THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Chemistry)

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FIELD	**DEPARTMENT**

TITLE: Synthesis of Key Intermediates to Antiviral Sterol Orthoesters and Synthesis of New Polyhydroxy Sterols with Anticancer Activity Evaluation

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THESIS

SYNTHESIS OF KEY INTERMEDIATES TO ANTIVIRAL STEROL ORTHOESTERS AND SYNTHESIS OF NEW POLYHYDROXY STEROLS WITH ANTICANCER ACTIVITY EVALUATION

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A Thesis Submitted in Partial Fulfillment of
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Sterol orthoesters which are marine natural products, isolated from the Caribbean sponge *Petrosia weinbergi*, exhibited *in vitro* activity against the feline leukemia virus (FeL V), mouse influenza virus (PR8) and mouse corona virus (A59). Up to the present, there has been no report on the synthesis of sterol orthoesters and their intermediates, so synthesis of antiviral sterol orthoesters intermediates has been investigated by us. Two key intermediates, 2α, 3α-epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one and 16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-pregn-20-ene for intramolecular strategy have been successfully synthesized in 10 and 13 steps with 11.9% and 0.12% overall yield, respectively by using tigogenin obtained from waste of *Agave sisalana* leaves or commercially available 3β-acetoxy-5-pregnen-20-one as starting material. Intermolecular strategy was also a tool for synthesizing the key intermediate, 3β-*tert*-butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene from cheaply available diosgenin in 8 steps with 0.55% overall yield.

Six new polyhydroxy sterols and their sulfated analogs have also been synthesized by using Grignard reaction as a key step. Their structures contain various functionalities in ring A such as monohydroxyl group at C-3 (β) or dihydroxyl group at C-2 (β) and C-3 (α) and differ on the side chain for studying the effect on cytotoxicity against two cancer cell lines, human epidermoid carcinoma (KB) and human small cell lung carcinoma (NCI-H187). The results showed that 3β, 20(*S*), 24-trihydroxy-5α-cholestane bearing trihydroxyl group at C-3, C-20 and C-24 exhibited the strongest activity against both cell lines (IC_{50} (μg/ml) = 5.39 (KB) and 2.11 (NCI-H187)) whereas 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane containing extra hydroxyl group at C-2 in ring A was inactive against both cell lines.

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Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Associate Professor Boonsong Kongkathip, whose teaching and guidance were a source of great inspiration to me. His invaluable helpful suggestions, valuable enlightening explanations and assistance were indispensable throughout the course of my graduate study at Kasetsart University.

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Potjamarn Bunyathaworn

May 2011

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LIST OF ABBREVIATIONS

Ac	=	Acetyl
Bn	=	Benzyl
DBU	=	1, 8-Diazabicyclo[5.4.0]undec-7-ene
DCM	=	Dichloromethane
DMAP	=	*N*, *N*-Dimethylpyridine
DMF	=	*N*, *N*-Dimethylformamide
IBX	=	Iodoxybenzoic acid
*m*CPBA	=	meta-Chloroperbenzoic acid
PCC	=	Pyridinium chlorochromate
Ph	=	Phenyl
TBS	=	*tert*-Butyldimethylsilyl
TES	=	Triethylsilyl
Tf	=	Trifluoromethanesulfonate
δ	=	Chemical shift (ppm)
J	=	Coupling constant
υ_{max}	=	Maximum absorption frequency
cm^{-1}	=	Reciprocal centimeter (wave number)
m	=	Multiplet
s	=	Singlet
t	=	Triplet
q	=	Quartet
d	=	Doublet
dd	=	Doublet of doublets
ddd	=	Doublet of doublet of doublets
Hz	=	Hertz
FTIR	=	Fourier transform infrared spectroscopy
MS	=	Mass spectroscopy
CI	=	Chemical ionization
EI	=	Electron impact
HRMS	=	High resolution mass spectroscopy

LIST OF ABBREVIATIONS (Continued)

m.p.	=	Melting point
m/z	=	A value of mass divided by charge
NMR	=	Nuclear magnetic resonance
h	=	Hour

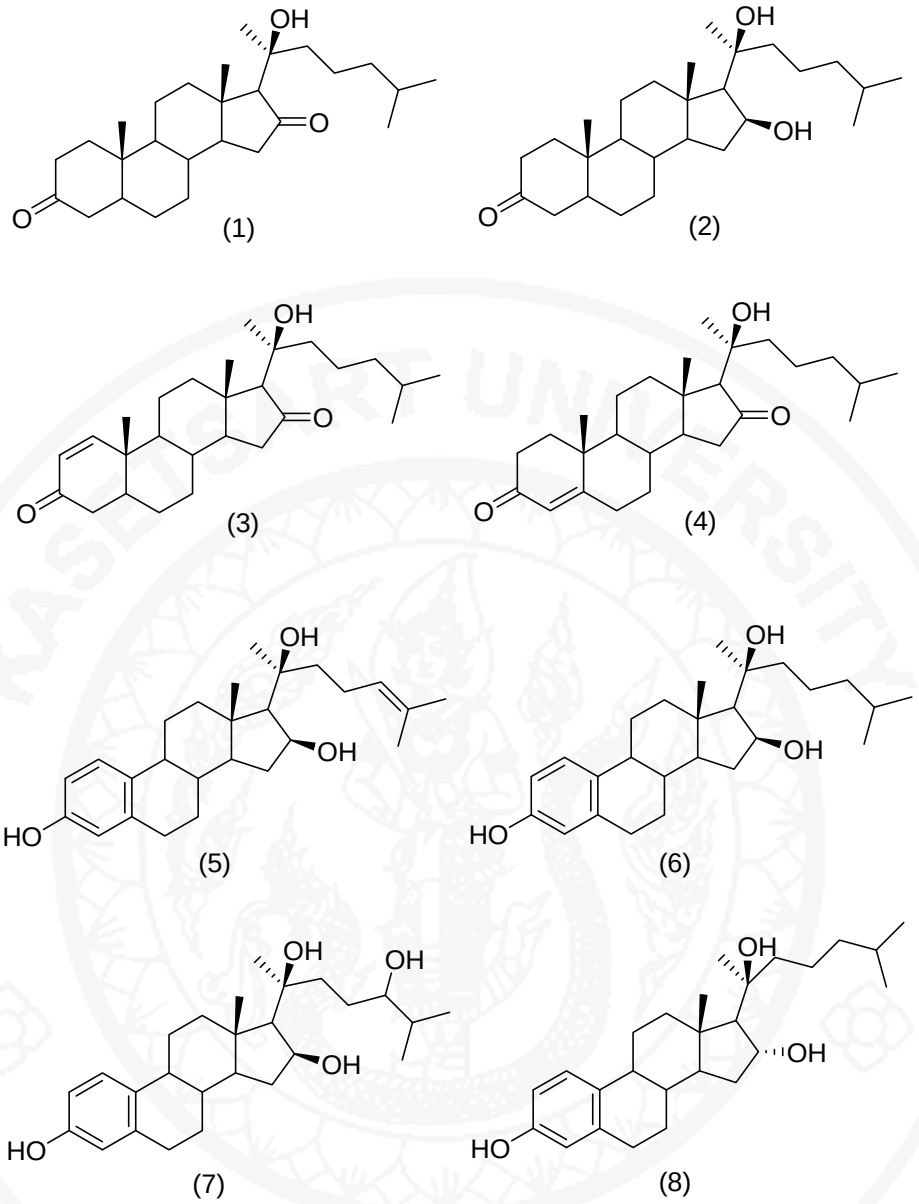
SYNTHESIS OF KEY INTERMEDIATES TO ANTIVIRAL STEROL ORTHOESTERS AND SYNTHESIS OF NEW POLYHYDROXY STEROLS WITH ANTICANCER ACTIVITY EVALUATION

INTRODUCTION

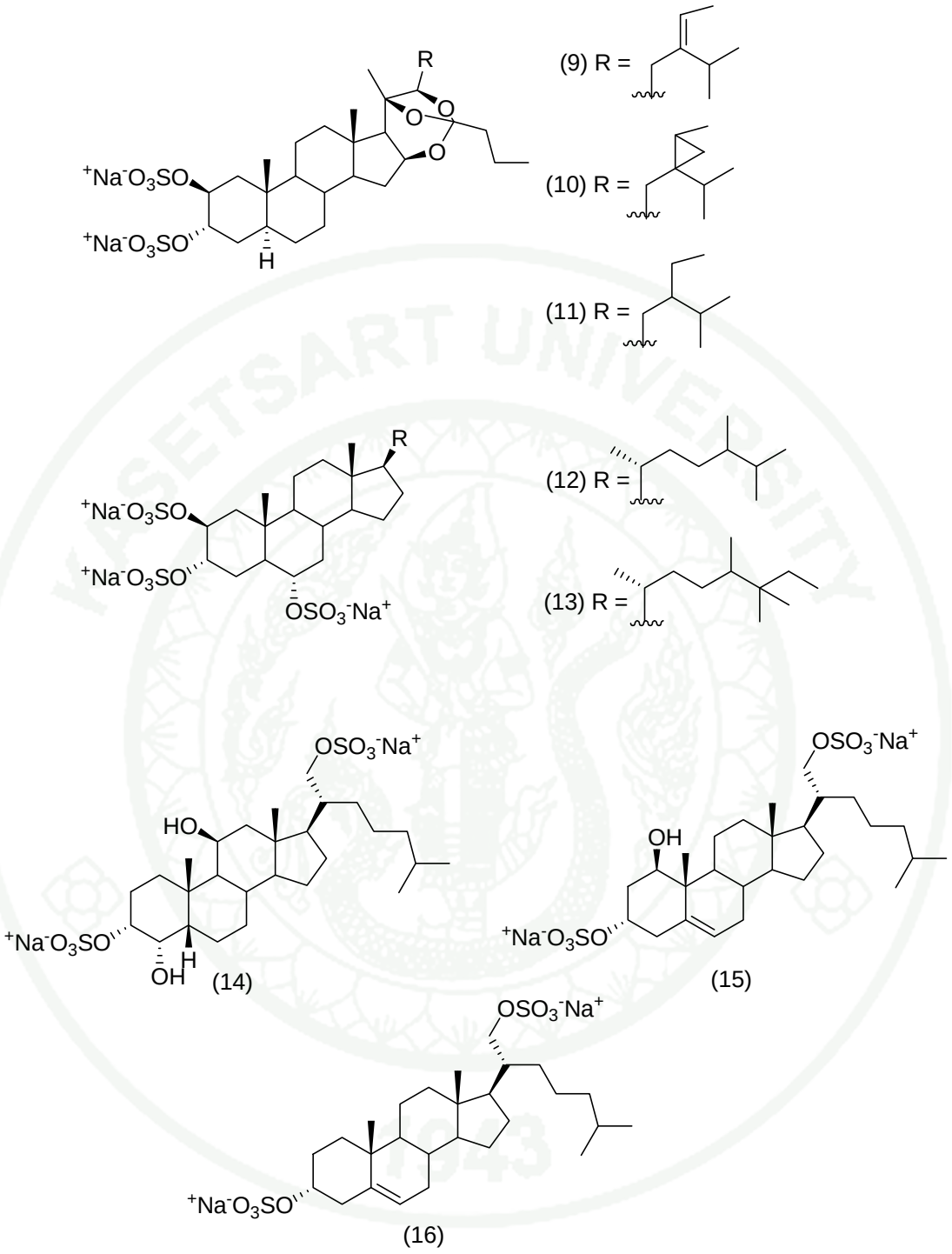
Marine organisms have historically been a rich source of novel sterol, particularly in term of unique side chain structures and unusual functionalization. The steroids isolated from sponges are sometimes very complex mixtures of highly functionalized compounds. Common structures of sponges steroids including additional oxygenation of both the nucleus and the side chain, side chain modified by alkylation and degradation, the occurrence of sulfate esters of polyoxygenated sterol have been documented.

Up to the present, two classes of interesting new steroids, polyoxygenated steroids and sulfated polyhydroxysterols, have attracted considerable attention because most of them have shown strong biological and pharmacological activities.

Polyoxygenated steroids are a large group that could be found in marine organism especially in sponges. Recently our group synthesized both of a series of polyoxygenated steroids **1**-**4** isolated from gorgonian *Leptogorgia sarmentosa* (Boonananwong *et al*., 2008a) and a series of new polyoxygenated aromatic steroid derivatives by various functionalities on side chain as saturated, unsaturated and free hydroxy cholesterol like side chains **5**-**8** which were evalutated the antitumor activity against three cell lines [human breast cancer cell line (MCF7), human lung cancer cell line (NCI) and human epidermoid carcinoma cell line (KB)] (Bunyathaworn *et al*., 2010).

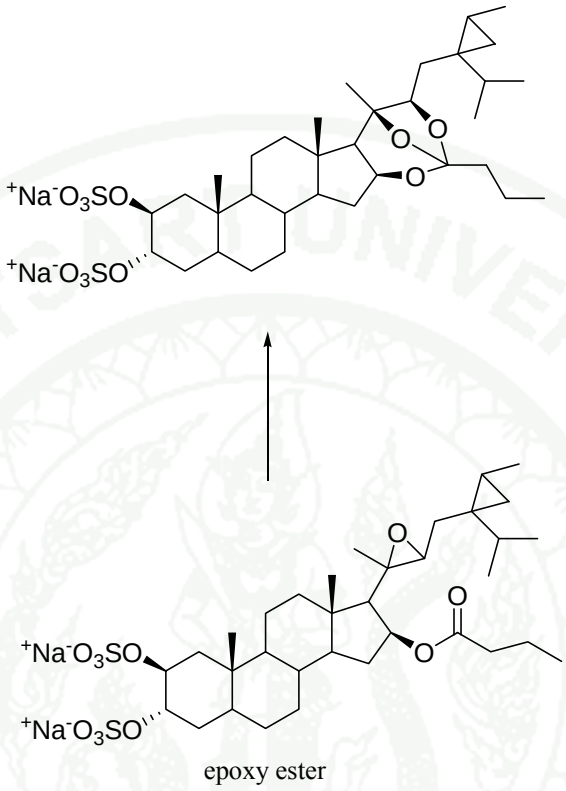


Sulfated polyhydroxysterols are naturally occurring metabolites in sponges and echinoderms (D' Auria *et al*., 1993). Most of these compounds have shown various biological activities such as antivirus (McKee *et al*., 1994) and inhibition of protein tyrosine kinase that result in control of cancer and other hyperproliferative conditions (Fu and Schmitz, 1994). For instance, Orthoesterol A (9), B (10) and C (11) exhibited *in vitro* activity against the feline leukemia virus (FeL V), mouse influenza virus (PR8) and mouse corona virus (A59) (Koehn *et al*., 1991), halistanol sulfates (12-13) showed anti-HIV activity (Bifulco *et al*., 1994) and sterol sulfate **14-16** inhibited Protein tyrosine kinase activity (Fu and Schmitz, 1994).



Orthoesterol A (9), orthoesterol B (10) and orthoesterol C (11) are marine natural products isolated from Caribbean sponge *Petrosia weinbergi*. Orthoesterols (A-C) contain a [3.2.1]-bicyclic orthoestyrate bridging the steroid side chain and ring D but they have a distinct side chain at C24-C28, i.e. orthoesterol A contains the olefinic function at this position whereas orthoesterol B and orthoesterol C possess the

cyclopropane ring and saturated function, respectively. Biosynthesis of orthoesterols may originate from an epoxy ester (Giner and Faraldos, 2002).



Biomimetic study of orthoester

.Our group has studied syntheses of several steroidal drugs, such as prednisolone (Mongkolsripatana, 1997), triamcinolone (Khunnavutimanotum, 1998), cyproterone acetate and its derivative (Sakee *et al*., 2003a; Sakee *et al*., 2003b), estradiol (Hasukunpaisarn, 2000), aglycones of OSW-1 (Chaosuansharoen, 2004), anticancer 9,11-secosterols (Hasukunpaisarn, 2005), and 3,16,20-polyoxygenated steroids (Boonananwong, 2007).

Since no report on synthesis of sulfated steroids, orthoesterol A, orthoesterol B and orthoesterol C, has been published. Therefore first synthesis of these steroids and their intermediates defies us to study using common steroids etc., tigogenin and diosgenin. Furthermore, polyoxygenated steroids that our group has studied on synthesis and evalution of their activities by construction and modification in steroids

skeleton and side chain bearing with D ring have shown the interesting results. For this reason prompts us to investigate by synthesizing a new series of polyoxygenated steroids as polyhydroxy sterols with different degrees of oxidation at C-2, C-3, C-20 and C-24 and their sulfated analogs were also synthesized. Finally, the anticancer and antiviral activities of these synthetic steroids will evaluate to gain insight into their structure-activity relationships (SARs).

OBJECTIVES

1. To synthesize sterol orthoesters key intermediates for intramolecular and intermolecular strategies

2. To synthesize new polyhydroxy sterols and their sulfated analogs

3. To evaluate anticancer activity of synthetic polyhydroxy sterols and sulfated steroid

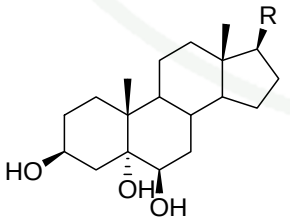
LITERATURE REVIEW

Early reports and biological activity of polyoxygenated steroids and polyhydroxy sterols

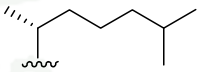
Recently, marine sponges have been known as a rich sources of steroids. Due to this reason, examination of isolation the naturally occurring compounds and evaluation of their biological activities have continued from many research groups.

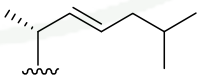
Polyoxygenated steroids and polyhydroxy sterols isolated from various organism, algae, marine invertebrate phyla such as Porifera, Coelenterata, Echinodermata and Tunicata and also in fish (D' Auria *et al*., 1993) is one class of active steroids.

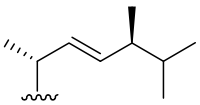
Marine sponge *Cliona copiosa*, collected from two different places of the Mediterranean sea, contained 5α-cholestane-3β, 5, 6β-triol (17), new five trihydroxy steroids, (22E)-5α-cholest-22-ene-3β, 5, 6β-triol (18), (22E, 24*S*)-24-methyl-5α-cholest-22-ene-3β, 5, 6β-triol (19), (22E, 24*R*)-24-methyl-5α-cholest-22-ene-3β, 5, 6β-triol (20), (24*R*)-24-ethyl-5α-cholestane-3β, 5, 6β-triol (21) and (24*S*)-24-ethyl-5α-cholestane-3β, 5, 6β-triol (22) (Notaro *et al*., 1991).

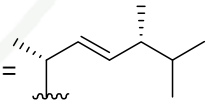


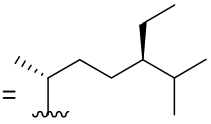
Occurrence : *Cliona copiosa*

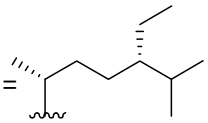
(17), R = 

(18), R = 

(19), R = 

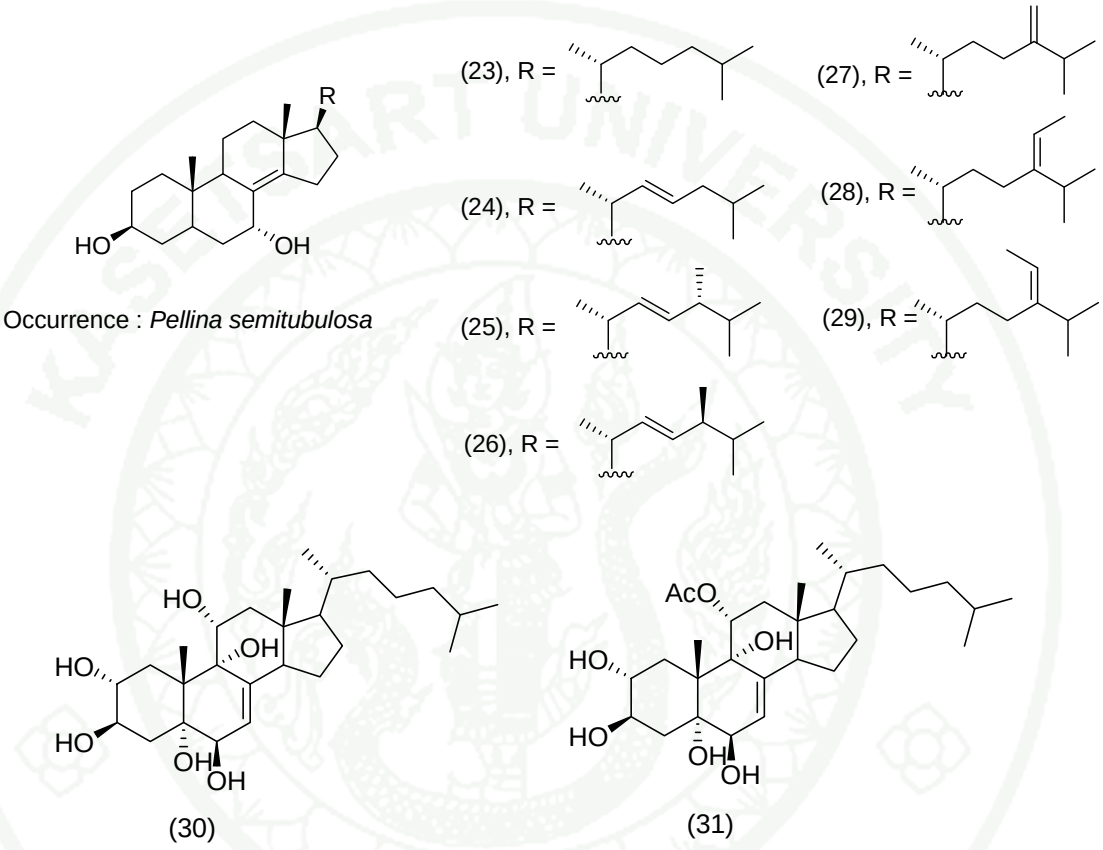
(20), R = 

(21), R = 

(22), R = 

Previously synthesized 5α-cholest-8(14)ene-3β, 7α-diol (23) and six new dihydroxy steroids **24**-**29** isolated from the marine sponge *Pellina semitubulosa* have been reported. Their structures were confirmed by synthesis of **23** starting from 5α-

cholest-7-ene-3β-ol (Notaro *et al*., 1992a). In this year, 1992, two new polyoxygenated steroids **30-31** were discovered from the Black sea sponge *Dysidea fragilis* (Milkova *et al*., 1992). Their structures and stereochemistry have been interpreted by analysis of spectral data.

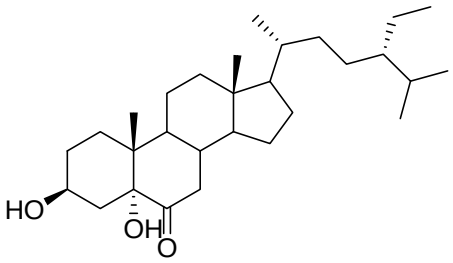


Occurrence : *Pellina semitubulosa*

(30) (31)

Occurrence : *Dysidea fragilis*

Chemical investigation of marine sponge *Spirastrella inconstans* led to new cholesterol derivative, (24*S*)-24-ethylcholesta-3β, 5α-diol-6-one (32) together with cholesterol, clionasterol and (24*S*)-24-ethylcholesta-3β, 5α, 6β-triol (33) (Das and Srinivas, 1992). The structure of **32** established from spectral data and confirmed by synthesis.



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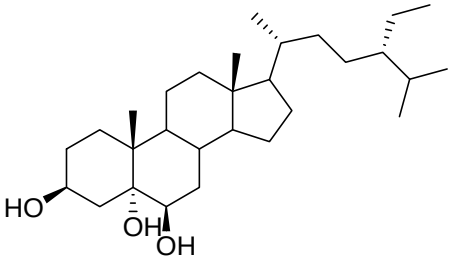
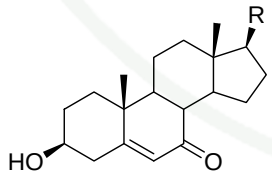
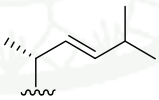


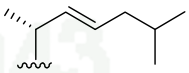
(33)

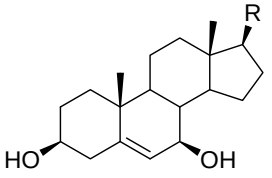
Occurrence : *Spirastrella inconstans*

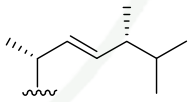
Polyoxygenated steroids **34**-**40**, isolated from marine sponge *Cliona copiosa*, were new compounds (Notaro *et al*., 1992b). Marine sponge *Spirastrella inconstans* contained new clionasterol derivative, (24*S*)-24- ethylcholesta-3β, 5α, 6α-triol (41) (Das *et al*., 1993). Five new highly oxygenated sterols, (20*S*, 22E)-cholesta-1,4,22-triene-18, 20-diol-3-one (42), (20*S*, 22E)-24-methylcholesta-1, 4, 22-triene-18, 20-diol-3-one (43), (20*S*)-cholest-4-ene-16β, 18, 20-triol-3-one (44), (20*S*, 22E)-cholesta-4, 22-diene-16β, 18, 20-triol-3-one (45) and (20*S*, 22E)-methylcholesta-4, 22-diene-16β, 18, 20-triol-3-one (46) were isolated from black coral *Antipathes subpinnata*. Compounds **43**, **44**, **45** and **46** showed unexpected toxicity toward the brine shrimp (Artemia salina) with LC_{50} of 55.6, 30.7, 7.2 and 139.4 μg/ml respectively (Aiello *et al*., 1992).

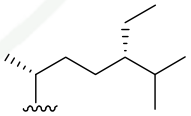


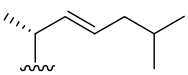
(34), R = 

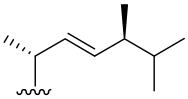
(35), R = 

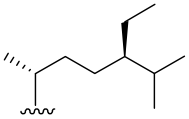


(36), R = 

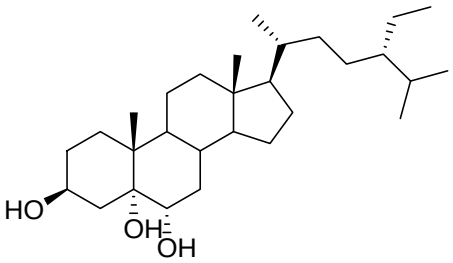
(37), R = 

(38), R = 

(39), R = 

(40), R = 

Occurrence : *Cliona copiosa*



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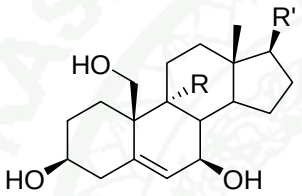
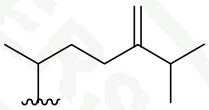
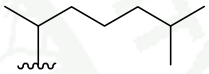
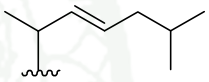
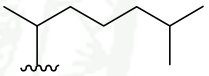
Occurrence : *Spirastrella inconstans*

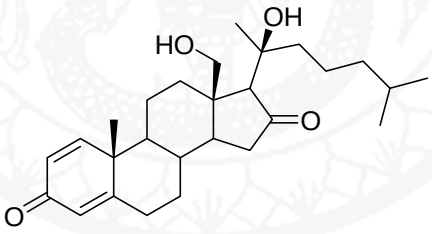


(42), R = H, R' = 

(43), R = H, R' = 

(44), R = H, R' = 

(45), R = OH, R' = 

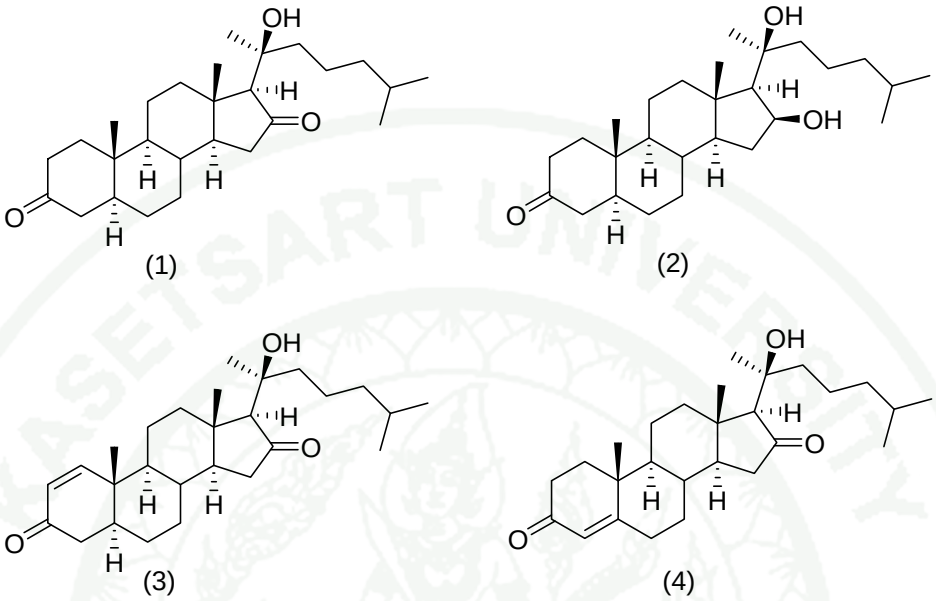


(46)

Occurrence : *Antipathes subpinnata*

A series of polyoxygenated steroids possessing uncommonly present oxidation both at C-16 and C-20 were previously studied from Mediterranean specimen of *L. sarmentosa* (Cimino *et al*., 1981, 1984; Benvegnu′ *et al*., 1982). In 2000, new cytotoxic metabolites **1-3** along with known compound **4** were isolated from marine organism, the gorgonian *Leptogorgia sarmentasa*. Compounds **1** and **2** showed significant cytotoxicity against P-388 suspension culture of mouse lymphoid neoplasm and the monolayer cultures of human lung carcinoma (A 549), human colon

carcinoma (HT 29), and human melanoma (MEL 28) exhibiting ED_{50} values of 1 μg/ml, in all cases (Garrido *et al*., 2000).



Occurrence : *Leptogorgia sarmentosa*

In 2008, our group reported first synthesis of 3, 16, 20-polyoxygenated cholestanes, new cytotoxic steroids from the gorgonian *Leptogorgia sarmentosa*. Four synthetic steroids, (20*S*)-20-hydroxycholestane-3, 16-dione (1), (16*S*, 20*S*)-16, 20-dihydroxycholestan-3-one (2), (20*S*)-20-hydroxycholest-1-ene-3, 16-dione (3) and (20*S*)-20-hydroxycholest-4-ene-3, 16-dione (4), were synthesized in 4 steps from tigogenin which could be obtained from the waste water of *Agave sisalana* industry. Compounds **3** and **4** showed strong activity against NCI (IC_{50} 6.16 and 10.51 μM) and moderate activity against MCF7 and KB (IC_{50} in range 30.65-47.22 μM). Compound **2**, being inactive in MCF7 and KB, exhibited moderate activity against NCI (IC_{50} 42.68 μM). Unfortunately, compound **1** showed no activity against in all cases (Boonananwong *et al*., 2008).

In continuation of our work on synthesis of polyoxygenated steroids for structure-actvity relationships (SARs) studies, we further investigated in the modification of steroid skeleton at ring A as aromatic and non-aromatic and functionality at C-16 as free hydroxy and ketone and side chain modification at C-20

as saturated and unsaturated cholesterol like side chain. The results showed that compounds with ring A as aromatic showed the significant potent cytotoxicities in all test cells [NCI, KB and MCF7] whereas the type of functional group and the stereochemistry at C-16 led to the different results. Moreover, the functional group on the side chain gave a selectivity on test cell lines such as unsaturated cholesterol like side chain showed no cytotoxicity against all tested cell lines and saturated cholesterol like side chain exhibiting no activity against both MCF7 and NCI but very active against KB cell lines as shown in Table 1 (Bunyathaworn *et al*., 2010).

Table 1 Cytotoxicity of synthetic polyoxygenated steroids against KB, NCI and MCF7

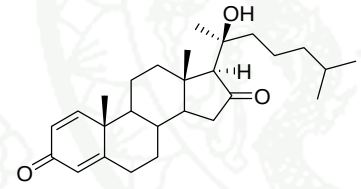
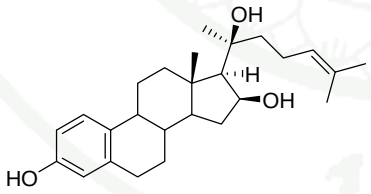
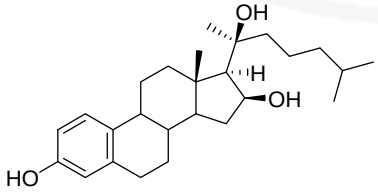
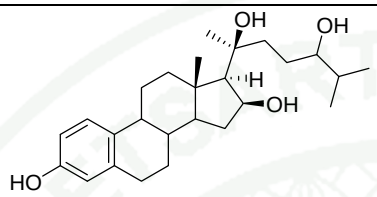
Compound	IC_{50} μM[a]		
	MCF7	NCI	KB
	55.53	55.75	48.40
	Inactive[b]	Inactive[b]	Inactive[b]
	Inactive[b]	Inactive[b]	11.73

Table 1 (Continued)

Compound	IC_{50} μM[a]		
	MCF7	NCI	KB
	42.43	Inactive[b]	20.41
Ellipticine[c]	ND	1.79	1.72
Doxorubicine[c]	1.51	0.16	0.33

MCF7, human breast adenocarcinoma; NCI, human small cell lung carcinoma; KB, human epidermoid carcinoma of cavity.

[a]; Data are typical values from six replicate experiments.

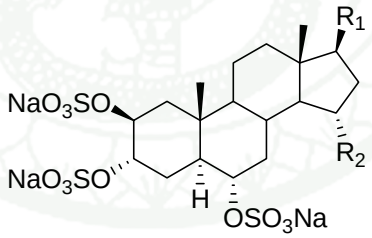
[b]; Inactive = inhibition < 50%.

[c]; Used as reference.

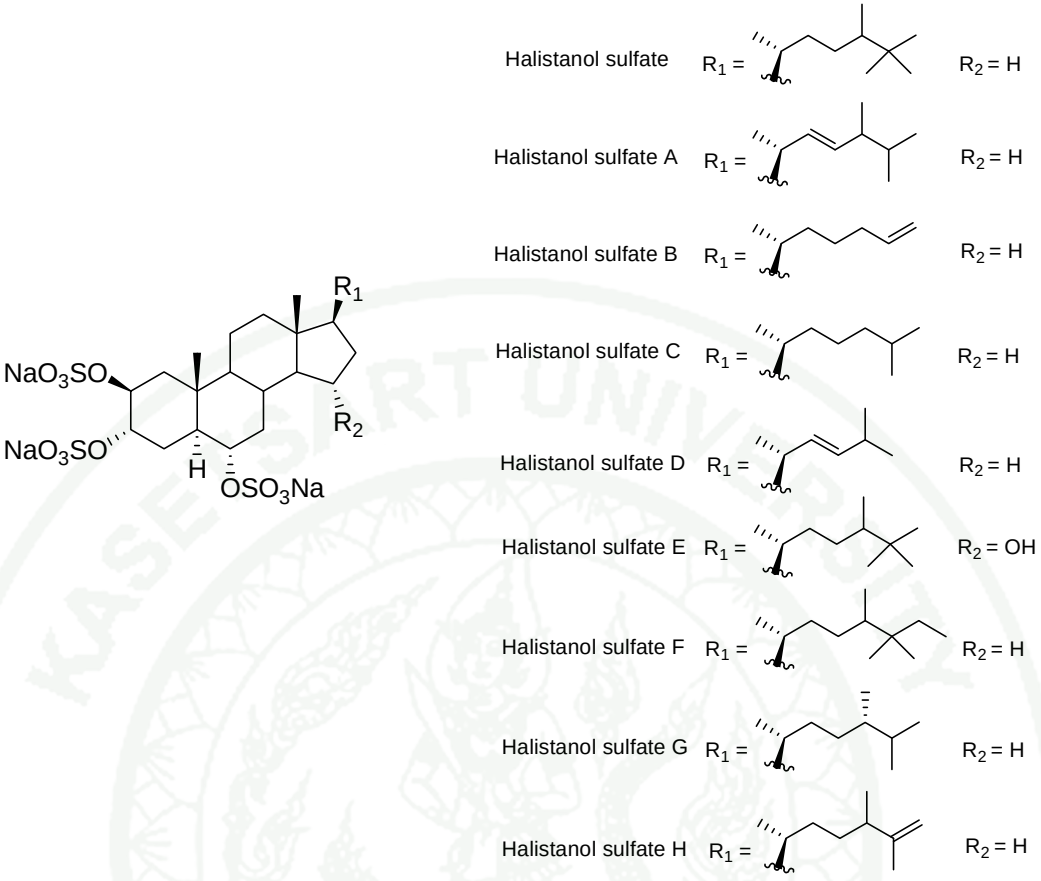
Early reports and biological activity of sulfated polyhydroxy steroids

Sulfated steroids have been recently found from a wide variety of marine organism, particularly sponges and echinoderms (Kerr and Barker, 1991). Most of the naturally occurring sulfated steroids are 2β, 3α, 6α-tri-O-sulfate, 2β, 3α,-di-O-sulfate and C-2 or C-3 and C-21 disulfate and contain a various of their side chain at C-20. These steroids exhibited a broad range of activities, HIV properties (McKee *et al*., 1994), cytotoxic action (Anderson *et al*., 1989), antimicrobial, hemolytic, ichthyotoxic (Fusetani *et al*., 1981), inhibition of protein tyrosine kinases (Fu and Schmitz, 1994).

Halistanol sulfate family is a large group of sulfated steroids from a vast sponges, Halichondriidae and Adociidae families, including the genera Halichondria (Fusetani *et al*., 1981; Makarieva *et al*., 1985), Trachyopsis (Makarieva *et al*., 1987), Toxadocia (Nakutsu *et al*., 1983) and Petrosia (Sun *et al*., 1991). They are very attractive because of their biological activity. Their common structure characterized by the same 2β, 3α, 6α-trisulfoxy function, differing only their side chain.

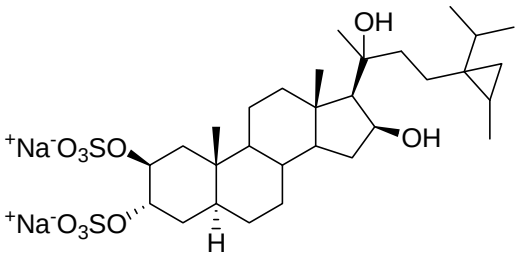


Halistanol sulfate, first isolated from Okinawan sponge *Halichondria* cf., showed the antimicrobial activity, hemolytic and ichthyotoxic activity. The structure of this compound was fully established by NMR technique of acid hydrolyzed form of halistanol sulfate (Fusetani *et al*., 1981). In 1992 new sulfated steroids, halistanol sulfate A-E were discovered from a marine sponge *Epipolasis* sp. These compounds exhibited anifungal and anti-thrombin activities (Kanazawa *et al*., 1992). Halistanol sulfate F-H were found from a sponge *Pseudoaxinissa digitata*, family Axinellidae in 1994. Halistanol sulfate F and G were proved to be cytoprotective against HIV-1 with EC_{50} 3 and 6 μg/ml, respectively (Bifulco *et al*., 1994).

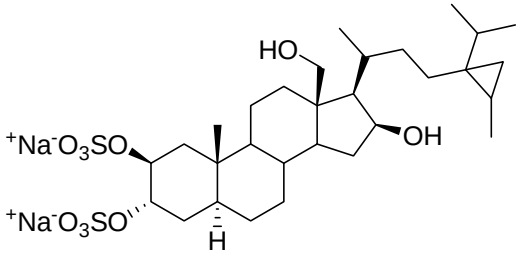


Moreover, new bioactive steroids were further screening from other sponges. The Caribbean sponge, *Petrosia weinbergi* is another species that gave unusual biologically active steroids, weinbersterols A and B including orthoesterol A-C. It was found that weinbersterols A and B showed *in vitro* activity against the feline leukemia virus (FeLV) with EC_{50} = 4.0 and 5.2 μg/ml, respectively and weinbersterols A also exhibited activity against the human immunodeficiency virus (EC_{50} = 1.0 μg/ml) (Sun *et al*., 1991).

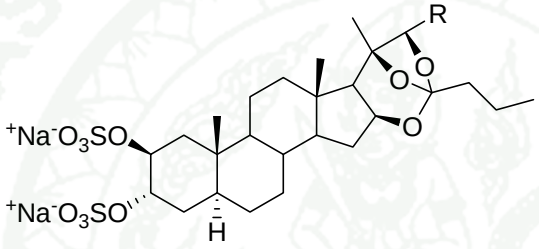
Orthoesterol A-C (9-11) isolated from marine sponge *Petrosia weinbergi* showed *in vitro* activities against feline leukemia virus (FeLV), influenza PR8 virus and mouse corona virus (Koehn *et al*., 1991). Orthoesterol A-C contain a [3.2.1]–bicyclic orthoestyrate bridging the steroid side chain and ring D but they have a distinct side chain at C24 – C25, i.e. orthoesterol A (9) contains the olefinic function at this position and orthoesterol C (11) has no functional group on side chain whereas orthoesterol B (10) contains the cyclopropane ring.



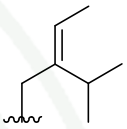
weinbersterol A



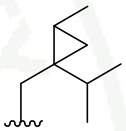
weinbersterol B



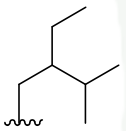
orthoesterol A, R =



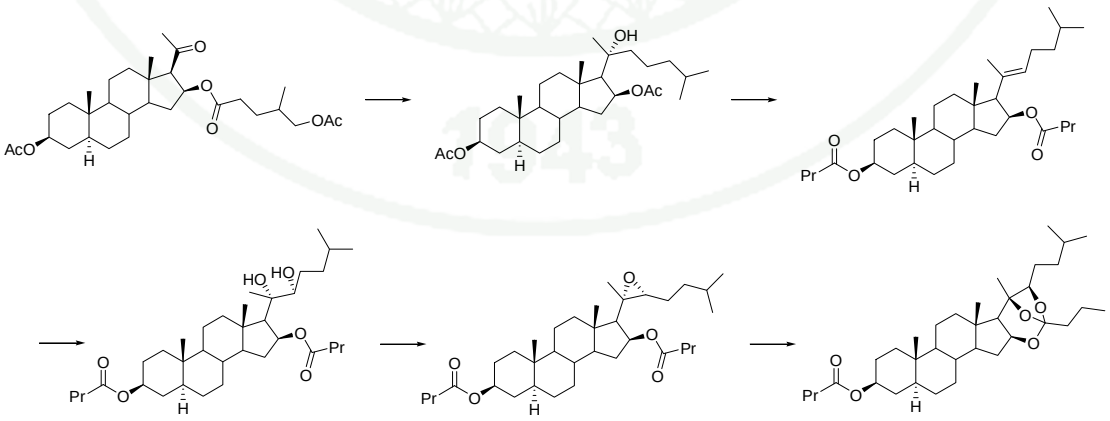
orthoesterol B, R =



orthoesterol C, R =

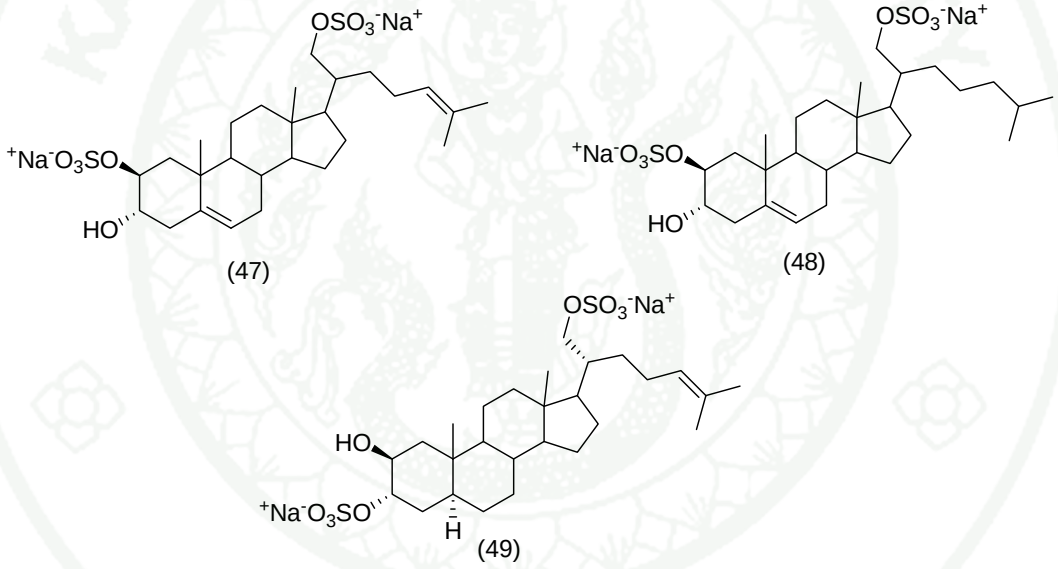


The hypothesis that othoesterol could be originated from epoxy ester was proved by construction [3.2.1]–bicyclic orthoestyrate bridging the steroid side chain under mild acidic catalysis as shown below.

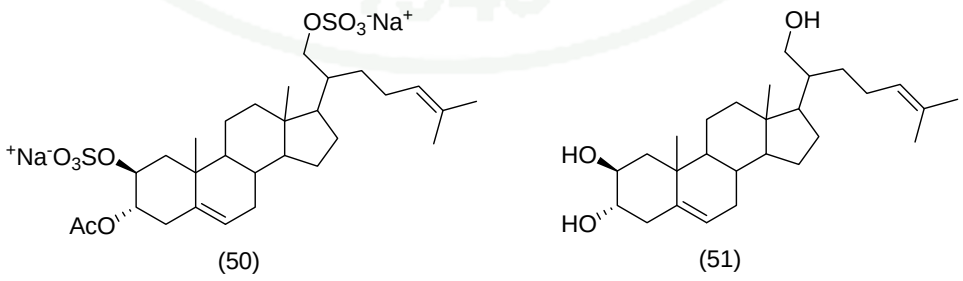


In order to gain insight into structure-activity relationships, synthetic sulfated polyhydroxysteroids were synthesized to compare evaluation of the antiviral activity

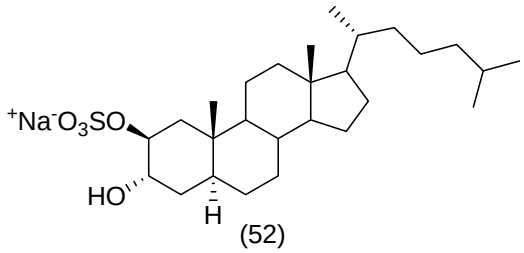
with the natural sulfated polyhydroxysteroids. For example, the natural disulfated steroids **47**-**49** and their synthetic analogs **50**-**51** containing sulfate at C-21 and C-2 (β) or C-3 (α) with additional hydroxyl group on ring A at C-2 (β) or C-3 (α) showed the most effective in their selective inhibitory action against herpes simplex virus (HSV-2) (Comin *et al*., 1999). These result led to synthesis of new synthetic sulfated steroids **52**-**53** from common 3β-hydroxy-5α-cholestane for testing the the activity. Compounds **52** and **53** were the most effective in their selective inhibitory action against herpes simplex virus (HSV-2) with IC_{50} = 19.3 and 23.9 μg/ml, respectively and compound **53** also inhibited against two pathogenic viruses, DEN-2 and JV (Garrido Santos *et al*., 2003).



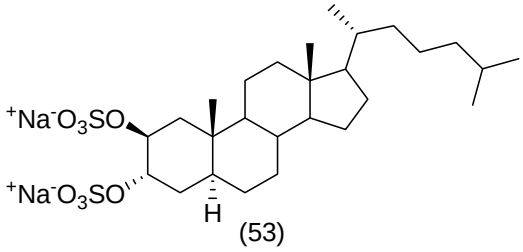
natural sulfated polyhydroxysteroids



synthetic sulfated polyhydroxysteroids



(52)



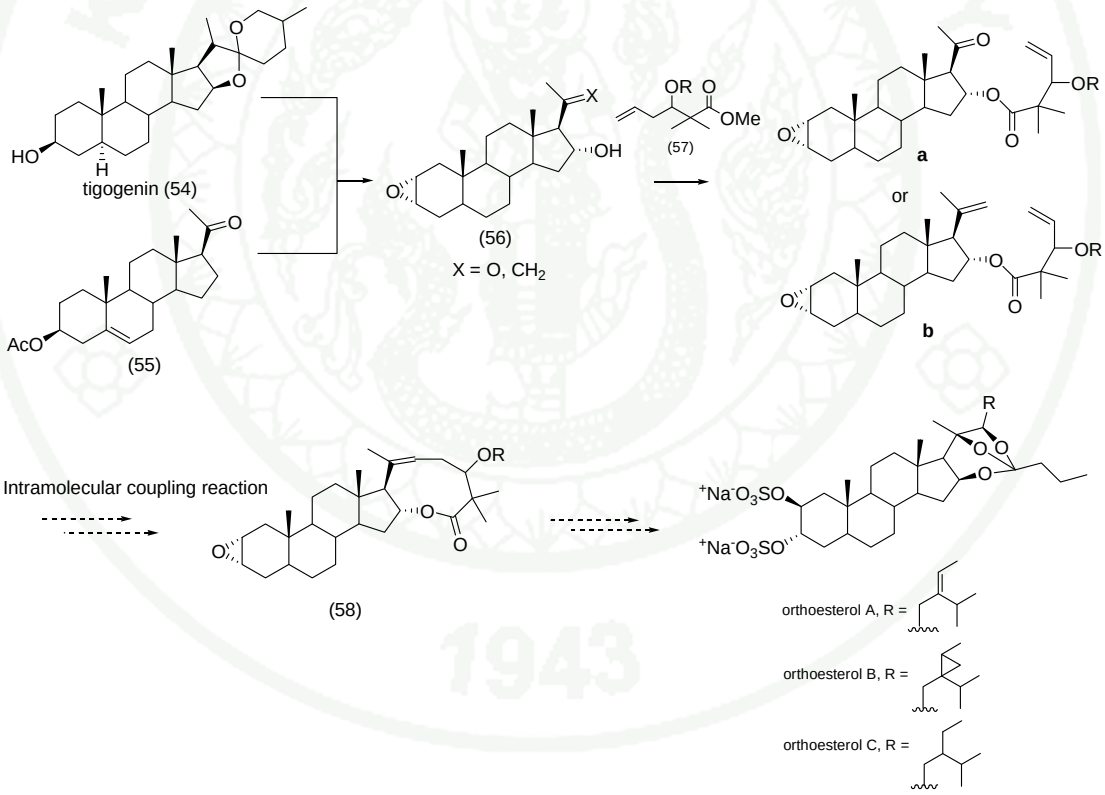
(53)

synthetic sulfated polyhydroxysteroids

Methodologies for synthesis of sterol orthoesters intermediate

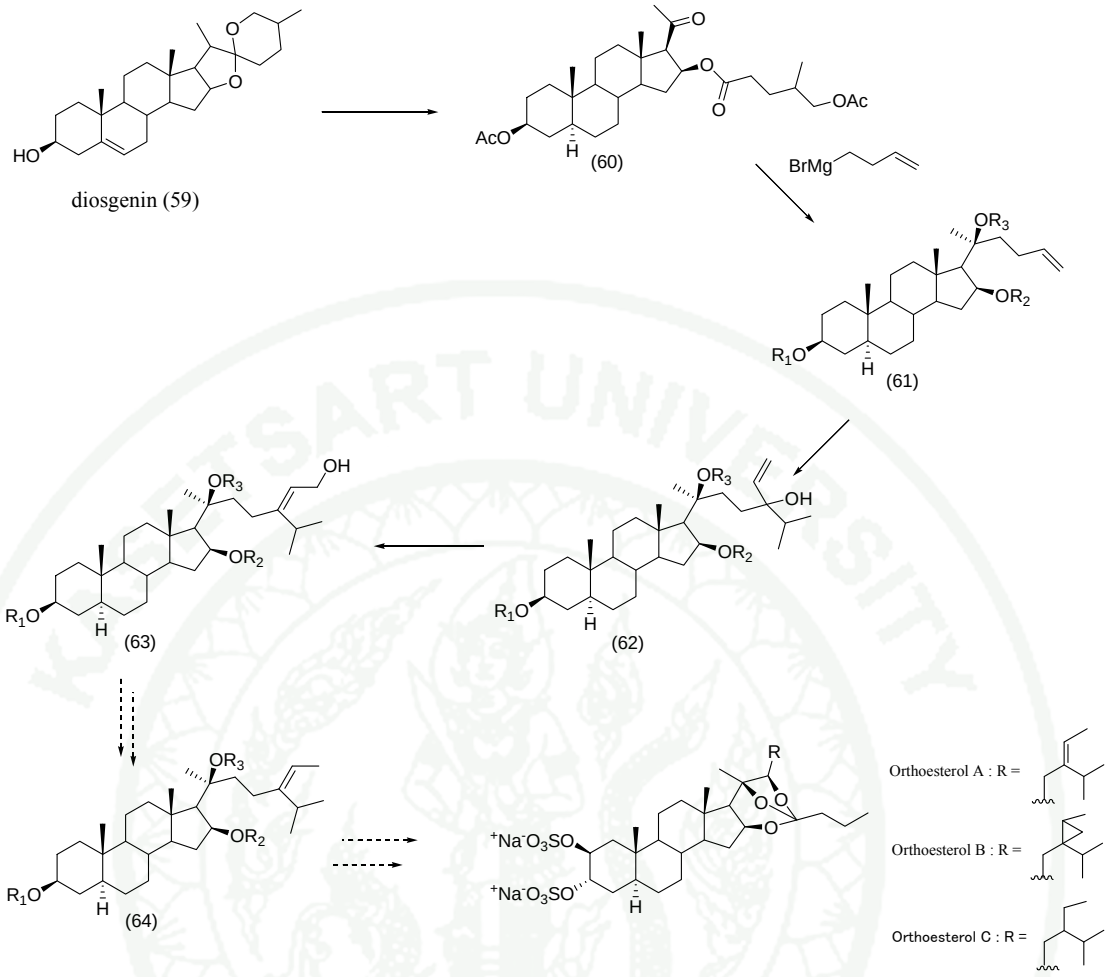
Due to no report on the synthesis of orthoesterol A-B and their intermediate has been published. Therefore, we were interested in investigation of synthesis of sterol orthoesters intermediates. Our strategies were devided into two routes consisting of intramolecular strategy and intermolecular strategy as shown in Scheme 1 and 2.

First route, we envisaged to incorporate steroid side chain of sterol orthoesters from 9-membered lactone ring **58** from key intermediates **a** and **b** *via* intramolecular Mc Murry coupling reaction and ring closing metathesis, respectively.



Scheme 1

Furthermore Grignard reaction of 20-keto steroid **60** and Grignard reagent followed by side chain extension was the alternatively synthetic method. Formation of orthoester will be followed the method described by Giner and Faraldos, (2002).

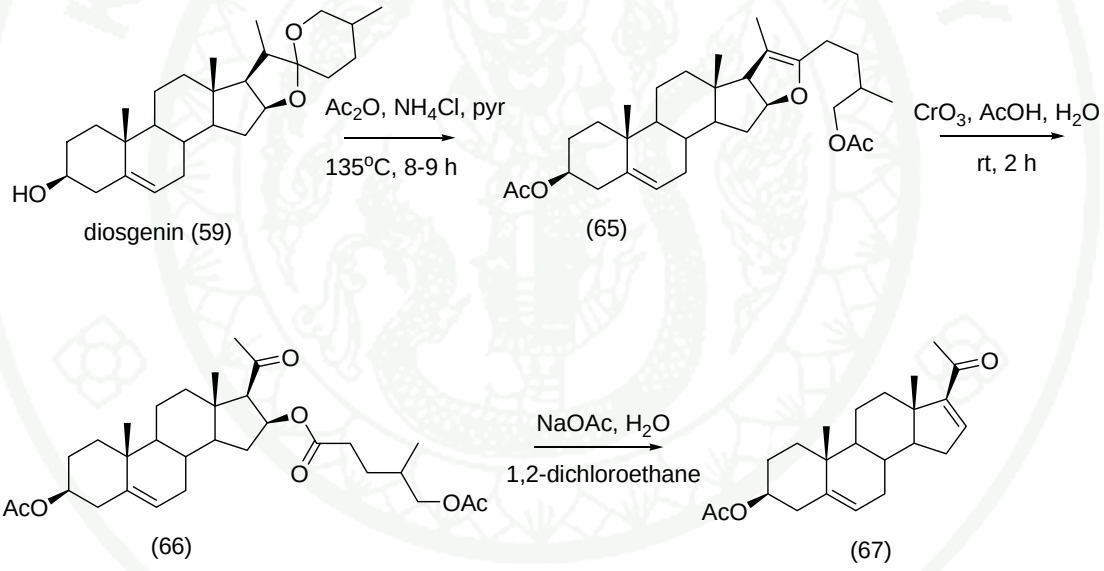


Scheme 2

Conversion of steroid sapogenin to Δ^{16}-pregn-3β-ol-20-one-3-acetate

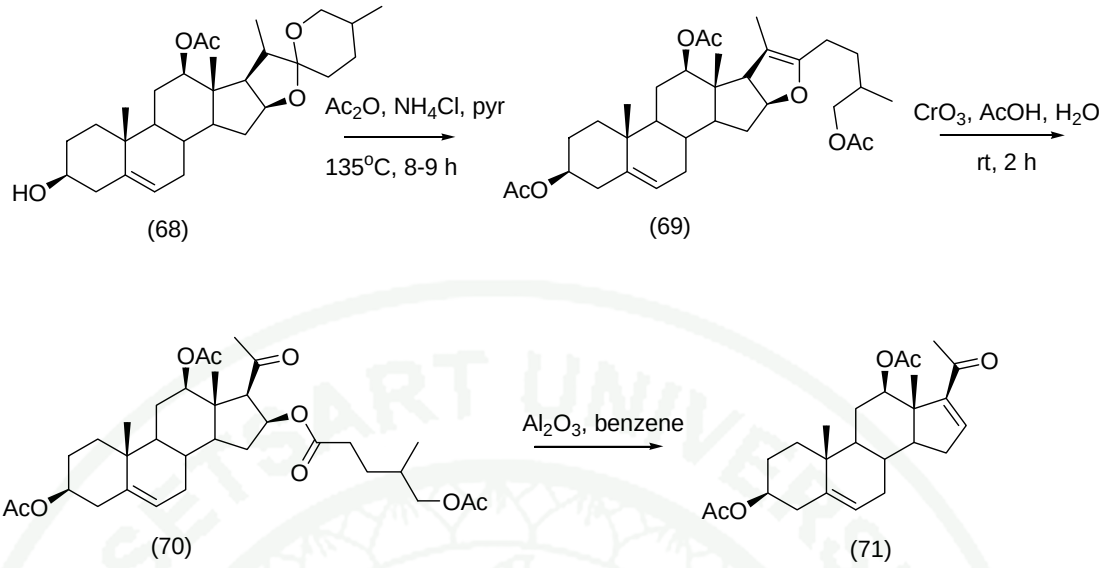
Δ^{16}-pregn-3β-ol-20-one-3-acetate is a key intermediate for the synthesis of many steroid drugs and steroid hormones. The synthetic methods have been documented by several groups such as Carmeron *et al*. (1955), Djerrassi *et al*. (1951) and Marker and Rohrmann (1940).

Mićović *et al*. (1990) displayed a simple one-pot preparation of 16-dehydropregnenolone acetate (67) from diosgenin (59) under normal pressure and reduced temperature. This efficient one-pot process was achieved as shown in Scheme 3.



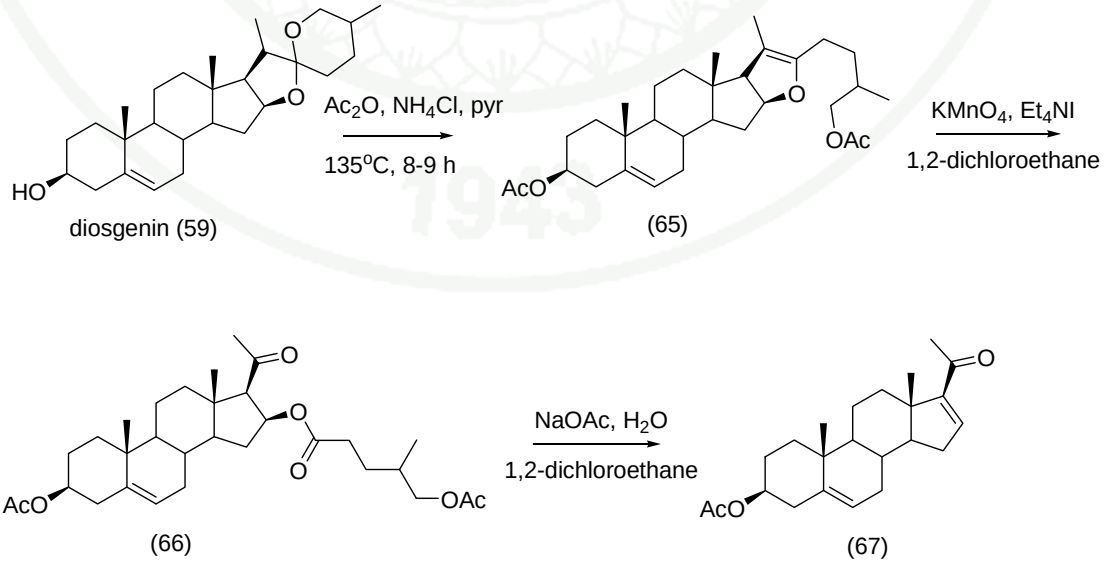
Scheme 3

The similar method for conversion of the rockogenin diacetate (68) to the enone compound **71** has been reported (Fuchs *et al*., (1999)). In the final step, they used the different method that used basic aluminium in benzene to generate the enone **71** from β-eliminaion of pentanoate side chain as shown in Scheme 4.



Scheme 4

Recently, new one-pot process has been developed for prepartation of 16-dehydropregnenolone acetate (67) from diosgenin (59) with more than 70% yield using potassium permanganate in the presence of tetraethylammonium iodide (Goswami *et al*., 2003). The reaction was undertaken in acidic condition (pH 3) at 0-5°C. Under this condition, only the double bond at furan ring was clevaged to form keto ester without breaking the double bond at C-5 as shown in Scheme 5.



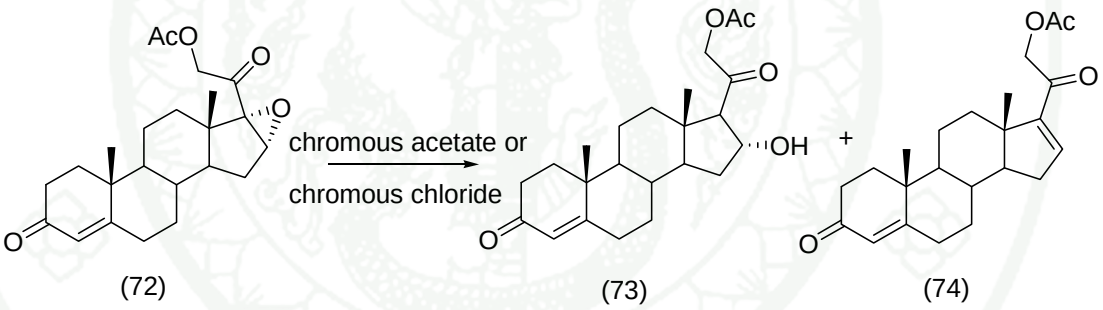
Scheme 5

Conversion of α, β-epoxy ketone to β-hydroxy ketone

Several methods to prepare β-hydroxy ketone from reductive ring opening of α, β-epoxy ketone have been reported because the β-hydroxy ketone species usually found for construction of many natural products.

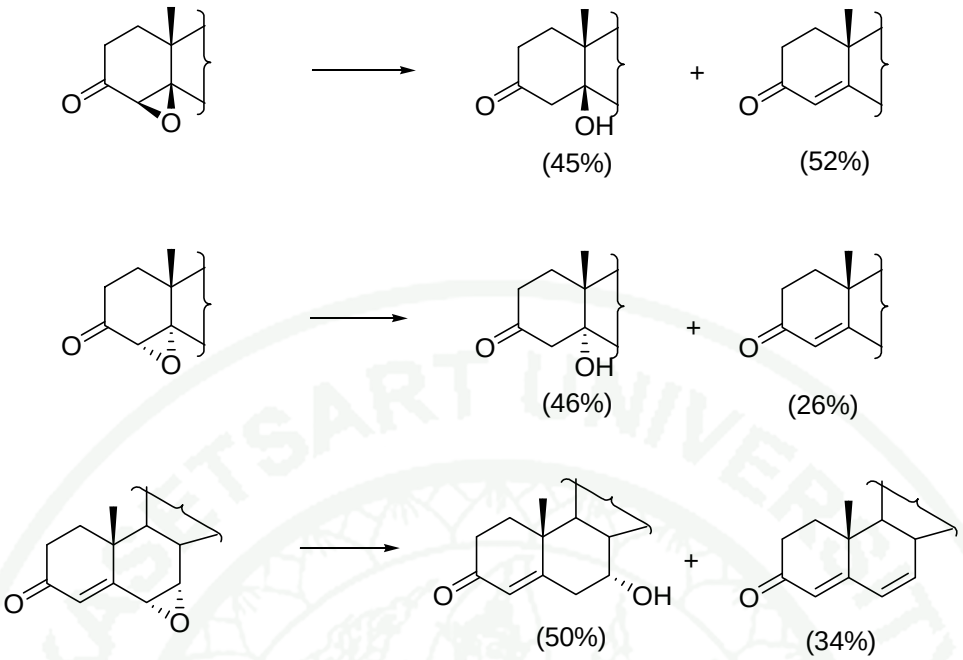
1. Chromous acetate

The first general method for preparation of the 16α-hydroxy-20-ketone steroid **73** from 16α, 17α-oxido-20-ketone **72** has been accomplished using chromous salt (Cole and Julian, 1953) as shown in Scheme 6. They found that using the chromous acetate led to a good yield of the desired product, β-hydroxy ketone, more than using chromous chloride .



Scheme 6

The scheme below are some examples of reductive ring opening of α, β-epoxy ketone to β-hydroxy ketone and other products using chromous acetate (Robinson and Henderson, 1972).



2. Alkali metal in liquid ammonia

The alternative sequences of reduction with lithium and liquid ammonia and protonation from proton source (ammonium chloride) from several α, β-epoxy ketone were displayed in Table 2.

Table 2 Reduction of epoxy ketones with lithium in liquid ammonia

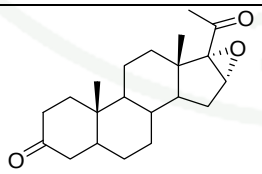
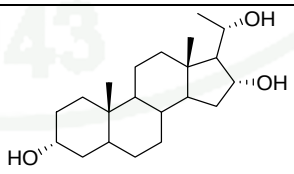
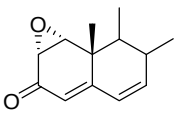
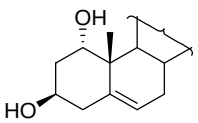
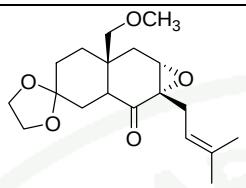
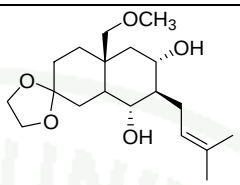
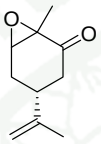
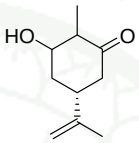
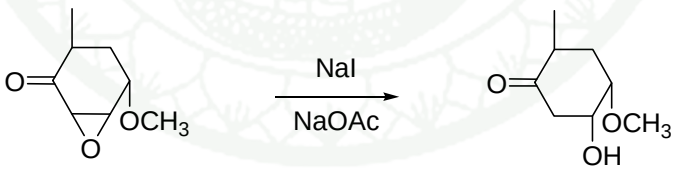
Entry	Epoxy ketone	Product	Reference
1			Hirschmann and Dans, 1959
2			Barton *et al*., 1973

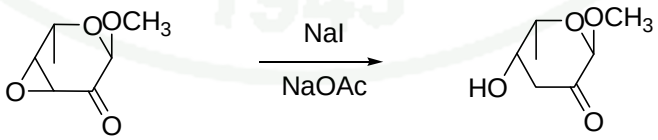
Table 2 (Continued)

Entry	Epoxy ketone	Product	Reference
3			Grieco *et al*., 1976
4			McChesney and Thompson, 1985

3. Sodium iodide

Reaction of α, β-epoxy ketone with sodium iodide, sodium acetate in the presence of acetic acid and acetone as solvent at ambient temperature for 5 min to provide the corresponding β-hydroxy ketone in 97% yield (Paulson *et al*., 1974).

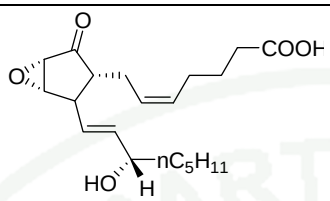
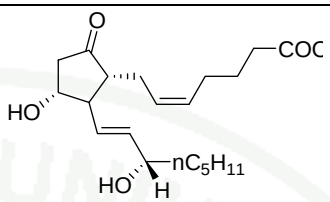
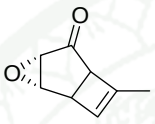
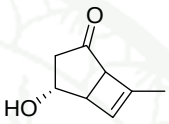
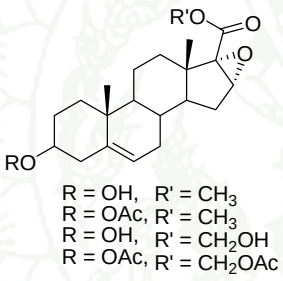
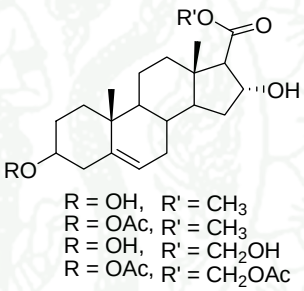




4. Aluminium amalgam (Al(Hg))

The varieties of conversion of epoxy ketones to β-hydroxy ketones using aluminium amulgum were carried out as shown in Table 3.

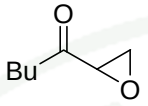
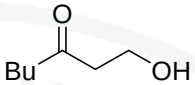
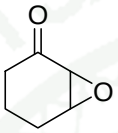
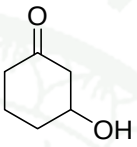
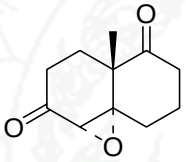
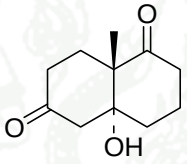
Table 3 Reduction of epoxy ketones with aluminium amalgam

Entry	Epoxy ketone	Product	Reference
1			Corey and Ensley, 1973
2			Moreno *et al*., 1993
3			Greene *et al*., 1982

5. Samarium iodide (SmI_2)

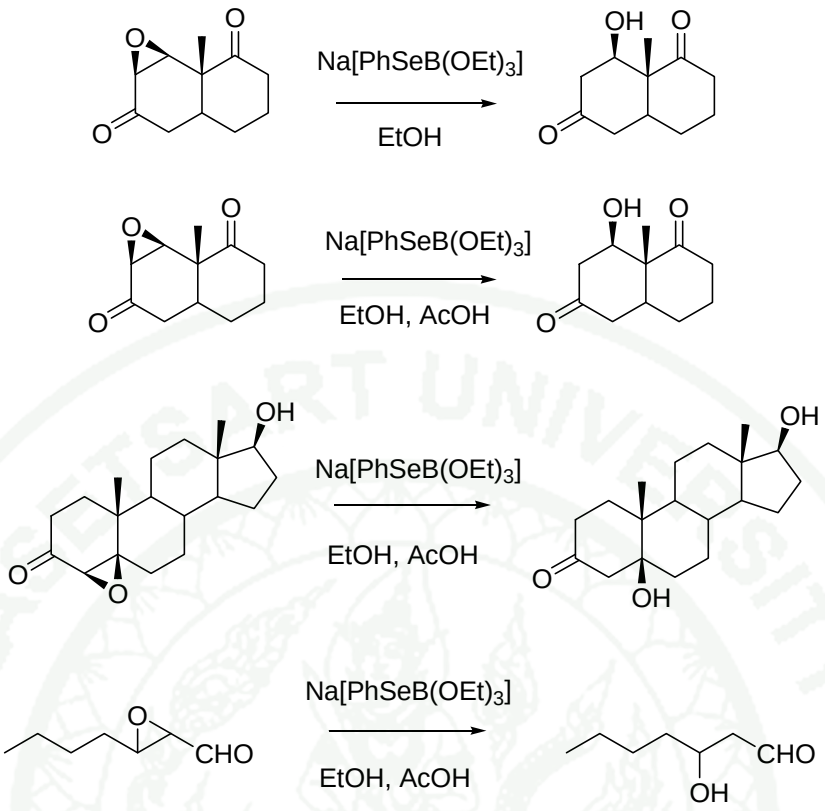
Reduction of α, β-epoxy ketone to β-hydroxy ketone with samarium iodide, generated *in situ* from samarium and diiodomethane, was displayed in Table 4 (Molander and Hahn, 1986).

Table 4 Reduction of α, β-epoxy ketone to β-hydroxy ketone with samarium iodide in THF and methanol at -90°C

Entry	Epoxy ketone	Product	% yield
1			97
2			82
3			76

6. Organoselenium

The investigation of reduction of α, β-epoxy ketone to β-hydroxy ketone by using organoselenium reagent was reported by Miyashita *et al*. (1997) as shown in Scheme 7. This reagent was easily prepared by reduction of diphenyldiselenide ($(PhSe)_2$) with sodium borohydride ($NaBH_4$) in ethanol and benzeneselenol (PhSeH).

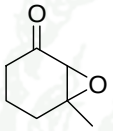
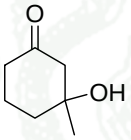
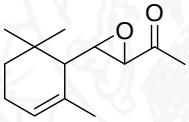
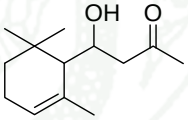
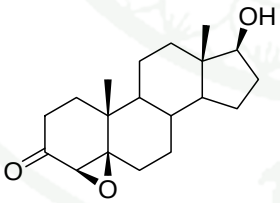
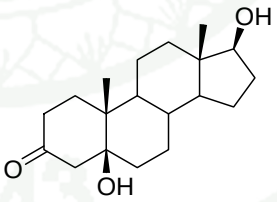
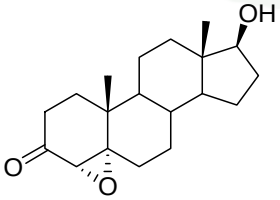
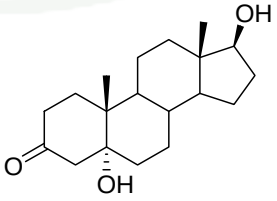


Scheme 7

7. Lithium naphthalenide

Lithium naphthalenide is a radical species, generated *in situ* from lithium and naphthalene in THF under dryness condition. This reagent is efficient to reduce α, β-epoxy ketone to corresponding β-hydroxy ketone (Jankowska *et al*., 1999). The results were shown in Table 5.

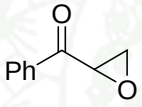
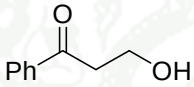
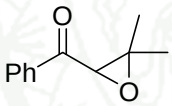
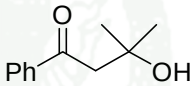
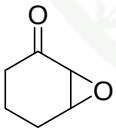
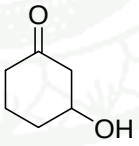
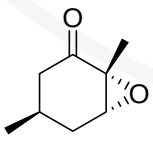
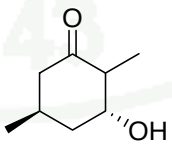
Table 5 Reductive cleavage of α, β-epoxy ketones to β-hydroxy ketones with lithium naphthalenide

Entry	Epoxy ketone	Product	% isolated yield (GC)
1			97
2			82
3			77
4			87

8. Low-valent titanium (III) complex (Cp_2TiCl)

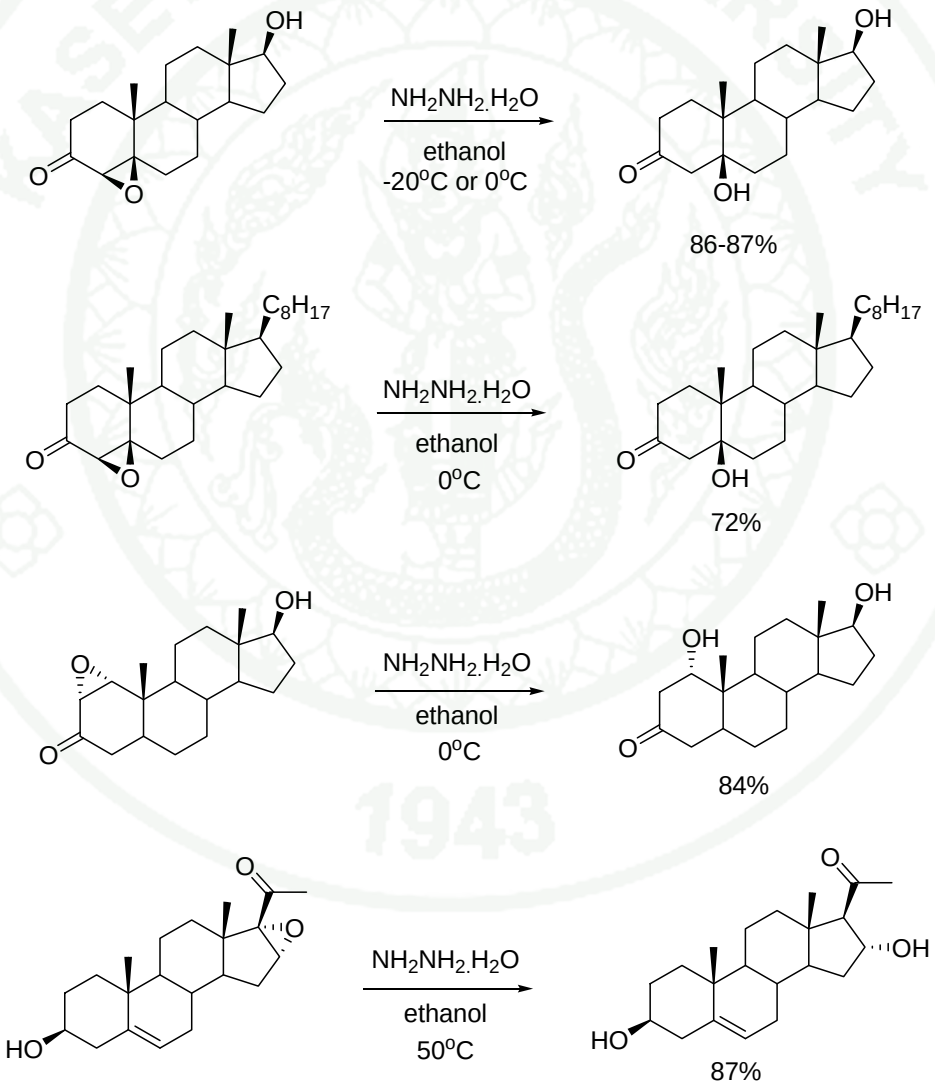
The application for reduction of α, β-epoxy ketone to β-hydroxy ketone using Cp_2TiCl, prepared *in situ* by reduction of Cp_2TiCl_2 with Zn powder, has been reported as shown in Table 6 (Hardouin *et al*., 2001). The results showed that the stereocenter at β-position was intact in all cases and the overall yields were satisfactory.

Table 6 Reductive cleavage of α, β-epoxy ketones to β-hydroxy ketones with Cp_2TiCl

Entry	Epoxy ketone	Product	% yield
1			86
2			74
3			80
4			65

9. Hydrazine hydrate

The development of the efficient and easily handle process using hydrazine hydrate in ethanol as solvent for reductive cleavage of α, β-epoxy ketone has been described (Salvador *et al*., 2005). They found that reaction temperature played an important role in this reaction after various reaction conditions were investigated. These are the examples that used this reagent with steroid compounds as shown in Scheme 8.



Scheme 8

Nucleophilic addition to 20-keto steroids

Construction of side chain from 20-keto steroids has been investigated, using Grignard reagent and other organometallic reagents in one or more steps sequences.

After 20-keto steroids underwent nucleophilic addition reaction, chiral center at C-20 was created with a variety of ratio of epimer at C-20 depending on the structure of the steroids, particularly the nature of substituents near C-20 and bulkiness of the reagent.

1. Grignard reagent (RMgX)

The investigation of nucleophilic addition of 20-keto steroids to tertiary alcohol by using Grignard reagent has been generally employed. This reagent could be generated easily by using activated magnesium and alkyl halide (Cl, Br and I) in dry THF or dry ether under dryness condition to give the black solution. Some examples were shown in Table 7.

Table 7 Nnucleophilic addition of 20-keto steroids to tertiary alcohols by using Grignard reagents

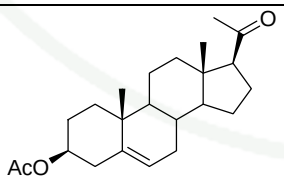
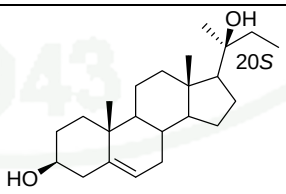
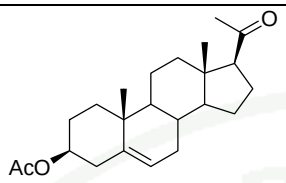
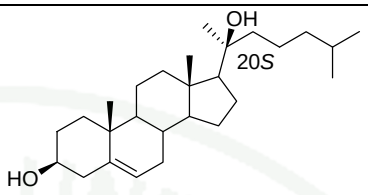
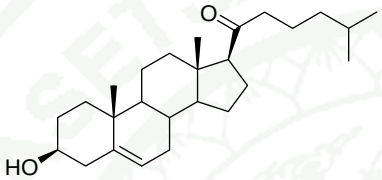
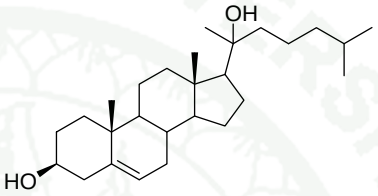
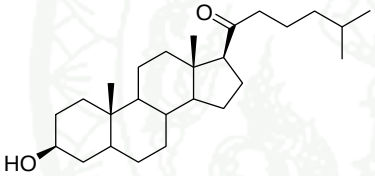
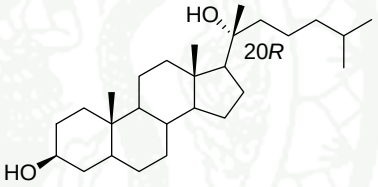
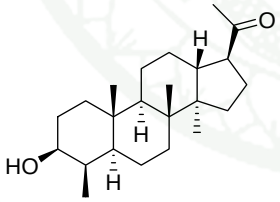
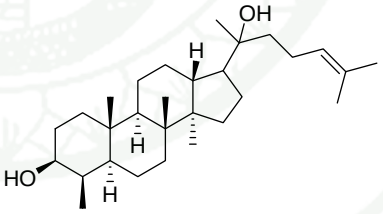
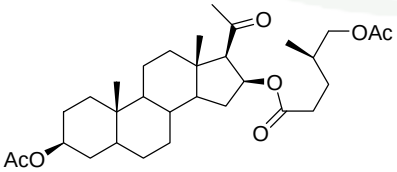
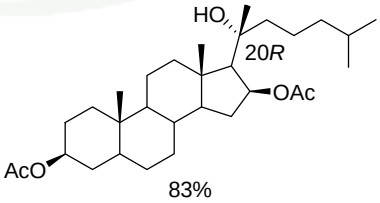
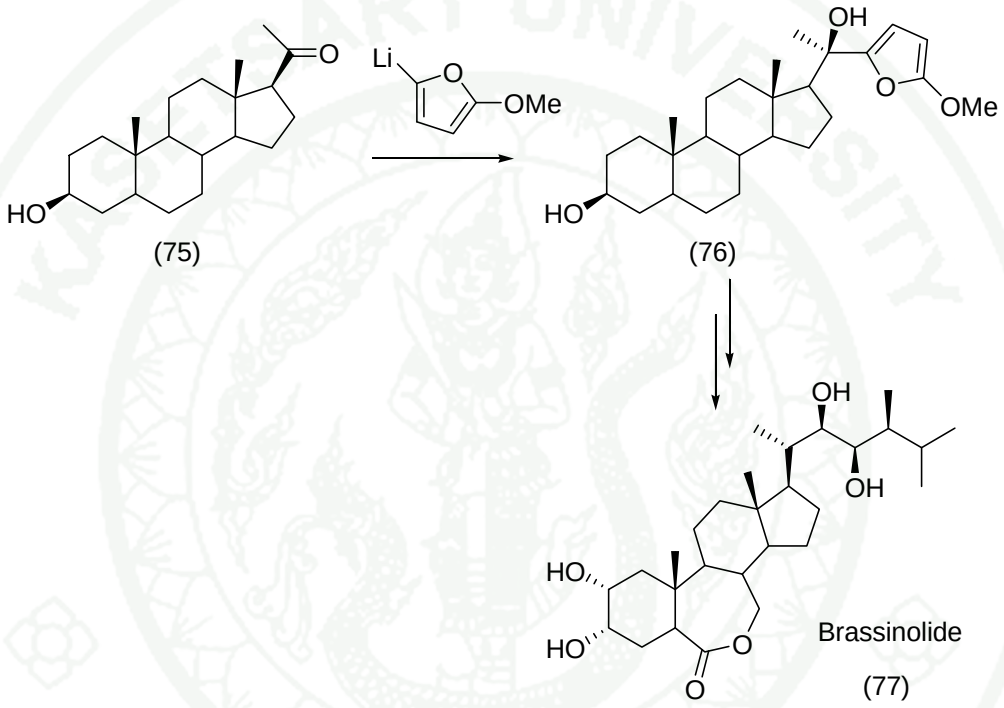
Entry	20-keto steroids	Product	Reference
1		 Yield not stated	Chaudhuri and Gut, 1969

Table 7 (Continued)

Entry	20-keto steroids	Product	Reference
2			Mijares *et al*., 1967
3		 20*S*:20*R* = 1:12	Nes and Varkey, 1976
4		 70%	Chaudhuri *et al*., 1969
5		 62% 20*S*:20*R* = 3:1	Johnson *et al*., 1999
6		 83%	Giner and Faraldos, 2002

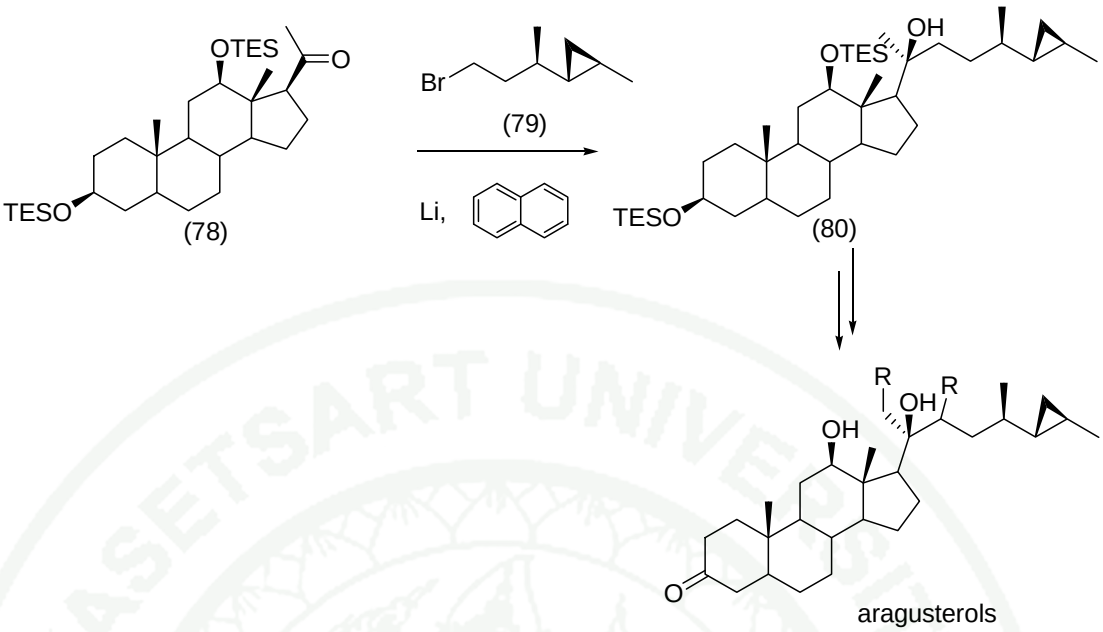
2. Alkyl lithium

The synthesis of brassinolide (77) reported by Kametani *et al*. (1986) has been accomplished *via* the stereocontrol addition of 5-lithi-2-methoxyfuran to pregnenolone (75) to provide only (20*S*)-epimer tertiary alcohol **76** as shown in Scheme 9.



