TOWARDS THE TOTAL SYNTHESIS OF WITHANOLIDE E AND PHYSACHENOLIDE C

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ABSTRACT

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***Keywords*: Total symmetric synthesis, withanolides, physachenolides, 14-hydroxywithanolide, steroid, natural product & stereochemistry.**

Withanolides are a class of ergostane natural products found in plants of family *Solanaceae*. Plants of this family are used in traditional medicine in Asia and South America. Recently, a series of 17β-hydroxy withanolides were identified from high-throughput screens as inhibitors of androgen-induced changes in gene expression of prostate cancer cells. Therefore, these compounds may have important applications as new therapies against prostate cancer. We have devised a synthetic route to members of this family and their analogues which allows stereoselective introduction of C14, C17 and C20 hydroxyl groups in separate steps. This will allow preparation of differentially hydroxylated analogues so as to identify which contributes to the potency and thus gain a better understanding of the SAR of this class of bioactive molecules. As part of this we have shown that the stereochemical outcome of the epoxidation of $\Delta^{14\text{-}15}$ cholestanes with *m*-CPBA is controlled by the steric bulk of a C17 substituent. When the C17 is in the β configuration, the epoxide is formed on the α face, whereas if the C17 is trigonal (flat) or the substituent is in the α configuration, the epoxide is formed on the β face. The presence of a hydroxyl substituent at C20 does not influence the stereochemical outcome of the epoxidation. We have successfully introduced aldehyde functionality to the lateral side chain 14 hydroxyl compound. This aldehyde compound is a key intermediate from which many of the withanolides can be made. We have also investigated the introduction of a hydroxyl at the C18 as an entry into the physachenolides. Finally, we have carried out an assessment of the potency of the synthesised compounds against hormone-insensitive prostate cancer cell line, PC-3.

ACKNOWLEDGMENT

I am grateful to my project supervisor Dr Kamyar Afarinkia for his expertise for supervision, patient guidance and continuous support to achieve my research objectives. I am also thankful to my co-supervisor Dr Victoria Vinader (she carried out the biology work presented in this thiesis) and Dr Klaus Pors for their continuous help. I express my heartiest thanks to all my teachers who taught me throughout my life and helped me to reach the position I am today.

Words are limited to express gratitude to my parents for their immeasurable support and encouragement. Their endless prayers have inspired me to achieve the best in life. I am greatly appreciative to my brother Mr Muhammad Attiq who made this destination possible by his support and affection, without him, my dream would not have become a reality. My wife has supported and helped me in every decision I have made. I am also thankful to my family and friends for their courage and motivation to achieve this goal.

Muhammad Anees

Institute of Cancer Therapeutics

University of Bradford

2020

DEDICATION

It is with my deepest gratitude and warmest affection that

I dedicate this Thesis to my

Late Father and my beloved mother

who have been a constant source of knowledge, inspiration and courage.

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LIST OF ABBREVIATIONS

AIBN:	Azobisisobutyronitrile
BuLi:	*n*-Butyllithium
COSY:	Correlation Spectroscopy
DBN:	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU:	l,8-diazabicyclo[5.4.0]undec-7-ene
DCM:	Dichloromethane
DDQ:	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DEPT (Q):	Distortionless Enhancement by Polarization Transfer (including Quaternary Carbon)
DIPEA:	*N,N*-Diisopropylethylamine
DMDO:	Dimethyldioxirane
DME:	1,2-Dimethoxyethane
DMF:	Dimethylformamide
DMPU:	*N,N*-dimethylpropyleneurea
DMSO:	Dimethyl Sulfoxide
glu:	Glucose
HMBC:	Heteronuclear Multiple Bond Correlation
HMPA:	Hexamethylphosphoramide
HMQC:	Heteronuclear Multiple Quantum Coherence
IBX:	2-Iodoxybenzoic Acid
IR:	Infrared Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
LDA:	Lithium diisopropylamide
LICHA:	Lithium isopropyl cyclohexyl amide
LiHMDS:	Lithium hexamethyldisilazide
m-CPBA:	*m*-Chloroperoxybenzoic acid
MEMCl:	2-Methoxyethoxymethyl chloride
MOMCl:	(Chloromethyl)methyl ether
MPO:	4-Methoxypyridine N-oxide
MS:	Mass Spectrometry

MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBS:	*N*-Bromosuccinamide
NCS:	*N*-Chlorosuccinamide
NMO:	*N*-Methylmorpholine N-oxide
NMR:	Nuclear Magnetic Resonance
NOESY:	Nuclear Overhauser Enhancement Spectroscopy
PDC:	Pyridinium dichromate
Py:	Pyridine
R_f:	Retention Factor
RT:	Room Temperature
TBDPSCl:	tert-Butyl(chloro)diphenylsilane
TBS:	*tert*-Butyldimethylsilyl ether
TBSCl:	*tert*-Butyldimethylsilyl chloride
TBSOTf:	*tert*-Butyldimethylsilyl trifluoromethanesulfonate
TFDO:	Methyl(trifluoromethyl)dioxirane
THF:	Tetrahydrofuran
TLC:	Thin Layer Chromatography
TMSI:	Iodotrimethylsilane
TMSOTf:	Trimethylsilyl trifluoromethanesulfonate
TOCSY:	Total Correlation Spectroscopy
TPAP:	Tetrapropylammonium perruthenate
TPP:	*meso*-Tetraphenylporphyrin
Triton B:	Benzyltrimethylammonium hydroxide
Ts:	*p*-Toluenesulfonyl

Publication and Conferences

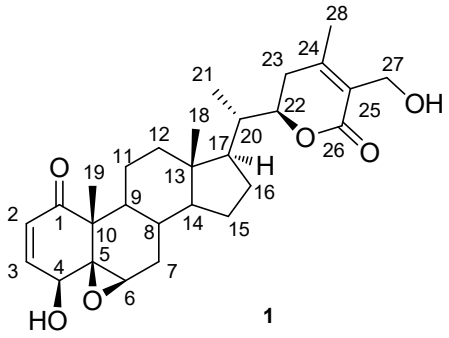
- **50th General Assembly & 47th IUPAC World Chemistry Congress, 5-12 July 2019** Paris, France
- **Anees, M**.; Nayak, S.; Afarinkia, K.; Vinader, V. Control of the stereochemistry of C14 hydroxyl during the total synthesis of withanolide E and physachenolide C. *RSC Adv.*, **2018**, 8, 39691 – 39695.
- **NCRI Cancer Conference, 5-7 November 2018** Scottish Event Campus, Glasgow, UK
- **RSC Organic Division North East Regional Meeting, 29th March 2017** Durham University, UK

CHAPTER 01

INTRODUCTION TO WITHANOLIDES AND THEIR TOTAL SYNTHESIS

1.1: Introduction

Naturally occurring withanolides belong to the C28 ergostane class of steroids which are further oxidised at C26 and C23, or at C26 and C22, to form either a γ or δ-lactone in their lateral side chain. The members of the withanolide family are known for their complex structure, and in particular presence of additional rings and diversity in the pattern of oxygenation. For example, most of these steroids also have epoxides at C5 and C6, or C6 and C7. The C1 is often found to be oxidised and indeed, more than 90% of reported withanolides are 1-ones.[1]



1

Figure 1.1: Structure of withaferin A.

The first withanolide, withaferin A (**1**, Figure 1.1), was isolated from *Withania somnifera* by Lavie *et al.* in 1965.[2] Since then more than five hundred withanolides have been isolated and characterised from plant sources. Withanolides are not widely distributed within the plant kingdom, the plant genera in which these compounds are found hail from around the world, both in the tropical and temperate zones. The majority of isolated withanolides are derived from plants belonging to the family Solanaceae. So far, phytochemical investigations on plant extracts or isolated withanolides are from 19 genera of Solanaceae family, which are *Acnistus, Datura, Deprea, Dunalis, Discopodium, Exodeconus, Hyoscyamus, Iochroma, Jaborosa, Larnax, Lycium, Nicandra, Physalis, Salpichroa, Trechonaetes, Tubocapsicum, Vassobia, Withania,* and *Witheringia.* There are other plant families which also are reported to yield withanolides, including Leguminosae *(Cassia siamea),* Labiatae *(Ajuga parviflora* and *Ajuga bracteosa),* Myrtaceae *(Eucalyptus globulus),* and Taccaceae *(Tacca plantaginea* and *Tacca chantrieri)*.

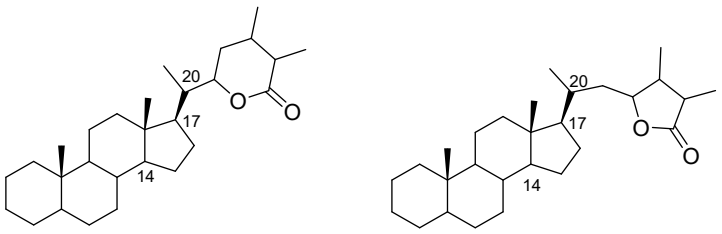
The withanolide natural products have shown antitumour, anti-inflammatory, cytotoxic, anti-anxiolytic, antifeedant, immunosuppressive and antimicrobial activities.[3] As a result,

researchers have developed an interest in this class of molecules for their potential in drug development.

In this chapter, first an overview of structural classification of this family of natural products is provided. Then a review on the biological activities reported in the members of this family and finally, a description of synthetic approaches to them is provided. This review is a compilation of recent studies and an update on previous reviews on the subject which are as follows: In 1981 Kirson and Glotter[4], reviewed classification and structural features of the withanolides. A 1991 review by Glotter[5] highlighted the classification, biosynthesis, bioactivities and synthetic approaches of withanolides. In a 1998 review, Anjaneyulu *et al.*[6] provided some spectroscopic characteristics of the withanolides and their biosynthesis. In 2005[7] structure and biosynthesis of some South American Solanaceae species were reviewed. In 2011 Chen *et al.*[1] reviewed classification, structures and biological activities of the withanolides.

1.2: Classification of Withanolides

From their structures, withanolides are divided into two main types, type A and B (Figure 1.2). Compounds with a six membered δ-lactone and δ-lactol side chain belong to type A, while compounds with a five membered γ-lactone and γ-lactol side chain belong to type B. The C17 side chain orientation can be β (which is common) or α (which is rare). In the latter case, mostly there is a hydroxyl group present at 17β position. It should be noted that whilst these are the two main types, there are a handful of withanolides which cannot fit into either type A or type B.[1]



Type A (17β configuration)

Type B (17β configuration)

Figure 1.2: Withanolides Type A and B

The majority of the withanolides isolated so far belong to type A, which is further divided into two subtypes. Withanolides with an unmodified skeleton (subtype I) and withanolides with modified skeleton (subtype II). The majority of withanolides in nature belong to the type A, subtype I family. Subtype I is further divided into 6 groups and subtype II is further divided into 13 groups.

There are fewer compounds of type B compared to type A. There are 5 subtypes for the type B withanolides. Now a very brief list of classes of withanolides and their generalised structures and subtypes are given below.

1.2.1 Type A Withanolides

Subtype I: Withanolides with an unmodified skeleton

There are following groups of withanolides in this subtype (Figure 1.3);

1. 5β, 6β-Epoxides

    5β, 6β-Epoxides with a 17α-side chain

    5β, 6β-Epoxides with a 17β-side chain

    5β, 6β-Epoxides with a 16-ene group

2. 5-Ene withanolides

    5-Enes with a 17β-side chain

    5-Enes with a 17α-side chain

3. 6α, 7α-Epoxides

    6α, 7α-Epoxides with a 17β-side chain

    6α, 7α-Epoxides with a 17α-side chain

    6α, 7α-Epoxides with a 17-ene group

4. Intermediate withanolides

    Intermediate withanolides with a 17β-side chain

    Intermediate withanolides with a 17α-side chain

    Intermediate withanolides with a 16-ene group

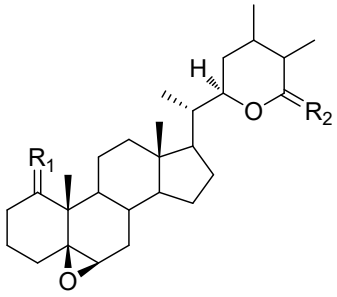
5. 5α, 6α-Epoxides

    5α, 6α-Epoxides with a 17β-side chain

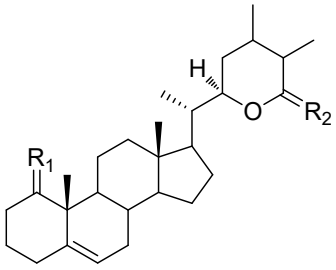
5α, 6α-Epoxides with a 17α-side chain

5α, 6α-Epoxides with a 16-ene group

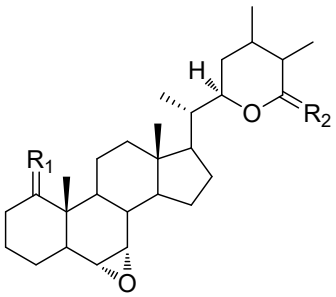
6. 6β, 7β-Epoxides



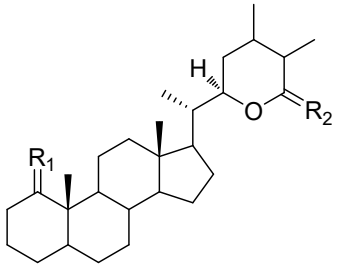
5β, 6β-Epoxides



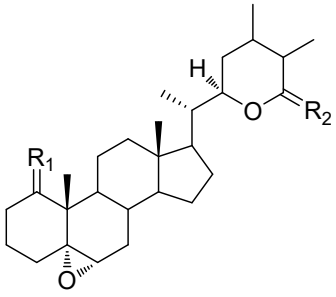
5-Ene Withanolides



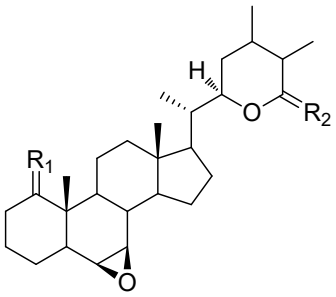
6α, 7α-Epoxides



Intermediate Withanolides



5α, 6α-Epoxides



6β, 7β-Epoxides

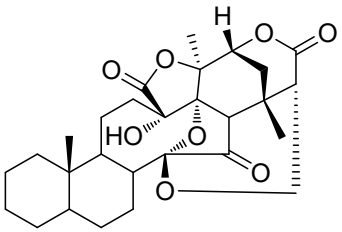
R_1 = H, OH or O and R_2 = H or OH

Figure 1.3: Subtype I: General structures of withanolides with an unmodified skeleton.

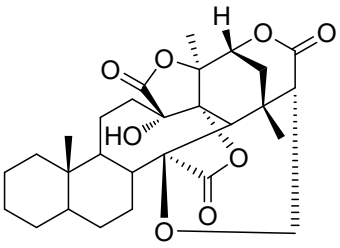
Subtype II: Withanolides with a modified skeleton

There are following groups of withanolides in this subtype (Figure 1.4);

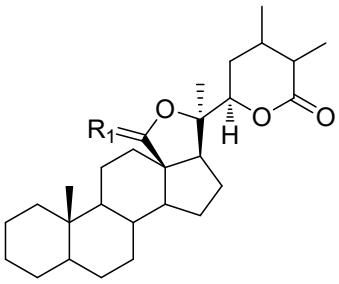
1. Physalins
2. Neophysalins
3. Withaphysalins
4. Acnistins and epiacnistins
5. Withajardins
6. Withametelins
7. Norbornane-type Withanolides
8. Sativolides
9. Spiranoid δ-lactones
10. 14α, 20α-Epoxides
11. Subtriflora-δ-lactones
12. Ring-A aromatic withanolides
13. Ring-D aromatic withanolides



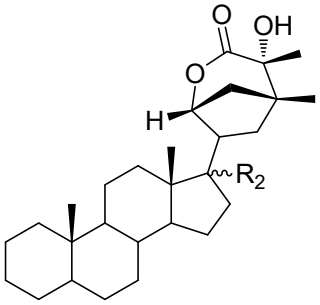
Physalins



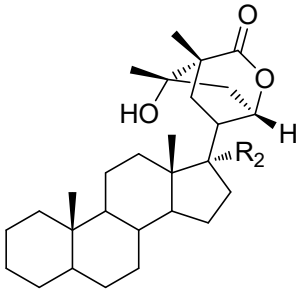
Neophysalins



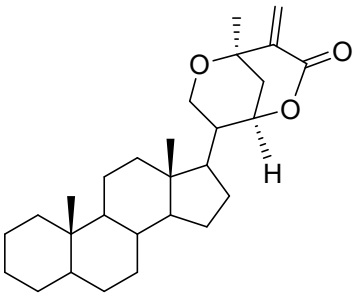
Withaphysalins



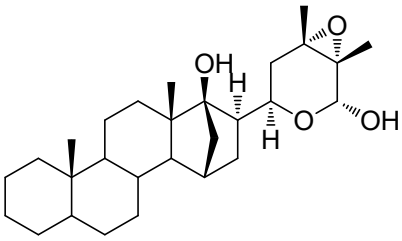
Acnistins and epiacnistins



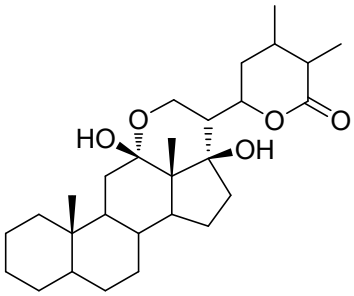
Withajardins



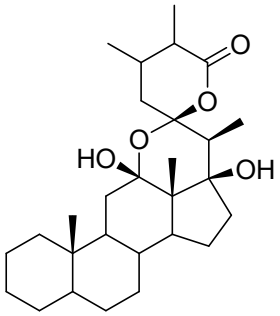
Withametelins



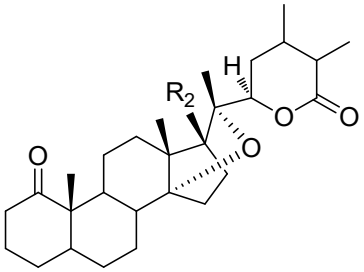
Norbornane-type Withanolides



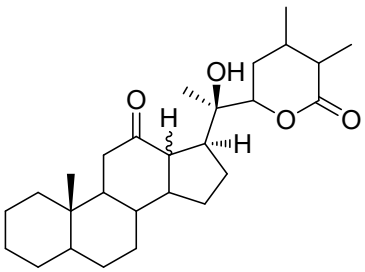
Sativolides



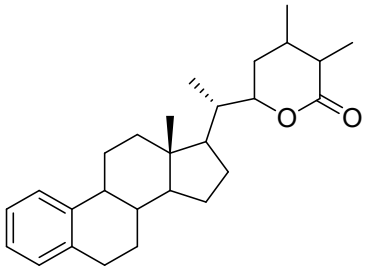
Spiranoid δ-lactones



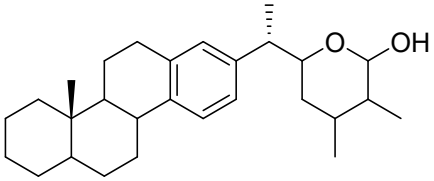
14α, 20α-Epoxides



Subtriflora-δ-lactones



Ring-A aromatic withanolides



Ring-D aromatic Withanolides

R_1 = H, OH or O and R_2 = H or OH

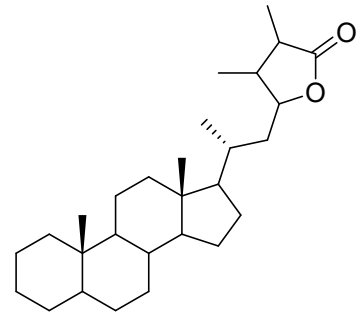
Figure 1.4: Subtype II: General structures of withanolides with a modified skeleton[1]

1.2.2: Type B Withanolides

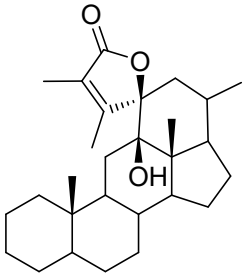
Figure 1.5 shows the withanolides in this type;

1. Ixocarpalactones 2. Spiranoid γ-lactones 3. Trechonolides

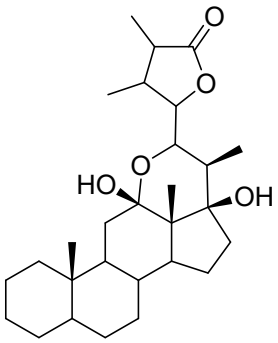
4. Subtriflora γ-lactones 5. Perulactones



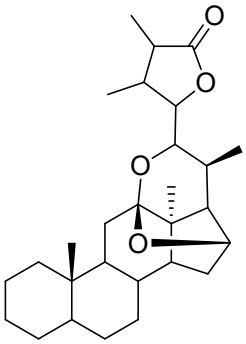
Ixocarpalactones



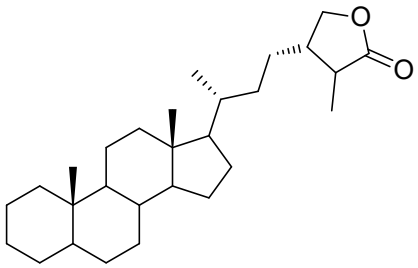
Spiranoid γ-lactones



Trechonolides



Subtriflora γ-lactones



Perulactones

Figure 1.5: Type B withanolides general structures[1]

1.3: Pharmacological Activities of Withanolides

Withanolides are also major constituent of plants and plant extracts which are widely used in traditional medicine. For example, extracts from *Withania somnifera*, which contains Withaferin A, and *Physalis angulate* have been used in different cultures for the treatment of many diseases and used as anticancer, anti-inflammatory, sedative, diuretic, antitussive and immunomodulatory agents.[8] There are also a number of isolated members of the withanolide family which have been tested in biological assays.

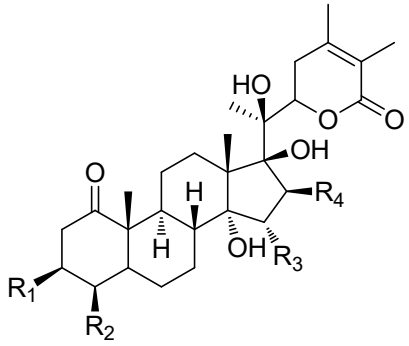
1.3.1: Antitumour activity

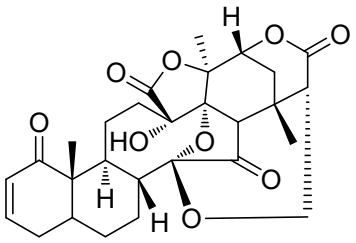
Withanolides and plants containing them have been extensively studied for their antitumour activities, including their effect on cell differentiation and cytotoxic activities.

1.3.1.1: Cytotoxic activity

The cytotoxic activity of many withanolide containing plant extracts has been studied *in vitro* on different tumour cell lines. For example extracts from *Physalis angulate* and *Physalis minima* have been used in traditional medicine for the treatment of cancer. As a result many withanolides isolated from *Physalis angulate* (Figure 1.6) have been studied for cytotoxic activity on human tumour cell lines. Some of the compounds like, physalins B, D, F, U and withaphysalins showed strong cytotoxicity against different tumour cell lines.[9, 10] Ten withanolides from *Physalis minima* (Figure 1.7) were studied by Hu and co-workers against NCI-H460 (lung) and HCT-116 (colon) cells. The compound which showed strongest cytotoxicity against both human cancer cell lines was withaphysalin C (Figure 1.7).[11] The study also led to some interesting conclusions about the relationship between the structure of the compounds and their cytotoxicity. By considering results from tumour cell lines it is clear that the 5β, 6β -epoxy group in ring B and 2,3-unsaturated ketone moiety in ring A are important for the cytotoxicity of these compounds. If one feature is missing from these withanolides, then the compound cytotoxicity is reduced and if both groups are missing then that compound possesses no cytotoxicity.

Several withaphysalin-type withanolides evaluated for the cytotoxic activity from *Acnistus arborescens* by Costa-Lotufo *et al*. Withaphysalins F, M, O and some other withanolides were found to be cytotoxic against many human cancer cell lines.[12] Withaferin A (**1**), along with many other of its biological activities, has antiproliferative activity. It has been shown that withaferin A has antiproliferative activity against pancreatic cancer cells *in vitro* (Panc-1, MiaPaca2 and BxPc3 with IC_{50} of 1.24, 2.93 and 2.78 μM respectively).[13] Some of these compounds showed inhibition of DNA synthesis and the others were cytotoxic for tumour cell lines. Some compounds in this family also seemed to have a role in inducing necrosis and apoptosis in tumour cells.[14] Withanolide D (**33**) showed *in vitro* cytotoxicity in the low micro molar range against tumour cell lines and selectivity was compared with normal human cell cultures.[15]





2 Δ^2; 5β, 6β-epoxy; $R_1 = H, R_2 = OH, R_3 = OH, R_4 = H$

3 5β, 6β-epoxy; $R_1 = H, R_2 = OH, R_3 = OCH_3, R_4 = H$

4 5α-OH, 6β-OH; $R_1 = H, R_2 = OH, R_3 = OCH_3, R_4 = H$

5 5β, 6β-epoxy; $R_1 = H, R_2 = OCH_3, R_3 = H, R_4 = H$

7 Δ^2; 5α-OH, 5β-OH; $R_1 = H, R_2 = OH, R_3 = H, R_4 = OH$

8 5β, 6β-epoxy; $R_1 = OCH_3, R_2 = H, R_3 = H, R_4 = OH$

18 Δ^2; 5β, 6β-epoxy; $R_1 = H, R_2 = OH, R_3 = H, R_4 = H$

19 Δ^2; 5β, 6β-epoxy; $R_1 = H, R_2 = H, R_3 = H, R_4 = H$

20 Δ^2; 5α-OH, 6β-OH; $R_1 = H, R_2 = OH, R_3 = H, R_4 = H$

10 Δ^5

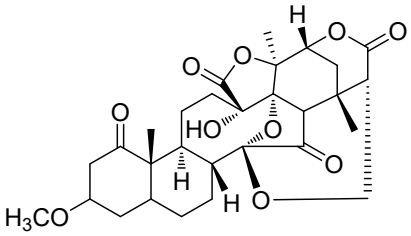
11 5α-OH, 6β-OH

12 5β, 6β-epoxy

13 Δ^4; 6α-OH

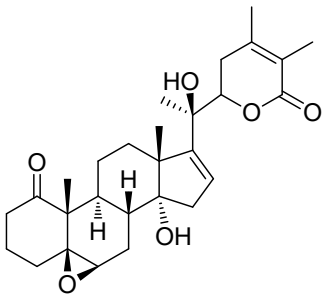
14 5α-OCH_3, 6β-OH

15 5α, 6α-epoxy



9 5αH

16 5β, 6β-epoxy



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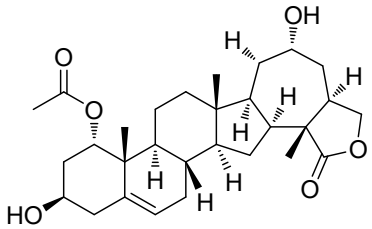
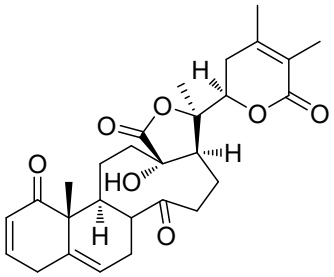


17

Figure 1.6: Withanolide from *Physalis angulate*. Withangulatins B-H (**2**-**8**), physalin B, D, F, G, I, J, U & W (**10** - **16** and **9**), physanolide A (**17**), 4β-hydroxywithanolide E (**18**), withanolide E (**19**) and withaperuvin (**20**).



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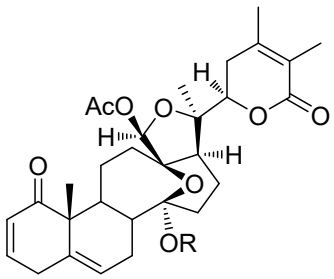
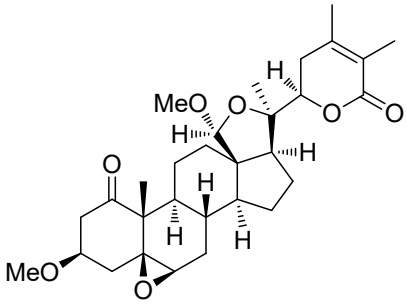


22 R = H

23 R = Ac



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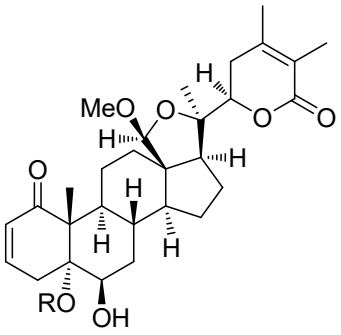
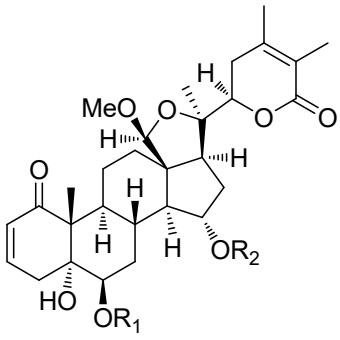


25 R = H

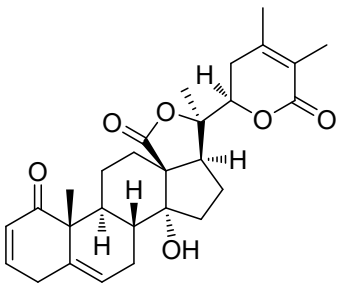
26 R = OMe



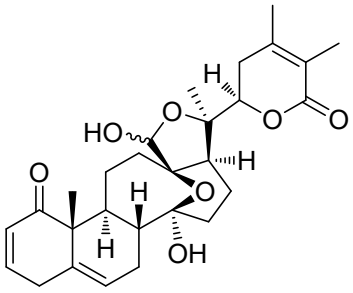
27 R_1 = H, R_2 = H

28 R_1 = H, R_2 = Ac

29 R_1 = Ac, R_2 = Ac



30



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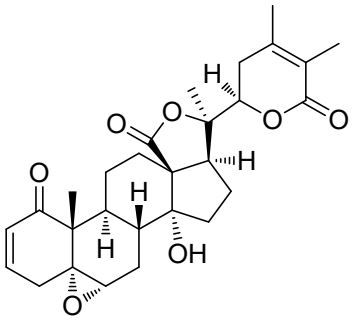


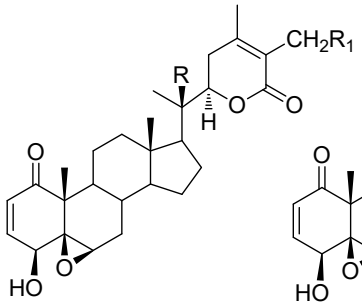
32

Figure 1.7: Withanolides isolated from the aerial part of *Physalis minima*. Withaphysalins **21** - **27**, withaphysalin A (**30**), withaphysalin C (**31**), and (5a,6a)-5,6-epoxywithaphysalin A (**32**)

1.3.1.2: Cell differentiation-inducing activity

There are a number of compounds in the withanolide family, which have been reported for their cell differentiation-inducing activity.[16-18]

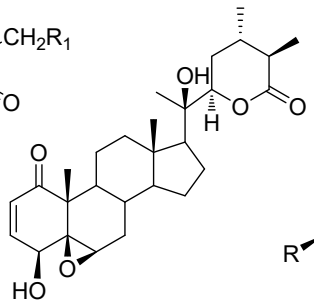
For instance, 16 withanolides from *Withania somnifera* (Figure 1.8) were tested by Kuroyanagi *et al*. against mouse myeloid leukemia (M1) cells for cell-differentiation-inducing activity.[19] Out of 16 compounds some showed very potent activity, including withaferin A (**1**), Withanolide D (**33**), dihydrowithanolide D (**36**) and 27-hydroxylwithanolide D (**34**) (Figure 1.8). These withanolides' potency for cell differentiation-inducing activity was proposed to be due to presence of 4β-hydroxy-5β,6β-epoxy-2-en-1-one side chain.



33 R = OH, R_1 = H

34 R = OH, R_1 = OH

35 R = H, R_1 = O-glu



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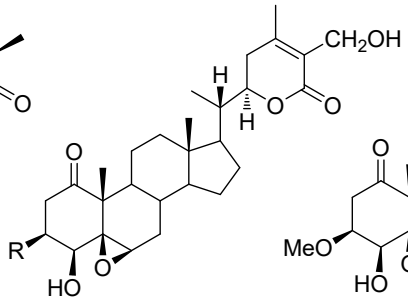
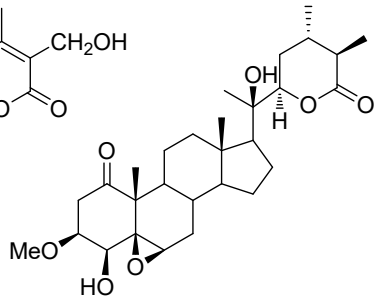


37 R = OMe

38 R= OH

39 R = H



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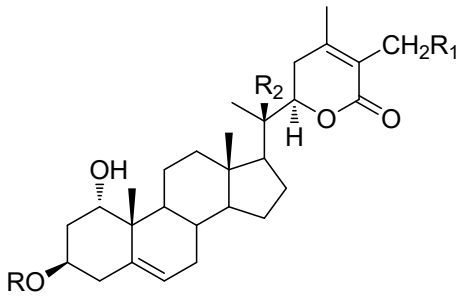
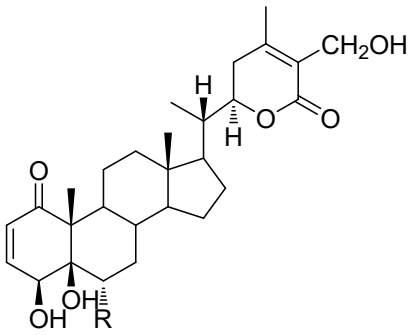


41 R = H, R_1 = H, R_2 = OH

42 R = H, R_1 = OH, R_2 = H

43 R = glu, R_1 = OH, R_2 = H

44 R = glu-glu, R_1 = R_2 = H



45 R = OH

46 R = Cl

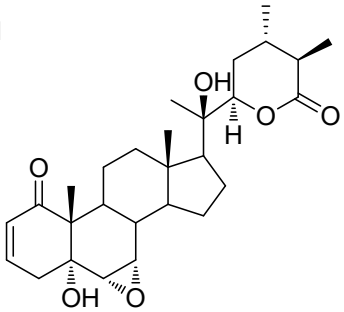


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Figure 1.8: Withanolides from *Withania somnifera*.

1.3.1.3: Radiosensitisation

Chemotherapy and radiotherapy have damaging side effects but these side effects are reported to be minimised by administering withanolides which can increase the sensitivity of the cancer cell and make it easy to destroy them with smaller radiotherapy doses.[20] Withaferin A from *Withania somnifera* along with many other significant biological roles has radiosensitising activity in radiotherapy on transplanted mouse melanoma and fibrosarcoma.

1.3.1.4: Pro-apoptotic

In the western blotting, withaferin A inhibited Hsp90 chaperone activity through an ATP - independent mechanism, which was reversed by the proteasomal inhibitor MG132. It results in Hsp90 client protein degradation and triggers *in vivo* anticancer activity against pancreatic cancer. It is also shown that withaferin A help in apoptosis pathways in cancer cells by mitochondrial dysfunction.[21]

1.3.2: Role of Withanolide in Prostate Cancer

Prostate cancer is the second most prevalent cancer for men in North America and the third most common in Western countries. [22, 23,24] Initial pathogenesis of prostate tumors is androgen-dependent, so hormonal deprivation therapy and castration can be used as a primary treatment.[25] There has been some success recently for prostate cancer therapies which target signalling of androgen receptors, like abiraterone[26] and second-generation antiandrogens, like enzalutamide[27] (Figure 1.9) but durable responses appear to be limited due to acquired resistance. Many patients eventually progress to hormone refractory, castration-resistant prostate cancer.[28] It has been shown that in androgen-insensitive prostate cancer expression of multiple androgen receptor genes resume expression, which suggests activation of transcriptional activity.[29-32] Therefore, it is important to target both androgen-sensitive and androgen-insensitive cancer cells.

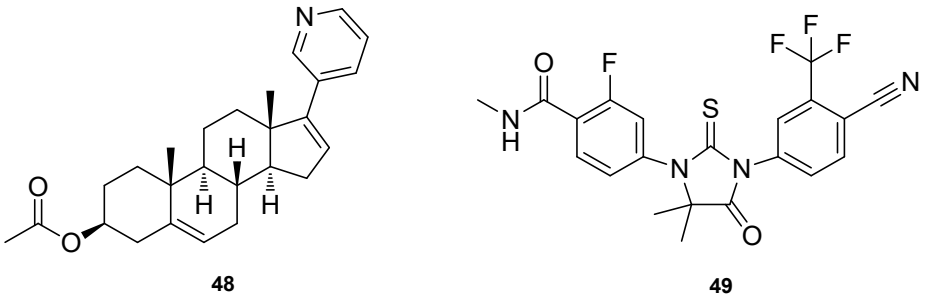


Figure 1.9: Structure of abiraterone acetate **48** and enzalutamide **49**.

The first association between withanolides and prostate cancer was reported by Roy *et al*. who observed mitotic catastrophe and growth arrest in prostate cancer cells after treatment with withaferin A (**1**, Figure 1.10) in vitro. [33] It was shown that the withaferin A induces cell cycle arrest at the G2/M phase and that this arrest may inhibit the growth of prostate cancer cells. Withaferin A associated cell cycle arrest was found to be associated with inhibition of Wee-1 expression, which results in mitotic arrest and eventually leads to cell death.

Later, Xu *et al*.[34] used application of high-throughput gene-expression profiling assay directly targeting genes of the androgen receptor pathway as a method to identify new leads for prostate cancer drugs. Screening of a natural products library led to the identification of 17β-hydroxywithanolides, from which physachenolide C **55** and physachenolide D **52** exhibited potent and selective *in vitro* activity against two PC cell lines, LNCaP and PC3. Physachenolide C **55** has shown higher potency and stability than physachenolide D **52**.

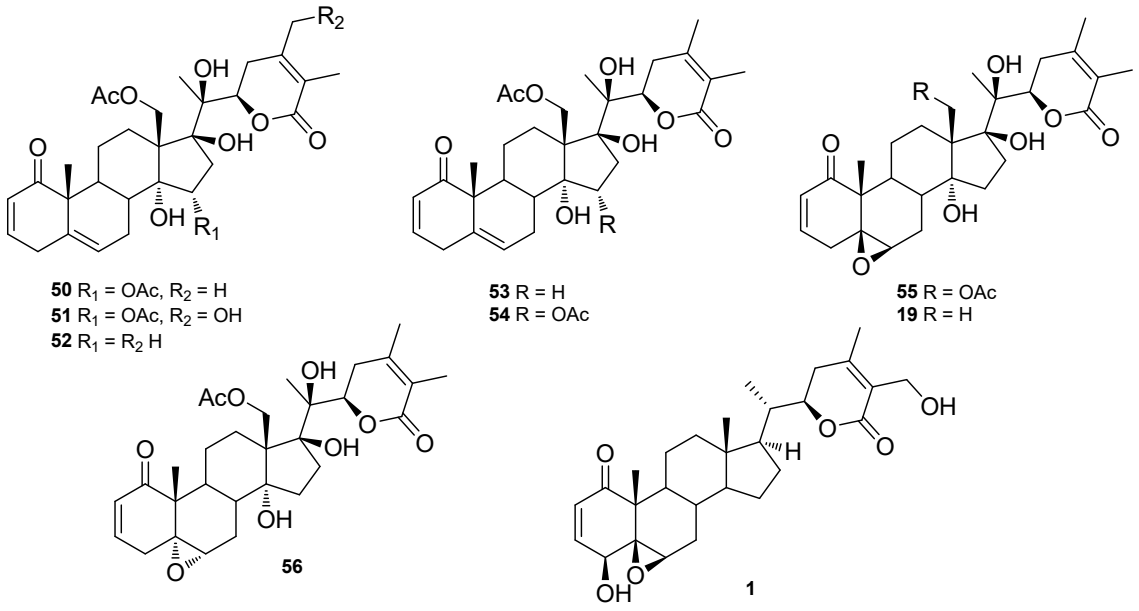


Figure 1.10: Structures of withanolides tested for prostate cancer cell lines.

A gene-expression profiling assay identifies changes in expression of gene sets in a cell, after treatment with different molecules. Dihydrotestosterone (DHT) is the main metabolite of the male hormone testosterone and it is a principal ligand for the androgen receptor, which plays a central role in prostate cancer development and progression. 13 out of 30 genes showed optimal signals for their expression in LNCaP cells with or without stimulation by DHT were selected for use in screening array. Those genes were *KLK2*, *KLK3*, *FKBP5*, *SPDEF*, *HSPA5*, *NDRG1*, *TPD52*, *STK39*, *SGK*, *NSMAF*, and *SIM2* up-regulated by DHT, and the genes *MYRIP* and *MIDI1* down-regulated by DHT. 4500 extracts from a 18000 member library of plant and microorganisms from the Sonoran desert were obtained. A high-throughput Array Plate assay provided 13500 fractions. Of the 18000 samples tested[34], an extract derived from the plant *Physalis crassifolia* showed significant inhibition of expression of the genes *KLK2*, *KLK3*, and *SPDEF* up-regulated by DHT (with IC_{50} = 4.11, 3.90, and 3.80 μM, respectively). These three genes were used for evaluating compounds during screening because of their good screening scores and this extract was selected for further screening. Physachenolide D exhibited the strong activity, with IC_{50} = 0.58, 0.75, and 0.77 μM for *KLK2*, *KLK3*, and *SPDEF*, respectively. The antiproliferative activities of 15α-acetoxyphysachenolide D **50**, 15α-acetoxy-28-hydroxyphysachenolide D **51**, 18-acetoxy-17-*epi*-withanolide K **53**, 15α,18-diacetoxy-17-*epi*-withanolide K **54**, and physachenolide D **52** were then determined in two PC cell lines, one that can be activated by androgen (LNCaP) and another that cannot be activated by androgen (PC3) (Figure 1.10). Physachenolide D **52** showed the strongest inhibition of expression of androgen-induced genes (IC_{50} = 0.70 μM), exhibited the most potent and selective antiproliferative activity against PC cell lines LNCaP and PC3, with IC_{50} = 70 and 130 nM respectively (Table 1.1).

For structure-activity relationship (SAR), data was compared with withaferin A, **1**, β-epoxidation of **52** gave **55** which had improved activity against the both PC cell lines while maintaining selectivity, whereas α-epoxidation caused significant loss of cytotoxic activity. Antiproliferative activity data (Table 1.1) suggested that the orientation of the side chain and/or the presence of the OH group at C17 may be partly responsible for their significant selectivity to the PC cell lines, especially LNCaP. In addition, the IC_{50} data of physachenolide C **55** and its 18-deacetoxy analogue, withanolide E **19**, suggested that the presence of the OAc group at C18 and/or the absence of the OH group at C4 in **55** caused 15-fold

enhancement in cytotoxic activity for LNCaP cell line. All 17β-hydroxywithanolides evaluated in this study also contained hydroxy groups at C14 and C20. These hydroxyl groups could be important for the activity of these compounds. In vivo study showed that physachenolide D **52** was capable of reducing tumor mass by 66% after 30 day treatment at a dose of 50 mg/kg[34].

Cytotoxicity (IC_{50} values in μM)		
Compound	LNCaP	PC3
1	0.87	0.41
50	0.44	2.0
51	3.3	>5.0
52	0.07	0.13
53	1.1	3.1
54	2.0	>5.0
55	0.02	0.09
56	0.32	1.2

Table 1.1: Cytotoxicity data of withanolides from *Physalis crassifolia* against LNCaP and PC3.[34]

More recently the same group reported withanolides extracted (Figure 1.11) from *Physalis peruviana* with selective cytotoxicity for the androgen-sensitive PC (LNCaP) and androgen-insensitive PC (22Rv1) cell lines.[22, 35] From all of the withanolides tested, only 17β-hydroxywithanolides (17-BHWs), **18**, **19**, **55** **64**, **65**, **68** - **72**, **74** and **75**, exhibited cytotoxicity to LNCaP cells at a concentration below 2.0 μM (Table 1.2). Withanolides **57** - **63**, **66**, **67**, **73**, and **76** - **79** have shown no activity up to 5.0 μM.

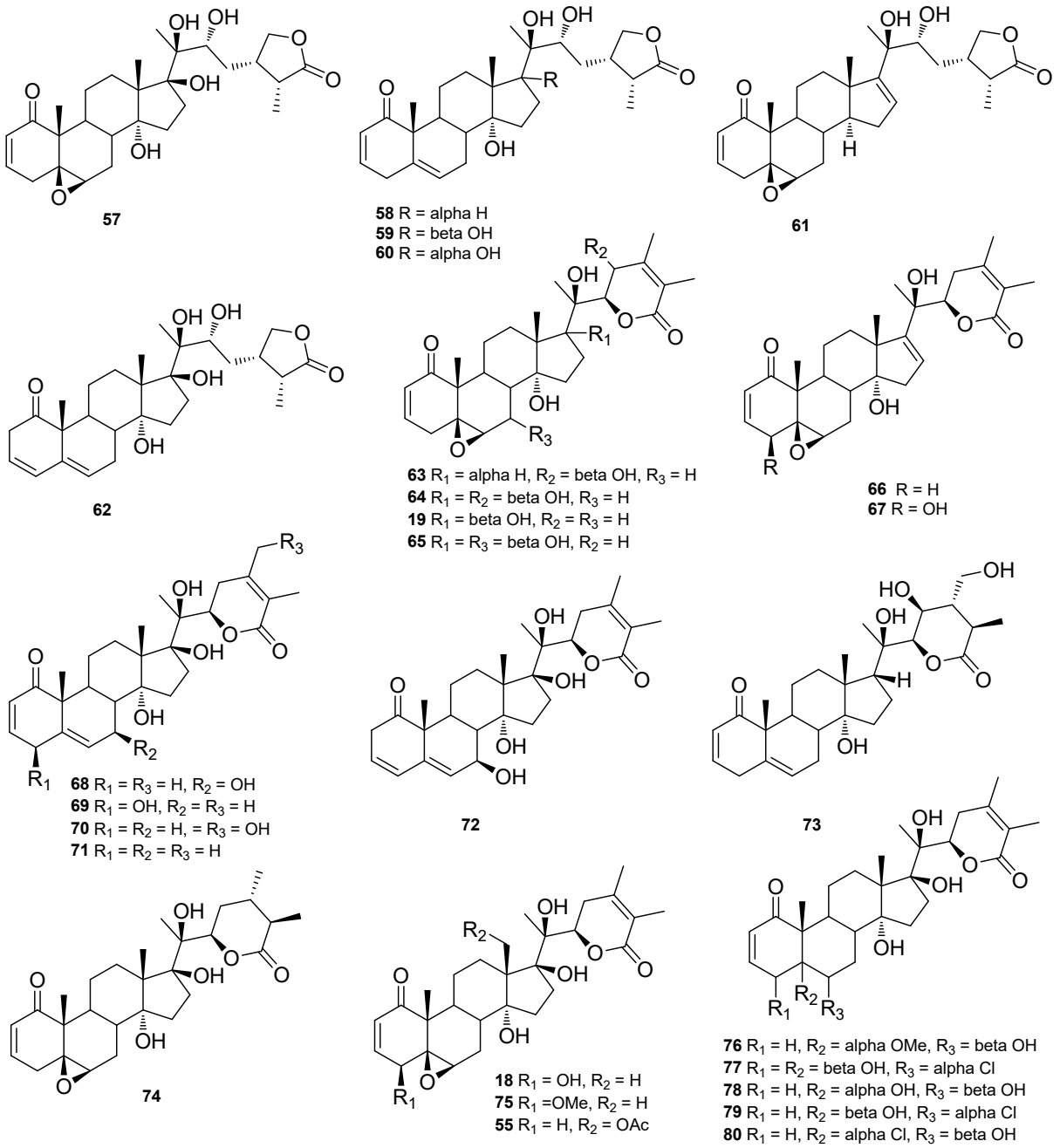


Figure 1.11: Structures of withanolides from *Physalis peruviana*.

