Master of Science in Pharmacy

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Synthesis of Novel Oxysterol Based Liver X-Receptor Regulators

45 study points

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Acknowledgements

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Fiona, you are the love of my life. I will always be thankful that I ended up as a pharmacist; I can see no other way I would have met you.

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Aleksander Kristensen

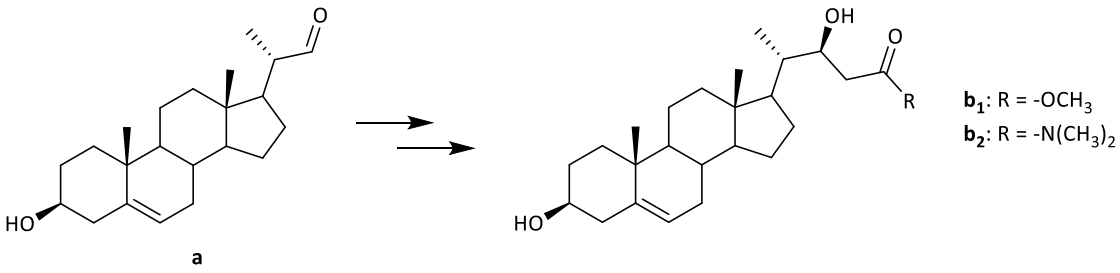
Abstract

The synthesis of cholene derivatives hold many intriguing possibilities for modulation of lipogenesis.[1] Compounds derived from **a** have been widely studied in our group.

Successful synthesis of $\mathbf{b_1}$ and $\mathbf{b_2}$ was achieved in this project through elegant use of Mukaiyama Aldol Addition[2] (**Scheme 1**, compound $\mathbf{b_1}$) and direct alkylation[3] of aldehyde **a** (**Scheme 1,** compound $\mathbf{b_2}$). Structure of compounds $\mathbf{b_1}$ and $\mathbf{b_2}$ was confirmed by X-ray crystal structure.

All intermediates were synthesized successfully and characterized. A new synthetic route was applied for the synthesis of achieving pure compound **a**.

All aims for this thesis were met.



Scheme 1 Graphical overview of thesis

ABBREVIATIONS

^{13}C-NMR	Carbon NMR
^{1}H-NMR	Proton NMR
22(R)-HC	22(R)-hydroxy cholesterol
22(S)-HC	22(S)-hydroxy cholesterol
ACSL1	Long-chain-fatty-acid—CoA ligase 1
aq	Aqueous
Ar-atm	Argon atmosphere
BF_3OEt_2	Boron trifluoride etherate
CD36	Fatty acid translocase (FAT) 1
$CDCl_3$	Deuterated chloroform
DCM	Dichloromethane
DIBAL	Diisobutylaluminium hydride
DMF	Dimethylformamide
DMP	Dess-Martion Periodane
DMSO-d_6	Deuterated DMSO
ee.	Enantiomeric excess
Et_2O	Diethyl ether
FF-MAS	Follicular fluid meiosis-activating sterol
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HOBT•H_2O	1-Hydroxybenzotriazole monohydrate
HR-MS	High Resolution Mass Spectra
$HSiMe_2Ph$	Dimethyl(phenyl)silane
IBX	2-Iodoxybenzoic acid
InI_3	Indium (III) iodide
J	Coupling constant
KOAc	Potasium acetate
LDA	Lithium diisopropyl amide
$LiAlH_4$/LAH	Lithium aluminium hydride

$LiOH \bullet H_2O$	Lithium hydroxide monohydrate
LXR	Liver X-receptor
LXRE	Liver X responsive element
MeOH	Methanol
$MgBrOEt_2$	Magnesiumbromide etherate
$MgSO_4$	Magnesium sulfate
MHz	Megahertz
min	Minute
N_2-atm	Nitrogen atmosphere
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
***n*-BuLi**	*n*-Butyllithium
NH_4Cl	Ammonium chloride
NMR	Nuclear magnetic resonance
NR1H2	Nuclear receptor subfamily 1, group H, member 2
NR1H3	Nuclear receptor subfamily 1, group H, member 3
ppm	Parts per million
rt	Room temperature
RXR	Retionoid X-receptor
sat	Saturated
SCD-1	Stearoyl-CoA desaturase-1
T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerol
TBAF	Tetra-*n*-butylammonium flouride
TBSO	*tert*-Butyldimethylsilyl-ether
TBSOTF	*tert*-Butyldimethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TsCl	Tosylatechloride
δ	Chemical shift

Index of Thesis

1. Introduction

Type 2 diabetes mellitus is a serious and chronic disease[4] which consists of two primary characteristics, insulin resistance in muscle cells and reduced insulin production in the pancreas.[5] Furthermore, a direct link between obesity and type 2 diabetes exists in the form of lipotoxicity[6], which upon progression to T2DM develops into a combined glucolipotoxic state.[7]

The World Health Organization estimates that 347 million people worldwide have diabetes (defined by a fasting glucose level of ≥7.0 mmol/L or taking an antidiabetic medication) and this is expected to increase due to the fact that more than 1.4 billion adults over 20 years of age were overweight or obese in 2008 (defined as having a body mass index or BMI of 25 kg/m^2 and above).[8]

The burdens of these diseases are severe and significant.[9] Continuous exposure to elevated levels of glucose and lipids in diabetic patients leads to several severe comorbidities, such as retinopathy, neuropathy, kidney disease or failure, cardiovascular disease, infection and ulcers in the extremities which can lead to amputation. Obesity causes or leads to other comorbidities such as cardiovascular disease, diabetes, musculoskeletal disorders and some cancers (endometrial, breast and colon).[8]

The rationale for further research into these diseases can be found in the lack of an effective intervention, pharmacological or other.[4, 10] While it should theoretically be possible to treat obesity by lifestyle related alterations[11] (eg. higher energy expenditure and lower energy intake) combined with insulin sensitizers, insulin secretagogues or insulin for diabetics. This has proven to be more complex as a large portion of patients never reach the recommended treatment goals.[4b] The commercial potential of an effective and safe treatment for obesity or diabetes is also huge.[12]

1.1 Oxysterols

Steroids consist of four fused rings (A, B, C and D). The A, B and C are 6-memebered rings while ring D is 5-membered. This basic skeleton is called cholestane. An oxysterols consist of a cholestane skeleton and a 3β-hydroxy-group in C-3. The numbering is as shown in **Figure 1**.[13]

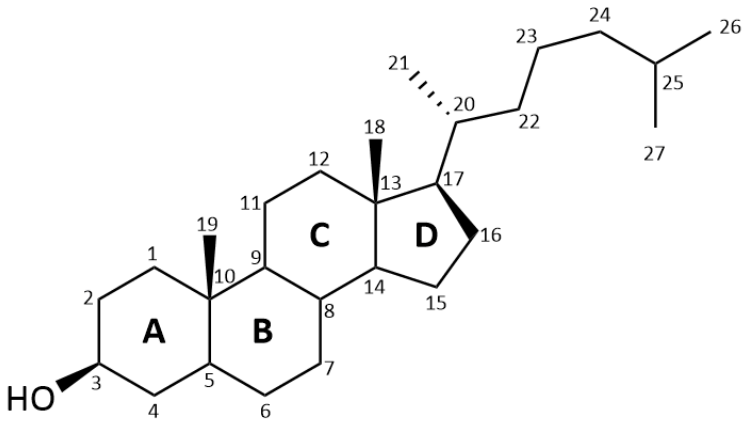


Figure 1. Basic numbering system of oxysterols.

1.2 Liver X receptor and oxysterols

The liver X-receptor belongs to the nuclear receptors superfamily and consist of the two isoforms LXR_{α} and LXR_{β} and is also known as NR1H3 and NR1H2 respectively. LXR is a ligand binding transcription factor that upon activation forms a heterodimer with retinoid X receptor (RXR), translocates to the nucleus and binds to liver X responsive element (LXRE) of the promoter in a target gene.[14] LXR was first found to be expressed in the liver and thereby its name. The isoform found to be expressed in the liver was predominantly LXR_{α} and this isoform has since also been shown to be expressed in adipose tissue, skeletal muscle, macrophages, kidney and small intestine.[14] In contrast, LXR_{β} is expressed ubiquitously.[15]

Janowski *et al*. reported that the oxysterols are the endogenous ligands for LXR.[16] Their studies revealed that for oxysterols (**Figure 2a**) to bind to LXRα, a 3β-hydroxyl moiety in C-3 and an additional hydroxyl group on the cholesterol side chain was required. The strongest LXRα activator found was the naturally occurring 22(R)-hydroxy cholesterol. Initially, 22(S)-hydroxy cholesterol was found to be completely inactive as an agonist, which illustrates the crucial nature of the stereochemistry in oxysterols.[16]

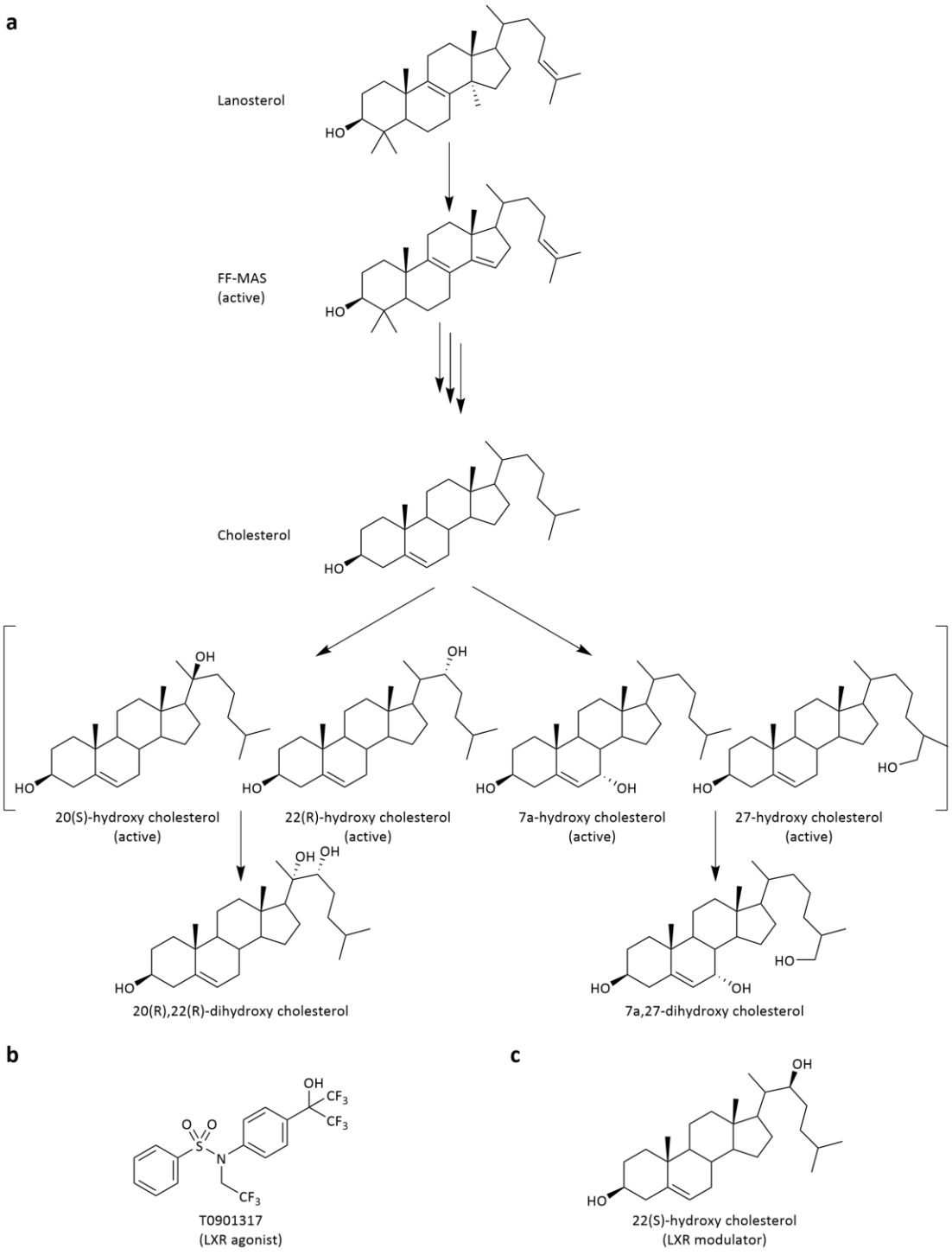


Figure 2. (a) Overview of metabolic pathway from lanosterol to oxysterols. (b) Structure of full LXR agonist T0901317. (c) Structure of 22(S)-hydroxy cholesterol. Adapted from references Janowski et al. 1996 and Shultz et al. 2000.

Kase *et al*. showed that 22(S)-HC is not an inactive ligand, but represses expression of enzymes such as CD36, ACSL1 and SCD-1. 22(S)-HC also represses the effects of the full agonist T0901317 when co-administered in cell cultures and should therefore be considered an antagonist. Their experiments did indicated that 22-HC may regulate lipogenesis through interactions with LXR.[1d]
T0901317 can through activation of LXR, induce a non-alcoholic fatty liver disease.[17] This has been shown to give an increased risk of cardiovascular disease.[18]

1.3 *In vitro* and *in vivo* effects of LXR modulation

To show that also 22(S)-HC is an antagonist in human cells from lean controls, obese and obese diabetics, they expanded upon that study. Kase *et al*. found that 22(S)-HC repressed *de novo* lipogenesis, reduced formation of cholesterol and lipids and in potentiated glucose transport and utilisation in all donor groups. 22(S)-HC also counteracted the effects of T0901317 in these donors. In addition, the study found that 22(R)-HC is a LXR partial agonist in human myotubes.[1c]

To show how 22(S)-HC theoretically docks with both LXR_{α} and LXR_{β}, Hessvik *et al*. conducted a docking experiment with T0901317 compared to 22(S)-HC. The model (**Figure 3 a** and **b**) shows how T0901317 interacts with amino acid residue His421/His435 and Trp443/Trp457, albeit somewhat altered compared to crystal structures. 22(S)-HC, however, is more shielded from interaction with His421/His435 and Trp443/457 and therefore is not an agonist of LXR.[1b]

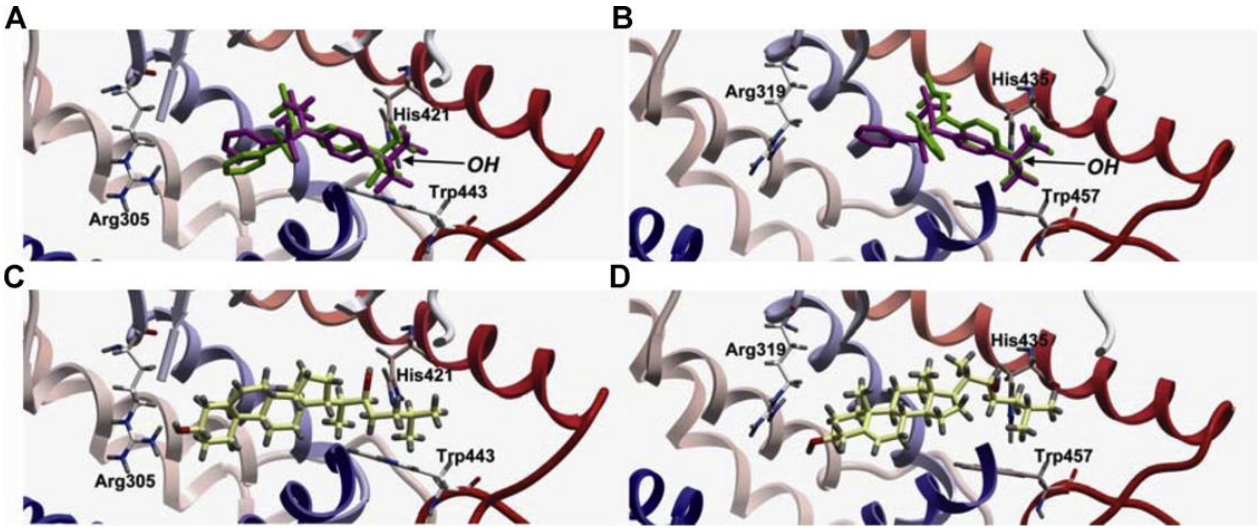


Figure 3. Models of T0901317 (A and B) and 22(S)-HC (C and D) bound to LXRa (A and C) and LXRb (B and D). Green symbols theoretically docked superimposed compound and purple shows co-crystallized compound. Reproduced from Hessvik et al. 2012.

Hessvik *et al*. also found that the effect of 22(S)-HC on lipid formation and glucose utilisation depends on what cell type is examined. In myotubes and hepatocytes, lipogenesis and lipid accumulation is reduced by 22(S)-HC. If this is an effect general to all non-adipose tissue, 22(S)-HC could be a potential pharmacological treatment for T2DM.[1b]

To further study the effects of *in vivo* administration of 22(S)-HC, Kase *et al*. used Male Wistar rats on high fat diet or normal diets with controls administered 30 mg/kg/day 22(S)-HC for three weeks. Regardless of diet, rats treated with 22(S)-HC gained a statistically significant less amount of weight as compared with controls. This was also significant for rats fed a normal diet, but not as early in the protocol. Further effects of 22(S)-HC administration was increased serum TAG and reduced liver TAG for rats fed a high fat diet.[1a]

It is evident that 22(S)-HC has many exiting characteristics. However, one major problem remains. However, 22(S)-HC does not fulfil the criteria for patent of being novel and unpublished. To successfully launch a pharmaceutical product based on 22(S)-HC, patent protection is highly important.

1.4 Retrosynthetic analysis

Considering the structure of 22(S)-HC (**Figure 2c**), a synthetically useful group reveals itself if a functional group interchange is done on 22-hydroxy group. When a disconnection is done between C-22 and C-23, reveals an aldehyde moiety. Aldehydes are useful for a wide range of synthetic pathways[2b, 19] However, to reach compound **5** from a commercially available starting material several more disconnections are needed (**Figure 4**).

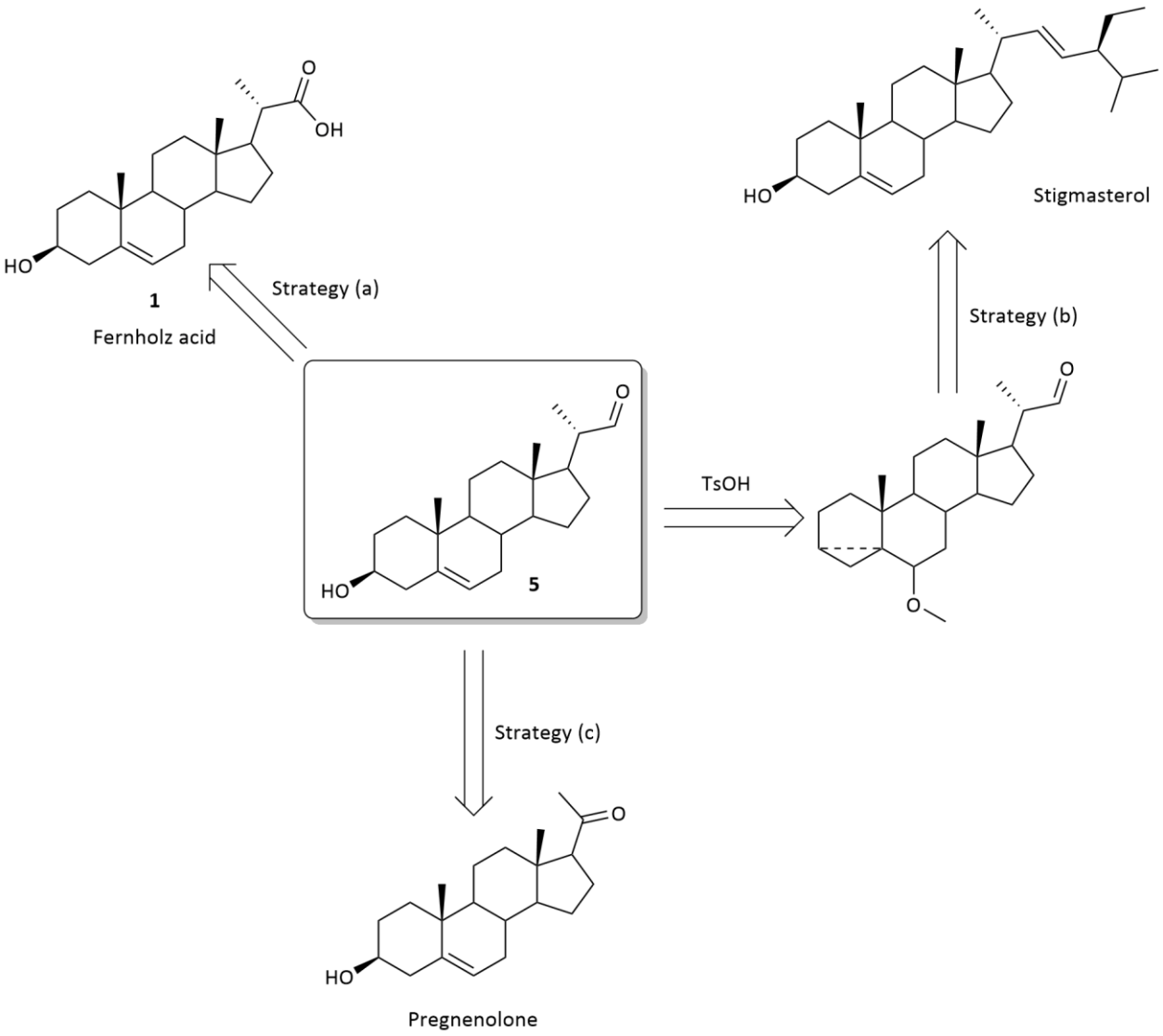
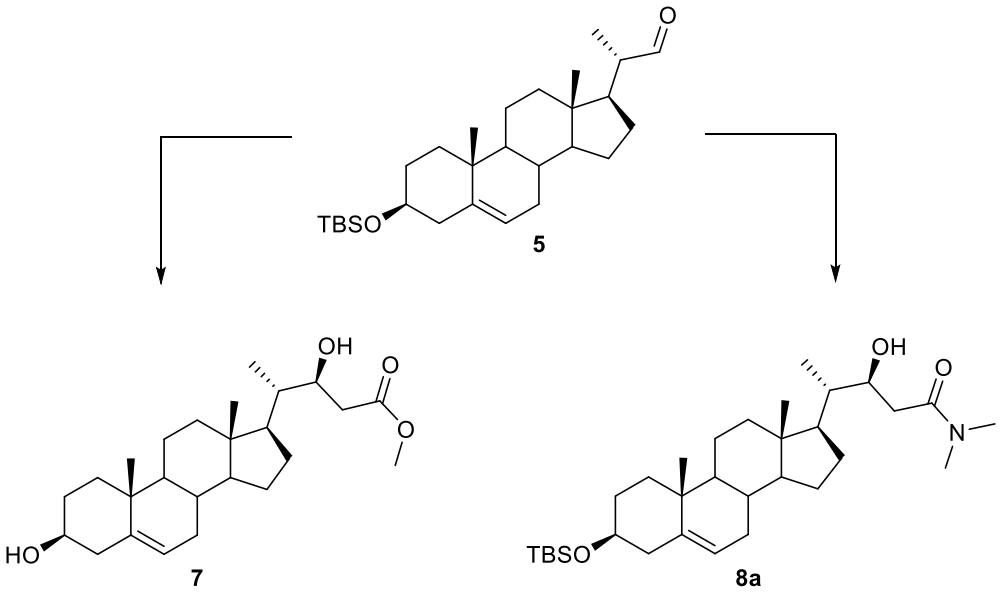


Figure 4. Retrosynthetic analysis of key intermediate 5 with three strategies to achieve the desired configuration from commercially available starting materials. (a) 1. Esterification, 2. 3(S)-OH protection (TBSOTf), 3. LAH or DIBAL, 4. Swern/IBX/DMP, 5. Deprotection. (b) 1. TsCl/pyridine, 2. KOAc/MeOH, 3. O_3 (c) 1. 3(S)-OH protection (TBSOTf), 2. Horner-Wittig, 3. Di-Cy-BH/NaOH/H_2O_2, 4. Deprotection.