Scheme 9

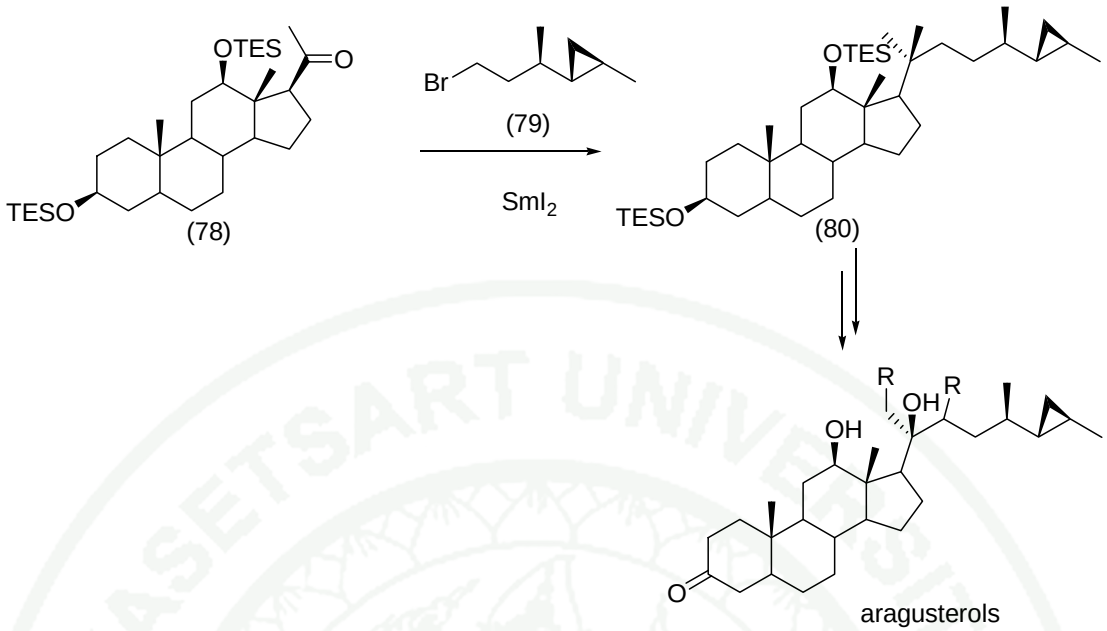
Yamada *et al*. (1995) reported the synthesis of aragusterols by using alkyl lithium generated from alkyl bromide **79** and lithium naphthalinide in THF at 0^{o}C to give the corresponding tertiary alcohol **80** with high stereoselectivity (20*S*:20*R* = 26:1; determined by 1H NMR analysis) as shown in Scheme 10.



Scheme 10

3. Samarium iodide (SmI_2)

The synthesis of aragusterols was also reported by Honda *et al.* (1996) using intermolecular coupling reaction between the 20-keto steroids and organometallic such as alkyl lithium, Grignard reagent and samarium iodide. The results showed that samarium iodide gave the better yield more than alkyl lithium and Grignard reagent as shown in Scheme 11.



Scheme 11

MATERIALS AND METHODS

Materials

Instrumenttation

The following analytical methods were used throughout this work, unless otherwise indicated.

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Varian Gemini 300 spectrophotometer and on 400 MHz Brucker Advance DPX-400. Chemical shifts were recorded as δ values in ppm. Spectra were acquired in $CDCl_3$ unless otherwise stated. The peak due to residual $CHCl_3$ (7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C) was used as the internal reference. Coupling constants (*J*) are given in Hz, and multiplicity is defined as followes : br = broad, s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplet, t = triplet, q = quartet, m = multiplet.

Infrared (IR) spectra were recorded in cm^{-1} on a Perkin-Elmer 2000 Fourier transform infrared spectrophotometer at the Department of Chemistry, Faculty of Science, Kasetsart University. Solid samples were analyzed as KBr disks.

Mass spectral were obtained on an Agilent Technology 1100 series LL/MSD Trap; the first number denoted *m/z* value and the ion assignment and abundance are given in parentheses and on a GCMS-QP-5050QA spectrometer in electron impact mode at 70 eV at the Kasetsart Agricultural and agro-Industrial Product improvement Institute (KAPI), Kasetsart University. Accurate masses (HRMS) were obtained from PERCH-CIC Mass Spectrometry Research Laboratory at Department of Chemistry, Faculty of Science, Chiangmai University.

Melting points (m.p.) were determined on a MeI-Temp electrothermal apparatus at the Department of Chemistry, Faculty of Science, Kasetsart University and were reported uncorrected in ^{o}C.

Chromatographic system

Analytical thin-layer chromatography (TLC) was conducted on aluminum-backed 0.2 mm thick silica gel 60 F254 plates (Merck) and the chromatograms were visualized under a 254 nm UV lamp and/or by spraying with a solution of vanillin (3% in ethanol with 3% sulfuric acid) followed by heating.

Flash column chromatography was conducted according to the method of Still and co-workers (1978) using silica gel 60 (mesh size 0.040-0.063 mm).

Column chromatography was performed on silica gel 60 (70-230 mesh, Merck) and the column was packed by slurry method.

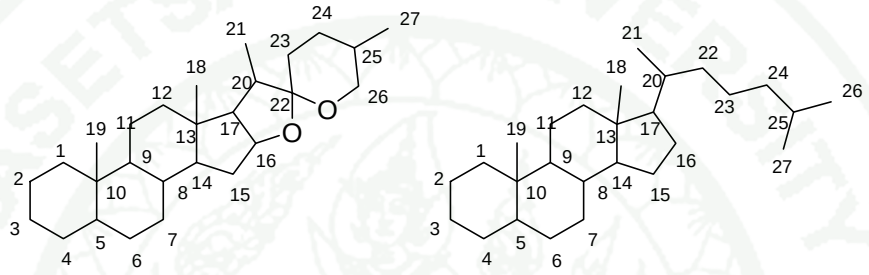
Chemical reagents

Solvents and reagents used for synthesis were obtained from commercial source and used directly without purification unless noted. Dry tetrahydrofuran (THF) was freshly distilled under nitrogen atmosphere from sodium with benzophenone ketyl as an indicator.

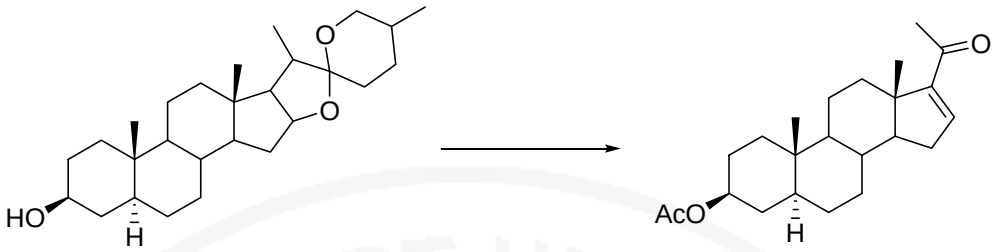
Methods

Numbering of tetracyclic steroid carbon skeleton

The spectral data of all synthesized compounds reported throughout this work were referred to the numbered of tetracyclic steroid carbon skeletons as shown below. The additional carbons would be numbered in each case.



3β-Acetoxy-5α-16-pregnen-20-one (81)



tigogenin (54) (81)

Step I

A mixture of tigogenin (54) (2.0 g, 4.6 mmol), acetic anhydride (31.6 mL), ammonium chloride (490 mg, 9.2 mmol) and pyridine (0.35 mL) was heated to 135°C and kept at this temperature for 16 h. After cooling down, the reaction mixture was added with cool water and extracted with CH_2Cl_2 (4×400 mL). The combined organic layers were neutralized with saturated aqueous $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was used in the next step without purification.

Step II

The crude product was dissolved in 1, 2-dichloroethane (11.5 mL), water (0.9 mL) and acetic acid (4.5 mL). The mixture was cooled to 0°C and added with a solution of chromium trioxide (964.5 mg) in water (1.2 mL) and acetic acid (0.43 mL) (This solution was kept below 7°C during addition). The mixture was allowed to warm to room temperature and stirred for 2 h. A solution of sodium chloride (3.9 g) in water (13 mL) and methanol (0.15 mL) was introduced and the resulting mixture was further stirred for additional 1 h. The reaction was added with cool water and extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were neutralized with saturated aqueous $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was used in the next step without purification.

Step III

The residue was dissolved in benzene (50 mL) and basic aluminar (6.7 g) was added and stirred for 16 h at room temperature. The resulting reaction was filtered and eluted with CH_2Cl_2. Filtrate was evaporated and further purified by flash column chromatography (10% ethyl acetate:hexane) to give 3β-acetoxy-5α-16-pregnen-20-one (81) (787.1 mg, 49%) as a white solid; m.p.153-155^{o}C. (Plattner *et al*., 1947, m.p. 152-156.5^{o}C)

3β-Acetoxy-5α-16-pregnen-20-one (81)

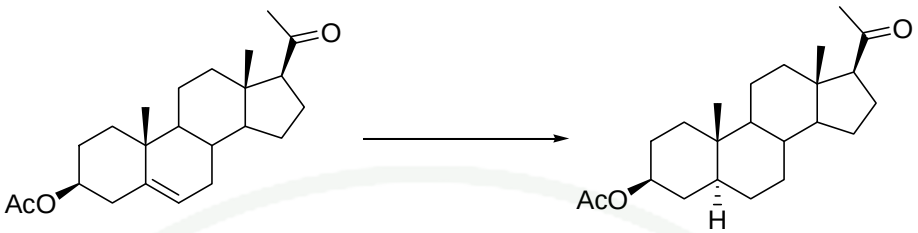
FTIR (KBr), ν_{max}, cm^{-1}: 2935, 1729, 1658, 1368, 1262, 1030

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.60 (dd, *J* = 3.3, 1.9 Hz, 1H, H-16), 4.60 (m, 1H, H-3), 2.22 (m, 2H, H-15), 2.17 (s, 3H, H-21), 1.94 (s, 3H, CH_3COO), 1.96-0.62 (4CH, 8CH_2), 0.80 (s, 3H, H-19), 0.78 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.0 (C-20), 170.9 (COO), 155.7 (C-17), 144.2 (C-16), 73.8 (C-3), 56.5 (CH), 54.9 (CH), 46.5 (C-13), 45.1 (CH), 36.7 (CH_2), 35.9 (C-10), 34.9 (CH_2), 34.2 (CH_2), 34.0 (CH), 33.4 (CH_2), 32.1 (CH_2), 28.6 (CH_2), 27.6 (CH_2), 27.3 ($\underline{C}H_3COO$), 21.6 (C-21), 21.2 (CH_2), 16.1 (C-19), 12.4 (C-18)

MS (EI), *m/z* (relative intensity): 358 (73), 343 (19), 315 (63), 298 (13), 207 (100)

3β-Acetoxy-5α-pregnan-20-one (82)



(55) (82)

To a solution of 3β-acetoxy-5-pregnen-20-one (55) (47 mg, 0.13 mmol) in ethyl acetate (2 mL) and methanol (6 mL) was added 5% Pd/C (6.5 mg, 2.62×10^{-3} mmol) and the suspension mixture was stirred at room temperature under hydrogen atmosphere for 16 h. Then, the reaction was filtered through celite and eluted with CH_2Cl_2, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give 3β-acetoxy-5α-pregnan-20-one (82) (43 mg, 91%) as a white solid; 149-150°C. (Karl *et al*., 1949, m.p. 150-152°C)

3β-Acetoxy-5α-pregnan-20-one (82)

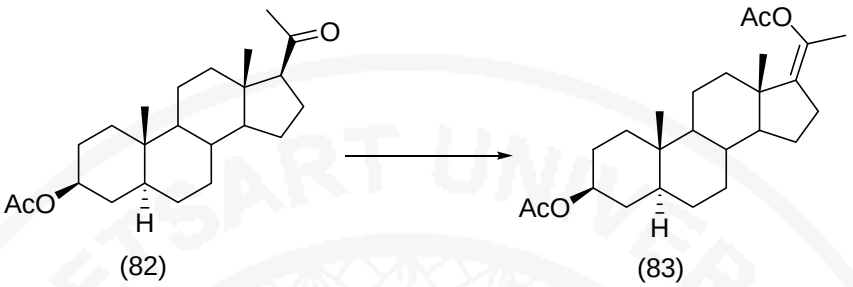
FTIR (KBr), ν_{max}, cm^{-1}: 2925, 1723, 1705, 1362, 1261, 1037

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.62 (m, 1H, H-3), 2.45 (t, J = 9.0 Hz, 1H, H-17), 2.08 (m, 1H, H-16), 2.04 (s, 3H, H-21), 1.95 (s, 3H, CH_3COO), 1.91 (m, 1H, H-12), 1.75 (m, 1H, CH of CH_2), 1.70-0.78 (H-12, CH of CH_2, 3CH, 7CH_2), 0.75 (s, 3H, H-19), 0.63 (m, 1H, H-9), 0.53 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.6 (C-20), 170.7 (COO), 73.6 (C-3), 63.8 (C-17), 56.6 (C-14), 54.1 (C-9), 44.6 (C-5), 44.2 (C-13), 39.0 (C-12), 36.7 (C-1), 35.5 (C-10), 35.4 (C-8), 33.9 (CH_2), 31.9 (CH_2), 31.5 (C-21), 28.4 (CH_2), 27.4 (CH_2), 24.7 (CH_2), 22.8 (C-16), 21.4 ($\underline{C}H_3COO$), 21.2 (CH_2), 13.4 (C-18), 12.2 (C-19)

MS (APCI), *m/z* (relative intensity): 301 (81), 283 (100)

3β-Acetoxy-5α-17-pregnenyl acetate (83)



(82) (83)

p-Toluenesulfonic acid (197 mg, 1.0 mL) was added to a stired solution of 3β-acetoxy-5α-pregnan-20-one (82) (367.9 mg, 1.0 mmol) in acetic anhydride (54 mL). After resulting reaction mixture was refluxed for 6 h, acetic anhydride was removed slowly by distillation. Then, the reaction was chilled and ice water was added. The residue was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (5% ethyl acetate:hexane) to provide 3β-acetoxy-5α-17-pregnenyl acetate (83) (272.1 mg, 65% (88% brsm)) as a white solid; m.p. 110-111^{o}C (Marshall *et al*., 1948, m.p.121.5-122.5^{o}C) and recovered starting material (82) (91.8 mg, 75% conversion).

3β-Acetoxy-5α-17-pregnenyl acetate (83)

FTIR (KBr), ν_{max}, cm^{-1}: 2933, 1738, 1370, 1243, 1029

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.61 (m, 1H, H-3), 2.06 (m, 2H, H-16), 2.02 (s, 3H, CH_3COO), 1.94 (s, 3H, CH_3COO), 1.72 (s, 3H, H-21), 1.81-0.81 (4CH and 9CH_2), 0.75 (s, 3H, H-19), 0.74 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.9 (COO), 170.2 (COO), 136.1 (C=C), 136.0 (C=C), 73.9 (C-3), 55.7 (CH), 54.2 (CH), 44.8 (CH), 44.4 (C-13), 36.9 (CH_2), 36.2 (CH_2), 35.1 (C-10), 35.0 (CH), 34.2 (CH_2), 32.0 (CH_2), 28.7 (C-16), 28.0 (CH_2), 27.7 (CH_2), 24.7 (CH_2), 21.6 ($\underline{C}H_3COO$), 21.5 (CH_2), 21.2 ($\underline{C}H_3COO$), 17.7 (CH_2), 17.1 (C-19), 12.4 (C-18)

3β-Acetoxy-17-bromo-5α-pregnan-20-one (84)

(83) (84)

To a solution of 3β-acetoxy-5α-17-pregnenyl acetate (83) (996 mg, 2.5 mmol) in CH_2Cl_2 (15 mL) was added a solution of bromine (0.1 mL, 2.5 mmol) in CH_2Cl_2 (5 mL). After reaction mixture was stirred at room temperature for 15 min, the reaction was quenched by addition of saturated aqueous $NaHSO_3$ solution. The mixture was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (3% ethyl acetate:hexane) to give 3β-acetoxy-17-bromo-5α-pregnan-20-one (84) (950.9 mg, 87%) as a white crystal after recrystallization (CH_2Cl_2-hexane); m.p. 138°C. (Marker *et al*., 1942, m.p. 155°C)

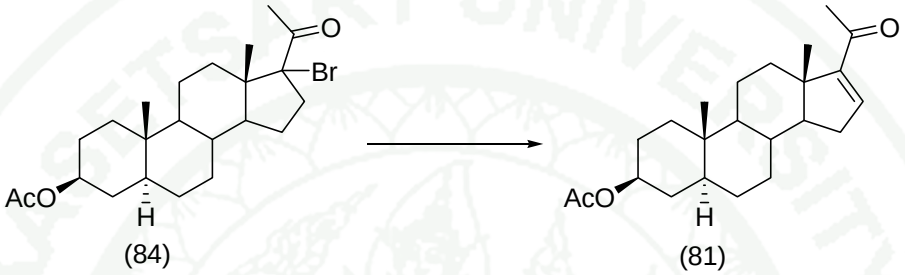
3β-Acetoxy-17-bromo-5α-pregnan-20-one (84)

FTIR (KBr), ν_{max}, cm^{-1}: 2936, 1739, 1703, 1355, 1238, 1030

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.67 (m, 1H, H-3), 3.03 (m, 1H, H-16), 2.35 (s, 3H, $C\underline{H}_3COO$), 2.24 (m, 1H, H-16), 2.00 (s, 3H, H-21), 1.97-0.92 (4CH and 8CH_2), 0.81 (s, 3H, H-19), 0.72 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.5 (C-20), 170.6 (COO), 86.4 (C-17), 73.5 (C-3), 53.3 (CH), 53.2 (CH), 44.5 (CH), 44.4 (C-13), 36.6 (CH_2), 35.9 (CH_2), 35.8 (CH), 35.6 (CH_2), 35.4 (C-10), 33.9 (CH_2), 31.7 (CH_2), 28.4 (CH_2), 27.4 (C-21), 27.3 (CH_2), 22.8 (CH_2), 21.4 ($\underline{C}H_3COO$), 21.2 (CH_2), 14.1 (C-19), 12.2 (C-18)

3β-Acetoxy-5α-16-pregnen-20-one (81)



(84) (81)

To a solution of 3β-acetoxy-5α-17-bromo-pregnan-20-one (84) (499 mg, 1.1 mmol) in toluene (60 mL) was added 1,5-diazabicyclo[5.4.0]undec-7-ene (DBU) (1.2 mL, 8.0 mmol). The resulting mixture was refluxed for 8 h. The reaction was quenched by addition of saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to afford 3β-acetoxy-5α-16-pregnen-20-one (81) (367.5 mg, 89%) as a white solid; m.p. 153-155^{o}C. (Plattner *et al*., 1947, m.p. 152-156.5^{o}C)

3β-Acetoxy-5α-16-pregnen-20-one (81)

FTIR (KBr), ν_{max}, cm^{-1}: 2935, 1729, 1658, 1368, 1262, 1030

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.60 (dd, J = 3.3, 1.9 Hz, 1H, H-16), 4.60 (m, 1H, H-3), 2.22 (m, 2H, H-15), 2.17 (s, 3H, H-21), 1.94 (s, 3H, CH_3COO), 1.96-0.62 (4CH, 8CH_2), 0.80 (s, 3H, H-19), 0.78 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.0 (C-20), 170.9 (COO), 155.7 (C-17), 144.2 (C-16), 73.8 (C-3), 56.5 (CH), 54.9 (CH), 46.5 (C-13), 45.1 (CH), 36.7 (CH_2), 35.9 (C-10), 34.9 (CH_2), 34.2 (CH_2), 34.0 (CH), 33.4 (CH_2), 32.1 (CH_2), 28.6 (CH_2), 27.6 (CH_2), 27.3 ($\underline{C}H_3COO$), 21.6 (C-21), 21.2 (CH_2), 16.1 (C-19), 12.4 (C-18)

MS (EI), *m/z* (relative intensity): 358 (73), 343 (19), 315 (63), 298 (13), 207 (100)

16α, 17α-Epoxy-3β-hydroxy-5α-pregnan-20-one (85)

(81) (85)

A suspension of 3β-acetoxy-5α-16-pregnen-20-one (81) (367 mg, 1.0 mmol) in methanol (25 mL) was cooled to 18°C. To this suspension was added 30% H_2O_2 (2.2 mL) and 2.5 M NaOH (2.2 mL). The resulting mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of saturated aqueous NaCl solution. After methanol was removed, the residue was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (10% ethyl acetate:hexane) to give 16α, 17α-epoxy-3β-hydroxy-5α-pregnan-20-one (85) (321.3 mg, 94%) as a white solid; m.p. 190-191°C. (Hirschmann *et al*., 1961, m.p. 186.5-188°C)

16α, 17α-Epoxy-3β-hydroxy-5α-pregnan-20-one (85)

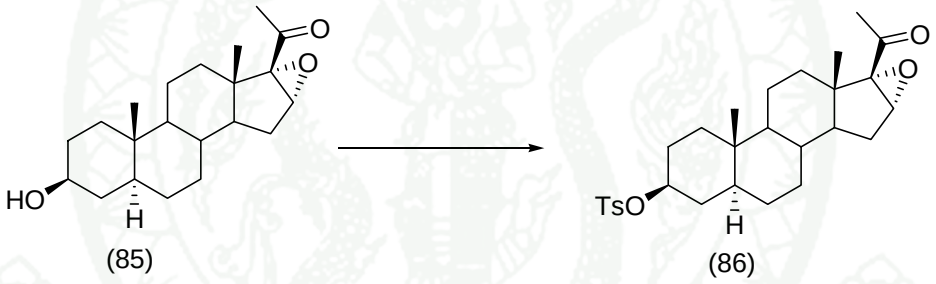
FTIR (KBr), ν_{max}, cm^{-1}: 2928, 1720, 1365, 1034

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.65 (m, 1H, H-16), 3.59 (m, 1H, H-3), 2.02 (s, 3H, H-21), 2.00-1.40 (4CH and 8CH_2), 1.02 (s, 3H, H-19), 0.82 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.2 (C-20), 72.3 (C-17), 70.7 (C-3), 61.5 (C-16), 45.5 (CH), 42.3 (CH), 41.5 (C-13), 39.2 (CH_2), 38.5 (CH_2), 36.5 (CH_2), 34.5 (C-10), 32.3 (CH_2), 31.6 (CH_2), 31.3 (CH), 29.2 (C-21), 26.5 (CH_2), 20.1 (CH_2), 19.6 (C-19), 16.7 (C-18)

MS (EI), *m/z* (relative intensity): 332 (34), 314 (18), 271 (59), 253 (28), 207 (100)

16α, 17α-Epoxy-3β-tosyloxy-5α-pregnan-20-one (86)



Method I

A solution of 16α, 17α-epoxy-3β-hydroxy-5α-pregnan-20-one (85) (310 mg, 0.9 mmol), *p*-toluenesulfonyl chloride (266 mg, 1.4 mmol), dimethylaminopyridine (126 mg, 1.0 mmol) in pyridine (0.54 mL) and CH_2Cl_2 (5 mL) was stirred at room temperature for 3 day. The reaction was quenched by addition of water and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with 10% HCl, saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to give 16α, 17α-epoxy-3β-tosyloxy-5α-pregnan-20-one (86) (192.7 mg, 43%, 92% (brsm)) as a white solid; m.p. 141-142^{o}C (Hirschmann *et al*., 1961, m.p. 165.5-168^{o}C with decomposition) and the recovered starting material (85) (143.6 mg, 46% conversion).

Method II

To a solution of 16α, 17α-epoxy-3β-hydroxy-5α-pregnan-20-one (85) (166.4 mg, 0.5 mmol) in dry CH_2Cl_2 (5 mL) under an argon atmosphere was added *p*-toluenesulfonyl chloride (215 mg, 1.13 mmol) and dry pyridine (1 mL). The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched by addition of water and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with 10% HCl, saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to give 16α, 17α-epoxy-3β-tosyloxy-5α-pregnan-20-one (86) (188 mg, 77%, 86% (brsm)) as a white solid; m.p. 141-142^{o}C (Hirschmann *et al*., 1961, m.p.165.5-168^{o}C with decomposition) and the recovered starting material (85) (16.7 mg, 90% conversion).

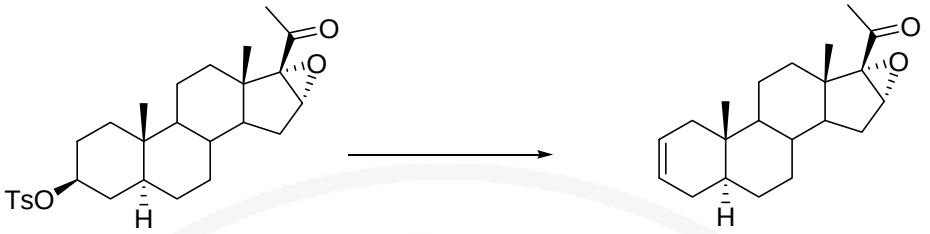
16α, 17α-Epoxy-3β-tosyloxy-5α-pregnan-20-one (86)

FTIR (KBr), ν_{max}, cm^{-1}: 2934, 1699, 1336, 1172, 1101

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (d, *J* = 8.3 Hz, 2H, H-2', H-6'), 7.29 (d, *J* = 8.3 Hz, 2H, H-3', H-5'), 4.37 (m, 1H, H-3), 3.62 (s, 1H, H-16), 2.41 (s, 3H, C$\underline{H}_3$-C_6H_4), 1.98 (s, 3H, H-21), 1.88 (dd, *J* = 13.3, 6.1 Hz, 1H, CH), 1.96-0.80 (4CH, 8CH_2), 0.96 (s, 3H, H-19), 0.75 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.8 (C-20), 144.3 (C-1'), 134.7 (C-4'), 129.7 (C-2', C-6'), 127.6 (C-3', C-5'), 82.2 (CH), 70.9 (C-17), 60.5 (CH), 54.3 (CH), 45.1 (CH), 44.8 (CH), 41.8 (C-13), 36.3 (CH_2), 35.3 (C-10), 34.8 (CH_2), 33.0 (CH), 31.4 (CH_2), 31.3 (CH_2), 28.2 (CH_2), 28.1 (CH_2), 27.3 (CH_2), 25.9 (C-21), 21.6 (CH_3-C-4'), 20.6 (CH_2), 15.3 (CH_3), 12.0 (CH_3)

16α, 17α-Epoxy-5α-2-pregnen-20-one (87)



(86) (87)

Lithium bromide (93 mg, 1.0 mmol) and lithium carbonate (80 mg, 1.0 mmol) were added to a solution of 16α, 17α-epoxy-3β-tosyloxy-5α-pregnan-20-one (86) (35 mg, 0.07 mmol) in *N,N*-dimethylformamide (3 mL). The reaction mixture was refluxed for 4 h before cooling to room temperature. The reaction was slowly poured to 10% HCl and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with water and saturated aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to provide 16α, 17α-epoxy-5α-2-pregnen-20-one (87) (17 mg, 75%) as a white solid; m.p. 116-118^{o}C.

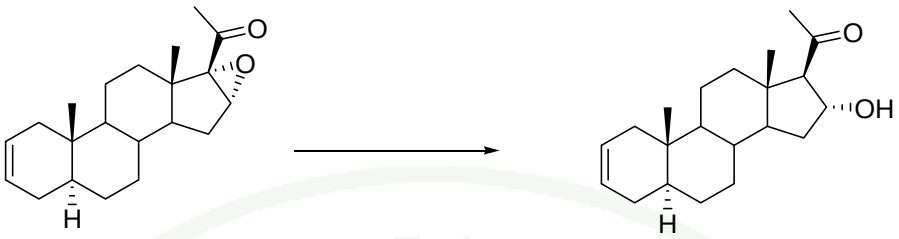
16α, 17α-Epoxy-5α-2-pregnen-20-one (87)

FTIR (KBr), ν_{max}, cm^{-1}: 2919, 1700, 1649

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.51 (m, 2H, H-2, H-3), 3.58 (s, 1H, H-16), 1.94 (s, 3H, H-21), 1.95-0.70 (4CH, 7CH_2), 0.96 (s, 3H, H-19), 0.69 (s, 3H, H-18)

MS (EI), *m/z* (relative intensity): 314 (45), 296 (26), 281 (56), 271 (79), 253 (49), 207 (100)

16α-Hydroxy-5α-2-pregnen-20-one (88)



(87) (88)

Hydrazine monohydrate (0.7 mL) was added to a solution of 16α, 17α-epoxy-5α-2-pregnen-20-one (87) (248.7 mg, 0.8 mmol) in ethanol (30 mL). The resulting mixture was stirred at room temperature for 3 day. The reaction was hydrolyzed by addition of 10% HCl and further stirred for 20 h. The residue was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (30% ethyl acetate:hexane) to provide 16α-hydroxy-5α-2-pregnen-20-one (88) (146 mg, 60%) as a white solid; m.p. 149-151^{o}C.

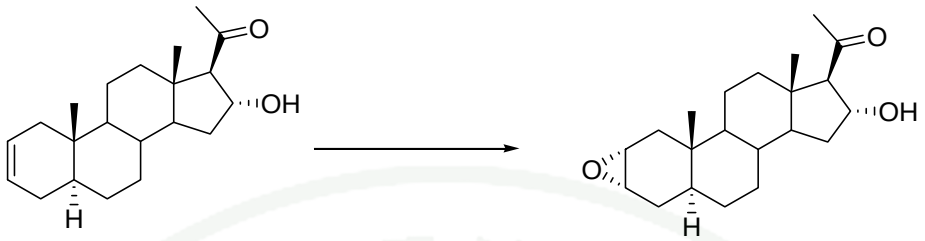
16α-Hydroxy-5α-2-pregnen-20-one (88)

FTIR (KBr), ν_{max}, cm^{-1}: 3421, 2909, 1699, 1638, 1048

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.52 (m, 2H, H-2, H-3), 4.75 (m, 1H, H-16), 2.45 (d, *J* = 6.6 Hz, 1H, H-17), 2.09 (s, 3H, H-21), 1.99-0.70 (4CH, 7CH_2), 0.68 (s, 3H, H-19), 0.56 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.7 (C-20), 125.8 (C=C), 125.6 (C=C), 73.9 (C-17), 72.0 (C-16), 54.3 (CH), 53.8 (CH), 45.0 (C-13), 41.4 (CH), 39.6 (CH_2), 39.0 (CH_2), 35.2 (CH_2), 35.1 (CH), 34.6 (C-10), 31.6 (C-21), 31.5 (CH_2), 30.2 (CH_2), 28.5 (CH_2), 20.5 (CH_2), 14.4 (CH_3), 11.6 (CH_3)

2α, 3α-Epoxy-16α-hydroxy-5α-pregnan-20-one (89)



(88) (89)

To a solution of 16α-hydroxy-5α-2-pregnen-20-one (88) (281 mg, 0.9 mmol) in CH_2Cl_2 (20 mL) was added water (10 mL) and sodium carbonate (350 mg, 3.3 mmol). The mixture was stirred vigorously before adding *m*-chloroperbenzoic acid (212 mg, 1.2 mmol). The reaction mixture was stirred at room temperature for 4 h. The aqueous layer was separated and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with 5% sodium sulfite solution, saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (25% ethyl acetate:hexane) to afford 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89) (109.5 mg, 64%) as a white needle after recrystallization (CH_2Cl_2-hexane); m.p. 176-177°C.

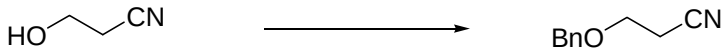
2α, 3α-Epoxy-16α-hydroxy-5α-pregnan-20-one (89)

FTIR (KBr), ν_{max}, cm^{-1}: 3453, 2915, 1697, 1181, 1054

^1H NMR ($CDCl_3$, 400 MHz) δ 4.76 (m, 1H, H-16), 3.12 (m, 1H, H-3), 3.07 (m, 1H, H-2), 2.47 (d, *J* = 6.6 Hz, H-17), 2.12 (s, 3H, H-21), 2.11-0.79 (4CH, 7CH_2), 0.71 (s, 3H, H-19), 0.56 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.7 (C-20), 73.7 (C-17), 72.0 (C-16), 54.1 (CH), 53.4 (CH), 52.3 (CH), 50.9 (CH), 44.9 (C-13), 38.8 (CH_2), 38.1 (CH_2), 36.1 (CH), 35.2 (CH_2), 35.1 (CH), 33.6 (C-10), 31.7 (C-21), 31.4 (CH_2), 28.9 (CH_2), 28.1 (CH_2), 20.4 (CH_2), 14.4 (CH_3), 12.9 (CH_3)

3-Benzyloxypropionitrile (91)



(90) (91)

NaH (531 mg, 22.1 mmol) was washed with dry THF (5 mL) and stirred for 10 min before removing solvent.

To a suspension of NaH in dry THF was slowly added 3-hydroxypropionitrile (90) (0.5 mL, 7.4 mmol) and the reaction mixture was stirred at room temperature for 45 min. Benzyl bromide was added to a stirred reaction mixture and stirred at room temperature for an additional 5 h. The reaction was quenched with saturated aqueous NH_4Cl solution. After THF was removed, the residue was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane to 5% ethyl acetate:hexane) to provide 3-benzyloxypropionitrile (91) (600 mg, 50%) as a yellow oil.

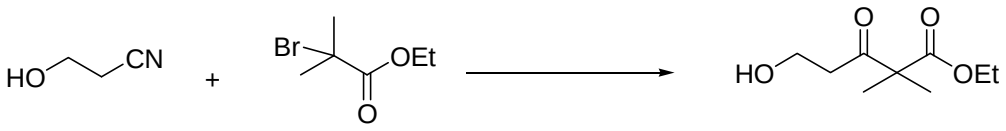
3-Benzyloxypropionitrile (91)

FTIR (neat), ν_{max}, cm^{-1}: 2250, 1454, 1410, 1105

^1H NMR ($CDCl_3$, 400 MHz) δ 7.26 (m, 5H, $C_6\underline{H}_5$), 4.49 (s, 2H, $C_6H_5C\underline{H}_2O$), 3.58 (t, J = 6.4 Hz, 2H, H-3), 2.52 (t, J = 6.4 Hz, 2H, H-2)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.2 (C_{AR}), 128.5 (CH_{AR}), 127.9 (CH_{AR}), 127.7 (CH_{AR}), 117.7 (CN), 73.2 ($C_6H_5\underline{C}H_2O$), 64.5 (C-3), 18.8 (C-2)

Ethyl 5-hydroxy-2, 2-dimethyl-3-oxopentanoate (93)



(90) (92) (93)

To a suspension of Zn powder (20.6 g, 300 mmol) in dry THF (80 mL) was added 3-hydroxypropionitrile (90) (1.35 mL, 20 mmol) and dropwised ethyl 2-bromo-2, 2-dimethylacetate (92) (30 mL, 200 mmol). The reaction mixture was sonicated for 2 h and then THF layer was decanted. The unreacted Zn was washed with THF (50 mL). The combined THF solutions were concentrated to 2 mL under reduced pressure to give the residue that was added with a mixture of ethyl acetate (100 mL) and 1 M HCl (100 mL) and stirred vigorously for 30 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (20% ethyl acetate:hexane) to give ethyl 5-hydroxy-2, 2-dimethyl-3-oxopentanoate (93) (2.6 g, 70%) as a pale yellow oil.

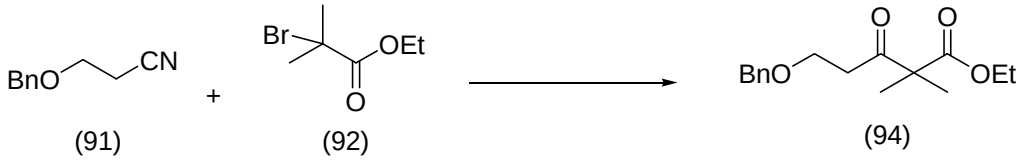
Ethyl 5-hydroxy-2, 2-dimethyl-3-oxopentanoate (93)

FTIR (neat), ν_{max}, cm^{-1}: 3438, 2984, 1720, 1710, 1387, 1268, 1150

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.12 (q, *J* = 7.1 Hz, 2H, $COOC\underline{H}_2CH_3$), 3.79 (t, *J* = 5.4 Hz, 2H, H-5), 2.66 (t, *J* = 5.4 Hz, 2H, H-4), 2.00 (s, 1H, OH), 1.31 (s, 6H, $C(C\underline{H}_3)_2$), 1.19 (t, *J* = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.3 (C-3), 173.3 (C-1), 61.4 ($COO\underline{C}H_2CH_3$), 58.0 (C-5), 55.6 (C-2), 40.1 (C-4), 21.7 ($2CH_3$), 13.9 ($COOCH_2\underline{C}H_3$)

Ethyl 5-benzyloxy-2, 2-dimethyl-3-oxopentanoate (94)



(91) (92) (94)

To a suspension of Zn powder (2.3 g, 37 mmol) in dry THF (10 mL) was added 3-benzyloxypropionitrile (91) (393.4 mg, 2.4 mmol) and added dropwise ethyl 2-bromo-2, 2-dimethylacetate (92) (1.8 mL, 12.2 mmol). The reaction mixture was sonicated for 2 h and then THF layer was decanted. The unreacted Zn was washed with THF (15 mL). The combined THF solutions were concentrated to 1 mL under reduced pressure to give the residue that was added with a mixture of ethyl acetate (10 mL) and 1 M HCl (10 mL) and stirred vigorously for 30 min. The organic layer was separated and the aqueous layer was back extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (5% ethyl acetate:hexane) to give ethyl 5-benzyloxy-2, 2-dimethyl-3-oxopentanoate (94) (418.5 mg, 61%) as a colorless oil.

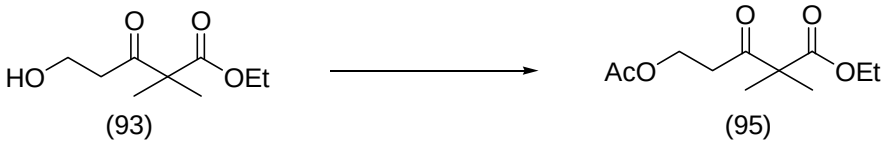
Ethyl 5-benzyloxy-2, 2-dimethyl-3-oxopentanoate (94)

FTIR (neat), ν_{max}, cm^{-1}: 2982, 1734, 1713, 1598, 1454, 1263, 1148

^1H NMR ($CDCl_3$, 400 MHz) δ 7.24 (m, 5H, $C_6\underline{H}_5$), 4.43 (s, 2H, $C_6H_5C\underline{H}_2O$), 4.10 (q, J = 7.1 Hz, 2H, $COOC\underline{H}_2CH_3$), 3.68 (t, J = 5.4 Hz, 2H, H-5), 2.70 (t, J = 5.4 Hz, 2H, H-4), 1.30 (s, 6H, $C(C\underline{H}_3)_2$), 1.16 (t, J = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.1 (CO), 173.4 (COO), 138.2 (C_{AR}), 128.3 (CH_{AR}), 127.6 (CH_{AR}), 73.2 ($C_6H_5\underline{C}H_2O$), 65.4 (C-5), 61.3 ($COO\underline{C}H_2CH_3$), 55.7 (C-2), 38.4 (C-4), 21.6 ($2CH_3$), 13.9 ($COOCH_2\underline{C}H_3$)

Ethyl 5-acetoxy-2, 2-dimethyl-3-oxopentanoate (95)



(93) (95)

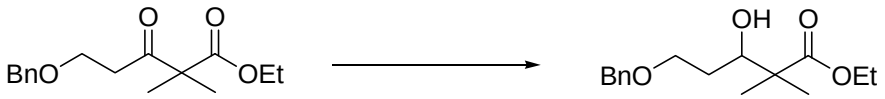
A solution of ethyl 5-hydroxy-2, 2-dimethyl-3-oxopentanoate (93) (1.0 g. 5.3 mmol), acetic anhydride (7 mL) and pyridine (2.5 mL) was stirred at room temperature for 16 h. The reaction was quenched with ice water and extrated with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give ethyl 5-acetoxy-2, 2-dimethyl-3-oxopentanoate (95) (726 mg, 70%) as a colorless oil.

Ethyl 5-acetoxy-2, 2-dimethyl-3-oxopentanoate (95)

FTIR (neat), ν_{max}, cm^{-1}: 2985, 1742, 1714, 1387, 1241, 1150

^1H NMR ($CDCl_3$, 400 MHz) δ 4.26 (t, *J* = 6.4 Hz, 2H, H-5), 4.12 (q, *J* = 7.1 Hz, 2H, $COOC\underline{H}_2CH_3$), 2.72 (t, *J* = 6.4 Hz, 2H, H-4), 1.94 (s, 3H, $C\underline{H}_3OCO$), 1.29 (s, 6H, $C(C\underline{H}_3)_2$), 1.19 (t, *J* = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$)

Ethyl 5-benzyloxy-3-hydroxy-2, 2-dimethylpentanoate (100)



(94) (100)

Sodium borohydride (24.7 mg, 0.6 mmol) was added to a solution of ethyl 5-benzyloxy-2, 2-dimethyl-3-oxopentanoate (94) (73.3 mg, 0.3 mmol) in methanol. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of saturated aqueous NaCl solution. After methanol was removed, the mixture was extracted with CH_2Cl_2 (3×25 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to give ethyl 5-benzyloxy-3-hydroxy-2, 2-dimethylpentanoate (100) (43.7 mg, 60%) as a colorless oil.

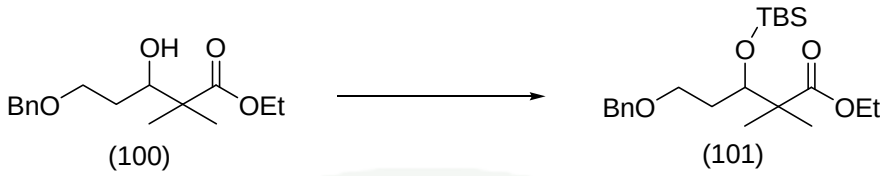
Ethyl 5-benzyloxy-3-hydroxy-2, 2-dimethylpentanoate (100)

FTIR (neat), ν_{max}, cm^{-1}: 3492, 2979, 1724, 1469, 1454, 1267, 1095

^1H NMR ($CDCl_3$, 400 MHz) δ 7.30 (m, 5H, $C_6\underline{H}_5$), 4.51 (s, 1H, $C_6H_5C\underline{H}_2O$), 4.50 (s, 1H, $C_6H_5C\underline{H}_2O$), 4.13 (q, J = 7.1 Hz, 2H, $COOC\underline{H}_2CH_3$), 3.88 (m, 1H, H-3), 3.68 (m, 2H, H-5), 3.14 (s, OH), 3.12 (s, OH), 1.67 (m, 2H, H-4), 1.23 (t, J = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.17 (s, 3H, CCH_3), 1.15 (s, 3H, CCH_3)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.4 (C-1), 136.0 (C_{AR}), 128.5 (CH_{AR}), 127.7 (CH_{AR}), 127.0 (CH_{AR}), 75.3 (C-3), 73.3 ($C_6H_5\underline{C}H_2O$), 69.2 (C-5), 60.6 ($COO\underline{C}H_2CH_3$), 46.8 (C-2), 31.5 (C-4), 21.6 (CCH_3), 20.4 (CCH_3), 14.1 ($COOCH_2\underline{C}H_3$)

Ethyl 5-benzyloxy-3-*tert*-butyldimethylsilyloxy-2, 2-dimethylpentanoate (101)



(100) (101)

To a cooled (0°C) solution of ethyl 5-benzyloxy-3-hydroxy-2, 2-dimethylpentanoate (100) (279.7 mg, 1.0 mmol) in dry CH_2Cl_2 was added 2, 6-lutidine (0.23 mL, 2.0 mmol) and *tert*-butyldimethylsilyltrifluoromethanesulfonate (0.5 mL, 1.9 mmol). The reaction was kept at 0°C and stirred for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (2% ethyl acetate:hexane) to afford ethyl 5-benzyloxy-3-*tert*-butyldimethylsilyloxy-2, 2-dimethylpentanoate (101) (221.6 mg, 61%) as a colorless oil.

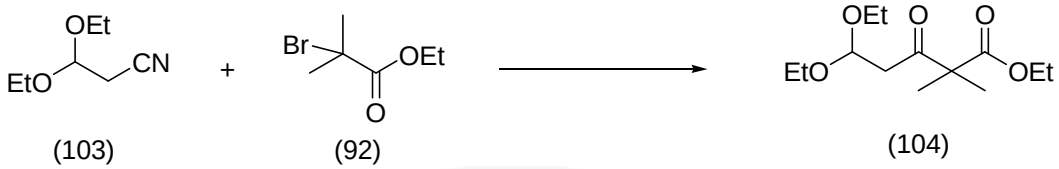
Ethyl 5-benzyloxy-3-*tert*-butyldimethylsilyloxy-2, 2-dimethylpentanoate (101)

FTIR (neat), ν_{max}, cm^{-1}: 2932, 1729, 1469, 1256, 1100, 836

^1H NMR ($CDCl_3$, 400 MHz) δ 7.31 (m, 5H, $C_6\underline{H}_5$), 4.67 (s, 1H, $C_6H_5C\underline{H}_2O$), 4.46 (s, 1H, $C_6H_5C\underline{H}_2O$), 4.06 (m, 3H, $COOC\underline{H}_2CH_3$ and H-3), 3.49 (m, 2H, H-5), 1.79 (m, 1H, H-4), 1.64 (m, 1H, H-4), 1.20 (t, J = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.14 (s, 3H, $CC\underline{H}_3$), 1.07 (s, 3H, $CC\underline{H}_3$), 0.84 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.02 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.0 (C-1), 138.5 (C_{AR}), 128.3 (CH_{AR}), 127.6 (CH_{AR}), 127.5 (CH_{AR}), 73.8 (C-3), 72.8 ($C_6H_5\underline{C}H_2O$), 67.7 (C-5), 60.3 ($COO\underline{C}H_2CH_3$), 48.2 (C-2), 40.0 (C-4), 26.0 ($SiC(\underline{C}H_3)_3$), 21.9 ($C\underline{C}H_3$), 20.2 ($C\underline{C}H_3$), 18.3 ($Si\underline{C}(CH_3)_3$) 14.0 ($COOCH_2\underline{C}H_3$), -4.1 ($SiCH_3$), -4.3 ($SiCH_3$)

Ethyl 5, 5-diethoxy-2, 2-dimethyl-3-oxopentanoate (104)



(103) (92) (104)

To a suspension of Zn powder (1.2 g, 20.0 mmol) in dry THF (5 mL) was added 3, 3-diethoxypropionitrile (103) (0.2 mL, 1.3 mmol) and added dropwise ethyl 2-bromo-2, 2-dimethylacetate (92) (1.0 mL, 6.6 mmol). The reaction mixture was sonicated for 30 min and then THF layer was decanted. The unreacted Zn was washed with THF (10 mL). The combined THF solutions were concentrated to 0.5 mL under reduced pressure to give the residue that was added with a mixture of ethyl acetate (10 mL) and 1 M HCl (10 mL) and stirred vigorously for 30 min. The organic layer was separated and the aqueous layer was back extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (2% ethyl acetate:hexane) to give ethyl 5, 5-diethoxy-2, 2-dimethyl-3-oxopentanoate (104) (297 mg, 86%) as a pale yellow oil.

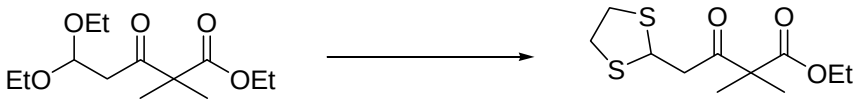
Ethyl 5, 5-diethoxy-2, 2-dimethyl-3-oxopentanoate (104)

FTIR (neat), ν_{max}, cm^{-1}: 2979, 1727, 1716, 1263, 1148, 1060

^1H NMR ($CDCl_3$, 400 MHz) δ 4.88 (t, *J* = 5.5 Hz, 1H, H-5), 4.10 (t, *J* = 7.1 Hz, 3H, $COOC\underline{H}_2CH_3$), 3.59 (m, 2H, $CH_3C\underline{H}_2O$), 3.46 (m, 2H, $CH_3C\underline{H}_2O$), 2.73 (d, *J* = 5.5 Hz, 2H, H-4), 1.28 (s, 6H, $C(C\underline{H}_3)_2$), 1.18 (t, *J* = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.10 (t, *J* = 7.1 Hz, 6H, $C\underline{H}_3CH_2O$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.9 (C-3), 173.2 (C-1), 100.3 (C-5), 62.8 ($CH_3\underline{C}H_2O$), 61.3 ($COO\underline{C}H_2CH_3$), 55.8 (C-2), 42.3 (C-4), 21.5 ($C(\underline{C}H_3)_2$), 15.2 ($\underline{C}H_3CH_2O$), 13.9 ($COOCH_2\underline{C}H_3$)

Ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (105)



(104) (105)

1, 2-ethanedithiol (0.5 mL, 4.9 mmol) and borontrifluoro etherate (0.5 mL, 3.9 mL) were added dropwise to a solution of ethyl 5, 5-diethoxy-2, 2-dimethyl-3-oxopentanoate (104) (1.2 g, 4.8 mmol) in dry CH_2Cl_2 (40 mL). The resulting solution was stirred at room temperature for 1 h. The reaction was poured into 10% aqueous KOH (40 mL) and extracted with CH_2Cl_2 (4×50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (2% ethyl acetate:hexane) to yield ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (105) (923.9 mg, 74%) as a colorless oil.

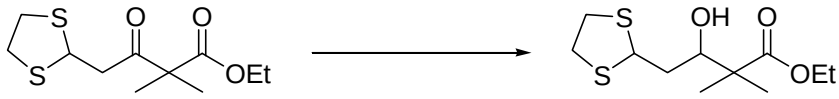
Ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (105)

FTIR (neat), ν_{max}, cm^{-1}: 2981, 1738, 1712, 1261, 1148

^1H NMR ($CDCl_3$, 400 MHz) δ 4.79 (t, *J* = 6.9 Hz, 1H, H-5), 4.11 (q, *J* = 7.1 Hz, 2H, $COOC\underline{H}_2CH_3$), 3.14 (s, 4H, $CH(SC\underline{H}_2)_2$), 2.99 (d, *J* = 6.9 Hz, 2H, H-4), 1.30 (s, 6H, $(C(C\underline{H}_3)_2$), 1.20 (t, *J* = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.5 (C-3), 173.2 (C-1), 61.5 ($COO\underline{C}H_2CH_3$), 55.3 (C-2), 48.2 ($CH(S\underline{C}H_2)_2$), 47.0 (C-5), 38.5 (C-4), 21.8 ($C(\underline{C}H_3)_2$), 14.0 ($COOCH_2\underline{C}H_3$)

Ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (106)



(105) (106)

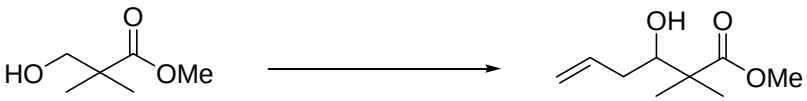
Sodium borohydride (78 mg, 2.0 mmol) was added to a cooled (0°C) solution of ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (105) (261.7 mg, 1.0 mmol) in methanol (4 mL). The reaction was stirred at room temperature for 1 h and quenched with saturated aqueous NaCl solution. Methanol was removed and the residue was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (3% ethyl acetate:hexane) to give an isomeric mixture of ethyl 4-(1, 3-dithiolan-2-yl)-3-hydroxy-2, 2-dimethylbutanoate (106) (110.2 mg, 42%) as a colorless oil.

Ethyl 4-(1, 3-dithiolan-2-yl)-3-hydroxy-2, 2-dimethylbutanoate (106)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, *J* = 9.4, 6.4 Hz, 1H, H-5), 4.10 (q, *J* = 7.2 Hz, 1H, COO$\underline{C}H_2CH_3$), 4.09 (q, *J* = 7.2 Hz, 1H, COOC$\underline{H}_2CH_3$), 3.71 (m, 1H, H-3) 3.17 (m, 4H, CH(SC$\underline{H}_2)_2$), 2.85, 2.84 (2s, 1H, OH), 1.81 (m, 2H, H-4), 1.20 (t, *J* = 7.1 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.13 (s, 3H, $CC\underline{H}_3$), 1.10 (s, 3H, $CC\underline{H}_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.3 (C-1), 75.8 (C-3), 60.7 ($COO\underline{C}H_2CH_3$), 50.8 (C-5), 46.7 (C-2), 41.5 (C-4), 38.4, 37.9 (CH(S$\underline{C}H_2)_2$), 22.2 (CCH_3), 20.2 (CCH_3), 14.1 ($COOCH_2\underline{C}H_3$)

Methyl 3-hydroxy-2, 2-dimethyl-5-hexenoate (109)



(108) (109)

Step I (Using PCC)

To a solution of methyl 3-hydroxy-2, 2-dimethylpropionoate (108) (812.4 mg, 6.2 mmol) in CH_2Cl_2 (10 mL) was added pyridinium chlorochromate (PCC) (1.9 g, 9.2 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was diluted with CH_2Cl_2 (25 mL) and allowed to stir for additional 15 min before filtered through a short pad of silica gel. The filtrate was concentrated under reduced pressure. The aldehyde intermediate was used in the next step without purification.

Step I (Using IBX)

To a solution of methyl 3-hydroxy-2, 2-dimethylpropionoate (108) (201.7 mg, 1.5 mmol) in dimethylsulfoxide (3 mL) was added 2-iodoxybenzoic acid (IBX) (1.8 g, 6.5 mmol). The resulting mixture was stirred at room temperature for 5 h. The reaction was diluted with water and stirred for 10 min. The residue was filtered through sintered glass funnel, the filtrate was extracted with diethyl ether (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure.The aldehyde intermediate was used in the next step without purification.

Step II

To a mixture of aldehyde intermediate in THF (20 mL) and saturated aqueous NH_4Cl solution (40 mL) was added Zn powder (564 mg, 8.3 mmol) and allyl bromide (0.7 mL, 8.3 mmol), respectively. The reaction mixture was gentle refluxed and

stirred for 20 min. THF was removed under reduced pressure and the residue was extracted with diethyl ether (4×50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (7% ethyl acetate:hexane) to yield methyl 3-hydroxy-2, 2-dimethyl-5-hexenoate (109) (320 mg, 30%) as a colorless oil.

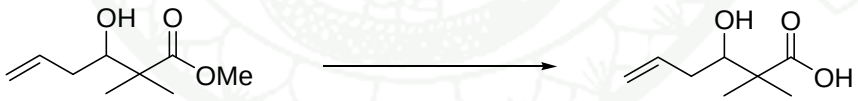
Methyl 3-hydroxy-2, 2-dimethyl-5-hexenoate (109)

FTIR (neat), ν_{max}, cm^{-1}: 3482, 2980, 1724, 1642, 1469, 1265, 1142, 1023

^1H NMR ($CDCl_3$, 400 MHz) δ 5.84 (m, 1H, H-5), 5.09 (m, 2H, H-6), 3.69 (dd, *J* = 10.4, 2.5, Hz, 1H, H-3), 3.67 (s, 3H, $COOCH_3$), 2.24 (m, 2H, H-4, OH), 2.03 (m, 1H, H-4), 1.17 (s, 6H, $C(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.8 (C-1), 135.5 (C-5), 117.5 (C-6), 75.6 (C-3), 51.9 ($COO\underline{C}H_3$), 46.8 (C-2), 36.5 (C-4), 22.0 ($C\underline{C}H_3$), 20.4 ($C\underline{C}H_3$)

3-Hydroxy-2, 2-dimethyl-5-hexenoic acid (110)



(109) (110)

1 M LiOH (12 mL) was slowly added to the solution of methyl 3-hydroxy-2, 2-dimethyl-5-hexenoate (109) (320 mg, 1.9 mmol) in methanol (7 mL) and stirred at room temperature for 16 h. The resulting mixture was diluted with ethyl acetate (10 mL). The mixture was acidified to pH 1 with aqueous 10% HCl solution, extracted with ethyl acetate (3×50 mL). The combine organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (30% ethyl acetate:hexane) to provide 3-hydroxy-2, 2-dimethyl-5-hexenoic acid (110) (205.6 mg, 70%) as a colorless oil.

3-Hydroxy-2, 2-dimethyl-5-hexenoic acid (110)

FTIR (neat), ν_{max}, cm^{-1}: 3418, 2981, 1706, 1642, 1472, 1264, 1068

^1H NMR ($CDCl_3$, 400 MHz) δ 5.80 (m, 1H, H-5), 5.08 (m, 2H, H-6), 3.19 (dd, *J* = 10.4, 2.5 Hz, 1H, H-3), 2.26 (m, 1H, H-4), 2.03 (m, 1H, H-4), 1.17 (s, 3H, C$\underline{H}_3$) 1.15 (s, 3H, C$\underline{H}_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.6 (C-1), 135.2 (C-5), 117.9 (C-6), 75.4 (C-3), 46.7 (C-2), 36.3 (C-4), 22.1 (C$\underline{C}H_3$), 20.1 (C$\underline{C}H_3$)

3-Acetoxy-2, 2-dimethyl-5-hexenoic acid (111)

(110) (111)

Acetic acid anhydride (3 mL) and pyridine (2 mL) were added to 3-hydroxy-2, 2-dimethyl-5-hexenoic acid (110) (205.6 mg, 1.3 mmol). The reaction mixture was stirred at room temperature for 6 h. The reaction was quenched with ice water, extracted with ethyl acetate (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (20% ethyl acetate:hexane) to give 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111) (167 mg, 61%) as a white solid; m.p. 66-67^oC.

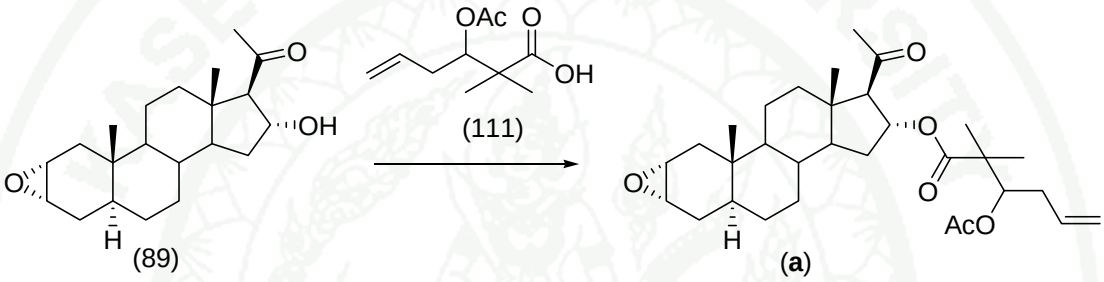
3-Acetoxy-2, 2-dimethyl-5-hexenoic acid (111)

FTIR (KBr), ν_{max}, cm^{-1}: 1738, 1694, 1469, 1228, 1026

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.70 (m, 1H, H-5), 5.25 (dd, *J* = 9.4, 3.3 Hz, 1H, H-3), 5.02 (m, 2H, H-6), 2.28 (m, 2H, H-4), 2.01 (s, 3H, $OCOCH_3$), 1.20 (s, 3H, CCH_3), 1.18 (s, 3H, CCH_3)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.7 (C-1), 170.5 (COO), 134.1 (C-5), 117.6 (C-6), 75.7 (C-3), 46.2 (C-2), 35.2 (C-4), 21.9 ($COO\underline{C}H_3$), 20.8 ($C\underline{C}H_3$), 20.3 ($C\underline{C}H_3$)

2α, 3α-Epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (a)



To a solution of 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111) (89 mg, 0.4 mmol) in benzene (2 mL) containing 1 drop of *N,N*-dimethylformamide was added oxalyl chloride (0.13 mL, 1.6 mmol) under nitrogen gas atmosphere. The reaction mixture was stirred at room temperature for 2.5 h before removing solvent under reduced pressure to give the residue which was diluted with dry CH_2Cl_2 (1 mL) and then slowly added to the solution of 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89) (51 mg, 0.15 mmol) and dimethylaminopyridine (195 mg, 1.6 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 30 min under nitrogen gas atmosphere and quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and further extracted with CH_2Cl_2 (3×40 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (7% ethyl acetate:hexane) to provide 2α, 3α-epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (**a**) (61 mg, 77%) as a colorless gum.

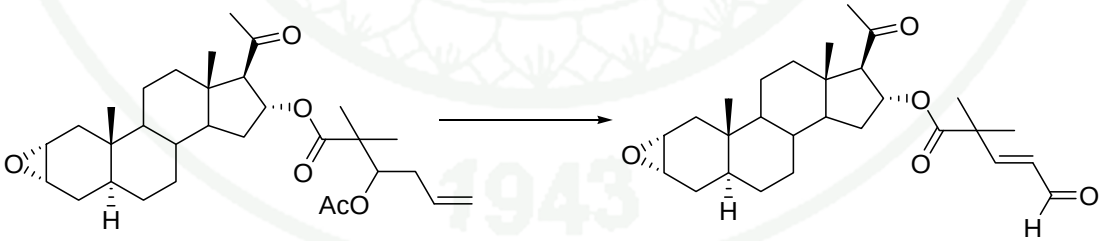
2α, 3α-Epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (a)

FTIR (neat), ν_{max}, cm^{-1}: 2931, 1737, 1705, 1649, 1234, 1153, 1022

^1H NMR ($CDCl_3$, 400 MHz) δ 5.60 (m, 1H, H-5'), 5.39 (m, 1H, H-16), 5.25 (dd, *J* = 9.8, 3.2 Hz, 1H, H-3'), 5.02 (m, 2H, H-6'), 3.06 (m, 2H, H-2, H-3), 2.61 (m, 1H, H-17), 2.17 (m, 2H, H-4'), 2.07 (m, 3H, $OCOCH_3$), 1.94 (s, 1H, H-21), 1.88 (s, 2H, H-21), 2.05-1.10 (4CH, $7CH_2$), 1.08 (m, 6H, $C(CH_3)_2$), 0.68 (m, 3H, CH_3), 0.55 (m, 3H, CH_3)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.4, 206.3 (CO), 175.1, 175.0 (COO), 170.3, 170.2 (COO), 134.2, 134.1 (CH), 117.5 (CH_2), 76.0, 75.8 (CH), 75.6 (CH), 69.8, 69.5 (CH), 54.2, 54.1 (CH), 53.4, 53.3 (CH), 52.3 (CH), 50.8 (CH), 46.1, 46.0 (Cq), 44.4 (Cq), 38.8, 38.7 (CH_2), 38.1 (CH_2), 36.1 (CH), 35.1 (CH_2), 35.0, 34.9 (CH), 33.6, 33.3 (CH_2), 33.1 (Cq), 31.4 (CH_2), 28.9, 28.8 (CH_2), 28.1, 28.0 (CH_2), 21.5, 21.3, 20.9, 20.8, 20.6 ($3CH_3$), 20.5 (CH_2), 20.2 (CH_3), 14.4 (CH_3), 12.7 (CH_3)

16α-(4-Formyl-2, 2-dimethyl-3-butenoate)-2α, 3α-epoxy-5α-pregnan-20-one (162)



(**a**) (162)

A stream of O_3 was bubbled through a solution of 2α, 3α-epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (**a**) (27 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) at -78°C until the solution turned blue, ozone addition was stopped. Triphenylphosphine (34 mg, 0.13 mmol) was added and the mixture was allowed to warm to room temperature for 2 h. The solution was concentrated under reduced pressure and the residue was purified by flash column chromatography (10% ethyl

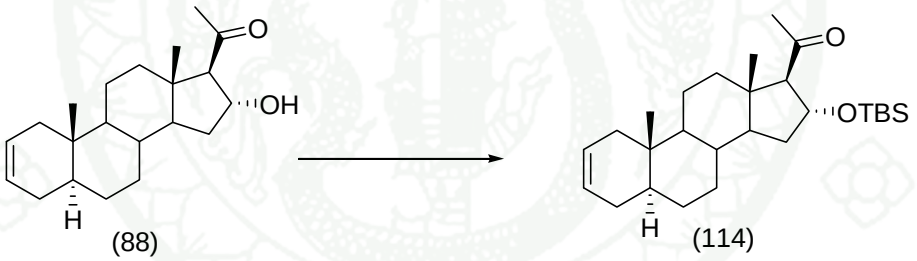
acetate:hexane) to give 16α-(4-formyl-2, 2-dimethyl-3-butenoate)-2α, 3α-epoxy-5α-pregnan-20-one (162) (19 mg, 80%) as a colorless gum.

16α-(4-Formyl-2, 2-dimethyl-3-butenoate)-2α, 3α-epoxy-5α-pregnan-20-one (162)

FTIR (neat), ν_{max}, cm^{-1}: 2925, 1720, 1686, 1649, 1623, 1149

^1H NMR ($CDCl_3$, 400 MHz) δ 9.48 (d, *J* = 7.6 Hz, 1H, CO<u>H</u>), 6.89 (d, *J* = 16.0 Hz, 1H, H-4'), 6.04 (dd, *J* = 16.0, 7.6 Hz, 1H, H-3'), 5.42 (m, 1H, H-16), 3.06 (m, 2H, H-2, H-3), 2.54 (d, *J* = 6.5 Hz, 1H, H-17), 2.07 (s, 3H, H-21), 1.92-0.77 (4CH, $7CH_2$), 1.29 (s, 6H, $C(CH_3)_2$), 0.68 (s, 3H, CH_3), 0.58 (s, 3H, CH_3)

16α-*tert*-Butyldimethylsilyloxy-5α-2-pregnen-20-one (114)



To a solution of 16α-hydroxy-5α-2-pregnen-20-one (88) (30 mg, 0.1 mmol) in CH_2Cl_2 (2 mL) was added *tert*-butyldimethylsilyl chloride (74 mg, 0.5 mmol), imidazole (18.3 mg, 0.3 mmol) and *N,N*-dimethylformamide (0.05 mL). The resulting mixture was stirred at room temperature for 16 h. Then, the reaction was quenched by addition of water. After separation, aqueous layer was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (2% ethyl acetate:hexane) to provide 16α-*tert*-butyldimethylsilyloxy-5α-2-pregnen-20-one (114) (40.8 mg, 95%) as a white prism after recrystallization (ethanol); m.p. 140-142°C.

16α-*tert*-Butyldimethylsilyloxy-5α-2-pregnen-20-one (114)

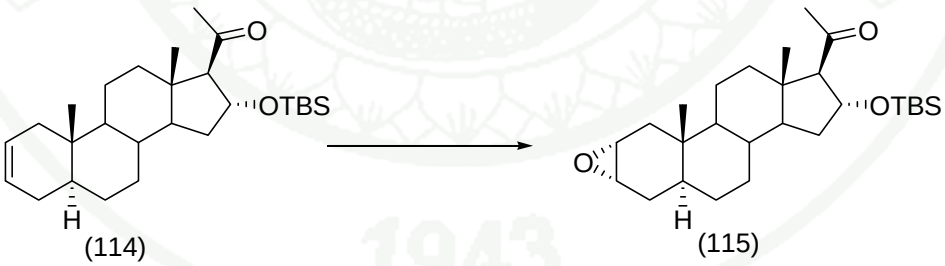
FTIR (KBr), ν_{max}, cm^{-1}: 2929, 1701, 1649, 1553, 1454, 1070, 1019, 831

^1H NMR ($CDCl_3$, 400 MHz) δ 5.56 (m, 2H, H-2, H-3), 4.73 (td, *J* = 7.8, 1.5 Hz, 1H, H-16), 2.58 (d, *J* = 6.3 Hz, 1H, H-17), 2.10 (s, 3H, H-21), 2.07-0.73 (4CH, 7CH_2), 0.82 (s, 9H, $SiC(CH_3)_3$), 0.72 (s, 3H, H-19), 0.58 (s, 3H, H-18), -0.01 (s, 3H, $SiCH_3$), -0.04 (s, 3H, $SiCH_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.6 (C-20), 125.9, 125.7 (C-2, C-3), 74.4 (C-17), 72.9 (C-16), 54.3 (CH), 53.7 (CH), 44.9 (C-13), 41.4 (CH), 39.6 (CH_2), 38.9 (CH_2), 36.2 (CH_2), 35.2 (CH), 34.6 (C-10), 32.4 (C-21), 31.6 (CH_2), 30.2 (CH_2), 28.5 (CH_2), 25.8 ($SiC(\underline{C}H_3)_3$), 20.6 (CH_2), 17.9 ($Si\underline{C}(CH_3)_3$), 14.6 (CH_3), 11.6 (CH_3), -4.7 ($SiCH_3$), -4.9 ($SiCH_3$)

HRMS *m/z*: $C_{27}H_{47}O_2NaSi$ $[M+Na]^+$, calcd 431.3345, found 431.3344

16α-*tert*-Butyldimethylsilyloxy-2α, 3α-epoxy-5α-pregnan-20-one (115)



(114) (115)

To a solution of 16α-*tert*-butyldimethylsilyloxy-5α-2-pregnen-20-one (114) (18.6 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) was added water (1 mL) and sodium carbonate (23.2 mg, 0.2 mmol). The mixture was stirred vigorously before *m*-chloroperbenzoic acid (10.1 mg, 0.06 mmol) was added slowly. The reaction mixture was stirred at room temperature for 4 h. The aqueous layer was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were washed with 5% sodium sulfite solution, saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous

Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give 16α-*tert*-butyldimethylsilyloxy-2α, 3α-epoxy-5α-pregnan-20-one (115) (10.5 mg, 59%) as a white needle after recrystallization (ethanol); m.p. 130-131°C.

16α-*tert*-Butyldimethylsilyloxy -2α, 3α-epoxy-5α-pregnan-20-one (115)

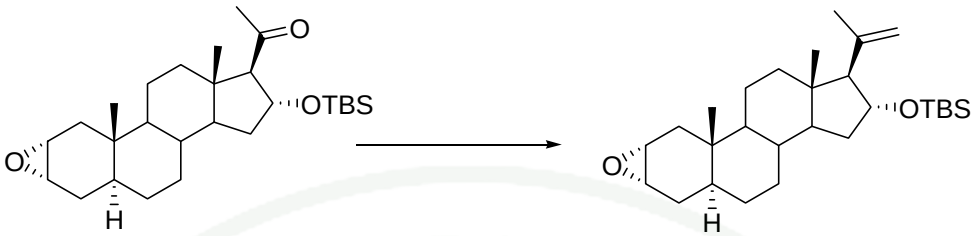
FTIR (KBr), ν_{max}, cm^{-1}: 2915, 1697, 1454, 1255, 1071, 835

^1H NMR ($CDCl_3$, 400 MHz) δ 4.71 (m, 1H, H-16), 3.13 (m, 1H, H-3), 3.08 (m, 1H, H-2), 2.57 (d, *J* = 6.4 Hz, 1H, H-17), 2.09 (s, 3H, H-21), 1.91-0.65 (4CH, 7CH_2), 0.81 (s, 9H, $SiC(CH_3)_3$), 0.72 (s, 3H, H-19), 0.56 (s, 3H, H-18), -0.02 (s, 3H, $SiCH_3$), -0.05 (s, 3H, $SiCH_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.6 (C-20), 74.0 (C-17), 72.9 (C-16), 54.1 (CH), 53.5 (CH), 52.3 (C-3), 50.9 (C-2), 44.8 (C-13), 38.8 (CH_2), 38.2 (CH_2), 36.2 (CH_2), 36.2 (CH), 35.2 (CH), 33.7 (C-21), 32.4 (C-10), 31.5 (CH_2), 29.0 (CH_2), 28.2 (CH_2), 25.8 ($SiC(CH_3)_3$), 20.0 (CH_2), 17.9 ($SiC(CH_3)_3$), 14.5 (CH_3), 12.1 (CH_3), -4.7 ($SiCH_3$), -4.9 ($SiCH_3$)

HRMS *m/z*: $C_{27}H_{47}O_3NaSi$ $[M+Na]^+$, calcd 447.3294, found 447.3293

2α, 3α-Epoxy-16α-*tert*-butyldimethylsilyloxy-20-methyl-5α-pregn-20-ene (116)



(115) (116)

To a cool (0°C) solution of methyltriphenylphosphonium iodide (160 mg, 0.4 mmol) in THF (3 mL) was added a solution of *n*BuLi in hexane (0.4 mL, 0.6 mmol). The mixture was stirred at 0°C for 15-20 min, warmed to room temperature, and stirred for an additional 1 h. A solution of 2α, 3α-epoxy-16α-*tert*-butyldimethylsilyloxy-5α-pregnan-20-one (115) (19 mg, 0.04 mmol) in THF (3 mL) was added dropwise to the above mixture and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of saturated aqueous NH_4Cl, and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (1% ethyl acetate:hexane) to give 2α, 3α-epoxy-16α-*tert*-butyldimethylsilyloxy-20-methyl-5α-pregn-20-ene (116) (3.1 mg, 17% (brsm)) as a white needle after recrystallization (ethanol); m.p. 150-152°C and recovered starting material (115) (10.3 mg, 46% conversion).

2α, 3α-Epoxy-16α-*tert*-butyldimethylsilyloxy-20-methyl-5α-pregn-20-ene (116)

FTIR (KBr), ν_{max}, cm^{-1}: 2929, 1649, 1557, 1461, 1078, 1015, 835

^1H NMR ($CDCl_3$, 400 MHz) δ 4.89 (m, 1H, H-22), 4.68 (m, 1H, H-22), 4.34 (td, *J* = 7.3, 2.0 Hz, 1H, H-16), 3.13 (m, 1H, H-3), 3.08 (m, 1H, H-2), 1.99 (d, *J* = 7.3 Hz, 1H, H-17), 1.87 (m, 2H, CH_2), 1.72 (s, 3H, H-21), 1.67-0.67 (4CH, 6CH_2), 0.82

(s, 9H, $SiC(CH_3)_3$), 0.72 (s, 3H, CH_3), 0.55 (s, 3H, CH_3), -0.02 (s, 3H, $SiCH_3$), -0.03 (s, 3H, $SiCH_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.3 (C-20), 111.4 (C-22), 75.2 (C-16), 67.9 (C-17), 53.8 (CH), 53.4 (CH), 52.4 (C-3), 51.0 (C-2), 43.8 (C-13), 38.4 (CH_2), 38.2 (CH_2), 36.3 (CH_2), 36.3 (CH), 35.5 (CH), 33.7 (C-10), 31.6 (CH_2), 29.0 (CH_2), 28.4 (CH_2), 25.9 ($SiC(\underline{C}H_3)$), 25.2 (C-21), 20.4 (CH_2), 18.0 ($Si\underline{C}(CH_3)$), 14.4 (CH_3), 12.9 (CH_3), -4.4 ($SiCH_3$), -4.7 ($SiCH_3$)

HRMS *m/z*: $C_{28}H_{49}O_2NaSi$ $[M+Na]^+$, calcd 445.3502 found 445.3505

2α, 3α-Epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117)

(116) (117)

To a solution of 2α, 3α-epoxy-16α-*tert*-butyldimethylsilyloxy-20-methyl-5α-20-pregnene (116) (6.07 mg, 0.02 mmol) in THF (2 mL) was added $TBAF.H_2O$ (100.2 mg, 0.3 mmol) at room temperature and stirred for 48 h. The reaction was quenched by dilution with diethyl ether and washing with water. The aqueous phase was extracted with diethyl ether (3×25 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to provide 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117) (1.6 mg, 32% (brsm)) as a white solid and recovered starting material (116) (2.0 mg, 70% conversion).

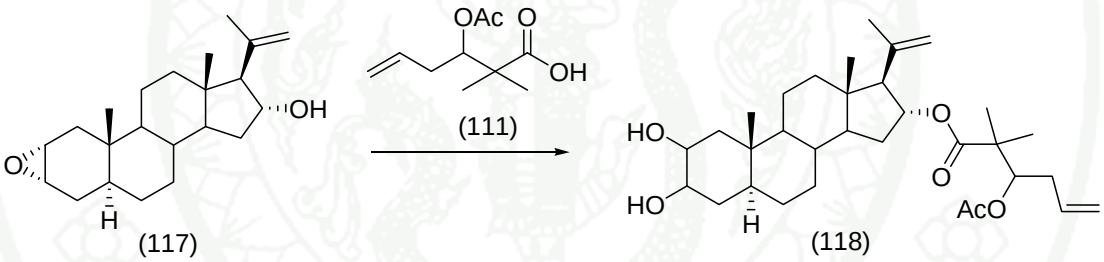
2α, 3α-Epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117)

FTIR (neat), ν_{max}, cm^{-1}: 3431, 2922, 1638, 1454, 1056

^1H NMR ($CDCl_3$, 400 MHz) δ 4.89 (m, 1H, H-22), 4.74 (m, 1H, H-22), 4.41 (m, 1H, H-16), 3.08, 3.04 (m, 2H, H-2, H-3), 1.92 (d, *J* = 7.2 Hz, 1H, H-17), 1.82 (m, 2H, CH_2), 1.72 (s, 3H, H-21), 1.70-0.61 (2CH, $7CH_2$), 0.68 (s, 3H, CH_3), 0.52 (s, 3H, CH_3)

HRMS *m/z*: $C_{22}H_{34}O_2Na$ $[M+Na]^+$, calcd 353.2457, found 353.2458

16α-(3-Acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-pregn-20-ene (118)



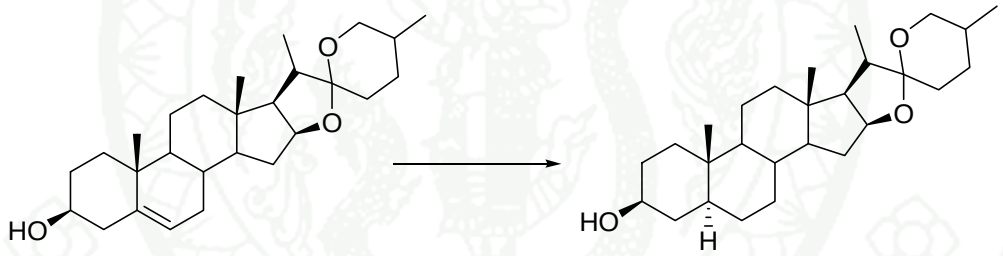
To a solution of 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111) (15.3 mg, 0.07 mmol) in benzene (1 mL) containing 1 drop of *N,N*-dimethylformamide was added oxalyl chloride (0.01 mL, 0.12 mmol) under nitrogen gas atmosphere. The reaction mixture was stirred at room temperature for 2.5 h and the solvent was removed under reduced pressure to give the residue that was diluted with CH_2Cl_2 (1 mL) and slowly added to a solution of 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-20-pregnene (117) (4.5 mg, 0.01 mmol) and dimethylaminopyridine (DMAP) in CH_2Cl_2 (1 mL). The reaction mixture was stirred at room temperature for 30 min under nitrogen gas atmosphere and quenched with saturated aqueous $NaHCO_3$ solution. The aqueous phase was back extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (7%

ethyl acetate:hexane) to give 16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-pregn-20-ene (118) (1.2 mg, 16%) as a white solid.

16α-(3-Acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-pregn-20-ene (118)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.64 (m, 1H, H-5'), 5.39, 5.31 (2m, 1H, H-16), 5.18 (m, 1H, H-6'), 4.94 (m, 1H, H-6') , 4.82, 4.80 (2m, 1H, H-22), 4.69, 4.65 (2s, 1H, H-22), 4.08, 4.02 (2m, 2H, H-2, H-3), 2.21 (s, 3H, $COOCH_3$), 1.94, 1.91 (2s, 3H, H-21), 2.24-1.19 (5CH, 8CH_2), 1.18 (m, 3H, CH_3), 1.09, 1.08 (m, 3H, CH_3), 0.98, 0.97 (2s, 3H, CH_3), 0.56, 0.55 (2s, 3H, CH_3)

Tigogenin (54)



To a solution of diosgenin (59) (221.4 mg, 0.5 mmol) in THF (5 mL) was added 5% Pd/C (24 mg, 10.6×10^{-3} mmol) under hydrogen gas atmosphere. The resulting mixture was stirred at room temperature for 48 h. After the reaction was filtered through Celite and eluted with CH_2Cl_2, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to give tigogenin (54) (179 mg, 82%) as a white needle after crystallization (hexane); m.p. 201-202^{o}C.

Tigogenin (54)

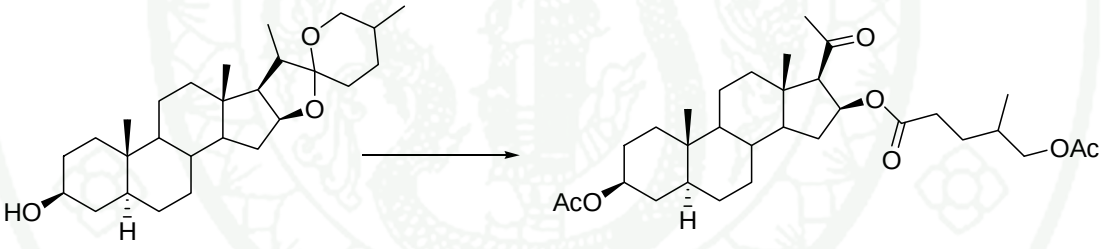
FTIR (KBr), ν_{max}, cm^{-1}: 3520, 3389, 2928, 1459, 1177, 1053, 1041

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.32 (m, 1H, H-16), 3.51 (m, 1H, H-3), 3.34 (ddd, *J* = 10.9, 4.2, 1.9 Hz, 1H, H-26), 3.30 (t, *J* = 10.9 Hz, 1H, H-26), 1.90 (m, 1H, H-25), 1.79 (t, *J* = 6.9 Hz, 1H, H-20), 1.75-0.78 (4CH, 10CH_2), 0.89 (d, *J* = 6.9 Hz, 3H, H-21), 0.75 (s, 3H, H-19), 0.72 (s, 3H, H-18), 0.69 (m, 1H, CH)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 109.2 (C-22), 80.8 (C-16), 71.3 (C-3), 66.8 (C-26), 62.2 (CH), 56.3 (CH), 54.3 (CH), 44.8 (CH), 41.6 (CH), 40.6 (C-13), 40.0 (CH), 38.2 (CH_2), 37.0 (CH_2), 35.6 (C-10), 35.1 (CH), 32.2 (CH_2), 31.7 (CH_2), 31.5 (CH_2), 31.4 (CH_2), 30.3 (CH), 28.8 (CH_2), 28.6 (CH_2), 21.1 (CH_2), 17.1 (C-27), 16.5 (C-18), 14.5 (C-21), 12.3 (C-19)

MS (ESI) *m/z*: $C_{27}H_{46}O_3$ $[M+H]^+$, calcd 417.3363 , found 417.3381

3β-Acetoxy-16β-(5-acetoxy-4-methylpentanoate)-5α-pregnan-20-one (60)



Step I

A mixture of tigogenin (54) (1.4 g, 3.2 mmol), acetic anhydride (12.1 mL), ammonium chloride (395 mg) and pyridine (3.1 mL) was heated to 135^{o}C and kept at this temperature for 16 h. After cooling down, the reaction mixture was added cool water and extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were neutralized with saturated aqueous $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was used in the next step without further purification.

Step II

The crude product was dissolved in 1, 2-dichloroethane (8 mL), water (0.6 mL) and acetic acid (3.1 mL). The mixture was cooled to 0°C and added with a solution of chromium trioxide (671 mg) in water (0.8 mL) and acetic acid (0.3 mL) (This solution was kept below 7°C during addition). The mixture was allowed to warm to room temperature and stirred for 2 h. A solution of sodium chloride (2.7 g) in water (11 mL) and methanol (0.13 mL) was introduced and the resulting mixture was further stirred for additional 1 h. The reaction was quenched with cool water and extracted with CH_2Cl_2 (4×100 mL).The combined organic layers were neutralized with saturated aqueous $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (20% ethyl acetate:hexane) to give 3β-acetoxy-16β-(5-acetoxy-4-methylpentanoate)-5α-pregnan-20-one (60) (732.3 mg, 43%) as a white solid; m.p. 98-99°C. (Boonananwong *et al*., 2008b, m.p. 97-98°C)

3β-Acetoxy-16β-(5-acetoxy-4-methylpentanoate)-5α-pregnan-20-one (60)

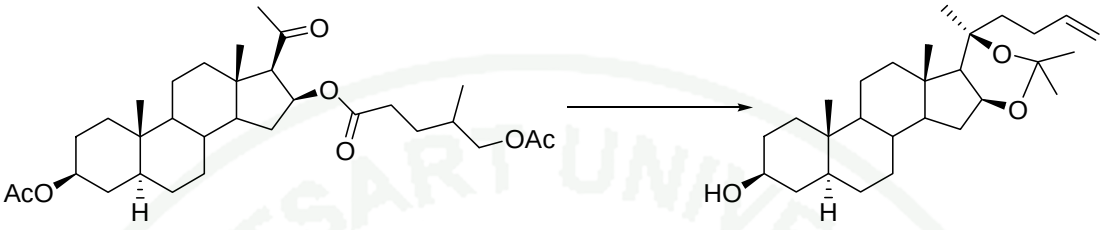
FTIR (KBr), ν_{max}, cm^{-1}: 2933, 1732, 1363, 1247, 1153, 1028

^1H NMR ($CDCl_3$, 400 MHz) δ 5.44 (dt, *J* = 7.8, 4.4 Hz, 1H, H-16), 4.61 (m, 1H, H-3), 3.83 (d, *J* = 6.1 Hz, 2H, H-5'), 2.33 (m, 1H, CH), 2.32 (d, *J* = 7.6 Hz, 1H, H-17), 2.22 (m, 2H, CH_2), 1.99 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.94 (s, 3H, CH_3), 1.78-0.78 (4CH, 9CH_2), 0.95 (s, 3H, H-19), 0.86 (d, *J* = 6.7 Hz, 3H, H-4'), 0.77 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.2 (C-20), 172.9 (COO), 171.0 (COO), 170.6 (COO), 74.3 (C-16), 73.5 (C-3), 68.7 (C-5'), 66.6 (C-17), 54.2 (CH), 53.8 (CH), 44.6 (CH), 42.5 (C-13), 38.1 (CH_2), 36.6 (CH_2), 35.5 (C-10), 35.0 (CH_2), 34.3 (CH), 33.8 (CH_2), 31.9 (CH), 31.8 (CH_2), 31.7 (CH_2), 30.6 (CH_3), 28.3 (CH_2), 28.2 (CH_2), 27.3 (CH_2), 21.3 (CH_3), 20.8 (CH_3), 20.4 (CH_3), 16.3 (CH_3), 13.5 (CH_3), 12.1 (CH_3)

HRMS *m/z*: $C_{31}H_{48}O_7Na$ $[M+Na]^+$, calcd 555.3298, found 555.3298

3β-Hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119)



(60) (119)

Step I

To a stirring mixture of Mg (302 mg, 12.4 mmol) and catalyst I_2 in dry THF (5 mL) was slowly added 4-bromobutene (1.0 mL, 9.8 mmol) under nitrogen atmosphere and stirred for 1 h. A solution of 3β-acetoxy-16β-(5-acetoxy-4-methylpentanoate)-5α-pregnan-20-one (60) (644.2 mg, 1.2 mmol) in dry THF (5 mL) was slowly added. After 20 min, the reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with ethyl acetate (4×100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was used in the next step without further purification.

