University of Puerto Rico

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A. DESIGN, SYNTHESIS, AND ANTI-TUBERCULAR ACTIVITY OF LENINGOSTEROL AND ITS DERIVATIVES

B. DESIGN, SYNTHESIS, AND ANTI-INFECTIVE ACTIVITY OF ISOTHIOCYANATE- AND ISOSELENOCYANATE-FUNCTIONALIZED AMPHILECTANE DITERPENES
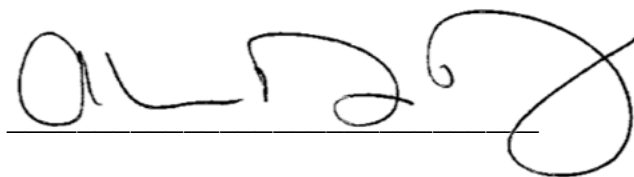
By

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A Thesis Submitted in Partial

Fulfillment of the Requirements

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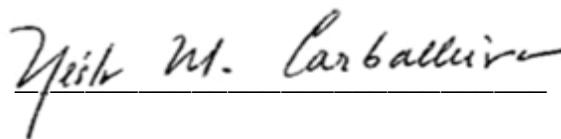
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*I dedicate this work
to my beloved parents,
María del R. Merced
and Carlos Nieves,
for their unconditional
love and support.*

ACKNOWLEDGMENTS

ABSTRACT

Saringosterol (**1.22**), a sterol isolated from the brown algae *Sargassum ringgoldianum* and *Lessonia nigrescens*, is a potent anti-tubercular agent with a minimum inhibitory concentration (MIC) of 0.25 µg/mL and low cytotoxicity towards the Vero cell line. However, its pharmacokinetic properties are hampered by its limited aqueous solubility. On the other hand, *abeo*-sterols, a sub-group of sterols, are also promising anti-tubercular agents. A small library of these compounds prepared by Rodríguez and co-workers suggests that the abeo-steroidal moiety is responsible for the activity. Based on these observations we designed a new molecule, named by us as leningosterol (**2.1**), which combines the structural features of *abeo*-sterols with those of saringosterol (**1.22**).

A synthetic strategy to access leningosterol (**2.1**) has been developed using 3β-hydroxy-5-cholenic acid as the starting material. A Weinreb amide, ozonolysis, aldol addition, and organometallic alkylations were the key steps during the synthesis. Leningosterol (**2.1**) was finally obtained in ten steps with an overall yield of 7%. Surprisingly, leningosterol (**2.1**) exhibited only a moderate MIC of 20 µg/mL against *Mycobacterium tuberculosis*.

Fifteen derivatives were synthesized and evaluated for anti-tubercular activity in this research. Structure activity relationship (SAR) studies were performed to determine the influence of same substituents at C-24 on the anti-tubercular potency. Furthermore, the aldehyde functionality at C-6 and the 5,7-

alkene moiety were modified as part of our efforts to optimize potency. MIC values ranged from 4.3 to >128 μg/mL with compound **3.14** being the most active.

In another project, the marine natural product (–)-8,15-diisocyano-11(20)-amphilectene, isolated from the Caribbean sponge *Svenzea flava*, was used as scaffold to synthetize five new products, all of which were tested *in vitro* against laboratory strains of *Plasmodium falciparum* and *Mycobacterium tuberculosis*. The scaffold along with its isothio- and isoselenocyanate analogs displayed low to sub-micro molar (0.0012–11.7669 μM) anti-plasmodial activity with the best derivative, diisoselenocyanate (**4.32**), showing an IC_{50} value of 0.0025 μM against a drug-sensitive strain 3D7. Of the compounds assayed against *M. tuberculosis* H_{37}Rv, compound **4.35** was found to be the most active, with a MIC of 2.1 μM.

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LIST OF ABBREVIATIONS

α	alpha
Am	Amikacin
Amx/Clv	Amoxicillin/clavulanate
ATP	Adenosine triphosphate
β	beta
CC	Column Chromatography
CDI	Carbonyldiimidazole
Cfz	Clofazimine
CHMP	Committee for Medicinal Products for Human Use
Clr	Clarithromycin
Cm	Capreomycin
^{13}C-NMR	Carbon Nuclear Magnetic Resonance
Cs	Cycloserine
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DR-TB	Drug Resistant Tuberculosis
DS-TB	Drug Susceptibility Tuberculosis
E	Ethanbutol
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimie

EMA	European Medicines Agency
Eto	Ethionamide
FDA	Food and Drug Administration
H	Isoniazid
HIV	Human Immunodeficiency Virus
^{1}H-NMR	Proton Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
Im	Imidazole
Ipm/cln	Imipenem/cilastatin
*i*PrLi	*iso*propyl lithium
Km	Kanamycin
Lfx	Levoflaxacin
log *P*	Partition Coefficient
LTBI	Latent Tuberculosis Infection
Lzd	Linezolid
M. africanum	*Mycobacterium africanum*
M. bovis	*Mycobacterium bovis*
M. canetti	*Mycobacterium canetti*
*m*CPBA	*meta*-Chloroperoxybenzoic acid
MDR-TB	Multidrug-Resistant Tuberculosis
Mfx	Moxiflozacin

MIC	Minimum Inhibitory Concentration
M. microti	*Mycobacterium microti*
MMPP	Magnesium Monoperoxypthalate
MPC	Manganese Phorphyn Complex
M. tuberculosis	*Mycobacterium tuberculosis*
NMO	*N*-Methyl-2-pyrrolidone
Ofx	Ofloxacin
ORTEP	Oak Ridge Thermal Ellipsoid Plot Program
PAS	*para*-aminosalicylic acid
PTC	Phase Transfer Catalyst
PPTS	Pyridinium *para*-toluene sulfonate
Pto	Prothionamide
pTSA	*para*-toluenesulfonic acid
R	Rifampicin
RNA	Ribonucleic acid
S	Streptomycin
SAR	Structure Activity Relationship
TB	Tuberculosis
TBS	*tert*-Butyldimethylsilyl
TBSCl	*tert*-Butyldimethylsilyl chloride
TEA	Triethylamine

TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thz	Thioacetazone
TMEDA	Tetramethylethylenediamine
Trd	Terizidone
TsCl	*para*-Toluenesulfonyl chloride
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis
Z	Pyrazinamide
μg/mL	micrograms/milliliters

STATEMENT OF THE PROBLEM

Tuberculosis and malaria are deadly diseases affecting the population worldwide, especially in Africa. The multi-drug resistance by these diseases generates an urgency for the discovery and/or development of new drugs. Among the most promising anti-tubercular agents are the sterols, such as saringosterol (**1.22**), reported by Timmerman and co-workers. Unfortunately, due to its low solubility and poor pharmacokinetic properties sterol **1.22** was not developed as a drug. Nonetheless, the work reported by Rodríguez and co-workers demonstrated that ring B abeo-sterols are potentially very promising anti-tubercular drugs. Another family of compounds having both anti-tubercular and anti-plasmodial properties is the amphilectane diterpenes. Since there are only a limited number of studies on the biological activity of both *abeo*-sterols and isothiocyanate-amphilectane diterpenes, we decided to design and synthesize several compounds to potentially treat tuberculosis and/or malaria.

Our hypothesis is that the conversion of saringosterol (**1.22**) to its ring B *abeo*-sterol analog will led to a powerful anti-tubercular agent. Also, the derivatization of (–)-8,15-diisocyano-11(20)-amphilectene (**4.30**) into its isothiocyanate- and isoselenocyanate-functionalized derivatives will enhance the anti-plasmodial and anti-mycobacterial properties of the amphilectane diterpenes, while decreasing its toxicity. In order to prove this hypothesis, we pursued the following specific aims:

1. *To synthesize and characterize leningosterol from 3β-hydroxyl-5-cholenic acid.*

2. *To synthesize and characterize leningosterol derivatives.*

3. *To perform biological assays to assess the anti-tubercular properties of leningosterol C_{24} epimers and its derivatives.*

4. *To synthesize and characterize isothiocyanate- and isoselenocyanate-functionalized amphilectane diterpenes from (–)-8,15-diisocyano-11(20)-amphilectene.*

5. *To perform biological assays to assess the anti-tubercular and anti-plasmodial properties of isothiocyanate- and isoselenocyanate-functionalized amphilectane diterpenes.*

A.   Design, Synthesis, and Anti-tubercular Activity of Leningosterol and its Derivatives

Chapter 1.　Sterols and *Abeo*-sterols as Promising Anti-tubercular Agents

1.1. Tuberculosis

1.1.1. Introduction

Tuberculosis (TB), an infectious disease caused mainly by the bacillus *Mycobacterium tuberculosis*, remains as a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV).[1] In 2014, 9.6 million new cases of TB emerged and 1.5 million people died from the disease including 400,000 people with HIV.[1] Currently, one third of the world's population is infected with the bacterium, with alarming rates of infection occurring every second.[2] People with weakened immune systems have a much greater risk of falling ill from the disease.

1.1.2. Causes and Symptoms

M. tuberculosis, a small, aerobic, nonmotile bacillus, is the main cause of TB.[3] Compared to other bacteria, which usually divide in less than an hour, it divides at an extremely low rate (every 16–20 hours).[4] Other known TB-causing mycobacteria are: *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti*.[5]

TB typically affects the lungs (pulmonary TB) but can affect other parts of the body such as the genitourinary tract, meninges, and bones (extrapulmonary TB).[6,7] It is an airborne bacterium, therefore it spreads from person to person through the air. When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets.[8] A single sneeze can release up to 40,000 droplets and only a few of these germs are needed to become

infected.[9] People infected with the bacterium have a lifetime risk of getting ill with TB of 10%.[10] However, persons with compromised immune systems, such as people living with HIV, malnutrition, or diabetes, have a much higher risk of falling ill. The symptoms when a person develops active TB include cough, fever, chills, night sweats, loss of appetite, weight loss, and fatigue.[8,11]

1.1.3. TB Classifications and Treatment

Tuberculosis can be classified in two phases, the latent and the active stage. The active stage is sub-divided in two groups: *1.* Drug-Susceptible Tuberculosis (DS-TB), *2.* Drug-Resistant Tuberculosis (DR-TB) which includes Multidrug-Resistant Tuberculosis (MDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB).[12] A brief description of the aforementioned groups is presented below.

1.1.3.1. Latent Tuberculosis Infection (LTBI)

Latent tuberculosis infection (LTBI) occurs when the body's immune system of a person that carries the TB bacterium keeps it under control and do not cause the disease.[13] The infected individuals do not feel sick, do not have any symptoms of TB, and cannot transmit the bacteria on to other people.[14] There is only a 10% lifetime chance that the latent infection will progress to overt active tuberculosis

disease.[15] This infection can be treated with isoniazid (H, **1.1**), rifampicin (R, **1.2**), and rifapentine (P, **1.3**) for several months (Figure 1.1).[16]

1.1.3.2. Drug-Susceptible Tuberculosis (DS-TB)

People with active TB disease show symptoms that may vary, as mentioned previously. They are usually treated with a standard six-months regimen of four first-line drugs which include isoniazid (H, **1.1**), rifampicin (R, **1.2**), ethambutol (E, **1.4**), and pyrazinamide (Z, **1.5**) (Figure 1.1).[17] Thus, this stage is called the drug-susceptible tuberculosis (DS-TB).
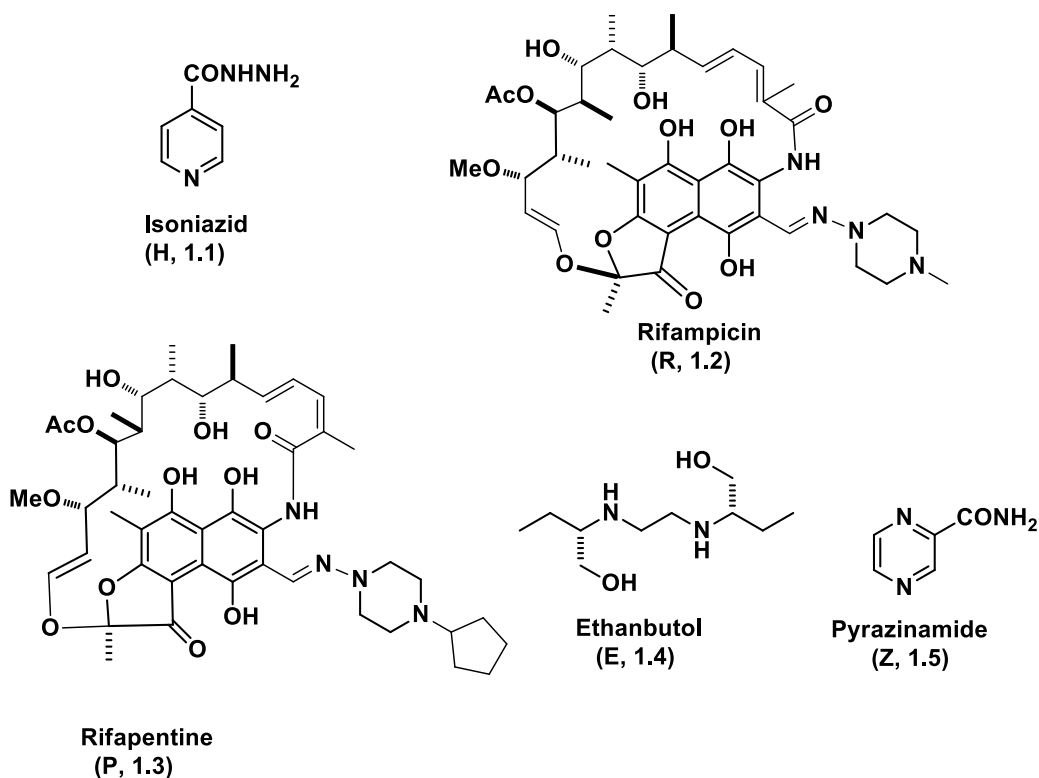
Figure 1.1 Molecular structures of anti-tubercular drugs used to treat LTBI and DS-TB.

According to the World Health Organization (WHO), treatment success rates of 90% or more for new cases are regularly reported.[18] Some side-effects of these drugs include rash, acute renal failure, nausea, orange-colored body fluids, and hepatitis.[19] The mechanism of action of these drugs is depicted in Figure 1.2. H (**1.1**) and E (**1.4**) inhibit the cell wall synthesis, while R (**1.2**) inhibits RNA synthesis. On the other hand, the exact target of Z (**1.5**) is unclear, but it is known that it disrupts the plasma membrane and energy metabolism.[19,20]
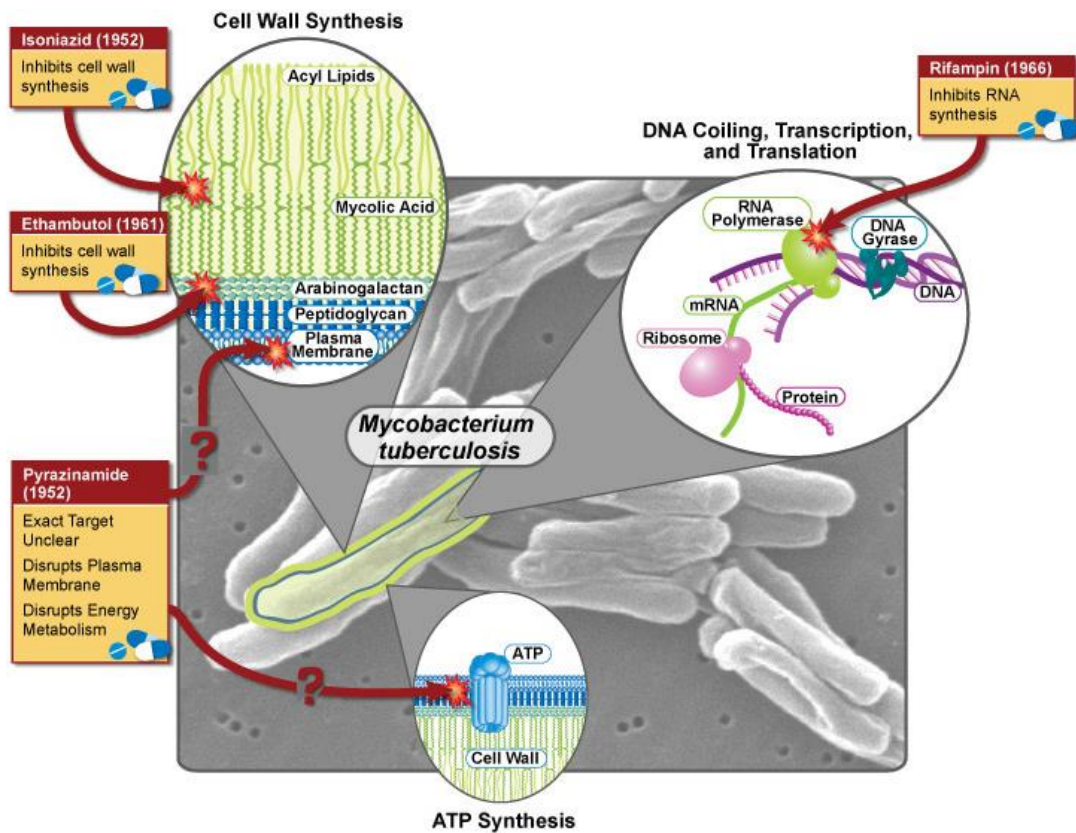
Figure 1.2 Mechanism of action of first-line anti-tubercular drugs used to treat DS-TB.[20]

1.1.3.3. Drug-Resistant Tuberculosis (DR-TB)

When TB bacteria become resistant to drugs, the person has developed drug-resistant tuberculosis (DR-TB). The main cause of this is the interruption, erratic, or improper use of antibiotics in chemotherapy of DS-TB patients.[21] DR-TB is divided into two groups, MDR-TB and XDR-TB, which are described in more detail below.

MDR-TB occurs when a *M. tuberculosis* strain is resistant to H (**1.1**) and R (**1.2**), the best antibiotics of the first-line drugs.[22] To treat and possibly cure MDR-TB, second-line drugs are used, but these may have more side effects, the treatment may last much longer, and the cost may be up to 100 times more than first-line therapy.[23] In 2014, about 480,000 incident cases of MDR-TB were reported and 190,000 deaths of this strain are estimated to have occurred.[1] The drugs used to treat MDR-TB are described in Table 1.1 and are classified by groups based on evidence of efficacy, potency, drug class, and experience of use. All the first-line TB drugs are in group 1, while groups 2–4 are considered second-line anti-TB drugs, and group 5 are the ones with limited clinical evidence or potentially limited efficacy.[24,25,26]

Table 1.1 <u>Group of drugs used to treat DR-TB</u>

Group	Drugs	Abbreviation
1: **First-line oral agents**	isoniazid	H
	rifampicin	R
	ethambutol	E
	pyrazinamide	Z
2: **Injectable agents**	kanamycin	Km
	amikacin	Am
	capreomycin	Cm
	streptomycin	S
3: **Fluoroquinolones**	levofloxacin	Lfx
	moxiflozacin	Mfx
	ofloxacin	Ofx
4: **Oral bacteriostatic second-line agents**	*para*-aminosalicylic acid	PAS
	cycloserine	Cs
	terizidone	Trd
	ethionamide	Eto

Group	Drugs	Abbreviation
	prothionamide	Pto
5: **Agents with an unclear role in the treatment of drug resistant-TB**	clofazimine	Cfz
	linezolid	Lzd
	amoxicillin/clavulanate	Amx/Clv
	thioacetazone	Thz
	imipenem/cilastatin	Ipm/Cln
	high-dosage isoniazid	high-dosage H
	clarithromycin	Clr

The mechanism of action of some of these drugs is depicted in Figure 1.3. From group 2, the aminoglycosides (Km, Am) and the cyclic peptide Cm inhibit protein synthesis in the bacterium.[23] The fluoroquinolones (group 3) inhibit the DNA Gyrase. From group 4, Cs and thioamides (Eto, Pto) inhibit the synthesis of the cell wall and PAS inhibits the synthesis of DNA precursors.
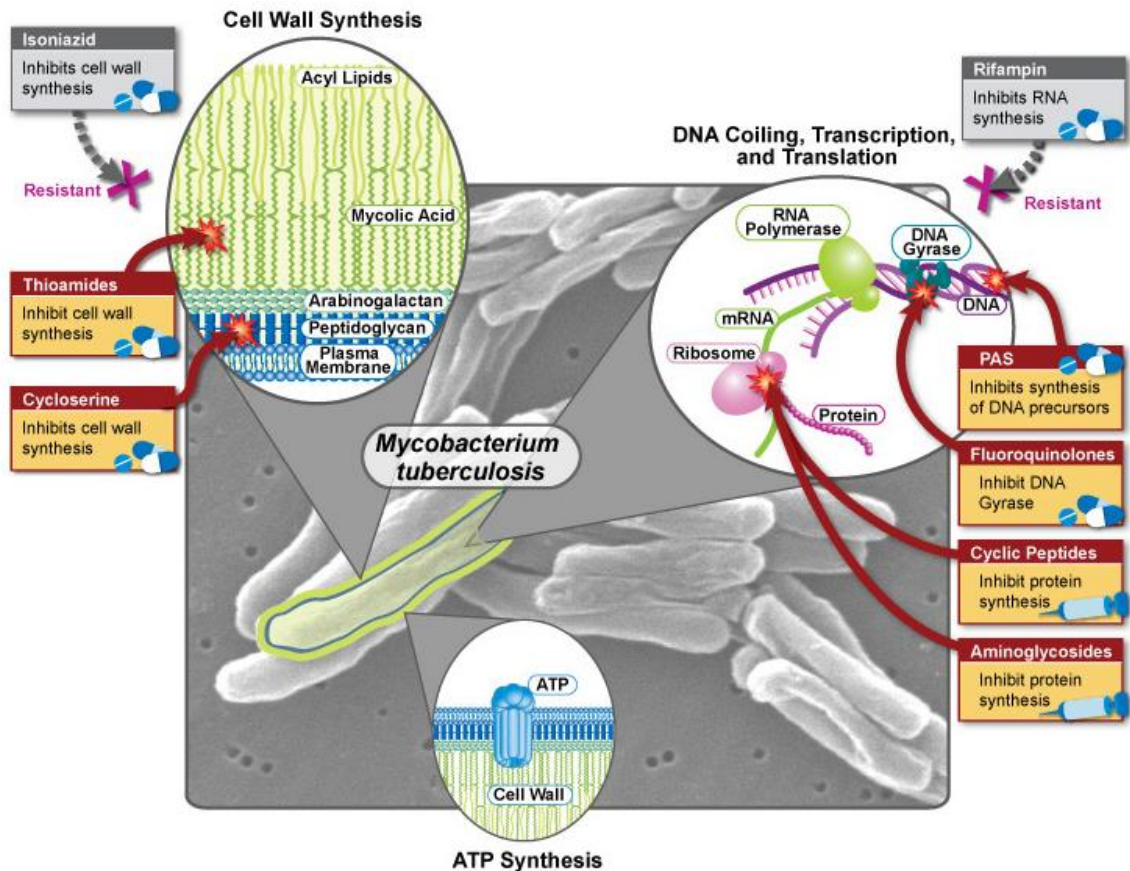
Figure 1.3 Mechanism of action of second-line anti-tubercular drugs used to treat MDR-TB.[23]

The worst case scenario occurs when the bacterium is resistant to the two most powerful first-line drugs (H and R), to any of the fluoroquines, and to at least one of three injectable second-line drugs (Km, Am, Cm). At this point, the person has developed XDR-TB which is extremely difficult to treat. The main cause for the development of XDR-TB is the misuse or mismanagement of second-line drugs. Patients with this disease can be cured, but it depends on the extent of the drug resistance, the severity of the disease, and whether the patient's immune system is compromised or not. XDR-TB patients infected with HIV may have a higher mortality. By 2015, 105 countries had reported at least one XDR-TB case.[1]

The fifth group of TB drugs includes some drugs whose effectiveness is not known. They are not recommended by WHO to treat DR-TB, but for patients with XDR-TB they can be used with consultation of an expert in the area.[26]

1.1.4. New TB Drugs Under Development

Currently, there are eight compounds in various stages of clinical development for TB.[1] Noteworthy is TBA-354, the first drug in six years to enter Phase I clinical trial (Table 1.2).

Table 1.2 <u>**List of TB drugs currently under development**</u>

Clinical Development		
Phase I	**Phase II**	**Phase III**
TBA-354	Sutezolid (PNU-100480)	Bedaquiline (TMC-207)
	SQ-109	Delamanid (OPC-67683)
	AZD5847	Rifapentine
	Rifapentine	Petromanid
	Bedaquiline	

Bedaquiline (TMC-207)

For the first time in over forty years, a new TB drug with a novel mechanism of action is available, Bedaquiline (**1.6**).[18] It is a diarylquinoline with a Minimum Inhibitory Concentration (MIC) ranging from 0.002 to 0.06 μg/mL, which was discovered by a team led by Koen Andries at Janssen Pharmaceutica with the trade name Sirturo™.[27,28] It was approved on December 2012 by the US Food and Drug Administration (FDA) as part of combination therapy to treat adults with MDR-TB, when no other alternatives are available.[29] In November 2013, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) recommended granting a conditional marketing authorization for bedaquiline with the same indication, which was formally approved in April 2014. Currently, it is in Phase III trials to investigate its safety and efficacy when used in combination with short MDR-TB regimes of nine and six months durations, respectively.[1] This is the first anti-tubercular drug to interfere with bacterial energy metabolism – it inhibits mycobacterial ATP synthase.[28,30]
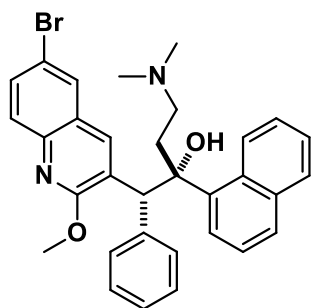
Bedaquiline (1.6)
MIC = 0.002-0.06 μg/mL
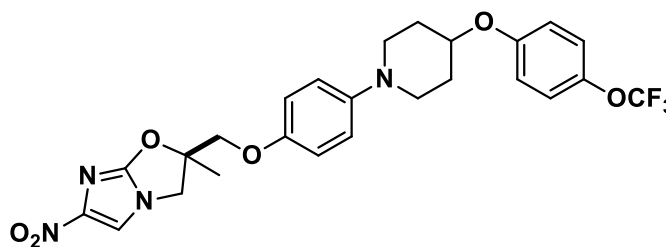
Delamanid (1.7)
MIC = 0.012 μg/mL

Figure 1.4 Molecular structures and MIC's of bedaquiline (**1.6**) and delamanid (**1.7**).

Delamanid (OPC-67683)

The second TB drug for the treatment of MDR-TB discovered in over 40 years is a member of the nitroimidazo-oxazole family, Delamanid (**1.7**). It exhibited a MIC of 0.012 μm/mL and was developed by the Otsuka pharmaceutical company, which has started a phase III trial.[31] In November 2013, the CHMP-EMA recommended the granting of a conditional marketing authorization for Delamanid (**1.7**) for use as part of an appropriate combination regimen for pulmonary MDR-TB in adult patients "when an effective treatment regimen cannot otherwise be composed for reasons of resistance or tolerability".[18] It works by blocking the synthesis of mycolic acids in *M. tuberculosis*, thus destabilizing its cell wall.[31,32]

Other New TB Drugs Under Development

SQ-109 is undergoing development for the treatment of DS-TB and DR-TB that unfortunately has not shown a benefit over E (**1.4**) in standard therapy for DS-TB.[18] On the other hand, Petromanid is still being tested for potential combination of regimen for the treatment of DS-TB. AZD5847 and Sutezolid have been in clinical trials for years, but have not progressed in the last two years.[1]

Existing Drugs Redeveloped or Repurposed for TB

Rifapentine (**1.3**), a semi synthetic form of rifamycin, is attractive as a possible TB drug for shortening treatment and for intermittent TB drug treatment.

Clinical trials are currently evaluating a daily high-dose of rifapentine for less than six months.[33]

The urgency for new anti-tubercular drugs is to improve the current treatment by shortening its total duration, to improve the DR-TB treatment, and to provide a more effective treatment for the LTBI.[34] The complex pathobiology of *M. tuberculosis*, which allows the bacterium to persist in a dormant stage for years, combined with the current lack of understanding about the mechanisms of mycobacterial metabolism in the vegetative state and site of infection, renders treatment difficult.

Scientists for many decades have been seeking and/or designing new drugs to treat TB; however, this has proved to be a challenge since only two drugs, bedaquiline (**1.6**) and delamanid (**1.7**), have been recently developed after forty years. Arguably, sterols and abeo-sterols should be considered also as potential candidates.

1.2. Anti-tubercular Sterols and *Abeo*-sterols

1.2.1. Sterols

Many sterols, a sub-group of steroids that have a hydroxyl group at C-3 of the A-ring and four fused rings in a 6-6-6-5 fashion, are promising anti-tubercular agents (Figure 1.5).
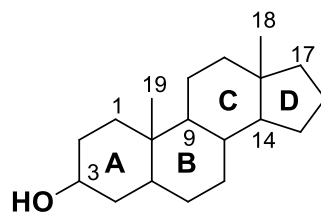
Figure 1.5 Sterol skeleton and numbering system.

Among the natural sterols with strong anti-tubercular activity are litosterol (**1.8**) and nephalsterol C (**1.9**), isolated from the Red Sea coral *Nephthea* sp. (Figure 1.6).[35,36] They exhibited MIC values of 3.13 and 12.5 µg/mL, respectively. Campesta-5,7,22-trien-3β-ol (**1.10**) was isolated from the plant *Morinda citrifolia* and was observed to undergo autoxidation to the endoperoxide **1.11**, which exhibited a MIC of 2.5 µg/mL.[37,38] A 2:1 mixture of ketosteroids **1.12** and **1.13** were isolated from the same plant with a MIC of 2.0 µg/mL.[38] From the plant *Sapium haematospermun* was isolated cycloartanol (**1.14**) which exhibited a MIC of 8.0 µg/mL.[39] Ergosterol-5,8-endoperoxide (**1.15**), isolated from the plant *Ajuga remota,* exhibited a MIC value of 1.0 µg/mL, while the synthetic acetylated derivative (**1.16**) was less potent with a MIC of 8.0 µg/mL using the BACTEC 460 system.[40]

Sterol **1.15** was also found in the plant *Radermachera boniana* and in the Argentinian plant *Ruprechtia triflora* with similar MIC values.[41,42] From *R. triflora* were also isolated peroxides **1.17** and **1.18** and ketosteroid **1.19** with MIC values ranging from 2.0 – 4.0 µg/mL. From the plant pathogen *Stereum hirsutum* was isolated compound **1.20** along with the known epiodoxysterol **1.21**, with MIC values of 16 µg/mL.[43] Saringosterol (**1.22**), a 1:1 mixture of C-24 epimers,

originally isolated from the brown algae *Sargassum ringgoldianum*, was re-isolated from *Lessonia nigrescens* by Timmermann and collaborators.[44,45] The MIC value for the mixture of epimers was 0.25 µg/mL (the MIC value of rifampicin was determined as 0.25 µg/mL in this assay) with low cytotoxicity towards the Vero cell line, half maximal inhibitory concentration (IC$_{50}$) >128 µg/mL. Pure samples of the 24*R* and 24*S* epimers of saringosterol (**1.22**) were obtained by normal phase HPLC. The 24*R* isomer exhibited eight times the anti-tubercular potency (MIC = 0.125 µg/mL) of that of the 24*S* isomer (MIC = 1 µg/mL).
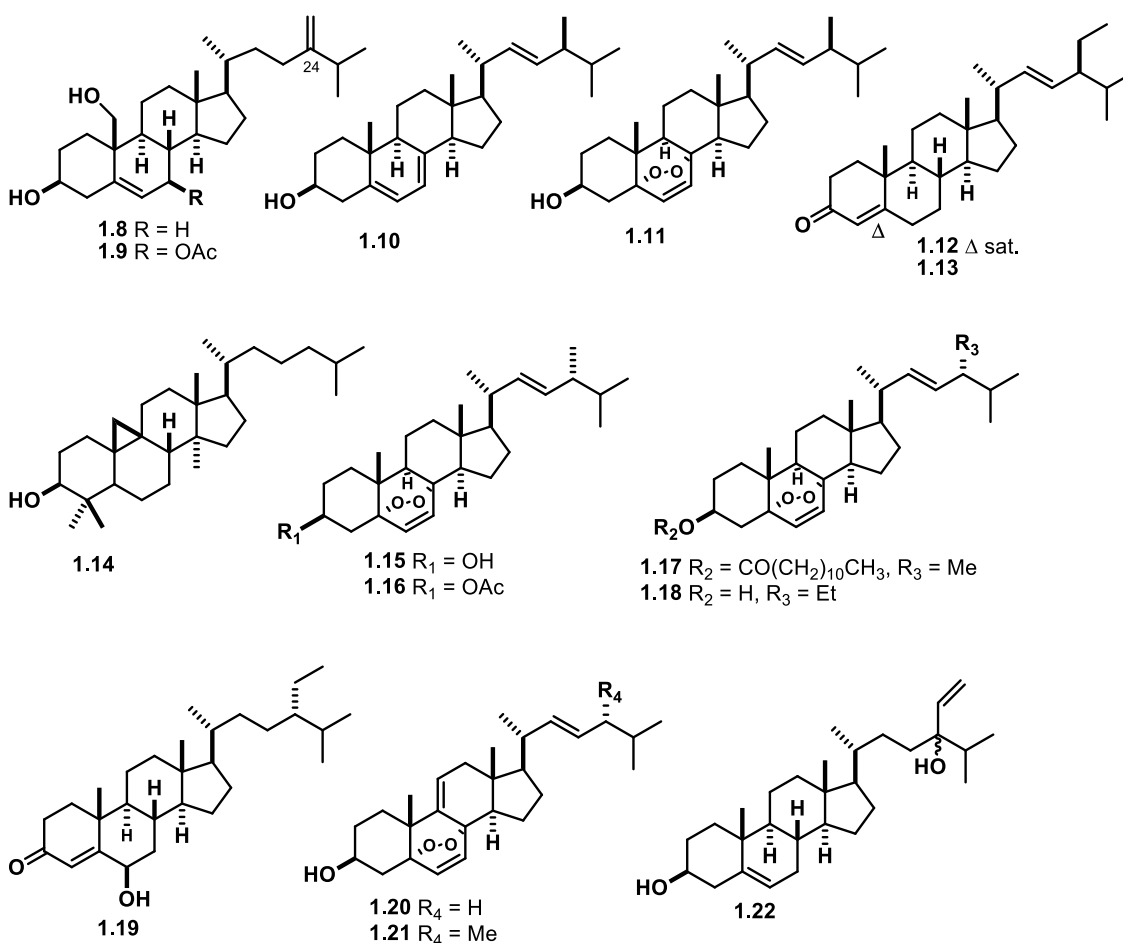
Figure 1.6 Molecular structures of selected sterols with strong anti-tubercular properties.

From these sterols, valuable structure-activity relationship (SAR) information can be obtained. For instance, acetylation of the hydroxyl group at C-3 reduces the anti-tubercular activity of sterols, such as **1.9** and **1.16**, suggesting that a free hydroxyl group at C-3 is a requirement for anti-tubercular activity. Ketosteroids such as **1.19**, wherein the hydroxyl group at C-3 appears to be oxidized, seem to be active. In general, methyl and ethyl groups at C-24 appear to be a common feature among active sterols. Saringosterol (**1.22**), on the other hand, is structurally similar to cholesterol (**1.23**), a commercially available sterol with no anti-tubercular activity, the only structural difference being the presence of a hydroxyl and vinyl groups at C-24. The remarkable anti-tubercular activity of **1.22** suggests that these groups are largely responsible for its activity. However, due to limited aqueous solubility, which severely hampers its pharmacokinetic properties, saringosterol (**1.22**) is not a drug *per se*.[35]

Saringosterol (1.22)   Cholesterol (1.23)
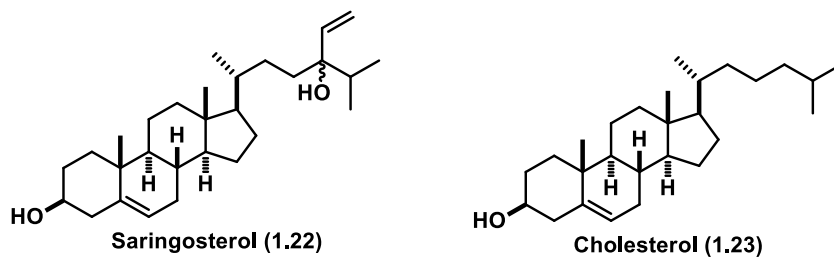
Figure 1.7 Comparison of the molecular structures of saringosterol (**1.22**) and cholesterol (**1.23**).

1.2.2. *Abeo*-sterols

A sub-group of sterols with promising anti-tubercular activity are the *abeo*-sterols. They comprise a group of sterols with four fused rings in a 6-5-6-5 fashion

as shown in Figure 1.8. The prefix *abeo* is used when a compound is considered to arise from a steroid by bond migration. The nomenclature is compiled as follows according to IUPAC: a numeral denoting the stationary end of the migrating bond (x) is followed by parenthesis enclosing the number denoting the original position (y), from which the other end of this bond has migrated, an arrow, and the number (z) denoting the new position to which the bond has moved. During the last ten years our research has focused on 5(6→7) *abeo*-sterols (*vide infra*).
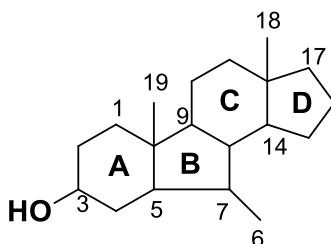
Figure 1.8 Abeo-sterol skeleton and numbering system.

In 2007, Rodríguez *et al.* reported two novel ring-B *abeo*-sterols as inhibitors of *M. tuberculosis* from the Caribbean Sea sponge *Svenzea zeai*.[46.47] The novel 5(6→7) *abeo*-sterols, which were named parguesterol A (**1.24**) and parguesterol B (**1.25**), exhibited MIC values for anti-tubercular activity of 7.8 and 11.2 µg/mL, respectively (Figure 1.9). Furthermore, from the same sponge, they found large amounts of known sterol **1.26**, a plausible biosynthetic precursor to parguesterols A and B with marginal anti-tubercular activity (MIC = 120 µg/mL). This finding suggested that the increased anti-mycobacterial activity of **1.24** and **1.25** might be related to an increase in the hydrophilic impact and rigidity of the steroidal backbone caused by the ring-B contraction.
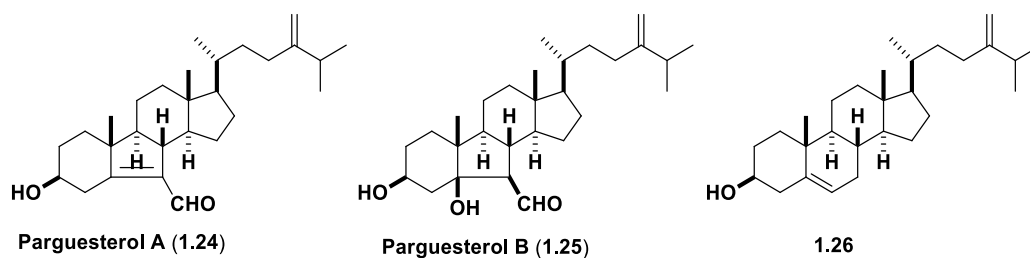
Figure 1.9 Molecular structures of sterols from the Caribbean Sea sponge *Svezea zeai*.

At the time, there were only a few examples of natural occurring ring-B *abeo*-sterols in the literature (Figure 1.10). They included six from terrestrial origin, taiwaniasterols A–D (**1.27** – **1.30**)[48] and hoodistanaloside A-B (**1.31** – **1.32**),[49] and one of marine origin, called orostanal (**1.33**).[50] The latter induces apoptosis in leukemia cell. However, the anti-tubercular activity of these naturally occurring metabolites was not assessed by the original investigators.
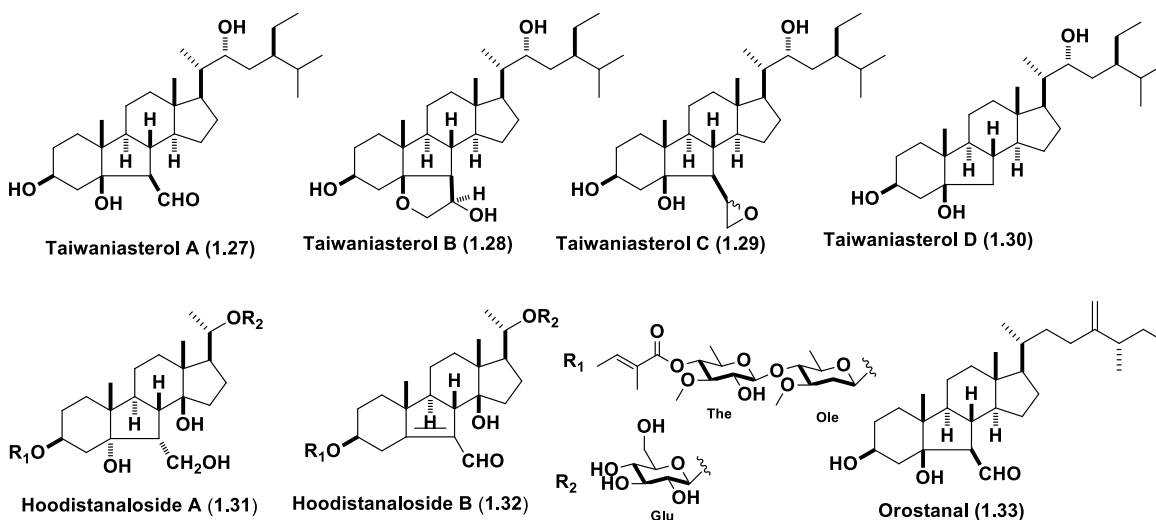
Figure 1.10 Molecular structures of natural occurring abeo-sterols.

1.3. Synthesis of *Abeo*-sterols

1.3.1. Synthesis of *Abeo*-cholesterol

During the determination of the absolute configuration of orostanal (**1.33**), the *abeo*-sterol analog of cholesterol was synthesized by Miyamoto *et al.* (Scheme 1.1).[50] KPN-2001 (**1.35**) was thus synthesized from the ozonolysis of cholesterol (**1.23**) followed by further aldol addition. Although the intention of this research group was to compare the circular dichroism (CD) spectrum of **1.33** with that of KPN-2001 (**1.35**) to determine its absolute configuration, this work represents the first attempt to synthesize the *abeo*-sterol framework.
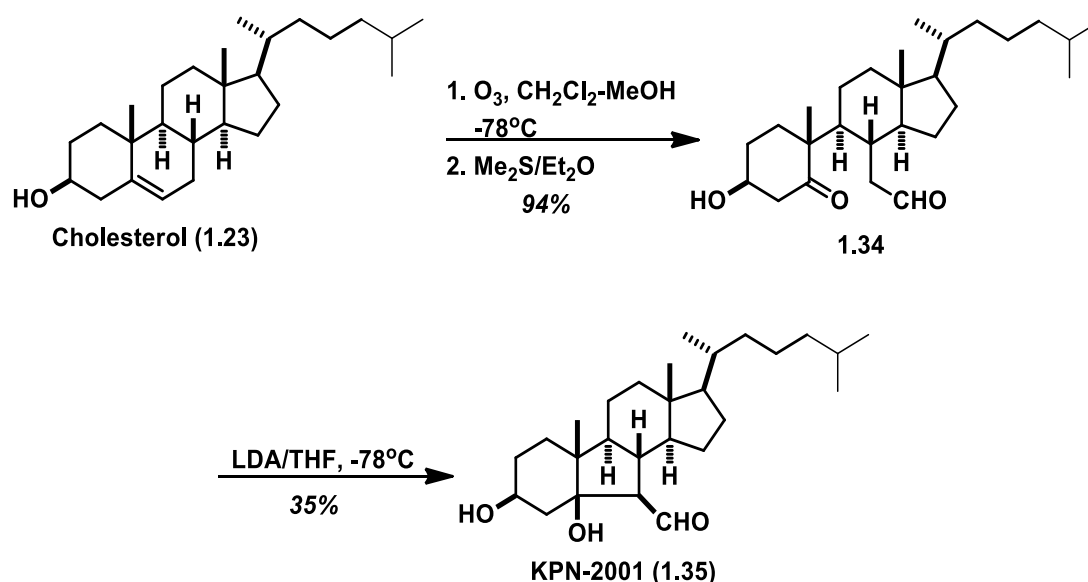
Scheme 1.1 Synthesis of abeo-cholesterol (KPN-2001, **1.35**).

1.3.2. Synthesis of Orostanal (1.33)

The first stereoselective synthesis of orostanal (**1.33**), reported by Liu and Zhou in 2002, has become the foundation for the synthesis of other *abeo*-sterols (Scheme 1.2).[51,52] The synthesis commences with methyl ester **1.37**, which was prepared from hydroxycholic acid methyl ester **1.36** in two steps following the procedure described by Bharucha *et al.*[53] Ozonolysis of **1.37** in 90% yield followed by the aldol addition of the generated keto-aldehyde **1.38** with neutral alumina, afforded *abeo*-sterol **1.39** in 83% yield. These reaction conditions are superior to the ones reported by Miyamoto *et al.* in the synthesis of KPN-2001 (**1.35**).[50] Liu and Zhou found that compound **1.38** was unstable and could slowly self-condensate during storage, thus two consecutive protections were performed. Aldehyde **1.39** was protected as the dimethyl acetyl by treatment with trimethyl orthoformate in absolute methanol catalyzed by NH_4Cl, followed by protection of the secondary alcohol **1.40** with *tert*-butyldimethylsilyl chloride (TBSCl). Reduction of methyl ester **1.40** was performed with lithium borohydride ($LiBH_4$) in 85% yield, since lithium aluminumhydride ($LiAlH_4$) removed the protecting group. Then, compound **1.44** was attained from the Swern oxidation of alcohol **1.42** followed by a Roush allylation with crotyl boronate (-)-**1.43** as chiral auxiliary. Reduction of alkene **1.44** produced **1.45,** which was then oxidized under Swern conditions to yield ketone **1.46**. Finally, to avoid racemization of the adjacent stereogenic center, the Lombardo's reagent was used for the methylenation of the carbonyl group, followed by removal of the protective groups under mild conditions to obtain

orostanal (**1.33**). The synthesis was completed in 18 steps with an overall yield of

7%.
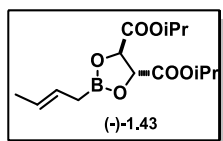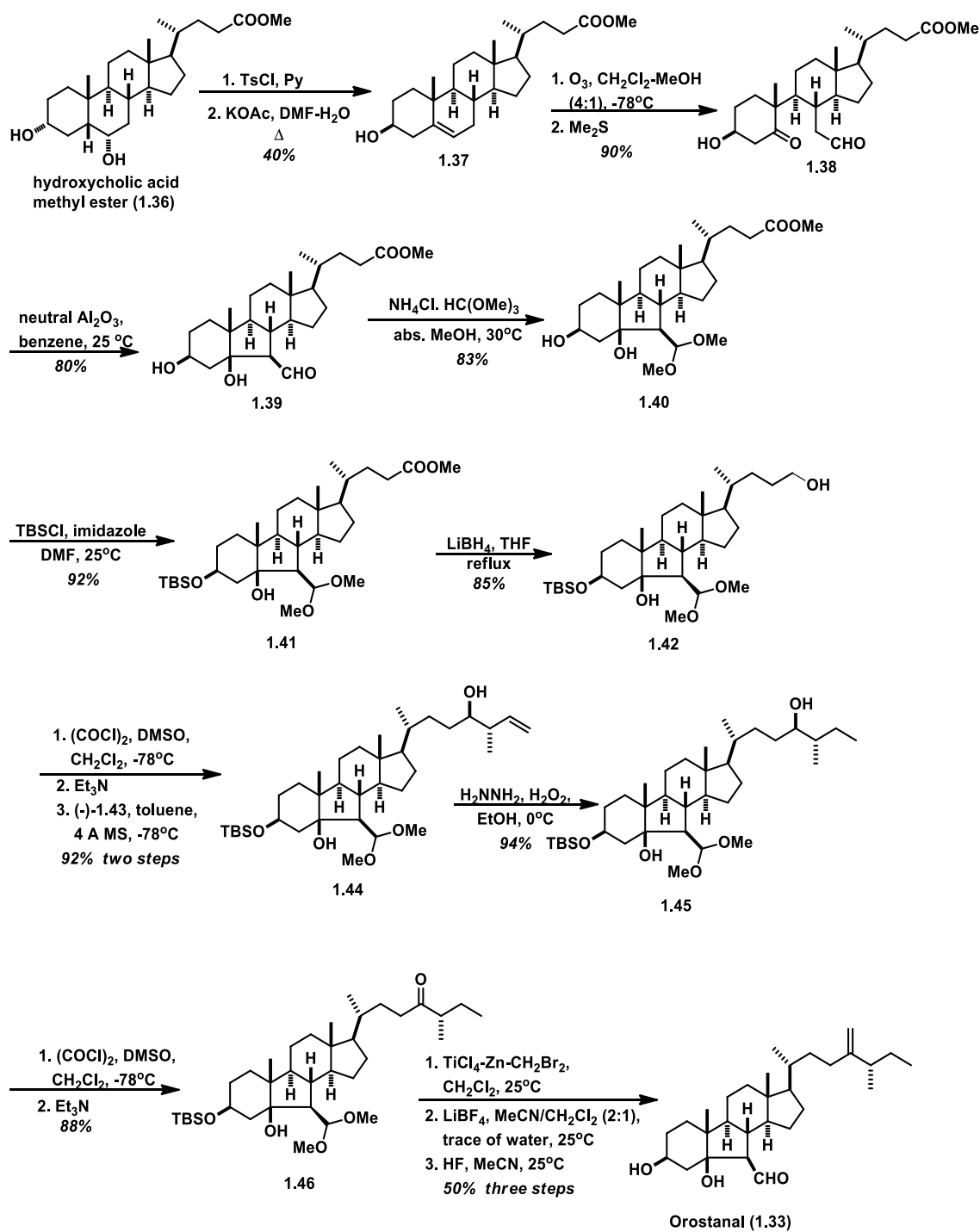
Scheme 1.2 Stereoselective synthesis of orostanal (**1.33**).

1.3.3. *Abeo*-sterols Library

The anti-tubercular activity of ring-B *abeo*-sterols has not been studied exhaustively. Nevertheless, based on the activity shown by parguesterol A and given the fact that no prior work has been conducted to explore the potential of 6-5-6-5 fused rings sterols as anti-tubercular agents, in 2008 Rodríguez and co-workers prepared a small library of 5(6→7) *abeo*-sterols and evaluated their *M. tuberculosis* inhibitory activity.[47] The abeo-sterol analogs were prepared in one-pot by reacting known 3β-hydroxy-Δ^5-cholestanes with ozone followed by an intramolecular aldol condensation in relatively low isolated yields (30% – 50%) (Scheme 1.3). Each of the starting 3β-hydroxy-Δ^5-cholestanes (**1.26**, **1.48**, **1.23**, **1.51**, and **1.53**) and all of the synthesized 5(6→7) *abeo*-sterols (**1.47**, **1.49**, **1.50**, **1.52**, and **1.54**) were screened for anti-mycobacterial activity. The activity results are shown in Table 1.3. The starting steroids exhibited MIC values > 120 µg/mL, and thus were considered inactive. They also lacked significant cytotoxicity (IC_{50}'s > 128 µg/mL) when screened against Vero cell lines. On the other hand, abeo-sterols showed MIC values ranging from 3.8 µg/mL to 15.0 µg/mL. Nonetheless, these analogs also exhibited increased cytotoxicity (IC_{50}'s 26.6 – 54.7 µg/mL) when compared to their respective precursors.

Noteworthy is the fact that even an inactive, commercially available sterol such as cholesterol (**1.23**) can be transformed into an active compound (**1.50**) after ring-B contraction. These data suggest that the 5(6→7) *abeo*-steroidal nucleus inherently enhances anti-mycobacterial activity. Also, the nature of the substituents at C-24 is an important structural feature that seems to influence

biological activity. Interestingly, ring-B *abeo*-sterols with a methyl or ethyl group at C-24 showed significantly higher activity than compound **1.47**, which has instead a carbonyl group in that position.

1. O₃, Et₂O or CH₂Cl₂, -78°C, 15 min
2. Me₂S, Et₂O, 25°C, 24h
3. 1.0 M KOH, MeOH, 25°C, 5 min

1.26 R =

1.47 R =

1.48 R =

1.49 R =

1.23 R =

1.50 R =

1.51 R =

1.52 R =

1.53 R =

1.54 R =
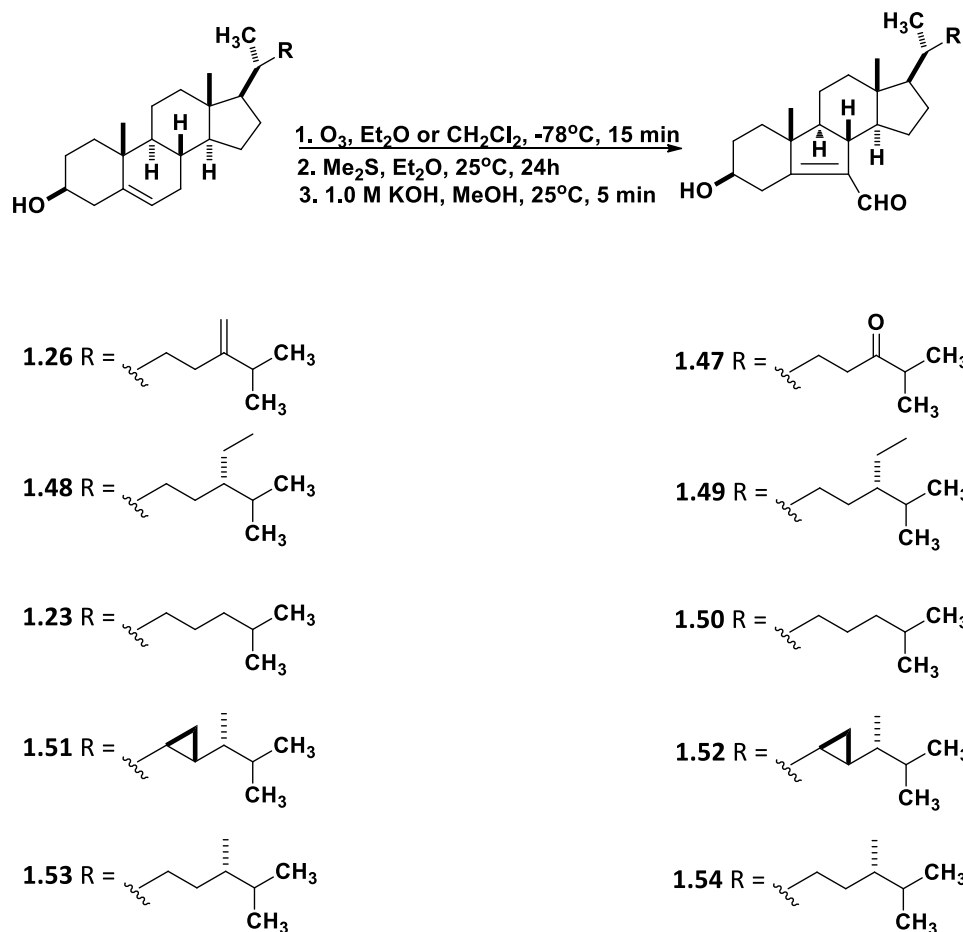
Scheme 1.3 Structures of the starting sterols and *abeo*-sterol analogs produced which were submitted for *in vitro* anti-mycobacterial and cytotoxicity screenings.

Table 1.3 __Anti-tubercular and cytotoxicity evaluation results of sterols 1.23,__
__1.26, and 1.47-1.54.__

Compound	MIC[a] (µg/mL)	IC$_{50}$[b] (µg/mL)
1.26	120.1	> 128.0
1.47	13.6	43.8
1.48	> 128.0	> 128.0
1.49	3.8	26.6
1.23	> 128.0	> 128.0
1.50	15.0	> 128.0
1.51	> 128.0	> 128.0
1.52	12.7	54.7
1.53	> 128.0	>128.0
1.54	3.9	42.3
RMP[c]	0.06	89.3

[a] Lowest drug concentration that effected an inhibition of ≥ 90% relative to untreated cultures.

[b] Cytotoxicity against VERO cells (ATCC CCL-81).

[c] Rifampicin was used as a positive control.

1.3.4. Synthesis of Parguesterol A and B

In 2009, the partial synthesis of anti-tubercular sterols Parguesterol A and B was depicted in the master's thesis of Ma (Scheme 1.4),[54] based on the synthesis of orostanal (**1.33**). Aldehyde **1.39** was protected as the dimethyl acetyl by treatment with trimethyl orthoformate in absolute methanol catalyzed by a 732 resin. Then, the protection of the secondary alcohol **1.40** with TBSCl attained

compound **1.41** in high yield. A Grignard reaction afforded isopropyl ketone **1.55,** which was further methylenated to generate compound **1.56**. A Wittig reaction or the Lombardo's reagent could be used for this transformation; however they reported higher yields with the first. Finally, the removal of the protecting groups in acid medium afforded parguesterol B (**1.25**) in 92% yield**.** Then, dehydration with methanolic-KOH was performed to obtain parguesterol A (**1.24**). The synthesis of parguesterol B (**1.25**) was completed in 11 steps with a total yield of 35%, while parguesterol A (**1.24**) was completed in 12 steps with a total yield of 29%.
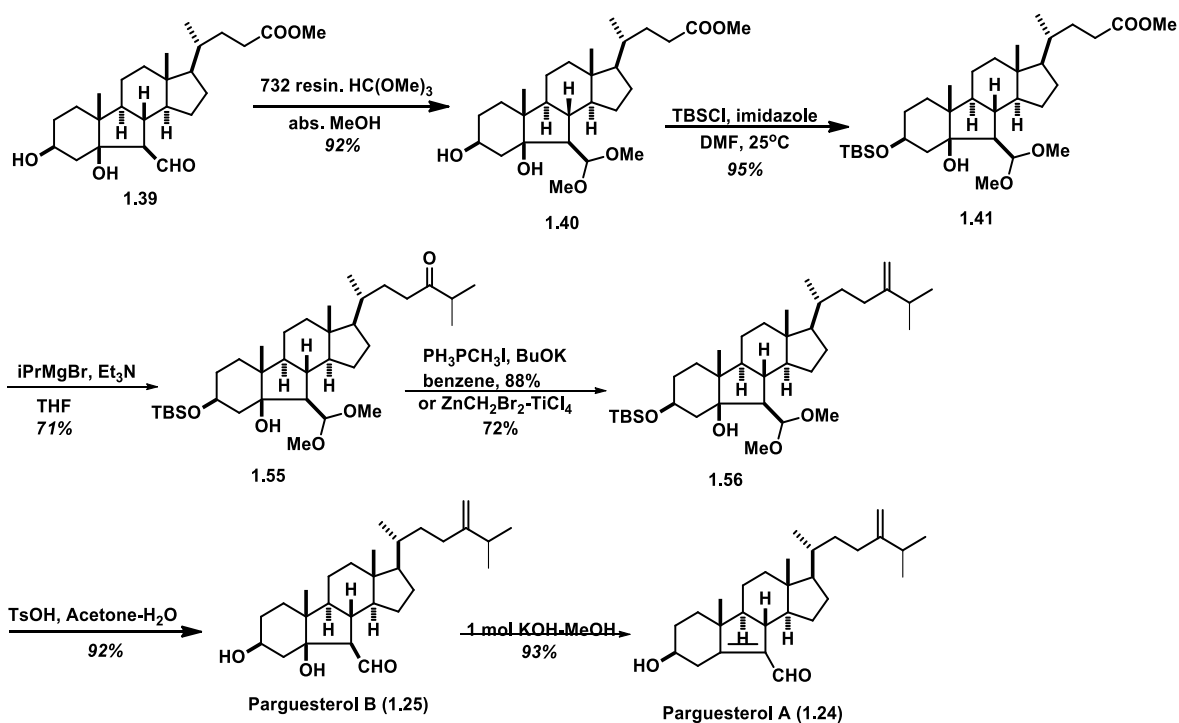
Scheme 1.4 Ma's partial synthesis of parguesterol A (**1.24**) and B (**1.25**).

1.3.1 Synthesis of Other *Abeo*-sterols

During the synthesis of parguesterol A and B, Ma also synthesized compounds **1.47** and **1.57**– **1.61** (Figure 1.11).
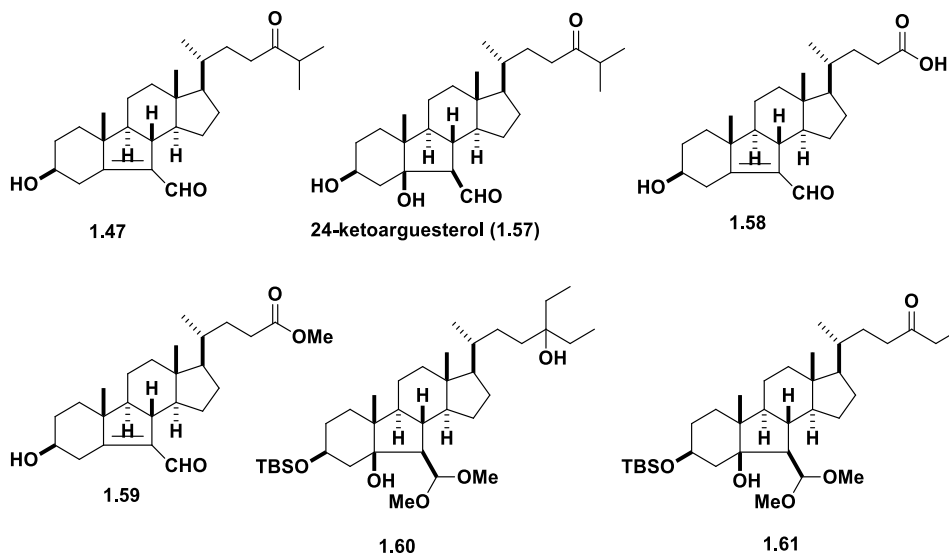
Figure 1.11 Molecular structures of other *abeo*-sterols.

As depicted in Scheme 1.5, 24-ketoarguesterol (**1.57**) can be generated from intermediate **1.55** after removal of the protecting groups in acid medium. Then, dehydration of **1.57** in methanolic KOH attained ketone **1.47**, which was previously synthesized from the naturally occurring sterol **1.26** by Rodríguez *et al.*[47]
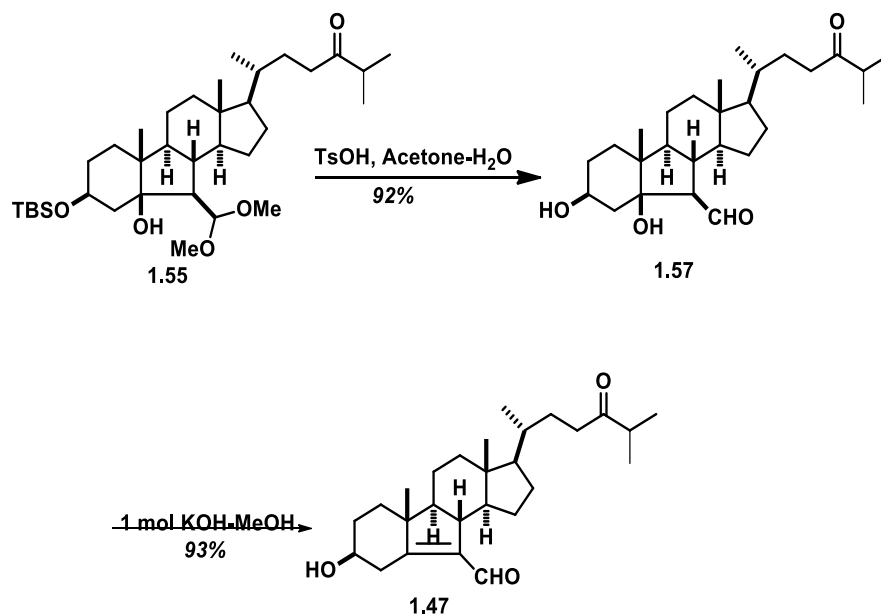
Scheme 1.5 Ma's synthesis of abeo-sterols **1.57** and **1.47**

Abeo-sterols **1.58** and **1.59** were obtained from the treatment of **1.39** with base (Scheme 1.6).
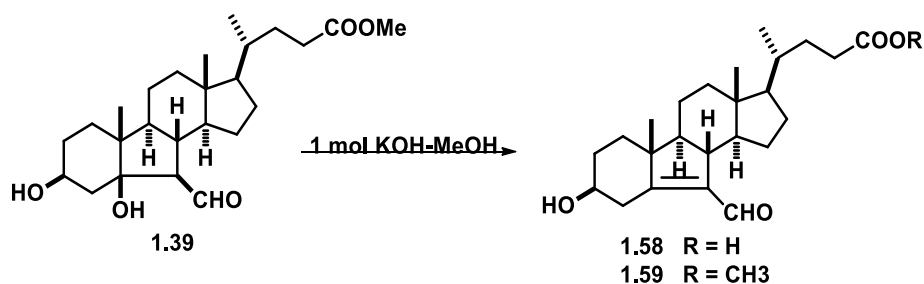
Scheme 1.6 Ma's synthesis of *abeo*-sterols **1.58** and **1.59**.

The alkylation of ester **1.41** with the Grignard reagent ethylmagnesium bromide generated compounds **1.60** and **1.61** in low yield (Scheme 1.7). At low temperature the mono-alkylation is favored, while refluxing leads mainly to the doubly alkylated product.
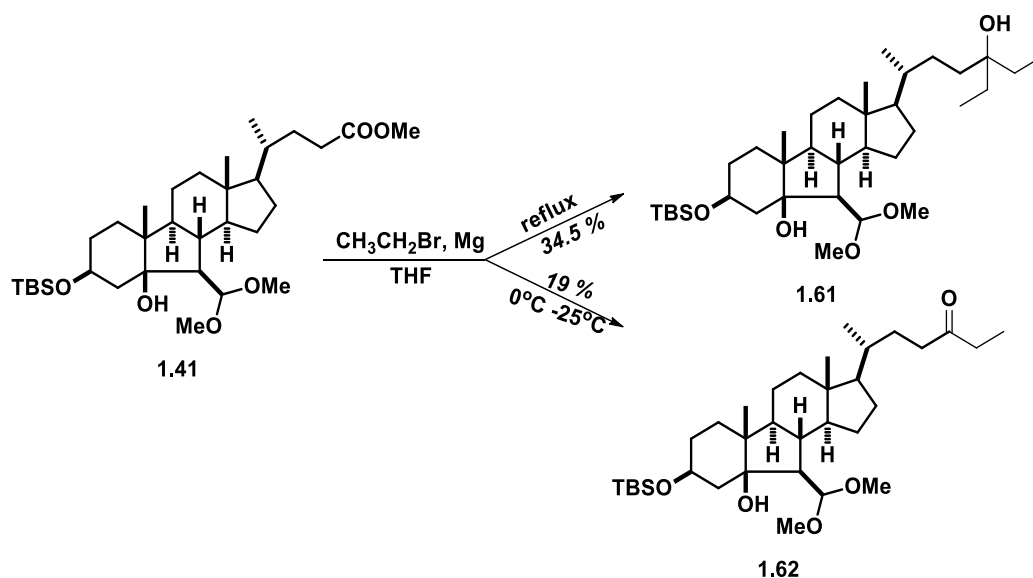
Scheme 1.7 Ma's synthesis of compounds **1.61** and **1.62**.

1.3.2 Synthesis of Orostanal Analogs

In 2012, inspired by the activity of orostanal (**1.33**) and KPN-2001 (**1.35**), Cui and co-workers synthesized a series of new steroidal derivatives in order to perform SAR studies.[55] The anti-proliferative activity was determined using HeLa (human cervical carcinoma), SMMC 7404 (human liver carcinoma), and MGC 7901 (human gastric carcinoma) cells.

Their initial aim was to determine if the aldehyde at C-6 was crucial for the anti-proliferative activity. For this reason they synthesized thiosemicarbazone (**1.67**) and triol (**1.68**) (Scheme 1.8). The synthesis commenced with the acetylation of cholesterol (**1.23**), followed by an oxidative cleavage with ozone to generate keto-aldehyde **1.64**. Aldol addition using neutral alumina in benzene afforded β-hydroxy aldehyde **1.65**. Then, reaction of **1.65** with thiosemicarbazide

and further deacetylation with methanolic KOH, attained compound **1.67**.
Compound **1.68** was obtained from the reduction of **1.65** with sodium borohydride.
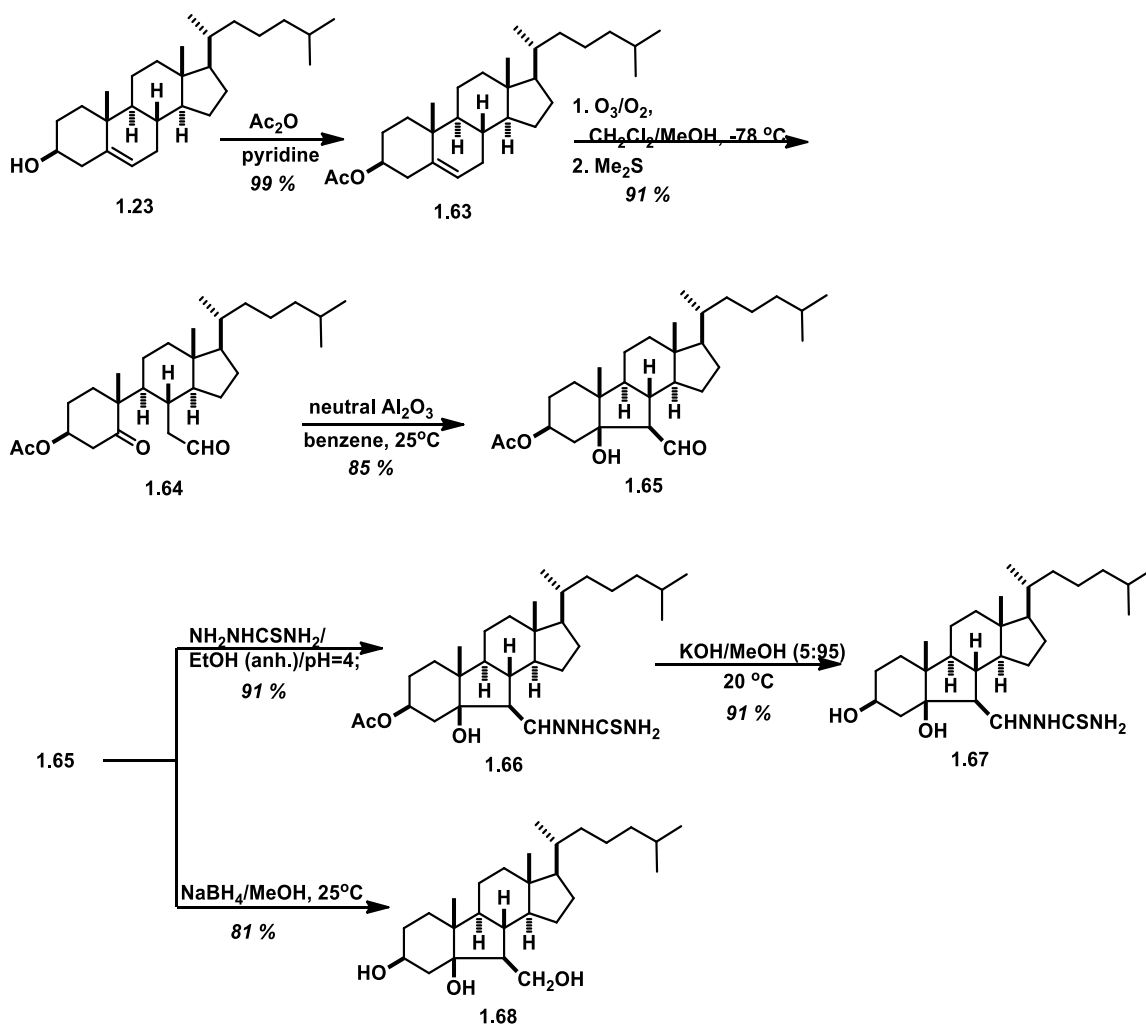
Scheme 1.8 Cui's synthesis of compounds **1.67** and **1.68**.

