Studies towards Steroidal Modulators
of the Hedgehog/Gli Signalling Pathway

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ABSTRACT

The modern age faces a progressive lengthening of life span, which is often associated with the onset of degenerative disorders such as Parkinson's, Alzheimer's and cancer. These diseases do not yet have a cure. The Hedgehog/Gli signalling pathway is one of the most promising pathways of cellular communication. Pharmacological modulation of this pathway would provide a remarkable approach for new potential therapeutics in regenerative medicine and cancer (**Chapter 1**).

Attention was focussed on coagulin L **I** and withanolide F **II**, two natural steroidal lactones featuring interesting reactive functionalities. The aims of this project (**Chapter 2**) were to investigate the chemical modifications of these natural products to prepare novel analogues for a structure-activity relationship study.
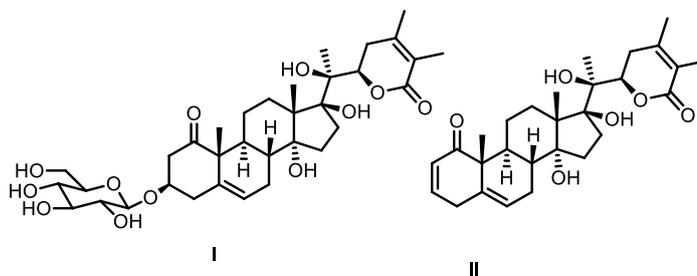
Leads compounds coagulin L **I** and withanolide F **II**.

3β-Methoxy-pregnenolone **III** is known as able to treat degenerative pathologies of the nervous system which could be implicated in Parkinson's and Alzheimer's diseases. The 3β-methoxy group also provides metabolic stability *in vivo*. Therefore, a library of steroidal analogues decorated differently on the right-hand side (**IV** and **V**) has been prepared by organometallic additions of heterocycles to the side-chain of this commercially-available steroid (**III**, **Chapter 3**).
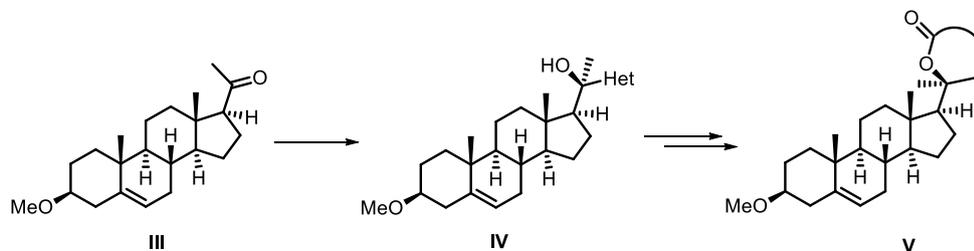
Chapter 3. Towards steroids with heterocyclic side-chains.

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The 14β-hydroxyl group is rarely found amongst withanolides, and no syntheses of withanolides with the *cis*-C/D ring rearrangement have been reported to date. Thus, the total synthesis of unnatural withanolide analogues **X** based on the Hajos and Parrish ketone derivative **IX** was investigated starting with readily available materials **VI**, **VII** and **VIII** (**Chapter 4**).
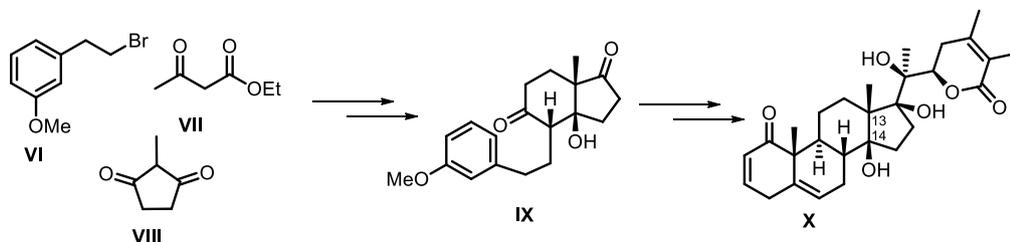
Chapter 4. Studies towards novel withanolide analogues.

Coagulin L **I** is the major metabolite extracted at AnalytiCon Discovery from plant material. Hence, its conversion into the more active withanolide F **II** *via* intermediate **XI** was explored (**Chapter 5**). Further elaboration of the left-hand side to provide additional novel compounds for our library of small molecules was also investigated.
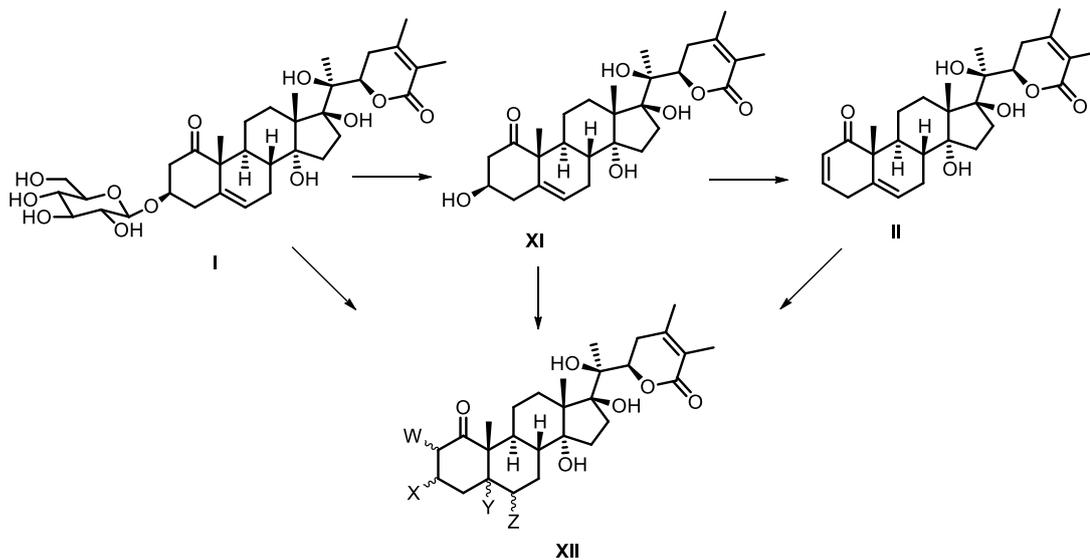
Chapter 5. Elaboration of coagulin L **I**.

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DECLARATION

The Research presented in this Thesis was carried out at the University of York and at AnalytiCon Discovery GmbH between November 2010 and October 2014. The work is, to the best of my knowledge, original except where due reference has been made to other workers.

CHAPTER 1.
INTRODUCTION: THE HEDGEHOG/GIi SIGNALLING

The progress of medical sciences and the widespread wealth in the developed world has had a major impact in lengthening of life expectancy. This has led to an increased number of age-associated loss of functionalities and diseases, including Parkinson's and Alzheimer's diseases, neurodegeneration and tumours. Although medical advances have provided improved knowledge on symptom management and palliative treatments, research into finding definitive cures for many of these patients remains an unmet need.

1.1. STEM CELLS: A CONCISE OVERVIEW

1.1.1. Embryonic and adult stem cells

In the last three decades attention has been given to understand the biology of stem cells and their crucial implications in organ formation, size, regeneration and homeostasis. Stem cells are unspecialised cells characterised by a long-term self-renewal capacity and an extensive differentiation potential, that is, to make copies of themselves and to develop into specialised cells.[1] Basically, two types of stem cells can be distinguished: embryonic stem cells (ESC) and adult stem cells. ESC and adult stem cells are both able to self-renew, but there is a difference. In fact, ESC are responsible for the very early stage of organ development and growth, and they can differentiate in all the cell lines within an organism, even under cultured conditions or after transplantation *in vivo*. On the contrary, adult stem cells can generate only specialised cells and are dedicated to mantain the homeostasis of their own tissues by replacing dead cells throughout life and/or to repair damages after injuries.[2]

1.1.2. Cancer stem cells

Genetic, age-related or inherited alterations of stem cells during life span may lead to loss of their functionalities and to the onset of degenerative diseases and cancer.[3] It is well known that solid tumours are made of clusters of different cells. Amongst them, a new type of cell has been recently identified: cancer stem cells (CSCs). These cells have the same properties of stem cells, self-renewal and plasticity, but they are able to regenerate primary tumours after inoculation *in vivo* and to give rise to a progeny of more specialised cancer cells. CSCs are believed to be responsible for initiating and maintaining cancer growth, and to be the common precursor from which all other cancer cells can arise.[4]

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1.1.3. Induced pluripotent stem cells

The interest in stem cell research increased remarkably in 2006, when the Nobel laureate Shinya Yamanaka discovered the genetic factors essential to converting back mature specialised cells into the pluripotent embryonic state. The newly formed cells, called induced pluripotent stem cells (iPSCs), possess both features typical of embryonic stem cells, *i.e.* the infinite self-renewal property and very high plasticity.[5] These findings completely changed the understanding of human biology. In fact, using iPSCs it became possible to make all the cell types existing in the human body directly from mature cells without destruction of the early embryos and so allowed ethical issues related to embryonic stem cell research to be sidestepped.[6]

1.2. APPLICABILITY OF STEM CELLS IN NEURODEGENERATION AND CANCER

The extraordinary advances in stem cell research is having a huge impact in regenerative medicine, cellular and genetic therapies and drug discovery. Their applications have helped, amongst others, to gain an understanding of the transformations of a fertilized egg into an organism, to achieve insights into molecular and genetic mechanisms of inherited diseases and cancer, to replace damaged or dead cells in disorders such as type II diabetes, stroke and neurodegeneration, to create models for drug testing, and to find out side effects in earlier stage of experimentation.[7] As a further advantage, iPS cells generated directly from an individual patient should be genetically identical to that patient, thus they could be transplanted with potentially no risk of rejection (**Figure 1.1**).[7, 8]
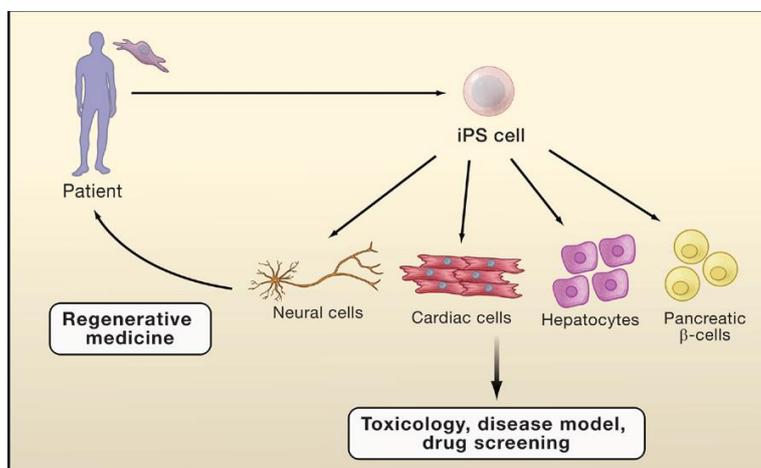
Figure 1.1. Yamanaka's representation of the possible applications of iPS cell technology (Figure reproduced from *Cell*, 2009, **137**, p. 13).[7]

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1.2.1. Parkinson's disease

Parkinson's disease (PD) is a debilitating neurodegenerative condition common amongst people aged over 65. It is reported that in the United Kingdom in 2012 more than 127000 people suffered from PD.[9] PD is caused by the progressive loss of the neurons producing dopamine in the substantia nigra, a deficit that leads to tremors, bradikinesia, rigidity and other neuropsychiatric symptoms such as depression, anxiety and sleep disturbances.[9] Current treatments of PD include pharmacological replacement with levodopa, brain stimulation and palliative care, with poor results especially in long-term therapy (along with additional side effects). Recently, cell-replacement by administration of dopaminergic cells derived from embryonic stem cells on *in vivo* PD animal models gave positive results in preclinical experiments. Nevertheless, continued research to elucidate the underlying mechanism of stem cell therapy is still needed before applications of PD cellular therapy are tested in clinical trials.[10]

1.2.2. Alzheimer's disease

Alzheimer's disease (AD) is a form of dementia characterised by a progressive decline of cognitive abilities which can lead to severe loss of memory, immobility and death. The Alzheimer's Society reports that in the UK 496000 people are affected by AD, and amongst them 17000 are under the age of 65.[11] There is currently no cure for AD, except for occupational and symptomatic therapies. Although the widespread degeneration of neurons and synaptic connectivity seemed to prevent the application of a cell-based replacement therapy, recent progress has been made in treating AD using stem cells. Transplantation of neural stem cells resulted in an increased synaptic density, suppression of inflammation and improvement of neurogenesis in a mouse model of AD. Furthermore, human AD modelling for genetic studies and drug testing have been realised using iPSCs.[12] The hope is that in the future iPSC technology will help to understand the basis of AD and resolve the numerous issues still related to this complex disease, hopefully providing personalised therapy to the patients.[12]

1.2.3. Cancer

Cancer is an aggressive disease in which a heterogeneous mass of cells acquires aberrant proliferative activity, does not respond to growth suppressors and apoptotic signals, and can spread to other tissues and organs by invasion and metastasis.[13] Despite many advances in tumour treatments, there are still a considerable number of patients not

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responsive to chemo- and radiation therapy. This seems to be due to the presence of CSC, a population of cells that can acquire treatment-resistance and can be involved in metastasis.[14] Thus, targeting CSC would provide a good possibility to fully eradicate cancer. Moreover, iPSC technology could help to understand the oncogenic molecular mechanisms, to design new drugs for selective tumour targeting and/or to replace blood cells and damaged organs and tissue in leukaemia or after chemotherapy.[15]

1.3. ABOUT CELL-TO-CELL INTERACTIONS: EMBRYONIC SIGNALLING PATHWAYS

During development and specialisation, intercellular cross-talk is of prime importance to channel stem cell differentiation into a selected cell lineage. Several mechanisms lie behind stem cell fate. Amongst others, developmental signalling pathways such as Hedgehog (Hh)/Gli, Wnt/β-catenin and Notch have been identified as key factors in the regulation of the two unique characteristics of stem cells: self-renewal and differentiation (**Figure 1.2**).[3, 16-18]

These three pathways are evolutionarily conserved, and strictly cooperate in the regulation of embryonic development, cell proliferation, migration and differentiation. Their aberrances have been related to the onset of several degenerative diseases and cancer.[17] Therefore, understanding the processes that regulate cell division, maturation and dissemination, and being able to manipulate them with selective small molecule modulators would help to develop new treatments and overcome the many issues still unresolved in the stem cell field.

1.3.1. Hh/Gli signalling pathway

The Hh/Gli signalling pathway was first identified in the fruit fly *Drosophila melanogaster* and takes the name from the evidence that mutation of the gene encoding for its biological agonist gave rise to a phenotype of larvae with particular hair similar to hedgehog spines.[19] In mammals, this pathway is activated after binding of Hh functional glycoproteins, termed Sonic (Shh), Indian (Ihh), and Desert (Dhh), to the 12-transmembrane receptor Patched1 (Ptch1, **Figure 1.2**, **C**). This entails the release of the 7-transmembrane receptor-like Smoothened (Smo), which in turn activates the intracellular complex of Costal2 (Cos2), Fused (Fu) and suppressor of Fused (Su(Fu)),

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and turns on the cascade of intracellular events ending with the translocation in the nucleus of the Gli-transcription factors.[20-22]
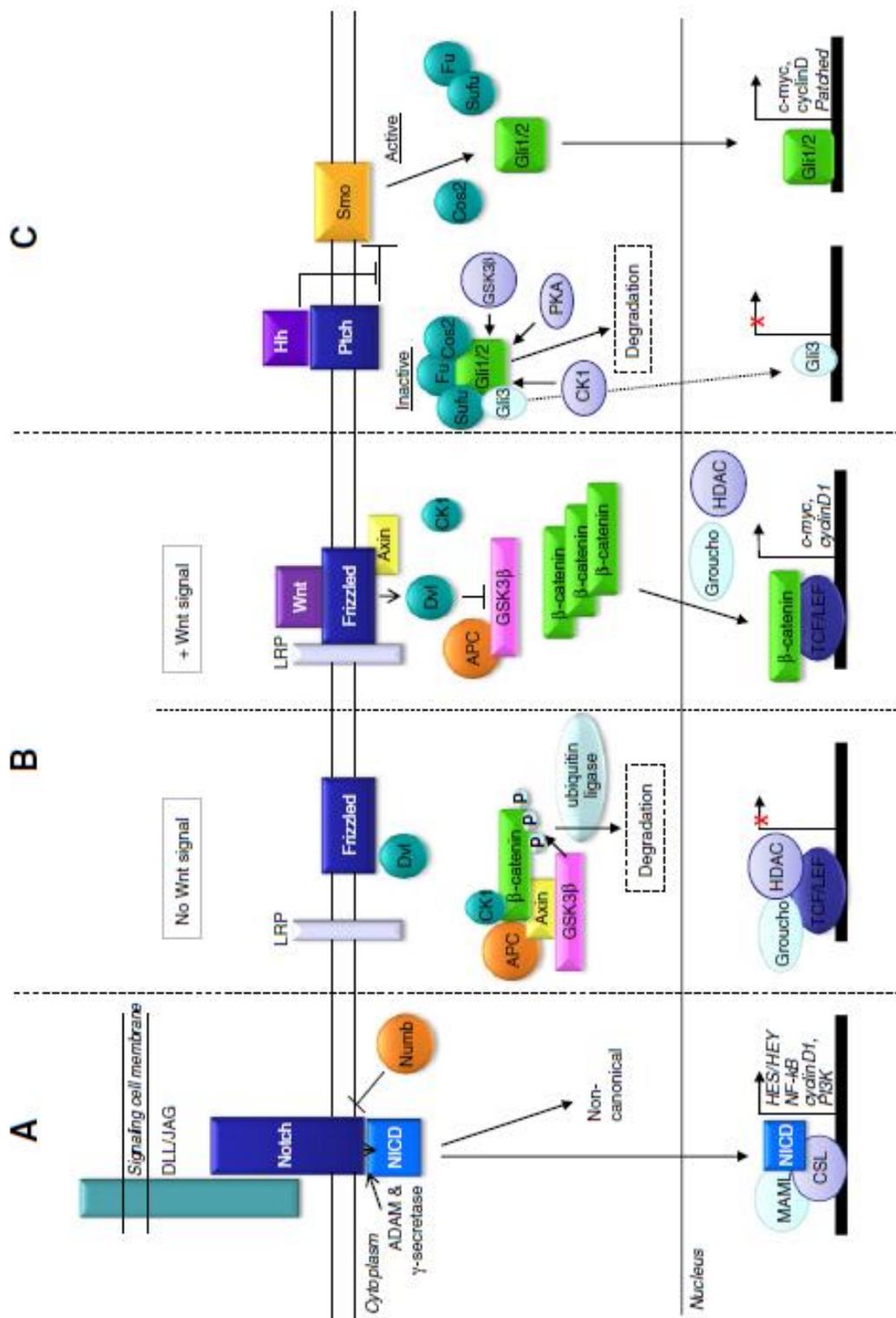
Figure 1.2. Overview of Notch (a), Wnt (b) and Hh/Gli (c) signalling pathways.[21] (Reproduced from *Biochim. Biophys. Acta*, **2013**, 1830, p. 2483).

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NANOG, the gene that encodes for the protein regulating the embryonic stem cells renewal and the re-programming of adult somatic cells to iPS cells, is one of the most important transcription factors controlled by the Hh/Gli signalling pathways.[23] Misregulation of the Hh/Gli cascade has been related to the generation and progression of several cancer types including medulloblastoma, leukemia, lung, pancreas, breast and colon cancer.[24] Moreover, a higher Hh signalling activity has recently been detected in CSCs than in normal adult cells, thus strengthening the hypothesis of its contribution to cancer maintenance and resistance (**Figure 1.2**, c).[16]

1.3.2. Wnt signalling pathway

The Wnt signalling pathway derives its name from the contraction of *wingless*, a gene responsible for segment polarity in *Drosophila*, and *Int-1*, its murine homologue.[25] The protein β-catenin is the major intracellular signal transducer in the Wnt cascade (**Figure 1.2**, b). When the pathway is not active, β-catenin undergoes a series of processes leading to its final proteosomal degradation. On the other hand, activation of the pathways by binding of Wnt glycoproteins to a 7-membered transmembrane G-protein-coupled receptor Frizzled (Fzd) results in the release of the downstream signal mediator Disheveld (Dvl), and in the final disassembling of the β-catenin destruction complex. The stabilised β-catenin is then accumulated in the cytoplasm, and eventually it can translocate into the nucleus to regulate gene expression in cooperation with other transcription factors.[21, 25]

It has been demonstrated that the Wnt/β-catenin signalling pathway is involved in self-renewal of ESC and adult stem cells and in the maintenance of their pluri- and multipotency.[26] Deregulated Wnt pathways after mutations of its elements and/or hyper-activation have been found in several types of cancer, and are associated with CSCs activity.[16, 17] Moreover, Wnt signalling also seems to be involved in the reprogramming of iPSCs, but its role in this regard needs to be further clarified.[27]

1.3.3. Notch signalling pathway

The Notch signalling pathway takes its name from the evidence that a genetic defect in the flies *Drosophila melanogaster* caused little notches at the end of their wings.[28] Unlike Hh/Gli and Wnt pathways, the Notch single transmembrane receptor is not activated by secreted glycoproteins, but by interaction with Delta, Serrate, Delta-like (Dll) or Jagged (Jag) ligands, which are transmembrane proteins in juxtaposed cells (**Figure 1.2**, a). Downstream events lead to the release of the Nicd transcription factor, its translocation

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into the nucleus and further activation of targeted genes.[28, 29] The Notch pathway has a crucial implication in the control and survival of adult stem cells and its aberrant regulation has an oncogenic effect,[16, 17] although less is known about its role in ESC and iPSCs.

1.4. Hh/Gli MODULATORS: THE STATE OF THE ART

In 2010 Mas and Ruiz i Altaba reported in a comprehensive review the known small molecule modulators of the Hh/Gli signalling pathway, and classified them according to whether they were from a natural or synthetic source, and their mode of action.[24] More recently Trinh *et al.* published an updated review of Hh inhibitors.[30] A summary of the most representative modulators of the Hh/Gli cascade is provided below.

1.4.1. Natural Hh/Gli antagonists

Given the significant role of Hh/Gli in tissue patterning and cell number maintenance, and its implications in tumour formation and development, small molecule inhibitors of this growth-factor pathway have proven valuable in providing leads for anticancer therapies, and they are still an active area of research.

1.4.1.1. The first Hh/Gli antagonists: jervine *1.1* and cyclopamine *1.2*

Jervine **1.1** and cyclopamine **1.2** are *C-nor-D-homo* steroids, otherwise called [14(13→12)]-*abeo*-steroids, extracted from the plants of *Veratrum* genus, and are recognised as the first naturally occurring inhibitors of Hh/Gli expression (**Figure 1.3**).[31] They act as Smo receptor antagonists, and they prevent the downstream cascade of intracellular events by blocking Smo in an inactive form. Compounds **1.1** and **1.2** feature an interesting 6-6-5-6 ring pattern, which differs from the standard steroidal 6-6-6-5 skeleton. Ring D is spirocyclic attached to a tetrahydrofuran, which is, in turn, fused to a substituted piperidine.
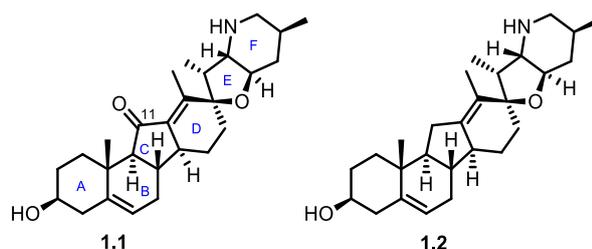
Figure 1.3. Structures of jervine **1.1** and its 11-deoxy derivative cyclopamine **1.2**.

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*1.4.1.2. Potent modulators from cyclopamine **1.2** derivatisation*

The potential of cyclopamine **1.2** as an anti-cancer drug has been confirmed by several experiments *in vitro* and *in vivo*.[24] However, its poor solubility and instability under acidic conditions along with a range of associated side effects drove scientists to synthesise novel cyclopamine prodrugs, with improved bioavailability and pharmacokinetics, and selective tumour targeting properties (**Figure 1.4**). KAAD-Cyclopamine **1.3**, the prostate-specific antigen peptide-cyclopamine conjugate **1.4**,[32] the water soluble carbohydrate-cyclopamine derivative **1.5**[33] and carboxylate cyclopamine tartrate **1.6**[34] are some representative examples (**Figure 1.4**). IPI-926 otherwise called saridegib (Infinity) **1.7** is a sulfonamide with improved potency and plasma half-life. Compound **1.7** is currently in phase II trials.[35]
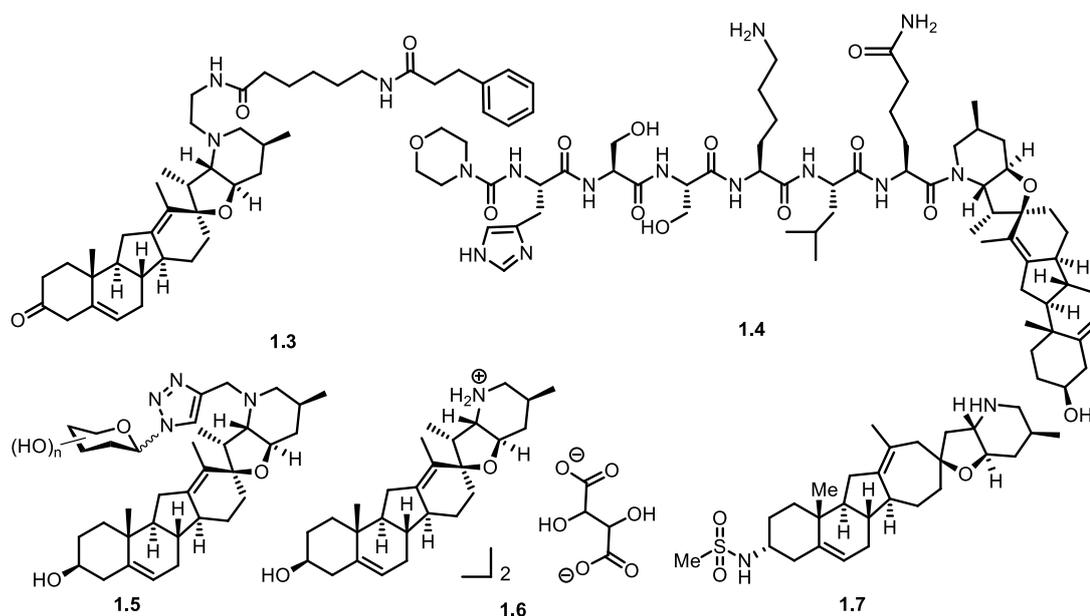
Figure 1.4. Representative examples of anticancer prodrugs based on cyclopamine **1.2**.

1.4.1.3. Additional natural Hh inhibitors from screening libraries

A wide range of other natural products have shown inhibitory activity by targeting the pathway at different sites (**1.8-1.19**, **Figure 1.5**). Curcumin **1.8** is a diferuloylmethane derivative extracted from *Curcuma longa*[36] which is able to downregulate Shh protein and the downstream expression of Gli1 in Hh/Gli, and to decrease the levels of β-catenin in Wnt signalling pathway. The cytotoxic effects in medulloblastoma cells are encouraging.[37]

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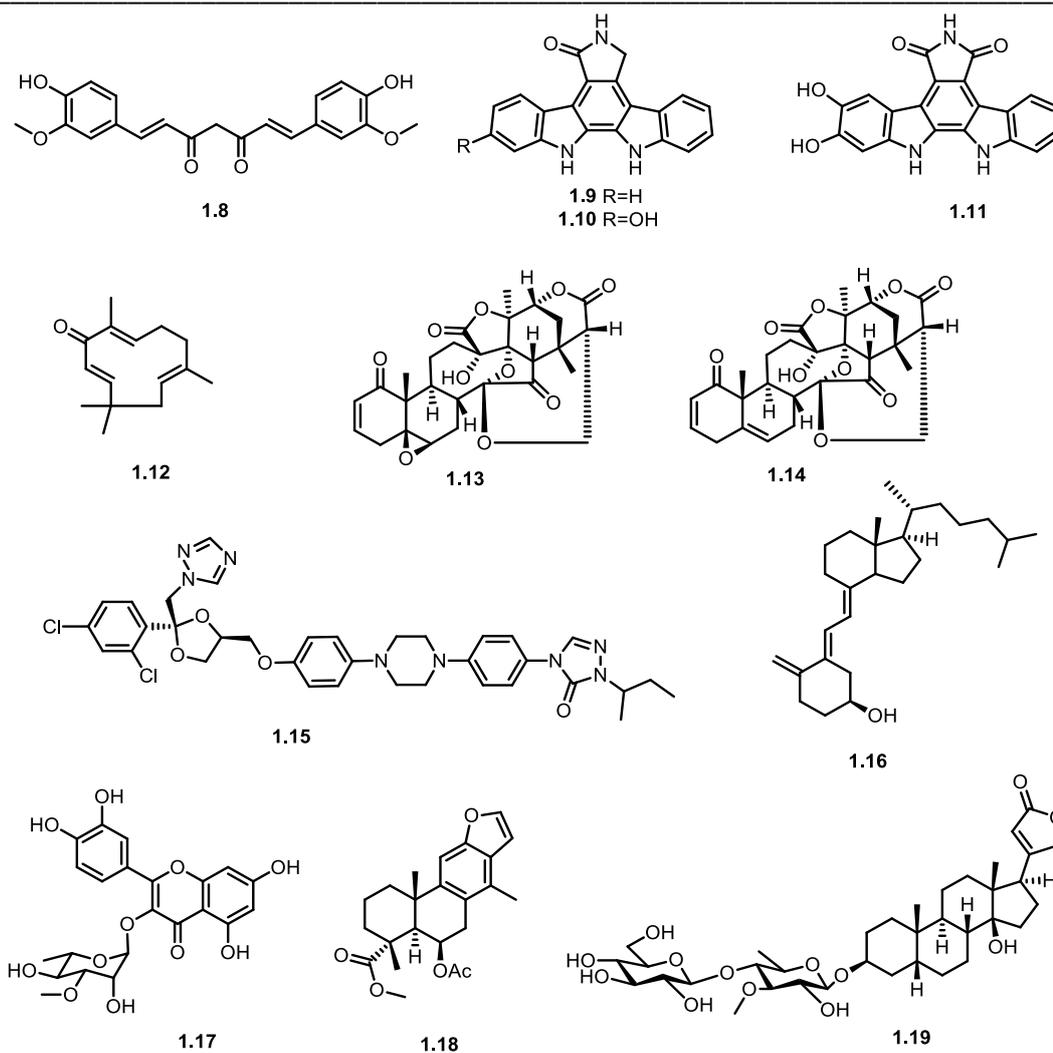
Figure 1.5. Examples of natural product inhibitors of Hh/Gli signalling.

The screening of a library of 94 natural products and 102 plant extracts on a cell-based assay allowed the identification of bisindole alkaloids staurosporinone **1.9**, 6-hydroxystaurosporinone **1.10**, 5,6-dihydroxyarcyriaflavin A **1.11**, sesquiterpene zerumbone **1.12**, withanolides physalin F **1.13** and physalin B **1.14** as potent inhibitors of the transcription activity of Gli1 with IC_{50} values of 1.8, 3.6, 6.9, 7.1, 0.66 and 0.62 μM, respectively (**Figure 1.5**). Compounds **1.9**, **1.12**, **1.13** and **1.14** also showed inhibitory activity on Gli2-mediated transcription (IC_{50} values of 7.1, 2.7, 1.4 and 1.5 μM, respectively) and reduction of the expression of anti-apoptotic protein Bcl2. Moreover, 13,14-*seco*-16,24-cyclosteroids **1.13** and **1.14** were remarkably cytotoxic in human pancreatic cancer cell lines PANC1, with IC_{50} values of 2.7 and 5.3 μM vs the standard cyclopamine **1.2** with IC_{50} value of 8.0 μM.[38]

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The triazole piperazine itraconazole **1.15** displayed Hh inhibitory activity *in vitro* even in the presence of Smo mutations that confer resistance, whereas, when used in combination with arsenic trioxide, it blocked the growth of medulloblastoma and basal cell carcinoma in a mouse cancer model.[39] Colecalciferol **1.16** and other vitamin D derivatives have also been reported as Hh pathways antagonists (**Figure 1.5**).[40]

Finally, cytotoxic natural product inhibitors of Gli1 mediated transcription in cell lines overexpressing Hh signalling have been extracted from *Excoecaria agallocha*, such as quercetine derivative **1.17**,[41] from *Acacia pennata*, such as taepeenin D **1.18**[42] and from *Adenium obesum*, such as cardiac glycoside **1.19** (**Figure 1.5**).[43]

1.4.2. Synthetic Hh/Gli antagonists

1.4.2.1. Synthetic Gli-mediated transcription inhibitors from compound library screening

It is well known that the combination of high-throughput chemistry (HTC) and high-throughput screening (HTS) can produce a range of bioactive lead compounds. In biological research and drug discovery, cell based assays are widely used to search for new bioactive compounds in a rapid manner, to evaluate drug toxicity and to study key molecular events in single cells. Amongst others, the luciferase assay system is a reporter gene assay that monitors cellular response at the transcription level by detecting emission of bioluminescence associated with increased activity of the enzyme *firefly luciferase*. A construction in which protein Gli binding sites have been coupled upstream to the gene encoding luciferase has been applied to compound libraries for primary screening and target validation of some of the most potent Hh/Gli pathway antagonists with activity downstream of Smo and Su(Fu). [44, 45]

Examples of such compounds are the symmetric hexahydro-pyrimidine GANT-61 **1.20** and the substituted thiophene GANT-58 **1.21**, two potent Gli1 antagonists (G =Gli and ANT = antagonist) identified from a library diversity set of 1990 compounds supplied by the USA National Cancer Institute;[46] HPI1 **1.22**, HPI2 **1.23**, HPI3 **1.24** and HPI4 **1.25** (HPI = Hh Pathway Inhibitors), which arose from the screening of 122755 small molecules;[47] and the 2,4-disubstuted thiazole derivative JK184 **1.26**, identified from a screen of 20000 heterocycles as a promising inhibitor of the Gli1 transcription activity (**Figure 1.6**). [48]

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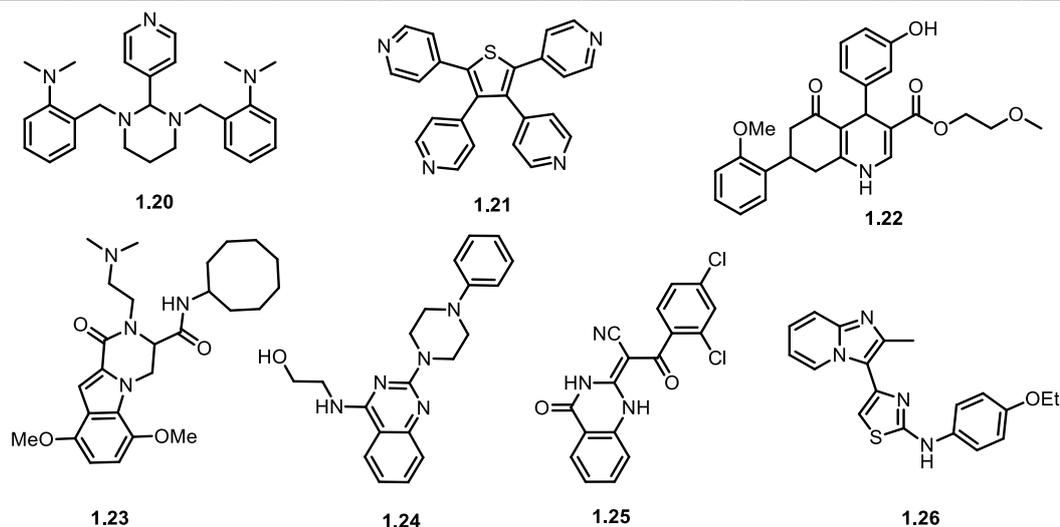
Figure 1.6. Synthetic inhibitors of Gli-mediated transcription from compound library screening.

1.4.2.2. Synthetic Smo inhibitors from compound library screening

Smo inhibitors currently represent the largest class of Hh antagonist. Structurally diverse compounds SANT1 **1.27**, SANT2 **1.28**, SANT3 **1.29** and SANT4 **1.30** (S = Smo and ANT = antagonist) were the first synthetic Smo antagonists identified when Beachy and co-workers screened a library of 10000 compounds during their mechanistic studies on the modulation of Smo activity (**Figure 1.7**).[49]
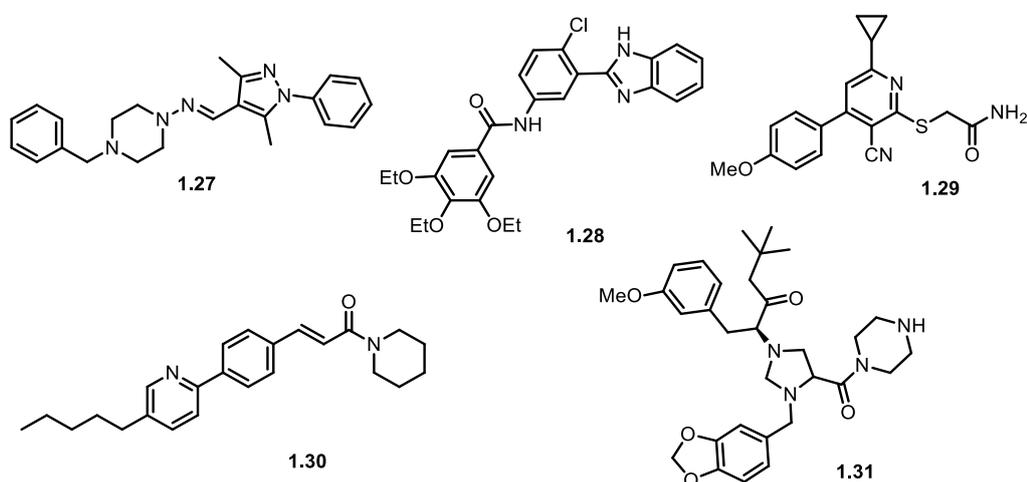
Figure 1.7. Some Smo inhibitors from compound library screening.

The aminoproline CUR61414 **1.31** (Curis) emerged from a HTS against 100000 small molecules on a Hh-responsive reported cell line (**Figure 1.7**). It is the first Smo antagonist to enter clinical trials. However, these studies were stopped because of undesirable pharmacokinetic issues [19, 50]

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1.4.2.3. Synthetic Smo inhibitors in clinical trials

Smo inhibitors are giving promising results in clinic trials. GDC-0449 **1.32** (Curis, Evotec, and Genentech), also known as vismodegib, is the first Hh pathway inhibitor approved by the Food and Drug Administration (FDA) and is currently in phase II for treatment of the medulloblastoma, basal cell carcinoma and a number of other solid advanced tumours (**Figure 1.8**). It was developed from lead compound **1.33**. Replacement of the benzimidazyl group with a pyridyl group and modification of the aromatic functionality attached to the amide group resulted in a compound with excellent potency, improved metabolic stability, low clearance and high absorption.[51, 52]
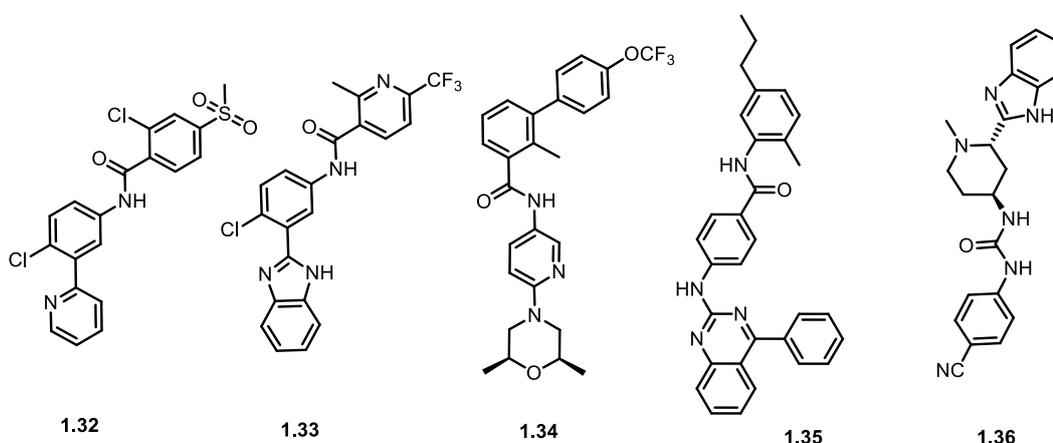
Figure 1.8. Synthetic Smo inhibitors in clinical phase I and II trials.

The potent and selective Smo antagonist NVP-LDE225 **1.34**, also known as erismodegib (Novartis), was discovered through a HTS of compound libraries generated by solid phase combinatorial chemistry (**Figure 1.8**).[53] This biphenyl-3-carboxamide led to dose-related tumour growth inhibition in a medulloblastoma allograft mouse model, and it is currently being evaluated in clinical development as a treatment for basal cell carcinoma, Gorlin's syndrome and pancreatic cancer.[52]

Quinazoline **1.35** (XL-139, Bristol-Myers Squibb and Exelixis) was disclosed as an oral Smo antagonist, and it is still progressing in clinical trials as a well-tolerated antineoplastic in medulloblastoma, basal cell carcinoma and chronic myeloid leukemia (**Figure 1.8**).[30, 52]

Based upon the evidence that benzimidazole derivatives gave promising results in inhibiting the activity of Gli/luciferase in a related assay, researchers have undertaken synthetic efforts to design a lead with improved pharmacokinetic properties. The resulting

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urea PF-04449913 **1.36** (Pfizer)[54] is currently being tested in humans as a chemotherapeutic agent for advanced solid tumours as well as for haematologic malignancies (**Figure 1.8**).[52]

*1.4.2.4. Robotnikinin **1.37**: joint target-based screening and combinatorial chemistry*

Robotnikinin **1.37** (**Figure 1.9**) is the first reported Hh/Gli antagonist active upstream of Smo, identified at Harvard University during a target-based screening against a library of 10000 structurally diverse compounds including 12-, 13- and 14-membered macrocycles. Its discovery stemmed from a novel investigative approach in the field of Hh pathway modulators, that is, the identification of compounds able to bind the known protein target of interest Shh/*N*-terminal peptide (ShhN) in small molecule microarrays (SMM), followed by library deconvolution.[30, 55, 56]
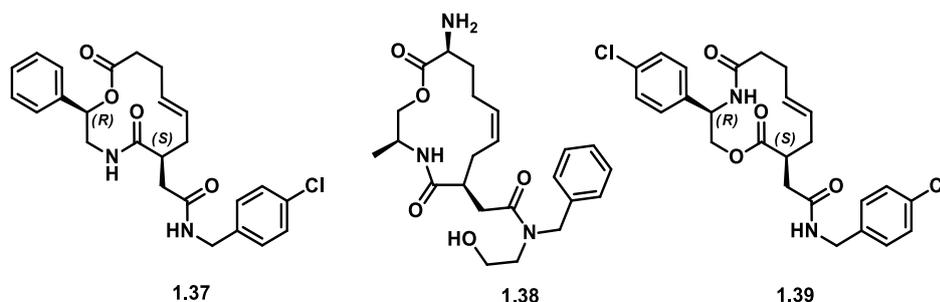
Figure 1.9. The direct Shh-binder robotnikinin **1.37**, the hit compound **1.38** and the Smo antagonist **1.39**.

A library of macrocycles was synthesised *via* a diversity-oriented synthesis (DOS) in the solid phase using the encoded one macrobead/one stock solution approach, and Evans' chiral auxiliaries, chiral 1,2-aminoalcohols, and unsaturated acids as readily available building blocks. Macrocycle **1.38** was identified as the best binder of ShhN in a concentration dependent manner.[55] Afterwards, a number of additional analogues were synthesised in liquid phase, and subjected to a secondary screening in cellular assays and structure-activity relationship (SAR) study. As the result, two novel Hh/Gli inhibitors with diverse mode of action were identified, *i.e.* the Hh ligand inhibitor robotnikinin **1.37**,[55] and the potent Smo antagonist BRD-6851 **1.39** (IC_{50} value of 0.4 μM vs 0.6 μM of cyclopamine **1.2**).[56] The authors pointed out that the presence of the inverted amino-alcohol was enough to diversify the bioactivity of analogues **1.37** and **1.39**, in spite of the retention of the 12-membered scaffold, the ene functionality and the lipophilic substituents with (*R*)-configuration (**Figure 1.9**).[56]

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In addition to the number of Hh antagonists descrived above, several other inhibitors have been identified. They include pyrrolopyridines, phthalazines, piperidines, piperazines, *N*-acylthioureas, *N*-acylureas, *N*-acylguanidines, triazoles, indazoles, estrone derivatives and many others arising from HTS of chemical libraries, rational design, chemical complexity or simplification of earlier hits. Some of these compounds are currently under investigation as potential anticancer drugs.[19, 24, 30]

1.4.3. Hh/Gli agonists

1.4.3.1. Synthetic Hh/Gli agonists from HTS

Despite the extensive findings concerning the Hh/Gli signalling pathway inhibitors, only a little is known about the related agonists. The first compound classified as a Smo agonist and named Hh-Ag 1.1 **1.40** was identified at Curis in 2002, and it emerged from the HTS of 140000 synthetic compounds in a mammalian cell based assay (**Figure 1.10**).[57]
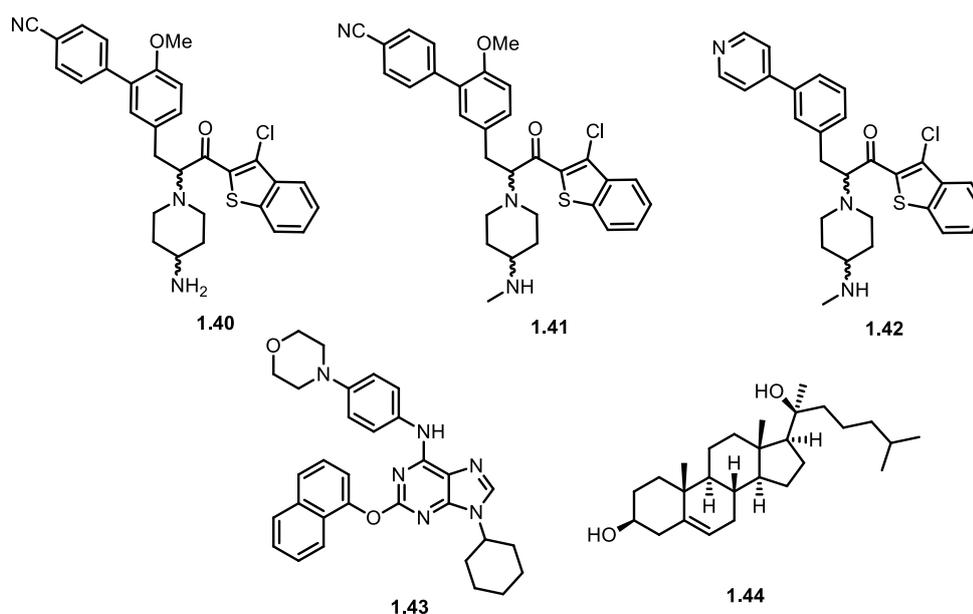
Figure 1.10. Hh/Gli agonists.

Structure-activity studies on hit **1.40** led to the synthesis of analogue Hh-Ag 1.2 **1.41** with better stability *in vitro* and *in vivo* and with less toxicity (**Figure 1.10**).[57] Chlorobenzothiophene **1.42** (named SAG, S = Smo and AG = AGonist) was studied in Beachy's group, during experiments to elucidate the biochemical mechanisms of Smo. It was shown that binding of SAG to Smo at low doses (about 3 nM) resulted in the activation of the pathways. By contrast, when the concentration was increased it acted as an inhibitor, maybe because of dual binding to both Smo and to a downstream effector

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that prevented the starting of the cascade. A few years later, a patent was placed on the prophylactic and therapeutic use of **1.40**, **1.41** and **1.42** to treat anxiety, depression, and cognitive and degenerative disorders because of the ability of such compounds to increase brain cell division.[58]

Purmorphamine **1.43**, a 2,6,9-trisubstituted purine containing a 4-morpholinoaniline is an agonist of Shh receptor Smo (**Figure 1.10**). It was discovered when researchers from The Scripps Research Institute and Novartis screened a library of 50000 heterocycles to search for compounds with osteogenesis-inducing activity.[59] In this assay, Smo agonist **1.43** induced differentiation in multipotent stem cells from fibroblast to osteocyte after 6 days of treatment at 2 μM.[59] Moreover, in a recent experiment it increased up to eight-fold the differentiation rate of mammalian dopamine-producing cells PC12 by activation of the oncogene SCL/TAL 1 interrupting locus.[60] Together, these results give value to the role of Hh/Gli agonists as promoters of stem cell differentiation, and indicate their use as possible therapeutics for Parkinson's and other degenerative diseases.

1.4.3.2. Natural Hh/Gli agonists: oxysterols

Oxysterols are oxidised derivatives of cholesterol, an endogenous steroid involved in Shh pathway activation and transduction. It is known that cholesterol participates in the autocatalytic activation process of Shh morphogen before its binding to Patch.[61] However, in the case of genetic mutations leading to deficiencies in sterol synthesis, Shh protein can still activate its pathways, although aberrancies in downstream events could be seen. Therefore, it appears that oxysterols play a central role in the Hh/Gli signalling transduction.[62] The most active steroid, 20(*S*)-hydroxycholesterol **1.44** exerts an osteoinductive effect in pluripotent mesenchymal cells by stimulation of Hh/Gli,[63] and activates Wnt and Notch target genes in bone marrow stromal cells.[64] The mechanism of action of oxysterols remains unclear, although recent experiments carried out using unnatural oxysterols supports the hypothesis of an allosteric interaction with Smo at a different site to the one used by cyclopamine **1.2** and other Smo regulators (**Figure 1.10**).[65, 66]

1.5. SUMMARY

Signalling pathways such as Hh/Gli, Wnt and Notch are being actively studied to find cures for debilitating degenerative diseases, non-healing wounds and cancer.[17] However,

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in spite of the encouraging results achieved, examples of drug resistance have been displayed during clinical trials of Hh/Gli antagonists, several side effects have been identified, and a clear correlation between kinds of tumours and dependence from Hh/Gli signalling is still not available, thus preventing personalised cures.[30, 52] Furthermore, the number of known Hh/Gli agonists is limited to few examples, and none of them have been progressed into clinical trials, although Curis are believed to be exploring applications of Hh agonists in Parkinson's, Alzheimer's and other diseases.[67] Hence, it appears that resaerch into the discovery of selective modulators of these signalling pathways is still a valuable research area.

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CHAPTER 2.
RESEARCH OBJECTIVES

The structural variety of Hh/Gli modulators and their potential medical applications have spurred our interest in the identification and synthesis of novel small molecule Hh/Gli agonists or antagonists to provide leads for anticancer drugs or regenerative therapies.

The objectives of this project are two-fold:

1. Identification of active lead compounds.
2. Conversion of the active lead compounds into drug candidates.

2.1. LEAD COMPOUNDS

Though synthetic small molecules provide an enormous contribution to the generation and identification of new drug candidates, one very successful drug discovery strategy is based on the exploitation of the potential diversity of active natural products.[68, 69]

Thus, we first sought novel potential Hh/Gli modulators amongst natural products *via* cell-based HTS. This was carried out at AnalytiCon Discovery GmbH (Postdam, Germany), one of the world's largest manufacture of pure natural product screening compounds, in cooperation with Prof. Altaba group (University of Geneva), a pioneer in developmental genetics with particular focus on the role of Gli proteins in embryo formation and diseases.

Amongst a number of natural products screened, attention has been focussed on two compounds isolated from *Withania adpressa*, coagulin L **2.1** and withanolide F **2.2** (**Figure 2.1**), which showed interesting activity in the modulation of embryonic signalling pathways. The detailed results of this collaboration are confidential.
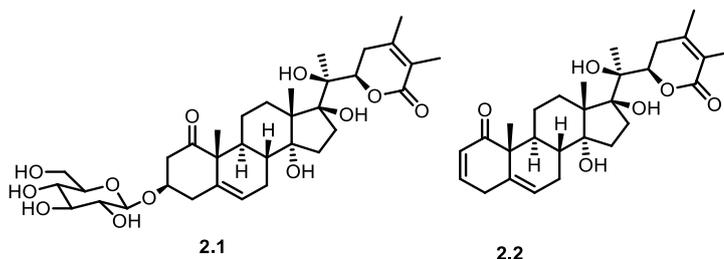
Figure 2.1. Coagulin L **2.1** and withanolide F **2.2**.

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Interestingly, these compounds have an oxygenated steroidal framework, which is also present in jervine **1.1** and cyclopamine **1.2** (**Figure 1.3**) and oxysterols such as compound **1.44** (**Figure 1.10**). Moreover, withanolide F **2.2** features an α,β-unsaturated carbonyl functionality, typical also of physalins F **1.13** and B **1.14** (**Figure 1.5**), and known to be an important anticancer pharmacophore.[70]

2.2. LEAD OPTIMISATION

In drug discovery it is important to structurally modify the lead compound in order to identify the pharmacophoric features. This is especially true for natural products, as they often bear functionalities that do not contribute to their biological activity and may lead to toxic side effects or compromise the metabolic stability.

Thus, with regards to withanolide F **2.2**, we aimed to synthesise a number of derivatives for SAR studies, as shown in **Figure 2.2**.
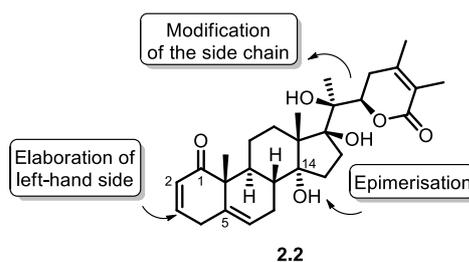
Figure 2.2 Intended modification in the hit to lead generation for withanolide F **2.2**.

There were three main aims:

1. We wanted first to investigate the influence of the right-hand side and the importance of the lactone ring in the modulation of the Hh/Gli signalling pathway. For that, we decided to elaborate the side-chain of a commercially available steroid with a number of heterocycles via organometallic additions or ring-closing metathesis reactions, in a diversity-driven approach (**Figure 2.3**). Details of this work are presented in Chapter 3.

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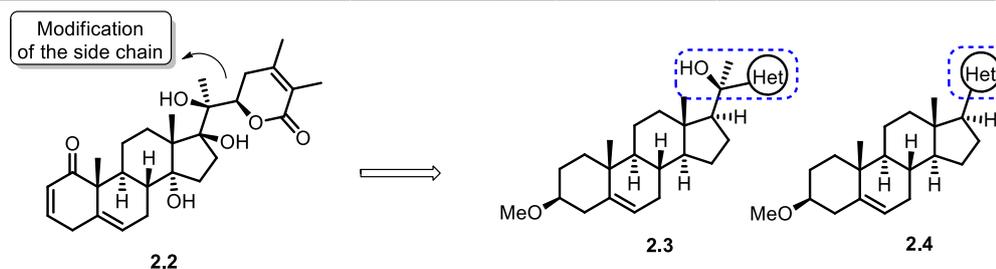
Figure 2.3. Elaborations of the side chain of a readily available steroid.

2. Changes in the configuration of the steroidal framework from a *trans*-C/D-ring fusion (common in sterols, the bile acids and nearly all types of steroids) to a *cis*-C/D-ring fusion (typical of the cardiac glycosides and related aglycones) could entail changes in bioactivity. Thus, we planned the asymmetric total synthesis of the unnatural withanolide F **2.5**, featuring the methyl group at C-13 and the hydroxyl group at C-14 *cis* to each other, starting from cheap commercially available materials (**Figure 2.4**). This research is outlined in Chapter 4.
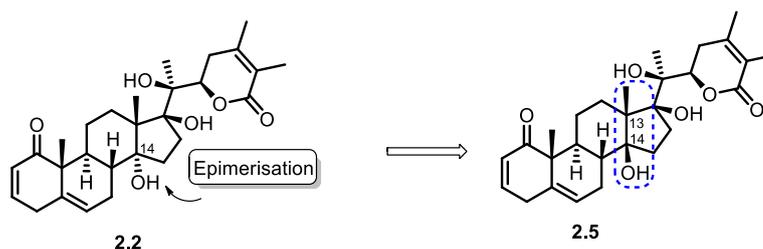
Figure 2.4. Modification of the lead compound **2.2** into its epimer **2.5**.

3. Finally, we wanted to investigate the importance of the 1-oxo-2,5-diene pattern in rings A and B towards the bioactivity. Therefore, we aimed to evaluate the reactivity of a series of withanolide derivatives **2.6** (**Figure 2.5**). This will be described in Chapter 5.
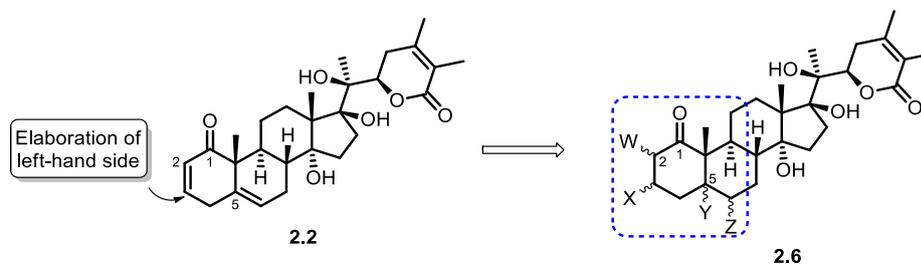
Figure 2.5. Modifications of rings A and B. W, X, Y, Z = H, C, O, S, N, halogen.

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CHAPTER 3.
TOWARDS STEROIDS WITH HETEROCYCLIC SIDE-CHAINS

3.1. THE SIDE-CHAIN OF STEROIDS

The female and male sex hormones such as estrone **3.1** and testosterone **3.2** lack a side-chain, and C-17 is oxidised to the ketone or alcohol level respectively, whilst progesterone **3.3** and corticosteroids such as cortisone **3.4** have a two carbon side-chain containing a keto group (**Figure 3.1**). Aliphatic side-chains are more common in bile acids such as cholic acid **3.5**, sterols such as cholesterol **3.6** and phytosterols such as stigmasterol **3.7**. Polyhydroxylated aliphatic side-chains decorate the more exotic plant hormones such as brassinolide **3.8** and cucurbitacine B **3.9**, which possess important effects in plant growth[71] and antitumour activity,[72] respectively, and in the insect hormones[73] such as ecdysone **3.10**.
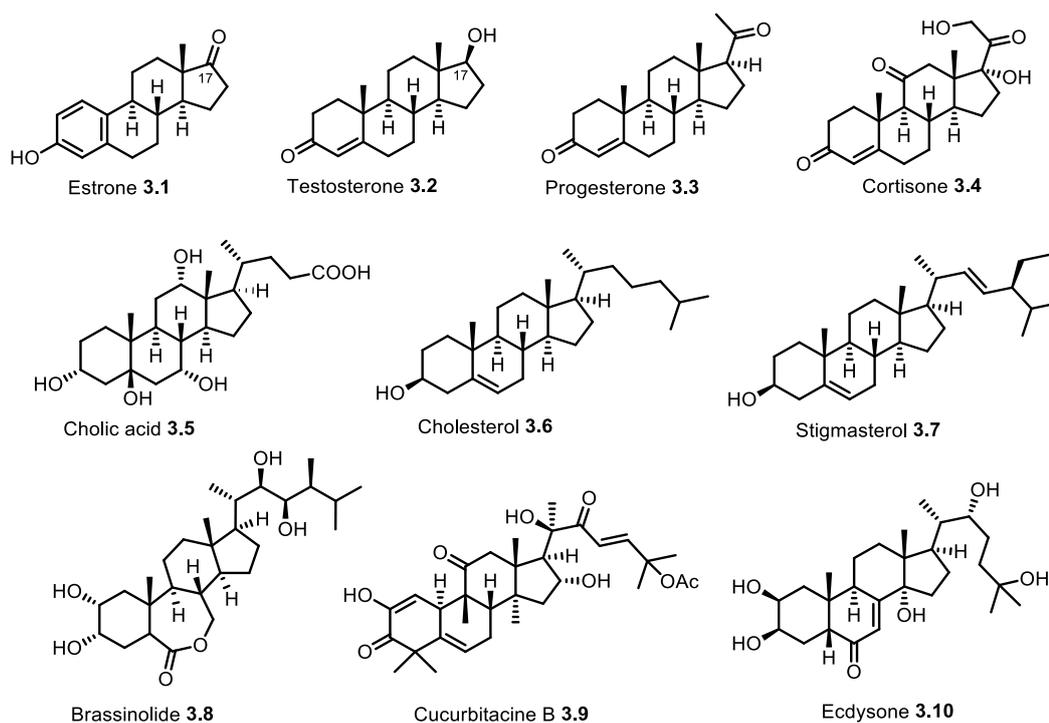
Figure 3.1. Selected examples of open-chain steroids.

Nature has also provided a number of steroids decorated with cyclic appendages in the form of oxygenated and, less commonly, nitrogen-containing heterocycles (**Figure 3.2**). Cardiac glycosides and aglycones such as digitoxigenin **3.11** and bufalin **3.12** possess an unsaturated γ- or δ-lactone, respectively, which is directly linked to C-17. Similar functionalities are present amongst withanolides such as withanolide B **3.13** and

ixocarpalactone A **3.14**, although a one or two carbon linker distances the heterocycle from the tetracyclic steroidal core. A unique spiro group consisting of two oxygenated rings fused at C-22 forms the side-chain of the sapogenins such as sarsasapogenin **3.15**. Finally, cyclic side-chains containing nitrogen are present in some steroidal alkaloids: tomatidine **3.16** and veratramine **3.17** are two examples of these compounds with 'normal' (6-6-6-5 tetracyclic) and 'modified' (6-6-5-6 tetracyclic) skeletons, respectively.

Digitoxigenin **3.11**   Bufalin **3.12**   Withanolide B **3.13**   Ixocarpalactone A **3.14**

Sarsasapogenin **3.15**   Tomatidine **3.16**   Veratramine **3.17**
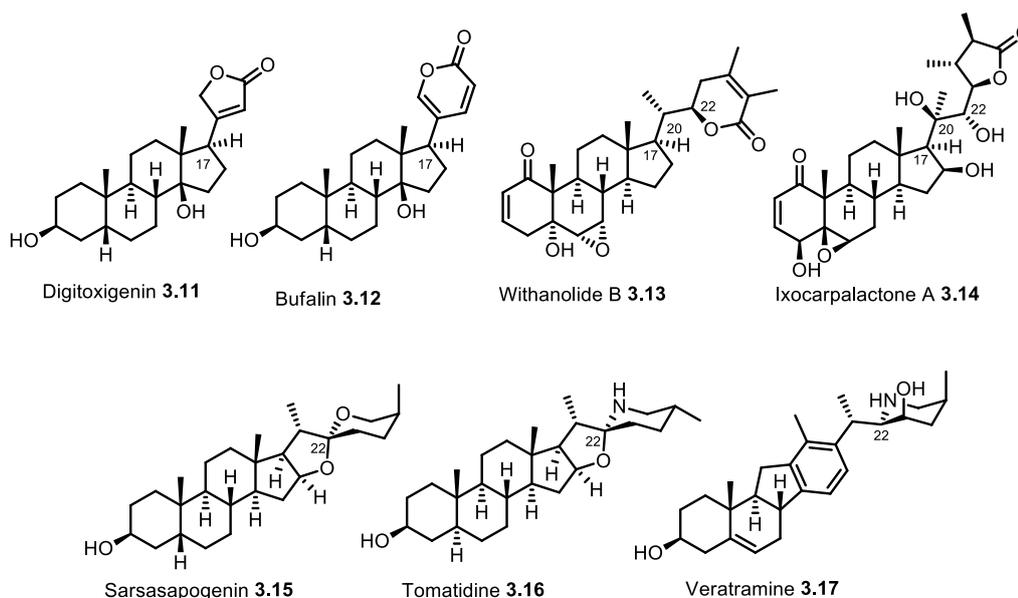
Figure 3.2. Selected steroids with cyclic side-chains.

3.1.1. The side-chain of steroidal modulators of Hh/Gli signalling pathway

As described in Chapter 1, steroidal compounds play a critical role in the modulation of the embryonic signalling pathways, particularly the Hh/Gli, but also the Wnt and Notch.[38, 43, 63, 66]

3.1.1.1. Steroidal Smo modulators

Cyclopamine **1.2** and its derivatives, featuring an alkaloid heterobicyclic side-chain, are Hh/Gli antagonists binding Smo in a narrow pocket between the 7-membered trans-membrane region and the extracellular domain.[66, 74] Oxysterols such as 20(*S*)-hydroxycholesterol **1.44** also bind Smo, and their binding site has been identified at a different place to cyclopamine **1.2** and mimics, *i.e.* at an extracellular domain rich in cysteines. Oxysterols are characterised by an aliphatic hydroxylated side-chain, and they are the only naturally occurring Hh/Gli activators known.[65, 66] Recently, researchers at the Harvard Medical School have developed the sterol **3.18** bearing a nitrogen-containing

aliphatic side-chain. Interestingly, this compound showed inhibition of Hh/Gli in mouse embryo fibroblast cells (NIH-3T3) with an IC_{50} value of 5 µM, by binding to Smo at the same site as oxysterols (**Figure 3.3**).[75]
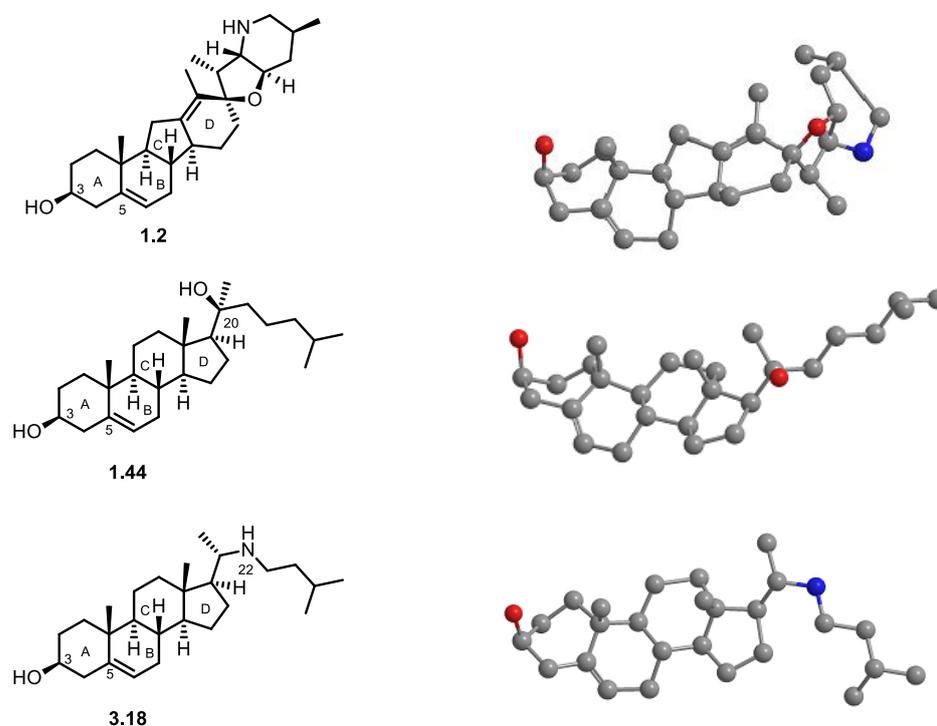
Figure 3.3. Spatial representations of cyclopamine **1.2**, 20(*S*)-hydroxycholesterol **1.44** and 22-azacholesterol **3.18**.

The Smo modulators **1.2**, **1.44** and **3.18** possess a common steroidal framework with *trans*-junctions between rings B and C, and C and D, and a 3β-hydroxyl-5-ene pattern in rings A and B (**Figure 3.3**). However, these compounds possess remarkably different mechanisms of action.[62, 75] Therefore, we could speculate that the orientation and the presence of heteroatoms in the side-chain rather than the steroidal skeleton could be the pharmacophore responsible for their different activities.

3.1.1.2. Steroidal Gli transcription factors modulators

Steroids are also found amongst the compounds which modulate the Gli transcription factor. In fact, two compounds belonging to the withanolide and the cardenolide class, physalin B **1.13** and β-D-glucosyl-(1→4)-β-D-thevetoside **1.19** (**Figure 3.4**), have shown interesting Gli transcription factor inhibition, with IC_{50} values of 1.4 and 0.11 µM, respectively.[38, 43] However, their binding site remains unclear.

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Physalin **1.13** and cardenolide **1.19** are structurally very different. The 1-oxo-2-ene system and the 5β,6β-epoxide present in the left-hand side of compound **1.13** are absent in compound **1.19**, while the 3β-hydroxyl group ether-linked to the sugar appendage in compound **1.19** is missing in withanolide **1.13**. (**Figure 3.4**). Thus, we imagined that the pharmacophore of the compounds involved in the inhibition of the Hh/Gli signalling might be the right-hand side side-chain containg a lactone-ring which is present in both compounds, although included in a complex cage-shaped structure in physalin **1.13** whilst easily accessible in cardenolide **1.19**.
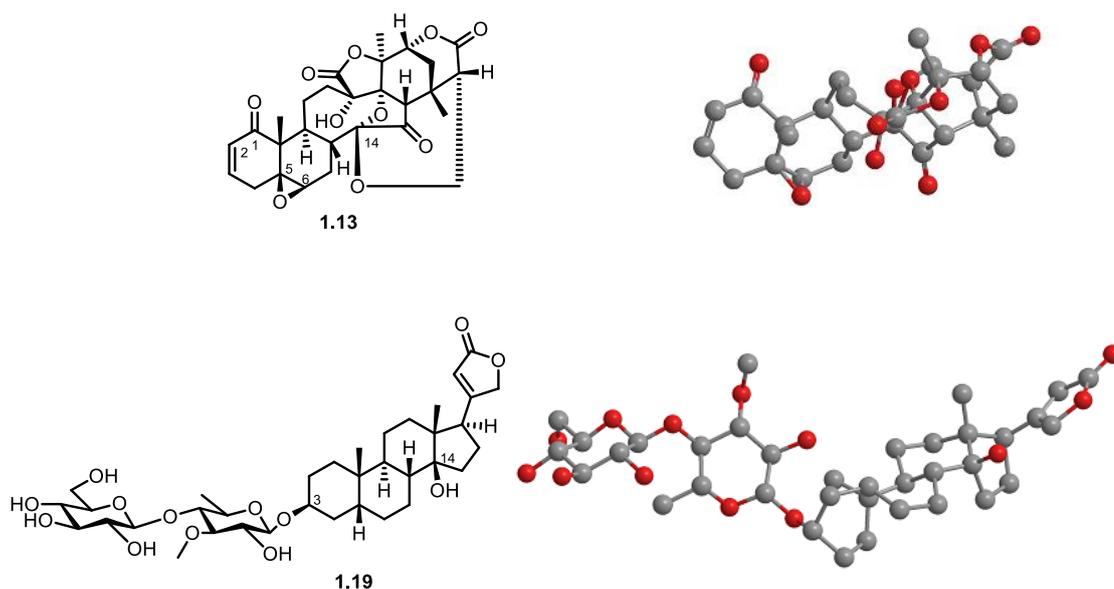
Figure 3.4. Spatial representations of the Gli transcription factors inhibitors **1.13** and **1.19**.

3.1.1.3. Structure-activity relationship studies of oxysterols

Preliminary SAR studies by Nachtergaele *et al.* on oxysterols showed that the stereochemistry at the chiral centres was crucial for the activation of the Hh/Gli pathway in Shh-responsive mouse embryonic fibroblast cell line (NIH-3T3 cells). 20(*S*)-Hydroxycholesterol **1.44** proved to be the most active compound (EC$_{50}$ value of 3 µM), while the 20(*R*)-diastereoisomer **3.19** and the enantiomer **3.20** were inactive. Compounds **3.21**-**3.27** lacking the C-20 hydroxyl were also inactive, even in the presence of hydroxyls distributed along the side-chain or in the tetracyclic scaffold (**Figure 3.5**).[65]

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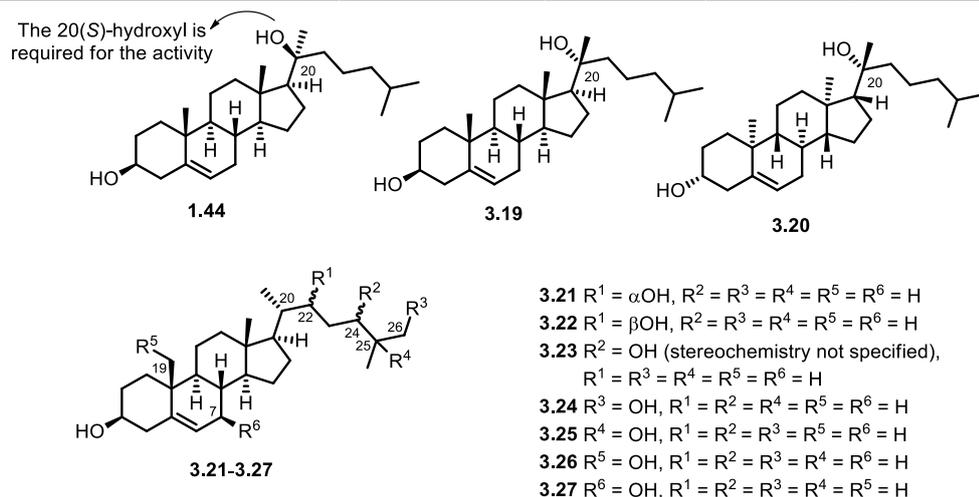
Figure 3.5. SAR of oxysterols as Hh/Gli activators in NIH-3T3 cells.[65]

A number of studies have highlighted the importance of oxysterols in Hh/Gli signalling,[61-66, 76, 77] and this class of steroids has also been recently evaluated for their selectivity towards the liver X receptor (LXR), a nuclear receptor implied in the homeostasis of cholesterol and identified as an inhibitor of Hh/Gli signalling.[78] 20(*S*)-Hydroxycholesterol **1.44**, 20(*S*),25-dihydroxycholesterol **3.28** and 23(*S*)-hydroxycholesterol **3.29** (**Figure 3.6**) demonstrated the best activation of the Hh/Gli signalling in the osteogenic pluripotent stem cell line (C3H10T1/2) with 91.8, 107.8 and 87.1 fold mRNA induction respectively over DMSO control, and 6.8, 4.1 and 17.8 fold Hh selectivity against LXR. Moreover, oxysterol **3.29** upregulated Gli1 and Patch expression in both C3H10T1/2 and murine bone stromal cell line (M2-10B4) with Ec_{50} values ranging between 0.54-0.65 µM, and 3 fold selectivity relative to LXR.[78] Compound **3.29** lacks the 20(*S*)-hydroxyl, thus the question whether this functionality is crucial seems to remain unanswered.
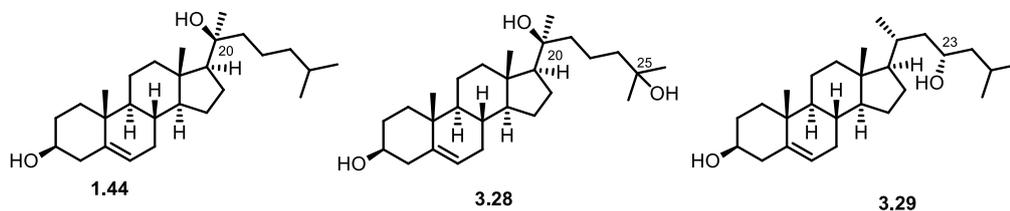
Figure 3.6. Oxysterols inducing osteogenic differentiation with highly Hh/Gli selective agonism relative to liver X receptor.[78]

After this PhD project began, Nedelcu *et al.* published SAR studies on the side-chains of oxysterols and azasterols.[75] With regard to oxysterols, it was noted that the stereochemistry at C-20 and the length of the side-chain had influence in the Hh/Gli

activation (**Figure 3.7**). Compounds **3.30**-**3.32** and **3.35**-**3.37** which possess linear side-chains of two, three or four carbon in length were not active in the luciferase assay on a cell line derived from mouse embryo fibroblast (Shh Light cells II), whether the C-20 hydroxyl was in the β- (**3.30**-**3.32**) or α-configuration (**3.35**-**3.37**). Compounds with linear side-chains of five (**3.33** and **3.34**) or six (**3.38** and **3.39**) carbons in length preserved the activity as long as the 20(*S*)-stereochemistry was maintained. For example steroids **3.33** and **3.34** were active, while the 20(*R*)-diastereoisomers **3.38** and **3.39** were inactive. Saturation of the C-5,C-6 double bond did not affect the bioactivity, and 20(*S*)- and 20(*R*)-steroids **3.40** and **3.41** were active and inactive, respectively, against the Hh/Gli signalling, as were the parent unsaturated 20(*S*)- and 20(*R*)-compounds **3.34** and **3.39**. Finally, it was shown that a free hydroxyl group at C-3 was required, since the 3β-methoxy-derivatives **3.42** and **3.43** exhibited reduced bioactivity.[75]

3.30 n = 0	**3.35** n = 0		
3.31 n = 1	**3.36** n = 1	**3.40** C-20: βOH, αMe	**3.42** C-20: βOH, αMe
3.32 n = 2	**3.37** n = 2	**3.41** C-20: αOH, βMe	**3.43** C-20: αOH, βOH
3.33 n = 3	**3.38** n = 3		
3.34 n = 4	**3.39** n = 4		
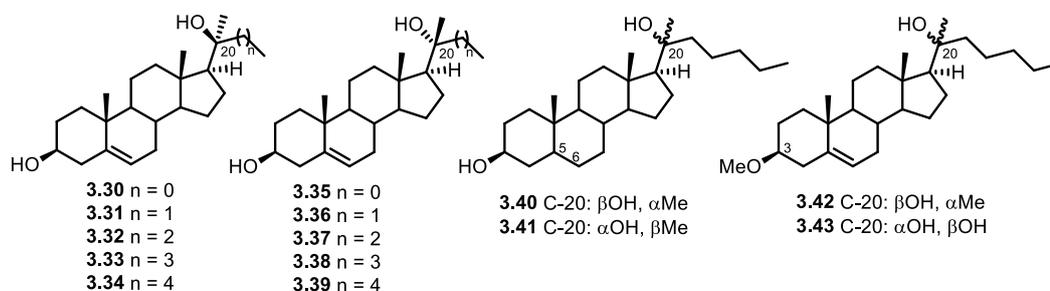
Figure 3.7. Oxysterol SAR for Hh/signalling activation by Nedelcu *et al.*[75]

For azasterols, the length of the side-chain and the stereochemistry at C-20 appear to be less important (**Figure 3.8**).[75] In fact, azasterols **3.44**, **3.45**, **3.48** and **3.49** retain the inhibitory activity of the lead 22-azasterol **3.18**, irrespective of the stereochemistry at C-20. Instead, hydroxylation at the terminal carbon such as in compounds **3.46**, **3.47**, **3.50** and **3.51** resulted in reduced activity [75]

3.18	**3.44** C-20: β-Me; R = H	**3.48** C-20: β-Me; R = H
	3.45 C-20: α-Me; R = H	**3.49** C-20: α-Me; R = H
	3.46 C-20: β-Me; R = OH	**3.50** C-20: β-Me; R = OH
	3.47 C-20: α-Me; R = OH	**3.51** C-20: α-Me; R = OH
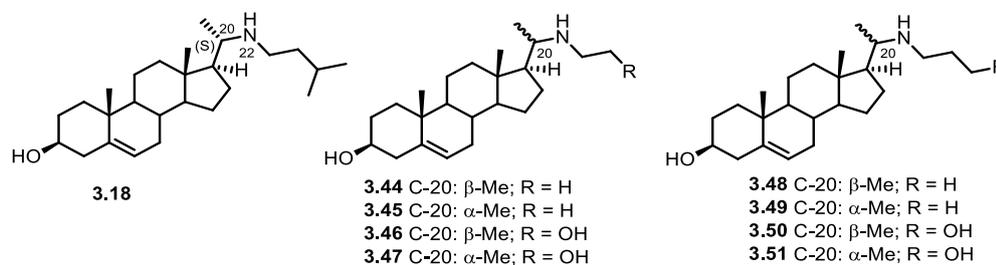
Figure 3.8. Azasterol SAR for Hh/signalling inhibition by Nedelcu *et al.*[75]

3.2 PROJECT AIM: TOWARDS Hh/Gli MODULATORS WITH HETEROCYCLIC SIDE-CHAINS

The chemical variety of steroidal Hh/Gli modulators reflects the diversity of their modes of action and, up to date, a comprehensive correlation of these structures with their activities is not available. In the light of the results shown in **Figures 3.3**-**3.8**, we first focussed our attention on an investigation of side-chain variations. To the best of our knowledge, no examples of heterocyclic analogues of steroids, in particular oxa-, thia- and aza- analogues, have been evaluated in embryonic signalling pathways. Thus, our aim was to synthesise a number of steroids bearing diverse side-chains and to test them in Hh/Gli signalling pathway in order to identify the structural features enhancing the biological activity.

Our interest in the preparation of a small library of heterocyclic steroids in a diversity-oriented manner drove a need for a flexible strategy in which a variety of side-chains could be attached to a common intermediate (**Scheme 3.1**).
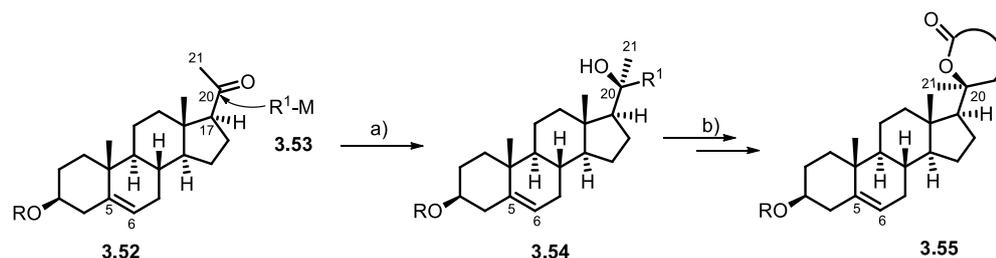
Scheme 3.1: General approach to the construction of heterocyclic side-chains. R = H or protecting group, R^1 = heterocycle or aliphatic chain.

It was envisaged that nucleophilic addition of organometallic reagents **3.53** to the C-20 ketone of readily available steroid **3.52** would be a feasible way to synthesise a number of novel compounds **3.54** featuring the C-20 β-hydroxyl group, which seems to be required for the bioactivity of oxysterols (**Scheme 3.1**, a).

Also of interest was the synthesis of potential steroidal Gli transcription inhibitors containing a lactone ring in the side-chain (generalised in compounds **3.55**), which could mimic members of the cardenolide, bufadienolide and withanolide classes. These compounds could be generated by further elaboration of compound **3.54** as described later (**Scheme 3.1**, b).

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3.3. RESULTS AND DISCUSSION

3.3.1. The scaffold: 3β-methoxy-pregnenolone 3.60

Commercially available pregnenolone **3.56** was chosen as a suitable building block as it possesses a cholesterol-like core and a ketone at C-20 for elaboration. We realised that the 3β-hydroxyl-5-ene pattern can be converted into progesterone **3.57** (a compound with strong hormonal activity)[79] by means of the enzyme 3β-hydroxysteroid dehydrogenase (HSD3B) in the presence of NAD$^+$. Moreover, 3β-hydroxysteroid sulfotransferases (HST), in the presence of 3'-phosphoadenosine-5'-phosphosulfate (PAPS), can give 3β-pregnenolone sulfate **3.58**, which is also biologically active (**Scheme 3.2**).[80]
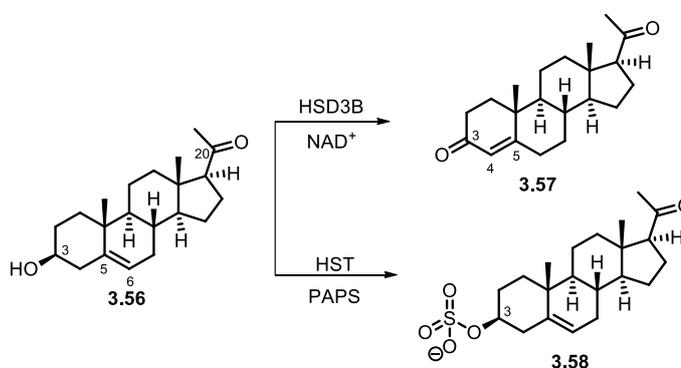
Scheme 3.2: Enzymatic transformation of pregnenolone **3.56**.

Although it is has recently be noted that a methoxy group at C-3 might be detrimental to activity against the Hh/Gli signalling,[75] Baulieu *et al.*[81] have shown that of 3β-methoxy-pregnenolone **3.60** might be useful to treat neurodegenerative lesions, thus providing a potential treatment for Parkinson's and Alzheimer's deseases. Therefore, we decided to convert the 3β-hydroxyl group of pregnenolone **3.56** into its 3β-methyl ether **3.60** before constructing the side-chain (**Scheme 3.3**).[81-84]
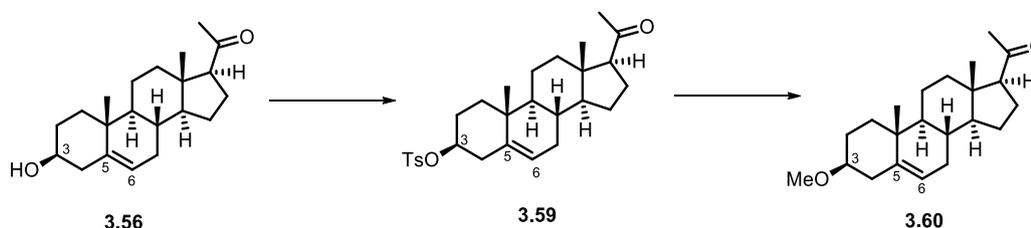
Scheme 3.3. Synthesis of 3β-methoxy-pregnenolone **3.60**.

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3.3.1.1. Neighbouring group participation in the synthesis of 3β-methoxy-pregnenolone **3.60**

The conversion of alcohol **3.56** into the 3β-methyl ether **3.60** with retention of configuration is a well-known reaction in the steroid field which occurs in two steps and requires the participation of the C-5,C-6 double bond as a neighbouring group (**Scheme 3.3**).[81-84] Thus, homoallylic alcohol **3.56** was first converted into tosylate **3.59** (**Table 3.1**).
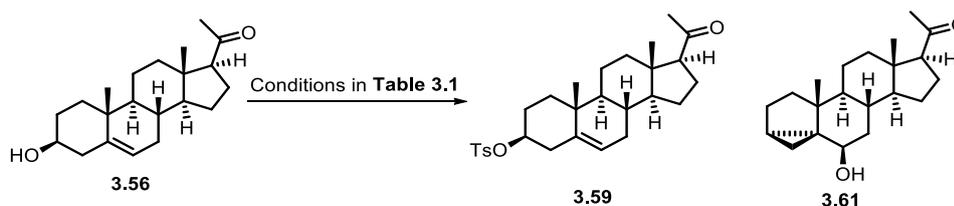
Entry	Conditions	Yield / Comments
1	*p*-TsCl (3.3 eq), py, rt	**3.59** 94%[a]
2	*p*-TsCl (5 eq), py, rt	**3.59** 79%[b]
3	*p*-TsCl (5 eq), DMAP (0.01 eq), py, rt	**3.59** 92%[a]
4	*p*-TsCl (5 eq), DMAP (0.01 eq), py, 30 °C	**3.59** 67%[a]
5	*p*-TsCl (5 eq), DMAP (0.1 eq), py, rt	**3.59** 64%,[a] **3.61** 7%[b]
6	*p*-TsCl (2 eq), DMAP (1 eq), Et$_3$N (3 eq), CH$_2$Cl$_2$, rt	**3.59** 99%

Table 3.1. Diverse reaction conditions for the synthesis of 3β-tosyl pregnenolone **3.59**. Reactions were performed on 500 mg – 5 g scale. Reaction time: 18 h. [a]Purification *via* crystallisation from water. [b]After extraction of the mother liquor with CH$_2$Cl$_2$, then column chromatography on silica gel.

Early attempts following published procedures led to tosylate **3.59** in yield up to 94% by exposure of pregnenolone **3.56** to an excess of *p*-toluenesulfonyl chloride in pyridine (**entries 1** and **2**),[81] and catalytic amounts of 4-dimethylaminopyridine (**entry 3**).[84] The yield dropped to 67% when the reaction mixture was stirred at 30 °C (**entry 4**). When the amount of 4-dimethylaminopyridine was increased to 10 mol% cyclic alcohol **3.61** was also isolated in 7% yield (**entry 5**).[83, 85] In order to avoid the vast amount of pyridine used and simplify the work-up, we tried to carry out the reaction using *p*-toluenesulfonyl chloride, triethylamine and 4-dimethylaminopyridine in dichloromethane as previously reported for the tosylation of an unhindered primary alcohol (**entry 6**).[86] Pleasingly, when these conditions were applied to our substrate, tosylate **3.59** was obtained in near-quantitative yield.

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3.3.1.2. i-Steroid formation

As mentioned above, in one example the *i*-steroid **3.61** was obtained in 7% yield (**entry 5**, **Table 3.1**). Compound **3.61** is known in the literature[83, 85] but the incomplete and conflicting published assignments of ^{1}H- and ^{13}C-NMR signals recorded at 200 MHz did not match our results. Therefore compound **3.61** was synthesised following published procedures for full characterisation and structural confirmation (**Scheme 3.4**).[83]
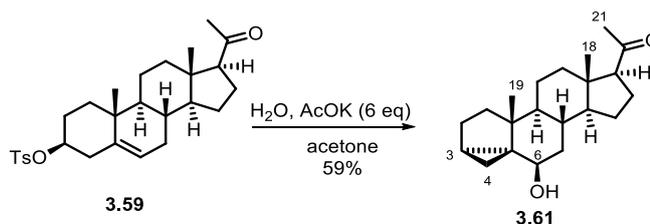
Scheme 3.4. Synthesis of cyclo-alcohol **3.61**.

Compound **3.61** prepared *via* the literature method was identical to the sample obtained from the tosylation procedure. On inspection of ^{1}H-, ^{13}C-, DEPT-NMR and 2D ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC and ^{1}H,^{1}H-COSY NMR spectra recorded at 500 MHz the signals of the C-4 cyclopropylic protons were identified at 0.28 and 0.51 ppm, while the proton at C-3 was observed at 1.07 ppm. These data are in contrast to the published values, which showed the three cyclopropylic protons as a multiplet between 0.24 and 0.49 ppm. A discrepancy was also found for the chemical shift of the C-19 methyl group at 1.03 ppm, against the published value of 1.18 ppm. On the other hand, the chemical shifts of the C-6 proton (3.24 ppm) and of the C-18 and C-21 methyl groups (0.65 and 2.09 ppm, respectively) matched the literature.[83]

The mechanism leading to *i*-steroids was studied first by Shoppee[87] and Winstein *et al.*[88-91] The reaction occurs in two steps, that is, first conversion of the 3β-hydroxyl group **3.6** into a leaving group such as a chloride or tosylate (compounds **3.62** and **3.63**), followed by alcoholysis or acetolysis to give steroids **3.64-3.66**. However, in the presence of a base, the 3β-alkyl ethers **3.64-3.66** were not produced, but 3,5-cyclosteroids also called *i*-steroids **3.67-3.70** were formed (**Scheme 3.5**).

