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Synthesis of ganoderic acid analogues to investigate their activity as anti-prostate cancer agents

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Declaration

I declare that the work embodied in this thesis is the result of my own investigations and no part has been submitted for any degree at the University of Nottingham or any other institutions.

Mohammed Muayad Shukur Al-Obaidi March 2019

Abstract

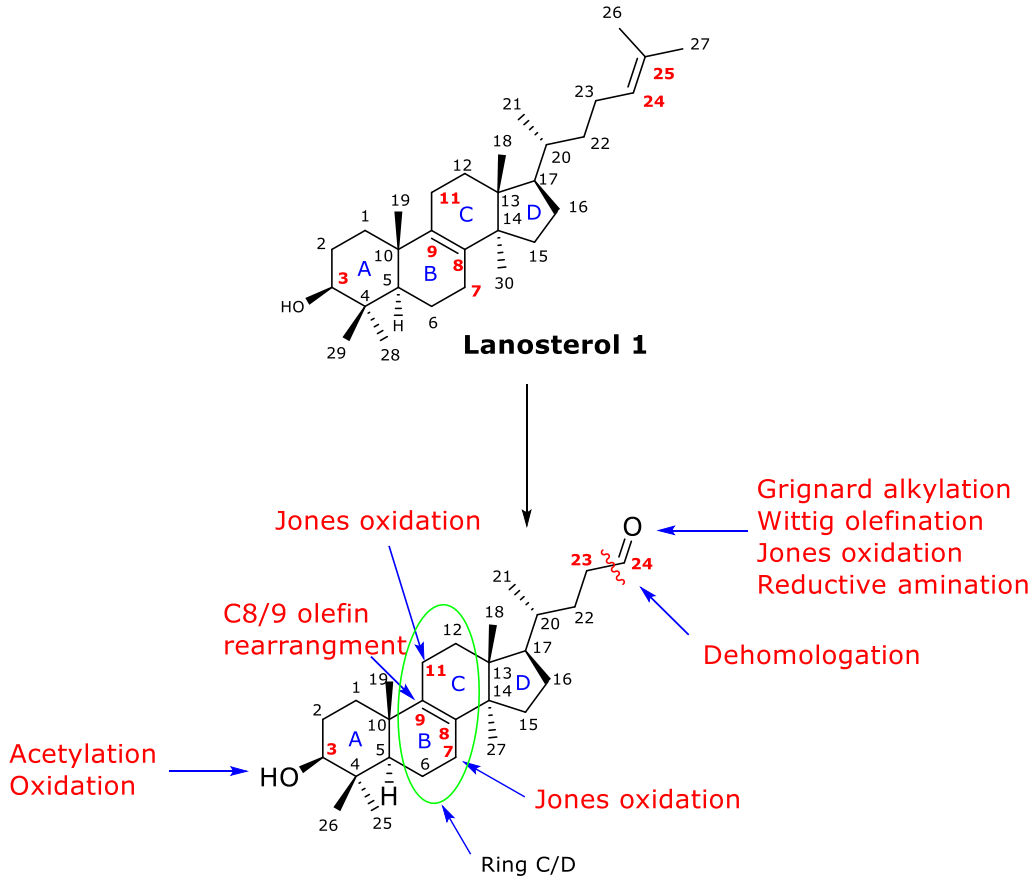
Cancers are one of the leading causes of premature human death globally. Prostate cancer is the second most common cancer in the world; chemoprevention can help decrease the proportion of fatal cases and prevent recurrence of the cancer.

Ganoderic acids (GAs) are a type of C30 lanostane triterpenoid produced by the mushroom *Ganoderma lucidum.* Analogues of GAs which have previously been tested showed biological activity as anti-cancer compounds. In this project, the synthesis of new ganoderic acid DM (GA-DM) analogues starting from lanosterol is reported as well as a preliminary evaluation of their biological activity as anti-prostate cancer agents. Lanosterol is a cheap natural starting material from sheep wool grease that is commercially available in 50-60% purity. From literature data, the sites that may enhance the biological activity of lanosterol and are modified in this study are: C3, C7, and C24.

This study outlines how aldehyde (10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-oxopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate was synthesised by Sharpless epoxidation of the C24/C25 double bond followed by oxidative cleavage. A series of molecules were then synthesized starting from this aldehyde intermediate including amines; azides; triazoles; lower homologues (olefin; diol and aldehyde) and GA-DM analogues.

The compounds were found to be insoluble in DMSO, therefore alternative solvent options were investigated. A significant number of

the compounds showed good solubility and low cancer cell line toxicity in ethyl acetate. The cytotoxicity of the ethyl acetate soluble compounds was evaluated against the PC3 and DU145 prostate cancer cell lines to assess their potential as anti-cancer drugs using MTT assay. Putting an acetate at C3 was found to greatly improve solubility and biological activity, whereas having a hydroxyl at C3 reduced both solubility and biological activity. The most effective compounds had values in the IC_{50} range 3-32 μM compared with methyl ganoderic acid-DM and ganoderic acid-DM with IC_{50} values of 0.3 and 40 μM respectively.



Side chain functionalization of commercial lanosterol 1, and proposed modifications on the key aldehyde 42.

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Abbreviation

ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
AMA	Amantadine hydrochloride
AR	Androgen receptor
ATR	Attenuated Total Reflection
anh.	Anhydrous
Da	Dalton
DCM	Dichloromethane
DCE	1,2-Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DIAD	Diisopropyl azodicarboxylate
DIPEA	*N,N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DPPA	Diphenyl phosphoryl azide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EI	Electrospray ionisation

Eq	equivalent
ESI^+	positive electrospray ionisation
Et	Ethyl
FD-MS	Field desorption mass spectrometry
FT-IR	Fourier Transform Infrared
HRMS	High Resolution Mass Spectrometry
GA-DM	Ganoderic acid-DM
GAs	Ganoderic acids
GC	Gas chromatography
G. lucidum	*Ganoderma lucidum*
GnRH	Gonadotropin-releasing hormone
h	hour
HPLC	High performance liquid chromatography
J	^{1}H-NMR coupling constant (H_z)
LAN	Lanosterol
LH	Luteinizing hormone
LNCaP	Lymph Node Carcinoma of the Prostate
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	Methyl

µM	micromolar
mmol	Millimoles
mp	Melting point
Ms	methanesulfonyl
nM	nanomolar
NMR	Nuclear magnetic resonance
N-Pr	n-Propyl
PC3	Prostate cancer cell lines
PCC	Pyridinium chlorochromate
PCR	polymerase chain reaction
PMA	Phosphomolybdic acid
PSA	Prostate specific antigen
Pyr	Pyridine
RIM	Rimantadine hydrochloride
ROS	Reactive Oxygen Species
r.t.	Room temperature
sat.	Saturated aqueous solution
SOD1	Superoxide dismutase 1
SOD2	Superoxide dismutase 2

SOD3	Superoxide dismutase 3
STAT3	Signal transducer and activator of transcription 3
TBAA	Tetrabutylammonium acetate
TBDMS	*Tert*-butyldimethylsilyl
ToF	Time of Flight
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TEA	Triethylamine

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1. Introduction

1.1. Prostate cancer frequency

Cancer is a term used for a number of conditions which can cause death; they are characterised by abnormal cell proliferation. Each individual cancer has a particular set of changes or mutations that causes the abnormal behaviour of the cells[1]. Cancer is the leading cause of death globally. Prostate cancer is the second most common cancer in the United States of America for men, after lung cancer. In 2010, the American Cancer Society reported an estimated 217,730 new cases in addition to 32,050 deaths[2]. Prostate cancer treatment can go through different therapy protocols, which give varied levels of success. Examples of these therapies would be surgery, radiation, chemotherapy, immunotherapy and hormone therapy. The prostate is a gland with limited growth and the growth speed is slow[3]. It is partially responsible for semen fluid production[4]. Furthermore, treatment and monitoring methods are decided by doctors. The prostate cancer risk rises with increasing age, especially individuals between 75 and 79 years old, who have the highest rate of prostate cancer development[3]. In a study by Hamdy *et al.*, 1098 men were monitored over 10 years, of the 553 had surgery 46 experienced metastasis, versus 112 from the 454 receiving only active monitoring[5].

1.2. Possible risk factors for prostate cancer:

Although prostate cancer is the second most prevalent cancer in men, little information is known about the fundamental causes of this type of cancer. The possible factors that can cause prostate cancer are:

Ageing: it is considered to be the dominant reason for the prostate cancer. Those under the age of 50 appear to be at a lower risk of prostate cancer.

Environment: statistical studies show that the percentage of men suffering from prostate cancer or who died because of it, is highest in the North American and northern European countries and is lowest in the Far East. Prostate cancer develops in black men earlier than white men. However, Chinese and Japanese have the lowest percentage of risk of prostate cancer, although prostate cancer prevalence is now increasing in China and Japan due to a change in diet and life style (Figure 1). The risk of getting prostate cancer for migrating men significantly increases when they move from low-risk areas to high-risk areas, both environmental and dietary changes are considered as key affecting factors.

Family history: around 9% of prostate cancer cases have a genetic basis. Prostate cancer development is linked to more than 30 genetic polymorphisms[6].

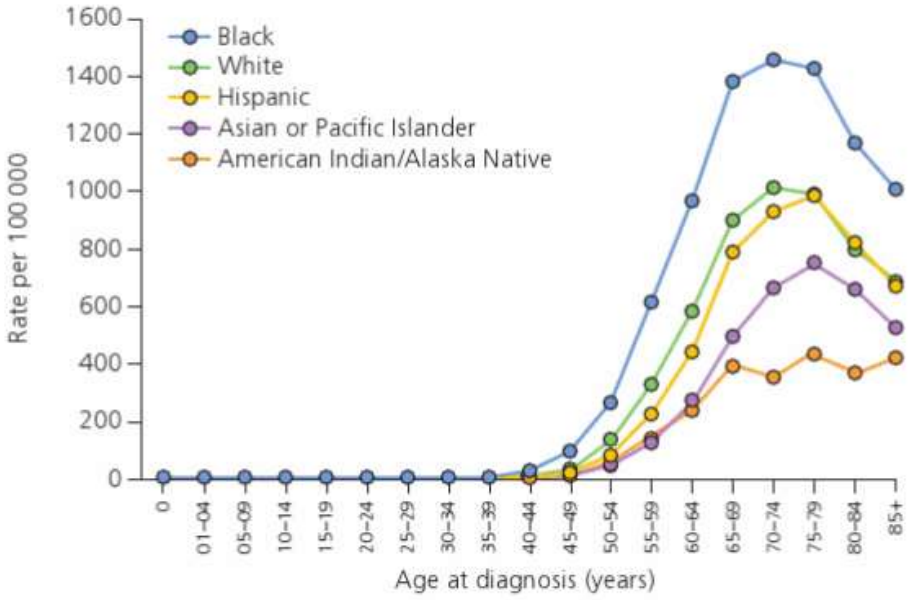
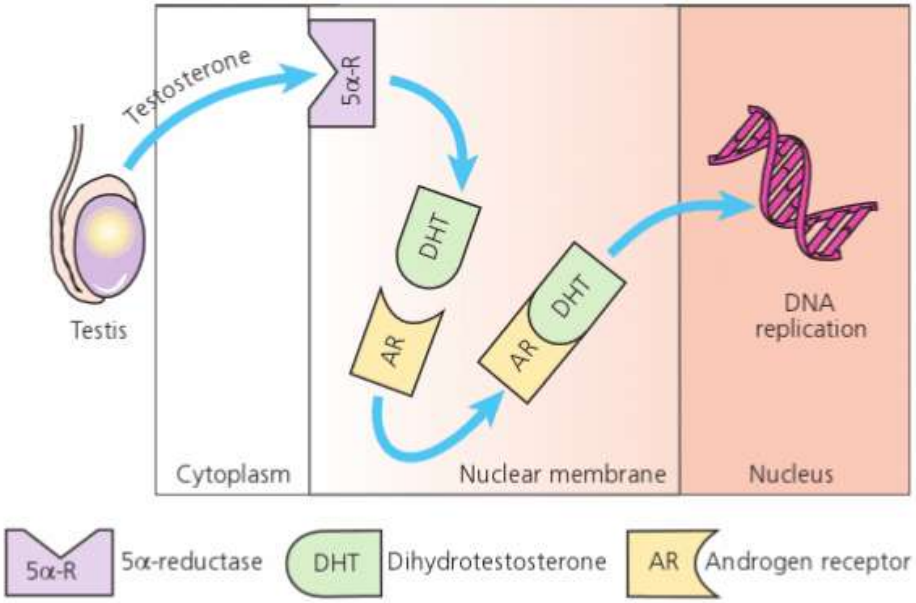


Figure 1. Age specific incidence of prostate cancer in the USA 1977-2004[6].

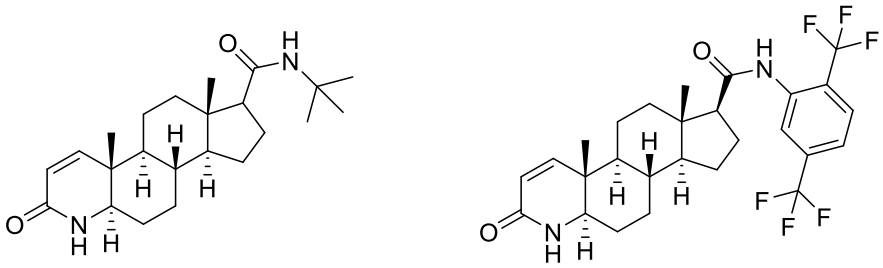
Hormones: testosterone is a male hormone which is converted to dihydrotestosterone (DHT) by 5 α-reductase (Scheme 1). There are two types of 5 α-reductase enzymes (type I and type II). DHT is very important in the normal prostate growth, but it is also linked to the development of prostate cancer. Prostate cancer development rarely happens in men who are castrated before their maturity as well as for men who have a 5 α-reductase deficiency. Many trials have investigated inhibition of the 5 α-reductase type II enzyme by using finasteride **1** and dutasteride **2** (Figure 2). The trials showed that nearly 25% of prostate cancer development decreased and suggested a significant role for DHT in its progression[6-9].

Obesity: The links between prostate cancer and body mass are as yet unclear. Some previous studies have stated that the risk of prostate cancer for obese people is higher than for non-obese people. However, conversely, recent studies showed that obese people have a lower risk

of prostate cancer which could be related to the low levels of prostate-specific antigen (PSA) and androgen as well. It is not clear if it has a links to prostate cancer[6, 10].



Scheme 1. Testosterone supports prostate cell function and simulates cell division[6].



1 **2**

Figure 2. Structure of finasteride 1 and dutasteride 2[6].

1.3. Prostate cancer stages

Prostate cancer is mostly classified by the Gleason system, which consists of five levels from least to most severe cancer:

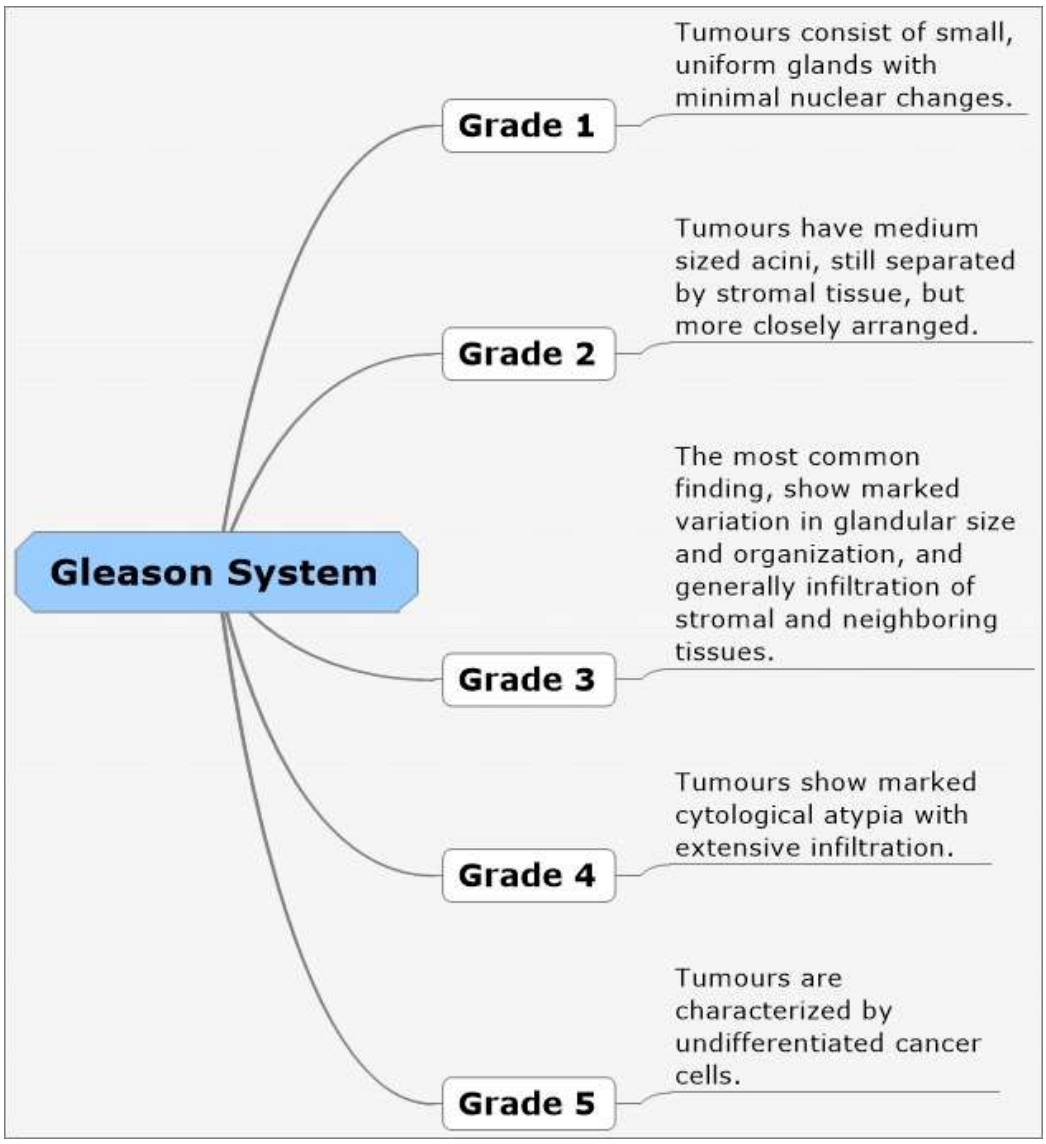


Figure 3. Gleason system classification[6].

1.4. Prostate cancer treatment

1.4.1. Prostate cancer diagnosis

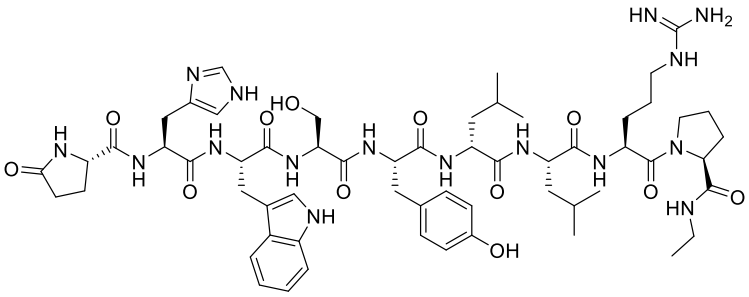
The common methods for prostate cancer diagnosis are the rectal examination and prostate-specific antigen (PSA) level evaluation in serum[11]. PSA is a marker to measure the abnormal growth of the prostate gland and high levels of PSA is an indicator for a possible prostate cancer risk[12, 13].

1.4.2. Early stage treatment

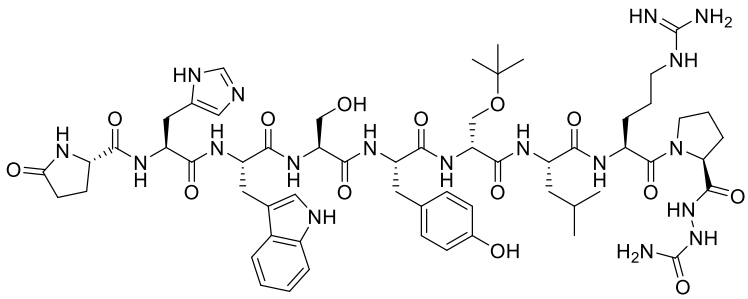
Early detection of prostate cancer gives a higher chance of effective treatment; the first option is surgery to remove the tumour. Radiotherapy treatment is another treatment option using either external beam therapy or by brachytherapy. Radioactive elements are used in radiotherapy such as Sr-89 or Sm-153. Studies have shown that Sr-89 or Sm-153 can control pain severity. These two radioactive isotopes are recommended in the early stage of prostate cancer treatment, the role of these isotopes are not only in pain control, but also in the prevention of metastasis. Sr-89 and Sm-153 have side effects such as haematological toxicity, and they are not given to any patient who may have suffered from cytotoxicity in pre-treatment with radioactive isotopes. Ra-223 is another radioactive isotope use in the treatment of early stage prostate cancer and now it has been approved as co-treatment with the current treatment for prostate cancer in the USA[14-18].

1.4.3. Chemotherapy treatment

Chemotherapy is used mainly in advanced prostate cancer therapy especially for metastatic prostate cancer patients[19-23]. There are two main types of chemotherapeutic agents: the first type are non-steroidal inhibitors such as Leuporlide **3** and Goserelin **4** (Figure 4). They work on the anterior pituitary gland by decreasing the release of luteinizing hormone (LH) which leads to a reduction in testosterone production through down-regulation of GnRH Receptors.



3

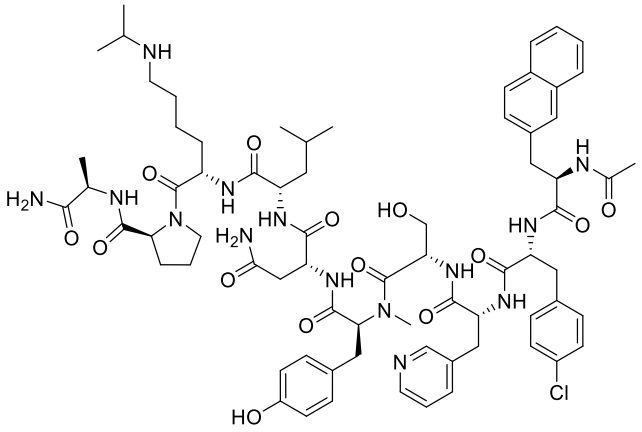


4

Figure 4. Structure of leuporlide 3 and goserelin 4.

Abarelix **5** is another non-steroidal drug which has been used to treat advanced-stage prostate cancer and works on the anterior pituitary gland. Unfortunately, anaphylaxis is one of its side effects[12, 24]. Anaphylaxis is a potentially fatal disorder which may cause blockage of the airways and lead to cardiac arrest and death[25-28]. Therefore, abarelix is no longer available in the US for prostate cancer treatment. Further

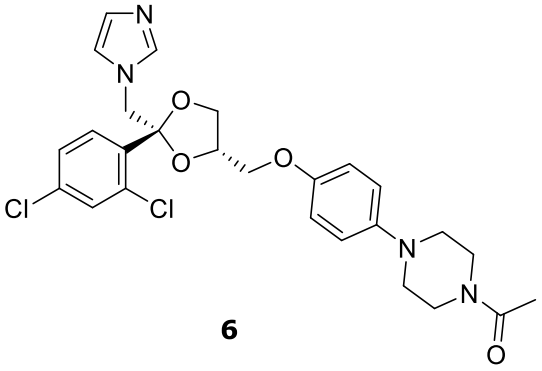
studies proved Abarelix reduces Follicle stimulating hormone (FSH) levels by 90 %, but this did not meet the criteria for prostate specific antigen (PSA) response [12, 24]. FSH is hormone secreted by the gonadotrophs, it has multiple roles but the main role for men is involved in the sperm production. PSA is considered to be the main biomarker for prostate cancer diagnoses[29, 30].



5

Figure 5. Structure of abarelix 5.

Ketoconazole **6** is an anti-prostate cancer drug which works on the adrenal gland by decreasing androgen synthesis. The patients should receive a course of steroid (hydrocortisone) alongside the treatment[31-35].



6

Figure 6. Structure of ketoconazole 6.

Flutamide **7**; Bicalutamide **8** and Nilutamide **9** are androgen receptor inhibitors, and they work on the prostate gland. The mechanism of action is competitive binding and antagonism of the androgen receptor. Gynecomastia, which an increase in breast size men and increased liver transaminases are the main side effects of these drugs on patients[31-34].

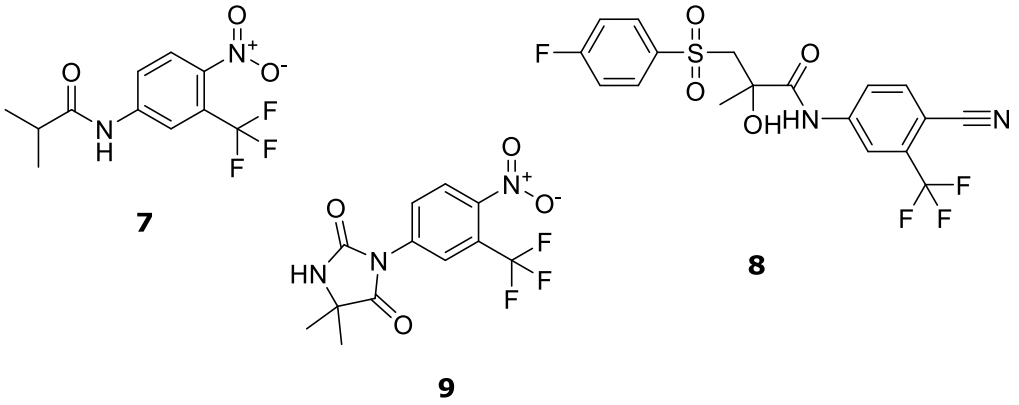


Figure 7. Structure of Flutamide 7; Bicalutamide 8 and Nilutamide 9[31].

One of the methods for treating prostate cancer is using enzyme inhibitors rather than receptor antagonists. Recent studies state that using finasteride, a 5 α-reductase inhibitor reduces prostate cancer by 24.8%, in comparison with using placebo for seven years[36]. Dutasteride is another enzyme inhibitor which inhibits 5 α-reductase. Studies have shown that using dutasteride as an inhibitor reduced the incidence of prostate cancer by 23%, 42 patients from 52 over a three month treatment period.

Table 1. Finasteride and dutasteride inhibition of 5 α-reductase[36].

Drug	5 α-reductase inhibition	Inhibition
Finasteride	Inhibits type II and low affinity for type I	24.8 %
Dutasteride	Inhibits type I and type II	23 %

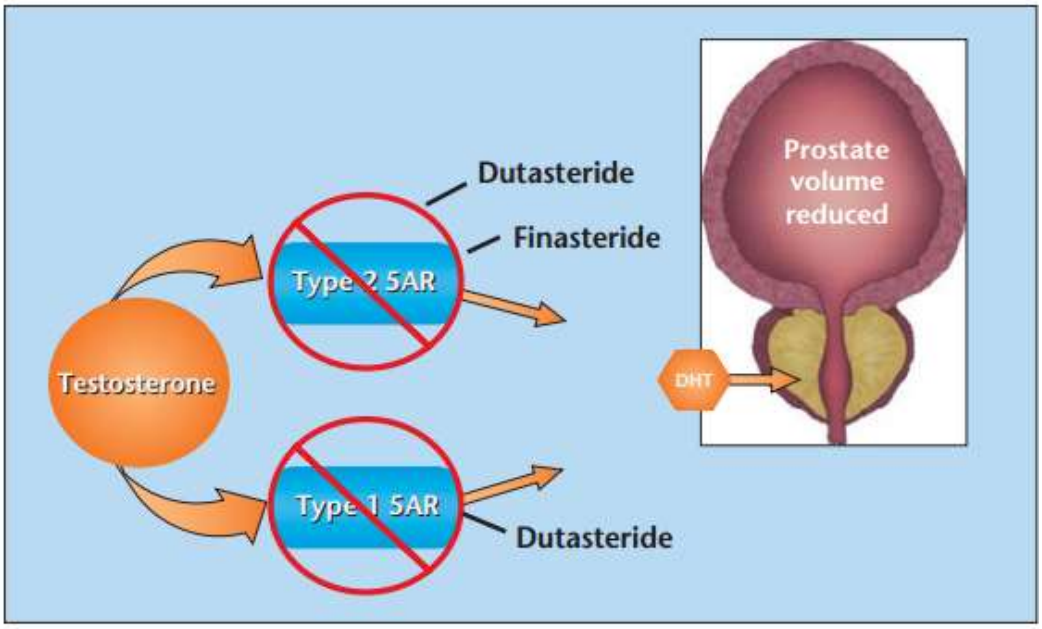
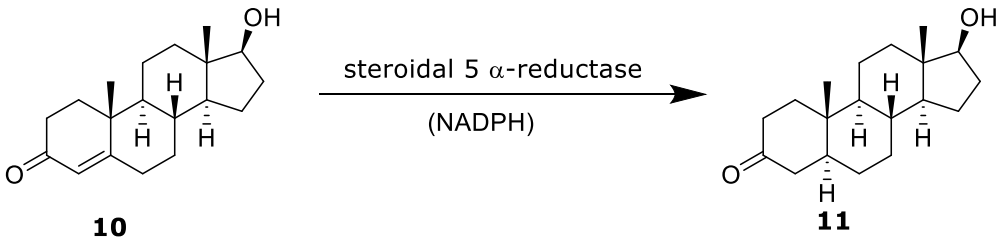


Figure 8. Reproduced from Nickel *et al.* "The 2 5α-reductase inhibitors (5ARIs), dutasteride and finasteride, suppress dihydrotestosterone (DHT) by inhibiting the conversion of testosterone to DHT. Finasteride inhibits only the Type 2 5AR isoenzyme, whereas dutasteride, the only dual 5ARI, selectively inhibits both Type 1 and Type 2 5AR isoenzymes''[36].



10 **11**

Scheme 2. Testosterone 10 reduction to DHT 11.

Previously Nickel *et al.* investigated short term comparative trials and similar non-comparative long-term trials of finasteride and dutasteride in terms of pharmacologic effect, safety and efficacy. Dutasteride **2** showed higher inhibition of DHT, long half-life and higher percent of prostate gland reduction, the study results are summarised in Table 2[36-38].

Table 2. Reproduced from Nickel *et al.* "5AR, 5α-reductase; DHT, dihydrotestosterone; PLESS, Proscar Long-Term Efficacy and Safety Study; MTOPS, Medical Treatment of Prostate Symptoms Study; Qmax, maximum flow rate; AUA-SI, American Urological Association-Symptom Index; AUR, acute urinary retention"[36].

	Dutasteride (0.5 mg)	Finasteride (5 mg)
DHT inhibition (serum) %	93%	70%
Half-life	5 weeks	6 to 8 hours
Prostate size reduction (from baseline)	-25.7% (2 y) -27.3% (4 y)	-18% (4 y, PLESS) -16% (4.5 y, MTOPS)
Urinary flow improvement (Qmax)	2.2 mL/sec (2 y) 2.7 mL/sec (4 y)	1.7 mL/sec (4 y, PLESS) 2.2 mL/sec (4.5 y,MTOPS)
Improvement in symptoms (AUA-SI)	-4.4 (2 y) -6.5 (4 y)	-3.3 (4 y, PLESS) -5.0 (4.5 y, MTOPS)
Reduction in AUR %	57% (2 y)	57% (4 y, PLESS) 79% (4.5 y, MTOPS)
Reduction in surgery %	48% (2 y)	55% (4 y, PLESS) 69% (4.5 y, MTOPS)

Steroidal 5 α-reductase is a nuclear membrane enzyme which reduces the double bond C4/C5 of testosterone and converts it to dihydrotestosterone by using the cofactor NADPH **12** (Figure 9) which is oxidised to $NADP^+$. High levels of 5 α-reductase can cause other diseases such as benign prostatic hyperplasia, prostate cancer, hirsutism, acne and male-patterned baldness. Over the last twenty years, many 5 α-reductase inhibitors have been developed, both steroidal and non-steroidal inhibitors. The steroidal compounds are considered to be interesting inhibitors from a drug development perspective because a small modification in the chemical structure may give higher biological activity.

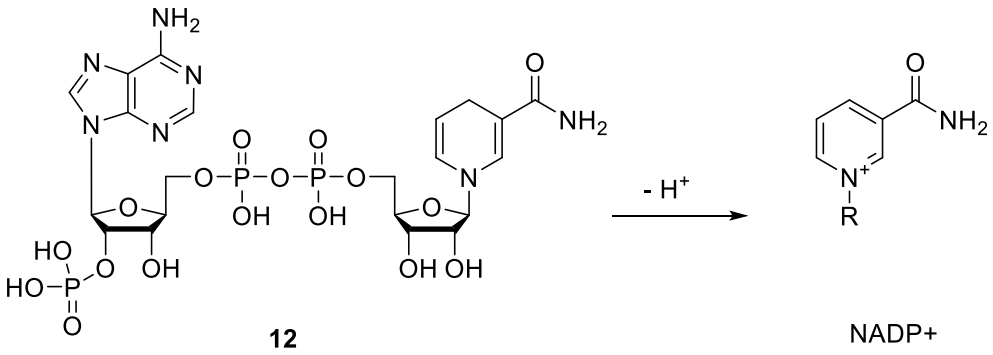
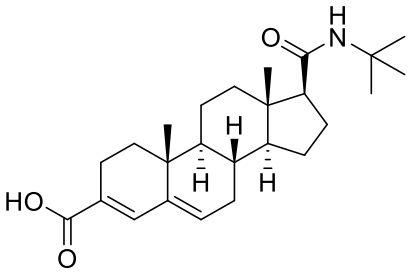


Figure 9. Structure of NADPH 12[39].

Epristeride **13** is another 5 α-reductase inhibitor which has a carboxy group at position 3, not observed in testosterone and DHT. It is this functional group which makes epristeride an interesting inhibitor, because it has a different pharmacophore. Moreover, epristeride significantly inhibits 5 α-reductase type II while it only slightly inhibits 5 α-reductase type I. It is used for treatment of benign prostatic hyperplasia.[6, 14, 40, 41].



13

Figure 10. Structure of epristeride 13.

1.5. Steroidal anti-prostate analogues

As part of previous work by Njar *et al.*, a library of steroidal imidazoles, 1,2,3-triazoles, and 1,2,4-triazoles were synthesized and tested against human and rat testicular $P450_{17\alpha}$. Compounds **14**, **15**, **17** showed potent inhibition with IC_{50} of 58 nM, 13 nM and 7 nM respectively. Compounds **16**, **17** and **18**, gave excellent 5 α-reductase inhibition, IC_{50}: 198 nM, 142 nM, 152 nM respectively[42].

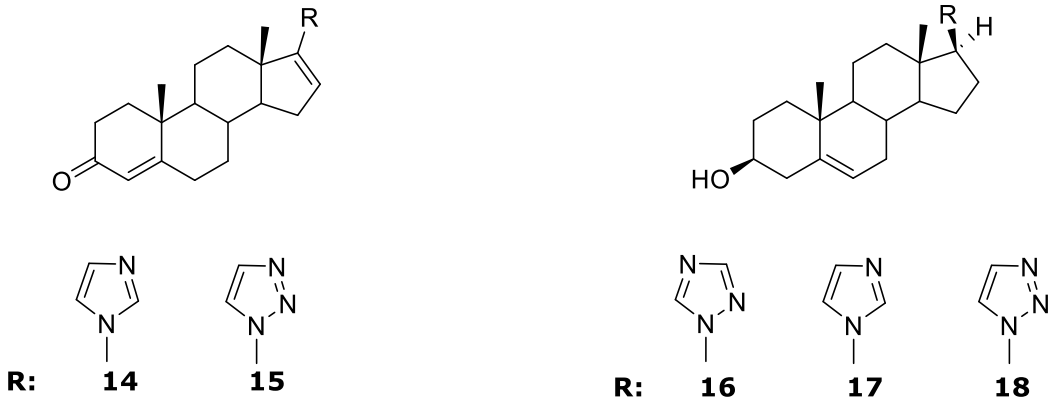


Figure 11. Structure of 14; 15; 16; 17 and 18.

A study by Penov-Gaši *et al.*, shown that one of the derivatives containing a tetrazole fused ring **19** was potent towards PC3 prostate cancer cells with an IC_{50} value of 15.3 μM compounds **20**, **21**, tetrazole

derivatives showed potent activity towards breast cancer inhibition with IC_{50} of 12.63 µM and 4.58 µM respectively[43].

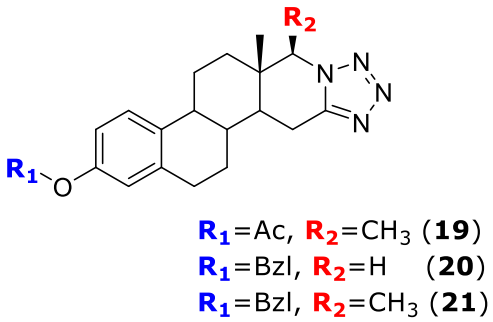


Figure 12. Structure of compounds 19, 20 and 21.

A study by Silva-Ortiz *et al.*, showed that three synthetic 6-dehydropregnenolone acetate derivatives with triazole rings, (**23**, **24**, **25**) gave IC_{50} values of 9.2 µM, 38.2 µM and 16.9 µM respectively on PC3 cells. The derivatives tested against 5 α-R1 inhibition, **22**, **24** showed excellent IC_{50} values of 88 nM and 320 nM respectively[44].

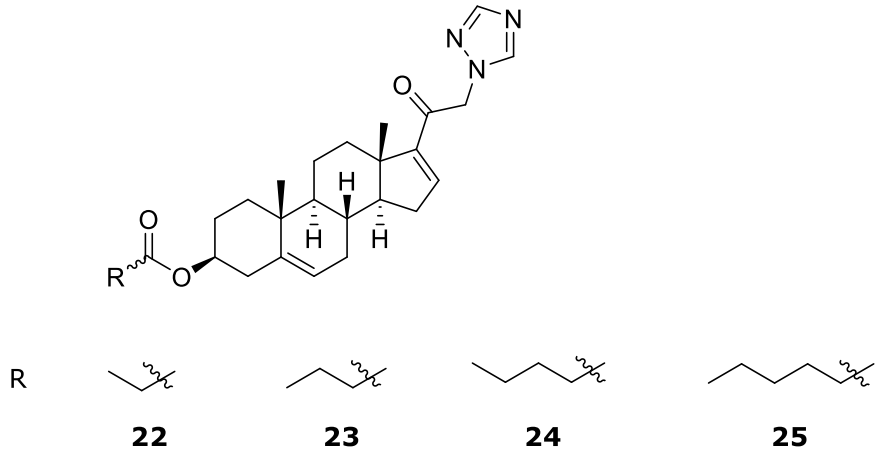
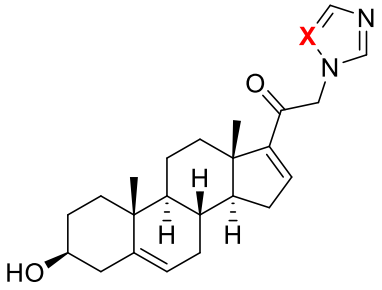


Figure 13. Structure of 22; 23; 24 and 25[44].

In a study by Silva-Ortiz *et al.*, synthesis of two novel pregnane derivatives with triazole and imidazole rings on the side chain **26** gave IC_{20} 310 nM and IC_{50} 17 µM on PC3 cells. However, derivative **27** gave an IC_{20} value of 4.3 nM and IC_{50} of 2 µM on PC3 cells. An *in silico* docking

study performed for the synthesized derivatives showed weak interaction with steroid receptors[45].



X= N, **26**
C, **27**

Figure 14. Structure of 26 and 27[45].

1.6. Medicinal Mushrooms

For hundreds of years mushrooms have been considered to be an important edible and medical resource[46]. Many molecules isolated from mushrooms have been identified as possessing a bioactive effect. Polysaccharides and triterpenes are regarded as the most active mushroom compounds with antitumour and immunomodulating features[46,47].

Mushrooms are considered as a valuable low-calorie food source. They also contain a high percentage of minerals, vitamins, fibres and essential amino acids. In general the gross composition of mushrooms is protein (2–40%), fat (2–8%), carbohydrates (1–55%), fibres (3–32%) and trace elements (8–10%) (mainly composed of salts, metals and so forth) with the remainder comprising water (up to 90%)[48].

Since the 1980s, medical and scientific researchers in Japan, China, Korea and the United States of America have increasingly demonstrated the efficient and unique features of mushroom-extracted compounds for the prevention and treatment of cancer. Contemporary clinical practice in Japan, Korea, China and other Asian countries still depends on mushroom derived preparations such as lentinan and schizophyllan (Figure 15)[49].

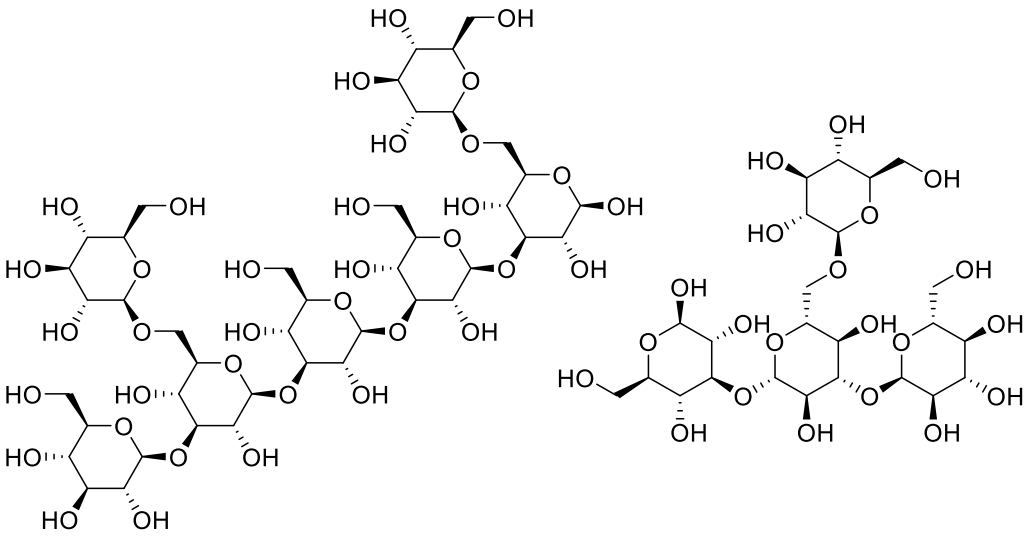


Figure 15. Structure of lentinan and schizophyllan[49].

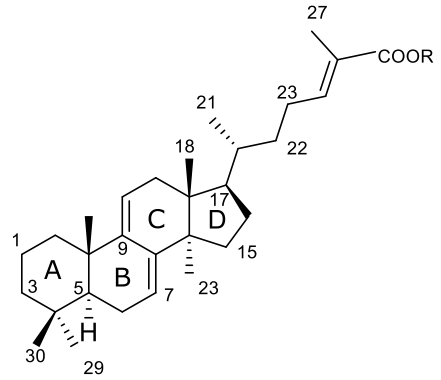
1.7. *Ganoderma lucidum*

Ganoderma is one of the natural sources of medicinal compounds that can be found mentioned in the earliest Chinese pharmacopeia. Ganoderma is also known as Lingzhi in Chinese[50] and Reishi in Japanese[51, 52]. In 1781, *Ganoderma lucidum* was described for the first time in the west by William Curtis based on materials grown in England and in 1934 in China.



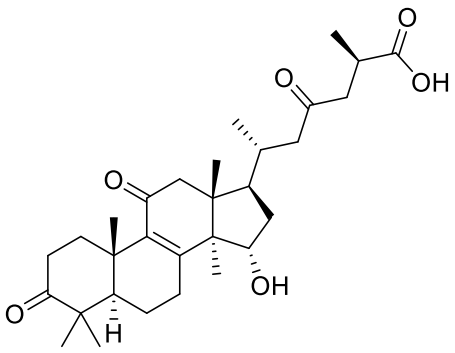
Figure 16. Ganoderma Lucidum fungus fruiting body[51].

The first scientific record of *Ganoderma lucidum* was made by Teng[53]. Various types of chemical compound with pharmacological activity have been isolated from the Ganoderma fruiting bodies and mycelium species. These include triterpenoids, polysaccharides, proteins, amino acids, nucleosides, alkaloids, steroids, lactones and fatty acids as well as enzymes. Triterpenoids and polysaccharides are considered as the essential medicinal components of *Ganoderma lucidum. Ganoderma lucidum* produces triterpenoids that contain mainly oxygenated functional groups on the lanostane skeleton **29** (Figure 17). More than 150 types of triterpenoid have been found in *Ganoderma* to date such as ganoderic, lucidenic, ganodermic, ganoderenic, ganolucidic and applanoxidic acids, lucidones, ganoderals and ganoderols such as Ganolucidic Acid A, Ganodermic acid S, Applanoxidic acid A and Ganoderenic acid A (Figure 18)[51].

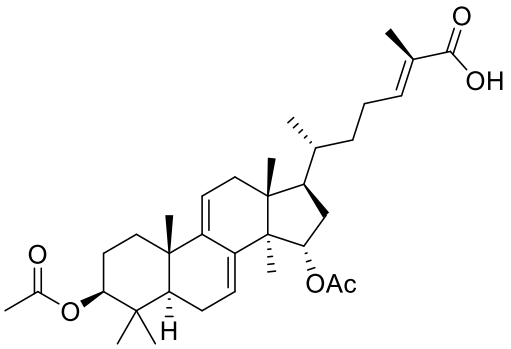


29

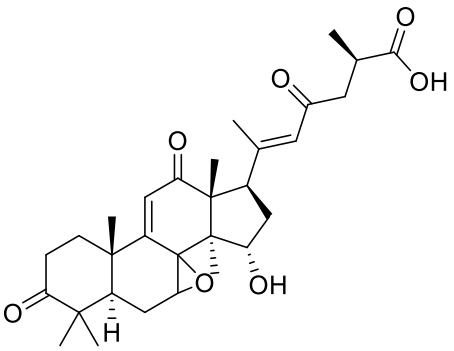
Figure 17. Lanostane A,B,C and D rings 29[54].



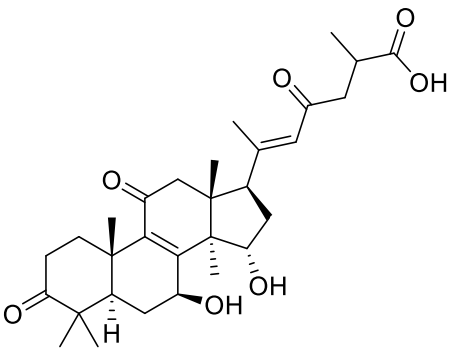
Ganolucidic Acid A



Ganodermic acid S



Applanoxidic acid A



Ganoderenic acid A

Figure 18. Ganolucidic Acid A, Ganodermic acid S, Applanoxidic acid A, Applanoxidic acid A[55].

Oxygenated triterpenoids with the side chains cleaved at C20 and C24 have been identified as well. Interestingly, mushrooms produce two isomers at C3 (α/β) which are stereoisomers and at C3/C15 which are positional isomers[56].

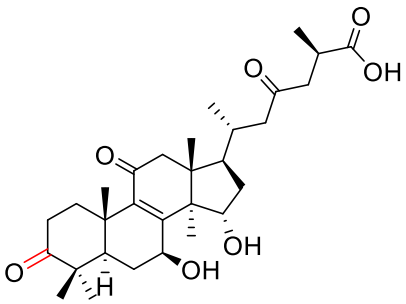
Ganoderma are commonly considered to be non-edible mushrooms due to their fruiting bodies being thick, corky, and tough[57]. An anticancer drug obtained from *Ganoderma lucidum* and produced in Japan is known by the name Krestin and is used to treat many cancer types, which accounts for 25 % of the world's total cancer drug marketing[47]. Different parts of *Ganoderma lucidum* mushroom have differing anti-cancer activities due to the different chemical compounds they contain[11, 53]. *Ganoderma lucidum* is used in Asia predominantly as a general health treatment; it can also be used as an anti-cancer agent in combination with chemotherapy treatment. Other medicinal uses would be as drugs to prevent obesity, reduce cholesterol, for the maintenance of gut health, reduction of hypertension, control of diabetes and the stimulation of probiotics amongst others[53].

Ganoderma lucidum has anti-cancer and anti-inflammatory therapeutic activity related to both direct and immune modulating properties[58].

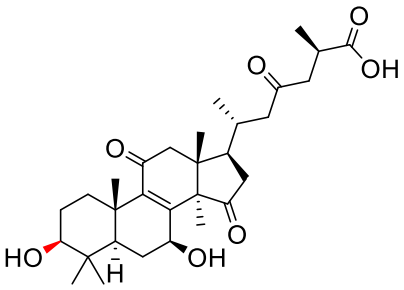
These have been tested against cell lines of most human and murine cancers including those of lung, liver, breast, prostate, colon, bladder and cervix as well as leukaemia and sarcoma. Polysaccharides and triterpenes are important bioactive components in producing the cytotoxic effect of *Ganoderma lucidum*[59].