Then, in order to determine the effect of the hydroxyl group at C-5 they synthesized compounds **1.69** and **1.71** (Scheme 1.9). β-hydroxy aldehyde **1.65** in methanolic KOH at 45 °C yielded α,β-unsaturated aldehyde **1.50** which was derivatized to compound **1.69** by reaction with thiosemicarbazide. Similarly, the extended conjugated aldehyde **1.70** was obtained from the dehydration of **1.65** in

methanolic KOH at 60 °C. Finally, the reaction of **1.70** with thiosemicarbazide produced compound **1.71**.
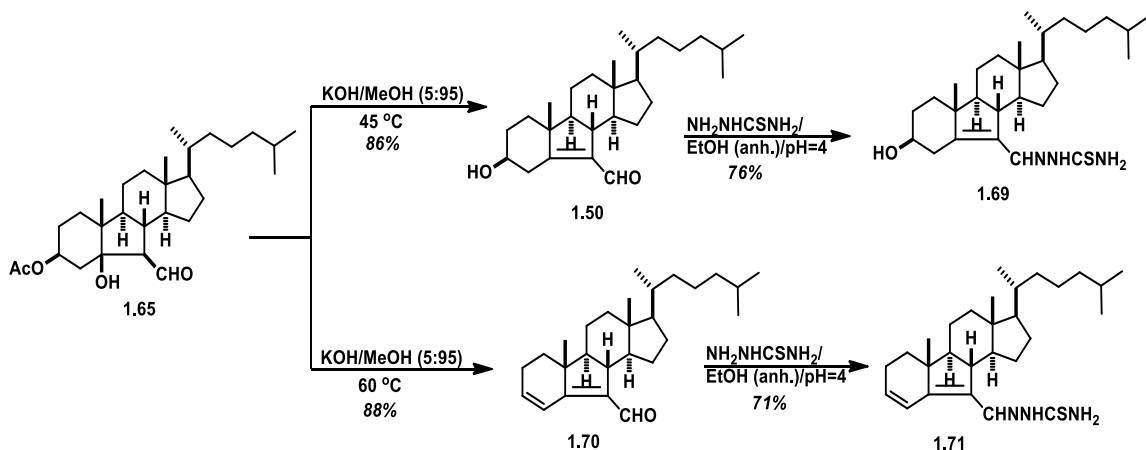
Scheme 1.9 Cui's synthesis of compounds **1.69** and **1.71**.

Compounds **1.84**, **1.85**, and **1.86** where synthesized by Cui and co-workers to determine the effect of various side chains on the anti-proliferative activity (Scheme 1.10). Acetylated β-sitosterol (**1.72**), dehydroisandrosterone (**1.73**), and pregnolone (**1.74**) where used as starting materials. The synthesis commenced with the ozonolysis and posterior aldol addition to generate abeo-sterols **1.78** – **1.80**. Further reaction with thiosemicarbazole followed by deacetylation afforded the desired compounds.
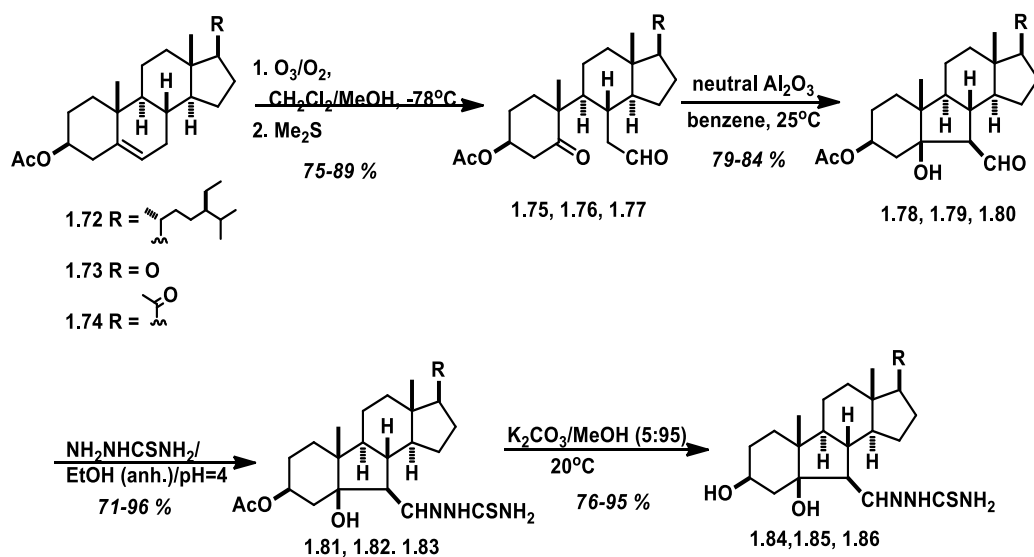
Scheme 1.10 Cui's synthesis of compounds **1.84 – 1.86**.

The same group also synthesized compounds **1.88** and **1.90**, which are depicted in Scheme 1.11. Reduction with sodium borohydride and further deacetylation of **1.82** and **1.83** generated the desired products.
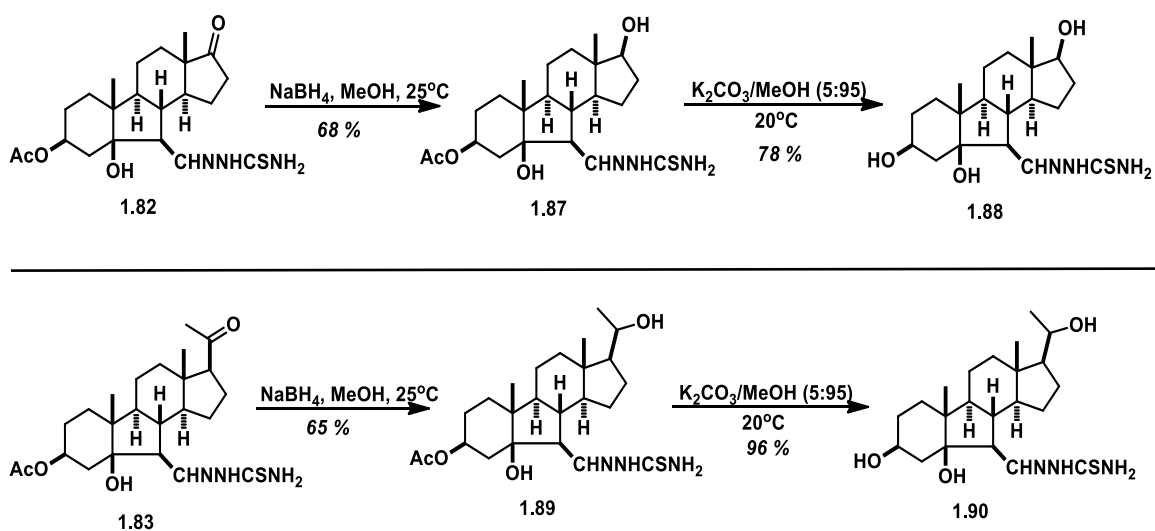
Scheme 1.11 Cui's synthesis of compounds **1.87 – 1.90.**

The *in vitro* anti-proliferative activity results are depicted in Table 1.4. They indicate that the presence of a cholesterol-type side chain and a thiosemicarbazone moiety at C-6 are important for the activity, since compounds **1.67**, **1.69**, and, **1.84** were the most active. Although the elimination of the hydroxyl group at C-5 has no obvious effect on the anti-proliferative activity, the removal of this group at C-3 markedly decreased it.

Table 1.4 *In vitro* anti-proliferative activities (IC$_{50}$ in μmol/L) of abeo-sterols 1.66–1.68, 1.71, and 1.81–1.90.

Compound	Cell Line		
	HeLa	7404	7901
1.66	57	40	36
1.67	19	8.6	8.4
1.68	61.3	25	19.9
1.69	13	11	9.0
1.71	>200	>200	>200
1.81	56	12	33
1.82	>200	>200	>200
1.83	119	>200	146
1.84	13	11	10
1.85	>200	>200	>200
1.86	>200	>200	>200
1.87	32	68	73

Compound	Cell Line		
	HeLa	7404	7901
1.88	71	110	158
1.89	77	85	75
1.90	>200	104	134
Cisplatin[a]	10	23	6.7

[d] Cisplatin was used as a positive control.

Interestingly, the number of *abeo*-sterols described in the literature is very limited, and only a few have been screened for anti-tubercular activity. For this reason the synthesis of additional analogs to conduct SAR studies was highly desirable.

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Chapter 2. Design, Synthesis, and Anti-tubercular Activity Evaluation of Leningosterol

2.1. Design of Leningosterol

Since bedaquiline (**1.6**) and delamanid (**1.7**) remain as the only two drugs approved in the last forty years, the urgency for new anti-tubercular drugs motivated us to design new, potentially useful anti-tubercular drugs. To date, saringosterol (**1.22**) has shown the lowest MIC value (0.25 µg/mL) of all the active sterols. It has also shown low cytotoxicity towards the Vero cell line.[1] The activity could be ascribed to the substituents at C-24, a vinyl and a hydroxyl group. Sadly, the pharmacokinetic properties of saringosterol (**1.22**) were hampered by its limited aqueous solubility.[2] On the other hand, parguesterol A (**1.24**) exhibited a MIC value of 7.8 µg/mL, while its plausible biosynthetic precursor (**1.26**) was inactive (120 µg/mL).[3,4] In this case the enhancement of anti-tubercular activity could be attributed to the 5(6→7) *abeo*-steroidal moiety and the increase in hydrophilicity due to the aldehyde at C-6. Based on these observations, we proceeded to design a new molecule that combined these structural features with the expectation that it would possess strong anti-tubercular activity and excellent pharmacokinetic properties. The synthesis of this hypothetical *abeo*-sterol, which we named leningosterol (**2.1**), is the main subject of this investigation (Scheme 2.1).

In order to predict the pharmacokinetic properties of leningosterol (**2.1**), we calculated its partition coefficient (log *P*) using ChemBioDraw Ultra 12.0. The resulting value was 5.3, very close to the cut-off number of 5.0 for "drug-like" molecules and less than the value for saringosterol (**1.22**, log *P* = 6.8), which give us high expectation about its pharmacokinetic properties.[5]
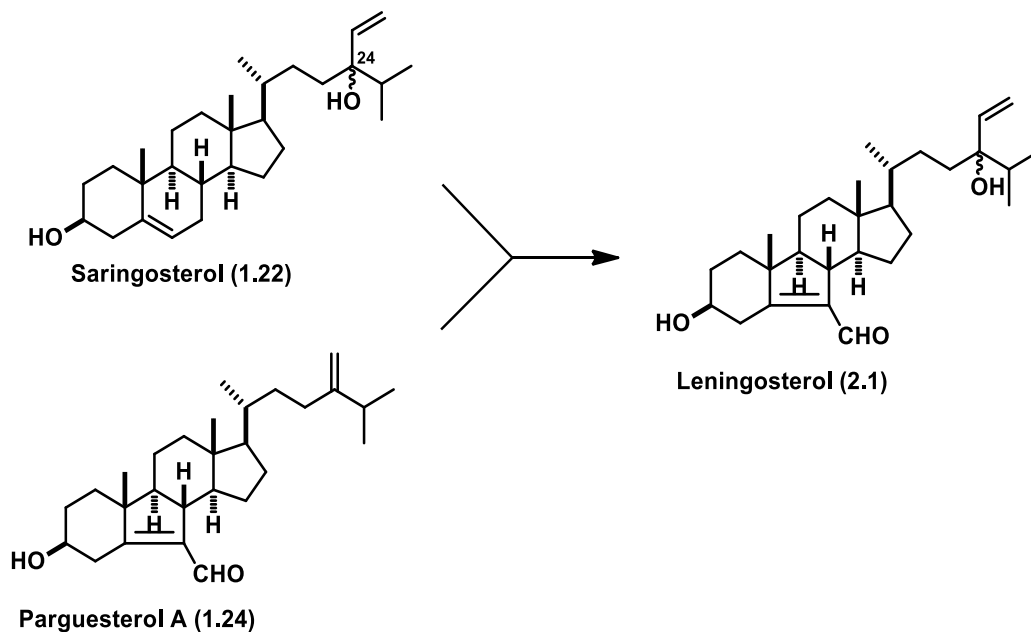
Scheme 2.1 Design of leningosterol (**2.1**) based on the key structural features of saringosterol (**1.22**) and parguesterol A (**1.24**).

2.2. Retrosynthetic Analysis

The retrosynthetic analysis of leningosterol (**2.1**) is depicted in Scheme 2.2. Ring-B *abeo*-sterol **2.1** has a hydroxyl and a vinyl group at C-24 that can be obtained from intermediate **2.2** through two distinct consecutive Grignard reactions. The contracted ring-B will be obtained from the aldol condensation of the ozonolyzed cholestane **2.2**. The precursor to intermediate **2.2** will be the commercially available sterol 3β-hydroxy-5-cholenic acid (**2.3**). Other starting materials were considered; yet, they were inaccessible or would not allow the incorporation of different *R* groups at C-24. The Weinreb amide can be generated from the ester of **2.3** after protection of the alcohol at C-3.
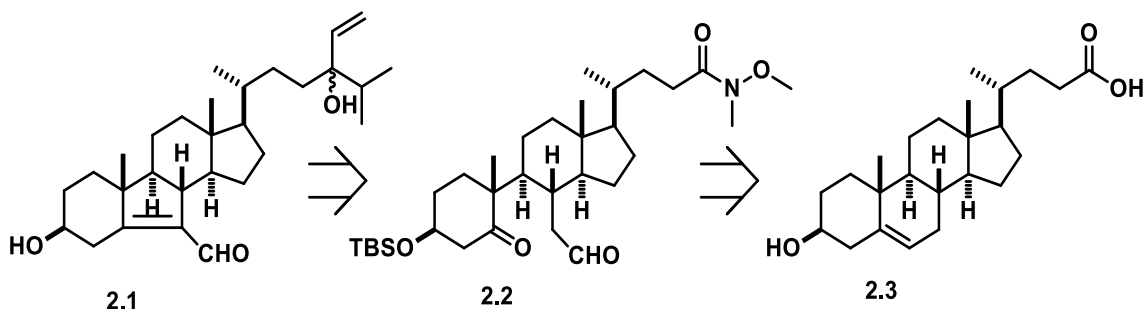
Scheme 2.2 Retrosynthetic analysis of leningosterol (**2.1**).

2.3. Total Synthesis of Leningosterol

2.3.1. First Attempt Towards the Synthesis of Leningosterol (2.1)

The synthesis of leningosterol (**2.1**) started with the methylation of the commercially available sterol 3β-hydroxy-5-cholenic acid (**2.3**) with diazomethane (CH_2N_2) in 99% yield, as depicted in Scheme 2.3.[6] Then, **1.37** was protected with *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole (Im) followed by the conversion of **2.4** into the Weinreb amide **2.5**.[7,8] An oxidative cleavage was performed with ozone to **2.5** in order to attain keto-aldehyde **2.2** in 85% yield.[4] Consecutively, **2.2** was submitted to an aldol condensation reaction using a phase transfer catalyst (PTC) to afford the deprotected *abeo*-sterol **2.6** as the minor product (15%) and the highly conjugated *abeo*-sterol **2.7** as the major product (65%).[9] Our rationale here is that the extended conjugation brings stability to compound **2.7** by resonance, favoring it over **2.6**. Presumably under these reaction conditions the protecting group was removed and the secondary alcohol at C-3 was dehydrated to afford **2.7** as the major product. These reaction

conditions were selected in an attempt to obtain better results than the 50% yield reported by Rodríguez and co-workers with methanolic-KOH.[4]
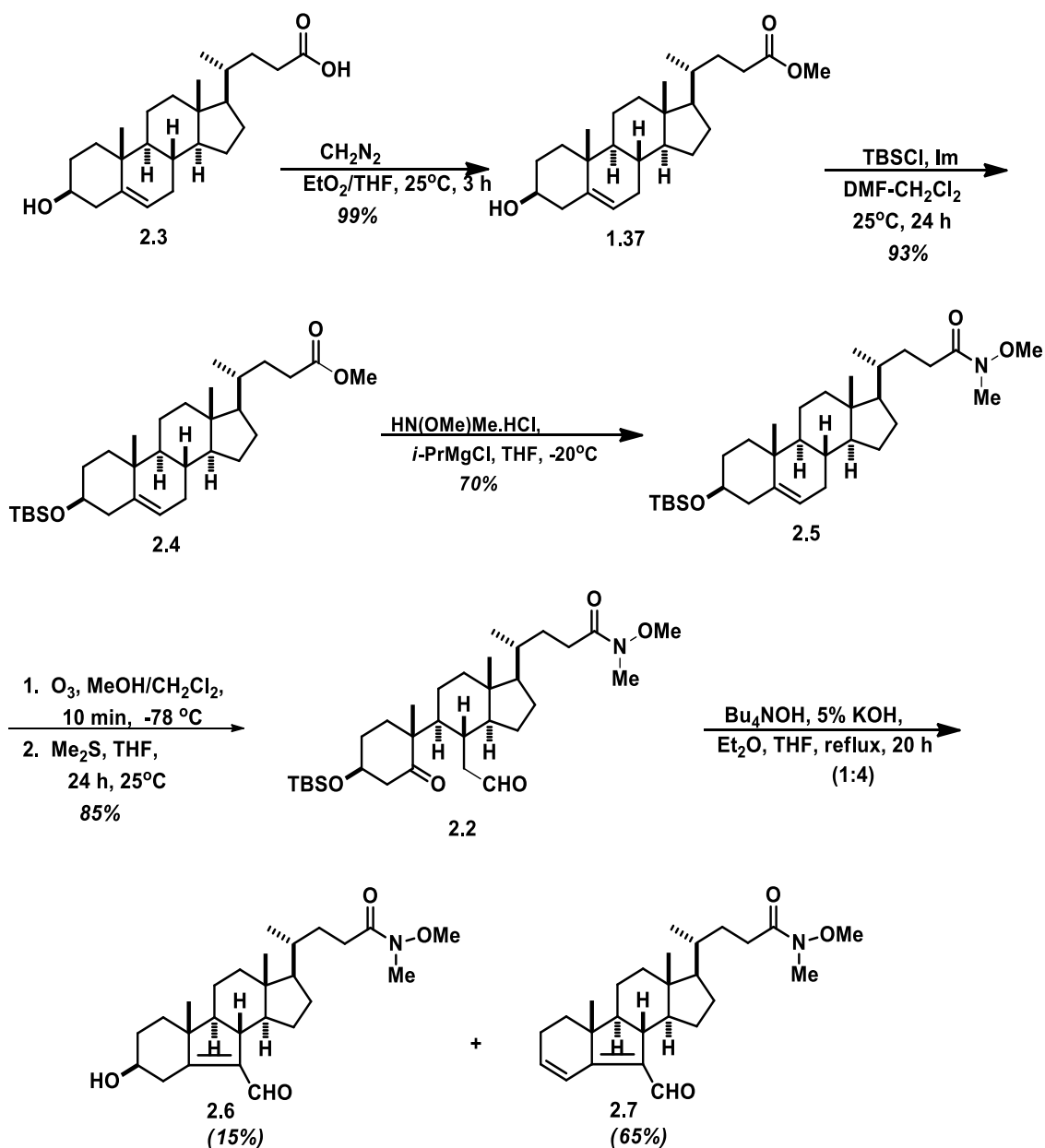
Scheme 2.3 Towards the synthesis of leningosterol (**2.1**) with TBSCl as the protecting group.

Other reaction conditions for the aldol condensation were explored in order to attain the ring-B *abeo*-sterol **2.6** in higher yield. *Seco*-cholesterol (**1.34**) was used as a model to find the ideal reaction conditions for this step (Table 2.1). When

using the PTC at 25 °C for 2 h the highly conjugated compound **1.70** was not observed, instead we obtained a mixture of compounds **1.35** and **1.50** (entry 1).[9] When changing the base to triethylamine (TEA) and heating at 35 °C for 24 h, compounds **1.50** and **1.70** were generated (entry 2). When milder reaction conditions were utilized, namely, pyrrolidine and benzoic acid, only *abeo*-sterol **1.35** was obtained. This occurred even at higher temperatures and upon adding PTC (entries 3-5).[10] When using KOH as the base, two scenarios were observed: 1) aqueous KOH only generated compound **1.35** (entries 6-10)[11], while 2) methanolic KOH for more than 24 h at 25 °C afforded compounds **1.50** and **1.70** (entries 11-12).[4] This result could be explained by the fact that in aqueous medium the equilibrium does not allow a second dehydration, while in methanolic medium the dehydration is more favorable. In the case of Rodríguez and co-workers, the reaction was performed in methanolic KOH for 15 min at 25 °C to obtain compound **1.50** in 50% yield; nonetheless, when attempting this procedure we obtained compound **1.35** as the sole product. The same results were obtained with NaOH (entry 13).[12] Other reaction conditions such as pyridinium *para*-toluene sulfonate (PPTS) and cobalt (II) acetate with 2,2'-bipyridine also generated β-hydroxy *abeo*-sterol **1.35** (entries 14 and 15).[13] The use of titanium tetrachloride (TiCl$_4$) with tetramethylethylenediamine (TMEDA) also generated compound **1.35** (entry 16); however, changing the base to TEA afforded compounds **1.35**, **1.50**, and **1.70** (entry 17).[14]

Table 2.1 Reaction conditions attempted for the aldol condensation of seco-cholesterol (1.34)
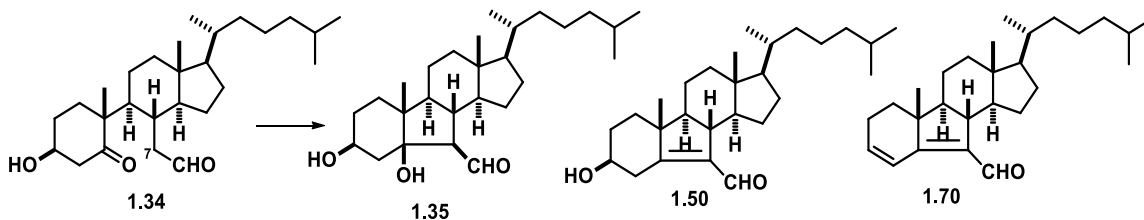
Entry	Reaction Conditions	Product
1	Bu$_4$NOH, 5% KOH$_{(aq)}$, THF, Et$_2$O, 25 °C, 2h	**1.35, 1.50**
2	Bu$_4$NOH, TEA, H$_2$O, THF, Et$_2$O, 35 °C, 24h	**1.50, 1.70**
3	pyrrolidine, benzoic acid, toluene, 25 °C, 24h	**1.35**
4	pyrrolidine, benzoic acid, toluene, 35 °C, 24h	**1.35**
5	pyrrolidine, benzoic acid, Bu$_4$NOH, THF, H$_2$O, Et$_2$O, 25 °C -35 °C, 48h	**1.35**
6	0.1 M KOH$_{(aq)}$, MeOH/CH$_2$Cl$_2$, 25 °C, 60h	**1.35**
7	0.5 M KOH$_{(aq)}$, MeOH/CH$_2$Cl$_2$, 25 °C -35 °C, 24h	**1.35, 1.50**
8	1.0 M KOH$_{(aq)}$, MeOH/CH$_2$Cl$_2$, 25 °C, 48h	**1.35**
9	1.0 M KOH$_{(aq)}$, TEA, MeOH/CH$_2$Cl$_2$, 25 °C, 24h	**1.35**
10	0.5 M KOH$_{(aq)}$, TEA, MeOH/CH$_2$Cl$_2$, 25 °C, 24h	**1.35**
11	1.0 M KOH-MeOH, TEA, 25 °C, 48h	**1.50, 1.70**

Entry	Reaction Conditions	Product
12	1.0 M KOH-MeOH, 25 °C, 48h	**1.50, 1.70**
13	1.0 M NaOH-MeOH, 25 °C, 12h	**1.50, 1.70**
14	PPTS, CH_2Cl_2, 25 °C, 24h	**1.35**
15	$Co(OAc)_2 \cdot 4H_2O$, 2,2'-bipyridine, DMF, 80 °C, 18h	**1.35**
16	$TiCl_4$, TMEDA, toluene, -50 °C -25 °C, 24h	**1.35**
17	$TiCl_4$, TEA, toluene, -10 °C-25 °C, 24h	**1.35, 1.50, 1.70**

^{1}H-NMR is a fast, useful tool to determine which product was generated (Figure 2.1). If the chemical shift was at 9.69 ppm, this meant that the β-hydroxy aldehyde (**1.35**) was produced. The conjugated systems, α,β-unsaturated aldehyde **1.50** and the highly conjugated aldehyde **1.70**, showed the same proton with chemical shifts at 9.96 ppm and 10.0 ppm, respectively.
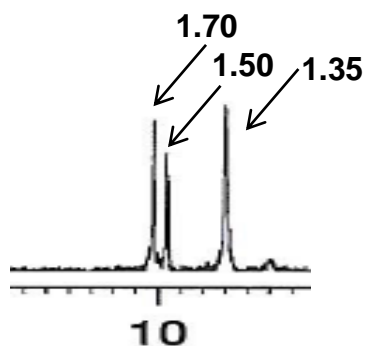
Figure 2.1 ^{1}H-NMR (CDCl3) chemical shifts of the aldehyde at C-6 of *abeo*-sterols **1.35**, **1.50**, and **1.70**.

A plausible explanation for the formation of these three products is that small bases in high concentration, such as KOH and NaOH, are strong and small enough to deprotonate H-7 leading to the condensation (Figure 2.2). While bases with steric hindrance such as TMEDA, 2,2'-bipyridine, PPTS, and pyrrolidine cannot promote dehydration, even at high temperatures. TEA seems to be small enough to fit and promote the condensation. We noticed that keto-aldehyde intermediate **1.34** is very unstable, and tends to slowly undergo an aldol addition as previously described by Liu and Zhou.[15,16] Also, the α,β-unsaturated aldehyde **1.50** can be obtained under very specific conditions, and once obtained, it quickly dehydrates to the more stable highly conjugated system **1.70**.
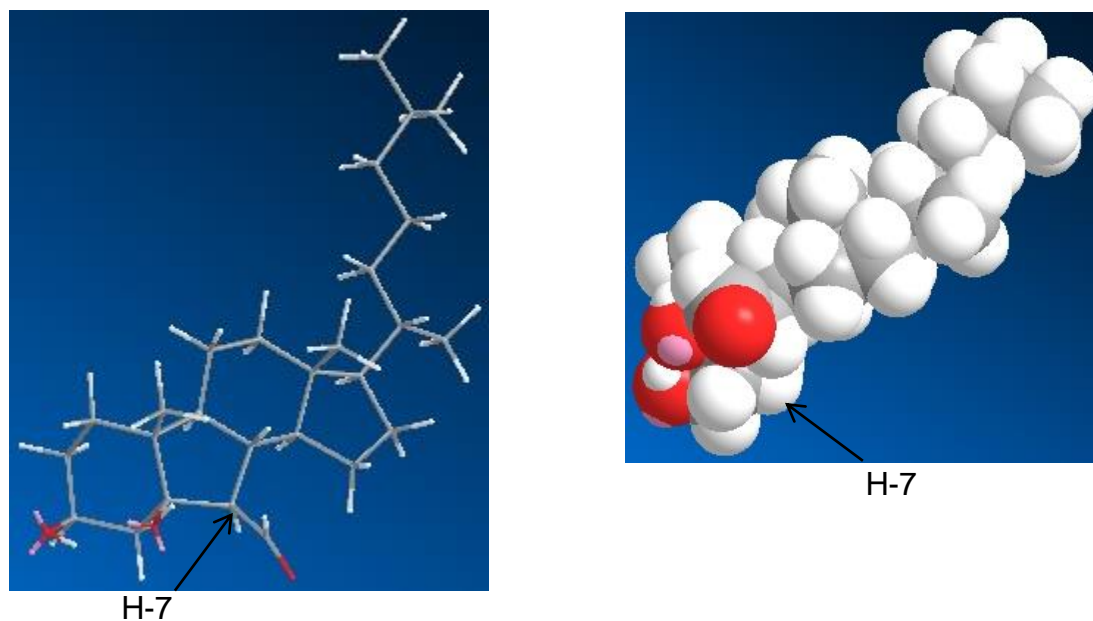
Figure 2.2 Molecular modeling of β-hydroxy abeo-sterol **1.35**.

2.3.1 Second Attempt Towards the Synthesis of Leningosterol (2.1)

Since none of the reaction conditions explored yielded the desired aldol in high yield, the protecting group was changed in order to minimize the formation of compound **2.7**. The new selected protecting group was a methyl residue (Scheme 2.4). Protection and methylation of **2.3** in a one-pot reaction led to the formation of two products, compounds **1.37** (60%) and **2.8** (30%).[17] Nevertheless, compound **2.8** was obtained in moderate yield from alcohol **1.37** using iodomethane and sodium hydride.[18] Then, methyl ester **2.8** was derivatized to the Weinreb amide **2.9**, followed by oxidative cleavage with ozone to afford keto-aldehyde **2.10**.[4,8] Finally, an aldol condensation was achieved with Bu_4NOH and TEA from 25 ºC to 30 ºC to attain three products, *abeo*-sterols **2.11**, **2.12**, and **2.7** in 50%, 5%, and 25% yield, respectively.[9]

The aim of protecting the alcohol at C-3 with a methyl group was to favor the generation of the α,β-unsaturated *abeo*-sterol **2.12** over the other two products. Unfortunately, once again **2.12** was obtained in low yield. If we compare these results to the aldol condensation of *seco*-cholesterol (**1.34**) and keto-aldehyde **2.2**, the products obtained can be rationalized. The methoxy group at C-3 is a more stable protecting group than the TBS group and thus diminishes the generation of the highly conjugated *abeo*-sterol **2.7**. Under these milder conditions (less reaction time and lower temperature) the β-hydroxy *abeo*-sterol **2.11** is favored. It is important to notice that even compound **2.7** is obtained in higher yield than the desired *abeo*-sterol **2.12**, confirming our hypothesis that the former is very unstable and tends to dehydrate very quickly.
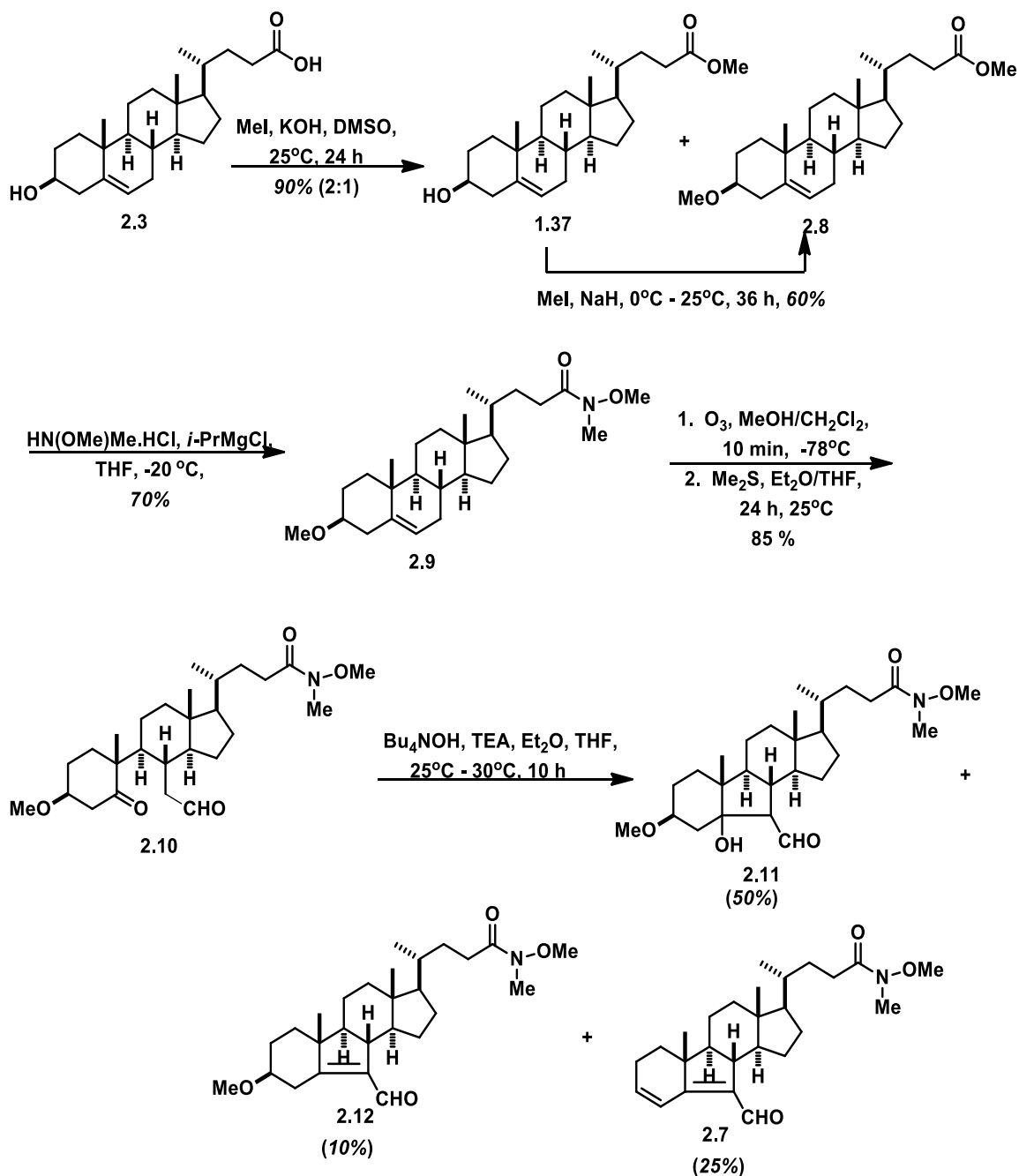
Scheme 2.4 Second attempt towards the synthesis of leningosterol (**2.1**) using a methyl group as the protecting group.

Notwithstanding the small amount of *abeo*-sterol **2.12** generated, we attempted to protect its α,β-unsaturated aldehyde several times (Table 2.2). We

used ethylene glycol and the traditional *para*-toluene sulfonic acid (pTSA),[19] triethyl orthoformate and tetrabutylammonium tribromide.[20] We also used hydrous ruthenium trichloride as catalyst (RuCl$_3$·3H$_2$O), which the authors claimed is a convenient and highly efficient methodology.[21] Sadly, due to the stability of the conjugated system we determined by NMR that neither procedure led to the formation of the desired product.

Table 2.2 <u>Second attempt towards the synthesis of leningosterol (2.1) using a methyl group as the protecting group.</u>
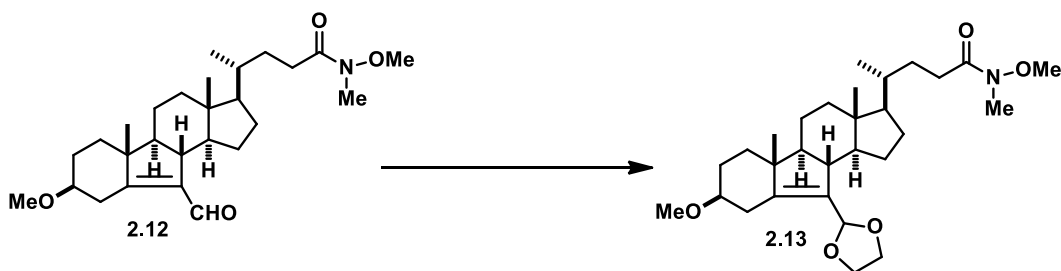
Entry	Reaction Conditions	Product
1	pTSA•H$_2$O, HO⌒OH , 25 °C, 24h	N.R.
2	pTSA•H$_2$O, HO⌒OH , toluene, 4Å MS, reflux, 24h	N.R.
3	0.1 mol% RuCl$_3$•3H$_2$O, HO⌒OH , Na$_2$SO$_4$, 25 °C, 15h	N.R.
4	HC(OEt)$_3$, HO⌒OH , Bu$_4$NBr$_3$, 25 °C, 24h	N.R.

2.3.2. Third Attempt Towards the Synthesis of Leningosterol (2.1)

Due to the difficulties confronted with the ring-B contraction and the α,β-unsaturated aldehyde protection, we decided to design a new synthetic route, as depicted in Scheme 2.5. Weinreb amide **2.5** will be subjected to two distinct consecutive alkylations to generate protected saringosterol (**2.14**), which then will undergo a selective oxidative cleavage with a manganese porphyrin complex (MPC) to keto-aldehyde **2.15**.[22] Finally, aldol condensation and deprotection will afford leningosterol (**2.1**). We expected the oxidative cleavage to be selective, since Liu and co-workers demonstrated that the internal double bond of (±)-limonene was cleaved to the corresponding carbonyl functions, while the terminal olefin remained intact.[22] They also found that simple α-olefins, terminal alkenes, alkynes, and α,β-unsaturated carbonyl compounds did not undergo the oxidative cleavage. The authors proposed that such a cleavage occurs through a pathway involving the formation of an epoxide intermediate.
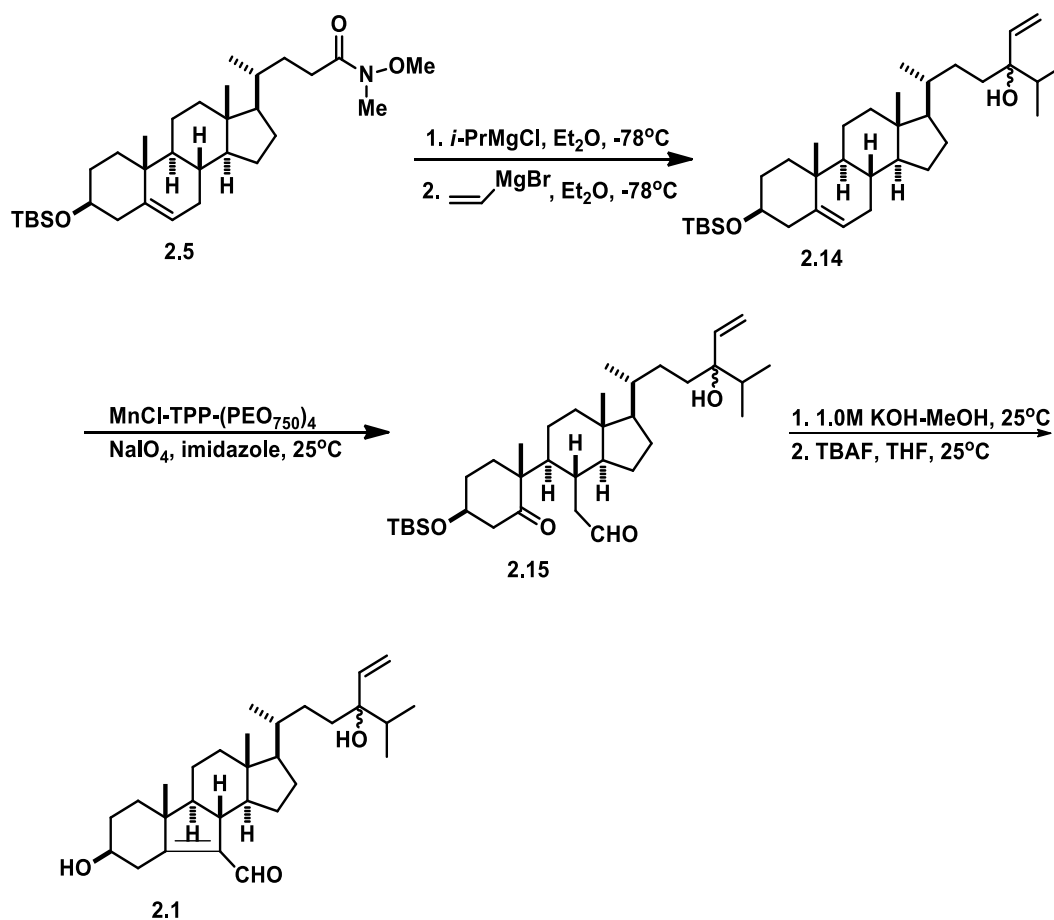
Scheme 2.5 Proposed alternate synthetic route for the synthesis of leningosterol (**2.1**) with manganese (III) porphyrin complex.

To embark in this new route, we decided to conduct a model study first using an abundant naturally occurring sterol already available in our laboratory, 25(26)-dehydroaplysterol (**2.16**) (Figure 2.3). The MPC was synthesized following the methodology described by Liu *et al.* (Scheme 2.6).[22] The commercially available 5, 10, 15, 20-tetrakis(4-methoxyphenyl)porphyrin [TPP-(OMe)$_4$] **2.17** was deprotected using boron tribromide (BBr$_3$) to afford TPP-(OH)$_4$ (**2.18**). Alongside, the hydroxyl group of the poly(ethylene oxide) methyl ether **2.19** was protected with *para*-toluenesulfonyl chloride (TsCl) to generate polymer **2.20**. Alkylation of

TPP-(OH)$_4$ (**2.18**) with **2.20** generated TPP-(PEO$_{750}$)$_4$ (**2.21**), which was finally treated with MnCl$_2$ to obtain the desired MPC **2.22** as a green oil.

2.16
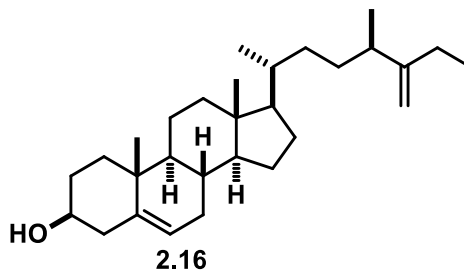
Figure 2.2 Molecular structure of 25(26)-dehydroaplysterol (**2.16**).
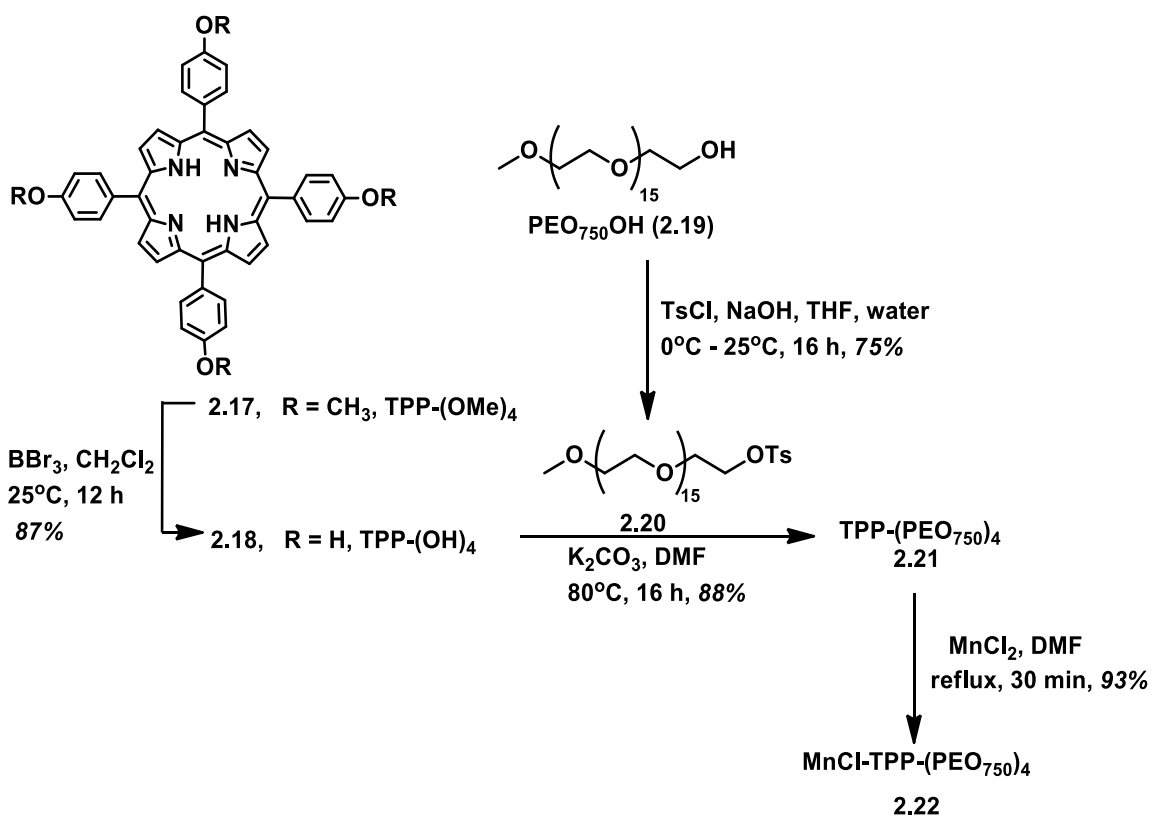
Scheme 2.6 Synthesis of the manganese porphyrin complex (MPC, **2.22**).

With the MPC in hand, we decided to perform the selective oxidative cleavage on 25(26)-dehydroaplysterol (**2.16**) (Scheme 2.7).   The reaction was performed in basic media at 25 °C; however, there was no reaction even at high temperatures and using more equivalents. We also tried the reaction with (*R*)-(+)-limonene, one of the examples included in Liu's publication, to determine the oxidative quality of MPC **2.22**, but there was no reaction. We noticed that they claim to obtain a green solid, while our pure MPC **2.22** was a green oil. In several occasions we endeavored to contact the authors, but unfortunately there was no response.
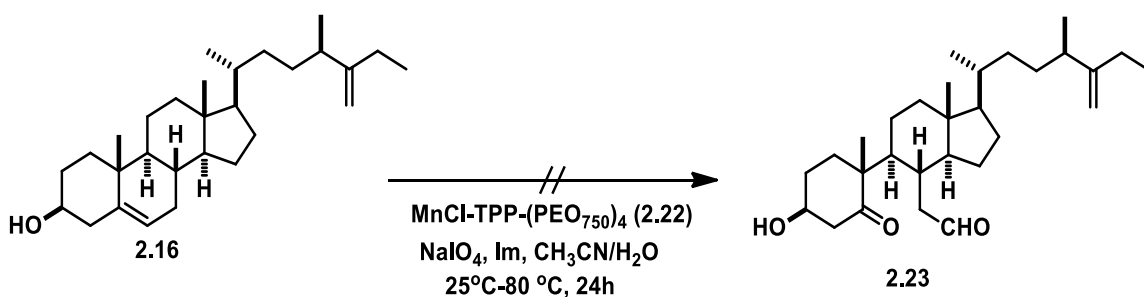
Scheme 2.7 Selective oxidation attempt of 25(26)-dehydroaplysterol (**2.16**) with MPC.

2.3.3. Fourth Attempt Towards the Synthesis of Leningosterol (2.1)

In an effort to resynthesize the α,β-unsaturated *abeo*-sterol moiety in higher yields, we decided to abandon the idea of using the methyl group as a protecting group since it was difficult to remove and the fact that it did not completely avert the formation of the highly conjugated system. The new approach started from ester **2.4**, but new reaction conditions were used for the Weinreb amide synthesis in order to improve its reproducibility and reaction yields.   We used

dimethylaluminum chloride (Me$_2$AlCl) and *N,O*-dimethylhydroxylamine hydrochloride (Scheme 2.8).[23] Surprisingly, the Weinreb amide was afforded with concomitant chlorination at C-3, as confirmed by NMR and X-ray diffraction analyses (Figure 2.3).[24] Nonetheless, when the reaction was performed adding protected ester **2.4** after 1h of stirring the same equivalents of Me$_2$AlCl and amine, compound **2.5** was generated in 90% yield.[25]
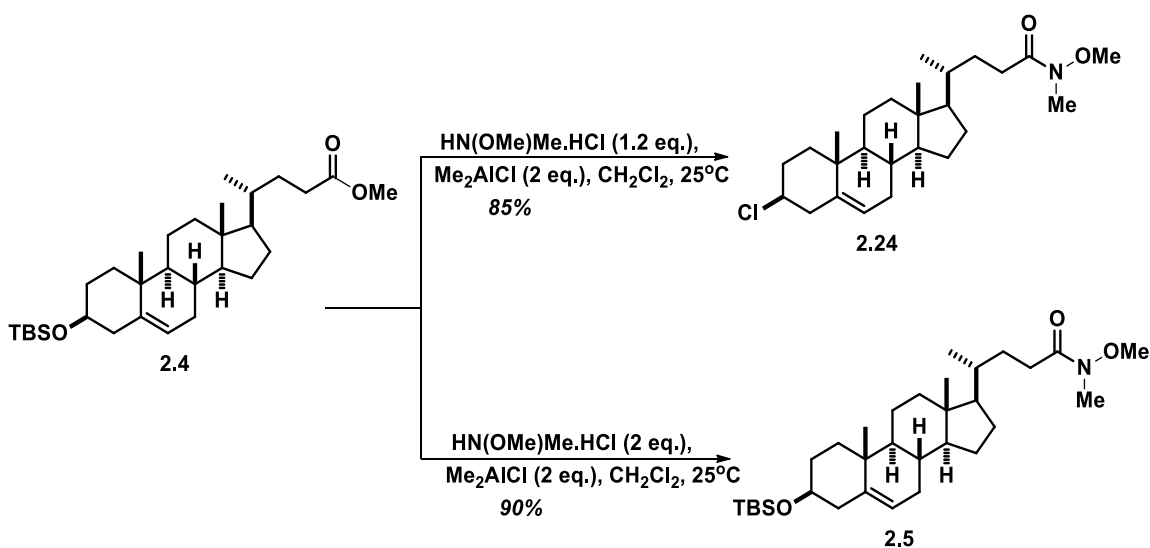
Scheme 2.8 Synthesis of chlorinated Weinreb amide **2.24** and Weinreb amide **2.5**.
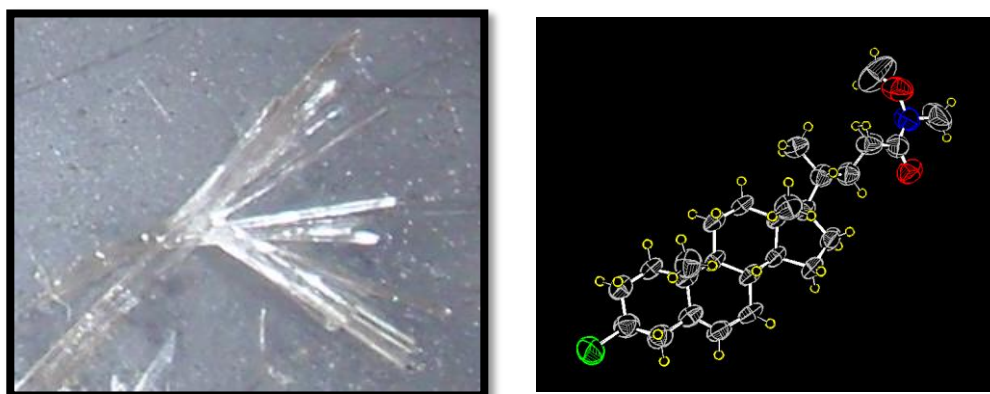
Figure 2.4 Crystals and ORTEP diagram obtained nfrom the X-ray diffraction analysis of chlorinated Weinreb amide **2.24**.

A plausible explanation for the former outcome is depicted in Scheme 2.9. When we add the same equivalents of Me$_2$AlCl and amine, Cl$_2$AlNMe(OMe) is formed *in situ*, which leads to Weinreb amide **2.5**.[25] However, when there is an excess of Lewis acid Me$_2$AlCl, it coordinates with the protected alcohol (**II**) to yield instead the chlorinated Weinreb amide **2.24**.[26]
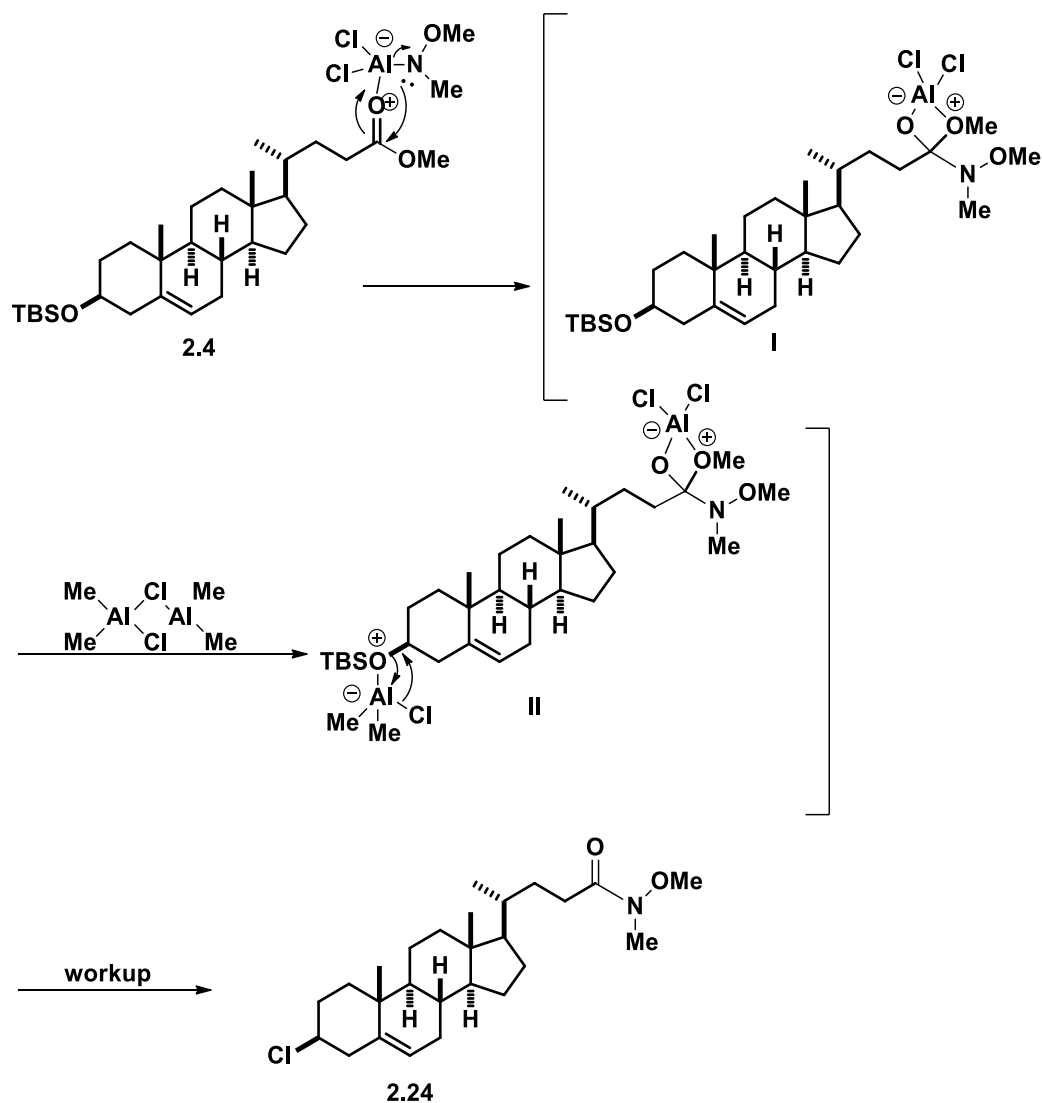
Scheme 2.9 Plausible mechanism for the synthesis of chlorinated Weinreb amide **2.24**.

Taking advance of the new chlorinated compound (**2.24**), we decided to synthesize the chlorinated analog of saringosterol (**1.22**), in order to perform structure-activity relationship (SAR) studies. At the same time, we wanted to explore the consecutive organometallic alkylation to avoid other unanticipated by-products. We explored two routes: in the first one we used *iso*propyllithium (*i*PrLi) followed by a Grignard reaction to afford 3β-chloro saringosterol (**2.25**) and, unexpectedly, methyl amide **2.26** (Scheme 2.10).[8] According to Graham and Scholz, when sterically hindered or highly basic nucleophiles are used, elimination of the methoxide moiety to release formaldehyde can occur as a significant side reaction (Scheme 2.11).[27] We also performed the reaction at lower temperature using organomagnesium reagents, but the yields and reproducibility were lower.
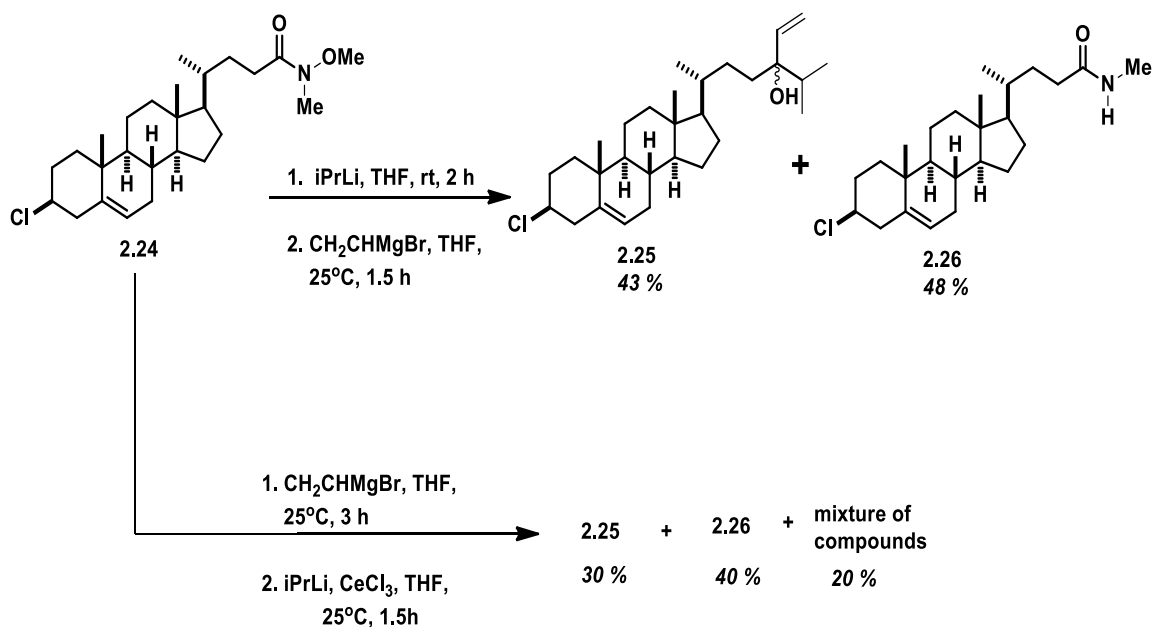
Scheme 2.10 Synthesis of 3β-chloro saringosterol (**2.25**) and compound **2.26**.
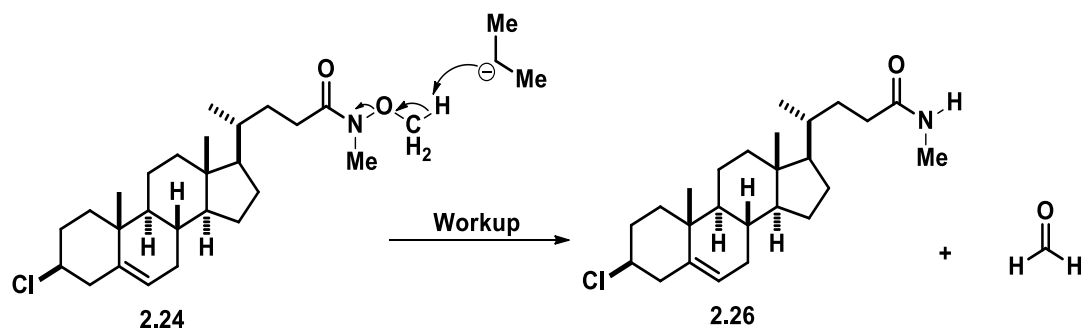
Scheme 2.11 Mechanism for the synthesis of methyl amide **2.26**.

In the second route, we inverted the order of addition (Scheme 2.10). First, we performed a Grignard alkylation with vinylmagnesium bromide and then an organolithium alkylation adding $CeCl_3$ to shun any possibility of the Michael addition product.[28] This time **2.25** was obtained in lower yield, the yield of **2.26** was not diminished, and a mixture of other compounds was obtained presumably due to the lability of the vinyl ketone intermediate.

Both routes generated the desired product albeit in low yield, suggesting that leningosterol (**2.1**) will be obtained in low yield due to the generation of methyl amide **2.26**.

2.3.4. Synthesis of Leningosterol (2.1)

Thus far, our attempts to synthesize leningosterol (**2.1**) have been hampered by the formation of unexpected by-products and insurmountable problems with the aldol condensation reaction. This motivated us to seek yet another route to finally obtain the desired target. Since we have learned that the

protection of the hydroxyl group at C-3 does not favor the formation of the α,β-unsaturated aldehyde, we decided to start the synthesis of the Weinreb amide without protecting the hydroxyl group at C-3.

Weinreb Amide Synthesis

For the synthesis of the Weinreb amide we decided to use a coupling agent to get the desired amide directly from the carboxylic acid, since the reaction conditions aforementioned were irreproducible.[25] Amongst the possible coupling agents that could be used, we selected *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI) and *N,N'*- carbonyldiimidazole (CDI) due to the good yields reported in the literature.[29,30] However, when using EDCI there was no reaction, while with CDI we obtained Weinreb amide **2.27** with a carbamate group at C-3 (Scheme 2.12). Taking advantage of this new product, we decided to make a model study with cholesterol in order to determine if this carbamate could be a protecting group to avert the formation of the highly conjugated system obtained previously.
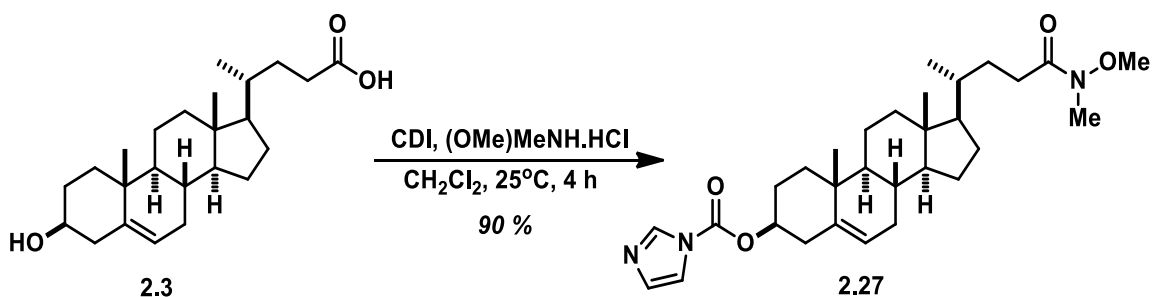
Scheme 2.12 Synthesis of carbamate Weinreb amide **2.27**.

Cholesterol carbamate **2.28** was obtained in high yield after reacting cholesterol (**1.23**) with CDI at 25 °C (Scheme 2.13).[30] Then an oxidative cleavage with ozone was performed to attain compound **2.29**.[4] Finally, after performing the aldol condensation reaction the undesired highly conjugated *abeo*-sterol **1.70** was obtained. We noticed that using the carbamate as a protecting group to avoid the highly conjugated product actually did the opposite. Apparently, the latter is a good leaving group accelerating the formation of **1.70**. No additional products were obtained, only unreacted starting material.
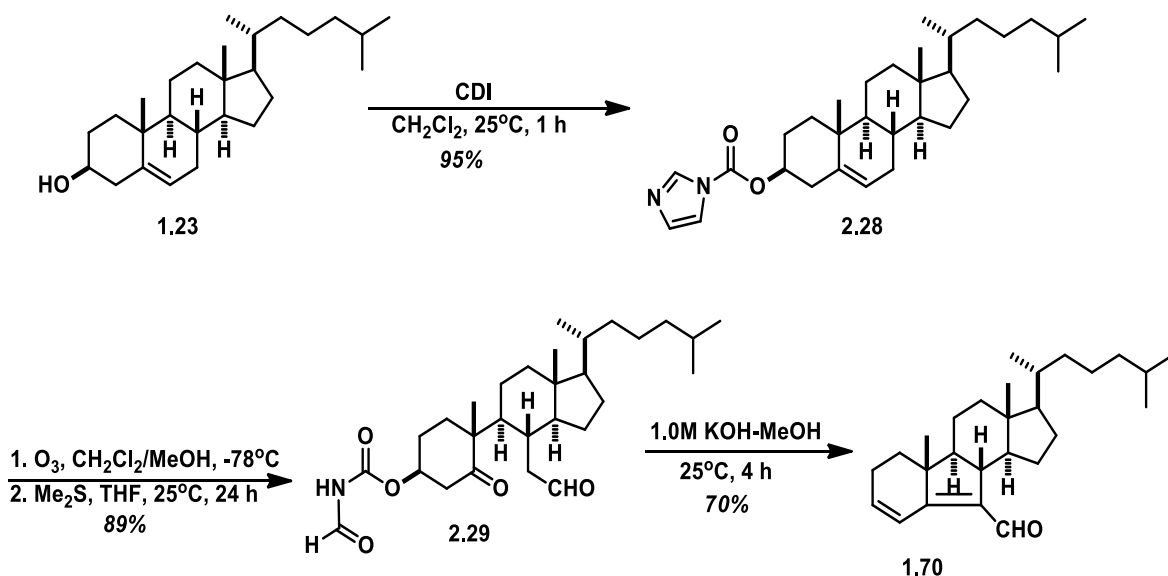
Scheme 2.13 Model study of carbamate on cholesterol (**1.23**).

After determining that the carbamate at C-3 was not a good protecting group, we decided to develop new reaction conditions that allow us to move forward in the presence of the free alcohol. We used acidic conditions with trifluoroacetic acid (TFA) at different concentrations, but there was no reaction.[31]

Finally, when we conducted the reaction under basic conditions using 1.0 M KOH-MeOH for ten min, free alcohol **2.30** was obtained in very high yield (Scheme 2.14).
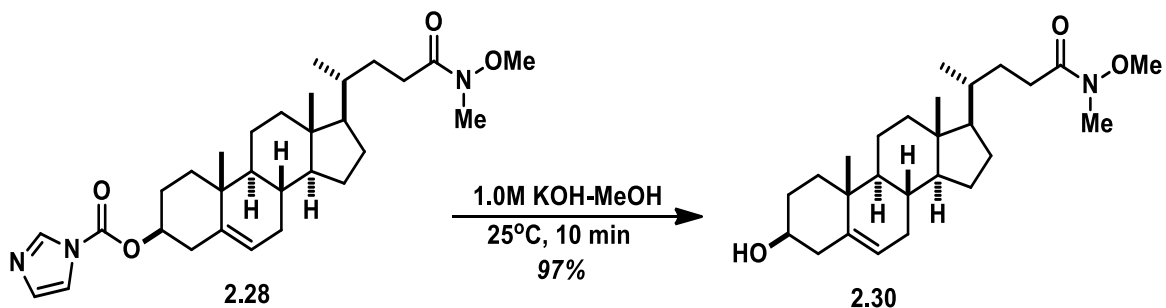
Scheme 2.14 Carbamate removal in basic medium.

Ozonolysis Reaction

After finding optimal reaction conditions for the carbamate removal, we proceeded with the ozonolysis.[4] The oxidative cleavage with ozone led us to keto-aldehyde **2.31** in good yield (Scheme 2.15).
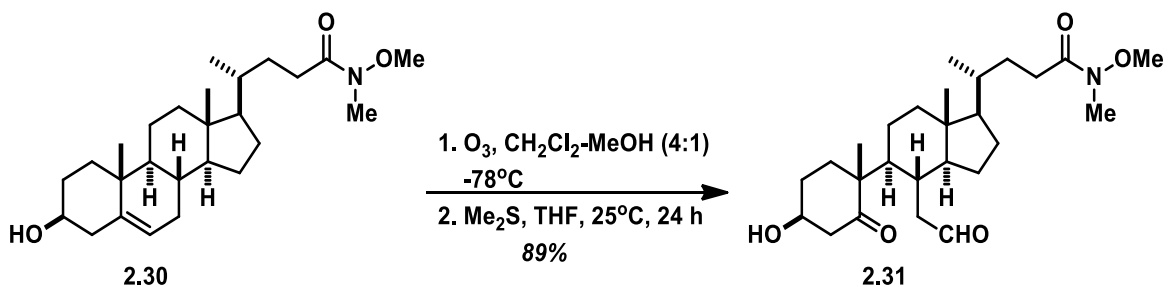
Scheme 2.15 Ozonolysis of Weinreb amide **2.30**.