The order of activity of these 17-BHWs (**19** > **69** > **71** > **18** > **70** > **74** > **68** > **75** > **65** > **72**) was found to be almost the same for both PC cell lines, LNCaP and 22Rv1, except for compounds **59** which have a β-OH group at C23 and it was active against 22Rv1 cells with an IC_{50} of 0.89 µM but there was no activity for LNCaP cells at 2.0 µM. Although these compounds have multiple functional groups which make it difficult to predict which functional group/s is responsible for activity but comparison of this data shows structure–activity relationship

(SAR) information for 17-BHWs. Cytotoxicity data for LNCaP cells of these 17-BHWs suggests that ring A 2,3-en-1-one and α-oriented unsaturated δ-lactone side chain and C17 β-OH are essential for their activity and acetylation at C18 leads to 3-fold enhancement of the activity (**55** vs **19**). SAR also provides information for inactivity in these compounds. Activity decreases due to (i) isomerization of the 2,3-ene to 3,4-ene (**68** vs **72**); (ii) β-hydroxylation at C4 (**18** vs **19**); (iii) β-methoxylation at C4 (**75** vs **19**); (iv) replacement of the 5β,6β-epoxide with 5,6-ene (**71** vs **19**); (v) β-hydroxylation at C7 (**75** vs **19** and **68** vs **71**); (vi) reduction of the 24,25-double bond of the side chain (**74** vs **19**); and (vii) hydroxylation at C28 (**70** vs **71**). Physachenolide C was the best compound with IC_{50} 0.02 and 0.03 for LNCaP and 22Rv1 respectively (Table 1.2).

Cytotoxicity (IC_{50} values in μM)		
Compound	LNCaP	22Rv1
18	0.16	0.09
19	0.06	0.07
55	0.02	0.03
64	>2	0.89
65	0.33	0.26
68	0.33	0.56
69	0.11	0.07
70	0.24	0.21
71	0.13	0.07
72	0.94	0.99
74	0.29	0.21
75	0.33	0.24

Table 1.2: Cytotoxicity data of withanolides extracted from *Physalis peruviana* against LNCaP and 22Rv1.[22]

The above results show that physachenolide C withanolide E with 17β-hydroxyl group have promising selective cytotoxicity for the androgen-sensitive PC (LNCaP) and androgen-insensitive PC (22Rv1) and PC3 cell lines.

1.3.3: Insecticidal and antifeedant activity

Salpichrolides A, B, C and G were studied for antifeedant properties by Mareggiani *et al*. in 2000.[36] The effect of salpichrolide A on the development of *Musca domestica* larvae was the largest among these withanolides. On the other hand salpichrolide G showed the most toxic effect on the larvae. *Tribolium castaneum* larvae development was delayed significantly by salpichrolide C, and salpichrolides A and G delayed development and caused lethal effects.[37] It is proposed that ring A having a 2-en-1-one and a hemiketal group are responsible for these antifeedant effects. The above results show that withanolides can be used for the crop protection from herbivore insects by destroying or disturbing the larval growth and development.

1.3.4: Trypanocidal and leishmanicidal activity

Trypanosoma cruzi is a protozoan that causes Chagas disease. This disease is responsible for tens of thousands of deaths and affecting millions of people each year in America.[38] A total of 215 compounds have been isolated and investigated for activity against epimastigote forms of *Trypanosoma cruzi*. Physalin F, withaphysalins M, O, and N from *Physalis angulate* and *Acnistus arborescens,* respectively, were studied for their activities and showed promising activity against epimastigote of *Trypanosoma cruzi*.[39] Nagafugi and co-workers tested many withanolides against epimastigote and trypomastigote of *Trypanosoma cruzi*,[40] hoping that the use of withanolides can potentially help to treat the Chagas disease.

Leishmaniasis is also a protozoal disease and it is distributed around the globe in 88 countries.[41] Millions of people are affected by this disease every year. Atta-ur-Rahman and coworkers studied the antileishmanial activities on nine physalins *in vitro*, which were extracted from *Physalis minima*.[42, 43] Some other withanolides were found significantly active against leishmaniasis, including isophysalin B, physalin F and physalin H.

1.3.5: Antifungal and antibacterial activity

Withania somnifera alcoholic extract and 4-deoxywithaperuvin were tested *in vitro* against eleven fungi and eight bacteria species for antimicrobial activity. It was found that *withania somnifera* alcoholic extract and 4-deoxywithaperuvin were effective against some species of bacteria but with a lower inhibitory action than chloramphenicol. Antifungal testing of 4-

deoxywithaperuvin against *Penicillium funiculosum*, *Penicillium waksmani*, *Aspergillus terreus* and *Aspergillus fumigatus* showed partial or complete inhibition but weaker effects than reference Trosyd.[44]

1.3.6: Anti-inflammatory activity

Physalis alkekengi var. franchetii, has been used as a traditional herbal medicine for its anti-inflammatory activities. Among many physalins tested for inhibition of nitric oxide synthase, four of them showed strong activity. Physalin A, B, F and O showed strong inhibition of NO production in lipopolysaccharide activated macrophages, although the effect of physalin F was the strongest.[45] Initial screening showed that physalins were the major active constituents of *Physalis alkekengi var. franchetii*. Structural features responsible for the activity of the compounds were the epoxy group or Δ^5 on the ring B and 2-en-1-one moiety on the ring A.

Withanolides have also been studied for their Immunoregulatory activities, [21, 46-50] effect on neurite outgrowth and synapses reconstruction, [51-53] inhibitory activity of cholinesterase, [54-58] diuretic activity. [59]

1.4: Previous Syntheses of Withanolides

As indicated above, a number of withanolides have been isolated and characterised from different plant species. Many of them are tested for various biological activities and have shown very promising results. To explore their potential medicinal value, different groups have synthesised some of these withanolides, either by total synthesis or semi synthesis.

Here we discuss the total syntheses of withanolides which have been reported so far, along with some semi syntheses. The focus of this review will be to highlight the synthetic strategy for introduction of key groups in withanolides. The key groups which are present in most of the withanolides require strategies for modification of ring A to introduce the enone moiety and introduction of hydroxyl groups in ring B at C5 and C6, modification of ring D to introduce hydroxyl group at C14, C15, C17, C18 and C20 and introduction of lactone moiety at C20.

1.4.1: Total Synthesis of Withanolides

In spite of the fact that withanolides have a diverse and interesting range of biological activities, there is relatively little reported on their synthesis. So far, total synthesis of only jaborosalactone A, B and D (**81** - **83**),[60] withaferin A (**1**) and 27-deoxywithaferin A (**84**),[61] withanolide D (**33**),[62] withanolide E (**19**),[63] minabeolide-3 (**85**)[64] and withanolide A (**86**)[65, 66] (Figure 1.12) have been reported, with the remaining preparative routes being derivatisation of natural withanolides (semi synthesis) and partial, incomplete syntheses.

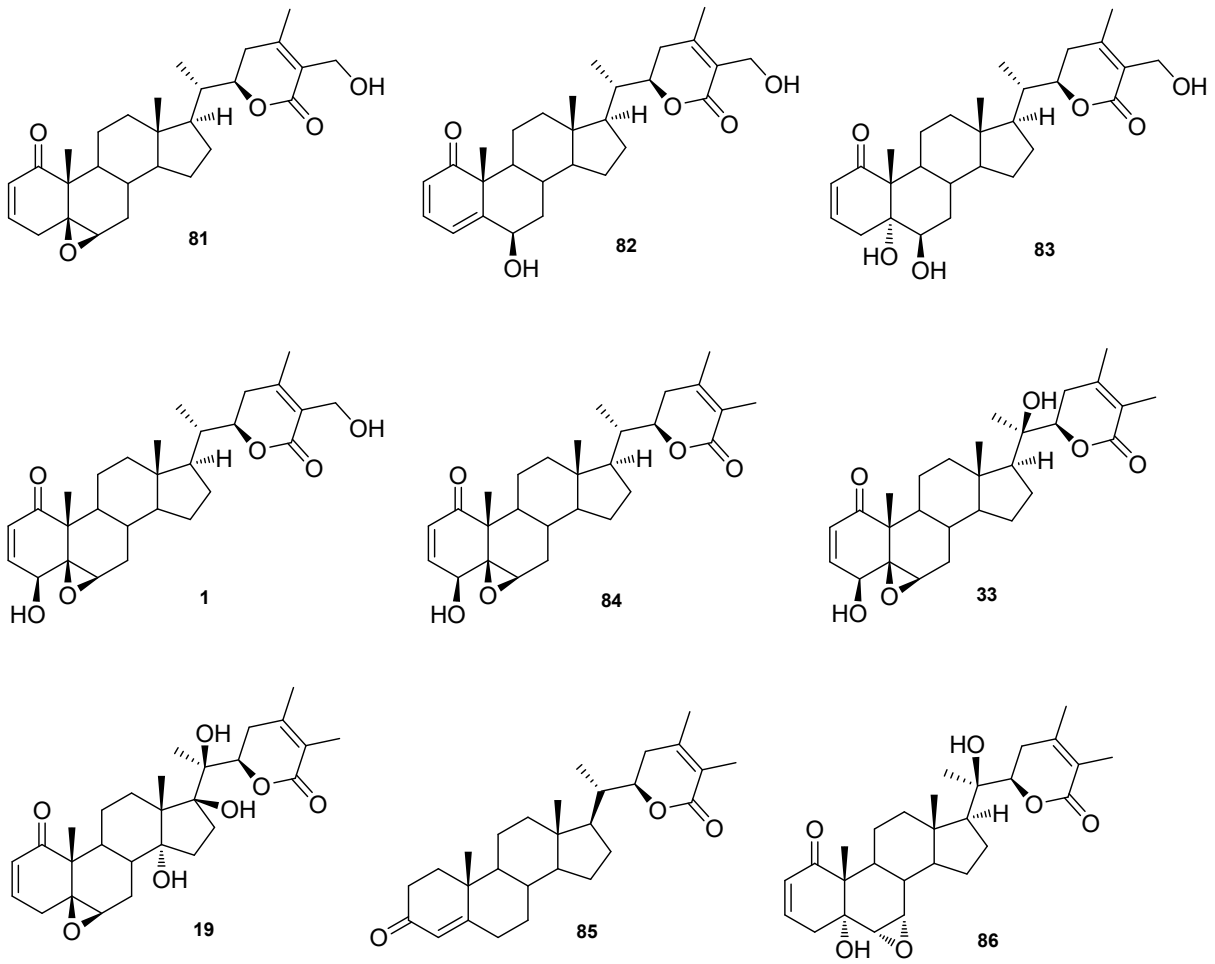
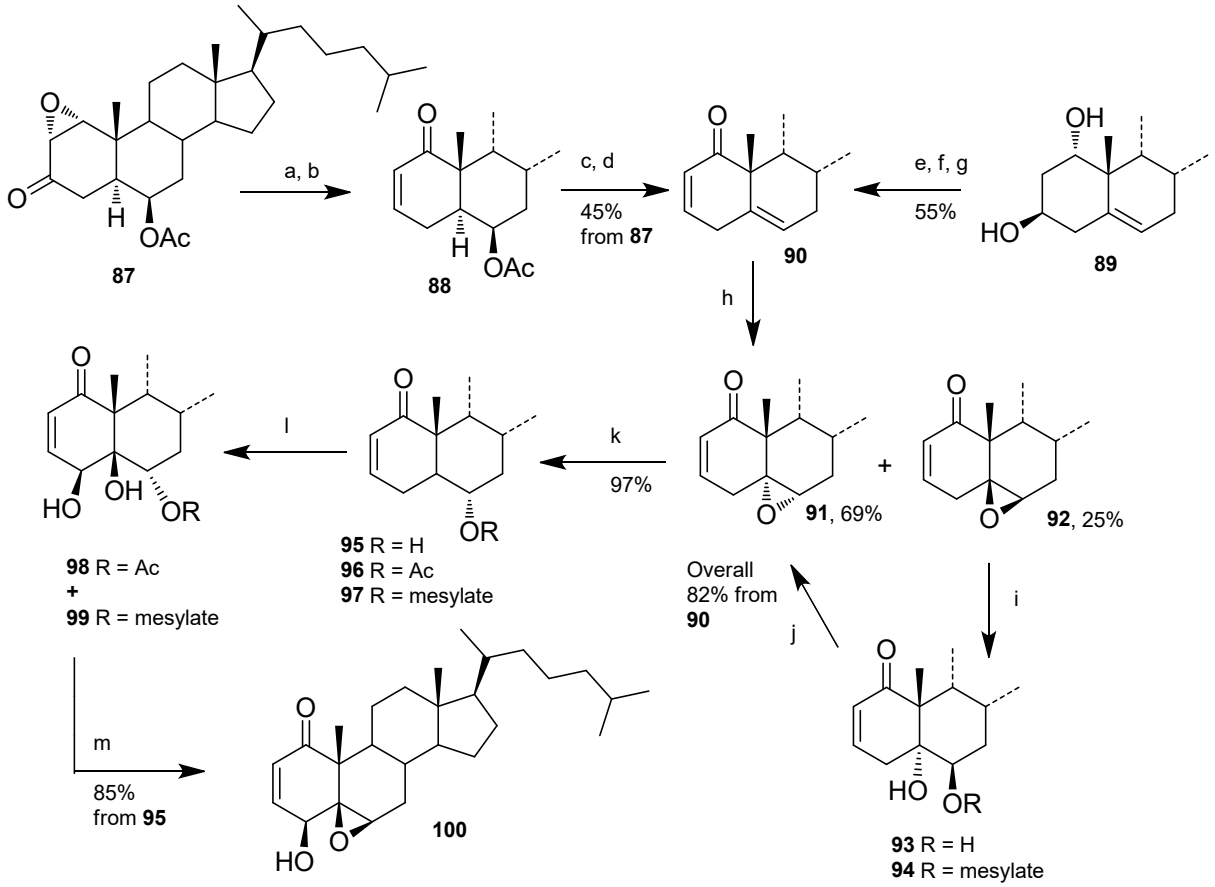


Figure 1.12: Structures of withanolides synthesised from commercially available compounds.

In 1974 Ishiguro *et al.* reported[67] the synthesis of 5β,6β-epoxy-1-oxocholest-2-en-4β-ol **100**. Ring A and B in **100** are the same as those present in many naturally accruing withanolides, including withaferin A (**1**), 27-deoxywithaferin A (**84**) and withanolide D (**33**). Therefore, this synthesis represents a model study for the synthesis of withanolides. Compound **100** was synthesised starting from 1α,2α-epoxy-3-oxo-5α-cholestan-6β-ol **87** over 6 chemical steps (Figure 1.13) and structure was confirmed by comparing NMR signals with those of withaferin A (**1**).



a) Hydrazine; b) Jones reagent; c) Hydrolysis; d) $POCl_3$-pyridine; e) Ac_2O-AcOH; f) Jones oxidation; g) NaOH, dioxane-H_2O; h) *m*-CPBA; i) $HClO_4$-THF; j) $NaHCO_3$-pyridine; k) NaOH-dioxane-H_2O, reflux; l) OsO_4; m) NaOH-pyridine, 70^oC.

Figure 1.13: Synthesis of 5β,6β-epoxy-1-oxocholest-2-en-4β-ol **100**.

In 1975, following the synthesis of **100** as a model of witheferin A (**1**), Ishiguro *et al*.[68] reported analogues of **100**. Compound **101** and **102** were the key intermediates, from which compound **103** - **108** were made. The common features of synthesised compounds are that they are stereoselective and all of them have α-hydroxyl-epoxide system connected to the cholest-2-en-1-one unit (Figure 1.14).

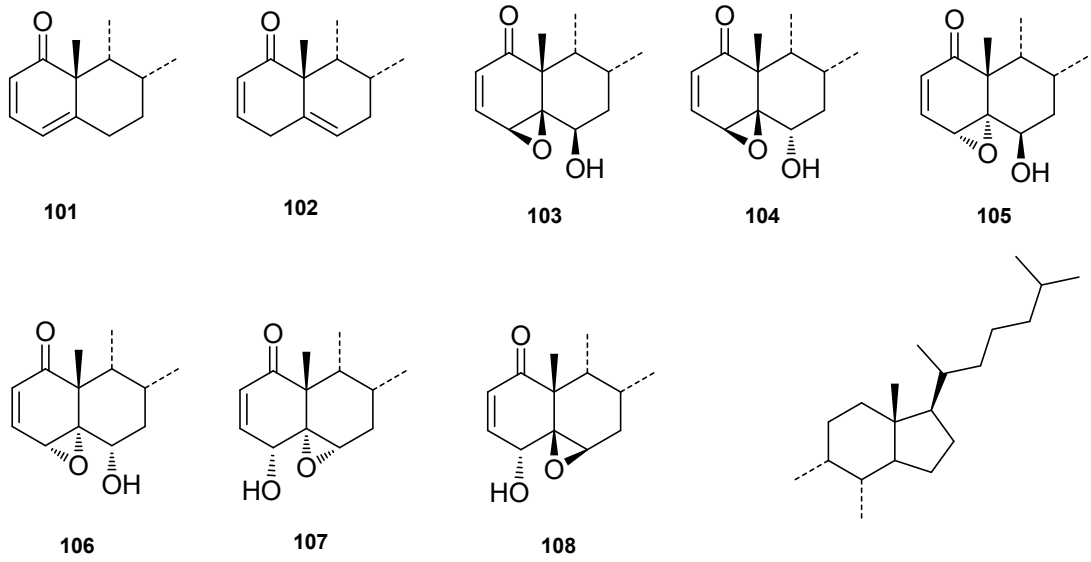


Figure 1.14: Synthesised analogues of withanolide **100**.

In 1977 Weissenberg *et al*.[69] reported construction of several cholestane derivatives. They also focused on modification of the ring A and B and made compounds with a variety of functional groups. They introduced functional groups in ring A and B which are present in certain withanolides (Figure 1.15), i.e. 2,5-diene-1-one present in withanolides G, H, J[70], F, and in physalins B **10** and C; 4β-hydroxy-2,5-diene-1-one, present in withanolide O; 5β,6β-epoxy-2-en-1-one, present in withanolide E **19** and jaborosalactone A; and 5β,6β-epoxy-4β-hydroxy-2-en-1-one, present in withaferin A **1** and withanolide D **33**.

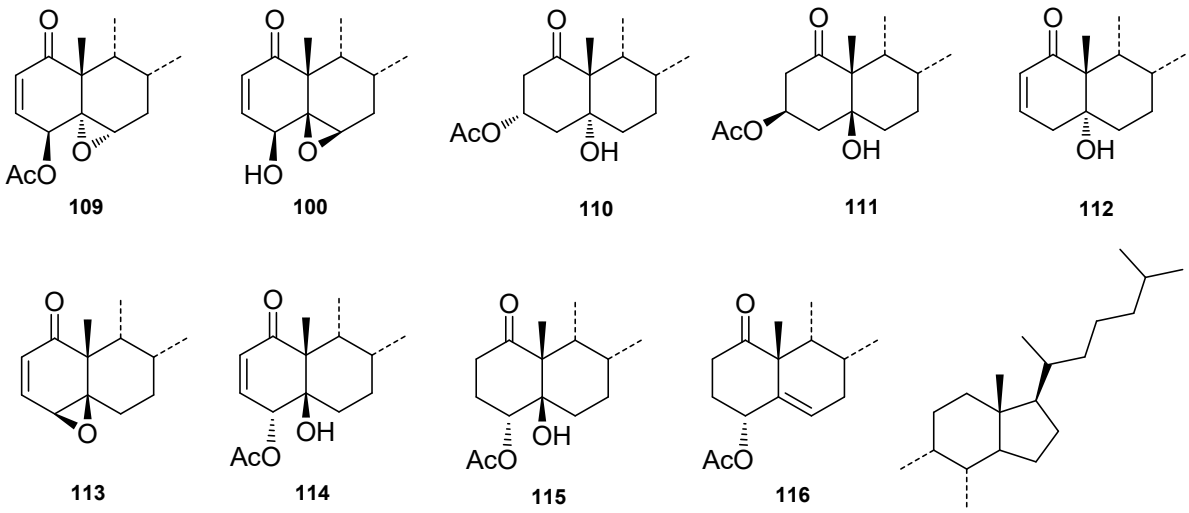
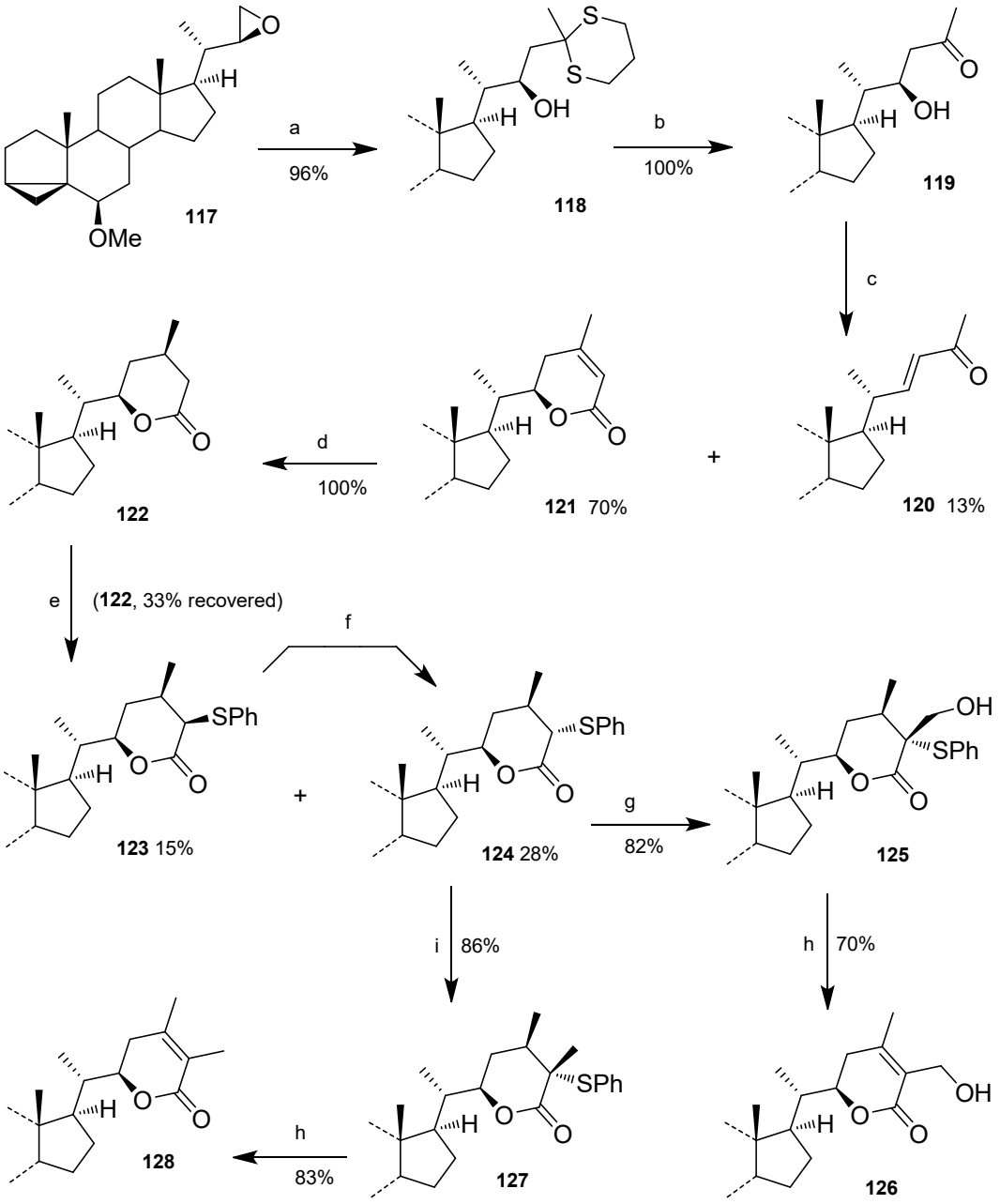


Figure 1.15: Modification of ring A and B.

Later, Hirayama, *et al*. reported the synthesis of the lactone moiety on ring D. [60, 71, 72] The lactone was made via key intermediate **119**, which was converted into **121** by ring closure

using bromoacetyl bromide. Compounds **126** and **128** which contained lactone moiety which is present in majority of naturally occurring withanolides were synthesised in 13 chemical steps (Figure 1.16). Hence this again represents a model study. Indeed, this lactone moiety is also present in jaborosalactone A, B and D **81** - **83**, withaferin A **1** and 27-deoxywithaferin A **84** (Figure 1.12) which were synthesised later by the same group.



a) 2-methyl-1,3-dithiane (BuLi), THF, - 78 ^{o}C, 2 h; b) HgO-BF_3·OEt_2, aq THF, room temperature, 15 min; c) i. $BrCH_2COBr$, Py-ether, 0 ^{o}C, 30 min, ii. $P(EtO)_3$, 100 ^{o}C, 50 min, iii. NaH, THF, room temperature, 30 min; d) H_2 (10% Pd-C, 1 atm), $NaHCO_3$, dioxane, room temperature; e) i. LICHA (2 equiv), THF, - 78 ^{o}C, 30 min, ii. $(PhS)_2$ (1 equiv), THF, HMPA, - 78 ^{o}C, 20 min; f) LICHA, 0 ^{o}C, 1 h; g) i. LICHA, THF, - 78 ^{o}C, 1h, ii. CH_2O, - 78 ^{o}C, 30 min; h) i. *m*-CPBA, $CHCl_3$, 0 ^{o}C,10 min, ii. neat,100 ^{o}C; i) i. LICHA, THF, - 78 ^{o}C, 1 h, ii. MeI, - 78 ^{o}C, 1 h.

Figure 1.16: Synthesis of **126** and **128**.

The above group reported first total synthesis of a withanolide in 1982 [60] when they reported the synthesis of jaborosalactone A, B and D **81** - **83** (Figure 1.17) from commercially available steroid **129** (Figure 1.13). First they introduced lactone moiety on ring D by using the method they reported earlier (Figure 1.16) and then modified ring A and B to complete the synthesis. Jaborosalactone A **81** was synthesised in total 35 steps, while jaborosalactone B **82** and D **83** were synthesised in 36 steps, starting from **129** as shown below (Figure 1.18).

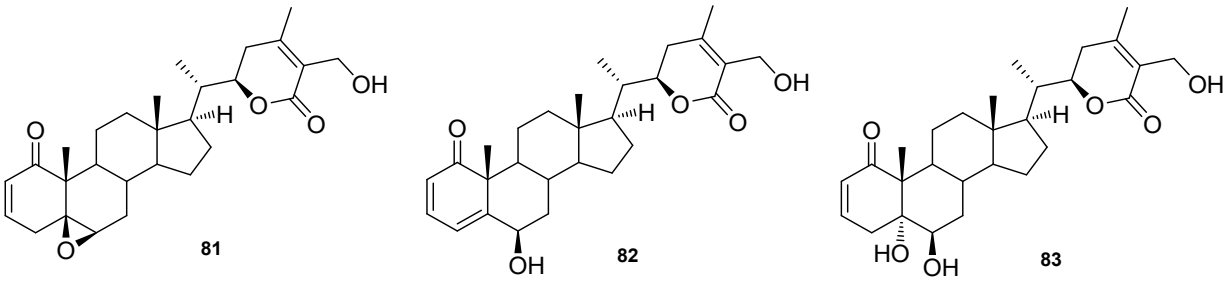
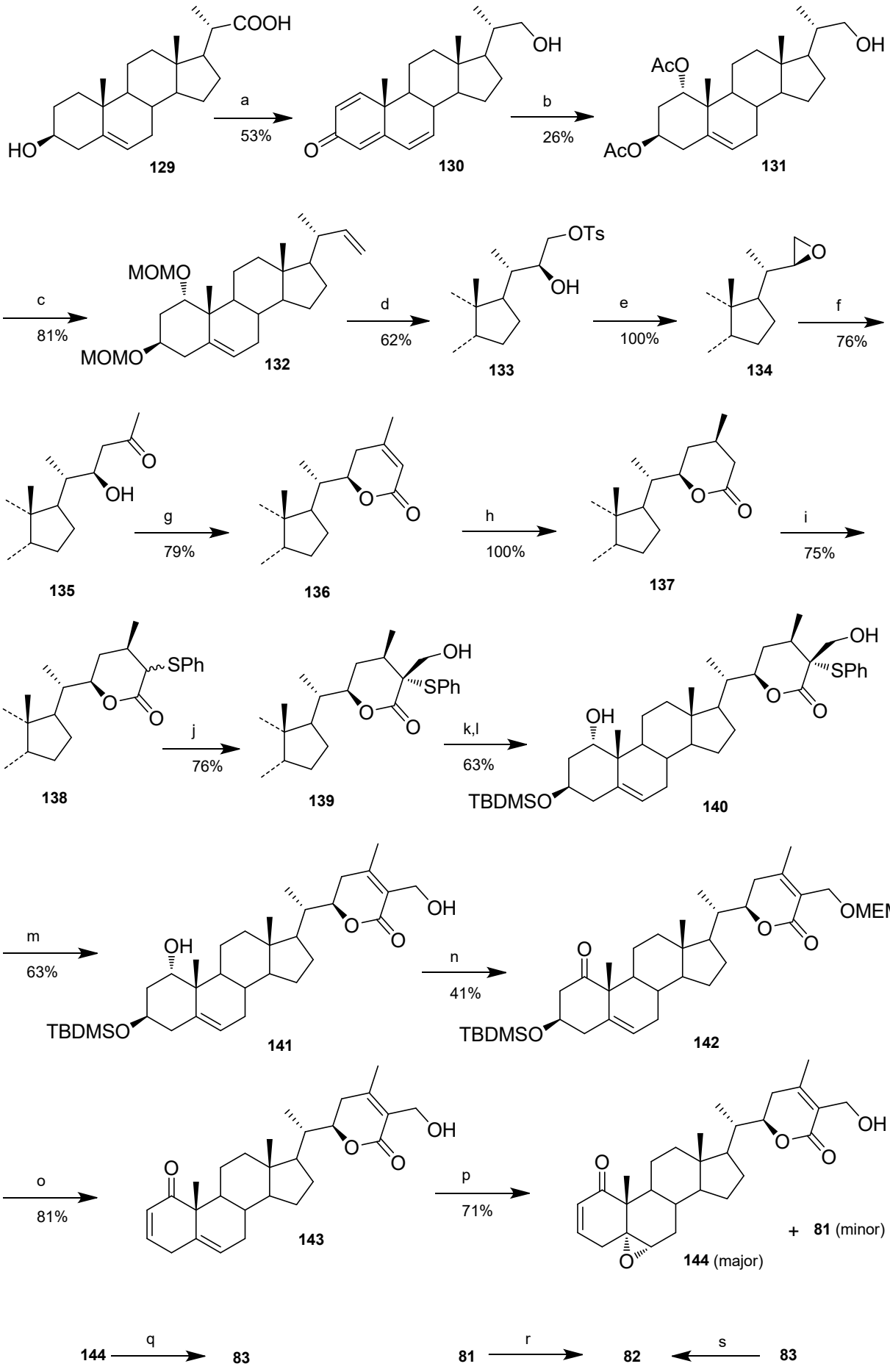


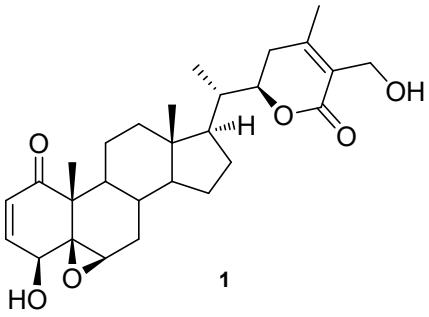
Figure 1.17: Structures of jaborosalactone A, B and D **81** - **83**.


COOH
HO
a
53%
OH
O
b
26%
AcO
AcO
OH
129
130
131
c
81%
MOMO
MOMO
d
62%
OTs
OH
e
100%
O
f
76%
132
133
134
O
OH
g
79%
O
O
h
100%
O
O
i
75%
135
136
137
SPh
O
O
j
76%
OH
SPh
O
O
k,l
63%
OH
SPh
O
O
OH
TBDMSO
138
139
140
m
63%
OH
O
O
OH
TBDMSO
n
41%
OMEM
O
O
O
TBDMSO
141
142
o
81%
O
O
OH
p
71%
O
O
OH
+ 81 (minor)
143
144 (major)
144
q
83
81
r
82
s
83

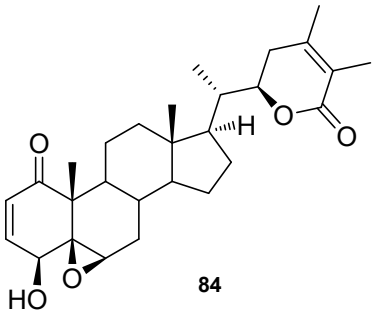
a) i. $LiAlH_4$, THF, ii. 4.2 equiv. DDQ, dioxane, reflux; b) five steps (Fuerst, A. **1978**); c) i. PCC, CH_2Cl_2, ii. $Ph_3P=CH_2$, ether, THF, iii. NaOH, MeOH, THF, iv. MOMCl, DIPEA, dioxane; d) i. OsO_4, N-methylmorpholine oxide, *t*-BuOH, THF, H_2O, ii. *p*-TsCl, pyridine.; e) K_2CO_3, MeOH; f) i. 2-Methyl-1,3-dithiane (BuLi), THF, -78 ^oC, ii. HgO, $BF_3.OEt_2$, THF-H_2O, room temperature; g) i. $BrCH_2COBr$, ether, pyridine, ii. $P(OEt)_3$ iii. NaH, THF; h) H_2, 5% Pd-C, $NaHCO_3$, dioxane; i) i. 2 equiv. LICHA, THF, -78 ^oC, ii. 1 equiv. of $(PhS)_2$, HMPA, THF, -78 ^oC; j) i. LICHA, THF, -78 ^oC, ii. CH_2O, THF, -78 ^oC; k) aq HCl, THF; l) TBDMSCl, imidazole, DMF; m) i. 1 equiv. *m*-CPBA, $CHCl_3$, -78 ^oC, ii. neat, 100 ^oC; n) i. MEMCl, DIPEA, CH_2Cl_2, ii. PDC, DMF; o) i. AcOH, H_2O, THF, ii. Ac_2O, pyridine, iii. Al_2O_3, C_6H_6; iv. H_2SO_4, THF; p) *m*-CPBA, CH_2Cl_2; q) 3% aq $HClO_4$, THF; r) 5% aq KOH, THF; s) *p*-Toluenesulfonic acid.

Figure 1.18: Total synthesis of jaborosalactone A, B and D **81** - **83**.

The same group also reported the total synthesis of withaferin A **1** and 27-deoxywithaferin A **84** (Figure 1.19) later in the same year. [61]



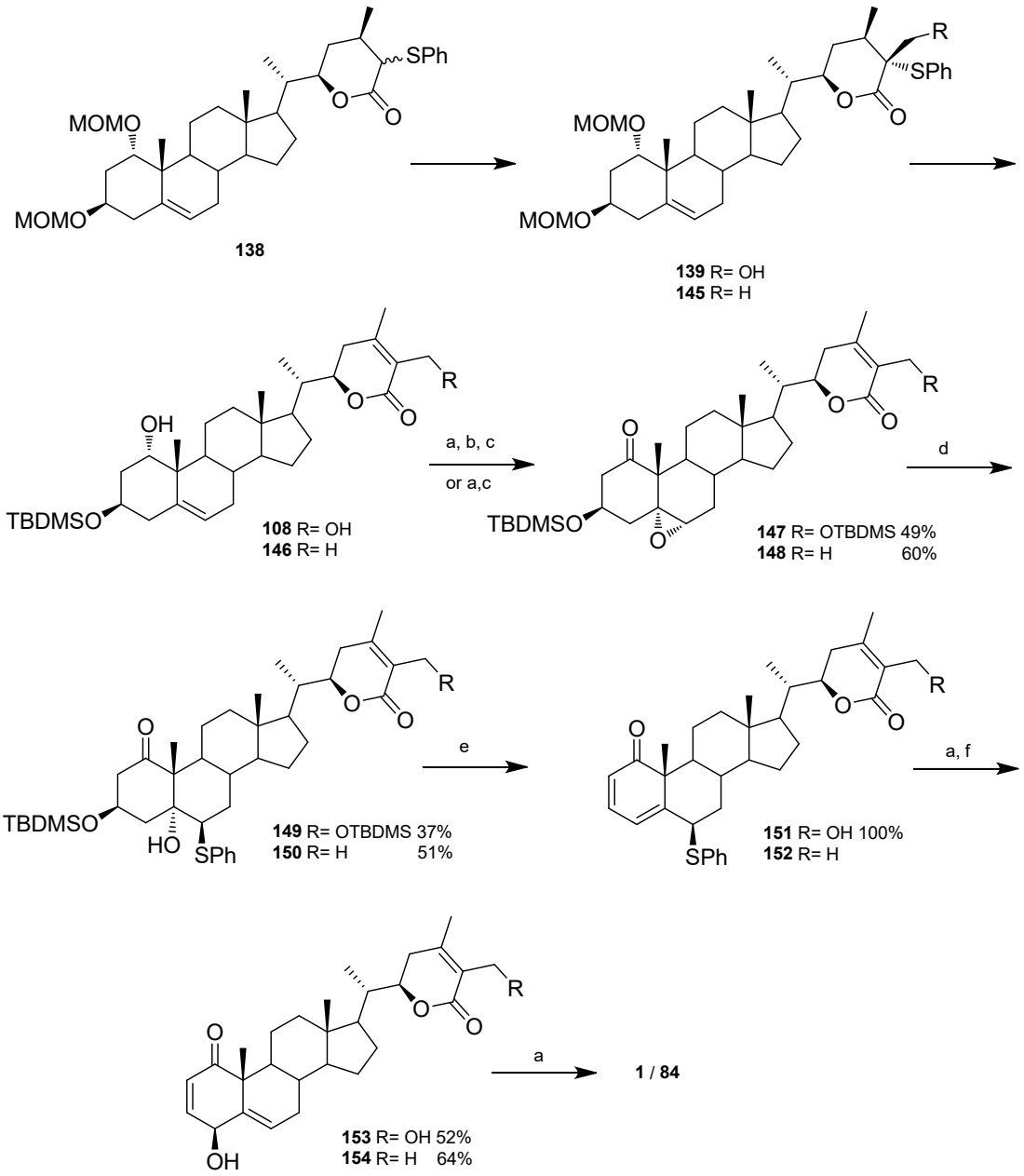
1



84

Figure 1.19: Structures of withaferin A **1** and 27-deoxywithaferin A **84**.

The synthesis starts from compound **138**, which was previously synthesised during the jaborosalactone A, B and D **81** - **83** synthesis and uses the same methodology for the introduction of lactone moiety on ring D (Figure 1.18). Withaferin A **1** was synthesised from **141** in 9 steps and from **129** in 37 steps while deoxywithaferin A **84** was synthesised from **146** in 8 steps and from **129** in 36 steps (Figure 1.18 and 1.20). The key reactions in this synthesis were introduction of lateral side chain and introduction of hydroxyl group at C4. Although ring A and B are exactly same as they are present in **100** (Figure 1.13), here different approach was used to introduce the C4 hydroxyl and C5 epoxide groups.



a) *m*-CPBA, $CHCl_3$; b) TBDMSCl, imidazole-DMF; c) PDC, DMF; d) PhSH, Al_2O_3, ether;
e) $TsOH \cdot H_2O$, C_6H_6, 60 °C; f) excess $P(OMe)_3$, MeOH-THF.

Figure 1.20: Synthesis of withaferin A **1** and 27-deoxywithaferin A **84**.

The same group also reported total synthesis of withanolides having C20 hydroxyl group.[62] Those were, withanolide D **33**, physalolactone B **155**, deacetylphysalolactone B **156** and 3β,20*R*-dihydroxy-1-oxowitha-5,24-dienolide **157** (Figure 1.21). Because of the requirement to introduce a hydroxyl group at C20, the established methodology for the introduction of the lactone ring had to be adapted. Therefore, a different approach was used to introduce the lateral lactone moiety. Aldehyde functionality was introduced on C20 and then ethyl 2,3-dimethyl-2-butenoate was added to make lactone moiety (Figure 1.22).

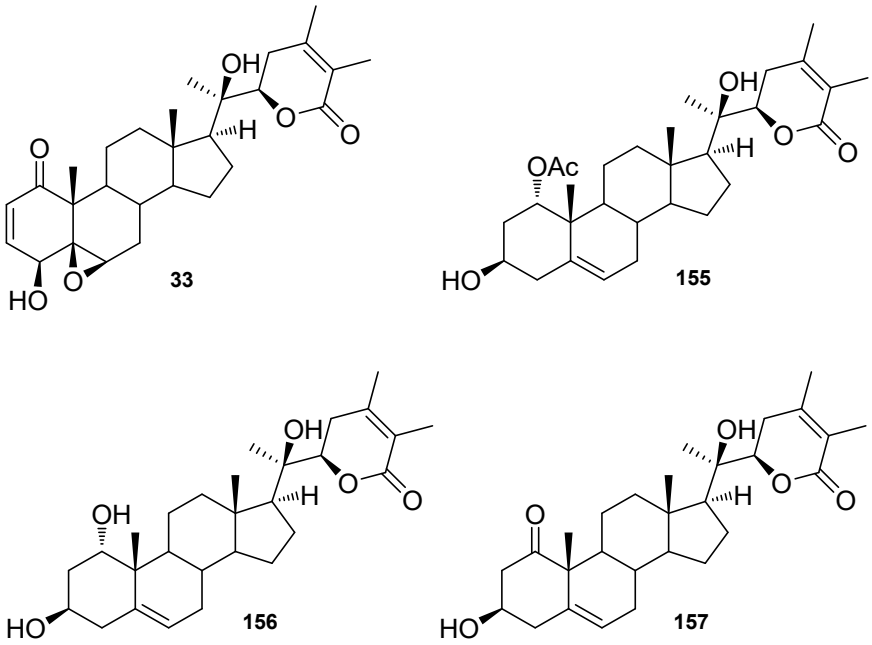
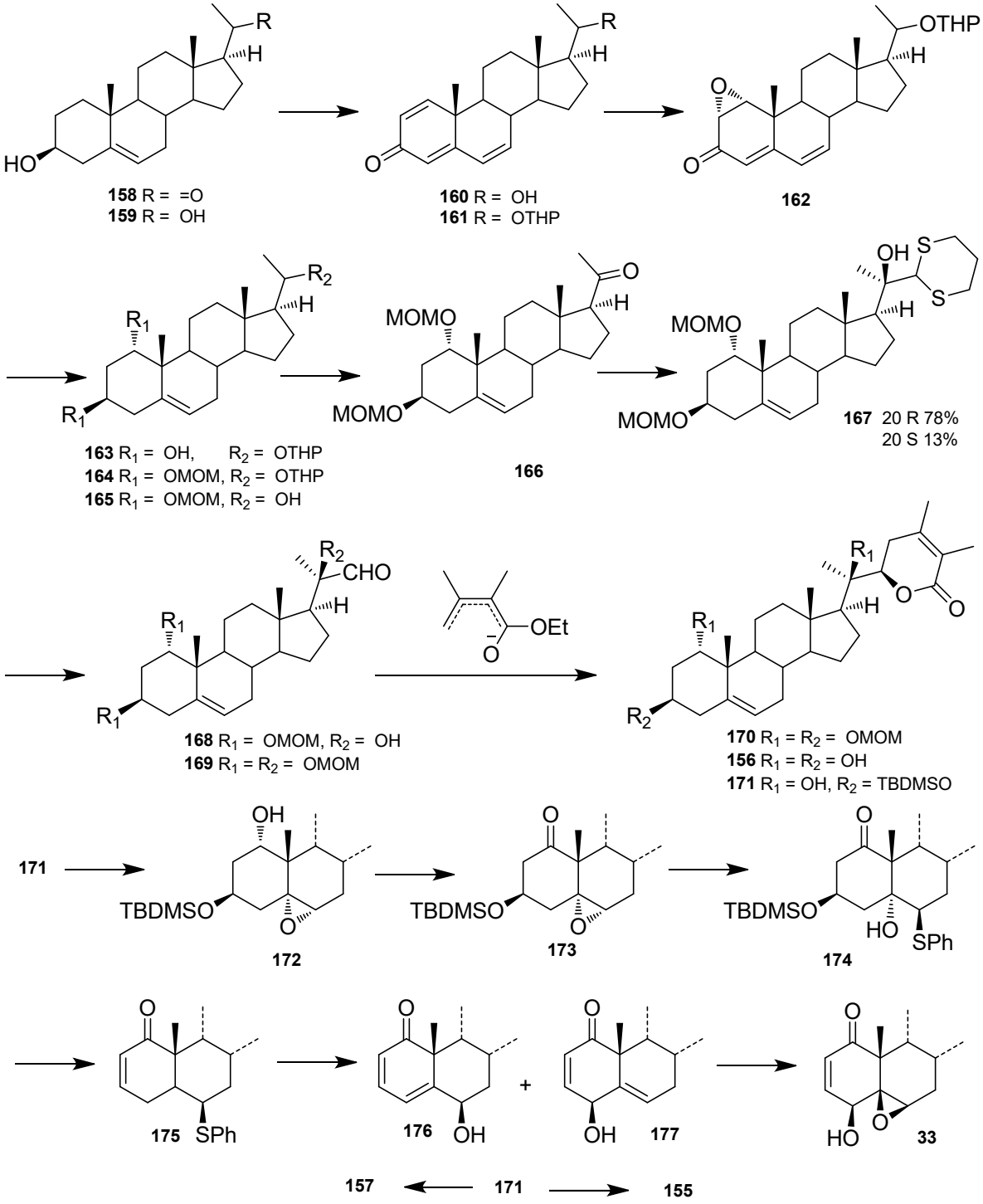


Figure 1.21: Structures of withanolide D **33**, physalolactone B **155**, deacetylphysalolactone B **156** and 3β,20*R*-dihydroxy-1-oxowitha-5,24-dienolide **157**.

The synthesis starts from commercially available pregnenolone **158**, which was converted into aldehyde **169** in 11 steps. Starting from pregnenolone **158**, deacetylphysalolactone B **156** was synthesised in 13 steps, physalolactone B **155** and 3β,20*R*-dihydroxy-1-oxowitha-5,24-dienolide **157** in 15 steps and withanolide D **33** was synthesised in 21 steps (Figure 1.22).