Step II

A mixture of the crude product, 2, 2-dimethoxypropane (8 mL) and *p*-toluenesulfonic acid (15 mg, 0.08 mmol) was stirred at room temperature for 4 h. the reaction mixture was quenched with triethylamine and water and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (15% ethyl acetate:hexane) to give 3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119) (168.1 mg, 32 % (2 steps)) as a white needle after recrystallization (hexane); m.p. 144-145 ^oC.

3β-Hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119)

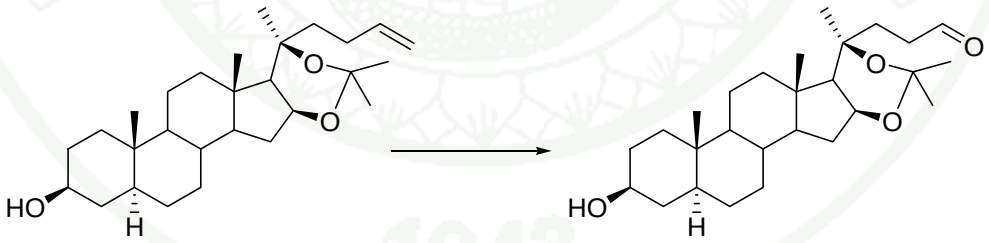
FTIR (KBr), ν_{max}, cm^{-1}: 3442, 2933, 1642, 1458 1374, 1244, 1196, 1041

^1H NMR ($CDCl_3$, 400 MHz) δ 5.74 (m, 1H, H-24), 4.94 (dd, *J* = 17.1, 1.7 Hz, 1H, H-25), 4.87 (dd, *J* = 10.6, 1.8 Hz, 1H, H-25), 4.39 (m, 1H, H-16), 3.51 (m, 1H, H-3), 2.07 (m, 3H, CH, CH_2), 1.89 (m, 2H, CH_2), 1.83-0.77 (4CH, 8CH_2), 1.38 (s, 3H, H-21), 1.26 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.02 (s, 3H, H-19), 0.75 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9 (C-24), 114.2 (C-25), 97.1 (Cq), 74.9 (C-20), 71.3 (CH), 68.6 (CH), 55.6 (CH), 54.7 (CH), 54.6 (CH), 44.9 (CH), 43.2 (CH_2), 42.4 (C-13), 40.0 (CH_2), 38.2 (CH_2), 36.9 (CH_2), 35.5 (C-10), 34.7 (CH), 33.8 (CH_2), 31.8 (CH_2), 31.8 (CH_2), 31.5 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 25.6 (CH_3), 24.1 (C-21), 20.7 (CH_2), 14.8 (CH_3), 12.3 (CH_3)

HRMS *m/z*: $C_{28}H_{46}O_3Na$ $[M+Na]^+$, calcd 453.3345, found 453.3354

24-Formyl-3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholane (120)



(119) (120)

A solution of 3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119) (27.1 mg, 0.06 mmol) in CH_2Cl_2 (5 mL) was cooled to -78°C and ozone was bubbled through the solution with stirring. When the solution turned blue, ozone addition was stopped. Nitrogen was passed through the solution until the blue color was discharged. The reaction was quenched at -78°C with triphenylphosphine (19.0 mg, 0.07 mmol). The resulting mixture was stirred and slowly allowed to warm for 1 h. The mixture was further to stir continuously at room temperature for 2 h, then

concentrated under reduced pressure and further to purify by flash column chromatography (3% ethyl acetate:hexane) to give 24-formyl-3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholane (120) (17.6 mg, 65%) as a white solid.

24-Formyl-3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholane (120)

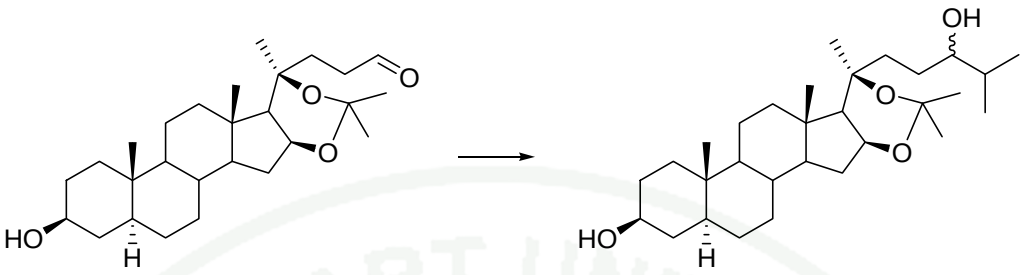
FTIR (KBr), ν_{max}, cm^{-1}: 3448, 2922, 1711, 1649, 1458, 1192, 1052

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.75 (s, 1H, H-24), 4.38 (m, 1H, H-16), 3.52 (m, 1H, H-3), 3.42 (s, 1H, OH), 2.52 (t, *J* = 7.6 Hz, 2H, H-23), 2.17 (m, 1H, CH), 2.08 (dt, *J* = 13.4, 7.7 Hz, 1H, CH), 1.90 (dt, *J* = 12.6, 3.6 Hz, 1H, CH), 1.73 (m, 1H, CH), 1.66-0.77 ($9CH_2$), 1.32 (s, 3H, H-21), 1.26 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.01 (s, 3H, H-19), 0.75 (s, 3H, H-18), 0.57 (m, 1H, CH)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.2 (C-24), 97.3 (Cq), 74.2 (C-20), 71.3 (CH), 68.7 (CH), 56.0 (CH), 54.6 (CH), 54.5 (CH), 44.9 (CH), 42.5 (C-13), 40.0 (CH), 38.9 (CH_2), 38.2 (CH_2), 36.9 (CH_2), 35.5 (C-10), 35.2 (CH_2), 34.7 (CH_2), 33.6 (CH_2), 31.8 (CH_2), 31.5 (CH), 31.4 (CH_2), 28.6 (CH_2), 25.9 (CH_3), 23.9 (CH_3), 20.7 (CH_2), 14.9 (CH_3), 12.3 (CH_3)

MS (APCI), *m/z* (relative intensity): 375 (33), 357 (83), 339 (100), 321 (25), 257 (69)

3β, 24-Dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (121)



(120) (121)

To a stirring mixture of Mg (100 mg, 4.2 mmol) and catalyst I_2 in dry THF (2 mL) was slowly added 2-bromopropane (1.0 mL, 10.2 mmol) under nitrogen atmosphere at room temperature. After stirring for 1 h, this mixture was cooled to -78°C and then a solution of 24-formyl-3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholane (120) (30.2 mg, 0.07 mmol) in dry THF (2 mL) was added slowly and kept temperature at -78°C for 1.5 h. The reaction was quenched by addition of saturated aqueous NH_4Cl, the resulting mixture was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatrography (20% ethyl acetate:hexane) to give 3β, 24-dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (121) (27.7 mg, 84%) as a white solid; m.p.169-174°C.

3β, 24-Dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (121)

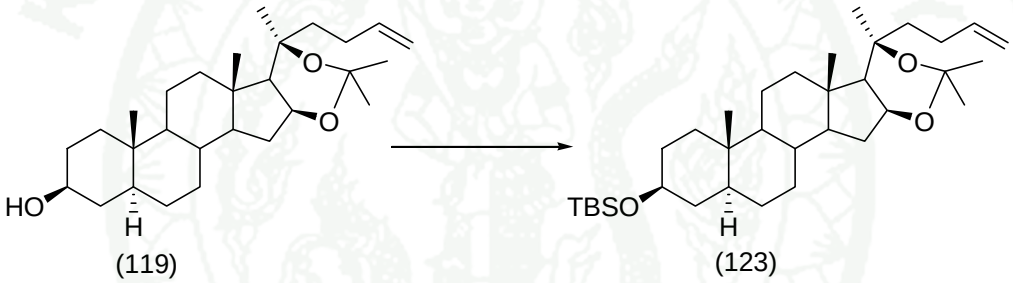
FTIR (KBr), ν_{max}, cm^{-1}: 3435, 1454, 1376, 1045

^1H NMR ($CDCl_3$, 400 MHz) δ 4.38 (m, 1H, H-16), 3.52 (m, 1H, H-3), 3.24 (m, 1H, H-24), 2.07 (m, 1H, CH), 1.90 (m, 1H, CH), 1.80-0.77 (6CH, 11CH_2), 1.36 (s, 3H, H-21), 1.27 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.01 (s, 3H, H-19), 0.86 (d, *J* = 6.5 Hz, 6H, H-26, H-27), 0.75 (s, 3H, H-18), 0.56 (m, 1H, CH)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 97.5, 97.2 (Cq), 77.5 (CH), 75.3 (C-20), 71.3 (CH), 68.8 (CH), 56.1, 55.9 (CH), 54.5 (CH), 44.9 (CH), 42.4 (C-13), 41.1, 41.0 (CH_2), 40.1, 40.0 (CH_2), 38.2 (CH_2), 36.9 (CH_2), 35.5 (CH), 34.7 (CH), 34.0 (CH_2), 33.6 (CH), 31.8, 31.6 (CH_2), 31.5 (CH_2), 28.6 (CH_2), 27.9 (CH_2), 25.8 (C-21), 24.4 (CH_3), 24.2 (CH_3), 20.7 (CH_2), 18.9, 18.7, 17.4, 17.0 (C-26, C-27), 15.0, 14.9 (CH_3), 12.3 (CH_3)

HRMS *m/z*: $C_{30}H_{52}O_4Na$ $[M+Na]^+$, calcd 499.3763, found 499.3765

3β-*tert*-Butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (123)



To a cooled (0°C) solution of 3β-hydroxy-(16*S*, 20*S*)-16,20-acetonide-5α-24a-homo-chol-24-ene (119) (284.3 mg, 0.66 mmol) in dry CH_2Cl_2 (5 mL) was added 2,6-lutidine (0.12 mL, 0.99 mmol) and *tert*-butyldimethylsilyltrifluoromethanesulfonate (0.23 mL, 0.95 mmol). The reaction mixture was stirred at 0°C for 30 min. Then the reaction was quenched by addition of saturated aqueous $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with water, the organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (2% ethyl acetate:hexane) to afford 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (123) (327.0 mg, 91%) as a white solid; m.p.96-98°C.

3β-*tert*-Butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (123)

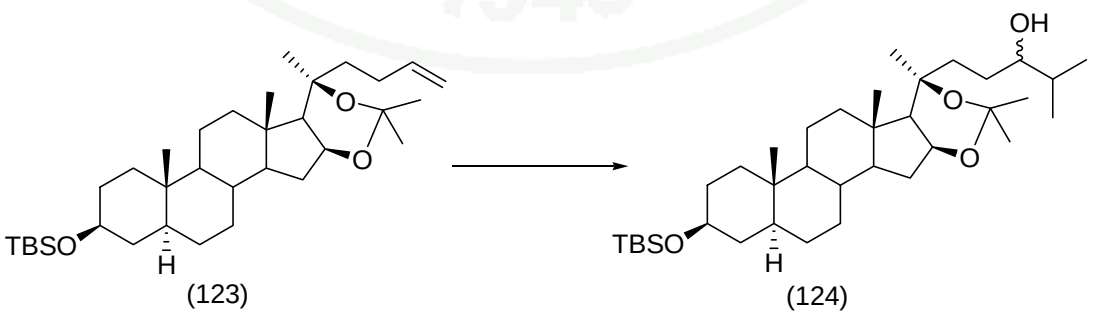
FTIR (KBr), ν_{max}, cm^{-1}: 2931, 1468, 1447, 1375, 1248, 1198, 1094, 834

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.76 (ddt, *J* = 17.0, 10.1, 6.6 Hz, 1H, H-24), 4.97 (dd, *J* = 17.1, 1.72 Hz, 1H, H-25), 4.89 (dd, *J* = 10.2, 1.8 Hz, 1H, H-25), 4.41 (m, 1H, H-16), 3.49 (m, 1H, H-3), 2.09 (m, 3H, C-17, H-23), 1.90 (m, 2H, 2CH), 1.62 (m, 2H, CH_2), 1.52-0.74 (2CH, 8CH_2), 1.40 (s, 3H, H-21), 1.28 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.04 (s, 3H, H-19), 0.84 (s, 9H, $SiC(CH_3)_3$), 0.76 (s, 3H, H-18), 0.56 (m. 1H, CH), 0.00 (s, 6H, $Si(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.8 (C-24), 114.2 (C-25), 97.1 (Cq), 74.9 (C-20), 72.1 (C-3), 68.6 (C-16), 55.6 (CH_2), 54.7 (CH), 54.6 (CH), 45.1 (CH), 43.2 (CH_2), 42.4 (C-13), 40.1 (CH_2), 38.6 (CH_2), 37.1 (CH_2), 35.5 (C-10), 34.7 (C-17), 33.7 (CH_2), 31.9 (2CH_2), 31.7 (CH_3), 28.7 (CH_2), 28.0 (C-23), 25.9 ($SiC(CH_3)_3$), 25.6 (CH_3), 24.1 (C-21), 20.7 (CH_2), 18.2 ($SiC(CH_3)_3$), 14.8 (C-19), 12.4 (C-18), -4.5 ($Si(CH_3)_2$)

MS (APCI), *m/z* (relative intensity): 487 (100), 469 (65), 337 (19), 257 (50)

3β-*tert*-Butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124)



(123) (124)

Step I

A solution of 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (123) (318.2 mg, 0.58 mmol) in CH_2Cl_2 (10 mL) was cooled to -78°C and ozone was bubbled through the solution with stirring. When the solution turned blue, ozone addition was stopped. Nitrogen was passed through the solution until the blue color was discharged. The reaction was quenched at -78°C with triphenylphosphine (160.8 mg, 0.59 mmol). The resulting mixture was stirred and slowly allowed to warm for 1 h. The mixture was further stirred at room temperature for 2 h, then concentrated under reduced pressure and furthered to purify by flash column chromatography (3% ethyl acetate:hexane) to give aldehyde intermediate (220 mg, 67%) as a white solid.

Step II

To a stirring of Mg (682 mg, 27.7 mmol) and catalyst I_2 in dry THF (15 mL) was slowly added 2-bromopropane (7.0 mL, 69 mmol) under nitrogen atmosphere at room temperature. After stirring for 1 h, this mixture was cooled to -78°C and then a solution of aldehyde intermediate (220 mg, 0.4 mmol) in dry THF (5 mL) was added slowly and kept temperature at -78°C for 1.5 h. The reaction was quenched by addition of saturated aqueous NH_4Cl, the resulting mixture was extracted with CH_2Cl_2 (4×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatrography (5% ethyl acetate:hexane) to give 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (102.9 mg, 44%) as a white solid.

3β-*tert*-Butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (Isomer I)

FTIR (KBr), ν_{max}, cm^{-1}: 3449, 2932, 1461, 1382, 1251, 1096

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.40 (m, 1H, H-16), 3.49 (m, 1H, H-3), 3.25 (m, 1H, H-24), 2.10 (t, *J* = 7.7 Hz, 0.5H, H-17), 2.07 (t, *J* = 7.7 Hz, 0.5H, H-17), 1.93 (m, 0.5H, CH_2), 1.90 (m, 0.5H, CH_2), 1.79 (m, 1H, CH), 1.70-0.73 (2CH, 10CH_2), 1.38 (s, 3H, H-21), 1.29 (CH_3), 1.25 (CH_3), 1.03 (s, 3H, H-19), 0.88 (d, *J* = 6.8 Hz, 6H, H-27, H-28), 0.83 (s, 9H, $SiC(CH_3)_3$), 0.76 (s, 3H, H-18), 0.56 (m, 1H, CH), 0.00 (s, 6H, $Si(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 94.2 (Cq), 77.5 (C-24), 75.3 (C-20), 72.1 (C-3), 68.8 (C-16), 54.9 (CH), 54.60 (CH), 54.57 (CH), 45.1 (CH), 42.4 (C-13), 41.1 (CH_2), 40.1 (CH_2), 38.6 (CH_2), 37.1 (CH_2), 35.5 (C-10), 34.7 (CH), 34.0 (C-17), 33.6 (CH_2), 31.9 (2CH_2), 31.6 (CH_3), 28.7 (CH_2), 27.9 (CH_2), 25.93 (3CH_3), 25.86 (CH_3), 24.2 (C-21), 20.7 (CH_2), 18.7 (C-27), 18.2 ($SiC(CH_3)_3$), 17.4 (C-28), 14.9 (C-19), 12.3 (C-18), -4.6 (2CH_3)

HRMS *m/z*: $C_{36}H_{66}O_4NaSi$ $[M+Na]^+$, calcd 613.4628, found 613.4634

3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (Isomer II)

FTIR (KBr), ν_{max}, cm^{-1}: 3448, 2936, 1654, 1458, 1382, 1249, 1064, 837, 775

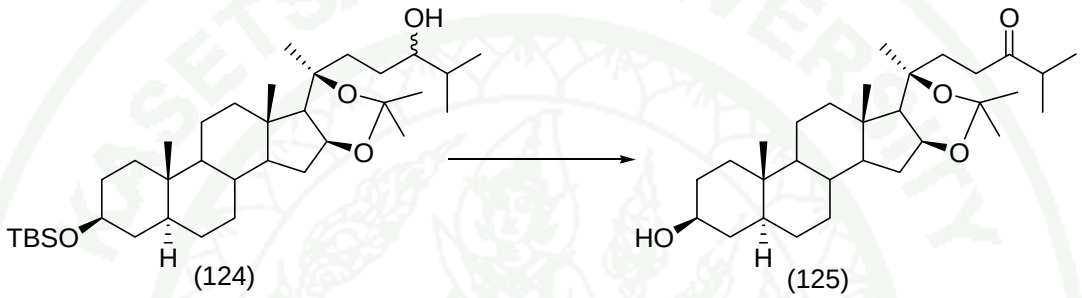
^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.42 (m, 1H, H-16), 3.51 (m, 1H, H-3), 3.28 (m, 1H, H-24), 2.12 (t, *J* = 7.7 Hz, 0.5H, H-17), 2.09 (t, *J* = 7.7 Hz, 0.5H, H-17), 2.03 (m, 1H, CH), 1.95 (m, 0.5H, CH_2), 1.92 (m, 0.5H, CH_2), 1.80-0.69 (2CH, 10CH_2), 1.43 (s, 3H, H-21), 1.31 (CH_3), 1.28 (CH_3), 1.05 (s, 3H, H-19), 0.88 (d, *J* = 6.7 Hz, 3H, H-27), (d, *J* = 6.6 Hz, 3H, H-28), 0.85 (s, 9H, $SiC(CH_3)_3$), 0.78 (s, 3H, H-18), 0.58 (m, 1H, CH), 0.00 (s, 6H, $SiC(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 97.5 (Cq), 77.3 (C-24), 75.1 (C-20), 72.1 (C-3), 68.8 (C-16), 56.2 (CH), 54.62 (CH), 54.56 (CH), 45.1 (CH), 42.5 (C-13), 41.0 (CH_2), 40.1 (CH_2), 38.6 (CH_2), 37.1 (CH_2), 35.5 (C-10), 34.7 (CH), 33.6 (CH_2), 33.5 (C-17), 31.94 (CH_2), 31.91 (CH_2), 31.5 (CH_3), 28.7 (CH_2), 28.3 (CH_2), 25.94

($SiC(CH_3)_3$), 25.90 (CH_3), 24.4 (C-21), 20.7 (CH_2), 19.0 (C-27), 18.2 ($Si\underline{C}(CH_3)_3$), 17.0,(C-28) 15.0 (C-19), 12.4 (C-18), -4.6 ($Si(CH_3)_2$)

HRMS *m/z*: $C_{36}H_{66}O_4NaSi$ $[M+Na]^+$, calcd 613.4628, found 613.4631

3β-*tert*-Butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125)



Method I

To a solution of 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (75 mg, 0.13 mmol) in CH_2Cl_2 (8 mL) was added sodium acetate (50 mg, 0.60 mmol) and pyridinium chlorochromate (PCC) (120 mg, 0.56 mmol). The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was filtered through celite and eluted with CH_2Cl_2 then the organic phase was concentrated under reduced pressure. The residue was purified by flash column chromatrography (2% ethyl acetate:hexane) to give 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125) (62.6 mg, 80%) as a white solid; m.p. 109-111^oC.

Method II

To a solution of 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (385 mg, 0.65 mmol) in CH_2Cl_2 (12 mL) was added pyridine (0.6 mL) and pyridinium chlorochromate (PCC) (600 mg, 2.8 mmol). The

reaction mixture was stirred at room temperature for 2 h. The resulting mixture was filtered through silica gel and eluted with CH_2Cl_2. Then the organic phase was concentrated under reduced pressure. The residue was purified by flash column chromatography (2% ethyl acetate:hexane) to give 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125) (363 mg, 90%).

3β-*tert*-Butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125)

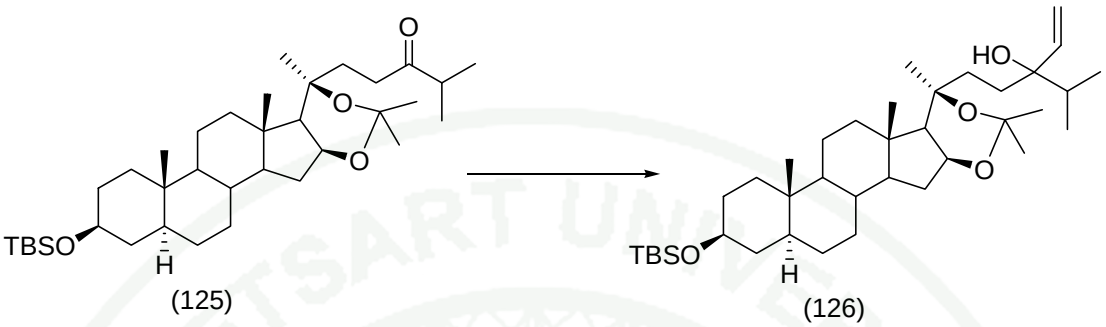
FTIR (KBr), ν_{max}, cm^{-1}: 2930, 1711, 1470, 1477, 1249, 1196, 1088, 1071, 870, 835, 778

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.42 (m, 1H, H-16), 3.50 (m, 1H, H-3), 2.58 (sept, *J* = 7.0 Hz, 1H, H-25), 2.53 (m, 2H, H-23), 2.17 (m, 1H, H-22), 2.11 (t, *J* = 7.2 Hz, 0.5H, H-17), 2.08 (t, *J* = 7.7 Hz, 0.5H, H-17), 1.94 (m, 0.5H, CH_2), 1.91 (m, 0.5H, CH_2), 1.57 (m, 1H, H-22), 1.72-0.73 (2CH, 8CH_2), 1.34 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.20 (s, 3H, H-21), 1.06 (d, *J* = 6.9 Hz, 6H, H-26, H-27), 1.03 (s, 3H, H-19), 0.84 (s, 9H, $SiC(CH_3)_3$), 0.76 (s, 3H, H-18), 0.57 (m, 1H, CH), 0.00 (s, 6H, $Si(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.6 (C-24), 97.0 (Cq), 74.3 (C-20), 72.1 (C-3), 68.6 (C-16), 55.9 (CH), 54.63 (CH), 54.56 (CH), 45.0 (CH), 42.4 (C-13), 41.0 (C-25), 40.0 (CH_2), 38.6 (CH_2), 37.1 (CH_2), 36.8 (CH_2), 35.5 (C-10), 34.7 (CH), 34.6 (C-23), 33.7 (CH_2), 31.9 (CH_2), 31.8 (CH_2), 31.6 (CH_3), 28.6 (CH_2), 25.9 ($SiC(CH_3)_3$), 25.7 (C-21), 23.9 (CH_3), 20.7 (CH_2), 18.4 (C-26), 18.3 (C-27), 18.2 ($SiC(CH_3)_3$), 14.8 (C-19), 12.3 (C-18), -4.6 ($Si(CH_3)_2$)

HRMS *m/z*: $C_{36}H_{64}O_4NaSi$ $[M+Na]^+$, calcd 611.4472, found 611.4472

3β-*tert*-Butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126)



To a solution of 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125) (62.2 mg, 0.11 mmol) in dry THF (10 mL) was added vinyl magnesium bromide (2.5 mL, 1M in THF, 2.6 mmol) at room temperature. The reaction mixture was refluxed for 2 h. Then, reaction was cooled to room temperature and quenched with saturated aqueous NH_4Cl. The resulting mixture was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatrography (5% ethyl acetate:hexane) to afford 3β-*tert*-butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126) (51.7 mg, 79%) as two isomers as a white solid.

3β-*tert*-Butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126) (Isomer I)

FTIR (KBr), ν_{max}, cm^{-1}: 3484, 2933, 1649, 1627, 1461, 1381, 1250, 1092, 835, 775

^1H NMR ($CDCl_3$, 400 MHz) δ 5.73 (dd, *J* = 17.4, 10.9 Hz, 1H, H-28), 5.15 (dd, *J* = 17.4, 1.5 Hz, 1H, H-29' *tran*), 5.12 (dd, *J* = 10.9, 1.5 Hz, 1H, H-29' *cis*), 4.37 (m, 1H, H-16), 3.49 (m, 1H, H-3), 2.07 (m, 1H, H-17), 1.92 (m, 1H, CH_2), 1.79-0.56 (5CH, 9.5 CH_2), 1.36 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.25 (s, 3H, H-21), 1.02 (s, 3H,

H-19), 0.86 (m, 6H, H-26, H-27), 0.83 (s, 9H, $SiC(CH_3)_3$), 0.76 (s, 3H, H-18), 0.00 (s, 6H, $Si(CH_3)_2$)

HRMS *m/z*: $C_{38}H_{68}O_4NaSi$ $[M+Na]^+$, calcd 640.4863, found 640.4865

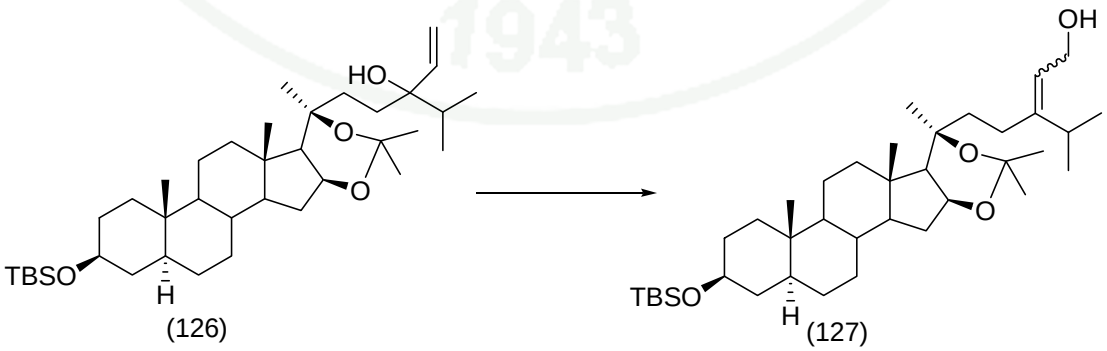
3β-*tert*-Butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126) (Isomer II)

FTIR (KBr), ν_{max}, cm^{-1}: 3448, 2933, 1654, 1631, 1461, 1382, 1093, 1063, 835, 775

^1H NMR ($CDCl_3$, 400 MHz) δ 5.73 (dd, *J* = 17.4, 10.9 Hz, 1H, H-28), 5.15 (dd, *J* = 17.4, 1.6 Hz, 1H, H-29' *tran*), 5.11 (dd, *J* = 10.9, 1.6 Hz, 1H, H-29' *cis*), 4.39 (m, 1H, H-16), 3.50 (m, 1H, H-3), 2.08 (m, 1H, H-17), 1.91 (m, 1H, CH_2), 1.82 (m, 1H, CH), 1.71-0.56 (4CH, 9.5CH_2), 1.40 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.24 (s, 3H, H-21), 1.02 (s, 3H, H-19), 0.87 (d, *J* = 6.9 Hz, 6H, H-26, H-27), 0.84 (s, 9H, $SiC(CH_3)_3$), 0.76 (s, 3H, H-18), 0.00 (s, 6H, $Si(CH_3)_2$)

HRMS *m/z*: $C_{38}H_{68}O_4NaSi$ $[M+Na]^+$, calcd 640.4863, found 640.4862

3β-*tert*-Butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127)



Step I

To a solution of 3β-*tert*-butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126) (35.1 mg, 0.06 mmol) in CH_2Cl_2 (5 mL) was added sodium acetate (60 mg, 0.73 mmol) and pyridinium chlorochromate (PCC) (120 mg, 0.56 mmol). The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was filtered through celite and eluted with CH_2Cl_2 then the organic phase was concentrated under reduced pressure. The crude product was used in the next step without purification.

Step II

To a solution of crude product from step I in ethanol (3 mL) was added sodium borohydride (27 mg, 0.71 mmol) and the mixture was stirred for 30 min. Then, ethanol was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with saturated aqueous NH_4Cl and brine. The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatrography (7% ethyl acetate:hexane) to give 3β-*tert*-butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127) (9.0 mg, 25.6%) as a white solid.

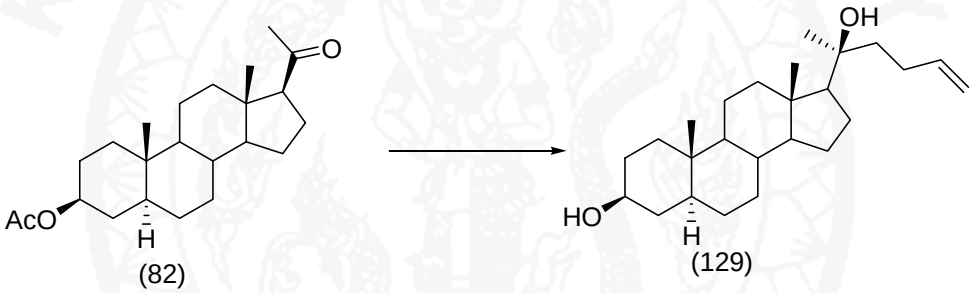
3β-*tert*-Butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.34 (t, *J* = 7.0 Hz, 1H, H-28), 4.37 (m, 1H, H-16), 4.12 (t, *J* = 6.0 Hz, 2H, H-29), 3.49 (m, 1H, H-3), 2.19 (m, 1H, CH), 2.08 (m, 2H, H-17, CH), 1.92 (m, 1H, CH_2), 1.80 (m, 1H, CH), 1.67-0.57 (3CH, 9.5CH_2), 1.42 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.03 (s, 3H, H-19), 1.00 (d, *J* = 6.8 Hz, 3H, H-26) 0.98 (d, *J* = 6.8 Hz, 3H, H-27), 0.83 (s, 9H, $SiC(CH_3)_3$), 0.76 (s, 3H, H-18), 0.00 (s, 6H, $Si(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.1 (C-24), 121.2 (C-28), 97.3 (Cq), 75.5 (C-20), 72.2 (C-3), 68.7 (C-16), 59.3 (C-29), 55.4 (C-17), 54.7 (C-9), 54.6 (C-14), 45.1 (C-5), 44.4 (CH_2), 42.5 (C-13), 40.2 (C-12), 38.6 (CH_2), 37.2 (C-1), 35.6 (C-10), 34.7 (CH), 34.0 (CH), 33.6 (CH_2), 31.9 (CH_2), 31.6 (CH_3), 28.7 (CH_2), 25.9 ($SiC(\underline{C}H_3)_3$), 25.8 (CH_3), 24.6 (CH_2), 24.5 (CH_3), 22.2, 22.0 (C-26, C-27), 20.7 (CH_2), 18.3 ($Si\underline{C}(CH_3)_3$), 15.0 (C-18), 12.4 (C-19), -4.6 ($Si(CH_3)_2$)

MS (APCI), *m/z* (relative intensity): 559 (11), 541 (100), 523 (86), 409 (48), 391 (37), 257 (10)

3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129)



(82) (129)

To a stirring of Mg (687 mg, 27 mmol) and catalyst I_2 in dry THF (20 mL) was slowly added 4-bromobutene (2.5 mL, 13.9 mmol) under nitrogen atmosphere at room temperature. After stirring for 1 h, a solution of 3β-acetoxy-5α-pregnan-20-one (82) (1.0 g, 2.8 mmol) in dry THF (15 mL) was added slowly. After 20 min, the reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to give 3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129) (639.8 mg, 61%) as a white needle after recrystallization (CH_2Cl_2); m.p. 119-122^{o}C.

3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129)

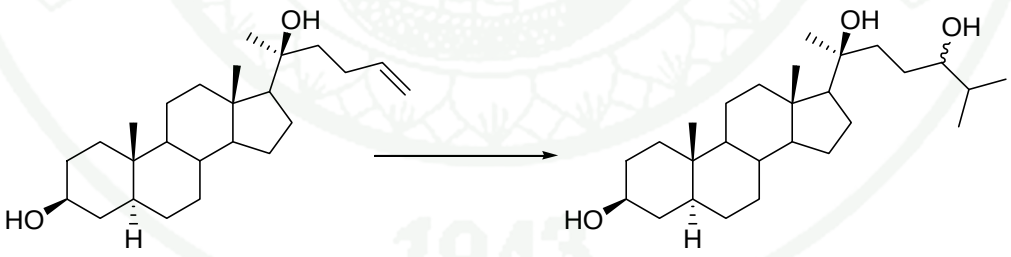
FTIR (KBr), ν_{max}, cm^{-1}: 3314, 2929, 1447, 1040, 895

^1H NMR ($CDCl_3$, 400 MHz) δ 5.80 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H, H-24), 4.99 (dd, *J* = 17.1, 1.9 Hz, 1H, H-25), 4.91 (dd, *J* = 10.2, 1.9 Hz, 1H, H-25), 3.56 (m, 1H, H-3), 2.04 (m, 4H, H-23, H-12), 1.81-0.82 (5CH, 9CH_2), 1.25 (s, 3H, H-21), 0.81 (s, 3H, H-18), 0.78 (s, 3H, H-19)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.1 (C-24), 114.2 (C-25), 75.1 (C-20), 71.3 (C-3), 58.2 (C-17, 56.7 (C-14), 54.3 (C-9), 44.9 (C-5), 43.0 (C-13), 42.6 (C-22), 40.1 (C-12), 38.2 (CH_2), 37.0 (C-1), 35.4 (C-10), 34.9 (C-8), 31.9 (CH_2), 31.5 (CH_2), 28.7 (C-23), 28.6 (CH_2), 26.2 (C-21), 23.7 (CH_2), 22.4 (CH_2), 21.5 (CH_2), 13.8 (C-19), 12.3 (C-18)

HRMS *m/z*: $C_{25}H_{42}O_2Na$ $[M+Na]^+$, calcd 397.3083, found 397.3083

3β, 20(*S*), 24-Trihydroxy-5α-cholestane (130)



(129) (130)

Step I

A mixture of 3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129) (700 mg, 1.94 mmol) and $NaHCO_3$ (796 mg) in CH_2Cl_2 (25 mL) and methanol (5 mL) was cooled to -78^oC and ozone was bubbled through the mixture with stirring. When the reaction mixture was turned blue, ozone addition was stopped. Nitrogen gas was

passed through the reaction until the blue color was discharged. The reaction was quenched by addition of triphenylphosphine (1.0 g, 3.8 mmol) at -78^{o}C. The reaction was slowly warmed to room temperature and further stirred for 1 h before concentration under reduced pressure. The residue was purified by flash column chromatography (20% ethyl acetate:hexane) to give aldehyde intermediate (468.3 mg, 68%) as a white solid.

Step II

To a stirring of Mg (1.1 g, 45 mmol) and catalyst I_2 in dry THF (20 mL) was slowly added 2-bromopropane (9.0 mL, 91.9 mmol) under nitrogen atmosphere at room temperature. After stirring for 2 h, this mixture was cooled to -78^{o}C. A solution of aldehyde intermediate (468.3 mg, 1.24 mmol) in dry THF (8 mL) was added slowly and kept at this temperature. After 20 min, the reaction mixture was allowed to slowly warm to room temperature and left at this temperature for 3 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with ethyl acetate (4×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (25% ethyl acetate:hexane) to give 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130) (231 mg, 75% (brsm)) as a white solid and recovered starting material (129) (187.2 mg, 61% conversion).

3β, 20(*S*), 24-Trihydroxy-5α-cholestane (130)

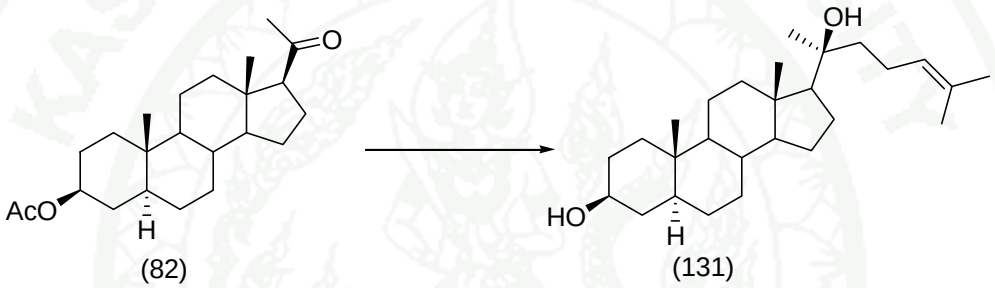
FTIR (KBr), ν_{max}, cm^{-1}: 3399, 2920, 1036

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.51 (m, 1H, H-3), 3.24 (m, 1H, H-24), 1.98 (m, 1H, H-12), 1.81-0.76 (6CH, 10CH_2, CH of H-12), 1.20 (s, 3H, H-21), 0.84 (m, 6H, H-26, H-27), 0.76 (s, 3H, H-18), 0.74 (s, 3H, H-19)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 77.1 (C-24), 75.0 (C-20), 71.3 (C-3), 58.9 (C-17), 56.6 (C-14), 54.3 (C-9), 44.8 (C-5), 43.0 (C-13), 40.4 (C-12), 39.8 (CH_2), 38.1 (CH_2), 37.0 (C-1), 35.4 (C-10), 34.8 (C-8), 33.6 (C-25), 31.9 (CH_2), 31.5 (CH_2), 28.7 (CH_2), 28.2 (CH_2), 26.0 (C-21) 23.7 (CH_2), 22.5 (CH_2), 21.1 (CH_2), 18.9, 17.1 (C-26, C-27), 13.8 (C-18), 12.3 (C-19)

HRMS *m/z*: $C_{27}H_{48}O_3Na$ $[M+Na]^+$, calcd 443.3501, found 443.3501

3β, 20(*S*)-Dihydroxy-5α-cholest-24-ene (131)



(82) (131)

To a stirring of Mg (101 mg, 3.9 mmol) and catalyst I_2 in dry THF (6 mL) was slowly added 5-bromo-2-pentene (0.43 mL, 4.16 mmol) under nitrogen atmosphere at room temperature. After stirring for 1 h, a solution of 3β-acetoxy-5α-pregnan-20-one (82) (150 mg, 0.42 mmol) in dry THF (5 mL) was added slowly. After 5 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with ethyl acetate (4×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (7% ethyl acetate:hexane) to give 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131) (60 mg, 36%) as a white solid.

3β, 20(*S*)-Dihydroxy-5α-cholest-24-ene (131)

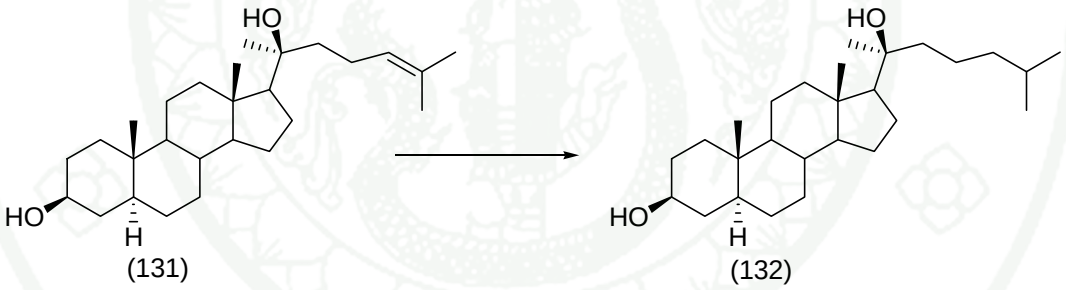
FTIR (KBr), ν_{max}, cm^{-1}: 3431, 2929, 1449, 1373, 1037

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.02 (m, 1H, H-24), 3.52 (m, 1H, H-3), 1.98 (m, 1H, H-12), 1.91 (m, 2H, H-23), 1.77-0.75 (4CH, 9CH_2, H-12), 1.61 (s, 3H, H-26), 1.54 (s, 3H, H-27), 1.20 (s, 3H, H-21), 0.77 (s, 3H, H-18), 0.73 (s, 3H, H-19), 0.55 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.5 (C-25), 124.5 (C-24), 75.2 (C-20), 71.3 (C-3), 58.1 (C-17), 56.6 (C-14), 54.3 (C-9), 44.9 (C-5), 43.5 (C-22), 42.9 (C-13), 40.4 (C-12), 38.2 (CH_2), 37.0 (CH_2), 35.4 (C-10), 34.9 (C-8), 31.9 (CH_2), 31.5 (CH_2), 28.7 (CH_2), 26.2 (C-21), 25.7, 17.6 (C-26, C-27), 23.7 (CH_2), 22.9 (CH_2), 22.4 (CH_2), 21.1 (CH_2), 13.8 (C-19), 12.3 (C-18)

MS (APCI), *m/z* (relative intensity): 385 (100), 367 (57)

3β, 20(*S*)-Dihydroxy-5α-cholestane (132)



To a solution of 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131) (20 mg, 0.05 mmol) in methanol (2 mL) was added 5% Pd/C (10 mg, 6.8×10^{-3} mmol). The black suspension was stirred under hydrogen atmosphere at room temperature for 2 h. After reaction mixture was filtered through celite and eluted with CH_2Cl_2, the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatrography (5% ethyl acetate:hexane) to give 3β, 20(*S*)-dihydroxy-5α-cholestane (132) (13 mg, 64%) as a white solid; m.p. 115-117^{o}C. (Chaudhuri *et al*., 1969, m.p. 125-127^{o}C (methanol))

3β, 20(*S*)-Dihydroxy-5α-cholestane (132)

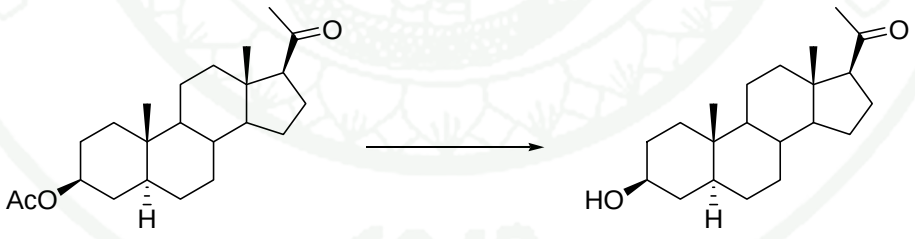
FTIR (KBr), ν_{max}, cm^{-1}: 3306, 2929, 1462, 1382, 1256, 1096, 836, 775

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.52 (m, 1H, H-3), 1.98 (dt, *J* = 12.2, 3.2 Hz, 1H, H-12), 1.73 (m, 1H, CH of CH_2), 1.68-0.84 (H-12, CH of CH_2, 5CH, 10CH_2) , 1.19 (s, 3H, H-21), 0.80 (d, *J* = 6.6 Hz, 6H, H-26, H-27), 0.77 (s, 3H, H-18), 0.74 (s, 3H, H-19), 0.55 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 75.2 (C-20), 71.4 (C-3), 57.8 (C-17), 56.6 (C-14), 54.3 (C-9), 44.9 (C-5), 44.2 (C-22), 42.9 (C-13), 40.4 (C-12), 39.6 (C-24), 38.2 (CH_2), 37.0 (C-1), 35.5 (C-10), 34.9 (C-8), 31.9 (CH_2), 31.5 (CH_2), 28.7 (CH_2), 27.9 (C-25), 26.4 (C-21), 23.7 (CH_2), 22.7, 22.6 (C-26, C-27), 22.4 (CH_2), 22.0 (CH_2), 21.1 (CH_2), 13.8 (C-18), 12.3 (C-19)

MS (APCI), *m/z* (relative intensity): 387 (29), 369 (100)

3β-Hydroxy-5α-pregnan-20-one (133)



(82) (133)

1M KOH (30 mL) was added slowly to a solution of 3β-acetoxy-5α-pregnan-20-one (82) (2.70 g, 7.5 mmol) in methanol (25 mL) and CH_2Cl_2 (25 mL) at room temperature and stirred for 6 h. Methanol and CH_2Cl_2 were removed under reduced pressure, then the residue was diluted with CH_2Cl_2 and washed with water. The aqueous layer was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with saturated aqueous NH_4Cl, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column

chromatrography (15% ethyl acetate:hexane) to give 3β-hydroxy-5α-pregnan-20-one (133) (2.1 g, 90%) as a white needle after recrystallization (CH_2Cl_2:hexane); m.p. 195-197°C. (Comin *et al*., 2004, m.p. 182-185°C (as white solid))

3β-Hydroxy-5α-pregnan-20-one (133)

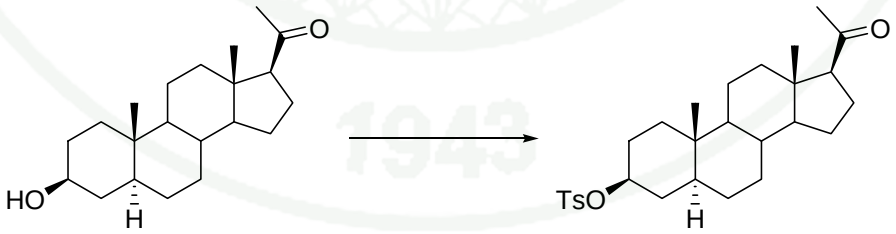
FTIR (KBr), ν_{max}, cm^{-1}: 3441, 2930, 1700, 1353, 1039

^1H NMR ($CDCl_3$, 400 MHz) δ 3.56 (m, 1H, H-3), 2.48 (t, J = 8.9 Hz, 1H, H-17), 2.12 (m, 1H, H-16)), 2.07 (s, 3H, H-21), 1.97 (m, 1H, H-12)), 1.81-0.82 (3CH, 7CH_2, H-12), H-16)), 0.77 (s, 3H, H-19), 0.65 (m, 1H, H-9), 0.57 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.6 (C-20), 71.2 (C-3), 63.8 (C-17), 56.7 (C-14), 54.2 (CH), 44.8 (CH), 44.2 (C-13), 39.0 (C-12), 38.1 (CH_2), 37.0 (C-1), 35.48 (C-10), 35.47 (CH), 32.0 (CH_2), 31.5 (C-21), 31.4 (CH_2), 28.6 (CH_2), 24.4 (CH_2), 22.8 (C-16), 21.2 (CH_2), 13.4 (C-18), 12.2 (C-19)

MS (APCI), *m/z* (relative intensity): 317 (11), 301 (100), 283 (22), 257 (7)

3β-Tosyloxy-5α-pregnan-20-one (134)



(133) (134)

To a solution of 3β-hydroxy-5α-pregnan-20-one (133) (1.5 g, 4.7 mmol) in dry CH_2Cl_2 (20 mL) and dry pyridine (8 mL) under nitrogen atmosphere was added a solution of *p*-tolulenesulfonyl chloride (3.42 g, 14.2 mmol) in dry pyridine (8 mL) and dry CH_2Cl_2 (10 mL). The resulting mixture was stirred at room temperature for 24 h. The reaction was quenched by addition of water and extracted with CH_2Cl_2

(3×150 mL). The combined organic layers were washed with 10% HCl, saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate:hexane) to give 3β-tosyloxy-5α-pregnan-20-one (134) (1.8 g, 81%) as a white needle after recrystallization (CH_2Cl_2:hexane); m.p. 126-127°C. (Fukushima *et al*., 1954, m.p. 133.5-135°C)

3β-Tosyloxy-5α-pregnan-20-one (134)

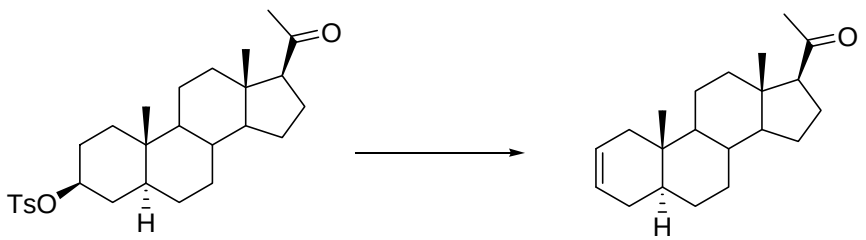
FTIR (KBr), ν_{max}, cm^{-1}: 2942, 1706, 1654, 1352, 1172, 1098, 935, 670, 556

^1H NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.25 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 4.35 (m, 1H, H-3), 2.43 (t, *J* = 8.6 Hz, 1H, H-17), 2.37 (s, 3H, C<u>H</u>$_3$-Ph), 2.12 (m, 1H, H-16)), 2.07 (s, 3H, H-21), 1.90 (m, 1H, H-12) 1.74-0.74 (3CH, 7CH_2, H-12, H-16), 0.70 (s, 3H, H-19), 0.57 (m, 1H, H-9), 0.51 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.5 (C-20), 144.3 (C-4'), 134.7 (C-1'), 129.7 (C-3', C-5'), 127.5 (C-2', C-6'), 82.3 (C-3), 63.7 (C-17), 56.5 (CH), 53.9 (CH), 44.7 (CH), 44.1 (C-13), 38.9 (CH_2), 36.7 (CH_2), 35.3 (CH), 35.2 (C-10), 34.7 (CH_2), 31.7 (CH_2), 31.4 (C-21), 28.3 (CH_2), 28.2 (CH_2), 24.3 (CH_2), 22.7 (CH_2), 21.6 (C-7'), 21.1 (CH_2), 13.4 (C-18), 12.0 (C-19)

HRMS *m/z*: $C_{28}H_{40}O_4SNa$ $[M+Na]^+$, calcd 495.2545, found 495.2548

2-Pregnen-20-one (135)



(134) (135)

Lithium bromide (3.2 g, 34 mmol) and lithium carbonate (3.0 g, 34 mmol) were added to a solution of 3β-tosyloxy-5α-pregnan-20-one (134) (1.8 g, 3.8 mmol) in *N,N*-dimethylformamide (60 mL). The resulting mixture was refluxed for 6 h. After reaction was allowed to cool at room temperature, the suspension was filtered and the filtrate was poured into 10% HCl, extracted with CH_2Cl_2 (3×150 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (1% ethyl acetate:hexane) to give 2-pregnen-20-one (135) (982 mg, 86%) as a white needle after recrystallization (ethanol); m.p. 124-125°C. (Comin *et al*., 2004, m.p. 90-92°C (as white solid))

2-Pregnen-20-one (135)

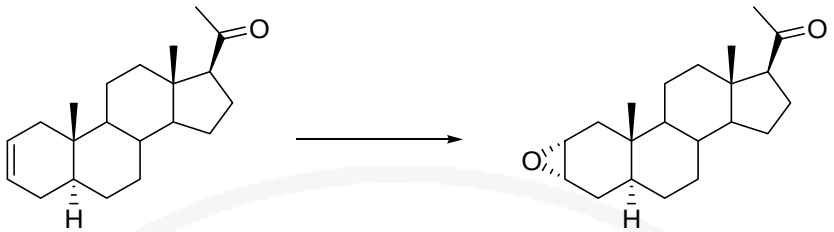
FTIR (KBr), ν_{max}, cm^{-1}: 2913, 1704, 1444, 1352, 1152

^1H NMR ($CDCl_3$, 400 MHz) δ 5.57 (m, 1H, H-2, H-3), 2.50 (t, J = 9.2 Hz, 1H, H-17), 2.13 (m, 1H, H-16), 2.09 (s, 3H, H-21), 1.98 (m, 1H, H-12), 1.87 (m, 2H, H-1), 1.75-0.82 (3CH, 5CH_2, H-16, H-12), 0.74 (m, 1H, H-14), 0.73 (s, 3H, H-19), 0.59 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.7 (C-20), 125.9 (CH), 125.7(CH), 63.9 (C-17), 56.7 (C-14), 53.9 (CH), 44.2 (C-13), 41.4 (CH), 39.8 (C-1), 39.1 (C-12), 35.6 (CH), 34.6 (C-10), 31.8 (C-16), 31.5 (C-21), 30.2 (CH_2), 28.6 (CH_2), 24.4 (CH_2), 22.8 (CH_2), 20.9 (CH_2), 13.3 (C-18), 11.7 (C-19)

HRMS *m/z*: $C_{21}H_{32}ONa$ $[M+Na]^+$, calcd 301.2531, found 301.2531

2α, 3α-Epoxy-5α-pregnan-20-one (136)



(135) (136)

To a solution of 2-pregnen-20-one (135) (1.71 g, 5.65 mmol) in CH_2Cl_2 (20 mL) was added water (10 mL) and sodium carbonate (3.02 g, 28.5 mmol). The reaction mixture was stirred vigorously and *m*-chloroperbenzoic acid (1.33 g, 7.7 mmol) was added slowly. The reaction mixture was stirred at room temperature for 4-6 h. The aqueous layer was separated and extracted with CH_2Cl_2 (3×200 mL). The combined organic layers were washed with 5% sodium sulfite solution, saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give 2α, 3α-epoxy-5α-pregnan-20-one (136) (744 mg, 54% (brsm)) as a white needle after recrystallization (hexane); m.p. 152-153^oC (Comin *et al*., 2004, m.p. 153-154^oC (as a white solid)) and the recovered starting material (135) (426 mg, 75% conversion)

2α, 3α-Epoxy-5α-pregnan-20-one (136)

FTIR (KBr), ν_{max}, cm^{-1}: 2940, 1699, 1653, 1450, 1357, 1185

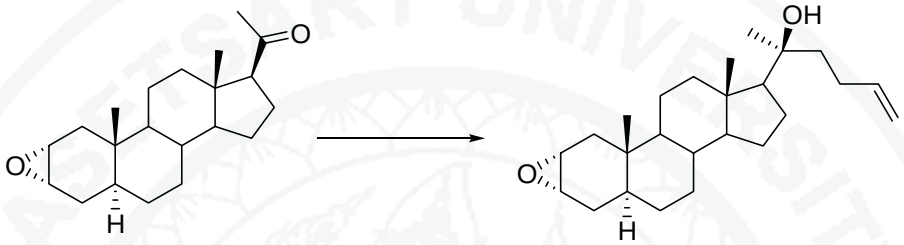
^1H NMR ($CDCl_3$, 400 MHz) δ 3.11 (m, 1H, H-3), 3.08 (m, 1H, H-2), 2.48 (t, *J* = 9.0 Hz, 1H, H-17), 2.10 (m, 1H, H-16), 2.07 (s, 3H, H-21), 1.97 (m, 1H, H-12), 1.87 (m, 2H, H-1), 1.67-1.00 (2CH, 6CH_2, H-12, H-16), 0.72 (s, 3H, H-19), 0.65 (m, 1H, H-9) 0.56 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.5 (C-20), 63.7 (C-17), 56.5 (CH), 53.6 (CH), 52.3 (C-3), 50.9 (C-2), 44.0 (C-13), 38.9 (CH_2), 38.2 (CH_2), 36.2 (CH), 35.6

(CH), 33.6 (C-10), 31.6 (CH_2), 31.5 (C-21), 29.0 (CH_2), 28.2 (CH_2), 24.3 (CH_2), 22.7 (C-16), 20.8 (CH_2), 13.3 (C-18), 12.9 (C-19)

HRMS *m/z*: $C_{21}H_{32}O_2Na$ $[M+Na]^+$, calcd 339.2300, found 339.2300

2α, 3α-Epoxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (137)



(136) (137)

To a stirring of Mg (191 mg, 7.64 mmol) and catalyst I_2 in dry THF (10 mL) was slowly added 4-bromobutene (1.10 mL, 6.1 mmol) under nitrogen atmosphere at room temperature. After stirring for 1 h, a solution of 2α, 3α-epoxy-5α-pregnan-20-one (136) (418.20 mg, 1.3 mmol) in dry THF (10 mL) was added slowly. After 20 min, the reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give 2α, 3α-epoxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (137) (249 mg, 50%) as a white needle after recrystallization (hexane); m.p. 254-256°C.

2α, 3α-Epoxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (137)

FTIR (KBr), ν_{max}, cm^{-1}: 3448, 2918, 1671, 1657, 1601, 1435, 1380, 1107

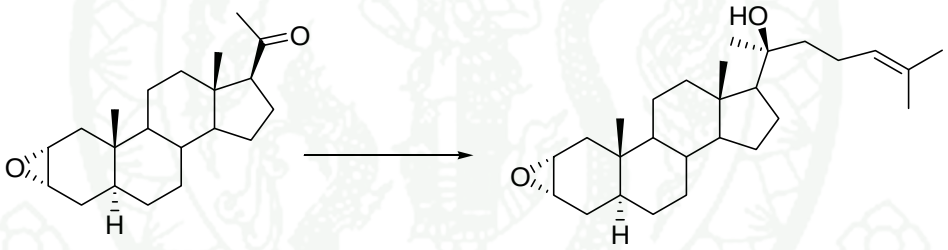
^1H NMR ($CDCl_3$, 400 MHz) δ 5.78 (ddt, *J* = 17.0, 10.2, 6.6 Hz, 1H, H-24), 4.98 (dd, *J* = 17.2, 1.6 Hz, 1H, H-25), 4.90 (dd, *J* = 10.2, 1.9 Hz, 1H, H-25), 3.11 (m,

1H, H-3), 3.07 (m, 1H, H-2), 2.03 (m, 3H, H-12, H-23), 1.86 (m, 2H, H-1), 1.73-0.88 (H-12, 4CH, 7CH_2), 1.24 (s, 3H, H-21), 0.77 (s, 3H, H-18), 0.72 (s, 3H, H-19), 0.58 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.0 (C-24), 114.2 (C-25), 75.0 (C-20), 58.1 (C-17), 56.4 (C-14), 53.6 (C-9), 52.4 (C-3), 51.0 (C-2), 42.7 (C-13), 42.6 (C-22), 40.2 (C-12), 38.2 (C-1), 36.2 (C-5), 35.0 (C-8), 33.6 (C-10), 31.5 (CH_2), 29.0 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 26.2 (C-21), 23.6 (CH_2), 22.3 (CH_2), 20.7 (CH_2), 13.6 (C-19), 12.9 (C-18)

HRMS *m/z*: $C_{25}H_{40}O_2Na$ $[M+Na]^+$, calcd 395.2926, found 395.2925

2α, 3α-Epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138)



(136) (138)

To a stirring of Mg (91 mg, 3.6 mmol) and catalyst I_2 in dry THF (4 mL) was slowly added 5-bromo-2-methyl-2-pentene (0.5 mL, 3.8 mmol) under nitrogen atmosphere at room temperature. After stirring for 15 min at room temperature, the mixture was warmed to 45^{o}C for 1 h before cooling to room temperature and the solution of 2α, 3α-epoxy-pregnan-20-one (136) (150 mg, 0.48 mmol) was added and stirred for 2 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and then extracted with diethyl ether (4×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (2% ethyl acetate:hexane) to yield 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138) (74.8 mg, 39%) as a colorless gum.

2α, 3α-Epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138)

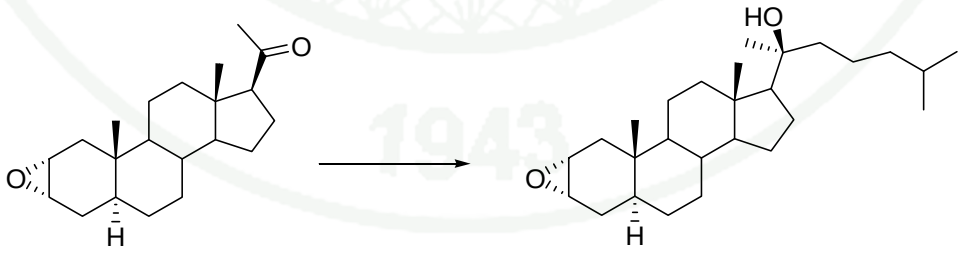
FTIR (neat), ν_{max}, cm^{-1}: 3441, 2923, 1672, 1653, 1603, 1435, 1382, 1027

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.06 (m, 1H, H-24), 3.12 (m, 1H, H-3), 3.08 (m, 1H, H-2), 2.03 (dt, *J* = 12.4, 3.2 Hz, 1H, H-12), 1.95 (dt, *J* = 8.0, 7.8 Hz, 2H, H-23), 1.88 (dd, *J* = 15.1, 5.9 Hz, 1H, H-1), 1.85 (m, 1H, CH of CH_2), 1.76-0.82 (H-12, H-1, CH of CH_2, 4CH, 7CH_2), 1.65, 1.58 (2s, 6H, H-26, H-27), 1.25 (s, 3H, H-21), 0.80 (s, 3H, H-18), 0.72 (s, 3H, H-19), 0.59 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.5 (C-25), 124.5 (C-24), 75.1 (C-20), 58.0 (C-17), 56.4 (C-9), 53.6 (C-14), 52.4 (C-3), 51.0 (C-2), 43.6 (C-22), 42.7 (C-13), 40.3 (C-12), 38.2 (C-1), 36.2 (C-5), 35.0 (C-8), 33.6 (C-10), 31.5 (CH_2), 29.0 (CH_2), 28.4 (CH_2), 26.1 (C-21), 25.7 (C-26 or C-27), 23.7 (CH_2), 22.9 (CH_2), 22.3 (CH_2), 20.7 (CH_2, 17.6 (C-26 or C-27), 13.6 (C-18), 12.9 (C-19)

MS (APCI), *m/z* (relative intensity): 401 (15), 383 (100), 365 (43), 283 (6), 257 (14)

2α, 3α-Epoxy-20(*S*)-hydroxy-5α-cholestane (139)



(136) (139)

To a stirring of Mg (91 mg, 3.6 mmol) and catalyst I_2 in dry THF (4 mL) was slowly added 1-bromo-4-methylpentane (0.5 mL, 3.8 mmol) under nitrogen atmosphere at room temperature. After stirring for 15 min at room temperature, the mixture was warmed to 45°C for 1 h before cooling to room temperature and the

solution of 2α, 3α-epoxy-pregnan-20-one (136) (152 mg, 0.48 mmol) was added and stirred for 2 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and then extracted with diethyl ether (4×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (3% ethyl acetate:hexane) to give 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (139) (97.2 mg, 50%) as a white solid; m.p. 121-123°C.

2α, 3α-Epoxy-20(*S*)-hydroxy-5α-cholestane (139)

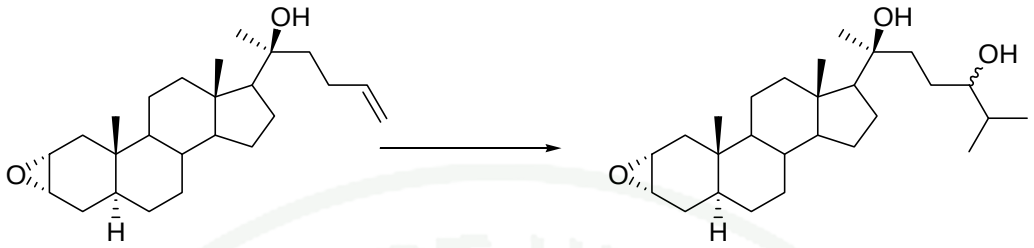
FTIR (KBr), ν_{max}, cm^{-1}: 2942, 1468, 1382

^1H NMR ($CDCl_3$, 400 MHz) δ 3.12 (m, 1H, H-3), 3.07 (m, 1H, H-2), 2.02 (m, 1H, H-12), 1.88 (dd, *J* = 12, 5.9 Hz, 1H, H-1), 1.84 (m, 1H, CH of CH_2), 1.73-0.87 (H-12, H-1, CH of CH_2, 5CH, 8CH_2), 1.23 (s, 3H, H-21), 0.84 (d, *J* = 6.6 Hz, 6H, H-26, H-27), 0.80 (s, 3H, H-18), 0.72 (s, 3H, H-19), 0.58 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 75.2 (C-20), 57.7 (C-17), 56.4 (C-14), 53.6 (C-9), 52.4 (C-3), 51.0 (C-2), 44.2 (C-22), 42.4 (C-13), 40.2 (C-12), 39.6 (C-24), 38.3 (C-1), 36.2 (C-5), 35.0 (C-8), 33.6 (C-10), 31.5 (CH_2), 29.0 (CH_2), 28.4 (CH_2), 27.9 (C-25), 26.4 (C-21), 23.7 (CH_2), 22.7, 22.5 (C-26, C-27), 22.3 (CH_2), 22.0 (CH_2), 20.7 (CH_2), 13.6 (C-18), 12.9 (C-19)

MS (APCI), *m/z* (relative intensity): 403 (4), 385 (76), 367 (100), 257 (13)

2α, 3α-Epoxy-20(*S*), 24-dihydroxy-5α-cholestane (140)



(137) (140)

Step I

A mixture of 2α, 3α-epoxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (137) (249 mg, 0.6 mmol) and $NaHCO_3$ (200 mg) in CH_2Cl_2 (10 mL) and methanol (2 mL) was cooled to -78°C and ozone was bubbled through the mixture with stirring. When the reaction mixture was turned blue, ozone addition was stopped. Nitrogen gas was passed through the reaction until the blue color was discharged. The reaction was quenched by addition of triphenylphosphine (418 mg, 1.6 mmol) at -78°C. The reaction was slowly warmed to room temperature and further stirred for 1 h before concentration under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give aldehyde intermediate (156 mg, 62%) as a white solid.

Step II

To a stirring of Mg (350 mg, 14.6 mmol) and catalyst I_2 in dry THF (20 mL) was slowly added 2-bromopropane (3 mL, 30.6 mmol) under nitrogen atmosphere at room temperature. After stirring for 2 h, this mixture was cooled to -78°C. A solution of aldehyde intermediate (156 mg, 0.42 mmol) in dry THF (10 mL) was added slowly and kept at this temperature. After 20 min, the reaction mixture was allowed to slowly warm to room temperature and left at this temperature for 3 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (4×50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced

pressure. The residue was purified by flash column chromatography (7% ethyl acetate:hexane) to give 2α, 3α-epoxy-20(*S*), 24-dihydroxy-5α-cholestane (140) (83.6 mg, 48% (brsm)) as a white solid and recovered starting material (41 mg, 74% conversion).

2α, 3α-Epoxy-20(*S*), 24-dihydroxy-5α-cholestane (140)

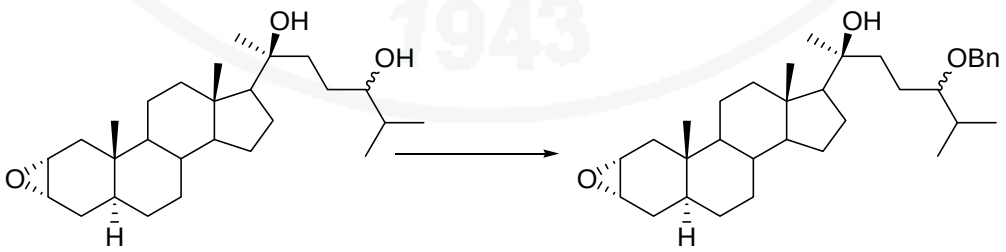
FTIR (KBr), ν_{max}, cm^{-1}: 3422, 2929, 1459, 1380, 1006

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.27 (m, 1H, H-24), 3.11 (m, 1H, H-3), 3.08 (m, 1H, H-2), 2.09-0.72 (6CH, 10CH_2), 1.22 (s, 3H, H-21), 0.87 (m, 6H, H-26, H-27), 0.78 (s, 3H, H-18), 0.71 (s, 3H, H-19), 0.57 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 76.7 (C-24), 75.1 (C-20), 58.8, 58.4 (C-17), 56.4 (C-14), 53.5 (C-9), 52.4 (C-3), 51.0 (C-2), 42.7 (C-13), 40.2 (C-12), 39.8 (C-22), 39.4 (CH_2), 38.2 (C-1), 36.2 (C-5), 35.0 (C-8), 33.6 (C-25), 33.5 (C-10), 31.4 (CH_2), 28.9 (CH_2), 28.3 (CH_2), 26.0 (C-21), 23.6 (CH_2), 22.3 (CH_2), 20.7 (CH_2), 18.9, 18.8, 17.4, 17.1 (C-26, C-27), 13.6 (C-18), 12.9 (C-19)

HRMS *m/z*: $C_{27}H_{46}O_3Na$ $[M+Na]^+$, calc 441.3345, found 441.3339

24-Benzyloxy-2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (141)



(140) (141)

NaH (155.7 mg, 3.8 mmol) was washed with dry THF (4 mL). The suspension was stirred vigorously for 10 min before removing solvent.

To the suspension of NaH in dry THF was slowly added a solution of 2α, 3α-epoxy-20(*S*), 24-dihydroxy-5α-cholestane (143) (165.3 mg, 0.38 mmol) in dry THF (5 mL) and the mixture was stirred under reflux for 2 h. After the reaction mixture was cooled down to room temperature, benzyl bromide (0.28 mL, 1.9 mmol) was added and stirred for an additional 30 min at room temperature and 4 h under reflux. The reaction was quenched by pouring into water and extracted with diethyl ether (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (3% ethyl acetate:hexane) to give 24-benzyloxy-2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (141) (124.2 mg, 64%) as a colorless gum.

24-Benzyloxy-2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (141)

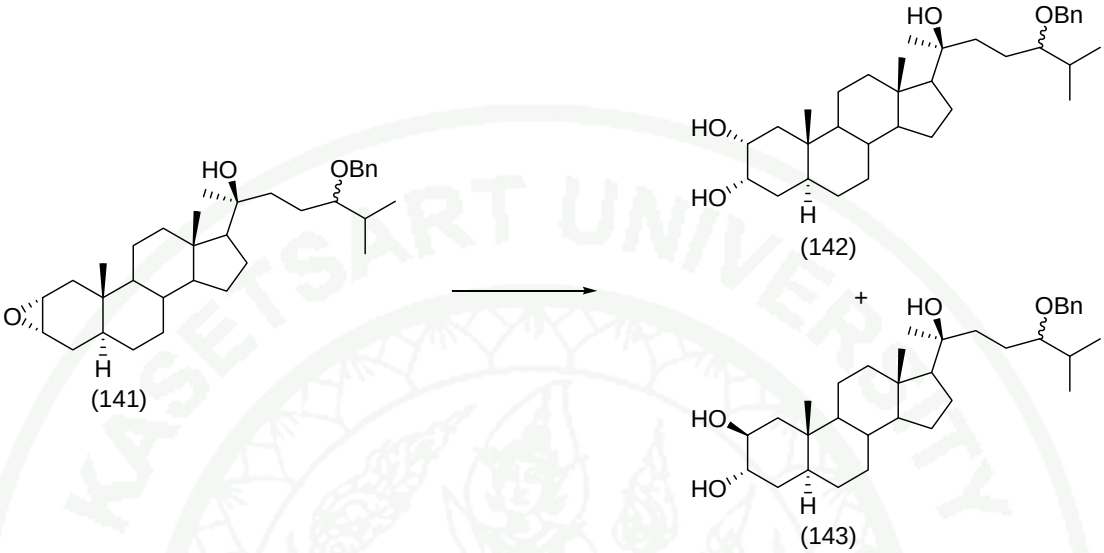
FTIR (neat), ν_{max}, cm^{-1}: 2930, 1455, 1382, 1069, 803, 735, 697

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27-7.25 (m, 5H, H_{AR}), 4.44, 4.43 (2s, 2H, H_{benzyl}), 3.08 (m, 1H, H-3), 3.03 (m, 2H, H-2, H-24), 1.97 (m, 1H, H-12), 1.82 (m, 3H, H-1, H-25, CH of CH_2), 1.68-0.78 (H-1, H-25, CH of CH_2, 5CH, 7CH_2), 1.17 (s, 3H, H-21), 0.87, 0.84 (2d, *J* = 6.8, 6.8 Hz, 6H, H-26, H-27), 0.74 (s, 3H, H-18), 0.68 (s, 3H, H-19), 0.54 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.1 (C_{AR}), 128.2 (CH_{AR}), 127.8 (CH_{AR}), 127.4 (CH_{AR}), 84.8 (C-24), 75.1 (C-20), 71.8 (C_{benzyl}), 58.2 (C-17), 56.4 (C-14), 53.6 (C-9), 52.4 (C-3), 51.0 (C-2), 42.7 (C-13), 40.3 (C-12), 39.4 (C-22), 38.2 (C-1), 36.2 (C-5), 35.0 (C-8), 33.6 (C-10), 31.5 (CH_2), 30.6 (C-25), 29.0 (CH_2), 28.4 (CH_2), 26.1 (C-21), 24.6 (CH_2), 23.6 (CH_2), 22.3 (CH_2), 20.7(CH_2), 18.6, 18.1 (C-26, C-27), 13.6 (C-18), 12.9 (C-19)

MS (APCI), *m/z* (relative intensity): 401 (5), 383 (100), 365 (34)

24-Benzyloxy-2α, 3α, 20(*S*)-trihydroxy-5α-cholestane (142) and 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (143)



A solution of 24-benzyloxy-2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (141) (99.2 mg, 0.195 mmol) in THF (4 mL) was treated with 1M H_2SO_4 (1.5 mL) and stirred for 24 h at room temperature. After neutralization with saturated aqueous $NaHCO_3$, the mixture was evaporated to fifth initial volume, diluted with water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (15% ethyl acetate:hexane) to give 24-benzyloxy-2α, 3α, 20(*S*)-trihydroxy-5α-cholestane (142) (3 mg) as a white solid; m.p. 156-160°C and 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (143) (45.3 mg) as a white solid; m.p. 146-150°C and the mixture of these steriods (10.7 mg).

24-Benzyloxy-2α, 3α, 20(*S*)-trihydroxy-5α-cholestane (142)

FTIR (KBr), ν_{max}, cm^{-1}: 3417, 2938, 1699, 1455, 1370, 1261, 1067, 1044, 733, 696

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27-7.25 (m, 5H, H_{AR}), 4.44, 4.43 (2s, 2H, H_{benzyl}), 3.82 (m, 1H, H-3), 3.53 (m, 1H, H-2), 3.04 (m, 1H, H-24), 1.98 (m, 2H, H-12, H-4), 1.83 (m, 1H, H-25), 1.75-0.77 (H-12, H-4, 5CH, 8 CH_2), 1.17 (s, 3H, H-21), 0.95 (s, 3H, H-19), 0.86, 0.85 (2d, *J* = 7.08, 7.04 Hz , 6H, H-26, H-27), 0.75 (s, 3H, H-18), 0.64 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9 (C_{AR}), 128.3 (CH_{AR}), 127.8 (CH_{AR}), 127.4 (CH_{AR}), 84.7 (C-24), 76.1 (CH), 74.9 (C-20), 71.8 (C_{benzyl}), 70.3 (CH), 58.2 (C-17), 56.8 (C-14), 55.1 (C-9), 43.9 (C-5), 42.9 (C-13), 40.3 (C-12), 39.2 (C-22), 35.8 (C-10), 34.8 (C-8), 32.1 (C-1), 31.6 (CH_2), 30.4 (C-25), 26.2 (C-21), 25.1 (CH_2), 24.5 (CH_2), 24.4 (CH_2), 23.7 (CH_2), 22.4 (CH_2), 20.0 (CH_2), 18.3, 18.1 (C-26, C-27), 14.1 (C-19), 13.7 (C-18)

MS (APCI), *m/z* (relative intensity): 401 (100), 383 (68), 365 (47)

24-Benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (143)

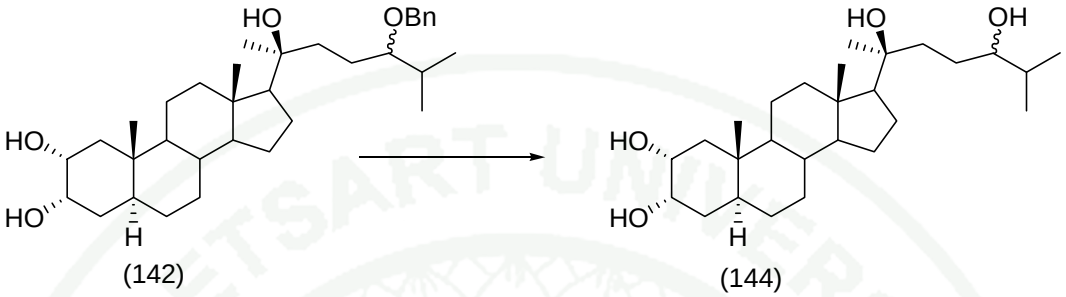
FTIR (KBr), ν_{max}, cm^{-1} : 3391, 2927, 1699, 1454, 1369, 1273, 1038, 742, 697

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27-7.25 (m, 5H, H_{AR}), 4.44, 4.43 (2s, 2H, H_{benzyl}), 3.81 (m, 1H, H-2), 3.78 (m, 1H, H-3), 3.04 (m, 1H, H-24), 1.98 (m, 1H, H-12), 1.83 (m, 2H, H-4, H-25), 1.74-0.78 (H-12, H-4, 5CH, 8CH_2), 1.17 (s, 3H, H-21), 0.91 (s, 3H, H-19), 0.86, 0.85 (2d, *J* = 7.0, 7.0 Hz , 6H, H-26, H-27), 0.75 (s, 3H, H-18), 0.63 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9 (C_{AR}), 128.3 (CH_{AR}), 127.8 (CH_{AR}), 127.4 (CH_{AR}), 84.7 (C-24), 74.9 (C-20), 71.8 (C_{benzyl}), 71.7 (C-2), 70.6 (C-3), 58.3 (C-17), 56.8 (C-14), 55.1 (C-9), 43.0 (C-13), 40.5 (C-1), 40.4 (C-12), 39.2 (C-22), 38.9 (C-5), 35.7 (C-10), 34.2 (C-8), 31.8 (CH_2), 31.7 (CH_2), 30.4 (C-25), 28.2 (CH_2), 26.1 (C-21), 24.3 (CH_2), 23.6 (CH_2), 22.4 (CH_2), 20.7 (CH_2), 18.4, 18.0 (C-26, C-27), 14.6 (C-19), 13.8 (C-18)

MS (APCI), *m/z* (relative intensity): 401 (100), 383 (69), 365 (28)

2α, 3α, 20(*S*), 24-Tetrahydroxy-5α-cholestane (144)



(142) (144)

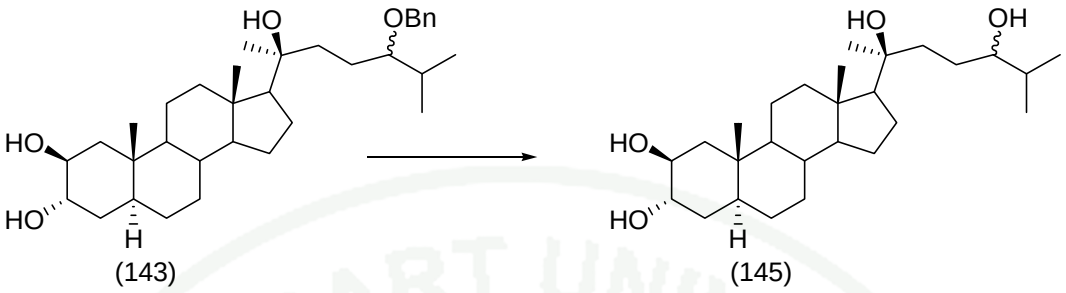
To a solution of 24-benzyloxy-2α, 3α, 20(*S*)-trihydroxy-5α-cholestane (142) (6 mg, 0.01 mmol) in methanol (1 mL) was added 5% Pd/C (4 mg). The black suspension was stirred under hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through celite and eluted with methanol. The filtrate was concentrated under reduced pressure to give 2α, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane (144) (4.2 mg, 90%) as a white solid.

2α, 3α, 20(*S*), 24-Tetrahydroxy-5α-cholestane (144)

FTIR (KBr), ν_{max}, cm^{-1}: 3394, 3363, 2932, 1699, 1540, 1456, 1385, 1263, 1042

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 (m, 1H, H-3), 3.53 (m, 1H, H-2), 3.24 (m, 1H, H-24), 2.04-0.97 (5CH, 10CH_2), 1.20 (s, 3H, H-21), 0.95 (s, 3H, H-19), 0.85 (m, 6H, H-26, H-27), 0.77 (s, 3H, H-18), 0.64 (m, 1H, H-9)

2β, 3α, 20(*S*), 24-Tetrahydroxy-5α-cholestane (145)



(143) (145)

To a solution of 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (143) (23 mg, 0.042 mmol) in methanol (2 mL) was added 5% Pd/C (15 mg). The black suspension was stirred under hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through celite and eluted with methanol. The filtrate was concentrated under reduced pressure to give 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane (145) (16.9 mg, 87%) as a white solid.

2β, 3α, 20(*S*), 24-Tetrahydroxy-5α-cholestane (145)

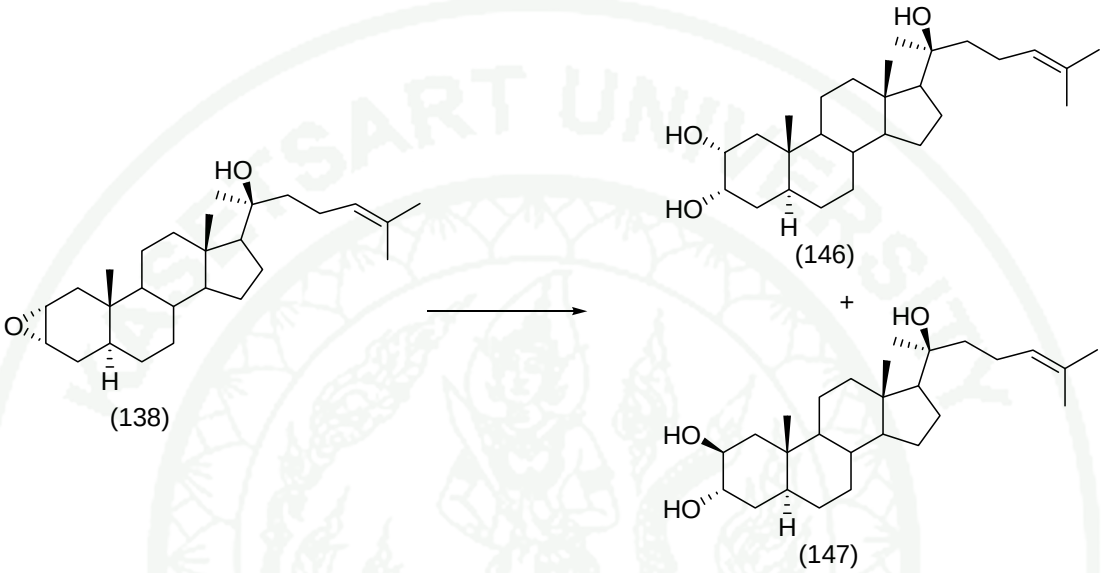
FTIR (KBr), ν_{max}, cm^{-1}: 3398, 2929, 1654, 1459, 1377, 1037

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.74 (m, 1H, H-2), 3.69 (m, 1H, H-3), 3.19 (m, 1H, H-24), 1.97 (m, 1H, H-12), 1.77 (m, 1H, H-4), 1.68-0.91 (H-12, H-4, 5CH, 8CH_2), 1.18, 1.17 (2s, 3H, H-21), 0.90 (s, 3H, H-19), 0.83 (m, 6H, H-26, H-27), 0.75, 0.74 (2s, 3H, H-18), 0.62 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 76.8, 76.7 (C-24), 75.0, 74.8 (C-20), 71.0 (C-2), 69.9 (C-3), 58.9, 58.1 (C-17), 56.4 (C-14), 54.9 (C-9), 42.8, 42.7 (C-13), 40.3, 40.2 (C-12), 39.8 (C-1), 39.5 (CH_2), 39.1 (CH_2), 38.7 (C-5), 35.4 (C-10), 34.1 (C-8), 33.4, 33.2 (C-25), 31.6 (CH_2), 31.2 (CH_2), 28.0, 27.7 (CH_2), 25.4, 25.2 (C-21), 23.4 (CH_2), 22.2, 22.1 (CH_2), 20.5 (CH_2), 18.6, 18.5, 17.3, 16.9 (C-26, C-27), 14.0 (C-19), 13.5, 13.4 (C-18)

MS (APCI), *m/z* (relative intensity): 419 (10), 401 (100), 383 (72), 365 (18)

2α, 3α, 20(*S*)-Trihydroxy-5α-cholest-24-ene (146) and 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147)



A solution of 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138) (59.1 mg, 0.15 mmol) in THF (2 mL) was treated with 1M H_2SO_4 (0.8 mL) and stirred for 24 h at room temperature. After neutralization with saturated aqueous $NaHCO_3$, the mixture was evaporated to fifth initial volume, diluted with water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (25% ethyl acetate:hexane) to give 2α, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (146) (3.6 mg, 6%) as a white solid and 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147) (32.9 mg, 53.1%) as a white solid; m.p. 163-165^{o}C and the mixture of these steriods (2 mg, 3.2%).