Aldol Addition Reaction

Synthesis of the Weinreb amide using CDI, followed by ozonolysis generated keto-aldehyde **2.31** in good yield and without complications.[4,30] As aforementioned, we encountered several difficulties trying to protect the α,β-unsaturated aldehyde, thus we decided to make an aldol addition instead. Different reaction conditions were explored and are depicted in Table 2.3. When using (*S*)-(-)-1-(2-pyrrolidinylmethyl)pyrrolidine[32] and lithium hydroxide (LiOH) 10 mol%[32] there was no reaction (entries 1 and 3). However, with L-proline,[32] alumina (Al$_2$O$_3$),[15,16] and pyrrolidine[10] β-hydroxy *abeo*-sterol **2.32** was obtained (entries 2 and 5-7). 1.0 M LiOH-MeOH was also used, but afforded a 1:1 mixture of compounds **2.32** and **2.6** (entry 4). Based on these results we decided to use pyrrolidine in CH$_2$Cl$_2$ due to the short reaction time and good yield.

Table 2.3 <u>Reaction conditions for the aldol addition leading to product 2.32</u>
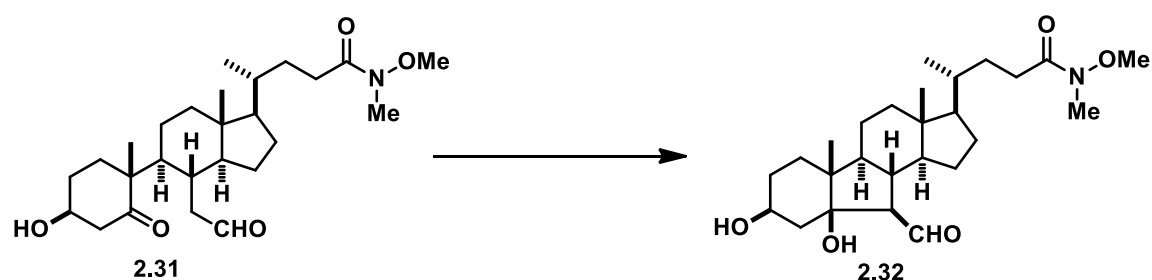
Entry	Reaction Conditions	Yield (%)
1	(*s*)-(-)-1-(2-pyrrolidinylmethyl)pyrrolidine, TFA, NMP, 0 °C-rt, 56h	0

Entry	Reaction Conditions	Yield (%)
2	L-proline 30 mol%, CH$_2$Cl$_2$, rt, 16h	70
3	LiOH 10 mol%, EtOH, H$_2$O, rt, 24h	0
4	1.0 M LiOH-MeOH, rt, 4h	50
5	Al$_2$O$_3$, benzene, rt, 24h	60
6	pyrrolidine, benzoic acid, toluene, rt, 4h	83
7	pyrrolidine, CH$_2$Cl$_2$, rt, 0.5h	90

Aldehyde Protection Reaction

The aldol addition using pyrrolidine generated the desired β-hydroxy *abeo*-sterol **2.32**, thus we decided to continue on with the aldehyde protection. From the reaction conditions tried previously (Table 2.2), we decided to use the classic conditions with ethylene glycol and *p*TSA to attain acetonide **2.33** (Scheme 2.16).[19]
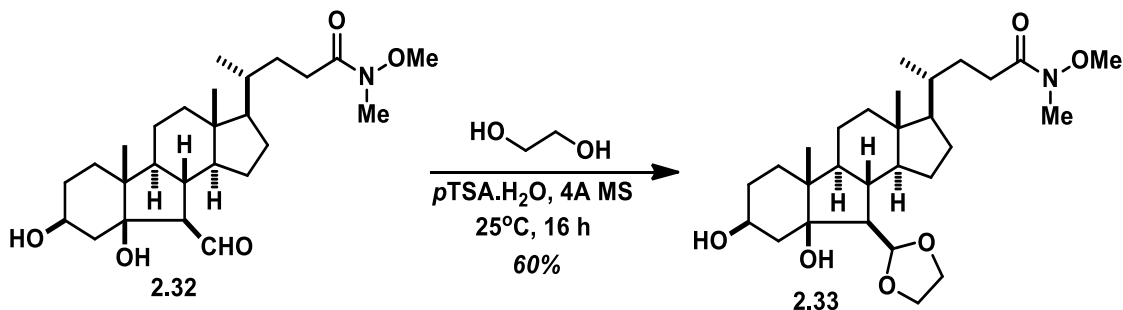
Scheme 2.16 Protection of aldehyde **2.33** with ethylene glycol and *p*TSA.

Alkylation Reactions

After acetonide **2.33** was obtained, we explored how to achieve two distinct alkylations and incorporate consecutively the alkyl groups at C-24. Based on the results obtained from the model study of the chlorinated Weinreb amide **2.24**, we decided to utilize *i*PrLi for the alkylation even if it generated the methylamide as a by-product.[8] Compound **2.33** was submitted under these reaction conditions and afforded the expected *iso*propylketone **2.34** and methylamide **2.35** in a roughly 1:1 ratio (Scheme 2.17). Then, a Grignard alkylation reaction was performed on **2.34** using vinylmagnesium bromide to generate compound **2.36** without use of any protecting group (Scheme 2.18).
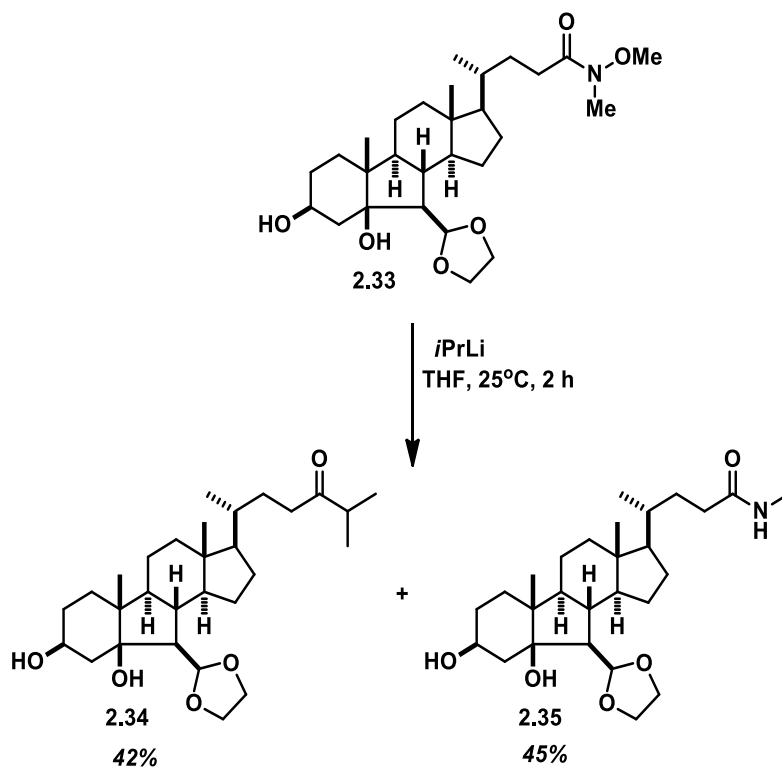
Scheme 2.17 Synthesis of isopropylketone **2.34** and methylamide **2.35**.
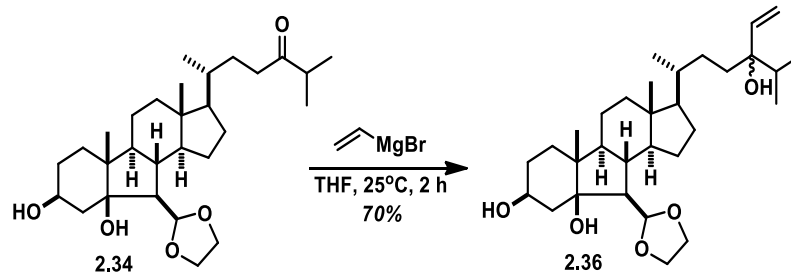
Scheme 2.18 Synthesis of compound **2.36**.

Deprotection and Dehydration Reactions

With compound **2.36** in hand, we were only two steps away from the target molecule. First, we performed an aldehyde deprotection utilizing *p*TSA in acetone-water to produce β-hydroxy leningosterol **2.37** (Scheme 2.19).[34] Then, a dehydration using methanolic KOH at 25 °C for a maximum of three hours with vigorous agitation generated leningosterol (**2.1**) as a 1:1 mixture of epimers at C-24.[35] However, the reaction yields were lower than those reported by Ma.[35]
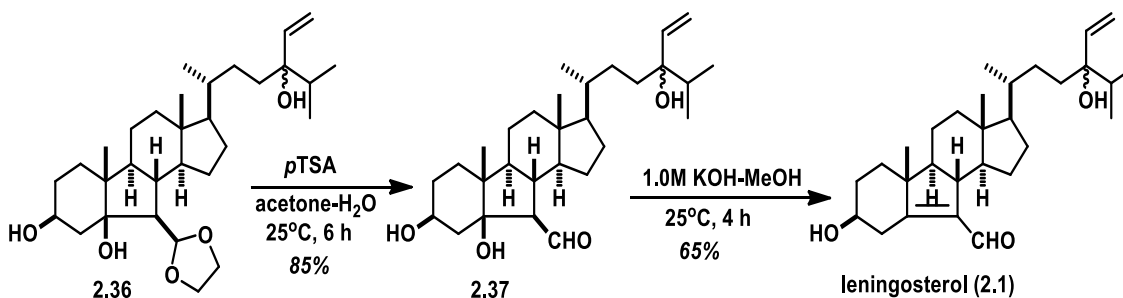
Scheme 2.19 Final steps of the synthesis of leningosterol (**2.1**).

In summary, leningosterol (**2.1**) was successfully synthesized in ten steps in 7% overall yield utilizing only one protecting group (Scheme 2.20). Key steps

include a Weinreb amide, ozonolysis, aldol addition, organometallic alkylations and dehydration. The Weinreb amide was generated in good yield with the use of the coupling agent CDI and a new methodology was developed for the removal of the concomitant C-3 carbamate. Furthermore, the insurmountable problem with the aldol condensation reaction was overcome with an aldol addition followed by subsequent dehydration.
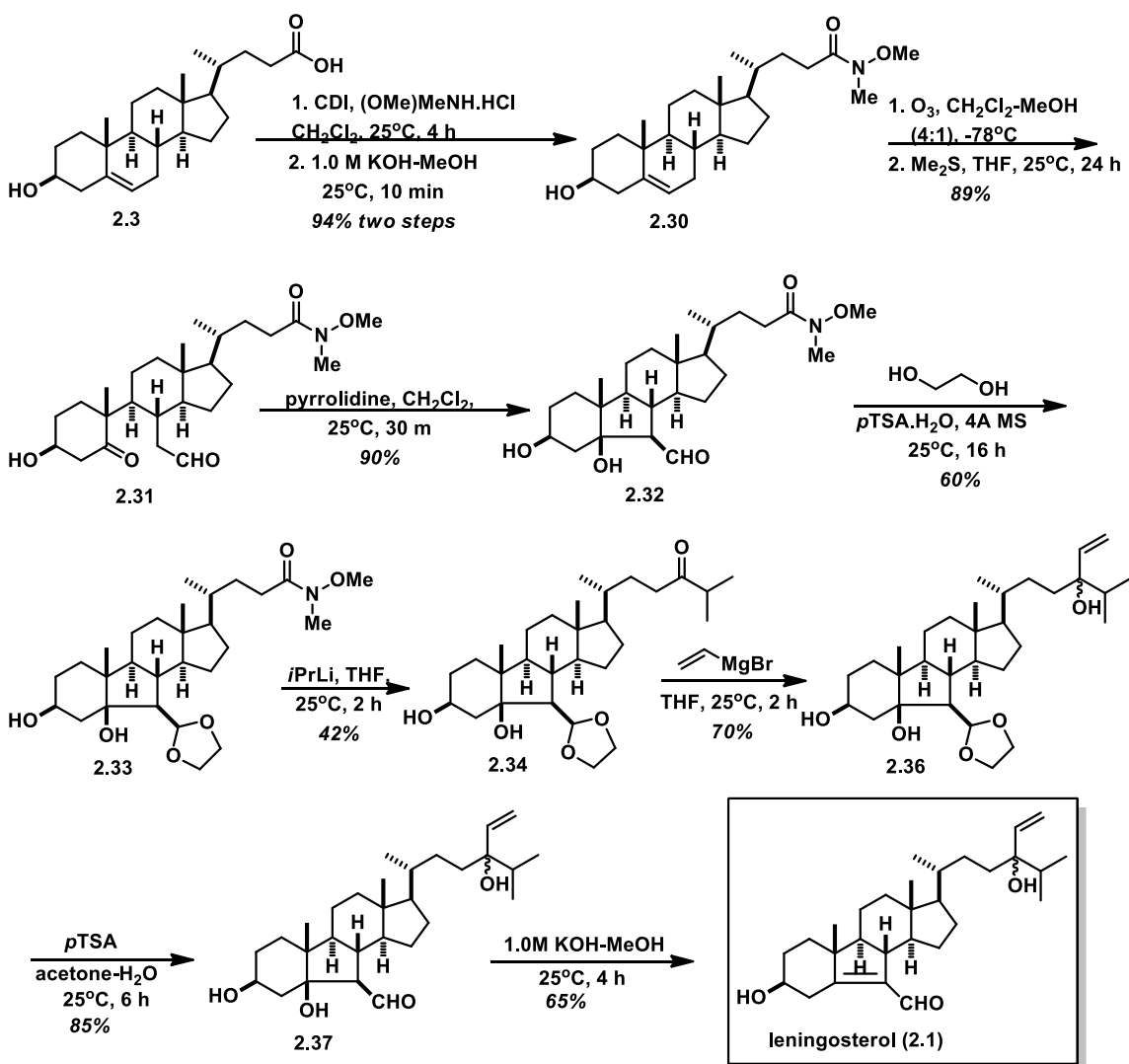
Scheme 2.20 Synthesis of leningosterol (**2.1**).

Epimers Separation and Absolute Configuration Determination

Epimers **2.1a** and **2.1b** were separated by RP-HPLC with 20% water in methanol with a Regis chiral column (Scheme 2.21). The absolute configuration of each epimer was determined by comparison of the ^{1}H-NMR of the side chain at C-17 with those published by Djerassi and co-workers for each saringosterol (**1.22**) C-24 epimer (Table 2.4).[36] The chemical shifts of protons 28 and 29 of leningosterol epimers **2.1a** and **2.1b** were almost an exact match with those reported in the literature for each C-24 epimer of **1.22**. At the same time, the determination of the absolute configuration of **1.22** epimers by Djerassi and co-workers was performed by chemical correlation with 24(28)-epoxides of fucosterol acetate (**2.38** and **2.39**) and *iso*fucosterol acetate (**2.40** and **2.41**) (Scheme 2.22).[37,38] Epoxides **2.38** – **2.41** were reduced to alcohols **2.42** and **2.43** while saringosterol (**1.22**) epimers were also reduced to the same alcohols to finally determine the absolute configuration by proton comparison. Sadly, to the best of our knowledge, a formal assignment of the ^{13}C-NMR data for each epimer has not been made to date.
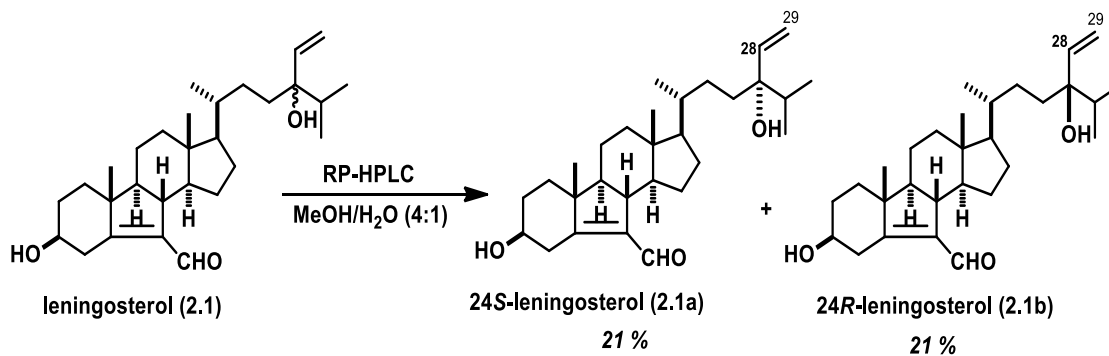
Scheme 2.21 Separation of leningosterol (**2.1**) epimers.

Table 2.4 Absolute configuration assignment of leningosterol (2.1) at C-24 by comparisons of the ^{1}H-NMR data (CDCl$_3$) with those for saringosterol (1.22).

Compound	H-28 (ppm)	H-29 (ppm)
24*S*-leningosterol (**2.1a**)	5.795	5.184, 5.132
24*S*-saringosterol	5.795	5.181, 5.130
24*R*- leningosterol (**2.1b**)	5.812	5.191, 5.140
24*R*-saringosterol	5.808	5.188, 5.136
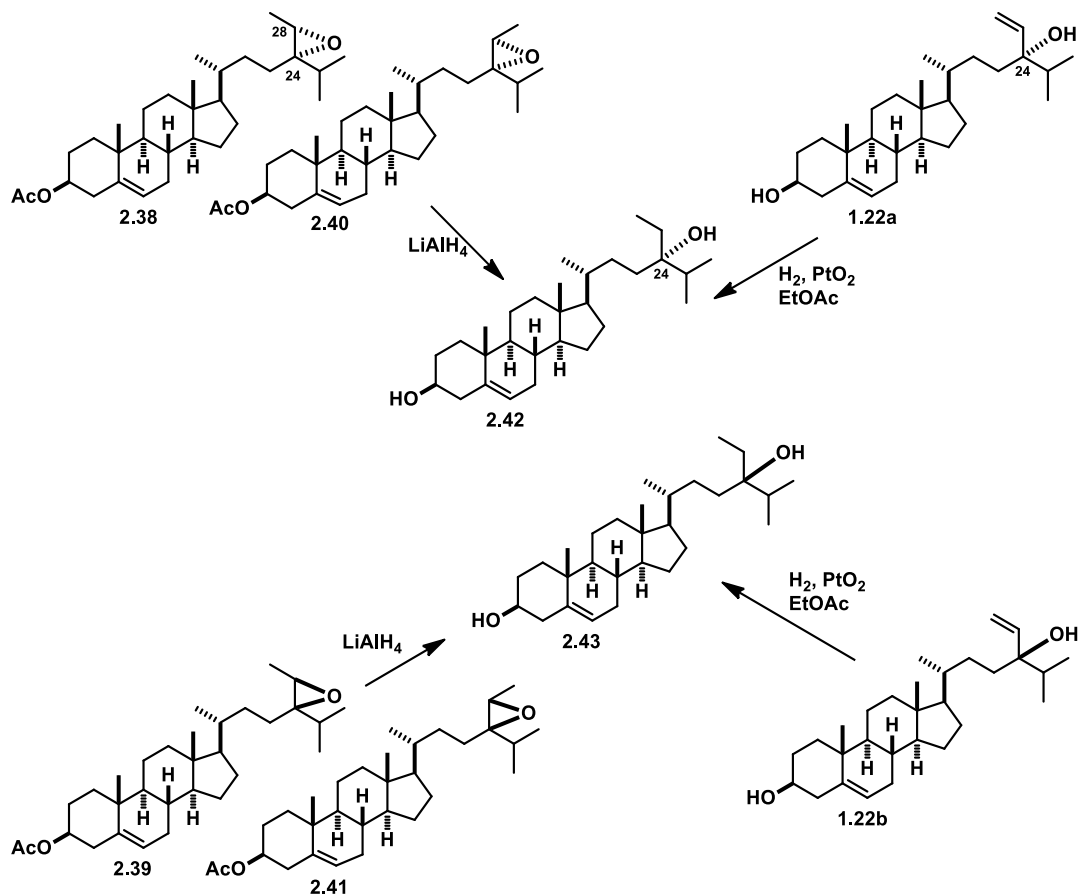
Scheme 2.22 Absolute configuration determination of saringosterol (**1.22**) epimers.

2.4. Synthesis of Saringosterol (1.22)

Taking advance of the synthetic route developed for leningosterol (**2.1**), we decided to synthesize saringosterol (**1.22**) to use it as a control for the anti-tubercular activity assay. Thus, Weinreb amide **2.30** was subjected to two distinct consecutive organometallic alkylations to afford saringosterol (**1.22**) as a mixture of epimers (Scheme 2.23).
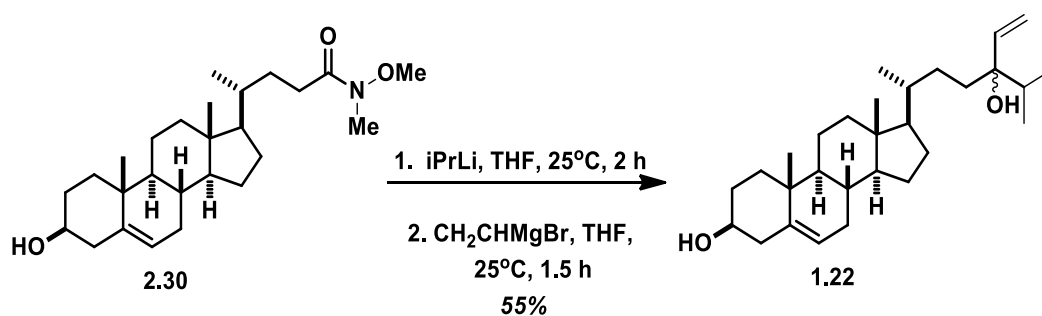
Scheme 2.23 Synthesis of saringosterol (**2.1**).

During the process of comparing the NMR data of our sample of synthetic saringosterol (**1.22**) with the values reported in the literature, we noticed some discrepancies in the ^{13}C-NMR chemical shifts corresponding to C-24. Ikekawa *et al.*,[39] Guyot *et al.*,[40] Catalan *et al.*,[36] Tang *et al.*,[41] Wang *et al.*,[42] Shi *et al.*,[43] Shi *et al.*,[44] Permeh *et al.*,[45] and Chen *et al.*[46] reported a chemical shift of 77.7 ppm for C-24 which is in accordance with the assignment we made. However, Ayyad *et al.*,[47] Huh *et al.*,[48] Kim *et al.*,[49] and Bouzidi *et al.*[50] reported a value of 89.8 ppm for the same position. This large chemical shift gap (12.1 ppm) can only be explained by the presence at C-24 of a hydroperoxy group instead of a hydroxyl group. As

reported by Guyot *et al.*,[40] Wang *et al.*,[42] and Shi *et al.*[43], the ^{13}C-NMR chemical shift of C-24 at 89.8ppm corresponds to 24-hydroperoxy-24-vinylcholesterol (**2.44**) and not to saringosterol (**1.22**) (Figure 2.5).

2.44
δ_{C-24} 89.8 ppm

1.22
δ_{C-24} 77.7 ppm
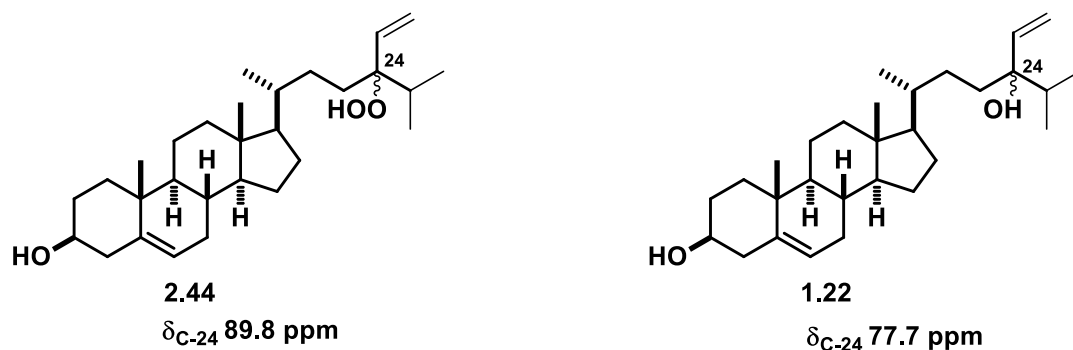
Figure 2.3 ^{13}C-NMR C-24 chemical shift comparison of 24-hydroperoxy-24-vinylcholesterol (**2.44**) and saringosterol (**1.22**).

2.5. Evaluation of Anti-tubercular Activity: Results and Discussion

The design and synthesis of leningosterol (**2.1**) was prompted by the anti-tubercular activity of two interesting natural products, namely, saringosterol (**1.22**) and parguesterol A (**1.24**).[1,4] Soon after we finished the synthesis of leningosterol (**2.1**) we sent a sample to our collaborators at the Institute for Tuberculosis Research, University of Illinois, Chicago for screening against the *Mtb* strain H$_{37}$Rv. For this purpose, they used the Microplate Alamar Blue Assay (MABA) with rifampicin (**1.2**) as a positive control.[51] We also sent our sample of synthetic saringosterol (**2.1**). The results are summarized in Table 2.5. We also included many other derivatives and by-products for evaluation to generate enough data on which to base our SAR studies.
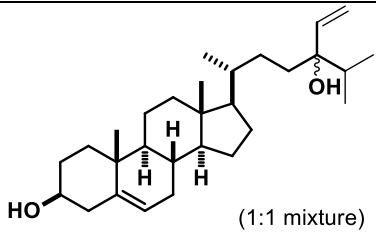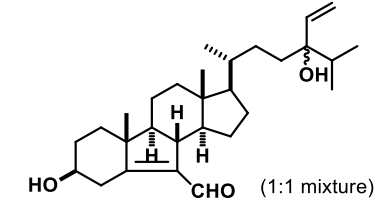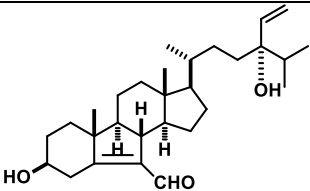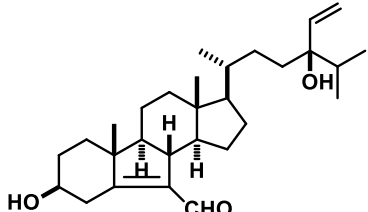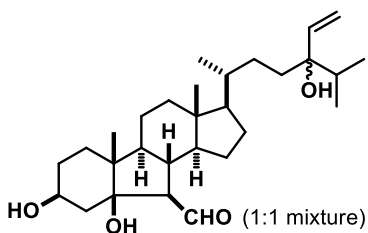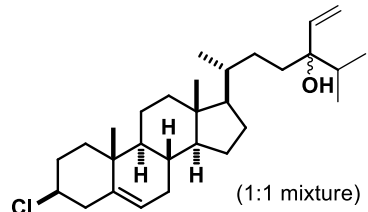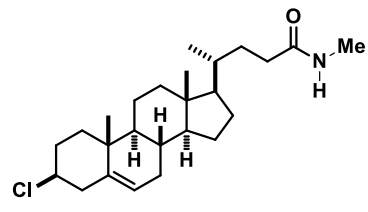
Surprisingly, leningosterol (**2.1**, mixture of epimers) exhibited moderate activity with a MIC of 20 μg/mL. Likewise, the separated epimers **2.1a** and **2.1b** showed MIC's values of 22 μg/mL and 24.0 μg/mL, respectively. Our hypothesis that perhaps one of the epimers was more active than the other was not supported by these results. β-hydroxy leningosterol (**2.37**, mixture of epimers) was even less active with a MIC of 53 μg/mL (entry 5). Furthermore, when they evaluated our sample of synthetic saringosterol (**1.22**) the MIC was 2 μg/mL (entry 1), a value much higher than that reported by Timmermann and co-workers (0.25 μg/mL).[1] It is important to remark here that both samples of saringosterols (**1.22**, our sample and that of Timmermann's) were evaluated at the same Institute although using different techniques. Back in 2001, the Institute of Tuberculosis Research utilized BACTEC [a radiometric drug susceptibility system that measures the $^{14}CO_2$ produced by metabolic breakdown of (1- ^{14}C) palmitic acid in a liquid Middlebrook 7H12 medium] for the evaluation of Timmermann's sample, whereas MABA was used (in 2012) for our sample.[51] The latter method consists of a dilution bioassay in which Alamar blue dye is used as an oxidation/reduction indicator that allows the growth/inhibition to be quantitated spectrophotometrically or fluorometrically. Presumably, there should not exist large discrepancies such as these depending on which technique is used, as has been demonstrated by Collins and Franzblau.[51] The differences in MIC values obtained suggest that perhaps the cells were not at their optimum conditions or that errors were made during sample preparations. As mentioned earlier, the pharmacokinetic studies of Timmermann's saringosterol (**1.22**) sample were hampered by solubility problems, thus there is a possibility that

solubility issues also affected our results.[1,2] Moreover, is surprising the moderate activity of leningosterol (**2.1**, MIC = 20 μg/mL) since it was carefully designed based on the structures of active compounds. Also, it is interesting that in the library of Rodríguez and co-workers the least active *abeo*-sterol was compound **1.50** (cholesterol *abeo*-sterol, MIC = 15 μg/mL) and leningosterol (**2.1**) was even less active.[4]

The chlorinated derivatives were also evaluated to determine the effect of this functional group on the anti-tubercular activity. However, 3β-chloro saringosterol (**2.25**) and 3β-chloro methyl amide **2.26** were inactive with MIC values > 128 μg/mL (entries 6 and 7) suggesting the importance of the hydroxyl group at C-3 for the detection of biological activity.

Table 2.5 **Screening results of anti-tubercular activity for compounds 1.22, 2.1, 2.25, 2.26 and 2.37.**

Entry	Compound	Structure	MIC[a] (μg/mL)
1	1.22	(1:1 mixture)	2[b]
2	2.1	(1:1 mixture)	20[b]

Entry	Compound	Structure	MIC[a] (μg/mL)
3	2.1a		22[b]
4	2.1b		24[b]
5	2.37	(1:1 mixture)	53[b]
6	2.25	(1:1 mixture)	>128[c]
7	2.26		>128[c]
8	RMP[d]		0.08

a Lowest drug concentration that effected an inhibition of P90% relative to untreated cultures.

b Values are means of several screenings

c Values of one screening.

d Rifampicin was used as a positive control.

2.6. Conclusions

The design of leningosterol (**2.1**) was strictly based on the purportedly anti-tubercular activity of saringosterol (**1.22**) and parguesterol A (**1.24**).[1,4] Its initial synthesis was plagued by the formation of unexpected by-products and insurmountable problems with the aldol condensation step. Several routes were explored to finally obtain the desired product in 10 steps with a 7% overall yield. Surprisingly, leningosterol (**2.1**) was significantly less potent than saringosterol (**1.22**). There were also serious experimental discrepancies with the anti-tubercular values reported for saringosterol (**1.22**) (between the one synthesized by us and that reported by Timmermann and co-workers).[1] The moderate anti-TB activity detected for the *abeo*-sterol analogs synthesized by us was certainly unexpected since their design was based carefully on the structures of active compounds. Also, some chlorinated derivatives were prepared and evaluated which turn out to be inactive. Specifically, 3β-chloro saringosterol (**2.25**) confirmed that the hydroxyl group is needed for the anti-tubercular activity. Nevertheless, it is important to synthesize additional derivatives with different *R* groups at C-24 to gauge the importance of the substituents at this position and whether or not they are pivotal for the anti-tubercular activity of *abeo*-sterols.

2.7. Experimental Section

2.7.1. General Experimental Methods

All of the reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an atmosphere of argon. Column chromatography (CC) was performed on silica gel (35–75 μm); reactions were followed by TLC analysis using glass pre-coated silica gel plates with fluorescent indicator (254 nm) and visualized with a UV lamp, I_2 vapors, or 10% ethanolic sulfuric acid followed by heating. Semipreparative RP-HPLC was performed using an UV detector set at 254 nm and a column with 5 μm, 250 x 4.6 mm size with a flow rate of 1.0 mL/min. Solvents and commercially available reagents were purchased and used as received without further purification. Starting material, 3β-hydroxy-5-cholenic acid (**2.3**), was obtained from Steraloids and TCI America (> 97% purity, CAS. No. 5255-17-4) and was used without further purification. Melting points were determined on a Melt Temp using 100 mm x 1mm capillary tubes. Optical rotations were recorded with a polarimeter using a 0.5 mL capacity cell with 1 dm path length. Infrared spectra were recorded using thin films supported on NaCl discs. UV were recorded on a UV-Vis spectrophotometer using quartz cuvettes and MeOH as solvent. ^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the specified field strength on a 700 or 500 MHz spectrometer. Spectra were obtained on CDCl$_3$ solutions in 5 mm diameter tubes, and chemical shifts are quoted in parts per million relative to the residual signals of CHCl$_3$ (δ_H = 7.26 ppm, δ_C = 77.0 ppm). Multiplicities in the ^{1}H NMR spectra are described as follows: s =

singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants are reported in Hertz. High-resolution mass spectrometry (HRMS) was performed using a quadrupole mass analyzer, and the data are reported with ion mass/charge (*m/z*) ratios as values in atomic mass units. Yields shown are based on recovered starting material. Product characterization was mainly established by NMR, new compounds have full data except if there was little amount of sample or the compound was labile. Elemental analysis was not performed due to the lability of the compounds with an aldehyde group. The anti-tubercular activity assay was performed at the Institute for Tuberculosis Research, University of Illinois, Chicago against the *Mtb* strain $H_{37}Rv$ by laboratory technicians Yuehong Wang, Baoji Wang, and Rui Ma. For this purpose, they used the Microplate Alamar Blue Assay (MABA) with rifampicin (**1.2**) as a positive control. Compounds with MIC's > 64 μg/mL are typically considered inactive.

2.7.2. Experimental Details

Methyl 3β-hydroxycholest-5-en-24-oate (1.37)
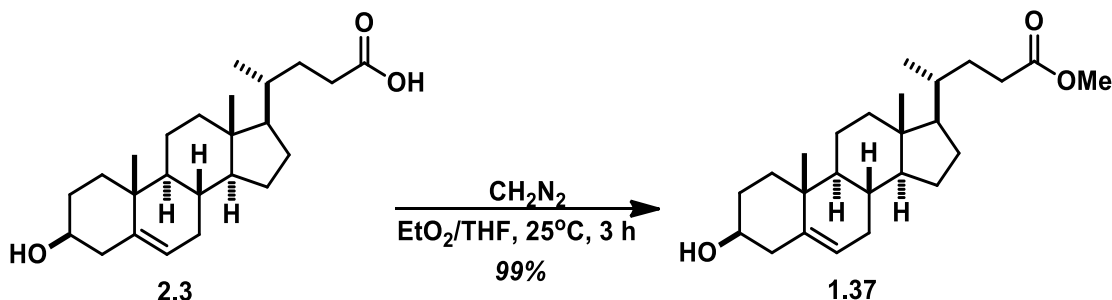
To a solution of 3β-hydroxy-5-cholenic acid (570 mg, 1.5 mmol) in THF (10 mL) was added freshly prepared diazomethane in diethyl ether (45 mL). The reaction mixture was stirred at 25 °C for 3 h and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (4:1)] generated methyl ester **1.37** as a white powder in 99% yield (585 mg). IR (film) $\tilde{\nu}_{max}$ 3426, 2937, 2848, 1738, 1436, 1377, 1048 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (br d, J = 5.2 Hz, 1H, H-6), 3.65 (s, 3H, H-OMe), 3.51 (m, 1H, H-3), 2.39-2.18 (br envelope, 4H), 2.10-0.9 (br envelope, 21H), 0.99 (s, 3H, H-19), 0.91 (d, J = 6.5 Hz, 3H, H-21), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 174.8 (C, C-24), 140.7 (C, C-5), 121.6 (CH, C-6), 71.7 (CH, C-3), 56.7 (CH, C-14), 55.7 (CH, C-17), 51.5 (CH$_3$, C-COOMe), 50.0 (CH, C-9), 42.3 (CH$_2$, C-4), 42.2 (C, C-13), 39.7 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.4 (C, C-10), 35.3 (CH, C-20), 31.8 (CH, C-8), 31.8 (CH$_2$, C-7), 31.6 (CH$_2$, C-2), 31.0 (CH$_2$, C-22), 30.9 (CH$_2$, C-23), 28.1 (CH$_2$, C-16), 24.2 (CH$_2$, C-15), 21.0 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 18.3 (CH$_3$, C-21), 11.8 (CH$_3$, C-18). The NMR data were in accordance with published data.[54]

Methyl 3β-dimethyl-*t*-butylsilyloxycholest-5-en-24-oate (2.4)
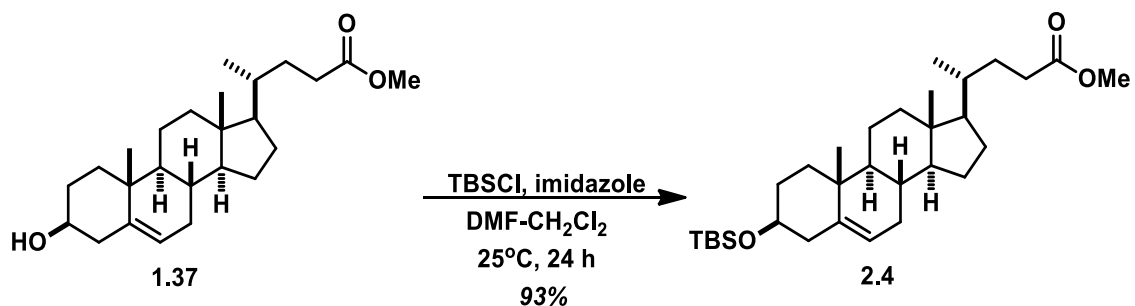
To a solution of methyl ester **1.37** (585 mg, 1.5 mmol) in dry CH_2Cl_2-DMF (25 mL, 4:1) was added imidazole (255 mg, 3.8 mmol) and TBSCl (1.0 M in CH_2Cl_2, 2.25 mL). After the reaction mixture was stirred for 24 h at 25 °C, it was dissolved in 20 mL of H_2O, and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (4:1)] afforded protected alcohol **2.4** as a white powder in 93% yield (704 mg). $[\alpha]_D^{20}$ − 7.0 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}_{max}$ 2939, 2880, 1744, 1438, 1252, 1101, 837, 777 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (br d, *J* = 5.2 Hz, 1H, H-6), 3.66 (s, 3H, H-OMe), 3.48 (m, 1H, H-3), 2.39-2.16 (br envelope, 4H), 2.02-0.90 (br envelope, 21H), 0.99 (s, 3H, H-19), 0.92 (d, *J* = 6.5 Hz, 3H, H-21), 0.89 (s, 9H, H-OTBS), 0.67 (s, 3H, H-18), 0.05 (s, 6H, H-OTBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.8 (C, C-24), 141.6 (C, C-5), 121.1 (CH, C-6), 72.6 (CH, C-3), 56.8 (CH, C-14), 55.8 (CH, C-17), 51.5 (CH_3, C-COOMe), 50.2 (CH, C-9), 42.8 (CH_2, C-4), 42.4 (C, C-13), 39.8 (CH_2, C-12), 37.4 (CH_2, C-1), 36.6 (C, C-10), 35.4 (CH, C-20), 32.1 (CH_2, C-2), 31.9 (CH, C-8), 31.9 (CH_2, C-7), 31.1 (CH_2, C-22), 31.0 (CH_2, C-23), 28.1 (CH_2, C-16), 25.9 (C, C-OTBS), 24.3 (CH_2, C-15), 21.1 (CH_2, C-11), 19.4 (CH_3, C-19), 18.3 (CH_3, C-21), 18.2 (CH_3, C-OTBS),

11.9 (CH_3, C-18), -4.6 (CH_3, C-OTBS). The NMR data were in accordance with published data.[53]

3β-Dimethyl-*t*-butylsilyloxy-*N*-methoxy, *N*-methylcholest-5-ene-24-carboxamide (2.5)
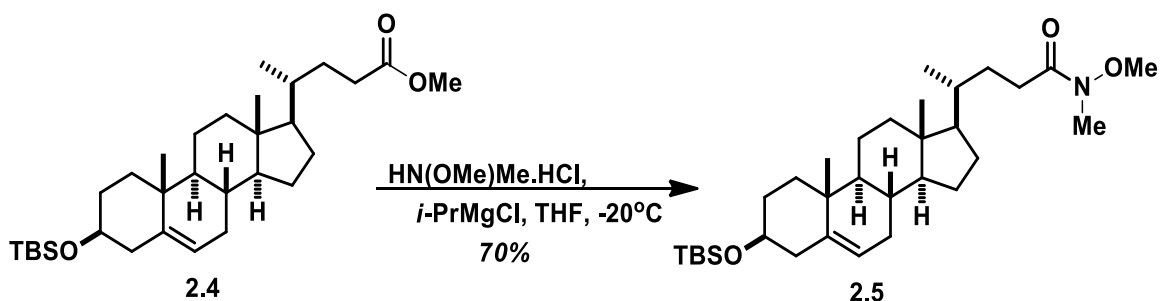
To a slurry of compound **2.4** (240 mg, 0.48 mmol) and (OMe)MeNH.HCl (70 mg, 0.72 mmol) in dry THF (6 mL) was added *i*PrMgCl (2.0 M in THF, 0.48 mL) dropwise at -20 °C over 15 min. The reaction mixture was stirred for 20 min at -10 ° C and (OMe)MeNH.HCl (70 mg, 0.72 mmol) and *i*PrMgCl (2.0 M in THF, 0.48 mL) were added at -20 °C. This procedure was repeated two more times. The reaction mixture was quenched with saturated aqueous NH_4Cl solution (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (4:1)] afforded Weinreb amide **2.5** as a white powder in 70% yield (178 mg). $[\alpha]_D^{20}$ - 18.0 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}_{max}$ 2935, 2881, 1647, 1470, 1383, 1256, 1088, 839, 771 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (br d, *J* = 5.2 Hz, 1H,H-6), 3.69 (s, 3H, H-NOMe), 3.48 (m, 1H, H-3), 3.17 (s, 3H, H-NMe),

2.49-2.13 (br envelope, 4H), 2.02-0.90 (br envelope, 21H), 0.99 (s, 3H,H-19), 0.95

(d, J = 6.5 Hz, 3H, H-21), 0.88 (s, 9H, H-OTBS), 0.68 (s, 3H, H-18), 0.05 (s, 6H,

H-OTBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 175.3 (C, C-24), 141.6 (C, C-5), 121.2

(CH, C-6), 72.7 (CH, C-3), 61.2 (CH$_3$, C-NOMe), 56.8 (CH, C-14), 55.9 (CH, C-

17), 50.2 (CH, C-9), 42.8 (CH$_2$, C-4), 42.4 (C, C-13), 39.8 (CH$_2$, C-12), 37.4 (CH$_2$,

C-1), 36.6 (C, C-10), 35.6 (CH, C-20), 32.2 (CH$_3$, C-NMe), 32.1 (CH$_2$, C-2), 31.9

(CH, C-8), 31.9 (CH$_2$, C-7), 30.8 (CH$_2$, C-22), 28.9 (CH$_2$, C-23), 28.1 (CH$_2$, C-16),

26.0 (C, C-OTBS), 24.3 (CH$_2$, C-15), 21.1 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 18.5

(CH$_3$, C-21), 18.3 (CH$_3$, C-OTBS), 11.9 (CH$_3$, C-18), -4.6 (CH$_3$, C-OTBS); ESI-

LRMS m/z [M + Na]$^+$ 554.4.

3β-Dimethyl-t-butylsilyloxy-N-methoxy, N-methyl-5,6-$seco$-5-oxo-cholest-6-al-24-carboxamide (2.2)
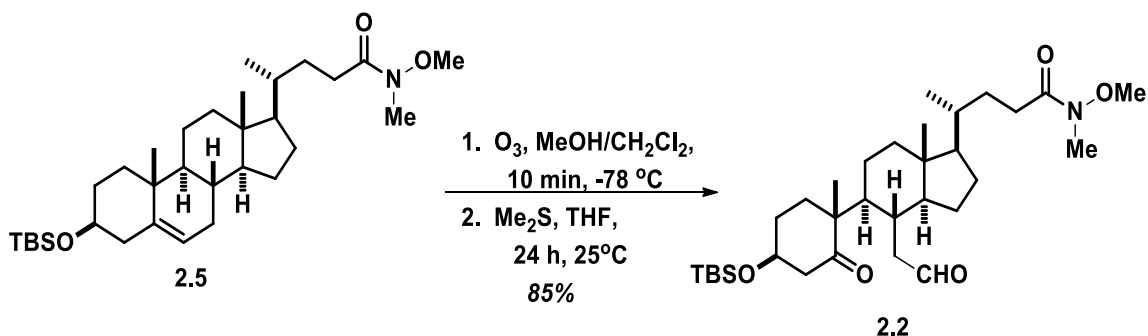
A stream of 2% O$_3$/O$_2$ was bubbled through a disposable pipet into a solution of

compound **2.5** (178 mg, 0.33 mmol) in CH$_2$Cl$_2$-MeOH (4:1, 10 mL) at -78 ° C, until

the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C,

the solvent was evaporated, and the residue obtained was stirred with a mixture

of dimethyl sulfide (0.4 mL, 5.5 mmol) and THF (10 mL) for 16 h at 25 °C. Then, it was concentrated *in vacuo* and purified by flash-Silica gel column chromatography [Hex/EtOAc (3:1)] to obtain keto-aldehyde **2.2** in 85% yield (160 mg) as a colorless oil. ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.58 (s, 1H, H-6), 4.36 (br s, 1H, H-3), 3.67 (s, 3H, H-NOMe), 3.15 (s, 3H, H-NMe), 2.93 (br d, J = 10.5 Hz, 1H, H-4β), 2.49-0.90 (br envelope, 24H), 0.98 (s, 3H,H-19), 0.93 (d, J = 6.5 Hz, 3H, H-21), 0.82 (s, 9H, H-OTBS), 0.66 (s, 3H, H-18), 0.01 (s, 3H, H-OTBS), -0.02 (s, 3H, H-OTBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 216.8 (C, C-5), 202.8 (CH, C-6), 175.3 (C, C-24), 71.3 (CH, C-3), 61.2 (CH$_3$, C-NOMe), 55.8 (CH, C-17), 54.0 (CH, C-14), 52.3 (C, C-10), 47.2 (CH$_2$, C-4), 44.0 (CH$_2$, C-7), 42.5 (C, C-13), 41.9 (CH, C-9), 39.8 (CH$_2$, C-12), 35.4 (CH, C-20), 34.7 (CH, C-8), 33.6 (CH$_2$, C-1), 32.1 (CH$_3$, C-NMe), 30.5 (CH$_2$, C-22), 28.8 (CH$_2$, C-2), 28.7 (CH$_2$, C-23), 27.7 (CH$_2$, C-16), 25.6 (C, C-OTBS), 25.2 (CH$_2$, C-15), 23.1 (CH$_2$, C-11), 18.3 (CH$_3$, C-OTBS), 17.9 (CH$_3$, C-21), 17.6 (CH$_3$, C-19), 11.4 (CH$_3$, C-18), -5.0 (CH$_3$, C-OTBS), -5.1(CH$_3$, C-OTBS).

Synthesis of compounds 2.6 and 2.7
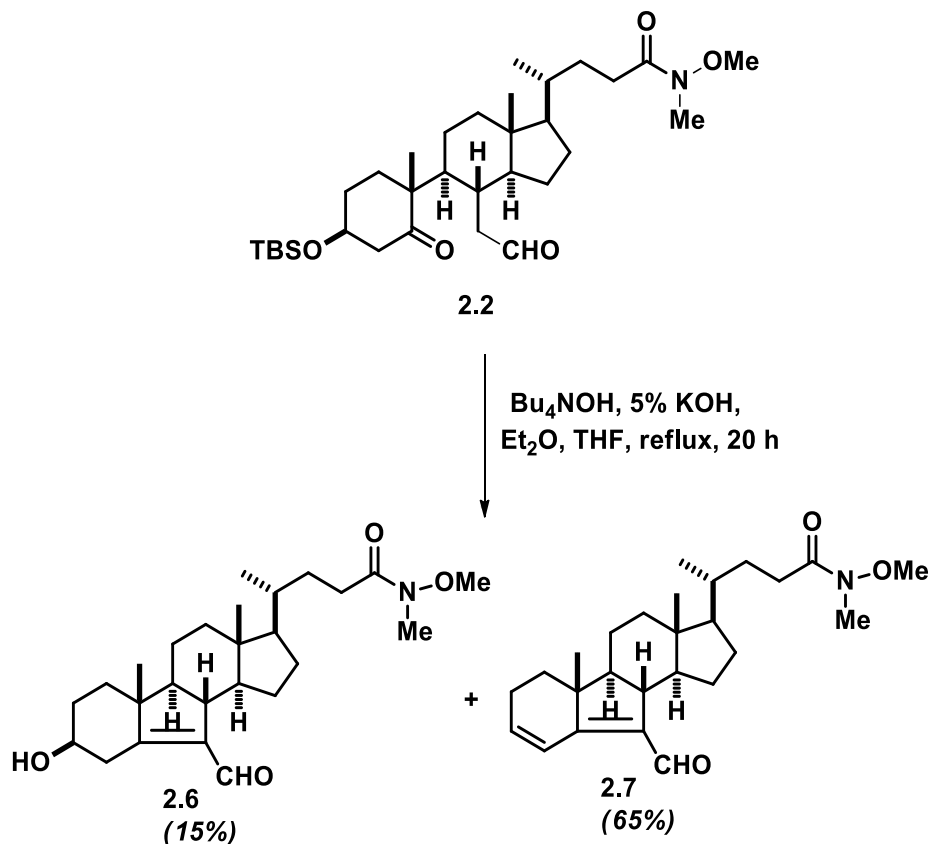
To a mixture of KOH (5% in H_2O, 2 mL) and Bu_4NOH (0.1 mL, 0.15 mmol) in THF (3 mL) was added compound **2.2** (50 mg, 0.09 mmol) in Et_2O (5 mL). The reaction mixture was refluxed for 20 h and after allowing it to reach 25 °C, it was poured onto water (7 mL) and extracted with Et_2O (3 x 7 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (9:1)] generated *abeo*-sterols **2.6** (6 mg) and **2.7** (24 mg) as colorless oils in 15% and 65% yield, respectively.

3β-Hydroxy-*N*-methoxy, *N*-methyl-5(6→7)-*abeo*-cholest-5-ene-6-al-24-carboxamide (2.6)

$[\alpha]_D^{20}$ − 21.0 (*c* 1.0, CHCl₃); IR (film) $\tilde{\nu}_{max}$ 3418, 2929, 2860, 1672, 1462, 1381 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.98 (s, 1H, H-6), 3.69 (s, 3H, H-NOMe), 3.69 (m, 1H, H-3), 3.46 (dd, *J* = 14.4, 2.6 Hz, 1H, H-4β), 3.17 (s, 3H, H-NMe), 2.55-0.90 (br envelope, 22H), 0.96 (d, *J* = 6.5 Hz, 3H, H-21), 0.92 (s, 3H, H-19), 0.74 (s, 3H, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ 189.6 (CH, C-6), 175.3 (C, C-24), 169.2 (C, C-5), 139.2 (C, C-7), 70.8 (CH, C-3), 61.2 (CH₃, C-NOMe), 60.1 (CH, C-9), 55.1 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.7 (CH₂, C-12), 36.1 (CH₂, C-1), 35.4 (CH, C-20), 33.8 (CH₂, C-4), 32.1 (CH₃, C-NMe), 31.2 (CH₂, C-2), 30.8 (CH₂, C-22), 28.7 (CH₂, C-23), 28.4 (CH₂, C-16), 26.5 (CH₂, C-15), 20.7 (CH₂, C-11), 18.7 (CH₃, C-21), 15.6 (CH₃, C-19), 12.5 (CH₃, C-18). ESI-LRMS *m/z* [M + Na]⁺ 454.5; [M + H]⁺ 432.5.

***N*-Methoxy, *N*-methyl-5(6→7)-*abeo*-cholest-2,5-diene-6-al-24-carboxamide (2.7)**

$[\alpha]_D^{20}$ − 32.8 (*c* 0.18, CHCl₃); UV (MeOH) λ_{max} 305 (ε 622), 201 (ε 7858) nm; IR (film) $\tilde{\nu}_{max}$ 2932, 2860, 1717, 1653, 1457, 1384 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 10.0 (s, 1H, H-6), 6.91 (br d, *J* = 9.7 Hz, 1H, H-4), 6.23 (br s, 1H, H-3), 3.69 (s, 3H, H-NOMe), 3.18 (s, 3H, H-NMe), 2.66 (br t, *J* = 10.6 Hz, 1H, H-8), 2.48-1.05 (br envelope, 20H), 0.98 (d, *J* = 6.6 Hz, 3H, H-21), 0.90 (s, 3H, H-19), 0.76 (s, 3H, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ 189.5 (CH, C-6), 175.3 (C, C-24), 163.6 (C, C-

5), 138.4 (CH, C-4), 135.8 (C, C-7), 120.7 (CH, C-3), 61.3 (CH$_3$, C-NOMe), 59.5 (CH, C-9), 55.1 (CH, C-17), 54.2 (CH, C-14), 45.5 (C, C-13), 45.0 (CH, C-8), 44.7 (C, C-10), 39.7 (CH$_2$, C-12), 35.4 (CH, C-20), 34.1 (CH$_2$, C-1), 32.2 (CH$_3$, C-NMe), 30.8 (CH$_2$, C-22), 28.7 (CH$_2$, C-23), 28.5 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 23.9 (CH$_2$, C-2), 20.6 (CH$_2$, C-11), 18.7 (CH$_3$, C-21), 14.8 (CH$_3$, C-19), 12.6 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 414.5.

Synthesis of compounds 1.35, 1.50, and 1.70

1.34   1.35   1.50   1.70
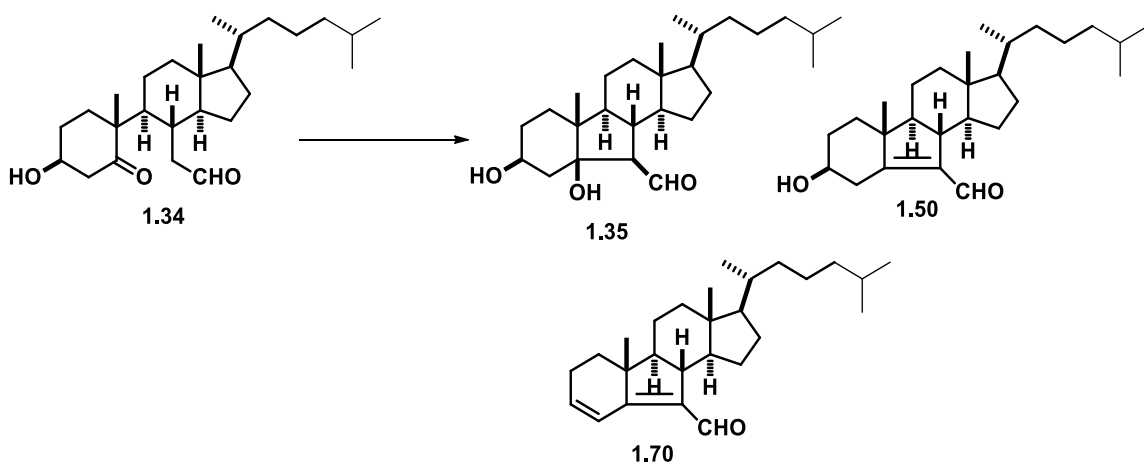
1. **General procedure for the aldol reaction with PTC**

To a mixture of KOH (5% in H$_2$O, 1 mL) and Bu$_4$NOH (0.05 mL, 0.08 mmol) in THF (2 mL) was added compound **1.34** (25 mg, 0.06 mmol) in Et$_2$O (3 mL). The reaction mixture was stirred at 25 °C for 2 h and then was dissolved in water (5 mL) and extracted with Et$_2$O (3 x 5 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*.

2. General procedure for the aldol reaction with pyrrolidine

To a solution of compound **1.34** (15 mg, 0.04 mmol) in toluene (1mL) was added pyrrolidine (0.7 μL, 0.01 mmol) and benzoic acid (0.4 μL, 0.004 mmol). The reaction mixture was stirred at 25 °C for 24 h and quenched with saturated aqueous NH_4Cl (3 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*.

3. General procedure for the aldol reaction with KOH(aq)

To a solution of compound **1.34** (20 mg, 0.05 mmol) in MeOH-CH_2Cl_2 (2 mL, 1:1) was added KOH (0.1M in H_2O, 2 mL). The reaction mixture was stirred at 25 °C for 60 h and quenched with saturated aqueous NH_4Cl (3 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*.

4. General procedure for the aldol reaction with methanolic KOH

To compound **1.34** (25 mg, 0.06 mmol) was added KOH (1.0 M in MeOH, 2 mL) and TEA (0.5 mL, 0.04 mmol). The reaction mixture was stirred at 25 °C for 48 h and quenched with saturated aqueous NH_4Cl (3 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*.

5. General procedure for the aldol reaction with PPTS

To a solution of compound **1.34** (20 mg, 0.05 mmol) in dry CH_2Cl_2 (3 mL) was added PPTS (12 mg, 0.05 mmol). The reaction mixture was stirred at 25 °C for 24 h filtered through a Silica gel column and concentrated *in vacuo*.

6. General procedure for the aldol reaction with Co(OAc)$_2$.4H$_2$O

To a solution of compound **1.34** (20 mg, 0.05 mmol) in dry DMF (3 mL) was added $Co(OAc)_2.4H_2O$ (3 mg, 0.01 mmol) and 2,2'-bipyridine (2 mg, 0.01 mmol). The reaction mixture was stirred at 80 °C for 18 h and quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*.

7. General procedure for the aldol reaction with TiCl$_4$

To a solution of compound **1.34** (20 mg, 0.05 mmol) in dry toluene (3 mL) was added $TiCl_4$ (1.0 M in toluene, 0.2 mL) and TMEDA (30 µL, 0.2 mmol) at -50 °C. The reaction mixture was stirred at 25 °C for 24 h and quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*.

3β, 5β-Dihydroxy-5(6→7)-*abeo*-cholest-6-al (1.35)

^{1}H NMR (500 MHz, CDCl$_3$) δ 9.68 (s, 1H, H-6), 4.11 (br s, 1H, H-3), 2.37-0.90 (br envelope, 26H), 0.93 (s, 3H, H-19), 0.92 (d, *J* = 6.5 Hz, 3H, H-21), 0.86 (d, *J* = 6.5

Hz, 6H, H-26, H-27), 0.71 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 204.8 (CH, C-6), 84.3 (C, C-5), 67.3 (CH, C-3), 63.9 (CH, C-7), 56.1 (CH, C-14), 55.7 (CH, C-17), 50.5 (CH, C-9), 45.5 (C, C-10), 44.7 (C, C-13), 44.3 (CH$_2$, C-4), 40.0 (CH, C-8), 39.7 (CH$_2$, C-12), 39.5 (CH$_2$, C-24), 36.2 (CH$_2$, C-22), 35.6 (CH, C-20), 28.3 (CH$_2$, C-16), 28.0 (CH, C-25), 27.9 (CH$_2$, C-2), 26.7 (CH$_2$, C-1), 24.6 (CH$_2$, C-15), 23.8 (CH$_2$, C-23), 22.8 (CH$_3$, C-27), 22.5 (CH$_3$, C-26), 21.6 (CH$_2$, C-11), 18.7 (CH$_3$, C-21), 18.4 (CH$_3$, C-19), 12.5 (CH$_3$, C-18). The NMR data were in accordance with published data.[54]

3β-Hydroxy-5(6→7)-*abeo*-cholest-5-ene-6-al (1.50)

IR (film) $\tilde{\nu}_{max}$ 3400, 2952, 2869, 1709, 1466, 1382 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.96 (s, 1H, H-6), 3.71 (m, 1H, H-3), 3.47 (ddd, J = 2.5, 4.0, 14.5 Hz, 1H, H-4β), 2.55 (dt, J = 4.0, 11.0 Hz, 1H, H-8), 2.09-0.90 (br envelope, 24H), 0.93 (s, 3H, H-19), 0.92 (d, J = 6.6 Hz, 3H, H-21), 0.87 (d, J = 6.6 Hz, 3H, H-27), 0.86 (d, J = 6.6 Hz, 3H, H-26), 0.73 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.7 (CH, C-6), 169.0 (C, C-5), 139.3 (C, C-7), 70.9 (CH, C-3), 60.1 (CH, C-9), 55.4 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.8 (CH$_2$, C-12), 39.5 (CH$_2$, C-24), 36.2 (CH$_2$, C-22), 36.1 (CH$_2$, C-1), 35.6 (CH, C-20), 33.9 (CH$_2$, C-4), 31.2 (CH$_2$, C-2), 28.5 (CH$_2$, C-16), 28.0 (CH, C-25), 26.6 (CH$_2$, C-15), 23.9 (CH$_2$, C-23), 22.8 (CH$_3$, C-27), 22.5 (CH$_3$, C-26), 20.7 (CH$_2$, C-11), 18.9 (CH$_3$, C-21), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18). The NMR data were in accordance with published data.[4,55]

5(6→7)-*abeo*-Cholest-3,5-diene-6-al (1.70)

^{1}H NMR (500 MHz, CDCl$_3$) δ 10.0 (s, 1H, H-6), 6.92 (br d, J = 9.7 Hz, 1H, H-4), 6.23 (br s, 1H, H-3), 2.68 (br t, J = 10.6 Hz, 1H, H-8), 2.42-1.00 (br envelope, 23H), 0.94 (d, J = 6.5 Hz, 3H, H-21), 0.93 (s, 3H, H-19), 0.86 (d, J = 6.6 Hz, 6H, H-26, H-27),0.76 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.7 (CH, C-6), 163.6 (C, C-5), 138.4 (CH, C-4), 135.9 (C, C-7), 120.8 (CH, C-3), 59.5 (CH, C-9), 55.4 (CH, C-17), 54.3 (CH, C-14), 45.4 (C, C-13), 45.0 (CH, C-8), 44.7 (C, C-10), 39.7 (CH$_2$, C-12), 39.5 (CH$_2$, C-24), 36.2 (CH$_2$, C-22), 35.6 (CH, C-20), 34.1 (CH$_2$, C-1), 28.5 (CH$_2$, C-16), 28.0 (CH, C-25), 26.6 (CH$_2$, C-15), 23.9 (CH$_2$, C-2), 23.9 (CH$_2$, C-23), 22.8 (CH$_3$, C-27), 22.6 (CH$_3$, C-26), 20.6 (CH$_2$, C-11), 18.9 (CH$_3$, C-21), 14.8 (CH$_3$, C-19), 12.5 (CH$_3$, C-18). The NMR data were in accordance with published data.[55]

Synthesis of compound 2.8
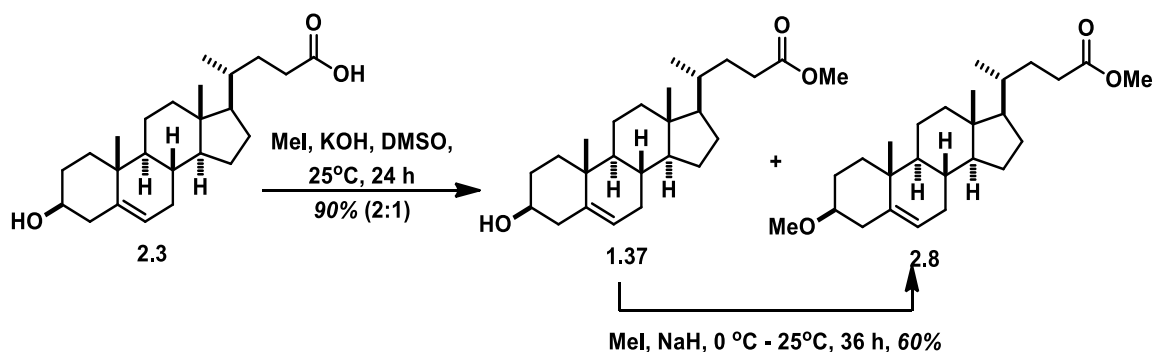
To a solution of KOH (272 mg, 4.3 mmol) in dry DMSO (10 mL) was added compound **2.3** (200 mg, 0.5 mmol) and MeI (0.13 mL, 2.1 mmol).The reaction mixture was stirred at 25 °C for 12 h, diluted with water (10 mL) and extracted with

EtOAc (3 x 20 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (4:1)] generated compounds **1.37** (130 mg) and **2.8** (65 mg) as white powders in 63% and 30% yield, respectively.

NaH (60% in mineral oil) was washed three times with dry hexane. To a solution of NaH (20 mg, 0.8 mmol) in dry THF (3 mL) was added compound **1.37** (130 mg, 0.3 mmol) in dry THF (10 mL) and was stirred for 30 min. The temperature was lowered to 0 °C and MeI (0.04 mL, 0.6 mmol) was added. The reaction mixture was stirred at 25 °C for 36 h. The reaction mixture was quenched with HCl (1.0 M, 10 mL) and extracted with EtOAc (2 x 15 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (9:1)] generated compound **2.8** (81 mg) as a white powder in 60% yield.

Methyl 3β-methoxycholest-5-ene-24-oate (2.8)

IR (film) $\tilde{\nu}_{max}$ 2935, 2849, 1736, 1433, 1377, 1196, 1102 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (br d, J = 5.0 Hz, 1H, H-6), 3.64 (s, 3H, H-COOMe), 3.33 (s, 3H,H-OMe), 3.03 (m, 1H, H-3), 2.39-0.90 (br envelope, 25H), 0.97 (s, 3H, H-19), 0.90 (d, J = 6.5 Hz, 3H, H-21), 0.66 (s, 3H,H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 174.7 (C, C-24), 140.8 (C, C-5), 121.8 (CH, C-6), 80.3 (CH, C-3), 56.7 (CH, C-14), 55.7 (CH, C-17), 55.5 (CH$_3$, C-OMe), 51.4 (CH$_3$, C-COOMe), 50.1 (CH, C-9), 42.3 (C,C-13), 39.7 (CH$_2$, C-12), 38.6 (CH$_2$, C-4), 37.1 (CH$_2$, C-1), 36.8 (C, C-10), 35.3 (CH,

C-20), 31.9 (CH$_2$, C-7), 31.8 (CH, C-8), 31.0 (CH$_2$, C-23), 30.9 (CH$_2$, C-22), 28.1 (CH$_2$, C-16), 27.9 (CH$_2$, C-2), 24.2 (CH$_2$, C-15), 21.0 (CH$_2$, C-11), 19.3 (CH$_3$, C-19), 18.3 (CH$_3$, C-21), 11.8 (CH$_3$, C-18).

3β-Methoxy-*N*-methoxy, *N*-methylcholest-5-ene-24-carboxamide (2.9)
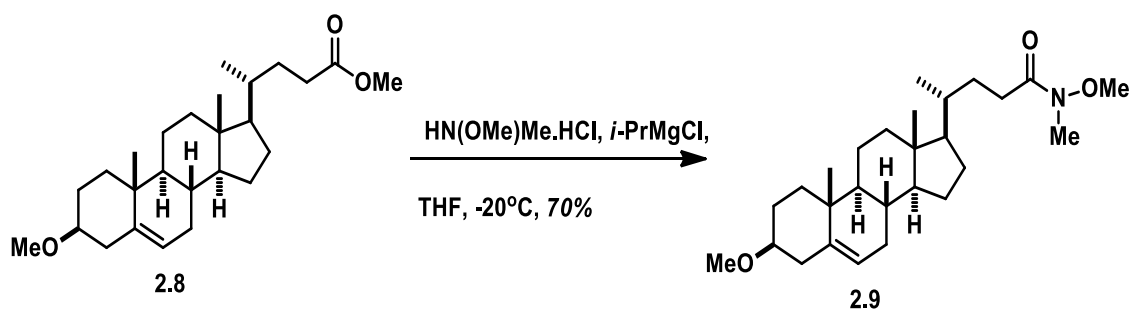
To a slurry of compound **2.8** (130 mg, 0.32 mmol) and (OMe)MeNH.HCl (47 mg, 0.48 mmol) in dry THF (5 mL) was added *i*PrMgCl (2.0 M in THF, 0.32 mL) dropwise at -20 °C over 15 min. The reaction mixture was stirred for 20 min at -10 ° C and (OMe)MeNH.HCl (47 mg, 0.48 mmol) and *i*PrMgCl (2.0 M in THF, 0.32 mL) were added at -20 °C. This procedure was repeated two more times. The reaction mixture was quenched with saturated aqueous NH$_4$Cl (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (9:1)] afforded Weinreb amide **2.9** as a white powder in 70% yield (98 mg). $[\alpha]_D^{20}$ + 20.0 (*c* 0.1, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.34 (br d, *J* = 5.0 Hz, 1H, H-6), 3.68 (s, 3H, H-NOMe), 3.34 (s, 3H, H-OMe), 3.16 (s, 3H, H-NMe), 3.05 (m, 1H, H-3), 2.49-0.90 (br envelope, 25H), 0.98 (s, 3H, H-

19), 0.94 (d, J = 6.5 Hz, 3H, H-21), 0.67 (s, 3H,H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 175.3 (C, C-24), 140.8 (C, C-5), 121.5 (CH, C-6), 80.3 (CH, C-3), 61.2 (CH$_3$, C-NOMe), 56.7 (CH, C-14), 55.8 (CH, C-17), 55.6 (CH$_3$, C-OMe), 50.1 (CH, C-9), 42.3 (C,C-13), 39.7 (CH$_2$, C-12), 38.6 (CH$_2$, C-4), 37.1 (CH$_2$, C-1), 36.8 (C, C-10), 35.5 (CH, C-20), 32.2 (CH$_3$, C-NMe), 31.9 (CH$_2$, C-7), 31.8 (CH, C-8), 30.7 (CH$_2$, C-22), 28.8 (CH$_2$, C-23), 28.1 (CH$_2$, C-16), 27.9 (CH$_2$, C-2), 24.2 (CH$_2$, C-15), 21.0 (CH$_2$, C-11), 19.3 (CH$_3$, C-19), 18.4 (CH$_3$, C-21), 11.8 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 432.6.

