รายงานวิจัยฉบับสมบูรณ์

การศึกษาวิธีการสังเคราะห์สาร Geodisterol และทดสอบการออกฤทธิ์ทางชีวภาพ

Studies toward the synthesis of Geodisterol and biological testing

โดย

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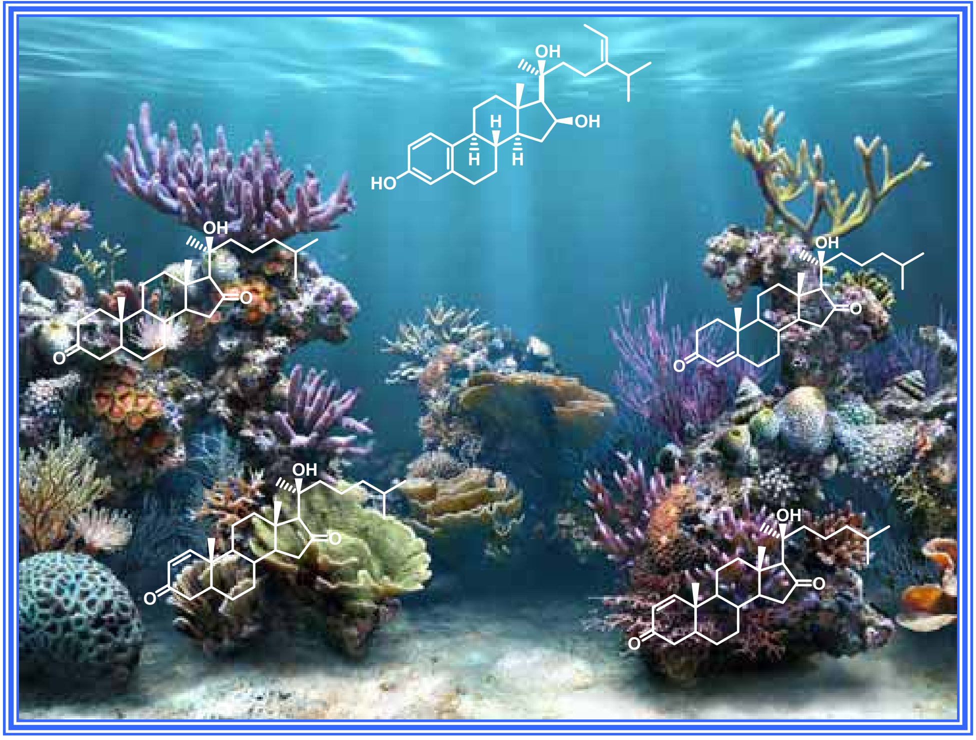

หน่วยปฏิบัติการวิจัยสารอินทรีย์ธรรมชาติและเคมีอินทรีย์สังเคราะห์
ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

พฤษภาคม 2551

สัญญาเลขที่ BGJ4680010

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Abstract

A series of C-3, C-16 and C-20 polyoxygenated steroids, with different degrees of unsaturation in ring A and variation of functional groups on the cholestane side chain, were successfully synthesized from tigogenin and diosgenin. The biological activity of these compounds against human epidermoid carcinoma (KB), human breast cancer (MCF 7) and lung cancer (NCI) cells depended on the degree of unsaturation in ring A, the cholesterol-type side chain and the existence and stereochemistry of hydroxyl group at C-16.

Keywords: Steroids, Geodisterol, Anti-cancer and Synthesis

รหัสโครงการ : BGJ4680010

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บทคัดย่อ

งานวิจัยนี้เกี่ยวกับการสังเคราะห์สารกลุ่มสเตียรอยด์ที่มีอะตอมออกซิเจนที่ตำแหน่งคาร์บอน 3, 16 และ 20 โดยสารอนุพันธ์ดังกล่าวมีระดับความไม่อิ่มตัวบนวงเอที่แตกต่างกันและมีจำนวนสายโซ่คาร์บอนเท่ากับคลอเรสเตอรอลแต่มีหมู่ฟังก์ชั่นบนสายโซ่แตกต่างกันไป โดยการสังเคราะห์จะเริ่มจากสารตั้งต้นทิโกจินินและไดออสจินิน การออกฤทธิ์ทางชีวภาพของสารกลุ่มนี้ต่อเซลล์มะเร็งช่องปาก (KB), มะเร็งทรวงอก (MCF 7) และมะเร็งปอด (NCI) ขึ้นอยู่กับระดับความไม่อิ่มตัวบนวงเอ, จำนวนสายโซ่คาร์บอนเท่ากับคลอเรสเตอรอล, การมีอยู่และสเตริโอเคมีของหมู่ไฮดรอกซีที่คาร์บอนตำแหน่ง 16

คำสำคัญ : สเตียรอยด์, จีโอไดสเตอรอล, สารออกฤทธิ์ต้านมะเร็ง และ การสังเคราะห์

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Boonsong Kongkathip
Ngampong Kongkathip
Suthinee Boonananwong
May 2008

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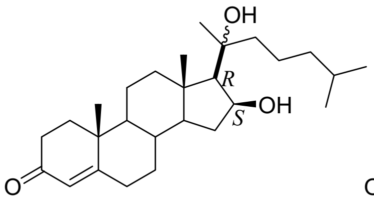
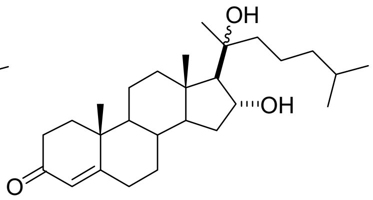
INTRODUCTION

Natural products derived from marine organisms are important sources of biologically active compounds. Those systems with a steroidal skeleton are usually isolated in large quantity from marine sponges and marine organisms. Polyhydroxysteroids have been found in algae, and virtually in every marine invertebrate phyla, such as Porifera, Coelenterata, Bryozoa, Molusca, Echinodermata, Arthropoda and Tunicata as well as in fish (M. V. D'Auria, 1993). Several polyoxgenated steroids with oxidation at C-3, C-16 and C-20 of the chloestane nucleus are found in marine organisms and most of them show biological activity. This brief review is concerned with polyoxygenated C-3, C-16 and C-20 sterols.

Guggulsterol (1) was first isolated from the resinous exudates of the tree *Commiphora mukul* and shown to possess activity anti-inflammatory, anti-rheumatic and hypocholesterolemic (Benvegnu *et al.*, 1982). Guggulsterol (1) together with two closely related steroids **2** and **3** were also isolated from the extracts of the Mediterranean gorgonian *Leptogorgia sarmentosa*.



Guggulsterl (1)



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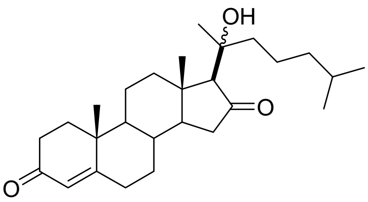
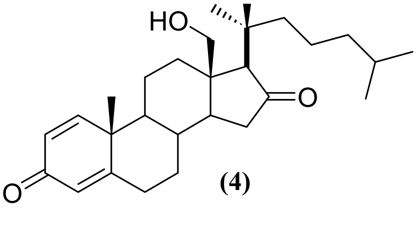


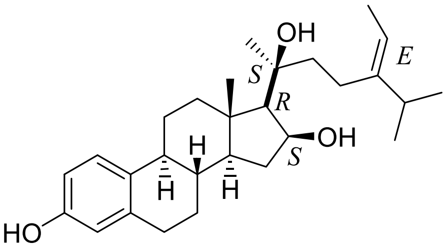
(3)

A polyoxygenated sterol with ring A quinone (4) was isolated from the marine black coral *Antipatbes subpinnata* and showed toxicity toward the brine shrimp (*Artemia salina*) with LC_{50} 139.4 µg/ml (Fatorusso, 1992).



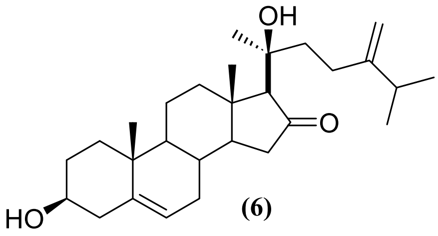
(4)

Geodisterol (5) was first isolated by Phil Crews as a major component of Indo-Pacific sponges of genus Geodia (Crew *et al.*, 1996). This compound represents the first polyoxygenated sterol with aromatic A ring, isolated from marine organisms. While sterols with an aromatic A rings have been obtained from terrestrial plants, animals and are ubiquitous as hormones (estradiol), all these polyoxygenated sterols have small side chain in comparison to geodisterol. The crude extract showed cytotoxicity against a human colon solid tumor cell line.



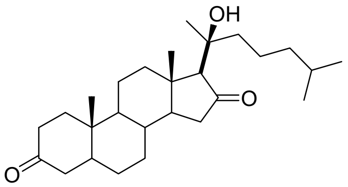
Geodisterol (5)

In 1998, (20*S*)-3β-20-dihydroxyergosta-5, 24(28)-diene-16-one (6) was isolated from the brown alga *Cystophora brownie* (Cytoseiraceae) by Bian and Altena.



(6)

Steroids **7-9** with the same side chain as **4** were isolated from the gorgonian *Leptogorgia sarmentosa* (Garrido *et al.* 2000). Compounds **7** and **8** 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) with ED_{50} values of 1 μg/ml, in all cases. A fraction contained compound **9** as the major component also showed cytotoxicity against the same four tumor cell lines as above mentioned (ED_{50} = 1 μg/ml).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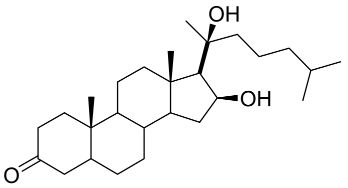
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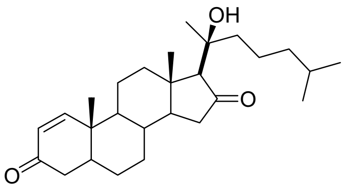
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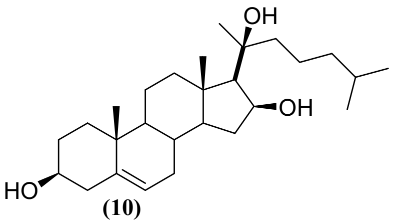
Meselhy (2003) the isolation of polyoxygenated steroids **10** and **11** from the oleogum resin of *Commiphora wightii.*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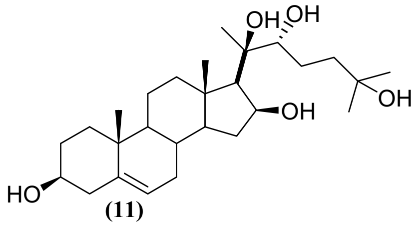
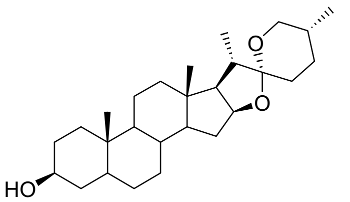
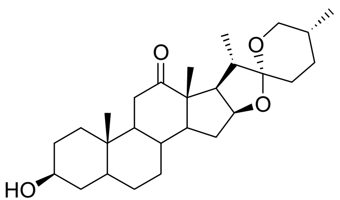
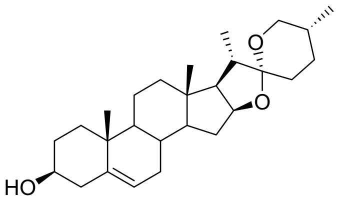
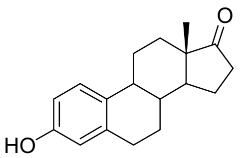

(11)

Our group has synthesized several steroids, such as prednisolone (Mongkolsripatana, 1997), triamcinolone (Khunnavutimanotum, 1998), cyproterone acetate and its derivatives (Sakee *et al.*, 2003a; Sakee *et al.*, 2003b), estradiol (Hasakunpaisan, 2000), OSW-1 aglycone (Chaosuansharoen, 2004), and betamethasone (Noimai, 2005) from naturally occurring steroidal sapogenin tigogenin (12), hecogenin (13) and diosgenin (14).


tigogenin (12)


hecogenin (13)


diosgenin (14)


estrone (15)

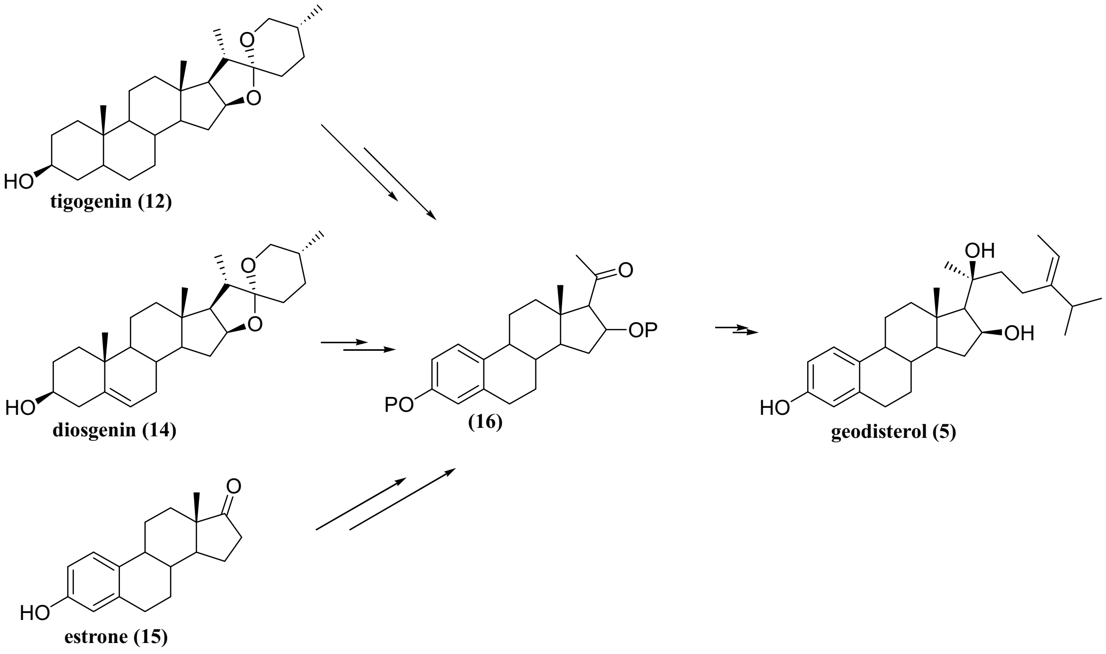
Since geodisterol and polyoxgenated sterols of this type showed anti-tumor activity and so far there are no reported on the synthesis in the literature. We were encouraged to synthesize and study the structure activity relationships of this type of compound.

The objectives of this research were to synthesize a series of C-3, C-16, C-20 polyoxygenated steroids and develop approaches geodisterol (5) starting from tigogenin (12), obtained from the waste of *Agavae sisalana* leaves, diosgenin (14), isolated from *Dioscorea* plant and a commercially available estrone (15), contained aromatic A ring. The synthesized compounds would then be evaluated for anti-cancer activity to gain insight into the structure-activity relationships (SARs).

LITERATURE REVIEWS

Synthetic methodology for the synthesis of 3, 16, 21- polyoxygenated steroids and synthetic intermediate towards geodisterol

We proposed to approach these types of compounds by synthesis of the core steroid intermediate **16**. Our strategies to synthesize **16** from tigogenin (12) or diosgenin (14) involved spiro ring degradation, aromatization at A ring and finally introduction of a hydroxyl group at C-16. The synthetic method starting from estrone (15), involved construction of an acetyl group at C-17 and introduction of a hydroxyl group at C-16 via an epoxy ketone intermediate. The construction of the alkyl side chain could be achieved by the nucleophilic addition of the C-20-ketone anion to appropriate electropliles (16).



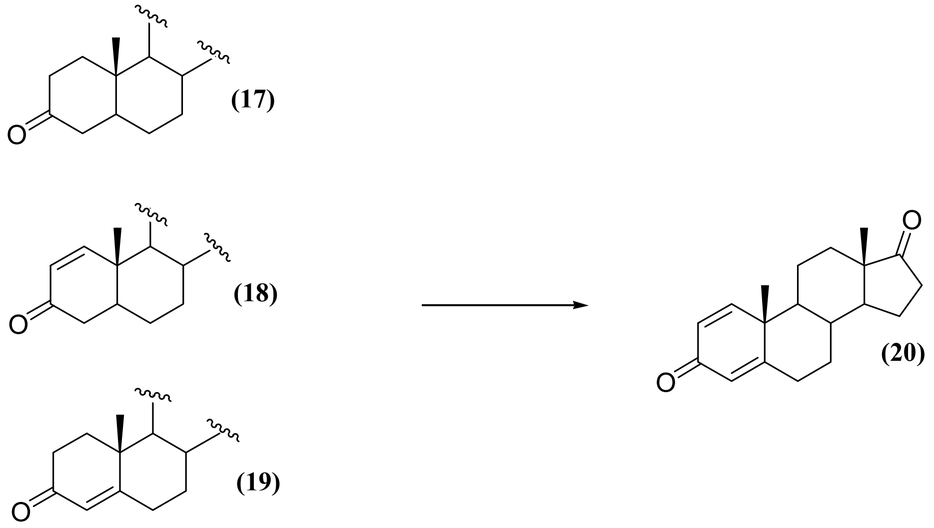
Scheme 1

1. Conversion of A ring to $\Delta^{1,4}$-steroids

Dehydrogenation of 3-hydroxy steroids and 3-keto-1- and 4-enes can be accomplished by several methods.

1.1 Microbiological dehydrogenation

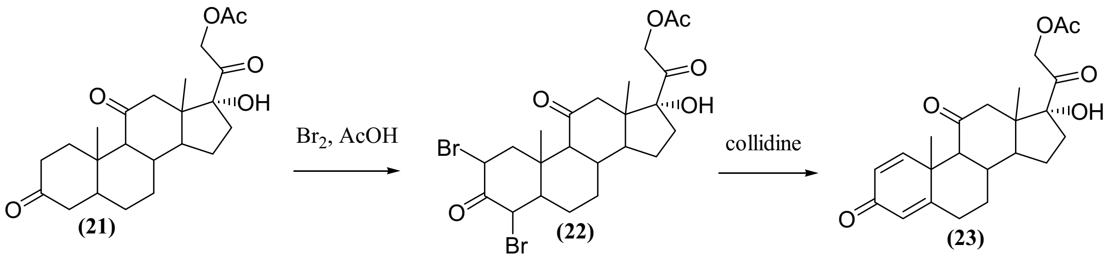
A number of reports have been published on the conversion of oxy pregnanes and androstanes into the corresponding $\Delta^{1,4}$-derivative using fermentation with fungi, actinomycetes and bacteria such as *Pseudomonas tertosteroni* (A.T.C.C. No. 11996), *Corynebacterium simplex* (A.T.C.C. 6946), *Bacillus lentus* and *Septomyxa affinis* (A.T.C.C. 6737) (Chaosuansharoen, 1999).



(17) **(18)** **(19)** **(20)**

1.2 Bromination-dehydrobromination

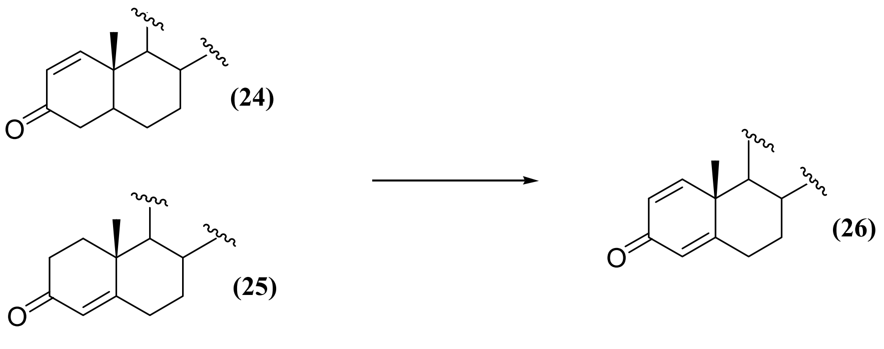
Bromination–dehydrobromination is another method widely used. Bromination of **21** with bromine in acetic acid followed by refluxing in collidine gave **23** (Herzog, 1995).



(21) **(22)** **(23)**

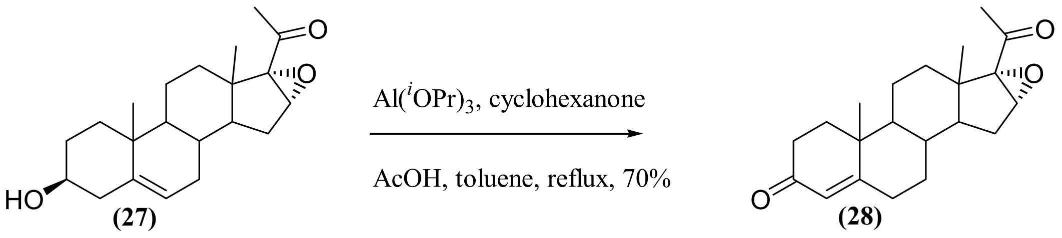
1.3 Thallium acetate

Thallium acetate in acetic acid effected dehydrogenation of **24** and **25** to **26** in 43-78% yield (Romeo and Ortar, 1972).

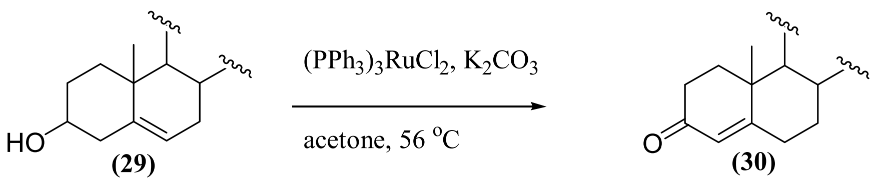


1.4 Oppenauer-type oxidation

In 1950, Julian reported the conversion of **27** to **28** by Oppenauer oxidation.

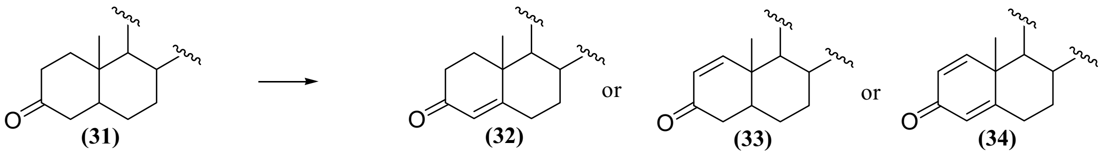


Almeida *et al* (1996) reported ruthenium-catalysed double bond isomerization-oxidation of steroidal 5-en-3β-ols to the corresponding 4-en-3-ones in 67-93% yield.



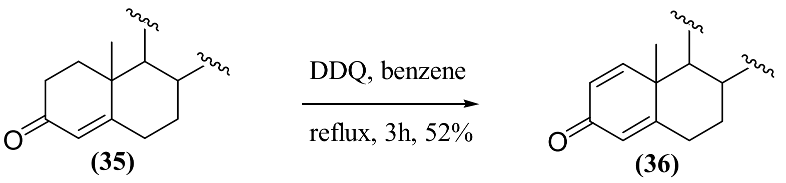
1.5 Selenium dioxide

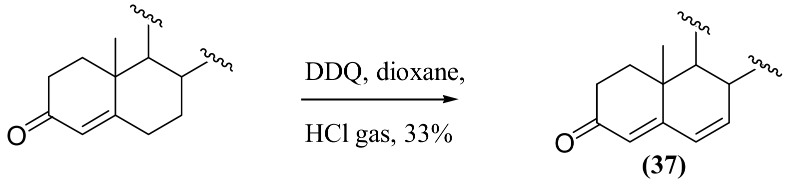
Selenium dioxide prove a versatile oxidizing agent to dehydrogenate 3-keto-steroids to Δ^{1}-3-keto-steroids or Δ^{4}-3-keto-steroids or $\Delta^{1,4}$-3-keto-steroids (Organon, 1957, Pfizer, 1958).



1.6 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ)

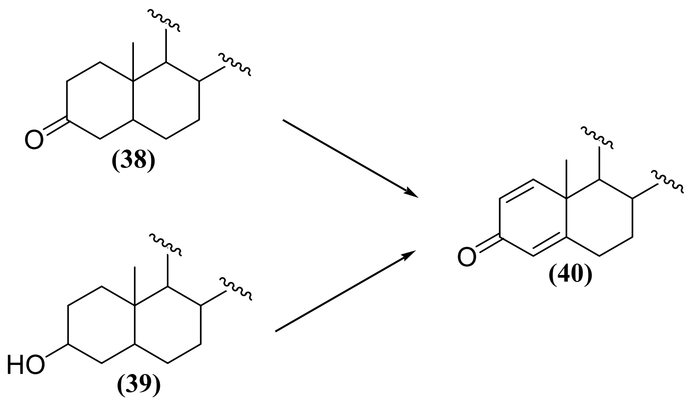
The DDQ dehydrogenation method has been used in dehydrogenation of steroid at A or B rings depending on the reaction conditions. Under neutral or weakly acidic condition, DDQ gives mostly $\Delta^{1,4}$-3-keto-steroids (Roux and Torossian, 1965), while in the presence of strong acid $\Delta^{4,6}$-3-keto-steroids are observed (Walker and Hiebert, 1966).





1.7 Diphenyl diselenide

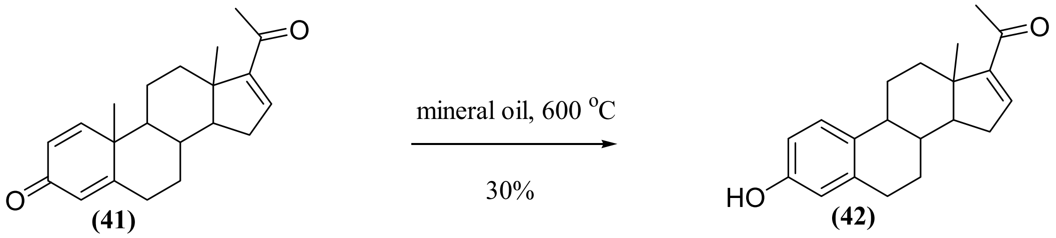
In 1984, the dehydrogenation of steroidal 3-keto (38) or 3-hydroxy steroids (39) to the corresponding 3-oxygenated $\Delta^{1,4}$-3-keto steroids **40** by benzeneseleninic anhydride and meta-iodoxybenzoic acid was reported by Barton (Scheme 2) (Barton and Motherwell, 1984, Chaosuansharoen, 1999).



Scheme 2

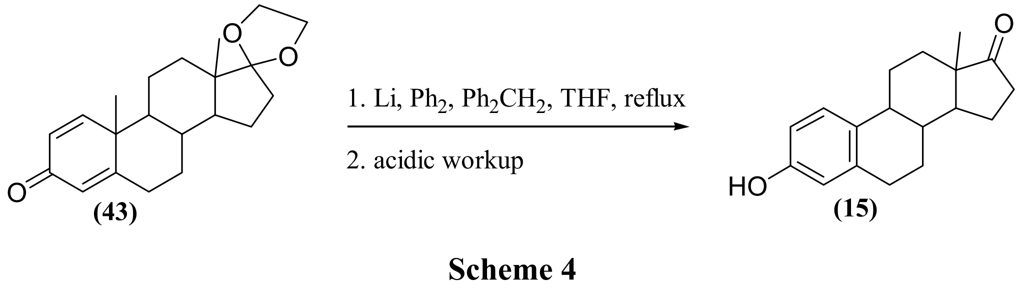
2. Conversion of A ring quinone to 19-nor $\Delta^{1,3,5(10)}$steroids

The preparation of 19-nor $\Delta^{1,3,5(10)}$-steroids was first reported by Djerassi (1951) from 1, 4, 16-trienyl-3-keto-steroids by pyrolysis in mineral oil-tetralin solution (Scheme 3).



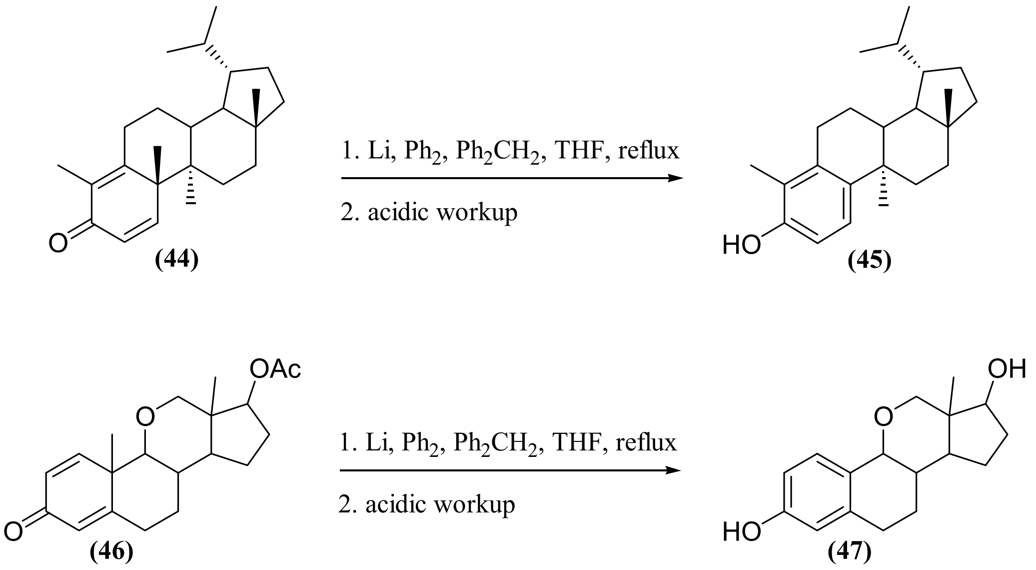
Scheme 3

Later, Dryden (1964) developed a new methodology to convert 1,4-dienyl-3-keto-17-ethylene ketal (43) to the corresponding estrone (15) by using lithium, biphenyl and biphenylmethane in boiling THF (Scheme 4). Sodium and potassium metals may replace lithium but the yield is lower.



Scheme 4

This strategy was later widely used as a general method for aromatization (Scheme 5) (Albrecht, 1989, Engel, 1989).



Scheme 5

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

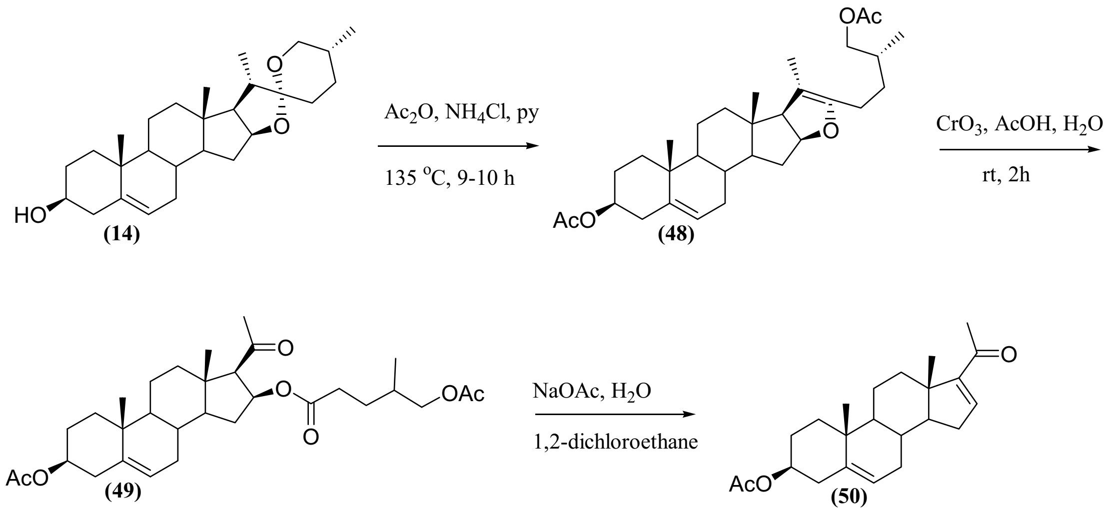
Δ^{16}-Pregn-3*β*-ol-20-one-3-acetate (50) is an important intermediate for the synthesis of many steroid drugs and hormones. Synthetic methods for this have been reported by several groups such as Carmeron *et al.*, (1955), Djerassi *et al.*, (1952) and Marker and Rohrmann (1940).

Mićović *et al.*, (1990) reported a simple one-pot preparation of 16-dehydropregnenolone acetate (50) from diosgenin (14) using normal pressure and reduced temperature. This efficient one-pot process was accomplished by the following reaction sequence.

a) Treatment of the steroidal sapogenin with acetic anhydride for 10 h at 135°C gave pseudosapogenin acetate (48).

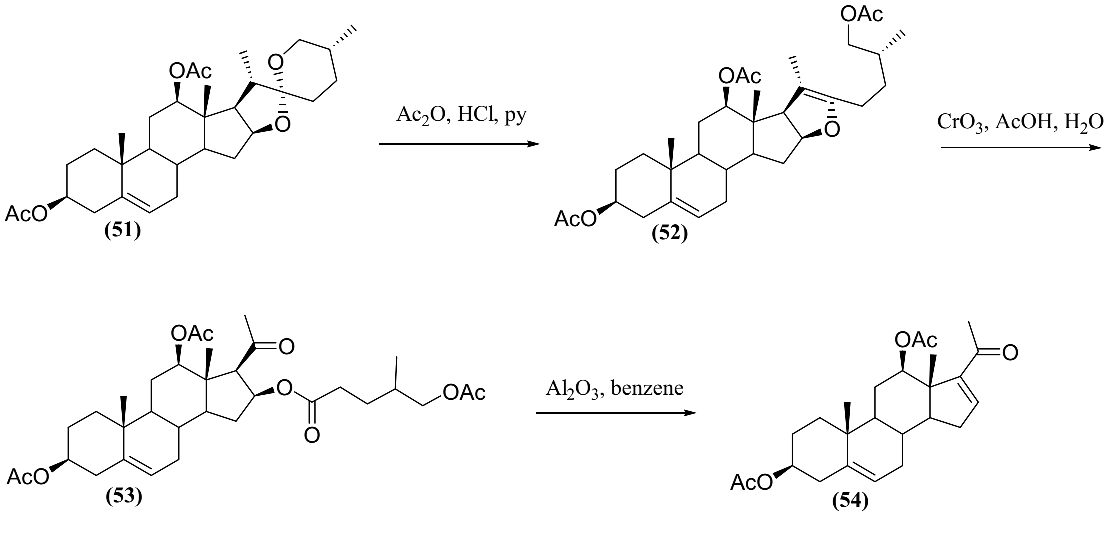
b) Oxidation using chromium trioxide in acetic acid gave **49**.

c) Heating compound (49) with dilute acid or alkali yielded 3*β*-acetoxypregna-5,16-dien-20-one (50).



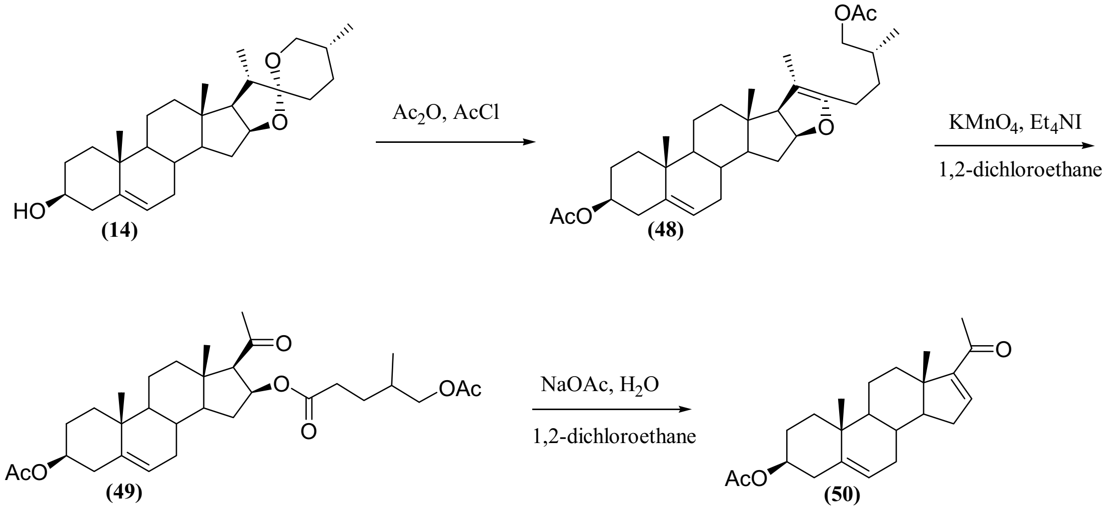
Scheme 6

Fuchs *et al.*, (1999) reported a similar conversion of rockogenin diacetate (51) to the desired enone **54** which differed slightly in the final step. Basic alumina in benzene was used in this step, effecting *β*-elimination of the pentanoate side chain to provide the desired enone **54** (Scheme 7).



Scheme 7

Recently, Goswami (2003) developed a new one-pot process for the preparation of 16-dehydropregnenolone acetate (50) from diosgenin (14) in more than 70% yield by replacing the highly toxic chromiumtrioxide with potassium permanganate in the presence of tetraethylammonium iodide. The reaction was carried out pH 3 and 0-5 °C. Under these conditions, the double bond in the substrate dihydro furan ring (48) was cleavaged to form keto ester (50) leaving the 5,6-double bond completely untouched.

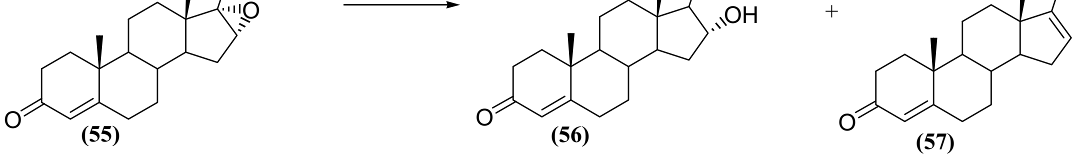


4. Reductive clevage of *α,β*-epoxy ketones to *β*-hydroxy ketones

The reduction of *α,β*-epoxy ketone to the corresponding *β*-hydroxy ketones is of the great interest for the synthesis of a variety of important of natural products. Several methods and reagents have been reported in the literature.

4.1 Chromous acetate

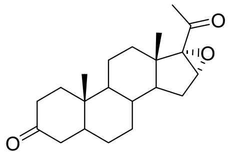
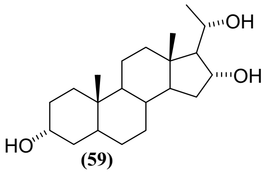
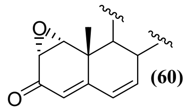
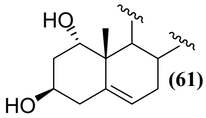
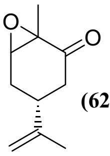
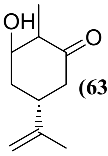
The first general method for the conversion of ring D epoxy-ketones to the corresponding *β*-hydroxy ketones was accomplished by using a chromous salt (Cole and Julian, 1954). The products from **55** depend on the chromous salt used. Using chromous acetate increased the yield of the desired *β*-hydroxy ketone **56** whilst chromous chloride led to α,β-unsaturated ketones e.g. **57**.



4.2 Alkali metal in liquid ammonia

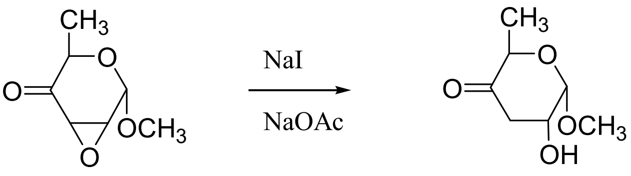
The reductive cleavage of the epoxy ketones with lithium in liquid ammonia in the presence of a proton source (ammonium chloride) provides an alternative sequence of reduction and protonation. Some of examples are shown in Table 1.

Table 1 Reductive cleavage of α,β-epoxy ketone by lithium in liquid ammonia.

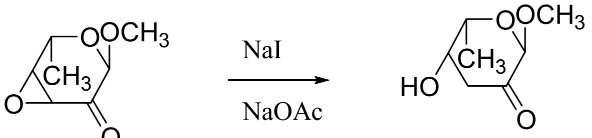
Entry	Epoxy ketone	Product	Reference
1	 **(58)**	 **(59)**	Hirschmann and Dans, 1959
2	 **(60)**	 **(61)**	Barton *et al*., 1973
3	 **(62)**	 **(63)**	Grieco *et al*., 1976

4.3 Sodium iodide

Treatment of an *α,β*-epoxy ketone **64** or **66** with sodium iodide and sodium acetate in the presence of acetic acid and acetone as solvent at ambient temperature for 5 min afforded the corresponding *β*-hydroxy ketone product **65** or **67** in 97% yield (Paulsen et al., 1974)



(64) **(65)**

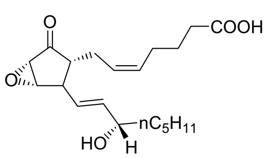
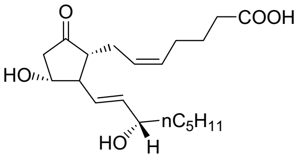
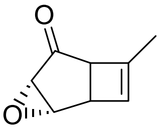
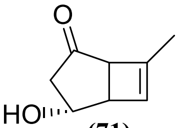
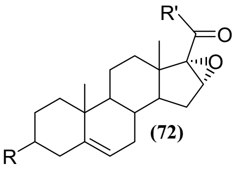
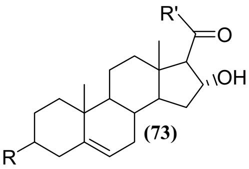


(66) **(67)**

4.4 Aluminium amalgam [Al(Hg)]

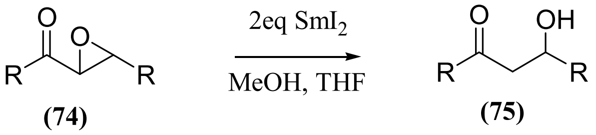
Reduction of epoxy ketones to the corresponding *β*-hydroxy ketones by aluminium amalgam under various reaction conditions has been investigated (Table 2).

Table 2 Reductive cleavage of α,β-epoxy ketone using aluminium amalgam.

Entry	Epoxy ketone	Product	Reference
1	 **(68)**	 **(69)**	Corey and Ensley, 1973
2	 **(70)**	 **(71)**	Moreno, 1993
3	 **(72)** R = OH R' = Me OAc = Me OH = CH_2OH OAc = CH_2OAc	 **(73)**	Greene, 1982

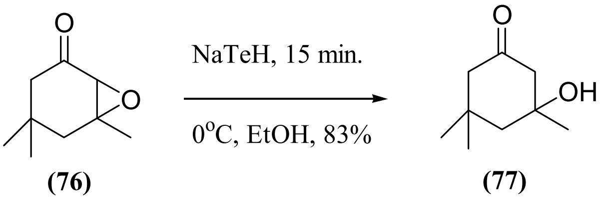
4.5 Samarium iodide (SmI_2)

Mukaiyama (2000) reported the reduction of alkyl or aryl oxiranyl ketones (74) with two equivalents of SmI_2 in the presence of protic solvent such as methanol to yield mono-aldol products **75** in 70-90% yield.



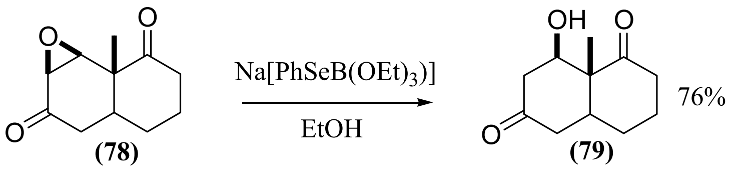
4.6 Sodium hydrogen telluride

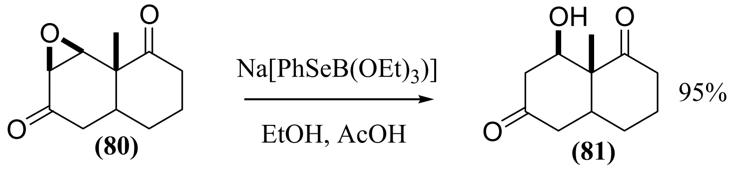
Sodium hydrogen telluride (NaTeH), which is readily prepared *in situ* from tellurium and sodium borohydride ($NaBH_4$), has been used for several chemoselective reductions of epoxy ketones to the corresponding *β*-hydroxyketones e.g. **76** to **77** (Osuka, 1984).

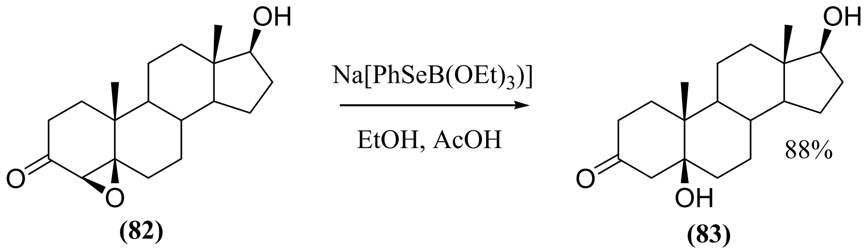


4.7 Organoselenium borates

Reduction of *α,β*-epoxy ketones to *β*-hydroxy carbonyl compounds by the use of sodium phenylseleno(triethyl)borate complex, $Na[PhSeB(OEt)_3]$, easily prepared by reduction of diphenyldiselenide with sodium borohydride in ethanol, was reported by Yoshikoshi (1987, 1997) to be an excellent reducing agent for these transformations.

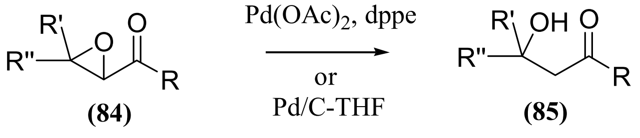






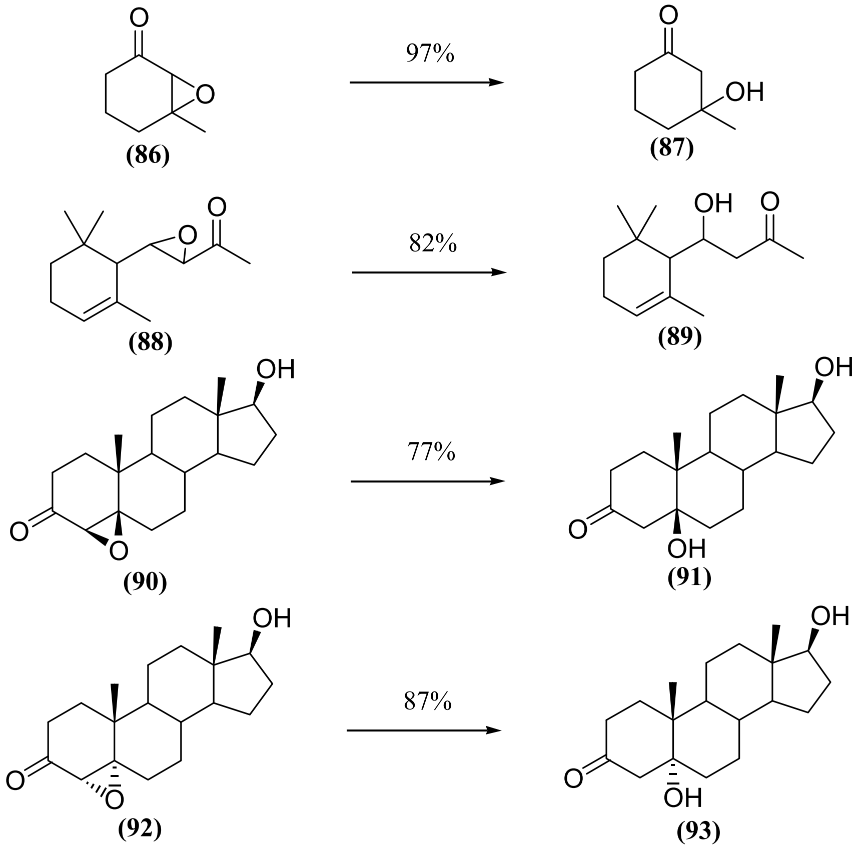
4.8 Palladium catalyzed rearrangement

The palladium catalyzed rearrangement of *α,β*-epoxy ketones to *β*-hydroxy ketones was reported by Torii (1989). The combination of ammonium formate and Pd(0) is known to work as a mild reducing agent and was used in the transformation of *α,β*-epoxy ketones or esters to *β*-hydroxy carbonyl compounds. Two procedures have been used for this conversion. One is a reduction with triethylammonium formate in the presence of palladium catalysts such as $Pd(OAc)_2$ and dppe and the second is hydrogenation in a Pd/C-THF system e.g. **84** to **85**.



4.9 Lithium naphthalenide

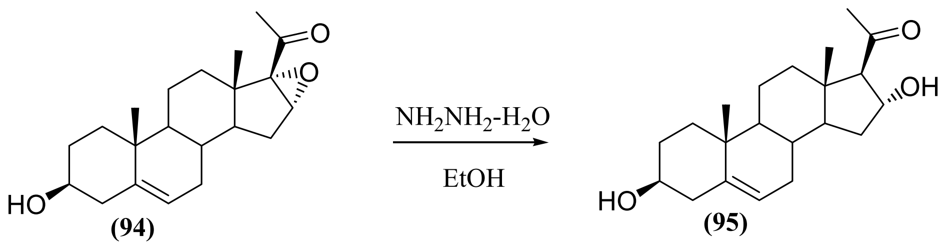
Lithium napthalenide, prepared *in situ* from lithium and naphthalene in THF is a mild and efficient reagent for cleavage *α,β*-epoxy ketones to *β*-hydroxy ketones (Jankowska et al. 1999). The generality of this procedure is evident from the results shown in (Scheme 8).



Scheme 8

4.10 Hydrazine hydrate

Salvador (2005) described an efficient and versatile procedure for the reductive cleavage of *α,β*-epoxy ketones **94** to the corresponding *β*-hydroxy ketones **95** under mild conditions using hydrazine hydrate in ethanol.

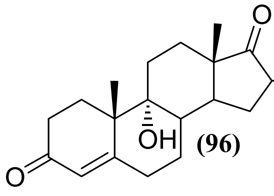
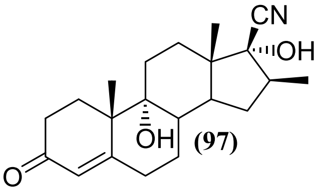
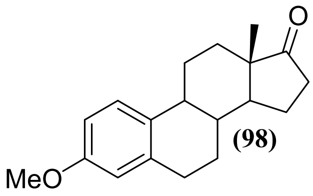
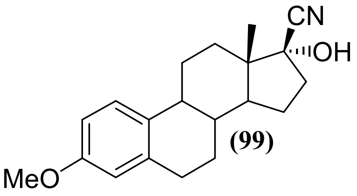


5. Conversion of 17-keto steroids to 17-acetyl steroids

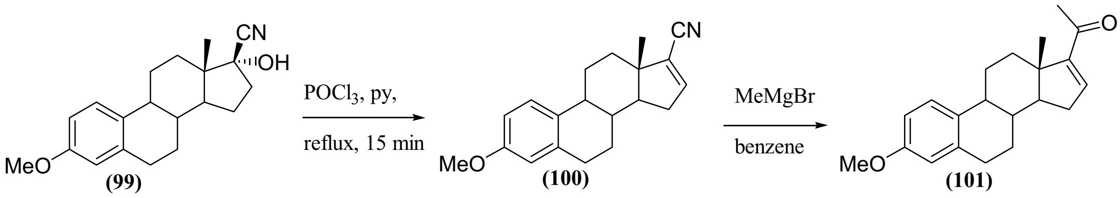
This process was accomplished by the addition of cyanide to the 17-keto steroid Table 13 followed by dehydration and additive of methylmagnesium bromide e.g. **99** to **101** and **102-104**.

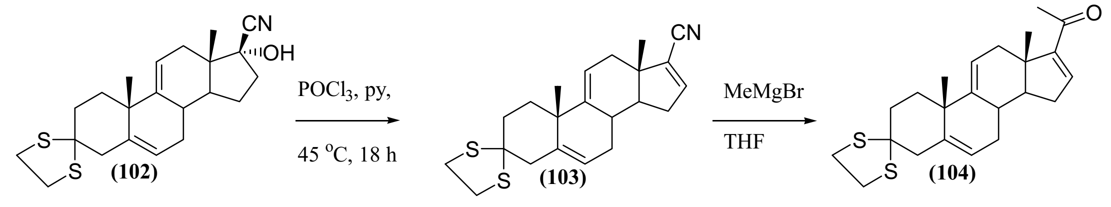
Conversion of 17-ketone steroids to cyanohydrin has been studied in various reaction conditions as shown in table 3.

Table 3 Conversion of 17-ketone steroids to cyanohydrin.

Entry	17-ketone steroid / reagents	Product	Reference
1	 **(96)** - KCN, AcOH, MeOH, 85% - KCN, AcOH, MeOH, CH_2Cl_2, 18h, 24% - KCN, MeOH, H_2O, AcOH, 87%	 **(97)**	Carruthers, 1992
2	 **(98)** TMSCN, ZnI_2, CH_2Cl_2, 88%	 **(99)**	McGuire, 1994

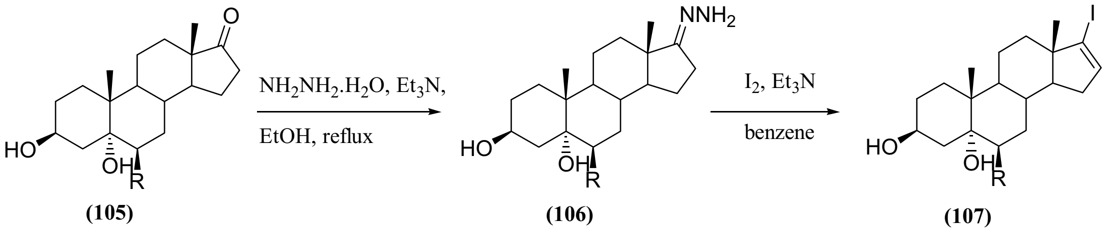
Dehydration of this cyanohydrin using phosphorous oxychloride in pyridine has been reported by several groups (McGuire, 1994, Lokos, 1993, Geurtz, 1997).





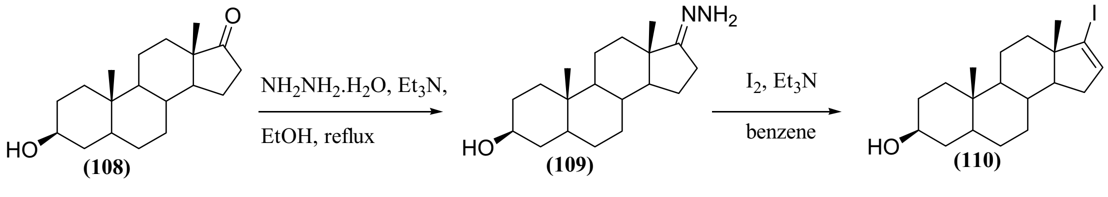
6. Conversion of 17-keto steroids to 16,17-unsaturated carbonitrile by palladium catalysts

In 2003, Marson reported the conversion of 17-ketone steroids to hydrazone using hydrazine hydrate in refluxing ethanol followed by reaction with iodine and triethylamine in benzene to afford the vinyl iodide product **105** to **106**.



R = H, 33% (2 steps)
= OH, 59% (2 steps)

This strategy was also used by Beaubien (2006) to convert to 110 in 91% yield over 2 steps.



There are several examples reported of cyanation of vinyl or aryl iodides to vinyl or aryl cyanides using various cyanide sources.

6.1 Impregnated cyanide reagent

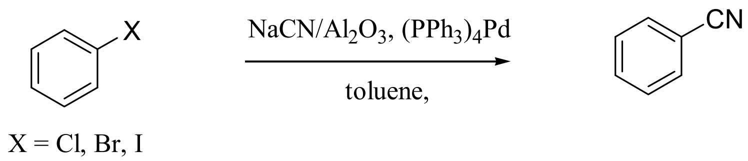
Regen (1979) reported a convenient method for carrying out cyanide displacement of organic halide using impregnated cyanide reagent which was prepared by stirring an aqueous solution of sodium cyanide with neutral alumina and the removal of the water under reduced pressure.

$$CH_3\text{-}(CH_2)n\text{-}CH_2\text{-}X \xrightarrow[\text{toluene, } 90\ ^{o}\text{C, 45h}]{NaCN/Al_2O_3} CH_3\text{-}(CH_2)n\text{-}CH_2\text{-}CN$$

n = 4-10
X- cl, Br, I

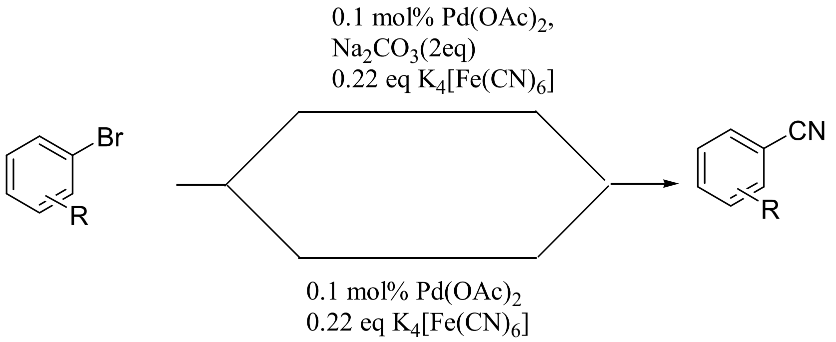
27-100%

He also reported a versatile strategy to prepare aryl cyanides by treatment of the aryl halide with the alumina impregnated cyanide in the presence of tetrakis(triphenylphosphine)palladium(0) in toluene.



6.2 Potassium hexacyanoferrate(II)

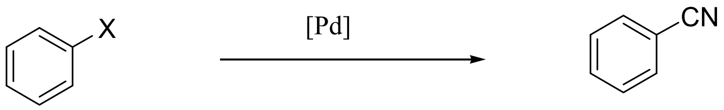
In 2004, Beller *et al.* discovered that benzonitriles were easily accessible via palladium-catalyzed cyanation of aryl halides using potassium hexacyanoferrate(II) as cyanating agent. Whilst, in the following year Weissman reported ligand-free cyanation of aryl halides utilizing palladium-catalysis in the combination with potassium hexacyanoferrate(II). The positive features of potassium hexacyanoferrate(II) are that all six cyanide groups are available for the reaction, it is inexpensive, easily handled, and nontoxic (Weissman, 2005).



6.3 Other cyanide sources: sodium cyanide (NaCN), potassium cyanide (KCN), trimethylsilyl cyanide (Me_3SiCN), zinc (II) cyanide ($Zn(CN)_2$ and tri-*n*-butyltin cyanide (*n*Bu_3SnCN)

Beller (2004), summarized the reaction conditions for palladium-catalyzed cyanation of aryl halide reported prior to 2000 (Table 4) and also described his own work ligands Pd catalysts.

Table 4 Palladium catalyzed cyanation of aryl halides with different cyanide sources and palladium catalysts.

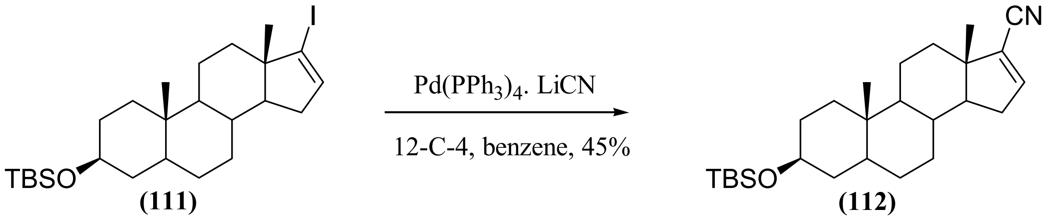


Entry	X	Catalyst (mol%)	Additive (mol%)	Cyanide (mol.eq)	Solvent	T (°C)/t(h)
1	I, Br	$Pd(OAc)_2$ (2)	-	KCN (2)	DMF	140/2-12
2	I	$Pd(PPh_3)_4$ (20)	-	KCN (1.5)	THF	Reflux/8
3	I, Br	$Pd(OAc)_2$ (1.5)	KOH(0.05), KI(9)	KCN (2)	HMPT	60-90/2-9
4	I, Br	$Pd(PPh_3)_4$ (10)	Al_2O_3	NaCN (5)	Toluene	80-100/2-40
5	I, Br	$Pd(PPh_3)_4$ (5)	-	KCN (1.5)	DMF	reflux/2-5
6	Cl	$Pd(PPh_3)_4$ (20)	18-C-6(40)	KCN (1)	Benzene	100/65
7	Br	$Pd(PPh_3)_4$ (2)	-	Me_3SiCN	Et_3N	reflux/0.17-0.5
8	I	$Pd(PPh_3)_4$ (1.5)	18-C-6(7.5), BuI(250)	KCN (250)	DMF	reflux/2

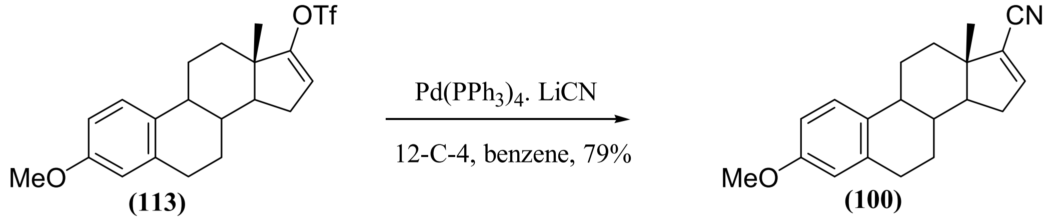
Table 4 cont'd

Entry	X	Catalyst (mol%)	Additive (mol%)	Cyanide (mol.eq)	Solvent	T (^{o}C)/t(h)
9	Br	$Pd(PPh_3)_4$ (not given)	-	*n*Bu_3SnCN (not give)	DMF	Not give
10	I	$PdCl_2(PPh_3)_2$ (2)	-	KCN (2)	DMF	reflux/2
11	Cl	$Pd_2(dba)_3$ $(CHCl_3)$(0.5),dppf (2)	-	KCN (2)	NMP	60-80/1-8
12	I	$Pd(PPh_3)_4$ (2-6)	-	$Zn(CN)_2$ (0.6)	DMF	80/0.5-7
13	I, Br	$Pd(PPh_3)_4$ (7)	-	$Zn(CN)_2$ (0.6)	NMP	90/20
14	I, Br, OTf	$Pd(PPh_3)_4$ (5)	Cu(I) (12)	NaCN (2)	MeCN	reflux/1-6
15	Br	$Pd_2(dba)_3$ (0.05-1), dppf	-	$Zn(CN)_2$ (0.6)	DMF (wet)	120/20
16	Cl	$Pd_2(dba)_3$ (2)	Zn(12)	$Zn(CN)_2$ (0.6)	DMF	120/1-12

However there are only a few examples involving cyanation of steroids (Beaubien) involving both a triflic enol ether or vinyl iodide, tetrakis(triphenylphosphine)palladium(0), lithium cyanide and 12-crown-4 in benzene e.g. **111** to **112** and **113-100**.



