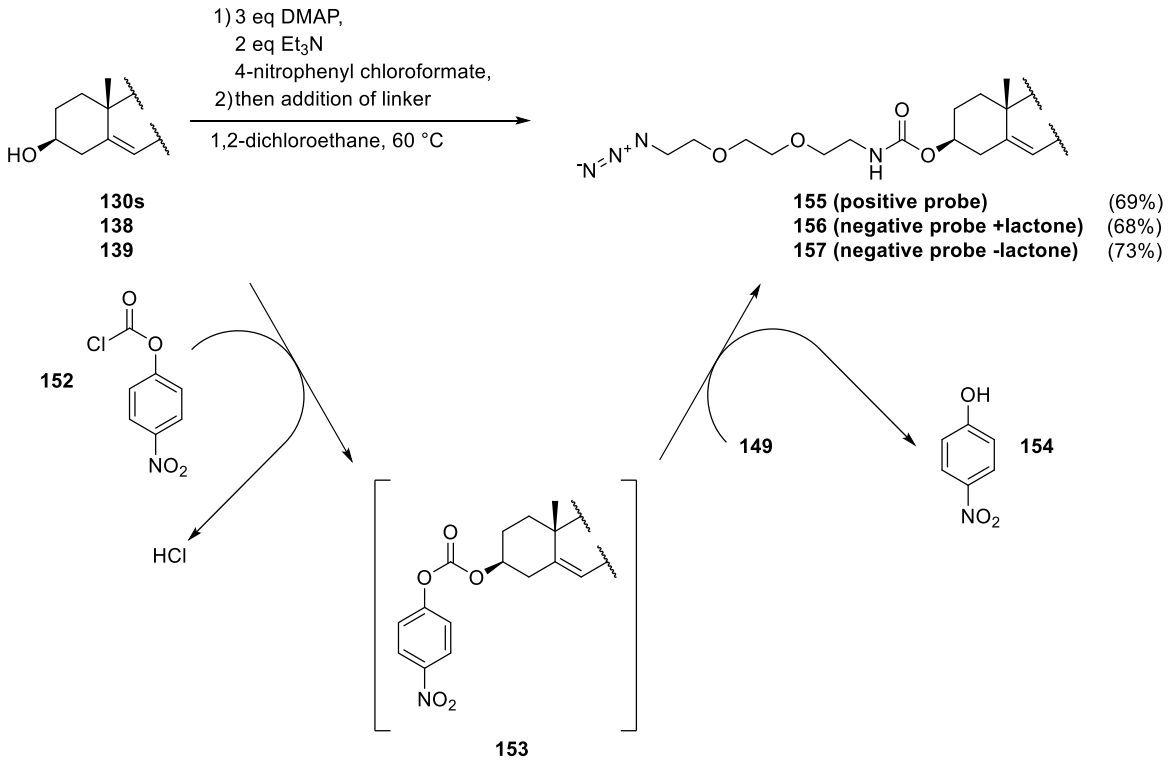
Scheme 25: Synthesis of the linker. (a) *p*-TsCl, KOH, CH_2Cl_2, RT; **(b)** NaN_3, TBAI, DMF, 80 °C; **(c)** PBu_3, *aq* HCl, toluene, RT; **(d)** bromoacetyl bromide, Na_2CO_3, CH_2Cl_2/H_2O, RT.

The attachment of the linker to the probe compounds via ether linkage was tested with deoxygenated compound **139** and (+)-dehydroisoandrosterone (**145**) (Scheme 26). All attempts to this transformation following procedures from the literature failed.[91-92] Probably the alcohol has to be deprotonated with a strong base prior to addition of the linker. Unfortunately however, strongly basic conditions could lead to side reactions with the enolizable substrate **138** or decomposition of sensitive compound **130s**.



Scheme 26: Attempted attachment of the linker via an ether.

As an alternative to the ether linkage it was decided to switch to carbamates (Scheme 27). The pulldown probes with carbamate linkage could be prepared from compounds **130s**, **138** and **139** together with azidoamine **149** under mild conditions, without strong bases or acids. The secondary alcohol of the steroid core first reacts with 4-nitrophenyl chloroformate (**152**), substituting chloride. When the first step is completed, the linker **149** is added and the amino group substitutes intensely yellow 4-nitrophenol (**154**).



Scheme 27: Carbamate formation for linker synthesis.

Probe molecules **155**, **156** and **157** underwent the Wnt-responsive Super-Topflash (STF) luciferase reporter assay in order to see the effect of the attached linker on Wnt inhibitory activity (Figure 25). As a control, **130s** was tested on the same plate and showed the expected strong activity with an IC_{50} of 0.19 µM. The positive probe **155** was an order of magnitude less active than **130s**, but still active enough for pulldown experiments. **156** showed an unexpected moderate IC_{50} of 4.36 µM. **157** was totally inactive with an IC_{50} of ~ 694 µM. In this case however, the data points could not be properly fitted and the IC_{50} is only an orientation value. Based on these results, **157** was decided to be used as the negative probe.

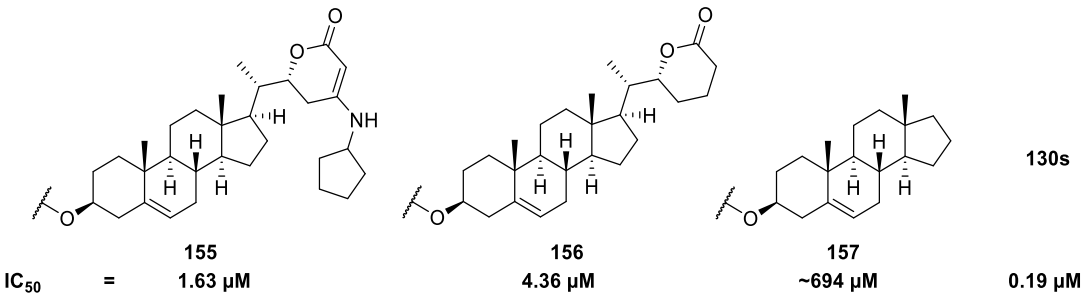
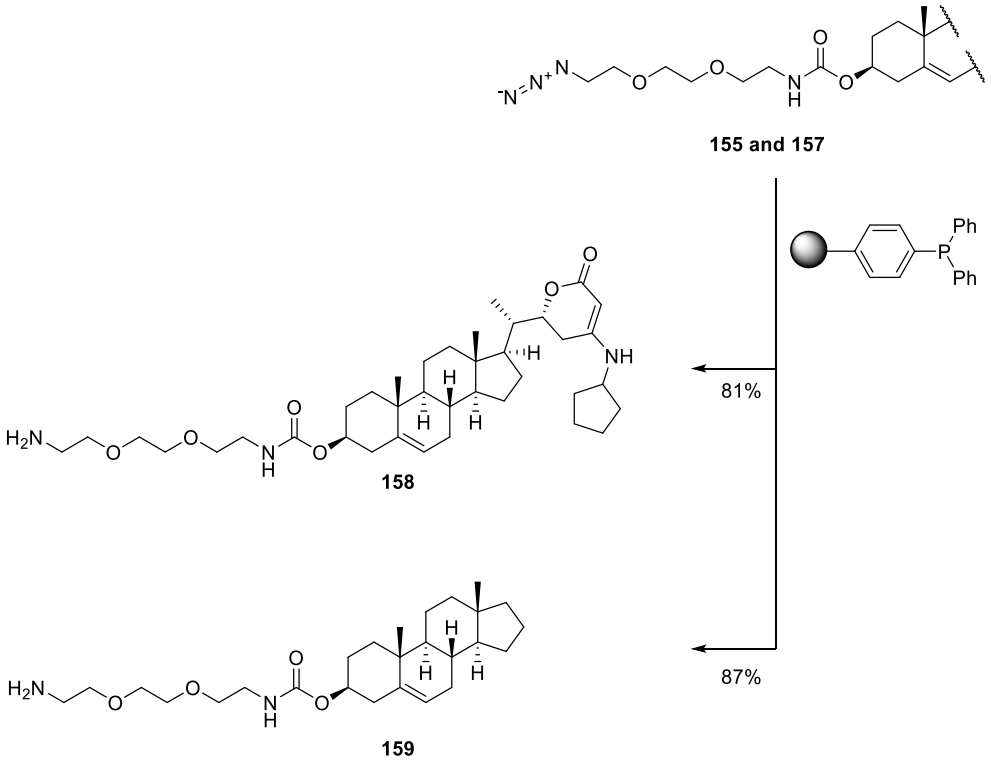


Figure 25: Probe compounds and their Wnt inhibitory activities.

For coupling to magnetic beads, the azide incorporated in the linker had to be reduced to the amine. The final Staudinger reactions were performed with polymer-supported triphenylphosphine. Initial experiments have shown that the reduction products are difficult to separate from triphenylphosphine oxide due to similar retention times in RP-HPLC (C4 column). Therefore, triphenylphosphine on solid support was used in order to avoid purification. The azides **155** and **157** were stirred with 5 equivalents of polymer-supported triphenylphosphine in THF/water. After full conversion was achieved, the reaction mixtures were filtered and concentrated to provide pulldown probes **157** and **159** (Scheme 28). Unfortunately, the products contained small amount of aromatic impurities originating from the polymer. However, this is of no consequence because the probes are coupled to beads and therefore separated from soluble impurities.



Scheme 28: The three pulldown probes.

3.1.5 Summary & Outlook

In summary, a collection of withanolide analogs bearing a full steroid scaffold was prepared. One subgroup of compounds contained potent and novel inhibitors of the Wnt signaling pathway. Deep biological investigations were performed for **130s**, the most potent compound. In future, target identification will provide insight into the molecular mechanism of action of **130s**. Besides this, the structural requirements for the biological activity will be further investigated. As mentioned above, the lactone appendage on the steroid core is required for Wnt pathway inhibition. This result proves the necessity of the presented multistep synthesis and validates the natural product based compound collection. However, it remains to be addressed whether the whole steroid is required for the observed biological activity. *In vivo*, the steroid scaffold bears the danger of poor selectivity due to the bulk of steroid-binding proteins. Therefore it would be desirable to truncate the steroid, under the condition that the molecules remain biologically active. In order to put this hypothesis to the test, it was planned to synthesize three truncated compounds **160**, **161** and **162** (Figure 26), which possess only the rings C and D of the steroid core. **160** is the truncated version of the most active Wnt pathway inhibitor **130s**, while **161** and **162** are related to two less active analogues **130i** and **130v**.

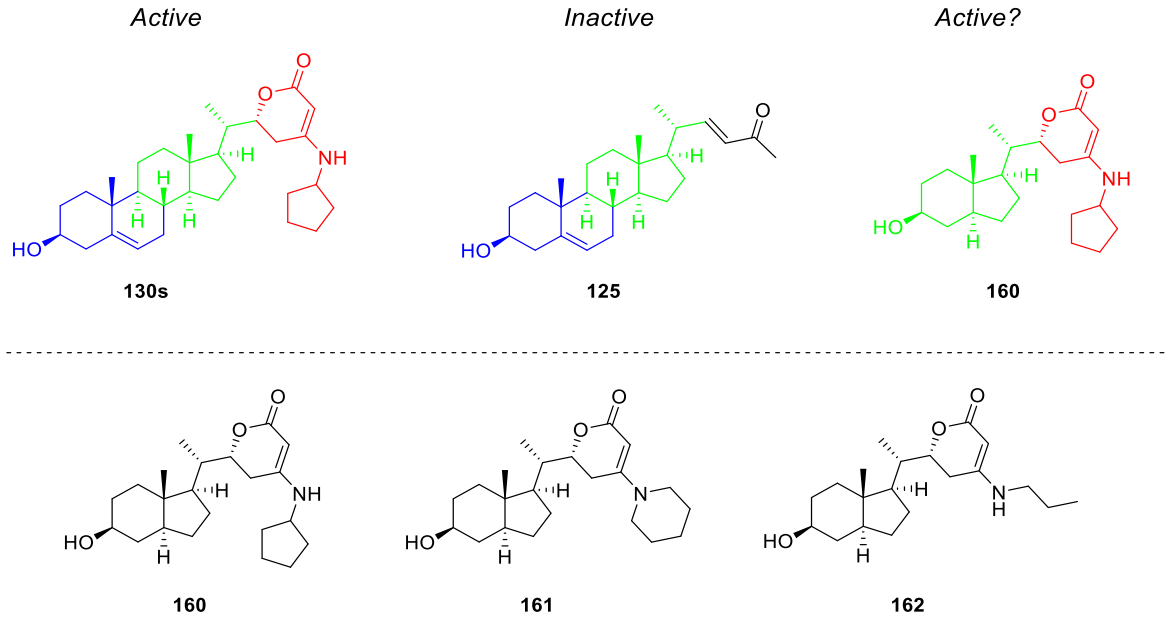
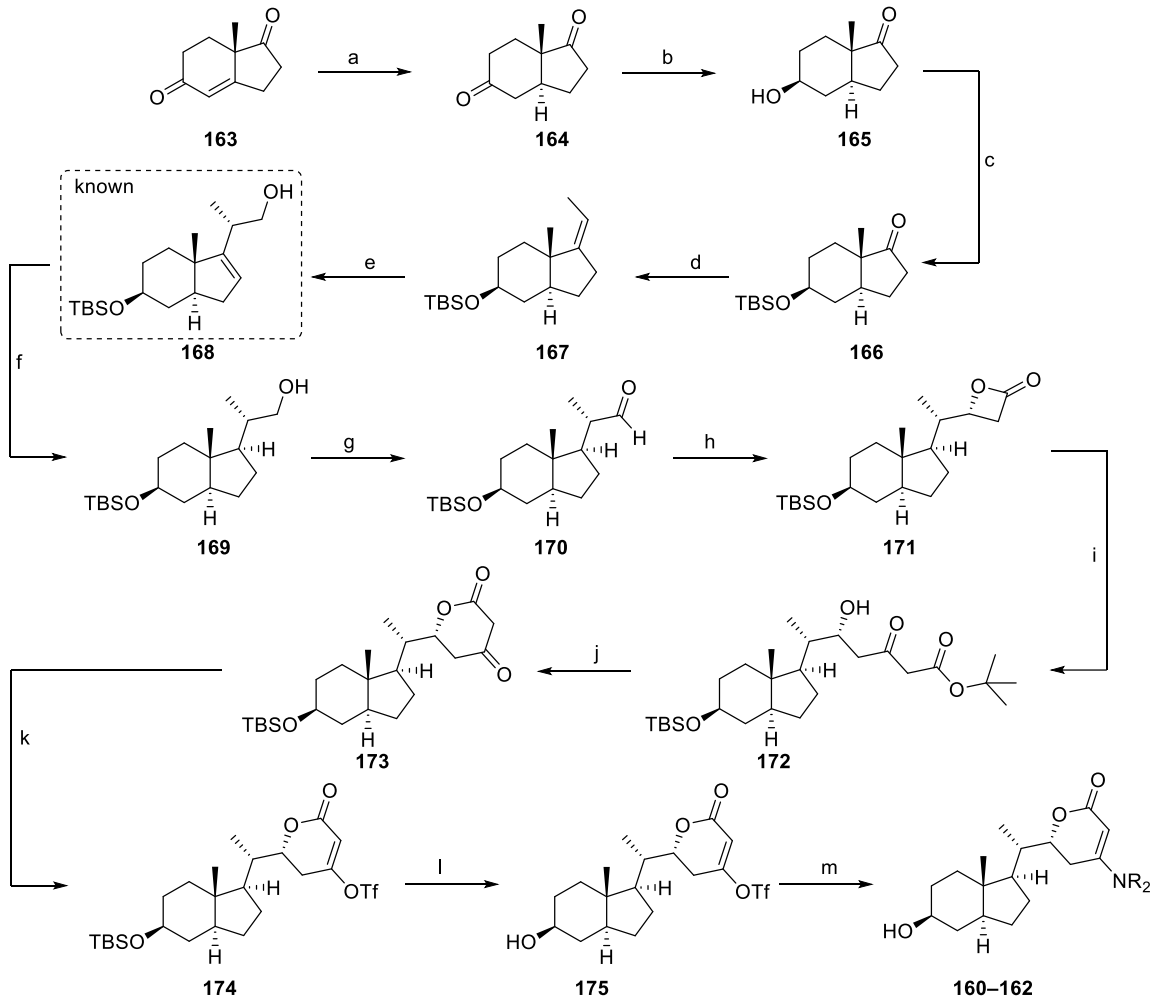


Figure 26: Structural requirements for activity and planned truncated compounds.

A proposal for the synthesis of such truncated compounds is shown in Scheme 29. The synthesis starts from Hajos-Parrish ketone (**163**) and leads in 13 steps to the desired products. Chemo- and stereoselective reductions of **163** to **164**[93], as well as **164** to **165** are described.[94] The sequence from **166** to **170** correcponds to Part B and proceeds over intermediate **168**, the last known compound of the proposed sequence[95]. From **170** the synthesis will follow the path discussed above.



Scheme 29: Proposed synthesis of truncated Wnt pathway inhibitors. (a) CuBr-DMS, *t*-BuLi, DIBAL-H, THF/HMPA, −78 °C; **(b)** $NaBH_4$, 2-propanol, 0 °C; **(c)** TBS-Cl, imidazole, CH_2Cl_2, RT; **(d)** ethyltriphenylphosphonium bromide, potassium *tert*-butoxide, THF, RT; **(e)** paraformaldehyde, $BF_3{\cdot}Et_2O$, CH_2Cl_2; **(f)** Pd/C, H_2; **(g)** PCC, CH_2Cl_2, RT; **(h)** acetyl chloride, *O*-TMS quinine, $LiClO_4$, *i*-Pr_2EtN, CH_2Cl_2/Et_2O, −78 °C; **(i)** *tert*-butyl acetate, LDA, THF; **(j)** TFA, CH_2Cl_2, RT; **(k)** trifluoromethanesulfonic anhydride, Et_3N, CH_2Cl_2, −78 °C; **(l)** triethylamine trihydrofluoride, CH_2Cl_2, RT; **(m)** amine, CH_2Cl_2, RT.

3.2 Part B: Truncated steroid analogues

The following chapter is related to:

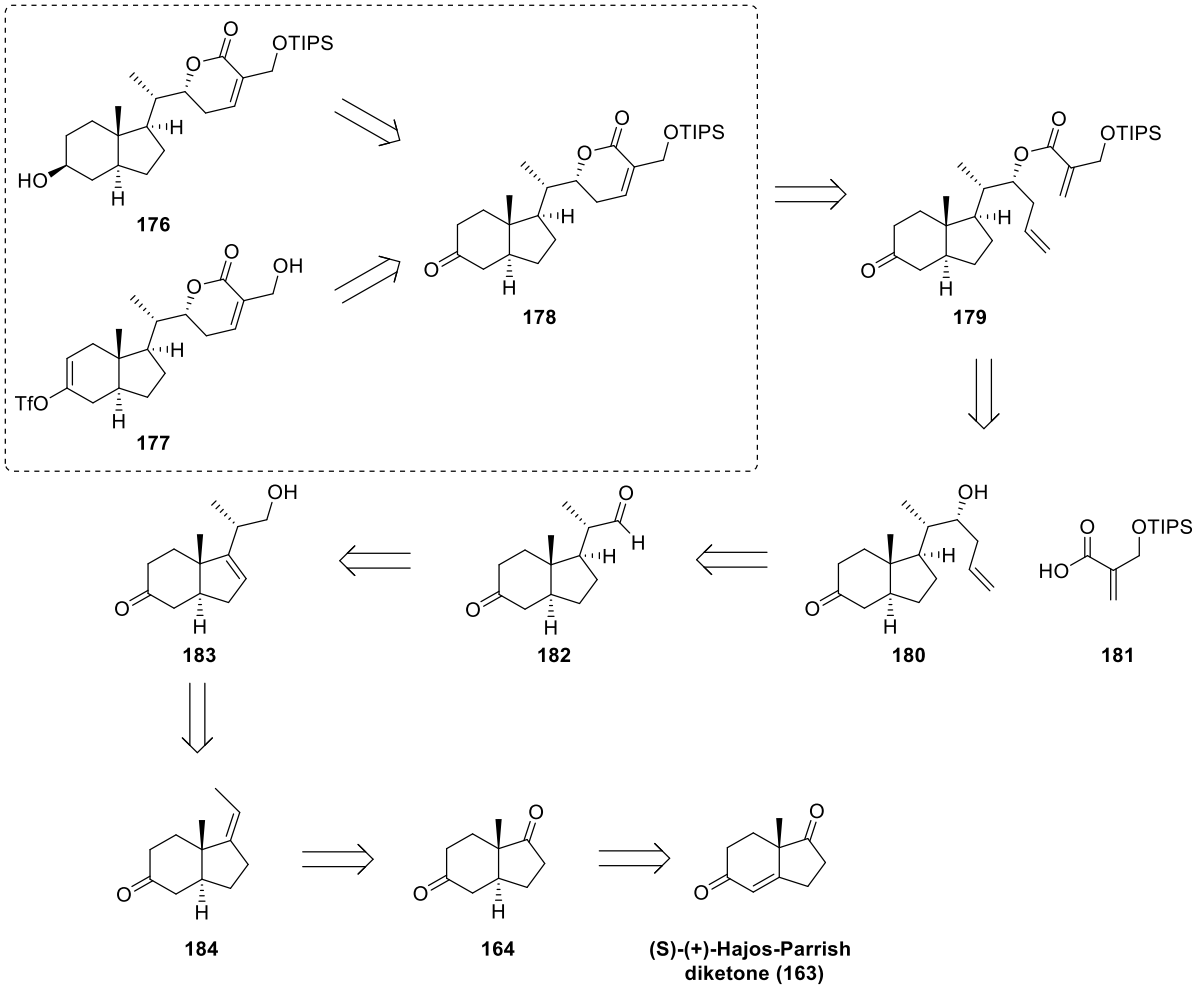
Biology-Oriented Synthesis of a Withanolide-Inspired Compound Collection Reveals Novel Modulators of Hedgehog Signaling

Jakub Švenda, Michael Sheremet, Lea Kremer, Lukáš Maier**,** Jonathan O. Bauer, Carsten Strohmann, Slava Ziegler, Kamal Kumar, Herbert Waldmann

Angew. Chem. Int. Ed. **2015**, *54*, 5596–5602; *Angew. Chem.* **2015**, *127*, 5688–5694

3.2.1 Synthesis Planning

The synthesis plan for the compound collection evolved around late-stage intermediates which bear a fully assembled unsaturated δ-lactone and possess a functional group amenable for diversification, leading to the preparation of a compound library (Scheme 30). The secondary alcohol **176**, the vinyl triflate **177** and the ketone **178** meet this criterion. Both the secondary alcohol and the enol triflate are accessible from the respective ketone **178**. The retrosynthetic analysis starts with the disassembly of the unsaturated lactone. Ring-opening leads to ester **179**, which can in turn be prepared by an esterification between homoallylic alcohol **180** and known carboxylic acid **181**. **180** is the product of a Brown allylation of aldehyde **182**. **182** contains a *trans*-hydrindane ring system and bears a short side chain. The side chain can be installed by a sequence of a distereoselective ene reaction, hydrogenation of the newly formed double bond and oxidation of the primary alcohol to the aldehyde (**184** to **182**). **184**, the substrate of the ene reaction, is derived from (*S*)-(+)-Hajos-Parrish diketone by a Wittig olefination and a conjugate reduction. Hence, the requirement of a C- and D-ring mimic is met by (*S*)-(+)-Hajos-Parrish diketone (**163**, CAS: 17553-86-5), which was used as the starting material for the synthesis of a compound library.

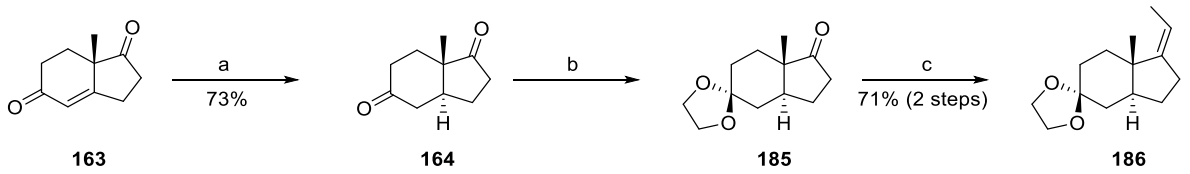


Scheme 30: Retrosynthetic analysis of truncated steroids.

3.2.2 Synthesis and initial biological Evaluation

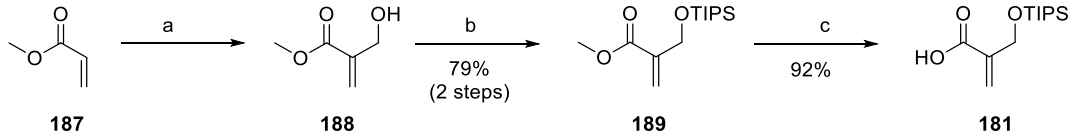
The synthetic strategy was partly developed by Ph.D. Jakub Švenda.

The synthesis commences from commercially available (*S*)-(+)-Hajos-Parrish diketone (Scheme 31). The first reaction is a diastereoselective conjugate reduction with DIBAL-H in the presence of *t*-BuLi and CuBr (**164**).[93] 73% of the pure diastereomer can be obtained on multigram scale. The more reactive ketone afterwards has to be protected as the acetal (**185**), followed by a Wittig reaction with ethyltriphenylphosphonium bromide. The Wittig reaction had a reproducible E/Z-selectivity of >95/5. These first three steps lead to described compound **186** and proceed with 52% total yield.[95-96]



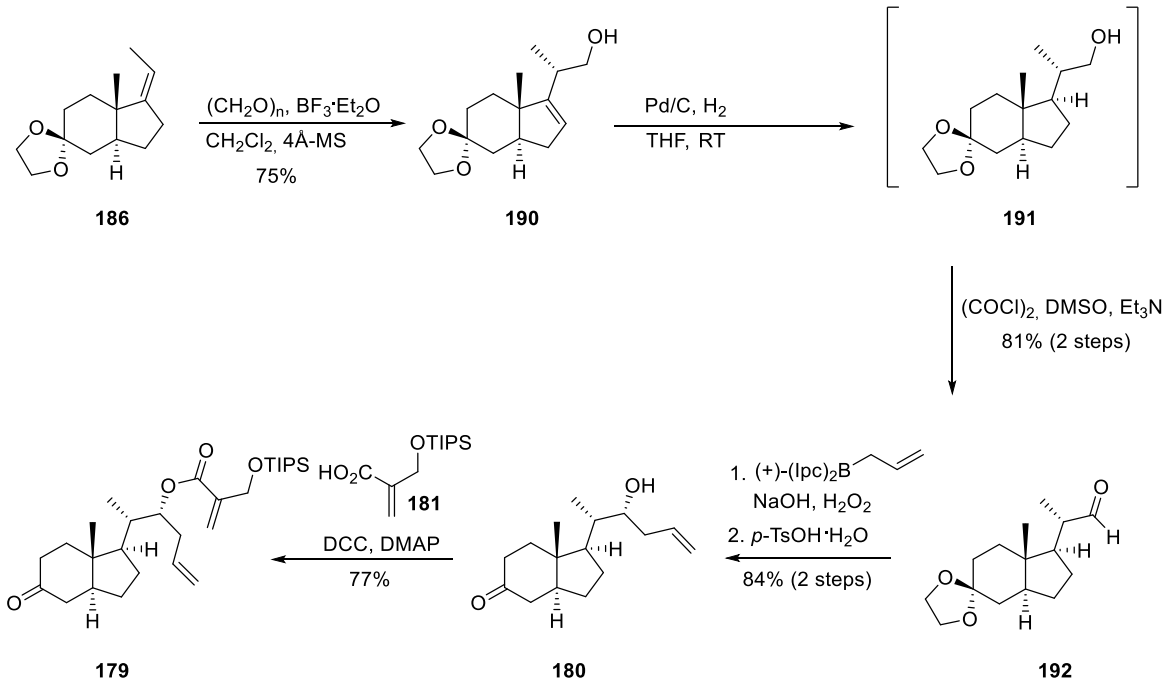
Scheme 31: (a) CuBr-DMS, *t*-BuLi, DIBAL-H, THF/HMPA, −78 °C; **(b)** ethylene glycol, oxalic acid, CH_3CN, RT; **(c)** ethyltriphenylphosphonium bromide, potassium *tert*-butoxide, THF, RT.

The synthesis of acrylic acid **181** was described by Liu *et al*. (Scheme 32).[97] Methyl acrylate is subjected to a Baylis–Hillman reaction with paraformaldehyde, followed by TIPS-protection of the primary alcohol and saponification of the methyl ester. All reactions can be performed on multigram scale. The yields given below are according to Liu *et al*. and were not determined in this work.



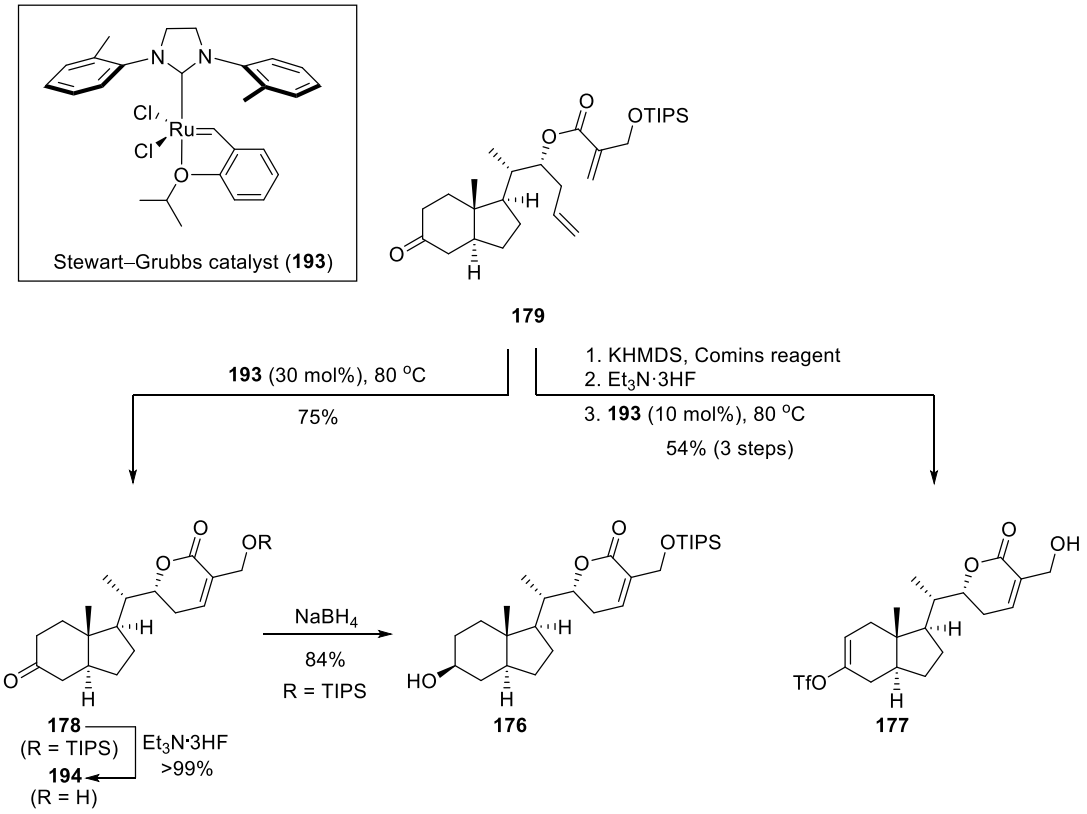
Scheme 32: (a) paraformaldehyde (10 equiv), DABCO (0.5 equiv), dioxane/H_2O (1:1), 72 h; (b) TIPS-Cl, imidazole, DMAP, CH_2Cl_2, 0°C to RT, 1 h; (c) LiOH, THF/H_2O (1:1), RT, 36 h. Yields according to Liu *et al*.[97]

Exposure of a mixture of **186** and paraformaldehyde to catalytic quantities of boron trifluoride etherate provided the corresponding homoallylic alcohol **190** in 75% yield (Scheme 33). The inclusion of activated 4-Å molecular sieves in the reaction was crucial in preventing the otherwise facile cleavage of the acetal protective group. Diastereoselective hydrogenation of the resulting homoallylic alcohol **190**, followed by standard conditions of Swern oxidation, provided aldehyde **192** in 81% yield over the two steps. As the aldehyde **192** proved unstable toward longer storage, a fresh batch of **192** was subjected to an asymmetric allylation reaction using (+)-*B*-allyldiisopinocampheylborane. Oxidative workup followed by acid-induced cleavage of the acetal protective group provided a mixture of the allylation product and (+)-isopinocampheol, from which the (*R*)-configured allylic alcohol **180** was isolated in 84% yield over 2 steps. Esterification of allylic alcohol **180** with acrylic acid derivative **181** yielded acrylic ester **179** in 77% yield, which served as a precursor to all planned δ-lactone intermediates (**176**, **177** and **178**).



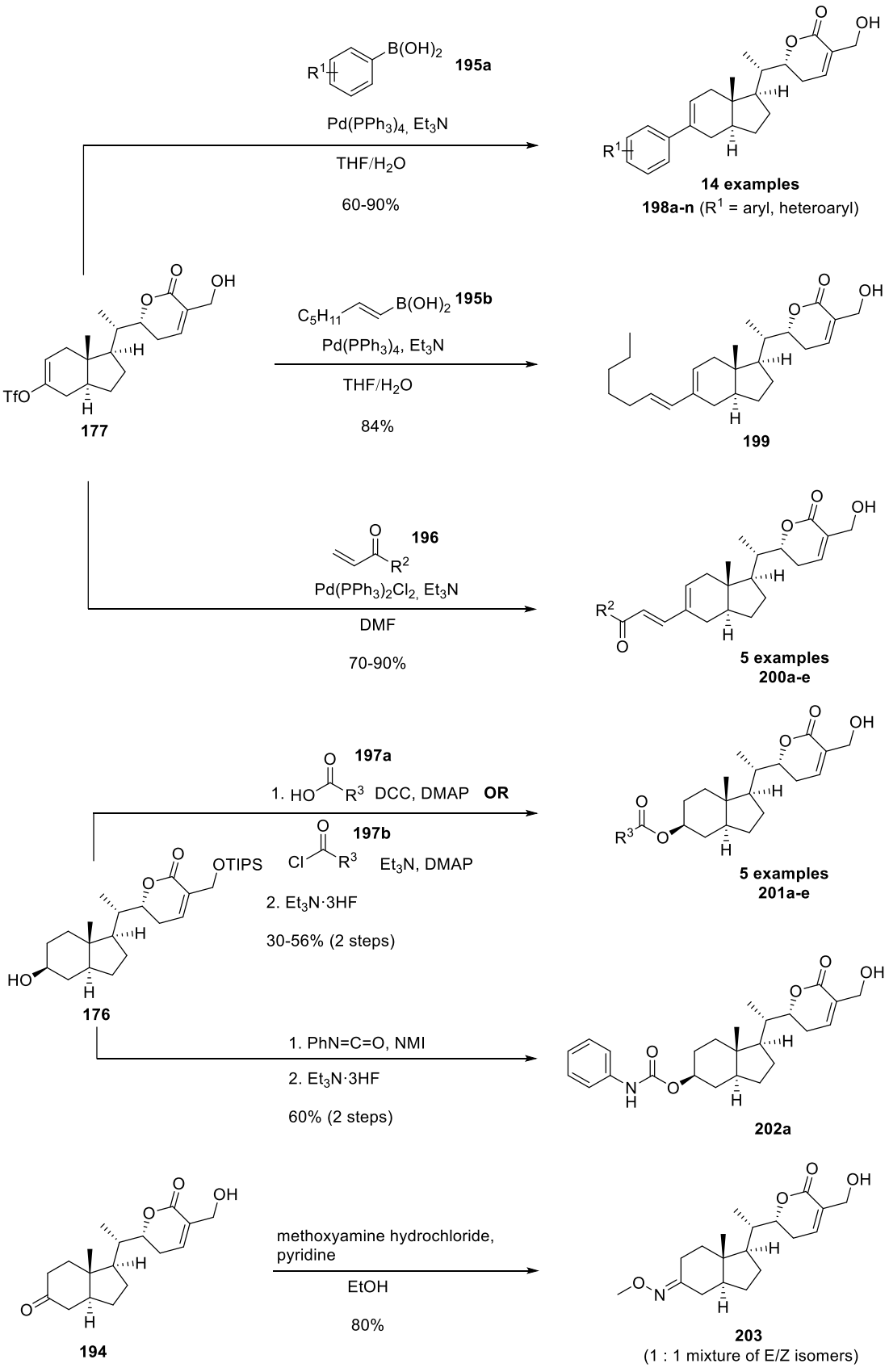
Scheme 33: Synthesis of common precursor 179.

Ring-closing metathesis of protected acrylic ester **179** catalyzed by 30 mol% of ruthenium(II) complex **193**[53, 98] followed by a deprotection step with triethylamine trihydrofluoride yielded δ-lactone **194** (29% over 8 steps from **186**). Chemo- and stereoselective[95] reduction of **178** with excess sodium borohydride provided access to the secondary alcohol **176** (25% over 8 steps from **186**). In order to access the enol triflate intermediate **177**, the ester **179** was treated with potassium hexamethyldisilazide and the resulting enolate was trapped with Commins reagent[99], leading to a single regioisomer of the enol triflate product.[100] TIPS-deprotection with excess triethylamine trihydrofluoride and ring-closing metathesis catalyzed by 10 mol% ruthenium(II) complex **193** were both fully compatible with the presence of the enol triflate. The δ-lactone product **177** was isolated in 54% yield over the three steps (21% over 9 steps from **186**).



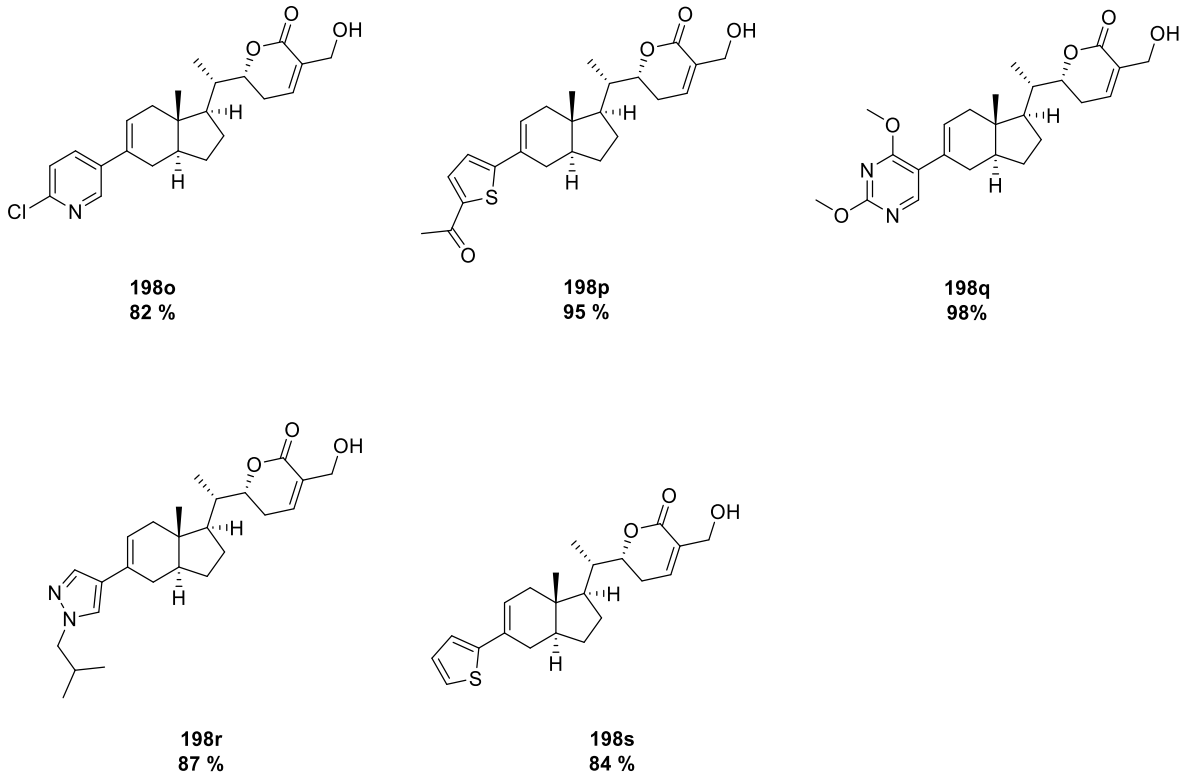
Scheme 34: Synthetic pathway from 179 to late-stage intermediates 194, 176 and 177.

Compounds **194**, **176** and **177** are the late-stage intermediates amenable for final diversifying transformations. TIPS-deprotected ketone **194** was used for an oxime formation with methoxyamine hydrochloride, alcohol **176** underwent a carbamate formation and esterifications, and enol triflate **177** was used for Suzuki and Heck cross couplings (Scheme 35). The following reactions were performed by Ph.D. Jakub Švenda.



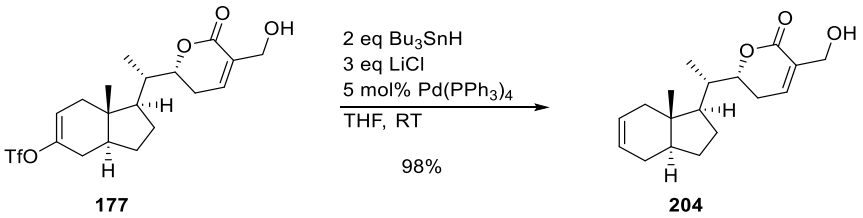
Scheme 35: Derivatization of trans-hydrindane dehydro-δ-lactone intermediates 176, 177 and 194.

In the course of the present work, five Suzuki coupling products with heteroaromatic boronic acids (Scheme 36) were prepared from **177**.



Scheme 36: The five prepared heteroaromatic coupling produts.

A new way of derivatization of the enol triflate was the reductive elimination to the unsubstituted olefin (Scheme 37).



Scheme 37: Reductive elimination of triflate group.

The initial compound collection of 28 compounds was evaluated in COMAS for modulation of Wnt and Hh cell signaling pathways. In the following section, a short introduction to Hh signaling is given. The Hedgehog signaling pathway was discovered in *Drosophila melanogaster*[101] and is evolutionarily conserved. "*Hedgehog*" was the name of a mutant phenotype of Drosophila larvae given by Nüsslein-Volhard and Eric F. Wieschaus. The mechanism and function of Hedgehog signaling has been reviewed in detail.[102] The main components of the mammalian Hh signaling pathway are the three secreted Hh

proteins Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH), the two membrane proteins Patched (PTCH) and Smoothened (SMO), and the glioma-associated oncogene transcription factors (GLI1, GLI2 and GLI3) (Figure 27). In the absence of the Hh ligands, the Hh receptor protein PTCH is localized in the cilium and inhibits SMO by preventing it from trafficking to the cilia. Upon binding of a Hh protein, PTCH is displaced from the cilia, alleviating SMO inhibition and allowing its activation and accumulation in the cilia. Activated SMO elicits a signal transduction pathway that releases GLI transcription factors from a protein complex with Suppressor of Fused (SUFU). Activated GLI proteins translocate to the nucleus and promote the expression of Hh target genes, one of which is PTCH1.[102b]

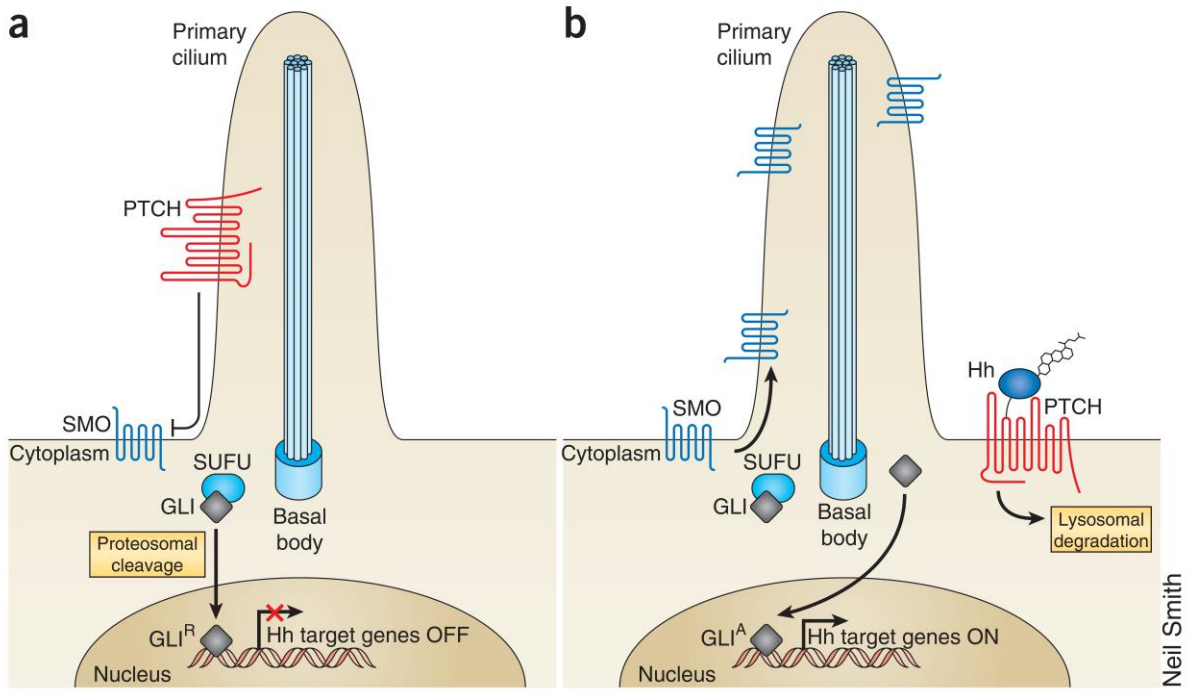


Figure 27: The mammalian Hh signaling pathway. Figure copied from [102b]. Reuse of this figure has been licensed by Nature Publishing Group.

Hh signaling has an important role in ontogenesis and its malfunction leads to aberrant development. In adults it is largely inactive, except for its function in tissue renewal.[103] Activation of the hedgehog pathway has been implicated in the development of cancers in various organs, especially basal cell carcinoma (BCC) of the skin.[104] It is known that mutated SMO can function as an oncogene in BCCs. BCC, a malignancy of the skin, is the most common cancer. It is rarely life-threatening and can most often be removed by surgical excision. Only in a few patients BCC progresses to a life-threatening, inoperable tumor or becomes metastatic.[105]

In the past decades a lot of effort was put in the development of Hh signaling inhibitors, with a major focus on targeting the Smoothened receptor (Figure 28).[106] Many natural products were identidied as modulators of the Hh signaling, notably also several withanolides belonging to the physalin subclass.[107] Physalin H (**205**) acts as an Hh signaling inhibitor by blockink GLI1-DNA-complex formation.[51] Another class of natural product-derived Hedgehog pathway inhibitors is based on vitamin D3 (**207**).[108] Many Hh pathway inhibitors can be grouped in three types according to their molecular target. Those are the Smoothened receptor, Hh proteins and GLI. Among all compounds targeting the Smoothened receptor, cyclopamine (**206**) is the most notable. The plant natural product cyclopamine, isolated from *Veratrum californicum*, inhibits Hh signaling by acting as an antagonist at the SMO receptor.[109] It has a modified steroid core and is biosynthesized from cholesterol.[110] Synthetic Hh signaling inhibitors include SANT-2 (**208**), discovered through small molecule screens.[111] In 2009, the first inhibitor of SHH, the extracellular signaling protein, was reported and coined robotnikinin (**209**). It was a macrocycle derived from a diversity oriented synthesis (DOS) library.[112] In the following, SAR studies on robotnikinin were performed.[113] Steroids are already known to play a role in vertebrate Hh signaling. SMO is endogenously activated by oxysterols, which are oxidized cholesterol derivatives. Oxysterol binding to vertebrate SMO is required for normal Hh signaling.[114] Nedelcu *et al*. developed azasterols that block Hh signaling triggered by the Hh ligand and by oxysterols. The azasterol 22-NHC (**210**) is an inhibitor of SMO and contains the cholesterol scaffold!

Besides Hh signaling inhibitors, Hh pathway activators are also known. In 2002, Wu *et al*. screened a combinatorial library of heterocycles for their ability to induce the differentiation of multipotent mesenchymal progenitor cells into osteoblasts. A substituted purine derivative, named purmorphamine (**211**), showed a strong activity.[115] Later it turned out that purmorphamine does so by activating the Hh pathway as a Smoothened agonist.[116] Another SMO agonist is SAG (**212**)[111a], for which extensive SAR-studies were performed.[117]

The first-in-class, commercial small-molecule Hh inhibitor is vismodegib (**213**) (Erivedge®, Genentech). It was approved in 2012 by the US FDA for treating locally advanced and metastatic basal-cell carcinoma (BCC).[118] Cyclopamine itself cannot be used as a drug because of poor aqueous solubility (ca. 5 µg/ml) and acid lability. It readily undergoes an acid-catalyzed rearrangement to an inactive compound with known structure.[119] While cyclopamine is still used for research purposes, no synthetic derivative thereof has so far been successful as drug candidate. A close derivative named Saridegib (also known as IPI-926)[120] was introduced to a phase II clinical trial in patients with myelofibrosis, but showed disappointing results.[121] Currently, many pharmaceutical companies are developing Hh inhibitors with varying targets.[122] The clinical application of single-agent SMO inhibitors is currently limited to BCC, which is almost solely dependent on Hh signaling. However, BCC rarely

requires systemic therapy. For other cancers, only a fraction of patients harbor mutations in Hh pathway genes.[102b]

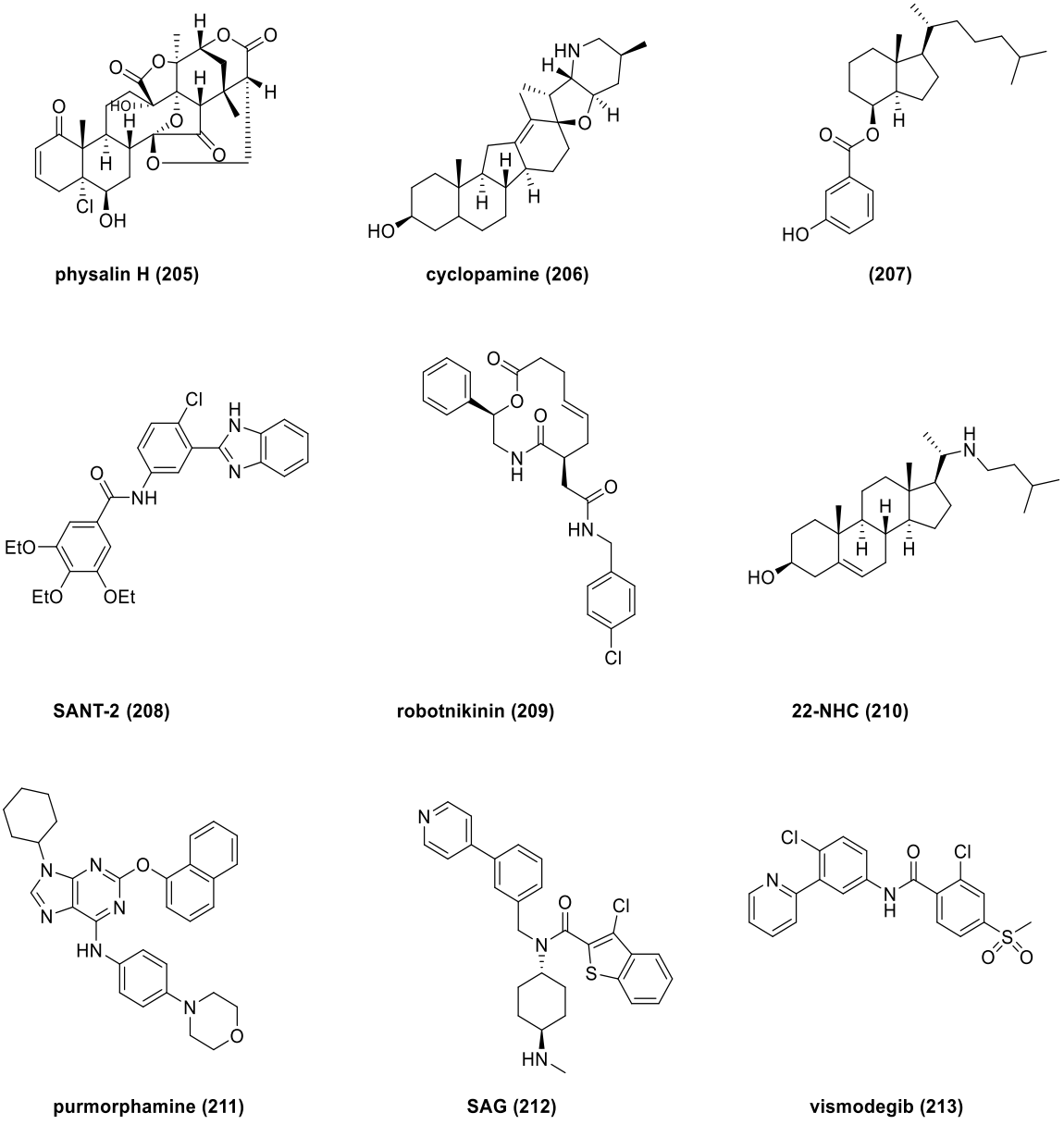
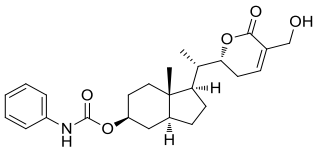
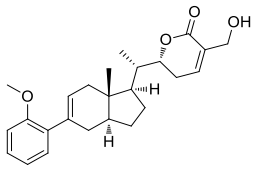
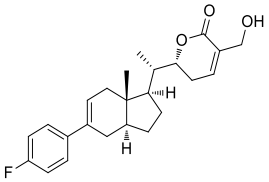
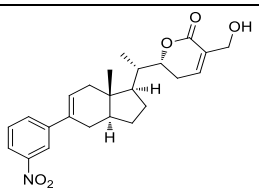
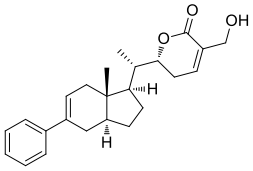


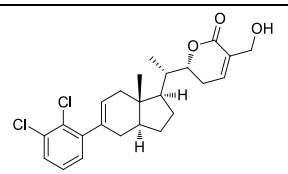
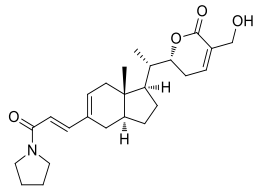
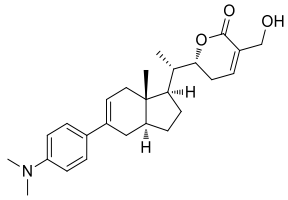
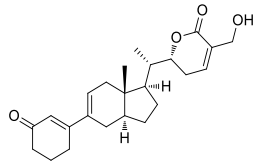
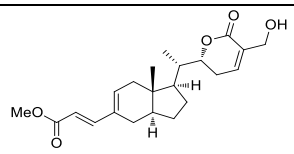
Figure 28: Known Hh signaling modulators.

In COMAS a cell-based assay was performed, which links Hedgehog pathway activity to the enzymatic activity of alkaline phosphatase. The assay uses mouse embryonic mesoderm fibroblast C3H10T1/2 cells. Upon treatment with the Hh signaling agonist purmorphamine (**211**), the cells differentiate into osteoblasts. During differentiation they express osteoblast-specific genes, such as alkaline phosphatase (ALK). The enzymatic activity of ALK is measured by monitoring the luminescence signal of the substrate hydrolysis product. Inhibition of the pathway results in a decrease of luminescence, while pathway activation would result in an increase of luminescence. Several compounds appeared

to significantly inhibit the Hh signaling pathway in the low µM range. The strongest Hh pathway inhibitor was carbamate **202a** (Entry 1) with a mean IC_{50} of 1.22±0.03 µM, while cross coupling and Heck coupling products were less active. Given that many Hh pathway inhibitors target the Smoothened receptor, the question was addressed whether the inhibitors found in the present work do so as well. Herefore, several of the compounds were sent to a SMO binding assay, performed by SB Drug Discovery. The underlying principle is the displacement of [^{3}H]-Cyclopamine from Smoothened membranes and the reference inhibitor SANT-2 (**208**) served as a control. The results are shown Table 4.

Table 4: Initial biological results. All comounds shown below were synthesized by Ph.D. Jakub Švenda. **[a]** Mean IC_{50} values ± s.d. (n ≥ 3) for inhibition of the Hedgehog pathway as determined in an osteogenesis assay. **[b]** Influencce on the viability of C3H10T1/2 cells as determined upon treatment with the compounds at 10 µM for 72 h using the CellTiter-Glo assay; >10 means, the cell viability at 10 µM was between 50-70% while inactive means that >70% cells were viable at 10 µM. **[c]** K_i values ± standard deviation (n≥2), as determined in a Smoothened competition assay using [^{3}H]-radiolabeled cyclopamine.

Entry	Structure	mean Hh IC_{50} [µM][a]	Hh Viability IC_{50} [µM][b]	K_i SMO Binding [µM][c]
1	 **202a**	1.8±0.6	inactive	0.057±0.01
2	 **198a**	1.89±0.53	inactive	10.99±2.96
3	 **198b**	1.98±0.37	inactive	No inhibition at 30 µM
4	 **198c**	2.16±0.7	inactive	6.12±4.04
5	 **198d**	2.32±0.53	> 10	No inhibition at 30 µM

6	 **198e**	2.67±1.07	> 10	No inhibition at 30 μM
7	 **200a**	2.69±0.49	inactive	nd
8	 **198f**	2.75±0.24	inactive	nd
9	 **200b**	2.92±1.12	inactive	nd
10	 **200c**	7.98±0.54	inactive	nd

Phenyl carbamate **202a** was a strong Hh pathway inhibitor and also a Smoothened receptor binder with an inhibition constant (K_i) of 57±10 nM! At the same time it showed no cytotoxicity. A deeper investigation of **202a** was started. The first goal was the synthesis of a sublibrary of carbamate analogues.

3.2.3 In-depth Analysis of Carbamates

Building on phenyl carbamate **202a,** a more focused library was prepared (Figure 29). Four variants of the lactone were prepared, including variants with and without the hydroxymethyl group, as well as with or without the unsaturation. In addition, compounds which completely lack the lactone were also prepared.