3β-Methoxy-*N*-methoxy, *N*-methyl -5,6-*seco*-6-oxo-cholest-6-al-24-carboxamide (2.10)
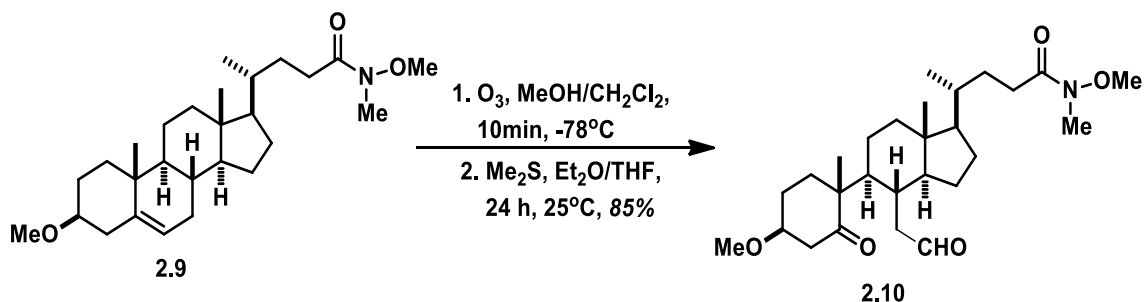
A stream of 2% O$_3$/O$_2$ was bubbled through a disposable pipet into a solution of compound **2.9** (98 mg, 0.32 mmol) in CH$_2$Cl$_2$-MeOH (4:1, 7 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of dimethyl sulfide (0.2 mL, 2.8 mmol) and THF (7 mL) for 16 h at 25 °C. Then, it was concentrated *in vacuo* and purified by flash-Silica gel column chromatography

[Hex/EtOAc (4:1)] to obtain keto-aldehyde **2.10** in 85% yield (89 mg) as a colorless

oil. ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.61 (br s, 1H, H-6), 3.85 (br s, 1H, H-3), 3.68 (s,

3H, H-NOMe), 3.26 (s, 3H, H-OMe), 3.16 (s, 3H,H-NMe), 2.92 (dd, J = 3.9, 13.7

Hz, 1H, H-4β), 2.51-0.90 (br envelope, 24H), 0.99 (s, 3H, H-19), 0.93 (d, J = 6.5

Hz, 3H, H-21), 0.68 (s, 3H,H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 216.9 (C, C-5),

202.8 (CH, C-6), 175.4 (C, C-24), 79.5 (CH, C-3), 61.2 (CH$_3$, C-NOMe), 55.8 (CH$_3$,

C-OMe), 55.8 (CH, C-17), 54.0 (CH, C-14), 52.4 (C, C-10), 44.0 (CH$_2$, C-7), 43.1

(CH$_2$, C-4), 42.6 (C,C-13), 42.0 (CH, C-9), 39.8 (CH$_2$, C-12), 35.5 (CH, C-20), 34.8

(CH, C-8), 34.0 (CH$_2$, C-1), 32.2 (CH$_3$, C-NMe), 30.5 (CH$_2$, C-22), 28.7 (CH$_2$, C-

23), 27.7 (CH$_2$, C-16), 25.2 (CH$_2$, C-2), 24.8 (CH$_2$, C-15), 23.1 (CH$_2$, C-11), 18.3

(CH$_3$, C-21), 17.5 (CH$_3$, C-19), 11.5 (CH$_3$, C-18).

Synthesis of compounds 2.11, 2.12, and 2.7
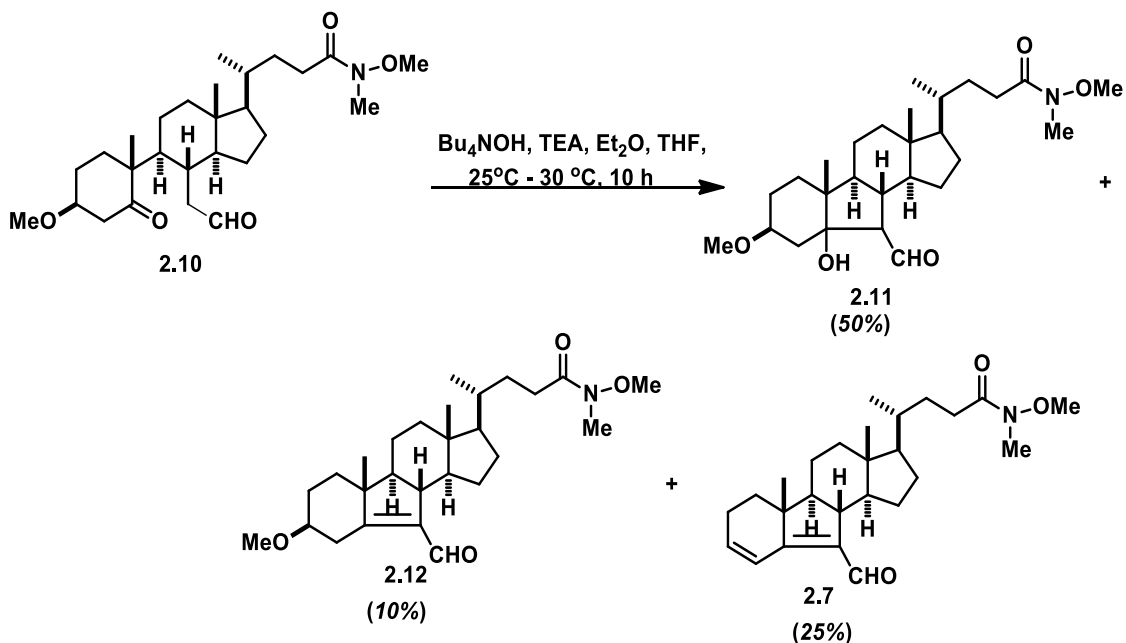
To a mixture of TEA (0.05 mL, 0.38 mmol) and Bu$_4$NOH (0.1 mL, 0.15 mmol) in THF (3 mL) was added compound **2.10** (89 mg, 0.19 mmol) in Et$_2$O (5 mL) at 25 ^{o}C. The reaction mixture was stirred at 30 ^{o}C for 10 h, then it was poured into water (7 mL) and extracted with Et$_2$O (3 x 10 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (9:1)] generated *abeo*-sterols **2.11** (44 mg), **2.12** (8 mg), and **2.7** (20 mg) as colorless oils in 50%, 10% yield, and 25%, respectively.

5β-Hydroxy-3β-methoxy-*N*-methoxy, *N*-methyl-5(6→7)-*abeo*-cholest-6-al-24-carboxamide (2.11)

IR (film) $\tilde{\nu}_{max}$ 3448, 2936, 2870, 1719, 1665, 1458, 1382, 1086 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.67 (d, *J* = 3.5 Hz, 1H, H-6), 3.68 (s, 3H, H-NOMe), 3.61 (m, 1H, H-3), 3.45 (s, -OH), 3.29 (s, 3H, H-OMe), 3.17 (s, 3H,H-NMe), 2.48-1.02 (br envelope, 25H), 0.95 (d, *J* = 6.5 Hz, 3H, H-21), 0.90 (s, 3H, H-19), 0.73 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 204.0 (CH, C-6), 175.3 (C, C-24), 84.1 (C, C-5), 76.4 (CH, C-3), 64.3 (CH, C-7), 61.2 (CH$_3$, C-NOMe), 56.1 (CH, C-14), 56.0 (CH$_3$, C-OMe), 55.5 (CH, C-17), 51.0 (CH, C-9), 45.7 (C, C-10), 44.7 (C,C-13), 42.2 (CH$_2$, C-4), 39.7 (CH$_2$, C-12), 38.7 (CH, C-8), 35.5 (CH, C-20), 32.1 (CH$_3$, C-NMe), 30.8 (CH$_2$, C-22), 28.9 (CH$_2$, C-23), 28.3 (CH$_2$, C-16), 27.1 (CH$_2$, C-1), 24.3 (CH$_2$, C-15), 23.6 (CH$_2$, C-2), 21.6 (CH$_2$, C-11), 18.6 (CH$_3$, C-21), 18.5 (CH$_3$, C-19), 12.5 (CH$_3$, C-18); ESI-LRMS *m/z* [M+H]$^+$ 464.5.

3β-Methoxy-*N*-methoxy, *N*-methyl-5(6→7)-*abeo*-cholest-5-ene-6-al-24-carboxamide (2.12)

IR (film) $\tilde{\nu}_{max}$ 2939,1717, 1665, 1461, 1382 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.96 (s,1H, H-6), 3.78 (m, 1H, H-3), 3.68 (s, 3H, H-NOMe), 3.27 (s, 3H, H-OMe), 3.16 (s, 3H,H-NMe), 2.48-1.02 (br envelope, 25H), 0.95 (d, *J* = 6.5 Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.73 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 189.6 (CH, C-6), 175.3 (C, C-24), 169.0 (C, C-5), 139.3 (C, C-7), 79.5 (CH, C-3), 61.2 (CH$_3$, C-NOMe), 60.1 (CH, C-9), 55.9 (CH$_3$, C-OMe), 55.4 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C,C-13), 39.7 (CH$_2$, C-12), 36.9 (CH$_2$, C-1), 35.5 (CH, C-20), 33.8 (CH$_2$, C-4), 32.2 (CH$_3$, C-NMe), 30.8 (CH$_2$, C-22), 28.8 (CH$_2$, C-23), 28.3 (CH$_2$, C-16), 27.8 (CH$_2$, C-2), 24.3 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 18.6 (CH$_3$, C-21), 18.0 (CH$_3$, C-19), 12.4 (CH$_3$, C-18); ESI-LRMS *m/z* [M + H]$^+$ 446.5.

N-Methoxy, *N*-methyl-5(6→7)-*abeo*-cholest-2,5-diene-6-al-24-carboxamide (2.7)

The characterization data for compound **2.7** are shown on page 84.

Synthesis of compounds 2.24 and 2.5
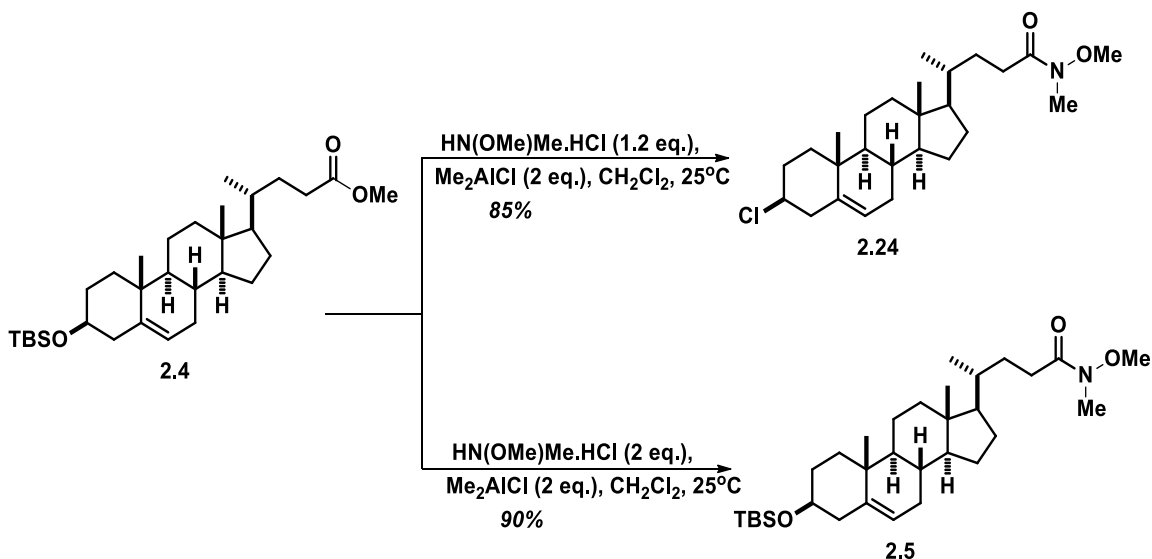
3β-Chloro-*N*-methoxy, *N*-methylcholest-5-ene-24-carboxamide (2.24)

To a solution of compound **2.4** (295 mg, 0.6 mmol) in CH_2Cl_2 (10 mL) was added

(OMe)MeNH·HCl (88 mg, 0.9 mmol) and Me_2AlCl (1.0 M in hexane, 1.5 mL). The

reaction mixture was stirred at 25 °C for 24 h, quenched with NaOH (1.0 M in H_2O,

10 mL), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were

dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash-Silica Gel column

chromatography [Hex/EtOAc (4:1)] afforded compound **2.24** (265 mg) as a

crystalline solid in 85% yield. mp 111-113 °C; $[\alpha]_D^{20}$ - 27.0 (*c* 1.0, $CHCl_3$); IR (film)

$\tilde{\nu}_{max}$ 2948, 1662, 1386, 994 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (br d, *J* = 5.0

Hz, 1H, H-6), 3.71 (m, 1H, H-3), 3.65 (s, 3H, H-NOMe), 3.13 (s, 3H, H-NMe), 2.53-

0.82 (br envelope, 25H), 0.98 (s, 3H, H-19), 0.91 (d, *J* = 6.5 Hz, 3H, H-21), 0.64

(s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.1 (C, C-24), 140.6 (C, C-5), 122.3

(CH, C-6), 61.1 (CH_3, C-NOMe), 60.1 (CH, C-3), 56.5 (CH, C-14), 55.8 (CH, C-

17), 49.9 (CH, C-9), 43.3 (CH$_2$, C-4), 42.2 (C, C-13), 39.5 (CH$_2$, C-12), 39.0 (CH$_2$, C-1), 36.2 (C, C-10), 35.4 (CH, C-20), 33.2 (CH$_2$, C-2), 32.1 (CH$_3$, C-NMe), 31.7 (CH$_2$, C-7), 31.6 (CH, C-8), 30.6 (CH$_2$, C-22), 28.7 (CH$_2$, C-23), 28.0 (CH$_2$, C-16), 24.1 (CH$_2$, C-15), 20.8 (CH$_2$, C-11), 19.1 (CH$_3$, C-19), 18.4 (CH$_3$, C-21), 11.7 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 446.3; ESI-HRMS m/z calcd for C$_{26}$H$_{43}$NO$_2$Cl [M + H]$^+$ 436.2982, found 436.2977.

3β-Dimethyl-t-butylsilyloxy-N-methoxy, N-methylcholest-5-ene-24-carboxamide (2.5)

A solution of Me$_2$AlCl (1.0 M in hexane, 1.0 mL) was added over a period of 5 min to a stirred suspension of (MeO)MeNH·HCl (100 mg, 1 mmol) in dry CH$_2$Cl$_2$ (2.5 mL) at 0 ^{o}C, and was stirred for 1 h, allowing the temperature to rise at 25 ^{o}C. Then, a solution of compound **2.4** (132 mg, 0.26 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added dropwise. The reaction mixture was stirred at 25 ^{o}C for 24 h and quenched with a phosphate buffer (pH 8.0, 5 mL). The mixture was diluted with CHCl$_3$ (5 mL) and filtered through a Celite pad and washed thoroughly with CHCl$_3$. The aqueous layer was extracted with CHCl$_3$ (2 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), dried (MgSO$_4$), and concentrated *in vacuo*. Purification by flash-Silica gel column chromatography [Hex/EtOAc (9:1)] afforded compound **2.5** (125 mg) as a white powder in 90% yield. The characterization data for compound **2.5** are shown on page 80.

Synthesis of compounds 2.25 and 2.26
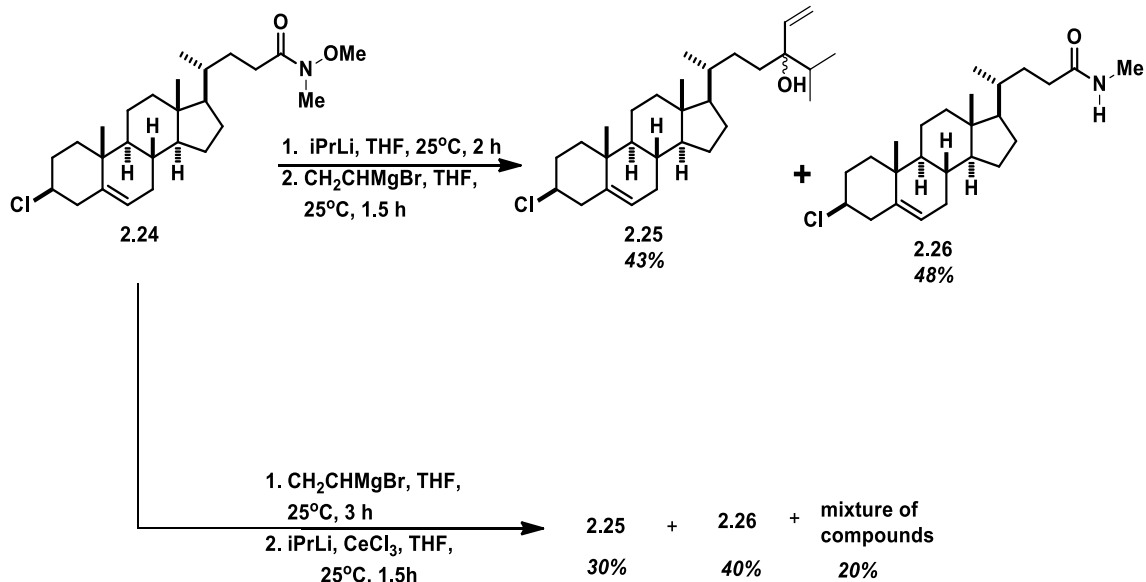
Method A: To a solution of compound **2.24** (25 mg, 0.06 mmol) in dry THF (1 mL) was added *i*PrLi (0.7 M in pentane, 0.9 mL). The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH_4Cl (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was dissolved in dry THF (1 mL) and $CH_2CHMgBr$ (1.0 M in THF, 0.3 mL) was added. The reaction mixture was stirred at 25 °C for 1.5 h, quenched with saturated aqueous NH_4Cl (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash-Silica Gel column chromatography [Hex/EtOAc (96:4)] afforded compounds **2.25** (10 mg) and **2.26** (10 mg) as white powders in 43% and 48% yield, respectively.

Method B: To a solution of compound **2.24** (22 mg, 0.05 mmol) in dry THF (1 mL) was added CH$_2$CHMgBr (1.0 M in THF, 0.5 mL) The reaction mixture was stirred at 25 °C for 3 h, quenched with saturated aqueous NH$_4$Cl (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. The crude was dissolved in dry THF (1 mL) and *i*PrLi (0.7 M in pentane, 0.9 mL) was added. The reaction mixture was stirred at 25 °C for 1.5 h, quenched with saturated aqueous NH$_4$Cl (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash-Silica Gel column chromatography [Hex/EtOAc (96:4)] afforded compounds **2.25** (7 mg) and **2.26** (8 mg) as white powders in 30% and 40%yield, respectively.

3β-Chloro-24-ethenyl-24-hydroxycholest-5-ene (2.25, mixture of epimers at C-24)

IR (film) $\tilde{\nu}_{max}$ 3479, 2943, 1465, 1378 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.81 (m, 1H, H-28), 5.37 (br d, J = 5.0 Hz, 1H, H-6), 5.13 (m, 2H, H-29$\alpha\beta$), 3.76 (m, 1H, H-3), 2.50 (m, 2H, H-4$\alpha\beta$), 2.08-0.97 (br envelope, 24H), 1.02 (s, 3H, H-19), 0.95-0.85 (br envelope, 9H, H-21,H-26, H-27), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 142.5/142.4 (C, C-28), 140.8 (C, C-5), 122.5 (CH, C-6), 112.9/112.8 (CH$_2$, C-29), 77.7/77.6 (C, C-24), 60.3 (CH, C-3), 56.7/56.6 (CH, C-14), 55.8 (CH, C-17), 50.0 (CH, C-9), 43.4 (CH$_2$, C-4), 42.3 (C, C-13), 39.7 (CH$_2$, C-12), 39.1 (CH$_2$, C-1), 36.4 (C, C-10), 36.1/36.0 (CH, C-25), 35.9 (CH, C-20), 34.8/34.5 (CH$_2$, C-23),

33.4 (CH$_2$, C-2), 31.8 (CH$_2$, C-7), 31.7 (CH, C-8), 29.1/29.0 (CH$_2$, C-22), 28.2/28.1 (CH$_2$, C-16), 24.2 (CH$_2$, C-15), 20.9 (CH$_2$, C-11), 19.2 (CH$_3$, C-19), 18.8/18.7 (CH$_3$, C-21), 17.6/17.5 (CH$_3$, C-27), 16.4 (CH$_3$, C-26), 11.8 (CH$_3$, C-18); ESI-LRMS *m/z* [M+Na]$^+$ 469.5.

3β-Chloro-*N*-methylcholest-5-ene-24-carboxamide (2.26)

IR (film) $\tilde{\nu}_{max}$ 2938, 1647, 1457 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.37 (br d, *J* = 5.0 Hz, 1H, H-6), 3.76 (m, 1H, H-3), 2.80 (d, *J* = 4.8 Hz, 1H, H-NMe), 2.59-0.82 (br envelope, 25H), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 174.2 (C, C-24), 140.6 (C, C-5), 122.4 (CH, C-6), 60.3 (CH, C-3), 56.6 (CH, C-14), 55.8 (CH, C-17), 50.0 (CH, C-9), 43.3 (CH$_2$, C-4), 42.3 (C, C-13), 39.6 (CH$_2$, C-12), 39.1 (CH$_2$, C-1), 36.3 (C, C-10), 35.5 (CH, C-20), 33.5 (CH$_2$, C-23), 33.3 (CH$_2$, C-2), 31.8 (CH$_2$, C-22), 31.8 (CH$_2$, C-7), 31.7 (CH, C-8), 28.1 (CH$_2$, C-16), 26.3 (CH$_3$, C-NMe), 24.2 (CH$_2$, C-15), 20.9 (CH$_2$, C-11), 19.2 (CH$_3$, C-19), 18.4 (CH$_3$, C-21), 11.8 (CH$_3$, C-18); ESI-LRMS *m/z* [M+H]$^+$ 406.5.

3β-Carbonylimidazole- *N*-methoxy, *N*-methylcholest-5-ene-24-carboxamide (2.27)
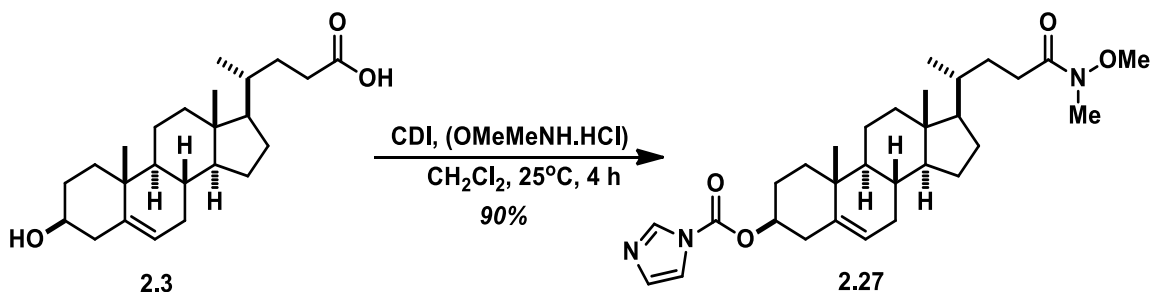
A heterogeneous solution of compound **2.3** (418 mg, 1.1 mmol) in dry CH_2Cl_2 (20 mL) and CDI (357 mg, 2.2 mmol) was stirred for 45 min until the solution became clear. Then, (OMe)MeNH·HCl (166 mg, 1.7 mmol) was added and the reaction mixture was stirred at 25 °C for 4 h, quenched with saturated aqueous NH_4Cl (20 mL), and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford carbamate **2.27** as a white powder in 90% yield (515 mg). $[\alpha]_D^{20}$ - 34.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 305 (ε 294), 204 (ε 29631) nm; IR (film) $\tilde{\nu}_{max}$ 3137, 2943, 1749, 1657, 1408, 1301 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.13 (s, 1H, H-2'), 7.41 (s, 1H, H-5'), 7.05 (s, 1H, H-4'), 5.43 (br d, *J* = 5.0 Hz, 1H, H-6), 4.82 (m, 1H, H-3), 3.68 (s, 3H, H-NOMe), 3.17 (s, 3H, H-NMe), 2.48 (br d, *J* = 7.9 Hz, 2H, H-4αβ), 2.78 (m, 2H, 23αβ), 2.10-0.90 (br envelope, 22H), 1.05 (s, 3H, H-19), 0.95 (d, *J* = 6.5 Hz, 3H, H-21), 0.69 (s, 3H,H-18); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 175.2 (C, C-24), 148.0 (C, C-COON), 138.6 (C, C-5), 137.1 (CH, C-2'), 130.4 (CH, C-4'), 123.6 (CH, C-6), 117.1 (CH, C-5'), 78.7 (CH, C-3), 61.2 (CH_3, C-NOMe), 56.5 (CH, C-14), 55.8 (CH, C-17), 49.9 (CH, C-9), 42.3 (C, C-13), 39.6 (CH_2, C-12), 37.8 (CH_2, C-4), 36.7 (CH_2, C-1), 36.5

(C, C-10), 35.5 (CH, C-20), 32.2 (CH_3, C-NMe), 31.8 (CH_2, C-7), 31.7 (CH, C-8),

30.7 (CH_2, C-22), 28.8 (CH_2, C-23), 28.1 (CH_2, C-16), 27.6 (CH_2, C-2), 24.2 (CH_2,

C-15), 21.0 (CH_2, C-11), 19.3 (CH_3, C-19), 18.4 (CH_3, C-21), 11.8 (CH_3, C-18);

ESI-LRMS *m/z* [M+H]$^+$ 512.6.

Cholest-5-en-3β-yl imidazole-1-carboxylate (2.28)
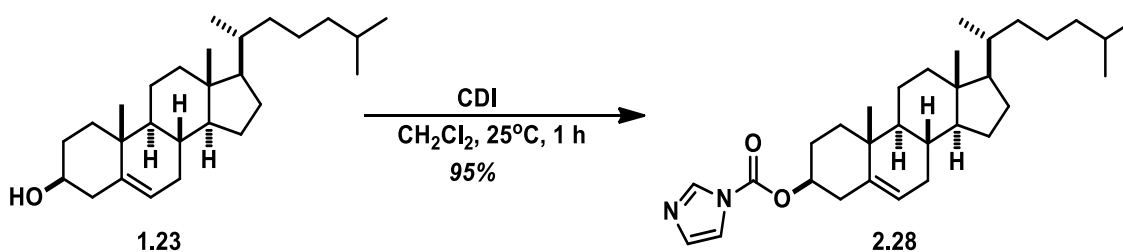
A solution of compound **1.23** (50 mg, 0.13 mmol) in dry CH_2Cl_2 (5 mL) and CDI (23

mg, 0.14 mmol) was stirred a 25 °C for 1 h. Then it was quenched with saturated

aqueous NH_4Cl (5 mL), and extracted with CH_2Cl_2 (3 x 5 mL). The combined

organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to produce

carbamate **2.28** as a white powder in 95% yield (59 mg). UV (MeOH) λ_{max} 306 (ε

253), 202 (ε 11829) nm; IR (film) $\tilde{\nu}_{max}$ 2943, 1465, 1749, 1658, 1408, 1302 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (s, 1H,H-2'), 7.40 (s, 1H, H-5'), 7.04 (s, 1H, H-

4'), 5.43 (br d, *J* = 5.0 Hz, 1H, H-6), 4.80 (m, 1H, H-3), 2.49 (d, *J* = 10.0 Hz, 1H,

H-4β), 2.03-0.97 (br envelope, 24H), 1.05 (s, 3H, H-19), 0.90 (d, *J* = 6.5 Hz, 3H,

H-21), 0.86 (d, *J* = 6.5 Hz, 3H, H-27), 0.85 (d, *J* = 6.5 Hz, 3H, H-26), 0.68 (s, 3H,

H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 148.0 (C, C-COON), 138.6 (C, C-5), 137.0

(CH, C-2'), 130.4 (CH, C-4'), 123.6 (CH, C-6), 117.1 (CH, C-5'), 78.7 (CH, C-3),

56.6 (CH, C-14), 56.1 (CH, C-17), 49.9 (CH, C-9), 42.3 (C, C-13), 39.6 (CH$_2$, C-12), 39.5 (CH$_2$, C-24), 37.8 (CH$_2$, C-1), 36.7 (C, C-10), 36.5 (CH$_2$, C-4), 36.1 (CH$_2$, C-22), 35.7 (CH, C-20), 31.9 (CH$_2$, C-7), 31.8 (CH, C-8), 28.2 (CH$_2$, C-16), 28.0 (CH, C-25), 27.6 (CH$_2$, C-2), 24.2 (CH$_2$, C-15), 23.8 (CH$_2$, C-23), 22.8 (CH$_3$, C-27), 22.5 (CH$_3$, C-26), 21.0 (CH$_2$, C-11), 19.3 (CH$_3$, C-19), 18.7 (CH$_3$, C-21), 11.8 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 480.3. The NMR data were in accordance with published data.[56,57]

N-Formyl-5,6-seco-6-oxo-cholest-6-al-3-carbamide (2.29)
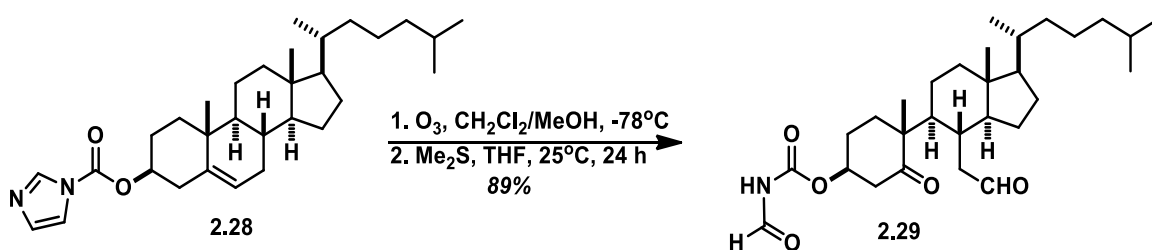
A stream of 2% O$_3$/O$_2$ was bubbled through a disposable pipet into a solution of compound **2.28** (61 mg, 0.13 mmol) in CH$_2$Cl$_2$-MeOH (4:1, 5 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of dimethyl sulfide (0.2 mL, 2.8 mmol) and THF (7 mL) for 16 h at 25 °C. Then, it was concentrated *in vacuo* and purified by flash-Silica gel column chromatography [Hex/EtOAc (4:1)] to obtain keto-aldehyde **2.29** in 89% yield (55 mg) as a colorless oil. [α]$_D^{20}$ + 57.0 (*c* 1.0, CHCl$_3$); UV (MeOH) λ_{max} 202 (ε 20382) nm; IR (film) $\tilde{\nu}_{max}$ 3270, 2952, 1710, 1468, 1379, 1208 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.59 (s,

1H, H-6), 8.89 (d, J = 10.1 Hz, 1H, H-2'), 8.30 (br s, 1H-NH), 5.43 (br s, 1H, H-3), 3.16 (dd, J = 4.3, 14.6 Hz, 1H, H-4β), 2.45-0.96 (br envelope, 27H), 0.99 (s, 3H, H-19), 0.89 (d, J = 6.6 Hz, 3H, H-21), 0.84 (d, J = 6.6 Hz, 3H, H-27), 0.83 (d, J = 6.6 Hz, 3H, H-26), 0.66 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 215.9 (C, C-5), 202.8 (CH, C-6), 162.6 (CH, C-2'), 151.7 (C, C-COON), 76.4 (CH, C-3), 56.1 (CH, C-17), 53.8 (CH, C-14), 52.4 (C, C-10), 43.7 (CH$_2$, C-7), 43.1 (CH$_2$, C-4), 42.5 (C, C-13), 42.0 (CH, C-9), 39.7 (CH$_2$, C-12), 39.4 (CH$_2$, C-24), 35.9 (CH$_2$, C-22), 35.7 (CH, C-20), 34.8 (CH, C-8), 34.2 (CH$_2$, C-1), 27.9 (CH, C-25), 27.7 (CH$_2$, C-2), 25.2 (CH$_2$, C-16), 25.0 (CH$_2$, C-15), 23.7 (CH$_2$, C-23), 23.1 (CH$_2$, C-11), 22.8 (CH$_3$, C-27), 22.5 (CH$_3$, C-26), 18.5 (CH$_3$, C-21), 17.6 (CH$_3$, C-19), 11.5 (CH$_3$, C-18); ESI-LRMS m/z [M+K]$^+$ 528.6.

5(6→7)-*abeo*-Cholest-5,6-diene-6-al (1.70)
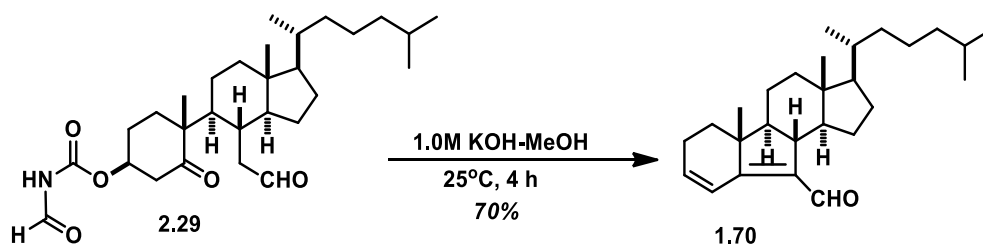
To neat compound **2.29** (55 mg, 0.11 mmol) was added KOH (1.0 M in MeOH, 2 mL) and was stirred at 25 °C for 3 h. Then it was quenched with saturated aqueous NH$_4$Cl (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo* to afford compound **1.70** in 70%

yield (30 mg) as a colorless oil. The characterization data for compound **1.70** are shown on page 89.

3β-hydroxy *N*-methoxy, *N*-methylcholest-5-ene-24-carboxamide (2.30)
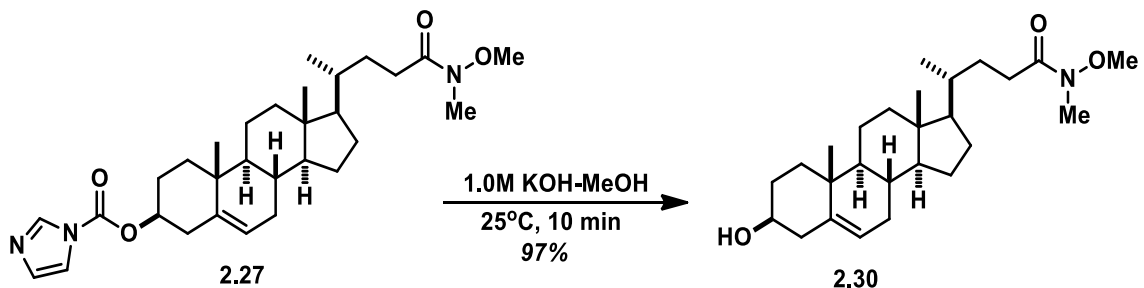
Compound **2.30** was obtained from the basic workup of **2.27** with KOH (1.0 M in MeOH, 3 mL). Then it was quenched with saturated aqueous NH_4Cl (15 mL), and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash-Silica Gel column chromatography [Hex/EtOAc (3:2)] afforded the Weinreb amide **2.30** as a white porder in 97% yield (408 mg). mp 119-120 °C; $[\alpha]_D^{20}$ - 42.0 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}_{max}$ 3397, 2942, 1634, 1454, 1383 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (br d, *J* = 5.0 Hz, 1H, H-6), 3.68 (s, 3H, H-NOMe), 3.49 (m, 1H, H-3), 3.16 (s, 3H, H-NMe), 2.47-0.89 (broad envelope, 25H), 0.99 (s, 3H, H-19), 0.94 (d, *J* = 6.5 Hz, 3H, H-21), 0.67 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.1 (C, C-24), 140.8 (C, C-5), 121.2 (CH, C-6), 71.3 (CH, C-3), 61.1 (CH_3, C-NOMe), 56.6 (CH, C-14), 55.7 (CH, C-17), 49.9 (CH, C-9), 42.2 (CH_2, C-4), 42.1 (C, C-13), 39.6 (CH_2, C-12), 37.1 (CH_2, C-1), 36.3 (C, C-10), 35.4 (CH, C-20), 32.0 (CH_3, C-NMe), 31.7

(CH$_2$, C-7), 31.7 (CH, C-8), 31.4 (CH$_2$, C-2), 30.6 (CH$_2$, C-22), 28.7 (CH$_2$, C-23), 28.0 (CH$_2$, C-16), 24.1 (CH$_2$, C-15), 20.9 (CH$_2$, C-11), 19.2 (CH$_3$, C-19), 18.3 (CH$_3$, C-21), 11.7 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 418.3; ESI-HRMS m/z calcd for C$_{26}$H$_{44}$NO$_3$ [M + H]$^+$ 418.3321, found 418.3318.

3β-Hydroxy *N*-methoxy, *N*-methyl-5,6-*seco*-5-oxo-cholest-6-al-24-carboxamide (2.31)
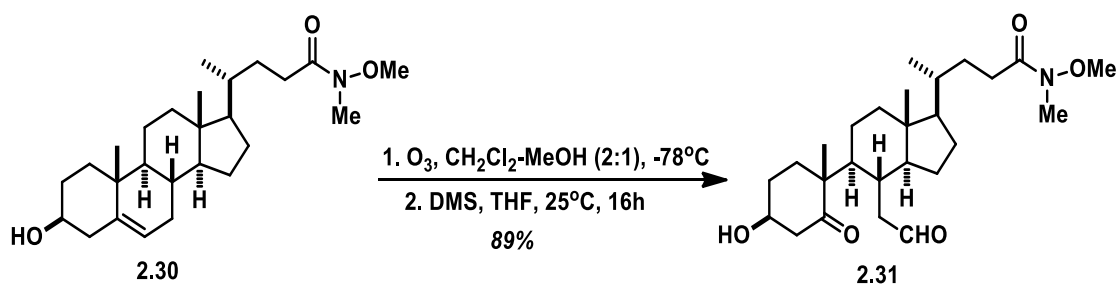
A stream of 2% O$_3$/O$_2$ was bubbled through a disposable pipet into a solution of compound **2.30** (408 mg, 0.67 mmol) in CH$_2$Cl$_2$-MeOH (4:1, 25 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of dimethyl sulfide (0.8 mL, 11.2 mmol) and THF (20 mL) for 16 h at 25 °C. Then, it was concentrated *in vacuo* and purified by flash-Silica gel column chromatography [Hex/Acetone (1:1)] to obtain keto-aldehyde **2.31** in 89% yield (391 mg) as a colorless oil. [α]$_D^{20}$ + 46.0 (*c* 1.0, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3429, 2940, 2871, 2721, 1719, 1699, 1649, 1460, 1385 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.60 (s, 1H, H-6), 4.46 (bs, 1H, H-3), 3.67 (s, 3H, H-NOMe), 3.15 (s, 3H, H-NMe), 3.08

(dd, J = 3.9, 13.7 Hz,1H, H-4β), 2.42-1.02 (br envelope, 8H), 1.00 (s, 3H, H-19), 0.92 (d, J = 6.5 Hz, 3H, H-21), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 217.5 (C, C-5), 202.6 (CH, C-6), 175.0 (C, C-24), 70.6 (CH, C-3), 61.1 (CH$_3$, C-NOMe), 55.7 (CH, C-17), 54.2 (CH, C-14), 52.4 (C, C-10), 46.6 (CH$_2$, C-4), 44.1 (CH$_2$, C-7), 42.4 (C, C-13), 42.1 (CH, C-9), 39.7 (CH$_2$, C-12), 35.3 (CH, C-20), 34.5 (CH, C-8), 33.9 (CH$_2$, C-1), 32.0 (CH$_3$, C-NMe), 30.3 (CH$_2$, C-22), 28.6 (CH$_2$, C-23), 27.6 (CH$_2$, C-2), 27.5 (CH$_2$, C-16), 25.2 (CH$_2$, C-15), 22.9 (CH$_2$, C-11), 18.2 (CH$_3$, C-21), 17.4 (CH$_3$, C-19), 11.4 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 450.3, [M + H − H$_2$O]$^+$ 432.3; ESI-HRMS m/z calcd for C$_{26}$H$_{44}$NO$_5$ [M + H]$^+$ 450.3219, found 450.3223.

Synthesis of compound 2.32

2.31   2.32
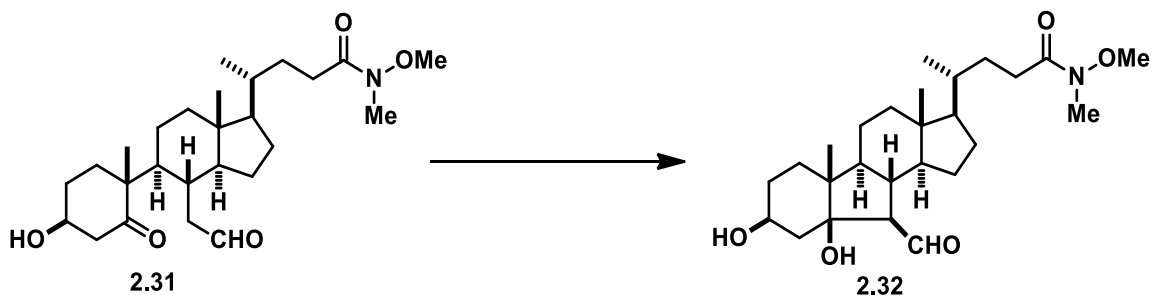
1. **General procedure for the aldol addition with (S)-(-)-1-(2-pyrrolidinylmethyl)pyrrolidine**

To a solution of compound **2.31** (50 mg, 0.11 mmol) in N-methyl-2-pyrrolidinone (1.3 mL) was added (S)-(-)-1-(2-pyrrolidinylmethyl)pyrrolidine·TFA (30 mol%, 0.01 mL) at 0 °C. The reaction mixture was stirred at 25 °C for 56 h and

quenched with a pH 7 phosphate buffer. Then, it was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine (3 x 10 mL). Finally, the combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*.

2. General procedure for the aldol addition with L-proline

To a solution of compound **2.31** (50 mg, 0.11 mmol) in dry CH$_2$Cl$_2$ (2 mL) was added L-proline (4 mg, 0.03 mmol). The reaction mixture was stirred at 25 °C for 16 h, diluted in water (5 mL), and extracted with CH$_2$Cl$_2$ (3 x 5 mL). The combined organic layers were dried (MγSO$_4$) and concentrated *in vacuo*.

3. General procedure for the aldol addition with aqueous LiOH

To a solution of compound **2.31** (50 mg, 0.11 mmol) in EtOH (2 mL) was added LiOH (10 mol% in H$_2$O, 0.5 mL). The reaction mixture was stirred at 25 °C for 24 h, diluted in water (5 mL), neutralized with HCl (2% in H$_2$O), and extracted with EtOAC (3 x 5 mL). The combined organic layers were washed with brine (2 x 5 mL), dried (MgSO$_4$) and concentrated *in vacuo*.

4. General procedure for the aldol addition with methanolic LiOH

To compound **2.31** (50 mg, 0.11 mmol) was added LiOH (1.0 M in MeOH, 2 mL). The reaction mixture was stirred at 25 °C for 4 h, quenched with saturated aqueous NH$_4$Cl (3 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*.

5. General procedure for the aldol addition with Al$_2$O$_3$

To a solution of compound **2.31** (50 mg, 0.11 mmol) in benzene (2 mL) was added Al$_2$O$_3$. The reaction mixture was stirred at 25 °C for 24 h, filtered, and passed through Celite washing thoroughly with CH$_2$Cl$_2$.

6. General procedure for the aldol reaction with pyrrolidine and benzoic acid

To a solution of compound **2.31** (50 mg, 0.11 mmol) in toluene (2 mL) was added pyrrolidine (2 µL, 0.02 mmol) and benzoic acid (1 µL, 0.01 mmol). The reaction mixture was stirred at 25 °C for 4 h and quenched with saturated aqueous NH$_4$Cl (3 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*.

7. General procedure for the aldol addition with pyrrolidine

To a solution of compound **2.31** (50 mg, 0.11 mmol) in dry CH$_2$Cl$_2$ (2 mL) was added pyrrolidine (0.9 µL, 0.01 mmol). The reaction mixture was stirred at 25 °C for 0.5 h, quenched with saturated aqueous NH$_4$Cl (5 mL), and extracted with CH$_2$Cl$_2$ (3 x 5 mL). The combined organic layers were dried (MgSO$_4$), concentrated *in vacuo*, and purified by flash-Silica gel column chromatography [Hex/Acetone (1:1)] to obtain compound **2.32** in 90% yield (45 mg) as a colorless oil.

3β,5β-Dihydroxy-*N*-methoxy, *N*-methyl-5(6→7)*abeo*-cholest-6-al-24-carboxamide (2.32)

[α]$_D^{20}$ + 32.0 (*c* 1.0, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3418, 2938, 2868, 2735, 1716, 1644, 1444, 1383 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.70 (d, *J* = 2.9 Hz, 1H, H-6), 4.12 (bs, 1H, H-3), 3.68 (s, 3H, H-NOMe), 3.53 (s, -OH), 3.17 (s, 3H, H-NMe), 2.46-1.09 (br envelope, 25H), 0.96 (d, *J* = 6.5 Hz, 3H, H-21) 0.92 (s, 3H, H-19), 0.72 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 204.3 (CH, C-6), 174.9 (C, C-24), 84.0 (C, C-5), 66.5 (CH, C-3), 63.8 (CH$_2$, C-7), 60.9 (CH$_3$, C-NOMe), 55.7 (CH, C-14), 55.0 (CH, C-17), 50.2 (CH, C-9), 45.2 (C, C-10), 44.4 (C, C-13), 43.9 (CH$_2$, C-4), 39.4 (CH$_2$, C-12), 38.8 (CH, C-8), 35.0 (CH, C-20), 31.9 (CH$_3$, C-NMe), 30.4 (CH$_2$, C-22), 28.5 (CH$_2$, C-23), 27.9 (CH$_2$, C-16), 27.4 (CH$_2$, C-2), 26.6 (CH$_2$, C-1), 24.0 (CH$_2$, C-15), 21.3 (CH$_2$, C-11), 18.3 (CH$_3$, C-19), 18.2 (CH$_3$, C-21), 12.2 (CH$_3$, C-18); ESI-LRMS *m/z* [M + H]$^+$ 450.3, [M + H – H$_2$O]$^+$ 432.3; ESI-HRMS *m/z* calcd for C$_{26}$H$_{44}$NO$_5$ [M + H]$^+$ 450.3215, found 450.3219.

6,6-(Ethylenedioxy)-3β,5β-dihydroxy *N*-methoxy, *N*-methyl-5(6→7)-*abeo*-cholest-24-carboxamide (2.33)

To a solution of **2.32** (285 mg, 0.63 mmol) in dry THF (20 mL) with 4Å molecular sieves was added ethylene glycol (42 μL, 0.76 mmol) and PTSA·H$_2$O (144 mg, 0.76 mmol). The reaction mixture was stirred a 25 °C for 16 h, quenched with saturated aqueous NaHCO$_3$ (10 mL), and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried (MgSO$_4$), concentrated under *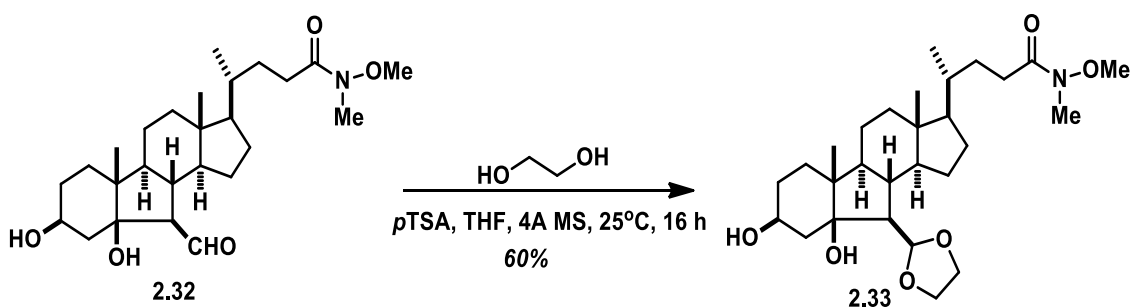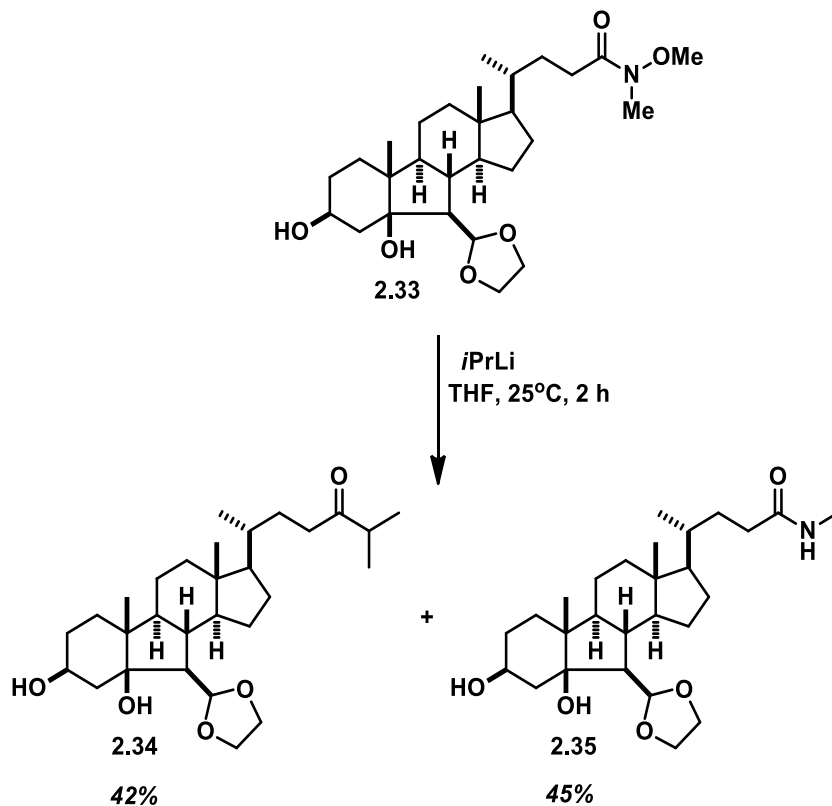vacuo*, and purified by flash-Silica gel column chromatography [Hex/EtOAc (2:3)] to produce **2.33** in 60% yield (188 mg) as a yellowish oil. [α]$_D^{20}$ + 30.0 (*c* 1.0, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3476, 2936, 1661, 1444, 1383 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.87 (d, *J* = 5.5 Hz, 1H, H-6), 3.92 (m, 3H, H-3, H-1'αβ), 3.82 (m, 2H, H-2'αβ), 3.67 (s, 3H, H-NOMe), 3.47 (s, -OH), 3.16 (s, 3H, H-NMe), 2.47-1.10 (br envelope, 24H), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.89 (s, 3H, H-19), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 175.1 (C, C-24), 105.3 (CH, C-6), 82.5 (C, C-5), 67.0 (CH, C-3), 64.5 (CH$_2$, C-1'), 64.4 (CH$_2$, C-2'), 61.2 (CH$_3$, C-NOMe), 57.0 (CH, C-14), 55.4 (CH, C-17), 54.8 (CH, C-7), 49.7 (CH, C-9), 45.4 (CH$_2$, C-4), 44.9 (C, C-13), 44.4 (C, C-10), 40.2 (CH, C-8), 40.0 (CH$_2$, C-12), 35.5 (CH, C-20), 32.2 (CH$_3$, C-NMe), 30.7 (CH$_2$, C-22), 28.9 (CH$_2$, C-23), 28.2 (CH$_2$, C-16), 28.0 (CH$_2$, C-2), 26.3 (CH$_2$, C-1), 25.4 (CH$_2$, C-15), 21.4 (CH$_2$, C-11), 18.6 (CH$_3$, C-19), 18.5 (CH$_3$, C-21), 12.7 (CH$_3$, C-18); ESI-LRMS *m/z* [M + H]$^+$ 494.3, [M + H – H$_2$O]$^+$ 476.3; ESI-HRMS *m/z* calcd for C$_{28}$H$_{48}$NO$_6$ [M + H]$^+$ 494.3482, found 494.3473.

Synthesis of compounds 2.34 and 2.35

To a solution of **2.33** (188 mg, 0.38 mmol) in dry THF (10 mL) was added *i*PrLi (0.7 M in pentane, 5.4 mL) over a period of 15 min. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH$_4$Cl (10mL), and extracted with EtOAC (3 x 15 mL). The combined organic layers were dried (MgSO$_4$), concentrated under *vacuo*, and purified by flash-Silica Gel column chromatography [Hex/EtOAc (2:3)] to afford **2.34** (76 mg) and **2.35** (80 mg) as yellowish oils in 42% and 45% yield, respectively.

6,6-(Ethylenedioxy)-3β,5β-dihydroxy-5(6→7)-*abeo*-24-oxo-cholestane (2.34)

$[\alpha]_D^{20}$ + 33.0 (*c* 1.0, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3487, 2933, 1710, 1466, 1382 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.88 (d, *J* = 5.5 Hz, 1H, H-6), 3.93 (m, 3H, H-3, H-1'αβ), 3.82 (m, 2H, H-2'αβ), 3.48 (s, -OH), 2.62-1.10 (br envelope, 25H), 1.09 (d, *J* = 6.9 Hz, 6H, H-26, H-27), 0.91 (d, *J* = 6.6 Hz, 3H, H-21), 0.90 (s, 3H, H-19), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 215.4 (C, C-24), 105.4 (CH, C-6), 82.5 (C, C-5), 67.1 (CH, C-3), 64.5 (CH$_2$, C-1'), 64.4 (CH$_2$, C-2'), 57.0 (CH, C-14), 55.5 (CH, C-17), 54.9 (CH, C-7), 49.8 (CH, C-9), 45.4 (CH$_2$, C-4), 44.9 (C, C-13), 44.4 (C, C-10), 40.8 (CH, C-25), 40.3 (CH, C-8), 40.0 (CH$_2$, C-12), 37.2 (CH$_2$, C-23), 35.3 (CH, C-20), 29.8 (CH$_2$, C-22), 28.3 (CH$_2$, C-16), 28.1 (CH$_2$, C-2), 26.3 (CH$_2$, C-1), 25.4 (CH$_2$, C-15), 21.4 (CH$_2$, C-11), 18.6 (CH$_3$, C-19), 18.6 (CH$_3$, C-21), 18.4 (CH$_3$, C-27), 18.3 (CH$_3$, C-26), 12.7 (CH$_3$, C-18); ESI-LRMS *m/z* [M + H]$^+$ 477.4; ESI-HRMS *m/z* calcd for C$_{29}$H$_{49}$O$_5$ [M + H]$^+$ 477.3580, found 477.3581.

6,6-(Ethylenedioxy)-3β,5β-dihydroxy-*N*-methly-5(6→7)-*abeo*cholest-24-carboxamide (2.35)

$[\alpha]_D^{20}$ + 38.0 (*c* 1.0, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3362, 2938, 1652, 1559, 1457cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.88 (d, *J* = 5.5 Hz, 1H, H-6), 3.94 (m, 3H, H-3, H-1'αβ), 3.82 (m, 2H, H-2'αβ), 3.48 (s, -OH), 2.80 (d, *J* = 4.8 Hz, 3H, H-NMe), 2.62-0.90 (br envelope, 25H), 0.92 (d, *J* = 6.5 Hz, 3H, H-21), 0.90 (s, 3H, H-19), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 174.2 (C, C-24), 105.4 (CH, C-6), 82.5 (C, C-

5), 67.1 (CH, C-3), 64.5 (CH$_2$, C-1'), 64.5 (CH$_2$, C-2'), 57.1 (CH, C-14), 55.5 (CH, C-17), 54.9 (CH, C-7), 49.8 (CH, C-9), 45.4 (CH$_2$, C-4), 45.0 (C, C-13), 44.4 (C, C-10), 40.3 (CH, C-8), 40.1 (CH$_2$, C-12), 35.5 (CH, C-20), 33.6 (CH$_2$, C-23), 31.9 (CH$_2$, C-22), 28.3 (CH$_2$, C-16), 28.1 (CH$_2$, C-2), 26.3 (CH$_3$, C-NMe), 26.3 (CH$_2$, C-1), 25.5 (CH$_2$, C-15), 21.5 (CH$_2$, C-11), 18.6 (CH$_3$, C-19), 18.6 (CH$_3$, C-21), 12.7 (CH$_3$, C-18); ESI-LRMS m/z [M + Na]$^+$ 486.3, [M + H – H$_2$O]$^+$ 446.3; ESI-HRMS m/z calcd for C$_{27}$H$_{45}$NO$_5$Na [M + Na]$^+$ 486.3195, found 486.3193.

24-Ethenyl-6,6-(ethylenedioxy)-3β,5β, 24ξ-trihydroxy-*N*-methly-5(6→7)-*abeo*cholestane (2.36, mixture of C-24 epimers)
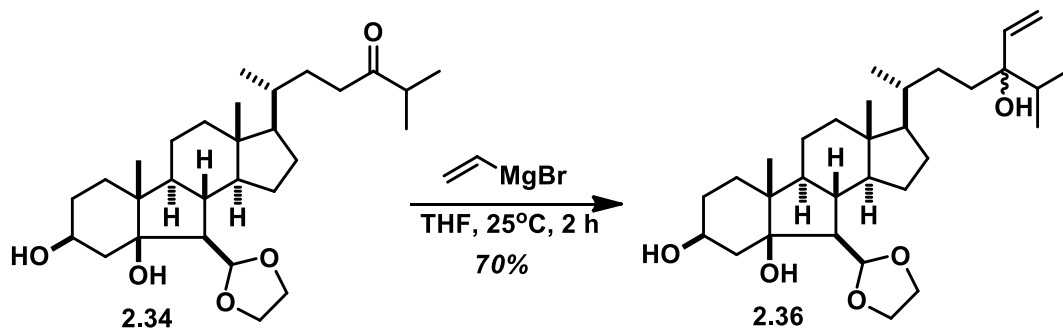
To a solution of **2.34** (76 mg, 0.16 mmol) in THF (7 mL) was added vinylmagnesium bromide (1.0 M in THF, 0.8 mL) over a period of 15 min. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH$_4$Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (MgSO$_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/EtOAc (1:1)] to generate **2.36** as a mixture of epimers at C-24 in 70% yield (58 mg) as a yellowish oil. IR (film) $\tilde{\nu}_{max}$ 3482, 2936, 1444, 1382

cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.79 (m, 1H, H-28), 5.15 (m, 1H, H-29αβ), 4.88

(d, J = 5.5 Hz, 1H, H-6), 3.93 (m, 3H, H-3, H-1'αβ), 3.82 (m, 2H, H-2'αβ), 3.48 (s,

-OH), 2.03-0.97 (br envelope, 25H), 0.92-86 (br envelope, 9H, H-21, H-27, H-26),

0.90 (s, 3H, H-19), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 142.5/142.4

(CH, C-28), 112.9/112.8 (CH$_2$, C-29), 105.4 (CH, C-6), 82.5 (C, C-5), 77.7/77.6 (C,

C-24), 67.1 (CH, C-3), 64.5 (CH$_2$, C-1'), 64.4 (CH$_2$, C-2'), 57.1/57.0 (CH, C-14),

55.4 (CH, C-17), 54.9 (CH, C-7), 49.8 (CH, C-9), 45.4 (CH$_2$, C-4), 44.9 (C, C-13),

44.4 (C, C-10), 40.3 (CH, C-8), 40.0 (CH$_2$, C-12), 36.1/36.0 (CH, C-25), 35.9/35.8

(CH, C-20), 34.8/34.6 (CH$_2$, C-23), 29.1/29.0 (CH$_2$, C-22), 28.3/28.2 (CH$_2$, C-16),

28.1 (CH$_2$, C-2), 26.3 (CH$_2$, C-1), 25.4 (CH$_2$, C-15), 21.4 (CH$_2$, C-11), 18.9 (CH$_3$,

C-21), 18.6 (CH$_3$, C-19), 17.5 (CH$_3$, C-27), 16.4 (CH$_3$, C-26), 12.7 (CH$_3$, C-18);

ESI-LRMS m/z [M + H]$^+$ 505.4; ESI-HRMS m/z calcd for C$_{31}$H$_{53}$O$_5$ [M + H]$^+$

505.3893, found 505.3889.

24-Ethenyl-3β,5β,24ξ-trihydroxy-5(6→7)*abeo*cholest-6-al (2.37, mixture of C-24 epimers)
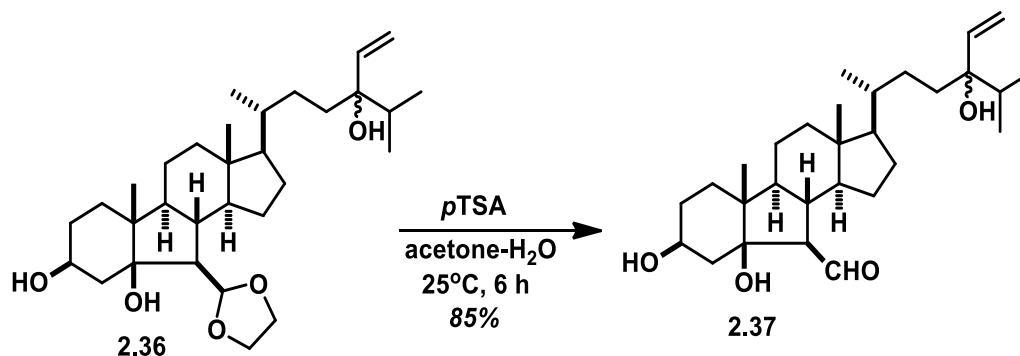
To a solution of **2.36** (58 mg, 0.11 mmol) in acetone-water (4:1, 5 mL) was added

pTSA·H_2O (31 mg, 0.17 mmol). The reaction mixture was stirred at 25 °C for 6 h,

quenched with saturated aqueous $NaHCO_3$ (5 mL), and extracted with EtOAc (3 x

10 mL). The combined organic layers were dried ($MgSO_4$), concentrated under

vacuo, and purified by flash-Silica gel column chromatography [Hex/EtOAc (6:4)]

to generate **2.37** as a colorless oil in 85% yield (45 mg). IR (film) $\tilde{\nu}_{max}$ 3448, 2942,

2872, 1716, 1457, 1382 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.69 (d, J = 2.9 Hz, 1H,

H-6), 5.79 (m, 1H, H-28), 5.15 (m, 2H, H-29αβ), 4.11 (m, 1H, H-3), 3.54 (s, -OH),

2.24-0.98 (br envelope, 27H), 0.92 (s, 3H, H-19), 0.90-0.85 (br envelope, 9H, H-

21, H-26, H-27), 0.70 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.6 (CH, C-

6), 142.5/142.4 (CH, C-28), 113.0/112.9 (CH_2, C-29), 84.2 (C, C-5), 77.7 (C, C-

24), 67.3 (CH, C-3), 63.9 (CH, C-7), 56.1 (CH, C-14), 55.4/55.3 (CH, C-17), 50.5

(CH, C-9), 45.5 (C, C-10), 44.7 (C, C-13), 44.3 (CH_2, C-4), 39.9 (CH, C-8), 39.7

(CH_2, C-12), 36.1/36.0 (CH, C-25), 35.9/35.7 (CH, C-20), 34.7/34.6 (CH_2, C-23),

29.1/29.0 (CH_2, C-22), 28.3/28.2 (CH_2, C-16), 27.9 (CH_2, C-2), 26.8 (CH_2, C-1),

24.5 (CH_2, C-15), 21.5 (CH_2, C-11), 18.8/18.7 (CH_3, C-21), 18.4 (CH_3, C-19), 17.5

(CH_3, C-27), 16.4 (CH_3, C-26), 12.5 (CH_3, C-18); ESI-LRMS m/z [M + H]$^+$ 461.4;

ESI-HRMS m/z calcd for $C_{29}H_{49}O_4$ [M + H]$^+$ 461.3631, found 461.3636.

**Leningosterol (2.1, mixture of epimers at C-24)**
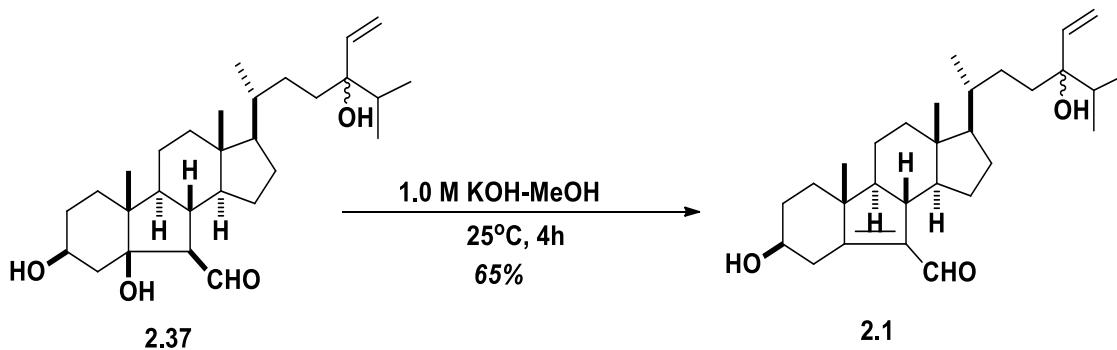
To neat **2.37** (45 mg, 0.1 mmol) was added KOH (1.0 M in MeOH, 3 mL) and was stirred at 25 °C for 4 h, quenched with saturated aqueous NH_4Cl (5 mL), and extracted with EtOAC (3 x 7 mL). The combined organic layers were dried ($MgSO_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/EtOAc (6:4)] to attain **2.1** as a colorless oil in 65% yield (28 mg). UV (MeOH) λ_{max} 254 (ε 4629), 201 (ε 8555), 192 (ε 4542) nm; IR (film) $\tilde{\nu}_{max}$ 3408, 2961, 1674, 1405 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.96 (s, 1H, H-6), 5.79 (m, 1H, H-28), 5.15 (m, 2H, H-29$\alpha\beta$), 3.70 (m, 1H, H-3), 3.46 (dd, J = 2.6, 14.4 Hz, 1H, H-4β), 2.57-0.98 (br envelope, 23H), 0.93 (s, 3H, H-19), 0.90-0.86 (br envelope, 9H, H-21, H-26, H-27), 0.72 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.6 (CH,C-6), 169.0 (C, C-5), 142.5/142.4 (CH, C-28), 139.3 (C, C-7), 113.0/112.9 (CH$_2$, C-29), 77.7 (C, C-24), 70.8 (CH, C-3), 60.1 (CH, C-9), 55.1/55.0 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.7 (CH$_2$, C-12), 36.1 (CH$_2$, C-1), 36.1/35.9 (CH, C-25), 35.8/35.7 (CH, C-20), 34.9/34.6 (CH$_2$, C-23), 33.8 (CH$_2$, C-4), 31.2 (CH$_2$, C-2), 29.1/29.0 (CH$_2$, C-22), 28.5/28.4 (CH$_2$, C-16), 26.5 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 19.0 (CH$_3$, C-21), 17.5

(CH$_3$, C-27), 16.4 (CH$_3$, C-26), 15.6 (CH$_3$, C-19), 12.4 (CH$_3$, C-18); EI-LRMS m/z [M]$^+$ 442 (50), 399 (78), 381 (66), 344 (65), 285 (65), 269 (70), 145 (59); EI-HRMS m/z calcd for C$_{29}$H$_{46}$O$_3$ [M]$^+$ 442.3447, found 442.3446.

Purification of leningosterol (2.1, mixture of epimers at C-24). The separation of leningosterol epimers **2.1a** and **2.1b** was performed using an HPLC equipped with a Diode Array Detector. A chiral Kromasil® Si gel column (Regis Technologies Inc., (*S,S*) Whelk-O 1, 5 μm, 100 Å, 25 cm x 4.6 mm i.d.) was used with 20% H$_2$O in MeOH at a flow rate of 1 mL/min. The retention times of the epimers were 29.5 min (24 *S*) and 30.7 min (24 *R*).

Leningosterol epimer 2.1a
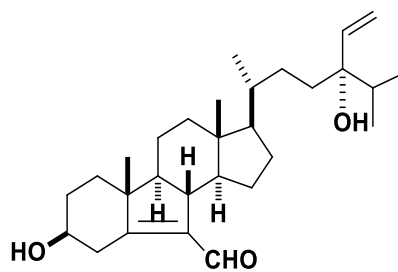
2.1a

Compound **2.1a** was obtained as a white powder in 21% yield (3 mg). [α]$_D^{20}$ - 93.0 (*c* 0.9, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3408, 2961, 1674, 1405 cm^{-1}; UV (MeOH) λ_{max} 254 (ε 4437), 201 (ε 7989), 192 (ε 4158) nm; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.97 (s, 1H, H-6), 5.80 (dd, *J* = 10.9, 17.4 Hz, 1H, H-28), 5.18 (dd, *J* = 1.5, 17.4 Hz, 1H, H-

29β), 5.13 (dd, J = 1.4, 10.9 Hz, 1H, H-29α), 3.70 (m, 1H, H-3), 3.46 (m, 1H, H-4β), 2.54 (m, 1H, H-8), 2.11-1,01 (br envelope, 22H), 0.93 (s, 3H, H-19), 0.92 (d, J = 6.5 Hz, 1H, H-21), 0.90 (d, J = 6.8 Hz, 1H, H-27), 0.87 (d, J = 6.9 Hz, 1H, H-26), 0.72 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.6 (CH,C-6), 168.8 (C, C-5), 142.6 (CH, C-28), 139.3 (C, C-7), 112.9 (CH$_2$, C-29), 77.7 (C, C-24), 70.9 (CH, C-3), 60.2 (CH, C-9), 55.1 (CH, C-17), 54.5 (CH, C-14), 46.3 (C, C-10), 46.2 (CH, C-8), 45.3 (C, C-13), 39.8 (CH$_2$, C-12), 36.2 (CH$_2$, C-1), 36.2 (CH, C-25), 35.8 (CH, C-20), 34.7 (CH$_2$, C-23), 33.9 (CH$_2$, C-4), 31.3 (CH$_2$, C-2), 29.2 (CH$_2$, C-22), 28.5 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 19.0 (CH$_3$, C-21), 17.5 (CH$_3$, C-27), 16.5 (CH$_3$, C-26), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 443.4, [M + H − H$_2$O]$^+$ 425.3; ESI-HRMS m/z calcd for C$_{29}$H$_{47}$O$_3$ [M + H]$^+$ 443.3525, found 443.3504.
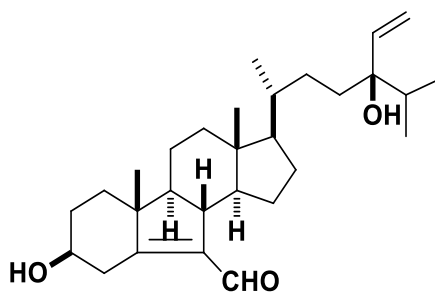
Leningosterol epimer 2.1b


2.1b

Compound **2.1b** was obtained as a white powder in 21% yield (3 mg). $[\alpha]_D^{20}$ -70 (c 0.4, CHCl$_3$); IR (film) $\tilde{\nu}_{max}$ 3408, 2961, 1674, 1405 cm^{-1}; UV (MeOH) λ_{max} 254 (ε

4437), 201 (ε 7989), 192 (ε 4158) nm; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.97 (s, 1H,

H-6), 5.82 (dd, J = 11.0, 17.4 Hz, 1H, H-28), 5.19 (dd, J = 1.4, 17.4 Hz, 1H, H-

29β), 5.14 (dd, J = 1.4, 10.9 Hz, 1H, H-29α), 3.70 (m, 1H, H-3), 3.46 (m, 1H, H-

4β), 2.55 (m, 1H, H-8), 2.11-1.01 (br envelope, 22H), 0.94 (d, J = 6.5 Hz, 1H, H-

21), 0.93 (s, 3H, H-19), 0.89 (d, J = 6.8 Hz, 1H, H-27), 0.87 (d, J = 6.9 Hz, 1H, H-

26), 0.72 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.6 (CH,C-6), 168.7 (C,

C-5), 142.5 (CH, C-28), 139.3 (C, C-7), 113.0 (CH$_2$, C-29), 77.7 (C, C-24), 70.9

(CH, C-3), 60.2 (CH, C-9), 55.2 (CH, C-17), 54.5 (CH, C-14), 46.3 (C, C-10), 46.2

(CH, C-8), 45.2 (C, C-13), 39.8 (CH$_2$, C-12), 36.2 (CH$_2$, C-1), 35.9 (CH, C-25), 35.9

(CH, C-20), 35.0 (CH$_2$, C-23), 33.9 (CH$_2$, C-4), 31.3 (CH$_2$, C-2), 29.2 (CH$_2$, C-22),

28.5 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 19.0 (CH$_3$, C-21), 17.5 (CH$_3$,

C-27), 16.4 (CH$_3$, C-26), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18);); ESI-LRMS m/z [M

+ H]$^+$ 443.4, [M + H − H$_2$O]$^+$ 425.3; ESI-HRMS m/z calcd for C$_{29}$H$_{47}$O$_3$ [M + H]$^+$

443.3525, found 443.3504.