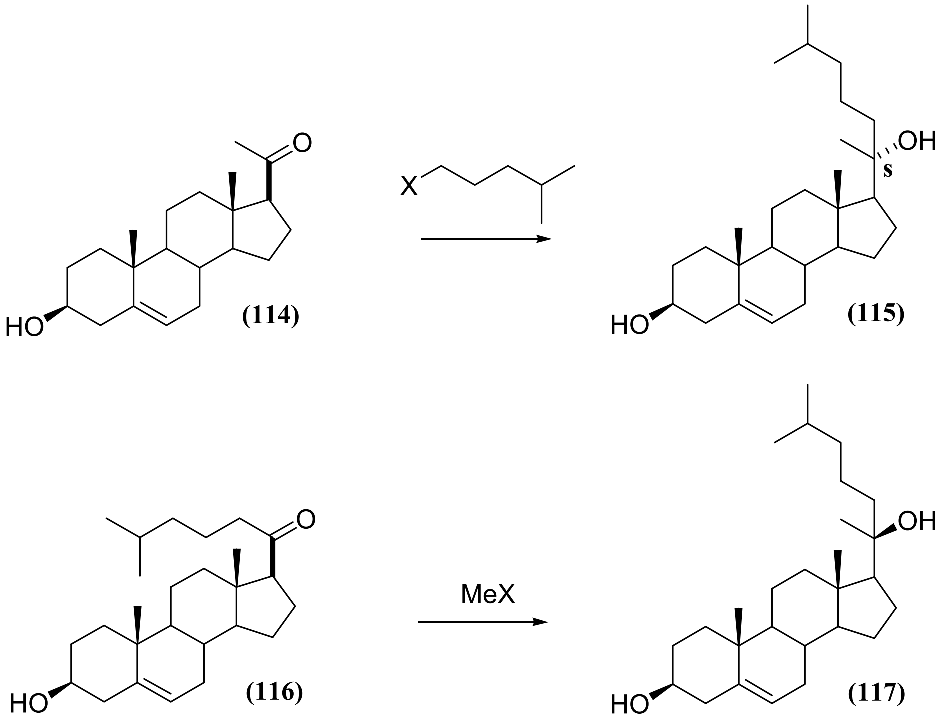
(111) **(112)**



(113) **(100)**

7. Nucleophilic addition of side chain to C-20 keto steroid

Nucleophilic addition to C-20 keto-steroids creates a new an asymmetric center at C-20, as illustrated in Scheme 9. The two epimers differ in the chemical shift of the 21-methyl proton of the 20α-hydroxy epimer (115) (1.17 ppm) which is downfield relative to that of the 21-methyl proton of the 20*β*-hydroxy epimer (117) (1.00 ppm) by about 0.17 (Chaudhuri, 1969).

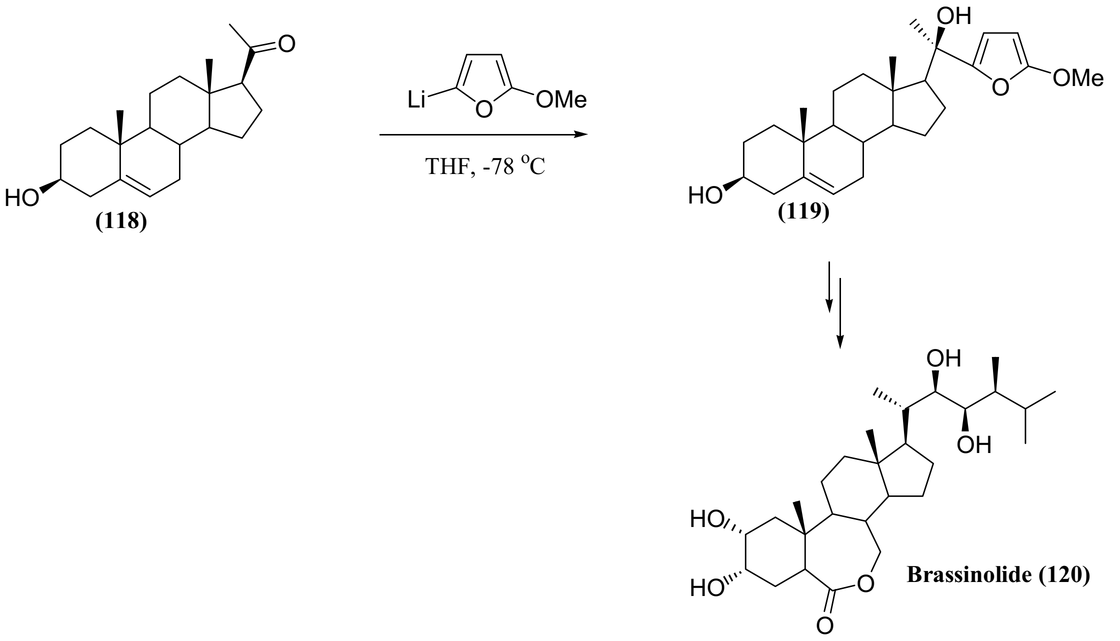


Scheme 9

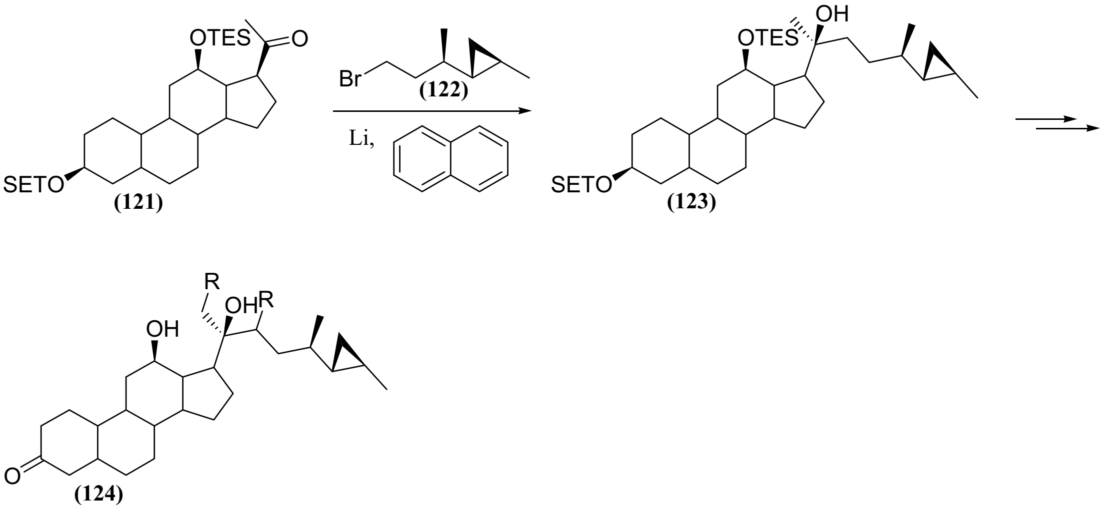
There are several methods reported for the addition of alkyl side chains to C-20 keto-steroids.

7.1 Alkyl lithium

Kametani reported the synthesis of brassinolide (120) utilizing a key stereocontrol led addition of 5-lithi-2-methoxyfuran to pregnenolone (118) to afford only the *S*-epimer **119**.



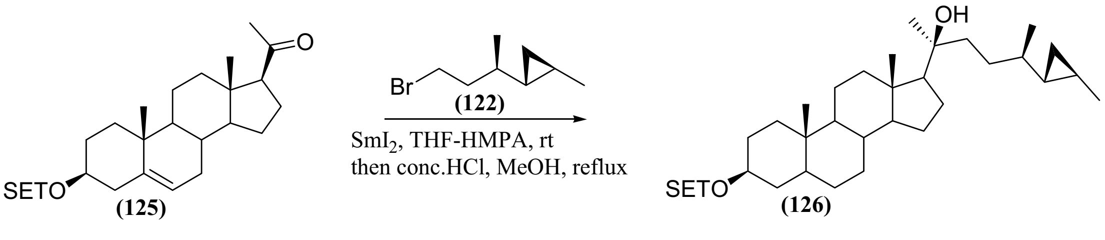
Yamada (1996) reported the synthesis of aragusterols (124) by utilizing the reaction of the 20-keto-steroid **121** with alkyl lithium prepared from bromide (122) with lithium naphthalinide in THF at 0°C which gave the corresponding alcohol (123) with high stereoselectivity (20*S* : 20*R* = 26 :1 ; determined by ^{1}H NMR analysis).



7.2 Sammarium(II)iodide

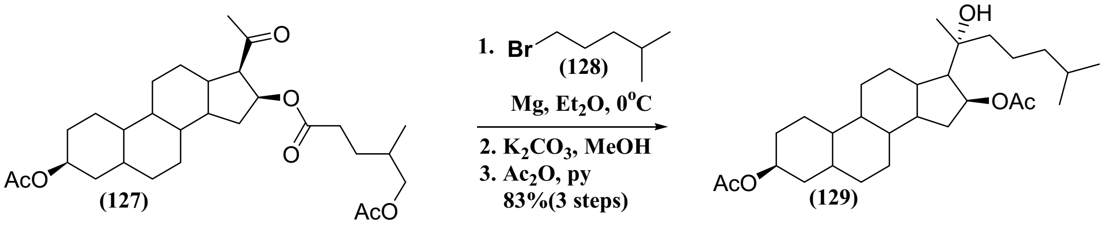
Aragusterols (124) were also synthesized by Honda *et al.*(1996), by coupling the 20-keto-steroid (125) with an alkyl lithium or Gridnard reagent, but yields of **126** were poor. However using samarium(II)iodide promoted coupling of **125** and

122 afforded **126** in 90% yield. The stereochemistry at the 20-position of product (126) was assumed to be the usually observed *S*-configuration.



7.3 Gridnard reagent

Recently, Giner (2002) employed the Gridnard addition of 1-bromo-4-methyl pentane (128) to tigone diacetate (127) to give *R*-**129**. However the explanation for assignment of the 20-C configuration is unclear.



MATERIALS AND METHODS

Materials

Instrumentation

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

Proton nuclear magnetic resonance (^1H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recoded on a Varian Gemini 300 spectrophotometer and on 400 MHz Brucker Advance DPX-400. Chemical shifts were recoded as δ values in ppm. Spectra were acquired in $CDCl_3$ unless otherwise state. The peak due to residual $CHCl_3$ (7.26 ppm for ^1H 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 follows: br = broad, s = singlet, d = doublet, dd = double of doublet, dt = double 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 Chemistry Department, Faculty of Science, Kasetsart University. Samples were analyzed as KBr disks.

Mass spectra were obtained on an Agilent Technology 1100 series LL/MSD Trap; the first number denotes *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 Mass spectrometry Reserch Laboratory, Department of Chemistry, Faculty of Science, Chiang Mai University.

Melting points (m.p) were determined on a Mel-Temp electrothermal apparatus at the Chemistry Department, Kasetsart University and are reported uncorrected in ºC.

Chromatographic system

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

Flash column chromatography was conducted according to the method of Still and co-workers (1978) using silica gel 60 (mesh size 0.040-0.063mm). Column chromatography was performed on silica gel 60 (70-230 mesh, Merck) and the column was packed by slurry method.

Chemical reagents

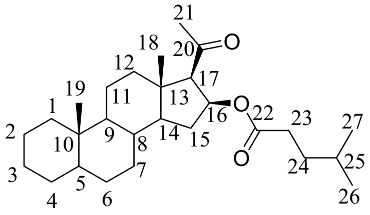
Analytical grade solvents and reagents used for synthesis were obtained from local commercial outlets. Dry tetrahydrofuran (THF) was freshly distilled under N_2 from sodium with benzophenone ketyl as an indicator.

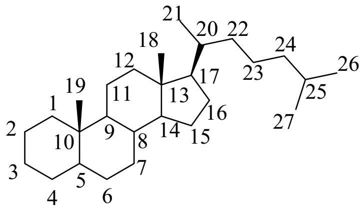
Experimental details of the reactions that failed have not been reported, although they are discussed.

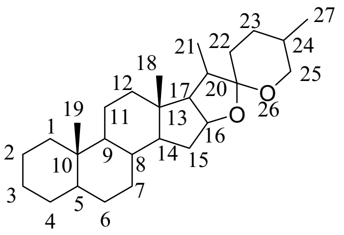
Methods

Numbering of tetracyclic steroid carbon skeleton

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

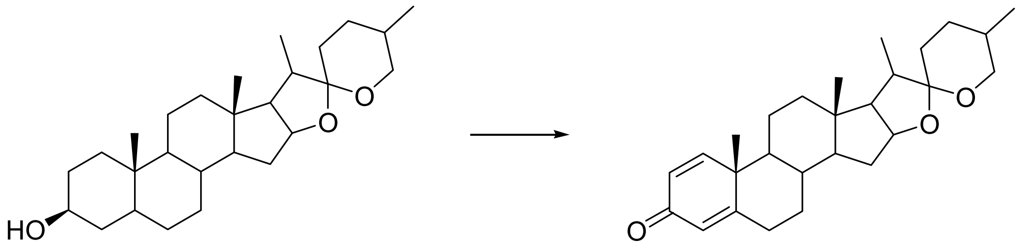






Synthetic intermediate towards geodisterol

Spirosta- $\Delta^{1,4}$-diene-3-one (130)



tigogenin (12) **(130)**

A mixture of meta-iodoxybenzoic acid (43 g, 153.85 mmol) and diphenyl diselenide (972 mg, 3.08 mmol) in toluene (50 ml) was refluxed until the yellow color of diphenyl diselenide disappeared. A solution of tigogenin (12) (6.4 g, 15.38 mmol) in toluene (50 ml) was introduced to this mixture. The reaction mixture was further heated under reflux for 7 h. The resulting mixture was cooled to room temperature and partitioned with methylene chloride and water. The organic layer was separated and dried over anhydrous sodium sulphate, filtered and concentrated under *vacuo*. The residue was purified by flash column chromatography (1 : 9 ethyl acetate : hexane) to provided **130** as a white solid (4.37 g, 70%), mp 182-184°C [lit 194-195 °C] (Sondheimer, 1954).

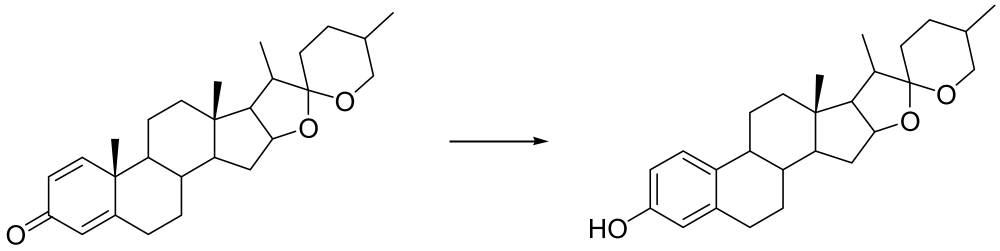
FTIR (KBr), ν_{max} , cm^{-1}: 1664 (C=O).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 6.97 (d, J=10.14 Hz, 1H, H-1), 6.2 (dd, J=10.2 Hz, 1.99 Hz, 1H, H-2), 5.99 (m, 1H, H-4), 4.31(m, 1H, H-16), 3.39 (m, 1H, H-26), 3.28 (m, 1H, H-26), 1.94 (m,1H, H-15), 1.27(m, 1H, H-15), 1.18 (s, 3H, H-19), 0.89 (d, J=7 Hz, 3H, H-27,), 0.78 (s, 3H, H-18), 0.71 (d, J=6.36 Hz, 3H, H-21).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 186.1 (C-3), 168.8 (C-10), 155.6 (CH-1), 127.3 (CH-4), 123.7 (CH-2), 109.6 (C-5), 80.3 (CH-16), 66.7 (CH_2-26), 61.9 (CH), 55.1 (CH), 52.2 (CH), 43.4 (C), 42.5 (CH), 40.3 (CH_2-15), 39.3 (C), 34.9 (CH), 33.6 (CH_2), 32.6 (CH_2), 31.8 (CH_2), 31.2 (CH_2), 30.1 (CH), 28.7(CH_2), 22.6 (CH_2), 18.6 (CH_3-19), 16.9 (CH_3-21), 16.7 (CH_3-18), 14.3 (CH_3-27).

MS (EI), *m/z* (relative intensity): 410(18), 351(14), 289(10), 181(28), 139(100).

19-Norpregna-$\Delta^{1,3,5(10)}$statriene-3-ol (131)



(130) **(131)**

Lithium was cut into small pieces and introduced to the mixture of biphenyl (17.3 g, 112.1 mmol) in dry tetrahydrofuran (100 ml) under N_2 atmosphere at room temperature. The mixture was stirred under reflux for 0.5 h when the solution had turned blue. The mixture was treated dropwise, under N_2, with a solution of **130** (6.5 g, 16.0 mmol) and diphenyl methane (7.8 ml) in dry tetrahydrofuran. After refluxing for 2 h and cooling, methanol was added to destroy the excess of lithium. The solvent was removed and residue was dissolved in 10% aq. HCl and extracted with methylene chloride. The organic layer was washed with water, dried over anhydrous sodium sulphate, filtered and the solvent was removed in *vacuo*. Purification of the crude product by flash column chromatography eluting with ethyl acetate : hexane (1:9) provided the desired product 19-Norpregna-$\Delta^{1,3,5(10)}$statriene-3-ol (131) as a white solid (2.47 g, 44%), mp 244-246°C [lit 245-247°C] (Sondheimer, 1954).

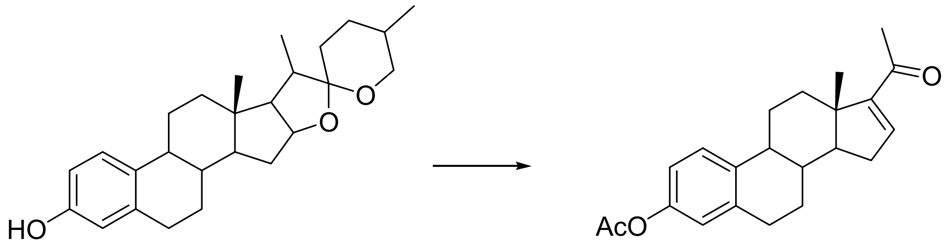
FTIR (KBr), ν_{max}, cm $^{-1}$: 3402 (OH), 3062 (CH-Ar), 1609.

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.06 (d, *J*=8.36 Hz, 1H, H-1), 6.54 (dd, *J*=2.76 Hz, 8.36 Hz, 1H, CH-2), 6.48 (d, *J*=2.76 Hz, 1H, CH-4), 4.67 (s, OH), 4.37 (m, 1H, H-16), 3.44 (m, 1H, H-26), 3.32 (t, *J*=10.83 Hz, 1H, H-26), 2.76 (m, 2H, H-6), 2.01 (m, 1H, H-15), 1.83 (m, 1H, H-17), 1.30 (m, 1H, H-15), 0.92 (d, *J*=6.84 Hz, 3H, H-27), 0.75 (s, 3H, H-18), 0.72 (d, *J*=6.32 Hz, 3H, H-21).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 153.7 (C-3), 138.3 (C-5), 132.8 (C-10), 126.3 (CH-1), 115.2 (CH-4), 112.6 (CH-2), 109.3 (C-22), 80.9 (CH-16), 66.9 (CH_2-26), 62.3 (CH), 55.3 (CH-14), 43.7 (CH-9), 41.6 (CH), 40.8 (C-13), 39.9 (CH_2-12), 38.3 (CH-8), 31.53 (CH_2-15), 31.40 (CH_2), 30.3 (CH), 29.5 (CH_2-6), 28.8 (CH_2), 27.8(CH_2-7), 26.4 (CH_2-11), 17.1 (CH_3-21), 16.4 (CH_3-18), 14.5 (CH_3-21).

MS (EI), *m/z* (relative intensity): 396(13), 282(48), 139(100).

3, 17-Acethyl-$\Delta^{1,3,5(10),16}$-estratetraene (132)



(131) **(132)**

A mixture of 19-nor-$\Delta^{1,3,5(10)}$-spirostatriene-3-ol (131) (300 mg, 0.78 mmol), acetic anhydride(5.4 ml), ammonium chloride (83.14 mg, 1.56 mmol) and pyridine (0.06 ml) was heated to 125-135 °C and kept at this temperature for 12 hours. After cooling down, the mixture was dissolved in 1,2-dichloroethane (0.7 ml), water (0.083 ml), acetic acid (0.25 ml) and acetone (2 ml) and cooled to 0 °C. A solution of chromium trioxide (105 mg, 1.05 mmol, 2.9 eq) in water (2.19 ml) and acetic acid (0.25 ml) was added (keeping the temperature below 7 degree celsius) then the reaction mixture was stirred in ice water until TLC indicated the completion of the reaction. Then a solution of sodium chloride (124 mg) in water (1.66 ml) and methanol (1.66 ml) was introduced and stirred for 1 h. The reaction mixture was neutralized using sodium bicarbonate and extracted with methylene chloride and the organic phase was washed with water, dried over anhydrous sodium sulphate, filtered and the filtrate concentrated. The residue was dissolved in benzene (30 ml) and basic aluminar (1 g) added and the mixture stirred for 16 h, filtered, the residue washed with methylene chloride and the filtrate evaporated. The residue was purified by flash column chromatography eluting with 1 : 9 ethyl acetate : hexane to give 3, 17-acetyl-$\Delta^{1,3,5(10),16}$-estratetraene (132) as a white solid (146.6 mg, 58%), mp 154-156°C [lit 161-162 °C] (Sondheimer, 1954).

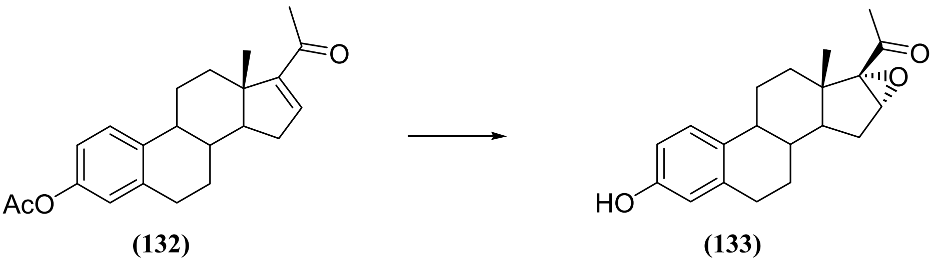
FTIR (KBr), ν_{max}, cm $^{-1}$: 3062, 1609 (C=O).

^{1}H NMR (CDCl$_3$, 400MHz) δ:7.32 (d, *J*=8.43Hz, 1H, CH-1), 6.88 (dd, *J*=8.4, 2.6Hz, 1H, CH-2), 6.83 (d, J=2.4Hz, H, CH-4), 6.78 (dd, *J*=3.3, 1.8Hz, 1H, CH-16), 2.93 (m , 2H, CH$_2$-6), 2.32 (s, 3H, Me), 2.32 (s, 3H, Me), 0.95 (s, 3H, Me).

^{13}C NMR (CDCl$_3$, 100MHz) δ: 196.7 (C=O), 169.8 (C=O), 155.4 (C-3), 148.4 (C-10), 148.4 (C-17), 144.1 7(CH-16), 137.9 (C-5), 126.1 (CH-1), 121.4 (CH-4), 118.5 (CH-2), 55.6 (C-13), 46.4 (CH), 44.3 (CH), 36.6 (CH), 34.7 (CH$_2$), 31.9 (CH$_2$), 29.3 (CH$_2$), 27.5 (CH$_2$), 27.1 (CH$_3$), 26.2 (CH$_2$), 21.0 (CH$_3$), 15.8 (CH$_3$).

MS (EI), *m/z* (relative intensity): 296(100), 159(94).

16α,17α-Epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (132)



(132) **(133)**

To a stirred solution of **132** (45 mg) in methanol (5 ml) was added 2.5 N potassium hydroxide (1 ml) at room temperature. The reaction mixture was cooled to 0 °C and then was treated with 30% hydrogen peroxide (1 ml). The mixture was stirred for 16 h. The solvent was removed, the residue extracted with methylene chloride, dried over anhydrous sodium sulphate and concentrated in *vacuo*. The crude product was purified by flash column chromatography eluting with 3 : 7 ethyl acetate : hexane to give 16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (133) as a white solid (37 mg, 90%), mp 224-226 °C [lit 234.6 °C] (Djerassi, 1985).

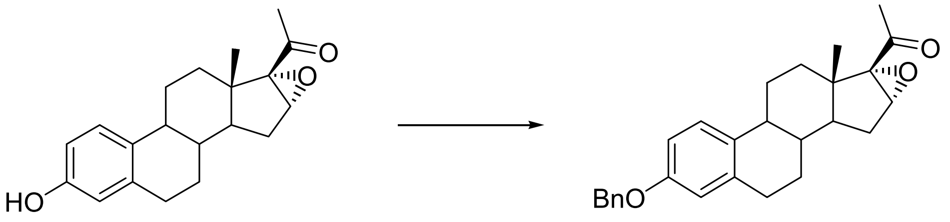
FTIR (KBr), ν_{max}, cm $^{-1}$: 3468 (OH), 3018 (CH-Ar), 1686 (C=O), 1620.

^{1}H NMR (CDCl$_3$, 400MHz) δ: 7.15 (d, J= 8.46 Hz , 1H, H-1), 6.65 (dd, *J*= 8.36, 2.74 Hz, 1H, H-2), 6.58 (d, *J*=2.66 Hz, 1H, H-4), 4.80 (s, 1H), 3.76 (s, 1H, H-16), 2.84 (m, 2H, H-6), 2.08 (s, 3H, H-21), 1.08 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 204.9 (C-20), 153.4 (C-3), 137.9 (C-10), 132,4 (C-5), 126.3 (CH-1), 115.2 (CH-4), 112.7 (CH-2), 71.1 (CH), 60.5 (CH), 44.5 (CH), 44.0 (CH), 42.1 (C-13), 36.3 (CH), 31.5 (CH_2), 29.4 (CH_2), 27.4 (CH_2), 27.2 (CH_2), , 26.0 (Me-21), 25.9 (CH_2), 15.3 (Me-18).

MS (EI), *m/z* (relative intensity): 312(15), 251(3).

3-Benzyloxy-16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (134)



(133) **(134)**

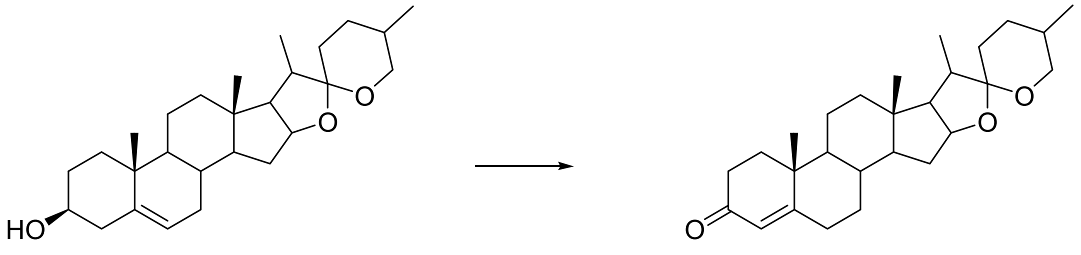
To a mixture of 16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (133) (20 mg, 0.064 mmol), K_2CO_3 (13 mg, 0.096 mmol) in acetone (2ml) was added benzyl bromide (0.01ml, 0.076 mmol) and refluxed for 6 h. The reaction mixture was quenched with saturated aqueous ammonium chloride and the solvent was removed under reduce pressure. The mixture was extracted with methylene chloride and the combined organic layer was washed with water, dried over anhydrous sodium sulfate, filtered and the filtrate concentrated under reduce pressure. The crude product was purified by flash column chromatography (1 : 4 ethyl acetate : hexane) to provide 3-benzyloxy-16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (134) as a colorless wax (25 mg, 97%), mp 100-101°C.

FTIR (KBr), ν_{max} , cm $^{-1}$: 1702 (C=O).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.36-7.23 (m, 5H, ArH), 7.10 (d, *J*=8.42 Hz, 1H, CH-1), 6.70 (dd, *J*=8.60, 2.68 Hz, CH-2), 1H), 6.63 (d, *J*=2.72 Hz, 1H, CH-4), 4.95 (s, 2H, CH_2-Ar), 3.64 (s, 1H, CH-16), 2.78 (m, 2H, CH_2-6), 1.98 (s, 3H, CH_3-21), 0.99 (s, 3H, CH_3-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 172.0 (C=O), 158.2 (C-3), 137.9 (C-10), 137.5 (C-Ar), 133.3 (C-5), 128.7 (CH-Ar), 128.1 (CH-Ar), 127.6 (CH-Ar), 126.3 (CH-1), 115.1 (CH-4), 112.4 (CH-2), 70.1 (CH_2OBn), 60.7 (CH-16), 56.2 (C-16), 44.7 (CH), 44.3 (CH), 42.2 (C-13), 36.5 (CH), 31.7 (CH_2), 29.8 (CH_2-6), 27.7 (CH_2), 27.4 (CH_2), 26.2 (Me-21), 26.1 (CH_2), 15.5 (Me-18).

Spirosta–4–ene-3-one (135)



diosgenin (14) **(135)**

A solutin of diosgenin (14) (10 g, 24.6 mmol) in dry toluene (80 ml) and cyclohaxanone (36 ml) was refluxed under N_2 atmosphere. It was then treated dropwise with a solution of aluminium isopropoxide (3.52 g) in dry toluene (50ml) over a period of 10 min. The reaction conversion was complete after 4 h. The reaction mixture was extracted with diethyl ether. The combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure. The crude was purified by flash column chromatography (1 : 9 ethyl acetate : hexane) to provide spirosta–4–ene-3-one (135) as a white solid (7.03 g, 70%), mp 180-182^{o}C [lit 180-182 ^{o}C] (Lee, 1998).

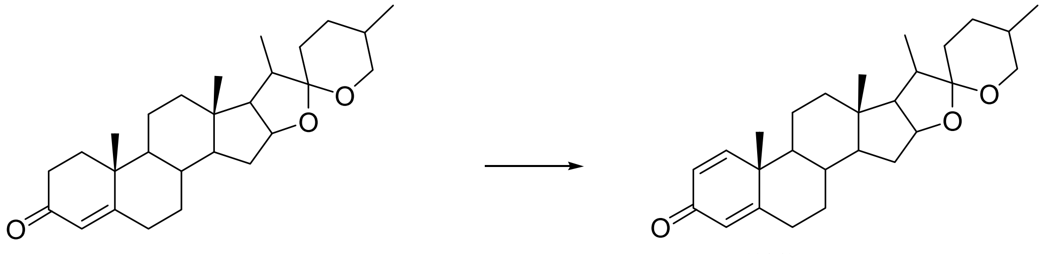
FTIR (KBr), ν_{max}, cm $^{-1:}$ 1671(C=O).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 5.65 (s, 1H, H-4), 4.32 (m, 1H, H-16), 3.40 (m, 1H, H-26), 3.29 (m, 1H, H-26), 1.13 (s, 3H, H-19), 0.88 (d, *J*=7.0 Hz, 3H, H-27), 0.75 (s, 3H, H-18), 0.72 (d, *J*=6.36 Hz, 3H, H-21).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 195.4 (C-3), 171.1 (C-5), 123.8 (CH-4), 109.2 (C-22), 80.6 (CH-16), 66.8 (CH2-26), 62.0 (CH), 55.6 (CH), 53.7 (CH), 41.6(CH), 40.3 (CH2), 39.6 (C), 38.6 (C), 35.6 (CH_2), 35.2 (CH), 33.9 (CH_2), 32.8 (CH_2), 32.7 (CH_2), 31.7 (CH_2), 31.3 (CH_2), 30.2 (CH), 28.8 (CH_2), 20.8 (CH_2), 17.4 (CH_3), 17.1 (CH_3), 16.3 (CH_3), 14.4 (CH_3)

MS (EI), *m/z* (relative intensity): 412(3.5), 298(46), 139(100).

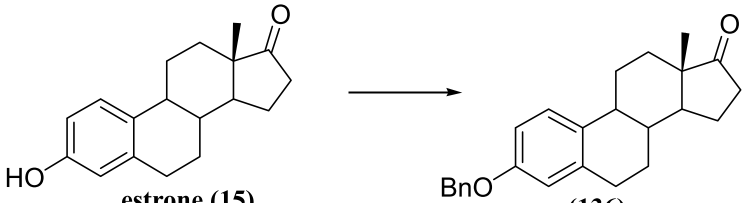
Spirosta- $\Delta^{1,4}$-diene-3-one (130)



(135) **(130)**

A mixture of meta-iodoxybenzoic acid (16.7 g, 59.6 mmol) and diphenyl diselenide (1.24 mg, 3.978 mmol) in toluene (100 ml) was refluxed until the yellow color of diphenyl diselenide disappeared. A solution of **135** (8.1 g, 19.85 mmol) in toluene (30 ml) was introduced to this mixture. The reaction was further heated under reflux for 7 h, cooled to room temperature and partitioned with methylene chloride and water. The organic layer was separated and dried over anhydrous sodium sulphate and concentrated under *vacuo*. The residue was purified by flash column chromatography (1 : 9 ethyl acetate : hexane) to provide **130** as a white solid (5.64 g, 70%), mp 182-184°C [lit 194-195 °C] (Sondheimer, 1954).

3-Benzyloxy-17-one-$\Delta^{1,3,5(10)}$-estratriene (136)



estrone (15) **(136)**

To a stirred solution of estrone (15) (1g, 3.7 mmol) in acetone (100 ml) was added potassium carbonate (5.1g, 37.0 mmol) and benzyl bromide (1.32 ml, 11.11 mmol) at room temperature. After refluxing for 6 h, the precipitate was filtered through a plug of siliga gel and the solid cake was washed with methylene chloride. The filtrate was concentrated, and the solid residue was stirred in methylene chloride and washed with water. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduce pressure. The residue was purified by flash column chromatography using ethylacetate : hexane (1: 9) to give benzyl estrone (136) (1.33 g, 100%) as a white solid, mp 128-130°C [lit 130-131°C] (Whitehead, 1964).

To a stirred solution of estrone (13) (2 mg, 7.40 mmol) in methylene chloride (10 ml) was added dropwise a solution of sodium hydroxide (711 mg, 17.8 mmol) in water (11 ml) and *n*-butylammonium bromide (2.65 g, 8.89 mmol) at room temperature. Benzyl bromide (1.32 ml, 11.1 mmol) was then added and the mixture and then refluxed for 5 h. The reaction mixture was cooled to room temperature and acidified with 10% aqueous hydrochloric acid. The combined organic layer was separated and the aqueous phase was extracted with methylene chloride. The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate : hexane (1: 9) to give benzyl estrone (136) as a white solid in quantitative yield (2.67 g, 100%), mp 128-130°C [lit 130-131 °C] (Whitehead, 1964).

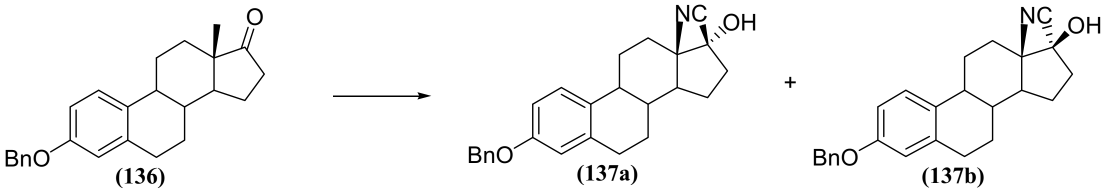
FTIR (KBr), ν_{max}, cm $^{-1:}$ 1737(C=O).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.36-7.24 (m, 5H, CH-Ar), 7.13 (d, *J*= 8.68 Hz, 1H, CH-1), 6.71 (dd, *J*= 8.6, 2.8 Hz, 1H. CH-2), 6.66 (d, *J*= 2.76 Hz, 1H, CH-4), 2.82 (m, 2H, CH_2-6), 2.43 (m, 1H, H-15), 2.08 (m, 1H, H-15), 0.83 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 220.9 (C=O), 156.8 (C-3), 137.8 (C-10), 132.3 (C), 128.5 (CH-5), 127.8 (CH-Ar), 127.4 (CH-Ar), 126.3 (CH-1), 114.9 (CH-4) , 112.4 (CH-2), 70.0 ($\underline{C}H_2$-OBn), 50.4 (CH-14), 48.0 (C-13), 44.0 (CH-9), 38.3 (CH-8), 35.8 (CH_2-16), 31.6 (CH_2), 29.6 (CH_2-6), 26.5 (CH_2), 25.9 (CH_2), 21.6 (CH_2), 13.8 (CH_3-18)

MS (EI), *m/z* (relative intensity): 361 (100).

17*α*-Hydroxy-3-benzyloxyestra-1,3,5(10)-triene-17*β*-carbonitrile (137)



Benzyl estone (136) (306 mg, 1 mmol) in methylene chloride (1.3 ml) and ethanol (1.3 ml) was treated with sodium cyanide (650 mg, 10 mmol) and acetic acid (0.57 ml, 10 mol) at room temperature. After stirring for 4 days, brine was added brine and the mixture extracted with methylene chloride. The aqueous phase was extracted with methylene chloride. The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduce pressure. The residue was purified by flash column chromatography using ethyl acetate : hHexane (15: 85) to give 17*α*-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17*β*-carbonitrile (137a) (272 mg, 0.7283 mmol**)** as a white solid in 73% yield, mp 146-148 °C [lit 186-186.5 °C] as a mixture of isomer] (Whitehead, 1964), and trace amount of 17*β*-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17*α*-carbonitrile (137b) and recovered starting material (136) (98 mg, 27%).

Compound 137b

FTIR (KBr), ν_{max}, cm $^{-1}$: 3409(OH), 2236(CN).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.36-7.24 (m, 5H, ArH), 7.12 (d, *J*=8.56 Hz, 1H, H-1), 6.71 (dd, *J*=8.60, 2.76 Hz, H-2,), 6.65 (d, *J*=2.64 Hz, 1H, H-4), 4.96 (s, 2H, CH_2Ar), 0.91 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 156.8 (C-3), 137.7 (C-10), 137.2 (C-Ar), 132.1 (C-5), 128.5 (CH-Ar), 127.8 (CH-Ar), 127.3 (CH-Ar), 126.2 (CH-1), 120.8 (C-16) , 114.9 (CH-4), 112.3 (CH-2), 78.1 (CN), 69.9 ($\underline{C}H_2$-OBn), 49.5 (C-13), 47.25 (CH), 43.2 (CH), 39.1 (CH), 38.2 (CH_2), 29.7 (CH_2), 27.8 (CH_2-6), 25.8 (CH_2), 23.6 (CH_2), 16.3 (CH_3-18).

MS (EI), *m/z* (relative intensity): 360(24), 281(4), 207(10), 91(100).

Compound 137a

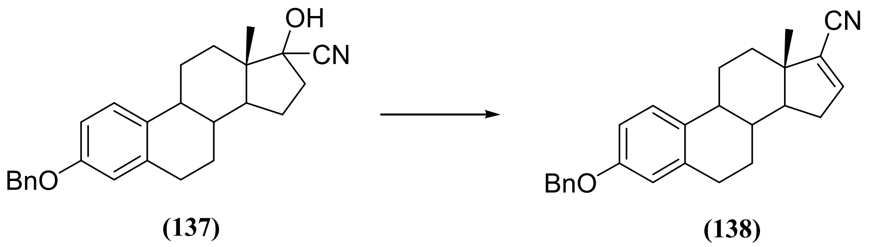
FTIR (KBr), ν_{max}, cm $^{-1}$: 3372(OH), 2236(CN).

^{1}**H NMR** ($CDCl_3$, 400MHz) δ: 7.38-7.25 (m, 5H, ArH), 7.13 (d, *J*=8.56 Hz, 1H, H-1), 6.73 (dd, *J*=8.60 Hz, 2.76 Hz, 1H, H-2), 6.65 (d, *J*=2.64 Hz, 1H, H-4), 4.97 (s, 2H, CH_2Ar), 2.8 (m, 2H, H-6), 0.84 (s, 3H, H-18).

^{13}**C NMR** ($CDCl_3$, 100MHz) δ: 156.8 (C-3), 137.7 (C-10), 137.2 (C-Ar), 132.1 (C-5), 128.5 (CH-Ar), 127.8 (CH-Ar), 127.4 (CH-Ar), 126.3 (CH-1), 122.1 (C-16) , 114.9 (CH-4), 112.4 (CH-2), 80.9 (CN), 69.9 ($\underline{C}H_2$-OBn), 49.7 (CH-14), 47.5 (C-13), 43.2 (CH-9), 39.1 (CH-8), 37.2 (CH_2), 33.3 (CH_2), 29.6 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 22.9 (CH_2), 12.1 (CH_3).

MS (EI), *m/z* (relative intensity): 360(23), 207(9), 91(100).

3-Benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138)



A solution of 17-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17-carbonitrile (137) (210 mg, 0.563 mmol) in pyridine (2 ml) was treated with phosohorus oxychloride (0.22 ml, 2.264 mmol) and heated to reflux for 9 h. The reaction mixture was cooled to room temperature, 10% aqueous hydrochloric acid-ice water was added and the mixture extracted with methylene chloride. The combined organic layers were washed with brine and water, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate : hexane (1: 19) to give 3-benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138) as a white solid (139mg, 70%), mp 160-162 °C [lit 168-168.5°C] (Whitehead, 1964).

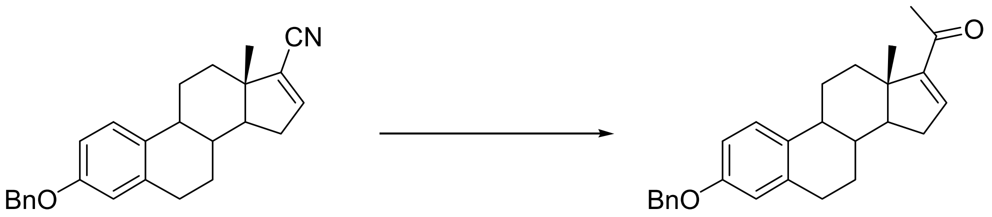
FTIR (KBr), ν_{max}, cm $^{-1}$: 2207(CN).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.36-7.20 (m, 5H, ArH), 7.11 (d, *J*=8.56 Hz, 1H, H-1), 6.7 (dd, *J*=8.52, 2.72 Hz, 1H, H-2), 6.60 (d, *J*=2.6 Hz, 1H, H-4), 6.50 (dd, J=3.82, 1.88 Hz, 1H, H-16), 4.96 (s, 2H, CH_2Ar), 2.8 (m, 2H, H-6), 0.87(s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 155.9 (C-3), 146.2 (C-16), 136.6 (C-10), 136.2 (C-Ar), 131.4 (C-5), 127.6 (CH-Ar), 126.9 (CH-Ar), 126.6 (C-17), 126.4 (CH-Ar), 125.0 (CH-1) , 114.8 (CN), 114.0 (CH-4), 111.4 (CH-2), 68.9 ($\underline{CH_2}$-OBn), 54.2 (CH), 47.5 (C-13), 43.2 (CH), 36.2 (CH), 33.2 (CH), 33.1 (CH_2), 31.7 (CH_2), 28.5 (CH_2), 26.7 (CH_2), 25.2 (CH_2), 15.4 (CH_3).

MS (EI), *m/z* (relative intensity): 369(16), 281(46), 207(100), 91(100).

17-Acetyl 3-benzyloxyestra -1,3,5(10), 16-tetraene (139)



(138) **(139)**

Methylmagnesium bromide (3M in diethyl ether, 0.1 ml, 0.271 mmol) was added at room temperature under nitrogen atmosphere to a stirred solution of 3-benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138) (50 mg, 0.1355 mmol) in dry benzene (7 ml). The mixture was heated at reflux for 9 h and then poured into 10% aq. HCl-ice water. The mixture was extracted with ethyl acetate. The combined organic layers were washed with brine and water, dried over anhydrous sodium sulphate, filtered and concentrated under reduces pressure. The residue was purified by flash column chromatography using ethyl acetate : hexane (1: 9) to give 17-acetyl-3-

benzyloxyestra-1,3,5(10), 16-tetraene (139) as a white amorphous-solid (36.6 mg, 70%), mp 114-116 °C [mp was not report] (Whitehead, 1964) .

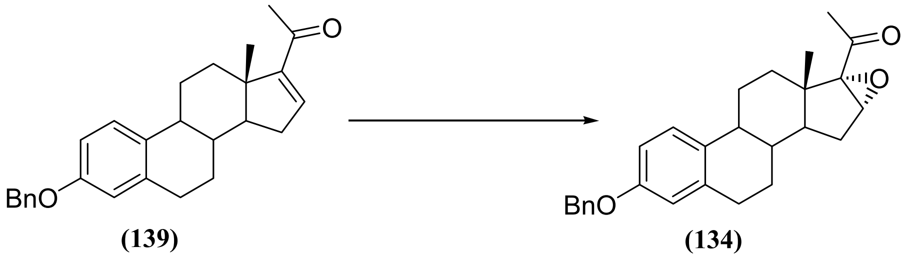
FTIR (KBr), ν_{max}, cm $^{-1}$: 1662 (C=O).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.36-7.21 (m, 5H, ArH), 7.13 (d, *J*=8.42 Hz, 1H, H-1), 6.71 (dd, J=8.55, 2.78 Hz, H-16, 1H), 6.65 (m, 2H, H-4, H-16), 4.96 (s, 2H, CH_2-Ar), 2.80 (m, 2H, H-6), 2.20 (s, 3H, H-21), 0.84 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 172.2 (C=O), 156.9 (C-3), 144.5 (CH-16), 138.0 (C-10), 137.5 (C-Ar), 133.3 (C-5), 128.5 (CH-Ar), 127.6 (CH-Ar), 127.6 (CH-Ar), 126.3 (CH-1), 115.1 (CH-4), 112.4 (CH-2), 70.2 (CH_2OBn), 55.8 (CH), 46.7 (C-13), 44.5 (CH), 37.2 (CH), 35.0 (CH_2), 32.2 (CH_2), 29.9 (CH_2), 27.9 (CH_2), 27.3 (CH_3), 26.6 (CH_2), 16.1 (CH_3).

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

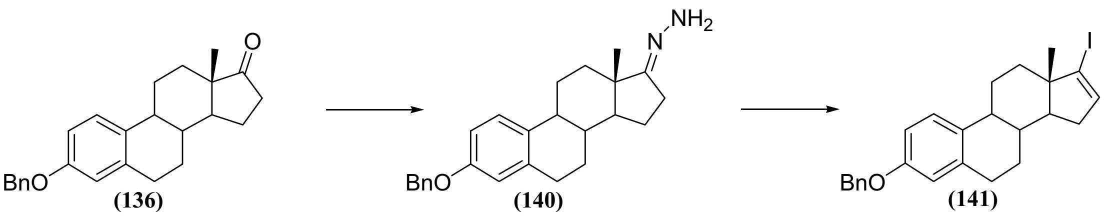
16α, 17α-Epoxy-3-benzyloxyestra -1,3,5(10)-triene (134)



To a stirred solution of 17-acetyl-3-benzyloxyestra-1,3,5(10), 16-tetraene (139) (100 mg, 0.2715 mmol) in methanol (4.1 ml) was added 2.5 N sodium hydroxide (0.54 ml) at room temperature. The reaction mixture was cooled to 0°C and then treated with 30 % H_2O_2 (10.5 ml). The mixture was stirred for 16 h. The solvent was removed, and the residue extracted with methylene chloride. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduce pressure. The crude product was purified by flash column chromatography using ethyl acetate : hexane (1 : 19 to give 3-benzyloxyestra-16α, 17α-epoxy-1,3,5(10)-triene (134) (95 mg, 90 %) as a white solid, mp 100-101 °C.

17-Iodo-3-benzyloxyestra-1,3,5(10),16- tetraene (141)

3-Benzyloxyestra-1,3,5(10)- triene hydrazone (140)



To a stirred solution of benzyl estrone (136) (100 mg, 0.2778 mmol) in ethanol (1.2 ml) were added hydrazine (0.1 ml, 2.1478 mol) and triethylamine (0.04 ml, 2.398 mmol) at room temperature and the mixture was refluxed for 3 h. After cooling to room temperature, the solvent was evaporated and water was added. The aqueous phase was extracted with methylenechloride and the combined organic layers were dried over anhydrous sodium sulphate, filtered and evaporated to give, 3-benzyloxyestra-1,3,5(10)- triene hydrazone (140) as a white solid which was used in the next step without further purification.

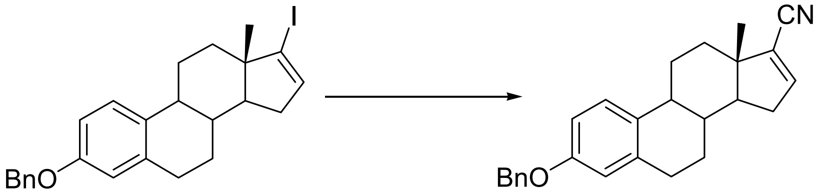
To a solution of **140** (57 mg, 0.153 mmol) in dry THF (1.5 ml) was added triethylamine (0.5 ml, 3.514 mmol) at room temperature under a nitrogen atmosphere. After stirring for 10 min solution of iodine (72.3 mg, 0.2846 mmol) in dry THF (1.2 ml) was added dropwise over 5 min. When the addition was completed, the mixture was washed successively with 10% aqueous hydrochloric acid, aqueous sodium thiosulfate, aqueous sodium hydrogencarbonate and water. The combined organic layers were dried over anhydrous sodium sulphate, filtered and evaporated. The crude product was purified by flash column chromatograph eluting with methylene chloride : hexane (5 : 95) to give 17-iodo-3-benzyloxyestra-1,3,5(10),16- tetraene (141) (26 mg, 67% over 2 steps) as a white solid, mp 126-127 ^{0}C.

FTIR (KBr) ν_{max}, cm $^{-1}$: 3028, 1244.

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.395-7.24 (m, 5H, Ar(H), 7.12 (d, *J*=8.56 Hz, 1H, H-1), 6.70 (dd, J=8.56, 2.67 Hz, 1H, H-2), 6.50 (d, *J*=2.72 Hz, 1H, H-4), 6.09(dd, *J*=3.24, 1.68 Hz, 1H, H-16), 4.95 (s, 2H, CH_2Ar), 2.80 (m, 2H, H-6), 0.93 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 156.8 (C-3), 137.8 (C-10), 137.4 (CH-16), 137.3 (C-Ar), 132.8 (C-5), 128.5 (CH-Ar), 127.8 (CH-Ar), 127.4 (CH-Ar), 126.1 (CH-1), 114.9 (CH-4), 112.9 (C-17), 112.3 (CH-2), 69.9 (CH_2OBn), 54.1 (CH), 50.3 (C-13), 44.2 (CH), 37.2 (CH), 36.3 (CH_2), 33.4 (CH_2), 29.6 (CH_2-6), 27.4 (CH_2), 26.4 (CH_2), 15.3 (CH_3).

General procedure for cyanation reaction



(140) **(138)**

Method A: entry 1,6,10,11

Under N_2 atmosphere sodium carbonate, CN source and Pd catalyst were placed in a round bottom flask. A needle was inserted into the round bottom flask and the solids were flushed with nitrogen for 10 minutes. Then a solution of **141** in solvent was added and the reaction was heated to require temperature After all of starting material was consumed (monitoring by TLC), the reaction was cooled to room temperature, diluted with ethyl acetate and filtered through a celite pad. The filtrate was evaporated. The residue was purified by flash column chromatograph using ethyl acetate : hexane (2.5 : 97.5) to give 3-cenzyloxyestra-1,3,5(10), 16-tetraene-17-barbonitrile (138) as a white solid.

Method B: entry 17,18

The substrate **141**, potassium cyanide, tetrakis(triphenylphosphine) palladium(0) and copper(I)halide were placed in a round bottom flask which was flushed with nitrogen gas. Solvent was added via syringe. The resulting mixture was heated to reflux under N_2 with vigorous agitation by a magnetic stirrer. After the starting material was consumed (monitoring by TLC), the reaction was cooled to room temperature, diluted with ethyl acetate and filtered through a celite pad. The filtrate was evaporated. The crude workup was purified by flash column chromatograph using ethyl acetate : hexane (2.5 : 97.5) to give 3-benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138) as a white solid.

Method C: entry 2,8,9

The substrate **141**, cyanide source, palladium(II)acetate and triphenylphosphine were placed in a round bottom flask which was flushed with nitrogen gas. Solvent was added via syringe. The resulting mixture was heated to reflux under N_2 with vigorous agitation by a magnetic stirrer. After all the starting material was consumed (monitoring by TLC), the reaction was cooled to room temperature, diluted with ethyl acetate and filtered through a celite pad. The filtrate was evaporated. The residue was purified by flash column chromatograph using ethyl acetate : hexane (2.5 : 97.5) to give 3-benzyloxyestra-1,3,5(10), 16-tetraene-17-barbonitrile (138) as a white solid.

Method D: entry 3,4,5,7,12,13,14,15,16

The substrate **141**, cyanide source and Pd catalyst were placed in a round bottom flask which was flushed with nitrogen gas. Solvent was added via syringe. The resulting mixture was heated to reflux under N_2 with vigorous agitation by a magnetic stirrer. After all the starting material was consumed (monitoring by TLC), the reaction was cooled to room temperature, diluted with ethyl acetate and filtered through celite pad. The filtrate was evaporated. The residue was purified by flash column chromatograph using ethyl acetate : hexane (2.5 : 97.5) to give 3-benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138) as a white solid

Preparation of impregnated of NaCN on Alumina

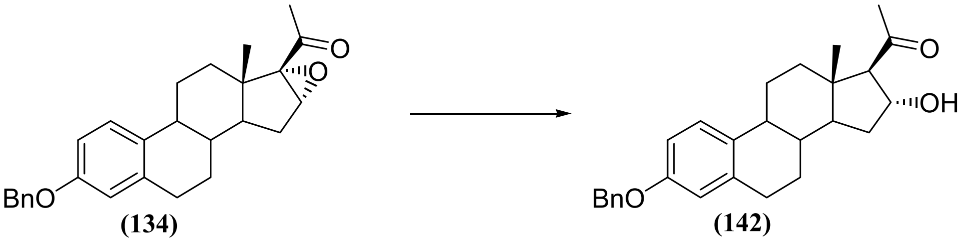
A 50 ml round bottom flask was charged with a solution of sodium cyanide (2.0 g, 40.8 mmol) in distilled water (5 ml), and neutral alumina (4.0 g) was added in one portion. The water was evaporated under reduced pressure, keeping the bath temperature below 65 ^{0}C. The impregnated alumina was then dried [4h, 110 °C (0.05 mm)].

Dehydration of potassium hexaferrocyanate.trihydrate ($K_4[Fe(CN)_6].3H_2O$)

$$K_4[Fe(CN)_6].3H_2O \longrightarrow K_4\{Fe(CN)_6]$$

($K_4[Fe(CN)_6].3H_2O$) was ground to a fine powder and dried in vacuum (ca. 2 mbar) at 80 °C for 16 h.

3-Benzyloxy- 16α-hydroxy -17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (142):



To a solution of **134** (26 mg, 0.0647 mmol) in ethanol (1 ml) and methylene chloride (0.2ml) was added hydrazine hydrate (0.24 ml, 4.975 mmol) at room temperature. After stirring for 5h, the solvent was evaporated and the residue stirred vigorously for 30 min with a mixture of ethyl acetate (3 ml) and 10% aqueous hydrochloric acid (3 ml). The organic layer was separated, and the aqueous layer was washed with ethyl acetate the combined organic layer, washed with saturated brine and water and dried over anhydrous sodium sulphate. Concentration of the dried organic extract under reduce pressure followed by purification by flash column chromatography using ethyl acetate : hexane (3 : 7) afforded the product, **142** (24 mg, 92%) as a white solid, mp 224-226 °C.

FTIR (KBr), ν_{max}, cm^{-1}: 3436(OH), 1699(C=O).

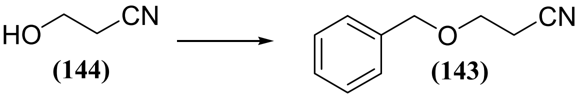
^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.46-7.30 (m, 5H, Ar(H)), 7.19 (d, *J*=8.40 Hz, 1H, H-1), 6.65 (dd, *J*=8.8, 2.8 Hz, 1H, H-2), 6.55 (d, *J*=2.80 Hz, 1H, H-4), 5.05 (s, 2H, CH_2Ar), 4.90 (m, 1H, H-16), 2.87 (m, 2H, H-6), 2.63 (d, *J*= 6.8 Hz, H-17), 2.15 (s, 3H, H-21), 0.68 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 100MHz) δ:177.0 (C=O), 156.8 (C-3), 137.9 (C-10), 137.3 (C-Ar), 133.1 (C-5), 128.7 (CH-Ar), 128.6 (CH-Ar), 127.4 (CH-Ar), 126.1 (CH-1), 114.9 (CH-4), 112.2 (CH-2), 72.1 (CH-16), 70.0 (CH_2OBn), 55.6 (CH), 46.5 (C-13), 44.2 (CH), 38.9 (CH), 37.0 (CH-17), 34.9 (CH_2), 32.0 (CH_2), 29.7 (CH_2-6), 27.7 (CH_2), 26.4 (CH_3), 26.2 (CH_2), 15.9 (CH_3).

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

HRMS: 405.2444 $C_{27}H_{33}O_3$ requires 405.2430.

3–Benzyloxy propionitrile (143)



To a stirred suspension of sodium hydride (1.78 g, 44.32 mmol) in dry THF (30 ml), a solution of 3-hydroxy propionitrile (144) (2 ml, 29.55 mmol) in dry THF (20 ml) was added dropwised at 0 °C. The reaction mixture was stirred for 30 min, and then benzylbromide (10.52 ml, 88.64 mmol) was added at room temperature. The reaction mixture was stirred at room temperature for 16 h, cooled to room temperature, quenched with water and extracted with diethyl ether. The combined organic layer were washed with water, dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo*. The residue was purified by flash column chromatography (1 : 9 ethyl acetate : hexane) to yield 3-benzyloxy propionitrile (143) (4.214 g, 88.6%) as a colorless oil (Thatavarathy-Rama, 2003).

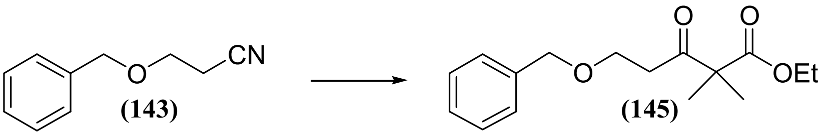
FTIR (neat), ν_{max}, cm^{-1}: 3031, 2251, 1496, 1104.

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.34 (s, 5H), 4.57 (s, 2H), 3.65 (t, *J*=6.22 Hz, 2H), 2.60 (t, *J*=6.22 Hz, 2H).

^{13}C NMR ($CDCl_3$, 100MHz) δ:140.9 (CN), 137.1 (C-Ar), 128.5 (CH-Ar), 128.0 (CH-Ar), 127.7 (CH-Ar), 126.9 (CH-Ar), 126.9 (CH-Ar), 73.2 (CH_2OBn), 64.5 (CH_2O), 18.8 (CH_2CN).

MS (APCI), *m/z* (relative intensity): 161 (32), 91(100).

5-Benzyloxy-2,2-dimethyl-3-oxo-pentanoic acid ethyl ester (145)



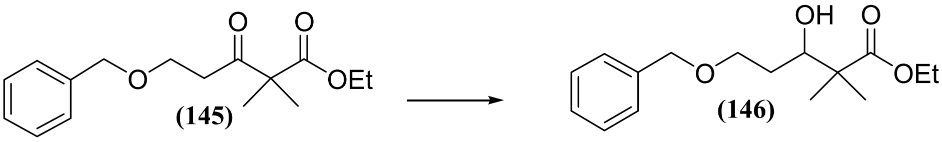
To a mixture containing of 3-benzyloxy propionitrile (143) (4.214 g, 26.174 mmol) and Zn powder (26 g, 39.26 mmol) in THF (86 ml) was added dropwise ethyl-α-bromoisobutylrate (10) (31 ml, 209 mmol) and the mixture was sonicated at 30 °C for 2 hours under N_2. The resultant mixture was left at 30 °C for another 2 hours and then the THF layer was decanted. The unreacted Zn was washed with THF and the filtrates combined. The combined THF solution was concentrated under reduce pressure and stirred vigorously for 30 min with a mixture of ethyl acetate (208 ml) and 1.0 M aqueous hydrochloric acid (312ml). The organic layer was separated and the aqueous layer was washed with ethyl acetate. The combined organic layers were washed with saturated sodium carbonate, brine and dried over anhydrous sodium sulphate. Concentration of the dried organic extracted under reduced pressure followed by purification by flash column chromatography (5 : 95 ethyl acetate : hexane) afforded the product, 5-benzyloxy-2,2-dimethyl-3-oxo-pentanoic acid ethyl ester (145), (4.88 g, 17.54 mmol) as a pale yellow oil in 67 % yield.

FTIR (neat), ν_{max}, cm $^{-1}$: 3070, 2982, 1755 (C=O), 1715 (C=O).

^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.3 (m, 5H), 4.50 (s, 2H), 4.13 (q, *J*=6.80 Hz, 2H), 3.74 (t, *J*=6.8 Hz, 2H), 2.77 (t, *J*=6.4 Hz, 2H), 1.37 (s, 6H), 1.22 (t, *J*=6.8 Hz, 3H).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 206.2 (C=O), 173.4 (C=O), 138.1 (C-Ar), 128.3 (CHAr), 127.6 (CHAr), 73.2 ($\underline{CH_2}$OBn), 65.3 (OCH_2), 61.3 (CH_2O), 55.7 (C), 38.4 (CH_2), 21.7 (CH_3X2), 13.9 (Me).