1.8. Ganoderic acid

Ganoderic acids are a type of highly oxygenated C_{30} lanostane triterpenoid produced by *Ganoderma lucidum*[60]. The first isolation of ganoderic acids analogues were achieved by Kupota *et al.* in 1982, when they discovered and separated analogues: Ganoderic acid A (7β,15α-Dihydroxy-3,11,23-trioxo-5α-lanost-8-en-26-oic acid) **30** and Ganoderic acid B (3β,7β-Dihydroxy-11,15,23-trioxo-5α-lanost-8-en-26-oic acid) **31** (Figure 19) from *Ganoderma lucidum*[61]. Since then, more than 316 triterpenes including 68 ganoderic acids have been isolated from the fruiting bodies, spores, gills, and mycelia of several Ganoderma mushrooms[62].



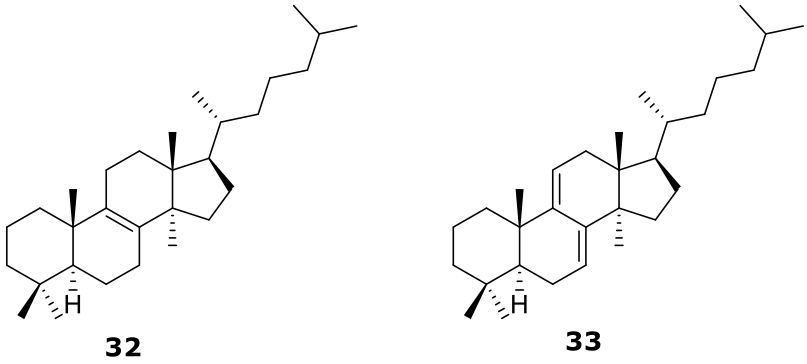
Ganoderic acid A **30**



Ganoderic acid B **31**

Figure 19. Structure of ganoderic acid A 30 and ganoderic acid B 31[62].

There are two generic structures for ganoderic acids which are Δ8(9)-lanostone and Δ7(8) **32**,9(11) **33**-lanostadiene (shown in Figure 20)[63].



32 **33**

Figure 20. 7(8) 32,9(11) 33-lanostadiene[54].

1.9. Ganoderic acids for treatment of cancer

Contemporary research mentions that the biological activity of particular ganoderic acid compounds such as GA-T **35** induces apoptosis of lung cancer cells, and GA-Me **34** inhibits tumour growth and lung metastasis[64]

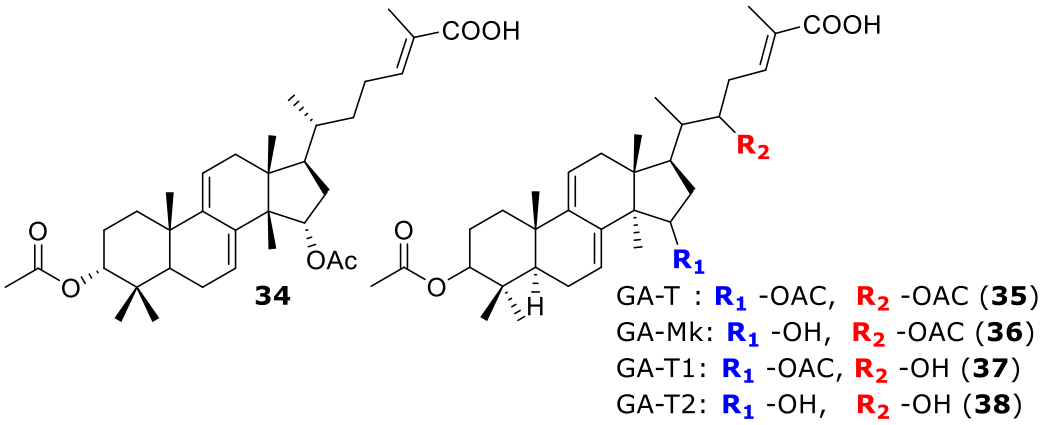


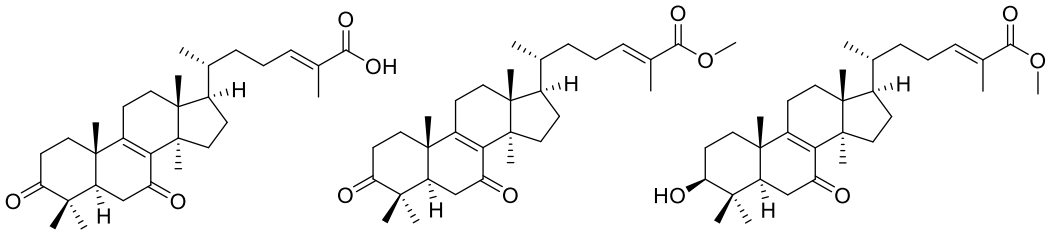
Figure 21. Structure of ganoderic acid Me 34; GA-T 35; GA-Mk 36; GA-T1 37 and GA-T2 38[64, 65].

Apoptosis and reactive oxygen species (ROS) production have been studied for four ganoderic acids: GA-T **35**, GA-Mk **36** and another two deacetylated derivatives of GA-T: GA-T1 **37** (3a,15abiacetoxy-22-hydroxy-5a-lanosta-7,9(11),24-trien-26-oic acid), and GA-T2 **38** (3α-

acetoxy-15a,22-bihydroxy-5α-lanosta-7,9(11),24-trien-26-oic acid). These were tested on the antioxidant defence system and were found to induce apoptosis *in vitro* in Hela cervical cancer cells lines. The order of strength of induced apoptosis, producing ROS and suppression of antioxidant defence system was: GA-T > GA-Mk > GA-T1 > GA-T2[65]. The modification of the C3 hydroxyl group by acetylation of GAs may contribute to the pro-oxidant activity of GAs and their cytotoxicity; this should be considered for future development of novel chemotherapy agents.

Ganoderic acid DM (GA-DM) **39** is considered to be the major component in *Ganoderma lucidum* and it has medicinal impact in different cases of anti-androgen and anti-proliferative activities. This study focused on the inhibitory effect of GA-DM **39** and its two analogues (Methyl GA-DM **40** and 7-oxo methyl GA-Z **41**) against osteoclastogenesis[66]. Osteoclastogenesis is a several-step procedure for cancer treatment by bone resorption and formation[67-69]. The results show that the blocking of osteoclastogenesis was achieved with 12.5 μM methyl GA-DM **40**; this was in conjunction with cytotoxicity lower than 30 %. Furthermore, the inhibition occurred at 50 μM GA-DM, which is a higher concentration than for methyl GA-DM. However, the 7-oxo methyl GA-Z (**41**) did not block the osteoclastogenesis even at a concentration as high as 100 μM. From the results of the study, it can be concluded that the carbonyl group at position C3 is quite important for inhibition, whereas the methyl group located on C26 is important to decrease the cytotoxicity to the osteoclast[66]. GA-DM showed varied

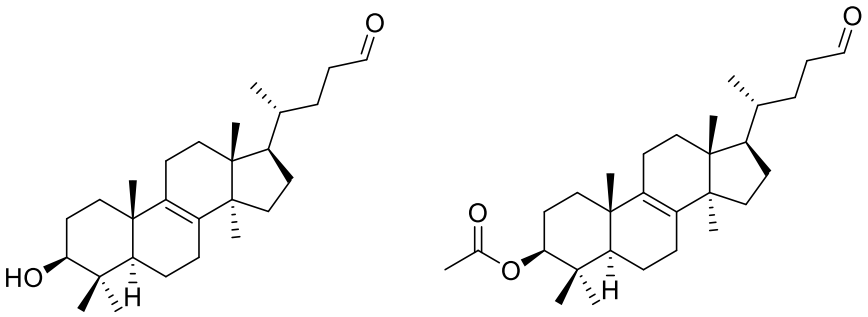
biological activates including inhibition of the 5α-reductase enzyme, cytotoxicity against prostate cancer cell lines, binding activity with the androgen receptor[70-73].



GA-DM **39** MeGA-DM **40** 7-oxo-MeGA-Z **41**

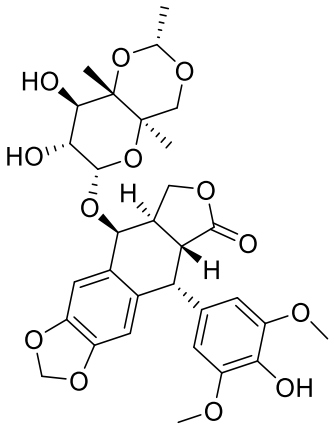
Figure 22. Structure of GA-DM 39, MeGA-DM 40 and 7-oxo-MeGA-Z 41[66].

New derivatives were prepared by O'Keeffe *et al.* using lanosterol **1** as a starting material to afford novel 24-amino-25, 26, 27-trinorlanost-8-enes. A variety of amines were used in the synthesis. The amino derivatives were prepared directly by reacting aldehydes **42** and **43** with various primary and secondary amines via reductive amination using sodium triacetoxyborohydride in 1,2-dichloroethane and the reactions proceeded to yield amines quickly. Also, no aldehyde reduction occurred. The cytotoxic and apoptotic potential against U937 cells was determined revealing excellent IC_{50} results for piperidine and 1-(2-hydroxyethyl)piperazine derivatives. Two of the compounds prepared had IC_{50} values of 1.9 μM and 2.7 μM in U937 cells, comparable to the marketed Etoposide **44** 1.5 μM.[74] Etoposide is used for treatment of different cancer kinds such as leukaemia, testicular cancer, and small-cell lung cancer[75].



42 **43**

Figure 23. Structure of aldehyde 42 and aldehyde 43[74].

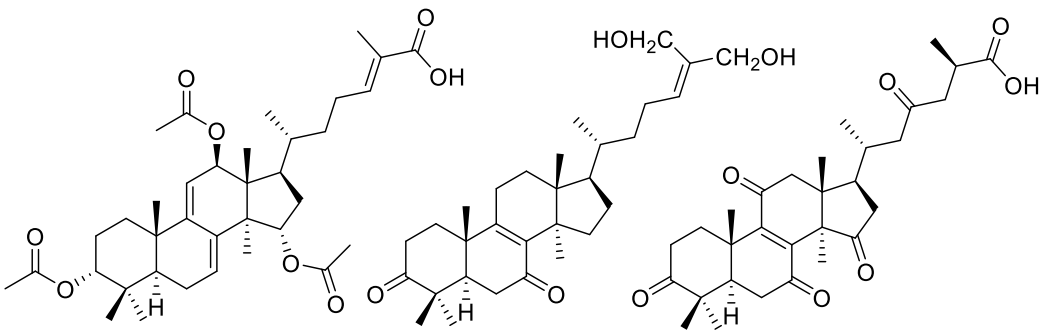


44

Figure 24. Structure of etoposide 44[75].

Separation of 18 compounds from a stock of *Ganoderma lucidum* mushrooms from Vietnam was performed in an anti-angiogenesis study. Two new lanostanes (one of them indicated as tri-acetoxy ganoderic acid), (3α,12β,15α-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid **45** and 5α-lanosta-8,24-diene-26,27-dihydroxy-3,7-dione) **46** with another sixteen known derivatives including 4 ganoderic acids, were isolated from the fruiting bodies of these mushrooms. Extensive structure characterization by IR, HR-EI-MS, 1D and 2D NMR analysis was performed. The cytotoxic activity of these compounds was tested against human non-small cell lung adenocarcinoma A549, breast

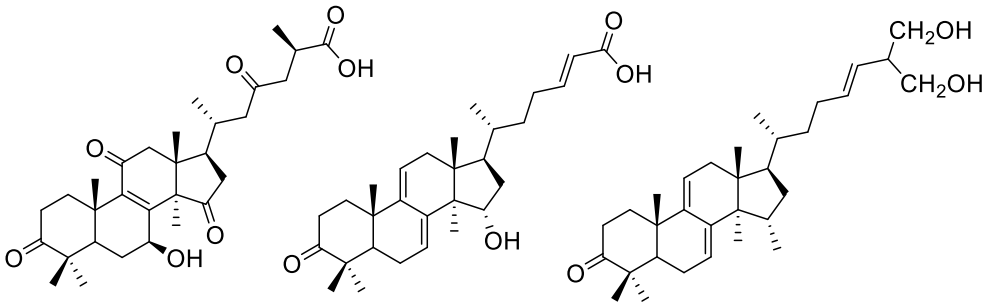
adenocarcinoma MCF7, and prostatic small cell carcinoma PC3. Compound **45** gave significant cytotoxic activity versus PC3 cells, with an IC_{50} of 11.5 μM. On the other hand, ganoderic acid F **47** was found to be the most active analogue to inhibit the formation of capillary-like structures of human umbilical vein endothelial cells, especially at concentrations between 3-30 μm[76].



45 **46** **47**

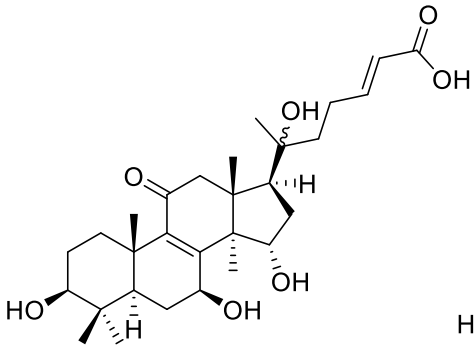
Figure 25. Structure of 45 , 46 , GA-F 47[76].

In a quantitative determination of five triterpenoid compounds, one was found to be ganoderma alcohol (ganodermatriol **50**) and the other four were ganoderma acids (GA-TR **48**, GA-DM **39**, GA-A **30**, and GA-D **49**). The study was undertaken at six different stages of growth of mushroom. In stage 5, ganoderic acids GA-DM **39** and GA-TR **48** were obtained with the highest concentration. These two also gave the strongest 5α reductase inhibitory activity with lower IC_{50} of 8.6 and 10.6 μM for ganoderic acid TR and ganoderic TM respectively. Conversely, other triterpenoids gave IC_{50} > 41.9 μM and this could be a good indicator of their activity in 5α reductase inhibition[77].

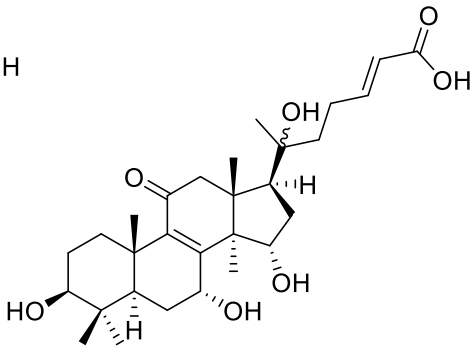


48 **49** **50**

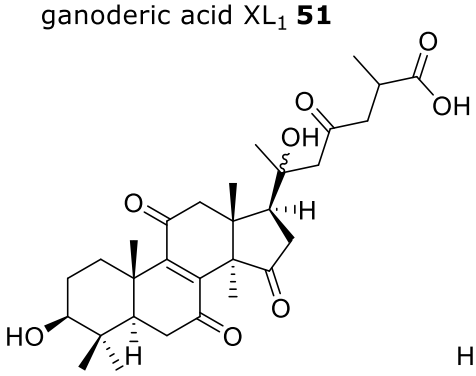
Figure 26. Structure of GA-TR 48, GA-D 49 and ganodermatriol 50[77].



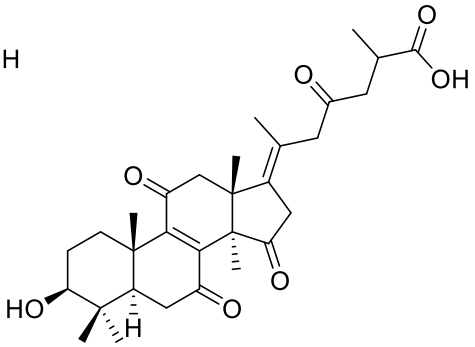
ganoderic acid XL_1 **51**



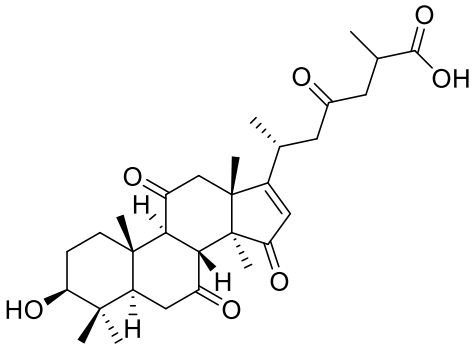
ganoderic acid XL_2 **52**



20-hydroxyganoderic acid AM_1 **53**



ganoderenic acid AM_1 **54**

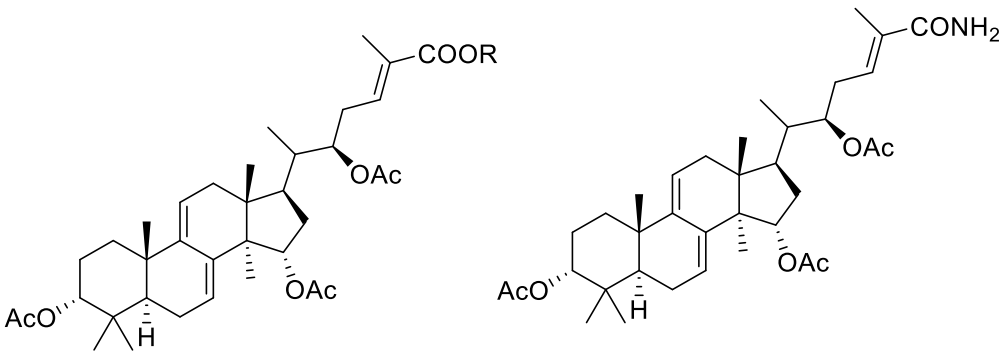


ganoderesin C **55**

Figure 27. Structure of ganoderic acid XL_1 51, ganoderic acid XL_2 52, 20-hydroxyganodeic acid AM_1 53, ganoderenic acid AM_1 54 and ganodersin C 55[78].

A hepatoprotective inhibitory study for five new ganoderic acids (GA-XL1 **51**, GA-XL2 **52**, 20-hydroxy Gap-AM1 **53**, GA-AM1 **54** and ganoderesin C **55** was performed, with another five known triterpenoids. Ganoderic acids **51**, **54** and **55** and two other analogues gave hepatoprotective inhibitory activity with a concentration of 10 μM[78].

In a clinical study for novel ganoderic acid derivatives, ganoderic acid-T (GA-T) **35** (22S,24E)-3α,15α,22-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid ethyl ester, (TLTO-Me) **56** (22S,24E)-3α,15α,22-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid methyl ester (TLTO-Ee) **57** (22S,24E)-3α,15α,22-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid, propyl ester (TLTO-Pe) **58** and (22S,24E)-3α,15α,22-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid amide (TLTO-A) **59** were tested for inhibitory effect and cytotoxicity. It was found that TLTO-A gave higher inhibitor activity and lower cytotoxicity for non-tumour cells when compared to the other derivatives. The reason for this may be due to the amino group at C26 which can undergo hydrogen bonding with intracellular macromolecules. The study concluded that the C26 carboxylic group is a potential modification site, but may not be the optimum position. The study results showed the following results for cytotoxicity: TLTO-A > GA-T ≈ TLTO-Me≈ TLTO-Ee ≈ TLTO-Pe[79].



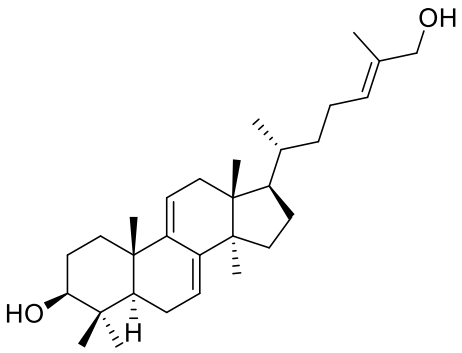
GA-T R: H **35**
TLTO-Me : R = CH_3 **56**
TLTO-Ee : R = Et **57**
TLTO-Pe : R = *n*-Pr **58**

TLTO-A **59**

Figure 28. Structure of GA-T 35, TLTO-Me 56, TLTO-Ee 57, TLTO-Pe 58 and TLTO-A 59[79].

Ganoderic acid DM **39** and four other analogues have been successfully extracted from *Ganoderma lucidum* by Jie *et al*. The extracted compounds have been tested for 5 α-reductase inhibition in PC3 cells. Methyl GA-DM **40** and GA-DM **39** showed the highest activity with IC_{50} values of 0.3 and 40 μM respectively. However, in addition to 5 α-reductase inhibition activity of GA-DM, it has other functions such as binding activity with the androgen receptor, with prostate cancer cell, and in proliferation and osteoclast differentiation.

Jie *et al.,* have reported the biological activity of ganoderol B **60** against 5 α–reductase as well as the ability of ganoderol B **60** to bind to the androgen receptor (AR) [80]. This may inhibit androgen-induced LNCaP cell growth and suppress regrowth of the ventral prostate induced by testosterone in rats. The down-regulation of AR signalling by ganoderol B provides an important mechanism for its anti-androgenic activity.



60

Figure 29. Structure of ganoderol 60.

In a docking study of the prostate cancer STAT3 pathway, Balraj *et al.* (2016) evaluated the antioxidant potential of ganoderic acid A **30**. The study was performed by using GLIDE 9.6 for different molecular docking isoforms and ADMET activities. Ganoderic acid A inhibits proliferation, viability, ROS, DPPH and the expression of superoxide dismutase SOD1, SOD2, and SOD3 measured by real-time PCR in a PC3 cell in a dose-dependent manner. Moreover, the binding mechanism of ganoderic acid A was checked in fifty different isoforms against STAT3, signalling has been explained through the molecular docking which inhibits the proliferation, viability and ROS in PC3 cells. Curcumin **61** was used as a natural activator in the modelling software with ganoderic acid A through the docking process. The molecular docking showed that protein-ligand interactions were between ganoderic acid and Gly 583 and Glu 582 of STAT3 while Gln 524, Arg 518 of STAT3 were involved in curcumin binding[81].

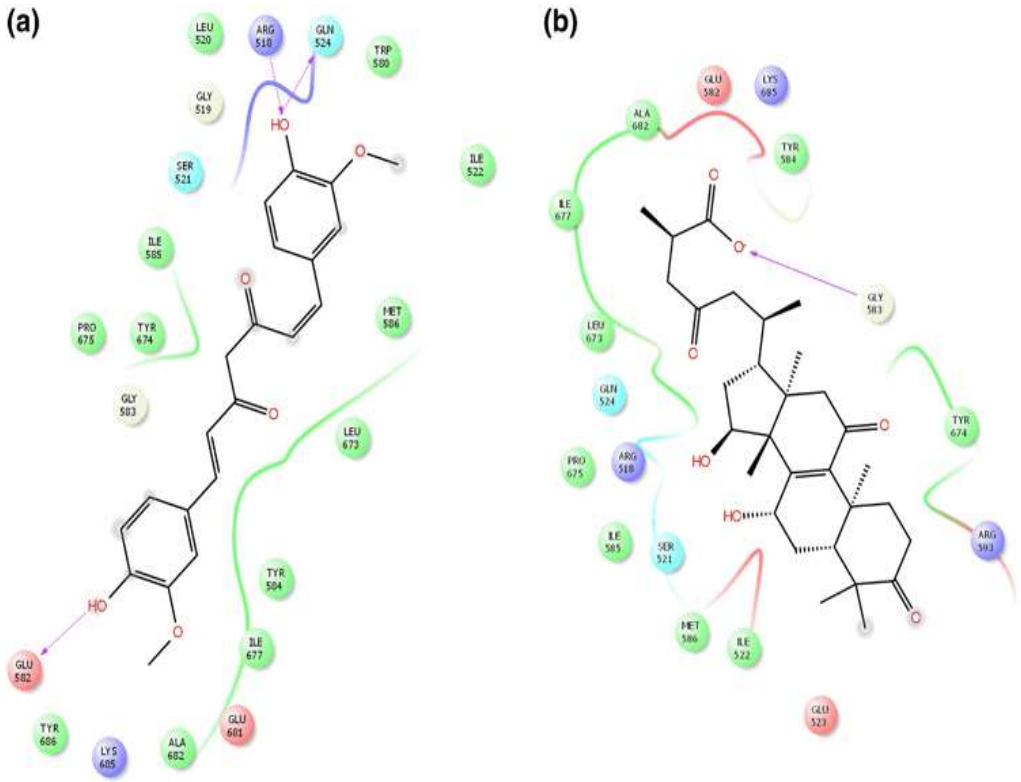
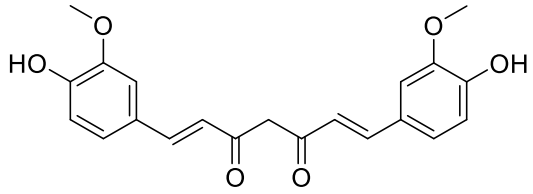


Figure 30. Reproduced from Gill *et al.* "Receptor-based molecular docking protein–ligands interactions profile of STAT3/DNA with ganoderic acid A 30 and curcumin 61. Protein ligand interactions revealed the involved in ganoderic acid A binding acid were Gly 583 and Glu 582, while Gln 524, Arg 518 were involved in curcumin binding"[81].



61

Figure 31. Structure of curcumin 61.

1.10. Summary for the best human 5 α-reductase inhibitors

Table 3. Best 5 α-reductase inhibitors[42, 77, 82, 83].

Compound	5 α-reductase inhibition type	5 α- reductase IC_{50}
Finasteride **1**	type I type II	360 nM 69 nM
Dutasteride **2**	type I type II	6 nM 7 nM
Epristeride **13**	type I type II type III	1.6 μM 0.18 nM 160 nM
Ganoderic acid DM **39**	Not specified	10.6 μM
ganoderic acid TR **48**	Not specified	8.5 μM
5a-Lanosta-7,9(11),24-triene-15a,26-dihydroxy-3-one	Not specified	41.9 μM
16	Not specified	198 nM
17	Not specified	142 nM
18	Not specified	152 nM

1.11. Aim and Objectives

The main aim of this project was to synthesise ganoderic acid (GA) analogues starting from lanosterol **1** in high yield and purity and biologically evaluate them against androgen-independent prostate cancer cell lines. Aldehydes **42** and **43** were used as key intermediates to prepare analogues by reactions including Wittig olefination, reductive amination, dehomologation or allylic oxidation, and to evaluate the biological activity of these.

Modifications on the key aldehydes **42** and **43** generally included formation of the β-hydroxyl, ketone and acetate variants at C3, in addition to modification of the B/C ring to generate a dione. Finally, modifications of the C24 side chain were performed including alterations to chain length and functionality.

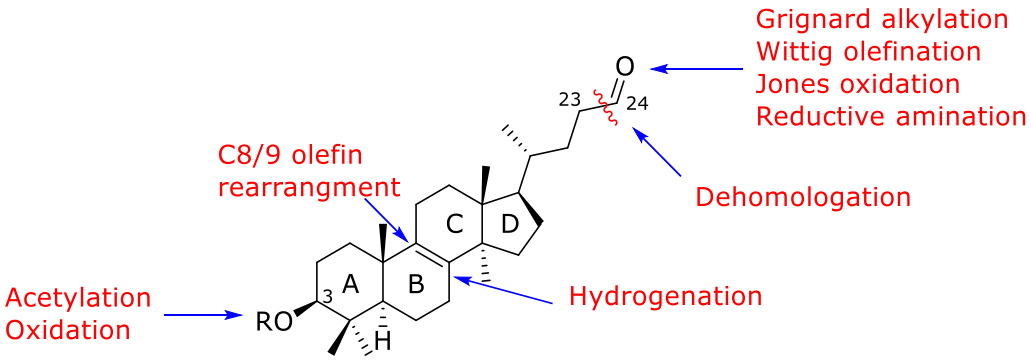
The second part of the project was to evaluate the biological activity of the synthesised derivatives and check their biological activity against multiple prostate cancer cell lines by using a cell survival assay. Prior to this evaluation it was necessary to test the solubility of the compounds in different solvents, to identify a good solvent in which the compounds could be effectively dissolved.

The objectives were:

- Synthesis of pure intermediate compounds in a high yield.
- Synthesis of key aldehyde intermediates with varied side chain length via Sharpless dihydroxylation route or dehomologation of aldehyde **43** via enamine route[84, 85].

- Synthesis amines with hydroxyl and acetate C3 analogues.
- Synthesis of GA-DM analogues with ketone, hydroxyl and acetate on C3.
- Synthesis of triazoles with varied side chain length on the triazole ring.
- Find the optimal convenient solvent (vehicle) to dissolve the compounds in order to assess their biological activity.
- Assess the biological activity for the synthesized compounds by MTT cell metabolic activity assay against prostate cancer cell lines PC3 and DU145.

  Based on the SAR for the bioactive compounds, synthesize more analogues in order to determine the functional groups that may affect the activity.



R H: **42**
Ac: **43**

Figure 32. Proposed modifications on the key aldehydes 42 and 43.

2. Results and Discussion

2.1. Ganoderic acid analogues

GA-H analogues were synthesized by modifying the side chain of lanosterol which has the same lanostane generic structure as GA-H **62**[86-88]. High purity lanosterol ≥93% is commercially available, but it is very expensive (£93.40 per milligram 2019, Sigma Aldrich) to use in synthetic preparations, so the low purity lanosterol (50-60%) which is also available at £1.36 per gram, (2019, TCI), is used. Lanosterol (50-60%) known as isocholesterol, is a mixture of sterols obtained from the non-saponifiable composition of the raw wool fat of sheep it includes (50-60%) lanosterol **1** (5α-lanosta-8,24-dien-3β-ol), (30-20%) dihydrolanosterol **63** (5α-lanost-8-en-3β-ol) , and small amounts of the conjugated dienes ~(10%) agnosterol **64** (5α-lanosta-7,9(11),24-trien-3β-ol) and dihydroagnosterol **65** (5α-lanosta-7,9(11)-dien-3β-ol) (as shown in Figure 33)[89, 90, 91].

It is difficult to purify lanosterol in high yield from the crude lanosterol (50-60%) due to the similarity in the physical and chemical properties of analogues in the crude mixture. A number of lanosterol purification systems have been recommended in the literature [89], nonetheless, their suitability may be limited by the use of toxic chemicals in the preparation, in addition low yields are common[89, 90, 91]. A method has been reported by O'Keeffe *et al.* in 2015 by acetate protection of **1** followed by epoxidation with *m*-CPBA in order to separate the dihydrolanosterol. The reported route has the advantage of lower costs, lower toxicity and higher yield to obtain lanosterol in purified form by

using the commercially available mixture to prepare the aldehyde intermediate **42**[74].

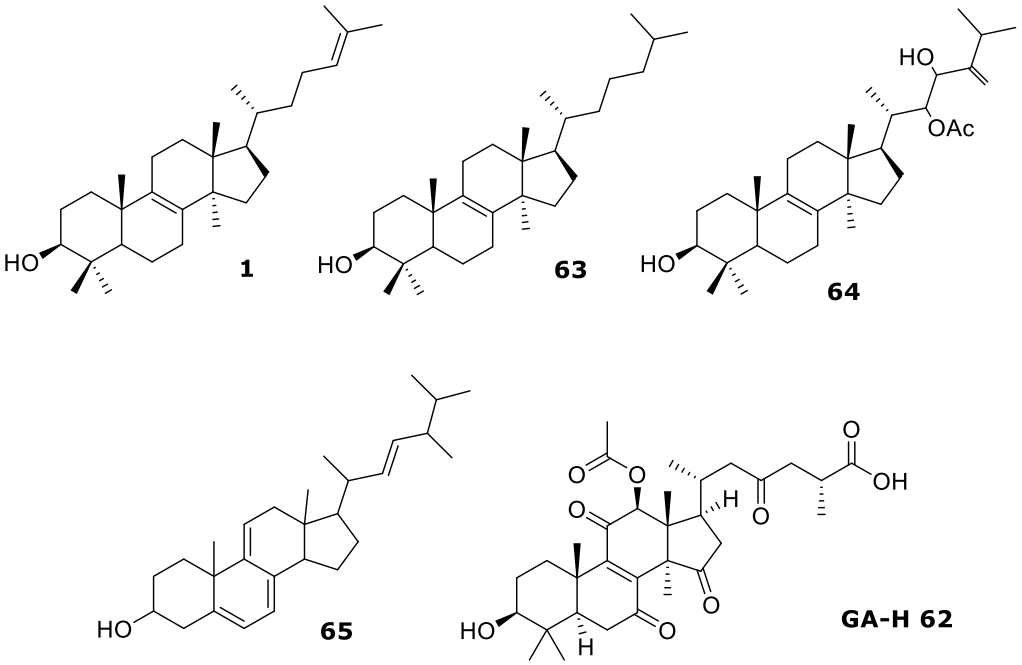
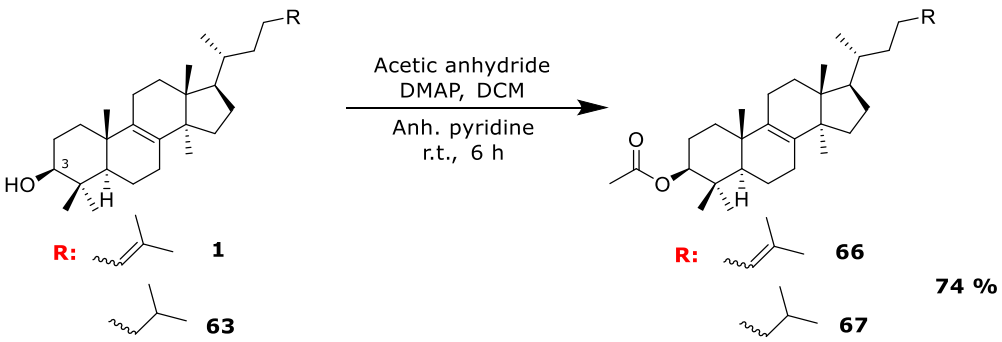


Figure 33. GA-H 62 structure and main constituents of isocholesterol: lanosterol 1 54%, dihydrolanosterol 63 36%, agnosterol 64 and dehydroergosterol 65 10%[90, 88, 91].

2.2. Acetylation of lanosterol 1

Lanosterol **1** has a β-hydroxyl at C3 which was acetylated using acetic anhydride **65** and dimethylaminopyridine (DMAP) as a catalyst in the presence of pyridine, producing a mixture mainly consisting of lanosterol acetate and dihydrolanosterol acetate in a yield of 74%. The acetate mixture was inseparable by column chromatography (diethyl ether/petroleum ether 1:39). The crude mixture of acetates can be carried on to the next step which is formation of an epoxide ring on C24/C25 via using *m*-CPBA. The C24/C25 epoxide intermediate was converted to the desired aldehyde **42** in 74% yield.



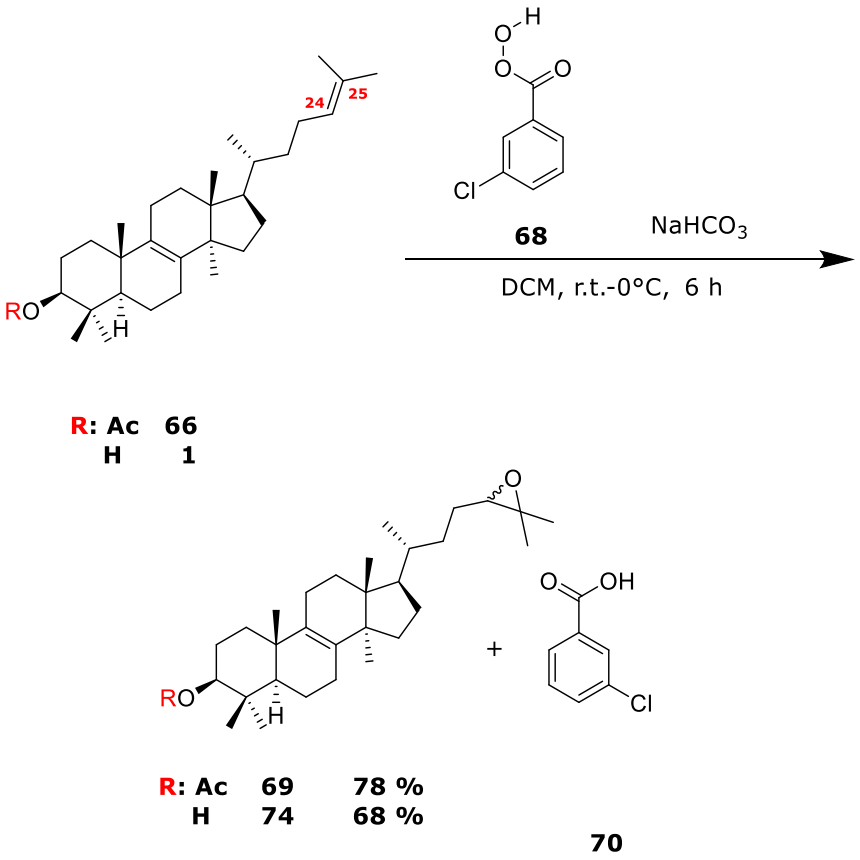
Scheme 3. Lanosterol 1 and dihydrolanosterol 63 acetylation.

2.3. Epoxidation of lanosterol acetate 66

The crude lanosterol acetate **66** and dihydrolanosterol acetate **67** mixture underwent epoxidation at C24/C25 by two steps of addition of *m*-CPBA **68** and $NaHCO_3$ through a Prilezhaev reaction to generate an inseparable mixture of 3β-acetoxy-5-α-24,25-epoxy-lanost-8-ene **69** and dihydrolanosterol acetate **67**.

The Prilezhaev reaction occurs more rapidly with compounds which are activated by electron donating groups. For lanosterol, the alkene located at C24/C25 is tertiary with two methyl groups donating electron density and thus increasing the rate of reaction. The solvent that was used was aprotic; therefore no hydrogen bonding will occur between *m*-CPBA and the solvent which may lead to a slower rate of reaction.

The epoxidation of C3 hydroxyl lanosterol **1** was undertaken by following the same reaction condition, unfortunately the crude was inseparable by column chromatography.

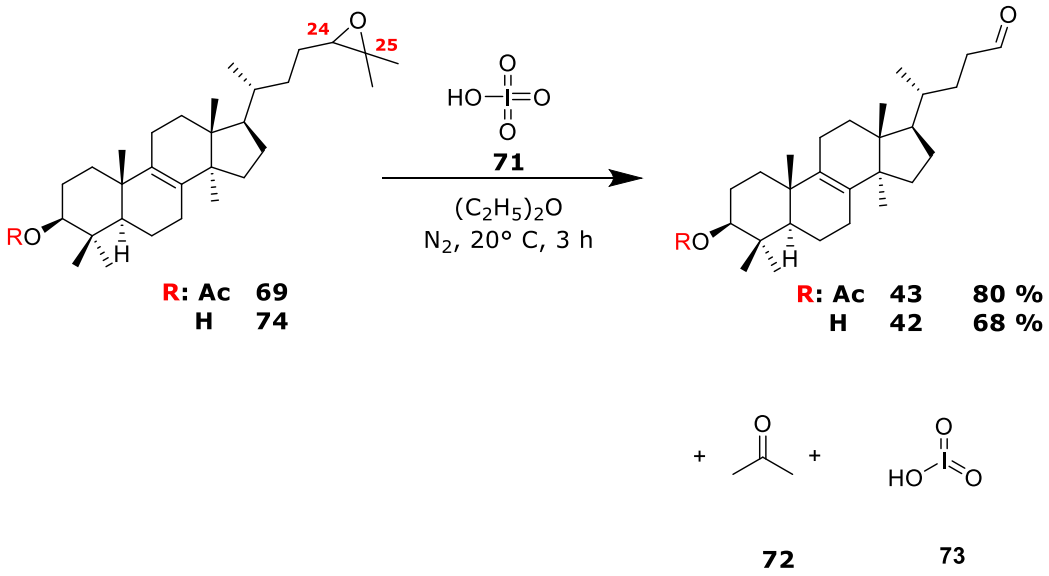


Scheme 4. Lanosterol C3 hydroxyl and C3 acetate C24/25 epoxidation

2.4. Oxidative cleavage of epoxides 69 and 74

The mixture of 3β-acetoxy-5-α-24,25-epoxy-lanost-8-ene **69** epimers and dihydrolanosterol acetate **67** was then carried through to an oxidative cleavage using periodic acid **71**. The reaction occurred via cyclic per iodate ester, the rearrangement happened due to cleavage of the C24/C25 epoxide ring. The resulting compounds products of the cleavage are: acetone; iodic acid HIO_3 **73** and the desired aldehyde **43** in a yield of 80 %. In this step the aldehyde was separated from the dihydrolanosterol epoxide disteroisomers **69** by column chromatography.

The mixture of 24(R,S)-3β-hydroxy-24,25-epoxy-5α-lanost-8-ene **74**, underwent oxidative cleavage by using periodic acid **71** and gave the pure aldehyde **42** in 68 % yield.



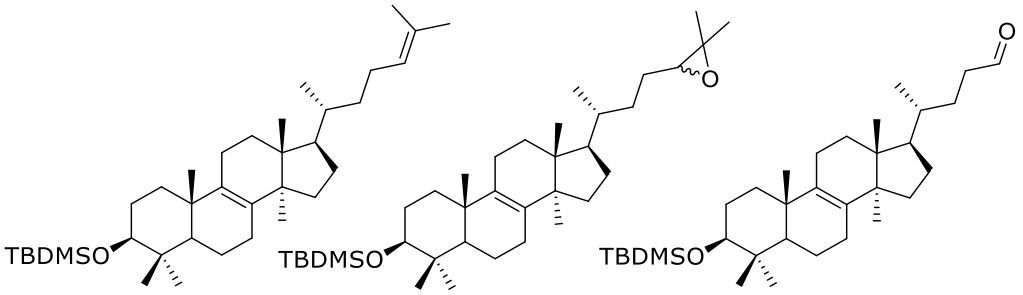
Scheme 5. Oxidative cleavage of epoxide to form the aldehyde 43.

2.5. 3-β hydroxyl protection

The hydroxyl group at 3-β was protected with *tert*-butyldimethylsilyl chloride (TBDMSCl group) to achieve the highest possible yield in the Grignard reaction. Grignard reagents act as nucleophiles in alkylation reaction with aldehydes. Furthermore, the Grignard reagent is also a strong base and can react with acidic hydrogens to abstract a proton from the hydroxyl group.

The reaction conditions for the protection were optimised through examination of the different procedures achieving a range of yields (15-80%). The protection was performed on lanosterol **1** and on aldehyde **42**, following 7 different procedures (see Table 4), the best conditions

identified for both was using DMF as a solvent; 3.73 equivalents of imidazole and warm reaction at 40 °C.



75 **76** **77**

Figure 34. Structure of 75, 76 and 77.

Reactant	**1**	**1**	**1**	**74**	**42**	**1**	**1**
Product	**75**	**75**	**75**	**76**	**77**	**75**	**75**
Conditions	DMF, 1.27 eq. imidazole r.t..	THF, Imidazole , DIPEA	DCE, imidazole, r.t.	DMF, 3.7 eq imidazole 40°C.	DMF, 1.27 eq. imidazole r.t..	DMF, 3.7 eq. imidazole r.t..	DMF, 3.7 eq. imidazole 40°C.
comments	Reaction was slow, 1.18 extra equivalent from the base added after 19h, reaction period	Reaction failed.	Reaction gave low yield	Reaction gave mixture of compounds	High percentage of yield achieved by this reaction, although reaction time was longer (42 h)	Reaction gave good percentage of yield, reaction time was longer (41 h)	High yield percentage , reaction was fast (24 h)
Yield%	**52**	**-**	**15**	**-**	**80**	**64**	**85**

Table 4. C3 Hydroxyl protection with acetate and TBDMS-Cl.

2.6. THP protection of lanosterol 1

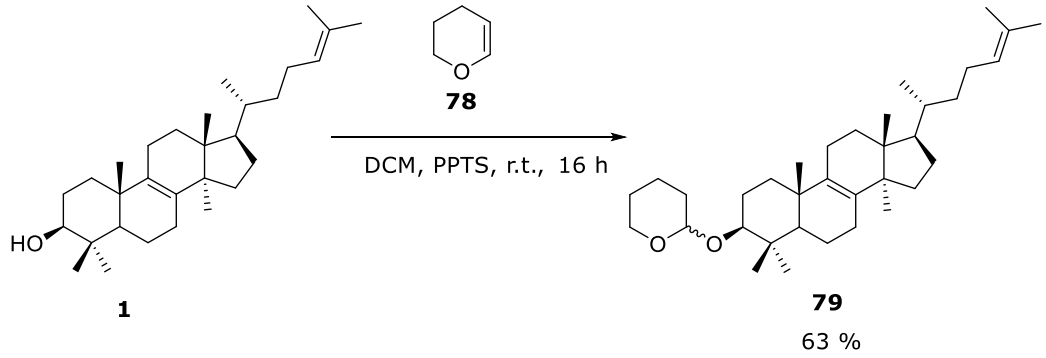


Figure 35. THP protection of lanosterol 1.

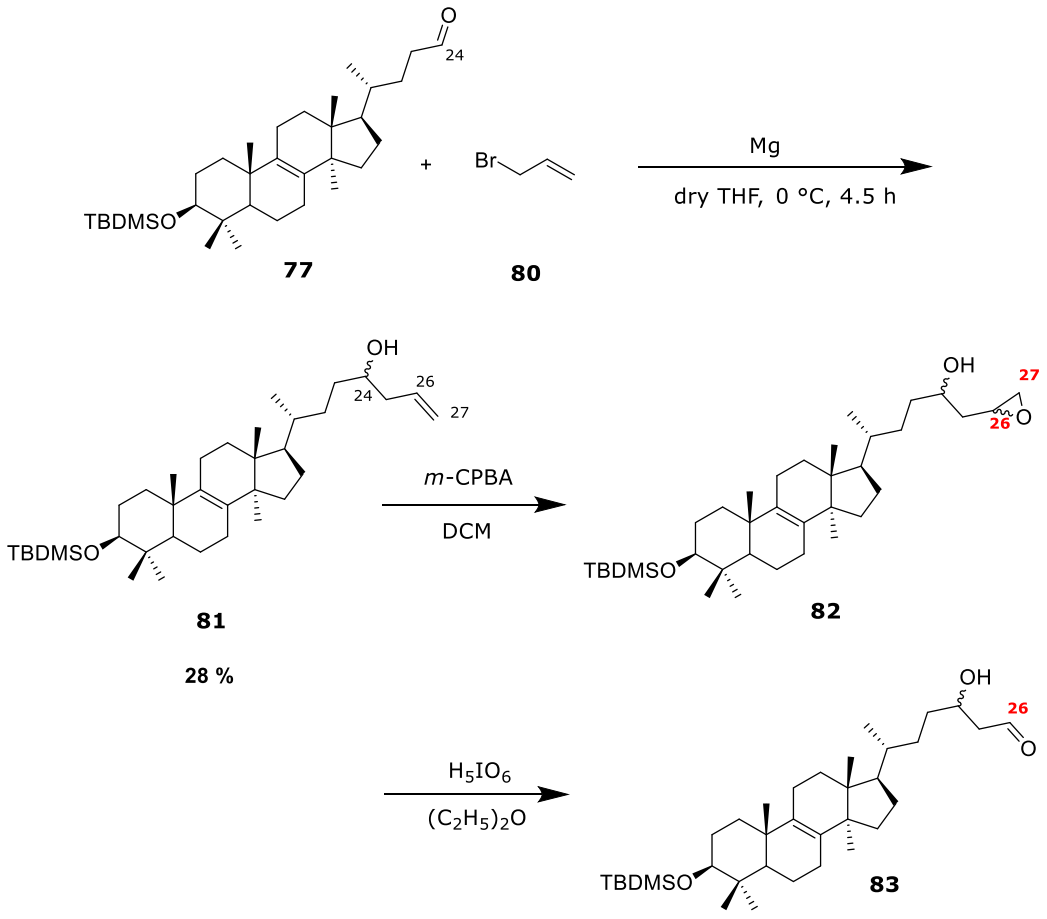
The C3 hydroxyl of lanosterol was protected successfully with THP in 63% yield. Tetrahydropyranyl (THP) ether is a good protecting group for alcohols, and is stable towards strongly basic conditions, hydrides, acylating reagents and alkylation reagents. THP ethers form under acidic conditions and are deprotected by acidic hydrolysis[92]. Lanosterol **1** was tetrahydropyranylated in the presence of pyridinium *p*-toluenesulfonate (PPTS) producing protected lanosterol **79** as diastereomeric mixtures in 63% yield because the starting alcohol possess a stereogenic centre. However the diastereoisomers were inseparable by flash column chromatography.