Saringosterol (1.22, mixture of C-24 epimers)
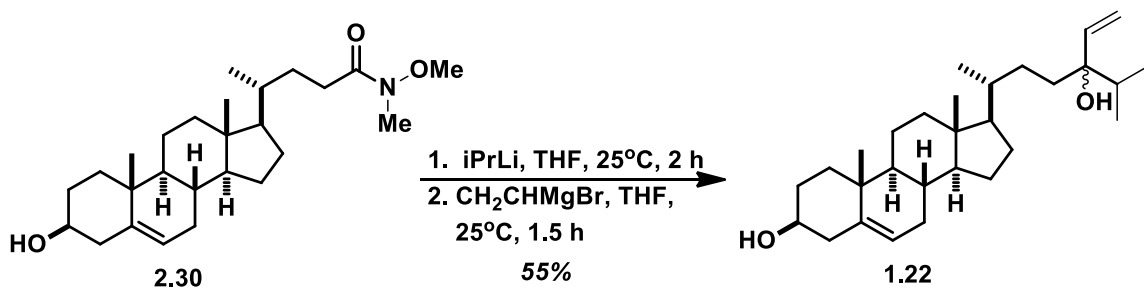
To a solution of **2.30** (50 mg, 0.12 mmol) in dry THF (3 mL) was added *i*PrLi (0.7 M in pentane, 1 mL) over a period of 15 min. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH_4Cl (5 mL), and extracted with EtOAC (3 x 8 mL). The combined organic layers were dried ($MgSO_4$) and then concentrated under *vacuo*. To a solution of the crude in THF (3 mL) was added vinylmagnesium bromide (1.0 M in THF, 0.8 mL) over a period of 15 min. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated $NH_4Cl_{(aq)}$ (5 mL), and extracted with EtOAc (3 x 8 mL). The combined organic layers were dried ($MgSO_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/EtOAc (7:3)] to produce **1.22** as a mixture of epimers at C-24 in 55% yield (28 mg). IR (film) $\tilde{\nu}_{max}$ 3366, 2935, 2868, 1465, 1377 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 5.80 (m, 1H, H-28), 5.32 (br d, $J = 5.0$ Hz, 1H, H-6), 5.15 (m, 2H, H-29$\alpha\beta$), 3.52 (m, 1H, H-3), 2.30-0.98 (br envelope, 26H), 1.00 (s, 3H, H-19), 0.95-0.86 (br envelope, 9H, H-21, H-26, H-27), 0.67 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 142.5/142.4 (CH, C-28), 140.7 (C, C-5), 121.7 (CH, C-6), 112.9/112.8 (CH_2, C-29), 77.7/77.5 (C, C-24), 71.8 (CH, C-3), 56.7/56.6 (CH, C-14), 55.8/55.7 (CH, C-17), 50.1 (CH, C-9), 42.3 (CH_2, C-4), 42.2 (C, C-13), 39.7 (CH_2, C-12), 37.2 (CH_2, C-1), 36.5 (C, C-10), 36.1/36.0 (CH, C-25), 35.9/35.8 (CH, C-20), 34.7/34.5 (CH_2, C-23), 31.9 (C, C-7), 31.9 (CH, C-8), 31.6 (CH_2, C-2), 29.1/29.0 (CH_2, C-22), 28.2/28.1 (CH_2, C-16), 24.2 (CH_2, C-15), 21.0 (CH_2, C-11), 19.4 (CH_3, C-19), 18.8/18.7 (CH_3, C-21), 17.5 (CH_3, C-27), 16.4 (CH_3, C-26), 11.8 (CH_3, C-18); ESI-LRMS m/z [M + Na]$^+$ 451.4; ESI-HRMS m/z calcd for $C_{29}H_{48}O_2Na$

[M+Na]$^+$ 451.3552, found 451.3546. The NMR data were in accordance with published data.[36,39-46]

2.7.3. Evaluation of Anti-tubercular Activity

M. tuberculosis H37Rv (ATCC 27294) was obtained from the American Type Culture Collection (Rockville,Md.). For the first three (of four) replicate experiments, H37Rv inocula were first passaged in radiometric 7H12 broth until the growth index (GI) reached 800 to 999. For the fourth replicate experiment, H37Rv was grown in 7H9GC-Tween. Cultures were incubated in 500 mL nephelometer flasks on a rotary shaker at 150 rpm and 37°C until they reached an optical density of 0.4 to 0.5 at 550 nm. The filtrates were aliquoted, stored at 280 °C, and used within 30 days. The microplate alamar blue assay (MABA) was performed in black, clear-bottomed, 96-well microplates in order to minimize background fluorescence as published.[50] Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. All the statistical analyses were performed with the program SAS (Statistical Analysis System). The anti-tuberculosis drug rifampicin (RMP) was used as a positive control during the assays.

2.7.4. Spectroscopic Data

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$
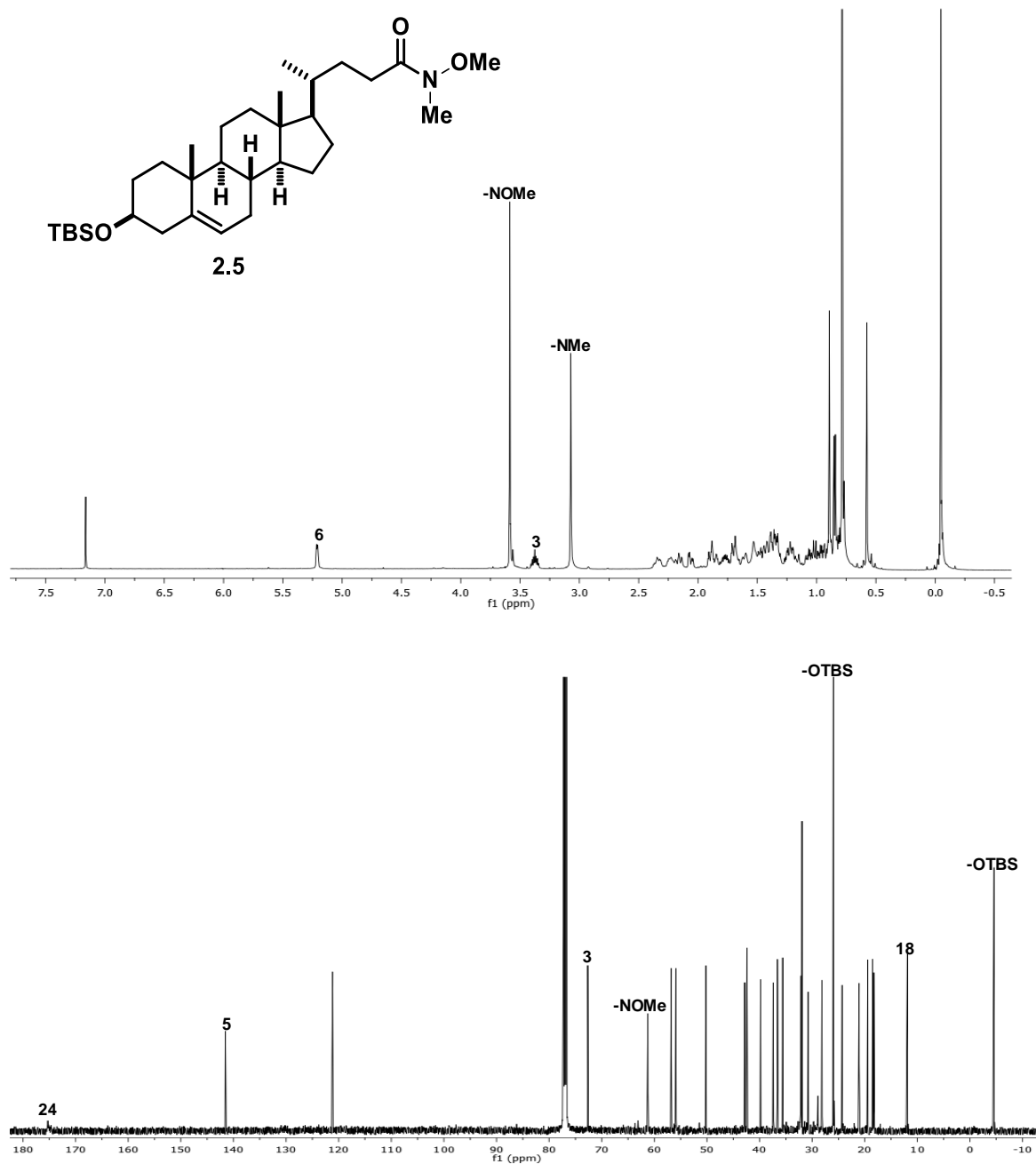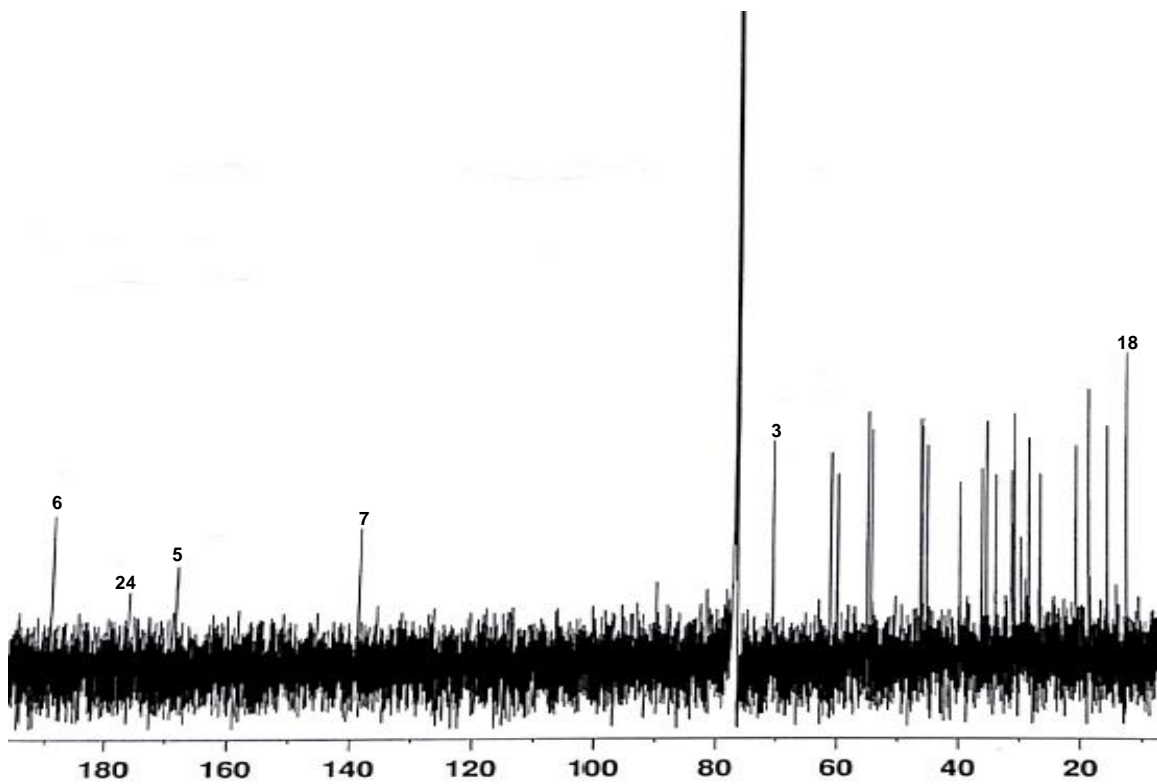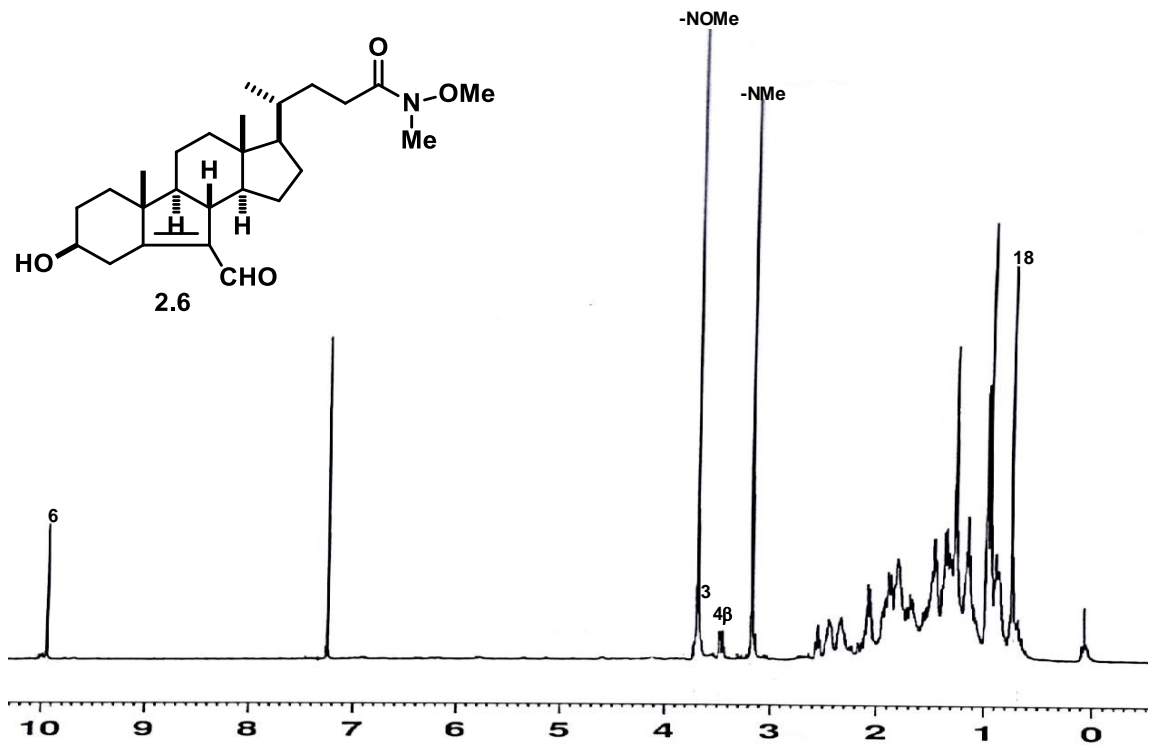
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

124

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$
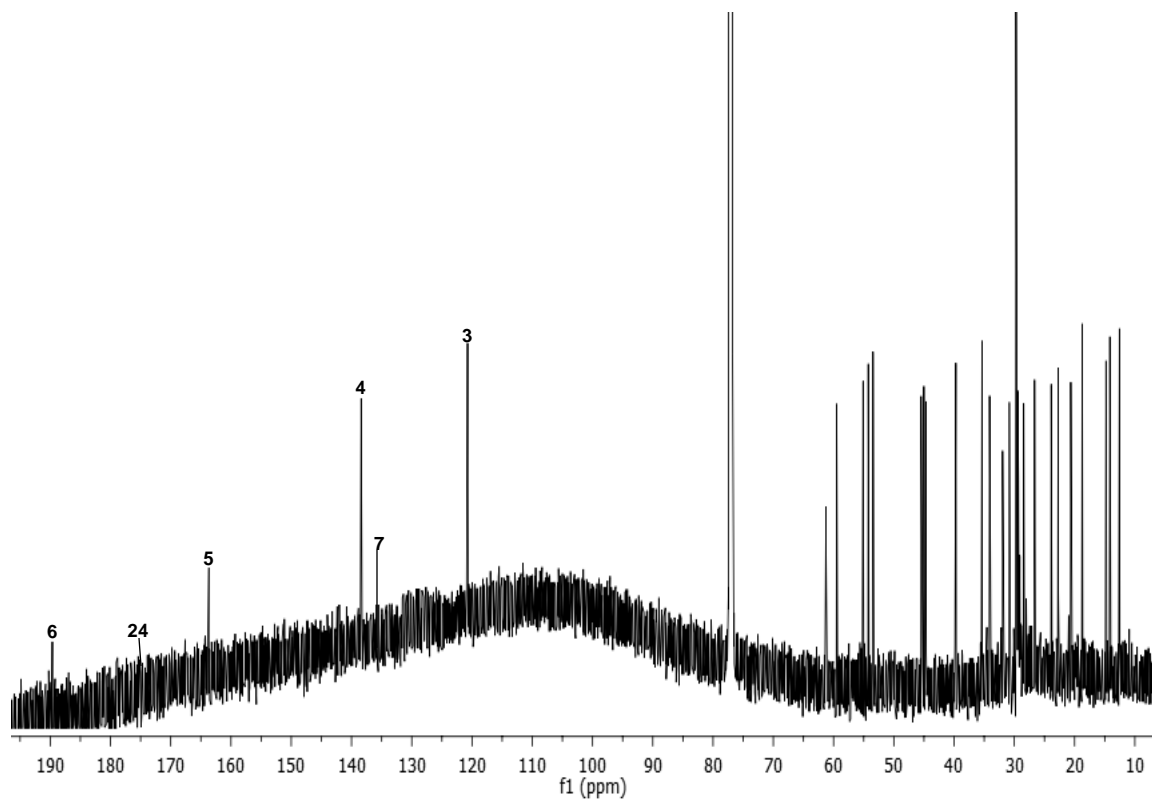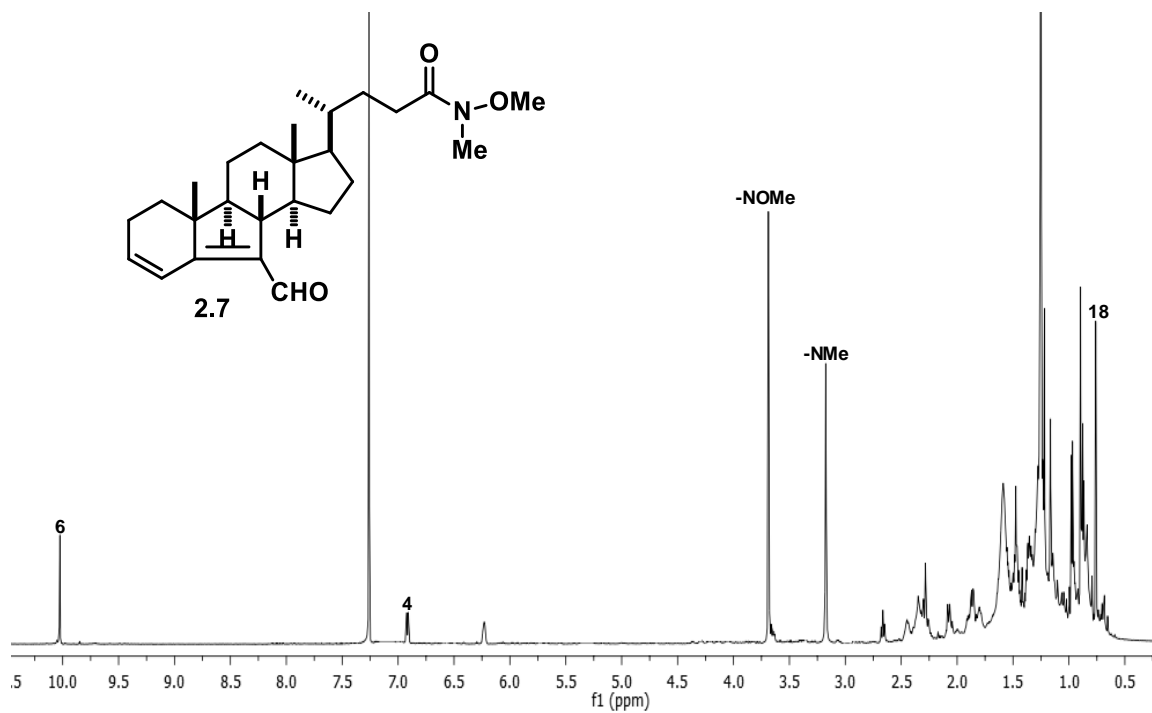
^1H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$
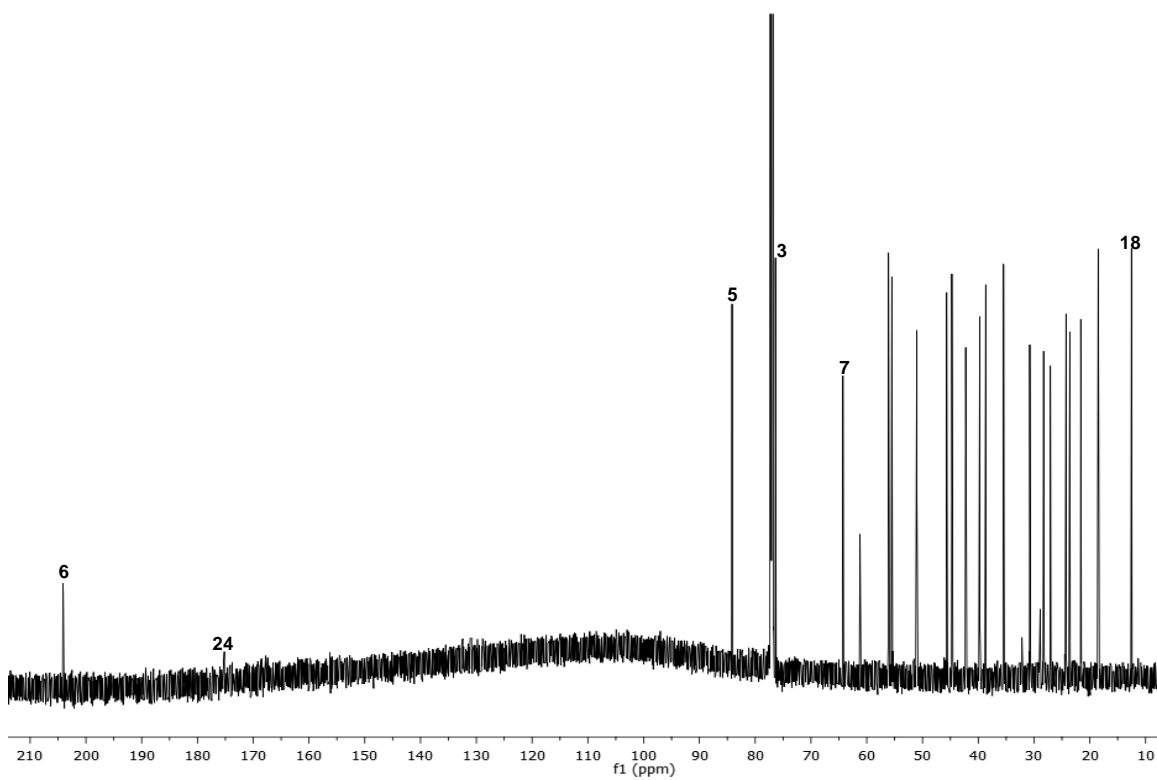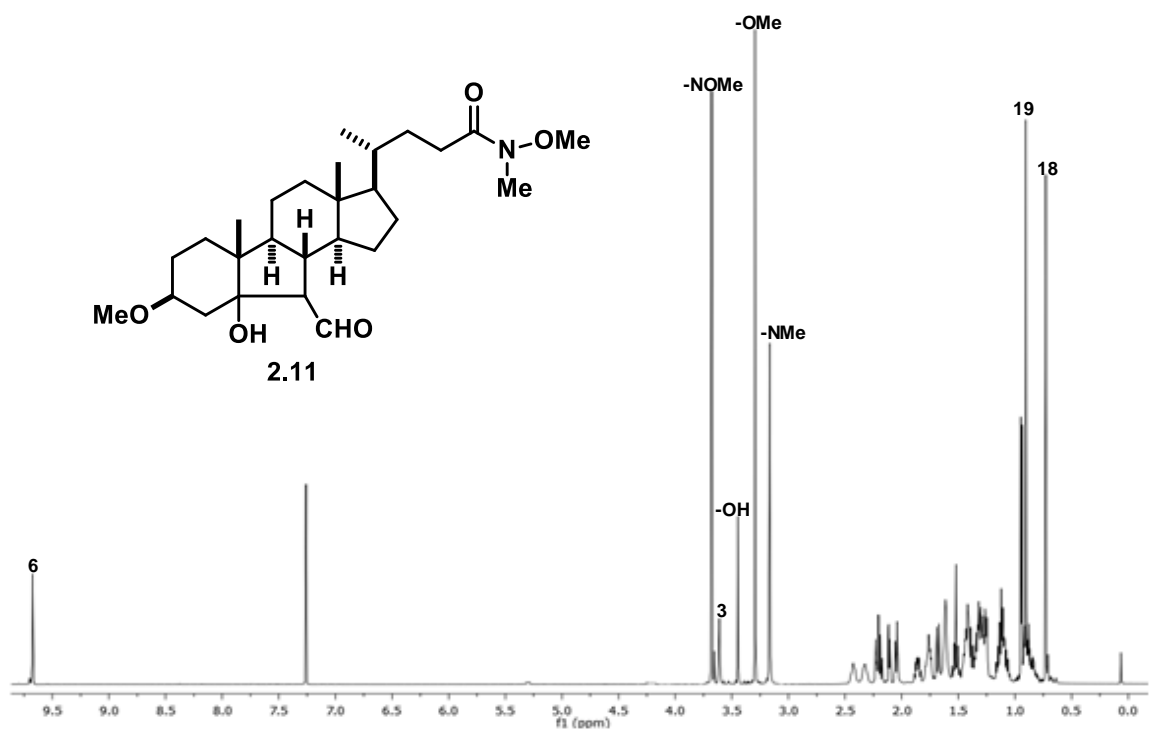
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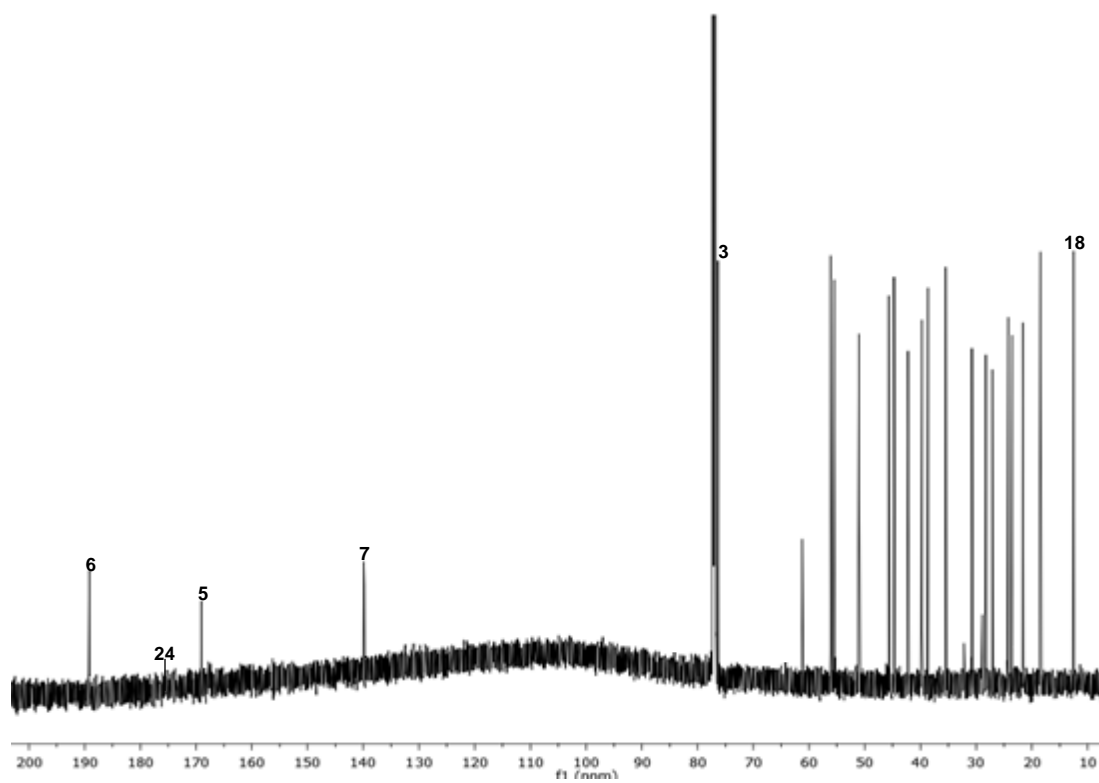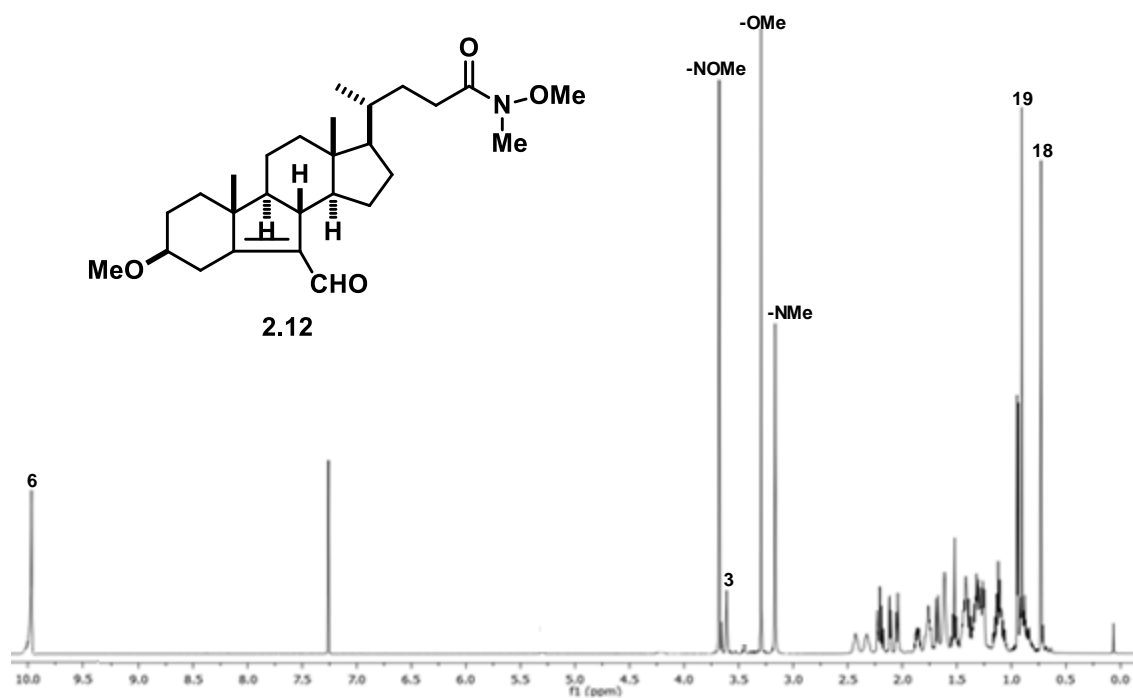
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

2.25
(mixture of epimers)

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

2.26

19
18
21
-NMe
6
3

24
5
6
3
18

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

¹H NMR (500 MHz) and ¹³C NMR (125 MHz) in CDCl₃

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

2.36
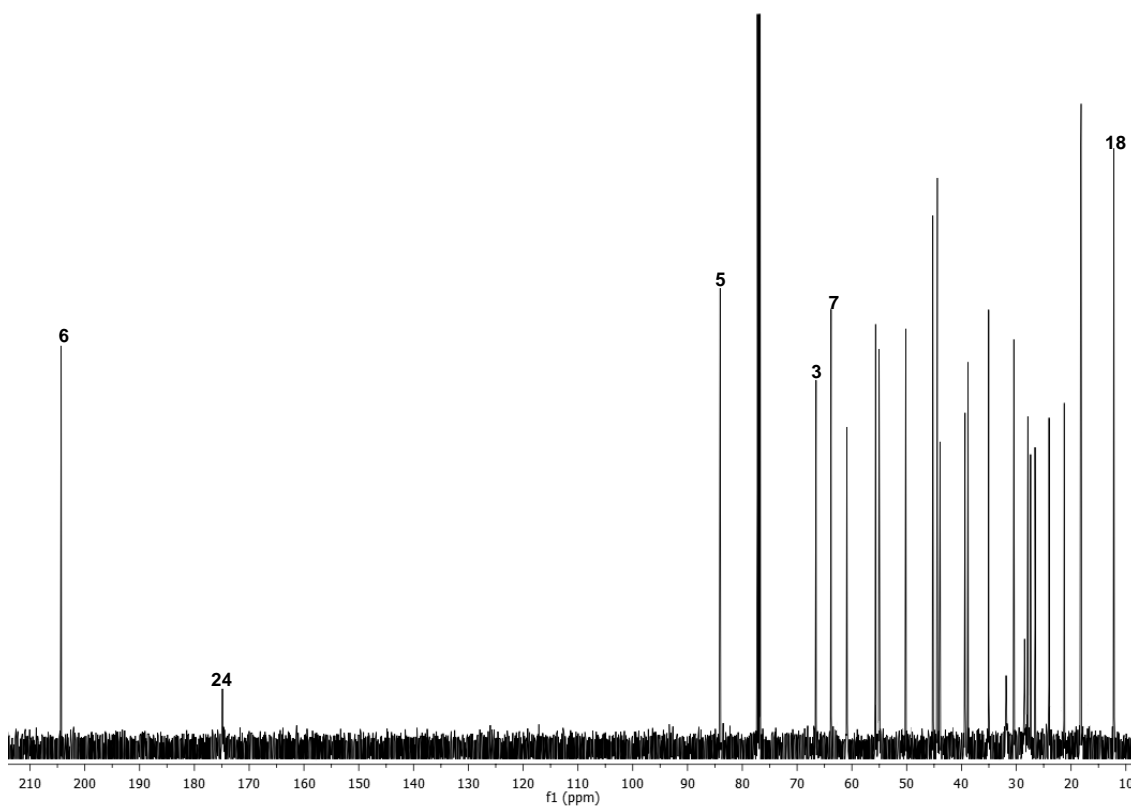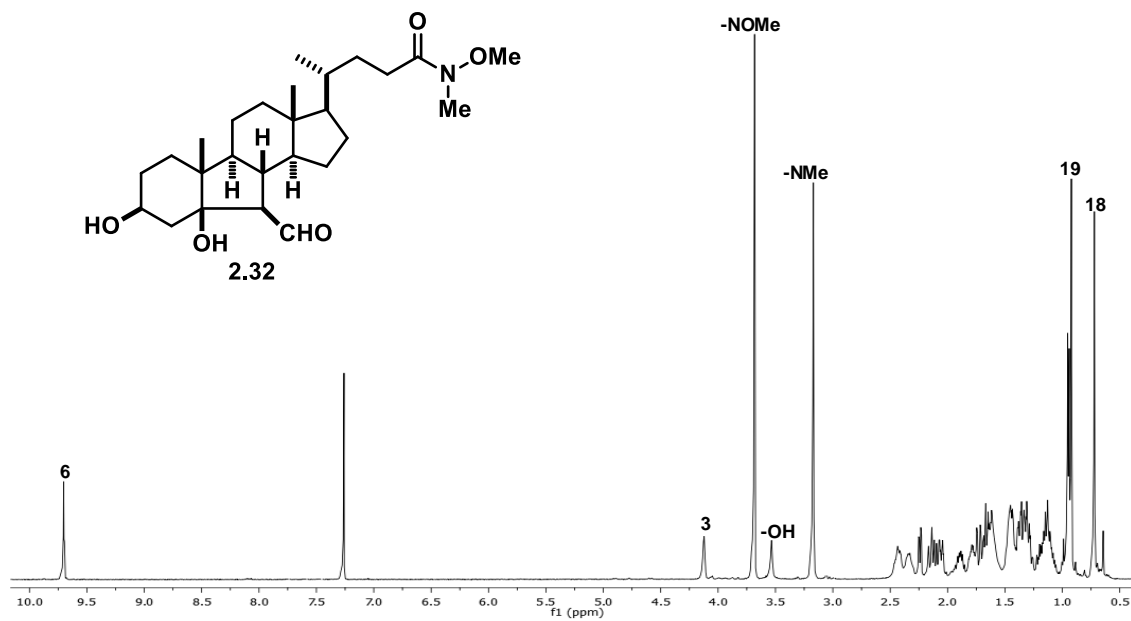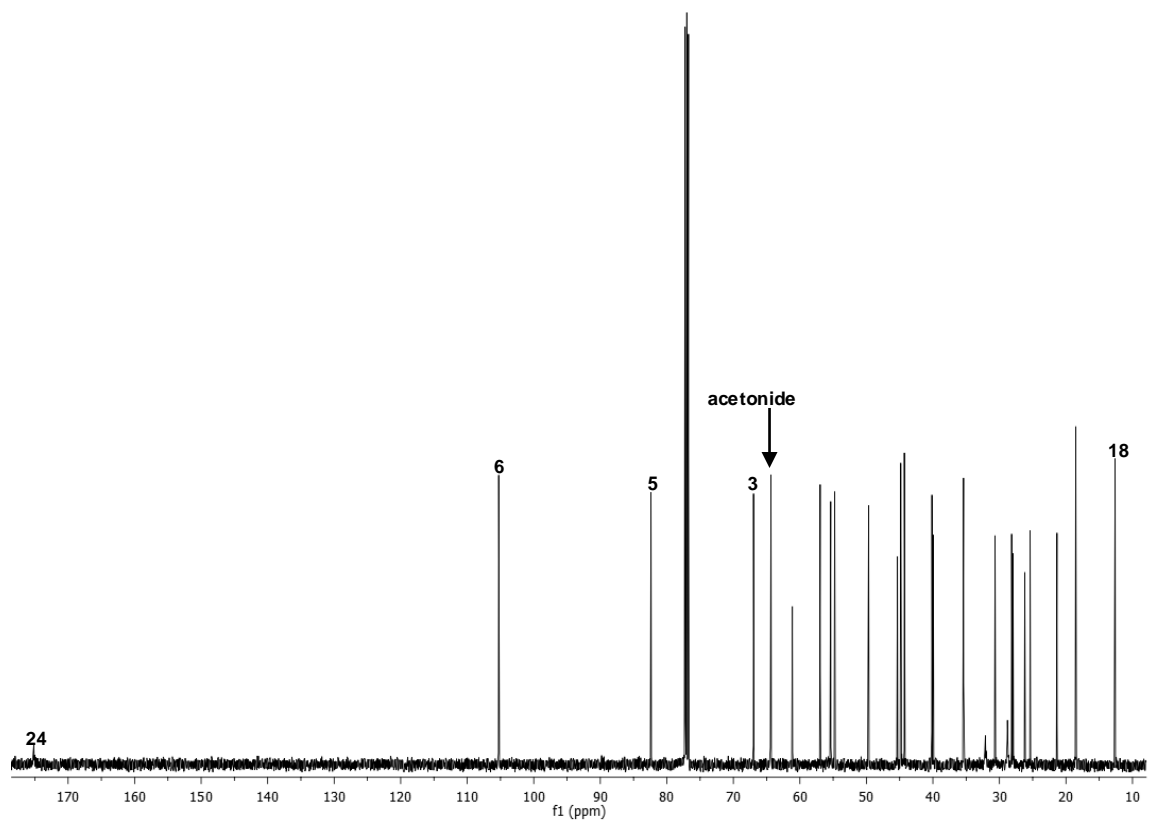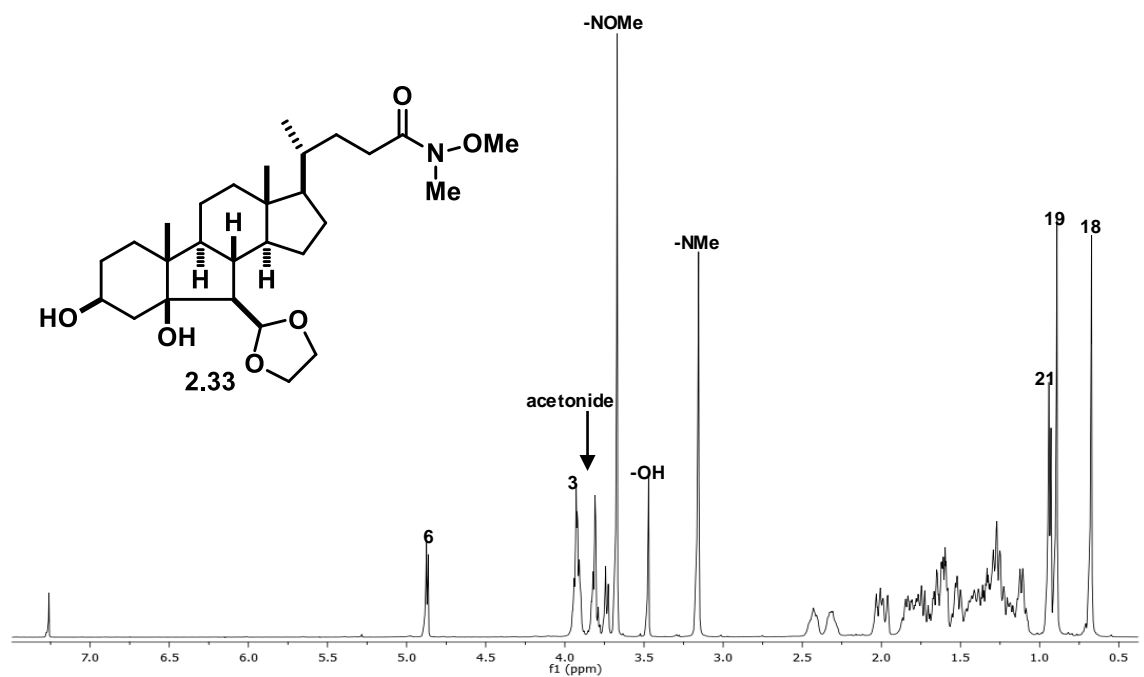
(mixture of epimers)

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

2.37
(mixture of epimers)
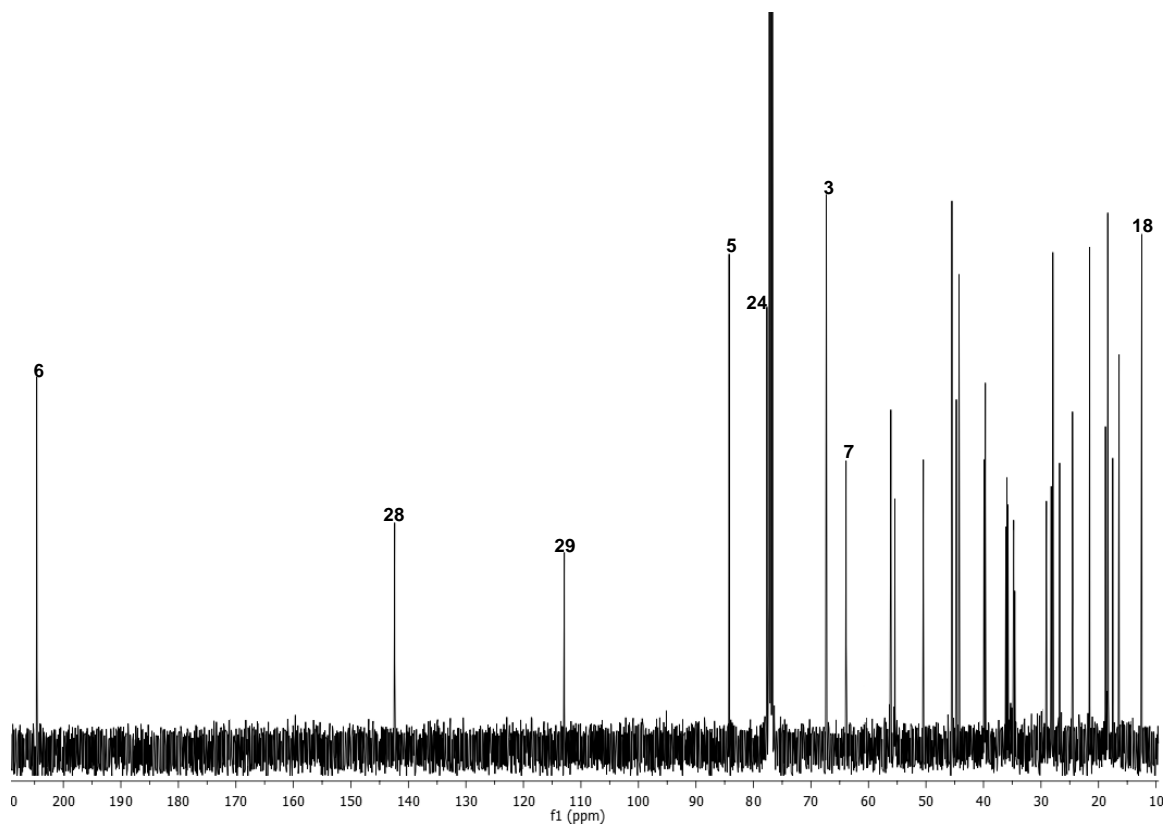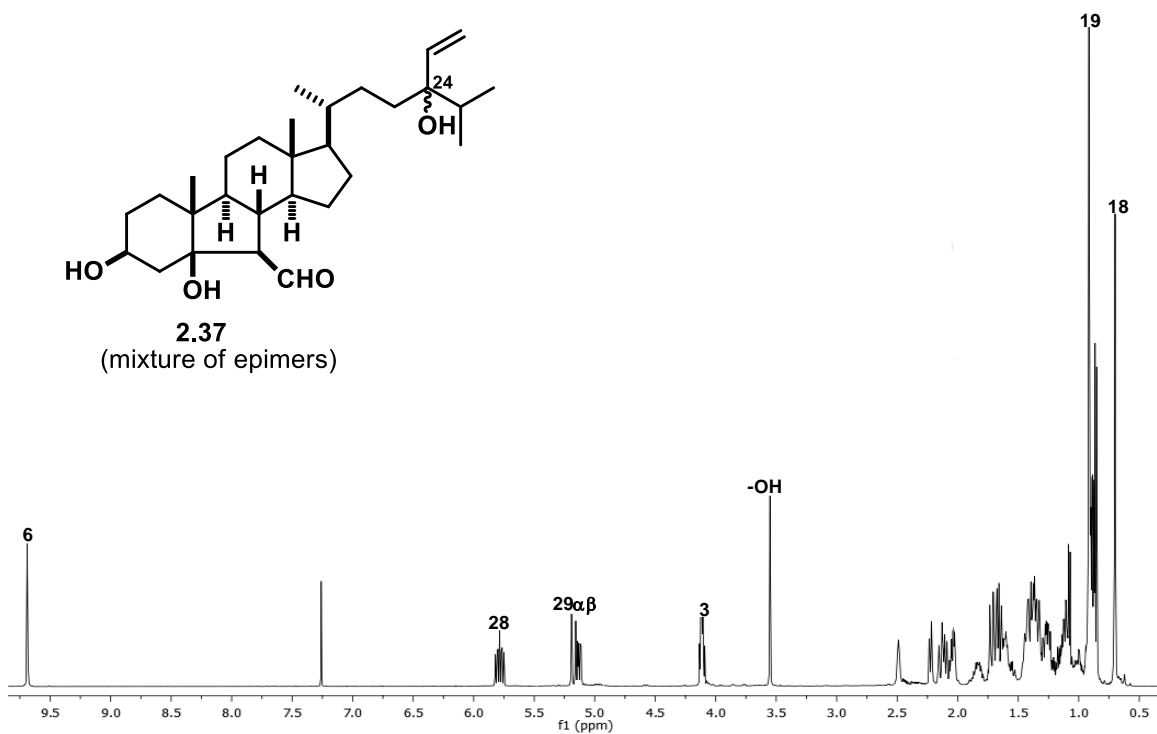
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

2.1
(mixture of epimers)
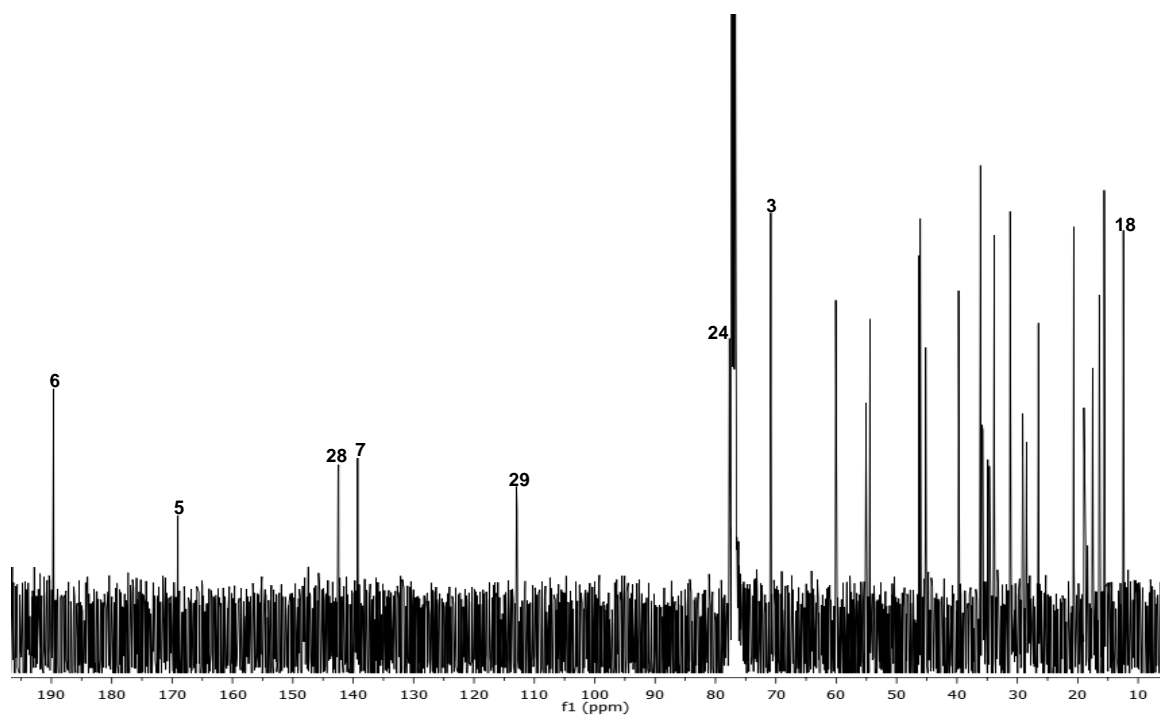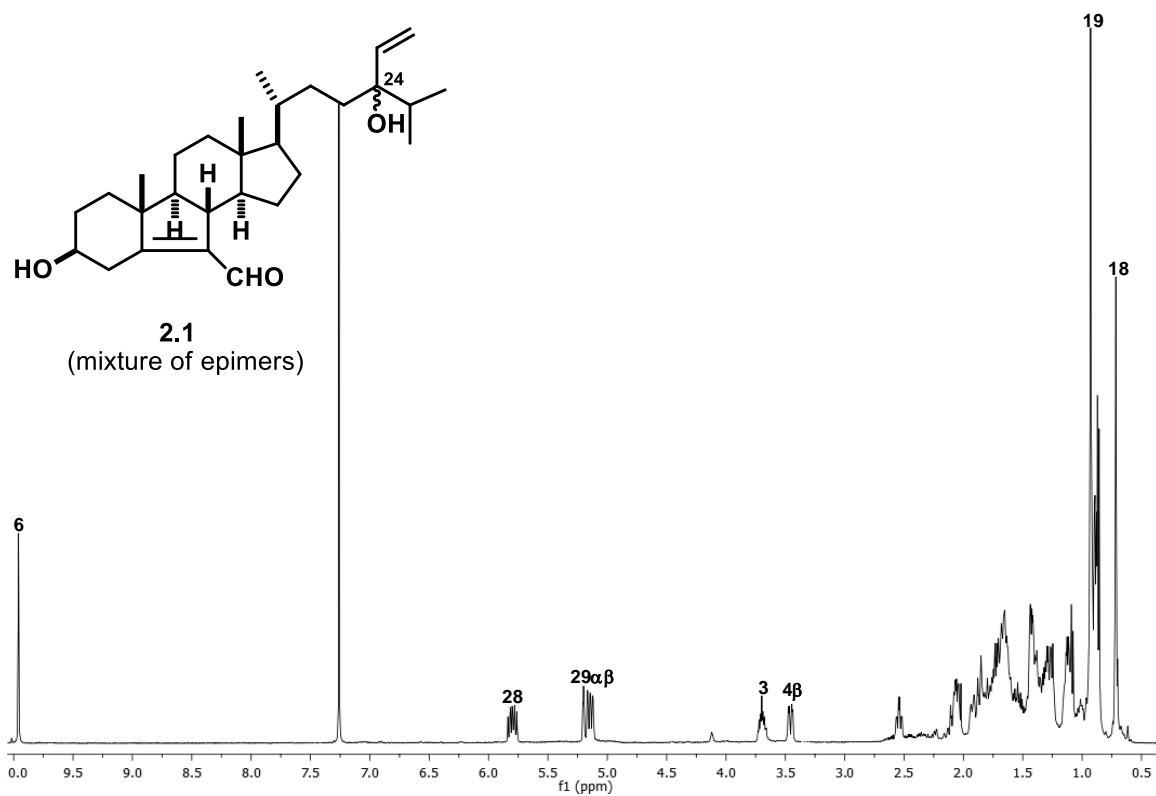
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$
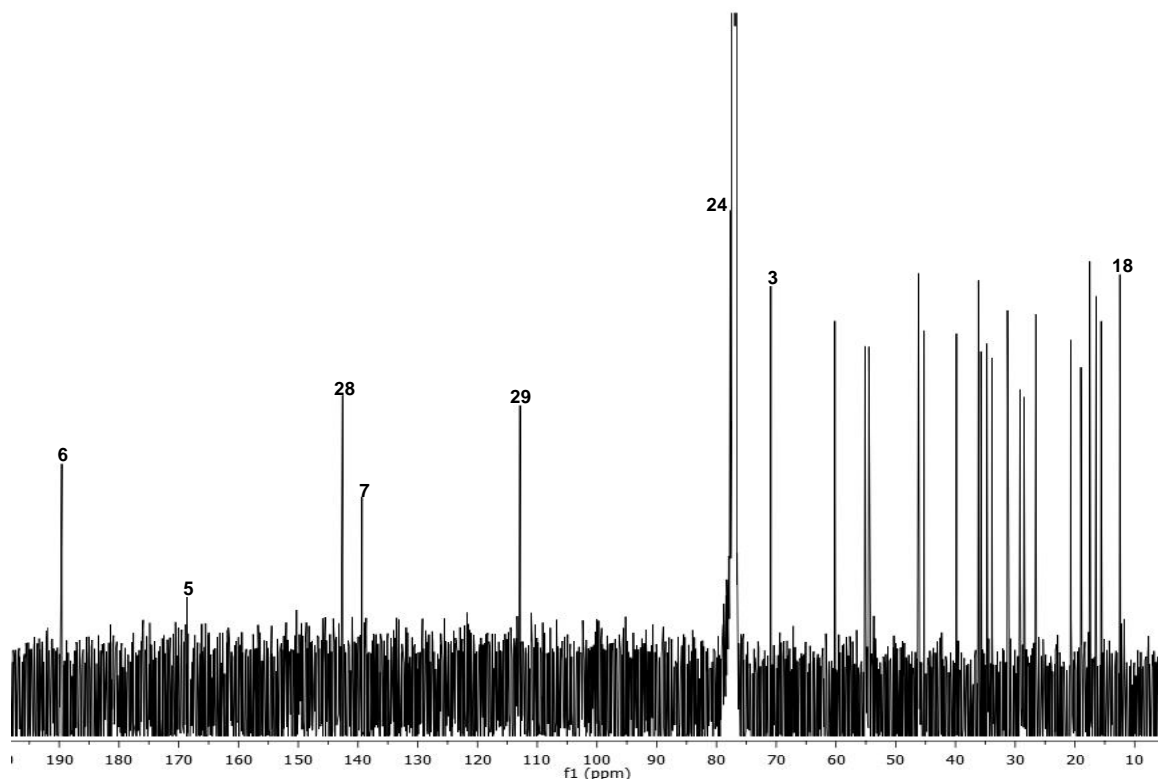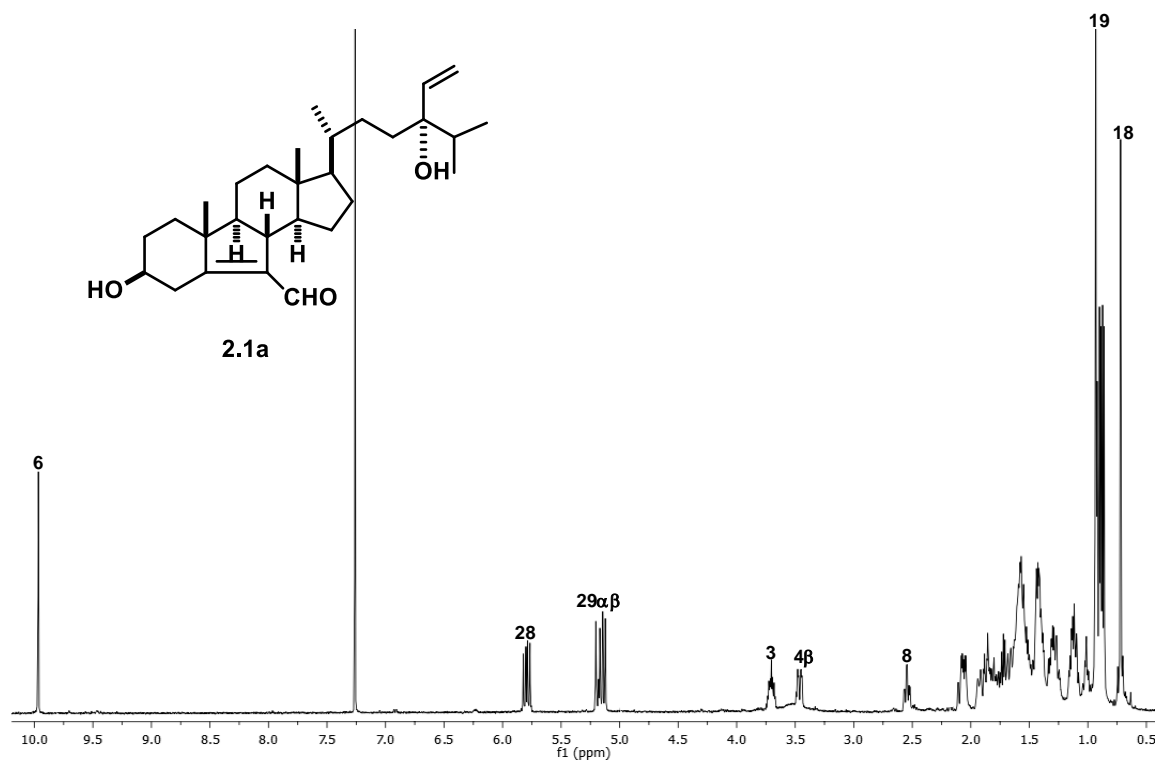
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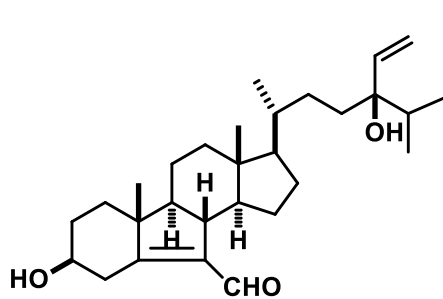
2.1b
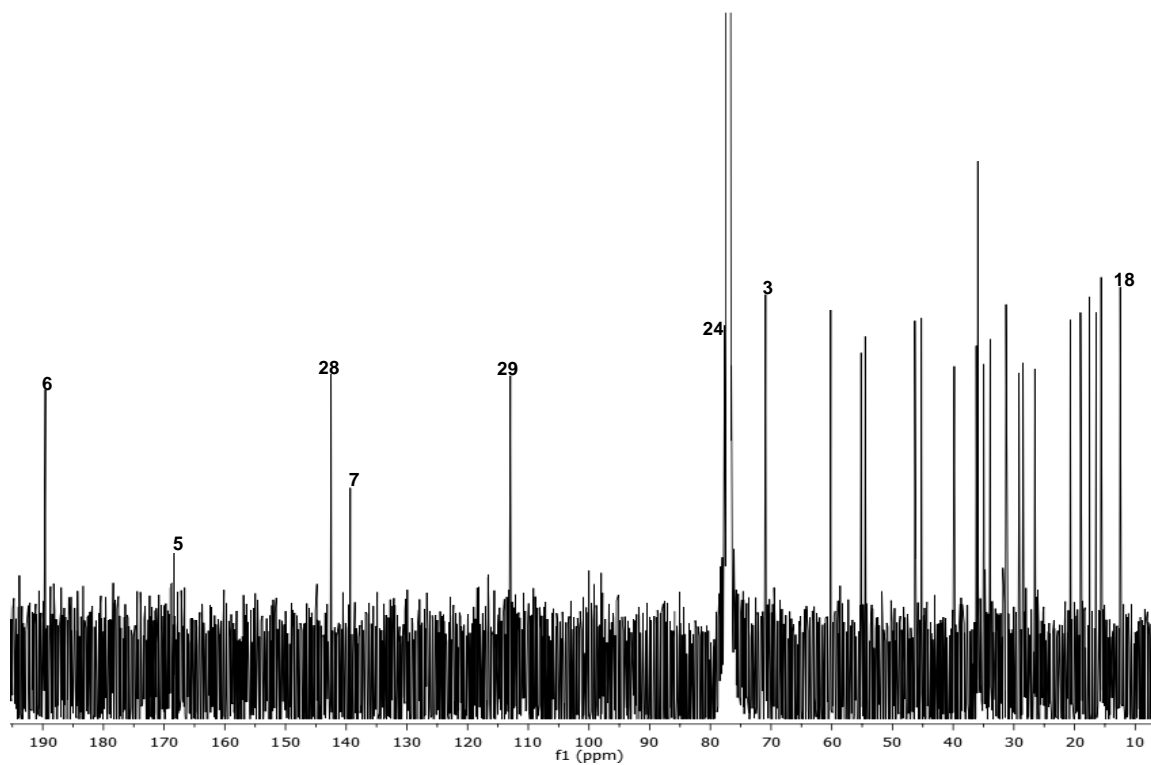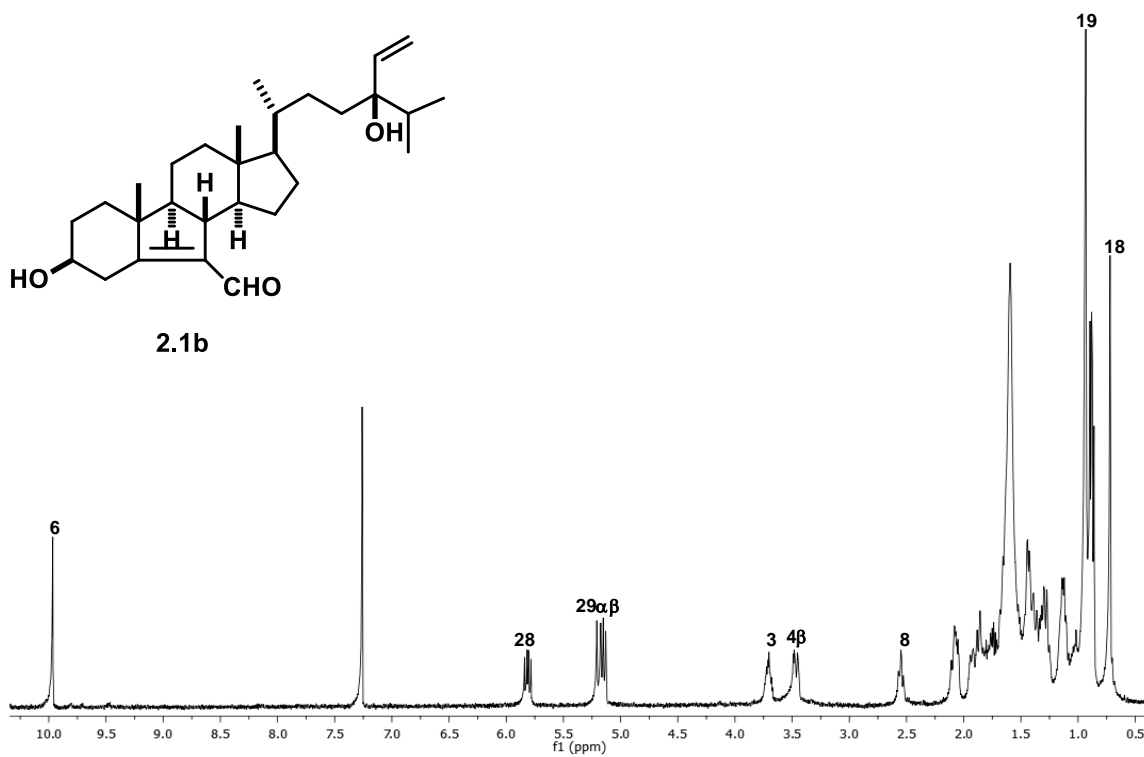
¹H NMR (700 MHz) and ¹³C NMR (175 MHz) in CDCl₃
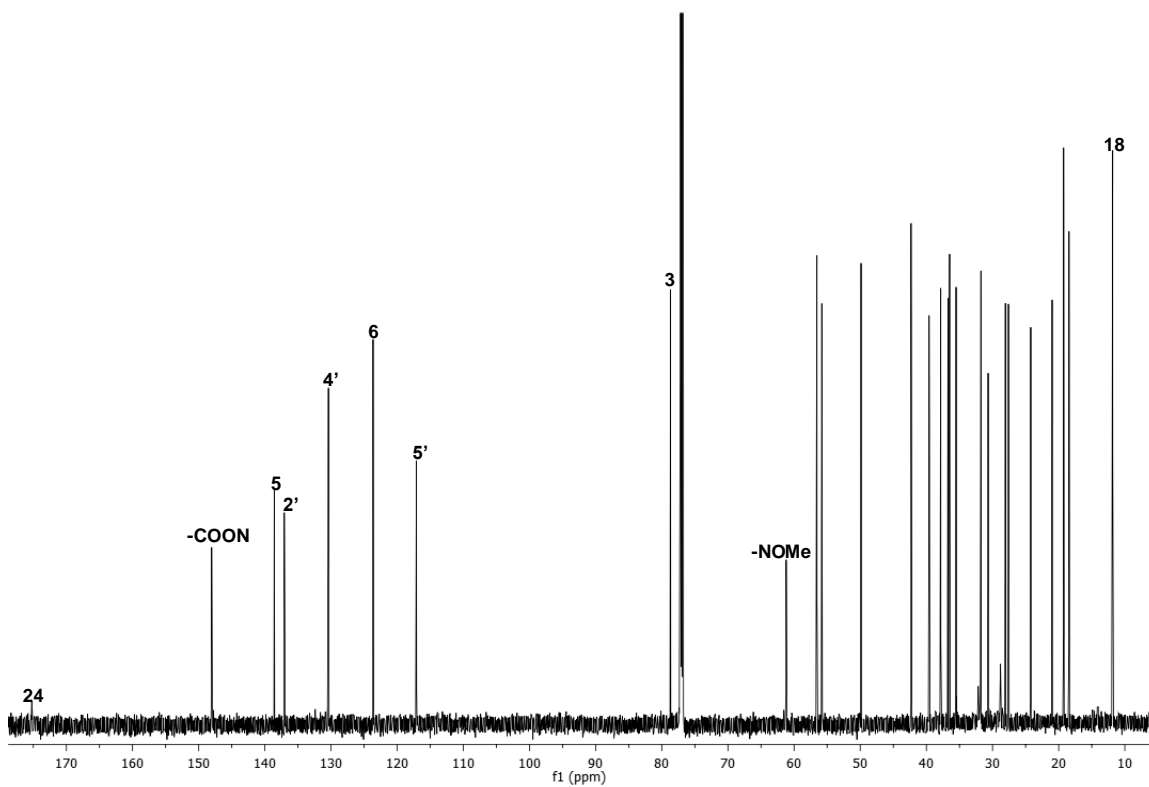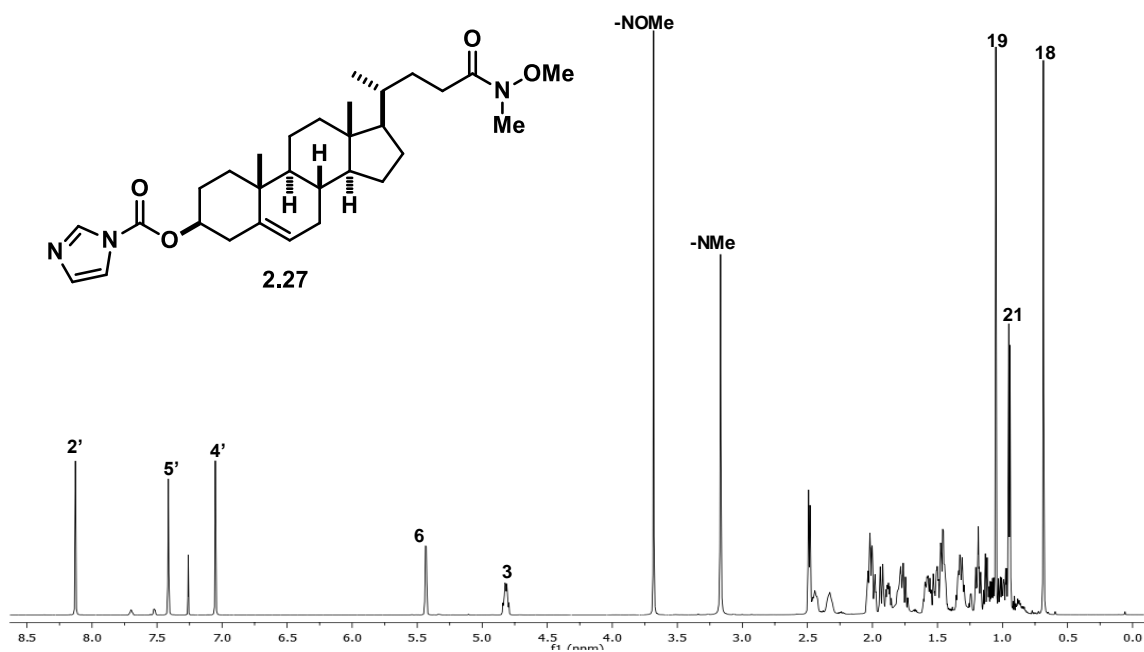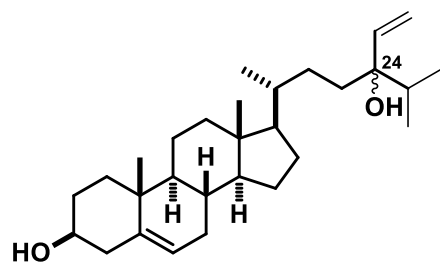

1.22
(mixture of epimers)
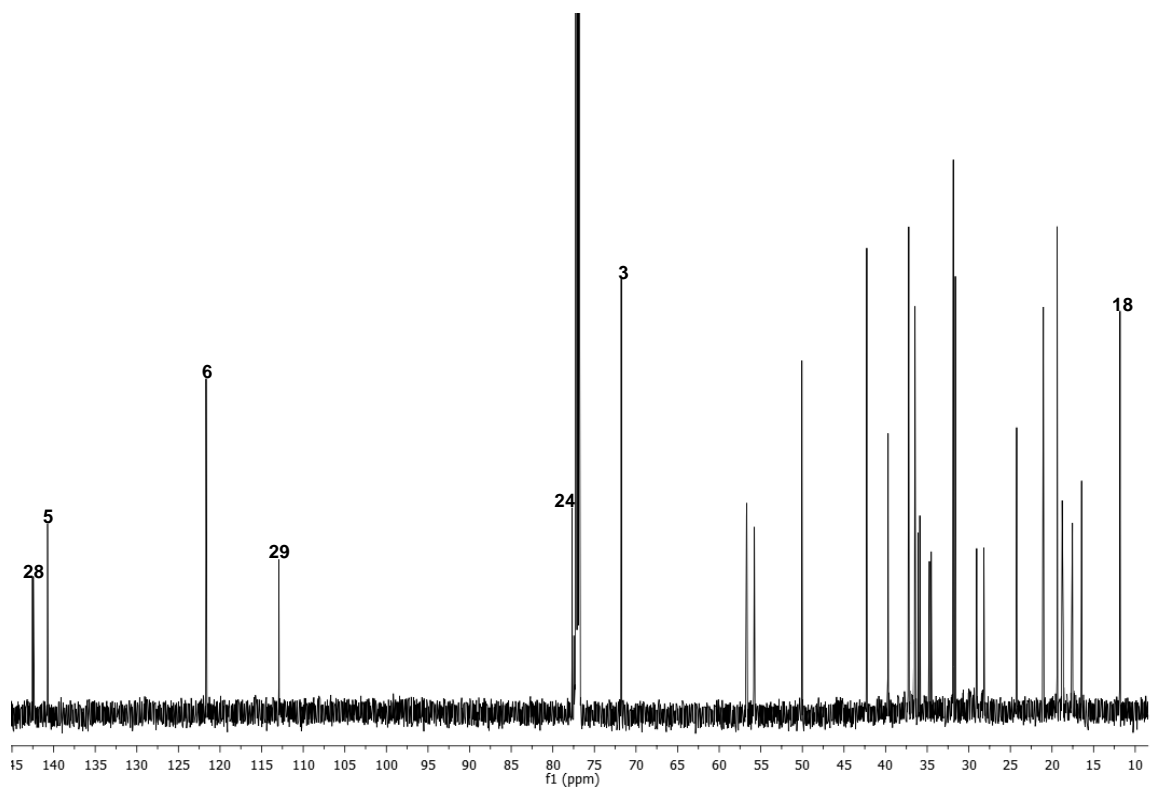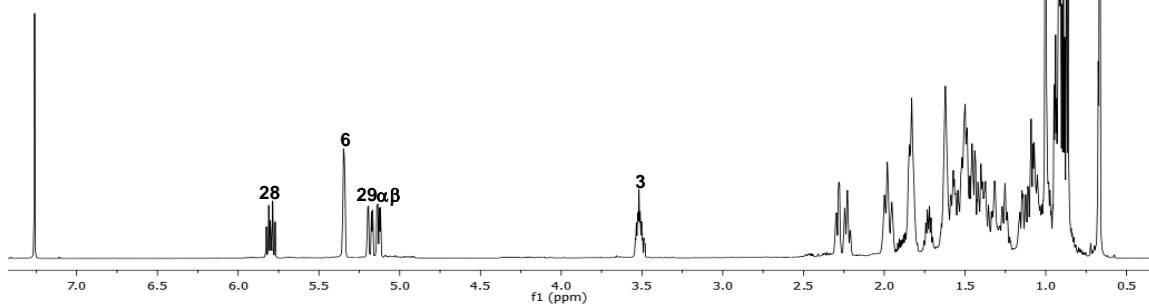
2.8. References

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Chapter 3. Design, Synthesis, and Anti-tubercular Activity Evaluation of Leningosterol Derivatives

3.1. Design of Leningosterol Derivatives

In the previous chapter, the synthesis of leningosterol (**2.1**) was depicted along with its anti-tubercular activity. Unfortunately, it was moderately active, thus it did not turn out to be the lead anti-tubercular agent we intended to develop. Nonetheless, the synthesis of leningosterol (**2.1**) and other derivatives helped us to establish that a hydroxyl group at C-3 is pivotal for the activity, but we need to explore what other structural features are essential. To achieve this goal, we decided to synthesize additional leningosterol derivatives.