1.4 Aim of Thesis

At the beginning, the first target was key intermediate, compound **5** because of its versatility in forming a variety of interesting and potential biologically active substituents.[1d, 16, 19c, 20]



Scheme 2. Target compounds for this study.

After the synthesis of compound **5**, the next targets were compounds **7** and **8a** taking into consideration their stereo selectivity at C-22 and C-3.[1]

2. Results and Discussion

2.1 Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5)

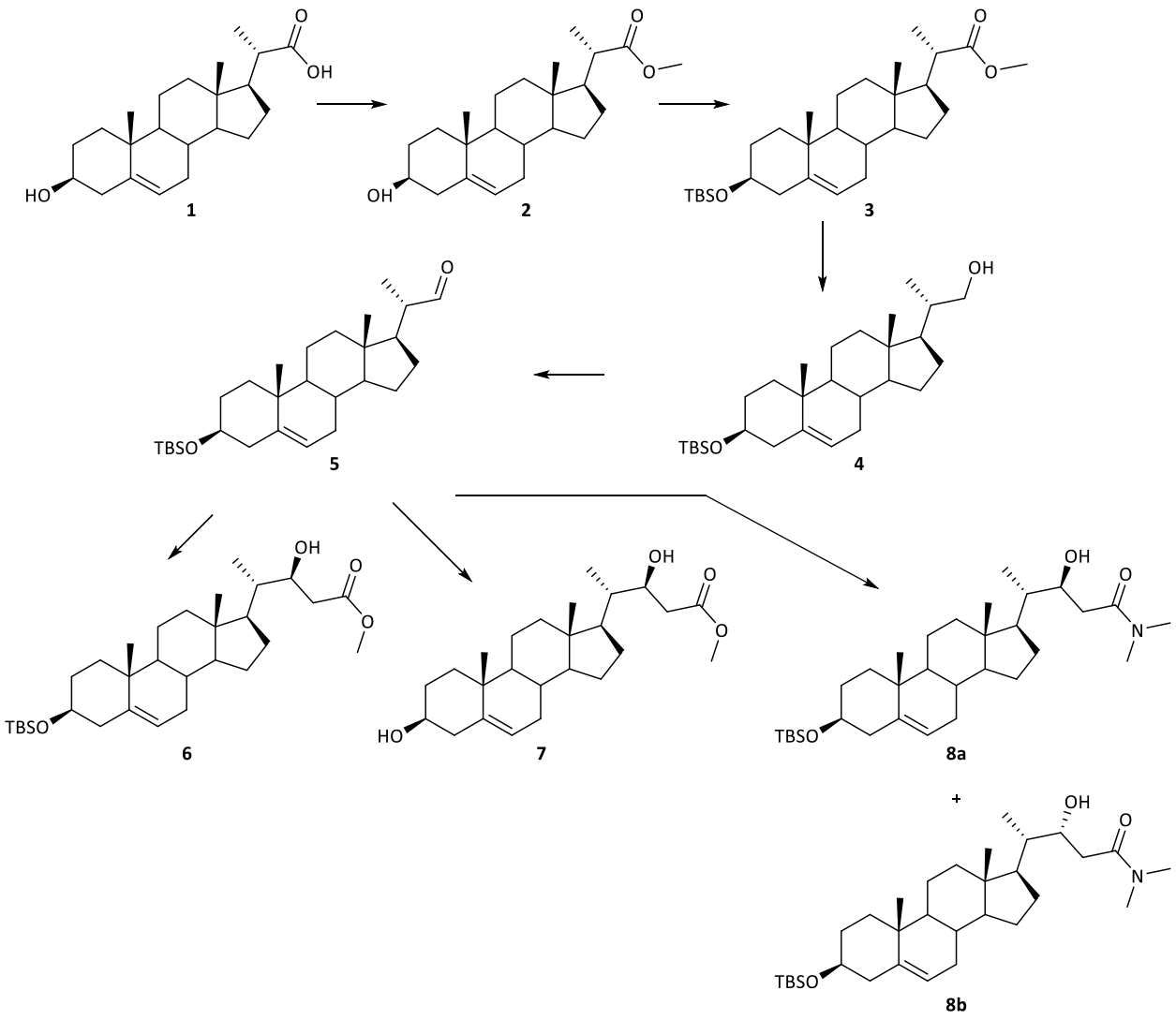
As seen from the overall retro synthetic analysis of key intermediate **5**, three possible strategies were theorized.

A custom synthesis agency, Synthetica AS, attempted to utilize strategy (b) from the retrosynthetic analysis (Figure 4) to reach intermediate **5**, however they were not successful (data not shown).

For this reason we envisioned that strategy (a) would be more successful as this has been reported previously.[19c, 20]

There is also literature that supports the synthesis of compound **1** to **4** as a one-pot reaction[21], but as Visbal *et al.* reports that that strategy has been difficult to adapt for the synthesis of compound **5.**[19c]

For these reasons and because of the availability of compound **1** as a pure enantiomer, strategy (a) was chosen (**Scheme 3**).

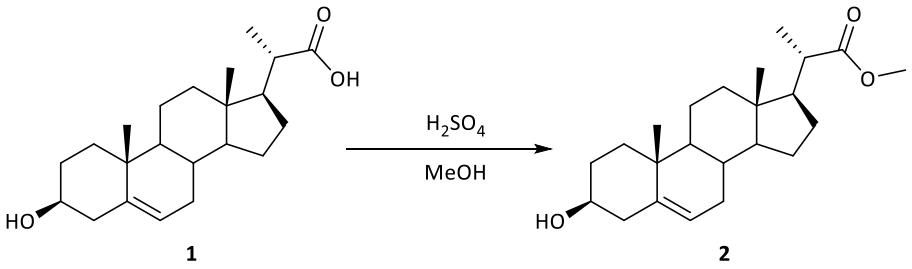


Scheme 3. Overview of strategy (a).

This section discusses the reactions in chronological order starting from the synthesis of compound **2** from compound **1**.

2.1.1 Synthesis of 23,24-bisnorchol-5-ene-3-ol-22-methyloate (2)

23,24-bisnorchol-5-ene-3-ol-22-methyloate **2** was synthesized from 23,24-bisnorchol-5-eneic acid **1**, also known as Fernholz acid[22], according to procedure published by Guerlet *et al.*[20], see **Scheme 4**. This procedure is shown in section **4.2.1** and yields and reaction conditions are reported in **Table 1**.



Scheme 4. Reaction conditions for synthesis of 23,24-bisnorchol-5-ene-22-methyloate (2)

Table 1 Reaction conditions and yields for synthesis of 23,24-bisnorchol-5-ene-22-methyloate (2)

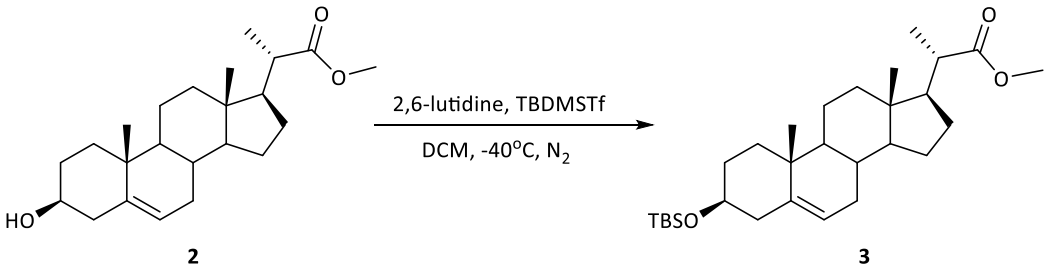
Entry	Reaction time	Catalyst (eq.)	Yields (%)
1	17.5 h	0.8	38.5
2	17.5 h	1.0	82.4
3	18.5 h	1.0	73.9

The synthesis of compound **2** has been reported by Guerlet *et al.* by starting from Fernholz acid (**1**) using sulphuric acid in methanol under reflux overnight. [20]

We first attempted this reaction with minimal amount of acid catalyst (**Table 1**, Entry 1). This gave a low yield of 38.5%. Increasing the amount of acid catalyst improved the reaction yield (**Table 1**, Entry 2). Increasing the reaction time however reduced the yield (**Table 1**, Entry 3). This might be caused by the reaction equilibrium, converting the desired compound back to its starting material **1** by ester-hydrolysis. This change is not easily observed on TLC as compound **1** was highly insoluble in organic solvents.

2.1.2 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-methyloate (3)

The protection of the hydroxy- group in C-3 on the steroid skeleton was essential to facilitate the selective oxidation of hydroxyl group in C-22 (**Scheme 7**). We employed the method previously reported by Guillame Guerlet *et al*.[20]



2 **3**

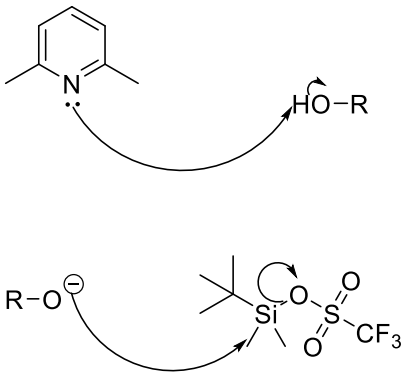
Scheme 5. Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-methyloate (3)

Table 2. Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-methyloate (3)

Entry	Reaction time	Reactant (eq.)	Catalyst (eq.)	Yield %
1	1h	1.2	1.9	80.9
2	1h	1.2	1.9	81.4
3	1h	1,3	2.0	93.5

The synthesis of compound **3** was attempted twice utilizing the same reaction conditions as Guerlet *et al*. This gave a good yield of 81% (**Table 2**, Entry 1 and Entry 2). However quantitative yield as Guerlet *et al*. reported was not observed. A better temperature control could have yielded the same results as reported by Guerlet *et al*., but due to a limitation of cooling equipment, a slight increment in reagents were attempted instead (**Table 2**, Entry 3).[20]

Increasing the amount of reagents improved the yield, because residual water in the reaction mixture might have partially quenched the intermediate anion of the alcohol (**Scheme 6**).

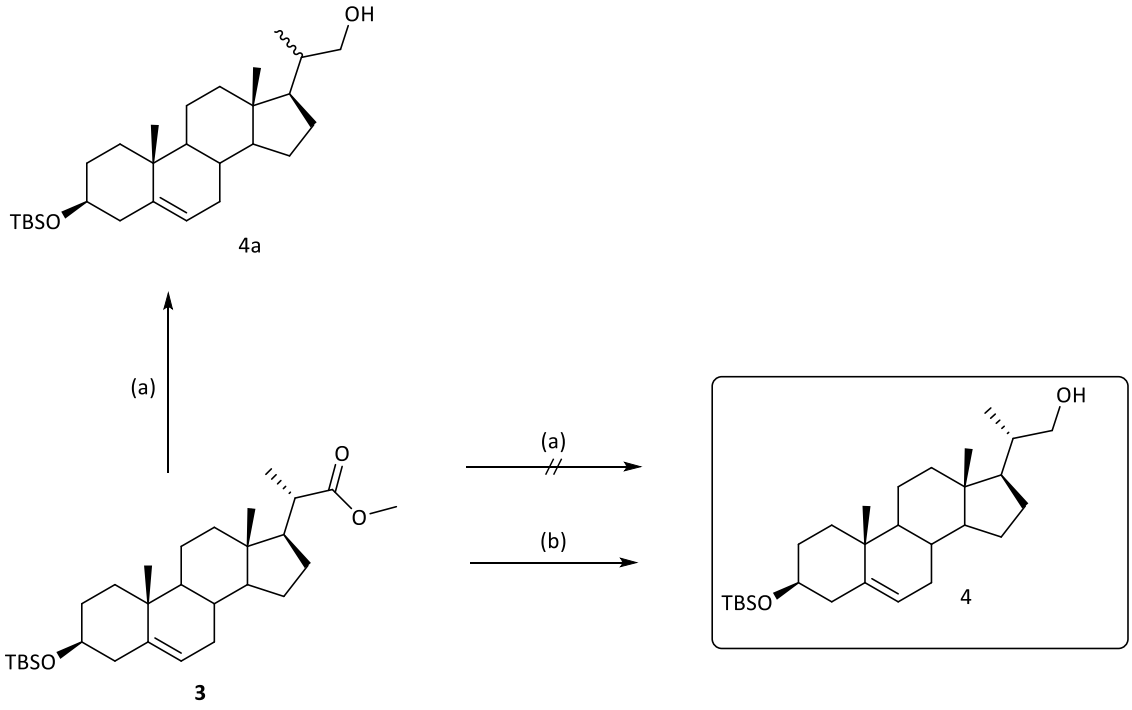


Scheme 6. Reaction mechanism of TBDMS-O-R ether protection by TDMSTf. If water is present in the reaction the intermediate R-O$^-$ would be quenched before it can react with TDMSTf.

Increasing the reagents causes the reaction to proceed favourably toward compound **3**. This attempt was proven useful and gave a excellent yield of 94%. At this point, we were satisfied and no further modification was done on this reaction (**Scheme 5**).

2.1.3 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-ol (4)

Two different routes were employed for the synthesis of compound **4** (**Scheme 7**).

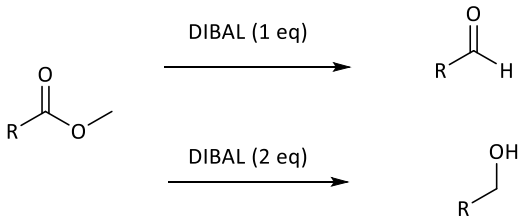


Scheme 7. Synthetic routes toward the synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-ol (4) Reagents and conditions: (a) $LiAlH_4$ (2.5 eq),Et_2O, H_2O, 2h, r.t., Ar-atm (b) 1)DIBAL, DCM, -78˚C, N_2-atm 2) Na-K-tartrate

Route I

First, reaction (a) was attempted according to Guillaume Guerlet *et al.* to synthesize compound **4** from compound **3**. (**Scheme 7**, reaction (a)). This reaction condition was attempted to synthesize compound **4.** Full conversion was observed by TLC in 2h, but an unwanted mixture of diastereomers **4** and **4a** was formed instead of quantitative yield of pure compound **4**. This formation of mixtures was unknown until later in the series because its ^{1}H-NMR region of 1-2ppm was complicated. When this problem was known, route II was employed as the new strategy (**Scheme 7**, reaction (b)).

Route II



Scheme 8. Reduction procedures for ester moiety.[23]

DIBAL for reduction of the ester moiety is widely reported.[23] When DIBAL was employed in excess to compound **3**, this gave us the alcohol (compound **4**) preserving the stereochemistry in C-20. (**Scheme 7**, reaction (b)).

Table 3. Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-ol (4) using DIBAL

Entry	Reaction time	Reactant (eq.)	Yield (%)
1	3h	4.6	66.1[a]
2	1h	4.0	94.1[b]

[a] yield after flash chromatography purification

[b] crude product yield

Employing DIBAL to compound **3** gave a moderate yield after purification (**Table 3**, Entry 1). The reaction was repeated and crude product NMR was pure, so purification was not necessary. This gave an excellent yield of 94% (**Table 3**, Entry 2)

Comparing route I and route II

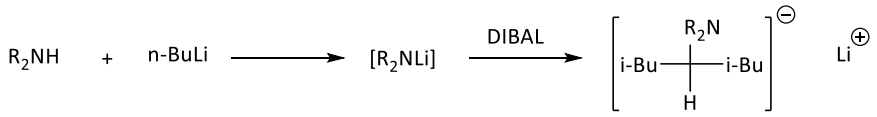
Analysis by ^{1}H NMR and ^{13}C NMR showed no splitting of carbonyl C-22 and this indicates that DIBAL reduction followed by oxidation may be a better option than the $LiAlH_4$ reduction with Feiser's workup.

Possible Improvements

DIBAL

DIBAL is known as a powerful and convenient way of reducing esters to aldehydes or alcohols.[23] In retrospect, this reaction should have been performed with 1 eq. of DIBAL (**Scheme 7**) and longer reaction time to allow full conversion to potentially compound **5**.

LDBPA



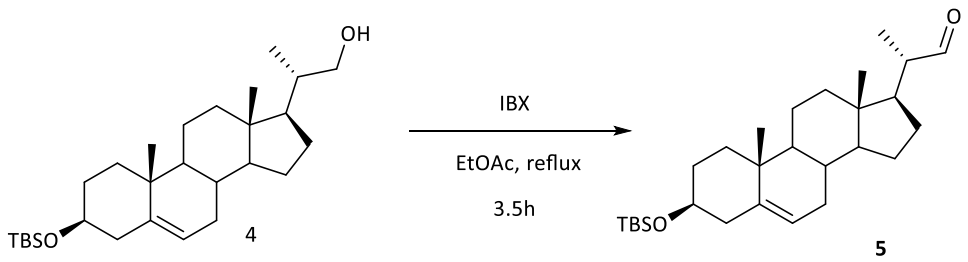
Scheme 9. Formation of LDBPA when R is piperidine.

There are also other reduction agents with the potential to convert an ester moiety to aldehyde. One such is LDBPA made from piperidine, *n*-BuLi and DIBAL (**Scheme 9**). Ahn *et al.* reported successful reduction of esters to aldehyde with LDBPA, where DIBAL reduced esters to the alcohols. As this could eliminate one step of the reaction pathway and improve the overall yield while being able to do the reaction at rt.; this warrants further investigation. [24]

2.1.4 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5)

Two strategies where employed for the synthesis of compound **5** (**Scheme 10** and **Scheme 12**).

Strategy I



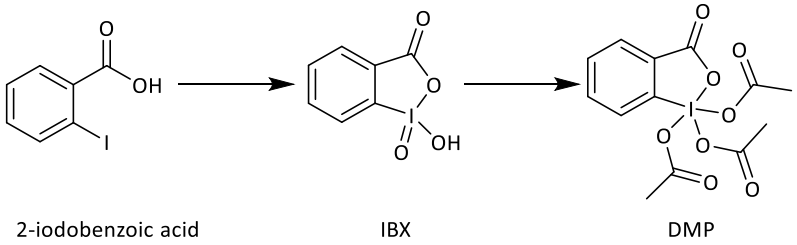
Scheme 10. Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5) by IBX oxidation

Guerlet *et al.* reaction conditions were employed, no purifications were required (**Scheme 10)**. This gave quantitative yield (**Table 4**).

Table 4. Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5) by IBX oxidation

Entry	Reaction time	IBX (eq.)	Yield (%)
1	3.5h	3.2	99.9

Dess-Martin Priodinane

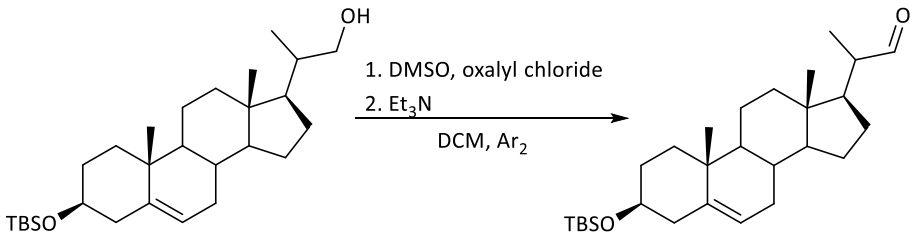


Scheme 11. Formation of DMP from 2-iodobenzoic acid via IBX. [25]

On a high scale, utilizing the Dess-Martin Periodinane (DMP) can be a practical solution. The main advantage of DMP is its high solubility in organic solvents such as DCM. Milder conditions are employed instead of reflux; this can be useful for more sensitive compounds.

Strategy II

Guerlet *et al*. reported that Swern' conditions had been successfully utilised to synthesis aldehyde **5** from primary alcohol **4**.[20] A standard procedure for Swern' oxidation [26] (Scheme 12) was employed instead because no supporting information on this synthesis was provided in the Guerlet et al. report.



4a **5a**

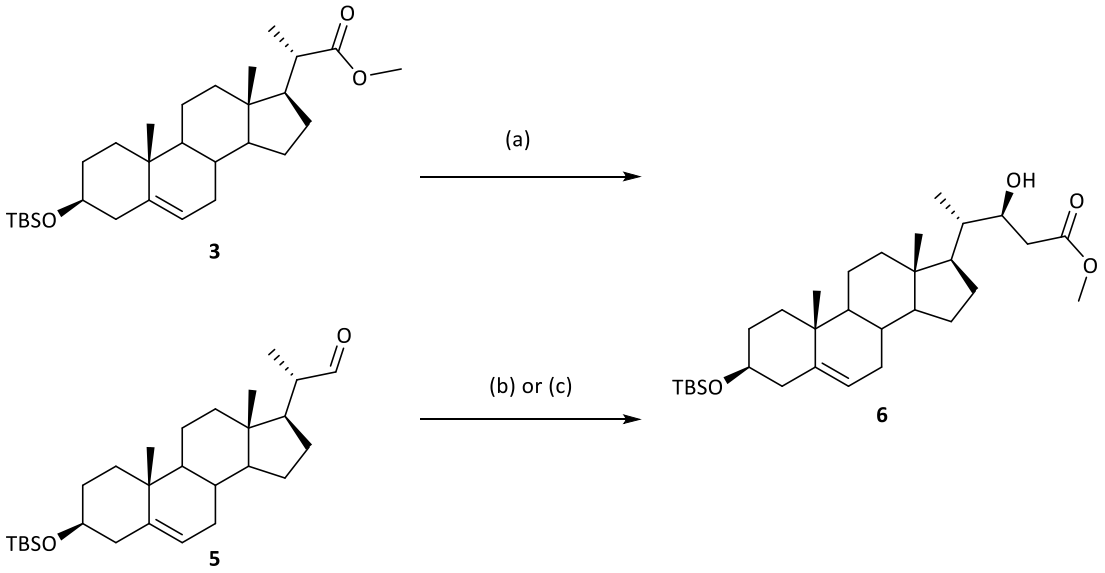
Scheme 12. Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5a) using Swern conditions

It was unknown that compound **4a** was actually a mixture until after the Swern oxidation reaction. The ^{1}H-NMR spectra of its product mixture **5a** brought this problem to light. The mixture was of 20R and 20S

in a ratio of 1 : 4.6. A recrystallization attempt was made, but its ratio only changed to a meagre 20R and 20S in the ratio of 1 : 5.3. Since Route I was fruitful, no further investigation was done on the mixture.