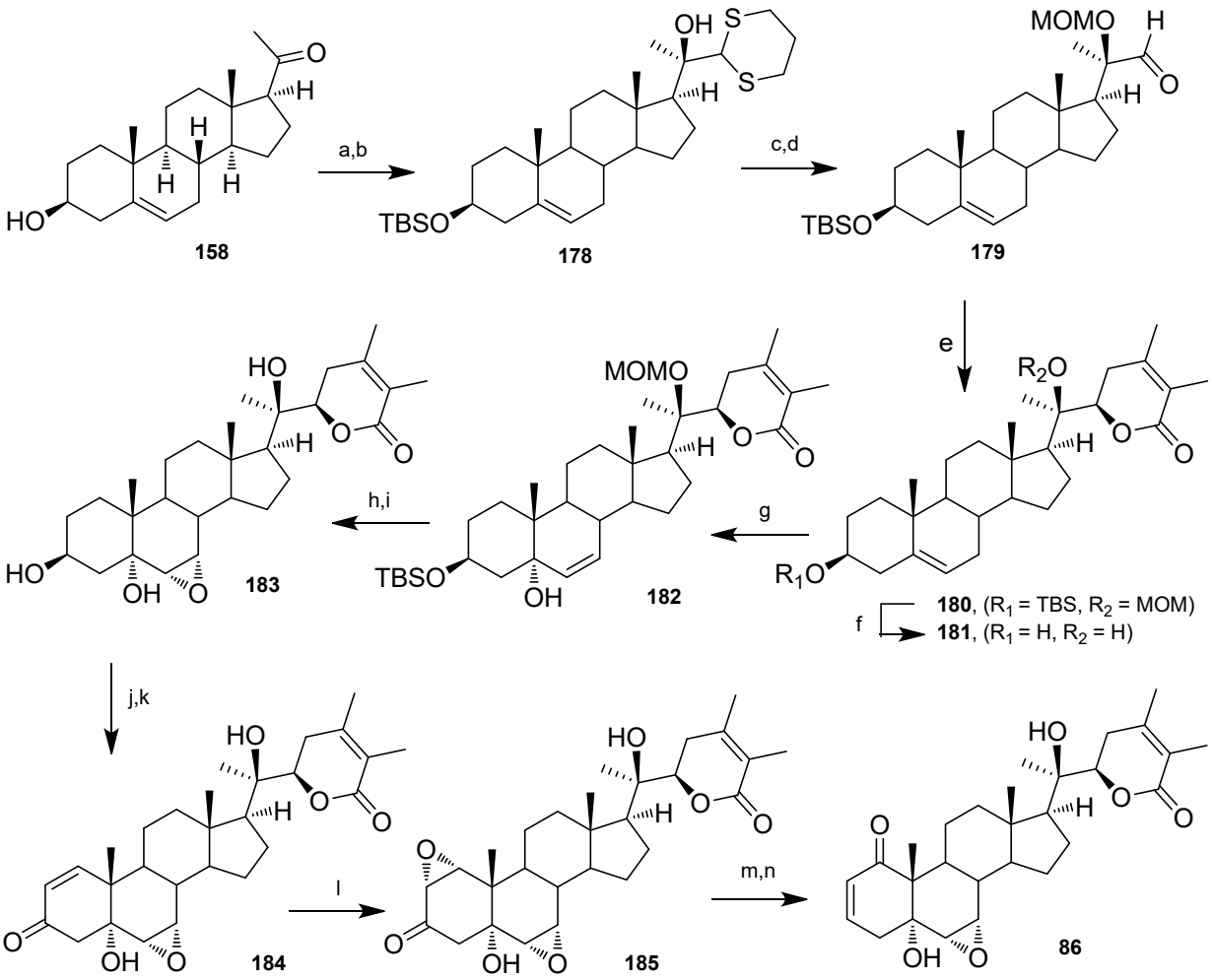


158 to **159** $NaBH_4$, MeOH-THF; **159** to **160** DDQ, dioxane, reflux, 8h, 61%; **160** to **161** Dihydropyran, TsOH, DCM, RT, 3h; **161** to **162** H_2O_2 (l), NaOH, MeOH, 15 ^oC, overnight; **162** to **163** CO_2 (s), NH_3, Li, NH_4Cl, THF (66% from **160**); **163** to **165** i. MOMCl, diethylcyclohexylamine, dioxane, reflux, 6h, ii. 2M HCl, MeOH, RT, 2h, 60%; **132** to **133** PCC, AcONa, DCM, RT, 3h, 80%; **166** to **167** 1,3-Dithiane, THF, BuLi, -5 ^oC, 8h, 20R 78%, 20S 13%; **167** to **168** HgO, BF_3-ether, THF, reflux, 30 min, 85%; **168** to **169** MOMCl, diethylcyclohexylamine, dioxane, reflux, 8h, 81%; **169** to **170** Ethyl-alpha,beta-dimethylcrotonate, LDA, HMPA, THF, - 78 ^oC, 7h, 86%; **170** to **156** 6M HCl, RT, 3h, 82%; **156** to **171** Imidazole, TBDMSCl, DMF, RT, 1h, 90%; **171** to **172** *m*-CPBA, $CHCl_3$, -5 ^oC, 40 min, 76%; **172** to **173** PDC, DMF, -5 ^oC, 8h, 80%; **173** to **174** alumina (Woelm N-20) in ether, thiophenol, RT, 20 min, ether-THF, 2h, 75%; **174** to **175** TsOH.H_2O, benzene, 60 ^oC, 1h, 84%; **175** to **176** and **177** i. *m*-CPBA, $CHCl_3$, -78 ^oC, 10 min, 76%, ii. Trimethyl phosphite, MeOH-THF, RT, dark, 10h, **176** 25% and **177** 55%; **177** to **33** . *m*-CPBA, $CHCl_3$, RT, 6h; **171** to **155** i. Ac_2O, py, RT, 4h, ii. aq AcOH, THF, 40 ^oC 1h, 68%; **171** to **157** i. PDC, DMF, -5 ^oC, 8h, ii. aq AcOH, THF, 40 ^oC 1h, 61%.

Figure 1.22: Total synthesis of withanolides with C20 hydroxyl group.

A similar methodology for the introduction of the lactone ring in the lateral side chain was used in the in the total synthesis of withanolide A **86**, by Jana *et al.*[65, 66] in 2011. Withanolide A was synthesised in 13 steps from commercially available pregnenolone **158** (Figure 1.23). The new feature in this total synthesis than that of previously reported synthesis is the introduction of epoxide at C6 position.

In the first step 3β-hydroxyl group in pregnenolone **158** was protected as a TBS ether and then Corey-Seebach umpolung method was used to make dithiane **178**. The 1,3-dithiane was then oxidatively hydrolysed to aldehyde and the tertiary hydroxyl group was protected as a MOM ether to give **179**. Lactone **180** was obtained stereoselectively by vinylogous aldol reaction from aldehyde **179** by treatment with ethyl 2,3-dimethylbut-2-enoate and LiHMDS in good yield (87%). Compound **180** was converted to allylic alcohol **182** by the reaction of singlet oxygen, which was produced in situ from O_2 under Na lamp irradiation and TPP was used as a sensitizer. Stereoselective epoxidation of **182** with 3-chloroperbenzoic acid at room temperature followed by deprotection of the hydroxyl groups by hydrochloric acid to obtain the triol **183**. The secondary hydroxyl group at C3 was oxidised into ketone by TPAP and NMO, followed by dehydrogenation by using IBX and MPO to afford **184**. The resulting enone **184** was treated with hydrogen peroxide and triton B to afford the epoxide **185**. Compound **185** was treated with hydrazine hydrochloride and trimethylamine to afford rearranged allylic alcohol, which was transferred into withanolide A **86** by subsequent oxidation by pyridinium dichromate (Figure 1.23).

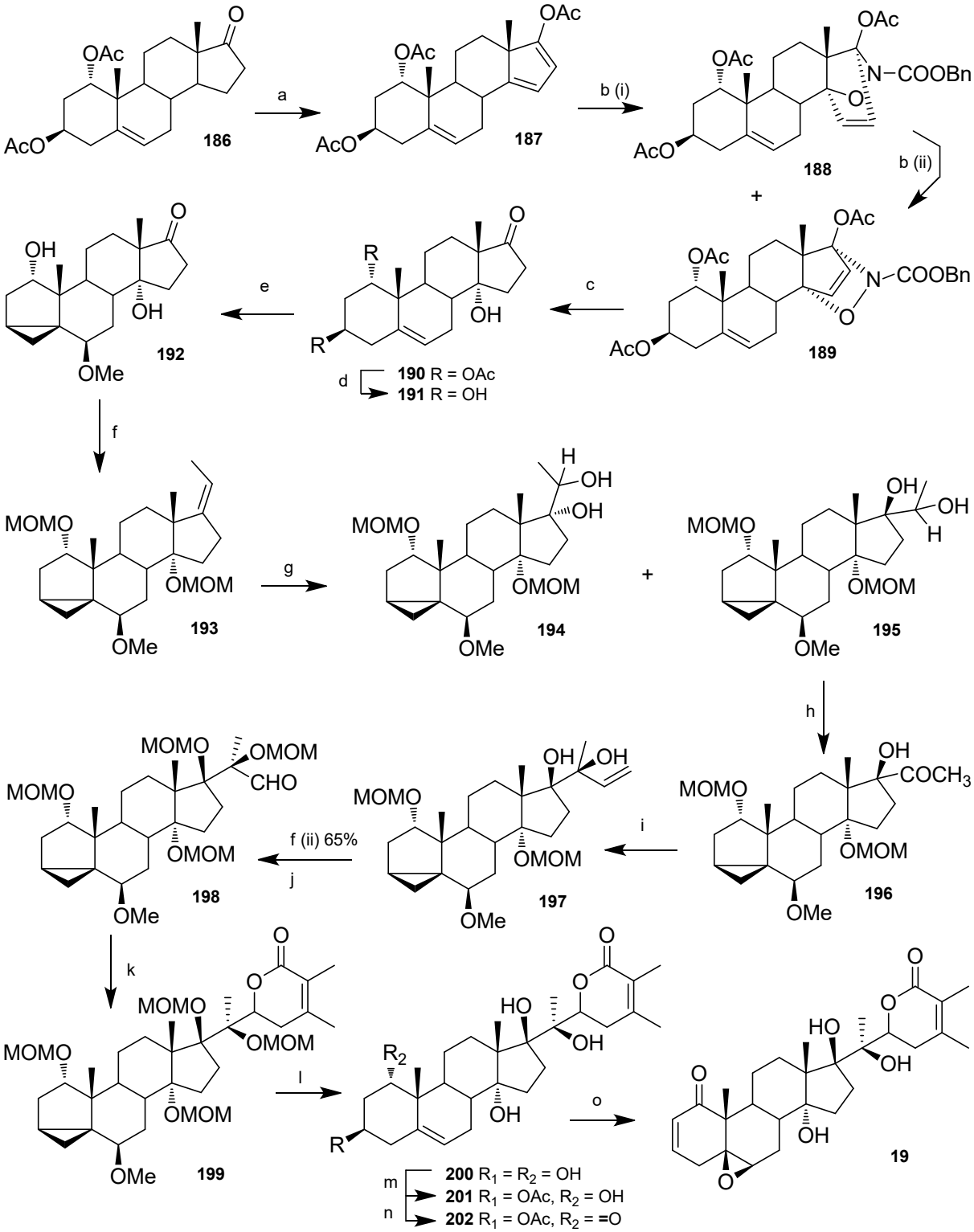


a) TBSCl, Imidazole, THF, RT, 98%; b) Dithiane, BuLi, THF, -78°C - RT, 84%; c) NCS, CH_2Cl_2, RT, 73%; d) MOMCl, NaI, DIPEA, DME, reflux, 94%; e) Ethyl 2,3-dimethylbut-2-enoate, LiHMDS, THF/DMPU, -78°C to RT, 87%; f) HCl, THF/H_2O, RT, 87%; g) O_2, TPP, Na light, pyridine, PPh_3, RT, 61%; h) 3-Chloroperbenzoic Acid, CH_2Cl_2, 0°C to RT, 96%; i) HCl, THF/H_2O, RT, 80%; j) TPAP, NMO, CH_2Cl_2, RT, 95%; k) IBX, MPO, DMSO, 40°C, 81%; l) H_2O_2, Triton B, THF, 0°C 60%; m) N_2H_5Cl, Et_3N, 0°C to RT, 62%; n) PDC, CH_2Cl_2, RT, 80%.

Figure 1.23: Synthesis of withanolide A **86**.

Total synthesis of withanolide E **19**, one of the more complex structures in the family with hydroxyl groups at the C14, C17 and C20 positions, was reported in 1991 by Perez-Medrano & Grieco.[63] Withanolide E was synthesised in 25 steps starting from diacetate **186** (Figure 1.24). It is the only reported total synthesis of a withanolide with the hydroxyl groups at C14, C17 and C20. Ring A has enone moiety while ring B has epoxide at C5 and these functional group has been introduced previously and are well established (Figure 1.13 and 1.18) but the main synthesis was carried out on ring D.

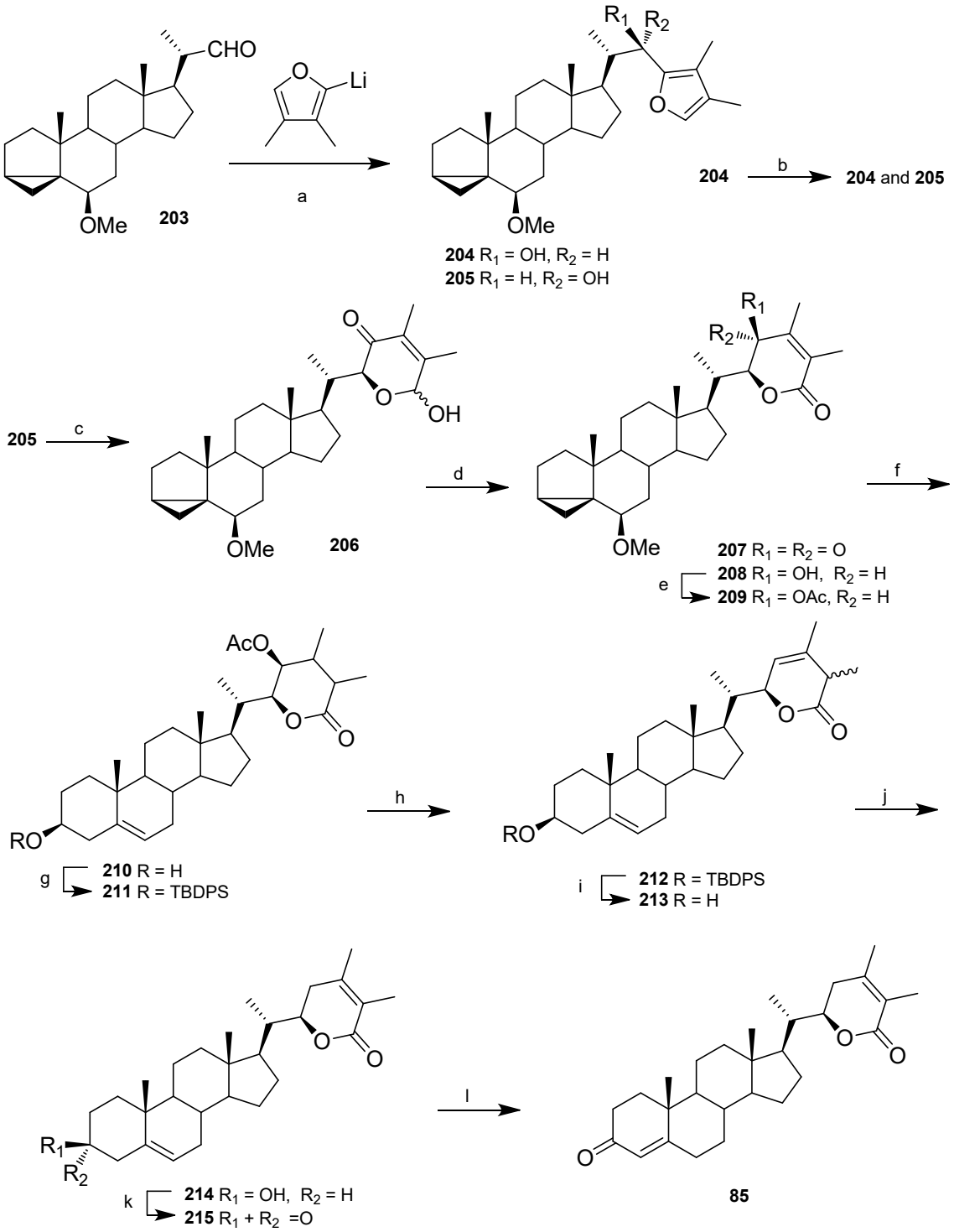
Treatment of **187** with benzyl nitrosoformate (generated in situ by oxidation of benzyl *N*-hydroxycarbamate with tetrabutylammonium periodate) afforded **188** and **189**. Cycloadduct **189** was transformed into the C14 α-hydroxy **191** by two step sequence, reduction with H_2, 5% Pd-BaSO, and then treatment with $CuCl_2.2H_2O$ and finally by hydrolysis using 5% KOH, MeOH, reflux. C14 hydroxyl was protected by using MOM group and then C17 and C20 hydroxyl groups were introduced before adding lateral lactone moiety by previously reported method (Figure 1.22). The key feature of this synthesis when compared to the previously reported synthesis was the addition of C14 and C17 hydroxyl groups and then introduction of lactone moiety.



a) i. TMSI, $(TMS)_2NH$, Et_3N, 1,2-dichloroethane, -23 ^oC, 45 min, ii. $Pd(OAc)_2$, K_2CO_3, CH_3CN, 12 h, iii. TsOH, isopropenyl acetate. 86%; b) i. benzyl-*N*-hydroxycarbamate, tetrabutylammonium periodate, **188** and **189** 1:2, ii. toluene, reflux, 20 min. **189** 85%; c) i. H_2, 5% Pd-$BaSO_4$, EtOH, 3 h, ii. $CuC1_2.2H_20$, H_20-THF, 4 h, 79%; d) 5% KOH, MeOH, reflux, 2 h, 100%; e) i. TsCI, py, 12 h, ii. TBDMSOTf, Et_3N, CH_2Cl_2, 0 ^oC, 30 min, iii. MeOH, KOAc, reflux, 12 h, iv. $Bu_4NF.THF$, 4 days. 50%; f) i. ethylidenetriphenylphosphorane, THF, ii. MOMCI, *i*-Pr_2NEt, dioxane, 80 ^oC, 24 h. 85%; g) OsO_4, py, **194** and **195** 1:1.4, 80%; h) TFAA, DMSO, Et_3N, CH_2Cl_2, -78 ^oC, 1 .5 h, 89%; i) vinyllithium in THF, -78 ^oC, 1 h, 97%; j) i. O_3, MeOH, -100 ^oC, ii. Me_2S, 30 min. 70%; k) Ethyl 2,3-dimethyl-2-butenoate, LDA, THF, HMPA, -78 ^oC to RT, 1.5 h; l) 2M H_2SO_4-dioxane, 36h; m) Ac_2O, DMAP, py, 15 h, 72%; n) Swern oxidation, 78%; o) i. DBN, CH_2Cl_2, 40 min, ii. *m*-CPBA, $NaHCO_3$, CH_2C1_2, 18 h, 71%.

Figure 1.24: Total synthesis of withanolide E **19**.

Finally in this section of note, that Tsubuki *et al*. reported total synthesis of minabeolide-3 **85** in 1992.[64] This synthesis reports a different method than previous synthesis of withanolides to introduce lactone moiety at C22. First a furan moiety was introduced[73] at C22 and then it was converted into lactone moiety by ring expansion. Minabeolide-3 **85** was synthesised in 12 steps from protected aldehyde **203** (Figure 1.25).

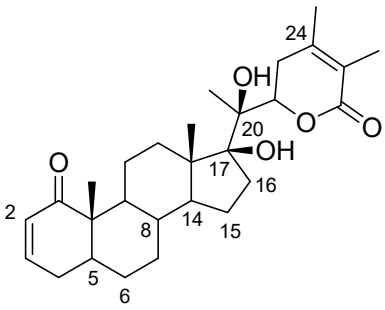


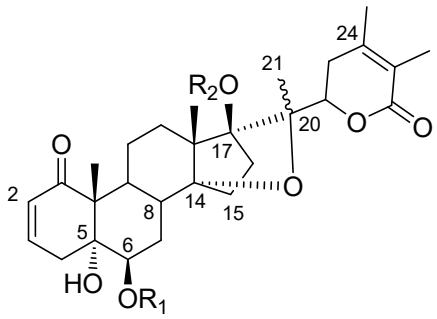
a) 3,4-dimethylfuran, *n*-BuLi, hexane, THF, -78°C **204** 60.7% and **205** 18.8%, 3.2:1; b) i. DDQ, dioxane, RT, 30 min, 61.4%, ii. $LiAlH_4$, THF, -78°C (**204** 5.8%, **205** 93.8%); c) NBS, NaOAc, THF-H_2O, 0°C, 30 min, 84.4%; d) i. PCC, NaOAc, CH_2Cl_2, RT, 2 h, ii. $NaBH_4$, MeOH, 0°C, 30 min, 73.4%; e) Ac_2O, Py, CH_2Cl_2, 0°C, 1 h, 95.4%; f) TsOH, dioxane-H_2O, 80°C, 1 h, 99.8%; g) TBDPSCl, triethylamine, 4-(dimethylamino)pyridine, CH_2Cl_2, reflux, 6 h; h) Zn(Hg), HCl=Et_2O, - 15°C, 15min, 80%; i) HF-MeCN, RT, 4 h, 93.3%; j) DBU, THF, RT, 2 h, 98.4%; k) Swern oxidation (oxalyl chloride, CH_2Cl_2, DMSO, -50°C, 45min), 80%; l) Oxalic acid, EtOH, 40°C, 3 h, 100%.

Figure 1.25: Total synthesis of minabeolide-3 **85**.

1.4.2: Semi- Syntheses of Withanolides

Glotter & Kirson *et al.*[74, 75] reported the acid catalysed modifications in the withanolide E **19** (Figure 1.9) and withanolide S **216** (Figure 1.26). Treatment of 2,3-dihydro derivative of **19** with trace of 8N-aqueous sulphuric acid in acetone for 1 hour, smoothly eliminated 14α-OH to give **217** (83%). Treatment of **19** with large amount of 8N-aqueous sulphuric acid in acetone for 4 hours provided **218** (75%) and **220** (20%). The ratio between **218** and **220** changed to 3 : 7 when the reaction was performed with 98% sulphuric acid in acetone. Withanolide S also gave the same results. Acetylation of **220** by using acetic anhydride at room temperature provided monoacetate **221** while at higher temperature the amount of 6,17-diacetate **222** increased (Figure 1.26).





216 5α-OH, 6β-OH, 14α-OH

217 5β, 6β-epoxy Δ^{14}

218 5α-OH, 6β-OH, Δ^{14}

219 5α-OH, 6β-OAc, Δ^{14}

220 $R_1 = R_2 = H$

221 $R_1 = Ac$ $R_2 = H$

222 $R_1 = R_2 = Ac$

Figure 1.26: Acid catalysed modifications in the withanolide E **19**.

There are some other notable synthesis and modifications of withanolides which are mentioned below but these are not discussed in detail.

Keinan and Sahai gave a chemoselective method for the conversion of withaferin A **1** into jaborosalactone and 4β-acetoxywithanolide E into withaperuvin C by eliminating oxygen functionalities with palladium-mediated reduction.[76] α, β-Unsaturated side chain, δ-lactone analogues of the withanolides were synthesised by condensation and decarboxylation of aldehydes at C_{21} and C_{22} by Anna *et al*.[77] Different withanolide artifacts were reported when some of the natural withanolides from *Physalis longifolia* were left in methanol.[78] Twenty

withanolide derivatives were synthesised from jaborosalactone 5 by single step synthesis.[79] A series of withanolide derivatives were synthesised with modifications at carbons 5, 6, and 7 in ring B of withanolides.[80]

In summary, there is significant precedence for the introduction of the pyrone-containing lateral side chain and for the manipulation of rings A and B for the synthesis of withanolides (and by extension physachenolides). However, with the exception of a report by Grieco, there are no methods for the introduction of hydroxyls on ring D. Furthermore, Grieco's method introduces hydroxyls at C14 and 17 simultaneously and hence is not suitable if hydroxylation at only one of those positions were required.

CHAPTER 02

REVIEW:

FUNCTIONALISATION

OF

C14 AND C18 IN

CHOLESTANE MOLECULES

2.1: Hydroxylation at C14 in Steroids

As described in the previous chapter, hydroxylation at C14, C17, C20 are common in both withanolides and physachenolides and in addition, hydroxylation at C18 is a feature of physachenolides. Indeed, hydroxylation at each of these positions individually or collectively is seen in a number of steroid natural products. Any total syntheses of these natural products, in particular withanolides and physachenolides must include methodologies for regio- and stereoselective introduction of these hydroxyls. Therefore, in this chapter existing methods for these hydroxylations are surveyed.

A summary on introduction of C14 hydroxyl group is now presented. To note is that apart from regioselectivity, a key feature of any methodology used to introduce hydroxylation at C14 position must ensure stereoselectivity. In other words, methods used to introduce C14 hydroxyl should not only be regioselective but also give only the α or the β isomer.

2.1.1: Hydroxylation at C14 by using Chemical Oxidants

Generally speaking, methods for the introduction of the C14 hydroxyl fall into two broad categories: those where a chemical reagent is used and those where either an intact microorganism or an isolated enzyme from that microorganism is used. Chemical methods for introduction of C14 hydroxyl are further split into three categories: those involving direct conversion of unactivated H14 to 14-OH; those where H14 is activated or C14 is part of a double bond, and finally those involving a Norrish type I/Prins reaction. In 1986 H.W. Hoppe and P. Welzel starting from deoxycholic acid **223**, synthesised bufalin acetate **225** in 12 steps .[81] The scheme for introduction of C14 hydroxyl group is given below (Figure 2.1). The key point and challenge in the synthesis was introduction of the C14 hydroxyl group. This was achieved by the conversion of **223** to **226** which was then converted to **228** via a Norrish type I fragmentation and subsequent reduction of unstable aldehyde intermediate, **227**. Conversion of the phenylsufide to sulfone and elimination afforded the α-pyrone **229**. Presumably the construction of α-pyrone succeeded the Norish reaction as it is a UV absorbing aromatic ring. Alcohol **228** was converted to mesylate **230** to make it a better leaving group and treatment with acidic water/acetone mixture resulted in a conjugate displacement/cylisation to **224**. After hydrolysis bufalin acetate **225** was obtained. Although

this was an overall a short synthesis, the yield of key steps were low making it a relatively inefficient synthesis.

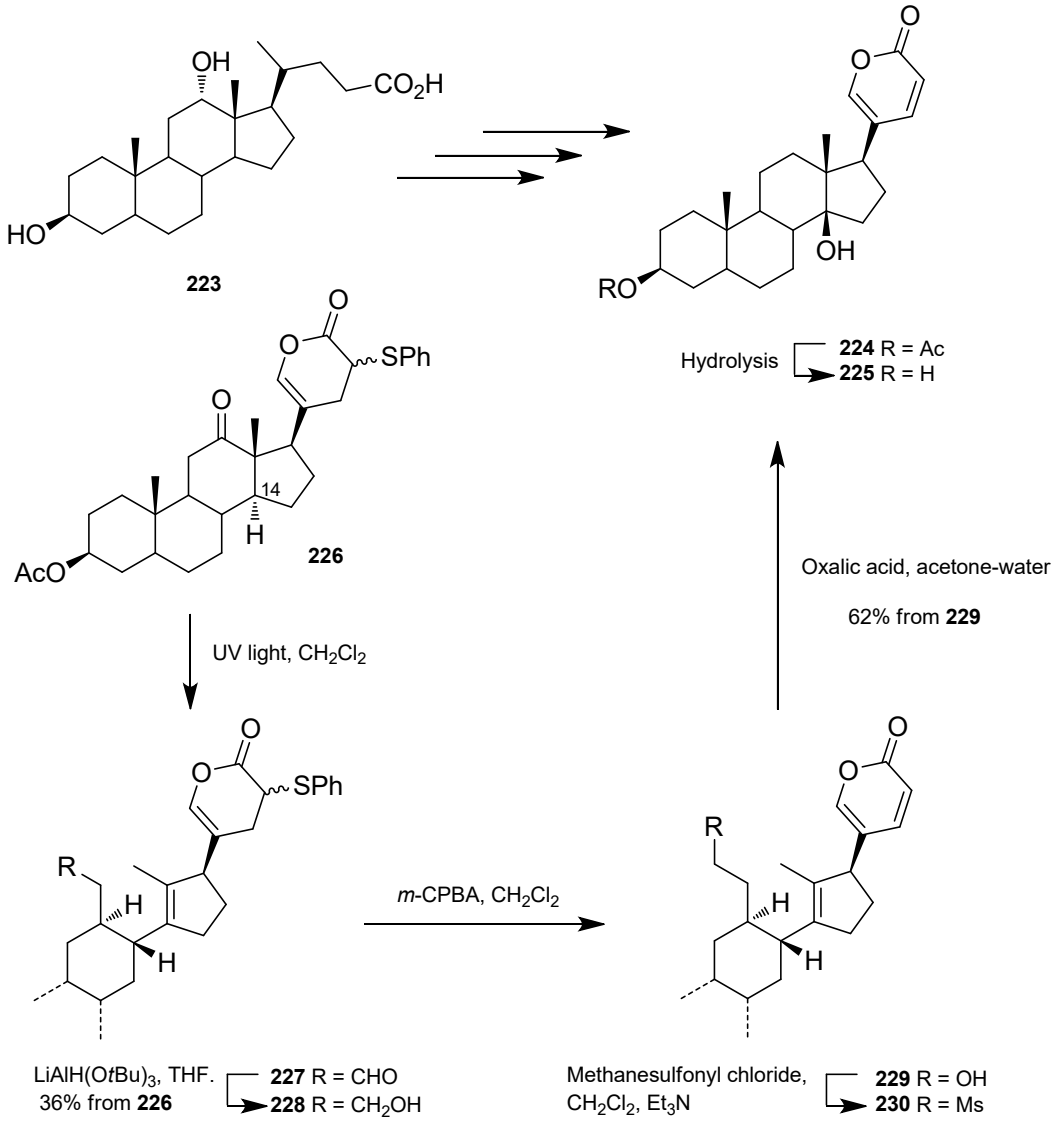


Figure 2.1: Introduction of C14 hydroxyl group during synthesis of bufalin acetate **225**.

A similar approach was used LaCour *et al.*[82] and Geoffroy *et al*.[83] who showed that irradiation of a dioxane solution of hecogenin acetate **231** with 75% acetic acid (25 °C, 24 h) quantitatively and smoothly affects the intramolecular Prins reaction to yield a mixture of diols **232** 14α/14β (~5:1). Upon Jones oxidation, the crude mixture gave keto alcohol **233** in 94% overall yield. In the reaction scheme they have shown C14 in **233** as β isomer but they have written it as α isomer and it is an error in writing (Figure 2.2).

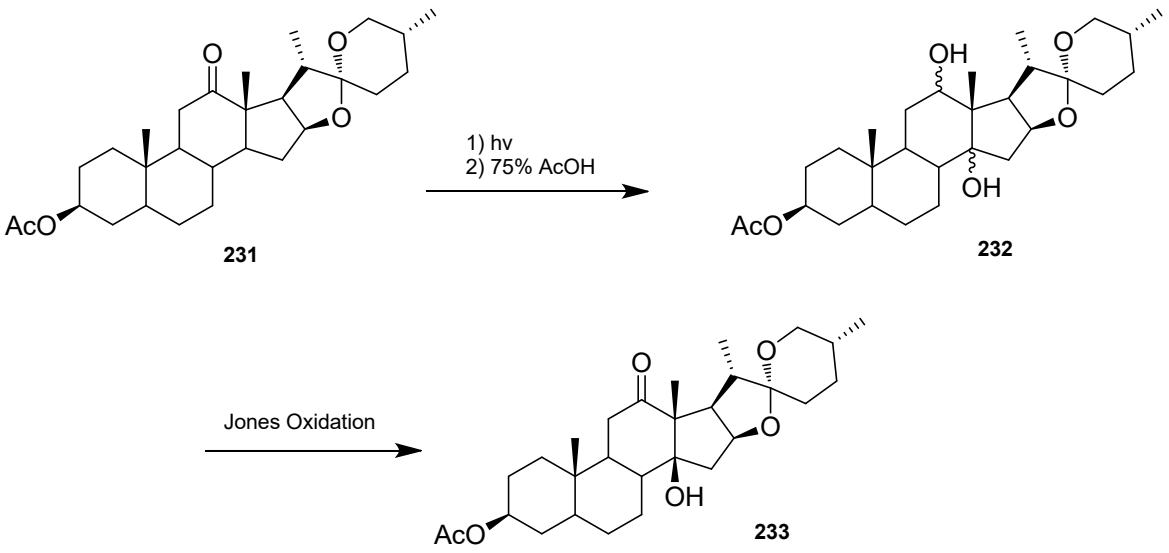


Figure 2.2: Prins reaction to make **233**.

Unlike enzymatic methods, introduction of a hydroxyl group to unactivated carbon atoms by chemical reagents is usually quite a challenging problem and often proceeds without regiospecificity. For example, C14 hydroxylation of **234** has been shown by using manganese (III) salen *N,N*-bis-(salicylideneamino)ethane) complexes [84], however, a mixture of C12 oxidised by-products were obtained in addition to the desired one. Interestingly reactions were stereoselective and reported to afford the α isomer only. The best yield was 42% of **235**, 7% of **236** and 2% of **237** (Figure 2.3). Compound **238** gives similar results as **234** (Figure 2.4).

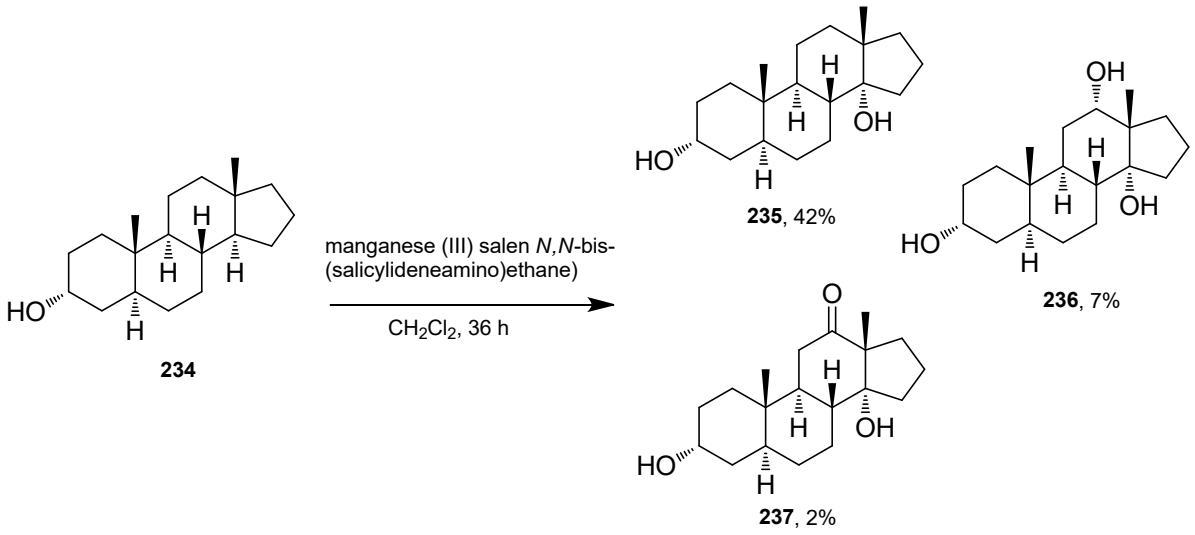


Figure 2.3: Oxidation of unactivated carbons of 5α-androstan-3α-ol by using manganese (III) salen *N,N*-bis-(salicylideneamino)ethane).

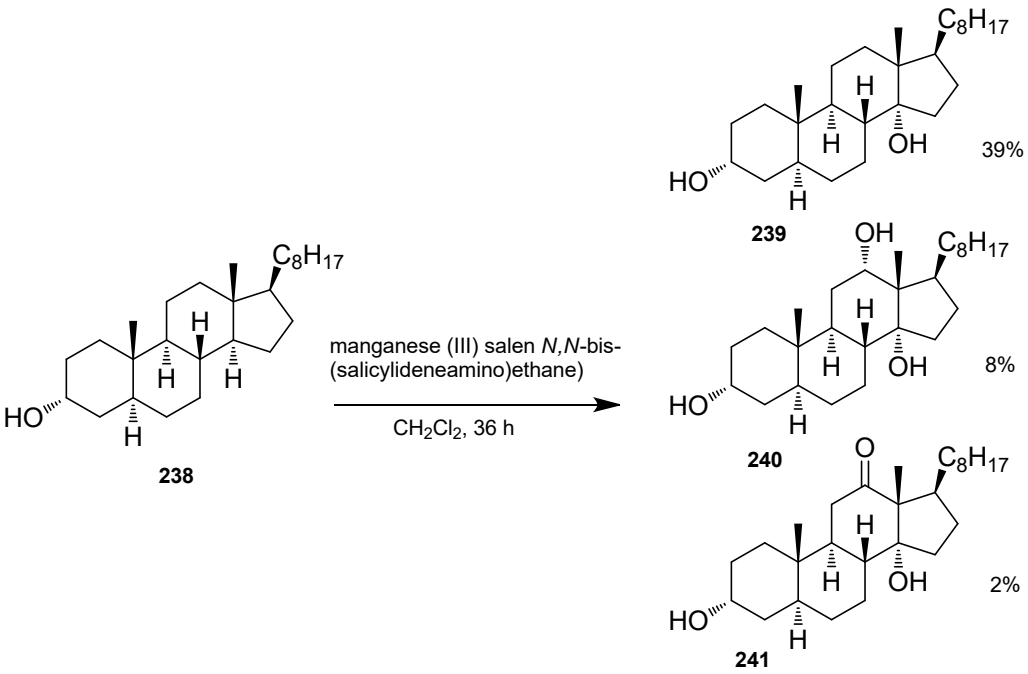


Figure 2.4: Oxidation of unactivated carbons of 5α-cholestan-3α-ol by using manganese (III) salen *N,N*-bis-(salicylideneamino)ethane).

Oxidation of steroids by using manganese porphyrin was reported to give multiple products.[85] C14 α hydroxyl product was obtained as a minor product and the best yield reported was **246** (11%), although the reaction did proceed stereoselectively (Figure 2.5).

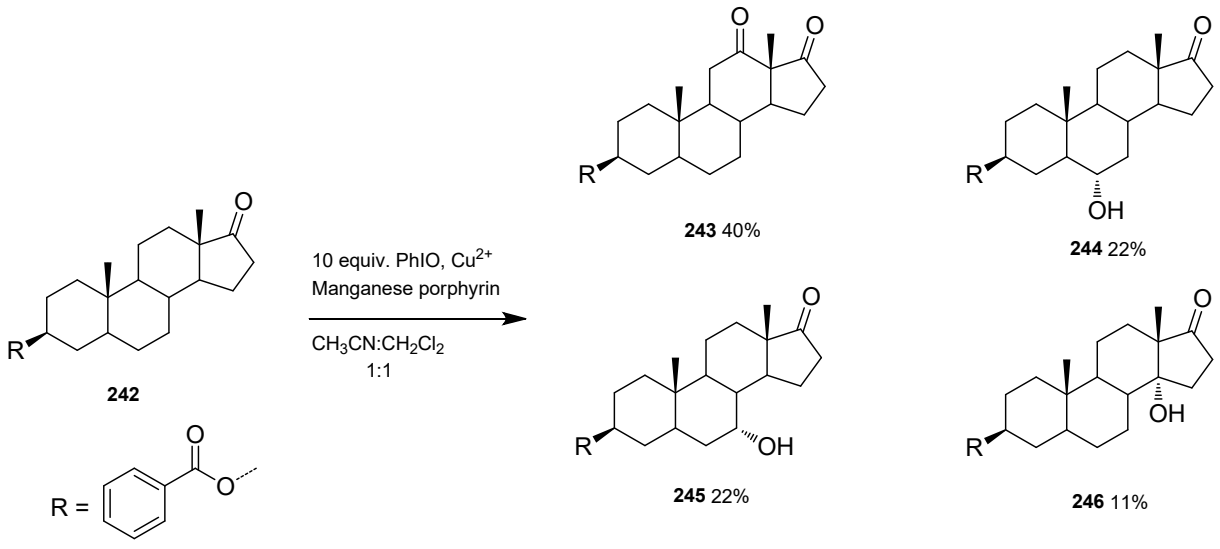


Figure 2.5: Hydroxylation of **242** by using manganese porphyrin.

Non-metal based organic reagents can also be used to affect C14 hydroxylation. Reaction of the unactivated methine carbon of methyl 3α,7β-diacetoxy-5β-cholan-24-oate **247** by using dimethyldioxirane (DMDO) gives C14α hydroxylation but also 4 other oxidised products, shown in Fig 2.6.[86-92] C14α hydroxylation has also been carried out on androstane triacetate **253**, by using methyl(trifluoromethyl)dioxirane (TFDO) (Figure 2.7).[93] Interestingly in both examples, the hydroxyl is again reported to have been introduced from the α face. This is because the group at C17 is on the β face which directs the incoming hydroxyl group at α position. Stereochemistry at C14 is explained in detail later in this thesis.

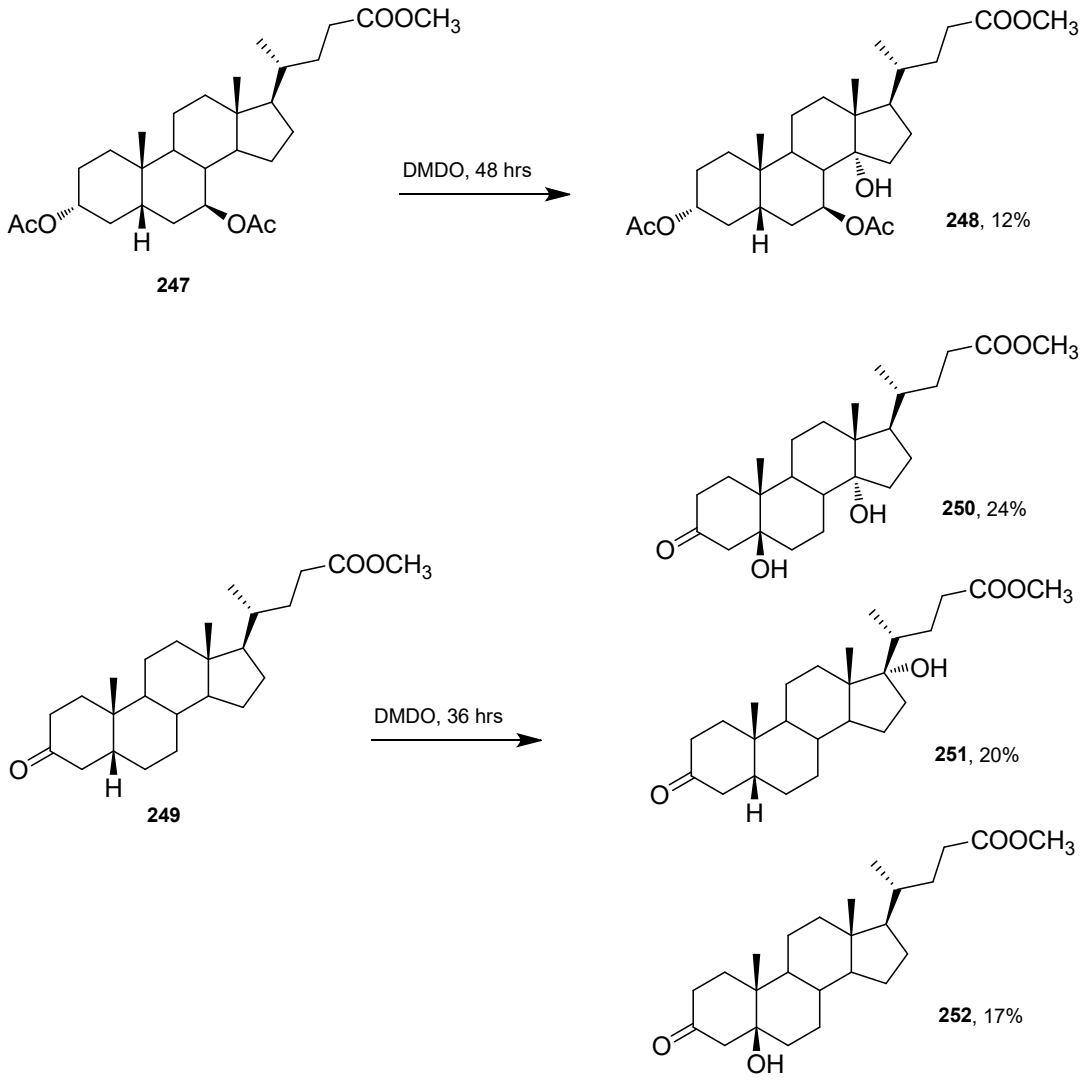


Figure 2.6: Hydroxylation of **247** and **249** by DMDO.

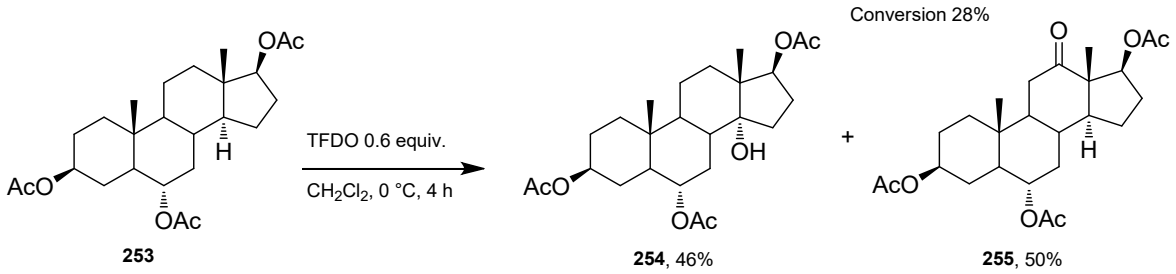


Figure 2.7: Hydroxylation of **253** by TFDO.

Introduction of C14 hydroxyl to activated C14 or when C14 is part of an alkene is perhaps the most common chemical route to these compounds. For example γ-isobufalin **260** was synthesised starting from testosterone **256** via this method (Figure 2.8).[94] C15 hydroxylated compound **257** was regioselectively dehydrated by using thionyl chloride in pyridine to give C14-15 unsaturated compound **258**. Unsaturated **258** was converted into C14β hydroxyl **259**, both stereo- and regioselectively. The reaction proceeds via hydrobromination across C14-C15 double bond followed by reductive debromination at C15. In contrast to previous examples, the C14 is reported to be in the β face of the molecule.