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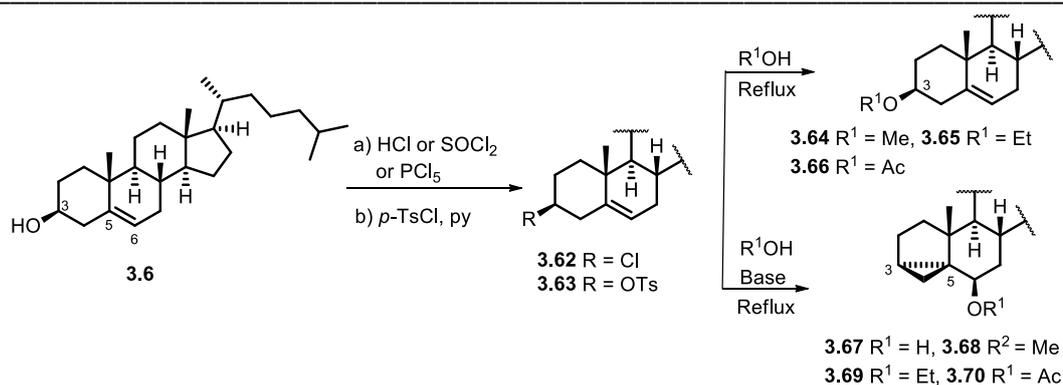
Scheme 3.5. Neighbouring group participation in the nucleophilic substitution at C-3 of cholesterol **3.6**.[88, 90]

Looking at the mechanism (**Scheme 3.6**), the first step is the unimolecular displacement of the leaving group to give the cyclopropylic cation **3.71**, which is in equilibrium with the unsaturated form **3.72**. In the presence of nucleophiles such as methanol, the more reactive carbocation **3.71** readily gives the kinetic product **3.68**. However, the instability of the *i*-steroids under slightly acidic conditions enables the reverse reaction, thus leading to the formation of the thermodynamic product **3.64**. On the contrary, when a buffer such as potassium acetate is added to the reaction mixture, cyclosteroid **3.68** is formed. [88, 91]
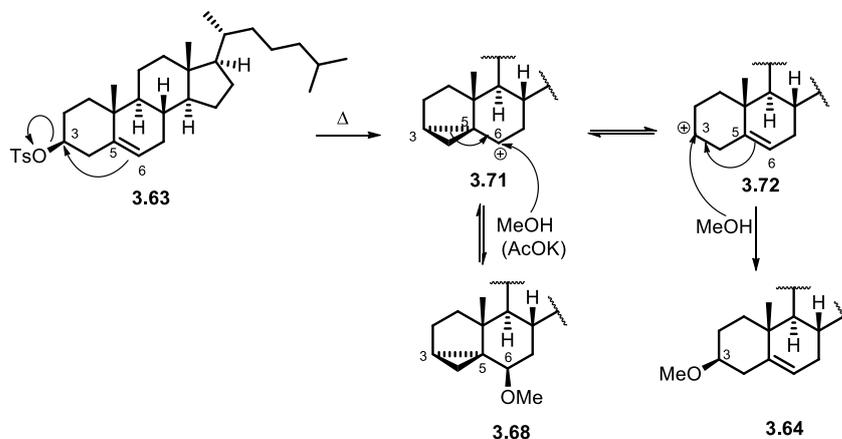
Scheme 3.6. Neighbouring group participation in homoallylic steroids.

3.3.1.3. Synthesis of 3β-methoxy-pregnenolone *3.60* from tosyl pregnenolone *3.59*

With 3β-tosyl pregnenolone **3.59** in hand, we were in a position to carry out its stereoselective conversion to the 3β-methoxy-derivative **3.60** (**Table 3.2**).
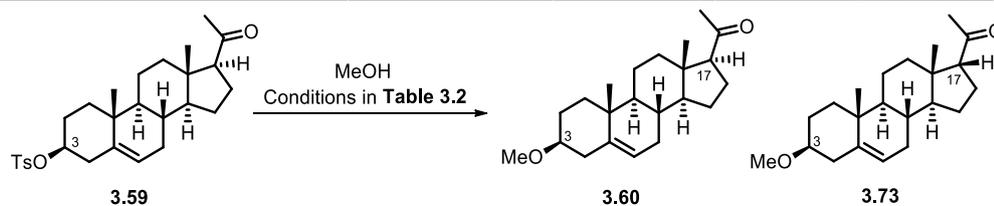
Entry	Conditions	Results
1	**3.59** 0.1 M, reflux, 4 h	**3.60**/**3.73** = 81/19, 99%
2	**3.59** 0.01 M, reflux, 18 h	**3.60**/**3.73** = 76/24[a]
3	**3.59** 0.1 M reflux, 18 h	**3.60**/**3.73** = 80/20[a]
4	**3.59** 0.01 M, rt, 18 h	Sm **3.59**[a]
5	**3.59** 0.1 M, rt, 18 h	Sm **3.59**[a]
6	**3.59** 0.01 M, 30 °C, 18 h	Mainly sm **3.59**, trace of **3.60**[a]
7	**3.59** 0.1 M, 30 °C, 18 h	Sm **3.59** and **3.60** = 1/1[a]
8	**3.59** 0.03 M, reflux, 1.5 h	**3.60**/**3.73** = 89/11, 100%

Table 3.2. Diverse reaction conditions for the synthesis of 3β-methoxy-pregnenolone **3.60**. All the reactions were performed in MeOH as reagent and solvent, on a 50 mg scale. [a]Ratio determined by NMR analysis of the crude product.

In spite of the stereospecificity and excellent yields published,[81, 82, 84] we found that methyl ether **3.60** was not isolated as a single diastereoisomer, but with its 17α-epimer **3.73** in an 81/19 ratio as determined by NMR analysis (**entry 1**). Attempts to minimise the formation of the undesired isomer by changing the reaction time, temperature, molarity and purification methods proved ineffective (**entries 2**-**8**).

However, the major and desired isomer **3.60** could be isolated by recrystallisation from methanol leaving the filtrate enriched up to 47/53 in favour to the 17α-isomer **3.73**. Crystallographic studies confirmed the structure of compound **3.60** (**Figure 3.9**).
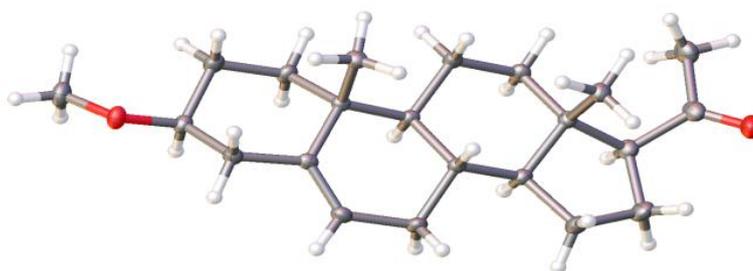
Figure 3.9. X-Ray crystallography of 3β-methoxy-pregnenolone **3.60** (CCDC 1021885, **Appendix VII**).

The 17α-isomer **3.73** is known in the literature, although it has never been fully characterised. Rodig *et al.*[92] described it as a secondary product of the synthesis of 3β-methoxy-pregnenolone **3.60** from tosylate **3.59**. Its structure was proposed after a

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process of elimination which involved the comparison of infrared, optical rotation, optical rotatory dispersion and elemental analysis data with previously reported compounds 3α-methoxy-pregnenolone **3.74** and 6β-methoxy-cyclo-steroid **3.75** (**Figure 3.10**).[92]

3.73　　　　　　**3.74**　　　　　　**3.75**
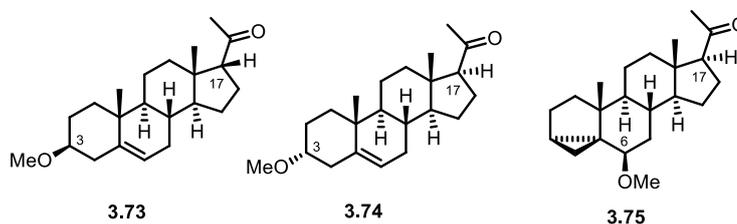
Figure 3.10. Structures of 17α,3β-methoxy-pregnenolone **3.73**, 3α-methoxy-pregnenolone **3.74** and 6β-methoxy-cyclo-pregnenolone **3.75**.

We were therefore interested in obtaining a pure sample of the 17α-isomer **3.73**. Since its isolation by recrystallisation was unsuccessful,[92] we tried to epimerise the C-17 position of the 17β-isomer **3.60** by treatment with acid (**entries 1-4**, **Table 3.3**) or base (**entries 5 and 6**).[93]

3.60　　　　Conditions in **Table 3.3**　　　　**3.73**
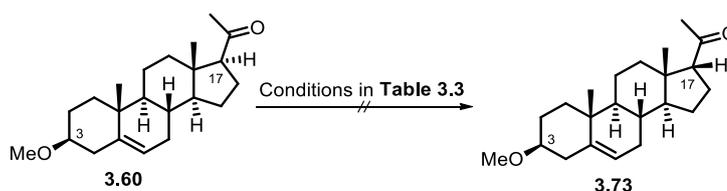
Entry	Conditions	Results
1	AcOH, THF, rt, 20 h	Sm **3.60**
2	AcOH, THF, reflux, 20 h	Sm **3.60**
3	AcOH, MeOH, reflux, 20 h	Sm **3.60**
4	PTSA, MeOH, reflux, 18 h	Sm **3.60** and decomposition
5	LiHMDS, -78 °C, 2h	Sm **3.60**
6	KOH, MeOH, reflux, 18 h	Mixture **3.60/3.73** = 4/1

Table 3.3. Attempted isomerization of 3β-methoxy-pregnenolone **3.60** to the 17α-epimer **3.73**.

Unfortunately, the only success was achieved when the β-isomer **3.60** was exposed to refluxing methanol containing potassium hydroxide followed by a quench with acetic acid (**entry 6**). This provided a 4/1 ratio of compound **3.60** to **3.73**, but the diastereoisomers could not be separated and no further work was carried out.

3.3.1.4. Alternative routes to 3β-methoxy-pregnenolone 3.60

Alternative conditions to obtain 3β-methoxy-pregnenolone **3.60** in one step from pregnenolone **3.56** were also explored (**Table 3.4**).

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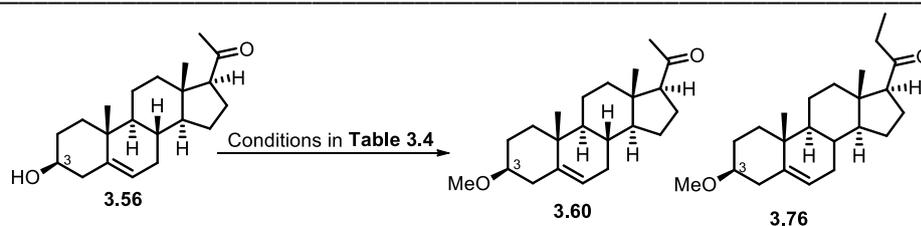
Entry	Conditions	Yield	
1	MeI (20 eq), Ag$_2$O (1.5 eq), MS 4 Å, Et$_2$O/THF, 40 °C, 18 h	**3.60**	70%
2	MeI (5 eq), NaH (1.2 eq), Et$_2$O/THF, rt, 18 h	**3.60**	80%
3	MeI (5 eq), NaH (1.5 eq), Et$_2$O/THF, rt, 3 d	**3.60**	63%
		3.76	5%

Table 3.4. Alternative routes to 3β-methoxy-pregnenolone **3.60**.

Treatment of pregnenolone **3.56** with freshly prepared silver(I) oxide and an excess of methyl iodide led to diasteromerically pure methyl ether **3.60** in 70% yield compared with the 57% published yield (**entry 1**).[94] Methylation using sodium hydride and methyl iodide, conditions applied in the synthesis of a 3β-methoxy derivative of cholesterol with a saturated aliphatic side-chain,[95] was also successful (**entry 2**). The major secondary product of the reaction was identified as the ethyl derivative **3.76** (**entry 3**),[96] formed *via* an α-carbonyl alkylation of the 3β-methyl ether **3.60** with excess methyl iodide. Compound **3.76** is known in the literature although it has never been fully characterised.[96] Its complete structural assignment is provided in the experimental section.

Finally, we synthesised *i*-pregnenolone-methyl-ether **3.75** by refluxing tosyl pregnenolone **3.59** in methanol containing potassium acetate.[82, 97] As expected, cyclo-steroid **3.75** was exclusively obtained in 79% yield (**Scheme 3.7**).
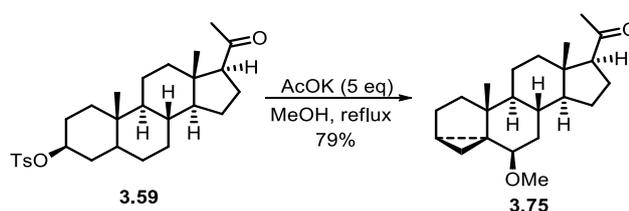
Scheme 3.7. Synthesis of *i*-pregnenolon-methyl ether **3.75**.

3.3.1.5. ^{1}H- and ^{13}C-NMR analysis

Although 3β-methoxy-pregnenolone **3.60,** 17α,3β-methoxy-pregnenolone **3.73**, tosyl pregnenolone **3.59**, ethyl ketone **3.76**, cyclo-3β-hydroxy-steroid **3.61** and cyclo-3β-methoxy-steroid **3.75** (**Figure 3.11**) are reported in several papers, no detailed

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NMR data are available. Therefore, we ran 1D- and 2D-NMR experiments for the full assignment of these compounds using a 500 MHz spectrometer. In particular, we found it convenient to narrow the spectral window from 5.5 to 0 ppm to obtain a better resolution of the overlapping low field signals in COSY, HSQC and HMBC techniques. The chemical shifts and spectra for these compounds are reported in **Appendices I-III**.
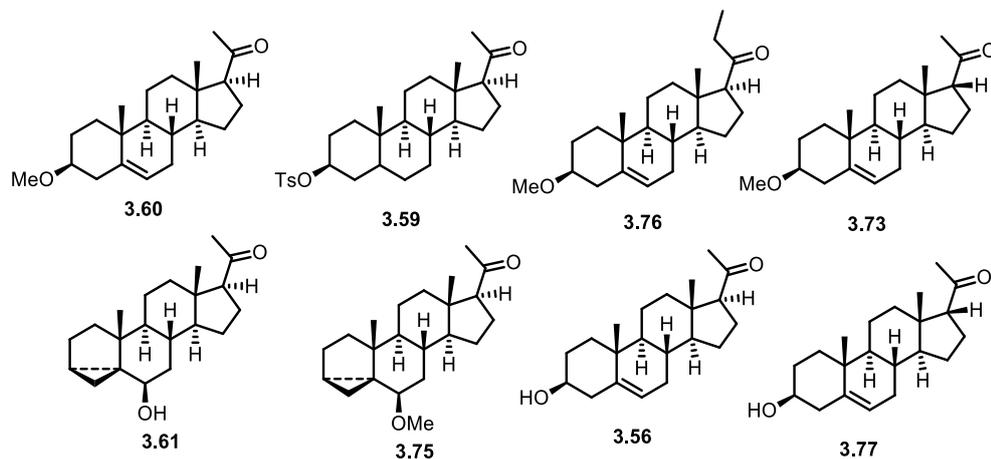
Figure 3.11. Compounds discussed in **Appendices I-III**.

3.3.2. Stereoselective addition of organolithium reagents to the side-chain of steroids

The reaction of organometallic reagents with C-20-keto steroids is known as a valuable method to build side-chains with aliphatic or aromatic appendages whose diversity is reflected in a wide range of biological activities.[98, 99]

3.3.2.1. Thiazolyl-steroids

Encouraged by earlier work confirming that addition of lithiated thiazole and pyridine to C-20 ketones would give preferably the corresponding β-tertiary alcohols,[99] we wanted to evaluate the outcome of these reactions on 3β-methoxy-pregnenolone **3.60**.

Although metallation at C-2 of thiazole is often carried by lithium halogen exchange of 2-bromothiazole,[99, 100] examples of direct lithiation can be also found. [100-102] Thus, 2-lithiothiazole was prepared *in situ* by the reaction of thiazole **3.78** with *n*-butyllithium at −30 °C, and 3β-methoxy-pregnenolone **3.60** was added (**Table 3.5**).
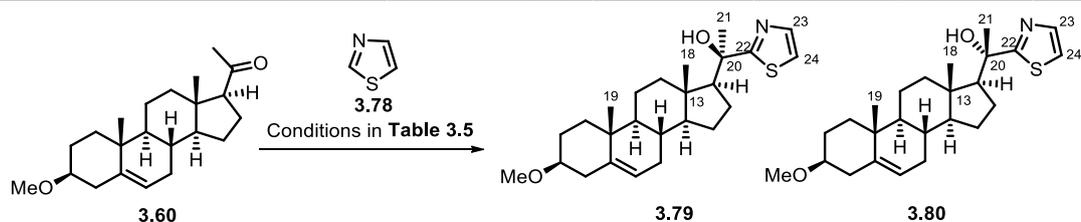
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Table 3.5. Synthesis of thiazolyl derivative **3.79**.

Entry	Conditions	Yield
1	i) **3.78** (1.1 eq), *n*-BuLi (1.1 eq), -30 °C, 30 min	**3.79**/**3.80** = 9/1: 35%
	ii) **3.60**, -20 °C to rt, 4 h	Sm **3.60**: 51%
2	i) **3.78** (2.5 eq), *n*-BuLi (2.5 eq), -30 °C, 30 min	**3.79**/**3.80** = 9/1: 84%
	ii) **3.60**, -20 °C to rt, 2 h	

We found that when a stoichiometric amount of organolithium was used, thiazolyl steroid **3.79** was obtained in poor yield along with recovered starting material (**entry 1**). In the presence of an excess of lithiothiazole, the yield could be increased up to 84% and in a shorter reaction time (**entry 2**). In both cases, analysis by NMR spectroscopy showed the presence of an inseparable compound identified as the C-20 epimer **3.80** (**3.79**/**3.80** = 9/1). However, pure 20(*R*)-thiazole **3.79** could be obtained in 48% yield by recrystallisation of the epimeric mixture from methanol, leaving the filtrate as a 77/23 mixture of diastereoisomers **3.79** and **3.80**.

The proton NMR spectrum of thiazolyl steroid **3.79** showed three singlets at 0.86, 0.99 and 1.70 ppm corresponding to the methyl groups at C-18, C-19 and C-21 respectively, which are in accordance to the data described by Shingata *et al.* for an analogous compound.[99] An exhaustive structural assignment was obtained by combining 1D- and 2D-NMR experiments (**Appendices IV-VI**).

Indicative signals for the minor isomer **3.80** were determined from the **3.79**/**3.80** mixture. The differences were the aromatic doublets at 7.69 and 7.24 ppm shifted downfield with respect to the (*R*)-isomer **3.79**, and the chemical shifts of methyl groups at C-18, C-19 and C-21, which slightly decreased to 0.84, 0.91 and 1.58 ppm, respectively. In the carbon NMR spectrum the atoms in ring D and the side-chain also showed differences. Of particular relevance was the decrease in the chemical shift of C-22 from 180.6 to 179.4 ppm and of C-13 from 43.3 to 42.8 ppm in the 20(*R*)- **3.79** and 20(*S*)-isomers **3.80**, respectively. Full NMR data for compounds **3.79** and **3.80** are reported in **Appendices IV-VI**.

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Conclusive evidence for the structure of thiazole **3.79** was obtained by X-ray diffraction, which confirmed the β-configuration of the C-20 hydroxyl group. In the solid state, thiazole-steroid **3.79** was observed as a dimer linked by hydrogen bonds between the tertiary hydroxyl group in the side-chain as donor and the heterocyclic nitrogen as acceptor. Two additional hydrogen bonds between the hydroxyl and two molecules of methanol used for recrystallisation were also observed (**Figure 3.12**).
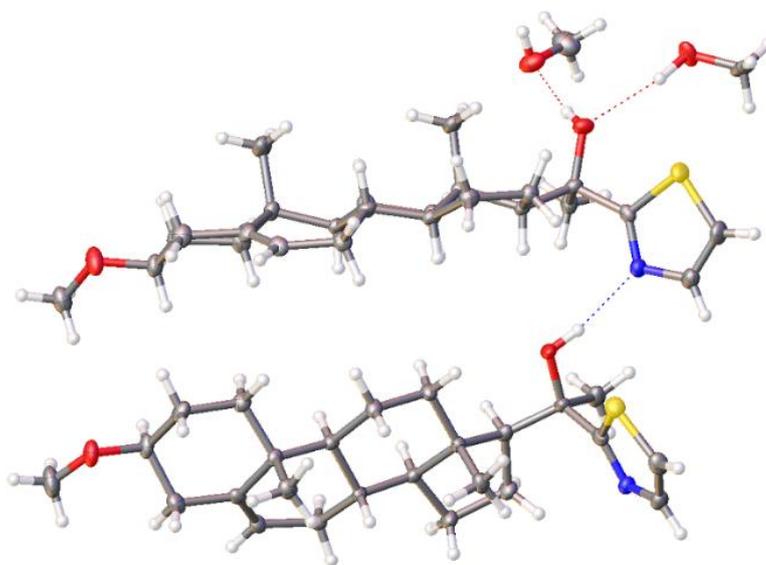
Figure 3.12. X-ray structure of thiazolyl-steroid **3.79** (CCDC 1019833, **Appendix VIII**).

3.3.2.2. Stereochemical rationale

It is precedented that organometallic additions to the C-20 ketone of pregnenolone analogues are highly stereoselective, providing predominantly the β-hydroxyl products.[98, 99, 103-105] It has also been shown that the carbonyl group in the side-chain adopts the preferred conformation as shown in compound **3.82** because of three factors: the presence of a chiral centre α to the ketone in which the small substituent is a proton, and the medium and large-sized groups are the ring D (as represented in **3.81**); the C-18 methyl group, whose steric hindrance pushes the ketone towards the outside of the molecule; the C-21 methyl group, which must be also staggered to avoid the bulky ring C (**Scheme 3.8**).[98, 105]

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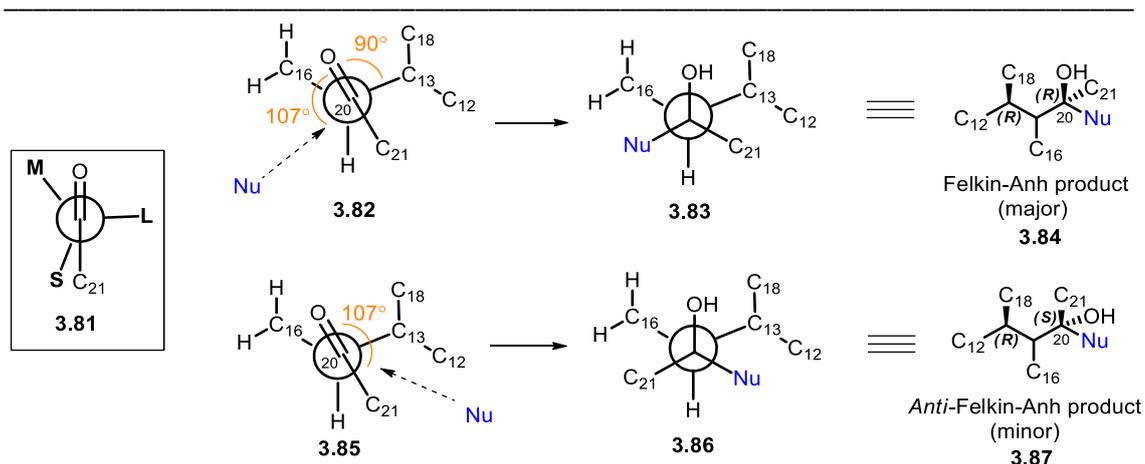
Scheme 3.8. Nucleophilic additions to the C-20 ketone: Felkin-Anh and *anti*-Felkin-Anh products.

With this in mind, it is easy to picture that the attack of a nucleophile occurs from the external side of the molecule according the Bürgi-Dunitz angle, thus giving compound **3.83** as the major compound, as predicted from the Felkin-Anh model. Therefore, the newly formed hydroxyl group would have predominantly a β-configuration and it would be in *syn*-relationship with the methyl group at C-18, as shown in **3.84**.[106] On the other hand, the addition of a nucleophile to the most hindered face of the carbonyl as in **3.85** would lead to the sterically unfavoured compound **3.86**, in which the C-20 hydroxyl group and the C-18 methyl group would be in *anti*-relationship and the C-20 chiral centre would have (*S*)-configuration as in the minor *anti*-Felkin-Anh product **3.87**.

These findings were in accordance with our results, and pleasingly the stereochemistry of the major compound isolated was in agreement with the 20β-configuration desired for our target molecules.

3.3.2.3. Addition to the 17α-analogue

We next explored addition to the 17α-epimer of 3β-methoxy-pregnenolone **3.73.** As expected, treatment of the epimeric mixture **3.60**/**3.73** (in 42/58 and 78/22 ratio) with lithiothiazole gave a corresponding mixture of diastereomeric products **3.79**/**3.88** (in 37/63 and 72/28 ratio, respectively) (**entries 1** and **2**, **Table 3.6**).
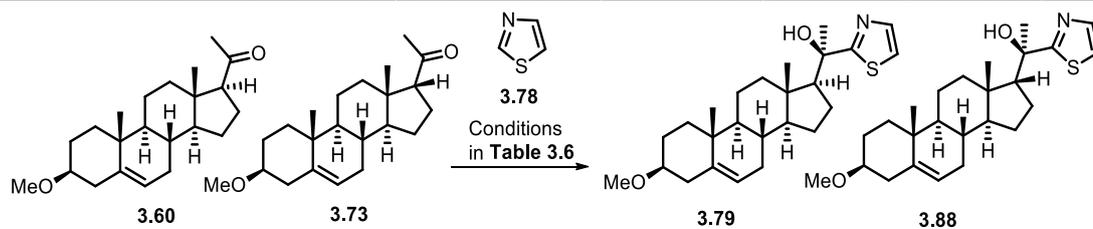
Entry	Conditions	Yield
1	i) **3.78** (1.1 eq), *n*-BuLi (1.1 eq), -30 °C, 30 min ii) **3.60/3.73** = 42/58, -20 °C to rt, 3 h	**3.79/3.88** = 37/63: 60%
2	i) **3.78** (2.5 eq), *n*-BuLi (2.5 eq), -30 °C, 20 min; ii) **3.60/3.73** = 78/22, -20 °C to -10 °C, 30 min	**3.79/3.88** = 72/28, 73%

Table 3.6. Synthesis of C-17 epimeric thiazolyl steroids **3.79** and **3.88**. THF was used as solvent.

The formation of diasteroisomer **3.88** was proven by NMR spectroscopic analysis of the complicated diastereomeric mixture **3.79/3.88** (**Appendices IV**-**VI**). However, it was possible to isolate **3.88** cleanly by recrystallisation of the diastereomeric mixture **3.79**/**3.88** (in a 72/28 ratio) from methanol. Indisputable evidence for the formation of diastereoisomer **3.88** was obtained by X-ray diffraction analysis (**Figure 3.13**).
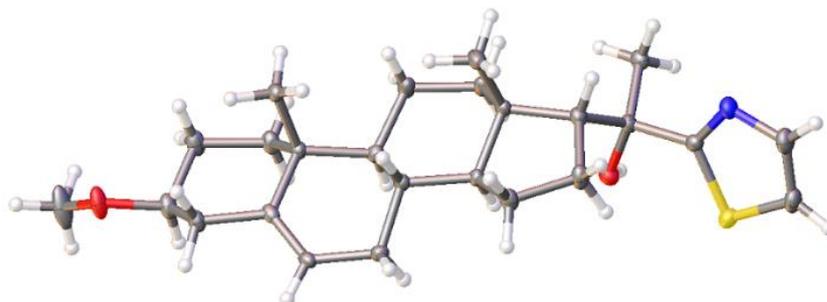
Figure 3.13. X-ray structure of thiazolyl-steroid **3.88** (CCDC 1019834, **Appendix IX**).

3.3.2.4. The preparation of 2-pyridyl analogues

The idea to prepare a steroid bearing a pyridyl group arose from the intention to insert a basic centre in the side-chain. Nitrogen-containing side-chains are a feature of cyclopamine **1.2** and azacholesterol **3.18**, two potent Hh/Gli which interact with different binding sites on the Smo receptor (**Figure 3.3**).

The stereoselective addition of 2-lithio-pyridine **3.90** to 17-keto and 20-keto steroids was described first by Heer and Hoffmann to occur with high stereoselectivity in favour of the 20(*R*)-isomer **3.91** (**Scheme 3.9**),[107] results recently confirmed by Shingate *et al.* [99]
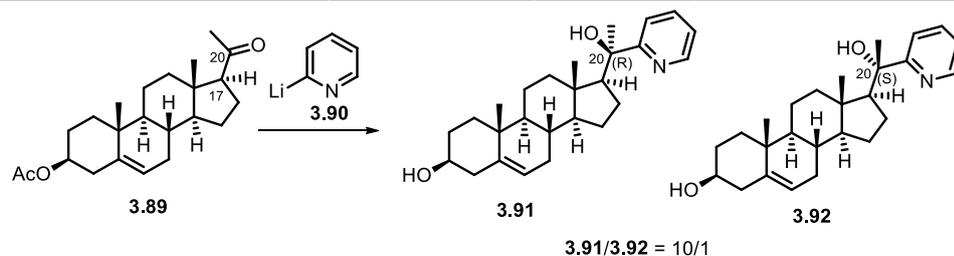
Scheme 3.9. Heer and Hoffmann's reaction of 2-lithio-pyridine **3.90** with a 20-keto steroid **3.89**.[107]

We aimed to achieve a similar conversion by reacting 3β-methoxy-pregnenolone **3.60** with 2-lithio-pyridine, which was freshly prepared by bromo-lithium exchange of 2-bromo-pyridine **3.93** and *n*-butyllithium.[108] As expected, the reaction resulted in a mixture of diastereoisomers **3.94**/**3.95** in a 9/1 ratio as determined by NMR spectroscopy, which after recrystallisation from methanol and dichloromethane gave pure 20(*R*)-isomer **3.94** in 53% yield, leaving the filtrate enriched 3/2 in favour of the minor isomer **3.95** (**Scheme 3.10**).
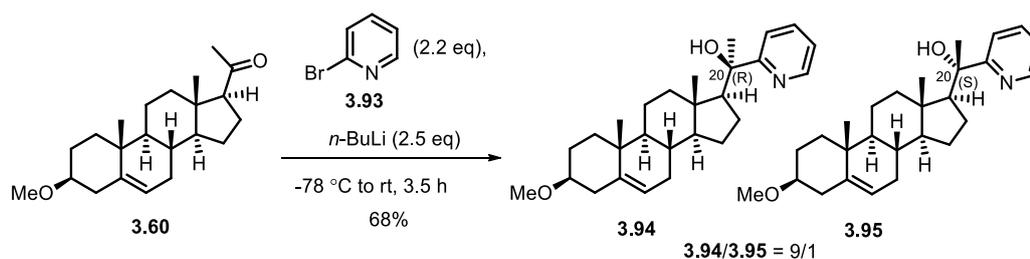
Scheme 3.10. Synthesis of pyridyl-steroid **3.94**.

NMR analysis of pyridyl steroid **3.94** showed three singlets at 0.95, 1.02 and 1.60 ppm corresponding to the protons at C-18, C-19 and C-21 respectively, as previously reported by Shingate *et al.*[99] for a similar compound. Indicative signals for the minor isomer **3.95** were found at 8.48 and 7.18 ppm for the aromatic protons, and at 3.31, 1.45, 0.87 and 0.73 ppm for the methyl groups. In accordance with the results of Heer and Hoffmann,[107] the newly formed hydroxyl of the major compound **3.94** was found in β-orientation (20*R*), and this was confirmed by crystallographic studies (**Figure 3.14**).
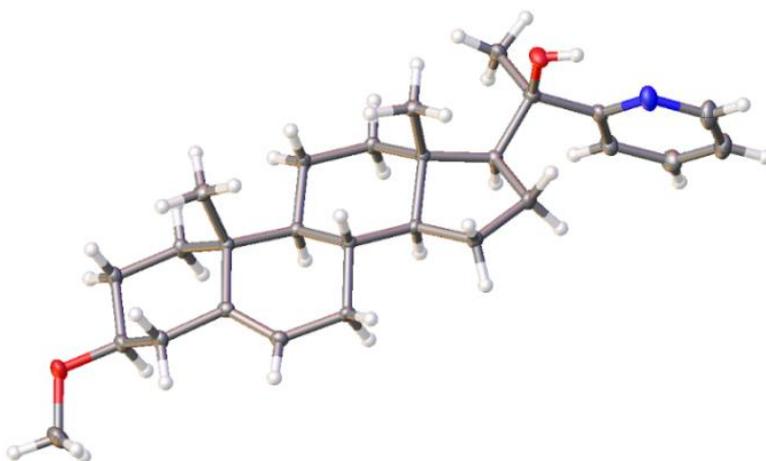
Figure 3.14. X-ray structure of pyridyl-steroid **3.94** (CCDC 1019835, **Appendix X**).

3.3.2.5. Thiophene analogue

We also wanted to synthesise a steroid featuring a thiophene group in the side-chain. This aromatic sulfur-containing heterocycle is bioisosteric with benzene. Thiophene-modified steroids are known in the literature (**3.96**-**3.98**, **Figure 3.15**).[109, 110]
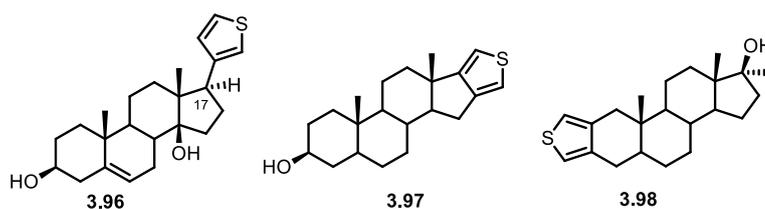
Figure 3.15. Selected steroids containing a thiophenyl group.[109, 110]

Nevertheless, to the best of our knowledge, no nucleophilic additions of 2-thiophenyllithium to C-20 steroidal ketones have been reported to date (**Table 3.7**).
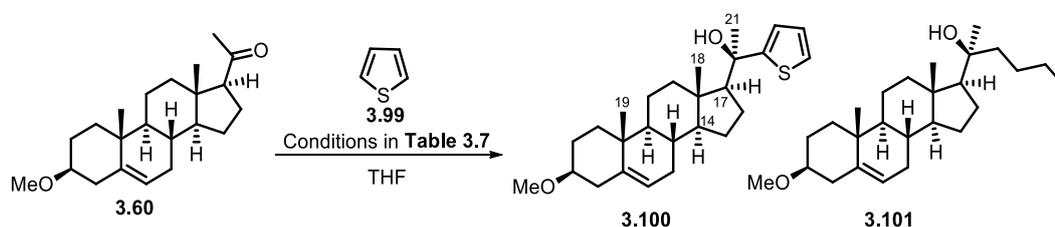
Entry	Conditions	Yield
1	i) **3.99** (2.2 eq), *n*-BuLi (2.5 eq), -40 to -10 °C, 20 min ii) **3.60**, 0 °C to rt, 2.5 h	**3.100/3.101** = 57/43: 48%
2	i) **3.99** (1.1 eq), *n*-BuLi (1.1 eq), TMEDA (1.1 eq), 0 °C to rt, 50 min ii) **3.60**, 0 °C to rt, 3 h	**3.100**: 51%

Table 3.7. Synthesis of thiophenyl-steroid **3.100**.

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Treatment of ketone **3.60** with an excess of lithiothiophene led to a complex mixture of compounds. After column chromatography, thiophene-steroid **3.100** was detected. However, an inseparable compound was also found, which was assigned as the novel *n*-butyl derivative **3.101** (on the basis of a similar compound described by Shingate *et al.*)[111] resulting from addition of *n*-butyllithium to 3β-methoxy-pregnenolone **3.60** (**entry 1**). However, compond **3.101** was not fully characterised

We thought the use *N,N,N',N',*-tetramethylethylenediamine[112] might increase the reactivity of the stoichiometric lithiothiophene, and avoid the formation of **3.101** (**entry 2**). Under these conditions, after column chromatography and recrystallisation from methanol, thiophene-steroid **3.100** was obtained diastereomerically pure in 51% yield. It should be noted that **3.100** readily undergoes dehydration (CD_2Cl_2 had to be employed for NMR studies as $CDCl_3$ caused dehydration).

The structure and stereochemistry of steroid **3.100** was elucidated by 1D- and 2D-NMR spectroscopy. The chemical shifts of C-14, C-17, C-18, C-19 and C-21 were in accordance to those above described for thiazolyl- and pyridyl-steroids **3.79** and **3.94**. The assignment of the 20(*R*)-stereochemistry therefore seems secure.

3.3.2.6. Oxa-heterocycles: furyl-steroids

Having validated this approach, we wanted to use furans to build oxygenated heterocyclic side-chains. Nucleophilic additions of lithiofurans to 17-keto steroids have precedent in the literature, and they have been extensively used by Kametani *et al.* during their studies towards the synthesis of the ecdysones and brassinolides.[113-118]

Of relevance in this regard, Kametani's group developed a novel strategy to introduce a lactone in the side-chain using an Achmatowicz rearrangement of furan **3.104** to dihydropyran **3.105** (**Scheme 3.11**).[115, 116] Stereoselective addition of lithiofuran **3.103** gave the tertiary alcohol **3.104**, which was treated with *m*-chloroperbenzoic acid to afford hemiacetal **3.105** in high yield. It was noted that the furyl derivative **3.104** was unstable, thus it was used without any further purification in the following oxidation to pyranone **3.106**. Hydrogenation of the side-chain resulted in a mixture of products **3.107**-**3.109**, whose distributions varied according to the hydrogenation conditions.[116]

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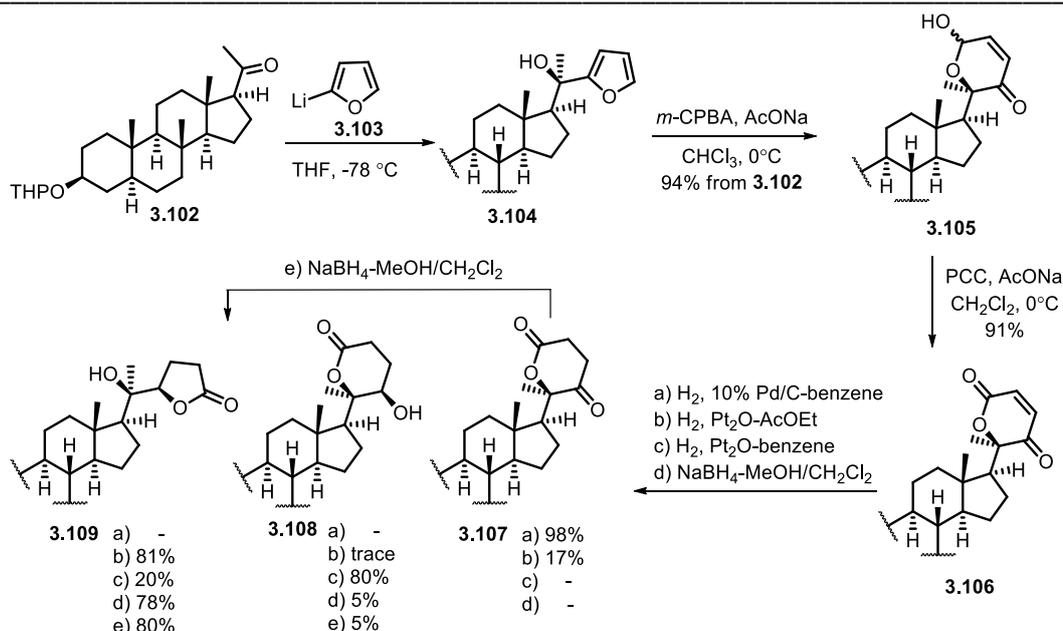
Scheme 3.11. Kametani's rearrangement of furan **3.104**.[116]

In order to investigate an Achmatowicz reaction and introduce a lactone functionality, we first had to add a furyl group to the C-20 ketone. Hence, our next challenge was the isolation of pure furyl steroid **3.111** (**Table 3.8**).
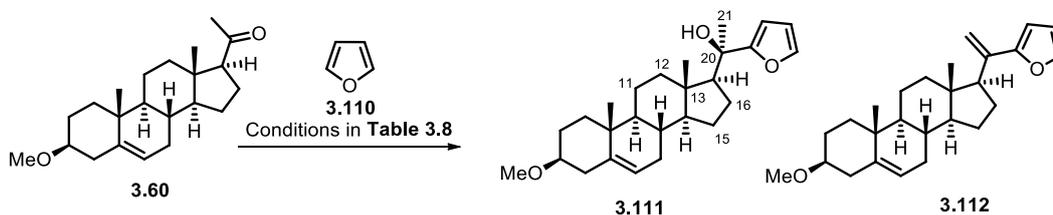
Entry	Conditions	Yield
1	i) **3.110** (2 eq), *n*-BuLi (2 eq), -10 °C, 30 min ii) **3.60**, -10 °C to rt, 2.5 h	**3.112**: 33 %
2	i) **3.110** (2 eq), TMEDA (2.5 eq), *n*-BuLi (2.1 eq), -50 °C, 40 min ii) **3.60**, -50 °C to -30 °C, 30 min	**3.111**: 84%

Table 3.8. Synthesis of furyl-steroid **3.111**. Reactions were performed in THF.

An initial attempt was carried out following a slightly modified procedure of Kametani[114, 116] but this failed and the novel styrene **3.112** was obtained (**entry 1**). We therefore began a screening of different reaction temperatures, quenches, work-ups, purifications and deuterated NMR solvents, informed by the findings for the thiophene steroid study described above. We discovered that the reaction works best when furan **3.110** is lithiated at –50 °C and the starting material **3.60** is added at the same temperature in the presence

of *N,N,N',N',*-tetramethylethylenediamine. Thin layer chromatography and column chromatography were performed on silica gel basified by adding 1% of triethylamine to the eluting system. Moreover, NMR experiments were run in deuterobenzene. These conditions proved sucessful in suppressing the formation of **3.112** and furyl steroid **3.111** was obtained in 84% yield along with traces of side-products which were not identified (**entry 2**). The stereochemistry of the 20(*R*)-hydroxyl in compound **3.111** was proved by NMR spectroscopy by comparison of the chemical shifts of the protons and carbons at C-11, C-12, C-13, C-15, C-16, C-20 and C-21 with those described above for thiazolyl- and pyridyl-steroids **3.79** and **3.94**, and further confirmed by crystallographic studies on derivative **3.124** as described below.

3.3.2.7. The Achmatowicz rearrangement of furans

With the furyl group successfully added, the Achmatowicz rearrangement could be investigated. The proposed mechanism[119] involved bromination of a furan (*e.g.* **3.113**, **Scheme 3.12**) which leads to bromonium ion **3.114**. Formation of oxonium ion **3.115** is followed by the addition of methanol which gives **3.116**. Displacement of bromide leads to a second oxonium ion **3.117** which is trapped by methanol to give dimethoxyacetal **3.118**. Finally, in the presence of acidic media, dimethoxyacetal **3.118** is readily hydrolysed to un unstable acyclic 1,4-dicarbonyl intermediate **3.119**, which in turn undergoes an intramolecular rearrangement to form the six-membered lactol **3.120**.

Scheme 3.12. Proposed mechanism for the Achmatowicz rearrangement.

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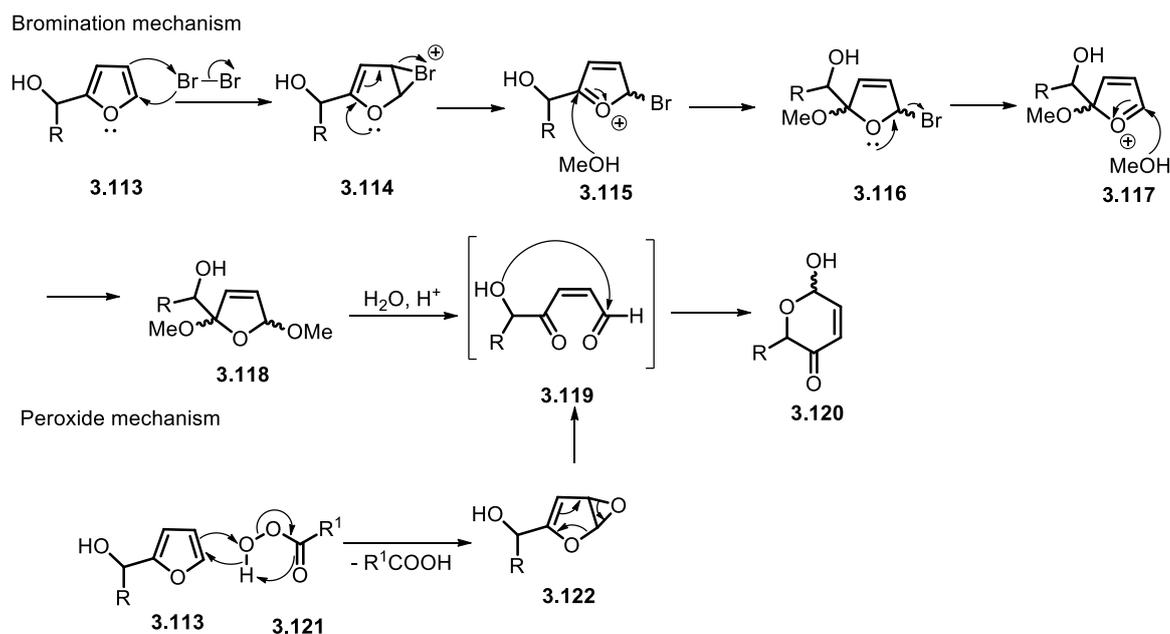
A number of reagents for the ring opening-cyclisation sequence have been published, including peroxyacids such as **3.121** (which lead to diketone **3.119** *via* the formation of epoxide **3.122** and further ring-opening, **Scheme 3.12**),[120-122] also in the presence of vanadium acetylacetonate catalyst,[123] dimethyldioxirane,[124] *N*-bromosuccinimide,[121, 125, 126] pyridinium chlorochromate.[122] More recently an example of Achmatowicz rearrangement by means of the enzyme laccase in the presence of oxygen and 4-acetamido-(2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (4-acetamido-TEMPO) as the mediator have been also reported.[127]

Amongst the number of methods available, we decided to use the milder *N*-bromosuccinimide for the furan ring opening-ring closure,[121] because of the C-5,C-6 double bond in **3.11** which might react with peroxyacids or bromine, and tetrapropylammonium perruthenate in the presence of *N*-methylmorpholine *N*-oxide[128] for the following oxidation of lactol **3.123** to lactone **3.124** (**Scheme 3.13**). These conditions proved successful, and 2-methyl-pyran-3,6-one **3.124** was obtained exclusively in two steps as a single diastereoisomer in 41% yield.
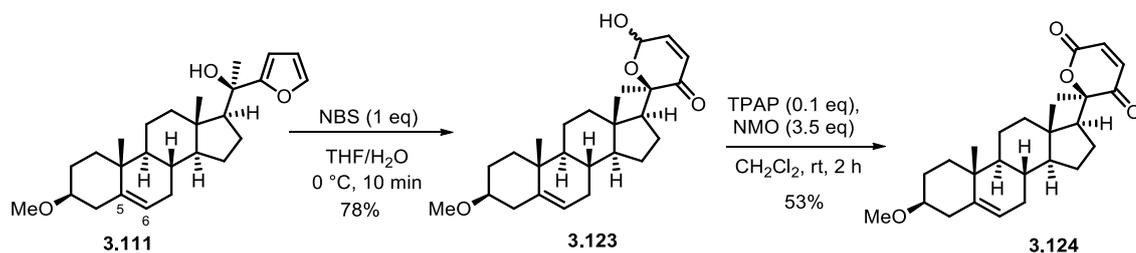
Scheme 3.13. Achmatowicz rearrangement of furyl-steroid **3.124**.

The structure and stereochemistry of compound **3.124** was confirmed by X-ray crystallography (**Figure 3.16**).
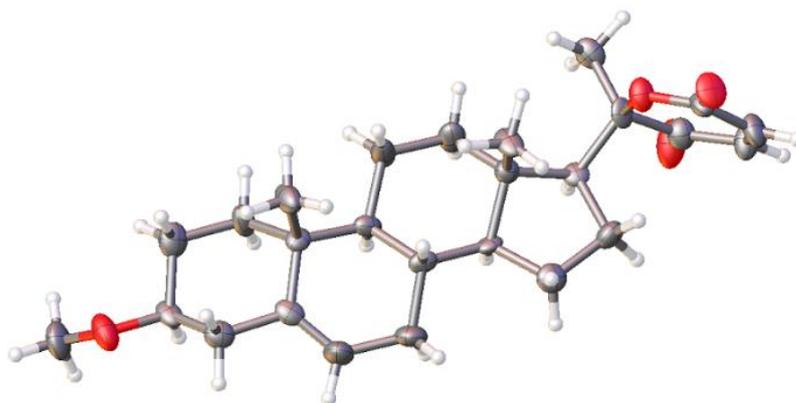
Figure 3.16. X-ray crystallography of lactone **3.124** (CCDC 1019836, **Appendix XI**).

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Having demonstrated the viability of the Achmatowicz rearrangement on furyl derivative **3.111** we speculated that a similar protocol could be applied to convert furfuryl steroid **3.125** into hemiacetal **3.126**, *i.e.* a steroid with a side-chain similar to withanolides **3.128**, featuring an oxygenated heterocycle at C-22 and retention of the β-hydroxyl group at C-20 (**Scheme 3.14**). In theory, two possible products **3.126** and **3.127** could result. We suspected that by using suitable protecting groups the reaction could be selectively driven to form the desired isomer **3.126**.
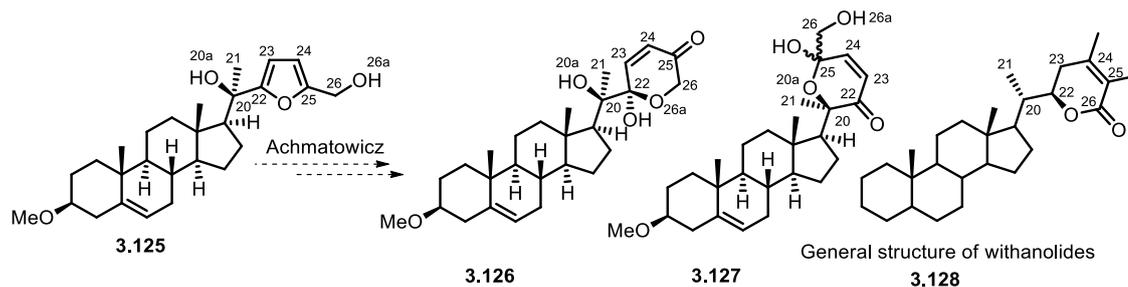
Scheme 3.14. Potential strategy to withanolide analogue **3.126**.

The attempted synthesis of steroid **3.125** started with the preparation of the *tert*-butyldimethylsilyl ether **3.131** in 75% yield (**Schemes 3.15** and **3.16**). Addition of the lithiated 5-hydroxymethyl-*tert*-butyldimethylsislyl ether **3.130**[129] to 3β-methoxy-pregnenolone **3.60** gave a single diastereoisomer which was assigned as 20(*R*) based on NMR comparison of the chemical shifts of the protons and carbons at C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-20 and C-21 with those previously described for furyl-steroid **3.111**.
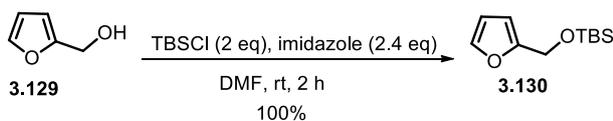
Scheme 3.15. TBS protection of furfuryl alcohol **3.130**.
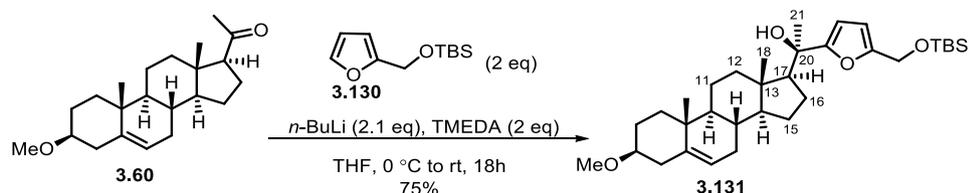
Scheme 3.16. Synthesis of adduct **3.131**

Next, protection of the secondary hydroxyl group of steroid **3.131** as an acetate was attempted using acetic anhydride (**entries 1**-**3**, **Table 3.9**) or acetyl chloride (**entries 4**

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and **5**). Unfortunately, acetate **3.132** could not be obtained and alkene **3.133** was isolated as a side-product (**entries 2**, **3**, and **4**).
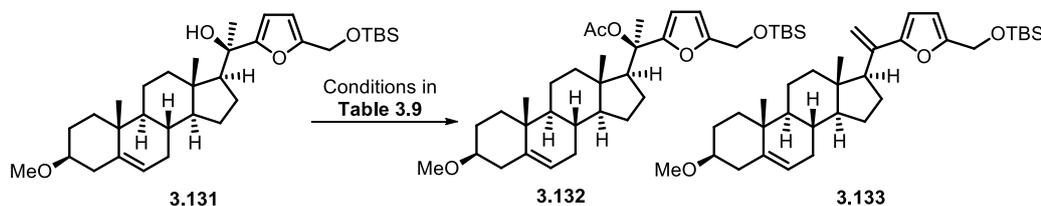
Entry	Conditions	Results/Yield
1	Ac$_2$O/py = 1/3, rt, 2 d	By-products
2	Ac$_2$O/py = 1/3, rt, 2 h	By-products, **3.131** and 8% of **3.133**
3	Ac$_2$O/py = 1/1, DMAP (cat), rt, 2 d	By-products and **3.133**[a]
4	AcCl (15 eq), py, rt, overnight	By-products and **3.133**[a]
5	AcCl (1.3 eq), CH$_2$Cl$_2$, rt, 30 min	Decomposition

Table 3.9. Attempted acetylation of tertiary alcohol **3.131**. [a]Detected by NMR spectroscopy of the crude product.

We next looked at the removal of the *tert*-butyldimethylsilyl ether (**Scheme 3.17**). On subjecting ether **3.131** to tetrabutylammonium fluoride[129] the formation of a single product was apparent by thin layer chromatography and confirmed by high resolution mass spectroscopy. However, the appearance of additional signals in the proton and carbon NMR spectra did not allow secure characterisation of this compound as alcohol **3.125**, therefore this study was abandoned.
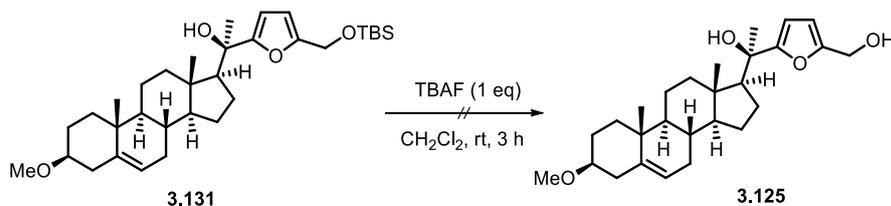
Scheme 3.17. Attempted deprotection of silyl ether **3.131**.

3.3.2.8. Benzo-fused heterocycles: benzofuranyl-, benzothianyl- and N-methyl-indolyl steroids

Based on the observation that aryl groups are common amongst Hh/Gli modulators, we next examined oxa-, thio- and aza-benzofused rings. We started our investigation on benzofuran, benzothiophene and *N*-methyl protected indole, commercially available heterocycles also identified in natural Hh/Gli antagonist taepeenin D **1.18** and staurosporinone **1.9**, and in the synthetic Hh/Gli agonist SAG **1.41** (**Figure 3.17**).

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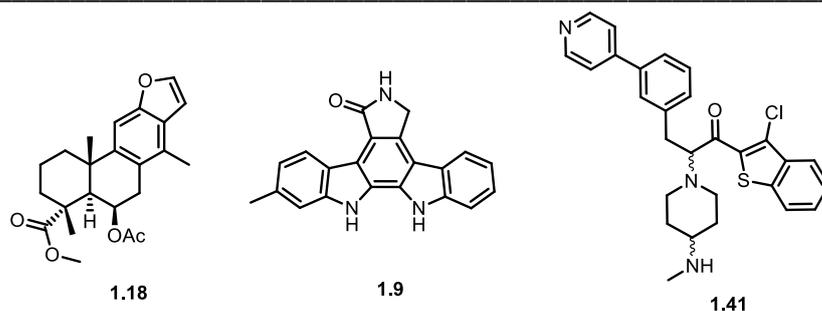
Figure 3.17. Examples of Hh/Gli modulator with benzo-fused heterocycles.

7-Benzofuranyl and 17-benzothianyl steroids have been previously synthesised by Suzuki coupling of 17-iodo steroids with the corresponding boronic acids in poor yields.[130] However, to the best of our knowledge no examples of organometallic addition of such heterocycles to 20-keto steroids have been reported.

Following modified literature procedures, benzofuran **3.134**[131] and benzothiophene **3.136**[132] were successfully lithiated and, upon reaction with ketone **3.60**, benzofuranyl and benzothianyl steroids **3.135** and **3.137** were obtained as single diastereoisomers in 56% and 47% yield, respectively (**Scheme 3.18**).
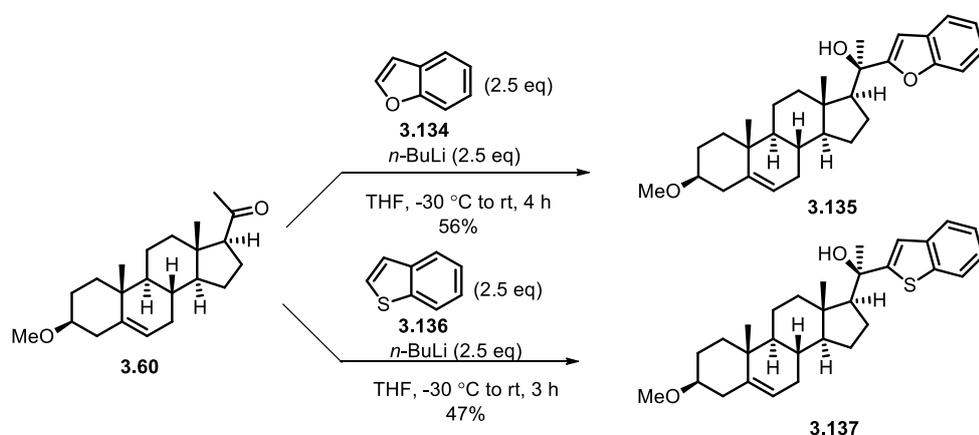
Scheme 3.18. Synthesis of benzofuranyl- and benzothianyl-steroids **3.135** and **3.137**.

Metallation of *N*-methyl indole **3.138** is known to require a strong base such as *tert*-butyllithium[133] or to occur at high temperature.[134] Thus, we performed the lithiation at reflux, whilst the subsequent addition was carried out at room temperature. Under these conditions indolyl derivative **3.139** was obtained, albeit in an unoptimised 12% yield (**Scheme 3.19**).

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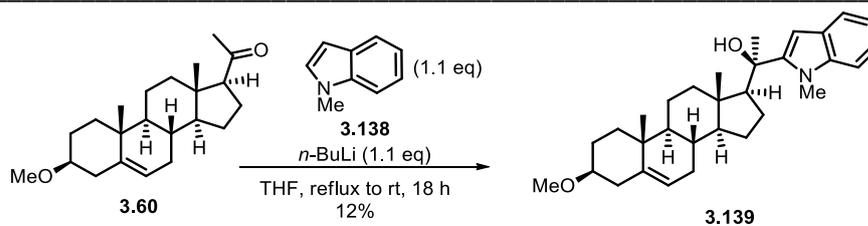
Scheme 3.19. Synthesis of *N*-methyl-indolyl steroid **3.139**.

In all these cases only one diastereoisomer was seen, which was assumed to be 20(*R*) based on NMR comparison with the adducts previously prepared.

3.3.2.9. Heterocyclic i-steroids: thiazolyl- and furanyl-derivatives

To expand the small library of heterocyclic steroids, we chose to synthesise thiazolyl cyclo-steroid **3.140** and furanyl cyclo-steroid **3.141** to establish the importance of whether the steroid is in an open or cyclic form, and of a substituent at C-3 and at C-6 in SAR studies (**Scheme 3.20**). Based on the results previously achieved, lithiation of thiazole **3.78** was carried out at -30°C, and after addition of ketone **3.75**, thiazolyl cyclo-steroid **3.140** was obtained in 58% yield. Lithiation of furan **3.110** worked better at lower temperature, and, in the presence of *N,N,N',N',*-tetramethylethylenediamine, furyl cyclo-steroid **3.141** was obtained in 47% yield.
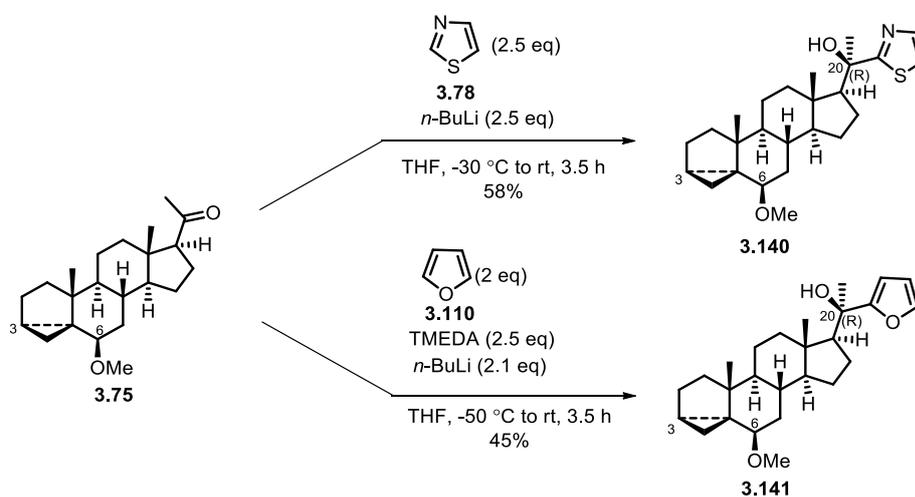
Scheme 3.20. Synthesis of thiazyl **3.140** and furyl **3.141** derivatives of *i*-pregnenolon-methyl ether **3.75**.

In both cases, a secondary product likely to be the 20(*S*)-diasteroisomer could be seen in the proton and carbon NMR spectra. However, the small amount detected did not allow their identification to be confirmed. According with the Felkin-Anh prediction, the newly formed stereocentre at C-20 was assigned as 20(*R*) for the major product, by comparison to the NMR spectra of the analogous compounds **3.79** and **3.111**, respectively.

3.3.2.10. Towards lactone side-chains via ring-closing metathesis

Previously we reported that an Achmatowicz rearrangement of furyl and furfuryl functionalities could lead to compounds featuring oxygenated side-chain such as **3.124** that resemble the withanolides **3.128** or the bufadienolides **3.142** (**Figure 3.18**). We thought it would be interesting to mimic these naturally occurring cyclic side-chains by synthesising α,β-unsaturated lactones such as **3.143**. These steroids could be further functionalised at the double bond for SAR studies
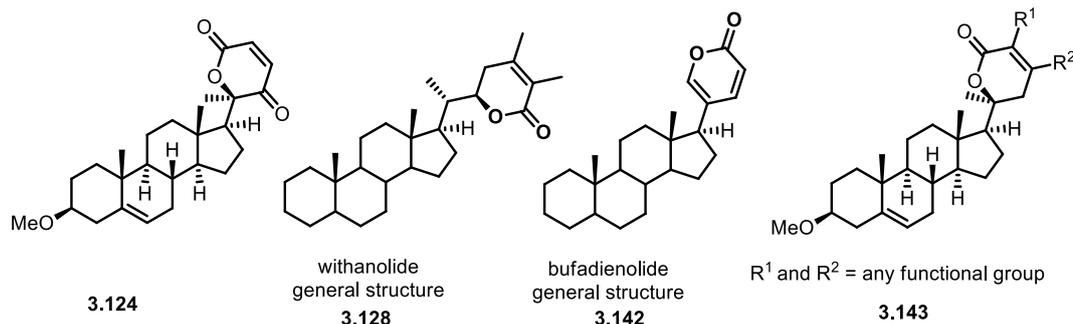
Figure 3.18. Similarities between diketopyranone **3.123**, withanolides **3.128**, bufadienolides **3.142**, and fuctionalised α,β-unsaturated lactones with general structure **3.143**.

We proposed that the lactone ring in **3.143** could be closed *via* an olefin metathesis of compound **3.144** (**Scheme 3.21**). One of the two terminal vinyl groups could be inserted *via* esterification of the tertiary alcohol **3.146** with the acyl chloride **3.145**. Grignard reaction of allylmagnesium bromide **3.147** to 3β-methoxy-pregnenolone **3.60** would lead to the installation of the first olefinic branch and give adduct **3.146** in which the newly formed hydroxyl group would have the desired β-configuration according to the Felkin-Ahn model (see **Scheme 3.8**).
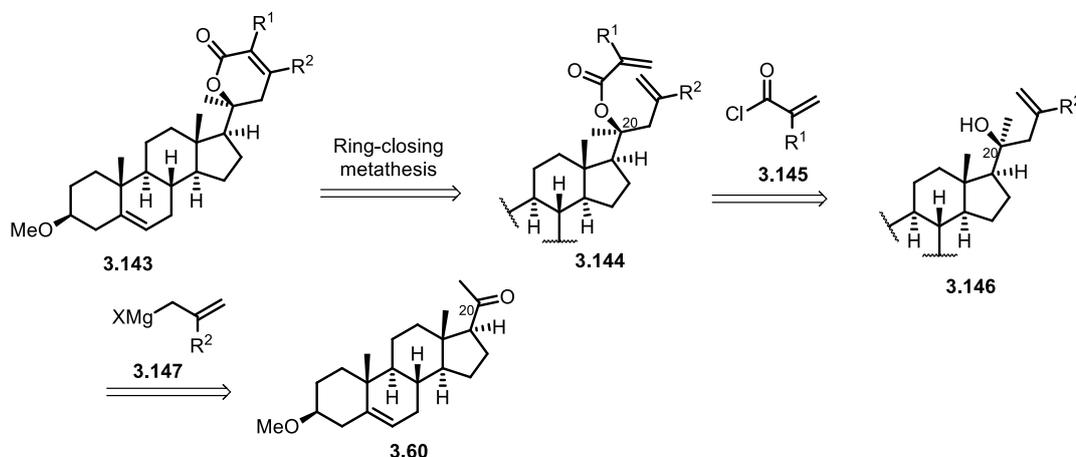
Scheme 3.21. Retrosynthetic plan to α,β-unsaturated lactones.

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Alkene metathesis has emerged as a potent tool in the steroid field, and all the ring-closing transformations, *i.e.* cross, enyne, ring-closing enyne, ring-closing dienyne and ring-opening metathesis, have found interesting applications in the total synthesis, hemisynthesis, dimerisation and side-chain modification of steroids.[135]

Despite this, to the best of our knowledge, the only example of ring-closing metathesis applied to the formation of withanolides has been published by Matsuya *et al.* during studies towards the synthesis of sorminone **3.157** and its analogues as novel anti-Alzheimer's disease candidates (**Scheme 3.22**).[136] The preparation of the substrate for the ring-closing metathesis started with the construction of the C-17 side-chain of commercially available 1α-hydroxy dehydroepiandrosterone **3.148** by Wittig olefination of the protected *tert*-butyldimethylsilyl ether **3.149**, followed by ene reaction with paraformaldehyde, reduction of the alkene **3.150** and further oxidation of the side-chain with pyridinium dichromate to obtain the C-20 aldehyde **3.151**.
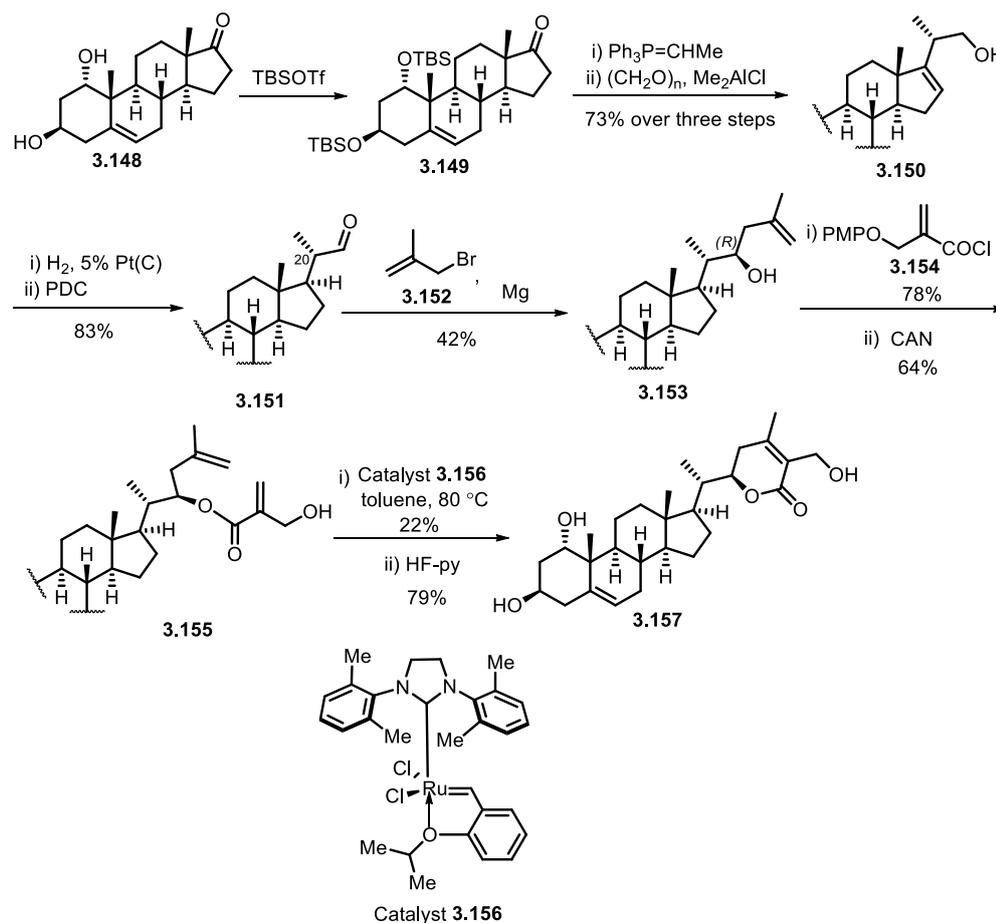
Scheme 3.22. Ring-closing metathesis approach of *Matsuya et al.* to sominone **3.157**.[136]

The first olefinic branch was inserted via a Barbier reaction involving 2-methylallyl bromide **3.152** and magnesium powder. The reaction resulted in poor stereoselectivity

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and the (*R*)-isomer **3.153** was obtained in 42% yield. The second olefinic branch was installed by esterification of alcohol **3.153** with 2-hydroxymethylacryloyl chloride protected as *para*-methoxyphenyl ether **3.154**, which was afterward released on treatment with cerium ammonium nitrate to give compound **3.155** in 50% yield over two steps. The *o*-tolyl variant of the second-generation Hoveyda-Grubbs' catalyst **3.156** was found to be the best reagent for the ring-closing metathesis. The use of this catalyst for the conversion of **3.155** to the hindered lactone **3.157** gave only 22% yield, but the unreacted starting material could be recovered and reused.

In 2012, Matsuya's group patented the synthesis and the therapeutic use of steroid **3.162**, characterised by an aromatic ring A and a δ-lactone in the side-chain monosubstituted with a hydroxymethyl group (**Scheme 3.23**). This compound was defined as 'compound having neurite-outgrowing activity' with applicability in the prevention and treatment of neurodegenerative diseases.[137] Its synthesis was also based on a ring-closing metathesis approach. Notably, the less hindered olefin **3.161**, which was prepared by oxidation of alcohol **3.158** with pyridinium dichromate followed by asymmetric allylboration and esterification of the resulting compound **3.159** with acryloyl chloride **3.160**, showed a better reactivity towards catalyst **3.156** compared to compound **3.155**, and the corresponding lactone **3.162** was obtained in 57% yield.
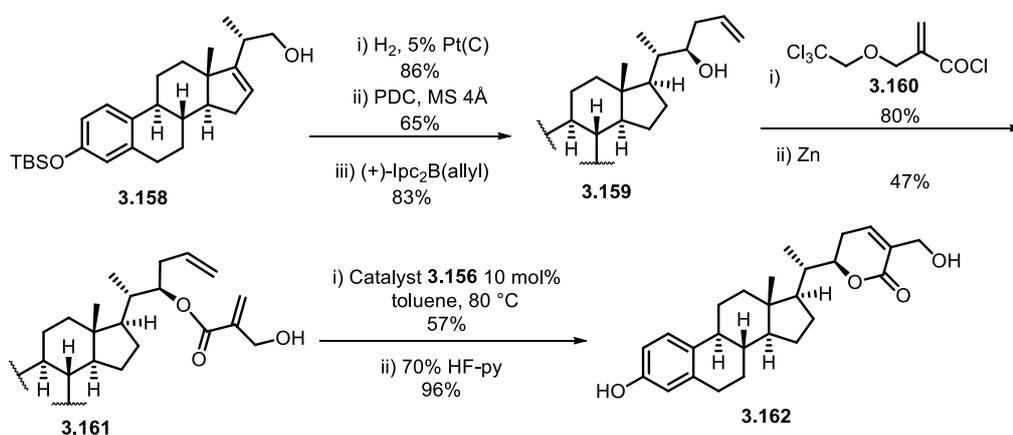
Scheme 3.23. Patented synthesis of steroid **3.162** by *Matsuya et al.*[137]

Inspired by these results, we targeted the simple unsubstituted C-20 lactone **3.168** (**Scheme 3.24**). The first step was the stereoselective addition of allylmagnesium bromide **3.163** to ketone **3.60**[138] which afforded exclusively the 20(*S*)-alcohol **3.164** in 72% yield. The following esterification of the sterically hindered tertiary alcohol **3.164** with commercially available acryloyl chloride **3.165**[138] was difficult and ester **3.166** was isolated in 33% yield, albeit along with recovered starting material. Afterwards, the use

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of second-generation Hoveyda-Grubbs catalyst **3.167** led to the formation of the α,β-unsaturated lactone **3.168** in excellent yield
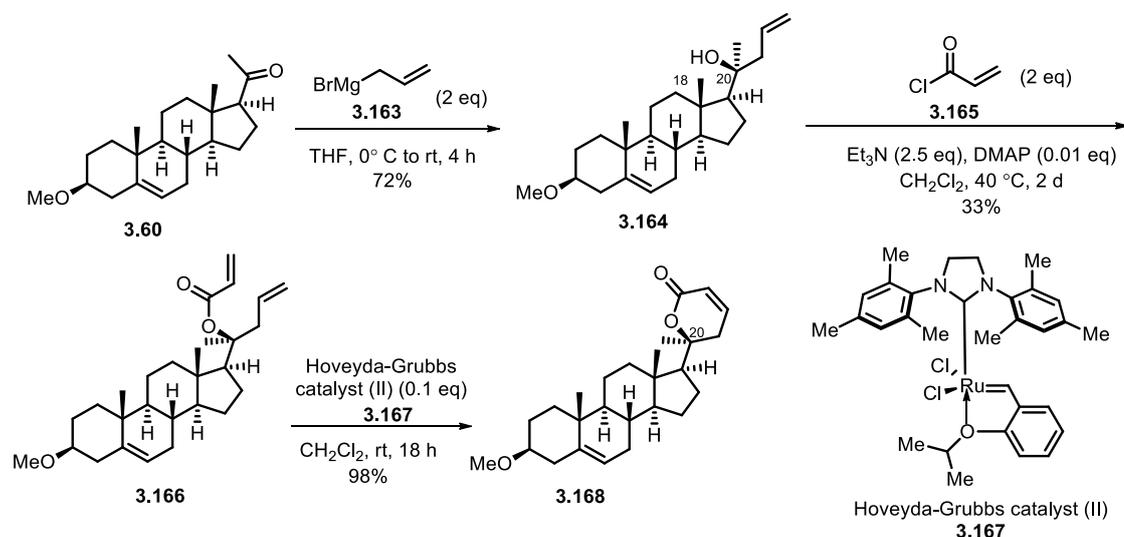
Scheme 3.24. Synthesis of the C-20 steroidal lactone **3.168**.

Structural confirmation of steroid **3.168** was obtained by X-ray diffraction, and it once again confirmed that the addition of the organometallic reagent to the C-20 keto group occurred under Felkin-Anh control, thus leading to a β-tertiary alcohol in a stereoselective fashion (**Figure 3.19**).
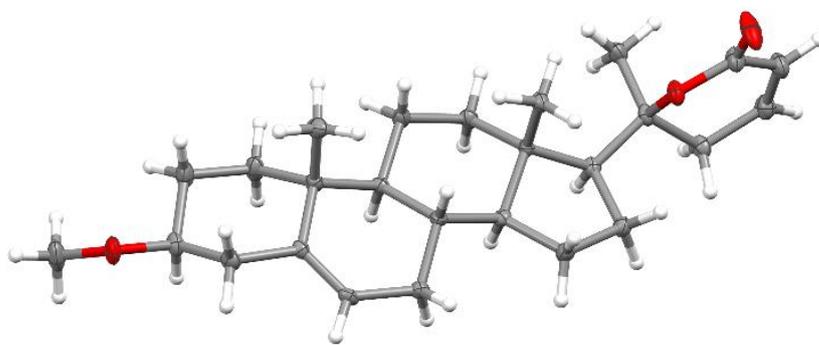
Figure 3.19. Crystal structure of steroidal lactone **3.168** (CCDC 1019837, **Appendix XII**).

Finally, we wanted to see whether similar reaction conditions could be applied to the synthesis of an α,β-dimethyl-α,β-unsaturated C-20-δ-lactone **3.173**, functionality typical of the withanolides (**Scheme 3.25**).

The addition of 2-methylallylmagnesium chloride **3.169** to ketone **3.60** at 0 °C gave steroid **3.170** in a mixture along with inseparable side-products. Therefore, a switch to a lower temperature was made, and this resulted in β-alcohol **3.170** which was obtained in 75% yield. Esterification in the presence of the bulky methyl-acryloyl chloride **3.171** gave

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diene **3.172** in 12% yield along with recovered starting material. Nonetheless, the steric hindrance did not prevent the intramolecular ring-closure, and, on treatment with the second-generation Hoveyda-Grubbs catalyst **3.167**, dimethyl-α,β-unsaturated lactone **3.173** was obtained in 24% yield.
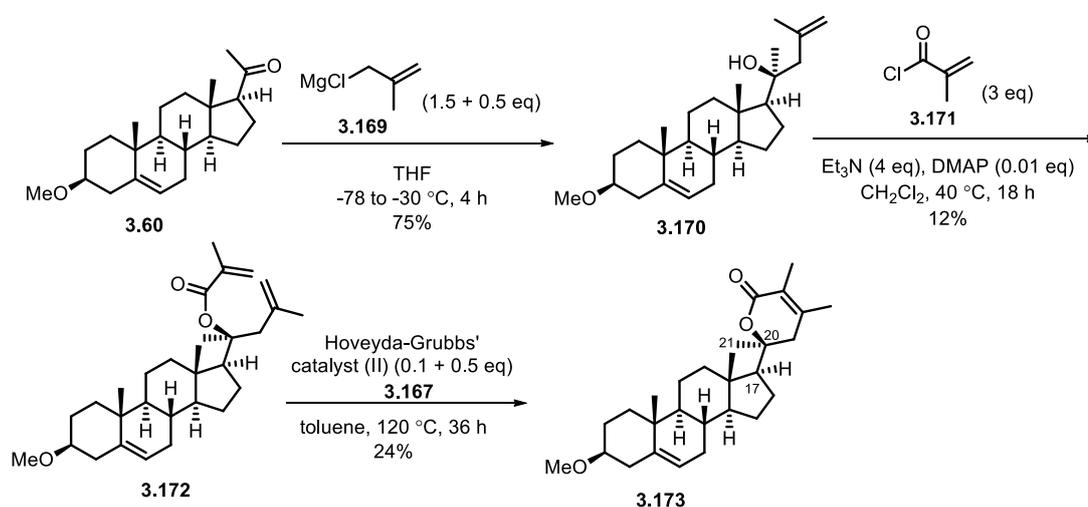
Scheme 3.25. Synthesis of the C-20 dimethyl lactone **3.173**.

The structure of steroid **3.173** was elucidated by NMR spectroscopy and the stereochemistry was secured by comparison of the chemical shift of C-17, C-20 and C-21 with those of the unsubstituted lactone **3.168**.

With regards to the lactone ring, the ^{1}H- and ^{13}C-NMR spectra of compound **3.173** were in reasonable agreement with those of withanolide F **2.2** (**Figure 3.20**).[139] Indeed, the ^{1}H-NMR spectrum showed singlets for the methyls C-6 and C-7 at 1.85 and 1.72 ppm, respectively, in withanolide **2.2**, while they were a unique singlet at 1.88 ppm in steroid **3.173**. Methylenic protons at C-2 appeared as a multiplet between 2.35 and 2.50 ppm in withanolide **2.2**, whereas they were spilt in a doublet at 2.71 ppm (J = 17.8 Hz) and in a multiplet between 2.05 and 1.69 ppm in steroid **3.173**. The ^{13}C-NMR showed a closer correspondence between the chemical shifts of C-1, C-3, C-4, C-5, C-6 and C-7 at 81.0, 150.7, 120.1, 165.9, 20.2 and 12.1 ppm for withanolide **2.2** and at 82.6, 146.5, 121.4, 165.9, 20.8 and 12.5 ppm for steroid **3.173**, respectively. However, the signals of C-8 and C-2 were shifted downfield from 19.1 and 34.3 ppm in compound **2.2** to 24.6 and 41.1 ppm in compound **3.173**, respectively. This result was expected, considering the positions C-8 and C-2 as the most affected by of proximity of the steroidal framework
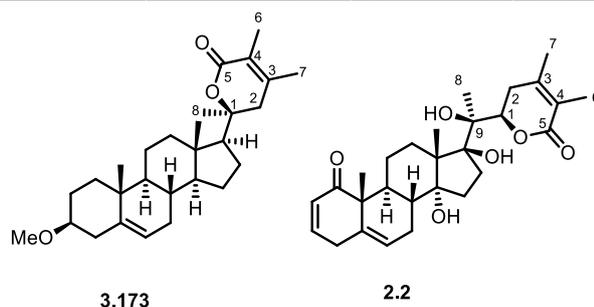
Figure 3.20. Structures of novel steroid **3.173** and withanolide F **2.2**.

3.4. SUMMARY

A number of novel steroids have been prepared as potential modulators of the Hh/Gli signalling pathway (**Figures 3.21-3.24**). The majority of these compounds feature a cholesterol-like skeleton and a heterocylic side-chain containing a 20(*R*)-hydroxyl group (**3.79**, **3.94**, **3.100**, **3.111**, **3.131**, **3.135**, **3.137**, **3.139**), or are C-20 vinylic analogues (**3.112** and **3.133**, **Figure 3.21**).
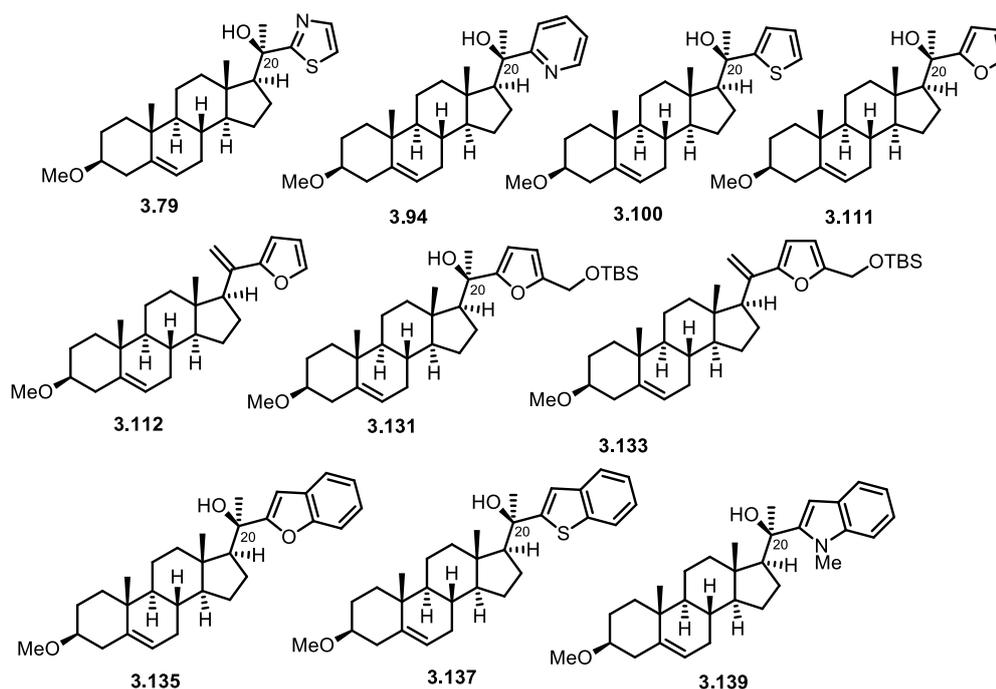
Figure 3.21. Novel 3β-methoxy-pregnenolone derivatives featuring a heterocylic side-chain and a 20(*R*)-hydroxyl or a C-20 vinyl group.

Isomeric compounds featuring a 20(*S*)-hydroxyl group (**3.80** and **3.95**), an α-oriented side-chain (**3.88**) or a cyclic ring A (**3.140** and **3.141**) have been also prepared (**Figure 3.22**).
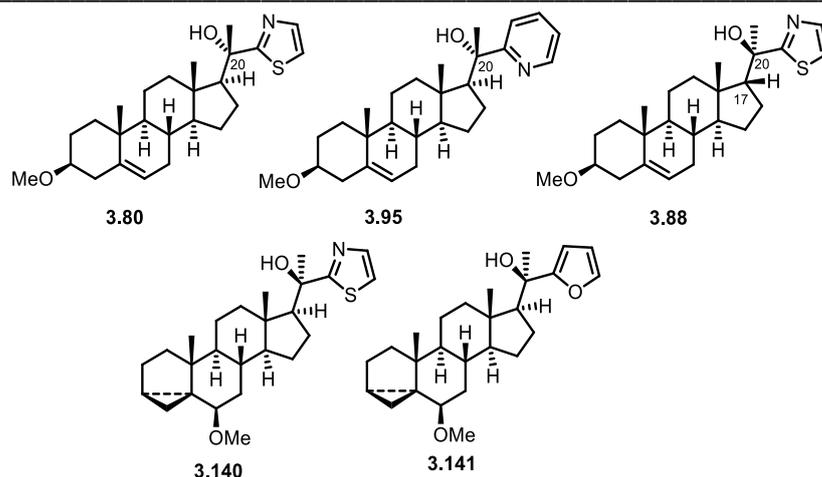
Figure 3.22. Novel isomeric 3β-methoxy-pregnenolone derivatives.

Novel intermediates with aliphatic linear and branched side-chains containing a free 20(*S*)-hydroxyl (**3.164** and **3.170**) or a C-20 ester (**3.166** and **3.172**) provide a wider chemical diversity to our small molecule library (**Figure 3.23**).
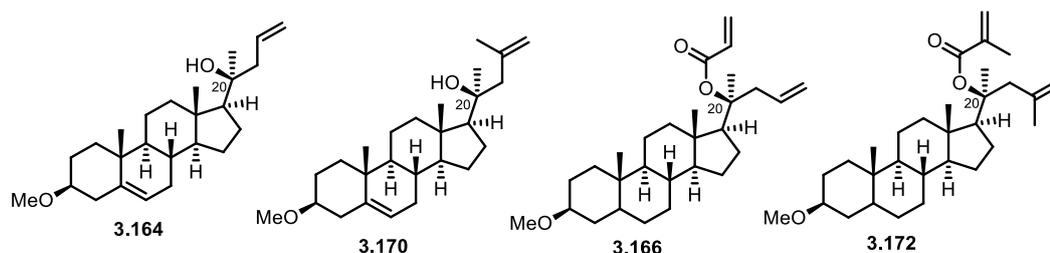
Figure 3.23. Novel steroids with acyclic side-chains

Finally, we successfully synthesised novel compounds with a δ-lactol (**3.123**) or δ-lactone (**3.124**, **3.168** and **3.173**) in the side-chain (**Figure 3.24**)
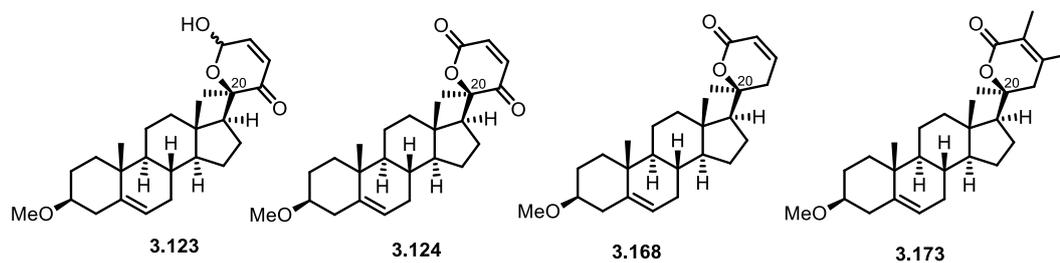
Figure 3.24. Novel lactonic steroids with a withanolide-like side-chain.

Lactonic side-chains are a typical feature of withanolides. An overview of the main characteristics of these natural products and our efforts towards the synthesis of an unnatural withanolide are provided in Chapter 4.

CHAPTER 4.
STUDIES TOWARDS NOVEL WITHANOLIDE ANALOGUES

4.1. INTRODUCTION TO WITHANOLIDES

In the history of medicine nature has always been a rich source of therapeutic agents, and many drugs that are now in commercial use are derived from plants, microbes or marine organisms.[140, 141]

Traditional medicine pre-dated Western medicine and its origins are earlier than recorded human history. Ayurveda is one of the oldest and most diffused forms of popular medicine born in India more than 4000 years ago. Studies on the folklore claim Ayurvedic practitioners have provided useful leads in drug discovery and they have inspired the development of important therapeutics currently used to treat several chronic diseases.[142]

Withania somnifera, also named Ashwagandha, Winter cherry or Indian ginseng, is an important plant in Ayurvedic healing that has been used since ancient times to enhance longevity and rejuvenation (**Figure 4.1**). It belongs to Rasayana, a group of treatments traditionally known to improve physical and mental abilities, to strengthen and give vigour to the body, to increase memory and acquire intelligence, to stimulate the defence system and to retard and arrest cell degeneration.[143]

Figure 4.1. Representations of *Withania somnifera*.

The chemical constitution of Aswagandha has been extensively investigated. Withanolides are the main bioactive components, localised in the leaves, where the concentration ranges between 0.001-0.5% of the dry weight, but are also present in the roots and fruits. These secondary metabolites are not directly involved in the normal growth, development or reproduction of the plants, but usually act as a defence system against animals, microorganisms or interspecies attacks.[144-146] Withaferin A **4.1** was the

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first withanolide isolated and it was found in the leaves of *Withania somnifera* by Lavie *et al.* in 1965 (**Figure 4.2**). It has been described as a white crystalline powder and its structure has been elucidated by spectroscopic methods and supported by chemical modifications.[147]
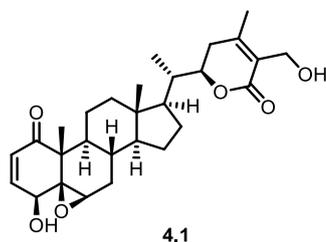
Figure 4.2. Withaferin A **4.1**

A number of additional withanolides were subsequently purified and characterised. Recent reviews report more than 650 withanolides, more than half of which have been isolated over the last 14 years.[148-150]

4.1.1. Natural occurrence of withanolides

The *Solanaceae* are a family of dicotyledonous flowering plants, herbs and shrubs present in every continent and consisting of nearly 100 genera and more than 3000 species, many of which are edible or with medicinal properties.[143, 150] Amongst them, plants belonging to *Withania*, *Jaborosa*, *Datura* and *Physalis* genera are the major source of withanolides.[150] The genus *Withania* contains 23 species distributed in the dry parts of the temperate and tropical zones of the Mediterranean area, spreading from the Canary Islands to Southwest Asia. *Jaborosa*, *Datura* and *Physalis* genera are endemic to the North and South America continents.[143]

As comprehensively reported by Misico *et al.*,[150] after their first appearance in *Withania somnifera*, withanolides have been extracted from many other species of plants belonging to the *Withania*, *Physalis*, *Jaborosa* and *Datura* genera.[150] Moreover, biologically interesting withanolides have been more recently extracted from other plants including *Leguminosae*, *Labiatae*, and *Taccaceae* and from marine microorganisms.[150-152]

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4.1.2. Structural features of withanolides

Withanolides are natural steroids characterised by a 28-carbon ergostane skeleton in which C-22 and C-26 or C-23 and C-26 are oxidised to form δ- or γ-lactone (such as in compounds **4.2** and **4.3**, respectively, **Figure 4.3**), or, less common, lactols.[149] The backbone consisting of the steroidal ABCD ring system and the oxygenated side-chain is generally designated the "withanolide skeleton".[153] Hydroxyls groups can typically decorate the framework at nearly every carbons, and they can be present as the free hydroxy or as epoxides, ethers, esters or more complex heterocycles.[148-150]
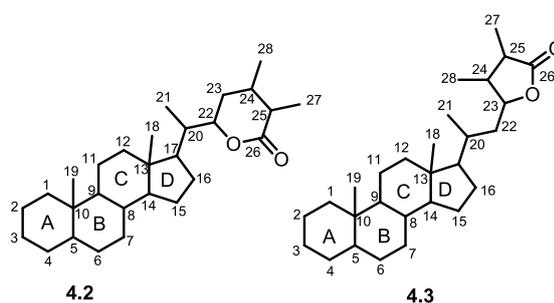
Figure 4.3: Basic structures of δ-lactone and γ-lactone withanolides.