2.1.5 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-chol-5-ene-22(S)-ol-24-methyloate (6) and chol-5-ene-3(S),22(S)-diol-24-methyloate (7)

Three different routes for the synthesis of compound **6** were attempted (**Scheme 13**).

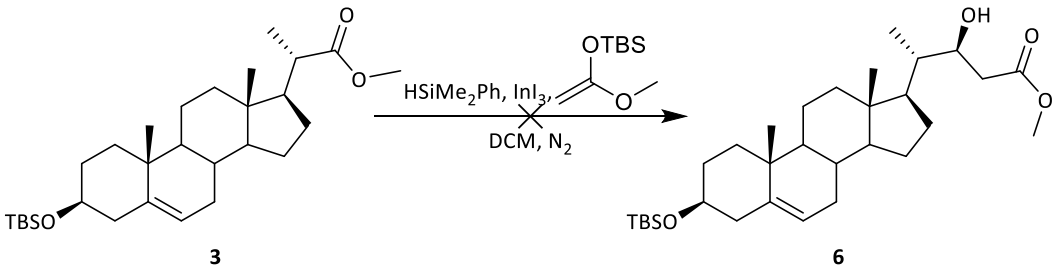


Scheme 13. Reaction conditions: (a) direct Mukaiyama Type Aldol reaction, Strategy I. (b) LDA, EtOAc, Strategy II. (c) One pot Mukaiyama aldol reaction, Strategy III

Strategy I

The most attractive strategy found at first sight was for direct Mukaiyama aldol reactions to an ester using silylenolates catalysed by InI_3.[27]

Inamoto *et al.* published their work in 2012,[27] and we attempted to reproduce these results using compound **3** as starting material. Our procedure for this reaction can be found in **section 4.2.5**.



3 6

Scheme 14. Attempted synthesis of chol-5-ene-3(S),22(S)-diol-24-methyloate using direct Mukaiyama Type Aldol reaction.[27]

No conversion was observed after 3h or overnight. There are many possible explanations for this lack of success and a key issue is here brought to attention.

The steroid skeleton structure leads to a severe steric hindered configuration. This potentially influences the transition states of the reaction, especially when InI_3 coordinates to the methoxy function of the ester moiety in compound **3** (**Figure 5a**) according to Inamoto *et al.* Adapting their suggestion of reaction mechanism to steroids, the InI_3 must completely occupy the hydrophobic pocket of the steroid skeleton to achieve this (**Figure 5b**).[27]

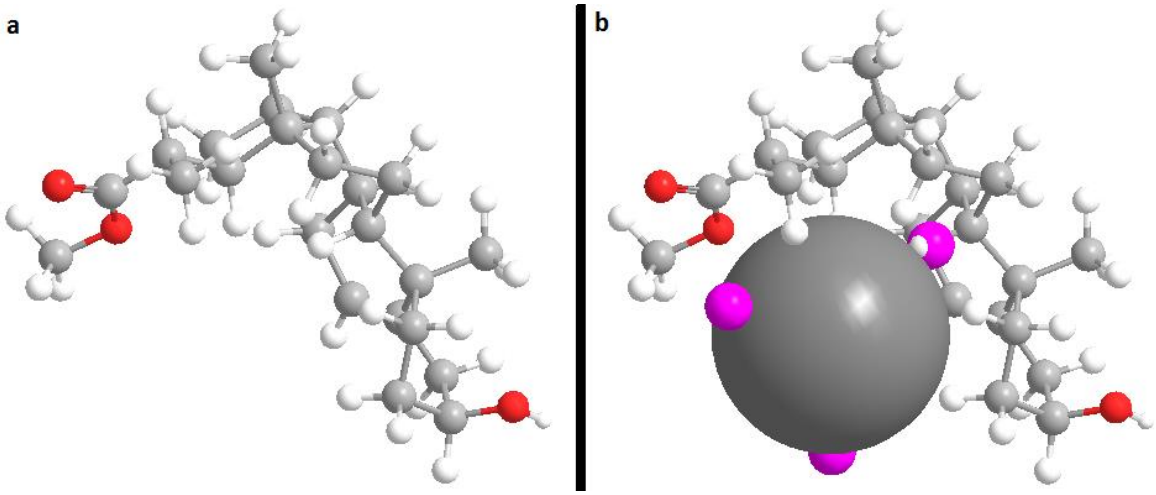


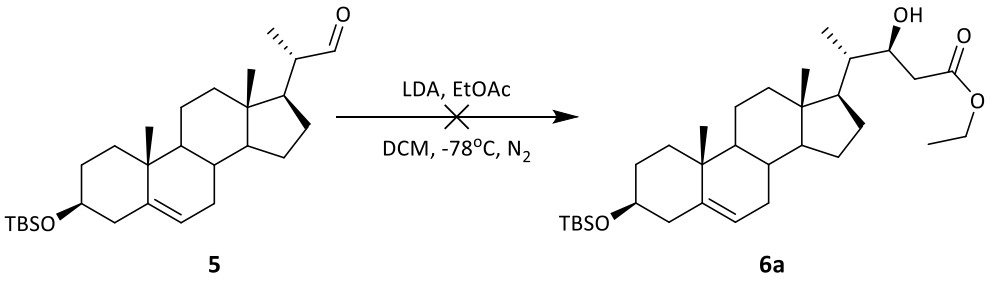
Figure 5. Three dimensional structure of (a) 23,24-bisnorchol-5-ene-22-methyloate and (b) 23,24-bisnorchol-5-ene-22-methyloate with space-filling model of InI3.

While InI_3 exist in several complexes none are less bulky than InI_3.[28] The model above illustrates the three dimensional structure of compound **3**. The sterically hindered nature of the cholesterol skeleton is a possible reason for the lack of reactivity of this type of reaction. Although this was out of scope for this

thesis; the nature of this reaction to form target molecule **6** is elegant, so further investigation and Lewis acid screening may be warranted.

Strategy II

The aldol reaction is a well-known and powerful way of forming C-C bonds from two carbonyl compounds which yields 1,3β-hydroxy carbonyl compound.[19b] This theoretically aligns very well with formation of target molecule **6** (**Scheme 15**).



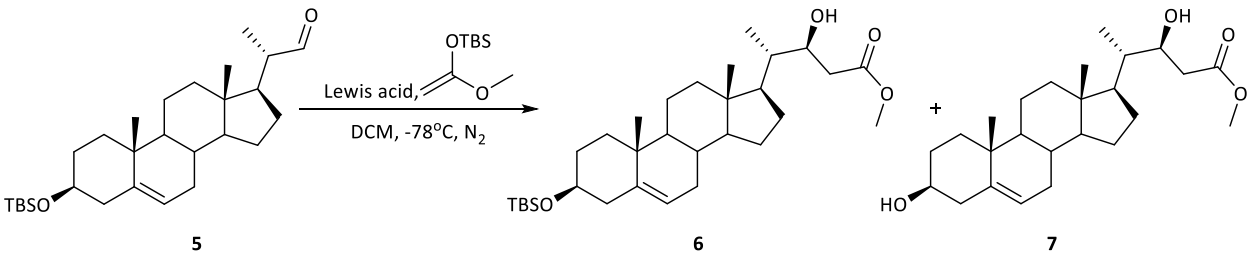
5 **6a**

Scheme 15. Attempted synthesis of chol-5-ene-3(S),22(S)-diol-24-methyloate (6a) using aldol type reaction.

No formation of compound **6a** was detected by TLC. A possible explanation for this is the quality of reactant used. Dry ethyl acetate was not available at the time. Though a newly opened bottle was used, small amounts of H_2O in the ethyl acetate probably quenched the lithium diisopropylamide. This would have prevented deprotonation of the ethyl acetate and the potential subsequent reaction to the aldehyde moiety. Due to the availability of a third strategy, this strategy was not pursued.

Strategy III

While the classic aldol reaction was not profitable, another possibility to synthesize the aldol product **6** from aldehyde **5** is the Mukaiyama aldol reaction discovered by Teruaki Mukaiyama and published in 1973.[2b] The reaction of an aldehyde with silyl enol ether in presence of a lewis acid would facilitate the formation of target molecule **6** (**Scheme 16**) and stereo selectivity has been reported to be excellent.[19d-g]



5 6 7

Scheme 16. Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-chol-5-ene-22(S)-ol-24-methyloate (6) and chol-5-ene-3(S),22(S)-diol-24-methyloate (7) using Mukaiyama Type Aldol addition catalysed by a lewis acid.

Three different Lewis Acids of varying strength were screened. The weakest one was attempted first in order to be conservative (**Table 5**).

Table 5. Lewis acid screening for synthesis of compound 6.[2-3, 19e-g]

Entry	Solvent	Lewis acid	Eq.	Reaction time	Temp	Yield 6 (%)	Yield 7 (%)
1	DCM	BF_3OEt_2	1.34	3h	-78°C	0	0
2	Toluene	$MgBrOEt_2$	3.34	35m	-15°C	12.7	0
3	DCM	$TiCl_4$	1.14	3h 45m	-78°C	7.9	1.1
4	DCM	$TiCl_4$	1.05	1h 10m	-78°C to rt.	7.3	6.8

Increasing strength of Lewis Acids

First, BF_3OEt_2 was attempted.[19e-g] After 3 hours, no conversion was observed but a full recovery of starting material. This could be due to the over-bulky nature of BF_3OEt_2 that do not coordinate to the aldehyde starting material (**Table 5**, Entry 1).

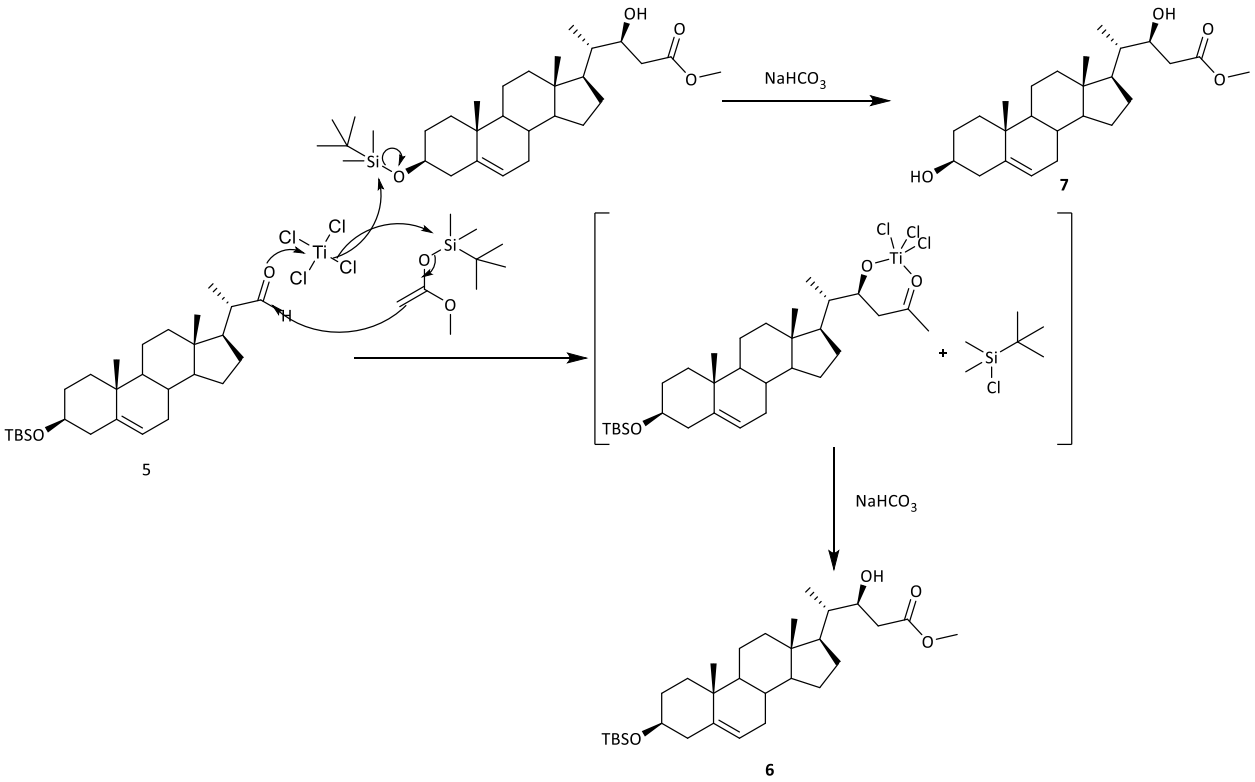
Second, an attempt using $MgBrOEt_2$[2a] showed full conversion after 35 minutes on TLC. The low yield motivated us to continue screening for another stronger Lewis acid (**Table 5**, Entry 2).

Third, $TiCl_4$ was employed as the Lewis acid.[2b, 3, 29] After almost 4 hours, no more starting material was observed on TLC, so the reaction mixture was quenched, worked up and purified by flash chromatography. This yielded 7.9 % of compound **6** and a small amount of compound **7** (**Table 5**, Entry 3).

Since the reaction using $TiCl_4$ as the Lewis acid yielded not only compound **6**, but also one of the main project targets, novel compound **7**, $TiCl_4$ was interesting and further investigated. This time a different temperature was studied. The reaction mixture was stirred at -78˚C to allow coordination of the $TiCl_4$ to

the starting material, compound **5**. Gradually increasing the reaction temperature to rt, was logical to hasten the reaction rate. After 1 hour of stirring at rt, no more starting material was observed, so the reaction was quenched and its crude product was purified by flash chromatography, yielding 7.3% of compound **6** and an immense improvement of yield in compound **7** at 6.8% (**Table 5**, Entry 4).

We suggest the following mechanism of action for the dual reactivity of entry 3 and 4 (**Table 5**).

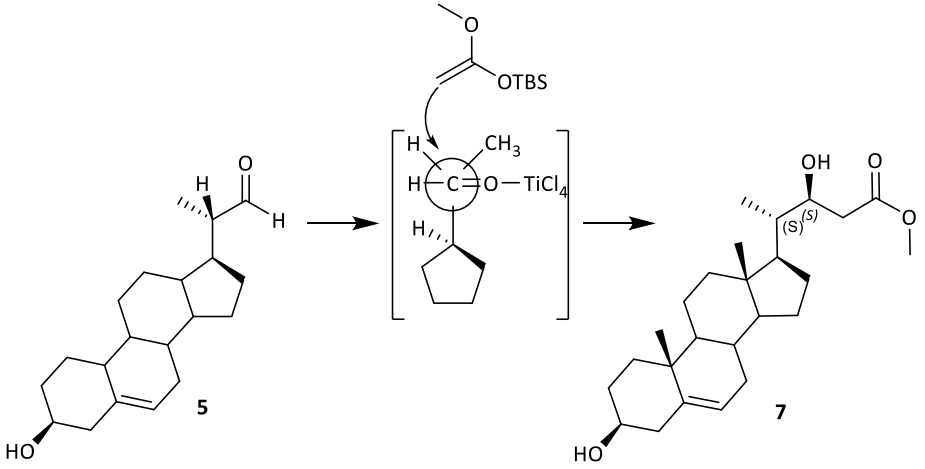


Scheme 17. Proposed mechanism of action. Compound 5 is activated by $TiCl_4$.[29b] This causes the silyl enol ether to react to position C-22. Denmark *et al*. and Reetz *et al*. have shown by NMR that a Cl-Si-R moeity is subsequently formed.[29b-29a]

The reaction of compound **5** most likely proceeds to an intermediary state (**Scheme 17**).[29b] Complexes suchs as R-CHO•$TiCl_4$ and would facilitate Cl-Si-R moieties as leaving groups for $TiCl_4$ catalysed Mukaiyama aldol additions.[29a] This would also explain how the reaction proceeds to de-protect the silyl ether function in C-3 of compound **6**, by forming free chloride ions that reacts with the silyl ether function.[30]

Another interesting aspect of this reaction is the excellent diastereomeric control exerted. We were unable to detect the 22(R)-hydroxy isomer. Evans and co-workers have several publications on this subject.[19d-g] The steric interference of substituents in α1,2 and β1,3 positions to the aldehyde, will

control the reaction to either 20(S),22(S) or 20(S),22(R) also known as Felkin or anti-Felkin control.[31] This control is also possible to reverse by introducing EWG in the β position.[29]



Scheme 18. Transition state of compound 5 to 7 based on Felkin model of torsional strain.[31] Transition state indicates aldehyde moiety with α1,2 and β1,3 substituent.

The formation of compound **7** follows traditional Felkin control of stereochemistry (**Scheme 18**) .[31] This is because of steric hindrance from the D-ring and C-21 methyl group of the cholene skeleton.

For X-ray crystals to confirm absolute stereochemistry of a new stereo centre (e.g. 22(S)-hydroxy of compound **7)** an existing stereo centre has to be present. For compound **7**, this is the 3(S)-hydroxy moiety. Since compound **7** has no acid protons in C-2 and C-4 position, racemisation in C-3 is highly unlikely as TBDMS-O- is a poor leaving group for SN2-inversion to occur. Therefore, the X-ray crystal structure of compound **6** (**Figure 6**) confirms 22(S) stereochemistry of compound **7**.

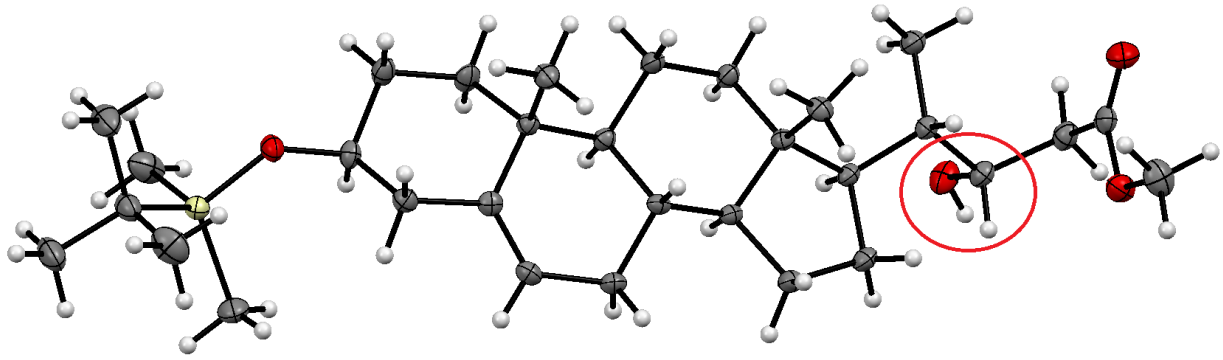


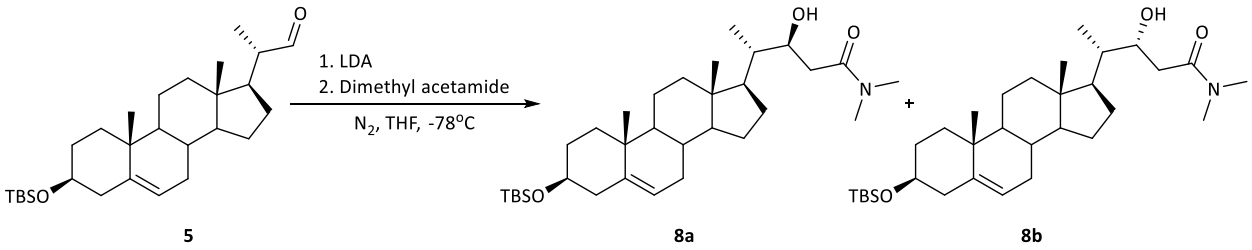
Figure 6. Crystal structure of 3β-(tert-butyldimethylsily)-oxy-chol-5-ene-22(S)-ol-24-methyloate (7) obtained from EtOAc. Highlighted area (red circle) indicates 22(S)-hydroxy group. Oxygen atoms coloured red and silicon is yellow.

Further optimizations should be conducted to selectively yield a 22(S)- or 22(R)-hydroxy group and ideally also control of the de-protection.

2.1.6 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-*N,N*-dimethyl amide (8a)

Synthesis of 1,3-hydroxy amides from aldehydes can be completed by treating dimethyl acetamide with LDA and subsequent addition to the respective aldehyde.[19a]

Using this strategy shown in **Scheme 19**, successful synthesis of target molecule **8a** was achieved with the reaction condition and yield (**Table 6**).



Scheme 19. Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-N,N-dimethyl amide (8a) using LDA and dimethyl acetamide

Table 6 Synthesis of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-N,N-dimethyl amide (8a) using LDA and dimethyl acetamide

Experiment	Reactant (eq.)	Temp	ee. (8a:8b)	Yield (%)
1	1.0	-78°C	68:32[a]	73.0

[a] Calculated from ^{1}H-NMR

Purification by flash chromatography was insufficient to separate the isomers of compound **8a** and **8b**. Its ee. was reported in the ratio of 68 : 32. We were thrilled that the recrystallization of compound **8a** from the mixture was successful. Recrystallization worked excellently and yielded needle like crystals from EtOAc (**Figure 7**).

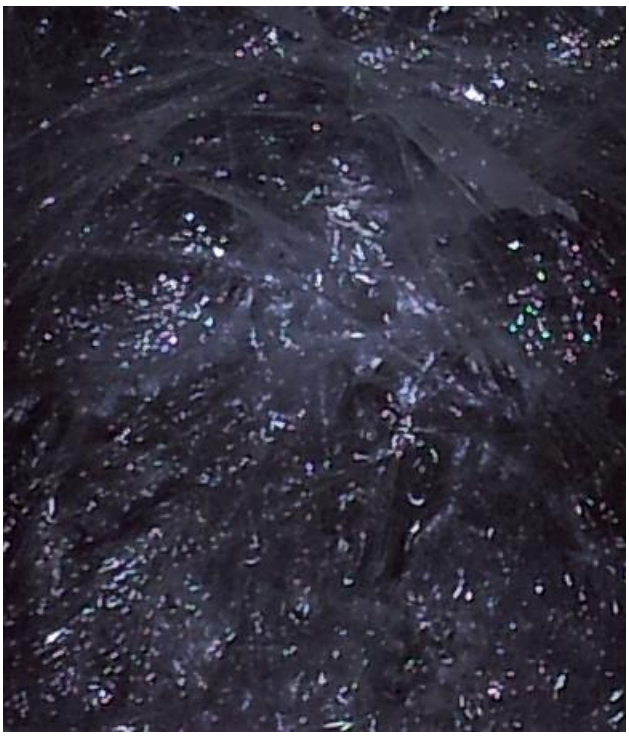


Figure 7. Compound 8a recrystallized from EtOAc in the form of needles.

The X-ray crystal structure obtained from these crystals (Figure 8) shows that the compound synthesized is indeed **8a**.