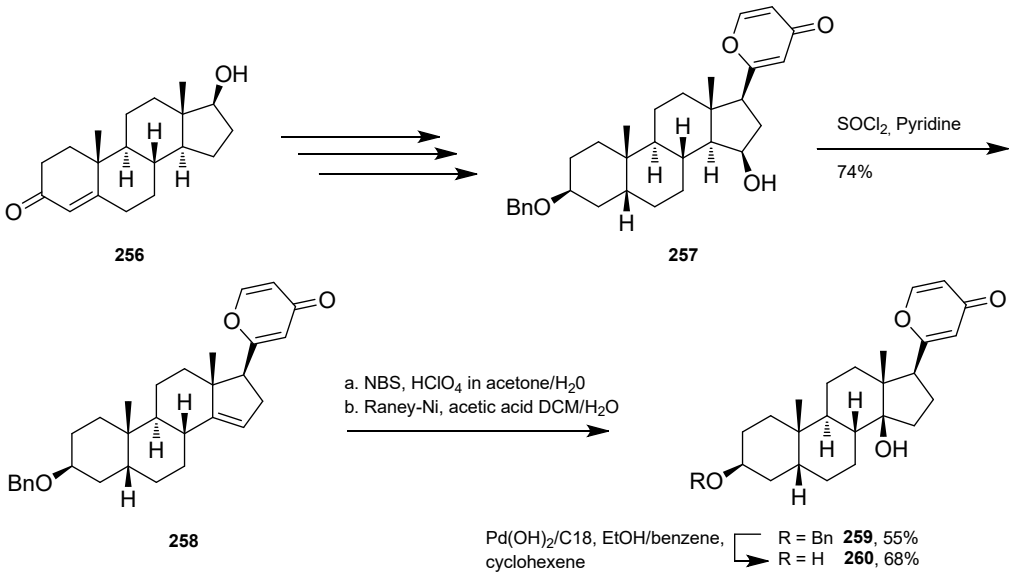


Figure 2.8: Synthesis of γ-Isobufalin **260**.

Another example is shown below (Figure 2.9), in this example, introduction of C14β hydroxyl group was achieved in three steps. [95] TBSOTf was used to introduce Δ^{14} which was epoxidised by using *m*-CPBA and after workup gave **264** and **265** with C14β hydroxyl. After 20 minutes of reaction **264** was obtained as the major product while **265** was the minor product, but after 10 hours only **265** was obtained in 87% yield.

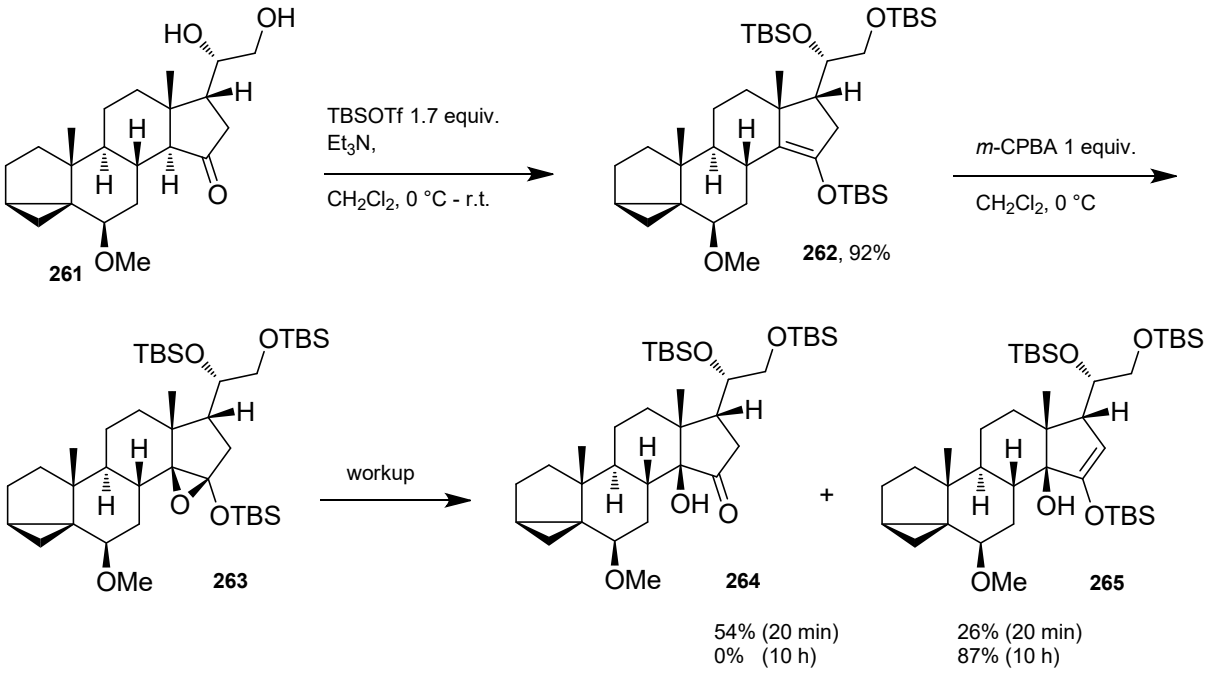


Figure 2.9: Introduction of C14β hydroxyl group.

Michalak *et al*.[96] reported introduction of both C14α & C14β hydroxyl group by using a Sharpless epoxidation and regioslective reduction of the resulting epoxides (Figure 2.10).

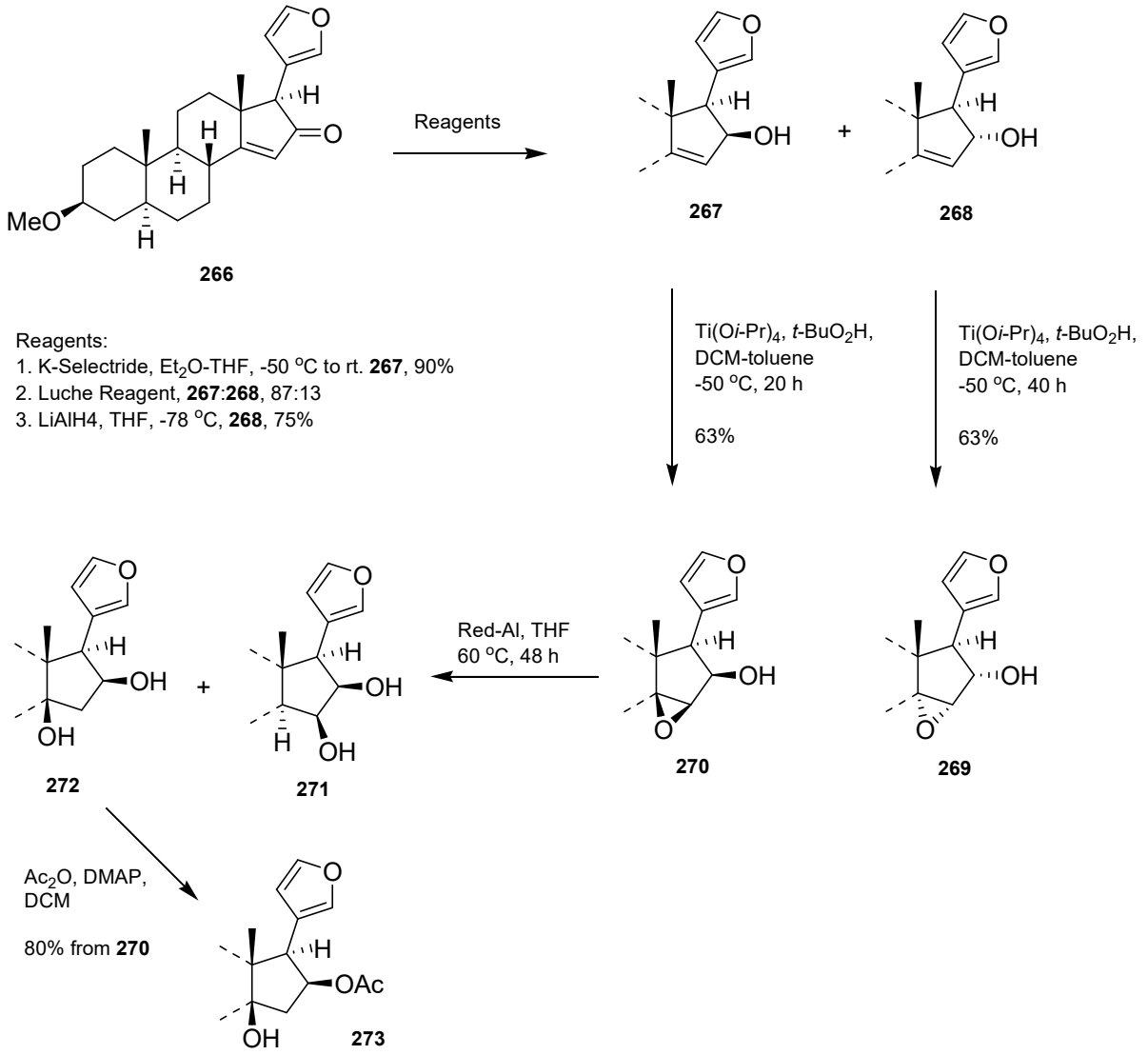


Figure 2.10: Introduction of C14β hydroxyl group.

Finally in this section, a regioselective dehydrogenation using Pd^{II} along with TMSOTf has been used to introduce $\Delta^{14\text{-}15}$ in **274**. Compound **275** was oxidised and then reduced to afford C14α hydroxyl compound **276** (Figure 2.11).[97, 98]

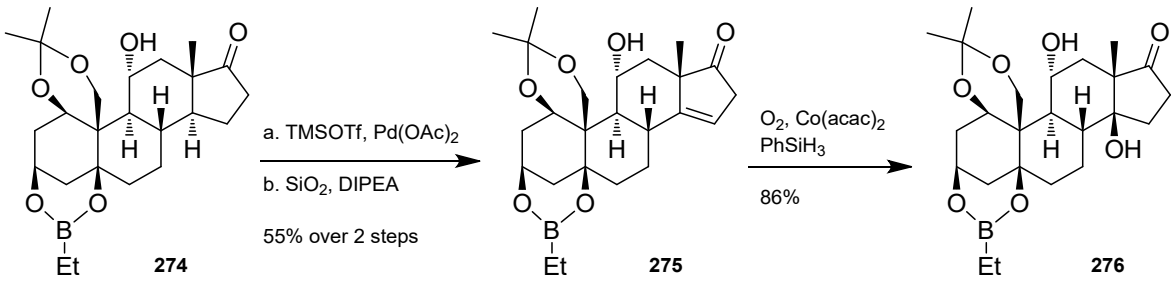


Figure 2.11: Introduction of C14β hydroxyl group.

2.1.2: Hydroxylation at C14 by using Microorganisms

Hydroxylation of steroids by both whole microorganisms (like fungus, yeast and bacteria) as well as isolated enzymes has been reported. These reactions typically proceed with regio- and stereo-selectivity due to the gross structural compatibilities between cholestane and the enzyme's active site. For instance, 14α hydroxylation was reported [99] by using fungal strains like *Thamnostylum piriforme, Mucor griseocyanus, Actinomucor elegans,* etc.

A good example of how structural features can influence the regio- and stereo-chemical preferences in hydroxylation of a cholestane substrate is shown during reactions by *T. piriforme, M. griseocyanus, A. elegans*. The result of hydroxylation of different substrates is given below (Figures 2.12-17). Progesterone **245** and testosterone **224** were found to be the best substrates for the production of 14α-hydroxylated derivative, but generally, a range of products were identified in the reactions with small yields (1-33%).

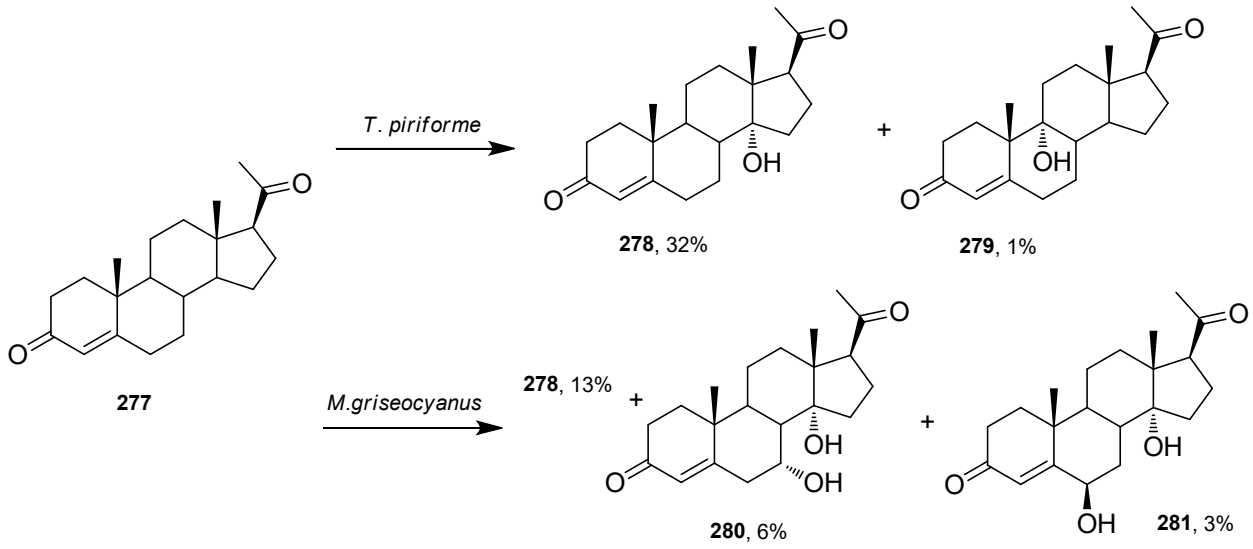
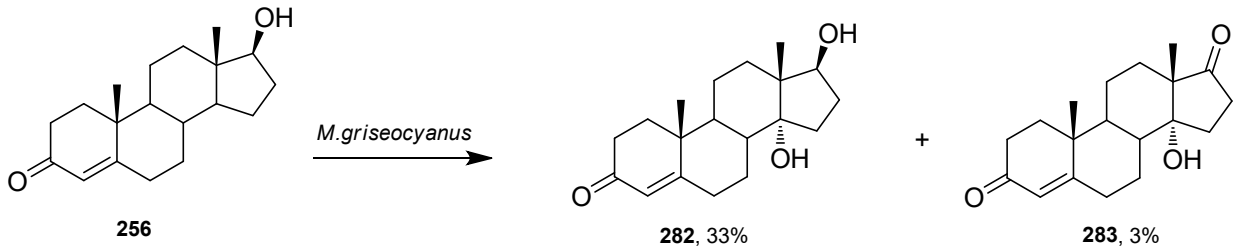


Figure 2.12: Transformation of progesterone **277**.

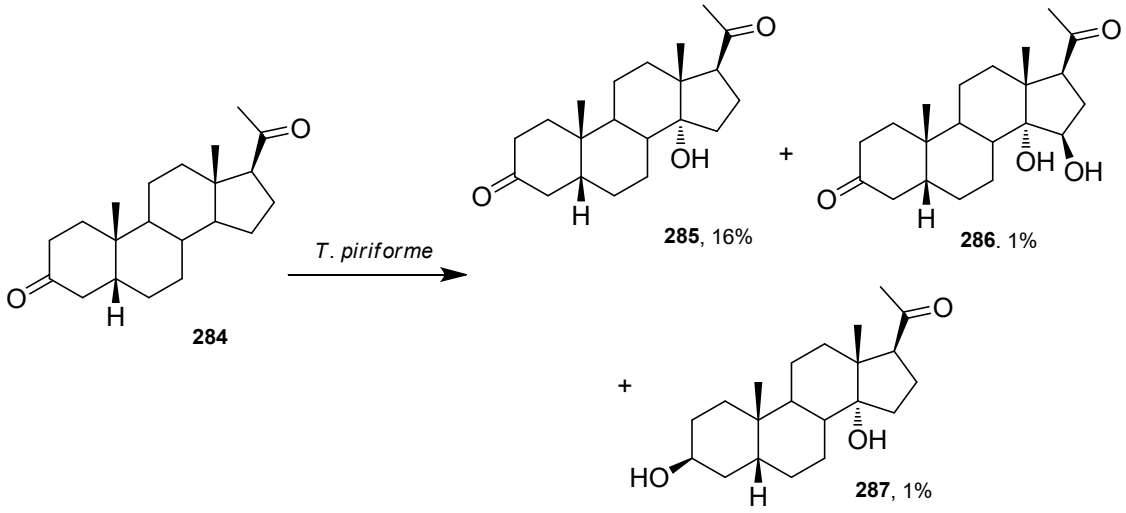


256

282, 33%

283, 3%

Figure 2.13: Transformation of testosterone **256**.



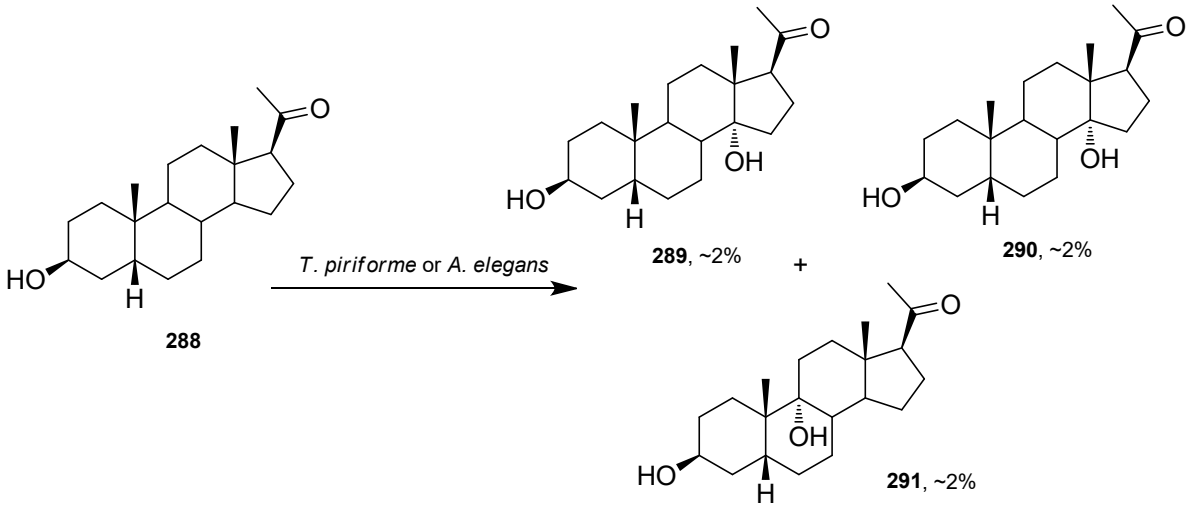
284

285, 16%

286. 1%

287, 1%

Figure 2.14: Transformation of 5β-pregnane-3,20-dione **284**.



288

289, ~2%

290, ~2%

291, ~2%

Figure 2.15: Transformation of 3β-hydroxy-5β-pregnane-20-one **288**.

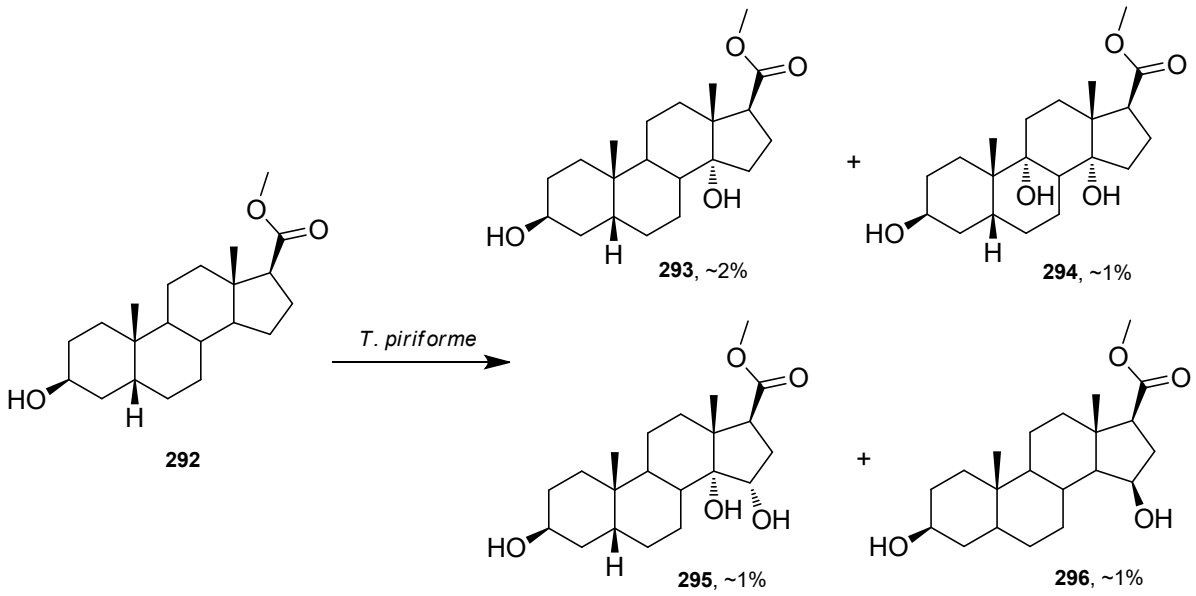


Figure 2.16: Transformation of 3β-hydroxy-5β, 17(αH)-etianic acid methyl ester **292**.

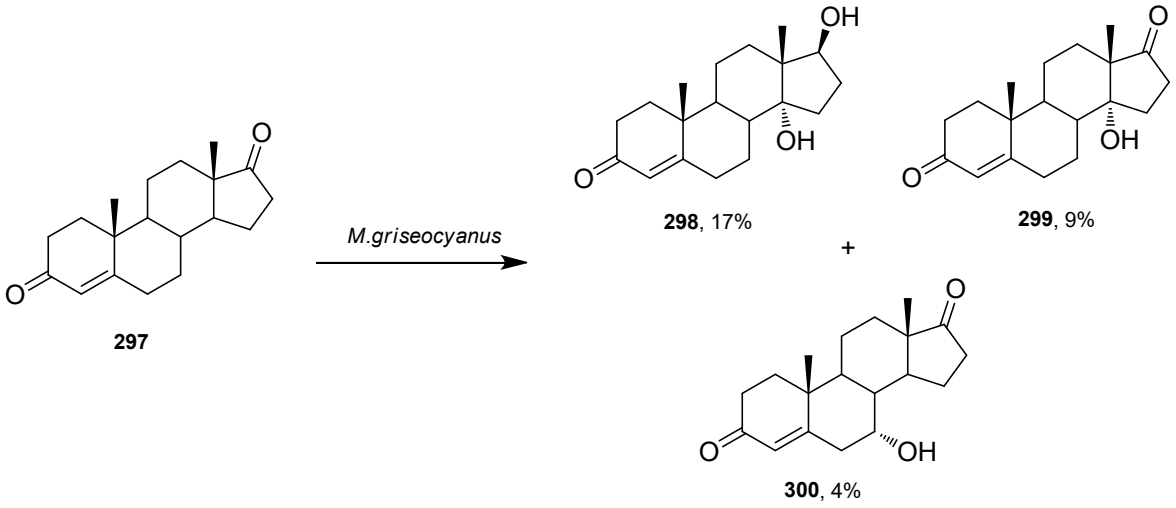


Figure 2.17: Transformation of androst-4-ene-3,17-dione **297**.

Boynton *et al.* (Fig 2.18) showed [100] introduction of 14α hydroxyl by using *Cephalosporium aphidicola*.

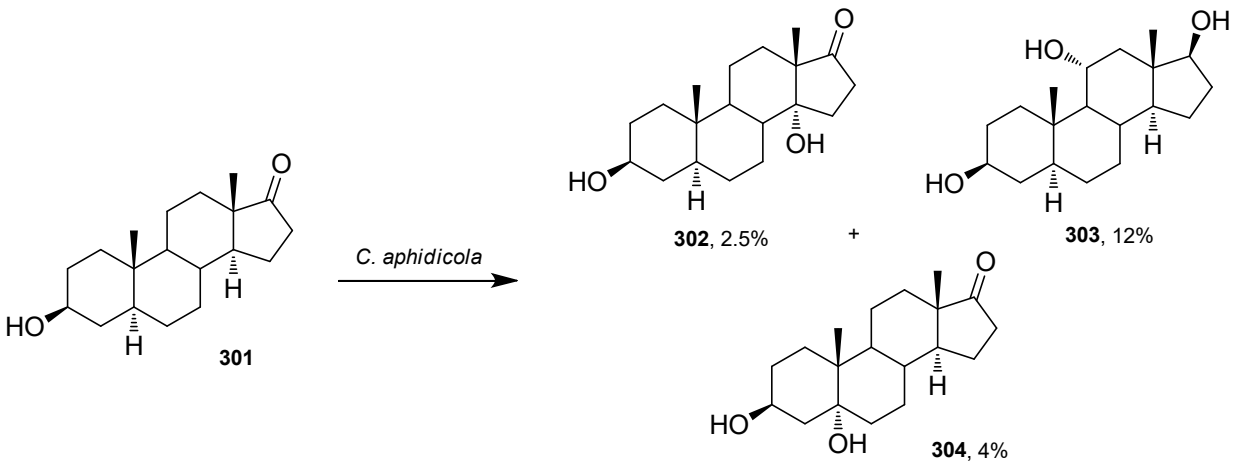


Figure 2.18: Transformation of 3β-hydroxy-5α-androstan-17-one **301**.

Herbert and co-workers[101] did an investigation on biotransformation of amino-steroids by using different fungal species, *Aspergillus ochraceus*, *Bacillus megaterium*, *Curvularia lunata*, and *Rhizopus arrhizus*. These species showed hydroxylation at different positions in steroids along with oxidation at C14. *Aspergillus ochraceus*, *Bacillus megaterium* and *Rhizopus arrhizu* did not give C14 hydroxylation in any compound used but *Curvularia lunata* did provide C14 hydroxylation in compounds shown (Figures 2.19-21).

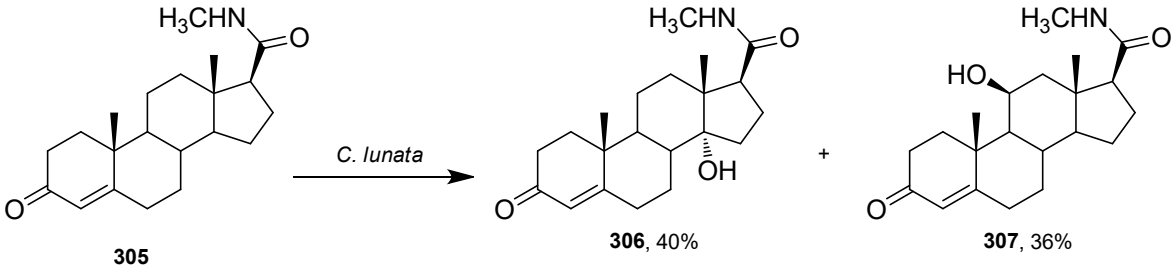


Figure 2.19: Transformation of *N*-methyl 3-ketoandrost-4-ene-17β-carboxamide **305**.

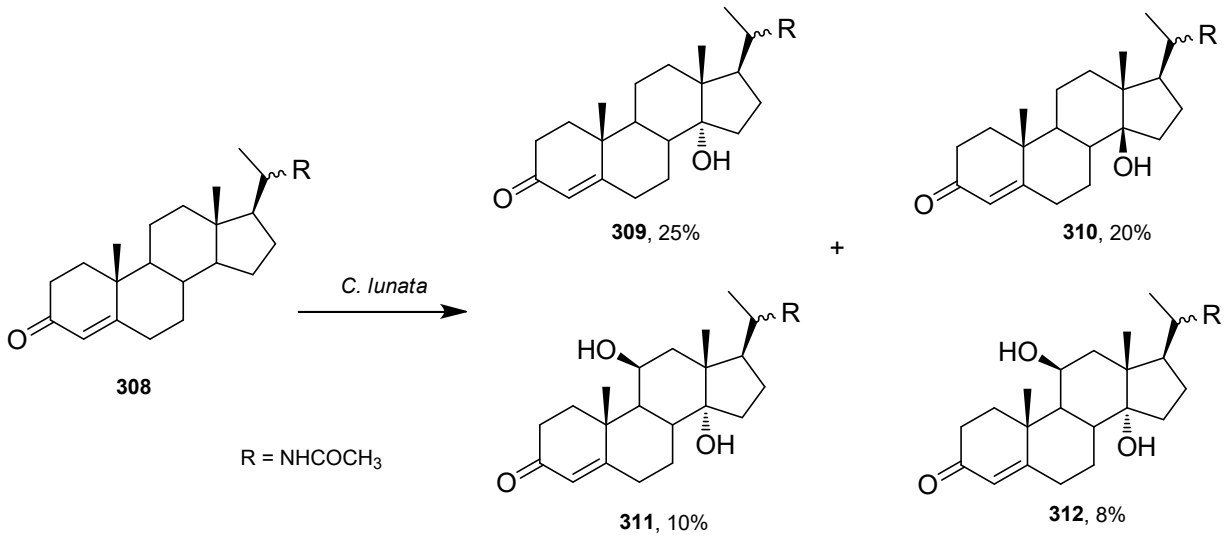


Figure 2.20: Transformation of 20α/β-acetylaminopregn-4-en-3-ones **308**.

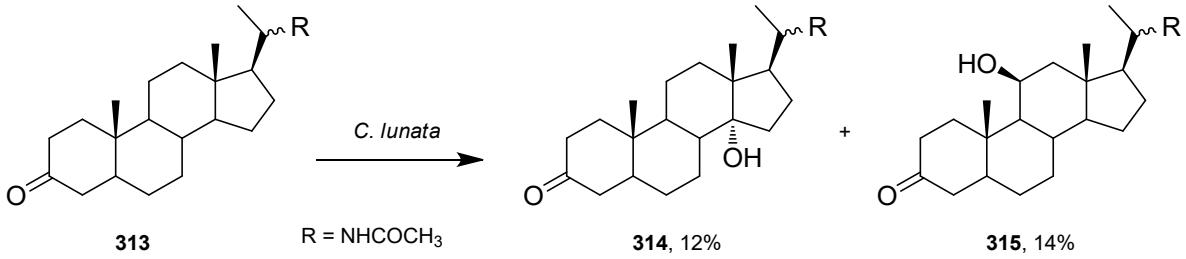


Figure 2.21: Transformation of 20α/β-acetylamino-5α-pregnan-3-one **313**.

5α-Androstane-3, 16-dione **316** was hydroxylated[102, 103] by using *Cephalosporium aphidicola* to give different hydroxylated products. The yield was very poor for 14 hydroxyl compounds (Figure 2.22).

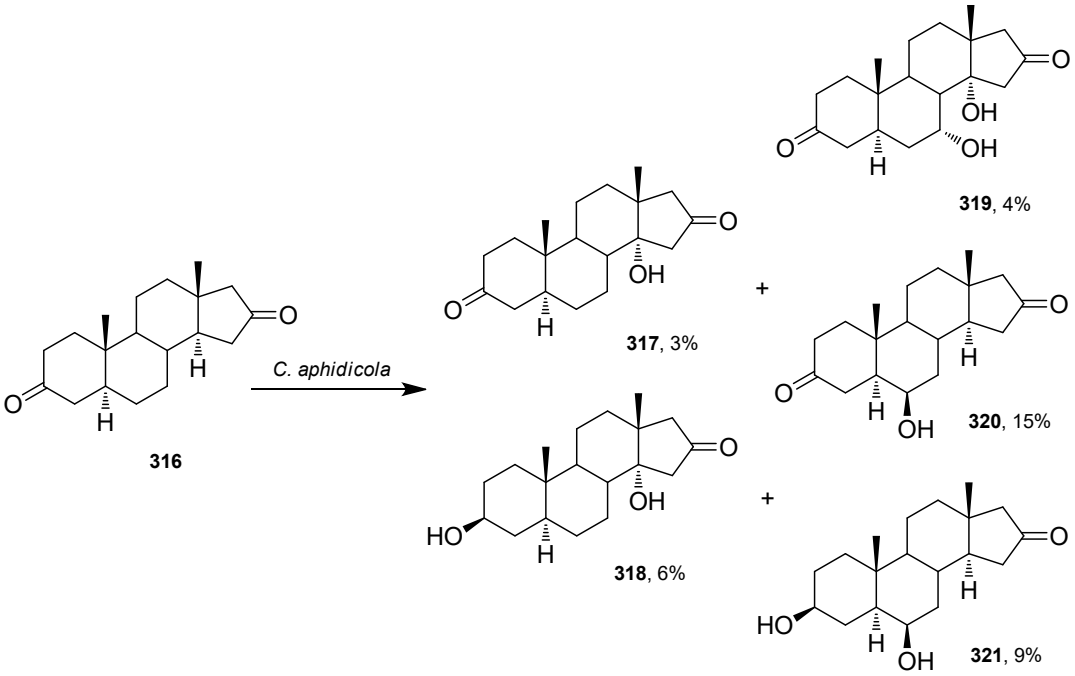


Figure 2.22: Transformation of 5α-androstane-3, 16-dione **316**.

Pregnane-3β,16β,20-triol was hydroxylated by using *Cunninghamella echinulata* [104] to give four products (Figure 2.23).

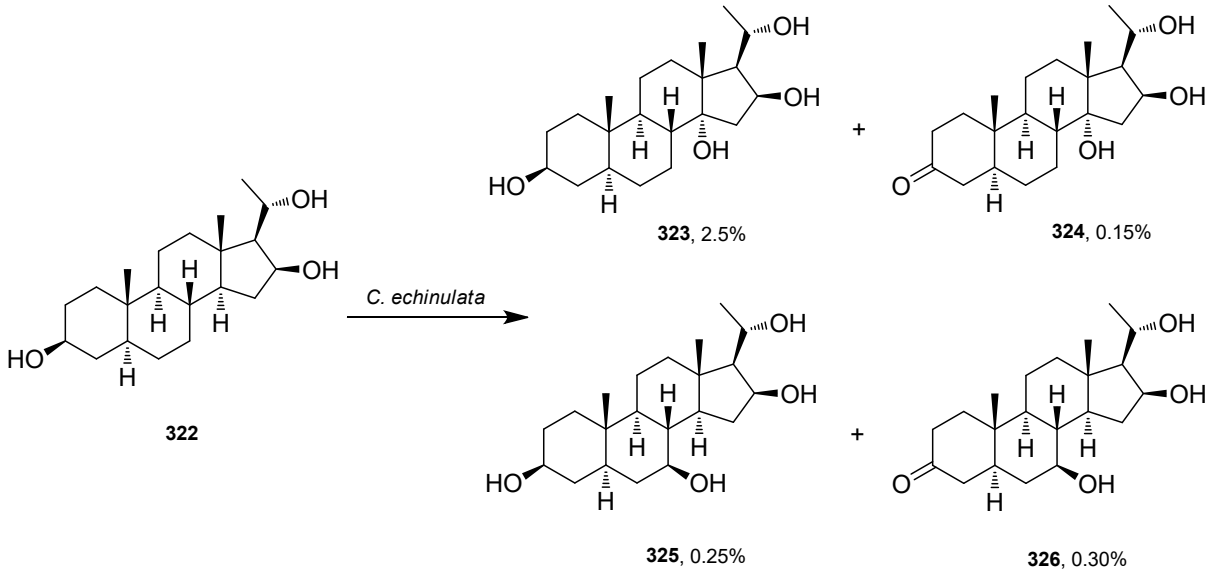


Figure 2.23: Transformation of pregnane-3β,16β,20-triol **322**.

In conclusion, although it has been shown that C14 hydroxyl group can be introduced by using microorganisms in different steroidal compounds, in most cases the yields are very poor, and there is no stereo and regioselectivity. The best yield for single hydroxylation at C14 was reported by Herbert and co-workers where C14α hydroxyl compound was obtained in 40% yield by *C. lunata* (Figure 2.19) but there was also another compound with C11β hydroxyl. Other notable C14 hydroxylations include hydroxylation of progesterone by using *T. piriforme,* in 32% yield with 3 other compounds (Figure 2.12) and hydroxylation of testosterone by using *M. griseocyanus,* in 33% with 3% C17 hydroxyl compound (Figure 2.13). Overall testosterone hydroxylation has been the best one with single hydroxylation.

2.2: Functionalisation of C18 in Steroids

There is a number of withanolides which have different functional groups at the C18 position. For example, physachenolides which is a subfamily of withanolides have acetoxy group present at C18. It is a challenge to introduce any functional group at C18 position because it is not very reactive, and also functionalisation of C18 requires a change of synthetic scheme. Here examples are mentioned, to show how different research groups have achieved C18 functionalisation.

Kerwin *et al.* reported chlorination of C18 in steroid hormone analogous.[105] C20 keto groups were converted into secondary amines which were then transformed into *N*-chloroamines **329**. *N*-chloroamines were dissolved in trifloroacetic acid and irradiated to displace chlorine to C18 and gave trifloroacetate salt **330**. Finally, by using different reagents like KOH, C18 was cyclised with C20 to give amino compound **331** (Figure 2.24).

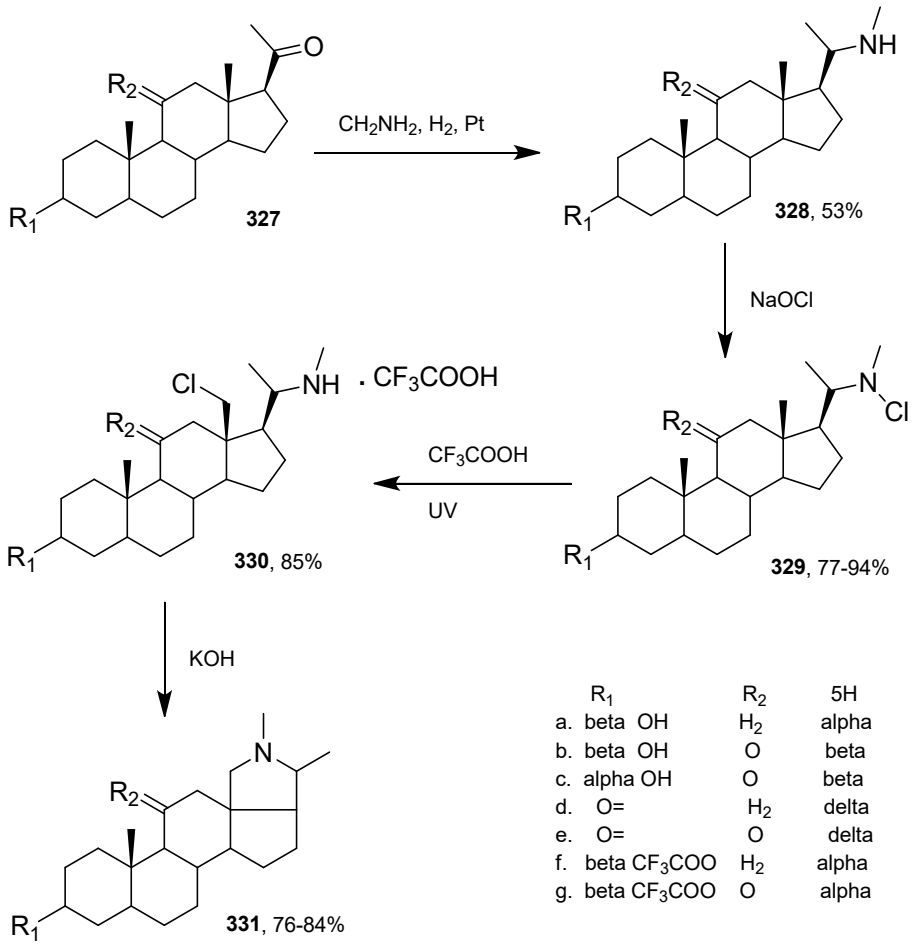
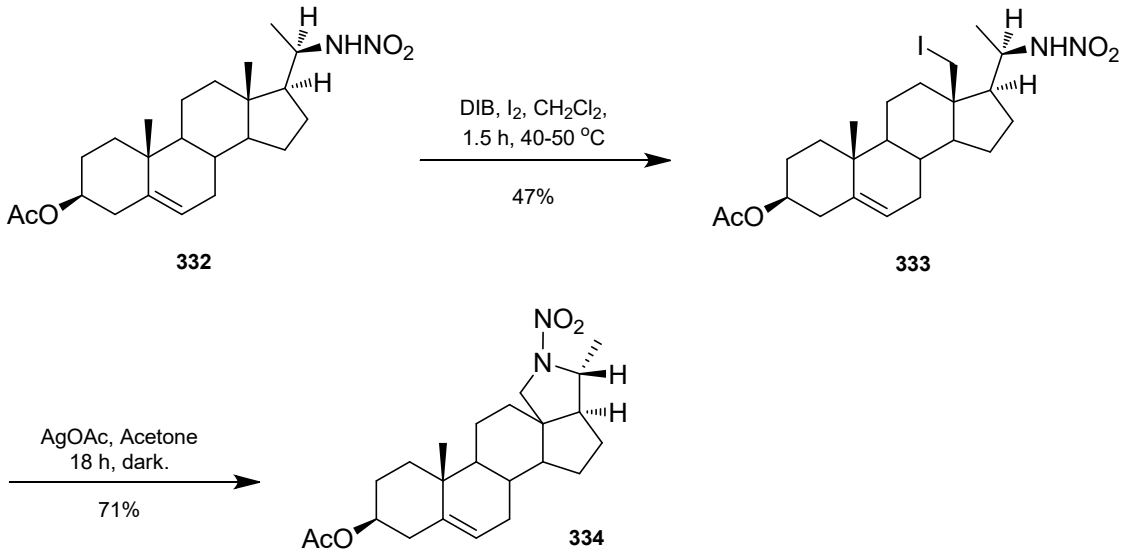


Figure 2.24: C18 chlorination and cyclisation of **327**.

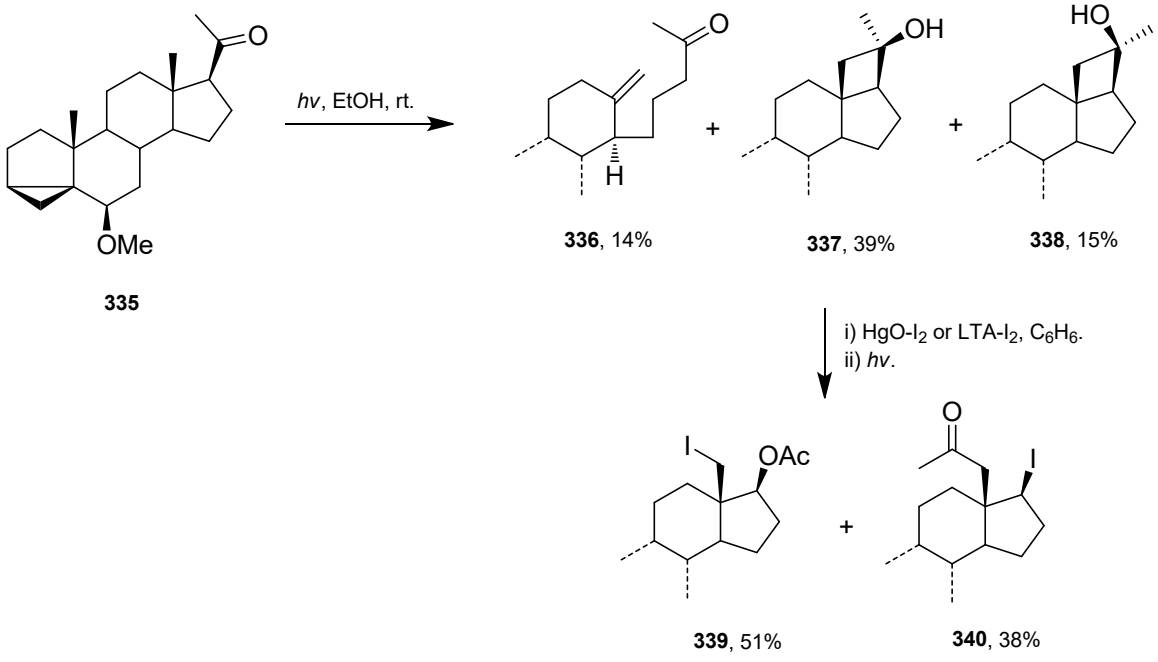
Iodation of C18 was done by Armas *et al*.[106, 107] on **332** by using DIB and iodine and then cyclisation of C18 by using AgOAc to give 5-membered cyclic nitro-amino compound **334** (Figure 2.25).



332 **333** **334**

Figure 2.25: C18 cyclisation of **332**.

Suginome *et al*.[108, 109] reported transformations of C18 functionalised androstanes. This reaction was non-regioselective and gave different products. Irradiation of **335** gave three products, from these products **335** was converted into iodo **339** and **340** (Figure 2.26).



335

336, 14% **337**, 39% **338**, 15%

339, 51% **340**, 38%

Figure 2.26: Synthesis of iodo compounds.

Shibuya *et al*.[110] reported oxygen-functionalisation of C18 methyl group. They proposed a mechanism for the radical reaction (Figure 2.27).

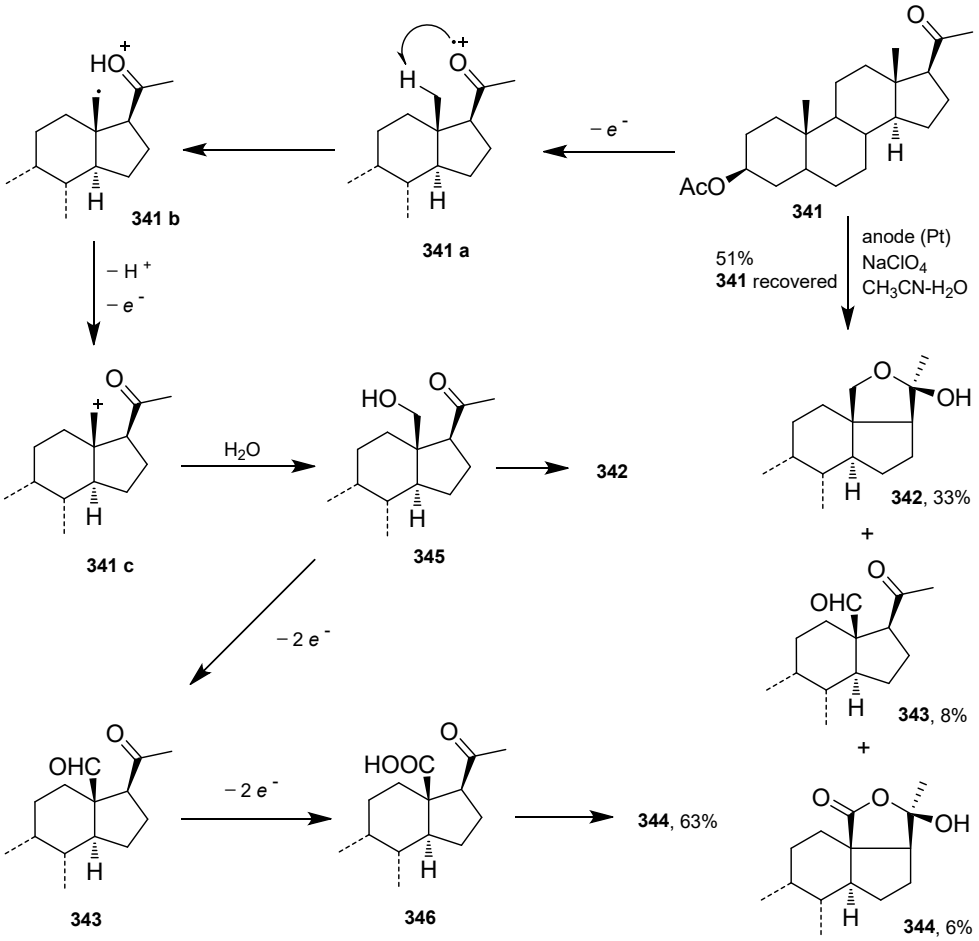


Figure 2.27: Radical reaction mechanism for C18 lactone synthesis.