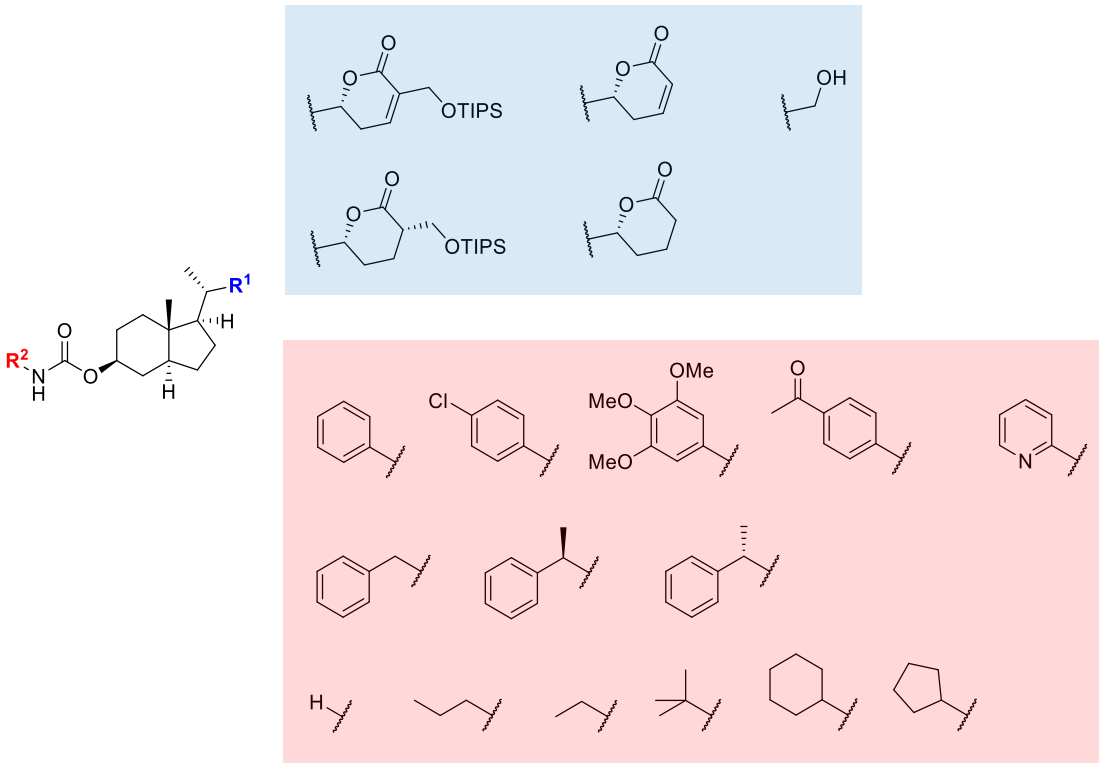
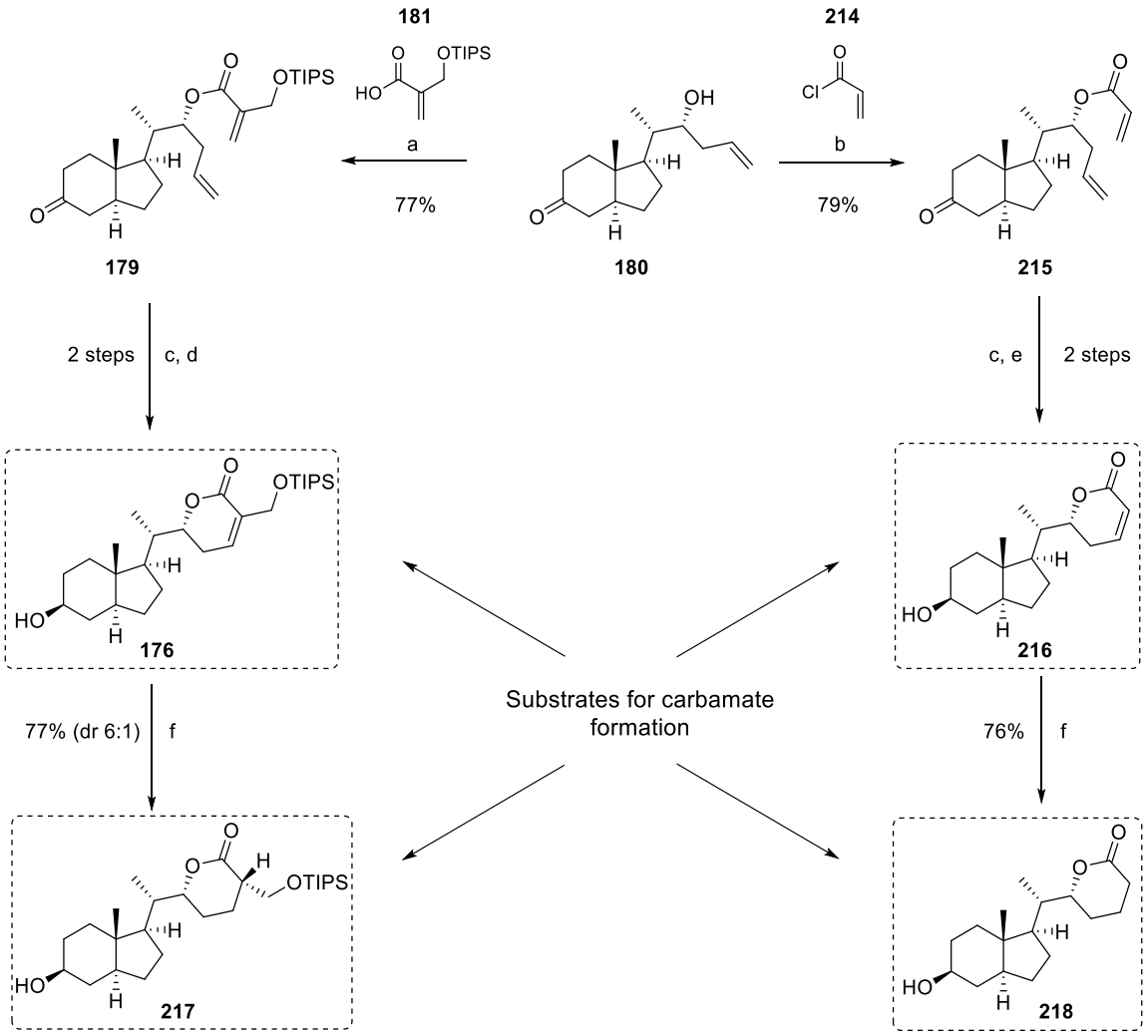


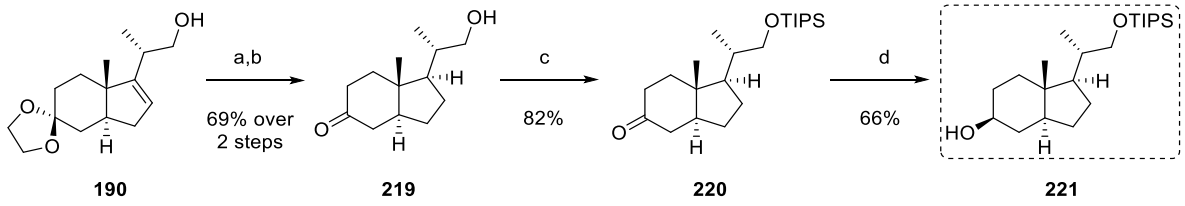
Figure 29: Synthesis of a focused carbamate library.

For the synthesis of all different δ-lactone intermediates, homoallylic alcohol **180** was used. Besides esterification with building block **181**, homoallylic alcohol **180** was also acylated with acryloyl chloride (**214**) (Scheme 38). In this case, the following RCM can be performed with 5 mol% catalyst only, compared to the RCM of **179**. For the following ketone reduction lithium *tri-tert*-butoxyaluminum hydride had to be used instead of $NaBH_4$. With $NaBH_4$ significant amounts of side products were formed, presumably due to reaction with the unsaturated lactone. In order to study the biological effects of unsaturation, compounds **176** and **216** were hydrogenated to produce saturated lactones **217** and **218**. In case of **176**, hydrogenation led to an inseparable epimeric mixture in a ratio of 6:1, with the major diastereomer as reported.[59a, 123] Hydrogenations of this system in the context of withanolide synthesis were studied by Iwadate *et al.* in detail.[124]



Scheme 38: Pathway from 180 to substrates for carbamate formation. (a) DCC, DMAP, RT; **(b)** Et_3N, RT; **(c)** Stewart-Grubbs ruthenium catalyst (**193**), toluene, 80 °C; **(d)** $NaBH_4$, 2-propanol, 0 °C; **(e)** $LiAlH[OtBu]_3$, THF, 0 °C; **(f)** Pd/C, H_2, THF, RT.

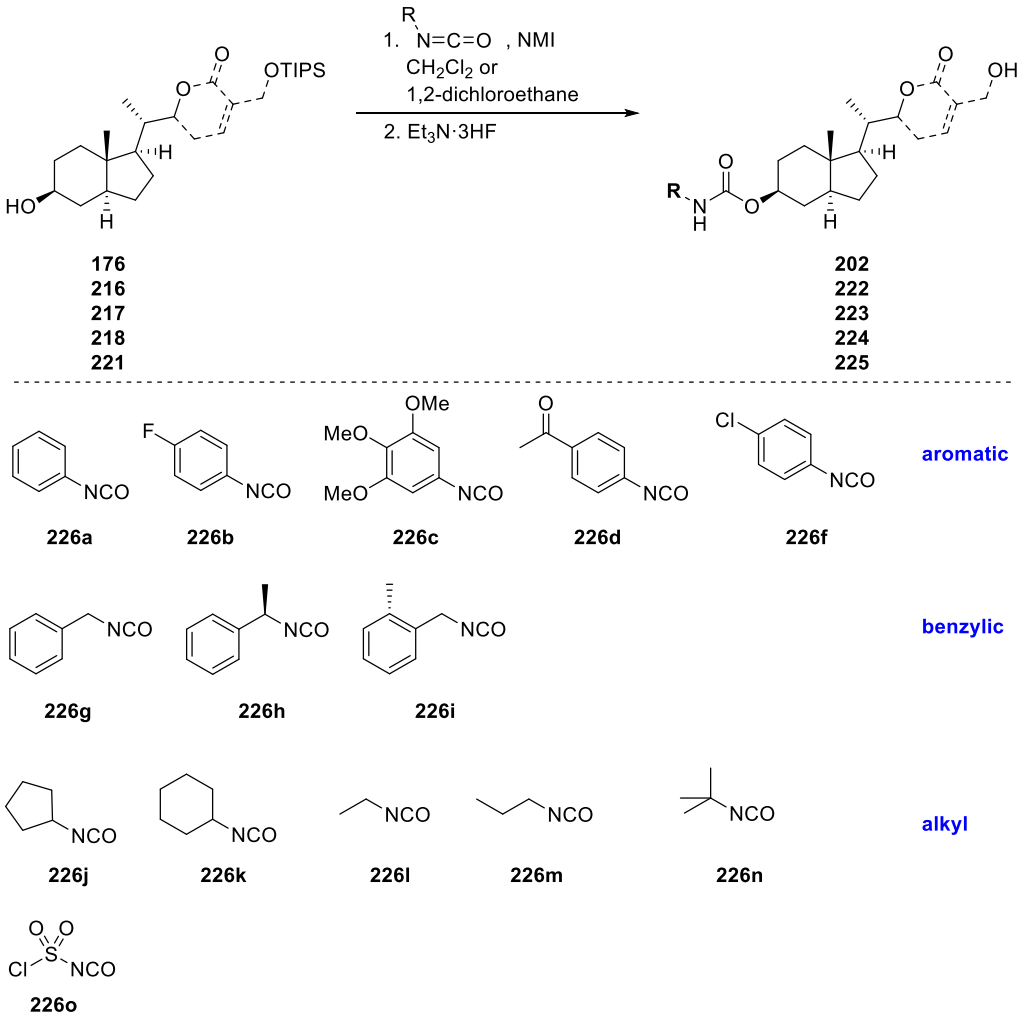
In order to address whether the lactone is a structural requirement for Hh inhibition, a series of analogues lacking the δ-lactone was synthesized. To this end, intermediate **190** was transformed into mono-TIPS-protected alcohol **221** in four steps (Scheme 39).



Scheme 39: Synthesis of compound 221. (a) Pd/C, THF, RT; **(b)** *p*-TsOH·H_2O, acetone/H_2O, 50 °C; **(c)** TIPS-Cl, Imidazole, CH_2Cl_2, RT; **(d)** $NaBH_4$, 2-propanol, 0°C.

Five series of carbamate analogues were synthesized from **176**, **216**, **217**, **218** and **221**. Electron-rich as well as electron-deficient aromatic isocyanates, benzyl isocyanates and alkyl isocyanates were used.

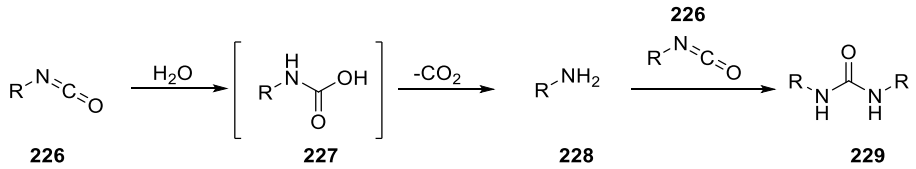
The carbamate formations were generally performed in DCM or in 1,2-dichloroethane in case higher temperature was necessary (Scheme 40). The number of equivalents of the isocyanate was adjusted according to their reactivity. 1-Methylimidazole (NMI) is an effective catalyst for this transformation.[125] In general, aromatic isocyanates are far more reactive than aliphatic analogues. Electron withdrawing groups on the phenyl ring do further increase the reaction rate, while electron donating ones do decrease the rate. The extremely unreactive *tert*-butyl isocyanate was used as a co-solvent. After carbamate formation the reaction mixture was worked up and the TBS-deprotection was performed in case of the carbamate series from **176**, **217** and **221**. A slightly different protocol was used for the carbamate formation with chlorosulfonyl isocyanate **226o**. In this case, after carbamate formation the chlorosulfonyl group is hydrolyzed to reveal the carbamoyl moiety.



Scheme 40: Carbamate synthesis from alcohols 176, 216, 217, 216 and 221.

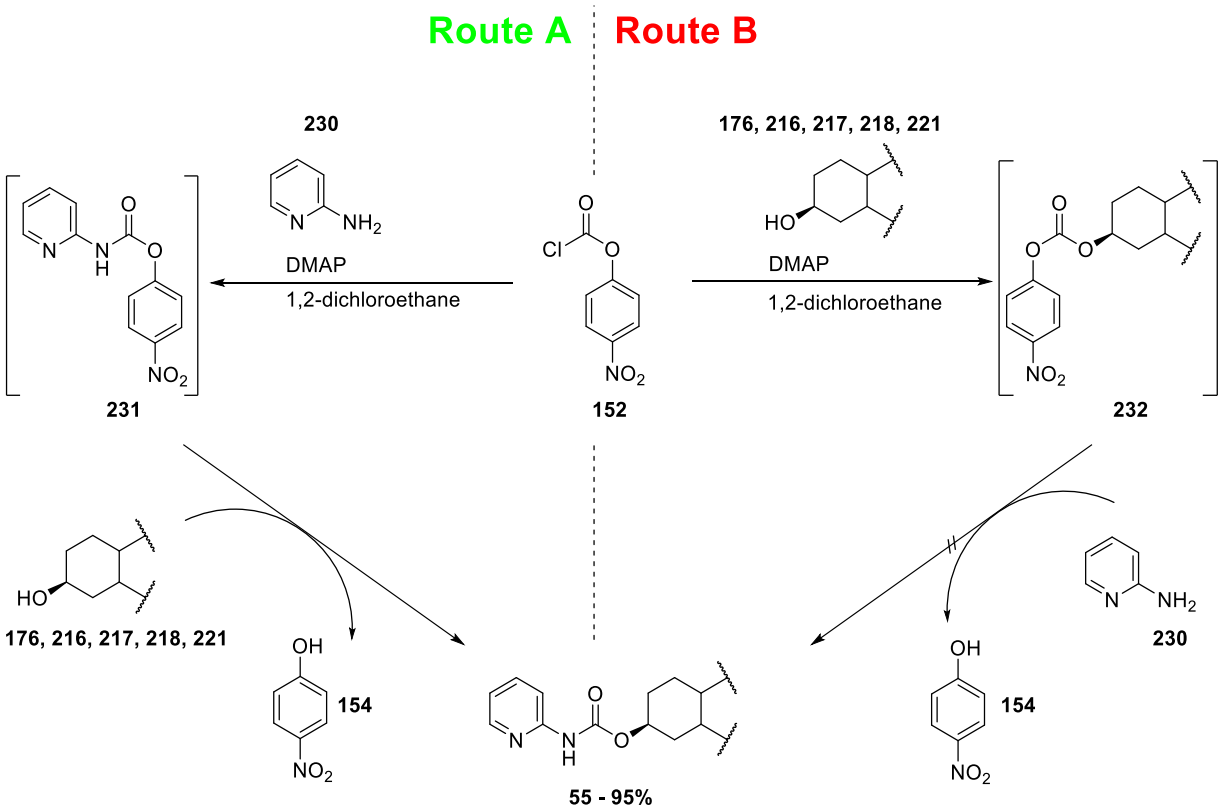
One reoccurring problem in the carbamate formations was the undesired formation of urea (Scheme 41), a known problem in the reaction between alcohols and isocyanates.[126] Even if the reaction was performed in anhydrous solvents, the problem remained because urea could form during aqueous

workup or on the column if no workup was performed. The isocyanates themselves also partly contained urea impurities. In some cases it was impossible to separate the desired product from the urea side product.



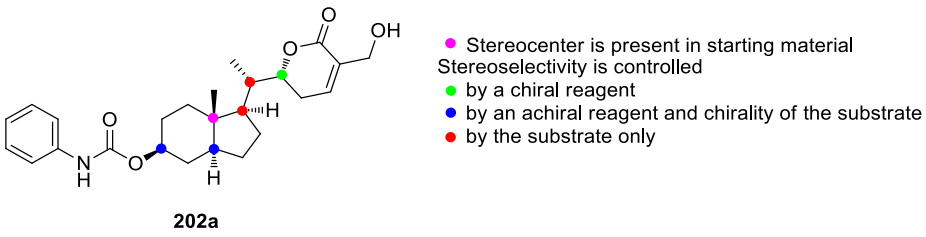
Scheme 41: Urea formation from an isocyanate in the presence of water.

Another method for carbamate formation was used for the synthesis of 2-pyridyl carbamates because the respective isocyanate is commercially not available (Scheme 42, Route A). 2-Aminopyridine (**230**) was treated with 4-nitrophenyl chloroformate (**152**) in the presence of DMAP to generate a reactive carbamate intermediate (**231**). This intermediate was added to a solution of the starting material and substitution of 4-nitrophenol yielded the desired product. This is a reverse way of carbamate formation compared to Scheme 27 (page 50). In the present case, 2-aminopyridine proved too unreactive to substitute 4-nitrophenol from the carbonate intermediate **232** (Route B).



Scheme 42: Synthesis of 2-pyridyl carbamates.

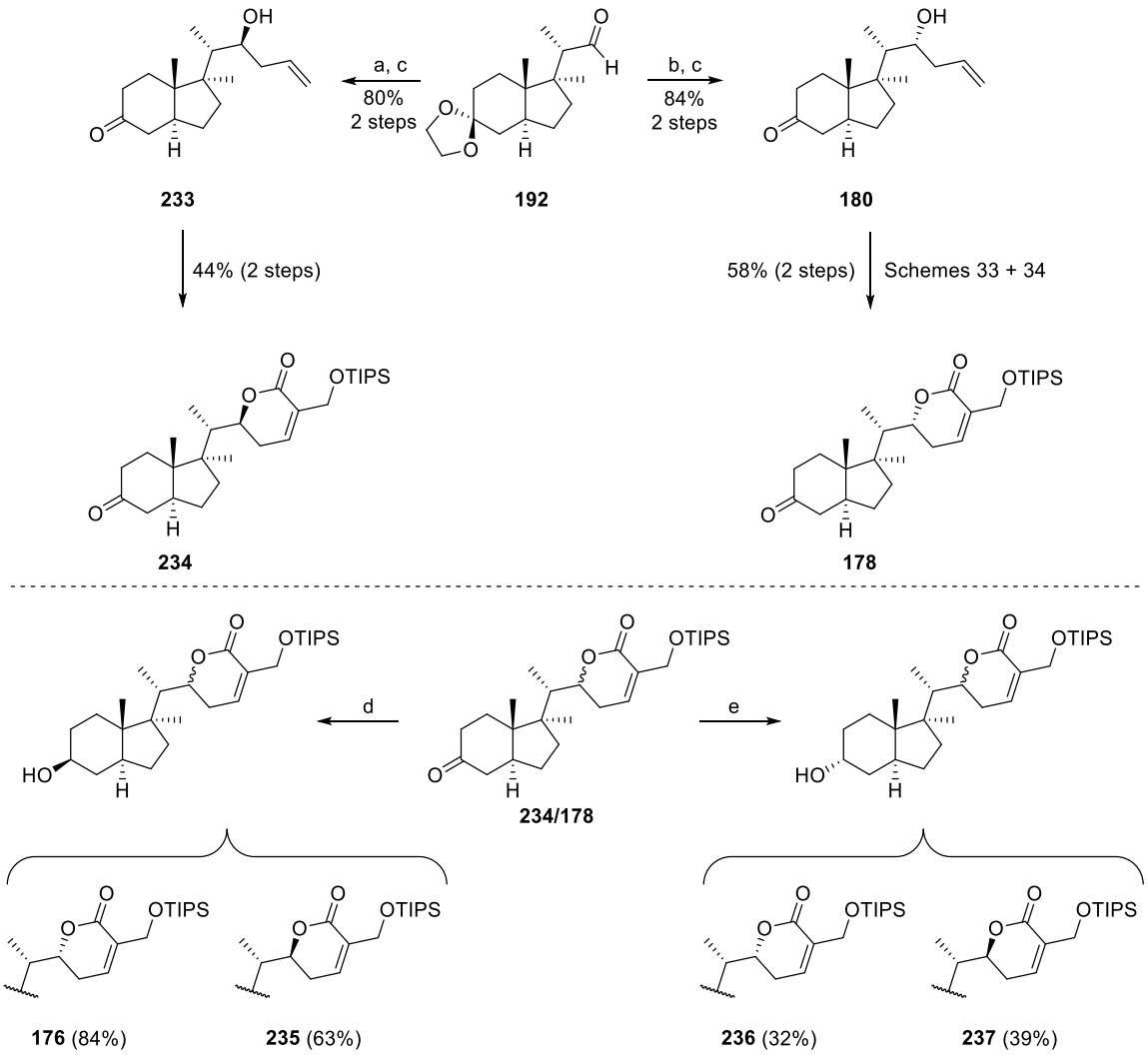
In order to probe the necessity of a stereoselective synthesis, an additional series of withanolide analogues with non-natural configuration at two carbon atoms was prepared. The two modified stereocenters are the one at the alcohol in the *trans*-hydrindane core (marked in blue, Figure 30) and the stereocenter within the lactone (marked in green). This was possible due to the fact that both stereocenters are introduced in reagent-controlled reactions. The other theroretically controllable stereocenter marked in blue was not varied because the switch from a *trans*-hydrindane to a *cis*-hydrindane would completely alter the geometry of the molecule. This would probably also change the diastereoselectivity in the introduction of the stereocenters marked in red, which are generated in purely diastereoselective reactions and cannot be controlled.



202a

Figure 30: Origin of the stereocenters

While Brown allylation of aldehyde **192** with (+)-Ipc_2B(allyl)borane yields a homoallylic alcohol with a configuration as it occurs in the lactone of naturally occurring withanolides (**180**), allylation with (-)-Ipc_2B(allyl)borane yields the epimer **233**. The two following steps are equal to Scheme 33 and Scheme 34 and lead to epimeric ketone **234**. The opposite configuration of the secondary alcohol was achieved by reduction of ketones **178** and **234** with L-Selectride (Scheme 43).[127]



Scheme 43: Generation of stereoisomeric analogues. (a) (-)-Ipc$_2$B(allyl)borane, H_2O_2, NaOH, THF; **(b)** (+)-Ipc$_2$B(allyl)borane, H_2O_2, NaOH, THF; **(c)** *p*-TsOH·H_2O, acetone/H_2O, 50 °C; **(d)** $NaBH_4$, 2-propanol, 0 °C; **(e)** L-Selectride, THF, -78 °C.

Besides the carbamates themselves, the parent substrates for carbamate formation **216** and **218** were also submitted to COMAS to undergo the Hh pathway inhibition assay. In case of **176**, **217** and **221** the respective TBS-deprotected versions **238**, **239** and **240** were prepared and assayed (Figure 31).

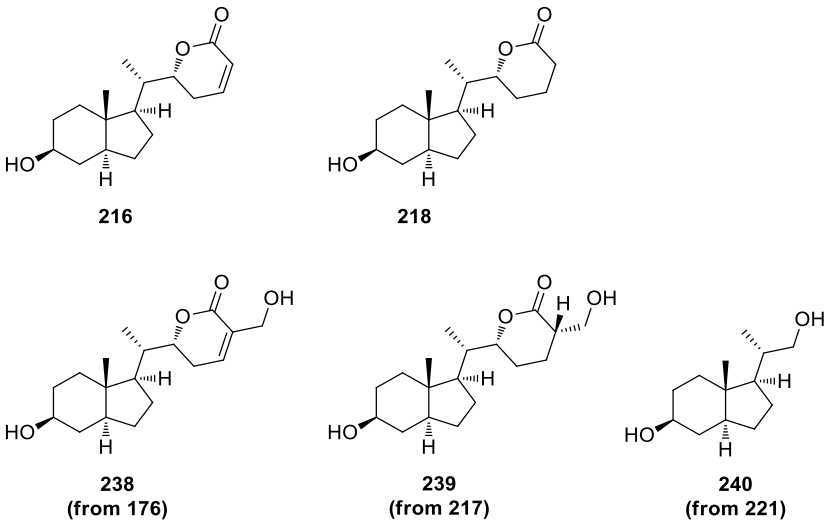


Figure 31: The five substrates for carbamate formation without TBS groups.

The compound collection was submitted to COMAS and underwent a cell-based screen for modulation of the Hh pathway. The most active compounds from the COMAS screen which were also not cytotoxic were again submitted to the Smoothened receptor binding assay performed at SB Drug Discovery©. The complete dataset is shown in Table 5.

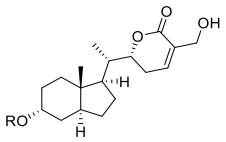
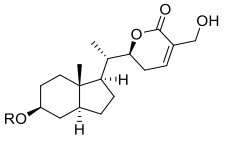
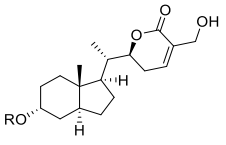
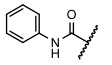
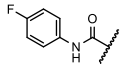
Table 5: Full dataset. [a] Mean IC_{50} values ± s.d. (n ≥ 3) for inhibition of the Hedgehog pathway as determined in an osteogenesis assay. **[b]** Influencce on the viability of C3H10T1/2 cells as determined upon treatment with the compounds at 10 µM for 72 h using the CellTiter-Glo assay; >10 means, the cell viability at 10 µM was between 50-70% while inactive means that >70% cells were viable at 10 µM. **[c]** K_i values ± standard deviation (n≥2) for **202a** and **202f**, otherwise n=1, as determined in a Smoothened competition assay using [^{3}H]-radiolabeled cyclopamine.

R	mean Hh IC_{50} [µM][a]	Hh Viability IC_{50} [µM][b]	**202** K_i Smo Binding [µM][c]	mean Hh IC_{50} [µM][a]	**223** Hh Viability IC_{50} [µM][b]	mean Hh IC_{50} [µM][a]	**222** Hh Viability IC_{50} [µM][b]	K_i Smo Binding [µM][c]	mean Hh IC_{50} [µM][a]	**224** Hh Viability IC_{50} [µM][b]	K_i Smo Binding [µM][c]	mean Hh IC_{50} [µM][a]	**225** Hh Viability IC_{50} [µM][b]	K_i Smo Binding [µM][c]
a	**1.8 ± 0.6**	inactive	0.057 ± 0.01	3.0 ± 0.6	inactive	2.7 ± 0.6	3.6	2.95	6.0 ± 1.1	inactive	6.10	inactive	8.2	14.1
b	2.4 ± 0.30	inactive												
c	2.5 ± 0.4	8.3		4.0 ± 0.3	inactive	3.6 ± 0.5	3.9		inactive	inactive		3.6 ± 0.3	4.1	
d	2.7 ± 0.2	9.3	0.26	6.6 ± 1.7	inactive	4.4 ± 0.4	4.9	0.54	> 10	inactive		3.6 ± 0.8	7.0	4.19
e	6.3 ± 2.1	inactive		5.8 ± 0.6	inactive	7.9 ± 1.3	4.1		inactive	inactive		inactive	> 10 µM	
f	2.3 ± 0.3	> 10	0.134 ± 0.06	3.4 ± 0.3	inactive				inactive	inactive		6.2 ± 1.3	3.6	5.32
g	4.1 ± 0.3	inactive	1.18						inactive	inactive				
h	3.4 ± 0.4	9.5	0.67									> 10 µM	inactive	
i	4.1 ± 0.2	9.1										6.6 ± 1.9	inactive	
j	3.6 ± 0.4	inactive	1.91			5.7 ± 0.7	6.8	2.59	inactive	inactive		inactive	inactive	10.3
k	2.4 ± 0.3	> 10	18.1											
l	inactive	inactive												
m	4.2 ± 0.9	inactive												
n	6.4 ± 1.4	inactive	0.46											
o						inactive	> 10							
	238			**239**		**207**			**209**			**240**		
	inactive	inactive	nd	inactive	inactive		toxic		inactive	inactive		inactive	inactive	
Number	**14 compounds**			**5 compounds**		**6 compounds**			**7 compounds**			**8 compounds**		

Total Number 40 compounds

The desired combination of properties for a compound is strong activity and low toxicity. The series of analogues with an unsaturated lactone and the hydroxymethyl group combines strong Hh pathway inhibition with low toxicity. The phenyl carbamate and substituted analogues are the most active. Hydrogenation of the lactone significantly reduces the activity while the compounds remain non-toxic. An intriguing observation is that compounds with an unsubstituted, unsaturated lactone are toxic, whereas those with a hydroxymethyl group are not. This observation is in accordance with the Michael acceptor reactivity trends explained above (page 20). Saturated analogues lacking the hydroxymethyl group are poor Hh pathway inhibitors and are not toxic. The truncated series of compounds **225** contains moderately active Hh pathway inhibitors, which are in part toxic. The molecules are very poor SMO binders in the micromolar range. The strongest Hh pathway inhibitor **202a** has an IC_{50} of 1.8±0.6 µM and is also a SMO binder with a K_i of 57±10 nM. SMO is a 7-transmembrane GPCR and a key element of the Hedgehog signaling. The diastereomers of **202a** are phenyl carbamates **241a**, **242a** and **243a**. All are significantly less potent as Hh pathway inhibitors (Table 6). This finding emphasizes the necessity of a stereoselective synthesis. **202a** possesses the C22-configuration of natural withanolides, and a deviation from the natural product diminishes the biological activity. 4-fluorophenyl carbamate **242b** is also significantly less active than **202a**.

Table 6: Diastereomeric carbamates. [a] Mean IC_{50} values ± s.d. (n ≥ 3) for inhibition of the Hedgehog pathway as determined in an osteogenesis assay. **[b]** Influencce on the viability of C3H10T1/2 cells as determined upon treatment with the compounds at 10 µM for 72 h using the CellTiter-Glo assay; >10 means, the cell viability at 10 µM was between 50-70% while inactive means that >70% cells were viable at 10 µM.

	mean Hh IC_{50} [µM][a]	**Hh Viability IC_{50} [µM][b]**	**mean Hh IC_{50} [µM][a]**	**Hh Viability IC_{50} [µM][b]**	**mean Hh IC_{50} [µM][a]**	**Hh Viability IC_{50} [µM][b]**
R	 **241**		 **242**		 **243**	
 **a**	9.40 ± 2.75	inactive	not determined	toxic (IC_{50} not determied)	inactive	inactive
 **b**	-		3.20 ± 0.4	> 10	-	

Further experiments were performed by Lea Kremer in order to link the observed Hh pathway inhibition in COMAS to the antagonistic effect at the SMO receptor (Figure 32). To this end, **202a** was compared to vismodegib, the only approved Smoothened antagonist drug. As a first step, a reporter gene assay was performed. NIH/3T3 cells were stably transfected with a GLI-responsive firefly luciferase reporter plasmid[128] and a pRL-TK constituitive *Renilla* luciferase expression vector (SHH-LIGHT2 cells[109c]). The cells were treated with **202a** or DMSO as a control in the presence of the Hh signaling agonist purmorphamine (**211**, page 62). Firefly luciferase/*Renilla* luciferase ratios were determined at different concentrations of **202a**. Apparently, **202a** inhibits GLI-dependent reporter gene expression, as seen by the relative decrease of firefly luciferase expression compared to *Renilla* luciferase (Figure 32a).

The expression of Hh target genes can be monitored by the quantification of mRNA (Figure 32b). NIH/3T3 cells were incubated with purmorphamine and 2 µM of **202a** or vismodegib and DMSO as controls for 48 h. After cell lysis and cDNA preparation, quantitative PCR was carried out employing oligonucleotides which are specific for PTCH1 or GAPDH as a reference gene. Compared to cells only treated with purmorphamine, **202a** decreases the expression of the Hedgehog target gene Patched 1 (PTCH1) in the same range as vismodegib.

The displacement of cyclopamine from SMO caused by **202a** can be visualized with the use of BODIPY-cyclopamine (Figure 32c). BODIPY-cyclopamine is a commercially available compound, in which the fluorophore BODIPY is covalently linked to cyclopamine. HEK293T cells, transfected with a SMO expression plasmid, were fixed and treated with **202a** or vismodegib and DMSO as controls in the presence of BODIPY-cyclopamine. In order to visualize DNA, the cells were then stained with 4',6-diamidino-2-phenylindole (DAPI, blue). The displacement of BODIPY-cyclopamine from SMO is visible from the decrease of green fluorescence in cells treated with **202a** or vismodegib.[116a] A comparable result was also obtained from flow cytometry (Figure 32d)[109d]. HEK293T cells were transfected with a SMO expression plasmid. Two days later, the cells were treated with **202a** or DMSO as control in the presence of 5 nM BODIPY-cyclopamine. The cells were then subjected to flow cytometry analysis to detect SMO-bound BODIPY-cyclopamine. Compared to DMSO-treated and unstained cells, both vismodegib and **202a** strongly interfere with the binding of BODIPY-cyclopamine to SMO. The effect of vismodegib is more pronounced than that of **202a**.

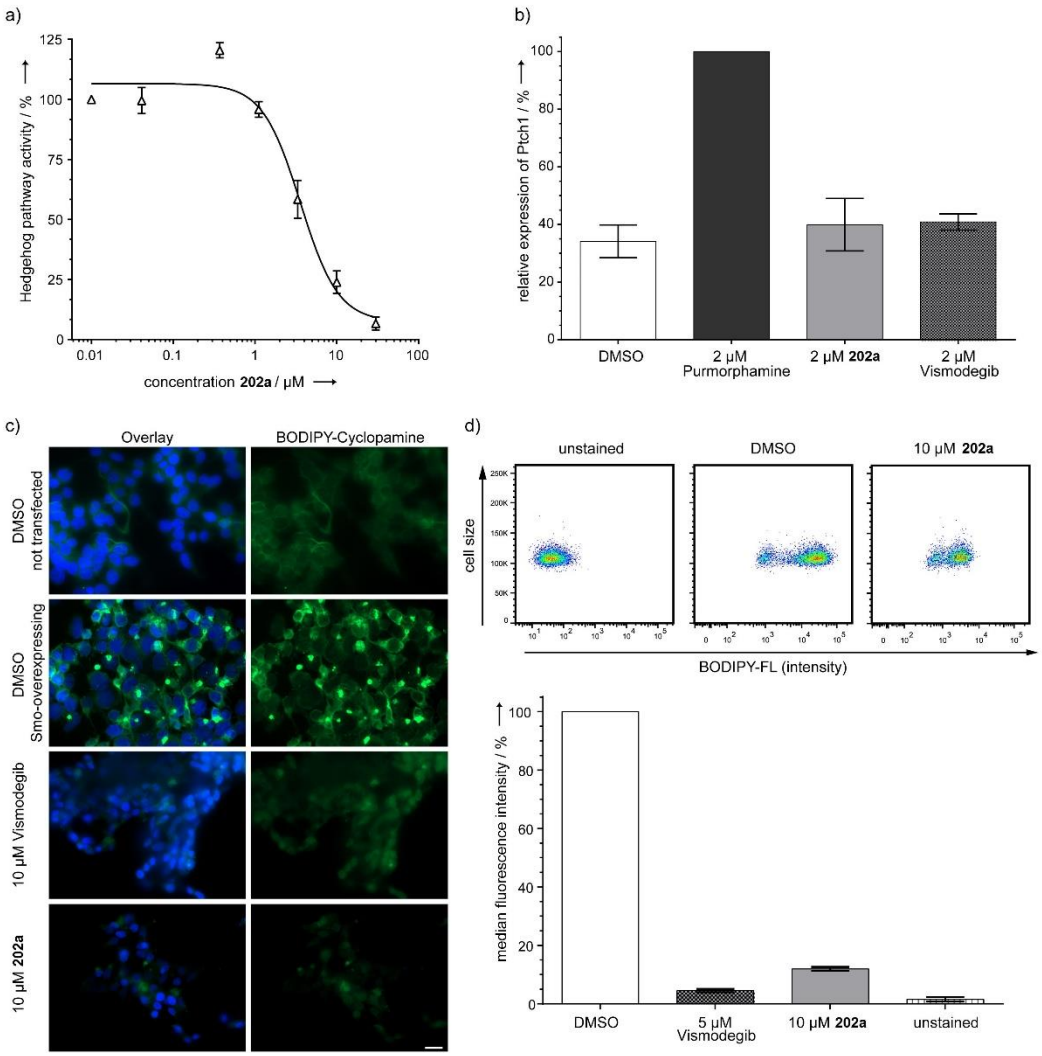


Figure 32: Biological experiments with 202a. (a) 202a inhibits GLI-dependent reporter gene expression. SHH-LIGHT2 cells were treated with 4 µM purmorphamine and different concentrations of **202a** or DMSO as control for 48 h. Firefly luciferase/*Renilla* luciferase ratios were determined. Values are expressed as percentage of DMSO-treated cells. Nonlinear regression was performed using a four parameter fit. Data are mean values (n = 3) ± s.d. **(b) 202a** decreases the expression of the Hedgehog target gene Patched 1 (PTCH1). NIH/3T3 cells were incubated with 2 µM purmorphamine and different concentrations of **202a** or vismodegib and DMSO as controls for 48 h. Upon cell lysis and cDNA preparation, quantitative PCR was carried out employing specific oligonucleotides for PTCH1 or GAPDH as a reference gene. Expression levels of PTCH1 were normalized to the levels of GAPDH and are depicted as percentage of purmorphamine-activated cells (100%). Data are mean values (n = 3) ± s.d. **(c and d)** Compound **202a** interferes with the binding of BODIPY-cyclopamine to SMO. **(c)** HEK293T cells were transfected with a SMO expression plasmid. 48 h later cells were fixed and treated with **202a** or vismodegib and DMSO as controls in the presence of 5 nM BODIPY-cyclopamine for 4 h. Cells were then stained with DAPI (blue) to visualize the DNA. Scale bar: 20 µm. **(d)** HEK293T cells were transfected with a SMO expression plasmid. Two days later, cells were treated with **202a** or DMSO as control in the presence of 5 nM BODIPY-cyclopamine for 5 hours. Cells were then subjected to flow cytometry analysis to detect SMO-bound BODIPY-cyclopamine. The graph shows the median BODIPY-cyclopamine fluorescence intensity of SMO-transfected cells upon treatment with the compounds. Data are mean values (n = 3) ± s.d. and are presented as percentage of DMSO-treated cells (100%). (Reuse of this figure has been licensed by Wiley.)

In summary, a compound collection based on the *trans*-hydrindane dehydro-δ-lactone scaffold was synthesized in a stereoselective manner. The synthesis strategy features the preparation and selective functionalization of three functionalized key intermediates. A biological investigation of the collection revealed novel and potent inhibitors of the Hedgehog signaling pathway. The molecular mechanism of action was shown to be the binding to the Smoothened protein.

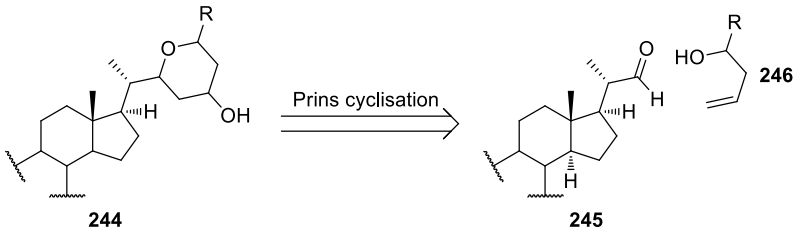
3.2.4 Discussion & Outlook

Targeted cancer chemotherpy has become an emergent concept in the past decades.[129] Standard chemotherapueutics act on all rapidly dividing normal and cancerous cells by interfering with ubiquitious structures like DNA or microtubules. A classic example of a generally cytotoxic drug is cisplatin. Cisplatin crosslinks DNA, thereby interfering with mitosis in all body cells.[130] In contratst to this, targeted therapies block the growth and spread of cancers by interfering with a specific molecular target (typically a protein) that has an essential role in tumour growth. One approach towards targeted therapy is the use of small molecule inhibitors of tumor-specific proteins. This has been already validated by clinic success as seen in the example of imatinib. Imatinib is a tyrosine-kinase inhibitor used in the treatment of chronic myelogenous leukemia (CML) ans is regarded a breakthrough in cancer treatment.[131] Another technique is the targeting of cell-surface receptors with monoclonal antibodies, possibly also conjugated to small molecule drugs.[132] An important example oft he latter is the monoclonal antibody Trastuzumab, which blockst he HER2 receptor on the cell surface of certain types of breast cancer.[133] Applied to the present work, a possible application of **202a** would be further developmet towards a drug in targeted cancer chemotherapy, indicated for patients with an overactive Hh signaling pathway.

The other major application of a bioactive compound is that as a chemical probe. Both for drugs and probes high potency is vital. Beyond that, drug candidates and tool compounds must meet different criteria.[134] For a drug to be successful, bioavailability, metabolism, cost of manufacture and so on are preconditions for success. On the other hand, selectivity for one target is not essential and the mechanism of action may even be unknwon. Contrary to this, among the prerequisites of a useful probe are a known mechanism of action and well-defined selectivity. Interaction with multiple targets is undesired for probes.[134b, 135] The question of selectivity of **202a** for SMO has not been addressed by the present work and would be the first question if follow-up studies should be conducted.

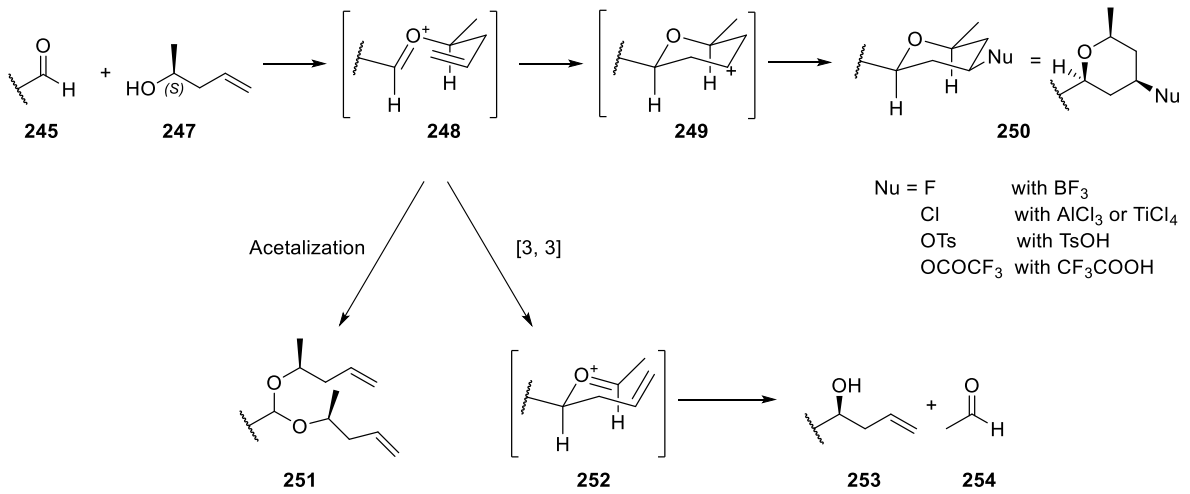
3.3 Part C: Prins cyclizations

In the projects described above, the α,β-unsaturated δ-lactone was constructed in a multistep procedure from an aldehyde. We were interested in finding a way how a lactone or a lactone mimic could be prepared quicker, using the intermediates described above. From literature it is known that Prins-type cyclizations are a powerful way for the stereoselective synthesis of substituted tetrahydropyran rings from aldehydes (Scheme 44). Its application in natural product synthesis is substantial and has been reviewed.[136]



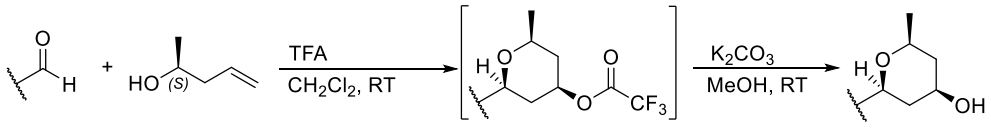
Scheme 44: Retrosynthetic analysis of a Prins cyclization.

The stereochemical outcome of the Prins cyclization is dictated by the orientation of substituents in the six-membered transition state (Scheme 45). Alkyl groups will generally adapt pseudoequatorial orientations in the transition state. The predictability of the stereochemical outcome adds to the synthetic value of this reaction. From the mechanism, typical side reactions are clearly visible. In the presence of an excess of homoallylic alcohol a Brønsted- or Lewis-acid-catalyzed acetalization can occur. Furthermore, the [3,3]-oxonia-Cope rearrangement leads to a scrambling of subunits.[136]



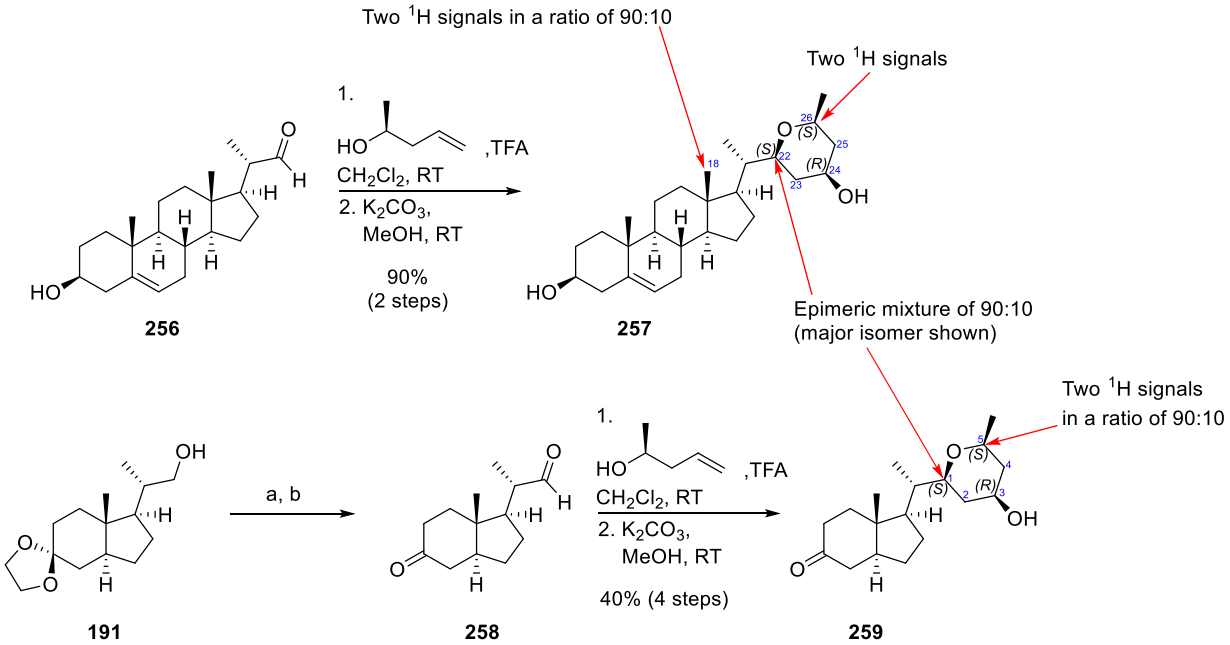
Scheme 45: The mechanism of the Prins cyclization and possible side reactions.

From all available literature procedures describing Prins cyclizations, in the present work a report from Barry *et al*. was used.[137] It uses TFA as Bronsted acids catalyst and results in a hydroxyl substituent para to the oxygen. Initially the trifluoroacetate anion reacts as nucleophile, followed by basic methanolysis of the labile ester (Scheme 46). The intermediate ester was not isolated.



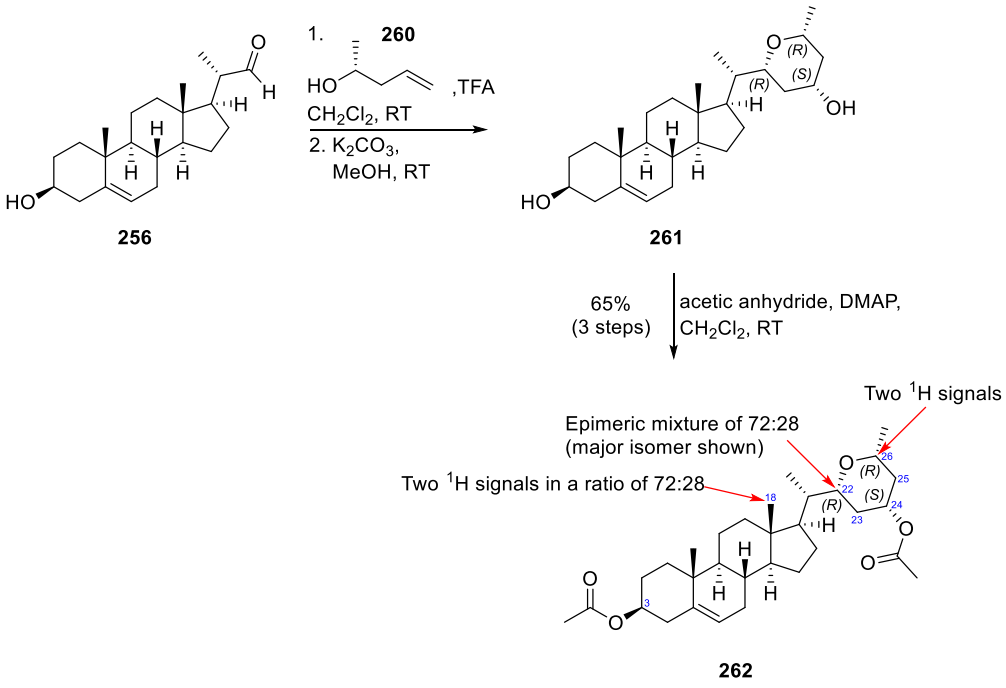
Scheme 46: TFA-catalyzed Prins cyclization.

The aldehydes **256** and **258** were used for Prins cyclizations (Scheme 47). While aldehyde **256** could be isolated and was benchstable, unstable aldehyde **258** was prepared from intermediate **191** by acetal deprotection and oxidation, and immediately used for Prins cyclization. Both aldehydes underwent Prins cyclization with (*S*)-(+)-4-penten-2-ol. The configuration of the newly formed stereocenters can be inferred from the mechanism above. However, both products appear to be mixtures of stereoisomers in a ratio of 90:10 at C-22 of **257** and the corresponding carbon atom C-1 in **259**. For **257**, the ratio was determined based on integration of the singlet proton signal at C-18. The proton signals at C-22 and C-26 are split and partly overlap, while the proton signal at C-24 is not split and does therefore represent a single stereoisomer at this position. For **259**, the ratio was determined based on integration of the proton signals at C-5. Here as well, the proton signal at C-3 is not split.



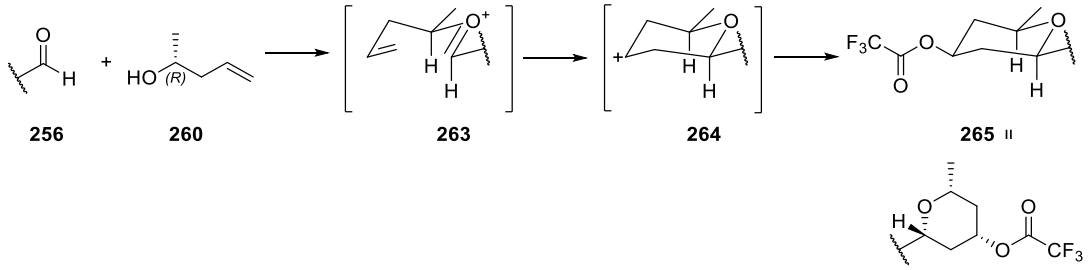
Scheme 47: Prins cyclizations with (*S*)-(+)-4-penten-2-ol. (a) *p*-TsOH·H_2O, acetone/H_2O, **(b)** PCC, CH_2Cl_2, RT.

256 was also subjected to Prins cyclization with (*R*)-(−)-4-penten-2-ol (Scheme 48). The product **261** was extremely poorly soluble in all common organic solvents and even crushed out from DMSO. It was therefore diacetylated to **262** in order to obtain a soluble compound. An inseparable mixture of two spots on TLC formed, which were isolated together. As before, ^{1}H and COSY NMR spectra were used to infer the stereochemical outcome at the two newly formed stereocenters at C-22 and C-24. The NMR shows a mixture of diastereomers in a ratio of 72:28 based on integration of the ^{1}H signals from C-18. The proton signals at C-3 and C-24 cannot be distinguished, but appear both as single signals. Compared to this, the signals at C-22 and C-26 are split, as before hinting towards an epimeric mixture at C-22. In accordance to the stereochemical model, comparing the proton signal to that of **257**, the configuration at C-22 appears to be the opposite one. Hence, the structure shown below most likely represents the major isomer of the product.



Scheme 48: Reaction of 256 with (*R*)-(−)-4-penten-2-ol.

The stereochemical outcome can be understood from the six-membered transition state of the reaction (Scheme 49). The scheme below shows the formation of the major isomer. The difference in the stereoselectivities in the reaction of **256** with (*S*)-(+)-4-penten-2-ol and (*R*)-(−)-4-penten-2-ol must be the consequence of the chirality of the substrate. Most likely the α-stereocenter of the aldehyde plays a major role.



Scheme 49: Six-membered transition state of the reaction of 256 with (*R*)-(−)-4-penten-2-ol.

In summary, Prins cyclizations were validated as a reasonable way for the synthesis of withanolide analogues. The obtained three products were prepared in moderate stereoselectivities. Probably larger groups R (Scheme 44, page 78) would exert a stronger stereoinductive effect in the six-membered transition state and improve stereoselectivities. Biological data for the compounds was not obtained.

IV Summary and Conclusions

In the present work a synthetic approach to the withanolide scaffold was developed and applied to the preparation of a compound collection. In order to cover a possibly broad chemical space, two complementary synthetic strategies were pursued, resulting in a collection of ~100 compounds. Collection A is based on full steroids and collection B on the *trans*-hydrindane scaffold (Figure 33).

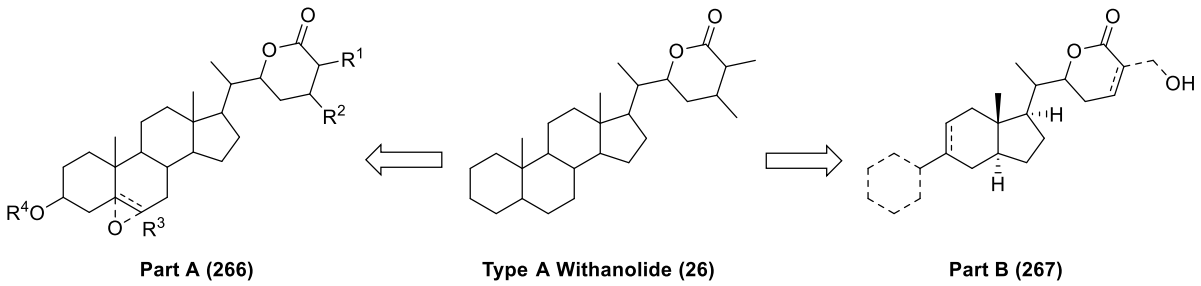
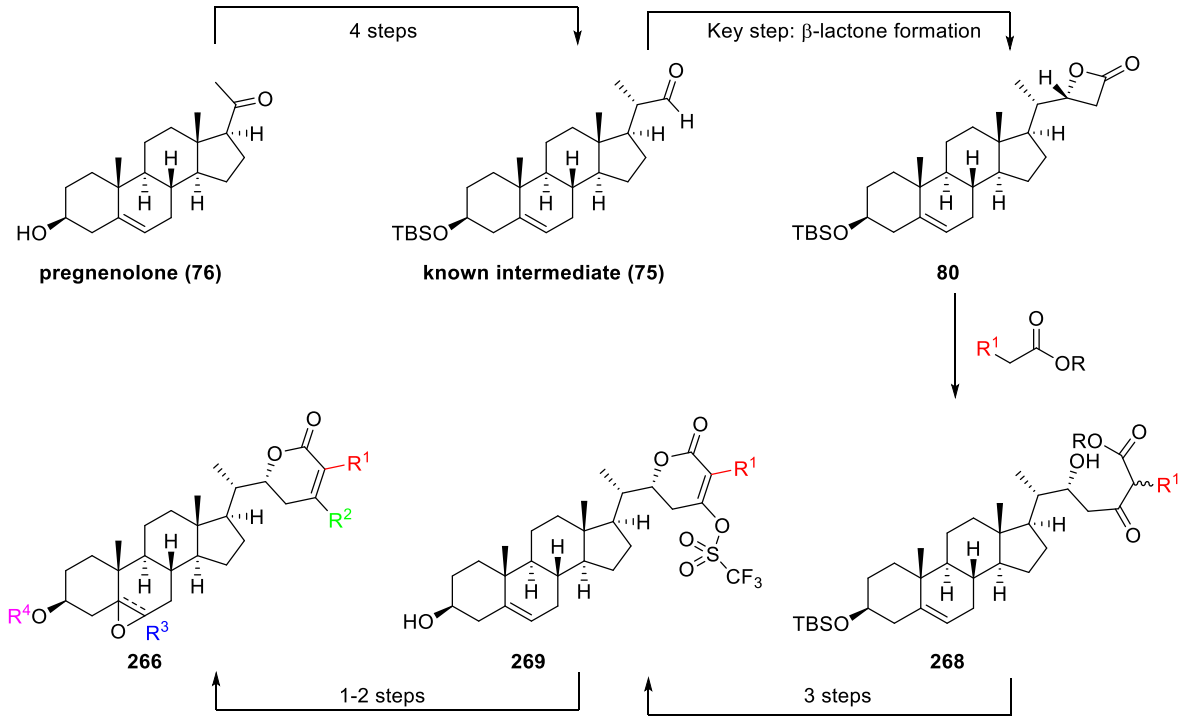


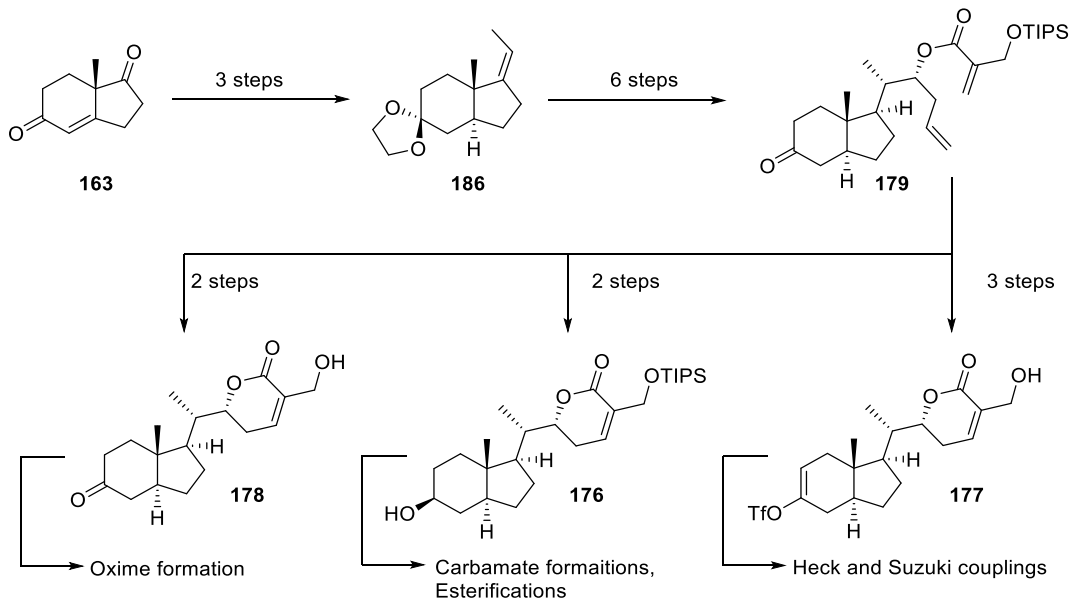
Figure 33: Outline of the two compound collections.