In light of the plethora of structures, a classification of withanolides have been proposed (**Figure 4.4**).[148-150]

According to the size of the side-chain, withanolides can be divided in:

- Withanolides with δ-lactone or δ-lactol side-chains.
- Withanolides with γ-lactone or γ-lactol side-chains.

Within each group, a second classification divides withanolides into subclasses depending on the similarities between their skeletons:

- Withanolides with unmodified skeleton, which are characterised by the parent skeleton of withaferin A **4.1** (general structure **4.4**).
- Withanolides with modified skeleton, which arise from different rearrangements of the side-chain and ring D mainly promoted by the hyperoxygenation (general structures **4.5**-**4.23**).

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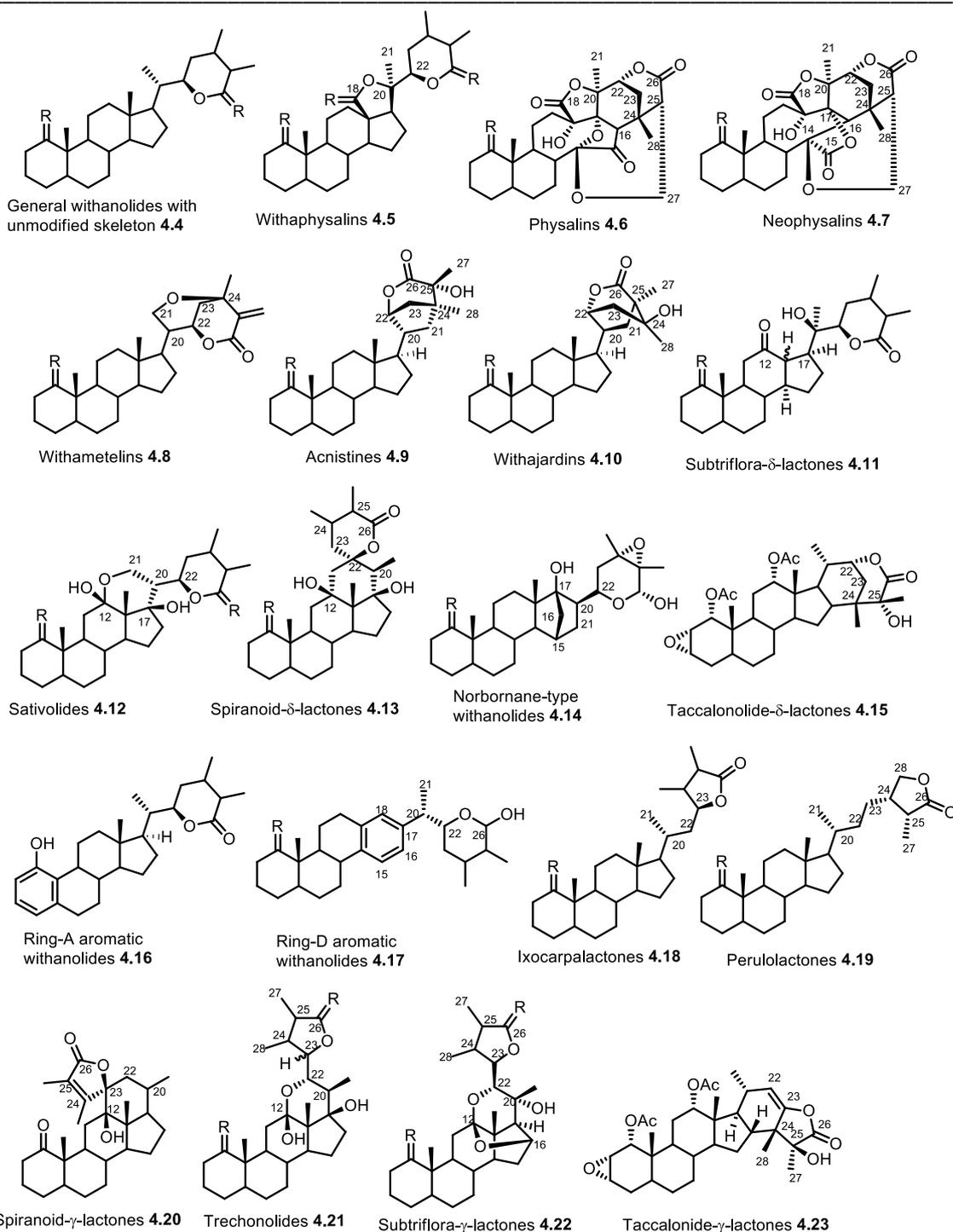
Figure 4.4. Classification of withanolides. R = H, OH; O.

4.1.3. Biosynthesis of withanolides

Although the biosynthetic route is not well established, it is commonly acknowledged that withanolides are likely to be naturally synthesised according to the same isoprene-pathway of natural triterpenes and sterols.[143, 154] In plants, the synthesis of isoprene occurs via two different routes both starting with D-glucose: the mavalonate pathway in cytosol

and the non-mevalonate pathway in plastids. By administration of $[^{13}C_1]$-D-glucose to *in vitro* producing withanolide shoots of *Withania somnifera*, Chaurasiya *et al.*[154] have recently demonstrated that both mevalonate and non-mevalonate pathways participate for the synthesis of withanolides, with a contribution of 75:25 in favour of the cytosolic route. An overview of the latter route leading to withaferin A **4.1** is shown in **Scheme 4.1**.
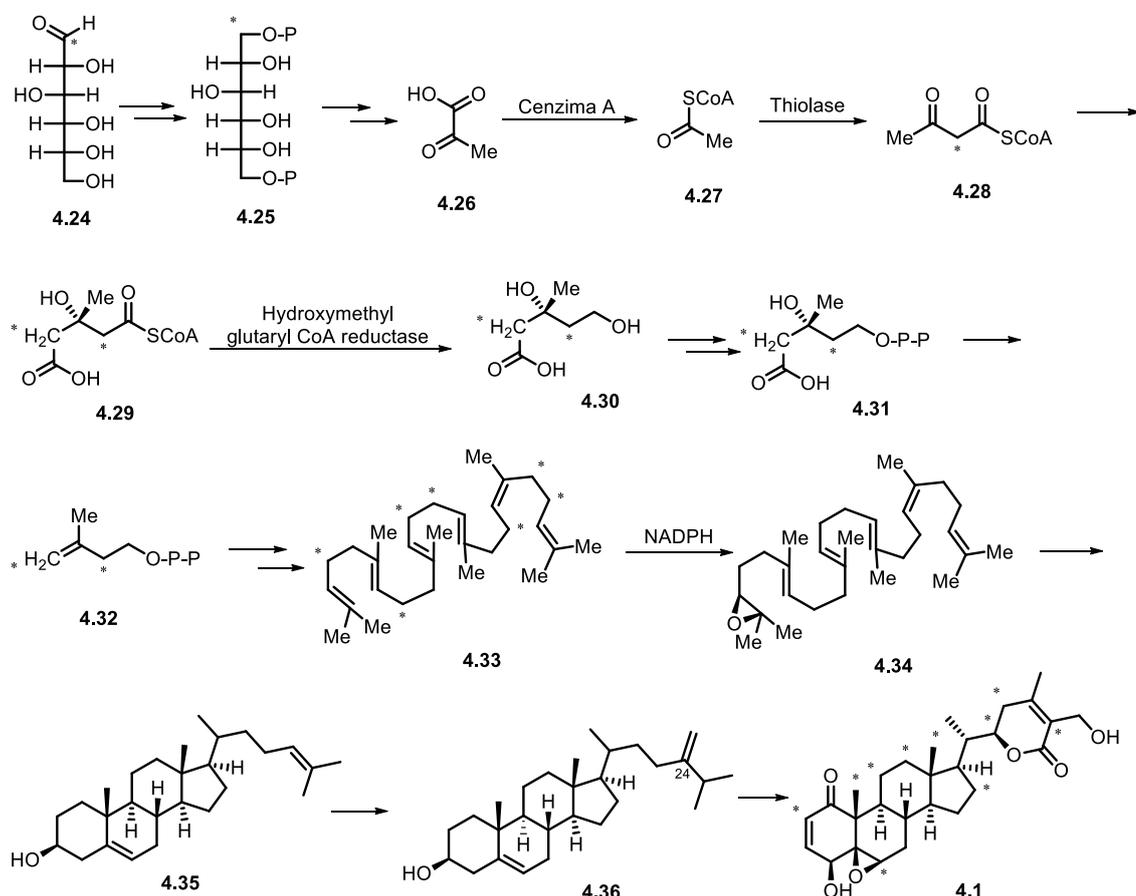
Scheme 4.1. Overview of biosynthesis of withaferin A **4.1**.[143, 154] Labelled intermediates relate to the work of Chaurasiya *et al.*[154]

The biosynthetic pathway starts with the enzymatic conversion of $[^{13}C_1]$-D-glucose **4.24** into pyruvate **4.26** in several steps including D-fructose-1,6-biphosphate **4.25** as intermediate. Two enzymatic transformations convert pyruvate **4.26** into acetyl Co-enzyme A **4.27** and acetoacetyl CoA **4.28**. Combination of metabolites **4.27** and **4.28** gives 3-hydroxy-3-methyl-glutaryl-Co-enzyme A **4.29**, which is in turn enzymatically metabolised to mevalonic acid **4.30**. Only the (*R*)-form of this acid proceeds along the route *via* first phosphorylation to mevalonate-5-pyrophosphate **4.31** followed by decarboxylation to isopentenyl-5-pyrophosphate **4.32**. Head-to-tail and head-to-head condensations of 6 units of pyrophosphate **4.32** lead to squalene **4.33**, which undergoes epoxidation to **4.34** in the presence of NADPH and mytochondrial oxygen. Subsequent

cyclisation leads to compound **4.35**, which can undergo methylenation by *S*-adenosylmethionine to 24-methylenecholesterol **4.36**, a well known intermediate in the synthesis of cholesterol and related steroids.[143, 154]

The relationship between 24-methylene cholesterol **4.36** and withanolides was first reported by Lockley in the 1970s, who showed that administration of [28-^{3}H]-24-methylenecholesterol to young leaves or the stem of *Withania somnifera* produced radioactive withanolides.[155, 156] Additional proof was found when Velde *et al.*, during studies on the constituents of *W. somnifera*[157] and *W. coagulans*,[158] isolated the novel steroids **4.37**,[157] **4.38**[158] and **4.39**,[158] which were identified as biogenetic precursors of withanolides (**Figure 4.5**).
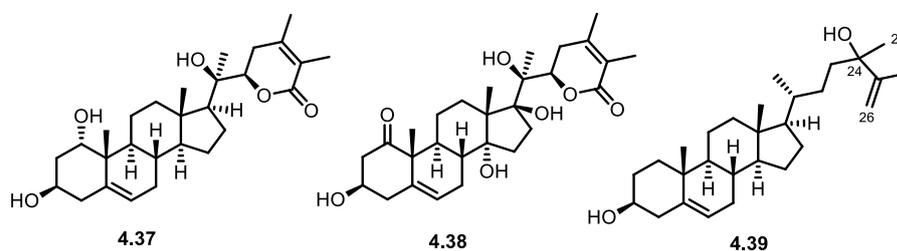
Figure 4.5. Potential intermediates of the withanolides' biosynthesis.[157, 158]

Valde proposed that the biosynthetic pathway could start with the oxidation of 24-methylene cholesterol **4.36** to sterol **4.39**, followed by C-22 hydroxylation to **4.40** (**Scheme 4.2**). Lactol **4.44** could be obtained in two ways. One way entailed the cyclisation of diol **4.40** to pyran **4.41** followed by hydroxylation. Otherwise, an allylic isomerisation of alcohol **4.40** to diol **4.42**, followed by oxidation to aldehyde **4.43** and subsequent ring-closure could be also considered. Final oxidation of lactol **4.44** led afterwards to the typical withanolide side-chain **4.45**.[158]
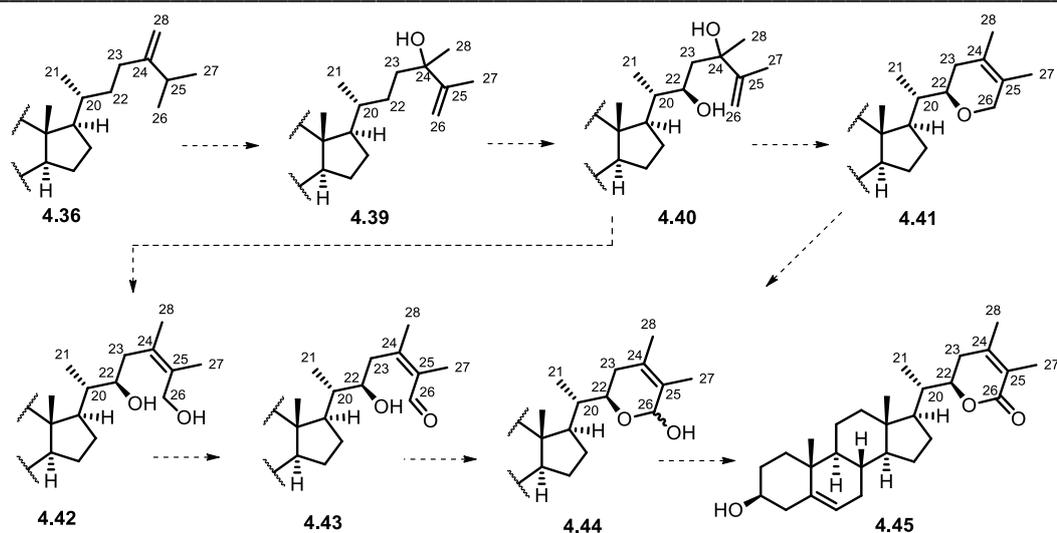
Scheme 4.2. Proposed biosynthesis of the withanolide side-chain.[158]

Velde suggested also that the biochemical elaboration of rings A and B leading to the recurrent 1-oxo-2,5-diene pattern could occur after the formation of the side-chain.[157] The simple sequence of C-1 hydroxylation to diol **4.46**, selective oxidation to 1-keto-3β-alcohol **4.47** and elimination to enone **4.48** has been confirmed by the isolation of the intermediates **4.45**-**4.48**, which were afterwards subjected to biomimetic reactions (**Scheme 4.3**).[158]
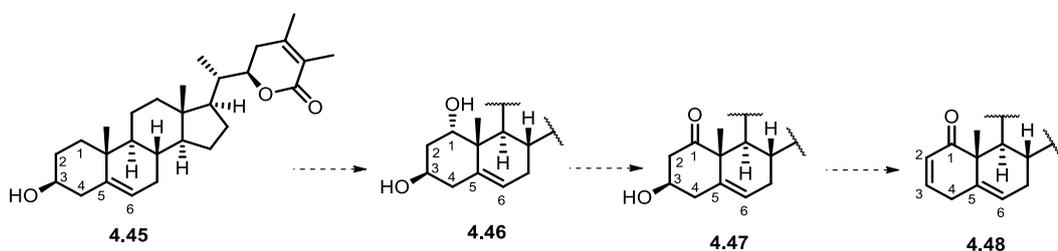
Scheme 4.3. Proposed biogenetical modifications of withanolides' ring-A.[157, 158]

The huge diversity of withanolide structures is reflected in the array of suggested biosyntheses. Particular attention has been given to the biosynthesis of withanolides with modified skeletons, including physalins, withametelins, acnistins, withajardins and withanolides with an aromatic ring D. These biogenetic pathways have in common the elaboration of hyperoxygenated precursor compounds in which the lactonic side-chain and the steroidal framework have been already preformed.[148-150] However, an in-depth analysis of these routes is beyond the scope of this work.

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4.1.4. Biological activities of withanolides

The pharmacological properties of withanolides have aroused the interest of the scientific community, especially in recent years. The list of their biological activities is wide and includes: phytotoxic, antifeedant, insecticidal, antimicrobial, antiparasitic, trypanocidal, leishmanicidal, anti-inflammatory, antistress, immunosuppressive, immunomodulatory effects; CNS-related activities on synaptogenesis, neurite outgrowth and inhibition of cholinesterase; and anticancer activities including cytotoxic, cancer chemopreventive, antiangiogenic and microtubule stabilisator agents.[148, 150]

4.1.4.1. Anticancer withanolides

With regard to cancer, in the last decades the cytotoxicity of withanolides has been widely investigated against a number of cancer cells lines, and several compounds have been considered as potential anticancer therapeutics. In recent work Zhang *et al.*[159] reported interesting antiproliferative activity of 15 withanolides with unmodified skeletons (IC$_{50}$ values in the range of 0.067-17.4 μM), either extracted from plant material or obtained via hemisyntheses, against 7 cancer cell lines including melanoma cell lines (murine B16F10 and human SKMEL28), human head and neck squamous cell carcinomas (HNSCC) cell lines (JMAR, MDA1986, DR081-1), human breast cancer cell lines (Hs578T) and non-malignant human renal epithelial cell line (MRC5), using cisplatin as a positive control. Combination of these results with precedent data allowed the authors to draw a SAR table for anticancer withanolides as follows (**Figure 4.6**):

- Δ^2-1-Oxo functionality is required in ring A.
- Hydroxylation of C-4 slightly reduces the potency; acetylation increases it.
- Replacement of the 5β,6β-epoxy group with the 4α-chloro-5β-hydroxy functionality does not decrease the cytotoxicity.
- Substitution at C-7 has essentially no effect on anti-cancer activity.
- Hydroxylation at C-11 and C-12 decreases the activity.
- 14α,15α-Epoxidation reduces the anticancer activity.
- Hydroxylation, acetylation and epoxidation at C-16 and C-17 slightly reduces the potential of withanolides.
- Hydroxylation or acetylation at C-18 and C-19 does not decrease the potency.
- Hydroxylation at C-20, C-23, C-24 and C-27 of the side-chain decreases the anticancer potential.

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- The δ-lactone is important in order to retain the antiproliferative activity.

- Glycosylation of withanolides decreases the cytotoxic effect.

- Withanolides with unmodified skeletons generally show the highest activity as antitumour agents compared to physalin, trechonolides and acnistine.
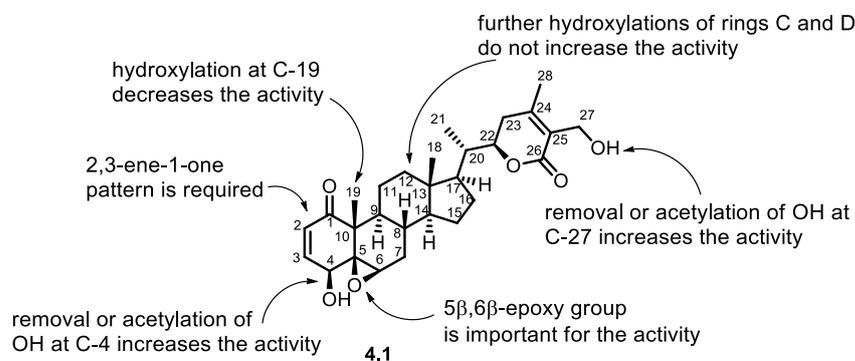
Figure 4.6: SAR of cytotoxic withanolides based on witaferin A **4.1**.[159]

Additionally, the necessary contribution of the 5β,6β-epoxide to the anticancer activity has been recently confirmed by Joshi *et al*. by replacement of this functionality with alcohols, thiirane and amino alcohols (see **Scheme 5.2** for synthetic details). In fact, the cytotoxicity of the resulting compounds appeared significantly reduced in tests against colon cancer (CACO-2), prostate cancer (PC-3), liver cancer (WRL-68) and breast cancer (MCF-7) cell lines (IC_{50} values > 85 μM), whilst the IC_{50} values of withaferin A **4.1** ranged between 3.8 and 5.4 μM.[160]

4.1.4.2. Withanolides with neurite outgrowth activity

Withanolide A **4.49** is a highly oxygenated steroidal lactone isolated first from the roots of *Withania somnifera*, which is known to possess interesting neuropharmacological activities in promoting neurite outgrowth, reversing neurite atrophy and aiding synapse reconstruction. After its successful total synthesis from pregnenolone,[161] Gademann and co-workers have recently investigated the influence of the 1-oxo-2-ene system towards the neurite outgrowth activity through the elaboration of ring A of withanolide A **4.49** and evaluation in human medulloblastoma cells (see **Schemes 5.3** and **5.4** for synthetic details).[162] The quantitative results of the biological screening were uncertain, however some conclusions could be drawn (**Figure 4.7**):

- 1α-2,3-allylic alcohol, 3α-1,2-allylic alcohol and 3β-1,2-allylic alcohols retain the activity of withanolide A **4.49**.

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- C-1 hydroxylamine derivative does not change the activity.

- A 2α,3α-epoxide or a 3α-1,2-allylic acetate showed less the activity then withanolide A **4.49**.

- C-1 methyl, allyl or benzyl oximes significantly decrease the activity.
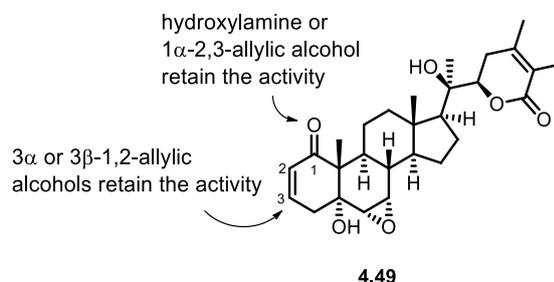
Figure 4.7: SAR of neurite-outgrowth withanolides based on withanolide A **4.49**.[162]

The authors assumed that the enone pattern in ring A might not be required for the bioactivity, and that the size of the substituents had more influence than the kind of functionality itself.[162]

4.1.4.3. Withanolides active in the Hh/Gli signalling pathway

Withanolides have also shown activity as inhibitors of the Hh/Gli signalling pathways. In 2008 Hosoya and coworkers screened 94 natural products, including terpenoids, flavonoids, phenylpropanoids, glycosides and bisindole alkaloids, and 192 tropical plant extracts in a cell-based reporter assay to identify potential Hh/Gli inhibitors. Amongst them, two withanolides extracted from *Physalia minima*, physalin F **1.13** and physalin B **1.14** (**Figure 4.8**), exhibited the highest inhibitory activity of the Gli-mediated transcription (IC_{50} = 0.66 and 0.62 µM, respectively) compared to cyclopamine **1.2** (IC_{50} > 40 µM). Additionally, physalins **1.13** and **1.14** showed potent cytotoxicity in human cancer pancreatic cell lines (PANC-1) expressing Hh/Gli components (IC_{50} = 2.7 and 5.3 µM, respectively). Again, cyclopamine gave weaker activity (IC_{50} 8.0. µM).[38]
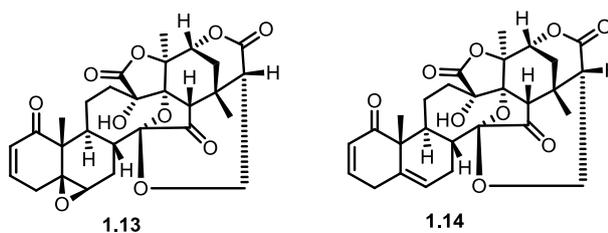
Figure 4.8. Withanolide inhibitors of the Hh/Gli signalling pathway.

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4.1.5. Chemical synthesis of withanolides

The large number of withanolides and their biological activities have motivated many researchers to embark on the synthesis of this class of natural products. Generally two strategies have been chosen: first, the transformation of a commercially available steroid into the target withanolide; secondly, a stereocontrolled total synthesis when no suitable starting material was readily available. In the first case, cholesterol **3.6** and pregnenolone **3.56** have been often used in model studies towards the synthesis of withanolides, as they bear a 3β-hydroxyl group and a C-5,C-6 double bond suitable for modification, and, in the case of pregnenolone **3.56**, a C-20 ketone on which the withanolide side-chain could be easily installed (**Figure 4.9**).[163]
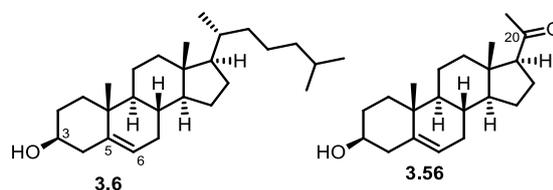
Figure 4.9. Cholesterol **3.6** and pregnenolone **3.56** as commercially available materials for studies on the right- and left-hand side elaboration of withanolides.

4.1.5.1. Earlier studies towards withanolide rings A and B

Amongst a wide number of possible patterns which can decorate rings A and B, the synthesis of the epoxide and quinoid-like moieties typical of withaferin A **4.1** have been widely investigated, both because it was the first withanolide isolated and it possesses a wide range of bioactivities, and because the decoration is quite recurrent amongst withanolides.[163-166]

In the mid seventies two independent groups, Ikekawa's group[164, 165] and Weissemberg *at al.*,[166] studied concurrently the synthesis of the 1-oxo-2-en-5β,6β-epoxy-4β-hydroxy pattern (*i.e.* pattern of rings A and B) using cholesterol derivatives as starting materials.

Ikekawa's route proceeded through the synthesis of the key intermediate **4.54** (whose 1-oxo-2,5-diene system is also present in rings A and B of withanolide F **2.2**). Elaboration of ring A began with the reduction of epoxide **4.50** with hydrazine, followed by C-1 oxidation to obtain the α,β-unsaturated ketone **4.52** (**Scheme 4.4**, pathway a). Afterwards the alkene in ring B was installed by alkaline hydrolysis of the acetate **4.52**, and subsequent dehydration with phosphoryl chloride. The second strategy used 1α,2β-

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hydroxycholesterol **4.55** as starting material, and led to intermediate **4.54** in three steps, that is the selective acetylation of its less hindered 3β-hydroxyl group, followed by Jones oxidation of 1α-hydroxyl group in compound **4.56** and, finally, alkaline α,β-elimination of keto ester **4.57** (**Scheme 4.4**, pathway b).[164, 165]

Scheme 4.4. Ikekawa's synthesis of the the 1-oxo-2,5-diene system **4.54**.[164, 165]
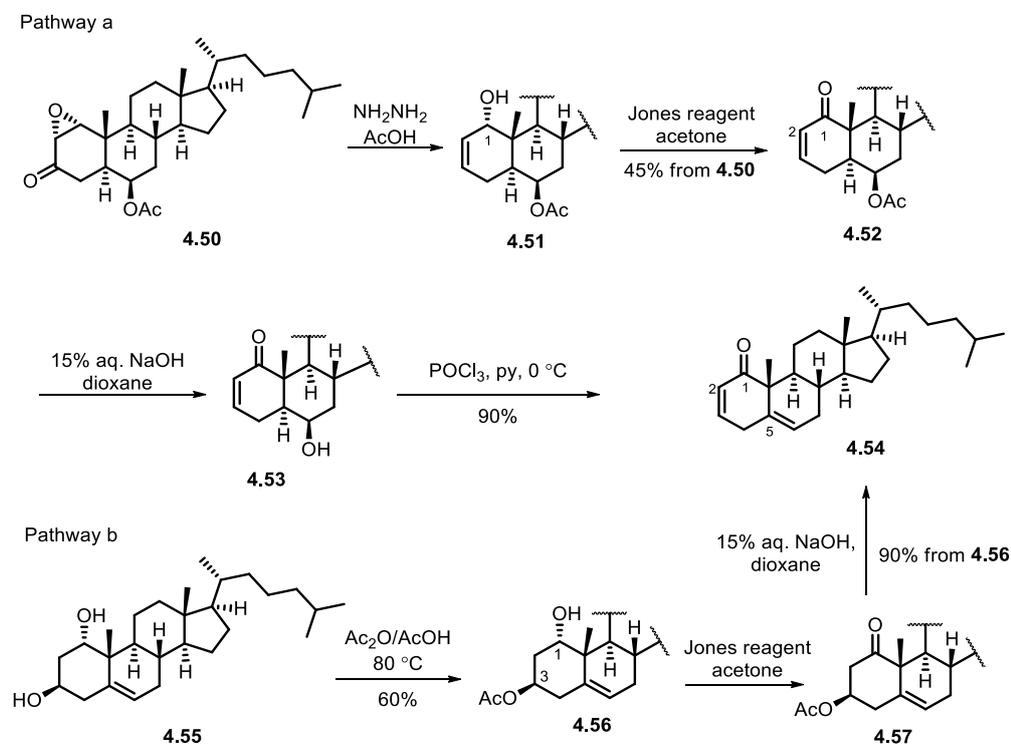
Once dienone **4.56** was in hand, further functionalisation was carried out by its epoxidation to compounds **4.58** and **4.59** with *m*-chloroperbenzoic acid, followed by a nearly quantitative ring opening-elimination sequence to allylic alcohol **4.60** (**Scheme 4.5**). The required 4β-hydroxyl group was installed as a glycol on treatment of α-mesylate **4.61** with osmium tetroxide, thus allowing the stereoselective epoxide formation to give 5β,6β-epoxy-4β-hydroxy-5β-cholest-2-en-1-one **4.63**.[164, 165]
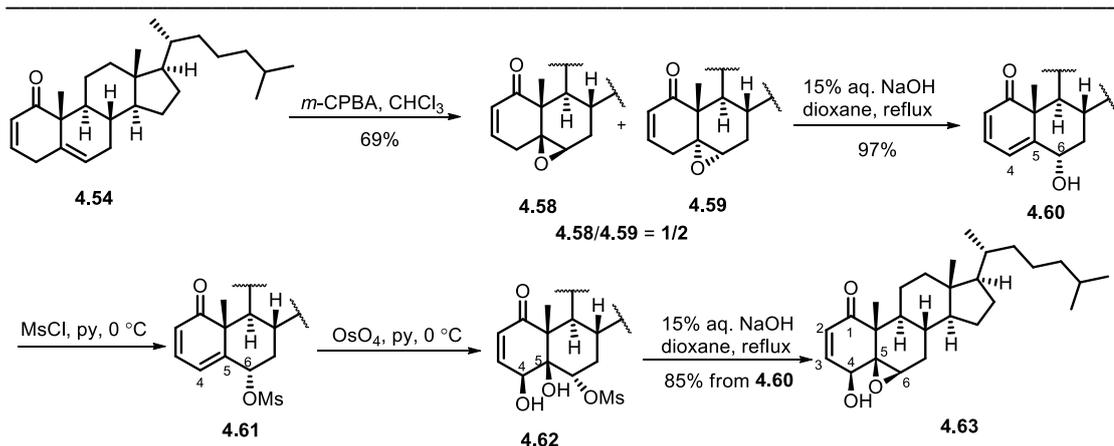
Scheme 4.5. Ikekawa's synthesis of the 5β,6β-epoxy-4β-hydroxy system **4.63**.

The procedure designed by Weissenberg *et al.*[166] has the advantage that it also give the 1-oxo-2,5-dien-4β-hydroxy system **4.74**, which is another of the most common substitution patterns of withanolide rings A and B (**Scheme 4.6**).
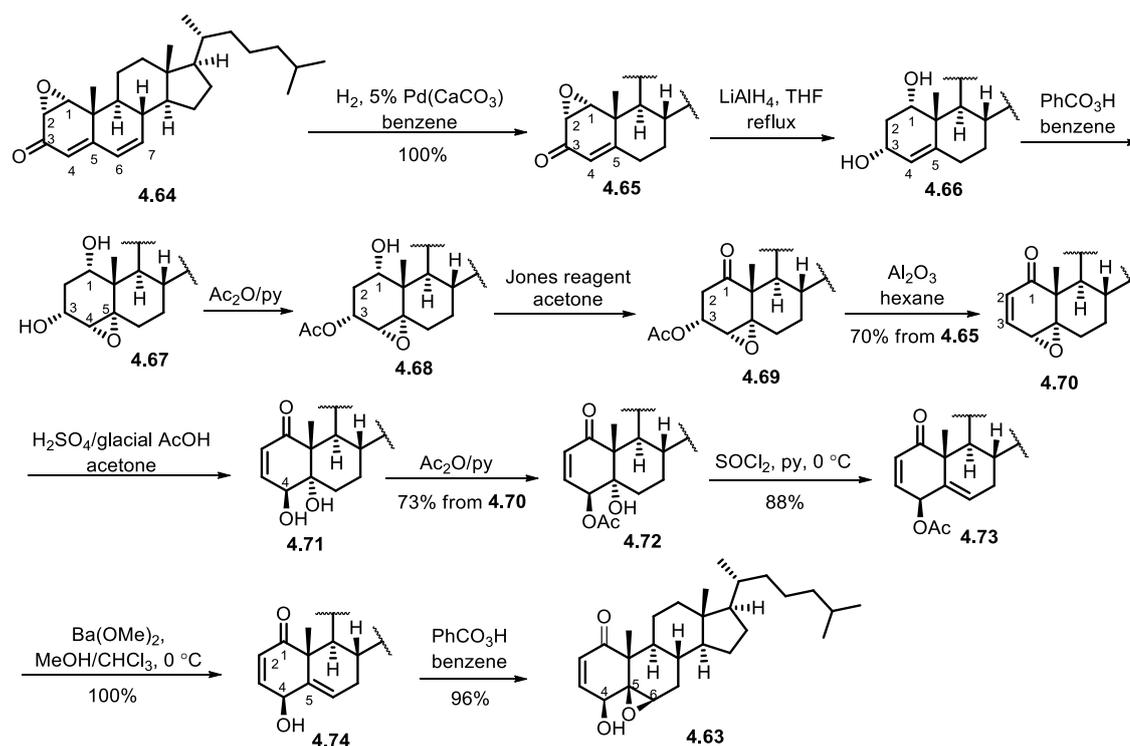
Scheme 4.6. Weissenberg's synthesis of the 5β,6β-epoxy-4β-hydroxy system **4.63**.[166]

The C-6,C-7 double bond in 1α,2α-epoxycholesta-4,6-dien-3-one **4.64** was selectively hydrogenated first. The formation of the 1-oxo-2-ene system began with the reduction of the 1α,2α-epoxide and 3-ketone in compound **4.65**, then epoxidation of the C-4,C-5 double bond in compound **4.66** followed by regioselective C-3 acetylation of **4.67** and C-1 oxidation of **4.68** to give ketone **4.69**. After alkaline α,β-elimination epoxyenone **4.70**

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was obtained in good yield. Acid-catalysed opening of epoxide **4.70** gave 4β-alcohol **4.71**. Finally the 4β,5β-epoxide was introduced smoothly by first dehydration of acetate **4.72** with thionyl chloride to dienone **4.73**, followed by a barium methoxide catalysed transesterification to allylic alcohol **4.74** and epoxidation in the presence of perbenzoic acid.[166]

4.1.5.2. Rings A and B elaboration: towards the synthesis of withanolide D **4.86**

Ten years later, Ikekawa's group described the first synthesis of withanolide D **4.86**, a compound with the same A and B ring functionality, starting from a pregnenolone **3.56**, through a different sequence of events (**Scheme 4.7**).[167]
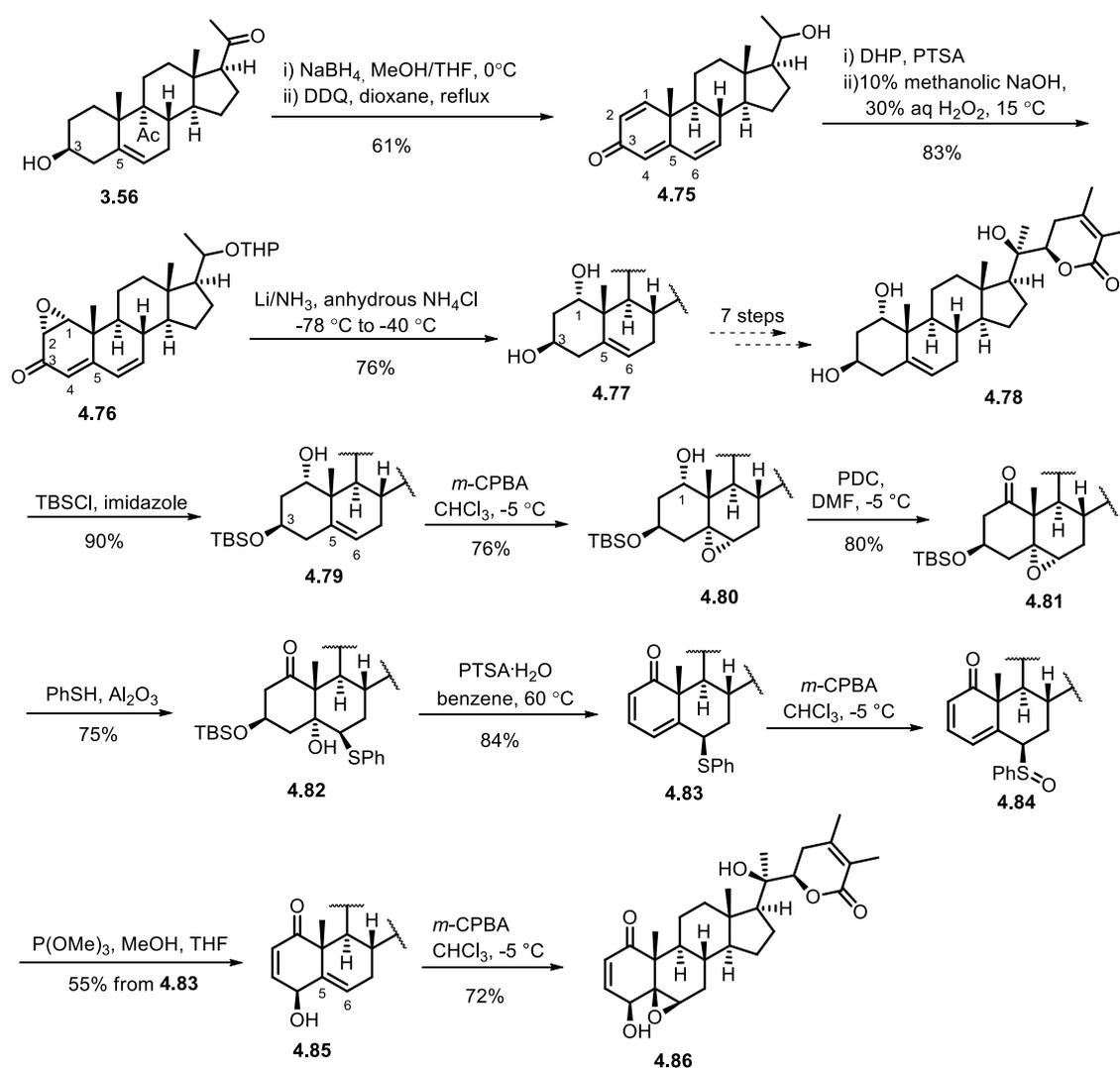
Scheme 4.7. Ikekawa's synthesis of withanolide D **4.86**.[167]

Elaboration of rings A and B of pregnenolone **3.56** started with the oxidation of its 3β-hydroxy-5-ene system to trienone **4.75** on treatment with 2,3-dichloro-5,6-dicyano-

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1,4-benzoquinone, followed by selective 1α,2α-epoxidation with alkaline hydrogen peroxide to intermediate **4.76**. Using lithium reduction in liquid ammonia, epoxydienone **4.76** was converted into 1α,3β-diol-5-ene **4.77**. After having installed the side-chain, the more accessible hydroxyl of diol **4.78** was protected as the silyl ether **4.79**, and the C,5-C,6 double bond selectively epoxidised to **4.80** before oxidation of the 1α-hydroxyl group to obtain ketone **4.81**. Epoxide ring opening with thiophenol and aluminium oxide furnished thioether **4.82**, which was dehydrated to dienone **4.83**, oxidised to allylic sulfoxide **4.84** and finally subjected to a 2,3-sigmatropic rearrangement to introduce the 4β-hydroxyl in compound **4.85**. Epoxidation with *m*-chloroperbenzoic acid afforded stereoselectively 5β,6β-epoxide **4.86** in good yield.[167]

4.1.5.3. Earlier studies towards the synthesis of withanolide δ-lactone

The first stereoselective construction of a 22(*R*)-δ-lactone in the steroid side-chain was achieved by Weihe and McMorris, during studies towards the synthesis of the fungi sexual hormone 23-deoxyantheridiol **4.96** (**Scheme 4.8**).[168]
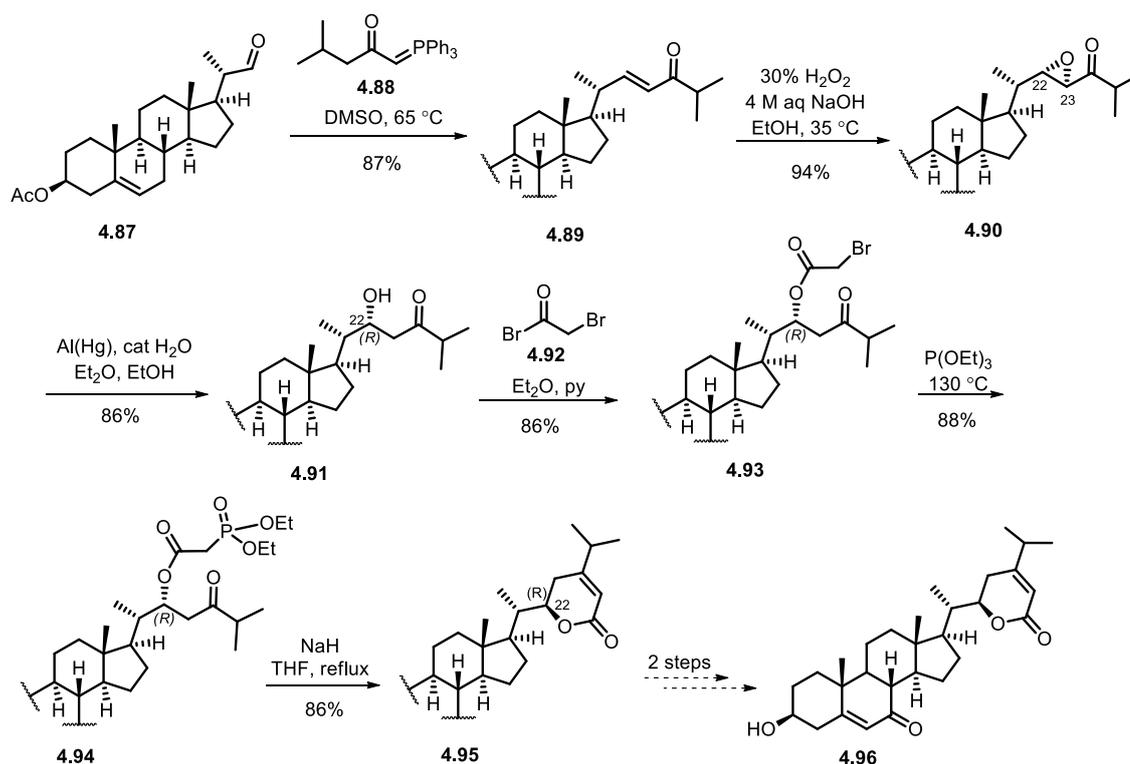
Scheme 4.8. Weihe and McMorris's synthesis of 23-deoxyantheridiol **4.96**.[168]

To obtain the natural 22(*R*)-configuration, aldehyde **4.87** was first converted into vinyl ketone **4.89** by Wittig reaction with ylide **4.88**, and afterwards stereoselectively epoxidised to the 22α,23α-epoxide **4.90**. Ring-opening of epoxide **4.90** on treatment with

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aluminium amalgam yielded exclusively the desired 22(R)-hydroxy ketone **4.91** in 86% yield. Ketol **4.91** was acetylated with α-bromoacetyl bromide **4.92** to compound **4.93**, which was in turn subjected to an Arbuzov reaction with triethyl phosphite to obtain phosphonate **4.94**. Ring-closure *via* an intramolecular Horner-Wadsworth-Emmons reaction gave finally δ-lactone **4.95** which featured the same 22(R)-configuration of the natural withanolide δ-lactone. [168]

4.1.5.4. Functionalisation of the withanolide δ-lactone

Amongst a number of studies towards the synthesis of withanolide, Ikekawa and co-workers also developed a strategy to functionalise the withanolide δ-lactone, *e.g.* by inserting a 27-hydroxyl group which is also a feature of withaferin A **4.1** (**Scheme 4.9**).[169]

Prior to its decoration, the six-membered lactone was installed following the procedure previously developed by McMorris,[168] although the substrate **4.106** was prepared *via* a different route (**Scheme 4.9**).[169]

Ikekawa's synthesis started with the conversion of commercially available compound **4.97** into olefin **4.100**. Reduction of acid **4.97** with lithium aluminium hydride furnished alcohol **4.98**, whose rings A and B were converted into acetate **4.99** before further elaboration of the side-chain. The C-20 hydroxyl group was oxidased with pyridinium chlorochromate, and afterwards subjected to a Wittig reaction with methylenetriphenylphosphorane to give olefin **4.100**. Osmium tetraoxide oxidation of **4.101** resulted in a mixture of 22(S)- and 22(R)-diols in 5/2 ratio. Only the desired 22(S)-isomer was progressed in the synthesis, and, after its tosylation to compound **4.102**, it was selectively epoxidised to compound **4.103** on treatment with potassium carbonate. An additional carbon was inserted in the side-chain *via* a Corey-Seebach umpolung reaction using 2-methyl dithiane **4.104**, and the dithioether **4.105** was released to ketone **4.106** by means of mercuric oxide and boron trifluoride etherate. On ketol **4.106**, the unsaturated δ-lactone **4.107** was then built in three steps in 79% yield following McMorris' procedure described in **Scheme 4.8**.

With the δ-lactone in hand, its functionalisation was achieved by first stereospecific hydrogenation of the unsaturated ring to give compound **4.108**, followed by α-alkylation with lithium *N-iso*propylcyclohexylamide and diphenyl sulfide to give a mixture of 25(S)- **4.109** and 25(R)-thioethers in 3/2 ratio. Alkylation of the desired isomer **4.109** by means of lithium *N-iso*propylcyclohexylamide and formaldehyde was highly

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stereoselective, and gave the intended 25(R)-isomer **4.110** in 68% yield. Finally, after elaboration of rings A and B to give compound **4.111**, thermal elimination of the corresponding sulfoxide **4.112** furnished α,β-unsaturated δ-lactone **4.113**, which featured the 22(R)-δ-lactone and the 27-hydroxyl group typical of withanolides and, in particular, of withaferin A **4.1**.[169]
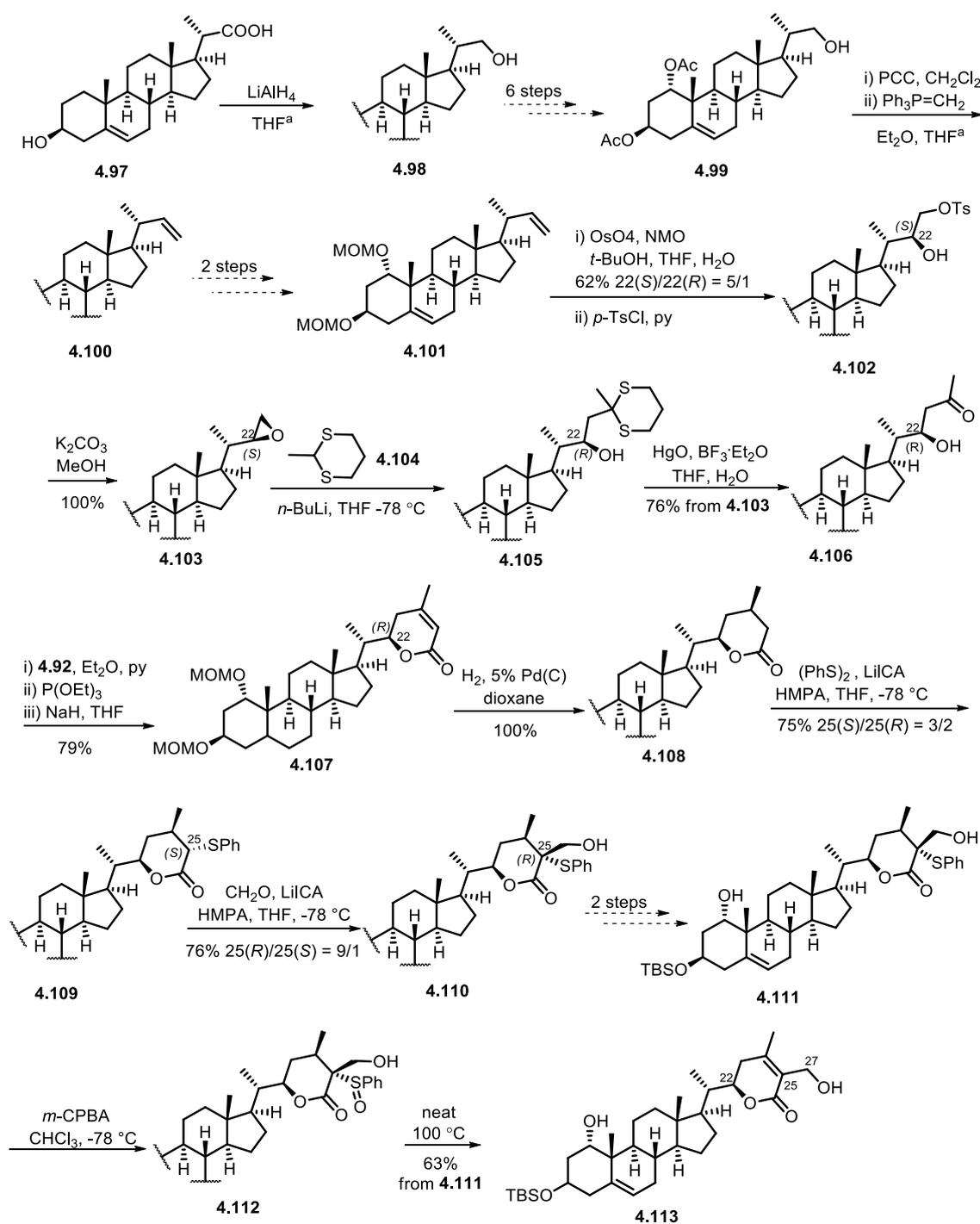
Scheme 4.9. Ikekawa's synthesis and C-25 functionalization of withanolide δ-lactone. [a]Yield not given for the specific reaction.[169]

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4.1.5.5. Synthesis of δ-lactone in 20(R)-hydroxyl withanolides

It was known that the reaction of 22-aldehyde **4.114** with the anion of the substituted ethyl crotonate **4.115** was stereoselective, and furnished exclusively the six-membered lactone with the unnatural 22(S)-configuration **4.116** (**Scheme 4.10**).[168, 170, 171]
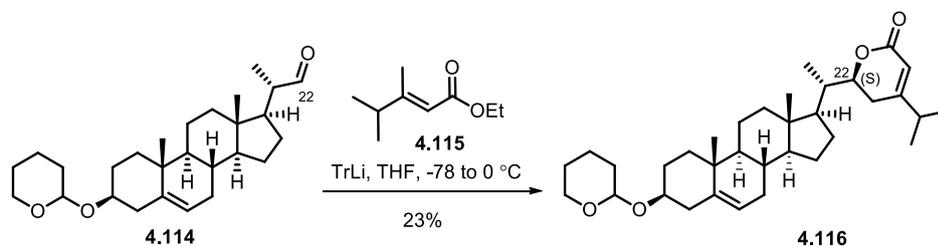
Scheme 4.10. Stereoselective aldol condensation towards unsaturated lactone **4.116**.[170, 171]

However, during the synthesis of withanolide D **4.86**, Ikekawa's group discovered that, in the presence of a hydroxyl group in α-position to the aldehyde, this reaction conversely resulted in a lactone ring with 22(R)-configuration (**Scheme 4.11**).[167]
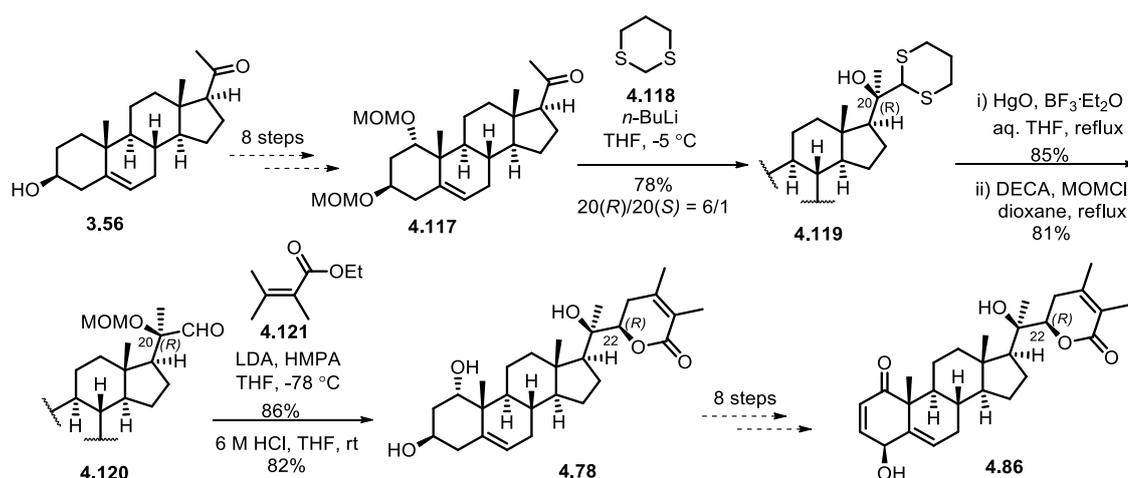
Scheme 4.11. Ikekawa's lactone ring formation during studies towards withanolide D **4.86**.[167]

To synthesise withanolide D **4.86**, pregnenolone **3.56** was chosen as starting material and firstly elaborated in ring A to give compound **4.117**. Construction of side-chain started with homologation *via* a Corey-Seebach umpolung reaction in the presence of dithiane **4.118**, giving a mixture of 20(R)-alcohol **4.119** and its 20(S)-isomer in 6/1 ratio. The aldehyde in compound **4.119** was released by treatment with mercuric oxide and boron trifluoride etherate and afterwards protected as its methoxymethyl derivative **4.120**. Finally, reaction of aldehyde **4.120** with the lithium enolate of α,β-dimethyl ethyl crotonoate **4.121** afforded lactone **4.78**, which was subsequently converted in eight steps

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into withanolide D **4.86** (see **Scheme 4.7**). In contrast with literature precedent (**Scheme 4.10**), the newly formed C-22 chiral centre was found in the natural (*R*)-configuration. The author assumed this was due to the steric hindrance of the 20β-methoxymethyl group, which selectively drove the attack of the enolate from the backside of the molecule and gave a 22(*R*)-lactone according to the Cram's cyclic model.[167]

*4.1.5.6. The α-orientated withanolide side-chain: total synthesis of withanolide E **4.137***

Although the side-chain can be either α or β-orientated, the number of withanolides bearing an α-orientated side-chain is slightly smaller and this pattern always occurs with a 17β-hydroxyl or, more rarely, a 17β-methyl group.[148-150] A typical example of withanolide featuring the 17α-side-chain and a 17β-hydroxyl group is withanolide E **4.137**, whose synthesis was accomplished by Perez-Medrano and Grieco starting from 17-keto steroid **4.122** (**Schemes 4.12** and **4.13**).[172]

Before starting the construction of the side-chain, ketone **4.122** was converted into diene **4.124** in good yield by a Seagusa reaction followed by acetylation with isopropenyl acetate **4.123** (**Scheme 4.12**). A 14α-hydroxyl was afterwards inserted *via* a hetero Deals-Alder reaction using benzyl nitrosoformate **4.125** as the dienophile, which in turn was generated *in situ* by oxidation of benzyl *N*-hydroxycarbamate with tetrabutylammonium periodate. The [4+2]-cycloaddition resulted in a mixture of isomers **4.126**/**4.127** in 1/2 ratio. However, the unwanted β-isomer **4.126** isomerised by boiling in toluene, thus giving cycloadduct **4.127** in 85% overall yield. Hydrogenation of steroid **4.127** and subsequent reaction with copper(II) chloride were used to release the C-14 hydroxyl group. Deacetylation of C-1 and C-3 under basic conditions led finally to triol **4.128** in 79% yield.[172]
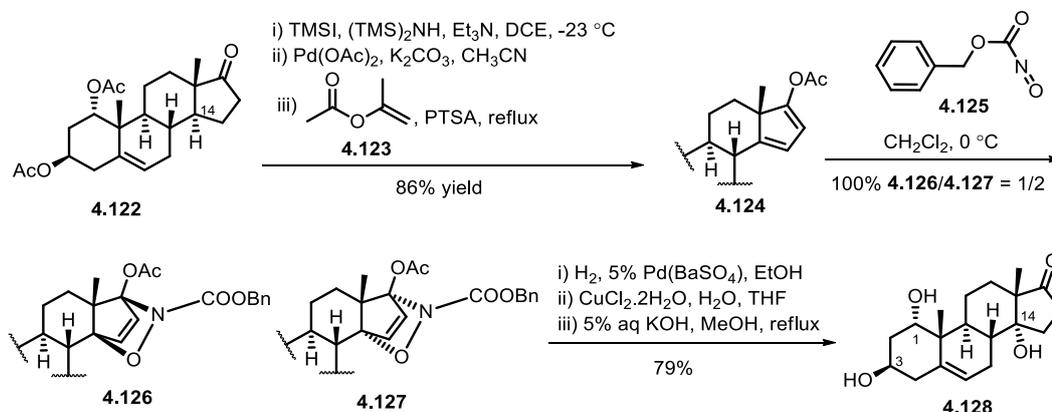
Scheme 4.12. C-14 α-hydroxylation in the synthesis of withanolide E **4.137**.[172]

In contrast to the precedented results that organometallics react with C-20 keto steroids to give 20β-alcohols, addition of nucleophiles to C-17 keto steroids proved unsuccessful and led to the 17α-isomers. To achieve the particular stereochemistry, the authors converted the C-17 ketone of compound **4.129** (whose ring A was converted into a cyclic form as protection) into alkene **4.130**, which was in turn di-hydroxylated on treatment with osmium tetraoxide in pyridine to obtain 17(*S*),20(*R*)-diol **4.131** and its 17(*R*),20(*S*)-diastereisomer in 1.4/1 ratio (**Scheme 4.13**). The desired α-isomer **4.131** was oxidised to 20-ketone **4.132** and then selectively alkylated with vinyllithium to the expected 20β-alcohol **4.133**. After protection of the hydroxyl groups as methoxymethyl ethers, ozonolysis of the vinyl group in compound **4.134** provided the 22-aldehyde **4.135** which was exclusively transformed into the 22(*R*)-lactone **4.136** on treatment with ethyl α,β-dimethylcrotonate **4.121**. The last steps were focused on the elaboration of rings A and B, and, after C-3 acetylation, C-1 Swern oxidation, elimination of the acetate and C-5,C-6 epoxidation, withanolide E **4.137** was obtained in good yield from pentaol **4.136**.[172]
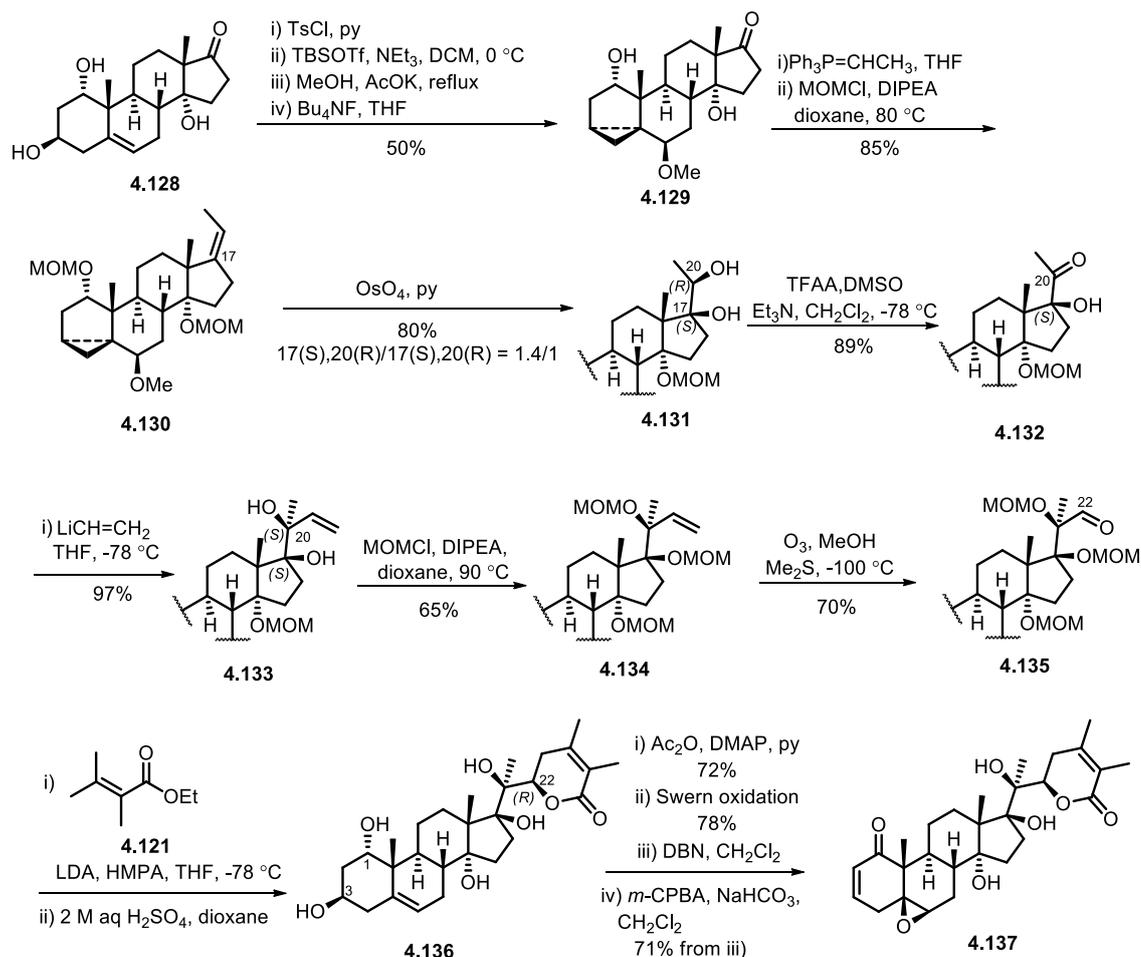
Scheme 4.13. Synthesis of the 17α-side-chain of withanolide E **4.137**.[172]

4.1.5.7. A modern approach to withanolides: total synthesis of withanolide A *4.49*

The synthesis of withanolide A **4.49** carried out by Gademann and co-workers in 2011 represents a modern approach to the total synthesis of withanolides, which occurred in fewer steps and was kept nearly free from protecting groups by using mild conditions and a well-designed order for installation of functionalities (**Scheme 4.14**). [161, 162]
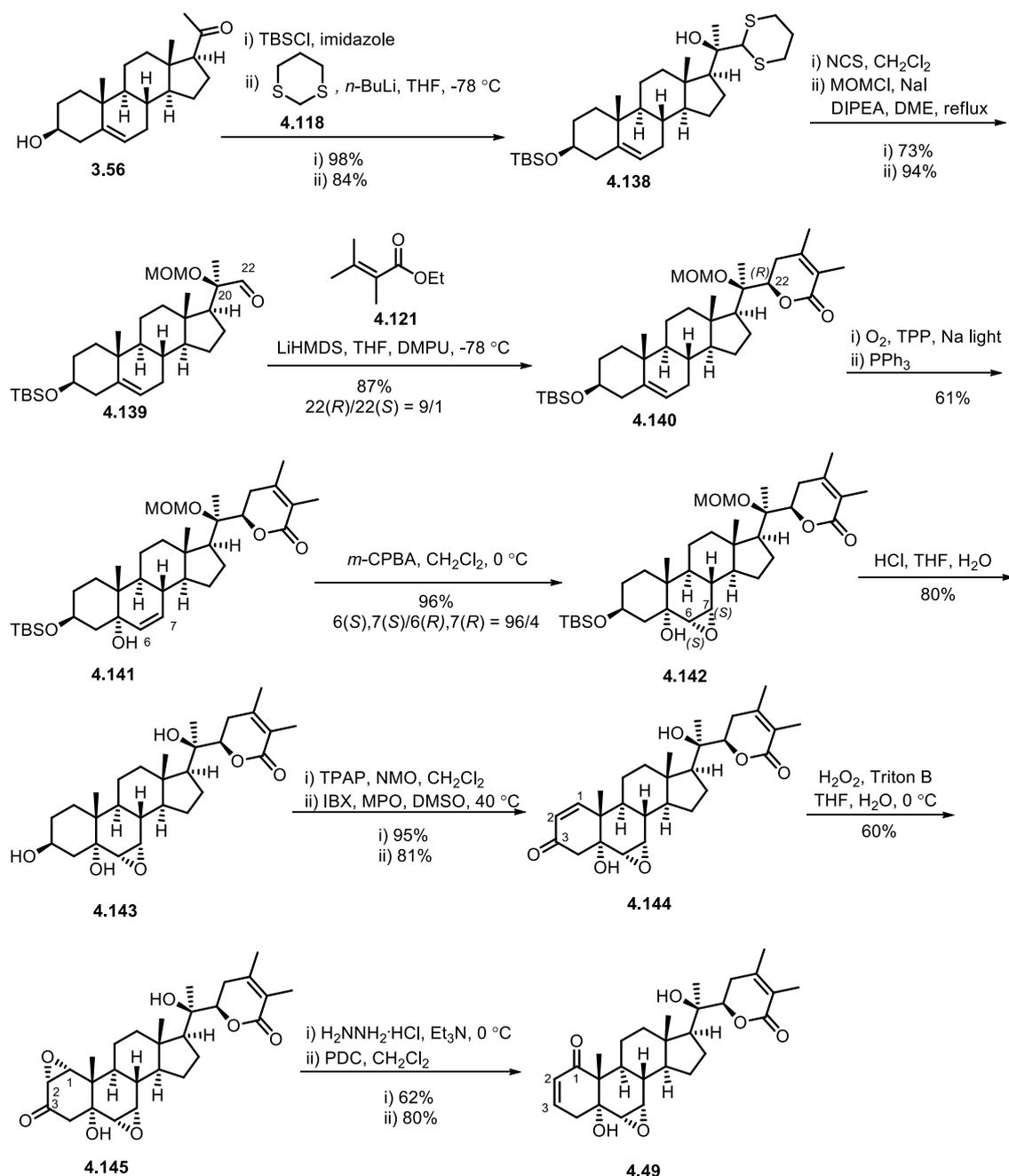
Scheme 4.14. Gademann's Total synthesis of Withanolide A **4.49**.[161, 162]

After protection of the hydroxyl group of pregnenolone **3.56**, construction of the side-chain was carried out followed classic conditions, *i.e.* Corey and Seebach umpolung

homologation by means of 1,3-dithiane **4.118** to intermediate **4.138** followed by the stereoselective vinylogous reaction of aldehyde **4.139** with ethyl 2,3-dimethylbut-2-enoate **4.121** and lithium hexamethyldisilazide, thus leading to lactone **4.140** in 87% yield and good stereoselectivity.

Attention was afterwards focussed on elaboration of rings A and B. On ring B, allylic alcohol **4.141** was prepared by a singlet-oxygen-mediated photooxygenative olefin migration in the presence of *meso*-tetraphenylporphyrin, and afterwards selectively epoxidised on treatment with *m*-cloroperbenzoic acid to compound **4.142**.[161, 162]

Acidic deprotection of the hydroxyl groups gave epoxy alcohol **4.143**, and then elaboration of ring A to the common 1-oxo-2,5-diene pattern was successfully achieved without the use of protecting groups. The 3-hydroxyl group was first converted into unsaturated ketone **4.144** by treatment with tetrapropylammonium perruthenate and *N*-methyl morpholine *N*-oxide, followed by 2-iodobenzoic acid oxidation. After a Triton B-catalysed 1,2-epoxidation using hydrogen peroxide, epoxide **4.145** was subjected to a Wharton carbonyl transposition in the presence of hydrazine hydrochloride and triethylamine. Final oxidation of the resulting alcohol yielded withanolide A **4.49** in good overall yield.[161, 162]

4.2. PROJECT AIM: STUDIES TOWARDS AN UNNATURAL WITHANOLIDE

Withanolide F **2.2** is natural steroid characterised by the 1-oxo-2,5-diene system, which is present in withanolides with cytotoxic acvivity and/or inhibitors of the the Hh/Gli signalling pathway. Withanolide F **2.2** also contains the less common α-orientated lactonic side-chain, along with the 17β-, the 20β- and the 14α-hydroxyl groups (**Figure 4.10**).
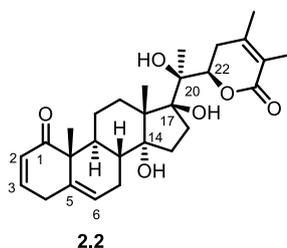
Figure 4.10. Structural features of withanolide F **2.2**.

With the aim of assessing the SAR of this class of compound towards the Hh/Gli signalling pathway, we wanted to first evaluate the influence of the 14-hydroxyl group.

A 14β-hydroxyl is a common feature of Hh/Gli inhibitors physalin F **1.13**, physalin **1.14** and cardiac glycoside **1.19** (**Figure 4.11**), and it is rare substitution amongst withanolides.
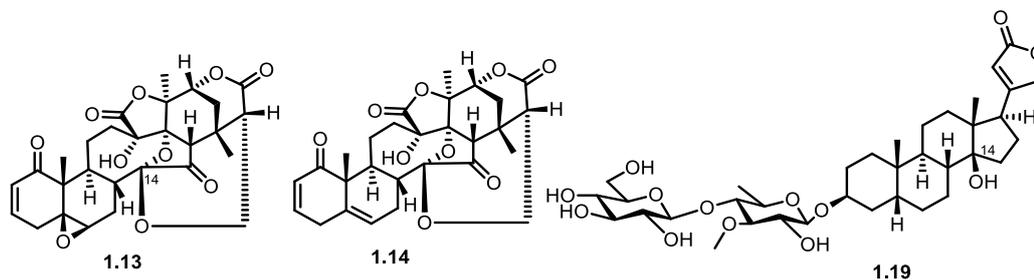
Figure 4.11. Natural Hh/Gli inhibitors.

Therefore, our first goal was the synthesis of the 14β-hydroxyl withanolide F analogue **2.5** with the C-13 methyl group and the C-14 hydroxyl group *cis* to each other, starting from cheap commercially available materials. The isolation or the total synthesis of the 14β-withanolide F **2.5** have no literature precedent. The key steps of the planned route are shown in **Scheme 4.15**.
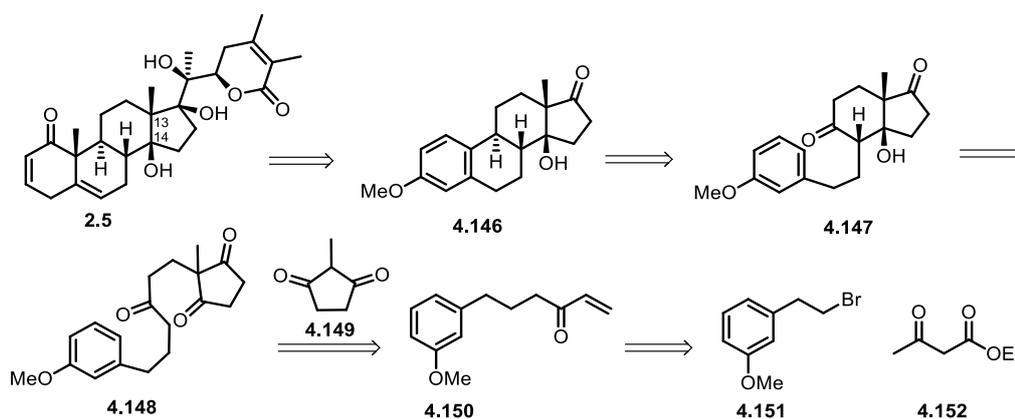
Scheme 4.15. Retrosynthesis of 14β-hydroxy withanolide F **2.5.**

We believed that the side-chain could be installed from intermediate **4.146** *via* a modification of Medrano's procedure for the synthesis of withanolide E **4.137**[172] by means of two consecutive Corey-Seebach umpolung reactions[173, 174] followed by a vinylogous aldol reaction with the lithium enolate of α,β-dimethylcrotonate. The aromatic ring A and ring B could be converted into the 1-oxo-2,5-diene pattern by first Birch reduction[175] followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone,[176] thus setting the basis for further elaboration under Ikekawa's conditions.[167]

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Steroid **4.146** was envisioned to come from an intramolecular Friedel-Crafts alkylation of the aromatic ring A with the proximal keto group· followed by reduction of the resulting benzylic alcohol, or the styrene double bond in case of dehydration.

The intramolecular asymmetric cyclisation of triketone **4.148** to diketoalcohol **4.147** is based on the Hajos-Parrish procedure.[177, 178] Triketone **4.148** has been prepared *via* a Mannich-Robinson extension of cyclopentanedione **4.149** with vinyl ketone **4.150** according to the procedure developed by Wang *et al.*[179] and, previously, by Smith *et al.*[180] Bromide **4.151**, ethyl acetoacetate **4.152** and cyclopentanedione **4.149** have been chosen as readily available materials for our planned synthesis.

Except for the reactions described in **Scheme 4.56**, all the reactions described in this Chapter were run at AnalytiCon Discovery GmbH (Potsdam, Germany).

4.3. RESULTS AND DISCUSSION

4.3.1. Ring A: synthesis of vinyl ketone 4.150

The first key intermediate vinyl ketone **4.150** is known in the literature as a useful intermediate for the synthesis of estrone and related steroids.[179-181] The first synthesis of vinyl ketone **4.150** was achieved by Hughes and co-workers in the 1960s (**Scheme 4.16**).[180, 182]
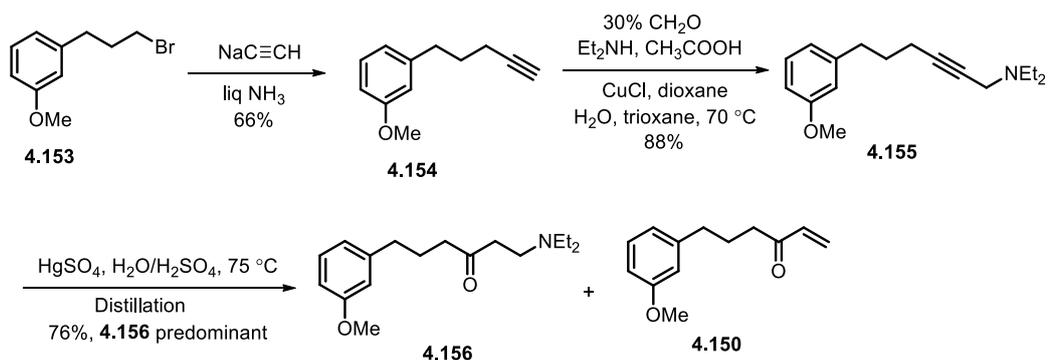
Scheme 4.16. Early steps of Hughes and Smith's synthesis of steroids.[180, 182]

The synthesis began with treatment of bromide **4.153** with sodium acetylide in liquid ammonia to give alkyne **4.154**, which was in turn elongated *via* a Mannich reaction to diethylamine **4.155** in good yield. To insert the keto group, the acetylenic bond was hydrated using mercuric sulfate in sulfuric acid and water, followed by purification by distillation. Under these conditions, ketoamine **4.156** underwent partial elimination, and

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it was isolated as a mixture along with vinyl ketone **4.150** (in a ratio depending on the degree of fractionation). The mixture of compounds **4.156** and **4.150** was not separated, but was used directly in the following condensation.[180]

Years later, Wang *et al.* described a different strategy to the synthsis of vinyl ketone **4.150** (**Scheme 4.17**). The procedure started with bromide **4.151** which reacted in the presence of the dianion of ethyl acetoaceate **4.152** to give, *via* a selective γ-alkylation, ketoester **4.157**. After protection of the keto group, ester **4.158** was reduced to alcohol **4.159**, and afterwards exposed to acidic conditions to promote dehydration and deprotection of the ketone. Thus, vinyl ketone **4.150** was easily obtained in 4 steps and 62% overall yield.[179]
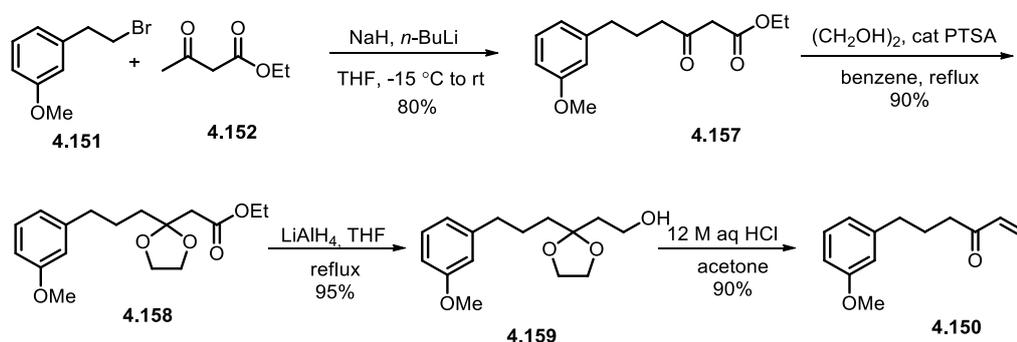
Scheme 4.17. Wang's synthesis of vinyl ketone **4.150**.[179]

The route of Wang *et al.*[179] seemed a straightforward way to prepare vinyl ketone **4.150**. Nevertheless, we were obliged to revise this strategy in order to achieve a satisfactory yield and a better reproducibility (**Scheme 4.18**).
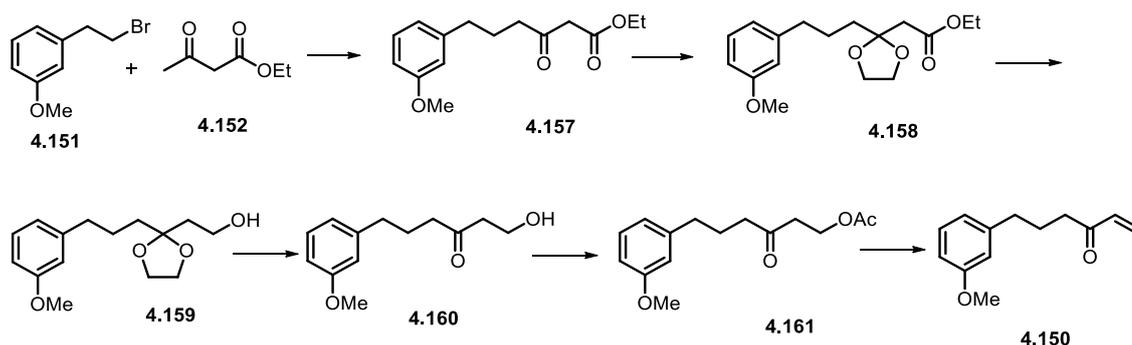
Scheme 4.18. Synthesis of vinyl ketone **4.150**.

4.3.1.1. Step 1. γ-Alkylation of β-ketoester **4.152** with 3-methoxyphenetyl bromide **4.151**

The starting step of the planned synthesis was the γ-alkylation of the dianion of ethyl acetoacetate **4.152** by means of phenethyl bromide **4.151** (**Table 4.1**). Early efforts were carried out following the procedure of Wang *et al.*,[179] *i.e.* first formation of the dianion

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with sodium hydride and *n*-butyllithium followed by addition of bromide **4.151** (**entries 1-4**). The first formation of monoanion was required because *n*-butyllithium is known to add also to the ester group of β-ketoesters, therefore protection of the carbonyl group from further nucleophilc attack was recommended.[183]

Entry	Conditions	Yield (4.157)
1	i) **4.152** (1.1 eq), NaH (1.3 eq), 0 °C, 5 min ii) *n*-BuLi (1.8 eq), -0 °C, 5 min iii) **4.151** (300 mg), 0 °C to rt, 30 min	49%
2	i) **4.152** (2.4 eq), NaH (2.8 eq), 0 °C, 10 min ii) *n*-BuLi (2.4 eq), -0 °C, 15 min iii) **4.151** (400 mg), 0 °C to rt, 30 min	90%
3	i) **4.152** (1.5 eq), NaH (1.6 eq), 0 °C, 10 min ii) *n*-BuLi (1.6 eq), -0 °C, 30 min iii) **4.151** (2.5 g), 0 °C to rt, 2h	77%
4	i) **4.152** (1.5 eq), NaH (1.6 eq), 0 °C, 15 min ii) *n*-BuLi (1.6 eq), -0 °C, 15 min iii) **4.151** (2.5 mg), 0 °C to rt, 30 min	48%
5	i) **4.152** (1.5 eq), LDA (3.2 eq), 0 °C to rt, 1 h ii) **4.151** (960 mg), 0 °C to rt, overnight	60%

Table 4.1. Synthesis of ketoester **4.157**. Styrene **4.162** was detected by NMR spectroscopy as the side-product in variable yield depending on the reaction conditions, but no efforts were made for its isolation.

However, the reaction did not give the expected results and ketoester **4.157** was obtained in lower yield than the published (**entry 1**). An excess of dianion compared to the alkylating agent gave the best yield (**entry 2**), altough this outcome could not be reproduced. Different equivalents of base, temperatures, reaction times and quenching conditions were therefore screened (**entries 3** and **4**), but the yield remained low. Good reproducible results were obtained when two equivalents of the non-nucleophilic lithium di*iso*propylamide[184] were used in the deprotonation step, thus leading to ketoester **4.157** in 60% yield (**entry 5**). Unfortunately, all attempts to improve the yield by increasing reaction times and temperatures led mainly to the formation of a compound which was likely to be methoxystyrene **4.162**, along with other by-products which were not fully characterised, whilst the use of lower temperatures and shorter reaction times did not allow the alkylation to take place.

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*4.3.1.2. Step 2. Protection of ketone **4.157** as its acetal **4.158***

The protection of ketone **4.157** as its acetal was required (**Table 4.2**). We again followed the procedure of Wang *et al.*,[179] and, on treatment with an excess of ethylene glycol and catalytic amounts of *p*-toluenesulfonic acid, acetal **4.158** was obtained in 75% yield, against the 90% yield published (**entry 1**)
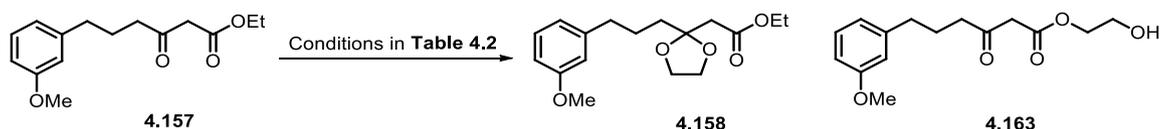
Entry	Conditions	Yield
1	$(CH_2OH)_2$ (5 eq), PTSA (0.01 eq), benzene, 90 °C,[a] 18 h	**4.158** 75%
2	$(CH_2OH)_2$ (5 eq), PTSA (0.01 eq), benzene, 95 °C,[a] 3 d	**4.163** 48%[b]

Table 4.2. Protection of ketone **4.158** as its acetal **4.159**. [a]Oil bath temperature. [b]Ester **4.158** was not observed.

Efforts aimed to improve the yield were carried out by using more than 0.01 equivalents of *p*-toluenesulfonic acid, 3 Å molecular sieves as dehydrating agent and/or the less toxic toluene as solvent. However, these conditions led only to more by-products, the major one of which was identified as the glycolic ester **4.163** (**entry 2**) arising from an acid-catalysed transesterification of ethyl ester **4.157** with ethylene glycol. Compound **4.163** is novel and it was fully characterised.

*4.3.1.3. Step 3. Synthesis of alcohol **4.159***

The reduction of ester **4.158** to the alcohol **4.159** is described by Wang *et al.* to occur in 95% yield using stoichiometric lithium aluminium hydride in refluxing tetrahydrofuran.[179] We prefered to use milder reaction conditions carrying out the reduction at lower temperature, which led to alcohol **4.159** in 88% yield (**Scheme 4.19**). Ketol **4.160** was the only by-product of the reaction. Therefore, the crude **4.159** could be used in the next step without any purification.
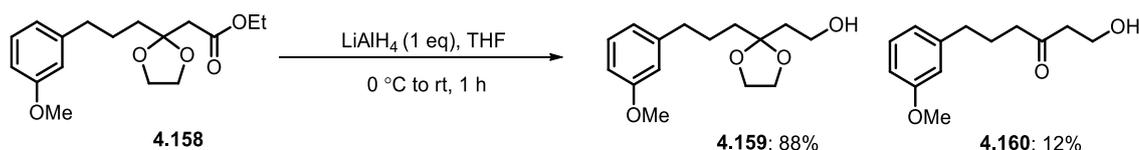
Scheme 4.19. Reduction of ester **4.158**.

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In order to improve the overall yield, glycolic ester **4.163** was converted into alcohol **4.159** on exposure to the same reaction conditions applied to ester **4.158** (**Scheme 4.20**). Thus, after protection of the ketone **4.163** as the acetal, ester **4.164** was reduced to alcohol **4.159** on treatment with lithium aluminium hydride in satisfactory yield.
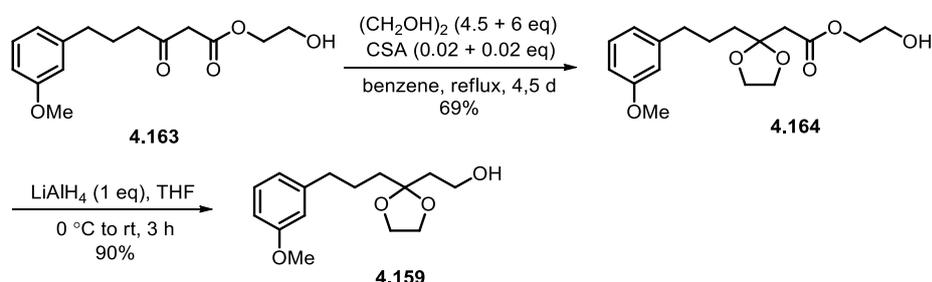
Scheme 4.20. Conversion of glycolic ester **4.163** into alcohol **4.159**.

4.3.1.4. Step 4. Deprotection of acetal 4.159

Wang's group also reported the direct conversion of alcohol **4.159** into vinyl ketone **4.150** on treatment with an excess of 12 M aqueous hydrochloric acid.[179] Indeed, this reaction allowed the removal of the acetal (**Scheme 4.21**). However, at the same time, the hydroxyl group was substituted for a chlorine atom and compound **4.165** was exclusively obtained.
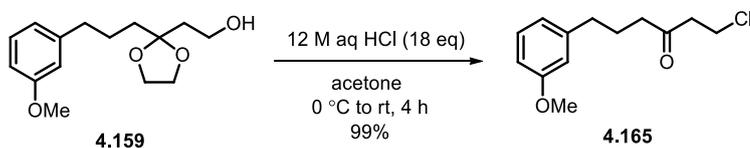
Scheme 4.21. Synthesis of chloro derivative **4.165**.

Nevertheless, when the concentration of hydrochloric acid was lowered to 1 M, the ketone **4.159** was exclusively deprotected, and ketoalcohol **4.160** was obtained in nearly quantitative yield (**Scheme 4.22**), although without elimination to alkene **4.150**.
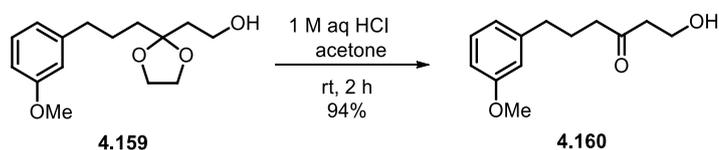
Scheme 4.22. Synthesis of keto alcohol **4.160**.

Chloride **4.165** is known in the literature[185] but no analytical data were published. Ketol **4.160** is novel. Both compounds were fully characterised.

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4.3.1.5. Step 5. Preparation of acetate 4.161

With alcohol **4.160** in hand, the preparation of olefin **4.150** by first conversion of the primary alcohol into acetate **4.161**, followed by a base-catalysed elimination was investigated. Indeed, novel acetate **4.161** was obtained in 61% on treatment with acetic anhydride and triethylamine (**Scheme 4.23**).
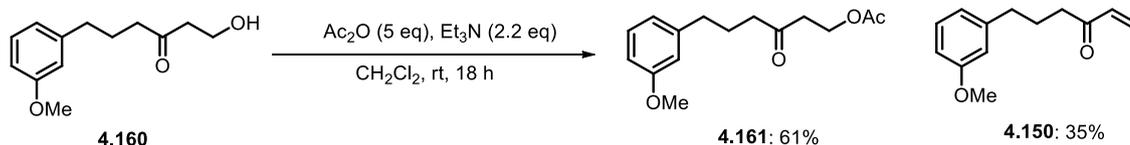
Scheme 4.23. Synthesis of acetate **4.161**.

We noticed that compound **4.150** was a by-product of the reaction, and so the crude product was used in the next step without further purification. All attempts to prepare vinyl ketone in one step from alcohol **4.160** failed when using a different base, higher temperatures or longer reaction times.

4.3.1.6. Step 6. Synthesis of vinyl ketone 4.150

The last step to the key intermediate **4.150** was the elimination of the acetate promoted by base, which procedeed rapidly and in nearly quantitative yield by stirring the crude acetate **4.161** in the presence of of 1,8-diazabicyclo[5.4.0]undec-7-ene (**Scheme 4.24**).
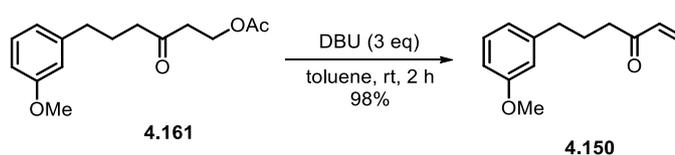
Scheme 4.24. Synthesis of vinyl ketone **4.150** from acetate **4.161**.

Under the same reaction conditions, also chloride **4.165** could be converted into vinyl ketone **4.150** (**Scheme 4.25**).
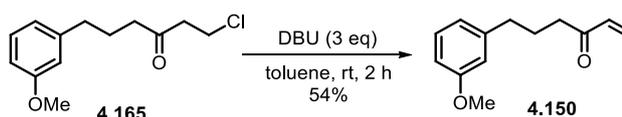
Scheme 4.25. Synthesis of vinyl ketone **4.150** from chloride **4.165**.

Once vinyl ketone **4.150** was in hand, its reaction with cyclopentanedione **4.149** was investigated.

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4.3.2. Rings C and D: synthesis of triketone 4.148 and its cyclisation to indanedione 4.147

4.3.2.1. Step 7. Synthesis of triketone 4.148

In the 1970s, Hajos and Parrish from Hoffman-La Roche synthesised triketone **4.167** *via* an acid-catalysed Michael addition of cyclopentanedione **4.149** to methyl vinyl ketone **4.166** (**Scheme 4.26**). The following intramolecular asymmetric cyclisation carried out in the presence of catalytic amounts of (*S*)-proline **4.168** as chiral inductor led exclusively to *cis*-diketoalcohol **4.169**, which in turn was exposed to acidic conditions and dehydrated to indenedione **4.170**.[177, 178, 186]
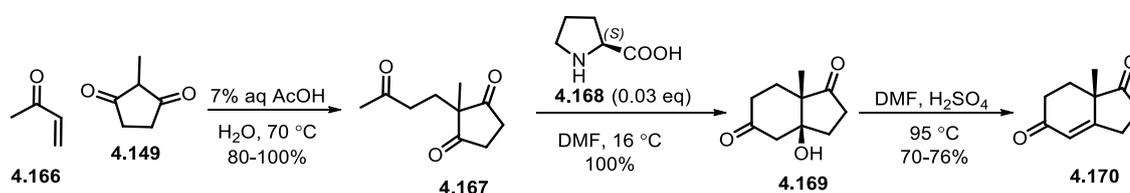
Scheme 4.26. Hajos and Parrish's asymmetric synthesis of indenedione **4.170**.[177, 178]

Concurrent studies by Eder, Sauer and Wiechert from Schering showed that the chiral homologue **4.172** could be obtained directly from triketone **4.171** by exposure to catalytic amounts of (*S*)-proline **4.168** containing a solution of organic or aqueous inorganic acids (**Scheme 4.27**).[186-188]
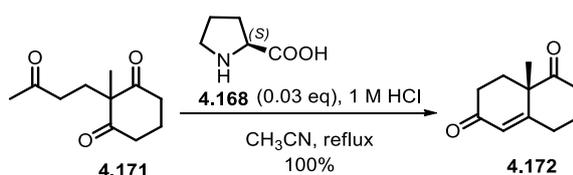
Scheme 4.27. Eder, Sauer and Wiechert's asymmetric synthesis of diketone **4.174**.[187]

The chiral bicycles **4.170** and **4.172** can be seen as the steroidal rings C and D, and A and B, respectively, and therefore have been extensively used in the preparation of diverse steroids.[186]

In light of these results, the synthesis of triketone **4.148** was first carried out under the Hajos and Parrish conditions[178] *via* an acid catalysed Michael addition of 2-methyl-1,3-cyclopentandione **4.149** to vinyl ketone **4.150**. Compound **4.148** was isolated, although in a low yield (**entry 1**, **Table 4.3**).