Bhandaru & Fuchs [111, 112] introduced hydroxyl group at C18 by using lead (IV) acetate, chromic acid and lithium aluminium hydride (Figure 2.28).

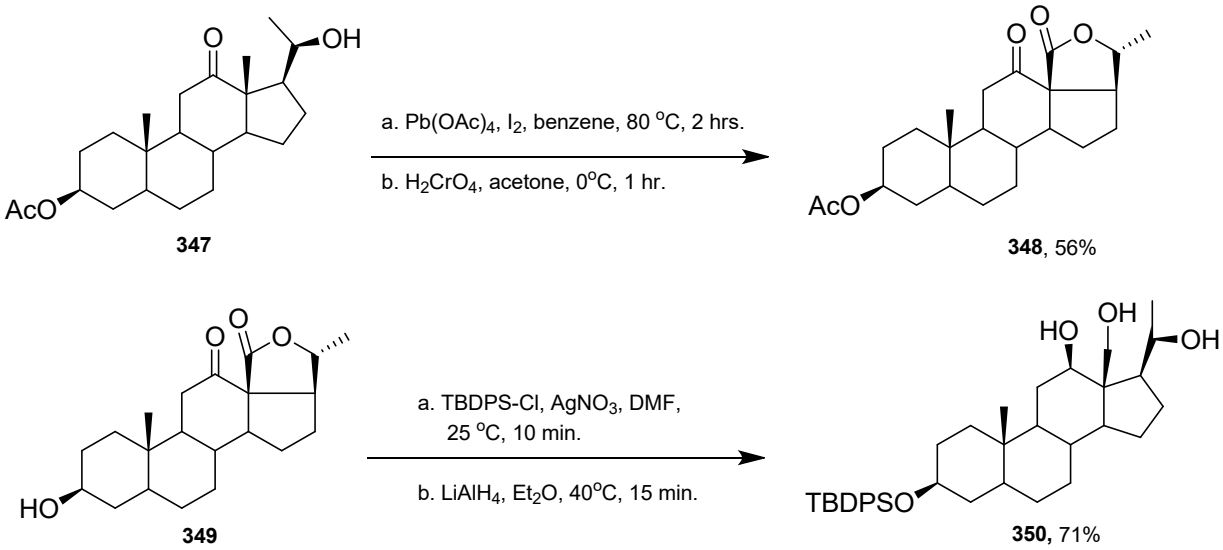


Figure 2.28: C18 hydroxylation of **347**.

Jiang *et al*.[113] introduced cyano group at C18 by using displacement reaction. First cyano group was introduced at C20 by using acetone cyanohydrin. The cyano group was displaced from C20 to C18 by using lead (IV) acetate, calcium carbonate and iodine (Figure 2.29).

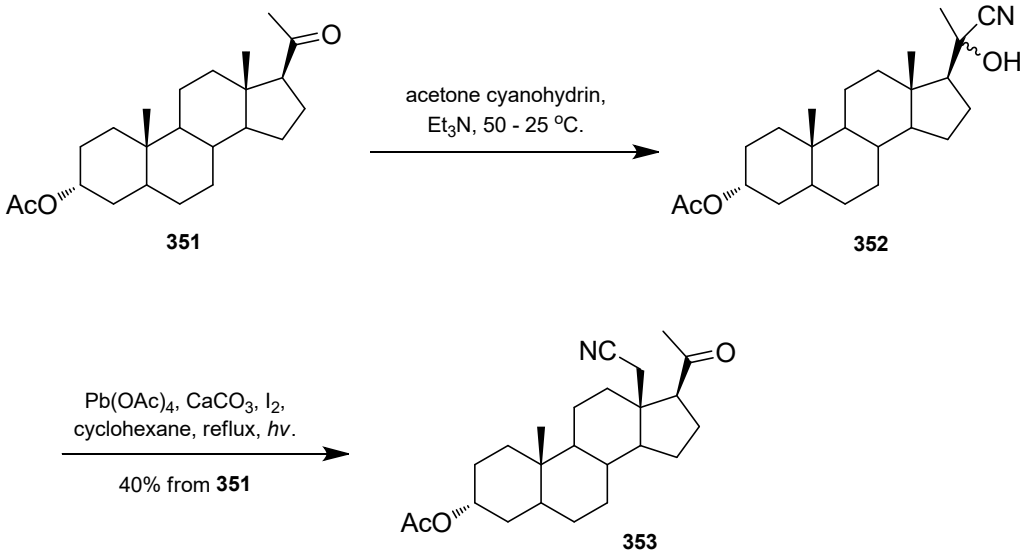


Figure 2.29: Introduction of cyano group at C18 by displacement reaction.

Tang & Yu [114, 115] reported a convenient method for the introduction of acetyl group at C18. Protected compound **354** was irradiated in the presence of DIB and iodine with a 300-W tungsten lamp, followed by oxidation with PCC, provided C18 iodo substituted **355**. Iodide **355** was hydrolysed with silver acetate to give hemiketal **356**,[116] which was reduced with $NaBH_4$ to give C18,20(R/S) diols, which were acetylated selectively with acetyl chloride to provide **357** (Figure 2.30).[117]

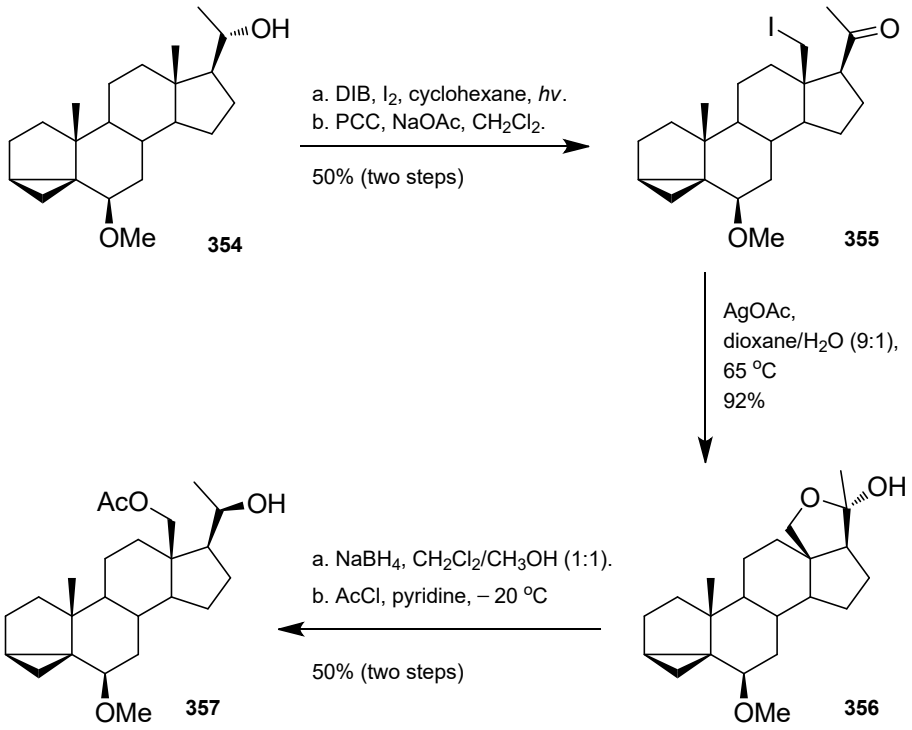


Figure 2.30: Acetylation of C18 in **354**.

Heusler *et al.*[118] treated **358** with lead (IV) acetate to get lactone **359**. In 2011 Shi *et al*.[119] and Gui *et al*.[120] also used same method to get **361** and **363** respectively. These lactones can easily be converted into C18 hydroxyl and C20 ketone by using $LiAlH_4$ (Figure 2.31).

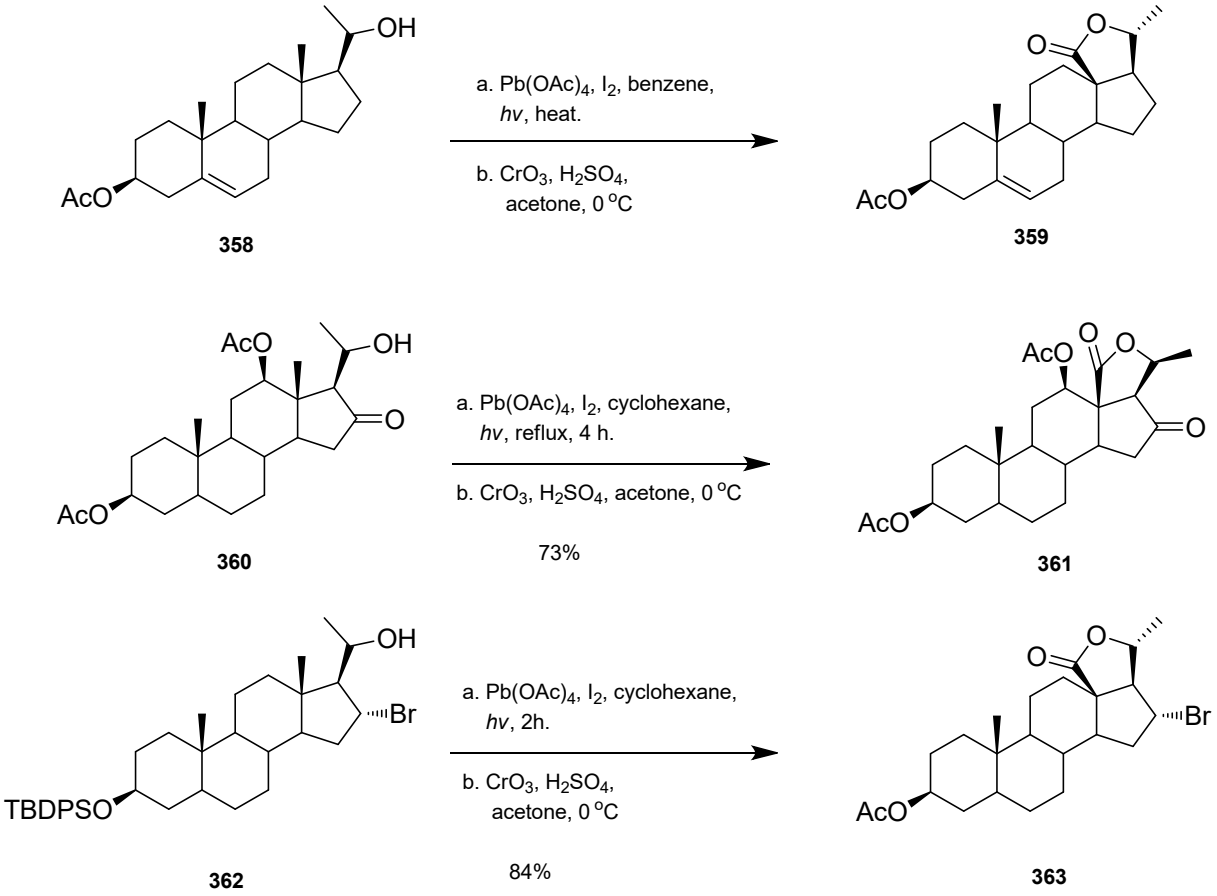


Figure 2.31: Lead (IV) acetate reaction to make C18 lactones.

Cheng *et al.*[121] reported a better yield of diol **366** by Suarez iodine (III) oxidation[122] ($PhI(OAc)_2/I_2$, *hv*) than Meystre's hypoiodite method[118] ($Pb(OAc)_4/I_2$, *hv*). Meystre's hypoiodite method gave the desired diol **366** in 40% yield after reduction with $LiAlH_4$ along with a cyclic ether as a by-product in 42% yield, but Suarez iodine (III) oxidation after reduction with $LiAlH_4$ gave the desired diol **366** in 78% yield (Figure 2.32).

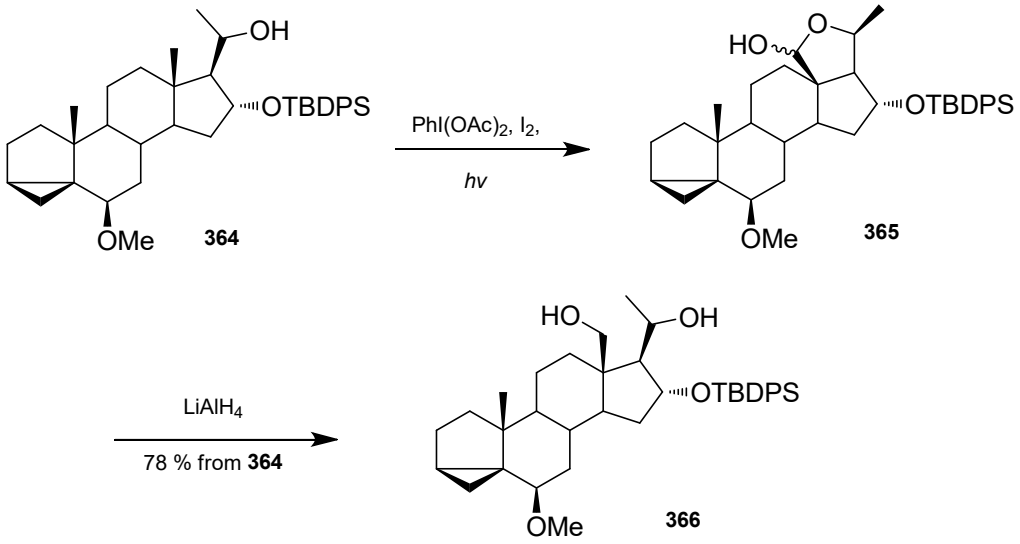


Figure 2.32: C18 hydroxylation of **364** by using $PhI(OAc)_2/I_2$.

Kudova *et al.*[123] also used Suarez iodine (III) oxidation[122] ($PhI(OAc)_2/I_2$, *hv*) on **367** to get **368**, but they also got a small amount of **369** & **370** mixture (Figure 2.33).

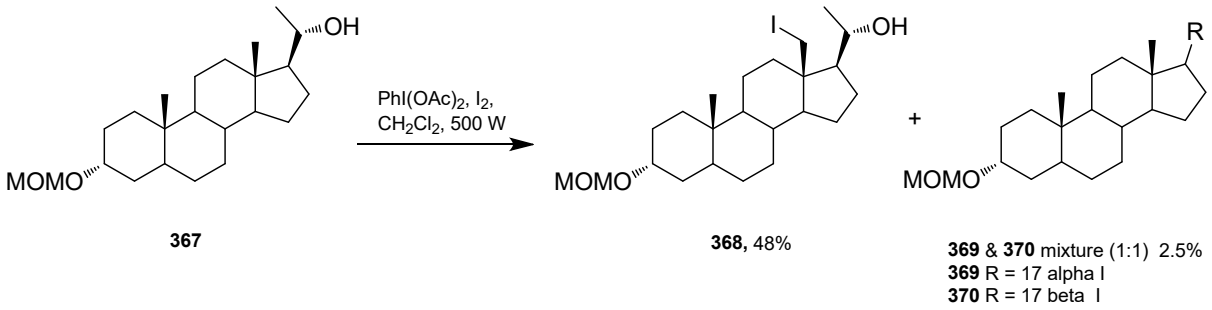


Figure 2.33: Iodation reaction of **367**.

There are some examples which can certainly be used to introduce acetyl group at C18 in physachenolides. The reaction reported by Tang & Yu (Figure 2.30) is a good example for the introduction of acetyl group at C18 but it should be noted that it was done on a simple molecule.

CHAPTER 03

AIMS

AND

OBJECTIVES

OF THE PROJECT

3.1: Background of the Project

Recently, screening of a 50,000 member library of natural and synthetic compounds identified a series of 17β-hydroxywithanolides as having potent antiproliferative activity, in prostate adenocarcinoma cell lines, LNCaP and PC-3M (metastatic variant of PC-3[124]).[125-128] Therefore, these compounds may have important applications as new therapies against prostate cancer.

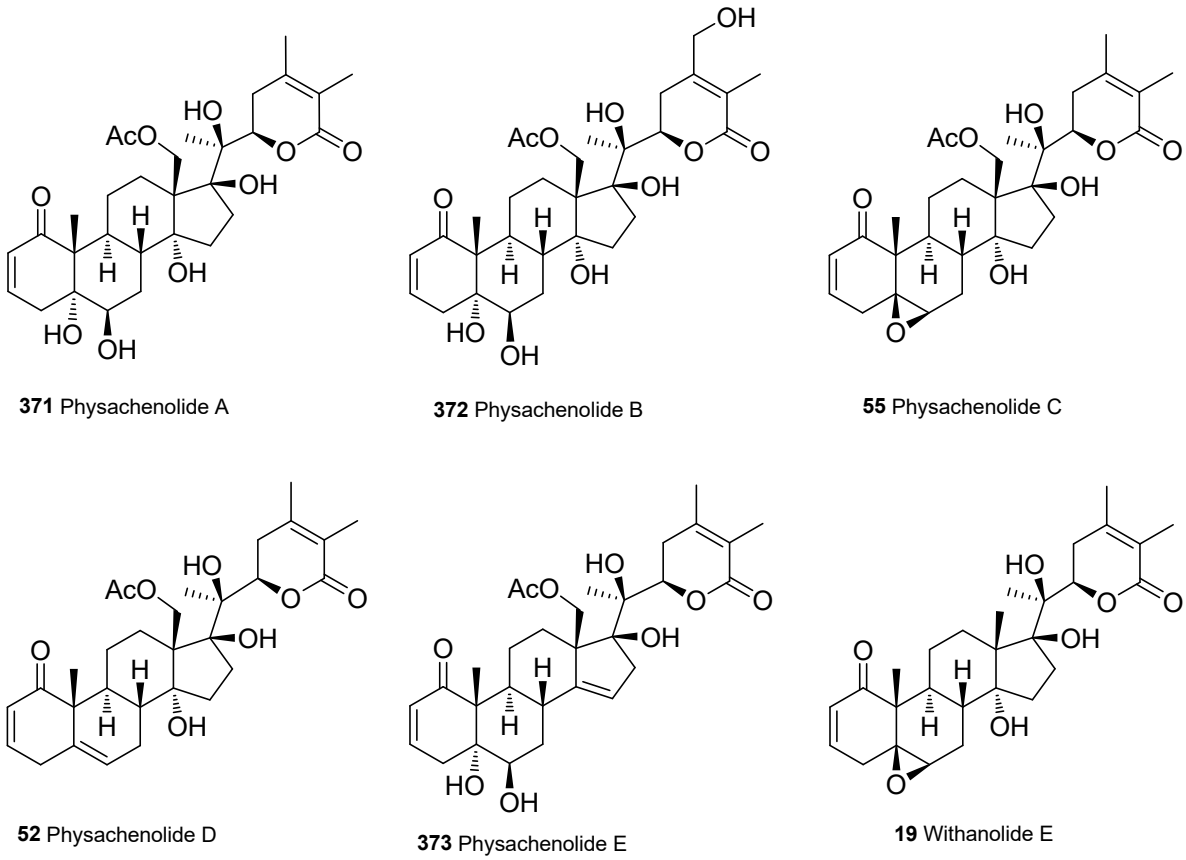


Figure 3.1: Target 17β-hydroxywithanolides.

However, before the value of this series of compounds can be evaluated as potential therapies, methods for the synthesis of various analogues should be developed so that a structure-activity relationship (SAR) can be ascertained and to better understand biological mechanisms targeted by these molecules. This exercise will provide information as to which elements of the withanolide structure contribute to its potency. So far, only limited information is available, mostly discerned from derivatisation of withanolides on the SAR in this class of molecules, and in particular the contribution of C14, C17, C18 and C20 hydroxyls to the potency. This is particularly relevant as the material cannot be isolated in large

quantities from natural sources and synthesis (particularly ones allowing the introduction of those hydroxyl groups) is long and inefficient.

3.2: Synthetic Route and Strategy

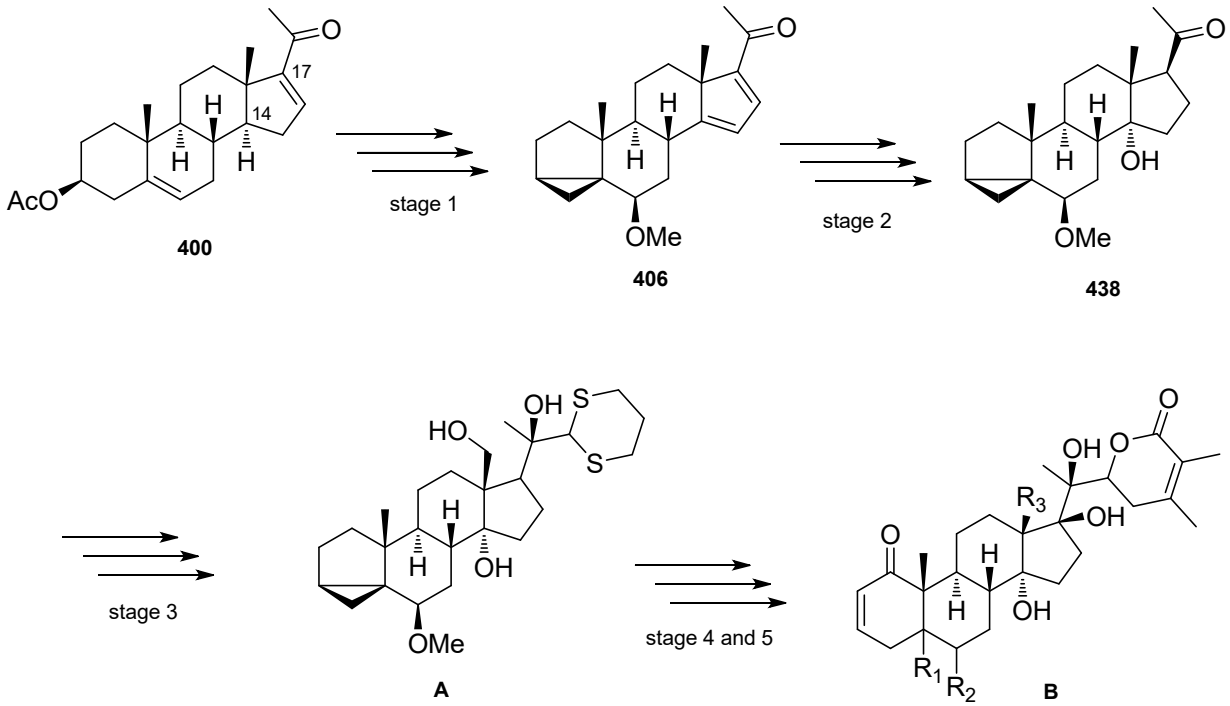


Figure 3.2: General route to make 17β-hydroxywithanolides

The objectives of this project are to develop a method for the introduction of C14, C17, C18 and C20 hydroxyls and thus to prepare physachenolides A-E as well as a series of hydroxylated withanolides such as withanolide E (Figure 3.1); and to assess their biological activity. As described previously in Chapter 1, except for a report by Grieco, there are no methods for the introduction of these hydroxyls and therefore, no a priori route for the synthesis of more complex withanolides. Furthermore, Grieco's method introduces hydroxyls at C14 and 17 simultaneously and hence is not suitable if hydroxylation at only one of those positions were required. As described previously in Chapter 1, outside of withanolide synthesis, methods for the introduction of hydroxyl at C14 have severe limitation and there are also discrepancies as regards to stereoselective outcomes of some of these hydroxylations. Therefore, a major objective of this project was to establish how these hydroxyl groups can be introduced stereoselectively.

The proposed synthesis is in five stages (Figure 3.2) and starts from commercially available 16-dehydropregnenolone acetate **400**. These steps effectively provide a protection for the Δ^5 group in ring B of the steroid. The double bond in **400** needs to be preserved in the earlier steps of the synthesis, so that at a later stage, rings A and B of the steroid can be modified. After protection of Δ^5, ring D $\Delta^{14\text{-}16}$ diene is transformed to **406** *en route* to the introduction of a hydroxyl at the C14 position **438**. The key point in this step is the control of stereoselectivity of the hydroxyl group. The next stage introduces a hydroxyl group at the C18 position and functionalises the keto group at position 20, to afford triol **A** as a prelude to the lateral lactone ring. In the fourth stage, the lateral side chain is elaborated and a hydroxyl group at C17 are introduced. Finally, in the closing stages of the synthesis, the Δ^5 group in ring B is unmasked and rings A and B are modified to afford the final physachenolide compounds, in particular physachenolide C and D (Figure 3.1).

The 17β-hydroxywithanolides will be tested *in vitro* at the Institute of Cancer Therapeutics in cell viability assays to determine their cytotoxicity. The bioactivity result from 17β-hydroxywithanolide will be used to have a better appreciation of structure-activity relationship in this series of compounds.

All of the synthesised compounds will be characterised and analysed in detail by spectroscopic techniques, NMR (^{1}H, ^{13}C, DEPTQ, HMQC, HMBC, COSY, TOCSY and NOESY), mass spectrometry & IR. In particular, the configuration of C14 hydroxylation is to be determined using either X-ray crystallography or NMR techniques.

CHAPTER 04

RESULTS

AND

DISCUSSION

4.1: Synthesis of Compounds

Commercially available 16-dehydropregnenolone acetate **400** was selected as the starting compound. This molecule has the Δ^5 and Δ^{16} both of which are required for the planned functionalisations in the synthesis of physachenolides. However, in order to be able to selectively manipulate the Δ^{16} without affecting Δ^5, the latter needed to be protected.

The Δ^5 was protected over three steps. Hydrolysis of **400** by KOH[129] gave secondary alcohol **401** in good yield (98%) (Figure 4.3: synthetic scheme I). Formation of product was confirmed by disappearance of CH_3CO signal at 2.05 ppm in proton NMR and up field shift of proton at C3 carbon atom. NMR values were in good agreement with the literature.[130] ^{13}C NMR confirmed the correct number of carbon atoms. In MS, a peak at 315.3 a.m.u. was observed, which was assigned as the molecular ion. There was another peak at 337.3 a.m.u. which was due to $M + Na^+$. IR showed broad OH stretch at 3369 cm^{-1}. Tosylation of **401** was done in pyridine by using tosyl chloride to give **402** in a satisfactory yield (87%). In proton NMR the singlet at 2.46 ppm integrates for 3 protons and it was assigned to the methyl of the tosyl group. The doublets at 7.34 & 7.81 ppm integrate for 2 protons each and these were assigned to the aromatic protons. ^{13}C NMR confirmed the right number of carbon atoms. The strong absorption bands at 798-907 cm^{-1} were assigned to S-O stretches.[131]

Mechanism for tosylation is illustrated in figure 4.1. In the first step, the hydroxyl group acts as a nucleophile attacking the sulfur to replace the chloride. The carbon with the stereogenic center is not involved in this step and its configuration retains. The pyridine is added as a base to deprotonate the intermediate and speed up the process of forming the toluenesulfonate ester (tosylate).

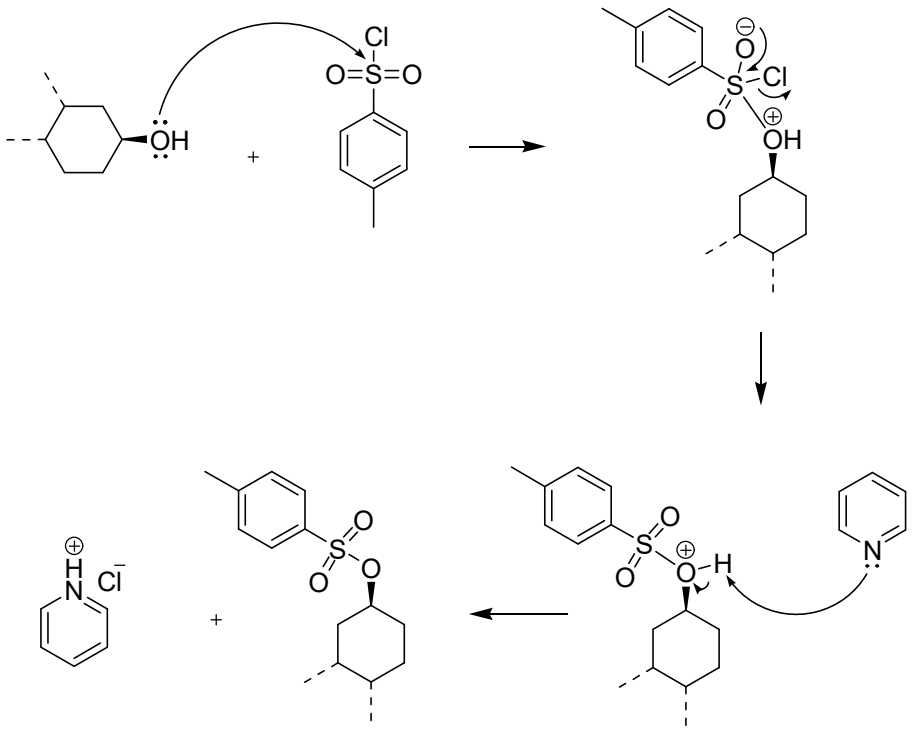


Figure 4.1: Mechanism for tosylation.

Heating **402** in methanol with pyridine (3 equivalent) at reflux provided a mixture of **403** and **404** [121] (Figure 4.3: synthetic scheme I). It was a big challenge to purify desired compound **403** because of very similar R_f values of **403** and **404** on silica gel chromatography. By using a long column with low polarity solvent system (3% EtOAc in PE) **403** (58%) and **404** (6%) were obtained, which was used for characterisation. For compound **403**, in proton NMR the new signal at 3.31 ppm appeared as a singlet which integrates for 3 protons of methyl group attach with oxygen. Further in proton NMR up field shift of H6 proton suggested that there was no double bond. Appearance of a double doublet at 0.41 ppm which integrates for one proton with a coupling constant of 8.0 & 5.2 Hz and an apparent triplet at 0.63 ppm integrates for one proton with a coupling constant of 4.4 Hz proved the three member ring with magnetically distinct protons of a CH_2 moiety. ^{13}C NMR confirmed the number of carbons. In MS the peak observed at 329.4 a.m.u. which was assigned to the molecular ion, the peak at 351.4 a.m.u. was due to $[M + Na]^+$ and the peak at 679.6 a.m.u. was ion of dimer with Na. The compound **404** was confirmed by comparison of NMR with **403** and **402**. MS and IR were quite similar as expected.

A mechanism for the conversion of **402** to **403** and **404** is proposed (Figure 4.2). This reaction does not proceed in the absence of pyridine as explained above (figure 4.1). To account for this observation, it can be argued that although pyridine is a relatively weak base (pK_b around 5.2), in refluxing methanol, it presumably can generate a small concentration of methoxide anions (pK_a around 15.2), which then undergo the reactions outlined. Methoxide anion attacks Δ^5 of **402** from above by giving its electrons to the p-orbital of C6. Electrons from double bond transfer to the C3 to make a sigma bond and tosylate group leaves to give C6 OMe **403**. Methoxide anion cannot displace tosylate directly by attacking at C3 because backside angle of approach is blocked (steric hindrance). Instead p-orbital at C5 can overlap, so conjugate addition occurs instead. As a result, predict the β OMe is protected. Reverse addition also happens when methoxide anion attacks C3 from above to give C3 OMe **404**.

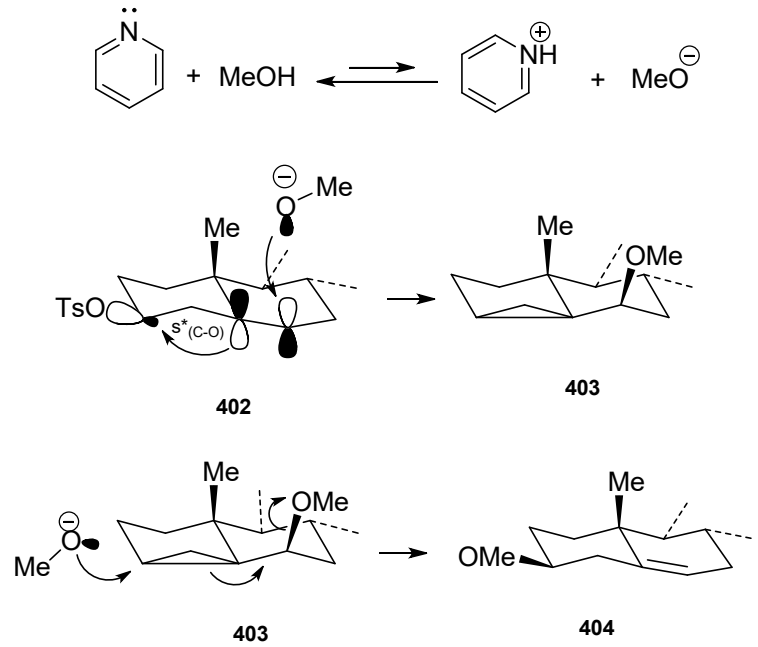
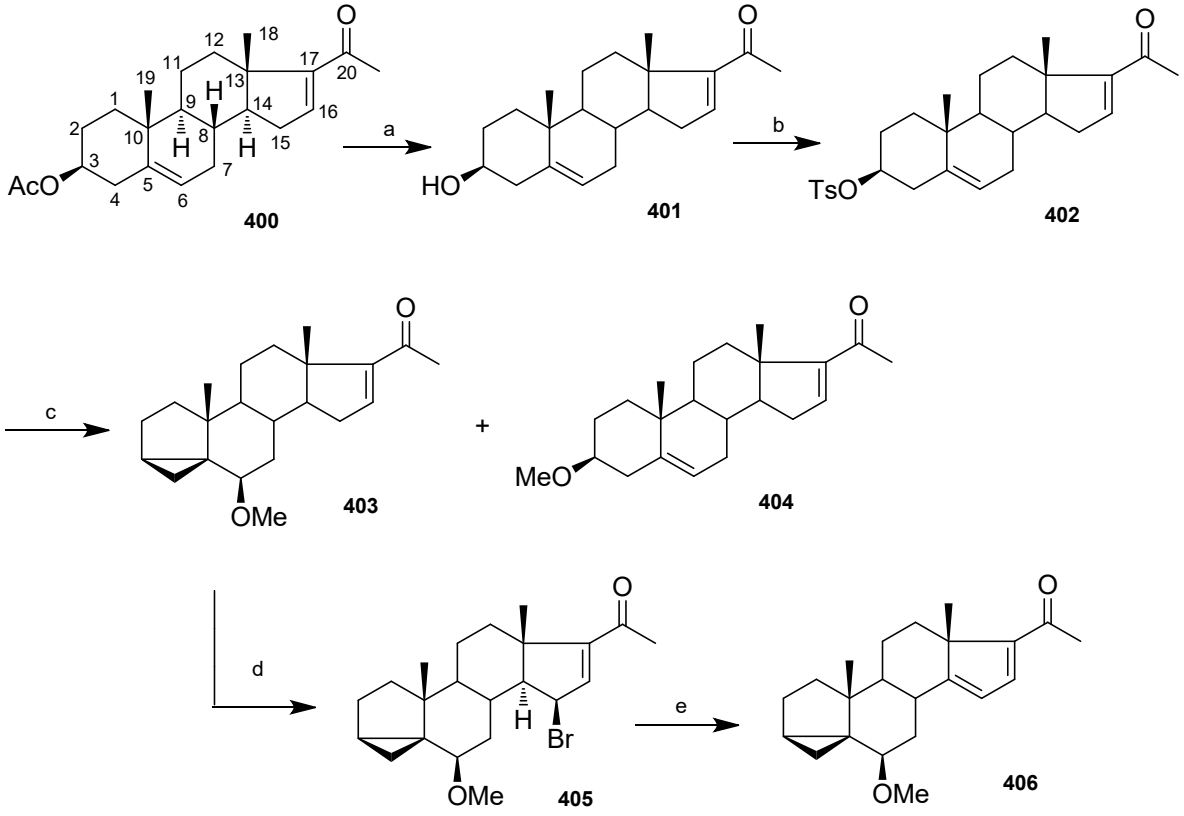


Figure 4.2: Reaction mechanism for formation of **403** and **404**.

The hydroxylation of C14 in the D ring was now investigated. Bromination of **403** with NBS, AIBN and EtOAc provided C15-bromo **405** and following dehydrobromination provided diene **406**[132] with a satisfactory yield (46%). Unreacted compound **403** and other brominated by-products were also recovered from the reaction mixture. These byproducts were close in R_f values to **406** and therefore, it was hard to purify the desired product. In proton NMR H16 signal shifted downfield and there was a new signal at 6.00 ppm as an apparent triplet with a coupling constant of 2.02 Hz and integrates for one proton, assigned to H15 of the new Δ^{14}.

^{13}C confirmed the number and types of carbon atoms. The peak observed in MS at 327.4 a.m.u. which was assigned to the molecular ion, the peak at 349.3 a.m.u. was due to M + Na^+ and the peak at 675.5 a.m.u. was ion of dimer with Na^+.

It is important to note here that for free redical reactions, innert solvent, like CCl_4 is normaly used because it doesn't react during free radical formation but it is a toxic solvent.[133] CCl_4 is one of the most potent hepatotoxins (toxic to the liver).[134] and exposure to high concentrations of CCl_4 (including vapor) can affect the central nervous system and degenerate the liver[135] and kidneys.[136] Prolonged exposure to CCl_4 may lead to coma or death.[133] Chronic exposure to CCl_4 can cause liver[137] and kidney damage and could result in cancer.[138] Due to toxicity of CCl_4, trials were performed using CCl_4 and EtOAc for **405** synthesis and both solvents provided the same yields. So a less toxic solvent, EtOAc was chosen in this project.

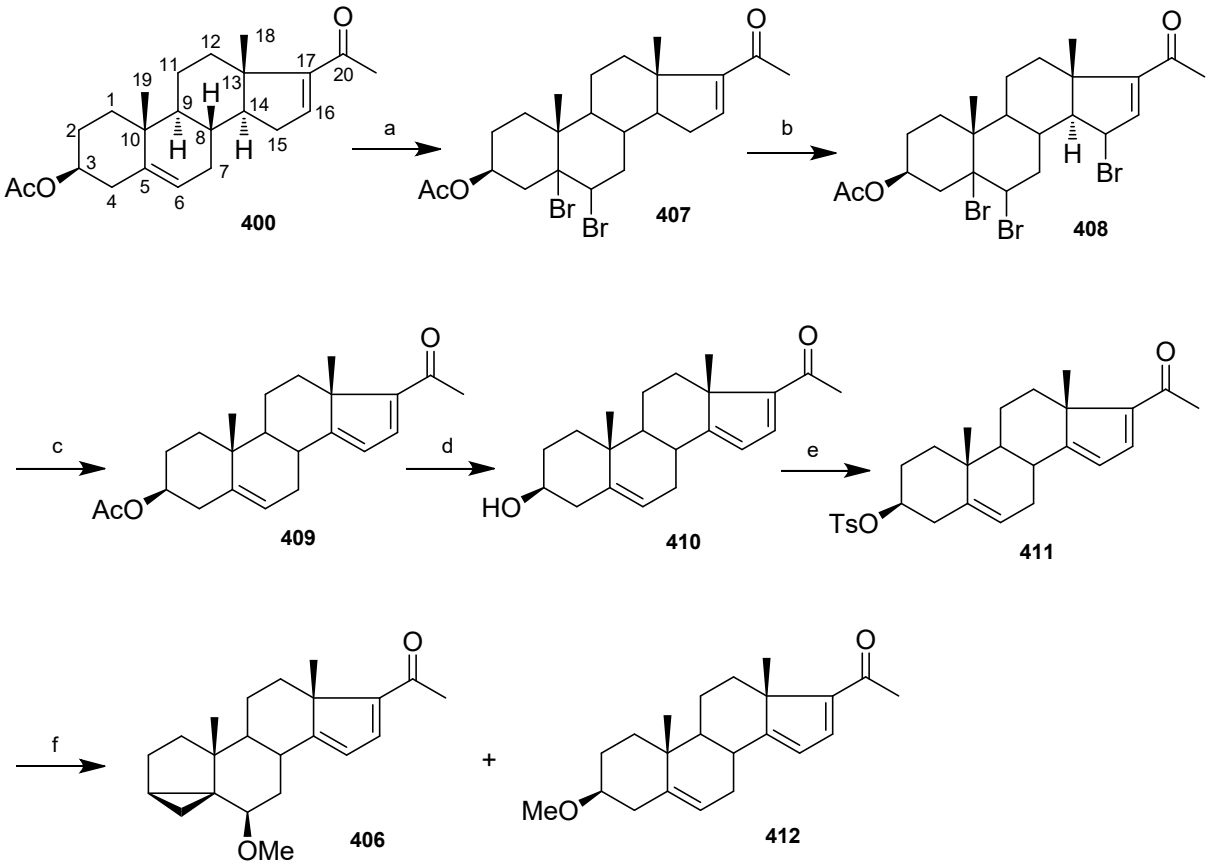


a) KOH, *t*-Butanol, 30°C, overnight, 98%; b) TsCl, py, 28 h, 87%; c) MeOH, py, reflux, 77%; d) NBS, AIBN, EtOAc, reflux; e) NaI, reflux, (46% over 2 steps).

Figure 4.3: Synthesis of compound **406**, synthetic scheme I.

The synthetic route outlined was far from ideal to produce large quantities of **406**. It was difficult to isolate **403** and **404**. Due to these challenges, it was decided to find a different route to make this compound. Solo & Singh reported synthesis of **409** [139] by bromination of Δ^5 **400** using bromine in acetic acid to give **407** and then bromination at C15 with NBS and AIBN followed by dehydrobromination with NaI to give $\Delta^{5,\ 14,\ 16}$-pregnatriene, **409**. Using this protocol, pure pregnatriene **409** (Figure 4.4: synthetic scheme II) was obtained in overall 35% yield over three steps. Corresponding proton and ^{13}C NMR signals in the NMR were in agreement when compared with **406** for Δ^{16} and rest of molecule with **400**. In MS, the peak observed at 355.3 a.m.u. which was assigned to the molecular ion, the peak at 377.3 a.m.u. was due to M + Na^+ and the peak at 731.37 a.m.u. was ion of dimer with Na^+.

Hydrolysis of **409** under the same reaction conditions as for **401** gave **410** quantitatively. NMR spectra were in the agreement with **401** except for signals due to Δ^{14}. In MS, the peak observed at 313.3 a.m.u. which was assigned to the molecular ion whereas the peak at 335.3 a.m.u. was due to M + Na^+. Tosylation of **410** provided **411** in the same way as the **402** was made and the product was confirmed by comparison of spectra with **402**. The product was also confirmed by MS, the molecular ion peak was observed at 467.4 a.m.u. Compound **411** was refluxed with pyridine in methanol to give **406** and **412** with good mass balance (95.5%). It was easier to isolate **406** (isolated in 84%) and **412** (isolated in 11.5%) by column chromatography than it was for **403** & **404**.



a) KOAc, Br_2, acetic acid, ether, 0^oC, quant; b) NBS, AIBN, EtOAc, reflux; c) NaI, reflux, (39% over 3 steps); d) KOH, *t*-butanol, 30^oC, overnight, quant; e) TsCl, py, 28 h, 87%; f) MeOH, py, reflux, **406** 84%, **412** 12%.

Figure 4.4: Synthesis of compound **406**, synthetic scheme II.

After having diene **406** in hand the next stage was to introduce hydroxyl group at C14. Our strategy involved stereoselective epoxidation at C14-C15 followed by selective reduction of epoxide at C15 to leave a C14 hydroxyl. Jung[140] has previously reported that epoxidation of $\Delta^{14\text{-}15}$ cholestane bearing alkyl lateral side chain with *m*-CPBA affords the α epoxide as a major product (10:1, α:β, Figure 4.5 A) and Bjelakovic[141] reported epoxidation of $\Delta^{14\text{-}15}$ cholestane bearing C5 α hydroxyl and alkyl lateral side chain with *m*-CPBA affords exclusively α epoxide (Figure 4.5 B). Bjelakovic has also reported α epoxide in an earlier publication[142]. However, neither author provides a rationale for this selectivity, particularly whether it is dictated by the configuration at C17 (which is β). A mechanism for *m*-CPBA epoxidation is provided in figure 4.6.

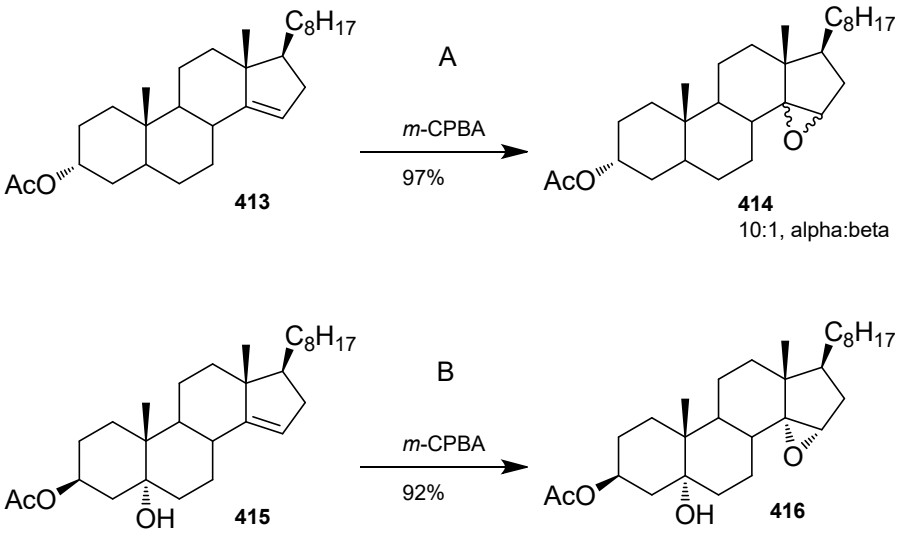


Figure 4.5: Epoxidation of **413** and **415**.

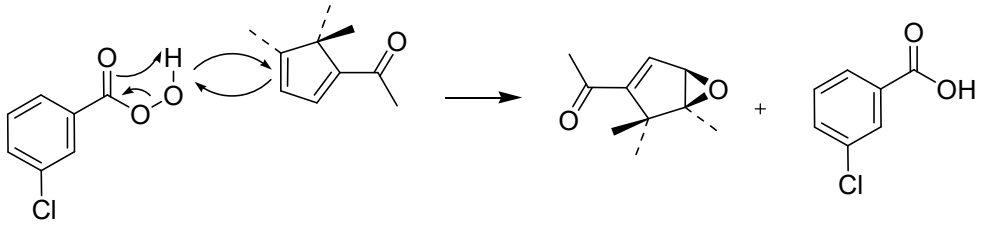


Figure 4.6: Mechanism for *m*-CPBA epoxidation.