5-Benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid ethyl ester (146)



To a stirred solution of 5-benzyloxy-3-oxo-2,2-dimethyl-pentanoic acid ethyl ester (145) (840 mg, 3.022 mmol) in methanol (45 ml) was added sodium borohydride (137 mg, 3.626 mmol) and the mixture was stirred at room temperature for 30 min. The solvent was removed and diluted with methylene chloride. The mixture was extracted with brine and washed with water. The combined organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduce pressure to afford 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid ethyl ester (146) as a colorless oil (846 mg, 100%) (Parmee, 1991).

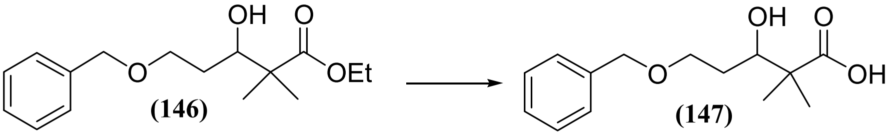
FTIR (neat), ν_{max}, cm^{-1}: 3493 (OH), 1724 (CO)

^{1}H NMR (400 MHz) δ : 7.35 (m, 5H, Ar(H)), 4.54 (s, 1H, O$\underline{CH_A}$$CH_B$Ar), 4.53 (s, 1H, $OCH_A$$\underline{CH_B}$Ar), 4.15 (q, *J*=7.2 Hz, 2H, O$\underline{CH_2}$$CH_3$), 3.92 (dd, *J*=10, 2 Hz, 1H, $\underline{CH}$OH), 3.72 (m, 2H, $\underline{CH_2}$O), 3.18 (bs, 1H, OH), 1.72 (m, 2H, CH_2), 1.25 (t, *J*=7.2 Hz, 3H, $OCH_2$$\underline{CH_3}$), 1.20 (s, 3H, CH_3), 1.18 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100MHz) δ: 177.3 (C=O), 137.9 (C-Ar), 128.41 (CH-Ar), 127.3 (CH-Ar), 126.8 (CH-Ar), 76.7 ($\underline{CH}$O), 73.2 ($\underline{CH_2}$OBn), 69.0 (CH_2O), 60.5 (O$\underline{CH_2}$$CH_3$), 46.8 (C), 31.4 ($CH_2$), 21.5 (Me), 20.4 (Me), 14.0 ($OCH_2$$\underline{CH_3}$).

MS (APCI), *m/z* (relative intensity): 281 (11), 173(77), 91 (100).

5-Benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid (147)



A stirred solution of 5-benzyloxy-3-hydroxy-2, 2-dimethyl-pentanoic acid ethyl ester (146) (500 mg, 1.79 mmol) in THF (41 ml) and water (21 ml) was cooled to 0 °C and 1N lithium hydroxide (18 ml) was added dropwise. The reaction temperature was allowed to rise to ambient temperature slowly and stirring continued at ambient temperature for 16 h. The solvent was removed under reduced pressure and the residue acidified (pH =2) with 2N aqueous hydrochloric acid. The mixture was diluted with ethyl acetate and washed with brine and with water, dried over anhydrous sodium sulphate, filtered and concentrated under reduce pressure. The residue was purified by flash column chromatography (1 : 1 ethyl acetate : hexane) to afford 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid (147) as a colorless oil (344 mg, 76%).

FTIR (neat), ν_{max}, cm^{-1}: 3438(OH), 1704(CO).

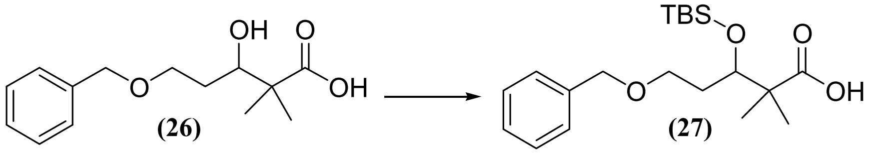
^{1}**H NMR** (400 MHz) δ : 7.35 (m, 5H, Ar(H)), 4.55 (s, 2H, $O\underline{CH_2}Ar$), 3.88 (m, 1H, $\underline{CH}OH$), 3.81(m, 1H, $\underline{CH_A}CH_BO$), 3.72 (m, 2H, CH_2O), 3.71 (m, 1H, $CH_A\underline{CH_B}O$), 1.79 (m, 2H, CH_2), 1.20 (t, *J*=7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 1.18 (s, 3H, CH_3), 1.18 (s, 3H CH_3).

^{13}**C NMR** ($CDCl_3$, 100MHz) δ: 181 (C=O), 137.5 (C-Ar), 128.5 (CH-Ar), 127.8 (CH-Ar), 127.1 (CH-Ar), 76.2 (CHOH), 73.4 (CH_2), 69.4 (CH_2), 46.5 (C), 31.0 (CH2), 22.0 (Me), 20.2 (Me).

MS (APCI), *m/z* (relative intensity): 275.1 (M+Na), 253.1 ($M+H^+$)

HRMS: 253.1450 $C_{14}H_{21}O_4$ requires 253.1440.

5-Benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2,2-dimethyl-pentanoic acid (148)



To a stirred solution of 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid (147) (180 mg, 0.72 mmol) in methylene chloride (15 ml) was added 2,6-lutidine (0.32 ml, 2.8 mmol) at room temperature. The resulting mixture was cooled to 0 °C, *tert*-butyl-dimethylsilyl methanesulfonate (0.8 ml, 1.8 mmol) added and stirred a further 2 h. Water was added and the reaction mixture was allowed to warmed up to room temperature and extracted with methylene chloride. The combined organic layer was dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure to afford bis-silylated material.

The material was dissolved in a mixture of methanol (13 ml) and THF (6.5 ml). Potassium carbonate (643 mg) dissolved in water (9 ml) was added and the mixture was stirred for 16 h at room temperature, diluted with brine and acidified to pH 2 (10% aqueous hydrochloric acid). The solution was extracted with methylene chloride and the combined extracts were dried over anhydrous sodium sulphate, filtered and concentrated reduce pressure. The crude mixture was purified by flash column chromatography (1 : 5 ethyl acetate : hexane) to afford 5-benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2,2-dimethyl-pentanoic acid (148) (154 mg, 58.6%) as a colorless oil.

FTIR (neat), ν_{max}, cm^{-1}: 2200-3500(OH), 1702(CO).

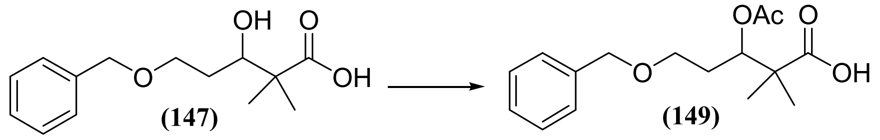
^{1}**H NMR** (400 MHz) δ : 7.29-7.18 (m, 5H, ArH), 4.46 (s, 2H, CH_2), 3.90 (dd, *J*=7.08, 3.32 Hz, 1H, CH), 3.45 (dd, *J*=6.16, 7.44 Hz, 2H, CH_2O), 1.10 (s, 3H, Me), 1.09 (s, 3H, Me), 0.8 (s, 9H, Me_3CSi), 0.00 (s, 6H, MeSi x 2).

^{13}**C NMR** (100 MHz) δ: 180.7 (CO), 137.4 (C(Ar)), 128.4, 128.4, 127.5, 127.5, 127.5 (CH(Ar)x5), 74.9 (CHO), 72.7 ($\underline{C}H_2Bn$), 66.9 ($\underline{C}H_2O$), 47.5 ($\underline{C}Me_2$), 33.6 (CH_2), 29.6 (CSi), 25.9 ($\underline{Me_3}CSi$ x 3), 22.1 (Me), 21.1 (Me), -4.38 (MeSi), -4.39 (MeSi).

MS (APCI), *m/z* (relative intensity): 186 (100), 91 (95).

HRMS: 295.154, $C_{16}H_{23}O_5$ requires 295.1545.

5-Benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149)



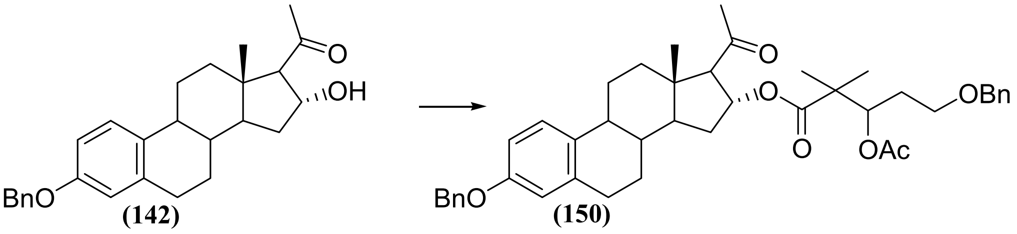
To a stirred solution of 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid (147) (3.16 g, 12.54 mmol) in pyridine (1 ml) was added acetic anhydride (1.8 ml) at room temperature. The resulting mixture was stirred at room temperature for 24 h. Water was added and stirring continued for 2 h. The reaction was diluted with methylene chloride and extracted with water. The combined extracts were dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure. The crude mixture was purified by flash column chromatography (1 : 1 ethyl acetate : hexane) to afford 5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149) (2.58 g, 70%) as a colorless oil.

FTIR (neat), ν_{max}, cm^{-1}: 3173(OH), 1741(C=O), 1705(C=O).

^{1}H NMR (400 MHz) δ: 7.25(m, 5H, CH-Ar), 5.26 (dd ,*J*=9.64, 2.92Hz, CHO), 4.39 (s, 2H, CH_2OBn), 3.40 (m, 2H, CH_2O), 1.93 (s, 6H, Me x 2), 1.78 (m, 2H, CH_2).

^{13}C NMR (100 MHz) δ: 181.3 (C=O), 170.6 (C=O), 138.1 (C-Ar), 128.3 (CH-Ar), 127.8 (CH-Ar), 127.6 (CH-Ar), 74.50 (CHO), 73.2 (CH_2), 67.5 (CH_2), 46.3 (C), 36.8 (CH_2), 21.7 (Me), 20.9 (Me), 20.4 (Me).

3-Benzyloxy-16*α*-(5-benzyloxy-3-acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (150)



(142)

(150)

To a stirred solution of 5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149) (683 mg, 2.32 mmol) in benzene (12 ml) was added oxalylchloride (0.33 ml, 3.48 mmol) and DMF (catalytic amount) and stirred at room temperature. for 4 h. Benzene was removed by using rotary evaporator and the crude acid chloride further used in the next step without purification.

To a stirred solution of **142** in methylene chloride (10 ml) was added DMAP (284 mg, 2.32 mmol). After being stirred for 3 min, a solution of the crude acid chloride in methylene chloride (10) was added at room temperature. After 30 minutes, the reaction mixture was quenched with water and extracted with methylene chloride. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude mixture was purified by flash column chromatography (1 : 9 ethyl acetate : hexane) to afford 3-benzyloxy-16*α*-(5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (150) as a white solid (526 mg, 100%) mp 106-108 °C.

FTIR (neat), ν_{max}, cm^{-1}: 1738(C=O), 1720 (C=O), 1701 (C=O).

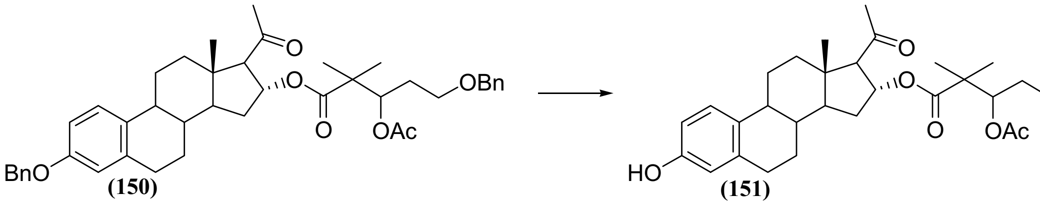
^{1}H NMR (400 MHz) δ : 7.36-7.17 (m, 10H, ArH), 7.10 (d, *J*=8.68 Hz, 1H, H-1), 6.70 (dd, *J*=8.52, 2.72 Hz, 1H, H-2), 6.63(m, 1H, H-4), 5.47 (m, 1H, H-16), 5.32 (m, 1H, $\underline{CH}$OAc), 4.69 (s, 2H, $\underline{CH_2}$O), 4.38 (s, 1H, $\underline{CH_A}H_B$OBn), 4.36 (d, *J*=2.48 Hz, 1H, $CH_A\underline{H_B}$OBn), 3.59 (m, 2H, CH_2O), 2.75 (m, 2H, H-6), 2.70 (d, *J*=6.44 Hz, H-17), 2.65 (d, *J*=6.56 Hz, H-17), 2.12 (s, 3H, H-21), 2.02 (s, 3H, H-21), 1.91 (s, 3H, H18), 1.88 (s, 3H, H-18), 1.08 (s, 6H, Me), 1.06 (s, 3H, Me), 1.05 (s, 3H, Me).

^{13}C NMR (100 MHz) δ: 175.1 (C), 170.4 (C), 156.8 (C), 138.3 (C), 137.8 (C), 137.2 (C), 132.3 (C), 128.5 (CH-Ar), 128.3 (CH-Ar), 127.2 (CH-Ar), 127.8 (CH-Ar), 127.8 (CH-Ar), 127.6 (CH-Ar), 127.4 (CH-Ar), 126.1 (CH), 114.9 (CH), 112.4 (CH), 75.9 (CH), 75.7 (CH), 74.5 (CH), 74.4 (CH), 74.2 (CH), 73.2 (CH_2), 73.2 (CH_2), 69.9 (CH_2), 69.8 (CH), 69.6 (CH), 67.4 (CH_2), 67.3 (CH_2), 55.5, 53.2, 46.2, 44.8, 44.2, 43.0, 38.7, 38.0, 36.9, 34.7, 33.0, 32.8, 31.4, 31.3, 30.8, 30.6, 29.5, 27.5, 27.10, 26.2, 21.4, 21.6, 20.5, 20.1, 15.8, 14.5.

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

HRMS: 703.3607 $C_{43}H_{52}O_7Na$ requires 703.3611.

3-Hydroxy-16*α*-(5-hydroxy-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (151):



To a stirred suspension of 5% Pd/C (97 mg, 0.041 mmol) in ethanol (11 ml) was added a solution of 3-benzyloxy-16*α*-(5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (150) (276 mg, 0.41 mmol) in ethylacetate (4 ml). A hydrogen balloon was attached and stirring continue for 16 hours. The mixture was filtered through celite pad, rinsed with ethyl acetate and the filtrate concentrated. The crude mixture was purified by flash column chromatography (4 : 6 ethyl acetate : hexane) to afford 3-hydroxy-16*α*-(5-hydroxy-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (151) (139.7 mg, 69%) as a white solid, mp 168-170 °C.

FTIR (neat), ν_{max}, cm^{-1}: 3410 (OH), 1736 (C=O), 1725 (C=O), 1708 (C=O).

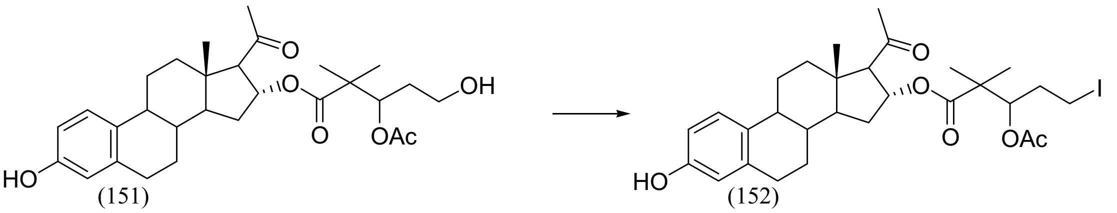
^{1}H NMR (400 MHz) δ : 7.04 (m, 1H, H-1), 6.58 (dd, *J*=8.40, 2.52 Hz, 1H, H-2), 6.49 (d, *J*=2.24 Hz, 1H, H-4), 5.47 (m, 1H, H-16), 5.24 (m, 1H, $\underline{CH}$OAc), 3.56 (m, 1H, $C\underline{H_A}H_BO$), 3.37 (m, 1H, $CH_A\underline{H_B}O$), 2.74 (m, 3H, CH_2-6, H-17), 2.14 (s, 3H, CH_3), 2.12 (s, 3H, H-21), 2.02 (s, 3H, H-21), 1.95 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.13 (s, 3H, H-18), 1.08 (s, 3H, Me), 1.06 (s, 3H, Me), 0.60 (s, 3H, Me), 0.58 (s, 3H, Me).

^{13}C NMR (100 MHz) δ : 206.9 (C), 206.7 (C), 175.1 (C), 172.3 (C), 172.0 (C), 153.9 (C), 137.8 (C), 131.4 (C), 126.1 (CH), 115.3 (CH), 112.8 (CH), 75.9 (CH), 75.6 (CH), 74.3 (CH), 74.1 (CH), 69.8 (CH), 69.5 (CH), 58.2 (CH_2), 58.1 (CH_2), 53.3 (CH), 53.2 (CH), 46.0 (C), 44.8 (CH), 43.3 (C), 37.7 (CH_2), 38.6 (CH_2), 38.0 (CH), 33.0 (CH_2), 32.8 (CH_2), 31.3 (CH_2), 29.3 (CH_2), 27.5 (CH_2), 26.2 (CH_2), 22.1 (CH_3), 20.9 (CH_3), 20.9 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 19.1 (CH_3), 14.4 (CH_3).

MS (APCI), *m/z* (relative intensity): 297 (100).

HRMS: 523.2673 $C_{29}H_{40}O_7Na$ requires 523.2672.

3-Hydroxy-16*α*-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (152)



(151)　(152)

The mixture of 3-hydroxy-16*α*-(5-hydroxy-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (151) (30 mg, 0.06 mmol), triphenylphosphine (24 mg, 0.09 mmol) and imidazole (24 mg, 0.35 mmol) was dissolved in toluene (1 ml) and stirred at room temperature. The solution of iodine (19 mg, 0.07 mmol) in benzene (2 ml) was added to the reaction mixture and then the reaction was reflux for 3 h. The reaction was cooled down to room temperature, diluted with ether and extracted with saturated $Na_2S_2O_3$. The combined organic phase was washed with water, brine and dried over anhydrous sodium sulphate. Concentration of organic extract using rotary evaporator followed by purification by flash column chromatography (15 : 85 ethyl acetate : hexane) afforded 3-hydroxy-16*α*-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (152) (20.6 mg, 56 %) as a pale yellow wax.

FTIR (neat), ν_{max}, cm^{-1}; 3428 (OH), 1739 (C=O), 1724 (C=O), 1708 (C=O).

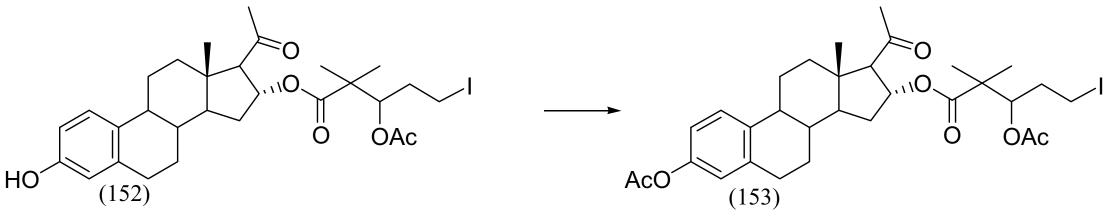
^{1}H NMR (400 MHz) δ : 7.05 (d, *J*= 8.4 Hz, 1H, H-1), 6.57 (dd, *J* =8.4,2.7Hz, 1H, H-2), 6.50 (m, 1H, H-4), 5.49 (m, 1H, H-16), 5.17 (m, 1H, CH-O), 3.05 (m, 1H, $\underline{\mathrm{CH}_A}\mathrm{H}_B$-I), 2.96 (m, 1H, $\mathrm{CH}_A\underline{\mathrm{H}_B}$-I), 2.75 (m, 2H, H-6), 2.70 (d, *J*=6.5 Hz, 1H, H-17), 2.13 (s, 3H, Me), 2.04 (s, 3H, Me), 1.92 (s, 3H, Me), 1.09 (s, 3H, Me), 1.07(s, 3H, Me), 0.61 (s, 3H, Me), 0.60 (s, 3H, Me).

^{13}C NMR (100 MHz) δ : 206.4 (C=O), 196.9 (C=O), 174.7 (C=O), 153.4 (C-3), 137.9 (C-10), 131.8 (C-5), 126.2 (CH-1), 115.3 (CH-4), 112.6 (CH-2), 76.2 (CH), 73.7 (CH), 70.6 (CH), 65.3 (CH_2), 55.5 (CH), 53.3 (C-13), 46.4 (C), 44.9 (CH), 43.4 (CH_2), 38.7 (CH_2), 38.0 (CH), 36.9 (CH_2), 34.7 (CH_2), 31.9 (CH_3), 29.4 (CH_2), 25.7 (CH_2), 21.9 (Me), 20.9 (Me), 20.4 (Me), 14.5 (Me).

MS (APCI), *m/z* (relative intensity): 610 (2), 297 (100).

HRMS: 633.1713 $C_{29}H_{39}O_6NaI$ requires 633.1689.

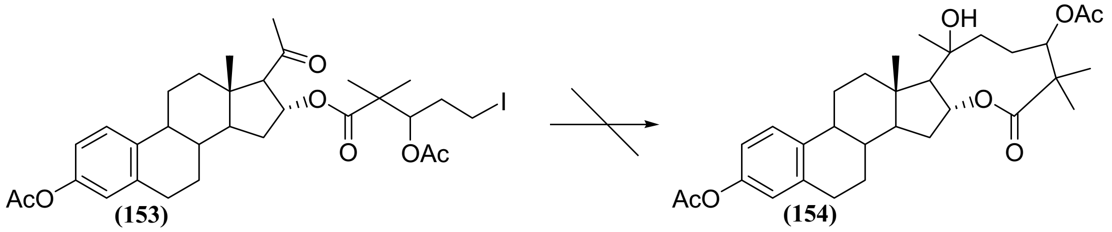
3-Acetoxy-16*α*-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (153)



To a stirred solution of 3-hydroxy-16*α*-(5-iodo-3-acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (152) (50 mg, 0.082 mmol) in py (0.1 ml) was added acetic anhydride (0.1) at 0 °C. The resulting mixture was continued stirring for 3 h. Ice-water was added and the reaction mixture was diluted with methylene chloride and extracted with water. The combined extracts were dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure. The crude mixture was purified by flash column chromatography (2 : 8 ethylacetate : hexane) to afford 3-acetoxy-16β-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (153) (49.4 mg, 92.5%) as a pale yellow wax. (**153** conversed to *α,β*-unsaturated ketone in $CDCl_3$)

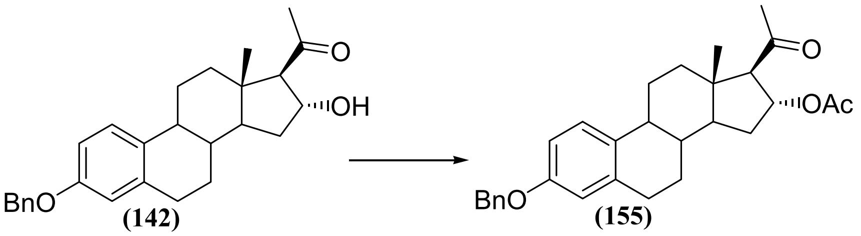
^{1}**H NMR** (400 MHz) δ : 7.19 (d, *J*=8.4Hz, 1H, H-1), 6.77 (dd, J=8.4, 2.4 Hz, 1H, H-2), 6.72(d, J=2.4 Hz, 1H, H-4), 5.49 (m, 1H, H-16), 5.23 (S, 2H, CH_2OBn), 5.17 (m, 1H, CHO), 3.0 5(m, 1H, $C\underline{H_A}H_B$-I), 2.95 (m, 1H, $CH_A\underline{H_B}$-I), 2.80 (m, 2H, H-6), 2.27 (d, *J*=6.48 Hz, H-17), 2.21 (s, 3H, Me), 2.13 (s ,3H ,Me), 2.01(s, 3H, Me), 1.95 (s, 3H ,Me), 1.09 (s, 3H, Me), 1.07 (s, 3H, Me), 0.62(s, 3H, Me), 0.59 (s, 3H, Me).

Compound 154



To a stirred slurry of Sm-powder (64 mg) in tetrahydrofuran (0.6 ml), a solution of 1,2-diiodoethane (90 mg) in tetrahydrofuran (1.2 ml) was slowly added at 0 °C. The resultant olive-green slurry was stirred at ambient temperature for 3 hours after which time the resultant dark blue slurry of samarium(II)iodide formed. The reaction was added the solution of 3-acetoxy-16*α*-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (153) (64 mg, 0.43mmol) in tetrahydrofuran (2 ml) at 0 °C and slowly warm up to room temperature. After 30 minutes then saturated sodium carbonate was added and stirred for 15 minutes. The resultant was extracted with methylene chloride. The combined organic layer was washed with water, dried over saturated sodium sulphate, filtered and concentrated under reduce pressure. Unfortunately none product was observed only starting material was recovered.

3-Benzyloxy-16α-acetoxy -17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (155):



To a stirred solution of 3-benzyloxy-16α-hydroxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (142) (404 mg, 1 mmol) in py (2 ml) was added acetic anhydride (2 ml) at 0 °C. The resulting mixture was continued stirring for 3 h. Ice-water was added and the reaction mixture was diluted with methylene chloride and extracted with water. The combined extracts were dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure. The crude mixture was purified by flash column chromatography (2 : 8 ethyl acetate : hexane) to afford 3-benzyloxy-16α-acetoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (155) (430.4 mg, 96.5%) as a white solid, mp 144-146 °C.

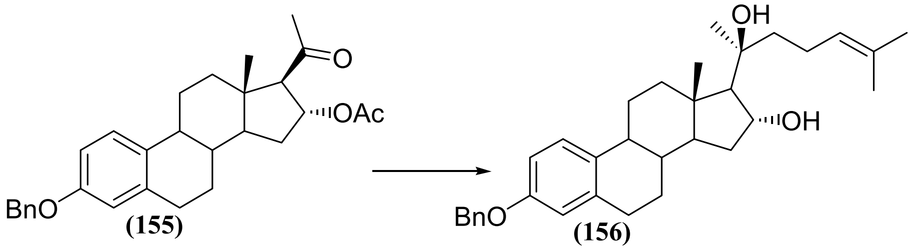
FTIR (neat), ν_{max}, cm^{-1}: 1736 (C=O), 1707 (C=O).

^{1}H NMR (400 MHz) δ : 7.35-7.23 (m, 5H, ArH), 7.10 (d, *J*=8.6 Hz, CH-1), 6.70 (dd, *J*=8.6, 2.4 Hz, 1H, CH-2), 6.64 (d, *J*=2.8 Hz, 1H, CH-4), 5.45 (m, 1H, CH-16), 4.95 (s, 2H, CH_2OBn), 2.78 (m, 2H, CH_2-6), 2.67(d, *J*=6.4Hz, 1H, CH-17), 2.11 (s, 3H, Me-21), 1.94 (s, 3H, Me), 0.61 (s, 3H, Me).

^{13}C NMR (100 MHz) δ : 206.4 (C=O), 170.6 (C=O), 156.8 (C-3), 137.7 (C-10), 137.2 (C-Ar), 132.2 (C-5), 128.5 (CH-Ar), 8 (CH-Ar), 3 (CH-Ar), 126.1 (CH-1), 114.8 (CH-4), 112.3 (CH-2), 75.7 (CH-16), 70.1 (CH-17), 69.9 (CH_2OBn), 53.3 (CH), 44.9 (CH), 43.5 (C-13), 38.8 (CH_2), 38.0 (CH), 33.1 (CH_2), 31.4 (Me), 25.5 (CH_2), 27.5 (CH_2), 26.2 (CH_2), 21.1 (CH_3), 14.5 (CH_3).

HRMS: 469.2363 $C_{29}H_{34}O_4Na$ requires 469.2355.

3-Benzyloxy-16*R*, 20*S*-dihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (156)



The mixture containing of Mg turning (181 mg, 7.53 mmol), I_2 (catalytic amount) and dry THF (30 ml) was added 5-bromo-2-methyl-pentene (0.72 ml, 5.38 mmol) at room temperature under N_2 atmosphere. The mixture was stirred at room temperature for 3 hours, and then the solution of 3-benzyloxy-16α-acetoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (155) (480 g, 1.08 mmol) in dry THF (20 ml) was added drowised at 0 °C. The reaction was stirred at 0 °c for 20 minutes and then quenched with ice-water followed by neutralize with dilute HCl. The mixture was then extracted with dichloromethane, the combined organic layer was washed with water, dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo*. The crude product was purified by flash column chromatography eluting with 3 : 7 ethyl acetate : hexane to provide 3-benzyloxy-16*R*,20*S*-dihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (156) (204 mg, 38.9 %) as a colorless wax.

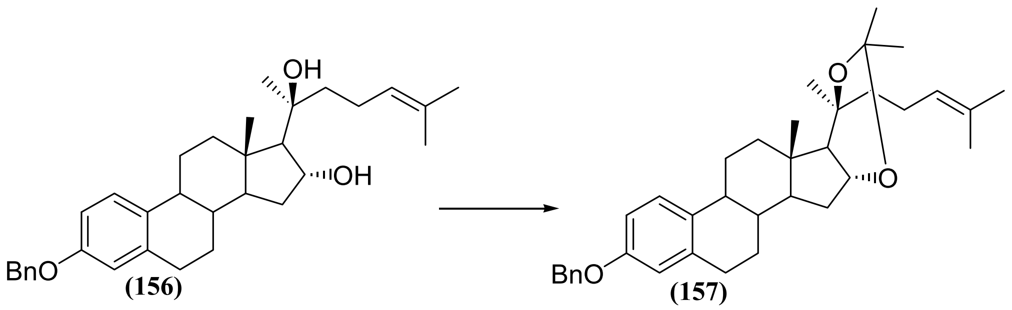
FTIR (neat), ν_{max}, cm^{-1}: 3411 (OH).

^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ : 7.31 (m, 5H, CH-Ar), 7.09(d, *J*=8.6Hz, 1H, CH-1), 6.70 (dd, *J*=8.6, 2.8Hz, 1H, CH-2), 6.64 (d, *J*=2.8 Hz, 1H, CH-4), 5.08 (m, 1H, CH=C), 4.96 (s, 2H, CH_2OBn), 4.49 (m, 1H, CH-16), 2.79 (m, 2H, CH_2-6), 1.64 (s, 3H, Me), 1.58 (s, 3H, Me), 1.27 (s, 3H, Me), 0.72 (s, 3H, Me).

^{13}C NMR (100 MHz, $CDCl_3$ and CD_3OD) δ: 156.7 (C-3), 138.0 (C-Ar), 137.3 (C-5), 133.0 (C-25), 131.8 (C-10), 128.5 (CH-Ar), 127.8 (CH-Ar), 127.4 (CH-Ar), 126.1 (CH-1), 124.4 (CH-24), 114.8 (CH-4), 112.3 (CH-2), 76.3 (C-20), 73.4 (CH-16), 69.9 (CH_2OBn), 69.5 (CH-17), 52.8 (CH-14), 45.2 (C-13), 43.9 (CH_2-22), 43.6 (CH-9), 40.0 (CH_2-12), 37.7 (CH-8), 34.6 (CH_2-15), 29.7 (CH_2-6), 27.2 (CH_2-7), 26.2 (CH_2-11), 25.7 (CH_3-21), 25.6 (CH_3-27), 22.1 (CH_2-23), 17.7 (CH_3-26), 15.2 (CH_3-18).

MS (APCI), *m/z* (relative intensity): 488 (28), 471 (100), 453 (73).

3-Benzyloxy-(16*R*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (157)



To a stirred solution of **156** (68 mg, 0.13 mmol) in 2,2-dimerthoxypropane (2 ml) was added *p*-toluenesulfonic acid (1.4 mg, 0.0074 mmol) at room temperature. The mixture was stirred for 2 h and triethylamine was added (1 drops). The mixture was added ice-water and extracted with dichloromethane. The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo*. The crude product was purified by flash column chromatography eluting with 1 : 9 ethyl acetate : hexane to provide 3-benzyloxy-(16*R*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (157) as a color wax (40 mg, 56.9%), mp 56-57 °C.

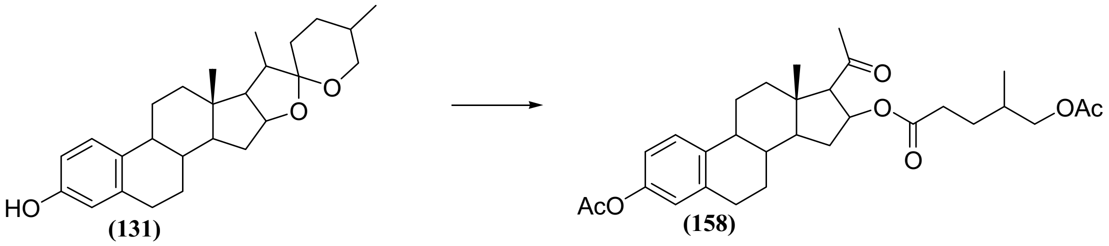
FTIR (neat), ν_{max}, cm^{-1}: 3025 (CH-Ar), 1608, 1498.

^{1}**H NMR** (400 MHz) δ : 7.35-7.14 (m, 6.5H, ArH), 7.13 (d, *J*=8.75 Hz, 0.3H, CH-1), 7.05 (d, *J*=8.76 Hz, 1H, CH-1), 6.69 (m, 1.3H, CH-2), 6.64 (d, *J*=2.72 Hz, 1H, CH-4), 6.60 (d, *J*=2.76 Hz, 0.3H, CH-4), 5.03 (m, 1.3H, CH-24), 4.95 (s, 2H, $\underline{CH_2}$OBn), 4.36 (m, 1.3H, CH-16), 2.76 (m, 2.6H, CH_2-6), 1.62 (s, 3H, MeC=), 1.56 (s, 3H, MeC=), 1.49 (s, 3H, Me), 1.41 (s, 3H, Me), 0.73 (s, 3H, Me-18).

^{13}**C NMR** (100 MHz) δ : 156.7 (C), 137.9 (C), 137.3 (C), 132.8 (C), 131.1 (C), 128.5 (CH-Ar), 128.5 (CH-Ar), 127.8 (CH-Ar), 127.4 (CH-Ar), 127.4 (CH-Ar), 125.99 (CH-1), 124.9 (CH-24), 114.8 (CH-4), 112.5 (CH-2), 100.0 (C), 79.0 (C), 69.9 (CH_2), 69.51 (CH_2), 59.4 (CH), 53.9 (CH), 44.5 7 (CH_2) , 44.1 (Me), 42.2 (C), 38.5 (CH_2), 37.6 (CH), 31.7 (CH), 29.6 (C), 27.5 (CH_2) , 25.7 (CH_2) , 25.7 (CH_2) , 25.6 (CH_2) , 24.9 (CH_2) , 21.7 (CH_3), 17.6 (CH_3), 14.9 (CH_3).

MS (APCI), *m/z* (relative intensity): 528 (4), 488 (36), 471 (100), 453 (72).

3-Acetoxy-16*β*-acetoxymethylvaleroyloxy-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (158)



A mixture of 19-nor-$\Delta^{1,3,5(10)}$-spirostatriene-3-ol (131) (300 mg, 0.78 mmol), acetic anhydride (5.4 ml), ammonium chloride (83.14 mg, 1.56 mmol) and pyridine (0.06 ml) was heated to 125-135 °C and kept at that temperature for 12 hours. After cooling down, the reaction mixture was neutralized with saturated sodium hydrogen carbonate then extracted with methylene chloride. The organic layer was washed with water, dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The crude residue was use in the next step without further purification.

The crude residue was dissolved in 1,2-dichloroethane (0.7 ml), water (0.1 ml) and actic acid (0.25 ml). The mixture was cooled to 0 °C. A solution of chromium trioxide (105 mg, 1.05 mmol) in water (2.19 ml) and acetic acid (0.25 ml) was added (the temperature was kept below 7 °C). The mixture was allowed to warm to room temperature and stirred for another 2 h. A solution of sodium chloride (124 mg) in water (1.66 ml) and methanol (1.66 ml) was introduced and the mixture was stirred for 1 h. The reaction mixture was neutralized using sodiumbicarbonate and extracted with methylene chloride and the organic phase washed with water, dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated and the crude residue was purified by flash column chromatography eluting with ethyl acetate : hexane (1:9) to provide 3-acetoxy-16*β*-acetoxymethylvaleroyloxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (151) (148 mg, 38%) as a pale yellow syrup.

FTIR (neat), ν_{max}, cm^{-1}:1756 (C=O), 1734 (C=O), 1712 (C=O).

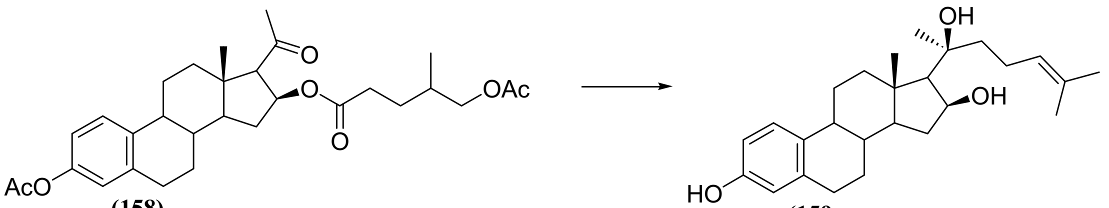
^{1}**H NMR** ($CDCl_3$, 400 MHz): 7.19 (d, *J*=8.48, 1H, CH-1), 6.76 (dd, *J*=8.48, 2.56, 1H, CH-2), 6.70 (d, *J*=2.48, 1H, CH-4), 5.49 (m, 1H, CH-16), 3.81 (d, 6.12, 2H, CH_2-26), 2.78 (m, 2H, CH_2-6), 2.46 (m, 1H, $C\underline{H_A}H_B$-15), 2.40 (d, 7.60, 1H, CH-17), 2.21 (m, 1H, CH-9), 2.20 (s, 3H, $\underline{CH_3}$C=O), 2.19 (m, 1H, $C\underline{H_A}H_B$-11), 2.15 (m, 1H, $C\underline{H_A}H_B$-7), 2.09 (m, 1H, $C\underline{H_A}H_B$-12), 2.01 (s, 3H, Me-21), 1.96 (s, 3H, $\underline{CH_3}$C=O).1.77 (m, 3H, CH_2-22, CH-27), 1.63 (m, 1H, $C\underline{H_A}H_B$-23), 1.50 (m, 2H, $CH_A\underline{H_B}$-7, CH-8), 1.37 (m, 1H, $CH_A\underline{H_B}$-15, $CH_A\underline{H_B}$-23), 1.35 (m, 1H, $CH_A\underline{H_B}$-11),), 1.19 (m, 1H, $CH_A\underline{H_B}$-12), 1.05 (m, 1H, CH-14), 0.99 (s, 3H, Me-18), 0.85 (s, 3H, Me-25).

^{13}**C NMR** ($CDCl_3$, 100 MHz): 260.0 (CO), 172.9 (CO), 171.1 (CO), 169.8 (CO), 148.45 (C-3), 137.9 (C-5), 137.6 (C-10), 126.24 (CH-1), 121.5 (CH-4), 118.61 (CH-2), 74.3 (CH-16), 68.7 (CH_2-26), 66.7 (CH-17), 53.0 (CH-14), 43.9 (CH-9), 42.6 (C-13), 37.97(CH_2-12), 37.1 (CH-8), 34.8 (CH_2-15), 32.0 ($\underline{CH_3}$C=O), 31.9 (CH_2-22), 30.5 (CH-24), 29.3 (CH_2-6),), 28.2 (CH_2-23), 27.3 (CH_2-7), 25.7 (CH_2-11), 21.0 (Me-21), 20.8 ($\underline{CH_3}$C=O), 16.3 (Me-25), 13.4 (Me-18).

MS (APCI), *m/z* (relative intensity): 512 (52), 339 (100), 297 (34).

HRMS: 535.2673 $C_{30}H_{40}O_7Na$ requires 535.2672.

3, 16*S*, 20*S*-Trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (159)



(158)

(159

The mixture of Mg turning (252 mg, 10.5 mmol), I_2 (catalytic amount) and dry THF (30 ml) was added 5-bromo-2-methyl-pentene (0.65 ml, 4.90 mmol) at room temperature under N_2 atmosphere. The mixture was stirred at room temperature for 3 h, and then a solution of **158** (351 g, 0.70 mmol) in dry THF (20 ml) was added drowised at 0 °C. The reaction was stirred at 0 °C for 20 minutes and then quenched with ice-water followed by neutralization with dilute hydrochloric acid. The mixture was then extracted with dichloromethane, the combined organic layer was washed with water, dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography eluting with 3 : 7 ethyl acetate : hexane to provide 3, 6*S*, 20*S*-16, 20-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (159) as a white solid (164.5 mg, 60 %), mp 220-222 °C.

FTIR (KBr), ν_{max}, cm^{-1}: 3427 (OH).

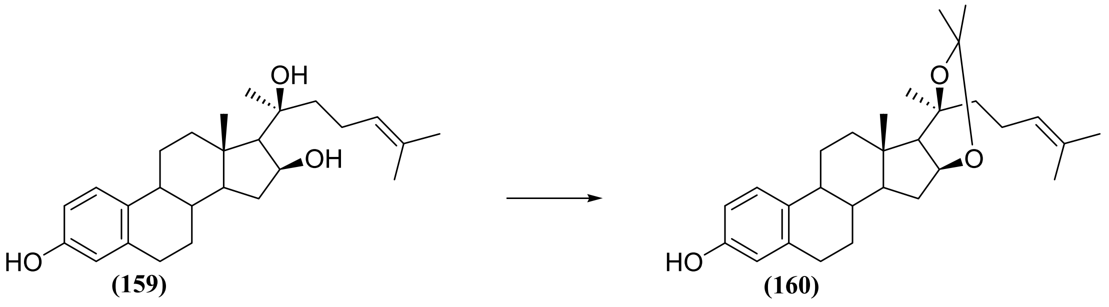
^{1}H NMR ($CDCl_3$ and CD_3OD, 400 MHz): 7.00 (d, *J*=8.32 Hz, 1H, H-1), 6.53 (dd, *J*=8.32, 2.56 Hz, 1H, H-2), 6.47 (d, *J*=2.56 Hz, 1H, H-4), 5.05 (m, 1H, H-24), 4.54 (m, 1H, H-16), 2.73 (m, 2H, H-6), 2.66 (m, 1H, H-15), 2.16 (m, 2H, H-9, H-12), 1.93 (m, 1H, H-11), 1.91 (m, 2H, H-23), 1.77 (m, 1H, H-22), 1.74 (m, 1H, H-7), 1.66 (s, 3H, H-26), 1.57 (m, 1H, H-22), 1.55 (s, 3H, H-27), 1.43 (m, 1H, H-8), 1.32 (m, 1H, H-17), 1.31 (m, 3H, H-7, H-11, H-15), 1.27 (m, 1H, H-12), 1.25 (s, 3H, H-21), 1.07 (s, 3H, H-18), 0.97 (m, 1H, H-14).

^{13}C NMR ($CDCl_3$ and CD_3OD, 100 MHz): 154.1 (C-3), 137.7 (C-5), 131.7 (Me-25), 131.3 (C-10), 126.0 (CH-1), 124.4 (CH-24), 112.5 (CH-2), 115.0 (CH-4), overlap with $CDCl_3$, C-20, 73.4 (CH-16), 59.8 (CH-17), 53.3 (CH-14), 43.6 (C-13), 43.5 (CH-9), 43.1(CH_2-22), 40.4 (CH_2-12), 37.6 (CH-8), 36.5 (CH_2-15), 29.4 (CH_2-6), 27.4 (CH_2-7), 26.3 (CH_2-11), 26.0 (Me27), 25.4 (Me-21), 23.2 (CH_2-23), 17.4 (Me-26), 14.6 (Me-18).

MS (APCI), *m/z* (relative intensity): 399 (4), 381 (98), 363 (100)

HRMS: 421.2721 $C_{26}H_3O_4Na$ requires 4212719.

3-Hydroxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (160)



(159) **(160)**

To a stirred solution of **159** (250 mg, 0.63 mmol) in 2,2-dimethoxypropane (7.7 ml) was added *p*-toluenesulfonic acid (6 mg, 0.032 mmol) at room temperature. The mixture was stirred for 2 h and triethylamine was added (3 drops). The mixture was added ice-water and extracted with dichloromethane. The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo*. The crude product was purified by flash column chromatography eluting with 1 : 9 ethyl acetate : hexane to provide 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (160) as a colorless wax (192.7 mg, 70.1%), mp 68-70 °C.

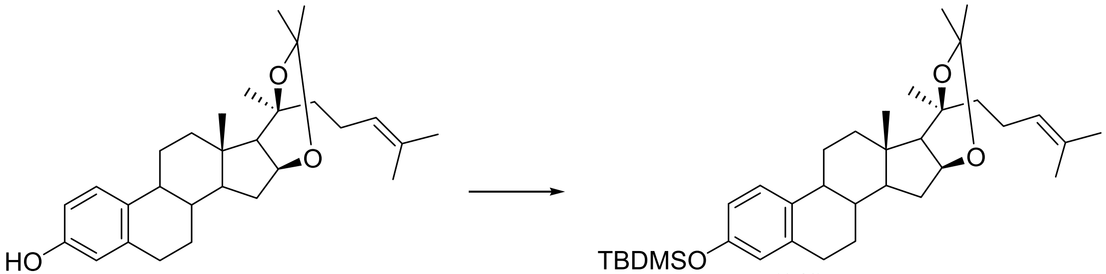
FTIR (KBr), ν_{max}, cm^{-1}: 3373 (OH).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.06 (d, *J*=8.40 HZ, 1H, H-1), 6.55(dd, *J*=8.40, 2.68 Hz, 1H, H-2), 6.48 (d, *J*=2.68 Hz, 1H, H-4), 5.01 (m, 1H, H-24), 4.44 (m, 1H, H-16), 2.75 (m, 2H, H-6), 2.19 (m, 1H, H-15), 2.14 (m, 1H, H-9), 2.12 (m, 1H, H-11), 2.09 (m, 1H, H-12), 1.97 (m, 2H, H-23), 1.83 (m, 1H, H-22), 1.80 (m, 2H, H-7), 1.62 (s, 3H, Me), 1.55 (s, 3H, Me), 1.50 (m, 1H, H-22), 1.46 (m, 2H, H-8, H-11), 1.42 (m, 1H, H-15), 1.41(s, 3H, H-21), 1.29 (m, 1H, H-12), 1.28 (s, 3H, H-27), 1.27 (s, 3H, H-26), 1.06 (s, 3H, H-18), 1.02 (m, 1H, H-14), 0.98 (m, 1H, H-17).

^{13}C NMR ($CDCl_3$, 100 MHz): 153.3 (C-3), 138.2 (C-5), 132.7 (C-10), 131.4 (Me-25), 126.4 (CH-1), 124.4 (CH-24), 115.2(CH-4), 112.6 (CH-2), 97.2 (C), 75.2 (C-20), 68.7 (CH-16), 55.6 (CH-17), 53.7 (CH-14), 44.2 (CH_2-22), 43.9 (CH-9), 42.6 (C-13), 40.0 (CH_2-12), 37.9 (CH-8), 33.5 (CH_2-15), 31.8 (CH_2-26), 29.6 (CH_2-6), 27.5 (CH_2-7) 26.2 (CH_2-11),), 25.7 (Me-27), 25.6 (Me), 25.2 (Me-21), 22.6 (CH_2-23), 17.7 (Me), 14.8 (Me-18).

MS (APCI), *m/z* (relative intensity): 439 (30), 381 (100), 363 (76).

3-*Tert*-butyl-dimethylsiloxy-(16*S*,20*S*)-16,20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (161)



(160) (161)

To a stirred solution of **160** (107 mg, 0.24 mmol) in DMF (0.4 ml) and methylene chlorine (5 ml) was added imidazole (50 mg, 0.73 mmol) and *tert*-butyldimethylsilylchloride (73mg, 0.49 mmol). The mixture was stirred at ambient temperature for 3 h, water was added (3 drops). The mixture was extracted with dichloromethane. The combined organic layer was dried over anhydrous sodiumsulphate, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography eluting with 2 : 98 ethyl acetate : hexane to provide 3-*tert*-butyl-dimethylsiloxy-(16*S*,20*S*)-16,20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (161) (106 mg, 79%) as colorless syrup.

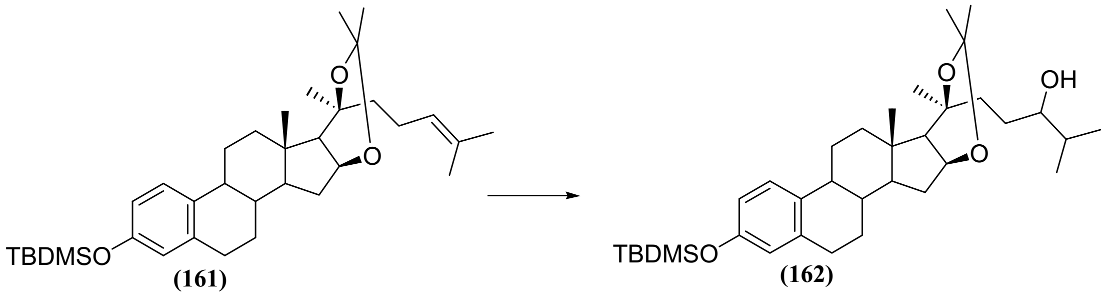
FTIR (KBr), ν_{max}, cm^{-1}: 1607, 1496, 1247.

^{1}H NMR (CDCl$_3$, 400 MHz): 7.03 (d, *J*=8.44 Hz, 1H, H-1), 6.53 (dd, *J*=8.44, 2.68 Hz, 1H, H-2), 6.47 (d, *J*=2.56 Hz, 1H, H-4), 5.01 (m, 1H, H-24), 4.45 (m, 1H, H-16), 2.73 (m, 2H, H-6), 2.20 (m, 1H, H-15), 2.15 (m, 1H, H-9), 2.14 (m, 2H, H-23), 1.97 (m, 1H, H-12), 1.93 (m, 1H, H-11), 1.83 (m, 2H, H-7), 1.81 (m, 1H, H-22), 1.62 (s, 3H, H-27), 1.56 (s, 3H, H-26), 1.44 (m, 1H, H-15), 1.43 (m, 1H, H-22), 1.42 (m, 1H, H-8), 1.40 (m, 4H, H-11, H-21), 1.29 (m, 1H, H-12), 1.06 (s, 9H, H-18, MeX2), 1.02 (m, 1H, H-14), 0.90 (s, 9H, Me$_3$CSi), 0.89 (m, 1H, H-17), 0.80 (s, 6H, Me$_2$Si).

^{13}C NMR ($CDCl_3$, 100 MHz): 153.3 (C-3), 137.8 (C-5), 131.4 (C-10), 131.1 (Me-25), 126.0 (CH-1), 124.5 (CH-24), 119.9 (CH-4), 117.1 (CH-2), 97.1 (C), 75.1 (C-20), 68.7 (CH-16), 55.6 (CH-17), 53.9 (CH-14), 44.2 (CH_2-22), 44.0 (CH-9), 42.6 (C-13), 40.0 (CH_2-12), 37.9 (CH-8), 33.5 (CH_2-15), 31.8 (Me), 29.6 (CH_2-6), 26.2 (CH_2-11), 27.5 (CH_2-7), 25.7 ($\underline{\text{Me}}$CSi), 25.7 ($\underline{\text{Me}}$CSi), 25.7 ($\underline{\text{Me}}$CSi), 25.7 (Me-27), 25.6 (Me-21), 24.2 (Me), 22.6 (CH_2-23), 18.2 (CH_2-26), 17.7 ($\underline{\text{C}}$Si), 14.8 (Me-18), -0.40 ($\underline{\text{Me}}$Si), -0.4 ($\underline{\text{Me}}$Si).

MS (APCI), *m/z* (relative intensity): 553 (100), 495 (30).

3-*tert*-Butyl-dimethylsiloxy-(16*S*,20*S*)-16,20-acetonide-24-ol-chlorestane-$\Delta^{1,3,5(10)}$-estratriene (162)



To a solution of **161** (106 mg, 0.20 mmol) in dry THF (5.6 ml) was added portionwise, with stirring, BH_3.THF complex (ca 1M in THF, 0.2 ml) during a period of 2 h at 0 °C. Hydrogen peroxide solution (35%, 0.6 ml) and 1N sodium hydroxide (0.8 ml) was then added and stirring continued for 16 h. Water was added and the mixture extracted with methylene chloride. The combined organic layer was dried over anhydrous sodiumsulphate, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography eluting with 1 : 19 ethyl acetate : hexane to provide 3-*tert*-butyl-dimethylsiloxy-(16*S*,20*S*)-16,20-acetonide-24-ol-chlorestane-$\Delta^{1,3,5(10)}$-estratriene (162) as a white solid (53 mg, 0.1 mmol) in 48.2 % yield, mp 69-71 °C.

FTIR (KBr), ν_{max} cm^{-1}: 3429 (OH).

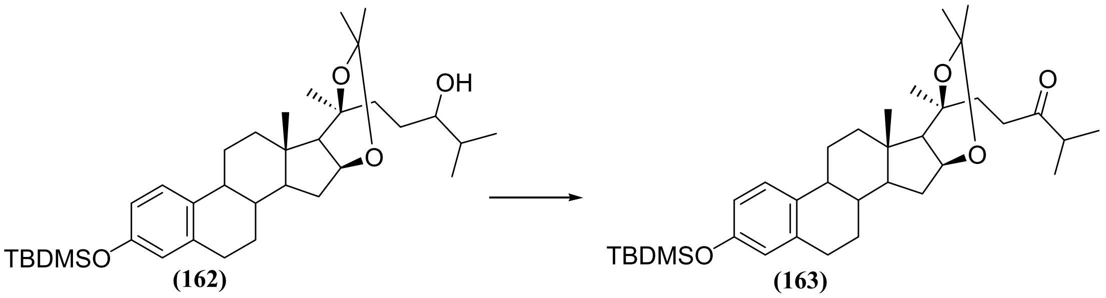
^{1}H NMR (CDCl3, 400 MHz): 6.90 (d, *J*=8.44 Hz, 1H, H-1), 6.42 (dd, *J*=8.44, 2.44 Hz, 1H, H-2), 6.36 (d, *J*=2.48, 1H, H-4), 4.33 (m, 1H, H-16), 3.13 (m, 1H, H-24), 2.26 (m, 2H, H-6), 2.06 (m, 2H, H-11, H-15), 2.05 (m, 1H, H-22), 2.00 (m, 1H, H-9), 1.93 (m, 1H, H-12), 1.67 (m, 2H, H-7), 1.49 (m, 1H, H-25), 1.42 (m, 2H, H-23), 1.37 (m, 2H, H-22), 1.30 (s, 3H, Me), 1.33 (m, 1H, H-8), 1.27 (s, 3H, Me), 1.16 (s, 3H, H-21), 1.13 (m, 1H, H-15), 1.11 (m, 1H, H-11), 1.10 (m, 1H, H-12), 1.08 (m, 1H, H-14), 0.95 (s, 3H, H-18), 0.86 (m, 1H, H-17), 0.79 (s, 9H, $\underline{Me_3}CSi$), 0.75 (s, 6H, H-26, H-27), 0.66 (s, 6H, Me_2Si).

^{13}C NMR (CDCl3, 100 MHz): 153.3 (C-3), 137.8 (C-5), 133.1 (C-10), 125.6 (CH-1), 119.9 (CH-4), 117.1 (CH-2), 97.5/97.3 (C), 77.6/77.3 (CH-24), 74.3 (C-20), 68.8 (CH-16), 56.0 (CH-17), 53.7 (CH-14), 44.0 (CH-9), 42.7 (C-13), 44.1 (CH_2-22), 40.1/40.0 (CH_2-12), 37.8 (CH-8), 33.9 (Me-25), 33.6/33.4 (CH_2-15), 31.6 (Me), 29.6 (CH_2-6), 28.4/28.0 (CH_2-23), 27.6(CH2-7), 26.1 (CH_2-11), 25.9 (Me-21), 25.7 (MeCSi), 25.7 (MeCSi), 25.7 (MeCSi), 24.4 (Me), 19.0/18.7 (Me-27), 15.0 (Me-18), 18.2 (CSi), 17.4 (CH_2-26), -0.40 (MeSi), -0.40 (MeSi).

MS (APCI), *m/z* (relative intensity): 571 (100), 495 (40).

HRMS: 593.4003 $C_{35}H_{58}O_4NaSi$ require 593.4002.

3-*Tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-one-chlorestane-$\Delta^{1,3,5(10)}$-estratriene (163)



To a solution of **162** (50 mg, 0.09 mmol) in dry methylene chloride (4 ml) was added sodium acetate (20 mg, 0.25 mmol) and PCC (60 mg, 0.28 mmol) during a period of 1 h. The reaction was filtered through celite pad and rinse with methylene chloride. Then the filtrate was concentrated in *vacuo*. The crude product was purified by flash column chromatography eluting with 1 : 19 ethyl acetate : hexane to provide

3-*tert*-butyl-dimethylsiloxy-(16*S*,20*S*)-16,20-acetonide-24-one-chlorestane-$\Delta^{1,3,5(10)}$-estratriene (163) as a colorless wax (30 mg, 60%) as a colorless wax, mp 54-55 °C.

FTIR (KBr), $\nu_{max,}$ cm^{-1}: 1708 (C=O).

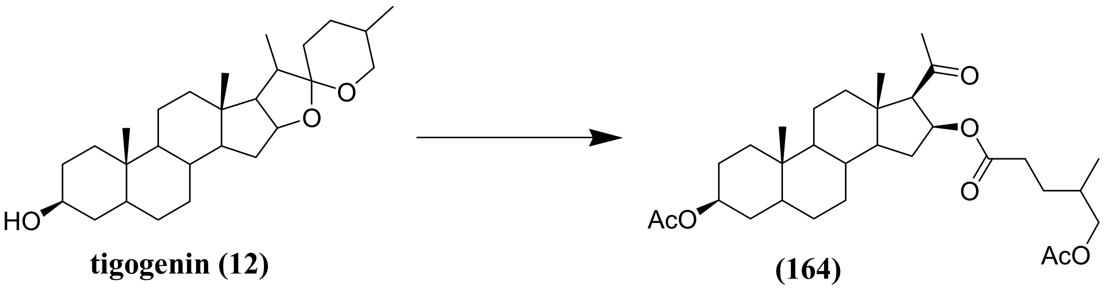
^{1}H NMR (CDCl$_3$, 400 MHz): 6.91 (d, *J*=8.44 Hz, 1H, H-1), 6.41 (dd, *J*=8.44, 2.64 Hz, 1H, H-2), 6.36 (d, *J*=2.64, 1H, H-4), 4.36 (m, 1H, H-16), 2.26 (m, 2H, H-6), 2.43 (d, *J*=6.90 Hz, 1H, H-25), 2.42 (m, 2H, H-23), 2.10 (m, 1H, H-22), 2.09 (m, 1H, H-15), 2.03 (m, 1H, H-11), 2.00 (m, 1H, H-9), 1.92 (m, 1H, H-12), 1.68 (m, 1H, H-7), 1.46 (m, 1H, H-22), 1.42 (m, 1H, H-11), 1.32 (m, 1H, H-8), 1.25 (m, 1H, H-15), 1.22 (s, 3H, Me), 1.19 (m, 1H, H-12), 1.15 (s, 3H, Me), 1.10 (s, 3H, H-21), 0.93 (s, 3H, H-18), 0.92 (d, *J*=6.9 Hz, 6H, H-26, H-27), 0.90 (m, 1H, H-14), 0.80 (m, 1H, H-17), 0.79 (s, 6H, Me_3CSi), 0.0 (s, 3H, Me_2Si).

^{13}C NMR (CDCl$_3$, 100 MHz): 214.8 (CH-24), 153.3 (C-3), 137.8 (C-5), 133.1 (C-10), 126.0 (CH-1), 117.1 (CH-4), 120.0 (CH-2), 97.15 (C), 74.3 (C-20), 68.7 (CH-16), 56.0 (CH-17), 53.8 (CH-14), 44.0 (CH-9), 42.7 (C-13), 41.9 (Me-25), 40.3 (CH$_2$-12), 37.9 (CH-8), 36.8 (CH$_2$-22), 34.7 (CH$_2$-23), 33.5 (CH$_2$-15), 31.7 (Me), 29.6 (CH$_2$-6), 27.6 (CH$_2$-7), 26.1 (CH$_2$-11), 25.8 (Me-21), 25.7 (MeCSi), 25.7 (MeCSi), 25.7 (MeCSi), 23.9 (Me), 18.5 (Me-27), 18.4 (CH$_2$-26), 18.2(CSi), 14.9 (Me-18), -0.40 (MeSi), -0.40 (MeSi).

MS (APCI), *m/z* (relative intensity): 569 (100), 493 (87).

Synthesis of C-3, C-16 and C-20 polyoxygenated steroids

3*β*-Acetoxy-16*β*-*γ*-acetoxymethylvaleroyloxy-5*α*-pregnan-20-one (164)



tigogenin (12) **(164)**

A mixture of tigogenin (12) (1g, 2.4 mmol), acetic anhydride (17 ml), ammonium chloride (256 mg, 4.8 mmol) and pyridine (1.4 ml) was heated to 135 °C and kept at that temperature for 16 h. After cooling down the reaction mixture, acetic acid (2 ml), 1,2-dichloroethane (95 ml) and water (0.5 ml) were added and the mixture cooled to 0 °C. A solution of chromium trioxide (424 mg) in water (0.6 ml) and acetic acid (0.2 ml) was added dropwise and the mixture then stirred at room temperature until the reaction was completed. Then a solution of sodium chloride (480 mg) in water (7.2 ml) and methanol (0.1 ml) was introduced and the mixture stirred for 1 h. The reaction mixture was extracted with methylene chloride and the organic phase washed with water and dried over anhydrous sodium sulphate. The residue from removal of the solvent in vacuo was purified by flash column chromatography (7 : 3 ethyl acetate : hexane) to provide **164** (580 mg, 45%) as a white solid, mp. 97-98 C [lit.99-100 C] (Carmeron, 1955).