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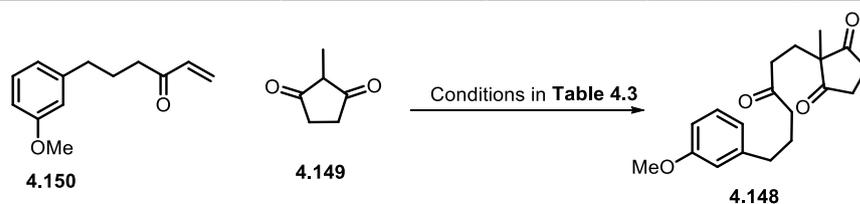
Entry	Conditions	Yield
1	**4.149** (1.7 eq), glacial AcOH/H_2O = 2/98, 70 °C, 18 h	25%
2	**4.149** (1.2 eq), glacial AcOH/H_2O = 6/1, 80 °C, 18 h	43%
3	**4.149** (3 eq), Et$_3$N (3.2 eq), THF, 40 °C, 1 d	90%

Table 4.3. Acid- and base-catalysed Michael additions for the synthesis of triketone **4.148**.

In the presence of an excess of acetic acid compared to water (6/1 ratio), the yield of triketone **4.148** was increased, probably with the increasing degree of solubility of the 1,3-cyclopentanedione **4.149** in the solvent mixture (**entry 2**).

It was described that the Hajos-Parrish ketone **4.170** could be also obtained by subjecting vinyl ketone **4.166** and cyclopentanedione **4.149** to basic conditions.[189] Moreover, Wang *et al.*[179] and Kurosawa *et al.*[190] reported that the prochiral triketone **4.148** could be synthesised in 75% and 80% yield, respectively, by stirring **4.150** and **4.149** in the presence of triethylamine for five days. The base-catalysed Michael addition was therefore addressed (**entry 3**). Pleasingly, and after a screening of reaction conditions, adduct **4.148** was obtained in 90% yield and in a considerably shorter reaction time compared to the literature procedures.[179, 190]

*4.3.2.2. Step 8. Intramolecular cyclisation of triketone **4.145***

During their studies, Clemente and Houk[188, 191] reported that the conversion of prochiral triketone **4.148** into (*S*)-enedione **4.174** ([α]$_D$ at rt = +181°)[187] occurred in 60% yield and 92% enantiomeric excess (under the conditions of Eder, Sauer and Wiechert) using (*S*)-phenylalanine **4.173** (**Scheme 4.28**, **a**), and in 76% yield and 45% enantiomeric excess using (*S*)-proline **4.168** (under the neutral conditions of Hajos and Parrish) (**Scheme 4.28**, **b**).
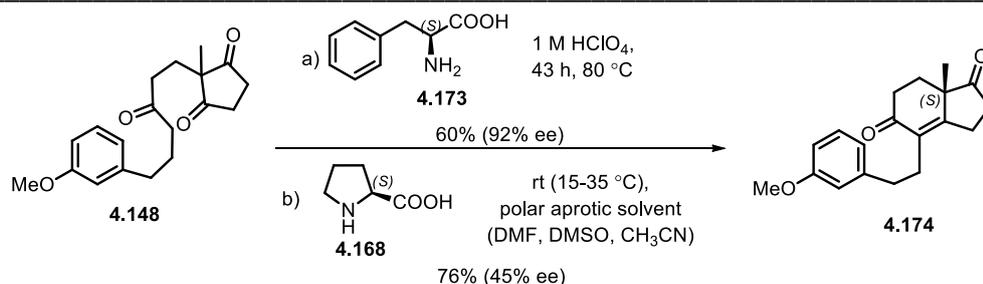
Scheme 4.28. Asymmetric ring-closure of triketone **4.148** to (*S*)-enedione **4.174** as described by Clemente and Houk.[188]

However, Hajos and Parrish reported in a patent that the compound isolated by heating triketone **4.148** in dimethylformamide in the presence of (*S*)-proline **4.168** was the "(+)-3aα-hydroxy-4β-(2-*m*-methoxyphenethyl)-7-aα-methyl-perhydroindan-1,5-dione" **4.175** (oil, [α]$_D$ at 25 °C = +30°) (**Scheme 4.29**), [191] which in turn in the presence of *p*-toluenesulfonic acid cyclised to compound (*R*)-**4.176** (oil, [α]$_D$ at 25 °C = +45.3°)[191] described as "den optischen Antipoden des bekannten (-)-3-methoxyestra-1,3,5(10),8,14-pentaen-17-one", *i.e.* the optical antipode of the known dienone (*S*)-**4.176** ([α]$_D$ at 23 °C = -105.3°) (**Figure 4.12**).[192]
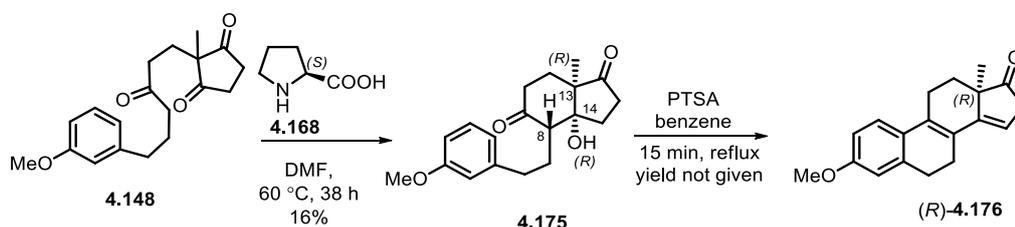
Scheme 4.29. Patented Hajos and Parrish's intramolecular asymmetric cyclo-dehydration of triketone **4.148**.[191]
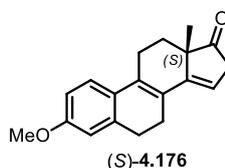
Figure 4.12. Structure of dienone (*S*)-**4.176**.

In light of these unclear results, and since no additional literature precedent was found for the amino acid-catalysed asymmetric intramolecular cyclisation of triketone **4.148**, the synthesis of the desired 13β-methyl-14β-hydroxy steroid **4.147** was attempted by treating prochiral triketone **4.148** at room temperature with catalytic amounts of (*S*)-proline **4.168** in dimethylformamide (**entry 1**, **Table 4.4**).[178, 191] However, in spite of the high yields reported in the literature, no traces of product **4.147** were seen, but only unreacted starting

material was recovered even when different catalysts, solvents or temperature were screened (**entries 2** and **3**).
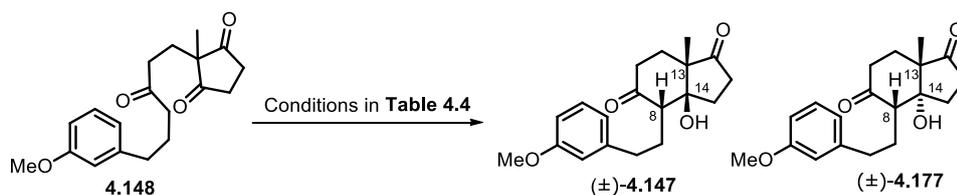
Entry	Conditions	Yield
1	(*S*)-proline **4.168** (0.03 eq), DMF, rt, 7 d	Sm **4.148**
2	(S)-proline **4.168** (0.03 eq), MeCN, rt to 70 °C, 8 d	Sm **4.148**
3	(S)-phenylalanine **4.173** (0.5 eq), MeCN, rt, 7 d	Sm **4.148**
4	Et$_3$N (1.2 eq), THF, rt to 70 °C, 5 d	7% **4.147** and **4.177** (3/1 ratio)[a]
5	DBU (0.5 eq), THF, rt, 5 d	37% **4.147** and **4.177** (3/1 ratio)[a]
6	DBU (0.3 eq), THF, 40 °C, 6 d	74% **4.147** and **4.177** (3/1 ratio)[a]

Table 4.4. The formation of ring C. Reactions were carried out on ca. 50 mg of triketone **4.148** as a 0.1-0.2 M solution in THF, except for **entry 6**, where ca. 2.5 g of starting material were used. [a]Ratio of keto alcohols **4.147** and **4.177** are approximately calculated by proton NMR spectroscopy.

In their studies towards the synthesis of estrone derivatives, Korosawa *et al.*[190] prepared the 11α,13β-dimethyl-carbinol **4.179** by stirring vinyl ketone **4.178** and cyclopentanedione **4.149** with triethylamine for seven days (**Scheme 4.30**). Triketone **4.180** was obtained as a side product. The *trans*-configuration of the C-11 and C-13 methyl groups in **4.179** was preferred to prevent 1,3-diaxial interactions. However, no consideration about the stereochemistry of the C-14 hydroxyl group was reported.
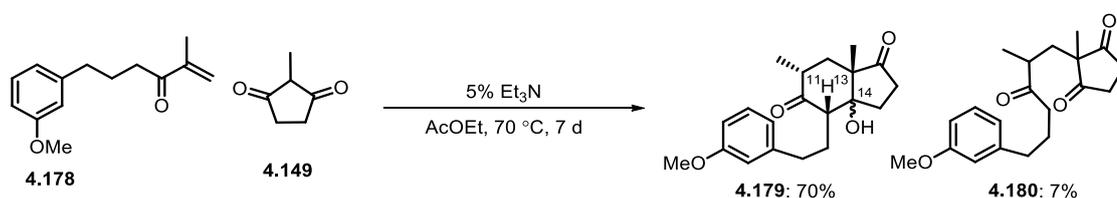
Scheme 4.30. Kurosawa's synthesis of 11α,13β-dimethyl-carbinol **4.179**.[190]

The intramolecular aldol reaction of **4.148** was therefore attempted in the presence of triethylamine (**entry 4**, **Table 4.3**), and, as an alternative, 1,8-diazabicyclo[5.4.0]undec-7-ene (**entries 5** and **6**). Pleasingly, the formation of racemic *cis*-ketoalcohol **4.147** was seen in both cases, although it was isolated as an inseparable mixture with the

trans-isomer **4.177** in about 3/1 ratio as detected by NMR spectroscopy. Efforts to achieve stereoselectivity were also carried out by adding (*S*)-proline **4.168** in a screening of bases, solvents and temperature. However, no stereochemical control was achieved, thus, the diastereomeric mixture of racemates **4.147** and **4.177** was carried through the next steps of the synthesis, leaving their potential chiral separation to a later stage.

Ketoacohols **4.147**[193] and **4.177**[194] are known in the literature, although little analytical data were provided. With the purpose of full characterisation, analytical amounts of the 3/1 mixture of carbinols **4.147** and **4.177**, were subjected to HPLC. The less polar peak corresponded to the *cis*-diastereoisomer **4.147** which was isolated as a white powder, followed by the *trans*-diasteroisomer **4.177** as an amorphous solid. In the NMR spectra, relevant signals were those of the C-18 methyl group, which was seen in the ^{1}H-NMR spectrum at 1.28 ppm in the *cis*-carbinol **4.147** and at 1.08 ppm in the *trans*-isomer **4.177**, in accordance with the literature values.[193, 194] In the ^{13}C-NMR spectra, the methyl group was shown at 12.7 ppm and at 18.1 ppm in compound **4.147** and **4.177**, respectively. Complete chemical shift assignment was supported by COSY, HMBC and HSQC experiments.

During attempts to obtain the stereoselective intramolecular aldol cyclisation, it was noticed that in the presence of stoichiometric base and proline triketone **4.148** was consumed after 18 h of stirring. However, carbinols **4.147** and **4.177** were not isolated (longer times being needed to complete the reaction). The major product obtained was identified as bicycle **4.182** (**Scheme 4.31**).
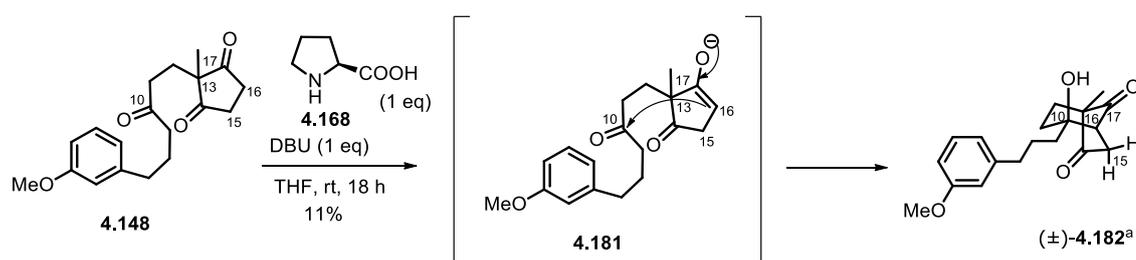
Scheme 4.31. Isolation of the kinetic product **4.182**. [a]In spite of the presence of (*S*)-proline **4.168** in the reaction mixture, compound **4.182** was assumend to be racemic based on the results shown in **Table 4.4**.

Bicyclic system **4.182** is novel, and its structure was elucidated by ^{1}H- and 2D-NMR spectroscopy. In the carbon NMR spectrum typical signals were found at 212.0 and 209.1 ppm for the two carbonyl groups, and at 83.8 ppm for the C-8 alcohol. In the ^{1}H-NMR spectrum the methyl group was seen at 1.06 ppm, while the C-15 axial and equatorial

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protons were found at 2.36 and 2.50 ppm, respectively, as assigned on the basis of the Hajos and Parrish investigation described below.[195]

In fact, Hajos and Parrish described the formation of the related bridged ketol **4.184** upon treatment of triketone **4.167** with piperidinium acetate **4.183** (**Scheme 4.32**).[195] Treatment of bicycle **4.184** with piperidine **4.185** followed by acetic acid gave the epimerised C-4 isomer **4.186**. The authors assigned the stereochemistry at C-4 on the basis of the chemical shift of the axial H-6, which was found to be particularly shielded in compound **4.186** (2.63 ppm) compared to isomer **4.184** (3.1 ppm) due to the *syn*-axial effect of the electronegative C-4 hydroxyl group. Comparison of these spectra with those of bicyclic compound **4.182** supported the assignment of its stereochemistry and connectivity.
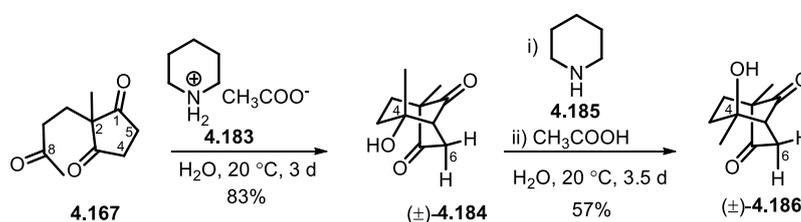
Scheme 4.32. Hajos and Parrish's synthesis of bridged ketols **4.184** and **4.186**.[195]

The bicyclic system **4.182** was imagined as the kinetic product of the reaction, which was formed first by nucleophilic attack of the enolate of the five-membered ring to the aliphatic ketone in intermediate **4.181** under basic conditions (**Scheme 4.31**). Indeed, when compound **4.182** was stirred with base for a longer time, a retro-aldol reaction on anion **4.187** to enolates **4.181** and **4.188** could occur, and led, after cyclisation of the latter, to the more stable keto alcohols **4.147** and **4.177** which were isolated in 40% yield and about 4/1 ratio (**Scheme 4.33**).

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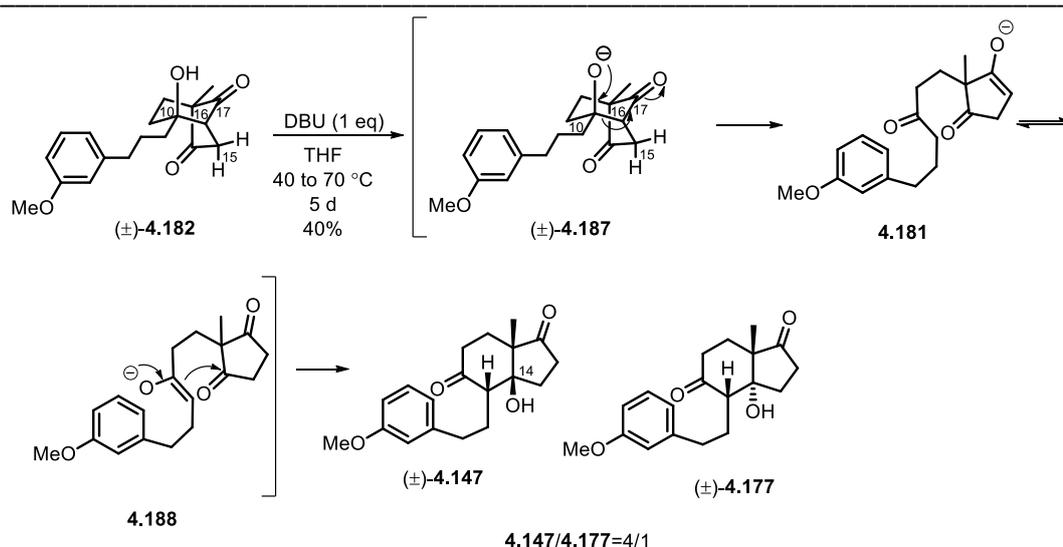
Scheme 4.33. Conversion of bridged ketol **4.182** into alcohols **4.147** and **4.177**.

4.3.3. Rings B: intramolecular Friedel-Crafts alkylation

The next step of the planned synthesis was the intramolecular Friedel-Crafts alkylation to give the tetracyclic steroidal framework (**scheme 4.34**). All the expected products **4.189**, **4.190** and **4.191** would be suitable intermediates, considering that the removal of the newly formed hydroxyl group in compounds **4.189** and **4.190** or the reduction of the double bond in compound **4.191** should be feasible.
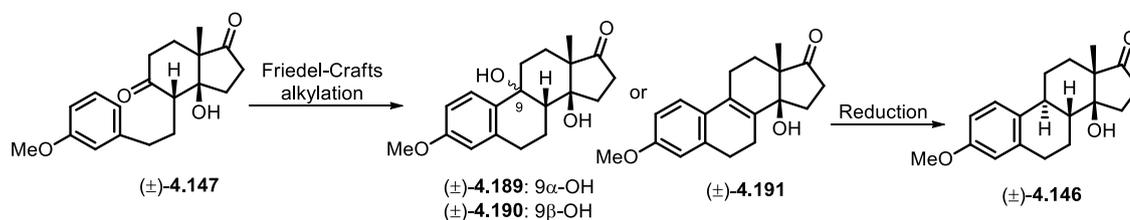
Scheme 4.34. Intended sequence to the steroidal ABCD-ring framework.

4.3.3.1. Intramolecular Friedel-Craft cyclisation with the unprotected 14-hydroxyl group

The cyclisation of carbinols **4.147** and **4.177** bearing the free 14-hydroxyl group was addressed first. Daniewski *et al.*[193] described that *cis*-carbinol **4.147** could be converted into dienone **4.176** *via* dihydroxy-ketone **4.189** on treatment with *p*-toluenesulfonic acid and acetic anhydride (**Scheme 4.35**). However, the supposed intermediate alcohol **4.189** was not isolated, due to its rapid dehydration to dienone **4.176**
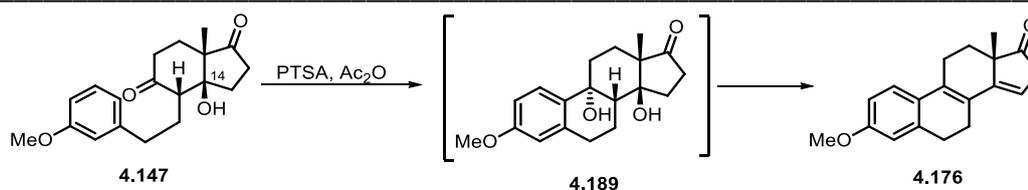
Scheme 4.35: Daniewski and Koćor's intramolecular cyclisation of diketoalcohol **4.147**.[193]

On the other hand, when steroid **4.192** with a substituent at C-11 was subjected to the same cyclisation, the triacetate **4.193** could be isolated in good yield (**Scheme 4.36**).[193, 196]
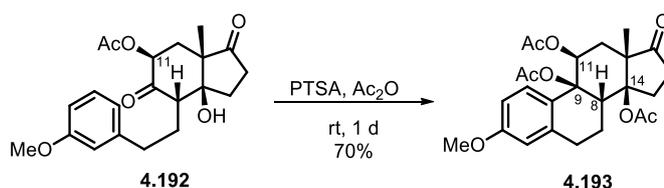
Scheme 4.36: Daniewski's intramolecular cyclisation of 11-acetyl-steroid **4.192**.[193, 196]

Daniewski supposed that the intramolecular cycloalkylation of carbinol **4.147** took place preferentially on the top face of molecule, thus leading to compound **4.194** with a *trans*-junction between rings B and C, which is common amongst steroids (**Scheme 4.37**).[193] Under acidic conditions, a *trans*-diaxial elimination to form the C-8,C-9 alkene **4.195** could occur. Finally, the newly formed unsaturation might take part in the acidic displacement of the C-14 acetate, leading to dienone **4.176** *via* cation **4.196**.
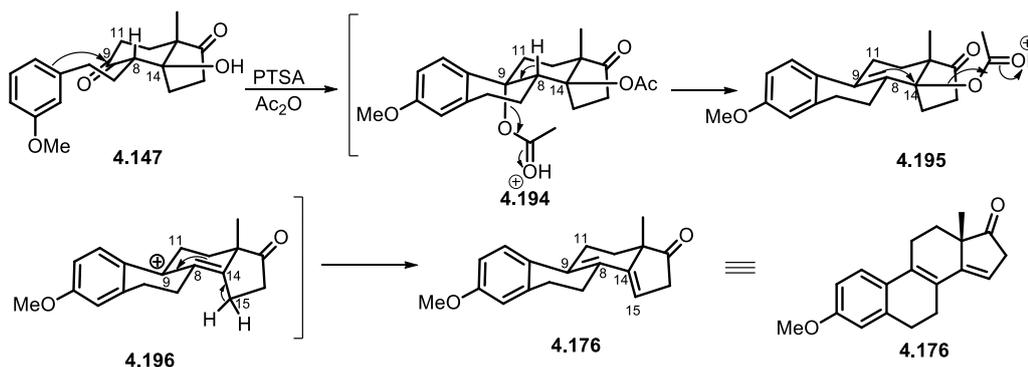
Scheme 4.37. 3D-model diagrams and possible explanation of Daniewski's intramolecular cyclisation of steroid **4.147**.[193]

On the other hand, the steric hindrance due to the 11β-acetyl group in compound **4.192** could drive the nucleophilic attack of the aromatic ring to occur from the bottom face of the molecule, thus forming acetate **4.193** with a *cis*-junction between rings B and C, which

in turn might prevent the subsequent elimination (**Scheme 4.38**). In the absence of the C,8-C,9 unsaturation, dienone **4.176** might not has been formed directly.[193]
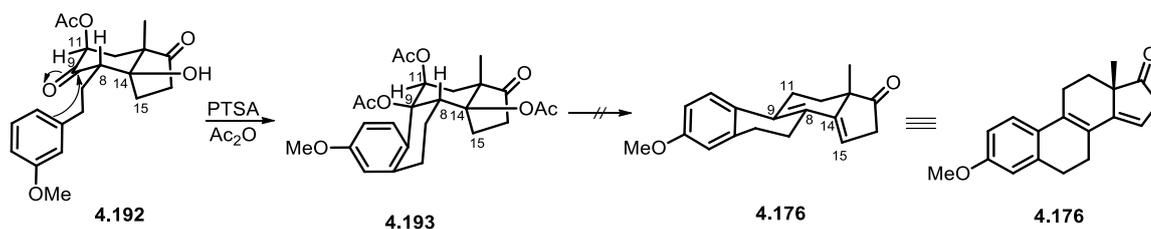
Scheme 4.38. 3D-model diagrams and possible explanation of Daniewski's intramolecular cyclisation in the presence of a C-11 substitution.[193]

cis-Alcohol **4.147** has no substitutent at C-11. However, it might be imagined that, by carrying out the cylisation under the Friedel-Crafts conditions, the Lewis acid might form a complex with the carbonyl group on the less hindered top face of the molecule (**Scheme 4.39**). As a consequence, nucleophilic attack of the aromatic ring might occur from below, and lead to the *cis*-junction between rings B and C and to the equatorial 9-hydroxyl group, such as in **4.190**, thus preventing elimination. However, to the best of our knowledge no precedent literature for this reaction was found.
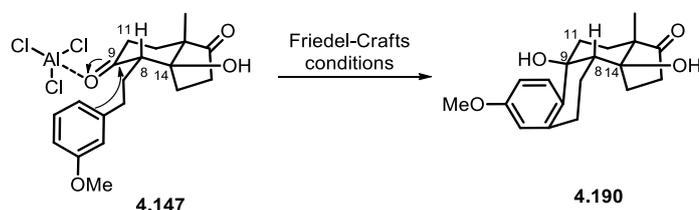
Scheme 4.39. 3D-model diagrams of the intended intramolecular Friedel-Crafts cycloalkylation.

Aluminium trichloride, boron trifluoride and iron trichloride were tried first (**Table 4.5**). Treatment of a 3/1 mixture of carbinols **4.147** and **4.177** with a slight excess of aluminium trichloride at 0 °C led solely to dehydration and the known open enone **4.174** was isolated in 37% yield (**entry 1**). In the presence of boron trifluoride (**entry 2**) and iron trichloride (**entry 3**) cyclisation occurred along with elimination, and led to the dienone **4.176** as the major product.

It is described that in a Friedel-Craft acylation the proton released during the re-aromatisation step might promote the elimination of water, an event that could be prevented by adding aluminium oxide as proton scavenger.[197] The Lewis acids tin tetrachloride (**entry 4**) and titanium tetrachloride (**entry 5**) were therefore tried in the

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presence of an excess of aluminium oxide. In both cases mainly dienone **4.176** and some decomposition were detected.
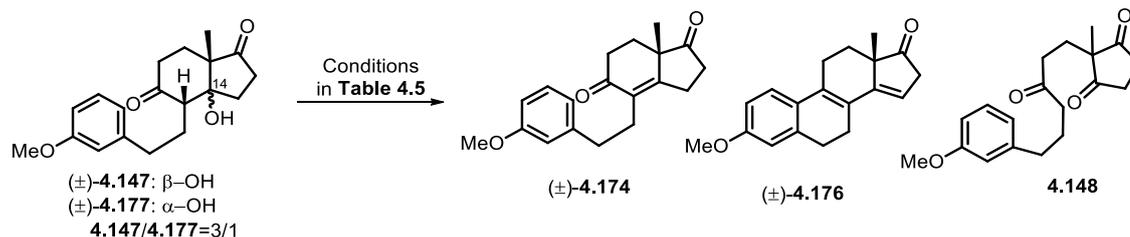
Entry	Conditions	Yield
1	AlCl$_3$ (1.1 eq), 0 °C to rt, 10 h	**4.174** 37%
2	BF$_3$·OEt$_2$ (1 eq), rt, 2 h	**4.176** 90%
3	FeCl$_3$ (1 eq), rt, 30 min	**4.176**[a]
4	1 M SnCl$_4$ (1.2 eq), Al$_2$O$_3$ (5 eq), -10 °C to rt, 2 h	**4.176** 50%
5	1 M TiCl$_4$ (1.1 eq), Al$_2$O$_3$ (5 eq), -15 °C to rt, 10 h	**4.176**[a]
6	1 M TiCl$_4$ (1.0 eq), -40 °C, 30 min	**4.176**[a]
7	1 M (*i*-PrO)$_3$TiCl (1.0 eq), 0 °C to rt, 18 h	**4.174** 50%
8	1 M (*i*-PrO)$_4$Ti (1.0 eq), rt, 18 h	Sm **4.147** and **4.177**
9	Sc(OTf)$_3$ (0.2 eq), -40 °C to rt, 18 h	Sm **4.147** and **4.177**
10	Sc(OTf)$_3$ (1 eq), -40 °C to 40 °C, 3 d	**4.174**[a]
11	PTSA (0.2 eq), 0 °C to rt, 1 d	Sm **4.147** and **4.177**, **4.174** and **4.176**[a]
12	MeOH (solvent), rt to 60 °C, 1 d	Sm **4.147** and **4.177**
13	MeOH (solvent), 140 °C, microwave, 2 h	Sm **4.147** and **4.177**, **4.176**, **4.148**[a]

Table 4.5: Attempted intramolecular cycloalkylation. Reactions performed in CH$_2$Cl$_2$, on *ca*. 10-50 mg of compounds **4.147** and **4.177** in approximately 3/1 ratio. [a]As detected by proton NMR spectroscopy and LC-MS spectrometry of the unpurified reaction mixture.

Next, titanium chloride and titanium isopropoxide were used (**entries 6**-**8**). Again, the cyclisation proved unsuccessful, and gave dienones **4.176** and **4.174**, and unreacted starting material, in accordance to the strength of the acid. Similar results were obtained in the presence of scandium trifluoromethanesulfonate.[198] In fact, under mild conditions the starting material did not react (**entry 9**), whereas by heating the reaction mixture to 40 °C, diketone **4.174** was the major product formed (**entry 10**).

The effects of a Brønsted acid and of a protic solvent were also evaluated, the latter chosen assuming that a hydrogen bond between the solvent and the ketone could promote the cyclisation. However, when catalytic amounts of *p*-toluenesulfonic acid were used dienone **4.176** was formed quickly at room temperature (**entry 11**). On the other hand, the starting material was found unreacted when it was heated in methanol (**entry 12**),

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whilst it was converted either into dienone **4.176** and into the open triketone **4.148** under microwave irradiation at high temperature (**entry 13**).

Two conclusions can be drawn. Under mild conditions, the cyclisation of **4.147** to form the steroidal framework is unlikely to occur. On the other hand, under harsh conditions the conjugated enedione **4.174** forms first. Afterwards, cyclisation can occur, but the unsaturation appears to promote the ready dehydration of the newly formed alcohol **4.197** thus giving dienone **4.176** (**Scheme 4.40**). These results are contrary to Daniewski's suggestions, which proposed the cyclisation to occur first, followed by dehydration (see *Section 4.3.3.6.* and **Table 4.8** for further evidence of the initial formation of diketone **4.174**).
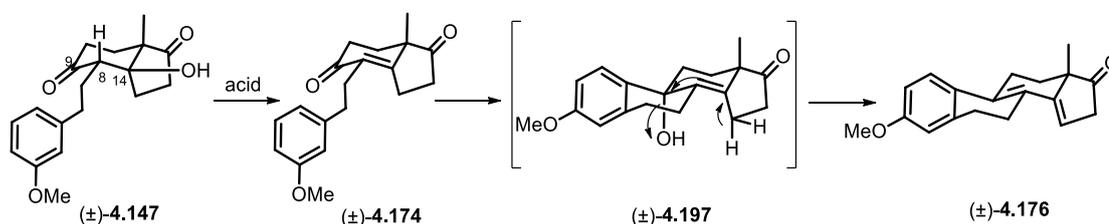
Scheme 4.40. Outcome of the intramolecular Friedel-Craft cycloalkylation.

Since the cyclisation with the retention of the free 14-hydroxyl group seemed difficult to achieve, its protection was considered to avoid the ready dehydration. In addition, chemical modification of the tertiary alcohol should provide a viable method to separate the C-13,C-14-*cis*- and *trans*- diastereoisomers.

4.3.3.2. Towards the 14-trimethylsilyl ether

The screening of the potential protecting groups started with the trimethylsilyl ether, a group installed by Sevillano *et al.* on the β-hydroxyl group of the Hajos-Parrish ketone in 79% yield using trimethylsilyl triflate and triethylamine.[199] Following these conditions, the 3/1 mixture of *cis*- and *trans*-carbinols **4.147** and **4.177** was treated with trimethylsilyl triflate (**Scheme 4.41**). A unique compound was isolated in 24% yield, which was identified as the 14- trimethylsilyl ether **4.198**.
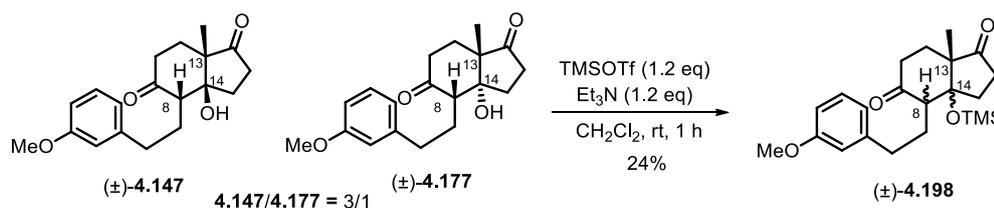
Scheme 4.41. Trimethylsilyl-protection of the 14-hydroxyl group.

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The order of addition of the reagents was crucial to the outcome of the reaction. The starting material decomposed rapidly when trimethylsilyl triflate was added before triethylamine. When trimethylsilyl chloride was used instead of the triflate only unreacted starting material was recovered.

The relative stereochemistry of the C-13 and C-14 groups in the novel trimethylsilyl derivative **4.198** was firstly tentatively assigned as *trans* (such as in compound **4.199**, **Figure 4.13**) by NMR spectroscopy on comparison with the chemical shifts of the angular methyl group at 1.05 ppm (in the ^{1}H-NMR spectrum) and at 18.3 ppm (in the ^{13}C-NMR spectrum), with those of the same group in the *trans*-carbinol **4.177**, which were shown at 1.08 and 18.3 ppm, respectively, and dissimilar to the signals at 1.28 ppm and at 12.9 ppm, respectively, of the *cis*-carbinol **4.147**.
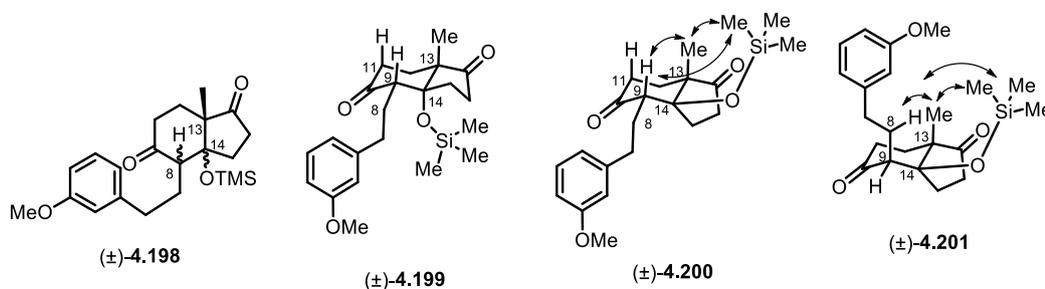
Figure 4.13. Potential configurations of trimethylsilyl ethers.

Nevertheless, nOe experiments showed a correlation between the C-13 methyl group and the C-14 silyl ether (such as in compounds **4.200** and **4.201**, **Figure 4.13**) which would be improbable if the two groups were *trans*-related. nOe Correlations were also observed between these groups and either the H-8 or H-9 proton, whose signals were overlapping. A configuration such as in compound **4.200** was excluded by comparison with the analytical data of an analogous triethylsilyl ether, whose relative stereochemistry was unambiguously assigned as described below (see *Section 4.3.3.6* and **Table 4.8** for further details). A highly hindered structure such as **4.201** could be supported by the nOe analysis, although it was likely to be energetically disfavoured by reason of the 1,3-axial interactions between the bulky C-9 chain and the C-13 methyl group. NMR spectroscopy studies to fully elucidate the relative stereochemistry at the C-9, C-13 and C-14 centres were therefore inconclusive.

To support the stereochemical studies, the release of the 14-hydroxyl group was attempted under diverse reaction conditions (**Table 4.6**) to enable comparison with the

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carbinols **4.147** and **4.177** whose structures have been assigned unambiguously. However, on treatment with acids (**entries 1** and **2**) or fluoride (**entry 3**) only decomposition or starting material was obtained.
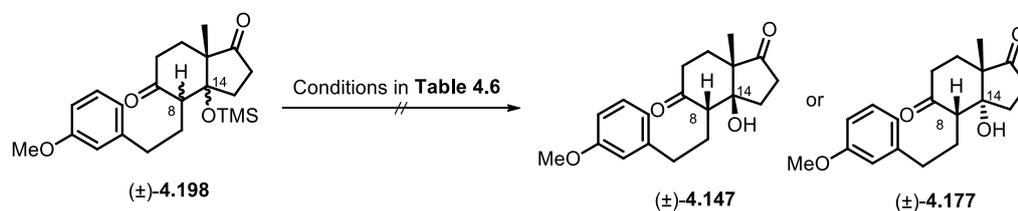
Entry	Conditions	Outcome
1	1 M aq HCl, dioxane, rt, 2h	Decomposition
2	THF/AcOH/H$_2$O = 1/1/1	Sm **4.198**
3	TBAF (1.2 eq), CH$_2$Cl$_2$, 0 °C to rt, 1 h	Decomposition

Table 4.6. Attempted release of the hydroxyl group. Reactions carried out on 10-50 mg scale.

Therefore, alternative protecting groups were considered.

4.3.3.3. Towards the 14-triethylsilyl ether

The introduction of a 14-triethylsilyl group was addressed next (**Table 4.7**).
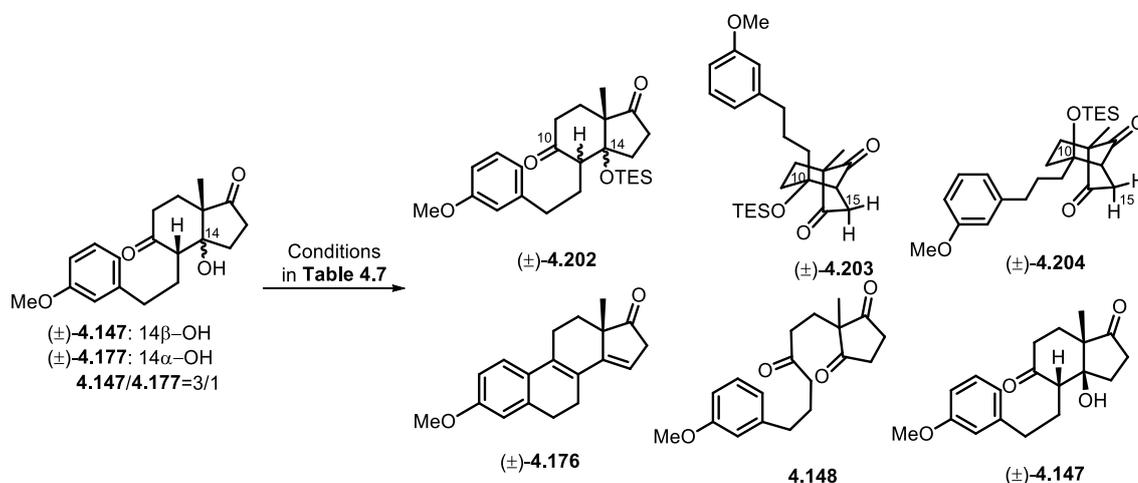
Entry	Conditions	Yield
1	TESOTf (2.2 eq), Et$_3$N (2.2 eq), CH$_2$Cl$_2$, 0 °C to rt, 30 min	**4.202** 20%
2	TESOTf (2.2 eq), Et$_3$N (2.2 eq), CH$_2$Cl$_2$, 0 °C, 30 min	**4.202** 7%, **4.203** 14%
3	TESOTf (1.2 eq), Et$_3$N (1.2 eq), CH$_2$Cl$_2$, 0 °C, 1 h	**4.202** 13%, **4.204** 6%, **4.176** 36%, **4.148** 11%, sm **4.147** 23%

Table 4.7: Protection of the 14β-hydroxyl group as its triethylsilyl ether.

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Following the protocol used to prepare the trimethylsilyl ether **4.198**, a mixture of carbinols **4.147** and **4.177** (in about 3/1 ratio) was treated with triethylamine and triethylsilyl triflate. The only compound isolated was the triethylslyl ether **4.202**, which was obtained in 20% yield (**entry 1**), in accordance to the results seen in the case of the trimethylsilyl ether **4.198** described above. Efforts to increase the yield were carried out by screening temperatures, reaction times and equivalents of base and triethylsilyl triflate. Of particular relevance were the results of the cyclisation attempted at 0 °C. In fact, after 30 minutes reaction time the formation of the triethylsilyl derivative **4.202** was minimal (**entry 2**), and the major product isolated was compound **4.203** with the substituents at C-10, *i.e.* the bulky chain and the triethylsilyl ether, in the axial and equatorial positions, respectively. However, when less equivalents of reagents were used and the reaction was stirred for a longer time (**entry 3**), a number of compounds were formed, including the silyl ether **4.202**, the isomeric bridged compound **4.204**, dienone **4.176** and triketone **4.148**, whilst only *cis*-carbinol **4.147** was recovered.

The complex mixture of compounds obtained reflects the high reactivity of carbinols **4.147** and **4.177** (**Scheme 4.42**).
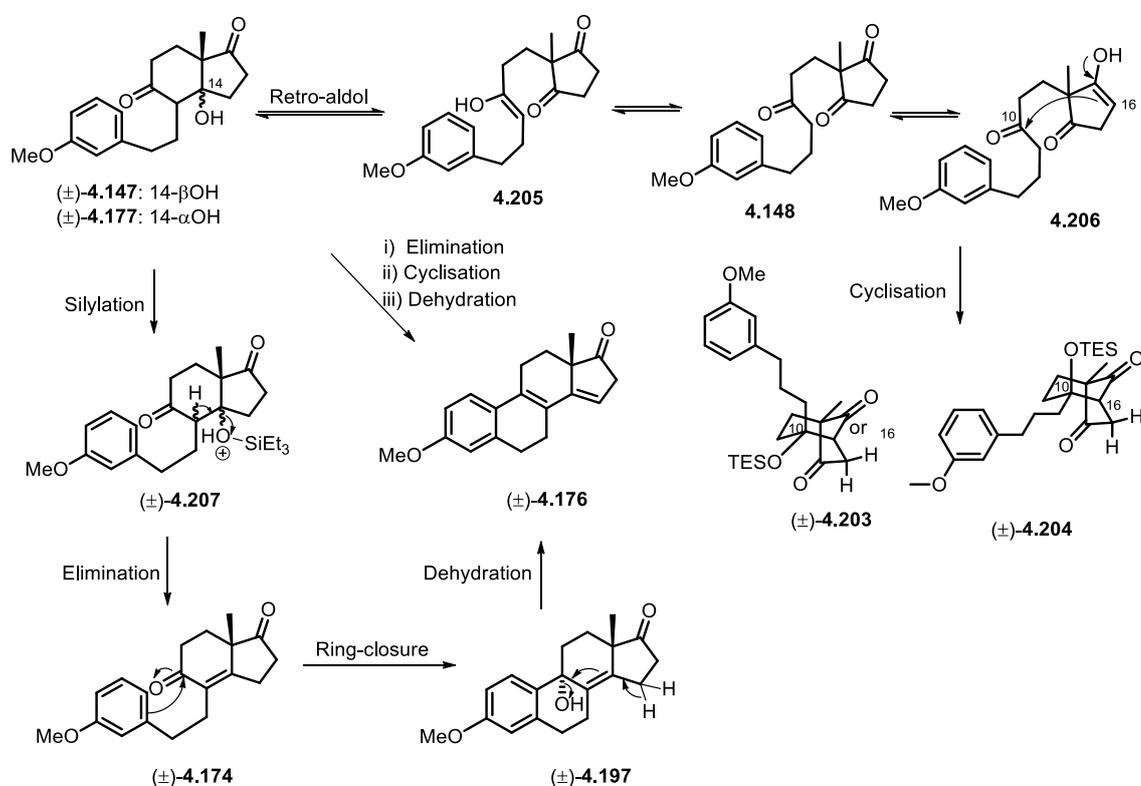
Scheme 4.42. Potential reactivity of carbinols **4.147** and **4.177**.

Indeed, under acidic conditions a retro-aldol ring-opening reaction to triketone **4.148** and its tautomers **4.205** and **4.206** could occur. In turn, enol **4.206** might undergo a further ring-closure reaction by nucleophilic attack of C-16 to the C-10 ketone followed by silylation, thus resulting in the isolation of bicyclic products **4.203** and **4.204**. Silylation of the C-14 hydroxyl group of carbinols **4.147** and/or **4.177** to silyl ether **4.207** could also take place. However, it might be followed by elimination to diketone **4.174** and further intramolecular cyclo-alkylation to form dienone **4.176** after dehydration of intermediate alcohol **4.197** which was not isolated. Moreover, carbinols **4.147** and/or **4.177** might also directly undergo a similar sequence of elimination and cyclisation reactions, thus giving dienone **4.176** which was isolated as the major compound.

The silyl ethers **4.202**, **4.203** and **4.204** are novel, and the connectivity of the atoms was fully assigned by means of 1D- and 2D-NMR spectroscopy. With regard to the 14-triethylsilyl ether **4.202**, the assignment of relative stereochemistry at C-9, C-13 and C-14 was controversial, as it was seen in the case of the trimethylsilyl ether **4.198**. The chemical shifts of the diagnostic angular methyl group at 1.09 and 18.3 ppm, in the proton and carbon NMR, respectively, would suggest a *trans*-connectivity (as represented in compound **4.208**, **Figure 4.14**) by comparison with the signals shown for the *trans*-carbinol **4.147**.
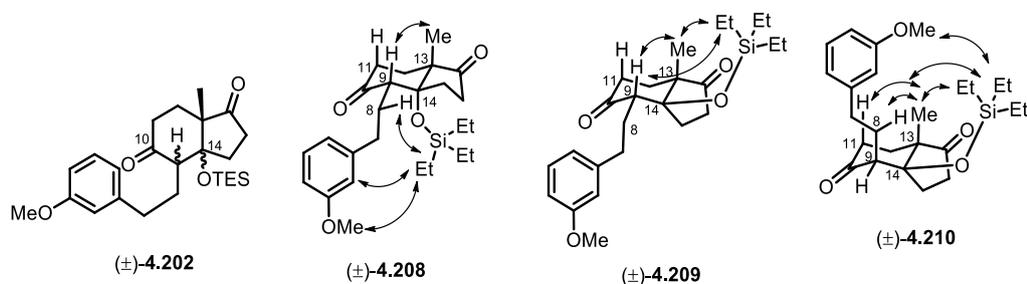
Figure 4.14. Possible structures of isomeric 14-silyl ethers.

However, nOe experiments acquired by irradiation of the angular methyl group showed a clear correlation with the triethylsilyl group and *vice versa*, thus suggesting the two groups having the same orientation with respect to the molecular plane (such as in isomers **4.209** and **4.210**). Silyl ether **4.209** was unambiguously described later as the product obtained from a different reaction (see *Section 4.3.3.6* and **Table 4.8** for further details), so **4.202** could not be identified as stereoisomer **4.209**. Moreover, correlations were seen between the aromatic protons, the methoxy group and the overlapping H-8 or H-9. Again, in light of the nOe experiments, silyl ether **4.202** could be tentatively assigned as the

sterically hindered and unfavoured compound **4.210**. However, additional experiments to confirm this hypothesis would be required.

With regard to the bridged silyl ethers **4.203** and **4.204**, the position of the substituents at C-10 was assigned on the basis of the chemical shifts of the C-15 axial and equatorial protons, which were seen at 2.94 and 2.45 ppm, respectively, in compound **4.203**, and overlapped at 2.45 ppm in compound **4.204**, in agreement with the literature models described by Hajos and Parrish and with the chemical shift values of bridged ketol **4.182** (see **Schemes 4.31** and **4.32**).[195]

4.3.3.4. Towards the 14-tert-butyldimethylsilyl ether

The formation of the more stable and bulkier 14β-*tert*-butyldimethylsilyl ether was also attempted in the presence of *tert*-butyldimethylsilyl triflate (**Scheme 4.43**), but it resulted again in a complex mixture of compounds. Amongst those, bis-silyl ether **4.211** and 17-silyl enol ether **4.212** were isolated in 6% and 9% yield, respectively, with traces of unidentified products, and they were fully characterised by means of 1D- and 2D-NMR spectroscopy. The formation of isomeric silyl ethers **4.213** and **4.214** was also detected by LC-MS and NMR spectroscopy, but they could not be fully characterised.
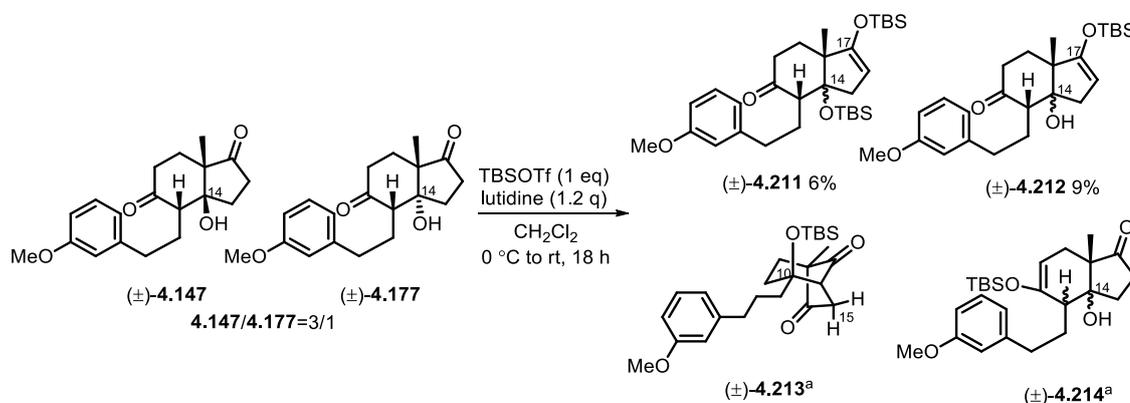
Scheme 4.43. Towards 14-*tert*-butyldimethylsilyl steroids. [a]Detected as the major compound of a complex mixture of products.

Treatment of carbinols **4.147** and **4.177** with *tert*-butyldimethylsilyl chloride in the presence of triethylamine or imidazole gave instead solely recovered starting material.

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4.3.3.5. Towards the 14-acetate

Considering the difficulties in the formation of 14-silyl ethers, the formation of a 14-acetate was also addresssed, which is known to be easy cleaved under acidic or basic conditions, but is stable enough for the Friedel-Crafts cyclisation.

The acetylation was attempted first in the presence of acetic anhydride in pyridine, but it failed, and it gave just unreacted starting material. However, when acidic conditions were used[175] a single compound identified as the acetyl derivative **4.215** was obtained in 48% yield, along with dienone **4.176** and a mixture of unidentified by-products (**Scheme 4.44**).
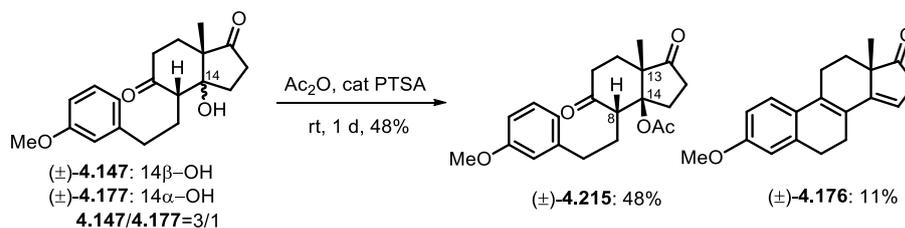
Scheme 4.44. Acetylation of the 14-hydroxyl group.

Acetate **4.215** is novel and it was fully characterised. In the proton NMR spectrum a singlet at 2.01 ppm and an apparent doublet at 3.46 ppm were assigned to the acetyl group and the proton at C-8, respectively. The signals of the angular methyl group were found at 1.28, and 14.2 ppm in the ^{1}H- and ^{13}C-NMR, respectively. This was in agreement with the values of the same group in the *cis*-carbinol **4.147**, which were observed at 1.29 and 12.9 ppm. The relative β-configuration of C-8, C-13 and C-14 were confirmed by nOe experiments, which showed correlations between the aforementioned groups irradiated at 3.46 ppm, 1.28 ppm and 2.01 ppm, respectively (**Figure 4.15**).
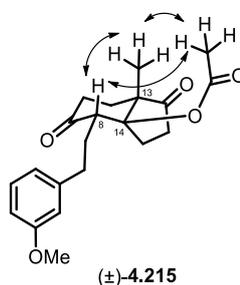
Figure 4.15. nOe Correlations on acetate **4.215**.

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*4.3.3.6. Intramolecular cyclo-alkylation of the 14-triethylsilyl ether **4.202***

Although the stereochemistry of 14-triethylsilyl ether **4.202** was unclear, its intramolecular cyclo-alkylation with Lewis acid was attempted (**Table 4.8**).
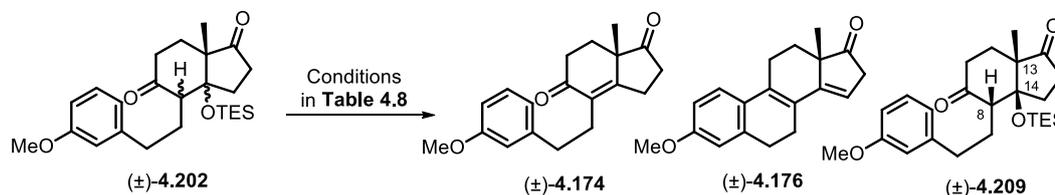
Entry	Conditions	Outcome
1	(i-PrO)$_3$TiCl (1 eq), CH$_2$Cl$_2$, rt, 10 h	Sm **4.202**
2	(i-PrO)$_3$TiCl (1 eq), toluene, rt to 80 °C, 4 h	Decomposition
3	AlCl$_3$ (1 eq), CH$_2$Cl$_2$, 0 °C to rt, 10 h	**4.174** (not isolated), **4.176** 46%, **4.209** 7%
4	AlMe$_3$ (1 eq), CH$_2$Cl$_2$, rt to 40 °C, 10 h	Sm **4.202**
5	AlMe$_3$ (1 eq), toluene, 40 °C, 18 h	Sm **4.202** and decomposition

Table 4.8. Attempted cyclisation of triethylsilyl ether **4.202**.

As described above (see **Scheme 4.40**), it was noticed that the first step in the formation of the tetracyclic steroidal framework is the formation of enedione **4.174**, followed by cyclisation and dehydration to the dienone **4.176**. It might be possible, under acidic conditions and in the presence of a protected 14-hydroxyl group, to induce cyclisation with no elimination. The medium stength acid chlorotitanium triisopropoxide was tried first (**entry 1**). However, under mild conditions only unreacted starting material was recovered, whereas changing the solvent and increasing the temperature up to 80 °C gave just decomposition of the starting material (**entry 2**).

The cyclisation using stoichiometric amounts of aluminium trichloride was tried next, and the outcome of the reaction was carefully monitored by LC-MS. It was seen that the formation of diketone **4.174** occurred first, even at low temperature, and it was followed by the formation of dienone **4.176** which was the major compound isolated (**entry 3**). Interestingly, a novel compound was isolated and it was clearly identified as the *cis*-triethylsilyl derivative **4.209**, which could have arisen from a sequence of desilylation-retro-aldol ring-opening of the starting material **4.202** to enol **4.205**, followed by aldol ring-closure and further silylation (**Scheme 4.45**).

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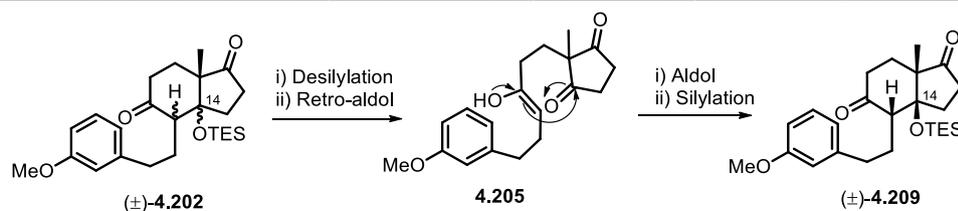
Scheme 4.45. Potential sequence leading to silyl ether **4.209**.

In a last attempt the milder trimethylaluminium was used (**entries 4** and **5**, **Table 4.8**). The reactions were again unsuccessful, and therefore this strategy was abandoned.

Silyl ether **4.209** is novel and it was fully characterised. The relative stereochemistry of the proton at C-8, of the methyl group at C-13 and of the silyl ether at C-14 was assigned by 1D- and 2D-NMR spectroscopy and confirmed by nOe experiments. The signals of the angular C-18 methyl were of particular relevance, and they were found at 1.29 and 14.3 ppm in the proton and carbon NMR spectra, respectively. These values are in accordance with those of the same methyl group in the *cis*-carbinol **4.147** and in the *cis*-acetate **4.215** which were shown at 1.28 and 1.29 ppm in the proton NMR spectrum, respectively, and at 12.9 ppm and 16.1 ppm in the carbon NMR spectrum, respectively.

4.3.3.7. Intramolecular cyclo-alkylation of the 14-acetate *4.215*

Finally, attention was focussed on the cyclisation of acetate **4.215** to give either compounds **4.216** or **4.217** (**Table 4.9**). *p*-Toluenesulfonic acid was chosen as a Brønsted acid (**entry 1**),[193] and aluminium trichloride as a Lewis acid (**entry 2**). However, both attempts failed to produce **4.216** or **4.217** and mainly dienone **4.176** was isolated (in agreement with the results of Daniewski, see **Scheme 4.37**),[193] along with some decomposition of the starting material.
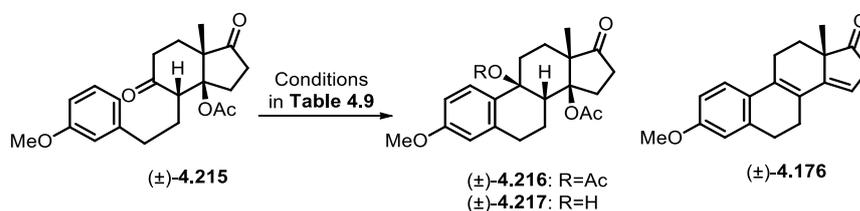
Entry	Conditions	Outcome
1	PTSA (1 eq), Ac2O, rt, 1 h	**4.176** 7% and decomposition
2	AlCl$_3$ (1 eq), CH$_2$Cl$_2$, 0 °C rt, 5 min	**4.176** 75%

Table 4.9. Intended Friedel-Crafts cycloalkylation of acetate **4.215**.

Hence, the strategy first planned towards the synthesis of the target 14β-withanolide F **2.5** was reconsidered.

4.3.4. Towards the reintroduction of the 14β-hydroxyl group on dienone 4.176

Given the apparent challenge of performing the cyclisation while retaining the 14-hydroxyl group and taking advantage of the easy formation of the dienone **4.176**, the reintroduction of the 14β-alcohol to give compound **4.146** after the formation of the steroidal framework was addressed next (**Scheme 4.46**).
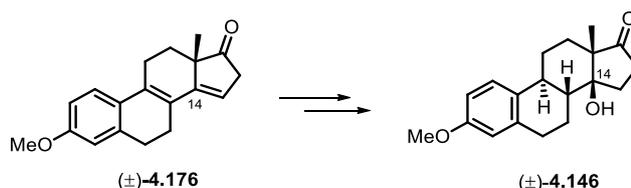
Scheme 4.46. Towards the reintroduction of the 14β-hydroxyl group.

4.3.4.1. Synthesis of dienone 4.176

Attention was focused first on increasing the yield of dienone **4.176** and shortening the full procedure by one step. During the attempted cycloalkylation of diketocarbinols **4.147** and **4.177**, dienone **4.176** was obtained as a by-product in yields up to 90% depending on the acid used. However, Wang *et al*. reported a synthesis of the same dienone occurring in 65% yield, by heating triketone **4.148** at reflux in benzene containing *p*-toluenesulfonic acid.[179] Indeed, under these conditions dienone **4.176** was isolated in 62% yield (**Scheme 4.47**). The two other products formed were the unsaturated ketones **4.218** and **4.174**, in 6% and 11% yield, respectively.
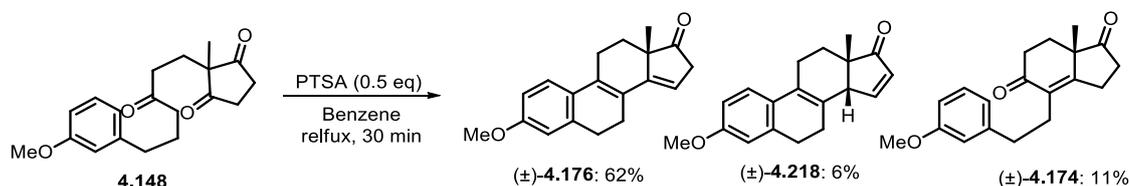
Scheme 4.47. Towards the cyclisation of triketone **4.148** to dienone **4.176**.

Both the enantionerically pure[192] and enantiomerically enriched (81% ee)[200] forms of compound **4.218** are known in the literature. The spectroscopic data of racemic **4.218** (proton and carbon NMR and IR spectra) were consistent with the published data.[192, 200]

Several more conditions were screened to improve the yield of steroid **4.176**, including the isomerization of enone **4.218** under basic conditions, but they proved to be unsuccessful.

4.3.4.2. Attempted cycloaddition to a 14-hydroxyl steroid

Kirsch *et al.* developed a method to introduce a steroidal 14β-hydroxyl group by means of a [4+2]-cycloaddition reaction between diene **4.219** and benzyl nitrosoformate, which was generated *in situ* by oxidation of benzyl *N*-hydroxycarbamate **4.220** with tetrabutylammonium iodide (**Scheme 4.48**). The mixture of isomeric adducts **4.221** and **4.222** was completely converted into the 14β-hydroxyl derivative **4.223** upon reflux in methanol. Palladium-catalysed hydrogenation led finally to carbinol **4.224** in 87% yield based on **4.219**.[201]
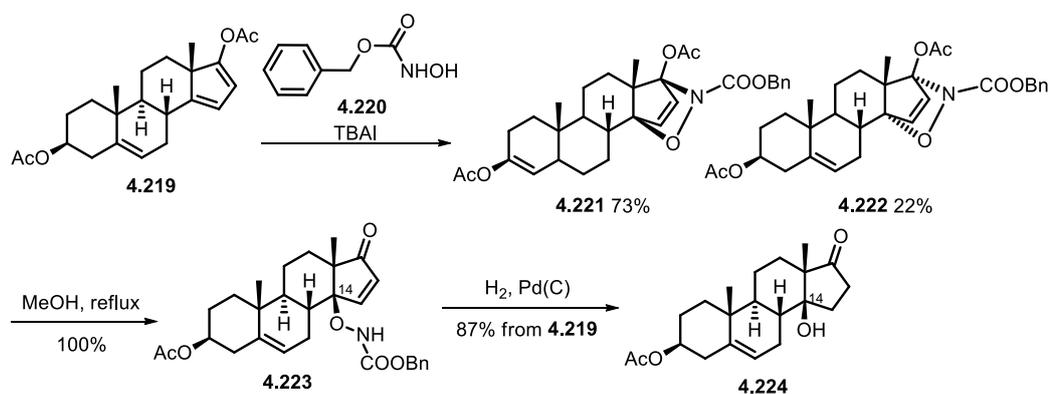
Scheme 4.48. Kirsch's stereoselective synthesis of 14β-hydroxysteroids.[201]

To follow Kirsch's procedure, we tried to convert dienone **4.176** into the known enol acetate **4.225**, which was prepared in 47% yield by Hughes and Smith in 1966 by heating at reflux dienone **4.176** in isopropenyl acetate **4.123** containing catalytic amounts of *p*-toluenesulfonic acid for 15 h.[202] The authors suggested the flask to be left open and protected from the light with aluminium foil during the reaction time. In our hands, the reaction turned quickly dark coloured and it resulted in just decomposition (**Scheme 4.49**).
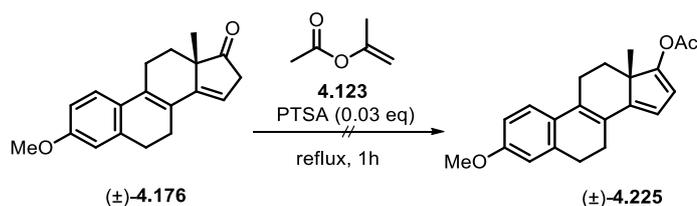
Scheme 4.49. Attempted acetylation of steroid **4.176**.

More attempts to make enol acetate **4.225** were tried. However, unsuccessful results were obtained when camphorsulfonic acid was used instead of *p*-toluenesulfonic acid, and when isopropenyl acetate was replaced by acetic anhydride in the presence of catalytic amounts of *N,N*-dimethylaminopyridine. The combination of acetic anhydride and catalytic amounts of *p*-toluenesulfonic acid gave instead unreacted starting material. Therefore, an alternative route was again considered.

4.3.4.3. Attempted C-14,C-15-epoxidation

Attention was turned to the selective epoxidation of the C-14,C-15 double bond which could lead to the desired 14β-hydroxyl group after reductive ring-opening. Although no literature precedent has been reported starting from dienone **4.176**, the stereoselective route established by Bull and Floor leading to 14β-diol **4.228** in two steps from alcohol **4.226** via epoxide **4.227** seemed attractive (**Scheme 4.50**).[203]
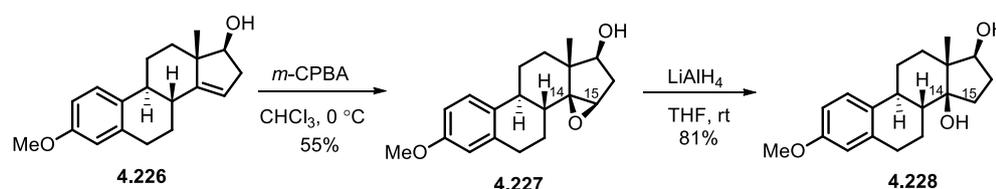
Scheme 4.50: Bull and Floor's stereoselective ring D elaboration.[203]

The preparation of **4.229** was first attempted using the conditions of Bull and Floor (**Scheme 4.51**).[203] However, no reaction was observed on treatment of dienone **4.176** with *m*-chloroperbenzoic acid at 0 °C, whereas the starting material decomposed when the oxidant was added at room temperature.
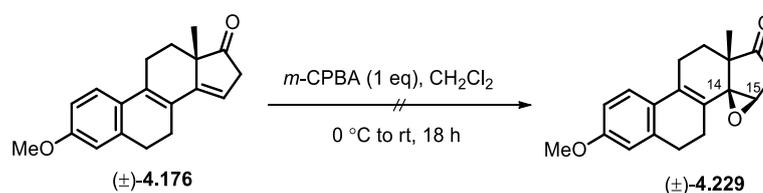
Scheme 4.51. Attempted epoxidation on ring D.

The formation of the epoxide was also attempted on treatment with *N*-bromosuccinimide and sodium methoxide, but in the presence of the brominating agent the starting material decomposed quickly.

Homo-allylic alcohol **4.230** could be imagined as a suitable substrate for the selective C-14,C-15-epoxidation using titanium *iso*propoxide as a transition metal catalyst and *tert*-

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butyl hydroperoxide as oxidising agent (**Scheme 4.52**).[204, 205] Reduction of dienone **4.176** with sodium borohydride[206] proceeded smoothly, and resulted in a single compound identified as the known 17β-homo-allylic alcohol **4.230**.[207, 208] However, the following epoxidation to obtain compound **4.231** failed and gave again just a complex mixture of products which were not isolated.
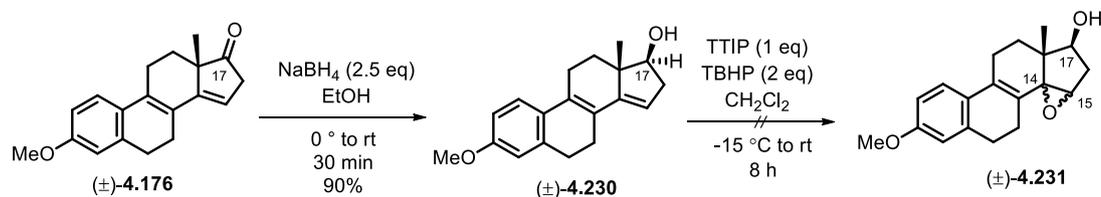
Scheme 4.52: Attempted epoxidation or ring D in the presence of titanium *iso*propoxide and *tert*-butyl hydroperoxide.

4.3.4.4. Attempted selective reduction of ring B

It was proposed that interruption of the conjugation between rings A-D in dienone **4.176** by selective reduction of the C-8,C-9 double bond would give compound **4.232**, whose ring D, with the remaining C-14,C-15 double bond, should be easier to elaborate to reintroduce the 14β-hydroxyl group and give ketoalcohol **4.146** (**Scheme 4.53**).
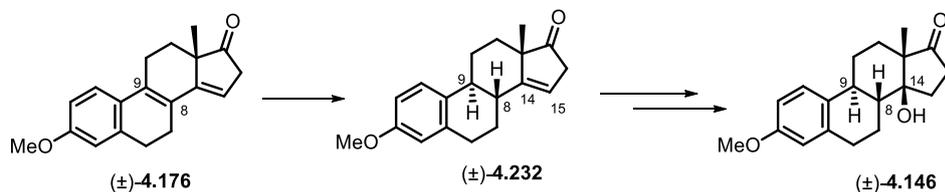
Scheme 4.53. Intended selective reduction of the benzylic double bond towards the ring D elaboration

Catalytic hydrogenation was tried first (**Table 4.10**). Although this reaction is known to proceed *via* a *syn* mechanism (thus forming an undesired *cis*-junction between rings C and D), it was seen a feasible method to address the selective reduction of the styrene double bond in the presence of palladium as metal catalyst. Ethyl acetate was chosen as the solvent, in order to decrease the reaction rate and hopefully favour the reduction of the styrene against the less substituted alkene. However, enone **4.234** was the only compound isolated (**entry 1**).

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Conditions
in **Table 4.10**

(±)-**4.176**   (±)-**4.233**   (±)-**4.234**
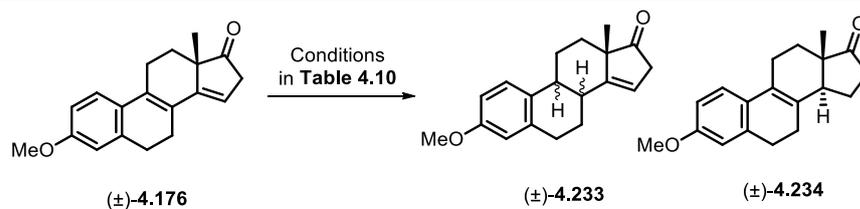
Entry	Conditions	Outcome
1	H$_2$, Pd(C), AcOEt, 30 min	**4.234** 25%
2	Et$_3$SiH (1 eq), H$_2$, 10% Pd(C),benzene, 0 °C rt, 18 h	**4.234** 56%

Table 4.10. Catalytic hydrogenation of steroid **4.176**.

Enone **4.234** is a known compound,[209, 210] which was also synthesised according to the procedure of Corey and co-workers *via* a catalytic hydrogenation of dienone **4.176** in benzene in the presence of triethylsilane (**entry 2**).[209] These results emphasised the challenge to achieve the internal reduction in a selective manner.

Next, the reduction of the aromatic steroid **4.176** under Birch conditions was tried. Firstly, the 17-keto group of **4.176** was protected as acetal **4.235** in high yield in the presence of ethylene glycol, trimethyl orthoformate and catalytic amounts *p*-toluenesulfonic acid (**Scheme 4.54**).[211] Acetal **4.235** is a known compound but no NMR spectra are provided,[212] therefore it was fully characterised.

(CH$_2$OH)$_2$ (2.5 eq)
CH(OCH$_3$)$_3$ (2 eq),
PTSA (0.01 eq)

30 °C, 18 h
91%

(±)-**4.176**   (±)-**4.235**
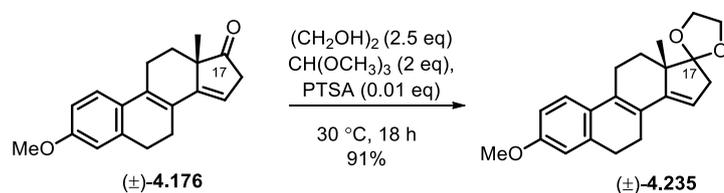
Scheme 4.54: Preparation of acetal **4.235**.

Compound **4.235** was afterwards treated with an excess of metallic sodium in liquid ammonia and tetrahydrofuran (**Scheme 4.55**). However, compound **4.236** was not seen, but the major product isolated was phenol **4.237**, with rings C and D fused in a *cis*-fashion.

i) Na (40 eq), NH$_3$ liq.
THF, -40 °C, 18 h

ii) MeOH

(±)-**4.235**   (±)-**4.236**   (±)-**4.237**: 48%
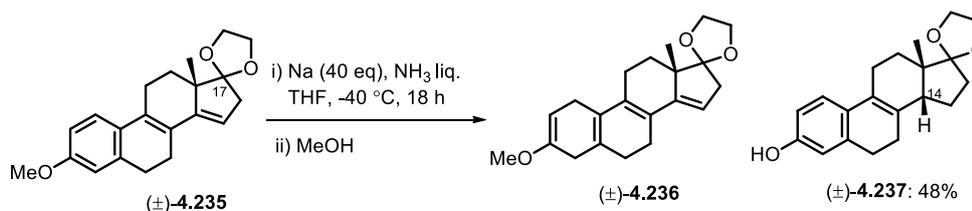
Scheme 4.55: Towards the Birch reduction of diene **4.235**.

Phenol **4.237** is novel and it was fully characterised. The stereochemistry at C-14 was assigned by nOe experiments (which showed a clear correlation between this proton and the angular C-18 methyl group), and it was confirmed by comparison with the analytical data of the same compound prepared as shown below (**Scheme 4.56**).

Ring D of the *cis*-fused dienone **4.218** was exhaustively reduced with sodium borohydride to obtain the 17α-hydroxyl compound **4.238**,[213] which is a known compound, but no analytical data are available (**Scheme 4.56**). The stereochemistry at C-17 was assigned by NMR spectroscopy on the basis of the chemical shift of the C-17 proton seen at 3.89 ppm, in accordance with literature examples,[207] and different from the chemical shift of the same atom in the 17β-alcohol **4.230** which was found at 4.05 ppm. Further oxidation of the 17-alcohol by means of Dess-Martin periodinane **4.239** resulted in the known *cis*-enone **4.240**,[210, 214] in which the relative stereochemistry between C-13 and C-14 was also confirmed by nOe experiments. Demethylation of the aromatic methoxy group in the presence of boron tribromide gave the known phenol **4.241**[215] in good yield. Finally, acetal protection of the C-17 ketone gave compound **4.237** in 56% yield, whose analytical data were in agreement with those of the same compound prepared by Birch reduction of acetal **4.235** as described in **Scheme 4.55**.
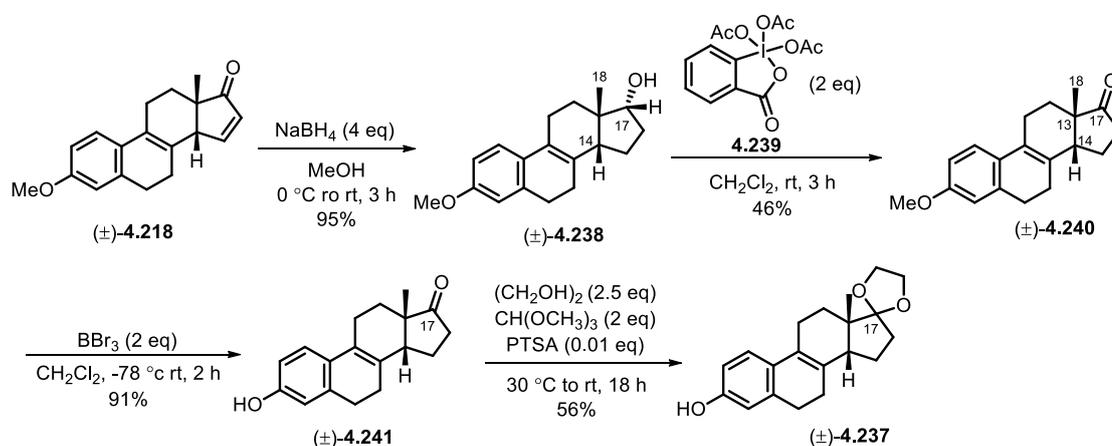
Scheme 4.56. Synthesis of rings C and D *cis*-fused steroid **4.219**.

It was noteworthy that reduction of steroidal ring D in the presence of a C-17 ketone produced compound **4.234** with a *trans*-junction between ring C and D (**Table 4.10**). By contrast, when the ketone was protected as its acetal, a *cis*-junction was obtained, such as in compound **4.237** (**Schemes 4.55**). Likewise, sodium borohydride reduction of dienone **4.176** furnished compound **4.230** with a 17β-hydroxyl group (**Schemes 4.52**). On the

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other hand, the same reduction performed on the conjugated ketone **4.218** produced the isomeric 17α-hydroxyl steroid **4.238**. (**Schemes 4.56**).

A screening of different solvent systems for the Birch reduction resulted in complex mixtures of products whose structures were not resolved, but none of them was compound **4.242**. However, using a mixture of tetrahydrofuran and *t*-butanol as solvents,[175] the isomeric dienedione **4.243**[216] was isolated in a low yield (**Scheme 4.57**).
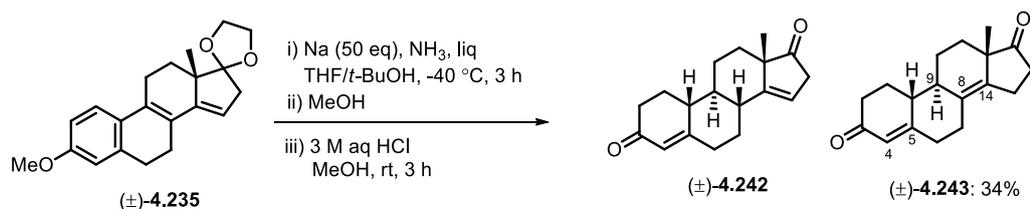
Scheme 4.57: Birch reduction of the steroidal framework.

Steroid **4.243** has been prepared in 27% yield by Re and co-workers in 1966 on treatment of acetal **4.235** with lithium metal in liquid ammonia, using tetrahydrofuran and diethyl ether as solvents, ethanol as proton source, and a mixture of acetic acid and 1 M hydrochloric acid for the following hydrolysis.[217] No ^{1}H- and ^{13}C-NMR data were available, therefore compound **4.243** was fully characterised.

It was proposed that a chemoselective epoxidation of the unconjugated C-8,C-14 double bond followed by epoxide ring-opening might allow the reintroduction of the 14β-hydroxyl group. Unfortunately, attempts to scale up the reaction failed giving just a complex mixture of products none of them having the mass spectrum required for compound **4.243**.

Disappointingly, the strategy was abandoned.

4.4. SUMMARY AND FUTURE WORK

The formidable challenge of synthesising the unnatural C-14 epimer of withanolide F **2.5** when starting from cheap commercially available materials was unsuccessful (**Scheme 4.58**). In spite of that, three of the four key intermediates have been effectively synthesised on the basis of literature procedures, which were modified to achieve a better yield and/or reproducibility.

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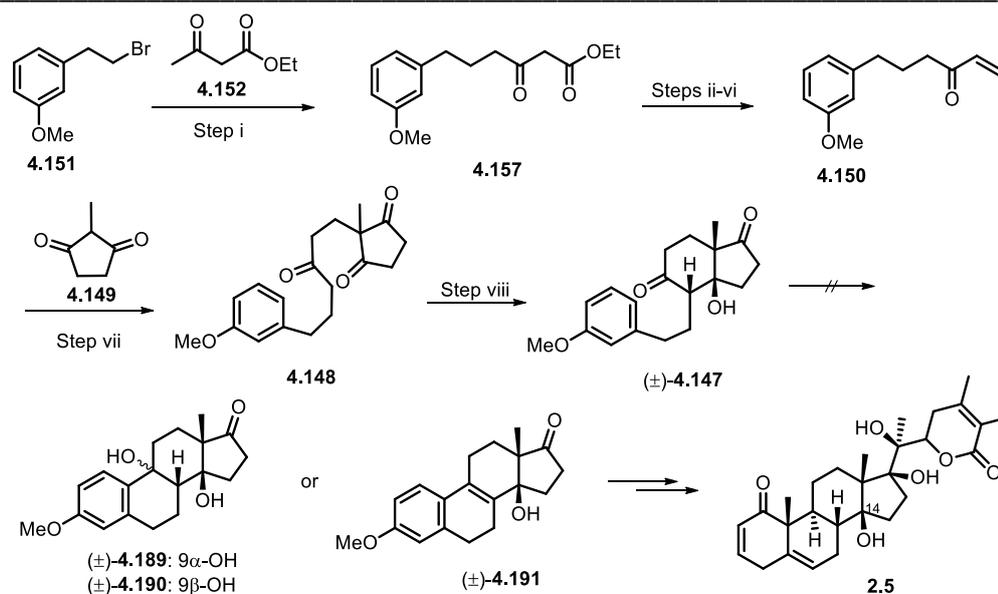
Scheme 4.58. Planned strategy to 14β-withanolide F **2.5**.

The first key intermediate, vinyl ketone **4.150** was obtained in 6 steps and 33% overall yield following the procedure developed by Wang *et al.*[179] Glycolic esters **4.163** and **4.164**, keto alcohol **4.160** and acetate **4.161** were novel, and have been completely characterised (**Figure 4.16**).
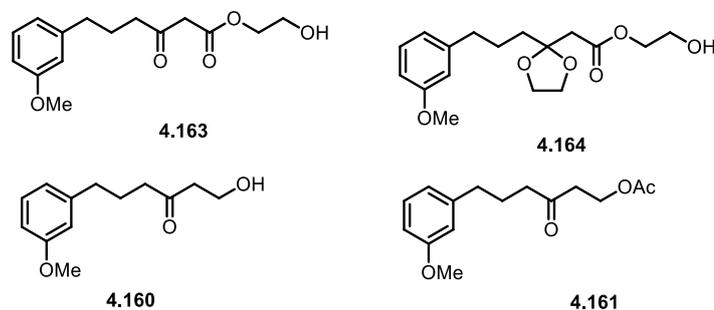
Figure 4.16. Novel intermediates isolated in the earlier steps of the target total synthesis.

The synthesis of the second key intermediate was improved, and triketone **4.148** was obtained in 90% yield after a one day reaction, against the published 80% yield after a 5 day reaction time.[179, 190]

The asymmetric amino-acid catalysed cylisation of triketone **4.148** to diketo carbinol **4.147** was disappointing using (*S*)-proline **4.168**. However, when the cyclisation was performed *via* an intramolecular aldol condensation, a mixture of diastereoisomers **4.147** and **4.177** in approximatively 3/1 ratio was obtained in 74% yield. The structures of

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compounds **4.147** and **4.177** were elucidated by 1D- and 2D-NMR spectroscopy after separation of an analytical sample by HPLC (**Figure 4.17).**
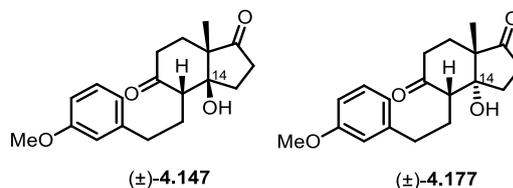
Figure 4.17. Epimeric 14-hydroxy steroids.

Unfortunately, the further cyclisation to complete the steroidal framework led first to the elimination of the 14-hydroxyl group to enone **4.174**, followed by ring B-closure and further dehydration to dienone **4.176** (**Scheme 4.59).**
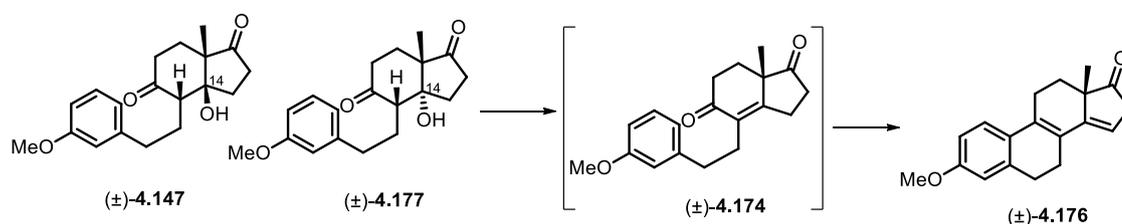
Scheme 4.59. Outcome of the intramolecular Friedel-Crafts cycloalkylation of the unprotected carbinols **4.174** and **4.177**.

Protection of the hydroxyl groups of the mixture of 14β- and 14α-isomers **4.147** and **4.177** proved difficult and additional bicyclic compounds arising from sequences of retro-aldol ring-opening and aldol-ring closing reactions were also isolated and fully characterised (**Figure 4.18**).
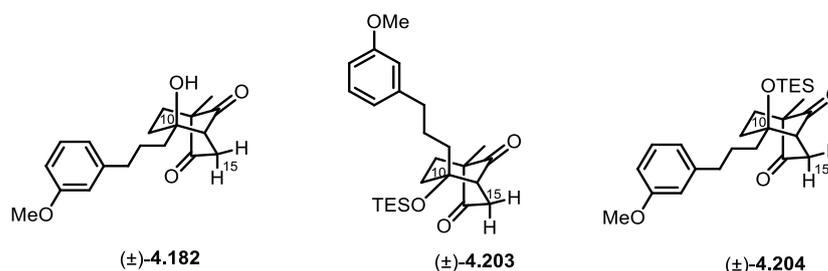
Figure 4.18. Structures of novel bicyclic systems.

Since dienone **4.176** was the major side-product obtained, attempts to reintroduce the 14β-hydroxyl group on **4.176** were also carried out, but they were unsuccessful and led to several by-products.

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In future work, the total synthesis of 14β-withanolide F **2.5** could be investigated starting from the commercially available dehydroepiandrosterone **4.244** (**Schemes 4.60**-**4.62**). Modification of ring A could be carried out by adapting the procedures of Ikekawa and co-workers[167] and Medrano and Grieco[172] which have been used in the synthesis of withanolide D (see **Scheme 4.7**) and E (see **Scheme 4.13**), respectively (**Scheme 4.60**).
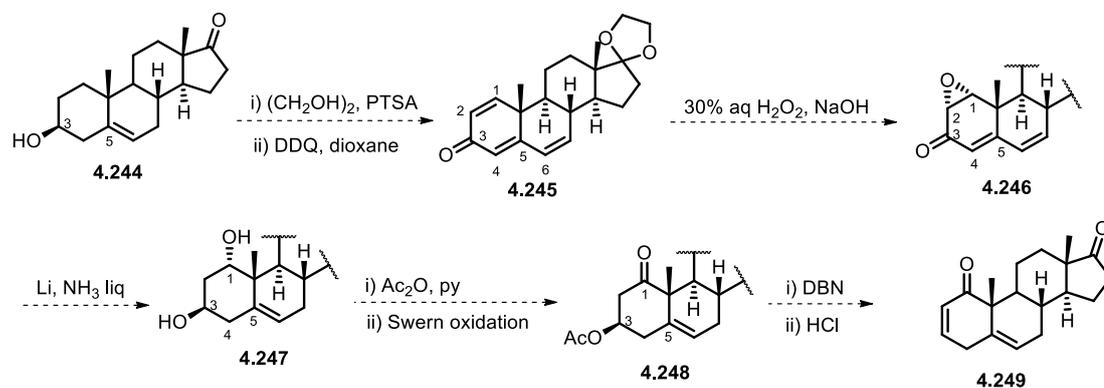
Scheme 4.60. Towards the 1-oxo-2,5-diene system starting from dehydroepiandrosterone **4.244.**

On dehydroepiandrosterone **4.244**, the reintroduction of the 14β-hydroxyl group could be addressed following the procedure of Kirsch *et al.*[201] as used in the stereoselective synthesis of 14β-hydroxysteroids (see **Scheme 4.48**, **Scheme 4.61**)**.**
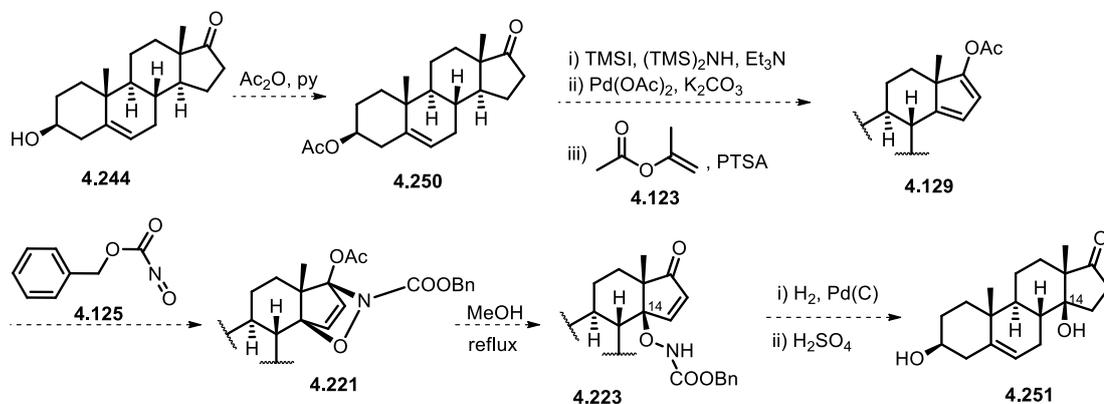
Scheme 4.61. Towards the reintroduction of the 14β-hydroxyl group in dehydroepiandrosterone **4.244.**

Finally, on dehydroepiandrsosterone **4.243** the lactonic side-chain containing the 17β- and 20β-hydroxyl groups could be installed following the procedure of Medrano and Grieco[172] which has been used in the synthesis of withanolide E (see **Scheme 4.13**, **Scheme 4.62**).
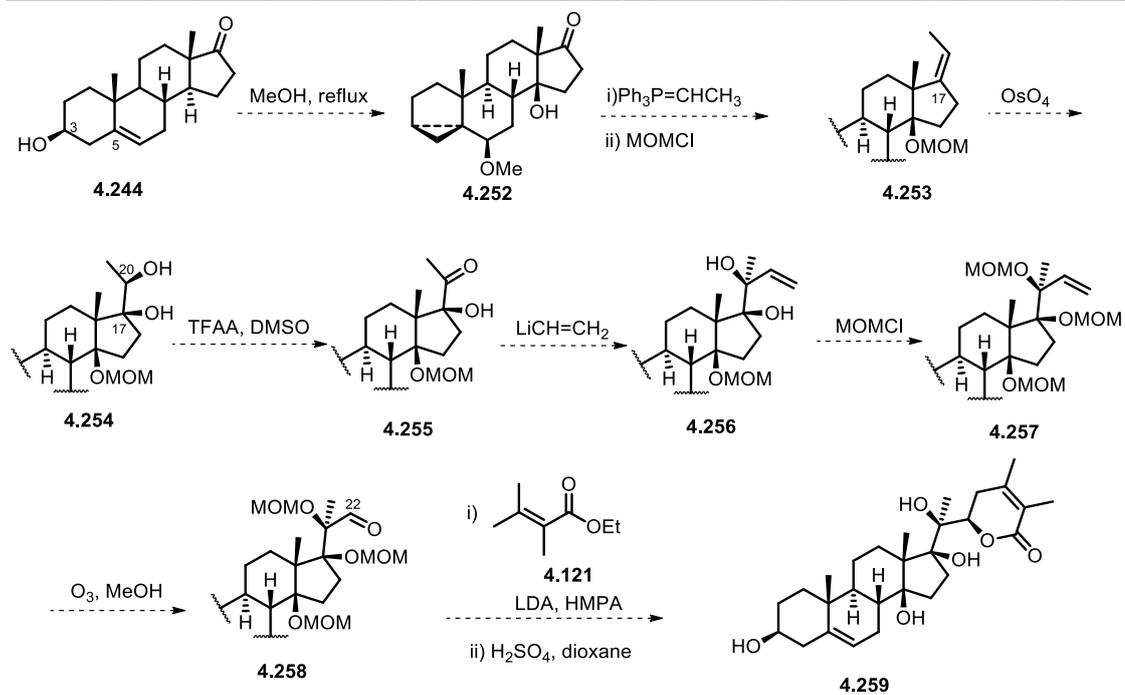
Scheme 4.62. Towards the installation of the lactonic side-chain in dehydroepiandrosterone **4.244**.