2.7. Alkylation of aldehyde 77

The alkylation reaction was attempted on aldehyde **77,** both with and without the protection of the 3-β hydroxyl group. The reaction failed with the unprotected hydroxyl group, whereas the TBDMS protected hydroxyl gave a yield of 28% of the β-hydroxyl alkene with elongation of the side chain. The reaction was performed by using allyl magnesium

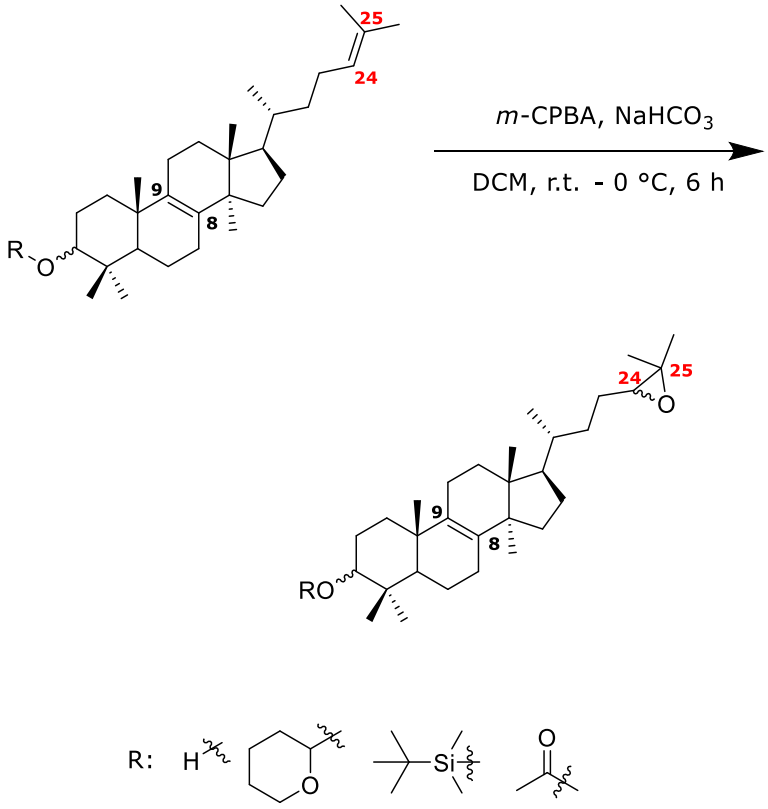
bromide **80** as the alkylating reagent. The Grignard reagent was prepared *in situ* before addition of aldehyde **77** using allyl bromide and magnesium turnings activated by iodine crystals. The reaction was successful in yielding the racemic homo allylic alcohol in 28%

Compound **81** can undergo further reaction with *m*-CPBA in the Prilezhaev reaction to form a major product or only one product of *syn* epoxide **82** due to the hydrogen bonding of the β-hydroxyl group[93]. This epoxide can be cleaved in the presence of periodic acid **71** to give an aldehyde **83** on the C26 atom with a chain that is two carbon atoms longer than the starting aldehyde **77**.



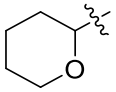
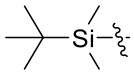
Scheme 6. Synthesis of aldehyde 83.

2.8. *m*-CPBA epoxidation of the protected lanosterol



m-CPBA was used to introduce an epoxide on to the side chain alkene at C24/C25. However, *m*-CPBA is a nonselective epoxidation reagent, another epoxidation also took place on C8/C9 and gave the di-epoxide by-product. The desired epoxide could not be successfully purified from the by-products. In order to get better separation, different protection groups were investigated.

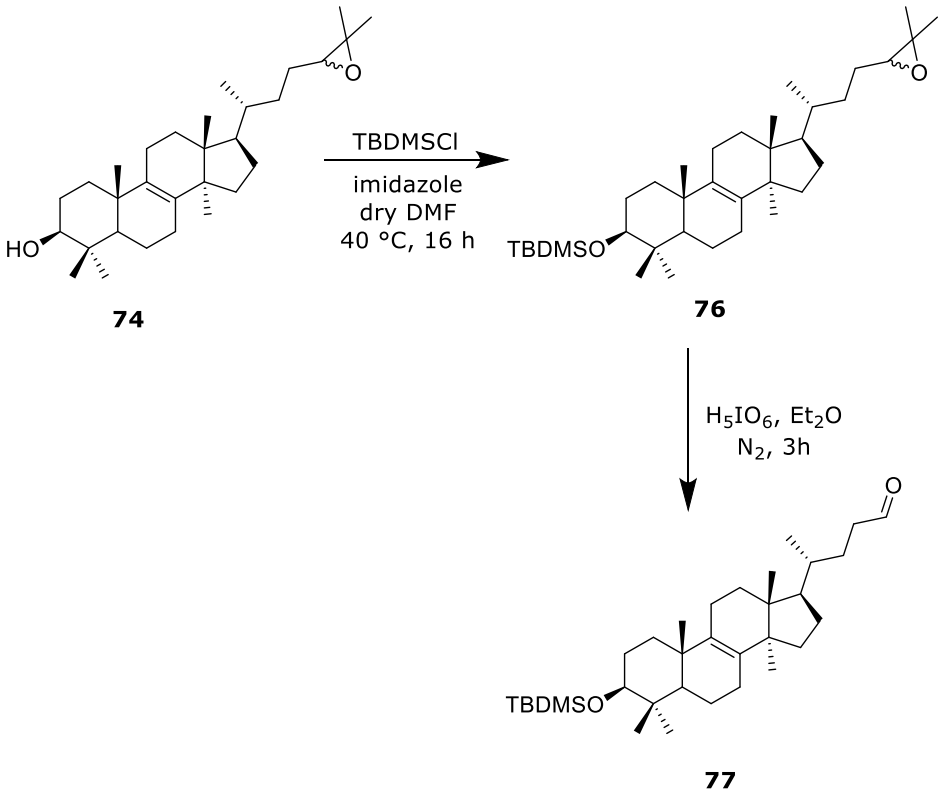
Table 5. Side chain epoxidation with different C3 protection.

R	Yield %	Comments
	67	Inseparable by-products
	41	Inseparable by-products
	-	Inseparable even with neat hexane
	78	Inseparable by-products

The purification of the crude was attempted, but all the crude compounds came out at the same time even with neat *n*-hexane as eluent in the column chromatography. Further purification carried out by recrystallization using *n*-hexane/DCM, did not yield the pure product.

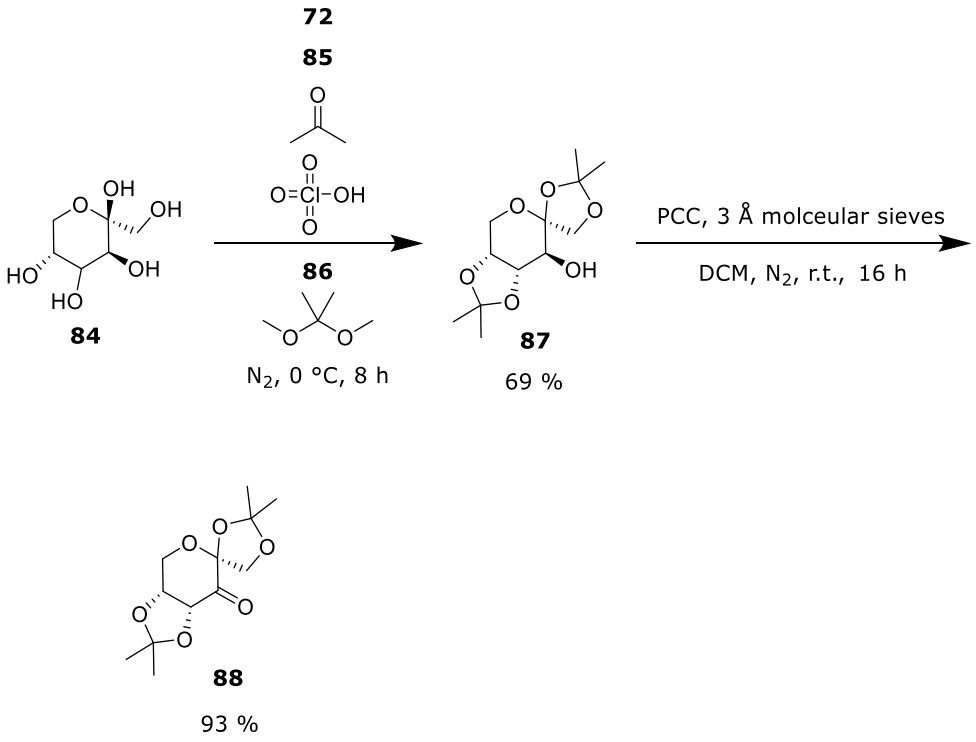
2.9. Synthesis of aldehyde 77 via the TBDMS route

Another route to make the aldehyde **77** was investigated by protecting the epoxide **74** via TBDMS. Prilezhaev epoxidation of lanosterol yielded epoxide **74** combined with some inseparable by-products. Conversion of the epoxide **74** to a TBDMS silyl ether protected epoxide **76** followed by oxidative cleavage gave an inseparable mixture of products.



Scheme 7. Synthesis of aldehyde 77.

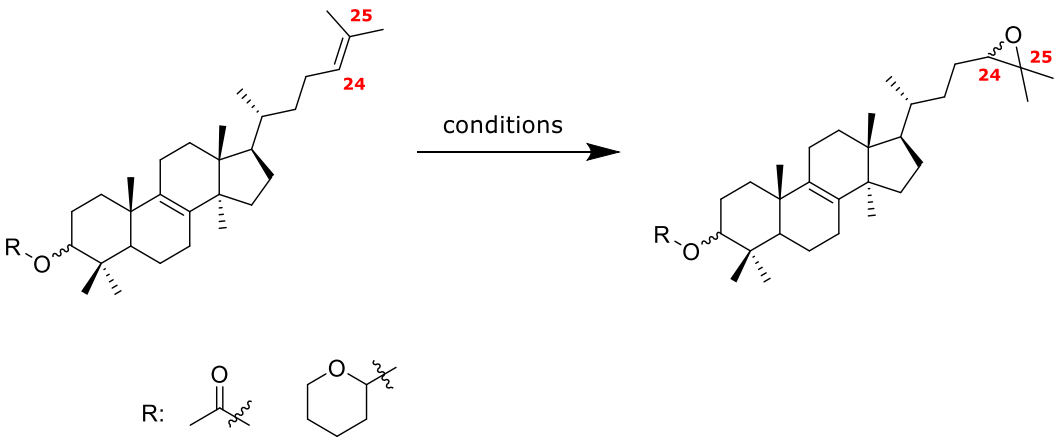
2.10. Synthesis of oxone chiral catalyst 88



Scheme 8. Synthesis of ketone 94.

The chiral catalyst **88** was prepared from the inexpensive starting material D-fructose over two steps. The first step was acetal protection of the hydroxyl groups followed by oxidation of the unprotected hydroxyl group by using Pyridinium Chloro Chromate (PCC) in an overall yield (69%). Ketone **88** was then used in asymmetric epoxidation with oxone. Ketone **88** has unique features; excellent stereochemical communication between the substrate and the **88** because of the reacting centre is close to the stereogenic centres. There is low possibility of epimerization of the stereogenic centers because the quaternary centre α to the carbonyl group and due to the presence of the fused ring. Moreover there are limited competing approach possibilities because one face of the catalyst is sterically blocked[94, 95].

2.11. Oxone Epoxidation of protected lanosterol

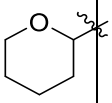


Scheme 9. Oxon epoxidation of protected lanosterol.

As shown in Table 6, eleven different trials of two different protected substrates was carried out using a Shi epoxidation with the aim of synthesising the C24/C25 epoxy lanosterol analogue. Five different ratios of catalyst investigated were carried out to identify the optimum catalyst loading conditions for the reaction (10%, 15%, 20%, 25%, and

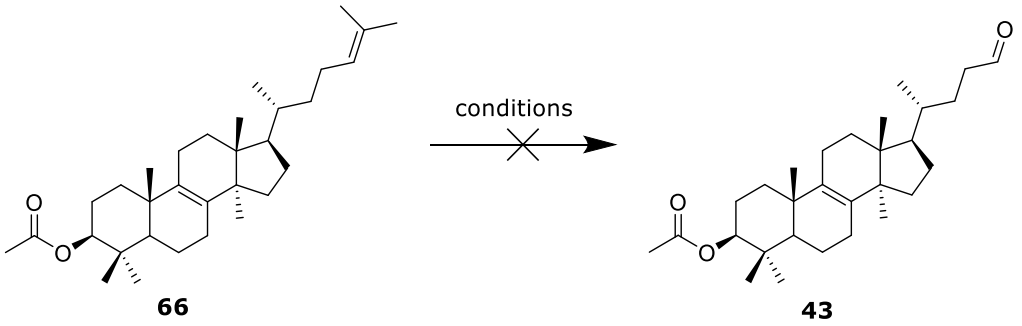
30%) for 30 min. Mass spectrometry detected the product but NMR did not show any significant peaks for the product.

Table 6. Screening of oxone epoxidation of 63 and 89.

R	Conditions	Yield %
	10-30% **88**, oxone, K_2CO_3, EDTA-Na_2, TBAA, MeCN, DME, r.t., 30 min	-
	5% **88**, oxone, K_2CO_3, EDTA-Na_2, TBAA, MeCN, DME, r.t., 16 h	-
	10% **88**, oxone, K_2CO_3, EDTA-Na_2, TBAA, MeCN, DME, r.t., 16 h	-

The reaction time was extended to 16 h using 10% w/v of ketone catalyst, however the reaction did not yield the desired product, therefore the reaction investigation was carried out using three different solvents; THF; 1,4-dioxane and EtOAc with the acetate protected substrate for 16 h reaction time. The solvent screening reactions did not yield the desired product. Another reaction were carried out using THF as solvent with THP protected substrate for 16 h, the crude analyzed by mass spectrometry and NMR showed no product formed. The reaction failed to yield the desired product. The reactions were carried in organic/aqueous phase using oxone, chiral ketone catalyst, K_2CO_3 aqueous solution, EDTA-Na_2 and tetrabutylammonium acetate (TBAA). The reason for choosing this method is the low cost and low toxicity of oxone, as well as the chiral ketone catalyst **88**. All the trials failed to produce the epoxide.

2.12. Ruthenium oxidative cleavage of 63



Scheme 10. Ruthenium oxidative cleavage of 66.

Table 7. Screening of 66 epoxidation by $RuCl_3.3H_2O$:$RuCl_3.3H_2O$:$NaIO_4$ equivalents to the starting material. * $NaIO_4$ can react to form the aldehyde or the $RuCl_3.3H_2O$ can react to form the diol first then react again with the diol to form the aldehyde.

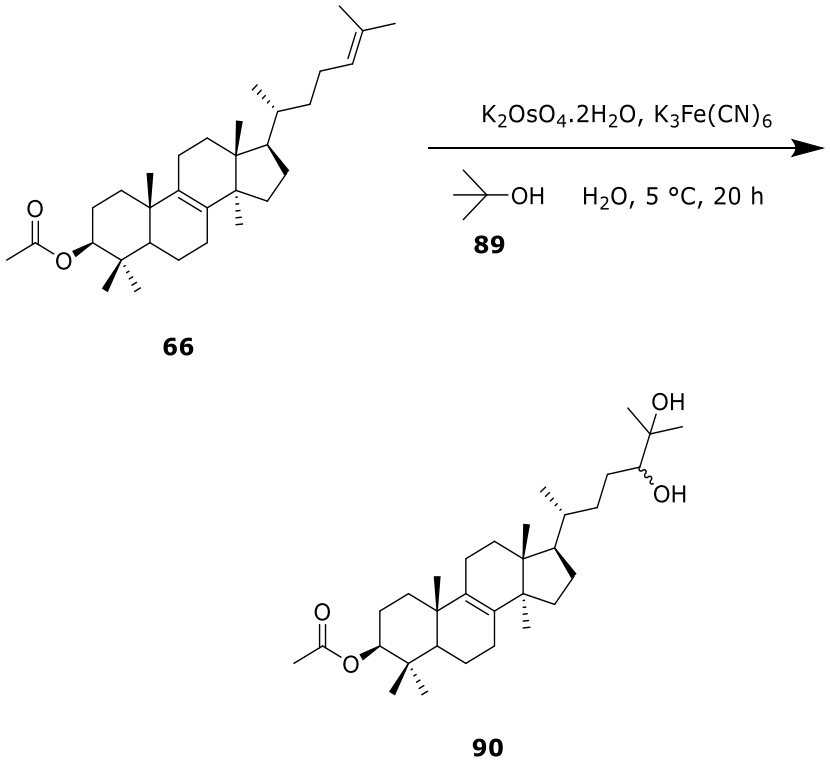
$RuCl_3.3H_2O$:$NaIO_4$*	Solvent	Conditions
0.04:3.94	CCl_4:MeCN 1:1	16 h, r.t., N_2
0.04:3.94	CCl_4:MeCN 1:1	60 h, r.t., N_2
0.04:3.94	CCl_4:MeCN:H_2O 1:1:0.3	16 h, r.t., N_2
0.04:3.94	CCl_4:MeCN:H_2O 1:1:2	16 h, r.t., N_2

In addition to the previously described approaches, an alternative synthetic pathway was investigated using sodium periodate as a co-oxidant and ruthenium chloride trihydrate. As ruthenium is a large metal, it works selectively on less sterically hindered alkenes[96]. The aim of using this catalyst was to perform selective dihydroxylation on the side chain alkene and not on the ring alkene, followed by oxidative cleavage by sodium periodate. Ruthenium compounds are less reactive

than osmium compounds, but are cheaper and less toxic. The impact of varying reaction conditions including time and solvent was investigated. Firstly, the reaction was attempted using acetonitrile and carbon tetrachloride (CCl_4) as a solvent system which failed to yield the desired product. Then water was used with acetonitrile and carbon tetrachloride in two different trials[97].

Unfortunately, the ruthenium mediated reaction did not work even after the reaction was further optimized using water.

2.13. Dihydroxylation of lanosterol 3-acetate 66.

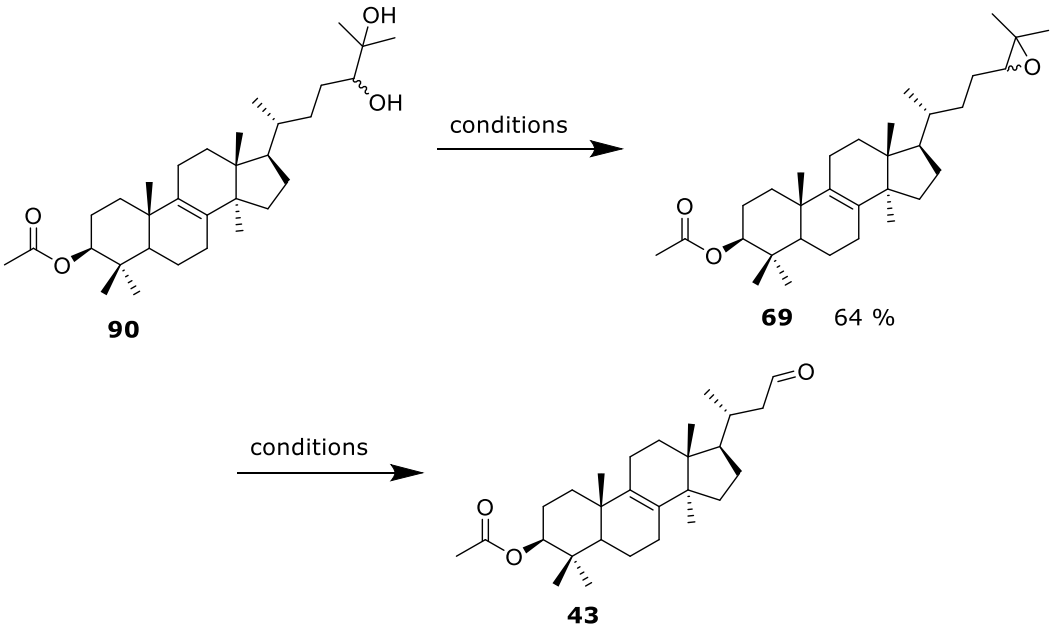


Scheme 11. Dihydroxylation of lanosterol acetate 66.

Another route to make the diol intermediate was investigated by using potassium ferrocyanide and potassium osmate(VI) dihydrate as stereoselective bulky catalyst following the Sharpless dihydroxylation method[98].

Osmium catalysts are expensive (Osmium tetroxide £128 per 250 mg, Sigma Aldrich, 2019) and also toxic, so a catalytic amount was used in the reaction alongside with potassium hexacyanoferrate(III) as a cheap and less toxic co-oxidant used to regenerate the osmium catalyst. It has been reported in the literature that olefin oxidation by osmium catalyst and potassium hexacyanoferrate(III) provides good yields for low molecular weight organic substrates, but for large molecules like steroids it produces lower yields. To improve the reaction's yield, the solvent system was optimised, tertiary butanol with water was used as a co-solvent system in 1:1 ratio. However, the reaction gave a mixture of inseparable compounds. ^{1}H NMR and ^{13}C NMR showed that the target compound has been produced, but because of the high toxicity and inseparable mixture, this method was not further investigated[99, 100].

2.14. Synthesis of 43 via intermediate 69



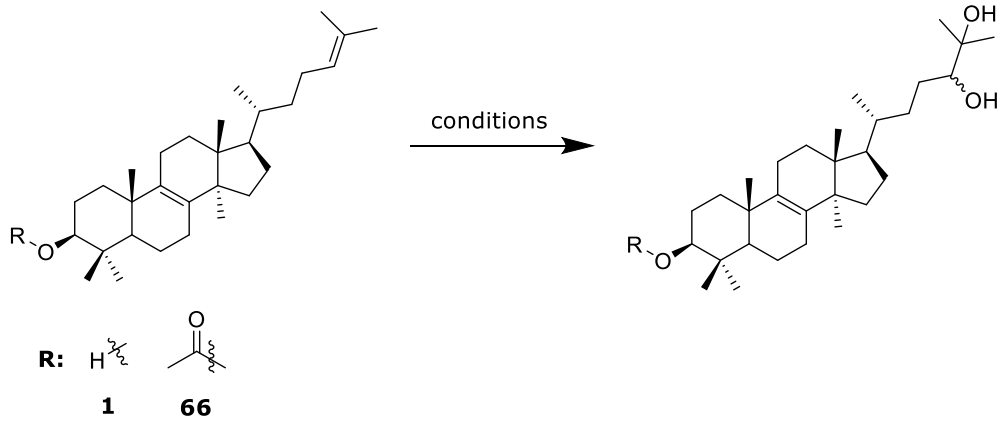
Scheme 12. Synthesis of aldehyde 43 from 96.

Table 8. Screening of the synthesis of 43.

Reactant	Product	Conditions	Yield
90	**69**	MsCl, DCM, pyridine, r.t., 5 h	64 %
69	**43**	H_5IO_6, Et_2O, r.t., 1.5 h	Inseparable by-products
69	**43**	H_5IO_6, Et_2O, r.t., 3 h	Inseparable by-products
69	**43**	H_5IO_4, water, acetone, r.t., 16 h	Inseparable by-products

Epoxidation of compounds mixture **90** was attempted using MsCl to convert the hydroxyl into a better leaving group and pyridine as a base using the procedure reported by Biao *et al*.[101]. This method gave the desired epoxide at 64% yield. The epoxide compound **69** underwent oxidative cleavage via two different reaction conditions firstly by using periodic acid and diethyl ether at r.t. for three hours under argon, secondly by using periodic acid; acetone and water as a solvent system the reaction mixture was left overnight at r.t. under argon. The purification of the crude product from the oxidative cleavage by column chromatography gave a mixture of inseparable aldehydes and ketones. As a result of this, the method was not investigated further.

2.15. Sharpless dihydroxylation of different protected lanosterol substrates



Scheme 13. Sharpless dihydroxylation of 1 and 66.

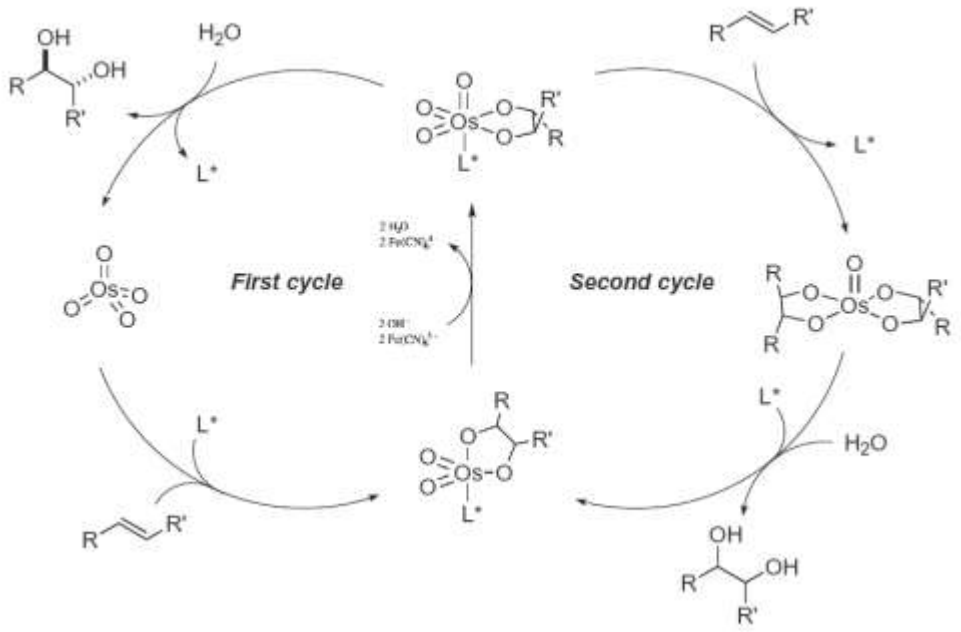
After the unsuccessful attempts to get pure epoxide/diol of lanosterol **1** by the aforementioned methods, Sharpless dihydroxylation was attempted as shown in Table 9 by using AD-mix-α and AD-mix-β as the catalyst with lanosterol **1** and lanosterol acetate **66**. The dihydroxylation **1** gave the diol product with a mixture of inseparable by-products. On the other hand, the dihydroxylation of **66** gave pure product when purified by column chromatography. AD-mix-α and AD-mix-β have the same molar ratio of mixture components, the only difference between them is the ligand.

Table 9. Dihydroxylation of lanosterol 1 and lanosterol acetate 66.

R	Conditions	Yield %
	AD-mix-α, methanesulfonamide, *tert*-butanol, water, r.t., 64 h	Inseparable mixture
	AD-mix-β, methanesulfonamide, *tert*-butanol, water, r.t., 64 h	Inseparable mixture
	AD-mix-α, methanesulfonamide, *tert*-butanol, water, r.t., 64 h	68
	AD-mix-β, methanesulfonamide, *tert*-butanol, water, r.t., 64 h	67

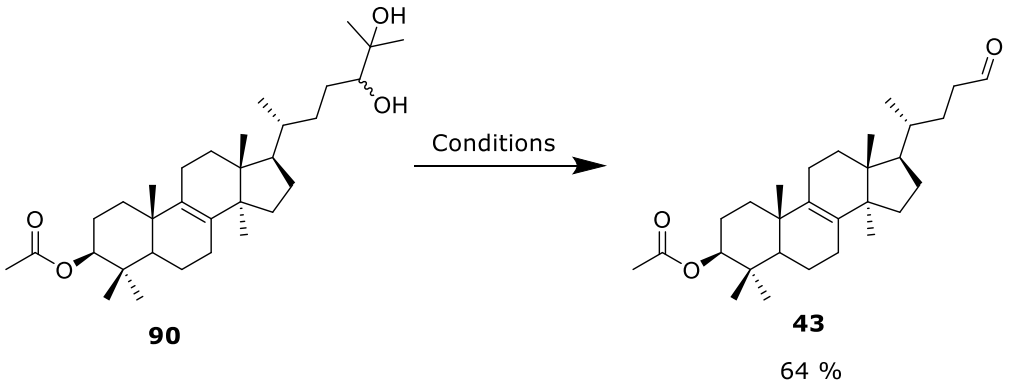
AD-mix-α contains 1.6mmol $(DHQ)_2PHAL$ as a ligand while AD-mix-β contains 1.6 mmol $(DHQD)_2PHAL$ as a ligand. Both of AD-mix-α and AD-mix-β contains 0.7 mmol of Osmium oxidant as a potassium osmate dehydrate. However it contains potassium ferricyanide 498.8 mmol and potassium carbonate 498.8 mmol as co-oxidant for the osmium to regenerate the oxidant catalyst. The solvent system used was *tert*-butanol/water (5:4); water and alcohol are miscible, and when the inorganic salts were added, the miscible solvents separated into two layers. The two phase solvent system is essential to the reaction cycle.

The polarity of lanosterol is low, hence it is insoluble in water, methanesulfonamide was used as a co-solvent and an acid catalyst (weak acid, pKa = 10.8) to transfer the hydroxide ions from the aqueous phase to the organic phase[102]. The first step of the reaction is osmylation of the lanosterol side chain alkene, the osmium is reduced from (VIII) to osmium ester (VI) in the organic phase. After transferring the hydroxyl ions by methanesulfonamide from the aqueous phase to the organic phase, the diol product formed and releasing osmium hydrate (VI) into the aqueous phase. Potassium ferricyanide and potassium carbonate re-oxidise osmium (VI) in the aqueous phase to regenerate the catalyst osmium tetroxide (VIII). Osmium (VIII) migrates to the organic phase to restart the dihydroxylation cycle of the olefin (shown in Scheme 14). The two oxygen atoms of the new diol came from the water aqueous phase (water). The fast and easy reduction/oxidation of osmium reduces the amount of the toxic waste and expensive cost of osmium used in the reaction. The yield from AD-mix-α and from AD-mix-β dihydroxylation of lanosterol acetate was 67 % for both. Next a dihydroxylation reaction was carried on using AD-mix-β only[103-105].



Scheme 14. Sharpless dihydroxylation of olefin cycles[103].

2.16. Diol Oxidative Cleavge



Scheme 15. Synthesis of aldehyde 43.

Using the method previously reported by O'Keeffe *et al.* [74] the oxidative cleavage of **90** to form **43** was investigated as shown in Table 10. The reaction temperature, solvent system and oxidant were optimised in eight different reactions. The best conditions found were using periodic acid as oxidant and diethyl ether as a solvent at room temperature under argon for two hours. Up on reaction completion a white precipitate formed on the reaction vessel and isolated in 81 % yield.

Table 10. Screening results of the synthesis of 43.

Oxidant	Conditions	Yield %
H_5IO_6	THF: DCM, 0-5 °C, 1 h	Inseparable mixture
H_5IO_6	THF: DCM; $^{-}10$-$^{-}5$ °C, 1 h	Inseparable mixture
H_5IO_6	THF: DCM, r.t., 1 h	Inseparable mixture
H_5IO_6	H_2O, acetone, r.t., 16 h	Inseparable mixture
$NaIO_4$	H_2O, acetone, r.t., 16 h	Inseparable mixture
H_5IO_6	Et_2O, r.t., 2 h	81

Moreover the seven investigated reactions gave the desired aldehyde with a mixture of inseparable by-products, ^{1}H NMR (9.52 and 9.34 ppm) and ^{13}C NMR (206 ppm) for the aldehyde C<u>H</u>O and <u>C</u>HO showed that the target compound was synthesised and the by-products were aldehydes and ketones.

2.17. Reductive amination of aldehydes 42 and 43.

Aldehydes **42** and **43** underwent reductive amination under mild conditions. The aldehydes react with secondary amines using sodium triacetoxyborohydride and 1,2-dichloroethane as the solvent. Secondary amines react with aldehydes to form an imine which can be reduced by using a mild reducing agent. Sodium triacetoxyborohydride is a selective reducing agent for imines used in reductive amination, and gave high yield. No aldehyde reduction was observed during the synthesis.

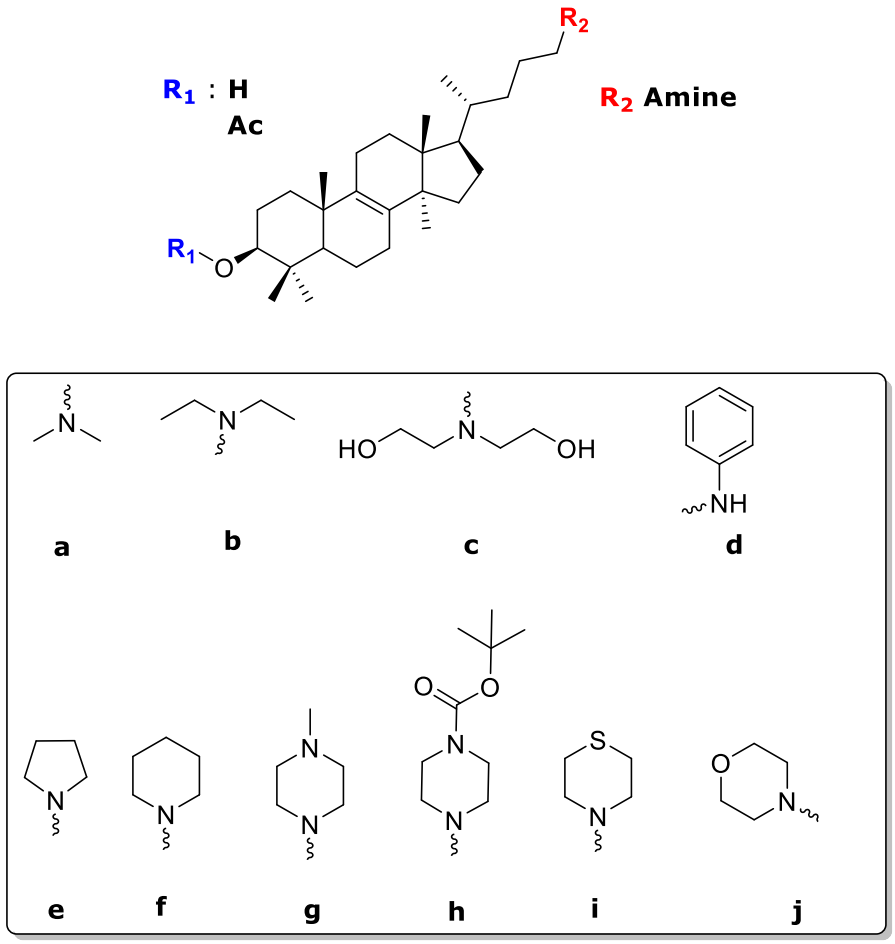
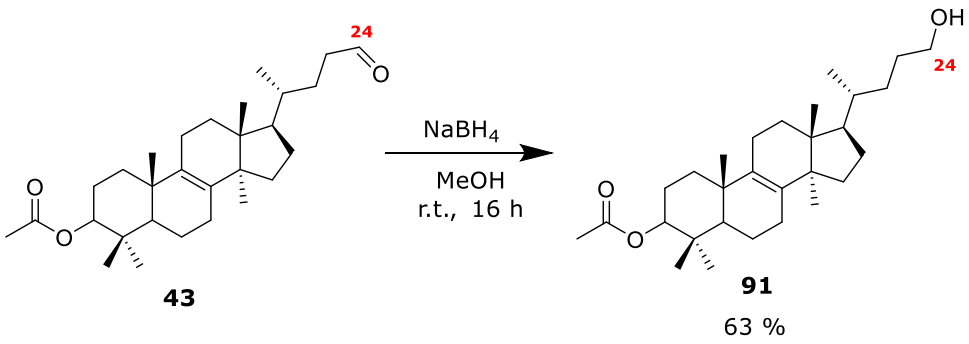


Figure 36. Reductive amination derivatives of 42 and 43.

Table 11. Reductive amination of the aldehydes 42, 43 with the amines i-j.

R_1	R_2	Yield %	Compound Number
OH	a	37	111
OAc	a	83	112
OH	b	34	113
OAc	b	45	114
H	c	78	115
OAc	c	72	116
OH	d	83	121
OAc	d	83	122
OH	e	64	117
OAc	e	68	118
OH	f	93	119
OAc	f	92	120
OH	g	71	128
OAc	g	82	129
OAc	h	84	130
OH	i	56	125
OAc	i	80	126
OH	j	70	123
OAc	j	57	124

2.18. Reduction of 43

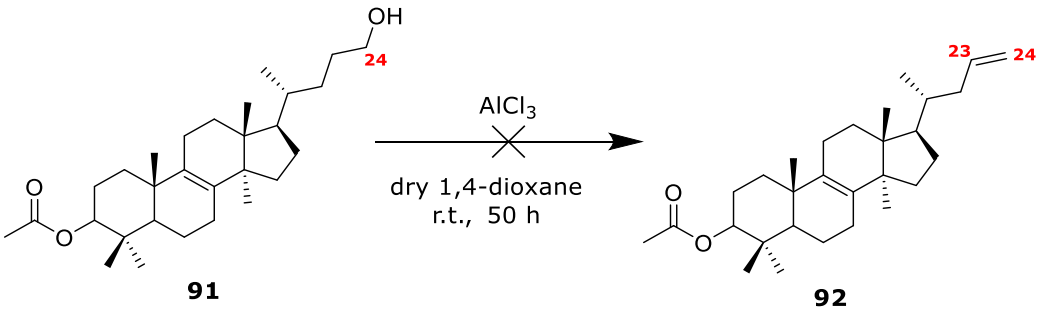


Scheme 16. Reduction of aldehyde 43 to 91.

Aldehyde **43** was successfully reduced using sodium borohydride in methanol as a mild selective reducing agent. Sodium borohydride is an efficient and selective reducing agent for aldehydes and ketones forming primary and secondary alcohols respectively in good yield[106]. Screening of the reaction was investigated by trying alternative solvents at milligram scale. As all of them gave approximately the same yield ratio, larger scale reactions were carried out using methanol.

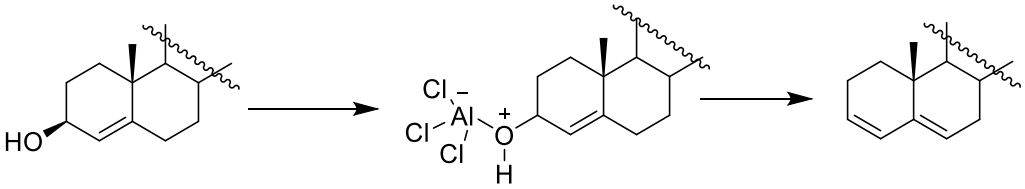
2.19. Dehydration of 91

2.19.1. Dehydration of 91 by $AlCl_3$



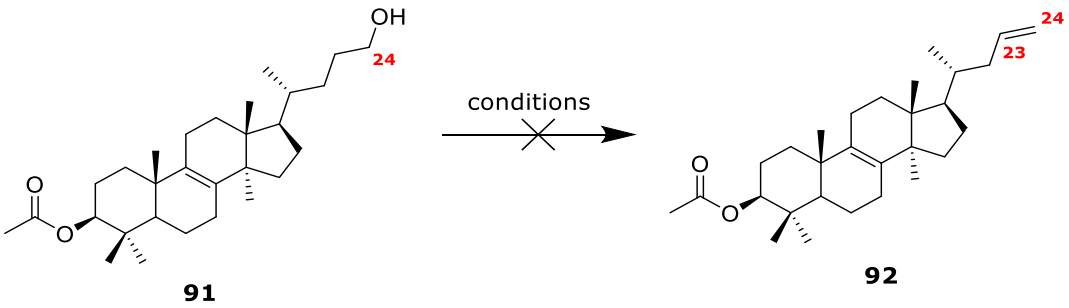
Scheme 17. Dehydration of 91 by AlCl3.

Using the method previously reported by De Marco *et al.*[107], the dehydration of **91** to form **92** was investigated. This approach utilises aluminium chloride to perform the dehydration step and was carried out over 50 h. The reaction failed to yield the desired product, and this was confirmed by TLC, mass spectrometry and NMR. A possible reason for the failure of this reaction is the nature of the substrates being used, the method reported in the literature used to dehydrate a secondary allylic hydroxyl steroid which favours elimination through the formation of a conjugated diene (Scheme 18). In contrast to this, **91** is a primary alcohol and so is not able to form the diene which drives the reaction hence the desired product was not obtained[107]. However, the reaction was carried out for 20 minutes in the literature, the reaction period of **91** was extended to 50 h and tracked by TLC over reaction time, with no sign of product formation.



Scheme 18. Dehydration of allylic alcohol to diene by $AlCl_3$[107].

2.19.2. Tosylate dehydration of 91



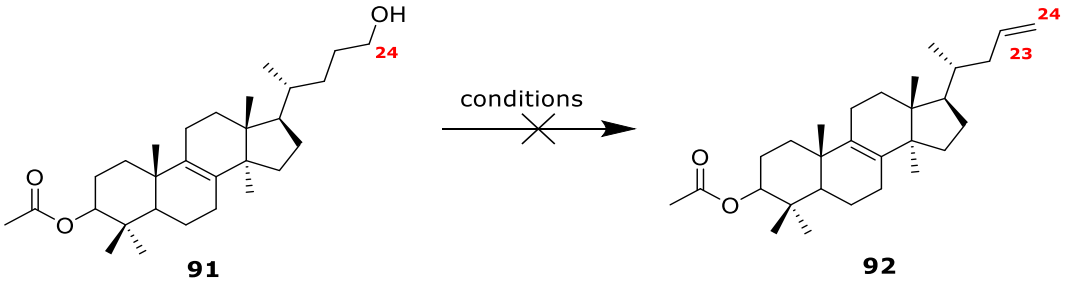
Scheme 19. Dehydration of 91 by tosylation.

Table 12. Optimising condition of tosylation-dehydration of 91.

Base	Conditions
Potassium *tert*-butoxide	TsCl, DCM, r.t., 48 h
NaH	TsCl, DCM, r.t., 48 h
NaH	TsCl, THF, r.t., 48 h

Using the method previously reported in literature by Biao *et al.*[101], the dehydration of **91** to synthesize **92** was attempted using tosyl chloride to convert the hydroxyl to tosylate. As shown in Table 12, three different conditions were investigated, involving two different bases and two different solvents. Potassium *tert*-butoxide and sodium hydride were trialled with DCM. Another trial investigated use of sodium hydride with THF as a polar solvent, as the substrate was insoluble in DCM. After monitoring the reactions progress for 48 hours, no product material was observed.

2.19.3. $SOCl_2$; $POCl_3$ Dehydration of 91



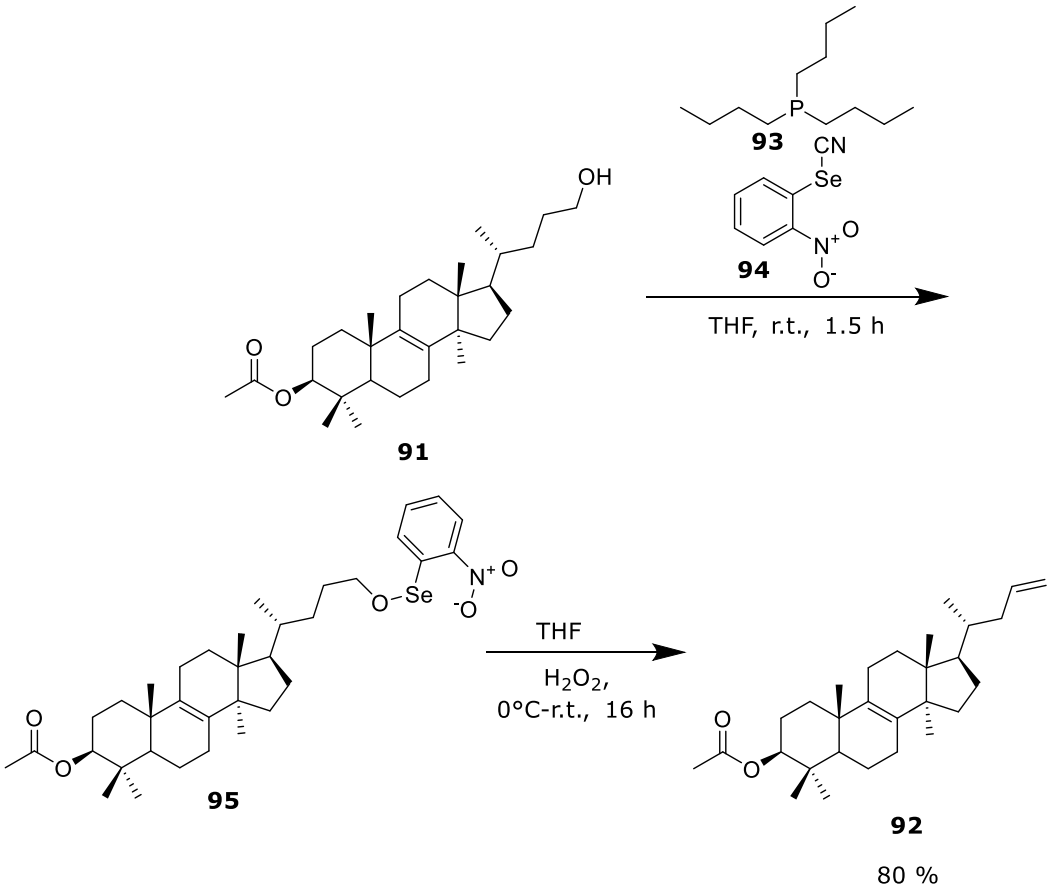
Scheme 20. Dehydration of 91 by $POCl_3$ route and $SOCl_2$ route.

Table 13. Dehydration of 91 conditions by $SOCl_2$ route and $POCl_3$ route.

Dehydrating agent	Conditions
$POCl_3$	Pyridine, r.t., 12 h
$SOCl_2$	Pyridine, 0 °C, 10 min

Young *et al.* have previously reported a dehydration method using thionyl chloride with pyridine. When attempted on **91** the reaction did not yield the desired product. Young *et al*[108]. has also reported a dehydration method using phosphoryl chloride with pyridine, this reaction was also unsuccessful at producing **92** from **91**.

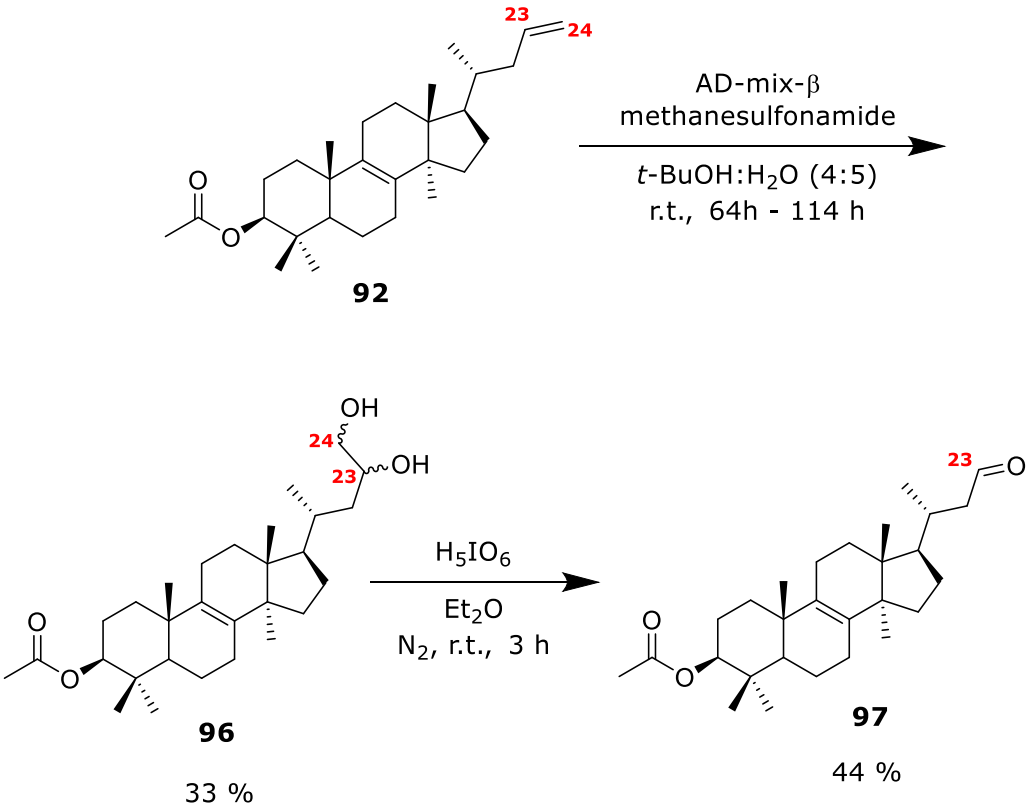
2.19.4. Grieco Dehydration of 91



Scheme 21. Synthesis of 92 using Greico elimination.

O-Nitrophenyl selenocyanate **94** is a very reactive compound often used in the dehydration of a primary alcohol[61]. The reaction is initiated by nucleophilic attack of tributylphosphine **93** on *O*-nitrophenyl selenocyanate to form selenide (selenium phosphonium salt) via a substitution on the electron-deficient selenium[109, 110]. The second step was oxidation of selenium by hydrogen peroxide to yield the desired alkene, triphenylphosphine oxide, and selenoxide. The yield over two steps was 67%.