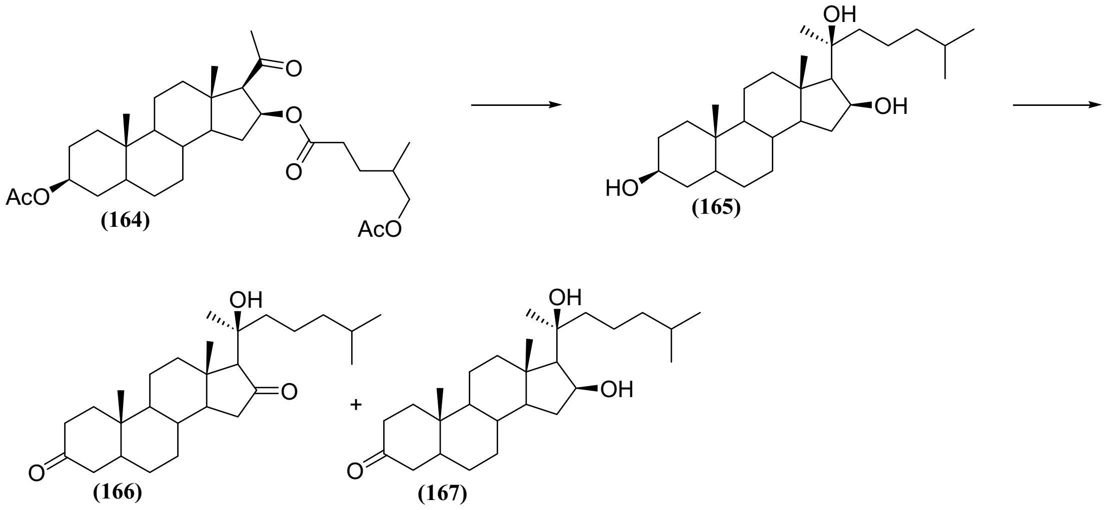
FTIR (KBr), υ_{max}, cm^{-1}: 1749 (C=O), 1739 (C=O), 1733 (C=O), 1717 (C=O).

^{1}H NMR ($CDCl_3$): 5.43 (dt, J=7.74, 4.36 Hz, 1H, H-16), 4.61 (m, 1H, H-3), 3.80 (d, J=6.12 Hz, 2H, H-27), 2.34 (, m, 2H, H-15), 2.32 (d, J=7.60 Hz, 1H, H-17), 2.0 (m, 2H, H-7), 1.99 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.95 (s, 3H, H-21), 1.80 (m, 1H, H-24), 1.70 (m, 1H, H-12), 1.68 (m, 1H, H-25), 1.65 (m, 1H, H-1), 1.62 (m, 2H, H-2, H-23), 1.60 (m, 1H, H-11), 1.56 (m, 1H, H-24), 1.50 (m, 3H, H-2, H-4), 1.40 (m, 1H, H-11), 1.38 (m, 1H, H-23), 1.24 (m, 1H, H-8), 1.22 (m, 2H, H-6), 1.10 (m, 1H, H-5), 0.95 (s, 3H, H-19), 0.94 (m, 1H, H-12), 0.85 (m, d, J=6.71 Hz, 3H, H-26), 0.84 (m, 2H, H-1, H-9), 0.77 (s, 3H, H-18), 0.62 (m, 1H, H-14).

^{13}C NMR ($CDCl_3$): 205.5(C=O), 172.6 (C=O), 171.1 (C=O), 17.7 (C=O), 74.4 (C-16), 73.5 (C-3), 68.7 (C-27), 66.6 (C-17), 54.2 (C-14), 53.9 (C-9), 44.6 (C-5), 42.5 (C-13), 38.1 (C-10), 36.6 (C-12), 35.5 (C-1), 35.0 (C-4), 34.3 (C-8), 33.9 (C-2), 31.9 (C-25), 31.8 (C-15), 31.7 (C-7), 30.6 (C-21), 28.3 (C-23), 28.2 (C-6), 27.4 (C-24), 21.4 (CH_3C=O), 20.8 (CH_3C=O), 2.5 (C-11), 16.4 (C-26), 13.6 (C-19), 12.2 (C-18).

MS (APCI), *m/z* (relative intensity): 352 (M^+, 51), 359 ($M^+ - C_8H_{13}O_4$, 100), 299 ($M^+ - C_8H_{13}O_4 - AcOH$, 44), 281 (17).

(20*S*)-20-Hydroxycholestane-3,16-dione (166) and (16*S*, 20*S*)-16, 20-dihydroxy-cholestan-3-one (167)



A mixture of Mg turnings (338 mg), I_2 (catalytic amount) and dry THF (30 ml) was added 1-bromo-4-methyl pentane (1.4 ml, 9.39 mmol) at room temperature under N_2. The mixture was stirred at room temperature for 3 h and then a solution of **164** (1 g, 1.87 mmol) in dry THF (20 ml) was added dropwise at 0 °C. The reaction was stirred at 0 °C for 20 min and then quenched with ice-water followed by neutralization with dilute HCl. The mixture was then extracted with dichloromethane, the combined organic layer were washed with water, dried over anhydrous sodium sulphate, and concentrated in *vacuo*. The crude product was filtered through a short column (eluting with 3 : 7 ethyl acetate : hexane) to provide product **165** which was used in the next step without further purification.

To a stirred solution of **165** in methylene chloride (50 ml) was added sodium acetate (596 mg, 7.27 mmol) and PCC (2 g, 7.97 mmol) at room temperature. After stirring for 3 h, the mixture was filtered through a celite pad. The filtrate was evaporated and purified by flash column chromatography (15 : 85; ethyl acetate : hexane) to yield **166** (195 mg, 25 % over 2 steps) as a white solid, mp 145-146 C (no mp was reported, Garrido, 2000) and 167 as a white solid (17 mg, 2.2% over steps).

(20*S*)-20-hydroxycholestane-3,16-dione (166)

FTIR (KBr), $\upsilon_{max,}$ cm^{-1}: 3440, 1727, 1717.

^{1}H NMR ($CDCl_3$): 0.80 (d, *J* = 6.6 Hz, 6H, H-26, H-27); 0.87 (s, 3H, H-18); 0.98 (s, 3H, H-19); 1.08 (m, 4H, H-7, H-9, H-24); 1.19 (m, 3H, H-21); 1.29 (m, 2H, H-23); 1.32 (m, 1H, H-1); 1.36 (m, 1H, H-14); 1.42 (m, 2H, H-11); 1.43 (m, 1H, H-12); 1.44 (m, 2H, H-22); 1.45 (m, 1H, H-25); 1.47 (m, 2H, H-6); 1.52 (m, 1H, H-8); 1.57 (m, 1H, H-5); 1.59 (m, 1H, H-7); 1.81 (dd, *J* = 18.5, 13.5 Hz, 1H, H-15); 2.04 (m, 1H, H-1); 2.02 (m, 1H, H-12); 2.06 (m, 1H, H-4); 2.13 (s, 1H, H-17); 2.20 (m, 1H, H-15); 2.27 (m, 1H, H-4); 2.32 (m, 1H, H-2); 2.37 (m, 1H, H-2).

^{13}C NMR ($CDCl_3$): 11.4 (C-19); 14.7 (C-18); 21.0 (C-11); 22.4 (C-23); 22.6 (C-26); 22.7 (C-27); 25.4 (C-21); 27.9 (C-25); 28.6 (C-6); 31.6 (C-7); 33.8 (C-8); 35.7 (C-10); 38.0 (C-2); 38.1 (C-1); 39.3 (C-12), 39.4 (C-15), 39.6 (C-24); 42.7 (C-13); 44.3 (C-22); 44.5 (C-4); 46.4 (C-5); 50.7 (C-14); 53.5 (C-9); 71.4 (C-17); 73.9 (C-20); 211.5 (C-3); 221.2 (C-16).

MS (APCI), *m/z* (relative intensity): 417 [$(M + 1)^+$**,** 7], 399 [$(M + H)^{+} - H_2O$, 100)]**,** 381**,** 331 ($M^{+} - C_6H_{13}$, 5), 289.

(16*S*, 20*S*)-16, 20-Dihydroxy- cholestan-3-one (167)

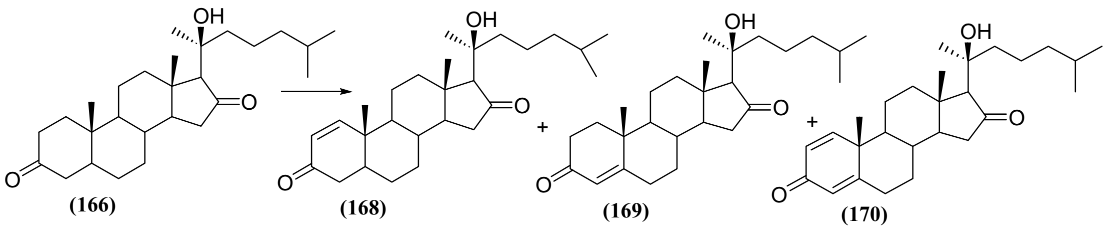
FTIR (KBr) υ_{max} cm^{-1}: 3439, 1700.

^{1}H NMR ($CDCl_3$): 0.66 (m, 1H, H-9); 0.77 (m, 1H, H-14); 0.80 (d, *J* = 6.6 Hz, 6H, H-26, H-27); 0.86 (m, 1H, H-7); 0.96 (s, 3H, H-19); 1.09 (s, 3H, H-18); 1.18 (m, 1H, H-15); 1.13 (m, 2H, H-24); 1.16 (m, 2H, H-12, H-17); 1.21 (s, 3H, H-21); 1.27 (m, 4H, H-6, H-23); 1.28 (m, 1H, H-1); 1.37(m, 1H, H-11); 1.41 (m, 1H, H-8), 1.42 (m, 1H, H-5); 1.46 (m, 1H, H-11); 1.47 (m, 1H, H-22); 1.48 (m, 1H, H-25); 1.64 (m, 1H, H-7); 1.70 (m, 1H, H-22); 1.96 (m, 1H, H-1); 2.00 (m, 2H, H-4); 2.07 (m, 1H, H-12); 2.17 (m, 1H, H-15); 2.20 (m, 1H, H-2); 2.31 (m, 1H, H-2); 4.51 (m, 1H, H-16).

^{13}C NMR ($CDCl_3$): 11.5 (C-19); 15.0 (C-18); 20.9 (C-23); 21.1 (C-11); 22.6 (C-26); 22.7 (C-27); 26.8 (C-21); 28.0 (C-25); 28.8 (C-6); 31.5 (C-7); 34.3 (C-8); 35.7 (C-10); 37.3 (C-15), 38.1 (C-2); 38.5 (C-1); 39.5 (C-24); 40.4 (C-12), 42.4 (C-22); 43.1 (C-13); 44.7 (C-4); 46.7 (C-5); 53.5 (C-9); 54.3 (C-14); 60.1 (C-17); 74.0 (C-16); 76.9 (C-20); 212.1 (C-3).

MS (APCI), *m/z* (relative intensity): 419 [$(M + H)^+$, 83], 410 ($M^{+}- H_2O$, 73), 383 (100), 333 ($M^{+}- C_6H_{13}$, 5), 316 ($M^{+}- C_6H_{14}O$, 15).

<u>(20S)-20-Hydroxycholest-1-ene-3,16-dione (168) , (20S)-20-hydroxy cholest-4-ene-3,16-dione (169) and (20S)-20-hydroxycholest-1,4-diene-3,16-dione (170)</u>



A mixture of *meta*-iodoxybenzoic acid (76 mg, 0.27 mmol) and diphenyl diselenide (8 mg, 0.0226 mmol) in toluene (5 ml) was refluxed until the yellow color of diphenyl diselenide disappeared. A solution of **166** (95 mg, 0.226 mmol) in toluene (3 ml) was introduced to this mixture. The reaction mixture was further heated under reflux for 3 h then cooled to room temperature and partitioned with methylene chloride and water. The organic layer was separated and dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo*. The crude residue was purified by flash column chromatography (2 : 8; ethyl acetate : hexane) to yield (20*S*)-20-hydroxycholest-1-ene-3,16-dione (168) as a colorless wax (17.8 mg, 18.9%) (mp was not reported, Garrido, 2000), (20*S*)-20-hydroxy cholest-4-ene-3,16-dione (169) as a colorless wax (12.1 mg, 12.9%) (mp was not reported, Benvegnu, 1982) and compound (20S)-20-hydroxycholest-1,4-diene-3,16-dione as a pale yellow wax (170) (29.2 mg, 31.2%).

<u>(20S)-20-Hydroxycholest-1-ene-3,16-dione (168)</u>

FTIR (KBr), υ_{max}, cm^{-1}: 3510, 1729, 1679.

^{1}H NMR ($CDCl_3$): 7.05 (1H, d, *J* = 10.2 Hz, H-1), 5.80 (1H, d, *J* = 10.2 Hz, H-2), 2.18 (m, 2H, H-4), 2.20 (m, 1H, H-15), 2.14 (s, 1H, H-17), 2.08 (m, 1H, H-12), 1.87 (m, 1H, H-5), 1.85 (m, 1H, H-11), 1.83 (dd, *J* = 18.5, 14.1 Hz, 1H, H-15), 1.82 (m, 1H, H-8), 1.59 (m, 2H, H-7), 1.55 (m, 2H, H-22), 1.48 (m, 1H, H-25), 1.47 (m, 2H, H-11, H-12), 1.45 (m, 2H, H-6), 1.40 (m, 1H, H-14), 1.30 (m, 2H, H-23), 1.19 (s, 3H, H-21), 1.10 (m, 1H, H-9), 1.08 (m, 2H, H-24), 1.03 (m, 1H, H-8), 0.98 (s, 3H, H-19), 0.89 (s, 3H, H-18), 0.81 (d, *J* = 6.6 Hz, 3H, H-27), 0.80 (d, *J* = 6.6 Hz, 3H, H-26).

^{13}C NMR ($CDCl_3$): 220.8 (C-16), 199.9 (C-3); 157.3 (C-1); 127.7 (C-2); 73.9 (C-20); 71.4 (C-17); 50.8 (C-14); 49.7 (C-9); 44.2 (C-5); 43.1 (C-13); 42.4 (C-22); 40.8 (C-4); 39.5 (C-24); 39.3 (C-12); 39.2 (C-15); 39.0 (C-10); 34.81 (C-8); 31.3 (C-7); 28.0 (C-25); 27.3 (C-6); 25.4 (C-21); 22.7 (C-27); 22.6 (C-26); 20.6 (C-23); 21.0 (C-11); 14.8 (C-18); 13.40 (C-19).

MS (APCI), *m/z* (relative intensity): 415 [$(M + H)^+$**,** 65], 397 [$(M + H)^+ - H_2O$, 100]**,** 329 (6)**,** 313 ($M^+ - C_6H_{13}$, 5), 287 (33).

(20S)-20-hydroxy cholest-4-ene-3,16-dione (169).

FTIR (KBr), υ_{max}, cm^{-1}: 3447, 1725, 1676.

^{1}H NMR ($CDCl_3$): 0.80 (d, 6H, *J* = 6.6 Hz, H-26, H-27); 0.90 (s, 3H, H-18); 1.02 (m, 1H, H-7); 1.08 (m, 2H, H-24); 1.09 (m, 1H, H-9); 1.15 (s, 3H, H-19); 1.19 (s, 3H, H-21); 1.29 (m,1H, H-1); 1.30 (m, 2H, H-23); 1.40 (m, 1H, H-14); 1.44 (m, 1H, H-11); 1.47 (m, 3H, H-8, H-12, H-25); 1.48 (m, 2H, H-22); 1.58 (m, 1H, H-7); 1.68 (m, 1H, H-11); 1.85 (dd, *J* = 18.4, 13.4 Hz, 1H, H-15); 1.96 (m, 1H, H-1); 2.08 (m, 1H, H-12); 2.13 (s, 1H, H-17); 2.22 (m, 2H, H-6); 2.24 (m, 1H, H-15); 2.27 (m, 1H, H-2); 2.37 (m, 1H, H-2); 5.68 (s, 1H, H-4)

^{13}C NMR ($CDCl_3$): 13.40 (C-19); 14.8 (C-18); 21.0 (C-11); 20.9 (C-23); 22.6 (C-26); 22.7 (C-27); 25.4 (C-21); 28.0 (C-25); 32.5 (C-7); 33.8 (C-6); 34.0 (C-8); 38.5 (C-10); 38.8 (C-1); 39.1 (C-15), 39.2 (C-12), 39.4 (C-24); 40.9 (C-2); 42.4 (C-22);

43.1 (C-13); 49.7 (C-9); 50.3 (C-14); 71.2 (C-17); 73.9 (C-20); 124.1 (C-4); 169.9 (C-5); 199.7 (C-3); 220.8 (C-16).

MS (APCI), *m/z* (relative intensity):: 415 [(M + H)$^+$, 100)], 397 [(M + H) $^+$- H$_2$O, 93], 345(45), 313 (M$^+$- C$_6$H$_{13}$, 21), 287 (23).

(20S)-20-hydroxycholest-1,4-diene-3,16-dione (170)

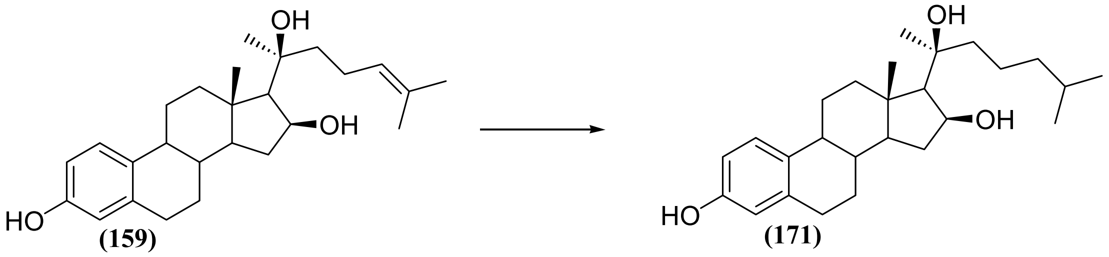
FTIR (KBr), υ_{max}, cm^{-1}: 3455 (OH), 1726 (C=O), 1662 (C=O).

^{1}H NMR (CDCl$_3$): 6.97 (d, *J*= 10.2 Hz, 1H, H-1), 6.19 (dd, *J*= 10.12, 1.88 Hz, 1H, H-2), 6.02 (d, m, 1H, H-4), 0.93 (s, 3H, H-18), 0.80 (d, 6H, *J* = 6.6 Hz, H-26, H-27).

^{13}C NMR (CDCl$_3$): 220.8 (C-16), 186.1 (C-3); 170.7 (C-5), 154.9 (CH-1); 127.8 (CH-4), 124.3 (CH-2), 73.8 (C-20); 71.4 (CH-17); 51.9 (CH-14); 49.9 (CH-9); 43.4 (C-10); 43.1 (C-13); 42.4 (CH$_2$-22); 39.4 (CH$_2$-24); 39.3 (C H$_2$-12); 38.9 (C H$_2$-15); 33.9 (C-8); 33.4 (CH$_2$-6); 32.0(CH$_2$-7), 28.0 (CH-25); 25.3 (C H$_3$-21); 22.7 (C H$_3$-27); 22.6 (C H$_3$-26); 22.2 (C H$_2$-11); 21.1 (C H$_2$-23); 18.7 (C H$_3$-19); 14.6 (C H$_3$-18).

MS (APCI), *m/z* (relative intensity): 413 (62), 412 (13), 395.2 (100), 285.2 (89).

3, 16*S*, 20*S*-Trihydroxy-cholestan-Δ$^{1,3,5(10)}$- estratriene (171)



To a stirred suspension of 5% Pd/C (3 mg, 0.0014 mmol) in ethanol (1ml) was added a solution of **159** (11 mg, 0.0276 mmol) in ethyl acetate (1 ml). The reaction mixture was treated with hydrogen balloon and stirred for 16 h. The mixture was filtered through celite pad and rinsed with ethyl acetate. The filtrate was concentrated by using rotary evaporator. The crude mixture was purified by flash column chromatography (3 : 7 ethyl acetate : hexane) to afford 3, 16*S*, 20*S*-trihydroxy-cholestan-$\Delta^{1,3,5(10)}$- estratriene (171) (11 mg, 100%) as a white solid, mp 245-246 °C.

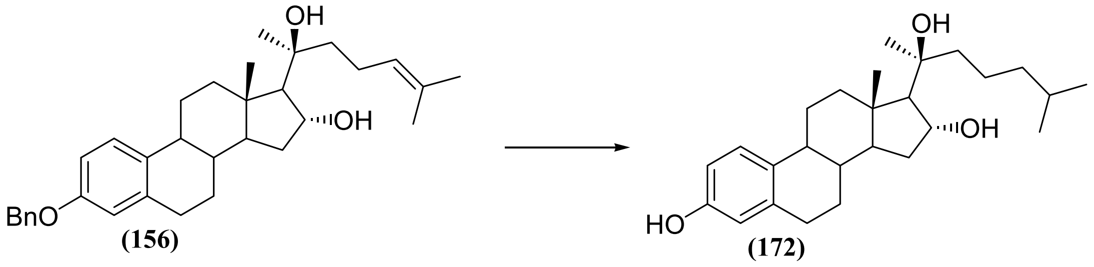
FTIR (KBr) υ_{max}, cm^{-1}: 3389 (OH).

^{1}H NMR (CDCl$_3$ and CD$_3$OD): 7.02 (d, *J*=8.4 Hz, 1H, H-2), 6.54 (dd, J= 8.4, 2.7 Hz, 1H, H-2), 6.47 (d, J= 2.6 Hz, 1H, H-4), 4.52 (m, 1H, H-16), 2.71 (m, 2H, H-6), 2.23 (m, 1H, H-12), 2.15 (m, 1H, H-11), 2.07 (m, 1H, H-9), 1,74 (m, 2H, H-7), 1.49 (m, 1H, H-11), 1.48 (m, 1H, H-), 1.43 (m, 1H, H-25), 1.40 (m, 2H, H-22), 1.29 (m, 2H, H-24), 1.27 (m, 1H, H-12), 1.23 (m, 1H, H-17), 1.22 (s, 3H, H-21), 1.18 (m, 2H, H-23), 1.11 (m, 2H, H-15), 1.07 (s, 3H, H-18), 0.98 (m, 1H, H-14), 0.80 (d, *J*= 6.6 Hz, 6H, H-26, H-27).

^{13}C NMR (CDCl$_3$ and CD$_3$OD): 154.0 (C-3), 137.5 (C-5), 131.4 (C-10), 125.8 (CH-1), 114.8 (CH-4), 112.3 (CH-2), 76.7 (C-20), 73.1 (CH-16), 59.4 (CH-17), 53.1 (CH-14), 44.0 (C-13), 43.5 (CH-9), 42.9 (CH$_2$-22), 40.2 (CH$_2$-12), 39.4 (CH$_2$-15), 37.5 (CH-8), 36.3 (CH$_2$-24), 29.3 (CH$_2$-6), 27.6 (CH-25), 27.3 (CH$_2$-7), 26.1 (CH$_2$-11), 25.7 (Me-21), 22.2 (Me X 2), 22.1 (CH$_2$-23), 14.3 (Me-18).

MS (APCI), *m/z* (relative intensity): 401 (7), 383 (90), 365 (100).

3-Benzyloxy, 16*R*, 20*S*-trihydroxy-cholestane-$\Delta^{1,3,5(10)}$- estratriene (172)



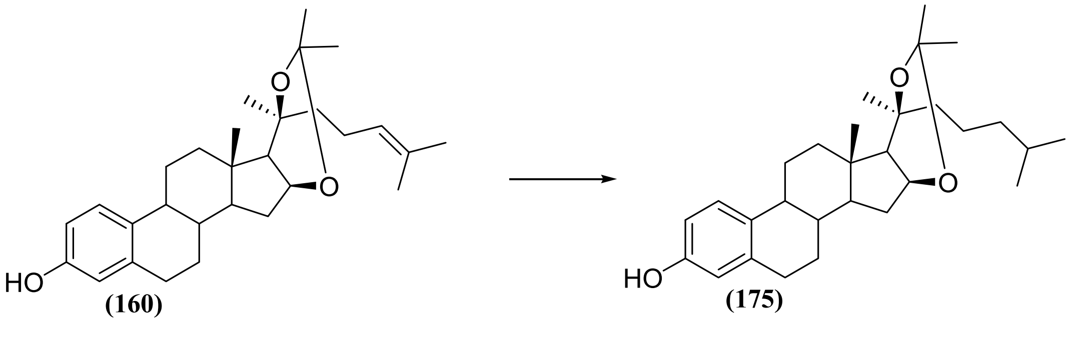
To a stirred suspension of 5% Pd/C (13.5 mg, 0.0064 mmol) in ethanol (3 ml) was added a solution of **156** (62 mg, 0.1269 mmol) in ethyl acetate (3 ml). The reaction mixture was treated with hydrogen ballon and stirred for 16 h. The mixture was filtered through celite pad and rinsed with ethylacetate. The filtrate was concentrated by using rotary evaporator. The crude mixture was purified by flash column chromatography (3:7 ethyl acetate : hexane) to afford 3-benzyloxy, 16*R*, 20*S*-trihydroxy-cholestane-$\Delta^{1,3,5(10)}$- estratriene (172) (50 mg, 99%) as a white solid, mp 94-96 °C.

FTIR (KBr), υ_{max} , cm^{-1}: 3347 (OH).

^{1}H NMR ($CDCl_3$ and CD_3OD): 7.00 (d, *J*=8.48, Hz, 1H, H-1), 6.53 (dd, *J*=8.36, 2.64 Hz, 1H, H-2), 6.47 (d, *J*=2.52 Hz, 1H, H-4), 4.42 (m, 1H, H-16), 2.72 (m, 2H, H-6), 2.16 (m, 1H, H-9), 2.14 (m, 1H, H-11), 1.94 (m, 1H, H-12), 1.74 (m, 1H, H-7), 1.62 (m, 2H, H-23), 1.61 (m, 1H, H-17), 1.60 (m, 2H, H-12, H-14), 1.58 (m, 3H, H-22, H-24), 1.47 (m, 1H, H-25), 1.43 (m, 2H, H-22), 1.38 (m, 1H, H-22), 1.34 (m, 1H, H-8), 1.29 (m, 1H, H-7), 1.22 (m, 3H, H-21), 1.20 (m, 2H, H-15), 0.81 (d, *J*=6.64 Hz, 6H, H-26, H-27), 0.71 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$ and CD_3OD): 154.9 (C-3), 137.6 (C-5), 131.3 (C-10), 125.8 (C-1), 114.9 (C-4), 112.4 (C-2), 75.5 (C-20), 72.8 (C-16), 67.6 (C-17), 52.6 (C-14), 43.5 (C-13), 43.4 (C-9), 43.3 (C-22), 39.7 (C-12), 39.3 (C-15), 37.6 (C-8), 34.3 (C-24), 29.3 (C-6), 27.8 (C-25), 27.3 (C-7), 25.8 (C-11), 24.7 (C-21), 22.3 (C-26), 22.2 (C-27), 20.6 (C-23), 14.8 (C-18).

3-Hydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$-estratriene (175) :



(160) (175)

To a stirred suspension of 5% Pd/C (2 mg, 8.32 μmol) in ethanol (1ml) was added a solution of **160** (8 mg, 0.0183 mmol) in ethylacetate (1 ml). The reaction mixture was treated with hydrogen ballon and stirred for 16 h. The mixture was filtered through a celite pad and rinsed with ethylacetate. The filtrate was concentrated by using rotary evaporator. The crude mixture was purified by flash column chromatography (3:7 ethyl acetate : hexane) to afford 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$-estratriene (175) (8 mg. 100%) as a colorless syrup.

FTIR (KBr) υ_{max} cm^{-1}: 3380(OH).

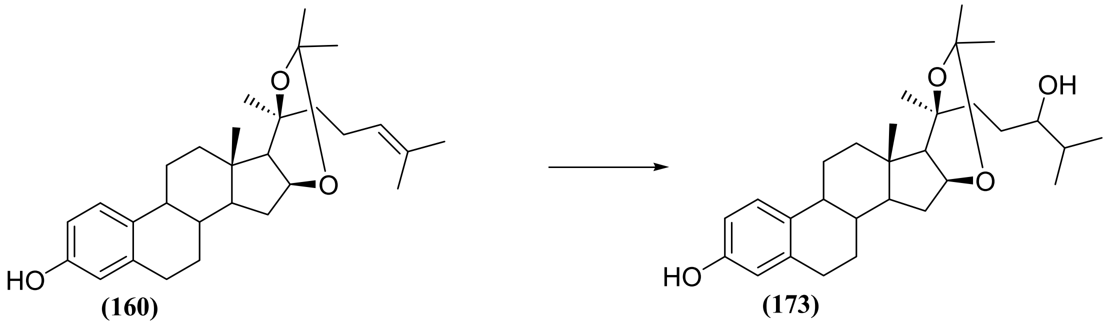
^{1}H NMR ($CDCl_3$): 7.06 (m, 1.6H, H-1), 6.55 (dd, *J*=8.4, 2.76 Hz, 1.6H, H-2), 6.49 (d, *J*=2.7 Hz, 1.6H, H-4), 4.59 (m, 0.6H, H-16'), 4.45 (m, 1H, H-16), 2.73 (m, 3.5H, H-6), 1.39 (s, 3H, Me-21), 1.27 (s, 7.8H, Me, Me, M-21'), 1.12 (s, 1.8H, Me-18'), 0.81 (d, J=6.6 Hz, 10H, Me, Me)

Remark : ^{1}H NMR show the mixture of **175** and **171** which came from acetonide group was removed during the period of ^{1}H NMR experiment.

^{13}C NMR ($CDCl_3$): 153.4 (C-3), 138.1 (C-5), 132.6 (C-10), 127.6 (CH-1), 115.2 (CH-4), 112.3 (CH-2), 78.3 (C-20), 74.1 (CH-16), 60.3 (CH-17), 53.5 (CH-14), 44.4 (C-13), 43.7 (CH-9), 43.3 (CH_2-22), 40.5 (CH_2-12), 39.6 (CH_2-15), 37.6 (CH-8), 37.1 (CH_2-24), 29.5 (CH_2-6), 27.9 (CH-25), 27.5 (CH_2-7), 26.9 (CH_2-11), 26.4 (Me-21), 22.7 (Me), 22.6 (Me), 22.4 (CH_2-23), 15.1 (Me-18).

Remark : ^{13}C NMR show **171,** came from completely conversion from **175** during the period of ^{13}C NMR experiment.

3,24-Dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$-estratriene (173)



(160) **(173)**

To a solution of **160** (100 mg, 0.228 mmol) in dry THF (5.8 ml) was added portionwise, with stirring, BH_3.THF complex (ca 1M in THF, 0.22 ml) during a period of 2 h at 0 °C. Hydrogen peroxide solution (35%, 1.2 ml) and 1N sodium hydroxide (1.6 ml) was then added and stirring continued for 16 h. Water was added and the mixture extracted with methylene chloride. The combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in *vacuo*. The crude product was purified by flash column chromatography eluting with 1:4 ethyl acetate : hexane to provide 3,24-dihydroxy-(16*S*,20*S*)-16,20-acetonide-cholestan-$\Delta^{1,3,5(10)}$-estratriene (173) as a colorless wax (67.1 mg, 64 %).

.

FTIR (KBr), υ_{max} , cm^{-1}: 3411 (OH).

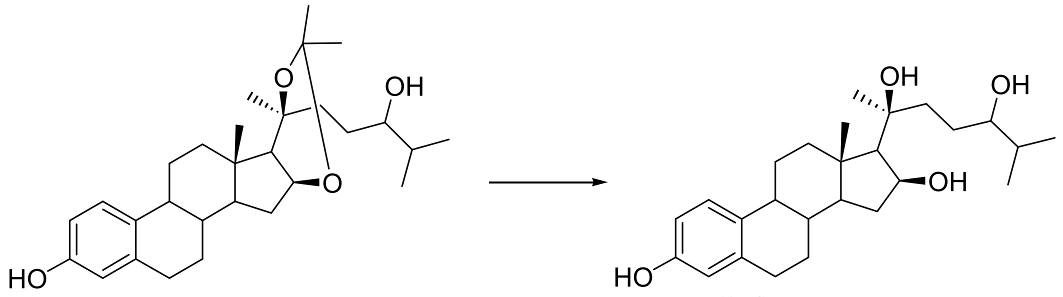
^{1}H NMR ($CDCl_3$): 7.05 (d, *J*=8.4 Hz, 1H, H-1), 6.55 (dd, *J*=8.32, 2.40 Hz, 1H, H-2), 6.49 (d, *J*=2.16 Hz, 1H, H-4), 4.43 (m, 1H, H-16), 3.26 (m, 1H, H-24), 2.73 (m, 2H, H-6), 2.18 (m, 1H, H-15), 2.15 (m, 1H, H-11), 2.08 (m, 1H, H-9), 2.04 (m, 2H, H-12), 1.64 (m, 2H, H-7), 1.60 (m, 1H, H-25), 1.52 (m, 1H, H-11), 1.43 (m, 1H, H-8), 1.41 (s, 3H, H-30), 1.34 (m, 6H, H-15, H-22, H-29), 1.17 (s, 3H, H-21), 1.05 (s, 3H, H-18), 1.02 (m, 1H, H-14), 1.0 (m, 1H, H-17), 0.87(m, 6H, H-26, H-27).

^{13}C NMR ($CDCl_3$): 153.6 (C-3), 138.1 (C-5), 132.5 (C-10), 126.3 (C-1), 115.3 (CH-4), 112.7 (CH-2), 97.6 (C), 77.4 (CH-24), 76.5 (C-20), 68.8 (CH-16), 56.2 (CH-17), 53.6 (CH-14), 43.8 (CH-9), 42.7 (C-13), 40.9 (CH_2-12), 40.0 (CH_2-22), 37.8 (CH-8), 33.5 (CH-25), 33.3 (CH_2-15), 31.5 (Me), 29.5 (CH_2-6), 28.3 (CH_2-23), 27.5 (CH_2-7), 26.2 (CH_2-11), 25.8 (CH_3-21), 24.4 (Me), 18.9 (Me-26), 17.0 (Me-27), 15.1 (Me-18).

MS (APCI), *m/z* (relative intensity): 457 (31), 456 (11), 381 (100).

HRMS: 479.3137 $C_{29}H_{44}O_4Na$ requires 479.3137.

3, 16*S*, 20*S*, 24-Tetrahydroxy-24-cholestan-$\Delta^{1,3,5(10)}$- estratriene (174).



(173) **(174)**

To a stirred solution of compound **173** (38 mg, 0.0833 mmol) in dioxane (4 ml) was added 70% aqueous acetic acid (4 ml) and the mixture was heated at 50 °C for 24 h. The mixture was neutralized with saturated aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The combined organic layer was washed with water and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (eluting with 1:1 ethyl acetate : hexane) to provide 3, 16*S*, 20*S*, 24-tetrahydroxy-24-cholestan-$\Delta^{1,3,5(10)}$-estratriene (174) (19.4 mg, 56%) as a white solid, mp 224-226°C.

FTIR (KBr), υ_{max}, cm^{-1}: 3366 (OH).

^{1}**H NMR** ($CDCl_3$ and CD_3OD): 7.02 (d, *J*=8.44 Hz, 1H, H-2), 6.54 (dd, *J*=8.4, 2.6 Hz, 1H, H-2), 6.48 (d, *J*=2.64, Hz, 1H, H-4), 4.55 (m, 1H, H-16), 3.24 (m, 1H, H-24), 2.76 (m, 2H, H-6), 2.22 (m, 1H, H-15), 2.16 (m, 1H, H-12), 2.11 (m, 1H, H-11), 2.08 (m, 1H, H-9), 1.52 (m, 1H, H-22), 1.75 (m, 1H, H-7), 1.60 (m, 1H, H-25), 1.58 (m, 1H, H-22), 1.52 (m, 1H, H-11), 1.45 (m, 1H, H-8), 1.40 (m, 1H, H-15), 1.30 (m, 1H, H-12), 1.29 (m, 2H, H-7, H-23), 1.27 (m, 1H, H-17), 1.25 (s, 3H, H-21), 1.20 (m, 1H, H-23), 1.07 (s, 1H, H-18), 0.95 (m, 1H, H-14), 0.85 (m, 6H, H-26, H-27)

^{13}**C NMR** ($CDCl_3$ and CD_3OD): 154.0 (C-3), 137.8 (C-5), 131.7 (C-10), 126.1 (CH-1), 115.1 (CH-4), 112.6 (CH-2), 77.2 (CH-24), 76.5 (C-20), 73.2 (CH-16), 61.1/60.0 (CH-17), 53.2 (CH-14), 43.6 (CH-9), 43.2 (C-13), 40.4 (CH_2-12), 39.8/39.7 (CH_2-22), 37.6 (CH-8), 36.5 (CH_2-15), 33.6/33.4 (CH-25), 29.5 (CH_2-6), 27.5 (CH_2-7), 26.3 (CH_2-11), 25.5 (CH_3-21), 23.3 (CH_2-23), 18.7/18.6 (CH_3-27), 17.7/17.3 (CH_3-26), 14.6/14.5 (CH_3-18).

MS (APCI), *m/z* (relative intensity): 417 (4), 381 (100).

HRMS: 439.2804.1450 $C_{26}H_{40}O_4Na$ requires 438.2824.

RESULTS AND DISCUSSION

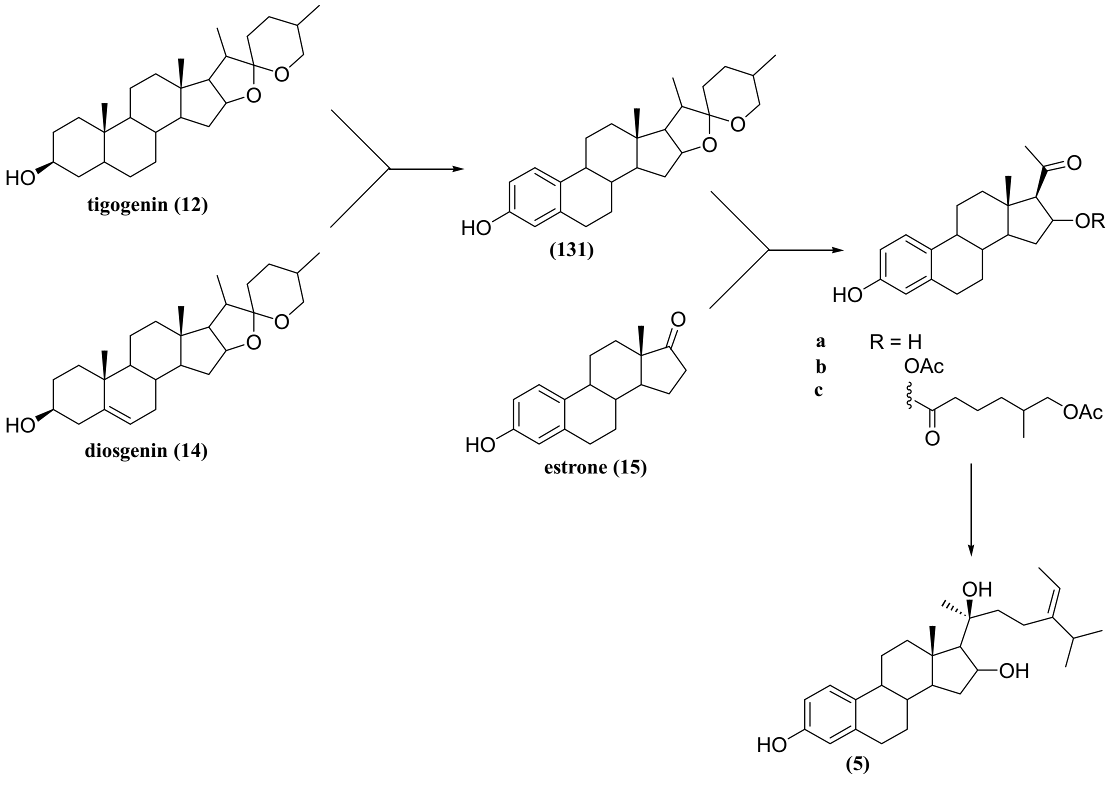
Results

Two examples of C-3, C-16, C-20 polyoxygenated steroids were successfully synthesized as follows;-

1) Synthesis of C-3, C-16, C-20 polyoxygenated steroid derivatives
2) Synthetic intermediate towards geodisterol
3) Evaluated the activity of these class of compound

1. Synthetic intermediate toward geodisterol

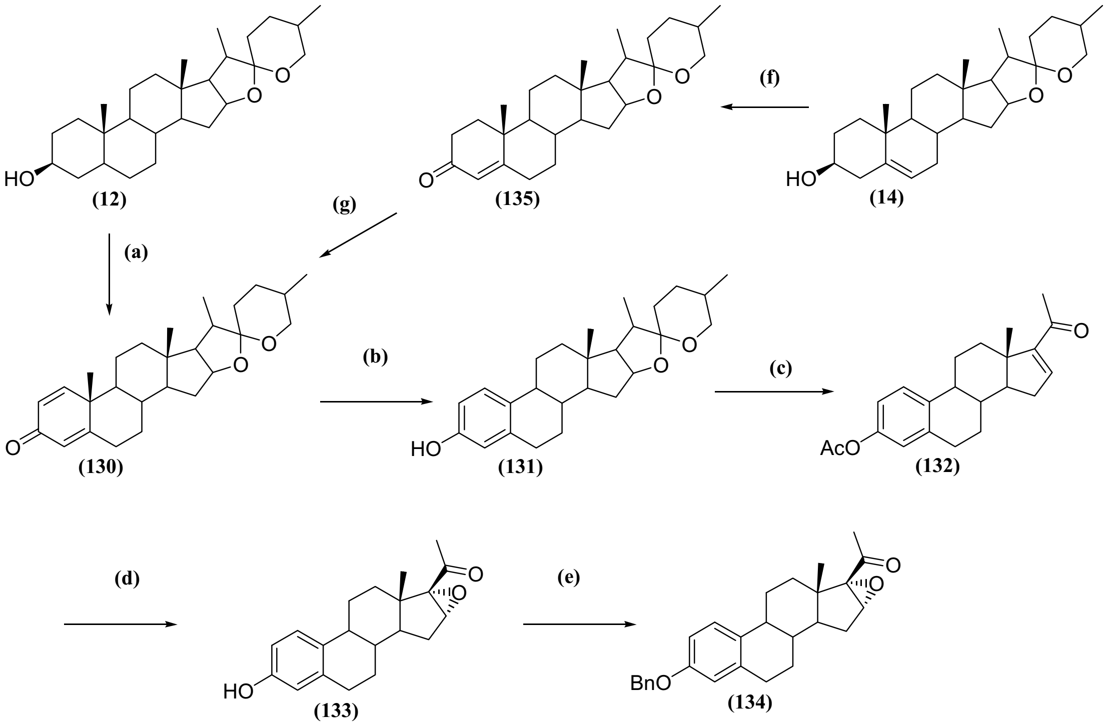
The synthesis of Geodisterol (5) from 3 different starting materials; tigogenin (12), diosgenin (14) and estrone (15) via the key intermediates **a**, **b** or **c** is summarized in Scheme 10.



Scheme 10

1.1 Preparation of 16α, 17α-epoxy-3-benzyloxyestra -1,3,5(10)-triene (134)

Conversion of tigogenin (12) and diosgenin (14) to 3-benzyloxyestra-16α, 17α-epoxy-1,3,5(10)-triene (134) is shown in Scheme 11.



Scheme 11

Reagents and conditions:

a) m-IBX, $(PhSe)_2$, toluene, reflux, 7 h, 70%

b) Li, Ph_2, Ph_2CH_2, THF, reflux, 2h, 44%

c) i) Ac_2O, Py, NH_4Cl, 135 ^{0}C, 12h

ii) CrO_3, AcOH, 1,2-dichloroethane, H_2O, 0^{o}C, 2h

iii) Al_2O_3, benzene, rt, 16h

58 % 3 steps

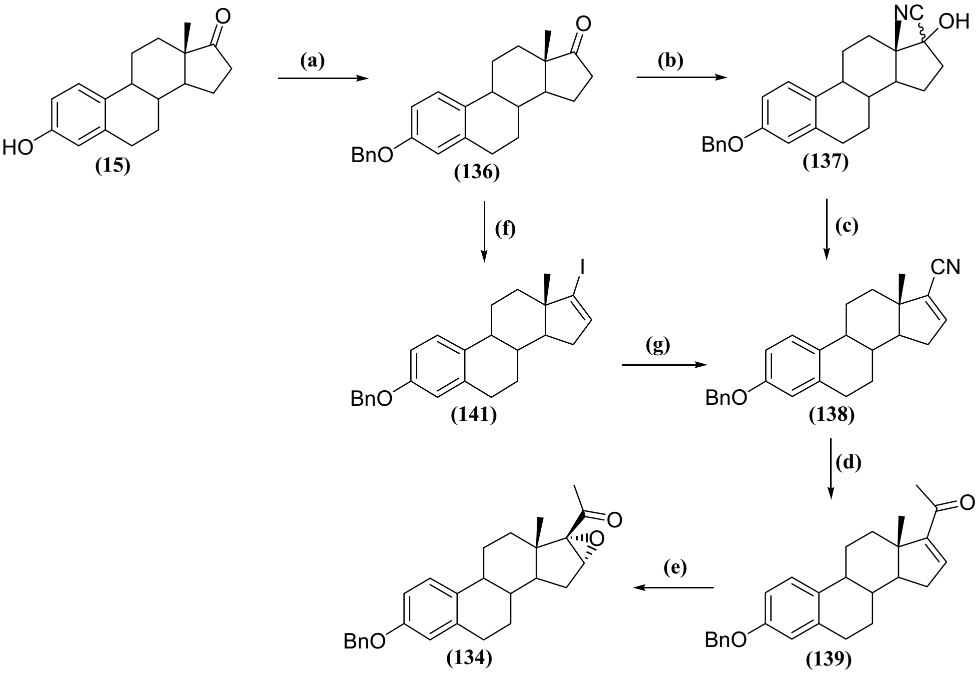
d) H_2O_2, NaOH, MeOH, rt, 16h, 90%

e) BnBr, K_2CO_3, acetone, reflux, 6h, 97%

f) Al(^{i}OPr)$_3$, cyclohexanone, toluene, reflux, 4h, 70%

g) m-IBX, $(PhSe)_2$, toluene, reflux, 7h, 70%

Conversion of estrone (15) to 16α, 17α-epoxy-3-benzyloxyestra -1,3,5(10)-triene (134) is shown in Scheme 12.



Scheme 12

Reagents and conditions:

a) BnBr, *n*BuNBr, NaOH, CH_2Cl_2, H_2O, reflux, 5h, 100%

or BnBr, K_2CO_3, acetone, reflux, 6h, 100%

b) NaCN, AcOH, EtOH, rt, 4 days, 73%

c) $POCl_3$, py, reflux, 9h, 70%

d) MeMgBr (3M in Et_2O), benzene, 50 ^{0}C, 9h, 70%

e) H_2O_2, NaOH, MeOH, rt, 16h, 90%

f) i) $NH_2NH_2.H_2O$, Et_3N, EtOH

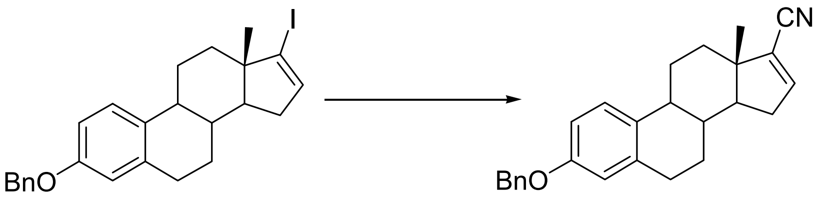
ii) I_2, Et_3N, THF, rt, 5 min.

67% 2 steps

g) [Pd], CN- source

Coupling reaction between 17-iodo-3-benzyloxyestra-1,3,5(10),16- tetraene (141) and variation of the cyanide source were performed by using palladium catalysts. The results are summarized in Table 5.

Table 5 Reaction conditions for the conversion of vinyl iodide (141) into *α,β*-unsaturated cyanide (138).



(141) **(138)**

Entry	Catalyst (eq)	Ligand (eq)	Additive (eq)	CN source (eq)	Solvent	Temp.(ºC) Time(h)	Results
1	$Pd(OAc)_2$ (0.2)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMAC	130	No reaction , starting material recovered
2	$Pd(OAc)_2$ (0.1)	PPh_3 (0.2)	-	$K_4Fe(CN)_6$ (6)	Benzene	80	No reaction , starting material recovered
3	$Pd(PPh_3)_4$ (0.1)	-	-	KCN (6)	DMF	100	Small amount of product, starting material recovered
4	$Pd(PPh_3)_4$ (0.1)	-	-	$K_4Fe(CN)_6$ (6)	CH_3CN	100	Small amount of product, starting material recovered
5	$Pd(PPh_3)_4$	-	-	KCN	CH_3CN	100	Small amount of product, starting material recovered

	(0.1)			(6)			
6	$Pd(OAc)_2$ (0.1)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMF	100	Unknown compound
7	$Pd(PPh_3)_4$ (0.1)	-	-	NaCN/ Al_2O_3	Toluene	110	No reaction , starting material recovered

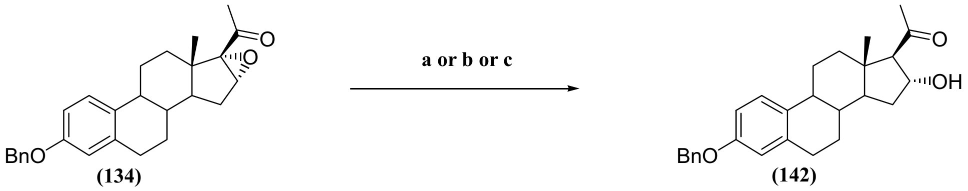
Table 5 (continued)

Entry	Catalyst (eq)	Ligand (eq)	Additive (eq)	CN source (eq)	Solvent.	Temp.(^{o}C) Time(h)	Results
8	$Pd(OAc)_2$ (0.1)	PPh_3 (0.2)	-	NaCN/ Al_2O_3	Toluene	110	No reaction , starting material recovered
9	$Pd(OAc)_2$ (0.1)	PPh_3 (0.2)	-	NaCN/ Al_2O_3	DMF	140	No reaction , starting material recovered
10	$Pd(OAc)_2$ (0.1)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMF	130	Small amount of product and unknown compound
11	$Pd(PPh_3)_4$ (0.1)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMF	130/7	Product (42%) and unknown product

12	Pd(PPh$_3$)$_4$ (0.1)	-	-	K$_4$Fe(CN)$_6$ (0.22)	DMF	130/3	Unknown product and starting material recovered
13	Pd(PPh$_3$)$_4$ (0.1)	-	-	K$_4$Fe(CN)$_6$ (0.22)	DMF	130/5	Unknown product
14	Pd(OAc)$_2$ (0.1)	-	-	KCN (1.2)	DMF	130	No reaction , starting material recovered
15	Pd(OAc)$_2$ (0.1)	-	-	K$_4$Fe(CN)$_6$ (0.22)	DMF	130	Unknown product
16	Pd(OAc)$_2$ (0.1)	-	-	K$_4$Fe(CN)$_6$ (0.22)	DMF	130/5	Unknown product and small amount of product
17	Pd(PPh$_3$)$_4$ (0.05)	-	CuI (0.1)	KCN (2)	CH$_3$CN	100	No reaction , starting material recovered
18	Pd(PPh$_3$)$_4$ (0.05)	-	CuBr (0.1)	KCN (2)	CH$_3$CN	100	No reaction , starting material recovered

Remark : DMAC (dimethylacetamide)
$NaCN/Al_2O_3$ (impregnated of NaCN on Alumina)
Method A : entry 1,6,10,11,
Method B : entry 17,18
Method C : entry 2,8,9
Method D : entry 3,4,5,7,12,13,14,15,16

Conversion of 16α, 17α-epoxy-3-benzyloxyestra -1,3,5(10)-triene (134) to key intermediate, 3-benzyloxy- 16α-hydroxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (142).



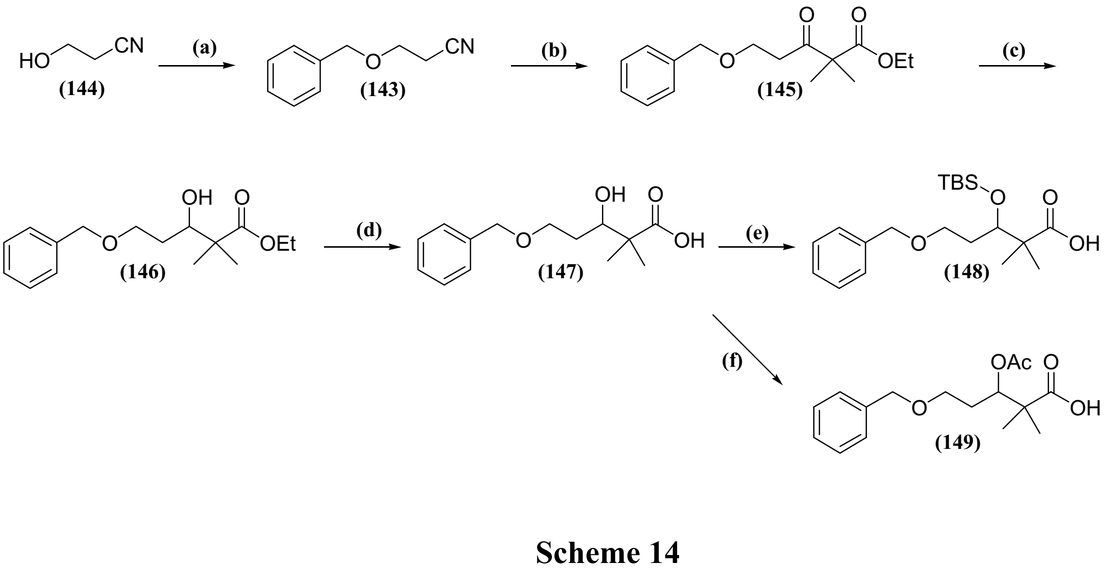
Scheme 13

Reagents and conditions:

a) SmI_2, THF, MeOH, 0 °C, 30 min, 74.5%
b) Al/Hg, $NaHCO_3$, EtOH, H_2O, sonicate, 31% (starting material recover 49%)
c) $NH_2NH_2.H_2O$, EtOH, CH_2Cl_2, rt, 30 min, 92%

1.2 Preparation of side chain moiety

The preparation of carboxylic acid side chain, 5-benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2,2-dimethyl-pentanoic acid (148) and 5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149) from 3-hydroxypropionitrile (144) is Shown in scheme 14.



Scheme 14

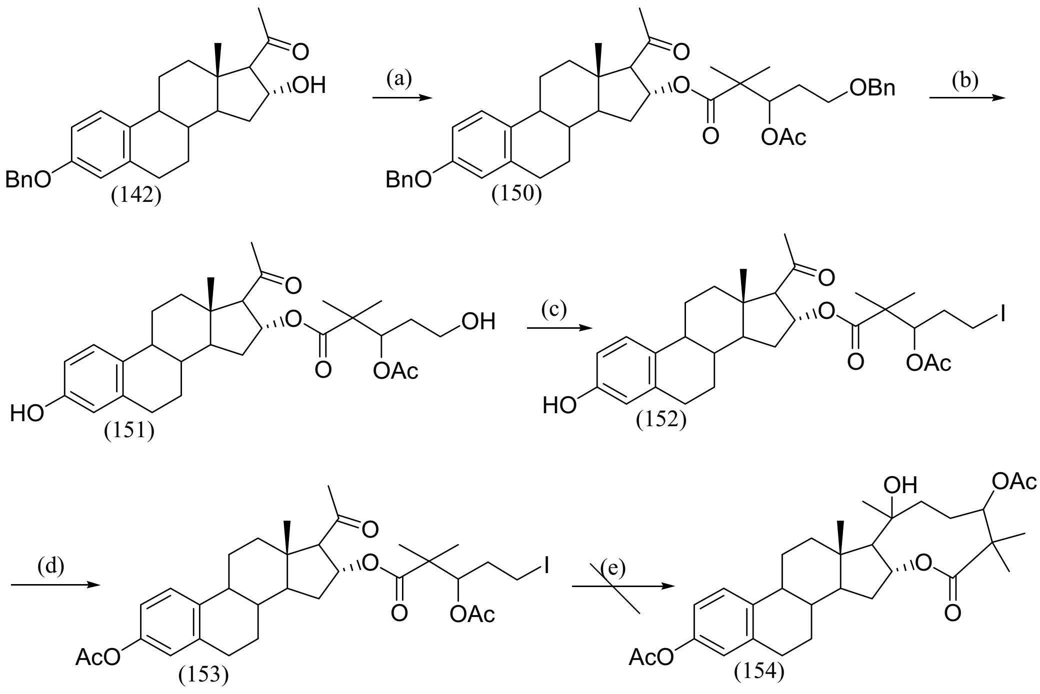
Reagents and conditions:

a) NaH, BnBr, THF, rt, 16h, 88.6%
b) i) ethyl-α-bromo isobutylrate, Zn, sonicate, 2h
  ii) EtOAc, 10% HCl
    67%
c) $NaBH_4$, MeOH, rt, 30 min., 100%
d) 1N LiOH, THF, H_2O, 0 °C-rt, 16h, 76%
e) i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 2h
  ii) K_2CO_3, MeOH, THF, H_2O, rt, 16h
    58.6% 2 steps
f) i) Ac_2O, py, rt, 24h
  ii) H_2O, rt, 2h
    70% 2 steps

1.3 Coupling reaction between side chain and steroid core

1.3.1 Intramolecular coupling reaction

Condensation of 3-benzyloxy- 16α-hydroxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (142) with 5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149) and intramolecular coupling reaction of alkyl halide side chain **153** is illustrated in Scheme 15.



Scheme 15

Reagents and conditions:

a) i) 3-Acetoxy-5-benzyloxy-2,2-dimethyl-pentanoic acid, oxalylchloride, benzene, rt, 4h

ii) **142**, DMAP, CH_2Cl_2, rt, 30 min.

100%

b) H_2, Pd/C (5%), EtOAc, EtOH, rt, 16h, 69%

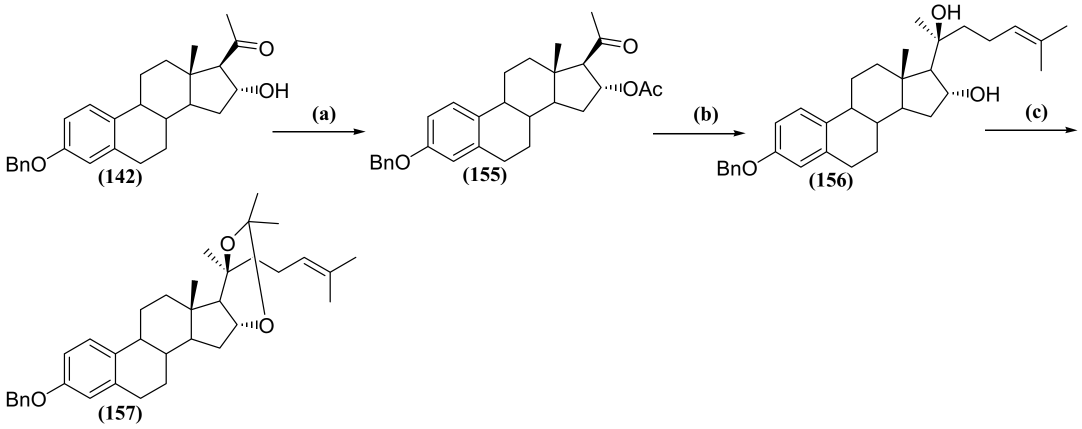
c) I_2, PPh_3, imidazole, toluene, reflux, 3h, 56%

d) Ac_2O, py, 0 °C, 3h, 92.5%

e) SmI_2, THF, 0 °C-rt

1.3.2 Intermolecular coupling reaction

Intermolecular coupling between 3-benzyloxy-16α-acetoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (155) and 5-bromo-2-methyl-2-pentene to provide 3-benzyloxy, 16*R*, 20*S*-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (156) is illustrated in Scheme 16.