Our derivatives will include a hydroxyl group at C-24 along with different *R* groups at the same position to determine the effect on the anti-tubercular activity and compared them with the small library of *abeo*-sterol analogs created by Rodríguez and co-workers.[1] The compounds we aimed to synthesize are depicted in Figure 3.1. Compounds **3.1** to **3.8** are pairs of sterols and *abeo*-sterols that have both a hydroxyl group and a distinct *R* group at the C-24 position. The *R* groups considered include an *iso*butenyl group (**3.1**, **3.2**) to determine how this sterically hindered substituent affects the anti-tubercular activity, an ethyl group (**3.3**, **3.4**) and a methyl group (**3.5**, **3.6**). It would be interesting to establish how these free rotating groups affect the activity. Finally, a hydrogen group (**3.7**, **3.8**) will be placed at C-24 to determine if an alkyl group is at all essential for the activity.
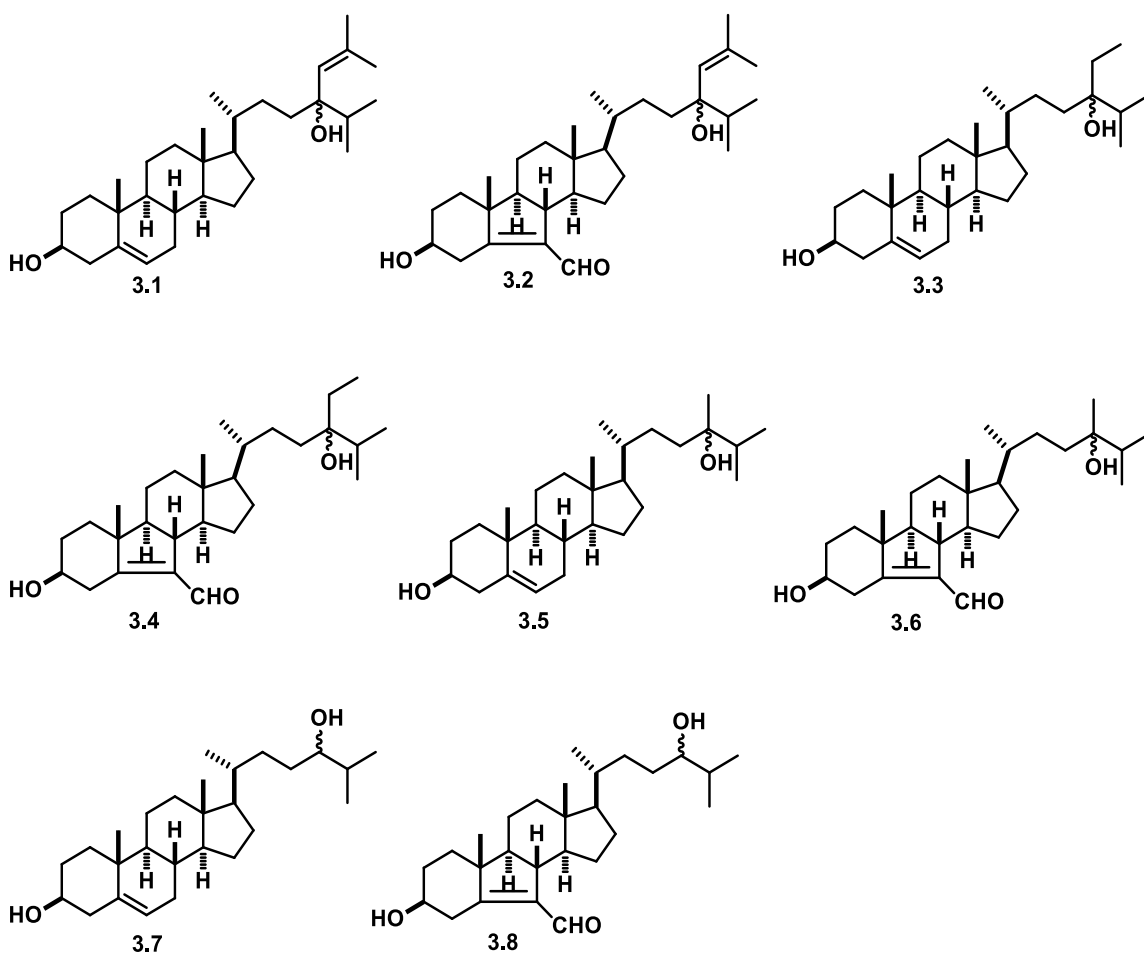
Figure 3.1 Molecular structures of compounds **3.1** - **3.8**.

Concomitant with these efforts, we will modify the 5,7-alkene and the aldehyde at C-6 of active *abeo*-sterols to determine its effect on the biological activity. Compounds **3.9** and **3.10** (epimers at C-24 of *abeo*-sterols **1.49** and **1.54**), synthesized in our laboratory by Dr. Wei, exhibited MIC values of 0.4 μg/mL and 3.7 μg/mL, respectively (Figure 3.2). These data (still unpublished) compared to their epimers **1.49** and **1.54** (MIC~4 μg/mL), respectively, helped us establish that the stereochemistry at C-24 in not a determining factor for anti-tubercular activity.[1] Also the anti-tubercular results of leningosterol epimers **2.1a** and **2.1b** (MIC~23

μg/mL), support this statement. In order to perform SAR studies, we will compare the anti-tubercular activity of *abeo*-sterols **3.9** and **3.10** to that of their analogs having either a hydroxymethylene (**3.11** and **3.12**) or a methyl group (**3.13** and **3.14**) at the C-6 position. Likewise, we will explore the effect of oxidizing the 5,7- and 5,6-alkene functionality (compounds **3.15** and **3.16**) on the biological activity of these derivatives.

1.49 *R* = Et
1.54 *R* = Me

3.9 *R* = Et
3.10 *R* = Me

3.11 *R* = Et
3.12 *R* = Me

3.13 *R* = Et
3.14 *R* = Me

3.15

3.16
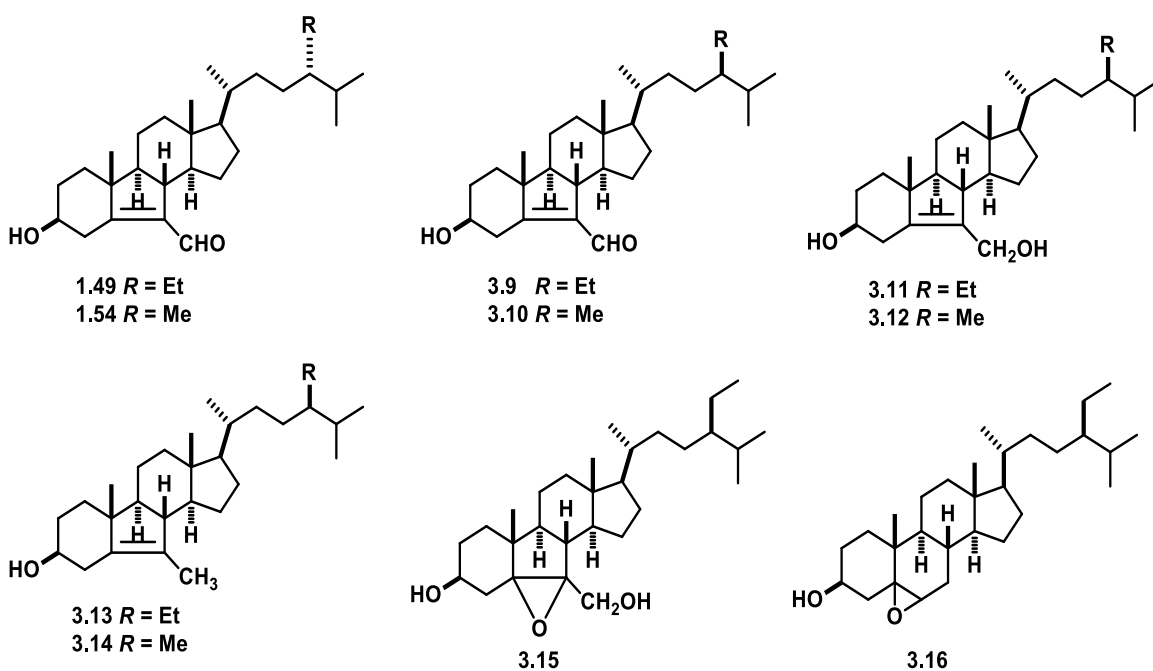
Figure 3.2 Molecular structures of compounds **1.49**, **1.54**, and **3.9 – 3.16**.

3.2. Retrosynthetic Analysis

The retrosynthetic analyses used to prepare the aforementioned compounds are depicted in Schemes 3.1 to 3.4. *Abeo*-sterols **3.4**, **3.6**, and **3.8** will be obtained from the ring-B contraction of sterols **3.3**, **3.5**, and **3.7**, respectively. Sterols **3.1**, **3.3**, **3.5**, and **3.7** will be attained from alkylation of Weinreb amide **2.30**. On the other hand, *abeo*-sterol **3.2** will be synthesized from compound **2.34**, due to synthetic incompatibilities with the olefin group at C-24 (Scheme 3.2).

3.4 *R* = Et
3.6 *R* = Me
3.8 *R* = H

3.1 *R* = ⟨
3.3 *R* = Et
3.5 *R* = Me
3.7 *R* = H

2.30
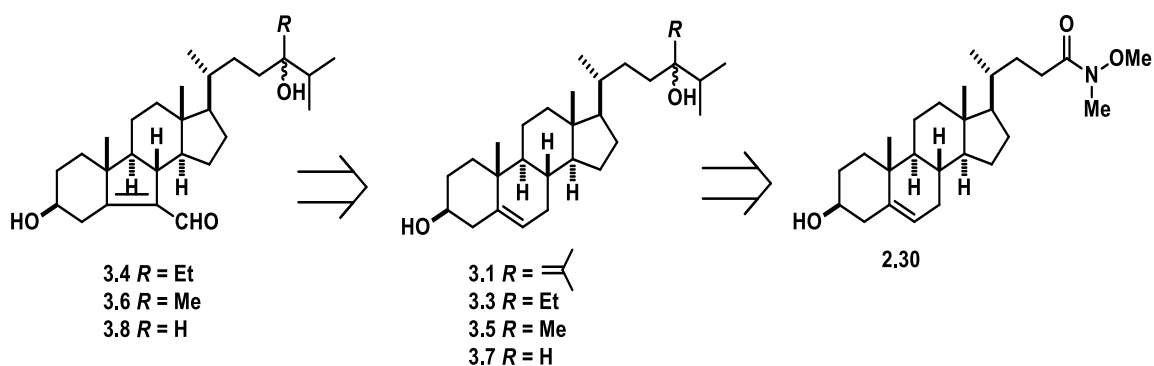
Scheme 3.1 Retrosynthetic analysis of compounds **3.1** and **3.3** – **3.8**.

3.2

2.34
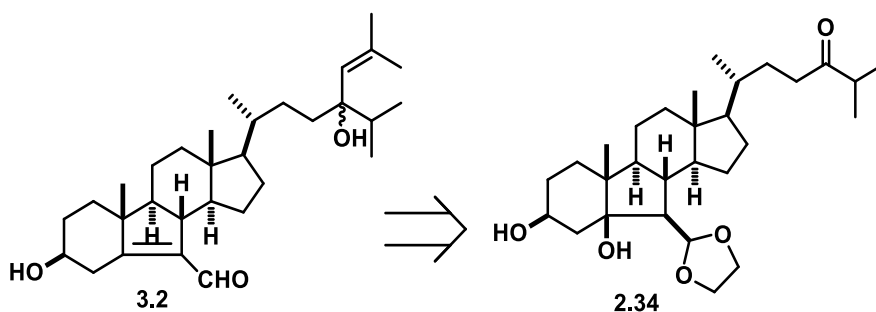
Scheme 3.2 Retrosynthetic analysis of compound **3.2**.

As part of our second study, *abeo*-sterols **3.9** and **3.10** will be the precursors of compounds **3.11** – **3.14** (Scheme 3.3). The starting material for the synthesis

will be a commercially available mixture of β-sitosterol (**3.17**), campesterol (**3.18**), and β-sitostanol (**3.19**).

3.11 R_1 = Et, R_2 = CH$_2$OH
3.12 R_1 = Me, R_2 = CH$_2$OH
3.13 R_1 = Et, R_2 = CH$_3$
3.14 R_1 = Me, R_2 = CH$_3$

3.9 R_1 = Et, R_2 = CHO
3.10 R_1 = Me, R_2 = CHO

3.17 R = Et, Δ^5 (70%)
3.18 R = Me, Δ^5 (15%)
3.19 R = Et (15%)
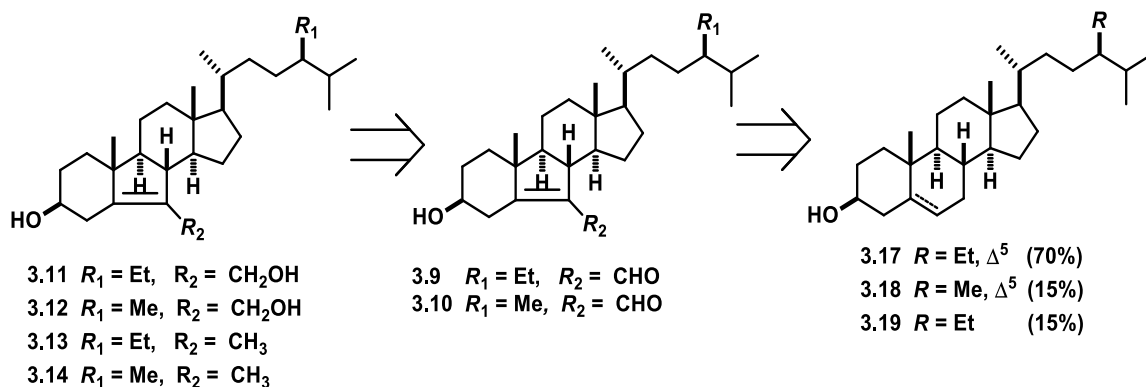
Scheme 3.3 Retrosynthetic analysis of compounds **3.11 – 3.14**.

Finally, epoxy *abeo*-sterol **3.15** will be obtained from the epoxidation of compound **3.11**; while compound **3.16** will be synthesized directly from the mixture of compounds **3.17 – 3.19.**

3.15

3.11

3.16

3.17 R = Et, Δ^5 (70%)
3.18 R = Me, Δ^5 (15%)
3.19 R = Et (15%)
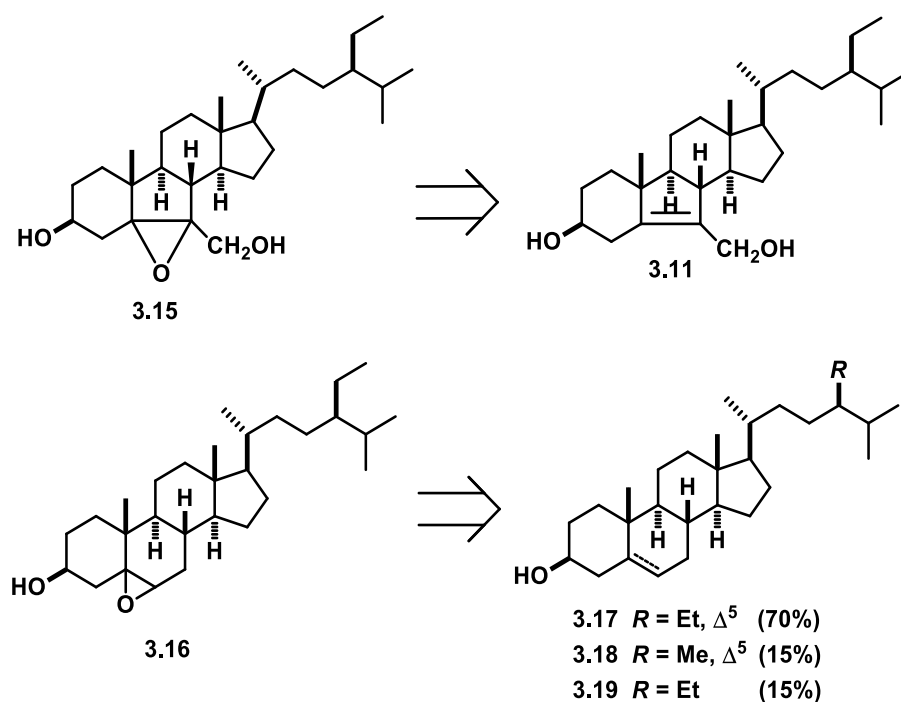
Scheme 3.4 Retrosynthetic analysis of compound **3.15** and **3.16**.

3.3. Synthesis of Leningosterol Derivatives

3.3.1. Synthesis of Compounds 3.1 and 3.3 - 3.8

The synthesis of sterols **3.1**, **3.3**, and **3.5** (Figure 3.1) commenced with the alkylation of Weinreb amide **2.30** with *i*PrLi followed by the appropriate Grignard reagent to introduce the *R* group of interest (Scheme 3.5).[2] Interestingly, sterol **3.1** could not be prepared following this methodology. It is well established that, during a nucleophilic attack by a Grignard reagent, sterically hindered ketones are prone to undergo side reactions, usually the reduction of the carbonyl group or the formation of an enolate.[3] In this case, sterol **3.1** is presumed to be an intermediate that promptly rearranges to sterol **3.20** due to the basicity of the Grignard reagent and steric hindrance (Scheme 3.6). The vinyl protons of the 2-methylallyl group of **3.20** resonate at 4.92 and 4.74 ppm, whereas the corresponding ^{13}C NMR signals appeared at 143 and 115 ppm (the corresponding signals for an *iso*butenly group appear at 135 and 125 ppm in the ^{13}C NMR spectrum and a singlet around 5.20 ppm in the ^{1}H-NMR spectrum).[4] On the other hand, sterol **3.7** was obtained as planned after treatment of **2.30** with *i*PrLi followed by a reduction with sodium borohydride (NaBH$_4$) (Scheme 3.5).[5]
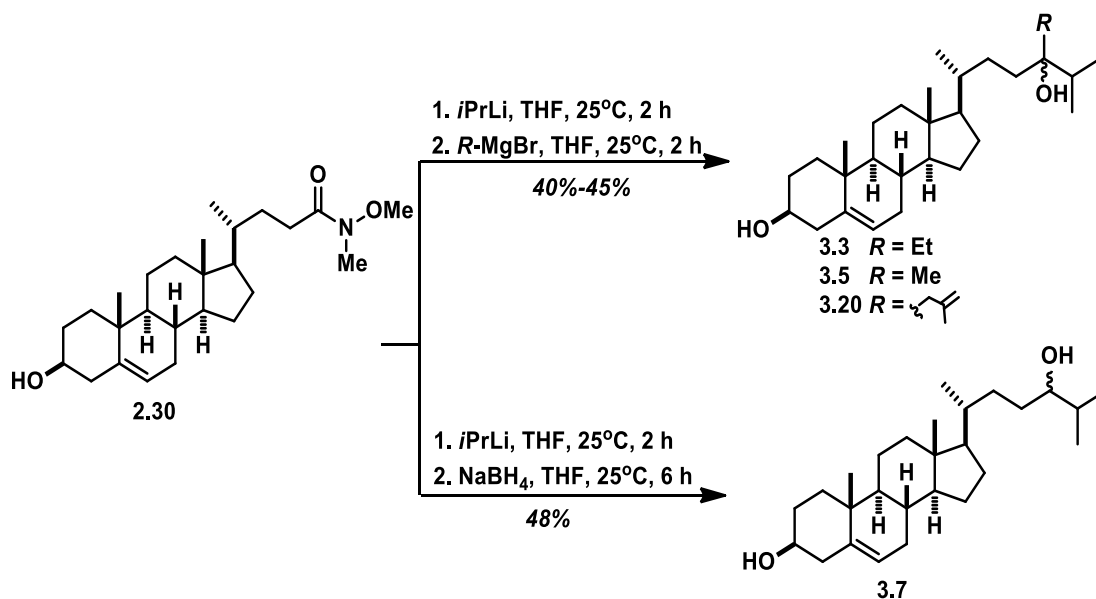
Scheme 3.5 Synthesis of compounds **3.3**, **3.5**, **3.7**, and **3.20**.
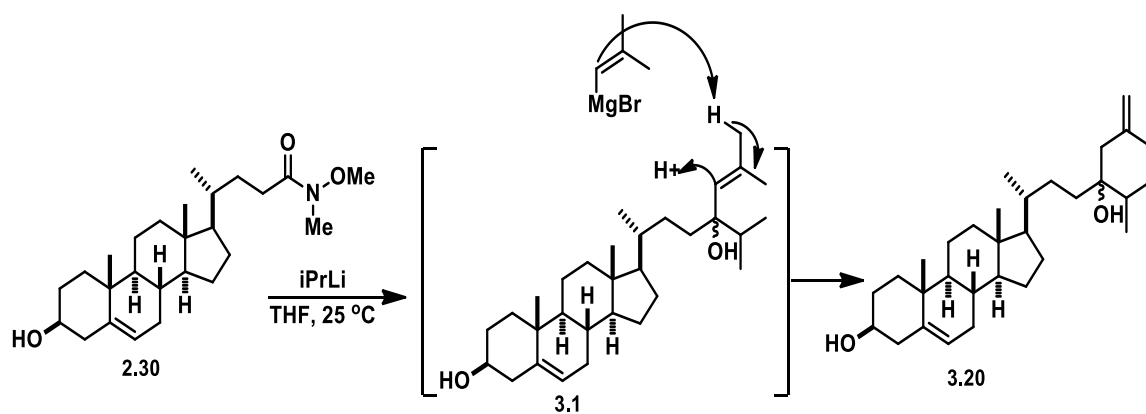
Scheme 3.6 Plausible mechanism for the formation of compound **3.20**.

Therefore, in order to convert compounds **3.3**, **3.5**, and **3.7** to their corresponding *abeo*-analogs a few steps were performed (Scheme 3.7). These compounds were submitted separately to an oxidative cleavage with ozone,

followed by intramolecular aldol condensation to afford *abeo*-sterols **3.4, 3.6**, and

3.8, respectively.[1,6,7]

1. O_3, CH_2Cl_2-MeOH, -78 °C
2. Me_2S, THF, 25°C
3. pyrrolidine, 25°C, 30min
4. 1.0M KOH-MeOH, 25°C, 3 h
40%

3.3 *R* = Et
3.5 *R* = Me
3.7 *R* = H

3.4 *R* = Et
3.6 *R* = Me
3.8 *R* = H
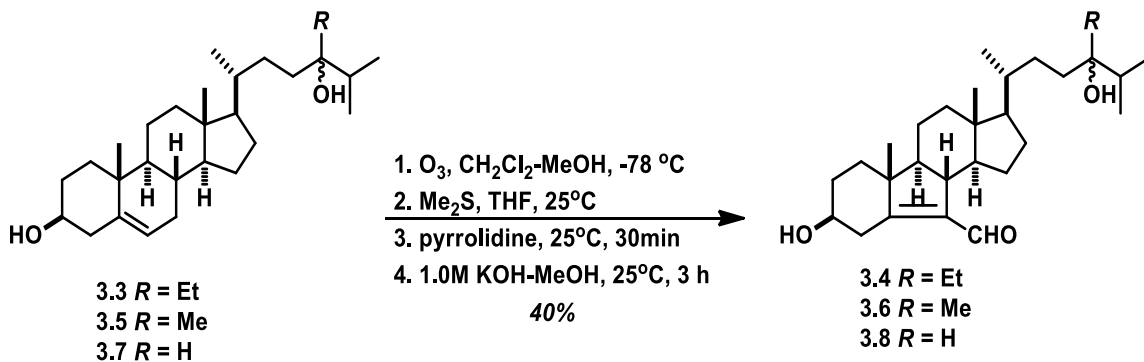
Scheme 3.7 Synthesis of compounds **3.4**, **3.6**, and **3.8**.

3.3.2. Synthesis of Compound 3.2

Due to the synthetic incompatibility of the olefin group at C-24 the synthesis of *abeo*-sterol **3.2** (Figure 3.1) was performed emulating the synthesis of leningosterol (**2.1**). Thus, the alkylation of ketone **2.34** followed by aldehyde deprotection and further dehydration led to *abeo*-sterol **3.21** in 38% yield instead of compound **3.2**.[2,7,8] Once again, as was the case with sterol **3.20**, the Grignard reagent acted as a base favoring the olefin rearrangement.[3]
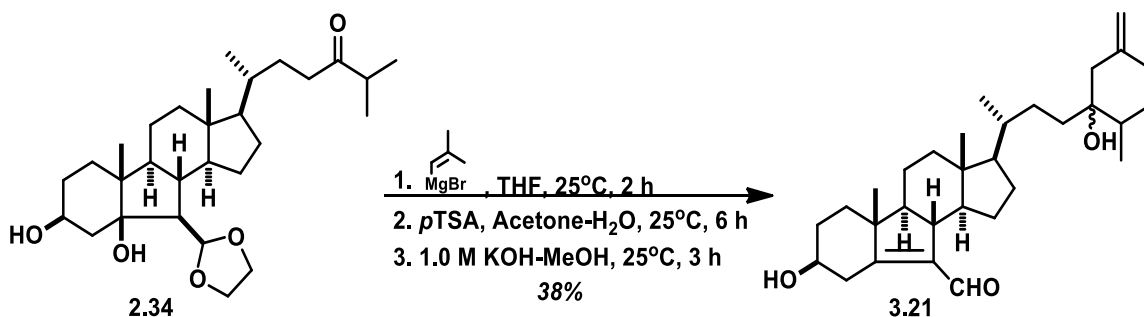
Scheme 3.8 Synthesis of compound **3.21**.

3.3.3. Synthesis of Compounds 3.11 – 3.14

A commercially available mixture of β-sitosterol (**3.17**, 70%), campesterol (**3.18**, 15%), and β-sitostanol (**3.19**, 15%) was selected as the starting material for the synthesis of *abeo*-sterols **3.11** – **3.14** (Schemes 3.9 – 3.11). This mixture underwent oxidative cleavage with ozone, followed by aldol condensation to afford *abeo*-sterols **3.9** and **3.10** as a mixture.[1,6,7] Then, reduction with NaBH₄ in THF produced alcohols **3.11** and **3.12** , but also the known epoxide **3.16a** (for characterization and anti-tubercular activity purposes these compounds were separated by column chromatography and HPLC).[5] The formation of this epoxide was surprising, and the stereoselectivity of the reaction even more so. It is known that the reduction of α,β-unsaturated carbonyl compounds by metal hydrides can follow two pathways: 1,2-addition (carbonyl reduction) or 1,4 addition (carbonyl and alkene reduction).[5,9] Specifically, the reduction of conjugated carbonyl compounds with NaBH₄ is highly solvent dependent, and it generally lacks regioselectivity.[5,10,11] Nonetheless, to the best of our knowledge, this is the first example where an epoxide is formed concomitantly with ring expansion. For this

reason an in-depth study of a plausible mechanism should be performed in the future. The reaction was also performed utilizing methanol as solvent; however, a complex mixture of compounds was obtained and the signals corresponding to the 5,7 alkene were not observed. In this case, sodium borohydride reacts with methanol to form methoxyborohydrides which are more reactive species.[9,12] The selective 1,2-reduction of α,β-unsaturated carbonyl compounds is commonly achieved by using modified tetrahydroborate agents; however it depends on the substrate since cyclic systems are more prone to 1,4-reduction than the acyclic analogs.[9,13-15] The stereochemistry of the epoxide was determined by comparing the ^{1}H and ^{13}C NMR chemical shifts of **3.16a** with those reported in the literature for the same compound.[16]
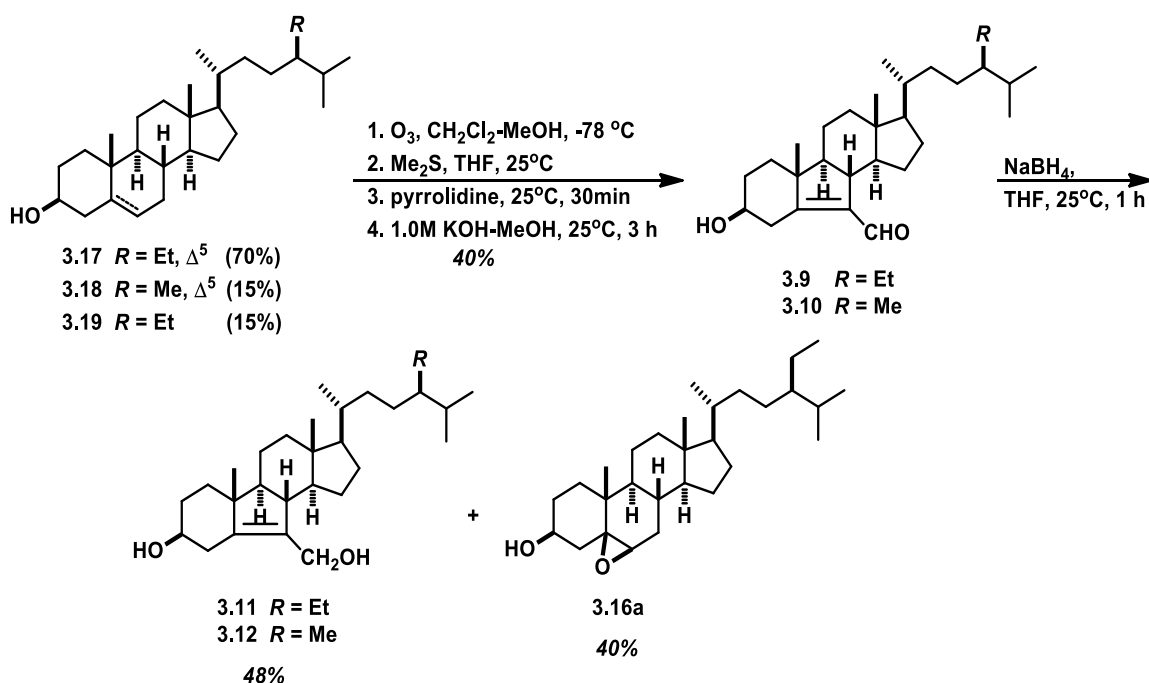
Scheme 3.9 Synthesis of compounds **3.11**, **3.12** and **3.16a**.

Finally, selective reduction of a mixture of primary alcohols **3.11** and **3.12** with sodium cyanoborohydride (NaBH$_3$CN) and boron trifluoride etherate (BF$_3$·Et$_2$O) led to a mixture of C-6 methyl substituted *abeo*-sterols **3.13** and **3.14** in 16% yield (Scheme 3.10).[17] Finally, these compounds were separated by HPLC for characterization and anti-tubercular activity purposes.

3.11 *R* = Et
3.12 *R* = Me

3.13 *R* = Et
3.14 *R* = Me
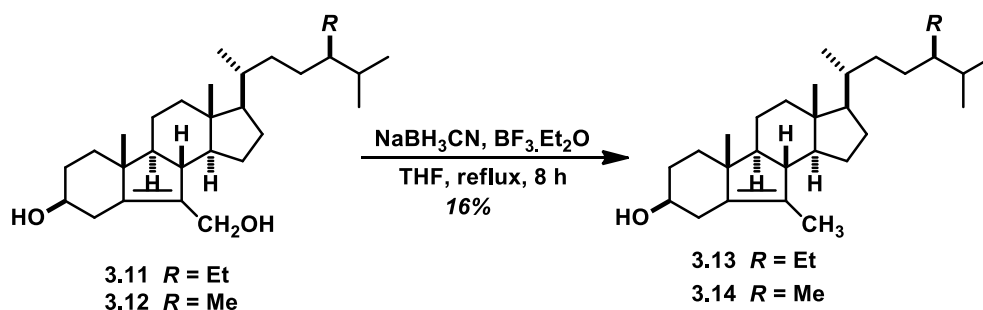
Scheme 3.10 Synthesis of compounds **3.13** and **3.14**.

3.3.4. Synthesis of Compounds 3.15 and 3.16b

Epoxides **3.15** and **3.16** (Figure 3.2) were obtained from the oxidation of compounds **3.11** and **3.17**, respectively (Scheme 3.11). Magnesium monoperoxyphthalate (MMPP) was chosen as the oxidizing agent since the yields obtained were higher in comparison with those obtained with *meta*-chloroperoxybenzoic acid (*m*CPBA).[18,19] The stereochemistry of the known epoxide **3.16b** was determined by comparing the ^{1}H and ^{13}C NMR chemical shifts with those reported in the literature for the same compound.[16] Epoxide **3.16b** was separated from unreacted sterol **3.19** by column chromatography and then purified

by HPLC. A small amount of the oxidized product of compound **3.18** was generated but it was consumed during purification by HPLC.
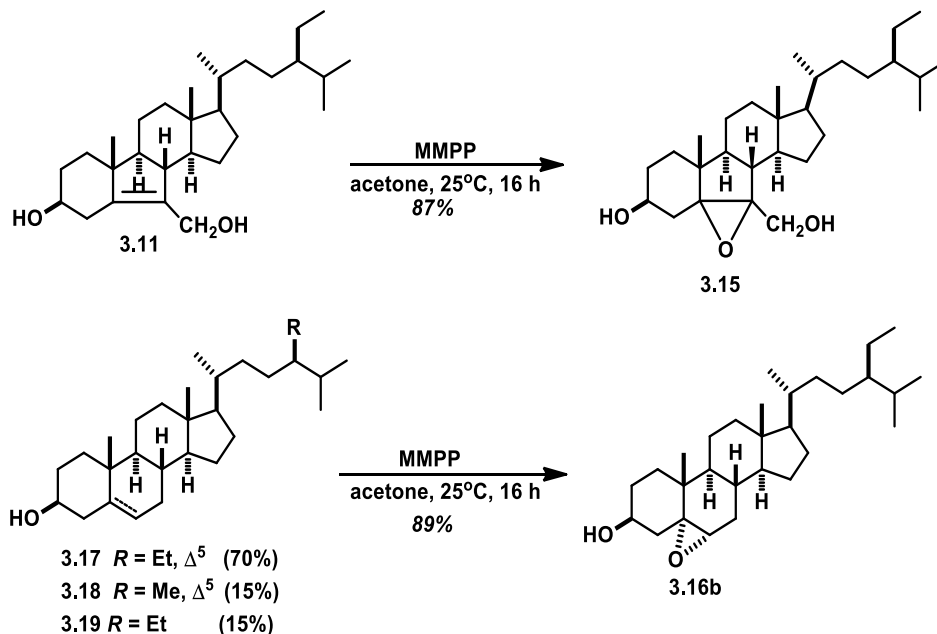
Scheme 3.11 Synthesis of compounds **3.15** and **3.16b**.

3.4. Evaluation of Anti-tubercular Activity: Results and Discussion

The aim of synthesizing compounds **3.3-3.8**, **3.11-3.16**, and **3.20-3.21** was to perform SAR studies and to find a lead anti-tubercular compound. The MIC values of these compounds were determined using an *in vitro* growth inhibition assay against a laboratory strain of *Mtb* H$_{37}$Rv with the anti-mycobacterial drug rifampicin as a positive control (Table 3.1).[20]
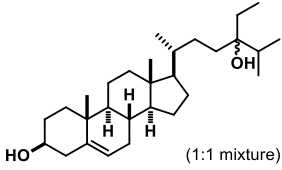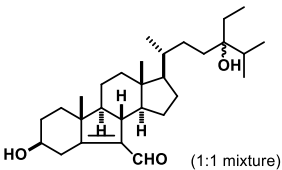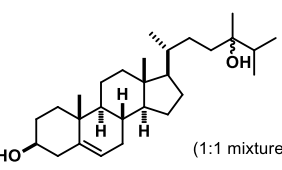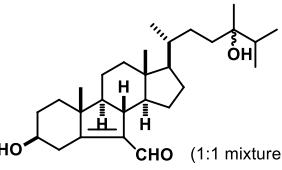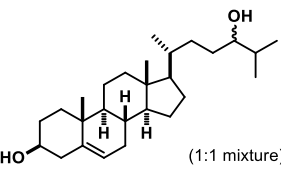
Sterols **3.3**, **3.5**, and **3.20** (entries 1, 3, and 7) were moderately active with MIC values of >35 - >38 μg/mL; while sterol **3.7** (entry 5) was inactive, MIC >64
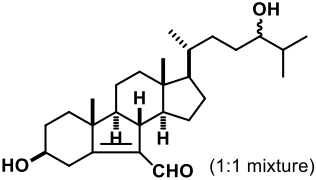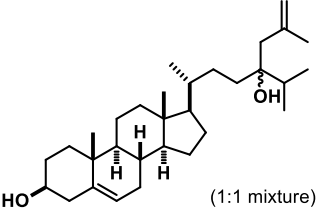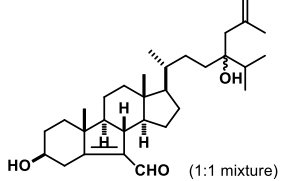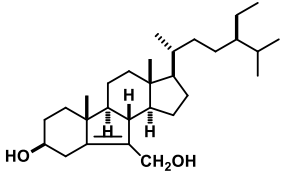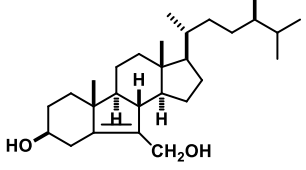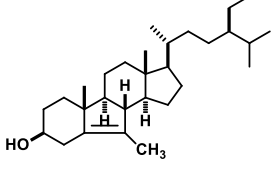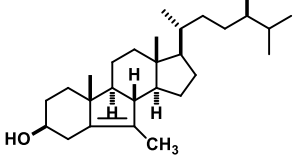
μg/mL. Likewise, *abeo*-sterols **3.4** and **3.6** (entries 2 and 4) exhibited moderate activity (MIC's 18 - >32 μg/mL), compound **3.21** (entry 8) showed modest activity (MIC >56 μg/mL) and compound **3.7** (entry 5) was inactive with a MIC >64 μg/mL. Valuable information can be obtained from these anti-tubercular screening results. An *R* group at C-24 is necessary for the activity, since when absent (compounds **3.7** and **3.8**) the anti-tubercular activity is completely lost. On the other hand, the identity of the *R* group at C-24 among sterols **3.3**, **3.5**, and **3.20** seems to have no significant effect on the biological activity. However, the effect of the *R* group at this position is considerable in the *abeo*-sterol series of compounds with an ethyl group being the most active (**3.4**), followed by the methyl group (**3.6**), and finally the 2-methylallyl group (**3.21**).
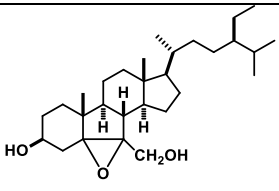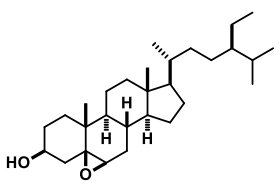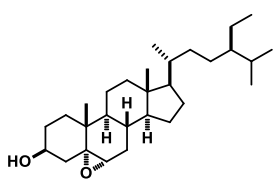
When comparing these anti-tubercular activity results with the small library of compounds made by Rodríguez and co-workers,[1] important conclusions can be reached. Cholesterol (**1.23**), a sterol with no anti-tubercular activity (MIC >128 μg/mL), becomes active (MIC = 15 μg/mL) when converted to its *abeo*-sterol **1.50** by ring-B contraction (Figure 3.3). However, when a hydroxyl group is added to the active *abeo*-sterol **1.50** at position C-24, the activity is completely lost (compound **3.8**, MIC > 64 μg/mL). A similar pattern is observed with 24α-methylcholesterol (**1.53**) and 24α-ethylcholesterol (**1.48**). These are inactive sterols (MIC's >128 μg/mL), but their corresponding *abeo*-sterol analogs (**1.54** and **1.49**) are very active (MIC's ~4 μg/mL). However, when a hydroxyl group is placed at C-24 the anti-tubercular activity is diminished (compound **3.6**, MIC > 32 μg/mL; compound **3.4**, MIC = 18 μg/mL). These data suggest that the hydroxyl group at

C-24 might be responsible for the reduced anti-tubercular activity in the *abeo*-sterol series of compounds.

Table 3.1 MIC values for compounds 3.3-3.8, 3.11-3.16, 3.20, and 3.21

Entry	Compound	Structure	MIC[a] (μg/mL)
1	3.3	(1:1 mixture)	>38[b]
2	3.4	(1:1 mixture)	18[b]
3	3.5	(1:1 mixture)	>35[b]
4	3.6	(1:1 mixture)	>32[b]
5	3.7	(1:1 mixture)	>64[c]

Entry	Compound	Structure	MIC[a] (μg/mL)
6	3.8	(1:1 mixture)	>64[c]
7	3. 20	(1:1 mixture)	>35[b]
8	3.21	(1:1 mixture)	>56[b]
9	3.11		12[b]
10	3.12		>27[b]
11	3.13		6.3[c]
12	3.14		4.3[c]

Entry	Compound	Structure	MIC[a] (µg/mL)
13	3.15		>54[b]
14	3.16a		>33[b]
15	3.16b		>42[b]
16	RMP[d]		0.01

a Lowest drug concentration that effected an inhibition of P90% relative to untreated cultures.

b Values are means of several screenings.

c Values of one screening.

d Rifampin was used as a positive control.
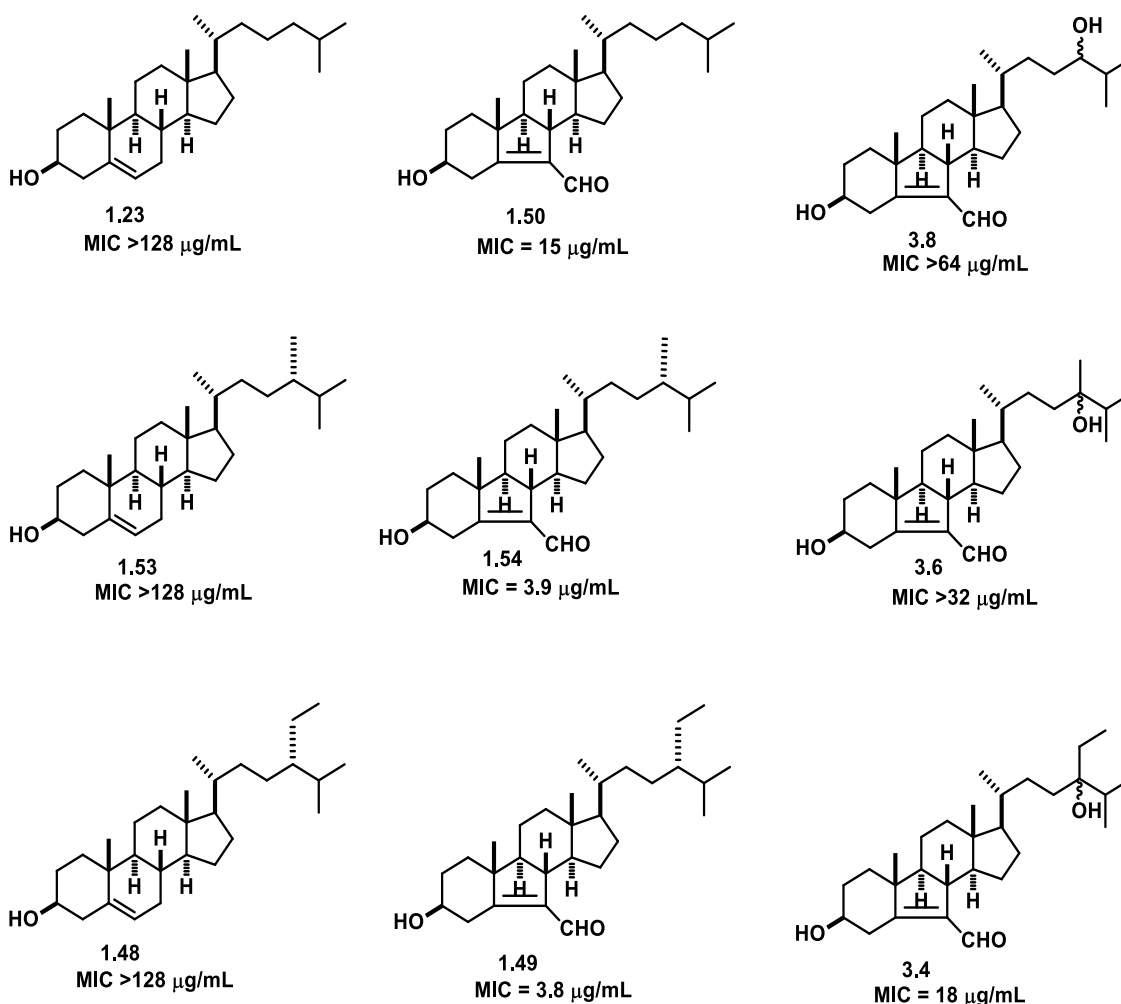
Figure 3.3 Comparison of the MIC values of some sterols and *abeo*-sterols.

In the second part of our study, we decided to synthesize compounds **3.11** - **3.16** based on the unpublished results of Dr. Wei in which *abeo*-sterols **3.9** and **3.10** (Scheme 3.9) exhibited potent anti-tubercular activity (MIC's of 0.4 and 4 μg/mL, respectively). These results stimulated an in-depth study of the role of the aldehyde at C-6 on the anti-tubercular activity. The reduction of the aldehyde functionality to give alcohols **3.11** and **3.12** diminished the anti-tubercular activity as shown on entries 9 and 10 (MIC's of 12 and >27 μg/mL, respectively).

Nonetheless, further reduction of the alcohol groups at C-6 to a methyl group enhanced the activity to 6.3 and 4.3 μg/mL for *abeo*-sterols **3.13** and **3.14**, respectively (entries 11 and 12). These results suggest that the presence of polar groups at C-6, such as alcohols, hampers the anti-tubercular activity of the *abeo*-sterols.

We also explored the epoxidation of the sterol and *abeo*-sterol framework, but this also resulted in the formation of modestly active analogs (entries 13-15). Epoxide **3.16a** (MIC >33 μg/mL) was slightly more active than its α-isomer **3.16b** (MIC >42 μg/mL). Moreover, both epoxy-sterols were more active than the abeo-sterol analog **3.15** (MIC >54 μg/mL). The comparison of **3.11** with **3.15** (entries 9 and 13), advocates that the oxidation of the 5,7-alkene reduces the anti-tubercular activity of *abeo*-sterols.

3.5. Conclusions

In this chapter, fifteen compounds were synthesized in order to perform SAR studies and establish a lead anti-tubercular sterol. However, none of them were as active as the positive control rifampicin (**1.2**) nor more active than saringosterol (**1.22**).[21] Nonetheless, we can conclude that the presence of a hydroxyl group at C-24 and the epoxidation of the 5,7-alkene functionality hamper the anti-tubercular activity in *abeo*-sterols. Moreover, the stereochemistry of the alkyl groups at C-24 has a nominal effect on the activity. Also, the aldehyde at C-6 is not essential for anti-tubercular activity, because when it was reduced to a

methyl group the resulting analogs exhibited similar potencies. Also, the one *R* group that consistently exhibits significant anti-tubercular activity is the ethyl group. Furthermore, the aforementioned data lead us to conclude that the ring-B contraction of a sterol with a hydroxyl group at C-24 does not enhance the anti-tubercular activity of an active (or inactive) sterol in and of itself.

Nevertheless, the synthetic methodologies described in these chapters can be applied to the future syntheses of other sterols and *abeo*-sterols with minimal usage of protecting groups. Also, the new methodologies developed herein, such as the carbamate removal in basic media to regenerate a free alcohol and the chlorination of a silyl ether with retention of configuration can be helpful to other researchers.

3.6. Proposed Recommendations

Sadly, the specific aim to synthesize a lead anti-tubercular sterol as potent as rifampicin (**1.2**) was not accomplished in this research. However, some future recommendations based on our conclusions can be made to achieve the ultimate goal of finding a lead compound to treat tuberculosis. The first recommendation is to synthesize (or isolate) the known sterol 24-vinylcholesterol (**3.22**) and its *abeo*-sterol analog (**3.23**) (Figure 3.4).[22] Since we have established that the hydroxyl group at C-24 diminishes the anti-tubercular activity of *abeo*-sterols, we conjecture that compound **3.23** (the 24-dehydroxy analog of leningosterol (**2.1**)) should be at least as active as compounds **1.49**, **1.54**, **3.9**, and **3.10** (Figure 3.2). On the other hand, the anti-tubercular activity of 24-vinylcholesterol (**3.22**) will be

helpful for SAR studies when compared to its corresponding 24-dehydroxy analog (i.e. saringosterol (**1.22**)). The second recommendation is to send leningosterol (**2.1**) and all the derivatives prepared herein for anti-proliferative evaluation, since orostanal (**1.35**) and the *abeo*-sterols described by Cui and co-workers were very active.[23,24]
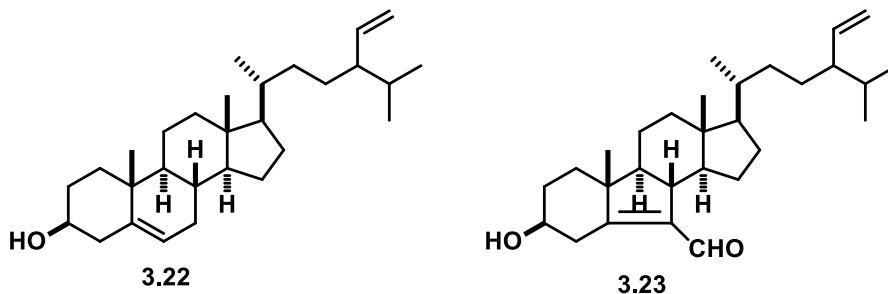
Figure 3.4 Molecular structures of compounds **3.22** and **3.23**.

3.7. Experimental Section

3.7.1. General Experimental Procedures

All of the reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an atmosphere of argon. Column chromatography (CC) was performed on silica gel (35–75 μm); reactions were followed by TLC analysis using glass pre-coated silica gel plates with fluorescent indicator (254 nm) and visualized with a UV lamp, I_2 vapors, or 10% ethanolic sulfuric acid followed by heating. Semipreparative RP-HPLC was performed using an UV detector set at 254 nm and a column with 5 μm, 250 x 4.6 mm size with a flow rate of 1 mL/min. Solvents and commercially available reagents were purchased and used as received without further purification. Starting material, β-sitosterol (**3.17**), was

obtained from Sigma Aldrich (> 70% purity, CAS. No. 83-46-5) and was used without further purification. Melting points were determined on a Melt Temp using 100 mm x 1mm capillary tubes. Optical rotations were recorded with a polarimeter using a 0.5 mL capacity cell with 1 dm path length. Infrared spectra were recorded using thin films supported on NaCl discs. UV were recorded on a UV-Vis spectrophotometer using quartz cuvettes and MeOH as solvent. ^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the specified field strength on a 700 or 500 MHz spectrometer. Spectra were obtained on CDCl$_3$ solutions in 5 mm diameter tubes, and chemical shifts are quoted in parts per million relative to the residual signals of CHCl$_3$ (δ_H = 7.26 ppm, δ_C = 77.0 ppm). Multiplicities in the ^{1}H NMR spectra are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants are reported in Hertz. High-resolution mass spectrometry (HRMS) was performed using a quadrupole mass analyzer, and the data are reported with ion mass/charge (*m/z*) ratios as values in atomic mass units. Yields shown are based on recovered starting material. Product characterization was mainly established by NMR, new compounds have full data except if there was little amount of sample or the compound was labile. Elemental analysis was not performed due to the lability of the compounds. The anti-tubercular activity assay was performed at the Institute for Tuberculosis Research, University of Illinois, Chicago against the *Mtb* strain H$_{37}$Rv by laboratory technicians Yuehong Wang, Baoji Wang, and Rui Ma. For this task, they used the Microplate Alamar Blue Assay (MABA) with rifampicin (**1.2**) as a positive control. Compounds with MIC's > 64 μg/mL are typically considered inactive.

3.7.2. Experimental Details

24-Ethyl-3β,24ξ-dihydroxycholest-5-ene (3.3, mixture of epimers at C-24)
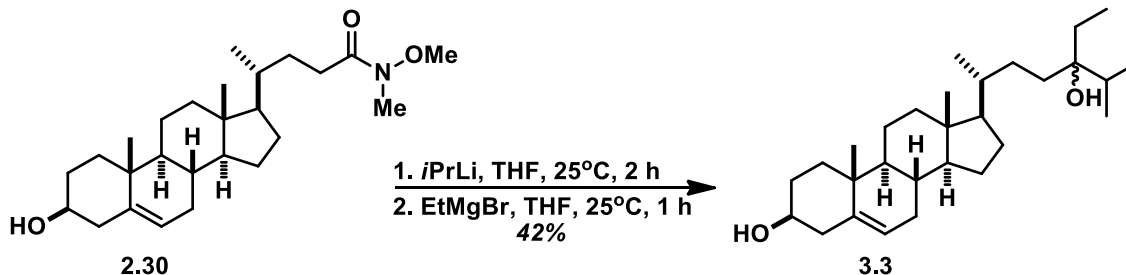
To a solution of **2.30** (120 mg, 0.29 mmol) in THF (5 mL) was added *iso*propyl lithium (0.7 M in pentane, 1.3 mL) dropwise. The reaction mixture was stirred at 25 °C for 2 h, quenched with aqueous saturated NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was dissolved in THF (5 mL) and then ethylmagnesium bromide (3.0 M in diethyl ether, 0.4 mL) was added dropwise. The reaction mixture was stirred at 25 °C for 1 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was purified by flash-Silica gel column chromatography [Hex/EtOAc (7:3)] to generate **3.3** as a white powder as a mixture of epimers at C-24 in 42% yield (52 mg). If the synthetic steps are inverted the yield increases to 65% (81 mg). IR (film) $\tilde{\nu}_{max}$ 3310, 2931, 2865, 1457, 1375cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 5.35 (d, *J* = 5.2 Hz, 1H, H-6), 3.53 (m, 1H,H-3), 2.26 (m, 2H, H-4αβ), 2.02-0.90 (br envelope, 26H), 1.01 (s, 3H, H-19), 0.94 (dd, *J* = 2.2, 6.5 Hz, 3H, H-21), 0.89 (d, *J* = 6.9 Hz, 3H, H-27), 0.88 (d, *J* = 6.8 Hz, 3H, H-26), 0.86 (dd, *J* = 7.6, 16.1 Hz, 3H, H-29), 0.68 (s, 3H, H-18);

^{13}C NMR (CDCl$_3$, 175 MHz) δ 140.8 (C, C-5), 121.7 (CH, C-6), 76.0/75.9 (C, C-24), 71.8 (CH, C-3), 56.7 (CH, C-14), 55.9/55.8 (CH, C-17), 50.1 (CH, C-9), 42.3 (CH$_2$, C-4), 42.2 (C, C-13), 39.8/39.7 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.5 (C, C-10), 36.4/36.3 (CH, C-20), 33.9 (CH, C-25), 31.9 (CH$_2$, C-7), 31.9 (CH, C-8), 31.8/31.7 (CH$_2$, C-23), 31.6 (CH$_2$, C-2), 29.1/29.0 (CH$_2$, C-22), 28.4 (CH$_2$, C-28), 28.3/28.2 (CH$_2$, C-16), 24.3 (CH$_2$, C-15), 21.1 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 18.8/18.7 (CH$_3$, C-21), 16.9/16.8 (CH$_3$, C-26), 16.7/16.6 (CH$_3$, C-27), 11.8 (CH$_3$, C-18), 7.7/7.6 (CH$_3$, C-29); EI-LRMS m/z [M]$^+$ 430 (3.7), 386 (72), 369 (52), 314 (100), 271 (51); EI-HRMS m/z [M]$^+$ calcd for C$_{29}$H$_{50}$O$_2$ 430.3811, found 430.3806. The NMR data were in accordance with published data.[25]

24-Ethyl-3β,24ξ-dihydroxy-5(6→7)abeocholest-5-en-6-al (3.4, mixture of epimers at C-24)
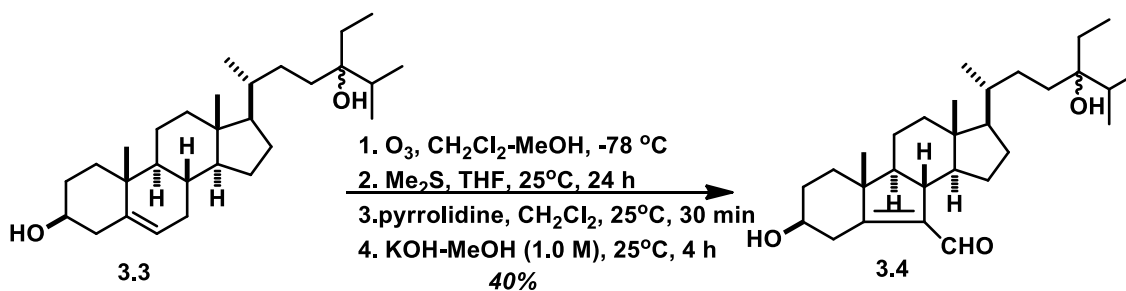
A stream of 2% O$_3$/O$_2$ was bubbled through a disposable pipet into a solution of compound **3.3** (52 mg, 0.12 mmol) in CH$_2$Cl$_2$-MeOH (4:1, 5 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of

dimethyl sulfide (0.4 mL, 5.5 mmol) and THF (5 mL) for 24 h at 25 °C. Then, it was concentrated *in vacuo*, dissolved in CH_2Cl_2 (5 mL), and then pyrrolidine (0.05 mL, 1.2 mmol) was added. The reaction mixture was stirred at 25 °C for 0.5 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. To the crude was added KOH (1.0 M in MeOH, 3 mL) and the mixture was stirred vigorously at 25 °C for 4 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAC (3 x 10 mL). The combined organic layers were dried ($MgSO_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/Acetone (4:1)] to generate **3.4** as a colorless oil in 40% yield (22 mg). UV (MeOH) λ_{max} 254 (ε 9389) nm; IR (film) $\tilde{\nu}_{max}$ 3410, 2962, 2872, 1674, 1462, 1380 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.97 (s, 1H, H-6), 3.70 (m, 1H, H-3), 3.46 (m, 1H, H-4β), 2.55 (m, 1H, H-8), 2.10-0.95 (br envelope, 24H), 0.95 (dd, J = 2.2, 6.5 Hz, 3H, H-21), 0.93 (s, 3H, H-19), 0.89 (m, 3H, H-27), 0.88 (m, 3H, H-26), 0.86 (m, 3H, H-29), 0.73 (s, 3H,H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 189.6 (CH, C-6), 169.0 (C, C-5), 139.3 (C, C-7), 76.0/75.9 (C, C-24), 70.9 (CH, C-3), 60.1 (CH, C-9), 55.1 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.8 (CH$_2$, C-12), 36.2 (CH, C-20), 36.1 (CH$_2$, C-1), 33.9 (CH, C-25), 33.8 (CH$_2$, C-4), 31.9/31.8 (CH$_2$, C-23), 31.2 (CH$_2$, C-2), 29.1/29.0 (CH$_2$, C-22), 28.6/28.5 (CH$_2$, C-16), 28.4/28.3 (CH$_2$, C-28), 26.5 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 19.0/18.9 (CH$_3$, C-21), 16.8 (CH$_3$, C-26), 16.7/16.6 (CH$_3$, C-27), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18), 7.7/7.6 (CH$_3$, C29); EI-LRMS *m/z* [M]$^+$ 444 (22), 400 (82), 383

(66), 353 (58), 269 (72), 175 (60), 161 (68.9), 145 (99); EI-HRMS *m/z* [M]+ calcd for $C_{29}H_{48}O_3$ 444.3603, found 444.3608.

3β,24ξ-Dihydroxy-24-methylcholest-5-ene(3.5, mixture of epimers at C-24)
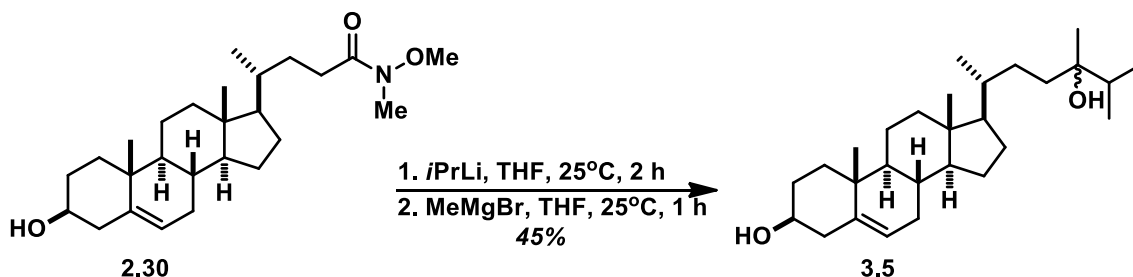
To a solution of **2.30** (120 mg, 0.29 mmol) in THF (5 mL) was added *iso*propyl lithium (0.7 M in pentane, 1.3 mL) dropwise. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was dissolved in THF (5 mL) and then methylmagnesium bromide (3.0 M in diethyl ether, 0.4 mL) was added dropwise. The reaction mixture was stirred at 25 °C for 1 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was purified by flash-Silica gel column chromatography [Hex/EtOAc (7:3)] to generate **3.5** as a white powder as a mixture of epimers at C-24 in 45% yield (54 mg). If the synthetic steps are inverted the yield increases to 68% (82 mg). IR (film) $\tilde{\nu}_{max}$ 3394, 2933,

1458, 1376 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 5.34 (d, J = 5.1 Hz, 1H, H-6), 3.51 (m, 1H, H-3), 2.25 (m, 2H, H-4αβ), 2.03-0.90 (br envelope, 24H), 1.06 (s, 3H, H-28), 0.99 (s, 3H, H-19), 0.92 (m, 3H, H-27), 0.90 (m, 3H, H-26), 0.87 (dd, J = 3.0, 6.8 Hz, 3H, H-21), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 140.7 (C, C-5), 121.6 (CH, C-6), 74.8/74.7 (C, C-24), 71.7 (CH, C-3), 56.7 (CH, C-14), 55.8/55.7 (CH, C-17), 50.1 (CH, C-9), 42.3 (CH$_2$, C-4), 42.2 (C, C-13), 39.7 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.5/36.1 (CH, C-25), 36.4 (C, C-10), 36.2/35.9 (CH, C-23), 36.1/36.0 (CH$_2$, C-20), 31.8 (CH$_2$, C-7), 31.8 (CH, C-8), 31.6 (CH$_2$, C-2), 29.1/29.0 (CH$_2$, C-22), 28.2 (CH$_2$, C-16), 24.2 (CH$_2$, C-15), 23.3 (CH$_3$, C-28), 21.0 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 18.8 (CH$_3$, C-21), 17.5/17.4 (CH$_3$, C-26), 16.9/16.8 (CH$_3$, C-27), 11.8 (CH$_3$, C-18); EI-LRMS m/z [M]$^+$ 416 (14), 398 (40), 373 (47), 355 (38), 314 (100), 271 (38), 145 (31); EI-HRMS m/z [M]$^+$ calcd for C$_{28}$H$_{48}$O$_2$ 416.3654, found 416.3656. The NMR data were in accordance with published data.[26]

3β, 24ξ-Dihydroxy-24-methyl-5(6→7)*abeo*cholest-5-en-6-al (3.6, mixture of epimers at C-24)
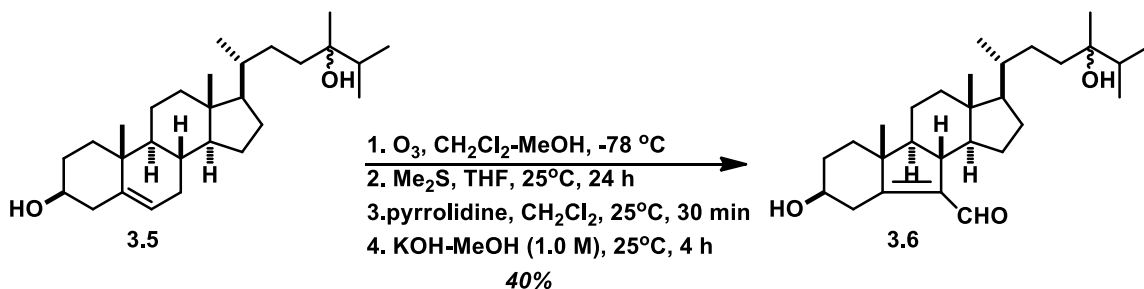
A stream of 2% O_3/O_2 was bubbled through a disposable pipet into a solution of compound **3.3** (54 mg, 0.13 mmol) in CH_2Cl_2-MeOH (4:1, 5 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of dimethyl sulfide (0.4 mL, 5.5 mmol) and THF (5 mL) for 24 h at 25 °C. Then it was concentrated *in vacuo*, dissolved in CH_2Cl_2 (5 mL), and then pyrrolidine (0.05 mL, 1.2 mmol) was added. The reaction mixture was stirred at 25 °C for 0.5 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. To the crude was added KOH (1.0 M in MeOH, 3 mL) and the mixture was stirred vigorously at 25 °C for 4 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAC (3 x 10 mL). The combined organic layers were dried ($MgSO_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/Acetone (4:1)] to afford **3.6** as a white powder in 40% yield (23 mg). mp 129-131 °C; UV (MeOH) λ_{max} 254 (ε 9337) nm; IR (film) $\tilde{\nu}_{max}$ 3392, 2959, 2870, 1673, 1462, 1379 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 9.97 (s, 1H, H-6), 3.70 (m, 1H, H-3), 3.46 (m, 1H, H-4β), 2.55 (m, 1H, H-8), 2.10-1.10 (br envelope, 22H), 1.07 (s, 3H, H-28), 0.94 (d, J = 6.5 Hz, 3H, H-21), 0.93 (s, 3H, H-19), 0.91 (d, J = 6.8 Hz, 3H, H-27), 0.88 (m, 3H, H-26), 0.73 (s, 3H,H-18); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 189.6 (CH, C-6), 169.1 (C, C-5), 139.3 (C, C-7), 74.8/74.7 (C, C-24), 70.8 (CH, C-3), 60.1 (CH, C-9), 55.1/55.0 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.8 (CH_2, C-12), 36.6/35.9 (CH, C-25), 36.3/36.0 (CH_2, C-23), 36.1 (CH_2, C-1), 36.1/35.9 (CH, C-20), 33.9 (CH_2, C-4),

31.2 (CH$_2$, C-2), 29.2/29.1 (CH$_2$, C-22), 28.5 (CH$_2$, C-16), 26.5 (CH$_2$, C-15), 23.3 (CH$_3$, C-28), 20.7 (CH$_2$, C-11), 19.0 (CH$_3$, C-21), 17.5/17.4 (CH$_3$, C-26), 16.9/16.8 (CH$_3$, C-27), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18); EI-LRMS m/z [M]$^+$ 430 (21), 386 (100), 368 (37), 269 (33), 175 (44), 145 (65).