CHAPTER 5.
ELABORATION OF COAGULIN L 2.1

5.1. INTRODUCTION TO COAGULIN L 2.1

5.1.1. Structural features

Coagulin L **2.1** is a withanolide first identified by Atta-Ur-Rahman *et al.* in 1998 during studies on the constituents of *Withania coagulans* Dunal, a plant endemic of South Asia which was named after the evidence of its milk coagulating properties. The authors reported that 30 mg of coagulin L **2.1** (1.02 x 10^{-5}%) were isolated from 25 Kg of dried plant material. Its structure was elucidated by spectroscopic and spectrometric techniques, and by comparison with related compounds.[218]

As with other withanolides, coagulin L **2.1** is characterised by a hyperoxygenated steroidal framework and a side-chain containing a δ-lactone (**Figure 5.1**). In addition, its 3β-hydroxyl group is ether-linked to a molecule of glucose. The aglycone of coagulin L **2.1**, 3β-hydroxy-2,3,-dihydro-withanolide F **5.1**, is a compound which was first isolated by Velde *et al.* and described as a potential intermediate in the biosynthesis of the withanolides.[158]
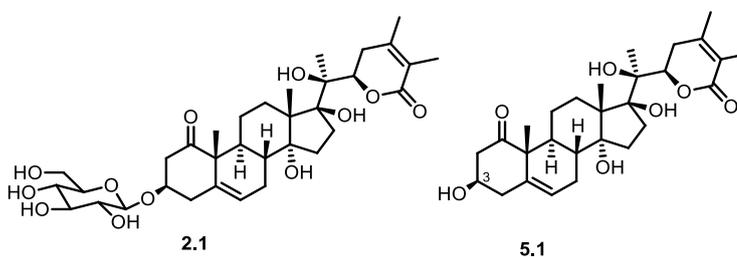
Figure 5.1. Structures of coagulin L **2.1** and its aglycone **5.1**.

5.1.2. Biological activities

In 2008 Maurya *et al*. investigated the constituents of *Withania coagulans* to seek compounds with antihyperglycemic activity, after earlier evidence of the cholesterol-lowering properties of the aqueous extracts of this plant when administrated to diabetic rats.[219] Amongst a number of withanolides isolated, coagulin L **2.1** was found to have the highest antihyperglicemic activity in promoting glucose tolerance by 29.8% in normoglycemic and by 23.3% in streptozotocin-induced diabetic rats (at a dose of 100 mg/kg body weight, compared with the value of 26.4% and 23.3 % of the standard drug

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metformine), and in decreasing by 22.7% the postprandial blood glucose level in type 2 diabetes model mice (at a dose of 50 mg/kg body weight, compared with the value of 18.6% of the standard drug metformine). A remarkable antidyslipidemic activity similar to fenofibrate was also observed.[219, 220]

Recently Beg *et al.* reported the antiadipogenetic properties of coagulin L **2.1**.[221] It is known that adipogenesis is modulated by the Wnt/β-catenin signalling pathway, whose activation in mesenchymal stem cells and 3T3-L1 pre-adipocyte cell lines prevents their differentiation into adipocytes.[222] Interestingly, it was shown that treatment of 3T3-L1 cells with coagulin L **2.1** remarkably inhibited the degradation of β-catenin and enhanced the expression of Wnt3a (a Wnt ligand) and its co-receptor LRP, with blockage of adipogenesis as the effects, thus, giving light to the potential applications of coagulin L **2.1** as treatment for metabolic syndrome and obesity.[221]

5.2. PROJECT AIM: TOWARDS THE ELABORATION OF COAGULIN L 2.1

Obesity and metabolic dysfunctions such as insulin resistance and type 2 diabetes are high risk factors of mortality, and coagulin L **2.1** has been shown to be a potential novel therapeutic to target these diseases.[221] Moreover, the study of Beg *et al*.[221] showed a clear correlation between coagulin L **2.1** and Wnt signalling, one of the three most studied embryonic signalling pathways. However, to the best of our knowledge, no studies of the biological effect of coagulin L **2.1** on the Hh/Gli pathway have been published.

AnalytiCon Discovery has reliable access to a large amount of plant material. Investigation of the chemical constituents of the powdered leaves of Withania *coagulans* and Withania *adpressa* resulted in the isolation of a substantial amount of coagulin L **2.1**. Around 5 g of this natural product were isolated from 5 kg of plant material, along with withanolide F **2.2**, which was obtained in a considerably lower yield (**2.1**/**2.2** = 50/1 ratio) (**Figures 5.2** and **5.3**).

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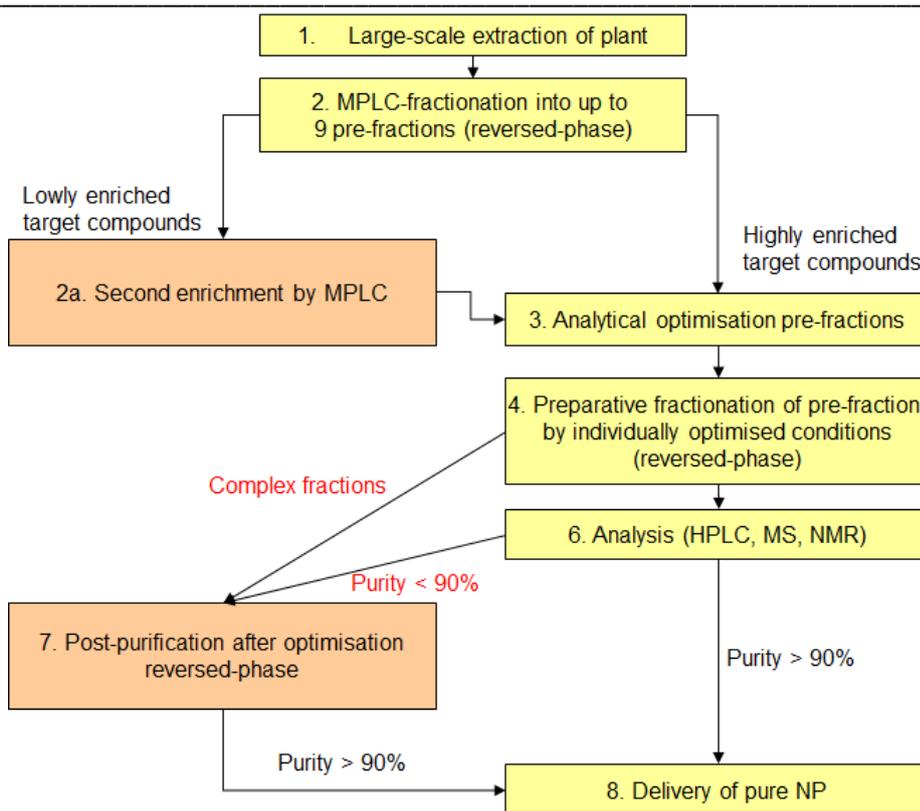
Figure 5.2. AnalytiCon's methodology for isolation of natural products.

The only structural differences between coagulin L **2.1** and withanolide F **2.2** can be found in ring A, where the elimination of glucose at C-3 from compound **2.1** followed by elimination of the resulting alcohol produces the C-2,C-3-unsaturation found in compound **2.2** (**Figure 5.3**).
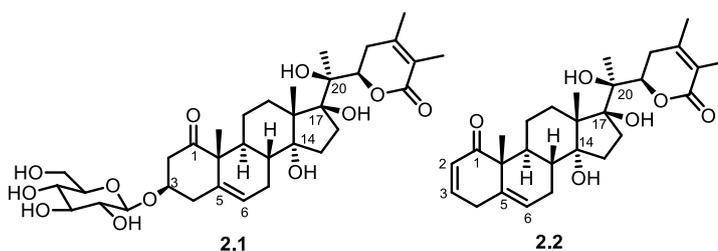
Figure 5.3. Structures of coagulin L **2.1** and withanolide F **2.2**.

Therefore, we considered that the deglycosylation of an original sample of coagulin L **2.1** would give withanolide **5.1** (**Scheme 5.1**), the right-hand side of which could be elaborated such as in **2.6** to provide additional analogues of withanolide F **2.2** for a structure-activity relationship study on the Hh/Gli signalling pathway, as well as potentially novel antihyperglycemic and antiobesity remedies.
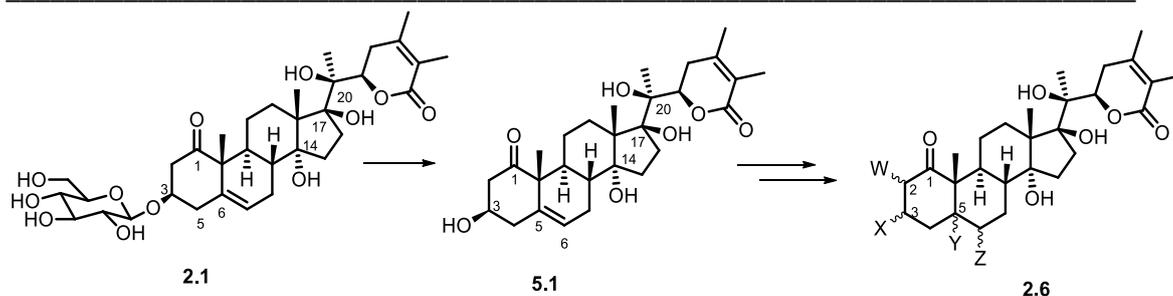
Scheme 5.1. Intended modifications of withanolide rings A and B. W, X, Y, Z = H, C, O, S, N, halogen.

5.3. RESULTS AND DISCUSSION

5.3.1. Recent literature elaboration of rings A and B of withanolides

In spite of the apparent fragility of the lactone ring, modifications of the withanolide framework after installation of the cyclic oxygenated side-chain have been widely reported, especially on withaferin A **4.1**,[160] and, recently on withanolide A **4.49**.[162]

With regards to withaferin A **4.1**, the most recent elaborations of the left-hand side have been reported by Joshi *et al.*,[160] who converted the 5β,6β-epoxide in ring B into the 5β-alcohol **5.2** by means of polymethylhydrosiloxane and a catalytic amount of iodine, and into the 5α,6α-thiirane **5.3** using cyanuric chloride **5.4** and ammonium thiocyanate (**Scheme 5.2**). The butyl-aminoalcohols **5.5** and **5.6**, and benzyl-aminoalcohol **5.7** were also prepared by treatment of compound **4.1** with the appropriate amines and water. In all these cases the lactone ring remained untouched.
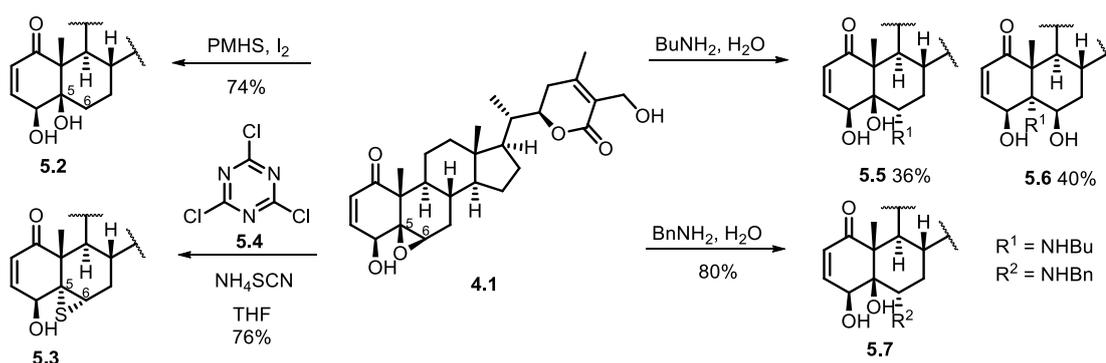
Scheme 5.2. Modification of the epoxide of withaferin A **4.1** by Joshi *et al.*[160]

Similar observations were also noted during the elaboration of withanolide A **4.49** by Gademann and co-workers,[162] who showed that only the ring A could be functionalised in spite of the presence of an epoxyalcohol in ring B and of the tetrasubstituted unsaturated lactone in the side-chain (**Schemes 5.3** and **5.4**).

In fact, the C,2-C,3 double bond was exclusively epoxidised to compound **5.8** when withaferin A **4.49** was treated with *tert*-butylhydroperoxide and tetrabutylammonium fluoride (**Scheme 5.3**). Moreover, the C-2,C-3 epoxide was solely converted into the 1α,3α-diol **5.9** *via* an organoselenium-mediated reduction (followed by treatment with sodium borohydride to reduce the C-1 ketone), and into the 3α-allylic alcohol **5.10** *via* a Wharton carbonyl transposition. The ring A of compound **5.10** was also selectively reduced to compound **5.11** *via* catalytic hydrogenation in the presence of Crabtree's catalyst, and transformed into the 3β-hydroxyl derivative **5.13** by a Mitsunobu reaction of 3α-alcohol **5.10** with *p*-nitrobenzoic acid **5.12**, diethylazodicarboxylate and triphenylphosphine followed by basic hydrolysis.
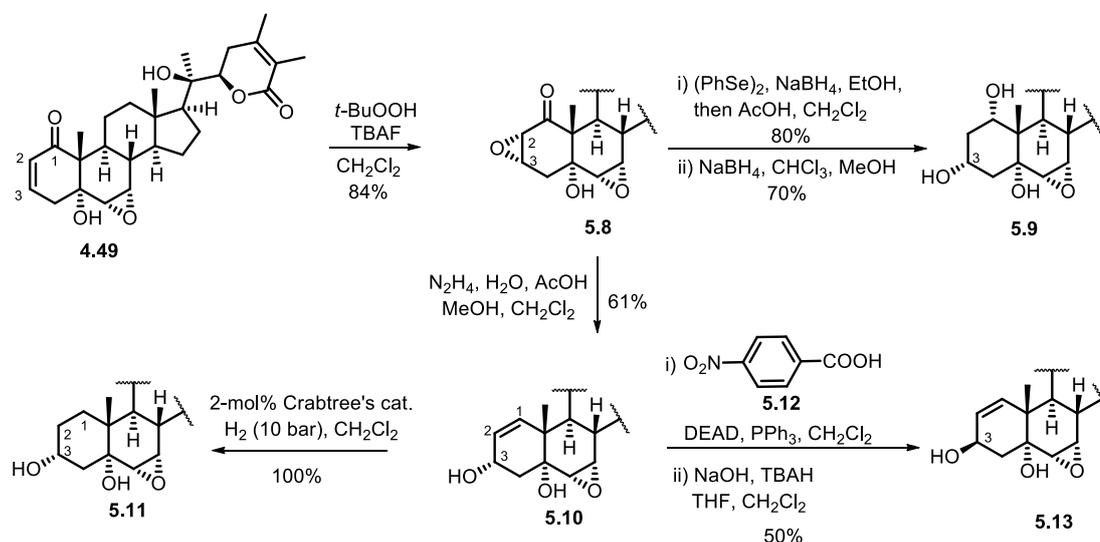
Scheme 5.3. Gademann's functionalisation of withanolide A **4.49**.[162]

The C-2,C-3 double bond and the C-1 ketone of withanolide A **4.49** could be also smoothly reduced to compounds **5.14** and **5.15**. However, further functionalization at C-1 proved to be trickier, and only oximes **5.16**-**5.19** were prepared (**Scheme 5.4**).[162]
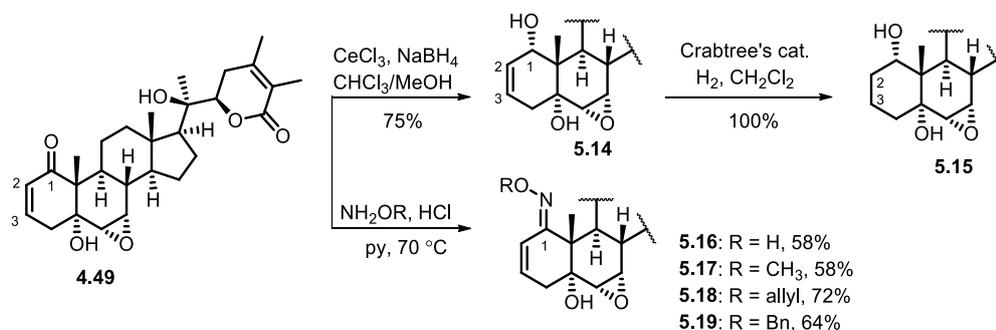
Scheme 5.4. Gademann's functionalisation of C-1 of withanolide A **4.49**.[162]

It is interesting to note that also in the case of coagulin L **2.1**, apart from its enzymatic deglycosylation, only the preparation of oxime **5.20** has been reported. It was prepared by heating compound **2.1** at reflux in the presence hydroxylamine hydrochloride (**Scheme 5.5**). The yield and additonal details of the reaction were not given.[220]
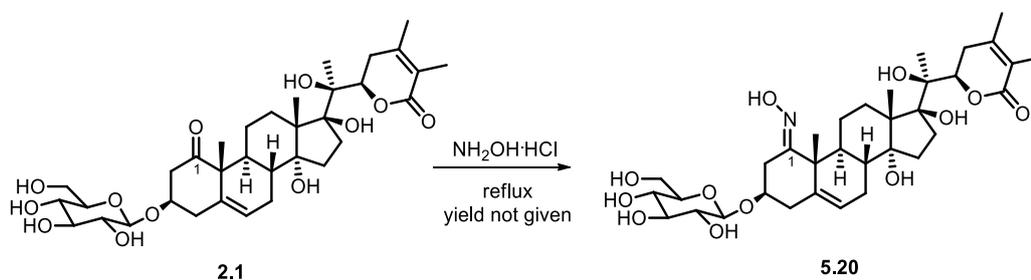
Scheme 5.5. Derivatisation of coagulin L **2.1** by Maurya *et al.*[220]

Inspired by these results, we believed that also the elaboration of rings A and B of coagulin L **2.1** could be selectively achieved, and that the two additional hydroxyl groups at C-14 and C-17 (compared with withaferin A **4.1** and withanolide A **4.49**) should not pose particular problems.

5.3.2. Towards withanolide F 2.2.

To further functionalise the left-hand side of coagulin L **2.1** there was the need to introduce a reactive functionality, for example, in ring A (**Scheme 5.6**). Thus, we expected that conversion of coagulin L **2.1** into withanolide F **2.2** would provide an α,β-unsaturated ketone, which could be envisaged as a building block to introduce a variety of functionalities at C-3 by means of Michael additions of diverse nucleophiles.
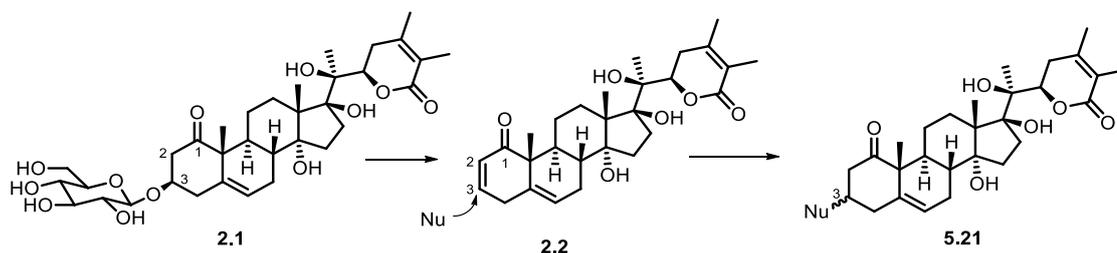
Scheme 5.6. Intended conjugated additions to withanolide F **2.2**. Nu = general carbon, sulfur, or nitrogen nucleophile.

Therefore, the deglycosylation of compound **2.1** followed by acetylation of the secondary alcohol in **5.1** and subsequent elimination of acetate **5.22** to obtain the unsaturated system **2.2** was first explored (**Scheme 5.7**).
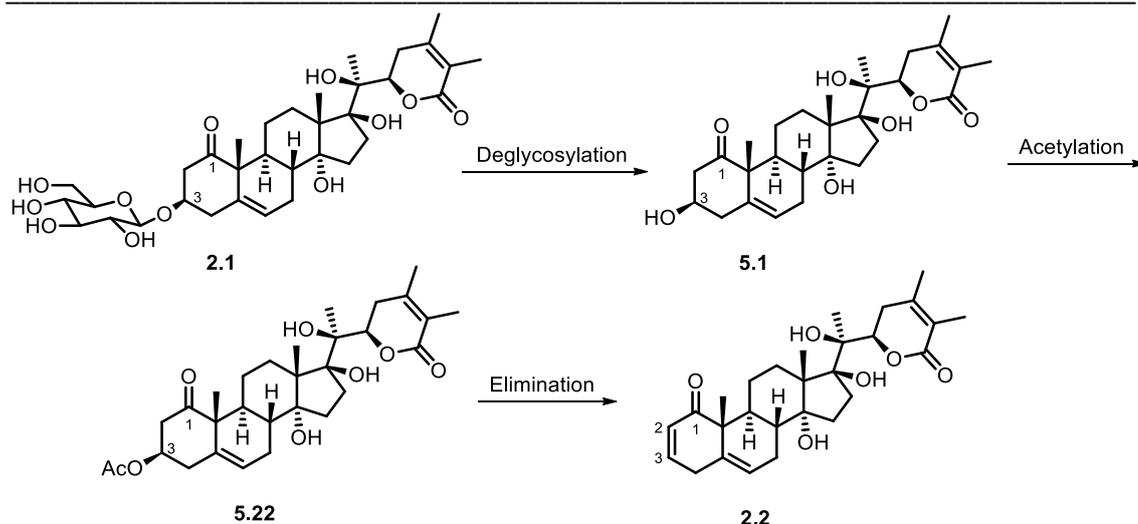
Scheme 5.7. Towards withanolide F **2.2** from coagulin L **2.1**.

5.3.2.1. Deglycosylation of coagulin L **2.1**

The enzymatic deglycosylation of coagulin L **2.1** was previously reported by Maurya and co-workers using the spores of *Aspergillus ochraceous* (the yield was not given).[220]

However, an extensive screening of reaction conditions performed at AnalytiCon Discovery established that the most reliable way to carry out this deglycosylation was by using the enzyme β-glucosidase from *Aspergillus niger*. The optimised procedure involved adding a solution of coagulin L **2.1** and β-glucosidase to a buffered solution of sodium acetate at pH 4, and incubation of the resulting mixture at 37 °C for one day. Under these conditions 3β-alcohol **5.1** was obtained in 61% yield (**entry 1**, **Table 5.1**).
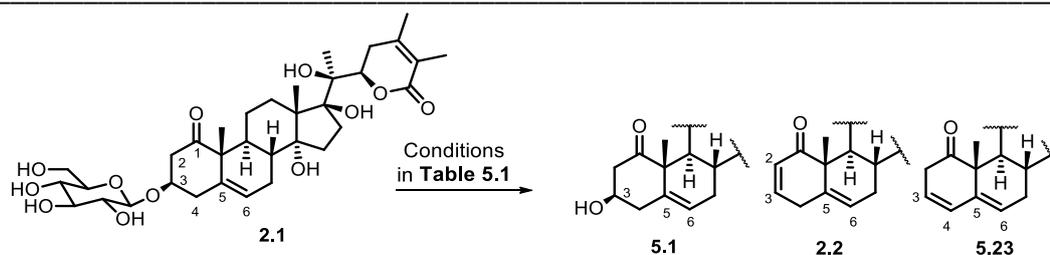
Entry	Conditions	Outcome
1	**2.1** (500 mg), enzyme, H_2O/MeOH = 1/1 (1100 mL), 37 °C, 1 d	**5.1** 61%[a]
2	**2.1** (200 mg), enzyme, H_2O/MeOH = 1/1 (440 mL), 37 °C, 18 h	**5.1** 43%[b]
3	**2.1** (50 mg), enzyme, H_2O/MeOH = 1/1 (110 mL), 37 °C, 18 h	**5.1** 50%[c]
4	**2.1** (20 mg), enzyme, H_2O/MeOH = 1/1 (44 mL), 37 °C, 18 h	**5.1** 100%[c]
5	10% aq HCl, dioxane/H_2O, rt to 40 °C, 3 h	Mainly **2.2**[d]
6	10% aq HCl, dioxane/H_2O, reflux, 10 min	Decomposition[d]
7	1 M methanolic HCl, reflux, 4 h	Decomposition[d]
8	1 M aq LiOH, THF/H_2O, rt 18 h	Decomposition[d]
9	0.1 M aq KOH, dioxane, rt 1 h	Mainly **2.2**[d]
10	DBU (1 + 1.2 eq), rt to 60 °C, 1 d	Mainly **5.23**[d]

Table 5.1. Deglycosylation of coagulin L **2.1**. Reactions in **entries 5**-**10** were carried out in 5-20 mg scale. [a]Reaction carried out in incubator at AnalytiCon Discovery. [b]Reaction carried out in incubator at the University of York. [c]Reaction carried out in an oil bath at the University of York. [d]As detected by NMR spectroscopy and ESI-MS of the crude material.

This deglycosylation proved to be sensitive to the amount of starting material used, and gave alcohol **5.1** in yields ranging between 43 and 100% starting from 200, 50 and 20 mg of coagulin L **2.1**, respectively (**entries 2-4**). However, despite the variable yields, the amount of compound **5.1** isolated enabled us to carry out subsequent transformations.

The deglycosylation was also attempted under acidic (**entries 5-7**) and basic (**entries 8-10**) conditions. However, only decomposition or a mixture of products including withanolide F **2.2** and its isomer **5.23** were detected.

5.3.2.2. Acetylation of 3β-hydroxy-coagulin L **5.1**

The acetylation of 3β-hydroxy-coagulin L **5.1** proceeded smoothly following the procedure of Vande Velde *et al.*[158] who used acetic anhydride in pyridine, and gave 3β-acetyl-coagulin L **5.22** in quantitative yield (**Scheme 5.8**).
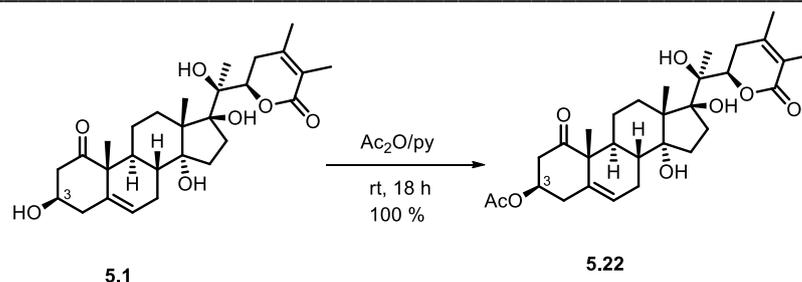
Scheme 5.8. Synthesis of 3β-acetyl withanolide **5.22.**

5.3.2.3. Elimination of acetate

The next step was the elimination of acetate from compound **5.22** to form the enone in ring A of withanolide F **2.2** (**Table 5.2**). The reaction was first attempted using an aqueous solution of KOH in dioxane,[158] conditions which led to the isolation of withanolide F **2.2** in 61% yield (**entry 1**).
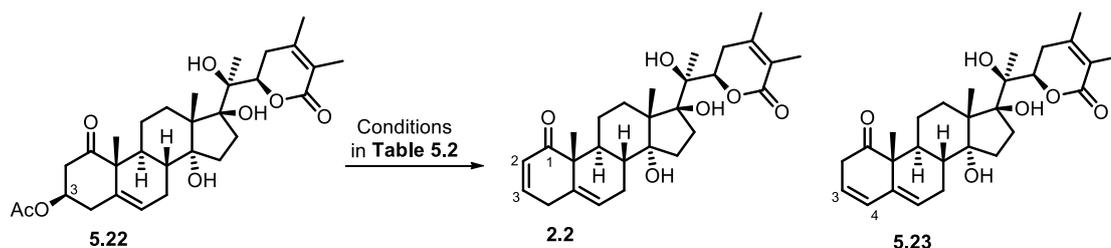
Entry	Conditions	Outcome
1	1 M aq KOH (2.5 eq), dioxane, rt, 15 min	**2.2** 61%[a]
2	DBU (1 eq), CH₂Cl₂, rt, 30 min	Sm **5.22** 25%, **2.2** 75%
3	DBU (1 eq), CH₂Cl₂, rt, 40 min	Starting material **5.22** 25%, **2.2** 58%, **5.23** 17%
4	DBU (1.5 eq), CH₂Cl₂, rt, 20 min	**5.23** 50%
5	DBU (1.5 eq), CH₂Cl₂, 0 °C, 2 h	Sm **5.22** 41%, **2.2** 59%
6	DBU (1.5 eq), CH₂Cl₂, 0 °C, 3 h	**2.2** 75%, **5.23** 25%

Table 5.2. Elimination of acetate **5.22**. Reactions were carried out on a 5-50 mg scale. [a]Reaction and purification performed at AnalytiCon Discovery.

We also carried out the elimination in the presence of the non-nucleophilic hindered base 1,8-diazabicyclo[5.4.0]undec-7-ene (**entries 2-6**). At room temperature, it was found that stoichiometric amounts of base gave withanolide F **2.2** in good yield along with unreacted starting material **5.22** (**entry 2**). When the reaction time was longer (**entry 3**) withanolide F **2.2** was formed along with the isomeric compound **5.23**.[158] A slight excess of base led mainly to compound **5.23** (**entry 4**). Similar results were seen when the reaction was carried out at 0 °C. A two hour reaction gave withanolide F **2.2** in 59% yield with 41%

of unreacted starting material (**entry 5**). By stirring the reaction mixture for a longer time the starting material was completely consumed, but again the isomeric compound **5.23** was obtained as well as withanolide F **2.2** (**entry 6**).

It is important to note that the products **2.2**, **5.23** and the starting material **5.22** have similar R_f values thus preventing their further separation (the course of the reactions had to be followed by NMR spectroscopy).

5.3.3. Michael addition of thiophenol 5.24 to withanolide F 2.2

We began the derivatisation of the ring A of withanolide F **2.2** by investigating the use of thiophenol **5.24** as a nucleophile (**Table 5.3**). Since the preparation of the starting material **2.2** proved difficult, a direct replacement of the sugar moiety of coagulin L **2.1** with thiophenol **5.24** was attempted first. However, when coagulin L **2.1** was treated with thiophenol and potassium hydroxide in pyridine (**entry 1**), a clean thiophenyl-derivative **5.25** could not be isolated, even when the reaction was carried out in the presence of stoichiometric amounts of *N*,*N*-dimethylaminopiridine (**entry 2**). The Michael additions attempted in the presence of triethylamine (**entry 3**), or 1,5-diazabicyclo(4.3.0)non-5-ene in methanol (**entry 4**) were also unsuccessful.[70]
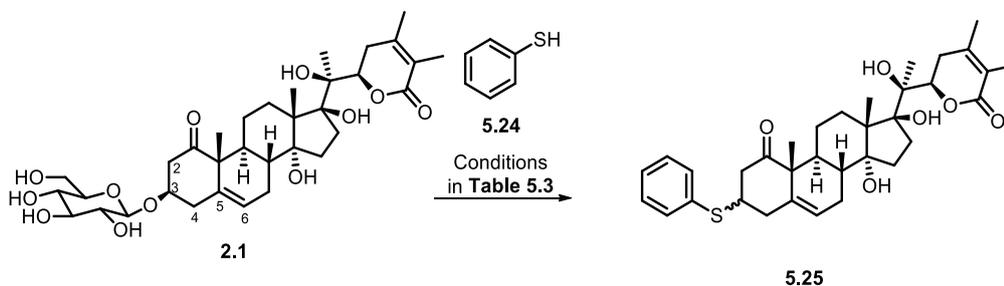
Entry	Conditions	Outcome
1	**5.24** (10 eq), 0.1 M aq KOH (10 eq), py, rt to 60 °C, 7 h	Traces of **5.25**
2	**5.24** (5 eq), DMAP (1 eq), py, rt, 18 h	Decomposition
3	**5.24** (1 eq), Et₃N (3 eq), MeOH, rt, 3 h	Decomposition
4	**5.24** (5 eq), DBN (1.5 eq), MeOH, rt to 60 °C, 7 h	Decomposition

Table 5.3. Attempted synthesis of thiophenyl ether **5.25** from coagulin L **2.2**. Rections were carried out on 5-20 mg of starting material. [a]Detected by NMR spectroscopy analysis of the crude material.

The addition of thiophenol **5.24** was next tried on the crude withanolide F **2.2** contaminated by acetate **5.22** (1/3, 11 mg) (**Scheme 5.9**). The formation of the thiophenyl ether **5.25** was detected by NMR spectroscopy and HRMS, however, the small scale and

the difficulties faced during the attempted purification process did not allow the isolation of a sufficient amount of material for its full characterisation (**Scheme 5.9**).
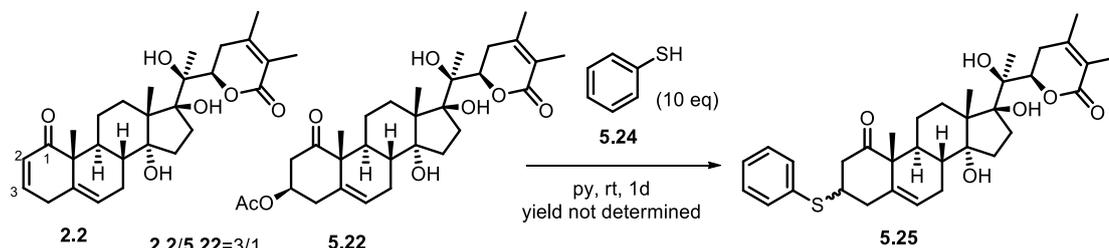
Scheme 5.9. Attempted synthesis of thiophenyl ether **5.25**.

Additional attempts to achieve achieve an adequate amount of pure phenylthioether **5.25** proved unsuccessful.

5.3.4. C-3 Esterification

Driven by the easy acetylation of the 3β-hydroxyl group of withanolide **5.1**, we wanted to check the feasibility of introducing other carboxylate esters on C-3. The benzoic ester was investigated first (**Table 5.4**).
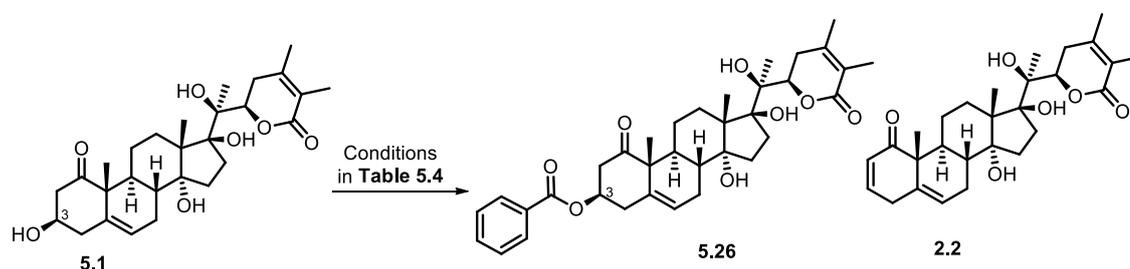
Entry	Conditions	Outcome
1	Benzoic acid (1.2 eq), T3P (2 eq), DIPEA, CHCl$_3$, rt to 40 °C, 18 h	Sm **5.1**
2	Benzoic acid (1.5 eq), T3P (1.3 eq), py, rt, 18 h	Sm **5.1**
3	Benzoic acid (1.5 eq), T3P (2 eq), py, 40 °C, 18 h	Sm **5.1, 2.2**[a]
4	Benzoic anhydride (10 eq), py, rt to 40 °C, 24 h	**5.26** 49%
5	Benzoyl chloride (10 eq), py, rt, 18 h	**5.26** 98%

Table 5.4. The formation of benzoic ester **5.26**. Reactions were carried out on a 5-50 mg scale. [a]Detected by NMR spectroscopy of the unpurified material.

Early efforts were carried out by using benzoic acid and propylphosphonic anhydride (T3P) as a coupling agent (**entries 1-3**). However, only unreacted starting material or a mixture of by-products including traces of **2.2** were detected by NMR spectroscopy of the crude material. Benzoic anhydride was tried next, following the protocol that, in the

acetylation (see **Scheme 5.8**), was successful. Indeed, by treating compound **5.1** with benzoic anhydride in pyridine, benzoic ester **5.26** was obtained in good yield (**entry 4**) which increased to 98% by replacing benzoic anhydride with benzoyl chloride (**entry 5**).

This strategy proved successful and reproducible, and led to the synsthesis of the (*E*)-cinnamic ester **5.28** in 89% yield on treatment of alcohol **5.1** with (*E*)-cinnamoyl chloride **5.27** in pyridine at room temperature (**Scheme 5.10**).
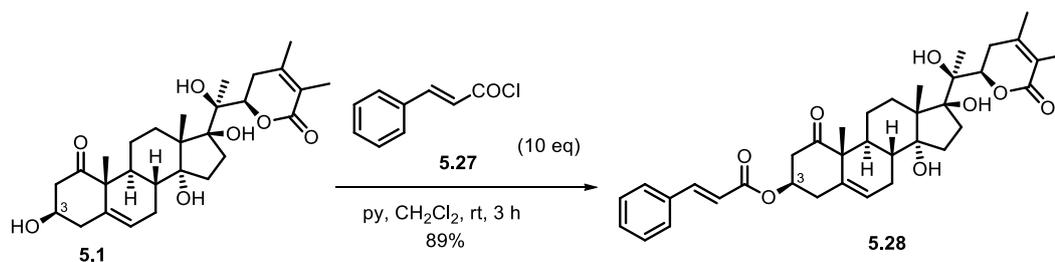
Scheme 5.10. Synthesis of (*E*)-cinnamic ester **5.28**

5.3.5. Attempted C-3 oxidation

The oxidation of C-3 to obtain the novel dienone **5.29** was attempted on 3β-hydroxy withanolide **5.1** (**Scheme 5.11**). However, when withanolide **5.1** was treated with Dess-Martin periodinane **4.239**, the starting material decomposed quickly, and no traces of the desired product were detected by analytical techniques. Similar results were seen when withanolide **5.1** was subjected to stoichiometric amounts of Jones reagent. This oxidation approach was therefore abandoned.
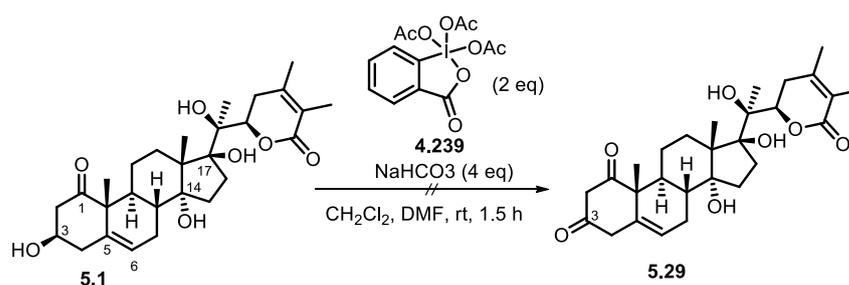
Scheme 5.11. Attempted oxidation of 3β-alcohol **5.1**.

5.3.6. Attempted epoxidation of ring B

Finally, the epoxidation of ring B was also attempted on acetate **5.22** in the presence of *m*-chloroperbenzoic acid and sodium hydrogen carbonate (**Scheme 5.12**). After one day of stirring at room temperature the starting material was consumed completely and LC-MS showed a peak corresponding to the mass of the intended compound. Two

consecutive purifications by flash column chromatography on silica gel were attempted using different solvent systems to isolate compound **5.30**. Unfortunately only decomposition was observed.
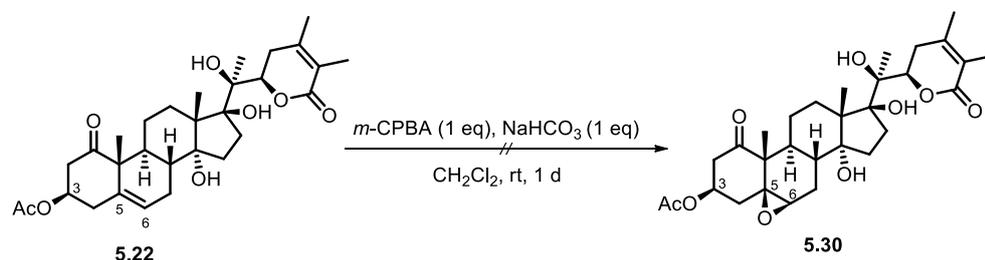
Scheme 5.12. Attempted epoxidation of acetate **5.21**.

5.4. SUMMARY AND FUTURE WORK

The glycoside withanolide coagulin L **2.1** was successfully deglycosylated on a small scale using the enzyme β-glucosidase from *Aspergillus niger*, following a procedure which was optimised at AnalytiCon Discovery.

The resulting 3β-hydroxy coagulin L **5.1** was converted in two steps, *via* acetate **5.22**, into the lead compound withanolide F **2.2** in satisfactory yield, although it was mainly isolated as a mixture with its isomer isowithanolide F **5.23** (**Scheme 5.13**). Since withanolide F **2.2** is extracted in minimal amounts from plant material compared to coagulin L **2.1** (which is instead present in gram amounts), a feasible way to obtain larger quantities of this natural product from coagulin L **2.1** has been provided.
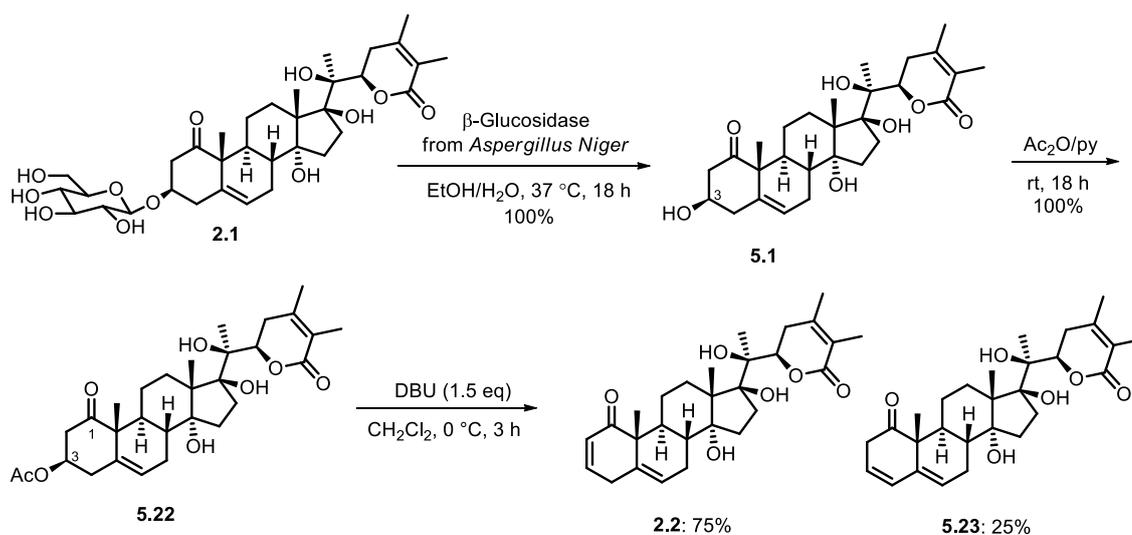
Scheme 5.13. Synthesis of withanolide F **2.2** from coagulin L **2.1**.

In addition to the known acetate **5.22**,[160] the preparation of the novel benzoate **5.26** and (*E*)-cinnamate **5.28** from 3β-hydroxy coagulin L **5.1** were also carried out in good yields (**Figure 5.4**). However, other transformations also proved difficult.
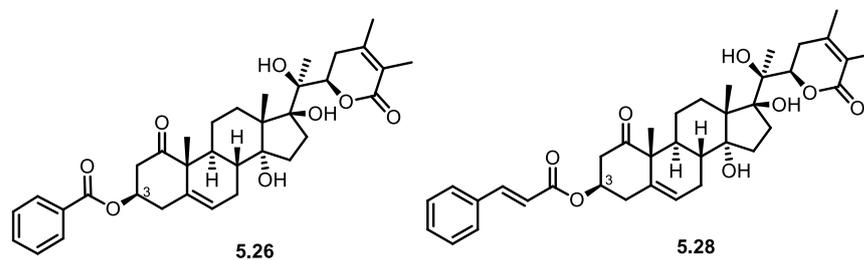
Figure 5.4. Structure of benzoate **5.26** and (*E*)-cinnamate **5.28**

In future work, conditions for the selective conversion of coagulin L **2.1** into withanolide F **2.2** will be developed, allowing the Michael additions to be explored under more controlled conditions.

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CHAPTER 6. EXPERIMENTAL

6.1. GENERAL DIRECTIONS

Reactions were monitored by Thin Layer Chromatography (TLC) and/or LC-MS (Liquid Chromatography-Mass Spectrometry). TLC was performed using pre-coated aluminium foil TLC-sheet Xtra SIL G/UV$_{254}$ (MACHERAY-NAGEL), layer 0.20 mm, silica gel 60 with fluorescent indicator UV$_{254}$, and on Merck silica gel 60F$_{254}$. Visualisation was carried out using UV light at 254 nm and basic aqueous potassium permanganate or ethanolic *p*-anisaldehyde as stains. Chemical reactions were carried out with magnetic stirring. All air and moisture sensitive reactions were performed in flame-dried glassware and under a nitrogen or argon atmosphere. Water refers to distilled water.

Reagents were purchased form commercial sources and used without any further purification except for:

- Pyridine, purchased anhydrous from commercial sources.
- Tetrahydrofuran, either purchased anhydrous from commercial sources or distilled from sodium-benzophenone ketyl immediately before use.
- Dichloromethane, either purchased anhydrous from commercial sources or obtained from an Innovative Technology Inc. PureSolv® solvent purification system.

Flash column chromatography was performed on a Biotage Isolera Four with a UV-VIS detector, using Fluka silica gel 60 (SiO$_2$) or using slurry packed Fluka silica gel, 35-70 μm, 60 Å, with the specific eluent. Petroleum ether (PE) is the fraction with the boiling point range 40-60 °C.

Melting points (m.p.) were determined using a Gallenkamp apparatus and are uncorrected.

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE spectrometer or on a Jeol ECX400 spectrometer operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C or at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts (δ) are quoted in parts per million (ppm) from tetramethylsilane calibrated to the residual nondeuterated solvent peak as internal standard.[223] Coupling constants (*J*) are quoted in Hertz. Structural assignment was verified by two dimensional NMR (HSQC, HMBC, COSY) and nOe

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where necessary. When required, deuterated chloroform was dried over oven-dried MgSO$_4$, stirred with K$_2$CO$_3$, filtered and stored over 4 Å molecular sieves.

High resolution mass spectra were obtained by the University of York Mass Spectrometry Service using electrospray ionisation (ESI) on a Bruker Daltonics, Micro-Tof spectrometer.

LC-MS spectra were recorded at AnalytiCon Discovery GmbH using API165, API150, API365, AB Sciex (UV, ELSD and DAD detectors), gradient A: 5mM ammonium formate + 0.1% formic acid, B: methanol/acetonitrile = 1/1 + ammonium monohydrogen carbonate.

CHN elemental analyses were obtained by the University of York CHN Service using an Exeter Analytical CE440 Elemental Anayser.

Optical Rotations were measured on a JASCO DIP-370 polarimeter using a sodium lamp and a 2 mL cell with 1 dm path length or a 1 mL cell with 10 mm path length. Data are reported as follows: $[\alpha]_D^T$ (c in g/100 mL, solvent).

Infrared spectra were recorded on a ThermoNicolet IR-100 spectrometer with NaCl plates as a thin film dispersed from a CH$_2$Cl$_2$ solution, or a PerkinElmer UATR spectrometer.

The naming of compounds conforms to IUPAC rules. The numbering on the structures do not always conform the IUPAC nomenclature but refers to the historic numbering of this class of compounds, and/or for purpose of characterisation.

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6.2. CHAPTER 3: EXPERIMENTAL DETAILS

(3β)-20-Oxopregn-5-en-3-yl 4-methylbenzenesulfonate 3.59
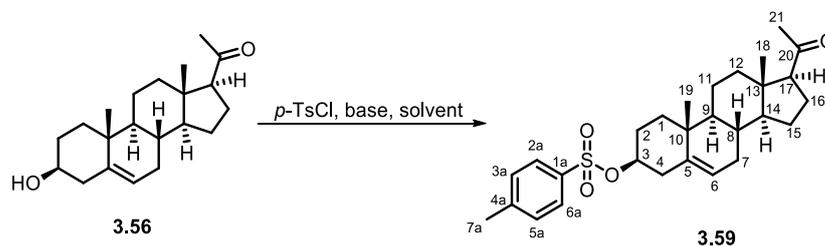
Synthesis of tosylate **3.59** using *p*-TsCl in py[81]

p-TsCl (2.00 g, 10.5 mmol) was dissolved in py (6 mL) and pregnenolone **3.56** (1.00 g, 3.16 mmol) was added in one portion. The reaction mixture was stirred at rt for 18 h and afterwards poured into an ice-bath cooled H_2O (20 mL). The solid obtained was collected by filtration and air dried to yield tosylate **3.59** (1.40 g, 94%) as white needles. **Ref. LNB0145-067**

R_f: 0.29 (PE/AcOEt = 9/1); **m.p.**: 139-140 °C (acetone/H_2O) [lit. 139-140 °C (acetone/H_2O)];[82] **[α]$_D^{24}$**: +12 (*c* 1.16, CHCl$_3$) [lit. +9 (*c* 68.55, CHCl$_3$)];[82] **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 7.78 (2H, d, *J* = 8.1 Hz, H-2a, H-6a), 7.32 (2H, d, *J* = 8.1 Hz, H-3a, H-5a), 5.30-5.27 (1H, m, H-6), 4.36-4.27 (1H, m, H-3), 2.5 (1H, "t", *J* = 8.8 Hz, H-17), 2.45-2.38 (1H, m, H-4), 2.43 (3H, s, 3H-7a), 2.29-2.23 (1H, m, H-4), 2.18-2.11 (1H, m, H-16), 2.09 (3H, s, 3H-21), 2.04-2.99 (1H, m, H-12), 1.99-1.92 (1H, m, H-7), 1.85-1.77 (2H, m, H-1, H-2), 1.73-1.36 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.25-1.15 (1H, m, H-15), 1.15-1.07 (1H, m, H-14), 1.06-0.98 (1H, m, H-1), 0.97-0.88 (1H, m, H-9), 0.95 (3H, s, 3H-19), 0.59 (3H, s, 3H-18); **^{13}C-NMR** (125 MHz, CDCl$_3$), δ: 209.5 (C, C-20), 144.5 (C, C-4a), 138.9 (C, C-5), 134.8 (C, C-1a), 129.9 (2CH, C-3a, C-5a), 127.7 (2CH, C-2a, C-6a), 123.3 (CH, C-6), 82.3 (CH, C-3), 63.7 (CH, C-17), 56.9 (CH, C-14), 49.8 (CH, C-9), 44.0 (C, C-13), 38.9 (CH$_2$, C-4), 38.8 (CH$_2$, C-12), 37.0 (CH$_2$, C-1), 36.4 (C, C-10), 31.8 (CH, C-7 + CH$_2$, C-8), 31.6 (CH$_3$, C-21), 28.7 (CH$_2$, C-2), 24.5 (CH$_2$, C-15), 22.9 (CH$_2$, C-16), 21.7 (CH$_3$, C-7a), 21.1 (CH$_2$, C-11), 19.2 (CH$_3$, C-19), 13.3 (CH$_3$, C-18); **IR** (neat): 2944, 2898, 2849, 1702, 1360, 1188, 1175, 937 cm^{-1}; **HRMS** (ESI): calculated for C$_{28}$H$_{38}$NaO$_4$S [MNa$^+$] requires 493.2388, found 493.2368 (3.1 ppm error). Data in accordance with those reported in the literature.[82, 84]

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Synthesis of tosylate **3.59** using *p*-TsCl and DMAP in py[84]

p-TsCl (4.65 g, 24.5 mmol) and DMAP (5.97 mg, 0.05 mmol) were added to a solution of pregnenolone **3.56** (1.55 g, 4.89 mmol) in dry py (30 mL). The reaction mixture was stirred at rt overnight before being poured into ice-bath cooled H_2O (75 mL). A white precipitate was formed, which was filtered, dissolved in CH_2Cl_2 (150 mL) and washed with H_2O (3 x 150 ml). The organic layer was separated, dried over $MgSO_4$, filtered, evaporated under reduced pressure and dried *in vacuo* to give tosylate **3.59** (2.11 g, 92%) as white needles. **Ref. LNB0161-139**

Synthesis of tosylate **3.59** using *p*-TsCl, Et_3N and DMAP in CH_2Cl_2[86]

Pregnenolone **3.56** (500 mg, 1.58 mmol) was dissolved in CH_2Cl_2 (8 mL), and Et_3N (660 µL, 4.75 mmol), DMAP (192 mg, 1.58 mmol) and *p*-TsCl (603 mg, 3.16 mmol) were added. The reaction mixture was stirred at rt overnight. The mixture was diluted with CH_2Cl_2 (40 mL) and H_2O (40 mL). The organic layer was separated, washed with $NaHCO_3$ (3 x 40 mL), H_2O (40 mL) and brine (40 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Recrystallisation of the crude product from acetone and water gave tosylate **3.59** (630 mg, 85%) as white needles. The mother liquor was evaporated under reduced pressure and the residual aqueous layer was extracted with CH_2Cl_2 (250 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1 to 4/1) to afford tosylate **3.59** (107 mg, 14%) as a white solid. The two batches were collected together to give tosylate **3.59** (737 mg, 99%) as a white solid. **Ref. LNB0162-090**

(3α, 5α, 6β)-6-Hydroxy-3,5-cyclopregnan-20-one 3.61

*Isolation of cyclosteroid **3.61** by-product of the synthesis of tosyl pregnenolone **3.59***
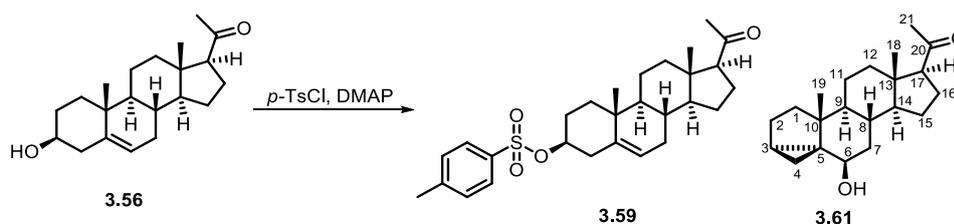
Pregnenolone **3.56** (2.05 g, 6.48 mmol) was dissolved in py (60 mL) and *p*-TsCl (6.16 g, 32.4 mmol) and DMAP (79.1 mg, 0.65 mmol) were added. The reaction mixture was

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stirred at rt overnight. H_2O (120 mL) was added and the mixture was allowed to crystallise at rt. The precipitate was collected by filtration, dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (3 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford tosylate **3.59** (1.96 g, 64%) as a white solid. The aqueous filtrate was then extracted with CH_2Cl_2 (3 x 50 mL). Combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 4/1) to afford cyclosteroid **3.61** (148 mg, 7%) as a white solid. **Ref. LNB0161-098**

***R*$_f$**: 0.28 (PE/AcOEt = 4/1); **m.p.**: 180-182 °C (hexane/AcOEt) [lit. 182 °C (hexane/AcOEt)];[83] **[α]$_D^{24}$**: +105 (*c* 0.34, $CHCl_3$) [lit. +107.4 (*c* 2.4, $CHCl_3$)];[83] **^{1}H-NMR** (500 MHz, $CDCl_3$), δ: 3.26-3.23 (1H, m, H-6), 2.51 (1H, "t", *J* = 9.0 Hz, H-17), 2.20-2.12 (1H, m, H-16), 2.09 (3H, s, 3H-21), 2.01 (1H, dd, *J* = 8.1, 2.7 Hz, H-12), 1.89-1.80 (2H, m, H-7, H-8,), 1.78-1.48 (6H, m, H-1, 2H-2, H-11, H-15, H-16), 1.44-1.36 (2H, m, H-11, H-12), 1.33-1.22 (1H, m, H-15), 1.22-1.12 (2H, m, H-7, H-14), 1.09-1.04 (1H, m, H-3), 1.03 (3H, s, 3H-19), 0.91-0.81 (2H, m, H-1, H-9), 0.65 (3H, s, 3H-18), 0.51 (1H, "t", *J* = 4.3 Hz, H-4), 0.28 (1H, dd, *J* = 8.1, 4.9 Hz, H-4); **^{13}C-NMR** (125 MHz, $CDCl_3$), δ: 209.7 (C, C-20), 73.6 (CH, C-6), 63.9 (CH, C-17), 56.8 (CH, C-14), 47.7 (CH, C-9), 44.4 (C, C-13), 43.0 (C, C-10), 39.3 (CH_2, C-12), 38.9 (C, C-5), 37.2 (CH_2, C-7), 33.3 (CH_2, C-1), 31.6 (CH_2, C-21), 30.0 (CH, C-8), 25.1 (CH_2, C-2), 24.5 (CH_2, C-15), 24.3 (CH, C-3), 23.0 (CH_2, C-16), 22.8 (CH_2, C-11), 20.3 (CH_3, C-19), 13.6 (CH_3, C-18), 11.7 (CH_2, C-4); **IR** (neat): 3517, 2928, 2913, 2859, 1693, 1015 cm^{-1}; **HRMS** (ESI): calculated for $C_{21}H_{32}NaO_2$ [MNa$^+$] requires 339.2295, found 339.2286 (2.3 ppm error). Data are consistent with those reported in the literature except for the ^{1}H-NMR assignments of H-3 and H-4.[83, 85]

*Synthesis of cyclosteroid **3.61** from tosylate **3.59**[83]*
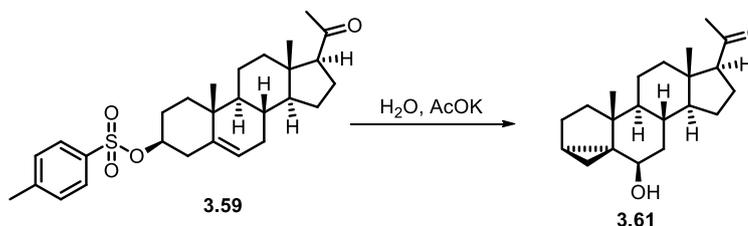
Tosylate **3.59** (107 mg, 0.23 mmol) was dissolved in a mixture of acetone/H_2O (5/1, 12 mL) and treated with AcOK (134 mg, 1.37 mmol). The mixture was stirred at reflux for

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1 d. The acetone was evaporated under reduced pressure and the residue was then diluted with H_2O (10 mL) and extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 4/1) to afford cyclosteroid **3.61** (43.0 mg, 59%) as a white solid. **Ref. LNB0162-092**

(3β)-3-Methoxypregn-5-en-20-one 3.60 and (3β)-3-methoxy-17α-pregnen-5-en-20-one 3.73[92]

*Synthesis of 3β-methoxy-pregnenolones **3.60** and **3.73** via rearrangement of tosylate **3.59**[82, 84]*
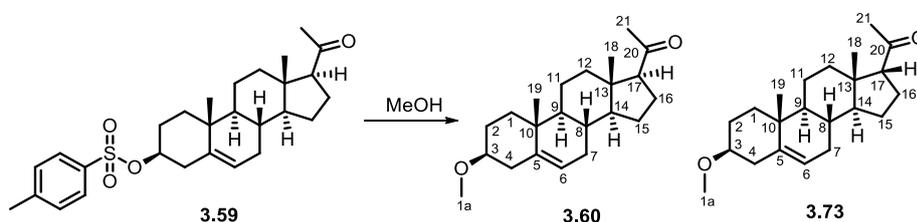
Tosylate **3.59** (2.11 g, 4.49 mmol) was suspended in MeOH (50 mL) and stirred at reflux for 4 h. After cooling down to rt, the methanol was evaporated under reduced pressure. The residue was dissolved in AcOEt (100 mL) and washed with H_2O (3 x 80 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford the diastereomeric mixture of methyl ethers **3.60/3.73** (81/19, 1.50 g, 99%) as a white solid. Recrystallisation from methanol gave methyl ether **3.60** (842 mg, 57%) as white needles. Evaporation of the filtrate gave a mixture of **3.60/3.73** (3/2, 658 mg) as a yellow solid. **Ref. LNB0161-141**

*Characterisation of **3.60**: R_f*: 0.33 (PE/AcOEt = 9/1); **m.p.**: 124 °C (MeOH) [lit. 123-124 °C (methanol/H_2O)];[82] **[α]$_D^{24}$**: +16 (*c* 1, CHCl$_3$) [lit. +18 (*c* 31.8, CHCl$_3$)];[82] **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 5.36-5.31 (1H, m, H-6), 3.34 (3H, s, 3H-1a), 3.09-3.00 (1H, m, H-3), 2.51 (1H, "t", *J* = 8.9 Hz, H-17), 2.38 (1H, ddd, *J* = 13.1, 4.5, 2.2 Hz, H-4), 2.23-2.09 (2H, m, H-4, H-16), 2.11 (3H, s, 3H-21), 2.06-1.95 (2H, m, H-7, H-12), 1.91 (1H, "dt", *J* = 12.6, 3.0 Hz, H-2), 1.86 (1H, "dt", *J* = 13.1, 3.3 Hz, H-1), 1.72-1.59 (m, 3H, H-16, H-15, H-11), 1.59-1.51 (1H, m, H-7), 1.51-1.36 (4H, m, H-2, H-8, H-11, H-12), 1.28-1.17 (1H, m, H-15), 1.17-1.09 (1H, m, H-14), 1.08-1.01 (1H, m, H-1), 1.00-0.93 (1H, m, H-9), 0.98 (3H, s, 3H-19), 0.62 (3H, s, 3H-18); **^{13}C-NMR** (125 MHz, CDCl$_3$), δ: 209.6 (C, C-20), 141.0 (C, C-5), 121.4 (CH, C-6), 80.4 (CH, C-3), 63.8 (CH,

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C-17), 57.1 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.2 (CH, C-9), 44.1 (C, C-13), 39.0 (CH$_2$, C-12), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 32.0 (CH, C-8), 31.9 (CH$_2$, C-7), 31.6 (CH$_3$, C-21), 28.1 (CH$_2$, C-2), 24.6 (CH$_2$, C-15), 22.9 (CH$_2$, C-16), 21.2 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.3 (CH$_3$, C-18); **^{1}H-NMR** (500 MHz, C$_6$D$_6$), δ: 5.36-5.32 (1H, m, H-6), 3.22 (3H, s, 3H-1a), 3.05 (1H, "tt", J = 11.1, 4.5 Hz, H-3), 2.55-2.47 (1H, m, H-4), 2.38-2.28 (2H, m, H-16, H-4), 2.12 (1H, "t", J = 8.9 Hz, H-17), 1.97-1.90 (1H, m, H-2), 1.90-1.79 (2H, m, H-7, H-12), 1.82 (3H, s, 3H-21), 1.71 (1H, "dt", J = 13.3, 3.5 Hz, H-1), 1.57-1.71 (5H, m, H-2, H-7, H-11, H-15, H-16), 1.36-1.23 (2H, m, H-8, H-11), 1.15-0.93 (3H, m, H-1, H-12, H-15,), 0.93-0.80 (2H, m, H-9, H-14), 0.89 (3H, s, 3H-19), 0.57 (3H, s, 3H-18); **^{13}C-NMR** (125 MHz, C$_6$D$_6$), δ: 206.8 (C, C-20), 141.2 (C, C-5), 121.5 (CH, C-6), 80.6 (CH, C-3), 63.8 (CH, C-17), 57.0 (CH, C-14), 55.5 (CH$_3$, C-1a), 50.5 (CH, C-9), 43.8 (C, C-13), 39.4 (CH$_2$, C-4), 39.0 (CH$_2$, C-12), 37.6 (CH$_2$, C-1), 37.2 (C, C-10), 32.2 (CH$_2$, C-7), 31.9 (CH, C-8), 31.2 (CH$_3$, C-21), 28.6 (CH$_2$, C-2), 24.7 (CH$_2$, C-15), 23.2 (CH$_2$, C-16), 21.4 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.3 (CH$_3$, C-18); **^{1}H-NMR** (400 MHz, CD$_2$Cl$_2$), δ: 5.38-5.33 (1H, m, H-6), 3.30 (3H, s, 3H-1a), 3.02 (1H, "tt", J = 11.2, 4.4 Hz, H-3), 2.52 (1H, "t", J = 9.0 Hz, H-17), 2.41-2.32 (1H, m, H-4), 2.18-1.95 (4H, m, H-4, H-7, H-12, H-16), 2.08 (3H, s, 3H-21), 1.94-1.83 (2H, m, H-1, H-2), 1.71-1.31 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16,), 1.28-1.10 (2H, m, H-14, H-15), 1.10-0.98 (2H, m, H-1, H-9), 0.99 (3H, m, 3H-19), 2.06 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CD$_2$Cl$_2$), δ: 209.3 (C, C-20), 141.4 (C, C-5), 121.5 (CH, C-6), 80.6 (CH, C-3), 63.9 (CH, C-17), 57.2 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.5 (CH, C-9), 44.2 (C, C-13), 39.2 (CH$_2$, C-12), 39.1 (CH$_2$, C-4), 37.6 (CH$_2$, C-1), 37.3 (C, C-10), 32.2 (CH, C-8), 32.2 (CH$_2$, C-7), 31.7 (CH$_3$, C-21), 28.4 (CH$_2$, C-2), 24.8 (CH$_2$, C-15), 23.1 (CH$_2$, C-16), 21.5 (CH$_2$, C-11), 19.6 (CH$_3$, C-19), 13.3 (CH$_3$, C-18); **IR** (neat): 2968, 2947, 2847, 1698, 1351, 1189, 1094, 945, 593 cm^{-1}; **HRMS** (ESI): calculated for C$_{22}$H$_{34}$NaO$_2$ [MNa$^+$] requires 353.2451, found 353.2435 (4.6 ppm error). Data are consistent with those reported in the literature.[82, 84]

*Characterisation of **3.73** within the diastereoisomeric mixture **3.60/3.73** in approximately 47/53 ratio (only signals of the 17α-isomer **3.73** are reported):* **R**$_f$: 0.31 (PE/AcOEt = 9/1); **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 5.36-5.30 (1H, m, H-6), 3.33 (3H, s, 3H-1a), 3.09-2.98 (1H, m, H-3), 2.79 (1H, dd, J = 8.3, 2.4 Hz, H-17), 2.40-2.33 (1H, m, H-4), 2.20-2.08 (1H, m, H-4), 2.10 (3H, s, 3H-21), 2.06-1.94 (12H, m, H-1, 2H-2, 2H-7, H-8, 2H-11, H-12, H-15, 2H-16), 1.28-1.08 (3H, m, H-12, H-14, H-15), 1.08-0.84 (2H, m,

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H-1, H-9), 0.97 (3H, s, 3H-19), 0.91 (3H, s, 3H-18); ^{13}**C-NMR** (125 MHz, CDCl$_3$), δ: 212.8 (C, C-20), 140.6 (C, C-5), 121.7 (CH, C-6), 80.3 (CH, C-3), 61.4 (CH, C-17), 55.7 (CH$_3$, C-1a), 50.7 (CH, C-14), 49.7 (CH, C-9), 45.6 (C, C-13), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 35.2 (CH$_2$, C-12), 32.9 (CH$_3$, C-21), 32.2 (CH$_2$, C-7), 31.2 (CH, C-8), 28.1 (CH$_2$, C-2), 26.1 (CH$_2$, C-15), 24.5 (CH$_2$, C-16), 21.1 (CH$_2$, C-11), 20.8 (CH$_3$, C-18), 19.4 (CH$_3$, C-19); **HRMS** (ESI): calculated for C$_{22}$H$_{34}$NaO$_2$ [MNa$^+$] requires 353.2451, found 353.2451 (0.5 ppm error).

*Synthesis of 3β-methoxypregnenolone **3.60** from pregnenolone **3.56***

3.56 MeI, base **3.60**
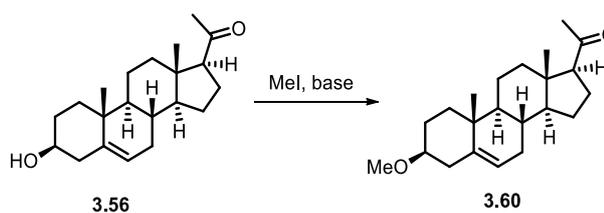
Preparation of silver(I) oxide[224]

A solution of AgNO$_3$ (850 mg, 5.00 mmol) in H$_2$O (1.5 mL) was added to a solution of NaOH (200 mg, 5.00 mmol) in H$_2$O (6 mL) under manual stirring. The brown precipitate formed was filtered, washed with H$_2$O and dried to give Ag$_2$O (540 mg, 48%) as a brown solid. **LNB0162-093**

Methylation of pregnenolone **3.56** with methyl iodide and silver(I) oxide[94]

Pregnenolone **3.56** (275 mg, 0.87 mmol) was added to a suspension of freshly prepared Ag$_2$O (303 mg, 1.31 mmol) and activated 4 Å molecular sieves in a mixture of Et$_2$O (15 mL) and THF (6 mL). MeI (1.08 mL, 17.4 mmol) was added and the reaction mixture was stirred at 40 °C for 2 d. After cooling down to rt, the reaction mixture was diluted with AcOEt (10 mL) and filtered through a pad of Celite$^®$. The filtrate was evaporated under reduced pressure. Purification of the residue by flash column chromatography (SiO$_2$, PE/AcOEt = 4/1) afforded methyl ether **3.60** (200 mg, 70%) as a white solid. **Ref. LNB0145-074**

Methylation of pregnenolone **3.56** with methyl iodide and sodium hydride[95]

Pregnenolone **3.56** (2.00 g, 6.33 mmol) was dissolved in dry THF (60 mL) and cooled to 0 °C. NaH (60% dispersion in mineral oil, 304 mg, 7.60 mmol) was added, and the mixture was stirred at 0 °C for 30 min. The ice bath was removed, MeI (1.97 mL, 31.7

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mmol) was added and the reaction mixture was stirred at rt overnight. The reaction was re-cooled to 0 °C, quenched with H_2O (200 mL) and extracted with AcOEt (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude was purified by flash column chromatography (SiO_2, PE/AcOEt = 4/1) to afford methyl ether **3.60** (1.59 g, 80%) as a white solid. **Ref. LNB0161-146**

1-[(3*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*S*)-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]propan-1-one 3.76

Isolation of ethyl-steroid 3.76 as by-product of the synthesis of 3β-methoxypregnenolone 3.60
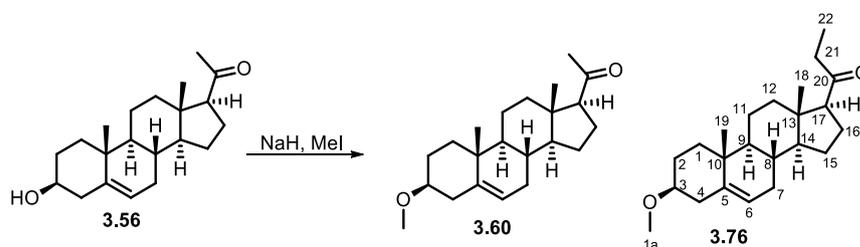
NaH (60% dispersion in mineral oil, 198 mg, 4.95 mmol) was suspended in THF (30 mL) and cooled to 0 °C. Pregnenolone **3.56** (1.04 g, 3.30 mmol) was added as a solid and the mixture was stirred at 0 °C for 20 min. MeI (1.03 mL, 16.5 mmol) was added, the ice bath was removed, and the reaction mixture stirred at rt for three d. The reaction was quenched with H_2O (30 mL) and extracted with AcOEt (3 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1 to 4/1) to afford methyl ether **3.60** (691 mg, 63%) as a single diastereoisomer and white solid. Also isolated was ethyl ketone **3.76** (54.0 mg, 5%) as a white solid. **Ref. LNB0145-073**

*Characterisation of ethyl ketone 3.76: **R**$_f$*: 0.40 (PE/AcOEt = 95/5); **m.p.**: 104-106 °C [lit. 118-119 °C (PE)];[96] **[α]$_D^{24}$**: +10 (*c* 0.34, $CHCl_3$) [lit. +17 (*c* 0.4, Et_2O)];[96] **^{1}H-NMR** (500 MHz, $CDCl_3$), δ: 5.36-5.30 (1H, m, H-6), 3.33 (3H, s, 3H-1a), 3.04 (1H, "tt", *J* = 11.2, 4.5 Hz, H-3), 2.50 (1H, "t", *J* = 8.9 Hz, H-17), 2.41-2.31 (3H, m, H-4, 2H-21), 2.21-2.09 (2H, m, H-4, H-16), 2.03-1.94 (2H, m, H-7, H-12), 1.90 (1H, "dt", *J* = 12.6, 3.0 Hz, H-2), 1.85 (1H, "dt", *J* = 13.1, 3.3 Hz, H-1), 1.72-1.35 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.27-1.17 (1H, m, H-15), 1.16-1.08 (1H, m, H-14), 1.07-0.91 (2H, m,

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H-1, H-9), 1.01 (3H, t, J = 7.2 Hz, 3H-22), 0.97 (3H, s, 3H-19), 0.59 (3H, s, 3H-18); [13]**C-NMR** (125 MHz, CDCl$_3$), δ: 212.1 (C, C-20), 141.0 (C, C-5), 121.4 (CH, C-6), 80.4 (CH, C-3), 62.8 (CH, C-17), 57.1 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.2 (CH, C-9), 44.2 (C, C-13), 39.0 (CH$_2$, C-12), 38.7 (CH$_2$, C-4), 37.4 (CH$_2$, C-21), 37.3 (CH$_2$, C-1), 37.0 (CH, C-10), 32.0 (CH, C-8), 31.9 (CH$_2$, C-7), 28.1 (CH$_2$, C-2), 24.7 (CH$_2$, C-15), 23.2 (CH$_2$, C-16), 21.2 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.5 (CH$_3$, C-18), 7.9 (CH$_3$, C-22); **IR** (neat): 2968, 2937, 2859, 1706, 1452, 1380, 1102, 1011, 800 cm^{-1}; **HRMS** (ESI): calculated for C$_{23}$H$_{37}$O$_2$ [MH$^+$] requires 345.2788, found 345.2782 (1.6 ppm error), calculated for C$_{23}$H$_{36}$NaO$_2$ [MNa$^+$] requires, 367.2608 found 367.2589 (4.5 ppm error). Data are consistent with those reported in the literature.[96]

(3α, 5α, 6β)-6-Methoxy-3,5-cyclopregnan-20-one 3.75[82, 97]
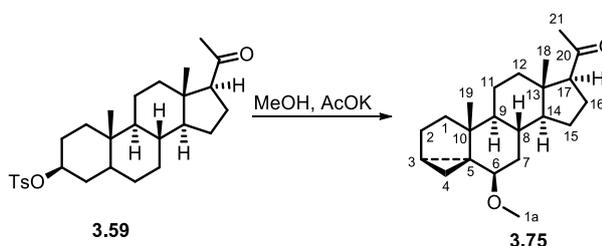
A solution of tosylate **3.59** (146 mg, 0.311 mmol) and potassium acetate (152 mg, 1.55 mmol) in methanol (35 mL) was stirred at reflux overnight. The mixture was cooled down to rt and then H$_2$O (20 mL) was added. The methanol was evaporated under reduced pressure and the aqueous phase was extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The crude material was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 9/1, 1% Et$_3$N) to afford the title compound **3.75** (81.0 mg, 79%) as a white solid. **Ref. LNB0161-034**

R_f: 0.47 (PE/AcOEt = 9/1, 1% Et$_3$N); **m.p.**: 124-125 °C (MeOH) [lit. 124-125 °C (Et$_2$O)];[82] **[α]$_D^{24}$**: +137.9 (*c* 1.12, CHCl$_3$) [lit. +132 (*c* 3.18, CHCl$_3$)];[82] **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 3.29 (3H, s, 3H-1a), 2.75 (1H, "t", J = 2.7 Hz, H-6), 2.51 (1H, "t", J = 9 Hz, H-17), 2.20-2.11 (1H, m, H-16), 2.09 (3H, s, 3H-21), 2.00 (1H, dd, J = 8.2, 2.8 Hz, H-12), 1.87 (1H, "dt", J = 13.4, 2.9 Hz, H-7), 1.79-1.57 (4H, m, H-2, H-8, H-15, H-16), 1.55-1.45 (3H, m, H-1, H-2, H-11), 1.45-1.35 (2H, m, H-11, H-12), 1.30-1.20 (1H, m, H-15), 1.20-1.12 (1H, m, H-14), 1.12-1.04 (1H, m, H-7), 1.00 (3H, s, 3H-19), 0.91-0.80 (3H, m, H-1, H-3, H-9), 0.67-0.61 (1H, m, H-4), 0.64 (3H, s, H-18), 0.42 (1H,

dd, J = 8.2, 5.1 Hz, H-4); **¹³C-NMR** (125 MHz, CDCl₃), δ: 209.6 (C, C-20), 82.3 (CH, C-6), 64.0 (CH, C-17), 56.8 (CH, C-14), 56.7 (CH₃, C-1a), 48.1 (CH, C-9), 44.5 (C, C-13), 43.5 (C, C-10), 39.4 (CH₂, C-12), 35.5 (C, C-5), 35.2 (CH₂, C-7), 33.5 (CH₂, C-1), 31.6 (CH₃, C-21), 30.6 (CH₂, C-8), 25.0 (CH₂, C-2), 24.5 (CH₂, C-15), 23.0 (CH₂, C-16), 22.86 (CH₂, C-11), 21.5 (CH, C-3), 19.4 (CH₃, C-19), 13.7 (CH₃, C-18), 13.2 (CH₂, C-4); **¹H-NMR** (500 MHz, C₆D₆), δ: 3.19 (3H, s, 3H-1a), 2.58 (1H, "t", J = 2.6 Hz, H-6), 2.41-2.31 (1H, m, H-16), 2.20-2.13 (1H, m, H-17), 1.92-1.85 (2H, m, H-7, H-8), 1.85-1.72 (2H, m, H-2, H-12,), 1.81 (3H, s, 3H-21), 1.61-1.43 (4H, m, H-1, H-2, H-7, H-15), 1.39-1.27 (2H, m, 2H-11), 1.23-1.13 (1H, m, H-15), 1.15 (3H, s, 3H-19), 1.09 (1H, "td", J = 12.1, 5.1 Hz, H-12), 0.97-0.71 (5H, m, H-1, H-3, H-7, H-9, H-14,), 0.63 (3H, s, 3H-18), 0.63-0.59 (1H, m, H-4), 0.40 (1H, dd, J = 7.8, 5.1 Hz, H-4); **¹³C-NMR** (125 MHz, C₆D₆), δ: 206.8 (C, C-20), 82.3 (CH, C-6), 63.9 (CH, C-17), 56.8 (CH, C-14), 56.6 (CH₃, C-1a), 48.4 (CH, C-9), 44.2 (C, C-13), 43.7 (C, C-10), 39.6 (CH₂, C-12), 35.7 (C, C-5), 35.5 (CH₂, C-7), 33.8 (CH₂, C-1), 31.1 (CH₃, C-21), 30.8 (CH, C-8), 25.4 (CH₂, C-2), 24.7 (CH₂, C-15), 23.3 (CH₂, C-16), 23.2 (CH₂, C-11), 21.7 (CH, C-3), 19.7 (CH₃, C-19), 13.6 (CH₃, C-18), 13.5 (CH₂, C-4); **IR** (neat): 2933, 1702, 1094, 1020 cm⁻¹; **HRMS** (ESI): calculated for $C_{22}H_{34}NaO_2$ [MNa⁺] requires, 353.2451, found 353.2439 (3.0 ppm error). Data in accordance to those reported in the literature.[82, 97]

(1R)-1-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-1-(1,3-thiazol-2-yl)ethanol 3.79 and 1-[(3b,17b)-3-methoxyandrost-5-en-17-yl]-1-(1,3-thiazol-2-yl)ethanol 3.80
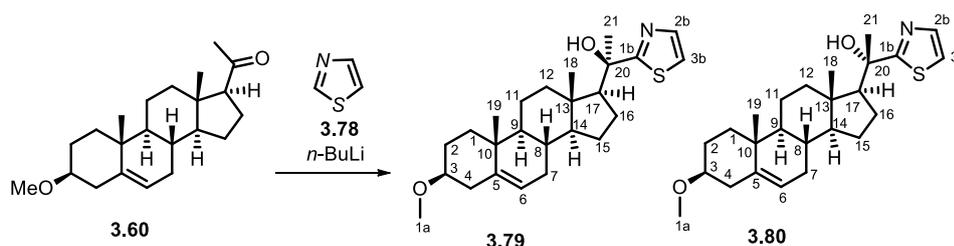
Thiazole **3.78** (54.0 µL, 0.76 mmol) was dissolved in THF (500 µL), cooled to -30 °C, and n-BuLi (2.5 M solution in hexane, 303 µL, 0.76 mmol) added dropwise. The reaction mixture was stirred for 30 min (during which time the temperature of the cooling bath reached -20 °C), before a solution of 3β-methoxy-pregnenolone **3.60** (100 mg, 0.32 mmol) in THF (1 mL) was added. The reaction mixture was stirred for 10 min at -20 °C, and then brought to rt and stirred for a further 2 h. The reaction was quenched with H₂O (20 mL) and extracted with AcOEt (3 x 20 mL). The combined organic layers were

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washed with brine (20 mL), dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 4/1) to afford the diasteromeric mixture of thiazolyl-steroids **3.79/3.80** (9/1, 105 mg, 84%) as white solids. Recrystallisation from methanol gave thiazolyl steroid **3.79** (64.0 mg, 48%) as white needles. Evaporation of the filtrate under reduced pressure gave a mixture of **3.79/3.80** (77/23, 35.0 mg) as a white solid. **Ref. LNB0162-099**

*Characterisation of 3.79: R*f: 0.14 (PE/AcOEt = 9/1); **m.p.**: 190-193 °C (MeOH); **[α]**$_D^{24}$ = -42 (*c* 0.40, CHCl$_3$); **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 7.65 (1H, d, *J* = 3.2 Hz, H-2b), 7.22 (1H, d, *J* = 3.2 Hz, H-3b), 5.35-5.31 (1H, m, H-6), 3.34 (3H, s, 3H-1a), 3.12 (1H, brs, OH), 3.09-3.01 (1H, m, H-3), 2.41-2.34 (1H, m, H-4), 2.19-2.07 (2H, m, H-4, H-12), 2.04 (1H, "t", *J* = 9.8 Hz, H-17), 1.99-1.88 (2H, m, H-4, H-7), 1.85 (1H, "dt", *J* = 13.3, 3.1 Hz, H-1), 1.82-1.73 (1H, m, H-16), 1.70 (3H, s, 3H-21), 1.60-1.22 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.18-0.97 (3H, m, H-1, H-14, H-15,), 0.99 (3H, s, 3H-19), 0.97-0.89 (1H, m, H-9), 0.86 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 180.6 (C, C-1b), 141.8 (CH, C-2b), 141.0 (C, C-5), 121.5 (CH, C-6), 118.9 (CH, C-3b), 80.4 (CH, C-3), 77.8 (C, C-20), 60.4 (CH, C-17), 56.8 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.2 (CH, C-9), 43.3 (C, C-13), 40.0 (CH$_2$, C-12), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 31.9 (CH$_2$, C-7), 31.4 (CH, C-8), 30.0 (CH$_3$, C-21), 28.1 (CH$_2$, C-2), 23.8 (CH$_2$, C-15), 22.9 (CH$_2$, C-16), 21.0 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.4 (CH$_3$, C-18); **IR** (neat): 3500, 2927, 1441, 1097 cm^{-1}; **HRMS** (ESI): calculated for C$_{25}$H$_{37}$NNaO$_2$S [MNa$^+$] requires 438.2437, found 438.2403 (8.0 ppm error); calculated for C$_{25}$H$_{38}$NO$_2$S [MH$^+$] requires 416.2618, found 416.2586 (7.3 ppm error);

*Characterisation of **3.80** within the diastereomeric mixture **3.79/3.80** = 77/23 (only relevant signals of isomer 3.80 are reported): R*f: 0.14 (PE/AcOEt = 9/1); **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 7.69 (1H, d, *J* = 3.2 Hz, H-2b), 7.25 (1H, d, *J* = 3.2 Hz, H-3b), 5.36-5.30 (1H, m, H-6), 3.32 (3H, s, 3H-1a), 3.12-2.97 (2H, m, H-3, OH), 2.38-2.32 (1H, m, H-4), 1.58 (3H, s, 3H-21), 0.91 (3H, s, 3H-19), 0.84 (3H, s, 3H-18); **^{13}C-NMR** (500 MHz, CDCl$_3$), δ: 179.4 (C, C-1b), 141.8 (CH, C-2b), 141.0 (C, C-5), 121.5 (CH, C-6), 118.8 (CH, C-3b), 80.4 (CH, C-3), 77.8 (C, C-20), 60.5 (CH, C-17), 56.7 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.1 (CH, C-9), 42.8 (C, C-13), 38.8 (CH$_2$, C-4), 37.9 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.9 (C, C-10), 31.9 (CH$_2$, C-7), 31.6 (CH$_3$, C-21), 31.4 (CH, C-8), 28.1 (CH$_2$, C-2), 22.8 (2CH$_2$, C-15, C-16), 20.9 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 13.1 (CH$_2$, C-18); **IR** (neat): 2923, 2853, 1097 cm^{-1}; **HRMS** (ESI): calculated for C$_{25}$H$_{37}$NNaO$_2$S

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[MNa$^+$] requires 438.2437, found 438.2414 (5.4 ppm error); calculated for C$_{25}$H$_{38}$NO$_2$S [MH$^+$] requires 416.2618, found 416.2592 (6.2 ppm error).

1-[(3β,17α)-3-Methoxyandrost-5-en-17-yl]-1-(1,3-thiazol-2-yl)ethanol 3.88
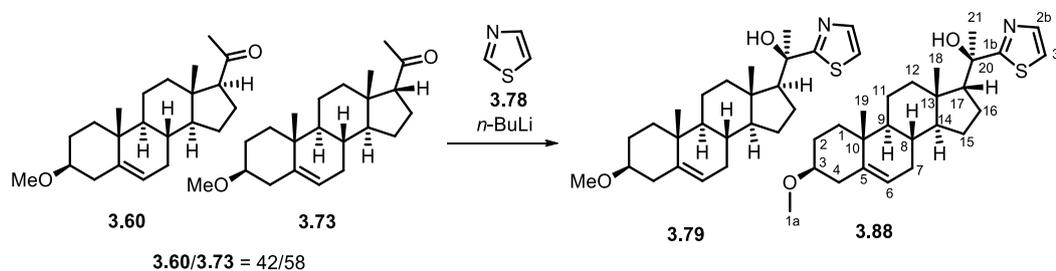
Thiazole **3.78** (38.0 μl, 0.54 mmol) was dissolved in THF (1 mL) and cooled to -40 °C, then *n*-BuLi (1.6 M in hexane, 339 μL, 0.54 mmol) was added. The mixture was stirred at -40 °C for 30 min, before a solution of diastereisomeric steroids **3.60/3.73** (42/58 ratio, 163 mg, 0.35 mmol) in THF (1 mL) was added. The reaction was allowed to reach rt and stirred for 3 h before being quenched with H$_2$O (5 mL) and extracted with AcOEt (3 x 5 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 9/1 to 7/3) to afford the mixture of thiazolyl-steroids **3.79/3.88** (37/63, 122 mg, 60%) as a white solid. **Ref. LNB0161-001**

*Characterisation of **3.88** as major isomer of the diastereomeric mixture **3.79/3.88** = 37/63 (only relevant signals of isomer **3.88** are reported):* **R**$_f$: 0.10 (PE/AcOEt = 9/1); **¹H-NMR** (500 MHz, CDCl$_3$), δ: 7.64 (1H, d, *J* = 3.2 Hz, H-2b), 7.25 (1H, d, *J* = 3.2 Hz, H-3b), 5.34-5.29 (1H, m, H-6), 3.33 (3H, s, 3H-1a), 3.07-3.00 (1H, m, H-3), 2.40-2.33 (1H, m, H-4), 2.32-2.25 (1H, brs, H-17), 1.68 (3H, s, 3H-21), 0.96 (3H, s, 3H-19), 0.86 (3H, s, 3H-18); **¹³C-NMR** (125 MHz, CDCl$_3$), δ: 180.7 (C, C-1b), 141.3 (CH, C-2b), 140.8 (C, C-5), 121.6 (CH, C-6), 119.2 (CH, C-3b), 80.4 (CH, C-3), 77.8 (C, C-20), 59.2 (CH, C-17), 55.6 (CH, C-1a), 53.3 (CH, C-14), 49.7 (CH, C-9), 44.6 (C, C-13), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 35.2 (CH$_2$, C-12), 32.4 (2C, CH$_2$, C-7, CH, C-8), 30.0 (CH$_3$, C-21), 28.1 (CH$_2$, C-2), 26.2 (CH$_2$, C-15), 25.9 (CH$_2$, C-16), 22.5 (CH$_3$, C-18), 21.4 (CH$_2$, C-11), 19.4 (CH$_3$, C-19); **IR** (liquid film): 3318, 2889, 1430, 1081 cm^{-1}; **HRMS** (ESI): calculated for C$_{25}$H$_{38}$NO$_2$S [MH$^+$] requires 416.2618, found 416.2600 (4.8 ppm error).

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(1*R*)-1-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-1-(pyridin-2-yl)ethanol 3.94 and 1-[(3b,17b)-3-methoxyandrost-5-en-17-yl]-1-(pyridin-2-yl)ethanol 3.95[108]
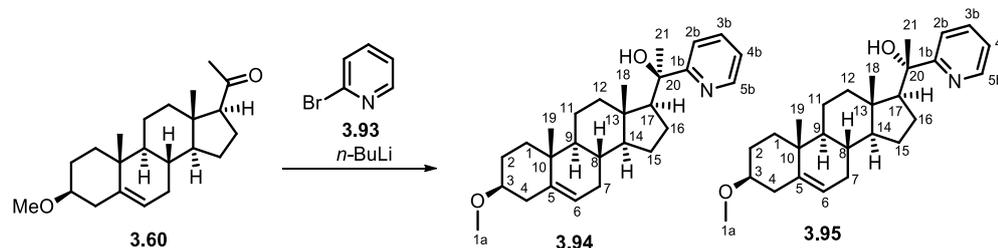
2-Bromopyridine **3.93** (64.0 µL, 0.67 mmol) was dissolved in THF (1 mL) and cooled to -78 °C, then *n*-BuLi (2.5 M solution in hexane, 303 µL, 0.76 mmol) was added. The mixture was stirred at -78 °C for 10 min, and then at 0 °C before a solution of steroid **3.60** (100 mg, 0.30 mmol) in THF (1 mL) was added. The reaction mixture was stirred at rt for 3.5 h, before being quenched with H_2O (20 mL) and extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, hexane/AcOEt = 9/1) to afford a mixture of **3.94/3.95** (9/1, 80.0 mg, 68%) as a white solid. Recrystallisation from MeOH and CH_2Cl_2 gave compound **3.94** (65.0 mg, 53%) as white needles, Evaporation of the filtrate under reduced pressure gave a mixture of **3.94/3.95** (2/3 ratio, 35.0 mg) as a white solid. **Ref. LNB0162-104**

Characterisation of isomer 3.94: **R**f: 0.24 (PE/AcOEt = 9/1); **m.p.:** 223-225 °C (CH_2Cl_2/MeOH); **[α]D^{24}:** -90 (*c* 0.49, $CHCl_3$); **^{1}H-NMR** (400 MHz, $CDCl_3$), δ: 8.46 (1H, d, *J* = 4.4 Hz, H-5b), 7.68 (1H, "td", *J* = 7.7, 1.5 Hz, H-3b), 7.33 (1H, d, *J* = 8.1 Hz, H-2b), 7.16 (1H, dd, *J* = 6.6, 5.1 Hz, H-4b), 5.53 (1H, s, OH), 5.37-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.13-3.00 (1H, m, H-3), 2.43-2.34 (1H, m, H-4), 2.23 (1H, "dt", *J* = 12.1, 3.3 Hz, H-12), 2.29-2.10 (1H, m, H-4), 2.19-1.84 (3H, m, H-1, H-2, H-7), 1.82-1.75 (1H, "t", *J* = 9.8 Hz, H-17), 1.67-1.27 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.59 (3H, s, 3H-21), 1.17-0.88 (4H, m, H-1, H-9, H-14, H-15), 1.02 (3H, s, 3H-19), 0.95 (3H, s, 3H-18), 0.88-0.76 (1H, m, H-16); **^{13}C-NMR** (100 MHz, $CDCl_3$), δ: 166.2 (C, C-1b), 146.7 (CH, C-5b), 141.1 (C, C-5), 137.1 (CH, C-3b), 121.6 (2CH, C-6, C-4b), 119.5 (CH, C-2b), 80.5 (CH, C-3), 75.1 (C, C-20), 60.5 (CH, C-17), 57.2 (CH, C-14), 55.8 (CH_3, C-1a), 50.4 (CH, C-9), 43.5 (C, C-13), 40.5 (CH_2, C-12), 38.8 (CH_2, C-4), 37.4 (CH_2, C-1), 37.1 (C, C-10), 32.0 (CH_2, C-7), 31.5 (CH, C-8), 28.9 (CH_3, C-21), 28.2 (CH_2, C-2), 23.9 (CH_2, C-15), 22.6 (CH_2, C-16), 21.2 (CH_2, C-11), 19.5 (CH_3, C-19),

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13.4 (CH$_3$, C-18); **IR** (liquid film): 3329, 2891, 2324, 1366, 1341, 1082 cm^{-1}; **HRMS** (ESI): calculated for C$_{27}$H$_{40}$NO$_2$ [MH$^+$] requires 410.3054, found 410.3054 (4.6 ppm error); **Elemental Analysis**: calculated for C$_{27}$H$_{39}$NO$_2$ requires C 79.17, H 9.60, N 3.42 found C 78.82, H 9.54, N 3.42.