In Part A, the steroid core was kept largely constant while the lactone side chain, which is typical of withanolides, was assembled with the incorporation of variable substituents R^1 and R^2 (Scheme 50). To this end, a synthetic route different from all so far described approaches towards withanolides was used. In order to further increase the variability of the collection, one-step modifications of the steroid core were performed in some cases. To this end, the olefin in the B-ring was epoxidized (R^3) or appendages on the secondary hydroxyl group attached (R^4). The important feature of the strategy is the assembly of a tetrasubstituted olefin in the lactone with a wide range of possible appendages. This has not been achieved by the currently known synthetic approaches towards withanolides.



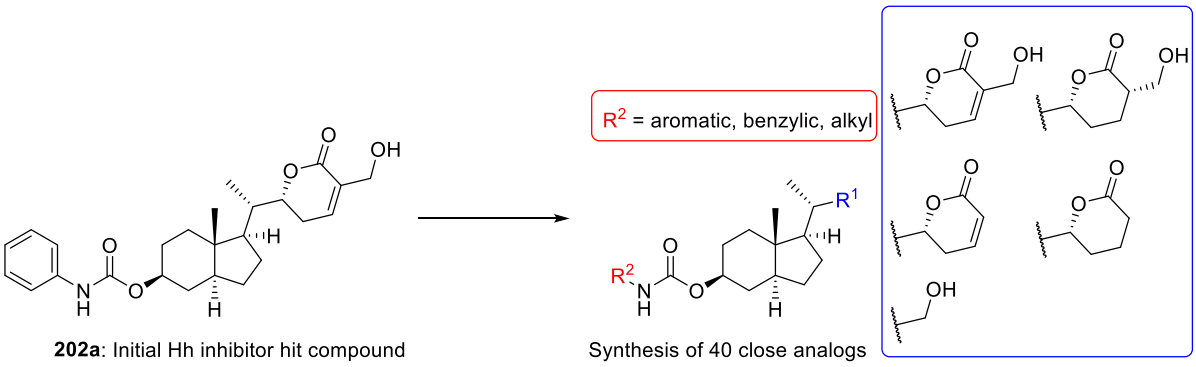
Scheme 50: Summary of the synthetic pathway of part A.

In Part B, a compound collection based on the *trans*-hydrindane dehydro-δ-lactone scaffold was prepared. Three fully assembled late-stage intermediates were prepared and derivatized by Suzuki- and Heck-type cross coupling reactions, esterifications, carbamate formations and oxime formations (Scheme 51). The CD-rings, as well as the typical α,β-unsaturated δ-lactone were kept constant, while mimics of the A-ring were attached to the C-ring.



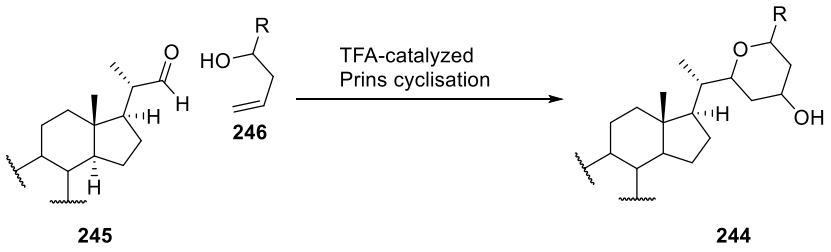
Scheme 51: Summary of the synthetic pathway of part B.

After the identification of an initial Hh pathway inhibitor, a focused library of 40 compounds was prepared. Variations of the lactone and the carbamate moiety were incorporated (Scheme 52).



Scheme 52: Synthesis of a focused library.

Finally, in part C an alternative approach to the synthesis of withanolide analogues was discussed (Scheme 53). Hereby, no lactone is assembled in multiple steps, but instead a Prins cyclization is used for the quick and stereoselective synthesis of a six-membered ring. Three products were prepared by this method.



Scheme 53: Prins cyclizations for the synthesis of tetrahydropyran rings as lactone mimics.

The compound library was submitted to a series of cell-based assays for the modulation of cellular signaling pathways. After the identification of one initial hit compound from each collection, two more focused sublibraries were synthesized in order to delineate a structure-activity relationship and obtain even more active compounds. Compound **130s**, derived from the collection of full steroidal analogues, is an inhibitor of the Wnt signaling pathway with an IC_{50} of 110±20 nM. It only modestly affects the TNKS1/2 enzymatic activity and has most likely another target. The target identification is currently ongoing. Compound **202a**, derived from collection B, is an inhibitor of the Hedgehog signaling pathway with an IC_{50} of 1.8±0.6 µM. It acts a cyclopamine-competitive antagonist at the Smoothened receptor with a K_i of 57±10 nM. Interestingly, the hit compound **202a** initially identified in COMAS remained the most active compound among all synthesized close analogues. Hence, the sublibrary did not yield a more active Hh inhibitor, but allowed to develop a SAR.

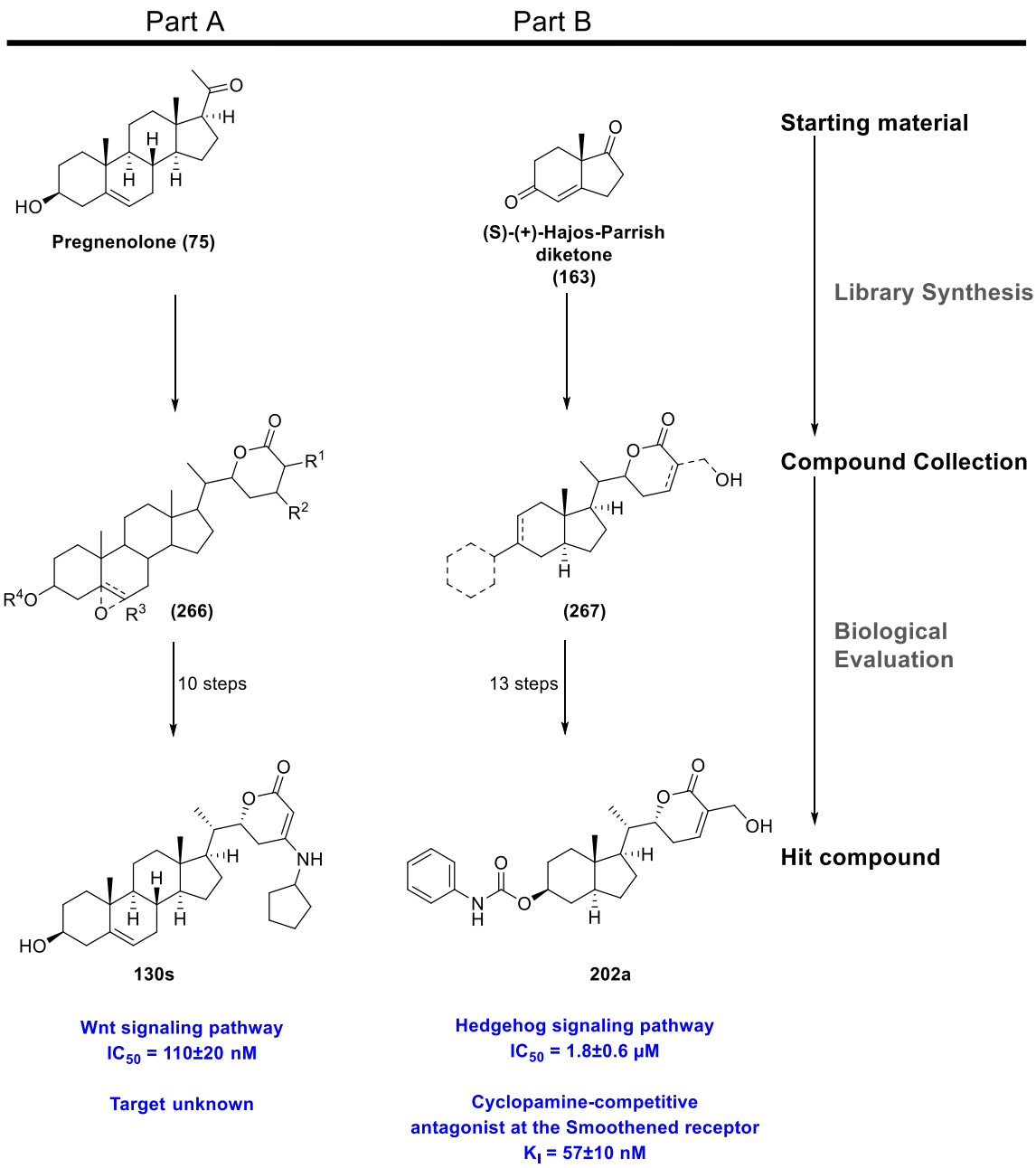


Figure 34: The two hit compounds 130s and 202a.

A possible application of compounds **130s** and **202a** would be the further development towards chemical probes, as tools for the study of biological systems. Alternatively, they could be used as drug candidates for targeted cancer chemotherapy, indicated for patients with aberrant Wnt- or Hh-pathways, respectively.

V Experimental Part

5.1 General

5.1.1 General Experimental Procedures

Reactions were carried out in standard glassware. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation at 40–60 °C.

Analytical thin-layer chromatography (TLC) was performed using aluminum plates pre-coated with silica gel (Silica gel 60 F_{254}, Merck KGA). TLC plates were visualized by exposure to ultraviolet light (UV), then were stained by submersion in aqueous ceric ammonium molybdate (CAM) or aqueous potassium permanganate solution ($KMnO_4$) followed by brief heating by a heat pistol. CAM solution was prepared as follows: 40 g of ammonium pentamolybdate + 1.6 g of cerium(IV) sulfate + 800 ml of diluted sulfuric acid (1:9, with water, v/v). $KMnO_4$ solution was prepared as follows: 1.5 g potassium permanganate + 10 g potassium carbonate + 1.25 ml 10% sodium hydroxide + 200 ml water.

Flash column chromatography was performed as described by Still *et al*.,[138] employing *silica gel Merck 60* (particle size 0.040–0.063 mm).

5.1.2 Materials

Chemicals were obtained from Sigma-Aldrich, Acros Organics, TCI or Alfa Aesar and were used without further purification. The molarity of solutions of *n*-butyllithium was determined by titration against diphenylacetic acid as an indicator.[139] Dry dichloromethane was prepared using a *MBRAUN MB-SPS-800* Solvent Purification Systems. Dry tetrahydrofuran, methanol, toluene and diethyl ether over molecular sieves were purchased from Sigma-Aldrich or Acros Organics.

5.1.3 Instrumentation

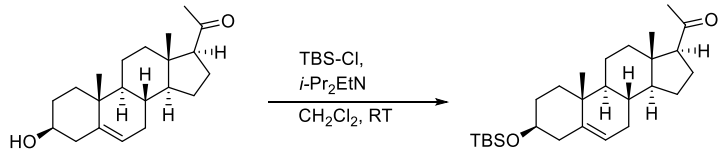
Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) spectra were recorded on *Varian Mercury 200* (200 MHz*), Bruker Avance DPX-300* (300 MHz), *Varian Mercury 400* (400 MHz), *Bruker Avance DRX 500* (500 MHz), *INOVA500* (500 MHz) and *Bruker AV600* (600 MHz) at ambient temperature. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26 ppm; CH_2Cl_2, δ 5.30 ppm; Acetone-d_6, 2.05; DMSO-d_6, 2.50 ppm, CD_3OD, 3.31 ppm). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant (*J*) in Hertz, integration, and assignment. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ 77.00; CD_2Cl_2, 54.00; Acetone-d_6, 206.26; DMSO-d_6, 39.52, CD_3OD, 49.00 ppm). **Fourier Transform Infrared spectra** (FTIR) were obtained from neat compounds using a *Bruker Tensor 27* FT-IR spectrometer. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (vs = very strong, s = strong, m = medium, w = weak, br = broad). **High resolution mass spectra** (HRMS) were recorded on a LTQ Orbitrap mass spectrometer coupled to an Acceka HPLC-System (HPLC column: Hypersyl GOLD, 50 mm x 1 mm, particle size 1.9 µm, ionization method: electron spray ionization). **Optical rotations** (α_D^{RT}) were measured in a *Schmidt + Haensch Polartronic HH8* polarimeter in cuvettes with a path length of 10 cm at ambient temperature. The concentration is given as g/100 ml. **Melting points** were measured in a *Büchi®* melting point apparatus *Model B-540*.

5.2 Experimental Part for Part A

5.2.1 Synthesis of Enol Triflates

Synthesis of known Aldehyde 75 from Pregnenolone

1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*S*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethan-1-one (77)[66b, 140]

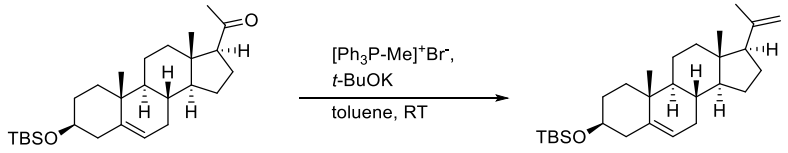


Pregnenolone (76) **77**

To a solution of pregnenolone (4.973 g, 15.71 mmol, 1 equiv) in dichloromethane (50 ml) were added *tert*-butyldimethylsilyl chloride (3.553 g, 23.57 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (10.95 ml, 62.85 mmol, 4 equiv). The resulting suspension was stirred at ambient temperature overnight. Then, dichloromethane and a saturated aqueous solution of sodium chloride were added. The organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 4–12% ethyl acetate in petroleum ether) provided TBS-protected pregnenolone **77** (6.467 g, 96%).

White solid**. TLC** (10% ethyl acetate in petroleum ether): R_f = 0.44 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.31 (m, 1H), 3.48 (m, 1H), 2.53 (t, *J* = 8.9 Hz, 1H), 2.32–2.13 (m, 3H), 2.12 (3H, s), 2.07–1.94 (m, 2H), 1.81 (dt, *J* = 13.2, 3.3 Hz), 1.76–1.37 (m, 9H), 1.30–0.90 (m, 4H), 0.99 (s, 3H), 0.88 (s, 9H), 0.62 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 209.55, 141.51, 120.84, 72.52, 63.71, 56.94, 50.04, 44.01, 42.75, 38.85, 37.37, 36.58, 32.02, 31.85, 31.79, 31.55, 25.92, 24.48, 22.79, 21.06, 19.41, 18.26, 13.21, -4.59. **FTIR** (neat), cm^{-1}: 2925 (br), 1699 (s), 1074 (vs), 829 (s), 773 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{46}O_2Si+H^+$): 431.33398, found: 431.33350. Elemental analysis: Calcd C 75.29, H 10.76; found C 75.1, H 11.0. **Melting point**: 164 °C (lit: 162–164 °C)[140]

tert-butyl(((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-dimethyl-17-(prop-1-en-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)dimethylsilane (78)[66b]

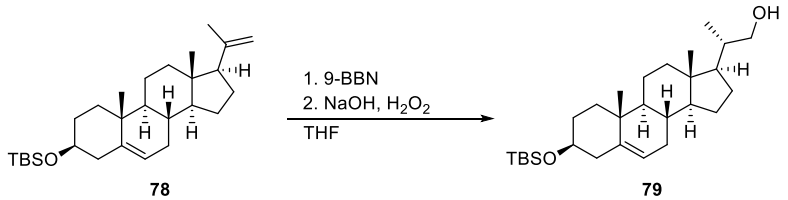


77 78

To a suspension of methyltriphenylphosphonium bromide (13.609 g, 38.1 mmol, 3 equiv) in dry toluene (120 ml) was added potassium *tert*-butoxide (4.275 g, 38.1 mmol, 3 equiv). The resulting yellow suspension was stirred vigorously at ambient temperature for 30 min. Then, TBS-protected pregnenolone **76** (5.470 g, 12.70 mmol, 1 equiv) was added. Resulting suspension was stirred for 1 h until full conversion. The reaction was quenched by addition of saturated an aqueous solution of sodium ammonium chloride. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 0–15% ethyl acetate in petroleum ether) to provide alkene **78** (4.890 g, 90%).

White solid**. ^{1}H NMR** ($CDCl_3$, 500 MHz,) δ: 5.33 (m, 1H), 4.85 (s, 1H), 4.71 (s, 1H), 3.48 (m, 1H), 2.27 (m, 1H), 2.17 (ddd, *J* = 13.3, 4.9, 2.2 Hz, 1H), 2.07–1.95 (m, 2H), 1.87 (dt, *J* = 12.3, 3.6 Hz, 1H), 1.84–1.62 (m, 5H) overlapping with 1.76 (s, 3H), 1.61–1.39 (m, 5H), 1.28–0.85 (m, 5H), 1.00 (s, 3H), 0.89 (s, 9H), 0.58 (s, 3H), 0.06 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 145.66, 141.61, 121.06, 110.67, 72.61, 57.26, 56.56, 50.37, 43.11, 42.83, 38.71, 37.41, 36.65, 32.24, 32.09, 31.86, 25.94, 25.42, 24.65, 24.26, 21.12, 19.46, 18.26, 12.68, -4.58. **FTIR** (neat), cm^{-1}: 2929 (br), 1251 (m), 1079 (vs), 887 (s), 837 (s). **Melting point**: 141 °C.

(*S*)-2-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)propan-1-ol (79)[66b, 140]



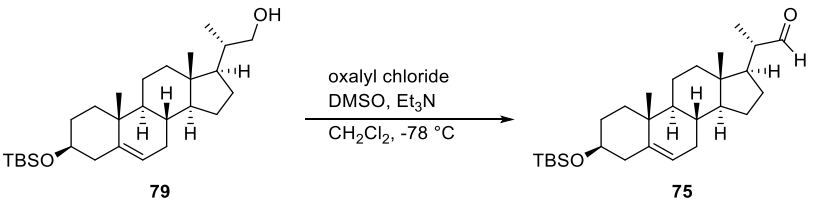
78 79

To a 0 °C of alkene **78** (5.172 g, 12.06 mmol, 1 equiv) in dry THF (90 ml) was added 9-BBN (0.5 M solution in THF, 48.25 ml, 24.13 mmol, 2 equiv) under argon. The ice-water cooling bath was removed and the resulting solution was stirred at ambient temperature for 3 h. The solution was again cooled to 0 °C before sodium hydroxide (2.0 M aqueous solution, 48.25 ml, 96.5 mmol, 8 equiv) and hydrogen peroxide (30% aqueous solution by weight, 9.38 ml, 96.5 mmol, 8 equiv) were added sequentially. The mixture was allowed to reach ambient temperature and then was stirred for 30 min. The mixture was diluted with dichloromethane and washed with a saturated aqueous solution of sodium chloride. The

washed organic phase was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The remaining residue was purified by flash-column chromatography (gradient elution with 10–50% ethyl acetate in petroleum ether) several times in order to remove the minor diastereomer to yield alcohol **79** (4.61 g, 85%).

White crystalline solid**. TLC** (40% ethyl acetate in petroleum ether): R_f = 0.55 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.32 (m, 1H), 3.63 (dd, *J* = 10.5, 3.2 Hz), 3.47 (m, 1H), 3.36 (dd, *J* = 10.5, 6.9 Hz), 2.27 (m, 1H), 2.16 (ddd, *J* = 13.3, 4.9, 2.1 Hz), 2.06–1.90 (m, 2H), 1.88–0.90 (m, 19H), 1.05 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.69 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 141.55, 121.06, 72.61, 68.01, 56.50, 52.37, 50.14, 42.79, 42.40, 39.61, 38.74, 37.36, 36.55, 32.05, 31.89, 27.72, 25.93, 24.37, 21.03, 19.42, 18.27, 16.75, 11.91, -4.60. **FTIR** (neat), cm^{-1}: 1470 (br), 1382 (m), 1255 (m), 1084 (s). The compound was not detectable by **HRMS**. Elemental analysis: Calcd C 75.27, H 11.28; found C 74.9, H 11.3. **Melting point**: 154 °C (lit: 153.5–155.5 °C)[140]

(*S*)-2-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)propanal (75)[66b]



79 **75**

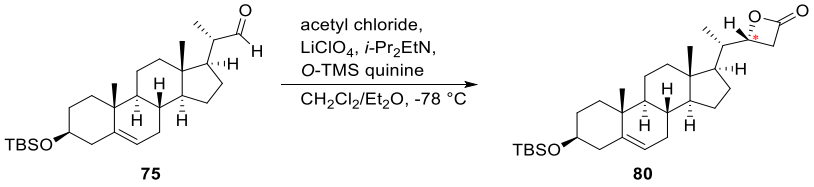
Dimethyl sulfoxide (1.75 ml, 24.6 mmol, 2.5 equiv) was added to a −78 °C solution of oxalyl chloride (1.35 ml, 15.77 mmol, 1.6 equiv) in dichloromethane (40 ml). After 30 min, a solution of alcohol **78** (4.40 g, 9.86 mmol, 1 equiv) in dichloromethane (100 ml) was added to the above mixture at −78 °C. The resulting suspension was stirred for 1 h, then triethylamine (4.95 ml, 35.5 mmol, 3.6 equiv) was added at −78 °C. The mixture was stirred for additional 15 min at −78 °C before allowed to warm to ambient temperature. The obtained clear solution was diluted with dichloromethane and the diluted solution was washed with saturated aqueous solution of sodium chloride. The washed organic phase was dried over anhydrous magnesium sulfate, filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 4–15% ethyl acetate in petroleum ether) provided aldehyde **75** (3.73 g, 85%).

White solid. **TLC** (5% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 9.57 (d, *J* = 3.2 Hz, 1H), 5.31 (m, 1H), 3.48 (m, 1H), 2.36 (m, 1H), 2.26 (m, 1H), 2.17 (ddd, *J* = 13.4, 4.9, 2.2 Hz, 1H), 2.02–1.92 (m, 2H), 1.92–1.84 (m, 1H), 1.83–1.76 (m, 1H), 1.75–1.32 (m, 9H), 1.28–0.90 (m, 5H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.00 (s, 3H), 0.89 (s, 9H), 0.72 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 205.03, 141.57, 120.94, 72.58, 56.07, 51.07, 50.22, 49.49, 42.99, 42.82, 39.52, 37.40, 36.61, 32.09, 31.93, 31.88, 27.06, 25.94, 24.67, 21.02, 19.43, 18.26, 13.46, 12.23, -4.57. **FTIR** (neat), cm^{-1}:

2932 (br), 1720 (vs), 1252 (m), 1078 (vs), 832 (s), 771 (s). The compound was not detectable by **HRMS**. **Elemental analysis**: Calcd C 75.61, H 10.88; found C 75.8, H 11.1.

β-Lactone Formation

(*R*)-4-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)oxetan-2-one (80)



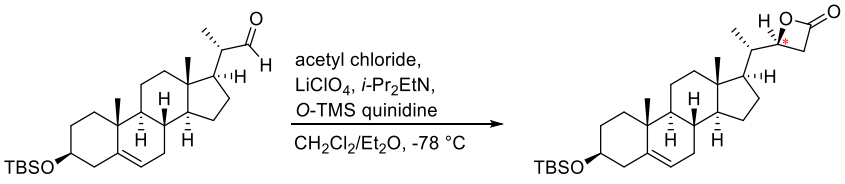
75 **80**

To a solution of *O*-TMS-quinine[68, 141] (241 mg, 607 µmol, 0.3 equiv) and aldehyde **75** (900 mg, 2.02 mmol, 1 equiv) in dry CH_2Cl_2 (15 ml) was added a solution of $LiClO_4$ (646 mg, 6.05 mmol, 3 equiv) in diethyl ether (6 ml) and the reaction mixture was cooled to −78 °C. To the resulting mixture was added *N*,*N*-diisopropylethylamine (2.11 ml, 12.1 mmol, 6 equiv). A solution of acetyl chloride (1 M solution in CH_2Cl_2, 10.1 ml, 10.1 mmol, 5 equiv) was then added over 4 h by syringe pump. The reaction mixture was diluted with dichloromethane and a saturated aqueous solution of ammonium chloride was added. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 4–25% ethyl acetate in petroleum ether) to provide the product **80** (841 mg, 85%) in an epimeric ratio of ~95:5 at the marked carbon atom.

The reaction does usually not run to completion and yields between 40 and 85% can be achieved. The remaining starting material can be reisolated without change and reused.

Crystalline white solid. **TLC** (15% ethyl acetate in petroleum ether): R_f = 0.40 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.32 (m, 1H), 4.49 (dd, *J* = 10.5, 5.8 Hz, 1H), 3.47 (m, 1H), 3.31 (dd, *J* = 16.4, 5.9 Hz, 1H), 3.15 (dd, *J* = 16.4, 4.6 Hz, 1H), 2.27 (m, 1H), 2.16 (ddd, *J* = 13.3, 4.9, 2.1 Hz, 1H), 2.10–1.90 (m, 3H), 1.85–1.37 (m, 10H), 1.27–0.92 (m, 9H), 1.00 (s, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.88 (s, 9H), 0.73 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 168.46, 141.55, 120.91, 73.83, 72.55, 56.11, 53.47, 50.11, 43.02, 42.76, 39.64, 38.70, 37.91, 37.35, 36.53, 32.03, 31.87, 31.82, 27.44, 25.92, 24.44, 21.01, 19.41, 18.26, 12.01, 11.98, -4.60. **FTIR** (neat), cm^{-1}: 2934 (s), 1840 (s), 1383 (m), 1255 (m), 1084 (vs), 836 (vs). **HRMS** (ESI): Calcd for ($C_{30}H_{50}O_3Si+H^+$): 487.36020, found: 487.35935. α_D^{RT}= -36.0 (c = 0.79 in CH_2Cl_2). **Melting point**: 173–174 °C.

(*S*)-4-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)oxetan-2-one (81)



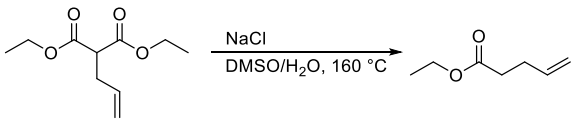
75 81

Epimeric β-lactone **81** was prepared using the same procedure as for β-lactone **80** with *O*-TMS quinidine[68, 141] (**83**) instead of *O*-TMS quinine (**82**). The product was isolated in 63% yield and an epimeric ratio of 90:10 at the marked carbon atom. Only the signals of the major diastereomer are listed.

White crystalline solid. **TLC** (15% ethyl acetate in petroleum ether): R_f = 0.35 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.31 (m, 1H), 4.40 (dd, *J* = 10.4, 5.8 Hz, 1H), 3.48 (m, 1H), 3.40 (dd, *J* = 16.4, 5.8 Hz, 1H), 3.21 (dd, *J* = 16.4, 4.4 Hz, 1H), 2.26 (m, 1H), 2.16 (ddd, *J* = 13.3, 4.8, 2.0 Hz, 1H), 2.08–1.90 (m, 2H), 1.85–1.59 (m, 5H), 1.58–0.84 (m, 12H), 1.09 (d, *J* = 6.7 Hz, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.72 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 168.46, 141.57, 120.90, 74.83, 72.57, 56.13, 53.04, 50.07, 42.85, 42.79, 41.37, 39.63, 39.37, 37.35, 36.54, 32.05, 31.94, 31.80, 27.64, 25.92, 24.35, 21.03, 19.40, 18.23, 13.40, 11.87, -4.59. **FTIR** (neat), cm^{-1}: 2934 (s), 1842 (s), 1811 (vs), 1383 (m), 1255 (m), 1083 (vs), 837 (vs). **HRMS** (ESI): Calcd for ($C_{30}H_{50}O_3Si+H^+$): 487.36020, found: 487.35993.

Synthesis of Ester 106

ethyl 4-pentenoate, CAS: 1968-40-7 (106)



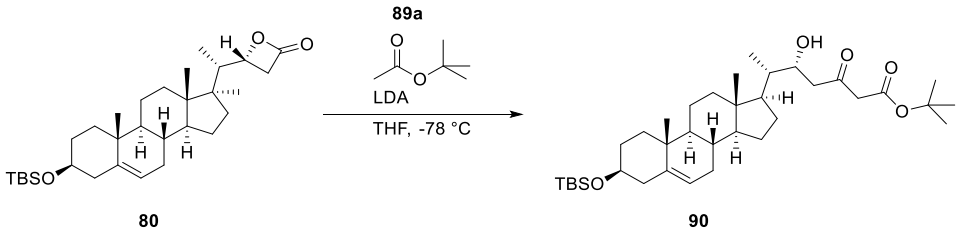
diethyl allylmalonate (108) **106**

Sodium chloride (13.331 g, 228 mmol, 3 equiv) was added to a solution of diethyl allylmalonate (15 ml, 76 mmol, 1 equiv) in dimethyl sulfoxide (47.5 ml) and water (2.5 ml). The solution was heated to 160 °C for 48 h. The reaction mixture was filtered through a plug of anhydrous magnesium sulfate and the resulting solution applied on a silica column. Purification by flash-column chromatography (gradient elution with 0–10% ethyl acetate in pentane) provided ethyl 4-pentenoate (**106**) (5.92 g, 61%).

Colourless oil. **TLC** (5% ethyl acetate in petroleum ether): R_f = 0.40 ($KMnO_4$). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.86–5.73 (m, 1H), 5.03 (dd, *J* = 17.2, 1.3 Hz, 1H), 4.97 (dd, *J* = 10.3, 1.0 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 1H), 2.40–2.31 (m, 4H), 1.22 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.91, 136.65, 115.32, 60.20, 33.52, 28.83, 14.15.

β-Lactone opening with substituted esters

***tert*-butyl (5*R*,6*S*)-6-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-5-hydroxy-3-oxoheptanoate (90)**

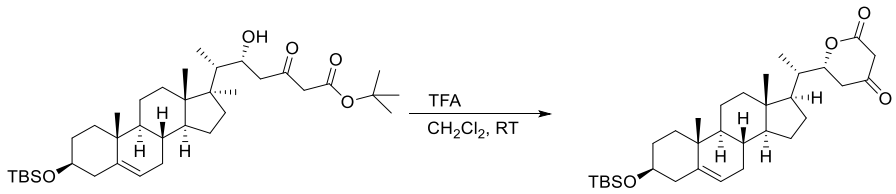


To a freshly prepared solution of LDA (0.5 M in THF, 5.55 ml, 2.77 mmol, 2.70 equiv) at −78 °C was added *tert*-butyl acetate (409 µl, 3.05 mmol, 3 equiv) dropwise. β-Lactone **80** (500 mg, 1.03 mmol, 1 equiv) was dissolved in dry THF (20 ml) and the solution cooled to −78 °C. The solution of the ester enolate was added to the starting material via syringe. The reaction mixture was quenched after 15 min by addition of saturated aqueous solution of ammonium chloride at −78 °C and the resulting mixture was diluted with dichloromethane. The separated organic phase was dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 7–25% ethyl acetate in petroleum ether) to yield product **90** (402 mg, 65%).

White amorphous solid**. TLC** (25% ethyl acetate in petroleum ether): R_f = 0.5 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.27 (m, 1H), 4.13 (dd, *J* = 7.5, 3.6 Hz, 1H), 3.44 (m, 1H), 3.37 (s, 2H), 2.76 (br s, 1H), 2.52 (m, 2H), 2.28–2.17 (m, 1H), 2.16–2.08 (m, 1H), 2.01–1.87 (m, 2H), 1.81–0.98 (m, 17H), 1.43 (s, 9H), 0.96 (s, 3H), 0.90 (d, *J* = 6.7 Hz, 3H), 0.85 (s, 9H), 0.66 (s, 3H), 0.01 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 204.91, 166.22, 141.57, 120.96, 82.20, 72.59, 68.88, 56.50, 53.15, 51.35, 50.21, 43.15, 42.79, 42.70, 40.73, 39.80, 37.37, 36.55, 32.06, 31.90, 31.88, 27.95, 27.38, 25.91, 24.29, 21.03, 19.38, 18.22, 12.56, 11.85, -4.60. **FTIR** (neat), cm^{-1}: 2932 (s), 2857 (m), 1739 (s), 1705 (s), 1250 (s), 1083 (vs). **HRMS** (ESI): Calcd for ($C_{36}H_{62}O_5Si+H^+$): 603.44393, found: 603.44444. $\boldsymbol{\alpha_D^{RT}}$= -20.4 (c = 1.43 in CH_2Cl_2). **Elemental analysis**: Calcd C 71.71, H 10.36; found C 71.5, H 10.0.

Procedure for Closure of β-Keto-δ-Ester Alcohol 90

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)dihydro-2*H*-pyran-2,4(3*H*)-dione (91)



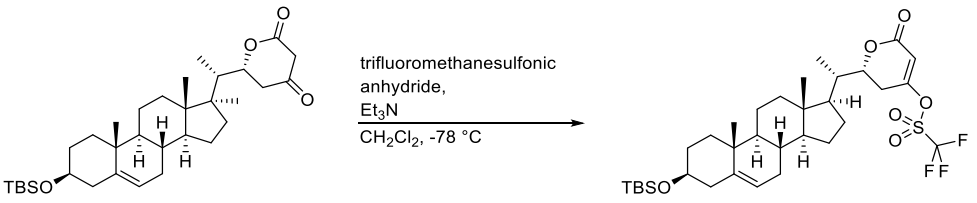
90 **91**

To a solution of **90** (265 mg, 440 µmol, 1 equiv) in dichloromethane (20 ml) was added trifluoroacetic acid (50 µl, 659 µmol, 1.5 equiv) and the solution was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane and a saturated aqueous solution of sodium bicarbonate was added. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered and the filtrate was concentrated in vacuo. The crude product **91** was used for the next transformation. A sample of the product was purified by column chromatography for analysis purposes.

White crystalline solid. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.5 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.31 (m, 1H), 4.67 (dt, *J* = 11.8, 3.1 Hz, 1H), 3.57 (d, *J* = 19.0 Hz, 1H), 3.47 (m, 1H), 3.40 (d, *J* = 19.0 Hz, 1H), 2.53 (dd, *J* = 18.1, 2.9 Hz, 1H), 2.46 (dd, *J* = 18.1, 11.8 Hz, 1H), 2.26 (m, 1H), 2.21–2.12 (m, 2H), 2.04–1.92 (m, 2H), 1.80 (dt, *J* = 12.9, 3.0 Hz, 1H), 1.75–0.85 (m, 15H), 1.04 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.74 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 200.67, 167.43, 141.61, 120.81, 77.52, 72.55, 56.33, 51.96, 50.08, 46.84, 42.88, 42.78, 39.67, 38.54, 37.63, 37.36, 36.55, 32.04, 31.91, 31.82, 27.45, 25.92, 24.28, 21.00, 19.40, 18.23, 12.42, 11.77, -4.59. **FTIR** (neat), cm^{-1}: 2931 (br), 1765 (s), 1727 (s), 1696 (s), 1092 (s), 835 (s). **HRMS** (ESI): Calcd for ($C_{32}H_{52}O_4Si+H^+$): 529.37076, found: 529.37069. $\boldsymbol{\alpha_D^{RT}}$ = +8.7 (c = 1.0 in CH_2Cl_2). **Melting point**: 169–170 °C.

Representative Procedure for Triflation of β-Keto Esters

(*R*)-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-6-oxo-3,6-dihydro-2*H*-pyran-4-yl trifluoromethanesulfonate (<u>109</u>)

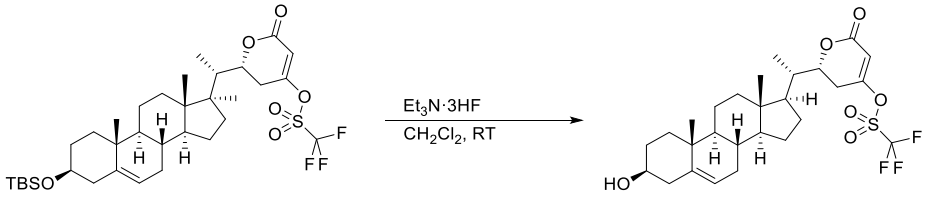


91 **109**

To the crude product of δ-lactone closure (procedure above) in dichloromethane (12 ml) at −78 °C was added triethylamine (123 µl, 879 µmol, 2 equiv relative to **90**). After 5 min, trifluoromethanesulfonic anhydride (74 µl, 440 µmol, 1 equiv relative to **90**) was added. The reaction was quenched after 15 min by addition of a saturated aqueous solution of sodium bicarbonate at −78 °C and the resulting mixture was diluted with dichloromethane. The separated organic phase was dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 5–10% ethyl acetate in petroleum ether) to yield enol triflate **109** as a colourless oil.

Representative Procedure for TBS-Deprotection

(*R*)-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-6-oxo-3,6-dihydro-2*H*-pyran-4-yl trifluoromethanesulfonate (<u>102e</u>)



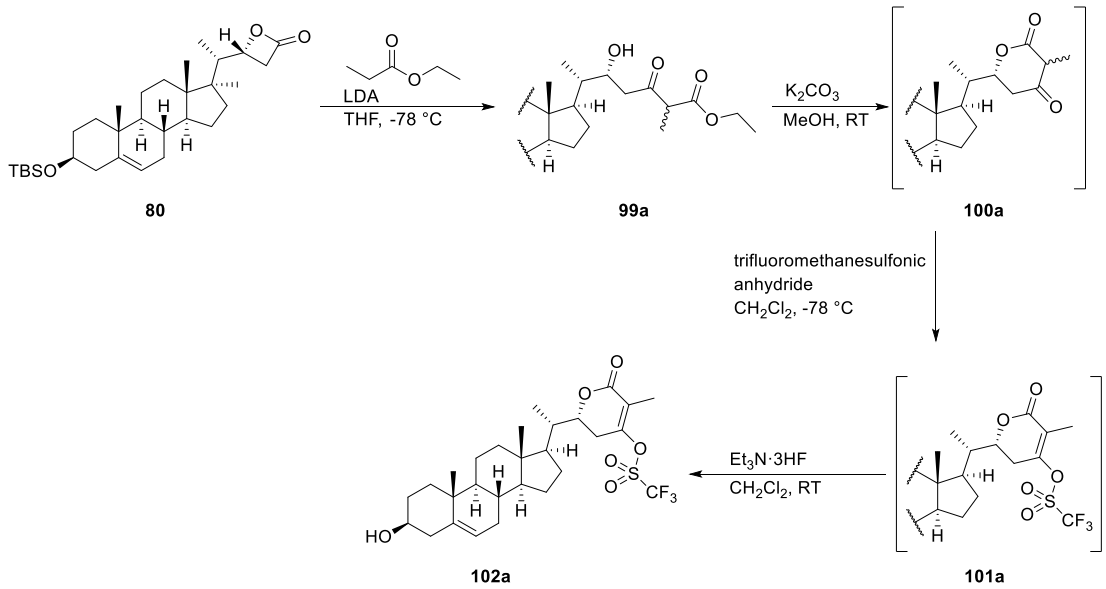
109 **102e**

To a solution of *tert*-butyldimethylsilyl ether **109** (procedure above) in dichloromethane (10 ml) was added triethylamine trihydrofluoride (2 ml). The reaction flask was sealed and stirring continued for 1 h. The reaction mixture was diluted with dichloromethane and the diluted mixture was neutralized by addition of a saturated aqueous solution of sodium bicarbonate. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 10–60% ethyl acetate in petroleum ether) to provide deprotected enol triflate **102e** (167 mg, 70% over three steps from **90**).

White solid**. TLC** (40% ethyl acetate in petroleum ether): R_f = 0.35 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.02 (d, *J* = 2.5 Hz, 1H), 5.34 (m, 1H), 4.57 (dd, *J* = 12.7, 3.6 Hz, 1H), 3.51 (m, 1H), 2.80 (ddd, *J* = 17.7, 12.9, 2.4 Hz, 1H), 2.36 (dd, *J* = 17.9, 3.6 Hz, 1H), 2.31–1.90 (m, 5H), 1.90–0.85 (m, 17H), 1.04 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 163.37, 162.48, 140.78, 121.29, 118.30 (q, *J* = 320.9 Hz), 109.85, 79.08, 71.62, 56.19, 51.73, 49.93, 42.83, 42.16, 39.56, 38.55, 37.17, 36.41, 31.83, 31.74, 31.52, 27.16, 26.76, 24.24, 20.95, 19.32, 13.13, 11.70. **FTIR** (neat), cm^{-1}: 2932 (br), 1740 (s), 1433 (s), 1209 (vs), 1136 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{37}F_3O_6S+H^+$): 547.23357, found: 547.23312. $\boldsymbol{\alpha_D^{RT}}$ = +23.4 (c = 1.0 in $CHCl_3$). **Melting point**: 86 °C.

Representative Procedure for the Synthesis of Enol Triflates 102a-d

(*R*)-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5-methyl-6-oxo-3,6-dihydro-2*H*-pyran-4-yl trifluoromethanesulfonate (102a)



To a freshly prepared solution of LDA (0.5 M in THF, 3.7 ml, 1.85 mmol, 9 equiv) at −78 °C was added ethyl propionate (234 µl, 2.03 mmol, 9.9 equiv) dropwise. β-Lactone **80** (100 mg, 205 µmol, 1 equiv) was dissolved in dry THF (10 ml) and the solution cooled to −78 °C. The solution of ester enolate was added to the starting material via syringe. The reaction was quenched after 15 min by addition of a saturated aqueous solution of ammonium chloride at −78 °C and the resulting mixture was diluted with dichloromethane. The separated organic phase was dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 5–30% ethyl acetate in petroleum ether) to yield the product.

To a solution of starting material in methanol (6 ml) was added potassium carbonate (19.9 mg, 144 µmol, 0.7 equiv relative to the β-lactone) and the solution was stirred for 45 min at room temperature. Methanol was evaporated and the residue dissolved in dichloromethane and a saturated

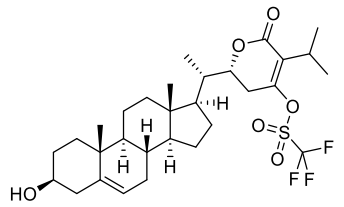
aqueous solution of ammonium chloride. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered and the filtrate was concentrated in vacuo. The crude product was used for the next transformation.

To a solution of β-keto ester **100a** in CH_2Cl_2 (15 ml) at −78 °C was added triethylamine (57.3 µl, 411 µmol, 2 equiv relative to the β-lactone). After 5 min, trifluoromethanesulfonic anhydride (34.6 µl, 205 µmol, 1 equiv relative to the β-lactone) was added. The reaction was quenched after 15 min by addition of saturated an aqueous solution of sodium bicarbonate at −78 °C and the resulting mixture was diluted with dichloromethane. The separated organic phase was dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The crude product was used for the next transformation.

To a solution of *tert*-butyldimethylsilyl ether **101a** in dichloromethane (5 ml) was added triethylamine trihydrofluoride (1.5 ml). The reaction flask was sealed and stirring continued for 1 h. The reaction mixture was diluted with dichloromethane and the diluted mixture was neutralized by addition of saturated an aqueous solution of sodium bicarbonate. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–50% ethyl acetate in petroleum ether) to provide deprotected enol triflate **102a** (66.2 mg, 57% over four steps).

White solid**. TLC** (30% ethyl acetate in petroleum ether): R_f = 0.40 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.34 (m, 1H), 4.52 (dd, *J* = 12.9, 3.5 Hz, 1H), 3.51 (m, 1H), 2.84 (ddd, *J* = 17.1, 13.2, 2.7 Hz, 1H), 2.39 (dd, *J* = 17.53, 2.50 Hz, 1H), 2.34–0.85 (m, 22H), 1.96 (d, *J* = 1.7 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 1H), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.23, 156.28, 140.78, 121.33, 120.12, 118.19 (q, *J* = 320.4 Hz), 78.40, 71.63, 56.14, 51.73, 49.94, 42.82, 42.18, 39.53, 38.55, 37.18, 36.43, 31.86, 31.74, 31.54, 27.15, 26.90, 24.26, 20.97, 19.33, 13.20, 11.70, 10.71. **FTIR** (neat), cm^{-1}: 3287 (br), 2933 (br), 1738 (s), 1209 (s), 1138 (s), 1057 (s). **HRMS** (ESI): Calcd for ($C_{28}H_{39}F_3O_6S+H^+$): 561.24922, found: 561.24974. $\boldsymbol{\alpha_D^{RT}}$ = +23.8 (c = 2.0 in CH_2Cl_2). **Melting point**: 170–171 °C.

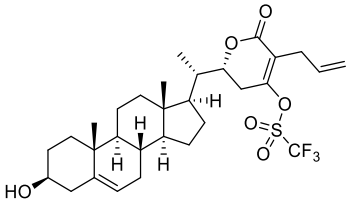
(*R*)-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5-isopropyl-6-oxo-3,6-dihydro-2*H*-pyran-4-yl trifluoromethanesulfonate (102b)



The product was synthesized according to the general procedure for the synthesis of enol triflates (page 96) in 61% yield over four steps (600 mg of starting material). The β-lactone was opened by 60 equiv of the lithium enolate generated from ethyl isovalerate.

Colourless foam**. TLC** (30% ethyl acetate in petroleum ether): R_f = 0.41 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.34 (m, 1H), 4.46 (dd, *J* = 12.8, 3.4 Hz, 1H), 3.52 (m, 1H), 3.06 (hept, *J* = 6.9 Hz, 1H), 2.82 (dd, *J* = 17.4, 13.0 Hz, 1H), 2.35 (dd, *J* = 17.4, 3.4 Hz, 1H), 2.31–0.9 (m, 22H), 1.29 (d, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 6.9 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 163.36, 154.94, 140.79, 128.87, 121.35, 118.10 (weak q, *J* = 320 Hz, CF_3), 78.04, 71.64, 56.14, 51.80, 49.96, 42.84, 42.20, 39.55, 38.50, 37.20, 36.44, 31.88, 31.76, 31.56, 27.21, 26.90, 26.48, 24.26, 20.98, 20.35, 19.78, 19.34, 13.27, 11.70. **FTIR** (neat), cm^{-1}: 2938 (br), 1730 (s), 1423 (m), 1214 (vs), 1139 (s). **HRMS** (ESI): Calcd for ($C_{30}H_{43}F_3O_6S+H^+$): 589.28052, found: 589.28033. $\boldsymbol{\alpha_D^{RT}}$ = +22.4 (c = 1.0 in CH_2Cl_2).

(*R*)-5-allyl-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-6-oxo-3,6-dihydro-2*H*-pyran-4-yl trifluoromethanesulfonate (102c)

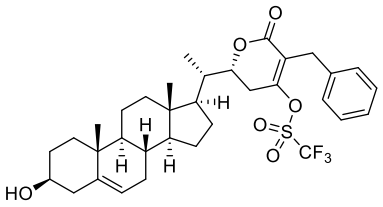


The product was synthesized according to the general procedure for the synthesis of enol triflates (page 96) in 48% yield over four steps. The β-lactone was opened of by 12 equiv of the lithium enolate generated from ethyl 4-pentenoate **(106)**.

Colourless foam. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.78 (m, 1H), 5.34 (m, 1H), 5.13 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.09 (dd, *J* = 10.1, 1.2 Hz, 1H), 4.52 (dt, *J* = 12.8, 3.5 Hz, 1H), 3.52 (m, 1H), 3.20 (dd, *J* = 13.5, 6.4 Hz, 1H), 3.13 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.86 (dd, *J* = 17.1, 13.3 Hz, 1H), 2.43 (dd, *J* = 17.5, 3.3 Hz, 1H), 2.38–2.16 (m, 3H), 2.13–1.92 (m, 3H), 1.88–1.78 (m, 2H), 1.74–0.84 (m, 14H), 1.03 (d, *J* = 6.7 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 164.41, 156.50, 140.76, 132.37, 122.20, 121.37, 118.17 (weak q, *J* = 320.4 Hz), 117.59, 78.30, 71.72, 56.15, 51.80, 49.98, 42.86, 42.15, 39.57, 38.55, 37.20, 36.44, 31.89, 31.76, 31.52, 29.04, 27.18,

26.83, 24.26, 20.99, 19.33, 13.24, 11.71. **FTIR** (neat), cm^{-1}: 2934 (br), 1728 (s), 1419 (s), 1214 (vs), 1136 (s). **HRMS** (ESI): Calcd for ($C_{30}H_{41}F_3O_6S+H^+$): 587.26487, found: 587.26579. $\boldsymbol{\alpha_D^{RT}}$ = +8.7 (c = 2.0 in CH_2Cl_2).

(*R*)-5-benzyl-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-6-oxo-3,6-dihydro-2*H*-pyran-4-yl trifluoromethanesulfonate (<u>102d</u>)



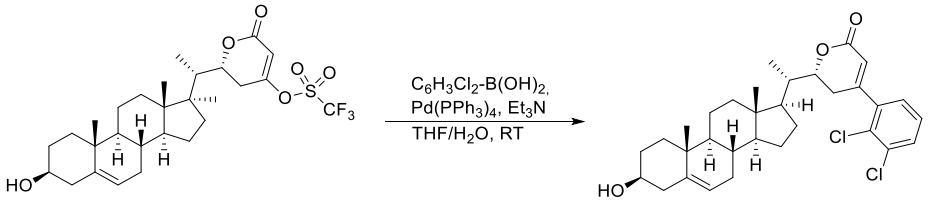
The product was synthesized according to the general procedure for the synthesis of enol triflates (page 96) in 46% yield over four steps. The β-lactone was opened of by 31 equiv of the lithium enolate generated from ethyl ethyl hydrocinnamate.

Colourless foam. **TLC** (35% ethyl acetate in petroleum ether): R_f = 0.4 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.32–7.19 (m, 5H), 5.34 (m, 1H), 4.49 (dt, *J* = 12.8, 3.5 Hz, 1H), 3.79 (d, *J* = 15.4 Hz, 1H), 3.75 (d, *J* = 14.2 Hz, 1H), 3.52 (m, 1H), 2.90 (dd, *J* = 17.1, 13.3 Hz, 1H), 2.47 (dd, *J* = 17.6, 3.4 Hz, 1H), 2.34–2.18 (m, 2H), 2.12–1.75 (m, 6H), 1.73–0.85 (m, 14H), 1.03 (d, *J* = 6.7 Hz, 3H), 1.01 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 164.51, 156.45, 140.79, 136.94, 128.72, 128.63, 126.83, 123.22, 121.34, 118.19 (weak q, *J* = 320.5 Hz), 78.18, 71.66, 56.13, 51.79, 49.98, 42.86, 42.19, 39.57, 38.52, 37.21, 36.44, 31.89, 31.75, 31.55, 30.50, 27.18, 26.83, 24.25, 20.99, 19.33, 13.26, 11.70. **FTIR** (neat), cm^{-1}: 2935 (br), 1727 (s), 1426 (s), 1213 (vs), 1135 (vs), 754 (s). **HRMS** (ESI): Calcd for ($C_{34}H_{43}F_3O_6S+H^+$): 637.28052, found: 637.28183. $\boldsymbol{\alpha_D^{RT}}$ = -16.5 (c = 2.0 in CH_2Cl_2).

5.2.2 Synthesis of Withanolide Analogues

Representative Procedure for Coupling of Enol Triflates 102a-e with Boronic Acids

(*R*)-4-(2,3-dichlorophenyl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130e)



102e **130e**

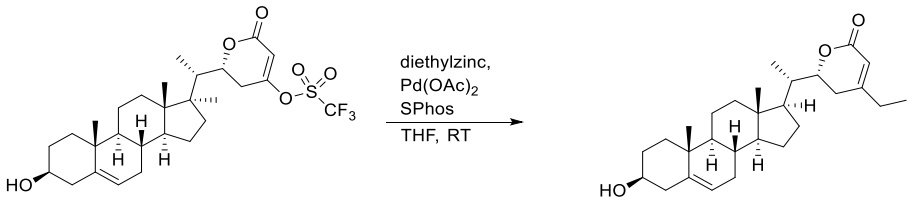
Palladium tetrakis(triphenylphosphine) (3.1 mg, 2.74 μmol, 0.05 equiv) was added to a stirred solution of enol triflate **102e** (30 mg, 54.9 μmol, 1 equiv), boronic acid (20.9 mg, 109.8 μmol, 2 equiv), and triethylamine (38.3 μL, 274 μmol, 5 equiv) in tetrahydrofuran/water solvent mixture (4:1, 2.5 ml) at ambient temperature. After 10 min, the reaction mixture was diluted with dichloromethane and washed with saturated an aqueous solution of ammonium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–65% ethyl acetate in petroleum ether) to provide the coupling product **130e** (29 mg, 99%).

*For coupling reactions with **102b** and **102d**, the reaction mixture was warmed to 50 °C.*

White solid**. TLC** (50% ethyl acetate in petroleum ether): R_f = 0.50 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.50 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.26 (t, *J* = 7.9 Hz, 1H), 7.12 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.00 (d, *J* = 2.4 Hz, 1H), 5.34 (m, 1H), 4.69 (dd, *J* = 12.7, 3.0 Hz, 1H), 3.51 (m, 1H), 2.67 (ddd, *J* = 16.9, 13.1, 2.3 Hz, 1H), 2.49 (dd, *J* = 17.7, 2.9 Hz, 1H), 2.27–0.84 (m, 22H), 1.07 (d, *J* = 6.5 Hz), 1.00 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.08, 156.27, 140.76, 139.88, 133.91, 130.96, 129.88, 127.87, 127.16, 121.41, 119.84, 80.50, 71.68, 56.28, 52.12, 49.98, 42.78, 42.19, 39.65, 38.90, 37.17, 36.43, 31.84, 31.79, 31.56, 27.70, 27.28, 24.31, 20.99, 19.35, 13.45, 11.71. **FTIR** (neat), cm^{-1}: 2930 (br), 1702 (vs), 1411 (m), 1057 (s), 1041 (s). **HRMS** (ESI): Calcd for ($C_{32}H_{40}Cl_2O_3$+H^+): 542.2355, found: 543.24216, calcd for ($C_{32}H_{40}Cl^{37}ClO_3$+H^+): 545.23978, found: 545.23926, calcd for ($C_{32}H_{40}{}^{37}Cl_2O_3$+$H^+$): 547.23683, found: 547.23630. $\boldsymbol{\alpha_D^{RT}}$ = +28.1 (c = 1.0 in $CHCl_3$). **Melting point**: 234 °C.

Representative Procedure for Coupling of Enol Triflates with Diethylzinc

(*R*)-4-ethyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130a)



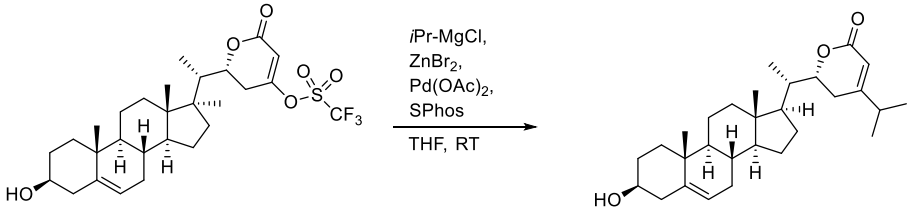
102e **130a**

To a solution of enol triflate **102e** (59 mg, 108 µmol, 1 equiv) in tetrahydrofuran (3 ml) at ambient temperature were added SPhos (4.43 mg, 10.8 µmol, 0.1 equiv) and palladium(II) acetate (1.21 mg, 5.4 µmol, 0.05 equiv), as solutions in tetrahydrofuran (500 µl) each. Then, diethylzinc solution (1.0 M in hexanes, 162 µl, 161 µmol, 1.5 equiv), was added. The reaction was stirred for 5 min until full consumption of the starting material was seen on the TLC. The reaction mixture was diluted with dichloromethane and washed with a saturated aqueous solution of ammonium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–65% ethyl acetate in petroleum ether). The neat product was extracted with a small amount of ethyl acetate in order to remove a yellow impurity, to provide the coupling product **130a** (37.3 mg, 81%).

White amorphous solid**. TLC** (40% ethyl acetate in petroleum ether): R_f = 0.28 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.76 (s, 1H), 5.33 (m, 1H), 4.40 (dt, *J* = 13.0, 3.3 Hz, 1H), 3.50 (m, 1H), 2.42–2.13 (m, 3H) overlapping with 2.25 (q, *J* = 7.3 Hz, 2H), 2.10–1.89 (m, 4H), 1.88–0.83 (m, 17H), 1.11 (t, *J* = 7.4 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.99 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 166.01, 162.71, 140.83, 121.31, 114.31, 79.59, 71.62, 56.31, 52.05, 50.00, 42.72, 42.16, 39.62, 38.77, 37.18, 36.42, 31.83, 31.79, 31.53, 29.74, 27.27, 27.01, 24.25, 20.97, 19.32, 13.34, 11.66, 10.78. **FTIR** (neat), cm^{-1}: 3481 (m), 2922 (br), 1701 (vs), 1290 (m). **HRMS** (ESI): Calcd for ($C_{28}H_{42}O_3$+H^+): 427.32067, found: 427.32028. $\boldsymbol{\alpha_D^{RT}}$ = +36.4 (c = 2.0 in $CHCl_3$). **Melting point**: 264 °C.

Representative Procedure for Coupling of Enol Triflate 102e with Isopropylmagnesium chloride

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-isopropyl-5,6-dihydro-2*H*-pyran-2-one (130c)



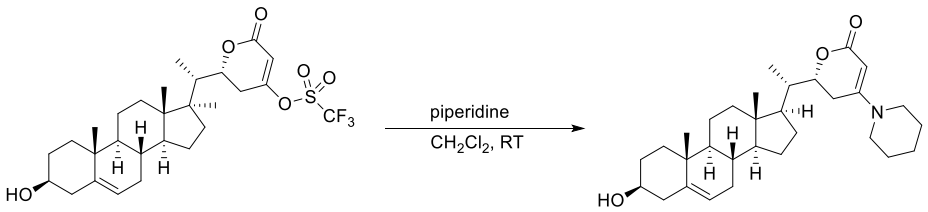
102e 130c

To neat zinc bromide (282 mg, 1.94 mmol, 18 equiv) were added tetrahydrofuran (5.67 ml) and an isopropylmagnesium chloride solution (2 M in tetrahydrofuran, 809 µl, 1.62 mmol, 15 equiv). In a separate flask, enol triflate **102e** was dissolved in tetrahydrofuran (3 ml). Palladium(II) acetate (1.21 mg, 5.4 µmol, 0.05 equiv) and SPhos (4.43 mg, 10.8 µmol, 0.1 equiv) were added as a solution in tetrahydrofuran (500 µl), each. 5 minutes later, the solution of the zinc reagent was added. The reaction mixture was stirred for 15 min at ambient temperature. The reaction mixture was diluted with dichloromethane and washed with a saturated aqueous solution of ammonium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–50% ethyl acetate in petroleum ether). A yellow impurity was extracted from the dry product with ethyl acetate to provide the coupling product **130c** (24.4 mg, 51%).