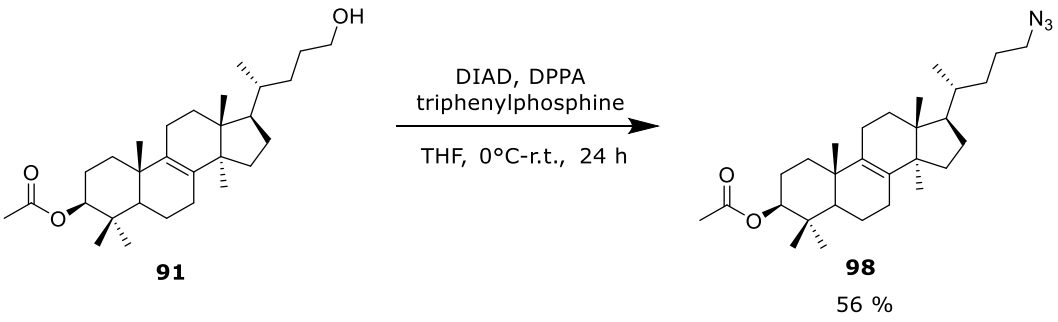
2.20. Synthesis of Aldehyde 97



Scheme 22. Synthesis of aldehyde 97.

Using a similar strategy to the synthesis of **90**, AD-mix-β was used to dihydroxylate the terminal alkene of **92** resulting in **96**. This diol then underwent oxidative cleavage of **96** to synthesis **97**. The dihydroxylation of **98** was slower than the dihyroxylation of **66** because Sharpless dihydroxylation is less efficient on a terminal alkene. The diol **96** oxidative cleavage was carried out by using periodic acid as oxidant under N_2 to synthesize **97**.

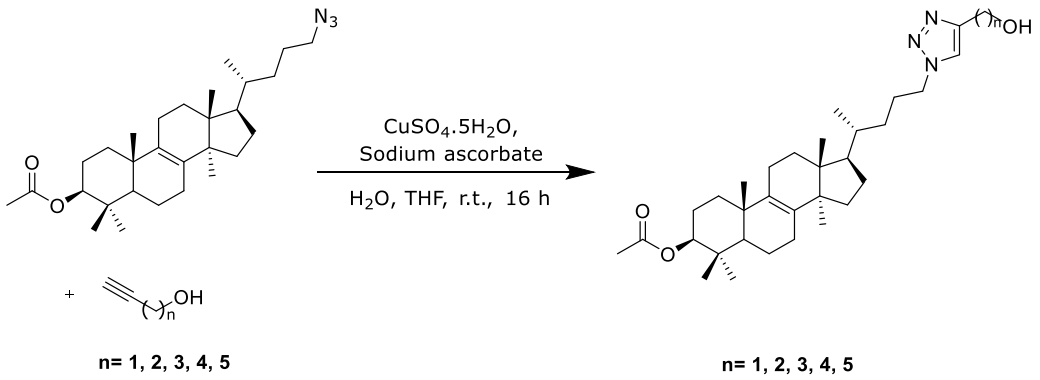
2.21. Synthesis of azide 98



Scheme 23. Synthesis of azide 98.

Azide compound **98** was easily synthesized from **91** by the modified Mitsunobu reaction, using triphenylphosphine; diphenyl phosphoryl azide (DPPA) as an azide source and diisopropylazodicarboxylate (DIAD). The reagents were added to the starting material solution at 0°C to reduce the reactivity because the reaction is exothermic. The azide product **98** yield was 56%, as some of the product was lost in the purification. Diisopropyl hydrazine-1,2-dicarboxylate, the reduced form of DIAD, sticks to the product. Different acid/ base washes and multi-column chromatography purification were attempted, however, the best yield achieved was 56%.

2.22. Synthesis of triazoles



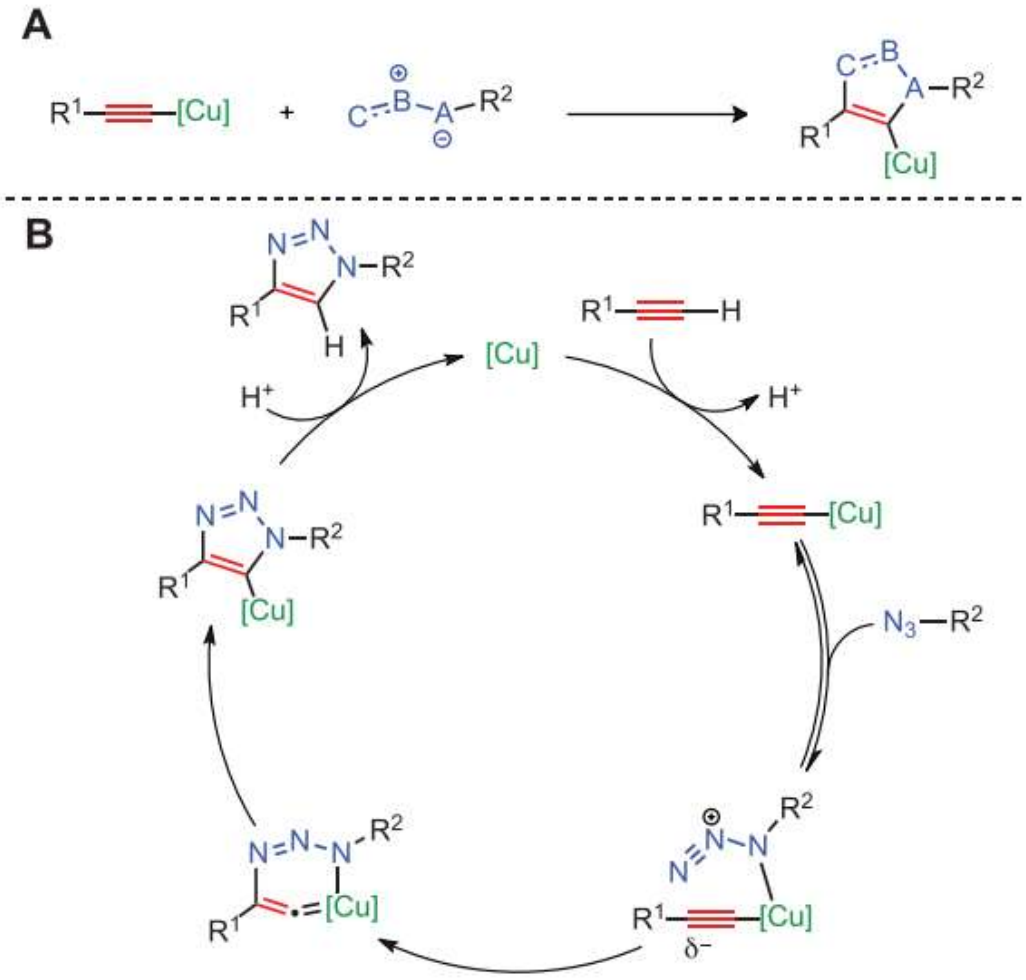
Scheme 24. Synthesis of triazoles.

Table 14. Side chain length of the triazoles synthesised.

N	1	2	3	4	5
Yield %	100	93	75	88	72

Triazole compounds were readily synthesized by using the best known click reaction, azide-alkyne cycloaddition. CuAAc is a copper catalysed cycloaddition of an azide to an alkyne. Copper sulphate pentahydrate ($CuSO_4.5H_2O$) and sodium ascorbate are combined to form Cu (I) are used as catalysts. The thermal *Huisgen* 1,3-dipolar cycloaddition produces a mixture of 1,4-triazole and 1,5-triazole regioisomers with a slow speed of reaction. The copper (I) catalysed reaction yields the 1,4-disubstituted-1,2,3-triazole isomer under ambient temperature with high yields and shorter reactions periods than the uncatalyzed reactions. Copper catalysed reactions are 10^7 to 10^8 faster than the uncatalysed reactions[111, 112]. Cu (I) coordinates to the alkyne forming copper acetylide, the azide coordinates to the alkyne and copper forming an unusual six membered ring (see Scheme 25). A five

membered triazole ring forms later with the copper substituent, protonolysis take place to afford the triazole derivative as a 1,4-disubstituted-1,2,3-triazole[113-117]. NMR analysis confirmed that the analogue synthesised was 1,4-disubstituted-1,2,3-triazole. Moreover, a ruthenium catalysed reaction can afford the 1,5-disubstituted-1,2,3-triazoles isomer selectively under ambient conditions[111, 112].



Scheme 25. CuAAC. (A) Generic form of the copper-catalyzed dipolar cycloaddition reaction. (B) Proposed mechanism of the CuAAC[116, 118, 119].

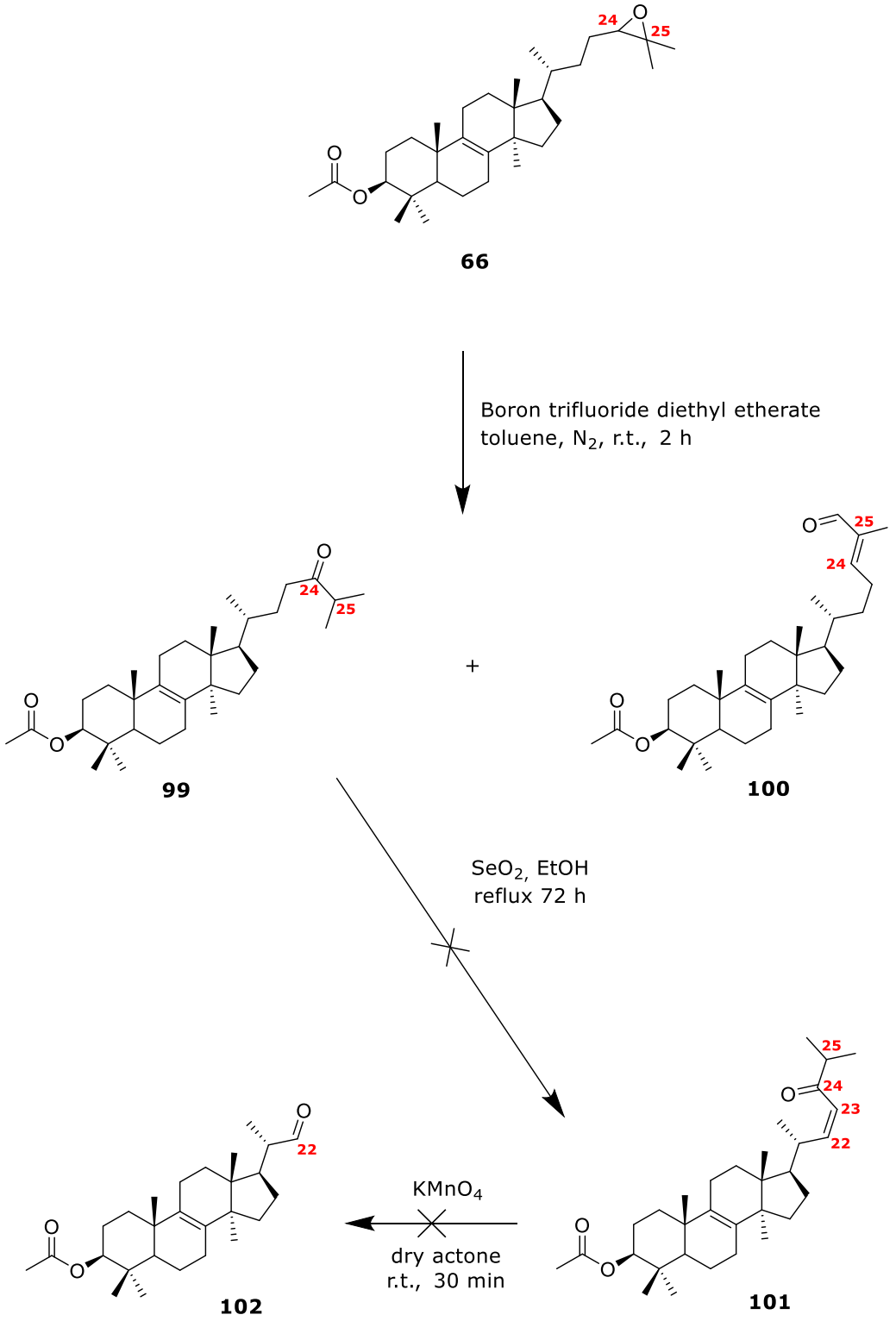
The 1,2,3-triazole 4,5-di -ol synthesis was undertaken by following the same synthesis procedure, unfortunately, the synthesis failed to produce the desired compound, in the literature different procedure

recommended to synthesise 1,5 di-ol and 1,5 di-acid triazoles using microwave or refluxing the reaction mixture by acetone or toluene[120-126]. Synthesis of triazoles has also been reported using a range of different metals including silver[127, 128], ruthenium[129, 130] and gold[131].

2.23. Synthesis of aldehyde 102

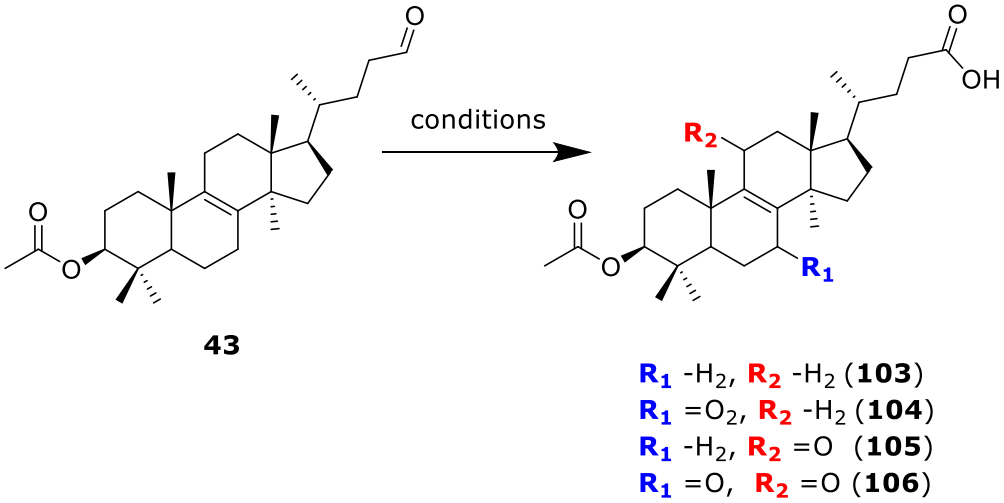
The rearrangement of lanosterol epoxide **69** was investigated using the method reported by Yoshihiro *et al.*[132]. The treatment of **69** with boron trifluoride diethyl etherate resulted in rearrangement to yield the aldehyde **100** and the ketone **99**. Starting material was recovered by column chromatography purification using *n*-hexane toluene (1:1) as an eluent with *Rf* 0.45. Further elution gave 10 mg of aldehyde **100** with *Rf* 0.24 and a mixture of aldehyde **100** and ketone **99** with *Rf* 0.13. The very small *Rf* difference between **100** and **99** and the low polarity of the eluent means the mixture was inseparable. The dehydration of the inseparable mixture of **99** and **100** was investigated using selenium dioxide (SeO_2) as the dehydrating agent in refluxing EtOH. This gave the α,β-unsaturated ketone **101**. The reaction afforded an inseparable mixture, HRMS showed the desired product peak. Synthesis of ketone **99**; aldehyde **100**; and conjugated ketone **101** was attempted once only; further optimisation will be required to get a better yield of **101**. Conjugated ketone **101** is an intermediate for the synthesis of the key aldehyde **102**. Aldehyde **102** is an interesting compound because it has lower molecular weight compared to its analogue **43**, therefore it obeys Lipinski's rules[133] regarding molecular weight (less than 500 Da), and

the possibility to use it in further reactions such as reductive amination, Wittig and Grignard Reactions.



Scheme 26. Synthesis of aldehyde 102.

2.24. Synthesis of Lucidenic acid 103



Scheme 27. Synthesis of lucidenic acid 103.

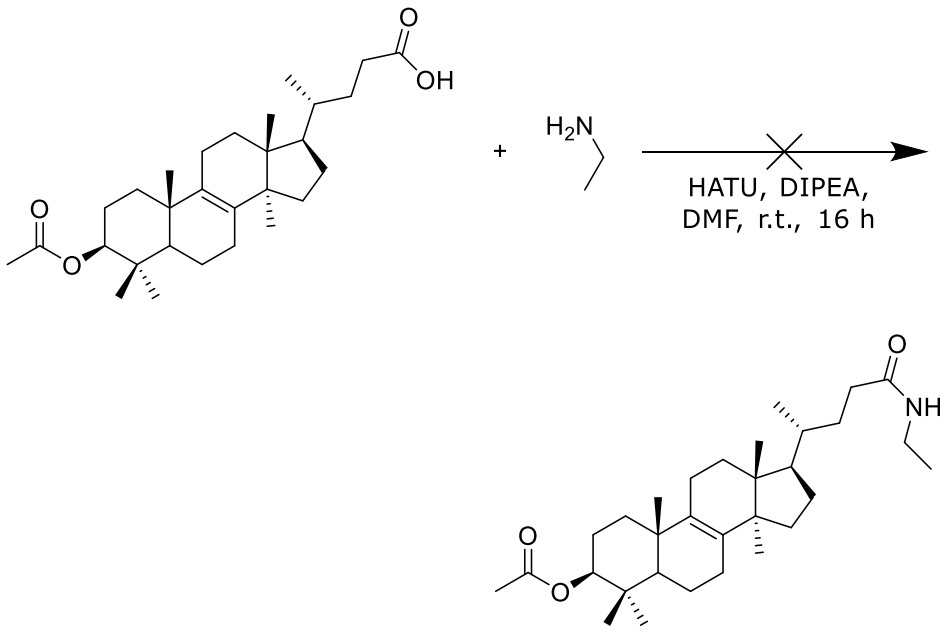
Table 15. Synthesis of lucidenic acid 103 optimizing conditions.

Oxidant	Conditions	Yield %
CrO_3	Acetone, r.t., 2 h	n/a
CrO_3	Dry acetone, r.t., 2 h	n/a
Oxone	DMF, r.t., 3 h	74 %

In order to synthesize lucidenic acid **103**, optimisation trials were carried out as shown in Table 15. Initially chromium (VI) trioxide (CrO_3) was used as the oxidant for the conversion of aldehyde **43** to carboxylic acid **103**-**106**. Another attempt was carried out using dry acetone, but this attempt was unsuccessful. The modified method was reported by Mazoir *et al.* to give quantitative yield of **103** from **43**. However, when

performed in this study the modified method gave **103** as intended but with **104**, **105** and **106** as by-products. However, in another attempt using oxone as oxidant and DMF to form lucidenic acid **103**, the reaction gave an excellent yield of 74 %.

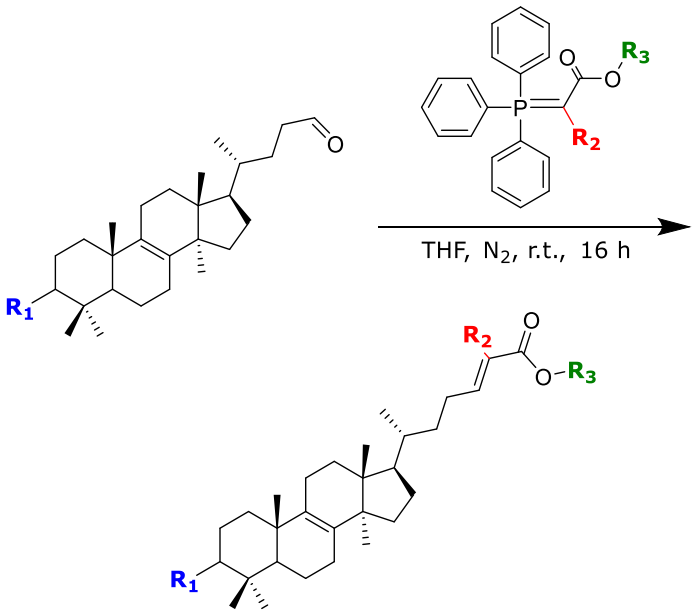
2.25. Synthesis of side chain amide



Scheme 28. Amide coupling of lucidenic acid.

Using the method previously reported by Hasserod*t et al.*[134] the amide coupling of the lucidenic acid to form the amide was investigated (as shown in Scheme 28). The reaction was monitored by mass spectrometry, TLC and NMR. After 16 hours of stirring, the product was detected by mass spectrometry only. The reaction was carried on for a further additional 96 hours with monitoring, TLC showed only the starting material and no product material was observed.

2.26. Synthesis of Ganoderic acid-DM analogues



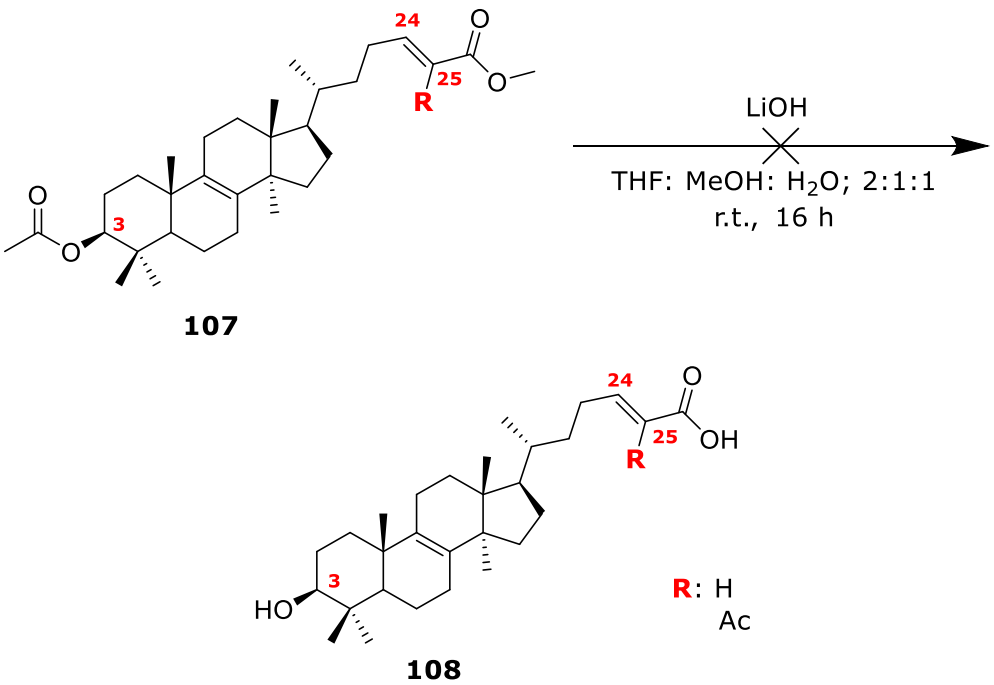
Scheme 29. Synthesis of ganoderic acid DM analogues

Table 16. Wittig olefination of the aldehyde 42, 43.

Compound number	R_1	R_2	R_3	Yield %
107	Acetate	H	CH_3	91
135	Hydroxyl	H	CH_3	56
137	Carbonyl	H	CH_3	62
140	Acetate	H	C_2H_5	80
138	Hydroxyl	H	C_2H_5	75
139	Carbonyl	H	C_2H_5	88
141	Hydroxyl	CH_3	C_2H_5	65

The synthesis of ganoderic acid DM analogues was attempted via Wittig olefination by following the method previously reported by Menozzi-Smarrito *et al.*[135]. The analogues were synthesised by using both aldehydes **42** and **43** with methyl and ethyl ester Wittig reagents. The reaction was performed under a N_2 stream to keep it dry and assure the reaction completion in a good yield. Ylides reaction produces olefins in two isomers, (*E*)-olefins and (*Z*) olefins[136-138]. The synthesis of the C3 ketone was performed by using Dess-Martin oxidation conditions. In order to achieve a higher yield and a faster reaction rate, water was used a as catalyst for the reaction (DCM:H_2O; 100:1)[139-143].

2.27. Synthesis of Lucidenic acid 108

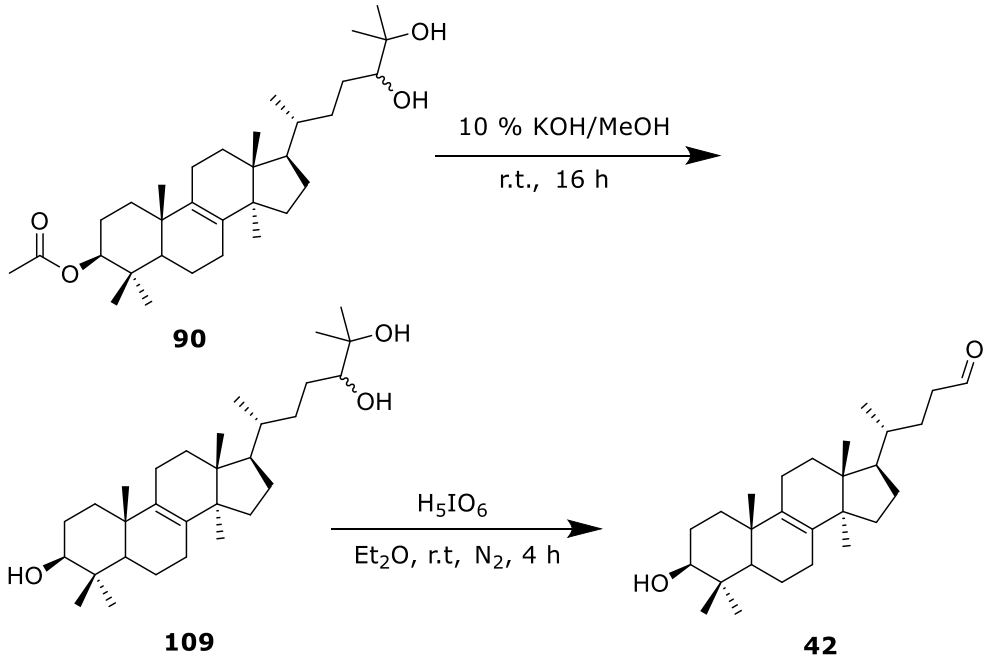


Scheme 30. Synthesis of lucidenic acid 108.

The synthesis of lucidenic acid was attempted first by using lithium hydroxide in THF: MeOH; H_2O (2:1:1), the reaction mixture was stirred

for 16 h at room temperature[135]. The reaction was monitored by mass spectrometry; TLC and NMR, unfortunately showed the reaction failed to yield the desired product. A second attempt was carried out by heating the reaction mixture at 65 °C, however the reaction failed to yield the desired product. The synthesis was attempted with two substrates, one with a hydrogen substituent on the C25 and other one with methyl group. The C3 ketone analogue of the free acid has been previously reported in the literature and patented as an active anti-HIV compound[144-147].

2.28. Synthesis of aldehyde 43 from the diol acetate 90

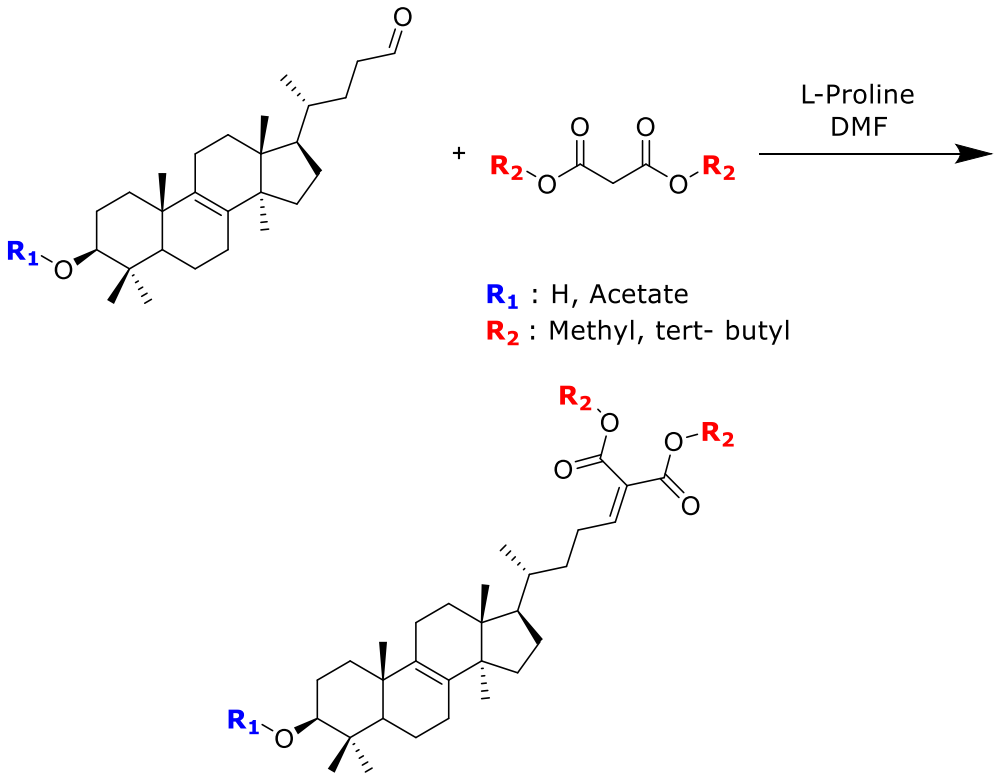


Scheme 31. Synthesis of aldehyde 42 from the diol acetate 90.

Another route to make the aldehyde **42** was investigated by deprotection of the C3 acetate of the diol by 10 % w/v alcoholic KOH, followed by diol oxidative cleavage with periodic acid. Ester hydrolysis

can be achieved by using acid or base catalysed reagents. The acetate deprotection was achieved in quantitative yield by using potassium hydroxide a strong base. This is able to hydrolyse the acetate group at room temperature. The diol underwent oxidative cleavage by using periodic acid as oxidant under N_2 to synthesis the aldehyde **42**.

2.29. Knövenagel Condensation



Scheme 32. Knövenagel Condensation of aldehydes 42 and 43.

Methyl ganoderic acid DM (Me GA-DM) is one of the active analogues of lanosterol with α-β unsaturated ester on the side chain. The IC_{50} of Me GA-DM on PC3 cell lines is 0.3 μM.

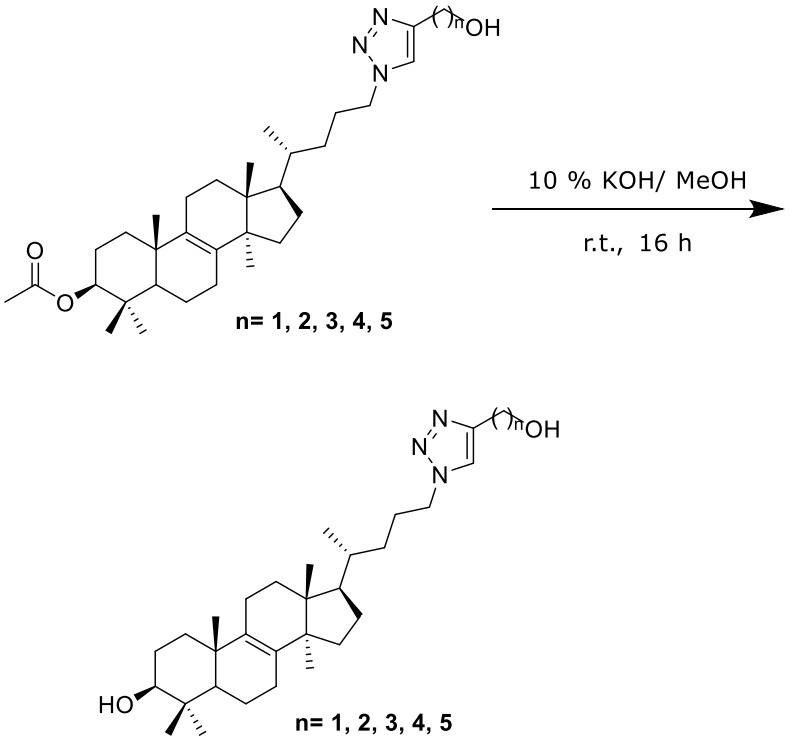
Table 17. Screening of Knövenagel condensation.

R_1	R_2	**Conditions**
H	Methyl	r.t., N_2, 16 h
H	Methyl	r.t., N_2, 120 h
Acetate	Methyl	r.t., N_2, 16 h
Acetate	Methyl	r.t., N_2, 120 h
Acetate	*Tert*-butyl	r.t., N_2, 16 h
Acetate	*Tert*-butyl	r.t., N_2, 120 h

The Knövenagel condensation synthesis approach investigated synthesis of the di-ester conjugated olefin in the side chain. The aim of the synthesis was to have di-ester terminal side chain analogues which might have higher activity compared to Me GA-DM.

The Knövenagel condensation was attempted twice by using the aldehyde **42** with DMF and L-proline as a base with dimethyl malonate and di-*tert*-butyl malonate. The reaction product was found in the mass spectrum only but not by TLC and NMR.

2.30. C-3 acetate deprotection of triazoles

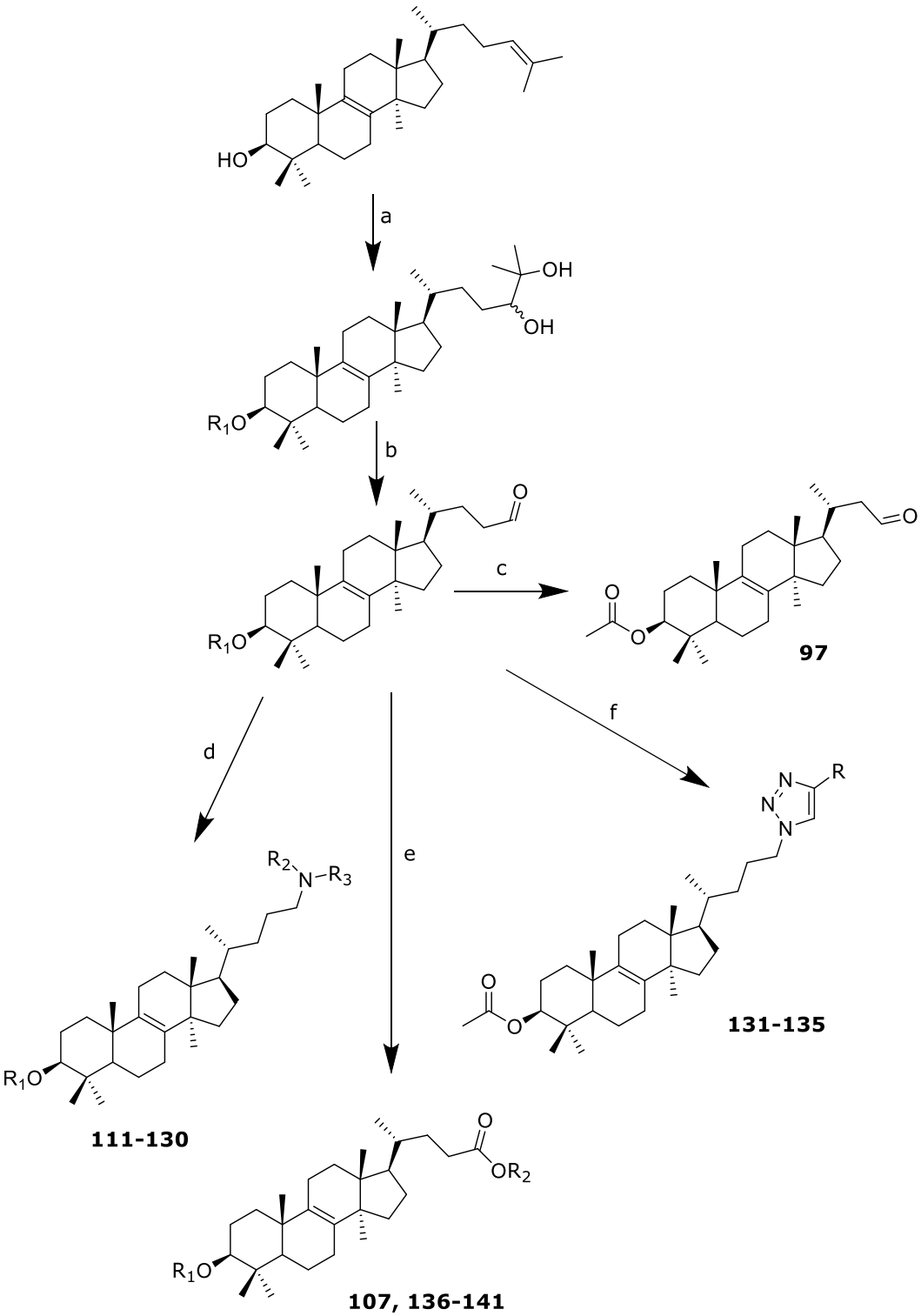


Scheme 33. C-3 Acetate deprotection of the synthesised triazoles.

The acetate deprotection at position 3 was attempted using 10 % alcoholic KOH. The desired product was detected by mass spectrometry. pH neutralization of the crude products was carried out by washing with 2 M HCl until it became acidic, however, the crude product was insoluble. The solubility issue was a limitation for not performing the NMR analysis and the biological activity.

The hydroxyl group on position 3 is more polar than the acetate; micelle formation could be the reason for the solubility issue. The aim from the C3 hydroxyl synthesis was to obtain lower molecular weight analogues compared to the reactant and the hydroxyl analogues have one extra hydrogen bond donor and one less hydrogen bond acceptor.

2.31. Summary of synthesised compounds



Scheme 34. Summary of the compounds synthesised.

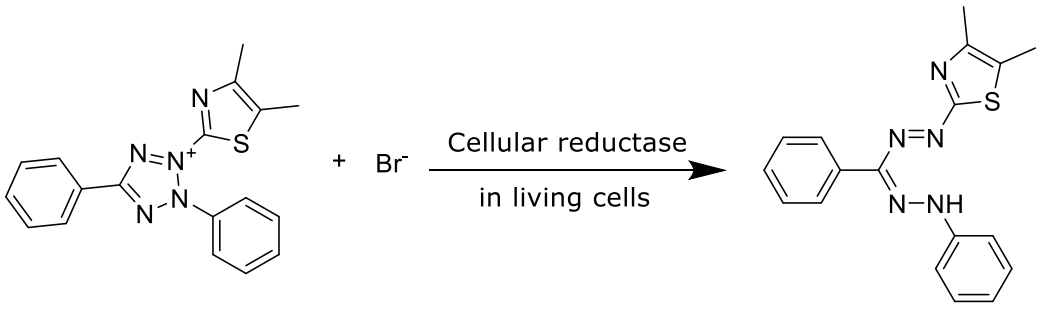
The compounds synthesised were designed based on an SAR study compared to the active compounds in the literature such as GA-DM. The final compounds listed in three main functional groups.

a. C24 Alkene dihydroxylation of hydroxyl C3 lanosterol and acetate C3 lanosterol.
b. Oxidative cleavage of the hydroxyl and acetate C3 lanosterol diol intermediates to produce the key aldehydes **42** and **43**.
c. Synthesis of one carbon atom shorter side chain aldehyde **97**.
d. Synthesis of incorporating secondary and tertiary amines on the side chain with hydroxyl and acetate C3 analogues.
e. Synthesis of GA-DM analogues with methyl ester, ethyl ester on the side chain.
f. Synthesis of C3 acetate 1,4-disubstituted-1,2,3-triazoles analogues.

3. Biological evaluation assays

3.1. Effect of the compounds on prostate cancer cell survival

The biological activity of the compounds synthesised was measured using an MTT assay to determine their IC_{50} values. The MTT assay is a colorimetric assay for *in vitro* biological evaluation which detects only living cells (cell viability) (see Scheme 35).



Scheme 35. MTT reduction to formazan.

In this study, the MTT assay was used to evaluate cell survival when treated with the ethyl acetate soluble compounds against PC3 and DU145 androgen-independent cancerous cell lines. The IC_{50} was measured at 72 h in 96 well plates at a total volume of 200 µl. The MTT assay was performed in duplicate in order to ensure validity of results.

3.2. Cell Density

At the beginning of the biological work, cell density screening was carried out for both PC3 and DU145 prostate cancer androgen-independent cell lines (used in previous work leading up to this study)[148]. Experiments were ran under identical conditions to the IC_{50} experiments. The 96 well plates were seeded with different cell densities (0 (control), 2, 3, 5, 6, 7.5, 10, 12.5, 15 and 20 X 10^3 cell/well) in 100

µl medium (RPMI for PC3 and DU145 for DMEM), day 0 followed by 100 µl media addition the next day. The control ensured any reading was a result of cell number alone. The cell density test was ran for 96h; 24h between seeding and extra media addition, equivalent to cell settling before treatment, and 72h of incubation following this media addition. The plates were checked and imaged by a bright field microscope on the final day.

It was found that the best cell density to achieve 80 % confluence near the final day was 3 X 10^3 cell/ well for the PC3 cell line and 4 X 10^4 cell/ well for the DU145.

Table 18. Cell density screening.

PC3 Cell count X 10^3/ well	0	2	3	5	6	7.5	10	12.5	15	20
DU145 Cell count X 10^3/ well	0	2	3	5	6	7.5	10	12.5	15	20

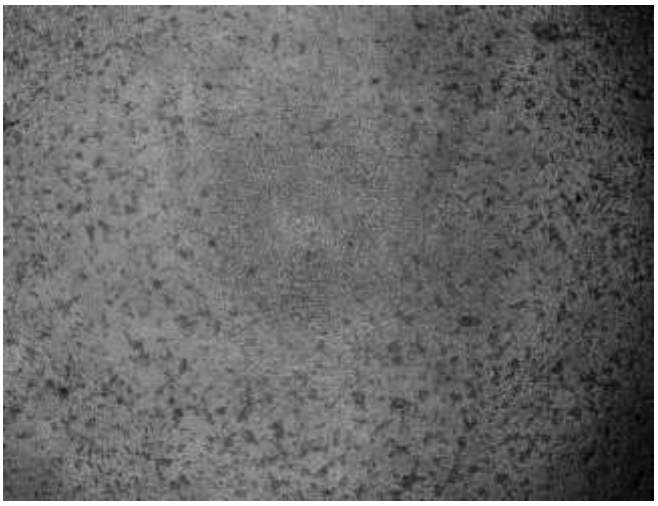


Figure 37. PC3 3 X 103 cell/well day 4.

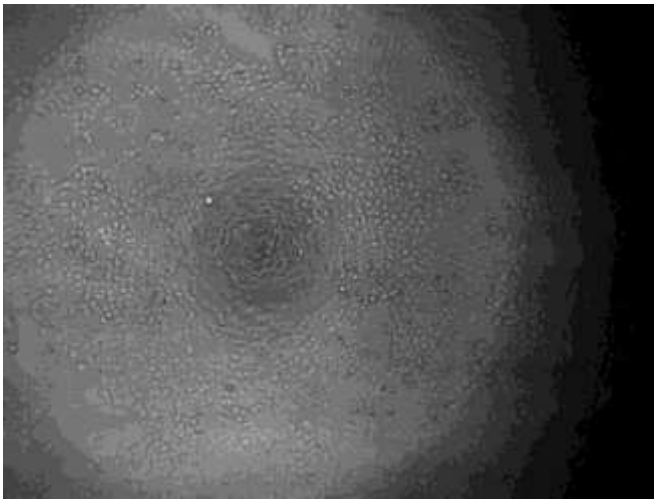


Figure 38. DU145 4 X 104 cell/well day 4.

3.3. Vehicle screening

DMSO is an aprotic polar organic solvent which dissolves both organic and non-organic compounds. In cell culture, DMSO is considered the standard solvent for organic compounds for both *in vitro* work and *in vivo* work. The maximum concentration for DMSO in *in vivo* work is 10 % v/v; higher concentrations can lead to cell death. Although DMSO is considered to be a very good solvent for organic compounds, the ganoderic acids synthesised in this thesis showed poor solubility in 1% v/v DMSO, due to their amphoteric nature and they gave cloudy suspensions.

A variety of solvents were screened for toxicity against PC3 and DU145 cell lines, with varying concentrations (1%, 3%, 6% and 10%) v/v in DMEM and RPMI medium. The solvents investigated were DMSO, acetone, methanol, ethanol, acetonitrile, ethyl acetate, DMF and water.

DMSO showed good solubility for most of the ganoderic acids at a 10 % v/v; however, concentrations higher than 3 % v/v were toxic and killed

the cells (see Figure 39). Screening of DMSO with 6 % v/v as vehicle leads to 50 % cell death, while using the 10 % v/v DMSO gave complete cell death by the fourth day of the experiment which is the typical period over which the cytotoxicity assay is run. This is consistent with findings in the literature that DMSO is not safe for *in vivo* work at these concentrations[149].

Ganoderic acids showed poor solubility in methanol, ethanol, water, acetonitrile, DMF and water even at 10% v/v (not shown). The ganoderic acid formed a cloudy suspension in these solvents even after mixing and warming at 37 °C.

Previously Maguaer *et al.* used ethyl acetate and a few acetone drops to dissolve the insoluble ganoderic acids in ethyl acetate.

Acetone showed poor solubility for the insoluble compounds in ethyl acetate (insoluble amines) even at 10 % v/v and gave complete cell death with the 10 % v/v.

Ethyl acetate 1% v/v showed good solubility for the compounds tested. This solvent alone result in cell survival of < 90 % in both cell lines indicating low solvent toxicity.

Based on these findings, ethyl acetate was used as the solvent to produce the stock solutions of the GA's at 200 µM.

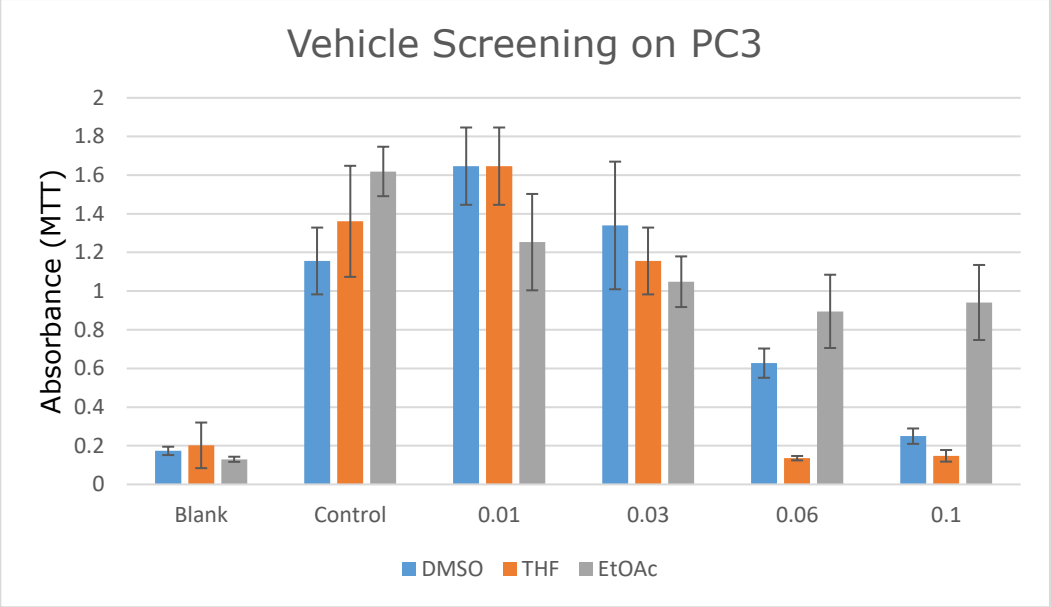
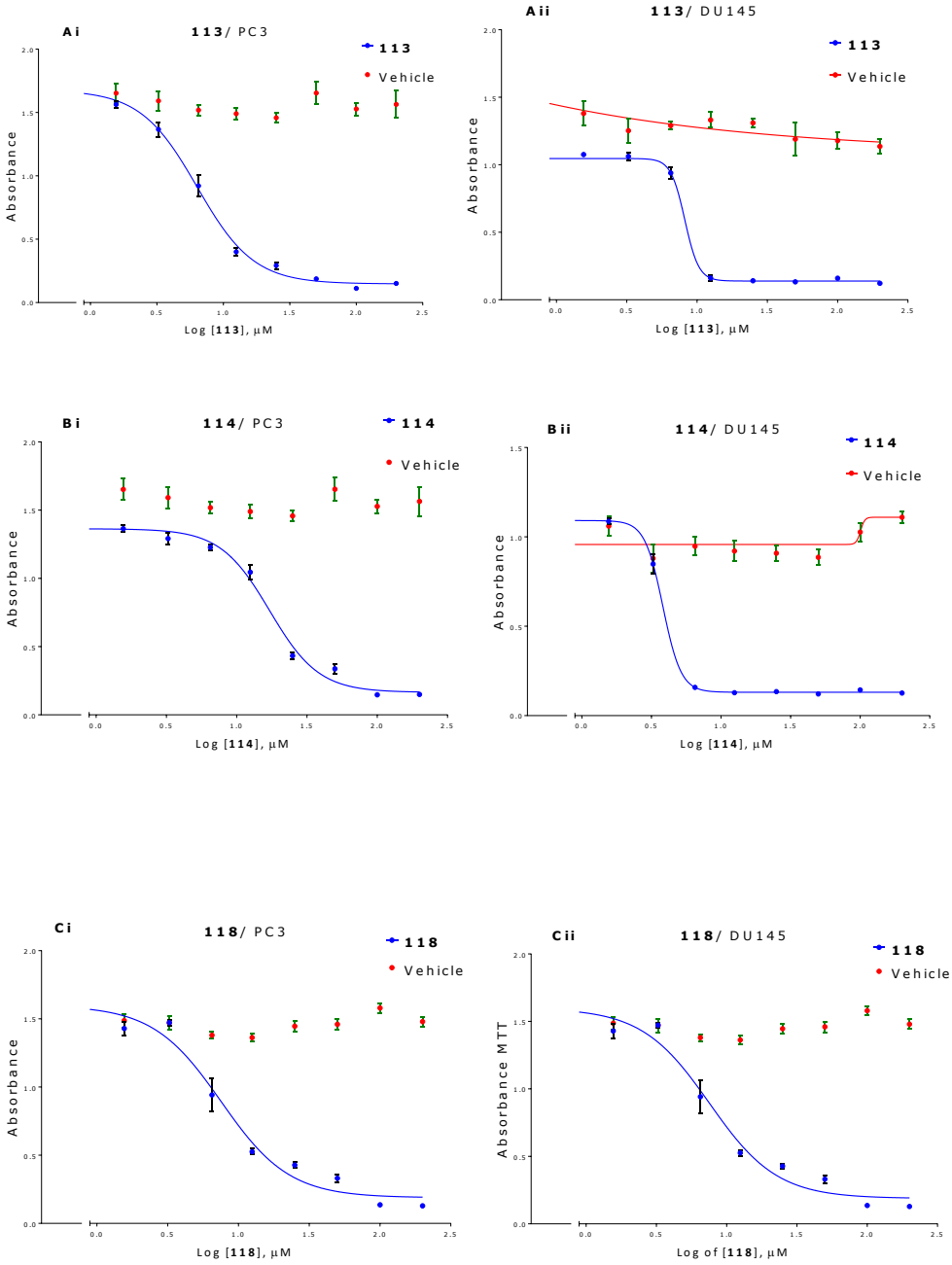


Figure 39. Survival of PC3 cells as measured by MTT assay following treatment with a range of concentrations of DMSO, THF and ethyl acetate.