Scheme 16

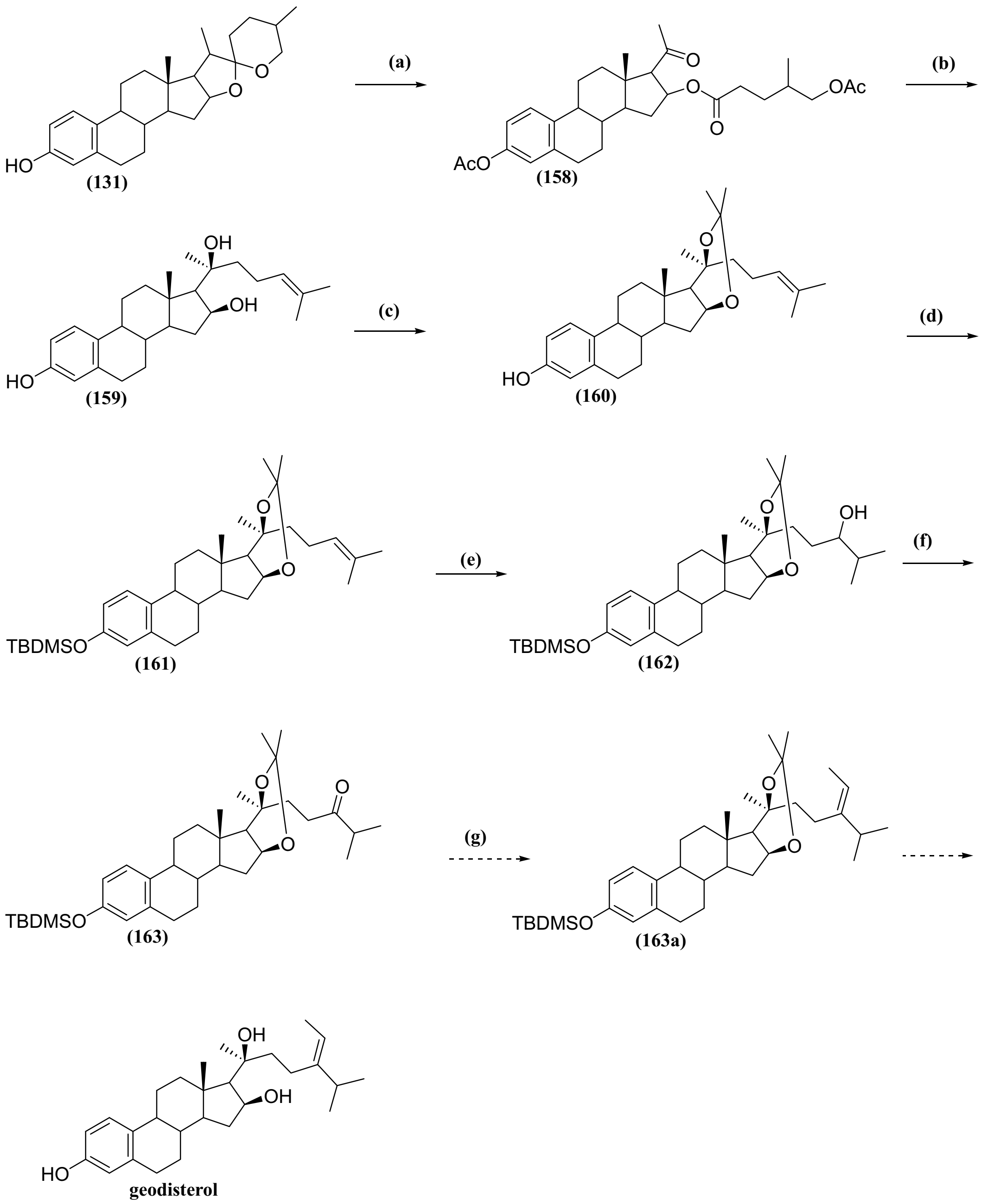
Reagents and conditions:

a) Ac_2O, py, 0 °C, 3h, 96.5%

b) 5-bromo-2-methyl-2-pentene, Mg, THF, 0 °C, 20 min, 38.9%

c) 2,2-dimethoxypropane, TsOH, rt, 2h, 56.9%

Intermolecular coupling of 3-acetoxy-16β-acetoxymethylvaleroyloxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (158) and 5-bromo-2-methyl-2-pentene to provide 3, 6*S*, 20*S*-16, 20-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (159) and its conversion to geodisterol (5) is illustrated in Scheme 17.



Scheme 17

<u>Reagents and conditions:</u>

a) i) Ac_2O, Py, NH_4Cl, 135 ^{0}C, 12h

ii) CrO_3, AcOH, 1,2-dichloroethane, H_2O, rt, 2h

38 %, 2 steps

b) 5-Bromo-2-methyl-2-pentene, Mg, THF, 0 °C, 20 min, 60%

c) 2,2-dimethoxypropane, TsOH, rt, 2h, 70.1%

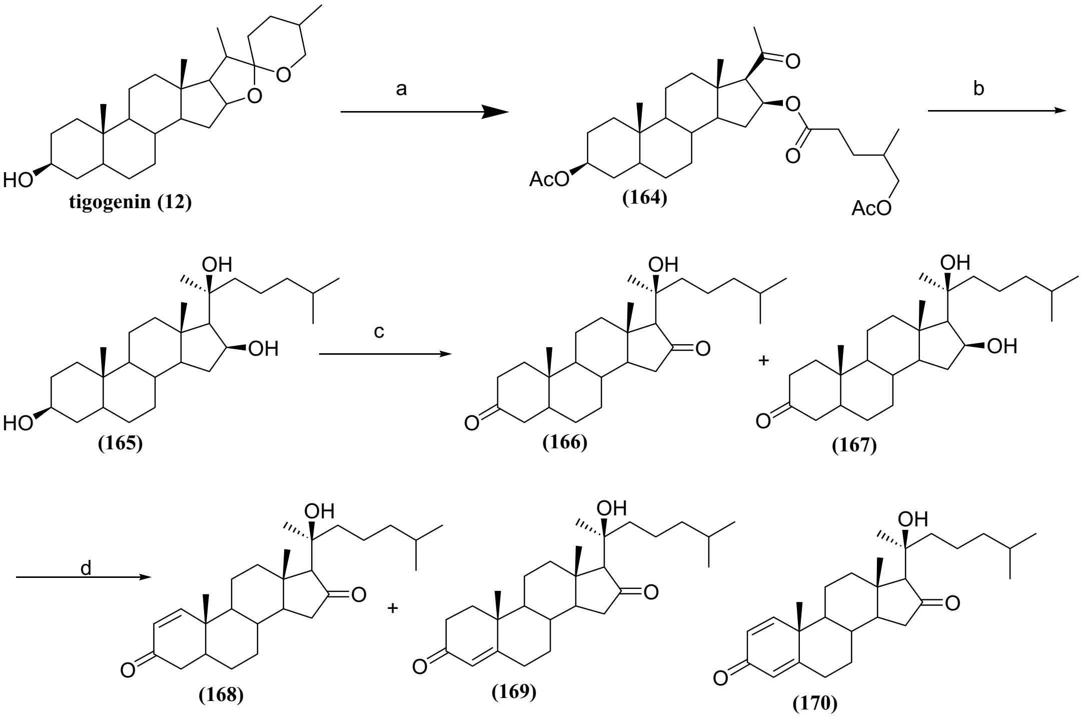
d) TBDMSCl, imidazole, DMF, CH_2Cl_2, rt, 3h, 79%

e) BH_3.THF (1M in THF), THF, 0 °C, 2h, then 35% H_2O_2, 1N NaOH%, 16h, 48.2%

f) NaOAc, PCC, CH_2Cl_2, rt, 1h, 60%

2. Synthesis of C-3, C-16 and C-20-polyoxygenated steroids

The C-3, C-16, C-20 polyoxygenated cholestanes, (20*S*)-20-hydroxycholestane-3,16-dione (166) and (16*S*, 20*S*)-16, 20-dihydroxy- cholestan-3-one (167) was synthesized starting from tigogenin (12), Subsequently, oxidation of compound **166** using *m*-IBX provide (20*S*)-20-hydroxycholest-1-ene-3,16-dione (168), (20*S*)-20-hydroxy cholest-4-ene-3,16-dione (169) and (20*S*)-20-hydroxycholest-1,4-diene-3,16-dione (170) as illustrated in Scheme 18.



Scheme 18

Reagents and conditions:

a) i NH_4Cl, py, Ac_2O, 135°C, 16h

ii CrO_3, AcOH, H_2O, $(CH_2Cl)_2$, rt, 2 h

45%; 2 steps

b) 1-bromo-4-methyl pentane, Mg, THF, 0 °C, 20 min,

c) PCC, NaOAc, CH_2Cl_2, rt, 3h;

25% 2 steps

d) Ph_2Se_2, *m*-iodoxybenzoic acid, toluene, reflux, 3h.

Hydrogenation of the 24, 25-double bond of 3, 6*S*, 20*S*-16, 20-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (159), 3-benzyloxy, 16*R*, 20*S*-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (156) and 3-hydroxy-(16*S*, 20*S*)-16,20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (160) provided 3, 16*S*, 20*s*-trihydroxy-cholestan-$\Delta^{1,3,5(10)}$-estratriene (171) and 3, 16*R*, 20*s*-trihydroxy-cholestan-$\Delta^{1,3,5(10)}$-estratriene (172) and 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$-estratriene (175) , respectively.

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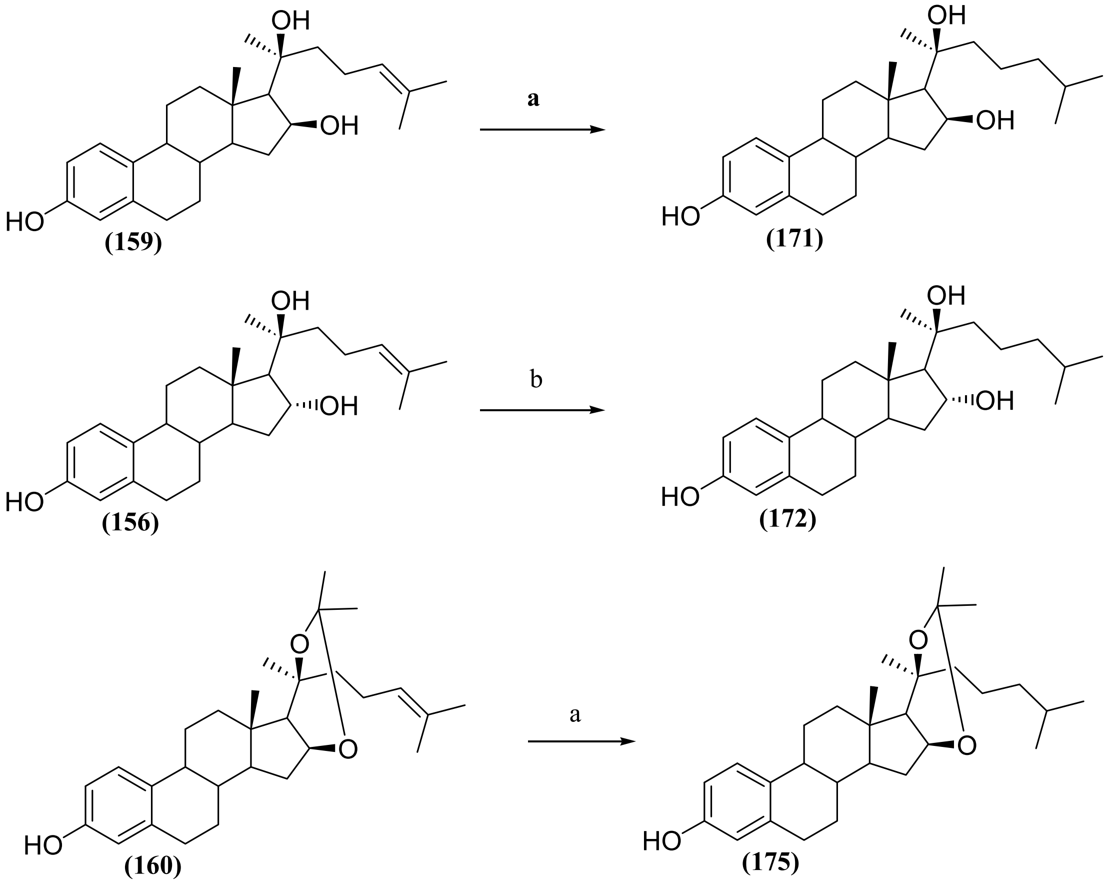
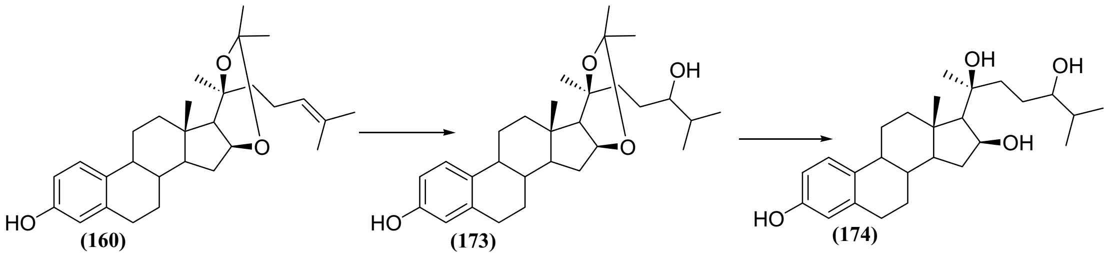


Scheme 19

Reagents and conditions:

a) H_2, Pd/C (5%), EtOAc, EtOH, rt, 16h, 100%
b) H_2, Pd/C (5%), EtOAc, EtOH, rt, 16h, 99%
b) H_2, Pd/C (5%), EtOAc, EtOH, rt, 16h, 100%

Conversion of 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$-estratriene (160) to 3, 16*S*, 20*S*, 24-tetrahydroxy-24-cholestan-$\Delta^{1,3,5(10)}$-estratriene (174) was accomplished by hydroboration-oxidation followed by removal of acetonide group.



Scheme 20

Reagents and conditions:

a) BH_3.THF (1M in THF), THF, 0 °C, 2h, then 35% H_2O_2, 1N NaOH%, 16 h, 64%
b) 70%AcOH, dioxane, 50 °C, 24 h, 56%

3. **Biological activity testing**

Cytotoxicity test against KB (Human epidermoid carcinoma), MCF7 (Breast cancer) and NCI-H187 (Lung cancer) were carried out at the Bioassay Laboratory, the National Center for Genetic Engineering and Biotechnolygy.

C-3, C-16 and C-20 Polyoxygenated steroids and the intermediates in the synthesis of geodisterol were evaluated for cytotoxicity against KB, MCF 7 and NCI. The results are summarized in Table 6.

KB (Human epidermoid carcinoma of cavity, ATCC CCL-17), MCF7 (Human breast adenocarcinoma, ATCC HTB-22) and NCI-H187 (Human small cell lung carcinoma, ATCC CRL-5804) were determined by resazurin microplate assay (REMA) which was a modified method of the use of a fluorescent dye for mammalian cell cytotoxicity according to Brien *et al*. (2000). Ellipticine and doxorubicin were used as positive controls. DMSO and sterile distilled water were used as negative controls. Briefly, cells at a logarithmic growth phase were harvested and diluted to10^5 cells/ml in fresh medium and gently mixed. Test compounds were diluted in culture medium in a ratio of 1:2 giving 8 concentrations. Five μl of test sample and 45 μl of cells were put into 384-well microtiter plates in total volume of 50 μl/well. Plates were incubated at 37 C, 5% CO_2, for 72 h for KB and MCF7, and 5 days for NCI-H187. After the incubation periods, 12.5 μl of resazurin solution was added to each well and the plates were incubated at 37 C for 4 h. The plates were then processed for optical density absorbance analysis using a Victor 3 Microplate reader at dual wavelengths of 530 and 590 nm (tested by National Centre for Genetic Engineering and Biotechnology).

Table 6 Cytotoxicity of C3-, C16- ans C-20-polyoxygenated steroids against KB, MCF 7 and NCI.

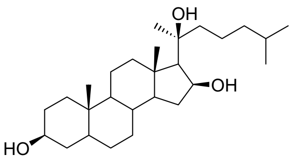
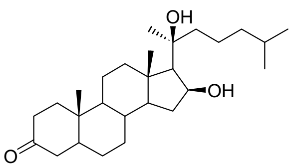
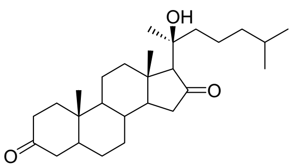
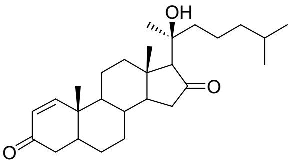
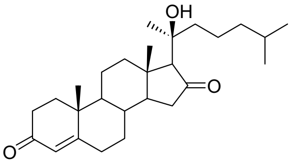
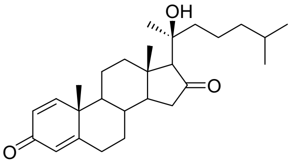
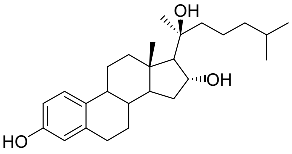
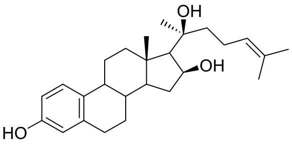
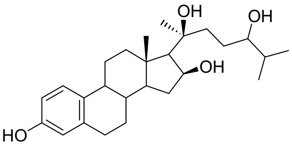
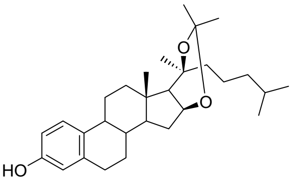
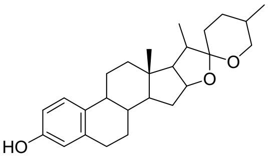
Entry	Compound	Cancer cell lines (IC$_{50}$ μg/ml) (μM)		
		MCF 7	NCI-H187	KB
1		50.0 (119.05)	11.04 (26.29)	10.22 (24.33)
2		Inactive	17.84 (42.68)	Inactive
3		Inactive	Inactive	Inactive

Table 6 (continued)

Entry	Compound	Cancer cell lines (IC$_{50}$ μg/ml) (μM)		
		MCF 7	NCI-H187	KB
4		12.69 (30.65)	2.549 (6.16)	19.55 (47.22)
5		13.01 (31.43)	4.347 (10.5)	17.29 (41.76)
6		22.88 (55.53)	22.97 (55.75)	19.94 (48.40)
8		12.08 (30.20)	4.776 (119.4)	15.78 (39.45)
9		Inactive	Inactive	Inactive
10		17.65 (41.43)	Inactive	8.49 (20.41)
11		5.57 (12.66)	22.52 (51.18)	7.32 (16.64)

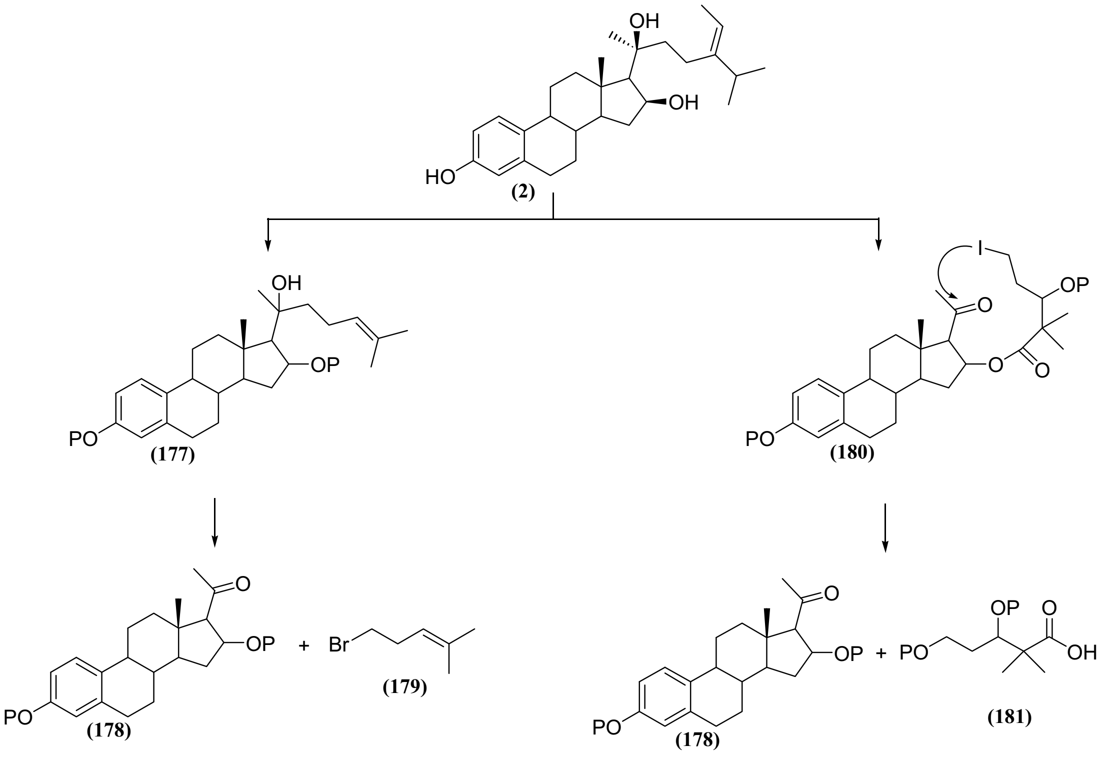
12		Inactive	Inactive	Inactive

Interpretation	% Inhibition	Activity
	< 50 %	Inactive
	≥ 50 %	Active (reported IC_{50} value from 2-fold dilution)

Discussion

1. Synthetic intermediate towards geodisterol

The synthesis of geodisterol (5) was divided into 2 pathways. The first pathway was involved intermolecular coupling reaction between 16-hydroxy-keto steroid (178) and 5-bromo-2-methyl-2-pentene (179). The second pathway was utilized intramolecular cyclization of **180**, synthesized from esterification of **178** and carboxylic acid side chain **181**. As illustrated in scheme 21, both methodologies were proposed to employ 16-hydroxy-keto steroid **178**. Compound **178** was synthesized from tigogenin (12), diosgenin (14) and estrone (15).



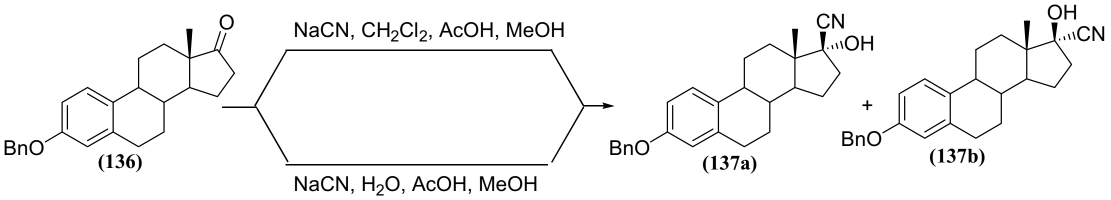
Scheme 21

1.1 Synthesis of the 16-hydroxy-keto steroid (178)

As shown in Scheme 11 aromatization of ring A was performed from quinone **130** by reaction with lithium a slightly excess of lithium radical anion was maintained during addition of the quinone **130** which was indicated by the deep purple color of the solution.

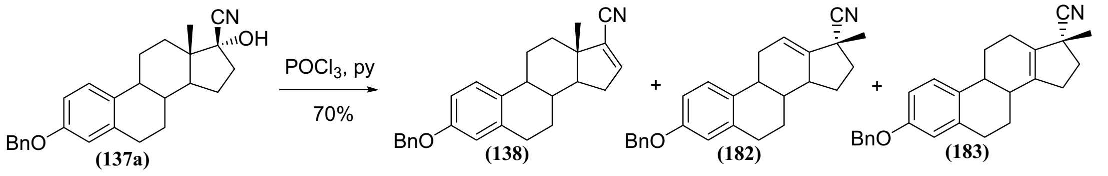
To convert the spiro intermediate **131** to **132** the spiro ring was degraded by a 3 steps sequence; pyran ring opening using acetic anhydride, ammonium chloride and pyridine followed by oxidative cleavage of the double bond using acidic chromium trioxide and finally *trans*-elimination which by treatment with basic alumina in benzene. The 16-O atom was introduced via epoxide ring formation using hydrogen peroxide and aqueous sodium hydroxide in methanol. Only the 16α-17α-epoxy ketone **133** was formed due to the steric effect of the 18β-angular methyl group.

Cyanide was introduced into estrone by the reaction with sodium cyanide in the presence of acetic acid to afford cyanohydrin **137** as described by Caruthers *et al.* Cyanohydrin **137b,** which was kinetically labile, was formed within the first two hours and then slowly equilibrated via **136** to the thermodynamically more stable isomer **137a**. However, when the reaction was carried out in the presence of water only **137b** was formed and no conversion to either starting material **136** or to **137a** was detected. Therefore, we adjusted the reaction condition by changing solvent to ethanol and prolong reaction time which resulted in a better yield (73% yield, 87% conversion) of main product **137a** and trace amount of **137b**.

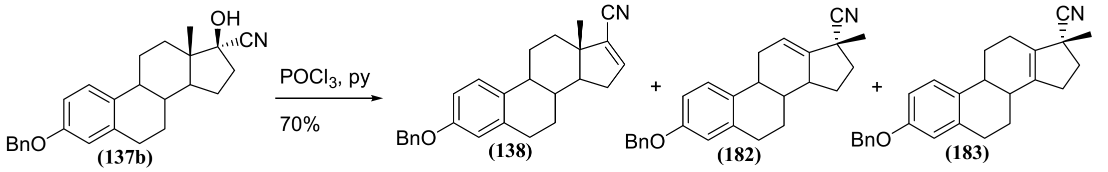


Many conditions for the dehydration of cyanohydrin of **137a** and **137b** were explored. The best conditions were 4 equivalents of phosphorus oxychloride and heating in pyridine at 135 ^{0}C for 9 hours. With **137a** the expected product compound **138** was obtained in 70% yield together with trace amounts of demethylated products

182 and **183**, whereas with **137b**, the product **138** and by product **182** were obtained in 1: 1 ratio with 70% yield.



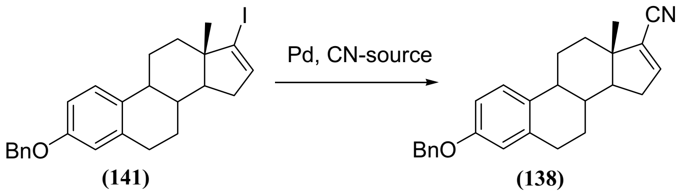
trace amount



1 : 1 trace amount

Furthermore, we also found that **137a** was converted to **138** easier than **137b**. The difficulty of elimination of 17*β*-hydroxy isomer **137b** might be due to the steric effect of the 18-methyl-group hindering reaction with phosphorus oxychloride.

Compound **138** has been prepared by palladium catalyzed coupling reaction of vinyl halide **141** with several of cyanide sources. Therefore, 17-keto steroid **136** was converted to the corresponding vinyl iodide **141** as described in the literature, Beaubien (2006).



We attempted to optimize the reaction conditions by starting from the general method for paladium-catalysed cyanation of aryl halides described by Weissman *et a*l and Schareina *et al.* The cyanide source of choice is potassium hexacyanoferrate (II) discovered by Beller *et al.*

The results clearly show that when palladium(II)acetate was used as catalyst and potassium hexacyanoferrate(II) as cyanide source both with or without sodium carbonate, the reaction did not give the product. In only one case was a small amount of product was detected (Table 7) (entry 3).

Table 7 Cyanation reaction of **141** to **138** using palladium(II)acetate.

Entry	Catalyst (eq)	Ligand (eq)	Additive (eq)	CN source (eq)	Solvent	Temp(°C) Time(h)	Results
1	$Pd(OAc)_2$ (0.2)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMAC	130	No reaction, starting material recovered
2	$Pd(OAc)_2$ (0.1)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMF	100	Unknown compound
3	$Pd(OAc)_2$ (0.1)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMF	130	Small amount of product and unknown compound
4	$Pd(OAc)_2$ (0.1)	-	-	$K_4Fe(CN)_6$ (0.22)	DMF	130	Unknown product
5	$Pd(OAc)_2$ (0.1)	-	-	KCN (1.2)	DMF	130	No reaction, starting material recovered
6	$Pd(OAc)_2$ (0.1)	PPh_3 (0.2)	-	NaCN/ Al_2O_3	Toluene	110	No reaction, starting material recovered
7	$Pd(OAc)_2$ (0.1)	PPh_3 (0.2)	-	NaCN/ Al_2O_3	DMF	140	No reaction, starting material recovered
8	$Pd(OAc)_2$ (0.1)	PPh_3 (0.2)	-	$K_4Fe(CN)_6$ (6)	Benzene	80	No reaction , starting material recovered
9	$Pd(OAc)_2$ (0.1)	-	-	$K_4Fe(CN)_6$ (0.22)	DMF	130/5	Unknown product and small amount of product

But when tetrakis(0)triphenylphosphine palladium(0) was used instead of palladium(II)acetate the product was obtained in moderated yield (entry 1). Changing cyanide source or solvent of the reaction did not improve the reaction yield (Regen *et al.*)

Table 8 Cyanation reaction of **141** to **138** using tetrakris(0)triphenylphosphine palladium(0).

Entry	**Catalyst** **(eq)**	**Ligand** **(eq)**	**Additive** **(eq)**	**CN source** **(eq)**	**Sovent**	**Temp(^{o}C)** **Time(h)**	**Results**
1	$Pd(PPh_3)_4$ (0.1)	-	Na_2CO_3 (1)	$K_4Fe(CN)_6$ (0.22)	DMF	130/7	Product (42%) and unknown product
2	$Pd(PPh_3)_4$ (0.1)	-	-	$K_4Fe(CN)_6$ (0.22)	DMF	130/5	Unknown product

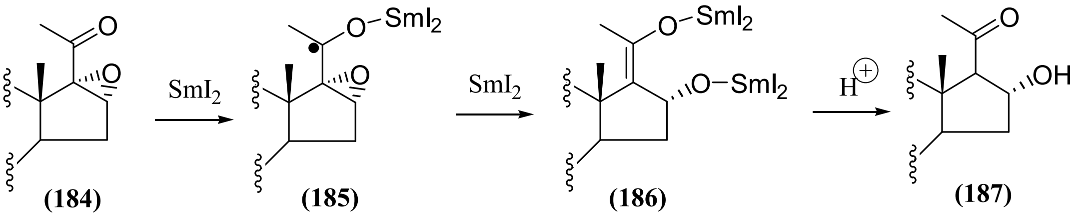
Table 8 (continued)

Entry	**Catalyst** **(eq)**	**Ligand** **(eq)**	**Additive** **(eq)**	**CN source** **(eq)**	**Sovent**	**Temp(^{o}C)** **Time(h)**	**Results**
3	$Pd(PPh_3)_4$ (0.1)	-	-	KCN (6)	DMF	100	Small amount of product, starting material recovered
5	$Pd(PPh_3)_4$ (0.1)	-	-	KCN (6)	CH_3CN	100	Small amount of product, starting material recovered
4	$Pd(PPh_3)_4$ (0.1)	-	-	$K_4Fe(CN)_6$ (6)	CH_3CN	100	Small amount of product, starting material recovered
5	$Pd(PPh_3)_4$ (0.1)	-	-	NaCN/ Al_2O_3	Toluene	110	No reaction, starting material recovered
7	$Pd(PPh_3)_4$ (0.05)	-	CuI (0.1)	KCN (2)	CH_3CN	100	No reaction, starting material recovered
8	$Pd(PPh_3)_4$ (0.05)	-	CuBr (0.1)	KCN (2)	CH_3CN	100	No reaction, starting material recovered
9	$Pd(PPh_3)_4$ (0.1)	-	-	$K_4Fe(CN)_6$ (0.22)	DMF	130/3	Unknown product and starting material recovered

In conclusion, all endeavors at cyanation using palladium catalysts and a variety of CN sources were unsuccessful. The best result (Table 8, entry 1) afforded the product in only 42% yield. One especially important point was the fact that an excess of cyanide ions in the reaction can react with palladium (II) species, forming inactive palladium (II) cyano complexes, which cannot be reduced to the active palladium (0) species. Potassium tetra cyano palladate(II) and palladium (II) cyanide were shown to be almost inactive in cyanation reaction. A dramatic solvent effect was observed which can be explained by the influence of the cyanide source solubility in different reaction media. The better the solubility of the cyanide salt, the lower the reaction outcome. The positive effect of sodium carbonate is believed to be due to it facilitating the reduction of palladium (II) species. Potassium hexacyanoferrate (II) seems to be a good CN source due to its properties; non-toxic, all six CN are available for reaction, inexpensive and easily handled. DMF is the solvent of choice because of can act as a ligand to accelerate the reaction.

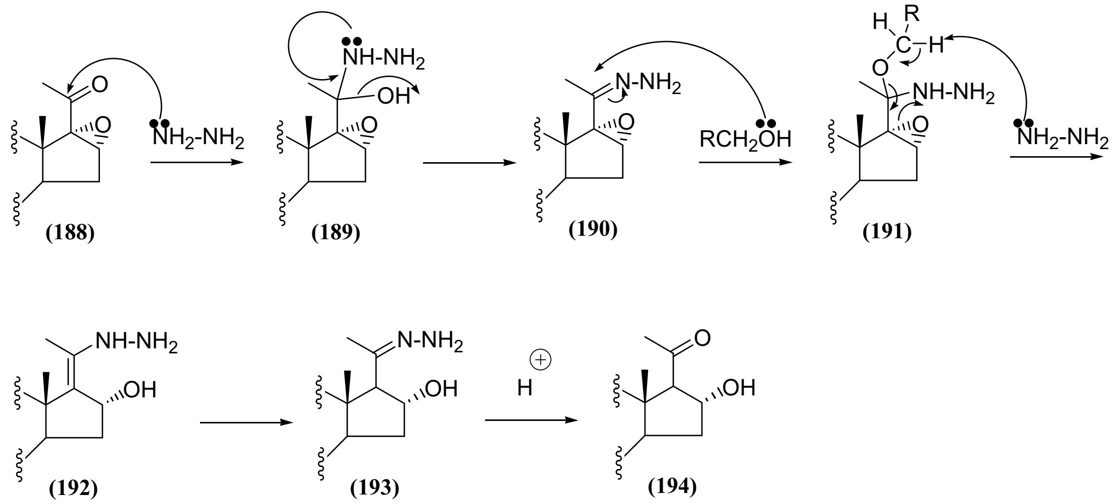
Grignard reaction of **138** with methyl magnesium bromide and subsequent acidic workup afforded **139** in excellent yield. Epoxidation of the phenolic protected steroid gave epoxy ketone in a good yield.

Opening of the epoxide ring to a *β*-hydroxy ketone has been explored (Scheme 13) by using three methodologies. The first method involved reduction of epoxy ketones with sumarium(II)iodide in the presence of a protic solvent, methanol, to afford mono-aldols probably via samarium enolate **186** as shown in following scheme 22. Although this reaction provided yield samarium metal and sumarium(II)iodide are quite expensive and difficult to handle.



Scheme 22

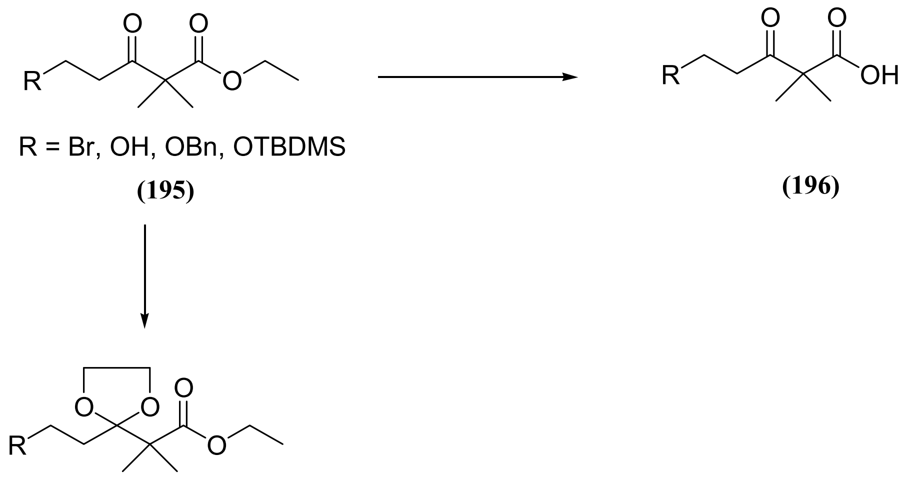
Opening of the epoxide ring using sonochemical reduction with aluminium amalgam furnished product in 31% yield with 51% conversion. Whereas hydrazine hydrate induced reductive cleavage of *α,β*-epoxy ketone provided the desired product in excellent yield. A plausible mechanism is shown in Scheme 23.



Scheme 23

1.2 Synthesis of the side chain (149)

Reformasky reaction of 3-hydroxypropionitrile and ethy-*α*-bromo isobutylate followed by acidic workup led to (**195**, R=OH)) (Scheme 24). Many attempts to hydrolye ester **195** (R=OH) to the corresponding carboxcylic acid **196** both by acidic and basic conditions were unsuccessful and lead to decarboxylation or elimination to give ketone or *α,β*-unsaturated carboxylic acid. Protecting the alcohol as a benzyl or silyl ether or changing to bromide (**195**, R= OBn, OTBDMS, Br) gave the same results. Due to this problem which we considered this came from the ketone group of **195**. We attempted to protect it as the cyclic ketal. Unfortunately the reaction did not proceed probably due to steric hindrance of the gem dimethyl group.



R = Br, OH, OBn, OTBDMS

(195)

(196)

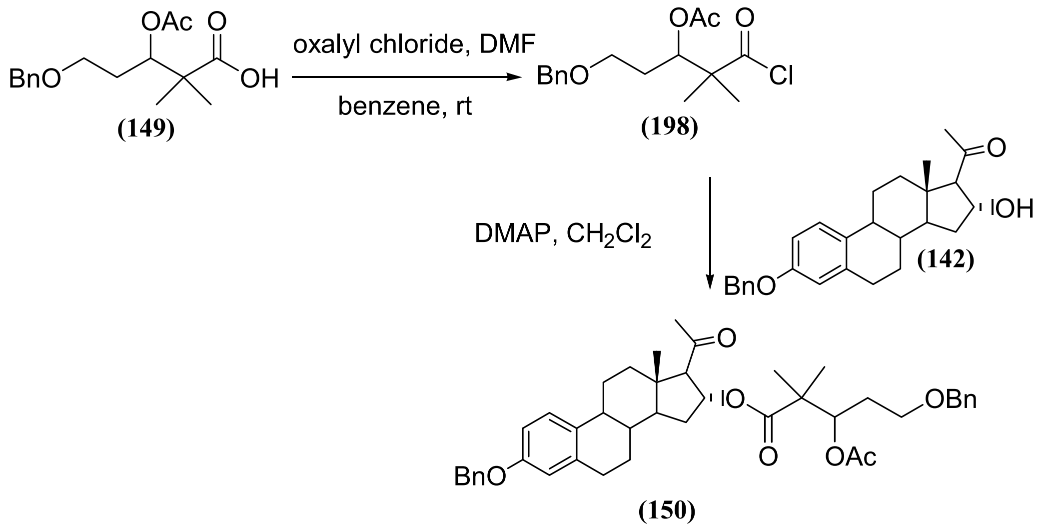
(197)

Scheme 24

We finally overcame this problem by reduction of the ketone group of **195** (R=OBn) to the corresponding hydroxy ester **146** then hydrolysis with base and protection as silyl ether **148** or acetate **149** as illustrated in scheme 14.

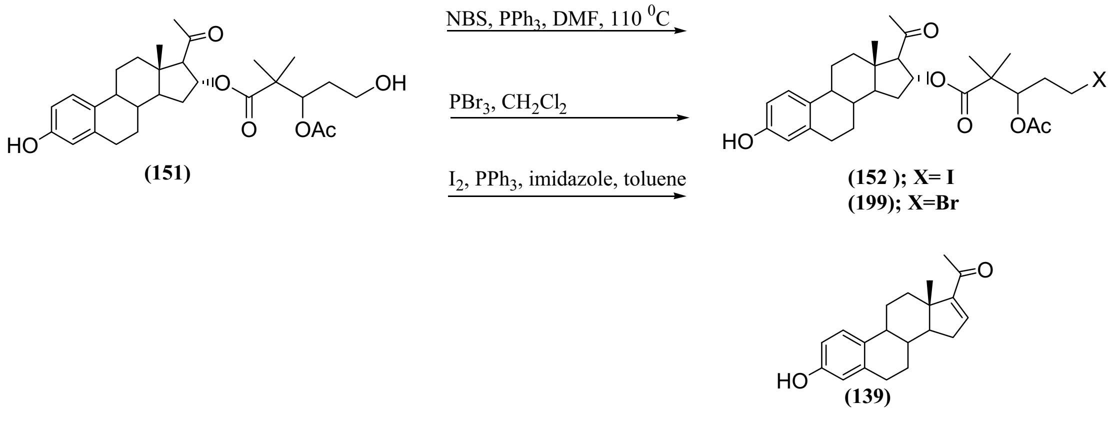
1.3 Intramolecular coupling reaction of (142) and (149) (Scheme 15)

Condensation between carboxylic acid **149** and *β*-hydroxy steroid **142** using DCC, CDI or EDCI under standard conditions did not provide the expected ester product **150**, only starting material was recovered. The failure is probably due to the activity and steric hindrance of the acylating agent generated from the reaction. We then turned to the more reactive acid chloride **198** which was generated by the reaction of carboxcylic acid **149** with oxalyl chloride and equal mole equivalent of DMAP to keep the reaction condition slightly basic.



Scheme 25

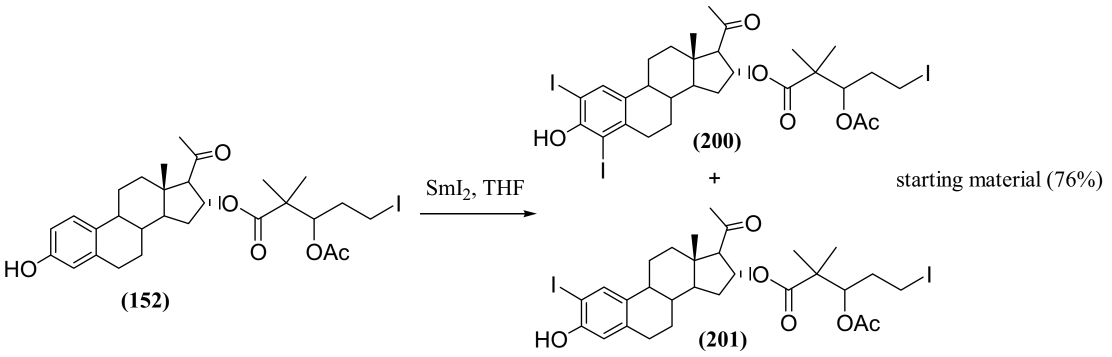
In order to investigate the intramolecular cyclization of **151**, primary alcohol group of **151** must be converted to halide. Bromination of **151** using NBS and triphenylphosphine in DMF did not give a bromide product, only starting alcohol was recovered. When **151** was treated with phosphorus tribromide, only elimination product **139** was obtained. However iodination using iodine and triphenylphosphine in toluene furnished iodide **152** in 56% yield.



Scheme 26

Intramolecular coupling reaction of **152** using sumarium(II)iodide was unsuccessful Instead electrophilic substitution on aromatic A ring to give **200** (15.4%)

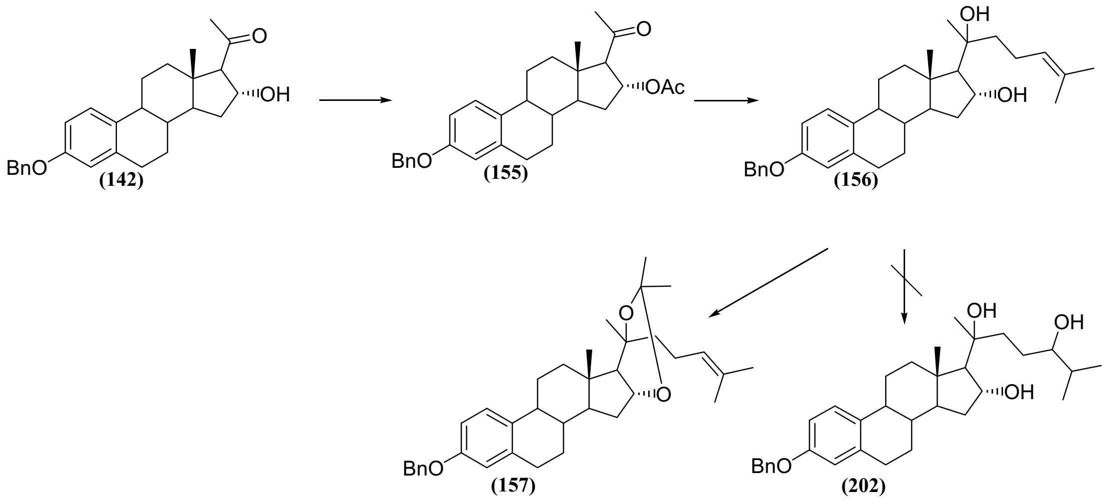
an **201** (8.3%) was observed (Scheme 27). To overcome this problem, acetylation of **152** was carried out to reduce the electron density of the aromatic A ring. However, several attempts at coupling a primary alkyl iodide to the C20 ketone using sumarium(II)iodide failed.



Scheme 27

1.4 Intermolecular coupling reaction of **142** and 5-bromo-2-methyl-2-pentene (179) (Scheme 16)

We turned our attention to introducing the side chain via reaction of **142** and 5-bromo-2-methyl pentene (179). Protection of the secondary alcohol at C16 of **142** as acetate to give **155** and subsequent, Grignard coupling reaction with 5-bromo-2-methyl pentene (179) afforded diol **156** in 38.9% yield. Without protection of tertiary alcohol at C-20, hydroboration of **156** followed by oxidation with hydrogen peroxide and sodium hydroxide proved unsuccessful. Therefore protection of the diol as acetonide was performed using 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid to provide **157** in 56.9% yield.



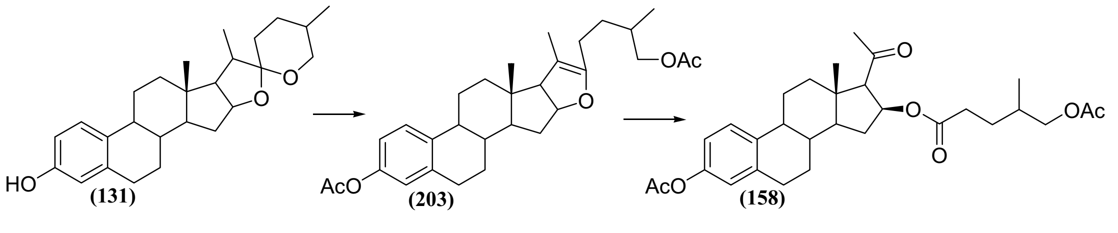
Scheme 27

The preparation of **157** by the above method involved many steps and the yield of each step was only moderate. So, we changed our strategy to synthesize geodisterol (5) from a precusor which already contains the *β*-configuration at C-16.

1.5 Intermolecular coupling reaction of **158** and **179** (Scheme 17)

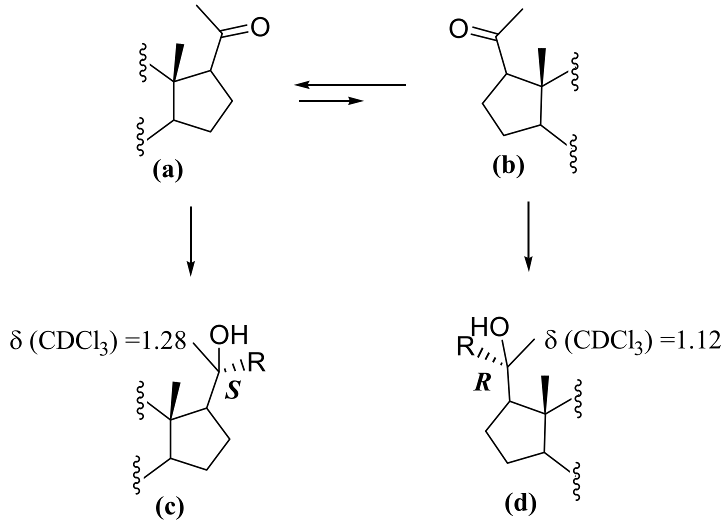
As previous described, aromatic steroid sapongenin **131** was prepared from the corresponding quinone **130**. Transformation of **131** to the keto ester **158** was achieved in a one-pot process using a modified Micovic's method.

Spiro ring degradation of **131** with acetic anhydride, ammonium chloride in pyridine at 135 ^{o}C provided pseudosapongenin acetate **203** which was subsequently oxidized with chromium trioxide, acetic acid in 1,2-dichloroethane at 0 ^{o}C to afford keto ester **158** in 38% yield after careful neutralization and rapid purification by flash column chromatography.



Scheme 28

The nucleophilic addition of Gridnard reagent to C-20 ketone group provided 20*S* (confirmed by comparison the 21-methyl proton chemical shift to the 20α-chloresterol) as the major product and 20*R* as the minor product in ratio 3 : 1 (confirmed by HPLC and ^{1}H NMR analysis). The result came from conformer **a** is present in the greater amount than **b** at the moment of reaction (Chaudhuri, 1969).



Scheme 29

As described above in scheme 27, protection of diol **159** is necessary. Protecion of phenolic alcohol of **160** as silyl enol ether gave **161** in good yield. Hydroboration of **161** followed by oxidation using hydrogen peroxide afforded **162**. Oxidation of **162** afforded intermediate ketone **163**. The intermediate **163** was proposed to be the valuable compound for the synthesis of geodisterol by olefination and deprotection of silyl and acetonide protecting group.

2. Biological activity testing

12 Compounds of C-3, C-16 and C-20 polyoxygenated steroids were tested for cytotoxicity against KB, MCF 7 and NCI. The results (table 6) indicated that cytotoxicity against cancer cell lines strongly depended on the different of degree of

unsaturation on A ring. The compounds containing *α,β*-unsaturated ketone on A ring showed strong cytotoxicity against NCI and moderate cytotoxicity against MCF 7 and KB (entry 4 and 5). While, compound with quinone on A ring showed less cytoloxicity (entry 6). Compounds possessing aromatic on A ring (entry 7 and 8) showed moderate to weak cytotoxicity against MCF 7 and KB but they are inactive against NCI except the compound containing α-hydroxy group (entry 8). However compound without side chain has no biological activity (entry 12).

It is noteworthy that compound containing hydroxyl group showed better biological activity than that containing keto group at the same position. For example, compound containing hydroxyl group at position 16 gave better cytotoxicity against NCI than that containing keto group at the same position (entry 2 and 3).

The results also showed that compound containing saturated side chain showed enhanced activity against KB and MCF7 (entry 7) whereas compound containing unsaturated side chain is inactive for all test cells.

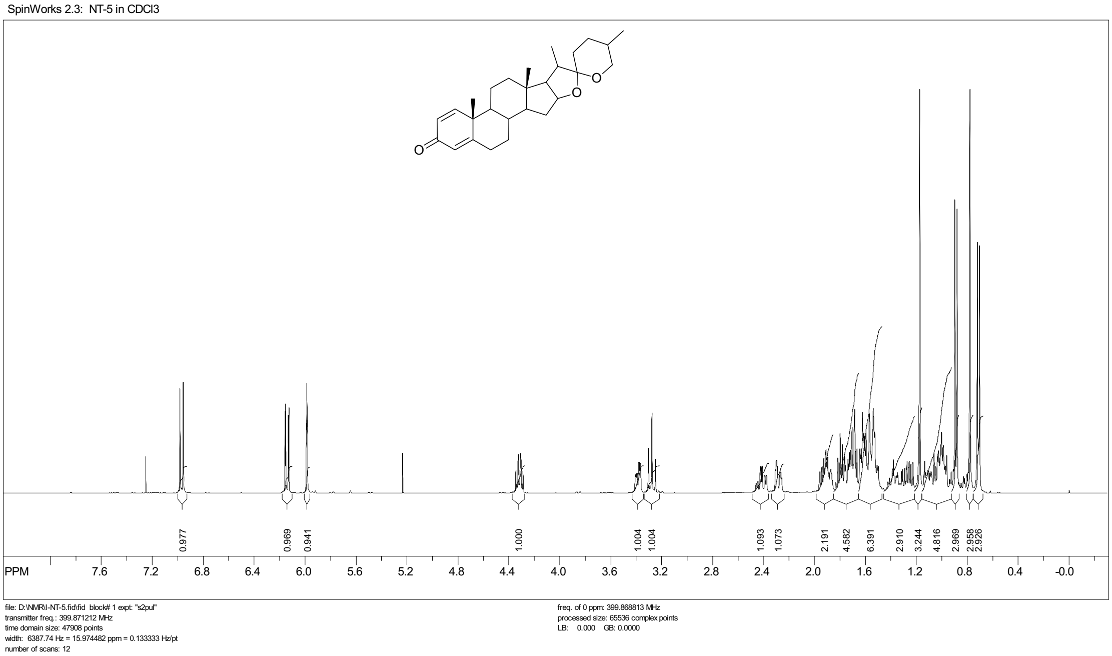
Compound with hydroxyl group at position 24 showed moderated cytotoxicity against MCF7 and KB but it is inactive against NCI (entry 10). Interestingly, compound with acetonide protecting group also improved activity against MCF7 and NCI (entry 11).

CONCLUSIONS

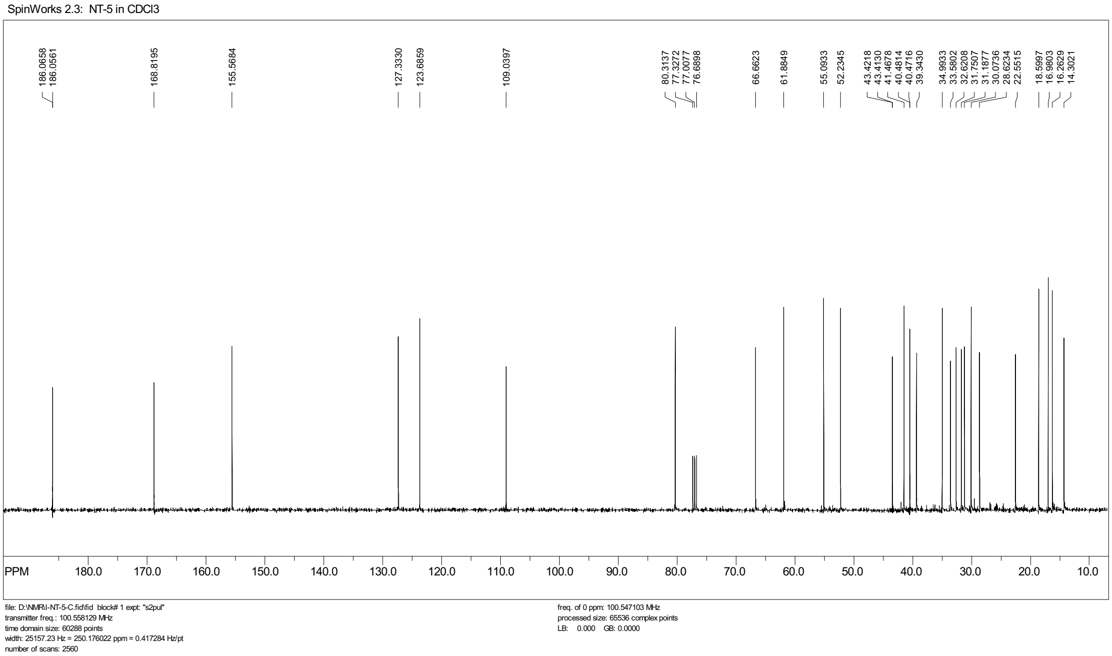
The series of C-3, C-16, C-20 polyoxygenated steroids were successfully synthesized starting from tigogenin and diosgenin. This class of compounds contained i) saturated A ring steroid, ii) 3-one steroids, iii) *α,β*-unsaturated steroids and iv) aromatic A ring steroid with cholestane or cholestene side chain.

The synthesized compounds were evaluated against human epidermoid carcinoma (KB), human breast cancer (MCF7) and Lung cancer (NCI). These initial structure-activity relationships studies have demonstrated that the different degree of unsaturation on A ring were significant in determining their biological activity. The existence of cholesterol-type side chain was also crucial for cytotoxicity activity. The presence and stereochemistry of OH at position 16 partly play the important role to the cytotoxic activity.