2α, 3α, 20(*S*)-Trihydroxy-5α-cholest-24-ene (146)

FTIR (KBr), ν_{max}, cm^{-1}: 3313, 2936, 1453, 1365, 1266, 1041

MS (APCI), *m/z* (relative intensity): 401 (38), 383 (28)

2β, 3α, 20(*S*)-Trihydroxy-5α-cholest-24-ene (147)

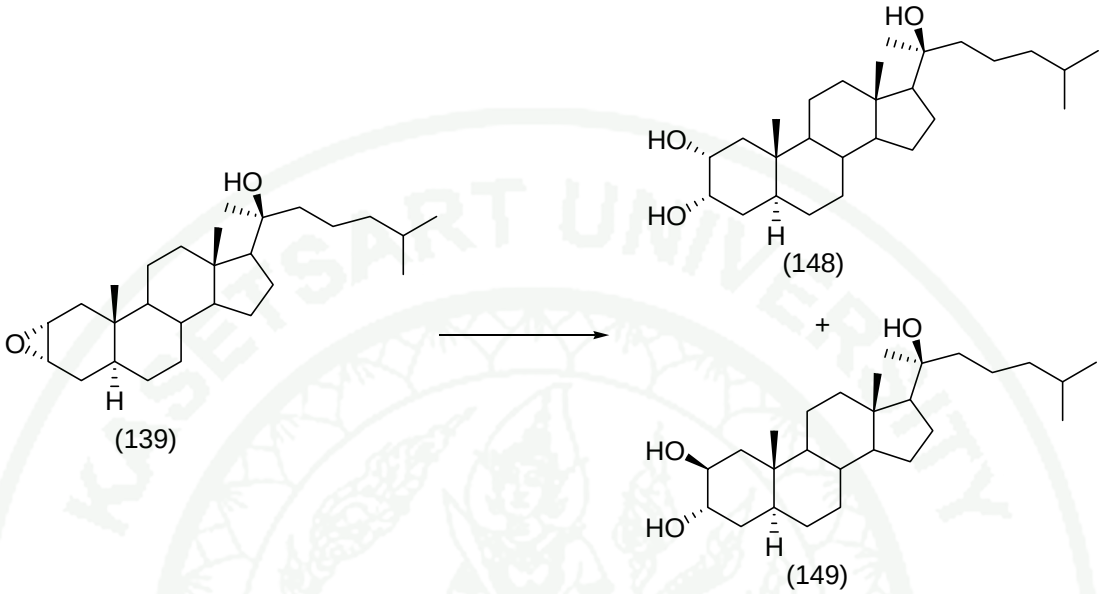
FTIR (KBr), ν_{max}, cm^{-1}: 3392, 2925, 1453, 1375, 1266, 1037

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.02 (m, 1H, H-24), 3.82 (m, 1H, H-2), 3.79 (m, 1H, H-3), 1.99 (m, 1H, H-12), 1.91 (dt, *J* = 7.9, 7.9 Hz, 2H, H-23), 1.82 (ddd, *J* = 13.9, 13.8, 3.2 Hz, 1H, H-4), 1.72-0.77 (H-12, H-4, 4CH, $7CH_2$), 1.61, 1.54 (2s, 6H, H-26, H-27), 1.21 (s, 3H, H-21), 0.92 (s, 3H, H-19), 0.76 (s, 3H, H-18), 0.63 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.5 (C-25), 124.5 (C-24), 75.2 (C-20), 71.8 (C-2), 70.6 (C-3), 58.0 (C-17), 56.6 (C-14), 55.1 (C-9), 43.5 (C-22), 43.0 (C-13), 40.5 (C-1), 40.4 (C-12), 38.9 (C-5), 35.7 (C-10), 34.2 (C-8), 31.8 (CH_2), 31.7 (CH_2), 28.2 (CH_2), 26.1 (C-21), 25.7 (C-26 or C-27), 23.6 (CH_2), 22.9 (C-23), 22.4 (CH_2), 20.7 (CH_2), 17.6 (C-26 or C-27), 14.6 (C-19), 13.8 (C-18)

HRMS *m/z*: $C_{27}H_{46}O_3Na$ $[M+Na]^+$, calcd 441.3345, found 441.3336

2α, 3α, 20(*S*)-Trihydroxy-5α-cholestane (148) and 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149)



A solution of 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (139) (64.4 mg, 0.17 mmol) in THF (3 mL) was treated with 1M H_2SO_4 (0.9 mL) and stirred for 24 h at room temperature. After neutralization with saturated aqueous $NaHCO_3$, the mixture was evaporated to fifth initial volume, diluted with water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (25% ethyl acetate:hexane) to give 2α, 3α, 20(*S*)-trihydroxy-5α-cholestane (148) (7 mg, 10%) as a white solid and 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149) (36.9 mg, 51.6%) as a white solid; m.p. 186-188^{o}C and the mixture of these steriods (10 mg, 14%).

2α, 3α, 20(*S*)-Trihydroxy-5α-cholestane (148)

FTIR (KBr), ν_{max}, cm^{-1}: 3394, 2937, 1450, 1362, 1258, 1107, 1040

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 (m, 1H, H-3), 3.53 (m, 1H, H-2), 1.98 (m, 2H, H-12, H-4), 1.75-0.84 (5CH, 9CH_2), 1.19 (s, 3H, H-21), 0.95 (s, 3H, H-19), 0.80 (d, *J* = 6.4 Hz, 6H, H-26, H-27), 0.77 (s, 3H, H-18), 0.63 (m, 1H, H-9)

2β, 3α, 20(*S*)-Trihydroxy-5α-cholestane (149)

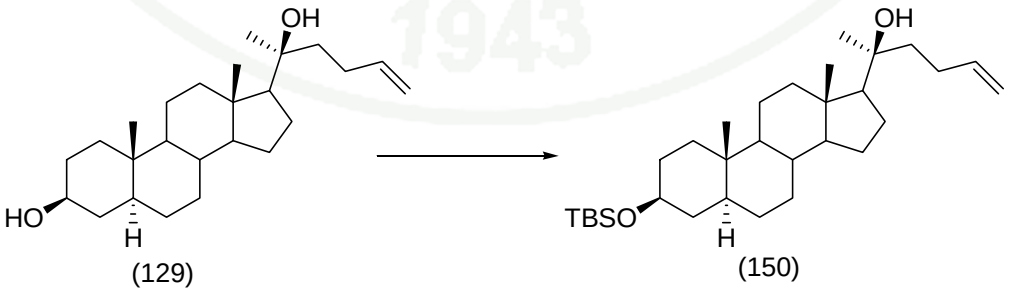
FTIR (KBr), ν_{max}, cm^{-1}: 3388, 2930, 1466, 1381, 1036

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 (m, 1H, H-2), 3.78 (m, 1H, H-3), 1.98 (m, 1H, H-12), 1.82 (ddd, *J* = 14.0, 13.2, 3.2 Hz, 1H, H-4), 1.71-0.84 (H-12, H-4, 5CH, 9CH_2), 1.19 (s, 3H, H-21), 0.92 (s, 3H, H-19), 0.80 (d, *J* = 6.6 Hz, 6H, H-26, H-27), 0.77 (s, 3H, H-18), 0.63 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 75.3 (C-20), 71.8 (C-2), 70.6 (C-3), 57.8 (C-17), 56.6 (C-14), 55.1 (C-9), 44.2 (C-22), 42.9 (C-13), 40.5 (C-1), 40.4 (C-12), 39.6 (C-24), 38.9 (C-5), 35.7 (C-10), 34.2 (C-8), 31.8 (CH_2), 31.7 (CH_2), 28.2 (CH_2), 27.9 (C-25), 26.3 (C-21), 23.6 (CH_2), 22.7, 22.5 (C-26, C-27), 22.3 (CH_2), 22.0 (CH_2), 20.7 (CH_2), 14.6 (C-19), 13.8 (C-18)

MS (APCI), *m/z* (relative intensity): 403 (75), 385 (100), 367 (76)

3β-*tert*-Butyldimethylsiloxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (150)



(129) (150)

To a cooled (0^{o}C) solution of 3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129) (126.7, 0.34 mmol) in dry CH_2Cl_2 (5 mL) was added 2, 6-lutidine (0.11 mL,

0.91 mmol) and *tert*-butyldimethylsilyltrifluoromethanesulfonate (0.11 mL, 0.50 mmol). The reaction mixture was stirred at 0°C for 30 min. Then the reaction was quenched by addition of saturated aqueous $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with water, the organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane) to afford 3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (150) (141 mg, 85%) as a white solid; m.p. 129-131°C.

3β-*tert*-Butyldimethylsiloxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (150)

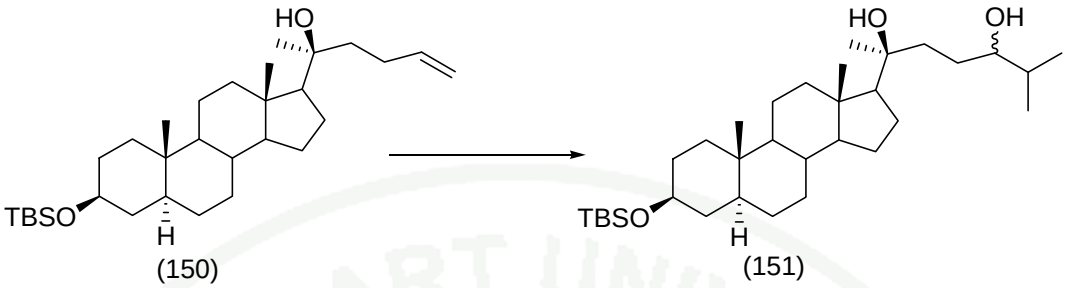
FTIR (KBr), ν_{max}, cm^{-1}: 2929, 1461, 1373, 1251, 1092, 835

^1H NMR ($CDCl_3$, 400 MHz) δ 5.80 (m, 1H, H-24), 5.00 (m, 1H, H-25), 4.92 (m, 1H, H-25), 3.52 (m, 1H, H-3), 2.04 (m, 4H, H-23, H-12), 1.73-0.81 (5CH, $9CH_2$), 1.25 (H-21), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.81 (H-18), 0.77 (H-19), 0.02 (s, 6H, $Si(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.1 (C-24), 114.2 (C-25), 75.1 (C-20), 72.2 (C-3), 58.2 (C-17), 56.7 (C-14), 54.4 (C-9), 45.0 (C-5), 43.0 (C-13), 42.6 (C-22), 40.5 (C-12), 38.7 (CH_2), 37.2 (C-1), 35.5 (C-10), 34.9 (CH_2), 32.0 (CH_2), 31.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 26.2 (C-21), 25.9 ($3CH_3$), 23.7 (CH_2), 22.4 (CH_2), 21.1 (CH_2), 18.2 (Cq), 13.8 (C-18), 12.4 (C-19), -4.6 ($2CH_3$)

HRMS *m/z*: $C_{31}H_{56}O_2NaSi$ $[M+Na]^+$, calcd 511.3947, found 511.3947

3β-*tert*-Butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (151)



Step I

A mixture of 3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-24a-homo-chlo-24-ene (150) (141 mg, 0.29 mmol) and $NaHCO_3$ (86.9 mg) in CH_2Cl_2 (5 mL) and methanol (1 mL) was cooled to -78°C and ozone was bubbled through the mixture with stirring. When the reaction mixture was turned blue, ozone addition was stopped. Nitrogen gas was passed through the reaction until the blue color was discharged. The reaction was quenched by addition of triphenylphosphine (151.9 mg, 0.58 mmol) at -78°C. The reaction was slowly warmed to room temperature and further stirred for 1 h before concentration under reduced pressure. The residue was purified by flash column chromatography (5% ethyl acetate:hexane) to give aldehyde intermediate (105 mg, 73%) as a white solid.

Step II

To a stirring of Mg (190.3 mg, 5.9 mmol) and catalyst I_2 in dry THF (10 mL) was slowly added 2-bromopropane (2.3 mL, 23.5 mmol) under nitrogen atmosphere at room temperature. After stirring for 2 h, this mixture was cooled to -78°C. A solution of aldehyde intermediate (105 mg, 0.21 mmol) in dry THF (8 mL) was added slowly and kept at this temperature. After 20 min, the reaction mixture was allowed to slowly warm to room temperature and left at this temperature for 3 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (4×30 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced

pressure. The residue was purified by flash column chromatography (7% ethyl acetate:hexane) to give 3β-*tert*-butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (151) (38 mg, 54% brsm) as a white solid and recovered starting material (39 mg, 62% conversion).

3β-*tert*-Butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (151)

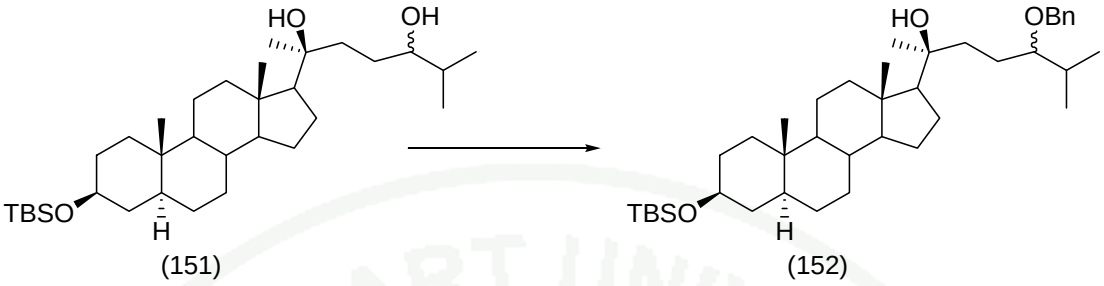
FTIR (KBr), ν_{max}, cm^{-1}: 3342, 2920, 1466, 1373, 1256, 1087, 830

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.52 (m, 1H, H-3), 3.30 (m, 1H, H-24), 2.02 (m, 1H, H-12), 1.76-0.83 (6CH, 10CH_2, H-12), 1.24 (s, 3H, H-21), 0.89 (m, 6H, H-26, H-27), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.80 (s, 3H, H-18), 0.77 (s, 3H, H-19), 0.02 (s, 6H, $Si(CH_3)_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 77.1 (C-24), 75.2 (C-20), 72.2 (C-3), 58.5 (C-17), 56.6 (C-14), 54.4 (C-9), 45.0 (C-5), 43.0 (C-13), 40.4 (C-12), 39.4 (C-22), 38.6 (CH_2), 37.2 (C-21), 35.5 (C-10), 34.9 (C-8), 33.5 (C-25), 32.0 (CH_2), 31.9 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 26.1 (C-21), 25.9 ($SiC(\underline{C}H_3)_3$), 23.7 (CH_2), 22.4 (CH_2), 21.1 (CH_2), 18.9, 17.4 (C-26, C-27), 18.2 ($Si\underline{C}(CH_3)_3$) 13.8 (C-18), 12.4 (C-19), -4.6 ($Si(CH_3)_2$)

HRMS *m/z*: $C_{33}H_{62}O_3NaSi$ $[M+Na]^+$, calcd 557.4366, found 557.4365

24-Benzyloxy-3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-cholestane (152)



(151) (152)

NaH (160 mg, 3.9 mmol) was washed with dry THF (4 mL). The suspension was stirred vigorously for 10 min before removing solvent.

To the suspension of NaH in dry THF was slowly added 3β-*tert*-butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (151) (209.5 mg, 0.39 mmol) and the mixture was stirred under reflux for 2 h. After the reaction mixture was cooled down to room temperature, benzyl bromide (0.28 mL, 1.9 mmol) was added and stirred for an additional 30 min at room temperature and 4 h under reflux. The reaction was quenched by pouring into water and extracted with diethyl ether (3×50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (100% hexane to 3% ethyl acetate:hexane) to give 24-benzyloxy-3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-cholestane (152) (183.2 mg, 75%) as a colorless gum.

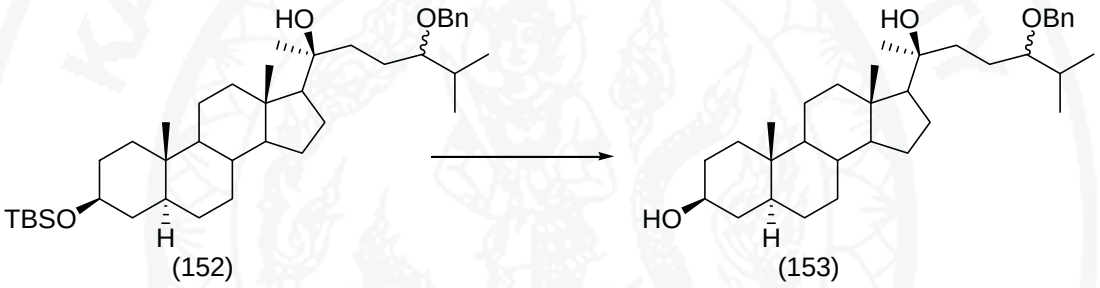
24-Benzyloxy-3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-cholestane (152)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29-7.27 (m, 5H, H_{AR}), 4.46, 4.47 (2s, 2H, H_{benzyl}), 3.50 (m, 1H, H-3), 3.06 (m, 1H, H-24), 1.98 (m 1H, H-12), 1.85 (m, 1H, H-25), 1.73-0.79 (H-12, 4CH, 10CH_2), 1.19 (s, 3H, H-21), 0.88 (m, 6H, H-26, H-27), 0.84 (s, 9H, SiC($C\underline{H}_3$)$_3$), 0.77 (s, 3H, H-18), 0.75 (s, 3H, s, H-19), 0.55 (m, 1H, H-9), 0.00 (s, 6H, Si($C\underline{H}_3$)$_2$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.0 (C_{AR}), 128.2 (CH_{AR}), 127.8 (CH_{AR}), 127.4 (CH_{AR}), 84.7 (C-24), 74.9 (C-20), 72.2 (C-3), 71.8 (C_{benzyl}), 58.2 (C-17), 56.7 (C-14), 54.4 (C-9), 45.0 (C-5), 43.0 (C-13), 40.4 (C-12), 39.2 (C-22), 38.7 (CH_2), 37.2 (CH_2), 35.5 (C-10), 34.9 (CH), 32.0 (CH_2), 31.9 (CH_2), 30.4 (CH), 28.7 (CH_2), 26.2 (C-21), 25.9 ($SiC(\underline{C}H_3)_3$), 24.4 (CH_2), 23.7 (CH_2), 22.5 (CH_2), 21.1 (CH_2), 18.3 ($Si\underline{C}(CH_3)_3$), 18.2, 18.1 (C-26, C-27), 13.7 (C-18), 12.4 (C-19), -4.5 ($Si\underline{C}H_3)_2$)

MS (APCI), *m/z* (relative intensity): 516 (4), 499 (100), 367 (64)

24-benzyloxy-3β, 20(*S*)-dihydroxy-5α-cholestane (153)



To a solution of 24-benzyloxy-3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-cholestane (152) (183.2 mg, 0.29 mmol) in THF (4 mL) was added 1M tetrabutylammonium fluoride (TBAF) in THF (1.46 mL, 1.46 mmol) and stirred under reflux for 3 h. The reaction was diluted with diethyl ether and washed with water. The aqueous was back extracted with diethyl ether (4×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product that was purified by flash column chromatography (10% ethyl acetate:hexane) to yield 24-benzyloxy-3β, 20(*S*)-dihydroxy-5α-cholestane (153) (122 mg, 81.5%) as a colorless gum.

24-benzyloxy-3β, 20(*S*)-dihydroxy-5α-cholestane (153)

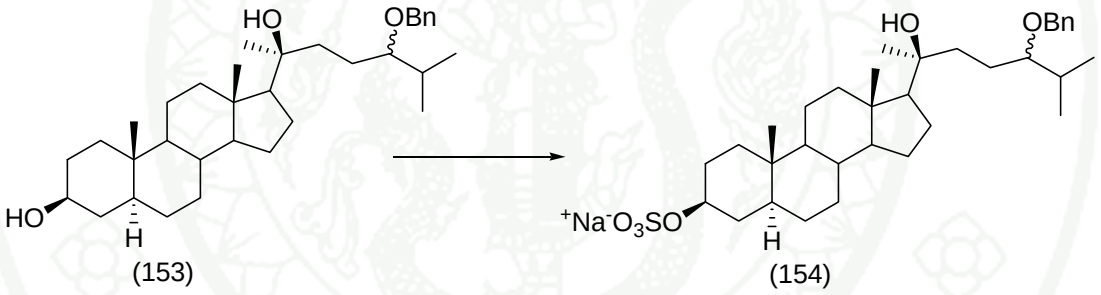
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27-7.25 (m, 5H, H_{AR}), 4.43 (m, 2H, H_{benzyl}), 3.50 (m, 1H, H-3), 3.03 (m, 1H, H-24), 1.97 (m, 1H, H-12), 1.83 (m, 1H, H-25), 1.76-

0.76 (H-12, 4CH, 10CH_2), 1.17, 1.16 (2s, 3H, H-21), 0.85 (m, 6H, H-26, H-27), 0.75 (s, 3H, H-18), 0.73 (s, 3H, H-19), 0.54 (m, 1H, H-9)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9 (C_{AR}), 128.2 (CH_{AR}), 127.8 (CH_{AR}), 127.4 (CH_{AR}), 84.8, 84.7 (C-24), 75.1, 74.9 (C-20), 71.8 (C_{benzyl}), 71.3 (C-3), 58.3 (C-17), 56.6 (C-14), 54.3 (C-9), 44.8 (C-5), 42.9 (C-13), 40.4 (C-12), 39.3, 39.1 (C-22), 38.2 (CH_2), 37.0 (C-1), 35.4 (C-10), 34.8 (C-8), 31.9 (CH_2), 31.5 (CH_2), 30.5, 30.4 (C-25), 28.7 (CH_2), 26.1, 26.0 (C-21), 24.6, 24.3 (CH_2), 23.7 (CH_2), 22.5, 22.4 (CH_2), 21.1 (CH_2), 18.6, 18.3, 18.1, 18.0 (C-26, C-27), 13.8, 13.7 (C-18), 12.3 (C-19)

MS (APCI), *m/z* (relative intensity): 403 (4), 385 (76), 367 (100), 257 (13)

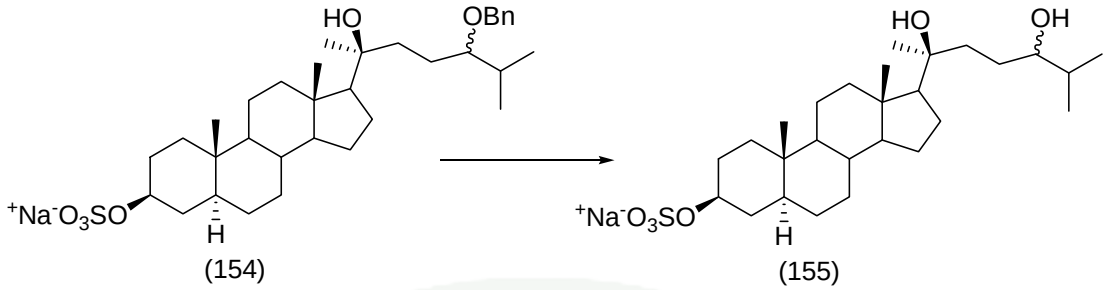
Sodium 3β, 20(*S*), 24-trihydroxy-5α-cholestane 3-sulfate (155)



(153) (154)

Step I

Triethylamine-sulfur trioxide complex (73.1 mg, 0.40 mmol) was added to a solution of 24-benzyloxy-3β, 20(*S*)-dihydroxy-5α-cholestane (153) (69.5 mg, 0.14 mmol) in *N,N*-dimethylformamide (2.5 mL). The reaction mixture was stirred at room temperature for 16 h and then quenched with water (2.5 mL). After evaporation to dryness, the residue was eluted through Amberlite CG-120 (Na form) with methanol. The crude product of sodium 24-benzyloxy-3β, 20(*S*)-dihydroxy-5α-cholestane 3-sulfate (154) was used in the next step without purification.



(154) (155)

Step II

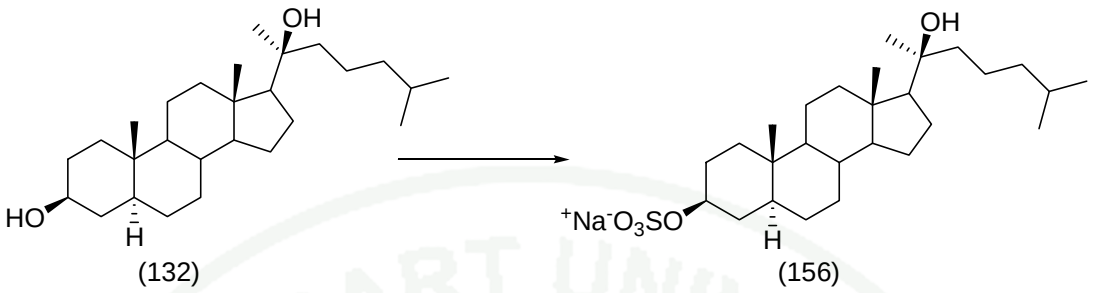
To a solution of sodium 24-benzyloxy-3β, 20(*S*)-dihydroxy-5α-cholestane 3-sulfate (154) in methanol (2 mL) was added 5% Pd/C (17.2 mg). The black suspension was stirred under hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through celite and eluted with methanol, the filtrate was concentrated under reduced pressure. The residue was further purified by column chromatography (4% methanol:CH_2Cl_2) to give sodium 3β, 20(*S*), 24-trihydroxy-5α-cholestane 3-sulfate (155) (30 mg, 42.5%) as a white solid.

Sodium 3β, 20(*S*), 24-trihydroxy-5α-cholestane 3-sulfate (155)

^{1}H NMR (CD_3OD, 400 MHz) δ 4.25 (m, 1H, H-3), 3.25 (m, 1H, H-24), 2.07 (m, 1H, H-12), 2.01 (m, 1H, H-14), 1.84-0.94 (H-12, H-14, 5CH, 9CH_2), 1.23 (s, 3H, H-21), 0.90 (m, 6H, H-26, H-27), 0.84 (s, 6H, H-18, H-19), 0.66 (m, 1H, H-9)

MS (APCI), *m/z* (relative intensity): 403 (3), 385 (79), 367 (100), 257 (3)

Sodium 3β, 20-dihydroxy-5α-cholestane 3-sulfate (156)



Triethylamine-sulfur trioxide complex (13.5 mg, 0.064mmol) was added to a solution of 3β, 20-dihydroxy-5α-cholestane (132) (13 mg, 0.032 mmol) in *N,N*-dimethylformamide (1 mL). The reaction mixture was stirred at room temperature for 24 h, and then quenched with water (1 mL). After evaporation to dryness, the residue was eluted through Amberlite CG-120 (Na form) with methanol. The residue was purified by flash column chromatrography (5% methanol:CH_2Cl_2) to give sodium 3β, 20-dihydroxy-5α-cholestane 3-sulfate (156) (10 mg, 61%) as a white solid.

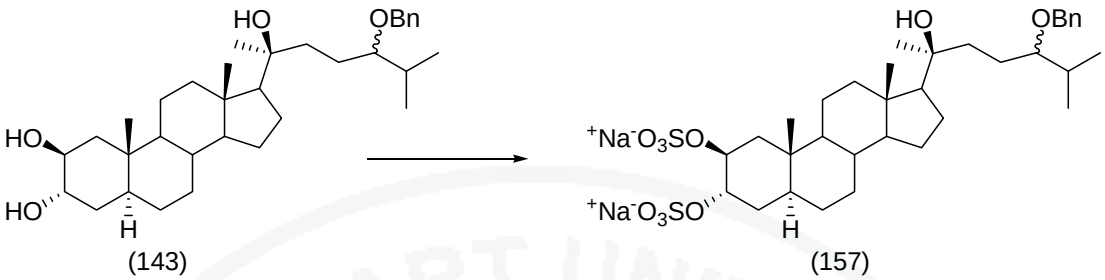
Sodium 3β, 20-dihydroxy-5α-cholestane 3-sulfate (156)

FTIR (KBr), ν_{max}, cm^{-1}: 2947, 1467, 1383, 1244, 1218, 1060, 972

^{1}H NMR (CD_3OD, 400 MHz) δ 4.26 (m, 1H, H-3), 2.03 (m, 1H, H-12), 1.83-0.90 (4CH, 11CH_2, H-12), 1.21 (s, 3H, H-21), 0.88 (d, *J* = 6.6 Hz, 6H, H-26, H-27), 0.83 (s, 6H, H-18, H-19), 0.69 (m, 1H, H-9)

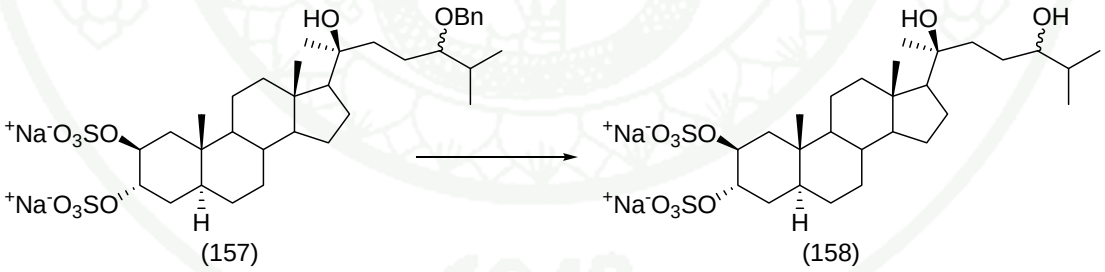
HRMS *m/z*: $C_{27}H_{46}O_3Na$ $[M+Na]^+$, calcd 483.3144, found 483.3144

Disodium 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane 2, 3-disulfate (158)



Step I

Triethylamine-sulfur trioxide complex (155.7 mg, 0.86 mmol) was added to a solution of 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (143) (45.3 mg, 0.86 mmol) in *N,N*-dimethylformamide (2 mL). The reaction mixture was stirred at 95°C for 16 h and then quenched with water (2 mL). After evaporation to dryness, the residue was eluted through Amberlite CG-120 (Na form) with methanol. The crude product of disodium 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane 2, 3-disulfate (157) was used in the next step without purification.



Step II

To a solution of disodium 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-5α-cholestane 2, 3-disulfate (157) in methanol (10 mL) was added 5% Pd/C (11 mg). The black suspension was stirred under hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through celite and eluted with methanol, the filtrate was concentrated under reduced pressure. The residue was further purified by flash

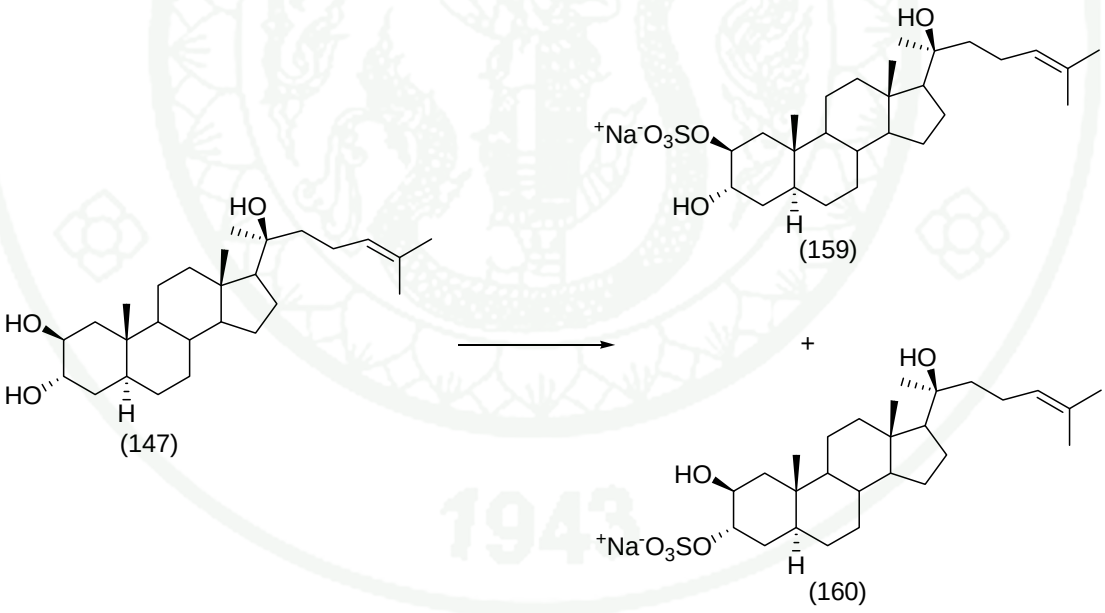
column chromatography (3% methanol:CH_2Cl_2) to give disodium 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane 2, 3-disulfate (158) (20 mg, 36%) as a white solid.

Disodium 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane 2, 3-disulfate (158)

^{1}H NMR (CD_3OD, 400 MHz) δ 4.70 (m, 2H, H-2, H-3), 3.20 (m, 1H, H-24), 2.18-0.63 (6CH, 10CH_2), 1.25 (s, 3H, H-21), 0.97 (s, 3H, CH_3), 0.87 (m, 6H, H-26, H-27), 0.51 (s, 3H, CH_3)

MS (APCI), *m/z* (relative intensity): 399 (69), 381 (47)

Sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 2-sulfate (159) and sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 3-sulfate (160)



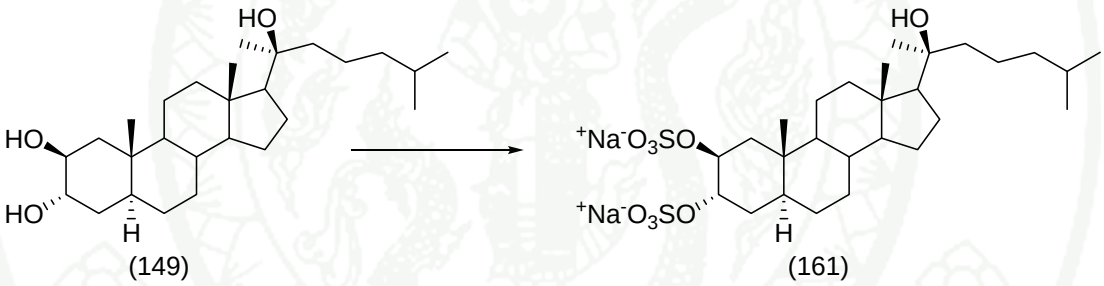
Triethylamine-sulfur trioxide complex (16.5 mg, 0.09 mmol) was added to a solution of 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147) (18.8 mg, 0.04 mmol) in *N,N*-dimethylformamide (2 mL). The reaction mixture was stirred at 95^{o}C for 16 h and then quenched with water (2 mL). After evaporation to dryness, the residue was eluted through Amberlite CG-120 (Na form) with methanol. The crude product was

purified by flash column chromatography (3% methanol:CH_2Cl_2) to give sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 2-sulfate (159) and sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 3-sulfate (160) (12 mg, 43.5%) as a pale yellow gum.

Sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 2-sulfate (159) and sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 3-sulfate (160)

^{1}H NMR (CD_3OD, 400 MHz) δ 5.09 (m, 1H, H-24), 4.42, 4.40, 4.07, 4.02 (m, 2H, H-2, H-3), 2.29-0.54 (5CH, 10CH_2), 1.66, 1.60 (2s, 6H, H-26, H-27), 1.24 (s, 3H, H-21), 0.99 (s, 3H, CH_3), 0.83 (s, 3H, CH_3)

Disodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane 2, 3-disulfate (161)



(149) (161)

Triethylamine-sulfur trioxide complex (56.6 mg, 0.31 mmol) was added to a solution of 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149) (21.9 mg, 0.05 mmol) in N,N-dimethylformamide (2 mL). The reaction mixture was stirred at 95^{o}C for 16 h and then quenched with water (2 mL). After evaporation to dryness, the residue was eluted through Amberlite CG-120 (Na form) with methanol. The crude product was purified by flash column chromatography (100% CH_2Cl_2 to 3% methanol:CH_2Cl_2) to give disodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane 2, 3-disulfate (161) as a white solid (15 mg, 46.1%)

Disodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane 2, 3-disulfate (161)

^{1}H NMR (CD_3OD, 400 MHz) δ 4.66, 4.62 (m, 2H, H-2, H-3), 2.05-0.99 (6CH, 11CH_2), 1.26 (s, 3H, H-21), 0.90 (s, 3H, CH_3), 0.80 (m, 6H, H-26, H-27), 0.46, 0.43 (2s, 3H, CH_3)

MS (APCI), *m/z* (relative intensity): 383 (46), 365 (20)

Biological assays

KB cell line (epidermoid carcinoma of oral cavity) and NCI-H187 (small cell lung carcinoma) were determined by resazurin microplate assay (REMA) method which was described by Brien *et al*. (2000). Briefly, cells at a logarithmic growth phase are harvested and diluted to 2.2×10^4 cells/ml for KB and 3.3×10^4 cells/ml for NCI-H187 in fresh medium. Successively, 5 µl of test sample diluted in 5% DMSO, and 45 µl of cell suspension are added to 384-well plates, incubated at 37°C in 5% CO_2 incubator. After the incubation period (3 days for KB and 5 days for NCI-H187), 12.5 µl of 62.5 µg/ml resazurin solution is added to each well, and the plates are then incubated at 37°C for 4 hours. Fluorescence signal is measured using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at excitation and emission wavelengths of 530 nm and 590 nm. Ellipticine and doxorubicin are used as a positive control and 0.5% DMSO and water are used as negative control.

Cytotoxicity against primate cell line (Vero) was determined by green fluorescent protein (GFP) detection method that was described by Hunt *et al*. (1999). The GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line with pEGFP-N1 plasmid (Clontech). The cell line is maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate and 0.8 mg/ml geneticin, at 37°C in a humidified incubator with 5% CO_2. The assay is carried out by adding 45 µl of cell suspension at 3.3×10^4 cells/ml to each well of 384-well plates containing 5 µl to test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days at 37°C incubator with 5% CO_2. Fluorescence signals are measure by using SpectraMax M5 microplate reader (Molecular Devices, USA) in the bottom reading mode with excitation and emission wavelengths of 485 and 535 nm. Ellipticine are used as appositive control and 0.5% DMSO are used as a negative control.

Before performing the anti-viral assay, compounds will be tested as a range of non-cytotoxic concentrations to evaluate their cytotoxic effect to host cells.

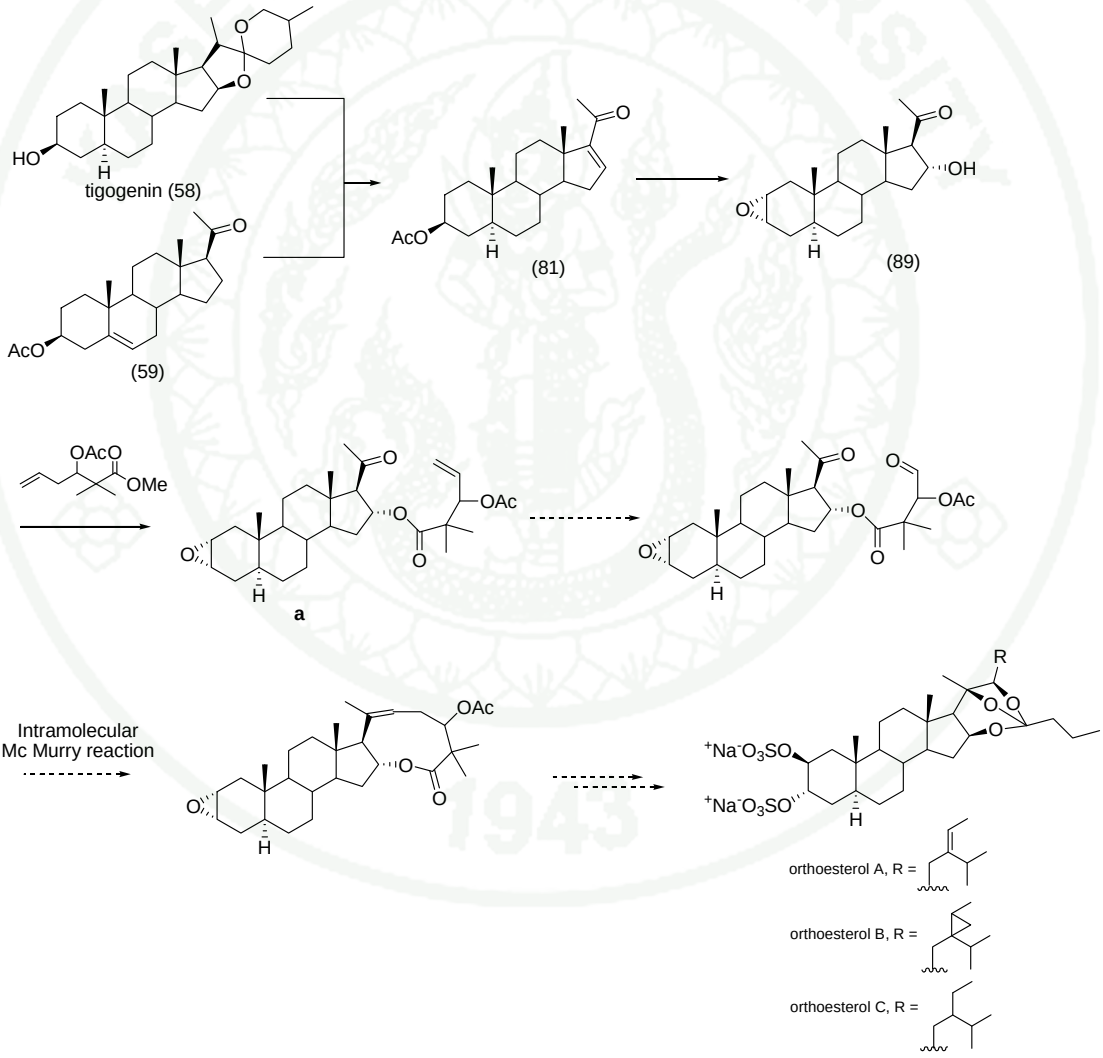
Sample solubilized in 10% DMSO are added to 96-well plates in the volume of 10 µl/well. Subsequently, 190 µl of GFP-expressing Vero cell suspension at 1×10^5 cells/ml premixed with 5×10^5 PFU/ml of HSV-1 inoculums is added to each well. The plates are then incubated in at 37°C humidified incubator with 5% CO_2 for 4 days. Fluorescence signals are measured on day zero and day 4 using SpectraMax M5 multi-detection microplate reader in the bottom-reading mode with excitation and emission wavelengths of 485 and 535 nm. Acyclovir and 0.5% DMSO are used as a positive and a negative controls, respectively.

RESULTS AND DISCUSSION

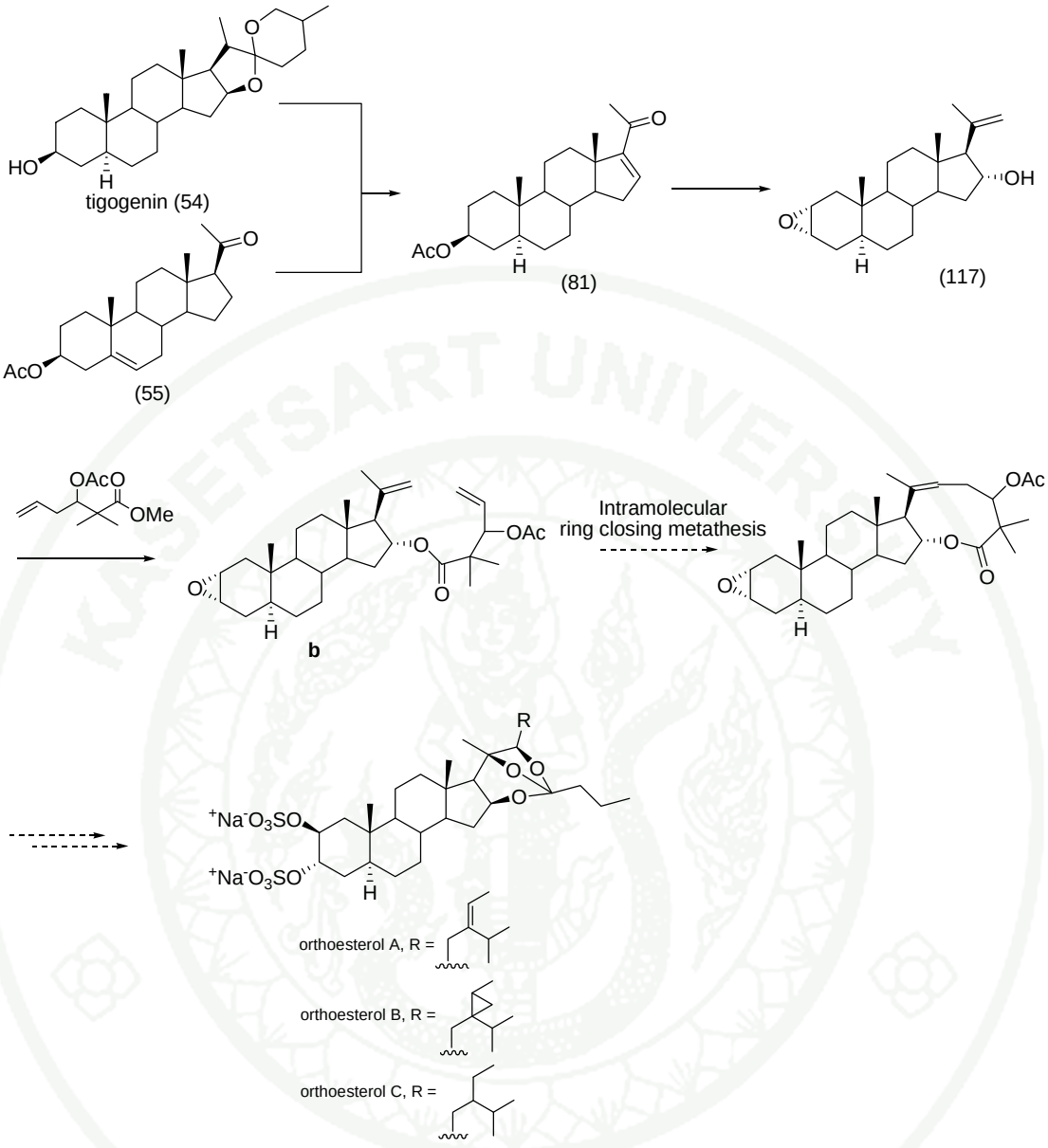
Results

1. Synthesis of sterol orthoesters intermediates

1.1 Synthesis of sterol orthoesters intermediates for intramolecular strategy (Scheme 12 and 13)



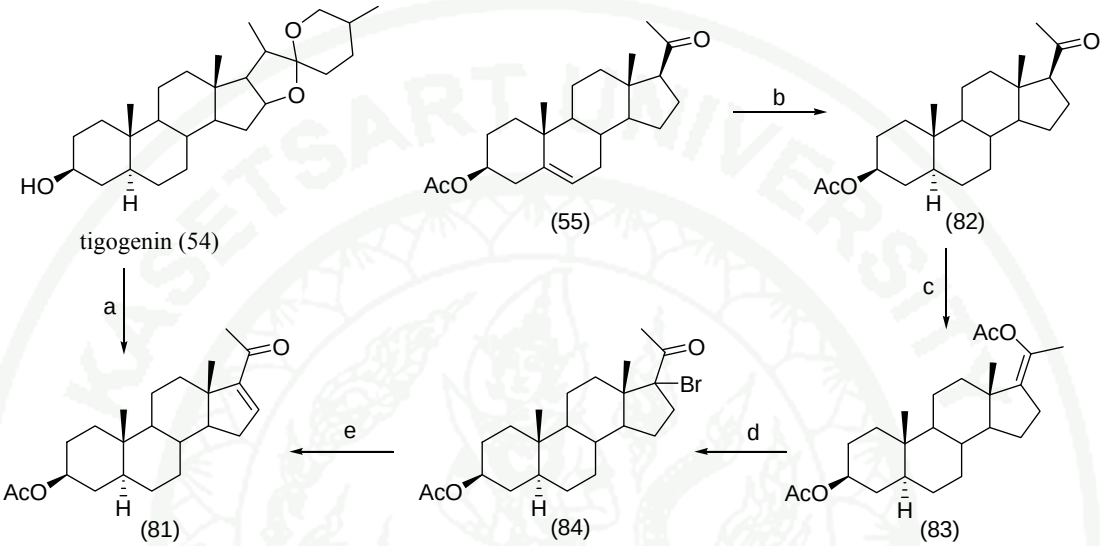
Scheme 12



Scheme 13

Preparation of 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89)

Conversion of tigogenin (54) and 3β-acetoxy-5-pregnen-20-one (55) to 3β-acetoxy-5α-16-pregnen-20-one (81) was shown in Scheme 14.



Scheme 14

Reagents and conditions:

a) i) Ac_2O, pyr, NH_4Cl, 135°C, 16h
   ii) CrO_3, AcOH, 1, 2-dichloroethane, H_2O, 0°C-rt, 2h
   iii) Al_2O_3, benzene, rt, 16h, 49% (3 steps)

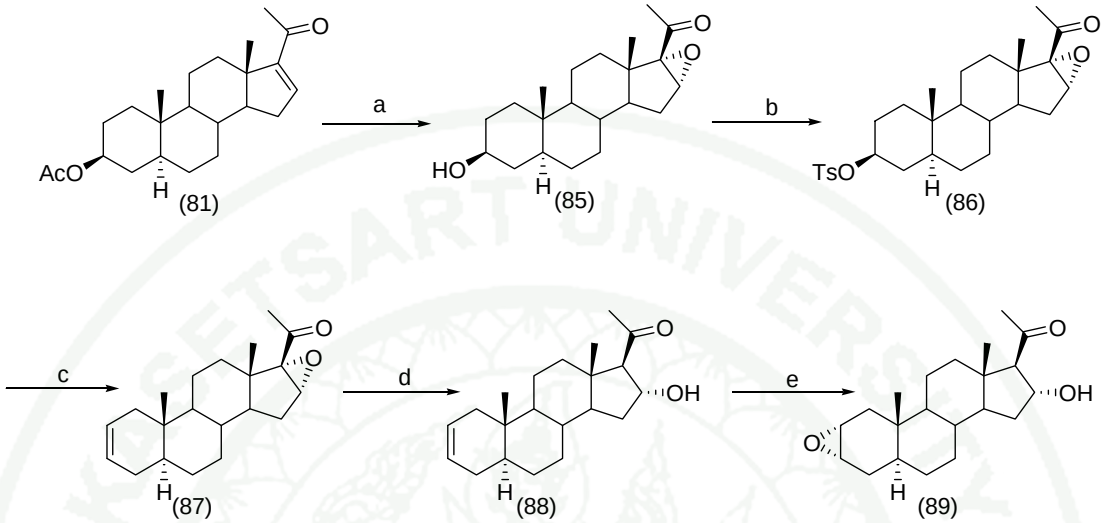
b) H_2, 5% Pd/C, ethyl acetate:methanol (1:3), rt, 16h, 91%

c) *p*-toluenesulfonic acid, Ac_2O, reflux, 6h, 88% (brsm), 75% conversion

d) Br_2/CH_2Cl_2, CH_2Cl_2, rt, 15 min, 87%

e) 1,5-diazabicyclo[5.4.0]undec-7-ene (DBU), toluene, reflux, 8h, 89%

Conversion of 3β-acetoxy-5α-16-pregnen-20-one (81) to 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89) was shown in Scheme 15.



Scheme 15

Reagents and conditions:

a) 30% H_2O_2, 2.5M NaOH, methanol, rt, 16h, 94%

b) *p*-toluenesulfonyl chloride, DMAP, pyr, CH_2Cl_2, rt, 3 day, 92% (brsm), 46% conversion

c) LiBr, Li_2CO_3, DMF, reflux, 4h, 75%

d) i) $NH_2NH_2.H_2O$, ethanol, rt, 3 day

ii) 10% HCl, 60%

e) *m*CPBA, Na_2CO_3, CH_2Cl_2, H_2O, rt, 4h, 64%

Table 8 Reductive cleavage of α, β-epoxy ketone **87** to β-hydroxy ketone **88**

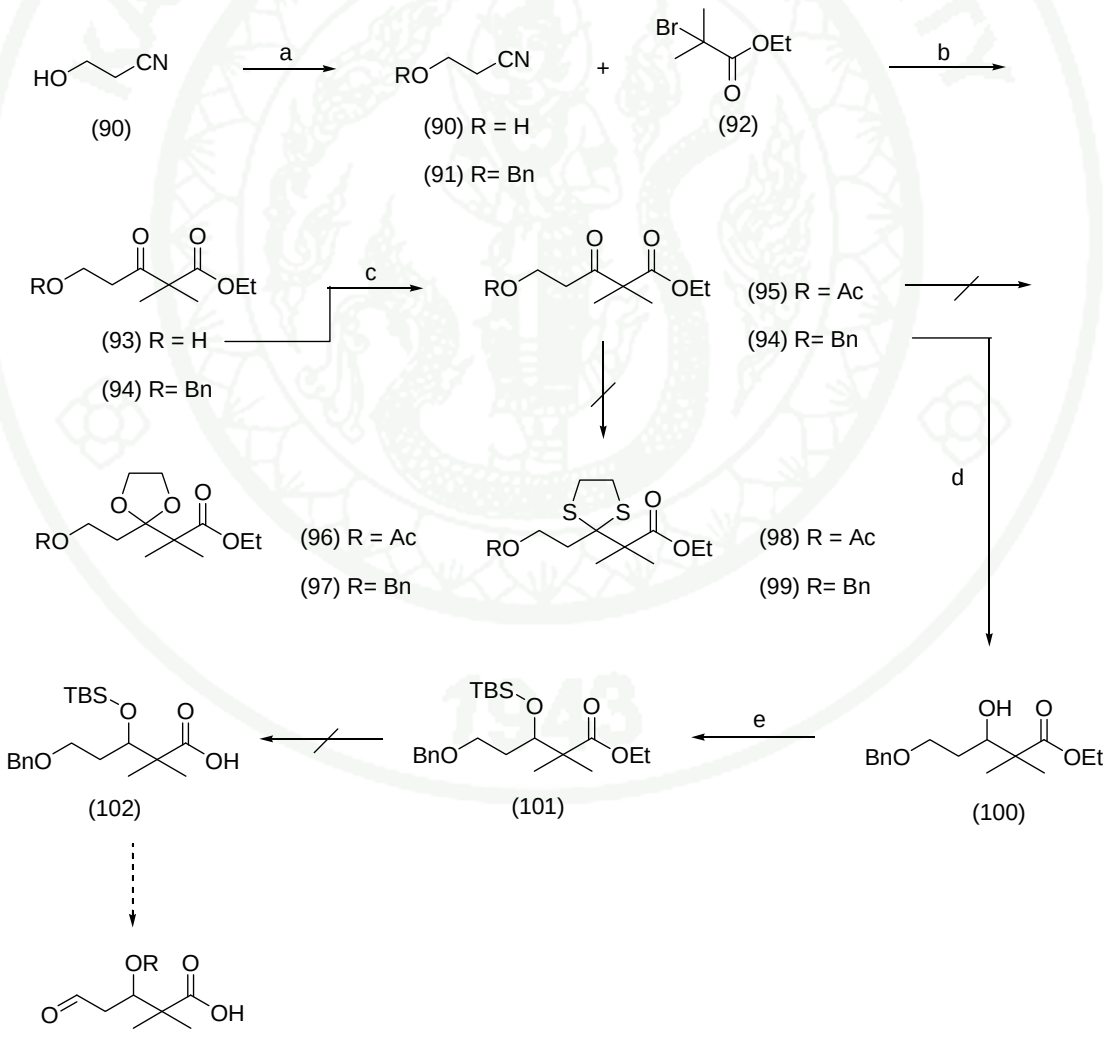
Entry	Reagent	% yield
1	Lithium naphthalenide	58
2	SmI_2	79

Table 8 (Continued)

Entry	Reagent	% yield
3	$NH_2NH_2.H_2O$	60

Preparation of side chain part

The alternative preparations of side chain moiety were described as shown in Scheme 16-18.



Scheme 16

Reagents and conditions:

a) NaH, BnBr, rt, 5h, 50%

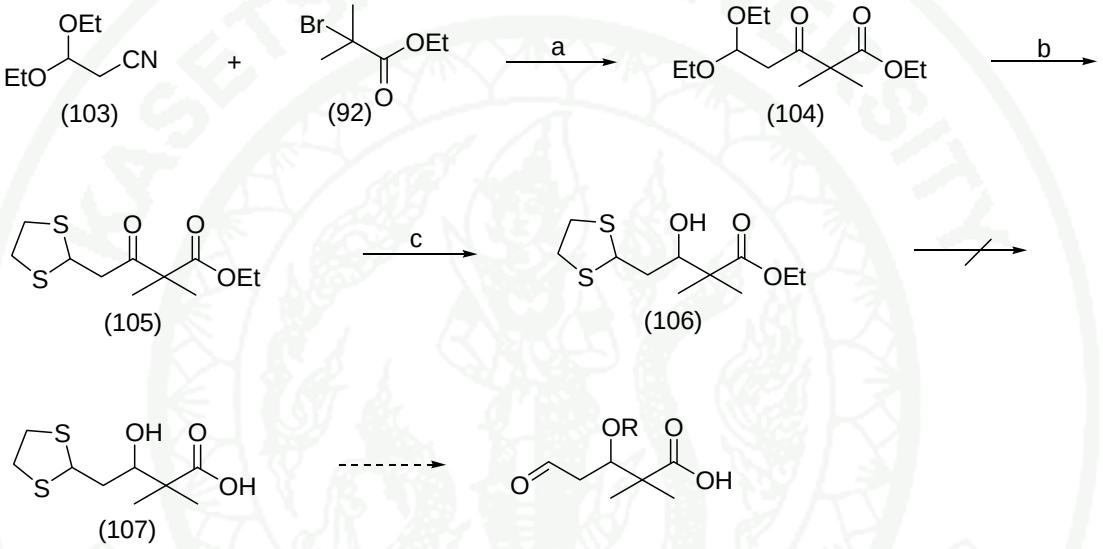
b) i) Zn (powder), THF, sonicate, 2h

ii) 1M HCl, ethyl acetate, rt, 30 min, 70% (93) and 61% (94)

c) Ac_2O, pyr, rt, 16h, 70%

d) $NaBH_4$, methanol, rt, 1h, 60%

e) TBSOTf, 2, 6-lutidine, CH_2Cl_2, 0^oC, 1h, 61%



Scheme 17

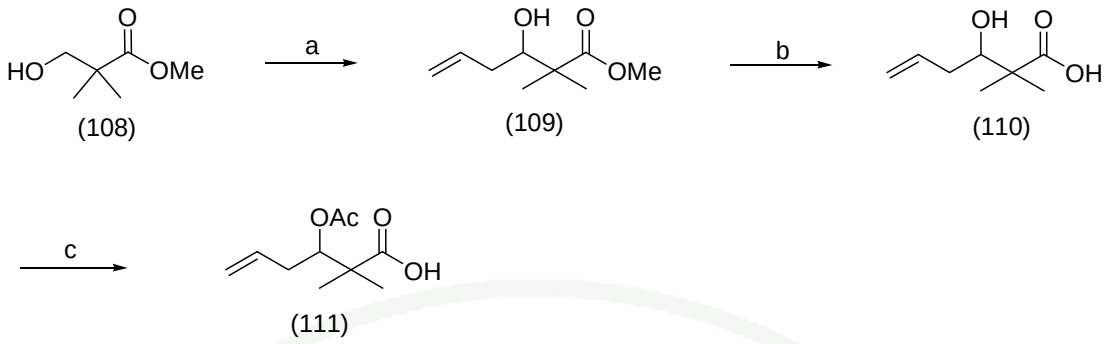
Reagents and conditions:

a) i) Zn (powder), THF, sonicate, 2h

ii) 1M HCl, ethyl acetate, rt, 30 min, 86%

b) 1, 2-ethanedithiol, $BF_3.OEt_2$, CH_2Cl_2, rt, 1h, 74%

c) $NaBH_4$, methanol, rt, 42%



(108) (109) (110) (111)

Scheme 18

Reagents and conditions:

a) i) PCC, CH_2Cl_2, rt, 16h or IBX, DMSO, rt, 5h

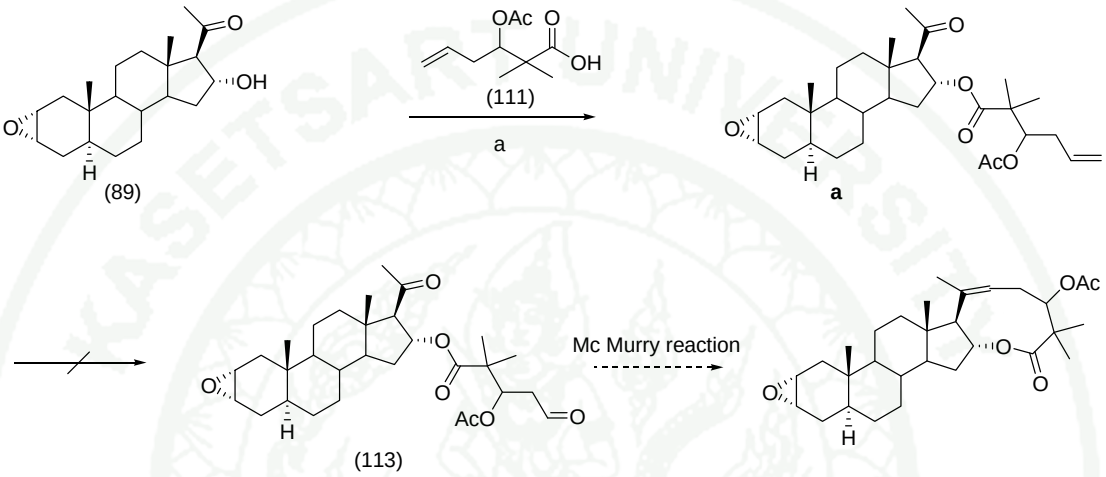
ii) Zn (powder), allyl bromide, Sat. NH_4Cl, reflux, 20 min, 30%

b) 1M LiOH, methanol, rt, 16h, 70%

c) Ac_2O, pyr, rt, 6h, 61%

Preparation of key intermediate **a**

Steroid part, 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89), coupled with side chain moiety, 3-acetoxy-2,2-dimethyl-5-hexenoic acid (111) to give intermediate **a** for intramolecular McMurry coupling reaction as shown in Scheme 19.



Scheme 19

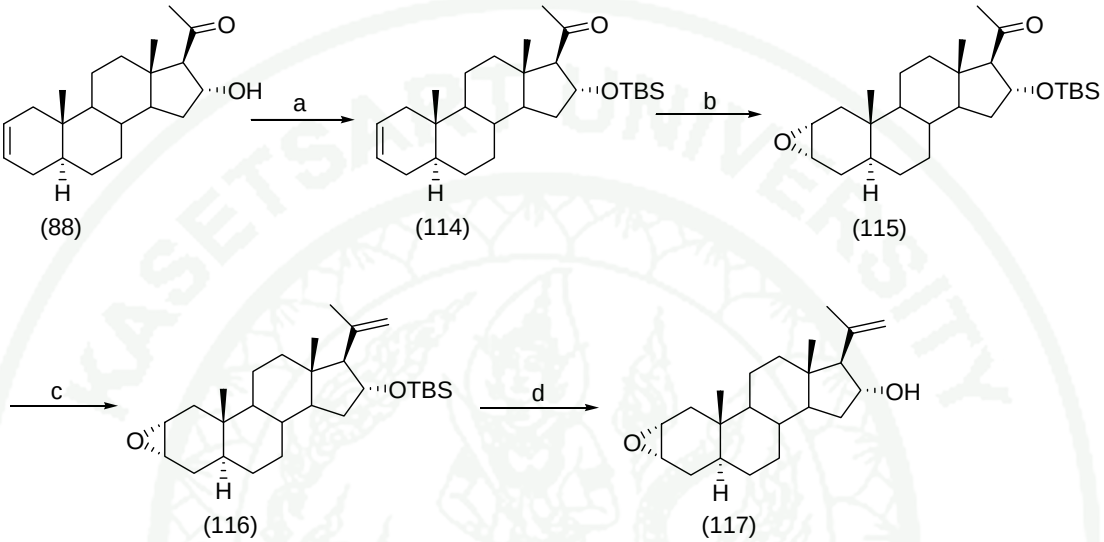
Reagents and conditions:

a) i) 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111), oxalyl chloride, DMF (1 drop), benzene, rt, 2.5h

ii) 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89) , DMAP, CH_2Cl_2, rt, 30 min, 77%

Preparation of 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117)

Conversion of 16α-hydroxy-5α-2-pregnen-20-one (88) to 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117) was shown in Scheme 20.



Scheme 20

Reagents and conditions:

a) TBSCl, imidazole, DMF, CH_2Cl_2, rt, 16h, 95%

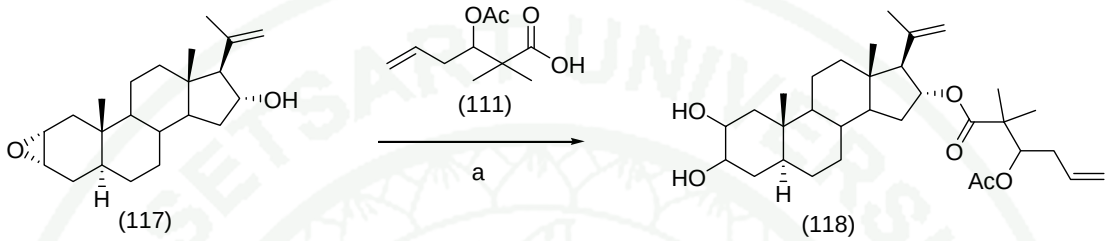
b) *m*CPBA, Na_2CO_3, CH_2Cl_2, H_2O, rt, 4h, 59%

c) $Ph_3P^+CH_3I^-$, *n*BuLi, THF, 0^oC-rt, 1.5h, 17% (brsm), 46% conversion

d) TBAF.H_2O, THF, rt, 48h, 32% (brsm), 70% conversion

Preparation of intermediate **b**

Steroid part, 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117), coupled with side chain moiety, 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111) to give **118** instead of intermediate **b** as presented in Scheme 21.



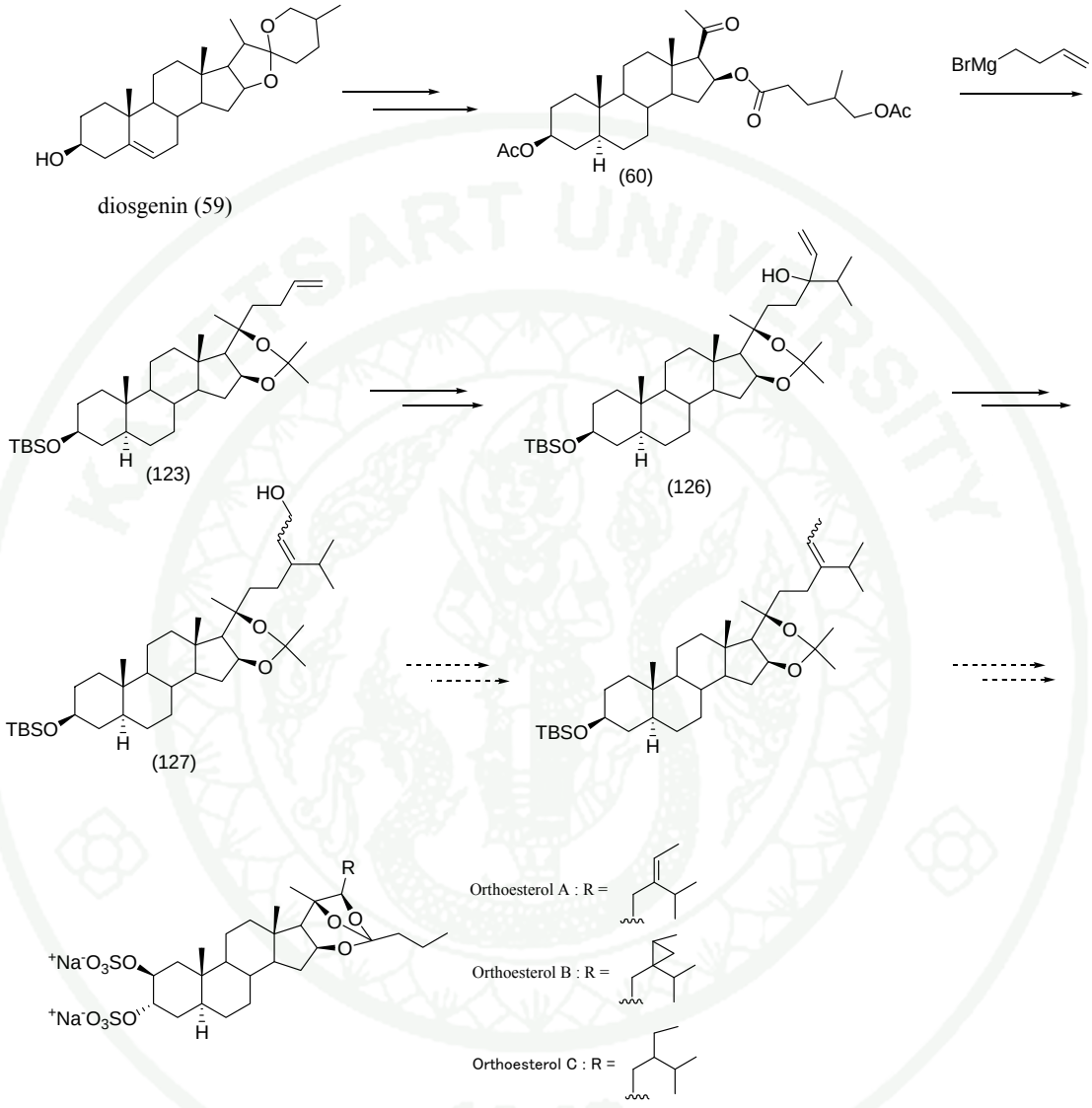
Scheme 21

Reagents and conditions:

a) i) 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111), oxalyl chloride, DMF (1 drop), benzene, rt, 2.5h

ii) 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117), DMAP, CH_2Cl_2, rt, 30 min, 16%

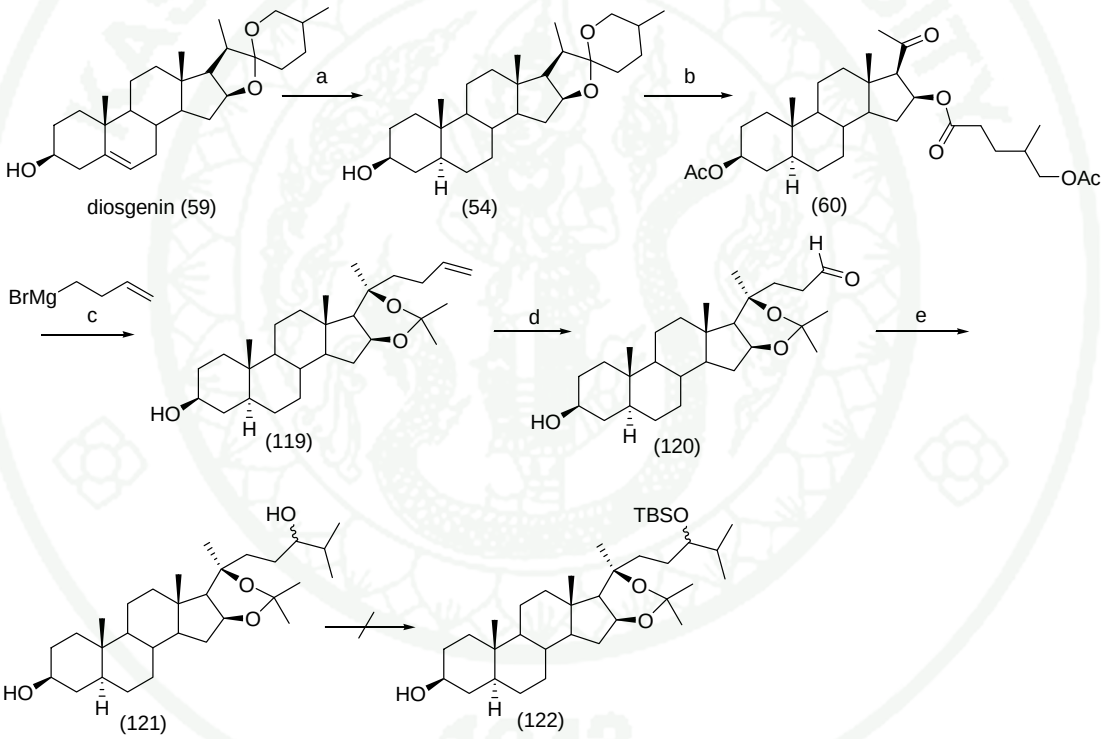
1.2 Synthesis of sterol orthoesters intermediates *via* intermolecular strategy (Scheme 22)



Scheme 22

Preparation of sterol orthoesters intermediates with intermolecular coupling reaction

Grignard reaction of 3β-acetoxy-16β-(5-acetoxy-4-methylpentanoate)-pregnan-20-one (60), synthesized from diosgenin (59), and butenyl magnesium bromide followed by 1, 3-diol protection to give 3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119) which was converted to 3β, 24-dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (121) in 2 steps and 3β-*tert*-butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127) in 5 steps as illustrated in Scheme 23 and 24, respectively.



Scheme 23

Reagents and conditions:

a) H_2, 5% Pd/C, THF, rt, 48h, 82%

b) i) Ac_2O, pyr, NH_4Cl, 135^oC, 16h

ii) CrO_3, AcOH, 1, 2-dichloroethane, H_2O, 0^oC-rt, 2h, 43% (2 steps)

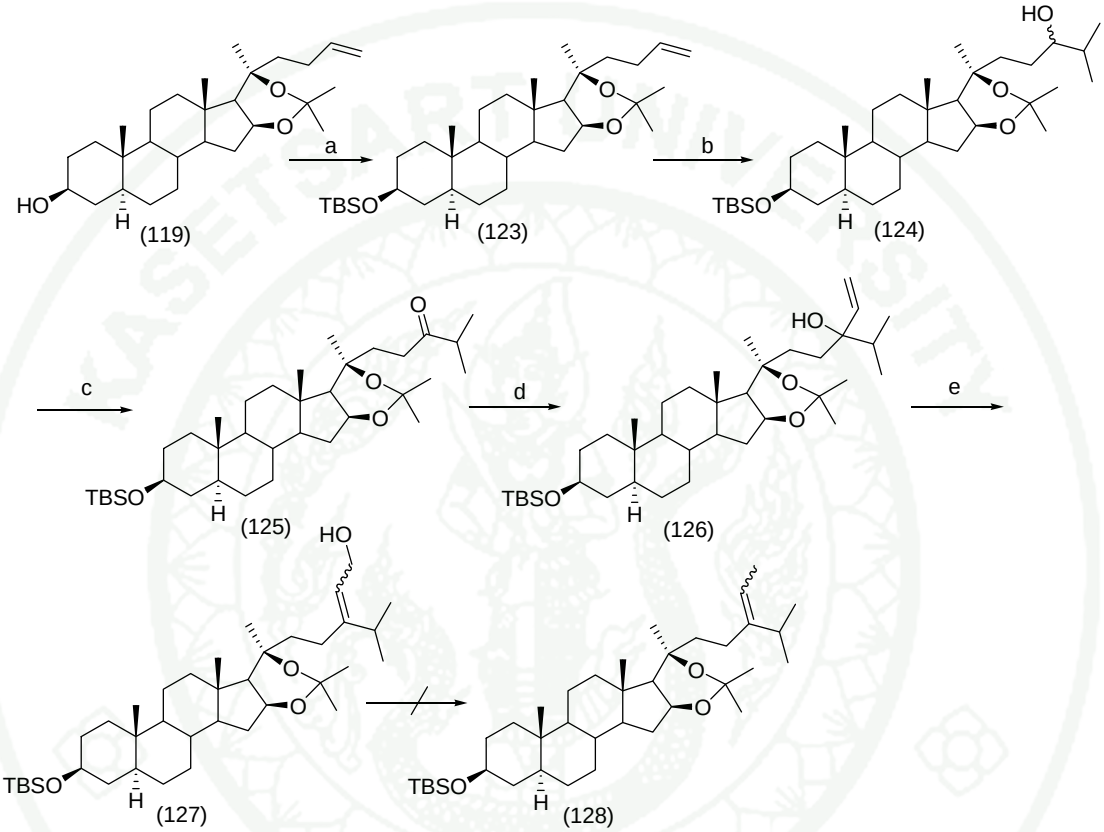
c) i) 4-bromobutene, Mg, I_2, THF, rt, 20 min

Reagents and conditions: (Continued)

ii) 2, 2-dimethoxypropane, *p*-toluenesulfonic acid, rt, 32% (2 steps)

d) O_3, CH_2Cl_2, PPh_3, -78°C, 3h, 65%

e) 2-bromopropane, Mg, I_2, THF, -78°C, 1.5h, 84%



Scheme 24

Reagents and conditions:

a) TBSOTf, 2, 6-lutidine, CH_2Cl_2, 0°C, 30 min, 91%

b) i) O_3, CH_2Cl_2, PPh_3, -78°C, 3h, 67%

ii) 2-bromopropane, Mg, I_2, THF, -78°C, 1.5h, 44%

c) PCC, pyr, CH_2Cl_2, rt, 2h, 90%

d) 1M vinyl magnesium bromide in THF, THF, reflux, 2h, 79%

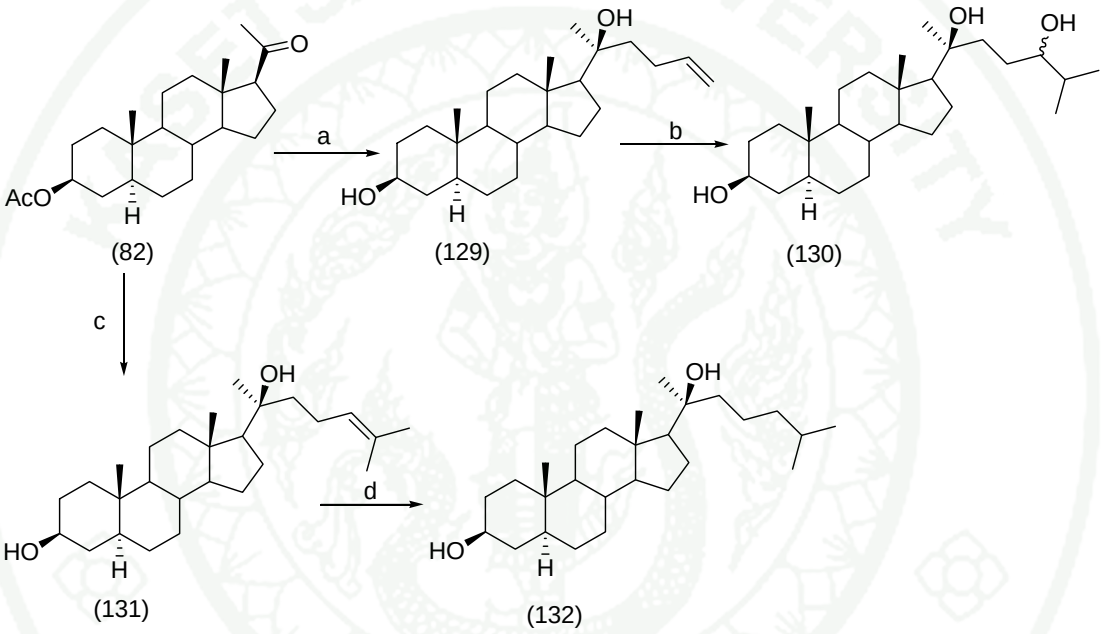
e) i) PCC, NaOAc, CH_2Cl_2, rt, 2h

ii) $NaBH_4$, ethanol, rt, 30 min, 25.6%

2. Synthesis of polyhydroxy sterols and their sulfated analogs

2.1 Synthesis of 3, 20-polyhydroxy sterols

Conversion of 3β-acetoxy-5α-pregnan-20-one (82) to 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130), 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131) and 3β, 20(*S*)-dihydroxy-5α-cholestane (132) was shown in Scheme 25.



Scheme 25

Reagents and conditions:

a) 4-bromobutene, Mg, I_2, THF, rt, 20 min, 61%

b) i) O_3, CH_2Cl_2, PPh_3, -78°C, 3h, 68%

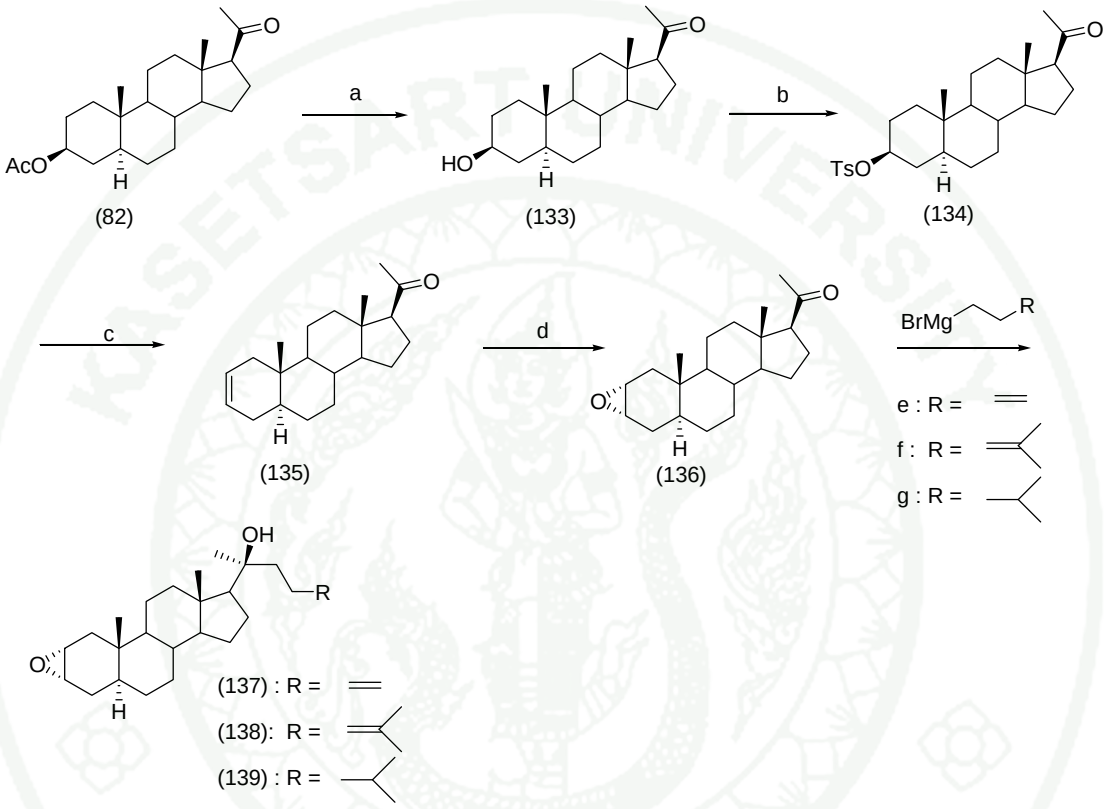
ii) 2-bromopropane, Mg, I_2, THF, -78°C, 1.5h, 75% (brsm), 61% conversion

c) 5-bromo-2-methyl-2-pentene, Mg, I_2, THF, rt, 5h, 36%

d) H_2, 5% Pd/C, methanol, rt, 2h, 64%

2.2 Synthesis of 2, 3, 20*S*-polyhydroxy sterols

Conversion of 3β-acetoxy-5α-pregnan-20-one (82) to 2, 3, 20(*S*), 24-tetrahydroxy-5α-cholestane was shown in Scheme 26 and 27.



Scheme 26

Reagents and conditions:

a) 1M KOH, MeOH, CH_2Cl_2 (1:1), rt, 6h, 90%

b) *p*-toluenesulfonyl chloride, CH_2Cl_2, pyr, rt, 24h, 81%

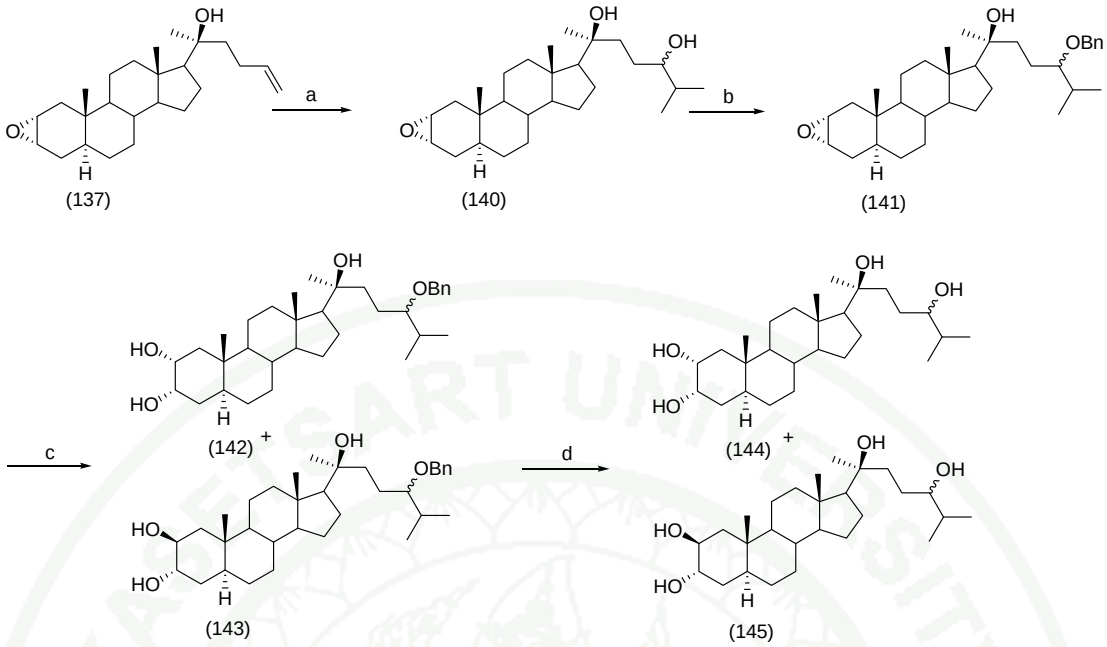
c) LiBr, Li_2CO_3, DMF, reflux, 6h, 86%

d) *m*CPBA, Na_2CO_3, CH_2Cl_2, H_2O, rt, 6h, 54% (brsm), 75% conversion

e) 4-bromobutene, Mg, I_2, THF, rt, 20 min, 50%

f) 5-bromo-2-methyl-2-pentene, Mg, I_2, THF, rt, 2h, 39%

g) 1-bromo-4-methylpentane, Mg, I_2, THF, rt, 2h, 50%



Scheme 27

Reagents and conditions:

a) i) O_3, $NaHCO_3$, CH_2Cl_2, PPh_3, -78^oC

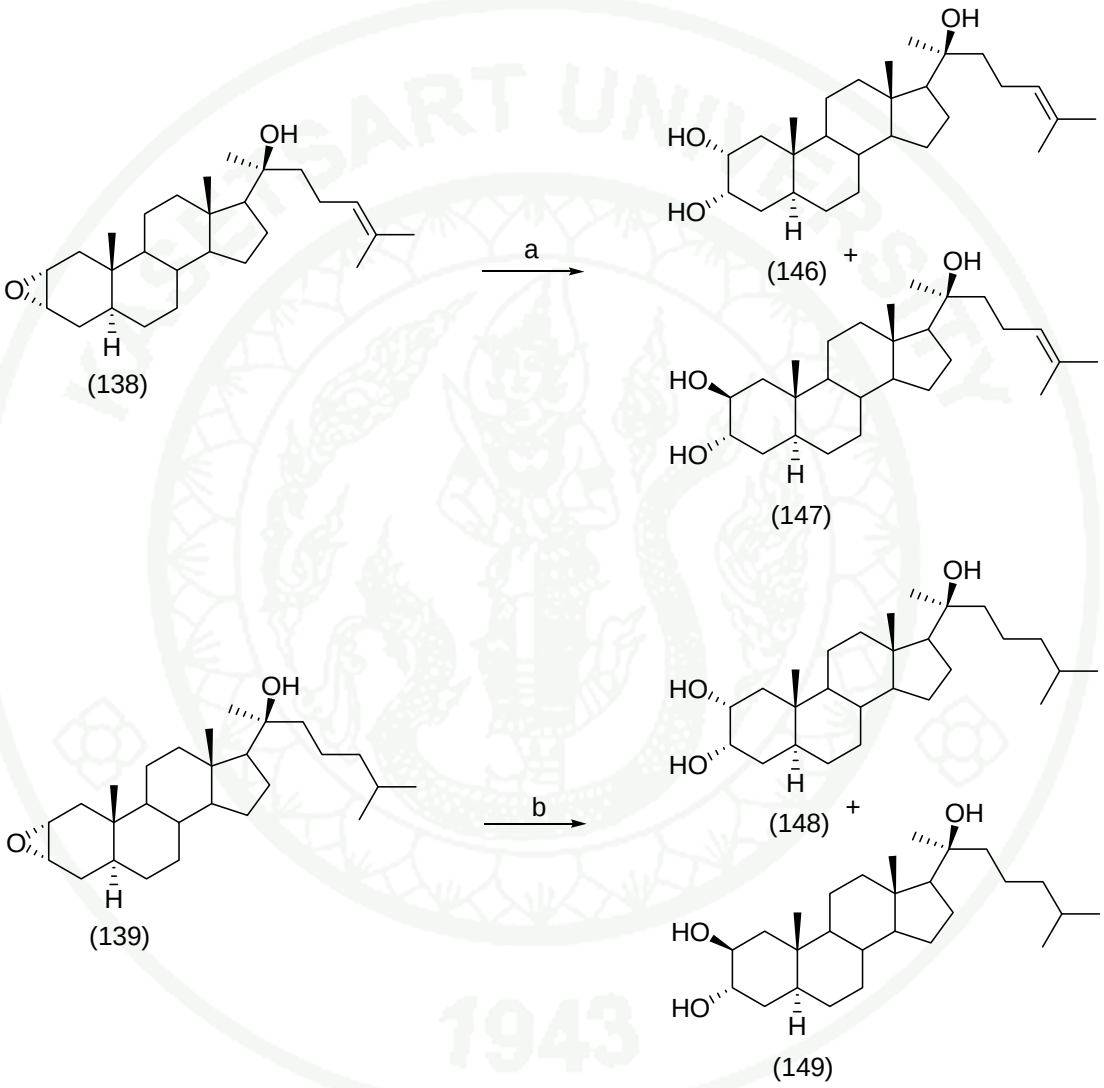
ii) 2-bromopropane, Mg, I_2, THF, -78^oC-rt, 3h, 48% (brsm), 74% conversion

b) BnBr, NaH, rt-reflux, 5h, 64%

c) 1M H_2SO_4, THF, rt, 24h, 57% (**142**:**143** = 1:15)

d) H_2, 5% Pd/C, MeOH, rt, 2h, 90% (**142** to **144**), 87% (**143** to **145**)

Hydrolysis of 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138) to 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147) and 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (139) to 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149) were shown in Scheme 28.