3.3.1. Assessment of amines activity

The biological activity of the analogues incorporating amines in the side chain was evaluated for compounds with both hydroxyl and acetate substitution at C3. Some of the amines showed biological activity with an IC_{50} of 3.5-21 μM on PC3 and DU145 cells. Diethylamine analogues showed activity for both hydroxyl and acetate substitution at C3, while the rest of the active amines showed activity for the C3 acetate substituted compounds only (Figure 40). The hydroxyl analogues showed no activity, possibly because of the polarity, which may affect cell membrane penetration (see Table 19).


Ai
113/ PC3
113
Vehicle
Absorbance
Log [113], µM
Aii
113/ DU145
113
Vehicle
Absorbance
Log [113], µM
Bi
114/ PC3
114
Vehicle
Absorbance
Log [114], µM
Bii
114/ DU145
114
Vehicle
Absorbance
Log [114], µM
Ci
118/ PC3
118
Vehicle
Absorbance
Log [118], µM
Cii
118/ DU145
118
Vehicle
Absorbance MTT
Log of [118], µM

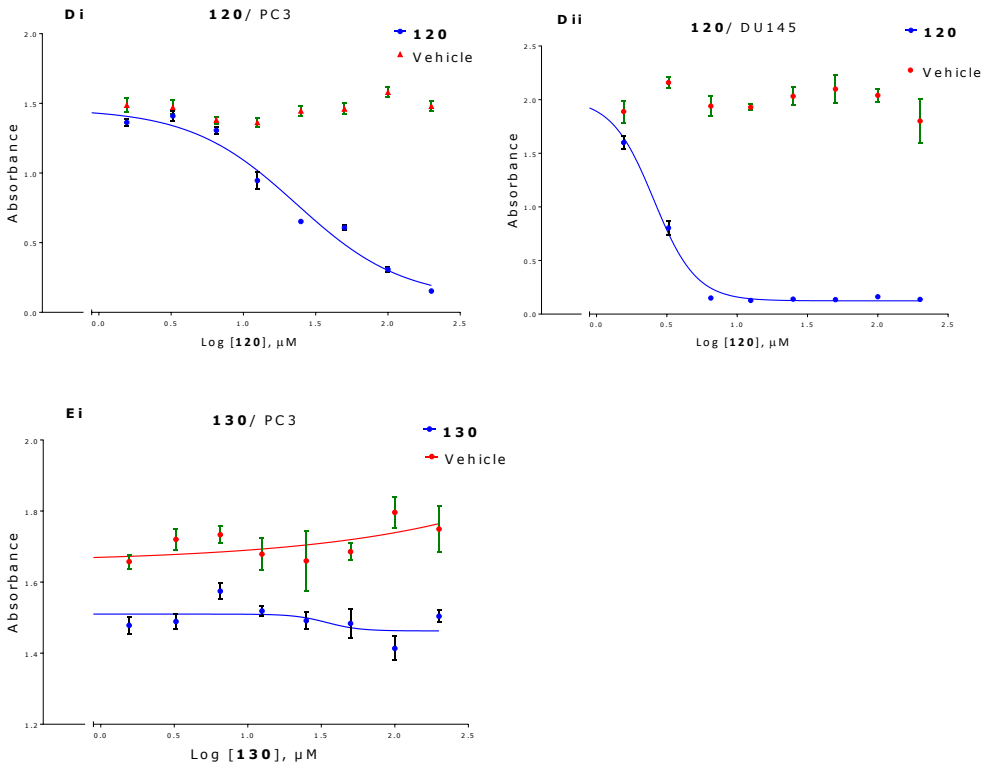


Figure 40. Effect of increasing concentrations of 113 (A), 114 (B), 118 (C), 120 (D) and 130 (E) inactive example on survival of PC3 (i) and DU145 (ii) cell lines measured by MTT assay.

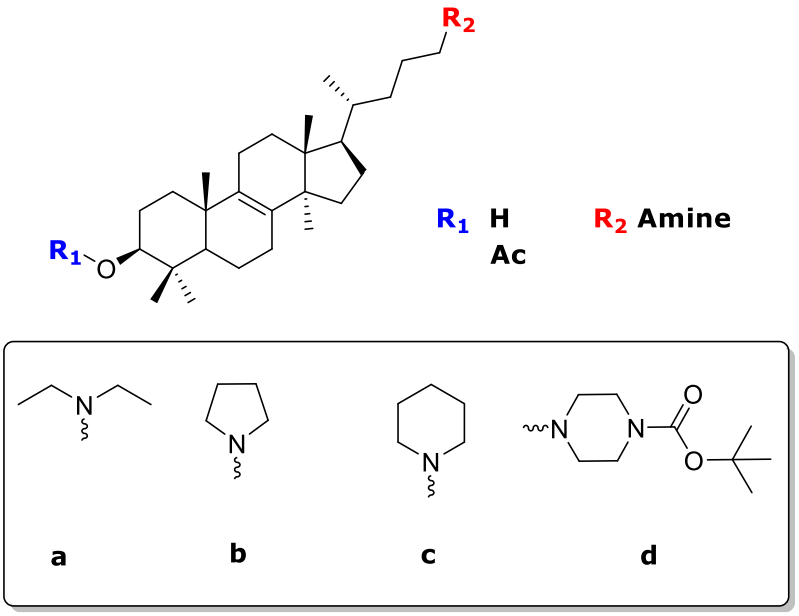


Figure 41. Biologically active amines structures.

Table 19. Biologically active amines IC_{50}.

R_1	R_2	Compound number	PC3 IC_{50} µM	DU145 IC_{50} µM
OH	a	**113**	6	3.5
OAc	a	**114**	11	4
OAc	b	**118**	5	3
OAc	c	**120**	13	5
OAc	d	**130***	-	-

* Inactive example

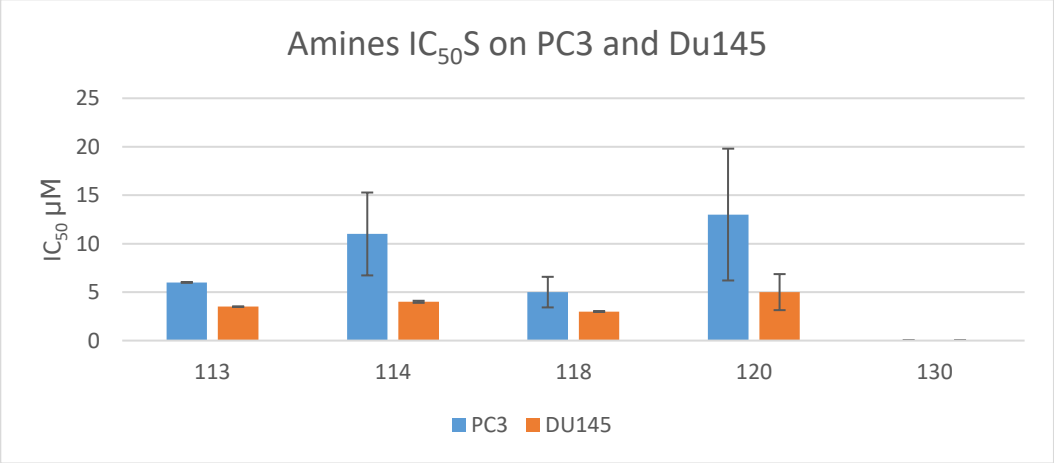


Figure 42. The IC_{50} of amines (113, 114, 118, 120 and 130 inactive example) against PC3 and DU145 cell lines measured by MTT assay.

Some of the soluble amines did not show activity on either cell line. The inactive amines were those with cyclic amine substitution on the side chain. The reason for their inactivity could be the steric hindrance of the ring with the binding site of the target. The amines were inactive with

both hydroxyl and acetate at R1 (C3) as shown in Figure 43 and Table 20.

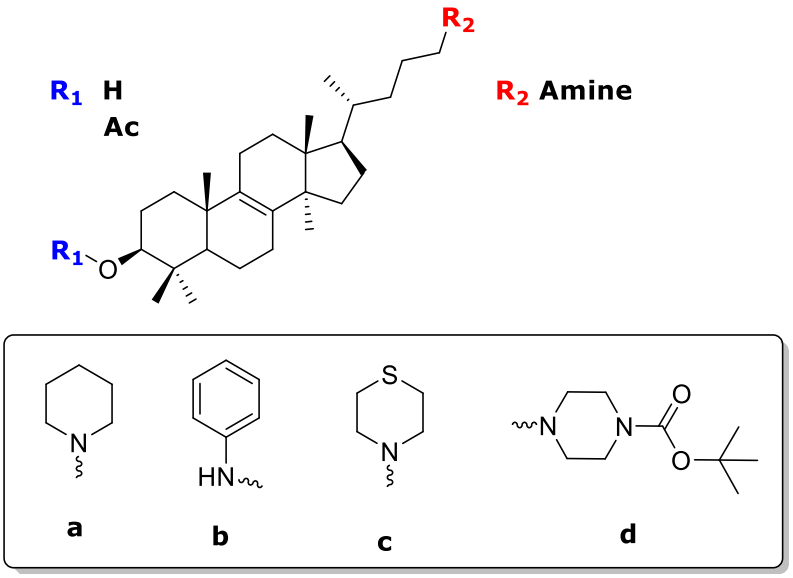


Figure 43. Biologically inactive amines structures.

Table 20. Biologically inactive amines structures.

R_1	R_2	Compound number	PC3 IC_{50} µM	DU145 IC_{50} µM
OH	a	**119**	-	-
OH	b	**121**	-	-
OAc	b	**122**	-	-
OH	c	**125**	-	-
OAc	c	**126**	-	-
OAc	d	**130***	-	-

* Inactive example

3.3.2. Assessment of triazoles activity

There are two types of triazoles, 1,2,3-triazoles and 1,2,4-triazoles[150, 151]. Triazoles are considered to be important bioisosteres in medicinal

chemistry because of their polarity, solubility and rigidity of the ring[136]. In addition the nitrogen atoms can act as hydrogen bond donors and acceptors; the triazole is a good replacement (bioisostere) for an amide group[94].

The first analogue designed was a 1,2,3-triazole, with a hydroxy methyl on C4 of the triazole ring **131**. This analogue had an IC_{50} value of 32 μM on both cell lines (PC3 and DU145). Another compound with a C4 hydroxy ethyl substitution on the triazole ring **132** was synthesised and tested against PC3 and DU145, but it did not show any activity on either cell line. After that the C4 hydroxy propyl on the triazole ring **133** was synthesised. The biological evaluation of the C4 hydroxy propyl analogue **133** showed an IC_{50} value of 20 μM on the DU145 cell line (and no activity on PC3 cells), nearly double that of the hydroxyl methyl analogue **131** against the same cell line.

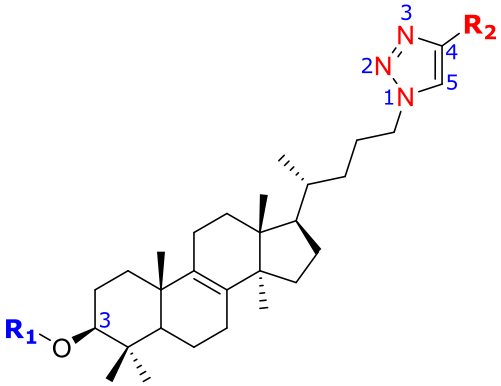
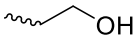
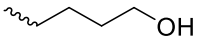


Figure 44. Biologically active triazoles.

Table 21. Biologically active triazoles.

R_1	R_2	Compound number	PC3 IC_{50} μM	DU145 IC_{50} μM
Acetate		**131**	32	32
Acetate		**133**	-	20

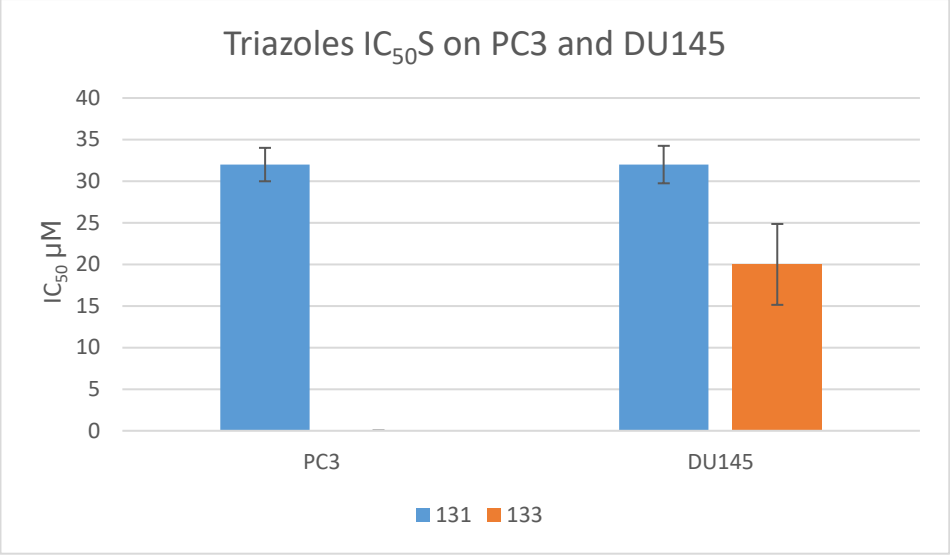


Figure 45. Biologically active triazoles IC_{50}.

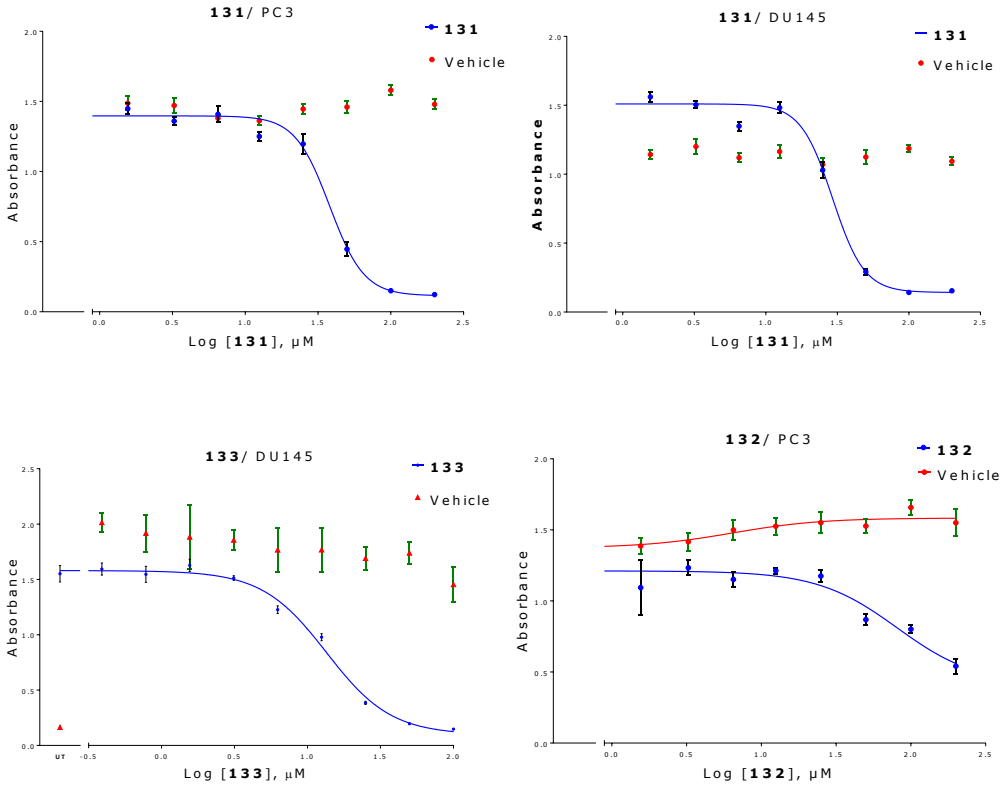


Figure 46. Effect of increasing concentrations of triazoles (131 on PC3 and DU145, 133 on PC3 and 132 on PC3[inactive example]) measured by MTT assay.

Analogues with a C-4 ethanol **132**, n-butanol **134** and n-pentanol **135** on the triazole ring were synthesised and tested on both cell lines. None

of these showed any activity on either cell line. This suggests that the side chain length is controlling the activity. The reason for this could be the number of rotation bonds, and the polarity or the distance approaching the receptor or the attraction site.

The 1,2,3-triazole 4,5-di-ol synthesis was unsuccessful, hence no biological evaluation was performed for the disubstituted triazoles.

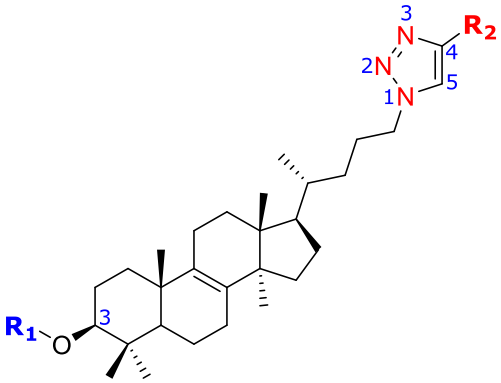
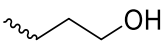
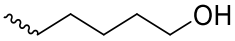
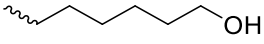


Figure 47. Triazole analouges generic structure.

Table 22. Biologically inactive triazoles.

R_1	R_2	Compound number	PC3 IC_{50} µM	DU145 IC_{50} µM
Acetate		**132**	-	-
Acetate		**134**	-	-
Acetate		**135**	-	-

3.3.3. Assessment of ganoderic acid-DM analogues activity

The ganoderic acid-DM analogues were all soluble in 1% v/v EtOAc. However, none of them showed activity against PC3 and DU145. The analogues synthesised were designed to be similar to methyl ganoderic

acid-DM with acetate, hydroxyl and ketone functional group at C3. The other difference is the ketone group at C7 which is present in methyl ganoderic acid-DM and not in the derivatives synthesized. From the biological evaluation we could conclude that the ketone at C7 is essential for activity. Biological evaluation of methyl ganoderic acid-DM against the PC3 cell line showed an IC_{50} of 0.3 μM while the ganoderic acid-DM showed an IC_{50} of 40 μM against the same cell line. The methyl group on the side chain of the ganoderic acid helps to reduce polarity and enhance cell membrane penetration[18].

The GA-DM analogues synthesised did not show any cytotoxicity, which confirms the importance of the ketone group on C7 ring B. The synthesis of this ketone group is very challenging as the intermediate is unstable (highly reactive) and forms to a diketone conjugated product C7/C9. However, the synthesis and the biological evaluation of the synthesised compounds in this study have not been previously performed.

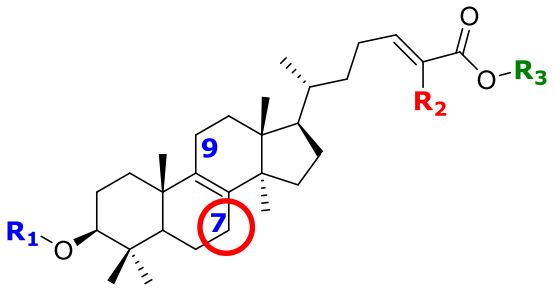


Figure 48. Ganoderic acid-DM analogues generic structure.

Table 23. Ganoderic acid-DM analogues substitutions/IC_{50}.

R_1	R_2	R_3	Compound number	PC3 IC_{50} µM	DU145 IC_{50} µM
Acetate	H	CH_3	**107**	-	-
H	H	CH_3	**136**	-	-
Carbonyl	H	CH_3	**137**	-	-
Acetate	H	C_2H_5	**140**	-	-
H	H	C_2H_5	**138**	-	-
Carbonyl	H	C_2H_5	**139**	-	-
Acetate	CH_3	C_2H_5	**142***	-	-
H	CH_3	C_2H_5	**141***	-	-
Carbonyl	CH_3	C_2H_5	**143***	-	-

* Compounds provided by previous PhD student.

3.4. Insoluble compounds

The amines mentioned in Figure 49 and Table 24 were insoluble by the solvent (EtOAc 1%) used in the biological evaluation. The reason for insolubility is the high polarity in both ends of the compounds, the side chain and at the C3 position. Alternative biological evaluation is possible by using drug delivery technique such as liposomes.

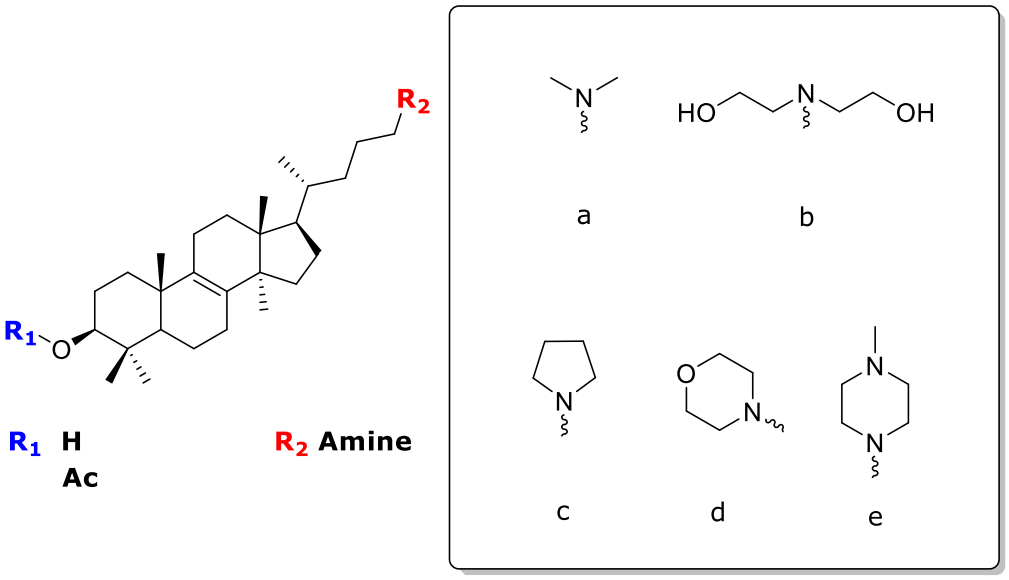


Figure 49. Insoluble amines compounds.

Table 24. Compounds biological activity was not evaluated (insoluble).

R_1	R_2	Compound number	PC3 IC_{50} µM	DU145 IC_{50} µM
OH	a	**111**	-	-
OAc	a	**112**	-	-
OH	b	**115**	-	-
OAc	b	**116**	-	-
OH	c	**117**	-	-
OH	d	**123**	-	-
OAc	d	**124**	-	-
OH	e	**128**	-	-
OAc	e	**129**	-	-

3.5. Conclusion

From the biological evaluation for the three groups (amines; triazoles and ganoderic acid-DM) of the synthesised analogues, it can be

concluded that low polarity aliphatic side chain amines showed biological activity on both cell lines, while higher polarity amines on the side chain did not show activity or were insoluble.

None of the synthesised analogues of ganoderic acid-DM showed activity. The difference in the generic structure between the natural ganoderic acid DM and the synthesised analogues is the ketone group on C7. The conclusion from the biological evaluation for the synthesised analogues is that the ketone group on C7 is essential for the biological activity of the ganoderic acid-DM analogues.

The odd short side chain on C4 of the triazole ring showed biological activity, while the even and long side chain did not show any activity.

C3 hydroxyl analogues of the triazole ring were insoluble, hence no biological evaluation was performed.

4. SAR Analysis

4.1. General SAR

We have investigated the structure-activity relationship (SAR) of the synthesized analogues. The modification areas of lanosterol **1** were C3 by performing hydroxyl acetylation or oxidation to a ketone, and C24 by any of three reactions: Wittig olefination, reductive amination or triazoles substitution. The first modifications on C24 were amines with aliphatic, aromatic and acyclic groups respectively; as well as high and low polarity amines. The other group of compounds were triazoles which according to the side chain length lengths attached to triazoles. The third group was GA-DM analogues with methyl and ethyl esters on the side chain but without the C7 ketone.

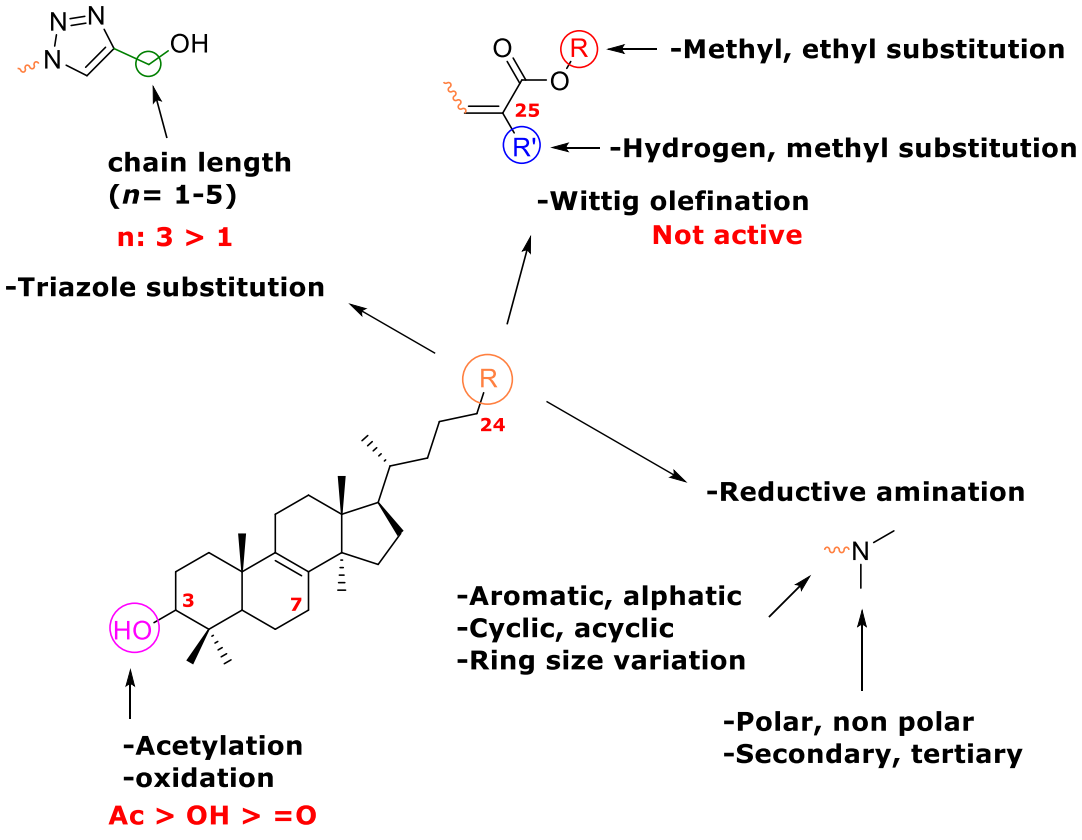


Figure 50. Areas of modification on lanosterol 1.

4.2. Amines

1. Compounds with substitution of dimethylamine **111** and **112**, diethanolamine **115** and **116**, morpholine **123** and **124** and methylpiperazine **128** and **129** at C24 were insoluble in DMSO and ethyl acetate with either hydroxyl or acetate at the C3 position; therefore, their biological activity was not evaluated.

2. The compound with substitution of pyrrolidine at C24 and hydroxyl at C3 **117** was insoluble.

3. The compound with substitution of pyrrolidine at C24 and acetate at C3 **118** was active against PC3 and DU145.

4. Compounds with dimethylamine substitution at C24 were active, with either hydroxyl **113** or acetate **114** at C3 against PC3 and DU145.

5. The compound with substitution of piperidine at C24 with a hydroxyl on C3 **119** was inactive, while the acetate analogue on C3 **120** was active against PC3 and DU145.

Table 25. Amines derivatives SAR.

Compound	Molecular Weight	No. of fused rings	H- Bond Donors	H- Bond Acceptors	[a]clogP
119	469.798	4	1	2	7.05
120	511.835	4	0	2	7.49
121	477.777	4	2	2	7.46
122	519.814	4	1	2	7.90
117	455.771	4	1	2	6.61
118	497.808	4	0	2	7.05
111	429.733	4	1	2	6.20
112	471.77	4	0	2	6.64
113	457.787	4	1	2	6.92
114	499.824	4	0	2	7.36
125	487.83	4	1	2	6.56
126	529.87	4	0	2	7.01
127	485.82	4	0	2	7.13
130	612.94	4	0	3	7.39
123	471.77	4	1	3	5.98
124	513.807	4	0	3	6.43
115	489.785	4	3	4	4.82
116	531.822	4	2	4	5.26
128	484.813	4	1	3	6.05
129	526.85	4	0	3	6.49

4.3. Triazoles

1. Triazole compounds with hydroxyl substitution at C3 were insoluble in DMSO and ethyl acetate.

2. Compounds with acetate at C3 and an odd number of carbons on the hydroxyl side chain of the triazole ring **131** and **133** showed biological activity, whereas those with an even numbers of carbons in the side chain **132** and **134** did not show any biological activity.

3. Compounds with acetate at C3 and amine or bromide on the side chain of the triazole ring were insoluble, therefore their biological activity was not evaluated.

Table 26. Triazoles derivatives SAR.

Compound	Molecular Weight	No. of fused rings	H- Bond Donors	H- Bond Acceptors	[a]clogP
131	525.778	4	1	1	5.792
132	539.805	4	1	1	6.034
133	553.832	4	1	1	6.316
134	567.859	4	1	1	6.764
135	581.886	4	1	1	7.20

4.4. GA-DM analogues

1. From literature, the IC_{50} on PC3 cell lines for GA-DM is 40 μM while the IC_{50} for the methyl GA-DM against the same cell lines is 0.3 μM[1].

2. A compound with similar structure to methyl GA-DM but without the ketone at C7, and in which the methyl group of the α-β unsaturated ester (C25) was replaced by a hydrogen atom was synthesised. The biological activity of the synthesised analogue was evaluated and did not show activity (**137**).

3. A compound with a similar structure to methyl GA-DM with an ethyl ester on the side chain, and without the ketone group on C7, as well as the methyl group on the α-β unsaturated ester (C25) being replaced by a hydrogen atom was synthesised. The biological activity of the synthesised compound was evaluated (**139**).

Table 27. GA-DM derivatives SAR.

Compound	Molecular Weight	No. of fused rings	H- Bond Donors	H- Bond Acceptors	[a]clogP
136	456.711	4	1	2	6.82
107	498.748	4	0	2	7.32
137	454.695	4	0	2	7.44
138	470.738	4	1	2	7.23
140	512.775	4	0	2	7.67
139	468.722	4	0	2	7.80
108	456.711	4	2	3	6.89

4.5. Structure-activity relationships (SARs) conclusion

We have investigated the SAR for the synthesised analogues. Taking aldehydes **42** and **43** as a starting point, the distinct difference was the C3 substitution. We replaced the C3 hydroxyl group with an acetate group and performed oxidation to produce ketone analogues. The enhancement in solubility associated with the C3 esterification indicates the importance of the low polarity on C3 for solubility and cell surveillance effect. Oxidation of the C3 hydroxyl shows no biological effect. Introduction of amines on the side chain at C24 showed activity for some compounds (**113**, **114**, **118**, **120**). The solubility issue associated with some of the polar amine analogues suggests that having polar groups on the side chain can reduce solubility and cell membrane penetration. The high potency of the aliphatic cyclic amine analogues with acetate at C3 suggests the importance of the low polarity for solubility and cell membrane penetration, compared with the C3 hydroxyl amines and the aromatic amines. Introduction of a 1,2,3-triazole ring resulted in high potency and solubility for the C3 acetate analogues, which suggests that having a heterocyclic aromatic ring on the side chain can enhance the solubility and the cell surveillance effect. The insolubility of the C3 hydroxyl triazoles confirms the importance of the acetate group for solubility.

The modification of the side chain with methyl and ethyl ester analogues via Wittig olefination led to a decrease in potency. The esters were synthesised as analogues of GA-DM with one functional group difference; the ketone on C7. This decrease in potency shows the importance of the ketone group on C7. Modifying the ester; amine and

triazole analogues with the ketone group on C7 can also possibly increase the potency many-fold.

5. Conclusions and future work

Using lanosterol **1**, which is commercially available in a reasonable purity of 50-60%, as a starting material, three different libraries of ganoderic acid analogues have been synthesised. These are amine, triazole and GA-DM analogues. The synthesis of these analogues was carried out by modifying **1** at the β-hydroxy on C3 and on the side chain at C24 (Figure 50).

The synthesis of aldehyde **43** gave a high yield of 67% via dihydroxylation and oxidative cleavage at the C24/25 double bond. After the oxidative cleavage, the desired aldehyde **42** was used to synthesise ganoderic acid analogues. Aldehydes **42** and **43** underwent reductive amination with secondary amines including diethylamine, piperdine and aniline, in high percentage yield. The protection of the hydroxyl group was attempted by following various different routes; the best protecting group in terms of synthesis stability and purification was the acetate. Aldehyde **98** was synthesised using Sharpless dihydroxylation and oxidative cleavage. This synthesis route was expensive and required seven steps, giving an overall low yield. As a result no further compounds used aldehyde **98** as an intermediate. Moreover triazole derivatives such as **131** were synthesised in excellent yield (100 %).

The biological activity of the compounds synthesised was evaluated by using PC3 and DU145 androgen-independent human prostate cancer cell lines using a MTT cell survival assay. The polarity and size of the substituents on C24 also affects the solubility and cytotoxicity of the compounds. A QSAR study showed that the acetate group at C3 is crucial for the cytotoxicity and good aqueous solubility. The ketone group at C7 is essential for the biological activity of the GA-DM analogues.

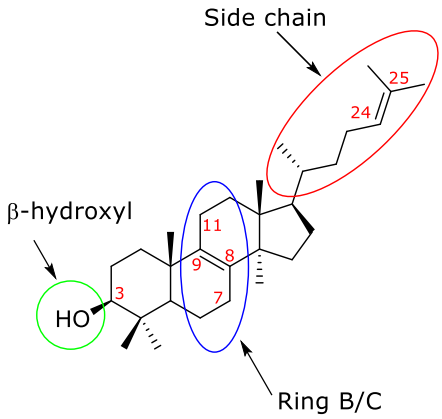
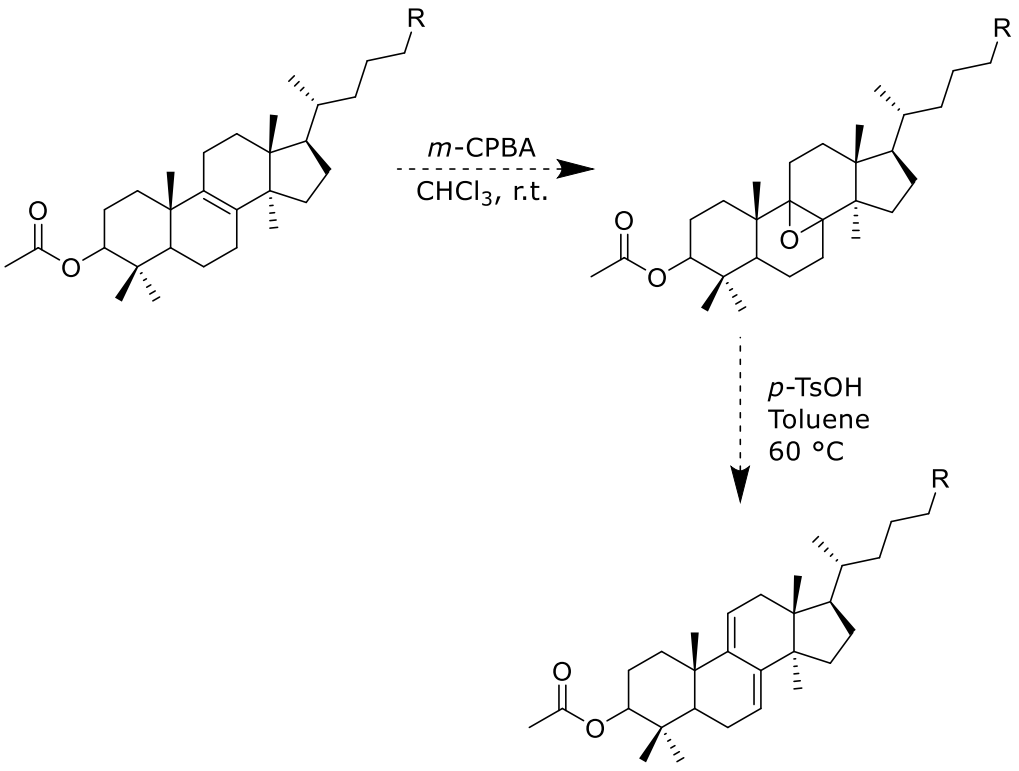


Figure 51. Possible functionalization of lanosterol 1.

Future work towards the synthesis of new analogues via Jones oxidation to get ketones at C7 and C11 would be interesting; the oxidation would occur on the allylic methylene groups C7 and C11. Future work might be able to achieve synthesis of C7/C8, C9/C11 diene moiety by forming an epoxide via the Prilezhaev reaction. The α8, α9-epoxy lanosterol, would undergo ring opening, the C-O would break, and then deprotonation would happen, forming the C7/C8, C9/C11 diene analogue (Scheme 36).



Scheme 36. Synthesis of C7/C8, C9/C11 diene analogue[152].

Further attempts at synthesis of secondary and tertiary amino derivatives at C24 starting with lanosterol **1**, is worth pursuing in future studies, as the resulting analogues may interact differently with a receptor active site.

Synthesis of new triazole and tetrazole side chain derivatives starting from aldehyde **43** is also worth more investigation. Additional attempts to synthesise aldehyde **97** and aldehyde **102** using selenium dioxide, with the aim of using them in reductive amination reactions and Wittig reactions.

Synthesis of new analogues from the active triazoles by etherification of the triazole ring side chain, would reduce the polarity, as a potential future route to improving biological activity.

Biological evaluation of any of these possible analogues should be done in further studies to check if they exhibit cytotoxicity or inhibitory effect, using cell proliferation assays, cell migration assays and bioavailability assays.

The active compounds also need to be tested in one of the non-cancerous lines e.g. epithelial RWPE1 prostate cell line to investigate their likely effects on healthy patient cells.

It could be useful to test the ethyl acetate insoluble compounds reported in this thesis (amines) by using a drug delivery system such as liposomes and to investigate the further mechanism of action of the biologically active compounds by the following assays:

- Reactive oxygen species (ROS) level evaluation
- Reactive nitrogen species (RNS) level evaluation.
- Measurement of 5α-reductase inhibitory activity.
- Androgen receptor binding assay.

6. Experimental

6.1. General Procedures

Chemicals purchased from Merck Millipore®, Sigma Aldrich®, Fischer Scientific®, TCI®, Fluorochem® and MP® were used without further purification. Commercial reagent grade solvents from Fischer Scientific® were used for all reactions. Anhydrous 1,4-dioxane and pyridine solvents were purchased from Fischer Scientific® and Sigma Aldrich®. All reactions were monitored by analytical thin layer chromatography (TLC) performed on Merck silica gel F_{254} percolated aluminium sheets, visualised by UV absorption (254 nm) and stained with potassium permanganate ($KMnO_4$), phosphomolybdic acid (PMA) solution, or sulphuric acid in ethanol.

Flash column chromatography was performed using Merck Kieselgel 35-70µ, 60 A°silica.

Melting points were determined with the Stuart Scientific Melting point apparatus and reported without correction. Infra-red (IR) spectra were recorded on a Bruker TENSOR 27 spectrometer; fitted with a diamond attenuated total reflectance (ATR) attachment.

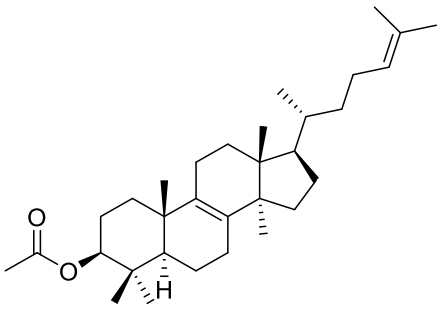
^{1}H NMR (400 MHz), ^{1}H NMR (500 MHz), ^{13}C NMR (100 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker™ DPX300MHz, AV400, or DPX400, AV3400HD, AV3500 spectrometers. They were recorded in solutions of deuterated chloroform ($CDCl_3$) and were processed in Mnova. Chemicals shifts δ, given in parts per million (ppm) and *J* values in Hertz (Hz). Multiplets were designated by the following notations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of

doublets combinations. All spectra are reported as observed and calibrated to the residual deuterated solvent peak ($CDCl_3$ σ_H 7.26 ppm, σ_c 77.28 ppm)

High resolution mass spectra (HRMS) were recorded on a Bruker™ micro ToF spectrometer using an electrospray ionisation (ESI positive ion) source. Values of mass-to-charge ratio, denoted m/z, given to four decimal places. The mass of counter ions; H^+; Na^+; $C_3H_4N_2^+$ and NH_4^+; 1.0078, 22.9898, 68.077 and 18.0384 respectively.

Compound names and structures were generated by ChemDraw Professional 16.0.

6.2. Synthesis of (5R,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[α]phenanthren-3-yl acetate 66[153]

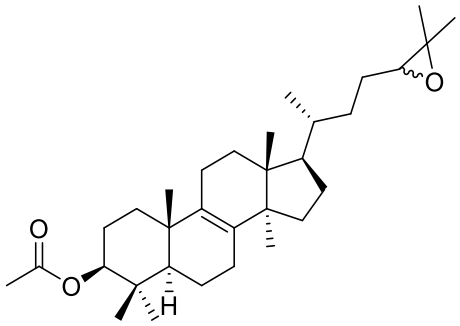


Commercial Lanosterol **1** (50-60%) (8 g, 9.37 mmol) was dissolved in dichloromethane (70 mL). DMAP (0.16 g, 1.30 mmol) and pyridine (12 mL, 149.0 mmol) was added to the solution, followed by the addition of acetic anhydride (3 mL, 31.7 mmol) to the reaction which was then

stirred for 6h at room temperature. The reaction mixture was added as colourless solution into dichloromethane (40 mL) then washed successively with hydrochloric acid (2 x 100 mL, 2M), sodium hydrogen carbonate (2 x 100 mL) (sat. aq. sol.) and brine (2 x 100 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and the filtrate collected. Solvent was removed under vacuum to yield a white residue. The crude product was purified by column chromatography using petroleum ether 40/60: diethyl ether (95:5) R_f = 0.3, to retrieve the purified mixture (lanosterol acetate and dehydrolanosterol acetate) (6.51g 13.9 mmol, 74 %).

M.p. 114-119 °C, Lit: 125-129°C[153]; FT-IR (ATR) V_{max}/cm^{-1} 2949, 1733, 1462, 1369, 1243 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 5.14- 5.10 (m, 1H), 4.52 (dd, 1H, *J*= 11.5,4.5 Hz) , 2.07 (s, 3H), 2.05-1.72 (m, 20H) 1.71 (s, 3H), 1.63 (s, 3H), 1.61-1.30 (m, 20 H), 1.28 (br s, 6 H), 1.22-1.15 (m, 1H), 1.13-1.04 (m, 2H), 1.03 (s, 3H), 0.94-0.92 (m, 5H), 0.90 (br s, 6H), 0.89-0.88 (m, 6H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 171.0 (C), 134.5 (C), 134.2 (C), 130.9 (C), 125.2 (CH), 80.9 (CH), 50.5 (C), 50.2 (CH), 50.3 (CH), 49.8 (C), 44.4 (C), 39.5 (C), 37.8 (C), 36.9 (C), 35.2 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 29.7 (CH_2), 28.2 (CH_2), 27.9 (CH_3), 26.4 (CH_2), 25.7 (CH_2), 24.9 (CH_2), 22.8 (CH_3), 21.3 (CH_2), 21 (CH_3), 19.1 (CH_2), 18.6 (CH_2), 18.1 (CH_3), 17.6 (CH_3), 16.5 (CH_2), 15.7 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 491.3842 $C_{32}H_{52}O_3$ required 491.3860.

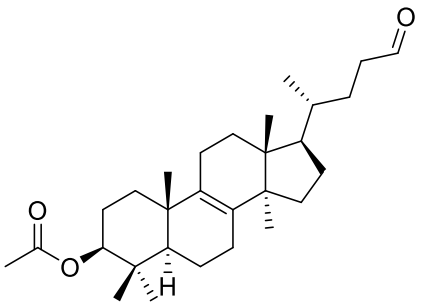
6.3. Synthesis of (5R,10S,13R,14R,17R)-17-((2R)-4-(3,3-dimethyloxiran-2-yl)butan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[α]phenanthren-3-yl acetate 69[74]



A mixture of 3β-acetoxy-5α-lanosta-8,24-diene and dihydrolanosterol acetate **66** (0.8 g, 1.7 mmol) was dissolved in dichloromethane (25 mL), *m*-CPBA (0.191 g, 1.11 mmol, 70-75%) and sodium hydrogen carbonate (0.145 g, 1.81 mmol) were mixed together and half of this mixture was added over 30 minutes at room temperature and the other half was added at 0 °C over 30 minutes. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was filtered then successively washed with sodium hydrogen carbonate (4 x 25 mL) (sat. aq. Sol.), and brine (1 x 25 mL). The aqueous layer was removed and the organic layer was dried over magnesium sulphate before filtration, solvent was removed under vacuum to yield the crude product as a white residue. The crude product was purified by flash chromatography petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.5, to afford a white residue (0.617 g 1.27 mmol, 78 %).

M.p. 179-184 °C, Lit: 184-187 °C[74]; FT-IR (ATR) V_{max}/cm^{-1} 2948, 1732, 1369, 1242, 1160 ; ^{1}H NMR (400 MHz, $CDCl_3$) δH 4.52 (dd, 1H, J= 11.5,4.5 Hz), 2.70 (t, 1H, J= 6.21), 2.07 (s, 3H), 2.06-1.92 (m, 20H) 1.78-1.64 (m, 20H), 1.62 (s, 6H), 1.61-1.34 (m, 20H), 1.33 (s, 3H), 1.29 (s, 3H) , 1.27-1.18 (m, 1H), 1.16-1.06 (m, 2H), 1.02 (s, 3H), 0.97-0.93 (m, 5H), 0.90 (br s, 6H), 0.89-0.88 (m, 6H) 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δc :171.0 (C), 134.4 (C), 134.2 (C), 80.9 (CH), 64.94 (CH), 64.80(C), 58.4 (C), 58.1 (C), 50.5 (C), 50.2 (CH), 49.8 (C), 44.5 (C), 37.8 (C), 36.9 (C), 36.2 (CH), 35.2 (CH_2), 30.8 (CH_2), 28.2 (CH_2), 27.9 (CH_3), 26.3 (CH_2), 24.9 (CH_3), 24.2 (CH_2), 24.1 (CH_3), 22.8 (CH_2), 21.3 (CH_2), 20.9 (CH_3), 19.1 (CH_2), 18.1 (CH_3), 16.5 (CH_2), 15.7 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 507.3820 $C_{32}H_{52}O_3Na$ required 507.3809.