*Characterisation of isomer **3.95** within the diastereomeric mixture **3.94/3.95** = 2/3 (only relevant signals of isomer **3.95** are reported):* **R**$_f$: 0.24 (PE/AcOEt = 9/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 8.48 (1H, d, *J* = 4.9 Hz, H-5b), 7.69-7.62 (1H, m, H-3b), 7.36-7.32 (1H, m, H-2b), 7.21-7.16 (1H, m, H-4b), 3.31 (3H, s, 3H-1a), 1.45 (3H, s, 3H-21), 0.87 (3H, s, 3H-19), 0.73 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 165.2 (C, C-1b), 146.6 (CH, C-5b), 140.9 (C, C-5), 136.5 (CH, C-3b), 121.9 (2CH, C-6, C-4b), 120.7 (CH, C-2b), 80.4 (CH, C-3), 74.9 (C, C-20), 60.6 (CH, C-17), 56.9 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.2 (CH, C-9), 42.6 (C, C-13), 38.8 (CH$_2$, C-4), 38.7 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.9 (C, C-10), 31.9 (CH$_2$, C-7), 31.3 (CH, C-8), 30.4 (CH$_3$, C-21), 28.1 (CH$_2$, C-2), 23.8 (CH$_2$, C-15), 23.1 (CH$_2$, C-16), 20.8 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 13.3 (CH$_3$, C-18); **IR** (neat): 3350, 2925, 2324, 1096 cm^{-1}; **HRMS** (ESI): calculated for C$_{27}$H$_{40}$NO$_2$ [MH$^+$] requires 410.3030, found 410.3026 (1.5 ppm error).

(1*R*)-1-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-1-(thiophen-2-yl)ethanol 3.100
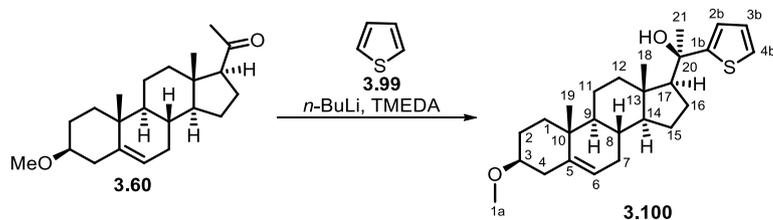
Thiophene **3.99** (19.0 µL, 0.24 mmol) and TMEDA (36.0 µL, 0.24 mmol) were dissolved in THF (0.5 mL) and cooled to 0 °C. *n*-BuLi (1.6 M solution in hexane, 150 µL, 0.24 mmol) was added and the reaction mixture was stirred for 10 min at 0 °C, it was then allowed to warm to rt and stirred for a further 50 min. The solution of 2-lithiothiophene was re-cooled again to 0 °C, and a solution of ketone **3.60** (72 mg, 0.22 mmol) in THF (1 mL) was added. The reaction mixture was stirred at rt for 3 h, before being quenched with H$_2$O (5 mL) and extracted with AcOEt (3 x 5 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 9/1) followed by

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recrystallisation in methanol to afford diastereomerically pure thiophenyl-steroid **3.100** (46.0 mg, 51%) as a white solid. **Ref. LNB0145-112**

R_f: 0.40 (PE/AcOEt = 9/1); **m.p.**: 190-192 °C (MeOH); $[\alpha]_D^{24}$: -67 (*c* 0.40, CH_2Cl_2); **¹H-NMR** (400 MHz, CD_2Cl_2), δ: 7.13 (1H, dd, *J* = 5.0, 1.1 Hz, H-4b), 6.92 (1H, dd, *J* = 5.0, 3.6 Hz, H-3b), 6.84 (1H, dd, *J* = 3.6, 1.1 Hz, H-2b), 5.37-5.30 (1H, m, H-6), 3.30 (3H, s, 3H-1a), 3.06-2.96 (1H, m, H-3), 2.39-2.21 (1H, m, H-4), 2.16-2.06 (1H, m, H-4), 2.05-1.81 (5H, m, H-1, H-2, H-7, H-12, H-17), 1.80-1.69 (1H, m, H-16), 1.68 (3H, s, H-21), 1.62-1.22 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.19-0.89 (4H, m, H-1, H-9, H-14, H-15), 1.00 (3H, s, 3H-19), 0.86 (3H, s, 3H-18); **¹³C-NMR** (100 MHz, CD_2Cl_2), δ: 156.6 (C, C-1b), 141.5 (C, C-5), 126. 9 (CH, C-3b), 123.2 (CH, C-4b), 121.8 (CH, C-2b), 121.6 (CH, C-6), 80.7 (CH, C-3), 76.6 (C, C-20), 61.9 (CH, C-17), 57.3 (CH, C-14), 55.7 (CH_3, C-1a), 50.5 (CH, C-9), 43.3 (C, C-13), 40.1 (CH_2, C-12), 39.1 (CH_2, C-4), 37.6 (CH_2, C-1), 37.2 (C, C-10), 32.2 (CH_2, C-7), 31.7 (CH, C-8), 31.4 (CH_3, C-21), 28.4 (CH_2, C-2), 23.9 (CH_2, C-15), 23.6 (CH_2, C-16), 21.3 (CH_2, C-11), 19.5 (CH_3, C-19), 13.4 (CH_3, C-18); **IR** (neat): 3454, 2932, 2902, 2861, 1096 cm⁻¹; **HRMS** (ESI): calculated for $C_{26}H_{38}NaO_2S$ [MNa⁺] requires, 437.2485, found 437.2470 (2.6 ppm error).

(3β,17β)-17-[1-(Furan-2-yl)ethenyl]-3-methoxyandrost-5-ene 3.112[116]
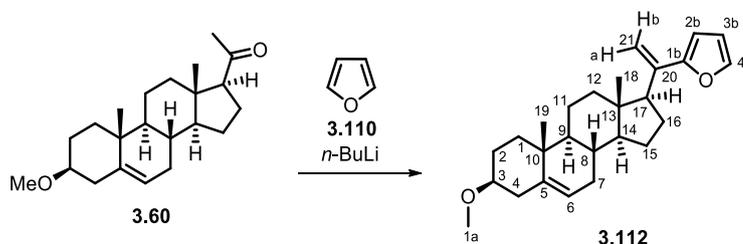
Furan **3.110** (65 μL, 0.90 mmol) was dissolved in THF (0.5 mL) and cooled to -10 °C. *n*-BuLi (1.6 M in hexane, 563 μL, 0.90 mmol) was added and the reaction mixture was stirred at -10 °C for 30 min before addition of a solution of steroid **3.60** (147 mg, 0.45 mmol) in THF (2 mL). The reaction mixture was stirred at -10 °C for 20 min, and then at rt for a further 2 h. The reaction was cooled to 0 °C, quenched with sat aq NH_4Cl (15 mL) and extracted with AcOEt (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1) to afford compound **3.112** (56.0 mg, 33%) as a white foam. **LNB0145-059**

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R_f: 0.38 (hexane/AcOEt = 9/1); $[\alpha]_D^{24}$: -51 (*c* 0.37, CHCl$_3$); **^{1}H-NMR** (400 MHz, C$_6$D$_6$), δ: 7.09 (1H, d, *J* = 1.6 Hz, H-4b), 6.25 (1H, d, *J* = 3.2 Hz, H-2b), 6.15 (1H, dd, *J* = 3.2, 1.6 Hz, H-3b), 5.92 (1H, brs, H-21b), 5.42-5.37 (1H, m, H-6), 5.07 (1H, brs, H-21a), 3.22 (3H, s, 3H-1a), 3.12-3.01 (1H, m, H-3), 2.62 (1H, "t", *J* = 9.6 Hz, H-17), 2.54 (1H, ddd, *J* = 13.1, 4.6, 2.1 Hz, H-4), 2.41-2.29 (1H, m, H-4), 2.01-1.87 (2H, m, H-2, H-7), 1.86-1.72 (2H, m, 2H-16), 1.72-1.64 (1H, m, H-1), 1.63-1.31 (6H, m, H-2, H-7, H-8, H-11, H-12, H-15), 1.31-1.18 (1H, m, H-11), 1.17-0.86 (5H, m, H-1, H-9, H-12, H-14, H-15), 0.89 (3H, s, 3H-19), 0.62 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, C$_6$D$_6$), δ = 156.9 (C, C-1b), 141.7 (CH, C-4b), 141.4 (C, C-5), 137.6 (C, C-20), 121.6 (CH, C-6), 111.2 (CH, C-3b), 110.6 (CH$_2$, C-21), 106.6 (CH, C-2b), 80.6 (CH, C-3), 57.0 (CH, C-14), 55.4 (CH$_3$, C-1a), 52.4 (CH, C-17), 50.9 (CH, C-9), 43.4 (C, C-13), 39.5 (CH$_2$, C-4), 39.0 (CH$_2$, C-12), 37.6 (CH$_2$, C-1), 37.3 (C, C-10), 32.8 (CH, C-8), 32.3 (CH$_2$, C-7), 28.6 (CH$_2$, C-2), 26.6 (CH$_2$, C-16), 24.5 (CH$_2$, C-15), 21.5 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.0 (CH$_3$, C-18); **IR** (thin film): 3337, 2892, 1246, 1081, 728 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{37}$O$_2$ [MH$^+$] requires 381.2788, found 381.2793 (-0.9 ppm error).

(1*R*)-1-(Furan-2-yl)-1-[(3β,17β)-3-methoxyandrost-5-en-17-yl]ethanol 3.111
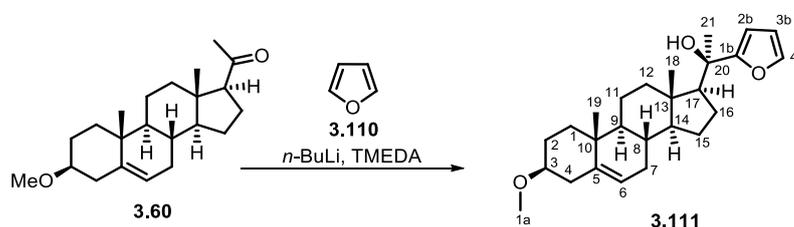
Furan **3.110** (162 µL, 2.24 mmol) and TMEDA (401 µL, 2.73 mmol) were dissolved in THF (0.2 mL) and the resulting solution was cooled to -50 °C. *n*-BuLi (1.6 M solution in THF, 1.47 mL, 2.35 mmol) was added and the reaction mixture was stirred at -50 °C for 40 min. A solution of steroid **3.60** (368 mg, 1.12 mmol) in THF (2 mL) was added, and the reaction mixture was stirred between -50 and -30 °C for 30 min, before being quenched with H$_2$O (10 mL) and extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 9/1, 1% Et$_3$N) to afford the title compound **3.111** (364 mg, 84%) as a white solid. **Ref. LNB0161-038**

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R_f: 0.30 (PE/AcOEt = 9/1, 1% Et$_3$N); **m.p.**: 152 °C (MeOH); **[α]$_D^{24}$**: -57 (*c* 0.82, CH$_2$Cl$_2$);

^{1}H-NMR (400 MHz, C$_6$D$_6$), δ: 7.08 (1H, brs, H-4b), 6.11 (1H, dd, *J* = 3.0, 1.6 Hz, H-3b), 6.06 (1H, d, *J* = 3.0 Hz, H-2b), 5.39-5.34 (1H, m, H-6), 3.22 (3H, s, 3H-1a), 3.11-2.99 (1H, m, H-3), 2.57-2.49 (1H, m, H-4), 2.41-2.30 (1H, m, H-4), 1.98-1.77 (5H, m, H-2, H-7, H-12, H-16, H-17), 1.71 (1H, "dt", *J* = 13.2, 3.3 Hz, H-1), 1.60-1.28 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-16), 1.54 (3H, s, 3H-21), 1.18-0.82 (5H, m, H-1, H-9, H-12, H-14, H-15), 0.93 (3H, s, 3H-19), 0.78 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, C$_6$D$_6$), δ: 162.1 (C, C-1b), 141.2 (C, C-5), 140.7 (CH, C-4b), 121.7 (CH, C-6), 110.4 (CH, C-3b), 104.0 (CH, C-2b), 80.6 (CH, C-3), 73.9 (C, C-20), 59.5 (CH, C-17), 57.0 (CH, C-14), 55.4 (CH$_3$, C-1a), 50.6 (CH, C-9), 42.8 (C, C-13), 39.7 (CH$_2$, C-12), 39.4 (CH$_2$, C-4), 37.6 (CH$_2$, C-1), 37.2 (C, C-10), 32.2 (CH$_2$, C-7), 31.7 (CH, C-8), 28.6 (CH$_2$, C-2), 27.6 (CH$_3$, C-21), 24.0 (CH$_2$, C-15), 23.4 (CH$_2$, C-16), 21.3 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.2 (CH$_3$, C-18); **IR** (thin film): 3378, 2889, 2856, 2806, 1347, 1138, 1081, 995 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{38}$NaO$_3$ [MNa$^+$] requires 421.2713, found 421.2699 (3.3 ppm error).

5-Hydroxy-6-[(3β,17β)-3-methoxyandrost-5-en-17-yl]-6-methyl-5,6-dihydro-2*H*-pyran-2-one 3.123[121]

3.111   NBS   **3.123**
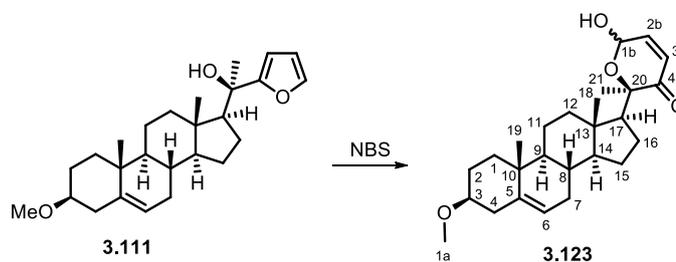
A solution of furyl steroid **3.111** (630 mg, 1.58 mmol) in a mixture of THF/H$_2$O (4/1 ratio, 12.5 mL) was cooled to 0 °C. NBS (281 mg, 1.58 mmol) was added portionwise (the yellow colour of the reaction mixture faded before addition of a second portion of NBS), while the temperature was carefully maintained at 0 °C. After the addition of the last portion of NBS the reaction was stirred for a further 10 min at 0 °C and diluted with AcOEt (20 mL). The organic phase was washed successively with aq 10% KI (20 mL), sat aq Na$_2$S$_2$O$_4$ (20 mL) and H$_2$O (20 mL), dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 4/1) to afford compound **3.123** as a mixture of diastereomers (at C-1b) (3/2, 510 mg, 78%) as a white solid. **Ref. LNB0161-152**

R_f: 0.14 (PE/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$, evident signals of the minor isomer starred), δ: 6.88-6.79 (1H, m, H-2b), 6.05 (0.67H, d, J = 10.5 Hz, H-3b), 6.00 (0.33H, d, J = 10.2* Hz, H-3b*), 5.70 (1H, brs, H-1b), 5.37-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.19 (1H, m, OH), 3.11-3.00 (1H, m, H-3), 2.42-2.33 (1H, m, H-4), 2.20-1.77 (8H, m, H-1, H-2, H-4, H-7, H-12, 2H-16, H-17), 1.64-1.35 (6H, m, H-2, H-7, H-8, 2H-11, H-15), 1.59 (3H, s, H-21), 1.34-1.18 (2H, m, H-12, H-15), 1.17-0.95 (2H, m, H-1, H-14), 0.99 (3H, s, 3H-19), 0.96-0.86 (1H, m, H-9), 0.82 (0.99H, s, 3H-18*); 0.80 (2.01H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$, evident signals of the minor isomer starred), δ: 119.4 (C, C-4b), 199.0 (C, C-4b*), 146.7 (CH, C-2b*), 143.5 (CH, C-2b), 141.0 (C, C-5), 140.9 (CH, C-5*), 127.7 (CH, C-3b*), 126.8 (CH, C-3b), 121.7 (CH, C-6*), 121.6 (CH, C-6), 87.7 (CH, C-1b*), 87.5 (CH, C-1b), 84.0 (C, C-20), 83.7 (C, C-20*), 80.5 (2CH, C-3, C-3*), 56.8 (CH, C-17), 56.6 (CH, C-14*), 56.5 (CH, C-14), 55.8 (CH, C-17*), 55.7 (2CH$_3$, C-1a, C-1a*), 50.3 (2CH, C-9, C-9*), 42.9 (C, C-13), 42.8 (C, C-13*), 40.2 (CH$_2$, C-12*), 40.1 (CH$_2$, C-12), 38.8 (2CH$_2$, C-4, C-4*), 37.3 (2CH$_2$, C-1, C-1*), 37.1 (2C, C-10, C-10*), 31.9 (2CH$_2$, C-7, C-7*), 31.5 (2CH, C-8, C-8*), 28.1 (2CH$_2$, C-2, C-2*), 26.9 (2CH$_3$, C-21, C-21*), 24.0 (2CH$_2$, C-15, C-15*), 22.6 (CH$_2$, C-16), 22.2 (CH$_2$, C-16*), 21.0 (2CH$_2$, C-11, C-11*), 19.5 (2CH$_3$, C-19, C-19*), 13.8 (CH$_3$, C-18), 13.7 (CH$_3$, C-18*); **IR** (neat): 3366, 2934, 1685, 1368, 1099, 1025, 944, 496 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{38}$NaO$_4$ [MNa$^+$] requires 437.2662, found 437.2655 (1.4 ppm error).

6-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-6-methyl-2H-pyran-2,5(6H)-dione 3.124[128]
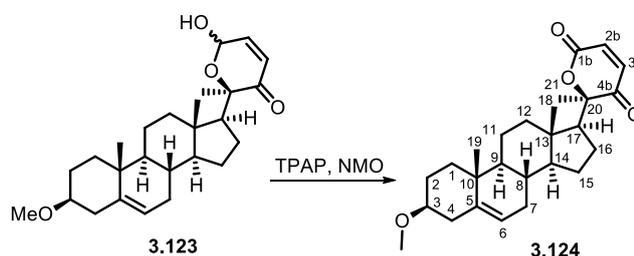
Lactol **3.123** (451 mg, 1.09 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL), NMO (446 mg, 3.82 mmol) was added and the mixture was stirred at rt for 10 min. TPAP (38.0 mg, 0.11 mmol) was added and the reaction mixture was stirred at rt for 2 h, before being diluted with CH$_2$Cl$_2$ (20 mL) and filtered through a pad of Celite$^®$. The filtrate was evaporated under reduced pressure and the residue was purified by flash column chromatography

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(SiO$_2$, PE/AcOEt = 9/1) to afford lactone **3.124** (240 mg, 53%) as a white solid. **Ref. LNB0161-160**

R$_f$: 0.28 (hexane/AcOEt = 4/1); **m.p.**: 240-250 °C (decomposition); **[α]$_D$^{24}**: -43 (*c* 0.46, CHCl$_3$); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ = 6.87 (1H, d, *J* = 10.1 Hz, H-2b), 6.67 (1H, d, *J* = 10.1 Hz, H-3b), 5.36-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 2.42-2.34 (1H, m, H-4), 2.20-2.09 (1H, m, H-4), 2.07-1.80 (5H, m, H-1, H-2, H-7, H-12, H-17), 1.70-1.29 (9H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, 2H-16), 1.64 (3H, s, 3H-21), 1.25-1.12 (1H, m, H-15), 1.09-0.96 (2H, m, H-1, H-14), 1.00 (3H, s, 3H-19), 0.96-0.89 (1H, m, H-9), 0.87 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 196.3 (C, C-4b), 160.8 (C, C-1b), 141.1 (C, C-5), 137.7 (CH, C-3b), 135.3 (CH, C-2b), 121.3 (CH, C-6), 92.4 (C, C-20), 80.4 (CH, C-3), 58.4 (CH, C-17), 56.3 (CH, C-14), 55.8 (CH$_3$, C-1a), 50.1 (CH, C-9), 43.3 (C, C-13), 39.8 (CH$_2$, C-12), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 31.8 (CH$_2$, C-7), 31.4 (CH, C-8), 28.1 (CH$_2$, C-2), 25.6 (CH$_3$, C-21), 23.8 (CH$_2$, C-15), 23.0 (CH$_2$, C-16), 21.0 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.8 (CH$_3$, C-18); **IR** (neat): 2971, 2948, 1685, 1721, 1683, 1301, 1101, 867 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{36}$NaO$_4$ [MNa$^+$] requires 435.2506, found 435.2511 (-0.8 ppm error).

2-Furanmethyl *tert*-butyldimethylsilylchloride 3.130[129]
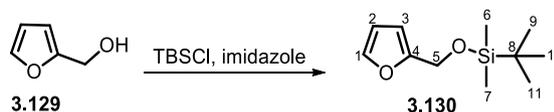
Furfuryl alcohol **3.129** (1.00 g, 10.2 mmol) was dissolved in DMF (20 mL) and imidazole (1.66 mg, 24.4 mmol) and TBSCl (3.68 mg, 20.4 mmol) were added. The resulting mixture was stirred at rt for 2 h, then diluted with Et$_2$O (120 mL) and washed with ice-cold H$_2$O (3 x 50 mL). The organic layer was dried over MgSO$_4$, filter and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 95/5) to afford ether **3.130** (2.16 g, 100%) as a yellow oil. **Ref. LNB0161-011**

R$_f$: 0.35 (PE/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.37 (1H, d, *J* = 1.4 Hz, H-1), 6.32 (1H, dd, *J* = 1.4, 2.9 Hz, H-2), 6.23 (1H, d, *J* = 2.9 Hz, H-3), 4.64 (2H, s, H-5), 0.90 (9H, s, 3H-9, 3H-10, 3H-111), 0.08 (6H, s, 3H-6, 3H-7). NMR data in accordance with those reported in the literature.[129]

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(1*R*)-1-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-1-(5-{[(2,3,3-trimethylbutan-2-yl)oxy]methyl furan-2-yl)ethanol 3.131
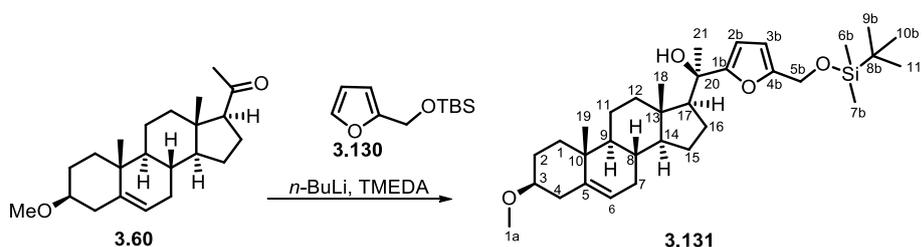
A solution of silyl ether **3.130** (720 mg, 3.39 mmol) and TMEDA (420 μL, 3.39 mmol) in THF (3 mL) was cooled to 0 °C and *n*-BuLi (1.6 M in hexane, 2.23 mL, 3.56 mmol) was added. The reaction mixture was stirred at 0 °C for 40 min and then a solution of 3β-methoxy-pregnenolone **3.60** (560 mg, 1.70 mmol) in THF (2 mL) was added. The reaction mixture was stirred for a further 5 min at 0 °C, and then at rt for 18 h. The reaction was quenched with H_2O (10 mL) and extracted with AcOEt (3 x 10 mL). Combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1) to afford furfuryl-steroid **3.131** (688 mg, 75%) as a yellow gum. **Ref. LNB0161-058**

*R*f: 0.11 (PE/AcOEt = 9/1); **[α]**$_D^{24}$: -32 (*c* 0.98, CHCl$_3$); **^{1}H-NMR** (400 MHz, C$_6$D$_6$), δ: 6.04 (2H, brs, H-2b, H-3b), 5.39-5.34 (1H, m, H-6), 4.51 (2H, s, 2H-5b), 3.21 (3H, s, 3H-1a), 3.10-3.00 (1H, m, H-3), 2.57-2.48 (1H, m, H-4), 2.40-2.30 (1H, m, H-4), 2.00-1.79 (5H, m, H-2, H-7, H-12, H-16, H-17), 1.77-1.68 (1H, m, H-1), 1.62-1.34 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-16), 1.57 (3H, s, 3H-21), 1.24-0.75 (5H, m, H-1, H-9, H-12, H-14, H-15), 0.98 (9H, s, 3H-9b, 3H-10b, 3H-11b), 0.94 (3H, s, 3H-19), 0.81 (3H, s, 3H-18), 0.09 (6H, s, 3H-6b, 3H-7b); **^{13}C-NMR** (100 MHz, C$_6$D$_6$), δ: 161.7 (C, C-1b), 153.0 (C, C-4b), 141.2 (C, C-5), 121.7 (CH, C-6), 108.2 (CH, C-3b), 104.6 (CH, C-2b), 80.6 (CH, C-3), 73.8 (C, C-20), 59.5 (CH, C-17), 58.4 (CH$_2$, C-5b), 57.1 (CH, C-14), 55.4 (CH$_3$, C-1a), 50.6 (CH, C-9), 42.8 (C, C-13), 39.7 (CH$_2$, C-12), 39.4 (CH$_2$, C-4), 37.6 (CH$_2$, C-1), 37.2 (C, C-10), 32.2 (CH$_2$, C-7), 31.7 (CH, C-8), 28.6 (CH$_2$, C-2), 27.6 (CH$_3$, C-21), 26.1 (3CH$_3$, C-9b, C-10b, C-11b), 24.0 (CH$_2$, C-15), 23.4 (CH$_2$, C-16), 21.3 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 18.5 (C, C-8b), 13.4 (CH$_3$, C-18), -5.0 (2CH$_3$, C-6b, C-7b); **IR** (neat): 3453, 2932, 1074, 835, 777, 497 cm^{-1}; **HRMS** (ESI): calculated for C$_{33}$H$_{54}$NaO$_4$Si [MNa$^+$] requires 565.3684, found 565.3674 (1.8 ppm error).

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(3β,17β)-17-{1-[5-({[*t*-Butyl(dimethyl)silyl]methyl)furan-2-yl]ethenyl-3-methoxyandrost-5-ene 3.133
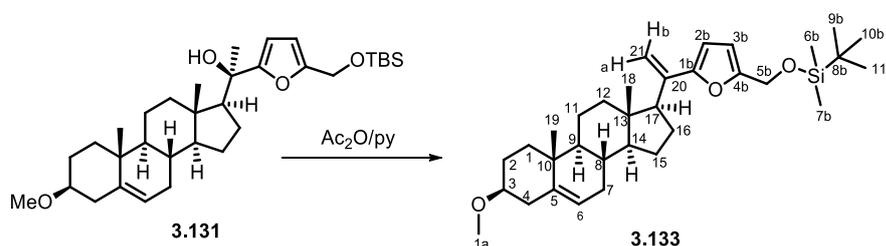
Alcohol **3.131** (50.0 mg, 0.09 mmol) was dissolved in a mixture of Ac_2O/py (1/3, 1 mL) and stirred at rt for 2 h. The solvents were evaporated under reduced pressure and the residue purified by flash column chromatography (SiO_2, PE/AcOEt = 10/0 to 9/1), which afforded alkene **3.133** (4.00 mg, 8%) as a yellow gum. **Ref. LNB0161-050**

*R*f: 0.48 (hexane/AcOEt = 9/1); **[α]**$_D^{24}$: -40 (*c* 1, $CHCl_3$); **^{1}H-NMR** (400 MHz, C_6D_6), δ: 6.22 (1H, d, *J* = 3.2 Hz, H-2b), 6.14 (1H, d, *J* = 3.2 Hz, H-3b), 5.94 (1H, brs, H-21b), 5.42-5.37 (1H, m, H-6), 5.07 (1H, brs, H-21a), 4.52 (2H, s, 2H-5b), 3.22 (3H, s, 3H-1a), 3.12-3.01 (1H, m, H-3), 2.62 (1H, "t", *J* = 9.7 Hz, H-17), 2.54 (1H, ddd, *J* = 13.1, 4.5, 2.1 Hz, H-4), 2.41-2.30 (1H, m, H-4), 2.02-1.65 (5H, H-1, H-2, H-7, 2H-16), 1.64-0.86 (12H, m, H-1, H-2, H-7, H-8, H-9, 2H-11, 2H-12, H-14, 2H-15, H-16), 0.97 (9H, s, 3H-9b, 3H-10b, 3H-11b), 0.90 (3H, s, 3H-19), 0.63 (3H, s, 3H-18), 0.09 (3H, s, 3H-6b or 3H-7b), 0.08 (3H, s, 3H-6b or 3H-7b); **^{13}C-NMR** (100 MHz, C_6D_6), δ: 156.3 (C, C-1b), 154.0 (C, C-4b), 141.4 (C, C-5), 137.6 (C, C-20), 121.6 (CH, C-6), 110.3 (CH_2, C-21), 108.9 (CH, C-3b), 107.3 (CH, C-2b), 80.6 (CH, C-3), 58.5 (CH_2, C-5b), 57.0 (CH, C-14), 55.4 (CH_3, C-1a), 52.2 (CH, C-17), 50.9 (CH, C-9), 43.4 (C, C-10), 39.5 (CH_2, C-4), 39.1 (CH_2, C-12), 37.6 (CH_2, C-1), 37.3 (C, C-10), 32.9 (CH, C-8), 32.3 (CH_2, C-7), 28.6 (CH_2, C-2), 26.6 (CH_2, C-16), 26.1 ($3CH_3$, C-9b, C-10b, C-11b), 24.5 (CH_2, C-15), 21.5 (CH_2, C-11), 19.5 (CH_3, C-19), 18.6 (C, C-8b), 13.1 (CH_3, C-13), -5.0 ($2CH_3$, C-6b, C-7b); **IR** (neat): 2935, 2953, 1463, 1252, 1092, 775, 740 cm^{-1}; **HRMS** (ESI): calculated for $C_{33}H_{53}O_3Si$ [M$^+$] requires 525.3758, found 525.3751 (2.6 ppm error), calculated for $C_{33}H_{52}NaO_3Si$ [MNa$^+$] requires 547.3578, found 547.3573 (4.9 ppm error).

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(1*R*)-1-(1-Benzofuran-2-yl)-1-[(3β,17β)-3-methoxyandrost-5-en-17-yl]ethanol 3.135
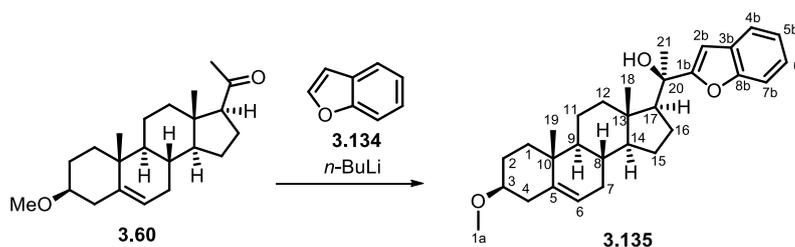
Benzofuran **3.134** (92.0 μL, 0.83 mmol) was dissolved in THF (1 mL) and cooled to -30 °C. *n*-BuLi (1.6 M in hexane, 520 μL, 0.83 mmol) was added and the reaction mixture was allowed to warm to -10 °C over 30 min. A solution of 3β-methoxy-pregnenolone **3.60** (100 mg, 0.33 mmol) in THF (1.5 mL) was added, and the mixture was allowed to reach rt and stirred for 4 h. The reaction was re-cooled to 0 °C, quenched with sat aq NH$_4$Cl (10 mL) and extracted with AcOEt (3 x 6 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 4/1) to afford benzofuranyl steroid **3.135** (83 mg, 56%) as a white solid. **Ref. LNB0145-079**

R$_f$: 0.30 (PE/AcOEt = 4/1); **m.p.**: 187-189 °C (MeOH/CH$_2$Cl$_2$); **[α]$_D^{24}$**: -37 (*c* 1, CHCl$_3$); **^{1}H-NMR** (400 MHz, C$_6$D$_6$), δ: 7.44 (1H, d, *J* = 7.1 Hz, H-4b), 7.40 (1H, d, *J* = 8.1 Hz, H-7b), 7.14-7.09 (2H, m, H-5b, H-6b), 6.41 (1H, s, H-2b), 5.39-5.31 (1H, m, H-6), 3.22 (3H, s, 3H-1a), 3.12-2.99 (1H, m, H-3), 2.58-2.48 (1H, m, H-4), 2.40-2.30 (1H, m, H-4), 2.04-1.79 (5H, m, H-2, H-7, H-12, H-16, H-17), 1.69 (1H, "dt", *J* = 13.2, 3.3 Hz, H-1), 1.62-1.27 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-16), 1.60 (3H, s, 3H-21), 1.23-1.12 (1H, m, H-12), 1.09-0.80 (4H, m, H-1, H-9, H-14, H-15), 0.92 (3H, s, 3H-9), 0.82 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, C$_6$D$_6$), δ: 165.1 (C, C-1b), 155.2 (C, C-8b), 141.2 (C, C-5), 129.1 (C, C-3b), 124 (C, C-6b), 123.1 (CH, C-5b), 121.6 (CH, C-6), 121.2 (CH, C4b), 111.4 (CH, C-7b), 100.9 (CH, C-2b), 80.6 (CH, C-3), 74.1 (C, C-20), 58.5 (CH, C-17), 57.0 (CH, C-14), 55.4 (CH$_3$, C-1a), 50.6 (CH, C-9), 42.9 (C, C-13), 39.9, (CH$_2$, C-12), 39.4 (CH$_2$, C-4), 37.6 (CH$_2$, C-1), 37.2 (C, C-10), 32.2 (CH$_2$, C-7), 31.7 (CH, C-8), 28.6 (CH$_2$, C-2), 27.8 (CH$_3$, C-21), 24.0 (CH$_2$, C-15), 23.3 (CH$_2$, C-16), 21.2 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.4 (CH$_3$, C-18); **IR** (thin film): 3371, 2873, 1431, 1078 cm^{-1}; **HRMS** (ESI): calculated for C$_{30}$H$_{40}$NaO$_3$ [MNa$^+$] requires 471.2870, found 471.2892 (-4.8 ppm error).

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(1*R*)-1-(1-Benzothiophen-2-yl)-1-[(3β,17β)-3-methoxyandrost-5-en-17-yl]ethanol 3.137
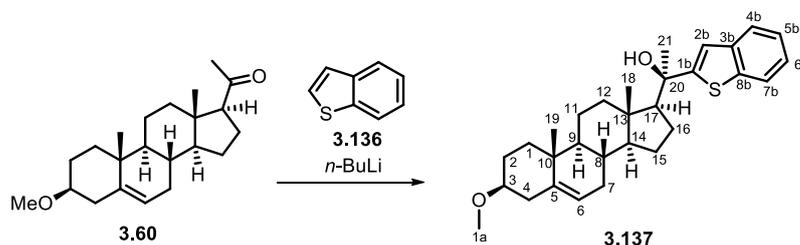
A solution of benzothiophene **3.136** (87.0 μL, 0.74 mmol) in THF (2 mL) was cooled to -30 °C, then *n*-BuLi (1.6 M in hexane, 463 μL, 0.74 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at rt for 2.5 h before adding a solution of 3β-methoxy-pregnenolone **3.60** (98.0 mg, 0.30 mmol) in THF (1 mL). The reaction was stirred at rt for 3 h, re-cooled to 0 °C, quenched with H_2O (10 mL) and extracted with AcOEt (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 95/5 to 4/1) followed by recrystallisation from methanol to afford benzothiophenyl steroid **3.137** (65.0 mg, 47%) as a white solid. **Ref. LNB0145-104**

R$_f$: 0.30 (PE/AcOEt = 4/1); **m.p.**: 194-196 °C (MeOH); **[α]$_D^{24}$**: -70 (*c* 0.61, $CHCl_3$); **^{1}H-NMR** (400 MHz, $CDCl_3$), δ: 7.78 (1H, d, *J* = 7.9 Hz, H-7b), 7.68 (1H, d, *J* = 7.7 Hz, H-4b), 7.35-7.23 (2H, m, H-5b, H-6b) 7.10 (1H, s, H-2b), 5.37-5.32 (1H, m, H-6), 3.36 (3H, s, 3H-1a), 3.12-3.02 (1H, m, H-3), 2.44-2.36 (1H, m, H-4), 2.20-2.06 (2H, m, H-4, H-12), 2.02-1.77 (5H, m, H-1, H-2, H-7, H-16, H-17), 1.76 (3H, s, 3H-21), 1.63-1.29 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.21-0.89 (4H, m, H-1, H-9, H-14, H-15), 1.01 (3H, s, 3H-19), 0.92 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, $CDCl_3$), δ: 156.7 (C, C-1b), 141.0 (C, C-3b), 130.1 (C, C-5), 139.1 (C, C-8b), 124.2 (CH, C-5b), 123.7 (CH, C-6b), 123.3 (CH, C-4b), 122.3 (CH, C-7b), 121.6 (CH, C-6), 117.9 (CH, C-2b), 80.3 (CH, C-3), 76.6 (C, C-20), 61.1 (CH, C-17), 57.0 (CH, C-14), 55.8 (CH_3, C-1a), 50.2 (CH, C-9), 43.2 (C, C-13), 40.0 (CH_2, C-12), 38.8 (CH_2, C-4), 37.3 (CH_2, C-1), 37.0 (C, C-10), 31.9 (CH_2, C-7), 31.4 (CH, C-8), 31.2 (CH_3, C-21), 28.1 (CH_2, C-2), 23.7 (CH_2, C-15), 23.3 (CH_2, C-16), 21.0 (CH_2, C-11), 19.5 (CH_3, C-19), 13.5 (CH_3, C-18); **IR** (thin film): 3361, 2887, 1411, 1343, 1074 cm^{-1}; **HRMS** (ESI): calculated for $C_{30}H_{40}NaO_2S$ [MNa$^+$] requires 487.2641, found 487.2657 (-3.2 ppm error).

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(1*R*)-1-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-1-(1-methyl-1*H*-indol-2-yl)ethanol 3.139
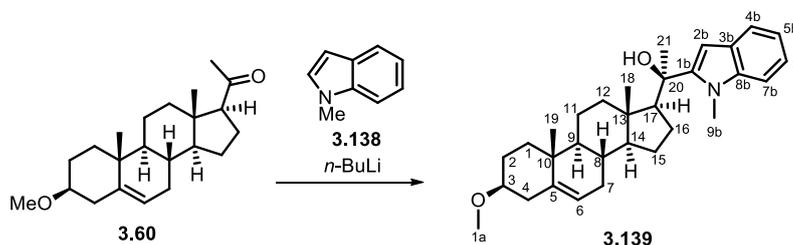
N-Methyl indole **3.138** (37.0 μL, 0.30 mmol) was dissolved in THF (1 mL) and *n*-BuLi (1.6 M in hexane, 0.27 mL, 0.30 mmol) was added. The mixture was heated at reflux for 4 h, cooled to rt and a solution of 3β-methoxy-pregnenolone **3.60** (90.0 mg, 0.27 mmol) in THF (1 mL) added. The reaction mixture was stirred at rt overnight, quenched with H_2O (3 mL) and extracted with AcOEt (3 x 4 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1 to 4/1) followed by recrystallisation from methanol to afford benzothiophenyl steroid **3.139** (16.0 mg, 12%) as a white solid. **Ref. LNB0145-125**

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*R*f: 0.20 (PE/AcOEt = 4/1); **m.p.** = 182-184 °C (MeOH); $[\alpha]_D^{24}$: -50 (*c* 0.21, CHCl₃);
¹H-NMR (400 MHz, C₆D₆), δ: 7.30 (1H, d, *J* = 7.3 Hz, H-4b), 7.33-7.22 (2H, m, H-5b, H-6b), 7.19 (1H, d, *J* = 8.1 Hz, H-7b), 6.33 (1H, s, H-2b), 5.39-5.32 (1H, m, H-6), 3.68 (3H, s, 3H-9b), 3.19 (3H, s, 3H-1a), 3.08-2.95 (1H, m, H-3), 2.55-2.46 (1H, m, H-4), 2.38-2.27 (1H, m, H-4), 2.18-2.10 (1H, m, H-17), 1.96-1.73 (4H, m, H-2, H-7, 2H-16), 1.62-1.27 (5H, m, H-1, H-2, H-7, H-8, H-15), 1.56 (3H, s, 3H-21), 1.10-0.61 (8H, m, H-1, H-9, 2H-11, 2H-12, H-14, H-15), 0.83 (3H, s, 3H-19), 0.71 (3H, s, 3H-18);
¹³C-NMR (100 MHz, C₆D₆), δ: 145.4 (C, C-1b), 141.4 (C, C-5), 139.0 (C, C-8b), 127.6 (C, C-3b), 121.9 (CH, C-6b), 121.5 (CH, C-6), 120.9 (CH, C-4b), 120.1 (CH, C-5b), 109.5 (C, C-7b), 100.2 (C, C-2b), 80.6 (CH, C-3), 74.1 (C, C-20), 58.6 (CH, C-17), 57.3 (CH, C-14), 55.4 (CH₃, C-1a), 50.7 (CH, C-9), 42.5 (C, C-13), 39.4 (CH₂, C-4), 37.7 (CH₂, C-12), 37.5 (CH₂, C-1), 37.1 (C, C-10), 32.3 (CH₃, C-9b), 32.2 (CH₂, C-7), 31.8 (CH, C-8), 28.5 (CH₂, C-2), 27.0 (CH₃, C-21), 23.9 (CH₂, C-15), 23.4 (CH₂, C-16), 20.9 (CH₂, C-11), 19.4 (CH₃, C-19), 13.3 (CH₃, C-18); **IR** (thin film): 3376, 2889, 1411, 1354, 1080, 742 cm⁻¹; **HRMS** (ESI): calculated for C₃₁H₄₃NNaO₂ [MNa⁺] requires 484.3186, found 484.3182 (0.6 ppm error).

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(1*R*)-1-[(3α,5α,6β,17β)-6-Methoxy-3,5-cycloandrostan-17-yl]-1-(1,3-thiazol-2-yl)ethanol 3.140
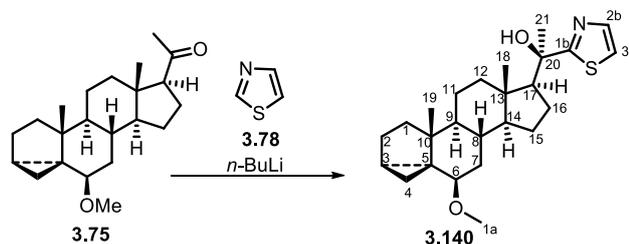
Thiazole **3.78** (54.0 μL, 0.76 mml) was dissolved in THF (1 mL), cooled to -30 °C, and *n*-BuLi (2.5 M in hexane, 303 μL, 0.76 mmol) was added. The reaction mixture was stirred at -30 °C for 30 min and then a solution of *i*-steroid **3.75** (100 mg, 0.30 mmol) in THF (1 mL) was added. The cold bath was removed and the reaction mixture was stirred at rt for 3.5 h, quenched with H_2O (10 mL), and extracted with AcOEt (3 x 10 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, hexane/AcOEt = 9/1 to 4/1) to afford the title compound **3.140** (73.0 mg, 58%) as a white solid. **Ref. LNB0162-105**

*R*f: 0.15 (hexane/AcOEt = 9/1); **m.p.**: 55-57 °C; **[α]$_D^{24}$**: +15 (*c* 1, CHCl$_3$); **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 7.65 (1H, d, *J* = 3.3 Hz, H-2b), 7.21 (1H, d, *J* = 3.3 Hz, H-3b), 3.30 (3H, s, 3H-1a), 3.12 (1H, brs, OH), 2.75 (1H, "t", *J* = 2.5 Hz, H-6), 2.10-2.02 (2H, m, H-12, H-17), 1.90-1.84 (1H, m, H-7), 1.80-1.67 (3H, m, H-2, H-8,H-16), 1.69 (3H, s, 3H-21), 1.62-0.97 (10H, m, H-1, H-2, H-7, 2H-11, H-12, H-14, 2H-15, H-16), 1.01 (3H, s, 3H-19), 0.92-0.75 (3H, m, H-1, H-3, H-9), 0.89 (3H, s, 3H-18), 0.63 (1H, "t", *J* = 4.4 Hz, H-4), 0.41 (1H, dd, *J* = 8.0, 5.0 Hz, H-4); **^{13}C-NMR** (125 MHz, CDCl$_3$), δ: 180.9 (C, C-1b), 141.8 (CH, C-2b), 118.8 (CH, C-3b), 82.4 (CH, C-6), 77.8 (C, C-20), 60.5 (CH, C-17), 56.7 (CH, C-14), 56.5 (CH$_3$, C-1a), 48.7 (CH, C-9), 43.7 (C, C-13), 43.5 (C, C-10), 40.4 (CH$_2$, C-12), 35.3 (C, C-5), 35.0 (CH$_2$, C-7), 33.4 (CH$_2$, C-1), 31.0 (CH, C-8), 29.9 (CH$_3$, C-21), 25.1 (CH$_2$, C-2), 23.7 (CH$_2$, C-15), 22.9 (CH$_2$, C-16), 22.7 (CH$_2$, C-11), 21.6 (CH, C-3), 19.4 (CH$_3$, C-19), 13.8 (CH$_3$, C-18), 13.2 (CH$_2$, C-4); **IR** (neat): 3339, 2928, 2865, 1099, 735 cm^{-1}; **HRMS** (ESI): calculated for $C_{25}H_{37}NNaO_2S$ [MNa$^+$] requires 438.2437, found 438.2432 (0.7 ppm error).

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(1*R*)-1-(Furan-2-yl)-1-[(3α,5α,6β,17β)-6-methoxy-3,5-cycloandrostan-17-yl]ethanol 3.141
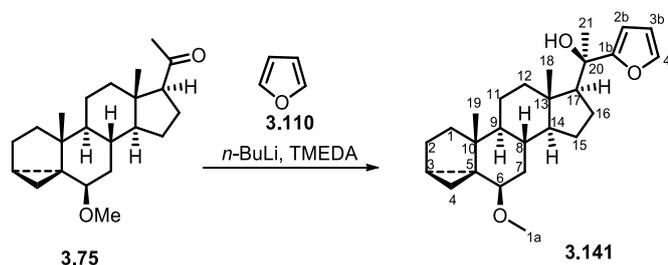
A solution of furan **3.110** (44.0 μL, 0.61 mmol) in THF (1 mL) was cooled to -50 °C and TMEDA (113 μL, 0.76 mmol) and *n*-BuLi (2.5 M in hexane, 254 μL, 0.64 mmol) were added. The reaction mixture was stirred for 40 min and then a solution of *i*-steroid **3.75** (100 mg, 0.30 mmol) in THF (1 mL) was added. The reaction mixture was stirred for 3.5 h during which time the temperature reached rt. The reaction was quenched with H_2O (10 mL), and extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1 to 4/1) to afford the title compound **3.141** (54.0 mg, 45%) as a yellow gum. **Ref. LNB0162-106**

*R*f: 0.24 (hexane/AcOEt = 4/1, 1% Et$_3$N); **[α]$_D^{24}$**: + 35 (*c* 0.35, CHCl$_3$); **^{1}H-NMR** (400 MHz, C$_6$D$_6$), δ: 7.08 (1H, d, *J* = 0.7 Hz, H-4b), 6.10 (1H, dd, *J* = 3.1, 0.7 Hz, H-3b), 6.06 (1H, d, *J* = 3.1 Hz, H-2b), 3.19 (3H, s, 3H-1a), 2.61-2.58 (1H, m, H-6), 2.01-1.70 (6H, m, H-2, H-7, H-8, H-12, H-16, H-17), 1.64-1.27 (6H, m, H-1, H-2, 2H-11, H-15, H-16), 1.53 (3H, s, 3H-21), 1.25-1.03 (2H, m, H-12, H-15), 1.18 (3H, s, 3H-19), 1.01-0.70 (5H, m, H-1, H-3, H-7, H-9, H-14), 0.83 (3H, s, 3H-18), 0.61 (1H, "t", *J* = 4.4 Hz, H-4), 0.39 (1H, dd, *J* = 7.9, 5.1 Hz, H-4); **^{13}C-NMR** (100 MHz, C$_6$D$_6$), δ: 162.0 (C, C-1b), 140.7 (CH, C-4b), 110.4 (CH, C-3b), 104.0 (CH, C-2b), 82.4 (CH, C-6), 73.9 (C, C-20), 59.6 (CH, C-17), 56.7 (CH, C-14), 56.6 (CH$_3$, C-1a), 48.4 (CH, C-9), 43.7 (C, C-13), 43.2 (C, C-10), 40.1 (CH$_2$, C-12), 35.7 (C, C-5), 35.4 (CH$_2$, C-7), 33.7 (CH$_2$, C-1), 30.3 (CH$_3$, C-21), 27.4 (CH, C-8), 25.4 (CH$_2$, C-2), 24.0 (CH$_2$, C-15), 23.4 (CH$_2$, C-16), 23.0 (CH$_2$, C-11), 21.5 (CH, C-3), 19.7 (CH$_3$, C-19), 13.6 (CH$_2$, C-4), 13.5 (CH$_3$, C-18); **IR** (neat): 3465, 2935, 2971, 1096 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{38}$NaO$_3$ [MNa$^+$] requires 421.2713, found 421.2719 (-1.2 ppm error).

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(3β)-3-Methoxychola-5,23-dien-20-ol 3.164[138]
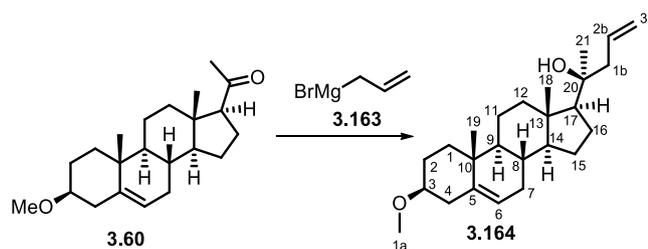
Methyl ether **3.60** (83.0 mg, 0.25 mmol) was dissolved in THF (1 mL), cooled to 0 °C and allylmagnesium bromide **3.163** (1 M solution in Et$_2$O, 503 μL, 0.50 mmol) added. The reaction mixture was stirred at 0 °C for 5 min, then at rt for 4 h. The reaction was quenched with H$_2$O (10 mL) and the resulting mixture was diluted with AcOEt (20 mL) and filtered through a pad of Celite®. The organic layer was isolated and the aqueous phase was extracted with AcOEt (3 x 5 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 9/1) to afford the title compound **3.161** (67.0 mg, 72%) as a white solid. **Ref. LNB0161-080**

R_f: 0.46 (hexane/AcOEt = 4/1); **m.p.**: 103-105 °C (methanol); **[α]$_D^{24}$**: -62 (*c* 0.27, CHCl$_3$); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 5.89-5.73 (1H, m, H-2b), 5.35 (1H, m, H-6), 5.14-5.02 (2H, m, 2H-3b), 3.34 (3H, s, 3H-1a), 3.11-2.99 (1H, m, H-3), 2.42-2.33 (1H, m, H-4), 2.23-2.04 (4H, m, H-4, H-12, 2H-1b), 2.02-1.34 (12H, m, H-1, 2H-2, 2H-7, H-8, 2H-11, H-15, 2H-16, H-17), 1.32-1.09 (2H, m, H-12, H-15), 1.28 (3H, s, H-21), 1.08-0.82 (3H, m, H-1, H-9, H-14), 0.99 (3H, s, H-19), 0.86 (3H, s, H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 141.0 (C, C-5), 134.5 (CH, C-2b), 121.6 (CH, C-6), 118.3 (CH$_2$, C-3b), 80.4 (CH, C-3), 74.7 (C, C-20), 58.0 (CH, C-17), 56.9 (CH, C-14), 55.7 (CH$_3$, C-1a), 50.2 (CH, C-9), 48.3 (CH$_2$, C-1b), 42.8 (C, C-13), 40.2 (CH$_2$, C-12), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 31.9 (CH$_2$, C-7), 31.4 (CH, C-8), 28.1 (CH$_2$, C-2), 26.7 (CH$_3$, C-21), 23.9 (CH$_2$, C-15), 22.4 (CH$_2$, C-16), 21.0 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.7 (CH$_3$, C-18); **IR** (neat): 3486, 2973, 2931, 2901, 2871, 2841, 1458, 1434, 1374, 1102, 909 cm^{-1}; **HRMS** (ESI): calculated for C$_{25}$H$_{40}$NaO$_2$ [MNa$^+$] requires 395.2921, found 395.2930 (-2.5 ppm error).

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(3β)-3-Methoxychola-5,23-dien-20-yl prop-2-enoate 3.166[138]
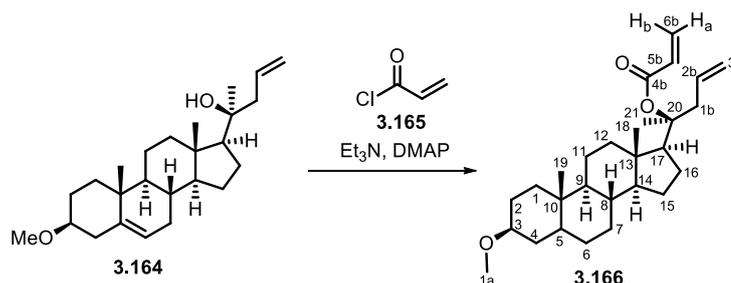
Alcohol **3.164** (474 mg, 1.27 mmol) was dissolved in THF (6 mL) and Et₃N (442 μL, 3.18 mmol), acryloyl chloride **3.165** (205 μL, 2.55 mmol) and DMAP (1.55 mg, 0.013 mmol) were added. The resulting suspension was stirred at 40 °C for 48 h, cooled to rt and quenched with H₂O (30 mL). The organic compounds were extracted with AcOEt (3 x 40 mL). The combined organic layers were dried over MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO₂, PE/AcOEt = 95/5 to 9/1) to afford ester **3.166** (176 mg, 33%) as a colourless gum. **Ref. LNB0161-155**

*R*f: 0.63 (hexane/AcOEt = 4/1); **[α]²⁴D**: -61° (*c* 0.26, CHCl₃); **¹H-NMR** (400 MHz, CDCl₃), δ: 6.28 (1H, dd, *J* = 17.3, 1.4 Hz, H-6bb), 6.02 (1H, dd, *J* = 17.3, 10.4 Hz, H-5b), 5.80-5.67 (1H, m, H-2b), 5.72 (1H, dd, *J* = 10.4, 1.4 Hz, H-6ba), 5.35 (1H, m, H-6), 5.14-5.00 (2H, m, 2H-3b), 3.34 (3H, s, 3H-1a), 3.14 (1H, dd, *J* = 13.5, 6.4 Hz, H-1b), 3.10-3.00 (1H, m, H-3), 2.42-2.28 (2H, m, H-4, H-1b), 2.20-2.09 (1H, m, H-4), 2.07-1.80 (5H, m, H-1, H-2, H-7, H-12, H-16), 1.79-1.66 (1H, m, H-17), 1.64 (3H, s, H-21), 1.57-1.09 (9H, m, H-2, H-7, H-8, 2H-11, H-12, 2H-15, H-16), 1.09-0.80 (3H, m, H-1, H-9, H-14), 0.99 (3H, s, 3H-19), 0.84 (3H, s, 3H-18); **¹³C-NMR** (100 MHz, CDCl₃), δ: 165.5 (C, C-4b), 141.0 (C, C-5), 134.0 (CH, C-2b), 130.7 (CH, C-5b), 129.4 (CH₂, C-6b), 121.6 (CH, C-6), 118.0 (CH₂, C-3b), 87.2 (C, C-20), 80.4 (CH, C-3), 57.0 (CH, C-17), 56.7 (CH, C-14), 55.7 (CH₃, C-1a), 50.1 (CH, C-9), 43.1 (CH₂, C-1b), 42.7 (C, C-13), 40.0 (CH₂ C-12), 38.8 (CH₂, C-4), 37.3 (CH₂, C-1), 37.0 (C, C-10), 31.9 (CH₂, C-7), 31.5 (CH, C-8), 28.1 (CH₂, C-2), 23.9 (CH₂, C-15), 23.7 (CH₃, C-21), 22.5 (CH₂, C-16), 21.0 (CH₂, C-11), 19.5 (CH₃, C-19), 14.1 (CH₃, C-18); **IR** (neat): 2937, 2901, 2853, 1720, 1398, 1205, 1102, 806 cm⁻¹; **HRMS** (ESI): calculated for C₂₈H₄₂NaO₃ [MNa⁺] requires 449.3026, found 449.3036 (-2.1 ppm error).

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6-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-6-methyl-5,6-dihydro-2*H*-pyran-2-one 3.168
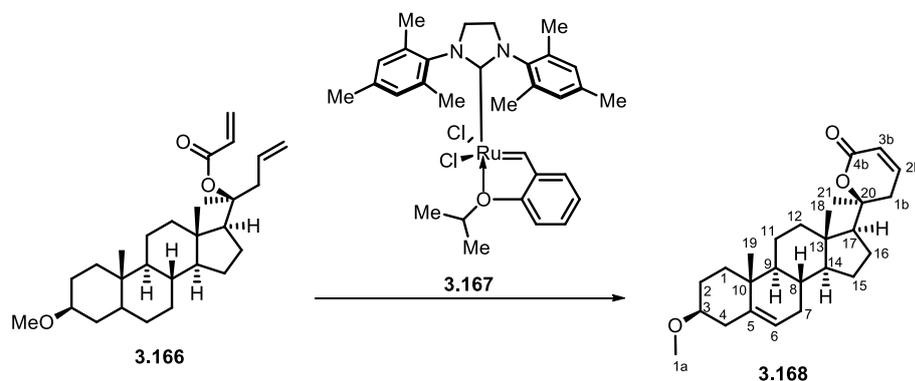
Diene **3.166** (10.0 mg, 0.02 mmol) was dissolved in CH$_2$Cl$_2$ (2 mL) and the 2nd generation Hoveyda-Grubbs catalyst **3.167** (1.50 mg, 0.002 mmol) was added. The reaction mixture was stirred at rt overnight, diluted with CH$_2$Cl$_2$ (20 mL) and filtered through a pad of Celite$^®$. The filtrate was evaporated under reduced pressure, and the residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 4/1) to afford **3.168** (9.00 mg, 98%) as a white solid. **Ref. LNB0161-078**

***R*f**: 0.16 (hexane/AcOEt = 8/2); **m.p.**: 177-179 °C; **[α]$_D^{24}$**: -54 (*c* 0.44, CHCl$_3$); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 6.71 (1H, ddd, *J* = 9.7, 5.9, 2.4 Hz, H-2b), 6.00 (1H, dd, *J* = 9.7, 1.8 Hz, H-3b), 5.38-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.10-2.99 (1H, m, H-3), 2.75-2.65 (1H, m, H-1b), 2.43-2.33 (1H, m, H-4), 2.22-2.06 (3H, m, H-4, H-12, H-1b), 2.05-1.72 (5H, m, H-1, H-2, H-7, 2H-16), 1.71-1.33 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-17), 1.51 (3H, s, 3H-21), 1.30-1.11 (2H, m, H-12, H-15), 1.10-0.82 (3H, m, H-1, H-9, H-14), 1.00 (3H, s, 3H-19), 0.91 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 163.9 (C, C-4b), 143.7 (CH, C-2b), 141.1 (C, C-5), 121.5 (CH, C-6), 120.9 (CH, C-3b), 84.9 (C, C-20), 80.4 (CH, C-3), 59.1 (CH, C-17), 57.1 (CH, C-14), 55.8 (CH$_3$, C-1a), 50.2 (CH, C-9), 43.0 (C, C-13), 40.2 (CH$_2$, C-12), 38.8 (CH$_2$, C-4), 37.3 (CH$_2$, C-1), 37.0 (C, C-10), 34.5 (CH$_2$, C-1b), 31.9 (CH$_2$, C-7), 31.4 (CH, C-8), 28.1 (CH$_2$, C-2), 24.8 (CH$_3$, C-21), 23.8 (CH$_2$, C-15), 23.5 (CH$_2$, C-16), 21.0 (CH$_2$, C-11), 19.5 (CH$_3$, C-19), 13.7 (CH$_3$, C-18); **IR** (neat): 2974, 2929, 2828, 1721, 1381, 1136, 1102 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{38}$NaO$_3$ [MNa$^+$] requires 421.2713, found 421.2709 (1.8 ppm error).

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(3β)-3-Methoxy-23-methylidenechol-5-en-20-ol 3.170
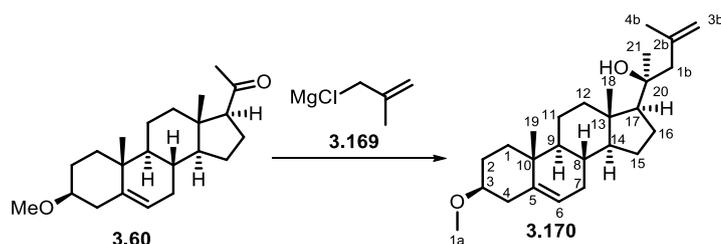
Methyl ether **3.60** (700 mg, 2.12 mmol) was dissolved in THF (20 mL) and cooled to -78 °C. 2-Methylallylmagnesium chloride **3.169** (0.5 M solution in THF, 6.36 mL, 3.18 mmol) was added and the reaction mixture was stirred for 2 h during which time the temperature reached -30 °C. Due to the presence of residual starting material, the reaction was re-cooled to -78 °C and a further portion of 2-methylallylmagnesium bromide (0.5 M solution in THF, 2.12 mL, 1.06 mmol) was added. The reaction mixture was stirred for a further 2 h during which time the temperature reached -30 °C, quenched with H_2O (20 mL) and extracted with AcOEt (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 95/5) to afford **3.170** (610 mg, 75%) as a white solid. **Ref. LNB0161-143**

R_f: 0.38 (hexane/AcOEt = 4/1); **m.p.**: 124 °C (MeOH); **[α]$_D^{24}$**: -50 (*c* 0.1, CHCl$_3$); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 5.33 (1H, m, H-6), 4.88 (1H, brs, H-3b), 4.69 (1H, brs, H-3b), 3.33 (3H, s, H-1a), 3.08-2.98 (1H, m, H-3), 2.40-2.32 (1H, m, H-4), 2.22 (1H, d, *J* = 13.5 Hz, H-1b), 2.18-2.06 (2H, m, H-4, H-12), 2.05 (1H, d, *J* = 13.5 Hz, H-1b), 2.00-1.33 (12H, m, H-1, 2H-2, 2H-7, H-8, 2H-11, H-15, 2H-16, H-17), 1.80 (3H, s, H-4b), 1.31-1.09 (2H, m, H-12, H-15), 1.26 (3H, s, H-21), 1.05-0.78 (3H, m, H-1, H-9, H-14), 0.98 (3H, s, H-19), 0.84 (3H, s, H-18) **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 142.9 (C, C-2b), 140.9 (C, C-5), 121.6 (CH, C-6), 114.9 (CH$_2$, C-3b), 80.4 (CH, C-3), 74.9 (C, C-20), 59.4 (CH, C-17), 57.0 (CH, C-14), 55.5 (CH$_3$, C-1a), 50.9 (CH$_2$, C-1b), 50.2 (CH, C-9), 42.9 (C, C-13), 40.3 (CH$_2$, C-12), 38.7 (CH$_2$, C-4), 37.2 (CH$_2$, C-1), 36.9 (C, C-10), 31.9 (CH$_2$, C-7), 31.4 (CH, C-8), 28.1 (CH$_2$, C-2), 26.8 (CH$_3$, C-21), 25.3 (CH$_3$, C-4b), 23.9 (CH$_2$, C-15), 22.7 (CH$_2$, C-16), 21.0 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 13.5 (CH$_3$, C-18); **IR** (neat): 3492, 2931, 2895, 2865, 1368, 1090, 902, 885, 734 cm^{-1}; **HRMS** (ESI): calculated for C$_{26}$H$_{42}$NaO$_2$ [MNa$^+$] requires 409.3077, found 409.3081 (-0.9 ppm error); **Elemental Analysis**: calculated for C$_{26}$H$_{42}$O$_2$ requires C 80.77, H 10.95, found C 80.86, H 11.05.

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(3β)-3-Methoxy-23-methylidenechol-5-en-20-yl 2-methylprop-2-enoate 3.172
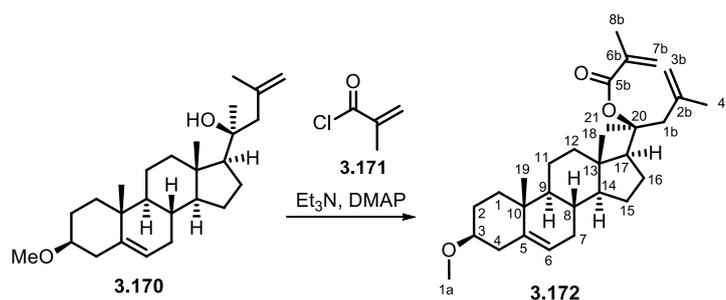
Alcohol **3.170** (90.0 mg, 0.23 mmol) was dissolved in CH_2Cl_2 (2 mL) and Et_3N (130 μL, 0.93 mmol), 2-methylacryloyl chloride **3.171** (68.0 μL, 0.70 mmol) and DMAP (0.28 mg, 0.002 mmol) were added. The reaction mixture was stirred at 40 °C for 18 h, cooled to rt and quenched with H_2O (10 mL). The organic layer was isolated, diluted with CH_2Cl_2 (5 mL) and then washed with sat aq $NaHCO_3$ (3 x 5 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 95/5) to afford the title compound **3.172** (13.0 mg, 12%) as a colourless gum. **Ref. LNB0161-109**

R_f: 0.65 (hexane/AcOEt = 4/1); **[α]$_D^{24}$**: -54 (*c* 0.13, $CHCl_3$); **^{1}H-NMR** (400 MHz, $CDCl_3$), δ: 6.00 (1H, brs, H-7b), 5.48 (1H, brs, H-7b), 5.38-5.32 (1H, m, H-6), 4.85 (1H, brs, H-3b), 4.78 (1H, brs, H-3b), 3.35 (3H, s, 3H-1a), 3.11-3.00 (2H, m, H-3, H-1b), 2.42-2.33 (2H, m, H-4, H-1b), 2.20-2.10 (1H, m, H-4), 2.09-1.58 (8H, m, H-1, H-2, H-7, H-12, H-15, 2H-16, H-17), 1.90 (3H, s, H-8b), 1.79 (3H, s, H-4b), 1.67 (3H, s, H-21), 1.57-1.38 (5H, m, H-2, H-7, H-8, 2H-11), 1.28-1.11 (2H, m, H-12, H-15), 1.08-0.96 (2H, m, H-1, H-14), 0.99 (3H, s, 3H-19), 0.96-0.81 (1H, m, H-9), 0.85 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, $CDCl_3$), δ: 166.8 (C, C-5b), 142.2 (C, C-2b), 141.03 (C, C-5), 138.4 (C, C-6b), 124.6 (CH_2, C-7b), 121.6 (CH, C-6), 115.5 (CH_2, C-3b), 88.0 (C, C-20), 80.4 (CH, C-3), 56.9 (CH, C-17), 56.7 (CH, C-14), 55.8 (CH_3, C-1a), 50.1 (CH, C-9), 46.0 (CH_2, C-1b), 42.8 (C, C-13), 39.8 (CH_2, C-12), 38.8 (CH_2, C-4), 37.3 (CH_2, C-1), 37.0 (C, C-10), 31.9 (CH_2, C-7), 31.5 (CH, C-8), 28.1 (CH_2, C-2), 24.7 (CH_3, C-21 or C-4b), 24.5 (CH_3, C-21 or C-4b), 23.9 (CH_2, C-15), 23.1 (CH_2, C-16), 21.0 (CH_2, C-11), 19.5 (CH_3, C-19), 18.8 (CH_3, C-8b), 14.2 (CH_3, C-18); **IR** (neat): 2933, 2829, 1710, 1171, 1144, 1101 cm^{-1}; **HRMS** (ESI): calculated for $C_{30}H_{46}NaO_3$ [MNa$^+$] requires 477.3339, found 477.3332 (1.3 ppm error).

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6-[(3β,17β)-3-Methoxyandrost-5-en-17-yl]-3,4,6-trimethyl-5,6-dihydro-2H-pyran-2-one 3.173
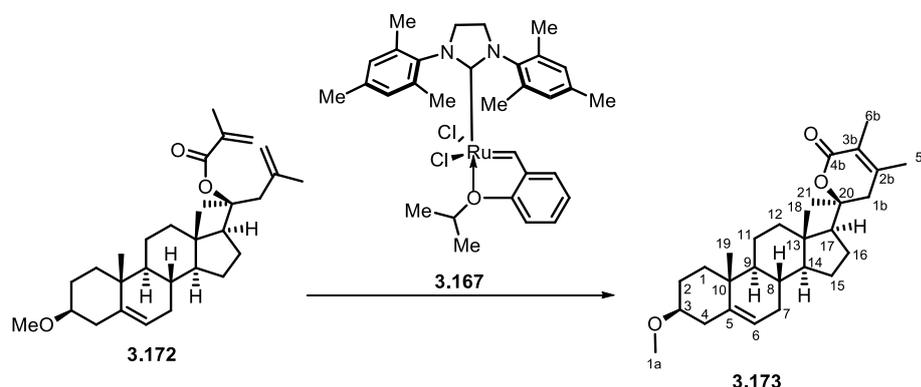
Diene **3.172** (58.0 mg, 0.13 mmol) was dissolved in toluene (10 mL) and 2nd generation Hoveyda-Grubbs catalyst **3.167** (8.00 mg, 0.01 mmol) was added. The reaction mixture was stirred at 120 °C for 24 h. At that time TLC still showed the presence of unreacted starting material, so another portion of catalyst (40 mg, 0.06 mmol) was added and the mixture was stirred at 120 °C for further 18 h. After cooling to rt, the mixture was diluted with CH_2Cl_2 (10 mL) and filtered through a pad of Celite®. The filtrate was evaporated under reduced pressure and the residue was purified by flash column chromatography (SiO_2, PE/AcOEt = 9/1 to 4/1) and then recrystallisation from methanol to afford lactone **3.173** (13.0 mg, 24%) as a colourless solid. **Ref. LNB0161-162**

R_f: 0.18 (hexane/AcOEt = 4/1); **m.p.**: 168-170 °C (methanol) $[\alpha]_D^{24}$: -65 (c 0.15, $CHCl_3$); **^{1}H-NMR** (400 MHz, $CDCl_3$), δ: 5.38-5.32 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.11-2.99 (1H, m, H-3), 2.71 (1H, d, J = 17.8 Hz, H-1b), 2.43-2.33 (1H, m, H-4), 2.22-2.05 (2H, m, H-4, H-12), 2.05-1.69 (6H, m, H-1, H-2, H-7, 2H-16, H-1b), 1.88 (6H, s, 3H-5b, 3H-6b), 1.69-1.36 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-17), 1.44 (3H, s, 3H-21), 1.31-1.09 (2H, m, H-12, H-15), 1.08-0.96 (2H, m, H-1, H-14), 1.00 (3H, s, H-19), 0.95-0.80 (1H, m, H-9), 0.90 (3H, s, H-18); **^{13}C-NMR** (100 MHz, $CDCl_3$), δ: 165.9 (C, C-4b), 146.5 (C, C-2b), 141.1 (C, C-5), 121.5 (CH, C-6), 121.4 (C, C-3b), 82.6 (C, C-20), 80.4 (CH, C-3), 59.0 (CH, C-17), 57.1 (CH, C-14), 55.8 (CH_3, C-1a), 50.2 (CH, C-9), 43.0 (C, C-13), 41.1 (CH_2, C-1b), 40.2 (CH_2, C-12), 38.8 (CH_2, C-4), 37.3 (CH_2, C-1), 37.0 (C, C-10), 31.9 (CH_2, C-7), 31.4 (CH, C-8), 28.1 (CH_2, C-2), 24.6 (CH_3, C-21), 23.8 (CH_2, C-15), 23.4 (CH_2, C-16), 21.0 (CH_2, C-11), 20.8 (CH_3, C-6b), 19.5 (CH_3, C-19), 13.7 (CH_3, C-18), 12.5 (CH_3, C-5b); **IR** (neat): 2925, 2850, 1704, 1463, 1381, 1102 cm^{-1}; **HRMS** (ESI): calculated for $C_{28}H_{42}NaO_3$ [MNa$^+$] requires 449.3026, found 449.3034 (-1.3 ppm error).

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6.3. CHAPTER 4: EXPERIMENTAL DETAILS

Ethyl 6-(3-methoxyphenyl)-3-oxohexanoate 4.157
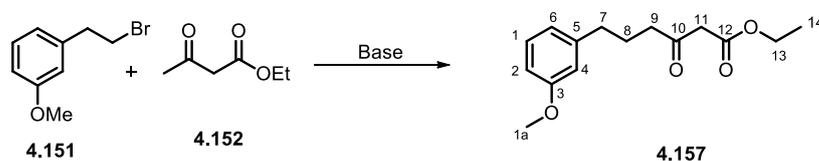
γ-Alkylation by means of NaH and *n*-BuLi[179]

NaH (60% dispersion in mineral oil, 210 mg, 5.25 mmol) was suspended in dry THF (25 mL) and cooled at 0 °C, then ethyl acetoacetate **4.152** (570 µL, 4.51 mmol) was added. The reaction mixture was stirred at 0 °C for 10 min, then *n*-BuLi (2.5 M in hexanes, 1.80 mL, 4.51 mmol) was added. The reaction mixture was stirred for a further 15 min in the ice-cold bath, then 3-methoxyphenethyl bromide **4.151** (0.30 mL, 1.90 mmol) was added. The reaction mixture was stirred at 0 °C for 15 min, then allowed to reach rt and stirred for another 30 min. The suspension was brought to pH 6 with 1 M aq HCl added under vigorous stirring, diluted with H_2O (50 mL) and extracted with AcOEt (3 x 150 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 25 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 4/1) to give ester **4.157** as a colourless oil (450 mg, 90%). **Ref. LNB0083-007**

*R*f: 0.29 (heptane/AcOEt = 4/1); **^{1}H-NMR** (500 MHz, CDCl₃), δ: 7.19 (1H, "t", *J* = 7.2 Hz, H-1), 6.80-6.68 (3H, m, H-2, H-4, H-6), 4.18 (2H, q, *J* = 7.2 Hz, 2H-13), 3.78 (3H, s, 3H, 1a), 3.40 (2H, s, 2H-11), 2.61 (2H, t, *J* = 7.4 Hz, 2H-7), 2.54 (2H, t, *J* = 7.4 Hz, 2H-9), 1.26 (2H, quint, *J* = 7.4 Hz, 2H-8), 1.26 (3H, t, *J* = 7.2 Hz, 3H-14); **^{13}C-NMR** (125 MHz, CDCl₃), δ: 202.7 (C, C-10), 167.3 (C, C-12), 159.7 (C, C-3), 143.1 (C, C-5), 129.4 (CH, C-1), 120.9 (CH, C-6), 114.2 (CH, C-4), 111.4 (CH, C-2), 61.4 (CH₂, C-13), 55.2 (CH₃, C-1a), 49.4 (CH₂, C-11), 42.1 (CH₃, C-9), 34.9 (CH₃, C-7), 24.8 (CH₃, C-8), 14.1 (CH₃, C-14); **IR** (neat): 2939, 1742, 1717, 1259, 1150, 1038 cm^{-1}; **HRMS** (ESI): calculated for $C_{15}H_{20}NaO_4$ [MNa$^+$] requires 287.1254, found 287.1240 (4.9 ppm error). Data are consistent with those reported in the literature.[179]

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γ-Alkylation by means of LDA

n-BuLi (2.5 M in hexanes, 5.76 mL, 14.4 mmol) was added dropwise to a solution of DIPA (2.03 mL, 14.4 mmol) in dry THF (20 mL) cooled to -78 °C and stirred under nitrogen atmosphere. The resulting yellow solution was allowed to reach -40 °C over 20 min, whereupon it was added to a solution of ethyl acetoacetate **4.149** (850 μL, 6.75 mmol) in dry THF (20 mL) previously cooled to 0 °C. The mixture turned dark red coloured. The cooling bath was removed and the reaction mixture was stirred at rt for 1 h. Finally, a solution of 3-methoxyphenethyl bromide **4.148** (0.96 g, 4.47 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture was stirred at rt overnight before being brought to pH 6 with 1M aq HCl, diluted with H_2O (100 mL) and extracted with AcOEt (3 x 150 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 50 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 7/3) to give ester **4.153** as a colourless oil (710 mg, 60%). **Ref. LNB0083-126**

Ethyl {2-[3-(3-methoxyphenyl)propyl]-1,3-dioxolan-2-yl}acetate 4.158[179]
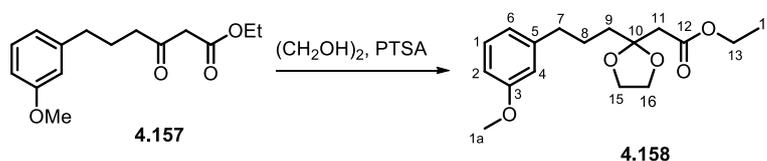
Ethylene glycol (3.44 mL, 61.7 mmol) and PTSA (23.5 mg, 0.12 mmol) were added to a solution of ketoester **4.157** (3.26 g, 12.3 mmol) in benzene (150 mL). The reaction mixture was heated to 90 °C and stirred overnight with a Dean-Stark water separator attached. After cooling to rt, the reaction mixture was quenched with a solution of $NaHCO_3$ (10.4 mg, 0.12 mmol) in H_2O (2 mL). Benzene was evaporated under reduced pressure. The residue was dissolved in AcOEt (200 ml) and washed with sat aq $NaHCO_3$ (3 x 150 mL). The organic layer was separated, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 100 g SiO_2 cartridge, toluene/AcOEt = 10/0 to 9/1) to give the acetal **4.158** (2.86 g, 75%) as a colourless oil. **Ref. LNB0083-143**

*R*f: 0.24 (heptane/AcOEt = 4/1); **¹H-NMR** (500 MHz, CDCl₃), δ: 7.16 (1H, "t", *J* = 7.6 Hz, H-1), 6.76 (1H, d, *J* = 7.6 Hz, H-6), 6.73-6.66 (2H, m, H-2, H-4), 4.11 (2H, q, *J* = 7.2 Hz, 2H-13), 4.00-3.88 (4H, m, 2H-15, 2H-16), 3.76 (3H, s, 3H-1a), 2.62 (2H, s, 2H-11),

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2.59 (2H, t, J = 7.9 Hz, 2H-7), 1.89-1.82 (2H, m, 2H-9), 1.77-1.68 (2H, m, 2H-8), 1.21 (3H, t, J = 7.2 Hz, 3H-14); **^{13}C-NMR** (125 MHz, CDCl$_3$), δ: 169.4 (C, C-12), 159.6 (C, C-3), 143.9 (C, C-5), 129.2 (CH, C-1), 120.8 (CH, C-6), 114.1 (CH, C-4), 111.0 (CH, C-2), 109.2 (C, C-10), 65.1 (2CH$_2$, C-15, C-16), 60.4 (CH$_2$, C-13), 55.0 (CH$_3$, C-1a), 42.7 (CH$_2$, C-11), 37.3 (CH$_2$, C-9), 35.9 (CH$_2$, C-7), 25.2 (CH$_2$, C-8), 14.1 (CH$_3$, C-14); **IR** (neat): 2953, 1728, 1259, 1157, 1038 cm^{-1}; **HRMS** (ESI): calculated for C$_{17}$H$_{24}$NaO$_5$ [MNa$^+$] requires 331.1516, found 331.1502 (3.8 ppm error). Data are consistent with those reported in the literature.[179]

2-Hydroxyethyl 6-(3-methoxyphenyl)-3-oxohexanoate 4.163
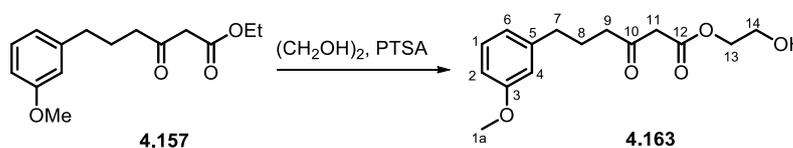
Ethylene glycol (11.1 mL, 0.20 mol) and PTSA (75.8 mg, 0.40 mmol) were added to a solution of ketoester **4.157** (10.5 g, 39.9 mmol) in benzene (250 mL). The reaction mixture was heated at reflux for 3 d, with a Dean-Stark apparatus in place. The mixture was quenched with sat aq NaHCO$_3$ (100 mL), then benzene was evaporated and the aqueous layer was extracted with AcOEt (3 x 100 mL). Combined organic layers were washed with brine (250 mL), dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 100 g silica cartridge, heptane/AcOEt = 10/0 to 8/2, then CH$_2$Cl$_2$/MeOH = 9/1, 1% AcOEt) to yield glycolic ester **4.163** (5.39 g, 48%) as a yellow oil along with recovered starting material **4.157** (3.63 g). **Ref. LNB0103-078**

Rf: 0.31 (hexane/AcOEt = 5/5); **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 7.8 (1H, "t", J = 7.8 Hz, H-1), 6.80-6.68 (3H, m, H-2, H-4, H-6), 4.33-4.21 (2H, m, 2H-13), 3.87-3.77 (5H, m, 2H-14, 3H-1a), 3.45 (2H, s, 2H-11), 2.61 (2H, t, J = 7.4 Hz, 2H-7), 2.54 (2H, t, J = 7.4 Hz, 2H-9), 1.94 (2H, quint, J = 7.4 Hz, 2H-8); **^{13}C-NMR** (125 MHz, CDCl$_3$), δ: 203.2 (C, C-10), 167.3 (C, C-12), 159.8 (C, C-3), 142.9 (C, C-5), 129.5 (CH, C-1), 121.0 (CH, C-6), 114.4 (CH, C-4), 111.4 (CH, C-2), 66.8 (CH$_2$, C-13), 60.9 (CH$_2$, C-14), 55.3 (CH$_3$, C-1a), 49.4 (CH$_2$, C-11), 42.3 (CH$_2$, C-9), 34.9 (CH$_2$, C-7), 24.8 (CH$_2$, C-8); **IR** (neat): 3481, 2943, 1738, 1710, 1259, 1147, 1038 cm^{-1}; **HRMS** (ESI): calculated for C$_{15}$H$_{20}$NaO$_5$ [MNa$^+$] requires 303.1203, found 303.1193 (3.1 ppm error).

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2-{2-[3-(3-Methoxyphenyl)propyl]-1,3-dioxolan-2-yl}ethanol 4.159
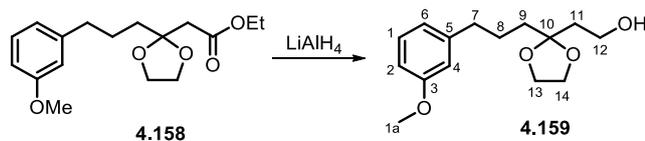
A solution of ethyl ester **4.158** (116 mg, 0.38 mmol) in dry THF (4 mL) was added to a suspension of LiAlH₄ (15.0 mg, 0.38 mmol) in dry THF (4 mL) cooled to 0 °C. The ice-bath was removed and the mixture was stirred at rt for 1 h. Excess LiAlH₄ was decomposed with ice-cold water (10 mL). The mixture was then brought to pH 4 with 1M aq. HCl and extracted with AcOEt (3 x 10 mL). Combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 5/5) to obtain alcohol **4.159** (89.0 mg, 88%) as a colourless oil. **Ref. LNB0083-009**

R_f: 0.17 (heptane/AcOEt = 5/5); **¹H-NMR** (500 MHz, $CDCl_3$), δ: 7.22-7.15 (1H, m, H-1), 6.77 (1H, d, J = 7.1 Hz, H-6), 6.75-6.70 (2H, m, H-2, H-4), 4.03-3.90 (4H, m, 2H-13, 2H-14), 3.80 (3H, s, 3H-1a), 3.75-3.70 (2H, m, 2H-12), 2.64-2.56 (2H, m, 2H-7), 2.40 (1H, brs, OH), 1.91 (2H, t, J = 5.6 Hz, 2H-11), 1.74-1.65 (4H, m, 2H-8, 2H-9); **¹³C-NMR** (125 MHz, $CDCl_3$), δ: 159.7 (C, C-3), 143.8 (C, C-5), 129.4 (CH, C-1), 120.1 (CH, C-6), 114.3 (CH, C-4), 112.2 (CH, C-2), 111.2 (C, C-10), 64.9 (2CH₂, C-13, C-14), 58.9 (CH₂, C-12), 55.2 (CH₃, C-1a), 38.3 (CH₂, C-11), 36.7 (CH₂, C-9), 36.0 (CH₂, C-7), 25.5 (CH₂, C-8); **IR** (neat): 3471, 2946, 1251, 1153, 1038 cm⁻¹; **HRMS** (ESI): calculated for $C_{15}H_{22}NaO_4$ [MNa⁺] requires 289.1410, found 289.1401 (3.3 ppm error). Data are consistent with those reported in the literature.[179]

2-Hydroxyethyl {2-[3-(3-methoxyphenyl)propyl]-1,3-dioxolan-2-yl}acetate 4.164
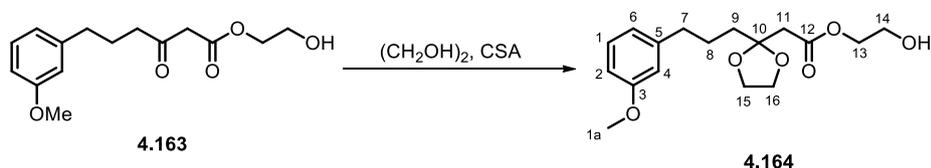
Ethylene glycol (3.07 mL, 55.2 mmol) and CSA (42.6 mg, 0.18 mmol) were added to a solution of ketoester **4.163** (5.15 g, 18.4 mmol) in benzene (150 mL). The reaction mixture was heated at 95 °C and stirred for 3 h with a Dean-Stark apparatus in place. Due to the presence of residual starting material, a further amount of ethylene glycol (1.5 mL,

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27.6 mmol) and CSA (21.0 mg, 0.09 mmol) were added and reaction mixture was heated at reflux for 1 h. After cooling to rt, the reaction was quenched with sat aq NaHCO$_3$ (10 mL). Benzene was evaporated and the residue was diluted with AcOEt (150 mL) and washed with sat aq NaHCO$_3$ (2 x 150 ml) and brine (150 mL). The organic phase was dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 100 g SiO$_2$, heptane/AcOEt = 4/1 to 5/5) to yield an inseparable mixture of acetal **4.164** and starting material **4.163** (about 1/1, 4.02 g). The crude product was dissolved in benzene (120 mL) and ethylene glycol (1.11 mL, 19.8 mmol) and CSA (15.3 mg, 0.066 mmol) were added. The reaction mixture was stirred at reflux for 3 d, and a further amount of ethylene glycol (1.11 mL, 19.8 mmol) and CSA (15.3 mg, 0.07 mmol) were added. The reaction mixture was stirred at reflux for an additional 1 d, before being worked up as described above. Purification of the crude material (Biotage SNAP 100 g SiO$_2$, CH$_2$Cl$_2$/MeOH = 9/1 + 1% AcOEt) gave acetal **4.164** (4.17 g, 69%) as a yellow oil. **Ref. LNB0103-089**

R$_f$: 0.33 (hexane/AcOEt = 5/5); **^{1}H-NMR** (500 MHz, CDCl$_3$), δ: 7.22-7.15 (1H, m, H-1), 6.78 (1H, d, *J* = 7.6 Hz, H-6), 6.75-6.69 (2H, m, H-2, H-4), 4.25-4.20 (2H, m, 2H-13), 4.03-3.91 (4H, m, 2H-15, 2H-16), 3.84-3.73 (5H, m, 2H-14, 3H-1a), 2.69 (2H, s, 2H-11), 2.60 (2H, t, *J* = 7.6 Hz, 2H-7), 1.90-1.82 (2H, m, 2H-9), 1.79-1.69 (2H, m, 2H-8); **^{13}C-NMR** (125 MHz, CDCl$_3$), δ: 169.8 (C, C-12), 159.7 (C, C-3), 143.9 (C, C-5), 129.4 (CH, C-1), 121.0 (CH, C-6), 114.3 (CH, C-4), 111.2 (CH, C-2), 109.4 (C, C-10), 66.2 (CH$_2$, C-13), 65.3 (2CH$_2$, C-15, C-16), 61.1 (CH$_2$, C-14), 55.3 (CH$_3$, C-1a), 43.0 (CH$_2$, C-11), 37.5 (CH$_2$, C-9), 35.9 (CH$_2$, C-7), 25.3 (CH$_2$, C-8); **IR** (neat): 3471, 2946, 1735, 1262, 1145, 1038 cm^{-1}; **HRMS** (ESI): calculated for C$_{17}$H$_{24}$NaO$_6$ [MNa$^+$] requires 347.1465, found 347.1472 (2.3 ppm error).

Convertion of glycolic ester 4.164 into alcohol 4.159
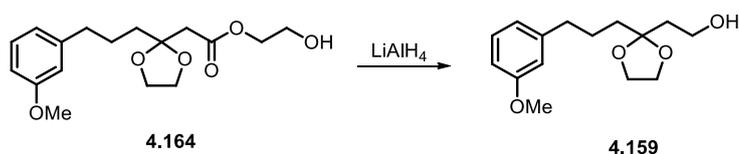
LiAlH$_4$ (494 mg, 13.0 mmol) was added portionwise to a solution of glycolic ester **4.164** (4.17 g, 13.0 mmol) in THF (150 mL) cooled to 0 °C. The ice-bath was removed and the mixture was stirred at rt for 3 h. Excess LiAlH$_4$ was decomposed with ice-cold water

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(200 mL). The mixture was brought to pH 5 with 1M aq HCl and extracted with AcOEt (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The title compound **4.155** was obtained as a colourless oil (3.09 g, 90%) without any further purification. **Ref. LNB0103-102**

1-Chloro-6-(3-methoxyphenyl)hexan-3-one 4.165[179]
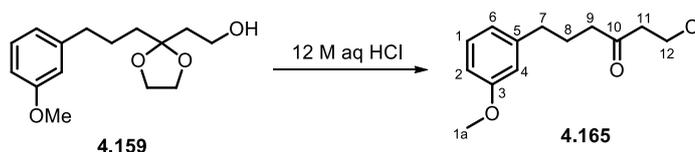
A solution of alcohol **4.159** (158 mg, 0.59 mmol) in acetone (20 mL) was cooled to 0 °C and acidified with 12 M aq HCl (891 μL, 10.7 mmol) added dropwise. The reaction mixture was stirred at 0 °C for 10 min, and at rt for a further 4 h. The reaction mixture was quenched with H_2O (20 mL) and the organic compounds were extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with sat aq $NaHCO_3$ (3 x 20 ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give chloride **4.165** (140 mg, 99%) as a colourless oil. **Ref. LNB0162-107**

R_f: 0.34 (heptane/AcOEt = 4/1); **¹H-NMR** (500 MHz, CDCl₃), δ: 7.20 (1H, "t", J = 7.9 Hz, H-1), 6.77-6.72 (3H, m, H-2, H-4, H-6), 3.80 (3H, s, 3H-1a), 3.73 (2H, t, J = 6.6 Hz, 2H-12), 2.84 (2H, t, J = 6.6 Hz, 2H-11), 2.61 (2H, t, J = 7.4 Hz, 2H-7), 2.45 (2H, t, J = 7.4 Hz, 2H-9), 1.93 (2H, quint, J = 7.4 Hz, 2H-8); **¹³C-NMR** (125 MHz, CDCl₃), δ: 207.3 (C, C-10), 159.9 (C, C-3), 143.2 (C, C-5), 129.5 (CH, C-1), 121.0 (CH, C-6), 114.4 (CH, C-4), 111.5 (CH, C-2), 55.3 (CH₃, C-1a), 45.2 (CH₂, C-11), 42.5 (CH₂, C-9), 38.4 (CH₂, C-12), 35.1 (CH₂, C-7), 24.9 (CH₂, C-8); **IR** (neat): 2925, 1710, 1255, 1154 cm⁻¹; **HRMS** (ESI): calculated for $C_{13}H_{17}{}^{35}ClNaO_2$ [MNa⁺] requires 263.0809, found 263.0804 (2.4 ppm error).