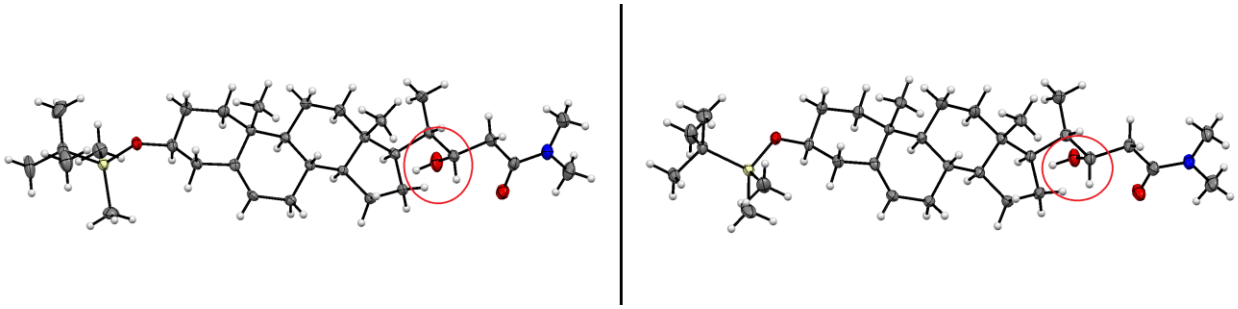


Figure 8. Crystal structure in different orientations of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-N,N-dimethyl amide (8a) obtained from EtOAc. Highlighted area (red circle) indicates the 22(S) hydroxy group. Nitrogen atoms are in blue, oxygen are atoms in red and silicon atoms are yellow.

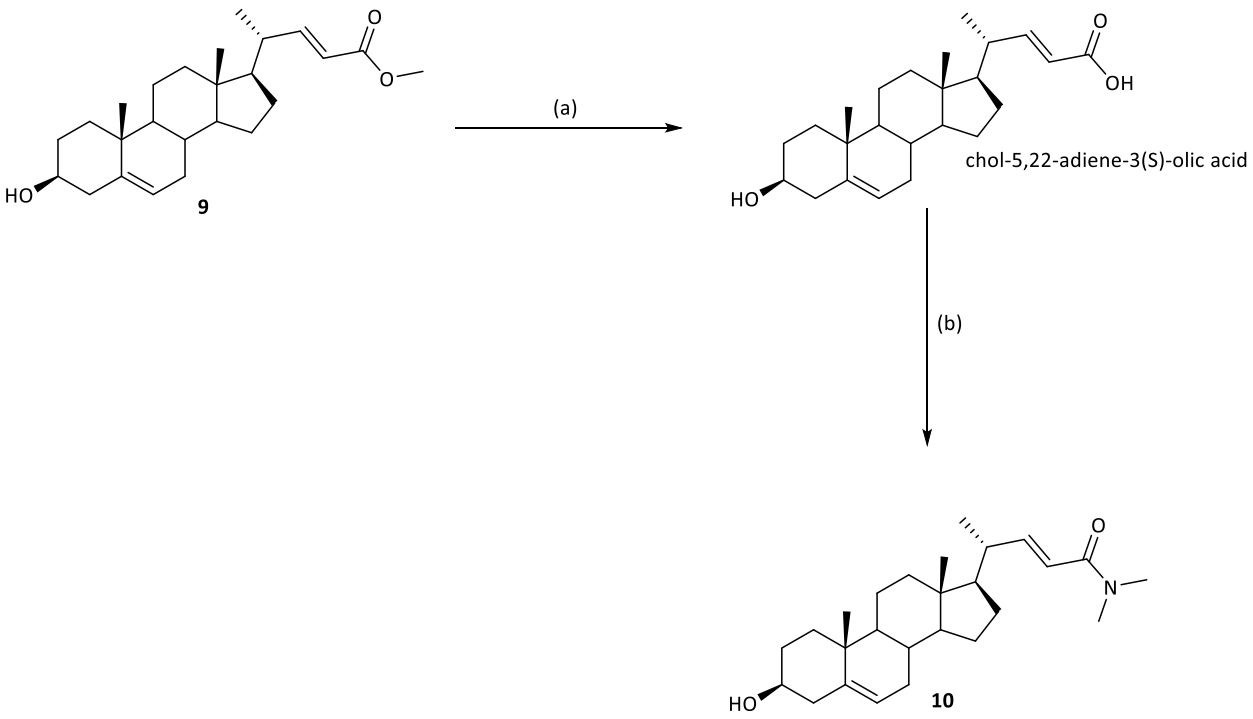
De-protection of compound **8a** was successfully completed by another member of our group (data not shown).

2.2 Modified workup for the synthesis of chol-5,22-adiene-3(S)-ol-24-*N,N*-dimethyl amide (10)

Target compound **10** is interesting because it structurally resembles 22(S)-HC, though without 22-hydroxy moiety (See section 1).

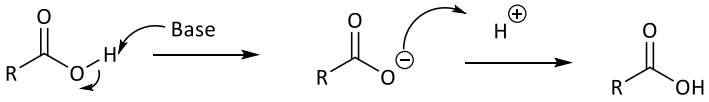
Custom synthesis laboratory Synthetica AS synthesized compound **9** as requested by our group. Compound **9** could also have been synthesized from **5** by a Wittig reaction[3], though that was not necessary. A conversion of compound **9** to **10** was also attempted, but only yielded a mere 15 % overall (data not shown).

Likely, their workup was less than ideal and therefore we attempted a modified workup of their reaction.



Scheme 20. Reaction conditions: (a) LiOH, THF, MeOH (b) DIPEA, DMA HCl, HATU, HOBT, DMF, 0°C to rt, 16h

After the first reaction (**Scheme 20**, reaction (a)) a meticulous workup strategy was applied to minimize the complex purification procedure of the intermediate chol-5,22-adiene-3(S)-olic acid. Base was added to the aq phase until pH ~10 to deprotonate the chol-5,22-adiene-3(S)-olic acid. This allows the starting material and other impurities to be extracted by the organic phase. The deprotonated chol-5,22-adiene-3(S)-olic acid is re-protonated by adding acid to the aq phase until pH ~4. This allows the chol-5,22-adiene-3(S)-olic acid to be extracted by the organic phase, evaporated and used as it is.



Scheme 21. Deprotonation/Reprotonation workup.

Standard amidation reaction method (from the group) was used as a simple way for the formation of target compound **10** with a subsequent flash purification that yielded 35 %. (**Scheme 20**, reaction (b))

2.2.1 Biological evaluation of chol-5,22-adiene-3(S)-ol-24-*N,N*-dimethyl amide (10)

Biological results for compound **10** were received on the 15th of May 2013. The structure of compound **10** is similar to that of de-protected **8a** and may be an indication of how compounds that were synthesized in this thesis will perform (**Figure 9** and **Figure 10**, entry S38)**,** if subjected to similar experiments**.** The results indicate that compound **10** exhibits improved *in vitro* characteristics on lipogenesis (**Figure 9**, entry S38) and genexpression (**Figure 10**, entry S38) compared with 22(S)-HC, the lead compound for this project.

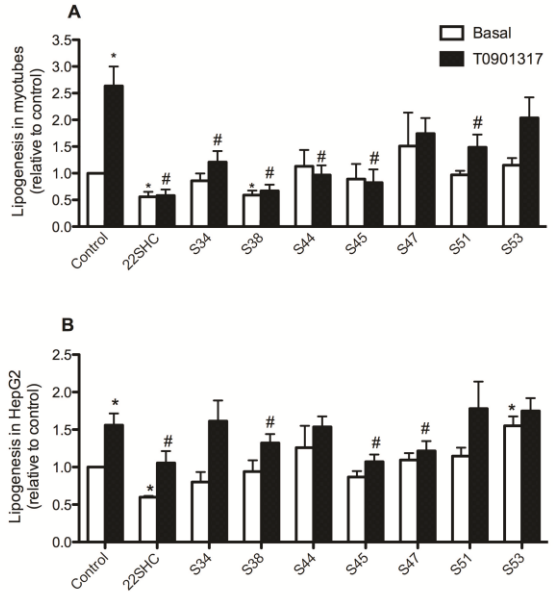


Figure 9. Lipogenesis in myotubes and HepG2 cells. Cells were treated with DMSO (0.1 %), 1 micro molar 22(S)-HC and test substances for 4 days for myotubes and 24 h for HepG2 cells. Values represent fold change relative to control given as means SEM (n=3-6) *P < 0.05 vs. control (DMSO) and #P < 0.05 for T0901317 vs treatment + T0901317. Entry S38 represents compound 10.

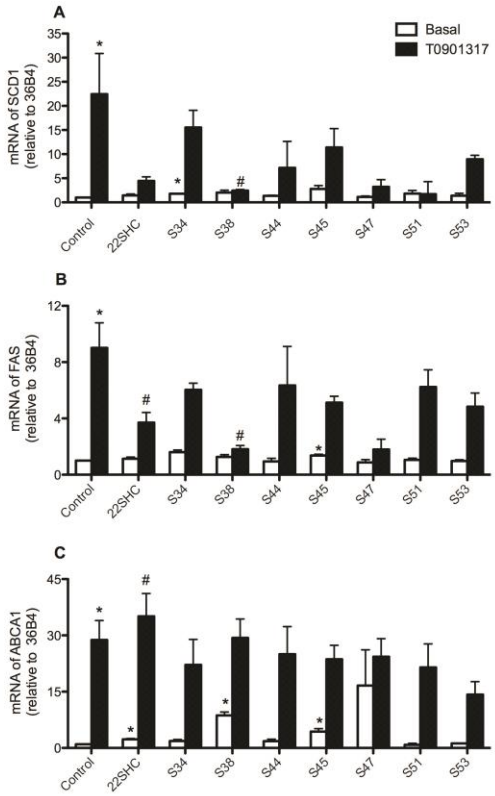


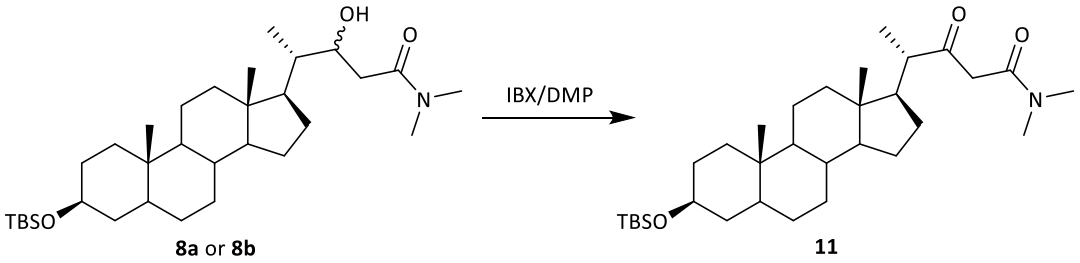
Figure 10. Gene expression in myotubes. Myotubes were treated with DMSO (0.1 %), 1 M T0901317, 10 M 22(S)-HC and test substances for 4 days. Values represent change relative to control given as means SEM (n=3-6). ABCA1, ATP-binding cassette transporter A1; FASN, fatty acid synthase; SCD1, Stearoyl-CoA desaturase 1. *P < 0.05 vs. control (DMSO) and #P < 0.05 for T0901317 vs. treatment + T0901317. Entry S38 represents compound 10.

2.3 Future Prospects

Some potential points of interest have already been raised in the discussion. We call some more to attention.

2.3.1 1,3-Ketoamides by oxidation

The stereochemistry of sterols have been shown to be important.[14] However, it would be interesting to examine the interaction of a 1,3-keto amide choline (compound **11**) on LXR. A direct comparison between compound **8a** and compound **11** in cells would provide important information on the limitations of oxygen moieties in C-22 (**Scheme 22**).



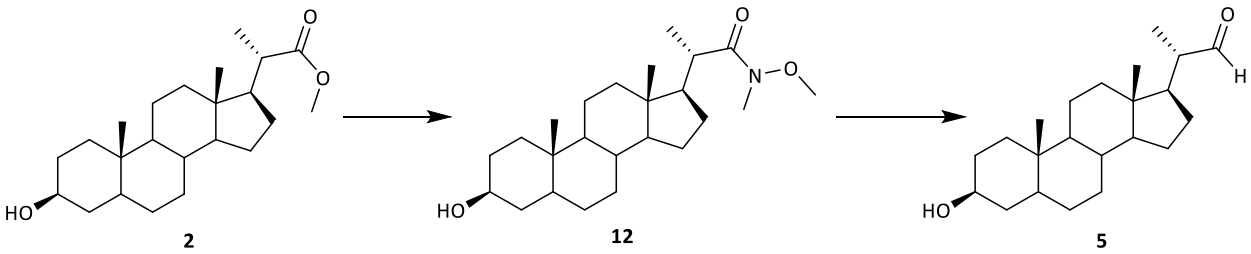
8a or **8b** **11**

Scheme 22. Proposed synthesis of 11 from 8a or 8b.

The oxidation compound **8a** or **8b** to compound **11** should be feasible based on reports of similar functional groups.[32] Preliminary work in our group was positive but not yet conclusive (data not shown).

2.3.2 Weinreb's amide

Weinreb's amide (compound **12**) has been found useful for synthesis of ketones and aldehydes.[33] If this can be applied towards the synthesis of compound **5** (**Scheme 23**), there is a potential to reduce the total synthesis by two steps compared to work done for this thesis. However, one must take into consideration the retention of stereochemistry on compound **5**. One limitation of this route (Scheme 23) is the use of LAH. The methodology developed in this thesis (**Scheme 8**) and suggested earlier is probably superior, but further investigation on the workup may be warranted.



Scheme 23. Proposed route for the synthesis of compound 5 by employing Weinreb's amide synthesis.[33]

3. Conclusion

All the aims for the project were accomplished. All three targets, key intermediate compound **5**, and novel compounds **7** and **8a**, were successfully accomplished in this short synthetic study. The crystal structure of compound **7** and **8a** was successfully characterized.

Four previously synthesized compounds **2**, **3**, **4** and **10** were also successfully synthesized in excellent or improved yields.

A new route was employed upon the synthesis of compound **4**. Most importantly the stereochemistry of compound **4** was successfully retained with DIBAL.

Novel intermediate, compound **6** was synthesized successfully and its X-ray crystal structure characterization was completed.

Compound **10** shows improved *it* vitro properties compared to 22(S)-HC, the lead compound for this thesis. Compounds **7** and de-protected **8a** were submitted for biological testing and the group is awaiting its results.

4. Experimental

4.1 General

All reagents were bought from Sigma-Aldrich except for 23,24-bisnorchol-5-ene-3(S)-olic acid (Steraloids), chol-5,22-adiene-3(S)-ol-24-methyloate (Synthetica AS) and IBX (synthesized according to procedure by Frigerio *et al*.[25] All reagents were used without further purification. All references to water in the section 4 are to type 2 ion exchange water. Merck 250 µm silica gel 60 F_{254}-plates for TLC and developed with potassium permanganate. All purification was done by flash chromatography with Merck silica 60 mesh (35-70 µm) unless otherwise noted.

NMR spectroscopy was recorded on a Bruker DPX 300 and Bruker AVII 400 instruments equipped with BACS-60 and BACS-120 sample changers. All NMR experiments were conducted at rt. (25^{o}C). $CDCl_3$ was used as the NMR solvent, except for the spectrum of starting material **1** (DMSO-d_6), and its spectra are referenced to residual peak at 7.26 ppm.

Assignment of peaks for the compounds has been done with reference to position of carbons on the cholan skeleton (Figure 11). All peak-splitting is reported as: s (singlet), d (doublet), t (triplet), m (multiplet) with coupling constants (*J*) in Hz where available. For example 1.20-1.18 (d, *J = 6Hz*, 3H, H-21 would refer to integration of three hydrogen atoms from C-21 methyl group.

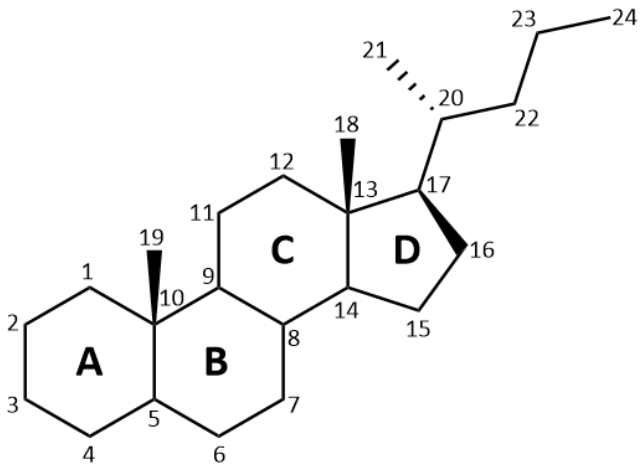
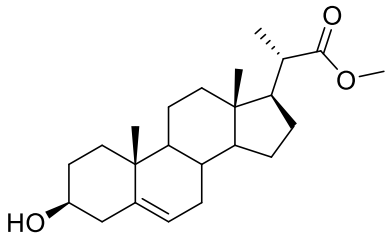


Figure 11. Numbering of carbons and rings in the cholan skeleton.

4.2 Methods

4.2.1 Synthesis of 23,24-bisnorchol-5-ene-3-ol-22-methyloate (2)



Molecular Weight: 360,54

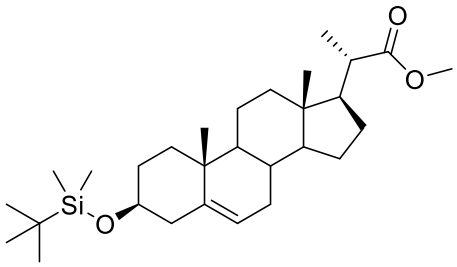
Fernholz acid (**1**) (519 mg, 1.50 mmol) was suspended in MeOH (47.5 mL) and 95 % sulphuric acid (2.5 mL). The mixture was refluxed for 17.5 hours. The resulting yellow reaction mixture was diluted with water (10 mL) and extracted with DCM (3x10 mL). The combined organic phases were dried with $MgSO_4$ and evaporated *in vacuo*. The resulting yellow solid was purified by flash chromatography 20 % EtOAc in hexanes yielding the desired product as a white solid (446 mg, 82.4 %).

^{1}H-NMR (300MHz, $CDCl_3$): δ 5.34-5.32 (m,1H,H-6),3.63 (s,3H,C$\underline{H}_3$O), 3.56-3.46 (m,1H,H-3), 2.46-2.39 (m, 1H, H-20), 2.32-2.19 (m,2H), 2.00-1.92 (m, 2H), 1.85-1.82 (m, 2H), 1.74-1.65 (m, 1H, H-17), 1.63-1.40 (m,9H), 1.36-1.23 (m, 3H), 1.20-1.18 (d, *J = 6Hz*, 3H, H-21), 1.13-1.05(m, 3H), 1.01(s, 3H, H-19), 0.98-0.92 (m, 1H), 0.69 (s,3H, H-18)

^{13}C-NMR (75MHz, $CDCl_3$): δ 177.34 ($\underline{C}$=O), 140.72 (C-5), 121.49 (C-6), 71.69 (C-3), 56.29 (C-14), 52.84 (C-24), 51.31 (C-17), 50.03 (C-9), 42.44 (C-20), 42.38 (C-13), 42.23 (C-4), 39.52 (C-12), 37.21 (C-10), 36.46 (C-1), 31.87 (C-7), 31.78 (C-8), 31.58 (C-2), 27.13(C-15), 24.3 (C-16), 20.99 (C-21), 19.36 (C-11), 17.09 (C-19), 11.99 (C-18)

^{1}H and ^{13}C-NMR are in accordance with literature.[20]

4.2.2 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-methyloate (3)



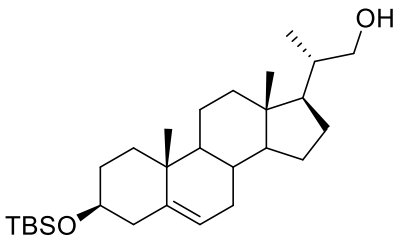
Molecular Weight: 474,80

2,6-lutidine (1.04 mL, X mmol) and *tert*-butyldimethylsilyl triflate (1.28 mL, X mmol) was added to a stirring solution of **2** (1.68 g, 4.67 mmol) in anhydrous DCM at -40°C under Ar-atm. The mixture was stirred for 15 minutes at -40°C, gradually warmed to rt. and stirred for one additional hour. The solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography 10 % EtOAc in hexanes yielding the desired product (2.07 g, 93.5 %, ee. >98 %) as a white solid.

^{1}H-NMR (300MHz, $CDCl_3$): δ 5.32-5.30 (m, 1H, H-6), 3.64 (s, 3H, C$\underline{H}_3$O-), 3.52-3.44 (m, 1H, H-3), 2.47-2.39 (m, 1H,H-20), 2.30-2.23 (m, 1H), 2.19-2.14 (m, 1H), 2.00-1.91 (m, 2H), 1.83-1.78 (dt, $J_1 = 3Hz$, $J_2 = 9Hz$,1H), 1.74-1.66 (m, 1H,H-17), 1.62-1.58 (m, 3H), 1.54 (s, 3H), 1.52-1.23 (m, 8H),1.20-1.18 (d, *J=6 Hz*, H-21), 1.15-1.02 (m, 3H), 1.00 (s, 3H, H-19), 0.89 (s, 9H, (C$\underline{H}_3$)$_3$), 0.69 (s, 3H, H-18), 0.06 (s, 6H, Si(C$\underline{H}_3$)$_2$)

^{1}H-NMR is in accordance with literature. [20]

4.2.3 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-ol (4)



Molecular Weight: 446,79

Method A

$LiAlH_4$ (177 mg, 4.48 mmol) was added portion wise to a stirring solution of compound **3** (851 mg, 1.79 mmol) in anhydrous Et_2O (21 mL) under Ar-atm. The mixture was stirred for 2 hours at rt. before the successive addition of water (0.20 mL), 15 % NaOH (aq, 0.20 mL), and water (0.60 mL). The mixture was stirred until a white precipitate was formed. The precipitate was filtered and washed with Et_2O. The filtrate was extracted with DCM (3x5 mL) and the combined organic phases were dried with $MgSO_4$ and evaporated *in vacuo* yielding a white solid (681 mg, 85.2 %). Compound **4** was used without further purification.

Method B

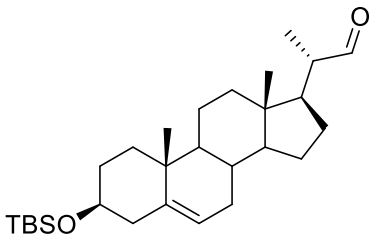
1M DIBAL in THF (17.44 mL) was added drop wise to a stirring solution of compound **3** (2.07g, 4.36 mmol) in anhydrous DCM (22 mL) at – 78°C under N_2-atm. After 1 hour sat. aq. $NaHCO_3$ (20 mL) was added to the mixture and stirred for 10 min. The reaction was quenched with solid Na-K-tartrate (5.29 g). The reaction mixture was extracted with Et_2O (3x10 mL). The combined organic phases were dried with

$MgSO_4$ and evaporated *in vacuo,* yielding a solid (1.83 g, 94.1 %, ee. >98 %). Compound **4** was used without further purification.