White solid**. TLC** (40% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.76 (s, 1H), 5.35 (m, 1H), 4.37 (dt, *J* = 13.1, 3.3 Hz, 1H), 3.52 (m, 1H), 2.44 (hept, *J* = 6.8 Hz, 1H), 2.38–2.14 (m, 3H), 2.10–0.83 (m, 21H), 1.11 (d, *J* = 6.8 Hz, 3H) overlapping with 1.11 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 1.01 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 166.65, 166.27, 140.84, 121.38, 113.44, 79.86, 71.69, 56.32, 52.19, 50.05, 42.77, 42.21, 39.66, 38.78, 37.21, 36.46, 34.77, 31.87, 31.83, 31.58, 27.30, 25.11, 24.29, 21.01, 20.39, 19.88, 19.35, 13.42, 11.71. **FTIR** (neat), cm^{-1}: 3489 (m), 2923 (br), 1699 (vs), 1061 (s). **HRMS** (ESI): Calcd for ($C_{29}H_{44}O_3$+H^+): 441.33632, found: 441.33622. $\boldsymbol{\alpha_D^{RT}}$ = +22.6 (c = 1.0 in $CHCl_3$). **Melting point**: 263 °C.

Representative Procedure for Addition-Elimination reaction of Enol Triflate 102e with Amines

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(piperidin-1-yl)-5,6-dihydro-2*H*-pyran-2-one (130i)



102e **130i**

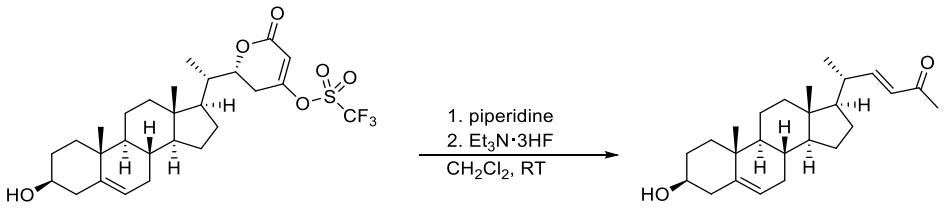
To a solution of **102e** (39.6 mg; 72 µmol, 1 equiv) in dichloromethane (2 ml) was added piperidine (30.8 mg, 35.8 µl, 263 µmol, 5 equiv) and the solution was stirred at room temperature for 10 min. The product was purified by flash-column chromatography (gradient elution with 80–100% ethyl acetate in petroleum ether, then 2–8% ethyl acetate in methanol) to yield the product **130i** (34 mg, quantitative yield).

Depending on the reactivity of the amine, the number of equivalents was varied. For reaction with primary amines, 10–50 equvalents amine were added. The products are instable to acid and should not be purified by HPLC if the water contains TFA.

White solid. **TLC** (100% ethyl acetate): R_f = 0.42 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.32 (m, 1H), 4.82 (s, 1H), 4.32 (dt, *J* = 12.8, 3.2 Hz, 1H), 3.50 (m, 1H), 3.35–3.15 (m, 4H), 2.37–0.83 (m, 30H), 1.02 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 169.42, 160.35, 140.88, 121.30, 84.69, 77.13, 71.62, 56.36, 52.24, 50.08, 47.22, 42.77, 42.19, 39.65, 38.84, 37.21, 36.45, 31.86, 31.83, 31.55, 27.30, 25.27, 24.77, 24.28, 24.14, 20.99, 19.33, 13.38, 11.67. **FTIR** (neat), cm^{-1}: 2918 (br), 1650 (vs), 1580 (s), 1261 (s), 799 (m). **HRMS** (ESI): Calcd for ($C_{31}H_{47}NO_3$+H^+): 482.36287, found: 482.36278. $\boldsymbol{\alpha_D^{RT}}$ = -26.3 (c = 1.0 in $CHCl_3$). **Melting point**: 202.7 °C.

Decomposition of aminated Products

(*R*,*E*)-5-((3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)hex-3-en-2-one (125)[142]



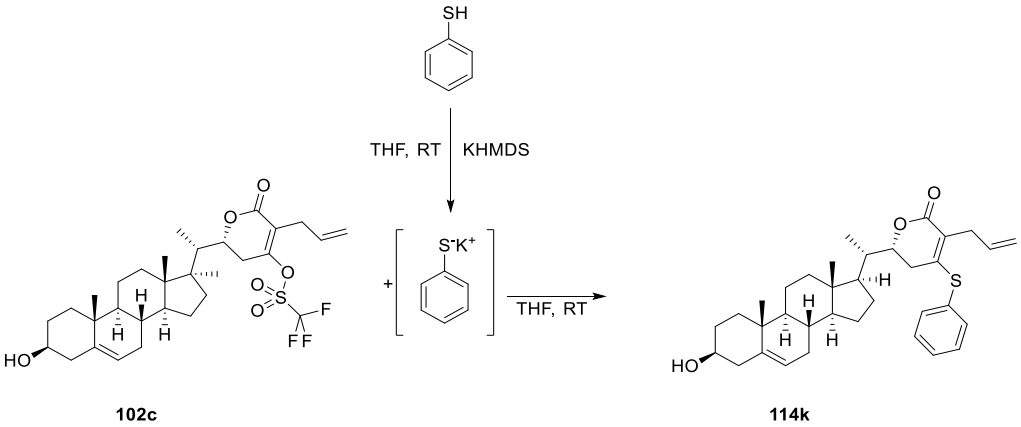
102e **125**

To enol triflate **102e** (28 mg, 51.2 µmol, 1 equiv) in dichloromethane (2 ml) at ambient temperature was added piperidine (51 µl, 512 µmol, 10 equiv). After stirring for 10 min, full conversion to **130i** (see procedure before) was achieved. Triethylamine trihydrofluoride (500 µl, 3.07 mmol, 60 equiv) was added and stirring was continued for 7 days. Then, dichloromethane and a saturated aqueous solution of sodium bicarbonate were added. The organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 10–50% ethyl acetate in petroleum ether) provided unsaturated ketone **125** (9.2 mg, 48%).

White crystalline solid. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.48 (CAM). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ: 6.65 (dd, *J* = 15.9, 8.8 Hz, 1H), 5.99 (d, *J* = 15.9 Hz, 1H), 5.34 (m, 1H), 3.52 (m, 1H), 2.35–2.12 (m, 3H) overlapping with 2.22 (s, 3H), 2.07–0.88 (m, 19H), 1.10 (d, *J* = 6.6 Hz, 3H), 1.01 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ: 199.20, 153.89, 140.74, 128.94, 121.51, 71.71, 56.52, 54.97, 50.04, 42.69, 42.23, 39.96, 39.59, 37.22, 36.46, 31.85, 31.79, 31.59, 28.14, 26.85, 24.26, 21.01, 19.37, 19.26, 12.10. **FTIR** (neat), cm^{-1}: 2931 (br), 1662 (vs), 1266 (s), 1063 (s), 979 (m). **HRMS** (ESI): Calcd for ($C_{25}H_{38}O_2$+H^+): 371.29446, found: 371.29454. $\boldsymbol{\alpha_D^{RT}}$ = -38.4 (c = 0.81 in CH_2Cl_2). **Melting point**: 167 °C (lit: 163–165 °C)[142].

Procedure for Addition-Elimination with Thiophenol

(*R*)-3-allyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(phenylthio)-5,6-dihydro-2*H*-pyran-2-one (114k)



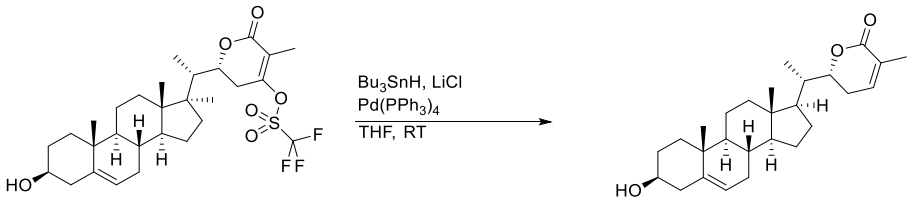
102c **114k**

Enol triflate **102c** (28.6 mg, 48.8 µmol, 1 equiv) was at ambient temperature dissolved in dry tetrahydrofuran (2 ml). Thiophenol (125 µl, 1.22 mmol, 25 equiv) and potassium hexamethyldisilazide (0.5 M solution in toluene, 1.95 ml, 975 µmol, 20 equiv) were dissolved in tetrahydrofuran (5 ml) in a separate flask at ambient temperature. The potassium thiophenolate solution (500 µl, 68.9 µmol, 1.4 equiv) was added to the solution of starting material. After 5 min, the mixture was diluted with dichloromethane and washed with a saturated aqueous solution of ammonium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–50% ethyl acetate in petroleum ether) to provide the product **114k** (26.3 mg, 98%).

Colourless foam. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.42 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.46–7.36 (m, 5H), 5.95–5.83 (m, 1H), 5.33 (m, 1H), 5.16 (dd, *J* = 17.1, 1.6 Hz, 1H), 5.08 (dd, *J* = 10.0, 1.4 Hz, 1H), 4.30 (dt, *J* = 13.0, 3.1 Hz, 1H), 3.50 (m, 1H), 3.39 (ddd, *J* = 14.6, 6.6, 1.1 Hz, 1H), 3.32 (dd, *J* = 14.7, 6.1 Hz, 1H), 2.34–2.16 (m, 3H), 2.00–0.68 (m, 21H), 0.98 (s, 3H), 0.89 (d, *J* = 6.7 Hz, 3H), 0.64 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 164.39, 151.86, 140.84, 134.26, 134.15, 130.17, 129.48, 129.28, 125.25, 121.33, 116.13, 79.09, 71.72, 56.13, 52.06, 49.98, 42.68, 42.23, 39.50, 38.57, 37.23, 36.43, 32.66, 31.83, 31.77, 31.60, 27.71, 27.05, 24.17, 20.96, 19.32, 13.42, 11.64. **FTIR** (neat), cm^{-1}: 2932 (br), 1708 (vs), 1692 (vs), 1378 (s), 1056 (s). **HRMS** (ESI): Calcd for ($C_{35}H_{46}O_3S+H^+$): 547.32404, found: 547.32346. $\boldsymbol{\alpha_D^{RT}}$ = +130.0 (c = 1.0 in $CHCl_3$).

Representative Procedure for Reductive Elimination

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-methyl-5,6-dihydro-2*H*-pyran-2-one (131d)



102a **131d**

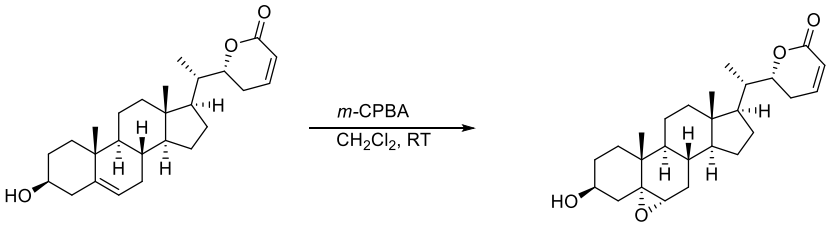
To a solution of enol triflate **102a** (109 mg, 194 µmol, 1 equiv) in tetrahydrofuran (7 ml) at ambient temperature were added lithium chloride (24.7 mg, 583 µmol, 3 equiv), tetrakis(triphenylphosphine) palladium(0) (11.2 mg, 9.72 µmol, 0.05 equiv) and tributyltin hydride (418 µl, 1.56 mmol, 8 equiv) dropwise. After 5 min, the mixture was diluted with dichloromethane and washed with a saturated aqueous solution of sodium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–70% ethyl acetate in petroleum ether) to provide the product **131d** (65.8 mg, 82%).

White amorphous solid. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.49 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.59 (d, *J* = 6.3 Hz, 1H), 5.34 (m, 1H), 4.44 (dt, *J* = 13.1, 3.4 Hz, 1H), 3.51 (m, 1H), 2.42–0.82 (m, 24H), 1.90 (s, 3H), 1.01 (d, *J* = 7.9 Hz, 1H), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 166.57, 140.81, 139.41, 128.19, 121.38, 80.31, 71.69, 56.33, 52.01, 50.02, 42.72, 42.21, 39.64, 38.89, 37.20, 36.44, 31.86, 31.81, 31.58, 27.27, 24.29, 23.27, 20.99, 19.35, 16.97, 13.45, 11.66. **FTIR** (neat), cm^{-1}: 3368 (br), 2932 (br), 1719 (vs), 1132 (m). **HRMS** (ESI): (Calcd for $C_{27}H_{40}O_3$+H^+): 413.30502, found: 413.30443. $\boldsymbol{\alpha_D^{RT}}$ = +12.3 (c = 1.0 in CH_2Cl_2).

5.2.3 Modification of Withanolide Analogues

Representative Procedure for Epoxidation

(*R*)-6-((*S*)-1-((3*S*,4a*R*,5a*S*,6a*S*,6b*S*,9*R*,9a*S*,11a*S*,11b*R*)-3-hydroxy-9a,11b-dimethylhexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-9-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130h)



143 **130h**

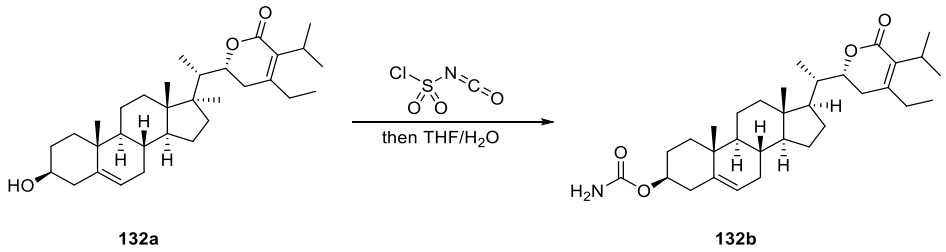
To a solution of alkene **143** (23 mg, 53.9 µmol, 1 equiv) in dichloromethane (2 ml) at ambient temperature was added *meta*-chloroperoxybenzoic acid (14 mg, 80.9 µmol, 1.5 equiv) and resulting solution was stirred for 30 min at ambient temperature. The mixture was diluted with dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–70% ethyl acetate in petroleum ether) to provide the epoxide **130h** (23.6 mg, 91%).

The product is a mixture of diasteromers in a ratio of 4:1 according to ^{1}H NMR.

White amorphous solid. **TLC** (40% ethyl acetate in dichloromethane): R_f = 0.36 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.90 (d, *J* = 9.1, 6.4, 1.7 Hz, 1H), 5.99 (dd, *J* = 9.7, 2.7 Hz, 1H), 4.46 (dt, *J* = 12.9, 3.5 Hz, 1H), 3.90 (m, 0.79H, *major diastereomer*), 3.69 (m, 0.21H, *minor diastereomer*), 3.05 (d, *J* = 2.2 Hz, 0.21H, *minor diastereomer*), 2.90 (d, *J* = 4.4 Hz, 0.79H, *major diastereomer*), 2.36 (m, 1H), 2.18–0.90 (m, 23H), 1.06 (s, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.68 and 0.65 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 164.94, 145.52, 121.26, 80.21, 68.66, 65.66, 59.12, 56.49, 51.80, 42.80, 42.53, 39.81, 39.34, 38.96, 34.84, 32.39, 31.07, 29.91, 28.75, 27.14, 24.05, 23.06, 20.60, 15.89, 13.36, 11.69. **FTIR** (neat), cm^{-1}: 2930 (br), 1714 (vs), 1386 (m), 1262 (s), 1040 (vs). **HRMS** (ESI): Calcd for ($C_{26}H_{38}O_4$+H^+): 415.28429, found: 415.28402. $\boldsymbol{\alpha_D^{RT}}$ = +19.1 (c = 1.0 in $CHCl_3$).

Representative Procedure for Carbamoylation

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-ethyl-5-isopropyl-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl carbamate (132b)



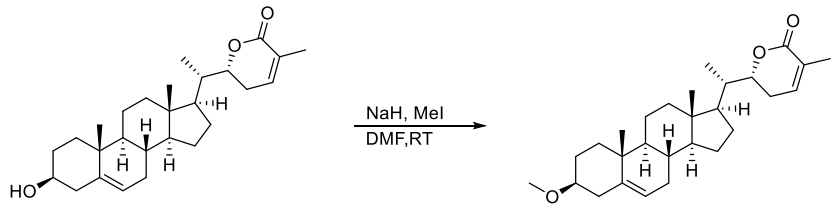
132a **132b**

To a 0 °C solution of alcohol **132a** (26.6 mg, 56.8 µmol, 1 equiv) in dry dichloromethane (3 ml) was added chlorosulfonyl isocyanate (7.41 µl, 85.1 µmol, 1.5 equiv) as a solution in dichloromethane (500 µl). Then, the ice-water cooling bath was removed and the resulting solution was stirred for 5 min at ambient temperature. Water (2 ml) and tetrahydrofuran (5 ml) were added and stirring was continued for 5 min. The reaction mixture was poured into a saturated aqueous solution of sodium bicarbonate and the product was extracted into dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 10–60% ethyl acetate in petroleum ether) to provide carbamate **132b** (22.8 mg, 78%).

Colourless amorphous solid. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.37 (m, 1H), 4.63 (s, 2H), 4.48 (m, 1H), 4.24 (dt, *J* = 13.1, 3.0 Hz, 1H), 2.89 (hept, *J* = 6.9 Hz, 1H), 2.42–1.77 (m, 10H), 1.72–0.83 (m, 15H), 1.24 (d, *J* = 7.0 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.07 (t, *J* = 7.5 Hz, 3H), 1.01 (s, 3H) overlapping with 1.01 (m, 3H), 0.70 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.23, 156.45, 153.52, 139.73, 130.90, 122.32, 78.42, 74.58, 56.26, 52.19, 49.97, 42.72, 39.62, 38.65, 38.33, 36.93, 36.50, 31.84, 31.82, 27.95, 27.66, 27.53, 27.39, 26.71, 24.28, 21.40, 20.97, 20.40, 19.28, 13.53, 12.40, 11.68. **FTIR** (neat), cm^{-1}: 2937 (br), 1704 (vs), 1459 (m), 1331 (m), 1053 (vs). **HRMS** (ESI): Calcd for ($C_{32}H_{49}NO_4$+H^+): 512.37344, found: 512.37299. $\boldsymbol{\alpha_D^{RT}}$ = +31.4 (c = 1.0 in $CHCl_3$).

Representative Procedure for Methylation

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-methyl-5,6-dihydro-2*H*-pyran-2-one (131e)



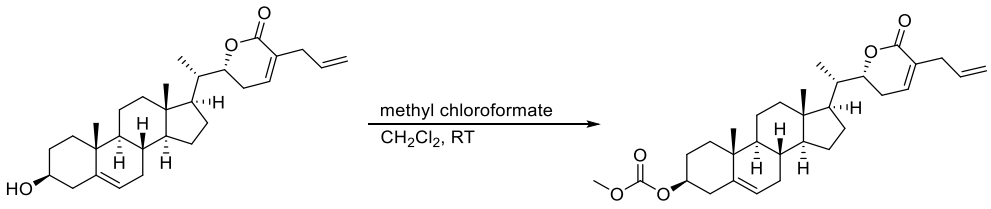
131d **131e**

To a solution of **131d** (10.6 mg, 25.7 µmol, 1 equiv) in dry dimethylformamide (2 ml) were added sodium hydride (60 % dispersion in mineral oil, 10.3 mg, 257 µmol, 10 equiv) and methyl iodide (32 µl, 514 µmol, 10 equiv) at ambient temperature. The reaction mixture was stirred for one hour. The reaction mixture was diluted with dichloromethane and quenched with 1M HCl solution. The separated aqueous layer was once again extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 5–25% ethyl acetate in petroleum ether) to provide the methyl ether **131e** (10.7 mg, 98%).

Colourless oil. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.44 (CAM). **^{1}H NMR** ($CDCl_3$, 600 MHz) δ: 6.59 (d, *J* = 6.5 Hz, 1H), 5.34 (m, 1H), 4.45 (dt, *J* = 13.2, 3.5 Hz, 1H), 3.35 (s, 3H), 3.05 (tt, *J* = 11.2, 4.5 Hz, 1H), 2.42–2.30 (m, 2H), 2.20–2.12 (m, 1H), 2.07 (ddd, *J* = 18.6, 6.5, 3.6 Hz, 1H), 2.04–1.80 (m, 5H), 1.91 (s, 3H), 1.72–1.57 (m, 2H), 1.56–0.85 (m, 12H), 1.02 (d, *J* = 6.7 Hz, 3H), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 151 MHz) δ: 166.63, 140.94, 139.45, 128.22, 121.33, 80.34, 80.29, 56.37, 55.59, 52.03, 50.10, 42.74, 39.66, 38.91, 38.62, 37.13, 36.85, 31.86, 27.93, 27.28, 24.30, 23.28, 20.99, 19.33, 16.99, 13.47, 11.68. **FTIR** (neat), cm^{-1}: 2931 (br), 1719 (vs), 1444 (m), 1378 (m), 1100 (s). **HRMS** (ESI): Calcd for ($C_{28}H_{42}O_3$+H^+): 427.32067, found: 427.32133.

Procedure for Carbonate Formation

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-5-allyl-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl methyl carbonate (114j)



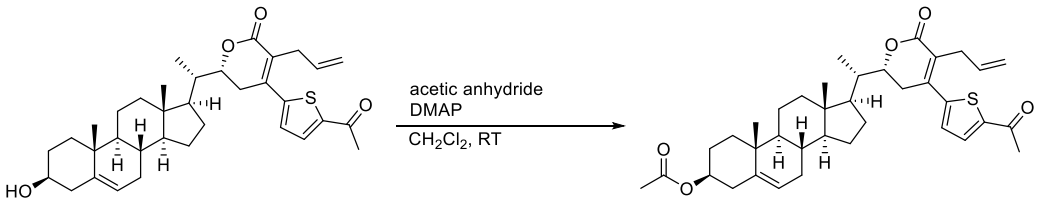
114i **114j**

To a solution of alcohol **114i** (16.2 mg, 36.9 µmol, 1 equiv) in dry dichloromethane (2 ml) were added methyl chloroformate (11.4 µl, 148 µmol, 4 equiv) and pyridine (5.95 µl, 73.9 µmol, 2 equiv) as a solution in dichloromethane (800 µl). The reaction was stirred at ambient temperature until full conversion was achieved. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous solution of sodium bicarbonate. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 5–25% ethyl acetate in petroleum ether) to provide the carbonate **114j** (15.3 mg, 83%).

TLC (15% ethyl acetate in petroleum ether): R_f = 0.38 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.59 (d, *J* = 5.5 Hz, 1H), 5.91–5.79 (m, 1H), 5.39 (m, 1H), 5.12 (m, 1H), 5.09 (s, 1H), 4.52–4.40 (m, 2H), 3.76 (s, 3H), 3.05 (d, *J* = 5.8 Hz, 2H), 2.45–2.31 (m, 3H), 2.11 (ddd, *J* = 18.0, 6.5, 3.4 Hz, 1H), 2.06–1.83 (m, 5H), 1.75–0.90 (m, 17H), 1.02 (d, *J* = 7.2 Hz, 3H) overlapping with 1.02 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.77, 155.16, 139.46, 139.44, 134.84, 131.01, 122.65, 117.09, 80.10, 77.79, 56.33, 54.45, 52.07, 49.99, 42.79, 39.65, 38.91, 38.01, 36.86, 36.53, 34.61, 31.87, 31.85, 27.67, 27.32, 24.30, 23.37, 21.00, 19.24, 13.48, 11.69. **FTIR** (neat), cm^{-1}: 2934 (br), 1737 (vs), 1705 (vs), 1271 (vs), 1256 (vs). **HRMS** (ESI): Calcd for ($C_{31}H_{44}O_5$+H^+): 497.32615, found: 497.32621. $\boldsymbol{\alpha_D^{RT}}$ = +5.0 (c = 1.0 in $CHCl_3$).

Representative Procedure for Acetylation

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(5-acetylthiophen-2-yl)-5-allyl-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (114d)



114c **114d**

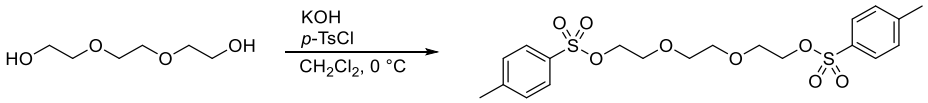
To a solution of **114c** (26.3 mg, 46.7 µmol, 1 equiv) in dichloromethane (2 ml) were added neat 4-dimethylaminopyridine (5.7 mg, 46.7 µmol, 1 equiv) and acetic anhydride (8.8 µl, 93.5 µmol, 2 equiv) as a solution in dichloromethane (500 µl). After 2 h, the reaction mixture was diluted with dichloromethane and saturated aqueous sodium bicarbonate. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 10–45% ethyl acetate in petroleum ether) to provide the product **114d** (25.8 mg, 91%).

Colourless amorphous solid. **TLC** (25% ethyl acetate in petroleum ether): R_f = 0.42 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.64 (d, *J* = 3.9 Hz, 1H), 7.28 (d, *J* = 4.0 Hz, 1H), 5.95 (m, 1H), 5.37 (m, 1H), 5.11 (d, *J* = 11.5 Hz, 1H), 5.07 (d, *J* = 17.9 Hz, 1H), 4.59 (m, 1H) overlapping with 4.54 (dt, *J* = 13.2, 3.0 Hz, 1H), 3.44 (dd, *J* = 15.6, 5.5 Hz, 1H), 3.28 (dd, *J* = 15.6, 5.1 Hz, 1H), 2.86 (dd, *J* = 16.8, 13.4 Hz, 1H), 2.58 (s, 3H), 2.46 (dd, *J* = 17.2, 2.6 Hz, 1H), 2.32 (m, 2H), 2.13–0.80 (m, 19H), 2.02 (s, 3H), 1.07 (d, *J* = 6.6 Hz, 3H), 1.02 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 190.54, 170.54, 165.89, 147.82, 145.48, 142.63, 139.66, 134.85, 132.20, 129.01, 127.17, 122.34, 116.24, 78.62, 73.85, 56.18, 52.03, 49.89, 42.78, 39.57, 38.81, 38.03, 36.92, 36.52, 32.44, 31.82, 31.78, 30.05, 27.69, 27.36, 26.73, 24.26, 21.41, 20.94, 19.26, 13.44, 11.69. **FTIR** (neat), cm^{-1}: 2938 (br), 1706 (vs), 1664 (vs), 1242 (vs). **HRMS** (ESI): Calcd for ($C_{37}H_{48}O_5S+H^+$): 605.32952, found: 605.33034. $\boldsymbol{\alpha_D^{RT}}$ = +27.5 (c = 1.0 in $CHCl_3$).

5.2.4 Synthesis of Pulldown Probes

Synthesis of the Linker

(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (147)[91]

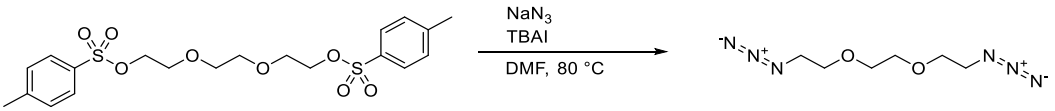


Triethylene glycol (146) 147

Potassium hydroxide (3.36 g, 60 mmol, 4 equiv) was added to an ice-cold solution of triethylene glycol (**146**) (2 ml, 15 mmol, 1 equiv) in dichloromethane (30 ml). 4-Toluenesulfonyl chloride (6.35 g, 33.3 mmol, 2.22 equiv) was added portionwise at 0 °C until full conversion was achieved. Then, water was added and the aqueous phase was extracted with dichloromethane. The separated dichloromethane solution was extracted with water, dried over $MgSO_4$ and concentrated. The remaining residue was purified by flash-column chromatography (gradient elution with 3–15% diethyl ether in dichloromethane) to provide the product **147** (5.2 g, 76%).

Colourless oil, which solidifies upon standing**. TLC** (50% ethyl acetate in petroleum ether): R_f = 0.36 ($KMnO_4$). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ: 7.76 (d, *J* = 8.4 Hz, 4H), 7.32 (d, *J* = 8.0 Hz, 4H), 4.11 (t, *J* = 4.7 Hz, 4H), 3.62 (t, *J* = 4.7 Hz, 4H), 3.49 (s, 4H), 2.42 (s, 6H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ: 144.78, 132.73, 129.74, 127.79, 70.50, 69.14, 68.56, 21.51. **FTIR** (neat), cm^{-1}: 2871 (w), 1346 (s), 1171 (vs), 1015 (s), 980 (s), 910 (vs). **HRMS** (ESI): Calcd for ($C_{20}H_{26}O_8S_2$+H^+): 459.11419, found: 459.11463.

1,2-bis(2-azidoethoxy)ethane (148)[91]

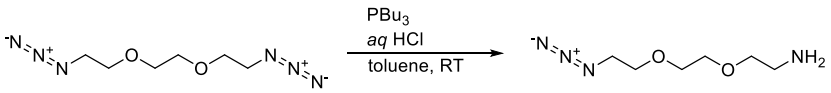


147 148

To a solution of **147** (5.08 g, 11.0 mmol, 1 equiv) in dimethylformamide (30 ml) were added sodium azide (2.88 g, 44.3 mmol, 4 equiv) and tetrabutylammonium iodide (204 mg, 554 µmol, 5 mol%). The reaction mixture was stirred at 80 °C for 6 h. Dimethylformamide was then evaporated under reduced pressure and the residue was suspended in diethyl ether. The solution was then extracted with 1M HCl and saturated NaCl. The organic layer was dried over $MgSO_4$ and the solvent evaporated to afford the product **148** (1.96 g, 89%).

Yellow oil**. TLC** (50% ethyl acetate in petroleum ether): R_f = 0.56 ($KMnO_4$). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ: 3.70–3.62 (m, 4H), 3.65 (s, 4H), 3.36 (t, *J* = 5.0 Hz, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ: 70.59, 70.00, 50.54. **FTIR** (neat), cm^{-1}: 2868 (br), 2092 (vs), 1283 (s), 1118 (s). **HRMS** (ESI): Calcd for ($C_6H_{12}N_6O_2$+H^+): 201.10945, found: 201.10978.

2-(2-(2-azidoethoxy)ethoxy)ethan-1-amine (149)[91]

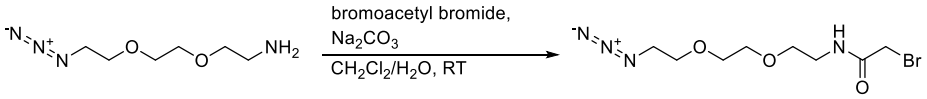


148 149

To a solution of **148** (1.9 g, 9.5 mmol, 1 equiv) in toluene (10 ml) were added aqueous hydrochloric acid (5%, 10 ml) and *tri-n*-butylphosphine (2.37 ml, 9.5 mmol, 1 equiv). The solution was stirred overnight at ambient temperature. Then, the reaction mixture was diluted with more toluene and water. The aqueous layer was separated, extracted with dichloromethane and then basified with excess solid sodium hydroxide to pH 14. The aqueous solution was extracted with dichloromethane, the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford the product **149** (935 mg, 57%).

Yellow oil. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ: 3.63–3.59 (m, 2H), 3.59–3.55 (m, 2H), 3.54–3.49 (m, 2H), 3.42–3.33 (m, 4H), 2.65 (t, *J* = 5.8 Hz, 2H), 1.57 (br s, 2H). **^{13}C NMR** (DMSO-d_6, 50 MHz) δ: 73.16, 69.71, 69.63, 69.34, 50.00, 41.39. **FTIR** (neat), cm^{-1}: 2864 (br), 2097 (vs), 1284 (m), 1117 (vs). **HRMS** (ESI): Calcd for ($C_6H_{14}N_4O_2$+H^+): 175.11895, found 175.11940.

N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-2-bromoacetamide (150)[91]



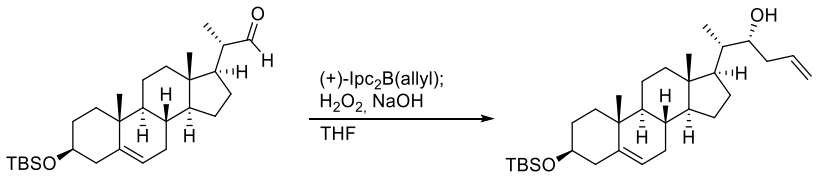
149 150

To a solution of **149** (538 mg, 3.09 mmol, 1 equiv) in a mixture of dichloromethane (15 ml) and saturated aqueous sodium carbonate (15 ml) was added bromoacetyl bromide (404 µl, 4.63 mmol, 1.50 equiv) at ambient temperature. The reaction mixture was stirred for 2h. Then, the reaction mixture was diluted with saturated aqueous sodium bicarbonate and the water phase was extracted with dichloromethane. The organic phase was washed with 1M HCl and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo to provide the product (818 mg, 90%).

Colourless oil**. ^{1}H NMR** (DMSO-d_6, 500 MHz) δ: 8.31 (s, 1H), 3.85 (s, 2H), 3.60 (t, *J* = 5.0 Hz, 1H), 3.58–3.55 (m, 2H), 3.55–3.51 (m, 2H), 3.44 (t, *J* = 5.8 Hz, 1H), 3.39 (t, *J* = 5.0 Hz, 1H). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ: 166.01, 69.59, 69.22, 68.73, 49.99, 39.09, 29.41. **FTIR** (neat), cm^{-1}: 2868 (br), 2098 (vs), 1656 (vs), 1533 (s), 1284 (s), 1116 (vs). **HRMS** (ESI): Calcd for ($C_8H_{15}BrN_4O_3$+H^+): 295.04003, found 295.04073, calcd for ($C_8H_{15}{}^{81}BrN_4O_3$+H^+): 297.03798, found 297.03818.

Synthesis of the negative Probes 138 and 139

(2*S*,3*R*)-2-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)hex-5-en-3-ol (140)[53]

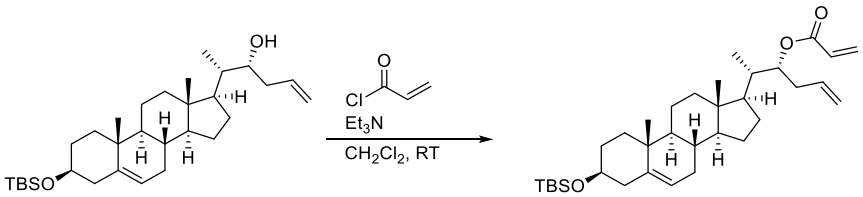


75 140

(+)-B-Allyldiisopinocampheylborane (1.0 M solution in pentane, 600 µL, 600 µmol, 1.3 equiv) was added dropwise to a −78 °C solution of aldehyde **75** (200 mg, 450 µmol, 1 equiv) in tetrahydrofuran (10 ml). After 25 min, the cooling bath was removed and sodium hydroxide (2.0 M aqueous solution, 450 µL, 899 µmol, 2 equiv) and hydrogen peroxide (35% aqueous solution by weight, 87 µl, 2 equiv) were added sequentially. The mixture was allowed to reach ambient temperature and then was stirred for 20 h. The oxidation mixture was diluted with dichloromethane and sequentially washed with water and saturated aqueous solution of sodium chloride. The washed organic phase was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The remaining residue was purified by flash-column chromatography (gradient elution with 5–15% ethyl acetate in petroleum ether) to provide the homoallylic product **140** (176 mg, 80%).

White crystalline solid. **TLC** (15% ethyl acetate in petroleum ether): R_f = 0.46 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.90–5.79 (m, 1H), 5.32 (m, 1H), 5.18–5.10 (m, 2H), 3.69 (m, 1H), 3.48 (m, 1H), 2.32–2.12 (m, 3H), 2.06–1.92 (m, 3H), 1.85–1.67 (m, 4H), 1.66–1.31 (m, 9H), 1.23–0.90 (m, 5H), 1.00 (s, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.89 (s, 9H), 0.71 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 141.62, 136.28, 121.02, 117.79, 72.62, 72.20, 56.46, 53.15, 50.25, 42.82, 42.70, 41.18, 39.83, 37.40, 36.59, 34.95, 32.09, 31.96, 31.94, 27.43, 25.94, 24.39, 21.08, 19.42, 18.26, 12.50, 11.85, -4.57. **FTIR** (neat), cm^{-1}: 2934 (s), 1470 (m), 1383 (m), 1255 (m), 1084 (s), 837 (s). **HRMS** (ESI): Calcd for ($C_{31}H_{54}O_2Si+H^+$): 487.39658, found 487.39424. α_D^{RT} = -31.1 (c = 1.0 in CH_2Cl_2). **Melting point**: 133 °C (lit: 125– 127 °C)[53]

(2*S*,3*R*)-2-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)hex-5-en-3-yl acrylate (141)



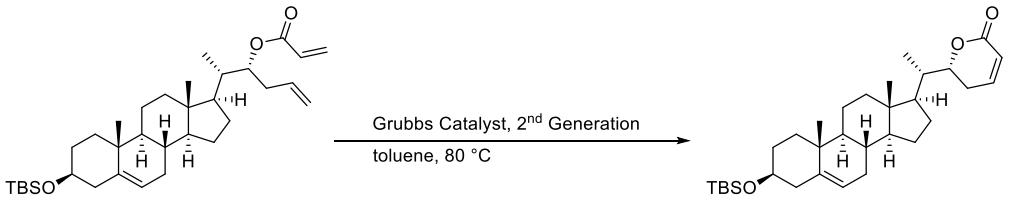
140 141

Acryloyl chloride (54 µl, 666 µmol, 2 equiv) and triethylamine (186 µl, 1.33 mmol, 4 equiv) were added to a solution of homoallylic alcohol **140** (162 mg, 333 µmol, 1 equiv) in dichloromethane (20 ml). The resulting light yellow solution was stirred at ambient temperature for 10 min. Then, dichloromethane

and a saturated aqueous solution of sodium bicarbonate were added. The organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 5–20% ethyl acetate in petroleum ether) provided acrylic ester **141** (140 mg, 78%).

White crystalline solid. **TLC** (5% ethyl acetate in petroleum ether): R_f = 0.46 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.37 (dd, *J* = 17.3, 1.5 Hz, 1H), 6.10 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.78 (dd, *J* = 10.4, 1.5 Hz, 1H) overlapping with 5.82–5.68 (m, 1H), 5.31 (m, 1H), 5.10–5.02 (m, 2H), 5.02–4.97 (m, 1H), 3.48 (m, 1H), 2.32–2.21 (m, 3H), 2.16 (ddd, *J* = 13.4, 4.7, 2.0 Hz, 1H), 2.02–1.93 (m, 2H), 1.90–1.38 (m, 11H), 1.30–0.84 (m, 6H), 0.99 (s, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.89 (s, 9H), 0.68 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.75, 141.54, 135.13, 130.08, 129.05, 121.03, 116.79, 76.08, 72.60, 56.47, 53.12, 50.22, 42.81, 42.75, 39.82, 39.16, 37.40, 36.57, 32.15, 32.09, 31.92, 27.16, 25.93, 24.34, 21.07, 19.41, 18.24, 13.15, 11.86, -4.58. **FTIR** (neat), cm^{-1}: 2933 (s), 1720 (s), 1196 (s), 1060 (vs), 836 (s). The compound was not detectable by **HRMS**. $\boldsymbol{\alpha_D^{RT}}$ = -17.2 (c = 1.0 in CH_2Cl_2). **Melting point**: 128-129 °C.

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (142)

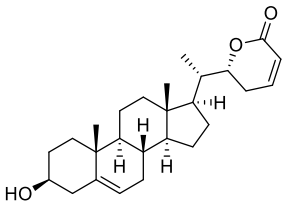


141 **142**

2nd Generation Grubbs catalyst (9.9 mg, 11.7 µmol, 0.05 equiv) was added to a solution of acrylate ester **141** (126 mg, 233 µmol, 1 equiv) in toluene (18 ml). The resulting mixture was stirred for 1 h at 80 °C. The dark brown mixture was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 0–10% ethyl acetate in dichloromethane) provided dehydrolactone **142** (100 mg, 84%).

White amorphous solid**. TLC** (dichloromethane): R_f = 0.25 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.93–6.86 (m, 1H), 5.99 (dd, *J* = 9.7, 2.3 Hz, 1H), 5.30 (m, 1H), 4.48 (dt, *J* = 12.9, 3.5 Hz, 1H), 3.47 (m, 1H), 2.42–2.21 (m, 2H), 2.20–2.08 (m, 2H), 2.07–1.91 (m, 3H), 1.90–1.30 (m, 10H), 1.27–0.90 (m, 6H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 0.88 (s, 9H), 0.71 (s, 3H), 0.04 (s, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 164.88, 145.43, 141.62, 121.30, 120.85, 80.25, 72.55, 56.42, 52.08, 50.16, 42.79, 39.72, 38.99, 37.36, 36.55, 32.04, 31.91, 31.86, 27.30, 25.91, 24.29, 23.07, 21.01, 19.38, 18.21, 13.41, 11.68, -4.60. **FTIR** (neat), cm^{-1}: 2933 (br), 1726 (s), 1698 (s), 1381 (s), 1246 (s), 1079 (s). **HRMS** (ESI): Calcd for ($C_{32}H_{52}O_3Si+H^+$): 513.37585, found 513.37537. $\boldsymbol{\alpha_D^{RT}}$ = +24.0 (c = 1.0 in CH_2Cl_2).

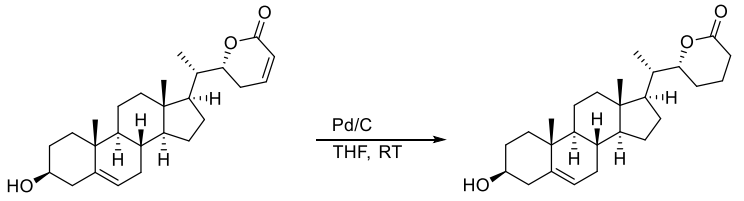
(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (143)



The product was prepared from **142** according to the general TBS-deprotection procedure (page 95) in 97% yield.

White crystalline solid. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ: 6.85 (m, 1H), 5.86 (dd, *J* = 9.7, 2.2 Hz, 1H), 5.20 (m, 1H), 4.38 (dt, *J* = 12.5, 3.2 Hz, 1H), 3.33 (m, 1H), 2.36–0.72 (m, 24H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 3H), 0.60 (s, 3H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ: 165.69, 146.34, 140.66, 121.00, 120.52, 80.40, 70.94, 56.05, 51.70, 49.78, 42.49, 41.53, 39.38, 38.74, 36.96, 36.19, 31.61, 31.52, 30.84, 26.99, 24.00, 22.75, 20.72, 19.01, 13.03, 11.36. **FTIR** (neat), cm^{-1}: 2930 (s), 1708 (vs), 1389 (s), 1267 (s), 804 (s). **HRMS** (ESI): Calcd for ($C_{26}H_{38}O_3$+H^+): 399.28937, found 399.28882. $\boldsymbol{\alpha_D^{RT}}$= +23.6 (c = 1.0 in CH_2Cl_2). **Melting point**: 234 °C.

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)tetrahydro-2*H*-pyran-2-one (138)



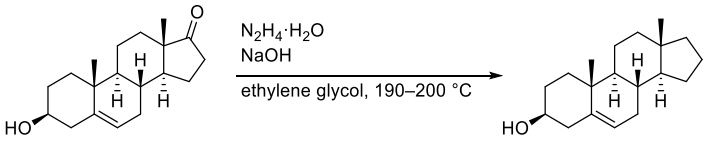
143 138

To a solution of unsaturated lactone **143** (62 mg, 156 µmol, 1 equiv) in tetrahydrofuran (5 ml) at ambient temperature was added 10% palladium-on-carbon (8.3 mg, 7.78 µmol, 0.05 equiv). The reaction flask was repeatedly evacuated-backfilled with dihydrogen, then a dihydrogen-filled balloon was attached. After vigorous stirring for 90 min at ambient temperature, the reaction mixture was filtered through cotton with dichloromethane and the filtrate was concentrated in vacuo to provide the crude hydrogenation product. Purification of the residue by flash-column chromatography (gradient elution with 30–100% ethyl acetate in petroleum ether) provided saturated lactone **138** (54 mg, 87%).

The hydrogenation time is not well reproducible! If the reaction is run for too long, hydrogenation of the olefin in the B-ring occurs. The starting material, the desired product and the overhydrogenation product all cospot on TLC and the reaction can be only monitored by NMR.The desired product and the overhydrogenation product cannot be separated by column chromatography!

White crystalline solid. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.35 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.34 (m, 1H), 4.34 (dt, *J* = 11.6, 3.1 Hz, 1H), 3.50 (m, 1H), 2.63–2.53 (m, 1H), 2.44–2.34 (m, 1H), 2.32–2.26 (m, 1H), 2.26–2.17 (m, 1H), 2.05–0.86 (m, 24H), 1.00 (s, 3H), 0.95 (d, *J* = 6.7 Hz, 1H), 0.70 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.15, 140.85, 121.37, 83.10, 71.68, 56.33, 52.17, 50.06, 42.74, 42.23, 39.65, 39.43, 37.22, 36.45, 31.88, 31.83, 31.59, 29.73, 27.29, 24.29, 21.08, 21.00, 19.34, 18.87, 12.83, 11.70. **FTIR** (neat), cm^{-1}: 3444 (br), 2931 (br), 1712 (vs), 1255 (s), 1051 (s). **HRMS** (ESI): Calcd for ($C_{26}H_{40}O_3$+H^+): 401.30502, found 401.30599. $\boldsymbol{\alpha_D^{RT}}$= -32.7 (c = 1.0 in CH_2Cl_2). **Melting point**: 220 °C.

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol (139)[90, 143]



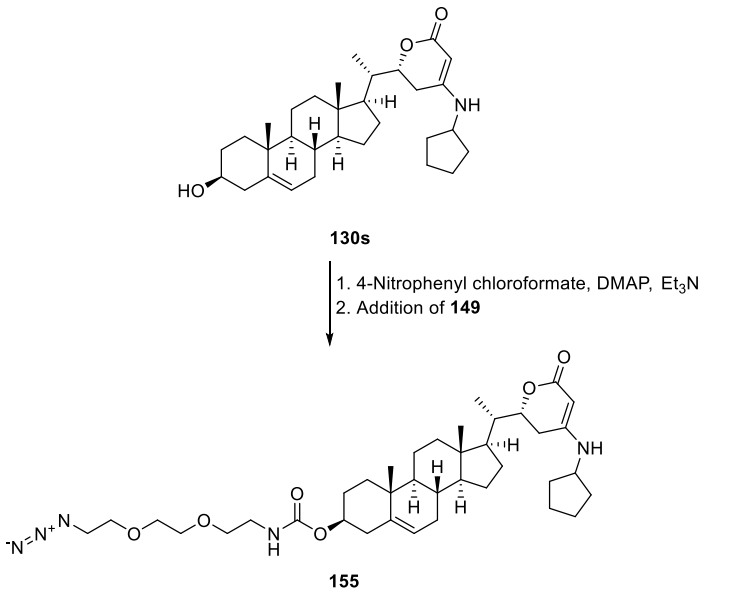
(+)-dehydroisoandrosterone (145) **139**

Hydrazine monohydrate (507 µl, 10.4 mmol, 6 equiv) and sodium hydroxide (408 mg, 10.20 mmol, 5.9 equiv) were added to a suspension of (+)-dehydroisoandrosterone (500 mg, 1.73 mmol, 1 equiv) in ethylene glycol (7 ml). The reaction mixture was stirred at 200 °C in a seled tube for 2 h. Then the tube was opened and stirring at 190 °C continued for further 20 h. Then, dichloromethane and a saturated aqueous solution of ammonium chloride were added. The organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 25–40% ethyl acetate in petroleum ether) provided product **139** (263 mg, 55%).

White crystalline solid. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.50 (CAM). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ: 5.34 (m, 1H), 3.52 (m, 1H), 2.35–2.15 (m, 3H), 2.08–1.94 (m, 1H), 1.91–0.82 (m, 18H) overlapping with 1.01 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ: 140.72, 121.71, 71.75, 54.80, 50.38, 42.27, 40.55, 40.23, 38.66, 37.29, 36.61, 32.16, 32.10, 31.63, 25.59, 21.10, 20.47, 19.42, 17.22. **FTIR** (neat), cm^{-1}: 3223 (br), 2931 (s), 1451 (m), 1376 (m), 1052 (vs). **HRMS** (ESI): Calcd for ($C_{19}H_{30}O$+H^+): 275.23694, found 275.23668. $\boldsymbol{\alpha_D^{RT}}$= -53.6 (c = 1.0 in CH_2Cl_2). **Melting point**: 129 °C (lit: 129–131 °C)[143].

Attachment of the Linker

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(cyclopentylamino)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl (2-(2-(2-azidoethoxy)ethoxy)ethyl)carbamate (155)

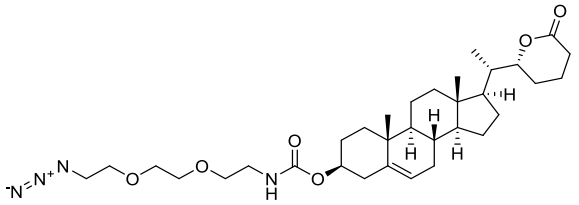


To a solution of **130s** (21.8 mg, 45.3 µmol, 1 equiv) in 1,2-dichloroethane (3 ml) at 60 °C were added 4-nitrophenyl chloroformate (36.5 mg, 181 µmol, 4 equiv), 4-dimethylaminopyridine (16.6 mg, 136 µmol, 3 equiv) and triethylamine (12.6 µl, 90.5 µmol, 2 equiv). The reaction was stirred at 60 °C until full consumption of the starting material was observed in TLC. Then the linker (**149**) was added dropwise and the conversion was monitored by TLC. The reaction was stirred at 60 °C until full conversion was achieved. The reaction mixture was diluted with dichloromethane and the solution extracted with a saturated aqueous solution of potassium carbonate until the organic layer was colourless. The organic solution was then dried over $MgSO_4$ and concentrated. Purification of the residue by flash-column chromatography (gradient elution with 50–100% ethyl acetate in petroleum ether) provided product **155** (21.4 mg, 69%).

*The starting material and the carbamate product co-spot on TLC. It is therefore essential to achieve full conversion to the carbonate intermediate before addition of the linker. Otherwise a mixture is formed. If the basic extraction is not complete, traces of 4-Nitrophenol remain in the product and cannot be removed by column chromatography. The product should not be purified by HPLC if the solvent contains TFA because otherwise fragmentation to compound **125** occurs.*

Colourless foam. **TLC** (ethyl acetate): R_f = 0.62 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.35 (m, 1H), 5.11 (m, 1H), 4.61 (s, 1H), 4.52 (d, *J* = 6.0 Hz, 1H), 4.40 (m, 1H), 4.33 (dt, *J* = 13.2, 3.2 Hz, 1H), 3.71 (m, 1H), 3.63 (t, *J* = 5.0 Hz, 2H), 3.61–3.56 (m, 4H), 3.51 (t, *J* = 5.2 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 2H), 3.29 (q, *J* = 5.2 Hz, 2H), 2.48 (m, 1H), 2.36–2.21 (m, 2H), 2.05–1.92 (m, 5H), 1.90–1.79 (m, 3H), 1.75–0.91 (m, 20H), 1.00 (s, 3H), 0.99 (d, *J* = 7.1 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 169.05, 156.61, 140.77, 122.63, 83.03, 77.78, 74.72, 71.10, 70.85, 70.63, 70.52, 57.05, 54.84, 52.91, 51.38, 50.71, 43.33, 41.31, 40.34, 39.51, 39.14, 37.64, 37.14, 33.56, 33.38, 32.47, 28.72, 27.89, 24.88, 24.59, 24.53, 21.61, 19.69, 13.70, 12.07. **FTIR** (neat), cm^{-1}: 3292 (br), 2938 (br), 2100 (s), 1655 (s), 1596 (s). **HRMS** (ESI): Calcd for ($C_{38}H_{59}N_5O_6$+H^+): 682.45381, found 682.45618. $\boldsymbol{\alpha_D^{RT}}$ = +32.1 (c = 1.5 in CH_2Cl_2).

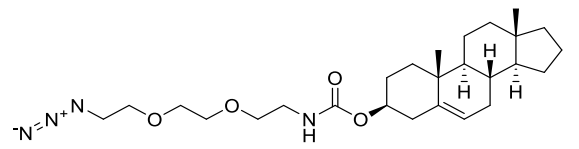
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-dimethyl-17-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl (2-(2-(2-azidoethoxy)ethoxy)ethyl)carbamate (156)



The compound was prepared according to the general procedure above in 68% yield.

Yellow oil, which solidifies at low temperature. **TLC** (70% ethyl acetate in petroleum ether): R_f = 0.37 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.36 (m, 1H), 5.11 (m, 1H), 4.48 (m, 1H), 4.33 (dt, *J* = 11.5, 3.0 Hz, 1H), 3.67 (t, *J* = 5.0 Hz, 2H), 3.63 (m, 4H), 3.55 (t, *J* = 5.0 Hz, 2H), 3.39 (t, *J* = 5.0 Hz, 2H), 3.36 (m, 2H), 2.59 (m, 1H), 2.45–2.32 (m, 2H), 2.27 (m, 1H), 2.05–0.85 (m, 23H), 1.00 (s, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.70 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.10, 156.14, 139.93, 122.12, 83.09, 74.17, 70.54, 70.24, 70.17, 70.03, 56.27, 52.15, 50.62, 49.95, 42.73, 40.63, 39.61, 39.44, 38.51, 36.97, 36.51, 31.85, 31.82, 29.74, 28.10, 27.28, 24.28, 21.07, 20.96, 19.26, 18.87, 12.81, 11.69. **FTIR** (neat), cm^{-1}: 2935 (br), 2109 (s), 1726 (vs), 1708 (vs), 1524 (s), 1239 (vs). **HRMS** (ESI): Calcd for ($C_{33}H_{52}N_4O_6$+H^+): 601.39596, found 601.39893. $\boldsymbol{\alpha_D^{RT}}$ = -15.3 (c = 1.0 in CH_2Cl_2).

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl (2-(2-(2-azidoethoxy)ethoxy)ethyl)carbamate (157)



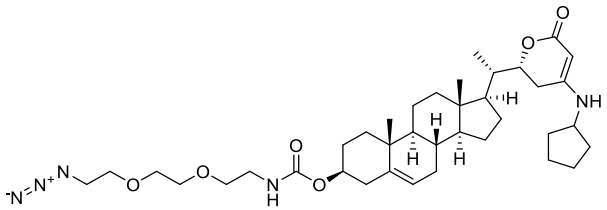
The compound was prepared according to the general procedure above in 73% yield.

Yellow oil, which solidifies at low temperature. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.30 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.36 (m, 1H), 5.11 (s, 1H), 4.49 (m, 1H), 3.67 (t, *J* = 5.0 Hz, 2H), 3.63

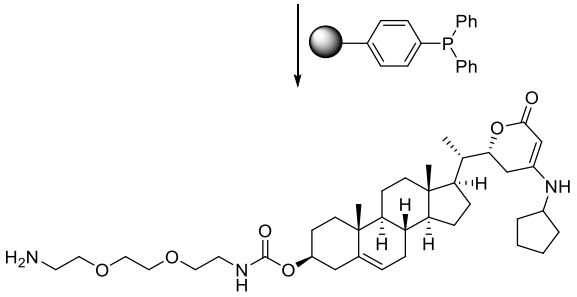
(m, 4H), 3.55 (t, *J* = 5.1 Hz, 2H), 3.40 (t, *J* = 5.0 Hz, 2H), 3.36 (m, 2H), 2.40–2.32 (m, 1H), 2.31–2.22 (m, 1H), 2.03–1.95 (m, 1H), 1.92–1.81 (m, 2H), 1.77–1.34 (m, 10H), 1.21–1.08 (m, 4H), 1.01 (s, 3H), 0.99–0.85 (m, 2H), 0.70 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 156.16, 139.84, 122.40, 74.27, 70.55, 70.25, 70.19, 70.04, 54.76, 50.63, 50.30, 40.65, 40.54, 40.24, 38.64, 38.55, 37.05, 36.68, 32.16, 32.10, 28.16, 25.58, 21.06, 20.47, 19.33, 17.22. **FTIR** (neat), cm^{-1}: 3333 (br), 2940 (br), 2092 (s), 1711 (vs), 1542 (s). **HRMS** (ESI): Calcd for ($C_{26}H_{42}N_4O_4$+H^+): 475.32788, found 475.32989. $\boldsymbol{\alpha_D^{RT}}$= -35.0 (c = 1.0 in CH_2Cl_2). **Melting point**: 68–69 °C.

Reduction of the Pulldown Probes

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(cyclopentylamino)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (158)



155



158

Polymer-supported triphenylphosphine[2] (1.4-2.0 mmol/g on polystyrene, 176 mg, 5 equiv) was four times washed with THF (3 ml). The starting material (40.9 mg, 60 µmol, 1 equiv) was added as a solution in THF (2 ml), followed by 300 µl water. The reaction was stirred at ambient temperature for 5 days. The reaction mixture was filtered through *CHROMAFIL® PET-45/15 MS* filters several times and concentrated to afford the product **158** in 81% yield.

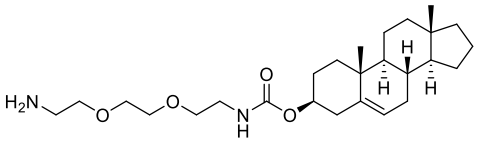
Polymer-supported triphenylphosphine can contain a substantial amount of unbound triphenylphosphine. It is therefore essential to wash it prior to usage. After complete reaction, polymer-supported triphenylphosphine/triphenylphosphine oxide can be only removed by multiple filtration.

Yellow foam. **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.45 (t, *J* = 5.5 Hz, 1H), 5.35 (m, 1H), 4.73 (m, N*H*), 4.60 (s, 1H), 4.40 (m, 1H), 4.32 (dt, *J* = 13.2, 3.1 Hz, 1H), 3.70 (m, 1H), 3.61–3.54 (m, 4H), 3.54–3.46 (m, 4H),

[2] Purchased from Alfa Aesar (L19478). The catalyst loading was assumed as 1.7 mmol/g.

3.34–3.22 (m, 2H), 2.85 (m, 2H), 2.47 (m, 1H), 2.35–2.23 (m, 2H), 2.05–0.78 (m, 31H), 1.00 (s, 3H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ 169.19, 156.73, 140.75, 125.96, 122.64, 82.83, 77.82, 70.86, 70.71, 70.62, 68.32, 57.04, 54.83, 52.91, 50.71, 43.33, 40.34, 39.51, 39.16, 37.64, 37.14, 33.52, 33.34, 32.47, 30.67, 28.74, 27.89, 26.15, 24.88, 24.60, 24.55, 21.61, 19.70, 13.72, 12.08. **FTIR** (neat), cm^{-1}: 3270 (br), 2931 (s), 1655 (vs), 1596 (vs), 1551 (vs), 1258 (vs). **HRMS** (ESI): Calcd for ($C_{38}H_{61}N_3O_6$+H^+): 656.46331, found 656.46624.