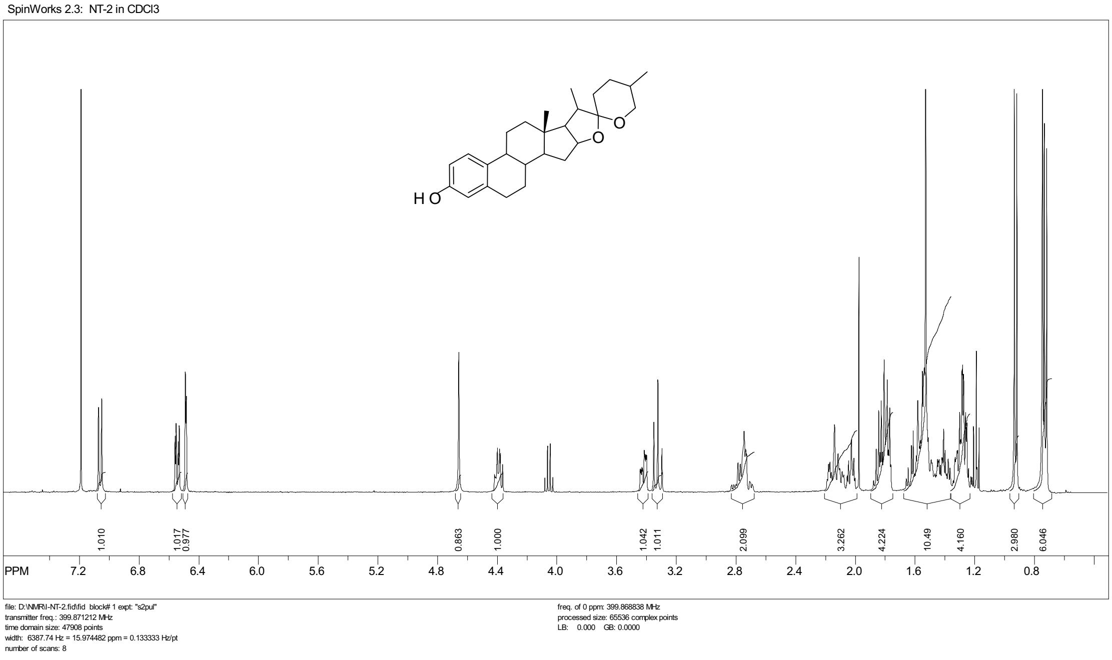
APPENDIX



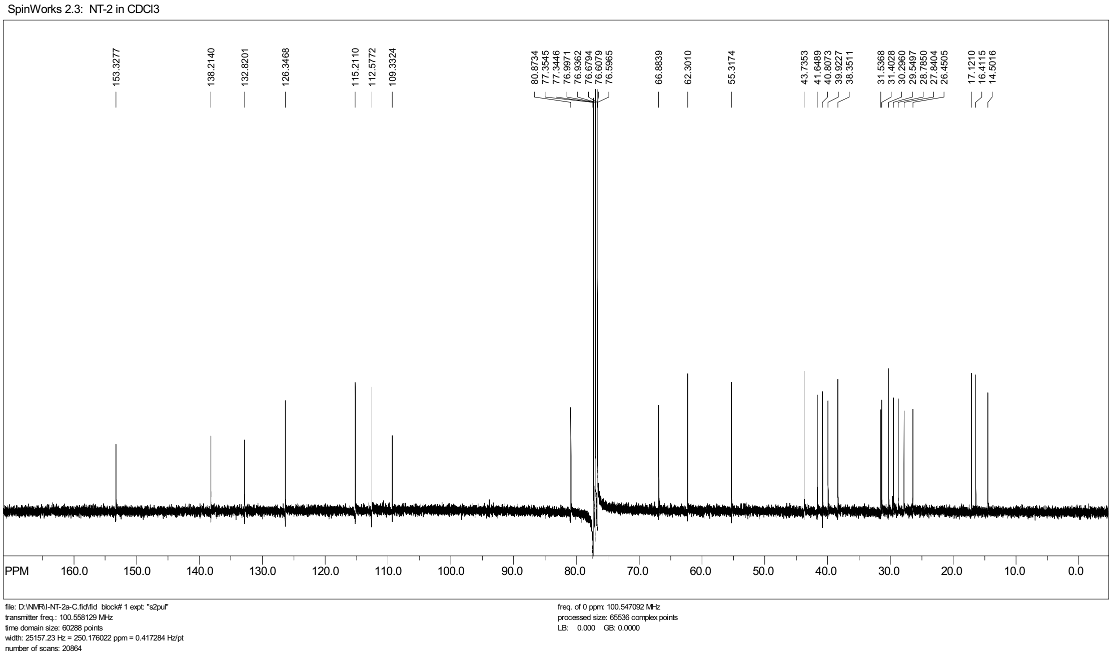
Apendix Figure 1 400 MHz ^{1}H NMR spectrum: Spirosta- $\Delta^{1,4}$-diene-3-one (130)



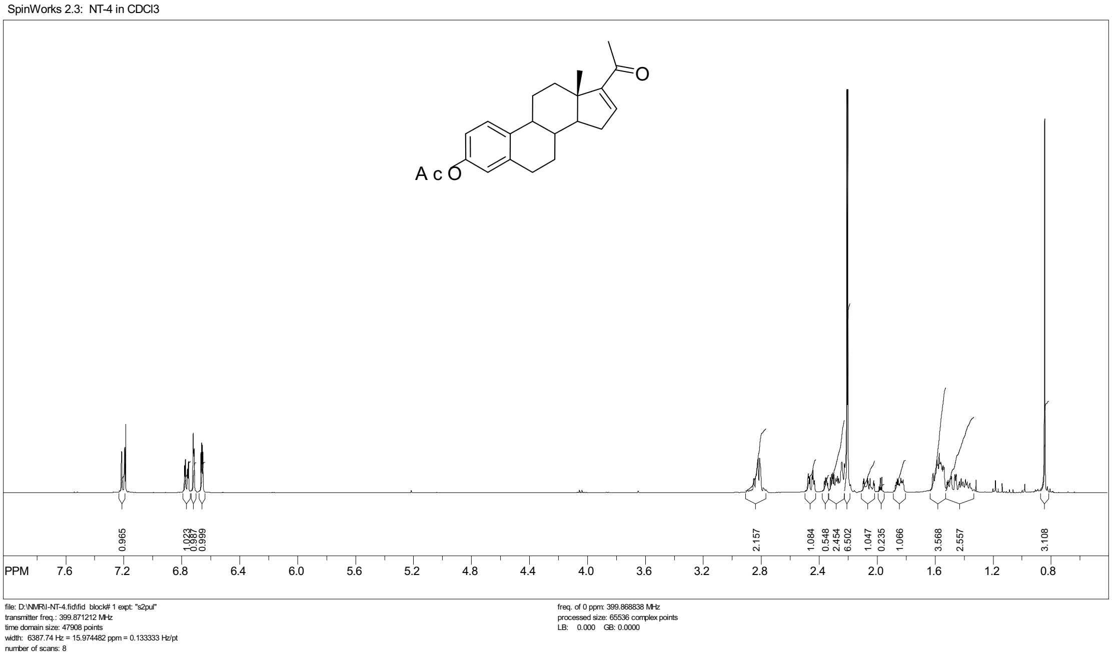
Apendix Figure 2 100 MHz ^{13}C NMR spectrum: Spirosta- $\Delta^{1,4}$-diene-3-one (130)



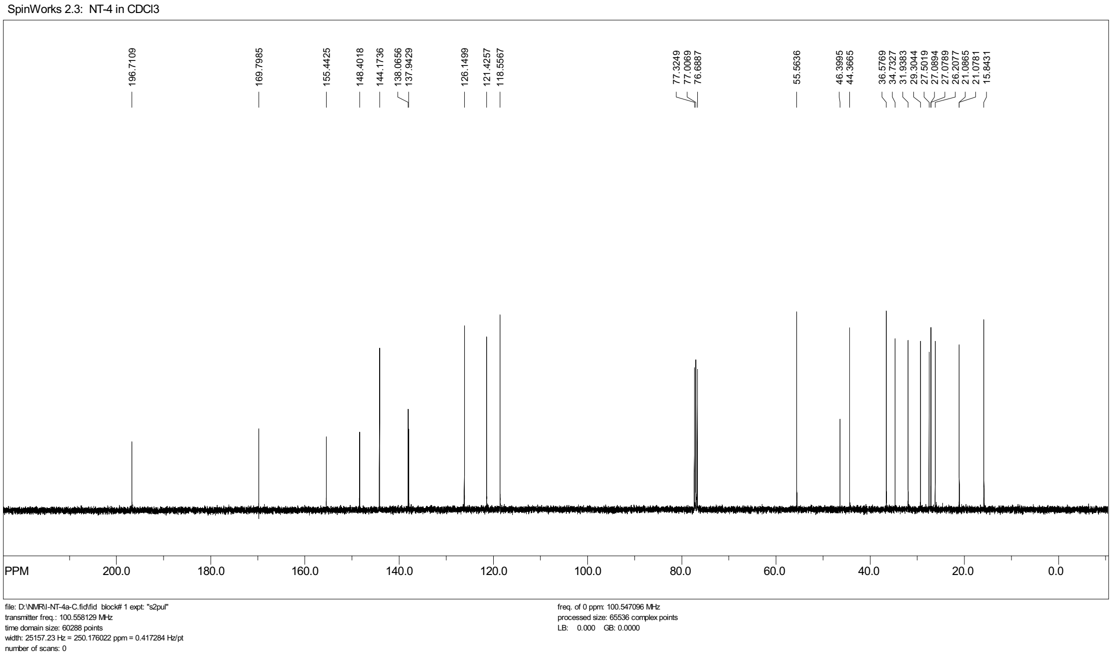
Apendix Figure 3 400 MHz ^{1}H NMR spectrum: 19-Norpregna-$\Delta^{1,3,5(10)}$statriene-3-ol (131)



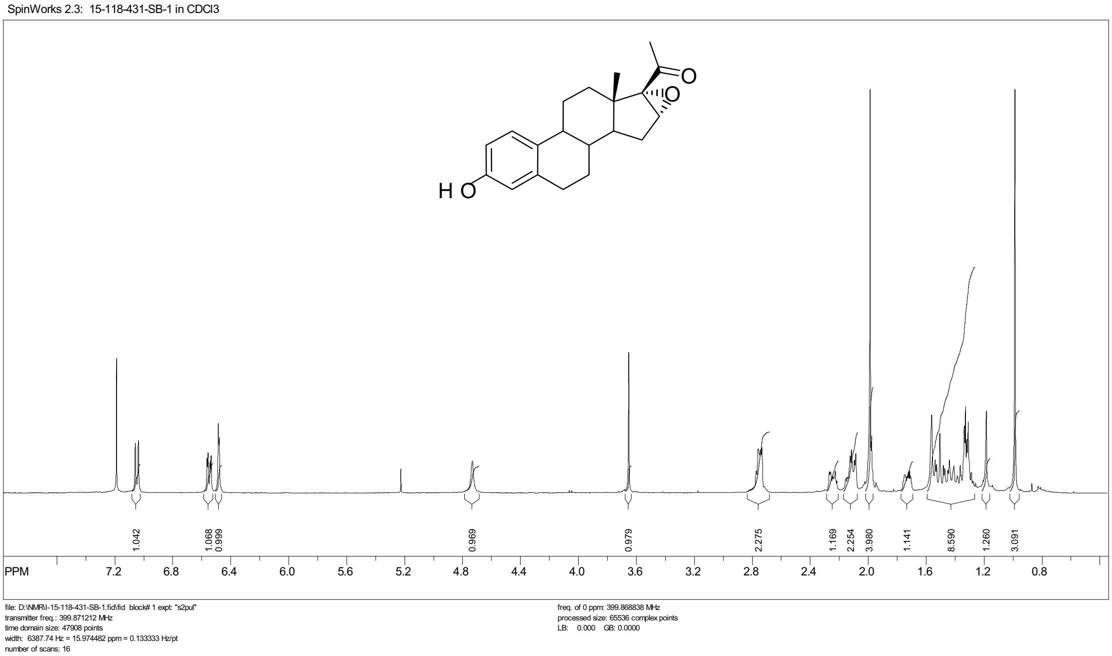
Apendix Figure 4 100 MHz ^{13}C NMR spectrum: 19-Norpregna-$\Delta^{1,3,5(10)}$statriene-3-ol (131)



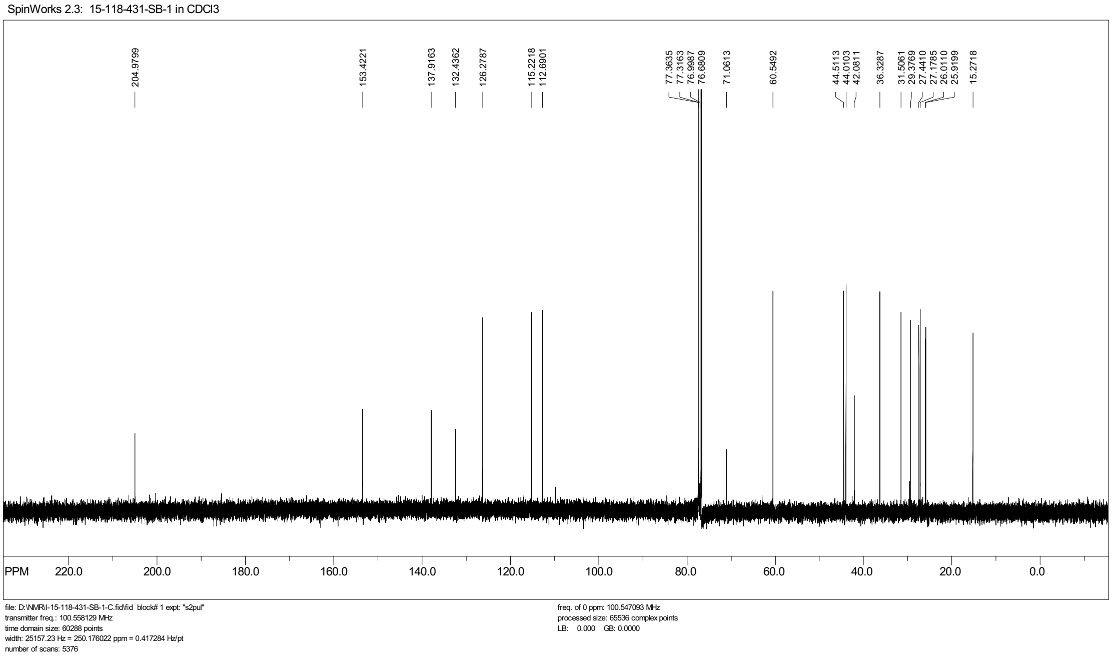
Apendix Figure 5 400 MHz ^{1}H NMR spectrum: 3, 17-acethyl-$\Delta^{1,3,5(10),16}$-estratetraene (132)



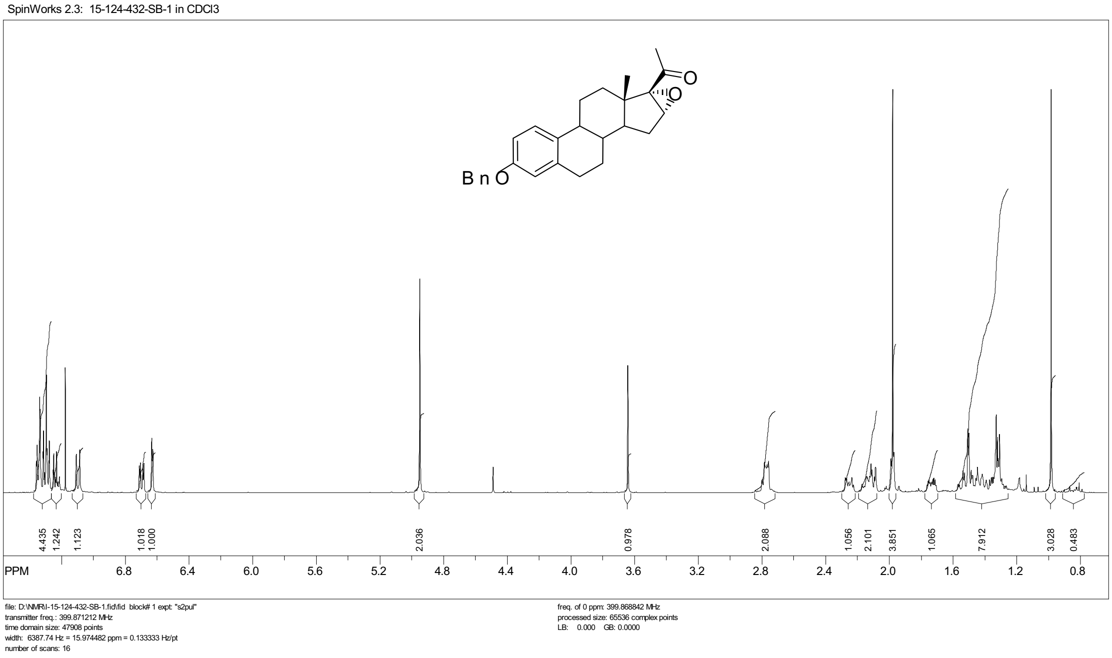
Apendix Figure 6 100 MHz ^{13}C NMR spectrum : 3, 17-acethyl-$\Delta^{1,3,5(10),16}$-estratetraene (132)



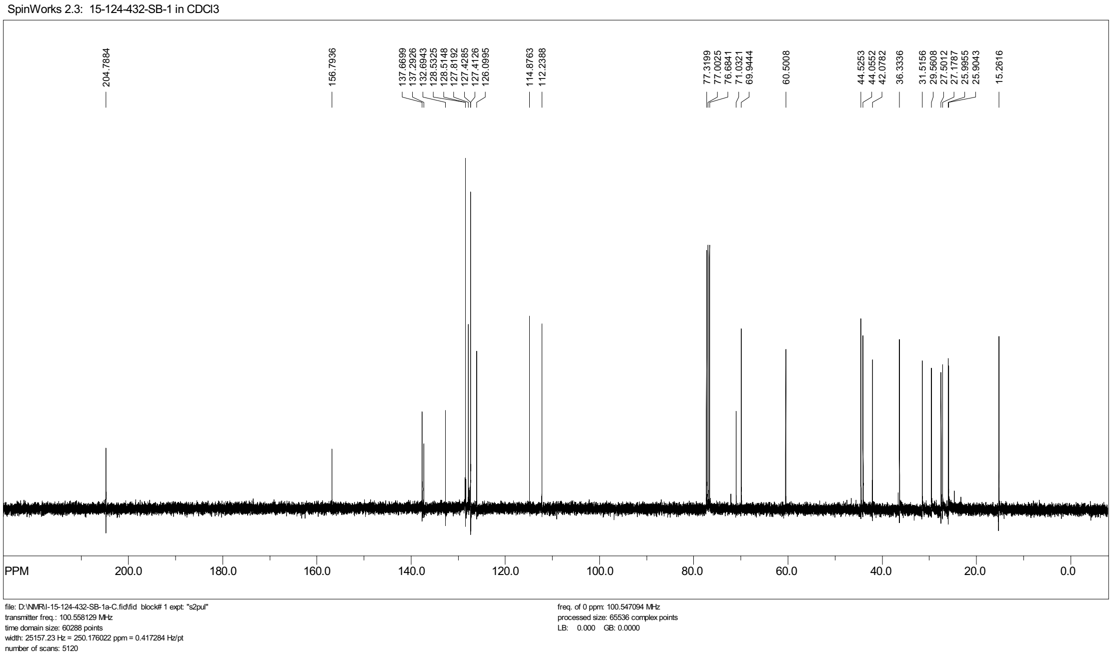
Apendix Figure 7 400 MHz ^{1}H NMR spectrum: 16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (132)



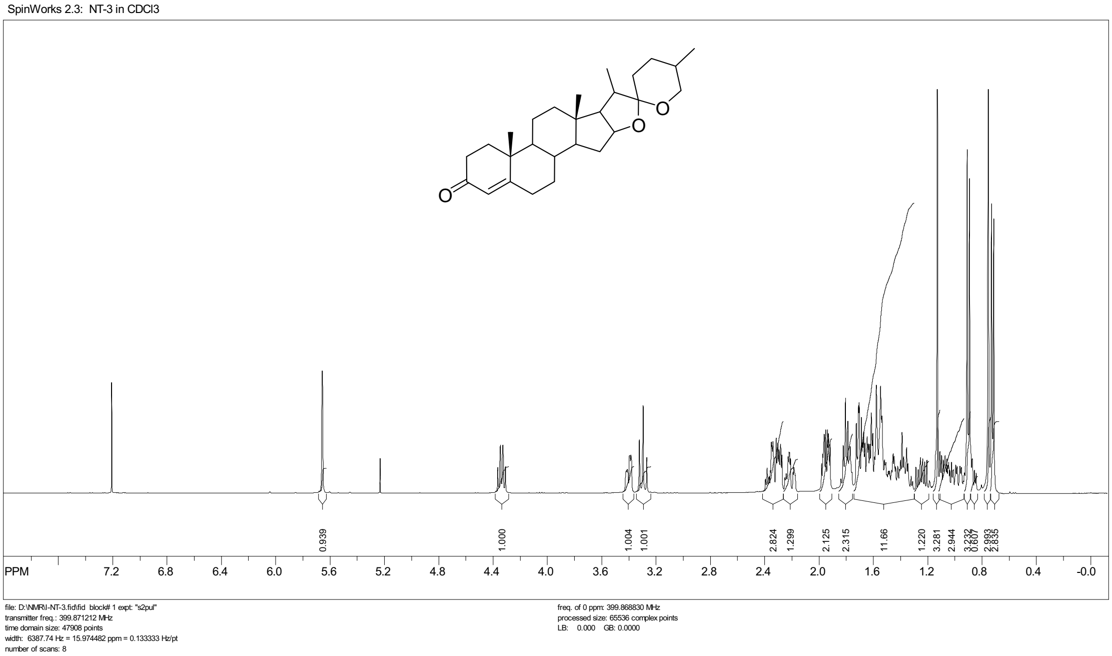
Apendix Figure 8 100 MHz ^{13}C NMR spectrum: 16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (132)



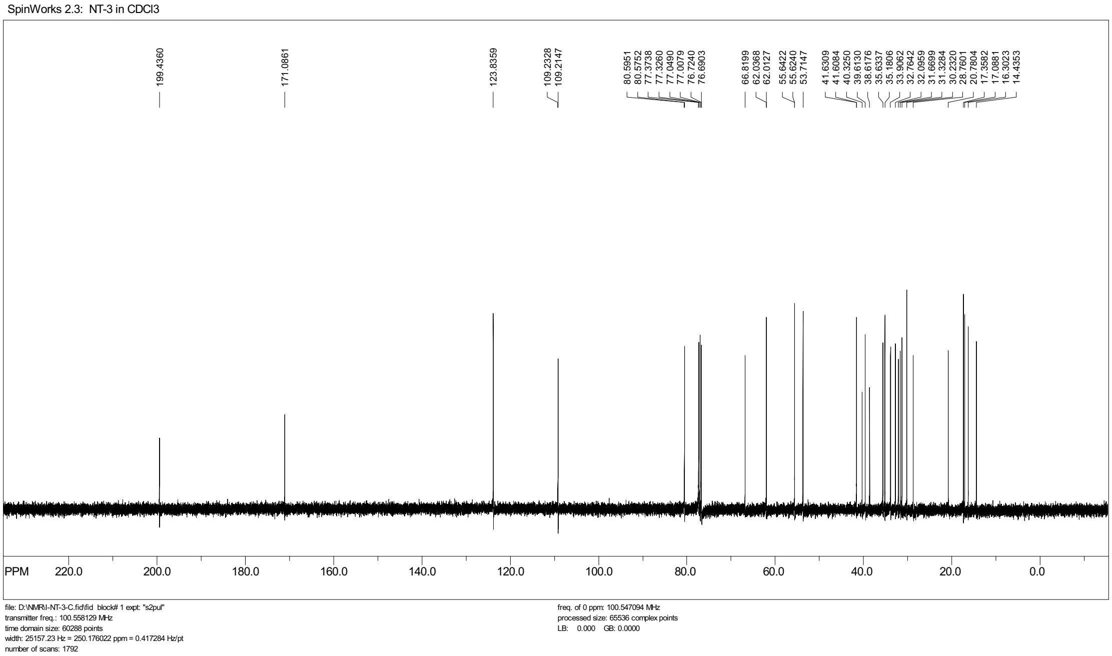
Apendix Figure 9 400 MHz ^{1}H NMR spectrum: 3-benzyloxy-16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (134)



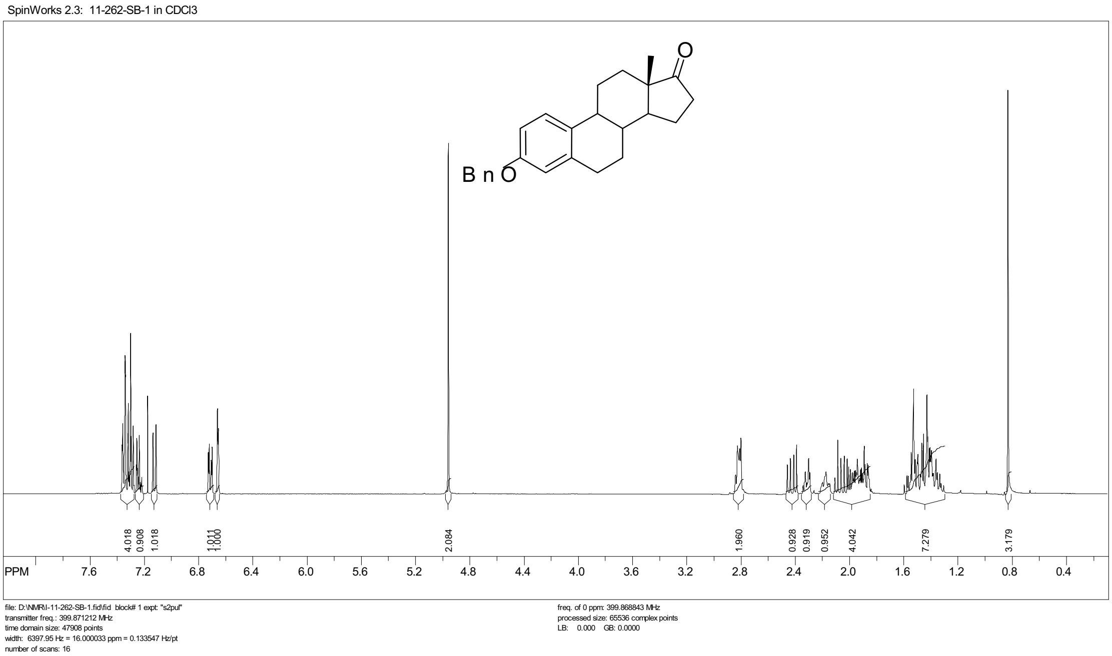
Apendix Figure 10 100 MHz ^{13}C NMR spectrum : 3-benzyloxy-16α,17α-epoxy-17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (134)



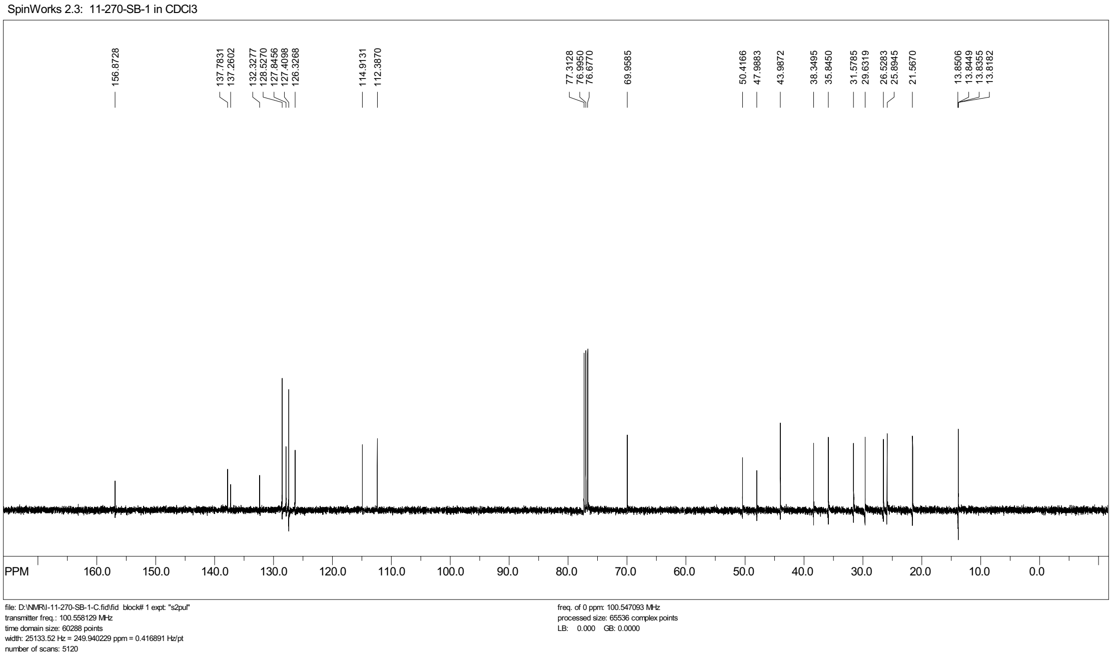
Apendix Figure 11 400 MHz ^{1}H NMR spectrum: Spirosta–4–ene-3-one (135)



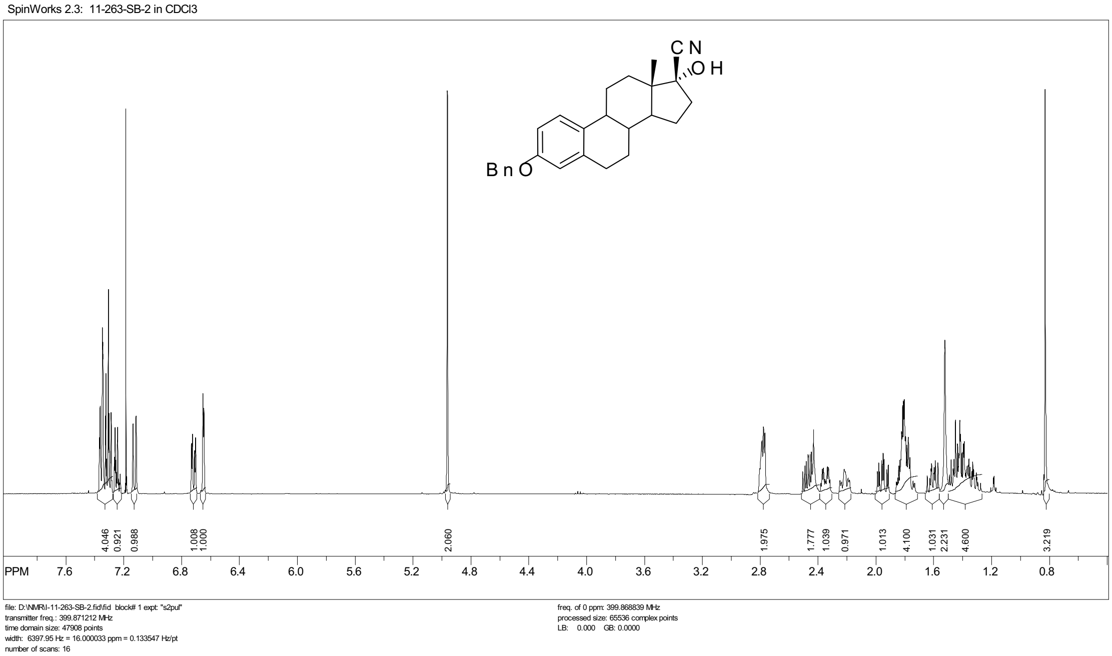
Apendix Figure 12 100 MHz ^{13}C NMR spectrum : Spirosta–4–ene-3-one (135)



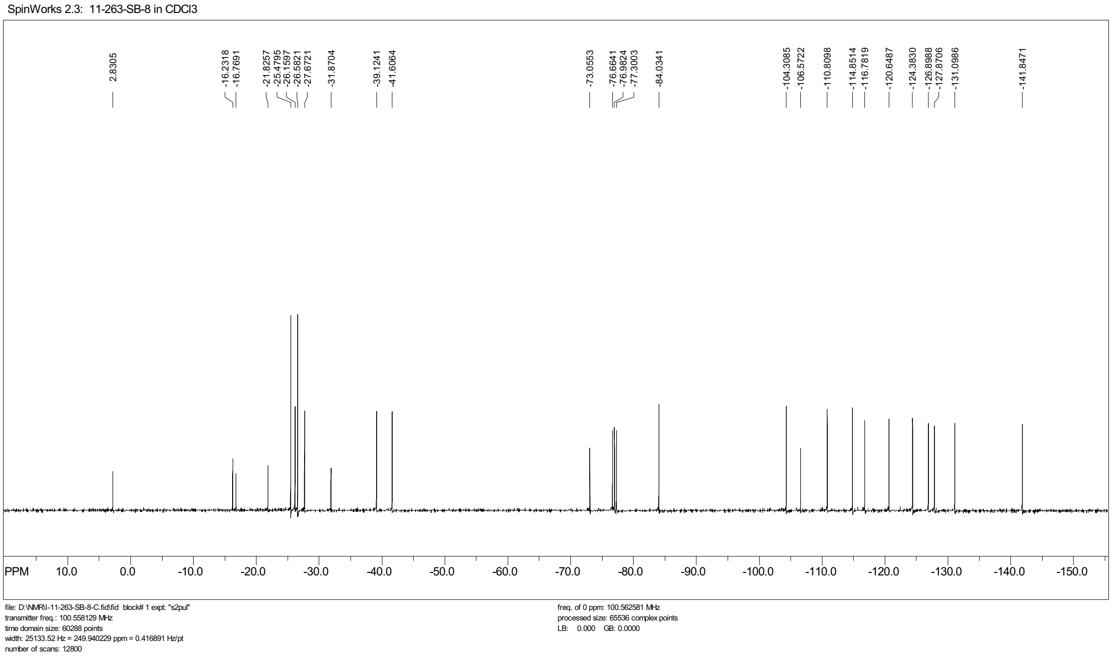
Apendix Figure 13 400 MHz ^{1}H NMR spectrum: 3-Benzyloxy-17-one-$\Delta^{1,3,5(10)}$-estratriene (136)



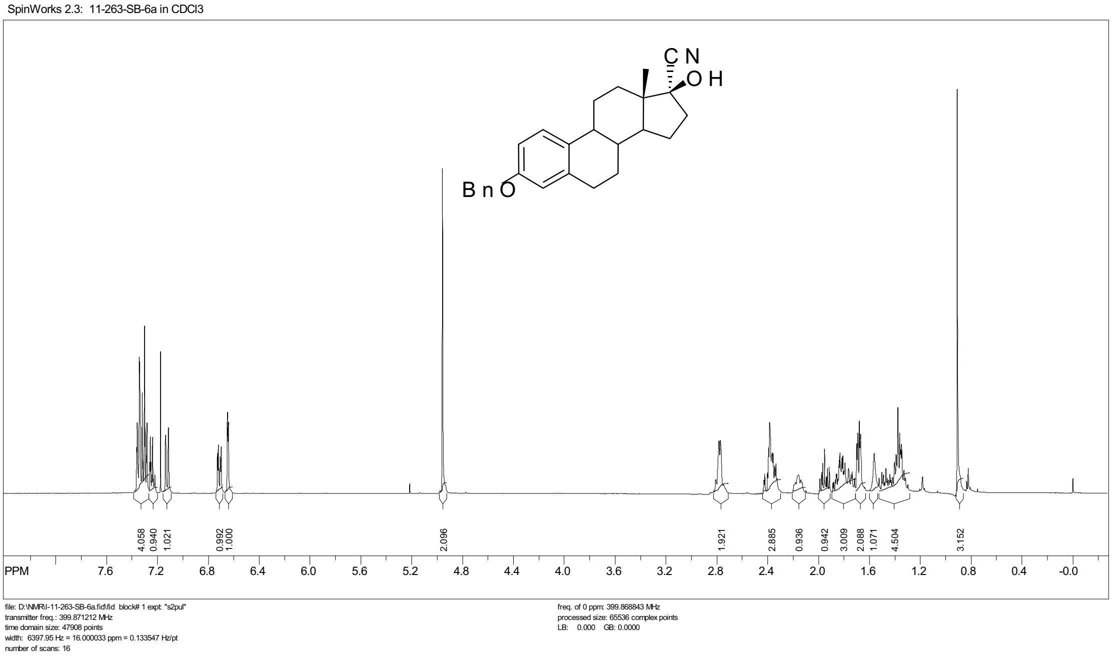
Apendix Figure 14 100 MHz ^{13}C NMR spectrum: 3-Benzyloxy-17-one-$\Delta^{1,3,5(10)}$-estratriene (136)



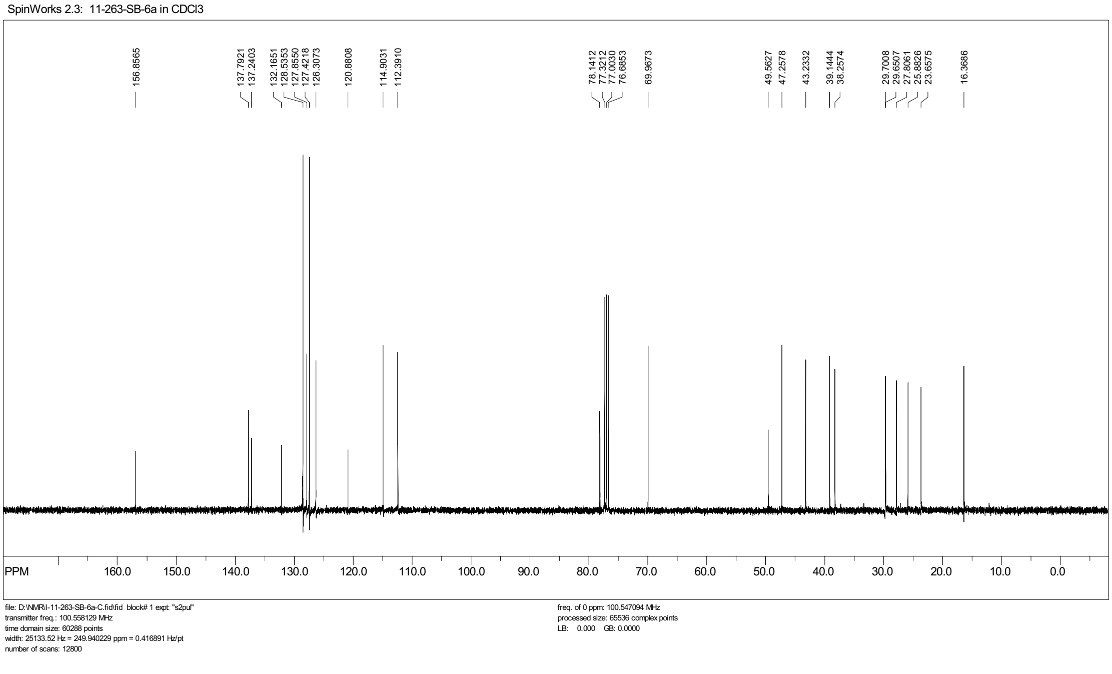
Apendix Figure 15 400 MHz ^{1}H NMR spectrum: 17α-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17β-carbonitrile (137b)



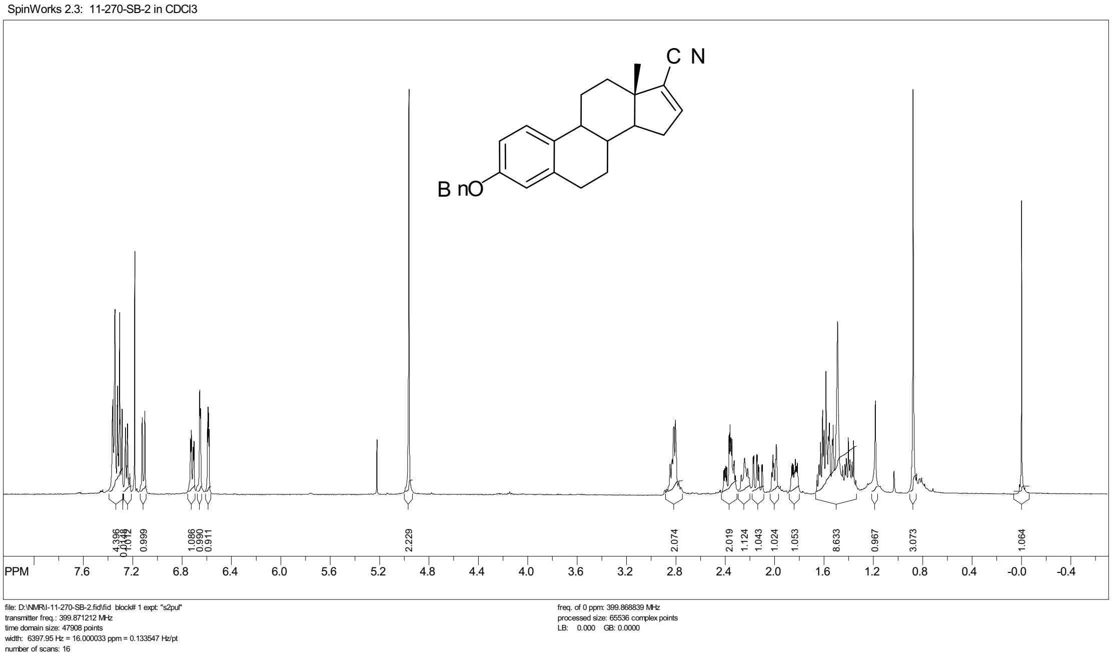
Apendix Figure 16 100 MHz ^{13}C NMR spectrum: 17α-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17β-carbonitrile (137b)



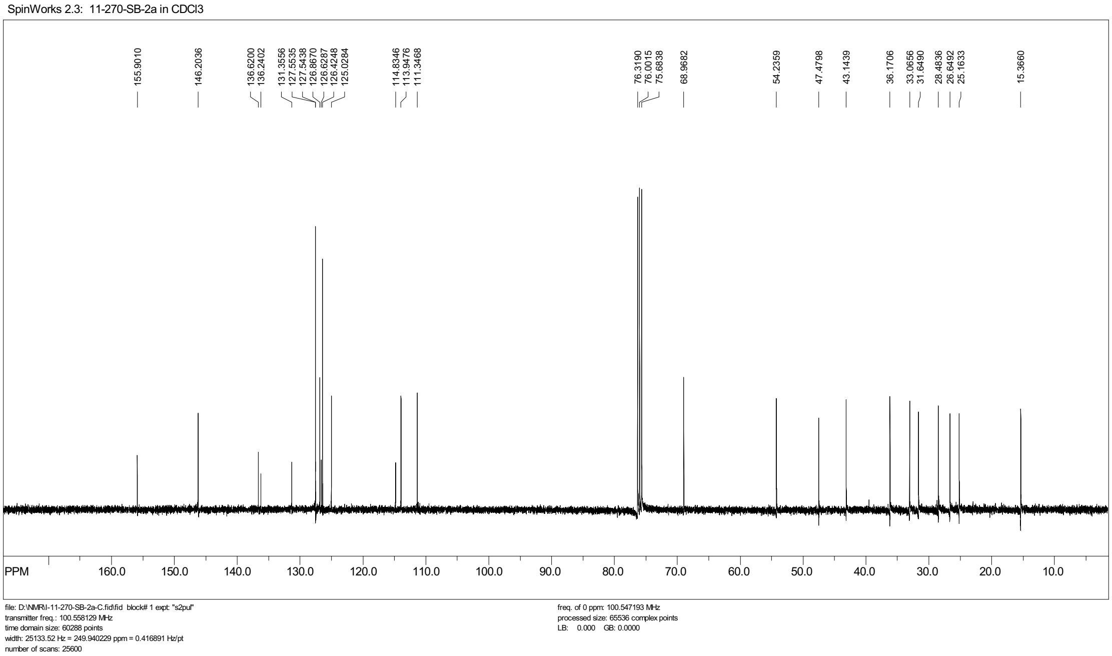
Apendix Figure 17 400 MHz ^{1}H NMR spectrum : 17β-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17α-carbonitrile (137a)



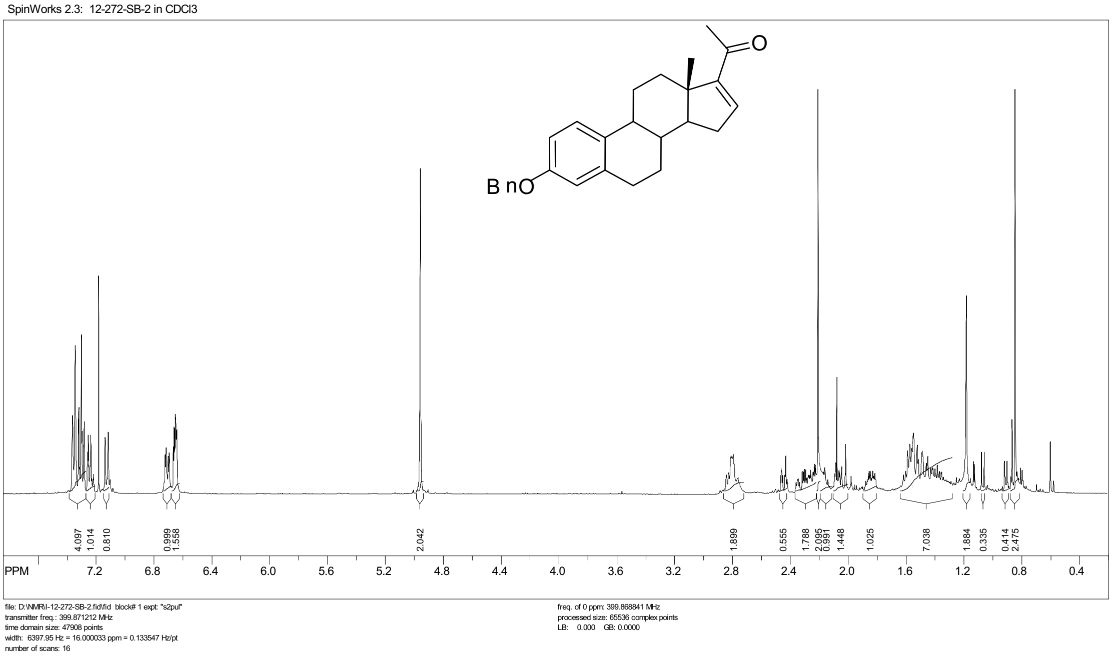
Apendix Figure 18 100 MHz ^{13}C NMR spectrum : 17β-hydoxy-3-benzyloxyestra-1,3,5(10)-triene-17α-carbonitrile (137a)



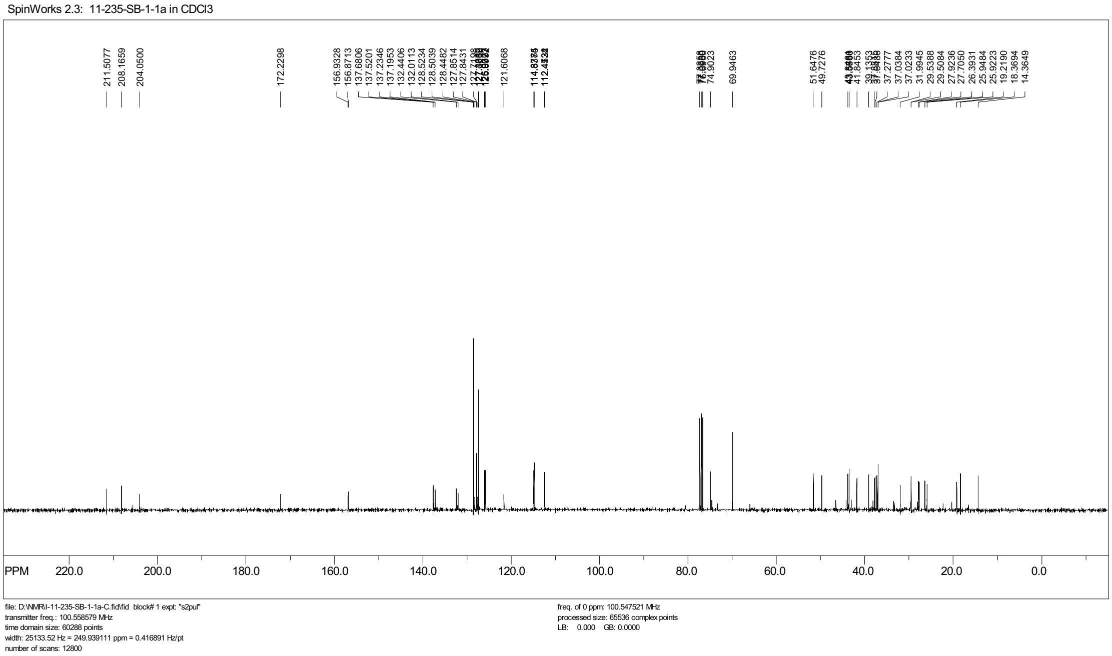
Apendix Figure 19 400 MHz ^{1}H NMR spectrum: 3-Benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138)



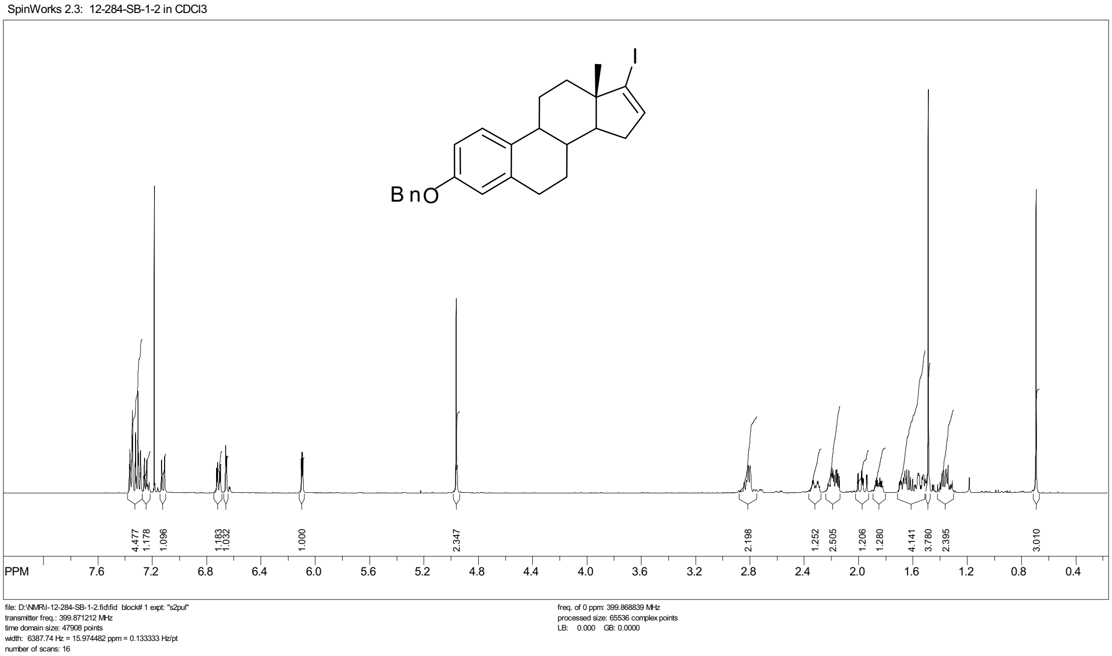
Apendix Figure 20 100 MHz ^{13}C NMR spectrum: 3-Benzyloxyestra-1,3,5(10), 16-tetraene-17-carbonitrile (138)



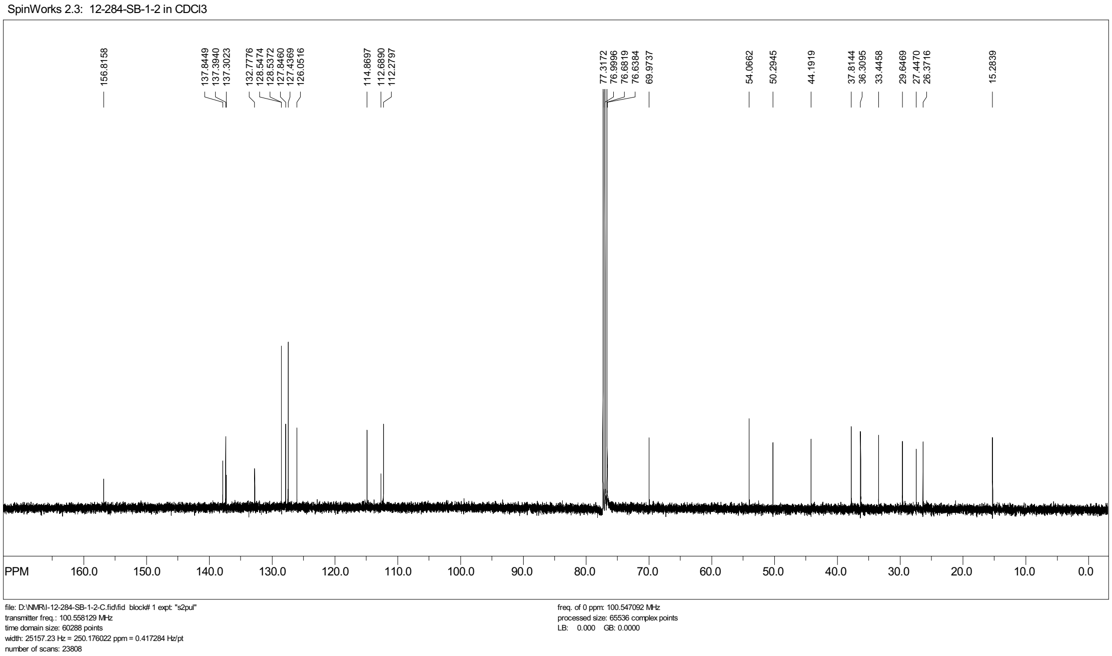
Apendix Figure 21 400 MHz ^{1}H NMR spectrum: 17-acetyl 3-benzyloxyestra - 1,3,5(10), 16-tetraene (139)



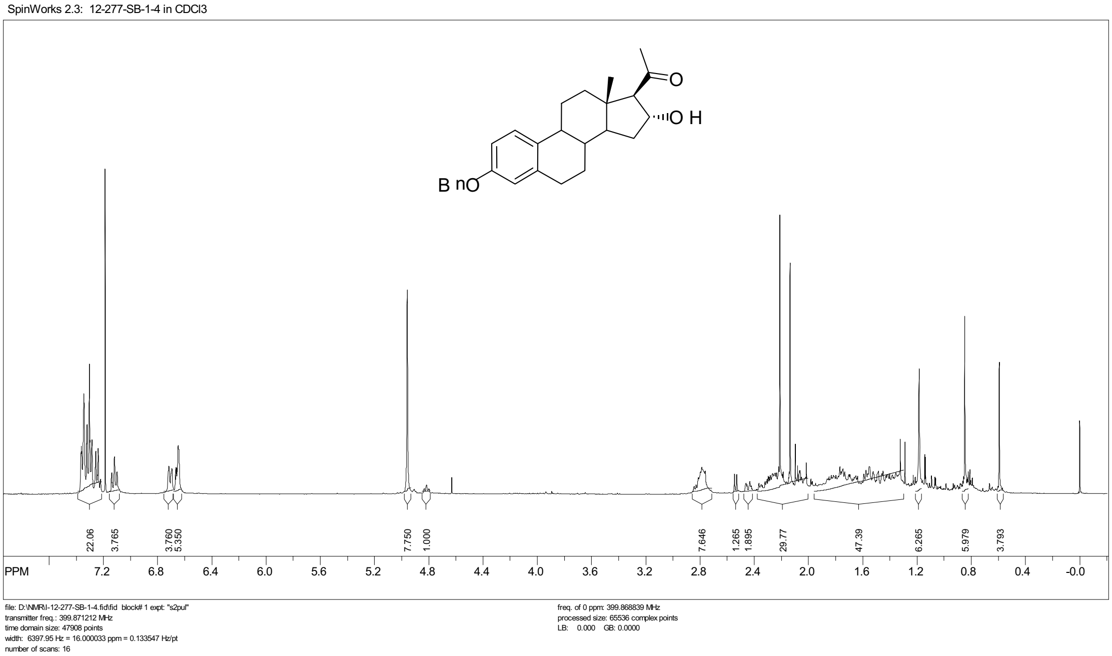
Apendix Figure 22 100 MHz ^{13}C NMR spectrum: 17-acetyl 3-benzyloxyestra - 1,3,5(10), 16-tetraene (139)



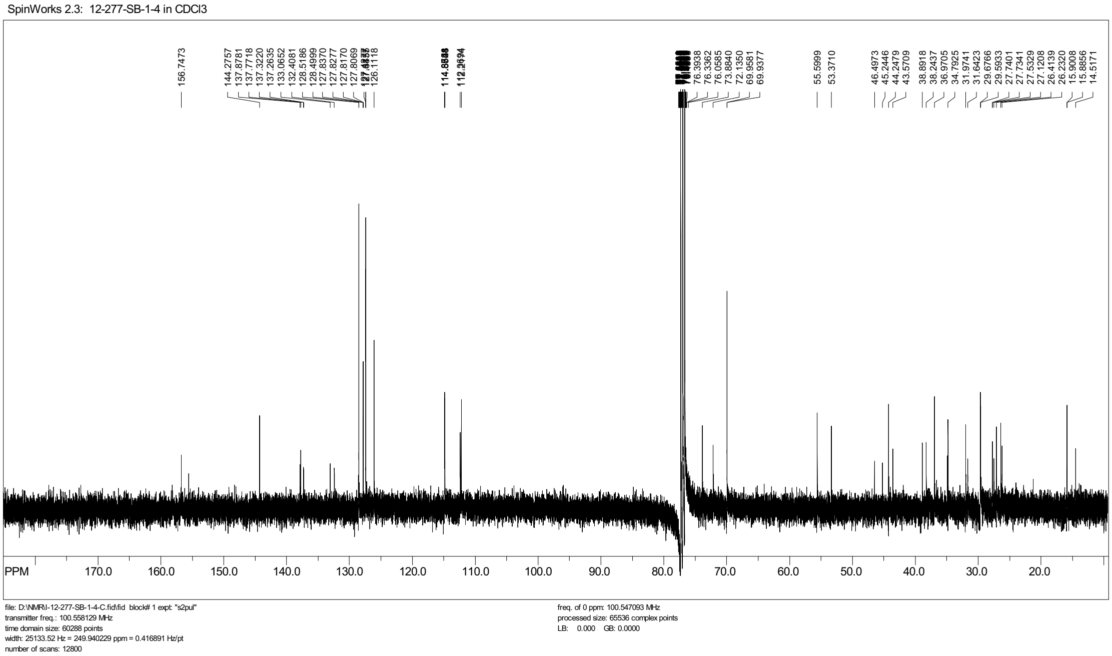
Apendix Figure 23 400 MHz ^{1}H NMR spectrum : 17-iodo-3-benzyloxyestra-1,3,5(10),16- tetraene (141)



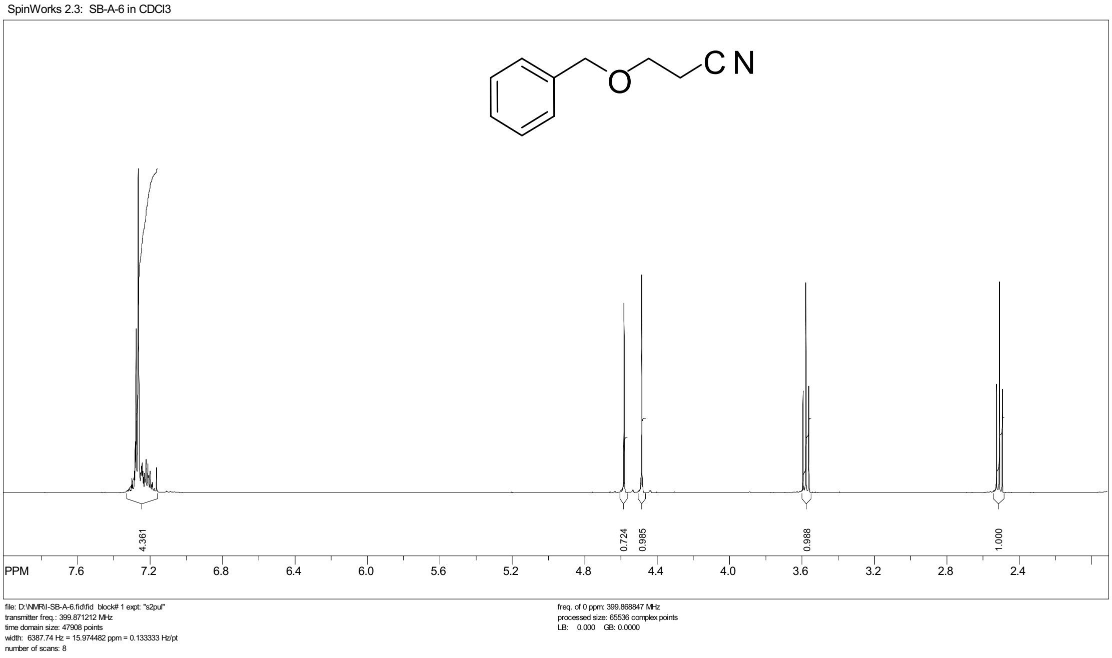
Apendix Figure 24 100 MHz ^{13}C NMR spectrum : 17-iodo-3-benzyloxyestra-1,3,5(10),16- tetraene (141)



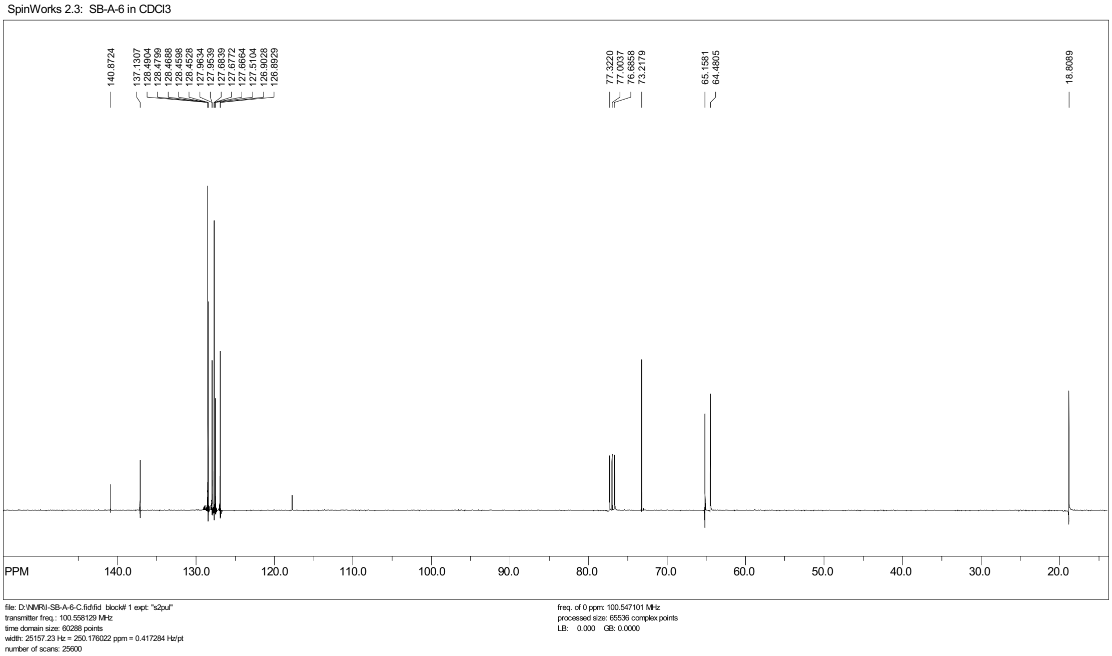
Apendix Figure 25 400 MHz ^{1}H NMR spectrum: 3-benzyloxy- 16α-hydroxy -17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (142)



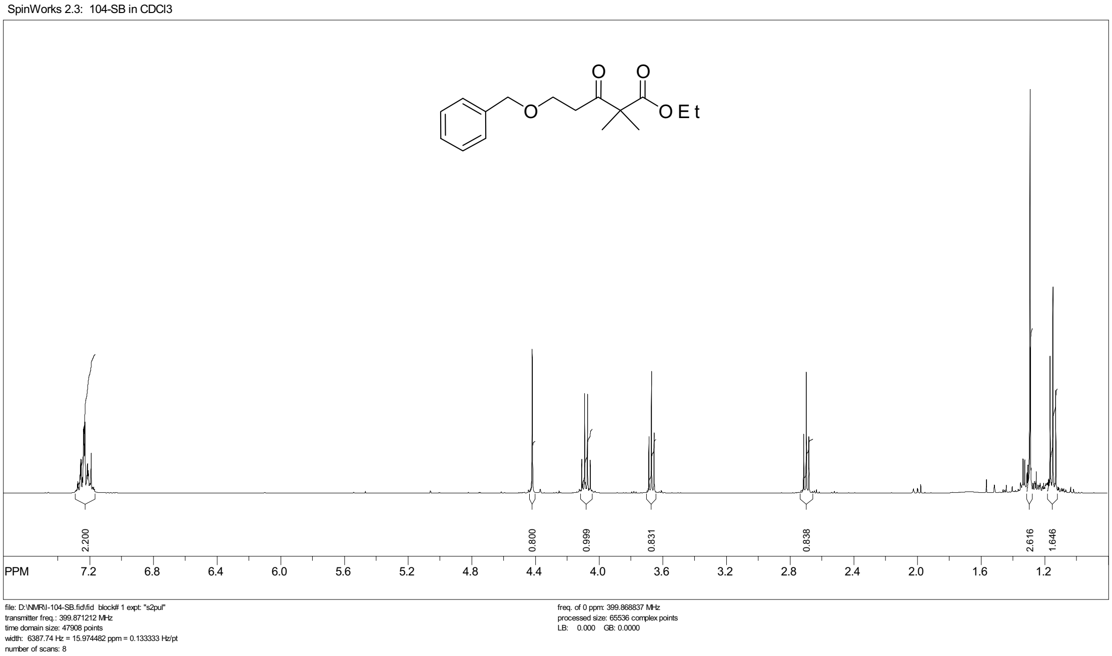
Apendix Figure 26 100 MHz ^{13}C NMR spectrum: 3-benzyloxy- 16α-hydroxy -17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (142)



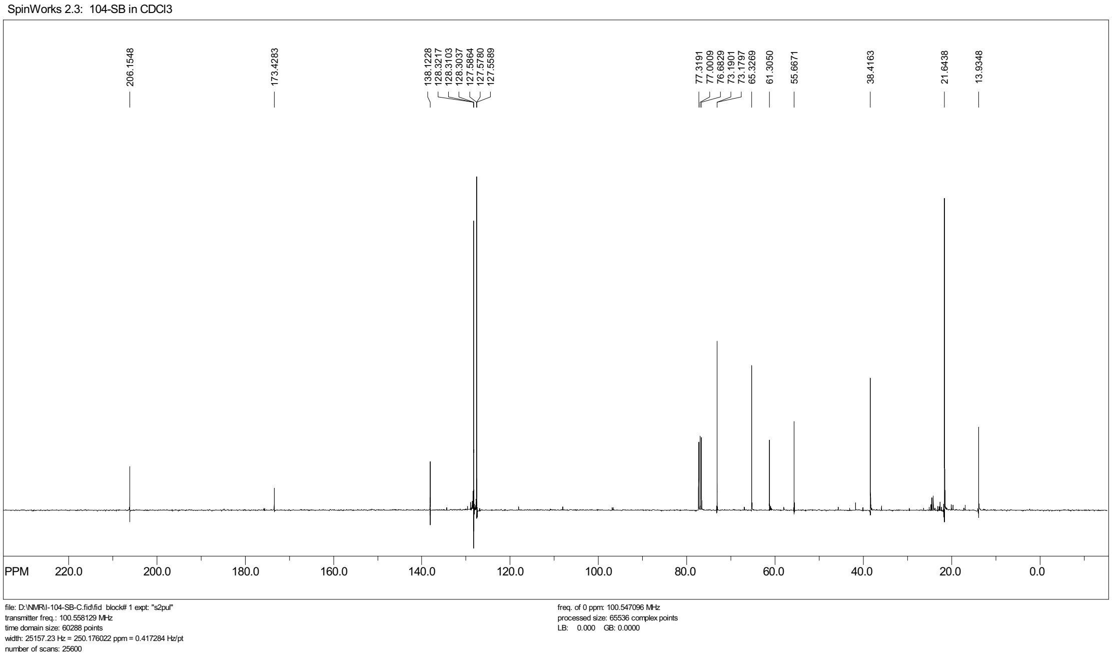
Apendix Figure 27 400 MHz ^{1}H NMR spectrum: 3–benzyloxy propionitrile (143)



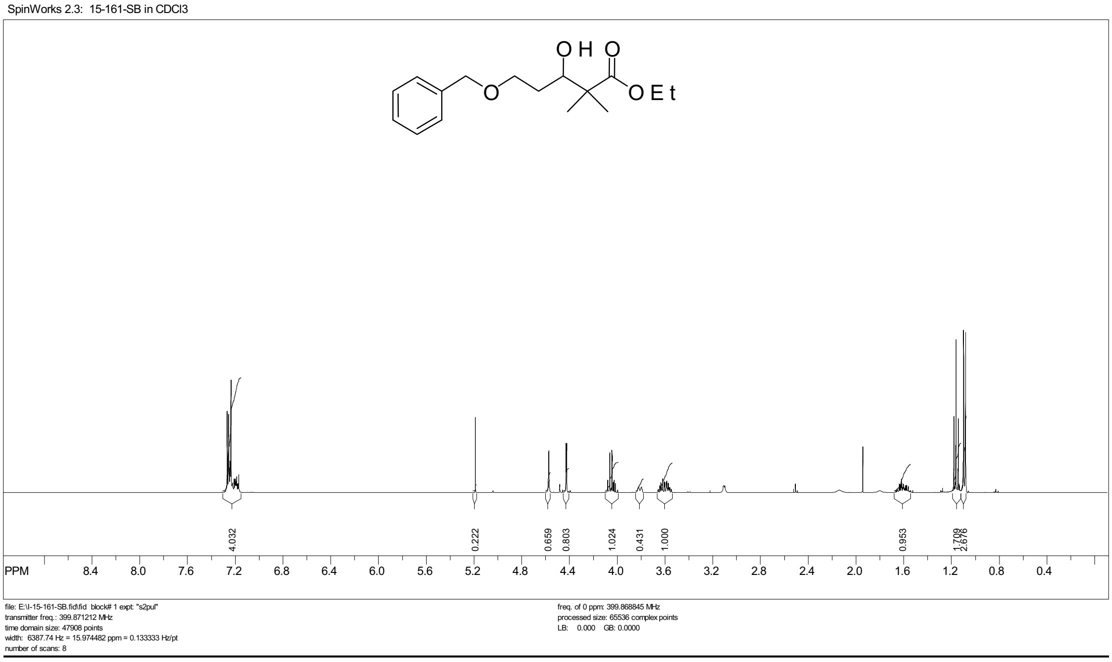
Apendix Figure 28 100 MHz ^{13}C NMR spectrum: 3–benzyloxy propionitrile (143)