Scheme 28

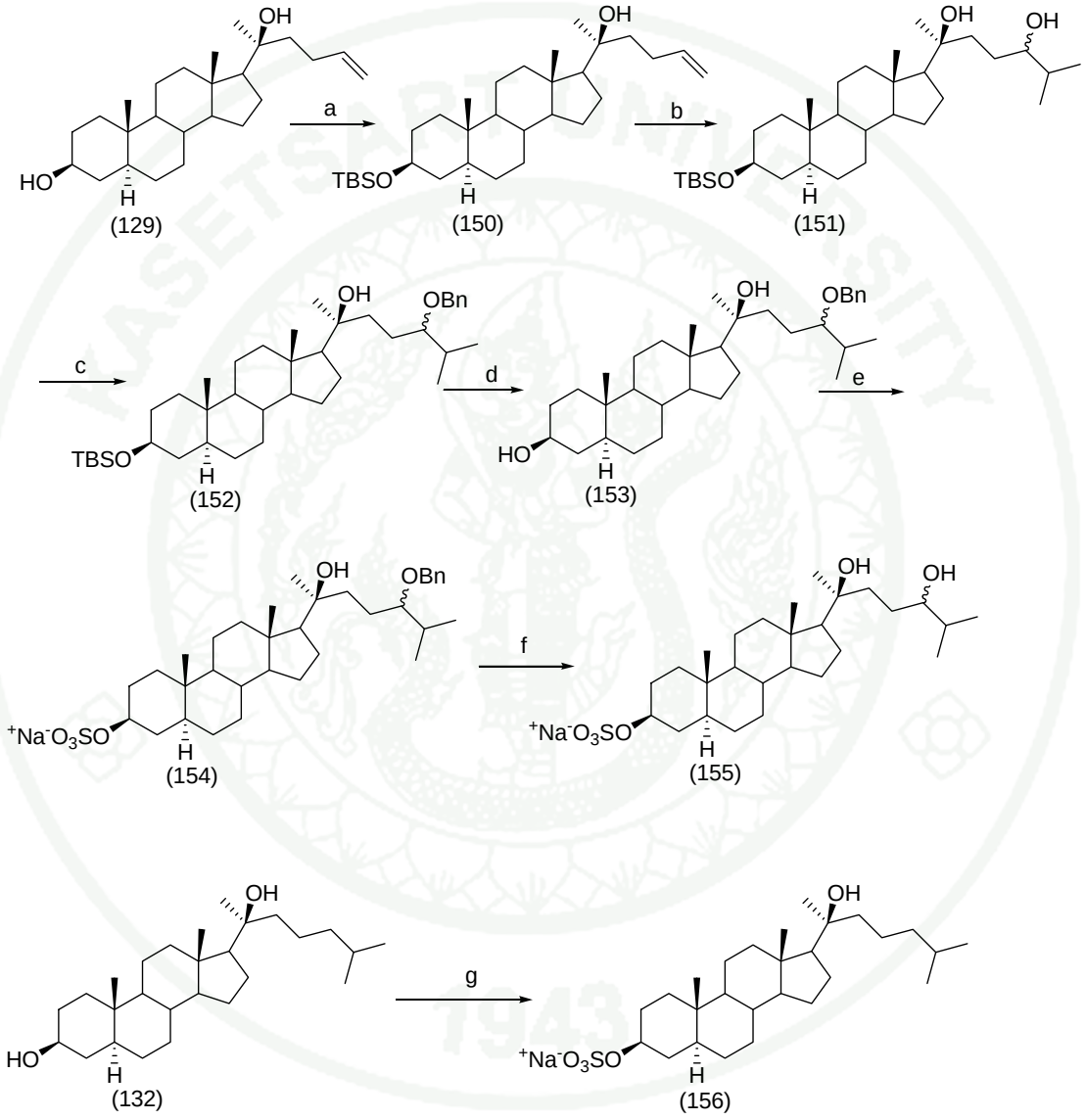
Reagents and conditions:

a) 1M H_2SO_4, THF, rt, 24h, 75.6% (**146**:**147** = 1:9)

b) 1M H_2SO_4, THF, rt, 24h, 62.3% (**148**:**149** = 1:5)

2.3 Synthesis of sulfated steroid analogs

Preparation of monosulfated steroid analogs **155** and **156** was shown in Scheme 29.



Scheme 29

Reagents and conditions:

a) TBSOTf, 2, 6-lutidine, CH_2Cl_2, 0°C, 30 min, 85%

b) i) O_3, $NaHCO_3$, CH_2Cl_2, PPh_3, -78°C, 68%

Reagents and conditions: (Continued)

ii) 2-bromopropane, Mg, I_2, THF, -78^{o}C-rt, 3.5h, 54% (brsm), 62% conversion

c) BnBr, NaH, THF, reflux, 4h, 75%

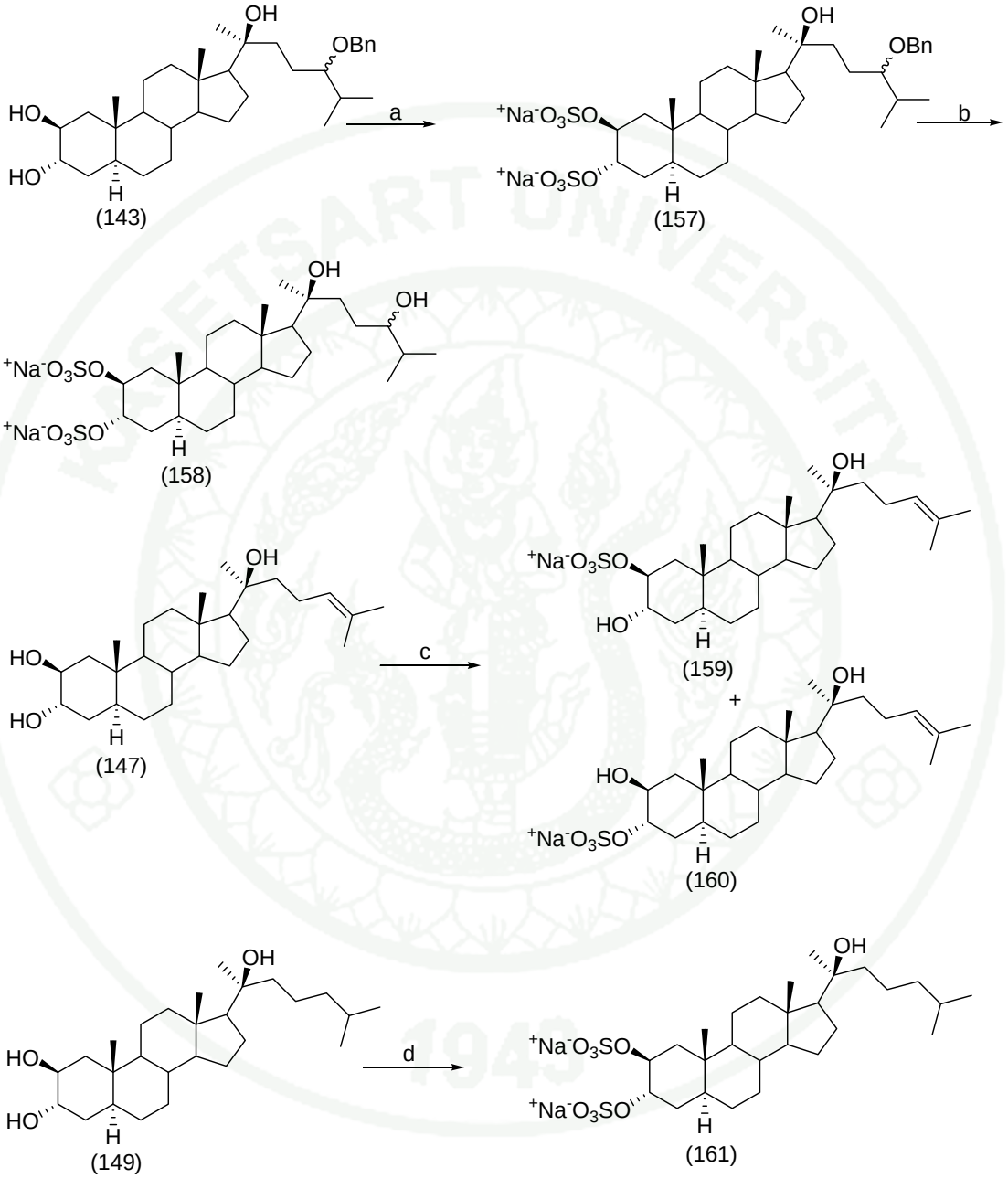
d) 1M of TBAF in THF, THF, reflux, 3h, 81.5%

e) $SO_3.NEt_3$, DMF, rt, 16h

f) H_2, 5% Pd/C, methanol, 2h, 42.5% (2 steps)

g) $SO_3.NEt_3$, DMF, rt, 24h, 61%

Preparation of disulfated steroid analogs **158** and **161** was shown in Scheme 30.



Scheme 30

Reagents and conditions:

a) $SO_3.NEt_3$, DMF, 95^{o}C, 16h

b) H_2, 5% Pd/C, methanol, 2h, 36% (2 steps)

Reagents and conditions: (Continued)

c) $SO_3.NEt_3$, DMF, 95°C, 16h, 43.5%

d) $SO_3.NEt_3$, DMF, 95°C, 16h, 46.1%

3. Evaluation of the anticancer and antiviral activities of synthetic polyhydroxy sterols and their sulfated analogs

3.1 Evaluation of the anticancer activity of synthetic polyhydroxy sterols and their sulfated analog (Table 9)

Table 9 Cytotoxicity of synthetic polyhydroxy sterols and their sulfated steroid against KB and NCI-H187

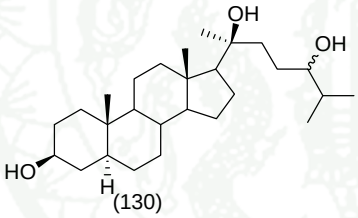
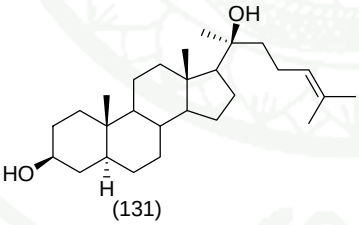
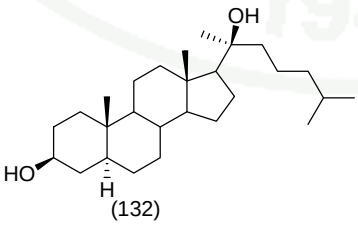
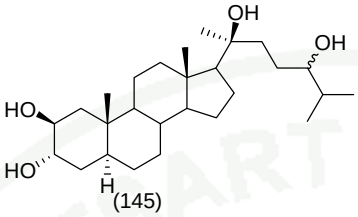
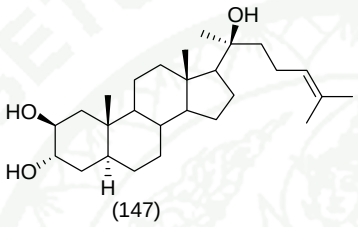
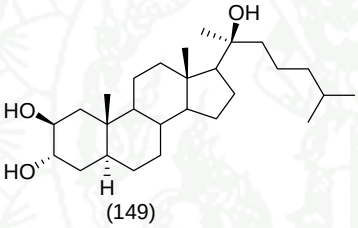
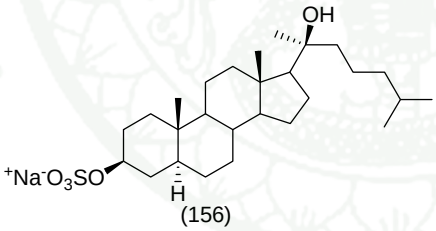
Compound	IC_{50} μg/ml[a]	
	NCI-H187	KB
 (130)	2.11	5.39
 (131)	4.24	39.12
 (132)	Inactive[b]	20.51

Table 9 (Continued)

Compound	IC_{50} µg/ml[a]	
	NCI-H187	KB
 (145)	Inactive[b]	Inactive[b]
 (147)	9.08	Inactive[b]
 (149)	9.59	10.14
 (156)	Inactive[b]	Inactive[b]
Ellipticine	0.57	0.393
Doxorubicine	0.43	0.16

NCI-H187, human small cell lung carcinoma; KB, human epidermoid carcinoma of cavity;

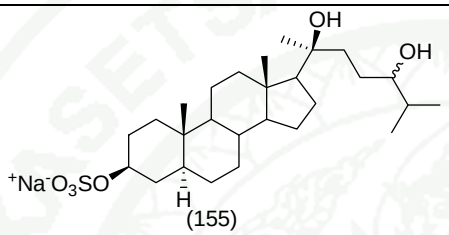
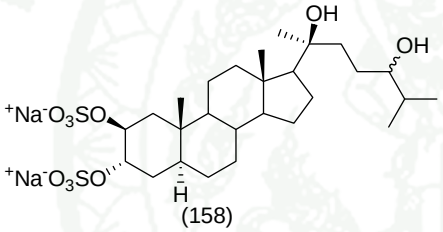
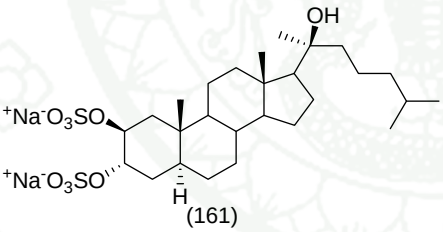
[a] ; Data are typical values from six replicate experiments

[b] ; Inactive = inhibition < 50%

[c] ; used as reference

3.2 Evaluation of the antiviral activity of sulfated steroids (Table 10)

Table 10 Antiviral HSV-1 activity and cytotoxicity against Vero cells of synthetic sulfated steroids

Compound	Anti-HSV-1 IC_{50} μg/ml[a]	Cytotoxicity against Vero cell[b]
 (155)	Inactive	Non-cytotoxic
 (158)	Inactive	Non-cytotoxic
 (161)	Inactive	Non-cytotoxic
Acyclovir[c]	1.5	-

Anti-HSV-1 (Herpes simplex virus type-1)

[a] ; Data are typical values from six replicate experiments

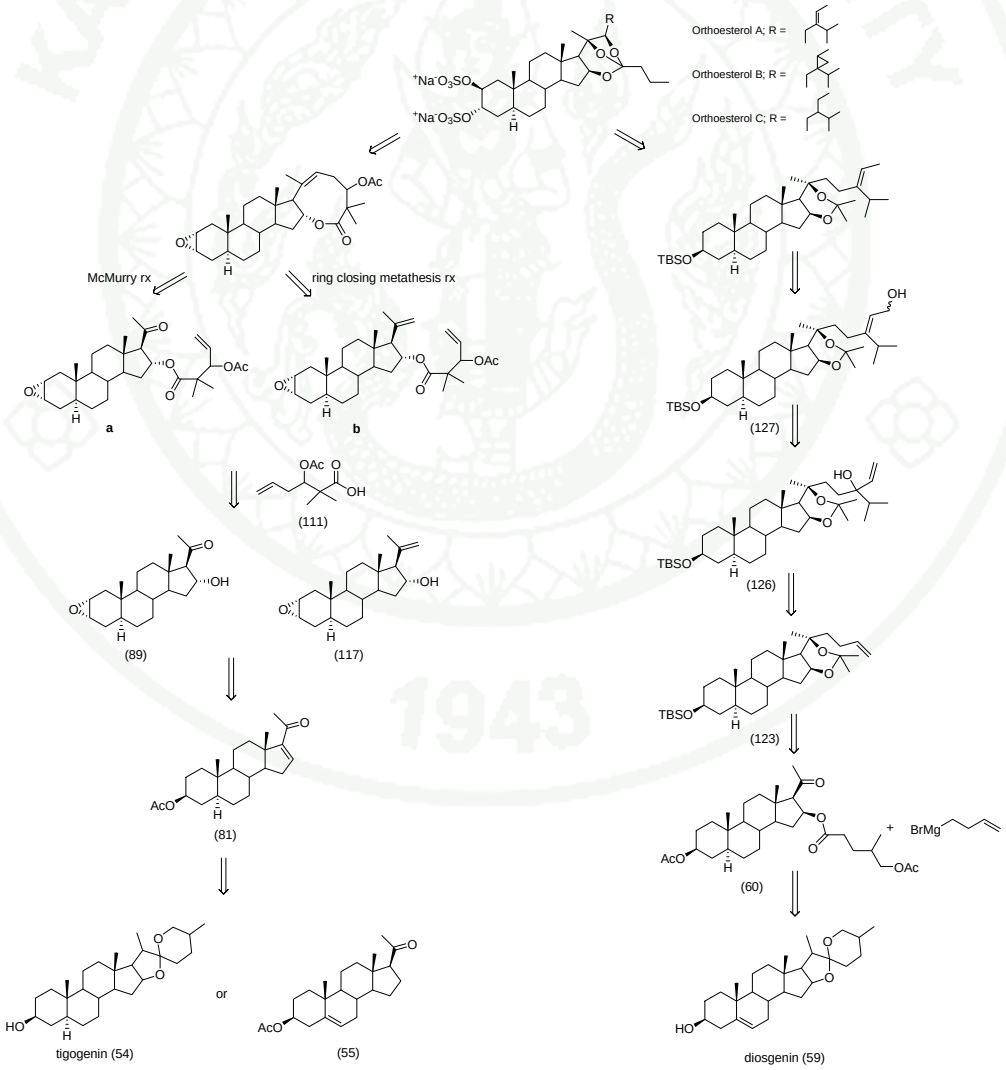
[b]; Cytotoxicity against Vero cells (African green monkey kidney)

[c]; used as reference

Discussion

Synthesis of sterol orthoesters intermediates

Herein we reported the synthesis of orthoesterol intermediates that were devided into two routes. The first route was performing key intermediates **a** and **b** to perform 9-membered lactone ring **58** for intramolecular strategy. The second route was intermolecular strategy to synthesize key intermediate **127** from Grignard reaction of steroid moiety (60) and Grignard reagent followed by side chain extension as shown in Scheme 31.



Scheme 31

Intramolecular strategy

The first route, both intermediate **a** for McMurry reaction and diene **b** for ring closing metathesis could be prepare from esterification of steroid (89) and steroid (117) with appropriate carboxylic acid (111). Tigogenin (54) and commercially available 20-keto steroid **55** were used as starting material.

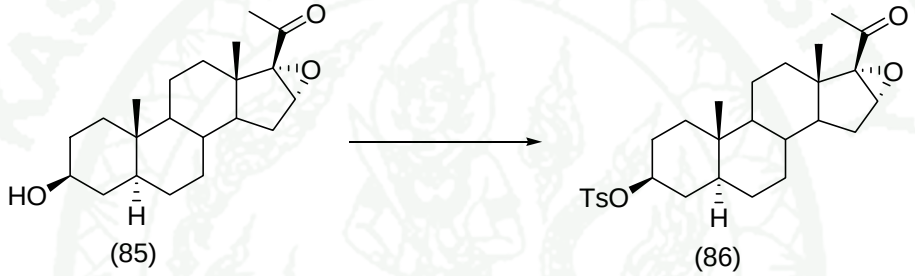
Synthesis of intermediate **a** and **b**

Preparation of key intermediate 16-hydroxy ketone **89**, we needed to perform α, β-unsaturted ketone **81** which could be synthesized from tigogenin (54) by the method described by Mićović *et al*. (1990). On the other hand, α, β-unsaturted ketone **81** could be obtained from commercially available steroid **55** in 4 steps sequences; hydrogenation using 5% Pd/C in methanol and ethyl acetate, transformation of ketone to enol acetate using acetic anhydride in the presence of *p*-toluenesulfonic acid under reflux, bromination and finally elimination with DBU in toluene under reflux. In the final step (Scheme 32), we found that mole amount of DBU and volume of solvent are significant to give a good yield. The suitable condition is 8 mole equivalent of DBU in 60 mL of toluene : 1 mmol of starting material and reflux for 8 h.

Scheme 32

Next steps were aimed at installing 16-hydroxy group from reductive cleavage of α, β-epoxy ketone and performing the diol group at ring A *via* opening epoxide ring that adapted the procedure reported by Garrido Santos *et al*. (2003) as shown in Scheme 15. Epoxidation reaction of **81** with H_2O_2 in the presence of NaOH in

methanol gave epoxide **85** in good yield**.** Under this condition acetate group at C-3 was hydrolyzed to give free hydroxyl group at C-3. Tosylation of **85** gave **86** which underwent elimination reaction to give **87**. In tosylation reaction (Scheme 33) with *p*-toluenesulfonyl chloride, dimethylaminopyridine and pyridine was not complete conversion although we tried to optimize the condition with adding more of *p*-toluenesulfonyl chloride and pyridine, leaving reaction longer. It was found that tosylation was complete conversion within 24 h when *p*-toluenesulfonyl chloride was dissolved in dichloromethane and pyridine before adding to a solution of **85** in a mixture of dichloromethane and pyridine.



Scheme 33

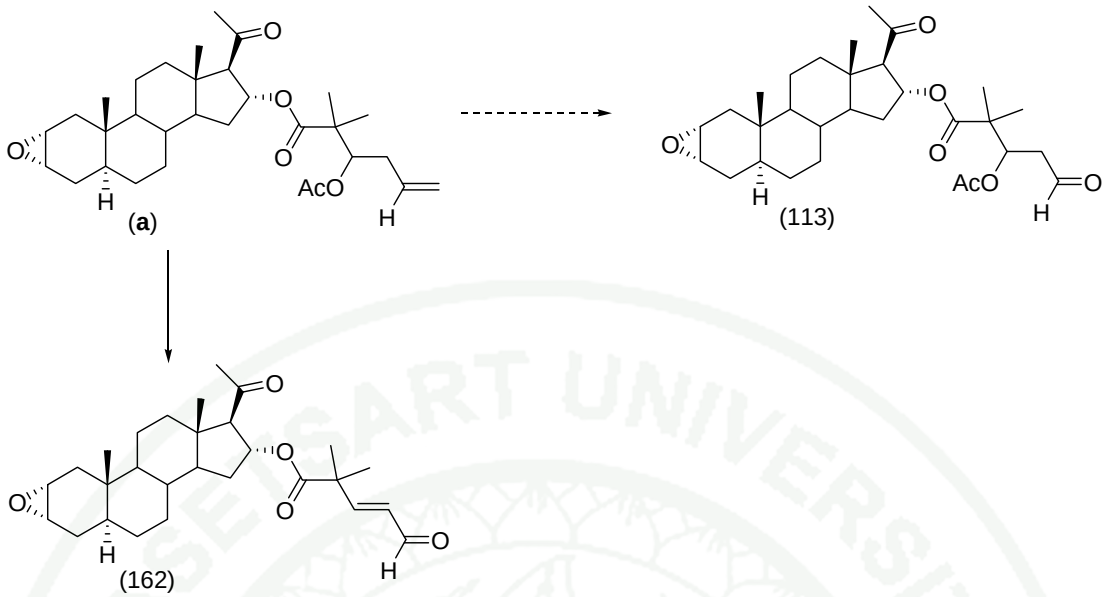
Elimination occured at C-2 and C-3 by comparison ^{1}H NMR spectra of **87** at both positions with the procedure reported by Garrido Santos *et al*. (2003). Reductive ring opening of α, β-epoxy ketone **87** was successfully performed in moderate yield by using hydrazine monohydrate in ethanol. Moreover, reduction with lithium naphthalenide and samarium iodide were also investigated for this reaction and the results were shown in Table 8. Although both conditions gave a satisfy yield and slightly better but both reagents have to generate *in situ* from lithium and samarium metals and they are very sensitive to oxygen and moisture.

Preparation of carboxylic side chain **111** for condensation with 16α-hydroxy steroid **89**

First method involved Blaise reaction of hydroxypropionitrile **90**, protected hydroxy propionitrile as benzyl **91**, acetoxy **95** and protected aldehyde propionitrile **103** with ethyl 2-bromo-2, 2-dimethylacetate (92) under sonication followed by acidic hydrolysis led to 1, 3-keto ester as shown in Scheme 16 and 17. Protection of ketone of 1, 3-keto ester derivatives was necessary because after hydrolysis of ester to carboxylic acid, 1, 3-keto acid could easily undergo decarboxylation. Although the attempts to protect this ketone as cyclic ketal and cyclic thiol ketal were investigated, the reaction could not proceed probably due to the sterric hindrance from geminal dimethyl group. Finally, this problem was solved by reduction of ketone to alcohol followed by silylation. Unfortunately, final step to hydrolysis ethyl ester to carboxylic acid was failure. We turned the attention to prepare with another route using inexpensive compound and short synthesis as shown in Scheme 18. The problem in this route was only the first step to generate aldehyde using PCC or IBX. It was found that aldehyde intermediate was unstable in purification step and obtained along with side products as complex mixture.

Condensation between steroid **89** and carboxylic acid side chain **111** was successful by generation of active species, acid chloride from **111** using oxalyl chloride in the presence of *N,N*-dimethylformamide, slightly excess mole equivalent of DMAP base on mole amount of acid side chain **111** to prevent the acidic condition that might lead to obtain elimination product.

However, attempts to oxidize the terminal olefin of steroid **a** with ozonolysis could not provide the expected **113**, only the by-product **162** was observed (Scheme 34).

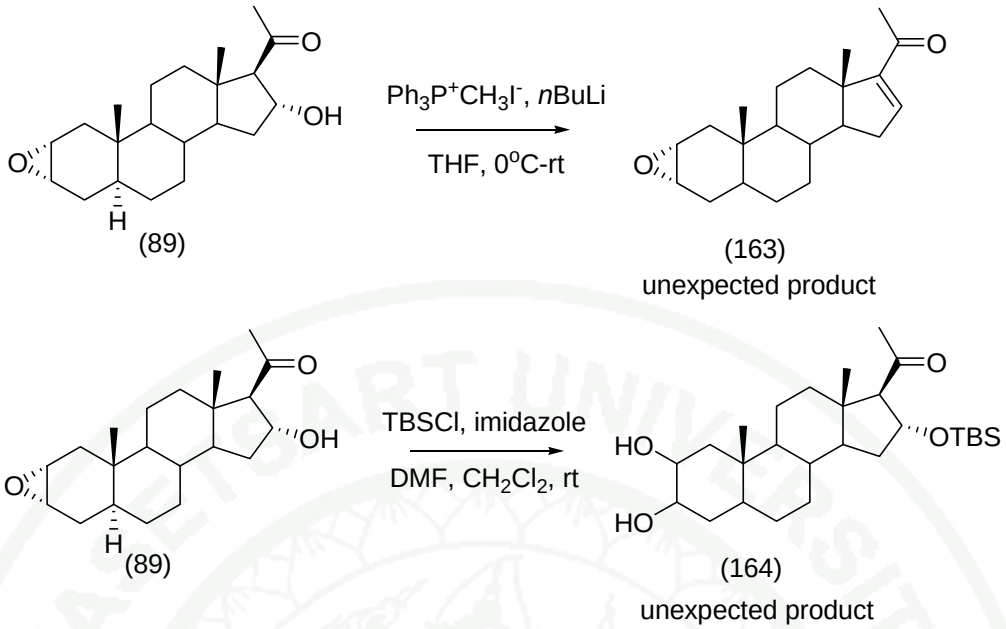


Scheme 34

After we could not synthesize **113** for McMurry reaction, we hoped to utilize ring closing metathesis to form 9-membered ring *via* diene **b** which could be prepared from condensation between steroid **117** and carboxylic acid side chain **111**.

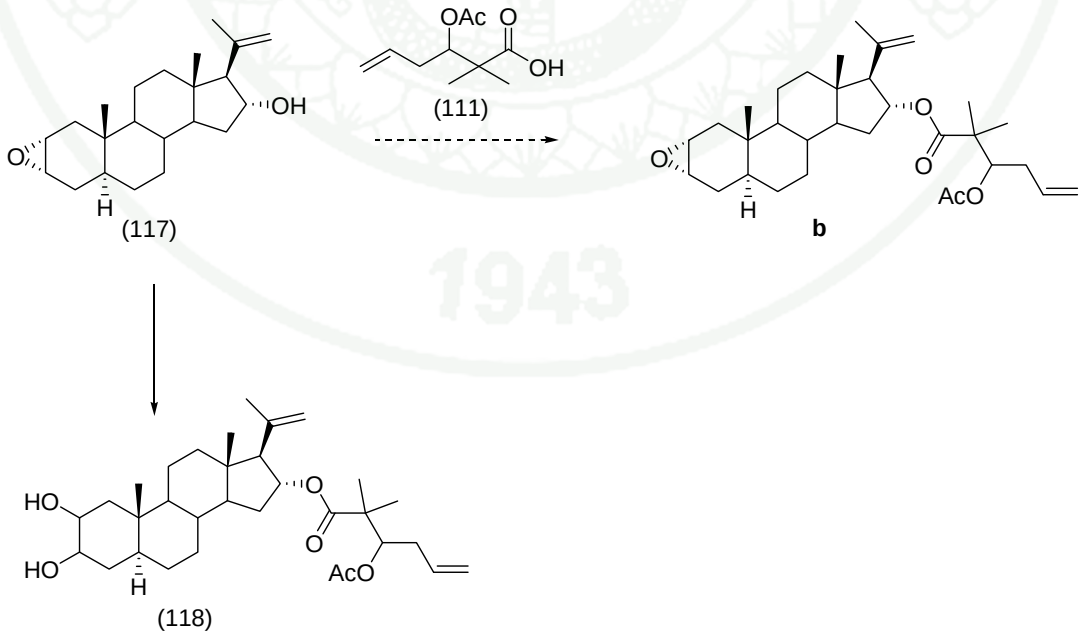
Preparation of 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-pregn-20-ene (117)

Firstly, steroid **89** underwent wittig reaction using $Ph_3P^+CH_3I^-$ and *n*BuLi in THF without protection of 16α-hydroxyl group to provide only unexpected the elimination product **163** instead of the desired olefinic steroid **117**. From this result, protection of 16α-hydroxyl group was necessary but silylation of steroid **89** gave unexpected diol **164** (Scheme 35). We needed to use steroid **88** for preparation of appropriate steroid **117** as shown in Scheme 20. Compound **115** underwent wittig reaction to provide desired product **116** in trace amount which was desilylated with TBAF.H_2O to give the corresponding 16α-hydroxy olefinic steroid **117**.



Scheme 35

Unfortunately condensation between steroid **117** and carboxylic acid side chain **111** by generation of active species, acid chloride from **111** to give unexpected **118** instead of intermediate **b** as shown in Scheme 36.

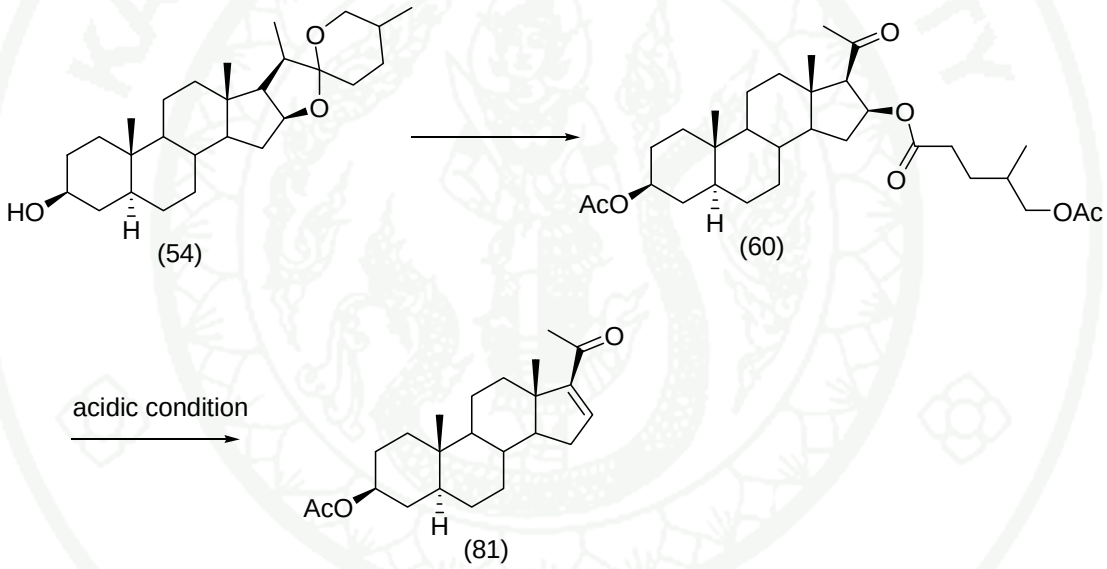


Scheme 36

Intermolecular strategy

Initially, we aimed at performing diol at C-2 (β) and C-3 (α) in ring A before completing side chain by selective protection at C-24 of steroid **121**.

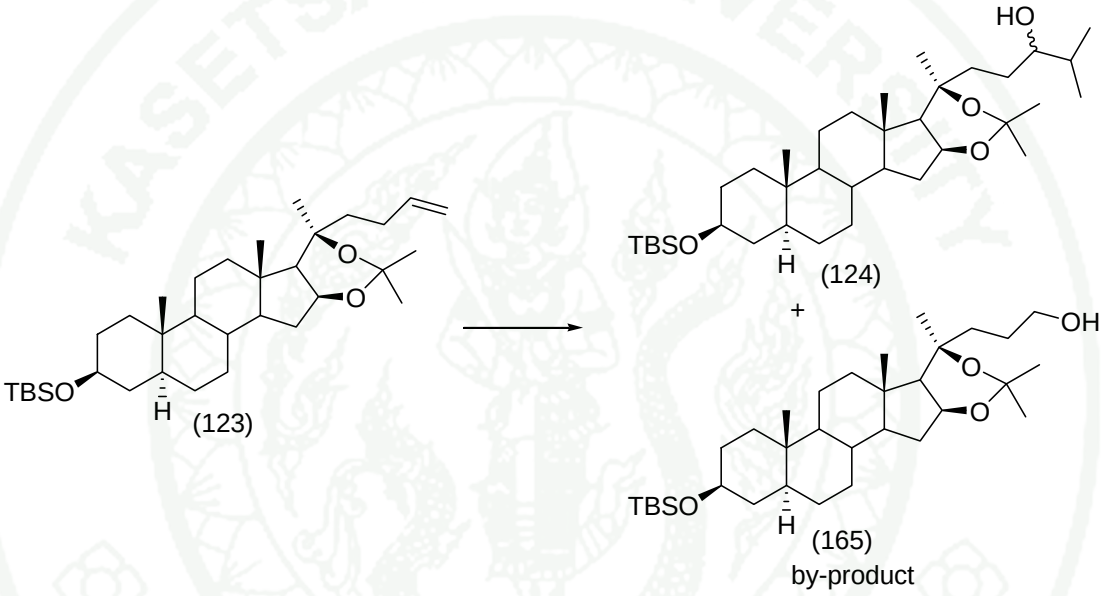
Preparation of 20-keto ester **60** had been accomplished from spiro ring degradation of tigogenin (54), synthesized from hydrogenation of diosgenin (59). It appeared that in work up step was crucial to avoid side product **81** from elimination reaction (Scheme 37) by neutralization with saturated sodium hydrogen carbonate until litmus testing was slightly basic (pH 7-8).



Scheme 37

Grignard reaction of compound **60** and butenyl magnesium bromide provided triol intermediate which was protected as cyclic ketal with 2, 2-dimethoxypropane in the presence of *p*-toluenesulfonic acid. Side chain extension was achieved *via* two steps sequences; ozonolysis of compound **119** followed by nucleophilic addition to provide compound **121**. However, attempts to protect selectively only at C-24 of **121** with *tert*-butyldimethylsilyl chloride could not confirm the expected silylether **122** because appearance products were obtained as very trace amount which could not further continue to prove the selectivity.

Since unexpected results were obsevered, we turned our attention to perform side chain firstly. Silylation of **119** at C-3 with *tert*-butyldimethylsilyltrifluoromethanesulfonate in the presence of 2, 6-lutidine gave silylether **123** which underwent ozonolysis followed by nucleophilic addition with Grignard reagent to provide 24-hydroxyl cholesterol like side chain **124**. Whereas the problems at the second step have been observed as by-product occurring **165** and not completing conversion (Scheme 38).

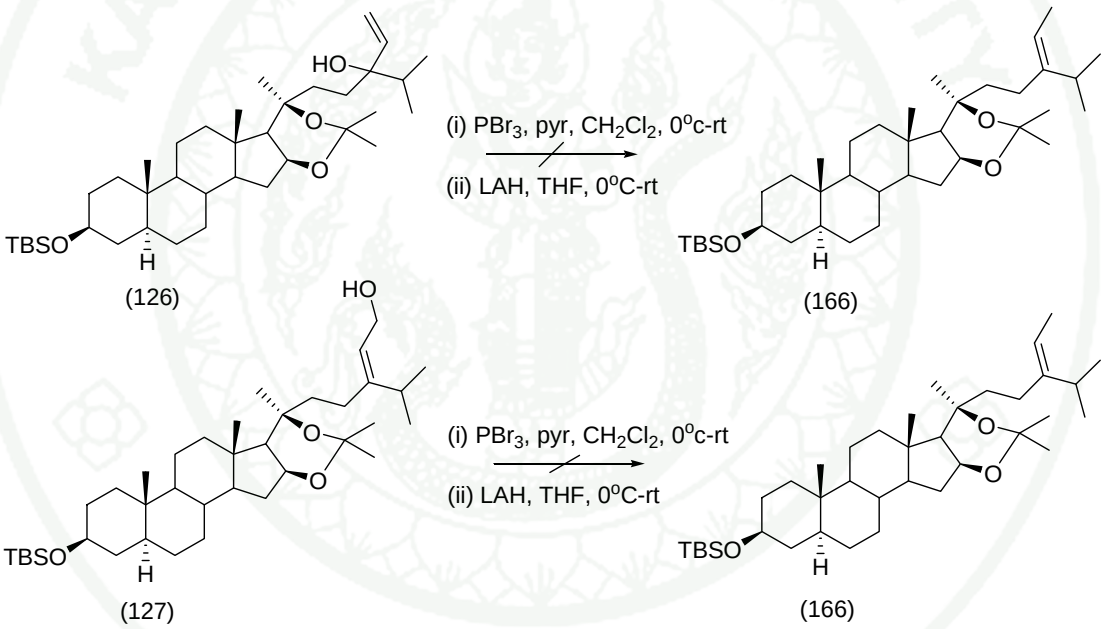


Scheme 38

After the problems have been considered, it was found that reaction temperature and rate of addition of aldehyde intermediate to generated Grignard reagent were crucial to solve the problems. This Grignard reaction should occur at low temperature (-78^{o}C) and slowly warm to room temperature and should control rate of addition to be dropwise. Oxidation of secondary alcohol of **124** with PCC in dichloromethane in the presence of NaOAc or pyridine was achieved in good yield. Nucleophilic addition of vinyl magnesium bromide to ketone **125** under refluxing provided tertiary alcohol **126** which was rearranged to allylic alcohol **127** in 2 steps; oxidation with PCC to α, β-unsaturated aldehyde intermediate and reduction of this

aldehyde with $NaBH_4$ to allylic alcohol. Many side products were observed in oxidation reaction of **129** with PCC to result in low yield of desired aldehyde product.

With tertiary alcohol **126** and allylic alcohol **127** in hand, deoxygenation of both would lead to sterol orthoester B side chain. However, treatment of **126** with phosphorus tribromide in the presence of pyridine in dichloromethane did not provide desired product **166** whereas only starting material was observed. Bromination of **127** with phosphorus tribromide in the presence of pyridine in dichloromethane followed by reduction using lithium aluminium hydride to provide the unstable product which decomposed after purification (Scheme 39).



Scheme 39

Synthesis of polyhydroxy sterols and sulfate analogs

Six new synthetic polyoxygenated steroids, 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130), 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131) and 3β, 20(*S*)-dihydroxy-5α-cholestane (132), 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane (145), 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147) and 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149), have been synthesized starting from commercially available 3β-acetoxy-5-pregnen-20-one (55).

Compound **129** was chosen to prove the configuration at C-20 by nuclear overhauser experiment (NOE) measurement. The *S* configuration at C-20 was indicated by NOE enhancement between H-12*eq* and Me-21.

For synthesis of 2, 3, 20-polyhydroxy sterols, compound **138**, **139** and **141** underwent epoxide ring opening under acidic condition to always provide two isomers. The stereochemistry of both at CH-2 and CH-3 was indicated by comparison of ^{1}H NMR (Cruz Silva *et al*., 2005). The desired isomer was obtained as a major product while the ratio of both was not reproducible.

Sulfation was performed using triethylamine-sulfur trioxide complex as the sulfating agent in *N,N*-dimethylformamide. Benzylation of hydroxyl group at C-24 was chosen as protecting group because the crude product after sulfation could dissolve only in ethanol or methanol and Pd/C was easy to remove without extraction and separation steps. Whereas purification of synthetic sulfated steroids was not successful. Unexpected peaks of proton signal were observed at δ 3.21 as triplet and 1.21 as quartet although purification was repeated by flash column chromatography and first eluted with dichloromethane before using solvent system as a mixture of methanol and dichloromethane in ratio 3:97 to elute target sulfated product. However, expected pattern could be found particularly proton signal at δ 4.20-4.30 for monosulfate and δ 4.60-4.70 for disulfate that indicated sulfation was performed.

Biological activity testing

Comparison on cytotoxicity of compounds which have the different functional groups on the cholesterol like side chain in the presence of hydroxyl group at C-3 (β) showed that compound **130** bearing the hydroxyl group at C-24 showed strong activities against all tested cells [KB (IC_{50} = 5.39 μg/ml) and NCI-H187 (IC_{50} = 2.11 μg/ml)] whereas compound **131** containing double bond at C-24 on side chain showed very potent cytotoxicity against NCI-H187 (IC_{50} = 4.24 μg/ml), low against KB (IC_{50} = 39.12 μg/ml) and compound **132** that has no functional group on side chain is inactive against NCI-H187 but moderate against KB (IC_{50} = 20.51 μg/ml).

Surprisingly, it was found that steroids which contain dihydroxy at C-2 (β) and C-3 (α) showed the different results which are i) compound **145** bearing the hydroxyl group at C-24 showed no cytotoxicity in all tested cells, ii) compound **147** containing double bond at C-24 on side chain showed decreasing the activity in all tested cells with no activity against KB and lower for NCI (IC_{50} = 9.08 μg/ml) and iii) compound **149** that has no functional group on side chain exhibited strong activity both of KB and NCI cell lines with IC_{50} = 10.14 and 9.59 μg/ml respectively.

Sulfated steroid **156**, having sulfate group at C-3 and no functional group on side chain, showed no cytotoxicity in all tested cells (Table 9).

Three synthetic sulfated steroids, **155**, **158** and **161** were tested for anti-viral activity HSV-1 (herpes simplex virus type-1). From the results (Table 10), all of them were inactive for HSV-1.

CONCLUSION

Sterol orthoesters which are marine natural products, isolated from the Caribbean sponge *Petrosia weinbergi*, exhibited *in vitro* activity against the feline leukemia virus (FeL V), mouse influenza virus (PR8) and mouse corona virus (A59). Recently, there has been no report on the synthesis of sterol orthoesters and their intermediates, therefore synthesis of their intermediates has been explored by us.

Our strategies to synthesize sterol orthoesters were devided into two routes, i) intramolecular coupling reaction using McMurry reaction or ring closing metathesis as key steps and ii) intermolecular coupling reaction using Grignard reaction as a key step.

First route, two key intermediates, 2α, 3α-epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (**a**) for McMurry reaction and 16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-pregn-20-en (118) for ring closing metathesis have been successfully synthesized in 10 and 13 steps with 11.9% and 0.12% overall yield, respectively by using tigogenin (54) which could be obtained from waste of *Agave sisalana* leaves or commercially available 3β-acetoxy-5-pregnen-20-one (55) as starting material.

2α, 3α-Epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (**a**), obtained from esterification of 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89) and 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111), would be further oxidized to prepare dicarbonyl intermediate for McMurry reaction and 16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-pregn-20-ene (118), synthesized from esterification of 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-20-pregnene (117) and 3-acetoxy-2,2-dimethyl-5-hexenoic acid (111), would be further cyclized *via* ring closing metathesis to form 9-membered lactone ring.

On the other hand, synthesis of 3β-tert-butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127) has been accomplished *via*

intermolecular strategy, Grignard reaction as the key step, using cheaply available diosgenin (59) as a starting material in 8 steps with 0.55% overall yield. Compound **127** can be used as a key intermediate for the synthesis of sterol orthoesters.

Furthermore, we were interested in the synthesis of new polyhydroxy sterols by various functionalities in ring A as monohydroxyl group at C-3 (β) or dihydroxyl group at C-2 (β) and C-3 (α) and differ on side chain and their sulfated analogs for studying the effect on cytotoxicity against two cancer cell lines, human epidermoid carcinoma (KB) and human small cell lung carcinoma (NCI-H187).

Six new polyhydroxy sterols, 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130), 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131), 3β, 20(*S*)-dihydroxy-5α-cholestane (132), 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane (145), 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147) and 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149) have been synthesized by using Grignard reaction as a key step. The results of their anticancer activity showed that most of synthetic polyhydroxy sterols exhibited biological activities against both KB and NCI-H187 cell lines. Especially, 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130) bearing trihydroxyl group at C-3, C-20 and C-24 showed the strongest activity against both cell lines (IC_{50} (μg/mL) = 5.39 (for KB) and 2.11 (for NCI-H187) whereas 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane (145) containing extra hydroxyl group at C-2 in ring A was inactive against both cell lines. Moreover, their sulfated analogs have been synthesized and evaluated for their antiviral activity against herpes simplex virus type-1 but both monosulfated **155** and disulfated **158** and **161** showed no activity against HSV-1.

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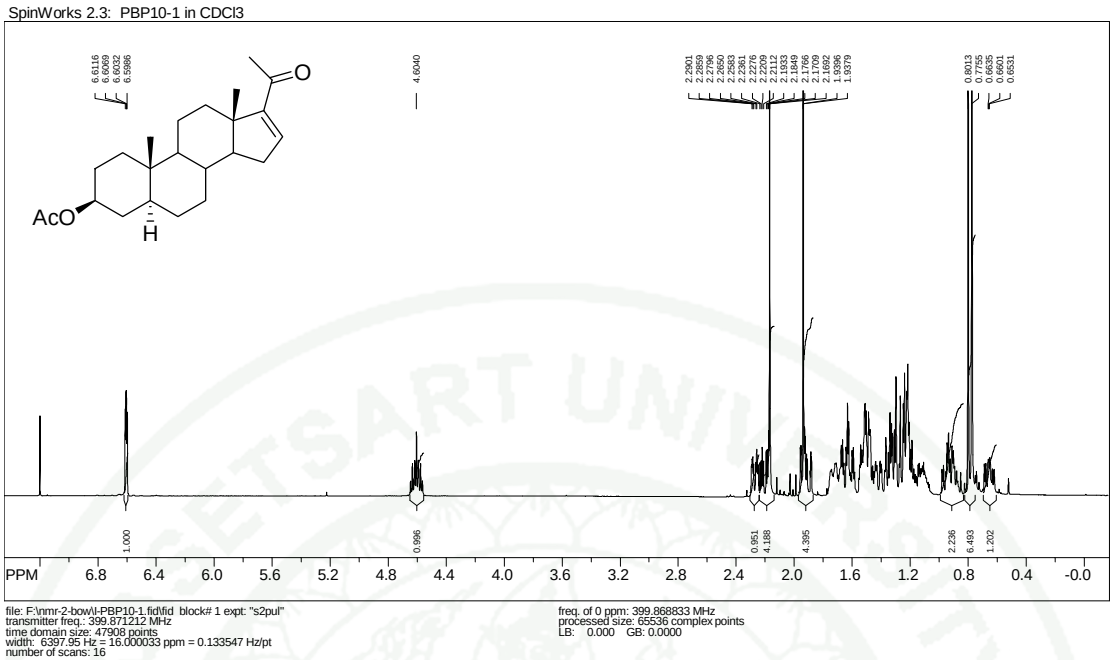
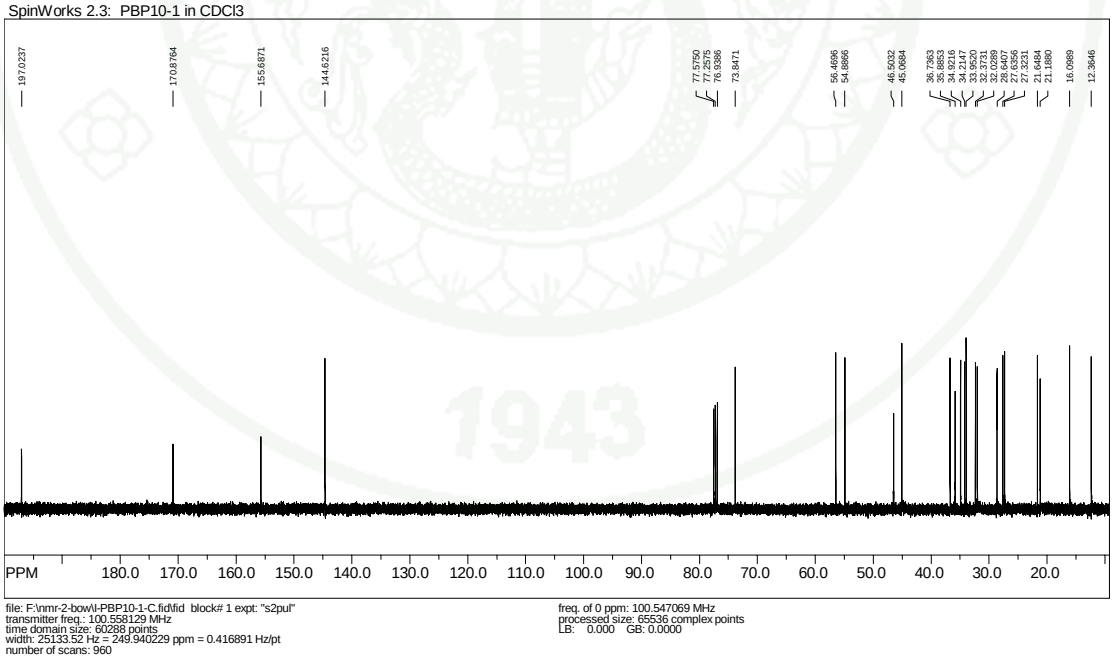
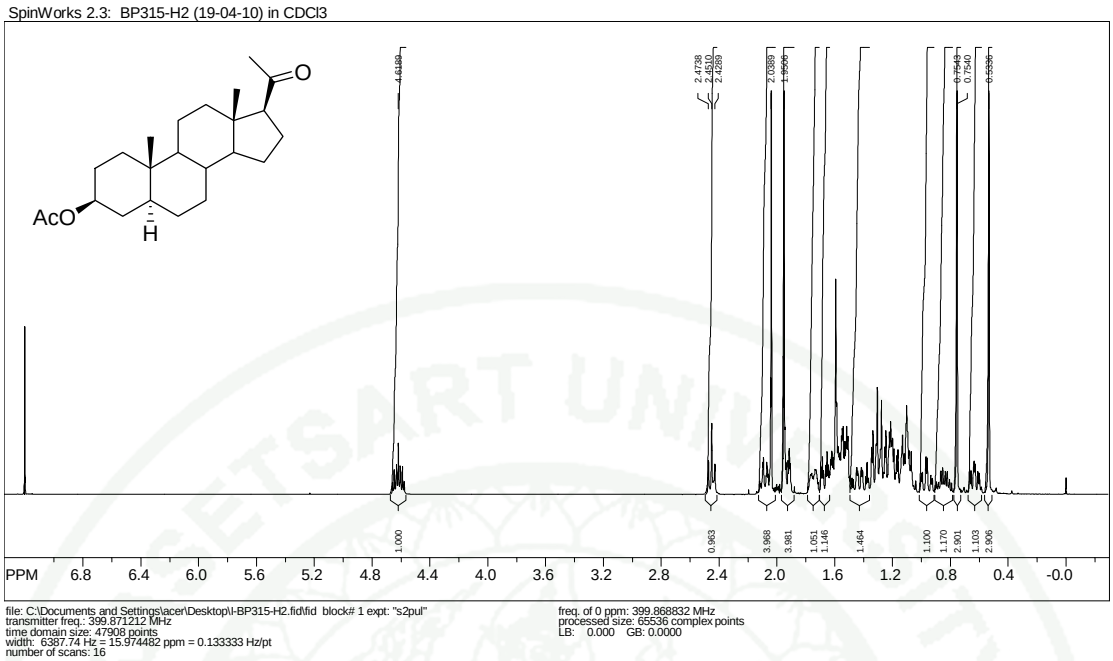
APPENDIX



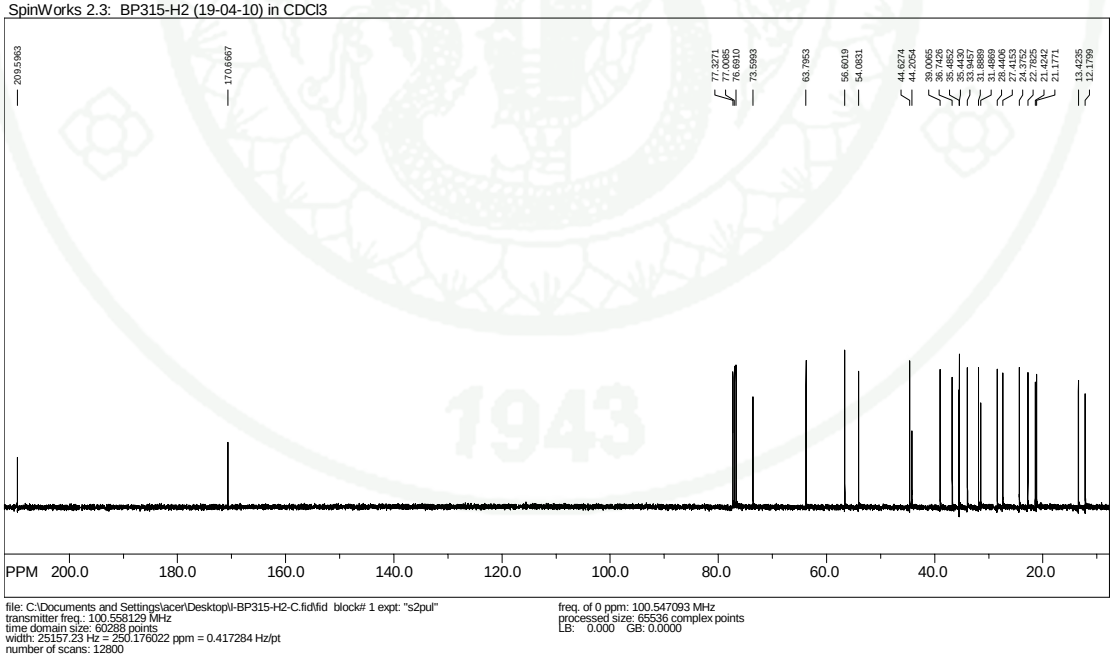
Appendix Figure 1 400 MHz ^{1}H NMR spectrum: 3β-acetoxy-5α-16-pregnen-20-one (81)



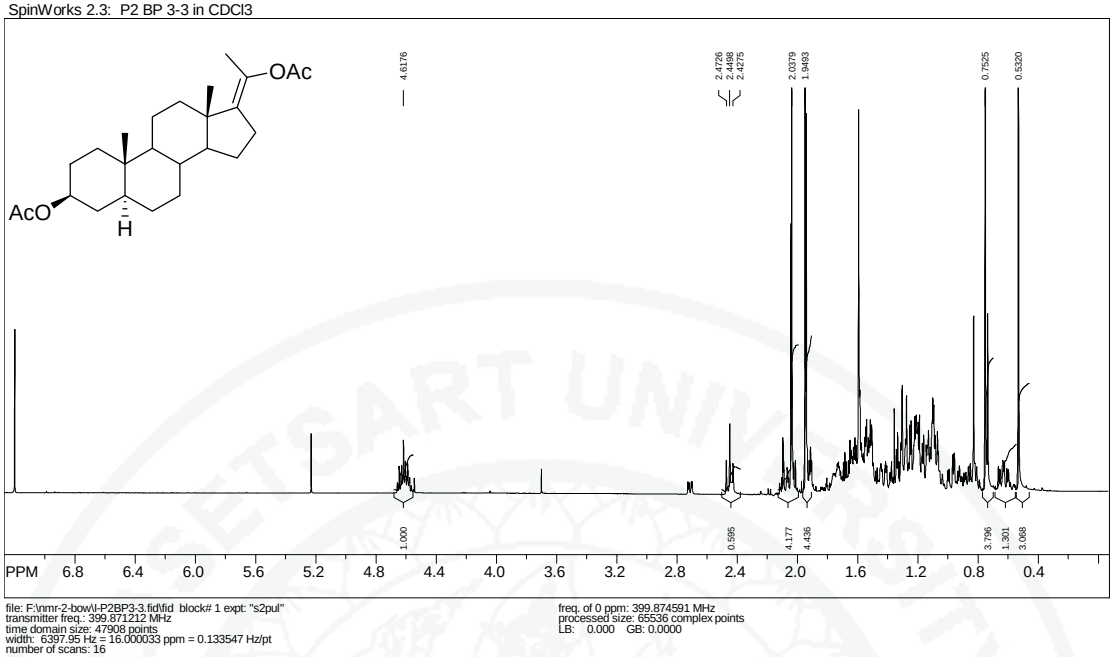
Appendix Figure 2 100 MHz ^{13}C NMR spectrum: 3β-acetoxy-5α-16-pregnen-20-one (81)



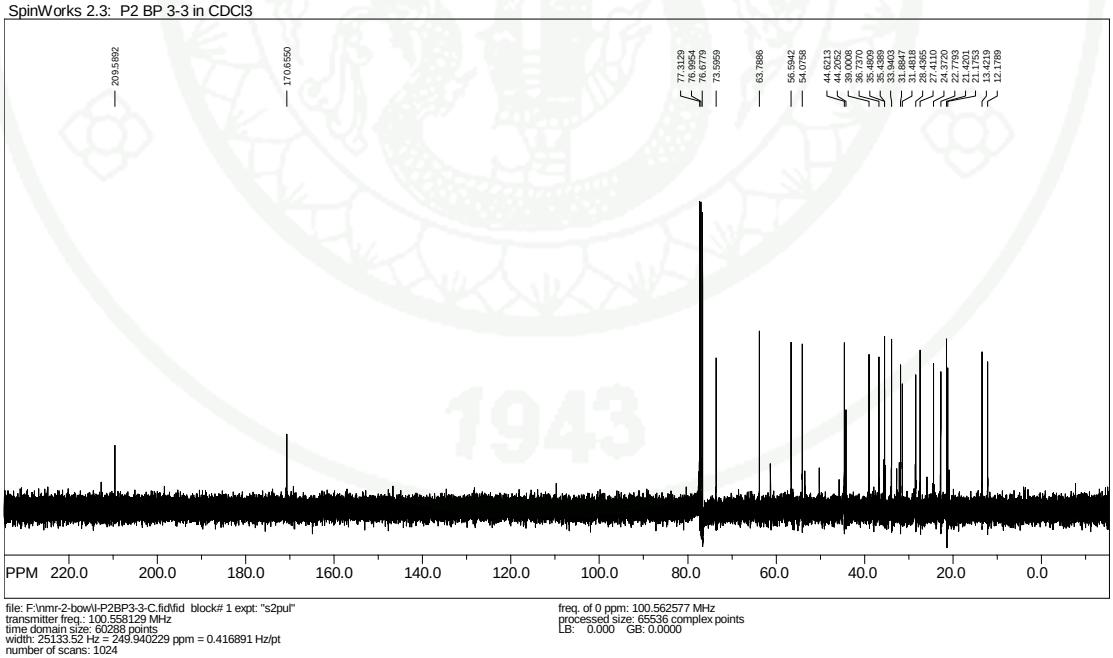
Appendix Figure 3 400 MHz ^{1}H NMR spectrum: 3β-acetoxy-5α-pregnan-20-one (82)



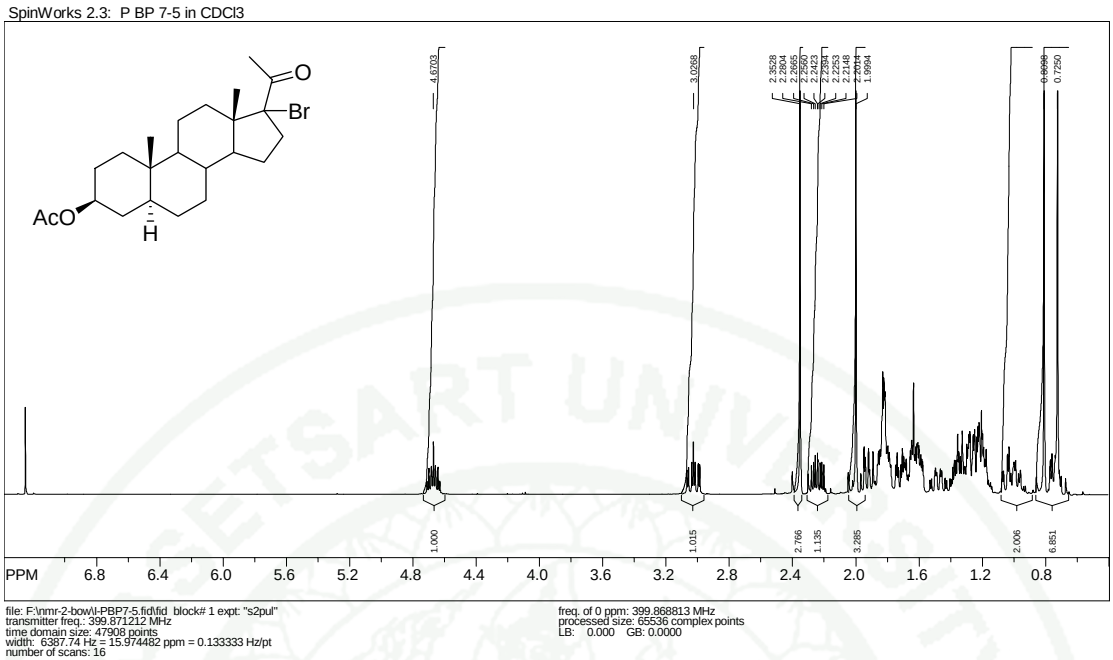
Appendix Figure 4 100 MHz ^{13}C NMR spectrum: 3β-acetoxy-5α-pregnan-20-one (82)



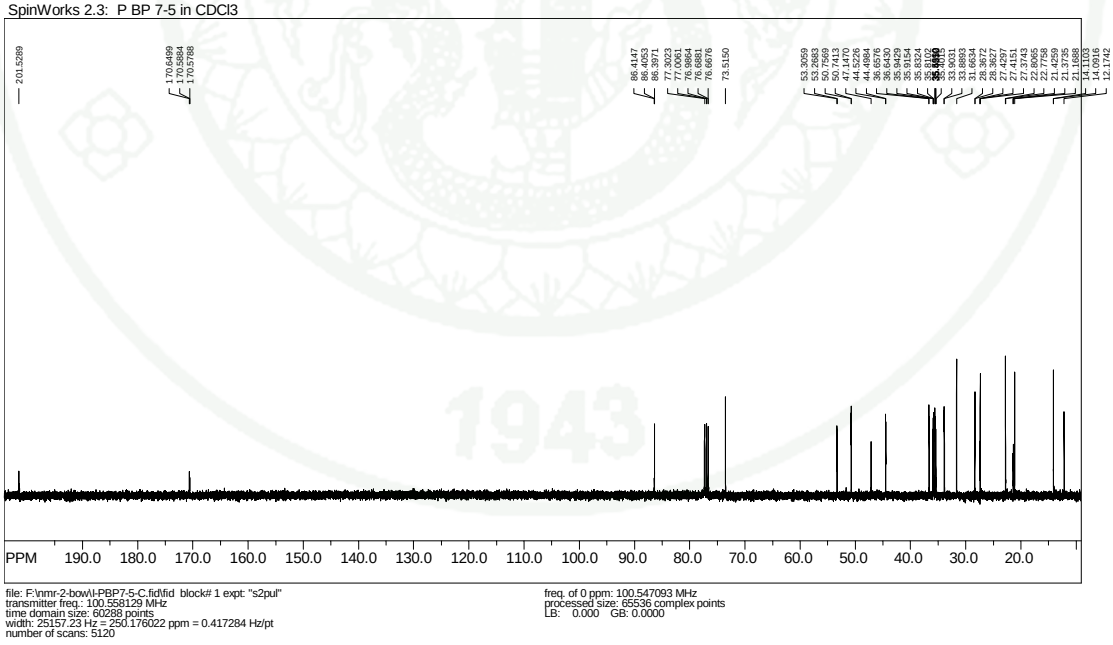
Appendix Figure 5 400 MHz ^{1}H NMR spectrum: 3β-acetoxy-5α-17-pregnenyl acetate (83)



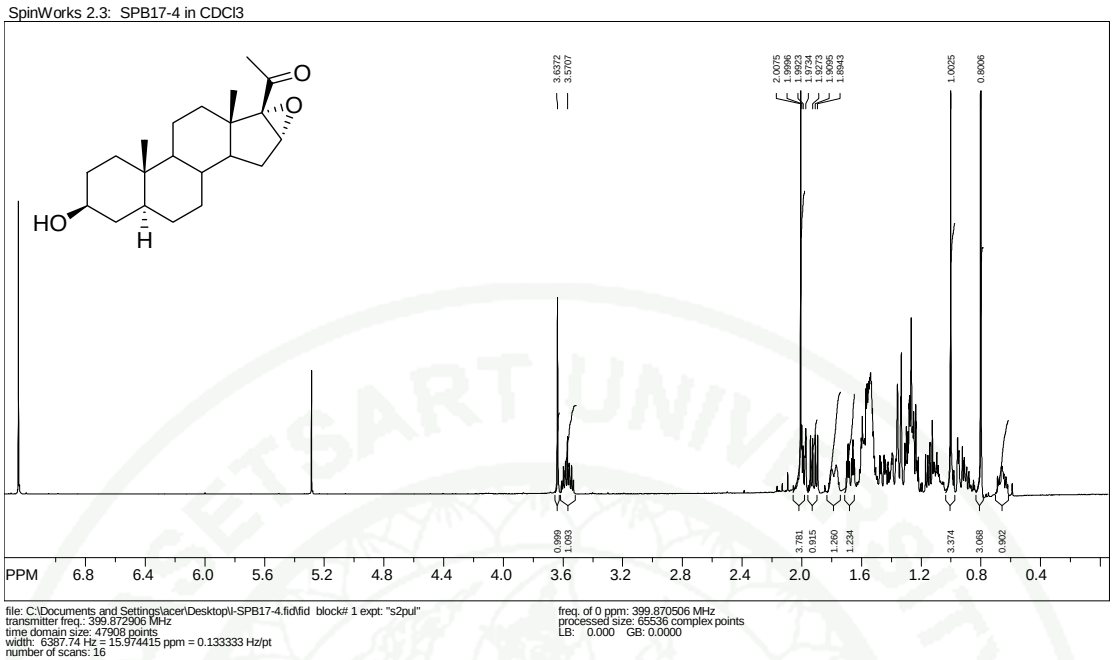
Appendix Figure 6 100 MHz ^{13}C NMR spectrum: 3β-acetoxy-5α-17-pregnenyl acetate (83)



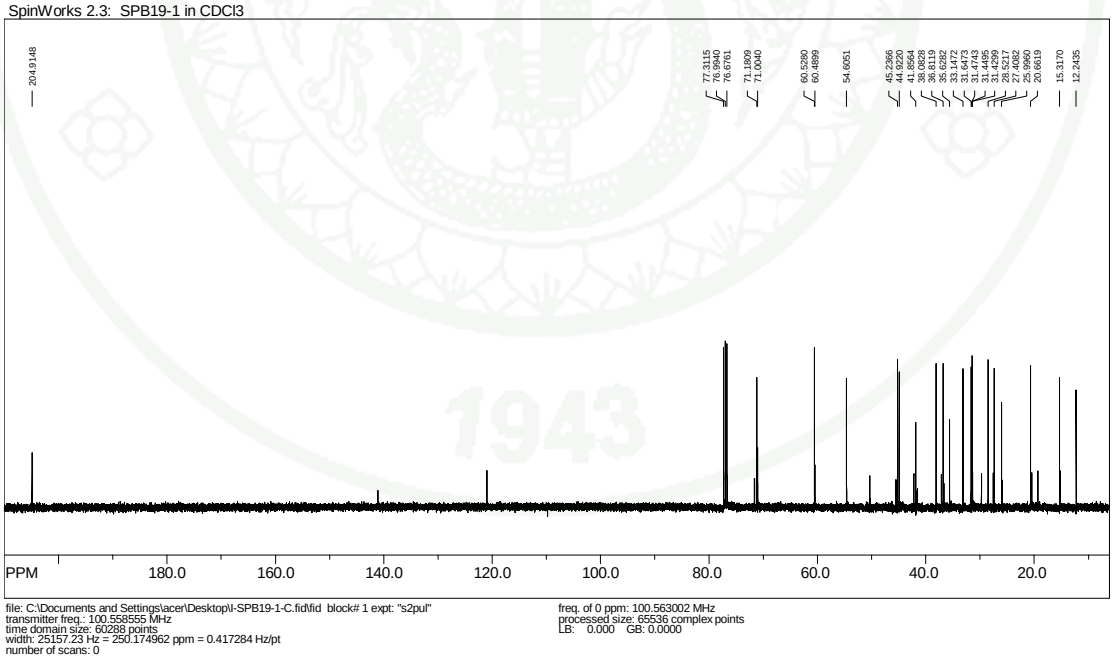
Appendix Figure 7 400 MHz ^{1}H NMR spectrum: 3β-acetoxy-5α-17-bromo-pregnan-20-one (84)



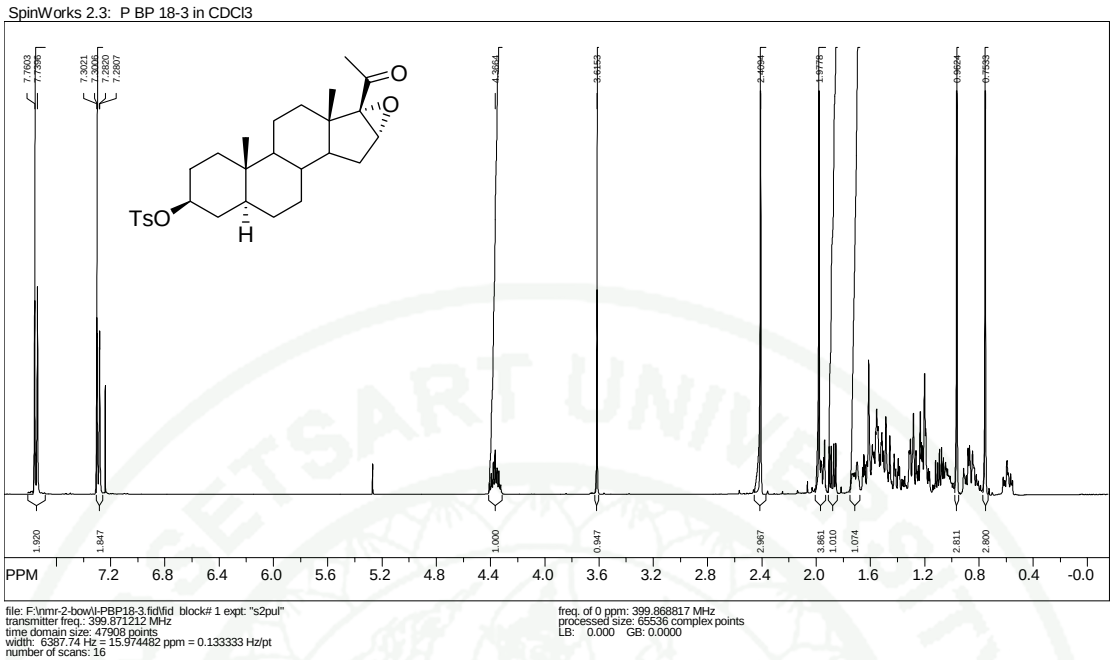
Appendix Figure 8 100 MHz ^{13}C NMR spectrum: 3β-acetoxy-5α-17-bromo-pregnan-20-one (84)



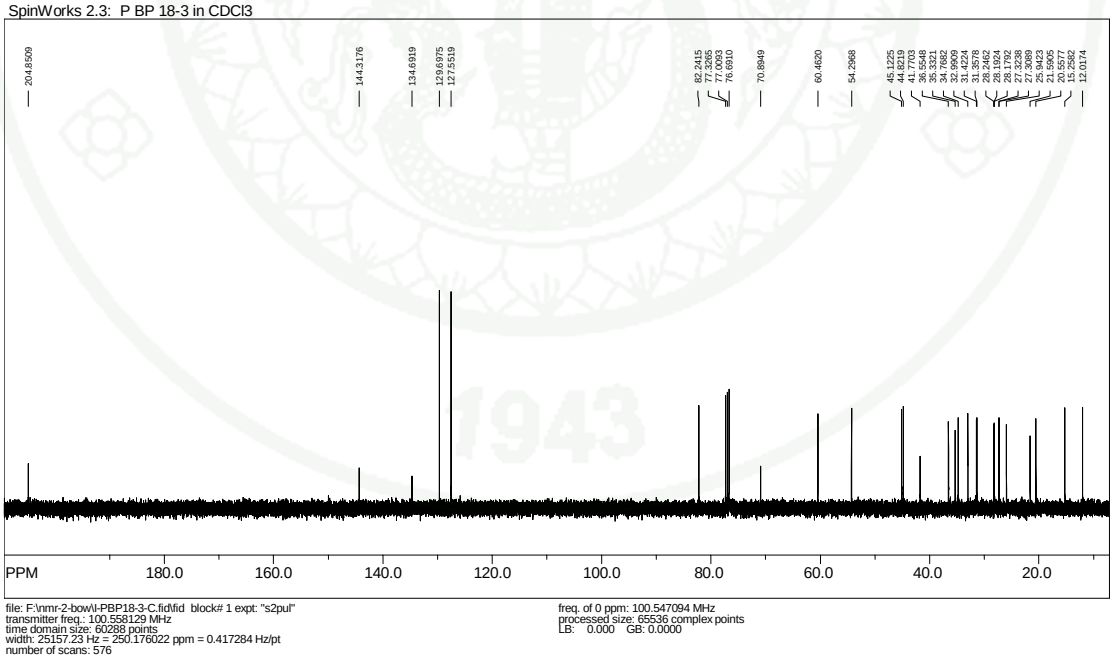
Appendix Figure 9 400 MHz ^{1}H NMR spectrum: 16α, 17α-epoxy-3β-hydroxy-5α-pregnan-20-one (85)



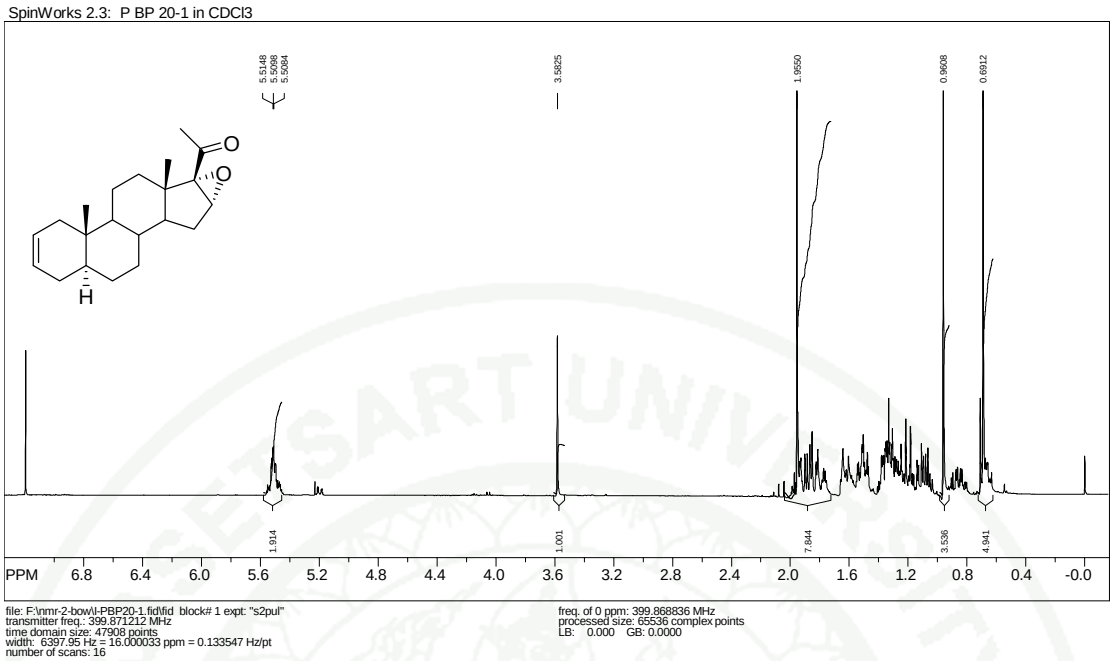
Appendix Figure 10 100 MHz ^{13}C NMR spectrum: 16α, 17α-epoxy-3β-hydroxy-5α- pregnan-20-one (85)



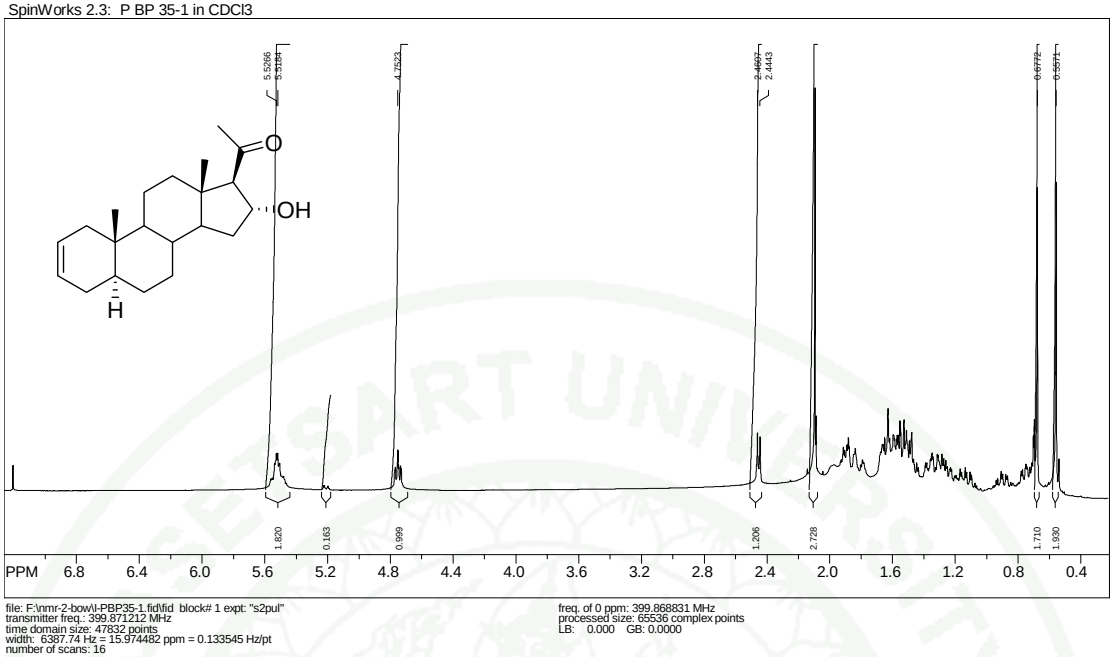
Appendix Figure 11 400 MHz ^{1}H NMR spectrum: 16α, 17α-epoxy-3β-tosyloxy-5α-pregnan-20-one (86)



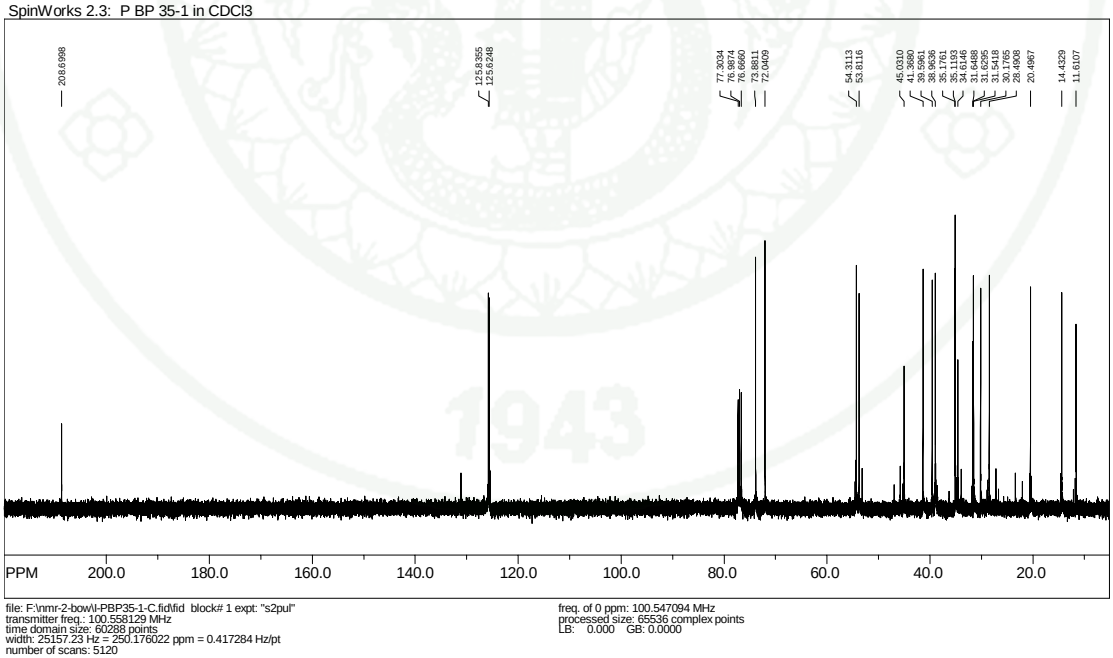
Appendix Figure 12 100 MHz ^{13}C NMR spectrum: 16α, 17α-epoxy-3β-tosyloxy-5α-pregnan-20-one (86)



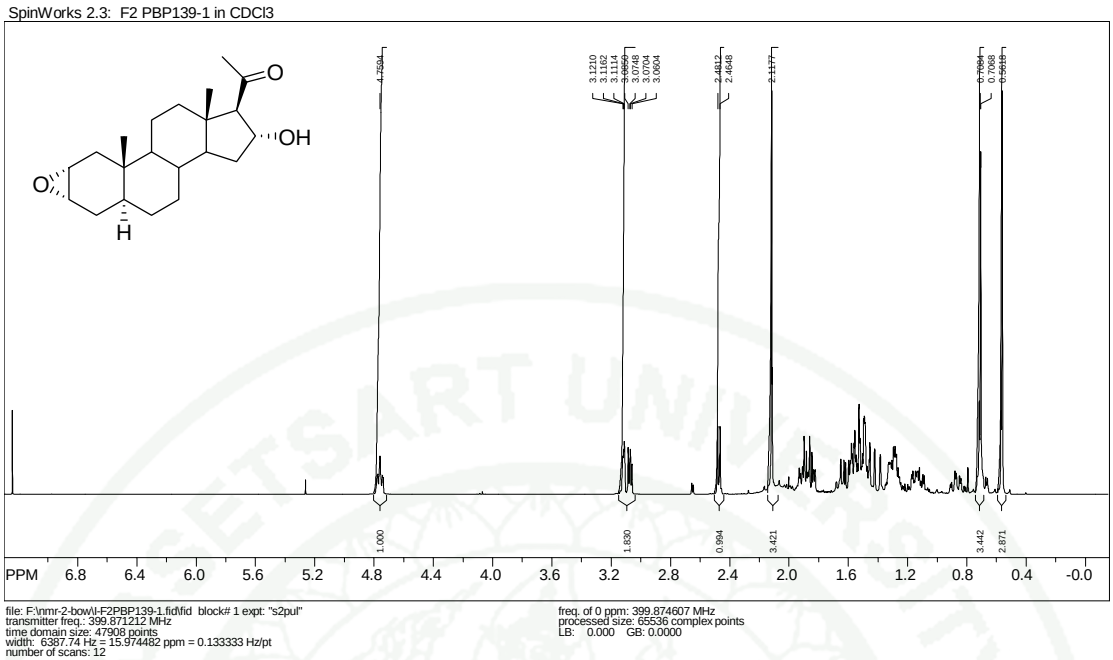
Appendix Figure 13 400 MHz ^{1}H NMR spectrum: 16α, 17α-epoxy-5α-2-pregnen-20-one (87)