6.4. Synthesis of (5R,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-oxopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[α]phenanthren-3-yl acetate 43[74]

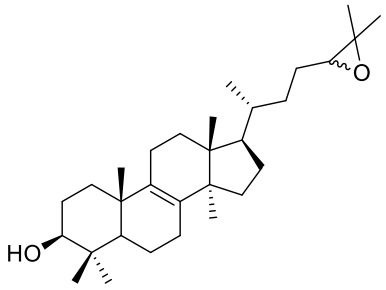


A mixture of 3β-acetoxy-5α-24, 25-epoxy-lanost-8-ene **69** (0.288 g, 0.594 mmol) was added to stirred solution of periodic acid (0.197 g,

0.858 mmol) in diethyl ether (55 mL) at room temperature, the solution was stirred under nitrogen atmosphere for 1.5 h. Water (28 mL) was added and the organic layer separated and washed with water (2 x 20 mL) to remove the precipitate HIO_3. The organic layer was dried over magnesium sulphate and the solvent was removed *in vacuo* to yield a white residue. The crude product was purified by flash chromatography using petroleum ether 40/60: diethyl ether (9:1) R_f = 0.1, to afford a white residue (0.216 g, 0.476 mmol, 80 %).

M.p. 157-163 °C, Lit: 153-156 °C[74]; FT-IR (ATR) V_{max}/cm^{-1} 2828, 1721 , 1451, 1366, 1250, 1182; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 9.79 (t,1H , *J*=1.9 Hz), 4.52 (dd, 1H, *J*= 11.8,4.7 Hz), 2.49-2.45 (m, 2H), 2.43-2.33 (m, 2H), 2.07 (s, 3H), 2.06-1.15 (m), 1.02 (s, 3H), 0.97-0.92 (m, 5H), 0.90 (br s, 6H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C : 203.0 (C), 171.0 (C), 134.37 (C), 134.32 (C), 80.9 (CH), 50.4 (C), 50.2 (CH), 49.8 (C), 44.5 (C), 41.1 (CH_2), 37.8 (C), 36.9 (C), 36.0 (CH), 35.2 (CH_2), 30.9 (CH_2), 30.7 (CH_2), 28.2 (CH_2), 28.1 (CH_3), 27.9 (CH_3), 26.3 (CH_2), 24.23 (CH_2), 24.17 (CH_2), 21.3 (CH_2), 20.9 (CH_3), 19.1 (CH_2), 18.4 (CH_2), 18.1 (CH_3), 16.5 (CH_2), 15.7 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 465.3343 $C_{29}H_{46}O_3Na$ required 465.3339.

6.5. Synthesis of (10S,13R,14R,17R)-17-((2R)-4-(3,3-dimethyloxiran-2-yl)butan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 74[74]

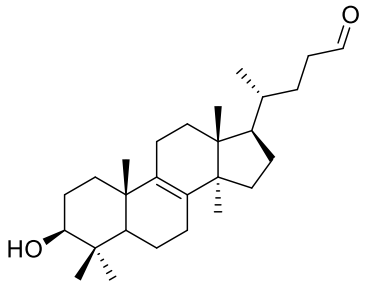


A mixture from lanosterol and dihydrolansterol (2 g, 4.69 mmol) was dissolved in dichloromethane (80 mL), *m*-CPBA (0.539 g, 3.12 mmol) and sodium hydrogen carbonate (0.407 g, 4.84 mmol, 70-75%) was mixed together and half of this mixture was added over 30 minutes at room temperature and the other half was added at 0 °C over 30 minutes. The reaction mixture was stirred at 0 °C for 4 h. The reaction mixture was filtered then successively washed with sodium hydrogen carbonate (4 x 25 mL) (sat. aq. Sol.), and brine (1 x 25 mL) and (1 x 25 mL). The aqueous layer was removed and the organic layer was dried over magnesium sulphate before filtration, solvent was removed under vacuum to yield the crude product as an off-white residue. Crude material was purified by column chromatography using gradient elution of petroleum ether 40/60: ethyl acetate (8:2 → 9:1 → 8:2) R_f = 0.6, to give the purified product (0.757 g, 1.71 mmol, 68 %) as an off-white solid.

M.p. 124-126 °C, Lit: 125-126 °C[74]. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H :3.26(dd, 1H, *J*= 11.55, 4.53 Hz), 2.71(t, 1H, *J*= 6.21 Hz), 2.08-1.35

(m, 20H) 1.33 (s, 3 H), 1.29 (s, 3H), 1.26-1.06 (m, 20 H), 1.02 (s, 3H), 1.0 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.90 (s, 6H), 0.88 (s, 3H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 134.4 (C), 134.3 (C), 78.9 (CH), 64.9 (C), 46.7(CH), 50.4 (C), 50.38 (CH), 50.34 (CH) , 49.8 (C), 44.5 (C), 38.9(C), 37.0 (C), 36.3 (C), 35.6 (CH_2), 31.0(CH_2), 30.9 (CH_2), 30.8 (CH_2), 28.2 (CH_2), 27.9 (CH_3), 26.5 (CH_2), 25.6 (CH_2), 24.9 (CH_2), 24.2 (CH_3), 20.9 (CH_3), 19.1 (CH_2), 18.7 (CH_2), 18.5 (CH_3), 18.1(CH_3), 16.5 (CH_2), 15.7 (CH_3), 15.4 (CH_3). Parent ion did not observed on HRMS or FDMS.

6.6. Synthesis of (4R)-4-((10S,13R,14R,17R)-3-hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanal 42[74]

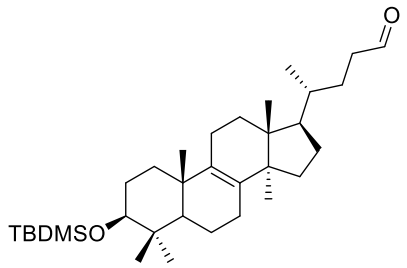


A mixture of 3β-Hydroxy-5α-24, 25-epoxy-lanost-8-ene **74** (0.615 g, 1.39 mmol) was added to stirred solution of periodic acid (0.456 g, 2.0 mmol) in diethyl ether (119 mL) at room temperature, the solution was stirred under nitrogen atmosphere. Water (68 mL) was added and the organic layer separated and washed with water (2 x 34 mL) to remove the precipitate HIO_3. The organic layer was dried over magnesium

sulphate before filtration. Filtrate was removed *in vacuo* to yield a white residue. The crude product was purified by flash chromatography using gradient elution of petroleum ether 40/60: diethyl ether (85:15 → 95:5) R_f = 0.2, to afford an off-white solid (0.379 g, 0.946 mmol, 68 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 9.8 (t,1H , *J*=1.9 Hz), 3.26 (dd, 1H, *J*= 11.69 , 4.68 Hz), 2.54-2.19 (m, 4H), 2.08-1.05 (m, 20H), 1.02 (s, 3H) , 1.0 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C : 203.2 (CH), 134.4 (C), 134.2 (C), 78.9 (CH), 50.4 (C), 50.2 (CH), 49.8 (C), 44.5 (C), 41.1 (CH_2), 38.9 (C), 37.0 (C), 36.0 (CH), 35.5 (CH_2), 31.0 (CH_2), 30.9 (CH_3), 30.8 (CH_2), 30.7 (CH),28.2 (CH_2), 28.1 (CH_3), 27.9 (CH_3), 26.4 (CH_2), 24.2 (CH_2), 20.9 (CH_3), 19.1 (CH_2), 18.4 (CH_2), 18.2 (CH_3), 15.7 (CH_2), 15.4 (CH_3). Parent ion did not observed on HRMS or FDMS.

6.7. Synthesis of (4R)-4-((10S,13R,14R,17R)-3-((tert-butyldimethylsilyl)oxy)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanal 77[154]

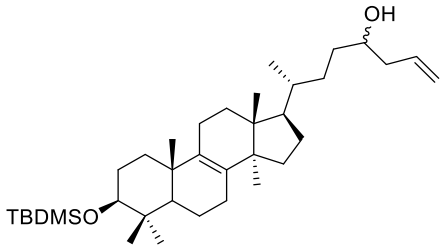


Dry DMF (7 mL) was added to aldehyde **42** (0.319 g 0.8 mmol) to form an off white suspension under argon at 0 °C, DMF (1.5 mL) was added

to imidazole (0.069 g, 1.01 mmol) and TBDMS-Cl (0.143 g, 0.947 mmol) under argon and transferred over a period of 10 minutes to the lanosterol suspension, the reaction mixture was allowed to cool to room temperature and stirred overnight. The reaction worked by dilution with 21 mL Et_2O, then washed successively with $NaHCO_3$ (3 X 11 mL), HCl (3 X 11 mL, 1 M) and water (3 X 11 mL), the solution was dried by anhydrous $NaSO_4$, filtrated, then concentrated using a rotary evaporator. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.1, to afford the pure product as a white solid (0.081 g, 0.157 mmol, 25 % yield).

M.p. 167-170 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 9.8 (t, 1H, *J*= 1.96), 3.23 (dd, 1H J = 11.0, 4.65 Hz), 2.54-2.33 (m, 4H), 2.05 – 1.03-1.00 (s, 3 H), 0.94 (s, 3H), 0.92 (s, 3H) , 0.90 (S, 9 H), 0.80 (S, 3 H), 0.72 (S, 3 H), 0.06 (d, 6 H, *J*= 2.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 203.4 (C), 79.4 (CH), 50.4 (CH), 50.3 (CH), 41.1 (CH_2), 36.0 (C), 35.5 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.4 (CH), 28.2 (CH_2), 26.5 (CH_2), 26.0 (C), 25.9 (3) (CH_3), 24.8 (CH_3), 24.2 (CH_3), 21 (CH_2), 19.2 (CH_3), 18.45 (CH_2), 18.4 (C), 15.9 (CH_3), 15.7 (CH_3), - 4.89 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 515.4274 required $C_{33}H_{58}O_2Si$ 515.4279. Parent ion did not observed on HRMS or FDMS.

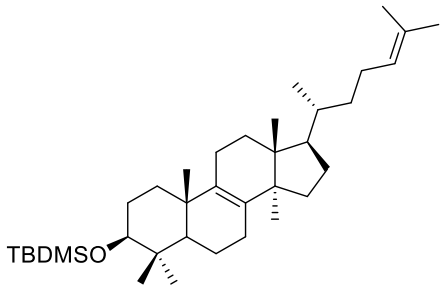
6.8. Synthesis of (7R)-7-((10S,13R,14R,17R)-3-((tert-butyldimethylsilyl)oxy)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)oct-1-en-4-ol 81



Magnesium turnings (63 mg, 2.59 mmol) and a spatula tip of iodine crystals were transferred into dry flask, dry THF (5.3 mL) was added through the septum, the temperature was raised to (45-50 °C) by water bath. The solution then cooled to 0 °C, allyl bromide (0.1 mL, 1.17 mmol) diluted in THF (0.3 mL) was added by cannula, aldehyde **77** (0.273 g, 0.53 mmol) was dissolved in dry THF (2.7 mL) and transferred as a solution to the reaction mixture by cannula, the mixture kept on stirring for 4.5 h at 0 °C. The reaction quenched by NH_4Cl (4.7 mL), THF was removed by rotary evaporator, the residue was extracted twice with a mixture of ethyl acetate (27 mL) and water (15 mL), washed successively with water (2 X 7 mL), brine (2 X 7 mL) and dried over anhydrous Na_2SO_4, filtrated, the solvent was removed under vacuum to give the crude product residue (211 mg). The crude product was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.2, to afford the pure product as a white solid (0.081 g 0.145 mmol, 28 % yield).

M.p. 141-144 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 5.9-5.8 (m, 1 H), 5.18-5.14 (m, 16 H), 3.6-3.5 (m, 1H), 3.23 (dd, 1H, *J* =11.0 , 4.6), 2.38-2.29 (m, 1 H), 2.21-2.11 (m, 1H), 2.04-2.03(m, 1H), 2.00-1.08(m, 20 H), 1.06-1.03 (m, 6H), 0.90 (s, 9H), 0.71 (s, 3H), 0.05 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.9 (CH), 134.6 (C), 134.2(C), 118.0 (CH_2), 79.4 (CH), 71.0 (CH), 50.4 (CH), 50.3 (CH), 49.8 (C), 44.5(C), 42.0 (CH_2), 39.4 (C), 36.9 (C), 35. 5 (CH), 33.5 (CH_2), 33.5 (CH_2), 32.1 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.3 (CH_2), 28.28 (CH_2), 28.25 (CH_2), 28.20 (CH_2), 26.5 (CH_2), 25.9 (CH_3), 24.2 (CH_2), 21.0 (CH_2), 19.02 (CH_3), 18.7 (C), 18.4 (CH_3), 15.9 (CH_3), -4.8 (CH_3). FDMS: found 556.4676 $C_{36}H_{64}O_2Si$ required 556.4670.

6.9. Synthesis of tert-butyldimethyl(((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)silane 75

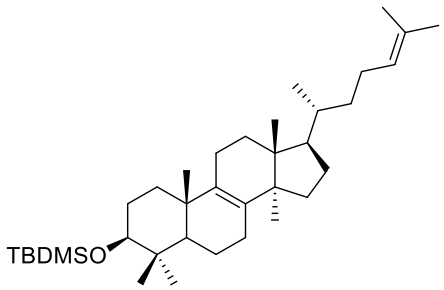


Lanosterol **1** (2 g, 4.69 mmol) was transferred into 100 mL preliminary calcined three-necked flask, flask content was put under a nitrogen atmosphere at 0 °C, anhydrous DMF (48 mL) was added by syringe to

form a suspension which was cooled to 0 °C, dry DMF (16 mL) was added to a vial containing imidazole (0.402 g, 5.9 mmol 1.26 eq.) and TBDMS-Cl (0.083 g 5.53 mmol 1.18 eq.) to get a turbid suspension. The suspension was transferred by cannula to the lanosterol suspension over 10 minutes, then allowed to cool to room temperature. The white suspension was stirred overnight, after 17 h. The reaction monitored by TLC, no additional spots appeared, then one equivalent of imidazole (0.32 g 4.7 mmol) in anhydrous DMF (2mL) added. The reaction was diluted after 42 h by diethyl ether, the resulting solution washed successively by $NaHCO_3$ (3 X 60 mL), HCl 1M (3 X 60 mL), water (3 X 60 mL), dried over anhydrous Na_2SO_4, filtrate, the solvent was removed under vacuum which gave a crude residue that purified by column chromatography using *n*-hexane R_f = 0.4, to afford a white solid (1.095 g, 2.02 mmol, 52 % yield).

M.p. 132-133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 5.03 (t, 1H, J = 7.09 Hz), 3.17 (dd, 1H J = 11.25, 4.89 Hz), 2.04 – 0.98 (m, 26 H), 0.95 (s, 3H), 0.88 (s, 9 H), 0.86(s, 3H) 0.84 (s, 3 H), 0.74 (s, 3 H), 0.66 (s, 3H), 0.01 (d, 6 H, *J*= 2.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.6 (C), 134.2 (C), 130.9 (C), 125.2 (C), 79.45 (CH), 50.47 (CH) , 50.43 (CH) , 49.8 (C), 44.5 (C) , 39.4 (C), 37.9 (C), 36.3 (CH), 36.2 (CH_2), 35.5 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.4 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 26.5 (CH_2), 25.9 (3) (CH_3), 25.7 (CH_3), 24.9 (CH_3), 24.2 (CH_2), 21 (CH_3), 19.2 (CH_2), 18.6 (CH_3), 18.4 (C), 18.1 (C), 17.6 (CH_3), 15.9 (CH_3), 15.7 (CH_3) - 4.9 2 X (CH_3). FDMS: found 540.4748 $C_{36}H_{64}O_2Si$ required 540.4721.

6.10. Synthesis of tert-butyldimethyl(((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)silane 75[155]

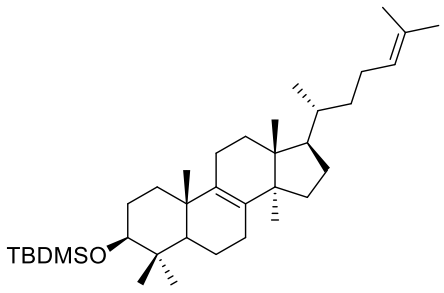


To lanosterol **1** (0.5 g 1.173 mmol) under nitrogen, dry DCE (1.5 mL) was added to form a white suspension, dry DCE (1.5 mL) added to imidazole (0.2 g 2.92 mmol 2.5 eq.) and TBDMS-Cl (0.21 g 1.4 mmol 1.2 eq.) and gave a turbid suspension which added to the lanosterol suspension. The suspension was left stirring overnight at room temperature. Reaction quenched by adding H_2O (0.5 mL) then diluted by DCE (3mL), the organic layer washed subsequently by 5% $KHSO_4$ (3 X 20 mL), $NaHCO_3$ (2 X 20 mL) and brine (2 X 20 mL). The organic layer dried by $NaSO_4$, filtered and then solvent was evaporated under vacuum. The crude mixture was purified by column chromatography using *n*-hexane R_f = 0.4, to afford a white solid (0.053 g, 0.097 mmol, 15 % yield).

M.p. 131-133 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 5.13 (t, 1H, J = 7.1Hz), 3.23 (dd, 1H J = 11.0 , 4.8 Hz), 2.05 – 1.73 (m, 20 H), 1.71 (S, 3 H), 1.63 (S, 3 H), 1.61- 1.03 (m 20 H), 1.07-1.03 (m, 6H), 1.00 (br S, 3

H), 0.95- 0.93 (m, 5H) , 0.92 (s, 9 H), 0.80 (s, 3 H), 0.71 (s, 3 H), 0.05 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.6 (C), 134.2 (C), 130.9 (C), 125.2 (C), 79.45 (CH), 50.5 (CH) , 50.4 (CH) , 49.8 (C), 44.5 (C) , 39.4 (C), 36.9 (C), 36.3 (CH), 36.2 (CH_2), 35.6 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.4 (CH_2), 28.0(CH_2), 26.5 (CH_2), 25.9 3 X (CH_3), 25.7 (CH_3), 24.9 (CH_3), 24.2 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 21 (CH_3), 19.2 (CH_2), 18.7 (CH_3), 18.6 (CH_3), 18.1 (C), 17.6 (CH_3), 15.9 (CH_3), 4.89 2 X (CH_3). Parent ion did not observed on HRMS or FDMS.

6.11. Synthesis of tert-butyldimethyl(((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)silane 75

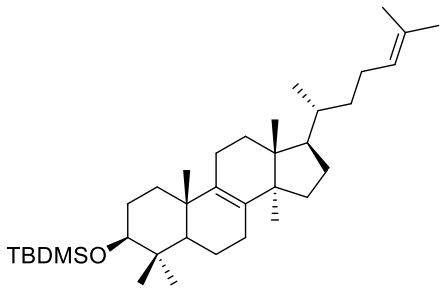


Dry DMF (42 mL) was added to lanosterol **1** (5 g 11.72 mmol) under argon at 0 °C to form an off-white suspension, imidazole (2.98 g 43.72 mmol) and TBDMS-Cl (2.598 g 17.27 mmol) was suspended in DMF (5 mL) then added as turbid white suspension to the lanosterol over 10 minutes at 0 °C. Reaction temperature was raised to room temperature. and stirred for 41 h. The reaction mixture was diluted with water (156

mL) and washed with DCM (3 X 78 mL), the organic layer was washed with water (3 X 78 mL) dried over anhydrous Na_2SO_4, filtrated then the solvent was removed under vacuum. The crude was purified by column chromatography using *n*-hexane R_f = 0.4, affording a white solid (4.78 g, 8.84 mmol, 64 % yield).

M.p. 131-133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 5.13 (t, 1H, *J*= 7.1Hz), 3.23 (dd, 1H J = 11, 4.8 Hz), 2.05 – 1.73 (m, 20 H), 1.71 (s, 3 H), 1.63 (s, 3 H), 1.61- 1.03 (m 20 H), 1.07-1.03 (m, 6H), 1.00 (br s, 3 H), 0.95- 0.93 (m, 5H) , 0.92 (S, 9 H), 0.80 (s, 3 H), 0.71 (s, 3 H), 0.05 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.6 (C), 134.2 (C), 130.9 (C), 125.2 (C), 79.45 (CH), 50.5 (CH), 50.4 (CH), 49.8 (C), 44.5 (C) , 39.4 (C), 36.9 (C), 36.3 (CH), 36.2 (CH_2), 35.6 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 28.0(CH_2), 26.5 (CH_2), 25.9 (3) (CH_3), 24.9 (CH_3), 24.2 (CH_2), 24.1 (CH_3), 22.8 (CH_3), 22.5 (CH_3), 21 (CH_3), 19.2 (CH_2), 18.7 (CH_3), 18.6 (CH_3), 18.4 (C), 18.1 (C), 17.6 (CH_3), 15.9 (CH_3), - 4.89 2 X (CH_3). Parent ion did not observed on HRMS or FDMS.

6.12. Synthesis of tert-butyldimethyl(((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)silane 75[156]

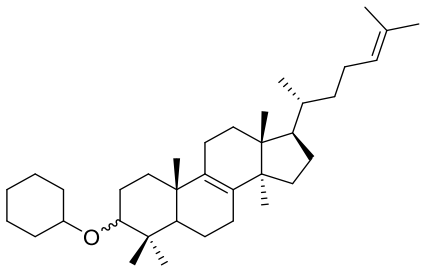


Dry DMF (42 mL) was added to lanosterol **1** (5 g 11.72 mmol) under argon at 0 °C to form an off-white suspension, imidazole (2.976 g 43.72 mmol) and TBDMS-Cl (2.598 g 17.27 mmol) was suspended in DMF (5 mL) then added as turbid white suspension to the lanosterol over 10 minutes at 0 °C. Reaction temperature was raised to 40 °C and stirred for 24 h. The reaction mixture was diluted with water (156 mL) and washed with DCM (3 X 78 mL), the organic layer was washed with water (3 X 78 mL) dried over anhydrous Na_2SO_4, filtered then the solvent was removed under vacuum. The crude was purified by column chromatography using *n*-hexane R_f = 0.4, affording a white solid (5.224 g, 9.66 mmol, 83 % yield).

M.p. 132-133 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 5.13 (t, 1H, J = 7.1Hz), 3.23 (dd, 1H J = 11, 4.8 Hz), 2.05 – 1.73 (m, 20 H), 1.71 (s, 3 H), 1.63 (s, 3 H), 1.61- 1.03 (m 20 H), 1.07-1.03 (m, 6H), 1.00 (br s, 3 H), 0.95- 0.93 (m, 5H) , 0.92 (s, 9 H), 0.80 (s , 3 H), 0.71 (s, 3 H), 0.05

(S, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.6 (C), 134.2 (C), 130.9 (C), 125.2 (C), 79.4 (CH), 50.5 (CH) , 50.4 (CH) , 49.8 (C), 44.5 (C) , 39.4 (C), 36.9 (C), 36.3 (CH), 36.2 (CH_2), 35.6 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.4 (CH_2), 28.0 (CH_2), 26.5 (CH_2), 25.9 3 X (CH_3), 25.7 (CH_3), 24.9 (CH_3), 24.2 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 21 (CH_3), 19.2 (CH_2), 18.7 (CH_3), 18.6 (CH_3), 18.1 (C), 17.6 (CH_3), 15.9 (CH_3), - 4.89 2X (CH_3). Parent ion did not observed on HRMS or FDMS.

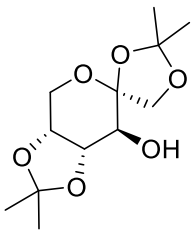
6.13. Synthesis of (10S,13R,14R,17R)-3-(cyclohexyloxy)-4,4,10,13,14-pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene 79[92]



3,4-Dihydro-2H-pyran **78** (0.23 mL, 2.34 mmol, 2 eq.) was added to a solution of **1** (500 mg, 1.17 mmol, 1 eq.) in dichloromethane (10 mL) and PPTS (43 mg, 0.17 mmol, 0.1 eq.) under nitrogen. The reaction mixture was then stirred for 16 at room temperature. The solvent was removed under *vacuo* and the crude residue was purified by column chromatography using toluene R_f = 0.5, to yield compound **79** as a white solid (378 mg, 0.74 mmol, 63 %)

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 (dddd, 1H, *J* = 6.4, 5.2, 2.7, 1.5 Hz), 4.72 (t, 1H, *J* = 4.7 Hz), 3.67 (ddd, 1H, *J* = 11.7, 6.5, 4.5 Hz), 3.49 (ddd, 1H, *J* = 11.6, 6.6, 4.5 Hz), 3.38 (dd, 1H, *J* = 5.8, 3.4 Hz), 2.21 – 2.06 (m, 2H), 2.08 – 2.01 (m, 1H), 2.05 – 1.94 (m, 2H), 1.98 – 1.86 (m, 1H), 1.89 – 1.41 (m, 27H), 1.42 – 1.29 (m, 2H), 1.21 (dtd, *J* = 12.5, 7.5, 5.9 Hz, 1H), 1.04 (s, 2H), 0.97 – 0.86 (m, 15H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.35 (C), 135.23 (C), 131.32 (C), 125.84(CH), 101.98 (CH), 84.93 (CH), 65.13 (CH_2), 51.80 (CH), 51.23 (CH), 47.17 (C), 44.72 (C), 38.59 (C), 37.72 (C), 36.86 (CH_2), 35.89 (CH_2), 35.40 (CH), 33.39 (CH_2), 31.93 (CH_2), 31.40 (CH_2), 27.49 (CH_2), 26.81 (CH_3), 26.48 (CH_2), 25.89 (CH_2), 25.75 (CH_2), 25.54 (CH_2), 24.56 (CH_3), 23.07 (CH_3), 21.65 (CH_2), 21.48 (CH_3), 20.71 (CH_2), 20.11 (CH_2), 19.91 (CH_3), 19.14 (CH_3), 18.59 (CH_3), 18.32 (CH_3). Parent ion did not observed on HRMS or FDMS.

6.14. Synthesis of (3a'R,4S,7a'S)-2,2,2',2'-tetramethyltetrahydrospiro[[1,3]dioxolane-4,6'-[1,3]dioxolo[4,5-c]pyran]-7'-ol 87[95]

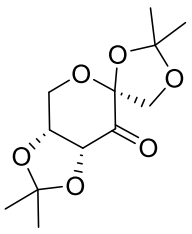


Perchloric acid **85** 70% (0.43 mL) was added to suspension of D-fructose **84** (1.842 g, 10.22 mmol, 1 eq.), in acetone **72** (37 mL) and 2,2-dimethoxypropane **86** (0.74 mL) at 0 °C. Reaction left on stirring

at 0°C under nitrogen for 6 h. Ammonium hydroxide 35 % was added until the mixture reached pH 7-8. The reaction mixture was stirred for 5 min at 0 °C. Solvent removed under *vacuo*, the yellow residue recrystallized from *n*-hexane/DCM (4:1 v/v) to yield **87** as a white needles 1.836 g, 7.05 mmol, 69%)

M.p. 117-119 °C, Lit: 117-118.5 °C[95]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 – 4.10 (4H, m), 4.09 – 3.95 (m, 2H), 3.69 (t, 1H, *J* = 5.8 Hz), 2.08 (d, 1H, *J* = 8.1 Hz), 1.56 (s, 3H), 1.54 (s, 3H), 1.46 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.86 (C), 109.43 (C), 104.52 (C), 77.31 (CH), 73.34 (CH), 72.33 (CH_2), 70.42 (CH), 60.75 (CH_2), 27.95 (CH_3), 26.44 (CH_3), 26.26 (CH_3), 25.35 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 283.1158 required $C_{12}H_{20}NaO_6$ 283.1152.

6.15. Synthesis of (3a'R,4S,7a'R)-2,2,2',2'-tetramethyldihydrospiro[[1,3]dioxolane-4,6'-[1,3]dioxolo[4,5-c]pyran]-7'(4'H)-one 88[95]

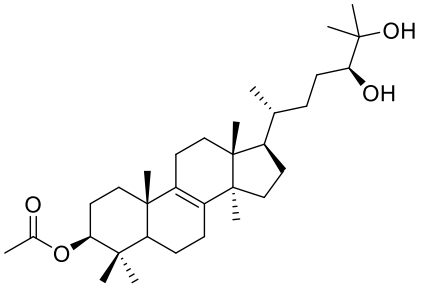


Compound **87** (1.836 g, 7.05 mmol, 1 eq.) was dissolved in dichloromethane (20 mL) under nitrogen, 3 Å molecular sieves (7.76 g, 19.05 mmol, 2.7 eq.) added to the reaction solution. PCC (4.108 g, 19.05 mmol, 2.7 eq.) was added in small portions over 15 min. Reaction mixture stirred for 16 h under nitrogen at room temperature. The reaction mixture filtered over Celite and washed carefully with ether.

The filtrate was concentrated under *vacuo*, the was residue purified by column chromatography using *n*-hexane: diethyl ether (1:1), affording a white residue. The residue was recrystallized from *n*-hexane/ dichloromethane R_f = 0.5, to yield the pure compound as white crystals (1.82 g, 7.04 mmol, 93%)

M.p. 101-103 °C, Lit: 101.5-102.5 °C[95]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (d,1H, J = 5.6 Hz), 4.63 (d, 1H, J = 9.5 Hz), 4.57 (ddd, 1H, J = 5.6, 2.2, 1.0 Hz), 4.41 (dd, 1H, J = 13.5, 2.2 Hz), 4.14 (dt, 1H, J = 13.5, 0.8 Hz), 4.01 (d, 1H, J = 9.5 Hz), 1.57 (d, 3H, J = 0.7 Hz), 1.48 (s, 3H), 1.44 – 1.40 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.93 (C), 113.82 (C), 110.64 (C), 104.12 (C), 77.93 (CH), 75.88 (CH), 70.01 (CH_2), 60.09 (CH_2), 27.16 (CH_3), 26.52 (CH_3), 26.07 (CH_3), 26.02 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 281.1002 required $C_{12}H_{18}NaO_6$ 281.0996.

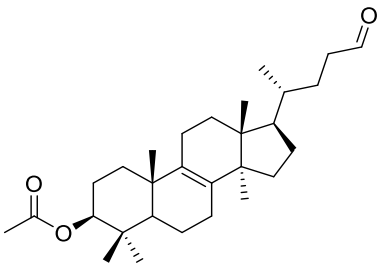
6.16. Synthesis of (3S,10S,13R,14R,17R)-17-((2R,5S)-5,6-dihydroxy-6-methylheptan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 109[105]



Compound **66** (548 mg, 1.16 mmol, 1 eq.), AD-mix-α (1.315 g) and methanesulfonamide (75 mg, 0.78 mmol, 0.77 eq.), was suspended in *tert*-butanol/water (22.5 mL) (5:4). The resulting reaction mixture left on stirring for 64 h at room temperature. Na_2SO_3 (1.25 g, 9.92 mmol) added and stirred for additional 30 min. The solvent was removed under *vacuo*, 75 mL of water added to the crude and extracted with ethyl acetate (3 X 60 mL). The organic phase dried over $MgSO_4$, filtrate and reduce under *vacuo*. The crude was purified by column chromatography using gradient elution of petroleum ether 40/60: ethyl acetate (8:2 → 100 % ethyl acetate) R_f = 0.3, yielding a white solid (214 mg 68 %)

M.p. 184-188 °C, Lit: 185-187 °C[105]; FT-IR (ATR) V_{max}/cm^{-1} 3662, 3604, 1730, 1196, 1044. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.81 (1H, s), 4.52 (dd, 1H, *J* = 11.5, 4.6 Hz), 3.31 (ddd, 1H, *J* = 10.1, 4.3, 2.1 Hz), 3.13 (s, 1H), 2.15 (d, 1H, *J* = 4.4 Hz), 2.11 – 1.88 (m, 7H), 1.83 – 1.54 (m, 7H), 1.54 – 1.35 (m, 1H), 1.44 – 1.30 (m, 2H), 1.33 – 1.24 (m, 1H), 1.26 – 1.03 (m, 10H), 1.00 (m, 5H), 0.96 – 0.87 (m, 11H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.06 (C), 134.46 (C), 134.26 (C), 80.96 (CH), 79.64 (CH), 73.24 (C), 60.41 (CH), 50.51 (CH), 50.44 (C), 49.81 (C), 44.49 (C), 37.81 (C), 36.91 (CH), 36.75 (CH_2), 35.28 (CH_2), 33.55 (CH_2), 30.99 (CH_2), 30.80 (CH_2), 28.72 (CH_2), 28.18 (CH_2), 27.92 (CH_3), 26.56 (CH_3), 26.38 (CH_2), 24.27 (CH_3), 24.18 (CH_3), 23.19 (CH_2), 21.34 (CH_3), 21.01 (CH_3), 19.19 (CH_2), 18.84 (CH_3), 18.13 (CH_3), 16.54 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 525.3902 required $C_{32}H_{54}NaO_4$ 525.3914. FDMS: found 502.4017 $C_{32}H_{54}O_4$ required 502.4019.

6.17. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-oxopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 43

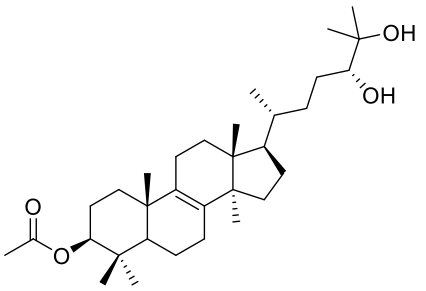


Compound **109** (200 mg, 0.4 mmol, 1 eq.) was added to stirred solution of periodic acid (106 mg, 0.465 mmol, 1.17 eq.) in dichloromethane (0.83 mL) and tetrahydrofuran (3.35 mL) at room temperature, the solution was stirred under nitrogen atmosphere. The reaction completion checked by TLC. Water (5 mL) was added and the organic layer separated and washed with water (2 x 5 mL) to remove the precipitate HIO_3. The organic layer was dried over magnesium sulphate and the solvent was removed *in vacuo* to yield a white residue. The crude product was purified by column chromatography using gradient elution of petroleum ether 40/60: diethyl ether (95:5 → 90:10) R_f = 0.4, to afford a white residue (109 mg, 0.246 mmol, 62 %).

M.p. 154-157 °C, Lit: 153-156 °C[74]. 1H NMR (500 MHz, $CDCl_3$) δ 9.80 (t, 1H, *J* = 1.9 Hz), 4.52 (dd, 1H, *J* = 11.7, 4.5 Hz), 2.49 (dddd, 1H, *J* = 16.9, 9.8, 5.3, 1.7 Hz), 2.45 – 2.33 (m, 1H), 2.07 (s, 1H), 2.12 – 1.91 (m, 3H), 1.89 – 1.56 (m, 8H), 1.58 – 1.11 (m, 8H), 1.02 (s, 3H), 0.99 – 0.86 (m, 13H), 0.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.33

(CH), 171.06 (C), 134.34 (C), 134.28 (C), 80.91 (CH), 50.47 (CH), 50.27 (CH), 49.81 (C), 44.52 (C), 41.16 (C), 37.80 (CH_2), 36.89 (C), 36.04 (CH_2), 35.25 (CH), 30.93 (CH_2), 30.78 (CH_2), 28.22 (CH_2), 28.16 (CH_2), 27.92 (CH_2), 26.37 (CH_3), 24.24 (CH_3), 24.17 (CH_3), 21.38 (CH_2), 20.98 (CH_3), 19.21 (CH_3), 18.41 (CH_2), 18.11 (CH_3), 16.55 (CH_3), 15.78 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 465.3323 required $C_{29}H_{46}NaO_3$ 465.3339.

6.18. Synthesis of (3S,10S,13R,14R,17R)-17-((2R,5R)-5,6-dihydroxy-6-methylheptan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 110[105]

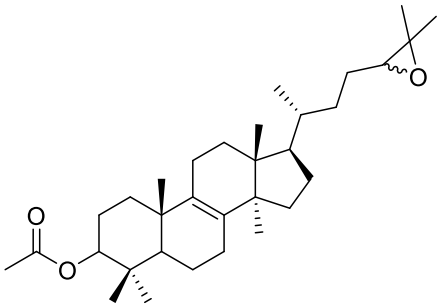


Compound **66** (478 mg, 1.02 mmol, 1 eq.), AD-mix-β (1.00 g), methanesulfonamide (75 mg, 0.78 mmol, 0.77 eq.), was suspended in *tert*-butanol/water (22.5 mL) (5:4). The resulting reaction mixture was stirred for 64 h at room temperature. Na_2SO_3 (1.25 g, 9.92 mmol) added and stirred for additional 30 min. The solvent was removed under *vacuo*, 75 mL of water added to the crude suspension and extracted with ethyl acetate (3 X 60 mL). The organic phase dried over $MgSO_4$, filtrate and reduce under *vacuo*. The crude was purified by column chromatography

using gradient elution of petroleum ether 40/60: ethyl acetate (8:2 → 100 % ethyl acetate) R_f = 0.3, yielding a white solid (188 mg, 0.374 mmol, 67 %)

M.p. 186-189 °C, Lit: 185-187°C[105]; FT-IR (ATR) V_{max}/cm^{-1} 3771, 3667, 2986, 1731, 1044. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.01 (s, 1H), 4.51 (dd, 1H, *J* = 11.7, 4.4 Hz), 3.39 – 3.32 (m, 1H), 3.12 (s, 1H), 2.23 (s, 1H), 2.15 (s, 1H), 2.07 (s, 3H), 2.10 – 1.91 (m, 2H), 1.82 (d, 1H, *J* = 16.5 Hz), 1.79 – 1.47 (m, 8H), 1.46 – 1.33 (m, 3H), 1.37 – 1.29 (m, 1H), 1.28 (ddt, 1H, *J* = 11.3, 4.7, 1.9 Hz), 1.24 – 1.12 (m, 8H), 1.02 (s, 3H), 0.99 – 0.86 (m, 11H), 0.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.14 (C), 134.43 (C), 134.22 (C), 80.98 (CH), 78.76 (CH), 73.21 (C), 50.56 (CH), 50.47 (CH), 49.81 (C), 44.49 (C), 37.80 (C), 36.88 (C), 36.32 (CH), 35.25 (CH_2), 33.13 (CH_2), 30.97 (CH_2), 30.80 (CH_2), 28.41 (CH_2), 28.31 (CH_2), 27.92 (CH_3), 27.92 (CH_3), 26.57 (CH_2), 26.37 (CH_2), 24.27 (CH_3), 24.16 (CH_2), 23.22 (CH_3), 21.38 (CH_3), 20.99 (CH_3), 19.20 (CH_2), 18.56 (CH_3), 18.11 (CH_3), 16.55 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 525.3901 required $C_{32}H_{54}NaO_4$ 525.3914. FDMS: found 502.4017 $C_{32}H_{54}NaO_4$ required 502.4019.

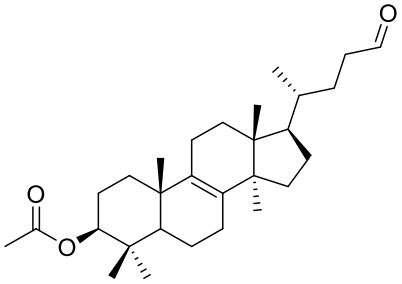
6.19. Synthesis of (10S,13R,14R,17R)-17-((2R)-4-(3,3-dimethyloxiran-2-yl)butan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 69



Compound **110** (537 mg, 1.14 mmol) was dissolved in dichloromethane (31 mL) at (0 °C), pyridine (1.85 mL) and methanesulfonyl chloride (0.62 mL) was added dropwise over 10 min. Reaction left on stirring for (5 h) at room temperature. Reaction quenched by adding sodium bicarbonate sat. solution (30 mL) to the reaction mixture, the crude extracted with chloroform (3 X 45 mL). The organic phase dried over $MgSO_4$, filtrate and the solvent was removed under *vacuo*. The crude suspended in methanol (30 mL), potassium carbonate solution (900 mg in 2.1 mL of H_2O) added to the crude suspension, the crude suspension left on stirring for (5 h) at room temperature. Water (30 mL) was added to quench the reaction, the mixture extracted with chloroform (3 X 60 mL), the organic layer dried over $MgSO_4$, filtrate and reduced under *vacuo*. The crude residue was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.7, affording **69** as off white solid (356 mg, 0.734 mmol, 64 %).

M.p. 176-181 °C, Lit: 184-187 °C[74]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (dd, *J* = 11.5, 4.5 Hz, 1H), 2.71 (t, *J* = 6.1 Hz, 1H), 2.14 – 1.86 (m, 9H), 1.82 – 1.67 (m, 3H), 1.71 – 1.48 (m, 4H), 1.52 – 1.28 (m, 3H), 1.33 (s, 4H), 1.29 (s, 4H), 1.26 – 1.08 (m, 3H), 1.02 (s, 3H), 1.00 – 0.83 (m, 14H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.00 (C), 134.42 (C), 134.28 (C), 80.93 (CH), 64.94 (CH), 58.12 (C), 50.51 (CH), 50.39 (CH), 49.82 (C), 44.50 (C), 37.81 (C), 36.91 (C), 36.36 (CH), 35.28 (CH_2), 32.82 (CH_2), 30.98 (CH_2), 30.81 (CH_2), 28.20 (CH_2), 27.92 (CH_2), 26.39 (CH_2), 25.94 (CH_2), 24.96 (CH_3), 24.24 (CH_3), 24.18 (CH_3), 21.34 (CH_3), 21.00(CH_2), 19.19 (CH_3), 18.66 (CH_3), 18.58 (CH_3), 18.12 (CH_2), 16.54 (CH_3), 15.78 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 507.3804 $C_{52}H_{32}O_3Na$ required 507.3809.

6.20. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-oxopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 43

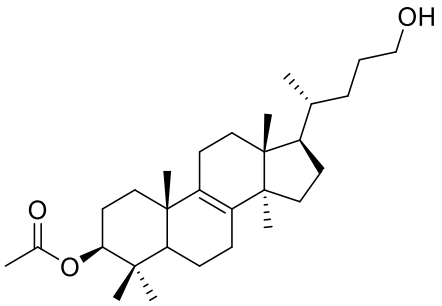


Compound **110** (1.04 g, 2.22 mmol) was added to stirred solution of periodic acid (675 mg, 2.96 mmol) in diethyl ether (178 mL) at room temperature, the solution was stirred under blanket of nitrogen. The reaction completion checked by TLC. Water (188 mL) was added and

the organic layer separated and washed with water (2 x 100 mL) to remove the precipitate HIO_3. The organic layer was dried over magnesium sulphate and the solvent was removed *in vacuo* to yield a white residue. The crude product was purified by flash chromatography using a solvent gradient of petroleum ether 40/60: diethyl ether (90:10 → 95:5) R_f = 0.9, to afford a white residue (632 mg, 1.43 mmol, 64 %).

M.p. 154-158 °C, Lit: 153-156 °C[74], Lit: 153-156 °C[74]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.79 (t, 1H, *J* = 1.9 Hz), 4.52 (dd, 1H, *J* = 11.7, 4.5 Hz), 2.49 (dddd, 1H, *J* = 16.9, 9.8, 5.4, 1.7 Hz), 2.44 – 2.33 (m, 1H), 2.07 (s, 4H), 2.12 – 1.91 (m, 3H), 1.89 – 1.75 (m, 1H), 1.79 – 1.72 (m, 1H), 1.75 – 1.67 (m, 2H), 1.71 – 1.56 (m, 2H), 1.53 (ddd, 1H, *J* = 12.8, 8.7, 4.9 Hz), 1.52 – 1.42 (m, 1H), 1.46 – 1.25 (m, 4H), 1.28 – 1.11 (m, 2H), 1.02 (s, 3H), 0.95 – 0.87 (m, 13H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.30 (CH), 171.05 (C), 134.34 (C), 134.28 (C), 80.90 (CH), 50.46 (CH), 49.81 (CH), 44.52 (C), 41.16 (C), 37.80 (C), 36.89 (CH_2), 36.04 (C), 35.25 (CH_2), 30.93 (CH), 30.77 (CH_2), 28.22 (CH_2), 28.16 (CH_2), 28.09 (CH_2), 27.92 (CH_2), 26.36 (CH_2), 24.24 (CH_3), 24.16 (CH_3), 21.37 (CH_3), 20.97 (CH_2), 19.20 (CH_3), 18.41 (CH_3), 18.10 (CH_2), 16.55 (CH_3), 15.78 (CH_3). HRMS m/z (ESI+) found $[M+ C_3H_5N_2]^+$ 511.3923 required $C_{32}H_{51}N_2O_3$ 511.3894. FDMS: found 442.3442 $C_{29}H_{46}O_3$ required 442.3434.

6.21. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-hydroxypentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 91[106]

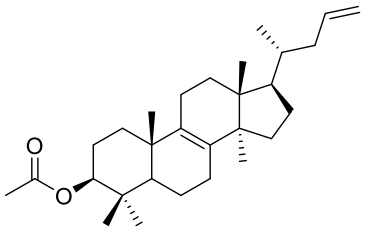


To a stirred suspension of aldehyde **43** (661 mg, 1.49 mmol, 1 eq.) in methanol (75 mL), sodium borohydride (1.251 g, 33.06 mmol, 22.18 eq.) was added. The suspension was stirred for 16 h at room temperature. The reaction worked up by removing solvent under *vacuo*. The residue filtered and washed with water to remove the unreacted sodium borohydride. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (8:2) R_f = 0.2, as an eluent, yielding **91** as a white solid (421 mg, 0.946 mmol, 63 %)

M.p. 178-184 °C; FT-IR (ATR) V_{max}/cm^{-1} 3662, 3058, 3008, 1730, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (dd, 1H, *J* = 11.5, 4.6 Hz), 3.71 – 3.56 (m, 2H), 2.06 (br s, 7H), 2.04 – 1.87 (m, 1H), 1.82 – 1.39 (m, 10H), 1.44 – 1.06 (m, 5H), 1.02 (s, 4H), 1.03 – 0.86 (m, 14H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.03 (C), 134.44 (C), 134.26 (C), 80.94 (CH), 63.60 (CH_2), 50.50 (CH), 50.37 (CH), 49.81 (CH), 44.48 (CH), 37.81 (CH), 36.90 (CH), 36.24 (CH), 35.27 (CH_2), 32.11 (CH_2), 30.98 (CH_2), 30.80 (CH_2), 29.62 (CH_2), 28.20 (CH_2), 27.92 (CH_2), 26.38

(CH_2), 24.25 (CH_3), 24.18 (CH_3), 21.34 (CH_3), 21.00 (CH_2), 19.19 (CH_3), 18.69 (CH_3), 18.13 (CH_2), 16.54 (CH_3), 15.77 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 467.3488 required $C_{29}H_{48}NaO_3$ 467.3496. FDMS: found 444.3598 $C_{29}H_{48}O_3$ required 444.3617.

6.22. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-pent-4-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 92[110]

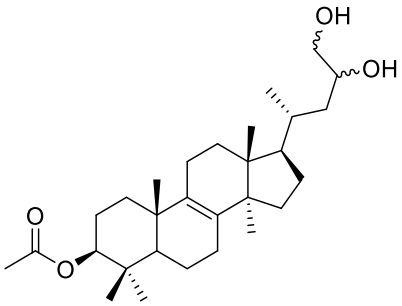


Alcohol **91** (454 mg, 1.02 mmol, 1 eq.) was dissolved in tetrahydrofuran (4.5 mL), 2-nitrophenylselenocynate (454 mg, 2.0 mmol, 1.95 eq.) and *tri*-n-butylphosphine (0.47 mL, 1.87 mmol, 1.83 eq.) were added to the stirred solution. Reaction mixture left on stirring for 90 min. The reaction suspension changed colour from dark brown to orange then reaction mixture was evaporated *in vacuo*. The orange residue filtered over sintered funnel and washed by DCM (3 X 10 mL). The solvent was evaporated *in vacuo*. The seleno crude compound was dissolved in tetrahydrofuran (13.44 mL), and cooled down to 0^{o}C. Hydrogen peroxide (30. wt % solution in water) (3.6 mL) added drop wise to the seleno crude compound at 0^{o}C. The reaction was allowed to warm to room temperature and stirred for 14 h. TLC showed some of the starting

material remained, hydrogen peroxide (1 mL) added and left on stirring for 4 h. Diethyl ether/ water (1:1) (100 mL) added to quench the reaction. The aqueous layer was extracted with diethyl ether (4X 50 mL). The organic phase was washed successively with water (3 X 20 mL), and brine (3 X 20 mL), dried over magnesium sulphate, filtrate, the solvent was removed under *vacuo*. The crude residue was then purified by column chromatography using petroleum ether 40/60: ethyl acetate (8:2) R_f = 0.6, as an eluent to yield **92** as a white solid (350 mg, 0.82 mmol, 80 %).

M.p. 134-138 °C; FT-IR (ATR) V_{max}/cm^{-1} 3072, 3027, 1731, 1602, 1467, 1017. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (dddd, 1H, J = 15.8, 11.1, 8.5, 6.1 Hz), 5.06 – 4.97 (m, 2H), 4.52 (dd, 1H, J = 11.7, 4.5 Hz), 2.27 – 2.18 (m, 1H), 2.08 (s, 4H), 2.12 – 1.91 (m, 3H), 1.86 – 1.69 (m, 4H), 1.72 – 1.51 (m, 4H), 1.51 (q, 2H, J = 3.5, 2.9 Hz), 1.44 – 1.26 (m, 2H), 1.25 – 1.13 (m, 2H), 1.02 (s, 3H), 1.00 – 0.87 (m, 14H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.01 (C), 137.56 (CH), 134.45 (C), 134.28 (C), 115.62 (CH_2), 80.93 (CH), 50.51 (CH), 50.07 (CH), 49.83 (C), 44.49 (C), 40.87 (CH_2), 37.82 (C), 36.91 (C), 36.44 (CH_2), 35.28 (CH), 30.87 (CH_2), 30.84 (CH_2), 28.13 (CH_2), 27.92 (CH_2), 26.39 (CH_2), 24.26 (CH_3), 24.19 (CH_3), 21.34 (CH_3), 20.99 (CH_2), 19.20 (CH_3), 18.61 (CH_3), 18.13 (CH_2), 16.54 (CH_3), 15.81 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 449.3391 required $C_{29}H_{46}NaO_2$ 449.3390.