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1-Hydroxy-6-(3-methoxyphenyl)hexan-3-one 4.160
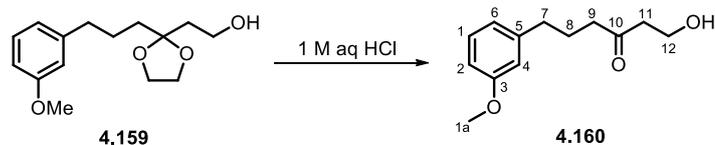
A solution of acetal **4.159** (2.90 g, 11.0 mmol) in acetone (50 mL) was treated with 1 M aq HCl (5.50 mL, 5.50 mmol). The reaction mixture was stirred at rt for 2 h, then the acetone was evaporated. The residue was diluted with AcOEt (200 mL) and washed with sat aq $NaHCO_3$ (3 x 200 mL). The organic layer was separated, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude oil was purified by flash column chromatography (Biotage SNAP 50 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 5/5) to give ketoalcohol **4.160** (2.30 g, 94%) as a colourless oil. **Ref. LNB0103-048**

R_f: 0.17 (heptane/AcOEt = 5/5); **^{1}H-NMR** (500 MHz, $CDCl_3$), δ: 7.20 (1H, "t", J = 7.8 Hz, H-1), 6.78-6.69 (3H, m, H-2, H-4, H-6), 3.82 (2H, t, J = 7.5 Hz, 2H-12), 3.80 (3H, s, 3H-1a), 2.66-2.57 (4H, m, 2H-11, 2H-7), 2.45 (2H, t, J = 7.4 Hz, 2H-9), 1.92 (2H, quint, J = 7.4 Hz, 2H-8); **^{13}C-NMR** (125 MHz, $CDCl_3$), δ: 211.5 (C, C-10), 159.7 (C, C-3), 143.2 (C, C-5), 129.5 (CH, C-1), 120.9 (CH, C-6), 114.4 (CH, C-4), 111.4 (CH, C-2), 58.0 (CH_2, C-12), 55.3 (CH_3, C-1a), 44.5 (CH_2, C-11), 42.5 (CH_2, C-9), 35.1 (CH_2, C-7), 24.9 (CH_2, C-8); **IR** (neat): 3471, 2932, 1710, 1580, 1255, 1150, 1038 cm^{-1}; **HRMS** (ESI): calculated for $C_{13}H_{18}NaO_3$ [MNa$^+$] requires 245.1148, found 245.1143 (1.9 ppm error).

6-(3-Methoxyphenyl)-3-oxohexyl acetate 4.161
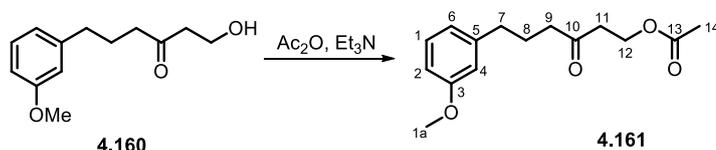
A solution of alcohol **4.160** (33.0 mg, 0.15 mmol), Ac_2O (70.0 μL, 0.74 mmol) and Et_3N (45.0 μL, 0.33 mmol) in CH_2Cl_2 (2 mL) was stirred at rt overnight. The reaction mixture was diluted with CH_2Cl_2 (1 mL) and washed with sat aq NH_4Cl (2 x 3 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g silica

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cartridge, heptane/AcOEt = 10/0 to 4/1) to give acetate **4.161** (24.0 mg, 61%) as a colourless oil. **Ref. LNB0083-020**

R_f: 0.17 (heptane/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.20 (1H, "t", J = 7.6 Hz, H-1), 6.76-6.71 (3H, m, H-2, H-4, H-6), 4.31 (2H, t, J = 6.2 Hz, 2H-12), 3.79 (3H, s, 3H-1a), 2.70 (2H, t, J = 6.2 Hz, 2H-11), 2.60 (2H, t, J = 7.5 Hz, H-7), 2.44 (2H, t, J = 7.5 Hz, H-9), 2.01 (3H, s, 3H-14), 1.92 (2H, quint, J = 7.5 Hz, H-8); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 207.7 (C, C-10), 170.8 (C, C-13), 159.8 (C, C-3), 143.0 (C, C-5), 129.3 (CH, C-1), 120.8 (CH, C-6), 114.2 (CH, C-4), 111.2 (CH, C-2), 59.8 (CH$_2$, C-12), 55.1 (CH$_3$, C-1a), 42.2 (CH$_2$, C-9), 41.3 (CH$_2$, C-11), 34.9 (CH$_2$, C-7), 24.8 (CH$_2$, C-8), 20.2 (CH$_3$, C-14); **IR** (neat): 2935, 1734, 1714, 1238, 1150, 1038 cm^{-1}; **HRMS** (ESI): calculated for C$_{15}$H$_{20}$NaO$_4$ [MNa$^+$] requires 287.1254, found 287.1241 (4.8 ppm error).

6-(3-Methoxyphenyl)hex-1-en-3-one 4.150

*Synthesis of vinyl ketone **4.150** from acetate **4.161***

4.161   **4.150**
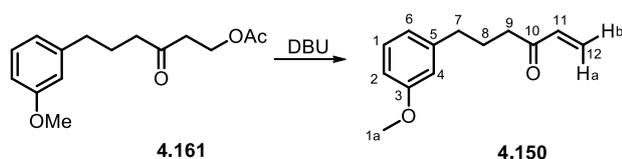
DBU (34.0 μL, 0.23 mmol) was added to a solution of acetate **4.161** (20.0 mg, 0.08 mmol) in toluene (4 mL). The reaction mixture was stirred at rt for 2 h. Toluene was evaporated and the residue was diluted with CH$_2$Cl$_2$ (20 mL) and washed with sat aq NH$_4$Cl (3x 8 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 4/1) to give vinyl ketone **4.150** (15.0 mg, 98%) as a colourless oil. **Ref. LNB0083-034**

R_f: 0.39 (heptane/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.20 (1H, "t", J = 8.1 Hz, H-1), 6.78-6.73 (3H, m, H-2, H-4, H-6), 6.34 (1H, dd, J = 17.1, 10.6 Hz, H-11), 6.18 (1H, d, J = 17.1 Hz, H-12a), 5.81 (1H, d, J = 10.6 Hz, H-12b), 3.80 (3H, s, C-1a), 2.65-2.58 (4H, m, 2H-7, 2H-9), 1.96 (2H, quint, J = 7.6 Hz, 2H-8); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 200.5 (C, C-10), 159.6 (C, C-3), 143.2 (C, C-5), 136.4 (CH, C-11), 129.3 (CH, C-1), 127.9 (CH$_2$, C-12), 120.8 (CH, C-6), 114.1 (CH, C-4), 111.2 (CH, C-2), 55.4 (CH$_3$, C-1a), 38.6 (CH$_2$, C-9), 35.0 (CH$_2$, C-7), 25.1 (CH$_2$, C-8); **IR** (neat): 2932, 1699, 1679,

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1580, 1259, 1147, 1038 cm^{-1}; **HRMS** (ESI): calculated for $C_{13}H_{16}NaO_2$ [MNa$^+$] requires 227.1043, found 227.1038 (1.7 ppm error). Data are consistent with those reported in the literature.[179]

*Synthesis of vinyl ketone **4.165** from chloride **4.150***
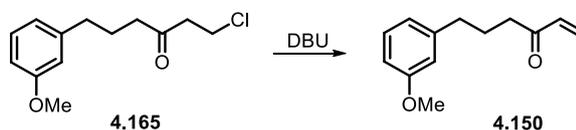
DBU (71.0 µL, 0.48 mmol) was added to a solution of chloride **4.165** (38.0 mg, 0.16 mmol) in toluene (10 mL). The reaction mixture was stirred at rt for 2 h. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with sat aq NH_4Cl (3 x 5 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 4/1) to afford vinyl ether **4.150** (17.0 mg, 54%) as a colourless oil. **Ref. LNB0083-036**

2-(6-(3-Methoxyphenyl)-3-oxohexyl)-2-methylcyclopentane-1,3-dione 4.148
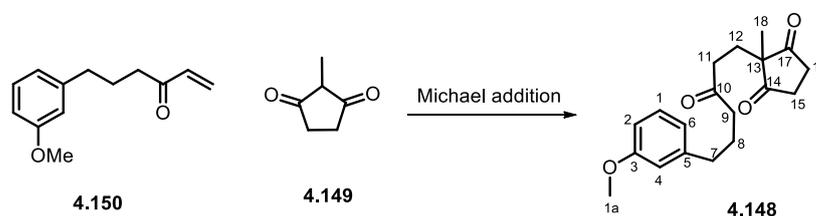
Acid-catalysed Michael addition[178]

Vinyl ketone **4.150** (220 mg, 1.10 mmol) was added to a solution of 2-methylcyclpentane-1,3-dione **4.149** (145 mg, 1.30 mmol) in a mixture of glacial AcOH (6 mL) and H_2O (1 mL). The reaction mixture was heated to 80 °C and stirred overnight. After cooling to rt, the mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, heptane/AcOEt = 3/2) to give triketone **4.148** (148 mg, 43%) as a yellow oil. **Ref. LNB0083-062.**

R$_f$: 0.33 (heptane/AcOEt = 5/5); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.19 (1H, "t", *J* = 7.9 Hz, H-1), 6.77-6.58 (3H, m, H-2, H-4, H-6), 3.79 (3H, s, 3H-1a), 2.90-2.70 (4H, m,

2H-15, 2H-16), 2.56 (2H, t, J = 7.6 Hz, 2H-7), 2.42-2.32 (4H, m, 2H-11, 2H-9), 1.90-1.80 (4H, m, 2H-12, 2H-8), 1.09 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 215.9 (2C, C-14, C-17), 209.9 (C, C-10), 159.8 (C, C-3), 143.2 (C, C-5), 129.5 (CH, C-1), 121.0 (CH, C-6), 114.3 (CH, C-4), 111.4 (CH, C-2), 55.3 (2C, C, C-13, CH$_3$, C-1a), 42.1 (CH$_2$, C-9), 36.6 (CH$_2$, C-11), 35.1 (CH$_2$, C-7), 34.8 (2CH$_2$, C-15, C-16), 28.0 (CH$_2$, C-12), 25.0 (CH$_2$, C-8), 19.3 (CH$_3$, C-18); **IR** (neat): 2932, 1710, 1266, 1150, 1038 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{24}$NaO$_4$ [MNa$^+$] requires 339.1567, found 339.1537 (8.4 ppm error). Data are consistent with those reported in the literature.[179]

<u>Base-catalysed Michael addition</u>

Et$_3$N (1.30 mL, 9.28 mmol) was added to a suspension of 2-methylcyclpentane-1,3-dione **4.149** (0.97 g, 8.70 mmol) in THF (20 mL). The reaction mixture was stirred at rt until complete dissolution, then a solution of vinyl ketone **4.150** (600 mg, 2.90 mmol) in THF (10 mL) was added. The reaction mixture was heated to 40 °C and stirred for 1 d. After cooling to rt, the mixture was diluted with AcOEt (100 mL) and washed with H$_2$O (3 x 100 mL). The combined aqueous layers were further extracted with AcOEt (2 x 200 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 50 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 3/2) to yield triketone **4.148** (828 mg, 90%) as a yellow oil. **Ref. LNB0083-144**

(14β)-14-Hydroxy-1-methoxy-9,10-secoestra-1(10),2,4-triene-9,17-dione 4.147 and 14-hydroxy-1-methoxy-9,10-secoestra-1(10),2,4-triene-9,17-dione 4.177
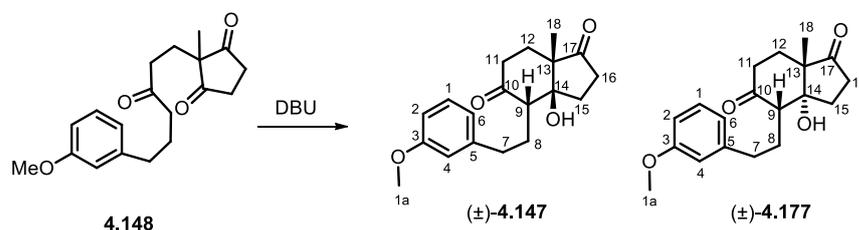
DBU (365 μL, 2.45 mmol) was added to a solution of triketone **4.148** (2.58 g, 8.16 mmol) in THF (40 mL). The reaction mixture was heated to 40 °C and stirred for 6 d under a nitrogen atmosphere. After cooling to rt, the reaction mixture was diluted with AcOEt (200 mL) and washed with H$_2$O (3 x 200 mL). The combined aqueous layers were further extracted with AcOEt (2 x 150 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by

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flash column chromatography (Biotage SNAP 100 g SiO$_2$ cartridge, heptane/AcOEt = 1/4 to 2/3) to yield a diastereomeric mixture of **4.147** and **4.177** (about 3/1, 1.92 g, 74%) as a white solid. **Ref. LNB0083-152**

Characterisation of alcohol 4.147: **R$_f$**: 0.33 (heptane/AcOEt = 5/5); **m.p.**: 128-130 °C (lit. m.p. of racemic **4.147**: 132-135.5 °C, m.p. of optically active **4.147**: 136-137 °C);[193] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.21 (1H, "t", *J* = 7.6 Hz, H-1), 6.80 (1H, d, *J* = 7.3 Hz, H-6), 6.78-6.72 (2H, m, H-2, H-4), 3.80 (3H, s, 3H-1a), 2.92-2.83 (1H, m, H-7), 2.61-2.48 (2H, m, H-9, H-11), 2.48-2.33 (4H, m, H-7, H-11, 2H-16), 2.22-2.11 (1H, m, H-8), 1.91-1.51 (5H, m, H-8, 2H-12, 2H-15), 1.28 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 218.1 (C, C-17), 208.3 (C, C-10), 159.9 (C, C-3), 143.7 (C, C-5), 129.6 (CH, C-1), 120.9 (CH, C-6), 114.3 (CH, C-4), 111.5 (CH, C-2), 85.0 (C, C-14), 56.3 (CH, C-9), 55.3 (CH$_3$, C-1a), 53.7 (C, C-13), 37.4 (CH$_2$, C-11), 34.9 (CH$_2$, C-7), 32.9 (CH$_2$, C-16), 30.5 (CH$_2$, C-12), 28.7 (CH$_2$, C-15), 24.0 (CH$_2$, C-8), 12.9 (CH$_3$, C-18); **IR** (neat): 3478, 2929, 1742, 1714, 1259 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{24}$NaO$_4$ [MNa$^+$] requires 339.1567, found 339.1592 (1.1 ppm error).

Characterisation of alcohol 4.177: **R$_f$**: 0.30 (heptane/AcOEt = 5/5); **m.p.**: 82-84 °C; **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.21 (1H, "t", *J* = 7.6 Hz, H-1), 6.77-6.67 (3H, m, H-2, H-4, H-6), 3.79 (3H, s, 3H-1a), 2.84-2.74 (1H, m, H-7), 2.54-2.44 (1H, m, H-16), 2.42-2.12 (6H, m, H-7, H-8, H-9, 2H-11, H-12), 2.09-1.83 (3H, m, 2H-15, H-16), 1.83-1.72 (1H, m, H-8), 1.71-1.62 (1H, m, H-12), 1.08 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 217.4 (C, C-17), 210.5 (C, C-10), 159.9 (C, C-3), 143.1 (C, C-5), 129.7 (CH, C-1), 121.6 (CH, C-6), 114.5 (CH, C-4), 111.6 (CH, C-2), 84.9 (C, C-14), 55.4 (CH$_3$, C-1a), 54.9 (CH, C-9) 53.7 (C, C-13), 37.8 (CH$_2$, C-11), 34.4 (CH$_2$, C-7), 33.8 (CH$_2$, C-16), 31.5 (CH$_2$, C-15), 29.8 (CH$_2$, C-12), 25.4 (CH$_2$, C-8), 18.3 (CH$_3$, C-18); **IR** (neat): 3468, 2922, 1742, 1711, 1259 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{24}$NaO$_4$ [MNa$^+$] requires 339.1568, found 339.1592 (0.5 ppm error).

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2-Hydroxy-2-[3-(3-methoxyphenyl)propyl]-5-methylbicyclo[3.2.1]octane-6,8-dione 4.182
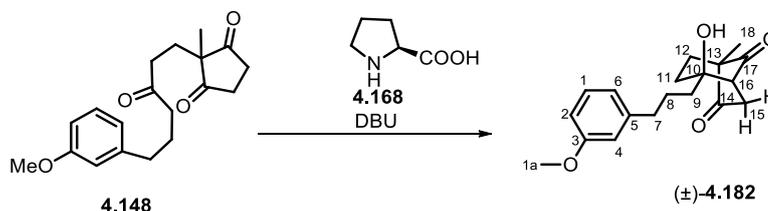
DBU (91.0 µL, 0.61 mmol) and (*S*)-proline **4.168** (70.0 mg, 0.61 mmol) were added to a solution of triketone **4.148** (193 mg, 0.61 mmol) in dry THF (10 mL) and the resulting mixture was stirred at rt overnight. The reaction was quenched with H_2O (10 mL) and the organic compounds were extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, hexane/AcOEt = 4/1 to 7/3) to give compound **4.182** (20.0 mg, 11%) as a yellow oil. **Ref. LNB0162-110**

R$_f$: 0.23 (hexane/AcOEt = 7/3); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.19 (1H, "t", *J* = 7.8 Hz, H-1), 6.78-6.68 (3H, m, H-2, H-4, H-6), 3.79 (3H, s, H-1a), 2.79 (1H, d, *J* = 6.6 Hz, H-16), 2.66-2.49 (3H, m, 2H-7, H-15), 2.42-2.31 (1H, m, H-15), 2.14-2.06 (1H, m, H-12), 1.89-1.66 (7H, m, 2H-8, 2H-9, 2H-11, H-12), 1.06 (3H, s, H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 214.1 (C, C-17), 211.2 (C, C-14), 159.8 (C, C-3), 143.3 (C, C-5), 129.5 (CH, C-1), 128.8 (CH, C-6), 114.4 (CH, C-4), 111.3 (CH, C-2), 82.6 (C, C-10), 58.8 (C, C-13), 55.5 (CH, C-16), 55.3 (CH$_3$, C-1a), 42.3 (CH$_2$, C-15), 40.0 (CH$_2$, C-9), 38.5 (CH$_2$, C-12), 35.9 (CH$_2$, C-7), 31.0 (CH$_2$, C-11), 24.3 (CH$_2$, C-8), 11.9 (CH$_3$, C-18); **IR** (neat): 3460, 2936, 1770, 1724 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{24}$NaO$_4$ [MNa$^+$] requires 339.1567, found 339.1555 (3.0 ppm error). In spite of the presence of (*S*)-proline **4.168** in the reaction mixture, compound **4.182** was assumed to be racemic (see **Table 4.4** and **Scheme 4.31**).

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*Conversion of bicycle **4.182** into carbinols **4.147** and **4.177***
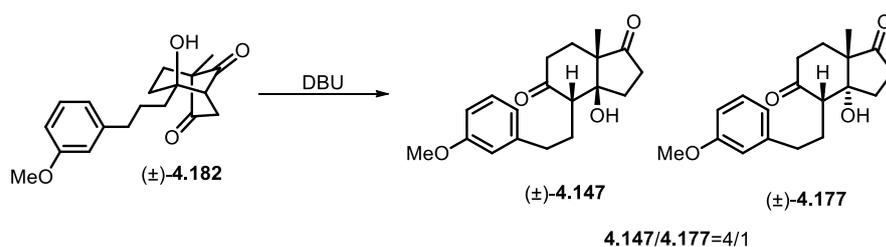
DBU (12.0 µL, 0.08 mmol) was added to a solution of bicycle **4.182** (102 mg, 0.32 mmol) in THF (1 mL), and the reaction mixture was stirred at 40 °C for 3 d. Due to the presence of residual starting material, a further amount of DBU (12.0 µL, 0.08 mmol) was added and the temperature was gradually increased to 70 °C during 2 d. The reaction mixture was diluted with AcOEt (10 mL) and washed with H_2O (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP SiO_2 cartridge, heptane/Et_2O = 1/1) to yield a diastereomeric mixture of **4.147** and **4.177** (about 4/1, 40.0 mg, 40%) as a white solid. **Ref. LNB0083-102**

1-Methoxy-9,10-secoestra-1(10),2,4,8(14)-tetraene-9,17-dione 4.174
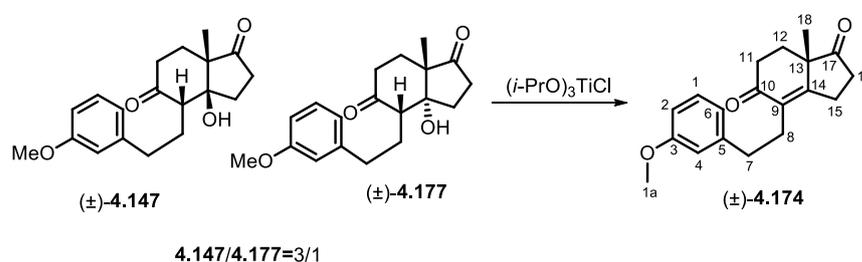
A diastereomeric mixture of carbinols **4.147** and **4.177** (about 3/1, 51.0 mg, 0.16 mmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C and (*i*-PrO)$_3$TiCl (1 M in hexane, 160 µl, 0.16 mmol) was added slowly. The reaction mixture was allowed to reach rt and stirred overnight. The reaction was quenched with sat aq $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP, silica cartridge, heptane/AcOEt = 4/1 to 3/2) to give the title diketone **4.174** (24.0 mg, 50%) as a yellow oil. **Ref. LNB0103-015**

*R*f: 0.37 (heptane/AcOEt = 5/5); **¹H-NMR** (400 MHz, CDCl₃), δ: 7.16 (1H, "t", *J* = 7.9 Hz, H-1), 6.74-6.70 (1H, m, H-2), 6.68 (1H, d, *J* = 7.9 Hz, H-6), 6.64 (1H, brs, H-4), 3.77

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(3H, s, 3H-1a), 2.72-2.65 (2H, m, 2H-7), 2.60-2.43 (6H, m, 2H-8, 2H-11, H-15, H-16), 2.27-2.15 (1H, m, H-15), 2.09-1.98 (2H, m, H-12, H-16), 1.79 (1H, "td", J = 13.6, 5.8 Hz, H-12), 1.19 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 217.6 (C, C-17), 197.6 (C, C-10), 164.4 (C, C-14), 159.3 (C, C-3), 143.3 (C, C-5), 132.7 (C, C-9) 129.9 (CH, C-1), 121.6 (CH, C-6), 115.1 (CH, C-4), 111.1 (CH, C-2), 55.1 (CH$_3$, C-1a), 49.0 (C, C-13), 35.7 (CH$_2$, C-16), 34.8 (CH$_2$, C-7), 33.2 (CH$_2$, C-11), 29.0 (CH$_2$, C-12), 27.6 (CH$_2$, C-8), 24.5 (CH$_2$, C-15), 21.2 (CH$_3$, C-18); **HRMS** (ESI): calculated for C$_{19}$H$_{22}$NaO$_3$ [MNa$^+$] requires 321.1461, found 321.1447 (3.7 ppm error). Data are consistent with those reported in the literature.[179]

3-Methoxyestra-1(10),2,4,8,14-pentaen-17-one 4.176
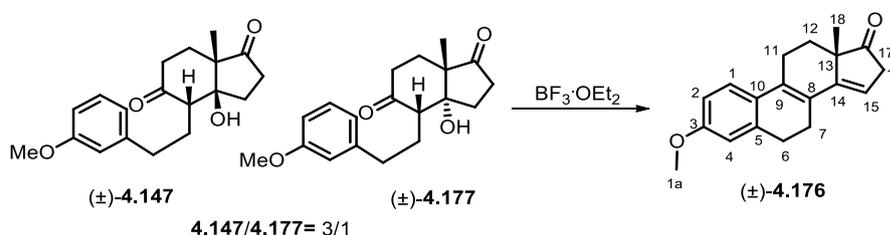
BF$_3$·OEt$_2$ (15.0 µL, 0.16 mmol) was added dropwise to a solution of of carbinols **4.147** and **4.177** (about 3/1, 50.0 mg, 0.16 mmol) in CH$_2$Cl$_2$ (1 mL) and the reaction mixture was stirred at rt for 2 h. The reaction was quenched with H$_2$O (5 mL). The organic layer was separated, and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 5 ml). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 9/1) to yield title compound **4.176** (40.0 mg, 90%) as a white solid. **Ref. LNB0083-149**

R$_f$: 0.48 (hexane/AcOEt = 4/1); **m.p.:** 111-113 °C (MeOH) [lit. 111-113 °C];[179] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.26-7.24 (1H, m, H-1), 6.78-6.69 (2H, H-2, H-4), 5.86 (1H, brs, H-15), 3.81 (3H, s, 3H-1a), 3.31 (1H, dd, J = 23.3, 3.0 Hz, H-16), 2.95 (1H, dd, J = 23.3, 3.0 Hz, H-16), 2.82-2.77 (2H, m, 2H-6), 2.65-2.57 (3H, m, H-7, 2H-11), 2.35-2.27 (1H, m, H-7), 2.06-2.01 (1H, m, H-12), 1.63-1.55 (1H, m, H-12), 1.14 (3H, s, H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 220.3 (C, C-17), 158.8 (C, C-3), 147.0 (C, C-14), 138.3 (C, C-5), 129.9 (C, C-9), 128.7 (C, C-10), 125.1 (C, C-8), 124.6 (CH, C-1), 114.4 (CH, C-15), 113.8 (CH, C-4), 111.3 (CH, C-2), 55.6 (CH$_3$, C-1a), 49.3 (C, C-13), 42.3 (CH$_2$, C-16), 28.6 (CH$_2$, C-6), 27.4 (CH$_2$, C-12), 23.1 (CH$_2$, C-11), 22.9 (CH$_2$, C-7),

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20.8 (CH₃, C-18); **HRMS** (ESI): calculated for $C_{19}H_{20}NaO_2$ [MNa⁺] requires 303.1356, found 303.1358 (0.5 ppm error). Data are consistent with those reported in the literature.[179]

1-Methoxy-14-[(trimethylsilyl)oxy]-9,10-secoestra-1(10),2,4-triene-9,17-dione 4.198
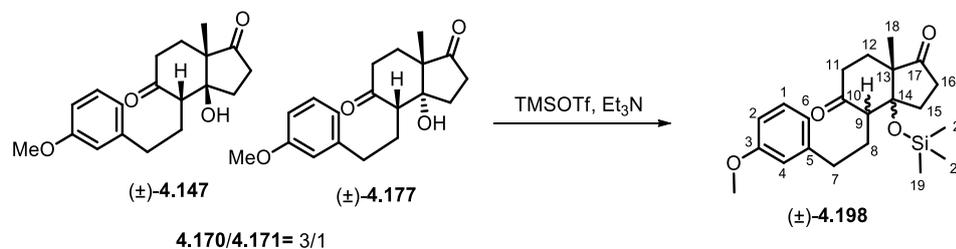
Et₃N (106 μL, 0.76 mmol) was added to a solution of carbinols **4.174** and **4.175** (about 3/1, 200 mg, 0.63 mmol) in dry CH_2Cl_2 (5 mL) under a nitrogen atmosphere. The mixture was cooled to 0 °C and TMSOTf (137 μL, 0.76 mmol) was added dropwise. Aftet 10 min, the ice bath was removed, and the reaction mixture was stirred at rt for an additional 1 h. The reaction was quenched with H₂O (5 mL). The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried over Na₂SO₄, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g silica cartidge, heptane/AcOEt = 10/0 to 4/1) to afford the title compound **4.198** (58.0 mg, 24%) as a colourless oil. **Ref. LNB0103-054**

*R*f: 0.29 (heptane/AcOEt = 4/1); **¹H-NMR** (400 MHz, CDCl₃), δ: 7.19 (1H, "t", *J* = 7.9 Hz, H-1), 6.76-6.66 (3H, m, H-2, H-4, H-6), 3.87 (3H, s, 3H-1a), 2.78-2.69 (1H, m, H-7), 2.48-2.28 (3H, m, H-7, H-11, H-16), 2.25-2.13 (3H, m, H-8, H-9, H-11), 2.06-1.90 (4H, m, H-12, 2H-15, H-16), 1.85-1.74 (1H, m, H-8), 1.67-1.55 (1H, m, H-12), 1.05 (3H, s, 3H-18), 0.12 (9H, s, 3H-19, 3H-20, 3H-21); **¹³C-NMR** (100 MHz, CDCl₃), δ: 217.5 (C, C-17), 210.4 (C, C-10), 159.9 (C, C-3), 143.2 (C, C-5), 129.6 (CH, C-1), 120.9 (CH, C-6), 114.4 (CH, C-4), 111.5 (CH, C-2), 87.6 (C, C-14), 56.3 (CH, C-9), 55.3 (CH₃, C-1a), 54.5 (C, C-13), 36.6 (CH₂, C-11), 34.2 (CH₂, C-7), 33.8 (CH₂, C-16), 31.0 (CH₂, C-15), 29.3 (CH₂, C-12), 26.9 (CH₂, C-8), 18.3 (CH₃, C-18), 2.48 (3CH₃, C-19, C-20, C-21); **IR** (neat): 2953, 1742, 1717, 1252, cm⁻¹; **HRMS** (ESI): calculated for $C_{22}H_{32}NaO_4Si$ [MNa⁺] requires 411.1962, found 411.1955 (0.7 ppm error).

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1-Methoxy-14-[(triethylsilyl)oxy]-9,10-secoestra-1(10),2,4-triene-9,17-dione 4.202
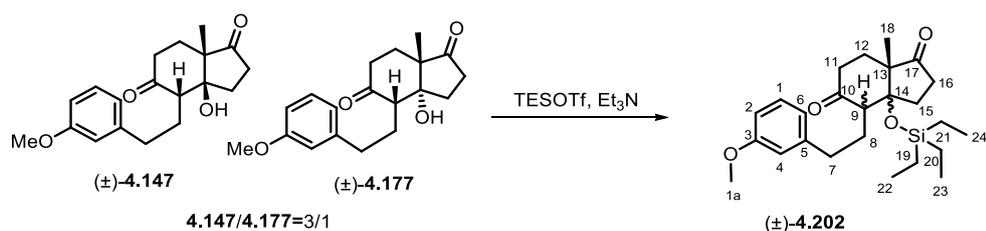
Et$_3$N (194 μL, 1.39 mmol) was added to a solution of carbinols **4.147** and **4.177** (about 3/1, 200 mg, 0.63 mmol) in dry CH$_2$Cl$_2$ (5 mL) stirred under an argon atmosphere. The reaction mixture was cooled at 0 °C, then TESOTf (314 μL, 1.39 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 10 min. The ice-bath was removed and the mixture was stirred at rt for a further 30 min before quenching with H$_2$O (5 mL). The organic layer was separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 10 mL). Combined organic layers were dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The residue oil was purified by flash column chromatography (Biotage SNAP 25 g SiO$_2$ cartridge, heptane/AcOEt= 10/0 to 4/1) to obtain the title compound **4.202** (54.0 mg, 20%) as a colorless oil. **Ref. LNB0103-055**

R$_f$: 0.31 (heptane/AcOEt = 4/1); **¹H-NMR** (400 MHz, CDCl$_3$), δ: 7.19 (1H, "t", *J* = 7.9 Hz, H-1), 6.76-6.66 (3H, m, H-2, H-4, H-6), 3.87 (3H, s, 3H-1a), 2.78-2.69 (1H, m, H-7), 2.48-2.28 (3H, m, H-7, H-11, H-16), 2.25-2.13 (3H, m, H-8, H-9, H-11), 2.06-1.90 (4H, m, H-12, 2H-15, H-16), 1.85-1.74 (1H, m, H-8), 1.67-1.55 (1H, m, H-12), 1.09 (3H, s, 3H-18), 0.92 (9H, t, *J* = 7.8 Hz, 3H-19, 3H-20, 3H-21), 0.58 (6H, q, *J* = 7.8 Hz, 2H-22, 2H-23, 2H-24); **¹³C-NMR** (100 MHz, CDCl$_3$), δ: 217.6 (C, C-17), 210.5 (C, C-10), 159.9 (C, C-3), 143.1 (C, C-5), 129.6 (CH, C-1), 121.0 (CH, C-6), 114.4 (CH, C-4), 111.6 (CH, C-2), 87.0 (C, C-14), 56.9 (CH, C-9), 55.3 (CH$_3$, C-1a), 54.7 (C, C-13), 36.3 (CH$_2$, C-11), 34.2 (CH$_2$, C-7), 33.7 (CH$_2$, C-16), 31.7 (CH$_2$, C-15), 29.5 (CH$_2$, C-12), 27.1 (CH$_2$, C-8), 17.9 (CH$_3$, C-18), 7.3 (3CH$_3$, C-22, C-23, C-24), 6.9 (3CH$_2$, C-19, C-20, C-21); **IR** (neat): 2957, 1742, 1721, 1259, 741 cm⁻¹; **HRMS** (ESI): calculated for C$_{25}$H$_{38}$NaO$_4$Si [MNa⁺] requires 453.2432, found 453.2422 (2.4 ppm error).

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2-[3-(3-Methoxyphenyl)propyl]-5-methyl-2-[(triethylsilyl)oxy]bicyclo[3.2.1]octane-6,8-dione 4.194
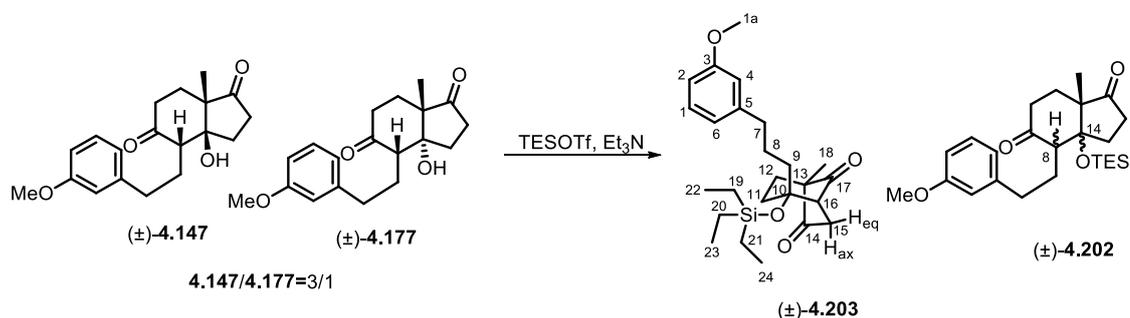
Carbinols **4.147** and **4.177** (about 3/1, 214 mg, 0.68 mmol) were dissolved in CH_2Cl_2 (6 mL) under an argon atmosphere, and Et_3N (207 µL, 1.49 mmol) was added. The reaction mixture was cooled at 0 °C, then TESOTf (336 µL, 1.49 mmol) was added slowly. The reaction mixture was maintained at 0 °C for 30 min then H_2O (10 mL) was added. After warming up to rt, the aqueous layer was separated and the organic phase was washed with H_2O (3 x 15 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatoghaphy (Biotage SNAP 25 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 4/1) to afford the bridged silylether **4.203** (40.0 mg, 14%), and the isomer **4.202** (20.0 mg, 7%) as clear oils. **Ref. LNB0103-087**

R_f: 0.66 (hexane/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.23-7.17 (1H, m, H-1), 6.78 (1H, d, J = 7.6 Hz, H-6), 6.76-6.71 (2H, m, H-2, H-4), 3.81 (3H, s, 3H-1a), 3.02-2.87 (2H, m, H-15$_{ax}$, H-16), 2.69-2.48 (3H, m, 2H-7, H-15$_{eq}$), 2.03-1.88 (1H, m, H-8), 1.81-1.41 (7H, m, H-8, 2H-9, 2H-11, 2H-12), 1.04 (3H, s, 3H-18), 0.92 (9H, t, J = 7.9 Hz, 3H-22, 3H-23, 3H-24), 0.63-0.48 (6H, m, 2H-19, 2H-20, 2H-21); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 213.1 (C, C-17), 211.7 (C, C-14), 159.8 (C, C-3), 147.3 (C, C-5), 129.4 (CH, C-1), 121.0 (CH, C-6), 114.3 (CH, C-4), 111.4 (CH, C-2), 81.6 (C, C-10), 58.8 (C, C-13), 56.1 (CH, C-16), 55.3 (CH$_3$, C-1a), 41.4 (CH$_2$, C-15), 38.5 (CH$_2$, C-9), 36.2 (CH$_2$, C-7), 36.0 (CH$_2$, C-12), 33.7 (CH$_2$, C-11), 24.7 (CH$_2$, C-8), 11.7 (CH$_3$, C-18), 7.26 (3CH$_3$, C-22, C-23, C-24), 6.76 (3CH$_2$, C-19, C-20, C-21); **IR** (neat): 2953, 2876, 1742, 1727, 1223, 729, 693 cm^{-1}; **HRMS** (ESI): calculated for C$_{25}$H$_{38}$NaO$_4$Si [MNa$^+$] requires 453.2425, found 453.2422 (1.9 ppm error).

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2-[3-(3-Methoxyphenyl)propyl]-5-methyl-2-[(triethylsilyl)oxy]bicyclo[3.2.1]octane-6,8-dione 4.204
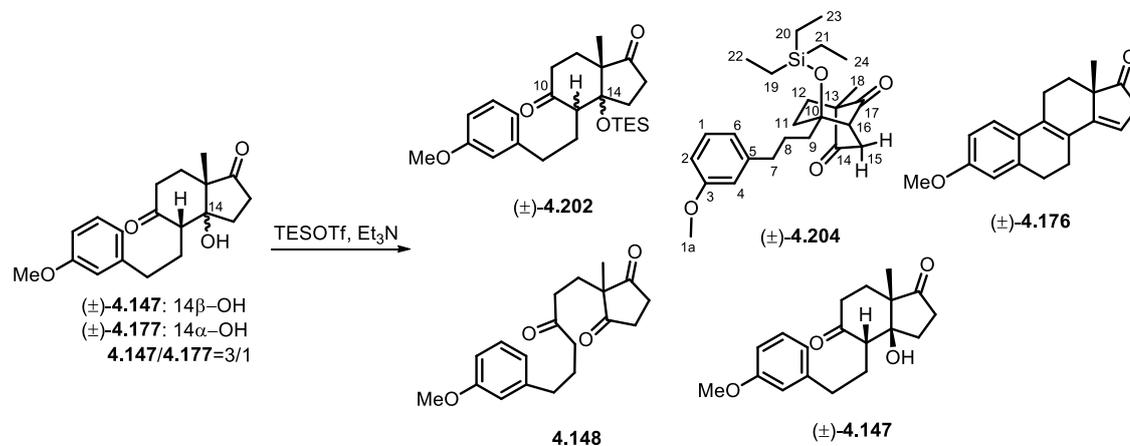
Et₃N (498 μL, 3.58 mmol) and TESOTf (808 μL, 3.58 mmol) were added to a solution of carbinols **4.147** and **4.177** (about 3/1, 942 mg, 2.98 mmol) in dry CH_2Cl_2 (10 mL) cooled at 0 °C and stirred under an argon atmosphere. The reaction mixture was stirred at 0 °C for 1 h, then it was quenched with H_2O (5 mL) and diluted with CH_2Cl_2 (10 mL). The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatoghaphy (Biotage SNAP 25 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 3/2) to afford silylether **4.202** (168 mg, 13%) as a clear oil, the bridged silylether **4.204** (8.00 mg, 6%) as a clear oil, dienone **4.176** (342 mg, 36%) as a white solid , triketone **4.148** (100 mg, 11%) as a clear oil and recovered *cis*-carbinol **4.174** (214 mg) as a white solid. **Ref. LNB0103-084**

*R*f: 0.55 (hexane/AcOEt = 4 /1); **¹H-NMR** (400 MHz, CDCl₃), δ: 7.21 (1H, "t", *J* = 7.2 Hz, H-1), 6.78-6.68 (3H, m, H-2, H-4, H-6), 3.80 (3H, s, 3H-1a), 2.86 (1H, d, *J* = 6.7 Hz, H-16), 2.62-2.53 (2H, m, 2H-7), 2.49-2.42 (2H, m, 2H-15), 2.02 (1H, td, *J* = 12.7, 6.4 Hz, H-12), 1.82-1.52 (7H, m, 2H-8, 2H-9, 2H-11), 1.04 (3H, s, 3H-18), 0.94 (9H, t, *J* = 7.9 Hz. 3H-22, 3H-23, 3H-24), 0.66-0.55 (6H, m, 2H-19, 2H-20, 2H-21); **¹³C-NMR** (100 MHz, CDCl₃), δ: 212.4 (C, C-17), 211.8 (C, C-14), 159.9 (C, C-3), 147.4 (C, C-5), 129.6 (CH, C-1), 120.9 (CH, C-6), 114.4 (CH, C-4), 111.3 (CH, C-2), 85.3 (C, C-10), 58.4 (C, C-13), 55.6 (CH, C-16), 55.3 (CH₃, C-1a), 42.4 (CH₂, C-15), 39.9 (CH₂, C-9), 38.0 (CH₂, C-12), 36.2 (CH₂, C-7), 32.2 (CH₂, C-11), 25.0 (CH₂, C-8), 12.1 (CH₃, C-18), 7.29 (3CH₃, C-22, C-23, C-24), 6.77 (3CH₂, C-19, C-20, C-21); **IR** (neat): 2953, 2884, 1742, 1772,

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1732, 1255, 1008, 741 cm^{-1}; **HRMS** (ESI): calculated for $C_{25}H_{38}NaO_4Si$ [MNa$^+$] requires 453.2432, found 453.2427 (1.2 ppm error).

(14ξ)-14,17-bis{[*tert*-Butyl(dimethyl)silyl]oxy}-1-methoxy-9,10-secoestra-1(10),2,4,16-tetraen-9-one 4.211 and (14ξ)-17-{[*tert*-butyl(dimethyl)silyl]oxy}-14-hydroxy-1-methoxy-9,10-secoestra-1(10),2,4,16-tetraen-9-one 4.212

Lutidine (26.0 µL, 0.23 mmol) and TBSOTf (44.0 µL, 0.19 mmol) were added subsequently to a solution of alcohols **4.147** and **4.177** (about 3/1, 61.0 mg, 0.19 mmol) dissolved in CH_2Cl_2 (800 µL) under an argon atmosphere. The reaction mixture was stirred at rt overnight, quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3 x 10 The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by repeated flash column chromatography (Biotage SNAP 10 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 7/3, then Biotage SNAP 10 g SiO_2 cartridge, heptane/AcOEt = 3/2) to give TBS ethers **4.211** (6.00 mg, 6% contaminated by unidentified by-products) and **4.212** (7.00 mg, 9% contaminated by unidentified by-products) as white solids. **Ref. LNB0103-097**

Characterisation of bis-silylether 4.211: R_f: 0.76 (heptane/AcOEt = 3/2); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.15-7.08 (1H, m, H-1), 6.75-6.63 (3H, m, H-4, H-6, H-2), 4.21 (1H, brs, H-16), 3.73 (3H, s, 3H-1a), 2.82-2.72 (1H, m, H-7), 2.50 (1H, d, *J* = 9.8 Hz, H-9), 2.38-2.10 (6H, m, H-7, H-8, 2H-11, 2H-15), 2.87-1.79 (1H, m, H-12), 1.70-1.53 (2H, m, H-8, H-12), 0.96 (3H, s, 3H-18), 0.86 (9H, s, 3H-22, 3H-23, 3H-24), 0.77 (9H, s, 3H-28, 3H-29, 3H-30), 0.09 (3H, s, 3H-19 or 3H-20), 0.07 (3H, s, 3H-25 or 3H-26), 0.07 (3H, s, 3H-19 or 3H-20), 0.01 (3H, s, 3H-25 or 3H-26); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 212.7 (C, C-10), 159.7 (C, C-3), 159.0 (C, C-17), 144.1 (C, C-5), 129.3 (CH, C-1), 121.6 (CH, C-6), 114.4 (CH, C-4), 111.2 (CH, C-2), 95.0 (CH, C-16), 87.0 (C, C-14), 55.4 (CH$_3$, C-1a), 55.3 (CH, C-9), 50.6 (C, C-13), 39.2 (CH$_2$, C-15), 36.2 (CH$_2$, C-11), 35.3 (CH$_2$, C-

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7), 30.4 (CH$_2$, C-12), 26.4 (CH$_2$, C-8), 26.3 (3CH$_3$, C-28, C-29, C-30), 25.6 (3CH$_3$, C-22, C-23, C-24), 21.0 (CH$_3$, C-18), 18.8 (C, C-27), 18.3 (C, C-21), -1.6 (CH$_3$, C-19 or C-20), -1.7 (CH$_3$, C-19 or C-20), -4.7 (CH$_3$, C-25 or C-26), -4.8 (CH$_3$, C-25 or C-26); **HRMS** (ESI): calculated for C$_{31}$H$_{52}$NaO$_4$Si$_2$ [MNa$^+$] requires 567.3296, found 567.3293 (-0.5 ppm error); **IR** spectrum could not be recorded because compound **4.211** decomposed.

*Characterisation of mono-silylether **4.212**:* **R**$_f$: 0.35 (heptane/AcOEt = 3/2); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.25-7.19 (1H, m, H-1), 6.83 (1H, d, *J* = 7.9 Hz, H-6), 114.4 (1H, brs, H-4), 111.7 (1H, d, *J* = 7.9 Hz, H-2), 4.35 (1H, brs, H-16), 3.80 (3H, s, 3H-1a), 2.85-2.74 (1H, m, H-7), 2.58 (1H, d, *J* = 8.5 Hz, H-9), 2.50-2.41 (1H, m, H-7), 2.37-2.25 (2H, m, H-11, H-15), 2.22-2.02 (3H, m, H-8, H-11, H-15), 1.92-1.79 (2H, m, H-12, H-8), 1.79-1.70 (1H, m, H-12), 1.14 (3H, s, 3H-18), 0.92 (9H, s, 3H-22, 3H-23, 3H-24), 0.16 (3H, s, 3H-19 or 3H-20), 0.14 (3H, s, 3H-19 or 3H-20); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 210.5 (C, C-10), 159.7 (C, C-3), 156.2 (C, C-17), 144.5 (C, C-5), 129.9 (CH, C-1), 121.3 (CH, C-6), 114.4 (CH, C-4), 111.7 (CH, C-2), 96.6 (CH, C-16), 82.2 (CH, C-14), 55.4 (CH$_3$, C-1a), 56.5 (CH, C-9), 51.9 (C, C-13), 39.1 (CH$_2$, C-15), 37.1 (CH$_2$, C-11), 35.2 (CH$_2$, C-7), 30.3 (CH$_2$, C-12), 25.8 (3CH$_3$, C-22, C-23, C-24), 25.2 (CH$_2$, -8), 18.5 (CH$_3$, C-18), 18.3 (C, C-21), -5.0 (CH$_3$, C-19 or C-20); **HRMS** (ESI): calculated for C$_{25}$H$_{38}$NaO$_4$Si [MNa$^+$] requires 453.2432, found 453.2435 (-0.4 ppm error); **IR** spectrum could not be recorded because compound **4.212** decomposed.

(14β)-1-Methoxy-9,17-dioxo-9,10-secoestra-1(10),2,4-trien-14-yl acetate 4.215

(±)-**4.147**: 14β–OH
(±)-**4.177**: 14α–OH
4.147/4.177=3/1
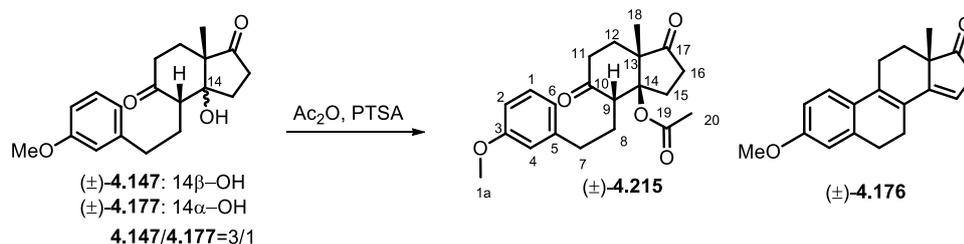
A solution of PTSA (5.77 mg, 0.03 mmol) in Ac$_2$O (113 µL) was added to a solution of carbinols **4.147** and **4.177** (about 3/1, 96.0 mg, 0.30 mmol) in Ac$_2$O (560 µL). The reaction mixture was stirred at rt under a nitrogen atmosphere atmosphere for 1 d. The reaction was quenched with H$_2$O (10 mL) and extracted with CH$_2$Cl$_2$ (3 x 10 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 25

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g SiO$_2$, heptane/AcOEt = 10/0 to 5/5) to give title compound **4.215** (52.0 mg, 48%) as a colourless oil and dienone **4.176** (9.00 mg, 11%) as a white solid. **Ref. LNB0103-012**

R$_f$: 0.35 (heptane/AcOEt = 5/5); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.21 (1H, "t", *J* = 7.6 Hz, H-1), 6.80 (1H, d, *J* = 7.6 Hz, H-6), 6.77-6.73 (2H, m, H-2, H-4); 3.81 (3H, s, 3H-1a), 3.48 (1H, d, *J* = 9.8 Hz, H-9), 2.86-2.75 (1H, m, H-7), 2.62-2.50 (2H, m, H-11, H-16), 2.47-2.38 (1H, m, H-7), 2.38-2.28 (2H, m, H-11, H-16), 2.25-2.10 (2H, m, H-8, H-15), 2.01 (3H, s, 3H-20), 1.96-1.88 (1H, m, H-15), 1.88-1.79 (1H, m, H-12), 1.73-1.57 (2H, m, H-8, H-12), 1.25 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 216.0 (C, C-17), 208.2 (C, C-10), 170.5 (C, C-9), 159.4 (C, C-3), 143.1 (C, C-5), 129.5 (CH, C-1), 121.0 (CH, C-6), 114.5 (CH, C-4), 111.3 (CH, C-2), 94.9 (C, C-14), 54.0 (CH, C-9), 55.3 (CH$_3$, C-1a), 53.8 (C, C-13), 37.2 (CH$_2$, C-11), 34.7 (CH$_2$, C-7), 33.8 (CH$_2$, C-16), 31.2 (CH$_2$, C-12), 28.5 (CH$_2$, C-15), 25.2 (CH$_2$, C-8), 16.1 (CH$_3$, C-18), 21.9 (CH$_3$, C-20); **IR** (neat): 2936, 1738, 1714, 1245, 1021 cm^{-1}; **HRMS** (ESI): calculated for C$_{21}$H$_{26}$NaO$_5$ [MNa$^+$] requires 381.1672, found 381.1682 (2.5 ppm error).

(14β)-1-Methoxy-14-[(triethylsilyl)oxy]-9,10-secoestra-1(10),2,4-triene-9,17-dione 4.209
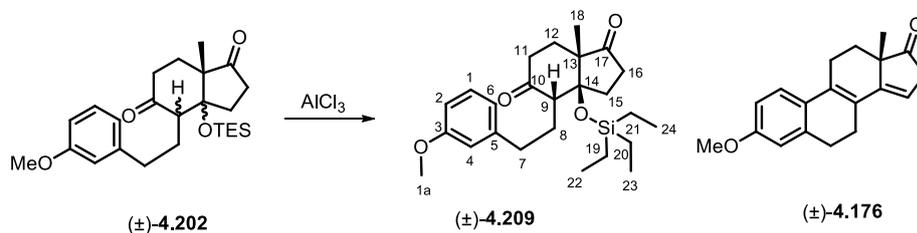
Triethylsilyl ether **4.202** (200 mg, 0.47 mmol) was dissolved in CH$_2$Cl$_2$ (6 mL) and the solution was cooled to 0 °C. AlCl$_3$ (62.0 mg, 0.47 mmol) was added portionwise and the reaction mixture was stirred at 0 °C for 2 h and at rt overnight. The reaction was quenched with H$_2$O (10 mL). The organic layer was separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 3 ml). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 25 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 4/1) to yield dienone **4.176** (60.0 mg, 46%) as a white solid and isomeric triethylsilyl ether **4.209** (15.0 mg, 7%) as a yellow gum. **Ref. LNB0103-086**

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R_f: 0.40 (heptane/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.22 (1H, "t", J = 7.9 Hz, H-1), 6.81 (1H, d, J = 7.9 Hz, H-6), 6.79-6.73 (2H, m, H-2, H-4), 3.81 (3H, s, 3H-1a), 2.82 (1H, "dt", J = 12.2, 4.3 Hz, H-7), 2.65 (1H, d, J = 11.0 Hz, H-9), 2.56 (1H, td, J = 13.7, 6.1 Hz, H-11), 2.47-2.27 (4H, m, H-7, H-11, 2H-16), 2.15-1.98 (1H, m, H-8), 1.94-1.83 (1H, m, H-15), 1.79-1.69 (2H, m, H-8, H-15), 1.68-1.60 (1H, m, H-12), 1.57-1.46 (1H, m, H-12), 1.29 (3H, s, 3H-18), 0.91 (9H, t, J = 7.9 Hz, 3H-22, 3H-23, 3H-24), 0.64-0.53 (6H, m, 2H-19, 2H-20, 2H-21); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 218.7 (C, C-17), 209.0 (C, C-10), 159.9 (C, C-3), 143.9 (C, C-5), 129.5 (CH, C-1), 120.9 (CH, C-6), 114.1 (CH, C-4), 111.6 (CH, C-2), 89.0 (C, C-14), 57.3 (CH, C-9), 55.3 (2C, C, C-13, CH$_3$, C-1a,), 37.4 (CH$_2$, C-11), 35.2 (CH$_2$, C-7), 33.2 (CH$_2$, C-16), 30.3 (CH$_2$, C-12), 28.9 (CH$_2$, C-15), 25.4 (CH$_2$, C-8), 14.1 (CH$_3$, C-18), 7.3 (3CH$_3$, C-22, C-23, C-24), 7.0 (3CH$_2$, C-19, C-20, C-21); **IR** (neat): 2963, 1745, 1721, 1714, 1073, 741 cm^{-1}; **HRMS** (ESI): calculated for C$_{25}$H$_{38}$NaO$_4$Si [MNa$^+$] requires 453.2432, found 453.2429 (0.7 ppm error).

(14β)-3-Methoxyestra-1,3,5(10),8,15-pentaen-17-one 4.218[179]
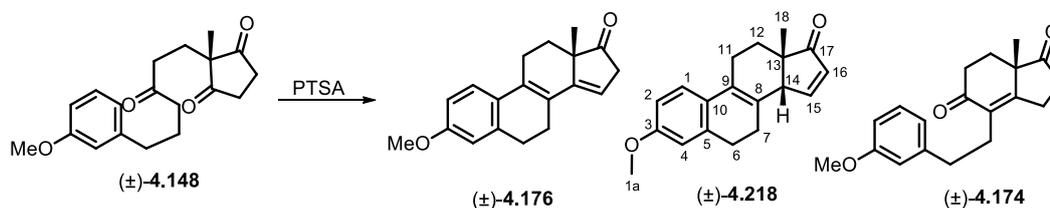
A solution of triketone **4.148** (1.00 g, 3.16 mmol) in benzene (150 mL) containing PTSA (301 mg, 1.58 mmol) was heated at 95 °C for 30 min with a Dean-stark water separator attached. The reaction mixture was allowed to reach rt and quenched with sat aq NaHCO$_3$ (30 mL). Benzene was evaporated under reduced pressure and the residual aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 30 ml). The combined organic layers were washed with H$_2$O (40 ml), dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 25 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 3/2) to yield dienone **4.176** (547 mg, 62%) as a white solid, dienone **4.218** (55.0 mg, 6% yield) as pink solid and enedione **4.174** (108 mg, 11%) as a yellow oil. **Ref. LNB0103-120**

R_f: 0.44 (heptane/AcOEt = 4/1); **m.p.**: 120-123 °C [lit. 133-135 °C, enatiomerically pure **4.202** isolated as a white solid];[192] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.64 (1H, dd, J = 5.5,

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2.4 Hz, H-15), 7.08 (1H, d, J = 8.5 Hz, H-1), 6.75-6.68 (2H, m, H-2, H-4), 6.15-6.10 (1H, m, H-16), 3.80 (3H, s, 3H-1a), 3.15 (1H, brs, H-14), 2.90-2.79 (1H, m, H-6), 2.78-2.70 (1H, m, H-6), 2.49-2.37 (2H, m, H-7, H-11), 2.30-2.20 (1H, m, H-7), 2.18-2.08 (1H, m, H-11), 1.98-1.87 (1H, m, H-12), 1.64-1.54 (1H, m, H-12), 1.20 (3H, s, 3H-18); **13C-NMR** (100 MHz, CDCl$_3$), δ: 214.5 (C, C-17), 162.7 (CH, C-15), 158.4 (CH, C-3), 136.8 (C, C-5), 130.7 (CH, C-16), 129.7 (C, C-9), 128.8 (C, C-8), 128.5 (C, C-10), 123.5 (CH, C-1), 113.7 (CH, C-4), 111.1 (CH, C-2), 55.6 (CH, C-14), 55.4 (CH$_3$, C-1a), 47.0 (C, C-13), 31.7 (CH$_2$, C-12), 28.8 (CH$_2$, C-6), 27.6 (CH$_2$, C-7), 22.6 (CH$_2$, C-11), 22.4 (CH$_3$, C-13); **IR** (neat): 2925, 2830, 1703, 1252 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{20}$NaO$_2$ [MNa$^+$] requires 303.1356, found 303.1343 (4.0 ppm error). Data are consistent with those reported in the literature for the enantiopure 13(*S*),14(*R*)-compound.[192]

(17β)-3-Methoxyestra-1,3,5(10),8,14-pentaen-17-ol 4.230[206]
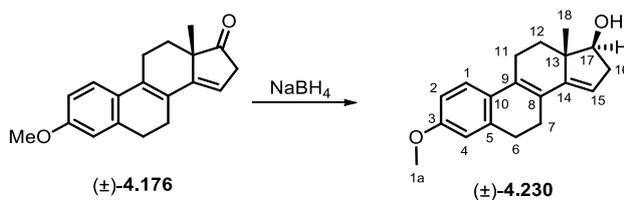
NaBH$_4$ (7.50 mg, 0.20 mmol) was added to a suspension of dienone **4.176** (22.0 mg, 0.08 mmol) in EtOH (1.5 mL) cooled at 0 °C. The reaction mixture was stirred at 0 °C for 5 min, then brought to rt and stirred for a further 30 min. The mixture was re-cooled to 0 °C and it was quenched with AcOH (7.00 μL). The solvents were evaporated under reduced pressure and the residue was purified by flash column chromatography (Biotage SNAP 10 g SiO$_2$ cartridge, heptane/AcOEt = 7/30) to give alcohol **4.230** (20.0 mg, 90%) as a white solid. **Ref. LNB0145-005**

R$_f$: 0.40 (heptane/AcOEt = 7/3); **m.p.**: 110-112 °C (MeOH) [lit. 110-111 °C (MeOH/H$_2$O)];[208] **1H-NMR** (400 MHz, CDCl$_3$), δ: 7.24 (1H, d, J = 8.1 Hz, H-1), 6.77-6.70 (2H, m, H-2, H-4), 5.50 (1H, brs, H-15), 4.09 (1H, "t", J = 8.3 Hz, H-17), 3.81 (3H, s, 3H-1a), 2.76 (2H, dd, J = 9.1, 6.6 Hz, 2H-6), 2.71-2.56 (3H, m, H-16, 2H-11), 2.56-2.47 (1H, m, H-7), 2.46-2.39 (1H, m, H-16), 2.29-2.19 (1H, m, H-7), 2.05 (1H, dd, J = 12.6, 4.0 Hz, H-12), 1.52 (1H, "dt", J = 12.1, 6.8 Hz, H-12), 0.97 (3H, s, 3H-18); **13C-NMR** (100 MHz, CDCl$_3$), δ: 158.5 (C, C-3), 147.3 (C, C-14), 138.2 (C, C-5), 129.4 (C, C-9), 129.3 (C, C-10), 126.0 (C, C-8), 124.1 (CH, C-1), 116.8 (CH, C-15), 113.6 (CH,

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C-4), 111.2 (CH, C-2), 82.4 (CH, C-17), 55.4 (CH$_3$, C-1a), 45.2 (C, C-13), 38.5 (CH$_2$, C-16), 34.0 (CH$_2$, C-12), 28.7 (CH$_2$, C-6), 23.8 (CH$_2$, C-11), 23.1 (CH$_2$, C-7), 14.9 (CH$_2$, C-18); **IR** (neat): 3383, 2921, 2834, 1605, 1497 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{22}$NaO$_2$ [MNa$^+$] requires 305.1512, found 305.1510 (1.0 ppm error). Data are consistent with those reported in the literature.[207, 208]

3-Methoxyestra-1,3,5(10),8-tetraen-17-one 4.234

(±)-**4.176**   (±)-**4.234**
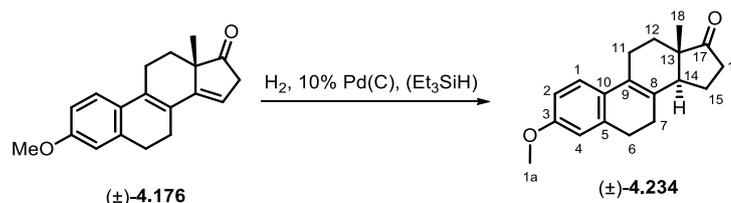
Catalytic hydrogenation without triethylsylane

Dienone **4.176** (20.0 mg, 0.07 mmol) was dissolved in AcOEt (3.2 mL) and 10% Pd(C) (5.00 mg) was added. The flask was evacuated and recharged with H$_2$ three times. The reaction mixture was then stirred at rt under a H$_2$ atmosphere for 30 min. The catalyst was filtered off through a pad of Celite® and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 4 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 4/1) to obtain ketone **4.234** (5.00 mg, 25%) as a white solid. **Ref. LNB0103-118**

R_f: 0.51 (hexane/AcOEt = 4/1); **m.p.**: 118-120 °C (MeOH) [lit. 120-123 °C (MeOH/Et$_2$O=3/1)];[210] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.14 (1H, d, J = 7.1 Hz, H-1), 6.75-6.70 (2H, m, H-2, H-4), 3.80 (3H, s, 3H-1a), 2.85-2.65 (3H, m, 2H-6, H-14), 2.62-2.52 (3H, m, 2H-11, H-16), 2.34-2.09 (4H, m, 2H-7, H-15, H-16), 2.07-2.00 (1H, m, H-12), 1.82-1.60 (2H, m, H-12, H-15), 0.90 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 219.9 (C, C-17), 158.2 (C, C-3), 137.3 (C, C-5), 130.7 (C, C-8), 128.8 (C, C-10), 126.6 (C, C-9), 123.2 (CH, C-1), 113.8 (CH, C-4), 110.9 (CH, C-2), 55.4 (CH$_3$, C-1a), 47.8 (C, C-13), 47.4 (CH, C-14), 36.7 (CH$_2$, C-16), 29.0 (CH$_2$, C-12), 28.7 (CH$_2$, C-6), 24.5 (CH$_2$, C-7), 23.9 (CH$_2$, C-11), 21.3 (CH$_3$, C-18); **IR** (neat): 2932, 1737, 1499, 1253 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{22}$NaO$_2$ [MNa$^+$] requires 305.1512, found 305.1511 (-4.4 ppm error). Data are consistent with those reported in the literature except for the ^{13}C-NMR assignment of C-8 and C-10.[210]

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Catalytic hydrogenation in the presence of triethylsylane[209]

A solution of dienone **4.176** (30.0 mg, 0.11 mmol) in benzene (3 mL) was cooled to 0 °C and 10% Pd/C (4.50 mg) and Et_3SiH (341 µL, 2.14 mmol) were subsequently added. The reaction mixture stirred was maintained at 0 °C, and the flask was evacuated and recharged with H_2 three times. The mixture was allowed to slowly reach rt and stirred under a H_2 atmosphere overnight. Solids were filtrated through a pad of Celite®, and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 4 g SiO_2 cartridge, heptane/AcOEt = 9/1) to obtain ketone **4.234** (17.0 mg, 56%) as a white solid. **Ref. LNB0103-146**

(13*S*)-3-Methoxy-13-methyl-6,7,11,12,13,16-hexahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolane] 4.235
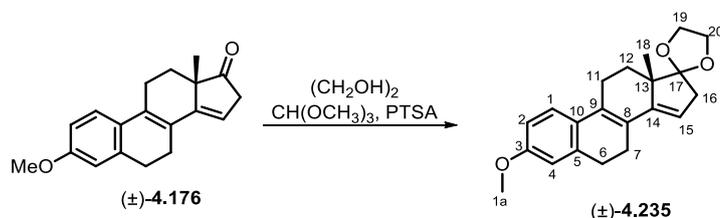
A mixture of dienone **4.176** (312 mg, 1.11 mmol), ethylene glycol (151 µL, 2.79 mmol), trimethyl ortoformate (243 mL, 2.23 mmol) and PTSA (2.1 mg, 0.001 mmol) was genlty warmed to 30 °C and stirred overnight. The mixture was cooled to rt and diluted with AcOEt (15 mL). The organic layer was washed with sat aq $NaHCO_3$ (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 25 g silica cartridge, heptane/AcOEt = 10/0 to 4/1) to afford the protected diene **4.235** (327 mg, 91%) as a white foam. **Ref. LNB0103-129**

*R*f: 0.38 (heptane/AcOEt = 4/1); **m.p.**: 91-95 °C (MeOH) (lit. 94-97.5 °C);[212] **¹H-NMR** (400 MHz, CDCl₃), δ: 7.26-7.23 (1H, brs, H-1), 6.76-6.69 (2H, m, H-2, H-4), 5.62 (1H, brs, H-15), 4.04-3.93 (4H, m, 2H-19, 2H-20), 3.81 (3H, s, 3H-1a), 2.84 (1H, d, *J* = 17.2 Hz, H-16), 2.78-2.71 (2H, m, 2H-6), 2.64-2.48 (4H, m, H-7, 2H-11, H-16), 2.29-2.16 (1H, m, H-7), 2.10-2.00 (1H, m, H-12), 1.61-1.53 (1H, m, H-12), 1.06 (3H, s, 3H-18); **¹³C-NMR** (100 MHz, CDCl₃), δ: 159.3 (C, C-3), 147.2 (C, C-14), 138.3 (C, C-5), 129.8 (C, C-9), 129.0 (C, C-10), 125.8 (C, C-8), 123.8 (CH, C-1), 116.1 (CH, C-15), 113.4 (CH, C-4), 111.4 (CH, C-2), 54.7 (CH₃, C-1a), 48.2 (C, C-13), 41.0 (CH₂, C-16), 28.6 (CH₂,

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C-6), 26.7 (CH$_2$, C-12), 23.3 (CH$_2$, C-11), 22.7 (CH$_2$, C-7), 18.0 (CH$_3$, C-18); **IR** (neat): 2928, 1549, 1493, 1243 cm^{-1}; **HRMS** (ESI): calculated for C$_{21}$H$_{24}$NaO$_3$ [MNa$^+$] requires 347.1618, found 347.1625 (-4.4 ppm error).

(13*S*,14*R*)-13-Methyl-6,7,11,12,13,14,15,16-octahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3-ol 4.237
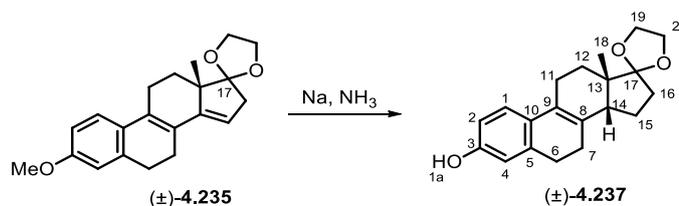
In a three necked round flask cooled to -40 °C liq NH$_3$ (15 mL) was trapped. Metallic sodium (360 mg, 15.7 mmol) was cut in small pieces and slowly added. After 30 min diene **4.235** (127 mg, 0.39 mmol) was dissolved in THF (2 mL) and added dropwise to the solution of sodium in ammonia. After 1 h of stirring at -40 °C, MeOH (2.4 mL) was added until the blue colour faded. NH$_3$ was evaporated under a stream of Ar and the reaction mixture was slowly allowed to reach rt. H$_2$O (10 mL) was added and the organic compounds were extracted with AcOEt (3 x 5 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 10 g SiO$_2$ cartridge, heptane/AcOEt = 4/1) to afford phenol **4.237** (58.5 mg, 48%) as a white foam. **Ref. LNB0103-138**

R$_f$: 0.30 (heptane/AcOEt = 4/1); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.08 (1H, d, *J* = 8.4 Hz, H-1), 6.65 (1H, dd, *J* = 8.4, 2.1 Hz, H-2), 6.61 (1H, brs, H-4), 4.01-3.91 (4H, m, 2H-19, 2H-20), 2.73-2.65 (2H, m, 2H-6), 2.39-2.31 (2H, m, 2H-11), 2.24-2.03 (5H, m, 2H-7, H-14, H-15, H-16), 1.89-1.81 (1H, m, H-16), 1.73-1.68 (1H, m, H-12), 1.65-1.55 (1H, m, H-12), 1.55-1.46 (1H, m, H-15), 0.90 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 153.9 (C, C-3), 138.1 (C, C-5), 134.5 (C, C-8), 129.7 (C, C-10), 123.9 (C, C-9), 123.4 (CH, C-1), 120.4 (C, C-17), 114.9 (CH, C-4), 111.3 (CH, C-2), 65.6 (CH$_2$, C-19 or C-20), 64.5 (CH$_2$, C-19 or C-20), 47.7 (CH, C-14), 44.5 (C, C-13), 33.1 (CH$_2$, C-16), 29.1 (CH$_2$, C-6), 28.7 (CH$_2$, C-7), 27.7 (CH$_2$, C-15), 27.3 (CH$_2$, C-12), 22.6 (CH$_2$, C-11), 16.4 (CH$_3$, C-18); **IR** (neat): 3361, 2936, 1610, 1493 cm^{-1}; **HRMS** (ESI): calculated for C$_{20}$H$_{24}$NaO$_3$ [MNa$^+$] requires 335.1618, found 335.1627 (-3.5 ppm error).

(14β,17α)-3-Methoxyestra-1,3,5(10),8-tetraen-17-ol 4.238
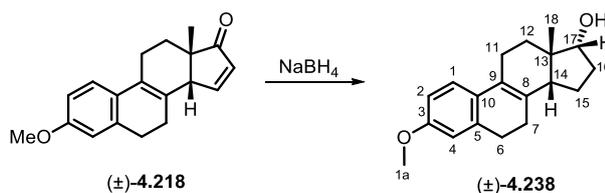
NaBH₄ (35.5 mg, 0.96 mmol) was added to a solution of dienone **4.218** (67.0 mg, 0.24 mmol) in methanol (15 mL) cooled to 0 °C. The reaction mixture was stirred at 0 °C for 5 min, then brought to rt and stirred for 3 h. The reaction mixture was re-cooled to 0 °C before being quenched with H_2O (30 mL). The pH was brought to 5 with 1 M aq HCl and the organic compounds were extracted with AcOEt (3 x 20 mL). The combined organic layers were washed with sat aq $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, heptane/AcOEt = 7/3) to give alcohol **4.238** (65.0 mg, 95%) as a white solid. **Ref. LNB0162-114**

*R*f: 0.33 (heptane/AcOEt = 7/3); **m.p.**: 131-133 °C (hexane) [lit. 131-133 °C (cyclohexane)];[213] **¹H-NMR** (400 MHz, CDCl₃), δ: 7.15 (1H, t, *J* = 8.8 Hz, H-1), 6.75-6.67 (2H, m, H-2, H-4), 3.89 (1H, t, *J* = 8.8 Hz, H-17), 3.80 (3H, s, 3H-1a), 2.77-2.68 (2H, m, 2H-6), 2.43-2.34 (2H, m, 2H-11), 2.23-1.99 (4H, m, 2H-7, H-15, H-16), 1.95 (1H, t, *J* = 8.7 Hz, H-14), 1.77-1.64 (1H, m, H-16), 1.59-1.48 (3H, m, 2H-12, H-15), 1.01 (3H, s, 3H-18); **¹³C-NMR** (100 MHz, CDCl₃), δ: 157.9 (C, C-3), 137.2 (C, C-5), 134.4 (C, C-8), 129.5 (C, C-10), 124.3 (C, C-9), 123.0 (CH, C-1), 113.6 (CH, C-4), 111.0 (CH, C-2), 82.4 (CH, C-17), 55.4 (CH₃, C-1a), 48.1 (CH, C-14), 41.8 (C, C-13), 30.6 (CH₂, C-16), 29.1 (CH₂, C-6), 28.1 (CH₂, C-7), 27.7 (CH₂, C-15), 23.2 (CH₂, C-12), 22.4 (CH₂, C-11), 22.1 (CH₃, C-18); **IR** (neat): 3381, 2933, 2876, 1610, 1497, 1249 cm⁻¹; **HRMS** (ESI): calculated for $C_{19}H_{24}NaO_2$ [MNa⁺] requires 307.1669, found 307.1665 (2.4 ppm error).

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(14β)-3-Methoxyestra-1,3,5(10),8-tetraen-17-one 4.240
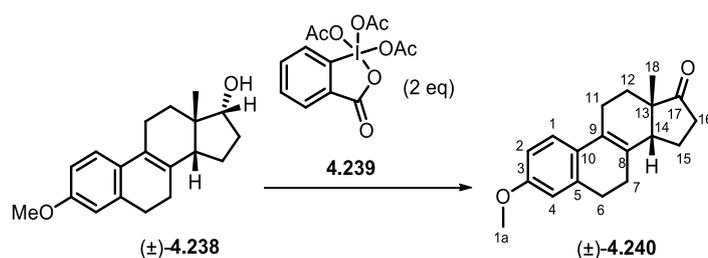
A solution of alcohol **4.238** (13.0 mg, 0.05 mmol) in CH_2Cl_2 (500 μL) was added to a suspension of Dess-Martin periodinane **4.239** (38.8 mg, 0.09 mmol) in CH_2Cl_2 (0.5 mL). The reaction mixture was stirred at rt for 3 h, then diluted with CH_2Cl_2 (15 mL) and 5% aq NaOH was added until the suspension turned into a solution. The organic layer was separated, washed with H_2O (2 x 5 mL) and brine (5 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (Biotage SNAP 4 g SiO_2 cartridge, heptane/AcOEt = 10/0 to 4/1) to give ketone **4.240** (6.00 mg, 46%) as a withe solid. **Ref. LNB0103-148**

***R*f**: 0.42 (heptane/AcOEt = 4/1); **m.p.**: 90-92 °C (MeOH) [lit. 88-90 °C (Et$_2$O/hexane)];[210] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.14 (1H, d, *J* = 8.1 Hz, H-1), 6.78-6.70 (2H, m, H-2, H-4), 3.80 (3H, s, 3H-1a), 2.83-2.75 (2H, m, 2H-6), 2.44 (1H, t, *J* = 7.8 Hz, H-14), 2.39-2.25 (6H, m, H-7, 2H-11, H-15, 2H-16), 2.23-2.11 (1H, m, H-7), 1.89-1.75 (2H, m, H-12, H-15), 1.56-1.47 (1H, m, H-12), 1.08 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 223.5 (C, C-17), 158.3 (C, C-3), 137.2 (C, C-5), 131.9 (C, C-8), 129.2 (C, C-10), 126.6 (C, C-9), 123.2 (CH, C-1), 113.6 (CH, C-4), 111.1 (CH, C-2), 55.4 (CH$_3$, C-1a), 48.8 (CH, C-14), 47.2 (C, C-13), 36.9 (CH$_2$, C-16), 28.9 (CH$_2$, C-6), 27.6 (CH$_2$, C-7), 27.2 (CH$_2$, C-12), 25.6 (CH$_2$, C-15), 22.1 (CH$_3$, C-11), 20.8 (CH$_3$, C-18); **IR** (neat): 2923, 1736, 1499, 1250 cm^{-1}; **HRMS** (ESI): calculated for C$_{19}$H$_{22}$NaO$_2$ [MNa$^+$] requires 305.1512, found 305.1513 (-0.7 ppm error). Data are consistent with those reported in the literature, except for the NMR spectroscopy assignment of the chemical shifts of H-11, H-15, C-8 and C-10.[210]

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(14β)-3-Hydroxyestra-1,3,5(10),8-tetraen-17-one 4.241

(±)-**4.240**   (±)-**4.241**
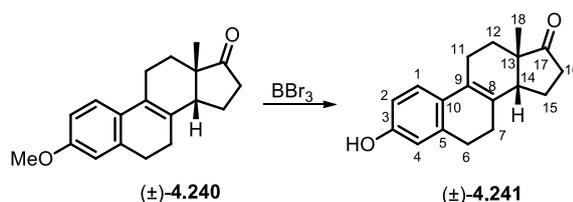
Boron tribromide (1 M sol in CH_2Cl_2, 312 μL, 0.31 mmol) was added to a solution of ketone **4.240** (44.0 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) cooled to -78 °C and stirred under an argon atmosphere. The cold bath was removed, and the reaction mixture was stirred at rt for 2 h. The reaction was cooled to 0 °C and it was quenched with H_2O (20 mL). After dilution with CH_2Cl_2 (20 mL), the aqueous layer was separated and the organic phase was washed with H_2O (3 x 20 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column cromathography (SiO$_2$, hexane/AcOEt = 7/3) to give phenol **4.241** (38.0 mg, 91%) as a white solid. **Ref. LNB0162-117**

R_f: 0.23 (hexane/AcOEt = 4/1); **m.p.**: 230-235 °C (MeOH) [lit. 202-204 °C (MeOH)];[215] **¹H-NMR** (400 MHz, CDCl$_3$), δ: 7.06 (1H, d, J = 8.4 Hz, H-1), 6.78-6.61 (2H, m, H-2, H-4), 2.80-2.66 (2H, m, 2H-6), 2.47-2.40 (1H, t, J = 6.9 Hz, H-14), 2.39-2.23 (6H, m, H-7, 2H-11, H-15, 2H-16), 2.19-2.07 (1H, m, H-7), 1.86-1.73 (2H, m, H-12, H-15), 1.60 (1H, brs, OH), 1.54-1.44 (1H, m, H-12), 1.08 (3H, s, 3H-18); **¹³C-NMR** (100 MHz, CDCl$_3$), δ: 223.6 (C, C-17), 154.1 (C, C-3), 137.5 (C, C-5), 131.9 (C, C-8), 129.4 (C, C-10), 126.5 (C, C-9), 123.2 (CH, C-1), 114.6 (CH, C-4), 111.8 (CH, C-2), 48.7 (CH, C-14), 47.3 (C, C-13), 36.9 (CH$_2$, C-16), 28.8 (CH$_2$, C-6), 27.5 (CH$_2$, C-7), 27.0 (CH$_2$, C-12), 25.5 (CH$_2$, C-15), 22.1 (CH$_2$, C-11), 20.7 (CH$_3$, C-18); **IR** (neat): 3377, 2933, 1724, 1501 cm^{-1}; **HRMS** (ESI): calculated for $C_{18}H_{20}NaO_2$ [MNa$^+$] requires 291.1356, found 291.1343 (-4.4 ppm error).

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*Protection of ketone **4.241** as acetal **4.237**:*
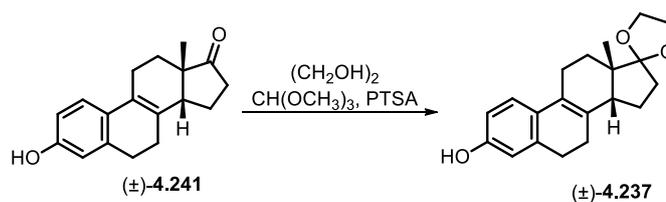
A mixture of ketone **4.241** (13.0 mg, 0.05 mmol), ethylene glycol (6.80 µL, 0.12 mmol), trimethyl orthoformate (10.7 µL, 0.10 mmol) and PTSA (0.09 mg, 0.015 mmol) was stirred at 30 °C until dissolution, and at rt overnight. The mixture was diluted with AcOEt (15 mL) and washed H_2O (3 x 6 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, heptane/AcOEt = 10/0 to 4/1) to afford the protected phenol **4.237** (8.50 mg, 56%) as a white foam. **Ref. LNB0162-118**

(9α,10β)-Estra-4,8(14)-diene-3,17-dione 4.243[175]
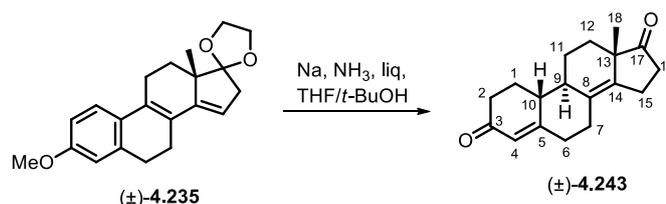
Liquid NH_3 (30 mL) was trapped in a dried three necked round flask cooled to -40 °C. Metallic sodium (558 mg, 24.3 mmol) was cut in small pieces and carefully added. The solution turned dark blue, and it was stirred at -40 °C for 30 min before addition of a solution of pentaene **4.235** (157 mg, 0.49 mmol) in THF/*t*-BuOH (3/1, 4 mL). The reaction was stirred at -40 °C for a further 2 h before being quenched with MeOH (20 mL). NH_3 was left to evaporate under a stream of argon. NH_4Cl (1.30 g) was then added and the mixture was stirred for 20 min at rt. The residue was dilute with H_2O (20 mL) and extracted with AcOEt (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was dissolved in MeOH (15 mL) and 3 M aq HCl (1.5 mL) was added. The mixture was stirred at rt for 3 h, then the pH was brought to 8 with sat aq $NaHCO_3$. Organic compounds were extracted with CH_2Cl_2 (3 x 50 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column

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chromatography (Biotage SNAP 25 g SiO$_2$ cartridge, heptane/AcOEt = 7/3) to give ketone **4.243** (44.0 mg, 34%) as a white solid. **Ref. LNB0145-027**

R$_f$: 0.23 (heptane/AcOEt = 7/3); **m.p.**: 126-128 °C [lit. 127-131 °C (EtOH)];[216] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 5.86 (1H, brs, H-4), 2.78-2.68 (2H, m, H-7, H-15), 2.64-2.50 (3H, m, H-6, H-15, H-16), 2.47-2.40 (1H, m, H-2), 2.32-2.14 (5H, m, H-1, H-2, H-6, H-16), 2.10-1.79 (4H, m, H-7, H-9, H-10, H-11, H-12), 1.71-1.62 (1H, m, H-1), 1.45-1.38 (1H, m, H-11), 1.30-1.21 (1H, m, H-12), 1.12 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 220.9 (C, C-17), 119.7 (C, C-3), 165.3 (C, C-5), 135.3 (C, C-14), 129.9 (C, C-8), 124.8 (CH, C-4), 47.8 (C, C-13), 45.4 (CH, C-10), 43.5 (CH, C-9), 36.5 (CH$_2$, C-2), 36.2 (CH$_2$, C-16), 35.6 (CH$_2$, C-6), 28.9 (CH$_2$, C-7), 28.7 (CH$_2$, C-12), 26.8 (CH$_2$, C-1), 25.0 (CH$_2$, C-11), 23.2 (CH$_2$, C-15), 22.6 (CH$_3$, C-18); **IR** (neat): 2929, 2856, 1736, 1671 cm^{-1}; **HRMS** (ESI): calculated for C$_{18}$H$_{22}$NaO$_2$ [MNa$^+$] requires 293.1512, found 293.1505 (2.1 ppm error).

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6.4. CHAPTER 5: EXPERIMENTAL DETAILS

3β-Hydroxy-2,3-dihydrowithanolide F 5.1
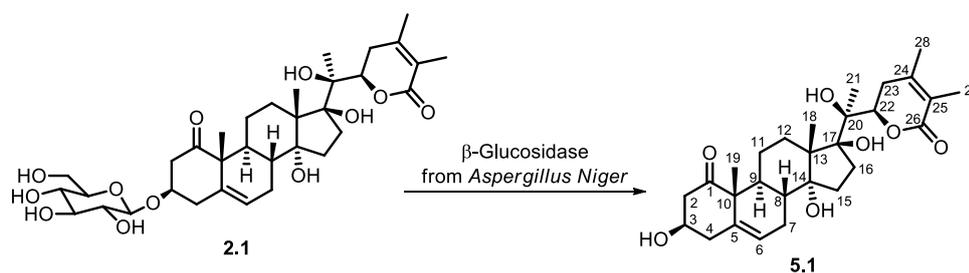
Preparation of 100 mM AcONa buffer (pH = 4)

Sodium acetate trihydrate (19.0 g, 0.14 mol) was dissolved in H_2O (1.12 L) and the pH of the resulting solution was brought to 4 with 1 M aq HCl. H_2O was then added to reach a total volume of 1.4 L. **Ref. LNB0145-044**

Enzymatic deglycosylation of coagulin L **2.1** carried out at AnalytiCon Discovery

A solution of coagulin L **2.1** (500 mg, 0.77 mmol) in 50% aqueous EtOH (100 mL) and a solution of β-glucosidase from *Aspergillus niger* (100 mg, 75 U) in H_2O (100 mL) were added to a buffered solution of 100 mM AcONa (900 mL) prepared as above. The mixture was incubated at 37 °C and with shaking for 1 d. The aqueous mixture was divided into three portions of 300 mL, and each portion was extracted with AcOEt (2 x 200 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography (Biotage SNAP 25 g SiO_2 cartridge, CH_2Cl_2/MeOH = 95/5) to give the title compound **5.1** (232 mg, 61%) as a white solid. **Ref. LNB0145-001**

***R*f**: 0.16 (CH_2Cl_2/MeOH = 95/5); **m.p.**: 261-263 °C (MeOH/H_2O) [lit. 260-261 °C (MeOH/acetone)];[158] **[α]$_D^{24}$**: +61 (*c* 0.11, CHCl$_3$) [lit. +61 (*c* 0.2, CHCl$_3$)];[158] **^{1}H-NMR** (400 MHz, DMSO-d6), δ: 6.70 (1H, brs, OH), 5.76 (1H, brs, OH), 5.57 (1H, brs, H-6), 5.13 (1H, brs, OH), 4.73-4.59 (2H, m, H-22, OH), 3.58 (1H, brs, H-3), 2.61-2.54 (1H, m, H-2), 2.47-2.33 (6H, m, H-2, 2H-4, H-16, 2H-23), 2.21-2.13 (1H, m, H-12), 2.09-2.01 (2H, m, H-7, H-9), 1.89 (3H, s, 3H-28), 1.78-1.70 (2H, m, H-7, H-8), 1.75 (3H, s, 3H-27), 1.61-1.50 (3H, m, H-11, H-15, H-16), 1.43-1.32 (2H, m, H-11, H-15), 1.25 (3H, s, 3H-21), 1.20 (3H, s, 3H-19), 1.18-1.12 (1H, m, H-12), 0.99 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, DMSO-d6), δ: 210.9 (C, C-1), 166.1 (C, C-26), 150.9 (C, C-24), 135.5 (C, C-5), 125.1 (CH, C-6), 120.1 (C, C-25), 87.4 (C, C-17), 81.3 (C, C-14), 80.9 (CH, C-22),

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78.2 (C, C-20), 67.9 (CH, C-3), 53.9 (C, C-13), 52.1 (C, C-10), 48.0 (CH$_2$, C-2), 40.9 (CH$_2$, C-4), 35.8 (CH$_2$, C-16), 35.4 (CH, C-8), 34.9 (CH$_2$, C-23), 34.3 (CH, C-9), 31.9 (CH$_2$, C-15), 29.9 (CH$_2$, C-12), 25.4 (CH$_2$, C-7), 21.7 (CH$_2$, C-11), 20.3 (2CH$_3$, C-18, C-28), 19.2 (CH$_3$, C-21), 18.5 (CH$_3$, C-19), 12.2 (CH$_3$, C-27); **IR** (neat): 3360, 2969, 2926, 2849, 1686, 1679, 1381, 1321, 1135, 1095, 1020 cm^{-1}; **HRMS** (ESI): calculated for C$_{28}$H$_{40}$NaO$_7$ [MNa$^+$] requires 511.2666, found 511.2681 (-2.8 ppm error).