^{1}H-NMR (300MHz, $CDCl_3$): δ 5.32-5.30(m, 1H, H-6), 3.65-3.62 (dd, $J_1 = 9Hz$, $J_2 = 3Hz$, 1H, H-8 or H-9), 3.50-3.44 (m, 1H, H-3), 3.39-3.34 (m, 1H, H-20,2.29-2.23 (t, $J = 9Hz$, 1H, H-9), 2.18-2.14 (m, 1H), 1.95-1.08 (m, 20H), 1.06-1.04 (m, 3H, H-21), 1.00 (s, 3H, H-19), 0.89 (s, 9H, $(C\underline{H}_3)_3$), 0.70 (s, 3H, H-18), 0.05 (s, 6H, $Si(C\underline{H}_3)_2$)

^{1}H-NMR is in accordance with literature. [20]

4.2.4 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5)



Molecular Weight: 444,77

Method A

DMSO (0.323 mL, 4.56 mmol) in anhydrous DCM (2 mL) was added drop wise to a stirring solution of oxalylchloride (0.261 mL, 3.04 mmol) in anhydrous DCM (10 mL) at -78°C under Ar_2-atm. The mixture was stirred for 20 minutes at -78°C before addition of compound **4** (681 mg, 1.52 mmol) in anhydrous DCM (2 mL). The mixture was stirred for an additional 20 minutes at -78°C before addition of NEt_3 (1.27 mL, 9.12 mmol). The mixture was gradually heated to rt., diluted with DCM (10 mL), washed with sat. aq. NH_4Cl (3x30 mL) and brine (20 mL). The organic phase was dried with $MgSO_4$ and evaporated *in vacuo.* The solid was purified by flash chromatography 10 % EtOAc in hexanes yielding a mixture of the desired product as a diastereomer (190 mg, 28.1 %, 1:4.6) which was further improved by precipitating the mixture from methanol (1:5.3).

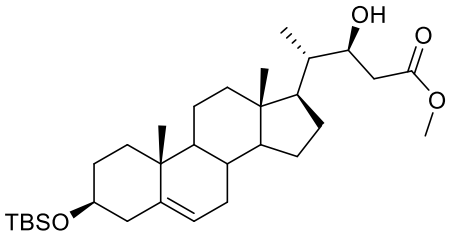
Method B

IBX (3.16 g, 10.5 mmol) was suspended in solution of EtOAc (110 mL) and compound **4** (1.56 g, 3.49 mmol). The suspension was refluxed under vigorous stirring for 3.5 hour and gradually cooled to rt. before the suspension was filtered. The solvent was evaporated *in vacuo* yielding the desired product (1.55 g, 99.9 %, ee. >98 %) as a white solid that was used without further purification.

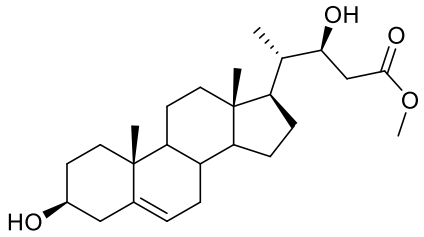
^{1}H-NMR (300MHz, $CDCl_3$): δ 9.58-9.57 (d, *J = 4Hz*, H-22), 5.32-5.30 (m, 1H, H-6), 3.52-3.44 (m, 1H,H-3),2.41-2.32(m, 1H, H-3), 2.30-2.24 (t, *J = 9Hz*, 1H, H-9), 2.18-2.15 (m, 1H, H-8 or H-14), 2.04-1.17 (m, 17H), 1.14-1.12 (d, *J = 6 Hz*, 3H, H-21), 1.04 (m, 1H), 1.01 (s, 3H, H-19), 0.89 (s, 9H, $(C\underline{H}_3)_3$), 0.73 (s, 3H, H-18), 0.06 (s, 6H, $Si(C\underline{H}_3)_2$)

^{13}C-NMR (100MHz, $CDCl_3$): δ 205.08 (H<u>C</u>=O), 141.57 (C-5), 120.94 (C-6), 72.58 (C-3), 56.06 (C-14), 50.20 (C-20), 49.50 (C-17), 42.98 (C-4), 42.81 (C-12), 39.51 (C-10), 37.39 (C-1), 36.60 (C-2), 32.07 (C-7),31.91 (C-8), 27.06 (-Si-$\underline{C}(CH_3)_3$), 25.94 (-Si-C($\underline{C}H_3)_3$), 24.66 (C-16), 19.43 (C-11), 21.01 (C-19), 13.46 (C-18), 12.23 (C-21), 4.58 (-Si($\underline{C}H_3)_2$)

4.2.5 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-chol-5-ene-22(S)-ol-24-methyloate and chol-5-ene-3(S),22(S)-diol-24-methyloate (7)



Molecular Weight: 518,85



Molecular Weight: 404,59

Method A

$HSiMe_2Ph$ (0.06 mL, 0.391 mmol) was added to a suspended solution of InI_3 (25 mg, 0.05 mmol), compound **3** (102 mg, 0.214 mmol) and 1-*tert*-butyldimethylsilyl-oxy-1-methoxyethene (0.09 mL, 0.412 mmol) in DCM (1.5 mL) at rt. under N_2-atm and left to stir for 18.5 hours. 1M TBAF in THF (5 mL) and 1M HCl (10 mL) was subsequently added. The mixture was extracted with Et_2O (3 x 10 mL). The combined organic phases were dried with $MgSO_4$, evaporated *in vacuo* and the crude was purified by flash chromatography 10-100 % EtOAc in hexanes. Only starting material was recovered.

Method B

2M LDA in THF (0.143, 0.285 mL) was diluted with anhydrous THF(1 mL) and cooled to -78˚C under N_2-atm. EtOAc (0.0253 mL, 0.259 mmol) and compound **5** (115 mg, 0.259 mmol) in anhydrous THF (1 mL) drop wise. The reaction was stirred for 4 hours before addition of sat. aq. NH_4Cl (2 mL) and dilution with water (10mL). The mixture was extracted with DCM (3 x 5 mL). The combined organic phases were dried with $MgSO_4$ and evaporated in *vacuo*. This gave full recovery of the starting material.

Method C

BF_3OEt_2 (0.03 mL, 0.229 mmol) was added drop wise to a stirring solution of 1-*tert*-butyldimethylsilyl-oxy-1-methoxyethene (0.06 mL, 0.275 mmol) and **5** (76 mg, 0.171 mmol) in anhydrous DCM (2 mL) at -78°C under N_2-atm. After 3 the reaction was quenched with sat. aq. $NaHCO_3$ (1 mL). The mixture was extracted with DCM (3 x 1.5 mL). The combined organic phases were dried with $MgSO_4$ and evaporated in *vacuo*. This gave full recovery of starting material.

Method D

A solution of compound **5** (94 mg, 0.211 mmol) in toluene (2 mL) was added drop wise to a stirring suspension of 1-*tert*-butyldimethylsilyl-oxy-1-methoxyethene (0.06 mL, 0.275 mmol) and $MgBrOEt_2$ (182 mg, 0.705 mmol) in toluene (2 mL) at -15°C under N_2-atm. The mixture stirred for 35 minutes and quenched with sat. aq. $NaHCO_3$ (2 mL) at 0°C. The mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried with $MgSO_4$ and evaporated in *vacuo*. The crude was purified by flash chromatography with a gradient of 5-20 % EtOAc in hexanes yielding the desired product **6** (0.014 mg, 12.7 %, ee. 93:7).

Method E

1M $TiCl_4$ in DCM (1.22 mL) was added drop wise to a stirring solution of compound **5** (0.514, 1.16 mmol) in anhydrous DCM (11 mL) at -78°C under N_2-atm followed by addition of 1-*tert*-butyldimethylsilyl-oxy-1-methoxyethene (0.253 mL, 1.16 mmol). The mixture was stirred at -78°C for 10 minutes before gradually heating to rt. and stirred for 1 hour. The reaction was quenched with sat. aq. $NaHCO_3$ (15 mL). The mixture was extracted with DCM (3 x 10 mL). The combined organic phases were dried with $MgSO_4$ and evaporated in *vacuo*. The crude product was purified by flash chromatography 5-25 % EtOAc in hexanes yielding desired products **6** (44 mg, 7.31 %, ee. >98 %) and **7** (32 mg, 6.82 %, ee. >98 %).

Compound 6

^{1}H-NMR (400MHz, $CDCl_3$): δ 5.32-5.30(d, *J = 8Hz*, 1H, H-6), 4.16-4.14 (d, *J = 8Hz*, 1H, O<u>H</u>), 3.70 (s, 3H, C<u>H</u>$_3$O), 3.51-3.44(m, 1H, H-3), 3.63-2.57 (dd, *J1 = 8Hz*, *J2 = 16Hz*, 1H, H-8), 2.31-1.04 (m, 24H), 1.04 (s, 3H, H-19), 0.95-0.93 (d, *J = 8 Hz*, 3H, H-21), 0.88 (s, 9H, CO(C<u>H</u>$_3$)$_3$), 0.67 (s, 3H, H-18), 0.05 (s, 6H, Si(C<u>H</u>$_3$)$_2$)

^{13}C-NMR (100MHz, $CDCl_3$): δ 173.86 (<u>C</u>=O), 141.54 (C-5), 121.08 (C-6), 72.26 (C-3), 69.53 (C-22), 56.62 (C-14), 52.35 (<u>C</u>H$_3$O), 51.73 (C-9), 50.14 (C-17), 42.82 (C-13), 42.21 (C-4), 40.62 (C-20), 39.81(C-13), 39.76 (C-12), 37.37 (C-23), 36.55 (C-1), 32.09(C-2), 31.97 (C-8), 31.87 (C-7), 27.68 (Si-<u>C</u>-(CH$_3$)$_3$), 25.93 (-C(<u>C</u>H$_3$)$_3$), 24.2 (C-16), 21.09 (C-11), 19.42 (C-19), 18.25 (C-10), 12.05 (C-21), 11.76 (C-18), -4.59 (-Si(<u>C</u>H$_3$)$_2$)

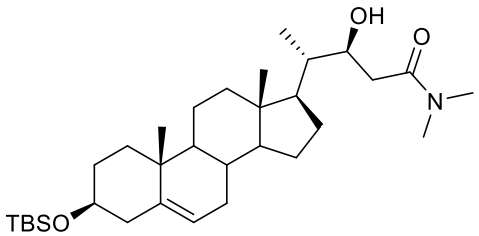
HR-MS Found 518.3777 calculated for $C_{31}H_{54}O_4Si$ 518.3791

Compound 7

^{1}H-NMR (400MHz, $CDCl_3$): δ 5.38-5.36 (m, 1H, H-6), 4.16-4.14 (d, *J = 8Hz*, 1H, -OH), 3.80-3.73 (m, 1H, OH), 3.71(s, 3H, CH_3O), 2.64-2.46(m, 3H), 2.31-1.05 (m, 30H), 1.03(s, 3H, H-19), 0.95-0.91 (d, *J = 8Hz*, 3H, H-21), 0.89 (m, 1H, H-17), 0.68 (m, 3H, H-18)

^{13}C-NMR (100MHz, $CDCl_3$): δ 173.89 (C=O), 140.80 (C-5), 122.41 (C-6), 69.81 (C-3), 60.30 (C-22), 56.51 (C-14), 52.33 (CH_3O), 51.76 (C-17), 49.98 (C-9), 43.39 (C-13), 42.20 (C-4), 40.62 (C-21), 39.80(C-12), 39.65 (C-23), 39.08 (C-10), 36.36 (C-1), 33.37 (C-7), 31.85 (C-8), 31.76 (C-2), 27.66 (C-15), 24.18 (C-16), 20.98 (C-11), 19.25 (C-19), 12.06 (C-21), 11.76 (C-18)

4.2.6 Synthesis of 3β-(*tert*-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-*N,N*-dimethyl amide (8a)



Molecular Weight: 531,90

Dimethylacetamide (1.04 mL, 11.2 mmol) was added drop wise to a stirring solution of *n*-BuLi (5.66 mL, 11.3 mmol) and diisopropylamine (1.59 mL, 11.2 mmol) in anhydrous THF (9 mL) at -78^{o}C under N_2-atm and stirred at that temperature for 30 minutes.

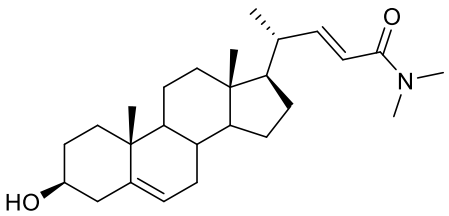
Lithiumdimethylacetamide (1.8 mL, 1.13 mmol) was added drop wise to a stirring solution of **5** (502 mg, 1.13 mmol) in anhydrous THF (7 mL) at -78^{o}C under N_2-atm. The mixture was stirred for 15 minutes and quenched with sat. aq. NH_4Cl (15 mL) upon which a white solid formed. The THF was and evaporated in *vacuo* before the aqueous phase was extracted with DCM (3 x 25 mL). The organic combined organic phases were dried with $MgSO_4$. The solvent was and evaporated in *vacuo*. The crude product was purified by flash chromatography 50 % EtOAc in hexanes yielding a mixture of **8a** and **8b** (439 mg, 73.0 %, ee. 68:32). The mixture (100 mg) was dissolved with minimal amount of boiling EtOAc and washed with ice-cold anhydrous Et_2O. This yielded compound **8a** (33 mg, 0.062 mmol).

^{1}H-NMR (400MHz, $CDCl_3$): δ 5.31-5.30 (m, 1H, H-6), 4.15-4.12 (d, *J = 12Hz*, 1H, OH), 3.87 (s, 1H), 3.51-3.43 (m, 1H, H-3), 2.99(s, 3H, -N-CH_3), 2.95 (s, 3H, -N-CH_3), 2.53-1.20 (m, 19H), 1.13-1.05(m, 3H, H-19),

0.99 (m, 3H, H-18 and H-21), 0.88 (s, 9H, -Si-C-($C\underline{H}_3$)$_3$), 0.67 (s, 6H, -Si-($C\underline{H}_3$)$_2$)

^{13}C-NMR (100MHz, $CDCl_3$): δ 173.30 ($\underline{C}$=O), 141.49 (C-5), 121.15 (C-6), 72.62 (C-3), 69.30 (C-22), 56.59 (C-14), 52.39 (C-17), 50.14 (C-9), 42.84 (C-13), 42.17 (C-4), 40.79 (C-12), 39.75 (C-10), 37.37 (C-17), 37.10 (C-20), 36.56 (C-23), 36.53 (C-2), 35.22 (C-1), 32.11 (C-7), 31.99 (C-8), 31.88 (-$\underline{C}$-(CH_3)$_3$), 27.76 (C-16), 25.95 (-C-($\underline{C}H_3$)$_3$), 24.23/21.11/19.43 (C-11), 18.26 (C-19), 12.59 (C-21), 11.75 (C-18)

4.2.7 Synthesis of chol-5,22-adiene-3(S)-ol-24-*N,N*-dimethyl amide (10)



Molecular Weight: 399,62

A solution of LiOH•H_2O (88mg, 2.10 mmol) in water (10 mL) was added to a stirring solution of compound **9** (500 mg, 1.29 mmol) in anhydrous THF (10 mL) and methanol (10.5 mL) at 0°C under Ar_2-atm. After 4 hours the mixture was diluted with water (50 mL), K_2CO_3 was added until pH ~10 and extracted with EtOAc (3 x 25 mL). Citric acid was added to the aq. Phase until pH ~4 and extracted with ether (3 x 25 mL). The organic phase was washed with brine (30 mL), dried with $MgSO_4$ and evaporated in *vacuo.* The solid (456 mg) was used without further purification.
Diisopropylethylamine (1.25 mL, 7.1 mmol) was added to a solution of dimethylamine hydrochloride (489 mg, 6.00 mmol), HATU (456 mg, 1.20 mmol), HOBT•H_2O (184 mg, 1.20 mmol) and chol-5,22-adiene-3(S)-olic acid (456 mg, 1.22 mmol) in anhydrous DMF (50 mL) at 0°C under Ar-atm. The reaction mixture was gradually heated to rt. and stirred for 16 hours. The mixture was diluted with water (500 mL) and extracted with EtOAc (3 x 50 mL). The organic phase was washed with 1M HCl (2 x 50 mL), sat. aq. $NaHCO_3$ (2 x 50 mL) and brine (50 mL). The organic phase was dried with $MgSO_4$ and evaporated in *vacuo*. The crude product was purified by flash chromatography 50-100 % EtOAc in hexanes yielding the desired product **10** (178 mg, 34.5 %) as a white solid.

^{1}H NMR (400 MHz, CDCl3) δ 6.72 (dd, J = 14.9, 9.0 Hz, 1H), 6.15 (d, J = 15.0 Hz, 1H), 5.34 (s, 1H), 3.52 (s, 1H), 3.02 (d, J = 28.9 Hz, 6H), 2.24 (dd, J = 20.7, 9.7 Hz, 3H), 1.99 (q, J = 15.2, 13.8 Hz, 2H), 1.84 (d, J = 10.9 Hz, 2H), 1.78 – 1.39 (m, 9H), 1.24 (q, J = 14.3, 10.1 Hz, 3H), 1.03 (td, J = 23.4, 22.8, 8.7 Hz, 10H), 0.72 (s, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 167.29 (s), 151.82 (s), 140.92 (s), 121.72 (s), 117.90 (s), 71.88 (s), 56.73 (s), 55.17 (s), 50.27 (s), 42.74 (s), 42.43 (s), 40.21 (s), 39.78 (s), 37.45 (d, J = 4.7 Hz), 36.66 (s), 35.81 (s), 32.16 – 31.71 (m), 28.45 (s), 24.45 (s), 21.22 (s), 19.76 (s), 19.55 (s), 12.27 (s)

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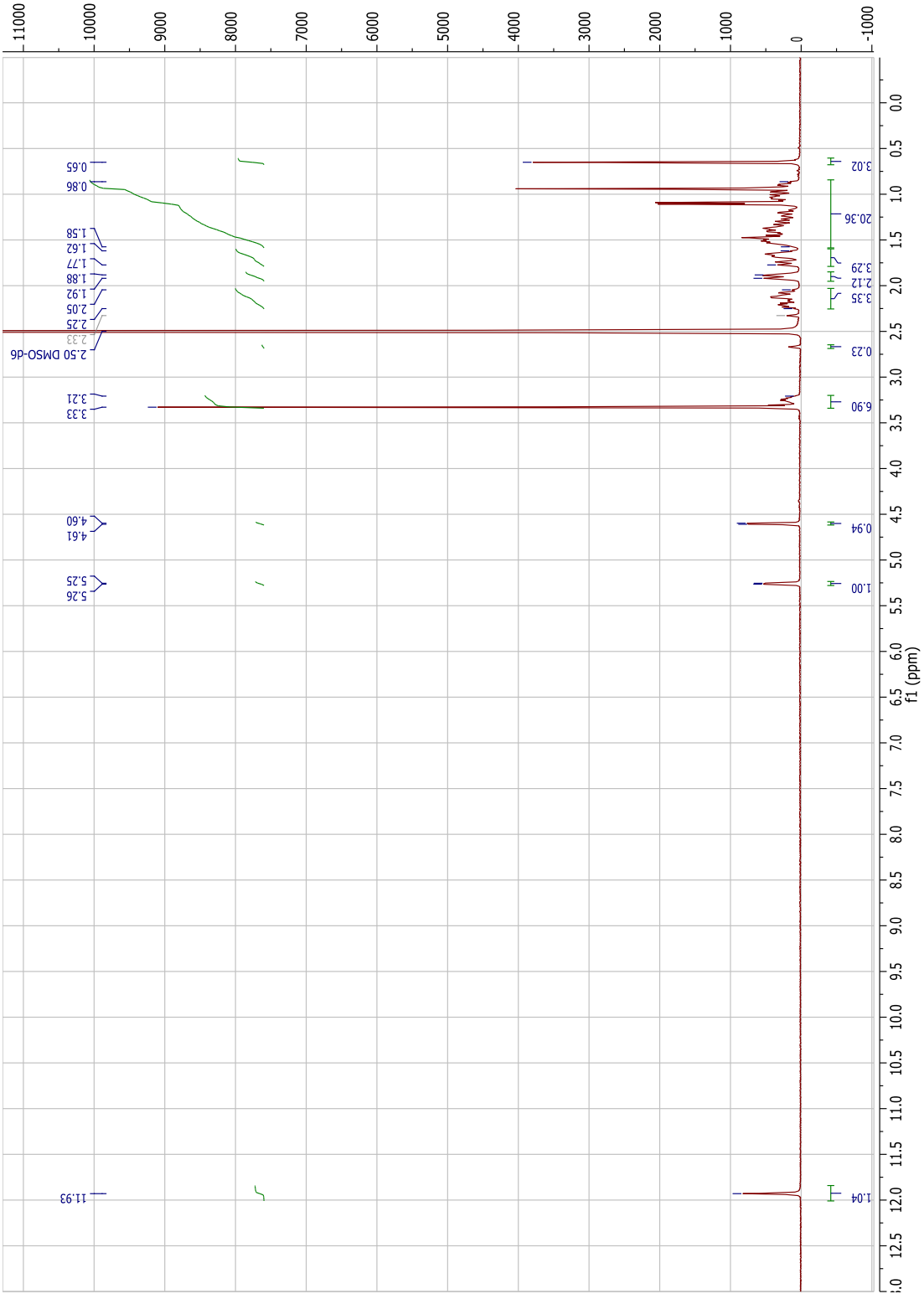
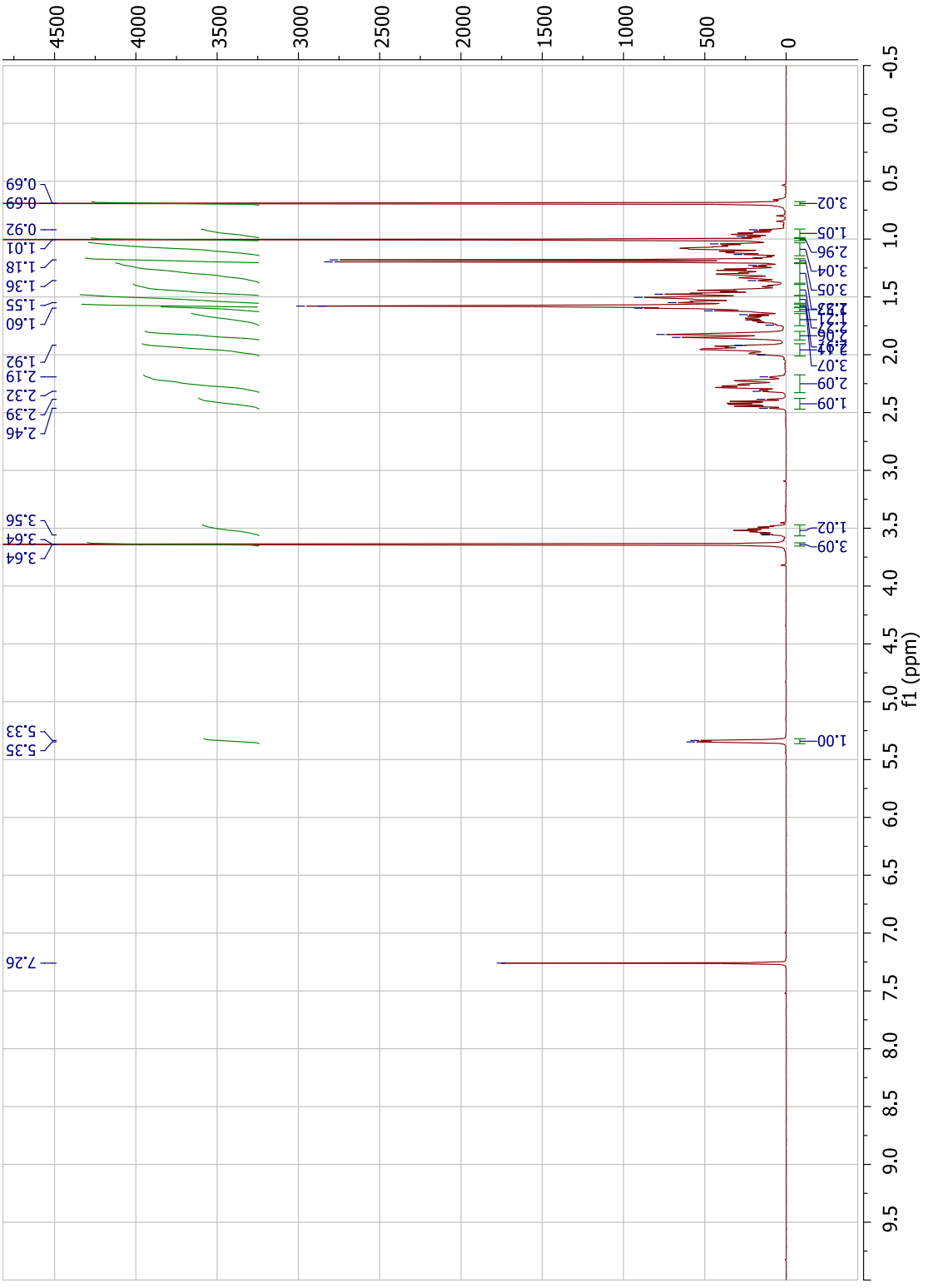
A. Spectrum of starting material