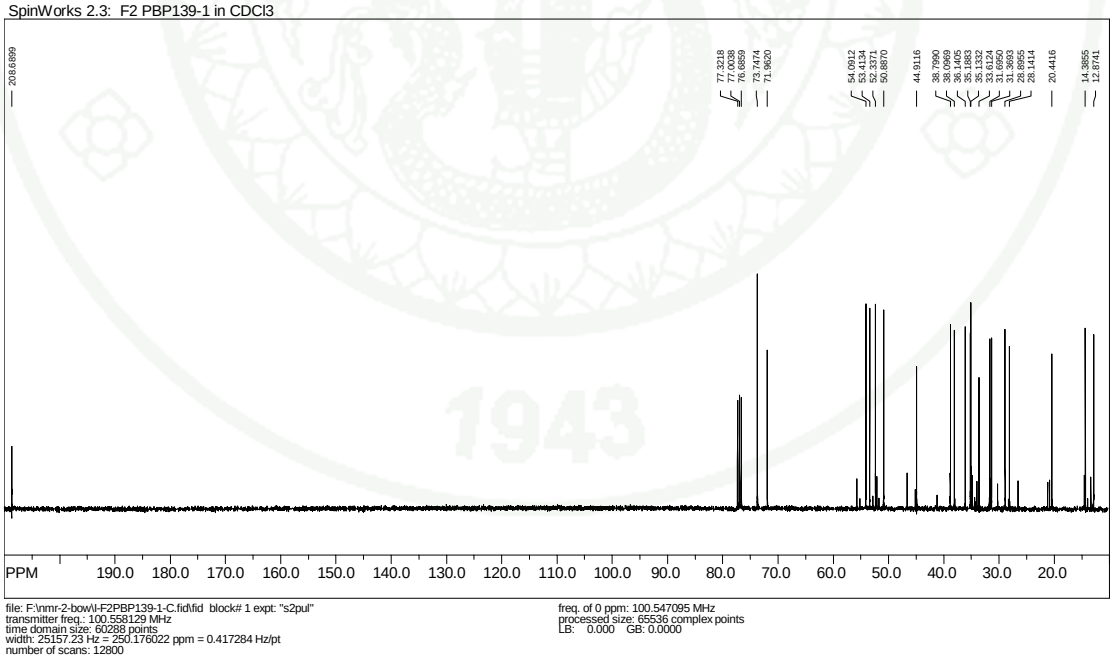
Appendix Figure 14 400 MHz ^{1}H NMR spectrum: 16α-hydroxy-5α-2-pregnen-20-one (88)



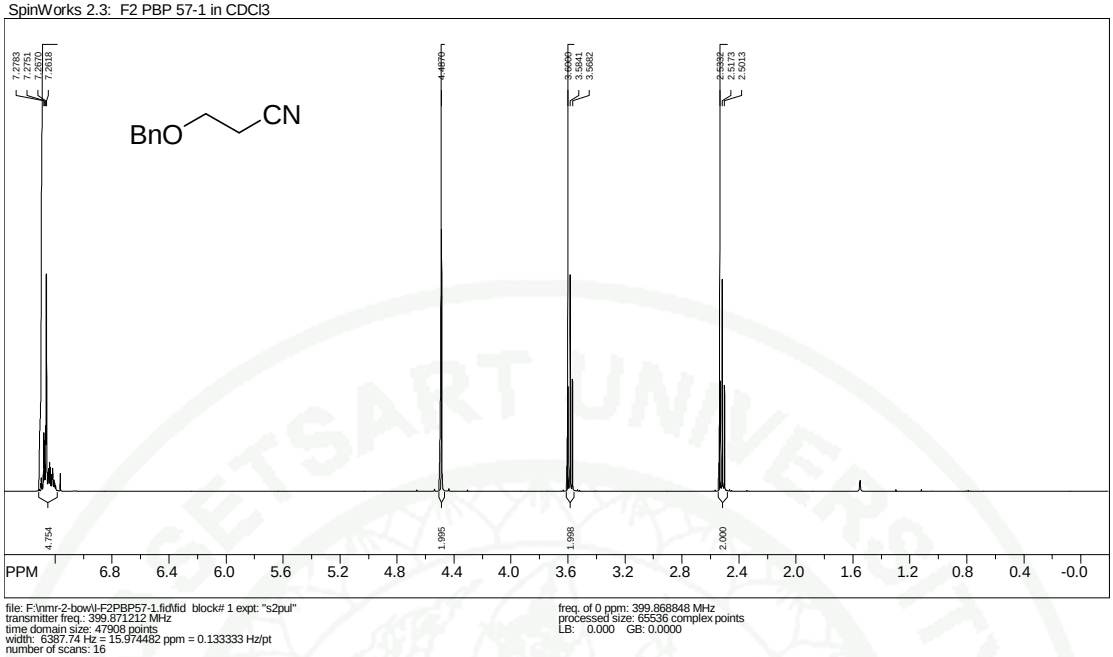
Appendix Figure 15 100 MHz ^{13}C NMR spectrum: 16α-hydroxy-5α-2-pregnen-20-one (88)



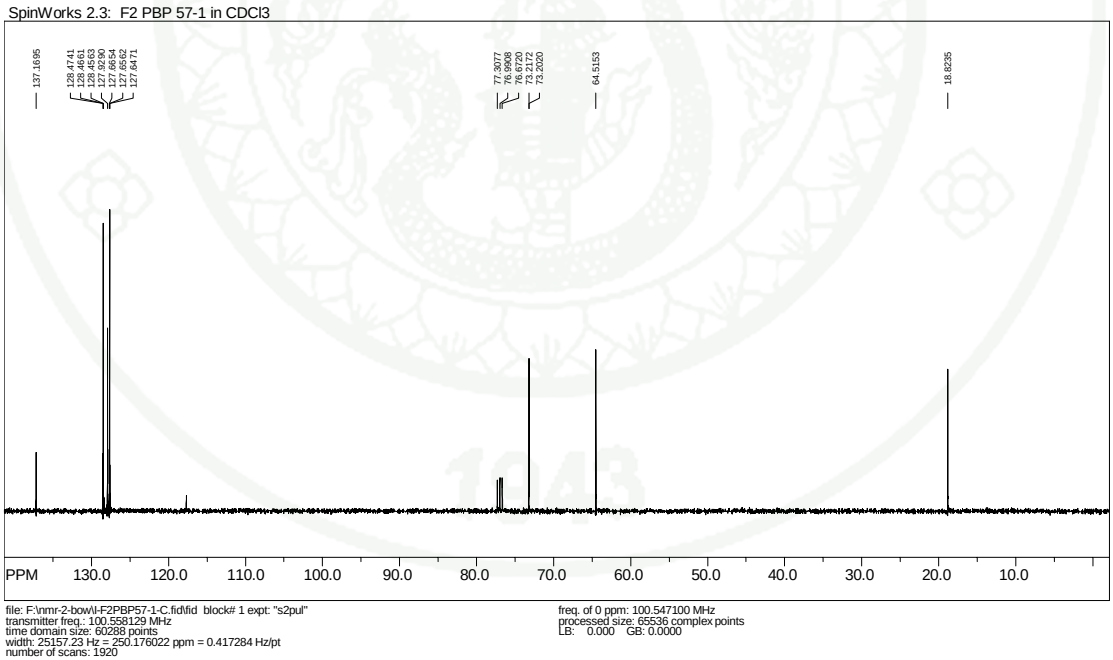
Appendix Figure 16 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89)



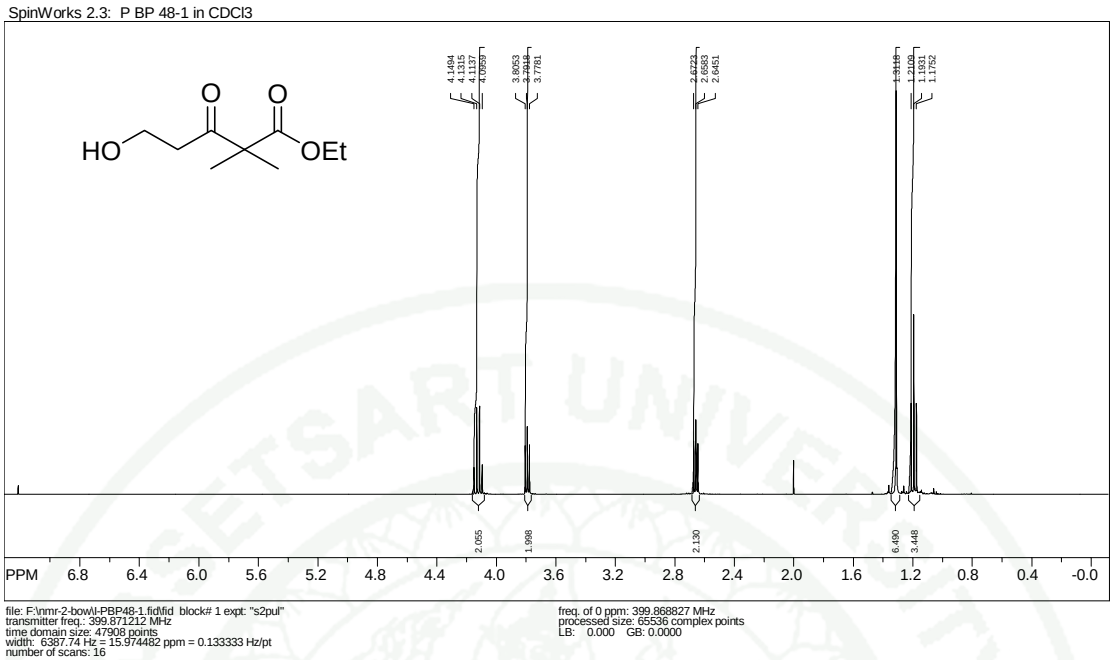
Appendix Figure 17 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-16α-hydroxy-5α-pregnan-20-one (89)



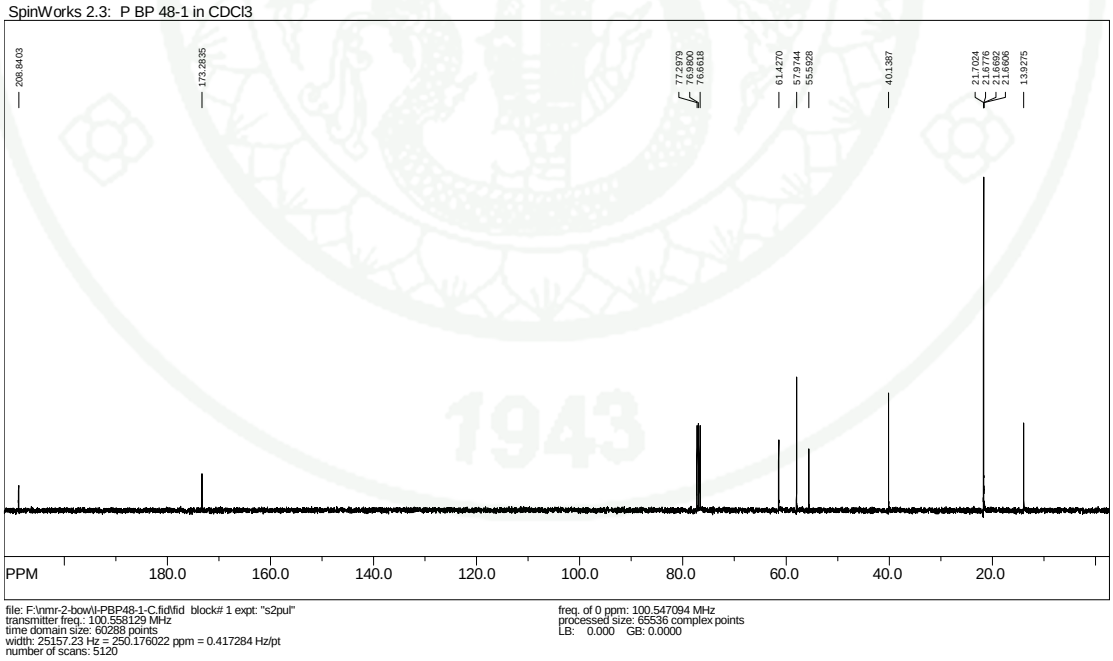
Appendix Figure 18 400 MHz ^{1}H NMR spectrum: 3-benzyloxypropionitrile (91)



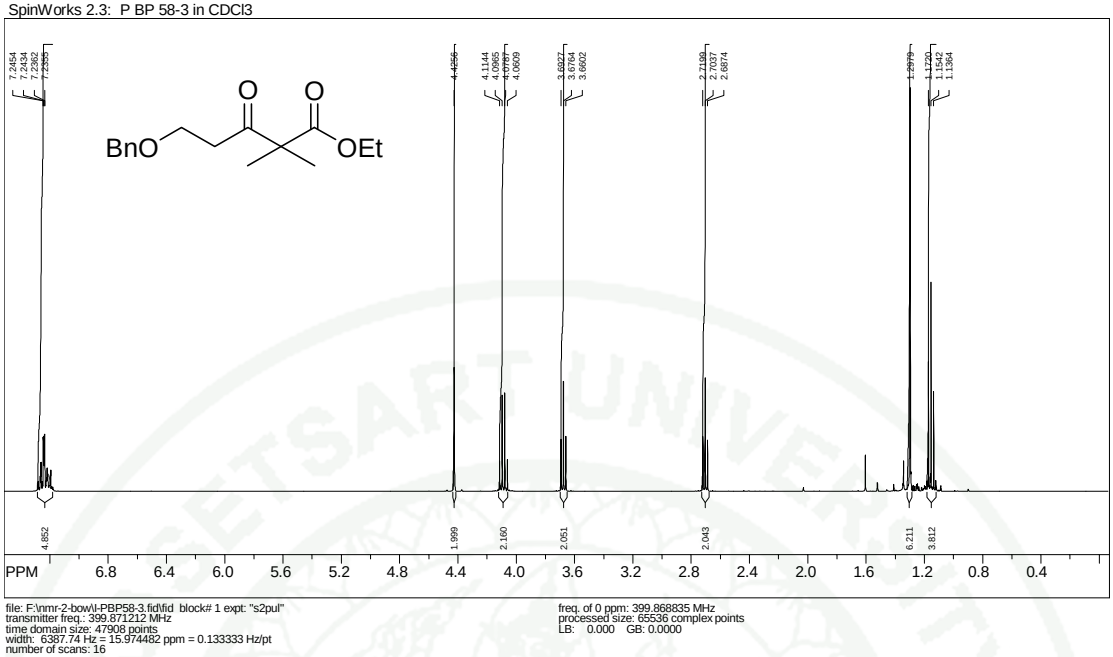
Appendix Figure 19 100 MHz ^{13}C NMR spectrum: 3-benzyloxypropionitrile (91)



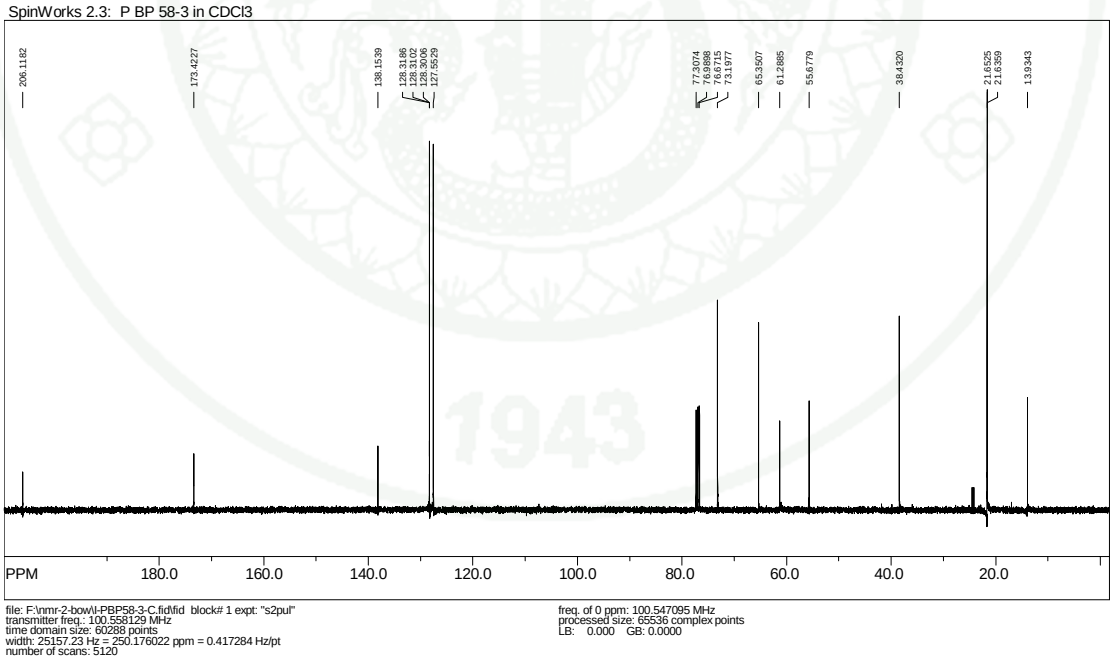
Appendix Figure 20 400 MHz ^{1}H NMR spectrum: ethyl 5-hydroxy-2, 2-dimethyl-3-oxopentanoate (93)



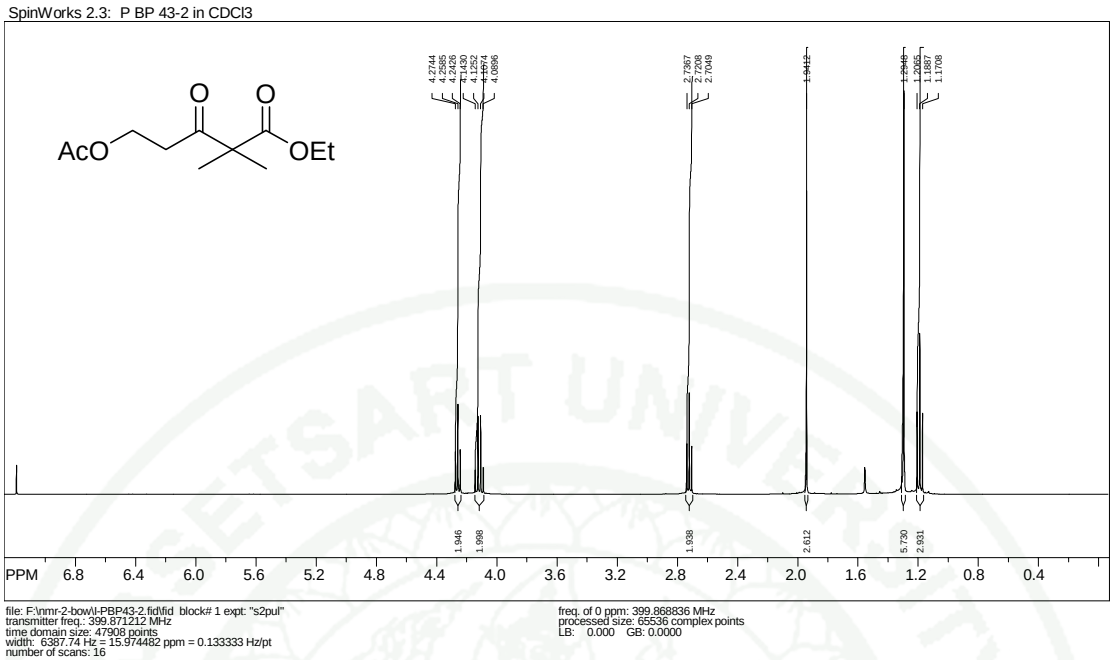
Appendix Figure 21 100 MHz ^{13}C NMR spectrum: ethyl 5-hydroxy-2, 2-dimethyl-3-oxopentanoate (93)



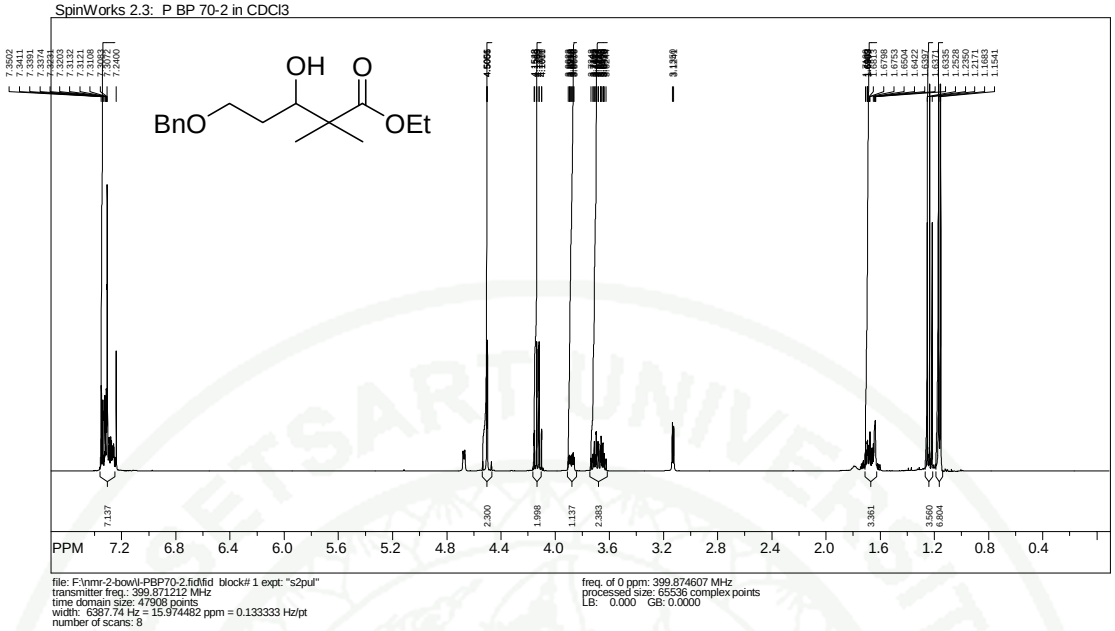
Appendix Figure 22 400 MHz ^{1}H NMR spectrum: ethyl 5-benzyloxy-2, 2-dimethyl-3-oxopentanoate (94)



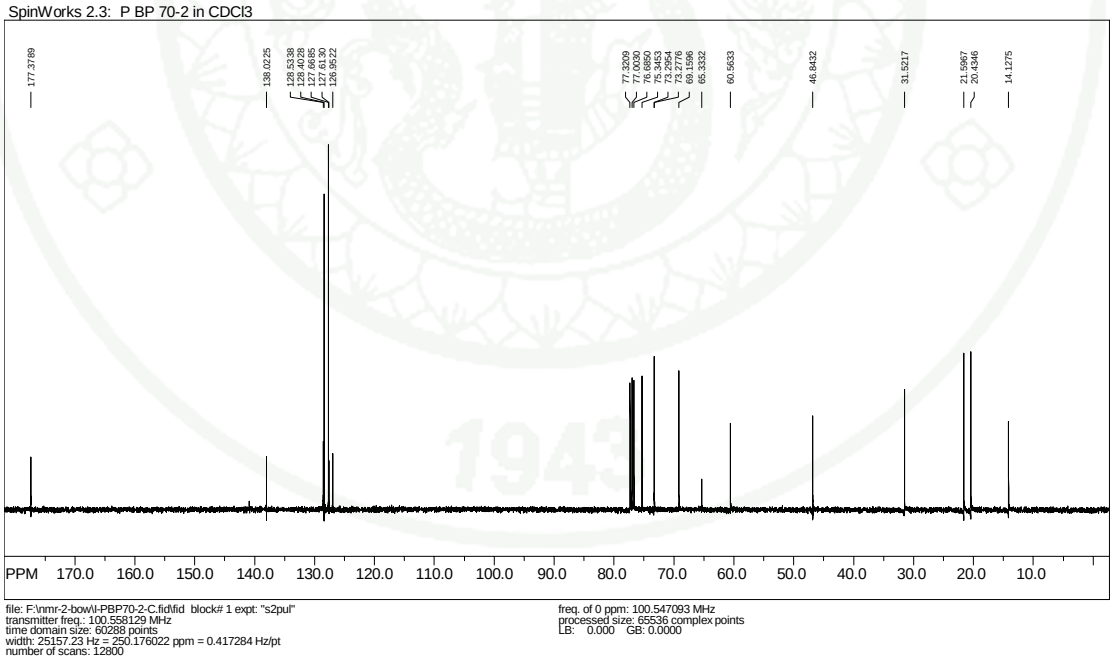
Appendix Figure 23 100 MHz ^{13}C NMR spectrum: ethyl 5-benzyloxy-2, 2-dimethyl-3-oxopentanoate (94)



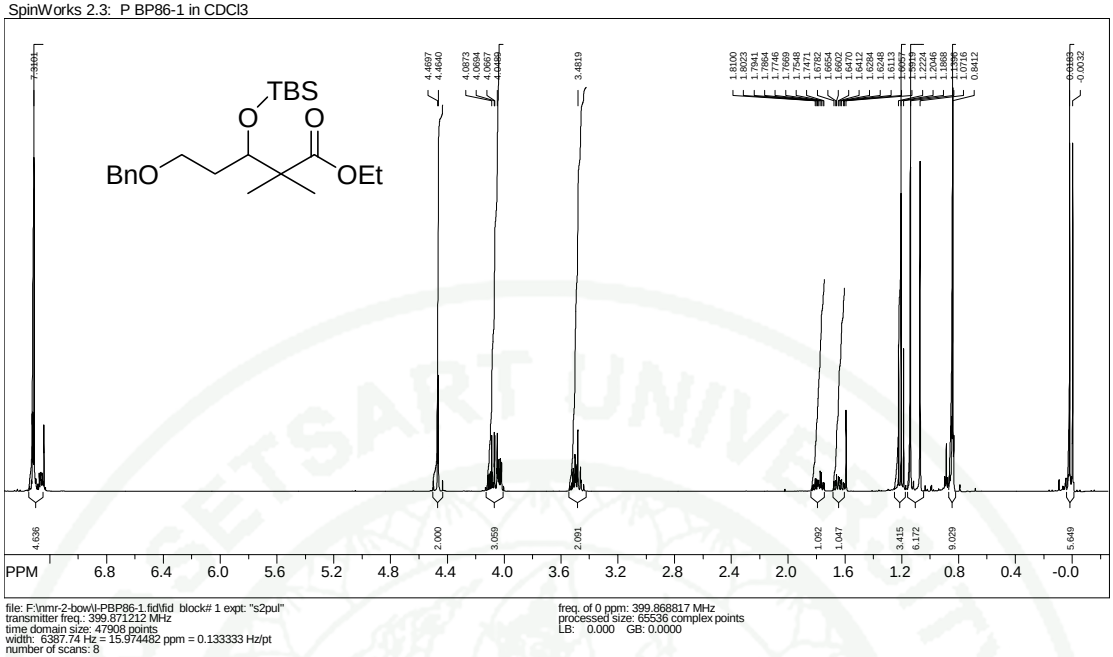
Appendix Figure 24 400 MHz ^{1}H NMR spectrum: ethyl 5-acetoxy-2, 2-dimethyl-3-oxopentanoate (95)



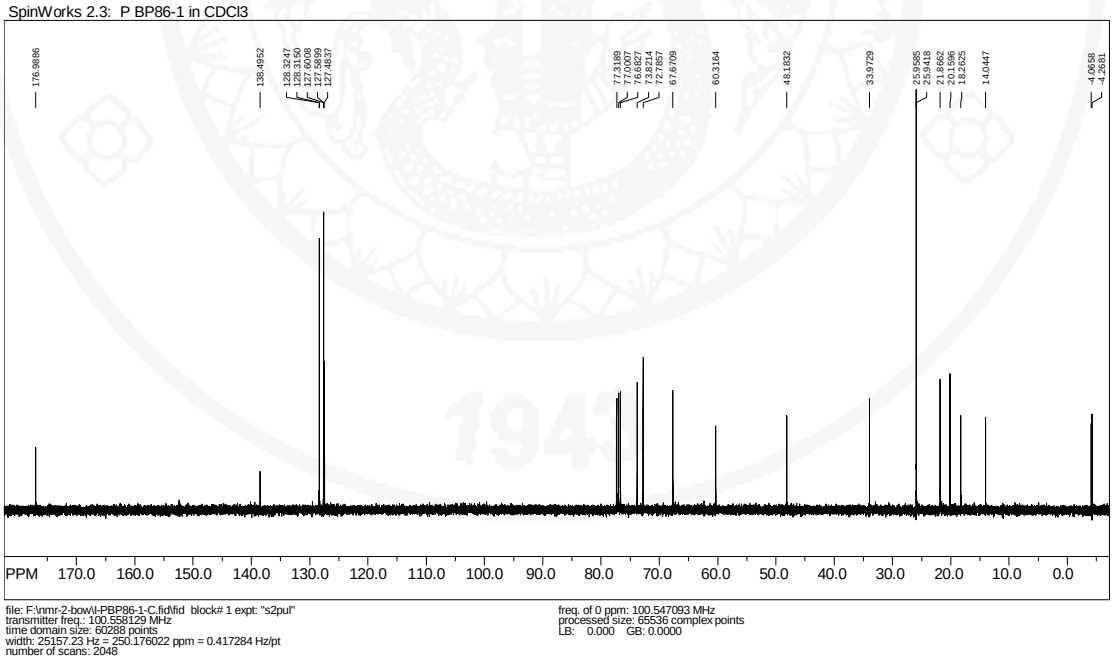
Appendix Figure 25 400 MHz ^{1}H NMR spectrum: ethyl 5-benzyloxy-3-hydroxy-2, 2-dimethylpentanoate (100)



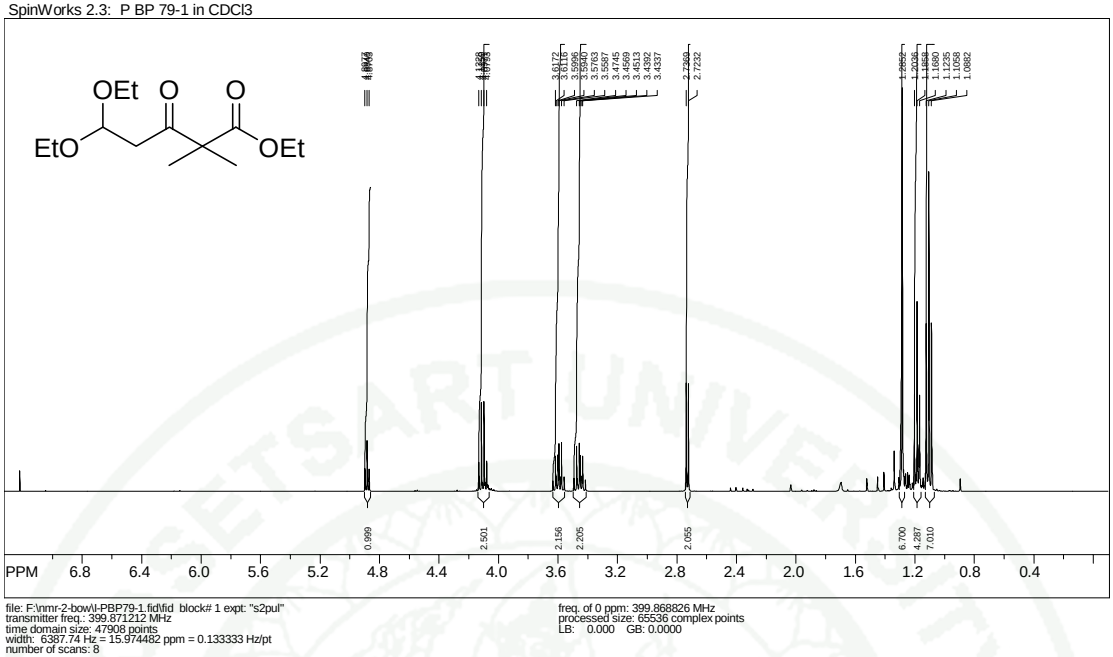
Appendix Figure 26 100 MHz ^{13}C NMR spectrum: ethyl 5-benzyloxy-3-hydroxy-2, 2-dimethylpentanoate (100)



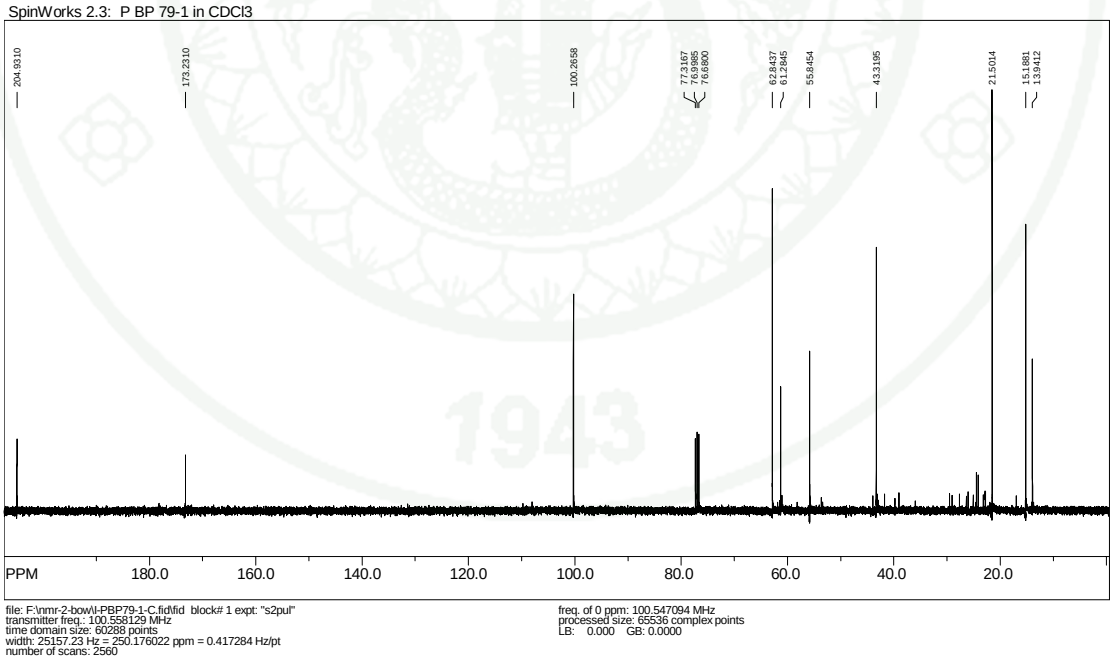
Appendix Figure 27 400 MHz ^{1}H NMR spectrum: ethyl 5-benzyloxy-3-*tert*-butyldimethylsilyloxy-2, 2-dimethylpentanoate (101)



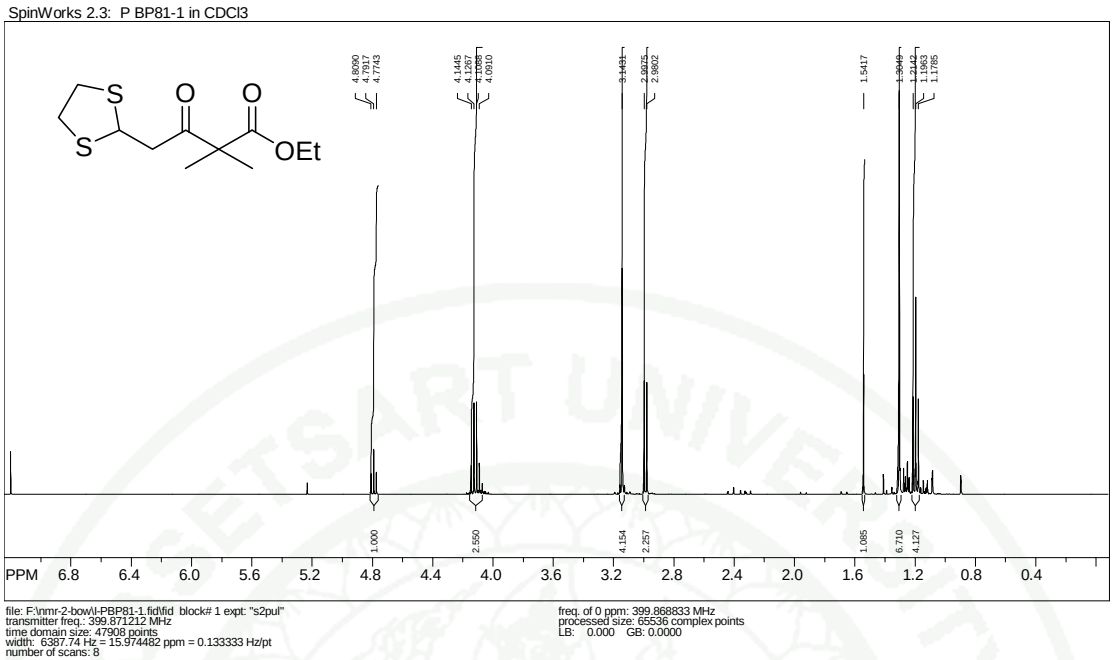
Appendix Figure 28 100 MHz ^{13}C NMR spectrum: ethyl 5-benzyloxy-3-*tert*-butyldimethylsilyloxy-2, 2-dimethylpentanoate (101)



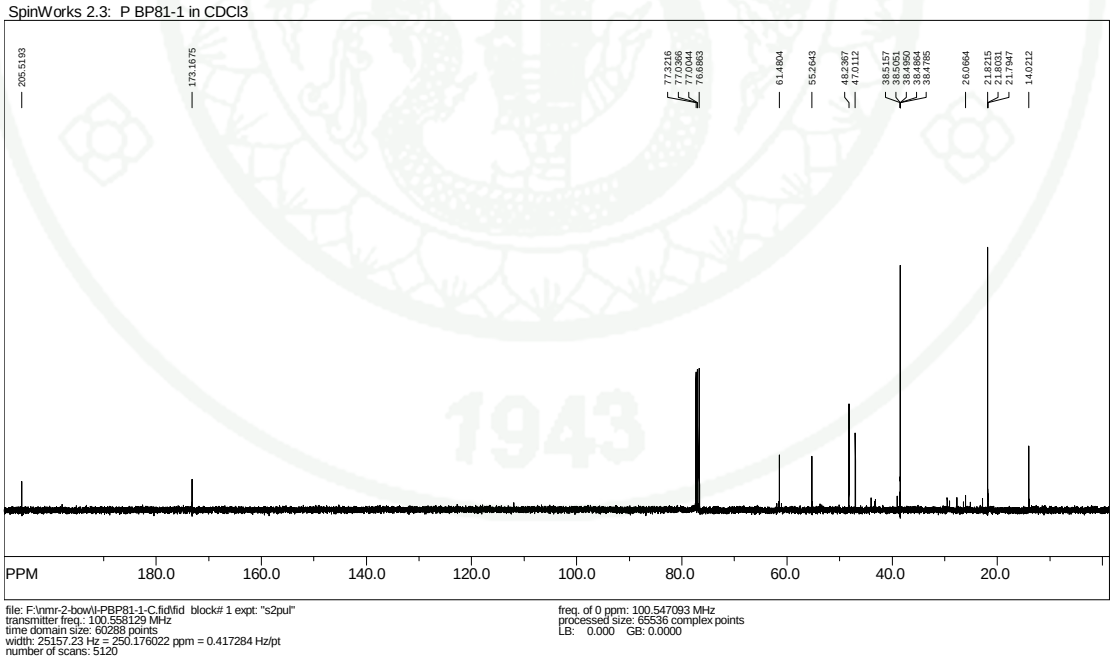
Appendix Figure 29 400 MHz ^{1}H NMR spectrum: ethyl 5, 5-diethoxy-2, 2-dimethyl-3-oxopentanoate (104)



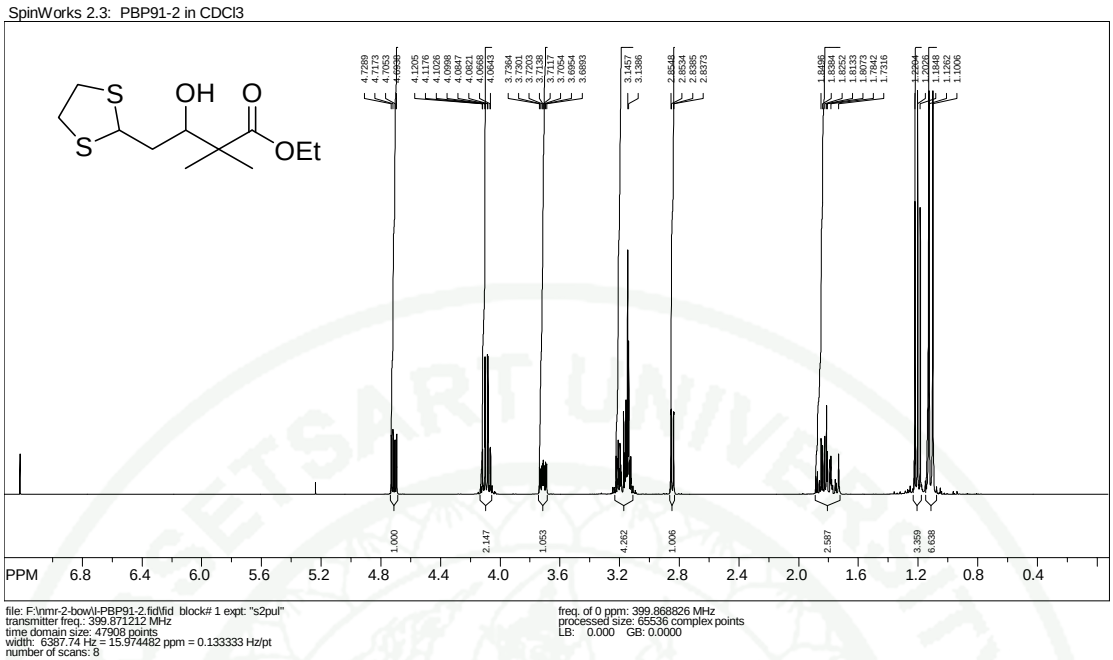
Appendix Figure 30 100 MHz ^{13}C NMR spectrum: ethyl 5, 5-diethoxy-2, 2-dimethyl-3-oxopentanoate (104)



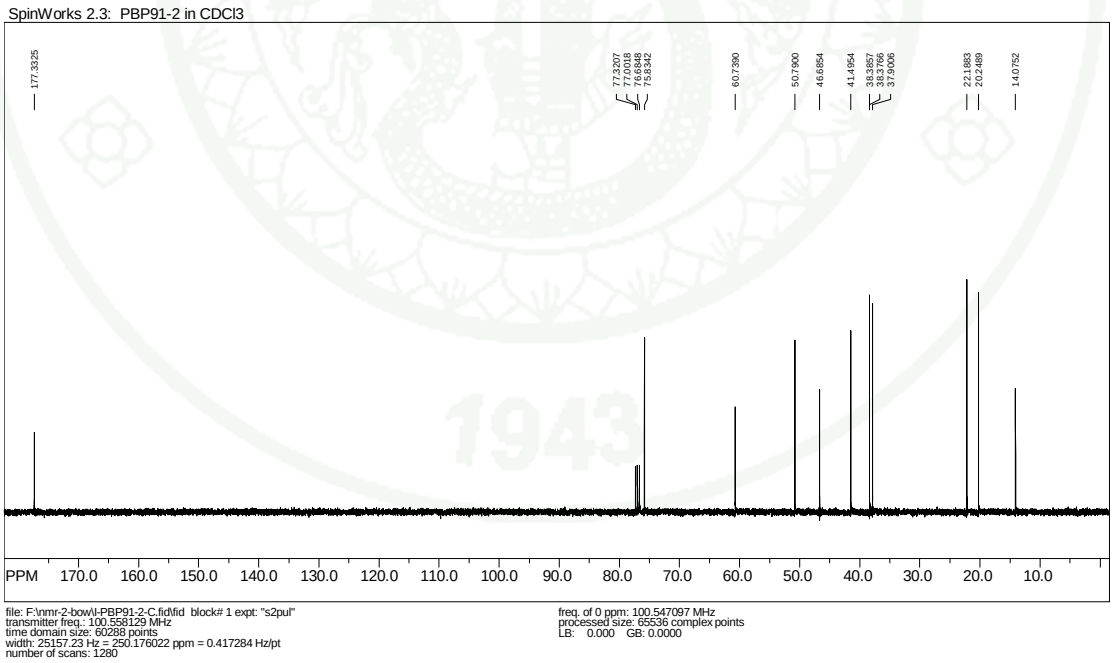
Appendix Figure 31 400 MHz ^{1}H NMR spectrum: ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (105)



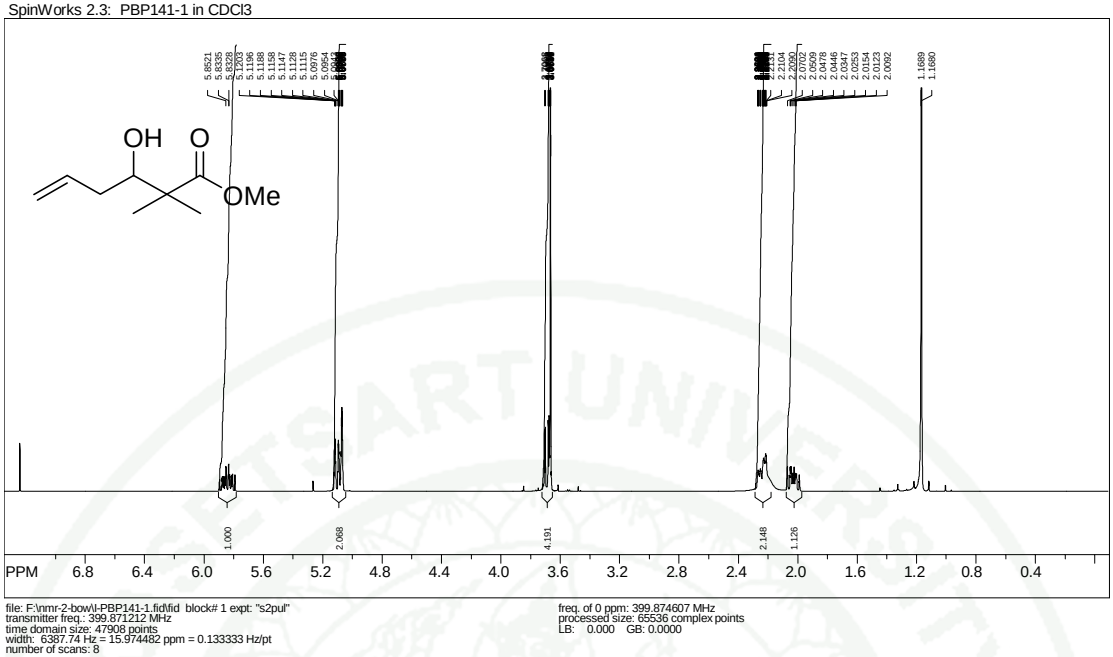
Appendix Figure 32 100 MHz ^{13}C NMR spectrum: ethyl 4-(1, 3-dithiolan-2-yl)-2, 2-dimethyl-3-oxobutanoate (105)



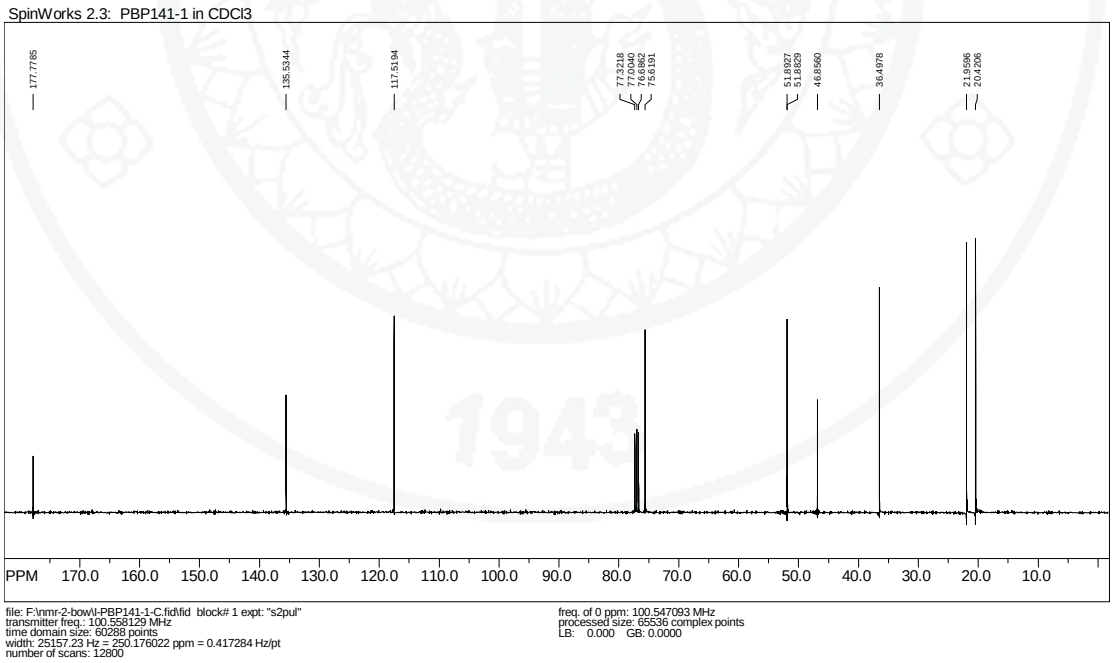
Appendix Figure 33 400 MHz ^{1}H NMR spectrum: ethyl 4-(1, 3-dithiolan-2-yl)-3-hydroxy-2, 2-dimethylbutanoate (106)



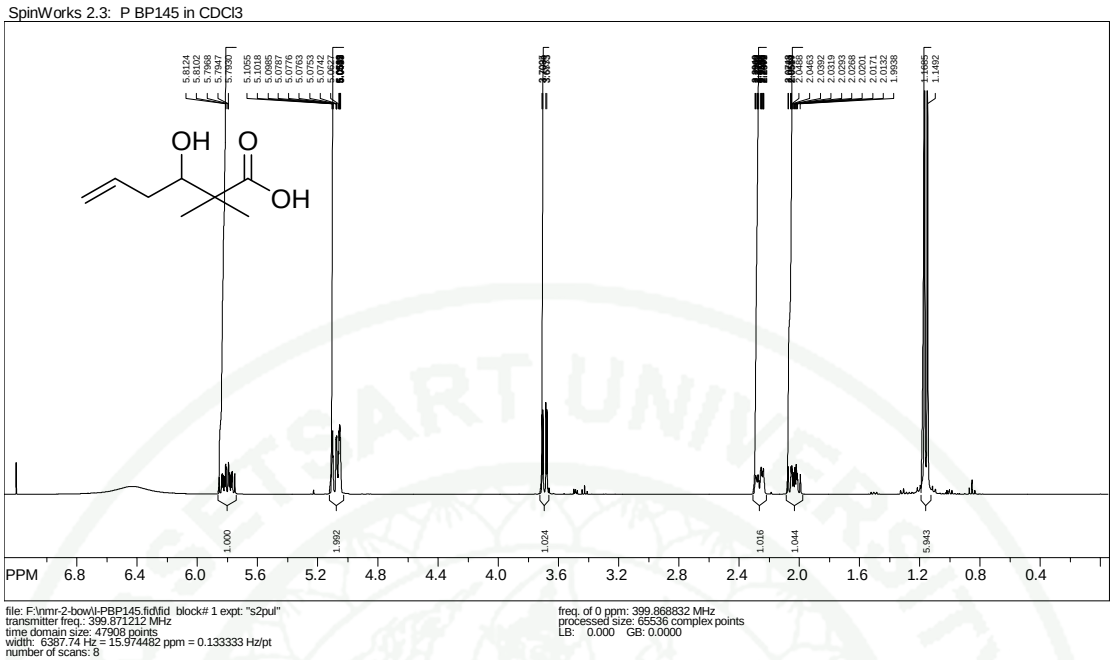
Appendix Figure 34 100 MHz ^{13}C NMR spectrum: ethyl 4-(1, 3-dithiolan-2-yl)-3-hydroxy-2, 2-dimethylbutanoate (106)



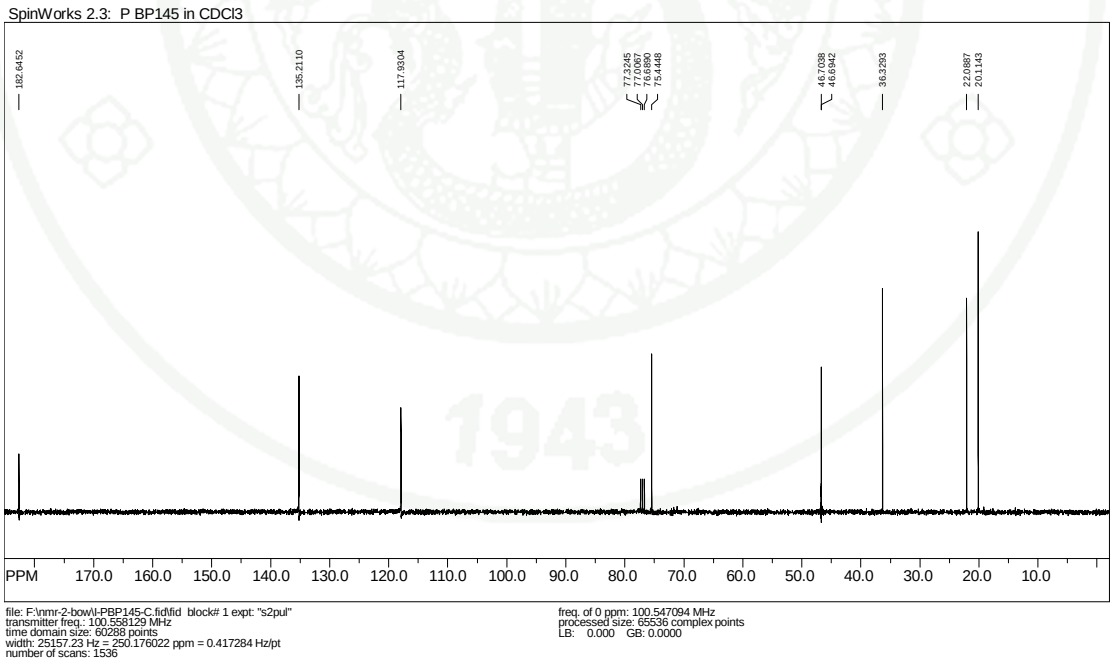
Appendix Figure 35 400 MHz ^{1}H NMR spectrum: methyl 3-hydroxy-2, 2-dimethyl-5-hexenoate (109)



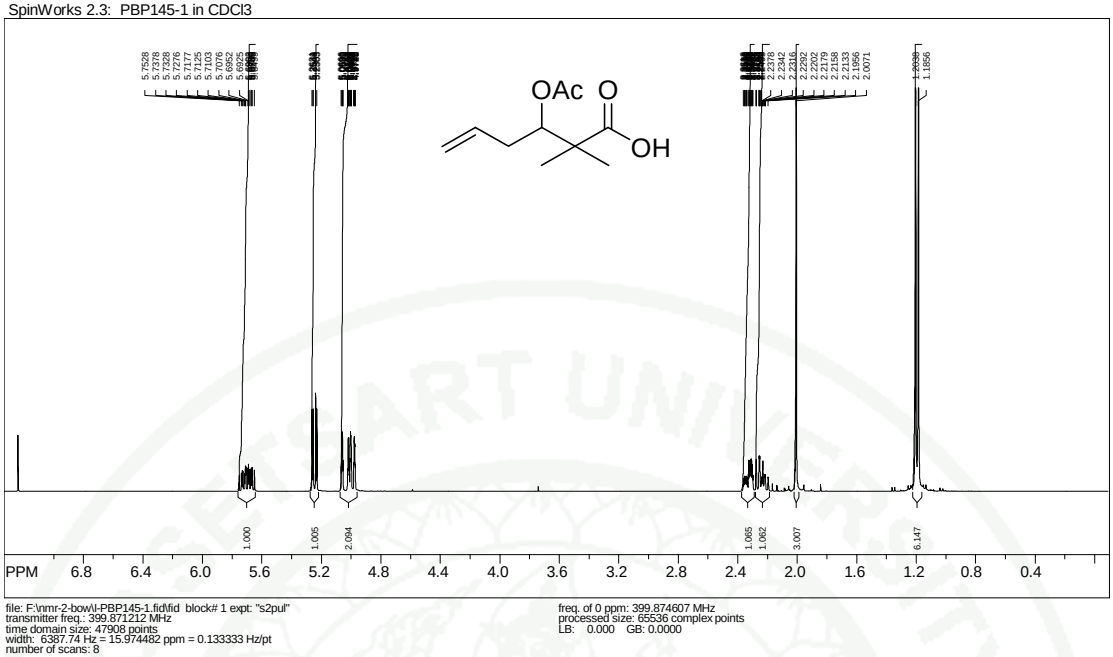
Appendix Figure 36 100 MHz ^{13}C NMR spectrum: methyl 3-hydroxy-2, 2-dimethyl-5-hexenoate (109)



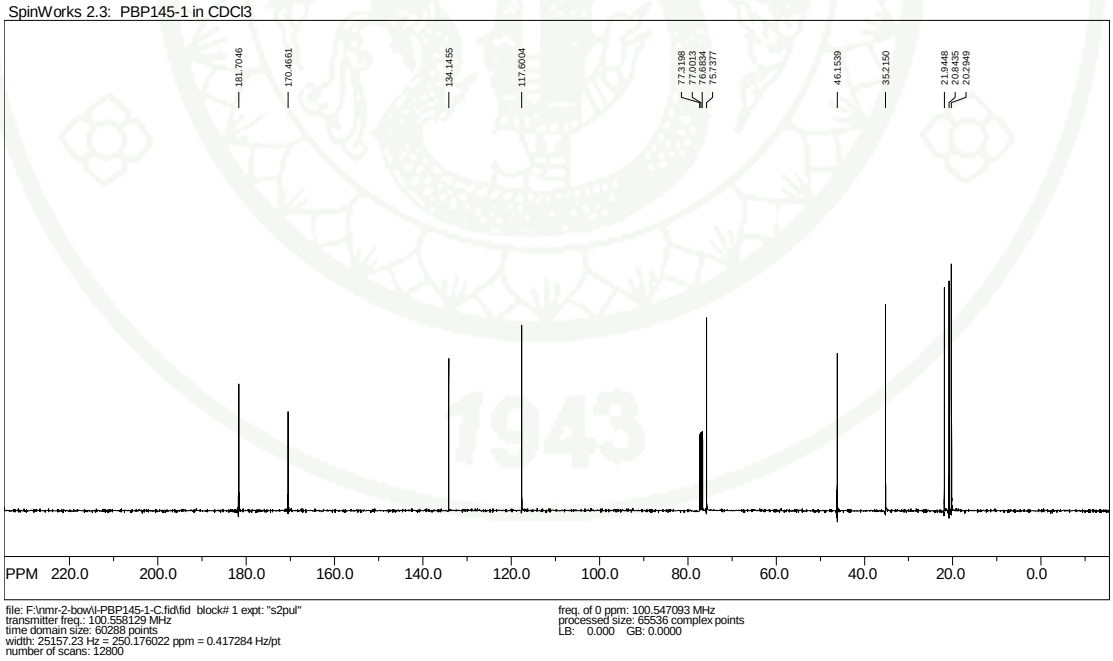
Appendix Figure 37 400 MHz ^{1}H NMR spectrum: 3-hydroxy-2, 2-dimethyl-5-hexenoic acid (110)



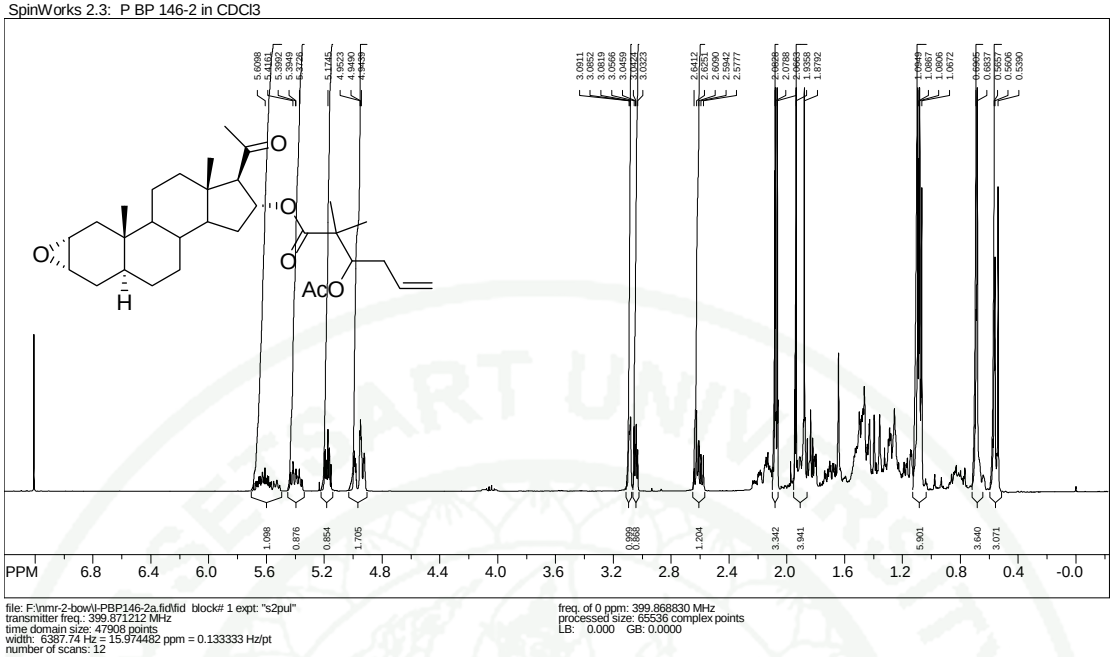
Appendix Figure 38 100 MHz ^{13}C NMR spectrum: 3-hydroxy-2, 2-dimethyl-5-hexenoic acid (110)



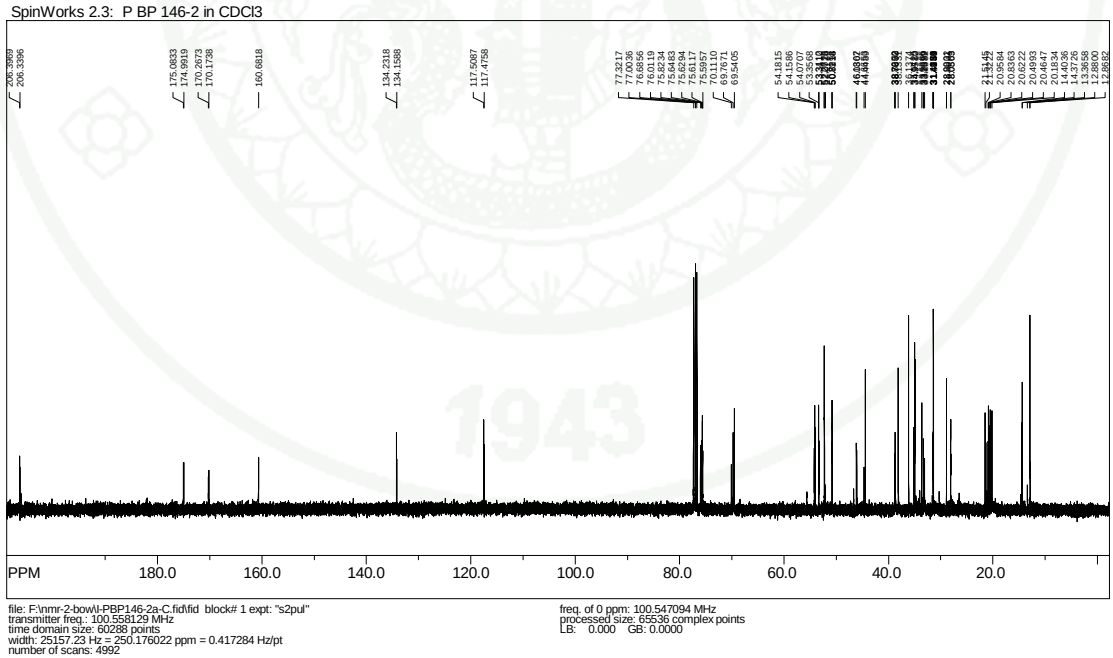
Appendix Figure 39 400 MHz ^{1}H NMR spectrum: 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111)



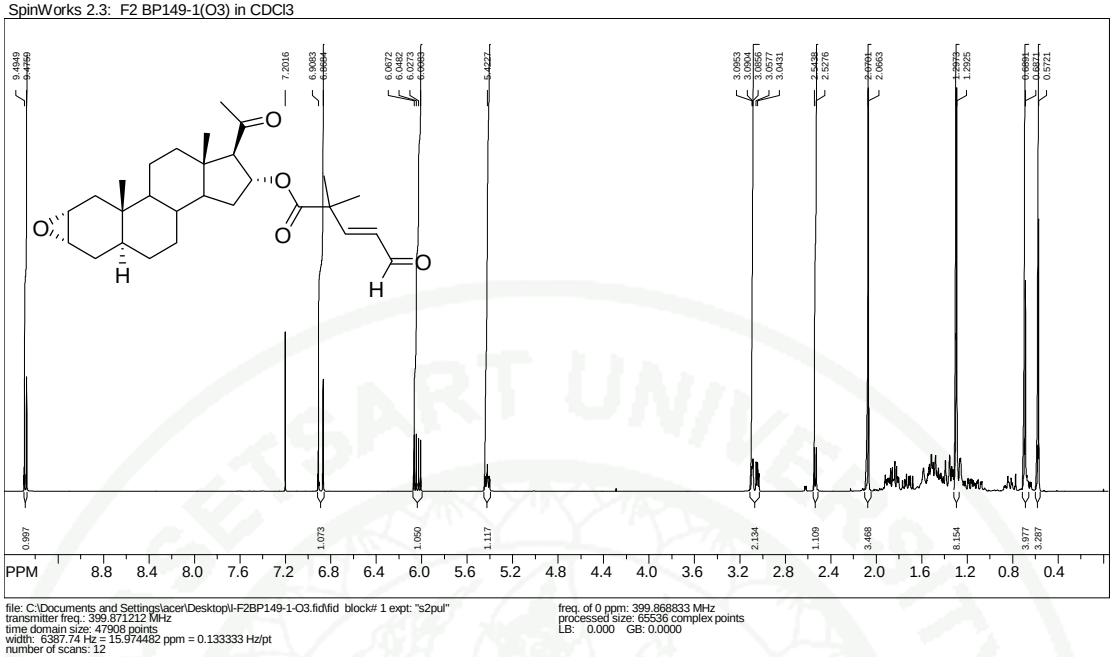
Appendix Figure 40 100 MHz ^{13}C NMR spectrum: 3-acetoxy-2, 2-dimethyl-5-hexenoic acid (111)



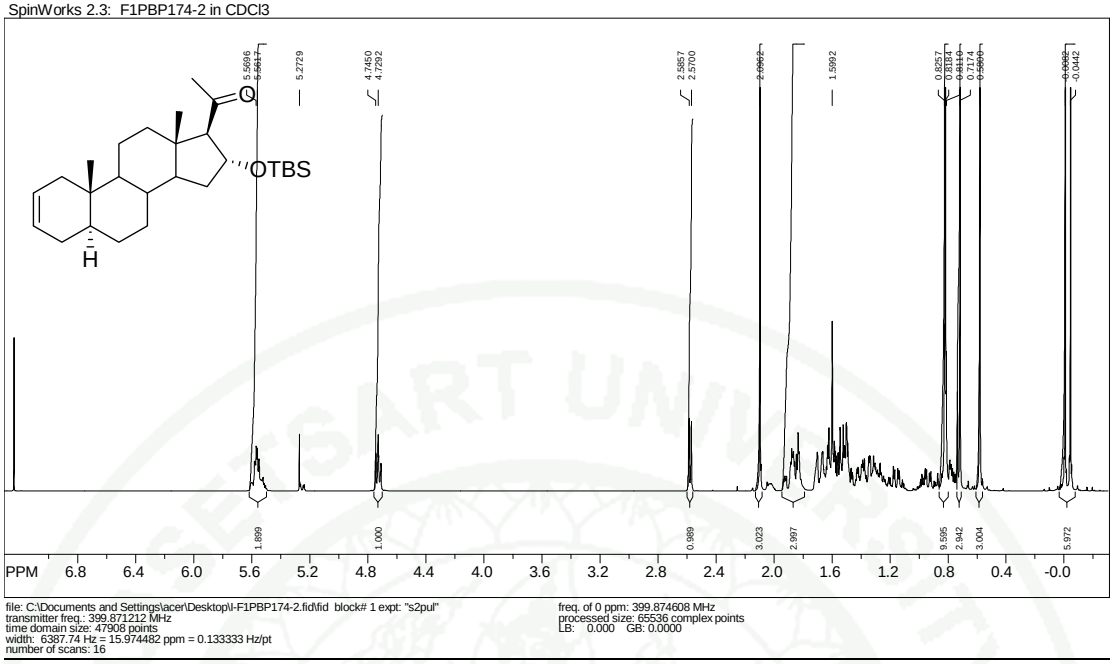
Appendix Figure 41 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (**a**)



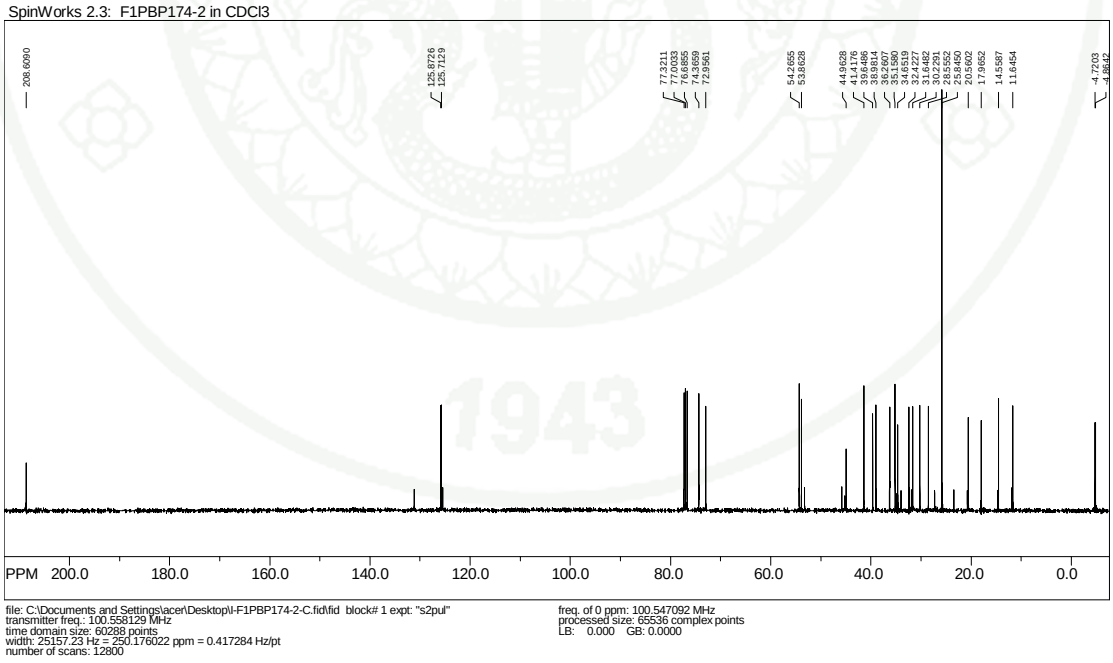
Appendix Figure 42 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-16α-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-5α-pregnan-20-one (**a**)



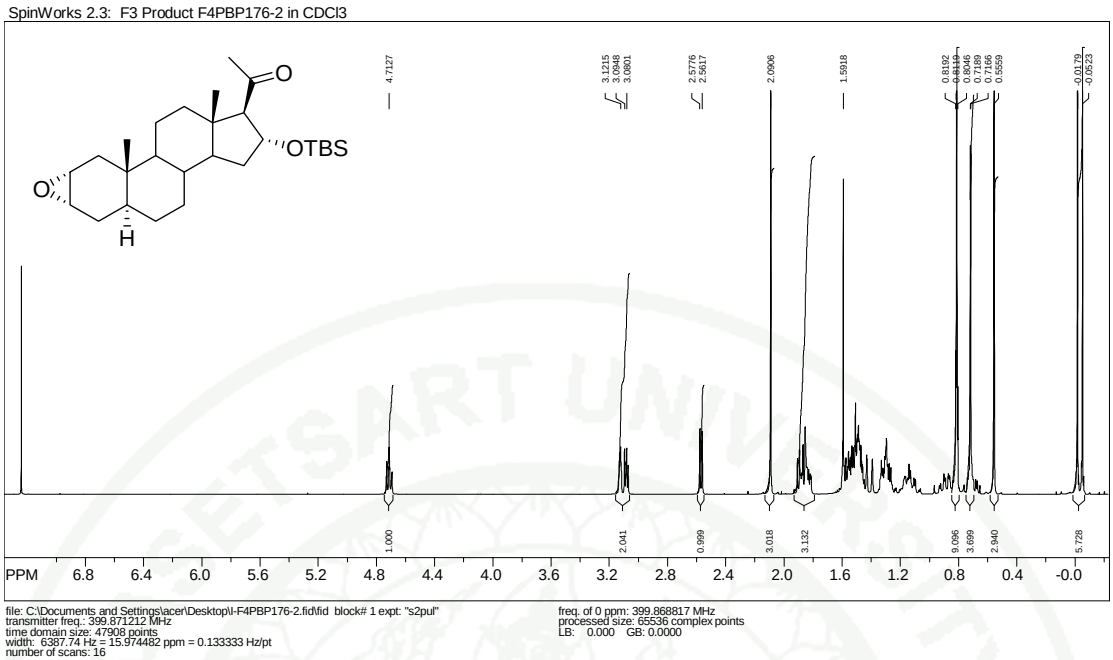
Appendix Figure 43 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-16α-(4-formyl-2, 2-dimethyl-3-butenoate)-5α-pregnan-20-one (162)



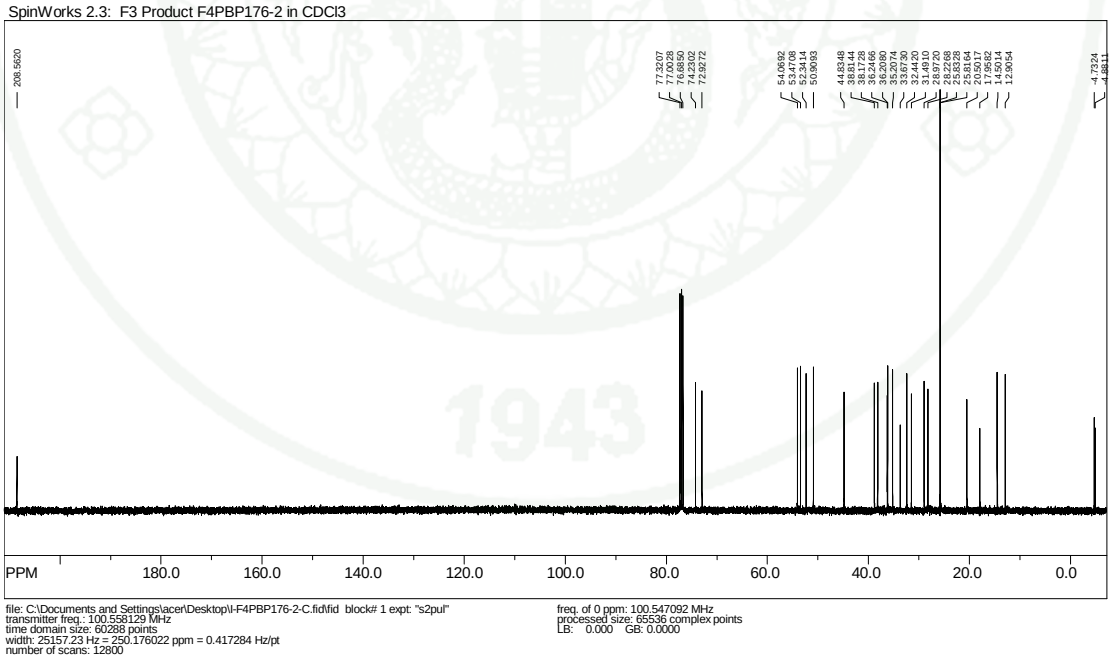
Apoendix Figure 44 400 MHz ^{1}H NMR spectrum: 16α-*tert*-butyldimethylsilyloxy-5α-2-pregnen-20-one (114)



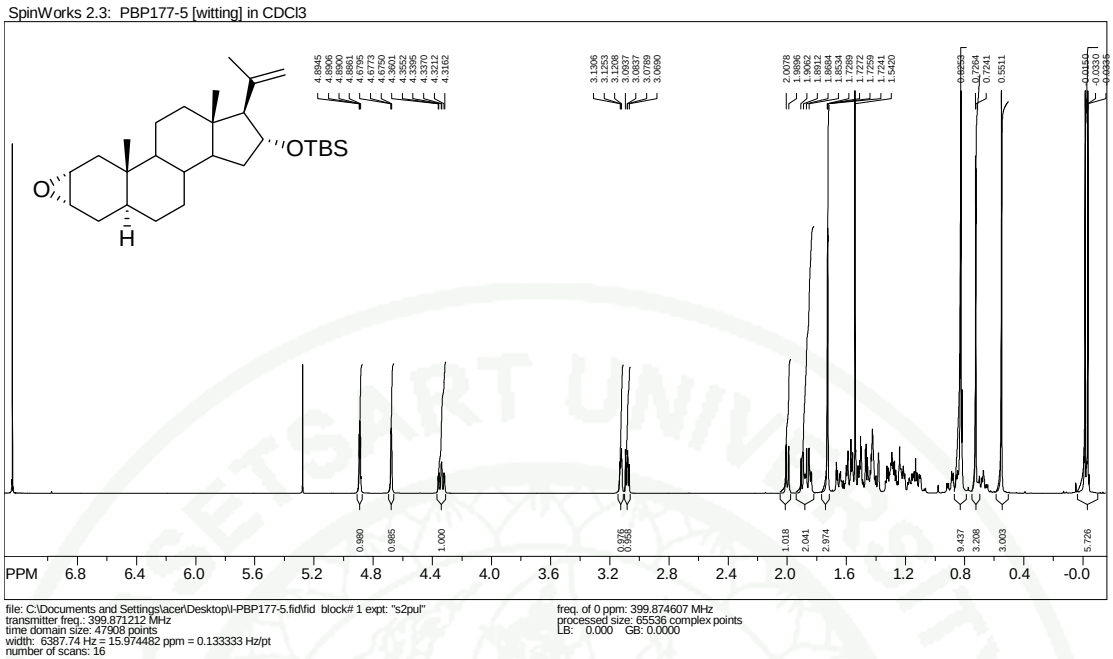
Appendix Figure 45 100 MHz ^{13}C NMR spectrum: 16α-*tert*-butyldimethylsilyloxy-5α-2-pregnen-20-one (114)



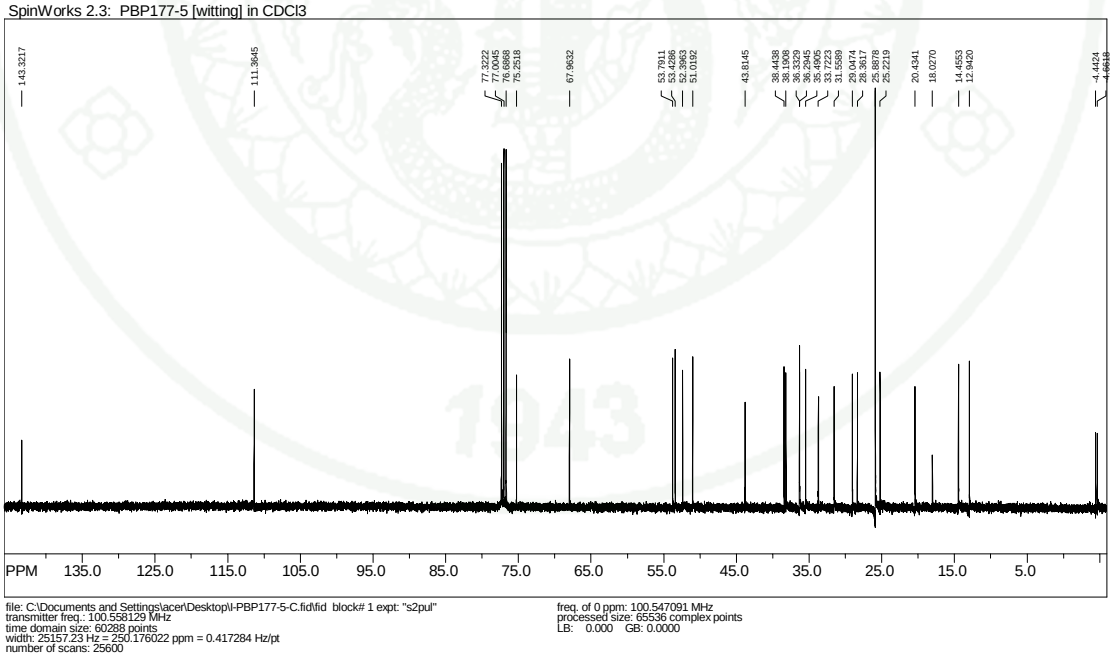
Appendix Figure 46 400 MHz ^{1}H NMR spectrum: 16α-*tert*-butyldimethylsilyloxy -2α, 3α-epoxy-5α-pregnan-20-one (115)



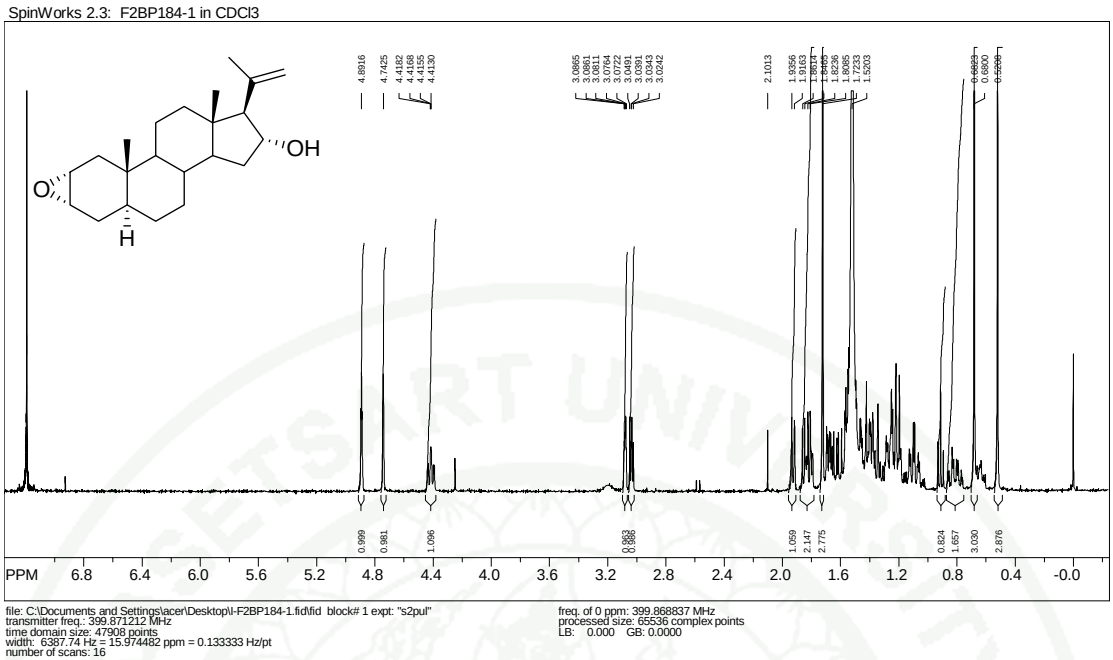
Appendix Figure 47 100 MHz ^{13}C NMR spectrum: 16α-*tert*-butyldimethylsilyloxy -2α, 3α-epoxy-5α-pregnan-20-one (115)



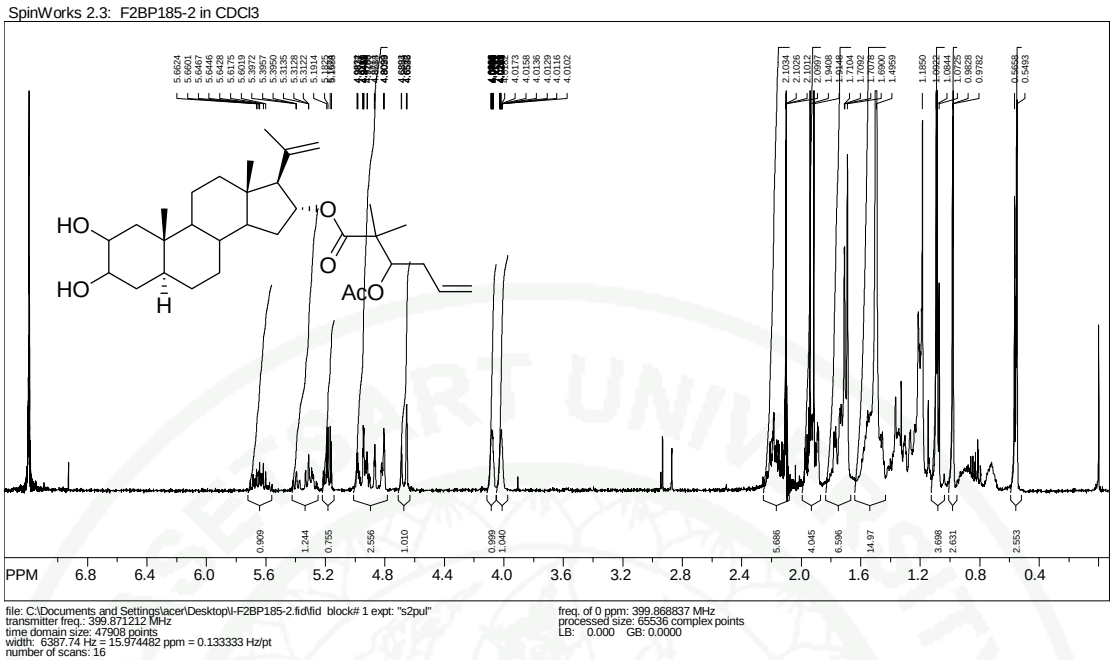
Appendix Figure 48 400 MHz ^{1}H NMR spectrum: 16α-*tert*-butyldimethylsilyloxy-2α, 3α-epoxy-20-methyl-5α-20-pregnene (116)



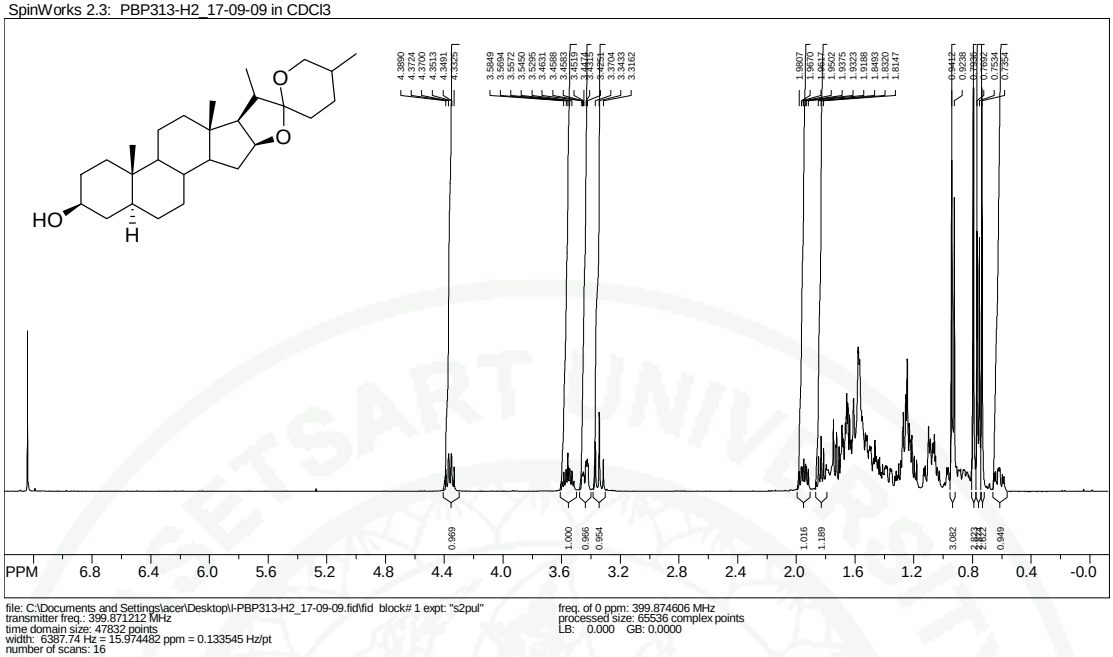
Appendix Figure 49 100 MHz ^{13}C NMR spectrum: 16α-*tert*-butyldimethylsilyloxy-2α, 3α-epoxy-20-methyl-5α-20-pregnene (116)