Since in compound **406**, the C17 is sp^2 hybridised and flat, we needed to establish the stereochemistry of the epoxidation. For *m*-CPBA expoxidation there are two ways oxygen atom can be introduced, either from above or below the molecule. In this case (figure 4.7) it is easier for *m*-CPBA to attack from above the molecule because attack from below is sterically hindered.

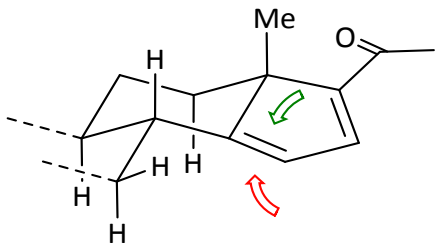


Figure 4.7: Diastereofacial selectivity in the epoxidation of compound **406**.

Oxidation of **406** with *m*-CPBA provided epoxide **417** in a good yield (81%) as a single isomer with 14% recovery of the starting material (Figure 4.8).[132] In proton NMR, H15 signal moved up field from 6.00 ppm to 3.69 ppm and it was a clear doublet with a coupling constant of 0.7 Hz, which confirmed the formation of epoxide. In MS, the peak observed at 343.3 a.m.u. was due to the molecular ion. The peak observed at 365.3 a.m.u. was assigned to M + Na^+ and the peak at 707.4 a.m.u. was ion of dimer with Na. Unfortunately, whilst we could confirm the regioselectivity of epoxidation for C14-C15, NMR techniques were not enough to unequivocally assign the configuration of epoxide **417**. However, a suitable crystal structure was obtained. This showed the epoxide to be at the β-face (Figure 4.9).

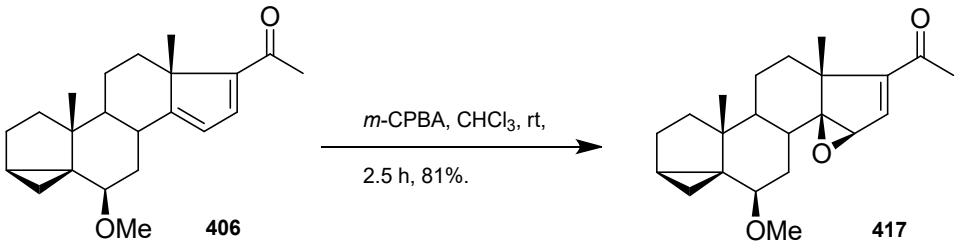


Figure 4.8: Epoxidation of **406**.

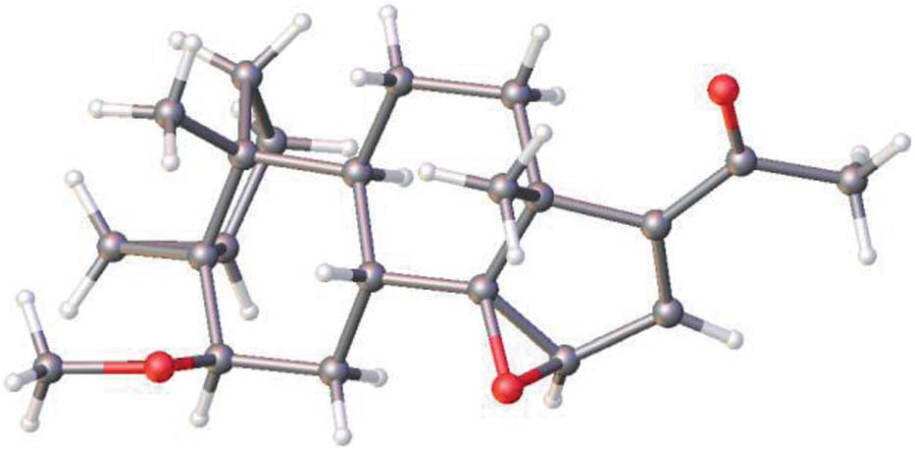


Figure 4.9: Crystal structure of **417**.

Regardless of this disappointing result (wrong stereochemistry), we decided to explore ring opening of the epoxide. This would allow us to understand the range of reagents available for this reaction. Hydrogenation of epoxide **417** for 4 hours by using Pd/C yielded **418** (17%), **419** (34%) and **420** (37%) after chromatography separation (Figure 4.10). In ^{13}C NMR, there were signals for 4 CH_3, 8 CH_2, 6 CH and 4 quaternary carbon atoms which confirmed the

structure of **418**. In MS, the peak observed at 331.4 a.m.u. was the molecular ion. The peak at 353.3 a.m.u. was due to M + Na^+ and the peak at 683.6 a.m.u. was ion of dimer with Na^+. For compound **419**, the disappearance of H16 and slight shift of H15 suggested the formation of the product. Appearance of a double doublet signal at 2.66 ppm with integration of one proton with a coupling constant of 10.2 and 7.5 Hz, suggests coupling of a CH to two magnetically different protons, this signal was assigned to CH17. In ^{13}C NMR a new methylene signal at 27.49 ppm was assigned to the CH_2-16 carbon atom and HMBC and COSY confirmed that these two carbons are linked. In MS, the peak observed at 367.3 a.m.u. was due to the molecular ion. The peak observed at 313.3 a.m.u. was assigned to M + Na^+ and the peak at 711.48 a.m.u. was ion of dimer with Na^+. Compound **420**, in proton NMR signal for H15 disappeared and triplet of H17 proton shifted downfield which suggests opening of the ring it was further confirmed by IR by appearance of OH stretch around 3450 cm^{-1}. The peak observed at 369.3 a.m.u. was assigned to M + Na^+ and the peak at 715.5 a.m.u. was ion of the dimer with Na^+.

Reduction of epoxide **417** with $LiAlH_4$ by refluxing in THF for 1 hour yielded **420** (42%) and a mixture of enantiotopes at C20 **421a** and **421b** (23%). Stirring **420** with $NaBH_4$ in methanol for 40 minutes yielded diastereomers **421a** and **421b** (75%) in the same ratio of enantiomers as reduction with $LiAlH_4$ (Figure 4.10).

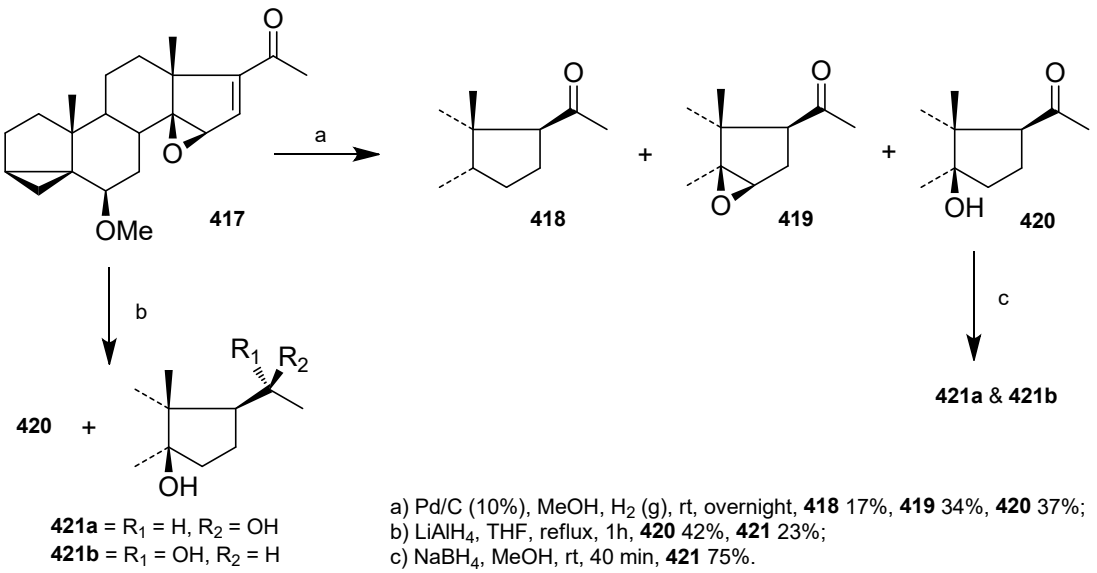


Figure 4.10: Opening of epoxide **417**.

Although we got the wrong configuration of epoxide (**417**, 14β), an extensive literature review pointed to a route for making the 14α configuration. It is precedented that using *m*-CPBA, if the group at C17 position is flat then the epoxide configuration[143] will be β-face (Figure 4.11) as in our case (Figure 4.7 & 4.8). However, if the group at C17 position is in β-face then the epoxidation with *m*-CPBA[141, 144] or DMDO[87] will be in the α-face (Figure 4.11).

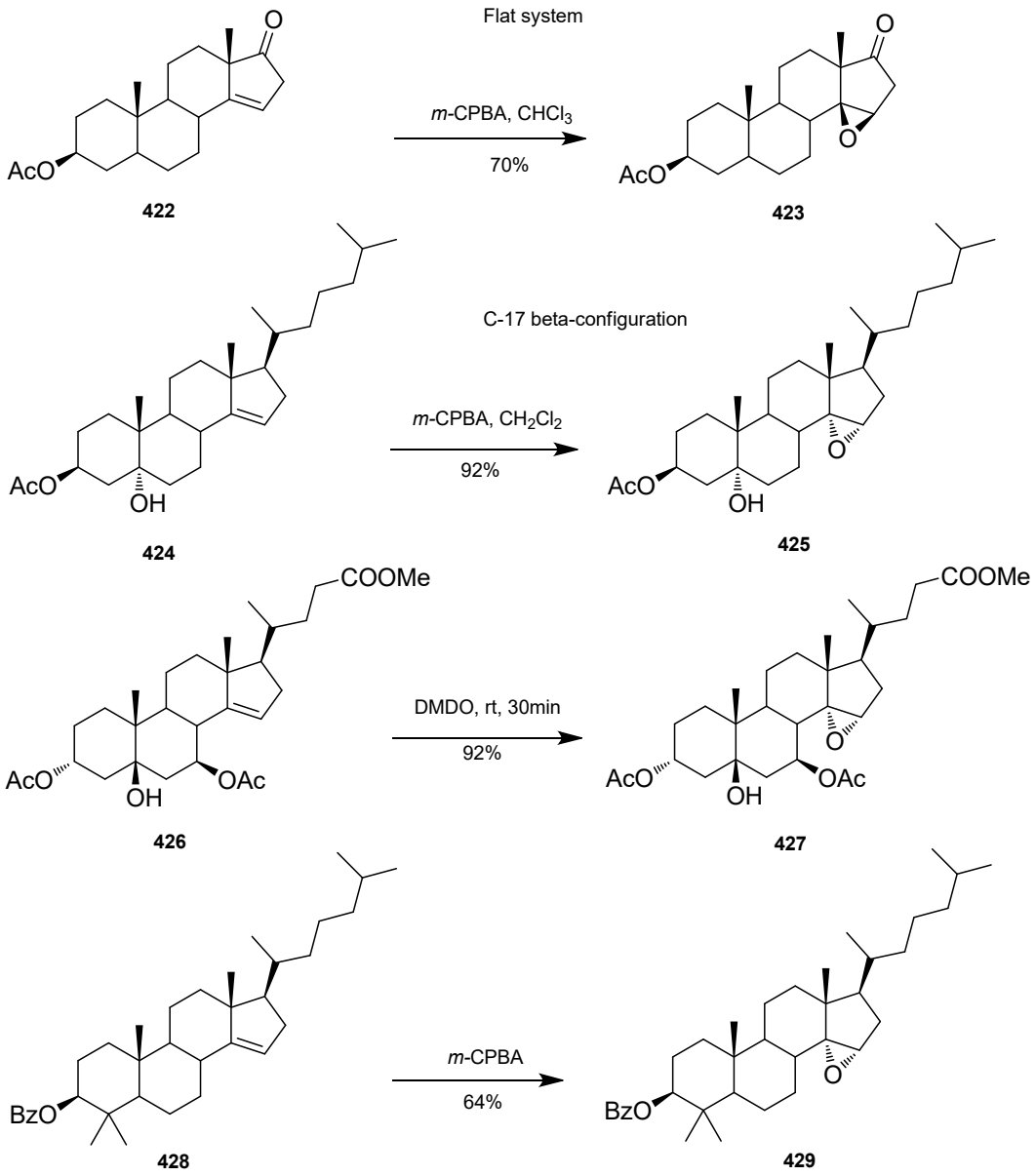
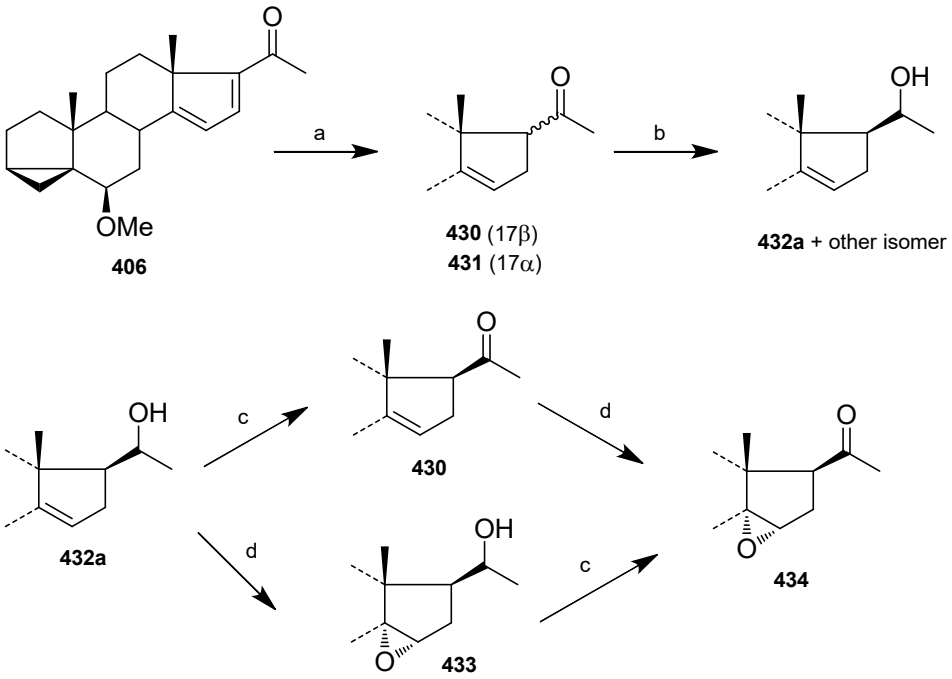


Figure 4.11: Configuration of epoxide at Δ^{14}.

We devised a strategy to selectively reduce Δ^{16} in order to get β-configuration at C17 and then using *m*-CPBA make 14α-epoxide. So we selectively reduced **406** by using triphenyltin hydride afforded a mixture of two C17 epimers, **430** with β-configuration at C17, and **431** with α-configuration at C17 (Figure 4.12). This was in contrast to a previous report that

indicated that a similar reduction was stereoselective.[145-147] Unfortunately, the two isomers, **430** and **431** were not easily separable on large scale by column chromatography. **430** was obtained in 19% yield from the mixture of **430** and **431**. In proton NMR, disappearance of H16 signal from 2.10 ppm confirmed the reduction of Δ^{16} in **430**. In HRMS, the peak observed at 329.2471 a.m.u. was due to the molecular ion. This isomeric mixture (**430** and **431**) was reduced with $NaBH_4$, affording a mixture of the four possible stereoisomers. The major isomer, **432a** was isolated by column chromatography and then converted back to **430** by treatment with PDC. It was much easier to get single isomer **430** from **432a** than directly from mixture of **430** and **431** by column chromatography.

Next step was to check what would be the configuration of epoxide at C14-C15, when acetyl and hydroxyl groups are present at C17 position when they are treated with *m*-CPBA. In epoxidations with *m*-CPBA, steric encumbrance can often be overcomed by directing the delivery of the oxygen atom through hydrogen bonding of the reagent to an adjacent hydroxyl group. So treatment of **430** or **432a** with *m*-CPBA afforded **434** and **433** respectively and both epoxides were α-configuration (Figure 4.12). It further proved that configuration of epoxide at C14-C15 does not depend on the type of side chain but configuration of C17. In proton NMR H15 signal shifted upfield which indicated formation of epoxide and also ^{13}C confirmed the number and types of carbon atoms. Compounds were further confirmed with HRMS and IR.



a) Ph_3SnH, AIBN, toluene, reflux, 10 h, 39%; b) $NaBH_4$, MeOH/DCM, rt, 40 min, **432a** 58%, other isomers 32%; c) PDC, DCM, rt, 48 h, 78%; d) *m*-CPBA, $CHCl_3$, rt, 3 h, **433** 84%, **434** 93%.

Figure 4.12: Synthesis of 14α-epoxide **433** and **434**.

Configuration of C14-C15 epoxide was confirmed by NMR analysis. A key feature in the NMR spectra that supports the assignment of the epoxide configuration in these epoxides is the value of the chemical shift for H18 protons. In both **433** and **434** (where the configuration of the C14-C15 epoxide is α) and in **430** (where there is no epoxide at C14-C15), the chemical shift for protons on C18 (H18) are similar and at 1.01, 0.81 and 0.89 ppm respectively. However, in compound **417** and **419** (where the configuration of epoxide is β) the protons on C18 are subject to magnetic anisotropy effect by the oxygen atom in the epoxide and therefore, their chemical shift is increased to 1.28 and 1.36 ppm respectively. These variations in the chemical shifts of C18 protons are consistent with those previously reported in similar structures.[148]

Another way in which NMR can be used to confirm the configuration of epoxides is by assessing the γ gauche effect in the molecules. It has been previously established that in steroids, a C14α hydroxyl group shields C12 (4-7 ppm) through γ gauche effect more than a C14β hydroxyl group does.[103, 149, 150] C12 is easily identifiable in NMR as it is the only methylene carbon correlating to H18 in an HMBC spectrum. The only limitation to this method is that we require a deoxy analogue to measure the changes in chemical shifts. For example for compound **435**, where there is no C14α hydroxylation and compound **436**

(figure 4.13), where there is C14α hydroxylation, the chemical shift for C12 decreases by 6.3 ppm (Table 4.1).[103]

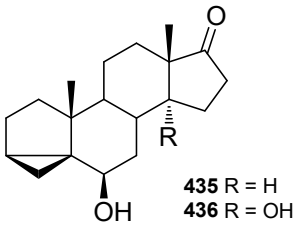
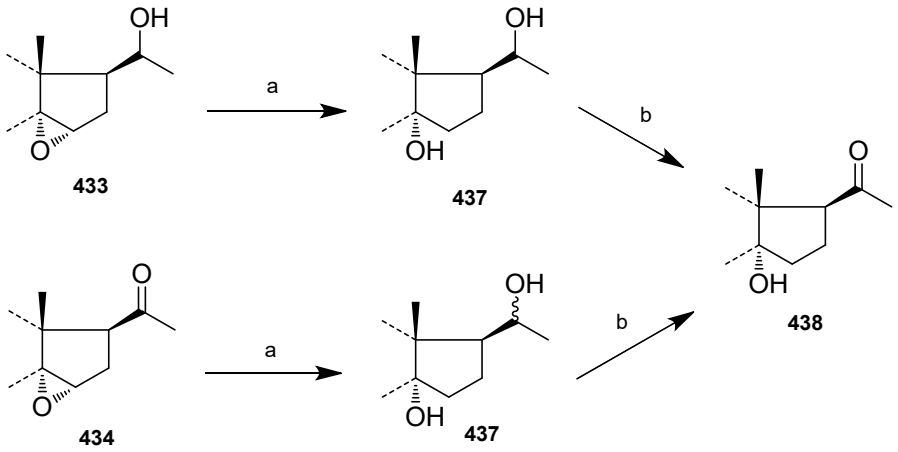


Figure 4.13: Compound **435** and **436**.

Epoxide **433** and **434** were first converted into **437** by treatment with $LiAlH_4$ and then to **438** by treatment with PDC (Figure 4.14). Now we compared the C12 chemical shifts in the carbon NMR of compounds **420** and **438** with those in compound **418** (Table 4.1), itself obtained as a byproduct from hydrogenation of **417**. The larger deshielding (3.3 ppm) in compound **438** compared to that in compound **420** (2.5 ppm) suggests the C14α hydroxyl configuration in compound **438**.



a) $LiAlH_4$, THF, reflux, 6 h, 61%; b) PDC, DCM, rt, 2 days, 77%.

Figure 4.14: Opening of epoxide **433** & **434**.

Position	^{13}C NMR chemical shifts for compounds							
	435	**436**	Δδ	**418**	**438**	Δδ	**420**	Δδ
δC-12 (ppm)	31.7	25.4	-6.3	28.6	31.9	-3.3	31.1	-2.5

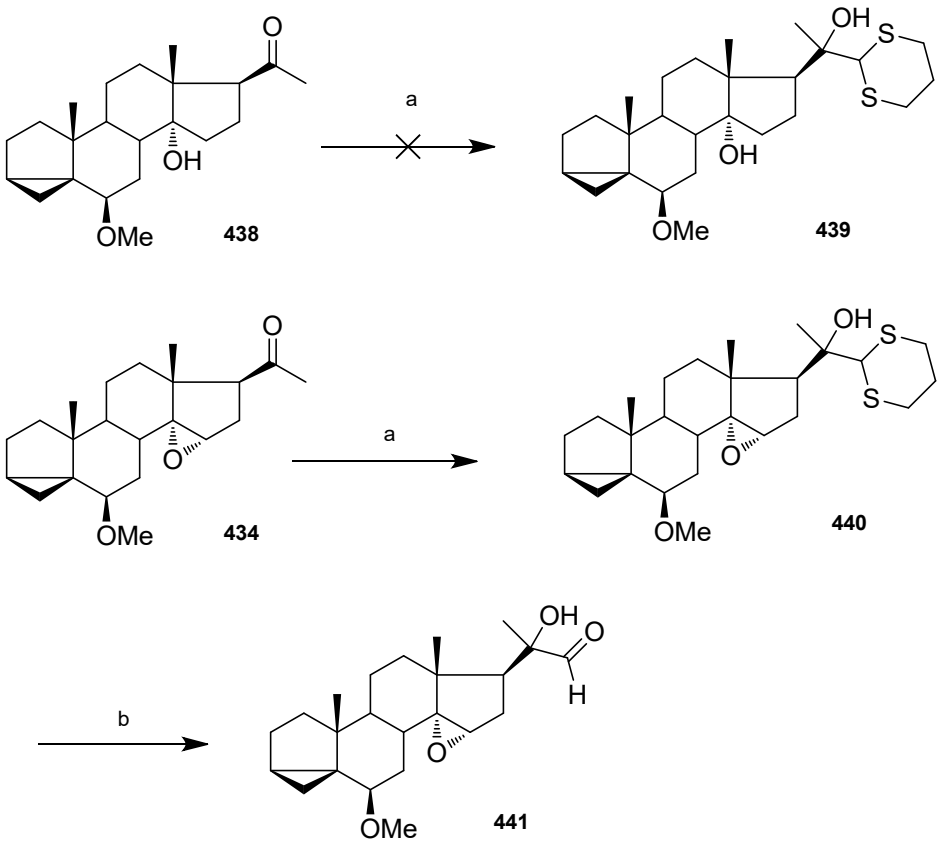
Table 4.1: ^{13}C NMR chemical shifts of C12 in compounds.

After getting the right configuration of epoxide we decided to modify the lateral side chain. Physachenolides and withanolide E (Figure 3.1) are from type A class of withanolides (Figure 1.2), which have lactone moiety on ring D. As mentioned before there are three ways in which lateral side chain has been introduced (Figure 1.16, 1.18, 1.22-1.24). It was decided to go with methodology used by Jana *et al*. (Figure 1.23) and Perez-Medrano & Grieco (Figure 1.24) to make withanolide A **53**. This method has fewer steps and simple than other methods and also different lateral side chain can be introduced easily because it you can add whole group in one step rather than building it on the molecule.

The reaction on 14α hydroxyl **438** with dithiane and *n*-butyllithium to get **439** did not work and starting material was recovered. We have shown that we can open epoxide to give 14α hydroxyl (**33**) so we decided to open it later in the synthesis. The reaction of **434** with dithiane and *n*-butyllithium worked smoothly to give **440** (55%, Figure 4.15). In proton NMR the multiplet at 2.90 ppm integrates for 5 protons and it was assigned to the protons of two methylenes on lateral side group and proton of C6. The singlet at 4.10 ppm integrates for 1 proton and this was assigned to the third methylene on lateral side group. ^{13}C NMR confirmed the right number of carbon atoms. In MS, we observed a peak at 465.4 a.m.u. which was assigned as molecular ion. There was another peak at 487.4 a.m.u. which was due to $M + Na^+$.

The final reaction done was conversion of dithiane **440** to aldehyde **441**. Stirring of dithiane **440** with *N*-chlorosuccinamide in dichloromethane and water[65, 66] (10:1) for 2 hours at room temperature provided aldehyde **441** as a single isomer on new stereocentre (53%, Figure 4.15). In proton NMR the apparent doublet at 9.41 ppm integrates for 1 proton and it was assigned to the proton of aldehyde. The signal at 4.10 ppm was not present for H22 protons which also indicates removal of dithiane moiety. The signal for H15 was found at 3.44 ppm which confirmed that epoxide was intact, also the signals at 0.44 ppm and 0.65 ppm were present which confirmed 3 member ring at ring A was also intact. In ^{13}C NMR the signal at 203.4 ppm was assigned to carbon atom of aldehyde. ^{13}C NMR also confirmed the right number of carbon atoms. In HRMS, the peak found at 375.2529 a.m.u. was assigned as molecular ion. Hirayama, *et al*. used $HgO.BF_3$-OEt_2 reagent for the removal of dithiane moiety (figure 1.16, 1.18 and 1.22) which contains Hg. Hg is toxic substance for aquatic life[151]

so a less toxic reagent, *N*-chlorosuccinamide which was used by Jana *et al*.[65, 66] during synthesis of the withanolide A (**86**) (figure 1.23), was used in above reaction.



a) Dithiane, BuLi, THF, -70^{o}C to 3^{o}C, overnight, 55%; b) NCS, DCM:H_2O, rt, 2h, 53%.

Figure 4.15: Synthesis of aldehyde **441**.

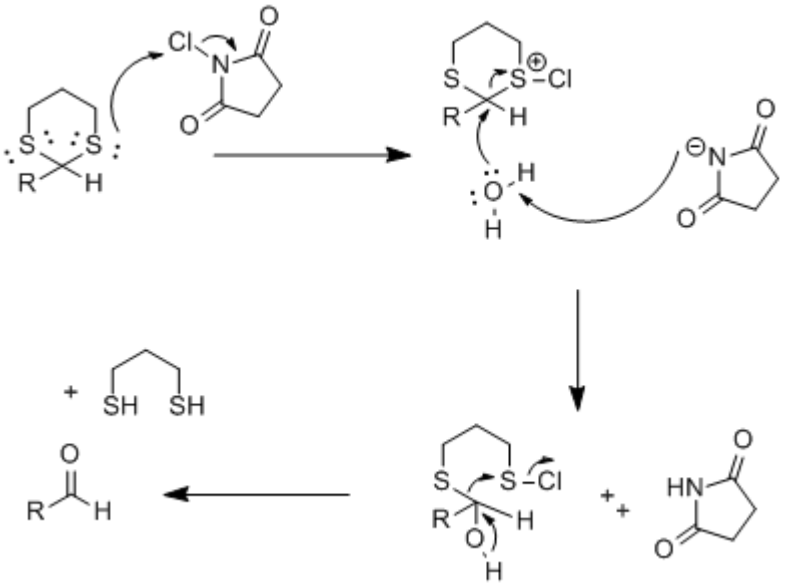
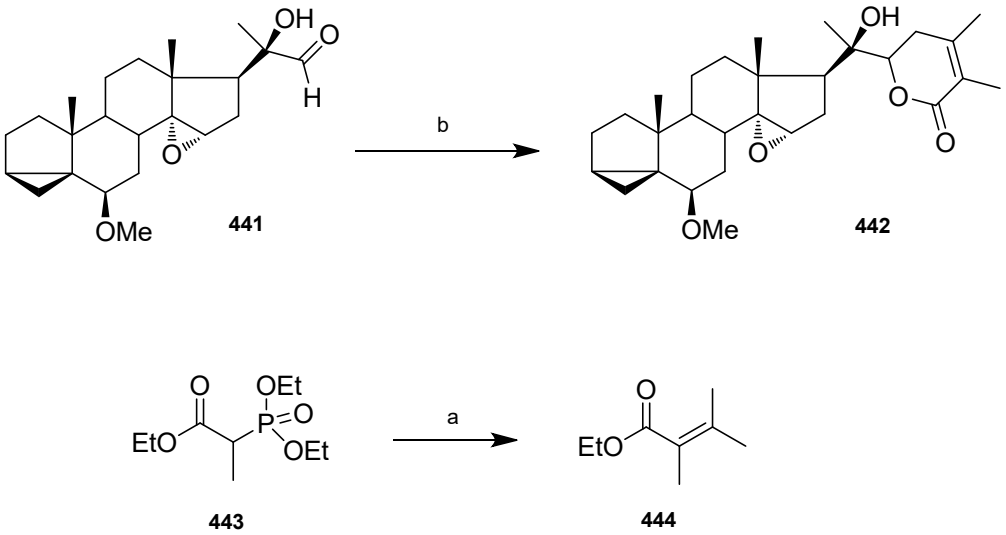


Figure 4.16: Reaction mechanism for *N*-chlorosuccinamide with dithiane.

Physachenolides, withanolide E (Figure 3.1) and other type A withanolides have different functional groups on lateral side chain and aldehyde **441** can be treated with any functional group containing reagent to make desired lateral side chain in one.

We have synthesised ester **444** which can be treated with aldehyde **441** by using Jana`s method mentioned earlier (Figure 1.23) to introduce lactone moiety (**442**), which is present in physachenolide A, C, D, E and withanolide E. Treatment of **443** with sodium hydride in dimethoxyethane at 0^{o}C provided ester **444** quantitatively (Figure 4.17).[152]



a) NaH, dimethoxyethane, acetone, 0^{o}C, quantitative; b) **444**, DMPU, LiHMDS, THF, -70^{o}C - rt, overnight;

Figure 4.17: Synthesis of ester **444**.

4.2: Biological activity of the synthesised compounds

The biology work presented in this section was carried out by Dr Victoria Vinader from Institute of Cancer Therapeutics, University of Bradford.

PC3 as a model for *in vitro* study

We chose androgen-insensitive human prostate adenocarcinoma PC3 cell line as a model for the initial *in vitro* study. PC3 cells do not express androgen receptor and prostate-specific antigen and their proliferation are independent of androgen. We also plan to assess the compounds in LnCap, a hormone sensitive prostate cancer cell line in due course.

MTT Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a colorimetric assay, which is used for evaluating cell metabolic activity.[153] Cellular reductase enzymes under physiologically balanced conditions correlate to the number of viable cells present. Reductase enzymes are capable of reducing the MTT, a yellow, water soluble tetrazolium dye to the water insoluble formazan, which is purple in colour (Figure 4.18). After the end of the experiment, removal of aqueous medium and addition of an organic solvent such as DMSO, dissolves the formazan. The absorbance of purple coloured formazan solution can be quantified by using a spectrophotometer. Higher absorbance means more cells were present.

Figure 4.18: Metabolism of MTT by reductase enzyme.

The advantages of MTT assay are that it is quick, affordable, and easy to do. Therefore, it is widely used to measure the loss of viable cells.

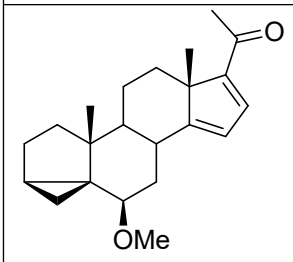
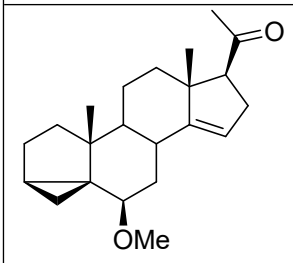
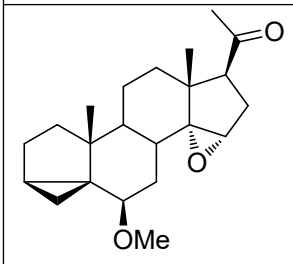
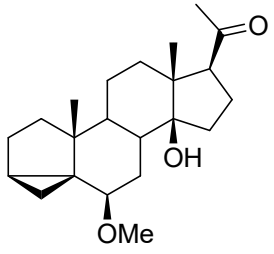
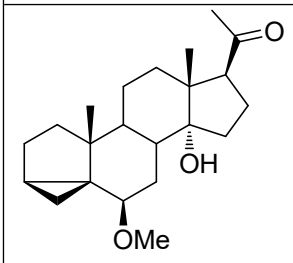
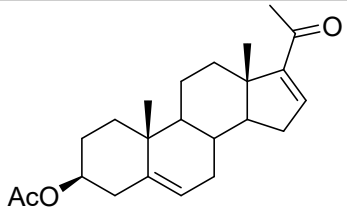
Conversion of MTT to formazan depends on cellular metabolic activity, so only metabolically active viable cells will work for MTT. The cells with low metabolism or metabolically inactive, will reduce very little MTT so it will affect the results. Therefore a major shortcoming of the assay is that it actually measures metabolically active, rather than "live" cells. Hence, it is very important that the cells are used in their log growth phase. Non-proliferating or quiescent cells do not give a proportional response in MTT assay. In addition MTT reagent is sensitive to light so you have to keep it in the dark if it is to be stored over a long period of time.

Structure–activity relationship

The structure-activity relationship (SAR) is the relationship between the chemical structure of a molecule and its biological activity. SAR analysis helps in determination of the functional group responsible for inducing a target biological effect in the organism.

The promising candidates of the synthesised compounds have been tested for their potency using PC3 cell line, and the obtained IC_{50} is shown in the Table 4.2. The compound **417** has shown the best potency among all compounds tested, against PC3 cell line with IC_{50} = 0.37 µM (Table 4.2). The reason could be that **417** is acting as Michael acceptor. On the other side aldehyde **441** has the shown least potency against PC3 cell line with IC_{50} = >100 µM and it could be due to hydrolysis of aldehyde. Compound **438** with C14 α-OH group has shown better potency then compound **420** with C14 β-OH group (IC_{50} = 50 µM and IC_{50} = 80 µM respectively). It shows that C14 α-OH group is better than C14 β-OH group for binding with target.

After completion of total synthesis more compounds will be available to test and also further testing on these compounds will help better understand which parts of the molecules are better for potency.

Structure	Compound number	IC_{50}				
		Run 1	Run 2	Run 3	Run 4	Average
	**406**	4 µM	15 µM	6 µM	10 µM	8.75 µM
	**430**	70 µM	100 µM	100 µM	90 µM	90 µM
	**434**	35 µM	35 µM	30 µM		33.3 µM
	**420**	80 µM	80 µM	80 µM		80 µM
	**438**	30 µM	50 µM	70 µM		50 µM
	**400**	20 µM	30 µM	50 µM		33.3 µM

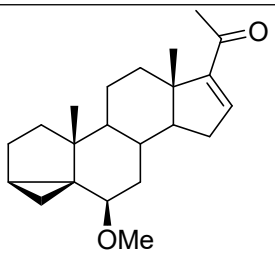
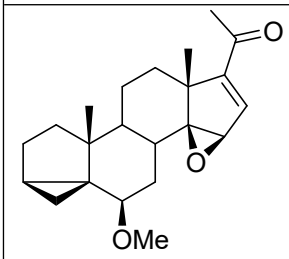
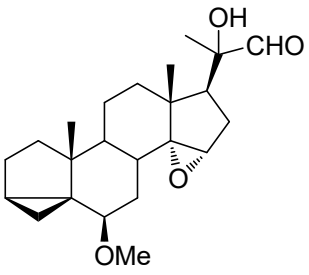
	**403**	30 µM	30 µM	30 µM		30 µM
	**417**	0.2 µM	0.5 µM	0.4 µM		**0.37 µM**
	**441**	70 µM	>100 µM	>100 µM		>100 µM

Table 4.2: Potency of synthesised compounds against PC3 cell line.

4.3: Conclusion and Future Work

We have successfully synthesised aldehyde **441** intermediate (Figure 4.17) from commercially available 16-dehydropregnenolone acetate **400**. Aldehyde **441** is a key intermediate which will be used to make different withanolides, especially physachenolides and withanolide E (figure 3.1). We have also shown that how hydroxyl group can be introduced stereoselectively at C14 position of steroids.

Work done on this project so far is shown in a detailed schematic diagram in figure 4.19. Encirculed work in figure 4.19 will be carried out to introduce C18 acetyl moiety. The dehdroxylation at C17 is precendent in literarure.[114, 115, 119, 154] Introduction of lactone moiety using LiHMDS and **444** (figure 4.17) is also precedent in literature as mentioned in introduction (figure 1.16, 1.18 and 1.22).

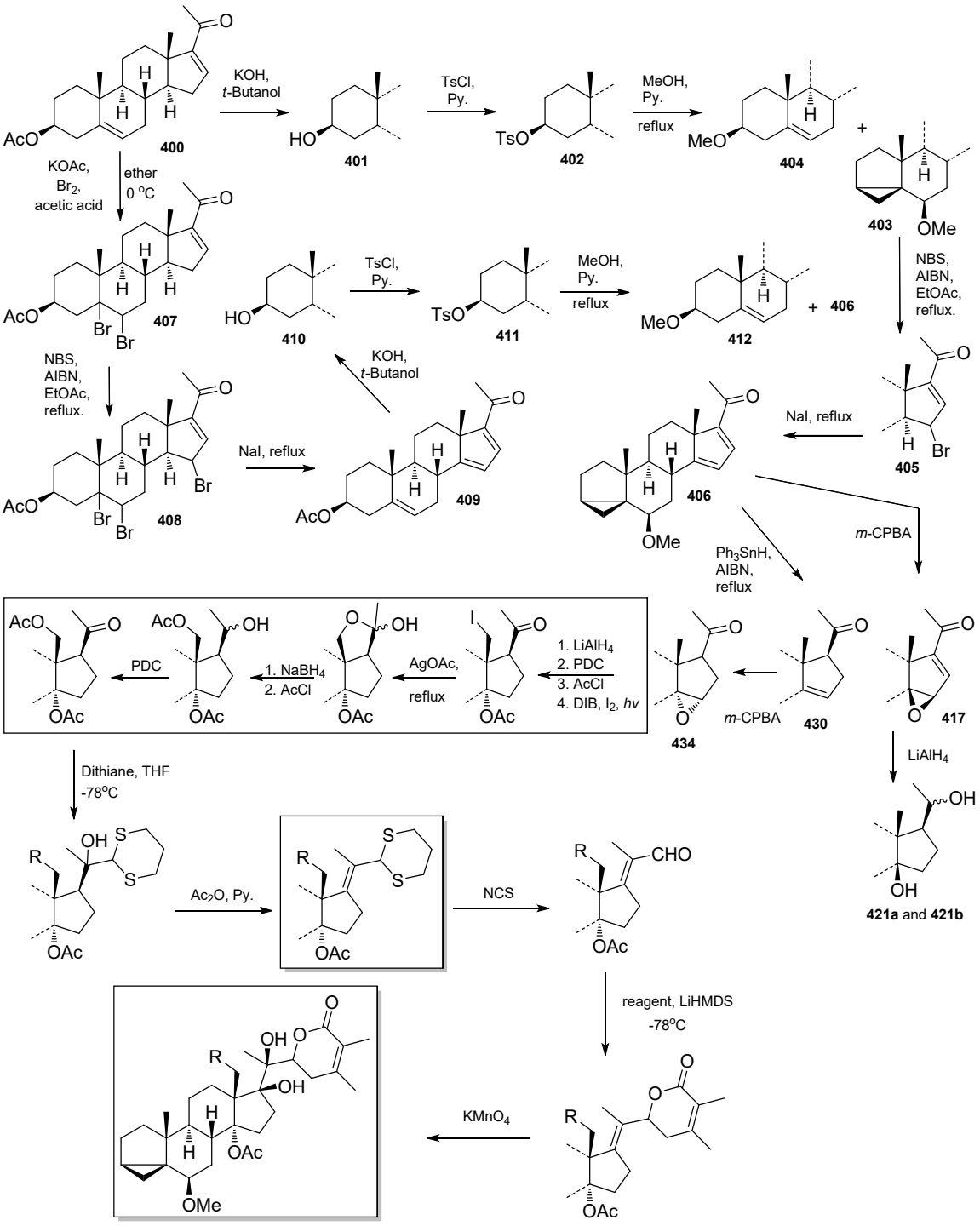


Figure 4.19: Synthetic route for the total synthesis of physachenolides.

The modification of ring A and B are very frequently done in the synthesis of withanolides and in general ergostane molecules. A proposed is shown in figure 4.20 and some examples are mentioned in the introduction chapter (Figure 1.13, 1.18 and 1.24) for the modification on ring A and B.

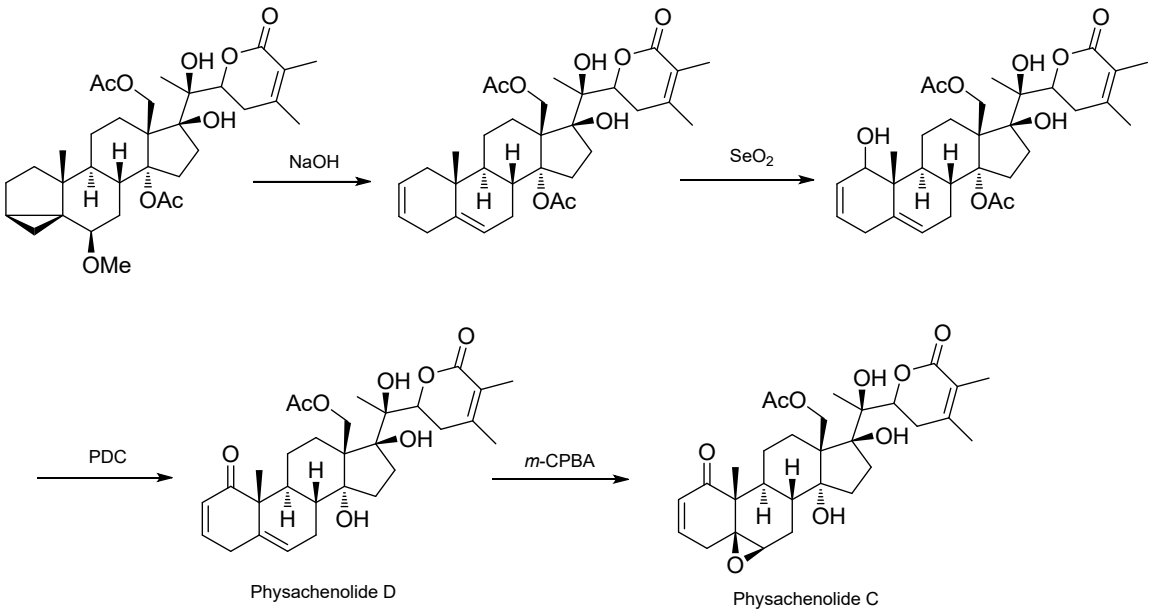


Figure 4.20: Modification of ring A and B to make complete physachenolide.

The work on this project is ongoing and would result in the total synthesis of withanolides. Completion of this total synthesis will open new windows for synthesis of more withanolides through this route.

CHAPTER 05

EXPERIMENTAL

5.1: Experimental Techniques, Materials and Instrumentation

General: All chemical reagents and starting materials used below were commercially available and purchased from Sigma-Aldrich, Alfa Aesar and Carbosynth. All the reagents were used as purchased without any additional purification unless otherwise stated. All solvents were of reagent grade, suited for general laboratory use. Petroleum ether (PE) refers to the fraction of petroleum spirit boiling in the range of 60 to 80 °C. Where stated, mixtures of solvents are referred to as volume-to-volume (v/v) ratios. Synthesised compounds were purified by flash chromatography using high-purity silica gel (Fluorochem: LC4025), pore size 40-63 µm (CAS Number: 7631-86-9). The analytical thin layer chromatography was conducted on Merck silica gel 60 F254 aluminium backed plates (catalogue number 1.05554.0001, VWR). TLC plates were visualised under ultraviolet light lamp (254 nm), or by dipping in basic potassium permanganate ($KMnO_4$) solution.

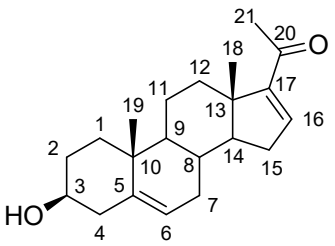
Proton and carbon nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded using Bruker AMX400 spectrometer operating at 400 MHz and 101 MHz respectively. Deuterated chloroform ($CDCl_3$) was used as the NMR solvent unless otherwise stated. Chemical shifts are reported in parts per million (δ, ppm). ^1H NMR chemical shifts are reported relative to an internal reference (tetramethylsilane) or residual proton signals of the solvent. Coupling constants (*J*) are expressed in Hertz (Hz). The splitting patters in NMR spectra are reported with the following abbreviations; singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint.), multiplet (m), double doublet (dd), double triplet (dt), double double doublet (ddd), triple double doublet (tdd) and broad (br). ^{13}C NMR chemical shifts are reported relative to the signal of the solvent. Where necessary, correlation spectroscopy (COSY), nuclear overhauser enhanced spectroscopy (NOESY), heteronuclear multiple quantum correlation spectroscopy (HMQC) for $^{13}C/^1H$ nuclei, and distortionless enhancement by polarization transfer (DEPT) technique were employed to confirm the assignment of NMR spectra.

Routine infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Samples were used directly (solid / liquid) without making sodium chloride plates or KBr discs. Spectra are reported in wavenumbers (cm^{-1}).

Routine LC-MS was used to monitor reactions, on Waters, Micromass Quattro Ultima in the electrospray ionization, in positive ion mode (ESI+). High resolution accurate mass (HRMS) determination was performed using a Thermo Orbitrap LTQ XL spectrometer.

5.2: Synthesised Compounds

5.2.1: Compound 401



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

To a stirred solution of 16-dehydropregnenolone acetate **400** (5.94 g, 16.7 mmol) in t-butanol (110 mL) was added KOH (4.67 g, 5 eq.) in H_2O (5 mL). The resulting mixture was stirred overnight at 30°C and then t-butanol was removed under reduced pressure. Water (200 mL) was added and the resulting solid was filtered, washed with plenty of water and dried under high vacuum to afford the title compound **401** as a white solid (5.12g, 98 %).