Enzymatic deglycosylation of coagulin L **2.1** carried out at The University of York

A solution of coagulin L **2.1** (20.0 mg, 0.03 mmol) in 50% aq EtOH (4 mL) and a solution of β-glucosidase from *Aspergillus niger* (36.0 mg, 31.0 U) in H$_2$O (4 mL) were added to a buffered solution of 100 mM AcONa (36 mL) prepared as above. The reaction mixture was maintained in an oil bath at 37 °C overnight. The mixture was extracted with AcOEt (3 x 20 mL). The combined organic layers were dried over MgSO$_4$, filtered and evaporated under reduced pressure. The crude was purified by flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH = 95/5 to 9/1) to give the title compound **5.1** (15.2 mg, 100%) as a white solid. **Ref. LNB0162-040**

3β-Acetyl-2,3-dihydrowithanolide F 5.22
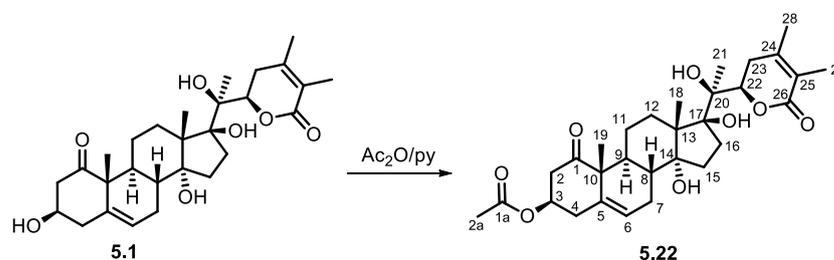
Alcohol **5.1** (20.0 mg, 0.04 mmol) was dissolved in a mixture of py/AcO$_2$ (2/1, 15 μL) and the reaction mixture was stirred at rt overnight. Sat aq NaHCO$_3$ (1 mL) was added and the mixture was vigourously stirred for 10 min. The mixture was extracted with AcOEt (3 x 500 μL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The crude was purified by flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH = 95/5) to give acetate **5.22** (22.0 mg, 100%) as a white solid. **Ref. LNB0162-016**

R$_f$: 0.33 (CH$_2$Cl$_2$/MeOH = 95/5); **m.p.**: 156-158 °C (AcOEt/heptane) [lit. 154-155 °C (AcOEt)][158] **[α]$_D^{24}$**: +38 (*c* 3.10, CHCl$_3$) [lit. +33 (*c* 0.15, CHCl$_3$)];[158] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 5.67 (1H, brs, H-6), 5.03 (1H, "dt", *J* = 14.1, 6.9 Hz, H-3), 4.88 (1H,

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dd, J = 9.6, 6.9 Hz, H-22), 2.80 (1H, dd, J = 13.5, 6.6 Hz, H-2), 2.74-2.65 (2H, m, H-4, H-16), 2.55-2.46 (2H, m, 2H-23), 2.45-2.24 (4H, m, H-2, H-4, H-7, H-12), 2.14-2.04 (1H, m, H-9), 2.02 (3H, s, 3H-21), 1.94-1.85 (2H, m, H-7, H-8), 1.93 (3H, s, 3H-28), 1.87 (3H, s, 3H-27), 1.79-1.52 (4H, m, 2H-11, 2H-15), 1.49-1.40 (1H, m, H-16), 1.42 (3H, s, 3H-21), 1.35-1.26 (1H, m, H-12), 1.23 (3H, s, 3H-19), 1.11 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 211.9 (C, C-1), 170.4 (C, C-1a), 166.1 (C, C-26), 150.7 (C, C-24), 134.7 (C, C-5), 125.4 (CH, C-6), 121.6 (C, C-25), 88.0 (C, C-17), 81.9 (C, C-14), 79.9 (CH, C-22), 79.2 (C, C-20), 70.2 (CH, C-3), 54.8 (C, C-13), 53.0 (C, C-10), 43.6 (CH$_2$, C-2), 38.1 (CH$_2$, C-16), 37.1 (CH$_2$, C-4), 36.0 (2CH, C-8, C-9), 34.3 (CH$_2$, C-23), 32.4 (CH$_2$, C-15), 30.0 (CH$_2$, C-12), 26.0 (CH$_2$, C-7), 22.0 (CH$_2$, C-11), 21.3 (CH$_3$, C-2a), 20.8 (CH$_3$, C-28), 20.5 (CH$_3$, C-18), 19.9 (CH$_3$, C-21), 17.4 (CH$_3$, C-19), 12.5 (CH$_3$, C-27); **IR** (neat): 3358, 2972, 1711, 1686, 1235 cm^{-1}; **HRMS** (ESI): calculated for C$_{30}$H$_{42}$NaO$_8$ [MNa$^+$] requires 533.2772, found 533.2755 (2.5 ppm error). ^{13}C-NMR values in accordance to those reported in the literature except for the assignment of signals for C-23, C-15 and C-12.[158]

Withanolide F 2.2
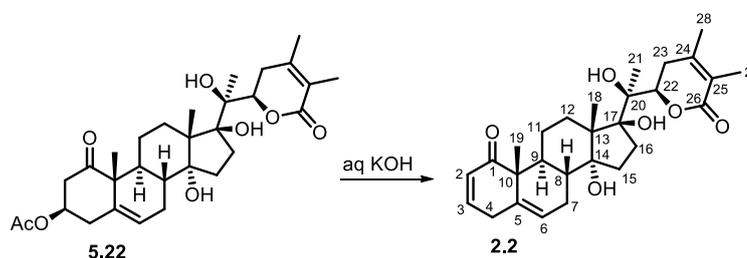
Acetate **5.22** (20.0 mg, 0.04 mmol) was suspended in dioxane (1 mL) and 1 M aq KOH (100 µL) was added. The reaction mixture was stirred at rt 15 min, diluted with AcOEt (5 mL) and washed with H$_2$O (3 x 5 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated under educed pressure. The residue was purified by consecutive flash column chromatography (Biotage SNAP 10 g SiO$_2$ cartridge, CH$_2$Cl$_2$/MeOH = 95/5 then Biotage SNAP 10 g SiO$_2$ cartridge, heptane/AcOEt = 10/0 to 0/10) to give withanolide F **2.2** (11.0 mg, 62% yield) as a white powder. **Ref. LNB0145-011**

R$_f$: 0.29 (CH$_2$Cl$_2$/MeOH = 95/5); **m.p.**: 189-193 (PE/AcOE) [lit. 191-192 °C (AcOEt)];[158] **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 6.80-6.74 (1H, m, H-3), 5.85 (1H, dd, J = 10.0, 3.7 Hz, H-2), 5.62-5.56 (1H, m, H-6), 4.95-4.86 (1H, m, H-22), 3.25 (1H, d, J = 20.7 Hz, H-4),

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2.81 (1H, dd, J = 20.7, 3.7 Hz, H-4), 2.75-2.63 (1H, m, H-16), 2.57-2.15 (6H, m, H-7, H-9, H-11, H-12, 2H-23), 1.95-1.76 (2H, m, H-7, H-8), 1.93 (3H, s, 3H-28), 1.87 (3H, s, 3H-27), 1.74-1.52 (3H, m, H-11, 2H-15), 1.49-1.28 (2H, m, H-12, H-16), 1.41 (3H, s, 3H-21), 1.22 (3H, s, 3H-19), 1.12 (3H, s, 3H-18); ^{13}**C-NMR** (100 MHz, CDCl$_3$), δ: 204.4 (C, C-1), 166.1 (C, C-26), 150.7 (C, C-24), 145.3 (CH, C-3), 135.1 (C, C-5), 128.1 (CH, C-2), 125.4 (CH, C-6), 121.6 (C, C-25), 88.1 (C, C-17), 82.2 (C, C-14), 80.2 (CH, C-22), 79.1 (C, C-20), 54.6 (C, C-13), 50.8 (C, C-10), 37.8 (CH$_2$, C-16), 37.6 (CH, C-8), 35.8 (CH, C-9), 34.3 (CH$_3$, C-23), 33.4 (CH$_2$, C-4), 32.6 (CH$_2$, C-15), 30.5 (CH$_2$, C-12), 25.8 (CH$_2$, C-7), 23.2 (CH$_2$, C-11), 20.8 (CH$_3$, C-28), 20.7 (CH$_3$, C-18), 20.1 (CH$_3$, C-21), 18.9 (CH$_3$, C-19), 12.7 (CH$_3$, C-27); **IR** (neat): 3356, 2968, 2925, 1683, 1670, 1139 cm^{-1}; **HRMS** (ESI): calculated for C$_{28}$H$_{38}$NaO$_6$ [MNa$^+$] requires 493.2561, found 493.2553 (1.5 ppm error). Data are in accordance with those reported in the literature.[158]

Isowithanolide F 5.23
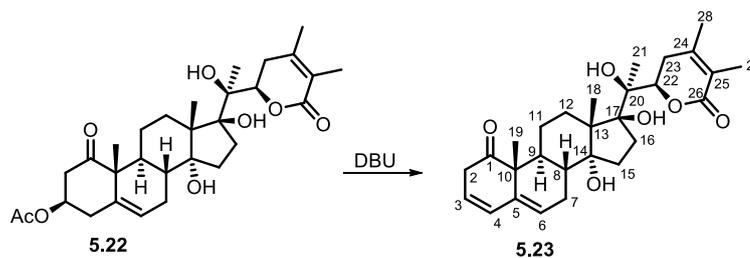
Acetate **5.22** (32.0 mg, 0.06 mmol) was dissolved in CH$_2$Cl$_2$ (600 µL) and DBU (13.5 µL, 0.09 mmol) was added. The reaction mixture was stirred at rt for 20 min, then diluted with CH$_2$Cl$_2$ (4 mL) and washed with sat aq NH$_4$Cl (3 x 4 mL). The organic layer was dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO$_2$, PE/AcOEt = 3/7) to afford withanolide **5.23** (13.9 mg, 50%) as an amorphous solid which could not be recrystallised. **Ref. LNB0162-045**

R_f: 0.32 (CH$_2$Cl$_2$/MeOH = 95/5); ^{1}**H-NMR** (400 MHz, CDCl$_3$), δ: 6.03 (1H, d, J = 8.4 Hz, H-4), 5.71-5.65 (1H, m, H-6), 5.62-5.58 (1H, m, H-3), 4.92 (1H, t, J = 4.9 Hz, H-22), 3.26 (1H, d, J = 20.0 Hz, H-2), 2.79-2.66 (2H, m, H-2, H-16), 2.55-2.43 (4H, m, H-9, H-12, 2H-23), 2.37-2.26 (1H, m, H-7), 2.05-1.84 (2H, m, H-7, H-8), 1.94 (3H, s, 3H-28), 1.88 (3H, s, 3H-27), 1.78-1.20 (6H, m, 2H-11, H-12, 2H-15, H-16), 1.43 (3H, s, 3H-21), 1.37 (3H, s, 3H-19), 1.12 (3H, s, 3H-18); ^{13}**C-NMR** (100 MHz, CDCl$_3$), δ: 210.7 (C, C-

1), 166.1 (C, C-26), 150.7 (C, C-24), 140.5 (C, C-5), 129.6 (CH, C-4), 128.1 (CH, C-6), 121.6 (C, C-25), 121.3 (C, C-3), 88.1 (C, C-17), 81.9 (C, C-14), 80.4 (CH, C-22), 79.1 (C, C-20), 53.8 (C, C-13), 52.3 (C, C-10), 39.9 (CH_2, C-2), 38.0 (CH_2, C-16), 36.3 (CH, C-8), 34.3 (CH_2, C-23), 34.1 (CH, C-9), 32.5 (CH_2, C-15), 30.3 (CH_2, C-12), 26.1 (CH_2, C-7), 22.0 (CH_2, C-11), 20.8 (CH_3, C-28), 20.7 (CH_3, C-18), 20.5 (CH_3, C-19), 20.1 (CH_3, C-21), 12.5 (CH_3, C-27); **IR** (neat): 3341, 2925, 1712, 1689, 1137 cm^{-1}; **HRMS** (ESI): calculated for $C_{28}H_{38}NaO_6$ [MNa$^+$] requires 493.2561, found 493.2539 (3.3 ppm error). Data are in accordance with those reported in the literature except for the assignment of the ^{13}C-NMR values of C-8, C-9, C-12, C-15 and C-23.[158]

3β-Benzoyl-2,3-dihydrowithanolide F 5.26
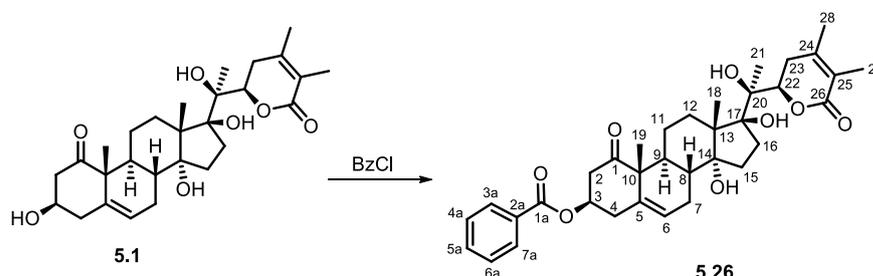
Alcohol **5.1** (5.0 mg, 0.01 mmol) was dissolved in py (150 μL) and benzoyl chloride (12.0 μL, 0.10 mmol) was added. The reaction mixture was stirred at rt overnight, then diluted with CH_2Cl_2 (2 mL) and washed with sat aq $NaHCO_3$ (3 x 1 mL) and H_2O (1 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC (SiO$_2$, CH_2Cl_2/MeOH = 95/5) to afford bezoyl derivative **5.26** (5.80 mg, 98%) as a white solid. **Ref. LNB0162-033**

R_f: 0.30 (CH_2Cl_2/MeOH = 95/5); **m.p.**: 180 °C (MeOH, decomposition); **[α]$_D^{24}$**: +37.9 (c 0.29, CHCl$_3$); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 8.00 (2H, d, J = 7.1 Hz, H-3a, H-7a), 7.55 (1H, "t", J = 7.4 Hz, H-5a), 7.42 (2H, "t", J = 7.7 Hz, H-4a, H-6a), 5.73 (1H, brs, H-6), 5.35-5.27 (1H, m, H-3), 4.90 (1H, dd, J = 10.3, 6.6 Hz, H-22), 2.94 (1H, dd, J = 13.6, 6.7 Hz, H-2), 2.85 (1H, dd, J = 13.6, 7.3 Hz, H-4), 2.81-2.69 (1H, m, H-16), 2.62-2.28 (6H, m, H-2, H-4, H-7, H-12, 2H-23), 2.16 (1H, "td", J = 11.5, 6.0 Hz, H-9), 1.98-1.84 (2H, m, H-7, H-8), 1.94 (3H, s, 3H-28), 1.88 (3H, s, 3H-27), 1.78-1.54 (4H, m, 2H-11, 2H-15), 1.49-1.42 (1H, m, H-16), 1.43 (3H, s, 3H-21), 1.40-1.30 (1H, m, H-12), 1.28 (3H, s, 3H-19), 1.13 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 211.7 (C, C-1), 165.9 (C, C-26), 165.8 (C, C-1a), 150.7 (C, C-24), 134.7 (C, C-5), 133.2 (CH, C-5a),

130.2 (C, C-2a), 129.8 (2CH, C-3a, C-7a), 128.5 (2CH, C-4a, C-6a), 126.6 (CH, C-6), 121.6 (C, C-25), 87.9 (C, C-17), 81.9 (C, C-14), 79.8 (CH, C-22), 79.2 (C, C-20), 70.8 (CH, C-3), 54.8 (C, C-13), 53.1 (C, C-10), 43.7 (CH$_2$, C-2), 38.1 (CH$_2$, C-16), 37.1 (CH$_2$, C-4), 36.0 (2CH, C-8, C-9), 34.3 (CH$_2$, C-23), 32.4 (CH$_2$, C-15), 30.0 (CH$_2$, C-12), 26.0 (CH$_2$, C-7), 22.0 (CH$_2$, C-11), 20.8 (CH$_3$, C-28), 20.5 (CH$_3$, C-18), 19.9 (CH$_3$, C-21), 17.4 (CH$_3$, C-19), 12.5 (CH$_3$, C-27); **IR** (neat): 3372, 2925, 1715, 1699, 1273 cm^{-1}; **HRMS** (ESI): calculated for C$_{35}$H$_{44}$NaO$_8$ [MNa$^+$] requires 615.2928, found 615.2924 (0.6 ppm error).

3β-(*E*)-Cinnamoyl-2,3-dihydrowithanolide F 5.28
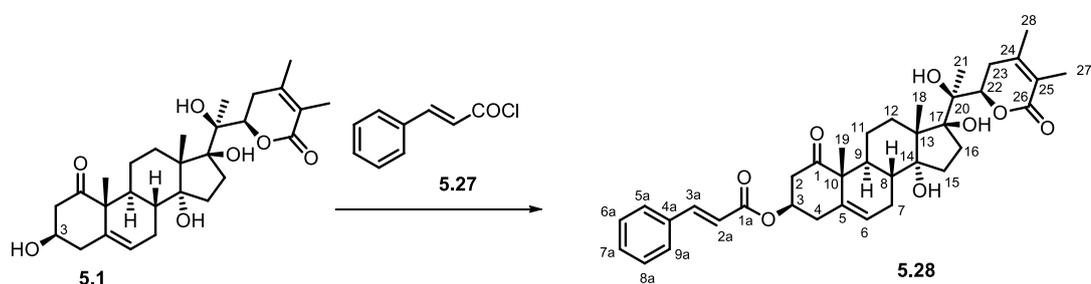
(*E*)-Cinnamic acid (62.0 mg, 0.42 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) and oxalyl chloride (54.0 µL, 0.63 mmol) and a catalytic amounts of DMF (3.50 µL, 0.04 mmol) were subsequently added. The mixture was stirred at rt for 2 h, then the solvent and the excess oxalyl chloride were evaporated. The crude (*E*)-cinnamoyl chloride **5.27** was dissolved in CH$_2$Cl$_2$ (10 mL) and added dropwise to a solution of alcohol **5.1** (20.0 mg, 0.04 mmol) in py (10 mL). The reaction mixture was stirred at rt for 3 h, then diluted with CH$_2$Cl$_2$ (20 mL) and washed with sat aq NaHCO$_3$ (3 x 20 mL), H$_2$O (20 mL) and brine (20 mL). The organic layer was separated, dried over MgSO$_4$, filtered and eveporated under reduced pressure. The residue was purified by preparative TLC (SiO$_2$, CH$_2$Cl$_2$/MeOH = 95/5) to give compound **5.28** contaminated by residual (*E*)-cinnamic acid. The crude **5.28** was diluted with AcOEt (30 mL) and washed with sat aq NaHCO$_3$ (3 x 20 mL). The organic layer was separated, dried over MgSO$_4$, filtered and evaporated under reduced pressure to afford (*E*)-cinnamoyl derivative **5.28** (23.0 mg, 89%) as a white solid. **Ref. LNB0162-135**

R$_f$: 0.29 (CH$_2$Cl$_2$/MeOH = 95/5); **m.p.**: 129-132 °C; **[α]$_D^{24}$**: + 31.7 (*c* 0.36, CHCl$_3$); **^{1}H-NMR** (400 MHz, CDCl$_3$), δ: 7.66 (1H, d, *J* = 16.0 Hz, H-3a), 7.55-7.48 (2H, m, H-5a, H-9a), 7.41-7.35 (3H, m, H-6a, H-7a, H-8a), 6.39 (1H, d, *J* = 16.0 Hz, H-2a), 5.71

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(1H, brs, H-6), 5.18 (1H, "quint", J = 7.0 Hz, H-3), 4.89 (1H, dd, J = 9.7, 6.6 Hz, H-22), 2.89 (1H, dd, J = 13.7, 6.8 Hz, H-2), 2.82-2.69 (2H, m, H-4, H-16), 2.57-2.47 (3H, m, H-2, 2H-23), 2.44-2.24 (3H, m, H-4, H-7, H-12), 2.20-2.08 (1H, m, H-9), 1.98-1.85 (2H, m, H-7, H-8), 1.94 (3H, s, 3H-28), 1.88 (3H, s, 3H-27), 1.76-1.53 (4H, m, 2H-11, 2H-15), 1.50-1.40 (1H, m, H-16), 1.43 (3H, s, 3H-21), 1.36-1.28 (1H, m, H-12), 1.27 (3H, s, 3H-19), 1.12 (3H, s, 3H-18); **^{13}C-NMR** (100 MHz, CDCl$_3$), δ: 211.8 (C, C-1), 166.2 (C, C-1a), 166.0 (C, C-26), 150.7 (C, C-24), 145.3 (CH, C-3a), 134.9 (C, C-5), 134.5 (C, C-4a), 130.5 (CH, C-7a), 129.8 (2CH, C-5a, C-9a), 128.3 (2CH, C-4a, C-6a), 126.4 (CH, C-6), 121.6 (C, C-25), 118.1 (CH, C-2a), 88.0 (C, C-17), 81.9 (C, C-14), 79.8 (CH, C-22), 79.2 (C, C-20), 70.3 (CH, C-3), 54.9 (C, C-13), 53.1 (C, C-10), 43.7 (CH$_2$, C-2), 38.1 (CH$_2$, C-16), 37.2 (CH$_2$, C-4), 36.1 (CH, C-8), 36.0 (CH, C-9), 34.4 (CH$_2$, C-23), 32.4 (CH$_2$, C-15), 30.0 (CH$_2$, C-12), 26.1 (CH$_2$, C-7), 22.0 (CH$_2$, C-11), 20.8 (CH$_3$, C-28), 20.6 (CH$_3$, C-18), 20.0 (CH$_3$, C-21), 17.4 (CH$_3$, C-19), 12.5 (CH$_3$, C-27); **IR** (neat): 3325, 2920, 2952, 1715, 1635, 1162 cm^{-1}; **HRMS** (ESI): calculated for C$_{37}$H$_{46}$NaO$_8$ [MNa$^+$] requires 641.3085, found 641.3098 (-2.8 ppm error).

APPENDICES

Appendix I. ^{1}H-NMR signals of 20-keto steroids
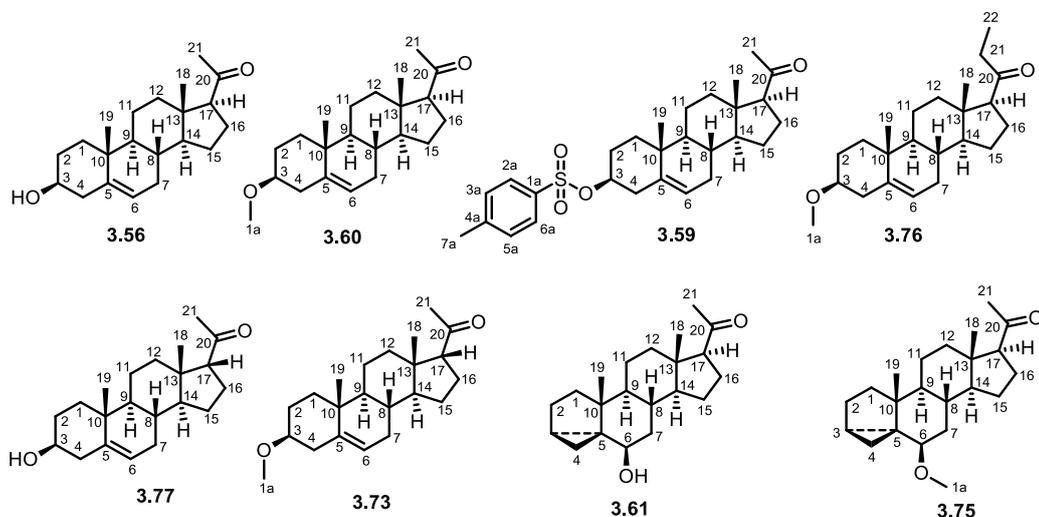
The NMR assignment of 3β-methoxy-pregnenolone **3.60** (recorded in CDCl$_3$, C$_6$D$_6$ and CD$_2$Cl$_2$), tosylate **3.59** (recorded in CDCl$_3$), and ethyl ketone **3.76** (recorded in CDCl$_3$) found correspondence in the complete NMR assignment of pregnenolone **3.56** (recorded in CDCl$_3$ and C$_6$D$_6$) by Szendi *et al.*,[225] with minor differences in the proton and carbon chemical shifts of the atom next to the hydroxyl, tosylate or methoxyl at C-3, and in the side-chain of ethyl ketone **3.76** The structural assignment of the 17α,3β-methoxy-pregnenolone **3.73** (recorded in CDCl$_3$) was carried out on the diastereomeric mixture **3.60/3.73** in 47/53 ratio, and compared to the NMR signals of 17α-pregnenolone **3.77** (recorded in CDCl$_3$) as reported by Černý.[226] Variations of chemical shifts were observed mainly for atoms in ring D. On the other hand, difference in the chemical shift of *i*-steroids **3.61** and **3.75** (recorded in CDCl$_3$ and in CDCl$_3$ and C$_6$D$_6$, respectively) compared with 3β-methoxy-pregnenolone **3.60** involved mainly atoms at rings A and B. The relevant signals are highlighted in **Table I.1.** and in the corresponding ^{13}C-NMR **Table II.1** reported in **Appendix II**.

Atom	3.56[225]		3.60			3.59	3.76
	CDCl₃[a]	C₆D₆[a]	CDCl₃[b]	C₆D₆[b]	CD₂Cl₂[a]	CDCl₃[b]	CDCl₃[b]
1	1.84; 1.08	1.65; 0.95	1.86; 1.04	1.71; 0.97	1.85; 1.04	1.81; 1.02	1.85; 1.02
2	1.84; 1.48	1.71; 1.30	1.91; 1.42	1.93; 1.52	1.89; 1.37	1.80; 1.70	1.90; 1.40
3	3.54	3.40	3.05	3.05	3.02	4.31	3.04
4	2.27; -	2.23; -	2.38; 2.14	2.51; 2.33	2.36; 2.13	2.41; 2.26	2.36; 2.13
5	-	-	-	-	-	-	-
6	5.34	5.31	5.34	5.34	5.34	5.29	5.33
7	1.97; 1.57	1.84; 1.43	1.99; 1.55	1.86; 1.47	1.98; 1.57	1.95; 1.50	1.98; 1.54
8	1.46	1.43	1.45	1.31	1.49	1.43	1.46
9	0.98	0.82	0.97	0.87	0.97	0.93	0.96
10	-	-	-	-	-	-	-
11	1.62; 1.47	1.38; 1.27	1.60; 1.45	1.42; 1.30	1.62; 1.44	1.54; 1.42	1.53; 1.44
12	2.04; 1.43	1.81; 1.08	2.04; 1.42	1.84; 1.12	2.02; 1.44	2.01; 1.40	1.98; 1.40
13	-	-	-	-	-	-	-
14	1.17	0.84	1.14	0.85	1.15	1.09	1.12
15	1.68; 1.23	1.46; 1.03	1.64; 1.13	1.50; 1.06	1.63; 1.17	1.65; 1.19	1.65; 1.21
16	2.19; 1.66	2.33; 1.47	2.18; 1.66	2.34; 1.50	2.12; 1.65	2.15; 1.63	2.18; 1.63
17	2.55	2.09	2.52	2.12	2.52	2.50	2.50
18	0.63	0.58	0.62	0.57	0.60	0.59	0.59
19	1.00	0.86	0.98	0.89	0.99	0.95	0.97
20	-	-	-	-	-	-	-
21	2.11	1.80	2.11	1.82	2.08	2.09	2.36
22	-	-	-	-	-	-	1.01
1a	-	-	3.34	3.22	3.30	-	3.33
2a	-	-	-	-	-	7.78	-
3a	-	-	-	-	-	7.32	-
4a	-	-	-	-	-	-	-
5a	-	-	-	-	-	7.32	-
6a	-	-	-	-	-	7.78	-
7a	-	-	-	-	-	2.43	-

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Atom	3.77[226]	3.73	3.61	3.75	
	$CDCl_3^a$	$CDCl_3^b$	$CDCl_3^b$	$CDCl_3^b$	$C_6D_6^b$
1	1.82; 1.05	1.84; 1.01	1.54; 0.86	1.48; 0.84	1.45; 0.85
2	1.80; 1.49	1.90; 1.40	1.72; 1.52	1.73; 1.50	1.76; 1.57
3	3.52	3.04	1.06	0.87	0.81
4	2.29; 2.22	2.36; 2.12	0.50; 0.28	0.61; 0.42	0.61; 0.40
5	-	-	-	-	-
6	5.34	5.33	3.24	2.75	2.58
7	2.00; 1.63	1.97; 1.60	1.82; 1.16	1.87; 1.08	1.87; 0.93
8	1.44	1.43	1.84	1.74	1.87
9	0.92	0.90	0.87	0.84	0.75
10	-	-	-	-	-
11	1.58; 1.49	1.55; 1.48	1.51; 1.41	1.48; 1.39	1.36; 1.34
12	1.77; 1.17	1.76; 1.17	2.01; 1.40	2.00; 1.39	1.81; 1.08
13	-	-	-	-	-
14	1.25	1.24	1.17	1.15	0.90
15	1.80; 1.25	1.79; 1.23	1.67; 1.26	1.67; 1.24	1.56; 1.18
16	1.92; 1.73	1.90; 1.71	2.15; 1.61	2.15, 1.61	2.36; 1.54
17	2.81	2.79	2.51	2.51	2.16
18	0.93	0.91	0.65	0.64	0.63
19	1.00	0.97	1.03	1.00	1.15
20	-	-	-	-	-
21	2.13	2.10	2.09	2.09	1.81
1a	-	3.33	-	3.29	3.19

Table I.1. ^{1}H-NMR signals of 3β-methoxy-pregnenolone **3.60**, tosyl pregnenolone **3.59**, ethyl ketone **3.76**, 17α,3β–methoxy-pregnenolone **3.73**, cyclo-hydroxy-steroid **3.61** and cyclo-methoxy-steroid **3.75** compared to pregnenolone **3.56**[225] and 17α-pregnenolone **3.77**.[226] [a]Spectrum recorded at 400 MHz. [b]Spectrum recorded at 500 MHz. Relevant signals highlighted.

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Appendix II. ^{13}C-NMR signals of 20 keto-steroids

Atom	3.56[225]			3.60			3.59	3.76
	CDCl$_3$^{a}	C$_6$D$_6$^{a}	CDCl$_3$^{b}	C$_6$D$_6$^{b}	CD$_2$Cl$_2$^{a}		CDCl$_3$^{b}	CDCl$_3$^{b}
1	37.3	37.4	37.3	37.6	37.6		37.0	37.3
2	31.7	32.1	28.1	28.6	28.4		28.7	28.1
3	71.7	71.6	80.4	80.6	80.6		82.3	80.4
4	42.3	42.9	38.8	39.4	39.1		38.9	38.7
5	140.9	141.4	141.0	141.2	141.4		138.9	141.0
6	121.4	121.3	121.4	121.5	121.5		123.3	121.4
7	31.8	32.2	31.9	32.2	32.2		31.8	31.9
8	31.9	32.1	32.0	32.1	32.2		31.8	32.0
9	50.1	50.5	50.2	50.5	50.5		49.8	50.2
10	36.6	36.8	37.0	37.2	37.3		36.4	37.0
11	21.1	21.4	21.2	21.4	21.5		21.1	21.2
12	38.9	39.0	39.0	39.0	39.2		38.8	39.0
13	44.0	43.8	44.1	43.8	44.2		44.0	44.2
14	57.0	57.0	57.1	57.0	57.2		56.9	57.1
15	24.5	24.7	24.6	24.7	24.8		24.5	24.7
16	22.9	23.3	22.9	23.2	23.1		22.9	23.2
17	63.8	63.7	63.8	63.6	63.9		63.7	62.8
18	13.2	13.3	13.3	13.3	13.3		13.3	13.5
19	19.4	19.5	19.5	19.5	19.6		19.2	19.5
20	209.4	206.6	209.6	206.8	209.3		209.5	212.1
21	31.5	31.1	31.6	31.2	31.7		31.6	37.4
22	-	-	-	-	-		-	7.9
1a	-	-	55.7	55.5	55.7		134.8	55.7
2a	-	-	-	-	-		127.7	-
3a	-	-	-	-	-		129.9	-
4a	-	-	-	-	-		144.5	-
5a	-	-	-	-	-		129.9	-
6a	-	-	-	-	-		127.7	-
7a	-	-	-	-	-		21.7	-

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Atom	3.77[226]	3.73	3.61	3.75	
	CDCl₃[a]	CDCl₃[b]	CDCl₃[b]	CDCl₃[b]	C₆D₆[b]
1	37.2	37.3	33.3	33.5	33.8
2	31.6	28.1	25.1	25.0	25.4
3	71.6	80.3	24.3	21.5	21.7
4	42.2	38.8	11.7	13.2	13.5
5	140.4	140.6	38.9	35.3	35.7
6	121.6	121.7	73.6	82.3	82.3
7	31.9	32.2	37.2	35.2	35.5
8	32.0	32.1	30.0	30.6	30.8
9	50.5	49.7	47.7	48.1	48.4
10	36.4	37.0	43.0	43.5	43.7
11	21.0	21.1	22.8	22.9	23.2
12	35.1	35.2	39.3	39.4	39.6
13	45.5	45.6	44.4	44.5	44.2
14	49.5	50.7	56.8	56.8	56.8
15	26.0	26.1	24.5	24.5	24.7
16	24.3	24.5	23.0	23.0	23.3
17	61.3	61.4	63.9	64.0	63.9
18	20.6	20.8	13.6	13.7	13.6
19	19.3	19.4	20.3	19.4	19.7
20	212.8	212.8	209.7	209.6	206.8
21	32.8	32.9	31.6	31.6	31.1
22	-	-	-	-	-
1a	-	55.7	-	56.7	56.6
2a	-	-	-	-	-
3a	-	-	-	-	-
4a	-	-	-	-	-
5a	-	-	-	-	-
6a	-	-	-	-	-
7a	-	-	-	-	-

Table II.1. ^{13}C-NMR signals of 3β-methoxy-pregnenolone **3.60**, tosyl pregnenolone **3.59**, ethyl ketone **3.76**, 17α,3β-methoxy-pregnenolone **3.73**, cyclo-hydroxy-steroid **3.61** and cyclo-methoxy-steroid **3.75** compared to pregnenolone **3.56**[225] and 17α-pregnenolone **3.77**.[227] [a]Spectrum recorded at 100 MHz. [b]Spectrum recorded at 125 MHz.

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Appendix III. ¹H- and ¹³C-NMR spectra of 20-keto steroids
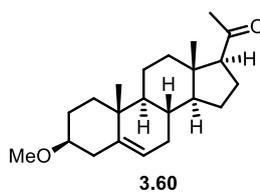
i4554h.001.esp
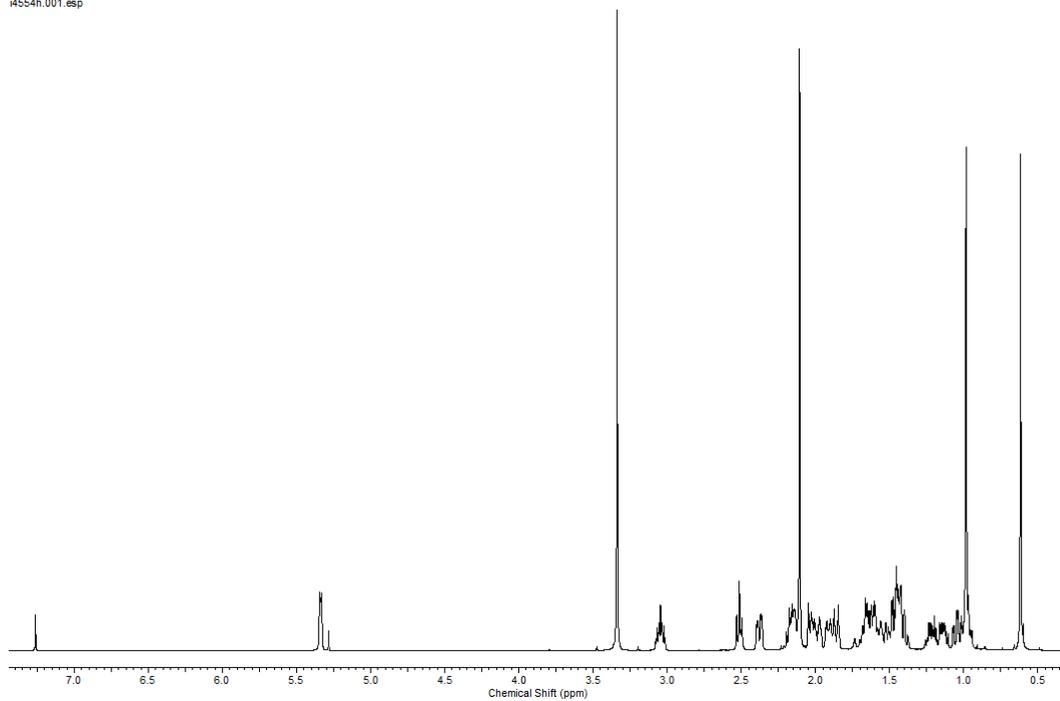
i4554c_1.003.esp
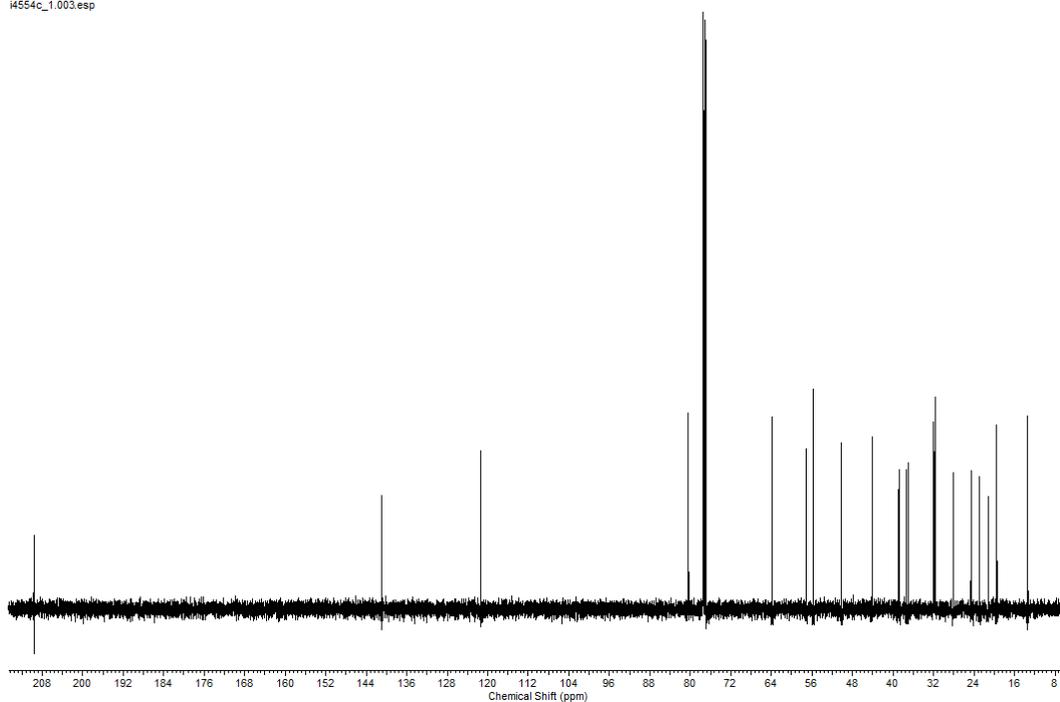
Figure III.1. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectra of 3β-methoxy-pregnenolone **3.60** recorded in CDCl₃.
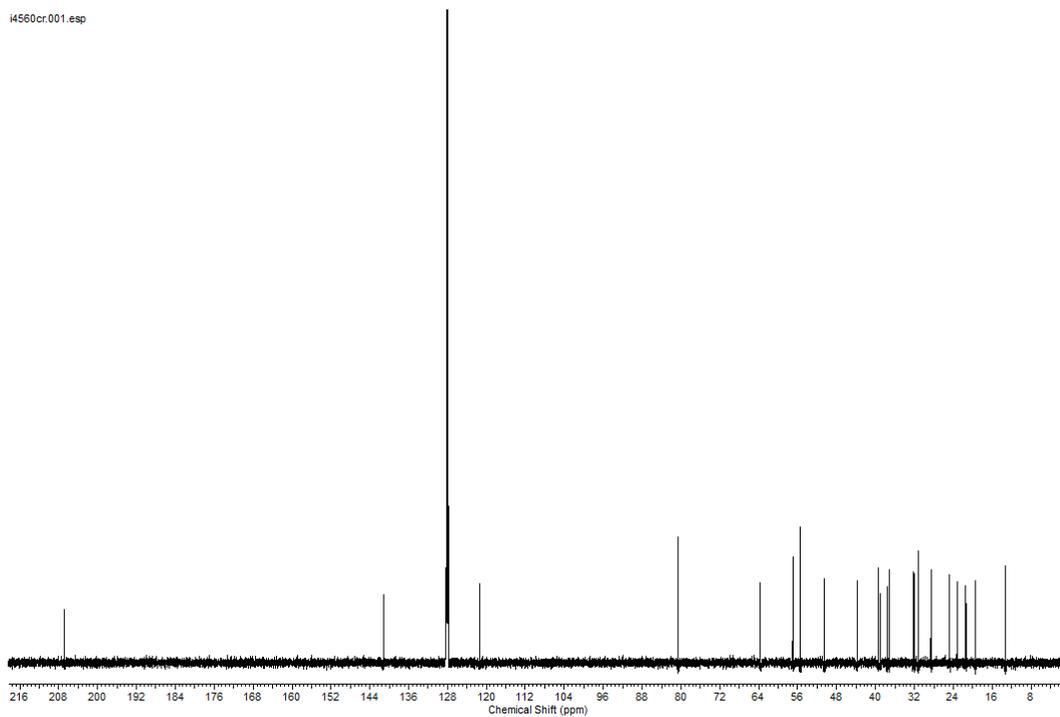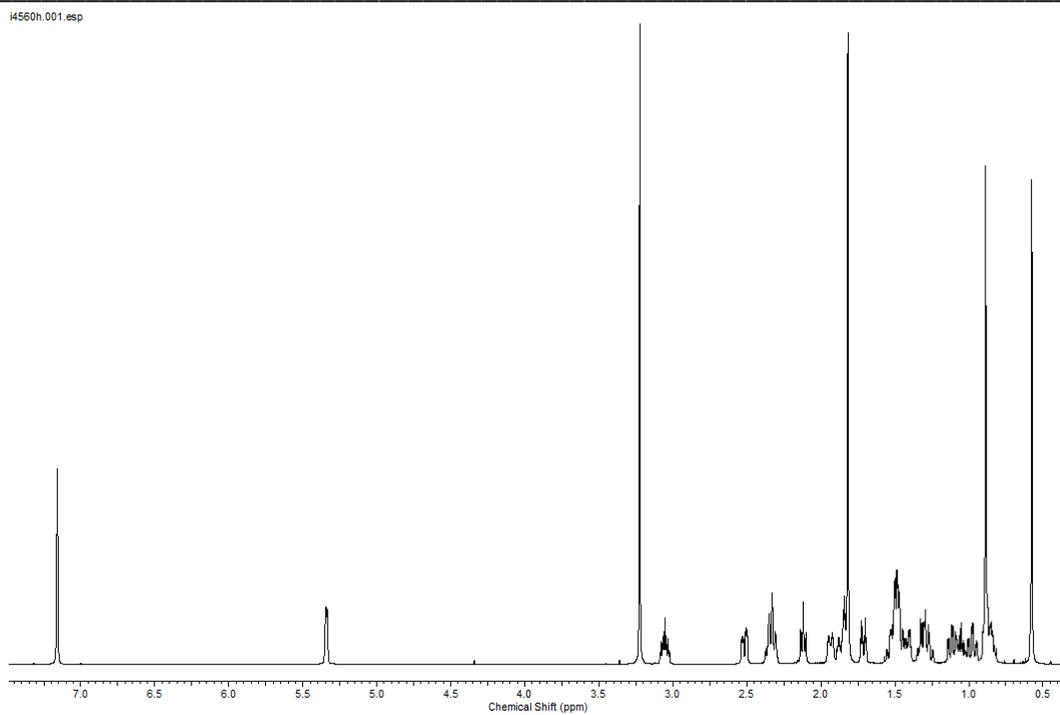
Figure III.2. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of 3β-methoxy-pregnenolone **3.60** recorded in C_6D_6.

k3221luv_PROTON-1.esp

j0770luv_CARBON-1.esp

Figure III.3. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of 3β-methoxy-pregnenolone **3.60** recorded in CH$_2$Cl$_2$
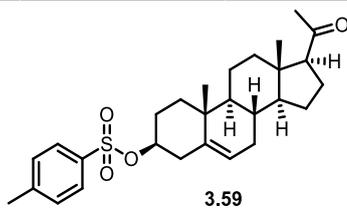
i4584h.001.esp
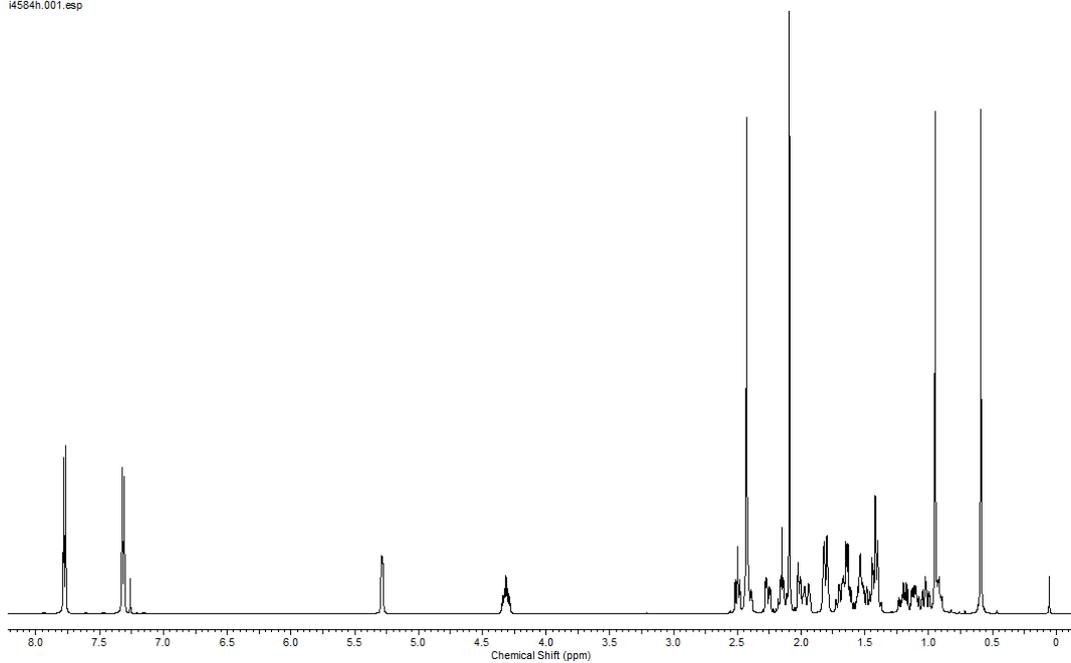
i4584c.001.esp
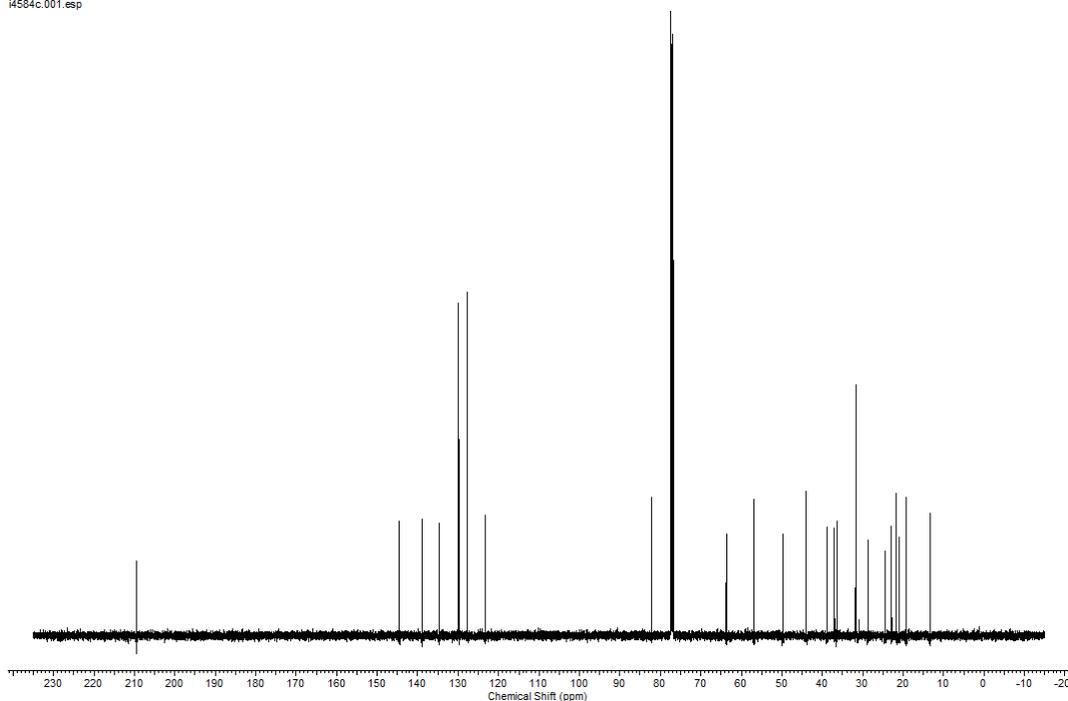
Figure III.4. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of tosylate **3.59** recorded in CDCl$_3$
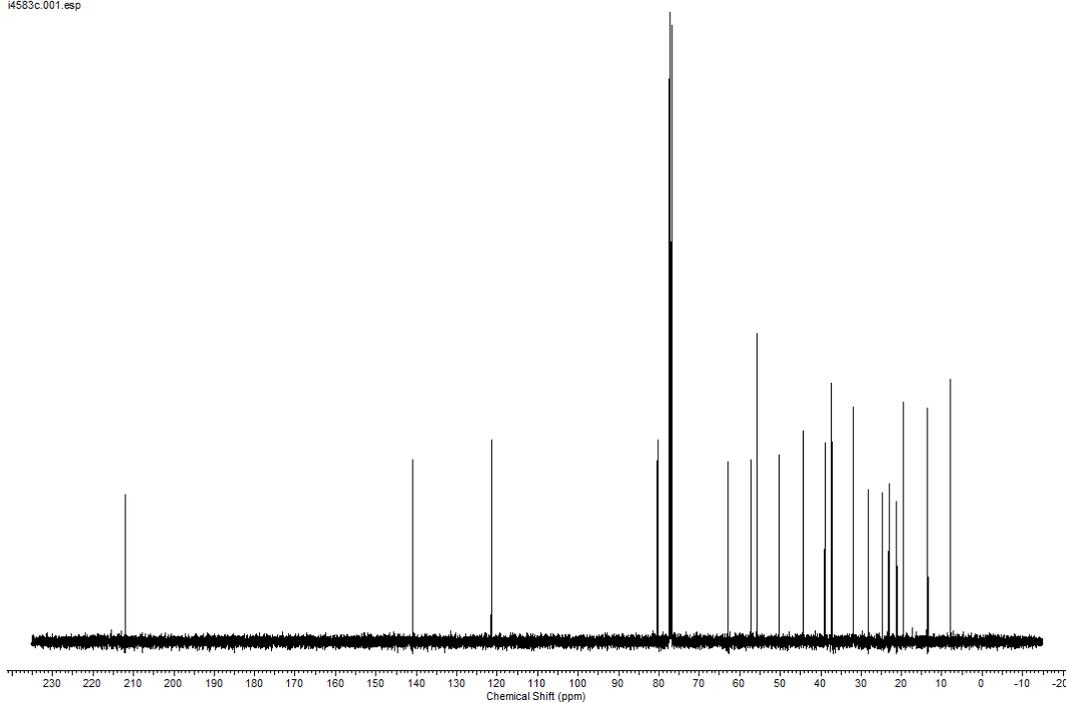
Figure III.5. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of ethyl-steroid **3.76** recorded in CDCl$_3$.
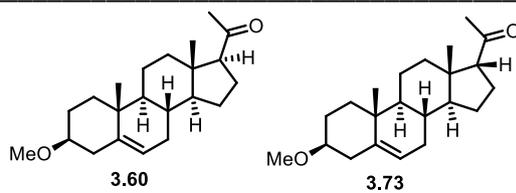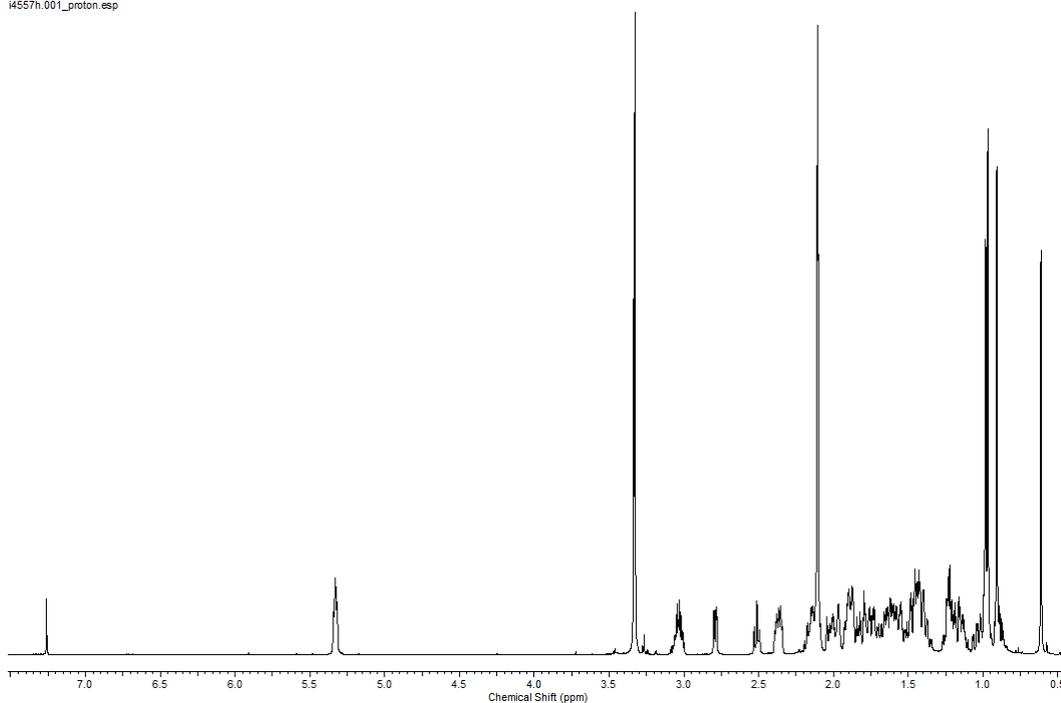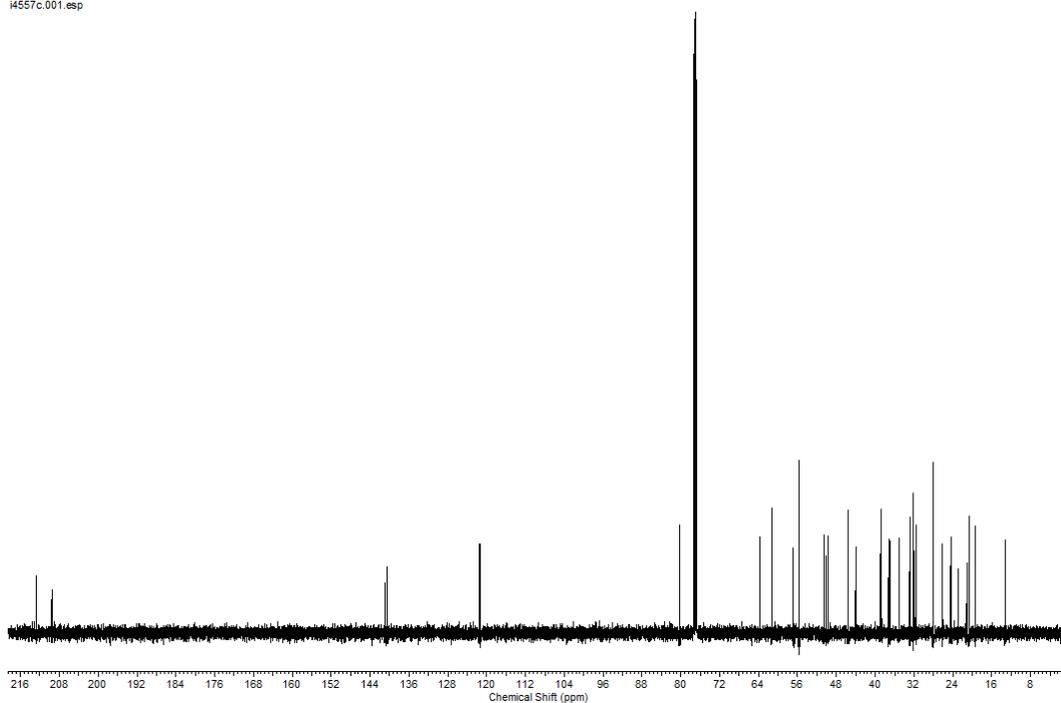
Figure III.6. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of the diastereomeric mixture of **3.60/3.73** in approximately 47/53 ratio recorded in CDCl$_3$.
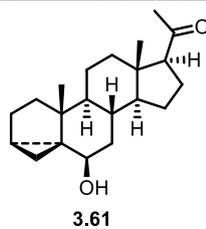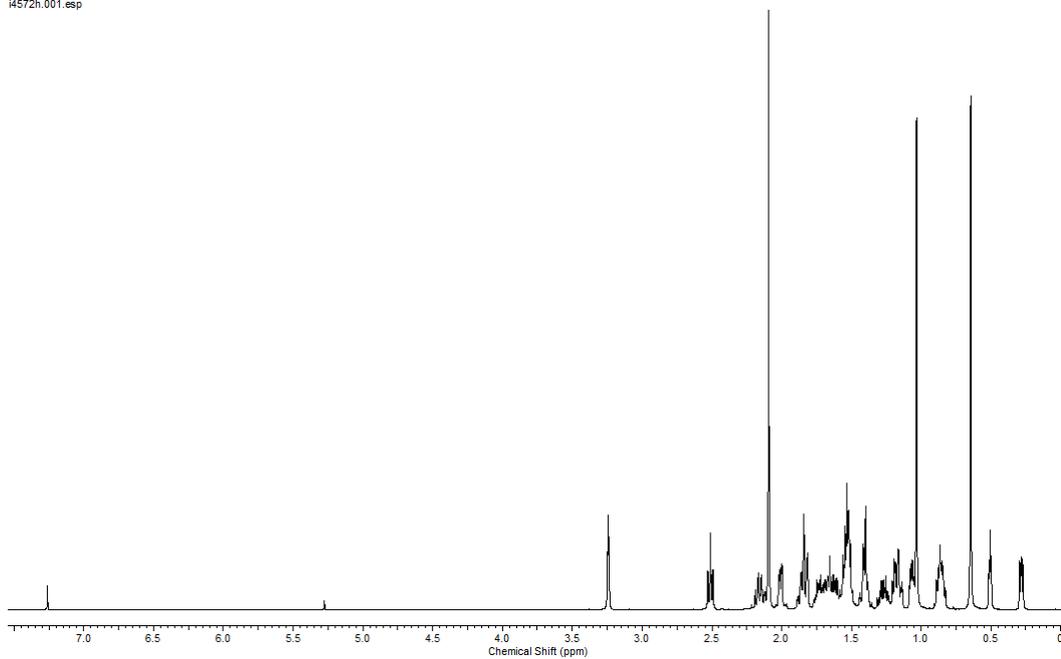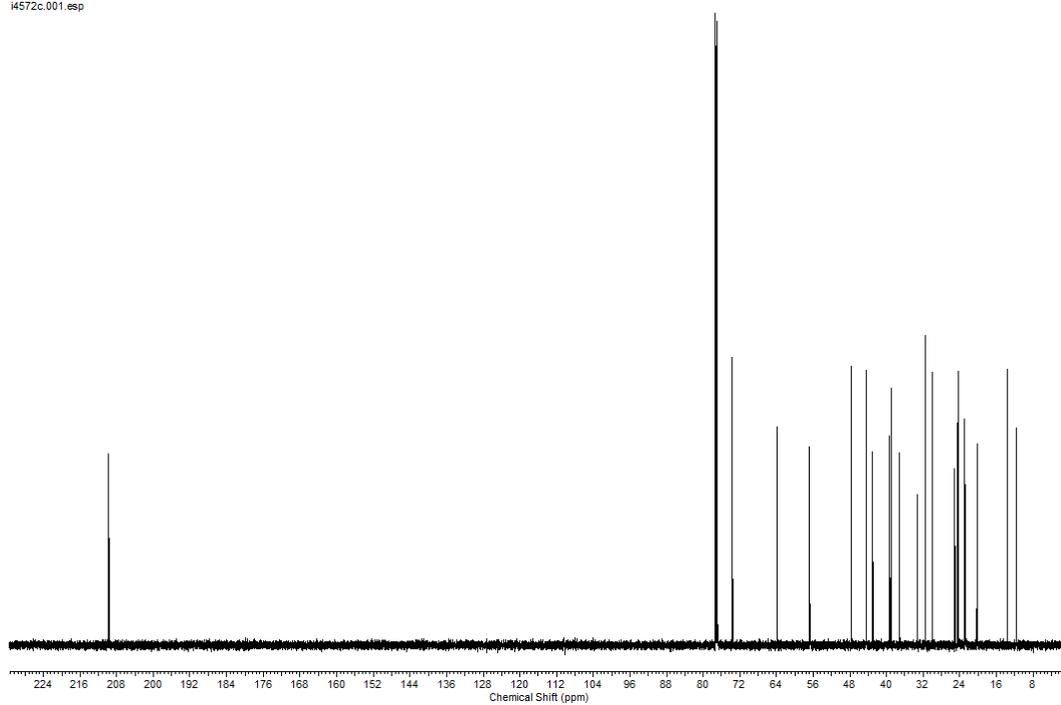
Figure III.7. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of cyclo-alcohol **3.61** recorded in CDCl$_3$.

Figure III.8. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of cyclo-ester **3.75** recorded in CDCl$_3$.
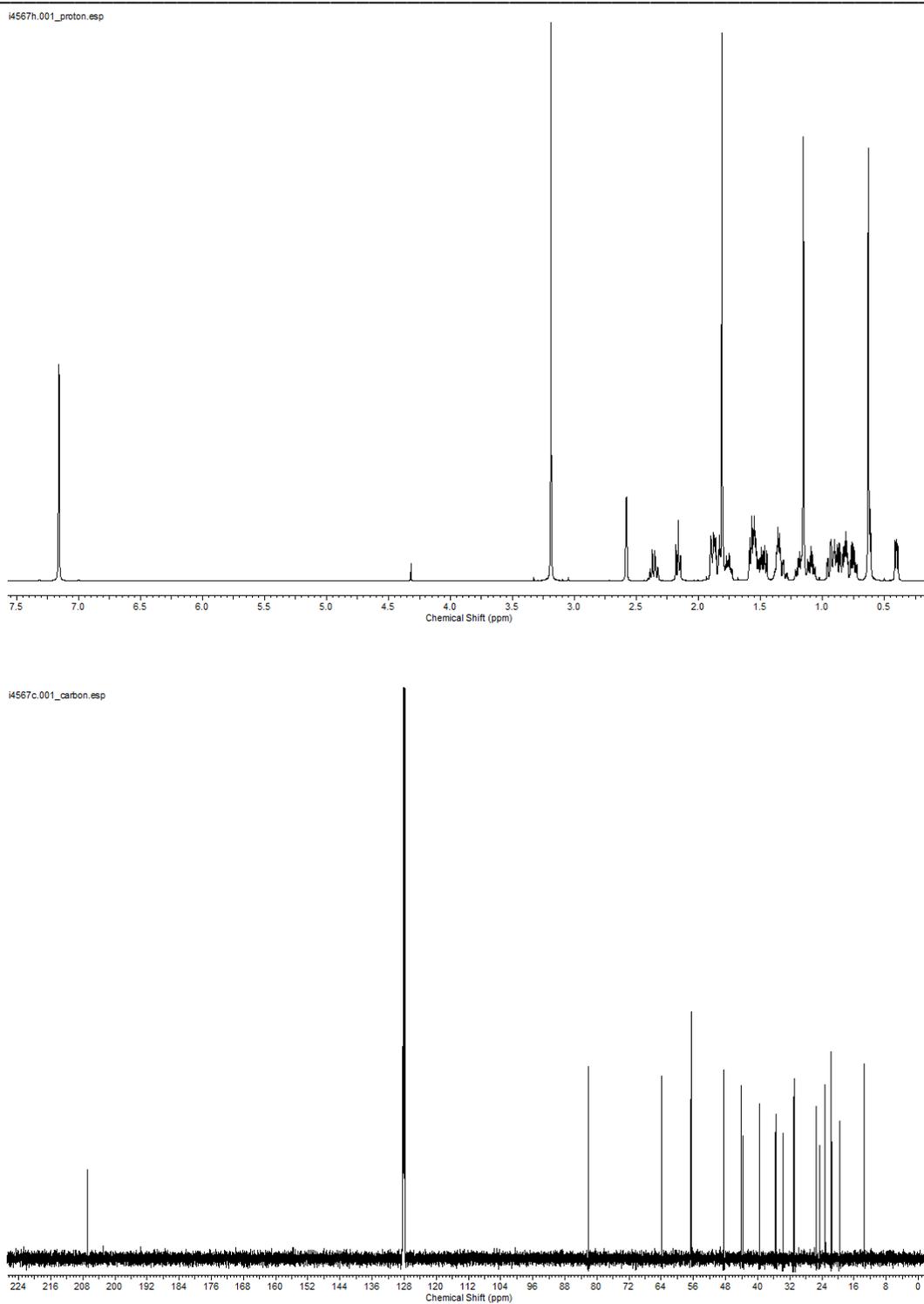
Figure III.9. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of cyclo-ester **3.75** recorded in C_6D_6.

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Appendix IV. ¹H-NMR signals of diastereomeric thiazole-steroids
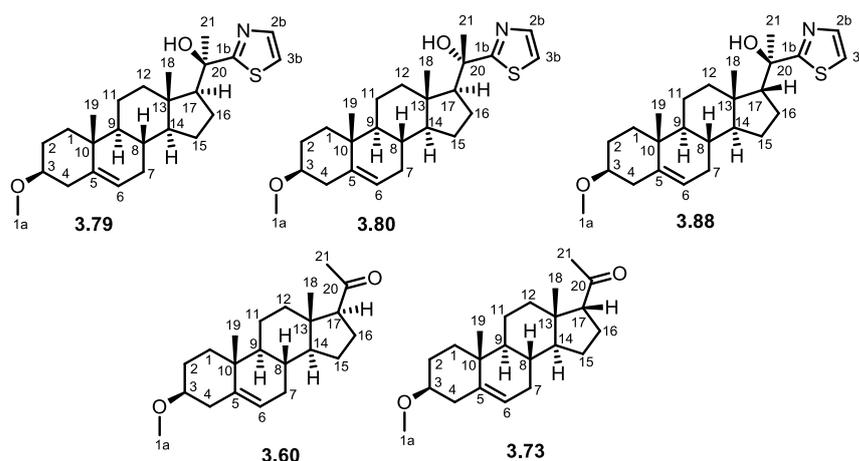
Atom	3.60	3.79	3.80	3.88	3.73
	CDCl₃	CDCl₃	CDCl₃	CDCl₃	CDCl₃
1	1.86; 1.04	1.85; 1.03	na	na	1.84; 1.01
2	1.91; 1.42	1.91; 1.40	na	na	1.90; 1.40
3	3.05	3.05	3.03	3.05	3.04
4	2.38; 2.14	2.37; 2.14	2.35; na	2.36; na	2.36; 2.12
5	-	-	-	-	-
6	5.34	5.34	5.31	5.31	5.33
7	1.99; 1.55	1.94; 1.49	na	na	1.97; 1.60
8	1.45	1.45	na	na	1.43
9	0.97	0.93	na	0.89	0.90
10	-	-	na	-	-
11	1.60; 1.45	1.52; 1.47	na	na	1.55; 1.48
12	2.04; 1.42	2.09; 1.35	na	na	1.76; 1.17
13	-	-	-	-	-
14	1.14	1.07	na	1.17	1.24
15	1.64; 1.13	1.55; 1.16	na	na	1.79; 1.23
16	2.18; 1.66	1.77; 1.26	na	na	1.90; 1.71
17	2.52	2.04	na	2.27	2.79
18	0.62	0.86	0.84	0.86	0.91
19	0.98	0.99	0.91	0.96	0.97
20	-	-	-	-	-
21	2.11	1.70	1.58	1.68	2.10
1a	3.34	3.34	3.32	3.33	3.33
1b	-	-	-	-	-
2b	-	7.65	7.69	7.64	-
3b	-	7.22	7.24	7.25	-

Table IV.1. ¹H-NMR signals of 20(*R*)-thiazolyl-steroid **3.79**, 20(*S*)-thiazolyl-steroid **3.80** and 17α-20(*R*)-thiazolyl-steroid **3.88** compared to 3β-methoxy-pregenenolone **3.60** and 17α,3β-methoxy-pregnenolone **3.73**. The structure of compound **3.80** and compound **3.88** were elucidated in diastereomeric mixtures of **3.79/3.80** and **3.79/3.88** in approximately 77/23 and 37/63 ratio, respectively. Relevant signals highlighted. na = Not assigned

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Appendix V. ^{13}C-NMR signals of diastereomeric thiazole-steroids.
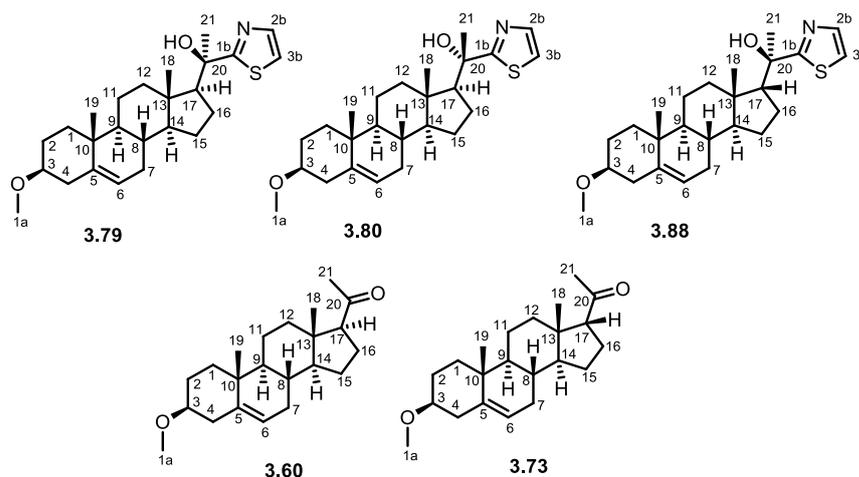
3.79   3.80   3.88

3.60   3.73

Atom	3.60	3.79	3.80	3.88	3.73
	CDCl$_3^b$	CDCl$_3^b$	CDCl$_3^b$	CDCl$_3^b$	CDCl$_3^b$
1	37.3	37.3	37.2	37.3	37.3
2	28.1	28.1	28.1	28.1	28.1
3	80.4	80.4	80.4	80.4	80.3
4	38.8	38.8	38.8	38.8	38.8
5	141.0	141.0	141.0	140.8	140.6
6	121.4	121.5	121.5	121.6	121.7
7	31.9	31.9	31.9	32.4	32.2
8	32.0	31.4	31.6	32.4	32.9
9	50.2	50.2	50.1	49.7	49.7
10	37.0	37.0	36.9	37.0	37.0
11	21.2	21.0	20.9	21.4	21.1
12	39.0	40.0	37.9	35.2	35.2
13	44.1	43.3	42.8	44.6	45.6
14	57.1	56.8	56.7	53.3	50.7
15	24.6	23.8	22.9	26.2	26.1
16	22.9	22.9	22.9	25.9	24.5
17	63.8	60.4	60.5	59.2	61.4
18	13.3	13.4	13.1	22.5	20.8
19	19.5	19.5	19.4	19.4	19.4
20	209.6	77.8	77.8	77.8	212.8
21	31.6	30.0	31.6	30.0	31.6
1a	55.7	55.7	55.7	55.7	55.7
1b	-	180.6	179.4	180.7	-
2b	-	141.8	141.8	141.3	-
3b	-	118.9	118.8	119.2	-

Table V.1. ^{13}C-NMR signals of 20(*R*)-thiazolyl-steroid **3.79**, 20(*S*)-thiazolyl-steroid **3.80** and 17α-20(*R*)-thiazolyl-steroid **3.88** compared to 3β-methoxy-pregenenolone **3.60** and 17α,3β-methoxy-pregnenolone **3.73**. The structure of compound **3.80** and compound **3.88** were elucidated in diastereomeric mixtures of **3.79/3.80** and **3.79/3.88** in approximately 77/23 and 37/63 ratio, respectively. Relevant signals highlighted.

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Appendix VI. ^{1}H- and ^{13}C-NMR spectra of diastereomeric thiazole-steroids.

3.79
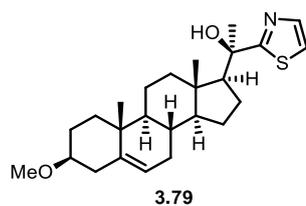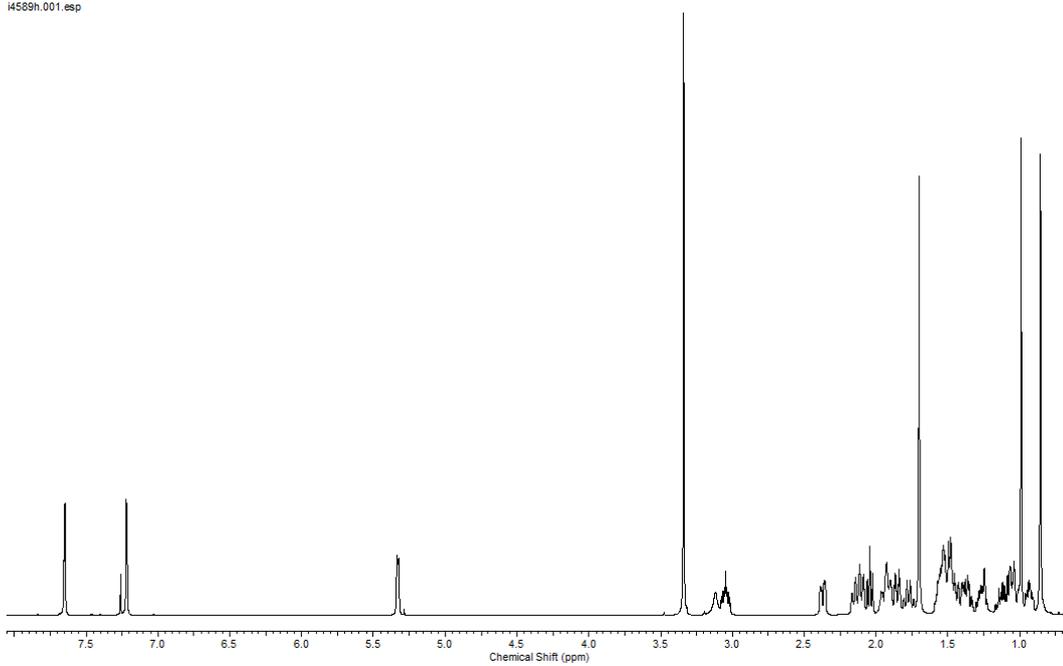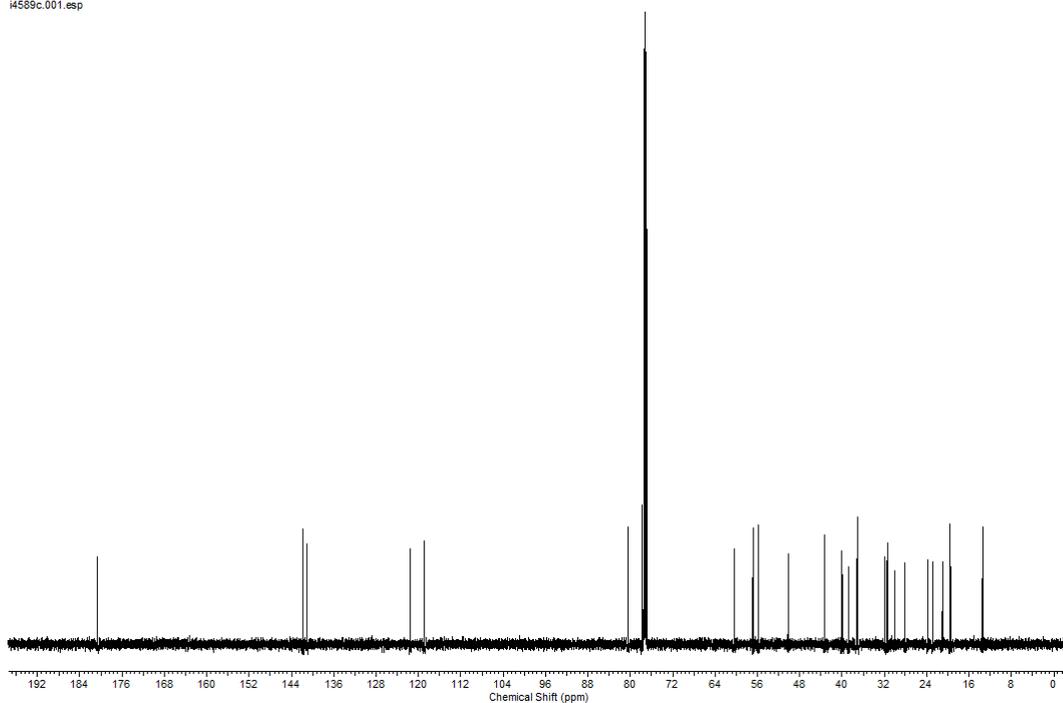
Figure VI.1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of 20(*R*)-thiazolyl-steroid **3.79** recorded in CDCl$_3$.

Figure VI.2. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of the diastereomeric mixture of **3.79/3.80** in approximately 77/23 ratio recorded in CDCl$_3$.
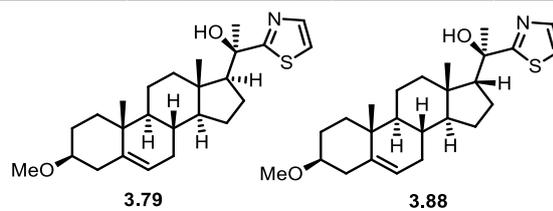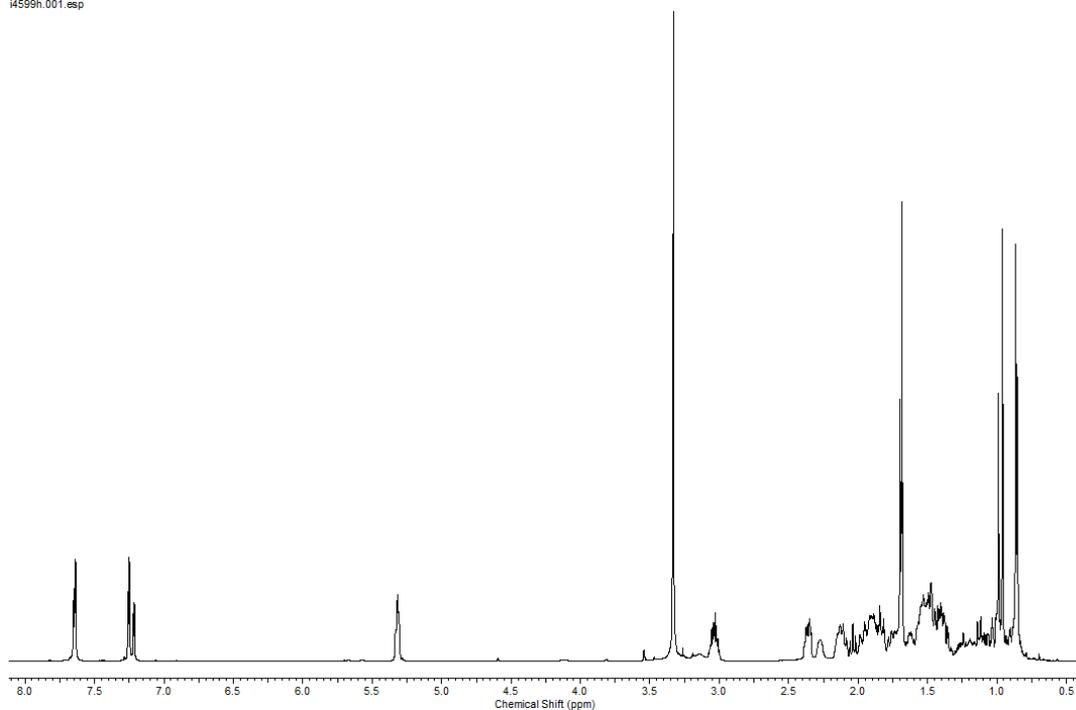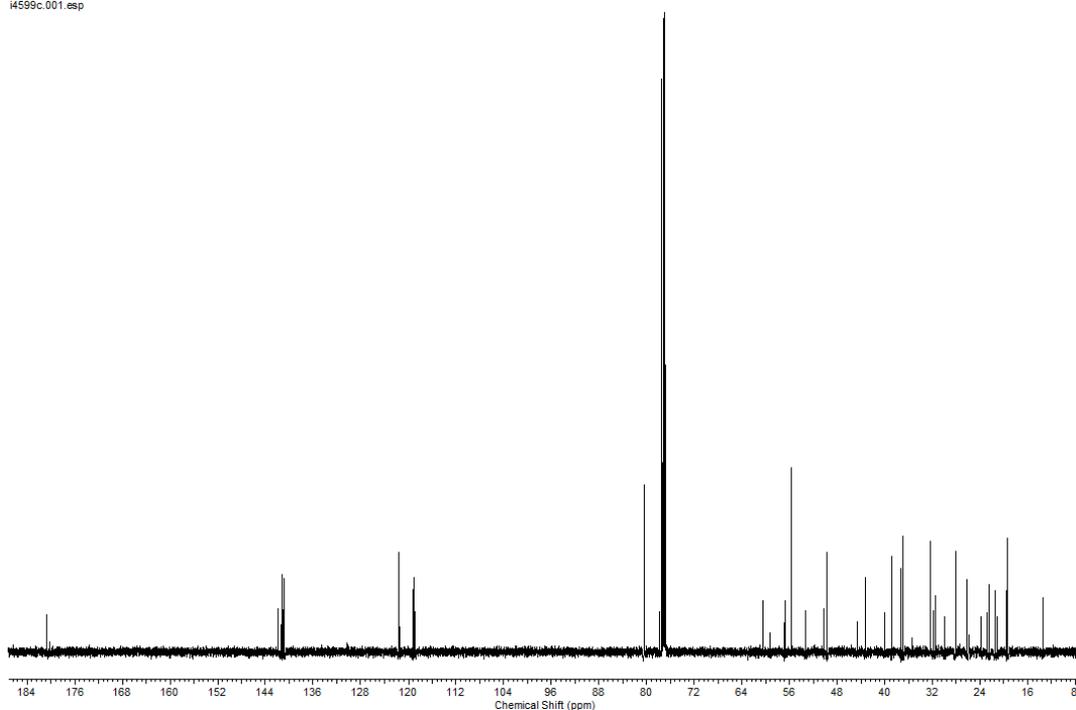
Figure VI.3. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of the diastereomeric mixture of **3.79/3.88** in approximately 37/63 ratio recorded in CDCl₃.

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Appendix VII. Crystallographic data – Compound 3.60 (CCDC 1021885)

Identification code	rjkt1303
Empirical formula	$C_{22}H_{34}O_2$
Formula weight	330.49
Temperature/K	110.0
Crystal system	monoclinic
Space group	$P2_1$
a/Å	6.66855(17)
b/Å	11.9626(3)
c/Å	12.0329(3)
α/	°90.00
β/°	105.676(3)
γ/°	90.00
Volume/Å^3	924.20(4)
Z	2
ρ_{calc}mg/mm^3	1.188
m/mm^{-1}	0.073
F(000)	364.0
Crystal size/mm^3	$0.274 \times 0.0684 \times 0.0545$
2Θ range for data collection	6.34 to 64.52°
Index ranges	$-9 \leq h \leq 10, -17 \leq k \leq 16, -17 \leq l \leq 17$
Reflections collected	8628
Independent reflections	5515[R(int) = 0.0287]
Data/restraints/parameters	5515/1/221
Goodness-of-fit on F^2	1.033
Final R indexes [I>=2σ (I)]	$R_1 = 0.0474$, w$R_2 = 0.1134$
Final R indexes [all data]	$R_1 = 0.0550$, w$R_2 = 0.1185$
Largest diff. peak/hole / e Å^{-3}	0.27/-0.23
Flack parameter	0.6(10)
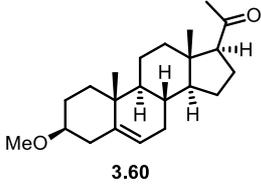
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Appendix VIII. Crystallographic data – Compound 3.79 (dimer including 2.MeOH) (CCDC 1019833)

Identification code:	rjkt1305
Empirical formula	$C_{51.5}H_{80}N_2O_{5.5}S_2$
Formula weight	879.30
Temperature/K	110.0
Crystal system	monoclinic
Space group	$P2_1$
a/Å	12.2620(3)
b/Å	11.8358(3)
c/Å	16.5376(4)
α/°	90.00
β/°	97.549(2)
γ/°	90.00
Volume/Å^3	2379.31(10)
Z	2
ρ_{calc}mg/mm^3	1.227
m/mm^{-1}	0.162
F(000)	958.0
Crystal size/mm^3	$0.195 \times 0.1099 \times 0.0557$
2Θ range for data collection	5.6 to 52.04°
Index ranges	$-15 \leq h \leq 15, -14 \leq k \leq 14, -19 \leq l \leq 20$
Reflections collected	10254
Independent reflections	7181[R(int) = 0.0319]
Data/restraints/parameters	7181/1/581
Goodness-of-fit on F^2	1.044
Final R indexes [I>=2σ (I)]	$R_1 = 0.0449$, w$R_2 = 0.0987$
Final R indexes [all data]	$R_1 = 0.0531$, w$R_2 = 0.1056$
Largest diff. peak/hole / e Å^{-3}	0.34/-0.29
Flack parameter	-0.11(6)
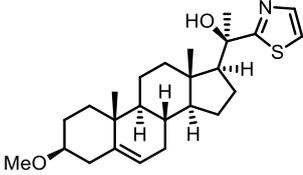
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Appendix IX. Crystallographic data – Compound 3.88 (CCDC 1019834)

Identification code:	rjkt1216
Empirical formula	$C_{25}H_{37}NO_2S$
Formula weight	415.62
Temperature/K	109.90(14)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.29333(17)
b/Å	9.5588(2)
c/Å	32.2458(7)
α/°	90.00
β/°	90.00
γ/°	90.00
Volume/Å^3	2248.03(9)
Z	4
ρ_{calc}mg/mm^3	1.228
m/mm^{-1}	0.165
F(000)	904.0
Crystal size/mm^3	$0.1443 \times 0.1268 \times 0.0636$
2Θ range for data collection	5.72 to 55.74°
Index ranges	$-5 \leq h \leq 9, -12 \leq k \leq 7, -19 \leq l \leq 42$
Reflections collected	6698
Independent reflections	4868[R(int) = 0.0229]
Data/restraints/parameters	4868/0/270
Goodness-of-fit on F^2	1.069
Final R indexes [I>=2σ (I)]	$R_1 = 0.0401, wR_2 = 0.0876$
Final R indexes [all data]	$R_1 = 0.0446, wR_2 = 0.0899$
Largest diff. peak/hole / e Å^{-3}	0.27/-0.22
Flack parameter	-0.04(7)
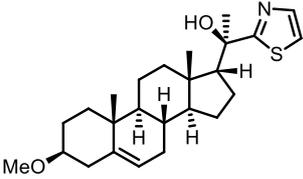
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Appendix X. Crystallographic data – Compound 3.94 (CCDC 1019835)

Identification code:	rjkt1217a
Empirical formula	$C_{27}H_{39}NO_2$
Formula weight	409.59
Temperature/K	110.00(10)
Crystal system	triclinic
Space group	P1
a/Å	6.1294(2)
b/Å	6.1292(2)
c/Å	15.9024(7)
α/°	90.341(3)
β/°	100.911(3)
γ/°	106.244(3)
Volume/Å^3	563.95(4)
Z	1
ρ_{calc}mg/mm^3	1.206
m/mm^{-1}	0.074
F(000)	224.0
Crystal size/mm^3	$0.2187 \times 0.1698 \times 0.1409$
2Θ range for data collection	6.92 to 60.16°
Index ranges	$-8 \leq h \leq 8$, $-8 \leq k \leq 8$, $-22 \leq l \leq 22$
Reflections collected	9826
Independent reflections	6370[R(int) = 0.0229]
Data/restraints/parameters	6370/3/279
Goodness-of-fit on F^2	1.089
Final R indexes [I>=2σ (I)]	$R_1 = 0.0380$, $wR_2 = 0.0982$
Final R indexes [all data]	$R_1 = 0.0401$, $wR_2 = 0.1004$
Largest diff. peak/hole / e Å^{-3}	0.33/-0.21
Flack parameter	-0.9(8)
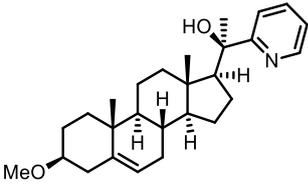
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Appendix XI. Crystallographic data – Compound 3.124 (CCDC 1019836)

Identification code:	rjkt1314_twin1_hklf4
Empirical formula	$C_{26}H_{36}O_4$
Formula weight	412.55
Temperature/K	110.05(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.468(4)
b/Å	10.075(6)
c/Å	29.082(17)
α/°	90.00
β/°	90.000
γ/°	90.000
Volume/Å^3	5188(2)
Z	4
ρ_{calc}mg/mm^3	1.252
m/mm^{-1}	0.083
F(000)	896.6
Crystal size/mm^3	$0.4066 \times 0.2616 \times 0.0229$
Radiation	MoKα (λ = 0.71070)
2Θ range for data collection	6.12 to 50.7°
Index ranges	$-5 \le h \le 8, -5 \le k \le 12, -34 \le l \le 28$
Reflections collected	5177
Independent reflections	3496[R_{int} = 0.0229, R_{sigma} = 0.1363]
Data/restraints/parameters	3496/0/275
Goodness-of-fit on F^2	1.027
Final R indexes [I>=2σ (I)]	R_1 = 0.0874, wR_2 = 0.1795
Final R indexes [all data]	R_1 = 0.1506, wR_2 = 0.2016
Largest diff. peak/hole / e Å^{-3}	0.22/-0.27
Flack parameter	2(4)
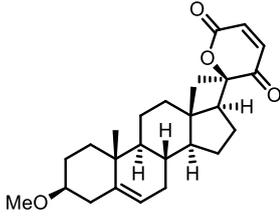
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Appendix XII. Crystallographic data – Compound 3.168 (CCDC 1019837)

Identification code	rjkt1313a
Empirical formula	$C_{26}H_{38}O_3$
Formula weight	398.56
Temperature/K	110.05(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	6.20811(17)
b/Å	11.6297(3)
c/Å	29.9007(7)
α/°	90.00
β/°	90.00
γ/°	90.00
Volume/Å^3	2158.78(10)
Z	4
ρ_{calc}mg/mm^3	1.226
m/mm^{-1}	0.078
F(000)	872.0
Crystal size/mm^3	$0.1979 \times 0.1378 \times 0.0197$
Radiation	Mo Kα ($\lambda = 0.7107$)
2Θ range for data collection	6.48 to 55.5°
Index ranges	$-7 \leq h \leq 8, -8 \leq k \leq 14, -39 \leq l \leq 24$
Reflections collected	5672
Independent reflections	3690[R(int) = 0.0207]
Data/restraints/parameters	3690/0/266
Goodness-of-fit on F^2	1.035
Final R indexes [I>=2σ (I)]	$R_1 = 0.0437, wR_2 = 0.0922$
Final R indexes [all data]	$R_1 = 0.0527, wR_2 = 0.0974$
Largest diff. peak/hole / e Å^{-3}	0.27/-0.21
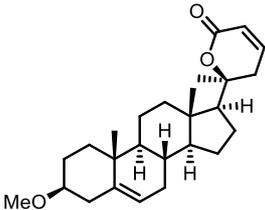
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ABBREVIATIONS

Ac	Acetyl
AD	Alzheimer's disease
aq	Aqueous
brs	Broad singlet
Bu	Butyl
Bn	Benzyl
c	Concentration
°C	Grade(s) Celsius (Centigrade)
CAN	Ceric ammonium nitrate
Cat	Catalytic
CCDC	Cambridge Crystallographic Data Centre
CoA	Coenzyme A
Cos	Costal
COSY	Correlation spectroscopy
CSA	Camphorsulfonic acid
CSCs	Cancer stem cells
δ	Chemical shifts in ppm
d	Day(s)
DAD	Diode array detector
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-Dichloroethane
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethylazodicarboxylate
DECA	*N,N*-Diethylcyclohexylamine
DEPT	Distortionless enhancement by polarization transfer
Dhh	Desert hedgehog
DHP	3,4-Dihydro-2*H*-pyran
DIPA	Di*iso*propylamine
DIPEA	*N,N*-Di*iso*propylethylamine

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Dll	Delta-like
DMAP	4-Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMPU	*N*,*N*′-Dimethylpropylene urea
DMSO	Dimethyl sulfoxide
DOS	Diversity-oriented synthesis
"dt"	Apparent doublet of triplets
Dvl	Disheveled
eq	Equivalents
ELSD	Evaporative Light Scattering Detector
ESC	Embryonic stem cells
ESI	Electrospray ionisation
Et	Ethyl
Fused	Fu
Fzd	Frizzled
g	Gram(s)
Gli	Glioma-associated oncogene homologue zinc finger protein
h	Hour(s)
Hh	Hedgehog
HMBC	Heteronuclear Multiple Bond Correlation
HMPA	Hexamethylphosphoramide
HRMS	High resolution mass spectrometry
HSD3B	3β-Hydroxysteroid dehydrogenase
HSQC	Heteronuclear single quantum correlation
HST	3β-Hydroxysteroid sulfotransferases
HTC	High-throughput chemistry
HTS	High-throughput screening
Hz	Hertz
IBX	2-Iodoxybenzoic acid
i	*Iso*
IC_{50}	Inhibition of cellular proliferation by 50%
Ihh	Indian hedgehog
Ipc	Isopinocampheyl

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Abbreviations

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iPS	Induced pluripotent stem
iPSCs	Induced pluripotent stem cells
IR	Infrared spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
J	Coupling constant in Hz
Jag	Jagged
L	Liter(s)
LC-MS	Liquid Chromatography – Mass Spectrometry
LDA	Lithium di*iso*propylamide
LiHMDS	Lithium bis(trimethylsilyl)amide
LiICA	Lithium *N-iso*propylcyclohexylamide
LXR	Liver X receptor
M	Molar
m	Multiplet
m	*Meta*
m-CPBA	*m*-Chloroperbenzoic acid
Me	Methyl
mg	Milligram(s)
mL	Millilitre(s)
MOM	Methoxymethyl
m.p.	Melting point
MPO	4-Methoxypyridine *N*-oxide
MS	Mass spectrometry
Ms	Mesyl
MHz	MegaHertz
μL	Microlitre(s)
min	Minute(s)
μM	Micromolar
MPLC	Medium pressure liquid chromatography
mRNA	Messenger ribonucleic acid
MS	Molecular sieves
n	Normal
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide

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NAD$^+$	Oxidised form of the coenzyme nicotinamide adenine dinucleotide
NADPH	Reduced form of the coenzyme icotinamide adenine dinucleotide phosphate
nM	Nanomolar
NMO	*N*-methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
NP	Natural product
nOe	Nuclear Overhauser effect
Nu	Nucleophile
p	*Para*
PAPS	3'-Phosphoadenosine-5'-phosphosulfate
PCC	Pyridinium chlorochromate
PD	Parkinson's disease
PDC	Pyridinium dichromate
PE	Petroleum ether, fraction with the boiling point range 40-60 °C
Ph	Phenyl
PMHS	Polymethylhydrosiloxane
PMP	*para*-Methoxyphenyl
ppm	Parts per million
pr	Propyl
py	Pyridine
Ptch	Patched
PTSA	*p*-Toluenesulfonic acid
q	Quartet
quint	Quintet
R	General alkyl group
R	*Rectus*
R_f	Retention factor
rt	Room temperature
S	*Sinister*
s	Singlet
SAR	Structure-activity relationship
Sm	Starting material
Smo	Smoothened

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Shh	Sonic hedgehog
ShhN	Shh/*N*-terminal peptide
SMM	Small molecule microarrays
Su(Fu)	Suppressor of Fused
t	*Tert*
"t"	Apparent triplet
T3P	Propylphosphonic anhydride
TBAF	Tetrabutylammonium fluoride
TBAH	Tetrabutylammonium hydroxide
TBAI	Tetrabutylammonium iodide
TBHP	*tert*-Butyl hydroperoxide
TBS	*tert*-Butyldimethylsilyl
"td"	Apparent triplet of doublets
TEMPO	(2,2,6,6-Tetramethyl-piperidin-1-yl)oxy
TES	Triethylsilyl
Tf	Trifluoromethanesulfonate
TFFA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMEDA	*N,N,N',N',*-Tetramethylethylenediamine
TMS	Trimethylsilyl
TPAP	Tetrapropylammonium perruthenate
TPP	Tetraphenylporphyrin
Tr	Triphenylmethyl
Ts	Tosyl, toluenesulfonyl
TTIP	Titanium *iso*propoxide
U	Enzyme activity
UV	Ultraviolet
UV-VIS	Ultraviolet-visible

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