3β,24ξ-Dihydrohycholest-5-ene (3.7, mixture of epimers at C-24)
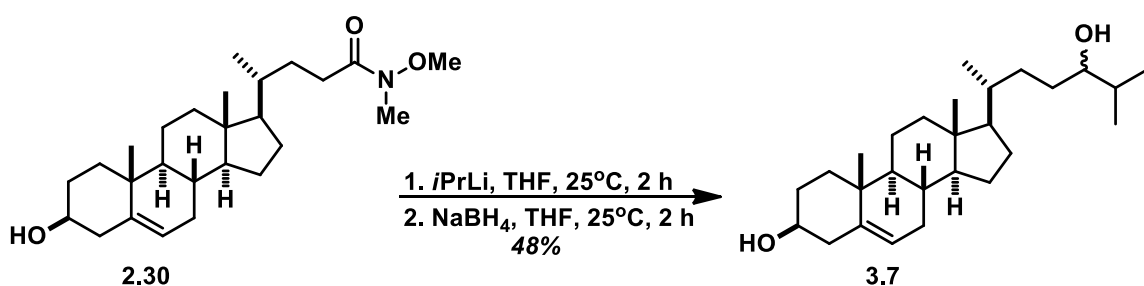
To a solution of **2.30** (100 mg, 0.24 mmol) in THF (5 mL) was added *iso*propyl lithium (0.7 M in pentane, 1.0 mL) dropwise. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH$_4$Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. The crude was dissolved in THF (5 mL) and then sodium borohydride (5 mg, 0.12 mmol) was added. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH$_4$Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. The crude was purified by flash-Silica gel column chromatography [Hex/Acetone (4:1)] to generate **3.7** as a white powder as a mixture of epimers at C-24 in 48% yield (46 mg). IR (film) $\tilde{\nu}_{max}$ 3384, 2939, 2870, 1457, 1379 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.35 (d, J = 5.1 Hz, 1H, H-6), 3.52

(m, 1H, H-3), 3.31 (m, 1H, H-24), 2.32-0.90 (br envelope, 26H), 1.00 (s, 3H, H-19), 0.92 (m, 9H, H-21, H-27, H-26), 0.68 (d, J = 2.2 Hz, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 140.7 (C, C-5), 121.7 (CH, C-6), 77.4/77.1 (C, C-24), 71.8 (CH, C-3), 56.7 (CH, C-14), 56.0/55.9 (CH, C-17), 50.1 (CH, C-9), 42.3 (CH$_2$, C-4), 42.2 (C, C-13), 39.7 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.5 (C, C-10), 35.9/35.7 (CH, C-20), 33.5/33.1 (CH, C-25), 32.2/32.0 (CH$_2$, C-22), 31.9 (CH$_2$, C-7), 31.9 (CH, C-8), 31.6 (CH$_2$, C-2), 30.7/30.5 (CH$_2$, C-23), 28.3/28.2 (CH$_2$, C-16), 24.3 (CH$_2$, C-15), 21.1 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 19.0/18.9 (CH$_3$, C-21), 18.8/18.6 (CH$_3$, C-26), 17.2/16.7 (CH$_3$, C-27), 11.9 (CH$_3$, C-18); ESI-LRMS m/z [M+K]$^+$ 441.6. The NMR data were in accordance with published data.[27]

3β,24ξ-Dihydroxy-5(6→7)$abeo$cholest-5-en-6-al (3.8, mixture of epimers at C-24)

1. O$_3$, CH$_2$Cl$_2$-MeOH, -78°C
2. Me$_2$S, THF, 25°C, 24 h
3. pyrrolidine, CH$_2$Cl$_2$, 25°C, 30 min
4. KOH-MeOH (1.0 M), 25°C, 4 h

40%

A stream of 2% O$_3$/O$_2$ was bubbled through a disposable pipet into a solution of compound **3.7** (46 mg, 0.11 mmol) in CH$_2$Cl$_2$-MeOH (4:1, 5 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of

dimethyl sulfide (0.4 mL, 5.5 mmol) and THF (5 mL) for 24 h at 25 °C. Then it was concentrated *in vacuo*, dissolved in CH_2Cl_2 (5 mL), and then pyrrolidine (0.05 mL, 1.2 mmol) was added. The reaction mixture was stirred at 25 °C for 0.5 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. To the crude was added KOH (1.0 M in MeOH, 3 mL) and the mixture was stirred vigorously at 25 °C for 4 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAC (3 x 10 mL). The combined organic layers were dried ($MgSO_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/Acetone (4:1)] to give **3.8** as a white powder in 40% yield (19 mg). mp 132-134 °C; UV (MeOH) λ_{max} 254 (ε 7092) nm; IR (film) $\tilde{\nu}_{max}$ 3379, 2955, 2869, 1675, 1465, 1381 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.97 (s, 1H, H-6), 3.70 (m, 1H, H-3), 3.46 (m, 1H, H-4β), 3.32 (m, 1H, H-24), 2.56 (m, 1H, H-8), 2.10-1.10 (br envelope, 22H), 0.92 (m, 9H, H-21, H-26, H-27), 0.94 (s, 3H, H-19), 0.73 (s, 3H,H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 189.7 (CH, C-6), 169.0 (C, C-5), 139.3 (C, C-7), 77.4/77.1 (C, C-24), 70.9 (CH, C-3), 60.1 (CH, C-9), 55.3/55.2 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.8 (CH$_2$, C-12), 36.2 (CH$_2$, C-1), 35.7/35.6 (CH, C-20), 33.9 (CH$_2$, C-4), 33.5/33.1 (CH, C-25), 32.2/32.1 (CH$_2$, C-22), 31.3 (CH$_2$, C-2), 30.8/30.7 (CH$_2$, C-23), 28.6/28.5 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 19.1/19.0 (CH$_3$, C-21), 18.9/18.8 (CH$_3$, C-26), 17.2/16.7 (CH$_3$, C-27), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18); ESI-LRMS *m/z* [M+H]$^+$ 417.6.
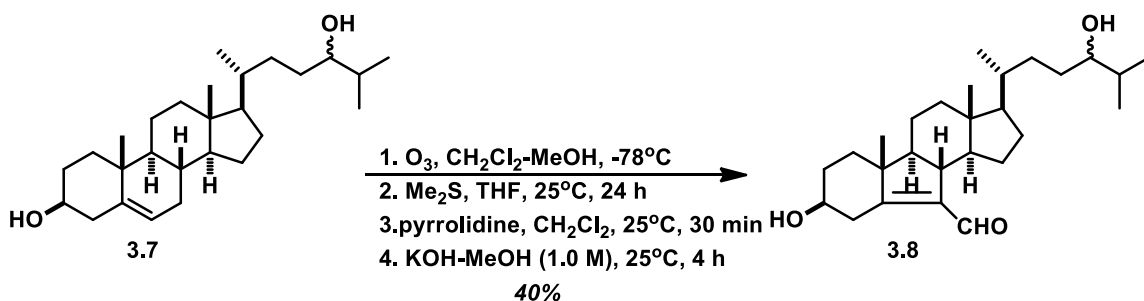
24-(2-Methyl-2-propenyl)-3β,24ξ-dihydroxycholest-5-ene (3.20, mixture of epimers at C-24)
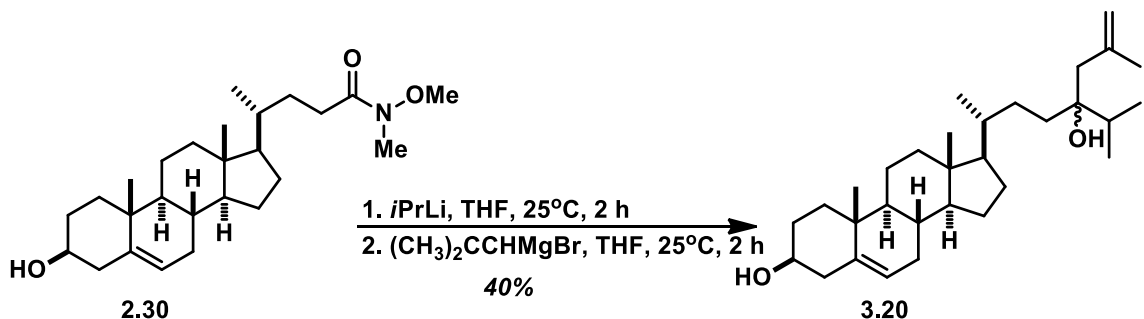
To a solution of **2.30** (50 mg, 0.12 mmol) in THF (5 mL) was added *iso*propyl lithium (0.7 M in pentane, 0.8 mL) dropwise. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was dissolved in THF (5 mL) and then 2-methyl-1-propenylmagnesium bromide (0.5 M in THF, 1 mL) was added. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated $NH_4Cl_{(aq)}$ (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was purified by flash-Alumina column chromatography [Hex/EtOAc (9:1)] to generate **3.20** as a colorless oil as a mixture of epimers at C-24 in 40% yield (22 mg). IR (film) $\tilde{\nu}_{max}$ 3389, 2935, 2869, 1464, 1377 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 5.35 (br d, J = 5.0 Hz, 1H, H-6), 4.92 (s, 1H, 30β), 4.74 (s, 1H, 30α), 3.52 (m, 1H, H-3), 2.30-0.93 (br envelope, 28H), 1.85 (s, 3H, H-31), 1.00 (s, 3H, H-19), 0.91 (m, 9H, H-21, H-26, H-27), 0.67 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 143.4/143.3 (C, C-29), 140.7 (C, C-5), 121.7 (CH,C-6), 114.8/114.7 (CH$_2$, C-30), 75.9/75.8 (C, C-24), 71.8 (CH, C-3),

56.7 (CH, C-14), 56.2/56.1 (CH, C-17), 50.1 (CH, C-9), 43.6/43.2 (CH$_2$, C-28), 42.3 (C, C-13), 42.2 (CH$_2$, C-4), 39.7 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.6/36.4 (CH, C-25), 36.5 (C, C-10), 34.3/34.2 (CH, C-20), 33.5/33.1 (CH$_2$, C-23), 31.9 (C, C-7), 31.9 (CH, C-8), 31.6 (CH$_2$, C-2), 29.2/28.9 (CH$_2$, C-22), 28.3 (CH$_2$, C-16), 25.3/25.1 (CH$_3$, C-31), 24.3 (CH$_2$, C-15), 21.1 (CH$_2$, C-11), 19.4 (CH$_3$, C-19), 18.7/18.6 (CH$_3$, C-21), 17.1/17.0 (CH$_3$, C-27), 16.9/16.8 (CH$_3$, C-26), 11.8 (CH$_3$, C-18); EI-LRMS m/z [M]$^+$ 456 (3), 413 (100), 383 (41), 271 (56), 127 (49); EI-HRMS m/z [M]$^+$ calcd for C$_{31}$H$_{52}$O$_2$ 456.3967, found 456.3972.

24-(2-Methyl-2-propenyl)-3β,24ξ-dihydroxy- 5(6→7)*abeo*cholest-5-en-6-al (3.21, mixture of epimers at C-24)
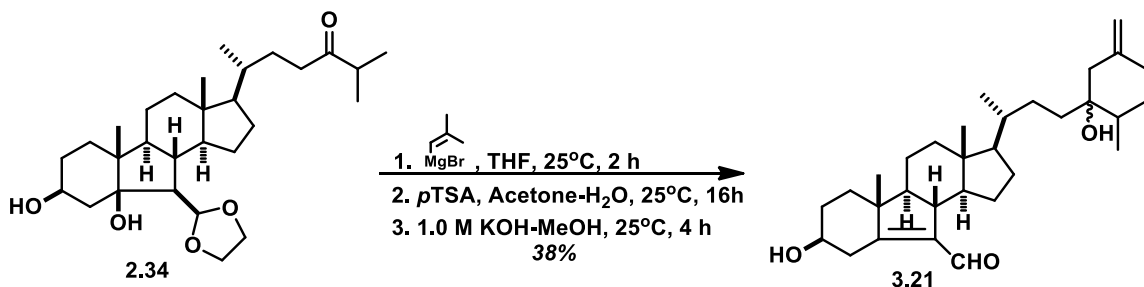
To a solution of **2.34** (50 mg, 0.10 mmol) in THF (5 mL) was added 2-methyl-1-propenylmagnesium bromide (0.5 M in THF, 1 mL) dropwise. The reaction mixture was stirred at 25 °C for 2 h, quenched with saturated aqueous NH$_4$Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (MgSO$_4$) and concentrated under vacuum. The crude was dissolved in acetone/H$_2$O (5 mL, 4:1) and then *p*TSA (17 mg, 0.10 mmol) was added. The

reaction mixture was stirred at 25 °C overnight, quenched with saturated aqueous NaHSO$_3$ (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. Finally, the crude was dehydrated using KOH (1.0 M in MeOH, 3 mL) at 25 °C for 4 h. It was quenched with saturated aqueous NH$_4$Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The crude was purified by flash-Alumina column chromatography [Hex/EtOAc (8:2)] to generate **3.21** as a colorless oil as a mixture of epimers at C-24 in 38% yield (19 mg); UV (MeOH) λ_{max} 254 (ε 4567) nm, 201 (ε 9171) nm; IR (film) $\tilde{\nu}_{max}$ 3401, 2958, 2871, 1675, 1462, 1380 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 9.96 (s, 1H, H-6), 4.91 (s, 1H, H-30β), 4.74 (s, 1H, H-30α), 3.70 (m, 1H, H-3), 3.46 (dd, J = 2.7, 14.4 Hz, 1H, H-4β), 2.54 (m, 1H, H-8), 2.25 (d, J = 13.9 Hz, 1H, H-28β), 2.14-0.98 (br envelope, 26H), 1.84 (s, 3H, H-31), 0.93 (s, 3H, H-19), 0.91 (m, 9H, H-21, H-26, H-27), 0.73 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 189.5 (CH,C-6), 169.1 (C, C-5), 143.4/143.3 (C, C-29), 139.3 (C, C-7), 114.8/114.7 (CH$_2$, C-30), 75.9/75.8 (C, C-24), 70.9 (CH, C-3), 60.1 (CH, C-9), 55.4/55.3 (CH, C-17), 54.4 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 43.6/43.2 (CH$_2$, C-28), 39.8 (CH$_2$, C-12), 36.4/36.2 (CH, C-25), 36.1 (CH$_2$, C-1), 34.3/34.2 (CH, C-20), 33.9 (CH$_2$, C-4), 33.6/33.1 (CH$_2$, C-23), 31.3 (CH$_2$, C-2), 29.3/29.0 (CH$_2$, C-22), 28.7/28.6 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 25.3/25.1 (CH$_3$, C-31), 20.7 (CH$_2$, C-11), 19.0/18.9 (CH$_3$, C-21), 17.1/17.0 (CH$_3$, C-27), 16.9/16.8 (CH$_3$, C-26), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18); EI-LRMS *m/z* [M]$^+$ 470 (10), 415 (76), 397 (70), 285 (38), 269 (29), 145 (37.4).

Synthesis of compounds 3.9 and 3.10

3.17 *R* = Et, Δ⁵ (70%)
3.18 *R* = Me, Δ⁵ (15%)
3.19 *R* = Et (15%)

1. O₃, CH₂Cl₂-MeOH, -78°C
2. Me₂S, THF, 25°C
3. pyrrolidine, 25°C, 30min
4. 1.0M KOH-MeOH, 25°C, 4 h
40%

3.9 *R* = Et
3.10 *R* = Me
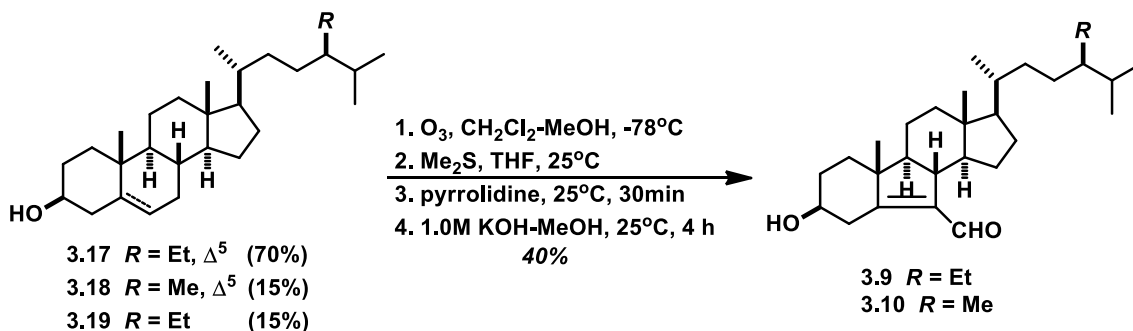
A stream of 2% O_3/O_2 was bubbled through a disposable pipet into a mixture of compounds **3.17**, **3.18**, and **3.19** (500 mg) in CH_2Cl_2-MeOH (4:1, 25 mL) at -78 °C, until the reaction mixture turned light blue. After allowing the reaction to warm to 25 °C, the solvent was evaporated, and the residue obtained was stirred with a mixture of dimethyl sulfide (0.8 mL, 11.0 mmol) and THF (5 mL) for 24 h at 25 °C. Then it was concentrated *in vacuo*, dissolved in CH_2Cl_2 (20 mL), and then pyrrolidine (0.25 mL, 6 mmol) was added. The reaction mixture was stirred at 25 °C for 0.5 h, quenched with saturated aqueous NH_4Cl (20 mL), and extracted with CH_2Cl_2 (3 x 20 mL).   The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. To the crude was added KOH (1.0 M in MeOH, 15 mL) and the mixture was stirred vigorously at 25 °C for 4 h, quenched with saturated aqueous NH_4Cl (20 mL), and extracted with EtOAC (3 x 20 mL). The combined organic layers were dried ($MgSO_4$), concentrated under vacuo, and purified by flash-Silica gel column chromatography [Hex/EtOAc (7:3)] to obtain a mixture of *abeo*-sterols **3.9** and **3.10** (200 mg) as a yellowish oil. The mixture was purified by HPLC with a SPHRI-5 C-18 column with 7% H_2O in MeOH at a flow rate of 1 mL/min. The retention times of the compounds were 20.8 min (**3.10**) and 22.5 min

(**3.9**). Compound **3.9** was obtained as a colorless oil in 40% yield (150 mg) and *abeo*-sterol **3.10** in 35% yield (28 mg).

24β-Ethyl-3β-hydroxy-5(6→7)*abeo*cholest-5-en-6-al (3.9)

$[\alpha]_D^{20}$ - 57.7 (*c* 1.00, CHCl$_3$); UV (MeOH) λ_{max} 255 (ε 55000) nm, 202 (ε 100000) nm; IR (film) $\tilde{\nu}_{max}$ 3383, 2958, 2870, 1676, 1464, 1380 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.97 (s, 1H, H-6), 3.70 (m, 1H, H-3), 3.49 (m, 1H, H-4β), 2.55 (m, 1H, H-8), 2.10-1.08 (br envelope, 25H), 0.94 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.84 (t, *J* = 7.5 Hz, 3H, H-29), 0.83 (d, *J* = 7.0 Hz, 3H, H-26), 0.81 (d, *J* = 7.0 Hz, 3H, H-27), 0.73 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.7 (CH, C-6), 168.9 (C, C-5), 139.3 (C, C-7), 70.9 (CH, C-3), 60.1 (CH, C-9), 55.3 (CH, C-17), 54.4 (CH, C-14), 46.3 (CH, C-10), 46.1 (C, C-8), 45.8 (CH, C-24), 45.2 (C, C-13), 39.8 (CH$_2$, C-12), 36.1 (CH$_2$, C-1), 36.0 (CH, C-20), 34.0 (CH$_2$, C-4), 33.9 (CH$_2$, C-22), 31.2 (CH$_2$, C-2), 29.1 (CH, C-25), 28.6 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 26.2 (CH$_2$, C-23), 23.0 (CH$_2$, C-28), 20.7 (CH$_2$, C-11), 19.8 (CH$_3$, C-26), 19.0 (CH$_3$, C-27), 18.9 (CH$_3$, C-21), 15.6 (CH$_3$, C-19), 12.5 (CH$_3$, C-18), 12.0 (CH$_3$, C-29); ESI-LRMS *m/z* [M+H]$^+$ 429.4; ESI-HRMS *m/z* calcd for C$_{29}$H$_{49}$O$_2$ [M+H]$^+$ 429.3733, found 429.3721.

3β-Hydroxy-24β-methyl -5(6→7)*abeo*cholest-5-en-6-al (3.10)

$[\alpha]_D^{20}$ - 35.0 (*c* 1.00, CHCl$_3$); UV (MeOH) λ_{max} 254 (ε 90000) nm, 202 (ε 60000) nm; IR (film) $\tilde{\nu}_{max}$ 3382, 1677, 1464, 1378 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.97 (s, 1H, H-6), 3.70 (m, 1H, H-3), 3.48 (m, 1H, H-4β), 2.55 (m, 1H, H-8), 2.12-1.05 (br envelope, 23H), 0.94 (s, 3H, H-19), 0.92 (d, *J* = 6.5 Hz, 3H, H-21), 0.85 (d, *J* = 6.5 Hz, 3H, H-27), 0.80 (d, *J* = 7.0 Hz, 3H, H-26), 0.77 (d, *J* = 6.0 Hz, 3H, H-28), 0.73 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 189.7 (CH, C-6), 168.9 (C, C-5), 139.3 (C, C-7), 70.9 (CH, C-3), 60.1 (CH, C-9), 55.3 (CH, C-17), 54.5 (CH, C-14), 46.3 (C, C-10), 46.1 (CH, C-8), 45.2 (C, C-13), 39.8 (CH$_2$, C-12), 38.8 (CH, C-24), 36.1 (CH$_2$, C-1), 35.7 (CH, C-20), 33.9 (CH$_2$, C-4), 33.7 (CH$_2$, C-22), 32.4 (CH, C-25), 31.3 (CH$_2$, C-2), 30.4 (CH$_2$, C-23), 28.6 (CH$_2$, C-16), 26.6 (CH$_2$, C-15), 20.7 (CH$_2$, C-11), 20.2 (CH$_3$, C-26), 18.9 (CH$_3$, C-21), 18.2 (CH$_3$, C-27), 15.6 (CH$_3$, C-19), 15.4 (CH$_3$, C-28), 12.5 (CH$_3$, C-18); ESI-LRMS *m/z* [M+H]$^+$ 415.4; ESI-HRMS *m/z* calcd for C$_{28}$H$_{47}$O$_2$ [M+H]$^+$ 415.3576, found 415.3580.

Synthesis of compounds 3.11, 3.12, and 3.16a

3.9 *R* = Et
3.10 *R* = Me

NaBH$_4$,
THF, 25°C, 1 h

3.11 *R* = Et
3.12 *R* = Me
48%

3.16
40%
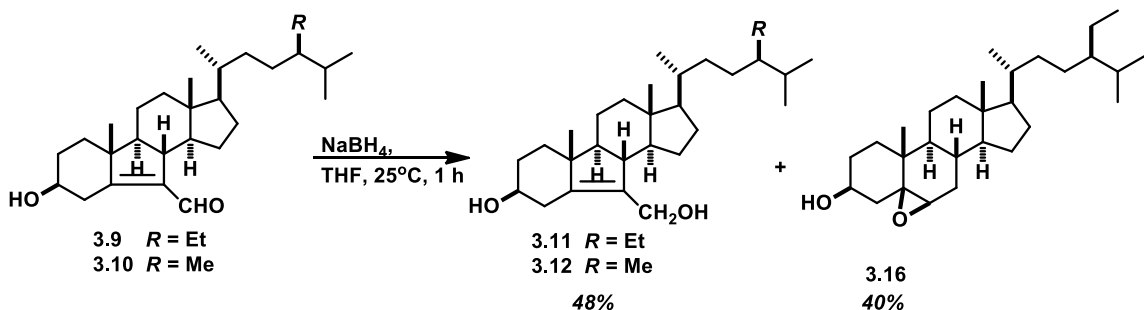
A solution of both *abeo*-sterols **3.9** and **3.10** (100 mg) in THF (10 mL) was stirred at 25 °C for 1 h with sodium borohydride (11 mg, 0.29 mmol). It was quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude was purified by flash-Silica gel column chromatography [Hex/EtOAc (1:1)] to generate epoxy-sterol **3.16** as a white powder (35 mg, 40% yield) and a mixture of *abeo*-sterols **3.11** and **3.12** (48 mg) as a colorless oil. The mixture of *abeo*-sterols was purified by HPLC with a SPHRI-5 C-18 column, with 5% H_2O in MeOH at a flow rate of 1 mL/min. The retention times of the compounds were 10.9 min (**3.12**) and 12.1 min (**3.11**), respectively. Compound **3.11** was obtained as a colorless oil in 34% yield (30 mg) and *abeo*-sterol **3.12** in 15% yield (2 mg).

24β-Ethyl-5(6→7)*abeo*cholest-5-en-3β,6-diol (3.11)

$[\alpha]_D^{20}$ +13.3 (*c* 0.60, $CHCl_3$); UV (MeOH) λ_{max} 203 (ε 88000) nm; IR (film) $\tilde{\nu}_{max}$ 3361, 2956, 1463, 1378, 1059, 1015 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.13 (dd, *J* = 12.0, 40.0 Hz, 2H, H-6αβ), 3.53 (m, 1H, H-3), 2.80 (dd, *J* = 2.0, 13.5 Hz, 1H, H-4β), 2.37 (m, 1H, H-8), 2.07-1.02 (br envelope, 25H), 0.95 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (s, 3H, H-19), 0.85 (t, *J* = 7.5 Hz, 3H, H-29), 0.84 (d, *J* = 7.0 Hz, 3H, H-26), 0.82 (d, *J* = 7.0 Hz, 3H, H-27), 0.70 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 145.8 (C, C-5), 136.8 (C, C-7), 71.6 (CH, C-3), 61.1 (CH, C-9), 57.4 (CH_2, C-6), 55.0 (CH, C-17), 53.8 (CH, C-14), 47.4 (CH, C-8), 45.8 (CH, C-24), 45.2 (C, C-13), 44.6 (C, C-10), 39.8 (CH_2, C-12), 37.0 (CH_2, C-1), 35.9 (CH, C-20), 34.0 (CH_2, C-

22), 33.3 (CH_2, C-4), 31.8 (CH_2, C-2), 29.1 (CH, C-25), 28.7 (CH_2, C-16), 26.1 (CH_2, C-23), 24.8 (CH_2, C-15), 23.0 (CH_2, C-28), 20.9 (CH_2, C-11), 19.8 (CH_3, C-26), 19.0 (CH_3, C-27), 18.9 (CH_3, C-21), 15.1 (CH_3, C-19), 12.4 (CH_3, C-18), 12.0 (CH_3, C-29); ESI-LRMS m/z [M+Na]$^+$ 453.4; ESI-HRMS m/z calcd for $C_{29}H_{50}O_2Na$ [M+Na]$^+$ 453.3709, found 453.3725.

24β-Methyl-5(6→7)*abeo*cholest-5-en-3β,6-diol (3.12)

[α]$_D^{20}$ -29.0 (*c* 1.00, CHCl$_3$); UV (MeOH) λ_{max} 203 (ε 88000) nm; IR (film) $\tilde{\nu}_{max}$ 3218, 2955, 2869, 1463, 1378, 1195, 1058 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.13 (dd, J = 11.5, 40.0 Hz, 2H, H-6 αβ), 3.54 (m, 1H, H-3), 2.80 (dd, J = 2.5, 13.0 Hz, 1H, H-4β), 2.37 (m, 1H, H-8), 2.06-1.00 (br envelope, 23H), 0.93 (d, J = 6.5 Hz, 3H, H-21), 0.87 (s, 3H, H-19), 0.85 (d, J = 7.5 Hz, 3H, H-26), 0.80 (d, J = 7.0 Hz, 3H, H-27), 0.77 (d, J = 7.0 Hz, 3H, H-28), 0.70 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 145.8 (C, C-5), 136.8 (C, C-7), 71.6 (CH, C-3), 61.1 (CH, C-9), 57.4 (CH_2, C-6), 55.1 (CH, C-17), 53.8 (CH, C-14), 47.4 (CH, C-8), 45.2 (C, C-13), 44.6 (C, C-10), 39.8 (CH_2, C-12), 38.8 (CH, C-24), 37.0 (CH_2, C-1), 35.6 (CH, C-20), 33.8 (CH_2, C-22), 33.3 (CH_2, C-4), 32.4 (CH, C-25), 31.8 (CH_2, C-2), 30.3 (CH_2, C-23), 28.8 (CH_2, C-16), 24.8 (CH_2, C-15), 20.9 (CH_2, C-11), 20.2 (CH_3, C-26), 18.9 (CH_3, C-21), 18.2 (CH_3, C-27), 15.4 (CH_3, C-28), 15.1 (CH_3, C-19), 12.4 (CH_3, C-18); ESI-LRMS m/z [M+Na]$^+$ 439.4; ESI-HRMS m/z calcd for $C_{28}H_{48}O_2Na$ [M+Na]$^+$ 439.3552, found 439.3562.

5,6α-Epoxy-5α-stigmastan-3β-ol (3.16a)

IR (film) $\tilde{\nu}_{max}$ 3396, 2931, 1457, 1378 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 3.70 (m, 1H, H-3), 3.06 (d, J = 2.2 Hz, 1H, H-6), 2.09 - 0.90 (br envelope, 28H), 0.99 (s, 3H, H-19), 0.90 (d, J = 6.5 Hz, 1H, H-21), 0.83 (m, 6H, H-26, H-29), 0.81 (d, J = 6.8 Hz, 1H, H-27), 0.64 (s, 3H, H-18), 0.60 (m, H-9); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 69.5 (CH, C-3), 63.7 (CH, C-6), 62.9 (C, C-5), 56.2 (CH, C-14), 56.1 (CH, C-17), 51.3 (CH, C-9), 45.8 (CH, C-24), 42.3 (C, C-13), 42.2 (CH$_2$, C-4), 39.8 (CH$_2$, C-12), 37.2 (CH$_2$, C-1), 36.1 (CH, C-20), 34.9 (C, C-10), 33.9 (CH$_2$, C-22), 32.6 (CH$_2$, C-7), 31.1 (CH$_2$, C-2), 29.8 (CH, C-8), 29.1 (CH, C-25), 28.2 (CH$_2$, C-16), 26.0 (CH$_2$, C-23), 24.2 (CH$_2$, C-15), 23.1 (CH, C-28), 22.0 (CH$_2$, C-11), 19.8 (CH$_3$, C-26), 19.0 (CH$_3$, C-27), 18.7 (CH$_3$, C-21), 17.1 (CH$_3$, C-19), 12.0 (CH$_3$, C-29), 11.8 (CH$_3$, C-18); ESI-LRMS m/z [M + H]$^+$ 431.6. The NMR data were in accordance with published data.[16]

Synthesis of compounds 3.13 and 3.14

3.11 *R* = Et
3.12 *R* = Me

NaBH$_3$CN, BF$_3$.Et$_2$O
THF, reflux, 8 h
16%

3.13 *R* = Et
3.14 *R* = Me
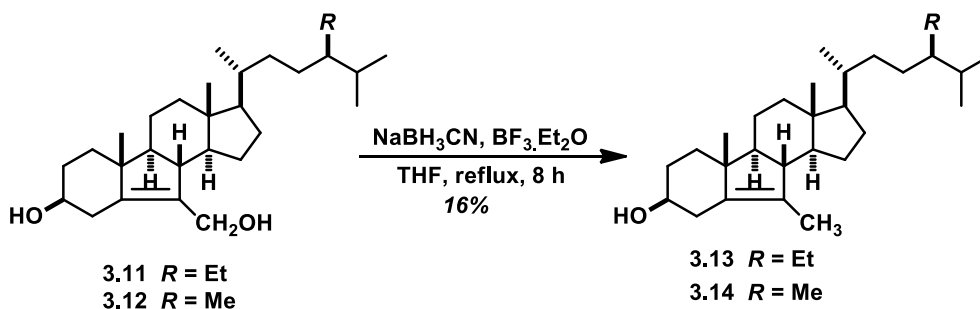
To a solution of a mixture of *abeo*-sterols **3.11** and **3.12** (300 mg) and BF$_3$·Et$_2$O (0.36 mL, 2.8 mmol) in THF (25 mL) was added sodium cyanoborohydride (129 mg, 2.1 mmol). The reaction mixture was refluxed for 8 h, quenched with saturated aqueous NaHCO$_3$ (30 mL), and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (2 x 30 mL), dried (MgSO$_4$) and concentrated *in vacuo*. The crude was purified by flash-Silica gel column chromatography [Hex/EtOAc (9:1)] to obtain a mixture of *abeo*-sterols **3.13** and **3.14** (46 mg, 16% yield) as a colorless oil. The mixture was purified by HPLC with a SPHRI-5 C-18 column, with 3% H$_2$O in MeOH at a flow rate of 1 mL/min. The retention times of the compounds were 15.5 min (**3.14**, 30 mg) and 17.5 min (**3.13**, 4 mg).

24β-Ethyl-6-methyl-5(6→7)*abeo*cholest-5-en-3β-ol (3.13)

IR (film) $\tilde{\nu}_{max}$ 3355, 2956, 1463, 1378cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 3.52 (m, 1H, H-3), 2.67 (m, 1H, H-4β), 2.15 (m, 1H, H-8), 2.04 - 0.95 (br envelope, 25H), 1.25(s, 3H, H-6), 0.93 (d, J = 6.5 Hz, 3H, H-21), 0.85 (t, J = 7.5 Hz, 3H, H-29), 0.82 (s, 3H, H-19), 0.79 (d, J = 7.0 Hz, 3H, H-26), 0.78 (d, J = 7.0 Hz, 3H, H-27), 0.68 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 139.5 (C, C-5), 133.8 (C, C-7), 71.8 (CH, C-3), 60.9 (CH, C-9), 55.0 (CH, C-17), 54.2 (CH, C-14), 49.6 (CH, C-8), 45.8 (CH, C-24), 45.3 (C, C-13), 44.3 (C, C-10), 40.0 (CH$_2$, C-12), 37.4 (CH$_2$, C-1), 35.9 (CH, C-20), 34.1 (CH$_2$, C-22), 33.3 (CH$_2$, C-4), 32.1 (CH$_2$, C-2), 29.1 (CH, C-25), 28.9 (CH$_2$, C-16), 26.2 (CH$_2$, C-23), 25.0 (CH$_2$, C-15), 23.1 (CH$_2$, C-28), 21.1 (CH$_2$,

C-11), 19.8 (CH$_3$, C-26), 19.0 (CH$_3$, C-27), 18.3 (CH$_3$, C-21), 15.1 (CH$_3$, C-19), 13.2 (CH$_3$, C-6), 12.4 (CH$_3$, C-18), 12.0 (CH$_3$, C-29).

6,24β-Dimethyl-5(6→7)*abeo*cholest-5-en-3β-ol (3.14)

^{1}H NMR (500 MHz, CDCl$_3$) δ 3.51 (m, 1H, H-3), 2.67 (m, 1H, H-4β), 2.19 – 0.90 (broad envelope, 24H), 1.25 (s, 3H, H-6), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.86 (d, *J* = 6.8 Hz, 3H, H-26), 0.82 (s, 3H, H-19), 0.80 (m, 6H, H-27, H-28), 0.68 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 139.5 (C, C-5), 133.8 (C, C-7), 71.8 (CH, C-3), 60.9 (CH, C-9), 55.2 (CH, C-17), 54.2 (CH, C-14), 49.6 (CH, C-8), 45.3 (C, C-13), 44.4 (C, C-10), 40.0 (CH$_2$, C-12), 38.9 (CH, C-24), 37.4 (CH$_2$, C-1), 35.6 (CH, C-20), 33.9 (CH$_2$, C-22), 33.4 (CH$_2$, C-4), 32.4 (CH, C-25), 32.1 (CH$_2$, C-2), 30.5 (CH$_2$, C-23), 28.9 (CH$_2$, C-16), 25.0 (CH$_2$, C-15), 21.1 (CH$_2$, C-11), 20.2 (CH$_3$, C-26), 18.9 (CH$_3$, C-21), 18.3 (CH$_3$, C-27), 15.4 (CH$_3$, C-28), 15.1 (CH$_3$, C-19), 13.1 (CH$_3$, C-6), 12.4 (CH$_3$, C-18).

5, 7-Epoxy-24β-ethyl-5(6→7)*abeo*cholest-3β,6-diol (3.15)
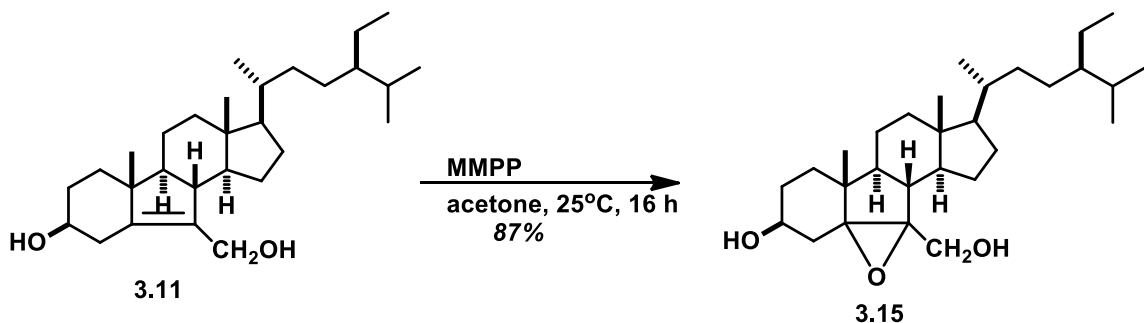
To a solution of compound **3.11** (10 mg, 0.02 mmol) in acetone (1 mL) was added MMPP (17 mg, 0.03 mmol, 80% purity). The reaction mixture was stirred at 25 °C for 16 h, filtered through a short Silica gel column, and concentrated *in vacuo* to afford compound **3.15** (9 mg) in 87%yield as a white powder. $[\alpha]_D^{20}$ + 26.0 (*c* 1.0, MeOH); IR (film) $\tilde{\nu}_{max}$ 3221, 2926, 2864, 1457, 1381cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 3.91 (m, 1H, H-3), 3.89 (d, *J* = 12.2 Hz, 1H, H-6β), 3.78 (d, *J* = 12.2 Hz, 1H, H-6α), 2.03 - 0.90 (br envelope, 23H), 0.92 (d, *J* = 6.5 Hz, 1H, H-21), 0.88 (s, 3H, H-19), 0.84 (m, 6H, H-26, H-29), 0.81 (d, *J* = 6.8 Hz, 1H, H-27), 0.65 (s, 3H, H-18); ^{13}C NMR (CDCl$_3$, 175 MHz) δ 74.4 (C, C-5), 70.0 (CH, C-3), 69.2 (C, C-7), 60.2 (CH$_2$,C-6), 55.0 (CH, C-17), 50.4 (CH, C-14), 49.0 (CH, C-9), 45.8 (CH, C-24), 45.1 (C, C-13), 42.4 (CH, C-8), 39.7 (CH$_2$, C-12), 39.2 (C, C-10), 36.0 (CH, C-20), 34.0 (CH$_2$, C-22), 32.8 (CH$_2$, C-4), 31.1 (CH$_2$, C-1), 30.6 (CH$_2$, C-2), 29.1 (CH, C-25), 28.7 (CH$_2$, C-16), 26.1 (CH$_2$, C-23), 24.9 (CH$_2$, C-15), 23.0 (CH, C-28), 20.8 (CH$_2$, C-11), 19.8 (CH$_3$, C-26), 19.0 (CH$_3$, C-27), 18.9 (CH$_3$, C-21), 15.6 (CH$_3$, C-19), 12.3 (CH$_3$, C-18), 12.0 (CH$_3$, C-29); ESI-LRMS *m/z* [M + Na]$^+$ 469.6.

5,6α-Epoxy-5α-stigmastan-3β-ol (3.16b)

3.17 R = Et, Δ^5 (70%)
3.18 R = Me, Δ^5 (15%)
3.19 R = Et   (15%)
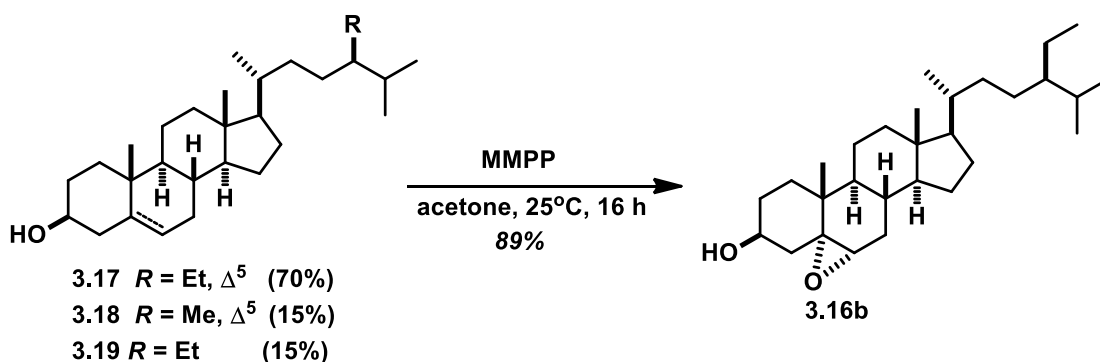
To a solution of a mixture of compounds **3.17**, **3.18**, and **3.19** (10 mg) in acetone (1 mL) was added MMPP (17 mg, 0.03 mmol, 80% purity). The reaction mixture was stirred at 25 °C for 16 h, filtered through a Silica gel column, and concentrated *in vacuo*.   The crude was purified by flash-Silica gel column chromatography [Hex/EtOAc (85:15)] and then by HPLC with a SPHRI-5 C-18 column, with 5% H_2O in MeOH at a flow rate of 1 mL/min. The retention time for compound **3.16b** (6.5 mg) was 11.1 min and was obtained as a white powder in 89% yield**.** $[\alpha]_D^{20}$ - 35.0 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}_{max}$ 3383, 2933, 2869, 1465, 1376 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 3.91 (m, 1H, H-3), 2.90 (d, J = 4.4 Hz, 1H, H-6), 2.07 (t, J = 12.1 Hz, 1H, H-4β), 1.96 - 0.90 (br envelope, 28H), 1.06 (s, 3H, H-19), 0.89 (d, J = 6.5 Hz, 1H, H-21), 0.83 (m, 6H, H-26, H-29), 0.81 (d, J = 6.8 Hz, 1H, H-27), 0.61 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 68.8 (CH, C-3), 65.7 (C, C-5), 59.3 (CH, C-6), 56.8 (CH, C-14), 55.8 (CH, C-17), 45.8 (CH, C-24), 42.5 (CH, C-9), 42.3 (C, C-13), 39.8 (CH_2, C-4), 39.4 (CH_2, C-12), 36.1 (CH, C-20), 34.8 (C, C-10), 33.9 (CH_2, C-22), 32.4 (CH_2, C-1), 31.1 (CH_2, C-2), 29.9 (CH, C-8), 29.1 (CH, C-25), 28.8 (CH_2, C-7), 28.1 (CH_2, C-16), 26.1 (CH_2, C-23), 24.0 (CH_2, C-15), 23.0 (CH,

C-28), 20.6 (CH$_2$, C-11), 19.8 (CH$_3$, C-26), 19.0 (CH$_3$, C-27), 18.7 (CH$_3$, C-21), 15.9 (CH$_3$, C-19), 12.0 (CH$_3$, C-29), 11.8 (CH$_3$, C-18); ESI-LRMS m/z [M + Na]$^+$ 453.6. The NMR data were in accordance with published data.[16]

3.7.3. Evaluation of Anti-tubercular Activity.

M. tuberculosis H37Rv (ATCC 27294) was obtained from the American Type Culture Collection (Rockville,Md.). For the first three (of four) replicate experiments, H37Rv inocula were first passaged in radiometric 7H12 broth until the growth index (GI) reached 800 to 999. For the fourth replicate experiment, H37Rv was grown in 7H9GC-Tween. Cultures were incubated in 500 mL nephelometer flasks on a rotary shaker at 150 rpm and 37 °C until they reached an optical density of 0.4 to 0.5 at 550 nm. The filtrates were aliquoted, stored at 280 °C, and used within 30 days. The microplate alamar blue assay (MABA) was performed in black, clear-bottomed, 96-well microplates in order to minimize background fluorescence as published.[20] Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. All the statistical analyses were performed with the program SAS (Statistical Analysis System). The anti-tuberculosis drug rifampicin (RMP) was used as a positive control during the assays

3.7.4. Spectroscopic Data

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.3 (mixture of epimers)

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.4
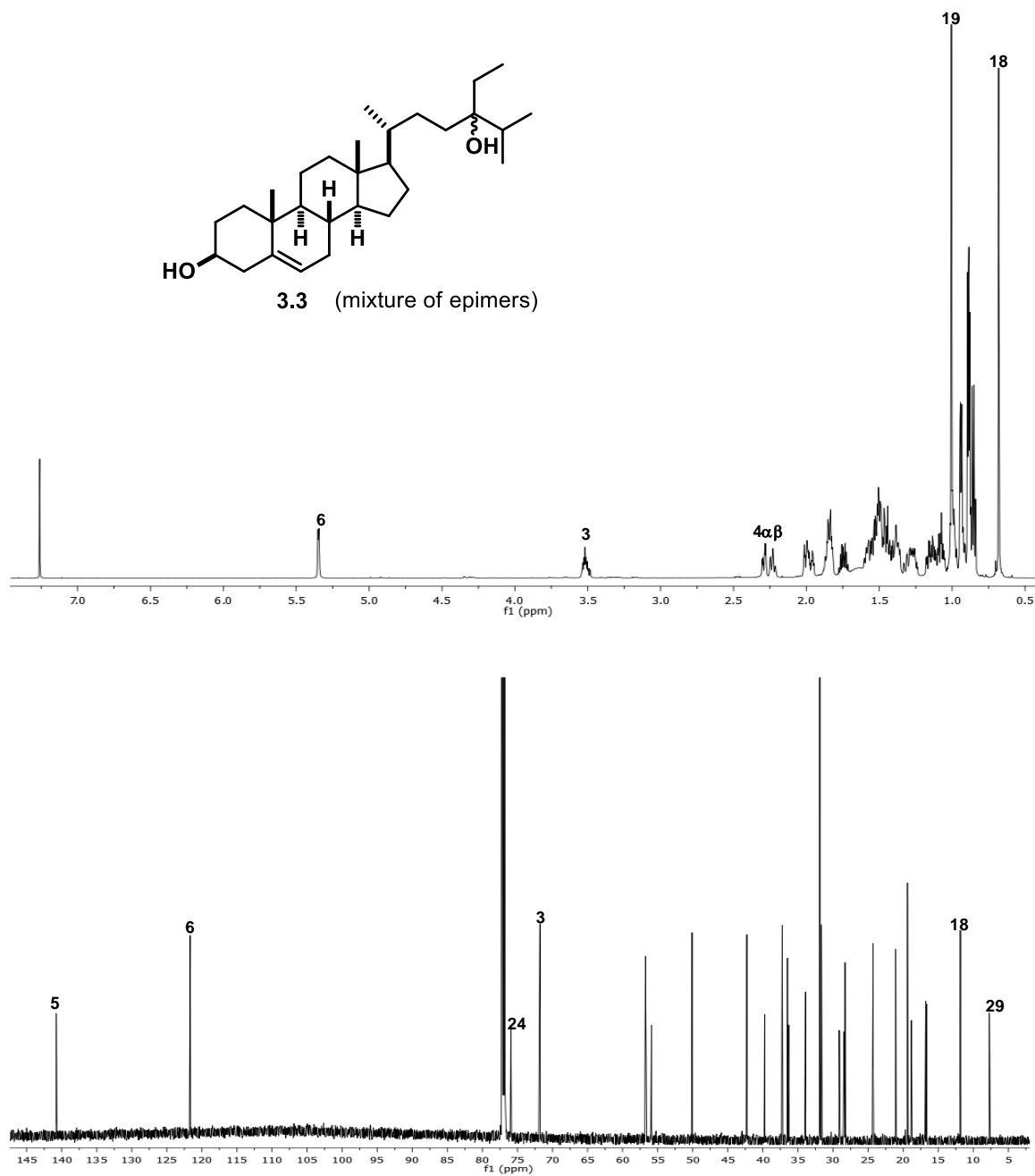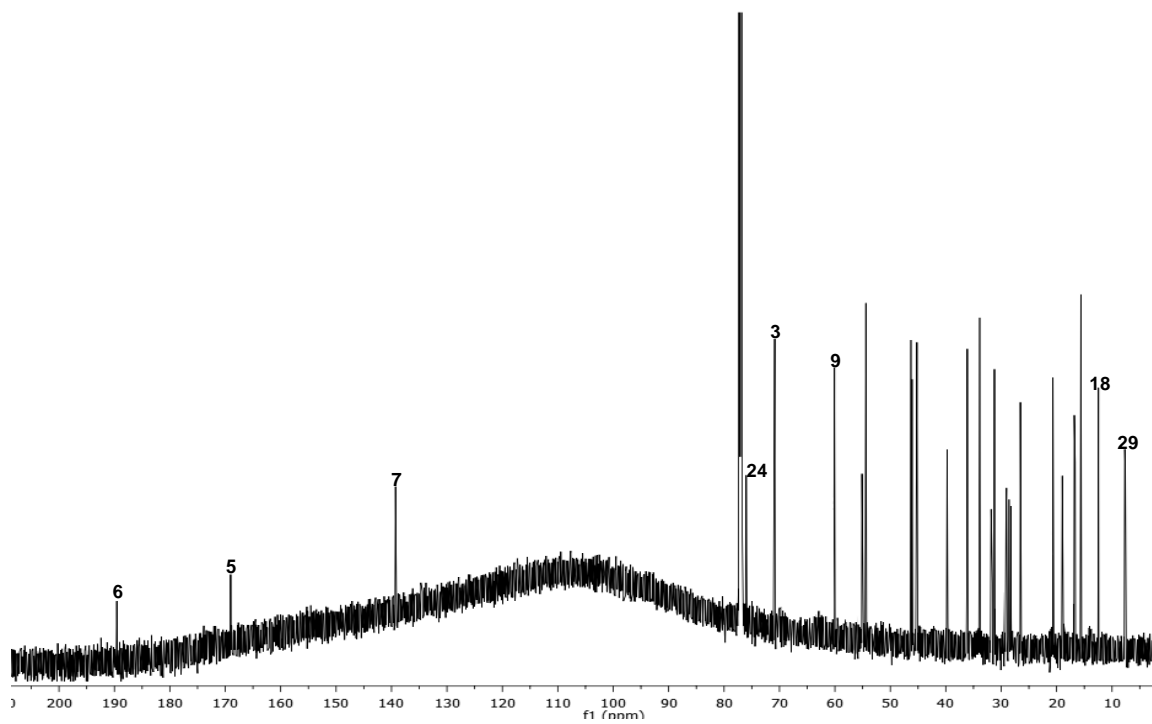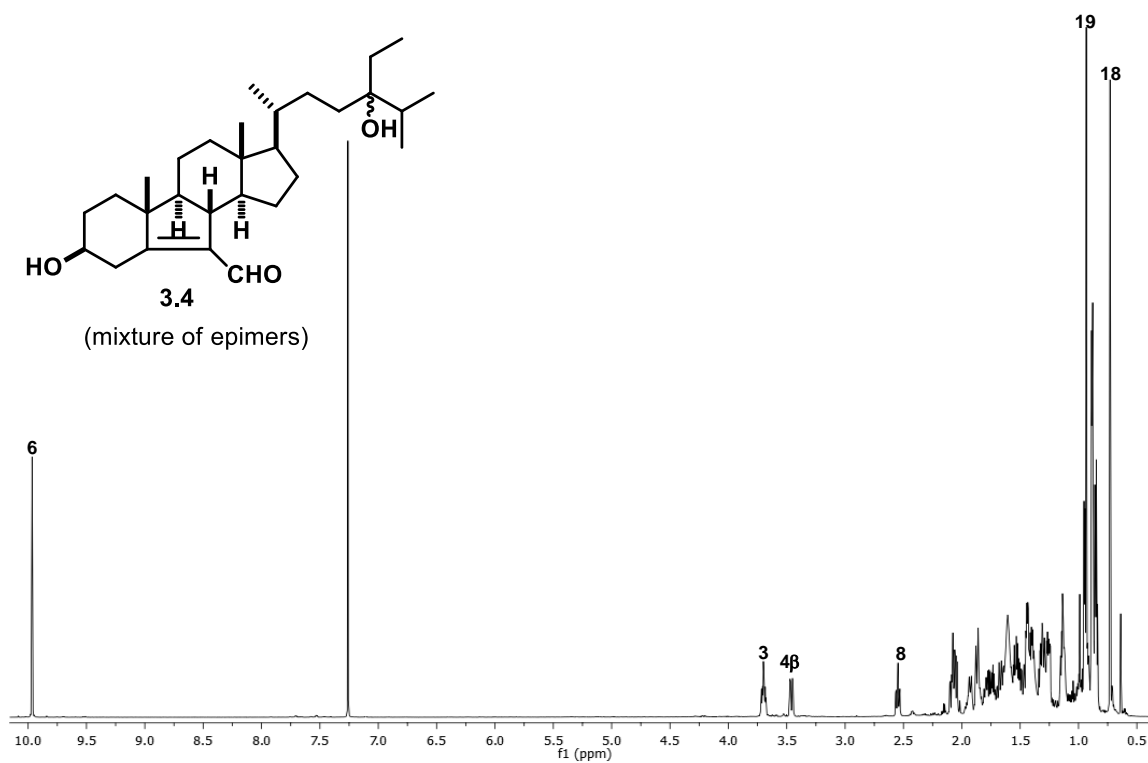
(mixture of epimers)

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.5 (mixture of epimers)

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.6

(mixture of epimers)

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

3.7 (mixture of epimers)

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.8

(mixture of epimers)

¹H NMR (700 MHz) and ¹³C NMR (175 MHz) in CDCl₃

1H NMR (700 MHz) and 13C NMR (175 MHz) in CDCl₃

3.20 (mixture of epimers)

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.21
(mixture of epimers)

¹H NMR (500 MHz) and ¹³C NMR (125 MHz) in CDCl₃

¹H NMR (500 MHz) and ¹³C NMR (125 MHz) in CDCl₃

¹H NMR (500 MHz) and ¹³C NMR (125 MHz) in CDCl₃

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.16a

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl$_3$

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.15
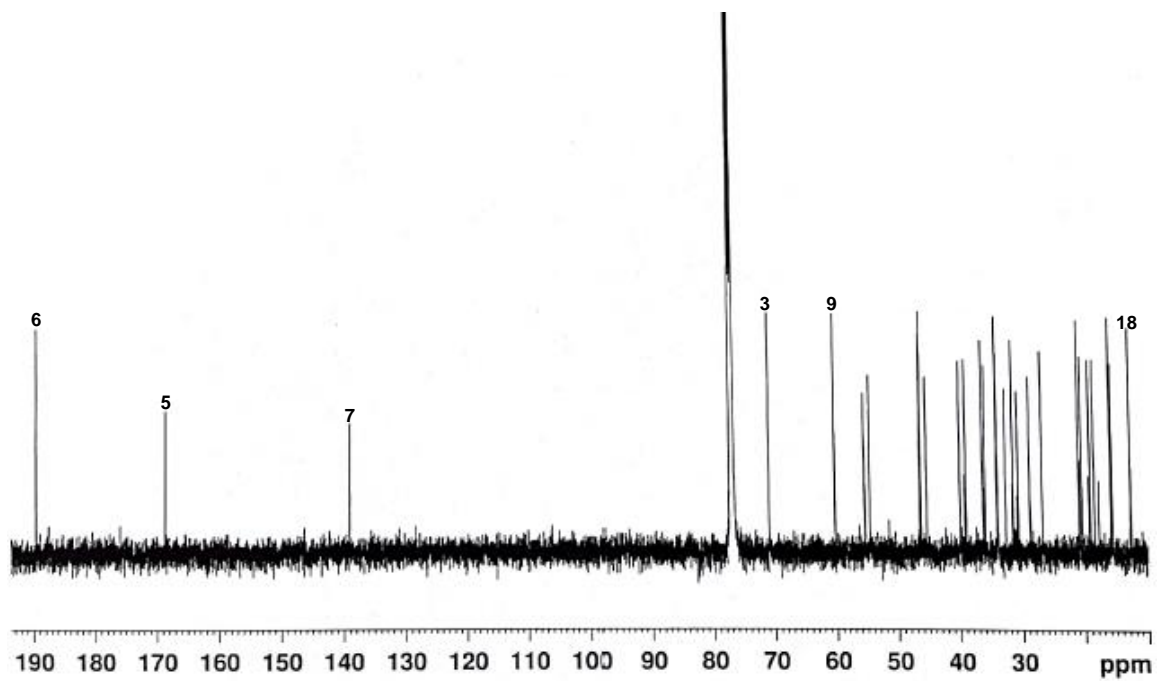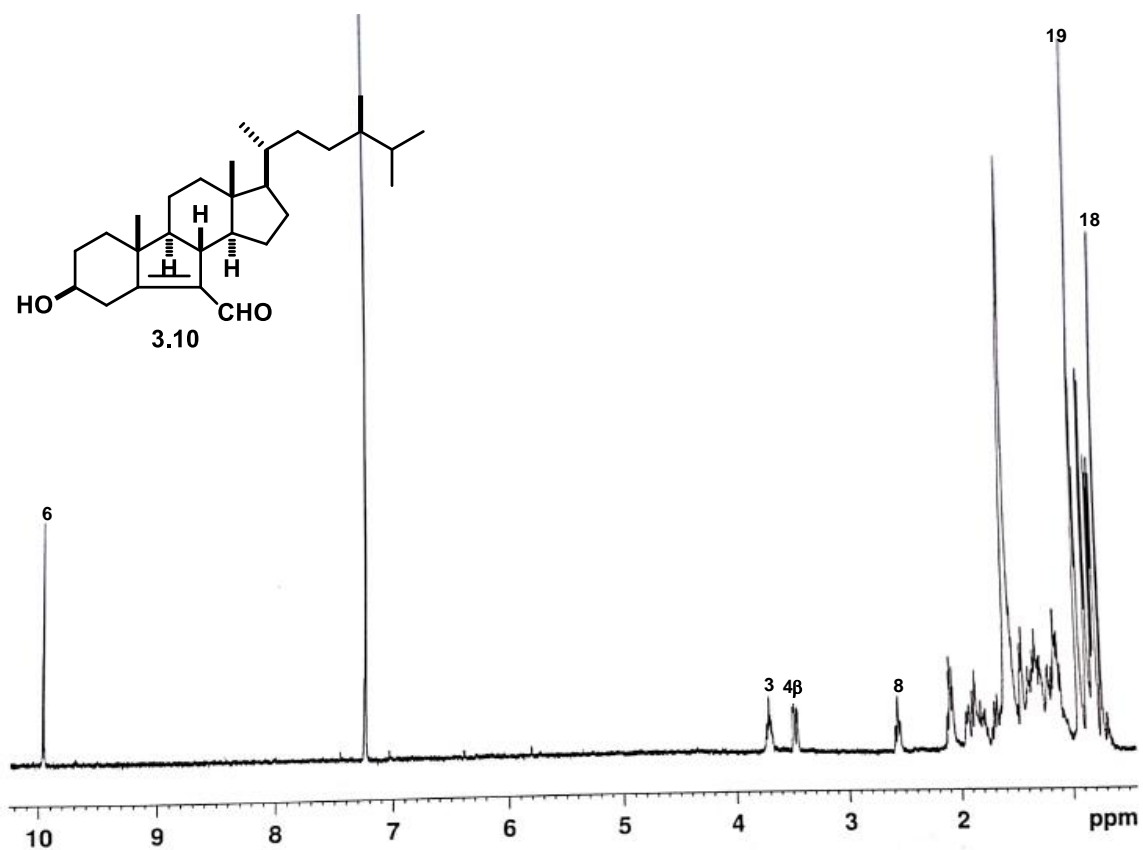
^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

3.8. References

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B. Design, Synthesis and Anti-infective Activity of Isothiocyanate- and Isoselenocyanate-functionalized Amphilectane Diterpenes

Chapter 4.   Natural Product-based Synthesis of Isothiocyanate- and Isoselenocyanate-functionalized Amphilectane Diterpenes as Novel Anti-infective Agents

In previous chapters we have described the synthesis of sterols and *abeo*-sterols with anti-tubercular activity. In this chapter, the synthesis of functionalized amphilectane diterpenes as novel anti-plasmodial and anti-tubercular agents will be described.

4.1. Malaria

4.1.1. Introduction

Malaria is an infectious disease caused by *Plasmodium* parasites through the bites of infected female *Anopheles* mosquitoes.[1] There are five parasite species that can infect and be spread by humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knoelesi*; however, the first two represent the greatest threat.[2] In 2015, 214 million new cases of malaria were reported and 430,000 people died from the disease.[3] Nonetheless, substantial progress has been made by decreasing the malaria incidence and the mortality rate, but still there are millions of people mainly in Africa not accessing the services they need to prevent and treat the disease.[3]

4.1.2. Symptoms

The symptoms of malaria, in a non-immune individual, appear usually 10-15 days after the infective mosquito bite.[4] Fever, headache, chills, and vomiting are the initial manifestations of the disease, which can resemble the flu.[1,5] Other

symptoms may include joint pain, hemolytic anemia, jaundice, hemoglobin in the urine, retinal damage, and convulsion.[6] Severe malaria is usually caused *by P. falciparum* and individuals with cerebral malaria exhibit neurological symptoms that include abnormal posturing, nystagmus, conjugate gaze palsy, opisthotonus, seizures, or coma.[5]

4.1.3. Treatment

The best available treatment, particularly for *P. falciparum* malaria, is artemisinin-based combination therapy (ACT) which is about 90% effective when used to treat uncomplicated malaria.[7,8] ACT includes artemisinin (**4.1**) with amodiaquine (**4.2**), lumefantrine (**4.3**), mefloquine (**4.4**), or sulfadoxine (**4.5**) - pyrimethamine (**4.6**) [Scheme 4.1].[9] Also recommended is a combination of dihydroartemisinin (**4.7**) and piperaquine (**4.8**).[10] Resistance of *P. falciparum* to previous generations of drugs [chloroquine (**4.9**) and sulfadoxine (**4.5**) – pyrimethamine (**4.7**)] was a major problem in the 70's and 80's. This problem is now resurging with the resistance to all the anti-malarial drugs available and therefore new drugs are urgently needed.[1,11]
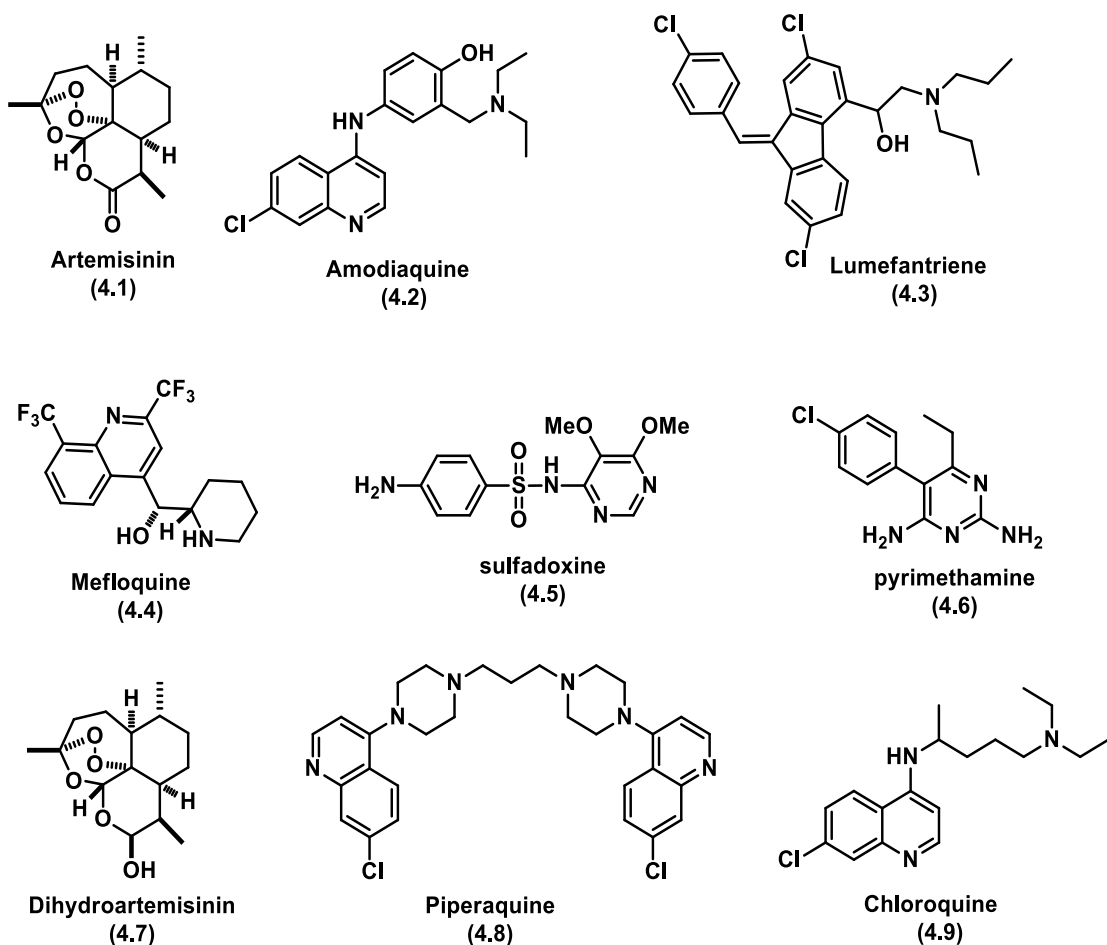
Figure 4.1 Molecular structures of anti-malarial drugs.

4.2. Anti-plasmodial and Anti-tubercular Amphilectane Diterpenes

Amphilectanes are tricyclic diterpenes (Figure 4.2) that have been isolated from marine sources and usually exhibit *in vitro* anti-plasmodial, anti-tubercular, anti-algal, anti-bacterial, anti-photosynthetic, anti-inflammatory, anti-proliferative, and anti-fouling activity if it contains an isocyanide or related functionalities.[12]
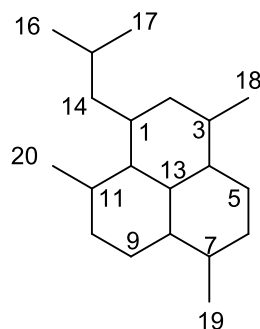
Figure 4.2 Amphilectane diterpene skeleton and numbering system.