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (159)

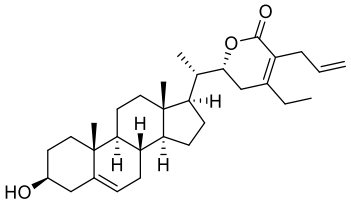


The product was prepared according to the general Staudinger reaction protocol (page 120) from **157** in 87% yield.

Yellow oil. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ: 7.04 (t, *J* = 5.5 Hz, 1H), 5.34 (m, 1H), 4.31 (m, 1H), 3.49 (m, 4H), 3.39 (t, *J* = 6.1 Hz, 2H), 3.36 (t, *J* = 5.8 Hz, 2H), 3.10 (dd, *J* = 11.8, 5.9 Hz, 2H), 2.66 (t, *J* = 5.7 Hz, 2H), 2.32–2.25 (m, 1H), 2.25–2.18 (m, 1H), 2.00–1.91 (m, 1H), 1.88–0.80 (m, 20H), 0.97 (s, 3H), 0.69 (s, 3H). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ: 155.68, 139.74, 121.80, 72.90, 72.59, 69.51, 69.08, 66.97, 54.22, 49.76, 41.13, 38.30, 38.10, 36.62, 36.19, 34.33, 31.62, 30.39, 27.87, 25.15, 25.09, 20.99, 20.59, 20.06, 19.01, 17.03. **FTIR** (neat), cm^{-1}: 3334 (br), 2931 (vs), 1698 (vs), 1454 (m), 1518 (s), 1250 (vs). **HRMS** (ESI): Calcd for ($C_{26}H_{44}N_2O_4$+H^+): 449.33738, found 449.33863.

5.2.5 Analytical Characterization of Withanolide Analogues

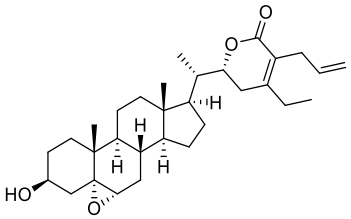
(*R*)-3-allyl-4-ethyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (114a)



The product was synthesized according to the general Negishi Coupling procedure (page 101) from **102c** in 59% yield. 3 equiv diethylzinc were added in one portion.

Pale yellow foam. **TLC** (40% ethyl acetate in petroleum ether): Rf = 0.46 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.84 (m, 1H), 5.34 (m, 1H), 5.01 (dd, *J* = 16.9, 1.2 Hz, 1H), 4.98 (dd, *J* = 9.9, 1.2 Hz, 1H), 4.34 (dt, *J* = 13.2, 3.2 Hz, 1H), 3.51 (m, 1H), 3.16 (dd, *J* = 15.3, 6.0 Hz, 1H), 3.06 (dd, *J* = 15.1, 5.8 Hz), 2.50–2.15 (m, 5H), 2.10–1.90 (m, 4H), 1.89–1.77 (m, 2H), 1.75–0.85 (m, 15H), 1.07 (t, *J* = 7.6 Hz, 3H), 1.01 (d, *J* = 8.0 Hz, 3H) overlapping with 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 166.58, 155.95, 140.83, 135.74, 123.51, 121.38, 114.98, 78.74, 71.68, 56.32, 52.11, 50.04, 42.73, 42.20, 39.64, 38.75, 37.20, 36.44, 31.86, 31.83, 31.57, 30.59, 27.33, 26.92, 24.28, 21.00, 19.35, 13.46, 11.82, 11.68. **FTIR** (neat), cm^{-1}: 2933 (br), 1687 (vs), 1129 (m), 1058 (m). **HRMS** (ESI): Calcd for (C31H46O3+H+): 467.35197, found: 467.35196.

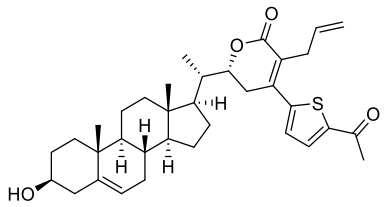
(*R*)-3-allyl-4-ethyl-6-((*S*)-1-((3*S*,4a*R*,5a*S*,6a*S*,6b*S*,9R,9a*S*,11a*S*,11b*R*)-3-hydroxy-9a,11b-dimethylhexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-9-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (114b)



The product was synthesized according to the general epoxidation procedure (page 107) of **114a** in 85% yield. The product is a mixture of diastereomers in a ratio of ~10:1. Only the signals of the major product are listed.

Colourless oil. **TLC** (60% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.89–5.78 (m, 1H), 5.05–4.95 (m, 2H), 4.32 (dt, *J* = 13.2, 3.3 Hz, 1H), 3.90 (m, 1H), 3.20–3.12 (m, 1H), 3.10–3.02 (m, 1H), 2.90 (d, *J* = 4.4 Hz, 1H), 2.45–0.80 (m, 26H), 1.09–1.06 (m, 6H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.64 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 166.55, 155.95, 135.76, 123.56, 115.00, 78.69, 68.66, 65.69, 59.14, 56.46, 51.88, 42.79, 42.53, 39.81, 39.34, 38.76, 34.84, 32.40, 31.07, 30.60, 29.92, 28.78, 27.20, 26.95, 26.92, 24.06, 20.60, 15.89, 13.41, 11.83, 11.70. **FTIR** (neat), cm^{-1}: 2935 (br), 1704 (vs), 1463 (m), 1183 (m), 1125 (s). **HRMS** (ESI): Calcd for ($C_{31}H_{46}O_4$+H^+): 483.34689, found: 483.34690.

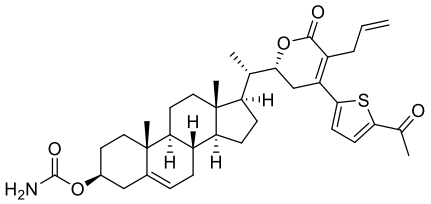
(*R*)-4-(5-acetylthiophen-2-yl)-3-allyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (114c)



The product was synthesized according to the general Suzuki Coupling procedure (page 100) from **102c** in 98% yield.

Orange foam. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.2 (CAM). **^{1}H NMR** (CDCl3, 400 MHz) δ: 7.63 (d, *J* = 4.0 Hz, 1H), 7.28 (d, *J* = 4.0 Hz, 1H), 5.94 (m, 1H), 5.33 (m, 1H), 5.10 (dd, *J* = 10.4, 1.2 Hz, 1H) overlapping with 5.06 (m, 1H), 4.53 (dt, *J* = 12.8, 2.9 Hz, 1H), 3.51 (m, 1H), 3.44 (dd, *J* = 15.6, 4.9 Hz, 1H), 3.27 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.85 (dd, *J* = 16.8, 13.4 Hz, 1H), 2.57 (s, 3H), 2.45 (dd, *J* = 17.2, 2.7 Hz, 1H), 2.34–2.16 (m, 2H), 2.13–1.08 (m, 20H), 1.06 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 190.57, 165.85, 147.84, 145.41, 142.60, 140.77, 134.82, 132.23, 129.01, 127.12, 121.33, 116.21, 78.59, 71.60, 56.20, 51.99, 49.93, 42.75, 42.14, 39.57, 38.76, 37.15, 36.40, 32.40, 31.83, 31.76, 31.51, 30.03, 27.32, 26.69, 24.23, 20.95, 19.32, 13.40, 11.66. **FTIR** (neat), cm^{-1}: 2923 (br), 1691 (vs), 1633 (s), 1276 (s). **HRMS** (ESI): Calcd for ($C_{35}H_{46}O_4S+H^+$): 563.31896, found: 563.31854. $\boldsymbol{\alpha_D^{RT}}$ = +41.0 (c = 1.0 in $CHCl_3$).

(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(5-acetylthiophen-2-yl)-5-allyl-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl carbamate (114e)

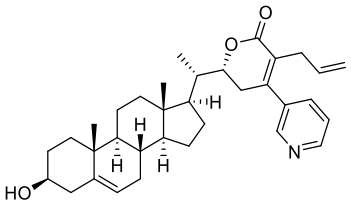


The product was synthesized according to the general carbamoylation procedure (page 108) from **114c** in 86% yield.

Pale yellow amorphous solid. **TLC** (70% ethyl acetate in petroleum ether): R_f = 0.40 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.73 (s, 1H), 7.64 (d, *J* = 3.9 Hz, 1H), 7.28 (d, *J* = 4.0 Hz, 1H), 5.95 (m, 1H), 5.40 (m, 1H), 5.11 (d, *J* = 10.5 Hz, 1H), 5.07 (d, *J* = 17.4 Hz, 1H), 4.62 (m, 1H), 4.54 (dt, *J* = 13.0, 3.0 Hz), 4.05 (s, 2H), 3.44 (dd, *J* = 15.5, 5.5 Hz, 1H), 3.28 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.86 (dd, *J* = 16.8, 13.4 Hz, 1H), 2.58 (s, 3H), 2.51–1.10 (m, 20H), 1.07 (d, *J* = 6.6 Hz, 3H), 1.02 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 190.64, 165.94, 149.56, 147.86, 145.48, 142.66, 138.85, 134.85, 132.26, 129.04, 127.18, 123.12, 116.26, 78.63, 77.68, 59.31, 56.14, 52.02, 49.79, 42.78, 39.52, 38.80, 37.83, 36.71, 36.44, 32.44, 31.78, 30.07, 27.58, 27.35, 26.73, 24.25, 20.95, 19.23, 13.45, 11.70. **FTIR** (neat), cm^{-1}: 2937 (br), 1703 (s), 1663

(s), 1166 (vs), 991 (s). **HRMS** (ESI): Calcd for ($C_{36}H_{47}NO_5S+H^+$): 606.32477, found: 606.32568. $\boldsymbol{\alpha_D^{RT}}$ = +27.5 (c = 1.0 in $CHCl_3$).

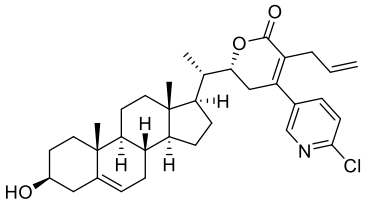
(*R*)-3-allyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(pyridin-3-yl)-5,6-dihydro-2*H*-pyran-2-one (<u>114f</u>)



The product was synthesized according to the general Suzuki Coupling procedure (page 100) from **102c** in 95% yield.

Colourless foam. **TLC** (70% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 8.62 (s, 1H), 8.54 (s, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.36 (dd, *J* = 7.6, 4.9 Hz, 1H), 5.88 (m, 1H), 5.32 (m, 1H), 5.02 (d, *J* = 10.2 Hz, 1H), 4.93 (d, *J* = 17.2 Hz, 1H), 4.58 (dt, *J* = 13.0, 3.1 Hz, 1H), 3.50 (m, 1H), 3.19 (dd, *J* = 15.1, 4.8 Hz, 1H), 2.91 (dd, *J* = 15.1, 5.8 Hz, 1H), 2.83 (dd, *J* = 16.8, 13.9 Hz, 1H), 2.34–0.82 (m, 23H) overlapping the following signals, 1.06 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.62, 149.55, 148.25, 147.58, 140.84, 135.54, 134.62, 127.63, 123.35, 121.30, 115.94, 78.94, 71.59, 56.28, 52.00, 49.97, 42.75, 42.19, 39.61, 38.82, 37.18, 36.42, 32.07, 31.83, 31.78, 31.55, 29.87, 27.40, 24.24, 20.96, 19.33, 13.45, 11.68. **FTIR** (neat), cm^{-1}: 2931 (br), 1707 (vs), 1391 (w), 715 (m). **HRMS** (ESI): Calcd for ($C_{34}H_{45}NO_3+H^+$): 516.34722, found: 516.34638. $\boldsymbol{\alpha_D^{RT}}$ = -10.0 (c = 1.0 in $CHCl_3$).

(*R*)-3-allyl-4-(6-chloropyridin-3-yl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (<u>114g</u>)

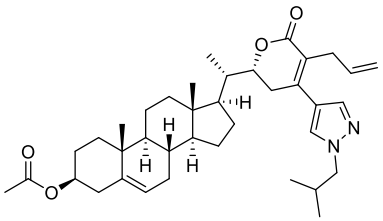


The product was synthesized according to the general Suzuki Coupling procedure (page 100) from **102c** in 98% yield.

Colourless foam. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.50 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.33 (d, *J* = 2.4 Hz, 1H), 7.56 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 1H), 5.94–5.82 (m, 1H), 5.33 (m, 1H), 5.04 (dd, *J* = 10.2, 1.2 Hz), 4.95 (dd, *J* = 17.2, 1.2 Hz, 1H), 4.58 (dt, *J* = 13.0, 3.2 Hz, 1H), 3.51 (m, 1H), 3.20 (dd, *J* = 15.3, 4.7 Hz, 1H), 2.90 (dd, *J* = 15.3, 5.8 Hz, 1H), 2.82 (dd, *J* = 16.7, 13.7 Hz, 1H), 2.33–2.18 (m, 3H), 2.14–1.90 (m, 3H), 1.87–1.78 (m, 2H), 1.75–0.91 (m, 15H), 1.06 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.37, 151.65, 147.79, 146.97, 140.81, 137.26, 135.40, 133.56, 128.19, 124.18, 121.35, 116.12, 78.95, 71.70, 56.32, 52.04, 50.02, 42.81, 42.20,

39.66, 38.85, 37.20, 36.44, 32.17, 31.87, 31.80, 31.57, 29.78, 27.44, 24.25, 20.99, 19.34, 13.46, 11.70. **FTIR** (neat), cm^{-1}: 3401 (br), 2931 (br), 1707 (vs), 1461 (s), 1107 (vs). **HRMS** (ESI): Calcd for ($C_{34}H_{44}ClNO_3$+H^+): 550.30825, found: 550.30770, calcd for ($C_{34}H_{44}{}^{37}ClNO_3$+$H^+$): 552.30530, found: 552.30485. $\boldsymbol{\alpha_D^{RT}}$ = -21.3 (c = 1.0 in $CHCl_3$).

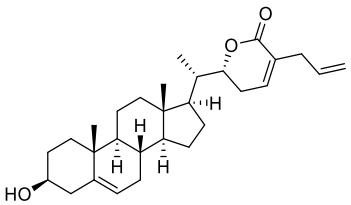
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-5-allyl-4-(1-isobutyl-1*H*-pyrazol-4-yl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (114h)



The product was synthesized according to the general Suzuki Coupling procedure (page 100), followed by acetylation (page 111) from **102c** in 86% yield over two steps.

Colourless oil. **TLC** (25% ethyl acetate in petroleum ether): R_f = 0.28 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.68 (s, 1H), 7.59 (s, 1H), 6.01–5.92 (m, 1H), 5.37 (m, 1H), 5.11 (dd, *J* = 10.3, 1.4 Hz, 1H), 5.06 (dd, *J* = 17.3, 1.4 Hz, 1H), 4.60 (m, 1H), 4.50 (dt, *J* = 13.0, 2.9, 2.9 Hz, 1H), 3.93 (d, *J* = 7.2 Hz, 2H), 3.39 (dd, *J* = 16.0, 4.8 Hz, 1H), 3.20 (dd, *J* = 16.2, 4.8 Hz, 1H), 2.76 (dd, *J* = 16.7, 13.5 Hz, 1H), 2.37 (dd, *J* = 17.1, 2.7 Hz, 1H), 2.34–1.10 (m, 22H) overlapping with 2.03 (s, 3H), 1.07 (d, *J* = 6.6 Hz, 3H), 1.02 (s, 3H), 0.92 (d, *J* = 6.6 Hz, 6H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 170.53, 167.02, 142.75, 139.68, 138.89, 135.28, 129.61, 122.34, 121.57, 119.20, 115.56, 78.34, 73.84, 59.98, 56.25, 52.07, 49.97, 42.79, 39.59, 38.93, 38.05, 36.96, 36.53, 32.22, 31.83, 29.60, 28.20, 27.70, 27.39, 24.28, 21.42, 20.96, 19.86, 19.27, 13.52, 11.69. **FTIR** (neat), cm^{-1}: 2938 (br), 1730 (s), 1694 (vs), 1240 (vs), 1025 (vs), 730 (vs). **HRMS** (ESI): Calcd for ($C_{38}H_{54}N_2O_4$+H^+): 603.41563, found: 603.41641. $\boldsymbol{\alpha_D^{RT}}$ = +23.0 (c = 1.0 in $CHCl_3$).

(*R*)-3-allyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (114i)

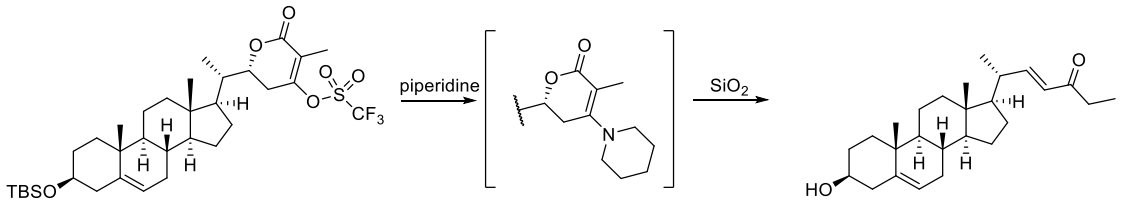


The product was synthesized according to the general procedure for reductive elimination of enol triflates (page 105) from **102c** in 81% yield. 7.5 equiv tributyltin hydride were added dropwise.

White solid. **TLC** (40% ethyl acetate in petroleum ether): Rf = 0.44 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.59 (d, *J* = 5.3 Hz, 1H), 5.85 (m, 1H), 5.35 (m, 1H), 5.11 (m, 1H), 5.09 (m, 1H), 4.44 (dt, *J* = 13.1, 3.5 Hz, 1H), 3.52 (m, 1H), 3.05 (d, *J* = 5.6 Hz, 2H), 2.42–2.17 (m, 4H), 2.11 (ddd, *J* = 18.1, 6.5, 3.5 Hz, 1H), 2.06–1.04 (m, 19H), 1.02 (d, *J* = 6.7 Hz, 3H) overlapping with 1.01 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126

MHz) δ: 165.80, 140.84, 139.48, 134.80, 130.95, 121.40, 117.10, 80.10, 71.71, 56.36, 52.04, 50.07, 42.77, 42.24, 39.67, 38.88, 37.23, 36.47, 34.59, 31.90, 31.83, 31.61, 27.30, 24.30, 23.34, 21.02, 19.35, 13.47, 11.68. **FTIR** (neat), cm^{-1}: 2931 (s), 1712 (vs), 1379 (m). **HRMS** (ESI): Calcd for ($C_{29}H_{42}O_3$+H^+): 439.32067, found: 439.32022. $\boldsymbol{\alpha_D^{RT}}$= +7.7 (c = 1.0 in CHCl3).

(*R*,*E*)-6-((3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)hept-4-en-3-one (120)

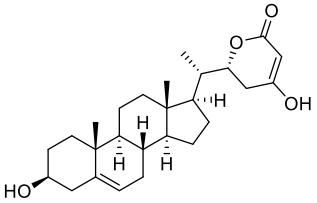


102a **116** **120**

The desired product **116** was attempted to be synthesized according to the general amination procedure (page 103) from **102a**. The reaction was performed in 1,2-dichloroethane at 50 °C. The desired product decomposed on silica to **120**, which was isolated in 72% yield.

White amorphous solid. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.55 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.68 (dd, *J* = 15.9, 8.9 Hz, 1H), 6.01 (dd, *J* = 15.9, 0.6 Hz, 1H), 5.34 (m, 1H), 3.52 (m, 1H), 2.54 (q, *J* = 7.4 Hz, 2H), 2.32–2.18 (m, 3H), 2.03–1.92 (m, 2H), 1.88–1.80 (m, 2H), 1.78–1.18 (m, 15H), 1.10 (d, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.3 Hz, 3H), 1.01 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 152.45, 140.74, 127.71, 121.55, 71.76, 56.55, 55.03, 50.09, 42.70, 42.27, 39.92, 39.62, 37.25, 36.50, 33.21, 31.89, 31.82, 31.63, 28.15, 24.29, 21.04, 19.39, 19.33, 12.13, 8.25. **FTIR** (neat), cm^{-1}: 3369 (br), 2930 (vs), 1669 (s), 1624 (s), 1458 (s), 1054 (vs). **HRMS** (ESI): Calcd for ($C_{26}H_{40}O_2$+H^+): 385.31011, found: 385.31127.

(*R*)-4-hydroxy-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (128)

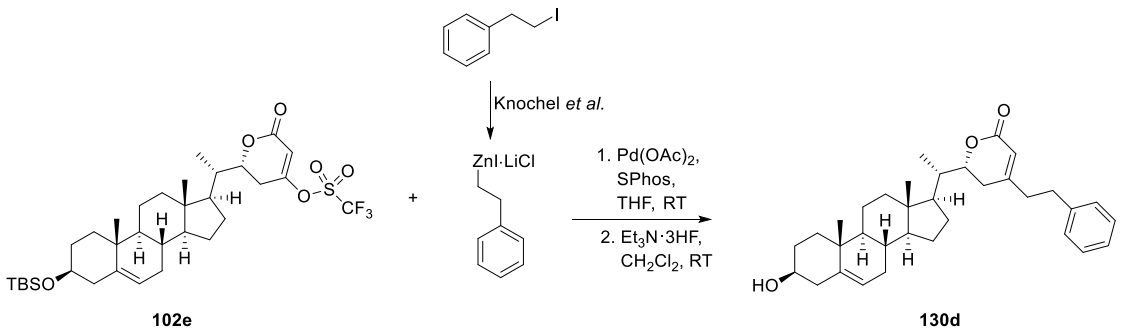


The product was prepared from **125** by TBS-deprotection (page 95), followed by δ-lactone closure (page 94) in 45% yield over 2 steps.

White crystalline solid**. TLC** (40% ethyl acetate in dichloromethane): R_f = 0.42 (CAM). **^{1}H NMR** (DMSO-d_6, 500 MHz) δ: 11.37 (s, 1H), 5.27 (m, 1H), 4.93 (s, 1H), 4.57 (m, 1H), 4.32 (d, *J* = 11.6 Hz, 1H), 3.25 (m, 1H), 2.46 (m, 1H), 2.20–0.85 (m, 22H), 0.95 (s, 3H) overlapping a (m, 3H), 0.69 (s, 3H). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ: 173.11, 141.27, 120.30, 90.59, 76.95, 69.98, 55.65, 51.26, 49.61, 42.20, 38.34, 36.92, 36.05, 31.48, 31.40, 31.33, 26.57, 23.98, 20.62, 19.11, 12.97, 11.50. **FTIR** (neat), cm^{-1}: 2932 (br),

1765 (s), 1719 (vs), 1374 (s), 1040 (s). **HRMS** (ESI): Calcd for ($C_{26}H_{38}O_4$+H^+): 415.28429, found: 415.28401. $\boldsymbol{\alpha_D^{RT}}$ = +24.4 (c = 0.52 in DMSO). **Melting point**: 181 °C.

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-phenethyl-5,6-dihydro-2*H*-pyran-2-one (130d)

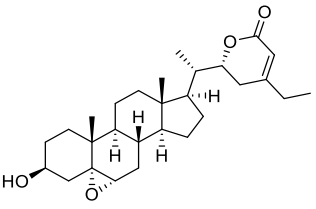


102e **130d**

The zinc reagent was prepared from (2-Iodoethyl)benzene according to a procedure from Knochel *et al*.[70] The coupling was performed as reported above for the coupling with diethyl zinc (page 101), followed by TBS-deprotection according to the general procedure (page 95) to provide the product **130d** in 45% yield over two steps.

White crystalline solid. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.5 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.30 (t, *J* = 7.5 Hz, 2H), 7.22 (t, *J* = 7.3 Hz, 1H), 7.17 (d, *J* = 7.4 Hz, 2H), 5.80 (s, 1H), 5.35 (m, 1H), 4.39 (dt, *J* = 13.1, 3.3 Hz, 1H), 3.52 (m, 1H), 2.89–2.79 (m, 2H), 2.56 (t, *J* = 7.8 Hz, 2H), 2.40–2.15 (m, 5H), 2.10–1.80 (m, 6H), 1.68–0.86 (m, 13H), 1.02 (s, 3H) overlapping with 1.02 (d, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.69, 160.26, 140.87, 140.12, 128.62, 128.17, 126.43, 121.39, 116.05, 79.58, 71.73, 56.40, 52.11, 50.10, 42.79, 42.26, 39.69, 38.83, 38.31, 37.25, 36.48, 33.07, 31.91, 31.86, 31.63, 27.33, 27.19, 24.30, 21.03, 19.37, 13.37, 11.69. **FTIR** (neat), cm^{-1}: 3478 (br), 2926 (br), 1698 (vs), 1262 (s), 1061 (s), 1018 (s). **HRMS** (ESI): Calcd for ($C_{34}H_{46}O_3$+H^+): 503.35197, found: 503.35137. $\boldsymbol{\alpha_D^{RT}}$ = +35.8 (c = 1.0 in $CHCl_3$). **Melting point**: 187–188 °C.

(*R*)-4-ethyl-6-((*S*)-1-((3*S*,4a*R*,5a*S*,6a*S*,6b*S*,9*R*,9a*S*,11a*S*,11b*R*)-3-hydroxy-9a,11b-dimethylhexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-9-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130b)

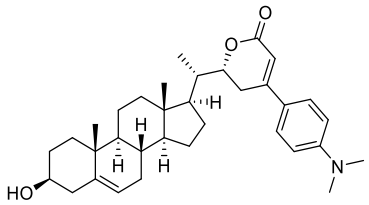


The product was synthesized according to the general epoxidation procedure (page 107) from **130a** in 80% yield. The product is a mixture of stereoisomers in a ratio of 4:1. Only the carbon signals of the major diastereomer are listed.

White solid. **TLC** (80% ethyl acetate in petroleum ether): R_f = 0.48 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.76 (m, 1H), 4.39 (dd, *J* = 13.1, 3.3 Hz, 1H), 3.89 (m, 0.8H, *major diastereomer*), 3.68 (m, 0.2H, *minor*

diastereomer), 3.05 (d, *J* = 2.1 Hz, 0.2H, *minor diastereomer*), 2.90 (d, *J* = 4.3 Hz, 0.8H, *major diastereomer*), 2.35 (m, 1H), 2.26 (q, *J* = 7.3 Hz, 2H), 2.12–0.90 (m, 23H), 1.12 (t, *J* = 7.4 Hz, 3H), 1.06 (s, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.67 and 0.65 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 166.01, 162.75, 114.34, 79.55, 68.64, 65.69, 59.13, 56.46, 51.82, 42.77, 42.49, 39.78, 39.30, 38.78, 34.82, 32.37, 31.04, 29.88, 29.78, 28.76, 27.15, 27.04, 24.04, 20.58, 15.89, 13.31, 11.69, 10.82. **FTIR** (neat), cm^{-1}: 3471 (br), 2936 (br), 1698 (vs), 1259 (m). **HRMS** (ESI): Calcd for ($C_{28}H_{42}O_4$+H^+): 443.31559, found: 443.31530. $\boldsymbol{\alpha_D^{RT}}$= +31.6 (c = 1.0 in $CHCl_3$).

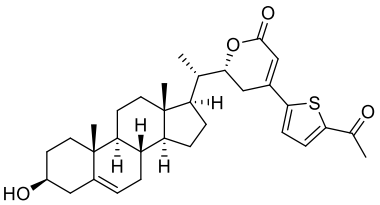
(*R*)-4-(4-(dimethylamino)phenyl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130f)



The product was synthesized according to the general Suzuki Coupling procedure (page 100) from **102e** in 98% yield.

Green-brown amorphos solid. **TLC** (60% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.48 (d, *J* = 9.0 Hz, 2H), 6.69 (d, *J* = 9.0 Hz, 2H), 6.22 (d, *J* = 1.8 Hz, 1H), 5.35 (m, 1H), 4.51 (dt, *J* = 12.2, 3.5 Hz, 1H), 3.53 (m, 1H), 3.03 (s, 3H), 2.71–2.50 (m, 2H), 2.35–0.85 (m, 22H), 1.10 (d, *J* = 6.6 Hz, 3H), 1.02 (s, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 167.20, 154.96, 151.84, 140.81, 127.40, 122.84, 121.43, 111.61, 109.63, 79.39, 71.73, 56.37, 52.26, 50.07, 42.80, 42.21, 40.08, 39.68, 38.97, 37.22, 36.46, 31.88, 31.84, 31.58, 27.32, 24.79, 24.30, 21.03, 19.37, 13.52, 11.72. **FTIR** (neat), cm^{-1}: 2931 (br), 1683 (vs), 1592 (vs), 1527 (s), 1365 (s). **HRMS** (ESI): Calcd for ($C_{34}H_{47}NO_3$+H^+): 518.36287, found: 518.36217. $\boldsymbol{\alpha_D^{RT}}$= +6.4 (c = 1.0 in $CHCl_3$).

(*R*)-4-(5-acetylthiophen-2-yl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130g)

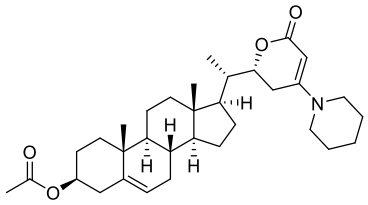


The product was synthesized according to the general Suzuki Coupling procedure (page 100) from **102e** in 72% yield.

White powder. **TLC** (60% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.65 (d, *J* = 4.0 Hz, 1H), 7.39 (d, *J* = 4.0 Hz, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 5.35 (m, 1H), 4.57 (dt, *J* = 12.8,

3.4 Hz, 1H), 3.53 (m, 1H), 2.74 (ddd, *J* = 17.1, 13.0, 2.4 Hz, 1H), 2.58 (s, 3H), 2.53 (dd, *J* = 17.4, 3.3 Hz, 1H), 2.34–2.19 (m, 2H), 2.17–0.92 (m, 20H), 1.09 (d, *J* = 6.7 Hz, 3H), 1.02 (s, 3H), 0.75 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 190.33, 165.24, 147.45, 147.26, 146.16, 140.86, 132.56, 127.51, 121.40, 114.97, 79.56, 71.72, 56.36, 52.18, 50.08, 42.89, 42.25, 39.71, 38.90, 37.24, 36.49, 31.93, 31.85, 31.62, 27.38, 26.76, 25.82, 24.31, 21.04, 19.37, 13.43, 11.74. **FTIR** (neat), cm^{-1}: 2926 (br), 2855 (m), 1701 (s), 1261 (s), 731 (s). **HRMS** (ESI): Calcd for ($C_{32}H_{42}O_4S+H^+$): 523.28766, found: 523.28789. $\boldsymbol{\alpha_D^{RT}}$= +69.7 (c = 1.0 in $CHCl_3$).

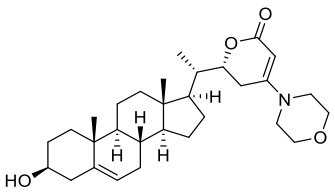
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-dimethyl-17-((*S*)-1-((*R*)-6-oxo-4-(piperidin-1-yl)-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130j)



The product was synthesized according to the general amination procedure (page 103) from **102e**, followed by acetylation (page 111) in 98% over two steps.

White crystalline solid. **TLC** (70% ethyl acetate in petroleum ether): R_f = 0.50 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.36 (m, 1H), 4.73 (s, 1H), 4.52 (m, 1H), 4.28 (dt, *J* = 12.8, 3.4 Hz, 1H), 3.32–3.16 (m, 4H), 2.34–2.24 (m, 3H), 2.15 (dd, *J* = 16.1, 3.3 Hz, 1H), 2.07–1.92 (m, 3H) overlapping with 1.97 (s, 3H), 1.90–1.77 (m, 2H), 1.75–1.33 (m, 13H), 1.30–0.92 (m, 7H), 1.02 (s, 3H) overlapping with 1.02 (d, *J* = 6.6 Hz, 3H), 0.73 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 170.79, 169.27, 161.03, 140.56, 122.83, 84.89, 77.62, 74.40, 57.03, 52.85, 50.74, 47.82, 43.37, 40.31, 39.70, 38.69, 37.61, 37.18, 32.49, 28.34, 27.89, 27.51, 25.93, 25.47, 24.90, 24.82, 21.72, 21.61, 19.68, 13.72, 12.09. **FTIR** (neat), cm^{-1}: 2935 (vs), 1673 (vs), 1571 (vs), 1440 (m), 1374 (m), 1236 (vs). **HRMS** (ESI): Calcd for ($C_{33}H_{49}NO_4+H^+$): 524.37344, found: 524.37411. $\boldsymbol{\alpha_D^{RT}}$= -23.8 (c = 1.0 in CH_2Cl_2). **Melting point**: 144 °C.

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-morpholino-5,6-dihydro-2*H*-pyran-2-one (130k)

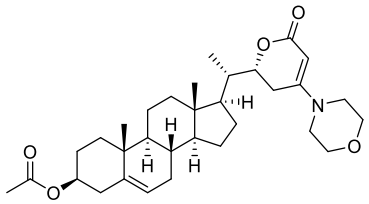


The product was synthesized according to the general amination procedure (page 103) from **102e** in 89% yield.

White crystalline solid. **TLC** (10% methanol in dichloromethane): R_f = 0.56 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.34 (m, 1H), 4.87 (s, 1H), 4.36 (dt, *J* = 12.9, 3.3 Hz, 1H), 3.75 (m, 4H), 3.51 (m, 1H), 3.34–3.25 (m, 2H), 3.23–3.16 (m, 2H), 2.40–2.18 (m, 3H), 2.14 (dd, *J* = 16.1, 3.2 Hz, 1H), 2.11–1.93 (m, 3H), 1.90–

1.77 (m, 2H), 1.75–0.85 (m, 15H), 1.04 (d, *J* = 6.6 Hz, 3H), 1.01 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 168.59, 160.69, 140.90, 121.34, 86.94, 77.30, 71.69, 66.05, 56.45, 52.29, 50.12, 46.03, 42.83, 42.24, 39.71, 38.86, 37.24, 36.48, 31.90, 31.87, 31.61, 27.37, 24.49, 24.31, 21.02, 19.36, 13.39, 11.70. **FTIR** (neat), cm^{-1}: 1651 (s), 1445 (m), 1258 (vs), 1172 (s), 1034 (s). **HRMS** (ESI): Calcd for ($C_{30}H_{45}NO_4$+H^+): 484.34214, found: 484.34153. $\boldsymbol{\alpha_D^{RT}}$= +4.4 (c = 0.64 in 1:1 CH_2Cl_2/ MeOH). **Melting point**: Colourless crystals gradually turn red-brown above 160 °C, followed by melting of the crystals at 234 °C.

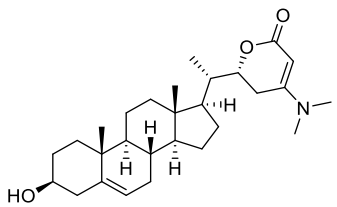
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-dimethyl-17-((*S*)-1-((*R*)-4-morpholino-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130l)



The product was synthesized according to the general amination procedure (page 103) from **102e**, followed by acetylation (page 111) in 88% yield over two steps. For reasons of solubility the NMR was measured in CD_3OD/CD_2Cl_2. The spectra are referenced to CD_2Cl_2.

White crystalline solid. **TLC** (ethyl acetate): R_f = 0.38 (CAM). **^{1}H NMR** (CD_3OD, 300 MHz) δ: 5.34 (m, 1H), 4.77 (s, 1H), 4.50 (m, 1H), 4.33 (dt, *J* = 12.8, 3.5 Hz, 1H), 3.77–3.61 (m, 4H) overlapping with C**H$_3$**OH, 3.38–3.12 (m, 4H), 2.42–2.23 (m, 3H), 2.18 (dd, *J* = 16.3, 3.6 Hz, 1H), 2.07–0.89 (m, 19H), 1.97 (s, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.99 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 75 MHz) δ: 171.61, 170.83, 162.41, 140.46, 122.89, 85.41, 78.26, 74.74, 66.62, 57.00, 52.69, 50.68, 46.70, 43.35, 40.25, 39.64, 38.59, 37.54, 37.14, 32.44, 28.25, 27.85, 25.05, 24.84, 21.65, 21.56, 19.60, 13.56, 12.00. **FTIR** (neat), cm^{-1}: 2938 (br), 1730 (m), 1665 (s), 1572 (s), 1238 (vs), 1025 (s). **HRMS** (ESI): Calcd for ($C_{32}H_{47}NO_5$ +H^+): 526.35270, found: 526.35331. $\boldsymbol{\alpha_D^{RT}}$= -9.7 (c = 0.84 in 1:1 CH_2Cl_2/MeOH). **Melting point**: 182 °C.

(*R*)-4-(dimethylamino)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130m)

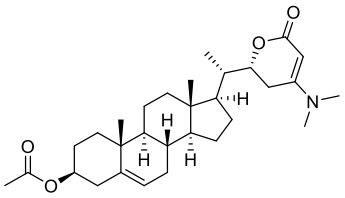


The product was synthesized according to the general amination procedure (page 103) from **102e** in 96% yield.

White crystalline solid. **TLC** (10% methanol in dichloromethane): R_f = 0.56 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.35 (m, 1H), 4.69 (s, 1H), 4.33 (dt, *J* = 12.8, 3.3 Hz, 1H), 3.52 (m, 1H), 2.95 (s, 6H), 2.42–1.90 (m, 9H), 1.89–1.06 (m, 15H), 1.04 (d, *J* = 6.7 Hz, 3H), 1.01 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz)

δ: 169.02, 161.03, 140.89, 121.40, 83.84, 77.08, 71.73, 56.44, 52.34, 50.14, 42.83, 42.26, 39.72, 39.30, 38.82, 37.26, 36.50, 31.92, 31.88, 31.63, 27.35, 24.83, 24.33, 21.04, 19.37, 13.43, 11.72. **FTIR** (neat), cm^{-1}: 3346 (br), 2918 (br), 1643 (vs), 1575 (vs), 1264 (s), 790 (s). **HRMS** (ESI): Calcd for ($C_{28}H_{43}NO_3$+H^+): 442.33157, found: 442.33129. $\boldsymbol{\alpha_D^{RT}}$= +12.2 (c = 0.98 in 1:1 CH_2Cl_2/MeOH). **Melting point**: 228.0 °C

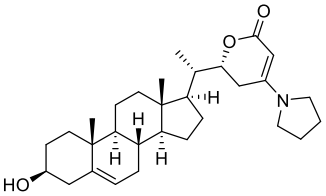
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(dimethylamino)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130n)



The product was synthesized according to the general amination procedure (page 103) from **102e**, followed by acetylation (page 111) in 82% yield over two steps.

TLC (ethyl acetate): R_f = 0.24 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.36 (m, 1H), 4.57 (s, 1H), 4.52 (m, 1H), 4.29 (dt, *J* = 12.8, 3.4 Hz, 1H), 2.91 (s, 6H), 2.37–2.15 (m, 4H), 2.06–1.92 (m, 3H) overlapping with 1.97 (s, 3H), 1.90–1.78 (m, 2H), 1.74–1.32 (m, 8H), 1.26–0.90 (m, 6H), 1.02 (d, *J* = 6.5 Hz, 1H), 1.01 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 170.82, 169.17, 161.96, 140.56, 122.82, 83.69, 77.61, 74.41, 57.01, 52.82, 50.73, 43.37, 40.29, 39.73, 39.64, 38.69, 37.61, 37.18, 32.48, 28.33, 27.89, 25.46, 24.89, 21.72, 21.60, 19.68, 13.71, 12.09. **FTIR** (neat), cm^{-1}: 2949 (br), 1733 (s), 1655 (vs), 1596 (vs), 1238 (vs). **HRMS** (ESI): Calcd for ($C_{30}H_{45}NO_4$+H^+): 484.34214, found: 484.34245. **Melting point**: Crystals gradually turn red-brown above 100 °C, followed by decomposition at 165 °C.

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(pyrrolidin-1-yl)-5,6-dihydro-2*H*-pyran-2-one (130o)

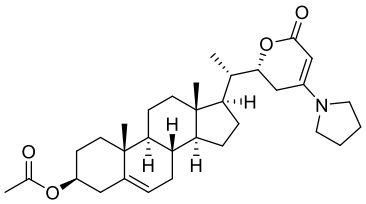


The product was synthesized according to the general amination procedure (page 103) from **102e** in quantitative yield. For solubility reasons the NMR was measured in a mixture of $CDCl_3$ and CD_3OD. The NMR is referenced to $CDCl_3$.

White crystalline solid. **TLC** (ethyl acetate): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.16 (m, 1H), 4.20 (dt, *J* = 12.9, 2.9 Hz, 1H), 4.13 (s, 1H) overlapping with CD_3O*H*, 3.28 (m, 3H), 3.07 (m, 2H), 2.30 (dd, *J* = 16.1, 13.7 Hz, 1H), 2.14–0.70 (m, 27H), 0.86 (d, *J* = 6.6 Hz, 3H) overlapping with 0.83 (s, 3H), 0.55 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 170.91, 159.71, 140.62, 120.92, 70.86, 56.02, 51.84, 49.80, 42.46, 41.43, 39.35, 38.54, 36.92, 36.15, 31.59, 31.48, 30.74, 27.01, 25.41, 24.85, 24.35, 23.94, 20.68, 18.90, 12.86, 11.28. **FTIR** (neat), cm^{-1}: 2923 (br), 2496 (m), 1644 (s), 1561 (vs), 1447 (m), 1037 (m). **HRMS**

(ESI): Calcd for ($C_{30}H_{45}NO_3$+H^+): 468.34722, found: 468.34730. α_D^{RT} = 34.3 (c = 1.0 in 1:1 CH_2Cl_2/MeOH). **Melting point**: Colourless crystals gradually turn red-brown above 190 °C, followed by decomposition.

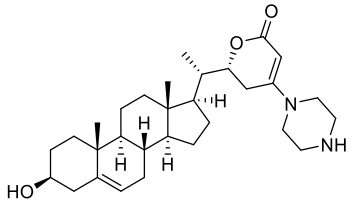
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-dimethyl-17-((*S*)-1-((*R*)-6-oxo-4-(pyrrolidin-1-yl)-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130p)



The product was synthesized according to the general amination procedure (page 103) from **102e**, followed by acetylation (page 111) in 80% yield over two steps.

White crystalline solid. **TLC** (ethyl acetate): R_f = 0.45 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.36 (m, 1H), 4.60 (m, 1H), 4.57 (s, 1H), 4.35 (dt, *J* = 12.8, 2.9 Hz, 1H), 3.42 (m, 2H), 3.21 (m, 2H), 2.43 (m, 1H), 2.31 (m, 2H), 2.17 (dd, *J* = 16.3, 2.9 Hz, 1H), 2.11–0.83 (m, 23H), 2.02 (s, 3H), 1.03 (m, 3H) overlapping with 1.01 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 170.55, 169.18, 158.68, 139.71, 122.33, 82.79, 77.20, 73.84, 56.33, 52.27, 50.01, 47.78, 42.75, 39.62, 38.73, 38.04, 36.96, 36.52, 31.81, 27.69, 27.31, 25.73, 25.26, 24.74, 24.28, 21.41, 20.95, 19.26, 13.39, 11.68. **FTIR** (neat), cm^{-1}: 2939 (br), 1731 (s), 1665 (s), 1560 (vs), 1443 (s), 1238 (vs), 1035 (s). **HRMS** (ESI): Calcd for ($C_{32}H_{47}NO_4$+H^+): 510.35779, found: 510.35799. α_D^{RT} = +23.5 (c = 1.0 in $CHCl_3$). **Melting point**: Colourless crystals gradually turn red-brown above 180 °C, followed by melting at 195 °C.

(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(piperazin-1-yl)-5,6-dihydro-2*H*-pyran-2-one (130q)

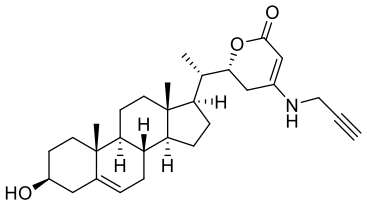


The product was synthesized according to the general amination procedure (page 103) from **102e** in 96% yield. For solubility reasons the NMR was measured in a mixture of $CDCl_3$ and CD_3OD. The NMR is referenced to CD_3OD.

White crystalline solid. **TLC** (20% methanol in dichloromethane): R_f = 0.35 (CAM). **^{1}H NMR** (CD_3OD, 500 MHz) δ: 5.33 (m, 1H), 4.81 (s, 1H), 4.36 (dt, *J* = 12.8, 3.4 Hz, 1H), 3.46–3.33 (m, 5H), 2.98–2.86 (m, 4H), 2.42 (dd, *J* = 16.1, 13.2 Hz, 1H), 2.32 (dd, *J* = 16.4, 3.5 Hz, 1H), 2.27–2.16 (m, 2H), 2.08–0.85 (m, 21H), 1.05 (d, *J* = 6.7 Hz, 3H), 1.01 (s, 3H), 0.75 (s, 3H). **^{13}C NMR** (CD_3OD, 126 MHz) δ: 172.16, 163.08, 141.82, 121.97, 84.62, 71.99, 57.23, 52.82, 51.15, 49.86, 46.99, 45.39, 44.15, 43.61, 42.60, 40.56, 40.03, 38.15, 37.31, 32.84, 32.63, 31.89, 28.10, 25.41, 25.07, 21.84, 19.78, 13.68, 12.14. **FTIR** (neat), cm^{-1}: 3342 (br), 2914 (br), 1636 (vs), 1563 (vs), 1441 (m), 1247 (s). **HRMS** (ESI): Calcd for ($C_{30}H_{46}N_2O_3$+H^+): 483.35812,

found: 483.35831. $\boldsymbol{\alpha_D^{RT}}$ = -6.1 (c = 0.82 in 1:1 CH_2Cl_2/MeOH). **Melting point**: Turns brown at 224 °C, then melting at 225.1 °C.

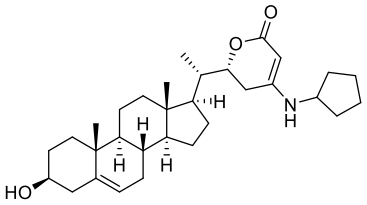
(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(prop-2-yn-1-ylamino)-5,6-dihydro-2*H*-pyran-2-one (130r)



The product was synthesized according to the general amination procedure (page 103) from **102e** in 71% yield.

White amorphous solid**. TLC** (ethyl acetate): R_f = 0.60 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.28 (m, 1H), 4.66 (s, 1H), 4.35 (dt, *J* = 13.4, 3.2 Hz, 1H), 3.82 (d, *J* = 2.3 Hz, 1H), 3.37 (m, 1H), 2.52–2.44 (m, 1H), 2.41 (t, *J* = 2.4 Hz, 1H), 2.24–2.10 (m, 2H), 2.07–1.87 (m, 4H), 1.85–0.75 (m, 18H), 0.97 (d, *J* = 7.7 Hz, 1H) overlapping with 0.96 (s, 3H), 0.69 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 171.49, 161.32, 141.80, 121.83, 82.26, 78.84, 72.95, 71.94, 57.13, 52.89, 50.96, 43.44, 42.52, 40.45, 39.63, 37.99, 37.17, 32.80, 32.64, 32.51, 31.85, 27.90, 27.12, 24.93, 21.71, 19.71, 13.61, 12.04. **FTIR** (neat), cm^{-1}: 2929 (br), 1643 (m), 1596 (vs), 1241 (m), 798 (m). **HRMS** (ESI): Calcd for ($C_{29}H_{41}NO_3$+H^+): 452.31592, found: 452.31629. $\boldsymbol{\alpha_D^{RT}}$ = +25.4 (c = 1.0 in 1:1 CH_2Cl_2/ MeOH).

(*R*)-4-(cyclopentylamino)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (130s)

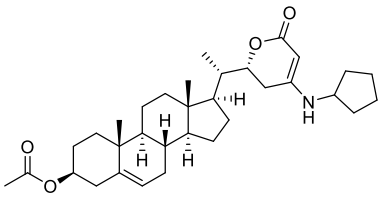


The product was synthesized according to the general amination procedure (page 103) from **102e** in 97% yield.

White crystalline solid. **TLC** (80% ethyl acetate in petroleum ether): R_f = 0.36 (CAM). **^{1}H NMR** (CD_3OD, 500 MHz) δ: 5.34 (m, 1H), 4.60 (s, 1H), 4.37 (dt, *J* = 13.5, 3.2 Hz, 1H), 3.73 (m, 1H), 3.41 (m, 1H), 2.49 (m, 1H), 2.28–0.84 (m, 33H), 1.03 (d, 3H) overlapping with 1.02 (s, 3H), 075 (s, 3H). **^{13}C NMR** (CD_3OD, 126 MHz) δ: 172.94, 162.46, 142.06, 122.06, 80.39, 79.17, 72.17, 57.47, 55.10, 53.25, 51.36, 43.69, 42.76, 40.79, 40.00, 38.31, 37.44, 33.27, 33.07, 32.99, 32.76, 32.05, 28.13, 27.52, 25.18, 24.84, 24.79, 21.97, 19.79, 13.69, 12.12. **FTIR** (neat), cm^{-1}: 3306 (br), 2935 (br), 1590 (vs), 1242 (s), 1040 (s). **HRMS** (ESI): Calcd for ($C_{31}H_{47}NO_3$+H^+): 482.36287, found: 482.36245. $\boldsymbol{\alpha_D^{RT}}$ = +30.0 (c = 1.0 in 1:1 CH_2Cl_2/MeOH).

Melting point: Colourless crystals gradually turn red-brown above 200 °C, followed by melting at 220 °C.

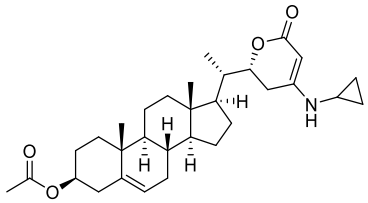
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(cyclopentylamino)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130t)



The product was synthesized according to the general acetylation procedure (page 111) of **130s** in 88% yield.

White crystalline solid. **TLC** (80% ethyl acetate in petroleum ether): R_f = 0.45 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.35 (m, 1H), 4.61 (s, 1H), 4.53 (m, 2H), 4.33 (dt, *J* = 13.2, 3.2 Hz, 1H), 3.71 (m, 1H), 2.48 (m, 1H), 2.28 (d, *J* = 7.8 Hz, 2H), 2.05–1.92 (m, 5H) overlapping with 1.97 (s, 3H), 1.90–1.77 (m, 3H), 1.75–0.81 (m, 20H), 1.01 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 170.82, 169.13, 159.32, 140.54, 122.81, 83.00, 77.80, 74.42, 57.04, 54.85, 52.91, 50.71, 43.34, 40.33, 39.51, 38.68, 37.62, 37.17, 33.56, 33.38, 32.46, 28.33, 27.89, 24.88, 24.59, 24.54, 21.73, 21.60, 19.67, 13.71, 12.08. **FTIR** (neat), cm^{-1}: 2939 (br), 1732 (m), 1653 (s), 1596 (s), 1238 (vs). **HRMS** (ESI): Calcd for ($C_{33}H_{49}NO_4$+H$^+$): 524.37344, found: 524.37418. α_D^{RT}= +31.2 (c = 1.0 in CH_2Cl_2). **Melting point**: Gradual decomposition above 130 °C, no clear melting.

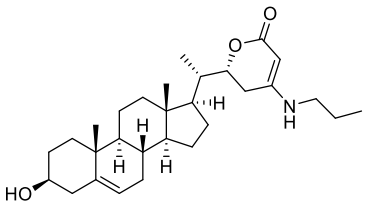
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(cyclopropylamino)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130u)



The product was synthesized according to the general amination procedure (page 103) from **102e**, followed by acetylation (page 111) in 74% yield over two steps.

Colourless amorphous solid. **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.36 (m, 1H), 4.97 (s, 1H), 4.65 (br s, 1H), 4.52 (m, 1H), 4.34 (dt, *J* = 13.2, 3.3 Hz, 1H), 2.50–2.38 (m, 2H), 2.32–2.24 (m, 2H), 1.97 (s, 3H) overlapping with 2.04–1.93 (m, 3H), 1.90–1.76 (m, 3H), 1.75–0.90 (m, 14H), 1.01 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.78–0.74 (m, 2H), 0.72 (s, 3H), 0.58–0.48 (m, 2H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 170.80, 168.78, 140.55, 122.81, 84.85, 77.90, 74.40, 57.02, 52.86, 50.71, 43.34, 40.31, 39.50, 38.69, 37.61, 37.17, 32.46, 32.45, 28.33, 27.87, 27.36, 25.01, 24.87, 21.73, 21.59, 19.67, 13.69, 12.07, 7.71, 7.36. **FTIR** (neat), cm^{-1}: 2940 (br), 1732 (s), 1637 (s), 1585 (s), 1541 (s), 1237 (vs). **HRMS** (ESI): Calcd for ($C_{31}H_{45}NO_4$+H$^+$): 496.34214, found: 496.34322. **Melting point**: Turns brown at 169–170 °C.

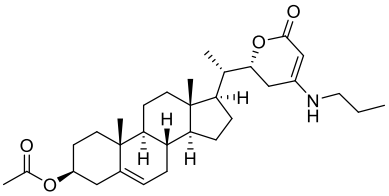
(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-4-(propylamino)-5,6-dihydro-2*H*-pyran-2-one (130v)



The product was synthesized according to the general amination procedure (page 103) from **102e** in 92% yield.

White crystalline solid**. TLC** (ethyl acetate): R_f = 0.36 (CAM). **^{1}H NMR** (CD_3OD, 500 MHz) δ: 5.34 (m, 1H), 4.58 (s, 1H), 4.37 (dt, *J* = 13.5, 3.2 Hz, 1H), 3.41 (m, 1H), 3.04 (t, *J* = 7.1 Hz, 1H), 2.52 (m, 1H), 2.30–0.90 (m, 26H), 1.03 (d, *J* = 6.9 Hz, 1H), 1.02 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** (CD_3OD, 126 MHz) δ: 172.94, 163.07, 142.01, 122.02, 79.69, 79.14, 72.13, 57.43, 53.20, 51.30, 45.49, 43.65, 42.71, 40.74, 39.94, 38.25, 37.40, 32.93, 32.72, 32.01, 28.09, 27.52, 25.13, 22.22, 21.93, 19.76, 13.65, 12.09, 11.73. **FTIR** (neat), cm^{-1}: 3324 (br), 2926 (br), 1655 (vs), 1609 (s), 1556 (s), 1259 (s), 1243 (s). **HRMS** (ESI): Calcd for ($C_{29}H_{45}NO_3$+H^+): 456.34722, found: 456.34726. $\boldsymbol{\alpha_D^{RT}}$= +22.3 (c = 0.49 in 1:1 CH_2Cl_2/MeOH). **Melting point**: Compound gradually turns brown above 200 °C, melting at 256 °C.

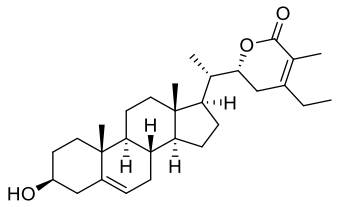
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-dimethyl-17-((*S*)-1-((*R*)-6-oxo-4-(propylamino)-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate (130w)



The product was synthesized according to the general amination procedure (page 103) from **102e**, followed by acetylation (page 111) in 84% yield over two steps.

White crystalline solid**. TLC** (80% ethyl acetate in petroleum ether): R_f = 0.54 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.36 (m, 1H), 4.60 (s, 1H), 4.53 (m, 1H) overlapping with 4.49 (m, 1H), 4.34 (dt, *J* = 13.2, 3.3 Hz, 1H), 3.02 (dd, *J* = 12.5, 7.1 Hz, 2H), 2.51 (m, 1H), 2.29 (d, *J* = 7.7 Hz, 2H), 2.05–1.77 (m, 6H) overlapping with 1.97 (s, 3H), 1.75–0.90 (m, 16H), 1.01 (s, 3H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.72 (s, 3H). **^{13}H NMR** (CD_2Cl_2, 126 MHz) δ: 170.81, 169.11, 160.02, 140.55, 122.81, 82.38, 77.83, 74.41, 57.04, 52.90, 50.71, 45.37, 43.34, 40.33, 39.51, 38.68, 37.62, 37.17, 32.46, 28.33, 27.89, 24.88, 22.35, 21.73, 21.60, 19.67, 13.71, 12.08, 11.80. **FTIR** (neat), cm^{-1}: 2932 (br), 1732 (s), 1598 (br), 1241 (vs). **HRMS** (ESI): Calcd for ($C_{31}H_{47}NO_4$+H^+): 498.35779, found: 498.35837. $\boldsymbol{\alpha_D^{RT}}$= +34.9 (c = 1.0 in CH_2Cl_2). **Melting point**: Compound gradually turns brown above 200 °C, melting at 228 °C.

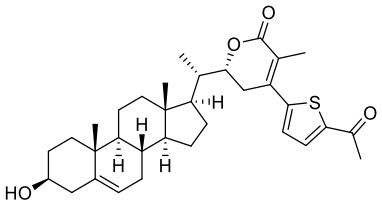
(*R*)-4-ethyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-methyl-5,6-dihydro-2*H*-pyran-2-one (131a)



The product was synthesized according to the general Negishi Coupling procedure from **102a** (page 101) in 76% yield. 2.5 equiv diethylzinc were added in three portions.

White solid. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.36 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.34 (m, 1H), 4.32 (dt, *J* = 13.2, 3.3 Hz, 1H), 3.51 (m, 1H), 2.45–2.13 (m, 5H), 2.07–1.75 (m, 6H) overlapping with 1.87 (s, 3H), 1.74–0.81 (m, 20H), 1.06 (t, *J* = 7.60 Hz, 3H), 1.87 (s, 3H), 1.01 (d, *J* = 6.1 Hz, 3H), 1.00 (s, 3H), 0.70 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 167.38, 154.29, 140.83, 121.36, 121.15, 78.71, 71.66, 56.32, 52.10, 50.04, 42.71, 42.20, 39.64, 38.77, 37.20, 36.44, 31.86, 31.82, 31.56, 27.32, 27.16, 26.93, 24.28, 20.99, 19.34, 13.44, 12.01, 11.66, 11.53. **FTIR** (neat), cm^{-1}: 3507 (br), 2940 (br), 1692 (vs), 1183 (m), 1133 (s), 1062 (s). **HRMS** (ESI): Calcd for ($C_{29}H_{44}O_3$+H^+): 441.33632, found: 441.33602. $\boldsymbol{\alpha_D^{RT}}$ = +40.3 (c = 1.0 in $CHCl_3$). **Melting point**: 218–220 °C.

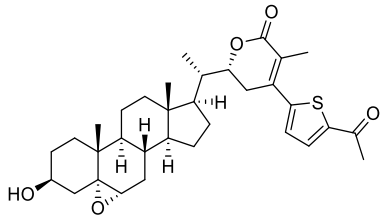
(*R*)-4-(5-acetylthiophen-2-yl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-methyl-5,6-dihydro-2*H*-pyran-2-one (131b)



The product was synthesized according to the general Suzuki Coupling procedure from **102a** (page 100) in 98% yield.