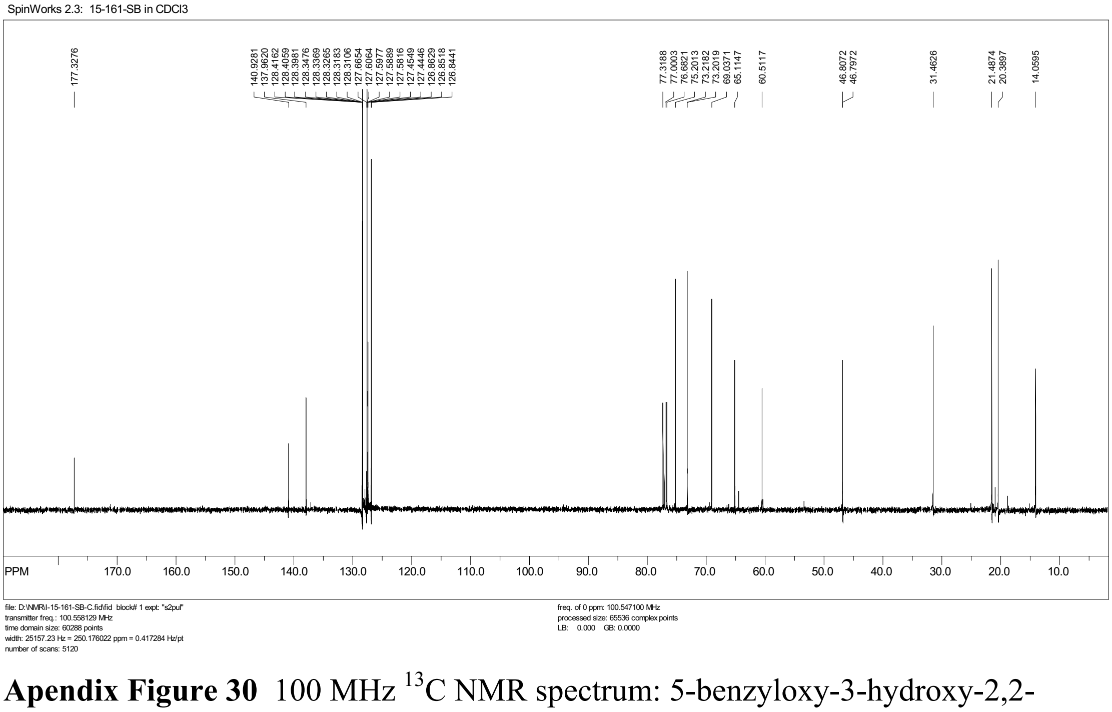
Apendix Figure 29 400 MHz ^{1}H NMR spectrum: 5-benzyloxy-2,2-dimethyl-3-oxo-pentanoic acid ethyl ester (145)



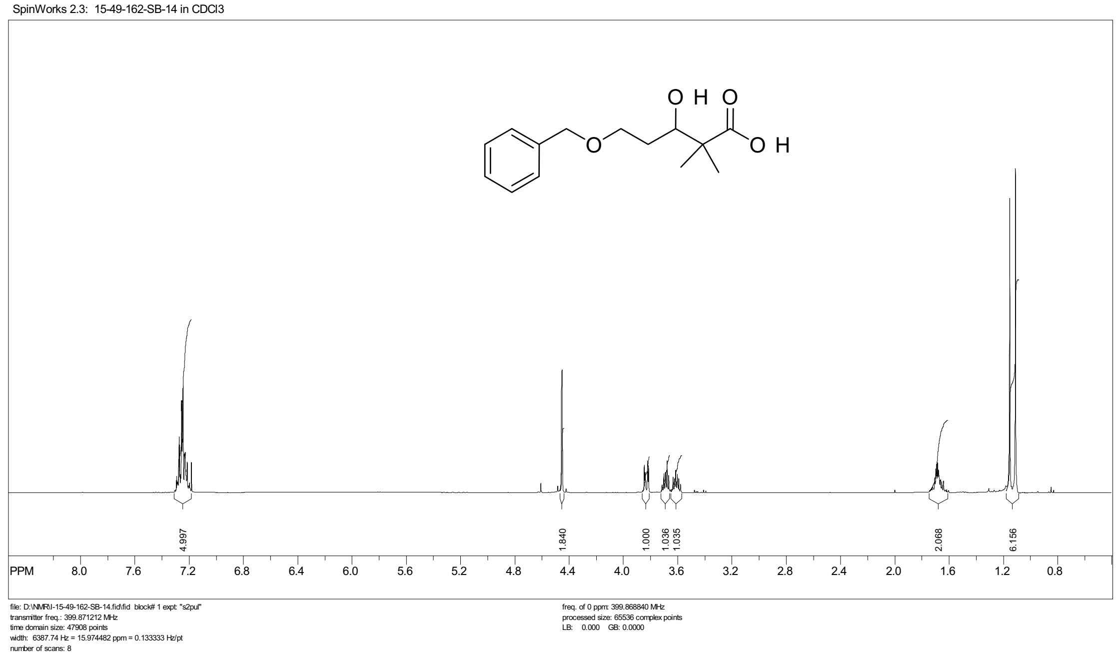
Apendix Figure 30 100 MHz ^{13}C NMR spectrum : 5-benzyloxy-2,2-dimethyl-3-oxo-pentanoic acid ethyl ester (145)



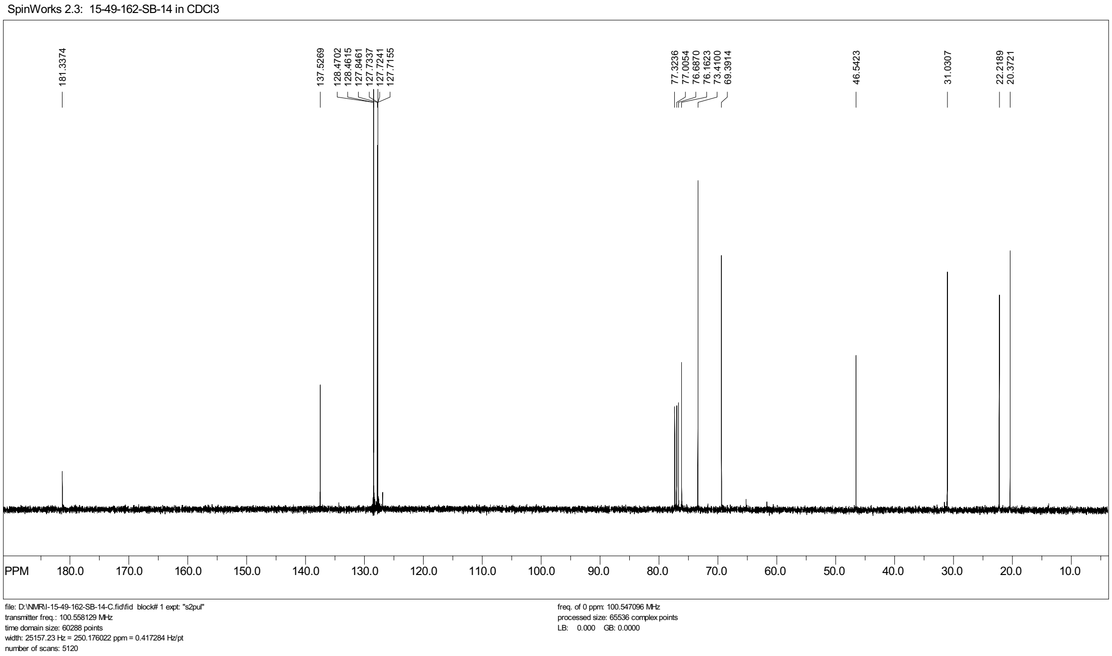
Apendix Figure 31 400 MHz ^{1}H NMR spectrum: 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid ethyl ester (146)



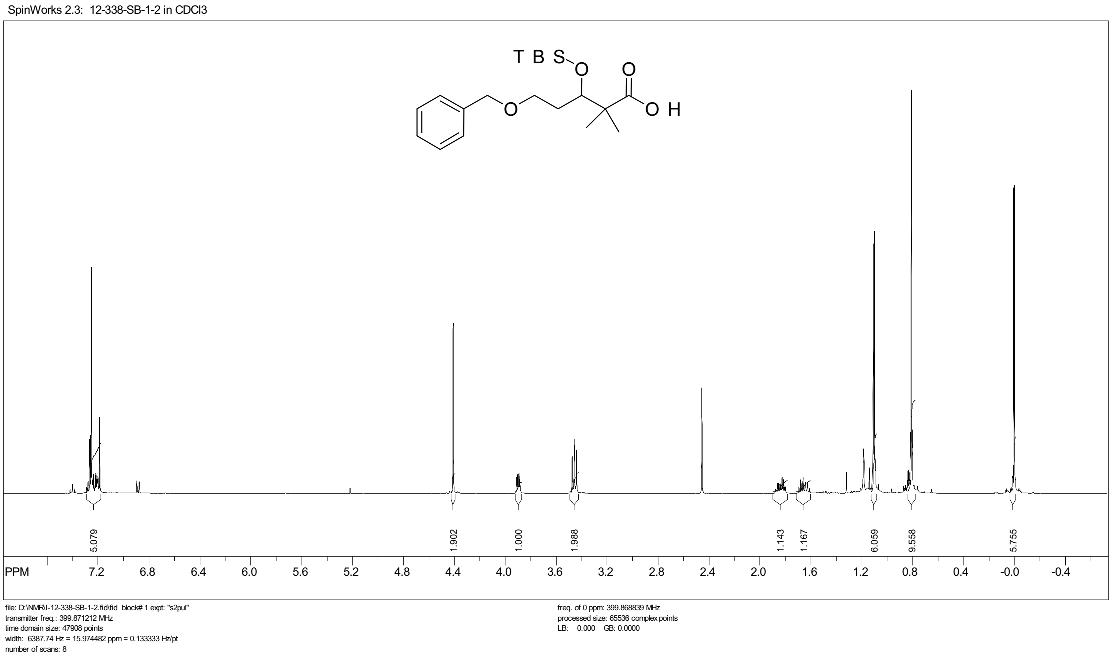
Apendix Figure 30 100 MHz ^{13}C NMR spectrum: 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid ethyl ester (146)



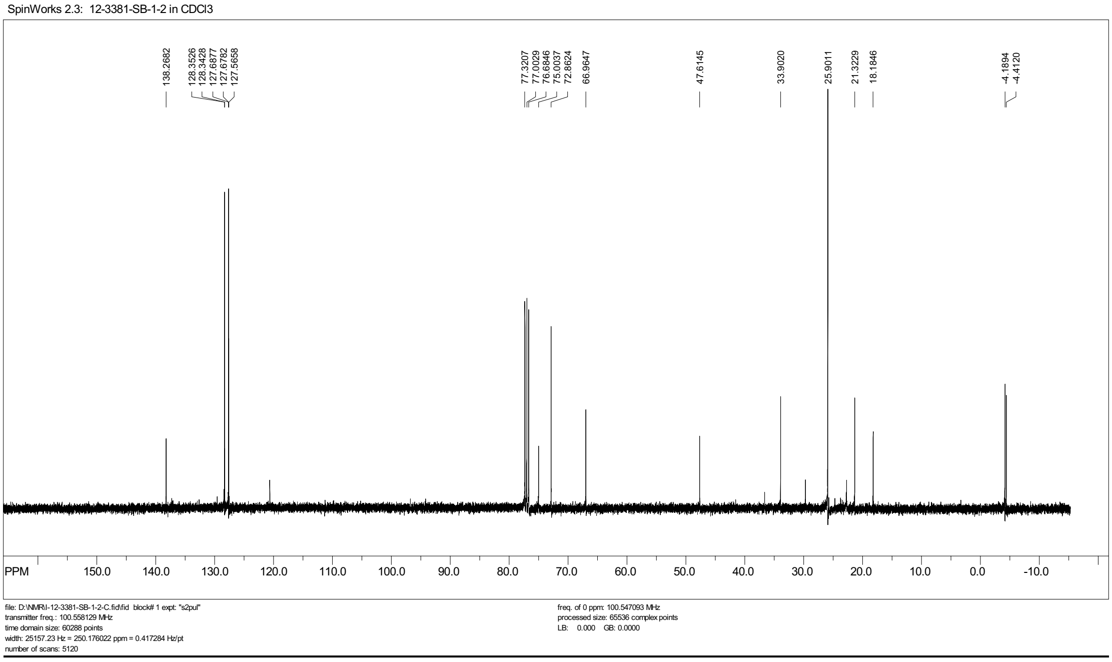
Apendix Figure 33 400 MHz ^{1}H NMR spectrum: 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid (26)



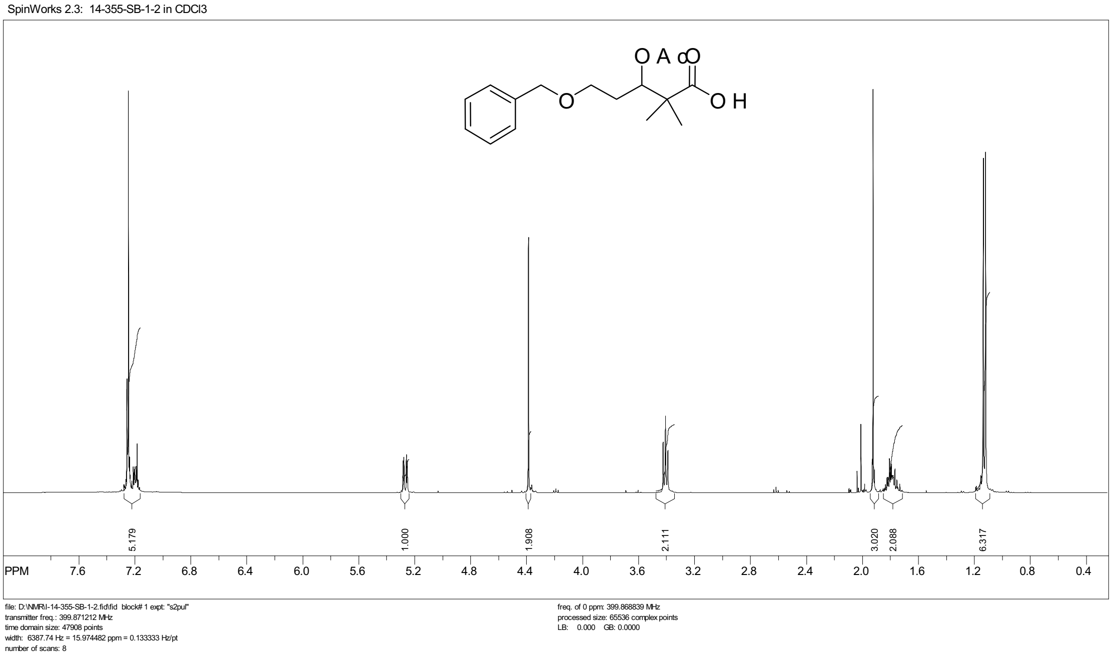
Apendix Figure 34 100 MHz ^{13}C NMR spectrum : 5-benzyloxy-3-hydroxy-2,2-dimethyl-pentanoic acid (26)



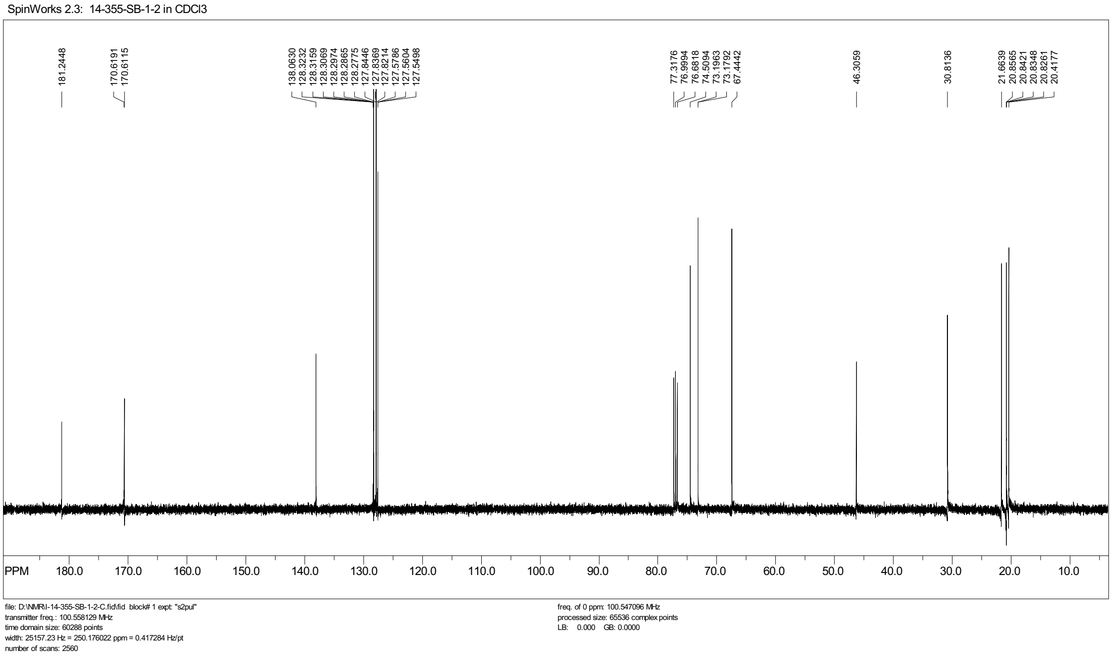
Apendix Figure 33 400 MHz ^{1}H NMR spectrum: 5-benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2,2-dimethyl-pentanoic acid (148)



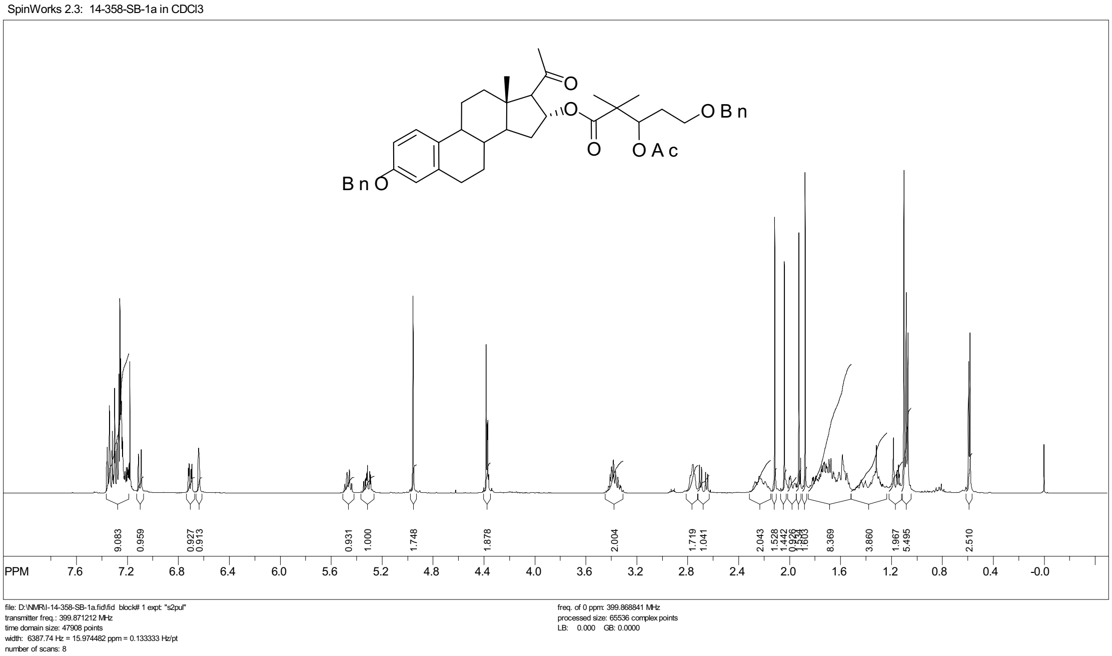
Apendix Figure 34 100 MHz ^{13}C NMR spectrum: 5-benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2,2-dimethyl-pentanoic acid (148)



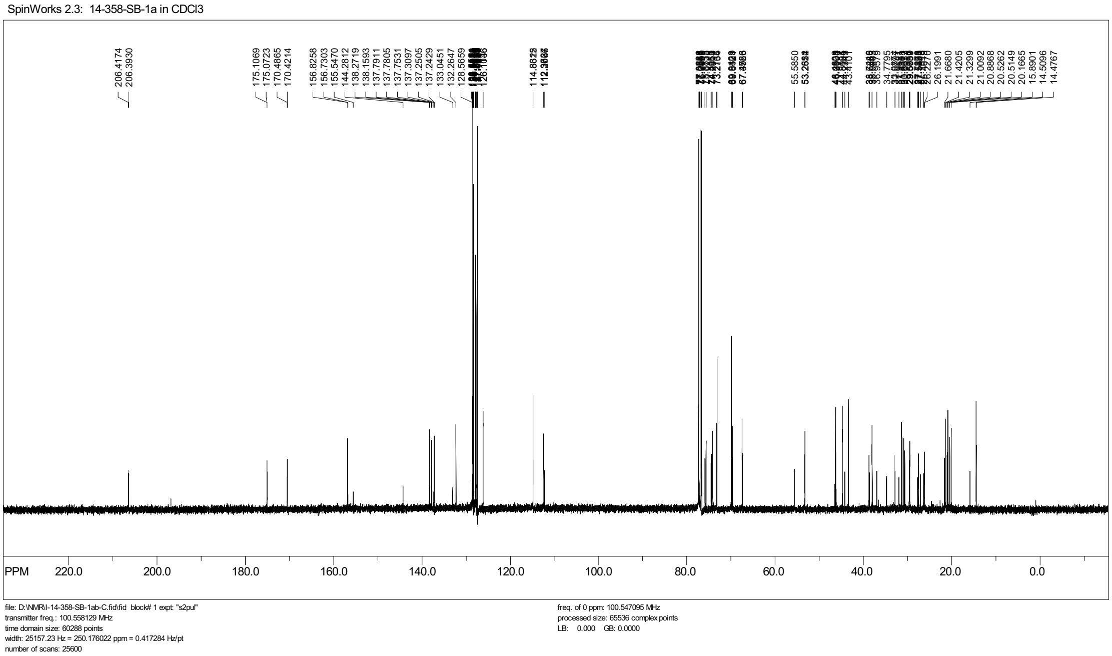
Apendix Figure 37 400 MHz ^{1}H NMR spectrum: 5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149)



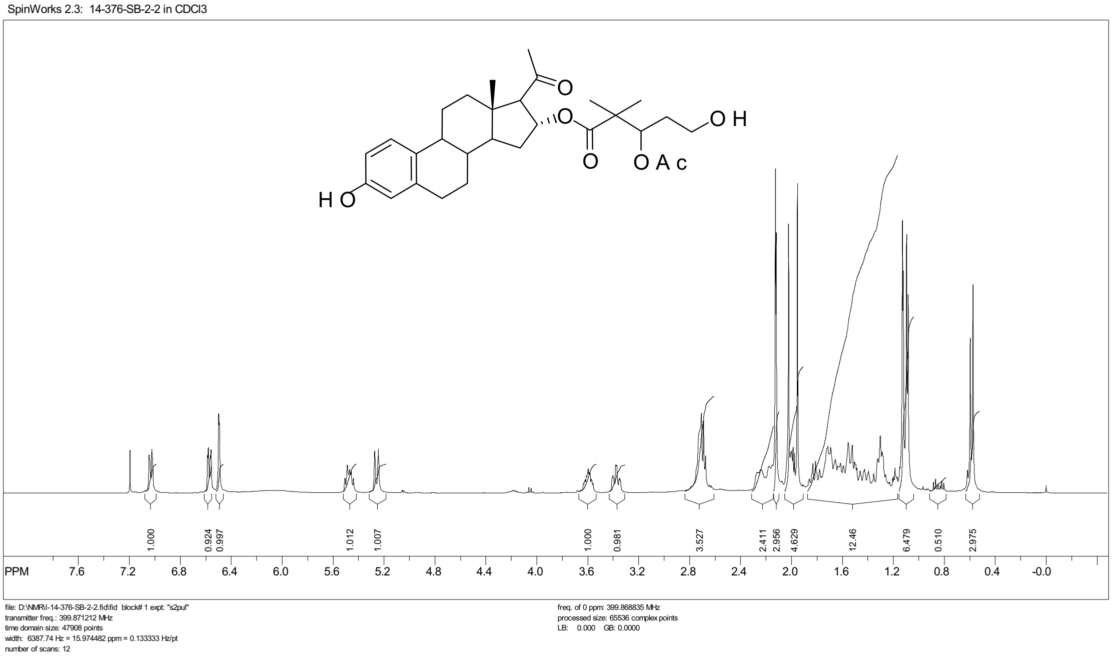
Apendix Figure 38 100 MHz ^{13}C NMR spectrum: 5-benzyloxy-3acetoxy-2,2-dimethyl-pentanoic acid (149)



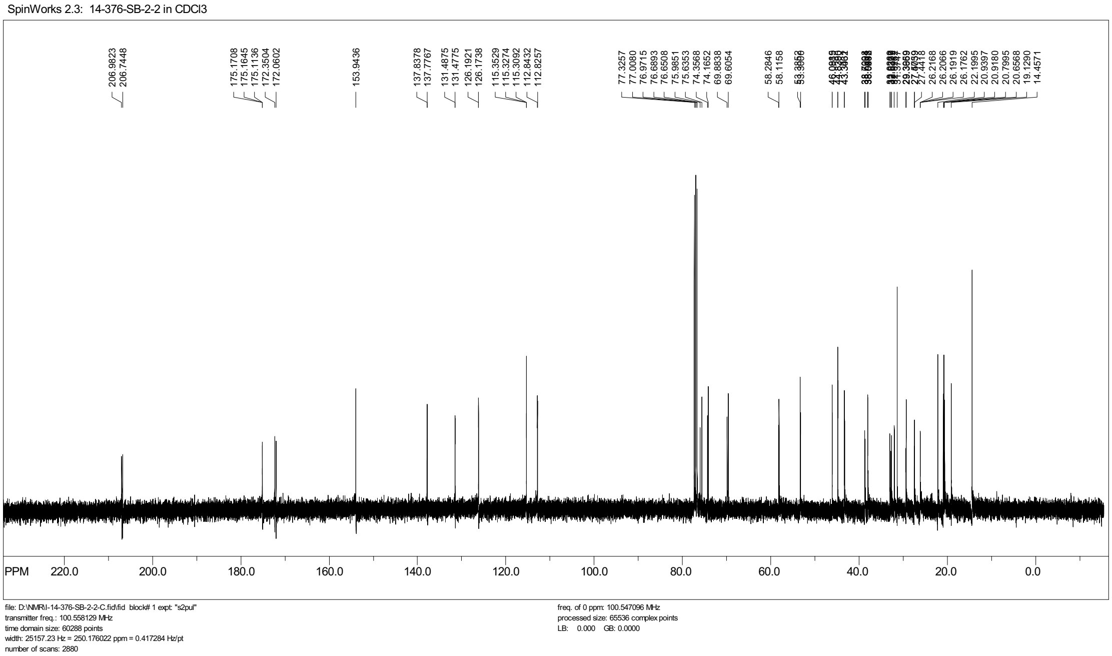
Apendix Figure 39 400 MHz ^{1}H NMR spectrum: 3-benzyloxy-16β-(5-benzyloxy-3-acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (150)



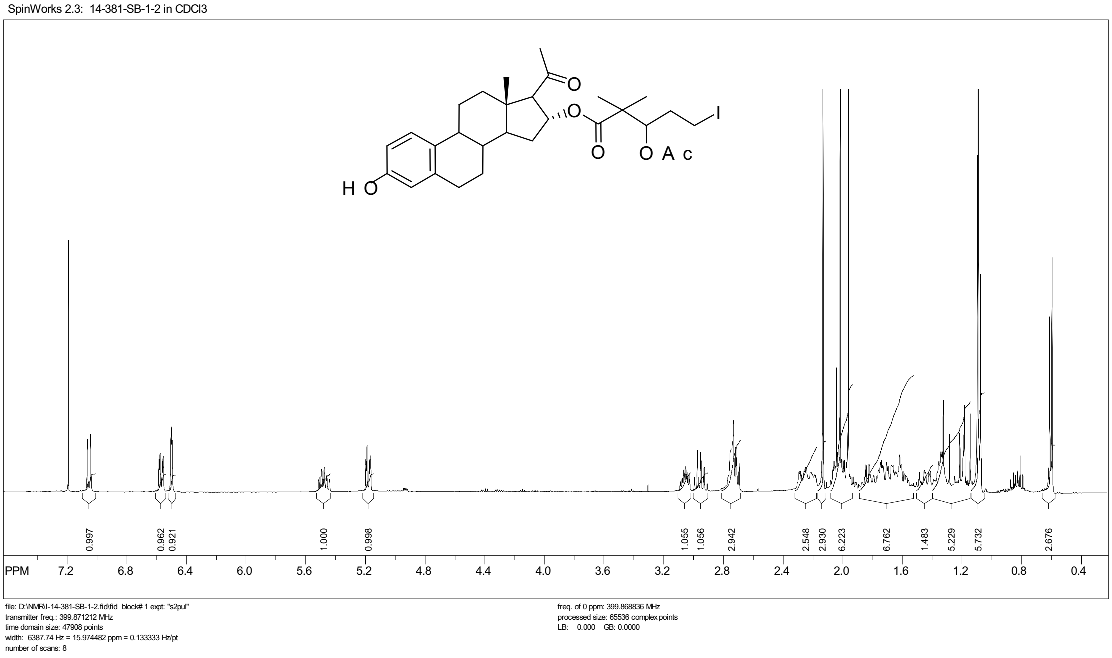
Apendix Figure 40 100 MHz ^{13}C NMR spectrum: 3-benzyloxy-16β-(5-benzyloxy-3-acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (150)



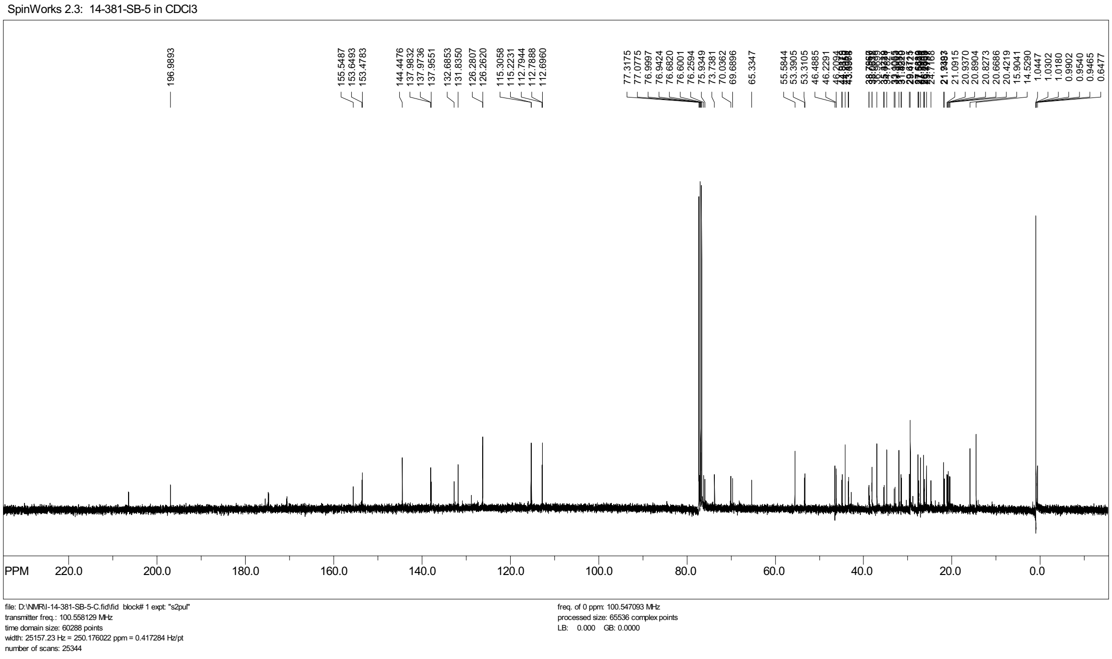
Apendix Figure 41 400 MHz ^{1}H NMR spectrum: 3-hydroxy-16β-(5-hydroxy-3-acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (151)



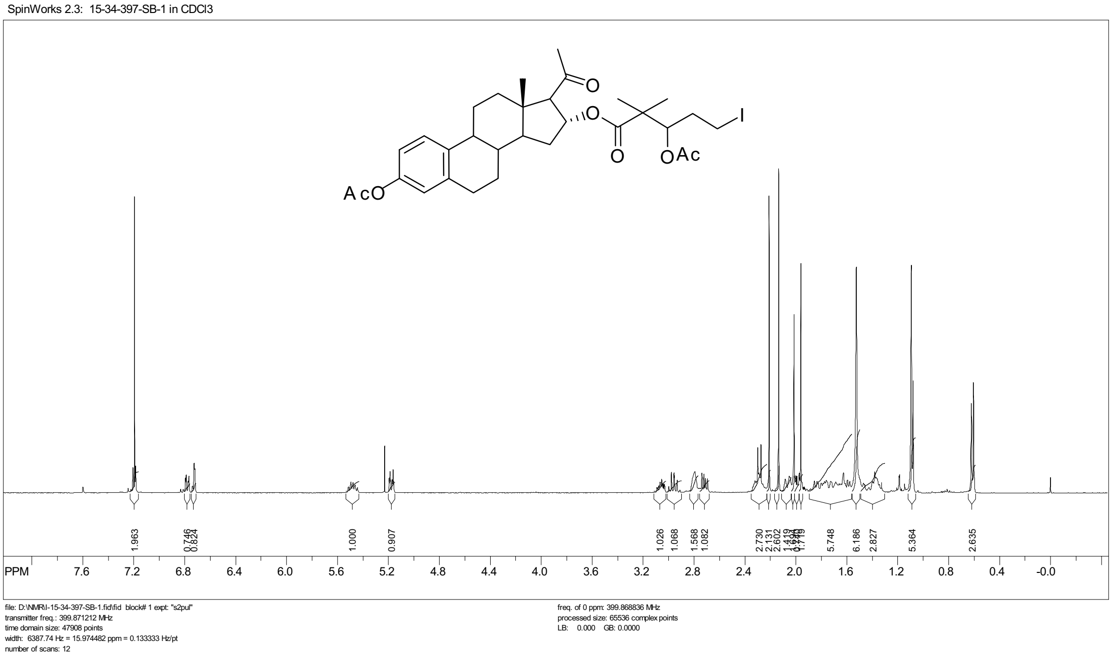
Apendix Figure 42 100 MHz ^{13}C NMR spectrum : 3-hydroxy-16β-(5-hydroxy-3-acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (151)



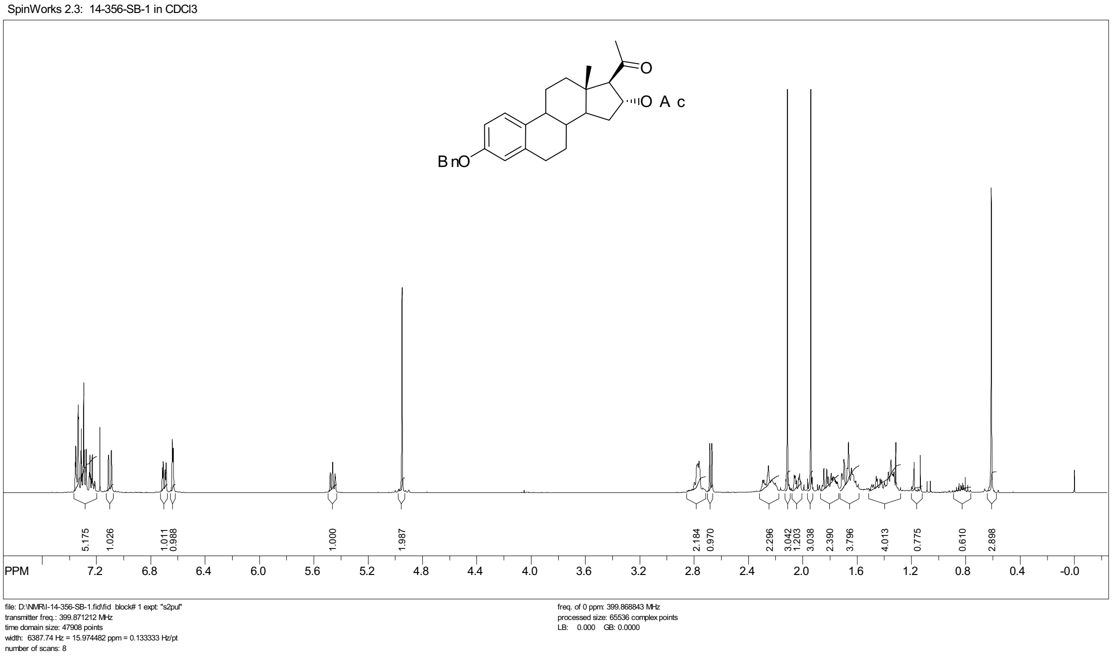
Apendix Figure 43 400 MHz ^{1}H NMR spectrum: 3-hydroxy-16β-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (152)



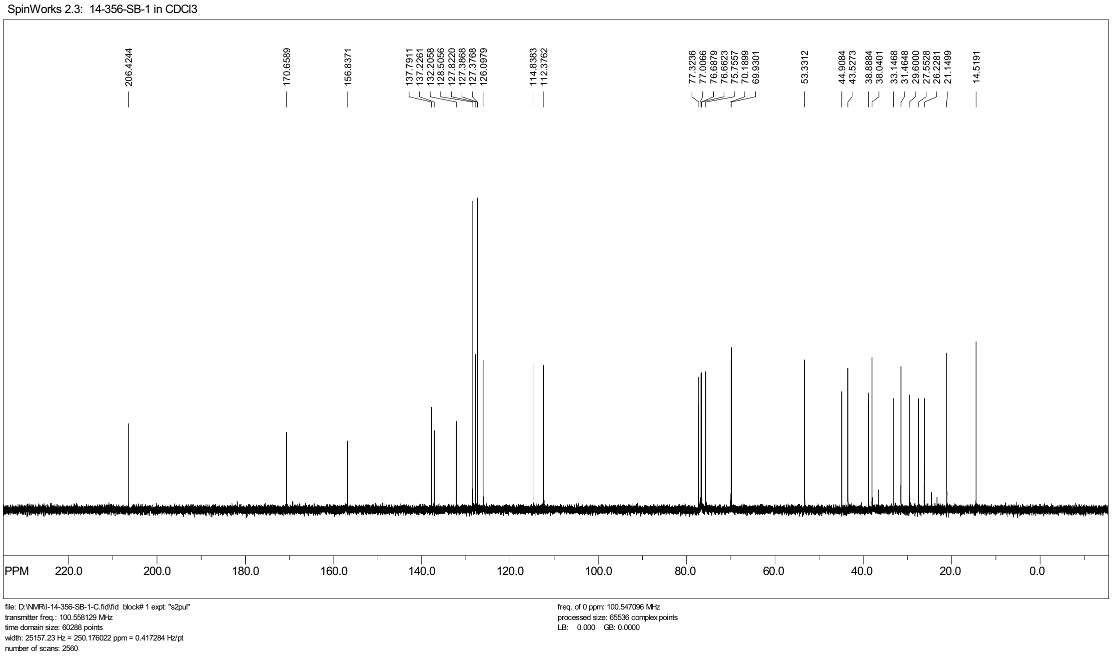
Apendix Figure 44 100 MHz ^{13}C NMR spectrum: 3-hydroxy-16β-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (152)



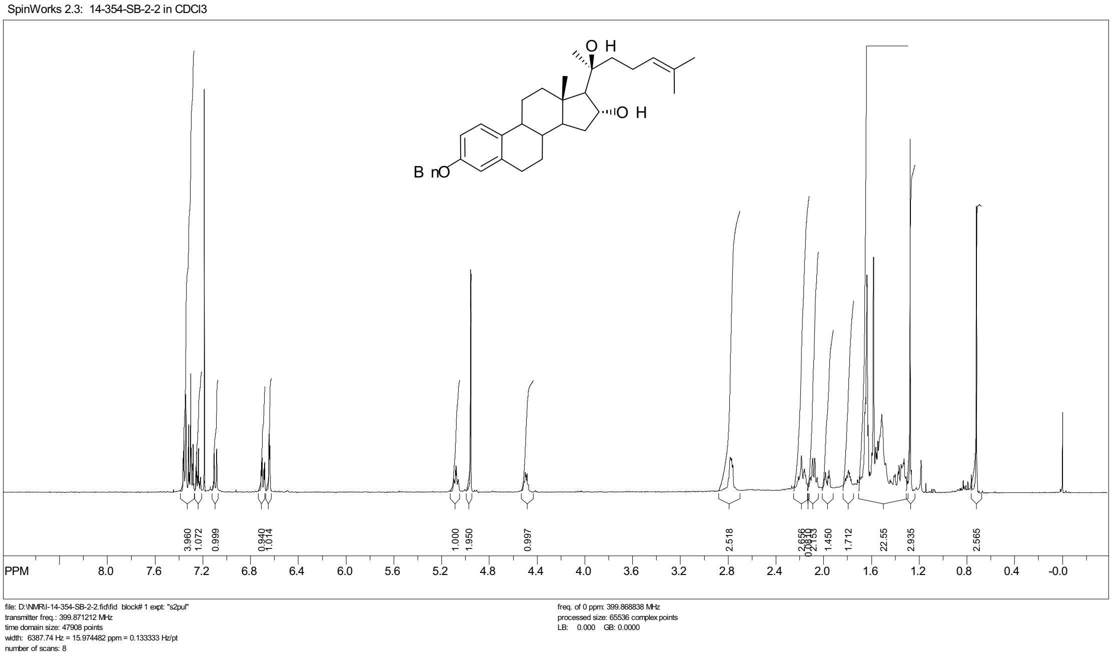
Apendix Figure 45 400 MHz ^{1}H NMR spectrum: 3-acetoxy-16β-(5-iodo-3acetoxy-2,2-dimethyl-pentanoate)-17-acetyl-$\Delta^{1,3,5(10)}$- estratriene (153)



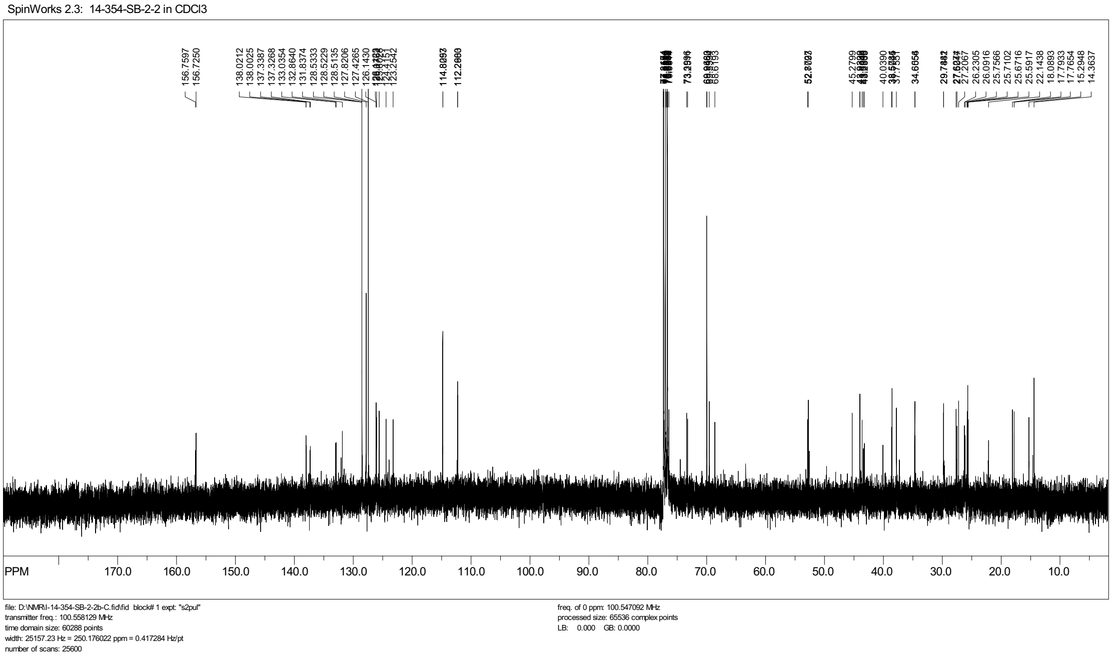
Apendix Figure 46 400 MHz ^{1}H NMR spectrum: 3-benzyloxy- 16α-acetoxy -17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (155)



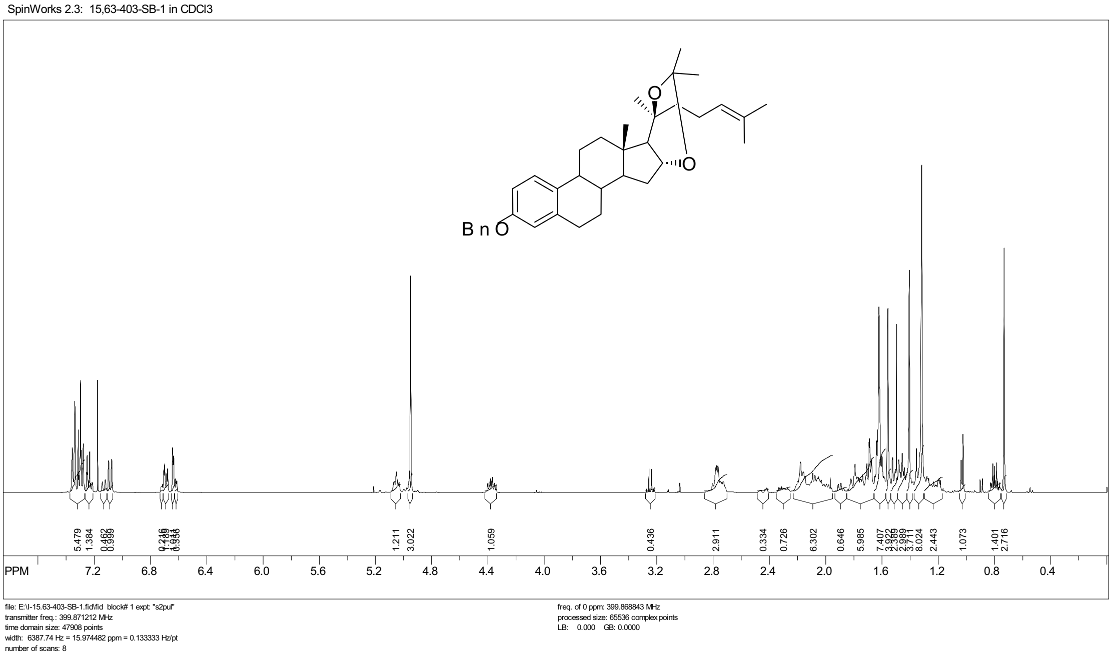
Apendix Figure 47 100 MHz ^{13}C NMR spectrum: 3-benzyloxy- 16α-acetoxy -17-acetyl-$\Delta^{1,3,5(10)}$-estratriene (155



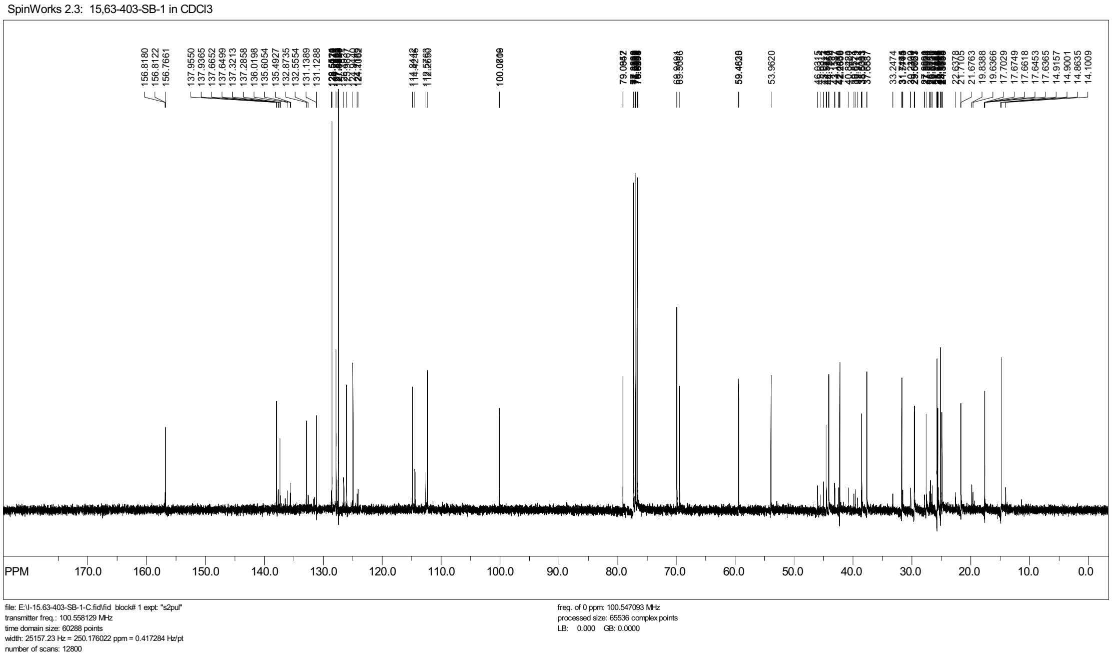
Apendix Figure 48 400 MHz ^{1}H NMR spectrum: 3-benzyloxy, 16*R*, 20*S*-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (156)



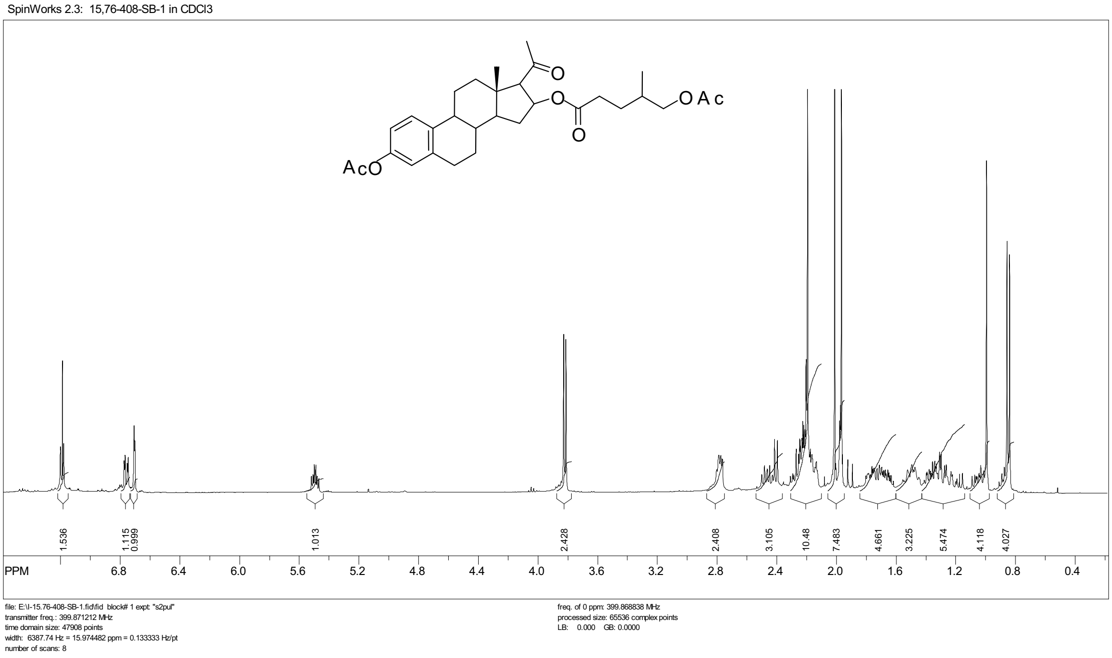
Apendix Figure 49 100 MHz ^{13}C NMR spectrum: 3-benzyloxy, 16*R*, 20*S*-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (156)



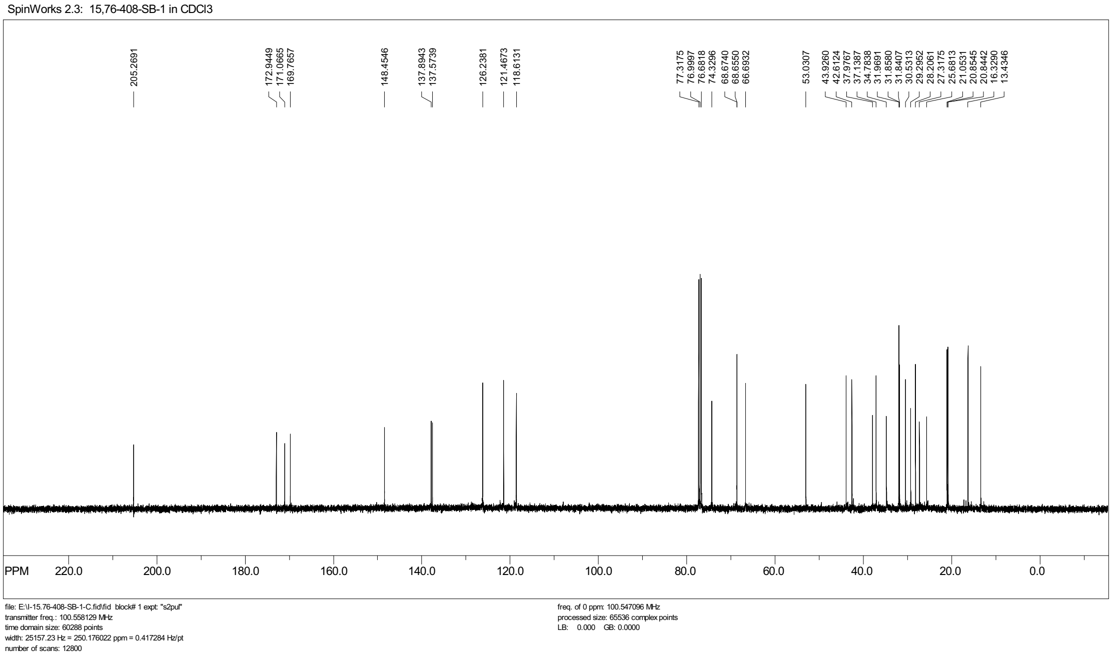
Apendix Figure 50 400 MHz ^{1}H NMR spectrum: 3-benzyloxy-(16*R*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (157)



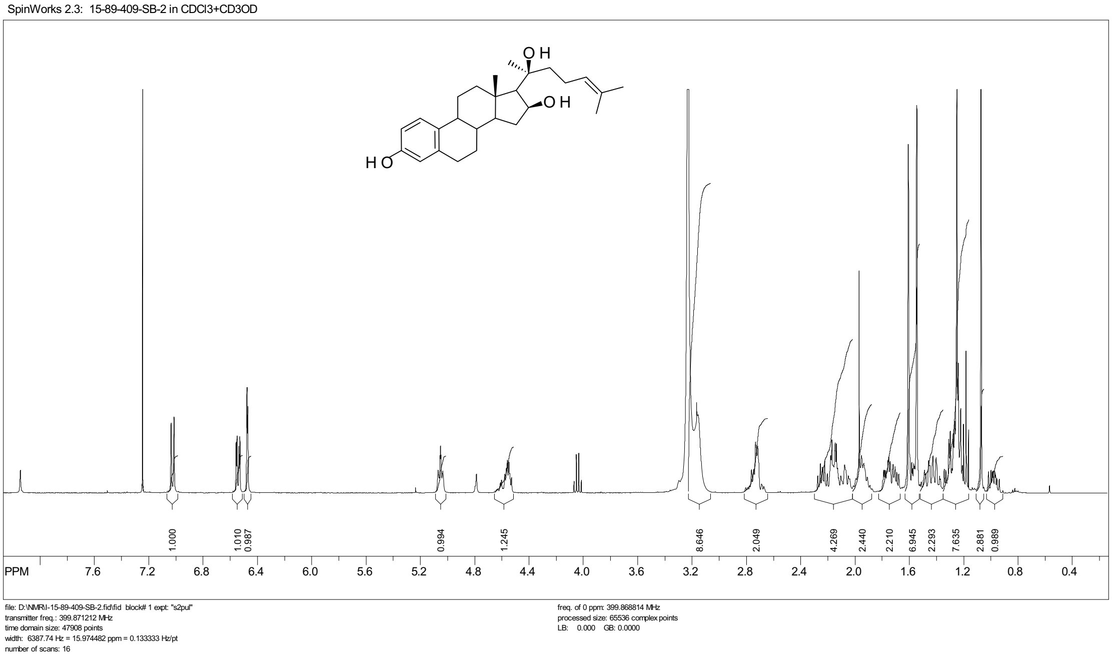
Apendix Figure 51 100 MHz ^{13}C NMR spectrum: 3-benzyloxy-(16*R*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (157)



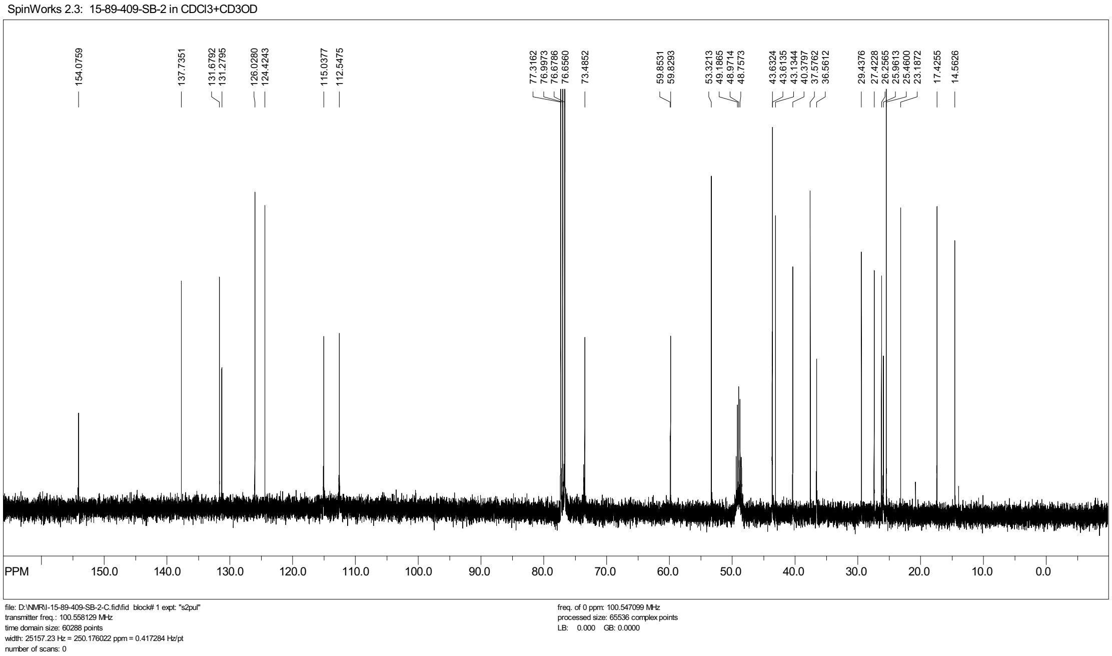
Apendix Figure 52 400 MHz ^{1}H NMR spectrum: 3-acetoxy-16β-acetoxymethylvaleroyloxy-17-acetyl-Δ$^{1,3,5(10)}$- estratriene (151)



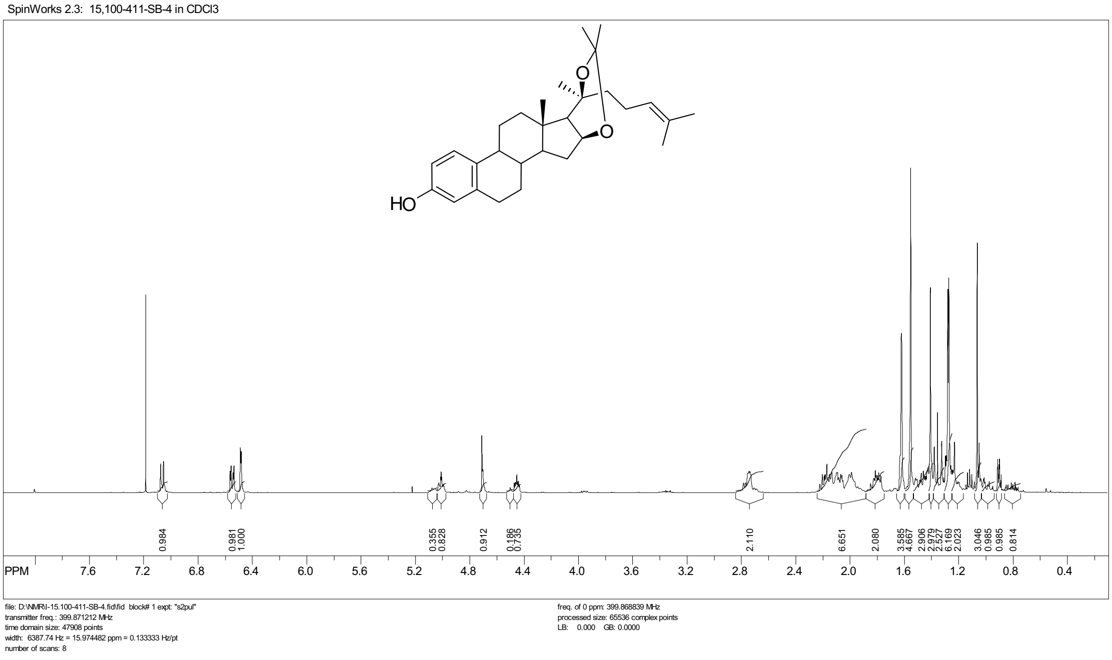
Apendix Figure 53 100 MHz ^{13}C NMR spectrum : 3-acetoxy-16β-acetoxymethylvaleroyloxy-17-acetyl-Δ$^{1,3,5(10)}$- estratriene (151)