A.1 ^{1}H NMR spectrum of 23,24-bisnorchol-5-eneic acid

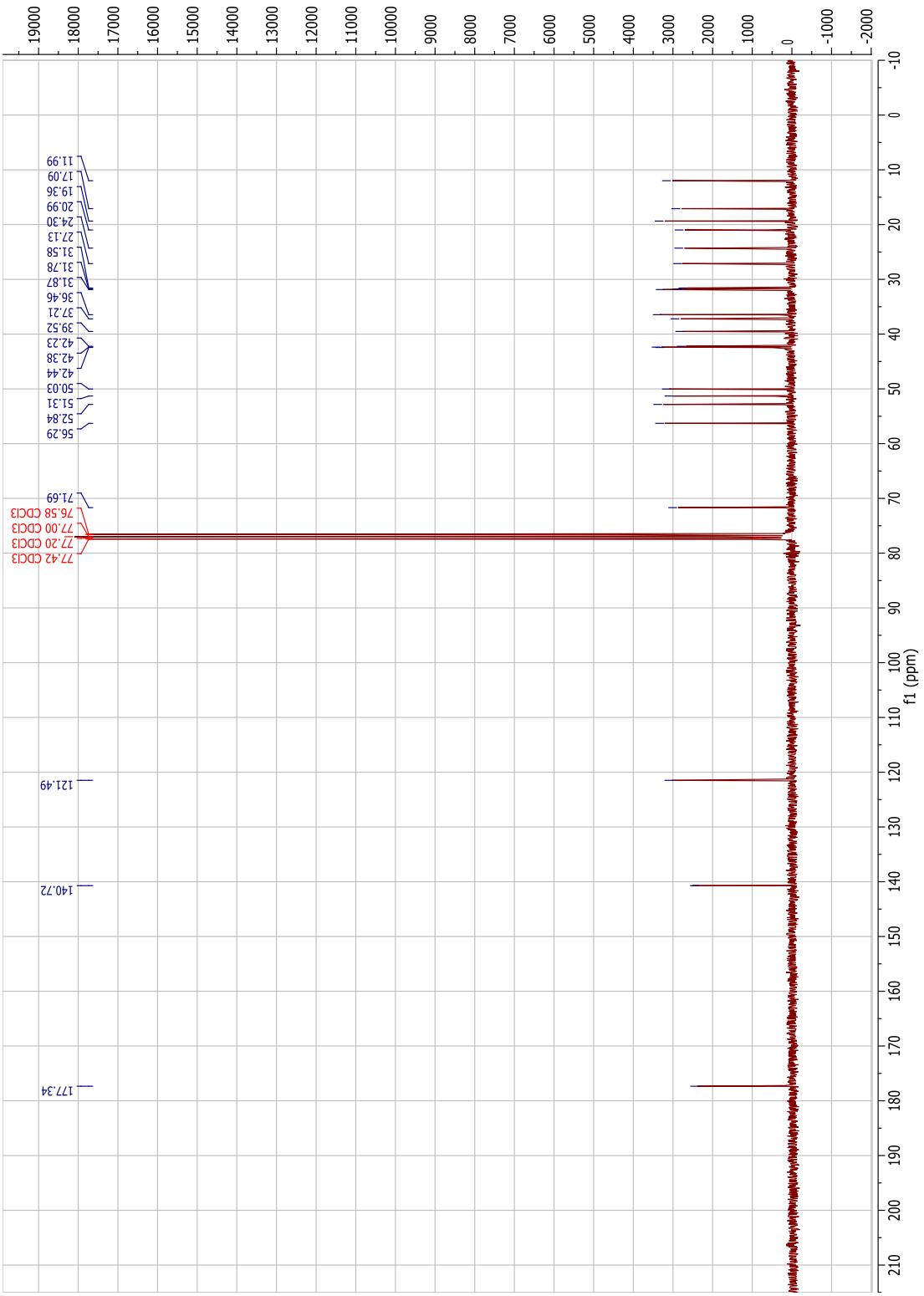


B. Spectra of Synthesized Compounds

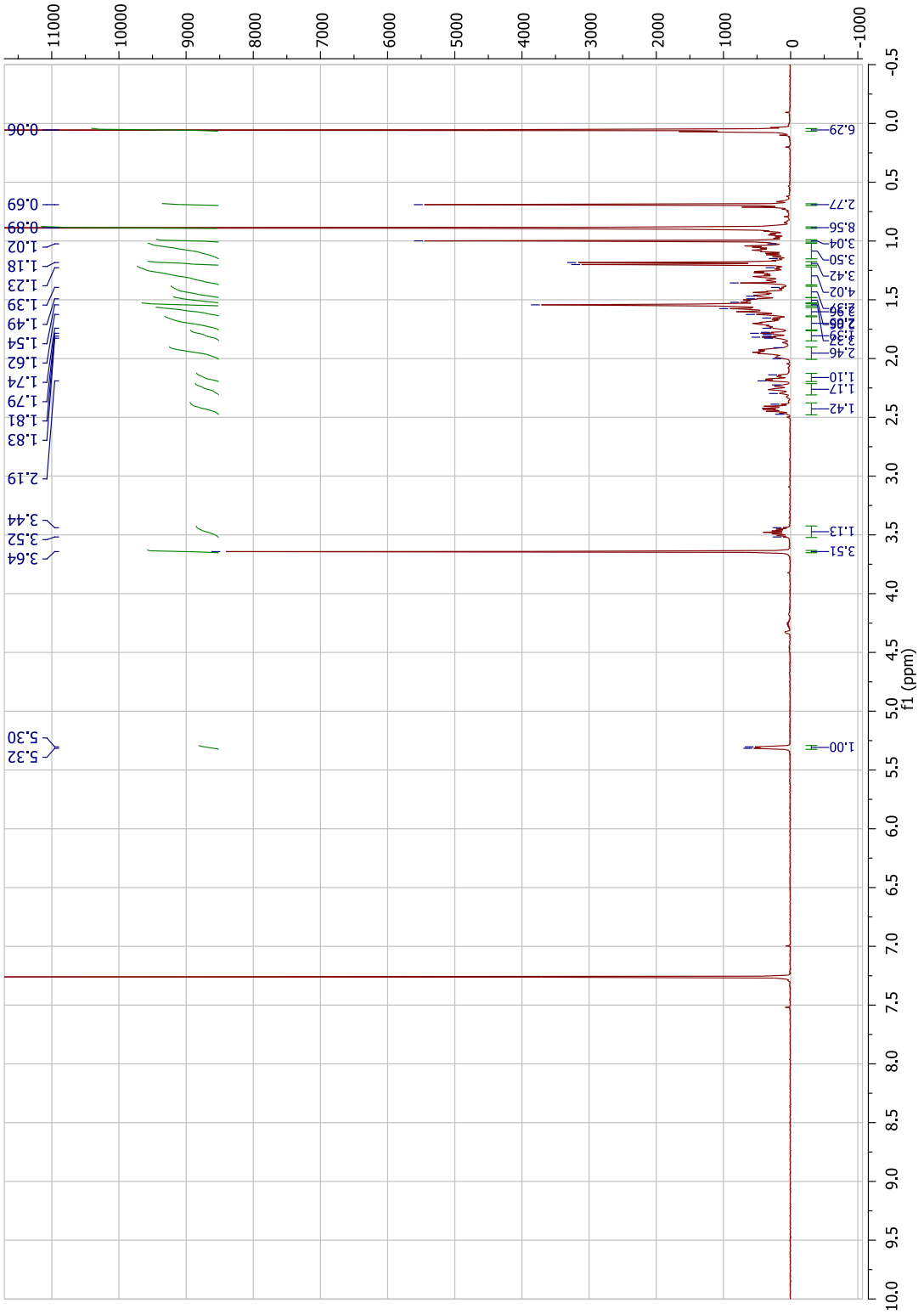
B.1 ^{1}H-NMR spectrum of 23,24-bisnorchol-5-ene-3-ol-22-methyloate (2)



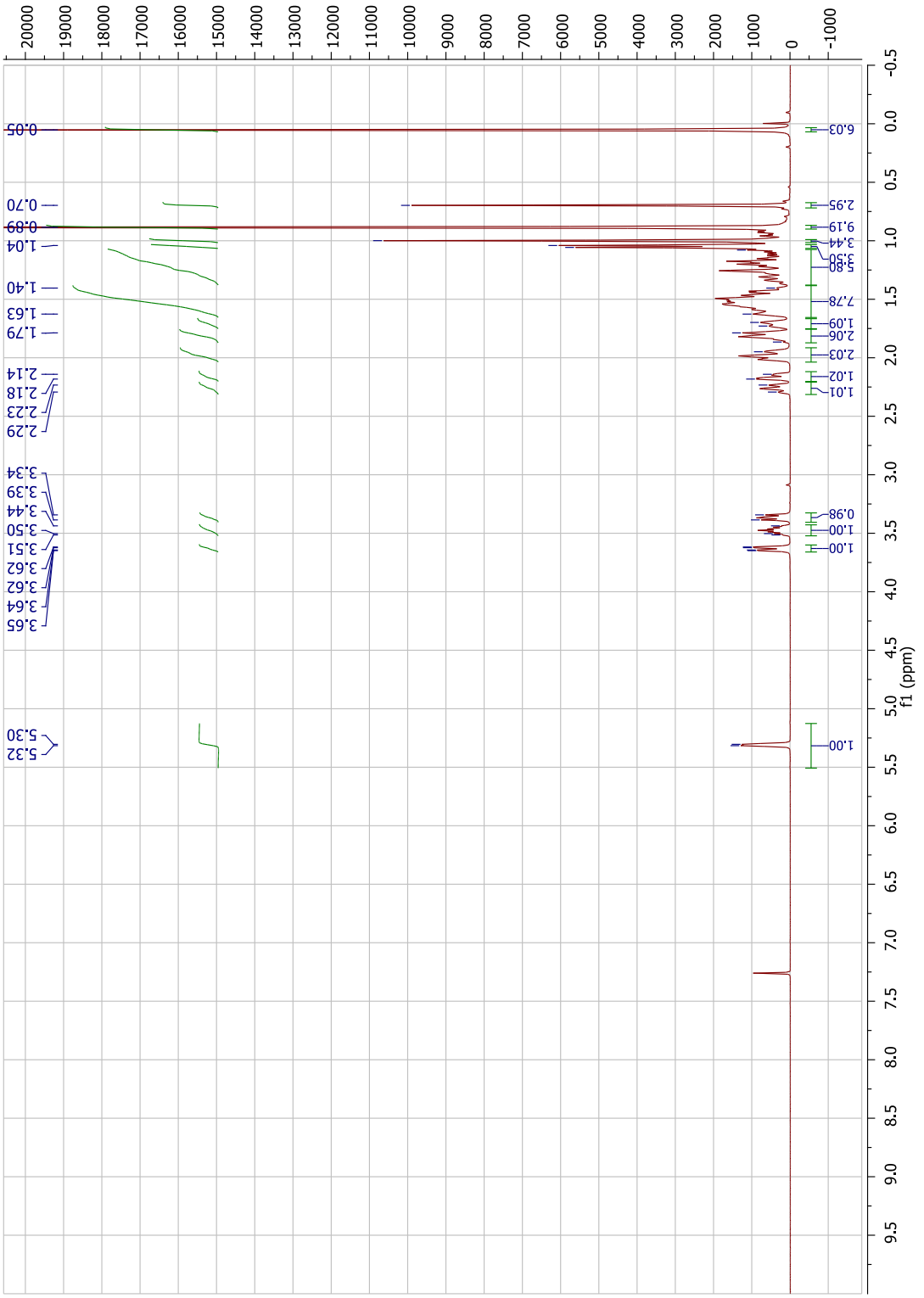
B.2 ^{13}C CPD spectrum of 23,24-bisnorchol-5-ene-3-ol-22-methyloate (2)



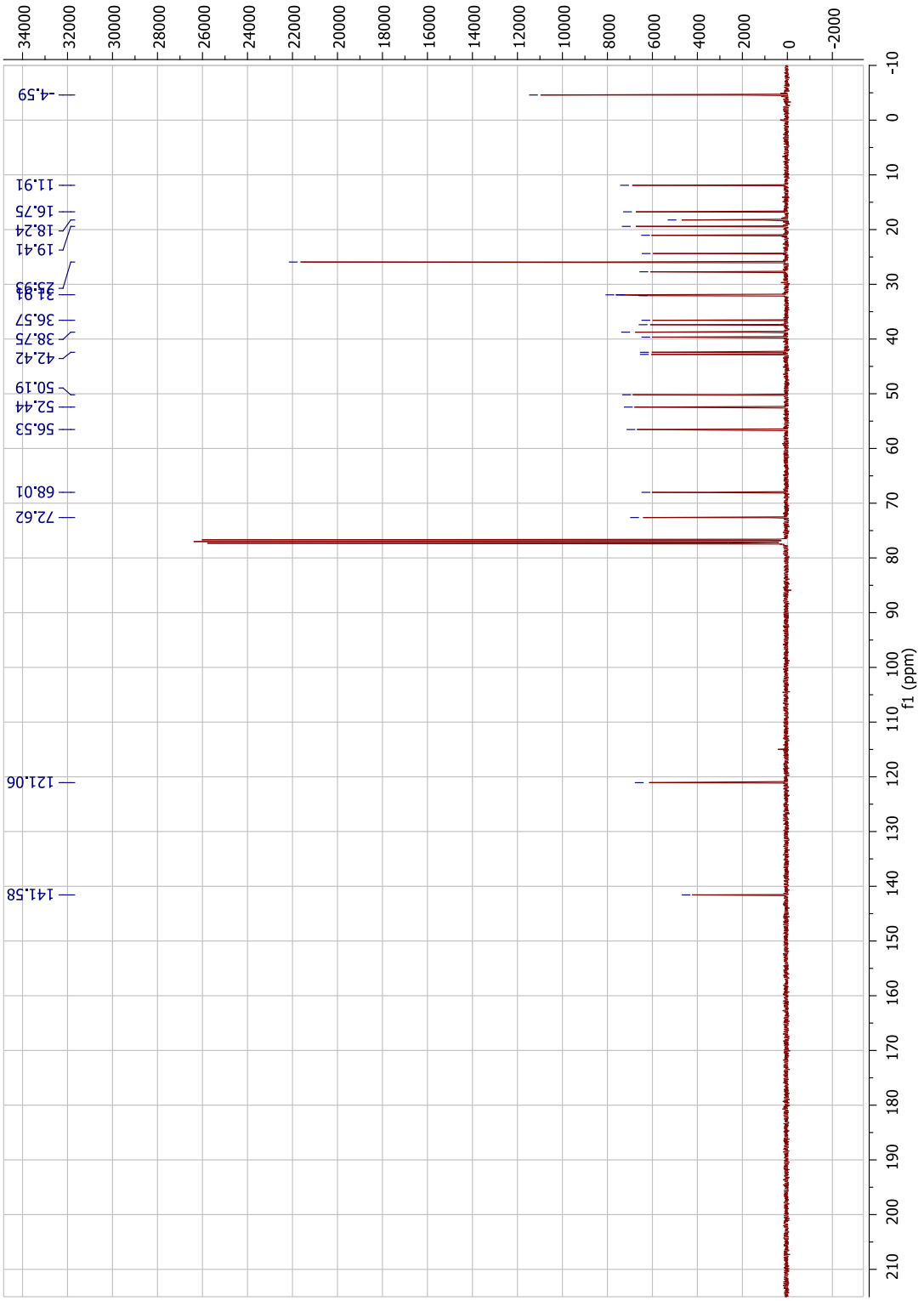
B.3 ^{1}H-NMR spectrum of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-methyloate (3)



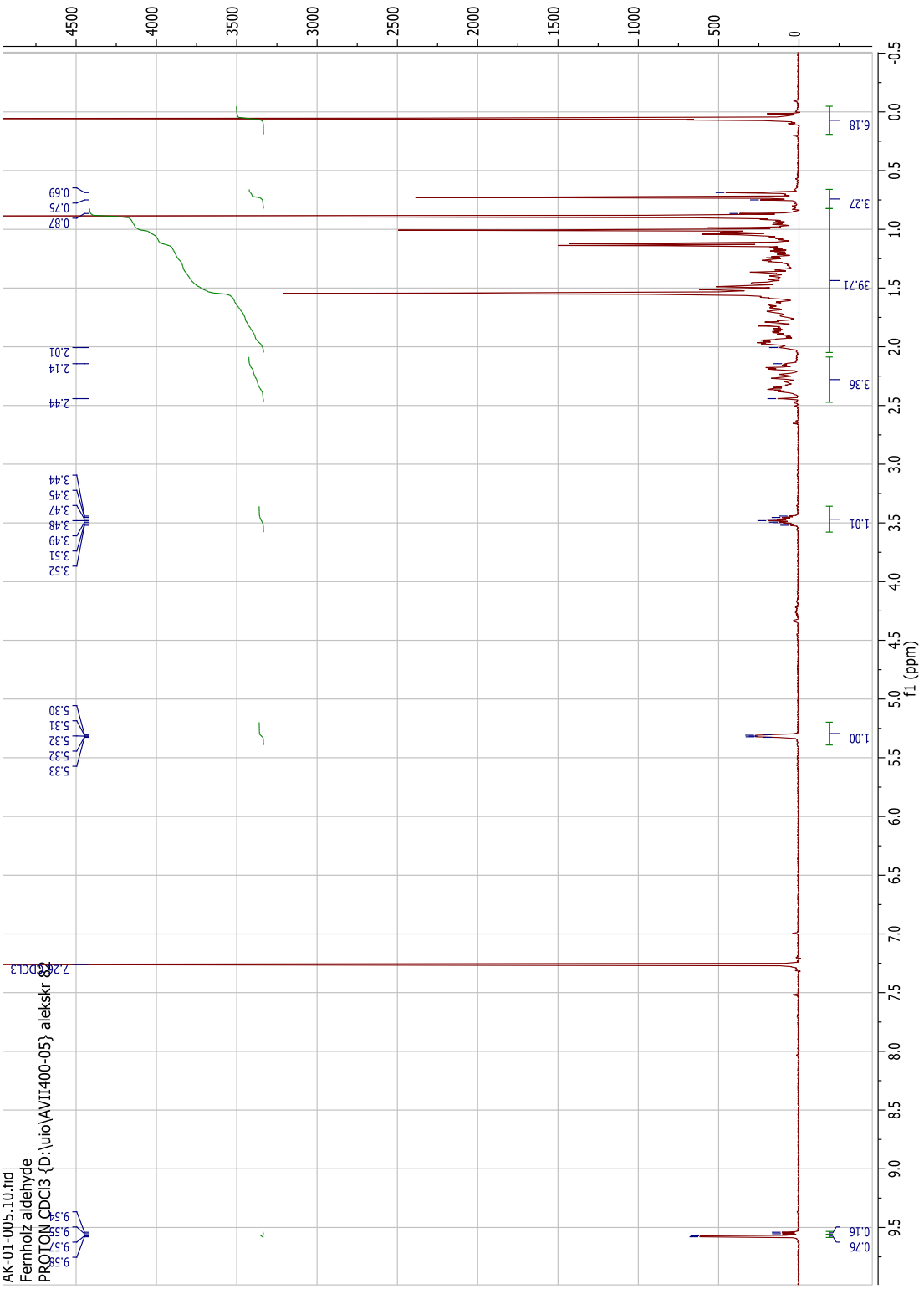
B.4 ^{1}H-NMR spectrum of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-ol (4)



B.5 ^{13}C CPD NMR spectrum of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-ol (4)



B.6 ^{1}H-NMR spectra of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5a)