White powder. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.35 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.66 (d, *J* = 4.0 Hz, 1H), 7.26 (d, *J* = 4.0 Hz, 1H), 5.34 (m, 1H), 4.51 (dt, *J* = 12.9, 3.1 Hz, 1H), 3.52 (m, 1H), 2.78 (m, 1H), 2.58 (s, 3H), 2.49 (m, 1H), 2.34–2.17 (m, 2H) overlapping with 2.21 (s, 3H), 2.12–0.82 (m, 20H), 1.06 (d, *J* = 6.7 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 190.62, 166.90, 148.24, 145.46, 140.80, 140.76, 132.08, 129.34, 124.99, 121.36, 78.57, 71.64, 56.26, 52.06, 49.99, 42.78, 42.19, 39.62, 38.79, 37.18, 36.44, 31.87, 31.79, 31.56, 29.33, 27.37, 26.73, 24.28, 20.99, 19.34, 15.43, 13.43, 11.69. **FTIR** (neat), cm^{-1}: 3236 (br), 2941 (br), 1698 (s), 1667 (s), 1057 (vs). **HRMS** (ESI): Calcd for ($C_{33}H_{44}O_4S$+H^+): 537.30331, found: 537.30270. $\boldsymbol{\alpha_D^{RT}}$ = +140.3 (c = 1.0 in $CHCl_3$). **Melting point**: 134 °C.

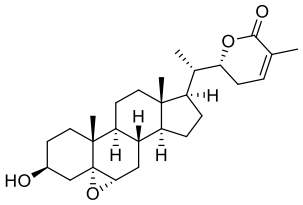
(*R*)-4-(5-acetylthiophen-2-yl)-6-((*S*)-1-((3*S*,4a*R*,5a*S*,6a*S*,6b*S*,9*R*,9a*S*,11a*S*,11b*R*)-3-hydroxy-9a,11b-dimethylhexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-9-yl)ethyl)-3-methyl-5,6-dihydro-2*H*-pyran-2-one (131c)



The product was synthesized according to the general epoxidation procedure from **131b** (page 107) in 90% yield. It is a mixture of stereoisomers in a ratio of 4:1. Only the carbon signals of the major stereoisomer are listed.

Colourless foam. **TLC** (80% ethyl acetate in petroleum ether): R_f = 0.38 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.67 (d, *J* = 4.0 Hz, 1H), 7.27 (m, 1H), 4.49 (dt, *J* = 12.9, 3.0 Hz, 1H), 3.90 (m, 0.8H, *major diastereomer*), 3.70 (m, 0.2H, *minor diastereomer)*, 3.06 (d, *J* = 1.8 Hz, 0.8H, *major diastereomer*), 2.90 (d, *J* = 4.3 Hz, 0.2H, *minor diastereomer*), 2.77 (m, 1H), 2.58 (s, 3H), 2.47 (d, *J* = 17.1 Hz, 1H), 2.20 (s, 3H), 2.12–0.94 (m, 22H), 1.06 (s, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.69 and 0.66 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 190.58, 166.88, 148.10, 145.55, 140.81, 132.02, 129.32, 124.97, 78.53, 68.62, 65.64, 59.10, 56.38, 51.81, 42.82, 42.44, 39.78, 39.28, 38.79, 34.83, 32.37, 31.04, 29.92, 29.26, 28.77, 27.22, 26.77, 24.06, 20.57, 15.88, 15.42, 13.41, 11.71. **FTIR** (neat), cm^{-1}: 3481 (m), 2922 (m), 1701 (vs). **HRMS** (ESI): Calcd for ($C_{33}H_{44}O_5S+H^+$): 553.29822, found: 553.29868. $\boldsymbol{\alpha_D^{RT}}$ = +126.7 (c = 1.0 in $CHCl_3$).

(*R*)-6-((*S*)-1-((3*S*,4a*R*,5a*S*,6a*S*,6b*S*,9*R*,9a*S*,11a*S*,11b*R*)-3-hydroxy-9a,11b-dimethylhexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-9-yl)ethyl)-3-methyl-5,6-dihydro-2*H*-pyran-2-one (131f)

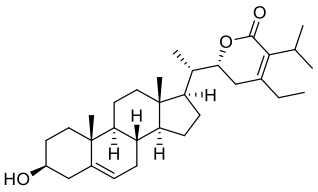


The product was synthesized according to the general epoxidation procedure (page 107) of **131d** in 84% yield. It is a mixture of stereoisomers in a ratio of 4:1. Only the carbon signals of the major stereoisomer are listed.

White powder. **TLC** (70% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.58 (d, *J* = 6.2 Hz, 1H), 4.42 (dt, *J* = 13.0, 3.3 Hz, 1H), 3.89 (m, 0.8H, *major diastereomer*), 3.68 (m, 0.2H, *minor diastereomer*), 3.05 (d, *J* = 1.8 Hz, 0.2H, *minor diastereomer*), 2.89 (d, *J* = 4.3 Hz, 0.8H, *major diastereomer*), 2.33 (m, 1H), 2.12–0.80 (m, 23H), 1.89 (s, 3H), 1.05 (s, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.66 and 0.64 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 166.55, 139.43, 128.16, 80.24, 68.62, 65.68, 59.12, 56.44, 51.74, 42.74, 42.49, 39.77, 39.29, 38.87, 34.81, 32.36, 31.03, 29.87, 28.73, 27.11, 24.03,

23.27, 20.57, 16.95, 15.87, 13.38, 11.66. **FTIR** (neat), cm^{-1}: 2931 (br), 1718 (vs), 1129 (m), 1037 (m). **HRMS** (ESI): Calcd for ($C_{27}H_{40}O_4$+H^+): 429.29994, found: 429.29943.

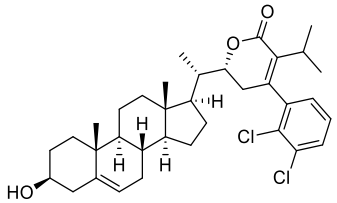
(*R*)-4-ethyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-isopropyl-5,6-dihydro-2*H*-pyran-2-one (132a)



The product was synthesized according to the general Negishi Coupling procedure from **102b** (page 101) in 87% yield. 19 equiv diethylzinc were added in four portions.

White amorphous solid. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.42 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.35 (m, 1H), 4.24 (ddd, *J* = 13.1, 3.1, 3.1 Hz, 1H), 3.52 (m, 1H), 2.89 (hept, *J* = 6.9 Hz, 1H), 2.40–0.85 (m, 26H), 1.25 (d, *J* = 7.0 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.07 (t, *J* = 7.6 Hz, 3H), 1.01 (s, 3H) overlapping with 1.01 (d, *J* = 6.4 Hz, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.23, 153.49, 140.84, 130.92, 121.41, 78.44, 71.71, 56.34, 52.22, 50.07, 42.74, 42.22, 39.67, 38.65, 37.22, 36.47, 31.89, 31.85, 31.60, 27.67, 27.56, 27.40, 26.72, 24.30, 21.40, 21.02, 20.40, 19.36, 13.55, 12.40, 11.69. **FTIR** (neat), cm^{-1}: 3448 (br), 2933 (br), 1696 (vs), 1057 (vs). **HRMS** (ESI): Calcd for ($C_{31}H_{48}O_3$+H^+): 469.36762, found: 469.36713. $\boldsymbol{\alpha_D^{RT}}$ = +35.8 (c = 1.0 in $CHCl_3$).

(*R*)-4-(2,3-dichlorophenyl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-isopropyl-5,6-dihydro-2*H*-pyran-2-one (132c)

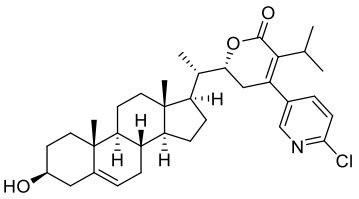


The product was synthesized according to the general Suzuki Coupling procedure from **102b** (page 100) in 98% yield. At ambient temperature in $CDCl_3$ the NMR shows a mixture of conformations in a ratio of 85:15. Only the ^{13}C signals of the major conformer are listed.

Colourless amorphous solid. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.26 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.46 (m, 1H), 7.25 (m, 1H), 7.01 (dd, *J* = 7.6, 1.4 Hz), 5.34 (m, 1H), 4.62 (dt, *J* = 12.6, 2.8 Hz, 0.85H, *major conformer*), 4.50 (dt, *J* = 13.3, 3.3 Hz, 0.15H, *minor conformer*), 3.50 (m, 1H), 2.92 (dd, *J* = 17.3, 13.4 Hz, 0.15H, *minor conformer*), 2.52 (dd, *J* = 17.4, 13.1 Hz, 0.85H, *major conformer*), 2.34 (m, 1H), 2.31–2.21 (m, 2H), 2.18 (dd, *J* = 17.4, 2.6 Hz), 2.10–0.84 (m, 20H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.11 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 164.66, 147.65, 140.78, 140.66, 134.60, 133.80, 130.09, 129.91, 127.75, 126.02, 121.45, 79.28, 71.70,

56.29, 52.17, 50.04, 42.75, 42.24, 39.68, 38.69, 37.20, 36.45, 31.88, 31.80, 31.59, 30.19, 29.31, 27.30, 24.33, 21.17, 21.01, 20.34, 19.35, 13.50, 11.70. **FTIR** (neat), cm^{-1}: 2935 (br), 1718 (vs), 1454 (m), 1056 (vs), 789 (vs). **HRMS** (ESI): Calcd for ($C_{35}H_{46}Cl_2O_3$+H^+): 585.28968, found: 585.29064, calcd for ($C_{35}H_{46}Cl^{37}ClO_3$+H^+): 587.28673, found: 587.28782, calcd for ($C_{35}H_{46}{}^{37}Cl_2O_3$+$H^+$): 589.28378, found: 589.28584. $\boldsymbol{\alpha_D^{RT}}$= +39.0 (c = 1.0 in $CHCl_3$).

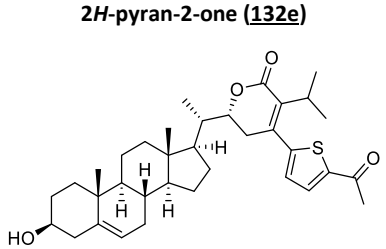
(*R*)-4-(6-chloropyridin-3-yl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-isopropyl-5,6-dihydro-2*H*-pyran-2-one (132d)



The product was synthesized according to the general Suzuki coupling procedure (page 100) from the TBS-protected enol triflate **101b** in 92% yield. The coupling product was then TBS-deprotected (page 95) in 90% yield.

White amorphous solid**. TLC** (40% ethyl acetate in petroleum ether): R_f = 0.40 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 8.26 (d, *J* = 2.1 Hz, 1H), 7.49 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 5.34 (m, 1H), 4.50 (dt, *J* = 12.9, 3.0 Hz, 1H), 3.51 (m, 1H), 2.67 (dd, *J* = 17.7, 13.2 Hz, 1H), 2.59 (hept, *J* = 6.9 Hz, 1H), 2.36–0.80 (m, 23H), 1.26 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 164.04, 151.26, 147.68, 145.28, 140.79, 137.36, 135.38, 134.30, 124.32, 121.36, 78.73, 71.66, 56.27, 52.04, 49.96, 42.75, 42.17, 39.60, 38.68, 37.16, 36.42, 31.83, 31.79, 31.54, 30.48, 29.52, 27.49, 24.26, 21.76, 20.96, 20.57, 19.34, 13.45, 11.69. **FTIR** (neat), cm^{-1}: 3403 (br), 2935 (br), 1709 (vs), 1461 (s), 1108 (m), 1055 (s). **HRMS** (ESI): Calcd for ($C_{34}H_{46}ClNO_3$+H^+): 552.32390, found: 552.32359, calcd for ($C_{34}H_{46}{}^{37}ClNO_3$+H+): 554.32095, found: 554.32156. $\boldsymbol{\alpha_D^{RT}}$= +54.2 (c = 1.0 in CH_2Cl_2).

(*R*)-4-(5-acetylthiophen-2-yl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-isopropyl-5,6-dihydro-2*H*-pyran-2-one (132e)

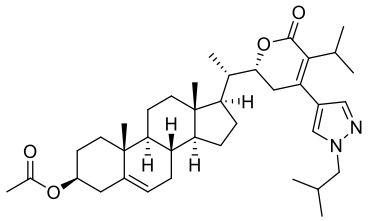


The product was synthesized according to the general Suzuki Coupling procedure from **102b** (page 100) in 98% yield.

Colourless foam. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.63 (d, *J* = 3.9 Hz, 1H), 7.10 (d, *J* = 3.9 Hz, 1H), 5.35 (m, 1H), 4.45 (dt, *J* = 12.7, 2.8 Hz, 1H), 3.51 (m,

1H), 3.06 (hept, *J* = 6.9 Hz, 1H), 2.69 (dd, *J* = 17.6, 13.0 Hz, 1H), 2.57 (s, 3H), 2.37 (dd, *J* = 17.7, 2.7 Hz, 1H), 2.33–2.18 (m, 2H), 2.09–1.92 (m, 3H), 1.88–0.83 (m, 17H), 1.39 (d, *J* = 6.9 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 190.62, 166.90, 148.24, 145.46, 140.80, 140.76, 132.08, 129.34, 124.99, 121.36, 78.57, 71.64, 56.26, 52.06, 49.99, 42.78, 42.19, 39.62, 38.79, 37.18, 36.44, 31.87, 31.79, 31.56, 29.33, 27.37, 26.73, 24.28, 20.99, 19.34, 15.43, 13.43, 11.69. **FTIR** (neat), cm^{-1}: 2932 (br), 1708 (vs), 1663 (vs), 1268 (vs), 1054 (vs). **HRMS** (ESI): Calcd for ($C_{35}H_{48}O_4S+H^+$): 565.33461, found: 565.33404. $\boldsymbol{\alpha_D^{RT}}$= +223.8 (c = 1.0 in $CHCl_3$).

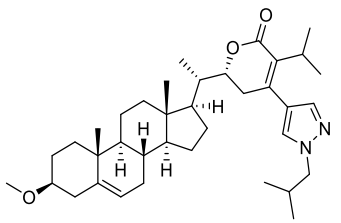
(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-((*S*)-1-((*R*)-4-(1-isobutyl-1*H*-pyrazol-4-yl)-5-isopropyl-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl acetate (132f)



The product was synthesized according to the general Suzuki Coupling procedure from **102b** (page 100), followed by acetylation (page 111) in 88% yield over two steps.

Colourless oil. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.60 (s, 1H), 7.45 (s, 1H), 5.37 (d, *J* = 4.5 Hz, 1H), 4.60 (m, 1H), 4.40 (dt, *J* = 13.0, 3.1 Hz, 1H), 3.96 (dd, *J* = 10.8, 4.5 Hz, 1H), 3.92 (dd, *J* = 10.8, 4.6 Hz, 1H), 3.06 (hept, *J* = 6.9 Hz, 1H), 2.56 (dd, *J* = 17.5, 13.1 Hz), 2.35–2.18 (m, 4H), 2.08–1.94 (m, 4H) overlapping with 2.03 (s, 3H), 1.92–1.42 (m, 11H), 1.40 (d, *J* = 6.9 Hz, 3H), 1.28–0.90 (m, 4H), 1.19 (d, *J* = 6.9 Hz, 3H), 1.04 (d, *J* = 7.0 Hz, 3H) overlapping with 1.03 (s, 3H), 0.94 (d, *J* = 6.7 Hz), 0.93 (d, *J* = 6.7 Hz), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 170.49, 165.29, 140.20, 139.77, 138.88, 130.82, 129.16, 122.35, 119.22, 77.95, 73.87, 59.97, 56.33, 52.25, 50.06, 42.83, 39.67, 38.76, 38.10, 37.01, 36.58, 31.90, 31.86, 29.64, 29.48, 28.91, 27.74, 27.52, 24.34, 21.39, 21.32, 21.00, 20.58, 19.88, 19.28, 13.61, 11.71. **FTIR** (neat), cm^{-1}: 2936 (br), 1698 (vs), 1241 (vs), 1024 (s). **HRMS** (ESI): Calcd for ($C_{38}H_{56}N_2O_4+H^+$): 605.43128, found: 605.43211. $\boldsymbol{\alpha_D^{RT}}$= +161.2 (c = 1.0 in $CHCl_3$).

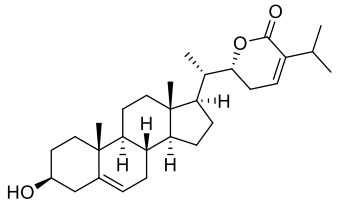
(*R*)-4-(1-isobutyl-1*H*-pyrazol-4-yl)-3-isopropyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (132g)



The product was synthesized according to the general Suzuki Coupling procedure from **102b** (page 100), followed by methylation (page 109) in 71% yield over two steps.

Colourless oil. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.60 (s, 1H), 7.46 (s, 1H), 5.36 (m, 1H), 4.40 (dt, *J* = 12.8, 2.9 Hz, 1H), 3.94 (dd, *J* = 7.3, 1.1 Hz), 3.35 (s, 3H), 3.05 (m, 2H), 2.55 (dd, *J* = 17.5, 13.1 Hz, 1H), 2.39 (m, 1H), 2.29 (dd, *J* = 17.7, 2.7 Hz, 1H), 2.25–0.8 (m, 21H), 1.40 (d, *J* = 7.0 Hz, 3H), 1.19 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 3H), 0.94 (d, *J* = 3.2 Hz, 3H), 0.92 (d, *J* = 3.2 Hz, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.31, 140.95, 140.17, 138.86, 130.73, 129.18, 121.30, 119.15, 80.25, 77.93, 59.93, 56.35, 55.57, 52.20, 50.14, 42.81, 39.66, 38.70, 38.63, 37.14, 36.85, 31.89, 29.63, 29.40, 28.87, 27.93, 27.50, 24.34, 21.31, 21.01, 20.55, 19.87, 19.33, 13.61, 11.71. **FTIR** (neat), cm^{-1}: 2933 (br), 1697 (vs), 1608 (m), 1539 (m), 1458 (s). **HRMS** (ESI): Calcd for ($C_{37}H_{56}N_2O_3$+H$^+$): 577.43637, found: 577.43699. $\boldsymbol{\alpha_D^{RT}}$ = +145.4 (c = 1.0 in $CHCl_3$).

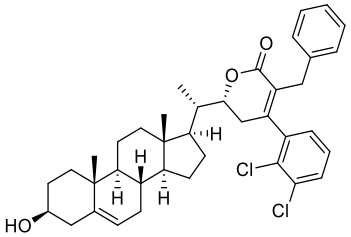
(*R*)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-3-isopropyl-5,6-dihydro-2*H*-pyran-2-one (<u>132h</u>)



The product was synthesized according to the general procedure for reductive elimination of enol triflates from **102b** (page 105) in 54% yield. 29 equiv tributyltin hydride were added dropwise.

Colourless crystals. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.28 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.53 (d, *J* = 6.4 Hz, 1H), 5.34 (m, 1H), 4.36 (dt, *J* = 13.0, 3.4 Hz, 1H), 3.51 (m, 1H), 2.84 (hept, *J* = 6.9 Hz, 1H), 2.40–2.16 (m, 3H), 2.09 (ddd, *J* = 18.0, 6.6, 3.4 Hz), 2.04–0.82 (m, 20H), 1.09 (d, *J* = 6.8 Hz, 3H), 1.05 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.6 Hz), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.82, 140.80, 138.49, 136.01, 121.40, 79.66, 71.69, 56.32, 52.02, 50.02, 42.73, 42.20, 39.63, 38.80, 37.20, 36.44, 31.87, 31.82, 31.57, 28.24, 27.30, 24.28, 23.07, 22.22, 21.07, 21.00, 19.35, 13.51, 11.66. **FTIR** (neat), cm^{-1}: 3481 (m), 2922 (m), 1701 (vs). **HRMS** (ESI): Calcd for ($C_{29}H_{44}O_3$+H$^+$): 441.33632, found: 441.33609. $\boldsymbol{\alpha_D^{RT}}$ = +12.5 (c = 1.0 in $CHCl_3$). **Melting point**: 228 °C.

(*R*)-3-benzyl-4-(2,3-dichlorophenyl)-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (<u>133a</u>)

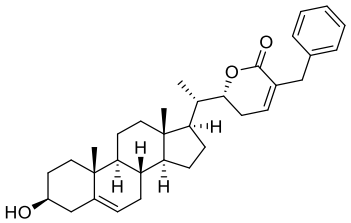


The product was synthesized from TBS-protected enol triflate **101d** according to the general Suzuki Coupling procedure (page 100) in 76% yield. The coupling product was deprotected according to the

general TBS-deprotecting procedure (page 95) in 84% yield. At ambient temperature in $CDCl_3$ the NMR shows a mixture of conformations in a ratio of 80:20. The ^{13}C signals of the major conformer are listed.

White amorphous solid. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.53–7.44 (m, 1H), 7.30–7.10 (m, 4H), 7.05–6.92 (m, 3H), 5.34 (m, 1H), 4.68 (dt, *J* = 12.8, 3.1 Hz, 0.75H, *major conformer*), 4.57 (dt, *J* = 13.0, 3.1 Hz, 0.25H, *minor conformer*), 3.79 (d, *J* = 14.5 Hz, 0.25H, *minor conformer*), 3.63 (d, *J* = 14.5 Hz, 0.75H, *major conformer*), 3.51 (m, 1H), 3.44 (d, *J* = 14.4 Hz, 0.75H, *major conformer*), 3.27 (d, *J* = 14.5 Hz, 0.25H, *minor conformer*), 3.05–2.95 (m, 0.25H, *minor conformer*), 2.65–2.51 (m, 0.75H, *major conformer*), 2.45–0.85 (m, 23H), 1.04 (d, *J* = 6.7 Hz, 3H), 1.00 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.95, 149.81, 140.72, 139.65, 138.58, 133.91, 130.42, 130.23, 129.16, 128.59, 128.44, 128.29, 128.22, 127.81, 126.88, 126.12, 121.47, 79.44, 71.69, 56.24, 52.12, 49.97, 42.74, 42.20, 39.64, 38.71, 37.16, 36.42, 33.47, 31.83, 31.78, 31.56, 29.13, 27.26, 24.32, 20.98, 19.35, 13.54, 11.69. **FTIR** (neat), cm^{-1}: 3393 (br), 2933 (br), 1709 (vs), 1188 (s), 728 (vs), 697 (vs). **HRMS** (ESI): Calcd for ($C_{39}H_{46}Cl_2O_3$+H^+): 633.28968, found: 633.28953, calcd for ($C_{39}H_{46}Cl^{37}ClO_3$+H^+): 635.28673, found: 635.28664, calcd for ($C_{39}H_{46}{}^{37}Cl_2O_3$+$H^+$): 637.28378, found: 637.28328. $\boldsymbol{\alpha_D^{RT}}$ = -5.3 (c = 1.0 in $CHCl_3$).

(*R*)-3-benzyl-6-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (133b)



The product was synthesized according to the general procedure for reductive elimination of enol triflates (page 105) from **102d** in 45% yield. 30 equiv tributyltin hydride were added dropwise.

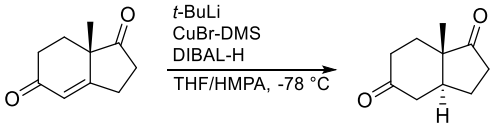
White crystalline solid. **TLC** 50% ethyl acetate in petroleum ether): R_f = 0.56 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.35–7.15 (m, 5H), 6.38 (d, *J* = 6.1 Hz, 1H), 5.34 (m, 1H), 4.42 (dt, *J* = 13.1, 3.4 Hz, 1H), 3.62 (s, 2H), 3.51 (m, 1H), 2.42–2.18 (m, 3H), 2.12–0.82 (m, 21H), 1.01 (d, *J* = 6.7 Hz, 3H) overlapping with 1.00 (s, 3H), 0.70 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.90, 140.77, 140.05, 138.38, 132.29, 129.23, 128.46, 126.39, 121.36, 80.01, 71.67, 56.26, 51.94, 49.98, 42.71, 42.16, 39.59, 38.81, 37.17, 36.46, 36.41, 31.83, 31.78, 31.53, 27.26, 24.25, 23.27, 20.97, 19.32, 13.46, 11.64. **FTIR** (neat), cm^{-1}: 2935 (br), 1711 (vs), 1381 (s), 1238 (m), 1057 (s). **HRMS** (ESI): Calcd for ($C_{33}H_{44}O_3$+H^+): 489.33632, found: 489.33562. $\boldsymbol{\alpha_D^{RT}}$ = +1.2 (c = 1.0 in $CHCl_3$). **Melting point**: 170–172 °C.

5.3 Experimental Part for Part B

5.3.1 Synthesis of Functionalized δ-Lactone Intermediates

Synthesis of known Intermediate 186

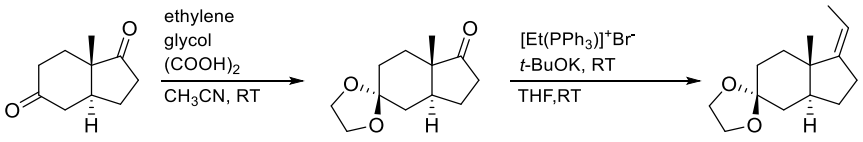
(3a*S*,7a*S*)-7a-methylhexahydro-1*H*-indene-1,5(4*H*)-dione (164)[144]



163 **164**

t-BuLi (1.7 M in pentane, 2.36 ml, 4.02 mmol, 0.33 equiv) was added dropwise to a suspension of copper(I) bromide dimethyl sulfide complex (751 mg, 3.65 mmol, 0.3 equiv) in THF (8 mL) at −78 °C and the whole mixture was stirred for 60 min at that temperature. HMPA (4.5 ml) was added to the mixture. Then a solution of the starting material (2 g, 12.2 mmol, 1 equiv) in THF (5 ml) was added dropwise at −78, followed by an addition of DIBAL-H (1.0 M in toluene, 18.3 ml, 18.3 mmol, 1.5 equiv) and HMPA (8.5 ml) over 4 hours via syringe pump. 5% HCl was added to the mixture and the whole mixture was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 20–40% ethyl acetate in petroleum ether) to provide **164** (1.48 g, 73%) as a pure stereoisomer. The measured NMR spectra match with reported data.[144]

(3a*S*,7a*S*,*Z*)-1-ethylidene-7a-methyloctahydrospiro[indene-5,2'-[1,3]dioxolane] (186)



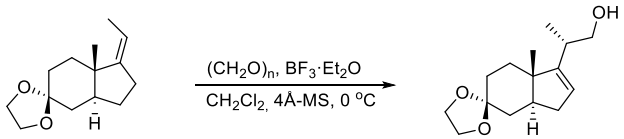
164 **185** **186**

The ketal protection was performed according to the procedure of Kotoku *et al*.[144], followed by a Wittig according to Minato *et al*.[72] in 71% yield over two steps.

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.13 (qt, *J* = 7.1, 2.0 Hz, 1H), 3.93 (s, 4H), 2.45–2.10 (m, 3H), 1.90–1.65 (m, 5H), 1.63 (dt, *J* = 7.1, 1.9 Hz, 3H), 1.61–1.47 (m, 2H), 1.35–1.27 (m, 1H), 0.92 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 148.66, 113.89, 109.76, 64.24, 64.11, 47.09, 43.42, 35.29, 33.90, 31.67, 31.52, 26.42, 15.05, 13.12.

Synthesis of protected Aldehyde 192

(*S*)-2-((3a*S*,7a*S*)-7a-methyl-3,3a,4,6,7,7a-hexahydrospiro[indene-5,2'-[1,3]dioxolan]-1-yl)propan-1-ol (190)

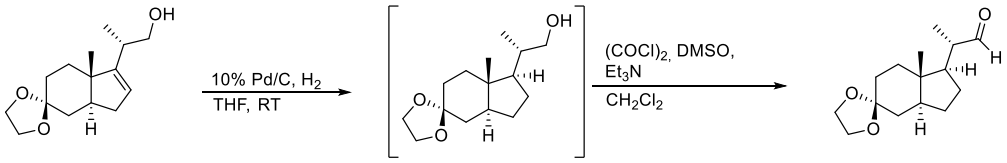


186 190

To a suspension of known alkene **186** (816 mg, 3.67 mmol, 1 equiv) and paraformaldehyde (331 mg, 11.01 mmol, 3 equiv) in dichloromethane (40 ml) at ambient temperature were added activated 4-Å molecular sieves (~2.5 g).[3] After stirring for 1.0 h at ambient temperature, the above mixture was placed into an ice-water cooling bath. Boron trifluoride-etherate (1.0 M solution in dichloromethane, 367 µl, 367 µmol, 0.1 equiv) was added dropwise and the reaction mixture was stirred for 10 h at 0 °C. Then, triethylamine (512 µL, 3.67 mmol, 1 equiv) was added at 0 °C followed, after 10 min, by addition of a saturated aqueous solution of sodium bicarbonate (20 ml). The resulting biphasic mixture was allowed to warm to ambient temperature. The organic phase was separated and the aqueous phase was extracted with dichloromethane (70 ml). The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 10–30% ethyl acetate in petroleum ether) to provide homoallylic alcohol **190** (693 mg, 75%[4]).

Colourless oil that solidified upon standing at ambient temperature. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.20 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 5.41 (m, 1H), 3.88 (m, 4H), 3.55 (dd, *J* = 10.6, 7.1 Hz, 1H), 3.46 (dd, *J* = 10.6, 6.4 Hz, 1H), 2.33 (m, 1H), 2.03–1.88 (m, 3H), 1.79–1.62 (m, 5H), 1.57–1.47 (m, 2H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.82 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 157.5, 123.8, 110.5, 67.1, 64.9, 64.7, 48.3, 47.1, 36.1, 35.2, 33.3, 32.8, 32.0, 18.5, 15.1. **FTIR** (neat), cm^{-1}: 3421 (br), 2931 (m), 1087 (m). **HRMS** (ESI): Calcd for ($C_{15}H_{24}O_3$+H^+): 253.1798, found: 253.1800. $\boldsymbol{\alpha_D^{RT}}$ = +4.3 (c = 1.0 in CH_2Cl_2).

(*S*)-2-((1*R*,3a*S*,7a*S*)-7a-methyloctahydrospiro[indene-5,2'-[1,3]dioxolan]-1-yl)propanal (192)



190 191 192

To a 23 °C solution of homoallylic alcohol **190** (530 mg, 2.10 mmol, 1 equiv) in tetrahydrofuran (21 ml) was added 10% palladium on carbon (75 mg). The reaction flask was repeatedly evacuated-backfilled

[3] Presence of activated 4-A molecular sieves was important to minimize undesired cleavage of the acetal protective group.

[4] The yield and stereoselectivity worsen if reaction is upscaled.

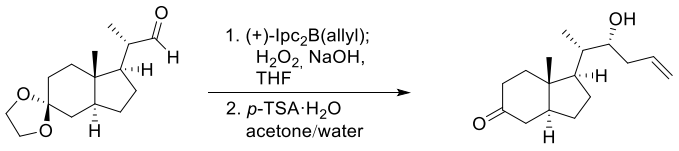
with dihydrogen (five cycles), then a dihydrogen-filled balloon was attached. After vigorous stirring for 11.5 h at ambient temperature, the reaction mixture was filtered through Celite® eluting with ethyl acetate and the filtrate was concentrated in vacuo to provide 535 mg of the crude hydrogenation product **191** as a colourless oil.

In a separate flask, a solution of dimethyl sulfoxide (344 μL, 4.84 mmol, 2.3 equiv) in dichloromethane (5 ml) was added to a −78 °C solution of oxalyl chloride (196 μL, 2.31 mmol, 1.1 equiv) in dichloromethane (10 ml) over 2 min. After 20 min, a solution of crude hydrogenation product **191** (535 mg, 2.10 mmol, 1 equiv; see paragraph above) in dichloromethane (10 ml) was added to the above mixture at −78 °C over 5 min. The resulting suspension was stirred for 30 min, then triethylamine (1.06 ml, 7.57 mmol, 3.6 equiv) was added dropwise over 5 min at −78 °C. The mixture was stirred for additional 1 h at −78 °C before allowed to warm to ambient temperature. The obtained clear solution was diluted with dichloromethane (120 ml) and the diluted solution was washed sequentially with water (20 ml) and saturated aqueous solution of sodium chloride (20 ml). The washed organic phase was dried over anhydrous magnesium sulfate, filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 10–15% ethyl acetate in petroleum ether) provided aldehyde **192** (429 mg, 81% over 2 steps).

Colourless oil. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.50 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 9.52 (d, *J* = 3.2 Hz, 1H), 3.86 (s, 4 H), 2.30 (m, 1H), 1.95–1.13 (m, 12H), 1.07 (d, *J* = 6.9 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 205.3, 110.1, 64.8, 64.7, 50.7, 50.1, 47.4, 43.0, 36.7, 35.9, 31.8, 28.1, 26.7, 13.8, 11.0. **FTIR** (neat), cm^{-1}: 2944 (s), 2873 (m), 1723 (vs), 1076 (vs). Calcd for ($C_{15}H_{24}O_3$+H^+): 253.1798, found: 253.1797. $\boldsymbol{\alpha_D^{RT}}$ = +8.5 (c = 1.0 in CH_2Cl_2).

Brown Allylation and Deprotection of 192

(1*R*,3a*S*,7a*S*)-1-((2*S*,3*R*)-3-hydroxyhex-5-en-2-yl)-7a-methyloctahydro-5*H*-inden-5-one (180)



192 **180**

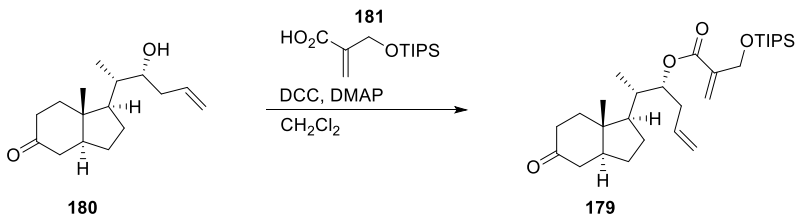
(+)-B-Allyldiisopinocampheylborane (1.0 M solution in pentane, 2.24 ml, 2.24 mmol, 1.1 equiv) was added dropwise to a −78 °C solution of aldehyde **192** (513 mg, 2.03 mmol, 1 equiv) in tetrahydrofuran (40 ml). After 25 min, the reaction mixture was allowed to warm to 0 °C and sodium hydroxide (2.0 M aqueous solution, 2.03 ml, 4.07 mmol, 2 equiv) and hydrogen peroxide (30% aqueous solution by weight, 461 μL, 2 equiv) were added sequentially. The mixture was allowed to reach ambient temperature and then was stirred for 11 h. The oxidation mixture was diluted with dichloromethane

(100 ml) and sequentially washed with water (40 ml) and saturated aqueous solution of sodium chloride (40 ml). Washed organic phase was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue comprising a closely running mixture of protected allylation product and (+)-isopinocampheol was dissolved in a 10:1 acetone-water mixture (22 ml) and p-toluenesulfonic acid monohydrate (193 mg, 1.02 mmol, 0.5 equiv) was added at ambient temperature. The resulting solution was warmed to 50 °C in an oil bath. After 30 min, the reaction mixture was allowed to cool down to ambient temperature, then was diluted with dichloromethane (100 ml). The diluted mixture was washed with saturated aqueous sodium bicarbonate (30 ml), the organic phase was separated and the aqueous phase was extracted with dichloromethane (50 ml). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the filtrate was concentrated in vacuo. The remaining residue was purified by flash-column chromatography (gradient elution with 10–30% ethyl acetate in petroleum ether) to provide the homoallylic product **180** (429 mg, 84% over 2 steps).

Colourless oil. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.15 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 5.83 (m, 1H), 5.17 (m, 1H), 5.14 (app s, 1H), 3.71 (ddd, *J* = 10.6, 3.2, 2.4 Hz, 1H), 2.48–2.12 (m, 6H), 2.04–1.75 (m, 4H), 1.70–1.48 (m, 3H), 1.33–1.16 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 211.9, 135.9, 118.1, 71.8, 51.9, 49.6, 42.7, 42.2, 41.0, 37.7, 37.5, 34.9, 28.2, 26.4, 12.4, 10.5. **FTIR** (neat), cm^{-1}: 3451 (br), 2947 (m), 1707 (s), 1014 (m). **HRMS** (ESI): Calcd for ($C_{16}H_{26}O_2$+H^+): 251.2006, found: 251.2002. $\boldsymbol{\alpha_D^{RT}}$= +41.8 (c = 2.0 in CH_2Cl_2).

Esterification of homoallylic Alcohols 180 and 233

(2*S*,3*R*)-2-((1*R*,3a*S*,7a*S*)-7a-methyl-5-oxooctahydro-1*H*-inden-1-yl)hex-5-en-3-yl 2-(((triisopropylsilyl)oxy)methyl)acrylate (179)



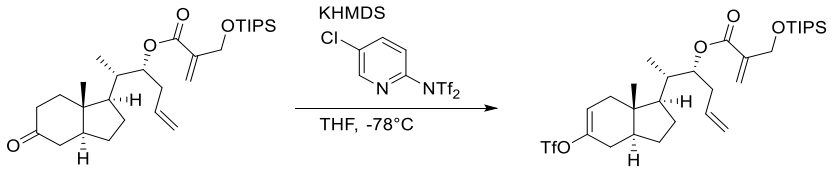
Dicyclohexylcarbodiimide (884 mg, 4.28 mmol, 2.5 equiv) and 4-dimethylaminopyridine (105 mg, 0.86 mmol, 0.5 equiv) were added sequentially to an ice-cold solution of homoallylic alcohol **180** (429 mg, 1.71 mmol, 1 equiv) and acrylic acid **181**[97] (1.11 g, 4.28 mmol, 2.5 equiv) in dichloromethane (20 ml). After 30 min, the ice-water cooling bath was removed and the resulting light yellow suspension was stirred vigorously at ambient temperature for 16 h. Then, dichloromethane (100 ml) and saturated aqueous solution of sodium bicarbonate (40 ml) were added. The organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was

concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 0.5–0.7% ethyl acetate in dichloromethane) provided acrylic ester **179** (649 mg, 77%).

Colourless oil. **TLC** (20% ethyl acetate in petroleum ether): R_f = 0.60 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.26 (d, *J* = 2.0 Hz, 1H), 5.97 (d, *J* = 2.1 Hz, 1H), 5.73 (m, 1H), 5.08–5.00 (m, 2H), 5.05 (ddd, *J* = 9.5, 3.5, 3.5 Hz, 1H), 4.46 (s, 2H), 2.45–2.23 (m, 6H), 2.14 (m, 1H), 2.00–1.91 (m, 2H), 1.85–1.52 (m, 4H), 1.36–1.04 (m, 22H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.92 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 211.6, 165.5, 140.0, 134.8, 123.3, 117.2, 75.7, 61.7, 52.0, 49.7, 42.7, 42.3, 39.0, 37.7, 37.5, 32.2, 28.0, 26.4, 18.0, 13.1, 12.0, 10.5. **FTIR** (neat), cm^{-1}: 2944 (s), 1711 (vs), 1096 (vs). **HRMS** (ESI): Calcd for ($C_{29}H_{51}O_4Si+H^+$): 491.3551, found: 491.3543. $\boldsymbol{\alpha_D^{RT}}$ = +26.5 (c = 1.0 in $CHCl_3$).

Triflation of 179

(2*S*,3*R*)-2-((1*R*,3a*S*,7a*S*)-7a-methyl-5-(((trifluoromethyl)sulfonyl)oxy)-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)hex-5-en-3-yl 2-(((triisopropylsilyl)oxy)methyl)acrylate (179-I)



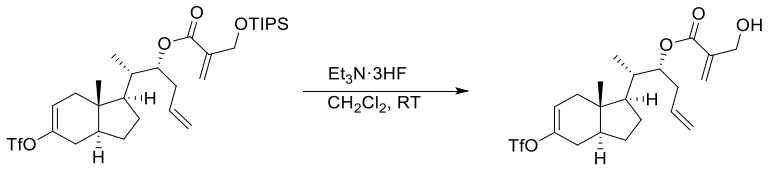
179 **179-I**

To a −78 °C solution of ketone **179** (400 mg, 0.82 mmol, 1 equiv) in tetrahydrofuran (16 ml) was added potassium hexamethyldisilazide (0.5 M solution in toluene, 1.96 ml, 0.98 mmol, 1.2 equiv). After 30 min, Comins reagent (384 mg, 0.98 mmol, 1.2 equiv) was added as a solution in tetrahydrofuran (4 ml) at −78 °C. The reaction was quenched after 15 min by addition of pH 7 phosphate buffer (10 ml) at −78 °C. The resulting mixture was allowed to warm to ambient temperature and then was diluted with dichloromethane (100 ml). The separated organic phase was dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 0–1% ethyl acetate in dichloromethane) to yield enol triflate **179-I** (410 mg, 81%).

Colourless oil. **TLC** (5% ethyl acetate in petroleum ether): 0.75 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.26 (d, *J* = 1.9 Hz, 1H), 5.97 (d, *J* = 2.0 Hz, 1H), 5.78–5.65 (m, 2H), 5.07–4.98 (m, 3H), 4.45 (s, 2H), 2.40–2.04 (m, 6H), 1.98–1.64 (m, 5H), 1.34–1.22 (m, 2H), 1.17–1.02 (m, 21H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.4, 148.6, 140.0, 134.7, 123.3, 119.0 (weak q, *J* = 319 Hz, CF_3), 118.1, 117.2, 75.6, 61.6, 51.8, 46.0, 41.2, 38.8, 38.6, 32.1, 30.5, 27.7, 25.8, 18.0, 12.8, 11.9, 10.9. **FTIR** (neat), cm^{-1}: 2945 (m), 1711 (m), 1417 (s), 1209 (s). **HRMS** (ESI): Calcd for ($C_{30}H_{49}F_3O_6SSi+H^+$): 623.3044, found: 623.3042.

General TBS-Deprotection Protocol

(2*S*,3*R*)-2-((1*R*,3a*S*,7a*S*)-7a-methyl-5-(((trifluoromethyl)sulfonyl)oxy)-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)hex-5-en-3-yl 2-(hydroxymethyl)acrylate (179-II)



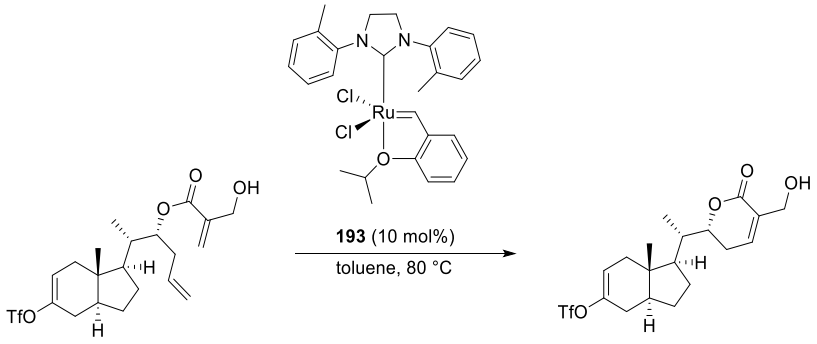
179-I **179-II**

To a 23 °C solution of *tri*-isopropylsilyl ether **179-I** (410 mg, 0.66 mmol, 1 equiv) in dichloromethane (8.8 ml) was added triethylamine-trihydrofluoride (2 ml). The reaction flask was sealed and stirring continued for 22 h. The reaction mixture was diluted with dichloromethane and the diluted mixture was carefully neutralized by addition of saturated aqueous solution of sodium bicarbonate. The organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 10–30% ethyl acetate in petroleum ether) to provide deprotected acrylate ester **179-II** (256 mg, 83%).

Colourless oil. **TLC** (15% ethyl acetate in petroleum ether): R_f = 0.18 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.26 (s, 1H), 5.81 (d, *J* = 0.9 Hz, 1H), 5.73 (m, 1H), 5.66 (m, 1H), 5.12–5.01 (m, 3H), 4.35 (d, *J* = 14.0 Hz, 1H), 4.29 (d, *J* = 13.5 Hz, 1H), 2.39–2.05 (m, 7H), 1.93 (m, 2H), 1.85–1.64 (m, 3H), 1.31 (m, 2H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.7, 148.6, 139.6, 134.8, 125.7, 118.5 (weak q, *J* = 319 Hz, CF_3), 118.0, 117.4, 76.1, 62.7, 51.9, 46.0, 41.3, 38.8, 38.6, 32.1, 30.5, 27.7, 25.8, 12.8, 10.9. **FTIR** (neat), cm^{-1}: 3450 (br), 2947 (m), 1710 (s), 1416 (vs), 1209 (vs), 1143 (vs). **HRMS** (ESI): Calcd for ($C_{21}H_{29}F_3O_6S+H^+$): 467.1710, found: 467.1700. $\boldsymbol{\alpha_D^{RT}}$ = +27.4 (c = 1.0 in CH_2Cl_2).

Ring-closing Metathesis of Ester 179-II

(1*R*,3a*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-5-yl trifluoromethanesulfonate (177)



179-II **177**

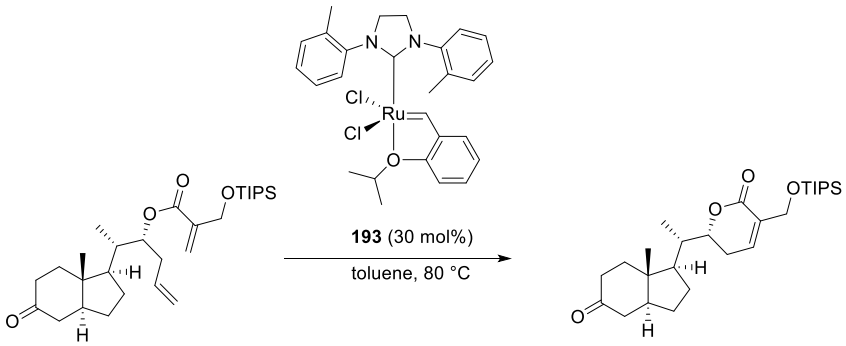
Note: Portionwise addition of ruthenium(II) catalyst **193** was employed in all ring-closing metathesis experiments detailed below.

First portion of Stewart-Grubbs ruthenium catalyst **193** (20 mg, 35.7 µmol, 0.05 equiv) was added to a 80 °C solution of acrylate ester **179-II** (333 mg, 714 µmol, 1 equiv) in toluene (135 ml; deoxygenated by bubbling a stream of argon through toluene solution for 45 min). After 2 h, the second portion of Stewart-Grubbs ruthenium catalyst **193** (20 mg, 35.7 µmol, 0.05 equiv) was added and stirring was continued for additional 2 h at 80 °C. The reaction mixture was allowed to cool down to ambient temperature, then a solution of potassium isocyanoacetate (90% purity, 79 mg, 0.64 mmol, 1 equiv) in methanol (6 ml) was added, and the resulting mixture was stirred for 1 h.[145] The dark yellow-brown mixture was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 30–50% ethyl acetate in petroleum ether) provided dehydrolactone **177** (250 mg, 80%).

White powder. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.18 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.85 (d, *J* = 6.0 Hz, 1H), 5.68 (app d, *J* = 5.7 Hz, 1H), 4.50 (ddd, *J* = 13.1, 3.4, 3.4 Hz, 1H), 4.32 (d, *J* = 14.5 Hz, 1H), 4.30 (d, *J* = 14.5 Hz, 1H), 2.48–2.00 (m, 8H), 1.78 (m, 3H), 1.50 (m, 1H), 1.36–1.20 (m, 2H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.7, 148.5, 140.4, 131.5, 118.5 (weak q, *J* = 319 Hz, CF_3), 117.9, 80.1, 61.7, 50.9, 46.0, 41.4, 38.7, 38.5, 30.6, 27.9, 25.8, 23.0, 13.1, 10.8. **FTIR** (neat), cm^{-1}: 3434 (br), 2943 (m), 1710 (vs), 1415 (vs), 1208 (vs), 1141 (vs). **HRMS** (ESI): Calcd for ($C_{19}H_{25}F_3O_6S$+H$^+$): 439.1397, found: 439.1391. $\boldsymbol{\alpha_D^{RT}}$ = +57.0 (c = 0.93 in CH_2Cl_2).

Ring-closing Metathesis of Ester 179 and Synthesis of 194

(*R*)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-7a-methyl-5-oxooctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)-5,6-dihydro-2*H*-pyran-2-one (178)

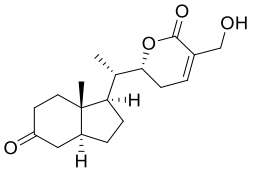


179 **178**

The first portion of Stewart-Grubbs ruthenium catalyst **193** (52.3 mg, 91.7 µmol, 0.15 equiv) was added to an 80 °C solution of acrylate ester **179** (300 mg, 611 µmol, 1 equiv) in toluene (200 ml; deoxygenated by bubbling a stream of argon into toluene for 45 min). After 3 h, the second portion of Stewart-Grubbs ruthenium catalyst **193** (52.3 mg, 91.7 µmol, 0.15 equiv) was added and stirring was continued for additional 4.5 h at 80 °C. The reaction mixture was allowed to cool down to ambient temperature, then a solution of potassium isocyanoacetate (90% purity, 167 mg, 1.22 mmol, 2 equiv) in methanol (8 ml) was added, and the resulting mixture was stirred for 1 h. The dark yellow-brown mixture was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 10–30% ethyl acetate in petroleum ether) provided unreacted acrylate ester **179** (56 mg, 19%) initially, followed by dehydrolactone **178** (211 mg, 75%).

White powder**. TLC** (20% acetone in petroleum ether): R_f = 0.27 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.96 (m, 1H), 4.60–4.37 (m, 2H), 4.47 (ddd, *J* = 13.3, 3.7, 3.7 Hz, 1H), 2.50–2.02 (m, 8H), 1.90–1.74 (m, 2H), 1.72–1.46 (m, 2H), 1.39–1.00 (m, 26H), 0.96 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 211.6, 165.0, 137.6, 131.7, 79.9, 60.6, 50.8, 49.6, 42.7, 42.2, 38.8, 37.6, 37.4, 28.1, 26.3, 22.9, 18.1, 13.4, 12.0, 10.4. **FTIR** (neat), cm^{-1}: 2944 (s), 1713 (vs), 1067 (m). **HRMS** (ESI): Calcd for ($C_{27}H_{47}O_4Si+H^+$): 463.3238, found: 463.3233. $\boldsymbol{\alpha_D^{RT}}$ = +76.7 (c = 1.0 in CH_2Cl_2).

(*R*)-3-(hydroxymethyl)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-7a-methyl-5-oxooctahydro-1*H*-inden-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (194)

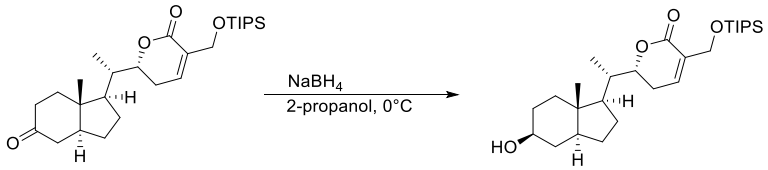


194 was prepared from **178** according to the general TBS-deprotection protocol (page 148) in >99% yield.

White amorphous solid. **TLC** (70% ethyl acetate in petroleum ether): R_f = 0.20 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.83 (d, *J* = 6.0 Hz, 1H), 4.51 (ddd, *J* = 13.2, 3.6, 3.6 Hz, 1H), 4.32 (d, *J* = 14.5 Hz, 1H), 4.29 (d, *J* = 14.5 Hz, 1H), 2.46–2.06 (m, 10H), 1.85–1.76 (m, 2H), 1.69–1.48 (m, 3H), 1.36–1.18 (m, 2H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.95 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 211.4, 165.8, 140.4, 131.4, 80.0, 61.5, 50.8, 49.6, 42.7, 42.2, 38.8, 37.6, 37.3, 28.1, 26.3, 23.1, 13.4, 10.4. **FTIR** (neat), cm^{-1}: 3434 (br), 2948 (w), 1707 (vs). **HRMS** (ESI): Calcd for ($C_{18}H_{27}O_4+H^+$): 307.1904, found: 307.1904. $\boldsymbol{\alpha_D^{RT}}$= +95.36 (c = 0.69 in $CHCl_3$).

Sodium Borohydride Reduction of Ketone 178 and Synthesis of 238

(*R*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)-5,6-dihydro-2*H*-pyran-2-one (176)



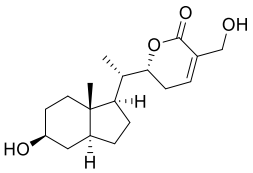
178 **176**

Sodium borohydride (19 mg, 0.51 mmol, 3 equiv) was added to an ice-cold solution of ketone **178** (78 mg, 0.17 mmol, 1 equiv) in 2-propanol (5 ml). The resulting suspension was stirred for 5 min at 0 °C, then for 15 min at ambient temperature. The obtained clear solution was diluted with dichloromethane (20 ml) and an excess of unreacted sodium borohydride was carefully quenched by dropwise addition of a saturated aqueous solution of ammonium chloride. The organic phase was separated and the aqueous phase was extracted further with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 10–30% ethyl acetate in petroleum ether) to provide alcohol **176** (66 mg, 84%; pure diastereomer[5]).

White foam. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.45 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.95 (m, 1H), 4.55 (m, 1H), 4.45 (ddd, *J* = 13.2, 3.6, 3.6 Hz, 1H), 4.42 (m, 1H), 3.61 (m, 1H), 2.38 (m, 1H), 2.19 (m, 1H), 2.03 (m, 1H), 1.92 (m, 1H), 1.86–1.05 (m, 32H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.1, 137.6, 131.6, 80.2, 71.5, 60.7, 51.3, 48.3, 42.3, 39.0, 37.5, 35.0, 31.5, 27.7, 25.8, 23.0, 18.0, 13.4, 12.0, 11.0. **FTIR** (neat), cm^{-1}: 3401 (br), 2940 (m), 1709 (s), 1066 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{48}O_4Si+H^+$): 465.3395, found: 465.3390. $\boldsymbol{\alpha_D^{RT}}$= +40.6 (c = 1.0 in CH_2Cl_2).

[5] A small amount of epimeric product (opposite alcohol configuration), separable from the major diastereomer, was also isolated (~10%).

(*R*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(hydroxymethyl)-5,6-dihydro-2*H*-pyran-2-one (238)

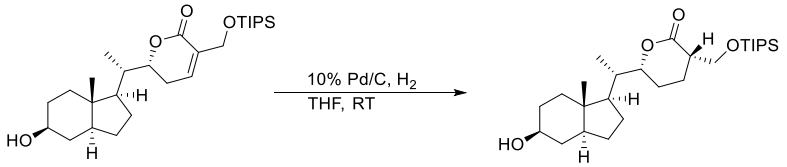


The product was prepared from **176** according to the general TBS-deprotection protocol (page 148) in 83% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.22 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.84 (d, *J* = 5.4 Hz, 1H), 4.49 (ddd, *J* = 13.1, 3.6, 3.6 Hz, 1H), 4.30 (s, 2H), 3.61 (m, 1H), 2.42 (m, 1H), 2.23 (m, 1H), 2.17 (m, 1H), 2.08–1.66 (m, 6H), 1.58–1.04 (m, 8H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.9, 140.7, 131.1, 80.3, 71.5, 61.7, 51.2, 48.3, 42.3, 38.9, 37.4, 35.0, 31.4, 27.7, 25.7, 23.0, 13.4, 11.0. **FTIR** (neat), cm^{-1}: 3369 (br), 2940 (m), 1701 (vs). **HRMS** (ESI): Calcd for ($C_{18}H_{29}O_4$+H_+): 309.2060, found: 309.2061. $\boldsymbol{\alpha_D^{RT}}$ = +66.79 (c = 0.76 in $CHCl_3$).

Synthesis of δ-Lactone Intermediate 217 and of 239

(3*S*,6*R*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)tetrahydro-2*H*-pyran-2-one (217)



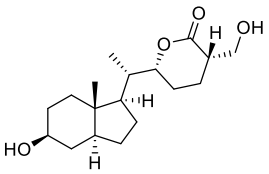
176 217

To a 23 °C solution of unsaturated lactone **176** (70 mg, 150 µmol, 1 equiv) in tetrahydrofuran (11 ml) was added 10% palladium-on-carbon (16 mg). The reaction flask was repeatedly evacuated-backfilled with dihydrogen (five cycles), then a dihydrogen-filled balloon was attached. After vigorous stirring overnight at ambient temperature, the reaction mixture was filtered through Celite® eluting with ethyl acetate and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 10–60% ethyl acetate in petroleum ether) provided saturated lactone **217** (54 mg, 77%, mixture of diastereomers 6:1).

Colourless oil**. TLC** (40% ethyl acetate in petroleum ether): R_f = 0.39 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 4.31 (m, 1H), 4.09 (dd, *J* = 9.6, 5.6 Hz, 0.15H, *minor stereoisomer*), 4.03 (dd, *J* = 9.8, 4.2 Hz, 0.85H, *major stereoisomer*), 3.93 (dd, *J* = 9.6, 3.3 Hz, 0.15H, *minor stereoisomer*), 3.88 (dd, *J* = 9.8, 7.7 Hz, 0.85H, *major stereoisomer*), 3.60 (m, 1H), 2.68 (ddd, *J* = 7.7, 7.6, 4.3, 0.85H, *major stereoisomer*), 2.53 (m, 0.15H, *minor stereoisomer*), 2.20–1.07 (m, 18H), 1.06–0.99 (m, 21H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 173.77, 81.26, 71.46, 64.22, 51.33, 48.23, 42.21, 41.01, 39.10, 37.31, 34.92, 31.37, 27.78, 25.70, 21.61, 19.47, 17.92, 17.90, 12.73, 11.81, 10.95. **FTIR** (neat), cm^{-1}:

3391 (br), 2941 (s), 2865 (s), 1726 (s), 1463 (m). **HRMS** (ESI): Calcd for ($C_{27}H_{50}O_4Si+H^+$): 467.35511, found: 467.35491.

(3*S*,6*R*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(hydroxymethyl)tetrahydro-2*H*-pyran-2-one (239)

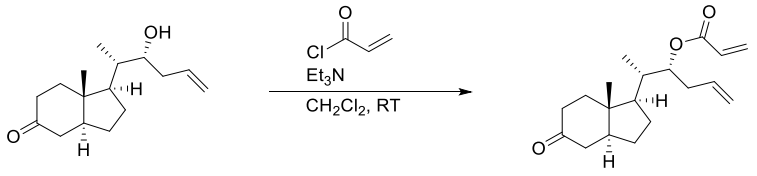


The product was synthesized according to the general TBS-deprotection protocol (page 148) from **217**. The yield was not determined.

White crystalline solid. **TLC** (80% ethyl acetate in dichloromethane): R_f = 0.24 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 4.34 (dt, *J* = 11.3, 3.3 Hz, 1H), 3.76 (dd, *J* = 11.5, 4.4 Hz, 1H) overlapping with 3.72 (dd, *J* = 11.5, 6.9 Hz, 1H), 3.62 (m, 1H), 2.69 (m, 1H), 2.07–1.06 (m, 19H), 0.97 (d, *J* = 6.7 Hz, 1H), 0.76 (s, 3H). **HRMS** (ESI): Calcd for ($C_{18}H_{30}O_4+H^+$): 311.22169, found: 311.22155.