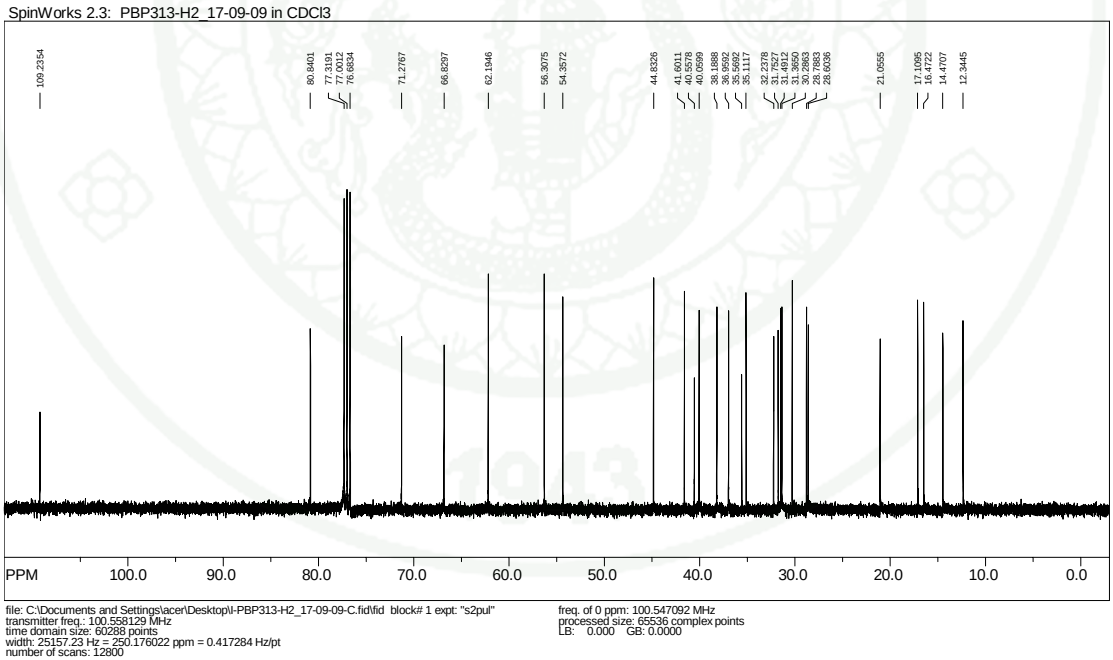
Appendix Figure 50 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-16α-hydroxy-20-methyl-5α-20-pregnene (117)



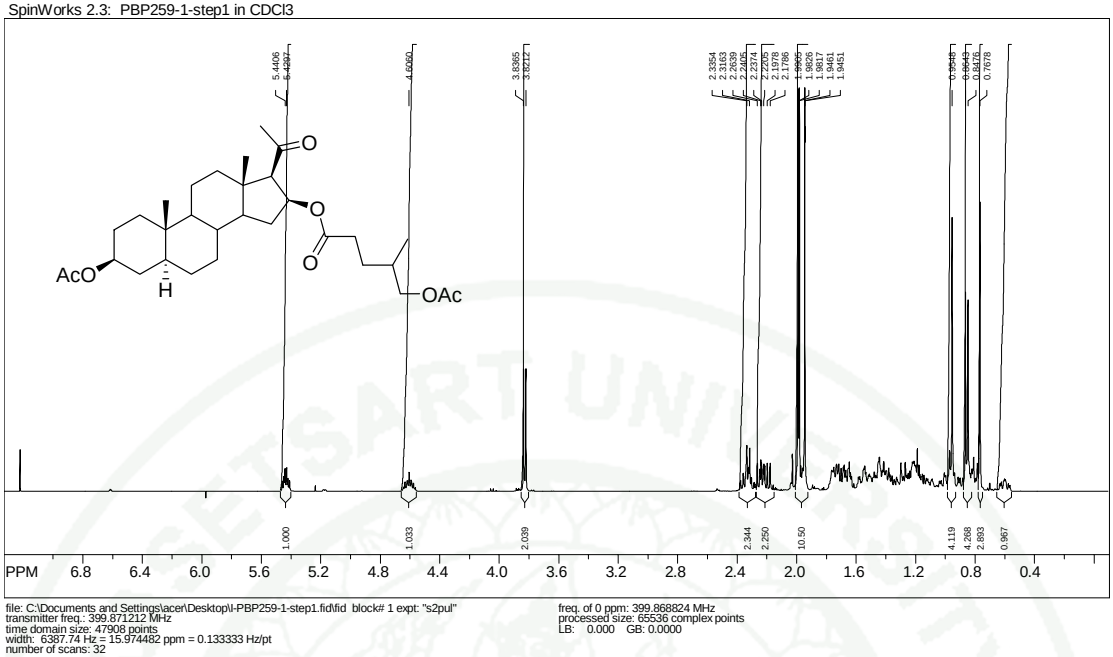
Appendix Figure 51 400 MHz ^{1}H NMR spectrum: 16β-(3-acetoxy-2, 2-dimethyl-5-hexenoate)-2, 3-dihydroxy-20-methyl-5α-20-pregnene (118)



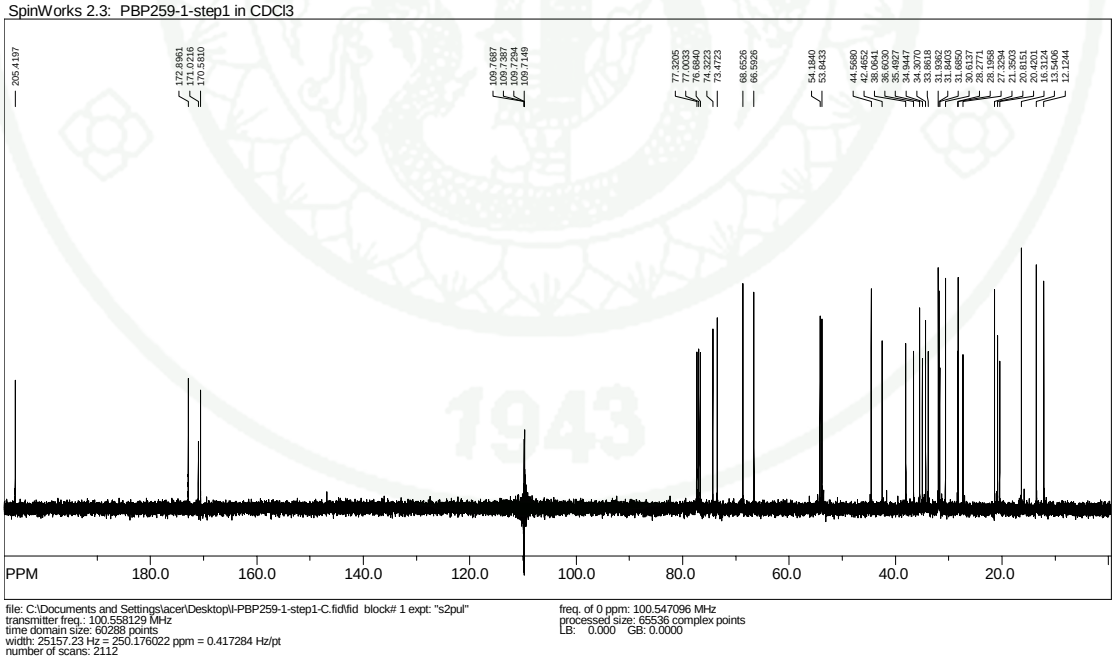
Appendix Figure 52 400 MHz ^{1}H NMR spectrum: tigogenin (54)



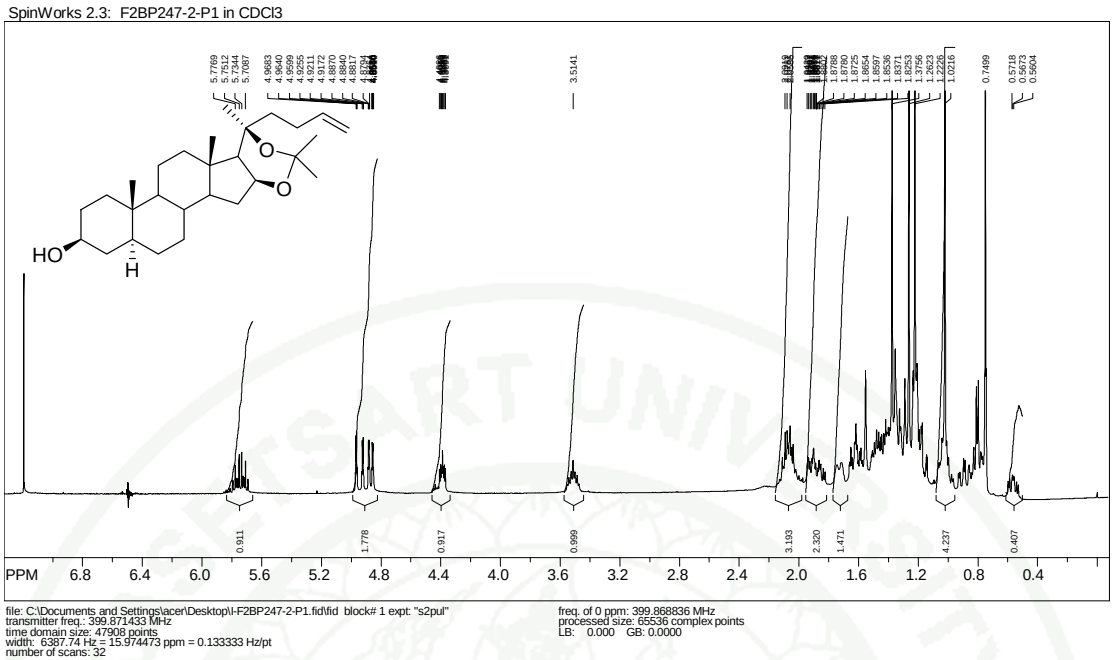
Appendix Figure 53 100 MHz ^{13}C NMR spectrum: tigogenin (54)



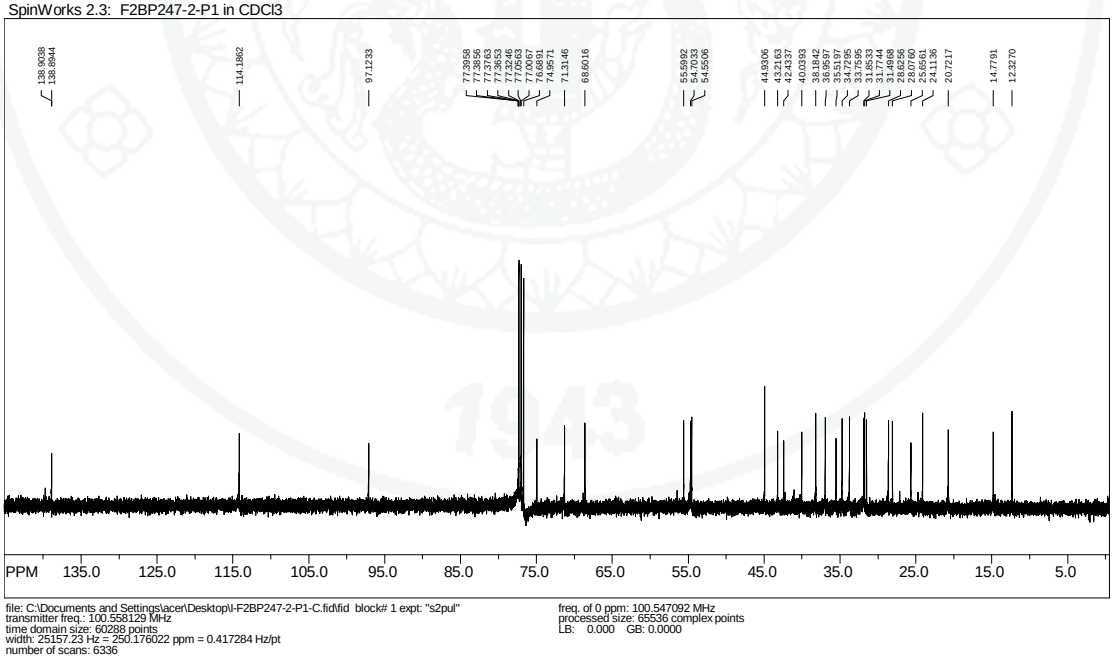
Appendix Figure 54 400 MHz ^{1}H NMR spectrum: 3β-acetoxy-16β-(5-acetoxy-4-methylpentanoate)-pregnan-20-one (60)



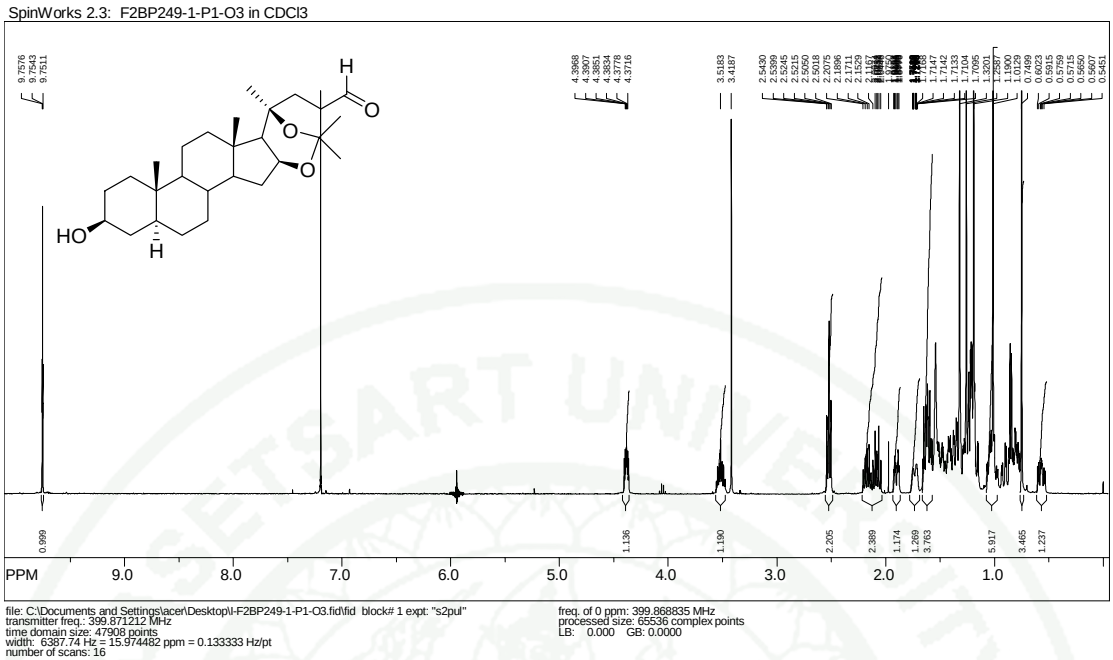
Appendix Figure 55 100 MHz ^{13}C NMR spectrum: 3β-acetoxy-16β-(5-acetoxy-4-methylpentanoate)-pregnan-20-one (60)



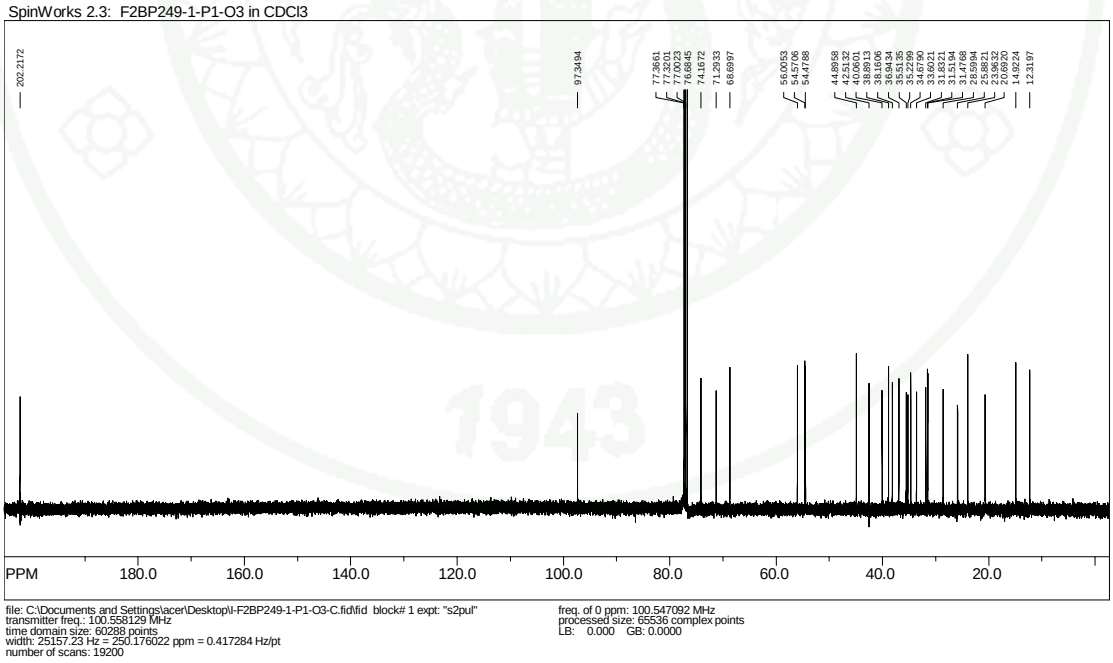
Appendix Figure 56 400 MHz ^{1}H NMR spectrum: 3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119)



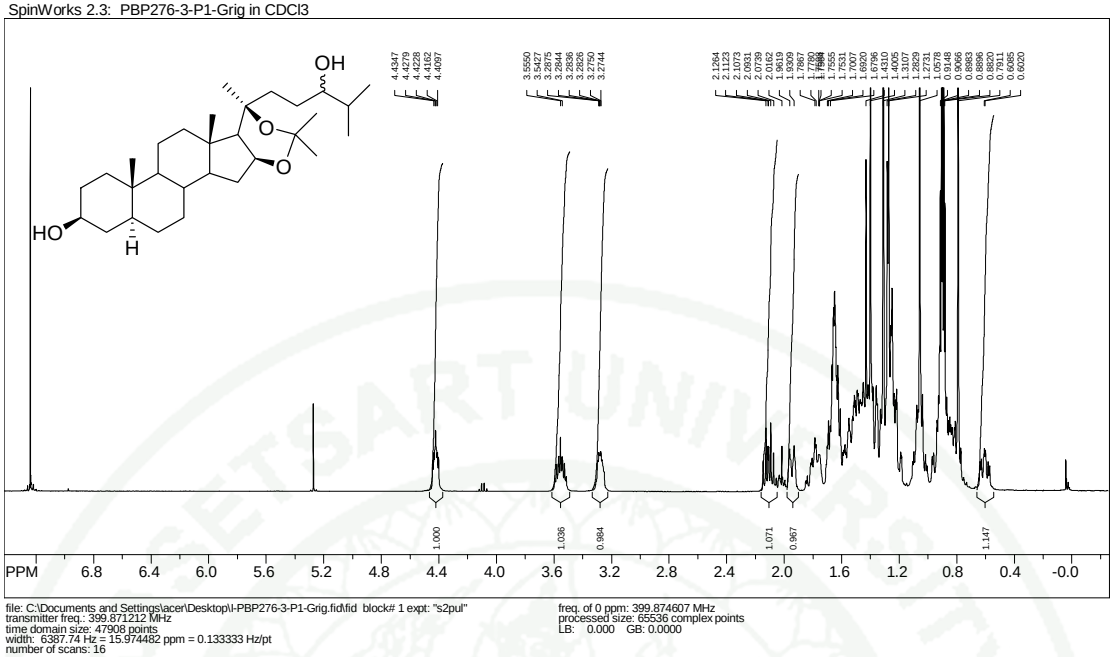
Appendix Figure 57 100 MHz ^{13}C NMR spectrum: 3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (119)



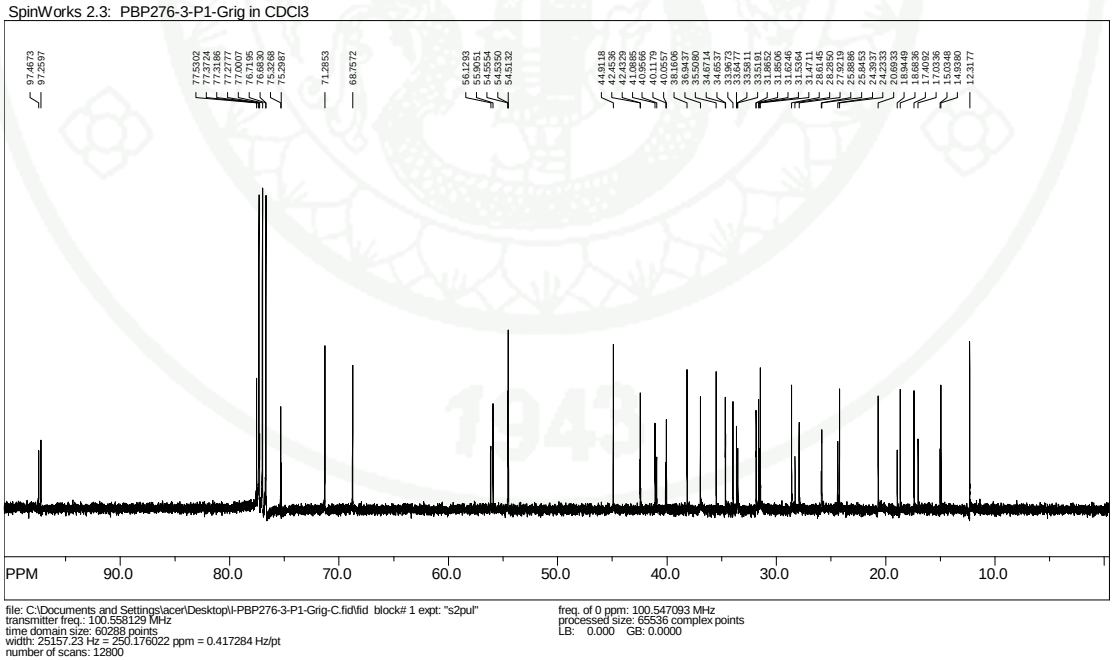
Appendix Figure 58 400 MHz ^{1}H NMR spectrum: 24-formyl-3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholane (120)



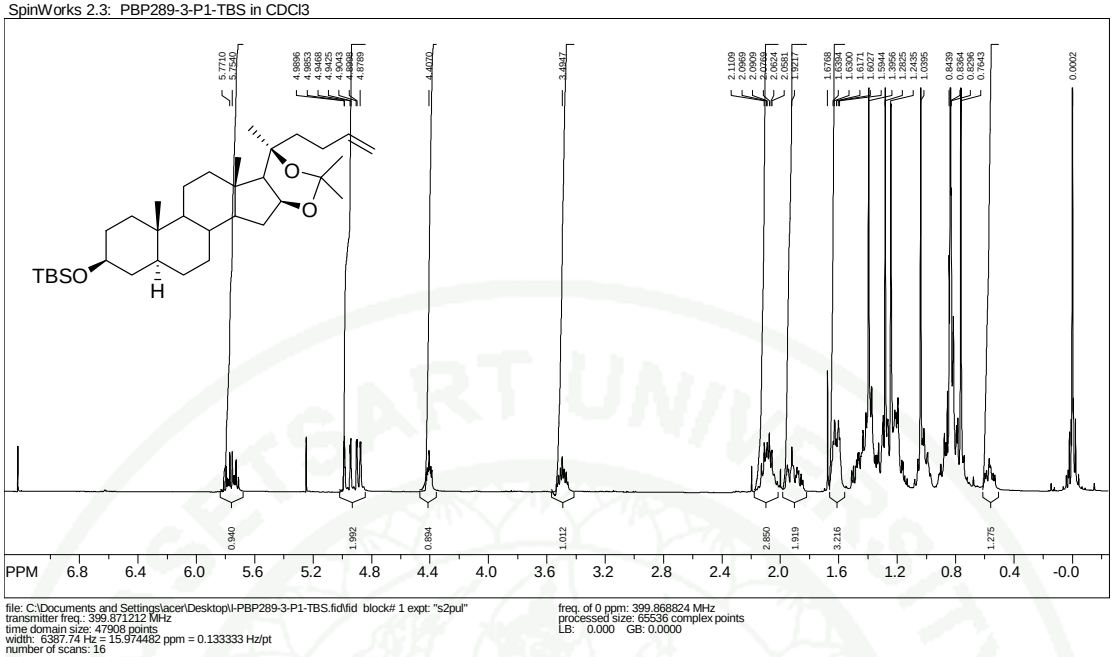
Appendix Figure 59 100 MHz ^{13}C NMR spectrum: 24-formyl-3β-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholane (120)



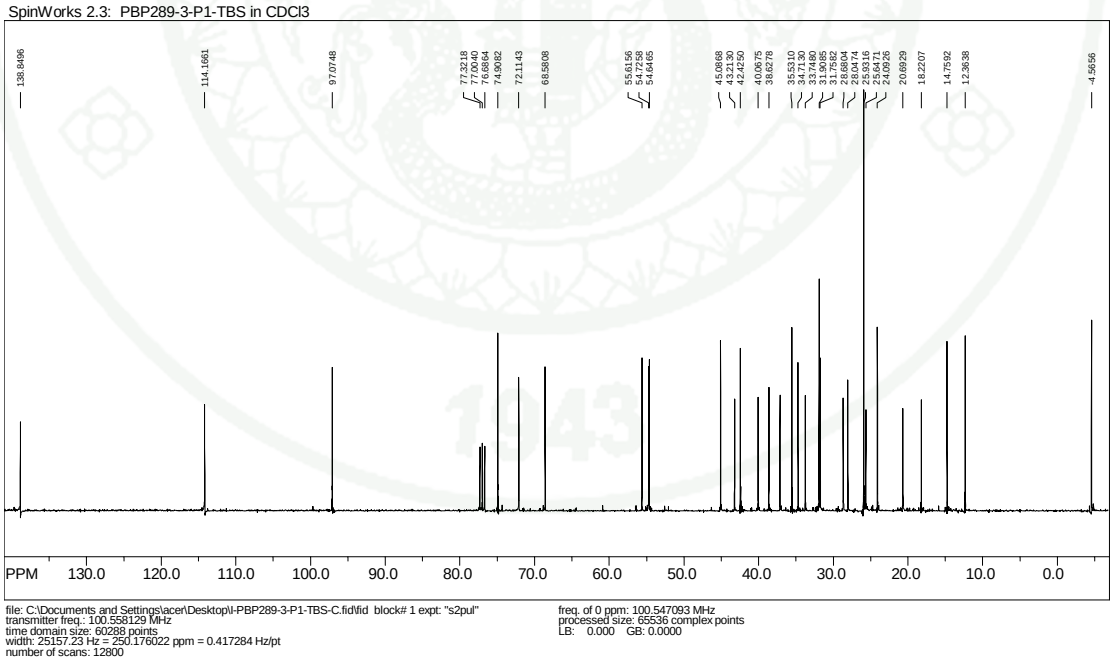
Appendix Figure 60 400 MHz ^{1}H NMR spectrum: 3β, 24-dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (121)



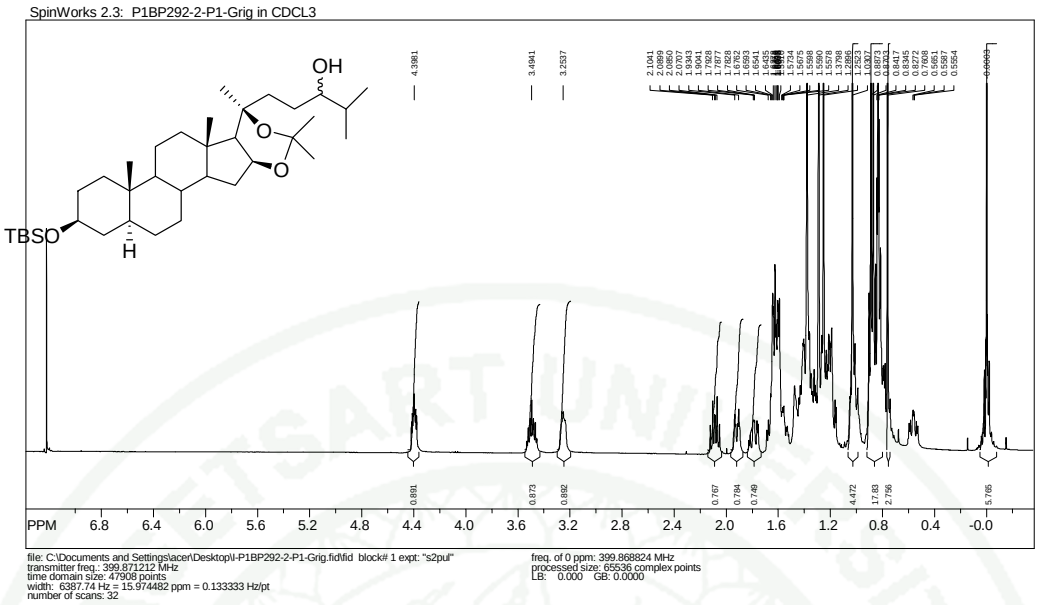
Appendix Figure 61 100 MHz ^{13}C NMR spectrum: 3β, 24-dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (121)



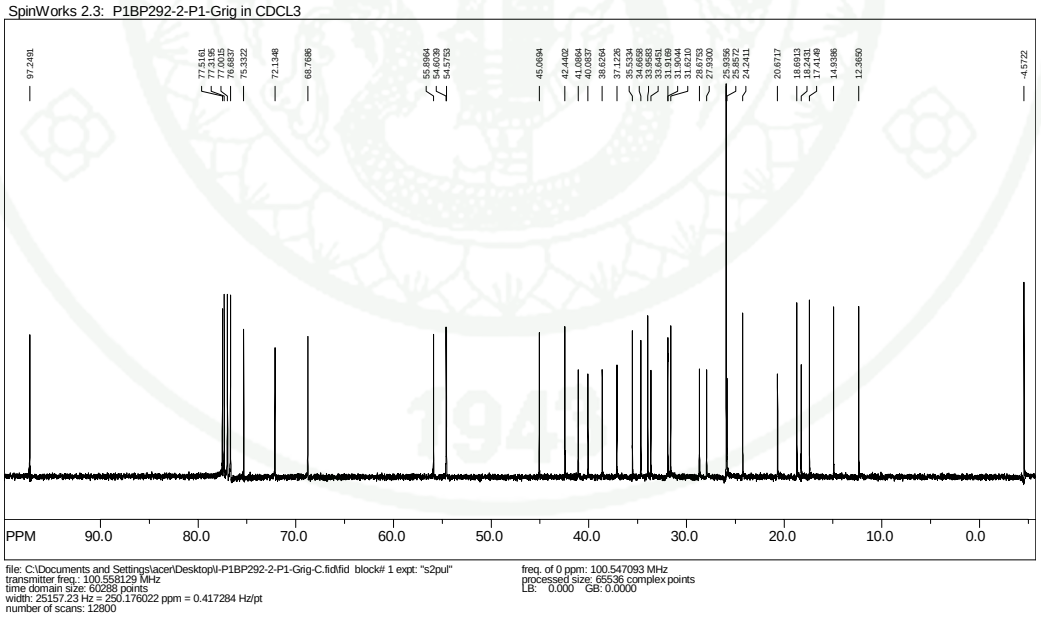
Appendix Figure 62 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (123)



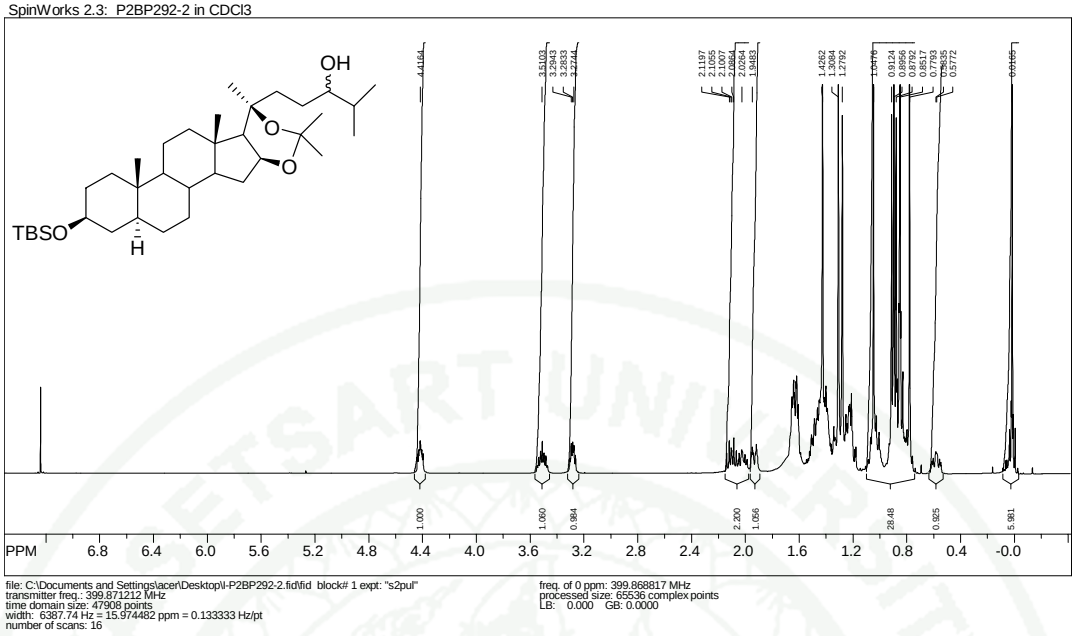
Appendix Figure 63 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-24a-homo-chol-24-ene (123)



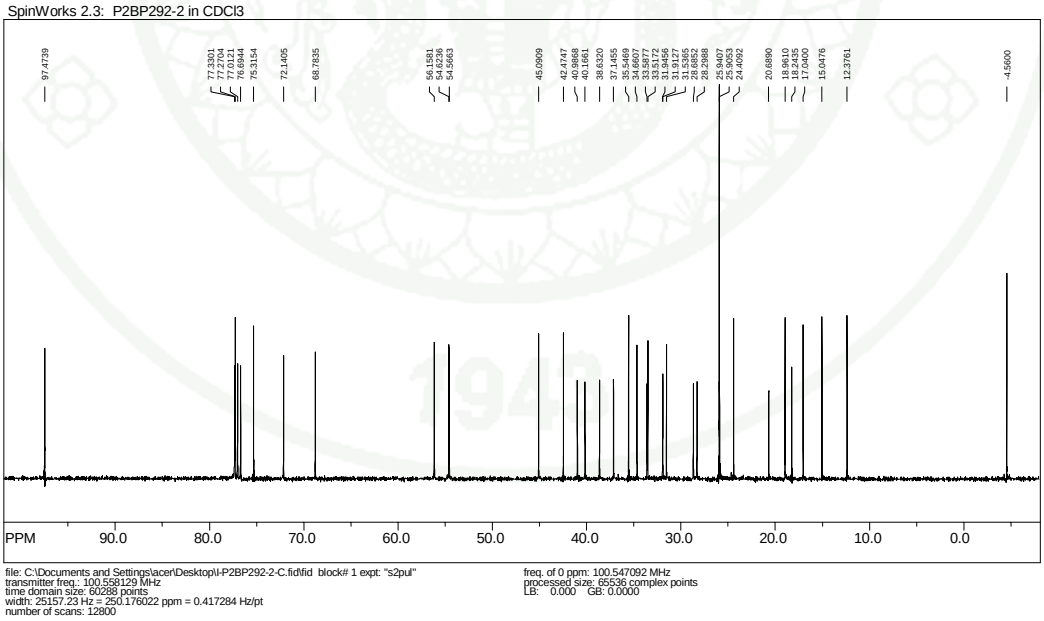
Appendix Figure 64 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (Isomer I)



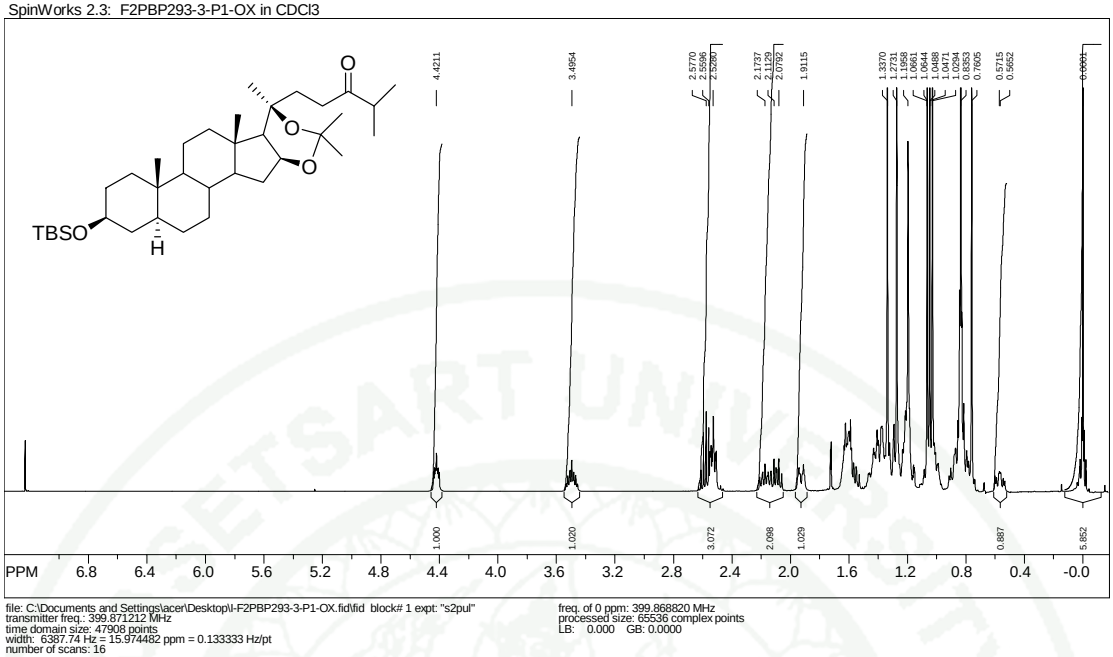
Appendix Figure 65 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (Isomer I)



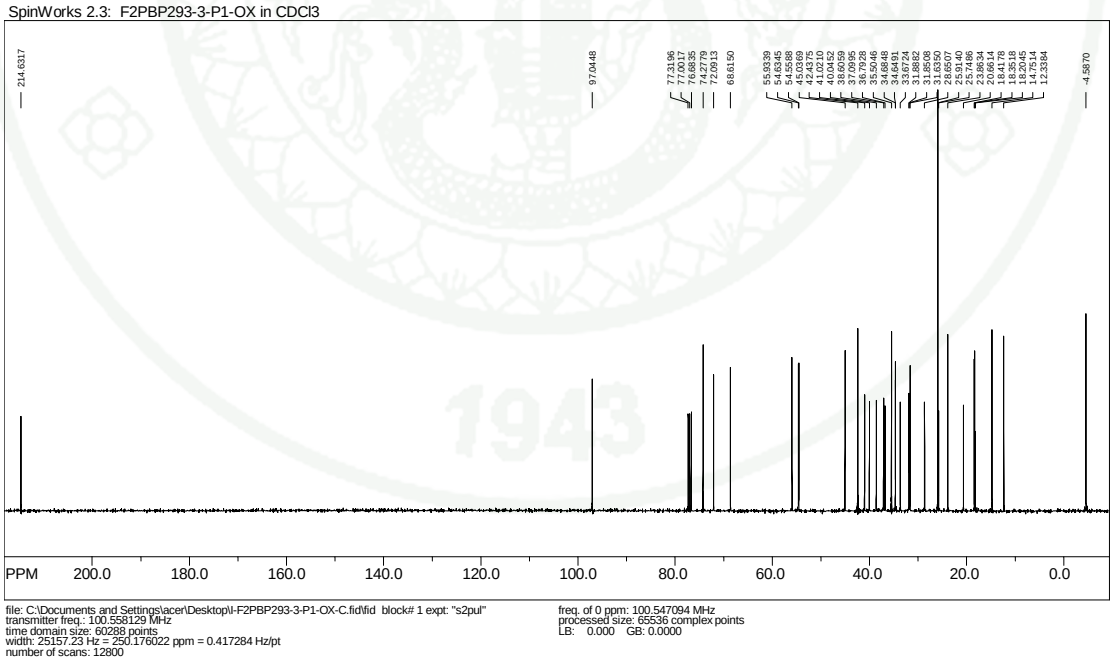
Appendix Figure 66 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (Isomer II)



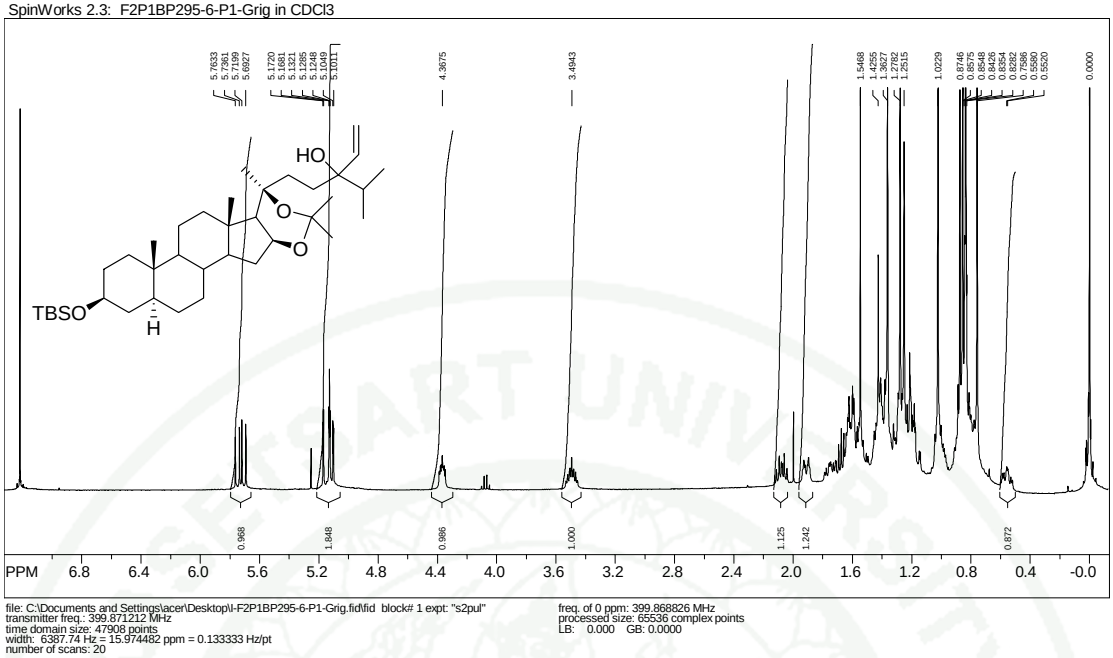
Appendix Figure 67 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (124) (Isomer II)



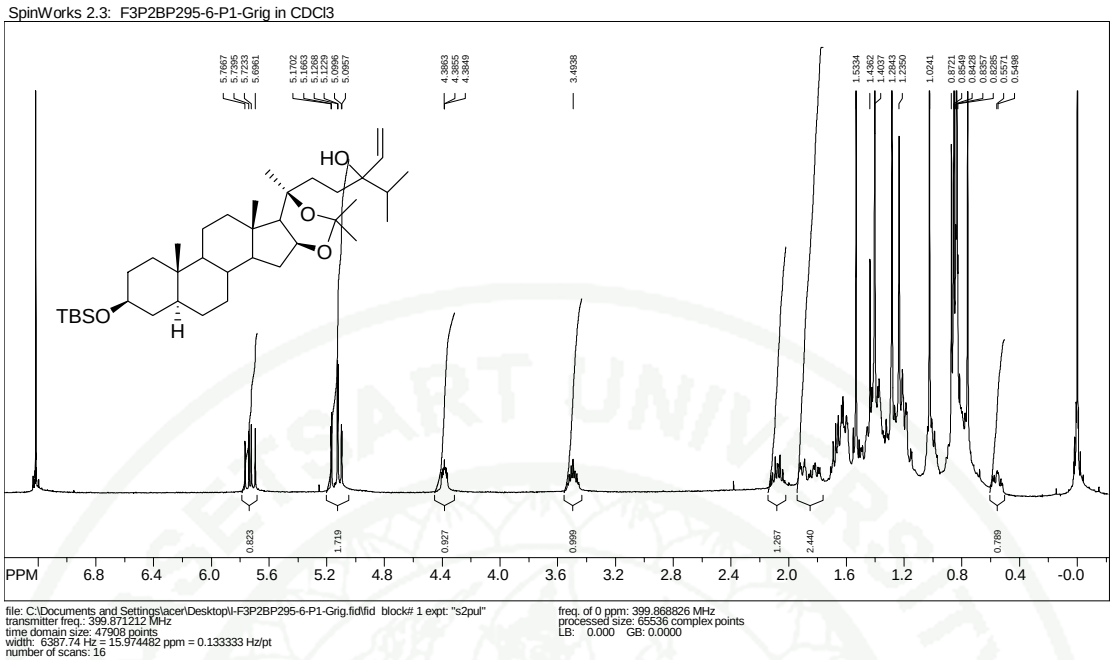
Appendix Figure 68 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125)



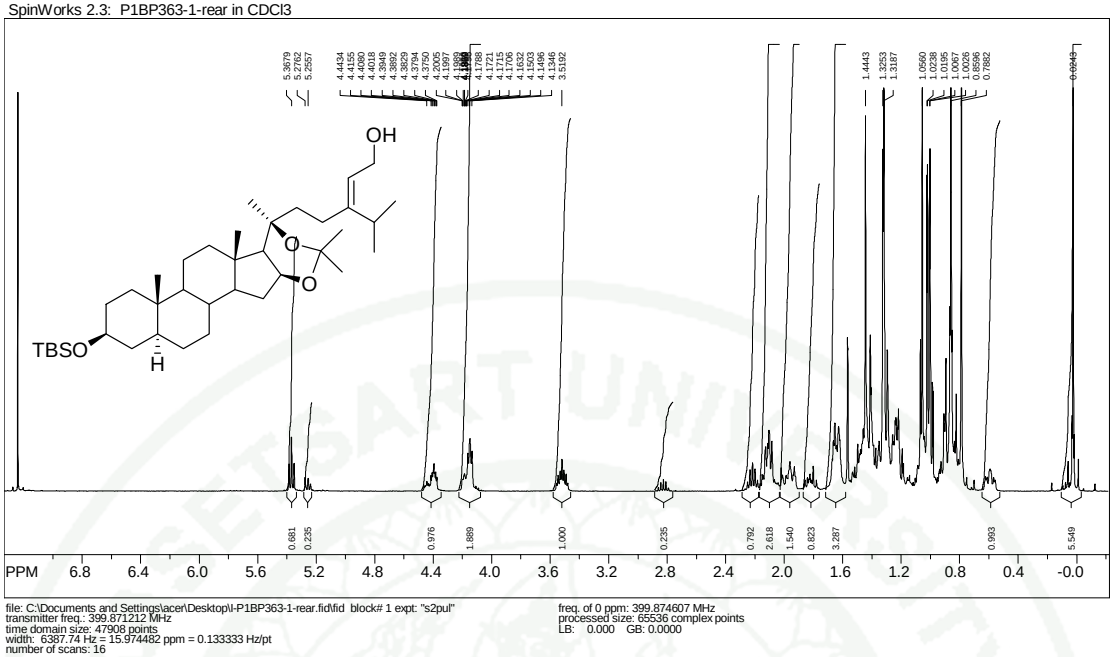
Appendix Figure 69 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestan-24-one (125)



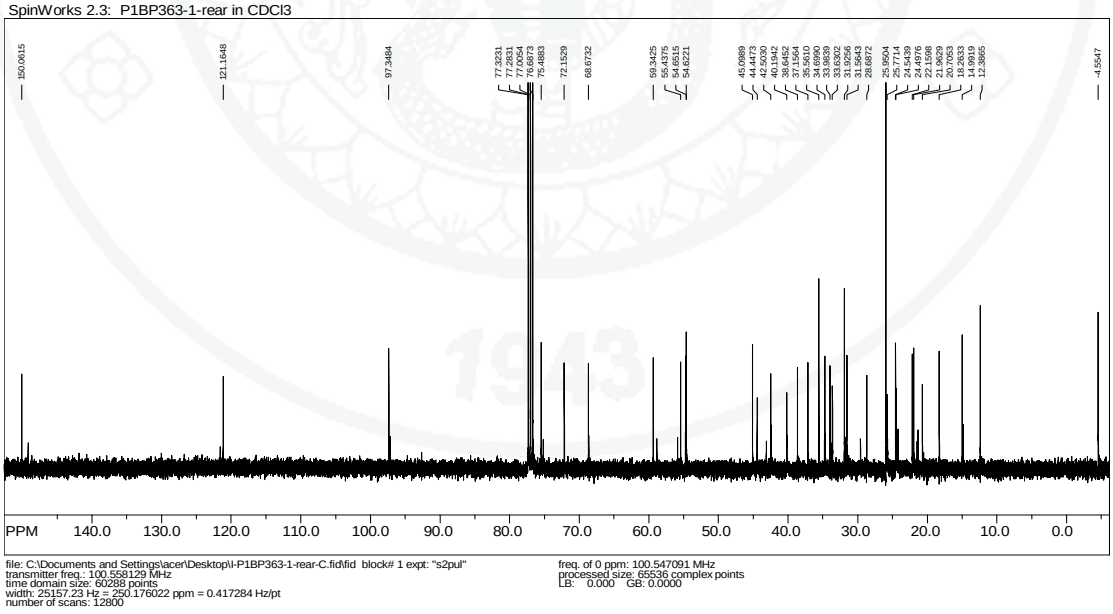
Appendix Figure 70 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126) (Isomer I)



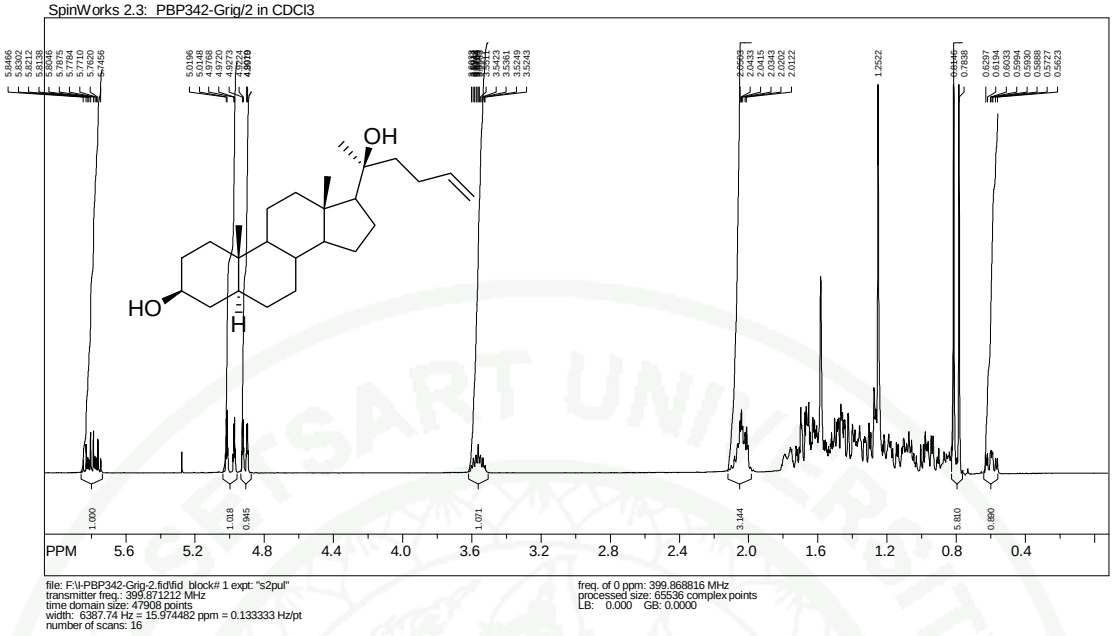
Appendix Figure 71 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-24-ethenyl-24-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholestane (126) (Isomer II)



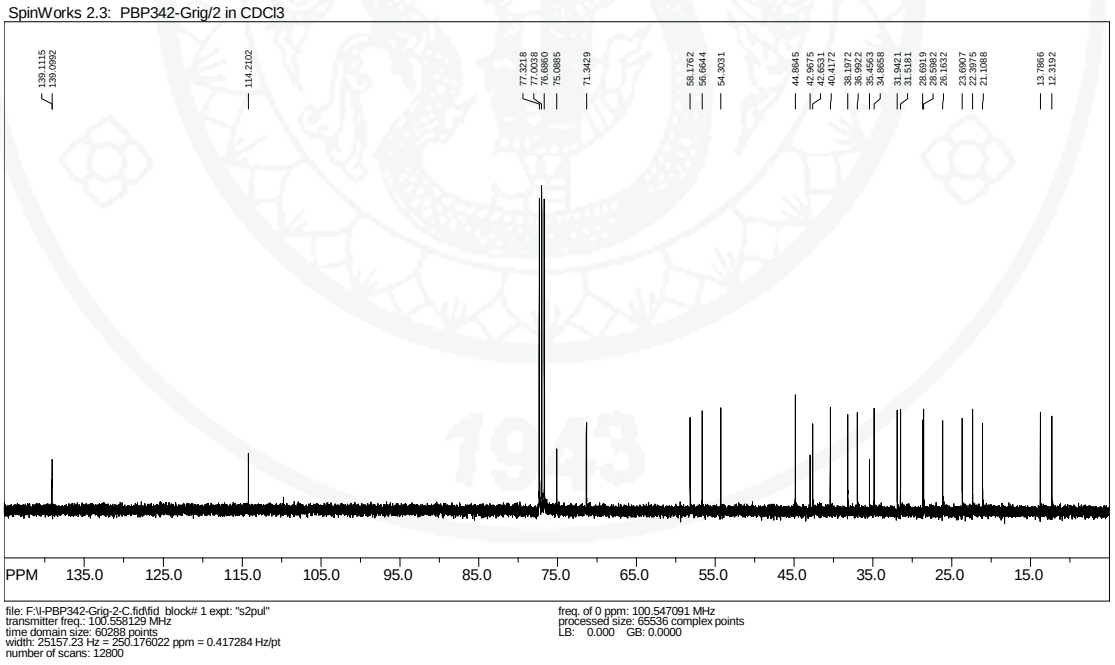
Appendix Figure 72 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127)



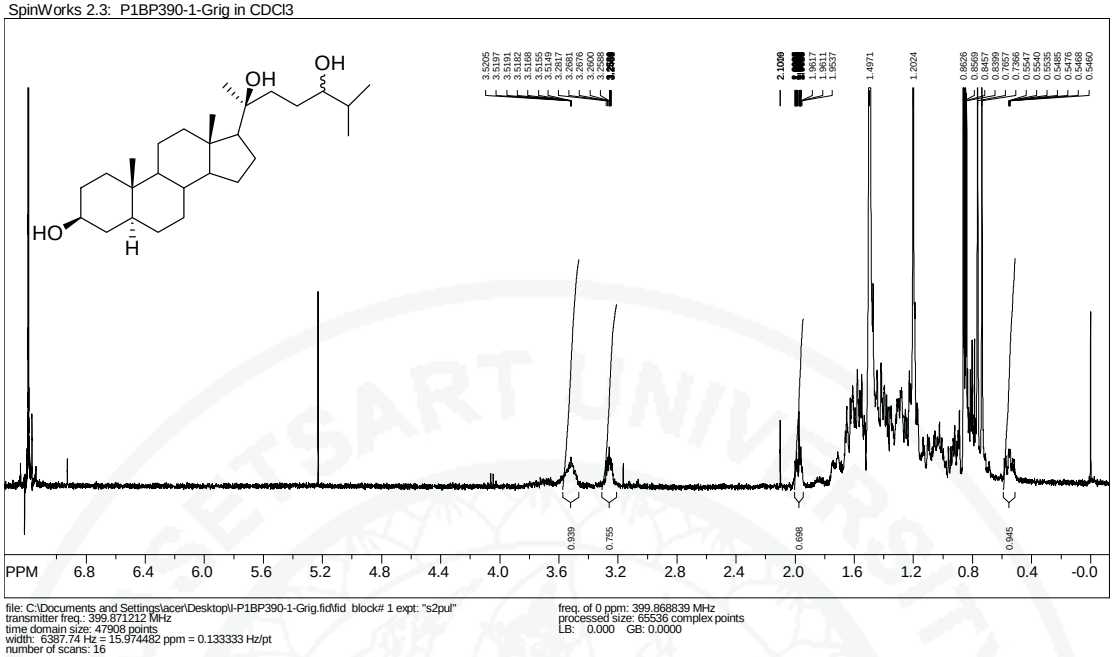
Appendix Figure 73 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-29-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-5α-cholest-24(28)-ene (127)



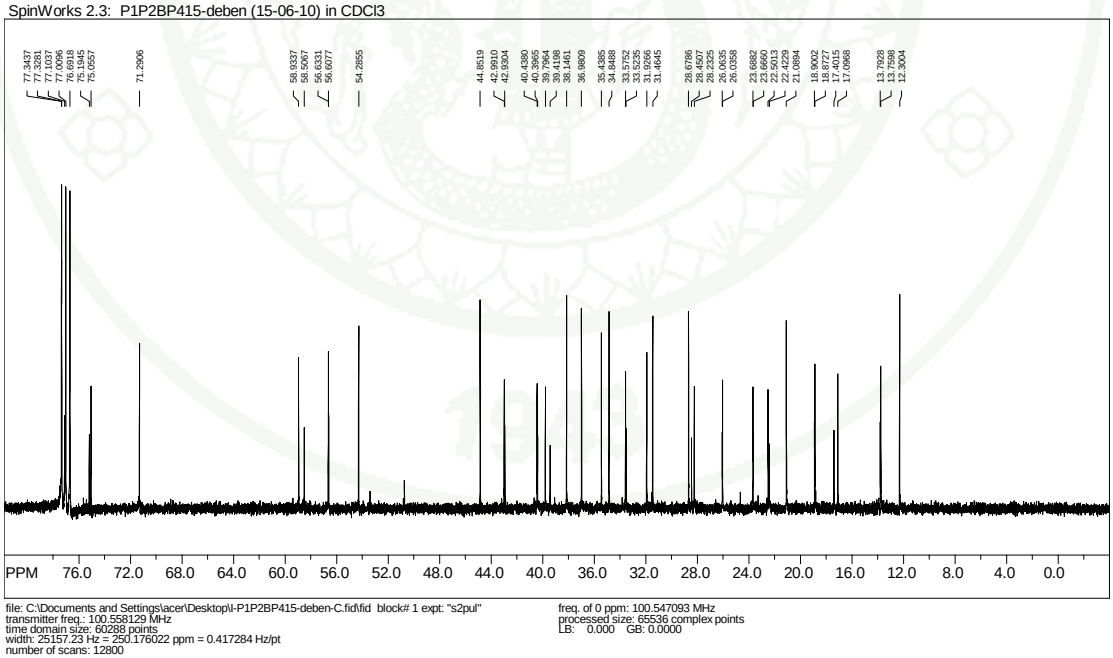
Appendix Figure 74 400 MHz ^{1}H NMR spectrum: 3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129)



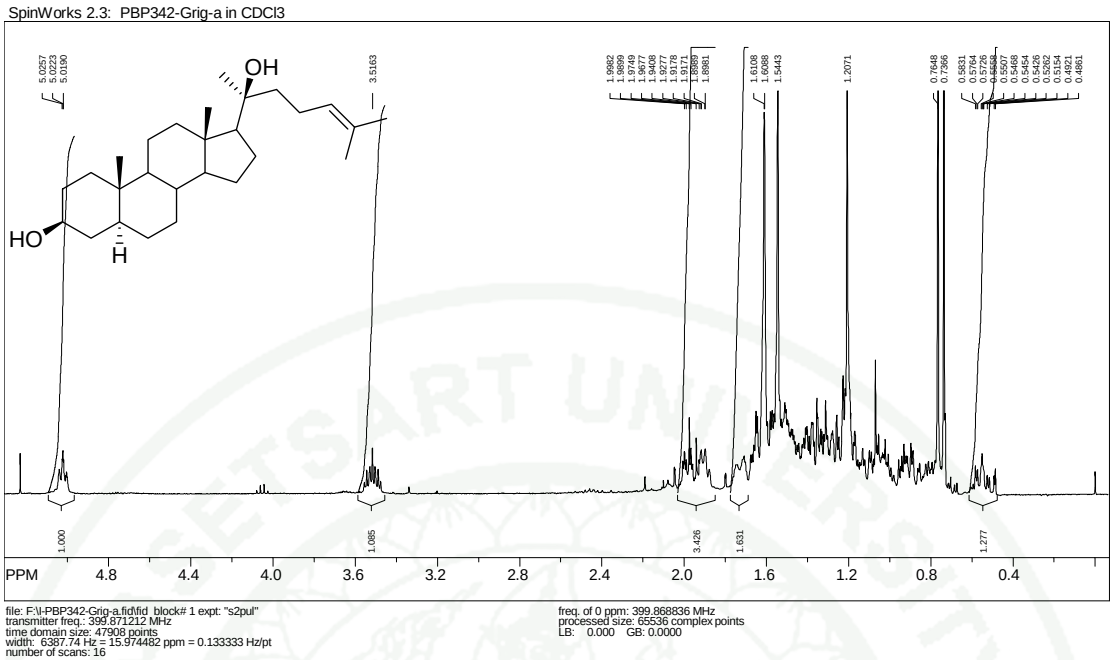
Appendix Figure 75 100 MHz ^{13}C NMR spectrum: 3β, 20(*S*)-dihydroxy-5α-24a-homo-chol-24-ene (129)



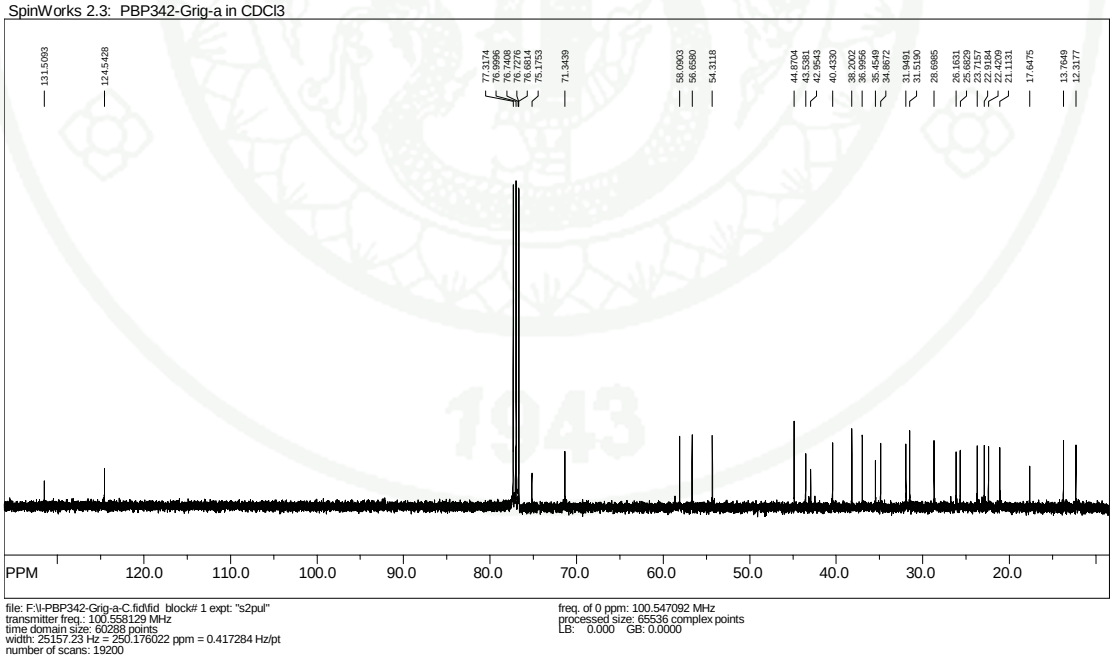
Appendix Figure 76 400 MHz ^{1}H NMR spectrum: 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130)



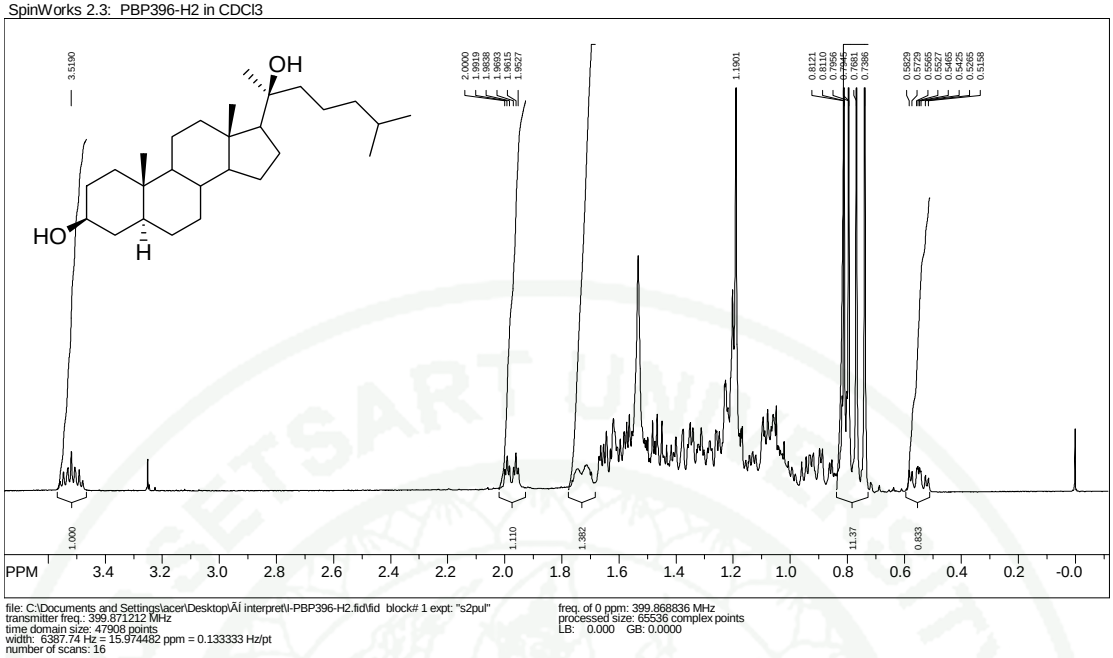
Appendix Figure 77 100 MHz ^{13}C NMR spectrum: 3β, 20(*S*), 24-trihydroxy-5α-cholestane (130)



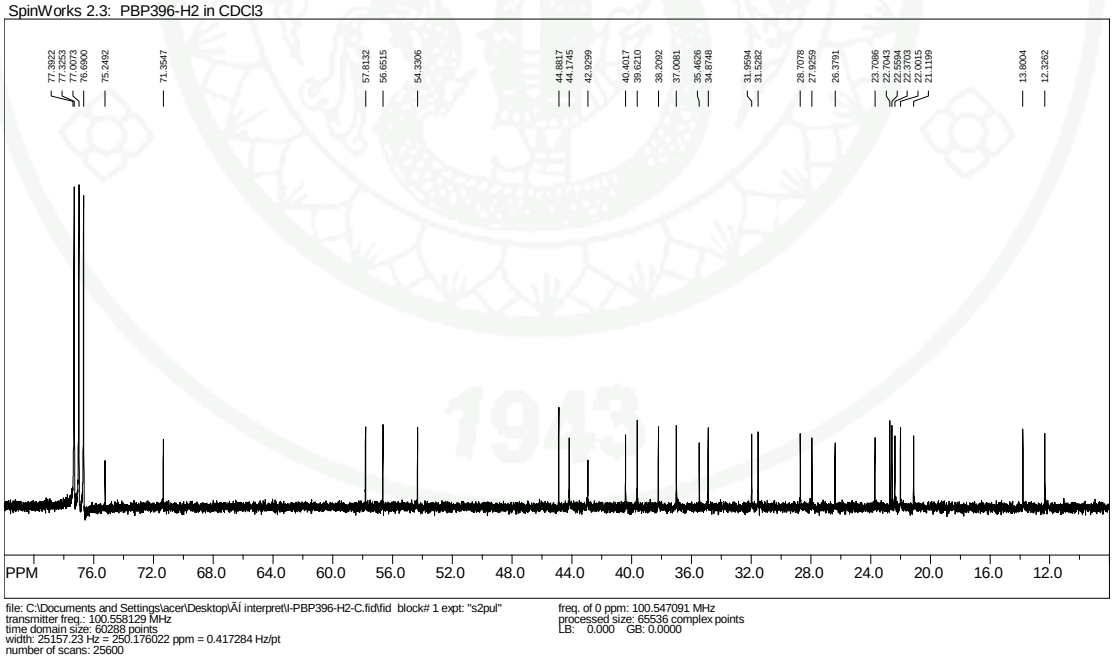
Appendix Figure 78 400 MHz ^{1}H NMR spectrum: 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131)



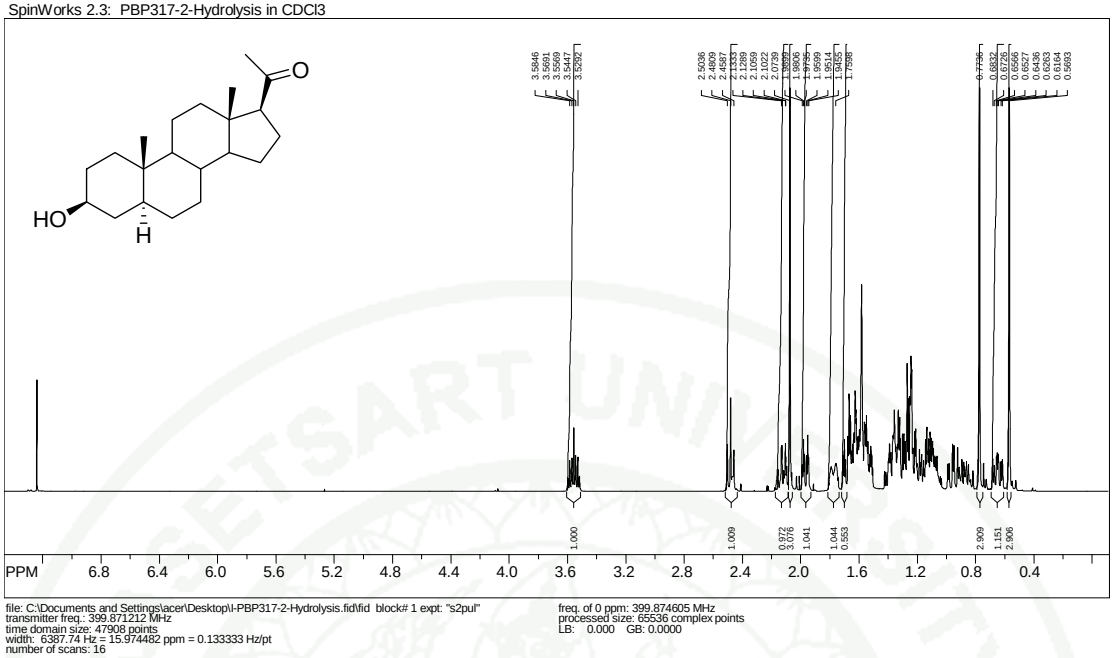
Appendix Figure 79 100 MHz ^{13}C NMR spectrum: 3β, 20(*S*)-dihydroxy-5α-cholest-24-ene (131)



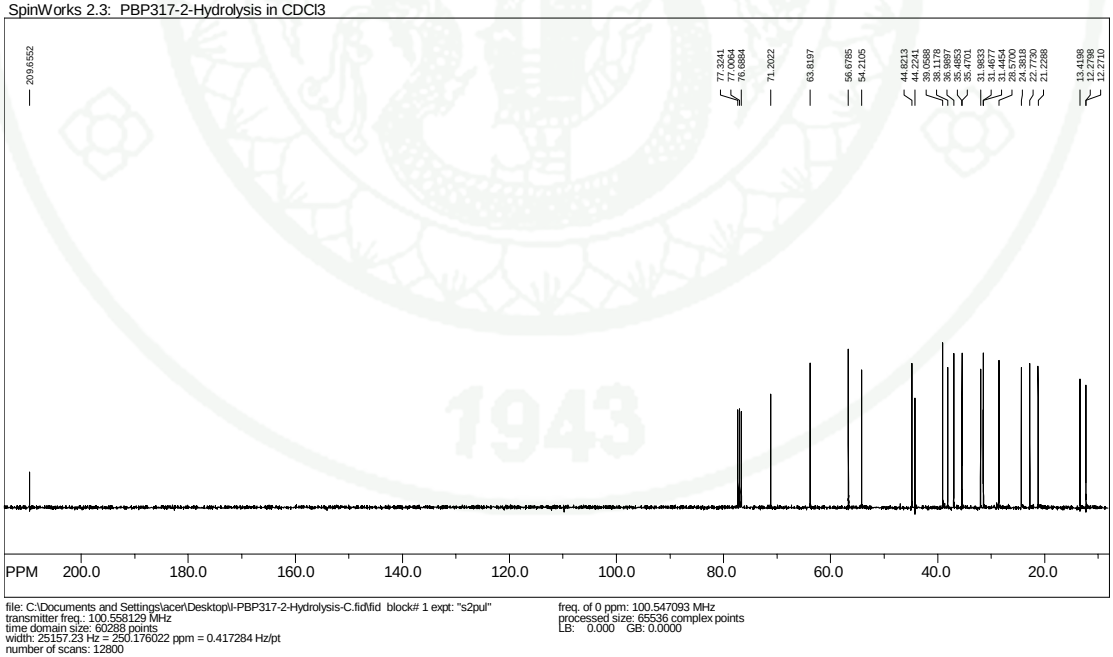
Appendix Figure 80 400 MHz ^{1}H NMR spectrum: 3β, 20(*S*)-dihydroxy-5α-cholestane (132)



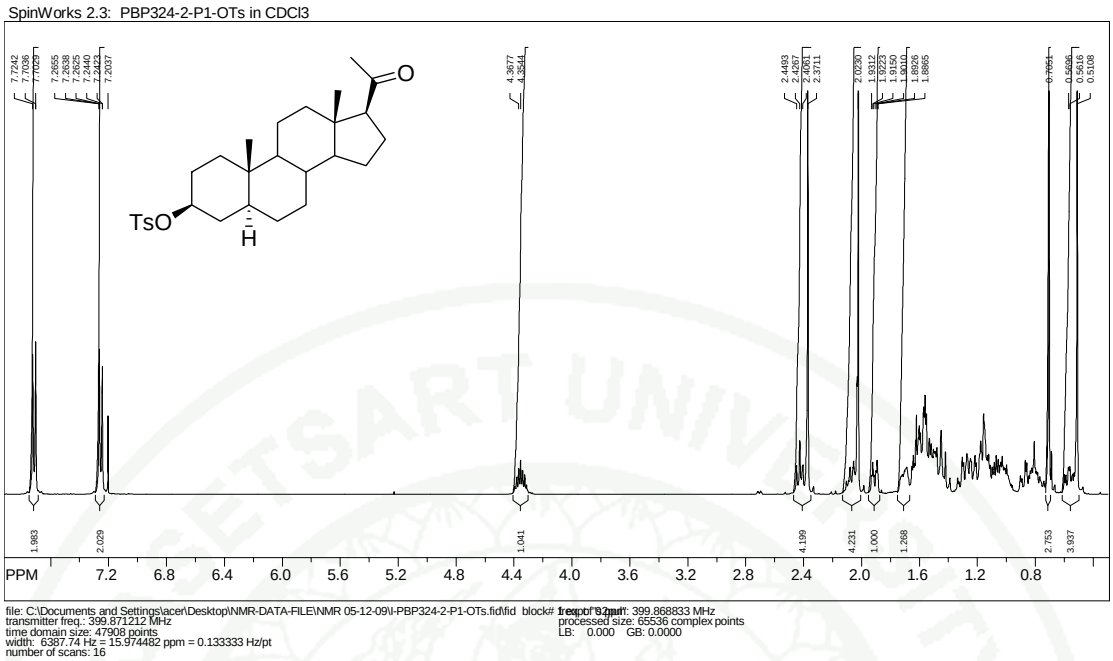
Appendix Figure 81 100 MHz ^{13}C NMR spectrum: 3β, 20(*S*)-dihydroxy-5α-cholestane (132)



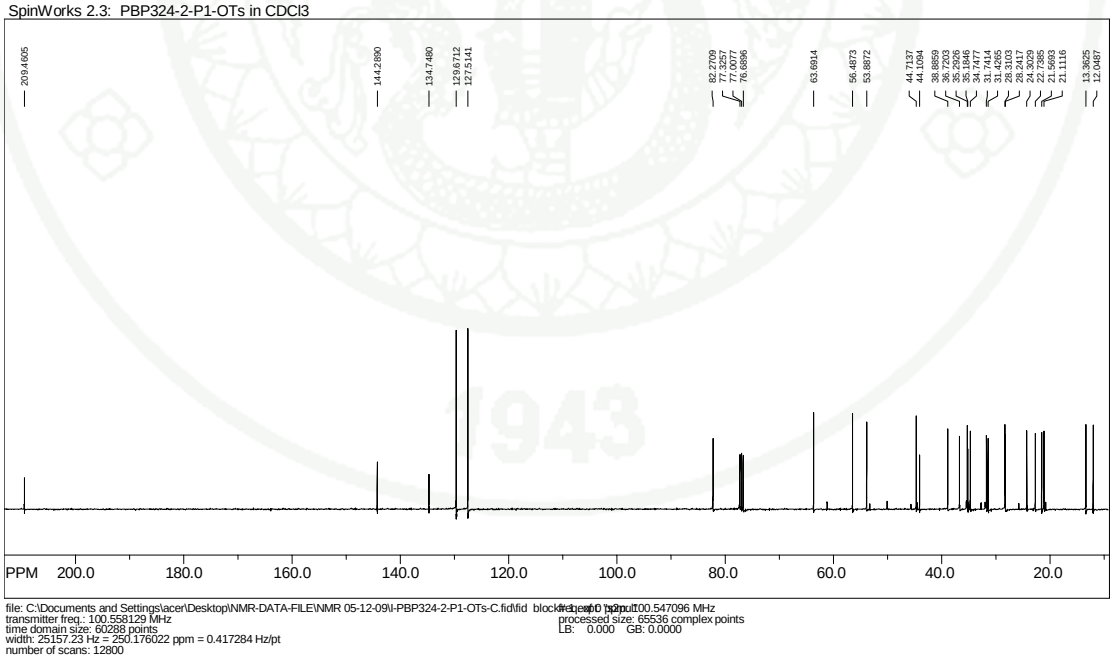
Appendix Figure 82 400 MHz ^{1}H NMR spectrum: 3β-hydroxy-5α-pregnan-20-one (133)



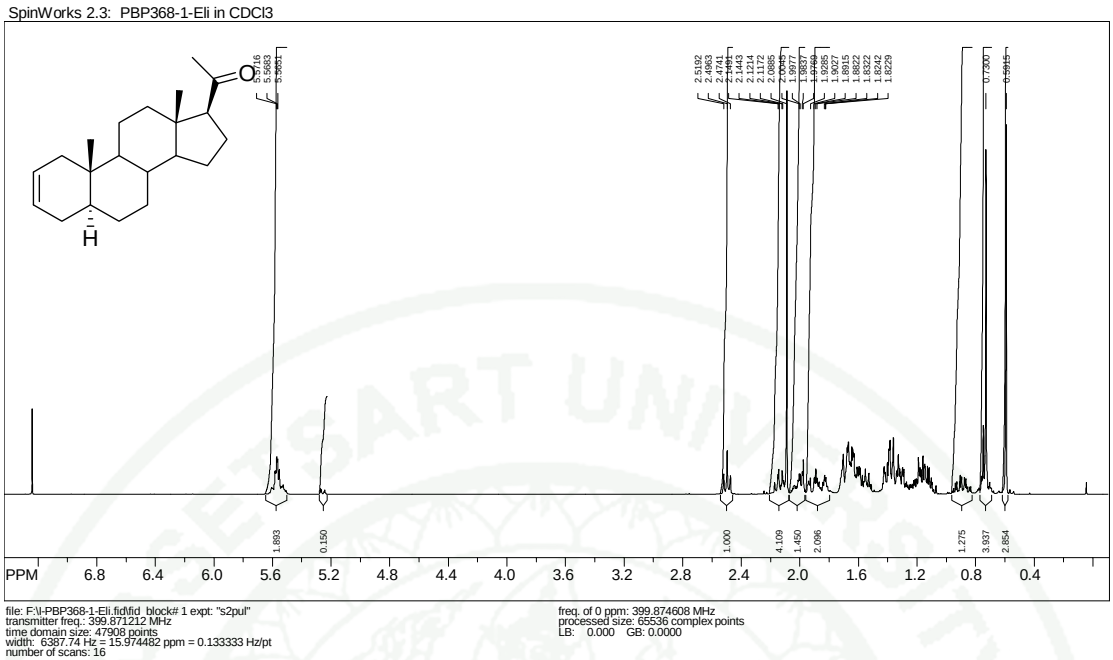
Appendix Figure 83 100 MHz ^{13}C NMR spectrum: 3β-hydroxy-5α-pregnan-20-one (133)



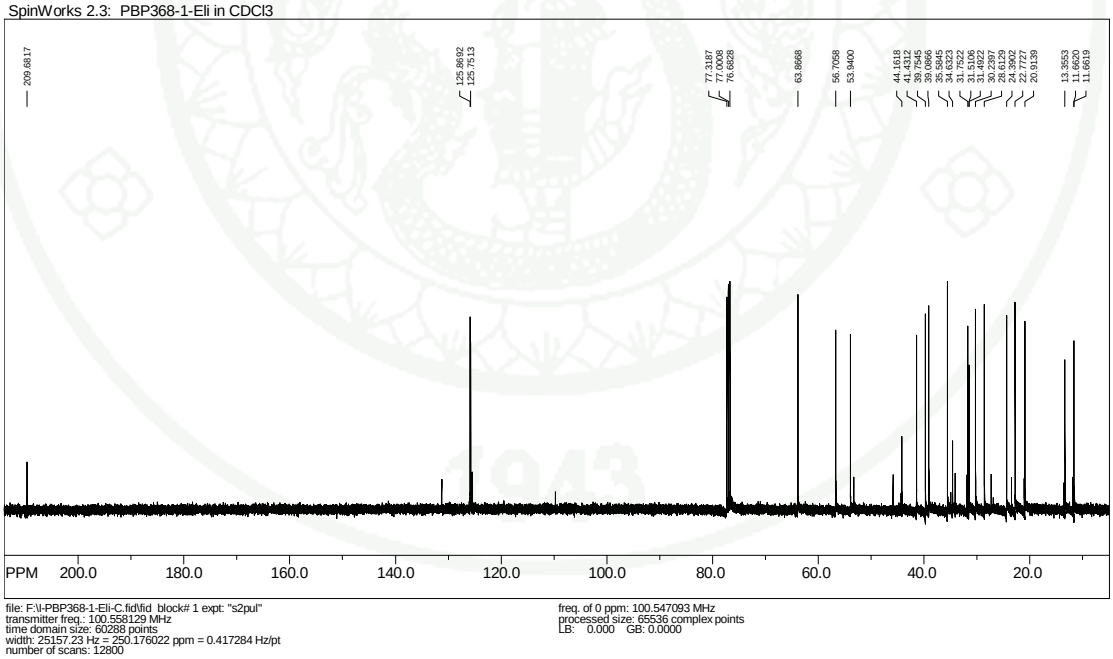
Appendix Figure 84 400 MHz ^{1}H NMR spectrum: 3β-tosyloxy-5α-pregnan-20-one (134)



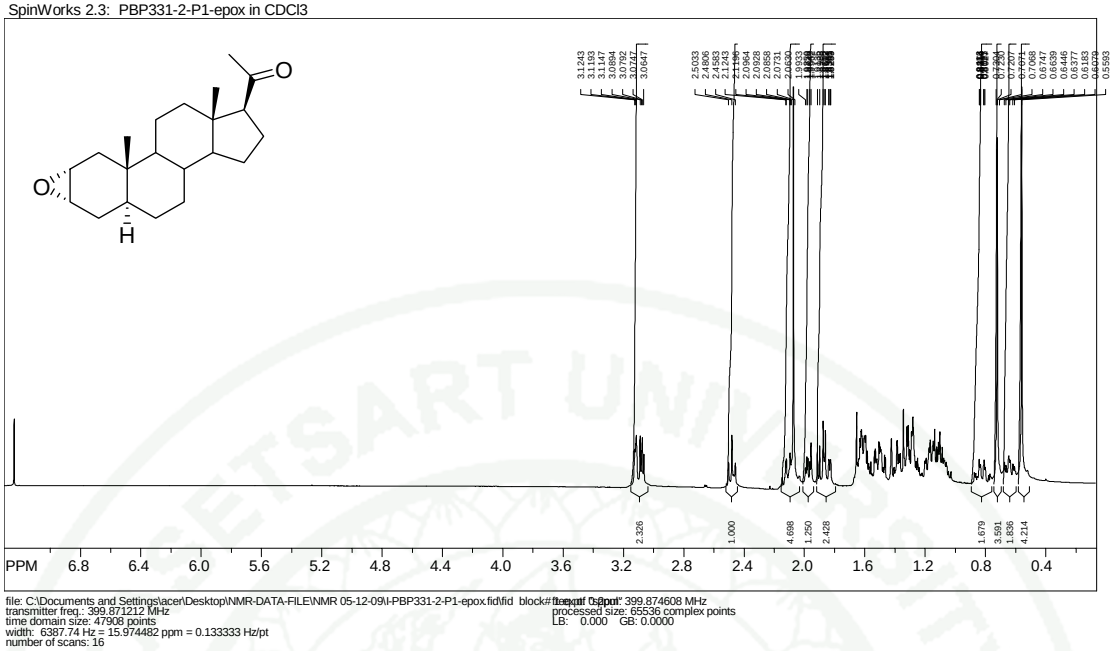
Appendix Figure 85 100 MHz ^{13}C NMR spectrum: 3β-tosyloxy-5α-pregnan-20-one (134)