1600
1500
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200
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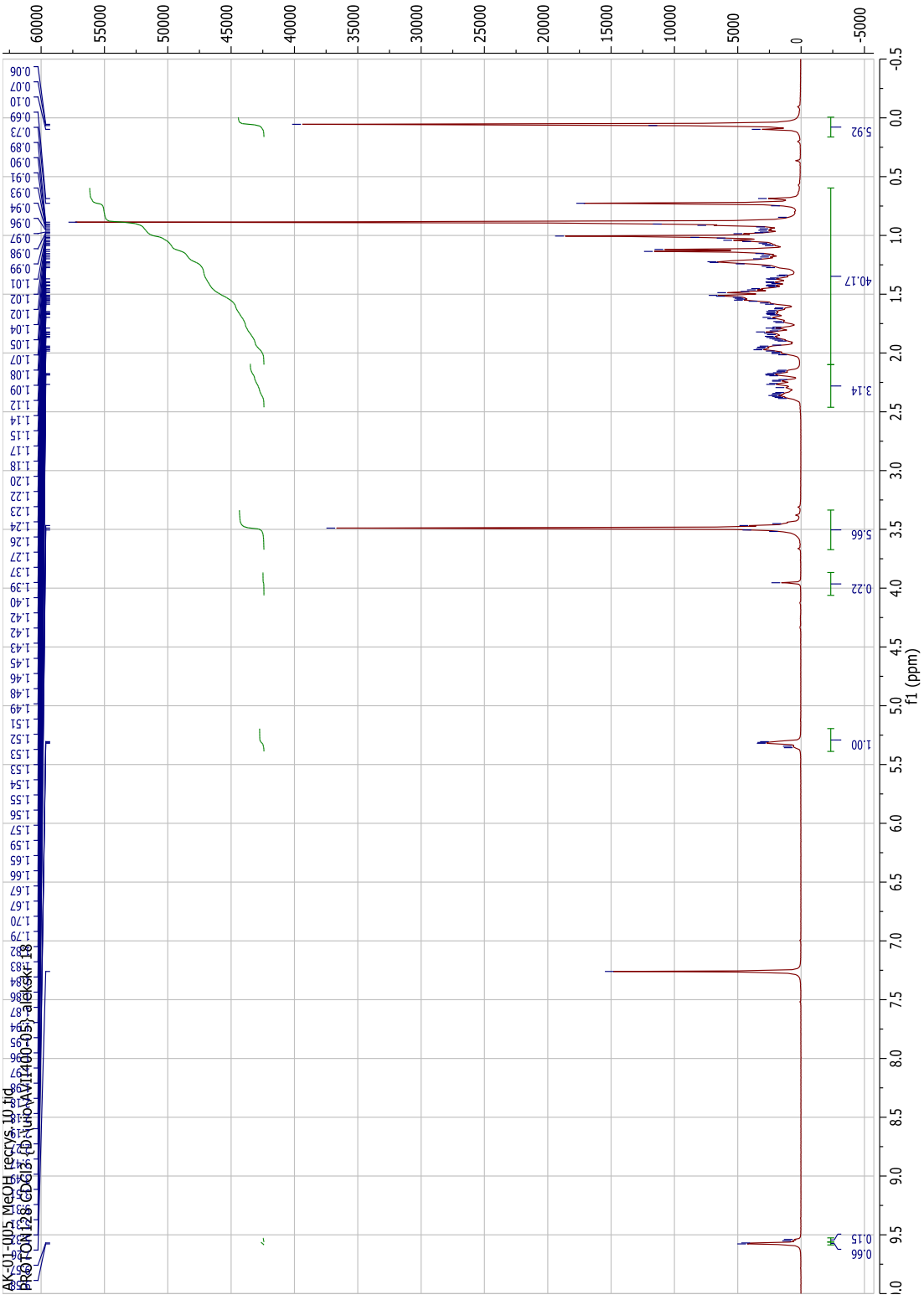
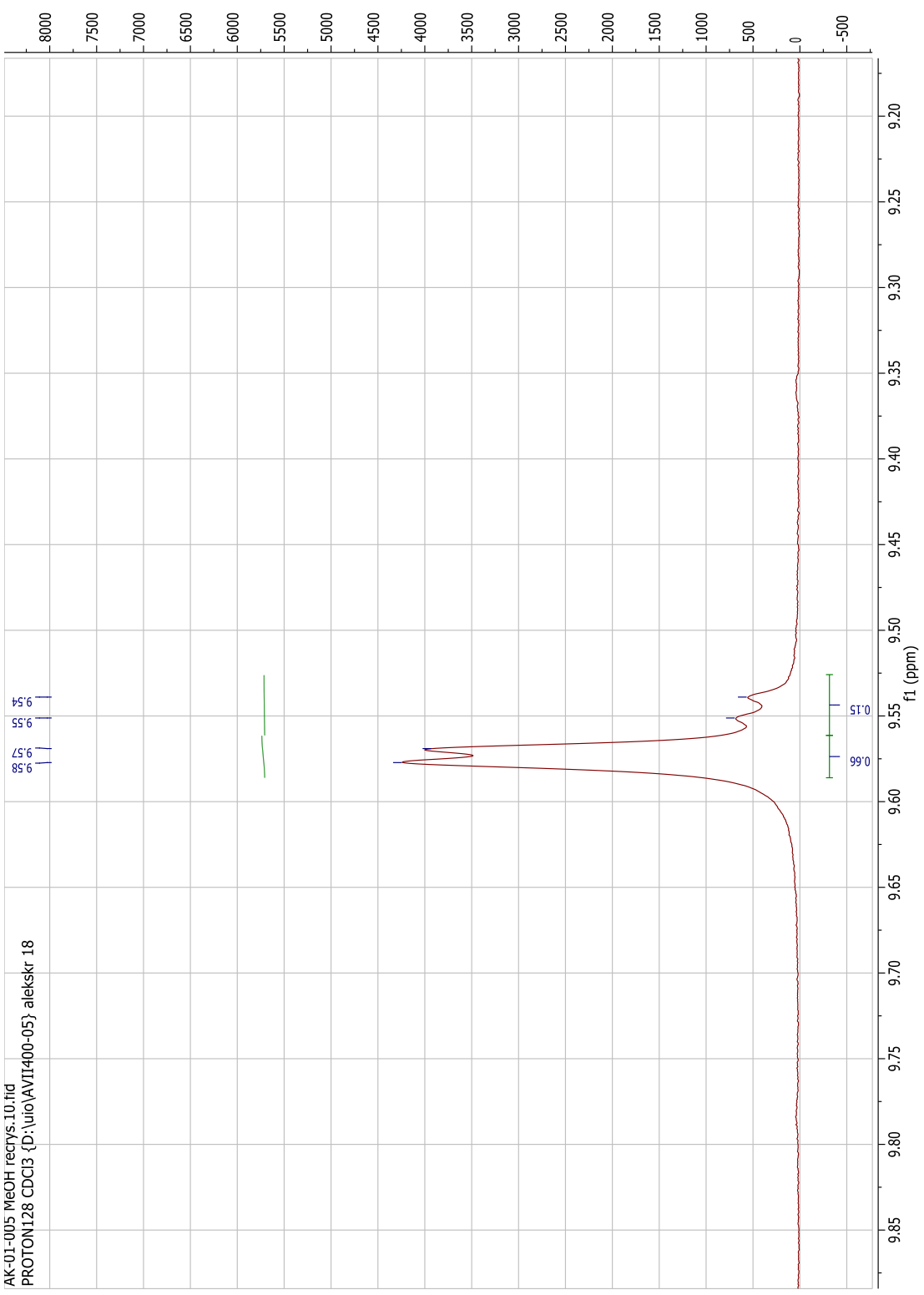
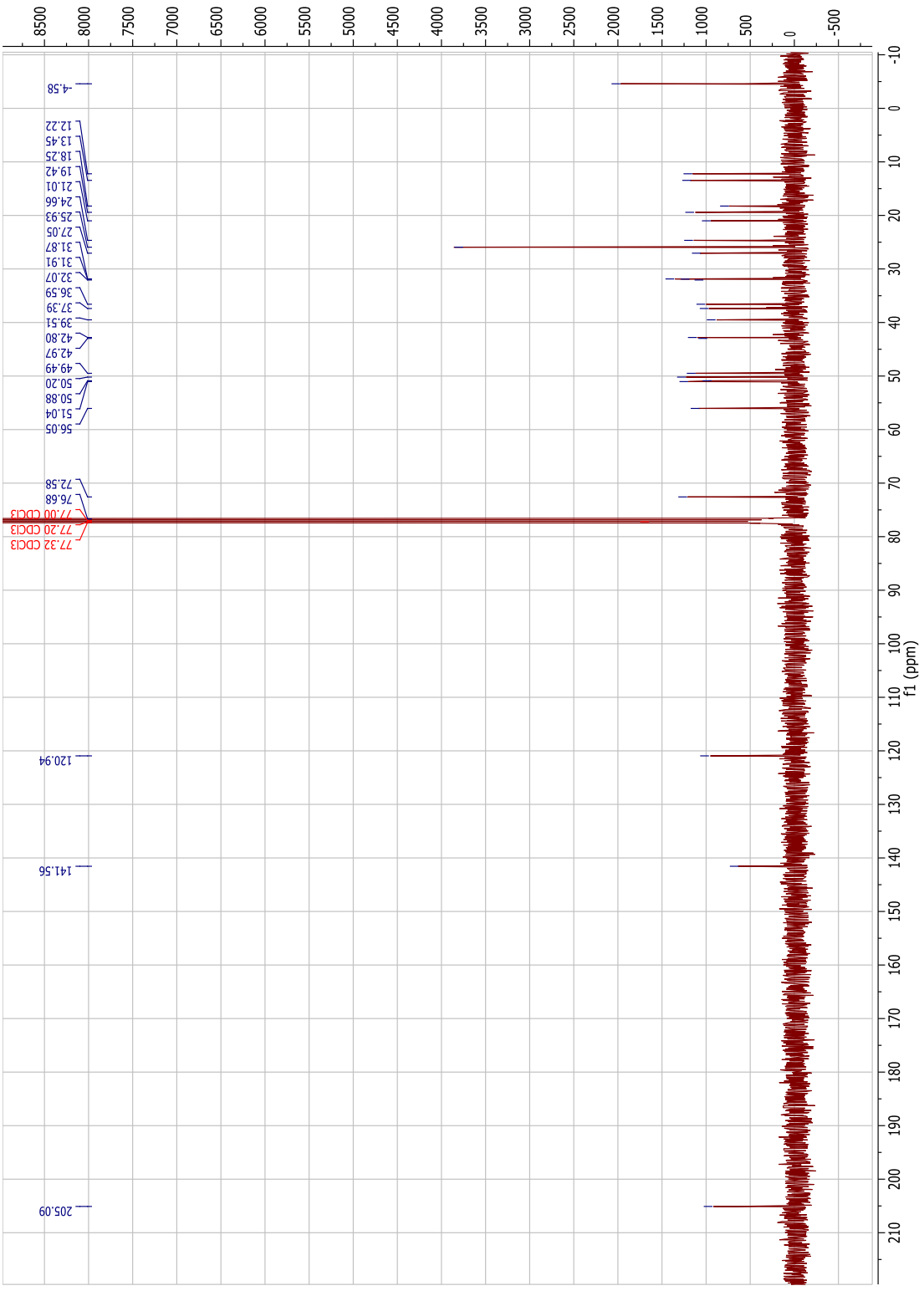
-100
9.20
9.25
9.30
9.35
9.40
9.45
9.50
9.55
9.60
9.65
9.70
9.75
9.80
f1 (ppm)
9.54
9.55
9.57
9.58
0.16
0.76

B.7 ^{1}H-NMR spectra of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al precipitated from MeOH (5a)

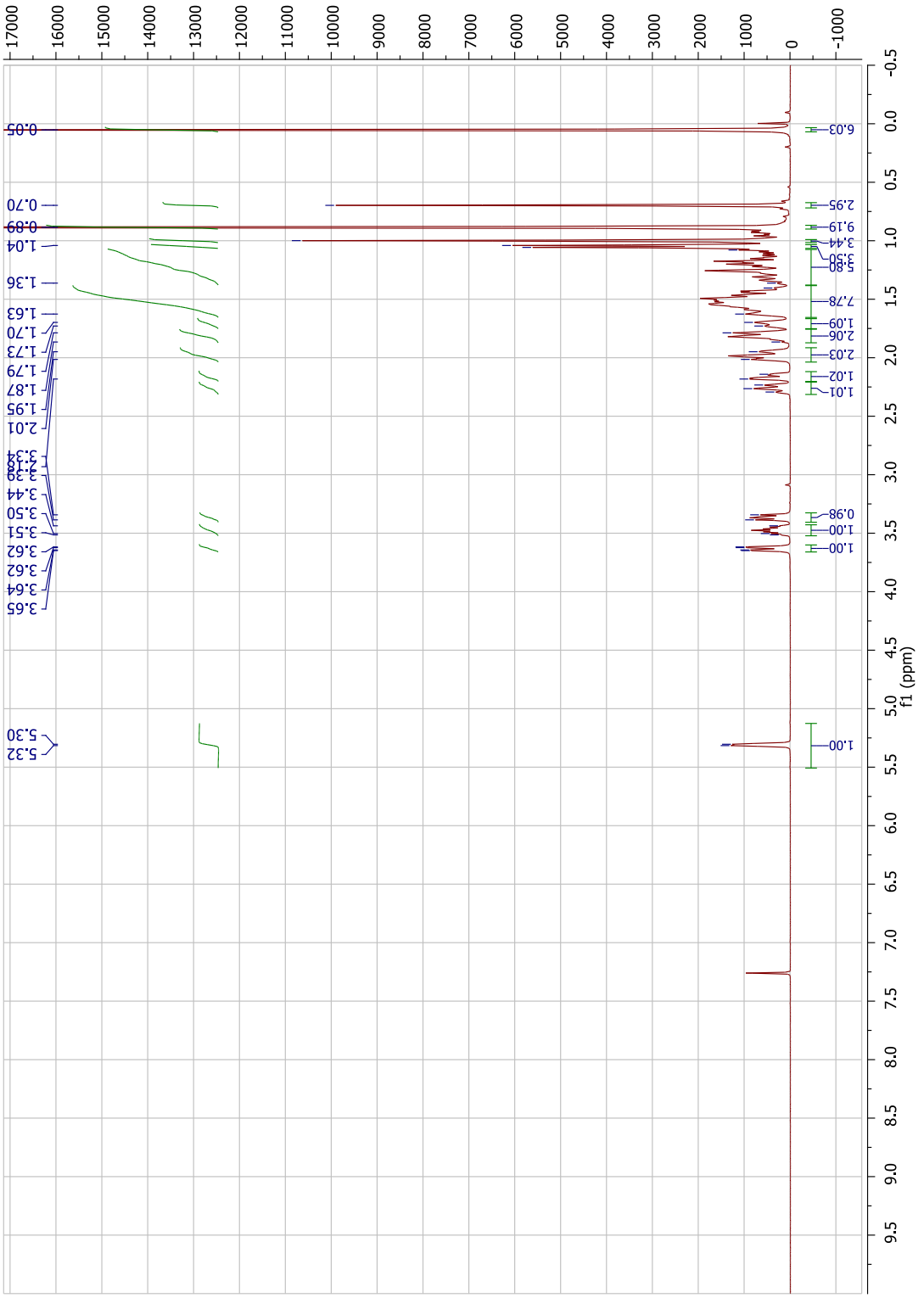



AK-01-005 MeOH recrys.10.fid
PROTON128 CDCl3 {D:\uio\AVII400-05} aleksk r 18
9.58
9.57
9.55
9.54
0.66
0.15
f1 (ppm)

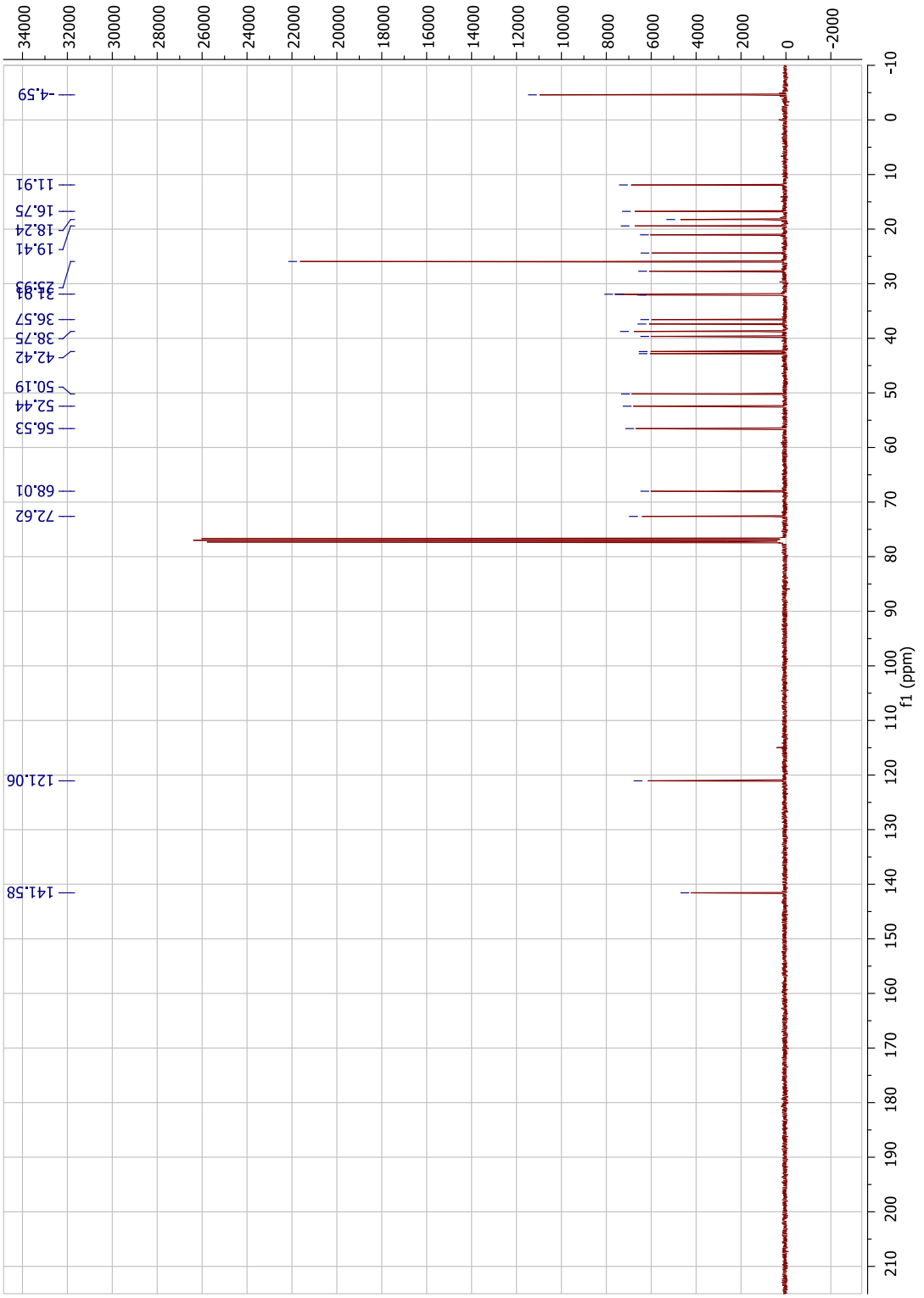
B.8 ^{13}C CPD spectrum of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al precipitated from MeOH (5a)



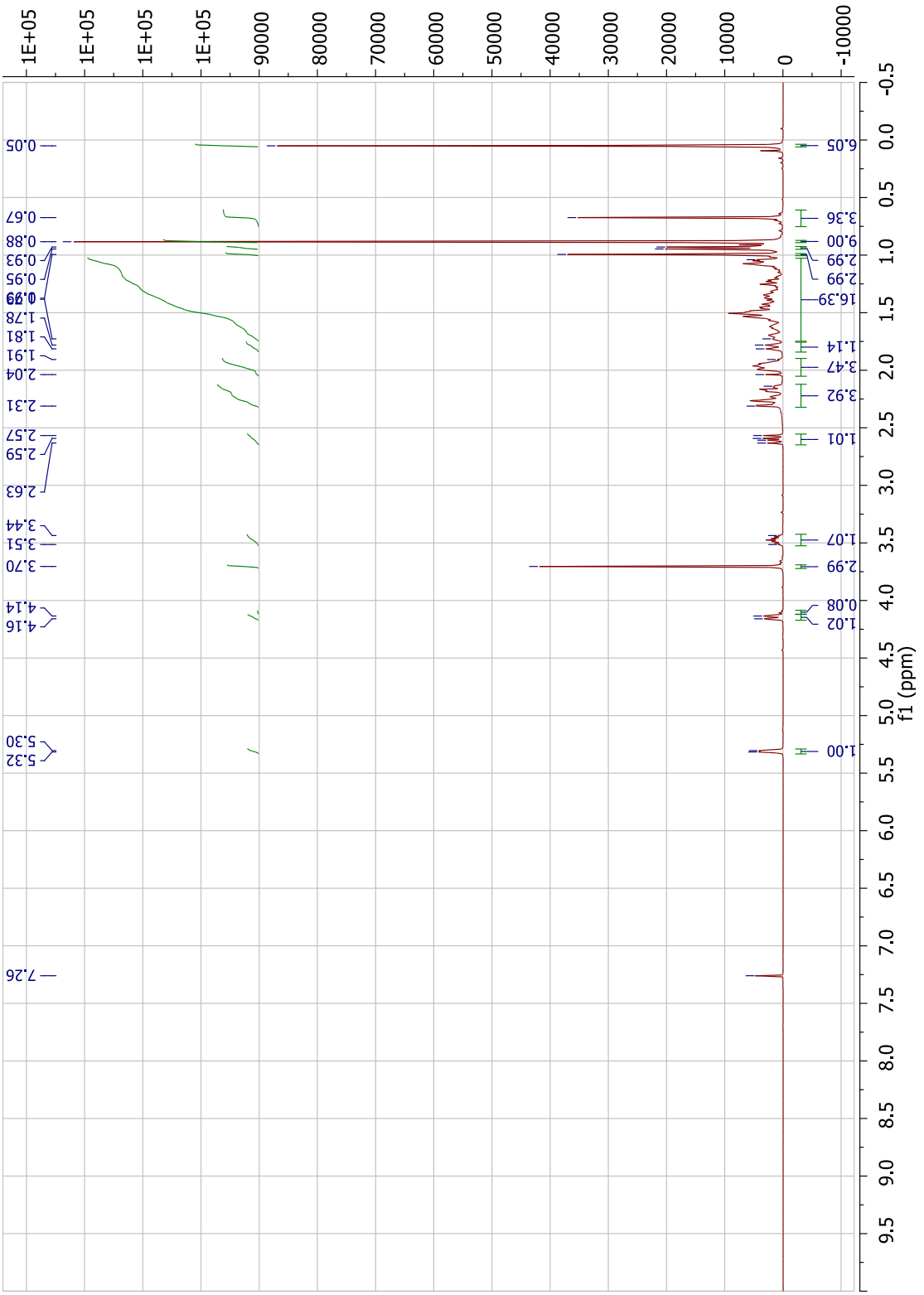
B.9 ^{1}H-NMR spectrum of of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5)