In 1996, Wright and co-workers reported six amphilectane diterpenes (**4.10** – **4.15**) with significant *in vitro* anti-plasmodial activity against *P. falciparum* strains D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) from the sponge *Cymbastela hooperi* (Figure 4.3); along with five isocycloamphilectanes (**4.16** – **4.20**), two cycloamphilectanes (**4.21** and **4.22**), and one isoneoamphilectane (**4.23**) also with significant bioactivity.[13] They performed a SAR analysis when comparing the IC_{50}'s of **4.16** (~4 ng/mL), **4.17** (~35 ng/mL), and **4.18** (~65 ng/mL) against *P. falciparum*; suggesting that the isocyanide group plays a pivotal role on the activity, while the isothiocyanate and isocyanate groups slightly renders the potency.[14,15] Likewise, **4.15** with an isothiocyanate at C-7, was the least active among all the derivatives with IC_{50} values of 797 ng/mL for D9 and 423 ng/mL for W2. They ascribed this decrease in anti-plasmodial potency to the modification of the isocyanide at C-7, since an impact on the cytotoxicity was also observed. They also claimed that the location of the isocyanide is pivotal, due to the increase in activity when the isocyanate and isocyanide groups are interchanged as in **4.18** (IC_{50}~65 ng/mL) and **4.19** (IC_{50}~3 ng/mL).[15]
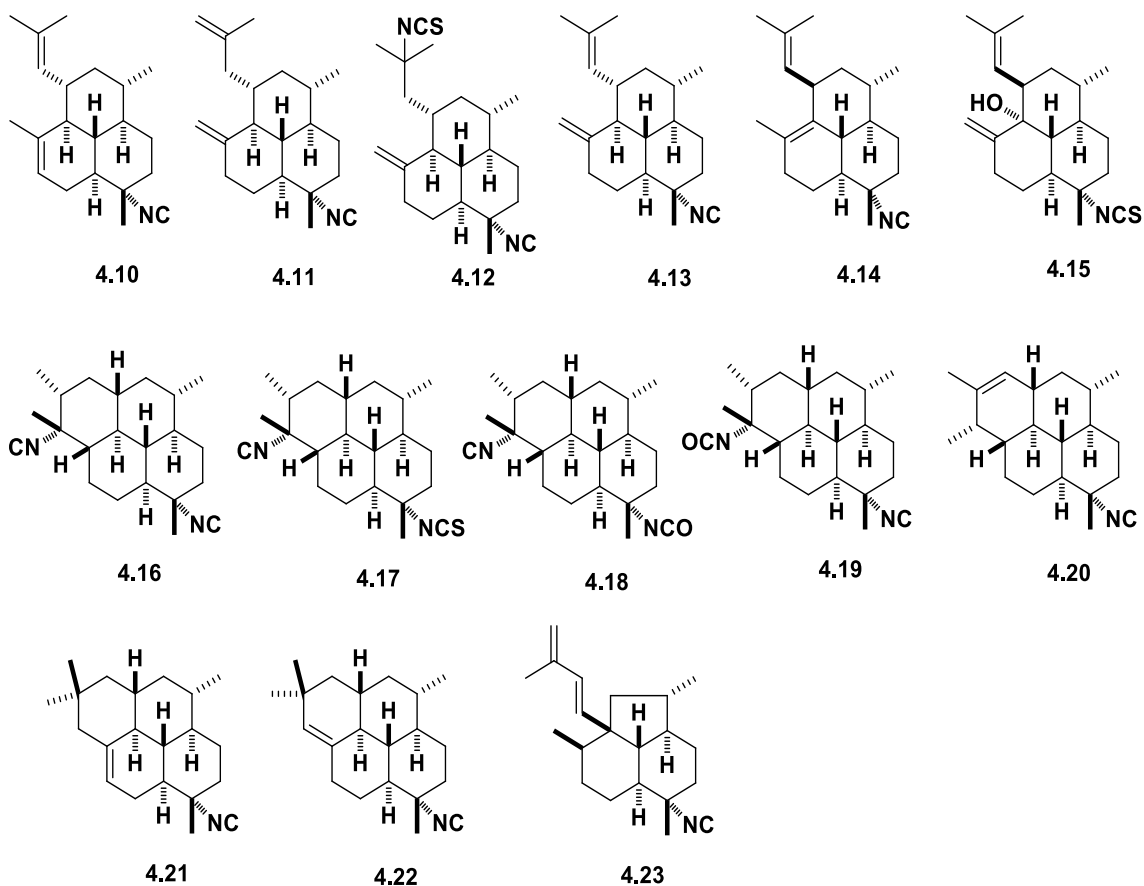
Figure 4.3 Molecular structures of compounds **4.10 – 4.23**.

More than a decade later, Wright and Lang-Unnash reported five diterpene formamides and their anti-plasmodial activity from the same sponge (Figure 4.4).[16] Compounds **4.24 – 4.28** were screened against the *P. falciparum* strain FCR3F86 and the IC$_{50}$ values oscillated from 0.2 μg/mL to >100 μg/mL. This reduced activity was consistent with the previous claim that a modification of the isocyanide group at C-7 hampers the anti-plasmodial activity.

4.24

4.25

4.26

4.27

4.28
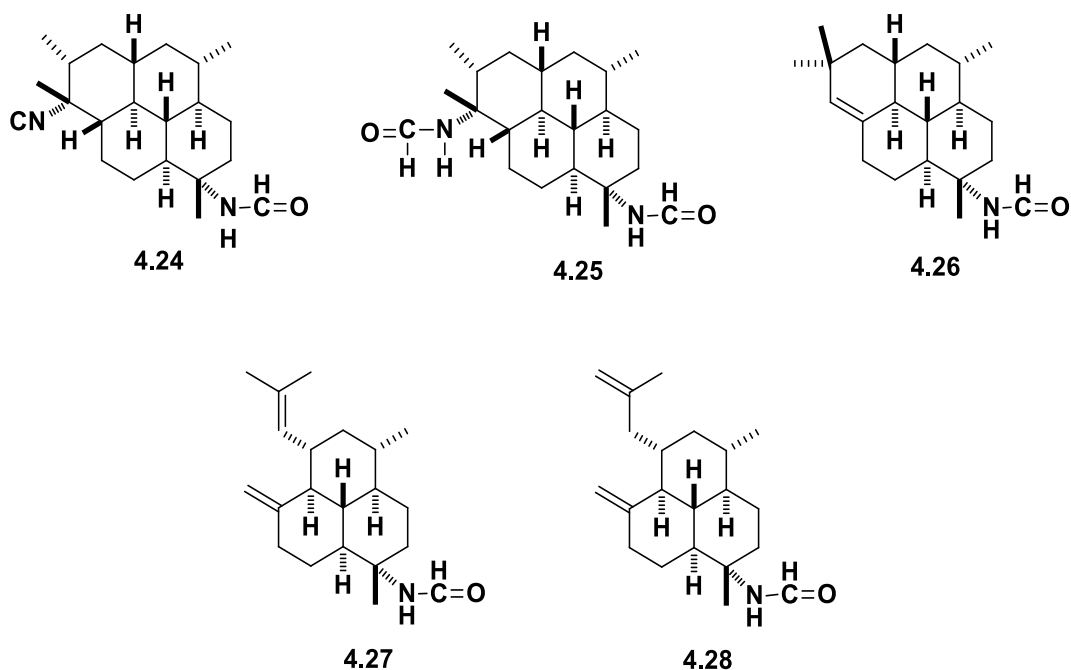
Figure 4.4 Molecular structures of compounds **4.24** – **4.28**.

In 2010, Avilés and Rodríguez reported the potent *in vitro* anti-infective activity of the marine sponge metabolites monamphilectane A (**4.29**) and (–)-8,15-diisocyano-11(20)-amphilectene (**4.30**) (Figure 4.5).[17] The latter compound was first reported in 1978 by Faulkner *et al.* from *Hymeniacidon amphilecta*.[18] The IC_{50} values of **4.29** and **4.30** against *P. falciparum* W2 strain were 0.6 and 0.4 μM, respectively; while the MIC values against *M. tuberculosis* H_{37}Rv were 15.3 and 3.2 μg/mL.[17] Several structurally related natural products as well as a small number of synthetic analogs prepared from diisocyanide **4.30** also exhibited anti-malarial and anti-mycobacterial potential.[19-21] Whilst comparison among their activities reveals that the biological activity is generally dependent on the presence of the isocyanide functionality, the structural features of the carbon backbone and

the location of the isocyanide groups also seem to play a pivotal role.[15] Notwithstanding, the observation that a plethora of sponge-derived isocyanide-, isothiocyanate-, isocyanate-, and formamide-containing diterpenoids based on amphilectane, cycloamphilectane, isocycloamphilectane, and isoneoamphilectane skeletons are often active (usually in the low nanomolar range), suggests that the biological activity does not depend strictly on the presence of the isocyanide functionality.[12,13,22,23] This observation implies that the metabolite's carbon skeleton can also modulate biological activity.
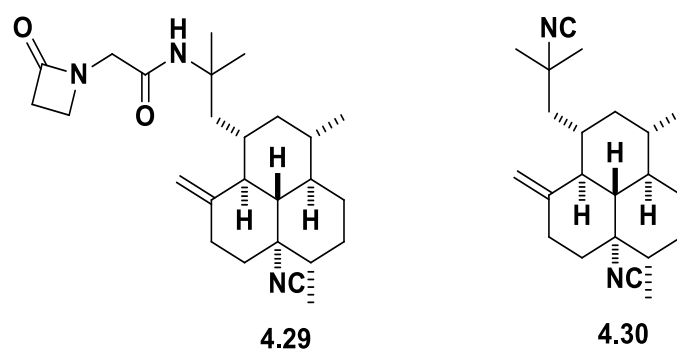
Figure 4.5 Molecular structures of monamplilectane A (**4.29**) and (–)-8,15-diisocyano-11(20)-amphilectene (**4.30**).

4.3. Design of Isothiocyanate- and Isoselenocyanate-functionalized Amphilectane Diterpenes

As part of our continued drug discovery program in search of new agents for the treatment of malaria and tuberculosis, we became interested in the synthesis of two amphilectane-based isothiocyanate (**4.31**) and isoselenocyanate

(**4.32**) diterpenes for biological evaluation (Scheme 4.1). Of the two classes of congeneric compounds, organic isoselenocyanates are of particular interest to us since so far they have received much less attention compared to their sulfur and oxygen analogs. We targeted diisocyanide **4.30** as a suitable starting material, a well-known anti-plasmodial and anti-mycobacterial pharmacophore accessible to us which contains both a rigid amphilectane skeleton and two isocyanide "handles" with potential for further synthetic elaboration.[17] We anticipated that comparison among the biological activities exhibited by the strictly related amphilectane analogs with those of **4.30** would reveal definite structure-activity relationships. While the isothiocyanate moiety is found in many natural products only two isothiocyanate-containing amphilectane diterpenoids with anti-plasmodial activity have been documented (**4.12** and **4.15**).[13] Remarkably, no studies assessing the potential anti-plasmodial or anti-mycobacterial properties of isoselenocyanate-containing compounds (synthetic or natural) have been reported so far.[24-26] Despite the high toxicity of many selenium compounds, organic derivatives of selenium have been previously synthesized for medical applications. As a result, selenium-containing compounds are of increasing interest because of their chemical properties and biological activities.[27,28]

In order to predict the pharmacokinetic properties of amphliectane diterpenes **4.31** and **4.32**, we calculated their partition coefficients (log P) using ChemBioDraw Ultra 12.0. The resulting values were 7.3 and 7.8, respectively. These values are distant form the cut-off number of 5.0 for "drug-like" molecules;

however, if their biological activity is remarkable, different formulation forms could be explored.[29]
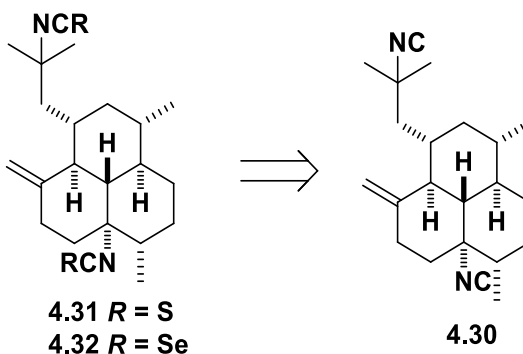
Scheme 4.1 Retrosynthetic analysis of compounds **4.31** and **4.32**.

4.4. Synthesis of Isothiocyanate- and Isoselenocyanate-functionalized Amphilectane Diterpenes

Since aliphatic isocyanides hardly react with elemental sulfur,[30,31] the desired diisothiocyanate **4.31** was synthesized via the isothiocyanation of **4.30** as outlined in Scheme 4.2. Thus, following a synthetic protocol previously described by Fujiwara *et al.*, treatment of diisocyanide **4.30** with S, Et$_3$N, and catalytic amounts of Se in refluxing THF afforded 8,15-diisothiocyano-11(20)-amphilectene (**4.31**) in 18% yield.[32] Surprisingly, the desired product was accompanied by large amounts of unreacted **4.30** along with smaller quantities of congeners **4.33** and **4.34** (53%), formed as a 2:3 mixture of regioisomers that was inseparable by chromatography (the integration of selected signals in the ^{1}H NMR spectra of the reaction products provided the isomer ratio). Addition of 2.5 mol% of S or increasing the refluxing time up to 16 h failed to afford full conversion to **4.31** or to

preclude the formation of **4.33** and **4.34**. These results suggest that in this case the reaction might exhibit a low catalytic activity of Se and that perhaps the amount of Se catalyst to isocyanide should be increased to >10 mol%. Even though the reaction was very sluggish, we were delighted to have these compounds at hand since their biological evaluation was at this point of outmost interest to us. As the only differences between **4.33** and **4.34** were a result of the –NCS and –NCSe functionalities switching positions, these isomers have nearly identical ^{13}C NMR shifts, apart from those at C-8 and C-15 (and their substituents). Nevertheless, we were able to distinguish the terpene isothiocyanate groups from its isoselenocyanate counterparts in **4.33** (minor) and **4.34** (major) by the ^{13}C chemical shift of the –NCS (129–132 ppm) vs –NCSe (121–125 ppm) group. Although these signals are typically of low intensity in the ^{13}C NMR spectra (during 1D spectroscopic acquisition an extended delay time (>5 s) and a 90° pulse angle are usually required to enhance their intensity) we detected them easily with a 700 MHz NMR spectrometer. These noticeable differences in ^{13}C NMR spectroscopic data, in combination with 2D NMR experiments (HSQC and HMBC spectra), allowed us to assign the structure of each isomer unambiguously.
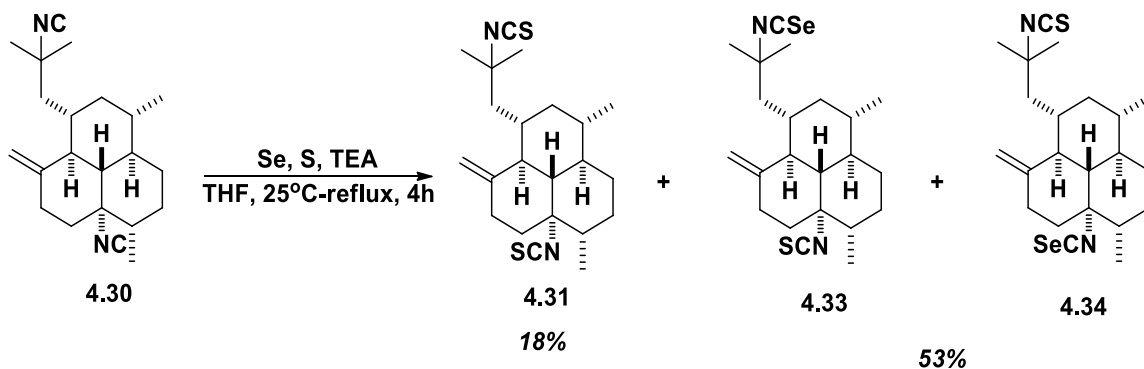
Scheme 4.2 Synthesis of isothiocyanate analogs **4.31**, **4.33**, and **4.34**.

Concomitantly with these efforts, we sought to achieve the isoselenocyanation of diisocyanide **4.30** to attain 8,15-diisoselenocyano-11(20)-amphilectene (**4.32**) in satisfactory yield.[33] The synthesis and biological evaluation of **4.32** was very appealing to us since natural products bearing the isoselenocyanate moiety have never been isolated.[34] Furthermore, synthetic isoselenocyanate-containing compounds apparently have never been investigated for potential anti-plasmodial or anti-mycobacterial activity. Thus, insertion of two selenium atom equivalents at C-21 and C-22 of diisocyanide **4.30** via an isoselenocyanation reaction with Se using TEA in THF led cleanly to **4.32** (Scheme 4.3, Procedure A). Gratifyingly, when the reaction was conducted in refluxing THF diisoselenocyanate **4.32** (50% yield) was accompanied by lesser quantities of isoselenocyanate **4.35** as a single regioisomer following purification by flash- and HPLC chromatography (Procedure B). In this fashion, the reaction proceeded with selective base-mediated decomposition of **4.32** at the more reactive C-15 isoselenocyanate group to give **4.35**, albeit in modest yield (33% yield). We

welcome the formation of **4.35** as it provided an opportunity to scrutinize its potential anti-infective properties.
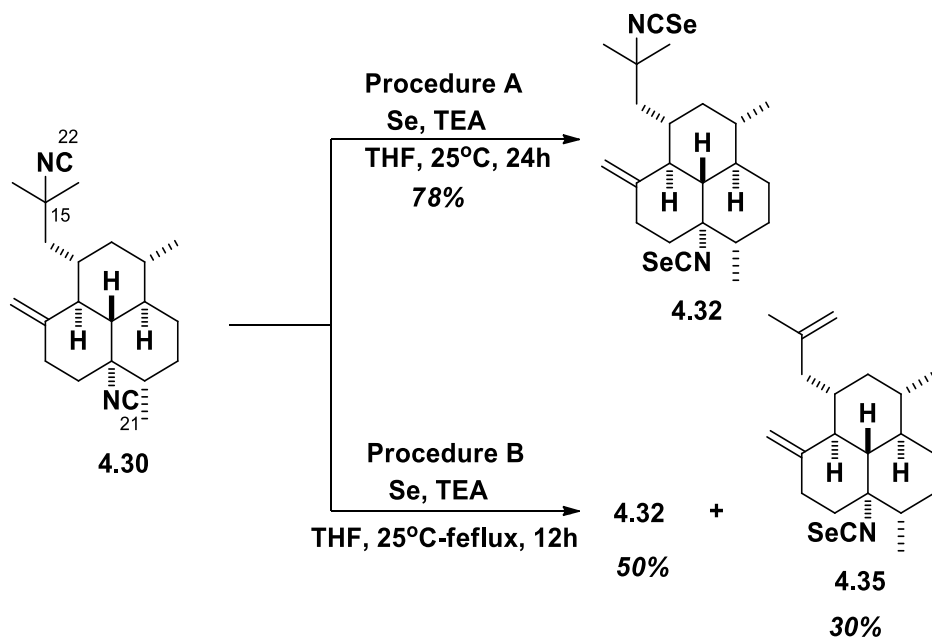
Scheme 4.3 Synthesis of isoselenocyanate analogs **4.32** and **4.35**.

As aforementioned, transformation of **4.30** to 8,15-diisothiocyano-11(20)-amphilectene (**4.31**) via Se-catalyzed isothiocyanation was characterized by poor yields of the expected product (≤18%) and the recovery of starting material. Thus, we developed a more efficient one-pot procedure based on diisoselenocyanation of **4.30** to **4.32** with Se (Scheme 4.3, Procedure A), followed by Se–S exchange in the presence of TEA to give **4.31** in 71% yield (Scheme 4.4). Conceivably, this minor modification to the original procedure reported Fijiwara *et al.*[32] should yield isothiocyanates in higher yields and shorter reaction times.
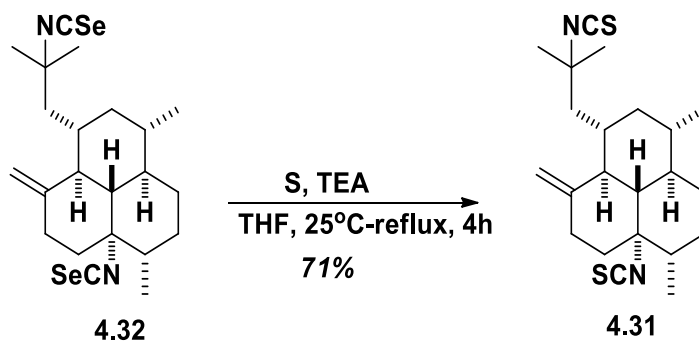
Scheme 4.4 Synthesis of diisothiocyanate **4.31** from diisoselenocyanate **4.32**.

4.5. Evaluation of Biological Activity: Results and Discussion

The synthesized compounds **4.31**- **4.35** were evaluated in an *in vitro* growth inhibition assay against two *P. falciparum* Dd2 (drug resistant) and 3D7 (chloroquine-sensitive) malaria parasite lines, using the anti-malarial drug chloroquine as reference standard. Concomitantly, compounds **4.31** – **4.35** were assayed against a laboratory strain of *Mtb* $H_{37}Rv$, using the anti-mycobacterial drug rifampicin as the control in the determination of the MIC value of each compound (Table 4.1). Active compounds were then assessed for potential cytotoxicity to human cells through the use of cultured Vero cells (Table 4.2). The values of Selectivity Index (SI) for each compound are shown in Table 4.2; a higher value indicates a higher degree of selectivity to *P. falciparum* and *Mtb* than to mammalian cells.

Except for the diisothiocyanate-functionalized amphilectane diterpene **4.31**, all of the isoselenocyanate hybrids (**4.32** – **4.35**) showed sub-micro molar *in vitro* anti-plasmodial activity (0.0025–0.3084 µM) against the two malaria parasite lines

screened.   Among these hybrids, only compound **4.32** having two isoselenocyanate functionalities showed more activity (Dd2, IC_{50} = 0.0066; 3D7, IC_{50} = 0.0025 μM) when compared to the standard drug chloroquine (Dd2, IC_{50} = 0.0519 μM; 3D7, IC_{50} = 0.0109 μM). Remarkably, hybrid **4.32** showed less toxicity (SI = 7356) than chloroquine (SI = 4518) against the drug resistant *P. falciparum* Dd2 strain.

In the end, however, starting scaffold **4.30** with two isocyanide groups proved to be the most promising compound of the series (Dd2, IC_{50} = 0.0031 μM; 3D7, IC_{50} = 0.0012 μM), which was manifold times more active and less toxic than the standard drug (Tables 4.1 and 4.2). Interestingly, as for inhibition against *P. falciparum*, the exchange of the isocyanide against the isocyanate group always results in a more significant drop in potency when compared to the –NC $\Rightarrow$ –NCS exchange.[13]   Still, our data suggest that switching the isocyanide for the isoselenocyanate functionality leads to no significant loss in antiparasitic activity.[35] Altogether, the most notable results obtained from this limited series of compounds are those for **4.32** – **4.35**.   To our knowledge, this is the first time that isoselenocyanate-functionalized inhibitors of *P. falciparum* have been described.

***Table 4.1 In vitro* anti-plasmodial and anti-mycobacterial activity of
compounds 4.30 – 4.35**

Compound	IC_{50} Dd2 (μM)	IC_{50} 3D7 (μM)	MABA MIC (μM)
4.30	0.0031	0.0012	9.8
4.31	11.5863	11.7669	99.1
4.32	0.0066	0.0025	3.9
4.33 and 4.34[a]	0.1433	0.3084	26.8
4.35	0.1490	0.1885	2.1
CQ	0.0519	0.0109	–
RMP	–	–	0.09

[a] Tested as a 2:3 mixture of regioisomers. CQ = chloroquine and RMP = rifampicin (+Ctrls).

The best (lowest) MIC values for *in vitro* activity against *Mtb* H_{37}Rv were determined for isoselenocyanate-functionalized hybrids **4.32** and **4.35** (3.9 and 2.1 μM, respectively). On the other hand, hybrids with an isothiocyanate moiety (**4.31**, **4.33**, and **4.34**) had the worst (highest) MIC's (26.8–99.1 μM) (Table 4.1). Interestingly, amphilectane-based diterpene **4.35** with a single isoselenocyanate moiety was identified as both the most potent (MIC = 2.1 μM) and the least toxic (SI value of 45.3) of the series (Table 4.2). Given its good MIC and SI, analog **4.35** is a potential candidate for efficacy studies in mice, and should future collaborations demonstrate that this isoselenocyanate-functionalized amphilectane diterpene has good pharmacokinetic properties, it could become a new anti-TB drug.

Table 4.2 __Comparison of selectivity indexes of compounds 4.30 – 4.35 with CQ and RMP__

Compound	IC$_{50}$ Vero cell μM	SI^a	SI^b	SI^c
4.30	99.74	32174	83117	10.2
4.31	>100	>9	>8	>1.0
4.32	48.55	7356	19420	12.4
4.33 and 4.34^d	78.14	545	253	2.9
4.35	95.22	639	505	45.3
CQ	234.47^e	4518	21511	–
RMP	>100	–	–	>1100

^{a} Selectivity index (SI) defined by the ratio: IC$_{50}$ (in mammalian Vero cell lines)/IC$_{50}$ of antiparasitic activity against Dd2 (CQ-resistant strain) cell line.

^{b} Selectivity index (SI) defined by the ratio: IC$_{50}$ (in mammalian Vero cell lines)/IC$_{50}$ of antiparasitic activity against 3D7 (CQ-sensitive strain) cell line.

^{c} Selectivity index (SI) defined by the ratio: IC$_{50}$ (in mammalian Vero cell lines)/MIC of antimycobacterial activity against *M. tuberculosis* H$_{37}$Rv cell line.

^{d} Tested as a 2:3 mixture of regioisomers.

^{e} Value obtained from Ref. 24. CQ = chloroquine and RMP = rifampicin (+Ctrls).

4.6. Conclusions

In the present work, the syntheses of analogs **4.31** – **4.35** were swiftly accomplished through the isothio- and isoselenocyanation of metabolite **4.30**, previously isolated by us from the marine sponge *Svenzea flava*.[17] All of the semi-synthetic derivatives exhibited strong to potent *in vitro* inhibition of *Plasmodium falciparum* Dd2 and 3D7 strains with some exhibiting greater anti-plasmodial activity than the standard drug chloroquine. Likewise, the new compounds have

shown sub-micromolar to low micromolar *in vitro* anti-mycobacterial activity. In order to assess their microbe-specific selectivity (i.e. whether the observed anti-microbial activity was a specific or general toxic effect) the cytotoxic effects of compounds **4.31** – **4.35** using a mammalian Vero cell line were also investigated. The results obtained are further evidence of the anti-infective potential of these novel amphilectane-based chemotypes. While based on a very limited library of hybrid compounds, this investigation demonstrates for the first time that isoselenocyanate-functionalized amphilectane diterpenes could become important anti-malarial and anti-tubercular pharmacophores.

4.7. Proposed Recommendations

The synthesis of compounds **4.31** – **4.35** demonstrated for the first time that isoselenocyanate-functionalized amphilectane diterpenes could become important anti-plasmodial and anti-tubercular agents and some future recommendations can be made.   The first recommendation is to synthesize isoselenocyanate-monamphilectane A (**4.36**) (Figure 4.6).   Since the anti-tubercular and anti-plasmodial activity of monamphilectane A (**4.29**) was comparable to that of diisocyanide **4.30**, we conjecture that compound **4.36** should be at least as active as compounds **4.35** (Scheme 4.3). The second recommendation is to send compounds **4.30** – **4.35** for anti-inflammatory and anti-proliferative evaluation, since usually amphilectane diterpenes are very active.[12]
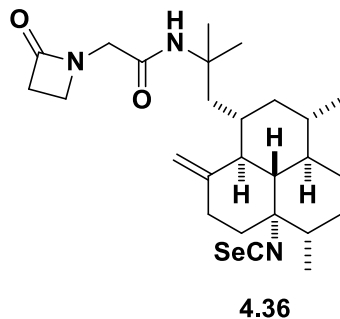
Figure 4.6 Molecular structure of compounds **4.36**.

4.8. Experimental Section

4.8.1. General experimental methods

All of the reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an atmosphere of argon. Column chromatography (CC) was performed on silica gel (35–75 μm); reactions were followed by TLC analysis using glass pre-coated silica gel plates with fluorescent indicator (254 nm) and visualized with a UV lamp, I_2 vapors, or 10% ethanolic sulfuric acid followed by heating. Semipreparative RP-HPLC was performed using an UV detector set at 254 nm and a column with 5 μm, 250 x 4.6 mm size with a flow rate of 1.0 mL/min. Solvents and commercially available reagents were purchased and used as received without further purification. Optical rotations were recorded with a polarimeter using a 0.5 mL capacity cell with 1 dm path length. Infrared spectra were recorded using thin films supported on NaCl discs. UV were recorded on a UV-Vis spectrophotometer using quartz cuvettes and MeOH as solvent. ^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the specified field

strength on a 700 or 500 MHz spectrometer. Spectra were obtained on $CDCl_3$ solutions in 5 mm diameter tubes, and chemical shifts are quoted in parts per million relative to the residual signals of $CHCl_3$ (δ_H = 7.26 ppm, δ_C = 77.0 ppm). Multiplicities in the ^{1}H NMR spectra are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants are reported in Hertz. High-resolution mass spectrometry (HRMS) was performed using a quadrupole mass analyzer, and the data are reported with ion mass/charge (*m/z*) ratios as values in atomic mass units. Elemental analysis was not performed due to the lability of the compounds. Yields shown are based on recovered starting material. The anti-plasmodial activity assay was performed by the research assistant Jacques Prudhomme at the University of California at Riverside, California against 3D7 and Dd2 strains of *P. falciparum* malaria using chloroquine (**4.9**) as a positive control. Compounds with IC_{50}'s > 200 μM are typically considered inactive. The anti-tubercular activity assay was performed at the Institute for Tuberculosis Research, University of Illinois, Chicago against the *Mtb* strain H_{37}Rv by laboratory technicians Yuehong Wang, Baoji Wang, and Rui Ma. For this task, they used the Microplate Alamar Blue Assay (MABA) with rifampicin (**1.2**) as a positive control. Compounds with MIC's > 64 μg/mL are typically considered inactive. The cytotoxicity assay was performed at the Institute for Tuberculosis Research, University of Illinois, Chicago with Vero cells (ATCC CRL-1586) by laboratory technician Yuehong Wang.

Animal material

The Caribbean sponge *Svenzea flava* (phylum Porifera; class Demospongiae; order Halichondrida; family Dictyonellidae) was collected at a depth of 89 feet by scuba off Mona Island, Puerto Rico, in July 2006. A voucher specimen (no. IM06-04) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The species *Svenzea flava* was originally classified as *Pseudoaxinella flava*.[36] Despite lacking dark granulous cells that are a signature characteristic of other species within the genus *Svenzea*, it has been accepted as *Svenzea flava*.[37]

Isolation and purification of (–)-8,15-diisocyano-11(20)-amphilectene (4.30)

The known sponge metabolite (–)-8,15-diisocyano-11(20)-amphilectene (**4.30**)[18] was obtained pure as white crystals (528 mg) from freshly collected sponge specimens as previously described (the sponge was originally reported by our group as *Hymeniacidon* sp.).[17] The structure characterization of **4.30** was established on the basis of IR, UV, $[\alpha]_D$, MS, X-ray crystallography, and ^{1}H and ^{13}C NMR spectroscopic analyses.

Synthesis of isothiocyanate-containing amphilectanes (4.31, 4.33, and 4.34)
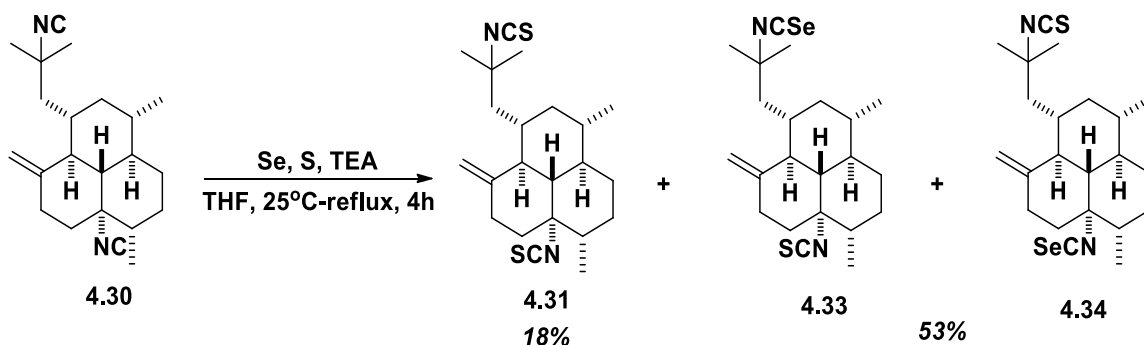
To a solution of diisocyanide **4.30** (37 mg, 0.11 mmol) in dry THF (5.0 mL) was added selenium (0.9 mg, 0.01 mmol), sulfur (4 mg, 0.12 mmol), and TEA (76 μL, 0.5 mmol) at 25 °C. After refluxing for 4 h the reaction mixture was allowed to cool to 25° C and then concentrated *in vacuo*. The crude oil obtained was purified by flash-silica gel CC with 100% hexane to afford unreacted **4.30** (23 mg) followed by another fraction consisting of a mixture of isothiocyanate-containing products that was purified by HPLC using 12% H_2O in MeOH as eluent. Retention times were 20.4 min for the more polar mixture of **4.33** and **4.34** (10 mg, 53%) and 23.8 min for the less polar compound **4.31** (3 mg, 18%).

8,15-Diisothiocyano-11(20)-amphilectene (4.31)

$[α]^{20}_D$ -108.0 (*c* 0.25, CHCl$_3$); UV (MeOH) $λ_{max}$ ($ε$) 202 (19471) nm; IR (film) $\tilde{ν}_{max}$ 2926, 2857, 2095, 1728, 1459, 1262 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 4.87 (br s, 1H, H-20β), 4.61 (br s, 1H, H-20α), 2.34 (m, 1H, H-9β), 2.26 (m, 2H, H-10), 2.16 (m, 1H, H-2β), 2.06 (dd, *J* = 1.3, 14.6 Hz, 1H, H-14β), 2.00 (m, 1H, H-5β), 1.89 (m,

1H, H-1), 1.75 (t, J = 10.8 Hz, 1H, H-12), 1.55 (m, 2H, H-6), 1.47 (s, 3H, H-17), 1.44 (s, 3H, H-16), 1.43–1.35 (br envelope, 2H, H-7, H-9α), 1.28 (m, 1H, H-14α), 1.11–1.00 (br envelope, 3H, H-3, H-4, H-13), 0.99 (d, J = 6.3 Hz, 3H, H-19), 0.94 (d, J = 6.1 Hz, 3H, H-18), 0.92–0.83 (br envelope, 2H, H-2α, H-5α); ^{13}C NMR (175 MHz, CDCl$_3$) δ 150.2 (C, C-11), 131.1 (C, C-21), 130.4 (C, C-22), 106.1 (CH$_2$, C-20), 69.6 (C, C-8), 60.6 (C, C-15), 56.9 (CH, C-13), 46.8 (CH$_2$, C-14), 46.7 (CH, C-12), 43.3 (CH, C-4), 42.5 (CH, C-7), 41.1 (CH$_2$, C-2), 40.1 (CH$_2$, C-9), 35.7 (CH, C-3), 34.1 (CH$_2$, C-10), 33.2 (CH, C-1), 31.9 (CH$_3$, C-17), 30.5 (CH$_2$, C-6), 29.9 (CH$_2$, C-5), 29.4 (CH$_3$, C-16), 19.9 (CH$_3$, C-18), 16.1 (CH$_3$, C-19); EI-LRMS m/z [M]$^+$ 388 (3), 330 (27), 329 (22), 271 (100), 255 (26), 215 (58), 201(30), 159 (31); EI-HRMS m/z calcd for C$_{22}$H$_{32}$N$_2$S$_2$ [M]$^+$ 388.2007, found 388.2007.

8-Isothiocyano-15-isoselenocyano-11(20)-amphilectene (4.33) and 8-Isoselenocyano-15-isothiocyano-11(20)-amphilectene (4.34) (2:3 mixture of isomers)

UV (MeOH) λ_{max} (ε) 192 (962), 202 (4457) nm; IR (film) $\tilde{\nu}_{max}$ 2920, 2870, 2255, 2096, 1453 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) (major isomer) δ 4.88 (br s, 1H, H-20β), 4.63 (br s, 1H, H-20α), 2.40–1.99 (br envelope, 6H, H-2β, H-5β, H-9β, H-10$\alpha\beta$, H-14β), 1.90 (m, 1H, H-1), 1.75 (m, 1H, H-12), 1.60–1.26 (br envelope, 5H, H-6$\alpha\beta$, H-7, H-9α, H-14α), 1.47 (s, 3H, H-17), 1.44 (s, 3H, H-16), 1.14–0.84 (br envelope, 5H, H-2α, H-3, H-4, H-5α, H-13), 1.00 (d, J = 6.4 Hz, 3H, H-19), 0.95 (d, J = 6.2 Hz, 3H, H-18); (minor isomer) δ 4.87 (br s, 1H, H-20β), 4.59 (br s, 1H, H-

20α), 2.40–1.99 (br envelope, 6H, H-2β, H-5β, H-9β, H-10$\alpha\beta$, H-14β), 1.90 (m, 1H, H-1), 1.75 (m, 1H, H-12), 1.60–1.26 (br envelope, 5H, H-6$\alpha\beta$, H-7, H-9α, H-14α), 1.51 (s, 3H, H-17), 1.44 (s, 3H, H-16), 1.14–0.84 (br envelope, 5H, H-2α, H-3, H-4, H-5α, H-13), 0.99 (d, J = 6.3 Hz, 3H, H-19), 0.96 (d, J = 6.1 Hz, 3H, H-18); ^{13}C NMR (175 MHz, CDCl$_3$) (major isomer) δ149.8 (C, C-11), 130.4 (C, C-22), 124.4 (C, C-21), 106.4 (CH$_2$, C-20), 71.0 (C, C-8), 60.6 (C, C-15), 56.7 (CH, C-13), 46.8 (CH$_2$, C-14), 46.6 (CH, C-12), 43.3 (CH, C-4), 42.3 (CH, C-7), 41.1 (CH$_2$, C-2), 39.8 (CH$_2$, C-9), 35.7 (CH, C-3), 33.9 (CH$_2$, C-10), 33.2 (CH, C-1), 31.9 (CH$_3$, C-17), 30.4 (CH$_2$, C-6), 29.8 (CH$_2$, C-5), 29.5 (CH$_3$, C-16), 19.8 (CH$_3$, C-18), 16.1 (CH$_3$, C-19); (minor isomer) δ 150.1 (C, C-11), 131.2 (C-21), 122.4 (C, C-22), 106.1 (CH$_2$, C-20), 69.6 (C, C-8), 61.3 (C, C-15), 56.9 (CH, C-13), 46.7 (CH, C-12), 46.5 (CH$_2$, C-14), 43.2 (CH, C-4), 42.5 (CH, C-7), 41.0 (CH$_2$, C-2), 40.1 (CH$_2$, C-9), 35.6 (CH, C-3), 34.0 (CH$_2$, C-10), 33.1 (CH, C-1), 31.6 (CH$_3$, C-17), 30.5 (CH$_2$, C-6), 29.9 (CH$_2$, C-5), 29.0 (CH$_3$, C-16), 19.9 (CH$_3$, C-18), 16.0 (CH$_3$, C-19); EI-LRMS m/z [M]$^+$ 436 (31), 330 (34), 329 (46), 271 (95), 270 (85), 255 (100), 215 (88), 201 (65), 159 (42); EI-HRMS m/z calcd for C$_{22}$H$_{32}$N$_2$S^{80}Se [M]$^+$ 436.1451, found 436.1459. Multiple attempts to separate the mixture of regioisomers **4.33** and **4.34** by normal- and reversed-phase HPLC proved unsuccessful.

Synthesis of isoselenocyanate-containing amphilectanes (4.32 and 4.35)
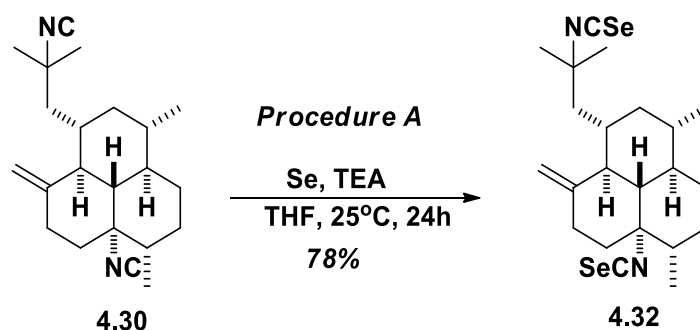
General Procedure A. To a solution of diisocyanide **4.30** (12 mg, 0.04 mmol) in dry THF (2.0 mL) was added selenium (6 mg, 0.08 mmol) and TEA (0.07 mL, 0.5 mmol) at room temprature. After stirring for 24 h the reaction mixture was concentrated *in vacuo* and the crude oil obtained was purified by flash-Silica gel CC using a 99:1 mixture of hexane/EtOAc to afford **4.32** as the sole product (14 mg, 78%).
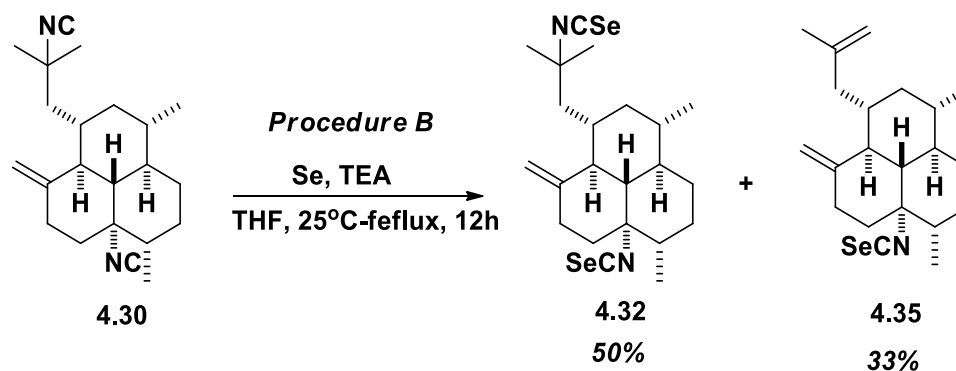
General Procedure B. To a solution of diisocyanide **4.30** (16 mg, 0.05 mmol) in dry THF (2.0 mL) was added selenium (8 mg, 0.1 mmol) and TEA (0.1 mL, 0.7 mmol) at 25 °C. After refluxing for 12 h the reaction mixture was allowed to cool to 25° C and then concentrated *in vacuo.* The crude oil obtained was purified by

flash-Silica gel CC using a 99:1 mixture of hexane/EtOAc to afford a fraction consisting of a mixture of two products that was subsequently purified by HPLC (MeOH/H$_2$O, 95/5). Retention times were 8.32 min for compound **4.32** (12 mg, 50%) and 9.29 min for compound **4.35** (6 mg, 33%).

8,15-Diisoselenocyano-11(20)-amphilectene (4.32)

[α]$^{20}_D$ -139.0 (*c* 1.0, CHCl$_3$); UV (MeOH) λ_{max} (ε) 202 (56143) nm; IR (film) $\tilde{\nu}_{max}$ 2921, 2870, 2258, 2110, 1455 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 4.89 (br s, 1H, H-20β), 4.60 (br s, 1H, H-20α), 2.38 (m, 1H, H-9β), 2.30 (m, 1H, H-10β), 2.24 (m, 1H, H-10α), 2.17 (m, 1H, H-2β), 2.10 (dd, *J* = 1.2, 14.7 Hz, 1H, H-14β), 2.02 (m, 1H, H-5β), 1.91 (m, 1H, H-1), 1.75 (t, *J* = 10.9 Hz, 1H, H-12), 1.52 (s, 3H, H-17), 1.49 (s, 3H, H-16), 1.48–1.32 (br envelope, 5H, H-6αβ, H-7, H-9α, H-14α), 1.14–1.02 (br envelope, 3H, H-3, H-4, H-13), 1.01 (d, *J* = 6.3 Hz, 3H, H-19), 0.96 (d, *J* = 6.3 Hz, 3H, H-18), 0.93–0.83 (br envelope, 2H, H-2α, H-5α); ^{13}C NMR (175 MHz, CDCl$_3$) δ 149.7 (C, C-11), 124.5 (C, C-21), 122.5 (C, C-22), 106.4 (CH$_2$, C-20), 70.9 (C, C-8), 61.3 (C, C-15), 56.6 (CH, C-13), 46.7(CH, C-12), 46.6 (CH$_2$, C-14), 43.3 (CH, C-4), 42.3 (CH, C-7), 41.0 (CH$_2$, C-2), 39.8 (CH$_2$, C-9), 35.6 (CH, C-3), 33.9 (CH$_2$, C-10), 33.2 (CH, C-1), 31.6 (CH$_3$, C-17), 30.4 (CH$_2$, C-6), 29.8 (CH$_2$, C-5), 29.1 (CH$_3$, C-16), 19.8 (CH$_3$, C-18), 16.1 (CH$_3$, C-19); EI-LRMS *m/z* [M]$^+$ 484 (28), 378 (23), 377 (21), 272 (45), 271 (100), 270 (86), 255 (97), 215 (94), 201 (82), 199 (65), 159 (68); EI-HRMS *m/z* calcd for C$_{22}$H$_{32}$N$_2$^{80}Se$_2$ [M]$^+$ 484.0896, found 484.0902.

8-Isoselenocyanoamphilecta-11(20),15-diene (4.35)

$[\alpha]^{20}_D$ + 70.0 (*c* 0.2, CHCl$_3$); UV (MeOH) λ_{max} (ε) 202 (9039) nm; IR (film) $\tilde{\nu}_{max}$ 2922, 2852, 2264, 2093, 1452 cm^{-1}; ^{1}H NMR (700 MHz, CDCl$_3$) δ 4.86 (s, 1H, H-20β), 4.76 (s, 1H, H-16β), 4.67 (s, 1H, H-16α), 4.66 (s, 1H, H-20α), 2.59 (d, *J* = 14.3 Hz, 1H, H-14β), 2.38 (m, 1H, H-9β), 2.29 (m, 2H, H-10), 2.01 (m, 1H, H-5β), 1.80 (m, 1H, H-2β), 1.76 (m, 2H, H-1, H-12), 1.72 (s, 3H, H-17), 1.54 (m, 3H, H-6$\alpha\beta$, H-14α), 1.41 (m, 2H, H-7, H-9α), 1.04 (m, 3H, H-3, H-4, H-13), 1.00 (d, *J* = 6.4 Hz, 3H, H-19), 0.90 (d, *J* = 5.9 Hz, 3H, H-18), 0.86 (m, 1H, 5α), 0.70 (m, 1H, 2α); ^{13}C NMR (175 MHz, CDCl$_3$) δ 149.7 (C, C-11), 144.2 (C, C-15), 123.8 (C, C-21), 111.3 (CH$_2$, C-16), 106.1 (CH$_2$, C-20), 70.9 (C, C-8), 56.7 (CH, C-13), 46.5 (CH, C-12), 43.6 (CH, C-4), 43.0 (CH$_2$, C-14), 42.3 (CH, C-7), 39.8 (CH$_2$, C-2), 39.6 (CH$_2$, C-9), 36.0 (CH, C-3), 33.9 (CH, C-1), 33.8 (CH$_2$, C-10), 30.5 (CH$_2$, C-6), 29.8 (CH$_2$, C-5), 22.6 (CH$_3$, C-17), 19.7 (CH$_3$, C-18), 16.1 (CH$_3$, C-19); EI-LRMS *m/z* [M]$^+$ 377 (17), 297 (7), 282 (18), 272 (42), 271 (100), 270 (83), 255 (94), 215 (81), 199 (60), 159 (55), 145 (48), 105 (52), 91 (59); EI-HRMS *m/z* calcd for C$_{21}$H$_{31}$N^{80}Se [M]$^+$ 377.1622, found 377.1626.

Synthesis of 8,15-diisothiocyano-11(20)-amphilectene (4.31) from diisoselenocyanate 4.32 with elemental sulfur

4.32   **4.31**
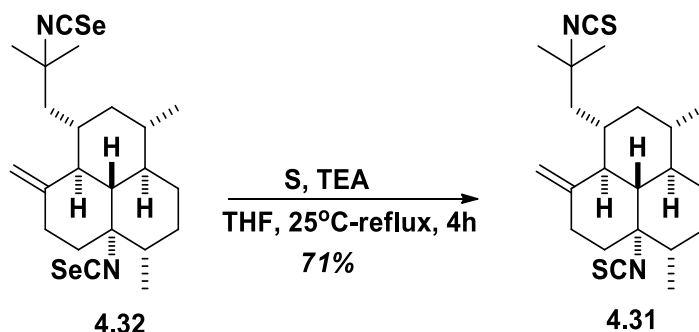
To a solution of diisoselenocyanate **4.32** (7 mg, 0.01 mmol) in dry THF (2.0 mL) was added sulfur (0.9 mg, 0.03 mmol) and TEA (9.7 μL, 0.07 mmol) at 25 °C. After refluxing for 4 h the reaction mixture was allowed to cool to 25° C and then concentrated *in vacuo*. The crude oil obtained was purified by flash-Silica gel CC with a 98:2 mixture of hexane/EtOAc to afford **4.31** (4 mg, 71%) as the sole product. The characterization data for compound **4.31** are shown on page 230.

Evaluation of Inhibition of *Plasmodium falciparum* Growth

The 3D7 and Dd2 strains of *P. falciparum* malaria (BEI Resources, MR4/ATCC, Manassas, VA) were cultured in human type O+ erythrocytes in complete medium consisting of RPMI 1640 (Cellgro), 0.043 mg/mL gentamicin (Gibco), 0.014 mg/mL hypoxanthine (Acros), 38.5 mM HEPES (Sigma), 0.18% sodium bicarbonate (Cellgro), 0.20% glucose (MP Biomedical), 0.003 mM NaOH (Sigma), 0.2% Albumax (Gibco), and 5% human serum as published.[38] Briefly, cultures were maintained in 25-cm^2 flasks (Corning) at a volume of 10 mL, gassed for 30 s with 3% CO_2, 1% O_2, and 96% N_2, and were finally incubated at 37 °C. The anti-plasmodial activity was determined with a SYBR Green based parasite

proliferation assay as published.[39] After 72 h of incubation in the presence of serial dilutions of compounds, the increase of parasite DNA contained in human red blood cells was measured. The relative fluorescence values were measured using a Molecular Devices SpectraMAX Gemini EM fluorimeter (excitation 495 nm, and emission 525 nm). Data were analyzed using Microsoft Excel and were plotted using SigmaPlot 10 (Systat).

4.8.2. Evaluation of Anti-tubercular Activity.

M. tuberculosis H37Rv (ATCC 27294) was obtained from the American Type Culture Collection (Rockville,Md.). For the first three (of four) replicate experiments, H37Rv inocula were first passaged in radiometric 7H12 broth until the growth index (GI) reached 800 to 999. For the fourth replicate experiment, H37Rv was grown in 7H9GC-Tween. Cultures were incubated in 500 mL nephelometer flasks on a rotary shaker at 150 rpm and 37 °C until they reached an optical density of 0.4 to 0.5 at 550 nm. The filtrates were aliquoted, stored at 280 °C, and used within 30 days. The microplate alamar blue assay (MABA) was performed in black, clear-bottomed, 96-well microplates in order to minimize background fluorescence as published.[40] Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of $\geq$50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. All the statistical analyses were

performed with the program SAS (Statistical Analysis System). The anti-tuberculosis drug rifampicin (RMP) was used as a positive control during the assays.

4.8.3. Cytotoxicity Assay

Vero cells (ATCC CRL-1586) were cultured in 10% fetal bovine serum (FBS) in minimum essential medium Eagle as outlined previously.[41] The cells were incubated at 37 °C under 5% CO_2 until confluent and then diluted with phosphate-buffered saline to 106 cells/mL. After incubation at 37 °C for 72 h, medium was removed and monolayers were washed twice with 100 mL of warm Hanks' balanced salt solution (HBSS). One hundred microliters of warm medium and 20 mL of freshly made MTS-PMS and phenylmetha-sulfazone (100:20) (Promega) were added to each well, plates were incubated for 3 h, and absorbance was determined at 490 nm.

4.8.4. Spectroscopic Data
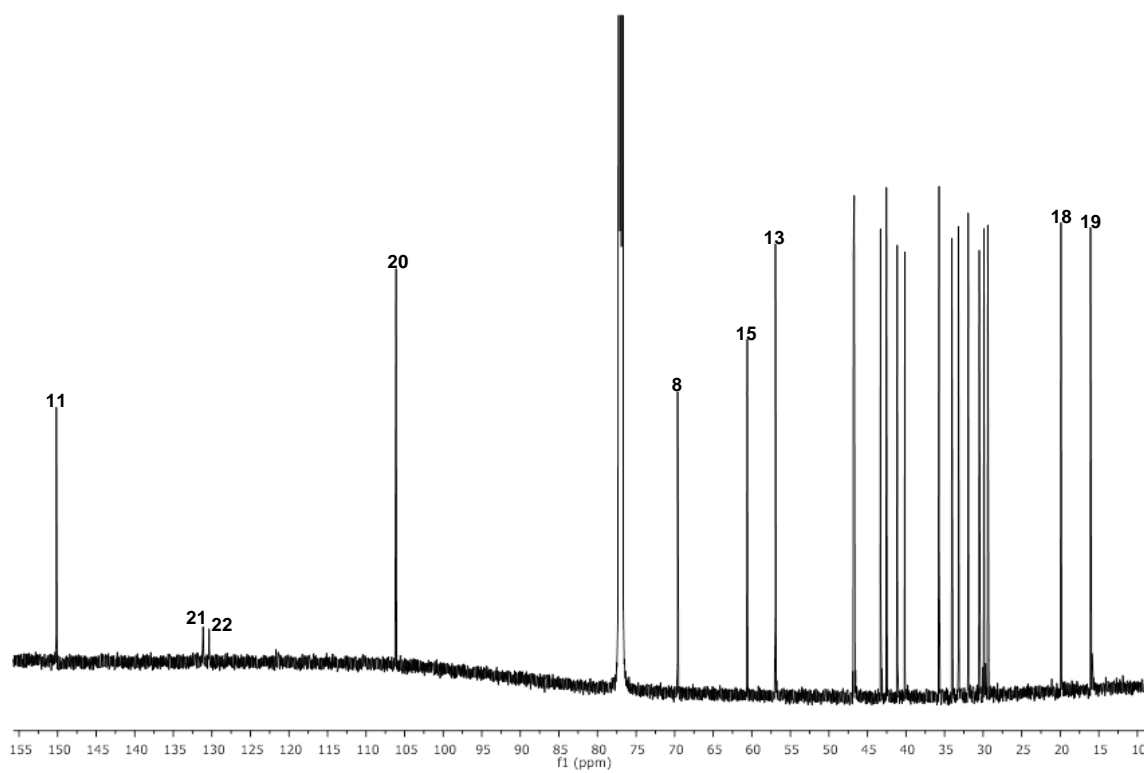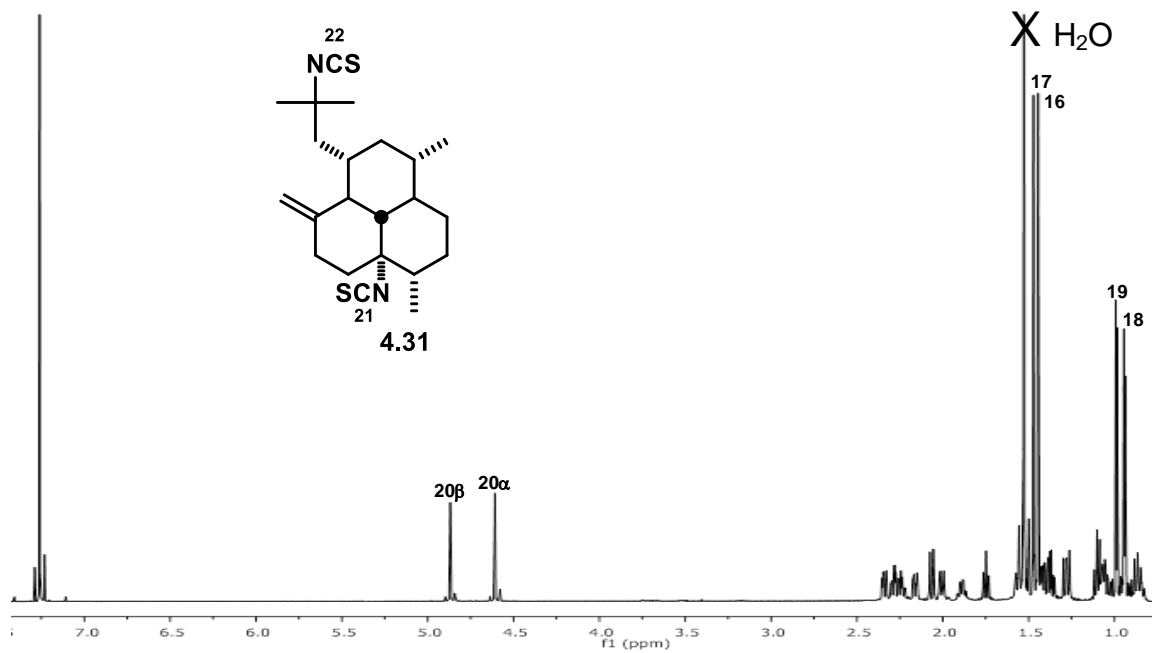
^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$
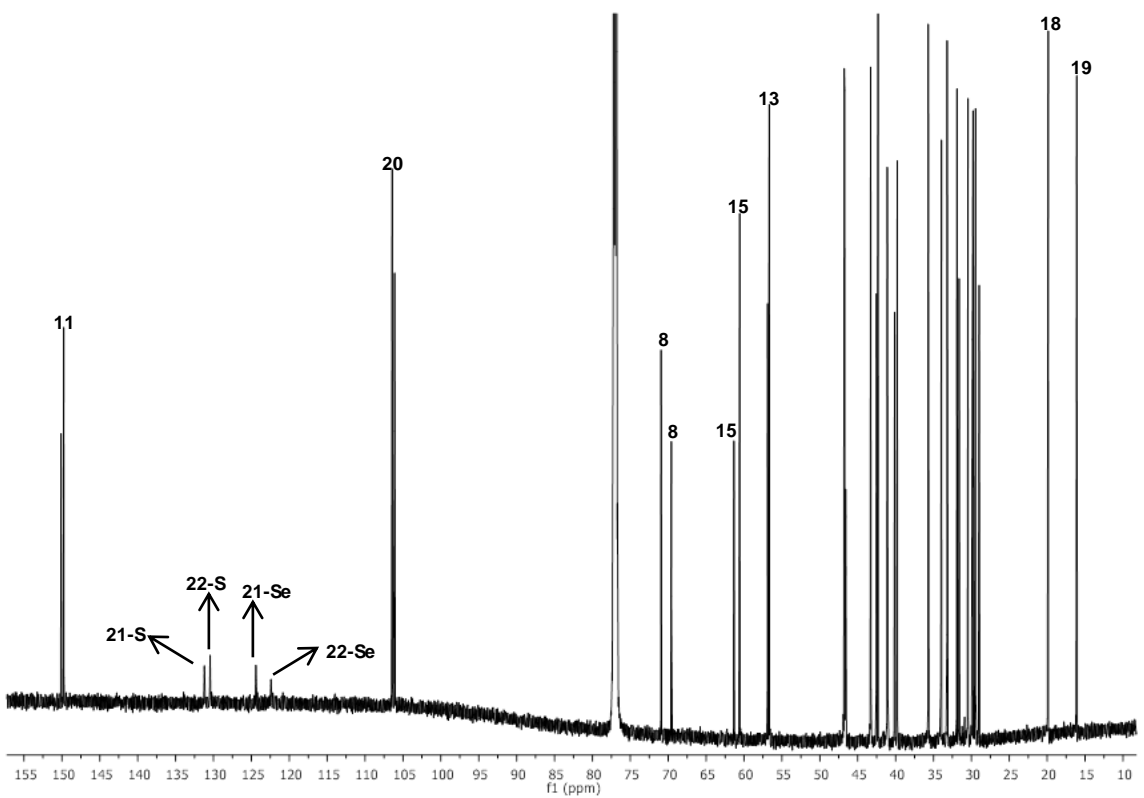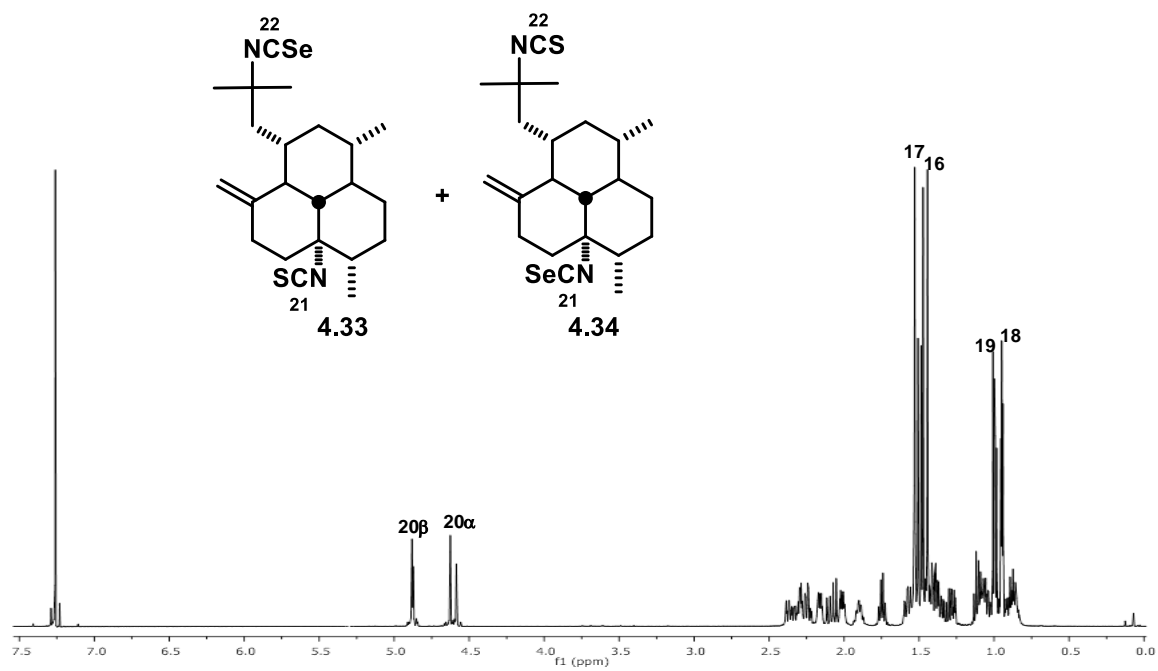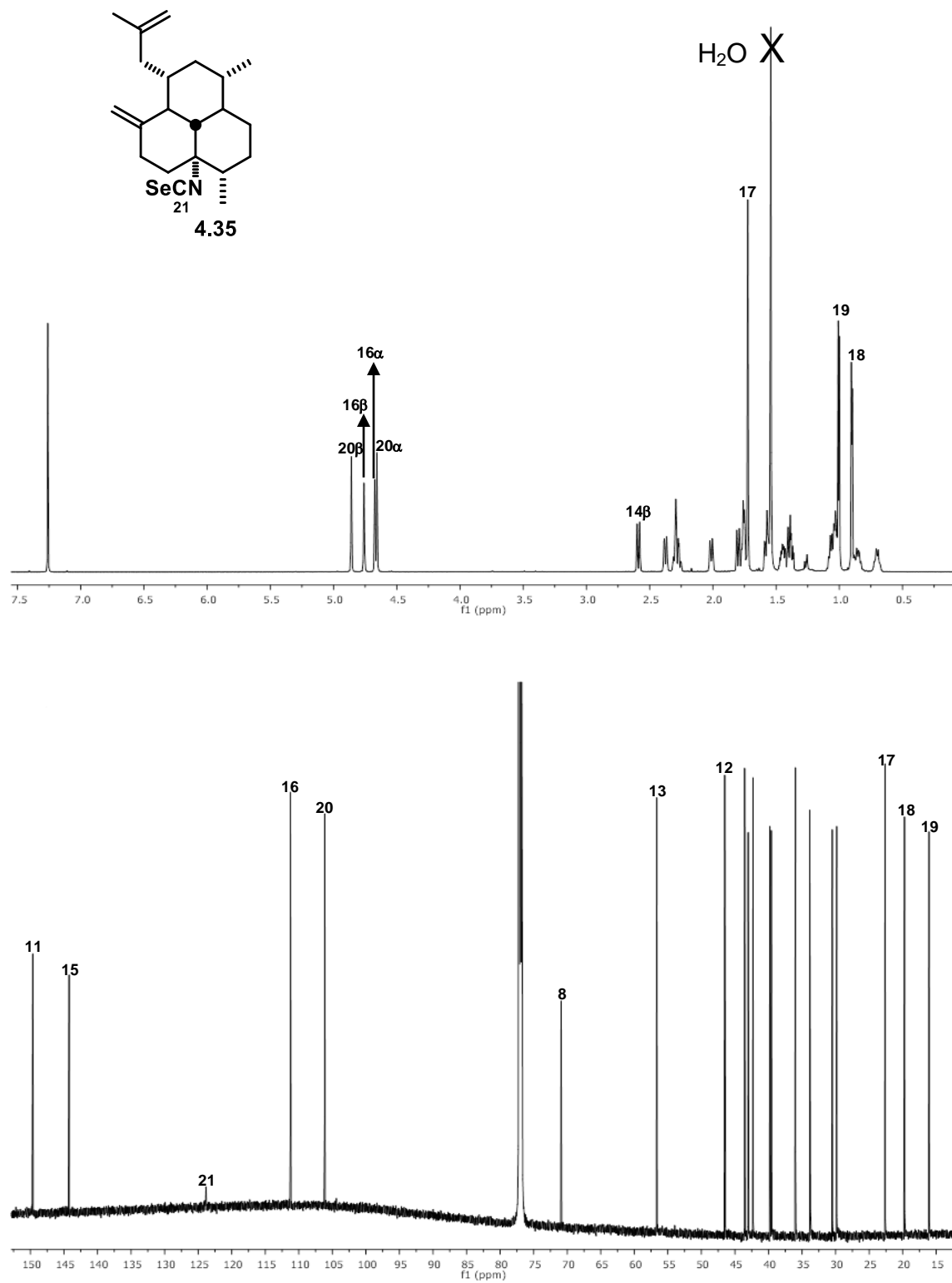
^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$
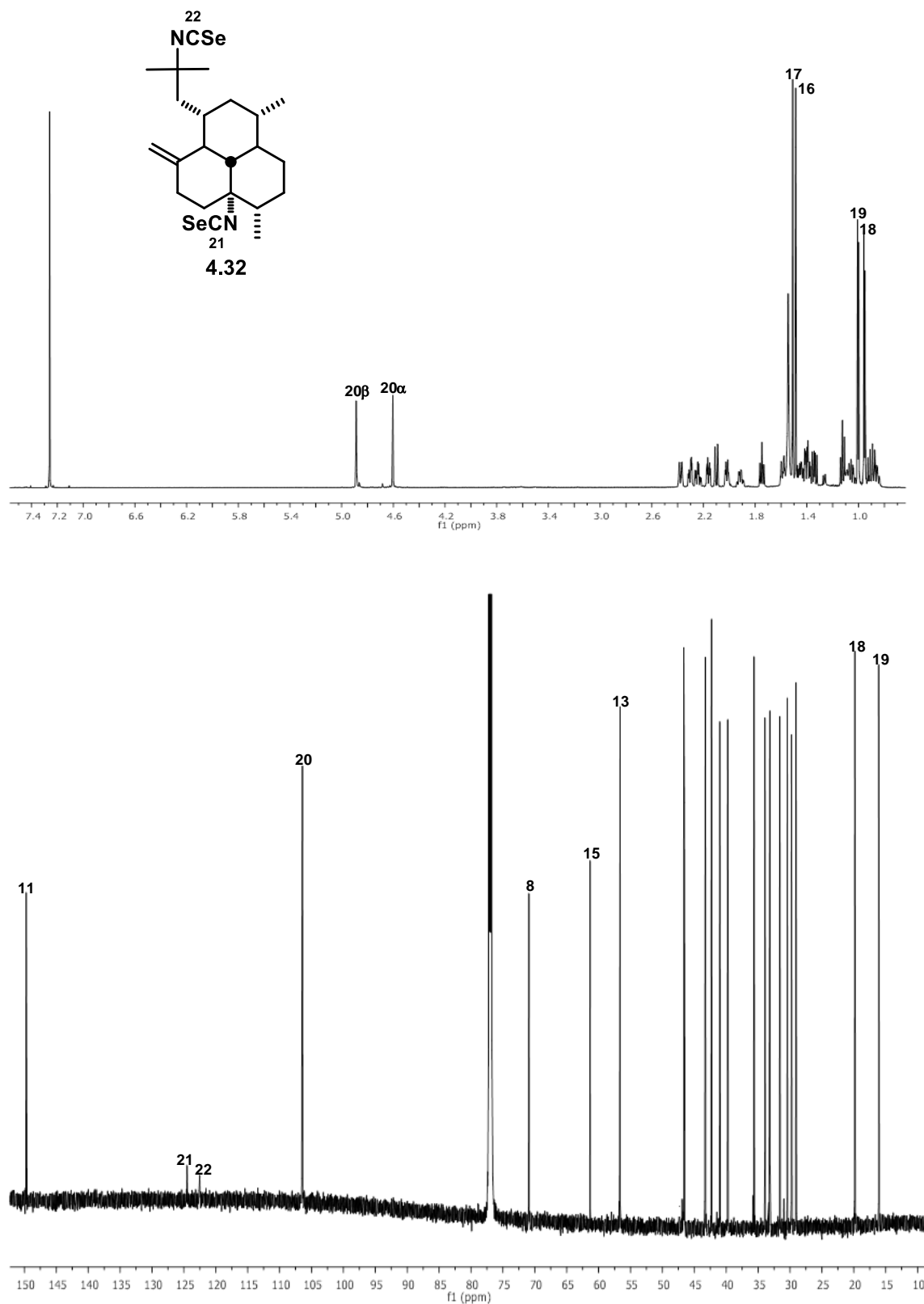
^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) in CDCl$_3$

Elemental composition report: EI-HRMS

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Mass        Calc. Mass    mDa     PPM     DBE     i-FIT     Formula
388.2007    388.2007      0.0     0.0     8.0     134.0     C22  H32  N2  S2 [a]
436.1459    436.1451      0.8     1.8     9.0     9.4       C22  H32  N2  S  80Se [b]
484.0902    484.0896      0.6     1.2     10.0    1116.0    C22  H32  N2  80Se2 [c]
377.1626    377.1622      0.4     1.1     8.0     58.8      C21  H31  N  80Se [d]
```

[a] Compound **4.31**, [b] Compounds **4.33:4.34**, [c] Compound **4.32**, [d] Compound **4.35**

4.9. References

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