Synthesis of δ-Lactone Intermediates 216 and 218

(2*S*,3*R*)-2-((1*R*,3a*S*,7a*S*)-7a-methyl-5-oxooctahydro-1*H*-inden-1-yl)hex-5-en-3-yl acrylate (215)

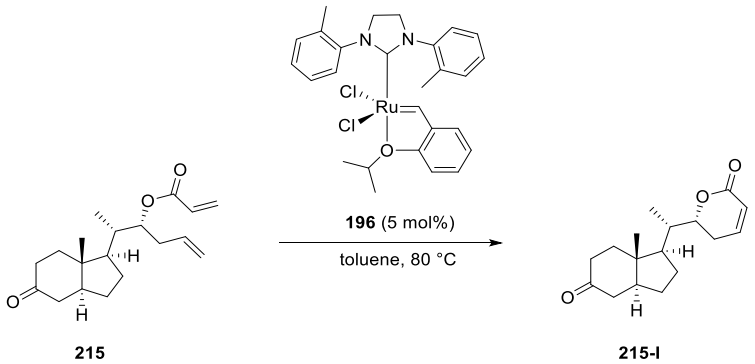


180 **215**

Acryloyl chloride (69 µl, 0.85 mmol, 2 equiv) and triethylamine (236 µl, 1.69 mmol, 4 equiv) were added to a solution of homoallylic alcohol **180** (106 mg, 0.42 mmol, 1 equiv) in dichloromethane (10 ml). The resulting light yellow solution was stirred at ambient temperature for 1 h. Then, dichloromethane (100 ml) and saturated aqueous solution of sodium bicarbonate (40 ml) were added. Organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 5–30% ethyl acetate in petroleum ether) provided acrylic ester **215** (92 mg, 71%).

Colourless oil. **TLC** (30% ethyl acetate in petroleum ether): R_f = 0.6 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.38 (dd, *J* = 17.3, 1.1 Hz, 1H), 6.10 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.80 (dd, *J* = 10.4, 1.1 Hz, 1H), 5.73 (m, 1H), 5.04 (m, 3H), 2.50–1.47 (m, 12), 1.38–1.18 (m, 3H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.91 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 211.78, 165.74, 134.75, 130.39, 128.82, 117.04, 75.62, 51.78, 49.63, 42.68, 42.15, 38.97, 37.60, 37.44, 32.02, 27.91, 26.32, 13.05, 10.49. **FTIR** (neat), cm^{-1}: 2958 (br), 1715 (vs), 1405 (m), 1193 (vs). **HRMS** (ESI): Calcd for ($C_{19}H_{28}O_3+H^+$): 305.21112, found: 305.21106. α_D^{RT} = +44.0 (c = 1.0 in CH_2Cl_2).

(R)-6-((S)-1-((1R,3aS,7aS)-7a-methyl-5-oxooctahydro-1H-inden-1-yl)ethyl)-5,6-dihydro-2H-pyran-2-one (215-I)

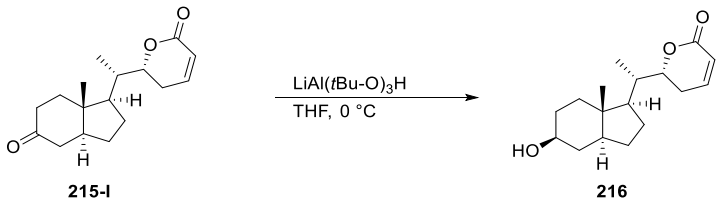


215 **215-I**

Stewart-Grubbs ruthenium catalyst **193** (24 mg, 42 µmol, 0.05 equiv) was added to a solution of acrylate ester **215** (260 mg, 854 µmol, 1 equiv) in toluene (65 ml). The resulting mixture was stirred for 1 h at 80 °C. The dark yellow-brown mixture was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 40–80% ethyl acetate in petroleum ether) provided dehydrolactone **215-I** (196 mg, 83%).

White powder. **TLC** (40% ethyl acetate in petroleum ether): R_f = 0.30 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.91 (ddd, *J* = 9.5, 6.6, 2.5 Hz, 1H), 6.00 (dd, *J* = 6.6, 2.8 Hz, 1H), 4.50 (dt, *J* = 13.0, 3.6 Hz, 1H), 2.46–2.05 (m, 8H), 1.86–1.50 (m, 5H), 1.36–1.18 (m, 2H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.95 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 211.4, 164.7, 145.3, 121.3, 79.8, 50.8, 49.6, 42.6, 42.2, 38.9, 37.6, 37.4, 28.1, 26.2, 23.0, 13.4, 10.3. **FTIR** (neat), cm^{-1}: 2948 (m), 1703 (vs), 1261 (s). **HRMS** (ESI): Calcd for ($C_{17}H_{24}O_3$+H^+): 277.1798, found: 277.1798. $\boldsymbol{\alpha_D^{RT}}$ = +102.78 (c = 1.28 in $CHCl_3$).

(*R*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (216)

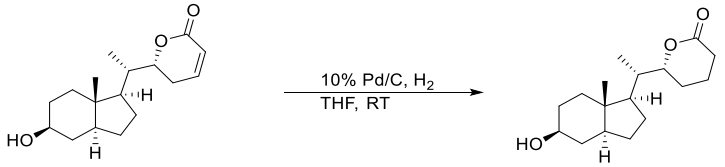


215-I **216**

Lithium *tri-tert*-butoxyaluminum hydride (168 mg, 662 µmol, 1 equiv) was added to an ice-cold solution of ketone **215-I** (183 mg, 662 µmol, 1 equiv) in THF (20 ml). The resulting solution was stirred for 5 min at 0 °C. The obtained clear solution was diluted with dichloromethane and an excess of unreacted lithium *tri-tert*-butoxyaluminum hydride was carefully quenched by dropwise addition of a 1M solution of hydrochloric acid (10 ml). The organic phase was separated and the aqueous phase was extracted further with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 50–100% ethyl acetate in petroleum ether) to provide alcohol **216** (146 mg, 79%; pure diastereomer).

TLC (40% ethyl acetate in petroleum ether): R_f = 0.15 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.90 (m, 1H), 6.01 (dd, *J* = 9.7, 2.8 Hz, 1H), 4.48 (dt, *J* = 13.0, 3.6 Hz, 1H), 3.62 (tt, *J* = 10.0, 4.9 Hz, 1H), 2.36 (m, 1H), 2.13 (ddd, *J* =18.3, 6.4, 3.5 Hz, 1H), 2.04 (m, 1H), 1.93 (dt, *J* = 13.0, 3.6 Hz, 1H), 1.85 – 1.70 (m, 3H), 1.70 – 1.18 (m, 7H), 1.09 (m, 1H), 1.02 (d, *J* = 6.7, 3H), 0.76 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 164.9, 145.5, 121.4, 80.1, 71.5, 51.3, 48.3, 42.4, 39.0, 37.4, 35.0, 31.5, 27.7, 25.7, 23.1, 13.4, 11.0. **FTIR** (neat), cm^{-1}: 3410 (br), 2940 (m), 1720 (vs), 1260 (m), 1029 (m). **HRMS** (ESI): Calcd for ($C_{17}H_{26}O_3$+H^+): 279.1955, found: 279.1954. $\boldsymbol{\alpha_D^{RT}}$= +105.79 (c = 0.16 in $CHCl_3$).

(*R*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)tetrahydro-2*H*-pyran-2-one (218)



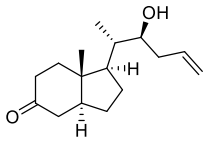
216 **218**

To a solution of unsaturated lactone **216** (92 mg, 330 µmol, 1 equiv) in tetrahydrofuran (10 ml) at ambient temperature was added 10% palladium on carbon (18 mg). The reaction flask was repeatedly evacuated-backfilled with dihydrogen (five cycles), then a dihydrogen-filled balloon was attached. After vigorous stirring overnight at ambient temperature, the reaction mixture was filtered through Celite® eluting with ethyl acetate and the filtrate was concentrated in vacuo to provide the crude hydrogenation product (colourless oil). Purification of the residue by flash-column chromatography (gradient elution with 50–80% ethyl acetate in petroleum ether) provided saturated lactone **218** (71 mg, 76%).

Colourless oil**. TLC** (70% ethyl acetate in petroleum ether): R_f = 0.29 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 4.32 (ddd, *J* = 11.60, 3.09, 3.09 Hz, 1H), 3.59 (m, 1H), 2.57 (ddd = 16.68, 7.14, 3.64 Hz, 1H), 2.37 (ddd = 17.20, 9.92, 7.28 Hz, 1H), 2.0–1.05 (m, 18H), 0.92 (d, *J* = 6.69 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 172.16, 82.97, 71.40, 51.30, 48.20, 42.21, 39.40, 37.29, 34.87, 31.32, 29.68, 27.68, 25.68, 20.99, 18.81, 12.71, 10.93. **FTIR** (neat), cm^{-1}: 3467 + 3398 (br), 2937 (m), 1724 (s), 1703 (s), 1255 (s). **HRMS** (ESI): Calcd for ($C_{17}H_{28}O_3$+H^+): 281.21112, found: 281.21051.

Synthesis of epimeric δ-Lactone Intermediate 234

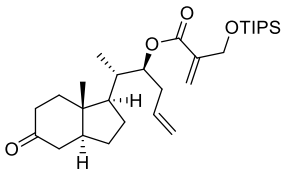
(1*R*,3a*S*,7a*S*)-1-((2*S*,3*S*)-3-hydroxyhex-5-en-2-yl)-7a-methyloctahydro-5*H*-inden-5-one (233)



The compound was prepared from **192** according to the Brown allylation and acetal deprotection protocol (page 145) in 80% yield. (-)-Ipc_2B(allyl)borane instead of (+)-Ipc_2B(allyl)borane was used for the Brown allylation.

TLC (30% ethyl acetate in petroleum ether): R_f = 0.23 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.79 (m, 1H), 5.13 (m, 1H), 5.09 (m, 1H), 3.72 (ddd, *J* = 8.6, 4.9, 1.5 Hz, 1H), 2.50–1.19 (m, 16H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.90 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 212.05, 135.41, 117.63, 72.03, 51.33, 49.82, 42.77, 41.70, 40.19, 40.00, 37.65, 37.50, 28.49, 26.24, 11.60, 10.40. **FTIR** (neat), cm^{-1}: 3429 (br), 2967 (m), 2940 (m), 2361 (m), 2342 (m), 1706 (s). **HRMS** (ESI): Calcd for ($C_{16}H_{26}O_2$+H^+): 251.20056, found: 251.19991. $\boldsymbol{\alpha_D^{RT}}$ = +44.8 (c = 1.0 in $CHCl_3$).

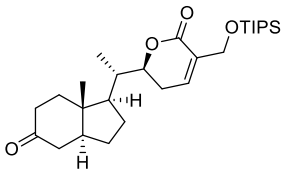
(2*S*,3*S*)-2-((1*R*,3a*S*,7a*S*)-7a-methyl-5-oxooctahydro-1*H*-inden-1-yl)hex-5-en-3-yl 2-(((triisopropylsilyl)oxy)methyl)acrylate (233-I)



The compound was prepared from **233** according to the esterification protocol described above (page 146) in 70% yield.

TLC (10% ethyl acetate in petroleum ether): R_f = 0.33 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.24 (m, 1H), 5.97 (m, 1H), 5.71 (m, 1H), 5.14–5.00 (m, 3H), 4.44 (s, 2H), 2.50–1.98 (m, 7H), 1.85–1.00 (m, 8H), 1.09–1.00 (m, 24H), 0.92 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 211.74, 165.41, 140.04, 133.85, 123.51, 117.73, 75.02, 61.70, 51.44, 49.81, 42.71, 41.77, 38.63, 37.61, 37.45, 36.80, 28.84, 26.28, 17.97, 12.83, 11.97, 10.34. **FTIR** (neat), cm^{-1}: 2944 (m), 2866 (m), 1711 (vs), 1266 (m), 1095 (vs). **HRMS** (ESI): Calcd for ($C_{29}H_{50}O_4Si$+H^+): 491.35511, found: 491.35456. $\boldsymbol{\alpha_D^{RT}}$ = +38.1 (c = 1.0 in $CHCl_3$).

(*S*)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-7a-methyl-5-oxooctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)-5,6-dihydro-2*H*-pyran-2-one (234)

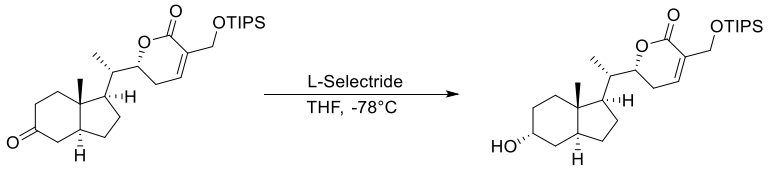


The compound was prepared according to the ring-closing metathesis protocol described above (page 150) in 63% yield. 11% of the starting material were isolated.

TLC (20% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.97 (d, *J* = 6.1 Hz, 1H), 4.60–4.45 (m, 2H), 4.44–4.35 (m, 1H), 2.67–2.53 (m, 1H), 2.48–1.10 (m, 17H), 1.10–1.02 (m, 21H), 0.91 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 211.62, 164.86, 137.88, 131.46, 79.63, 60.72, 50.14, 49.58, 42.71, 41.69, 39.53, 37.41, 37.36, 28.46, 27.26, 26.19, 17.98, 13.23, 11.91, 10.43. **FTIR** (neat), cm^{-1}: 2943 (m), 2865 (m), 1710 (vs), 1462 (m), 1068 (vs), 681 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{46}O_4Si+H^+$): 463.32381, found: 463.32405. $\boldsymbol{\alpha_D^{RT}}$= +10.0 (c = 1.0 in $CHCl_3$).

L-Selectride Reduction of Ketones 178 and 234, Synthesis of 235, 236 and 237

(*R*)-6-((*S*)-1-((1*R*,3a*S*,5*R*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)-5,6-dihydro-2*H*-pyran-2-one (236)

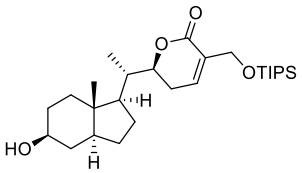


178 **236**

To a −78 °C solution of ketone **178** (200 mg, 432 µmol, 1 equiv) in dry tetrahydrofuran (8 ml) was added L-Selectride (1 M solution in tetrahydrofuran, 519 µl, 518 µmol, 1.2 equiv) dropwise. After stirring for 1 h at ambient temperature the reaction was quenched by addition of saturated aqueous ammonium chloride at −78 °C. The obtained solution was diluted with ethyl acetate and the diluted solution was washed with water. The washed organic phase was dried over anhydrous magnesium sulfate, filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 20–35% ethyl acetate in petroleum ether) provided epimeric alcohol **236** (65 mg, 32%).

Colourless oil**. ^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.99 (m, 1H), 4.58 (m, 1H), 4.49 (dt, *J* = 13.2, 3.6 Hz, 1H), 4.44 (m, 1H), 4.11 (m, 1H), 2.42 (m, 1H), 2.23 (m, 1H), 2.07 (m, 1H), 1.88–1.13 (m, 13H), 1.10 (m, 21H), 1.07 (d, *J* = 6.7 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.11, 137.68, 131.50, 80.24, 66.35, 60.71, 51.92, 42.63, 41.95, 38.96, 34.49, 33.21, 29.14, 26.81, 26.20, 22.85, 17.99, 13.49, 11.93, 9.86. **FTIR** (neat), cm^{-1}: **HRMS** (ESI): Calcd for ($C_{27}H_{48}O_4Si+H^+$): 465.33946, found: 465.33939.

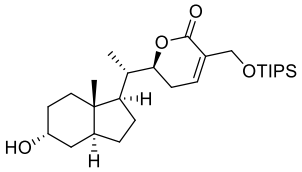
(*S*)-6-((*S*)-1-((1*R*,3a*S*,5*S*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)-5,6-dihydro-2*H*-pyran-2-one (235)



The compound was prepared from **234** according to the sodium borohydride reduction protocol described above (page 151) in 63% yield.

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 6.95 (m, 1H), 4.54 (d, *J* = 15.8 Hz, 1H), 4.47 (d, *J* = 12.9 Hz, 1H), 4.39 (d, *J* = 15.7 Hz, 1H), 3.62 (m, 1H), 2.58 (t, *J* = 15.8 Hz, 1H), 2.40–0.80 (m, 39H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.05, 138.00, 131.37, 79.87, 71.44, 60.72, 50.54, 48.40, 41.71, 39.60, 37.24, 35.04, 31.42, 28.10, 27.28, 25.62, 17.96, 13.15, 11.90, 10.97. **FTIR** (neat), cm^{-1}: 3402 (br), 2941 (s), 2865 (s), 1701 (s), 1062 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{48}O_4Si+H^+$): 465.33946, found: 465.33962.

(*S*)-6-((*S*)-1-((1*R*,3a*S*,5*R*,7a*S*)-5-hydroxy-7a-methyloctahydro-1*H*-inden-1-yl)ethyl)-3-(((triisopropylsilyl)oxy)methyl)-5,6-dihydro-2*H*-pyran-2-one (237)

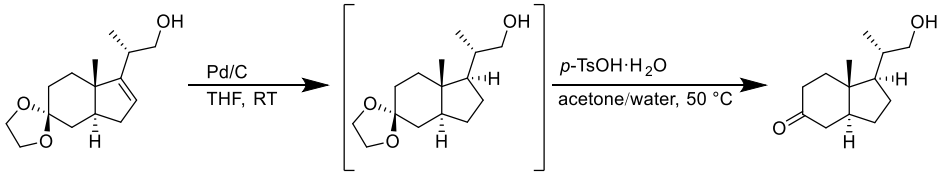


The product was prepared from **234** using the general L-Selectride reduction protocol (page 157) in 39% yield.

TLC (30% ethyl acetate in petroleum ether): R_f = 0.23 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.96 (m, 1H), 4.56 (m, 1H), 4.49 (m, 1H), 4.41 (m, 1H), 4.05 (m, 1H), 2.59 (m, 1H), 2.10 (m, 1H), 2.18–1.10 (m, 14H), 1.08 and 1.07 (m, 24H), 0.67 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.06, 137.93, 131.40, 80.11, 66.33, 60.74, 51.19, 42.10, 42.05, 39.67, 34.26, 33.28, 29.16, 27.20, 26.11, 18.00, 13.43, 11.90, 9.91. **FTIR** (neat), cm^{-1}: 2940 (m), 2865 (m), 1707 (vs), 1462 (w), 1061 (vs). **HRMS** (ESI): Calcd for ($C_{27}H_{48}O_4Si+H^+$): 465.33946, found: 465.33958.

Synthesis of functionalized Intermediate 221 and of 240

(1*R*,3a*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-5*H*-inden-5-one (219)

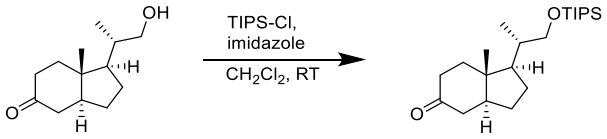


190 **191** **219**

190 was hydrogenated as described above (page 144). The hydrogenation product was not purified by column chromatography and the crude product subjected to acetal deprotection as described above (page 145) to yield **219**. The yield over two steps is 69%.

TLC (50% ethyl acetate in petroleum ether): R_f = 0.31 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 3.59 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.32 (dd, *J* = 10.5, 6.8 Hz, 1H), 2.39 (ddd, *J* = 16.1, 13.0, 7.0 Hz, 1H), 2.28–2.17 (m, 3H), 2.13 (ddd, *J* = 12.9, 7.1, 1.7 Hz, 1H), 1.93 (m, 1H), 1.79 (m, 1H), 1.72–1.39 (m, 5H), 1.27 (m, 2H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.90 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 212.02, 67.94, 51.85, 50.35, 43.27, 42.44, 39.27, 38.16, 38.02, 29.06, 26.99, 17.06, 10.87. **FTIR** (neat), cm^{-1}: 3412 (br), 2948 (s), 2870 (m), 1705 (vs), 1041 (m), 1013 (m), 985 (m). **HRMS** (ESI): Calcd for ($C_{13}H_{22}O_2$+H^+): 211.16926, found: 211.16907.

(1*R*,3a*S*,7a*S*)-7a-methyl-1-((*S*)-1-((triisopropylsilyl)oxy)propan-2-yl)octahydro-5*H*-inden-5-one (220)

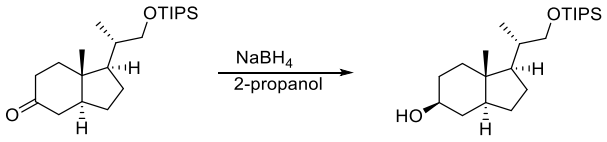


219 **220**

To a solution of alcohol **219** (200 mg, 951 µmol, 1 equiv) in dichloromethane (15 ml) were added Triisopropylsilyl chloride (303 µl, 1.43 mmol, 1.5 equiv) and imidazole (129 mg, 1.90 mmol, 2 equiv). The resulting suspension was stirred at ambient temperature overnight. Then, dichloromethane and saturated aqueous solution of sodium chloride were added. The organic phase was separated and dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash-column chromatography (gradient elution with 3–10% ethyl acetate in petroleum ether) provided TBS-protected product **220** (285 mg, 82%).

Colourless oil. **TLC** (10% ethyl acetate in petroleum ether): R_f = 0.49 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 3.67 (dd, *J* = 9.42, 3.30 Hz, 1H), 3.41 (dd, *J* = 9.43, 6.81 Hz, 1H), 2.47–2.10 (m, 5H), 2.02–1.89 (m, 1H), 1.86–1.74 (m, 1H), 1.73–1.18 (m, 6H), 1.13–1.00 (m, 24H), 0.92 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 212.14, 67.88, 51.26, 49.78, 42.79, 41.85, 39.20, 37.60, 37.55, 28.59, 26.51, 18.05, 17.02, 12.01, 10.58. **FTIR** (neat), cm^{-1}: 2945 (s), 2867 (s), 1716 (s), 1464 (m). **HRMS** (ESI): Calcd for ($C_{22}H_{42}O_2Si$+H^+): 367.30268, found: 367.30273. $\boldsymbol{\alpha_D^{RT}}$ = +36.7 (c = 1.0 in $CHCl_3$).

(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((triisopropylsilyl)oxy)propan-2-yl)octahydro-1*H*-inden-5-ol (221)

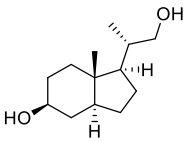


220 **221**

The compound was prepared from **220** according to the sodium borohydride reduction protocol described above (page 151) in 66% yield.

TLC (30% ethyl acetate in petroleum ether): $\mathbf{R_f}$ = 0.48 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 3.66 (dd, *J* = 9.4, 3.4 Hz, 1H) overlapping with 3.62 (m, 1H), 3.37 (dd, *J* = 9.3, 7.1 Hz, 1H), 1.99–1.70 (m, 4H), 1.60–1.08 (m, 10H), 1.06 (m, 21H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 71.76, 68.08, 51.80, 48.46, 41.92, 39.34, 37.41, 35.16, 31.60, 28.25, 25.97, 18.06, 16.98, 12.05, 11.22. **HRMS** (ESI): Calcd for ($C_{22}H_{44}O_2Si+H^+$): 369.31833, found: 369.31828. $\boldsymbol{\alpha_D^{RT}}$ = +14.5 (c = 1.0 in $CHCl_3$).

(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-ol (240)



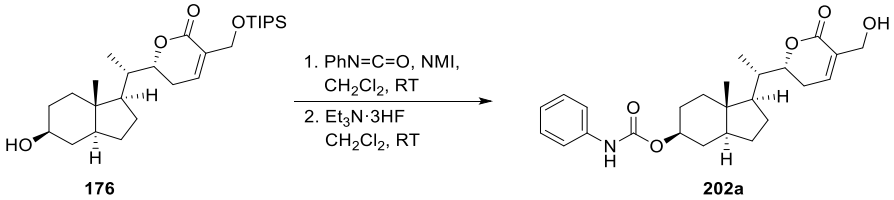
The product was synthesized according to the general TBS-deprotection protocol (page 148) from **221**. The yield was not determined.

Colourless oil. **TLC** (50% ethyl acetate in dichloromethane): R_f = 0.21 (CAM). **^{1}H NMR** (CD_2Cl_2, 300 MHz) δ: 3.57 (dd, *J* = 10.5, 3.3 Hz, 1H) overlapping with 3.55 (m, 1H), 3.28 (dd, *J* = 10.4, 6.9 Hz, 1H), 1.93–1.66 (m, 4H), 1.60–1.06 (m, 11H), 0.99 (d, *J* = 6.6 Hz, 1H), 0.71 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 75 MHz) δ: 72.04, 68.14, 52.25, 48.97, 42.45, 39.41, 37.92, 35.71, 32.15, 28.70, 26.43, 16.99, 11.48. **FTIR** (neat), cm^{-1}: 3312 (br), 2931 (m), 2866 (m), 1469 (w), 1444 (w), 1024 (s).

5.3.2 Synthesis of Withanolide Analogues

Representative Procedure for Carbamate Formation with Isocyanates

(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl phenylcarbamate (202a)



176 **202a**

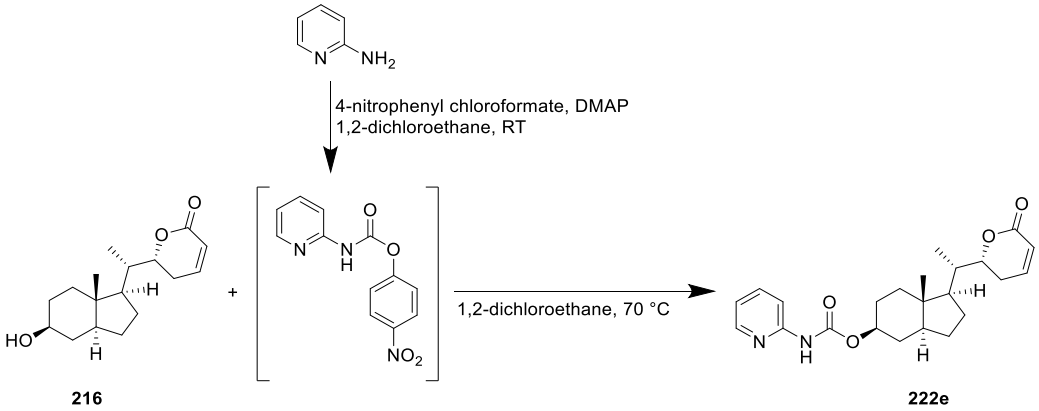
To a 23 °C solution of alcohol **176** (20 mg, 43 µmol, 1 equiv) and *N*-methylimidazole (0.7 µL, 8.6 µmol, 0.2 equiv) in dichloromethane (1.1 ml) was added phenyl isocyanate (9.4 µL, 86 µmol, 2 equiv) and the resulting clear solution was stirred for 18 h at ambient temperature. The reaction mixture was poured into a saturated aqueous solution of sodium bicarbonate (2 ml) and the product mixture was extracted into dichloromethane (2 × 30 ml). The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered and the filtrate was concentrated in vacuo. The obtained residue was filtered over a short plug of silica eluting with 10% ethyl acetate in petroleum ether to provide the crude, protected carbamate product (semisolid material), which was dissolved in dichloromethane (1 ml) and triethylamine-trihydrofluoride (200 µL). The mixture was stirred for 22 h at ambient temperature. Then, a saturated aqueous solution of sodium bicarbonate (2 ml) was added and the reaction mixture was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue was purified by flash-column chromatography (gradient elution with 30–50% ethyl acetate in petroleum ether) to provide carbamate **202a** (11 mg, 60%).

Depending on the reactivity of the amine, the number of equivalents and the reaction temperature were varied. For alkyl isocyanates more equivalents and a temperature of 60 °C in 1,2-dichloroethane is necessary.

Amorphous solid**. TLC** (55% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.41–7.26 (m, 4H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.85 (d, *J* = 5.6 Hz, 1H), 6.57 (s, 1H), 4.70 (m, 1H), 4.50 (dt, *J* = 13.1, 3.5 Hz, 1H), 4.33 (d, *J* = 14.4 Hz, 1H), 4.29 (d, *J* = 14.4 Hz, 1H), 2.43 (m, 1H), 2.19 (m, 1H), 2.10–1.52 (m, 8H), 1.47–1.09 (m, 6H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.79 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.93, 153.10, 140.72, 137.94, 131.30, 129.03, 123.29, 118.48, 80.27, 74.50, 61.72, 51.14, 48.00, 42.20, 38.89, 37.13, 31.35, 27.67, 27.55, 25.69, 23.05, 13.34, 10.89. **FTIR** (neat), cm^{-1}: 3322 (br), 2946 (m), 1700 (s), 1539 (m), 1224 (s). **HRMS** (ESI): Calcd for ($C_{25}H_{33}NO_5$+H^+): 428.2432, found: 428.2427. $\boldsymbol{\alpha_D^{RT}}$ = +43.78 (c = 0.41 in $CHCl_3$).

Representative Procedure for Synthesis of 2-Pyridylcarbamates

(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl pyridin-2-ylcarbamate (222e)



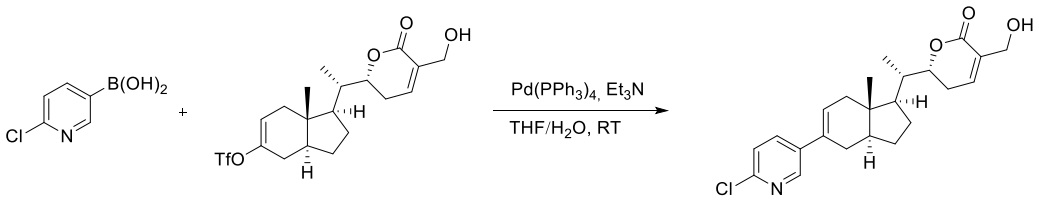
216 **222e**

4-dimethylaminopyridine (9.6 mg, 79 µmol, 1.5 equiv), 2-aminopyridin (37 mg, 393 µmol, 7.5 equiv) and 4-nitrophenyl chloroformate (64 mg, 315 µmol, 6 equiv) were suspended in 1,2-dichloroethane (2 ml). After 30 min, the suspension was added to a solution of alcohol **216** (14.6 mg, 52 µmol, 1 equiv) in 1 ml 1,2-dichloroethane at 70 °C. After 5 min, the reaction mixture was diluted with dichloromethane (50 ml) and washed with saturated aqueous solution of sodium chloride (30 ml). The separated aqueous layer was extracted with dichloromethane (25 ml). The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 0–30% ethyl acetate in dichloromethane) to provide carbamate (**222e**, 19.8 mg, 95%).

White solid. **TLC** (15% ethyl acetate in petroleum ether): R_f = 0.41 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 9.01 (br s, 1H), 8.31 (d, *J* = 4.4 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.70 (m, 1H), 6.99 (dd, *J* = 6.7, 5.5 Hz, 1H), 6.91 (m, 1H), 6.00 (dd, *J* = 9.7, 2.2 Hz, 1H), 4.73 (m, 1H), 4.49 (dt, *J* = 12.9, 3.5 Hz, 1H), 2.37 (m, 1H), 2.10–1.06 (m, 13H) , 2.15 (ddd, *J* = 18.3, 6.3, 3.5 Hz, 1H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.80 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 164.82, 153.03, 152.05, 147.17, 145.41, 138.65, 121.29, 118.45, 112.54, 80.04, 74.82, 51.27, 48.04, 42.22, 39.02, 37.17, 31.34, 27.67, 27.55, 25.68, 23.07, 13.33, 10.90. **FTIR** (neat), cm^{-1}: 1720 (vs), 1587 (s), 1541 (s), 1439 (s), 1223 (vs). **HRMS** (ESI): Calcd for ($C_{23}H_{30}N_2O_4$+H^+): 399.22783, found: 399.22628. $\boldsymbol{\alpha_D^{RT}}$ = +62.7 (c = 1.0 in $CHCl_3$).

Representative Procedure for Coupling of Enol Triflate 177 with Boronic Acids

(*R*)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-5-(6-chloropyridin-3-yl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)ethyl)-3-(hydroxymethyl)-5,6-dihydro-2*H*-pyran-2-one (<u>198o</u>)



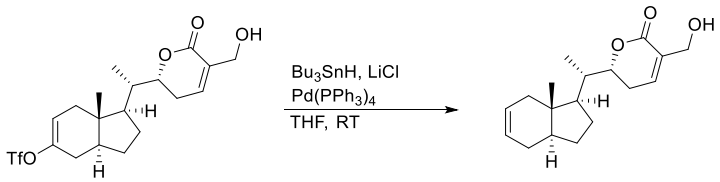
177 **198o**

Palladium tetrakis(triphenylphosphine) (3.3 mg, 2.85 µmol, 0.05 equiv) was added to a vigorously stirred solution of enol triflate **177** (25 mg, 57 µmol, 1 equiv), 6-chloro-3-pyridinylboronic acid (18 mg, 114 µmol, 2 equiv), and triethylamine (39.8 µL, 285 µmol, 5 equiv) in tetrahydrofuran-water solvent mixture (6:1, 1.4 ml) at ambient temperature. After 5 min, the reaction mixture was diluted with dichloromethane and washed with a saturated aqueous solution of ammonium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 30–60% ethyl acetate in petroleum ether) to provide the coupling product **198o** (18.7 mg, 82%).

TLC (80% ethyl acetate in petroleum ether): R_f = 0.42 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.41 (d, *J* = 2.5 Hz, 1H), 7.67 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 5.6 Hz, 1H), 6.10 (m, 1H), 4.53 (dt, *J* = 13.1, 3.6 Hz, 1H), 4.34 (d, *J* = 14.9 Hz, 1H), 4.31 (d, *J* = 14.9 Hz, 1H), 2.86 (br s, 1H), 2.53–2.38 (m, 3H), 2.27–2.04 (m, 4H), 1.85–1.68 (m, 3H), 1.52–1.23 (m, 3H), 1.08 (d, *J* = 6.7 Hz, 3H), 0.73 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.81, 148.77, 145.76, 140.49, 136.32, 135.58, 131.85, 131.47, 126.99, 123.88, 80.33, 61.67, 51.76, 45.46, 42.02, 41.06, 38.85, 29.78, 27.38, 26.48, 23.12, 13.09, 11.10. **FTIR** (neat), cm^{-1}: 3393 (br), 2922 (br), 1704 (vs), 1681 (vs). **HRMS** (ESI): Calcd for ($C_{23}H_{28}ClNO_3$+H^+): 402.18305, found: 402.18245, calcd for ($C_{23}H_{28}{}^{37}ClNO_3$+$H^+$): 404.18010, found: 404.17953. $\boldsymbol{\alpha_D^{RT}}$ = +65.1 (c = 1.0 in $CHCl_3$).

Reductive Elimination of Enol Triflate 177

(*R*)-3-(hydroxymethyl)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (204)



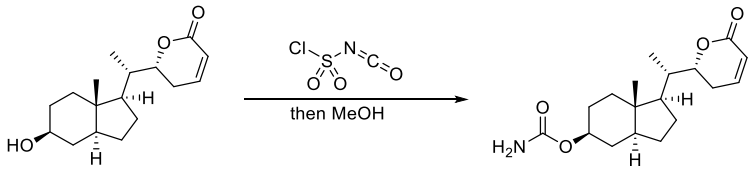
177 **204**

To a solution of enol triflate **177** (19.4 mg, 44.3 µmol, 1 equiv) in tetrahydrofuran (2 ml) at ambient temperature were added lithium chloride (5.6 mg, 132 µmol, 3 equiv), tetrakis(triphenylphosphine) palladium(0) (2.5 mg, 2.2 µmol, 0.05 equiv) and at last tributyltin hydride (23.8 µl, 88.5 mmol, 2 equiv). After 5 min, the mixture was diluted with dichloromethane and washed with saturated aqueous solution of sodium chloride. The separated aqueous layer was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 5–80% ethyl acetate in petroleum ether) to provide the product (12.6 mg, 98%).

White amorphous solid. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.45 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.85 (d, *J* = 6.2 Hz, 1H), 5.63 (m, 1H), 5.56 (m, 1H), 4.52 (dt, *J* = 13.2, 3.6 Hz, 1H), 4.33 (d, *J* = 15.0 Hz, 1H), 4.30 (d, *J* = 15.0 Hz, 1H), 2.44 (m, 1H), 2.38–1.10 (m, 13H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.68 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.96, 140.69, 131.38, 126.17, 126.02, 80.55, 61.76, 52.04, 44.95, 41.83, 41.33, 38.88, 28.21, 27.08, 26.63, 23.07, 13.04, 10.79. **FTIR** (neat), cm^{-1}: 2891 (br), 1701 (vs), 1397 (m), 1129 (s), 1042 (s). **HRMS** (ESI): Calcd for ($C_{18}H_{26}O_3$+H^+): 291.19547, found: 291.19489. $\boldsymbol{\alpha_D^{RT}}$ = +127.7 (c = 0.6 in $CHCl_3$).

Carbamoylation Procedure

(1*R*,3a*S*,5S,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxo-3,6-dihydro-2H-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl carbamate (222o)



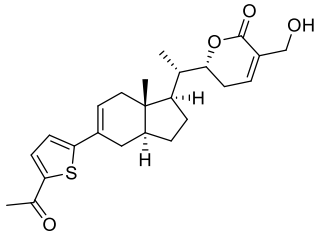
216 **222o**

To a solution of alcohol **216** (14.6 mg, 52.4 µmol, 1 equiv) in dichloromethane (1 ml) was added a solution of chlorosulfonyl isocyanate (6.85 µl, 78.7 µmol, 1.5 equiv) as a solution in dichloromethane (500 µl). The resulting solution was stirred for 5 min at ambient temperature. Methanol (300 µl) was added and stirring was continued for several more minutes. The reaction mixture was poured into a saturated aqueous solution of sodium chloride and the product mixture was extracted into dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (gradient elution with 0–6% methanol in ethyl acetate) to provide carbamate **222o** (16 mg, 95%).

Colourless oil. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.92 (m, 1H), 6.01 (dd, *J* = 9.7, 2.4 Hz, 1H), 4.72 (m, 1H), 4.48 (dd, *J* = 12.8, 3.3 Hz), 4.04 (s, 2H, *NH_2*), 2.37 (m, 1H), 2.23–0.81 (m, 14H) overlapping with 1.01 (d, *J* = 6.6 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.04, 149.75, 145.58, 121.22, 71.36, 51.10, 47.81, 42.08, 38.94, 36.90, 30.87, 27.59, 27.09, 25.55, 23.01, 13.33, 10.86. **FTIR** (neat), cm^{-1}: 2949 (br), 1694 (s), 1461 (m), 1378 (s), 1166 (s).**HRMS** (ESI): Calcd for ($C_{18}H_{27}NO_4$+H^+): 322.20128, found: 322.20163. $\boldsymbol{\alpha_D^{RT}}$ = +42.1 (c = 1.0 in CH_2Cl_2).

5.3.3 Analytical Characterization of Withanolide Analogues

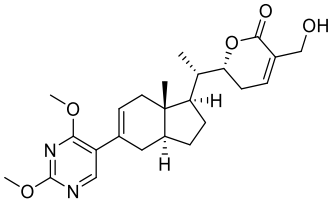
(*R*)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-5-(5-acetylthiophen-2-yl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)ethyl)-3-(hydroxymethyl)-5,6-dihydro-2*H*-pyran-2-one (198p)



The product was synthesized according to the general Suzuki coupling procedure (page 163) from **177** in 95% yield.

TLC (80% ethyl acetate in petroleum ether): R_f = 0.45 (CAM). **^{1}H NMR** (CD_2Cl_2, 100 MHz) δ: 7.54 (d, *J* = 4.0 Hz, 1H), 6.97 (d, *J* = 4.0 Hz, 1H), 6.84 (d, *J* = 5.8 Hz, 1H), 6.28 (m, 1H), 4.49 (dt, *J* = 13.1, 3.6 Hz, 1H), 4.26 (d, *J* = 15.0 Hz, 1H), 4.22 (d, *J* = 15.0 Hz, 1H), 2.46 (s, 3H) overlapping a m (3H), 2.25–1.95 (m, 4H), 1.83–1.66 (m, 3H), 1.50–1.20 (m, 4H), 1.04 (d, *J* = 6.7 Hz, 3H), 0.71 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 190.91, 166.25, 155.16, 141.70, 141.13, 133.69, 131.81, 130.84, 127.80, 122.80, 80.82, 61.98, 52.18, 45.78, 42.46, 41.75, 39.42, 30.51, 27.80, 26.95, 26.79, 23.57, 13.36, 11.43. **FTIR** (neat), cm^{-1}: 3376 (br), 1713 (vs), 1622 (vs), 1287 (vs), 1044 (vs). **HRMS** (ESI): Calcd for ($C_{24}H_{30}O_4S+H^+$): 415.19376, found: 415.19339. α_D^{RT} = +120.3 (c = 1.0 in $CHCl_3$).

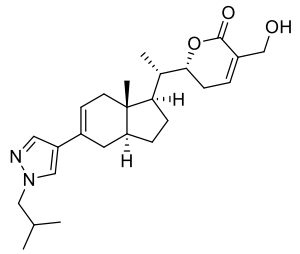
(*R*)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-5-(2,4-dimethoxypyrimidin-5-yl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)ethyl)-3-(hydroxymethyl)-5,6-dihydro-2*H*-pyran-2-one (198q)



The product was synthesized according to the general Suzuki coupling procedure (page 163) from **177** in 98% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.3 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 8.04 (s, 1H), 6.86 (d, *J* = 5.9 Hz, 1H), 5.76 (d, *J* = 4.9 Hz, 1H), 4.52 (dt, *J* = 13.0, 3.4 Hz, 1H), 4.33 (d, *J* = 14.4 Hz, 1H), 4.30 (d, *J* = 14.4 Hz, 1H), 3.98 (s, 3H), 3.97 (s, 3H), 2.52–2.00 (m, 7H), 1.80–1.62 (m, 3H), 1.48–1.12 (m, 4H), 1.06 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 168.33, 165.90, 163.87, 155.80, 140.61, 131.34, 130.63, 127.12, 117.97, 80.42, 61.60, 54.72, 53.92, 51.78, 45.39, 41.92, 40.97, 38.83, 30.83, 27.35, 26.45, 23.04, 13.04, 11.05. **FTIR** (neat), cm^{-1}: 3369 (br), 2923 (br), 1708 (s), 1467 (s), 1393 (vs). **HRMS** (ESI): Calcd for ($C_{24}H_{32}N_2O_5$ +H^+): 429.23840, found: 429.23787. $\boldsymbol{\alpha_D^{RT}}$ = +76.3 (c = 1.0 in $CHCl_3$).

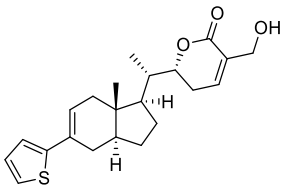
(*R*)-3-(hydroxymethyl)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-5-(1-isobutyl-1*H*-pyrazol-4-yl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (198r)



The product was synthesized according to the general Suzuki coupling procedure (page 163) from **177** in 87% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.25 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.55 (s, 1H), 7.30 (s, 1H), 6.86 (d, *J* = 6.0 Hz, 1H), 5.86 (m, 1H), 4.52 (dt, *J* = 13.1, 3.5 Hz, 1H), 4.33 (d, *J* = 14.9 Hz, 1H), 4.30 (d, *J* = 14.9 Hz, 1H), 3.87 (d, *J* = 7.2 Hz, 2H), 2.53–1.95 (m, 8H), 1.80–1.65 (m, 3H), 1.50–1.15 (m, 4H), 1.06 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 6H), 0.71 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.89, 140.56, 135.49, 131.41, 127.55, 125.31, 123.84, 119.89, 80.46, 61.59, 59.72, 51.84, 45.29, 41.62, 41.41, 38.88, 30.35, 29.65, 27.35, 26.58, 23.08, 19.92, 13.02, 10.98. **FTIR** (neat), cm^{-1}: 3162 (br), 2956 (br), 1717 (vs), 1396 (s). **HRMS** (ESI): Calcd for ($C_{25}H_{36}N_2O_3$+H^+): 413.27987, found: 413.27934. $\boldsymbol{\alpha_D^{RT}}$ = +93.3 (c = 1.0 in $CHCl_3$).

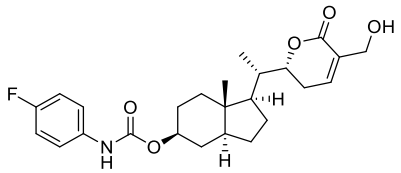
(*R*)-3-(hydroxymethyl)-6-((*S*)-1-((1*R*,3a*S*,7a*S*)-7a-methyl-5-(thiophen-2-yl)-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (198s)



The product was synthesized according to the general Suzuki coupling procedure (page 163) from **177** in 84% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.4 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.10 (dd, *J* = 4.7, 1.3 Hz, 1H), 6.96 (m, 2H), 6.86 (d, *J* = 5.8 Hz, 1H), 6.07 (d, *J* = 5.3 Hz, 1H), 4.53 (dt, *J* = 13.1, 3.6 Hz 1H), 4.33 (d, *J* = 14.8 Hz, 1H), 4.30 (d, *J* = 14.8 Hz, 1H), 2.60–1.20 (m, 14H), 1.07 (d, *J* = 6.7 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.90, 146.32, 140.64, 131.39, 130.38, 127.17, 123.10, 122.86, 121.26, 80.43, 61.69, 51.78, 45.40, 41.78, 41.40, 38.86, 30.34, 27.40, 26.51, 23.09, 13.03, 11.05. **FTIR** (neat), cm^{-1}: 3401 (br), 2936 (br), 1703 (vs), 1392 (m), 1045 (m). **HRMS** (ESI): Calcd for ($C_{22}H_{28}O_3S$+H^+): 373.18319, found: 373.18325. $\boldsymbol{\alpha_D^{RT}}$ = +107.4 (c = 1.0 in $CHCl_3$).

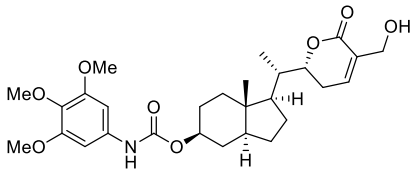
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (4-fluorophenyl)carbamate (202b)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 77% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.27 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.32 (m, 2H), 6.99 (t, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 5.9 Hz, 1H), 6.56 (s, 1H), 4.69 (m, 1H), 4.50 (dt, *J* = 13.1, 3.5 Hz, 1H), 4.33 (d, *J* = 14.4 Hz, 1H), 4.29 (d, *J* = 14.4 Hz, 1H), 2.43 (m, 1H) overlapping a broad s(1H), 2.19 (ddd, *J* = 18.3, 6.3, 3.5 Hz, 1H), 2.10–1.05 (m, 13H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.78 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.91, 158.86 (d, *J* = 242.6 Hz), 153.28, 140.69, 133.94, 131.31, 120.32, 115.62 (d, *J* = 22.5 Hz), 80.26, 74.61, 61.69, 51.13, 47.98, 42.19, 38.88, 37.11, 31.34, 27.65, 27.54, 25.68, 23.04, 13.33, 10.88. **FTIR** (neat), cm^{-1}: 2949 (br), 1510 (s), 1213 (vs), 1059 (s), 730 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{32}FNO_5$+H^+): 446.23373, found: 446.23350. $\boldsymbol{\alpha_D^{RT}}$ = +26.7 (c = 1.0 in $CHCl_3$).

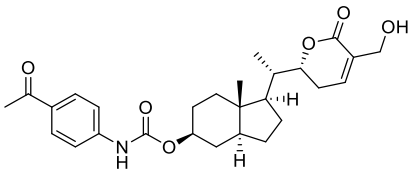
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (3,4,5-trimethoxyphenyl)carbamate (202c)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 97% yield.

Brown amorphous solid. **TLC** (80% ethyl acetate in petroleum ether): R_f = 0.34 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.85 (d, *J* = 6.0 Hz, 1H), 6.67 (s, 2H), 6.60 (s, 1H), 4.67 (m, 1H), 4.49 (dt, *J* = 12.7, 3.3 Hz, 1H), 4.32 (d, *J* = 14.2 Hz, 1H), 4.29 (d, *J* = 14.2 Hz, 1H), 3.83 (s, 6H), 3.79 (s, 3H), 2.58 (br s, 1H (OH)), 2.43 (m, 1H), 2.19 (ddd, *J* = 18.2, 6.0, 3.3 Hz, 1H), 2.60–0.8 (m, 14H) 1.02 (d, *J* = 6.6 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.90, 153.34, 153.18, 140.68, 134.12, 133.75, 131.26, 95.98, 80.23, 74.56, 61.63, 60.95, 55.97, 51.08, 47.97, 42.16, 38.85, 37.08, 31.32, 27.62, 27.52, 25.65, 23.02, 13.32, 10.87. **FTIR** (neat), cm^{-1}: 3331 (br), 2942 (br), 1699 (vs), 1218 (vs), 1122 (vs). **HRMS** (ESI): Calcd for ($C_{28}H_{39}NO_8$+H^+): 518.27484, found: 518.27525. $\boldsymbol{\alpha_D^{RT}}$ = +28.4 (c = 1.0 in $CHCl_3$).

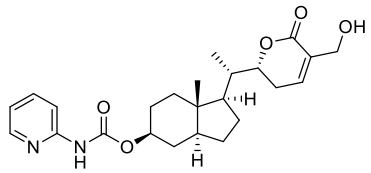
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (4-acetylphenyl)carbamate (202d)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 96% yield.

White crystalline solid. **TLC** (80% ethyl acetate in petroleum ether): R_f = 0.40 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.92 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 8.6 Hz, 2H), 6.98 (s, 1H), 6.86 (d, *J* = 5.95 Hz, 1H), 4.71 (m, 1H), 4.49 (dt, *J* = 13.0, 3.5 Hz, 1H), 4.33 (d, *J* = 14.0 Hz, 1H), 4.29 (d, *J* = 14.0 Hz, 1H), 2.56 (s, 3H) overlapping with a br s (1H), 2.42 (m, 1H), 2.19 (ddd, *J* = 18.2, 6.2, 3.5 Hz), 2.11–1.83 (m, 4H), 1.80–1.07 (m, 9H), 1.02 (d, *J* = 6.62 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 196.95, 165.91, 152.67, 142.53, 140.71, 131.95, 131.24, 129.83, 117.46, 80.23, 74.98, 61.58, 51.06, 47.91, 42.14, 38.83, 37.04, 31.24, 27.61, 27.45, 26.37, 25.63, 23.01, 13.32, 10.86 **FTIR** (neat), cm^{-1}: 3303 (br), 2946 (m), 1719 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{35}NO_6$+H^+): 470.25371, found: 470.25364. $\boldsymbol{\alpha_D^{RT}}$ = +38.1 (c = 1.0 in $CHCl_3$).

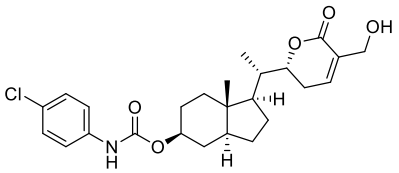
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl pyridin-2-ylcarbamate (202e)



The product was synthesized according to the general procedure for the synthesis of 2-pyridylcarbamates (page 162) from **176** in 63% yield.

TLC (80% ethyl acetate in petroleum ether): R_f = 0.31 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 8.96 (s, 1H), 8.30 (d, *J* = 4.8 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.68 (m, 1H), 6.98 (dd, *J* = 6.8, 5.5 Hz, 1H), 6.83 (d, *J* = 6.0 Hz, 1H), 4.71 (m, 1H), 4.47 (dt, *J* = 13.1, 3.5, 3.5 Hz, 1H), 4.25 (d, *J* = 14.9 Hz, 1H), 4.22 (d, *J* = 15.1 Hz, 1H), 2.52 (br s, 1H (OH)) overlapping with 2.39 (m, 1H), 2.18 (ddd, *J* = 18.2, 6.3, 3.6 Hz, 1H), 2.10–1.08 (m, 13H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.80 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 166.24, 153.55, 152.90, 148.26, 141.08, 138.86, 131.85, 118.93, 112.70, 80.74, 75.27, 62.02, 51.70, 48.54, 42.74, 39.52, 37.69, 31.89, 28.14, 26.27, 23.58, 13.65, 11.24. **FTIR** (neat), cm^{-1}: 2947 (br), 1727 (s), 1698 (s), 1591 (s), 1222 (vs). **HRMS** (ESI): Calcd for ($C_{24}H_{32}N_2O_5$+H^+): 429.23840, found: 429.23775. $\boldsymbol{\alpha_D^{RT}}$ = +49.8 (c = 0.82 in $CHCl_3$).

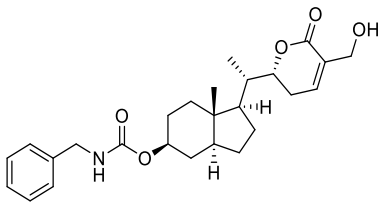
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (4-chlorophenyl)carbamate (202f)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 82% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.44 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.32 (d, *J* = 8.5 Hz, 2H), 7.25 (m, 2H), 6.85 (d, *J* = 5.8 Hz, 1H), 6.65 (s, 1H), 4.69 (m, 1H), 4.49 (dt, *J* = 13.1, 3.5 Hz, 1H), 4.33 (d, *J* = 14.5 Hz, 1H), 4.30 (d, *J* = 14.6 Hz, 1H), 2.50 (br s, 1H), 2.43 (ddd, *J* = 17.7, 13.2, 1.6 Hz, 1H), 2.19 (ddd, *J* = 18.2, 6.2, 3.3 Hz, 1H), 2.10–1.05 (m, 13H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.78 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.87, 153.03, 140.62, 136.61, 131.34, 128.98, 128.24, 119.75, 80.24, 74.75, 61.63, 51.14, 47.98, 42.18, 38.88, 37.12, 31.32, 27.65, 27.52, 25.67, 23.06, 13.32, 10.88. **FTIR** (neat), cm^{-1}: 3317 (br), 2947 (br), 1697 (vs), 1219 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{32}ClNO_5$+H^+): 462.20418, found: 462.20420, calcd for ($C_{25}H_{32}{}^{37}ClNO_5$+$H^+$): 464.20123, found: 464.20229. $\boldsymbol{\alpha_D^{RT}}$= +34.0 (c = 1.0 in $CHCl_3$).

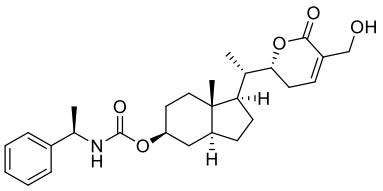
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl benzylcarbamate (202g)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 85% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.34 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.30 (m, 5H), 6.85 (d, *J* = 5.9 Hz, 1H), 4.96 (m, 1H), 4.63 (m, 1H), 4.49 (dt, *J* = 13.1, 3.5 Hz, 1H), 4.35 (d, *J* = 5.6 Hz, 2H), 4.30 (m, 2H), 2.50 (br s, 1H (OH)), 2.42 (m, 1H), 2.18 (ddd, *J* = 18.3, 6.3, 3.5 Hz, 1H), 2.10–1.05 (m, 13H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.90, 156.25, 140.67, 138.53, 131.26, 128.59, 127.44, 127.39, 80.25, 74.14, 61.61, 51.10, 47.98, 44.94, 42.16, 38.85, 37.13, 31.40, 27.64, 27.59, 25.66, 23.01, 13.30, 10.85. **FTIR** (neat), cm^{-1}: 3340 (s), 2945 (m), 1519 (m), 1693 (vs), 1234 (vs). **HRMS** (ESI): Calcd for ($C_{26}H_{35}NO_5$+H^+): 442.25880, found: 442.25880. $\boldsymbol{\alpha_D^{RT}}$= +35.4 (c = 1.0 in $CHCl_3$).

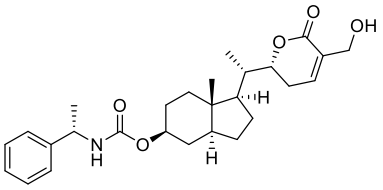
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl ((*R*)-1-phenylethyl)carbamate (202h)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 76% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.36–7.21 (m, 5H), 6.84 (d, *J* = 5.9 Hz, 1H), 4.89 (br m, 1H), 4.82 (br s, 1H), 4.57 (m, 1H), 4.48 (dt, *J* = 13.1, 3.5 Hz, 1H), 4.32 (d, *J* = 14.9 Hz, 1H), 4.29 (d, *J* = 14.9 Hz, 1H), 2.42 (ddd, *J* = 18.0, 13.1, 1.7 Hz, 1H) overlapping a br s(1H), 2.17 (ddd, *J* = 18.0, 6.4, 3.5 Hz, 1H), 2.07–1.04 (m, 13H) overlapping with 1.47 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.84, 155.42, 143.73, 140.54, 131.40, 128.57, 127.22, 125.87, 80.27, 74.04, 61.64, 51.18, 50.55, 48.04, 42.21, 38.91, 37.20, 31.43, 27.68, 25.68, 23.07, 22.52, 13.30, 10.86. **FTIR** (neat), cm^{-1}: 3326 (br), 2946 (br), 1695 (vs), 1234 (s), 750 (vs). **HRMS** (ESI): Calcd for ($C_{27}H_{37}NO_5$+H^+): 456.27445, found: 456.27455. $\boldsymbol{\alpha_D^{RT}}$ = +53.2 (c = 1.0 in $CHCl_3$).

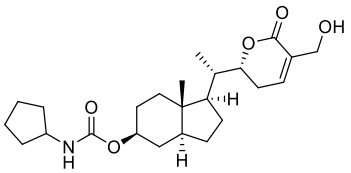
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl ((*S*)-1-phenylethyl)carbamate (202i)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 72% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.27 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.36–7.22 (m, 5H), 6.84 (d, *J* = 6.0 Hz, 1H), 4.89 (m, 1H), 4.81 (br s, 1H), 4.57 (m, 1H), 4.48 (dt, *J* = 13.1, 3.4 Hz, 1H), 4.32 (d, *J* = 14.8 Hz, 1H), 4.29 (d, *J* = 14.8 Hz, 1H), 2.41 (m, 1H) overlapping br s(1H), 2.18 (ddd, *J* = 18.2, 6.3, 3.4 Hz, 1H), 1.47 (d, *J* = 6.8 Hz, 3H) overlapping with 2.10–1.05 (m, 13H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.88, 140.62, 131.35, 128.58, 127.23, 125.88, 80.28, 74.00, 61.66, 51.15, 50.53, 48.01, 42.19, 38.90, 37.17, 31.40, 27.67, 27.60, 25.68, 23.05, 22.49, 13.30, 10.85. **FTIR** (neat), cm^{-1}: 3337 (br), 2945 (br), 1694 (vs), 1234 (s), 1047 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{37}NO_5$+H^+): 456.27445, found: 456.27445. $\boldsymbol{\alpha_D^{RT}}$ = +13.9 (c = 1.0 in $CHCl_3$).

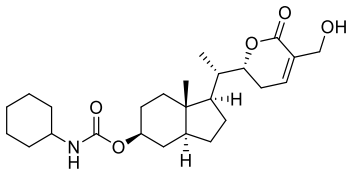
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl cyclopentylcarbamate (202j)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 73% yield.