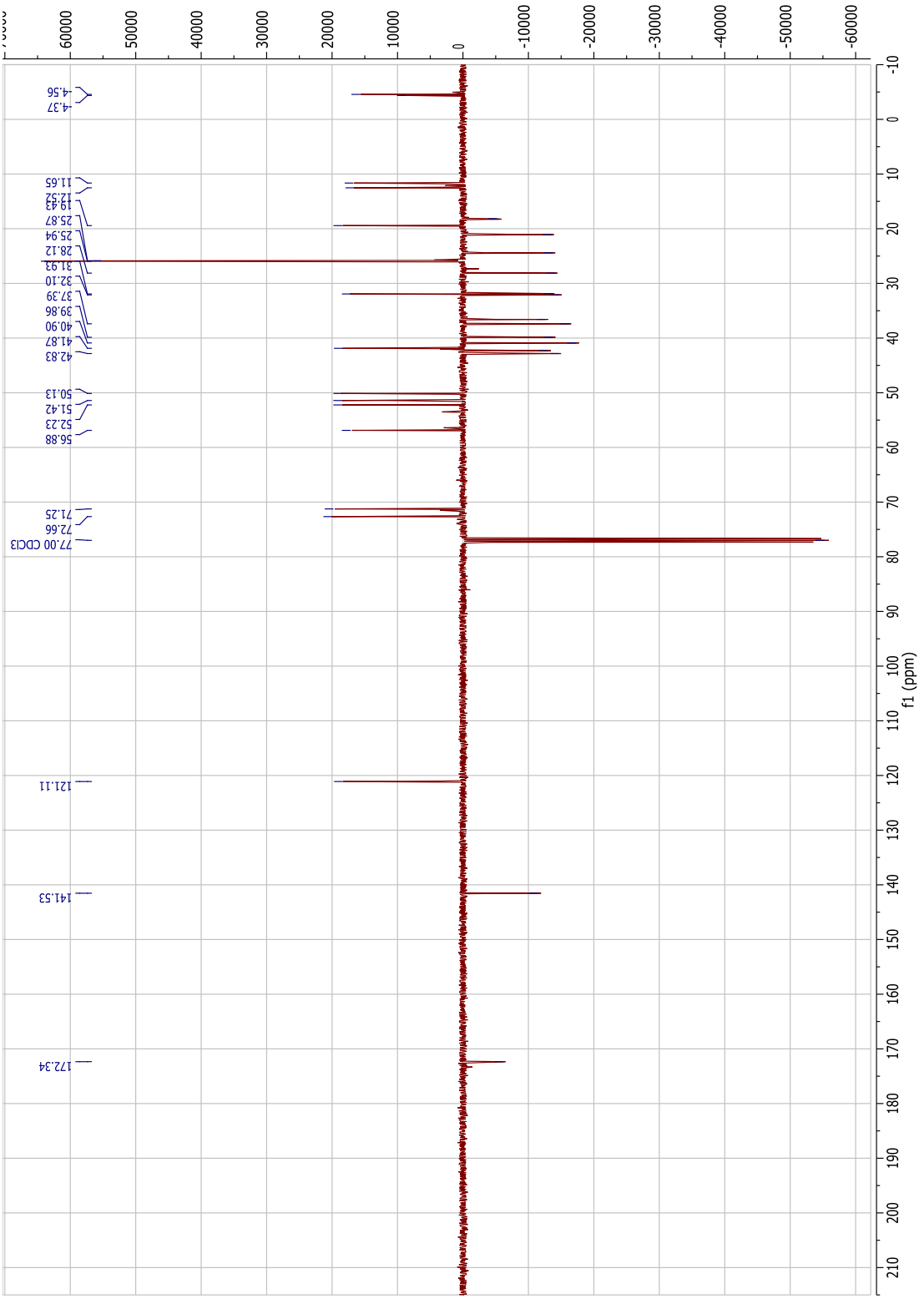
B.10 ^{13}C CPD NMR spectrum of of 3β-(tert-butyldimethylsilyl)-oxy-23,24-bisnorchol-5-ene-22-al (5)



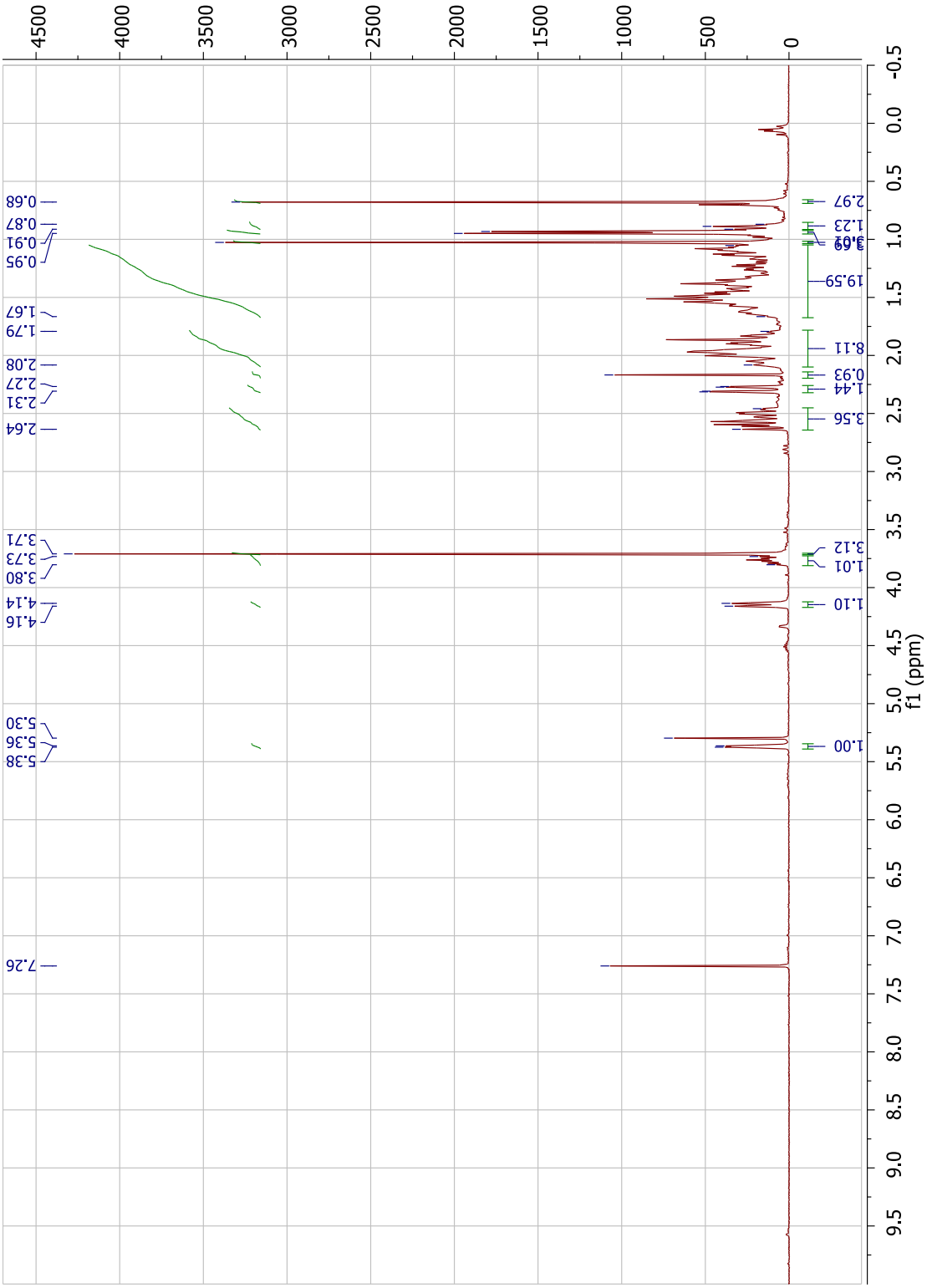
B.11 ^{1}H-NMR spectrum of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22(S)-ol-24-methyloate (6)



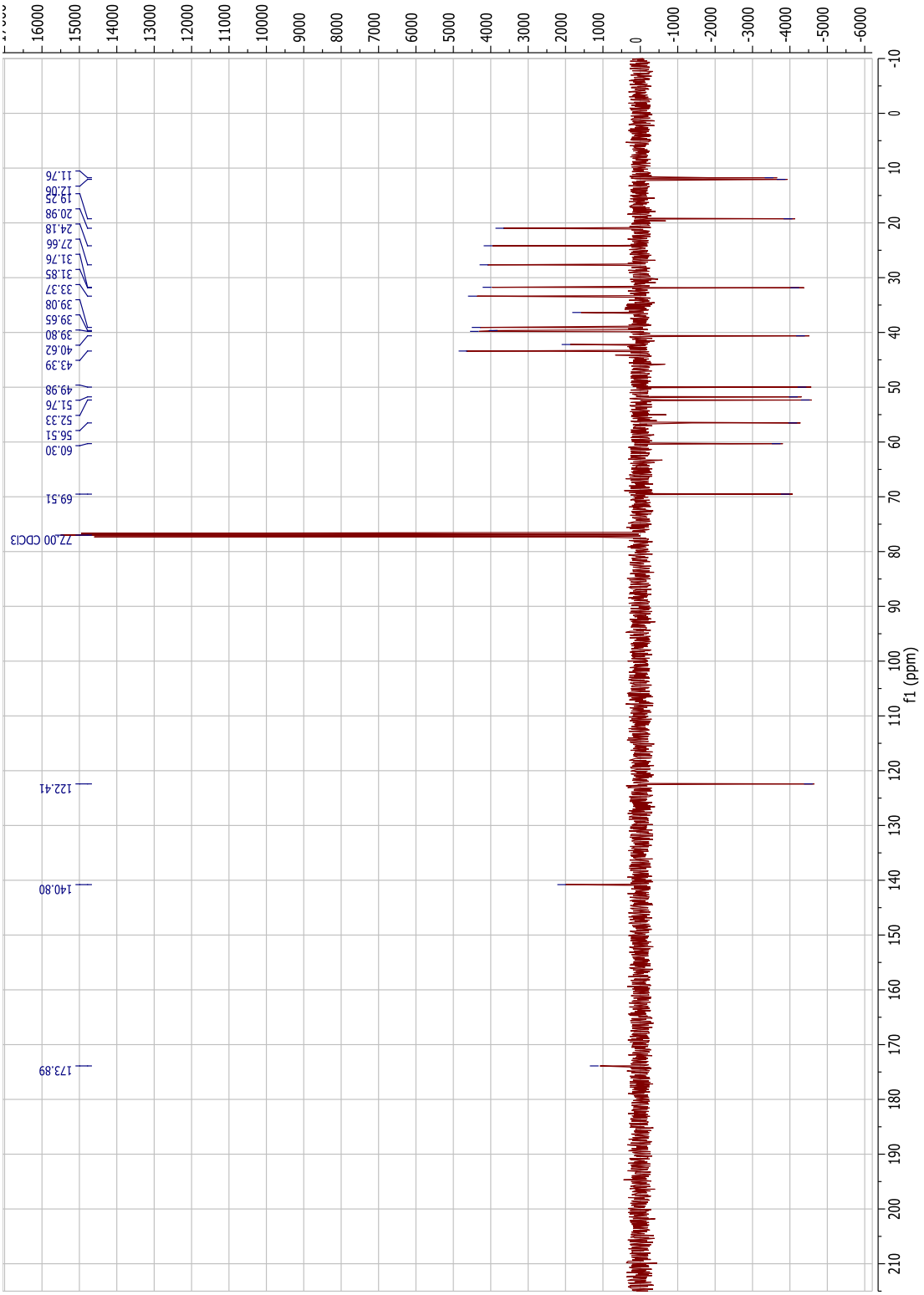
B.12 ^{13}C APT spectrum of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22(S)-ol-24-methyloate (6)



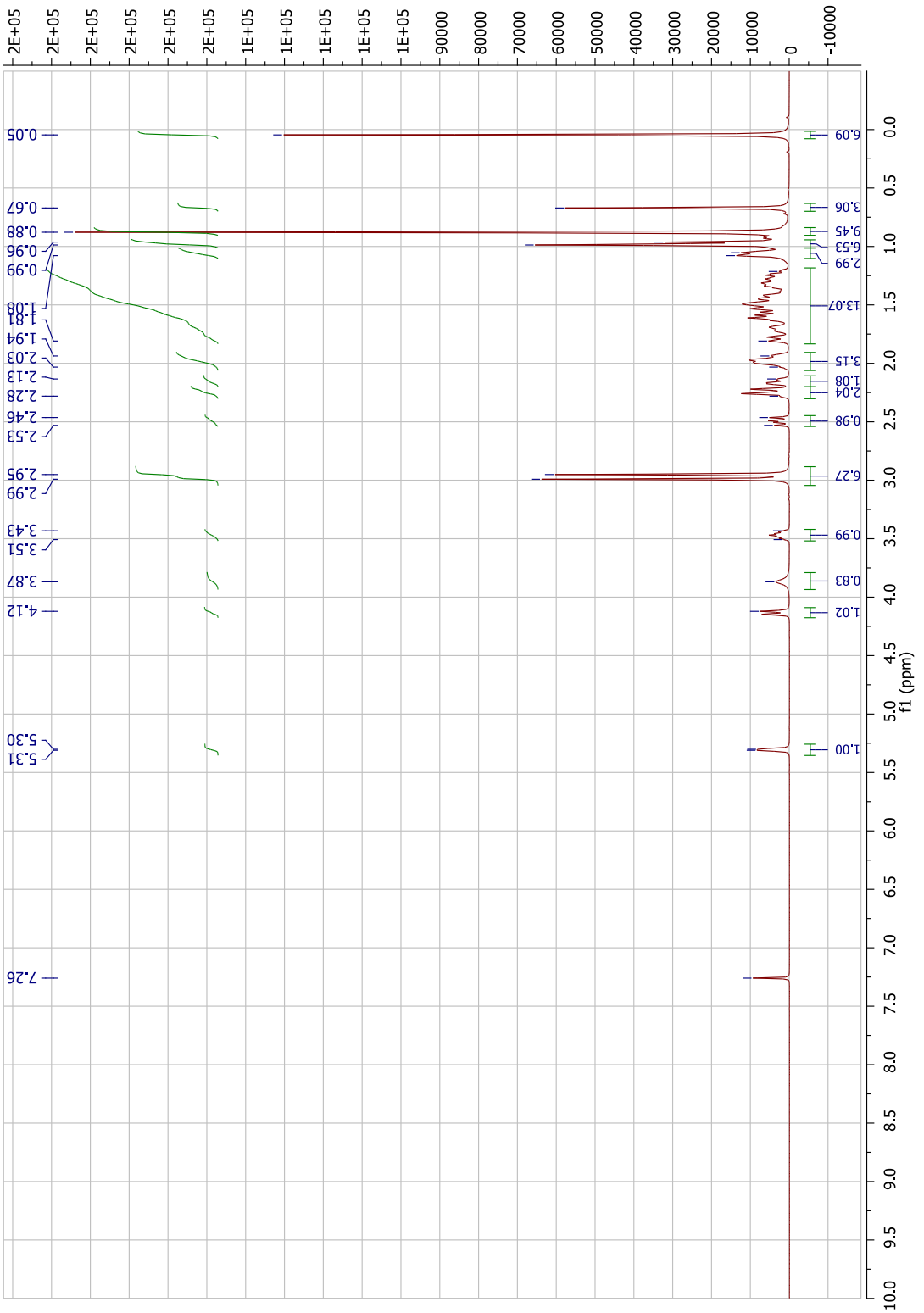
B.13 ^{1}H-NMR spectrum of chol-5-ene-3(S),22(S)-diol-24-methyloate (7)



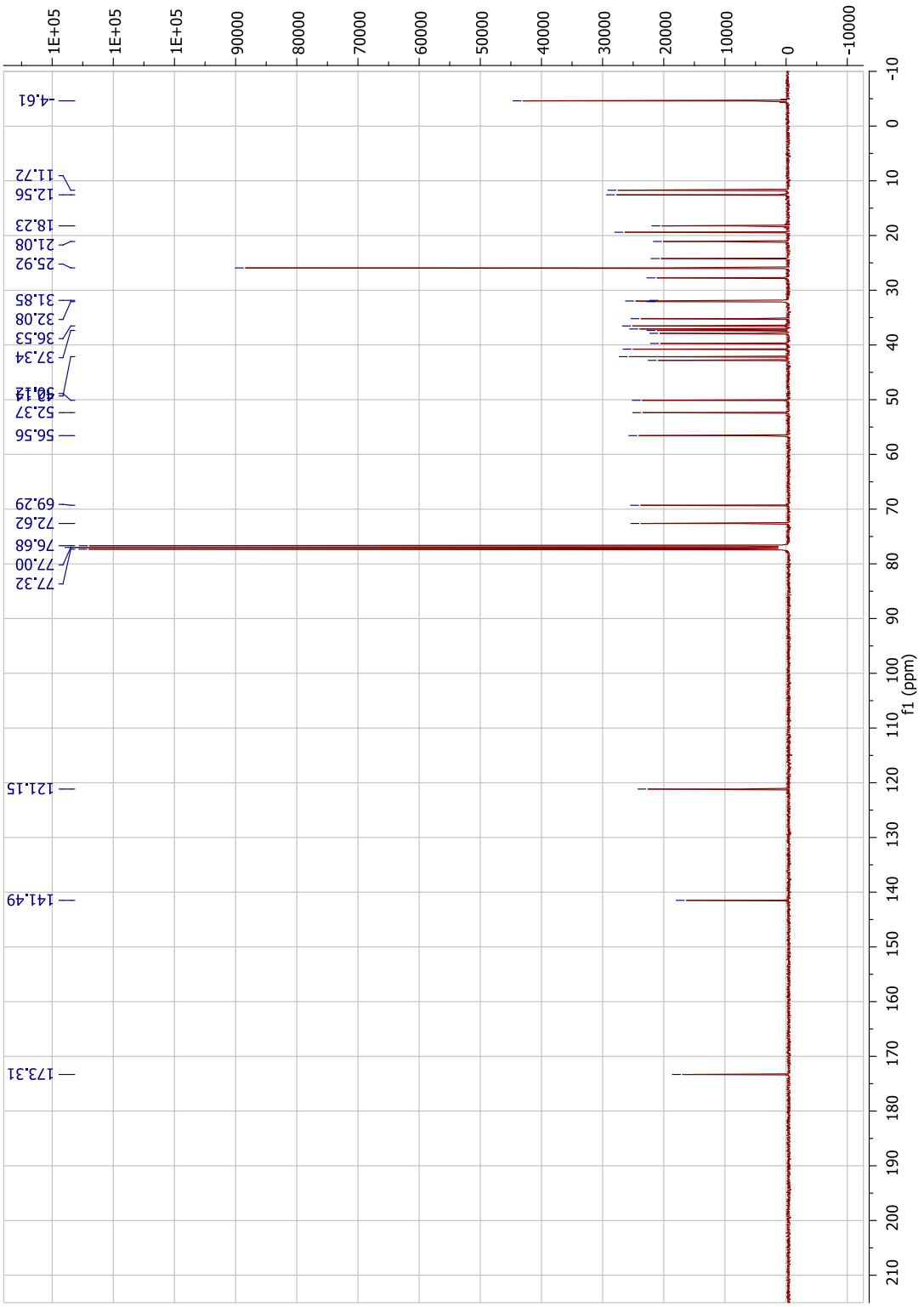
B.14 ^{13}C APT spectrum of chol-5-ene-3(S),22(S)-diol-24-methyloate (7)



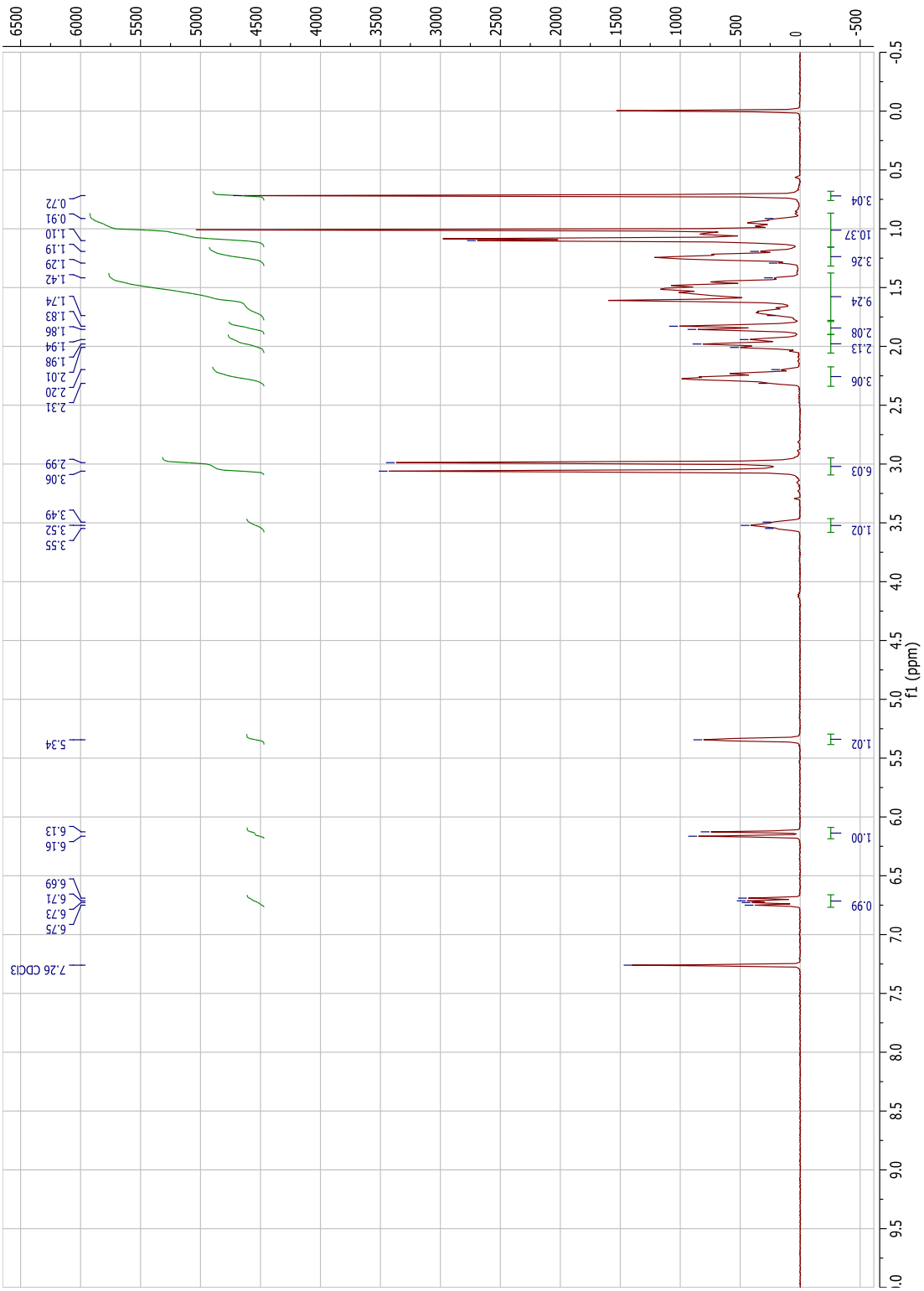
B.15 ^{1}H-NMR spectrum of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-*N*,*N*-dimethyl amide (8a)



B.16 ^{13}C CPD spectrum of 3β-(tert-butyldimethylsilyl)-oxy-chol-5-ene-22-(S)-ol-24-*N*,*N*-dimethyl amide (8a)



B.17 ^{1}H-NMR spectrum of chol-5,22-adiene-3(S)-ol-24-N,N-dimethyl amide (10)



B.18 ^{13}C CPD spectrum of chol-5,22-adiene-3(S)-ol-24-N,N-dimethyl amide (10)