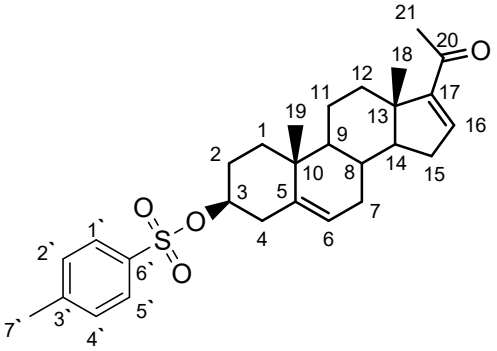
^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.92 (3H, s, CC$\underline{H}_3$ - 18), 0.98 - 1.13 (2H, m, C$\underline{H}$ - 9, C$\underline{H}_2$ - 1), 1.04 (3H, s, CC$\underline{H}_3$ - 19), 1.30 - 1.75 (6H, m, C$\underline{H}_2$ - 12, C$\underline{H}$ - 14, C$\underline{H}_2$ - 7, C$\underline{H}_2$ - 11, C$\underline{H}$ - 8), 1.82 - 1.87 (1H, m, C$\underline{H}_2$ - 1), 1.98 - 2.10 (3H, m, C$\underline{H}_2$ - 7, C$\underline{H}_2$ - 2), 2.23 - 2.34 (4H, m, C$\underline{H}_2$ - 15, C$\underline{H}_2$ - 4), 2.26 (3H, s, CC$\underline{H}_3$ - 21), 2.40 (1H, dt, *J* = 12.7, 3.7 Hz, C$\underline{H}_2$ - 12), 3.48 - 3.55 (1H, m, C$\underline{H}$ - 3), 5.36 (1H, dd, *J* = 5.2 Hz, C$\underline{H}$ - 6), 6.70 (1H, dd, *J* = 3.2, 1.9 Hz, C$\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 16.1 (C$\underline{C}H_3$, 18), 19.6 (C$\underline{C}H_3$, 19), 21.0 ($\underline{C}H_2$, 11), 27.5 (C$\underline{C}H_3$, 21), 30.5 ($\underline{C}$H, 8), 31.9 ($\underline{C}H_2$, 7), 32.0 ($\underline{C}H_2$, 2), 32.6 ($\underline{C}H_2$, 15), 35.0 ($\underline{C}H_2$, 12), 37.0 ($\underline{C}$, 10), 37.5 ($\underline{C}H_2$, 1), 42.6 ($\underline{C}H_2$, 4), 46.4 ($\underline{C}$, 13), 50.8 ($\underline{C}$H, 9), 56.8 ($\underline{C}$H, 14), 72.1 ($\underline{C}$H, 3), 121.4 ($\underline{C}$H, 6), 141.7 ($\underline{C}$, 5), 144.7 ($\underline{C}$H, 16), 155.7 ($\underline{C}$, 17), 197.2 ($\underline{C}$, 20).

Mass Spectrum: (ESI+), *m/z*, 315.34 $[M + H]^+$, 337.34 $[M + Na]^+$, 297.45 $[M - H_2O]^+$.

IR Spectrum: 3369 cm^{-1} (OH stretch), 1655 (C=O stretch), 1042 cm^{-1} (C-O stretch).
In agreement with previously reported literature for this compound.[155, 156]

5.2.2: Compound 402



(3*S*,10*R*,13*S*)-17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 4-methylbenzenesulfonate

To a solution of 16-dehydropregnenolone **401** (4.73 g, 15.0 mmol) in pyridine (35 mL) was added TsCl (8.60 g, 45.0 mmol) and stirred at room temperature for 28 hours. Water (200 mL) was added to the reaction mixture and the resulting solid was filtered and washed with plenty of water. Product was dried under high vacuum to afford compound **402** (6.11 g, 87 %) as an off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, s, CC$\underline{H}_3$ - 18), 0.93 - 1.05 (2H, m, C$\underline{H}$ - 9, C$\underline{H}_2$ - 1), 1.00 (3H, s, CC$\underline{H}_3$ - 19), 1.25 - 1.42 (2H, m, C$\underline{H}_2$ - 12, C$\underline{H}$ - 14), 1.50 – 1.74 (4H, m, C$\underline{H}_2$ - 11, C$\underline{H}_2$ - 8, C$\underline{H}$ – 7), 1.76 - 1.84 (3H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ - 2), 1.96 - 2.08 (1H, m, C$\underline{H}_2$ - 7), 2.25 (3H, s, CCH_3 - 21), 2.25 - 2.33 (4H, m, C$\underline{H}_2$ - 15, C$\underline{H}_2$ - 4), 2.36 - 2.49 (1H, m, C$\underline{H}_2$ - 12), 2.44 (3H, s, CC$\underline{H}_3$ – 7'), 4.28 - 4.36 (1H, m, C$\underline{H}$ - 3), 5.31 - 5.33 (1H, m, C$\underline{H}$ - 6), 6.69 (1H, dd, *J* = 3.2, 1.8 Hz, C$\underline{H}$ - 16), 7.32 (2H, d, *J* = 8.10 Hz, 2' & 4'), 7.79 (2H, d, *J* = 8.83 Hz, 1' & 5').

^{13}C NMR (101 MHz, $CDCl_3$) δ 16.0 ($\underline{C}H_3$, 18), 19.4 ($\underline{C}H_3$, 19), 20.9 ($\underline{C}H_2$, 11), 22.0 ($\underline{C}H_3$, 7), 27.5 ($\underline{C}H_3$, 21), 28.9 ($\underline{C}H_2$, 2), 30.4 ($\underline{C}H$, 8), 31.8 ($\underline{C}H_2$, 7), 32.5 ($\underline{C}H_2$, 15), 34.9 ($\underline{C}H_2$, 12), 36.9 ($\underline{C}$, 10), 37.1 ($\underline{C}H_2$, 1), 39.3 ($\underline{C}H_2$, 4), 46.4 ($\underline{C}$, 13), 50.6 ($\underline{C}H$, 9), 56.6 ($\underline{C}H$, 14), 82.5 ($\underline{C}H$, 3), 123.2 ($\underline{C}H$, 6), 128.0 ($\underline{C}H$, 1' & 5'), 130.0 ($\underline{C}H$, 2' & 4'), 135.0 ($\underline{C}$, 3), 139.8 ($\underline{C}$, 5), 144.7 ($\underline{C}H$, 16), 144.8 ($\underline{C}$, 6'), 155.6 ($\underline{C}$, 17), 197.1 ($\underline{C}$, 20).

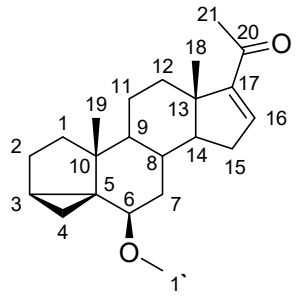
Mass Spectrum (ESI+), *m/z*, 297.45 [M –TsOH, $C_{21}H_{29}O$] $^{+}$.

IR Spectrum: 1657 cm^{-1} (C=O stretch), 1328 & 1172 cm^{-1} (S=O stretch), 907-798 cm^{-1} (S-O stretch).

HRMS: (ESI+), *m/z*, found = 469.2435, calculated for $C_{28}H_{37}O_4S$ [M + H] $^{+}$ = 469.2413.

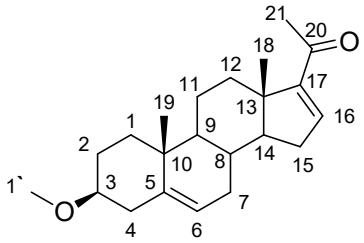
In agreement with previously reported literature for this compound.[154]

5.2.3: Compound 403 and 404



1-((1a*R*,3a*R*,5a*S*,10*R*,10a*R*)-10-methoxy-3a,5a-dimethyl-1,1a,2,3,3a,3b,4,5,5a,8,8a,8b,9,10-tetradecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

403



1-((3*S*,10*R*,13*S*)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethanone

404

To a solution of compound **402** (12.80 g, 27.0 mmol) in anhydrous methanol (100 ml) was added pyridine (6.52 ml, 81.0 mmol) at room temperature and refluxed for 4 hours. The reaction mixture was cooled and saturated $NaHCO_3$ aqueous solution (300 ml) was added and extracted with CH_2Cl_2 (200 ml x 3). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (3-5 % EtOAc in PE) afforded a mixture of **403** and **404** (6.88 g, 77 %) as a white solid. The mixture was subjected to silica gel column chromatography (3-5 % EtOAc in PE) to get **403** (5.24 g, 58 %) & **404** (0.56 g, 6 %).

Compound 403

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.39 (1H, dd, *J* = 8.0 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.61 (1H, app. t, 4.4 Hz C$\underline{H}_2$ - 4), 0.70 – 0.87 (3H, m, C$\underline{H}$ - 9, C$\underline{H}$ - 3 & CH_2 - 1), 0.88 (3H, s, CC$\underline{H}_3$ - 18), 0.99 (3H, s, CC$\underline{H}_3$ - 19), 1.05 - 1.31 (2H, m, C$\underline{H}_2$ - 7, C$\underline{H}_2$ - 12), 1.32 – 1.55 (4H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ - 2, C$\underline{H}_2$ – 11, C$\underline{H}$ –

14), 1.62 - 1.75 (1H, m, C$\underline{H}_2$ - 2), 1.80 - 2.07 (3H, m, C$\underline{H}_2$ – 7, C$\underline{H}$ – 8, C$\underline{H}_2$ - 15), 2.19 (3H, s, CC$\underline{H}_3$ - 21), 2.26 - 2.30 (2H, m, C$\underline{H}_2$ - 12, C$\underline{H}_2$ - 15), 2.74 (1H, t, *J* = 2.7 Hz, C$\underline{H}$ - 6), 3.29 (3H, s, OC$\underline{H}_3$ – 1'), 6.64 (1H, dd, *J* = 3.2, 1.9 Hz, C$\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.4 (C$\underline{H}_2$, 4), 16.4 (C$\underline{H}_3$, 18), 19.4 (C$\underline{H}_3$, 19), 21.5 (C$\underline{H}$, 3), 22.5 (C$\underline{H}_2$, 11), 25.1 (C$\underline{H}_2$, 2), 27.3 (C$\underline{H}_3$, 21), 29.1 (C$\underline{H}$, 8), 32.4 (C$\underline{H}_2$, 15), 33.3 (C$\underline{H}_2$, 1), 35.2 (C$\underline{H}_2$, 12), 35.4 (C$\underline{}$, 10), 35.5 (C$\underline{H}_2$, 7), 43.7 (C$\underline{}$, 5), 46.6 (C$\underline{}$, 13), 48.6 (C$\underline{H}$, 9), 56.6 (C$\underline{H}$, 14), 56.8 (C$\underline{H}_3$, 1'), 82.3 (C$\underline{H}$, 6), 144.5 (C$\underline{H}$, 16), 155.8 (C$\underline{}$, 17), 196.8 (C$\underline{}$, 20).

Mass Spectrum: (ESI+), *m/z*, 329.44 $[M + H]^+$, 351.44 $[M + Na]^+$, 297.45 $[C_{21}H_{29}O]^+$, 679.59 $[Mx2 + Na]^+$.

IR Spectrum: 1655 cm^{-1} (C=O stretch), 1097 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 329.2494, calculated for $C_{22}H_{32}O_2$ $[M + H]^+$ = 329.2481.

In agreement with previously reported literature for this compound.[154, 157]

Compound 404

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.91 (3H, s, CC$\underline{H}_3$ - 18), 0.92 - 1.09 (2H, m, C$\underline{H}$ - 9, C$\underline{H}_2$ - 7), 1.03 (3H, s, CC$\underline{H}_3$ - 19), 1.27 - 1.48 (3H, m, C$\underline{H}_2$ - 2, C$\underline{H}_2$- 12, C$\underline{H}_2$ - 14), 1.48 - 1.76 (3H, m, C$\underline{H}$ - 8, C$\underline{H}_2$ - 11), 1.81 - 2.21 (6H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 4, C$\underline{H}_2$ – 7, C$\underline{H}$ - 15), 2.25 (3H, s, CC$\underline{H}_3$ - 21), 2.26 – 2.46 (3H, m, C$\underline{H}_2$ – 1, C$\underline{H}_2$ – 12, C$\underline{H}_2$ - 15), 3.05 (1H, dt, *J* = 11.3, 4.5 Hz, C$\underline{H}$ - 3), 3.34 (3H, s, OC$\underline{H}_3$ - 1'), 5.31 - 5.41 (1H, m, C$\underline{H}$ - 6), 6.70 (1H, dd, *J* = 3.3, 1.9 Hz, C$\underline{H}$ - 16).

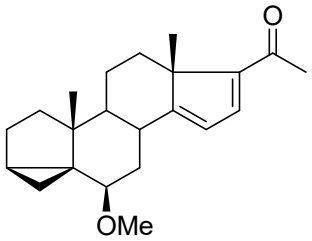
^{13}C NMR: (101 MHz, $CDCl_3$) δ 16.0 (C$\underline{C}H_3$, 18), 19.6 (C$\underline{C}H_3$, 19), 21.0 (C$\underline{H}_2$, 11), 27.5 (C$\underline{C}H_3$, 21), 28.2 (C$\underline{H}_2$, 2), 30.5 (C$\underline{H}$, 8), 31.9 (C$\underline{H}_2$, 15), 32.6 (C$\underline{H}_2$, 4), 34.9 (C$\underline{H}_2$, 12), 37.3 (C$\underline{H}_2$, 7), 37.4 (C$\underline{}$, 10), 39.1 (C$\underline{H}_2$, 1), 46.4 (C$\underline{}$, 13), 50.8 (C$\underline{H}$, 9), 55.9 (C$\underline{H}_3$, 1'), 56.7 (C$\underline{H}$, 14), 80.6 (C$\underline{H}$, 3), 121.2 (C$\underline{H}$, 6), 141.8 (C$\underline{}$, 5), 144.8 (C$\underline{H}$, 16), 155.7 (C$\underline{}$, 17), 197.2 (C$\underline{}$, 20).

Mass Spectrum: (ESI+), *m/z*, 329.44 $[M + H]^+$, 351.44 $[M + Na]^+$, 297.45 $[C_{21}H_{29}O]^+$.

IR Spectrum: 1656 cm^{-1} (C=O stretch), 1096 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 329.2496, calculated for $C_{22}H_{32}O_2$ $[M + H]^+$ = 329.2481.

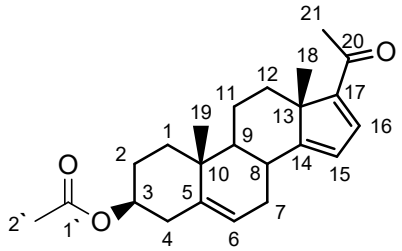
5.2.4: Compound 406



1-((1a*R*,3a*R*,5a*S*,10*R*,10a*R*)-10-methoxy-3a,5a-dimethyl-1,1a,2,3,3a,3b,4,5,5a,8b,9,10-dodecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

NBS (3.20 g, 17.96 mmol) & AIBN (20 mg) were added to a solution of **403** (2.95 g, 8.98 mmol) in EtOAc (50 ml) and the resulting mixture was heated to reflux under nitrogen atmosphere for 1 hour. The reaction mixture was cooled to room temperature and solvent was removed under reduced pressure. The resulting residue was dissolved in diethyl ether (150 ml), filtered and solvent was removed under reduced pressure to afford **405**. Sodium iodide (6.0 g) was added to a solution of **405** in acetone (40 ml) and heated to reflux for 3.5 hours under nitrogen atmosphere. The acetone was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 (400 ml) and aqueous $Na_2S_2O_3$ solution until most of iodine was removed. Combined organic extract was dried over $MgSO_4$ and solvent was removed under reduced pressure to afford a brown solid. Resulting solid was subjected to column chromatography (3-5 % EtOAc in PE) to and afforded a pale yellow solid (1.35 g, 46 %) but it was not pure compound **406**.

5.2.5: Compound 409



(3*S*,10*R*,13*S*)-17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13-decahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate

To a solution of 16-dehydropregnenolone acetate **400** (10.0 g, 28.05 mmol) in dry ether (300 mL) was added anhydrous potassium acetate (15.0 g) in glacial acetic acid (130 mL) slowly by

stirring. The mixture was cooled to 0 ^{o}C in an ice bath and bromine (4.49 g, 28.05 mmol) in acetic acid (100 mL) was added dropwise over a period of 3-4 hours. The reaction mixture was stirred at 0 ^{o}C in an ice bath for further 2 hours and then at room temperature overnight. The reaction mixture was partitioned between diethyl ether (400 mL) and water (500 mL). Organic layer was then extracted with aqueous potassium carbonate (500 mL × 2). Combined organic extract was dried over $MgSO_4$ and solvent was removed under reduced pressure to afford the white foam **407**. It was used for the next step without further purification.

NBS (9.99 g, 56.10 mmol) & AIBN (40 mg) were added to a solution of **407** in EtOAc (150 ml) and the resulting mixture was heated to reflux under nitrogen atmosphere for 1 hour. The reaction mixture was cooled to room temperature and solvent was removed under reduced pressure. The resulting residue was dissolved in diethyl ether (500 ml), filtered and solvent was removed under reduced pressure to afford white foam **408**. Sodium iodide (20.0 g) was added to a solution of **408** in acetone (120 ml) and heated to reflux for 3.5 hours under nitrogen atmosphere. The acetone was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 (400 ml) and aqueous $Na_2S_2O_3$ solution until most of iodine was removed. Combined organic extract was dried over $MgSO_4$ and solvent was removed under reduced pressure to afford brown solid. Resulting solid was subjected to column chromatography (5-10 % EtOAc in PE) to afford compound **409** (3.92 g, 39 %) as a pale yellow solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.81 (1H, td, *J* = 12.9, 5.1 Hz, C$\underline{H}_2$ - 12), 1.04 (1H, td, *J* = 11.4, 5.3 Hz, C$\underline{H}$ - 9), 1.09 – 1.20 (1H, m, C$\underline{H}_2$ - 1), 1.14 (3H, s, CC$\underline{H}_3$ - 19), 1.19 (3H, s, CC$\underline{H}_3$ - 18), 1.56 - 1.70 (3H, m, C$\underline{H}_2$ – 11, C$\underline{H}_2$ - 2), 1.83 - 1.93 (2H, m, C$\underline{H}_2$ - 2, C$\underline{H}_2$ - 1), 2.03 (3H, s, CC$\underline{H}_3$ - 2'), 2.18 – 2.27 (2H, m, C$\underline{H}_2$ - 7), 2.32 (3H, m, CC$\underline{H}_3$ – 21), 2.35 (2H, dd, *J* = 12.1, 3.4 Hz, C$\underline{H}_2$ - 4), 2.43 – 2.53 (2H, m, C$\underline{H}$ - 8, C$\underline{H}_2$ - 12), 4.60 (1H, ddd, *J* = 10.8, 8.4, 4.7 Hz, C$\underline{H}$ - 3), 5.45 – 5.49 (1H, m, C$\underline{H}$ - 6), 6.02 (1H, t, *J* = 2.1 Hz, C$\underline{H}$ - 15), 7.22 (1H, d, *J* = 2.3 Hz, C$\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 18.7 (C$\underline{C}H_3$, 18), 19.8 (C$\underline{C}H_3$, 19), 21.0 ($\underline{C}H_2$, 11), 21.7 (CC$\underline{H}_3$ - 2'), 27.0 (CC$\underline{H}_3$ - 21), 28.0 ($\underline{C}H_2$, 2), 29.2 ($\underline{C}H_2$, 7), 32.6 ($\underline{C}$H, 8), 35.9 ($\underline{C}H_2$, 12), 37.6 ($\underline{C}H_2$, 1), 37.8 ($\underline{C}$H, 10), 38.3 ($\underline{C}H_2$, 4), 53.8 ($\underline{C}$, 13), 54.3 ($\underline{C}$H, 9), 74.0 ($\underline{C}$H, 3), 119.5 ($\underline{C}$H, 15), 122.0 ($\underline{C}$H, 6), 139.7 ($\underline{C}$, 5), 142.1 ($\underline{C}$H, 16), 155.1 ($\underline{C}$, 17), 170.8 ($\underline{C}$ - 1'), 173.3 ($\underline{C}$ - 14), 192.9 ($\underline{C}$ - 20).

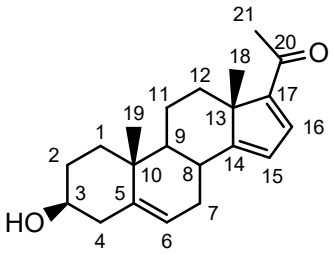
Mass Spectrum: (ESI+), *m/z*, 355.34 $[M + H]^+$, 377.34 $[M + Na]^+$, 295.35 $[C_{21}H_{27}O]^+$, 731.37 $[Mx2 + Na]^+$.

IR Spectrum: 1727 cm^{-1} (C=O stretch), 1032 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 355.2292, calculated for $C_{23}H_{31}O_3$ $[M + H]^+$ = 355.2273.

In agreement with previously reported literature for this compound.[158]

5.2.6: Compound 410



1-((3*S*,10*R*,13*S*)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13-decahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethanone

To a stirred solution of **409** (3.92 g, 11.06 mmol) in t-butanol (50 ml) was added KOH (3.10 g, 5 eq.) in H_2O (5 ml). The resulting mixture was stirred at 30 °C overnight. Solvent was removed under reduced pressure and water (300 ml) was added. The resulting suspension was filtered and washed with water. The solid was dried under high vacuum overnight to afford compound **410** (3.45 g, 100 %) as an off-white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.81 (1H, td, *J* = 12.8, 5.0 Hz, C$\underline{H}_2$ - 12), 1.04 (2H, ddd, *J* = 22.9, 12.5, 4.6 Hz, C$\underline{H}$ – 9, C$\underline{H}_2$ - 1), 1.13 (3H, s, CC$\underline{H}_3$ - 19), 1.19 (3H, s, CC$\underline{H}_3$ - 18), 1.47 - 1.72 (3H, m, C$\underline{H}_2$ – 2, C$\underline{H}_2$ - 11), 1.80 - 1.94 (2H, m, C$\underline{H}_2$ - 2, C$\underline{H}_2$ - 1), 2.14 – 2.38 (4H, m, C$\underline{H}_2$ – 4, C$\underline{H}_2$ - 7), 2.32 (3H, m, CC$\underline{H}_3$ – 21), 2.48 (2H, ddd, *J* = 9.9, 6.5, 3.2 Hz, C$\underline{H}$ – 8, C$\underline{H}_2$ - 12), 3.53 (1H, ddd, *J* = 16.0, 11.1, 4.9 Hz, C$\underline{H}$ - 3), 5.45 (1H, d, *J* = 2.4 Hz, C$\underline{H}$ - 6), 6.01 (1H, d, *J* = 1.8 Hz, C$\underline{H}$ - 15), 7.22 (1H, d, *J* = 2.2 Hz, C$\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 18.7 (C$\underline{C}H_3$, 18), 19.8 (C$\underline{C}H_3$, 19), 21.0 (C$\underline{H}_2$, 11), 27.0 (CC$\underline{H}_3$ - 21), 29.2 (C$\underline{H}_2$, 7), 31.9 (C$\underline{H}_2$, 2), 32.7 (C$\underline{H}$, 8), 36.0 (C$\underline{H}_2$, 12), 37.7 (C$\underline{}$, 10), 37.8 (C$\underline{H}_2$, 1), 42.5 (C$\underline{H}_2$, 4), 53.8 (C$\underline{}$, 13), 54.4 (C$\underline{H}$, 9), 72.0 (C$\underline{H}$, 3), 119.4 (C$\underline{H}$, 15), 121.1 (C$\underline{H}$, 6), 140.8 (C$\underline{}$, 5), 142.2 (C$\underline{H}$, 16), 155.1 (C$\underline{}$, 17), 173.6 (C$\underline{}$ - 14), 193.0 (C$\underline{}$ - 20).

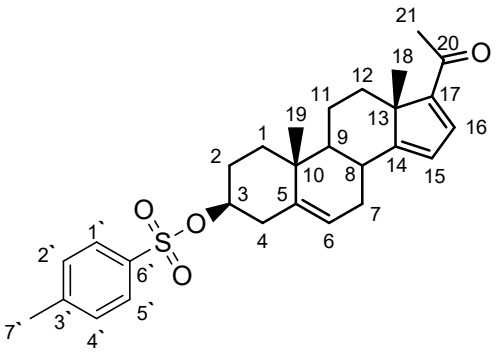
Mass Spectrum: (ESI+), *m/z*, 313.34 $[M + H]^+$, 335.34 $[M + Na]^+$, 295.35 $[C_{21}H_{27}O]^+$.

IR Spectrum: 3425 cm^{-1} (OH stretch), 1631 cm^{-1} (C=O stretch), 1027 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 313.2184, calculated for $C_{21}H_{29}O_2$ $[M + H]^+$ = 313.2168.

In agreement with previously reported literature for this compound.[158]

5.2.7: Compound 411



(3*S*,10*R*,13*S*)-17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13-decahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 4-methylbenzenesulfonate

Tosyl chloride (2.39 g, 12.55 mmol) was added to a stirred solution of **410** (1.31 g, 4.18 mmol) in pyridine (10 ml) at room temperature. After 28 hours, water (300 ml) was added. The organic mixture was extracted with CH_2Cl_2 (50 ml × 3). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to afford compound **411** (1.62 g, 83 %) as an off-white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.78 (1H, td, *J* = 13.1, 4.3 Hz, C$\underline{H}_2$ - 12), 0.93 – 1.08 (1H, m, C$\underline{H}$ – 9, C$\underline{H}_2$ - 1), 1.09 (3H, s, CC$\underline{H}_3$ - 19), 1.18 (3H, s, CC$\underline{H}_3$ - 18), 1.49 - 1.65 (2H, m, C$\underline{H}_2$ – 11), 1.70 - 1.89 (3H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ - 7), 2.11 – 2.39 (3H, m, C$\underline{H}_2$ – 2, C$\underline{H}_2$ - 4), 2.32 (3H, m, CC$\underline{H}_3$ – 21), 2.39 – 2.53 (3H, m, C$\underline{H}_2$ – 4, C$\underline{H}$ – 8, C$\underline{H}_2$ - 12), 4.39 – 4.28 (1H, m, C$\underline{H}$ - 3), 5.38 – 5.48 (1H, m, C$\underline{H}$ - 6), 6.00 (1H, app. t, *J* = 2.1 Hz, C$\underline{H}$ - 15), 7.21 (1H, d, *J* = 2.4 Hz, C$\underline{H}$ - 16), 7.33 (2H, d, *J* = 8.2 Hz, 2' & 4'), 7.86 (2H, d, *J* = 8.5 Hz, 1' & 5').

^{13}C NMR: (101 MHz, $CDCl_3$) δ 18.7 (C$\underline{C}H_3$, 18), 19.6 (C$\underline{C}H_3$, 19), 21.0 ($\underline{C}H_2$, 11), 22.0 (CC$\underline{H}_3$ - 7'), 27.0 ($\underline{C}H_3$, 21), 28.8 ($\underline{C}H_2$, 7), 29.2 ($\underline{C}H_2$, 2), 32.5 ($\underline{C}H$, 8), 35.9 ($\underline{C}H_2$, 12), 37.5 ($\underline{C}H_2$, 1), 37.6 ($\underline{C}$, 10), 39.2 ($\underline{C}H_2$, 4), 53.8 ($\underline{C}$ - 13), 54.2 ($\underline{C}H$, 9), 82.3 ($\underline{C}H$, 3), 119.6 ($\underline{C}H$, 15), 123.0 ($\underline{C}H$, 6), 128.0

(CH, 1' & 5'), 130.1 (CH - 2' & 4'), 135.0 (C - 3'), 139.0 (C - 5), 142.0 (CH, 16), 144.8 (C - 6'), 155.2 (C, 17), 173.0 (C - 14), 193.0 (C - 20).

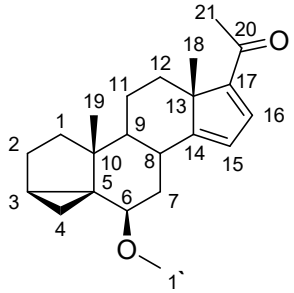
Mass Spectrum: (ESI+), *m/z*, 467.43 $[M + H]^+$, 295.45 $[C_{21}H_{27}O]^+$.

IR Spectrum: 1640 cm^{-1} (C=O stretch), 1342 & 1166 cm^{-1} (S=O stretch), 932-810 cm^{-1} (S-O stretch).

HRMS: (ESI+), *m/z*, found = 467.2283, calculated for $C_{28}H_{35}O_4S$ $[M + H]^+$ = 467.2256.

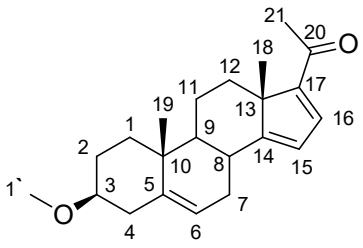
Previously unreported.

5.2.8: Compound 406 and 412



1-((1a*R*,3a*R*,5a*S*,10*R*,10a*R*)-10-methoxy-3a,5a-dimethyl-1,1a,2,3,3a,3b,4,5,5a,8b,9,10-dodecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

406



1-((3*S*,10*R*,13*S*)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13-decahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)ethanone

412

Pyridine (0.82 ml, 10.17 mmol) was added to a stirred solution of compound **411** (1.58 g, 3.39 mmol) in anhydrous methanol (10 ml) at room temperature and the mixture was heated to reflux for 4 hours. The reaction mixture was cooled to room temperature and extracted with saturated $NaHCO_3$ solution (300 ml) and CH_2Cl_2 (200 ml × 3). The organic

extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (3-5 % EtOAc in PE) afforded compound **406** (928 mg, 84 %) as an off-white solid and compound **412** (127 mg, 12 %) as a pale yellow solid.

Compound 406

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.50 (1H, dd, *J* = 8.0 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.68 (1H, app. t, 4.4 Hz C$\underline{H}_2$ - 4), 0.74 – 1.01 (4H, m, CH2 - 12, C$\underline{H}$ – 9, C$\underline{H}$ – 3, C$\underline{H}_2$ - 1), 1.14 (3H, s, CC$\underline{H}_3$ - 19), 1.22 (3H, s, CC$\underline{H}_3$ - 18), 1.40 - 1.78 (6H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ – 11, C$\underline{H}_2$ – 7, C$\underline{H}_2$ – 2), 2.21 (4H, dt, *J* = 13.3, 2.9 Hz, C$\underline{H}_2$ - 7), 2.31 (3H, s, CC$\underline{H}_3$ - 21), 2.47 (1H, dt, *J* = 3.1, 3.1 C$\underline{H}_2$ - 12), 2.75 (1H, app. t, *J* = 11.63 Hz, C$\underline{H}$ - 8), 2.91 (1H, t, *J* = 2.8 Hz, C$\underline{H}$ – 6), 3.37 (3H, s, OC$\underline{H}_3$ – 1'), 5.98 (1H, app. t, *J* = 2.02 Hz, C$\underline{H}$ - 15), 7.22 (1H, d, J = 2.3 Hz, C$\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.6 (C$\underline{H}_2$, 4), 19.2 (C$\underline{H}_3$, 18), 19.7 (C$\underline{H}_3$, 19), 21.8 (C$\underline{H}$, 3), 23.0 (C$\underline{H}_2$, 11), 25.2 (C$\underline{H}_2$, 2), 27.0 (C$\underline{H}_3$, 21), 31.9 (C$\underline{H}$, 8), 33.4 (C$\underline{H}_2$, 7), 34.2 (C$\underline{H}_2$, 1), 35.2 (C$\underline{}$, 10), 36.8 (C$\underline{H}_2$, 12), 44.4 (C$\underline{}$, 5), 52.6 (C$\underline{H}$, 9), 54.1 (C$\underline{}$, 13), 57.1 (C$\underline{H}_3$, 1'), 82.3 (C$\underline{H}$, 6), 118.4 (C$\underline{H}$, 15), 142.2 (C$\underline{H}$, 16), 155.1 (C$\underline{}$, 17), 174.9 (C$\underline{}$, 14) 192.9 (C$\underline{}$, 20).

Mass Spectrum: (ESI+), *m/z*, 327.35 $[M + H]^+$, 349.34 $[M + Na]^+$, 295.34 $[M - OMe]^+$, 675.49 $[M\times2 + Na]^+$.

IR Spectrum: 1637 cm^{-1} (C=O stretch), 1084 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 327.2339, calculated for $C_{22}H_{31}O_2$ $[M + H]^+$ = 327.2324.

In agreement with previously reported literature for this compound.[159]

Compound 412

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.81 (1H, td, *J* = 12.8, 5.1 Hz, C$\underline{H}_2$ - 12), 0.97 – 1.10 (2H, m, C$\underline{H}$ – 9, C$\underline{H}_2$ - 1), 1.12 (3H, s, CC$\underline{H}_3$ - 19), 1.20 (3H, s, CC$\underline{H}_3$ - 18), 1.38 - 1.52 (1H, m, C$\underline{H}_2$ – 2), 1.56 - 1.71 (2H, m, C$\underline{H}_2$ - 11), 1.88 (1H, t, *J* = 3.4 Hz, C$\underline{H}_2$ – 1), 1.94 (1H, dd, *J* = 9.8, 7.0 Hz, C$\underline{H}_2$ – 1), 2.14 – 2.24 (3H, m, C$\underline{H}_2$ – 4, C$\underline{H}_2$ - 7), 2.32 (3H, C$\underline{H}_3$ - 21), 2.37 – 2.53 (3H, m, C$\underline{H}_2$ – 4, C$\underline{H}$ – 8, C$\underline{H}_2$ – 12), 3.00 - 3.11 (1H, m, C$\underline{H}$ – 3), 3.35 (3H, s, OC$\underline{H}_3$ – 1'), 5.45 (1H, app. d, *J* = 4.9 Hz, C$\underline{H}$ - 6), 6.01 (1H, app .t, *J* = 2.0 Hz, C$\underline{H}$ - 15), 7.22 (1H, d, *J* = 2.3 Hz, C$\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 18.7 (C$\underline{C}H_3$, 18), 19.8 (C$\underline{C}H_3$, 19), 21.0 (C$\underline{H}_2$, 11), 27.0 (CC$\underline{H}_3$ - 21), 28.2 (C$\underline{H}_2$, 2), 29.3 (C$\underline{H}_2$, 7), 32.7 (C$\underline{H}$, 8), 36.1 (C$\underline{H}_2$, 12), 37.8 (C$\underline{H}_2$, 1), 38.2 (C$\underline{}$, 10), 39.0

($\underline{C}H_2$, 4), 53.9 ($\underline{C}$, 13), 54.6 ($\underline{C}H$, 9), 56.0 ($\underline{C}H_3$ - 1'), 80.5 ($\underline{C}H$, 3), 119.4 ($\underline{C}H$, 15), 121.0 ($\underline{C}H$, 6), 141.0 ($\underline{C}$, 5), 142.1 ($\underline{C}H$, 16), 155.1 ($\underline{C}$, 17), 173.6 ($\underline{C}$ - 14), 192.6 ($\underline{C}$ - 20).

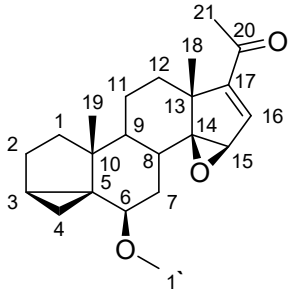
Mass Spectrum: (ESI+), *m/z*, 327.44 $[M + H]^+$, 349.34 $[M + Na]^+$, 295.35 $[C_{21}H_{27}O]^+$.

IR Spectrum: 1637 cm^{-1} (C=O stretch), 1057 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 327.2341, calculated for $C_{22}H_{31}O_2$ $[M + H]^+$ = 327.2324.

Previously unreported.

5.2.9: Compound 417



1-((2a*R*,4b*R*,6a*R*,7a*R*,8*R*,9b*S*,10a*R*)-8-methoxy-2a,4b-dimethyl-3,4,4a,4b,5,6,6a,7,8,9,9a,10a-dodecahydro-2a*H*-cyclopropa[1',7a']indeno[4',5':6,7]indeno[1,7a-*b*]oxiren-2-yl)ethanone

m-CPBA (75 %, 520 mg, 3.01 mmol) was added to a stirred solution of compound **406** (740 mg, 2.27 mmol) in $CHCl_3$ (25 ml) at room temperature. After 2.5 hours, $Na_2S_2O_3$ aqueous solution (0.5 M, 50 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (100 ml × 3) and the combined organic extracts were washed with saturated $NaHCO_3$ aqueous solution (100 ml). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (5 – 10 % EtOAc in PE) afforded epoxide **417** (632 mg, 81 %) as a white solid as well as unreacted compound **406** (73 mg, 10 %).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.41 (1H, dd, *J* = 8.0 & 5.2 Hz, $C\underline{H}_2$ - 4), 0.59 (1H, app. t, *J* = 4.4 Hz, $C\underline{H}_2$ - 4), 0.75 – 1.06 (6H, m, $C\underline{H}_2$ - 1, $C\underline{H}$ – 3, CH_2 – 11, $C\underline{H}_2$ – 7, $C\underline{H}$ – 8, $C\underline{H}_2$ - 12), 0.99 (3H, s, $CC\underline{H}_3$ - 19), 1.28 (3H, s, $CC\underline{H}_3$ - 18), 1.29 - 1.53 (3H, m, $C\underline{H}_2$ - 11, $C\underline{H}_2$ – 1, $C\underline{H}_2$ – 2), 1.67 (1H, tdd, *J* = 12.1, 7.9, 4.2, $C\underline{H}_2$ - 2), 1.93 (1H, dt, *J* = 13.2, 2.9 Hz, $C\underline{H}_2$ - 7), 2.18 (3H, s, $C\underline{H}_3$ - 21), 2.34 (1H, dt, *J* = 3.3, 3.3 Hz, $C\underline{H}_2$ – 12), 2.40 (1H, td, *J* = 12.1, 2.7 Hz, $C\underline{H}$ - 9), 2.73 (1H, t, *J* = 2.8 Hz, $C\underline{H}$ – 6), 3.24 (3H, s, $OC\underline{H}_3$ – 1'), 3.69 (1H, d, *J* = 0.7 Hz, $C\underline{H}$ - 15), 6.82 (1H, s, $C\underline{H}$ - 16).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.1 ($\underline{C}H_2$, 4), 16.9 ($\underline{C}H_3$, 18), 19.2 ($\underline{C}H_3$, 19), 21.6 ($\underline{C}H_2$, 11), 21.8 ($\underline{C}H$, 3), 24.9 ($\underline{C}H_2$, 2), 27.4 ($\underline{C}H_3$, 21), 28.5 ($\underline{C}H$, 9), 29.9 ($\underline{C}H_2$, 7), 33.4 ($\underline{C}H_2$, 1), 35.2 ($\underline{C}$, 10), 36.7 ($\underline{C}H_2$, 12), 43.8 ($\underline{C}$, 5), 46.7 ($\underline{C}H$, 8), 48.1 ($\underline{C}$, 13), 56.6 ($\underline{C}H_3$, 1'), 59.2 ($\underline{C}H$, 15), 74.7 ($\underline{C}$, 14), 81.6 (CH, 6), 140.5 (CH, 16), 158.4 (C, 17) 196.1 ($\underline{C}$, 20).

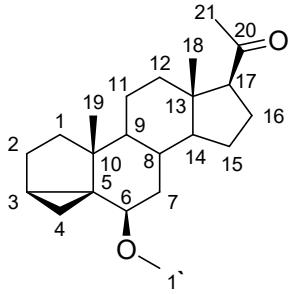
Mass Spectrum: (ESI+), *m/z*, 343.34 $[M + H]^+$, 365.34 $[M + Na]^+$, 311.34 $[C_{21}H_{27}O_2]^+$, 707.38 $[Mx2 + Na]^+$.

IR Spectrum: 1665 cm^{-1} (C=O stretch), 1089 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 343.2286, calculated for $C_{22}H_{31}O_3$ $[M + H]^+$ = 343.2273.

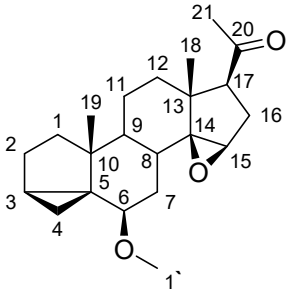
Previously unreported.

5.2.10: Compound 418, 419 and 420



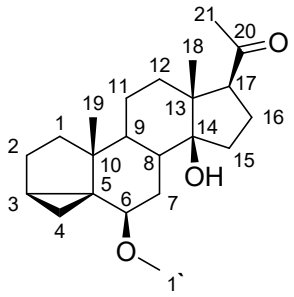
1-((1a*R*,3a*R*,5a*S*,6*S*,10*R*,10a*R*)-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

418



1-((2*S*,2a*R*,4b*R*,6a*R*,7a*R*,8*R*,9b*S*,10a*R*)-8-methoxy-2a,4b-dimethyltetradecahydro-1*H*-cyclopropa[1',7a']indeno[4',5':6,7]indeno[1,7a-*b*]oxiren-2-yl)ethanone

419



1-((1a*R*,3a*R*,5a*R*,6*S*,8a*S*,10*R*,10a*R*)-8a-hydroxy-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

420

To a solution of epoxide **417** (200 mg, 0.58 mmol) in methanol (7 ml) was added Pd/C (5%, 30 mg) and stirred under H_2 (g) overnight. The reaction mixture was filtered and concentrated. The residue was subjected to column chromatography (3-40 % EtOAc in PE) to afford compound **418** as a pale yellow oil (33 mg, 17 %), compound **419** as a clear oil (68 mg, 34 %) and compound **420** as a white solid (75 mg, 37 %).

Compound 418

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 8.0 & 5.1 Hz, C$\underline{H}_2$ - 4), 0.64 (1H, *J* = 9.7, 5.1 Hz, C$\underline{H}_2$ - 4), 0.77 – 0.93 (2H, m, C$\underline{H}$ – 3, C$\underline{H}_2$ - 1), 0.99 (3H, s, CC$\underline{H}_3$ - 19), 0.99 – 1.82 (13H, m, C$\underline{H}$ - 9, C$\underline{H}_2$ - 7, C$\underline{H}$ - 8, C$\underline{H}_2$ - 11, C$\underline{H}_2$ - 1, C$\underline{H}_2$ - 2, C$\underline{H}$ - 14, C$\underline{H}_2$ - 12, C$\underline{H}_2$ - 15), 1.27 (3H, s, CC$\underline{H}_3$ – 18), 2.02 - 2.18 (3H, m, C$\underline{H}_2$ - 7, C$\underline{H}_2$ - 16), 2.13 (3H, s, CC$\underline{H}_3$ - 21), 2.75 (2H, t, *J* = 9.2 Hz, C$\underline{H}$ - 17), 2.79 (1H, t, *J* = 2.8 Hz, C$\underline{H}$ - 6), 3.33 (3H, s, OC$\underline{H}_3$ – 1').

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.6 (C$\underline{H}_2$, 4), 19.4 (C$\underline{H}_3$, 19), 21.7 (C$\underline{H}$, 3), 23.1 (C$\underline{H}_2$, 11), 23.3 (C$\underline{H}_2$, 16), 23.6 (C$\underline{H}_2$, 15), 24.9 (C$\underline{H}_3$, 18), 25.3 (C$\underline{H}_2$, 2), 28.6 (C$\underline{H}$, 12), 29.0 (C$\underline{H}_2$, 7), 32.2 (C$\underline{H}_3$, 21), 33.9 (C$\underline{H}_2$, 1), 35.3 (C$\underline{}$, 5), 36.0 (C$\underline{H}_2$, 8), 40.0 (C$\underline{H}$, 9), 43.6 (C$\underline{}$, 10), 44.6 (C$\underline{}$, 13), 52.7 (C$\underline{H}$, 14), 57.0 (C$\underline{H}_3$, 1'), 64.9 (C$\underline{H}$, 17), 82.9 (C$\underline{H}$, 6), 210.6 (C$\underline{}$, 20).

Mass Spectrum: (ESI+), *m/z*, 331.44 $[M + H]^+$, 353.34 $[M + Na]^+$, 299.34 $[C_{21}H_{31}O]^+$, 683.59 [MX2 + Na] $^+$.

IR Spectrum: 1705 cm^{-1} (C=O stretch), 1100 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 331.2651, calculated for $C_{22}H_{35}O_2$ $[M + H]^+$ = 331.2637.

Previously unreported.

Compound 419

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.43 (1H, dd, *J* = 8.0 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.61 (1H, app. t, *J* = 4.4 Hz, C$\underline{H}_2$ - 4), 0.77 – 1.05 (4H, m, C$\underline{H}_2$ - 1, C$\underline{H}$ – 3, C$\underline{H}_2$ – 7, C$\underline{H}$ – 8), 1.02 (3H, s, CC$\underline{H}_3$ - 19), 1.09 – 1.26 (2H, m, C$\underline{H}_2$ - 12), 1.36 (3H, s, CC$\underline{H}_3$ - 18), 1.28 – 1.56 (4H, m, C$\underline{H}_2$ – 1, C$\underline{H}_2$ – 11, C$\underline{H}_2$ - 2), 1.71 (1H, tdd, *J* = 12.1, 7.9, 4.3 Hz, C$\underline{H}_2$ - 2), 1.88 – 1.98 (2H, m, C$\underline{H}_2$ – 7, C$\underline{H}_2$ - 16), 2.11 (3H, s, C$\underline{H}_3$ - 21), 2.06 – 2.18 (1H, m, C$\underline{H}_2$ - 16), 2.38 (1H, td, *J* = 12.0, 2.7 Hz, C$\underline{H}$ - 9), 2.64 (1H, dd, *J* = 10.2, 7.5 Hz, C$\underline{H}$ - 17), 2.75 (1H, t, *J* = 2.9 Hz, C$\underline{H}$ – 6), 3.28 (3H, s, OC$\underline{H}_3$ – 1'), 3.35 (1H, s, C$\underline{H}$ - 15).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.4 (C$\underline{H}_2$, 4), 19.5 (C$\underline{H}_3$, 19), 20.0 (C$\underline{H}_3$, 18), 22.1 (C$\underline{H}$, 3), 22.2 (C$\underline{H}_2$, 11), 25.2 (C$\underline{H}_2$, 2), 27.8 (C$\underline{H}_2$, 16), 28.5 (C$\underline{H}$, 9), 29.9 (C$\underline{H}_2$, 7), 31.5 (C$\underline{H}_2$, 12), 32.1 (C$\underline{H}_3$, 21), 33.7 (C$\underline{H}_2$, 1), 35.5 (C$\underline{}$, 10), 43.3 (C$\underline{}$, 13), 43.9 (C$\underline{}$, 5), 47.2 (C$\underline{H}$, 8), 56.9 (C$\underline{H}_3$, 1'), 57.7 (C$\underline{H}$, 17), 58.4 (CH, 15), 73.1 (C, 14), 81.9 (CH, 6) 208.8 (C$\underline{}$, 20).

Mass Spectrum: (ESI+), *m/z*, 367.34 $[M + Na]^+$, 313.34 $[C_{21}H_{29}O_2]^+$, 711.48 $[Mx2 + Na]^+$.

IR Spectrum: 1704 cm^{-1} (C=O stretch), 1091 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 345.2450, calculated for $C_{22}H_{33}O_3$ $[M + H]^+$ = 345.2430.

Previously unreported.