6.23. Synthesis of (3S,10S,13R,14R,17R)-17-((2R)-4,5-dihydroxypentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 96[105]

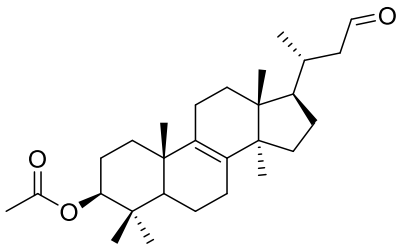


Compound **92** (174 mg, 0.407 mmol, 1 eq.), AD-mix-β (400 mg), methanesulfonamide (30 mg, 0.315 mmol, 0.77 eq.), was suspended in *tert*-butanol/water (9 mL) (5:4). The resulting reaction mixture left on stirring for 64 h at room temperature. Na_2SO_3 (0.5 g, 3.97 mmol) added and stirred for an additional 30 min. The solvent was removed under *vacuo*, water (30 mL) was added to the crude and extracted with ethyl acetate (3 X 24 mL). The organic phase dried over $MgSO_4$, filtrate and reduce under *vacuo*. The crude product was purified by column chromatography using gradient elution of petroleum ether 40/60: ethyl acetate (80:20 → 100 % ethyl acetate) R_f = 0.8, yielding **96** as a white solid (59 mg, 0.128 mmol, 33 %)

M.p. 128-130 °C; FT-IR (ATR) V_{max}/cm^{-1} 3662, 3605, 2985, 1720, 1044, 1029. ^1H NMR (400 MHz, $CDCl_3$) δ 4.92 (br s, 1H), 4.51 (dd, 1H, *J* = 11.6, 4.6 Hz), 3.85 (ddd, 1H, *J* = 11.1, 6.6, 3.6 Hz), 3.76 – 3.58 (m, 1H), 3.43 (ddd, 1H, *J* = 11.9, 7.7, 4.6 Hz), 3.13 (s, 1H), 2.22 – 2.17 (m, 2H), 2.09 – 1.87 (m, 8H), 1.83 – 1.41 (m, 6H), 1.44 – 1.22 (m, 5H), 1.25 – 1.10 (m, 2H), 1.05 – 0.92 (m, 6H), 0.97 – 0.82 (m, 10H),

0.73 (d, 3H, *J* = 14.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.10 (C), 134.38 (C), 134.30 (C), 80.97 (CH), 71.02 (CH), 69.61 (CH), 67.82 (CH_2), 66.47 (CH_2), 51.07 (CH), 50.50 (CH), 44.61 (C), 40.23 (C), 39.57 (CH_2), 37.81 (C), 36.90 (C), 35.27 (CH_2), 34.47 (CH_2), 32.86 (CH_2), 31.93 (CH), 29.71 (CH_2), 28.54 (CH_2), 27.92 (CH_2), 24.21 (CH_3), 24.17 (CH_3), 21.35 (CH_3), 20.99 (CH_2), 19.53 (CH_3), 19.20 (CH_3), 18.62 (CH_2), 18.11 (CH_3), 16.54 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 483.3462 required $C_{29}H_{48}NaO_4$ 483.3445

6.24. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-4-oxobutan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 97

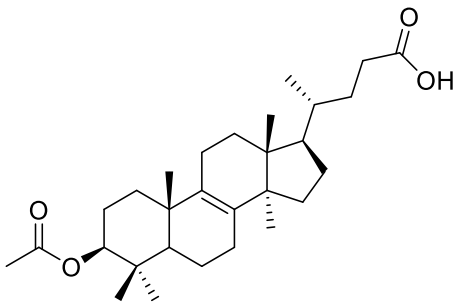


Compound **96** (280 mg, 0.607 mmol) was added to stirred solution of periodic acid (200 mg, 0.875 mmol) in (53 mL) diethyl ether at room temperature, the solution was stirred under blanket of nitrogen. The reaction completion checked by TLC. Water (25 mL) was added and the organic layer separated and washed with water (2 x 18 mL) to remove the precipitate HIO_3. The organic layer was dried over magnesium sulphate and the solvent was removed *in vacuo* to yield a white residue. The crude product was purified by flash chromatography using gradient

elution of petroleum ether 40/60: diethyl ether (90:10 → 95:5) R_f = 0.6, to afford a white residue (115 mg, 0.268 mmol, 44 %).

M.p. 163-166 °C; FT-IR (ATR) V_{max}/cm^{-1} 3662, 3029, 3009, 1722, 1048. ^1H NMR (400 MHz, $CDCl_3$) δ 9.80 (t, 1H, *J* = 1.9 Hz), 4.52 (dd, 1H, *J* = 11.5, 4.5 Hz), 2.55 – 2.31 (m, 1H), 2.11 – 1.90 (m, 6H), 1.89 – 1.68 (m, 3H), 1.66 – 1.58 (m, 5H), 1.56 – 1.42 (m, 1H), 1.41 – 1.31 (m, 2H), 1.28 (s, 6H), 1.25 – 1.18 (m, 1H), 1.03 (s, 2H), 0.94 – 0.86 (m, 13H), 0.74 – 0.69 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.23 (CH), 171.01 (C), 134.36 (C) 134.31 (C), 80.91 (CH), 53.43 (CH), 50.49 (CH), 50.30 (C), 49.82 (C), 44.54 (C), 41.15 (CH_2), 37.81 (C), 36.91 (CH_2), 36.04 (CH), 35.27 (CH_2), 30.95 (CH_2), 29.71 (CH_2), 28.24 (CH_2), 27.92 (CH_2), 26.38 (CH_3), 24.24 (CH_3), 24.17 (CH_3), 21.35 (CH_3), 20.98 (CH_3), 19.20 (CH_3), 18.41 (CH_2), 18.11 (CH_3), 16.54 (CH_3), 15.79 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 451.3173 required $C_{28}H_{44}NaO_3$ 451.3183.

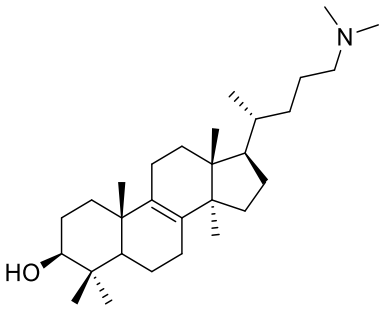
6.25. Synthesis of (4R)-4-((3S,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid 103[12]



The title compound was synthesised using a procedure adapted from the literature[12]. Aldehyde **43** (192 mg, 0.433 mmol, 1.0 eq.) was suspended in DMF (4.7 mL). Oxone (266 mg, 0.865 mmol, 2 eq.) was added to the **43** suspension in DMF. The reaction mixture was stirred at room temperature for 3 h. TLC monitoring using petroleum ether 40/60: ethyl acetate (3:1) showed reaction completion. The reaction was diluted with hydrochloric acid (1M aq. sol.) to dissolve the salt and ethyl acetate (10 mL X 3) used to extract the product. The organic phases were combined and washed with HCl (1 M aq. sol., 3 X 10 mL); (brine 2 X 10 ml). The organic phase was dried over Na_2SO_4, the solvent was removed under *vacuo*. The crude was then purified using column chromatography petroleum ether 40/60: ethyl acetate (3:1) R_f = 0.6, to yield the final product as a white powder (147 mg, 0.32 mmol, 74 %)

M.p. 199-206 °C, Lit: 199-205 °C[157]; FT-IR (ATR) V_{max}/cm^{-1} 3653, 3591, 2923, 1745, 1692, 1008. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (dd, 1H, J = 11.6, 4.5 Hz), 2.43 (ddd, 1H, J = 15.5, 10.1, 5.2 Hz), 2.29 (ddd, 1H, J = 15.9, 9.6, 6.5 Hz), 2.11 – 1.93 (m, 7H), 1.99 – 1.81 (m, 1H), 1.78 – 1.14 (m, 13H), 1.02 (s, 4H), 0.97 – 0.82 (m, 14H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.26 (C), 171.10 (C), 134.39 (C), 134.28 (C), 80.96 (CH), 50.49, 50.31 (CH), 49.82 (C), 44.53 (C), 37.81 (C), 36.90 (C), 36.01 (CH), 35.26 (CH_2), 31.21 (CH_2), 31.02 (CH_2), 30.96 (CH_2), 30.78 (CH_2), 29.71 (CH_2), 28.07 (CH_2), 27.92 (CH_2), 26.38 (CH_3), 24.23 (CH_3), 24.17 (CH_3), 21.34 (CH_2), 20.99 (CH_3), 19.20 (CH_3), 18.27 (CH_3), 18.12 (CH_2), 16.54 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 481.3266 required $C_{29}H_{46}NaO_4$ 481.3288.

6.26. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(dimethylamino)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 111

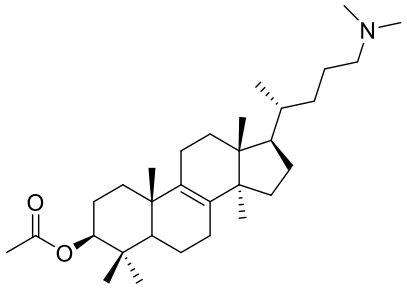


To a solution of aldehyde **42** (150 mg, 0.374 mmol) in 1,2-dichloroethane (15 mL), sodium triacetoxyborohydride (150 mg, 0.707

mmol) and dimethylamine (0.3 mL) was added under argon. Reaction was on stirred for 5.5 h at room temperature. Sodium bicarbonate (20 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (2 X 15 mL), wash with brine (2 X 15 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to yield the final product as white solid (59 mg, 0.137 mmol, 37 % yield).

M.p. 176-179 °C; FT-IR (ATR) V_{max}/cm^{-1} 3423, 3310, 2925, 1732, 1075, 977. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.25 (dd, 1H, *J* = 11.6, 4.6 Hz), 2.26 (s, 9H), 2.12 – 1.12 (m, 19H), 1.10 – 0.99 (m, 8H), 0.97 – 0.85 (m, 7H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 134.43 (C), 134.39 (C), 78.97 (CH), 60.40 (CH_2), 50.42 (CH), 50.36 (CH), 49.82 (C), 45.40 2 X (CH_3), 44.48 (C), 38.91 (C), 37.04 (C), 36.44 (CH), 35.61 (CH_2), 33.95 (CH_2), 30.99 (CH_2), 30.85 (CH_2), 28.22 (CH_2), 27.98 (CH_3), 27.88 (CH_2), 26.51 (CH_2), 24.40 (CH_2), 24.26 (CH_3), 21.01 (CH_2), 19.16 (CH_3), 18.74 (CH_3), 18.27 (CH_2), 15.76 (CH_3), 15.44 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 430.4057 required $C_{29}H_{52}NO$ 430.4043.

6.27. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(dimethylamino)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 112

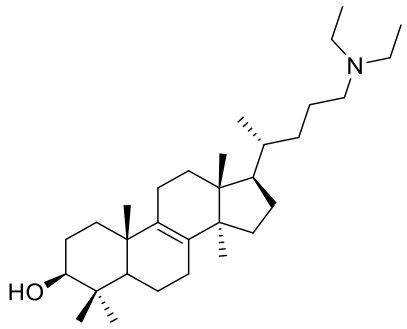


To a solution of aldehyde **43** (200 mg, 0.452 mmol) in 1,2-dicloroethane (20 mL), sodium triacetoxyborohydride (200 mg 0.943 mmol) and diethylamine (0.4 mL) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (15 mL), then extracted with ethyl acetate (2 X 15 mL), organic phase washed with brine (2 X 15 mL), dried by $MgSO_4$, filtrate and the solvent was removed *in vacuo.* The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to yield the final product as white solid in (176 mg, 0.354 mmol, 83 %).

M.p. 168-170 °C; FT-IR (ATR) V_{max}/cm^{-1} 3214, 2951, 1078, 1010. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.51 (dd, 1H, *J* = 11.7, 4.5 Hz), 2.28 (s, 9H), 2.11 – 1.87 (m, 7H), 1.79 – 1.11 (m, 18H), 1.02 (s, 3H), 0.92 (d, 3H, *J* = 6.4 Hz), 0.89 (s, 6H), 0.88 (s, 3H), 0.70 (s, 3H). ^{13}C NMR(125 MHz, $CDCl_3$): δ (ppm) 171.01 (C), 134.46 (C), 134.26 (C), 80.93 (CH), 60.32 (CH_2), 50.50 (CH), 50.36 (CH), 49.81 (C), 45.29 2 X (CH_3), 44.47 (C),

37.81 (C), 36.90 (C), 36.43 (CH), 35.27 (CH_2), 33.89 (CH_2), 30.96 (CH_2), 30.81 (CH_2), 28.22 (CH_2), 27.92 (CH_3), 26.39 (CH_2), 24.28 (CH_2), 24.24 (CH_3), 24.18 (CH_2), 21.35 (CH_3), 21.00 (CH_2), 19.19 (CH_3), 18.72 (CH_3), 18.13 (CH_2), 16.54 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 472.4174 required $C_{31}H_{54}NO_2$ 472.4149.

6.28. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(diethylamino)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 113[74]

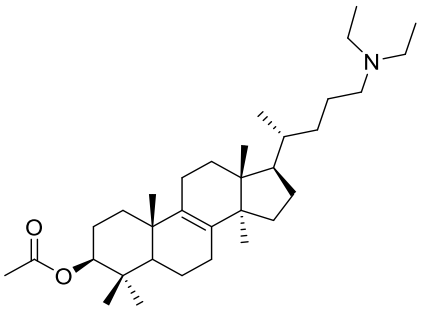


To a solution of aldehyde **42** (25 mg, 0.062 mmol) in 1,2-dichloroethane (2.5 mL), sodium triacetoxyborohydride (19 mg, 0.088 mmol) and diethylamine (6.5µL 0.063 mmol) was added under argon. Reaction was on stirred for 6 h at room temperature. Sodium bicarbonate (2.5 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (2 X 10 mL), wash with brine (2 X 10 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate

(100 %) as an eluent to afford a white solid (9.7 mg, 0.021 mmol, 34 % yield).

M.p. 135-139 °C, Lit: 135- 137 °C[74]; FT-IR (ATR) V_{max}/cm^{-1}; 3342; 2918, 1739, 1664. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 3.25 (dd, 4H, *J*= 7.0, 4.53 Hz), 2.66 (d, 4H *J*= 6.7 HZ), 2.48 (br. s, 2H), 2.22 -2.11 (m, 4H), 2.05-1.9 (m, 20 H), 1.78-1.14(m, 20H), 1.1 (t, 6H, *J*= 7.02Hz), 1.02(s, 3H), 1.0 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.4 (C), 134.3 (C), 78.9 (CH), 60.3 (CH_2), 50.4 (CH), 50.3 (C), 49.8 (CH), 46.6 (CH_2), 44.4 (C), 38.8 (C), 37.0 (C), 36.3 (CH), 35.6 (CH_2), 34.0 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 29.7 (CH_2), 28.2 (CH_2), 27.9 (CH_2), 27.8 (CH_2), 26.5 (CH_2), 24.2 (CH_3), 22.9 (CH_3), 21.06 (CH_2), 21.0 (CH_2), 19.1 (CH_3), 18.7 (CH_3), 18.2 (CH_3), 15.7 (CH3), 14.2 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 430.4022 $C_{31}H_{56}NO$ required 430.4043.

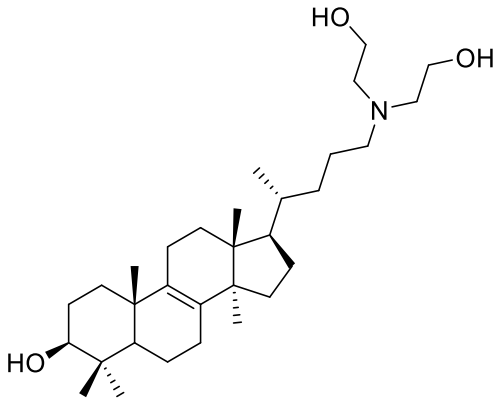
6.29. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(diethylamino)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 114[74]



To a solution of aldehyde **43** (50 mg, 0.113 mmol) in 1,2-dicloroethane (5 mL), sodium triacetoxyborohydride (67 mg 0.365 mmol) and diethylamine (11.5 µL 0.115 mmol) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (5 mL), then extracted with ethyl acetate (2 X 10 mL), organic phase washed with brine (2 X 10 mL), dried by $MgSO_4$, filtrate and the solvent removed *in vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (25.5 mg, 0.051 mmol, 45 %).

M.p. 89-92 °C, Lit: 87-89 °C[74]; FT-IR (ATR) V_{max}/cm^{-1} 3514, 2949, 1733, 1714. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 4.51 (dd,J=7.0, 4.68 HZ), 2.65-2.58 (m, 4H), 2.04-1.88 (m, 20 H), 1.76-1.14(m, 20H), 1.2 (t, 6H, *J*= 7.0 Hz), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 171.0 (C), 134.4 (C), 134.2 (C), 80.9 (CH), 53.2 (CH_2), 50.5 (CH), 50.3 (C), 49.8 (CH), 46.7 (CH_2), 44.4 (C), 37.8 (C), 36.8 (C), 36.3 (CH), 35.2 (CH_2), 34.0 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 28.1 (CH_2), 27.9 (CH_2), 26.3 (CH_2), 24.2 (CH_3), 24.1 (CH_3), 23.1(CH_3), 22.6 (CH_3), 21.3 (CH_2), 21.0 (CH_3), 19.1 (CH_3), 18.7 (CH_3), 18.1 (CH_3), 16.5 (CH_3), 15.7 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 472.4156, $C_{33}H_{58}NO_2$ required 472.4149.

6.30. Synthesis of (4R)-4-((3S,10S,13R,14R,17R)-3-hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanal 115



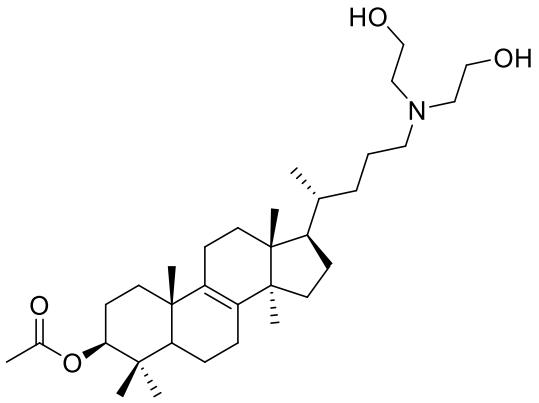
To a solution of aldehyde **42** (55 mg, 0.137 mmol) in 1,2-dichloroethane (6 mL), sodium triacetoxyborohydride (84 mg, 0.396 mmol) and diethanolamine (10 µL) was added under argon. Reaction was on stirred for 6 h at room temperature. Sodium bicarbonate (12 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (2 X 20 mL), wash with brine (2 X 20 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid (952 mg, 0.106 mmol, 78 % yield).

M.p. 186-189 °C; FT-IR (ATR) V_{max}/cm^{-1} 3355, 2951, 1080, 983.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.25 (dd, 1H, *J* = 11.6, 4.5 Hz), 2.88 – 2.27 (m, 12H), 2.23 – 1.04 (m, 21H), 1.02 (s, 3H), 1.00 (s, 3H), 0.92 (d, 3H, *J* = 6.3 Hz), 0.89 (s, 3H), 0.83 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (125

MHz, $CDCl_3$): δ (ppm) 134.43 (C), 134.38 (C), 78.98 (CH), 59.27 2 X (CH_2), 55.08 (CH_2), 53.20 2 X (CH_2), 50.27 (CH), 49.82 (C), 46.02 (CH), 44.48 (C), 38.91 (C), 37.03 (C), 36.36 (CH), 35.60 (CH_2), 34.00 (CH_2), 30.98 (CH_2), 30.85 (CH_2), 28.20 (CH_2), 27.98 (CH_3), 27.88 (CH_2), 26.51 (CH_2), 24.27 (CH_3), 23.56 (CH_2), 21.01 (CH_2), 19.16 (CH_3), 18.76 (CH_3), 18.27 (CH_2), 15.75 (CH_3), 15.44 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 490.4282 required $C_{31}H_{56}NO_3$ 490.4255.

6.31. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(bis(2-hydroxyethyl)amino)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 116

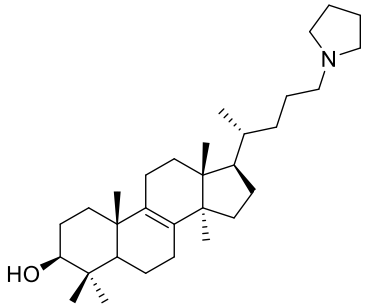


To a solution of aldehyde **43** (60 mg, 0.135 mmol) in 1,2-dicloroethane (6 mL), sodium triacetoxyborohydride (84 mg, 0.396 mmol) and diethanolamine (10 µL) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (12 mL), then extracted with ethyl

acetate (2 X 20 mL), organic phase washed with brine (2 X 20 mL), dried by $MgSO_4$, filtered and the solvent was removed *in vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (55 mg, 0.103 mmol, 76 %).

M.p. 178-183 °C; FT-IR (ATR) V_{max}/cm^{-1} 3513, 3351, 2921, 1713, 1037, 983. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (dd, 1H, *J* = 11.5, 4.5 Hz), 3.78 (t, 3H, *J* = 5.2 Hz), 3.72 – 3.62 (m, 3H), 3.01 – 2.60 (m, 4H), 2.15 – 1.85 (m, 9H), 1.81 – 1.07 (m, 15H), 1.02 (s, 3H), 0.98 – 0.80 (m, 14H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.04 (C), 134.40 (C), 134.31 (C), 80.93 (CH), 70.55 (CH_2), 58.61 (CH_2), 56.73 (CH_2), 55.49 (CH_2), 50.50 (CH), 50.44 (C), 49.82 (CH), 44.53 (C), 37.82 (C), 36.91 (C), 36.37 (CH), 35.27 (CH_2), 33.57 (CH_2), 30.97 (CH_2), 30.80 (CH_2), 29.72 (CH_2), 28.26 (CH_2), 27.92 (CH_2), 26.39 (CH_2), 24.26 (CH_2), 24.18 (CH_3), 21.35 (CH_2), 20.99 (CH_2), 19.20 (CH_3), 18.69 (CH_3), 18.12 (CH_3), 16.54 (CH_3), 15.79 (CH_3), 14.14 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 532.4393 required $C_{33}H_{58}NO_4$ 532.4360.

6.32. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(pyrrolidin-1-yl)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 117



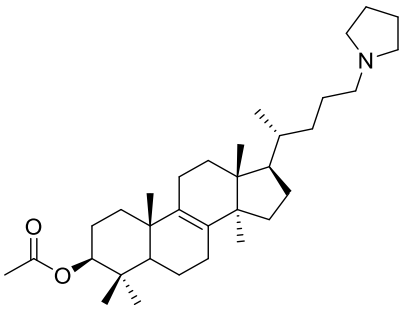
To a solution of aldehyde **42** (150 mg, 0.374 mmol) in 1,2-dichloroethane (15 mL), sodium triacetoxyborohydride (150 mg, 0.707 mmol) and pyrrolidine (100 µL) was added under argon. Reaction was on stirred for 6 h at room temperature. Sodium bicarbonate (15 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (2 X 20 mL), wash with brine (2 X 15 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid (108 mg, 0.236 mmol, 64 % yield).

M.p. 195-199 °C; FT-IR (ATR) V_{max}/cm^{-1} 3332, 2944, 1076, 987.

^1H NMR (500 MHz, $CDCl_3$) δ 3.25 (dd, 1H, *J* = 11.6, 4.5 Hz), 2.59 – 2.49 (m, 5H), 2.42 (t, 2H, *J* = 7.8 Hz), 2.11 – 1.89 (m, 6H), 1.81 (dq, 4H, *J* = 6.8, 3.0 Hz), 1.77 – 1.03 (m, 16H), 1.02 (s, 3H), 1.00 (s, 3H), 0.93

(d, 3H, *J* = 6.4 Hz), 0.89 (s, 3H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 134.42 (C), 134.39 (C), 78.97 (CH), 57.20 (CH_2), 54.23 2 X (CH_2), 50.42 (CH), 50.30 (CH), 49.82 (C), 44.47 (C), 38.91 (C), 37.03 (C), 36.37 (CH), 35.60 (CH_2), 34.16 (CH_2), 30.98 (CH_2), 30.85 (CH_2), 28.21 (CH_2), 27.98 (CH_3), 27.88 (CH_2), 26.51 (CH_2), 25.69 (CH_2), 24.26 (CH_3), 23.41 2 X (CH_2), 21.01 (CH_2), 19.16 (CH_3), 18.74 (CH_3), 18.27 (CH_2), 15.76 (CH_3), 15.44 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 456.4205 required $C_{31}H_{54}NO$ 456.4200.

6.33. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(pyrrolidin-1-yl)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 118

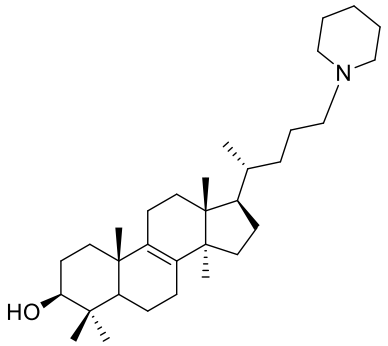


To a solution of aldehyde **43** (50 mg, 0.113 mmol) in 1,2-dicloroethane (5 mL), sodium triacetoxyborohydride (80 mg, 0.377 mmol) and pyrrolidine (10 µL) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (5 mL), then extracted with ethyl acetate (2 X 10 mL), organic phase washed with brine (2 X 10 mL), dried by $MgSO_4$, filtrate and the solvent was removed *in vacuo*. The

crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (38 mg, 0.076 mmol, 68 %).

M.p. compound decomposed; FT-IR (ATR) V_{max}/cm^{-1} 3380, 2949, 1732, 1028, 981. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.25 (dd, 1H, *J* = 11.6, 4.5 Hz), 2.57 (s, 3H), 2.30 – 1.99 (m, 6H), 1.98 – 1.03 (m, 22H), 1.01 (d, 7H, *J* = 10.7 Hz), 0.93 (d, 3H, *J* = 6.2 Hz), 0.91 – 0.87 (m, 5H), 0.83 (s, 4H), 0.70 (s, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.04 (C), 134.46 (C), 134.32 (C), 78.98 (CH), 59.06 (CH_2), 53.90 2 X (CH_2), 50.40 (CH), 50.33 (CH), 49.82 (C), 44.52 (C), 38.90 (C), 37.03 (C), 36.27 (CH), 35.59 (CH_2), 33.73 (CH_2), 30.97 (CH_2), 30.83 (CH_2), 29.72 (CH_2), 28.21 2 X (CH_2), 27.98 (CH_3), 27.86 (CH_2), 26.50 (CH_2), 24.27 (CH_3), 23.41 (CH_2), 21.35 (CH_3), 21.00 (CH_2), 19.16 (CH_3), 18.67 (CH_3), 18.26 (CH_2), 15.77 (CH_3), 15.43 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 498.4327 required $C_{33}H_{56}NO_2$ 498.4306.

6.34. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(piperidin-1-yl)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 119[74]

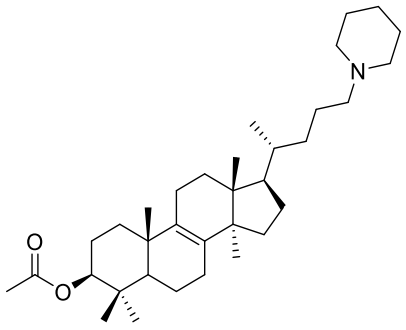


To a solution of aldehyde **42** (500 mg, 0.125 mmol) in 1,2-dichloroethane (5 mL), sodium triacetoxyborohydride (34 mg 0.158 mmol) and piperidine (11.5 µL, 0.115 mmol) were added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (5 mL), then extracted with ethyl acetate (2 X10 mL), wash with brine (2 X 10 mL) dried over $MgSO_4$, filtrate and the solvent was removed *in vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (58.6 mg, 0.125 mmol, 92 % yield).

M.p. 120-124 °C, Lit: 119-121°C[74]; FT-IR (ATR) V_{max}/cm^{-1} 3289, 2929,1450, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 3.25 (dd 1 H, j= 6.9, 4.6 Hz), 2.45-2.29 (m, 4H) , 2.07-2.0(m, 6H), 1.98-1.88 (m, 20 H), 1.781-05 (m, 20 H), 1.02 (s, 3H), 1.00 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.89 (s, 6H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 134.4(C), 134.3(C), 78.9 (CH), 59.9 (CH_2), 54.5 (CH_2), 50.4 (CH), 50.2

(CH), 49.8 (C), 44.4 (C), 38.9 (C), 37.0 (C), 36.3 (CH), 35.6 (CH_2), 34.1 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 28.1 (CH_2), 27.9 (CH_2), 27.8 (CH_2), 26.5 (CH_2), 25.7 (CH_2), 24.3 (CH_2), 24.2 (CH_3), 23.3 (CH_3), 21.0 (CH_2), 19.1 (CH_2), 18.7 (CH_3), 18.2 (CH_3), 15.7 (CH_3), 15.4 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 470.4412 $C_{33}H_{56}NO$ required 470.4356.

6.35. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(piperidin-1-yl)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 120[74]

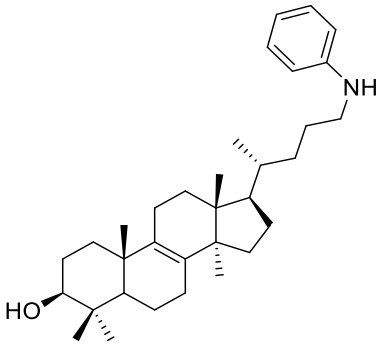


To a solution of aldehyde **43** (100 mg, 0.226 mmol) in 1,2-dicloroethane (10 mL), sodium triacetoxyborohydride (67 mg 0.316 mmol) and piperidine (23 µL, 0.23 mmol) was added under argon. Reaction was stirred for 4.5 h at room temperature, reaction monitored by TLC. 20 mL of sodium bicarbonate added to quench the reaction, then extract with ethyl acetate (2 X 20mL), wash with brine (2 X 20 mL), dried by $MgSO_4$ anhydrous, filtrate and the solvent was removed under reduce pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then

ethyl acetate (100 %) as an eluent to afford a white solid in (0.107 g, 0.21 mmol, 92 % yield).

M.p. 148-150 °C, Lit: 150-151°C[74]; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 4.52 (dd 1 H, *j*= 7.02, 4.68 Hz), 2.48-2.29 (m, 4H) , 2.04-1.90 (m, 20 H), 1.78-1.04 (m, 20 H), 1.02 (s, 6H), 0.93 (s, 3H), 0.92 (s, 3H), 0.90 (s, 6H), 0.88 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 171.0 (C), 134.4(C), 134.2(C), 80.9 (CH), 59.8 (CH_2), 54.4 (CH_2), 50.5 (CH), 50.2 (CH), 49.8 (C), 44.4 (C), 37.8 (C), 36.9 (C), 36.3 (CH), 35.2 (CH_2), 34.0 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 28.1 (CH_2), 27.9 (CH_2), 26.3 (CH_2), 25.5 (CH_2), 24.2 (CH_2), 24.1 (CH_3), 21.3 (CH_3), 20.9 (CH_3), 19.1 (CH_2), 18.7 (CH_3), 18.1 (CH_3), 16.5 (CH_3), 15.7 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 512.4504 $C_{34}H_{58}NO_2$ required 512.4462.

6.36. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(phenylamino)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 121[74]

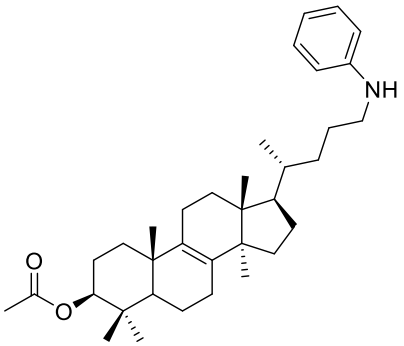


To a solution of aldehyde **42** (100 mg, 0.25 mmol) in 1,2-dichloroethane (10 mL), sodium triacetoxyborohydride (74 mg, 0.35 mmol) and aniline

(23 µL, 0.25 mmol) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Sodium bicarbonate (10 mL) added to quench the reaction, then extract with ethyl acetate (2 X 10 mL), wash with brine (2 X 10 mL) dried over $MgSO_4$ anhydrous, filtrated and the solvent was removed under reduce pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford an off-white solid in (0.01 g, 0.21 mmol, 84 % yield).

M.p, 134-137 °C; FT-IR (ATR) V_{max}/cm^{-1} 3403, 2928. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 7.22-7.16 (m, 2H), 6.74-6.69 (m, 1H), 6.66-6.61 (m, 2H), 3.26 (dd, J= 3.27, 3.25 Hz), 3.17-3.03 (m, 2H), 2.2-1.9 (m, 20 H), 1.8-1.04 (m, 20 H), 1.03(S, 3H) 1.01 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.90 (s, 3H), 0.84 (s, 3H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 148.5 (C), 134.4 (C), 134.3 (C), 129.2 (CH), 117.1 (CH), 112.7 (CH), 78.9 (CH), 50.4 (CH), 50.3 (CH), 49.8 (C), 44.59 (CH_2), 44.51 (C), 38.9 (C), 37.0 (C), 36.3 (CH), 35.6 (CH_2), 33.6 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 28.2 (CH_2), 27.9 (CH_2), 27.8 (CH_2), 26.5 (CH_2), 26.4 (CH_3), 24.2 (CH_3), 21.0 (CH_2), 19.1 (CH_2), 18.7 (CH_3), 18.2 (CH_3), 15.7 (CH_3), 15.4 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 478.4061 $C_{33}H_{52}NO$ required 478.4043.

6.37. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(phenylamino)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 122[74]

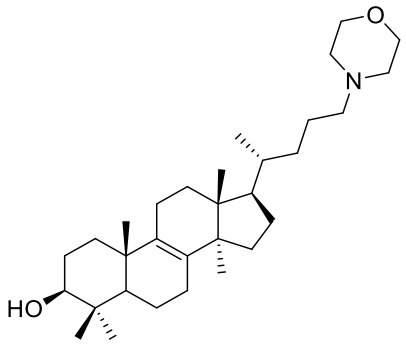


To a solution of aldehyde **43** (50 mg, 0.113 mmol) in 1,2-dicloroethane (5 mL), sodium triacetoxyborohydride (67 mg, 0.316 mmol) and aniline (10.5 µL, 0.115 mmol) were added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Sodium bicarbonate (5 mL) added to quench the reaction, then extract with ethyl acetate (2 X 10 mL), wash with brine (2 X 10 mL), dried over $MgSO_4$ anhydrous, filtrate and the solvent was removed under reduce pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford an off- white solid in (0.053 g, 0.102 mmol, 91 % yield).

M.p. 138-141 °C; FT-IR (ATR) V_{max}/cm^{-1} 2948, 1731, ^1H NMR (400 MHz, $CDCl_3$) δ_H : 7.22-7.17 (m, 2H), 6.73-6.69 (m, 1H), 6.64-6.64 (m, 2H), 4.53 (dd 1H, J= 7.02, 4.68 Hz), 3.16-3.04 (m, 2H), 2.08 (S, 3H), 2.05-1.90 (m, 20 H), 1.81-1.01 (m, 20 H), 1.03 (S, 6H), 0.98 (S, 3H), 0.94 (S, 3H), 0.9 (S, 3H), 0.89 (S, 3H), 0.72 (S, 3H). ^{13}C NMR (100 MHz,

$CDCl_3$) δC: 171.0 (C), 148.5 (C), 134.4 (C), 134.2 (C), 129.2 (CH), 117.1 (CH), 112.7 (CH), 80.9 (CH), 50.5 (CH), 50.3 (CH), 49.8 (C), 44.59 (CH_2), 44.50 (C), 37.8 (C), 36.9 (C), 36.3 (CH), 35.2 (CH_2), 33.6 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 28.2 (CH_2), 27.9 (CH_2), 27.9 (CH_2), 26.4 (CH_2), 26.3 (CH_3), 24.2 (CH3), 24.1 (CH_2), 21.3 (CH_2), 21.0 (CH_3), 19.1 (CH_2), 18.7 (CH_3), 18.1 (CH_3), 16.5 (CH_3), 15.7 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 520.4180 required $C_{35}H_{54}NO_2$ 520.4149.

6.38. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-morpholinopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 123

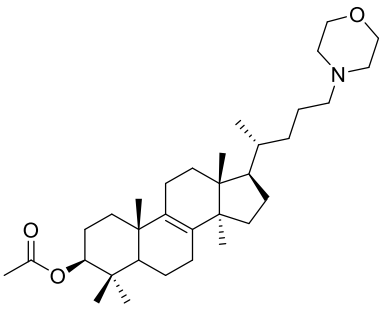


To a solution of aldehyde **42** (55 mg, 0.137 mmol) in 1,2-dichloroethane (6 mL), sodium triacetoxyborohydride (84 mg, 0.396 mmol) and morpholine (15 µL) was added under argon. Reaction was on stirred for 5.5 h at room temperature. Sodium bicarbonate (12 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (2 X 20 mL), wash with brine (2 X 20 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure.

The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a yellow oily compound (45 mg, 0.095 mmol, 70 % yield).

M.p. oily compound; FT-IR (ATR) V_{max}/cm^{-1} 3481, 3268, 2921, 1074, 978. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (t, 4H, *J* = 4.7 Hz), 3.17 (dd, 1H, *J* = 11.5, 4.5 Hz), 2.43 – 2.15 (m, 6H), 2.16 – 0.94 (m, 22H), 0.91 (s, 3H), 0.88 – 0.76 (m, 7H), 0.74 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 134.44 (C), 134.38 (C), 79.00 (CH), 67.02 2 X (CH_2), 59.73 (CH_2), 53.83 2 X (CH_2), 50.42 (CH), 50.30 (CH), 49.83 (C), 44.49 (C), 38.91 (C), 37.04 (C), 36.36 (CH), 35.60 (CH_2), 33.93 (CH_2), 30.98 (CH_2), 30.85 (CH_2), 29.72 (CH_2), 28.21 (CH_2), 27.98 (CH_3), 27.87 (CH_2), 26.51 (CH_2), 24.27 2 (CH_3), 21.01 (CH_2), 19.16 (CH_3), 18.76 (CH_3), 18.27 (CH_2), 15.76 (CH_3), 15.43 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 472.4162 required $C_{31}H_{54}NO_2$ 472.4149.

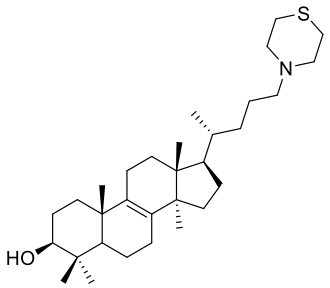
6.39. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-morpholinopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 124



To a solution of aldehyde **43** (60 mg, 0.135 mmol) in 1,2-dicloroethane (6 mL), sodium triacetoxyborohydride (84 mg, 0.396 mmol) and morpholine (15 µL) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (12 mL), then extracted with ethyl acetate (2 X 20 mL), organic phase washed with brine (2 X 20 mL), dried by $MgSO_4$, filtrate and the solvent was removed *in vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (43 mg, 0.084 mmol, 57 %).

M.p. 141-146 °C, Lit: 135-137 °C[74]; FT-IR (ATR) V_{max}/cm^{-1} 3445, 3387, 2947, 1734, 1071, 980. ^1H NMR (400 MHz, $CDCl_3$) δ 4.43 (dd, 1H, *J* = 11.5, 4.5 Hz), 3.66 (t, 4H, *J* = 4.7 Hz), 2.48 – 2.13 (m, 6H), 2.04 – 0.90 (m, 25H), 0.91 – 0.72 (m, 12H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ_C: 171.04 (C), 134.45 (C), 134.28 (C), 80.94 (CH), 67.00 2 X (CH_2), 59.72 2 X (CH_2), 53.81 (CH_2), 50.51 (CH), 50.29 (CH), 49.82 (C), 44.48 (C), 37.82 (C), 36.91 (C), 36.36 (CH), 35.28 (CH_2), 33.93 (CH_2), 30.96 (CH_2), 30.82 (CH_2), 28.21 (CH_2), 27.93 (CH_3), 26.39 (CH_2), 24.26 (CH_3), 24.18 (CH_2), 23.25 (CH_2), 21.35 (CH_3), 21.00 (CH_2), 19.20 (CH_3), 18.75 (CH_3), 18.13 (CH_2), 16.55 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 514.4279 required $C_{33}H_{56}NO_3$ 514.4255.

6.40. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-thiomorpholinopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 125

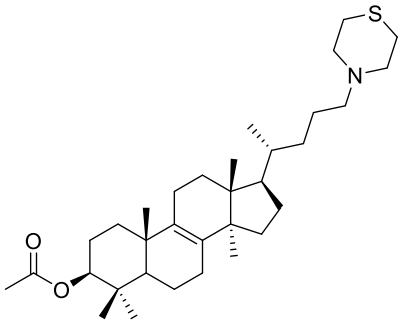


To a solution of aldehyde **42** (150 mg, 0.374 mmol) in 1,2-dichloroethane (15 mL), sodium triacetoxyborohydride (120 mg, 0.566 mmol) and thiomorpholine (39 mg 0.374 mmol) was added under argon. Reaction was on stirred for 6 h at room temperature. Sodium bicarbonate (15 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (3 X 15 mL), wash with brine (2 X 25 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid (146 mg, 0.3 mmol, 80 % yield).

M.p. 133-135 °C, Lit: 131-134 °C[74]; FT-IR (ATR) V_{max}/cm^{-1} 3258, 2949, 1037, 980. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.25 (dd, 1H, *J* = 11.5, 4.6 Hz), 2.82 – 2.58 (m, 9H), 2.47 – 2.15 (m, 2H), 2.14 – 1.83 (m, 5H), 1.83 – 1.11 (m, 13H), 1.11 – 0.96 (m, 8H), 0.94 – 0.87 (m, 6H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}NMR($CDCl_3$, 100 MHz): δ (ppm) 134.44 (C), 134.37 (C),

78.98 (CH), 59.99 (CH_2), 55.08 2 X (CH_2), 50.42 (CH), 50.31 (CH), 49.82 (C), 44.49 (C), 38.90 (C), 37.04 (C), 36.33 (CH), 35.60 (CH_2), 33.95 (CH_2), 30.98 (CH_2), 30.85 (CH_2), 28.21 (CH_2), 28.00 2 X (CH_2), 27.98 (CH_3), 27.87 (CH_2), 26.51 (CH_2), 24.27 (CH_3), 23.17 (CH_2), 21.01 (CH_2), 19.16 (CH_3), 18.76 (CH_3), 18.27 (CH_2), 15.76 (CH_3), 15.43 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 488.3929 required $C_{31}H_{54}NOS$ 488.3921.

6.41. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-thiomorpholinopentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 126

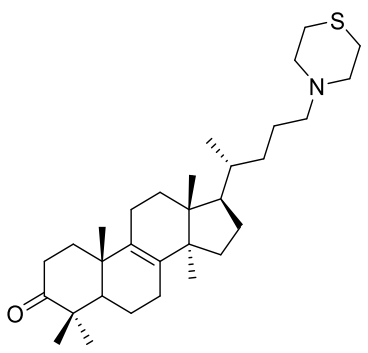


To a solution of aldehyde **43** (60 mg, 0.135 mmol) in 1,2-dicloroethane (6 mL), sodium triacetoxyborohydride (84 mg 0.396 mmol) and thiomorpholine (45 mg 0.436 mmol) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (12 mL), then extracted with ethyl acetate (2 X 20 mL), organic phase washed with brine (2 X 20 mL), dried by $MgSO_4$, filtrate and the solvent was removed *in vacuo*. The crude was purified by column chromatography using

petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (40 mg, 0.075 mmol, 56 %).

M.p. 136-139 °C, Lit: 137-139 °C[74]; FT-IR (ATR) V_{max}/cm^{-1} 3320, 2943, 1654, 1021. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (dd, 1H, *J* = 11.5, 4.5 Hz), 3.40 – 2.60 (m, 10H), 2.59 – 2.16 (m, 2H), 2.07 (s, 5H), 1.83 – 1.04 (m, 15H), 1.02 (s, 4H), 1.00 – 0.80 (m, 15H), 0.71 (d, 3H, *J* = 2.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.04 (C), 134.44 (C), 134.28 (C), 80.93 (CH), 59.88 (CH_2), 54.98 2 X (CH_2), 50.51 (CH), 50.31 (CH), 49.82 (C), 44.48 (C), 37.82 (C), 36.91 (C), 36.33 (CH), 35.27 (CH_2), 33.88 (CH_2), 33.79 (CH_2), 30.96 (CH_2), 30.81 (CH_2), 29.72 2X (CH_2), 28.21 (CH_2), 27.93 (CH_3), 26.39 (CH_2), 24.26 (CH_3), 24.18 (CH_2), 21.35 (CH_3), 21.00 (CH_2), 19.20 (CH_3), 18.74 (CH_3), 18.13 (CH_2), 16.55 (CH_3), 15.77 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 530.4056 required $C_{33}H_{56}NO_2S$ 530.4026.

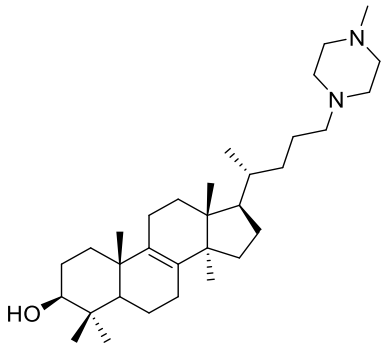
6.42. Synthesis of (10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-thiomorpholinopentan-2-yl)-1,2,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-3-one 127



To a solution of **125** (60 mg, 0.135 mmol) in DCM (4.65 mL) and H_2O (3 µL, 0.04 mmol) Dess-Martin periodinane (55 mg, 0.129 mmol) was added. Reaction stirred for 16 h as white suspension at room temperature. The solvent was removed under reduce pressure. The residue dissolved in 20 mL EtOAc, the crude washed by $Na_2S_2O_3$:$NaHCO_3$ (sat. aq. sol.) 1:1 (1 X 20 mL), brine (1 X 10 mL). The aqueous phase extracted with EtOAc (5 X 25 mL), dried by Na_2SO_4, filtrate and the solvent removed under reduce pressure to afford an off white solid. The crude residue purified by column chromatography using petroleum ether 40/60: ethyl acetate (92:8) R_f = 0.7, affording **127** (58 mg, 0.119 mmol, 97 %) as off white solid.

M.p. 89-93 °C; FT-IR (ATR) V_{max}/cm^{-1} 3456, 3310, 2945, 1734, 1028, 982. ^1H NMR (400 MHz, $CDCl_3$) δ 3.01 – 2.31 (m, 9H), 2.26 – 1.27 (m, 21H), 1.26 – 0.96 (m, 9H), 0.91 (m, 9H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 217.88 (C), 135.31 (C), 133.18 (C), 59.83 (CH_2), 54.95 X 2 (CH_2), 51.24 (CH), 50.30 (CH), 49.93 (C), 47.41 (C), 44.48 (C), 36.92 (C), 36.31 (CH), 36.08 (CH_2), 35.29 (CH_2), 34.64 (CH_2), 33.85 (CH_2), 30.94 (CH_2), 30.91 (CH_2), 29.71 (CH_2), 28.17 2X (CH_2), 26.35 (CH_2), 26.21 (CH_3) 24.32 (CH_3), 21.31 (CH_3), 21.09 (CH_2), 19.45 (CH_2), 18.74 (CH_3), 15.86 (CH_3), 15.77 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 486.3775 required $C_{31}H_{52}NOS$ 481. 486.3764.

6.43. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(4-methylpiperazin-1-yl)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol 128



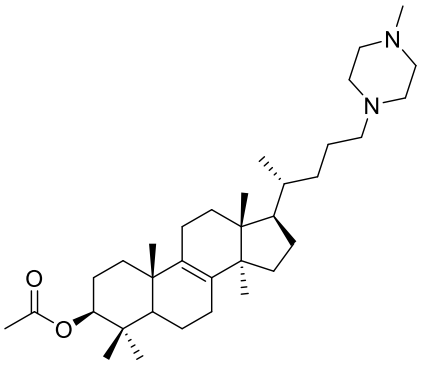
To a solution of aldehyde **42** (55 mg, 0.137 mmol) in 1,2-dichloroethane (6 mL), sodium triacetoxyborohydride (84 mg, 0.396 mmol) and 1-methylpiperazine (35 µL) was added under argon. Reaction was on stirred for 6 h at room temperature. Sodium bicarbonate (12 mL) was added to quench the reaction, the aqueous phase was extracted with ethyl acetate (2 X 20 mL), wash with brine (2 X 20 mL) dried over magnesium sulphate, filtrate and the solvent was removed under reduced pressure. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid (47 mg, 0.097 mmol, 71 % yield).