TLC (80% ethyl acetate in petroleum ether): R_f = 0.35 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 6.82 (d, *J* = 6.2 Hz, 1H), 4.61 (br s, 1H) overlapping with 4.52 (m, 1H) overlapping with 4.45 (dd, *J* = 13.1, 3.5 Hz, 1H), 4.24 (d, *J* = 15.0 Hz, 1H), 4.20 (d, *J* = 15.8 Hz, 1H), 3.88 (1, *J* = 6.5 Hz, 1H), 2.47 (br s, 1H (OH)) overlapping with 2.38 (ddd, *J* = 17.8, 13.2, 1.8 Hz, 1H), 2.16 (ddd, *J* = 18.3, 6.4, 3.6 Hz, 1H), 2.05–1.05 (m, 21H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 166.26, 156.14, 141.10, 131.83, 80.77, 74.09, 62.03, 53.16, 51.70, 48.55, 42.73, 39.52, 37.73, 33.71, 32.04, 28.26, 28.14, 26.29, 24.02, 23.57, 13.62, 11.20. **FTIR** (neat), cm^{-1}: 3295 (br), 2945 (br), 1703 (vs), 1681 (vs), 1542 (m). **HRMS** (ESI): Calcd for ($C_{24}H_{37}NO_5$+H$^+$): 420.27445, found: 420.27425. $\boldsymbol{\alpha_D^{RT}}$= +53.9 (c = 1.0 in $CHCl_3$).

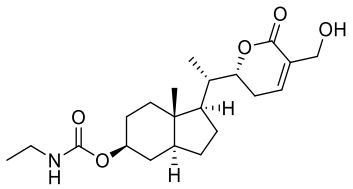
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl cyclohexylcarbamate (202k)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 74% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.29 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.84 (d, *J* = 6.0 Hz, 1H), 4.57 (m, 1H), 4.49 (dd, *J* = 13.0, 3.5 Hz, 1H) overlapping a br s(1H), 4.32 (d, *J* = 15.0 Hz, 1H), 4.29 (d, *J* = 14.8 Hz, 1H), 3.45 (m, 1H), 2.42 (m, 1H), 2.18 (ddd, *J* = 18.3, 6.3, 3.7 Hz, 1H) overlapping a broad s(1H), 2.10–1.05 (m, 23H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.85, 155.43, 140.55, 131.41, 80.29, 73.63, 61.64, 51.21, 49.67, 48.06, 42.23, 38.92, 37.24, 33.43, 31.49, 27.69, 27.68, 25.71, 25.50, 24.75, 23.08, 13.31, 10.87. **FTIR** (neat), cm^{-1}: 2929 (m), 2928 (m), 1692 (vs), 1550 (m), 1041 (s). **HRMS** (ESI): Calcd for ($C_{25}H_{39}NO_5$+H$^+$): 434.29010, found: 434.28998. $\boldsymbol{\alpha_D^{RT}}$= +40.3 (c = 0.9 in $CHCl_3$).

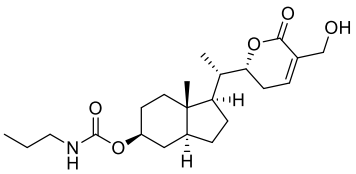
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl ethylcarbamate (202l)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 68% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.22 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.85 (d, *J* = 5.9 Hz, 1H), 4.57 (m, 1H), 4.49 (dd, *J* = 13.2, 3.6 Hz, 1H) overlapping a br s(1H), 4.32 (d, *J* = 14.8 Hz, 1H), 4.29 (d, *J* = 13.8 Hz, 1H), 3.19 (q, *J* = 6.9 Hz, 2H), 2.42 (ddd, *J* = 17.8, 13.2, 1.6 Hz, 1H), 2.18 (m, 1H), 2.13–1.15 (m, 14H), 1.12 (t, *J* = 7.2 Hz, 3H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.89, 156.15, 140.64, 131.35, 80.29, 73.87, 61.66, 51.18, 48.04, 42.21, 38.91, 37.21, 35.84, 31.48, 27.69, 27.66, 25.70, 23.07, 15.25, 13.32, 10.87. **FTIR** (neat), cm^{-1}: 3307 (br), 2936 (br), 1687 (vs), 1261 (s). **HRMS** (ESI): Calcd for ($C_{21}H_{33}NO_5$+H^+): 380.24315, found: 380.24331. $\boldsymbol{\alpha_D^{RT}}$= +58.3 (c = 0.5 in $CHCl_3$).

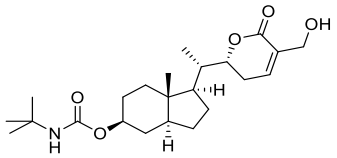
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl propylcarbamate (202m)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 86% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.34 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.85 (d, *J* = 6.1 Hz, 1H), 4.64–4.52 (m, 2H), 4.49 (dt, *J* = 13.0, 3.5 Hz, 1H), 4.32 (d, *J* = 14.28 Hz, 1H), 4.28 (d, *J* = 14.64 Hz, 1H), 3.12 (q, *J* = 6.4 Hz, 2H), 2.61 (s, br, 1H), 2.42 (m, 1H), 2.18 (ddd, *J* = 18.2, 6.3, 3.5 Hz, 1H), 2.10–1.00 (m, 15H), 1.01 (d, *J* = 6.63 Hz, 3H), 0.90 (t, *J* = 7.40 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 165.91, 156.24, 140.66, 131.27, 80.26, 73.71, 61.61, 51.11, 47.99, 42.58, 42.17, 38.86, 37.15, 31.43, 27.65, 27.62, 25.67, 23.18, 23.01, 13.30, 11.20, 10.85. **FTIR** (neat), cm^{-1}: 3305 (br), 2960 (m), 2959 (w), 1682 (s), 1534 (m). **HRMS** (ESI): Calcd for ($C_{22}H_{35}NO_5$+H^+): 394.25880, found: 394.25785. $\boldsymbol{\alpha_D^{RT}}$= +55.9 (c = 1.0 in $CHCl_3$).

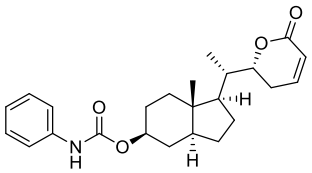
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl *tert*-butylcarbamate (202n)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **176** in 92% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.40 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 6.84 (d, *J* = 5.6 Hz, 1H), 4.57 (m, 2H), 4.48 (dd, *J* = 13.1, 3.6 Hz), 4.31 (d, *J* = 15.0 Hz, 1H), 4.28 (d, *J* = 15.0 Hz, 1H), 2.42 (m, 1H) overlapping a br s (1H), 2.18 (ddd, *J* = 18.2, 6.4, 3.6 Hz), 1.30 (s, 9H) overlapping a m (13H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 165.83, 154.73, 140.52, 131.40, 80.28, 73.23, 61.62, 51.19, 50.23, 48.10, 42.23, 38.92, 37.26, 31.50, 28.98, 27.69, 27.67, 25.71, 23.08, 13.30, 10.87. **FTIR** (neat), cm^{-1}: 3368 (br), 2951 (br), 1699 (vs), 1267 (m). **HRMS** (ESI): Calcd for ($C_{23}H_{37}NO_5$+H^+): 408.27445, found: 408.27423. $\boldsymbol{\alpha_D^{RT}}$ = +39.9 (c = 1.0 in $CHCl_3$).

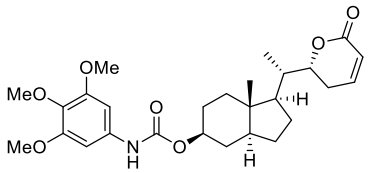
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl phenylcarbamate (222a)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **216** in 98% yield.

TLC (40% ethyl acetate in petroleum ether): R_f = 0.24 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 7.36 (d, *J* = 7.8 Hz, 2H), 7.27 (t, *J* = 7.9 Hz, 2H), 7.02 (t, *J* = 7.3 Hz, 1H), 6.90 (m, 1H), 6.72 (br s, 1H), 5.93 (dd, *J* = 9.7, 2.7 Hz, 1H), 4.67 (m, 1H), 4.46 (dt, *J* = 12.9, 3.6 Hz, 1H), 2.34 (m, 1H), 2.13 (ddd, *J* = 18.3, 6.2, 3.6 Hz, 1H), 2.05–1.05 (m, 13H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.78 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 165.22, 153.62, 146.34, 138.89, 129.46, 123.59, 121.48, 118.98, 80.59, 75.03, 51.75, 48.53, 42.73, 39.66, 37.69, 31.90, 28.14, 26.27, 23.58, 13.63, 11.23. **FTIR** (neat), cm^{-1}: 2938 (br), 1724 (s), 1689 (s), 1223 (vs). **HRMS** (ESI): Calcd for ($C_{24}H_{31}NO_4$+H^+): 398.23258, found: 398.23201. $\boldsymbol{\alpha_D^{RT}}$ = +60.2 (c = 1.0 in $CHCl_3$).

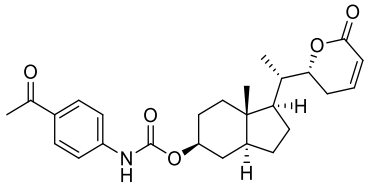
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl (3,4,5-trimethoxyphenyl)carbamate (222c)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **216** in 84% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.33 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 6.90 (m, 1H), 6.67 (s, 1H) overlapping with 6.66 (s, 2H), 5.92 (dd, *J* = 9.7, 2.7 Hz, 1H), 4.65 (m, 1H), 4.46 (dt, *J* = 12.9, 3.6 Hz, 1H), 3.77 (s, 6H), 3.70 (s, 3H), 2.33 (m, 1H), 2.13 (ddd, *J* = 18.4, 6.3, 3.6 Hz, 1H), 2.05–1.05 (m, 12H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.95–0.80 (m, 1H), 0.77 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 165.19, 153.98, 153.66, 146.32, 134.92, 134.35, 121.48, 96.70, 80.57, 75.07, 61.04, 56.45, 51.74, 48.53, 42.73, 39.65, 37.68, 31.90, 28.14, 28.13, 26.26, 23.58, 13.63, 11.22. **FTIR** (neat), cm^{-1}: 2941 (br), 1716 (vs), 1217 (vs), 1126 (vs), 749 (vs). **HRMS** (ESI): Calcd for ($C_{27}H_{37}NO_7$+H$^+$): 488.26428, found: 488.26449. $\boldsymbol{\alpha_D^{RT}}$ = +31.7 (c = 1.0 in $CHCl_3$).

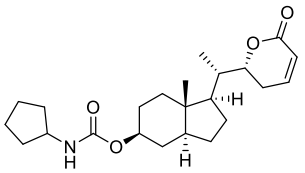
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl (4-acetylphenyl)carbamate (222d)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **216** in 96% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.47 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.92 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 8.6 Hz, 2H), 6.98 (br s, 1H), 6.92 (ddd, *J* = 8.5, 6.2, 1.3 Hz, 1H), 6.01 (dd, *J* = 9.7, 2.6 Hz, 1H), 4.71 (m, 1H), 4.48 (dd, *J* = 12.9, 3.4 Hz, 1H), 2.56 (s, 3H), 2.37 (m, 1H), 2.15 (ddd, *J* = 18.3, 6.2, 3.5 Hz, 1H), 2.08–1.07 (m, 13H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 196.88, 164.92, 152.67, 145.54, 142.54, 131.96, 129.82, 121.21, 117.46, 80.03, 74.97, 51.16, 47.93, 42.15, 38.97, 37.05, 31.26, 27.62, 27.46, 26.37, 25.63, 23.01, 13.32, 10.86. **FTIR** (neat), cm^{-1}: 3304 (m), 2931 (br), 1722 (s), 1592 (s), 1219 (vs). **HRMS** (ESI): Calcd for ($C_{26}H_{33}NO_5$+H$^+$): 440.24315, found: 440.24292. $\boldsymbol{\alpha_D^{RT}}$ = +52.6 (c = 1.0 in $CHCl_3$).

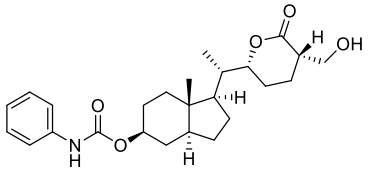
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl cyclopentylcarbamate (222j)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **216** in 90% yield.

TLC (60% ethyl acetate in petroleum ether): Rf = 0.47 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 6.94–6.87 (m, 1H), 6.00 (dd, *J* = 9.7, 2.5 Hz, 1H), 4.57 (m, 2H), 4.47 (dt, *J* = 13.0, 3.5 Hz, 1H), 3.95 (m, 1H), 2.36 (m, 1H), 2.14 (ddd, *J* = 18.3, 6.3, 3.5 Hz, 1H), 2.05–1.05 (m, 21H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 164.90, 155.68, 145.52, 121.22, 80.07, 73.62, 52.57, 51.21, 48.02, 42.17, 39.00, 37.18, 33.23, 31.47, 27.65, 25.67, 23.47, 23.01, 13.30, 10.85. **FTIR** (neat), cm^{-1}: 3353 (m), 2948 (br), 1719 (m), 1696 (s), 1518 (m). **HRMS** (ESI): Calcd for ($C_{23}H_{35}NO_4$+NH_4^+): 407.29043, found: 407.29235. $\boldsymbol{\alpha_D^{RT}}$= +57.2 (c = 1.0 in $CHCl_3$).

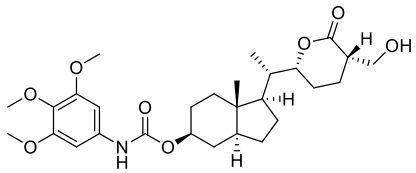
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((2*R*,5*S*)-5-(hydroxymethyl)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl phenylcarbamate (223a)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **217** in 52% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.38 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 7.35 (d, *J* = 7.8 Hz, 2H), 7.27 (t, *J* = 8.0 Hz, 2H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.67 (br s, 1H), 4.67 (m, 1H), 4.32 (dt, *J* = 11.5, 3.3 Hz, 1H), 3.67 (d, *J* = 6.1 Hz, 2H), 2.73 (br s, 1H), 2.65 (m, 1H), 2.05–1.00 (m, 17H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.78 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 176.53, 153.68, 138.96, 129.52, 123.68, 119.10, 80.69, 75.15, 63.09, 51.99, 48.61, 42.79, 40.99, 39.54, 37.78, 31.99, 28.32, 28.22, 26.35, 20.37, 20.28, 13.07, 11.32. **FTIR** (neat), cm^{-1}: 3392 (w), 2950 (br), 2360 (w), 1720 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{35}NO_5$+H^+): 430.25880, found: 430.25859. $\boldsymbol{\alpha_D^{RT}}$= -8.4 (c = 0.5 in $CHCl_3$).

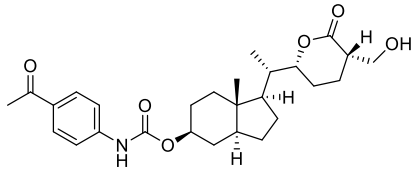
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((2*R*,5*S*)-5-(hydroxymethyl)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (3,4,5-trimethoxyphenyl)carbamate (223c)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **217** in 73% yield.

TLC (80% ethyl acetate in petroleum ether): R_f = 0.29 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 6.65 (s, 3H), 4.65 (m, 1H), 4.32 (dt, *J* = 11.3, 3.3 Hz, 1H), 3.78 (s, 6H), 3.70 (s, 3H), 3.67 (d, *J* = 5.9 Hz, 2H), 2.75 (br s, 1H), 2.65 (m, 1H), 2.05–1.10 (m, 17H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 176.51, 154.09, 153.74, 134.98, 134.63, 97.02, 80.69, 75.19, 63.09, 61.10, 56.57, 51.98, 48.61, 42.79, 40.99, 39.54, 37.77, 31.99, 28.31, 28.22, 26.35, 20.37, 20.29, 13.07, 11.31. **FTIR** (neat), cm^{-1}: 2941 (br), 1724 (vs), 1510 (m), 1220 (vs), 1127 (vs). **HRMS** (ESI): Calcd for ($C_{28}H_{41}NO_8$+H^+): 520.29049, found: 520.29093. $\boldsymbol{\alpha_D^{RT}}$ = -11.6 (c = 1.0 in $CHCl_3$).

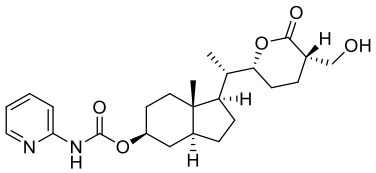
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((2*R*,5*S*)-5-(hydroxymethyl)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (4-acetylphenyl)carbamate (223d)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **217** in 71% yield.

TLC (80% ethyl acetate in petroleum ether): R_f = 0.39 (CAM). **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 10.00 (s, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.58 (d, *J* = 8.9 Hz, 2H), 4.67 (t, *J* = 5.4 Hz, 1H), 4.63 (m, 1H), 4.35 (dt, *J* = 11.3, 3.4 Hz), 3.62 (m, 1H), 3.51 (m, 1H), 2.67 (m, 1H), 2.10–1.10 (m, 20H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ: 196.30, 173.47, 152.81, 143.75, 130.89, 129.44, 117.17, 79.72, 73.83, 61.32, 50.61, 47.20, 41.59, 40.34, 38.51, 36.45, 31.08, 27.28, 27.05, 26.28, 25.34, 20.39, 18.97, 12.44, 10.67. **FTIR** (neat), cm^{-1}: 3329 (w), 2960 (br), 1588 (m), 1217 (s), 1184 (m). **HRMS** (ESI): Calcd for ($C_{27}H_{37}NO_6$+H^+): 472.26936, found: 472.26927. $\boldsymbol{\alpha_D^{RT}}$ = -14.0 (c = 0.4 in DMSO).

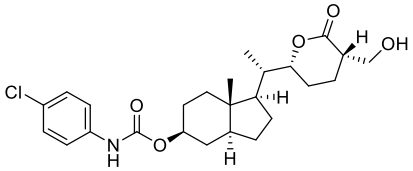
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((2*R*,5*S*)-5-(hydroxymethyl)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl pyridin-2-ylcarbamate (223e)



The product was synthesized according to the general procedure for the synthesis of 2-pyridylcarbamates (page 162) from **217** in 55% yield.

TLC (50% ethyl acetate in dichloromethane): R_f = 0.33 (CAM). ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 8.43 (s, 1H), 8.26 (d, *J* = 4.4 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.67 (t, *J* = 7.4 Hz, 1H), 6.97 (m, 1H), 4.70 (m, 1H), 4.32 (dd, *J* = 11.3, 3.4 Hz, 1H), 3.67 (d, *J* = 5.6 Hz, 2H), 2.74 (br s, 1H), 2.65 (m, 1H), 2.10–1.10 (m, 17H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.79 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 176.53, 153.53, 152.82, 148.36, 138.83, 119.06, 112.72, 80.69, 75.42, 63.09, 52.00, 48.63, 42.80, 41.00, 39.54, 37.78, 31.96, 28.32, 28.19, 26.35, 20.39, 20.29, 13.08, 11.33. **FTIR** (neat), cm^{-1}: 2945 (br), 1718 (vs), 1585 (m), 1538 (m), 1224 (s). **HRMS** (ESI): Calcd for ($C_{24}H_{34}N_2O_5$+H$^+$): 431.25405, found: 431.25346. $\boldsymbol{\alpha_D^{RT}}$ = -13.5 (c = 0.5 in $CHCl_3$).

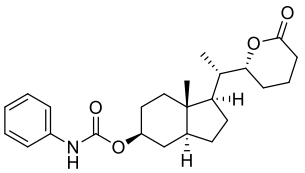
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((2*R*,5*S*)-5-(hydroxymethyl)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (4-chlorophenyl)carbamate (223f)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **217** in 48% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.35 (CAM). ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.33 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.68 (br s, 1H), 4.66 (m, 1H), 4.31 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.66 (m, 2H), 2.72 (m, 1H), 2.65 (m, 1H), 2.05–1.05 (m, 17H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 176.53, 153.57, 137.70, 129.47, 128.48, 120.35, 80.68, 75.41, 63.09, 51.98, 48.59, 42.78, 40.99, 39.53, 37.75, 31.95, 28.31, 28.18, 26.34, 20.38, 20.28, 13.08, 11.3. **FTIR** (neat), cm^{-1}: 2944 (br), 1725 (s), 1512 (s), 1207 (s), 1048 (s). **HRMS** (ESI): Calcd for ($C_{25}H_{34}ClNO_5$+H$^+$): 464.21983, found: 464.21980, calcd for ($C_{25}H_{34}{}^{37}ClNO_5$+H$^+$): 466.21688, found: 466.21733. $\boldsymbol{\alpha_D^{RT}}$ = +6.4 (c = 0.4 in $CHCl_3$).

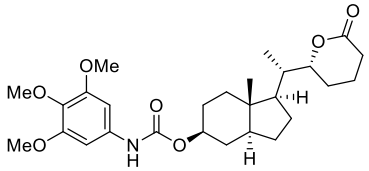
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl phenylcarbamate (224a)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **218** in 68% yield.

TLC (40% ethyl acetate in petroleum ether): R_f = 0.25 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.37 (d, *J* = 7.8 Hz, 2H), 7.30 (t, *J* = 7.9 Hz, 2H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.56 (br s, 1H), 4.70 (m, 1H), 4.34 (dd, *J* = 11.6, 3.0 Hz, 1H), 2.60 (m, 1H), 2.40 (ddd, *J* = 17.4, 10.0, 7.3 Hz, 1H), 2.05–1.10 (m, 17H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.06, 153.14, 137.99, 129.01, 123.27, 118.52, 82.92, 74.60, 51.37, 48.01, 42.20, 39.51, 37.16, 31.39, 29.75, 27.69, 27.59, 25.72, 21.11, 18.90, 12.76, 10.92. **FTIR** (neat), cm^{-1}: 3286 (br), 2941 (br), 1703 (s), 1542 (m), 1223 (s). **HRMS** (ESI): Calcd for ($C_{24}H_{33}NO_4$+H^+): 400.24824, found: 400.24756. $\boldsymbol{\alpha_D^{RT}}$ = +10.0 (c = 0.7 in $CHCl_3$).

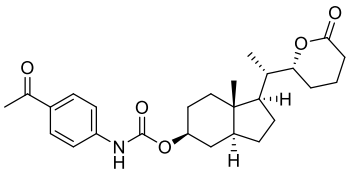
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl (3,4,5-trimethoxyphenyl)carbamate (224c)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **218** in 59% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.47 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 6.65 (s, 2H), 6.61 (s, 1H), 4.65 (m, 1H), 4.31 (dd, *J* = 11.5, 3.0 Hz), 3.78 (s, 6H), 3.70 (s, 3H), 2.52 (m, 1H), 2.33 (m, 1H), 2.00–1.08 (m, 17H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 172.33, 154.07, 153.71, 134.94, 134.57, 96.93, 83.32, 75.22, 61.09, 56.54, 51.97, 48.59, 42.77, 40.19, 37.76, 31.98, 30.36, 28.20, 26.34, 21.64, 19.47, 13.11, 11.30. **FTIR** (neat), cm^{-1}: 2943 (br), 1719 (vs), 1606 (s), 1218 (vs), 1126 (vs). **HRMS** (ESI): Calcd for ($C_{27}H_{39}NO_7$+H^+): 490.27993, found: 490.28023.

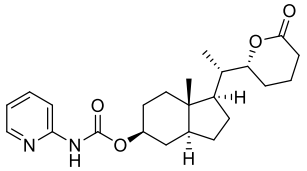
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl (4-acetylphenyl)carbamate (224d)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **218** in 66% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.45 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 7.88 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.02 (br s, 1H), 4.69 (m, 1H), 4.31 (dt, *J* = 11.8, 3.1 Hz, 1H), 2.60–2.45 (m, 1.6H), 2.34 (ddd, *J* = 17.1, 9.7, 7.2 Hz, 1H), 2.05–1.05 (m, 17H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 197.10, 172.38, 153.19, 143.32, 132.51, 130.18, 117.92, 117.84, 83.31, 75.58, 51.86, 48.46, 42.68, 40.11, 37.63, 31.83, 30.32, 28.12, 28.07, 26.27, 21.55, 19.41, 13.06, 11.25. **FTIR** (neat), cm^{-1}: 2947 (br), 1724 (s), 1587 (s), 1529 (s), 1212 (s). **HRMS** (ESI): Calcd for ($C_{26}H_{35}NO_5$+H$^+$): 442.25880, found: 442.25951. $\boldsymbol{\alpha_D^{RT}}$= +6.2 (c = 0.8 in $CHCl_3$).

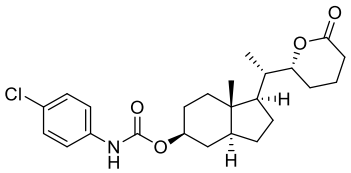
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl pyridin-2-ylcarbamate (224e)



The product was synthesized according to the general procedure for the synthesis of 2-pyridylcarbamates (162) from **218** in 74% yield.

TLC (20% ethyl acetate in petroleum ether): R_f = 0.49 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.91 (s, 1H), 8.30 (d, *J* = 4.4 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.71 (m, 1H), 6.99 (t, *J* = 6.1 Hz, 1H), 4.73 (m, 1H), 4.34 (dt, *J* = 11.3, 2.8 Hz, 1H), 2.60 (m, 1H), 2.40 (ddd, *J* = 17.2, 9.7, 7.2 Hz, 1H), 2.05–1.85 (m, 5H), 1.85–1.10 (m, 12H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.79 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.01, 152.99, 151.98, 147.02, 138.75, 118.46, 112.58, 82.89, 74.92, 51.37, 48.02, 42.19, 39.49, 37.15, 31.34, 29.74, 27.68, 27.55, 25.71, 21.11, 18.89, 12.77, 10.93. **FTIR** (neat), cm^{-1}: 2947 (br), 1720 (vs), 1586 (m), 1535 (m), 1223 (vs). **HRMS** (ESI): Calcd for ($C_{23}H_{32}N_2O_4$+H$^+$): 401.24348, found: 401.24190. $\boldsymbol{\alpha_D^{RT}}$= +7.6 (c = 0.87 in CH_2Cl_2).

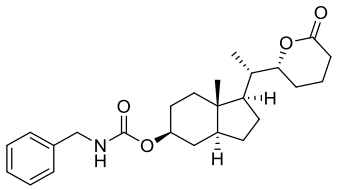
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl (4-chlorophenyl)carbamate (224f)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **218** in 45% yield.

TLC (50% ethyl acetate in petroleum ether): R_f = 0.43 (CAM). **^{1}H NMR** (Acetone-d_6, 500 MHz) δ: 8.67 (br s, 1H (NH, lost by deuteration)), 7.60 (d, *J* = 8.8 Hz, 2H), 7.30 (d, *J* = 8.9 Hz, 2H), 4.66 (m, 1H), 4.37 (dd, *J* = 11.7, 3.2 Hz, 1H), 2.53 (m, 1H), 2.34 (ddd, *J* = 16.7, 9.3, 7.3 Hz, 1H), 2.02–1.17 (m, 17H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.80 (s, 3H). **^{13}C NMR** (Acetone-d_6, 126 MHz) δ: 171.80, 154.22, 139.58, 129.69, 127.84, 120.75, 82.93, 75.17, 52.40, 48.90, 43.12, 40.69, 38.14, 32.43, 30.43, 28.62, 28.45, 26.72, 21.93, 19.66, 13.28, 11.43. **FTIR** (neat), cm^{-1}: 3295 (br), 2944 (br), 1708 (vs), 1219 (vs). **HRMS** (ESI): Calcd for ($C_{24}H_{32}ClNO_4$+H$^+$): 434.20926, found: 434.20914, calcd for ($C_{24}H_{32}{}^{37}ClNO_4$+H$^+$): 436.20631, found: 436.20662. $\boldsymbol{\alpha_D^{RT}}$= +3.9 (c = 0.89 in acetone).

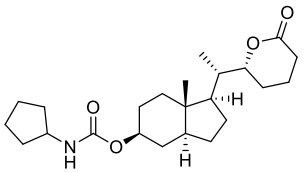
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl benzylcarbamate (224g)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **218** in 57% yield.

TLC (50% ethyl acetate in petroleum ether): R_f = 0.38 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.35–7.23 (m, 5H), 4.95 (m, 1H), 4.63 (m, 1H), 4.34 (m, 3H), 2.59 (m, 1H), 2.39 (ddd, *J* = 17.8, 10.0, 7.2 Hz), 2.05–1.05 (m, 17H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.05, 156.25, 138.61, 128.59, 127.44, 127.38, 82.92, 74.20, 51.34, 48.00, 44.97, 42.17, 39.49, 37.17, 31.45, 29.73, 27.68, 27.63, 25.70, 21.08, 18.87, 12.73, 10.88. **FTIR** (neat), cm^{-1}: 3337 (br), 2945 (br), 1710 (vs), 1515 (m), 1237 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{35}NO_4$+H$^+$): 414.26389, found: 414.26412. $\boldsymbol{\alpha_D^{RT}}$= +10.2 (c = 1.0 in CH_2Cl_2).

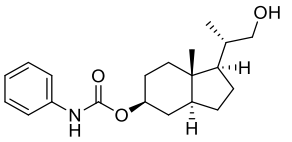
(1*R*,3a*S*,5*S*,7a*S*)-7a-methyl-1-((*S*)-1-((*R*)-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)octahydro-1*H*-inden-5-yl cyclopentylcarbamate (224j)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **218** in 89% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.46 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 4.55 (m, 2H), 4.33 (dd, *J* = 11.6, 3.1 Hz, 1H), 3.95 (m, 1H), 2.59 (m, 1H), 2.39 (ddd, *J* = 17.3, 10.0, 7.3 Hz, 1H), 2.00–1.05 (m, 25H), 0.93 (d, *J* = 6.70 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 172.06, 155.77, 82.94, 73.70, 52.60, 51.36, 48.02, 42.17, 39.49, 37.20, 33.25, 31.51, 29.74, 27.68, 25.71, 23.49, 21.08, 18.88, 12.73, 10.88. **FTIR** (neat), cm^{-1}: 2949 (br), 1707 (vs), 1524 (m), 1237 (vs). **HRMS** (ESI): Calcd for ($C_{23}H_{37}NO_4$+H^+): 392.27954, found: 392.27917. $\boldsymbol{\alpha_D^{RT}}$= +10.0 (c = 1.0 in $CHCl_3$).

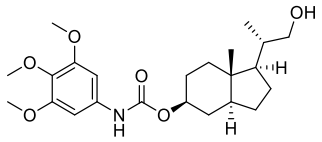
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl phenylcarbamate (225a)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 45% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.64 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 7.37 (d, *J* = 7.9 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.62 (s, 1H), 4.70 (m, 1H), 3.64 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.37 (dd, *J* = 10.5, 6.9 Hz), 2.05–1.80 (m, 4H), 1.70–1.13 (m, 10H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 153.17, 138.00, 128.99, 123.19, 118.48, 74.78, 67.76, 51.51, 48.06, 41.80, 38.71, 37.05, 31.43, 28.09, 27.62, 25.80, 16.63, 11.09. **FTIR** (neat), cm^{-1}: 3315 (br), 2946 (m), 1698 (s), 1539 (s), 1223 (vs). **HRMS** (ESI): Calcd for ($C_{20}H_{29}NO_3$+H^+): 332.22202, found: 332.22173. $\boldsymbol{\alpha_D^{RT}}$= +4.4 (c = 0.63 in $CHCl_3$).

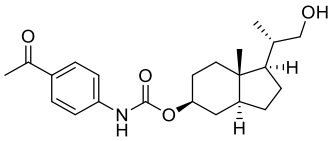
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl (3,4,5-trimethoxyphenyl)carbamate (225c)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 55% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.42 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 6.65 (s, 2H), 6.60 (s, 1H), 4.64 (m, 1H), 3.78 (s, 6H), 3.69 (s, 3H), 3.58 (dd, *J* = 10.4, 3.2 Hz, 1H), 3.30 (dd, *J* = 10.4, 6.9 Hz, 1H), 2.00–1.80 (m, 4H), 1.65–1.10 (m, 10H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 153.99, 153.67, 134.93, 134.35, 96.69, 75.33, 68.08, 61.04, 56.46, 52.17, 48.66, 42.35, 39.38, 37.66, 31.99, 28.61, 28.21, 26.38, 16.97, 11.40. **FTIR** (neat), cm^{-1}: 3324 (br), 2944 (m), 1607 (s), 1219 (vs), 1126 (vs). **HRMS** (ESI): Calcd for ($C_{23}H_{35}NO_6$+H^+): 422.25371, found: 422.25335. $\boldsymbol{\alpha_D^{RT}}$ = +41.5 (c = 0.39 in Acetone).

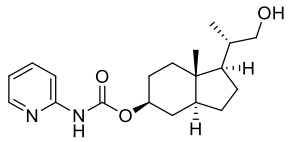
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl (4-acetylphenyl)carbamate (225d)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 84% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.44 (CAM). **^{1}H NMR** (CD_2Cl_2, 126 MHz) δ: 7.89 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.04 (br s, 1H), 4.69 (m, 1H), 3.59 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.31 (dd, *J* = 10.5, 6.9 Hz, 1H), 2.51 (s, 3H), 2.00–1.82 (m, 4H), 1.67–1.15 (m, 10H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 197.10, 153.32, 143.45, 132.64, 130.26, 118.04, 75.87, 68.14, 52.31, 48.71, 42.42, 39.43, 37.73, 31.99, 28.65, 28.21, 26.74, 26.44, 17.04, 11.46. **FTIR** (neat), cm^{-1}: 2949 (br), 1665 (m), 1592 (s), 1530 (s), 1216 (vs). **HRMS** (ESI): Calcd for ($C_{22}H_{31}NO_4$+H^+): 374.23258, found: 374.23267. $\boldsymbol{\alpha_D^{RT}}$ = +2.5 (c = 1.0 in Acetone).

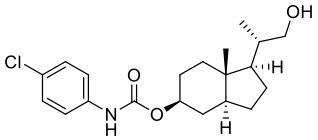
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl pyridin-2-ylcarbamate (225e)



The product was synthesized according to the general procedure for the synthesis of 2-pyridylcarbamates (page 162) from **221** in 69% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.4 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 9.01 (s, 1H), 8.30 (d, *J* = 4.9 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.68 (m, 1H), 6.97 (m, 1H), 4.71 (m, 1H), 3.59 (dd, *J* = 10.4, 3.1 Hz, 1H), 3.31 (dd, *J* = 10.4, 6.9 Hz, 1H), 2.03–1.80 (m, 4H), 1.70–1.10 (m, 10H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 153.59, 152.98, 148.25, 138.85, 118.87, 112.71, 75.48, 68.06, 52.19, 48.68, 42.36, 39.40, 37.68, 31.98, 28.62, 28.22, 26.39, 16.99, 11.42. **FTIR** (neat), cm^{-1}: 2946 (br), 1725 (vs), 1438 (s), 1219 (vs). **HRMS** (ESI): Calcd for ($C_{19}H_{28}N_2O_3$+H$^+$): 333.21727, found: 333.21712. $\boldsymbol{\alpha_D^{RT}}$ = +15.0 (c = 0.57 in Acetone).

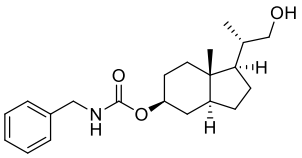
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl (4-chlorophenyl)carbamate (225f)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 82% yield.

TLC (40% ethyl acetate in petroleum ether): R_f = 0.34 (CAM). **^{1}H NMR** (Acetone-d_6, 500 MHz) δ: 8.67 (br s, 1H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.30 (d, *J* = 8.9 Hz, 2H), 4.65 (m, 1H), 3.56 (d, *J* = 9.9 Hz, 1H), 3.33 (m, 1H), 2.02–1.82 (m, 4H), 1.66–1.16 (m, 10H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.77 (s, 3H). **^{13}C NMR** (Acetone-d_6, 126 MHz) δ: 154.24, 139.61, 129.70, 127.84, 120.76, 75.32, 67.54, 52.95, 49.15, 42.80, 40.12, 38.21, 32.51, 28.97, 28.68, 26.84, 17.50, 11.64. **FTIR** (neat), cm^{-1}: 3393 (br), 2931 (m), 1550 (s), 1243 (vs), 830 (s). **HRMS** (ESI): Calcd for ($C_{20}H_{28}ClNO_3$+H$^+$): 366.18305, found: 366.18299, calcd for ($C_{20}H_{28}{}^{37}ClNO_3$+H$^+$): 368.18010, found: 368.18013.

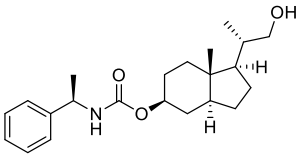
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl benzylcarbamate (225g)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 60% yield.

TLC (30% ethyl acetate in petroleum ether): R_f = 0.26 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.36–7.24 (m, 5H), 4.92 (br s, 1H), 4.64 (m, 1H), 4.36 (d, *J* = 5.4 Hz, 2H), 3.63 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.36 (dd, *J* = 10.5, 6.9 Hz, 1H), 1.97–1.80 (m, 4H), 1.62–1.13 (m, 10H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz δ: 156.30, 138.66, 128.62, 127.49, 127.40, 74.45, 67.83, 51.59, 48.12, 45.01, 41.84, 38.76, 37.14, 31.55, 28.12, 27.73, 25.83, 16.64, 11.10. $\boldsymbol{\alpha_D^{RT}}$= hardly any signal (c = 0.75 in $CHCl_3$).

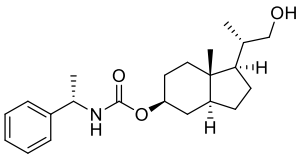
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl ((*R*)-1-phenylethyl)carbamate (225h)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 74% yield.

TLC (50% ethyl acetate in petroleum ether): R_f = 0.37 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 7.35–7,18 (m, 5H), 4.98 (br s, 1H), 4.74 (m, 1H), 4.51 (m, 1H), 3.56 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.28 (dd, *J* = 10.5, 7.0 Hz, 1H), 2.00–1.05 (m, 14H) overlapping with 1.41 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.71 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 155.85, 144.88, 129.01, 127.58, 126.35, 74.70, 68.08, 52.18, 51.06, 48.65, 42.34, 39.40, 37.69, 32.06, 28.61, 28.28, 26.38, 23.11, 16.96, 11.38. **FTIR** (neat), cm^{-1}: 3298 (br), 2947 (br), 1685 (vs), 1242 (vs), 698 (vs). **HRMS** (ESI): Calcd for ($C_{22}H_{33}NO_3$+H^+): 360.25332, found: 360.25339. $\boldsymbol{\alpha_D^{RT}}$= +40.2 (c = 0.89 in Acetone).

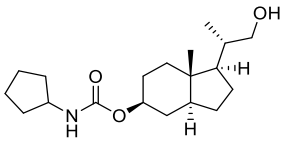
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl ((*S*)-1-phenylethyl)carbamate (225i)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 65% yield.

TLC (50% ethyl acetate in petroleum ether): R_f = 0.37 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 7.36–7.18 (m, 5H), 5.01 (br s, 1H), 4.75 (m, 1H), 4.51 (m, 1H), 3.56 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.28 (dd, *J* = 10.5, 7.0 Hz, 1H), 1.95–1.68 (m, 4H), 1.60–1.05 (m, 10H) overlapping with 1.42 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.71 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 155.85, 129.01, 127.58, 126.36, 74.69, 68.07, 52.19, 51.05, 48.65, 42.34, 39.40, 37.68, 32.06, 28.62, 28.27, 26.39, 23.11, 16.97, 11.38. **FTIR** (neat), cm^{-1}: 3313 (br), 2948 (br), 1686 (vs), 1242 (vs), 698 (s). **HRMS** (ESI): Calcd for ($C_{22}H_{33}NO_3$+H^+): 360.25332, found: 360.25335. $\boldsymbol{\alpha_D^{RT}}$= -29.7 (c = 1.0 in $CHCl_3$).

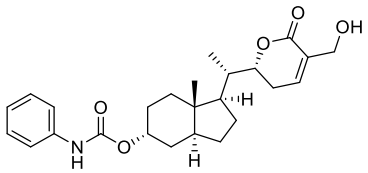
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyloctahydro-1*H*-inden-5-yl cyclopentylcarbamate (225j)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **221** in 72% yield.

TLC (50% ethyl acetate in petroleum ether): R_f = 0.34 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 4.59 (br s, 1H) overlapping with 4.52 (m, 1H), 3.89 (m, 1H), 3.57 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.29 (dd, *J* = 10.5, 6.9 Hz, 1H), 1.95–1.10 (m, 22H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.72 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 156.16, 74.31, 68.09, 53.21, 52.21, 48.69, 42.36, 39.41, 37.73, 33.73, 32.14, 28.62, 28.34, 26.41, 24.03, 16.97, 11.39. **FTIR** (neat), cm^{-1}: 2946 (m), 2869 (w), 1685 (s), 1529 (m), 1243 (s). **HRMS** (ESI): Calcd for ($C_{19}H_{33}NO_3$+H^+): 324.25332, found: 324.25299. $\boldsymbol{\alpha_D^{RT}}$= +6.4 (c = 0.7 in $CHCl_3$).

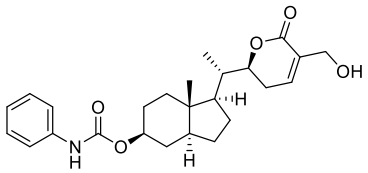
(1*R*,3a*S*,5*R*,7a*S*)-1-((*S*)-1-((*R*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl phenylcarbamate (241a)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **236** in 71% yield.

TLC (70% ethyl acetate in petroleum ether): R_f = 0.45 (CAM). **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ: 7.37 (d, *J* = 7.8 Hz, 2H), 7.28 (t, *J* = 8.0 Hz, 2H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 5.5 Hz, 1H), 6.68 (br s, 1H), 5.01 (m, 1H), 4.48 (dd, *J* = 13.1, 3.6 Hz, 1H), 4.24 (m, 2H), 2.50–2.35 (m, 2H), 2.18 (ddd, *J* = 18.3, 6.5, 3.6 Hz, 1H), 2.02 (m, 1H), 1.87–1.08 (m, 12H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.73 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 126 MHz) δ: 166.28, 153.60, 141.06, 139.00, 131.97, 129.54, 123.62, 118.97, 80.94, 71.36, 62.10, 52.47, 43.49, 42.94, 39.62, 35.74, 31.05, 27.34, 27.02, 26.70, 23.67, 13.82, 10.41. **FTIR** (neat), cm^{-1}: 2941 (br), 1696 (vs), 1540 (s), 1443 (m), 1221 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{33}NO_5$+H^+): 428.24315, found: 428.24293. $\boldsymbol{\alpha_D^{RT}}$= +51.5 (c = 1.0 in $CHCl_3$).

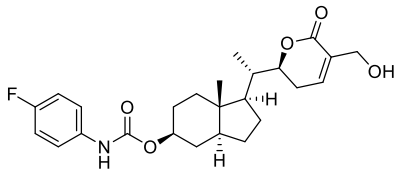
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*S*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl phenylcarbamate (242a)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **235** in 75% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.28 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 7.36 (d, *J* = 7.8 Hz, 2H). 7.28 (m, 2H), 7.02 (t, *J* = 7.3 Hz, 1H), 6.83 (d, *J* = 6.0 Hz, 1H), 6.70 (s, 1H), 4.68 (m, 1H), 4.49 (ddd, *J* = 13.0, 3.3, 1.0 Hz, 1H), 4.23 (m, 2H), 2.58 (ddd, *J* = 18.1, 13.2, 2.0 Hz, 1H), 2.48 (m, 1H), 2.25–1.05 (m, 14H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 166.18, 153.59, 141.42, 138.91, 131.71, 129.46, 123.56, 118.97, 80.57, 75.11, 62.10, 51.31, 48.66, 42.18, 40.00, 37.63, 32.01, 28.50, 28.20, 27.90, 26.16, 13.56, 11.18. **FTIR** (neat), cm^{-1}: 3336 (m), 2940 (br), 1717 (s), 1693 (s), 1541 (s), 1227 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{33}NO_5$+H^+): 428.24315, found: 428.24294. $\boldsymbol{\alpha_D^{RT}}$= -13.9 (c = 1.0 in $CHCl_3$).

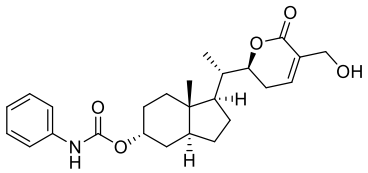
(1*R*,3a*S*,5*S*,7a*S*)-1-((*S*)-1-((*S*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl (4-fluorophenyl)carbamate (242b)



The product was synthesized according to the general procedure for carbamate formation (page 161) from **235** in 87% yield.

TLC (60% ethyl acetate in petroleum ether): R_f = 0.28 (CAM). **^{1}H NMR** (CD_2Cl_2, 400 MHz) δ: 7.33 (m, 2H), 6.98 (t, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 5.8 Hz, 1H), 6.70 (s, 1H), 4.67 (m, 1H), 4.49 (dd, *J* = 13.1, 2.7 Hz, 1H), 4.23 (m, 2H), 2.58 (ddd, *J* = 17.9, 13.2, 1.8 Hz, 1H), 2.48 (m, 1H), 2.30–1.05 (m, 14H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.74 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 100 MHz) δ: 166.19, 159.24 (d, *J* = 241.0 Hz), 153.75, 141.42, 135.03, 131.71, 120.77, 115.97 (d, *J* = 22.6 Hz), 80.57, 75.23, 62.09, 51.30, 48.65, 42.17, 40.00, 37.61, 32.00, 28.50, 28.18, 27.90, 26.15, 13.56, 11.17. **FTIR** (neat), cm^{-1}: 3341 (m), 2947 (br), 1726 (s), 1693 (s), 1211 (vs). **HRMS** (ESI): Calcd for ($C_{25}H_{32}FNO_5$+H^+): 446.23373, found: 446.23372. $\boldsymbol{\alpha_D^{RT}}$ = -13.5 (c = 1.0 in $CHCl_3$)

(1*R*,3a*S*,5*R*,7a*S*)-1-((*S*)-1-((*S*)-5-(hydroxymethyl)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl phenylcarbamate (243a)

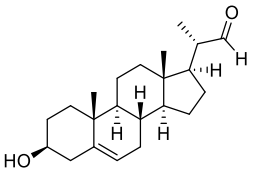


The product was synthesized according to the general procedure for carbamate formation (page 161) from **237** in 52% yield.

TLC (70% ethyl acetate in methylene chloride): R_f = 0.38 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.46 (d, *J* = 7.9 Hz, 2H), 7.29 (t, *J* = 7.9 Hz, 2H), 7.21 (br s, 1H), 7.03 (t, *J* = 7.9 Hz, 1H), 6.90 (d, *J* = 4.9 Hz, 1H), 5.06 (m, 1H), 4.54 (ddd, *J* = 12.9, 3.6, 1.0 Hz, 1H), 4.36 (d, *J* = 13.8 Hz, 1H), 4.32 (d, *J* = 13.8 Hz, 1H), 2.63 (m, 1H) overlapping a br s (1H), 2.12 (ddd, *J* = 18.1, 6.3, 3.6 Hz, 1H), 1.97–1.87 (m, 1H), 1.87–1.62 (m, 6H), 1.61–1.44 (m, 4H), 1.34–1.07 (m, 2H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.68 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 166.06, 153.26, 140.91, 138.38, 131.20, 128.92, 122.94, 118.38, 80.20, 70.36, 61.41, 51.16, 42.92, 41.67, 39.73, 34.89, 30.49, 27.33, 27.25, 26.48, 25.87, 13.11, 10.00. **HRMS** (ESI): Calcd for ($C_{25}H_{33}NO_5$+H^+): 428.24315, found: 428.24295.

5.4 Experimental Part for Part C

(*S*)-2-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)propanal (256)[146]

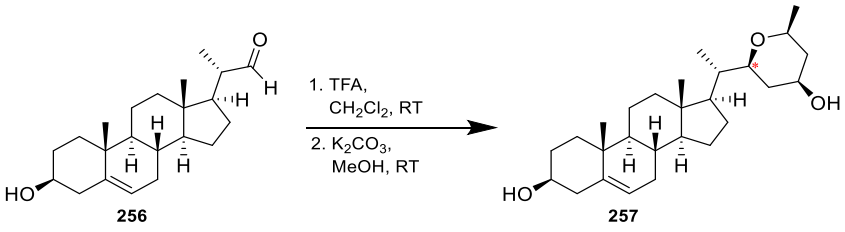


The compound was prepared from **74** according to the general TBS-deprotection protocol (page 95) in 95% yield.

TLC (30% ethyl acetate in petroleum ether): R_f = 0.32 (CAM). **^{1}H NMR** (CD_2Cl_2, 300 MHz) δ: 9.52 (d, *J* = 3.2 Hz, 1H), 5.33 (m, 1H), 3.44 (m, 1H), 2.39–0.87 (m, 22H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.99 (s, 3H), 0.71 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 75 MHz) δ: 205.51, 141.51, 121.83, 72.13, 56.53, 51.52, 50.69, 50.06, 43.43, 42.82, 39.99, 37.80, 37.01, 32.40, 32.38, 32.20, 27.57, 25.14, 21.54, 19.73, 13.78, 12.48. **FTIR** (neat), cm^{-1}: 3419 (br), 2932 (s), 1718 (vs), 1442 (m), 1376 (m), 1056 (vs). **HRMS** (ESI): Calcd for ($C_{22}H_{34}O_2$+H^+): 331.26316, found: 331.26238. **Melting point**: 153–154 °C (lit: 152–153 °C)[146].

5.4.1 General Procedure for Prins Cyclization

(4*S*,6*S*)-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-6-methyltetrahydro-2*H*-2λ^3-pyran-4-ol (257)



To aldehyde **256** (40 mg, 121 µmol, 1 equiv) in dichloromethane (4 ml) was added (*S*)-(+)-4-penten-2-ol (24.9 µl, 242 µmol, 2 equiv) and trifluoroacetic acid (463 µmol, 6.05 mmol, 50 equiv). The reaction was stirred at ambient temperature until full conversion was achieved and then quenched by addition of saturated aqueous sodium bicarbonate. The resulting mixture was diluted with dichloromethane. The separated organic phase was dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The intermediate was dissolved in methanol (10 ml) and potassium carbonate (33.4 mg, 242 µmol, 2 equiv) was added. Within several minutes the intermediate ester was cleaved. The reaction mixture was diluted with dichloromethane and washed with a saturated aqueous solution of ammonium chloride. The separated aqueous layer was extracted

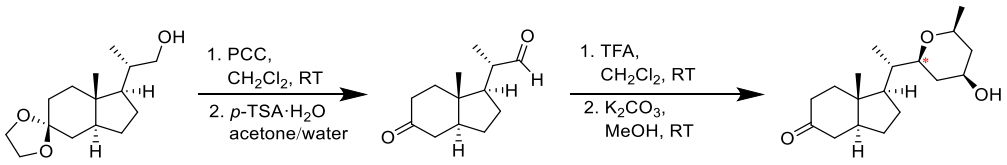
with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, the dried solution was filtered, and the filtrate was concentrated in vacuo. The obtained residue was purified by flash-column chromatography (gradient elution with 10–100% ethyl acetate in petroleum ether) to provide the product **257** (37.3 mg, 90%).

According to ^{1}H NMR, the product appears to be a 90:10 mixture of epimers at the marked carbon atom. Below, only the carbon signals of the major stereoisomer are given.

White solid. **TLC** (60% ethyl acetate in petroleum ether): R_f = 0.36 (CAM). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 5.34 (m, 1H), 3.76 (m, 1H), 3.51 (m, 1H), overlapping with 3.45–3.40 (m, 0.1H, *minor stereoisomer*) 3.37–3.23 (m, 1.9H, *major stereoisomer*), 2.35–2.15 (m, 2H), 2.03–1.70 (m, 8H), 1.65–0.85 (m, 17H), 1.17 (d, *J* = 6.2 Hz, 3H), 0.99 (s, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.66 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ: 140.75, 121.61, 77.22, 71.69, 71.63, 68.89, 56.48, 52.33, 50.06, 43.20, 42.22, 42.07, 40.26, 39.64, 38.36, 37.20, 36.45, 31.94, 31.83, 31.58, 27.57, 24.17, 21.81, 21.07, 19.36, 13.44, 11.75. **FTIR** (neat), cm^{-1}: 3347 (br), 2932 (br), 1447 (m), 1361 (m), 1042 (s). **HRMS** (ESI): Calcd for ($C_{27}H_{44}O_3$+H^+): 417.33632, found: 417.33596. **Melting point**: 190–191 °C.

5.4.2 Analytical Characterization of Withanolide Analogues

(1*R*,3a*S*,7a*S*)-1-((*S*)-1-((2*S*,4*R*,6*S*)-4-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)ethyl)-7a-methyloctahydro-5*H*-inden-5-one (259)

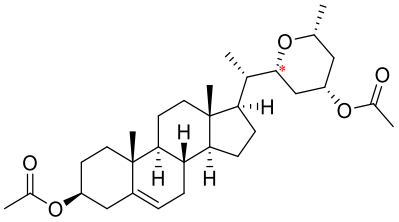


191 **258** **259**

The acetal of crude compound **191** was deprotected (page 145), the primary alcohol oxidized (page 145) to **258** and the product then subjected to the conditions described above. The yield over four steps is 40%. According to ^{1}H NMR, the product appears to be a 90:10 mixture of epimers at the marked carbon atom. Below, only the carbon signals of the major stereoisomer are given.

Colourless oil. **TLC** (50% ethyl acetate in petroleum ether): R_f = 0.44 (CAM). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 3.77 (m, 1H), 3.49–3.40 (m, 0.1H, *minor stereoisomer*), 3.37–3.27 (m, 1.9H, *major stereoisomer*), 2.45–2.20 (m, 4H), 2.12 (ddd, *J* = 13.0, 7.0, 1.9 Hz, 1H), 2.00–1.55 (m, 8H), 1.50–1.32 (m, 3H), 1.29–1.00 (m, 2H), 1.17 (d, *J* = 6.2 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 3H). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ: 212.34, 76.85, 71.69, 68.79, 51.13, 49.77, 43.20, 42.82, 41.58, 40.23, 38.33, 37.60, 37.55, 28.39, 26.26, 21.77, 13.33, 10.42. **FTIR** (neat), cm^{-1}: 3400 (br), 2947 (br), 2869 (s), 1707 (vs), 1137 (s). **HRMS** (ESI): Calcd for ($C_{18}H_{30}O_3$+H^+): 295.22677, found: 295.22653.

(4*R*,6*R*)-2-((*S*)-1-((3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-3-acetoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethyl)-6-methyltetrahydro-2*H*-2λ³-pyran-4-yl acetate (262)



The product was synthesized from **256** according to the general procedure for Prins cyclization (page 189), followed by acetylation (page 111) in 65% yield over three steps. According to ^{1}H NMR, the product appears to be a 72:28 mixture of epimers at the marked carbon atom. Below, only the carbon signals of the major stereoisomer are given.

Colourless oil. **TLC** *minor stereoisomer* (15% ethyl acetate in petroleum ether): R_f = 0.50 (CAM). **TLC** *major stereoisomer* (15% ethyl acetate in petroleum ether): R_f = 0.48 (CAM). **^{1}H NMR** ($CDCl_3$, 600 MHz) δ: 5.36 (m, 1H), 4.85 (m, 1H), 4.59 (m, 1H), 3.55–3.47 (m, 0.72H, *major stereoisomer*), 3.39 (ddd, *J* = 11.4, 3.3, 1.5 Hz, 0.72H, *major stereoisomer*), 3.37–3.33 (m, 0.28H, *minor stereoisomer*), 2.35–2.26 (m, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 –0.84 (m, 23H), 1.20 (d, *J* = 6.3 Hz, 3H), 1.00 (s, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.69 (s, 2.16H, *major stereoisomer*), 0.65 (s, 0.84H, *minor stereoisomer*). **^{13}C NMR** ($CDCl_3$, 151 MHz) δ: 170.58, 170.54, 139.61, 122.52, 73.92, 71.23, 71.10, 56.28, 52.53, 49.94, 42.57, 39.84, 39.63, 39.27, 38.05, 36.92, 36.52, 31.82, 29.78, 27.71, 27.33, 24.29, 21.76, 21.42, 21.34, 20.94, 19.24, 13.49, 11.80. **FTIR** (neat), cm^{-1}: 2937 (br), 1733 (s), 1448 (w), 1364 (m), 1237 (vs), 1027 (s). **HRMS** (ESI): Calcd for ($C_{31}H_{48}O_5$+Na^+): 523.33940, found: 523.34082.

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VII Appendix

7.1 Abbreviations

aq	aqueous
ALK	Alkaline phosphatase
APC	Adenomatous polyposis coli
9-BBN	9-borabicyclo(3.3.1)nonane
BCC	basal cell carcinoma
BIOS	Biology-oriented synthesis / Biologie-orientiete Synthese
Calcd	calculated
CK1α	Casein kinase 1α
CoA	coenzyme A
COMAS	Compound Management and Screening Center
CRC	colorectal cancer
CYPs	Cytochrome P450
δ	chemical shift
DABCO	1,4-diazabicyclo[2.2.2]octane
DAPI	4',6-diamidino-2-phenylindole
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCM	dichloromethane
DHH	Desert hedgehog
DMAP	4-dimethylaminopyridine
DMSO	dimethylsulfoxide

DNP	Dictionary of Natural Products
DOS	diversity oriented synthesis
EC_{50}	half maximal effective concentration
equiv	equivalent(s)
Et	ethyl
FAP	familial adenomatous polyposis
FCC	flash column chromatography
FDA	U.S. Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
g	gram(s)
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLI1, GLI2 and GLI3	Glioma-associated oncogene transcription factors
GSK3β	Glycogen synthase kinase 3β
Hh	Hedgehog
HMPA	hexamethylphosphoramide
HRMS	high resolution mass spectrometry
IC_{50}	half maximal inhibitory concentration
IHH	Indian hedgehog
LDA	lithium diisopropylamide
LRP-5/6	LDL-related proteins 5/6
KHMDS	potassium bis(trimethylsilyl)amide
K_i	inhibition constant
Me	methyl
MeOH	methanol
mg	milligram
MMOA	molecular mechanism of action
ml	millilitre
n-BuLi	*n*-butyllithium
NMR	nuclear magnetic resonance
PTCH	Patched

p-TsCl	*para*-toluenesulfonyl chloride
p-TsOH·H_2O	*para*-toluenesulfonic acid monohydrate
RCM	ring-closing metathesis
RT	room temperature
SAR	structure activity relationship
SCONP	Structural classification of natural products
s.d.	standard deviation
SHH	Sonic hedgehog
SM	secondary metabolite
SUFU	Suppressor of Fused
SV40	Simian vacuolating virus 40
TBAI	tetrabutylammonium iodide
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TMS	trimethylsilyl
TLC	thin layer chromatography
Wnt3a-CM	Wnt3a conditioned medium

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7.3 Eidesstattliche Versicherung (Affidavit)

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Belehrung:	**Official notification:**
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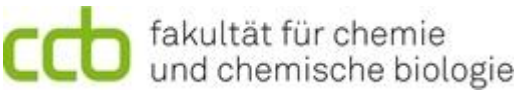
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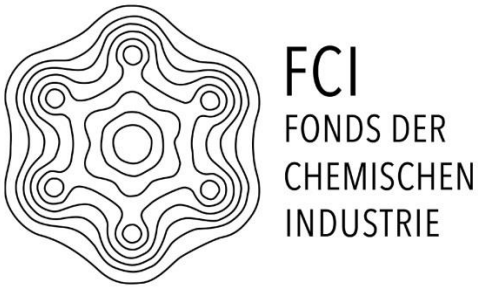
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