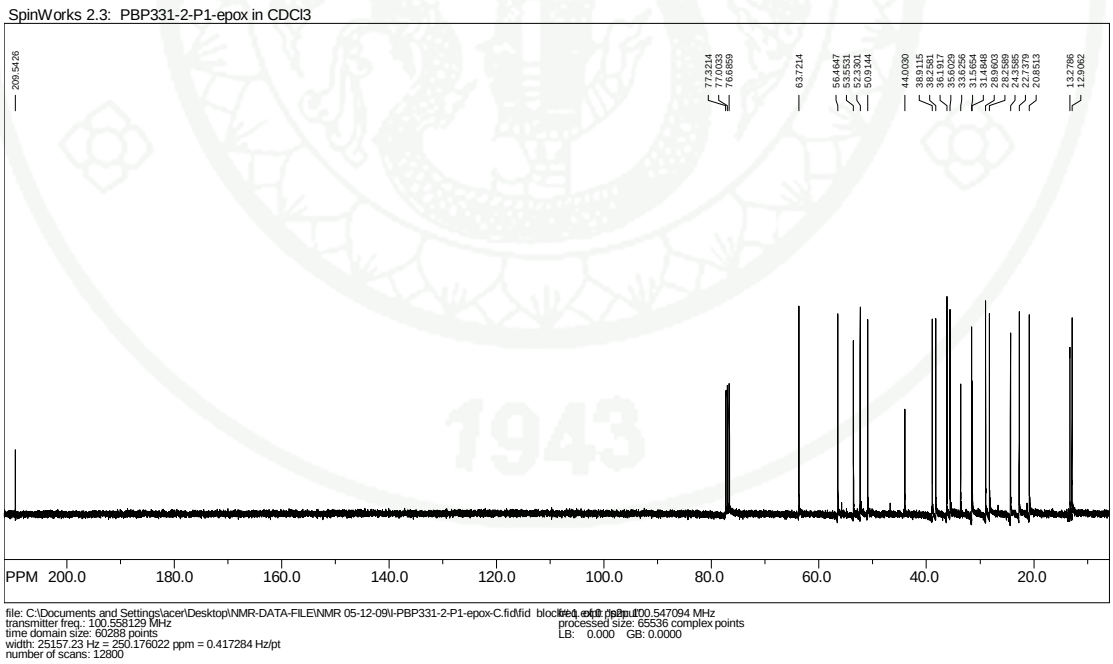
Appendix Figure 86 400 MHz ^{1}H NMR spectrum: 2-pregnen-20-one (135)



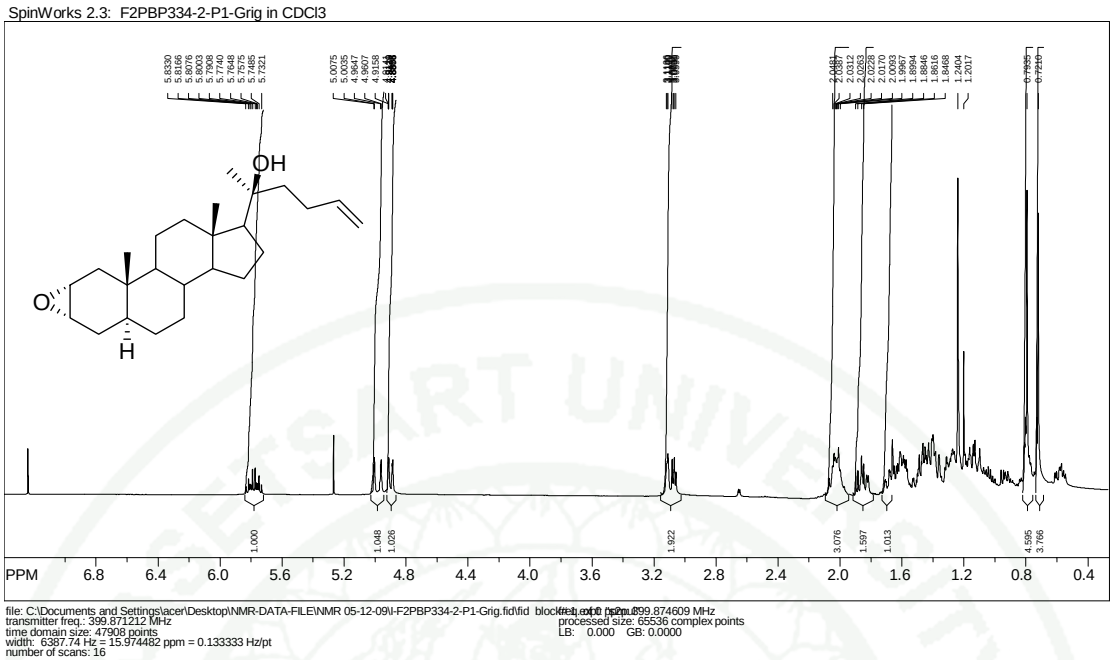
Appendix Figure 87 100 MHz ^{13}C NMR spectrum: 2-pregnen-20-one (135)



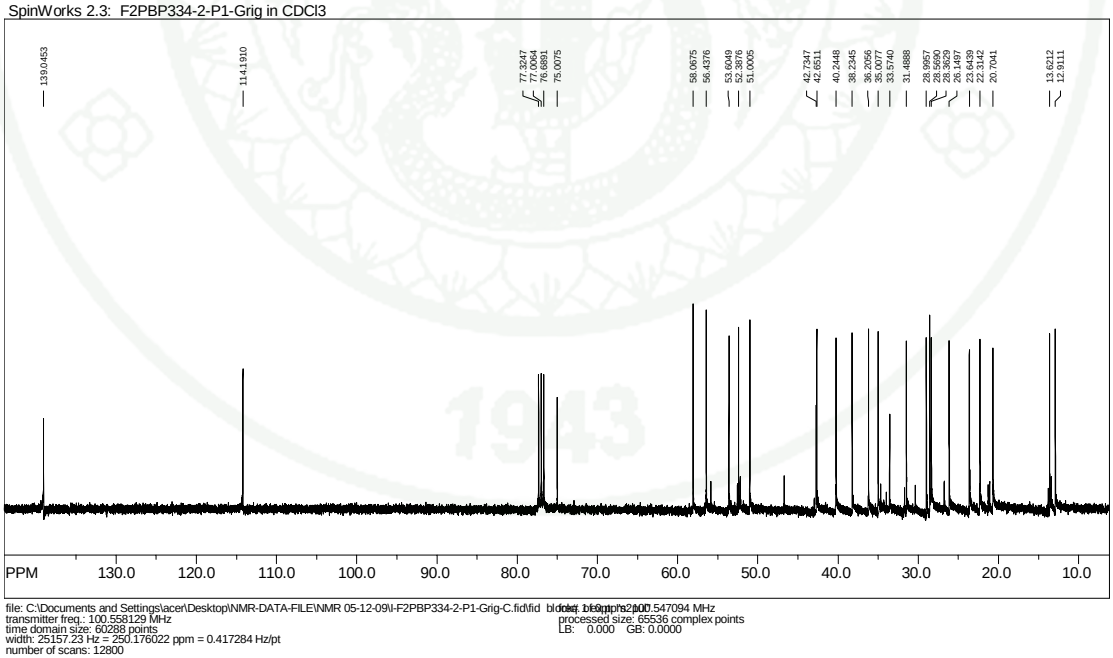
Appendix Figure 88 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-5α-pregnan-20-one (136)



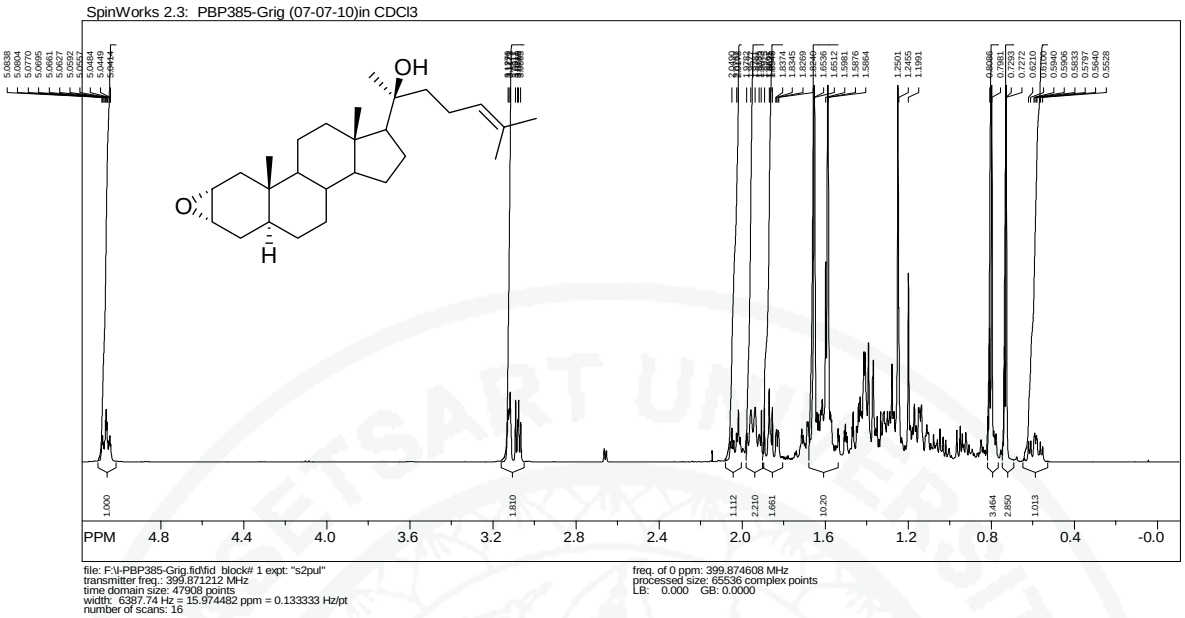
Appendix Figure 89 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-5α-pregnan-20-one (136)



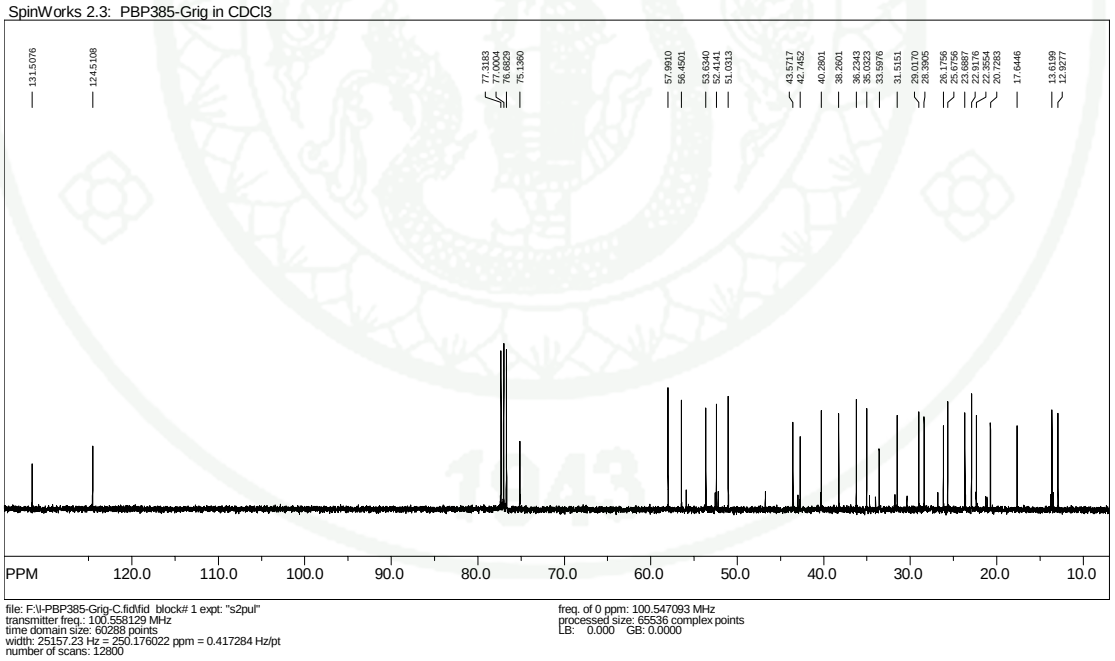
Appendix Figure 90 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (137)



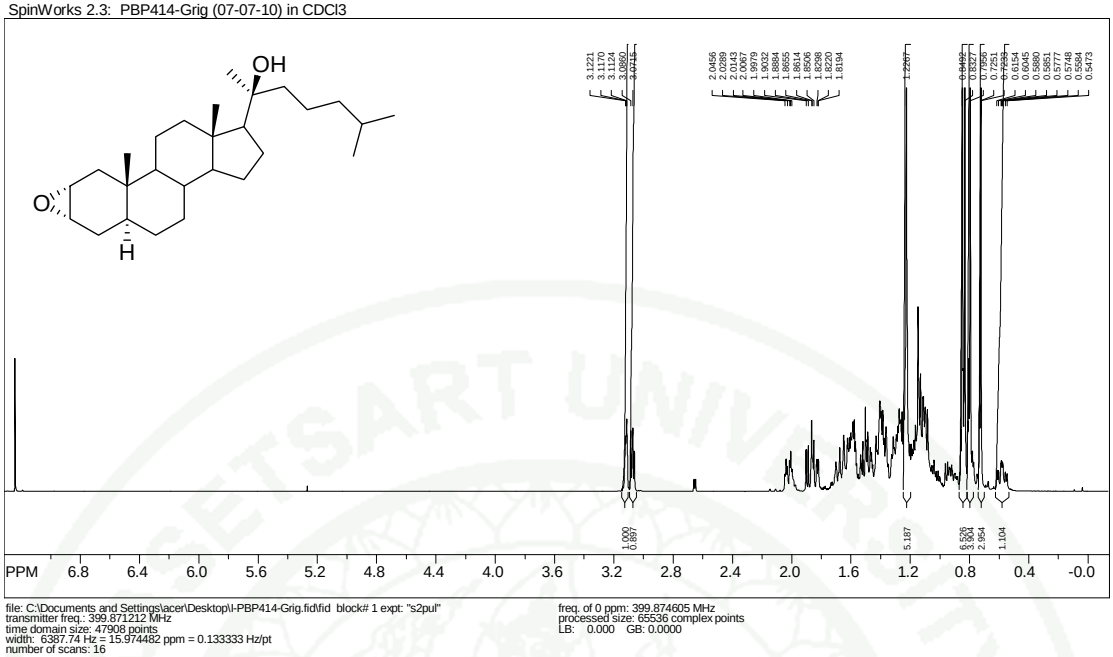
Appendix Figure 91 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-20(*S*)-hydroxy-5α-24a-homo-chol-24-ene (137)



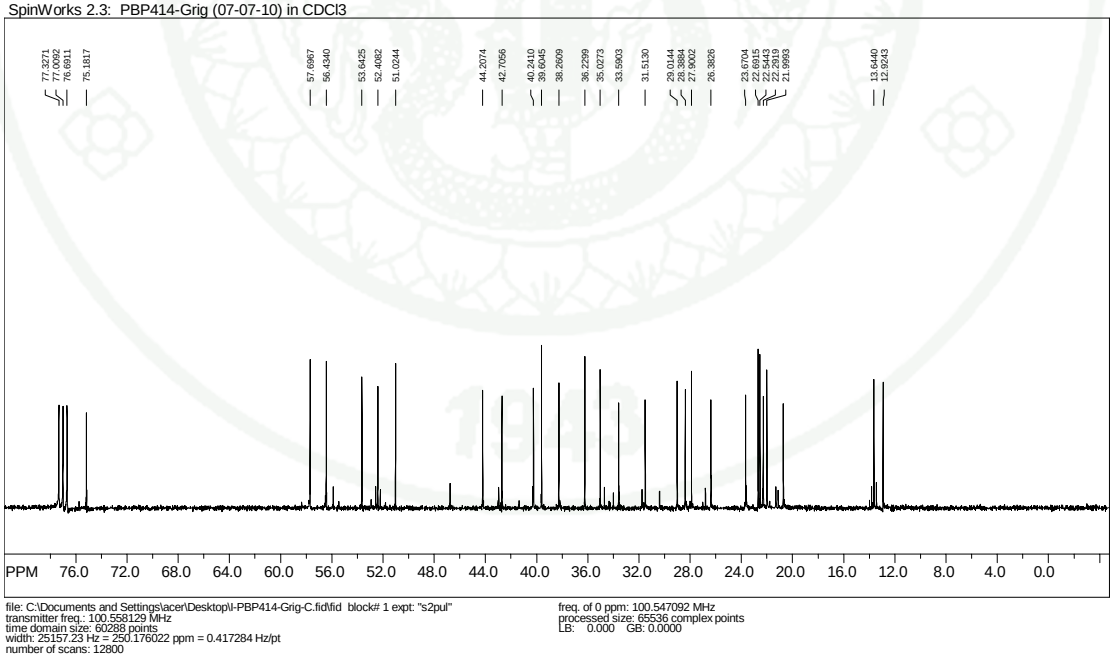
Appendix Figure 92 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138)



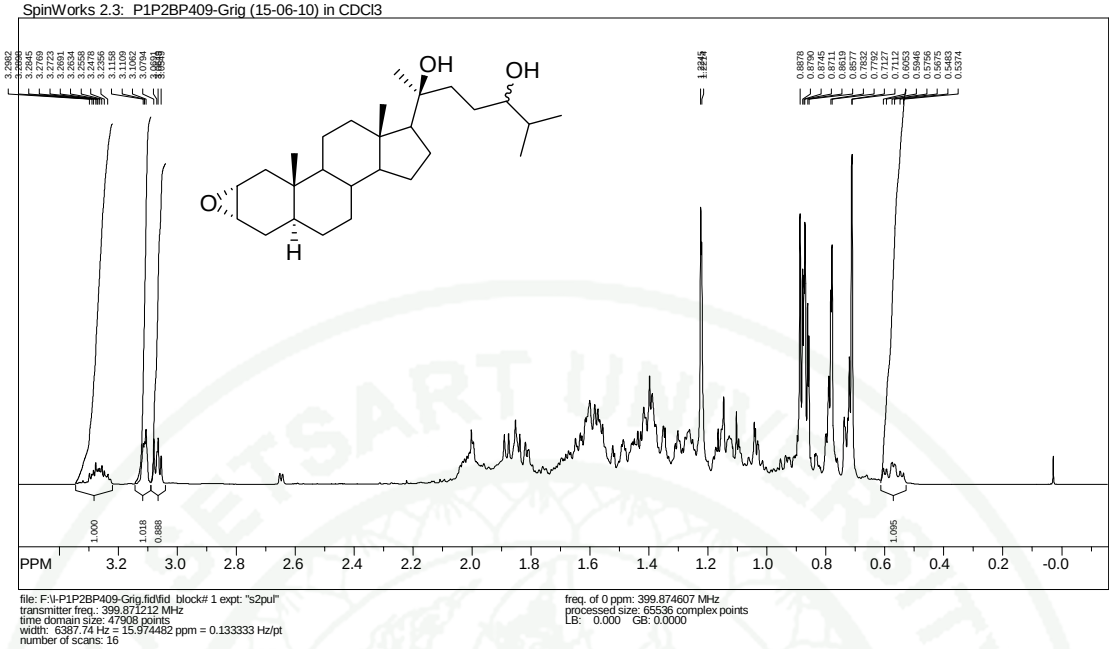
Appendix Figure 93 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholest-24-ene (138)



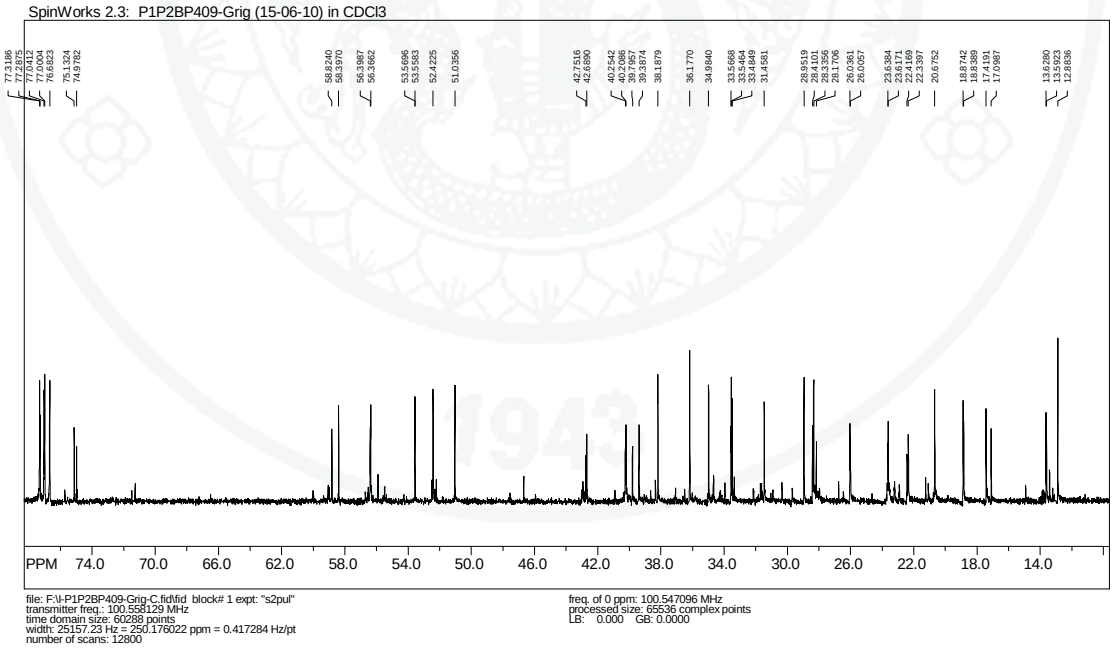
Appendix Figure 94 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (139)



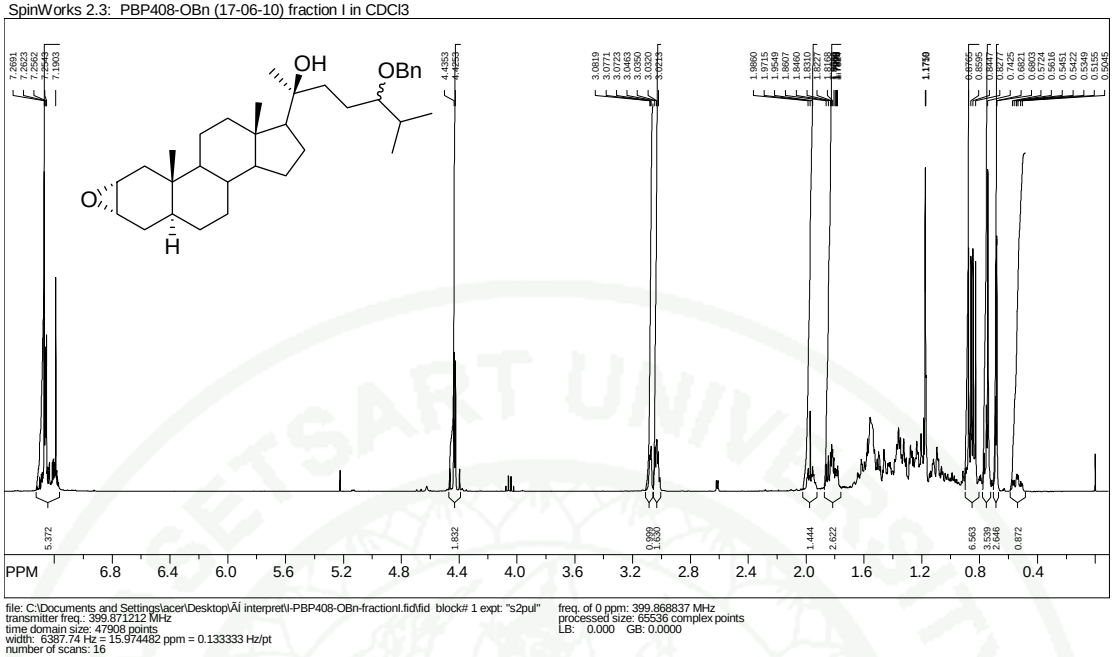
Appendix Figure 95 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (139)



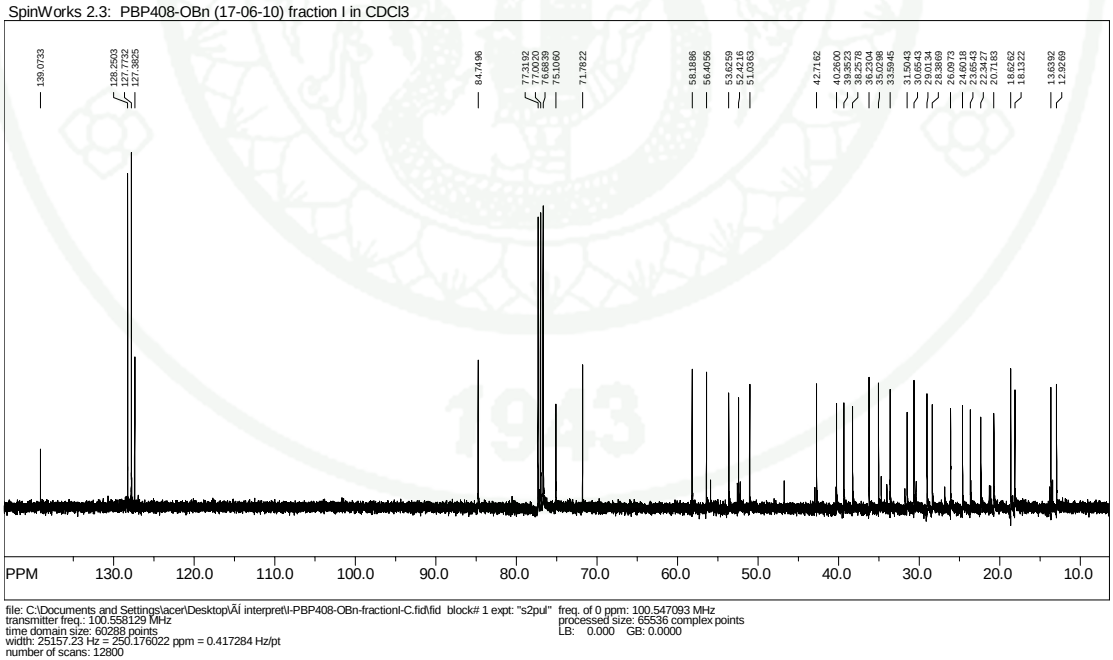
Appendix Figure 96 400 MHz ^{1}H NMR spectrum: 2α, 3α-epoxy-20(*S*), 24-dihydroxy-5α-cholestane (140)



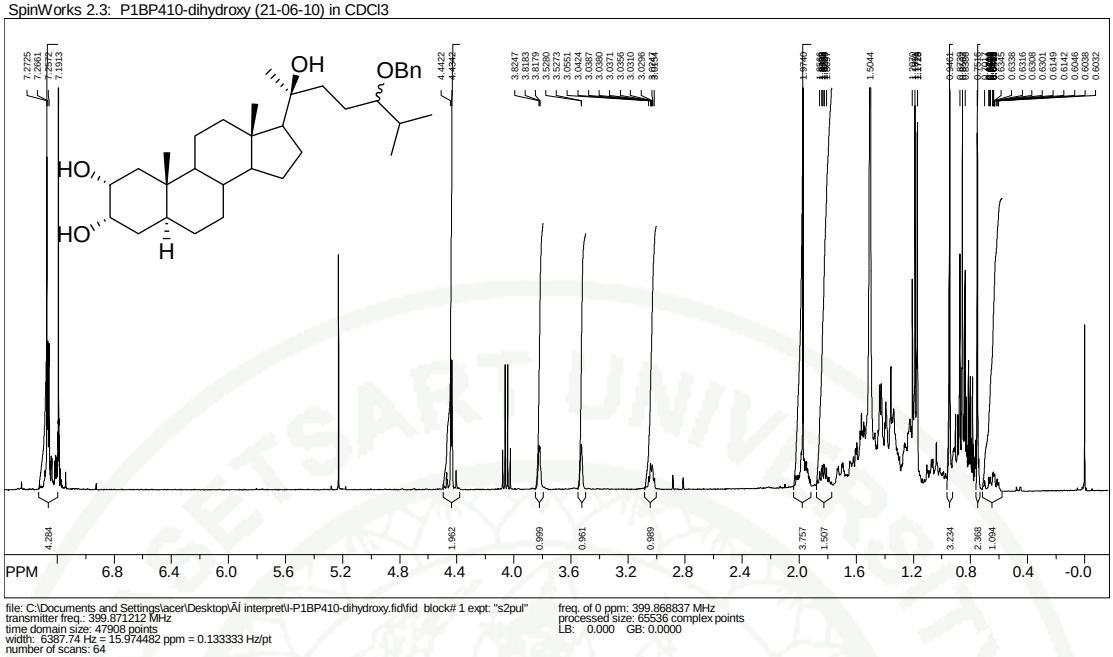
Appendix Figure 97 100 MHz ^{13}C NMR spectrum: 2α, 3α-epoxy-20(*S*), 24-dihydroxy-5α-cholestane (140)



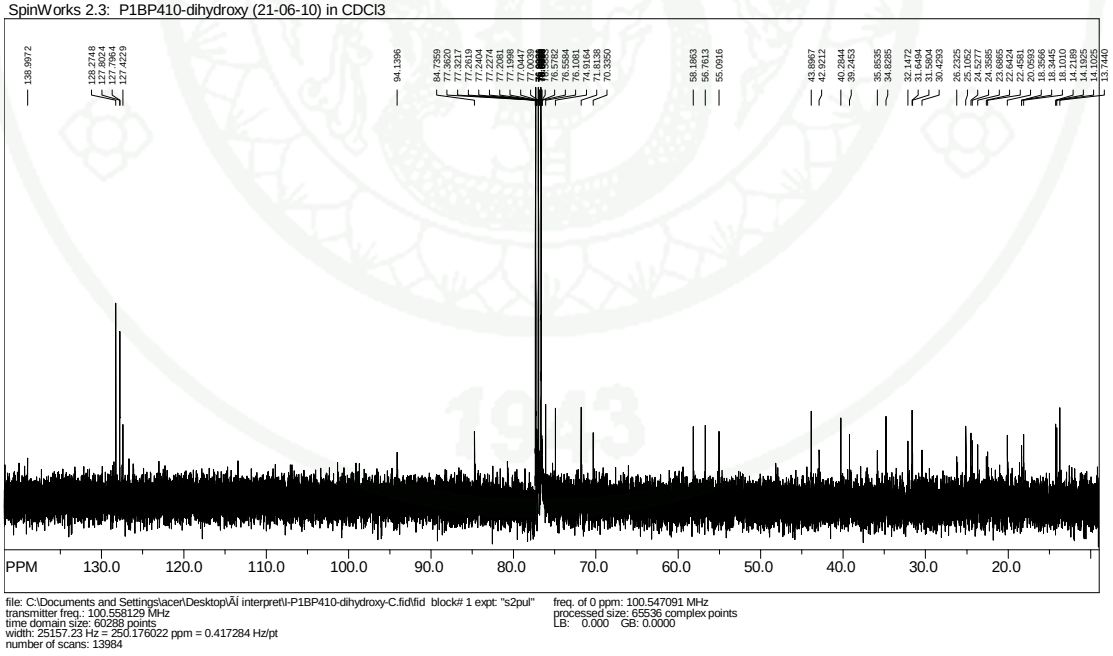
Appendix Figure 98 400 MHz ^{1}H NMR spectrum: 24-benzyloxy-2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (141)



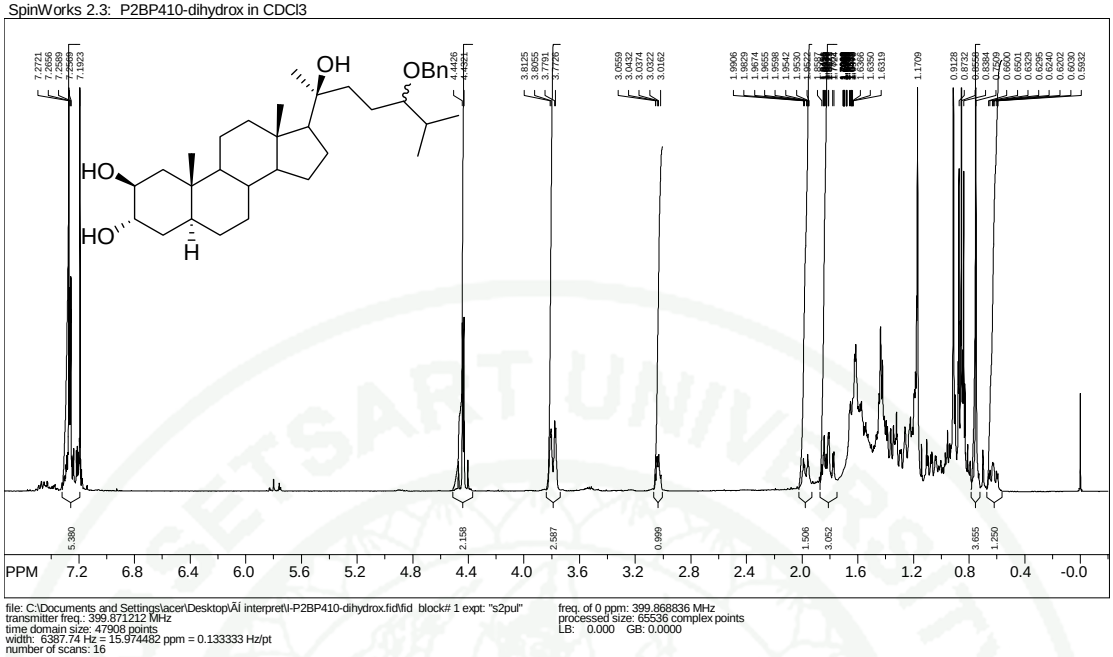
Appendix Figure 99 100 MHz ^{13}C NMR spectrum: 24-benzyloxy-2α, 3α-epoxy-20(*S*)-hydroxy-5α-cholestane (141)



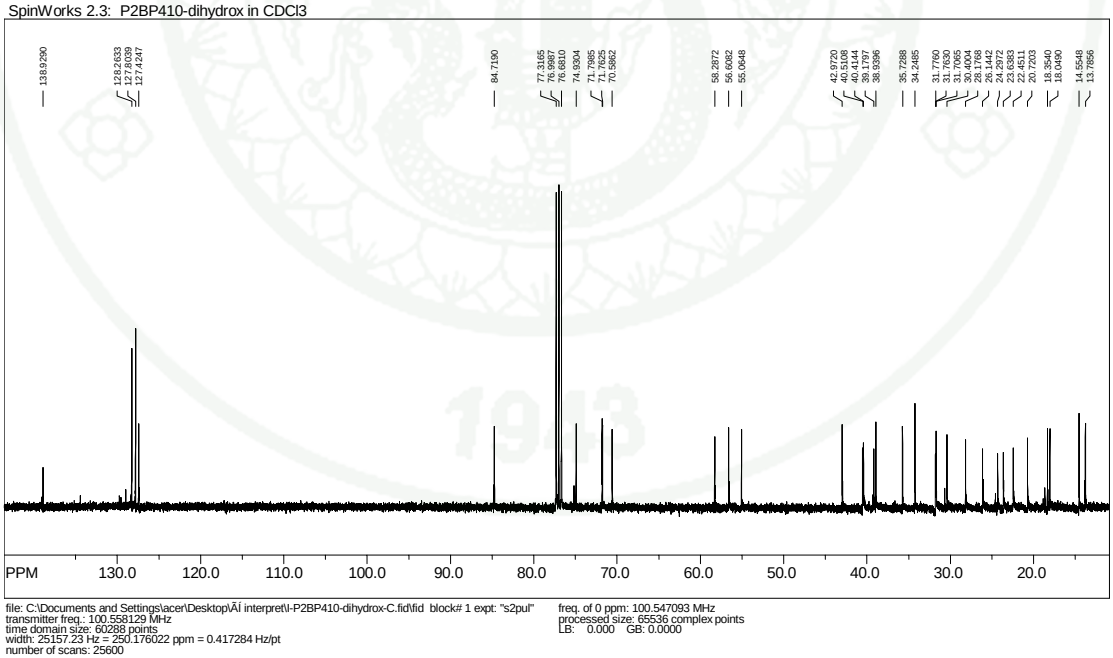
Appendix Figure 100 400 MHz ^{1}H NMR spectrum: 24-benzyloxy-2α, 3α, 20(*S*)-trihydroxy-cholestane (142)



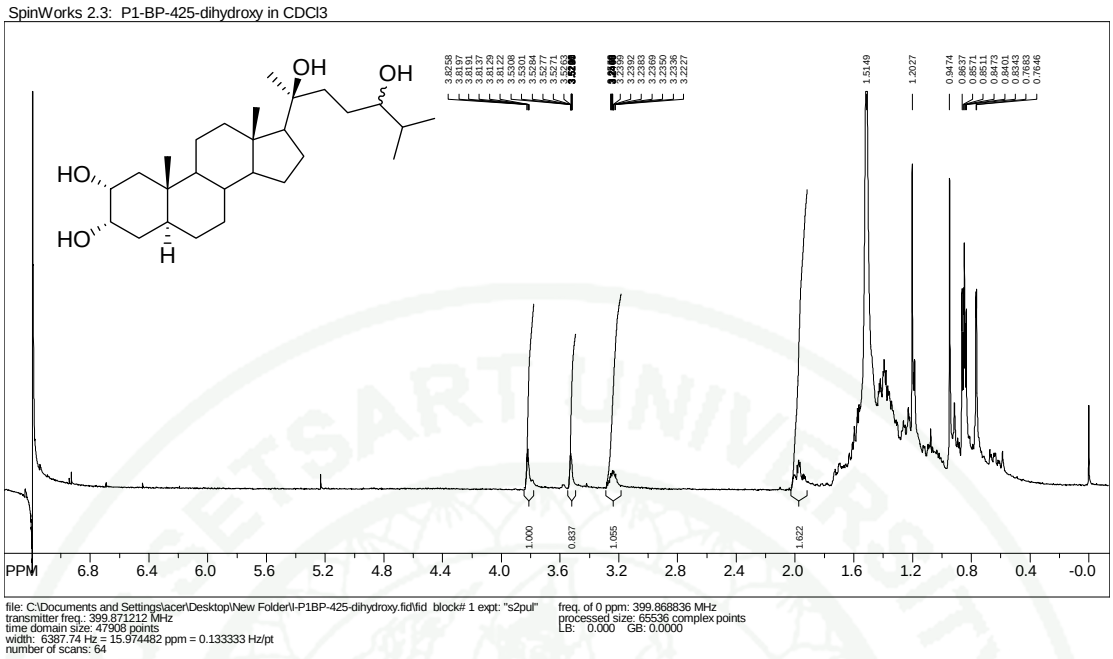
Appendix Figure 101 100 MHz ^{13}C NMR spectrum: 24-benzyloxy-2α, 3α, 20(*S*)-trihydroxy-cholestane (142)



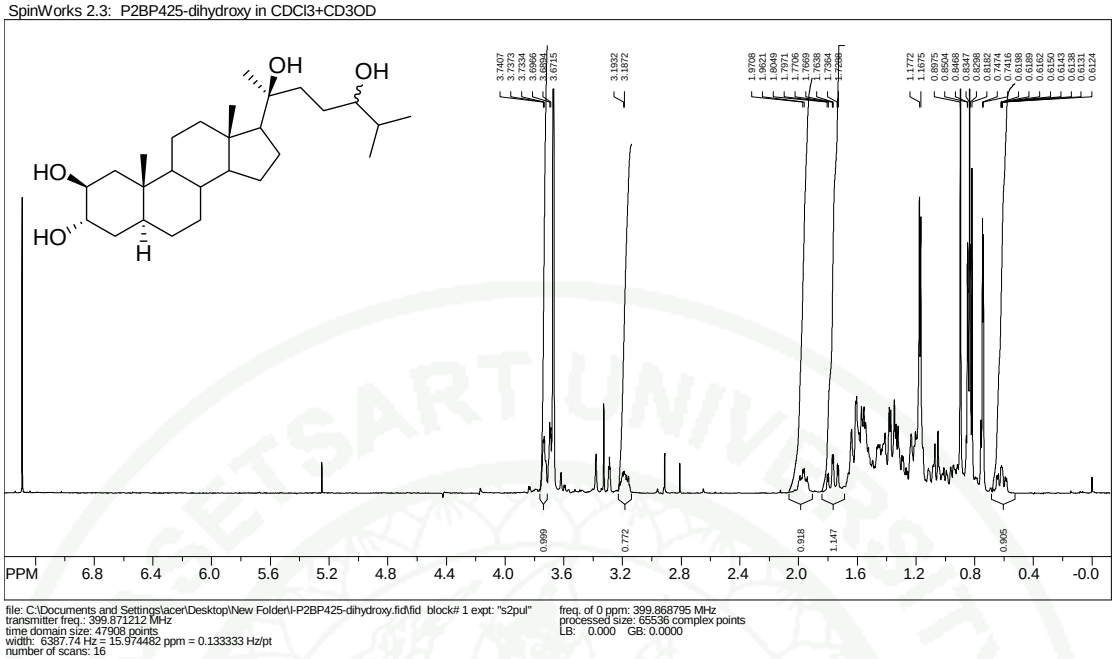
Appendix Figure 102 400 MHz ^{1}H NMR spectrum: 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-cholestane (143)



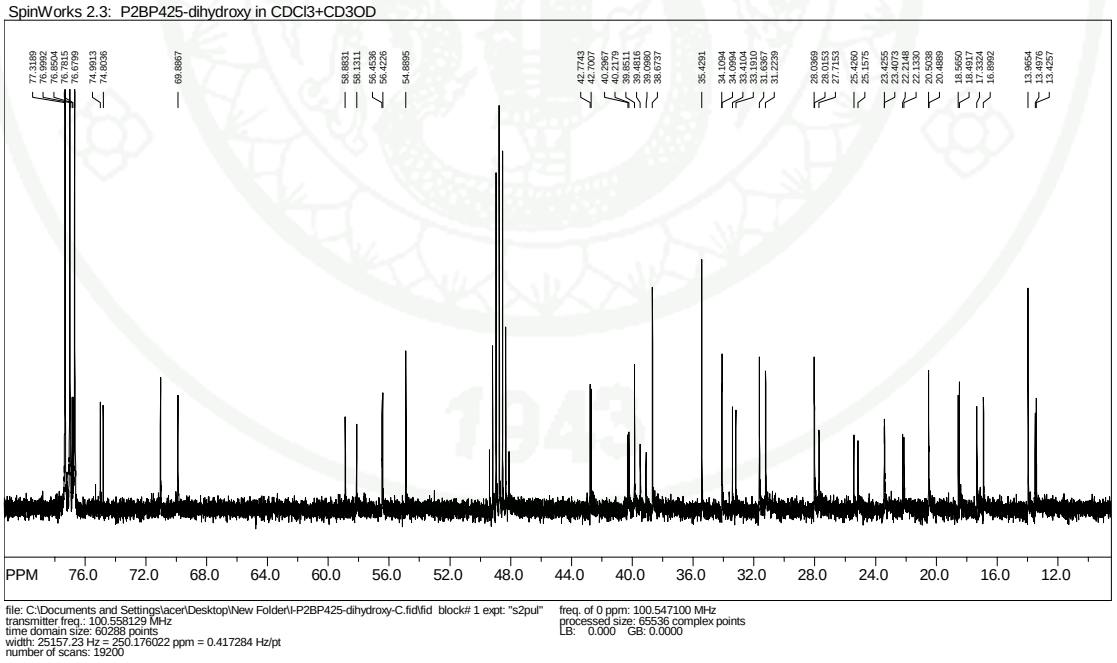
Appendix Figure 103 100 MHz ^{13}C NMR spectrum: 24-benzyloxy-2β, 3α, 20(*S*)-trihydroxy-cholestane (143)



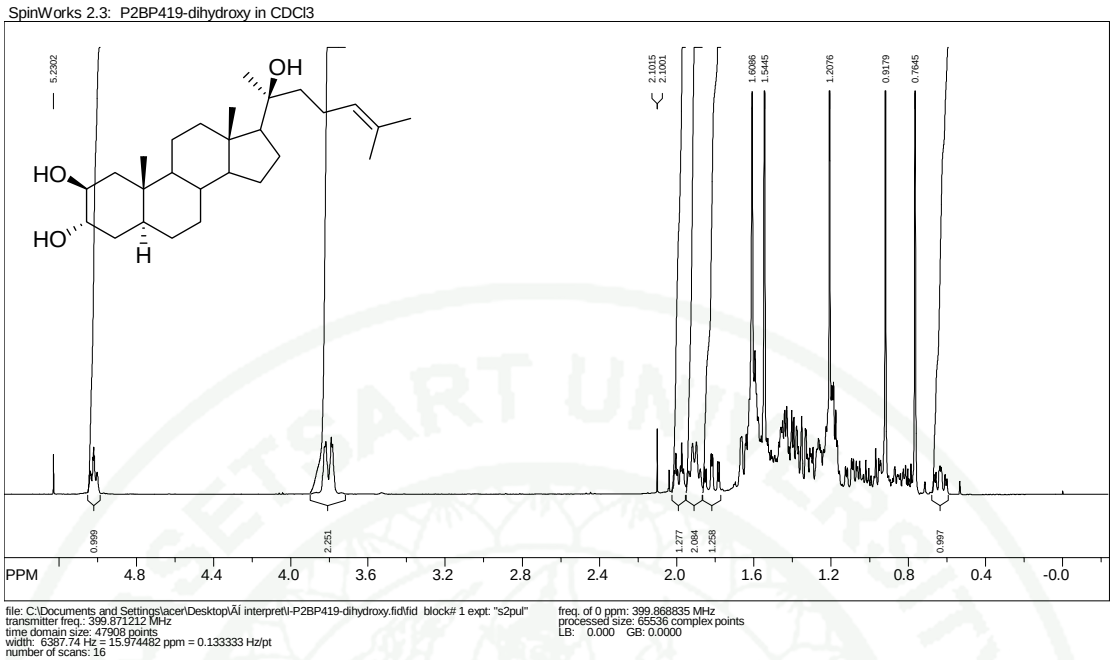
Appendix Figure 104 400 MHz ^{1}H NMR spectrum: 2α, 3α, 20(*S*), 24-tetrahydroxy-cholestane (144)



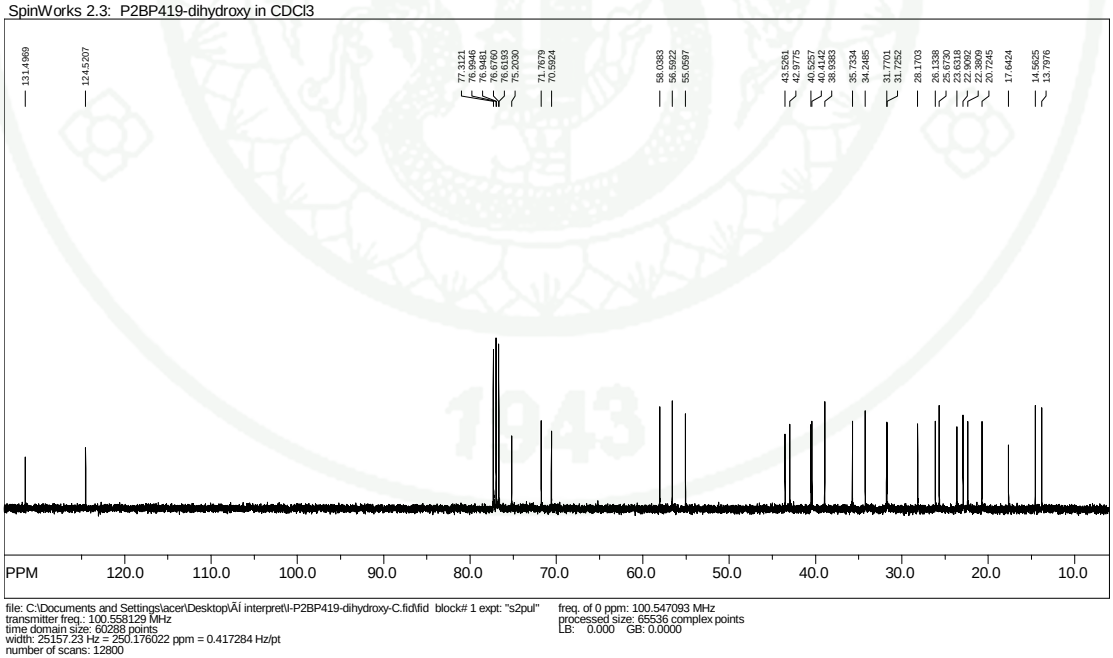
Appendix Figure 105 400 MHz ^{1}H NMR spectrum: 2β, 3α, 20(*S*), 24-tetrahydroxy-cholestane (145)



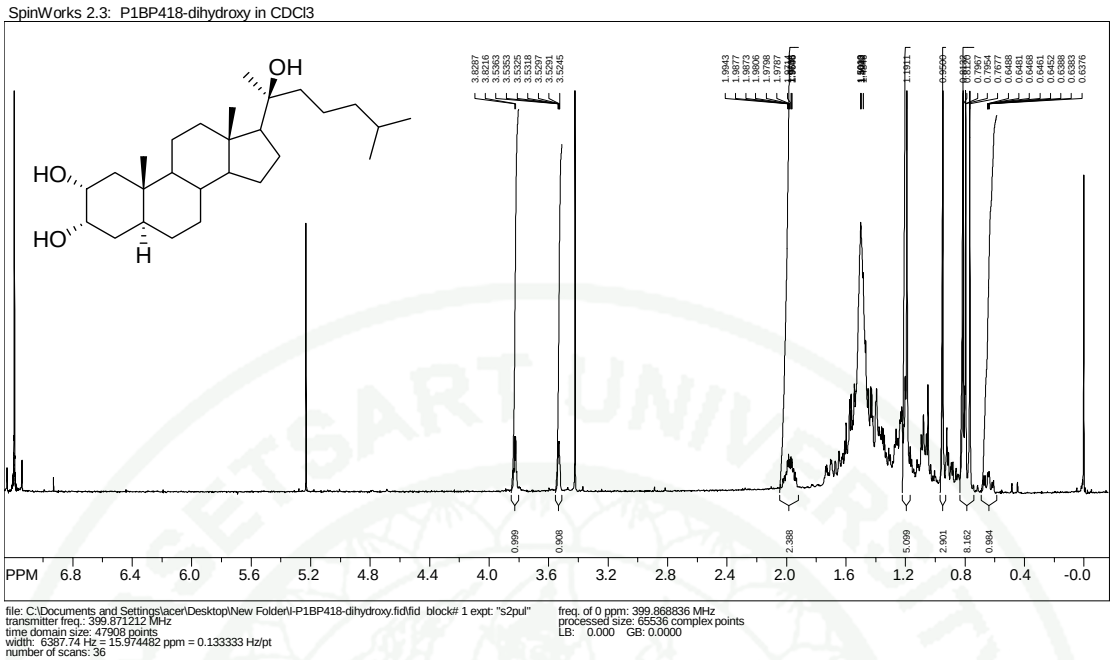
Appendix Figure 106 100 MHz ^{13}C NMR spectrum: 2β, 3α, 20(*S*), 24-tetrahydroxy-cholestane (145)



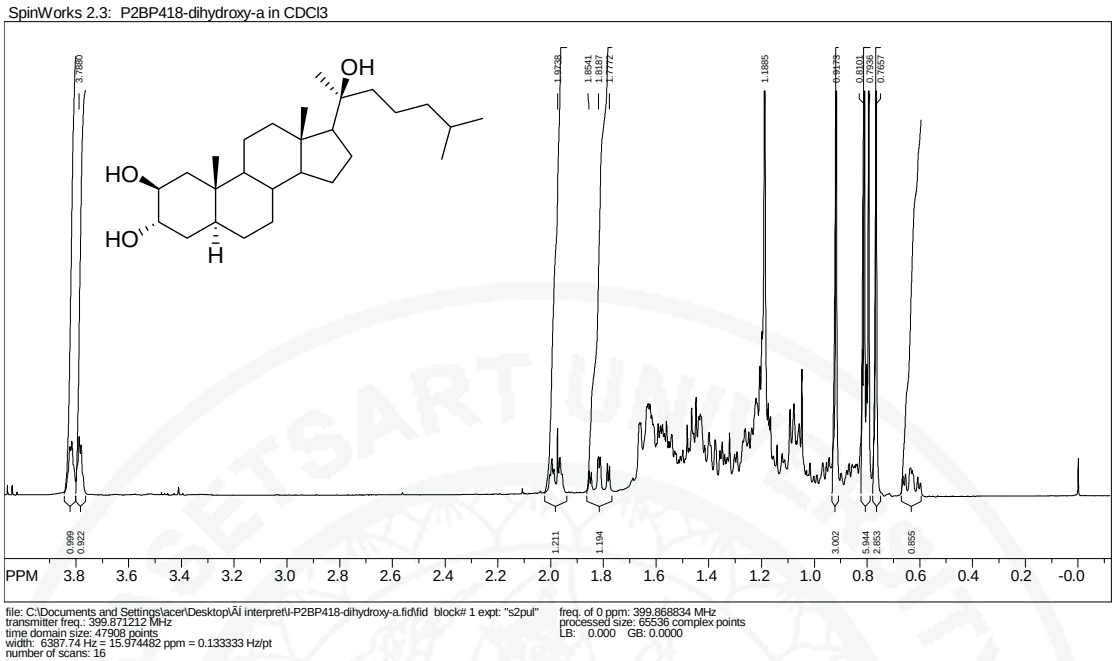
Appendix Figure 107 400 MHz ^{1}H NMR spectrum: 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147)



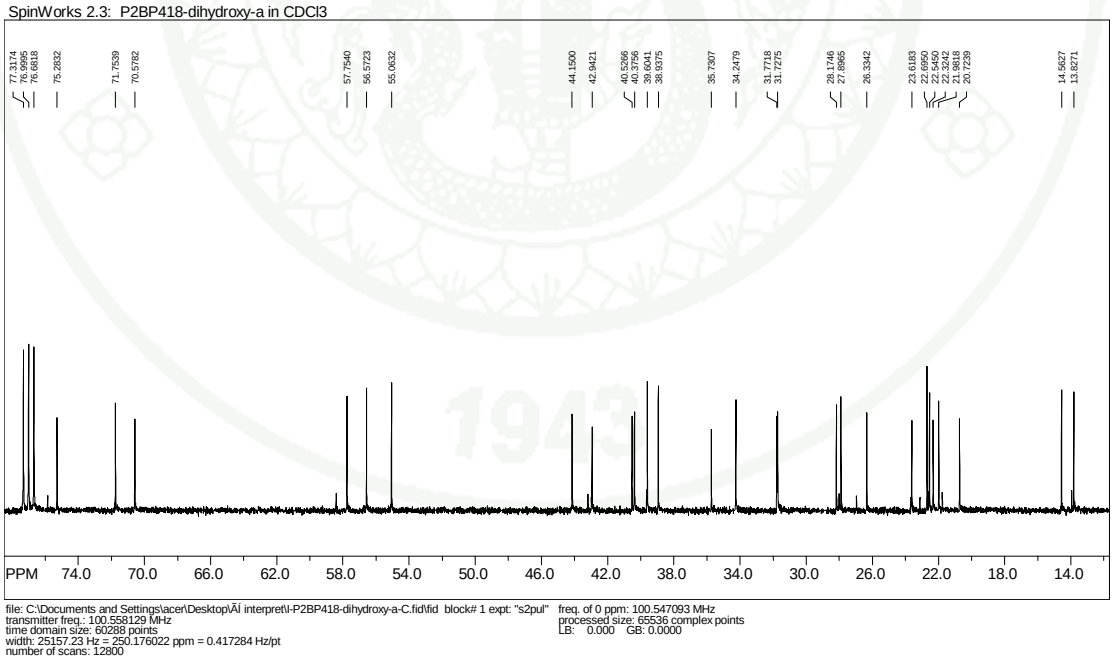
Appendix Figure 108 100 MHz ^{13}C NMR spectrum: 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene (147)



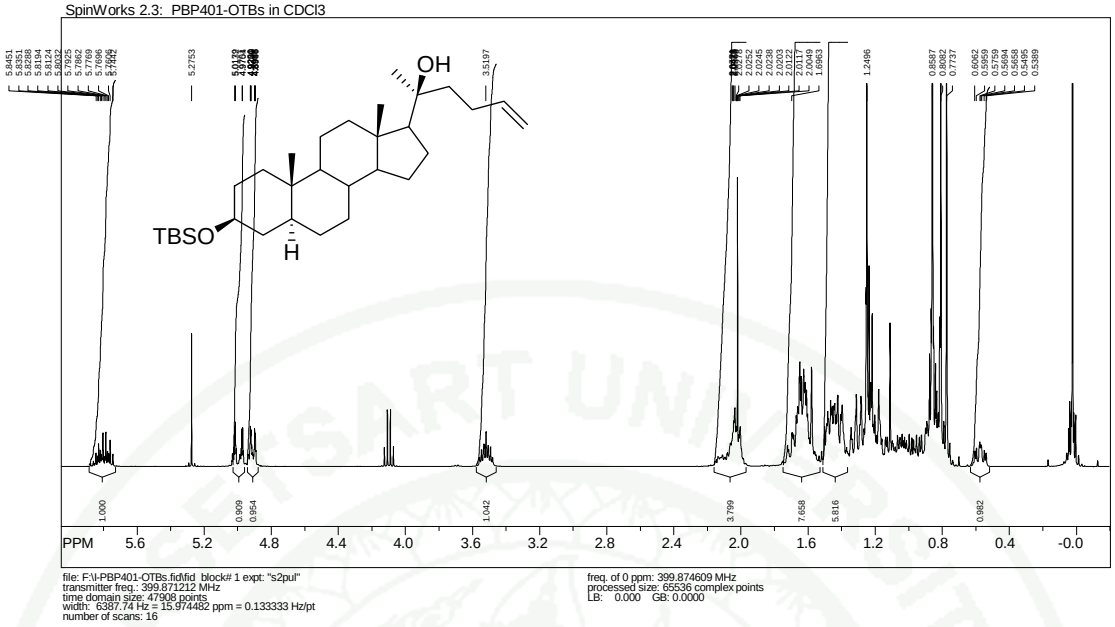
Appendix Figure 109 400 MHz ^{1}H NMR spectrum: 2α, 3α, 20(*S*)-trihydroxy-5α-cholestane (148)



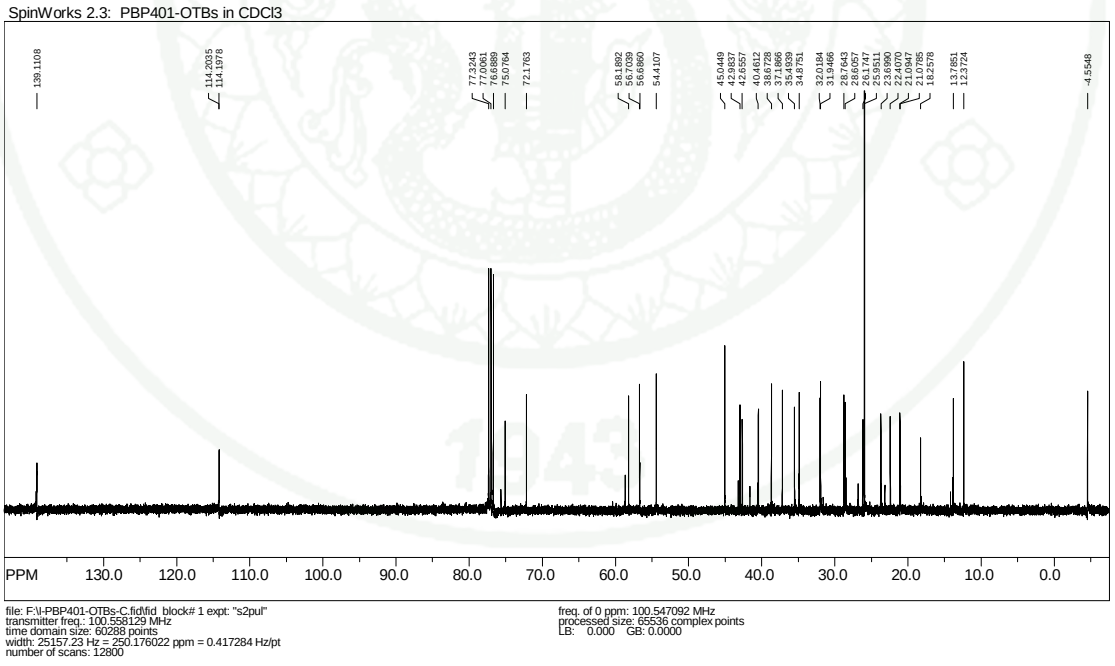
Appendix Figure 110 400 MHz ^{1}H NMR spectrum: 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149)



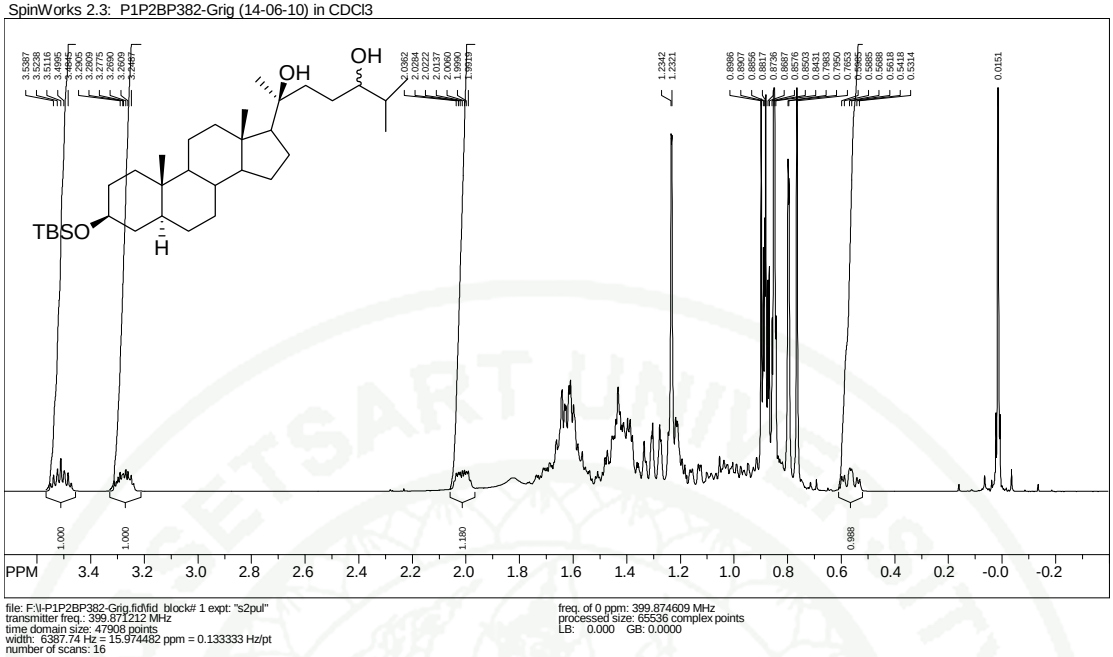
Appendix Figure 111 100 MHz ^{13}C NMR spectrum: 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane (149)



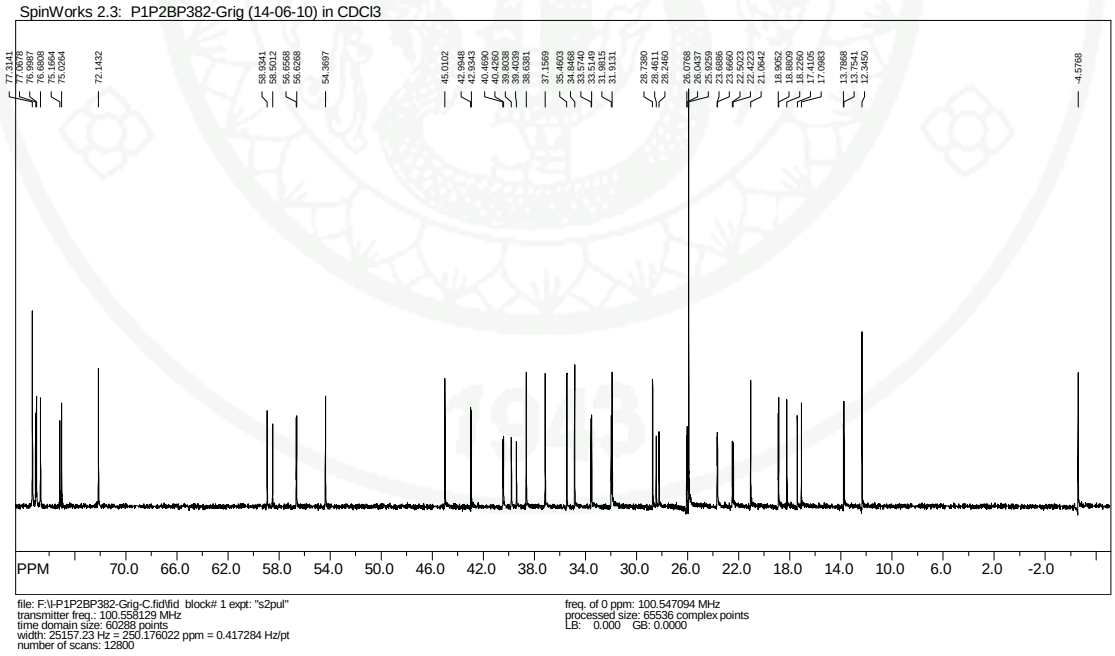
Appendix Figure 112 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-24a-homo-chlo-24-ene (150)



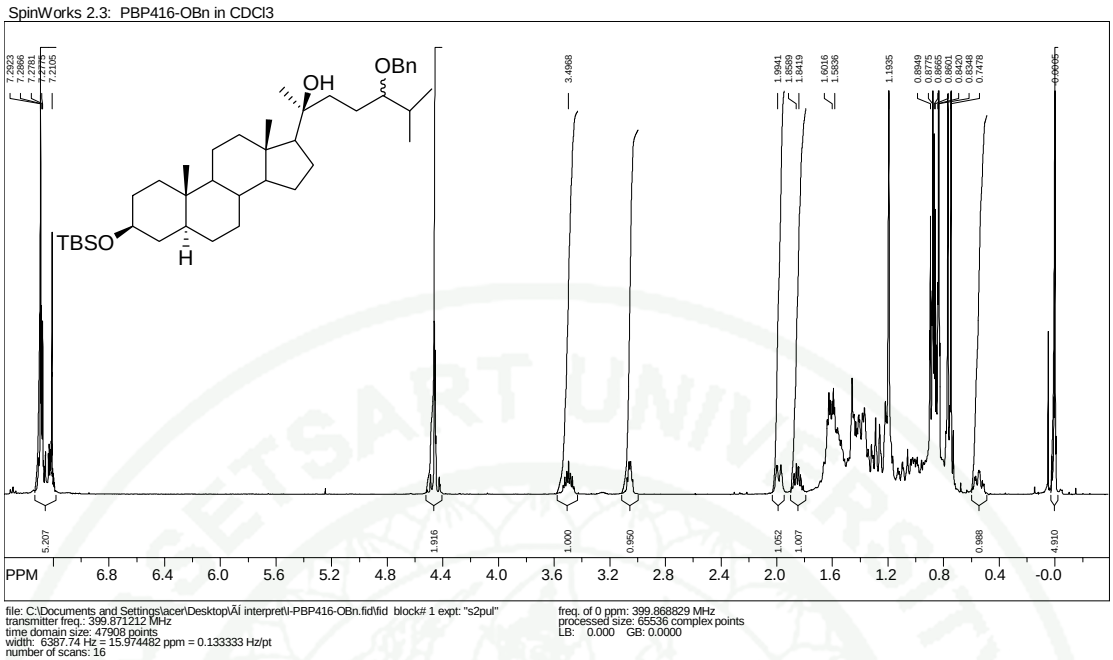
Appendix Figure 113 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-24a-homo-chlo-24-ene (150)



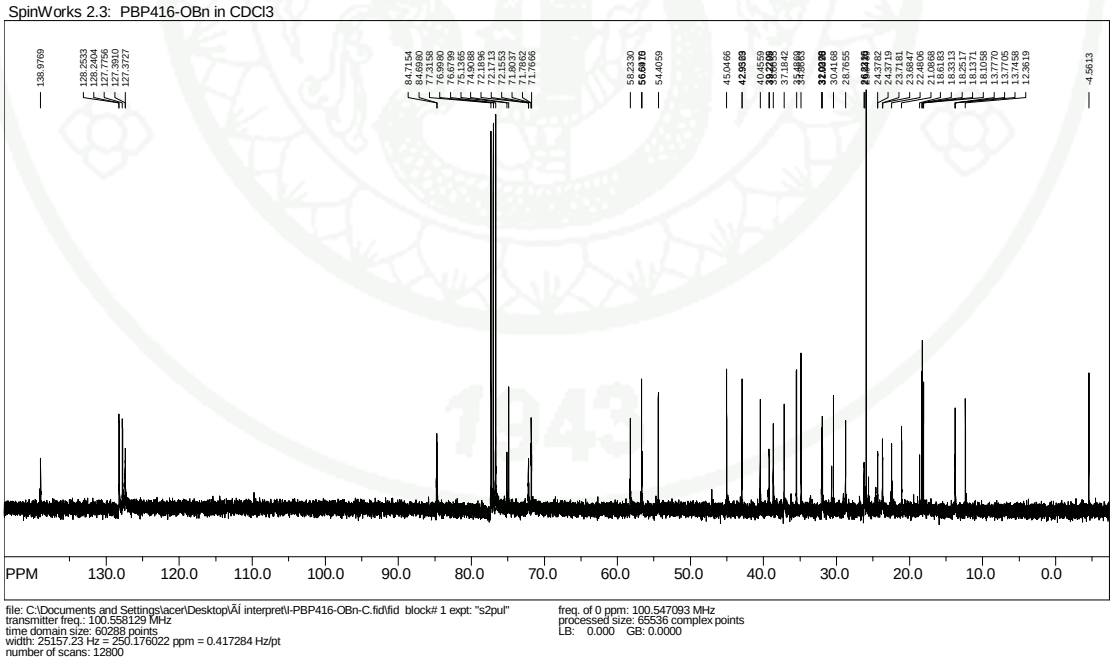
Appendix Figure 114 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (151)



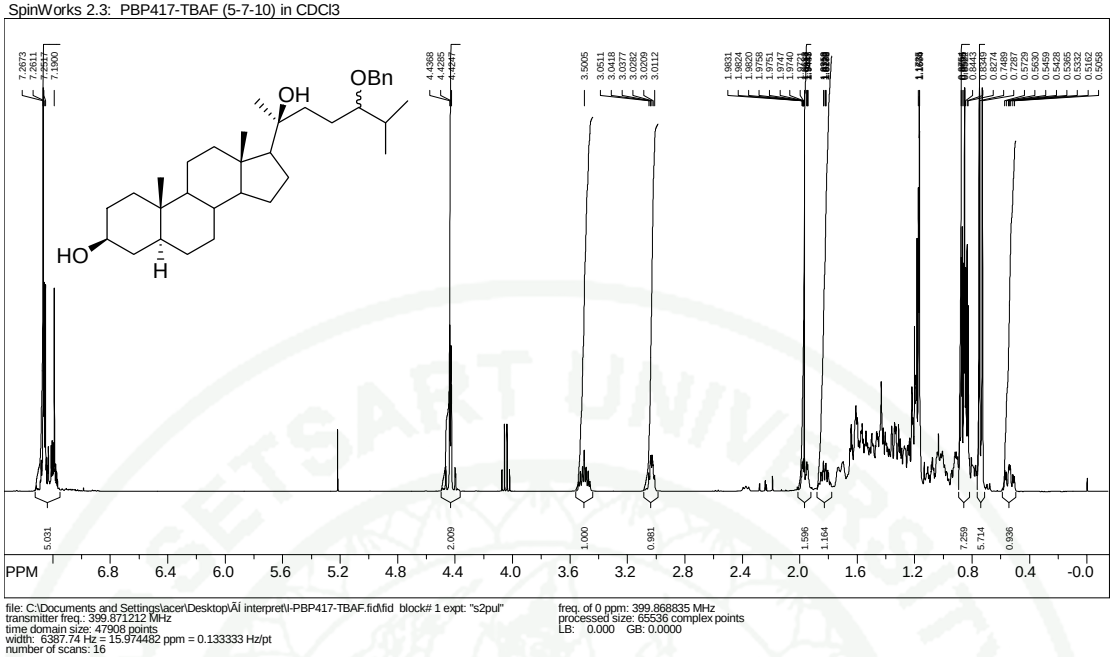
Appendix Figure 115 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (151)



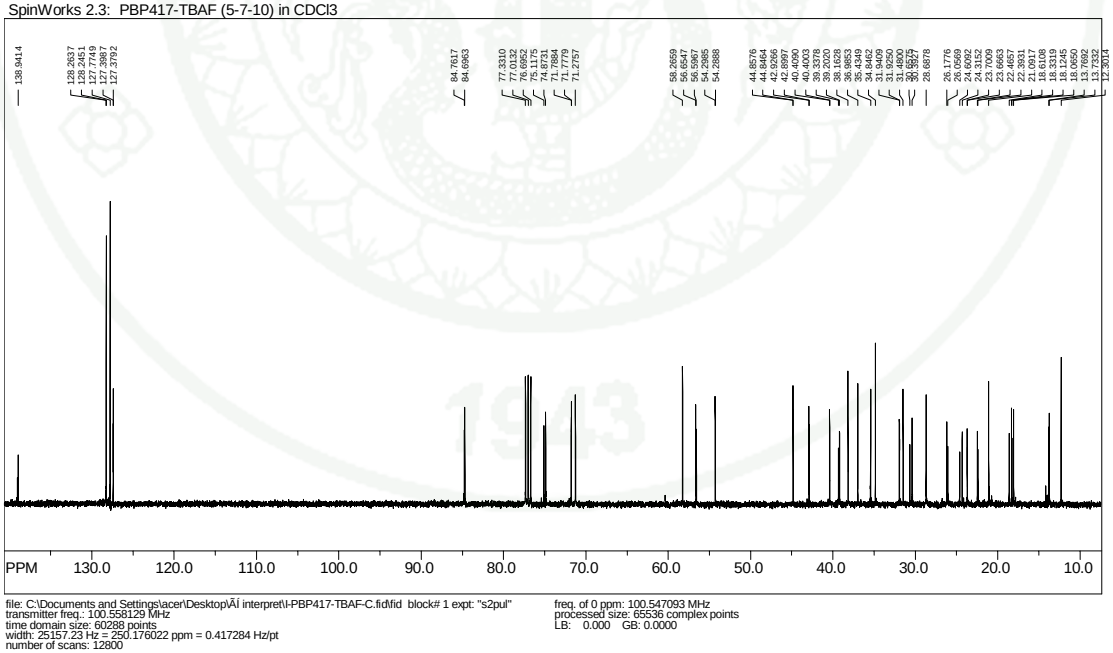
Appendix Figure 116 400 MHz ^{1}H NMR spectrum: 24-benzyloxy-3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-cholestane (152)



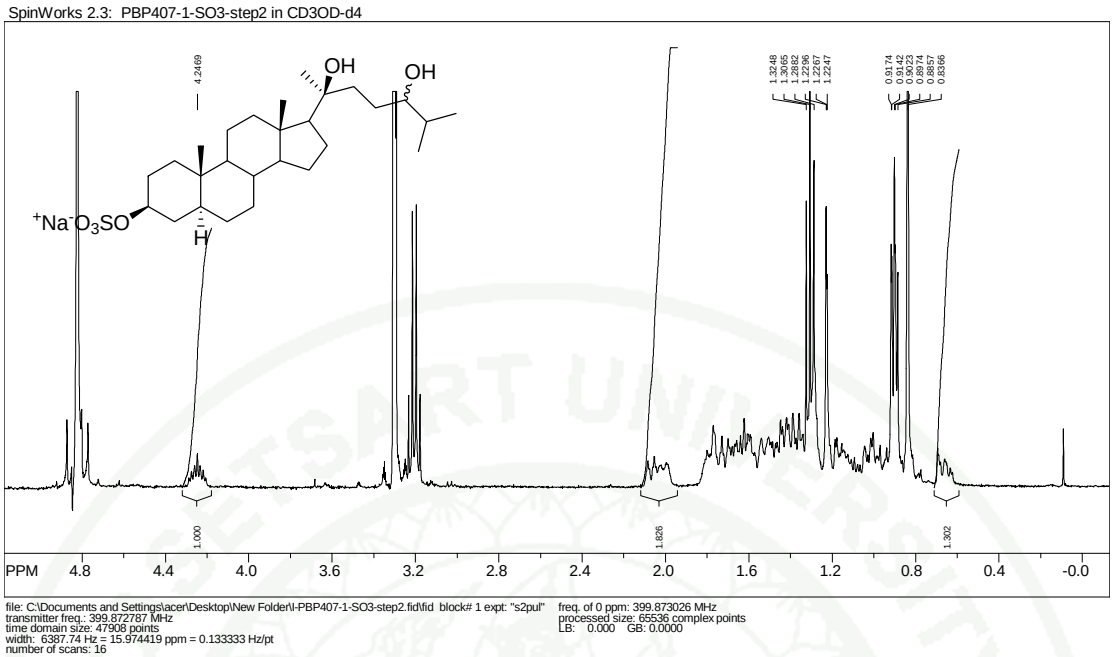
Apendix Figure 117 100 MHz ^{13}C NMR spectrum: 24-benzyloxy-3β-*tert*-butyldimethylsiloxy-20(*S*)-hydroxy-5α-cholestane (152)



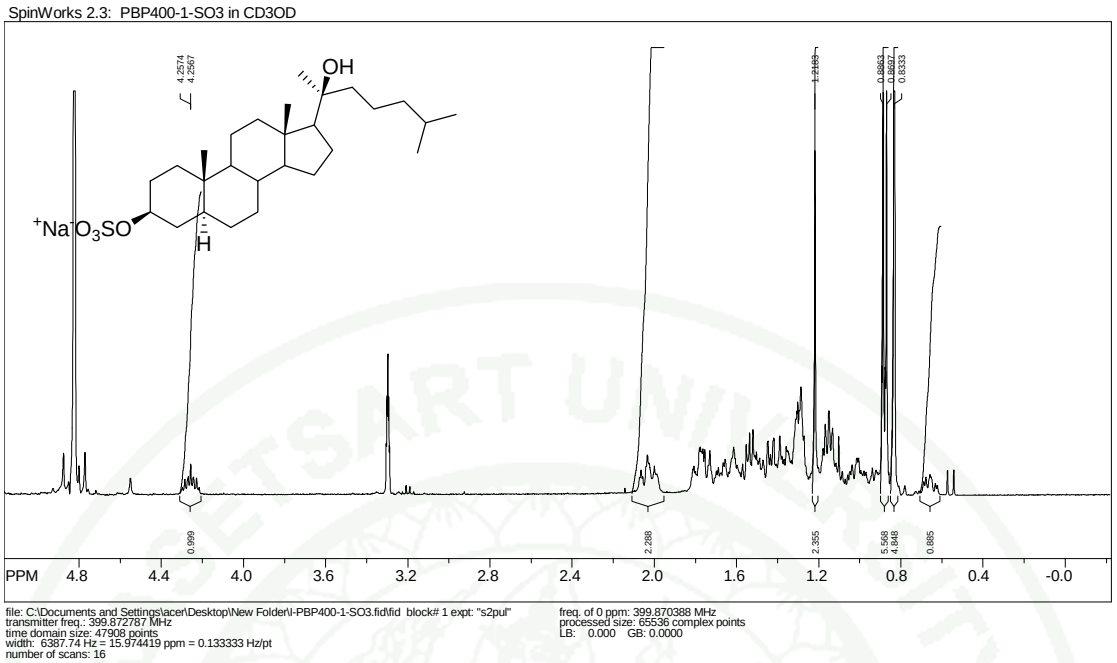
Appendix Figure 118 400 MHz ^{1}H NMR spectrum: 3β-*tert*-butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (153)



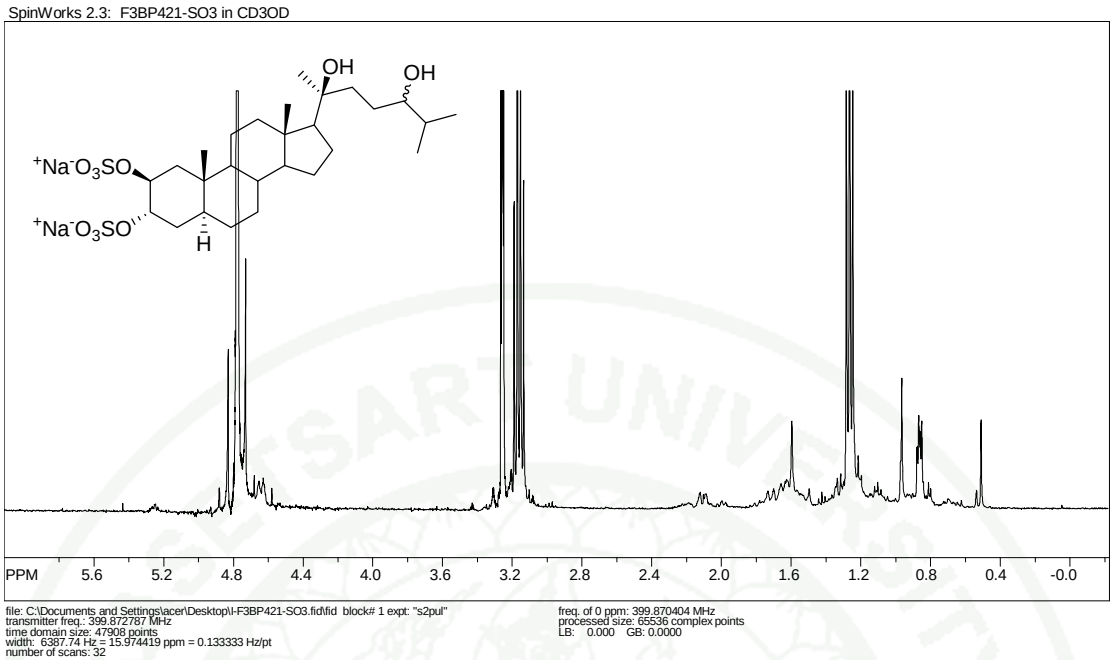
Appendix Figure 119 100 MHz ^{13}C NMR spectrum: 3β-*tert*-butyldimethylsiloxy-20(*S*), 24-dihydroxy-5α-cholestane (153)



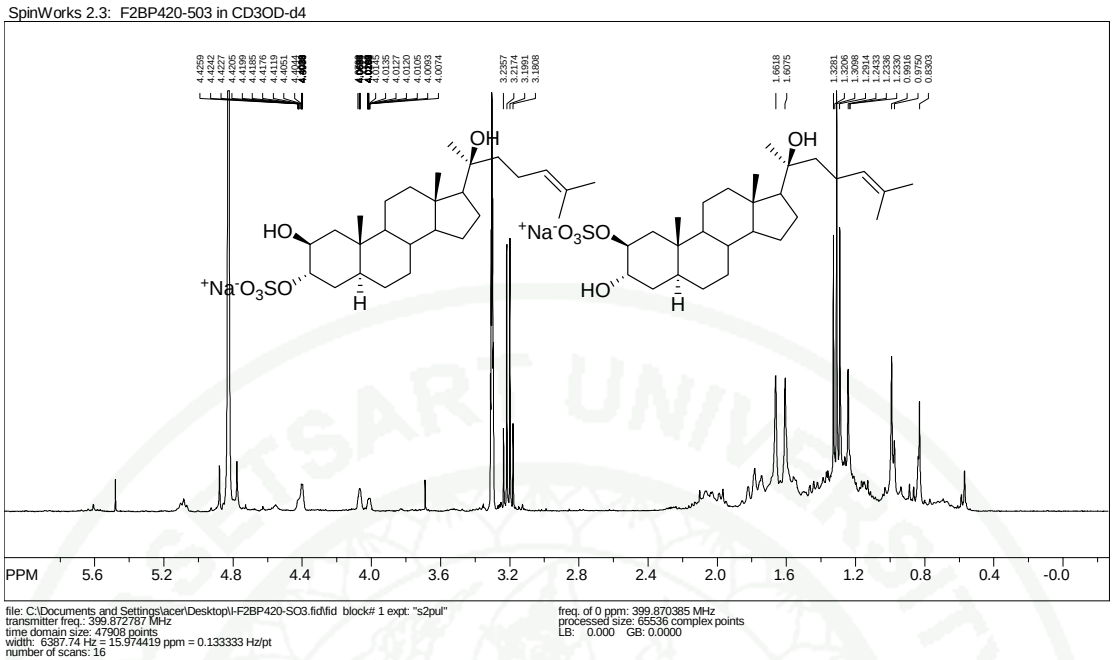
Appendix Figure (120) 400 MHz ^{1}H NMR spectrum: sodium 3β, 20(*S*), 24-trihydroxy-5α-cholestane 3-sulfate (155)



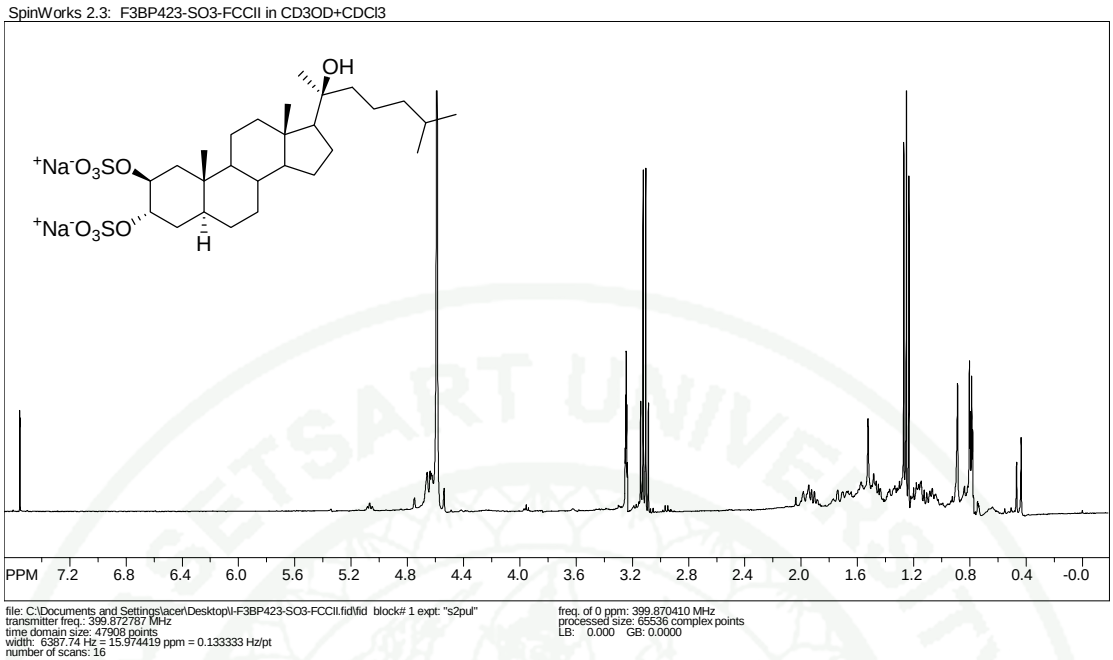
Apendix Figure 121 400 MHz ^{1}H NMR spectrum: sodium 3β, 20-dihydroxy-5α-cholestane 3-sulfate (156)



Appendix Figure 122 400 MHz ^{1}H NMR spectrum: disodium 2β, 3α, 20(*S*), 24-tetrahydroxy-5α-cholestane 2, 3-disulfate (158)



Apendix Figure 123 400 MHz ^{1}H NMR spectrum: sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 2-sulfate (159) and sodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholest-24-ene 3-sulfate (160)



Apendix Figure 124 400 MHz ^{1}H NMR spectrum: disodium 2β, 3α, 20(*S*)-trihydroxy-5α-cholestane 2, 3-disulfate (161)

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PUBLICATION : Bunyathaworn, P., S. Boonananwong, B. Kongkathip and N. Kongkathip. 2010. Further study on synthesis and evaluation of 3, 16, 20-polyoxygenated steroids of marine origin and their analogs as potent cytotoxic agents. **Steroids** 75: 432-444.

: Bunyathaworn, P., B. Kongkathip and N. Kongkathip. 2011. Synthesis and cytotoxicity studies of polyhydroxysterols and their sulfate analogs. **Kasetsart J. (Nat Sci.).** 45(4).