M.p. 209-214 °C; FT-IR (ATR) V_{max}/cm^{-1} 3234, 2935, 1600, 1037, 971.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (t, 5H, *J* = 5.3 Hz), 3.17 (dd, 1H, *J* = 11.6, 4.5 Hz), 2.70 – 2.40 (m, 5H), 2.13 – 1.04 (m, 20H), 1.02 – 0.88 (m, 7H), 0.87 – 0.79 (m, 7H), 0.74 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (125

MHz, $CDCl_3$): δ (ppm) 134.45 (C), 134.36 (C), 79.00 (CH), 59.47 2 X (CH_2), 56.27 2 X (CH_2), 55.40 (CH_2), 50.44 (CH), 50.41 (CH), 49.82 (C), 44.51 (C), 38.91 (C), 37.03 (C), 36.43 (CH), 35.59 (CH_2), 33.78 (CH_2), 30.99 (CH_2), 30.84 (CH_2),28.27 (CH_2), 27.98 2 X (CH_3), 27.86 (CH_2), 26.51 (CH_2), 24.29 (CH_3), 23.72 (CH_2), 21.01 (CH_2), 19.16 (CH_3), 18.75 (CH_3), 18.26 (CH_2), 15.77 (CH_3), 15.43 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 485.4491 required $C_{32}H_{57}N_2O$ 485.4465.

6.44. Synthesis of (3S,10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-17-((R)-5-(4-methylpiperazin-1-yl)pentan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 129

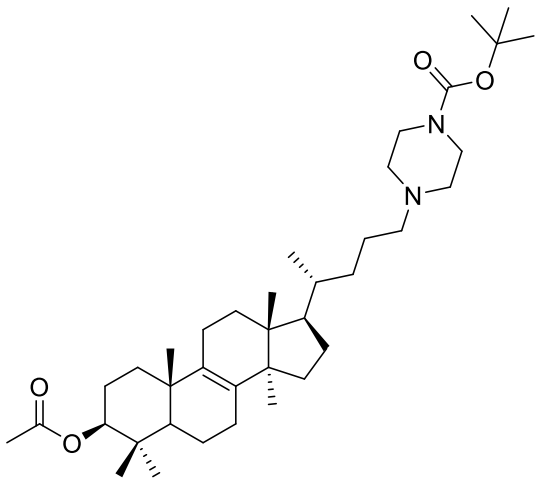


To a solution of aldehyde **43** (60 mg, 0.135 mmol) in 1,2-dicloroethane (6 mL), sodium triacetoxyborohydride (84 mg, 0.396 mmol) and 1-Methylpiperazine (35 µL) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (12 mL), then extracted with ethyl acetate (2 X 20 mL), organic phase washed with brine (2 X 20 mL),

dried by $MgSO_4$, filtrate and the solvent was removed *in vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (58 mg, 0.11 mmol, 82 %).

M.p. 120-124 °C; FT-IR (ATR) V_{max}/cm^{-1} 3423, 3310, 2925, 1732, 1075, 977. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.43 (dd, 1H, *J* = 11.5, 4.5 Hz), 2.23 (s, 5H), 2.02 – 1.71 (m, 9H), 1.72 – 1.03 (m, 14H), 0.93 (s, 3H), 0.90 – 0.74 (m, 12H), 0.61 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.02 (C), 134.47 (C), 134.26 (C), 80.94 (CH), 59.29 (CH_2), 55.13 2 X (CH_2), 53.25 2 X (CH_2), 50.51 (CH), 50.27 (CH), 49.81 (C), 46.05 (CH_3), 44.47 (C), 37.82 (C), 36.91 (C), 36.37 (CH), 35.28 (CH_2), 34.01 (CH_2), 30.95 (CH_2), 30.82 (CH_2), 28.19 (CH_2), 27.92 (CH_3), 26.39 (CH_2), 24.26 (CH_3), 24.19 (CH_2), 23.61 (CH_2), 21.35 (CH_3), 21.00 (CH_2), 19.19 (CH_3), 18.76 (CH_3), 18.13 (CH_2), 16.54 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 527.4599 required $C_{34}H_{59}N_2O_2$ 527.4571.

6.45. Synthesis of isopropyl 4-((4R)-4-((3S,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentyl)piperazine-1-carboxylate 130

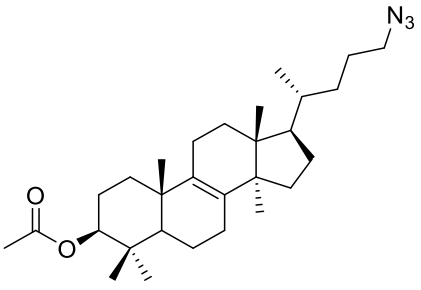


To a solution of aldehyde **43** (400 mg, 0.9 mmol) in 1,2-dicloroethane (40 mL), sodium triacetoxyborohydride (286 mg 1.264 mmol) and 1-Boc-piperazine (168 mg, 0.9 mmol) was added under argon. Reaction was stirred for 6 h at room temperature, reaction monitored by TLC. Reaction was quenched with sodium bicarbonate (40 mL), then extracted with ethyl acetate (2 X 40 mL), organic phase washed with brine (2 X 80 mL), dried by $MgSO_4$, filtrate and the solvent was removed *in vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) then ethyl acetate (100 %) as an eluent to afford a white solid in (444 mg, 0.724 mmol, 80 %).

M.p. 177-180 °C; FT-IR (ATR) V_{max}/cm^{-1} 3437, 3369, 1730, 1058, 977. ^1H NMR (400 MHz, $CDCl_3$) δ 4.51 (dd, 1H, *J* = 11.6, 4.5 Hz), 3.46 (t,

4H, *J* = 5.1 Hz), 2.28 – 1.06 (m, 39H), 1.02 (s, 3H), 0.90 (q, 14H, *J* = 6.1 Hz), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.01 (C), 154.76 (C), 134.45 (C), 134.28 (C), 80.92 (CH), 79.59 (C), 59.24 (CH_2), 53.02 2 X (CH_2), 50.50 (CH), 50.29 (CH), 49.81 (C), 44.47 (C), 37.81 (C), 36.90 (C), 36.34 (CH), 35.27 (CH_2), 33.92 (CH_2), 30.95 (CH_2), 30.81 (CH_2), 29.71 2 X (CH_2), 28.44 3 X (CH_3), 28.20 (CH_2), 27.92 (CH_3), 26.39 (CH_2), 24.26 (CH_3), 24.18 (CH_2), 23.42 (CH_2), 21.34 (CH_3), 21.00 (CH_2), 19.19 (CH_3), 18.74 (CH_3), 18.13 (CH_2), 16.54 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 613.495509 required $C_{38}H_{65}N_2O_4$ 613.493885.

6.46. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-azidopentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 98[158]

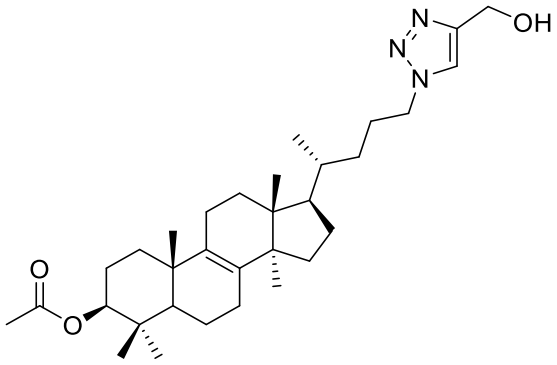


Diisopropylazidocarboxylate (0.11 mL, 0.56 mmol, 1.6 eq.) and diphenylphosphoryl azide (0.12 mL, 0.56 mmol, 1.6 eq.) were added

to a solution of **91** (158 mg 0.35 mmol, 1 eq.) and triphenylphosphine (141 mg, 0.53 mmol, 1.51 eq.) in THF (4 mL) at 0 °C for 10 min before it allowed to warm to 25 °C. The reaction mixture left on stirring for 24 h. The solvent was removed under *vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (92:8) R_f = 0.6, as an eluent to afford a yellow solid (93 mg, 0.198 mmol, 56%).

M.p. 127-129 °C; FT-IR (ATR) V_{max}/cm^{-1} 3710, 3679, 2943, 1723, 1012, 980, 968. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (dd, 1H, *J* = 11.6, 4.5 Hz, 1-CH), 3.34 – 3.18 (m, 2H), 2.12 – 1.88 (m, 7H), 1.72 (dddt, 4H, *J* = 18.4, 13.3, 7.1, 3.0 Hz), 1.67 – 1.40 (m, 4H), 1.44 – 1.07 (m, 10 H), 1.03 (s, 3H), 0.99 – 0.81 (m, 13H), 0.78 – 0.69 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.99 (C), 134.41 (C), 134.28 (C), 80.90 (CH), 51.97 (CH), 50.50 (CH), 50.30 (CH_2), 49.82 (C), 44.50 (C), 37.81 (C), 36.91 (C), 36.15 (CH), 35.27 (CH_2), 33.19 (CH_2), 30.97 (CH_2), 30.79 (CH_2), 28.20 (CH_2), 27.92 (CH_2), 26.38 (CH_2), 25.75 (CH_2), 24.25 (CH_3), 24.18 (CH_3), 21.34 (CH_2), 20.99 (CH_3), 19.20 (CH_3), 18.64 (CH_2), 18.12 (CH_3), 16.54 (CH_3), 15.77 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 492.3568 required $C_{29}H_{47}N_3O_2Na$ 492.3560

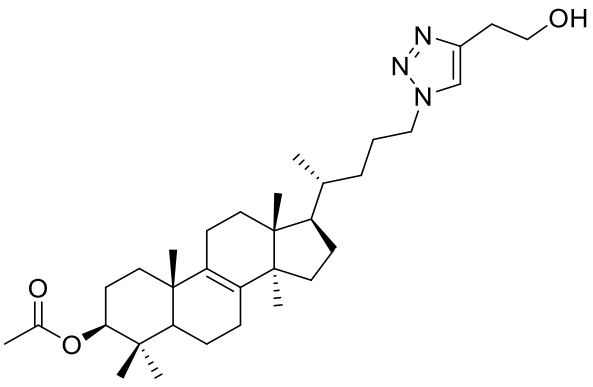
6.47. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 131[159]



The title compound was synthesised using a procedure adapted from literature. Azide **98** (93 mg, 0.197 mmol, 1 eq.) and propargyl alcohol (13 µL, 0.214 mmol, 1.08 eq.) were dissolved in THF/H_2O (3 mL, 1:1). $CuSO_4.5H_2O$ (4 mg, 0.02 mmol, 0.1 eq.) and (+)-Sodium L-ascorbate (5 mg, 0.02 mmol, 0.1 eq.) were added and the reaction mixture and stirred for 3 h at room temperature. TLC showed some of the starting material remained, 0.2 eq. of $CuSO_4.5H_2O$ and 0.2 eq. of (+)-Sodium L-ascorbate added and left on stirring for 13 h. After 13 h TLC showed reaction completion. The solvent removed under *vacuo*, the crude suspension was extracted by dichloromethane and water, the organic phase dried over $MgSO_4$, filtrate and the solvent was removed under *vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (7:3) R_f = 0.3, as an eluent to yield the final product as yellow solid (103 mg, 0.196 mmol, 100%).

M.p. 159-163 °C; FT-IR (ATR) V_{max}/cm^{-1} 3512, 3311, 2922, 2340, 1713, 1096, 986. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 4.82 – 4.76 (m, 2H), 4.50 (ddd, 1H, *J* = 11.5, 4.5, 1.6 Hz), 4.38 – 4.24 (m, 2H), 2.11 – 1.92 (m, 7H), 1.91 – 1.78 (m, 1H), 1.81 – 1.66 (m, 4H), 1.63 – 1.37 (m, 5H), 1.37 – 1.21 (m, 4H), 1.26 – 1.02 (m, 3H), 1.00 (d, 3H, *J* = 1.5 Hz), 0.96 – 0.83 (m,14H), 0.68 (d, 3H, *J* = 1.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.03 (C), 134.34 (C), 134.28 (C), 125.00 (CH), 80.91(CH), 56.35 (CH_2), 50.87(CH), 50.47 (CH), 50.18 (CH_2), 49.79 (C), 44.49 (C), 37.79 (C), 36.88 (C), 36.01 (CH), 35.24 (CH_2) , 32.94 (CH_2), 30.93 (CH_2), 30.75 (CH_2), 28.13 (CH_2), 27.91 (CH_2), 27.28 (CH_2), 26.36 (CH_2), 24.22 (CH_3), 24.15 (CH_3), 21.33 (CH_2), 20.96, 19.18 (CH_3), 18.57 (CH_3), 18.10 (CH_2), 16.53 (CH_3), 15.75 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 526.4003 required $C_{32}H_{52}N_3O_3$ 526.4003

6.48. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 132[159]

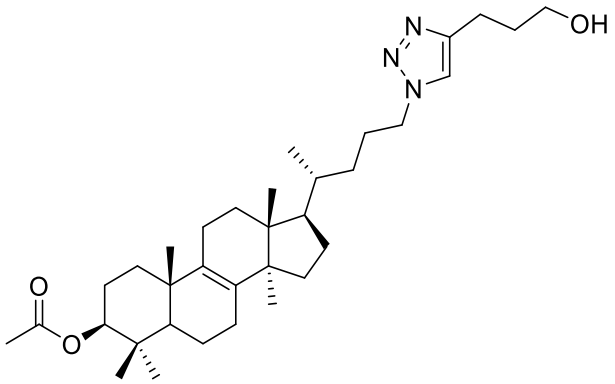


The title compound was synthesised using a procedure adapted from literature. Azide **98** (100 mg, 0.219 mmol, 1 eq.) and 3-butyn-1-ol (17 µL, 0.236 mmol, 1.08 eq.) were dissolved in THF/H_2O (3.33 mL, 1:1). $CuSO_4.5H_2O$ (5.5 mg, 0.022 mmol, 0.1 eq.) and (+)-sodium L-ascorbate (4.5 mg, 0.022 mmol, 0.1 eq.) were added and the reaction mixture and stirred for 3 h at room temperature. TLC showed some of the starting material remained, 0.2 eq. of $CuSO_4.5H_2O$ and 0.2 eq. of (+)-sodium L-ascorbate added and left on stirring for 13 h. After 13 h TLC showed reaction completion. The solvent was removed under *vacuo*, the crude suspension was extracted by dichloromethane and water, the organic phase dried over $MgSO_4$, filtrate and the solvent removed under *vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (7:3) R_f = 0.8, as an eluent to yield the final product as yellow solid (110 mg, 0.204 mmol, 93%).

M.p. 129-134 °C; FT-IR (ATR) V_{max}/cm^{-1} 3381, 2944, 2318, 1719, 1032, 982. ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 4.52 (dd, 1H, *J* = 11.5, 4.5 Hz), 4.38 – 4.24 (m, 2H), 3.98 (t, 2H, *J* = 5.8 Hz), 3.00 – 2.94 (m, 2H), 2.63 (s, 1H), 2.27 – 1.39 (m, 17H), 1.38 – 1.04 (m, 7H), 1.02 (s, 3H), 0.92 (d, 3H, *J* = 6.0 Hz), 0.90 (s, 7H), 0.88 – 0.87 (m, 3H), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.04 (C), 145.49 (C), 134.37 (C), 134.31 (C), 121.25 (CH), 80.92 (CH), 61.75 (CH_2), 50.77 (CH_2), 50.49 (CH), 50.18 (CH), 49.81 (C), 44.51 (C), 37.81 (C), 36.90 (C), 36.02 (CH), 35.26 (CH_2), 32.95 (CH_2), 30.94 (CH_2), 30.76 (CH_2),

28.65 (CH_2), 28.14 (CH_2), 27.92 (CH_3), 27.28 (CH_2), 26.37 (CH_2), 24.24 (CH_3), 24.17 (CH_2), 21.35 (CH_3), 20.97 (CH_2), 19.20 (CH_3), 18.59 (CH_3), 18.11 (CH_2), 16.54 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 540.4144 required $C_{33}H_{54}N_3O_3$ 540.4160.

6.49. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(4-(3-hydroxypropyl)-1H-1,2,3-triazol-1-yl)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 133[159]

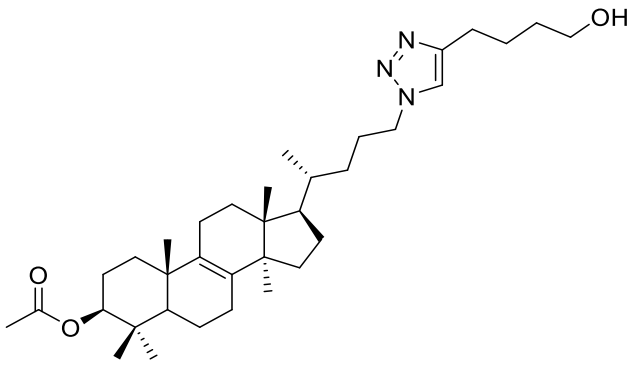


The title compound was synthesised using a procedure adapted from literature. Azide **98** (100 mg, 0.219 mmol, 1 eq.) and 4-pentyn-1-ol (22 µL, 0.236 mmol, 1.08 eq.) were dissolved in THF/H_2O (3.33 mL, 1:1). $CuSO_4.5H_2O$ (5.5 mg, 0.022 mmol, 0.1 eq.) and (+)-sodium L-ascorbate (4.5 mg, 0.022 mmol, 0.1 eq.) were added and the reaction mixture and stirred for 3 h at room temperature. TLC showed some of the starting material remained, 0.2 eq. of $CuSO_4.5H_2O$ and 0.2 eq. of (+)-sodium L-ascorbate added and left on stirring for 13 h. After 13 h TLC showed reaction completion. The solvent was removed under *vacuo*, the crude suspension was extracted by dichloromethane and

water, the organic phase dried over $MgSO_4$, filtrate and the solvent removed under *vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (7:3) R_f = 0.9, as an eluent to yield the final product as yellow solid (91 mg, 0.164 mmol, 75%).

M.p. 108-113 °C; FT-IR (ATR) V_{max}/cm^{-1} 3335, 2931, 2163, 1732, 1036, 983. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (s, 1H), 4.51 (dd, 1H, J = 11.7, 4.5 Hz), 4.34 – 4.26 (m, 2H), 3.74 (t, 2H, J = 6.1 Hz), 2.86 (t, 2H, J = 7.2 Hz), 2.07 (s, 6H), 1.91 – 1.05 (m, 19H), 1.02 (s, 3H), 0.99 – 0.89 (m, 12H), 0.88 – 0.82 (m, 3H), 0.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.06 (C), 147.38 (C), 134.37 (C), 134.31 (C), 120.65 (CH), 80.93 (CH), 61.98 (CH_2), 50.72 (CH_2), 50.49 (CH), 50.17 (CH), 49.81 (C), 44.50 (C), 37.81 (C), 36.90 (C), 36.02 (CH), 35.26 (CH_2), 32.94 (CH_2), 31.97 (CH_2), 30.94 (CH_2), 30.76 (CH_2), 28.14 (CH_2), 27.92 (CH_3), 27.29 (CH_2), 26.37 (CH_2), 24.24 (CH_3), 24.17 (CH_2), 22.17 (CH_2), 21.35 (CH_3), 20.97 (CH_2), 19.20 (CH_3), 18.60 (CH_3), 18.11 (CH_2), 16.54 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 554.4315 required $C_{34}H_{56}N_3O_3$ 554.4316.

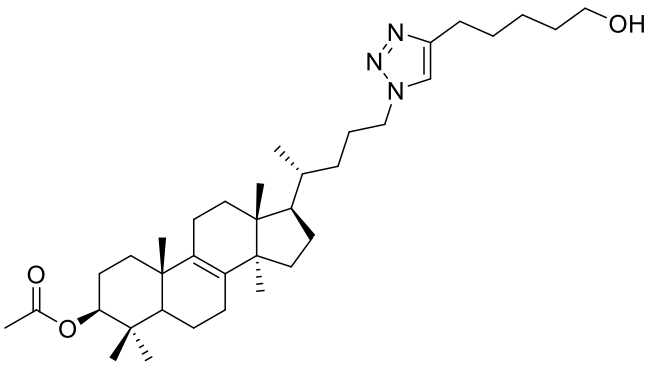
6.50. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(4-(4-hydroxybutyl)-1H-1,2,3-triazol-1-yl)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 134 [159]



The title compound was synthesised using a procedure adapted from literature. Azide **98** (150 mg, 0.328 mmol, 1 eq.) and 5-hexyn-1-ol (38 µL, 0.344 mmol, 1.08 eq.) were dissolved in THF/H_2O (5 mL, 1:1). $CuSO_4.5H_2O$ (8.3 mg, 0.033 mmol, 0.1 eq.) and (+)-sodium L-ascorbate (7 mg, 0.033 mmol, 0.1 eq.) were added and the reaction mixture and stirred for 3 h at room temperature. TLC showed some of the starting material remained, 0.2 eq. of $CuSO_4.5H_2O$ and 0.2 eq. of (+)-sodium L-ascorbate added and left on stirring for 13 h. After 13 h TLC showed reaction completion. The solvent was removed under *vacuo*, the crude suspension was extracted by dichloromethane and water, the organic phase dried over $MgSO_4$, filtrate and the solvent removed under *vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (7:3) R_f = 0.6, as an eluent then ethyl acetate: methanol (9:1) to yield the final product as yellow solid (160 mg, 0.281 mmol, 88%).

M.p. 126-129 °C; FT-IR (ATR) V_{max}/cm^{-1} 3613, 3338, 2934, 2368, 1732, 1035, 982. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (s, 1H), 5.32 (s, 0H), 4.51 (dd, 1H, *J* = 11.7, 4.5 Hz), 4.36 – 4.23 (m, 2H), 3.70 (t, 2H, *J* = 6.4 Hz), 2.78 (t, 2H, *J* = 7.5 Hz), 2.09 – 1.92 (m, 7H), 1.91 – 1.03 (m, 23H), 1.01 (s, 3H), 0.92 (d, 3H, *J* = 6.1 Hz), 0.90 (s, 6H), 0.87 (s, 3H), 0.69 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.05 (C), 147.98 (C), 134.37 (C), 134.30 (C), 120.47 (CH), 80.92 (CH), 62.50 (CH_2), 50.68 (CH_2), 50.49 (CH), 50.17 (CH), 49.81 (C), 44.50 (C), 37.81 (C), 36.90 (C), 36.02 (CH), 35.26 (CH_2), 32.94 (CH_2), 32.17 (CH_2), 30.94 (CH_2), 30.76 (CH_2), 28.14 (CH_2), 27.92 (CH_3), 27.31 (CH_2), 26.37 (CH_2), 25.61 (CH_2), 25.31 (CH_2), 24.24 (CH_3), 24.17 (CH_2), 21.35 (CH_3), 20.97 (CH_2), 19.19 (CH_3), 18.60 (CH_3), 18.11 (CH_2), 16.54 (CH_3), 15.76 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 568.4488 required $C_{35}H_{58}N_3O_3$ 568.4473.

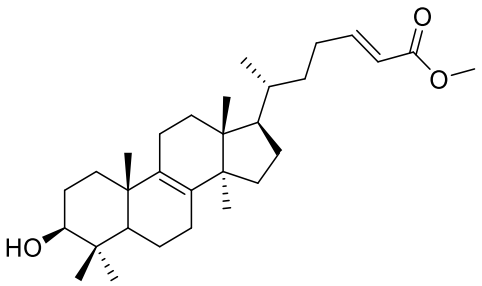
6.51. Synthesis of (3S,10S,13R,14R,17R)-17-((R)-5-(4-(5-hydroxypentyl)-1H-1,2,3-triazol-1-yl)pentan-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate 135[159]



The title compound was synthesised using a procedure adapted from literature. Azide **98** (200 mg, 0.438 mmol, 1 eq.) and 6-heptyn-1-ol (48 µL, 0.474 mmol, 1.08 eq.) were dissolved in THF/H_2O (6.66 mL, 1:1). $CuSO_4.5H_2O$ (11 mg, 0.044 mmol, 0.1 eq.) and (+)-sodium L-ascorbate (9 mg, 0.044 mmol, 0.1 eq.) were added and the reaction mixture and stirred for 3 h at room temperature. TLC showed some of the starting material remained, 0.2 eq. of $CuSO_4.5H_2O$ and 0.2 eq. of (+)-sodium L-ascorbate added and left on stirring for 13 h. After 13 h TLC showed reaction completion. The solvent was removed under *vacuo*, the crude suspension was extracted by dichloromethane and water, the organic phase dried over $MgSO_4$, filtrate and the solvent removed under *vacuo*. The crude was purified by column chromatography using petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.9, as an eluent then ethyl acetate: methanol (9:1) to yield the final product as yellow solid (191 mg, 0.328 mmol, 75 %).

M.p. 109-112 °C; FT-IR (ATR) Vmax/cm-1 3581, 3517, 2932, 1712, 1037, 983. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28 (td, 2H, *J* = 7.1, 3.9 Hz), 3.75 (d, 1H, *J* = 6.3 Hz), 3.65 (t, 2H, *J* = 6.5 Hz), 2.72 (t, 2H, *J* = 7.6 Hz), 2.25 – 1.07 (m, 32H), 1.05 – 0.79 (m, 16H), 0.67 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.06 (C), 148.05 (C), 134.35 (C), 134.28 (C), 120.46 (CH), 80.92 (CH), 62.63 (CH_2), 50.47 (CH), 50.16 (CH), 49.79 (C), 44.48 (C), 37.79 (C), 36.88 (C), 36.00 (CH), 35.24 (CH_2), 32.92 (CH_2), 32.41 (CH_2), 30.92 (CH_2), 30.75 (CH_2), 29.16 (CH_2)z, 28.11 (CH_2), 27.90 (CH_3), 27.28 (CH_2), 26.35 (CH_2), 25.6 (CH_2), 25.54 (CH_2), 25.30 (CH_2), 24.22 (CH_3), 24.15 (CH_2), 21.33 (CH_3), 20.96 (CH_2), 19.18 (CH_3), 18.59 (CH_3), 18.09 (CH_2), 16.53 (CH_3), 15.74 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 604.4482 required $C_{36}H_{59}N_3NaO_3$ 604.4449.

6.52. Synthesis of methyl (6R,E)-6-((3S,10S,13R,14R,17R)-3-hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate 136

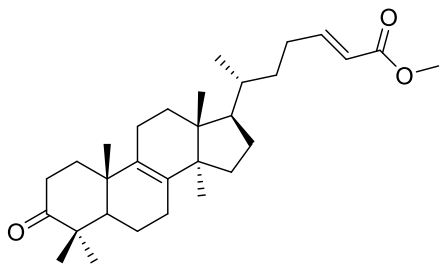


To a solution of aldehyde **42** (259 mg, 0.646 mmol) in THF (2.3 mL), (methoxycarbonylmethyl)triphenylphosphorane (432 mg, 1.292 mmol) was added. Reaction was stirred for 16 h at room temperature. The solvent was removed under reduced pressure. The crude was then purified using column chromatography petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.1, to yield the final product as an off white powder (163 mg, 0.357 mmol, 56 %)

M.p. 137-138 °C; FT-IR (ATR) V_{max}/cm^{-1} 3512, 2937, 2873, 1711, 1077, 985. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (dt, 1H, *J* = 15.6, 6.9 Hz), 5.84 (dt, 1H, *J* = 15.6, 1.6 Hz), 3.74 (s, 3H), 3.25 (dd, 1H, *J* = 11.5, 4.5 Hz), 2.36 – 1.87 (m, 7H), 1.80 – 1.04 (m, 16H), 1.02 (s, 3H), 1.00 (s, 3H), 0.93 (d, 3H, *J* = 6.1 Hz), 0.89 (d, 3H, *J* = 0.9 Hz), 0.83 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 167.26 (C), 150.35 (CH), 134.46 (C), 134.32 (C), 120.57 (CH), 78.97 (CH), 51.40 (CH_3), 50.41 (CH), 50.35 (CH), 49.82 (C), 44.55 (C), 38.90 (C), 37.03 (C),

36.14 (CH), 35.60 (CH_2), 34.54 (CH_2), 31.00 (CH_2), 30.82 (CH_2), 29.25 (CH_2), 28.18 (CH_2), 27.98 (CH_3), 27.85 (CH_2), 26.50 (CH_2), 24.26 (CH_3), 21.00 (CH_2), 19.16 (CH_3), 18.46 (CH_3), 18.26 (CH_2), 15.79 (CH_3), 15.44 (CH_3). HRMS m/z (ESI+) found $[M+NH_4]^+$ 474.3937 required $C_{30}H_{52}NO_3$ 474.3942.

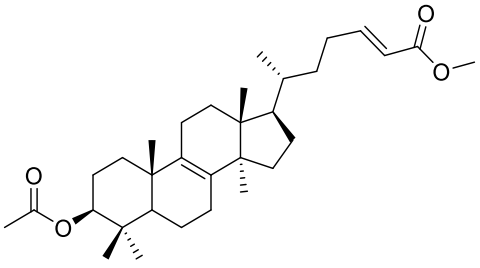
6.53. Synthesis of methyl (6R,E)-6-((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-3-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate 137



To a solution of ester **136** (60 mg, 0.13 mmol) in DCM (5.6 mL) and H_2O (3.5 µL, 0.04mmol) Dess-Martin periodinane (66 mg, 0.15 mmol) was added. Reaction was stirred for 16 h as white suspension at room temperature. The solvent removed under reduce pressure. The crude extracted by $Na_2S_2O_3$:$NaHCO_3$ (sat. aq. sol.) 1:1 (1 X 20 mL). The solvent was removed under reduce pressure to afford an off white solid. The crude residue was purified by column chromatography using petroleum ether 40/60: ethyl acetate (92:8) R_f = 0.2, affording **137** (37 mg, 0.08 mmol, 62 %) as an off white solid.

M.p. 94-98 °C; FT-IR (ATR) V_{max}/cm^{-1} 2934, 2854, 1724, 1703, 1075, 1655, 1039, 986. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (dt, 1H, *J* = 15.6, 6.9 Hz), 5.84 (dt, 1H, *J* = 15.6, 1.6 Hz), 3.75 (s, 3H), 2.68 – 1.86 (m, 6H), 1.85 – 1.16 (m, 17H), 1.14 (s, 3H), 1.12 (s, 3H), 1.09 (s, 3H), 0.99 – 0.83 (m, 6H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 217.89 (C), 167.26 (C), 150.29 (CH), 135.27 (C), 133.19 (C), 120.62 (CH), 51.41 (CH), 51.23 (CH), 50.36 (CH_3), 49.93 (C), 47.41 (C), 44.54 (C), 36.92 (C), 36.13 (CH), 36.07 (CH_2), 34.64 (CH_2), 34.52 (CH_2), 30.96 (CH_2), 29.72 (CH_2), 29.25 (CH_2), 28.15 (CH_2), 26.35 (CH_2), 26.21 (CH_3), 24.31 (CH_3), 21.31 (CH_3), 21.08 (CH_2), 19.45 (CH_2), 18.70 (CH_3), 18.46 (CH_3), 15.89 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 477.3342 required $C_{30}H_{46}NaO_3$ 477.3339.

6.54. Synthesis of methyl (6R,E)-6-((3S,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate 107

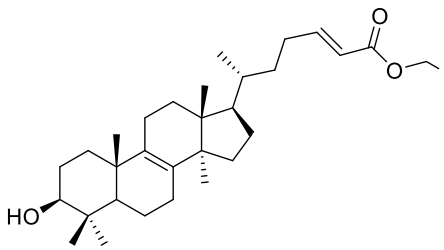


To a solution of aldehyde **43** (100 mg, 0.226 mmol) in THF (0.8 mL), (methoxycarbonylmethyl)triphenylphosphorane (150 mg, 0.452 mmol) was added. Reaction was stirred for 16 h at room temperature. The solvent was removed under reduced pressure. The crude was then

purified using column chromatography petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.3, to yield the final product as an off white powder (103 mg, 0.207 mmol, 91 %).

M.p. 113-116 °C; FT-IR (ATR) V_{max}/cm^{-1} 2950, 2926, 1723, 1651, 1281, 1075, 984. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (dt, 1H, *J* = 15.6, 7.0 Hz), 5.84 (dt, 1H, *J* = 15.6, 1.6 Hz), 4.52 (dd, 1H, *J* = 11.6, 4.5 Hz), 3.74 (s, 3H), 2.36 – 1.85 (m, 7H), 1.82 – 1.06 (m, 16H), 1.02 (s, 4H), 0.93 (d, 3H, *J* = 6.2 Hz), 0.90 (d, 11H, *J* = 3.5 Hz), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.02 (C), 167.26 (C), 150.34 (CH), 134.41 (C), 134.30 (C), 120.58 (CH), 80.92 (CH), 51.39 (CH_3), 50.50 (CH), 50.35 (CH), 49.82 (C), 44.54 (C), 37.81 (C), 36.91 (C), 36.13 (CH), 35.27 (CH_2), 34.53 (CH_2), 30.98 (CH_2), 30.78 (CH_2), 29.25 (CH_2), 28.17 (CH_2), 27.92 (CH_3), 26.38 (CH_2), 24.24 (CH_3), 24.18 (CH_2), 21.35 (CH_3), 20.99 (CH_2), 19.20 (CH_3), 18.45 (CH_3), 18.12 (CH_2), 16.54 (CH_3), 15.79 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 521.3569 required $C_{32}H_{50}NaO_4$ 521.3601.

6.55. Synthesis of ethyl (6R,E)-6-((3S,10S,13R,14R,17R)-3-hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate 138

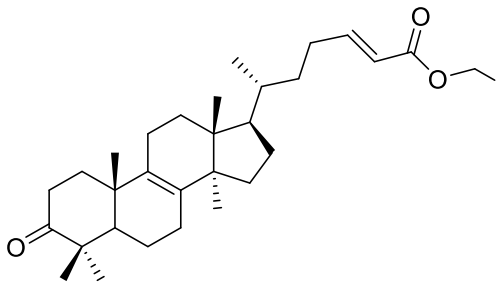


To a solution of aldehyde **42** (43 mg, 0.107 mmol) in THF (0.2 mL), (ethoxycarbonylmethyl)triphenylphosphorane (70 mg, 0.2 mmol) was added. Reaction was stirred for 16 h at room temperature. The solvent was removed under reduced pressure. The crude was then purified using column chromatography petroleum ether 40/60: ethyl acetate (9:1) R_f = 0.1, to yield the final product as an off white powder (38 mg, 0.081 mmol, 75 %)

M.p. 153-154 °C; FT-IR (ATR) V_{max}/cm^{-1} 2948, 2833, 1713, 1655, 1445, 1035, 985. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (dt, 1H, *J* = 15.6, 6.9 Hz), 5.83 (dt, 1H, *J* = 15.6, 1.6 Hz), 4.20 (q, 2H, *J* = 7.1 Hz), 3.26 (dd, 1H, *J* = 11.7, 4.5 Hz), 2.05 (td, 5H, *J* = 12.1, 11.5, 3.6 Hz), 1.86 – 1.12 (m, 20H), 1.07 (dd, 1H, *J* = 12.7, 2.2 Hz), 1.01 (d, *J* = 9.9 Hz, 6H), 0.94 (d, 3H, *J* = 6.2 Hz), 0.90 (d, 3H, *J* = 9 Hz), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 166.86 (C), 150.02 (CH), 134.45 (C), 134.34 (C), 120.99 (CH), 79.00 (CH), 60.13 (CH_2)z, 50.41 (CH), 50.35 (CH), 49.82 (C), 44.55 (C), 38.91 (C), 37.04 (C), 36.17

(CH), 35.60 (CH_2), 34.56 (CH_2), 31.00 (CH_2), 30.82 (CH_2), 29.23 (CH_2), 28.18 (CH_2), 27.98 (CH_3), 27.86 (CH_2), 26.51 (CH_2), 24.26 (CH_3), 21.00 (CH_2), 19.16 (CH_3), 18.48 (CH_3), 18.26 (CH_2), 15.79 (CH_3), 15.43 (CH_3), 14.30 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 493.3668 required $C_{31}H_{50}NaO_3$ 493.3652.

6.56. Synthesis of ethyl (6R,E)-6-((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-3-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate 139

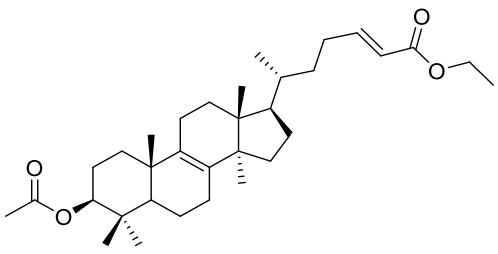


To a solution of **138** (70 mg, 0.149 mmol) in DCM (5.9 mL) and H_2O (3.7 µL, 0.04 mmol) Dess-Martin periodinane (75 mg, 0.177 mmol) was added. Reaction stirred for 16 h as white suspension at room temperature. The solvent was removed under reduce pressure. The residue dissolved in 20 mL EtOAc, the crude washed by $Na_2S_2O_3$:$NaHCO_3$ (sat. aq. sol.) 1:1 (1 X 20 mL), brine (1 X 10 mL). The aqueous phase was extracted with EtOAc (5 X 25 mL), dried by Na_2SO_4, filtrate and the solvent removed under reduce pressure to afford an off-white solid. The crude residue purified by column chromatography using

petroleum ether 40/60: ethyl acetate (92:8) R_f = 0.2, affording **139** (35 mg, 0.074 mmol, 50 %) as an off white solid.

M.p. 93-96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (dt, 1H, J = 15.6, 6.9 Hz), 5.83 (dt, 1H, J = 15.6, 1.6 Hz), 4.20 (q, 2H, J = 7.1 Hz), 2.61 (ddd, 1H, J = 15.7, 11.2, 7.1 Hz), 2.48 – 2.24 (m, 2H), 2.20 – 1.15 (m, 23H), 1.14 (s, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 0.94 (d, 3H, J = 6.2 Hz), 0.91 (d, 3H, J = 0.9 Hz), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 217.86 (C), 166.84 (C), 149.94 (CH), 135.28 (C), 133.19 (C), 121.03 (CH), 60.14 (CH_2), 51.23 (CH), 50.36 (CH), 49.93 (C), 47.41 (C), 44.54 (C), 36.92 (C), 36.15 (CH), 36.07 (CH_2), 34.63 (CH_2), 34.53 (CH_2), 30.96 (CH_2), 30.89 (CH_2), 29.22 (CH_2), 28.14 (CH_2), 26.35 (CH_2), 26.21 (CH_3), 24.31 (CH_3), 21.31 (CH_3), 21.08 (CH_2), 19.45 (CH_2), 18.70 (CH_3), 18.47 (CH_3), 15.89 (CH_3), 14.30 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 469.3659 required $C_{31}H_{49}O_3$ 469.3676.

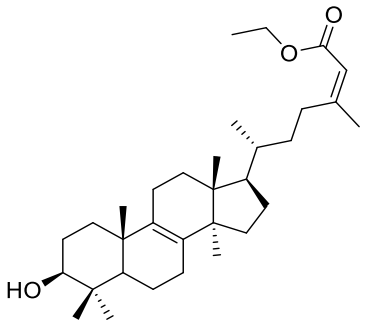
6.57. Synthesis of ethyl (6R,E)-6-((3S,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate 140



To a solution of aldehyde **42** (200 mg, 0.45 mmol) in THF (1.8 mL), (ethoxycarbonylmethyl)triphenylphosphorane (400 mg, 1.148 mmol) was added. Reaction was stirred for 16 h at room temperature. The was solvent removed under reduced pressure. The crude was then purified using column chromatography petroleum ether 40/60: ethyl acetate (92:8) R_f = 0.4, to yield the final product as an off white powder (193 mg, 0.376 mmol, 80 %)

M.p. 96-99 °C; FT-IR (ATR) V_{max}/cm^{-1} 2943, 2874, 1727, 1714, 1206, 1035, 983. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (dt, 1H, *J* = 15.7, 6.9 Hz), 5.83 (dt, 1H, *J* = 15.6, 1.6 Hz), 4.52 (dd, 1H, *J* = 11.7, 4.5 Hz), 4.20 (q, 2H, *J* = 7.2 Hz), 2.38 – 1.89 (m, 10H), 1.81 – 1.05 (m, 18H), 1.02 (s, 3H), 0.93 (d, 3H, *J* = 6.3 Hz), 0.90 (s, 7H), 0.89 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.05 (C), 166.86 (C), 150.02 (CH), 134.41 (C), 134.29 (C), 120.99 (CH), 80.94 (CH), 60.13 (CH_2), 50.50 (CH), 50.34 (CH), 49.82 (C), 44.54 (C), 37.81 (C), 36.90 (C), 36.16 (CH), 35.27 (CH, 34.54 (CH_2), 30.97 (CH_2), 30.79 (CH_2), 29.22 (CH_2), 28.17 (CH_2), 27.92 (CH_3), 26.38 (CH_2), 24.25 (CH_3), 24.18 (CH_2), 21.35 (CH_3), 20.99 (CH_2), 19.20 (CH_3), 18.46 (CH_3), 18.12 (CH_2), 16.54 (CH_3), 15.79 (CH_3), 14.30 (CH_3). HRMS m/z (ESI+) found $[M+Na]^+$ 535.3745 required $C_{33}H_{52}NaO_4$ 535.3758.

6.58. Synthesis of ethyl (6R,Z)-6-((3S,10S,13R,14R,17R)-3-hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-3-methylhept-2-enoate 141



To a solution of aldehyde **42** (200 mg, 0.5 mmol) in THF (1.74 mL), (carbethoxyethylidene)triphenylphosphorane (283 mg, 0.78 mmol) was added. Reaction was stirred for 16 h at room temperature. The solvent was removed under reduced pressure. The crude was then purified using column chromatography petroleum ether 40/60: ethyl acetate (8:2) R_f = 0.3, to yield the final product as an off white powder (154 mg, 63 %).

M.p. 123-126 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 – 6.71 (m, 1H), 4.21 (q, 2H, *J* = 7.1 Hz), 3.85 – 3.71 (m, 1H), 3.26 (dd, 1H, *J* = 11.6, 4.5 Hz), 2.33 – 2.00 (m, 6H), 2.00 – 1.86 (m, 2H), 1.85 (d, 3H, *J* = 1.4 Hz), 1.80 – 1.05 (m, 17H), 1.02 (s, 3H), 1.00 (s, 3H), 0.96 (d, 3H, *J* = 6.3 Hz), 0.91 – 0.89 (m, 3H), 0.83 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 168.38 (C), 142.91 (CH), 134.45 (C), 134.35 (C), 127.40 (C), 78.99 (CH), 60.38 (CH_2), 50.41 (CH), 50.31 (CH), 49.83 (C), 44.54 (C), 38.91 (C), 37.04 (C), 36.39 (CH), 35.60 (CH_2), 34.98

(CH_2), 31.00 (CH_2), 30.84 (CH_2), 28.21 (CH_2), 27.98 (CH_3), 27.86 (CH_2), 26.51 (CH_2), 25.66 (CH_2), 24.26 (CH_3), 21.01 (CH_2), 19.16 (CH_3), 18.55 (CH_3), 18.26 (CH_2), 15.78 (CH_3), 15.43 (CH_3), 14.32 (CH_3), 12.31 (CH_3). HRMS m/z (ESI+) found $[M+H]^+$ 485.3970 required $C_{32}H_{53}O_3$ 485.3989.

7. Biological methods

7.1. Cell maintenance:

- T75 flasks were used to grow the cells, with final media volume 10-12 mL.
- Splitting: 1:3; 1:4; 1:5 depending on the experiments.
- Media used :
    - RPMI (Sigma R0883) +10% FBS (Sigma F7524) + 1% l-glutamine (Sigma T3924) for PC3 cell lines.
    - DMEM (Sigma D5671)+10% FBS (Sigma F7524) + 1% l-glutamine (Sigma T3924) for DU145 cell lines.
- HBS (Sigma H8264) 5 mL was used to remove serum in order to detach cells.
- Trypsin (Sigma T3924) 5 mL was used to to detach cells 5-15 min incubation 37 °C, 5% CO_2.
- 5 mL media added to the detached cells to neutralise trypsin, final volume 10 mL.
- 300 g; 5 min used to get the cells pellets, the supernatant dissposed and the pellets resuspended with fresh media to split new passage/ flask.

7.2. Cell Density:

- A plate seeded with (Control, 2, 3, 5, 6, 7.5, 10, 12.5, 15, 20) X 10^3 cell/ well 100 µl seeding day followed with 100 µl media next day. Contorl is media without cells.

- The plate allowed to grow for 4 days: day 0 seeding; day 1 media addition as treatment day followed by three days as 72h of incubation.
- A picture of the plate was taken by the brightfield microscope (Figure 37 and Figure 38).
- The best seeding density for PC3 was 3 X 10^3/ well, DU145 4 X 10^4.

7.3. Vehicle scanning:

- Different vehicle scanned in order to to check the tocicty on cells.
- Solvent used: DMSO; EtOH; MeOH; EtOAc; THF; Et_2O.
- The veicle used was: 1%, 3%, 6% and 10% in medium with positive and negative controls: media without cells, Cells without treatment respectively.
- The plates were treated in the next day after seeding.
- The treatment was for 72 h.
- MTT: 50 µl (2mg/ml) prepared with cell media used to stain the plates for (4h) after 72 h of treatment.
- The media with MTT was removed by pump aspirator, 75 µl DMSO/ well added to dissolve the formazan crystals.
- The plate absorbance recorded at 550 nm.

7.4. Cell counting:

Cell counting: 40 µl cell suspension + 40 µl trypan blue. Mixed in micro tube then 10 µl used to fill side of the hemocytometer. 10X magnification used in cell counting.

7.5. Cell freezing:

- Cryovials used to freeze a stock of cells in – 80 °C, using Mr. frosty.
- 10 % DMSO/ media used in freezing cells.
- Cells pellets suspended in the DMSO/ media, confluent flask cells stored in 1 or 2 vials.
- After suspension of the pellets, the vials was frozen imediately.

7.6. Cell Thawing:

- Vials took out from the freezer, and thawed in the water bath (maxium 1 min), then diluted with 11 mL media and spinned at 300 g for 5 min. The supernatent was dissposed and the pellets was diluted with fresh media then transferred into T75 flask with the same passage number on the vial.
- The media changed in the next day.
- Cells growing was monitered by microscope and media change or cells spliting performed dependence on the flask confluency.

7.7. Vehicle plate running conditions:

- The vehicle used to dissolve the componds is Ethyl Acetate Extra pure from *Fisher*, product no. 11418243.
- All concentration was perforemd in 6 replicates B-G/ column.
- The 36 edge wells were filled with 175 µl media to reduce the evaporation. A1-12; H1-12; B1-G1; B12-G12.

- The Control (Cont.) was media without cells 100 µl on seeding day followed with the addition of 100 µl on treatment day, final volume (200 µl).
- A vehicle plate is runned with each day of IC_{50} experments in order to compare the effect of the vehicle on cells from the same flask used for seeding.
- The maximium vehicle concentration used is 1% and the minimum is 0.0039 %.
- Vehicle concentrations were prepared in 1:2 dilution.
- The plates treated in the next day after seeding.
- Treatment was for 72 h.
- MTT: 50 µl (2mg/ml) preperd with cell media used to stain the plates for (4h) after 72 h of treatment.
- The media with MTT was removed by pump aspirator, 75 µl DMSO/ well added to dissolve the formazan crystals.
- The plate absorbance recorded at 550 nm.

7.8. Compound plate running conditions:

- The vehicle used to dissolve the componds is Ethyl Acetate **Extra pure** from *Fisher*, pro. no. 11418243.
- All concentration performed in 6 replicates B-G/column.
- The 36 edge wells were filled with 175 µl media to reduce the evaporation. A1-12; H1-12; B1-G1; B12-G12.

- The Control (Cont.) was media with nontreated cells, 100µl of cells in seeding day follwed with 100µl of media in treatment day, the final volume was (200µl).
- A vehicle plate is runned with each day of IC_{50} experments in order to compare the effect of the vehicle on cells from the same flask used for seeding.
- The maximium compound concentraion used is 200 µm and the minium is 0.78 µm.
- The compounds concentrations were prepared in 1:2 dilution.
- The maximium vehicle concentration used is 1% and the minimum is 0.0039%.
- The plates were treated in the next day after seeding.
- The treatment was for 72h.
- MTT: 50 µl (2mg/ml) preperd with cell media used to stain the plates for (4h) after 72h of treatment.
- The media with MTT was removed by pump aspirator, 75µl DMSO/ well added to dissolve the formazan crystals.
- The plate absorbance recorded at 550 nm.
- The experiments repeated two times for each compound/cell line.

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