Compound 420

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.41 (1H, dd, *J* = 8.0 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.59 (1H, app. t, *J* = 4.4 Hz, C$\underline{H}_2$ - 4), 0.82 (4H, tdd, *J* = 11.8, 7.8, 3.6 Hz, C$\underline{H}_2$ - 1, C$\underline{H}$ – 3), 0.94 (3H, s, CC$\underline{H}_3$ - 19), 0.97 (2H, dd, *J* = 12.6, 3.9 Hz, C$\underline{H}$ - 8), 1.10 – 1.22 (3H, m, C$\underline{H}_2$ – 7, C$\underline{H}_2$ - 12),1.18 (3H, s, CC$\underline{H}_3$ - 18), 1.29 – 1.53 (6H, m, C$\underline{H}_2$ – 1, C$\underline{H}_2$ – 11, C$\underline{H}_2$ – 16, C$\underline{H}_2$ – 15, C$\underline{H}_2$ - 2), 1.62 – 1.97 (3H, m, C$\underline{H}_2$ – 2, C$\underline{H}$ – 9, C$\underline{H}_2$ - 15), 2.04 (1H, dd, *J* = 9.0, 3.7 Hz, C$\underline{H}_2$ – 16), 2.08 (3H, s, C$\underline{H}_3$ - 21), 2.14 (1H, dt, *J* = 13.6, 2.9 Hz, C$\underline{H}_2$ – 7), 2.79 (1H, t, *J* = 2.8 Hz, C$\underline{H}$ – 6), 3.20 (1H, t, *J* = 9.3 Hz, C$\underline{H}$ - 17) 3.27 (3H, s, OC$\underline{H}_3$ – 1').

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.3 (C$\underline{H}_2$, 4), 19.2 (C$\underline{H}_3$, 19), 19.2 (C$\underline{H}_3$, 18), 21.4 (C$\underline{H}$, 3), 21.6 (C$\underline{H}_2$, 11), 24.8 (C$\underline{H}_2$, 16), 24.9 (C$\underline{H}_2$, 2), 30.6 (C$\underline{H}_2$, 7), 30.7 (C$\underline{H}_2$, 15), 31.1 (C$\underline{H}_2$, 12), 32.0 (C$\underline{H}_3$, 21), 33.6 (C$\underline{H}_2$, 1), 34.8 (C$\underline{}$, 10), 36.5 (C$\underline{H}$, 9), 43.6 (C$\underline{}$, 5), 43.7 (C$\underline{H}$, 8), 48.4 (C$\underline{}$, 13), 56.6 (C$\underline{H}_3$, 1'), 61.4 (CH, 17), 82.2 (CH, 6), 86.4 (C14) 210.6 (C$\underline{}$, 20).

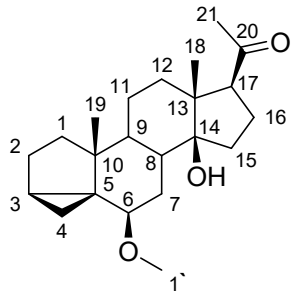
Mass Spectrum: (ESI+), *m/z*, 369.34 [M + Na] $^{+}$, 315.34 [$C_{21}H_{31}O_2$] $^{+}$, 297.35 [$C_{21}H_{30}O$] $^{+}$, 715.48 [Mx2 + Na] $^{+}$.

IR Spectrum: 3449 cm^{-1} (OH stretch), 1703 cm^{-1} (C=O stretch), 1075 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 369.2422, calculated for $C_{22}H_{34}O_3$ [M + Na] $^{+}$ = 369.2406.

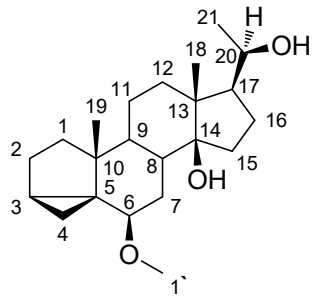
Previously unreported.

5.2.11: Compound 420, 421a and 421b



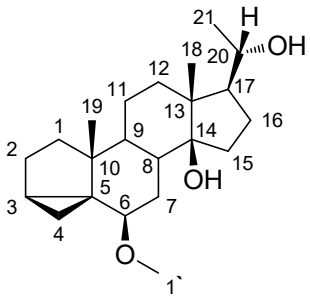
1-((1a*R*,3a*R*,5a*R*,6*S*,8a*S*,10*R*,10a*R*)-8a-hydroxy-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

420



(1a*R*,3a*R*,5a*R*,6*S*,8a*S*,10*R*,10a*R*)-6-((*R*)-1-hydroxyethyl)-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-8a-ol

421a



(1a*R*,3a*R*,5a*R*,6*S*,8a*S*,10*R*,10a*R*)-6-((*S*)-1-hydroxyethyl)-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-8a-ol

421b

The epoxide **417** (200 mg, 0.58 mmol) was added to dry THF (15 ml) containing $LiAlH_4$ (200 mg) and the mixture was refluxed under N_2 for 1 hour. The reaction mixture was cooled to room temperature and filtered. Solvent was removed under reduced pressure and residue extracted with diethyl ether (30 ml × 3) and water (100 ml). The residue was purified by column chromatography (5-40 % EtOAc in PE) to afford compound **420** (84 mg, 42 %) and a mixture of **421a** and **421b** (47 mg, 23 %).

To a solution of compound **420** (84 mg, 0.24 mmol) in methanol (5 ml) was added $NaBH_4$ (92 mg) and stirred at room temperature for 30 minutes. Water (100 ml) was added to the reaction mixture and extracted with 1M HCl (10 ml) and CH_2Cl_2 (20 ml × 3) to afford a mixture of **421a** and **421b** (63 mg, 75 %) as a clear gummy solid.

Compound 421a and 421b

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.46 (1H, dd, *J* = 8.0 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.64 (1H, app. t, *J* = 4.4 Hz, C$\underline{H}_2$ - 4), 0.84 – 1.05 (5H, m, C$\underline{H}_2$ - 1, C$\underline{H}$ – 3, C$\underline{H}$ – 8, C$\underline{H}_2$ – 12, C$\underline{H}_2$ - 7), 1.01 (3H, s, CC$\underline{H}_3$ - 19), 1.16 (3H, s, CC$\underline{H}_3$ – 21 R/S), 1.18 (3H, s, CC$\underline{H}_3$ – 21 R/S), 1.19 (3H, s, CC$\underline{H}_3$ – 18), 1.20 – 1.36 (1H, m, C$\underline{H}_2$ – 2), 1.39 – 1.57 (2H, m, C$\underline{H}_2$ – 1, C$\underline{H}_2$ - 2), 1.65 (1H, dt, *J* = 13.0, 3.2 Hz, C$\underline{H}_2$ – 12), 1.70 – 1.93 (4H, m, C$\underline{H}_2$ – 15, C$\underline{H}_2$ - 16), 2.017(1H, q, *J* = 9.5 Hz, C$\underline{H}$ - 17), 2.23 (1H, dt, *J* = 13.5, 2.9 Hz, C$\underline{H}_2$ – 7), 2.84 (1H, t, *J* = 2.7 Hz, C$\underline{H}$ – 6), 3.33 (3H, s, OC$\underline{H}_3$ – 1'), 3.73 (1H, dq, *J* = 10.3, 6.1 Hz, CH – 20 R/S), 3.87 (1H, p, *J* = 6.2 Hz, CH – 20 R/S), 5.29 (1H, s, OH).

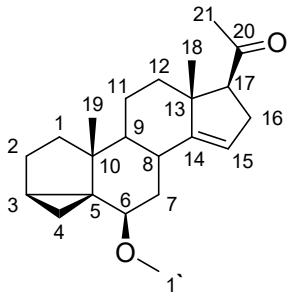
^{13}C NMR (101 MHz, $CDCl_3$) δ 13.6 (C$\underline{H}_2$, 4), 19.6 (C$\underline{H}_3$, 19), 20.6 (C$\underline{H}_3$, 18), 21.9 (C$\underline{H}$, 3), 22.0 (C$\underline{H}_2$, 11), 24.4 (C$\underline{H}_2$, 16), 24.5 (C$\underline{H}_3$, 21), 25.3 (C$\underline{H}_2$, 2), 30.5 (C$\underline{H}_2$, 12), 30.6 (C$\underline{H}_2$, 7 & 15), 33.9 (C$\underline{H}_2$, 1), 35.4 (C$\underline{}$, 10), 36.6 (C$\underline{H}$, 9), 44.0 (C$\underline{}$, 5), 44.3 (C$\underline{H}$, 8), 47.1 (C$\underline{}$, 13), 56.2 (C$\underline{H}$, 17), 56.9 (C$\underline{H}_3$, 1'), 68.4 (CH, 20 R/S), 70.1 (CH, 20 R/S), 82.7 (CH - 6) 86.8 (C$\underline{}$, 14).

Mass Spectrum (ESI+), *m/z*, 371.44 $[M + Na]^+$.

IR Spectrum: 1705 cm^{-1} (C=O stretch), 1076 cm^{-1} (C-O stretch).

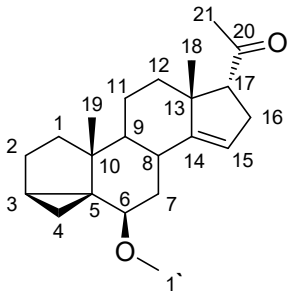
Previously unreported.

5.2.12: Compound 430 and 431



1-((1a*R*,3a*R*,5a*R*,6*S*,10*R*,10a*R*)-10-methoxy-3a,5a-dimethyl-1,1a,2,3,3a,3b,4,5,5a,6,7,8b,9,10-tetradecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

430



1-((1a*R*,3a*R*,5a*R*,6*R*,10*R*,10a*R*)-10-methoxy-3a,5a-dimethyl-1,1a,2,3,3a,3b,4,5,5a,6,7,8b,9,10-tetradecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

431

To a stirred solution of **406** (5.0 g, 15.31 mmol) in anhydrous toluene (80 ml) was added triphenyltin hydride (10.77 g, 30.63 mmol) & AIBN (30 mg) and the reaction mixture was refluxed under nitrogen for 10 hours with addition of AIBN (15 mg) every hour. Solvent was removed under reduced pressure and methanol was added to the resulting solid. Silica gel column chromatography (3 % EtOAc in PE) afforded mixture of **430** and **431** (1.94 g, 39 %) as a white solid as well as mixture of **430** and **431** and unreacted compound **406** (2.1 g, 42 %).

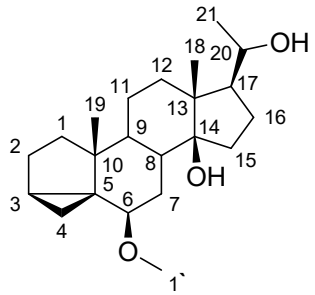
Mass Spectrum: (ESI+), *m/z*, 329.45 $[M + H]^+$, 351.45 $[M + Na]^+$, 679.49 $[M\times2 + Na]^+$, 297.45 $[M - OMe]^+$.

IR Spectrum: 1707 cm^{-1} (C=O stretch), 1090 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 329.2471, calculated for $C_{22}H_{33}O_2$ $[M + H]^+$ = 329.2480.

Previously unreported.

5.2.13: Compound 432



(1a*R*,3a*R*,5a*R*,6*S*,8a*S*,10*R*,10a*R*)-6-((*S*)-1-hydroxyethyl)-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-8a-ol

432a

To a stirred solution of **430** and **431** (1.04 g, 3.15 mmol) in methanol (30 ml) & CH_2Cl_2 (10 ml) was added $NaBH_4$ (238 mg 6.29 mmol) at room temperature. After 40 minutes, dilute HCl (200 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (100 ml × 3) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (5 % EtOAc in PE) afforded **432a** (602 mg, 58 %) as a white solid and an inseparable mixture of **432a**, **432b**, **432c & 432d** (330 mg, 32 %) as a white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.45 (1H, dd, *J* = 5.2 & 5.1 Hz, C$\underline{H}_2$ - 4), 0.65 (1H, app. t, *J* = 4.2 Hz, 4.5 Hz C$\underline{H}_2$ - 4), 0.80 – 0.93 (3H, m, C$\underline{H}_2$ - 9, C$\underline{H}$ – 1, C$\underline{H}$ – 3), 1.04 (3H, s, CC$\underline{H}_3$ - 19), 1.06 (3H, s, CC$\underline{H}_3$ - 18), 1.17 & 1.18 (3H, s, CC$\underline{H}_3$ - 21), 1.24 - 1.34 (1H, m, C$\underline{H}_2$ – 12), 1.36 - 1.55 (6H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ – 7, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 11), 1.70 - 1.80 (2H, m, C$\underline{H}$ - 17, C$\underline{H}_2$ – 2), 1.83 - 1.92 (1H, m, C$\underline{H}_2$ - 16), 2.05 – 2.20 (2H, m, C$\underline{H}_2$ – 12, C$\underline{H}_2$ – 16), 2.41 (1H, app. t, *J* = 11.9, 11.8 Hz, C$\underline{H}$ - 8), 2.84 (1H, t, *J* = 3.0 Hz, C$\underline{H}$ - 6), 3.34 (3H, s, OC$\underline{H}_3$ – 1'), 3.89 – 3.97 (1H, m, C$\underline{H}$ – 20), 5.14 (1H, app. q, *J* = 2.2 Hz, C$\underline{H}$ - 15).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.5 ($\underline{C}H_2$, 4), 18.9 ($\underline{C}H_3$, 18), 19.3 ($\underline{C}H_3$, 19), 21.8 ($\underline{C}H$, 3), 23.9 ($\underline{C}H_2$, 11), 24.0 ($\underline{C}H_3$, 21), 25.3 ($\underline{C}H_2$, 2), 30.5 ($\underline{C}H$, 8), 33.6 ($\underline{C}H_2$, 7), 34.0 ($\underline{C}H_2$, 1), 34.0 ($\underline{C}H_2$, 16), 35.2 ($\underline{C}$, 10), 43.2 ($\underline{C}H_2$, 12), 43.9 ($\underline{C}$, 5), 47.7 ($\underline{C}$, 13), 47.9 ($\underline{C}H$, 9), 57.0 ($\underline{C}H_3$, 1'), 61.1 ($\underline{C}H_2$, 17), 69.8 ($\underline{C}$, 20), 82.6 ($\underline{C}H$, 6), 116.8 ($\underline{C}H$, 15), 155.7 ($\underline{C}$, 14).

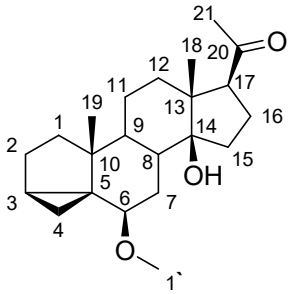
Mass Spectrum: (ESI+), *m/z*, 353.45 $[M + Na]^+$, 299.35 $[M - OMe]^+$, 281.35 $[C_{21}H_{29}]^+$.

IR Spectrum: 3425 cm^{-1} (OH stretch), 1084 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 299.2388, calculated for $C_{22}H_{33}O_2$ $[M – OMe]^+$ = 299.2375.

Previously unreported.

5.2.14: Compound 430



1-((1a*R*,3a*R*,5a*R*,6*S*,8a*S*,10*R*,10a*R*)-8a-hydroxy-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

To a stirred solution of **432a** (100 mg, 0.30 mmol) in CH_2Cl_2 (5 ml) was added PDC (228 mg, 0.60 mmol) at room temperature and stirred for 2 days. Saturated $Na_2S_2O_3$ aqueous solution (200 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (50 ml × 3) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (3 % EtOAc in PE) afforded **430** (78 mg, 78 %) as a white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.47 (1H, dd, *J* = 5.1 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.66 (1H, app. t, *J* = 4.4 Hz, C$\underline{H}_2$ - 4), 0.84 – 0.95 (3H, m, C$\underline{H}_2$ - 1, CH – 9, C$\underline{H}$ – 3), 0.89 (3H, s, CC$\underline{H}_3$ - 18), 1.04 (3H, s, CC$\underline{H}_3$ - 19), 1.37 - 1.60 (6H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ – 7, C$\underline{H}_2$ – 12, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 11), 1.69 – 1.80 (1H, m, C$\underline{H}_2$ - 2), 2.10 – 2.24 (3H, m, C$\underline{H}_2$ - 7, C$\underline{H}_2$ – 12, C$\underline{H}_2$ – 16), 2.39 (1H, app. t, *J* = 11.5, 11.5 Hz, C$\underline{H}$ - 8), 2.15 (3H, s, CC$\underline{H}_3$ - 21), 2.73 – 2.82 (1H, m, C$\underline{H}_2$ – 16), 2.85 (1H, t, *J* = 2.9 Hz, C$\underline{H}$ - 6), 2.93 (1H, dd, *J* = 8.1, 8.2 Hz, C$\underline{H}$ - 17), 3.34 (3H, s, OC$\underline{H}_3$ – 1'), 5.16 (1H, app. q, *J* = 2.2 Hz, C$\underline{H}$ - 15).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.5 ($\underline{C}H_2$, 4), 19.0 ($\underline{C}H_3$, 18), 19.3 ($\underline{C}H_3$, 19), 21.8 ($\underline{C}H$, 3), 24.0 ($\underline{C}H_2$, 11), 25.2 ($\underline{C}H_2$, 2), 30.5 ($\underline{C}H$, 8), 31.6 ($\underline{C}H_2$, 16), 31.7 ($\underline{C}H_3$, 21), 33.6 ($\underline{C}H_2$, 7), 34.0 ($\underline{C}H_2$, 1), 35.0 ($\underline{C}$, 10), 42.6 ($\underline{C}H_2$, 12), 43.9 ($\underline{C}$, 5), 48.1 ($\underline{C}H$, 9), 48.8 ($\underline{C}$, 13), 56.0 ($\underline{C}H_3$, 1'), 65.9 ($\underline{C}H_2$, 17), 82.5 ($\underline{C}H$, 6), 117.2 ($\underline{C}H$, 15), 152.3 ($\underline{C}$, 14), 209.8 ($\underline{C}$, 20).

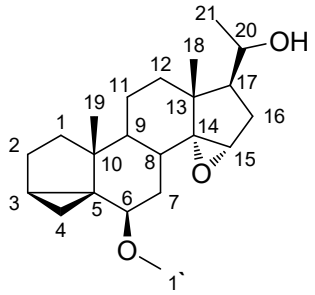
Mass Spectrum: (ESI+), *m/z*, 351.35 $[M + Na]^+$, 297.35 $[M - OMe]^+$.

IR Spectrum: 1707 cm^{-1} (C=O stretch), 1090 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 329.2471, calculated for $C_{22}H_{33}O_2$ $[M + H]^+$ = 329.2480.

Previously unreported.

5.2.15: Compound 433



(1*S*)-1-((2*S*,2a*R*,4b*R*,6a*R*,7a*R*,8*R*,9b*R*,10a*S*)-8-methoxy-2a,4b-dimethyltetradecahydro-1*H*-cyclopropa[1',7a']indeno[4',5':6,7]indeno[1,7a-*b*]oxiren-2-yl)ethanol

m-CPBA (75 %, 70 mg, 0.41 mmol) was added to a stirred solution of compound **432a** (41 mg, 0.12 mmol) in $CHCl_3$ (3 ml) at room temperature. After 3 hours, $Na_2S_2O_3$ aqueous solution (0.5 M, 50 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (100 ml × 3) and the combined organic extracts were washed with saturated $NaHCO_3$ aqueous solution (100 ml). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (10 % EtOAc in PE) afforded epoxide **433** (36 mg, 84 %) as a white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 5.2 & 5.2 Hz, $C\underline{H}_2$ - 4), 0.65 (1H, app. t, *J* = 4.5 Hz, $C\underline{H}_2$ - 4), 0.85 – 0.95 (2H, m, $C\underline{H}_2$ - 1, CH – 3), 1.01 (3H, s, $CC\underline{H}_3$ - 18), 1.06 (3H, s, $CC\underline{H}_3$ - 19), 1.14 & 1.15 (3H, s, $CC\underline{H}_3$ - 21), 1.17 – 1.26 (2H, m, $C\underline{H}$ - 9, $C\underline{H}_2$ - 7), 1.38 - 1.59 (8H, m, $C\underline{H}_2$ - 16, $C\underline{H}$ – 17, $C\underline{H}_2$ – 1, $C\underline{H}_2$ – 2, $C\underline{H}_2$ – 11, $C\underline{H}_2$ – 12), 1.78 – 1.86 (1H, m, $C\underline{H}_2$ – 2), 1.87 – 1.96 (2H, m, $C\underline{H}_2$ – 12, $C\underline{H}_2$ – 7), 2.54 (1H, dt, *J* = 11.0 Hz, $C\underline{H}$ - 8), 2.79 (1H, t, *J* = 2.9 Hz, $C\underline{H}$ - 6), 3.34 (3H, s, $OC\underline{H}_3$ – 1'), 3.38 (1H, app. s, $C\underline{H}$ - 15), 3.66 – 3.74 (1H, m, $C\underline{H}$ – 20).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.5 ($\underline{C}H_2$, 4), 15.5 ($\underline{C}H_3$, 18), 19.4 ($\underline{C}H_3$, 19), 21.4 ($\underline{C}H$, 3), 22.7 ($\underline{C}H_2$, 11), 24.0 ($\underline{C}H_3$, 21), 25.1 ($\underline{C}H_2$, 2), 27.8 ($\underline{C}H$, 8), 30.3 ($\underline{C}H_2$, 16), 30.7 ($\underline{C}H_2$, 7), 33.7 ($\underline{C}H_2$, 1), 34.7 ($\underline{C}$, 10), 36.2 ($\underline{C}H_2$, 12), 41.6 ($\underline{C}$, 13), 43.8 ($\underline{C}$, 5), 44.4 ($\underline{C}H$, 9), 51.3 ($\underline{C}H$, 17), 56.9 ($\underline{C}H_3$, 1'), 58.3 ($\underline{C}H$, 15), 69.3 ($\underline{C}H$, 20), 74.3 ($\underline{C}$, 14), 82.4 ($\underline{C}H$, 6).

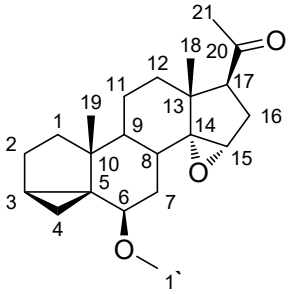
Mass Spectrum: (ESI+), *m/z*, 347.44 $[M + H]^+$, 315.45 $[M - OMe]^+$, 297.35 $[M - OMe, - H_2O]^+$, 279.35 $[M - OMe, - 2H_2O]^+$.

IR Spectrum: 3488 cm^{-1} (OH stretch), 1073 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 347.2597, calculated for $C_{22}H_{35}O_3$ $[M + H]^+$ = 347.2586.

Previously unreported.

5.2.16: Compound 434



1-((2*S*,2a*R*,4b*R*,6a*R*,7a*R*,8*R*,9b*R*,10a*S*)-8-methoxy-2a,4b-dimethyltetradecahydro-1*H*-cyclopropa[1',7a']indeno[4',5':6,7]indeno[1,7a-*b*]oxiren-2-yl)ethanone

From **430**

m-CPBA (75 %, 100 mg, 0.58 mmol) was added to a stirred solution of compound **430** (78 mg, 0.24 mmol) in $CHCl_3$ (5 ml) at room temperature. After 3 hours, $Na_2S_2O_3$ aqueous solution (0.5 M, 50 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (100 ml × 3) and the combined organic extracts were washed with saturated $NaHCO_3$ aqueous solution (100 ml). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (10 % EtOAc in PE) afforded epoxide **434** (76 mg, 93 %) as a white solid.

From **433**

To a stirred solution of **433** (23 mg, 0.07 mmol) in CH_2Cl_2 (3 ml) was added PDC (160 mg, 0.42 mmol) at room temperature and stirred for 2 days. Saturated $Na_2S_2O_3$ aqueous solution (50 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (30 ml × 3) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (10 % EtOAc in PE) afforded **434** (18 mg, 78 %) as a white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 5.2 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.65 (1H, app. t, *J* = 4.5 Hz, C$\underline{H}_2$ - 4), 0.81 (3H, s, CC$\underline{H}_3$ - 18), 0.85 – 0.95 (2H, m, C$\underline{H}_2$ - 1, CH – 3), 1.05 (3H, s, CC$\underline{H}_3$ - 19), 1.27 (1H, app. dt, 11.9, 11.9 Hz, C$\underline{H}$ - 9), 1.37 - 1.64 (6H, m, C$\underline{H}_2$ - 7, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 1, C$\underline{H}_2$ – 11), 1.69 – 1.87 (2H, m, C$\underline{H}_2$ – 12, C$\underline{H}_2$ - 2), 1.89 – 1.96 (1H, m, C$\underline{H}_2$ – 16), 1.98 (1H, t, *J* = 3.4 Hz, C$\underline{H}_2$ - 12), 2.00 – 2.07 (1H, m, C$\underline{H}_2$ – 16), 2.08 (3H, s, CC$\underline{H}_3$ - 21), 2.47 – 2.56 (2H, m, C$\underline{H}$ – 8, C$\underline{H}$ – 17), 2.79 (1H, t, *J* = 2.9 Hz, C$\underline{H}$ - 6), 3.32 (3H, s, OC$\underline{H}_3$ – 1'), 3.44 (1H, app. s, C$\underline{H}$ - 15).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.6 (C$\underline{H}_2$, 4), 16.4 (C$\underline{H}_3$, 18), 19.4 (C$\underline{H}_3$, 19), 21.5 (C$\underline{H}$, 3), 23.0 (C$\underline{H}_2$, 11), 25.2 (C$\underline{H}_2$, 2), 27.8 (C$\underline{H}$, 8), 28.1 (C$\underline{H}_2$, 16), 30.4 (C$\underline{H}_2$, 7), 31.6 (C$\underline{H}_3$, 21), 33.8 (C$\underline{H}_2$, 1), 34.8 (C$\underline{}$, 10), 35.4 (C$\underline{H}_2$, 12), 42.7 (C$\underline{}$, 13), 43.8 (C$\underline{}$, 5), 44.4 (C$\underline{H}$, 9), 57.0 (C$\underline{H}_3$, 1'), 57.3 (C$\underline{H}$, 17), 58.2 (C$\underline{H}$, 15), 73.4 (C$\underline{}$, 14), 82.3 (C$\underline{H}$, 6), 209.2 (C$\underline{}$, 20).

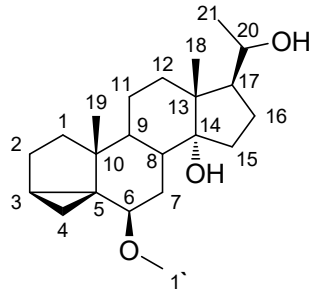
Mass Spectrum: (ESI+), *m/z*, 313.35 $[M - OMe]^+$, 295.35 $[M - OMe, - H_2O]^+$, 277.25 $[M - OMe, - 2H_2O]^+$.

IR Spectrum: 1704 cm^{-1} (C=O stretch), 1098 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 345.2442, calculated for $C_{22}H_{33}O_3$ $[M + H]^+$ = 345.2430.

Previously unreported.

5.2.17: Compound 437



(1a*R*,3a*R*,5a*R*,6*S*,8a*R*,10*R*,10a*R*)-6-((*S*)-1-hydroxyethyl)-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-8a-ol

From **433** and **434**

The epoxide **433** (300 mg, 0.87 mmol) was added to dry THF (5 ml) containing $LiAlH_4$ (33 mg, 0.87 mmol) and the mixture was refluxed under N_2 for overnight. The reaction mixture was cooled to room temperature and filtered. Solvent was removed under reduced pressure and residue extracted with diethyl ether (30 ml × 3) and water (50 ml). The residue was purified by column chromatography (30 % EtOAc in PE) to afford compound **437** (185 mg, 61 %) as a white solid and **433** (55 mg, 18 %) was also recovered.

Same procedure was used for **434** and similar yield of **437** was obtained with no starting material recovery.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 5.2 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.65 (1H, app. t, *J* = 4.5 Hz, C$\underline{H}_2$ - 4), 0.84 – 0.95 (2H, m, C$\underline{H}_2$ - 1, CH – 3), 0.96 (3H, s, CC$\underline{H}_3$ - 18), 1.06 (3H, s, CC$\underline{H}_3$ - 19), 1.17 & 1.19 (3H, s, CC$\underline{H}_3$ - 21), 1.20 – 1.89 (14H, m, C$\underline{H}_2$ – 16, C$\underline{H}$ - 9, C$\underline{H}_2$ - 11, C$\underline{H}_2$ – 1, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 7, C$\underline{H}_2$ – 15, C$\underline{H}_2$ – 12), 2.00 (1H, q, *J* = 9.4 Hz, C$\underline{H}$ – 17), 2.14 (1H, dt, *J* = 12.0 Hz, C$\underline{H}$ - 8), 2.84 (1H, t, *J* = 2.9 Hz, C$\underline{H}$ - 6), 3.33 (3H, s, OC$\underline{H}_3$ – 1'), 3.74 – 3.81 (1H, m, C$\underline{H}$ – 20).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.6 (C$\underline{H}_2$, 4), 16.9 (C$\underline{H}_3$, 18), 19.6 (C$\underline{H}_3$, 19), 21.7 (C$\underline{H}_2$, 11), 21.8 (C$\underline{H}$, 3), 24.3 (C$\underline{H}_3$, 21), 25.0 (C$\underline{H}_2$, 16), 25.3 (C$\underline{H}_2$, 2), 31.0 (C$\underline{H}_2$, 7), 32.8 (C$\underline{H}_2$, 12), 33.4 (C$\underline{H}_2$, 15), 33.5 (C$\underline{H}$, 8), 33.8 (C$\underline{H}_2$, 1), 35.6 (C$\underline{}$, 10), 41.0 (C$\underline{H}$, 9), 44.0 (C$\underline{}$, 5), 47.1 (C$\underline{}$, 13), 53.8 (C$\underline{H}$, 17), 57.0 (C$\underline{H}_3$, 1'), 70.7 (C$\underline{H}$, 20), 82.9 (C$\underline{H}$, 6), 86.0 (C$\underline{}$, 14).

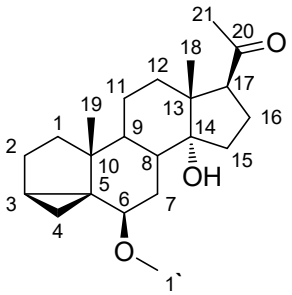
Mass Spectrum: (ESI+), *m/z*, 371.44 $[M + Na]^+$, 299.45 $[M - OMe, - H_2O]^+$, 281.45 $[M - OMe, - 2H_2O]^+$.

IR Spectrum: 3403 cm^{-1} (OH stretch), 1075 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 371.2549, calculated for $C_{22}H_{36}O_3Na$ $[M + Na]^+$ = 371.2562.

Previously unreported.

5.2.18: Compound 438



1-((1a*R*,3a*R*,5a*R*,6*S*,8a*R*,10*R*,10a*R*)-8a-hydroxy-10-methoxy-3a,5a-dimethylhexadecahydrocyclopenta[*a*]cyclopropa[2,3]cyclopenta[1,2-*f*]naphthalen-6-yl)ethanone

To a stirred solution of **437** (73 mg, 0.21 mmol) in CH_2Cl_2 (3 ml) was added PDC (394 mg, 1.01 mmol) at room temperature and stirred overnight. Saturated $Na_2S_2O_3$ aqueous solution (50 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (30 ml × 3) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (20 % EtOAc in PE) afforded **438** (56 mg, 77 %) as a white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 5.2 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.64 (1H, app. t, *J* = 4.5 Hz, C$\underline{H}_2$ - 4), 0.77 (3H, s, CC$\underline{H}_3$ - 18), 0.81 – 0.93 (2H, m, C$\underline{H}_2$ - 1, C$\underline{H}$ – 3), 1.03 (3H, s, CC$\underline{H}_3$ - 19), 1.33 – 1.57 (7H, m, C$\underline{H}$ - 9, C$\underline{H}_2$ – 1, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 7, C$\underline{H}_2$ – 15, C$\underline{H}_2$ – 16), 1.61 - 1.85 (5H, m, C$\underline{H}_2$ - 11, C$\underline{H}_2$ – 2, C$\underline{H}_2$ - 7, C$\underline{H}_2$ – 12), 1.90 – 1.98 (1H, m, C$\underline{H}_2$ – 12), 2.09 (3H, s, CC$\underline{H}_3$ - 21), 2.10 – 2.17 (1H, m, C$\underline{H}$ - 8), 2.22 – 2.31 (1H, m, C$\underline{H}_2$ – 16), 2.83 (1H, t, *J* = 2.9 Hz, C$\underline{H}$ - 6), 3.19 (1H, t, *J* = 8.75 Hz, C$\underline{H}$ – 17), 3.31 (3H, s, OC$\underline{H}_3$ – 1').

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.5 (C$\underline{H}_2$, 4), 17.8 (C$\underline{H}_3$, 18), 19.5 (C$\underline{H}_3$, 19), 21.8 (C$\underline{H}$, 3), 21.8 (C$\underline{H}_2$, 11), 21.9 (C$\underline{H}_2$, 16), 25.3 (C$\underline{H}_2$, 2), 31.1 (C$\underline{H}_2$, 7), 31.7 (C$\underline{H}_3$, 21), 31.9 (C$\underline{H}_2$, 12), 33.4 (C$\underline{H}_2$, 15), 33.5 (C$\underline{H}$, 8), 33.8 (C$\underline{H}_2$, 1), 35.4 (C$\underline{}$, 10), 40.8 (C$\underline{H}$, 9), 43.9 (C$\underline{}$, 5), 48.6 (C$\underline{}$, 13), 56.9 (C$\underline{H}_3$, 1'), 60.0 (C$\underline{H}$, 17), 82.6 (C$\underline{H}$, 6), 86.2 (C$\underline{}$, 14), 210.9 (C$\underline{H}$, 20).

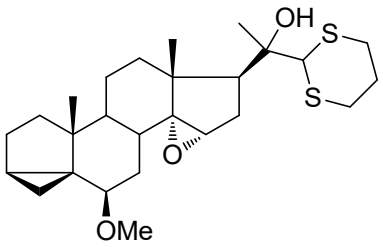
Mass Spectrum (ESI+), *m/z*, 369.44 $[M + Na]^+$, 297.35 $[M - OMe, - H_2O]^+$, 279.35 $[M - OMe, - 2H_2O]^+$.

IR Spectrum: 3547 cm^{-1} (OH stretch), 1691 cm^{-1} (C=O stretch), 1096 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 369.2397, calculated for $C_{22}H_{34}O_3Na$ [M + Na] $^+$ = 369.2406.

Previously unreported.

5.2.19: Compound 440



1-(1,3-dithian-2-yl)-1-((2*S*,2a*R*,4b*R*,6a*R*,7a*R*,8*R*,9b*R*,10a*S*)-8-methoxy-2a,4b-dimethyltetradecahydro-1*H*-cyclopropa[1',7a']indeno[4',5':6,7]indeno[1,7a-*b*]oxiren-2-yl)ethanol

To a two-necked round bottom flask, containing dithiane (101 mg, 0.84 mmol, 1.5 eq.), was added dry THF (5 ml) under N_2 and the resulting solution was cooled to – 70 ^{o}C. *n*-BuLi (2.5 M in hexane, 0.40 ml, 1.01 mmol, 1.8 eq.) was added to the solution dropwise and it was stirred at – 70 ^{o}C for 2 hours. **434** (194 mg, 0.56 mmol, 1 eq.) in dry THF (5 ml) was added to the reaction mixture dropwise at – 70 ^{o}C. The reaction mixture was left in cold room (3 ^{o}C) overnight. Cold saturated NH_4Cl aqueous solution (20 ml) was added and THF was removed under reduce pressure. The aqueous phase was extracted with CH_2Cl_2 (20 ml × 3) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (10 % EtOAc in PE) afforded **440** (140 mg, 53 %) as a pale yellow solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 5.2 & 5.2 Hz, C$\underline{H}_2$ - 4), 0.65 (1H, app. t, *J* = 4.5 Hz, C$\underline{H}_2$ - 4), 0.82 – 0.92 (2H, m, C$\underline{H}_2$ - 1, C$\underline{H}$ – 3), 1.06 (3H, s, CC$\underline{H}_3$ - 19), 1.12 (3H, s, CC$\underline{H}_3$ - 18), 1.21 - 1.28 (2H, m, C$\underline{H}$ - 9, C$\underline{H}_2$ - 7), 1.38 (3H, s, CC$\underline{H}_3$ – 21), 1.40 - 1.62 (4H, m, C$\underline{H}_2$ - 1, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 11, C$\underline{H}_2$ – 12, C$\underline{H}_2$ – 7), 1.74 – 1.86 (3H, m, C$\underline{H}_2$ – 2, C$\underline{H}_2$ – 16, C$\underline{H}_2$ – 24), 1.93 – 2.13 (4H, m, C$\underline{H}_2$ – 16, C$\underline{H}_2$ – 12, C$\underline{H}$ – 17 ,C$\underline{H}_2$ – 24), 2.56 (1H, dt, *J* = 11.0 Hz, C$\underline{H}$ - 8), 2.77 - 3.00 (5H, m, C$\underline{H}$ – 6, C$\underline{H}_2$ – 23, C$\underline{H}_2$ – 25), 3.33 (3H, s, OC$\underline{H}_3$ – 1'), 3.43 (1H, app. s, C$\underline{H}$ - 15), 4.10 (1H, s, C$\underline{H}$ – 22).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.6 (C$\underline{H}_2$, 4), 16.5 (C$\underline{H}_3$, 18), 19.5 (C$\underline{H}_3$, 19), 21.6 (C$\underline{H}$, 3), 22.8 (C$\underline{H}_2$, 11), 24.3 (C$\underline{H}_3$, 21), 25.2 (C$\underline{H}_2$, 2), 26.3 (C$\underline{H}_2$, 24), 27.1 (C$\underline{H}_2$, 16), 27.4 (C$\underline{H}$, 8), 30.2 (C$\underline{H}_2$, 7), 31.3 (C$\underline{H}_2$, 25), 31.8 (C$\underline{H}_2$, 23), 33.8 (C$\underline{H}_2$, 1), 34.9 (C$\underline{}$, 10), 36.6 (C$\underline{H}_2$, 12), 42.4 (C$\underline{}$, 13), 43.8

(C, 5), 44.3 (CH, 9), 48.4 (CH, 17), 57.0 (CH_3, 1'), 57.9 (CH, 15), 61.7 (CH, 22), 74.2 (C, 14), 76.4 (C, 20), 82.5 (CH, 6).

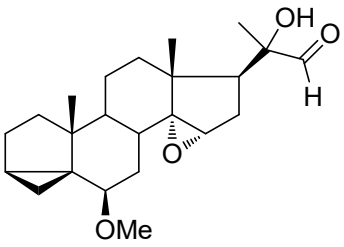
Mass Spectrum (ESI+), *m/z*, 465.44 $[M + H]^+$, 487.44 $[M + Na]^+$, 951.43 $[M \times 2 + Na]^+$, 447.44 $[M - OH]^+$, 433.44 $[M - OMe]^+$, 415.44 $[M - OMe, - H_2O]^+$, 397.43 $[M - OMe, - 2H_2O]^+$.

IR Spectrum: 3409 cm^{-1} (OH stretch), 2928 cm^{-1} (CH stretch), 1078 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 465.2477, calculated for $C_{26}H_{41}O_3S_2$ $[M + H]^+$ = 465.2497.

Previously unreported.

5.2.20: Compound 441



2-hydroxy-2-((2*S*,2a*R*,4b*R*,6a*R*,7a*R*,8*R*,9b*R*,10a*S*)-8-methoxy-2a,4b-dimethyltetradecahydro-1*H*-cyclopropa[1',7a']indeno[4',5':6,7]indeno[1,7a-*b*]oxiren-2-yl)propanal

To a stirred solution of **440** (140 mg, 0.30 mmol) in CH_2Cl_2/H_2O (10:1, 10 ml) was added N-chlorosuccinimide (80 mg, 0.60 mmol, 2 eq.) in portions. The reaction mixture was stirred at room temperature for 2 hours. Saturated $Na_2S_2O_3/NaHCO_3$ aqueous solution (1:1, 20 ml) was added and the reaction mixture was extracted with CH_2Cl_2 (20 ml × 3) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel column chromatography (10 % EtOAc in PE) afforded **441** (69 mg, 61 %) as a white solid.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 0.44 (1H, dd, *J* = 5.2 & 5.2 Hz, CH_2 - 4), 0.65 (1H, app. t, *J* = 4.5 Hz, CH_2 - 4), 0.82 – 0.94 (2H, m, CH_2 - 1, CH - 3), 1.07 (3H, s, CCH_3 - 19), 1.08 (3H, s, CCH_3 - 18), 1.21 - 1.28 (1H, m, CH - 9), 1.31 (3H, s, CCH_3 – 21), 1.37 - 1.70 (9H, m, CH_2 – 7, CH_2 - 1, CH_2 – 2, CH_2 – 11, CH_2 – 12, CH_2 – 16), 1.76 – 1.86 (2H, m, CH_2 – 2, CH_2 – 17), 1.98 – 2.04 (1H, m, CH_2 – 12), 2.55 (1H, dt, *J* = 11.5 Hz, CH - 8), 2.79 (1H, t, *J* = 2.9 Hz, CH - 6), 3.22 (1H, app. d, *J* =

1.20 Hz, OH), 3.33 (3H, s, OC<u>H</u>$_3$ – 1'), 3.44 (1H, app. s, C<u>H</u> - 15), 9.41 (1H, app. d, *J* = 1.1 Hz, C<u>H</u>O - 22).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 13.6 (<u>C</u>H$_2$, 4), 16.7 (<u>C</u>H$_3$, 18), 19.5 (<u>C</u>H$_3$, 19), 21.5 (<u>C</u>H, 3), 22.8 (<u>C</u>H$_2$, 11), 22.9 (<u>C</u>H$_3$, 21), 25.2 (<u>C</u>H$_2$, 2), 27.4 (<u>C</u>H, 8), 27.6 (<u>C</u>H$_2$, 16), 30.3 (<u>C</u>H$_2$, 7), 33.8 (<u>C</u>H$_2$, 1), 34.8 (<u>C</u>, 10), 36.6 (<u>C</u>H$_2$, 12), 42.4 (<u>C</u>, 13), 43.9 (<u>C</u>, 5), 44.4 (<u>C</u>H, 9), 47.6 (<u>C</u>H, 17), 57.0 (<u>C</u>H$_3$, 1'), 58.1 (<u>C</u>H, 15), 74.1 (<u>C</u>, 14), 78.8 (<u>C</u>, 20), 82.4 (<u>C</u>H, 6), 203.4 (<u>C</u>HO, 22).

Mass Spectrum (ESI+), *m/z*, 375.45 $[M + H]^+$, 397.45 $[M + Na]^+$, 357.44 $[M - OH]^+$, 343.45 $[M - OMe]^+$.

IR Spectrum: 3351 cm^{-1} (OH stretch), 1732 cm^{-1} (C=O stretch), 1074 cm^{-1} (C-O stretch).

HRMS: (ESI+), *m/z*, found = 375.2529, calculated for $C_{23}H_{35}O_4$ $[M + H]^+$ = 375.2535.

Previously unreported.

5.3: Biological Evaluation

Cells and reagents: Human prostate PC-3 cell line was obtained from the ATCC (Middlesex, TW11 0LY, UK). The cells were cultured as monolayers at 37 °C and 5% CO2 atmosphere in RPMI1640 medium (Sigma-Aldrich, catalogue number R5886), supplemented with 10% foetal bovine serum (FBS), 1 mM sodium pyruvate (sigma-Aldrich, catalogue number, S8636) and 2 mM L-glutamine (sigma-Aldrich, catalogue number G7513). This cell line was last authenticated in Nov 2017. All compounds were initially dissolved in DMSO (10-100 µL) to make a 100 mM solution which was then diluted x100 folds in PBS to make a stock solution. The stock solutions were then diluted as required for the concentration range of the cell viability experiment. MTT solution was prepared by dissolving 100 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in complete RPMI medium (20 mL) and the resulting solution was filtered through a 20 micron filter to remove any insoluble materials. The MTT solution was stored in the dark and at 4 °C before and in between use.

Cell viability assay: Into each well of the 96 well plates, 180 µl of PC-3 cell suspension were seeded at a concentration of 5 x 10^4 cells/ml. 20 µl of either PBS (as control) or the solution of compounds in increasing concentrations were also added (see outline of cell viability plate design, Figure x). Readings for each concentration were carried out in quadruplicate. Plates were incubated at 37 °C, 5% CO_2, for 24-96 hours. Upon completion of the incubation time of 96 hours, the growth medium was aspirated, replaced with MTT solution and incubated at 37 °C, 5% CO2 for 4 hours. After the incubation period, the MTT was carefully removed and DMSO added to dissolve the (MTT) crystals. The Absorbance of the 96 well plates was measured at 540 nm using a Multiscan Ex 96 well microplate reader (Thermo Electron Corporation, United Kingdom). The experiment was repeated in triplicate (n=3).

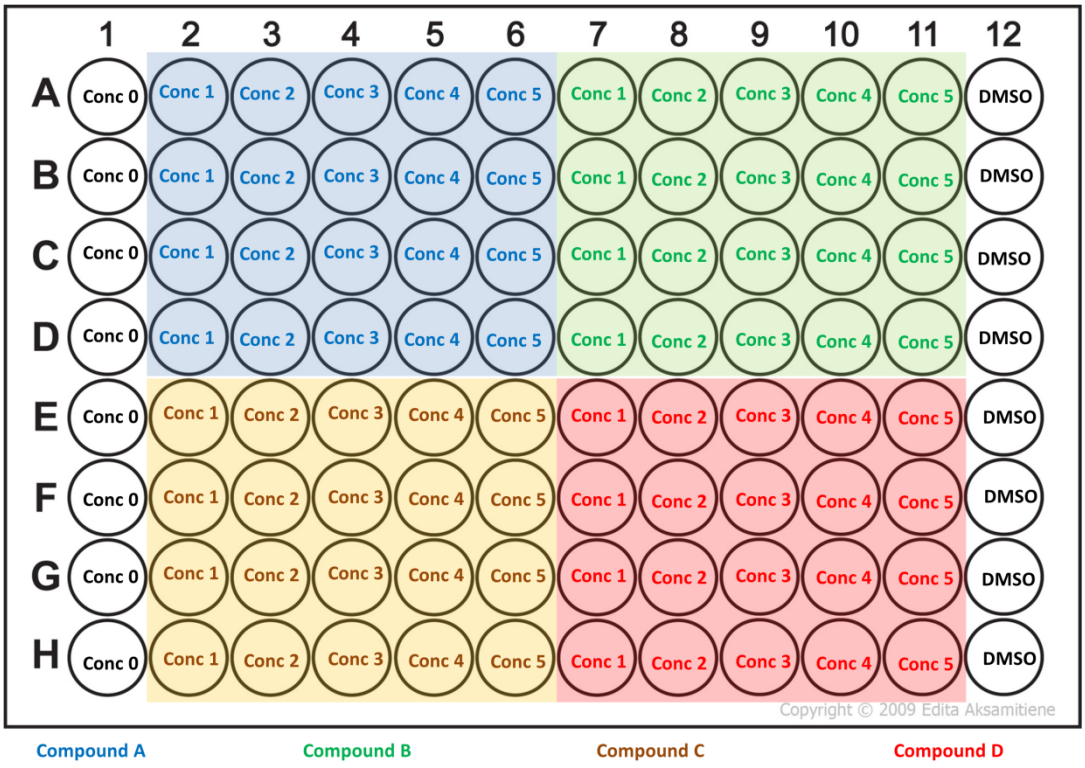


Figure 5.1: Outline of cell viability plate design.

Analysis: All data is presented as the mean ±standard deviation of at least 3 independent experiments. Graph construction and statistical analysis was performed using GraphPad Prism 6.

The biological experiments were carried out by Dr Victoria Vinader at the Institute of Cancer Therapeutics, University of Bradford.

CHAPTER 06

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