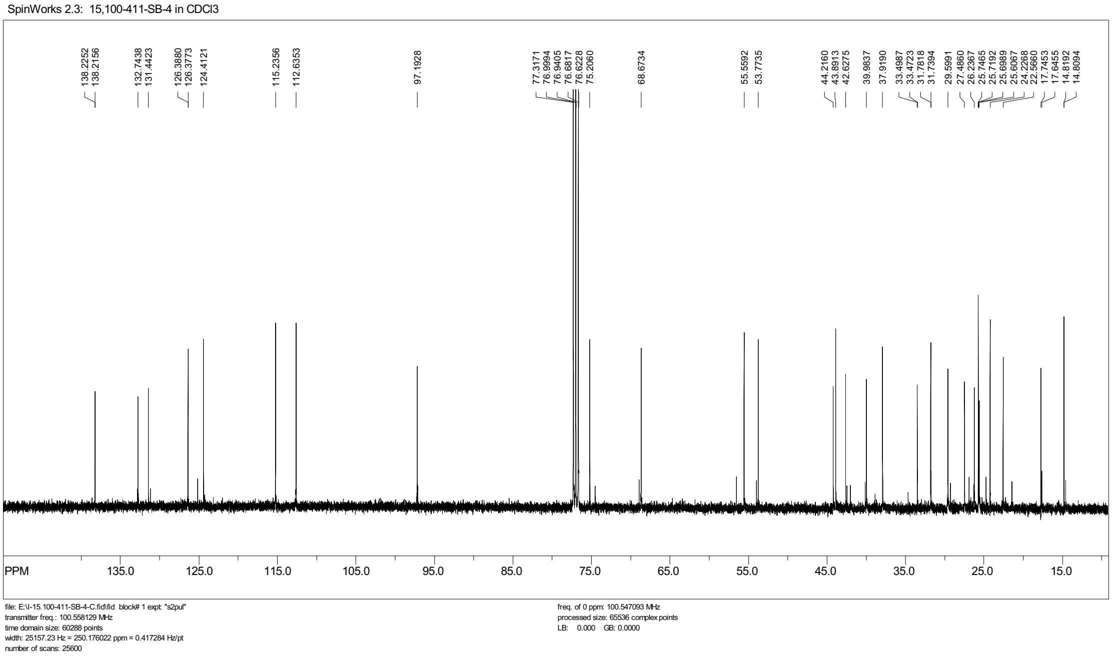
Apendix Figure 54 400 MHz ^{1}H NMR spectrum: 3, 6*S*, 20*S*-16, 20-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (159)



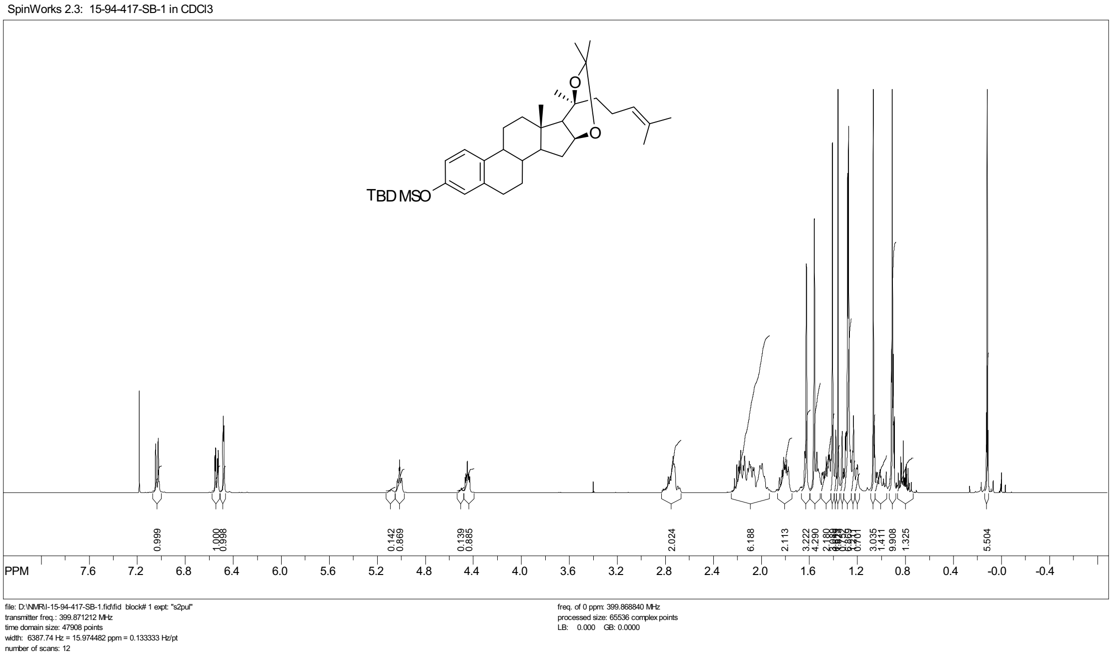
Apendix Figure 55 100 MHz ^{13}C NMR spectrum : 3, 6*S*, 20*S*-16, 20-trihydroxy-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (159)



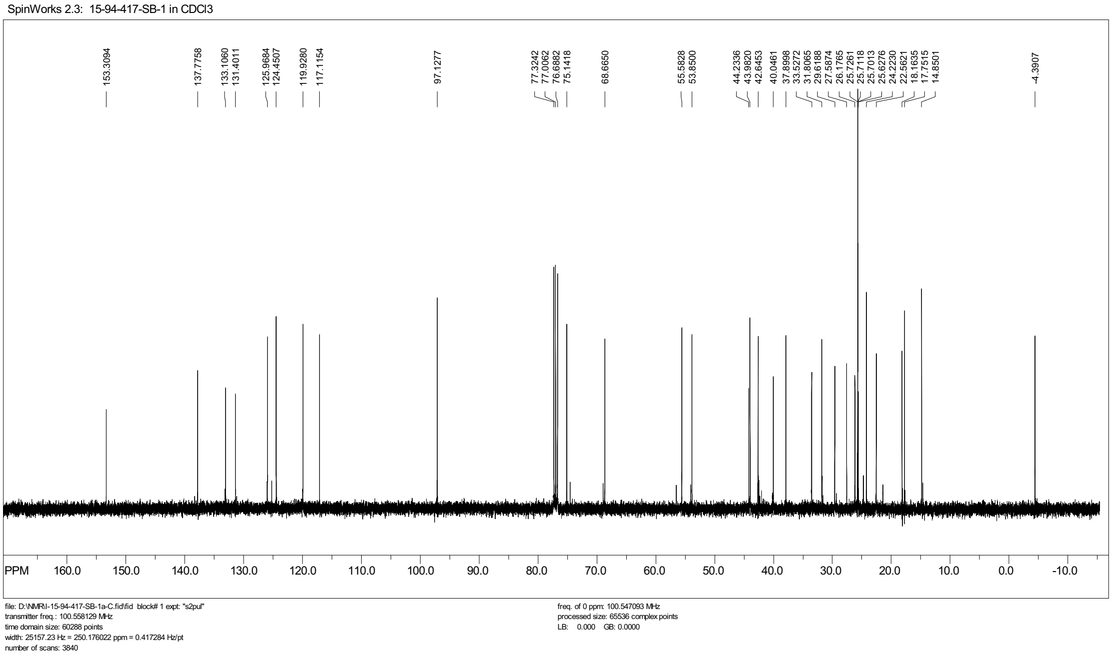
Apendix Figure 56 400 MHz ^{1}H NMR spectrum: 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (160)



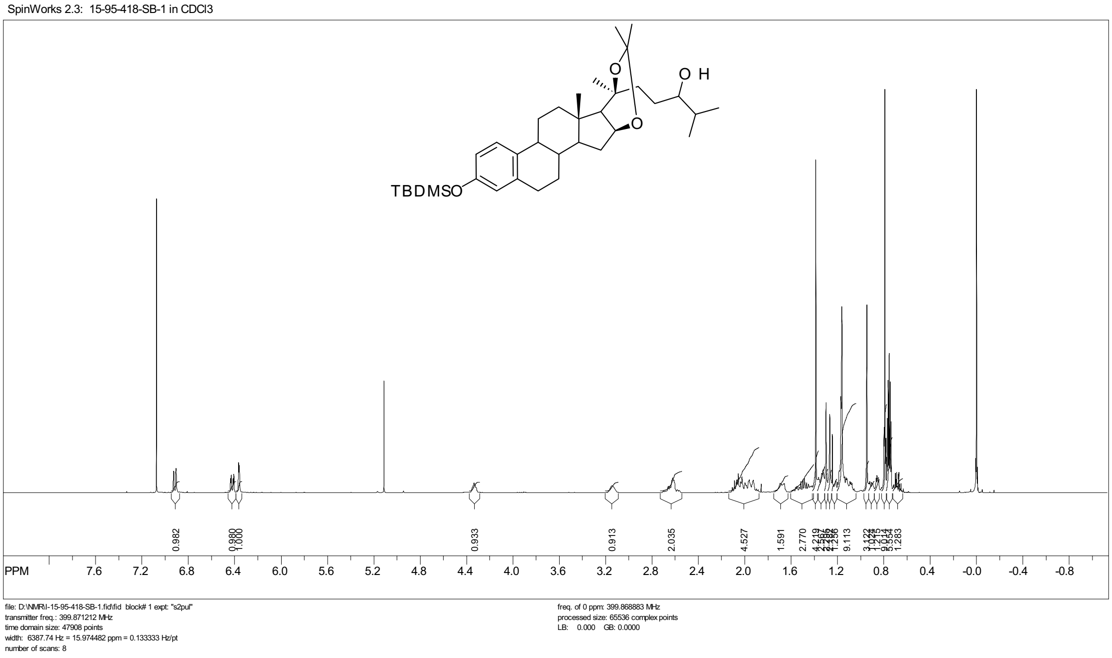
Apendix Figure 57 100 MHz ^{13}C NMR spectrum: 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (160)



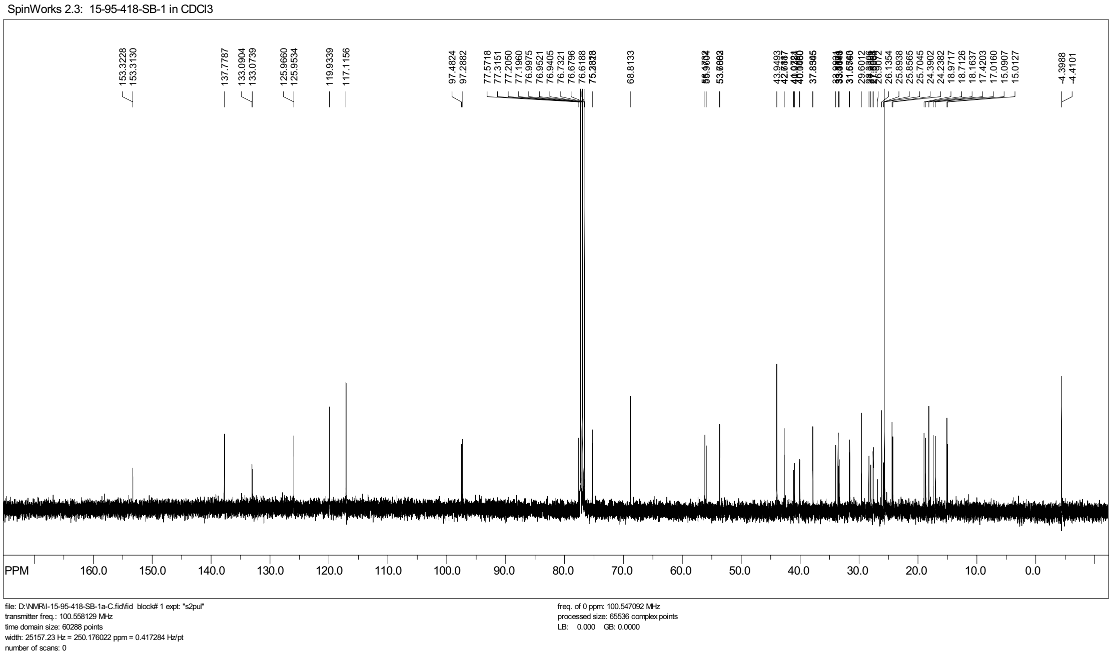
Apendix Figure 58 400 MHz ^{1}H NMR spectrum: 3-*tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (161)



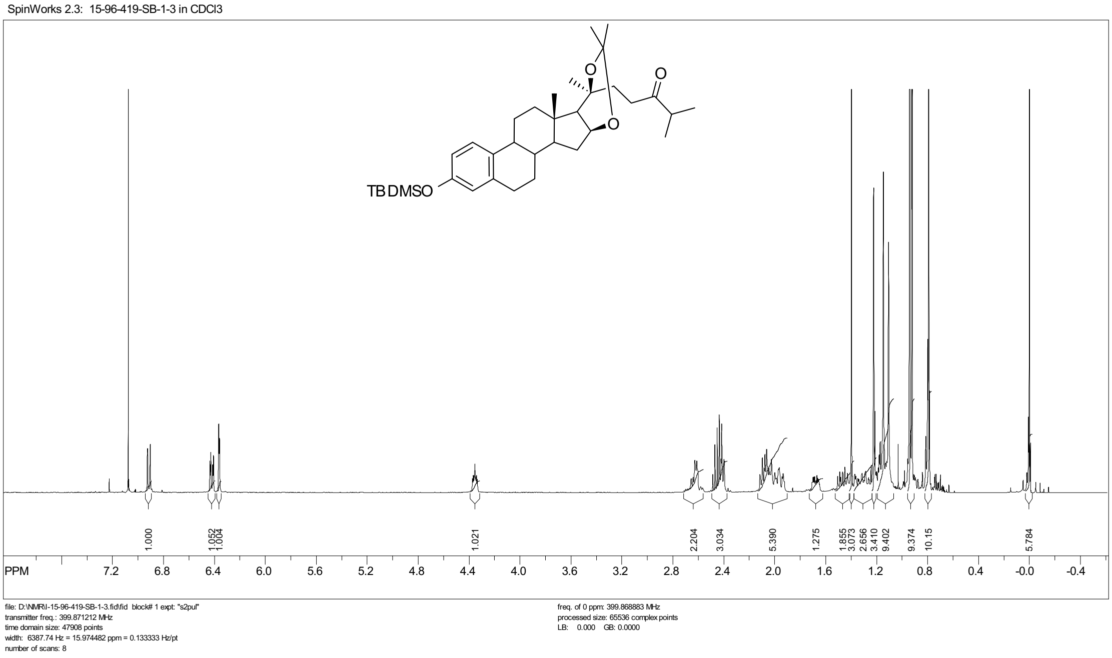
Apendix Figure 59 100 MHz ^{13}C NMR spectrum: 3-*tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-cholestene-$\Delta^{1,3,5(10)}$- estratriene (161)



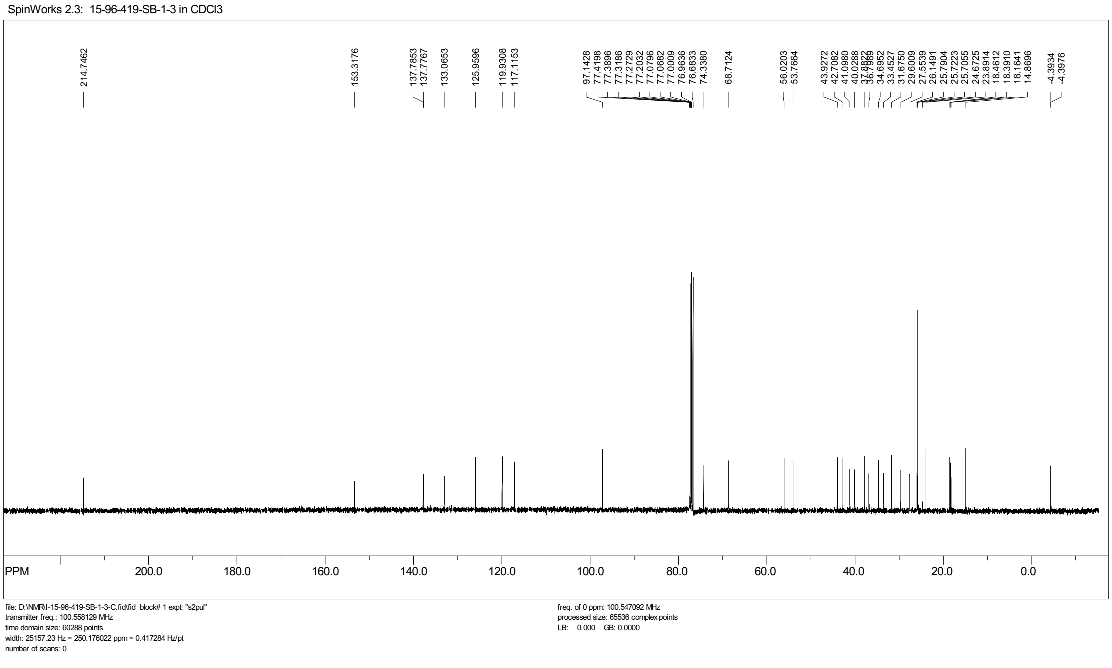
Apendix Figure 60 400 MHz ^{1}H NMR spectrum: 3-*tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-ol-chlorestane-$\Delta^{1,3,5(10)}$- estratriene (162)



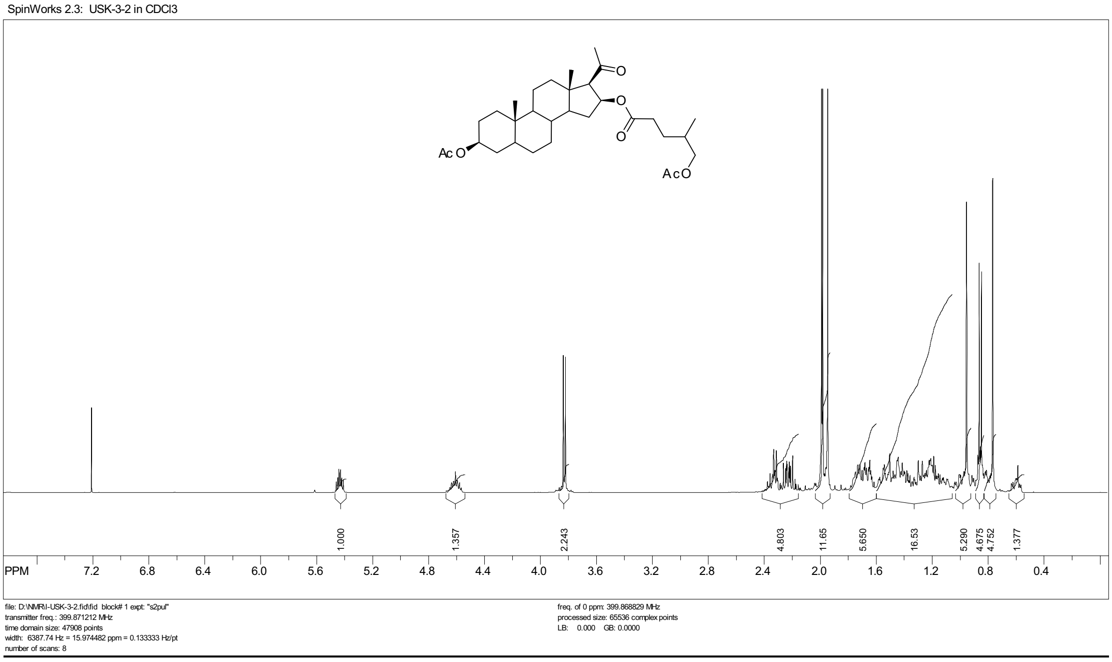
Apendix Figure 61 100 MHz ^{13}C NMR spectrum: 3-*tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-ol-chlorestane-$\Delta^{1,3,5(10)}$- estratriene (162)



Apendix Figure 62 400 MHz ^{1}H NMR spectrum: 3-*tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-one-chlorestane-$\Delta^{1,3,5(10)}$- estratriene (163)



Apendix Figure 63 100 MHz ^{13}C NMR spectrum: 3-*tert*-butyl-dimethylsiloxy-(16*S*, 20*S*)-16, 20-acetonide-24-one-chlorestane-$\Delta^{1,3,5(10)}$- estratriene (163)



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freq. of 0 ppm: 399.868829 MHz
processed size: 65536 complex points
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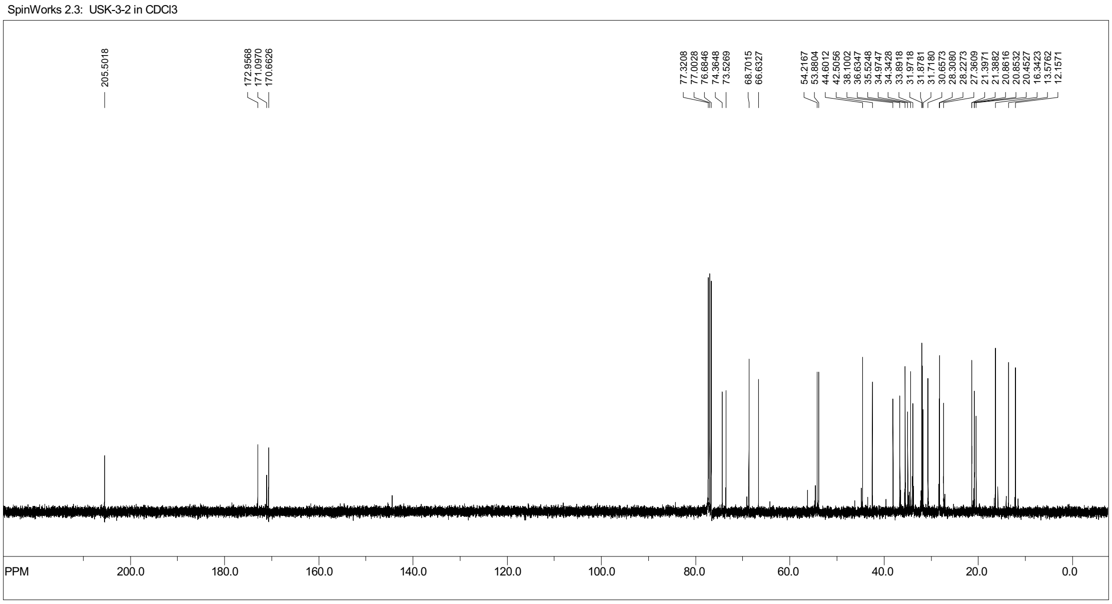
Apendix Figure 64 400 MHz ^{1}H NMR spectrum: 3β-Acetoxy-16β-γ-acetoxymethylvaleroyloxy-5α-pregnan-20-one (164)



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number of scans: 5120

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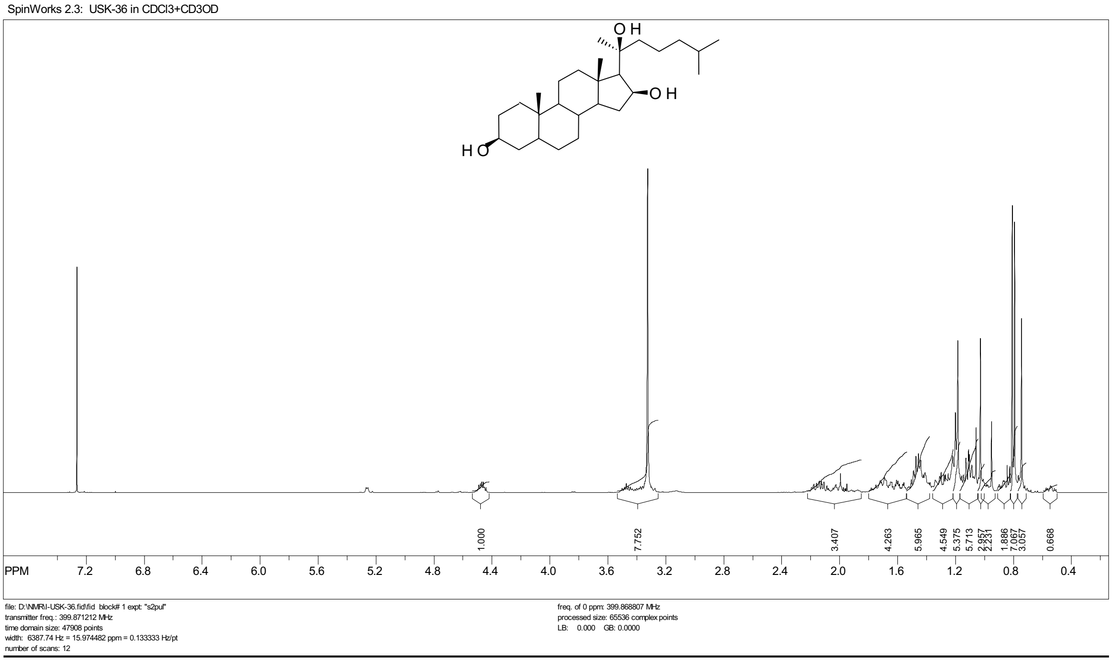
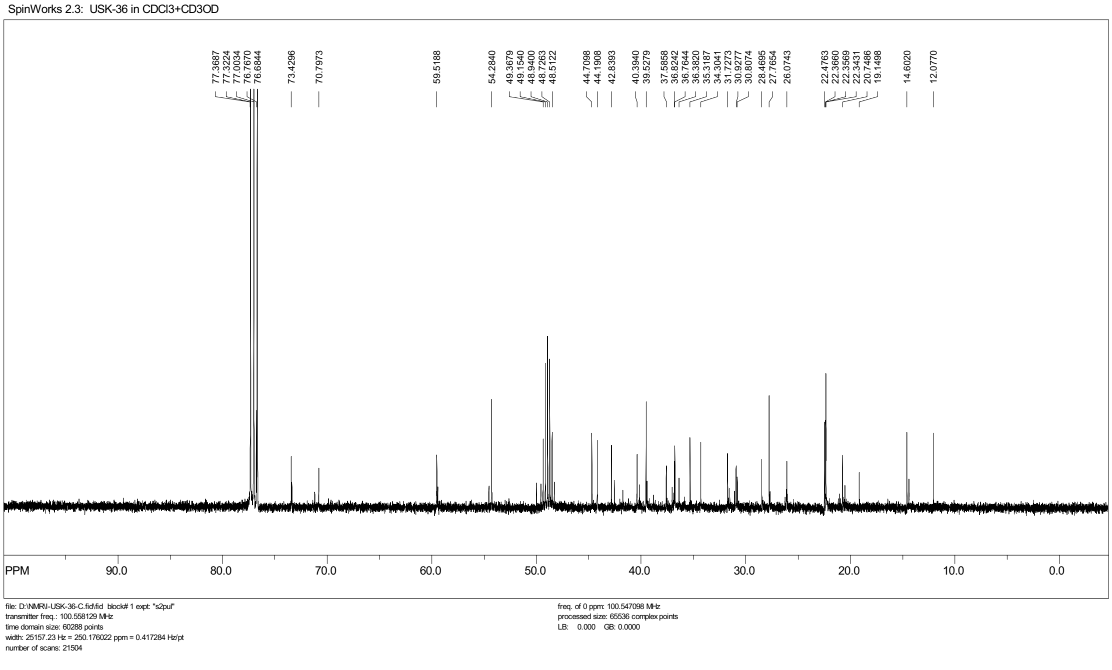
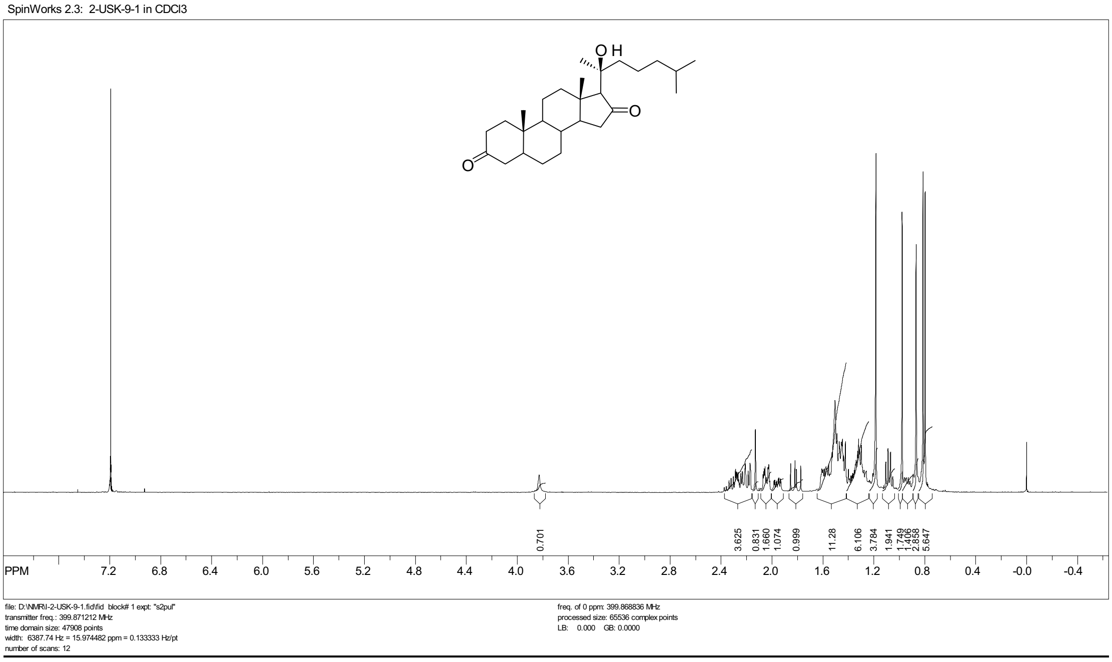
Apendix Figure 65 100 MHz ^{13}C NMR spectrum: 3β-Acetoxy-16β-γ-acetoxymethylvaleroyloxy-5α-pregnan-20-one (164)



Apendix Figure 66 400 MHz ^{1}H NMR spectrum: (20S)-3, 16, 20-trihydroxycholestane (165)



Apendix Figure 67 100 MHz ^{13}C NMR spectrum: (20*S*)-3, 16, 20-trihydroxycholestane (165)



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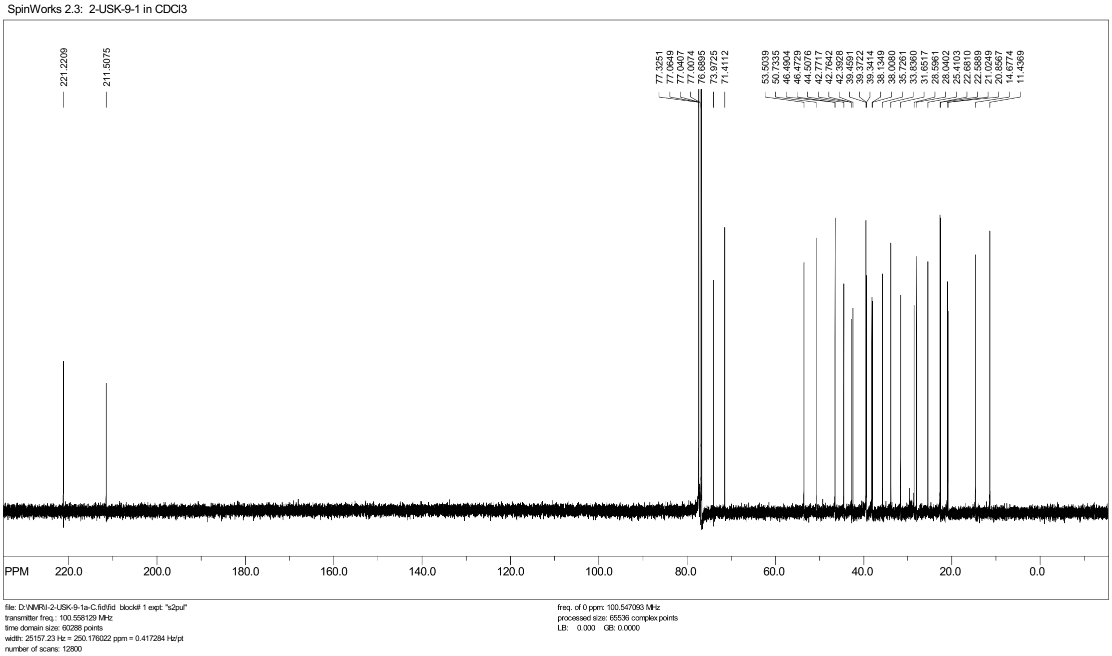
Apendix Figure 68 400 MHz ^{1}H NMR spectrum: (20S)-20-hydroxycholestane-3,16-dione (166)



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number of scans: 12800

freq. of 0 ppm: 100.547093 MHz
processed size: 65536 complex points
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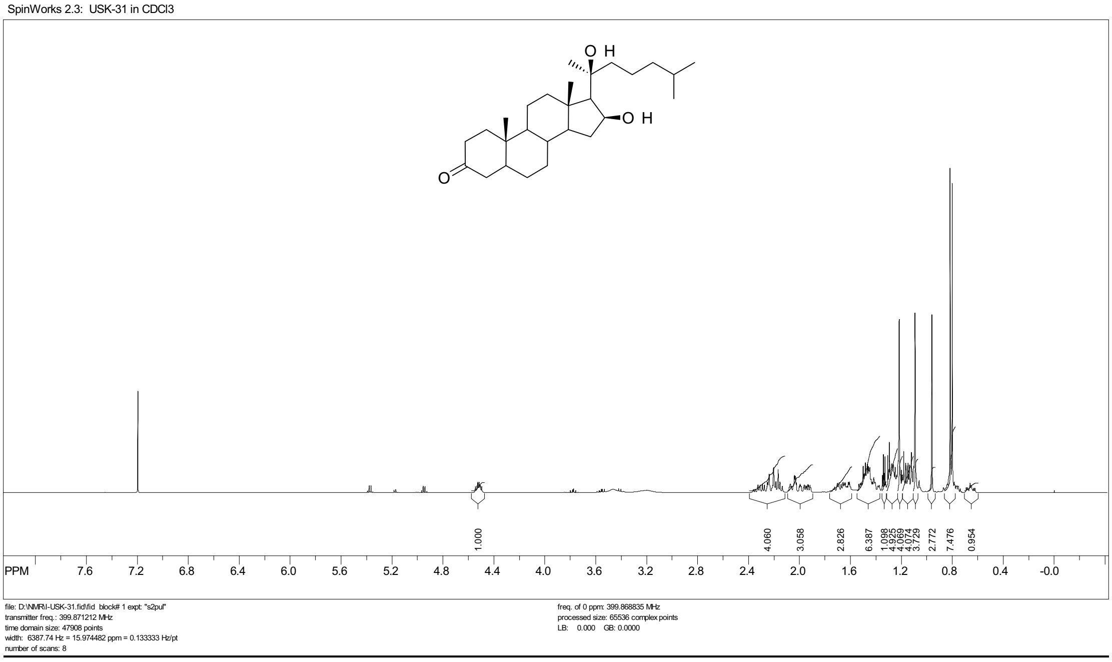
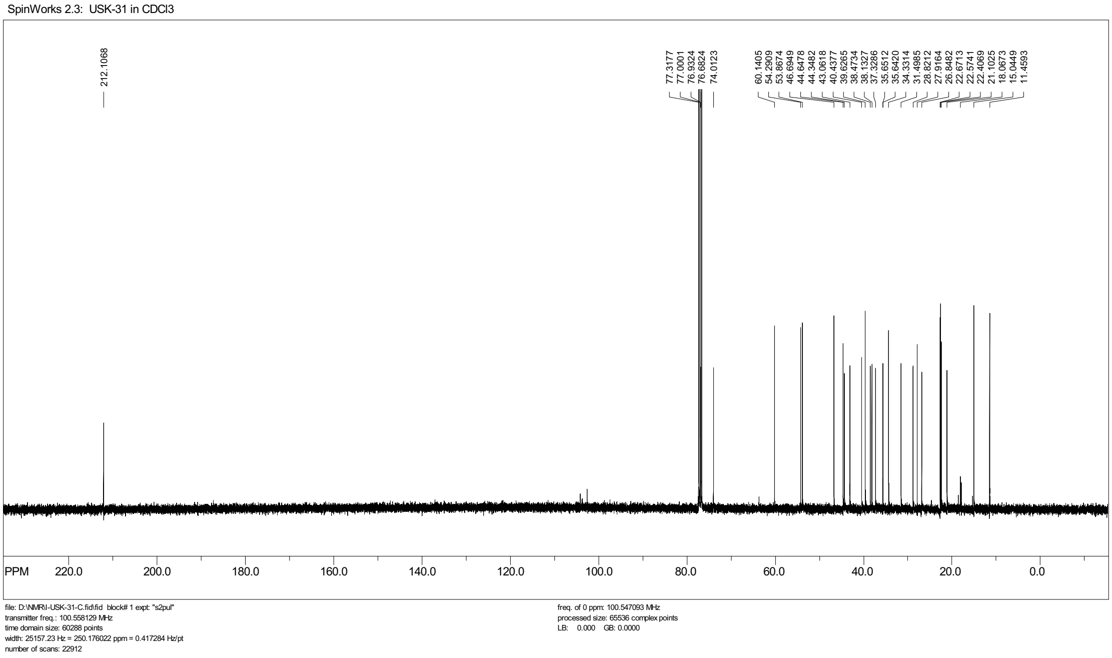
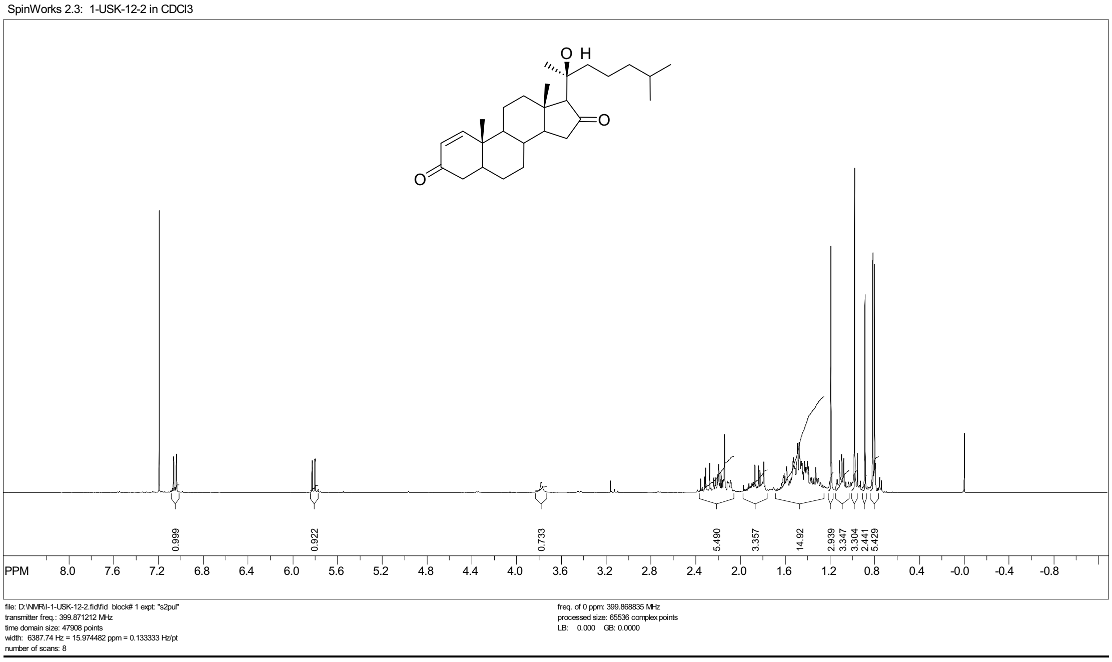
Apendix Figure 69 100 MHz ^{13}C NMR spectrum: (20S)-20-hydroxycholestane-3,16-dione (166)



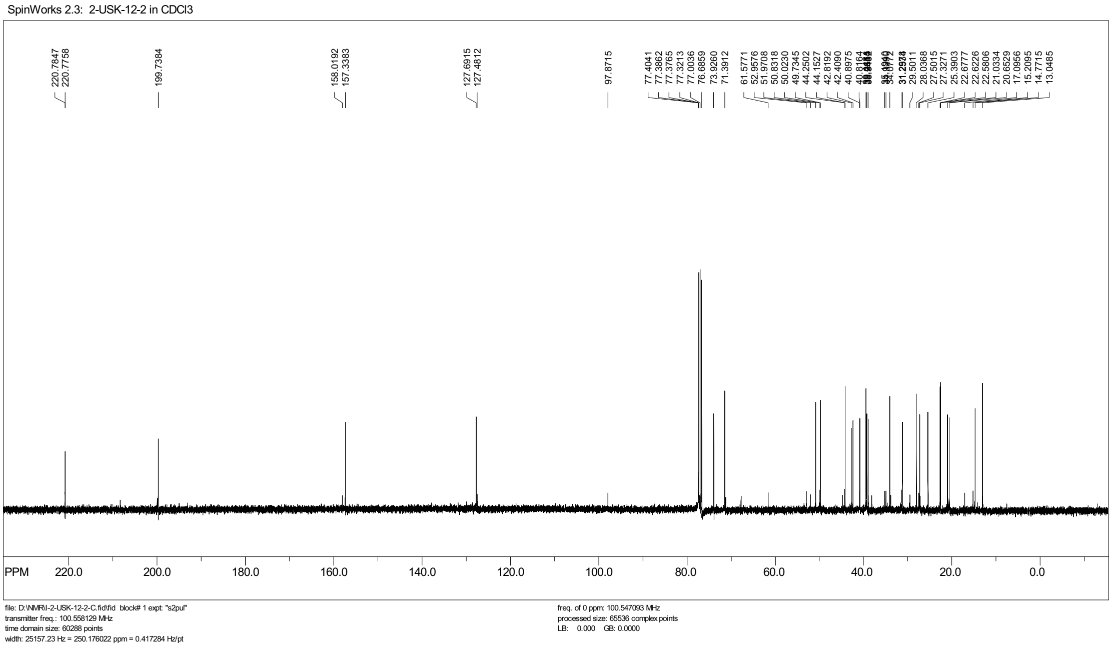
Apendix Figure 70 400 MHz ^{1}H NMR spectrum: (16S, 20S)-16, 20-dihydroxy-cholestan-3-one (167)



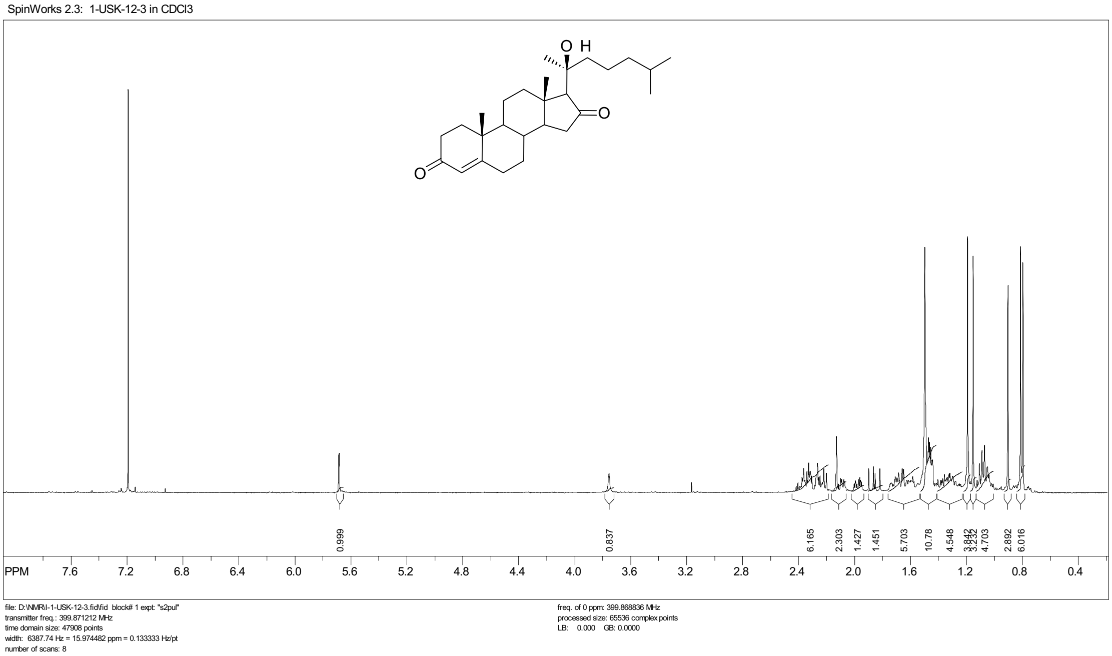
Apendix Figure 71 100 MHz ^{13}C NMR spectrum: (16S, 20S)-16, 20-dihydroxy-cholestan-3-one (167)



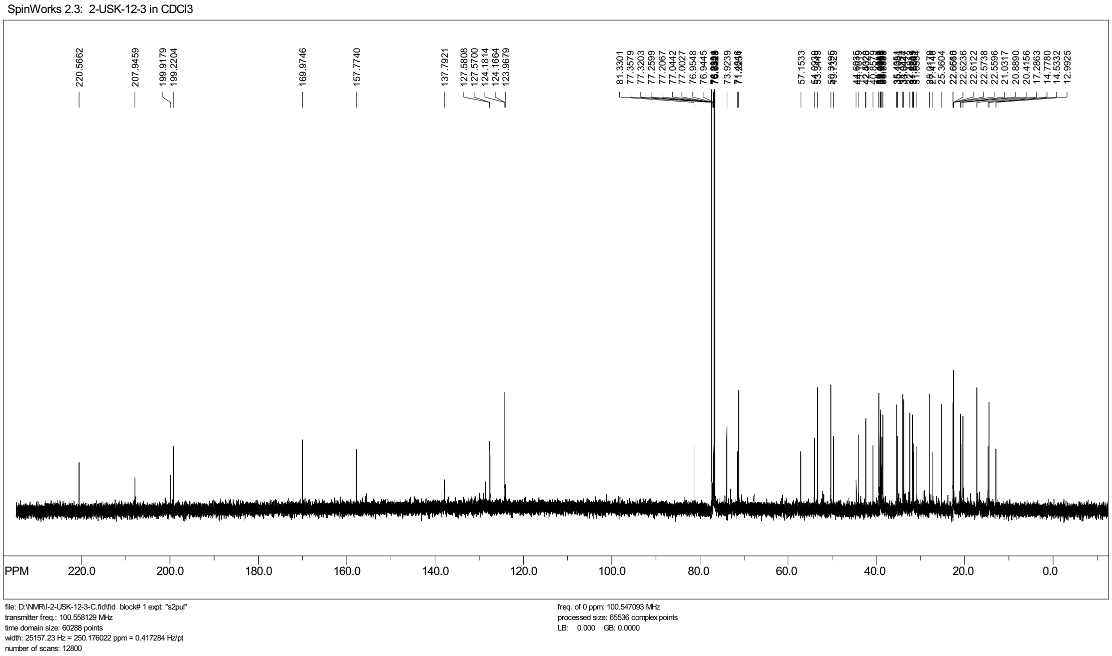
Apendix Figure 72 400 MHz ^{1}H NMR spectrum: (20S)-20-Hydroxycholest-1-ene-3,16-dione (168)



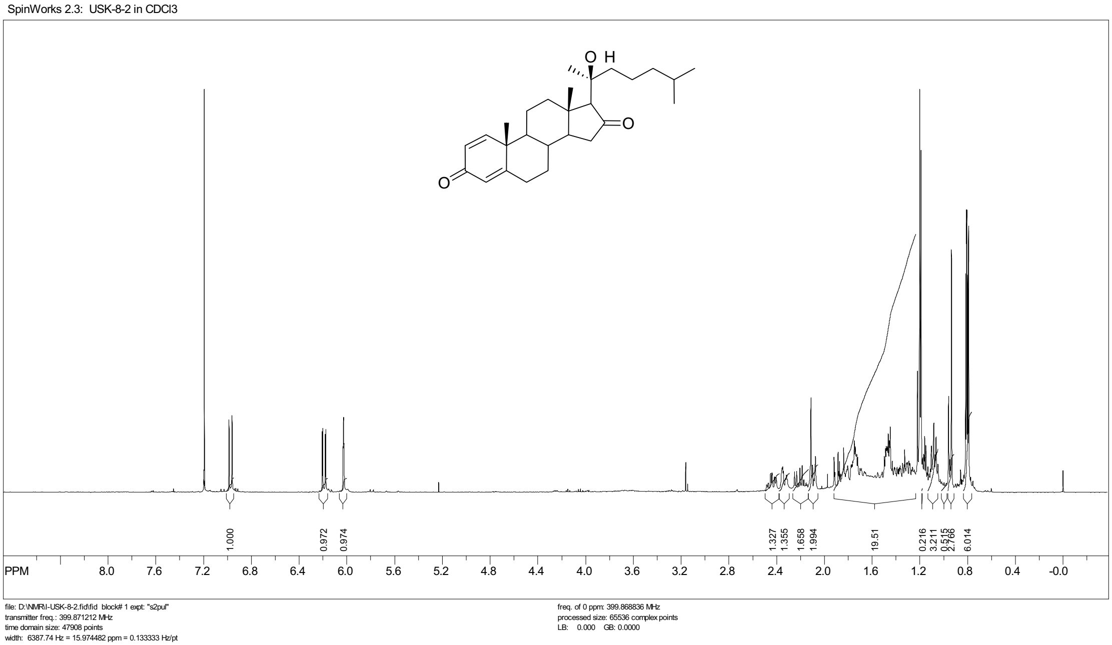
Apendix Figure 73 100 MHz ^{13}C NMR spectrum: (20S)-20-Hydroxycholest-1-ene-3,16-dione (168)



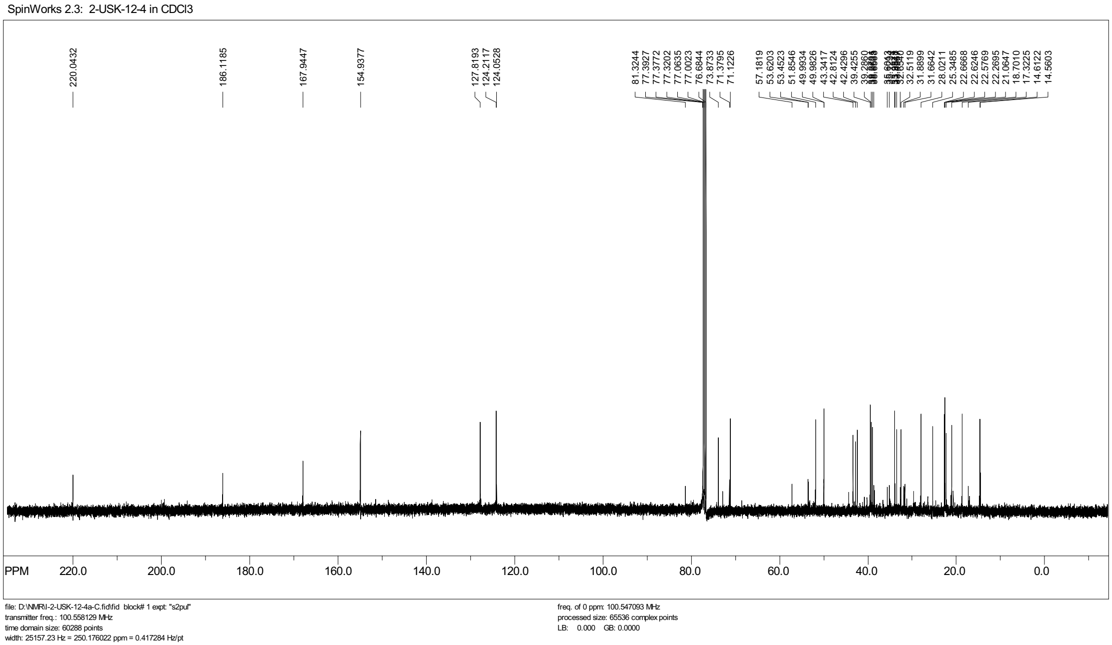
Apendix Figure 74 400 MHz ^{1}H NMR spectrum: (20S)-20-hydroxy cholest-4-ene-3,16-dione (169)



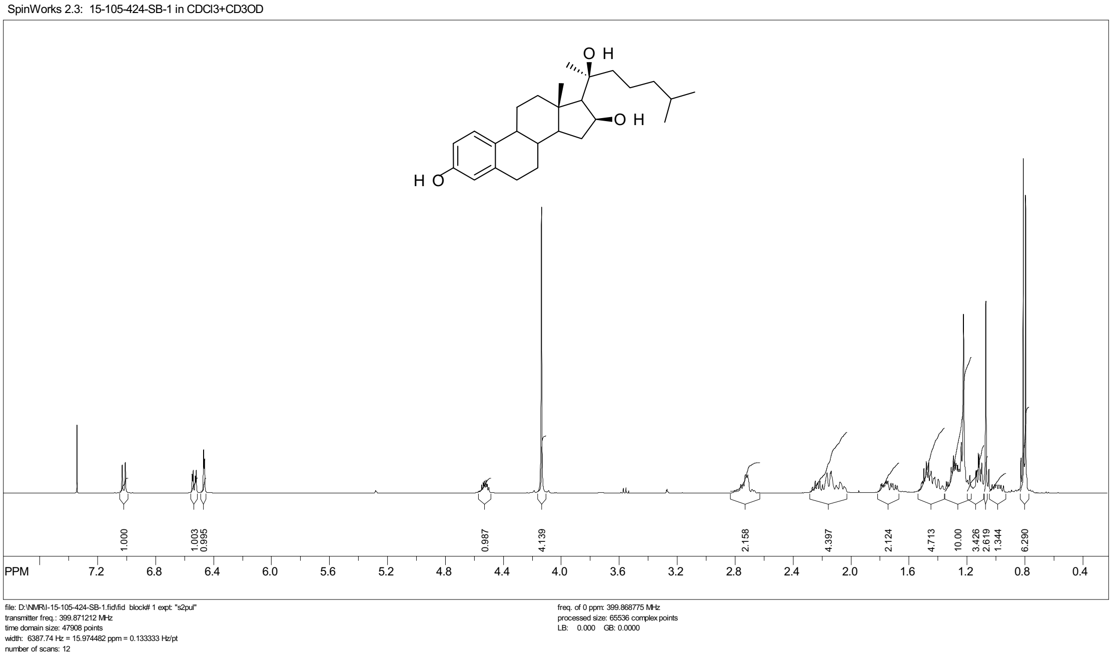
Apendix Figure 75 100 MHz ^{13}C NMR spectrum: (20S)-20-hydroxy cholest-4-ene-3,16-dione (169)



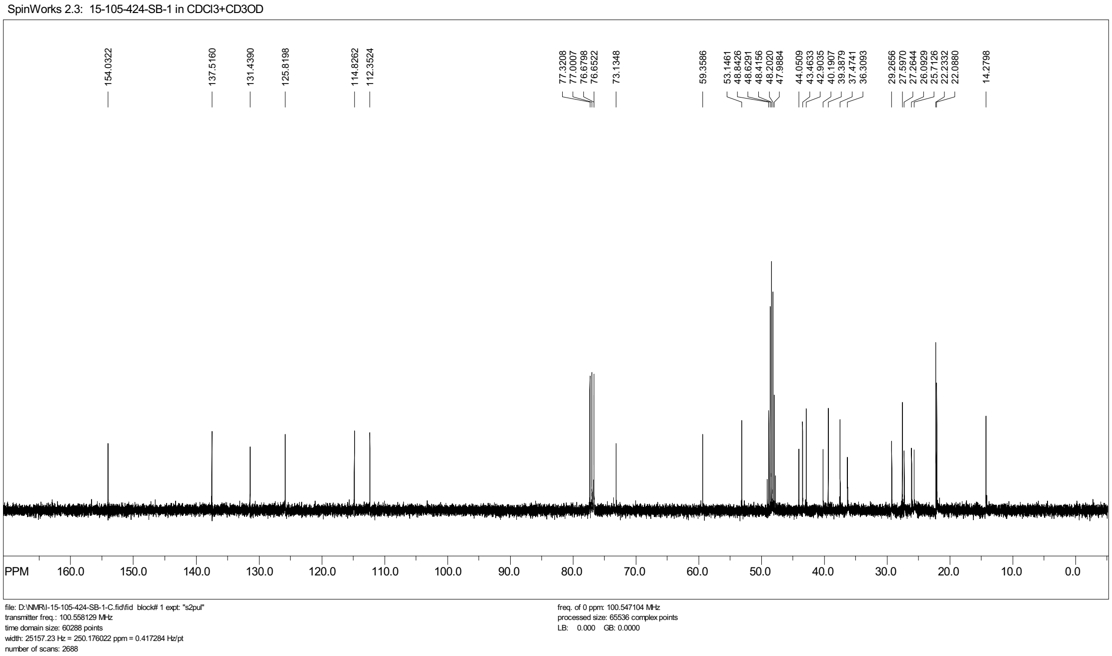
Apendix Figure 76 400 MHz ^{1}H NMR spectrum: (20S)-20-hydroxycholest-1,4-diene-3,16-dione (170)



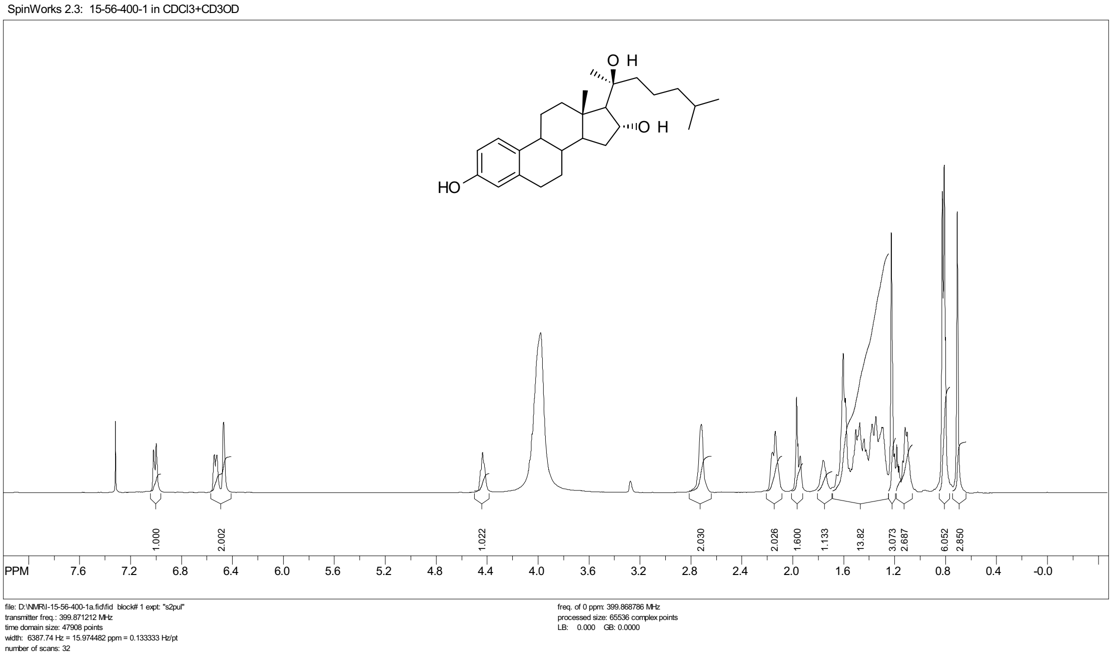
Apendix Figure 77 100 MHz ^{13}C NMR spectrum: (20S)-20-hydroxycholest-1,4-diene-3,16-dione (170)



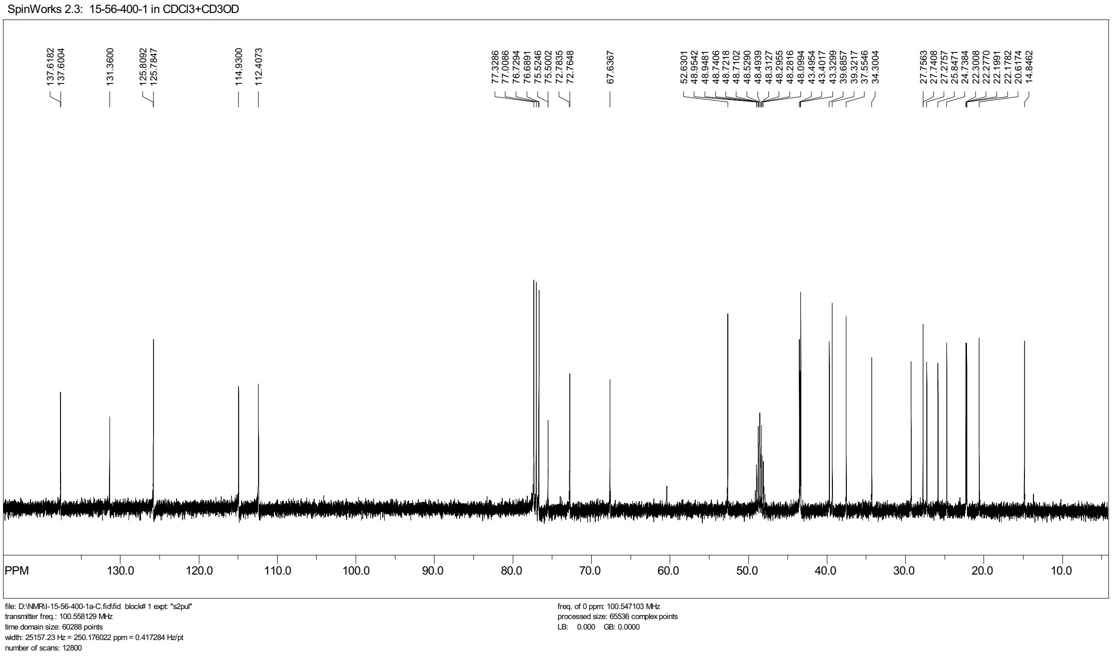
Apendix Figure 78 400 MHz ^{1}H NMR spectrum: 3, 16*s*, 20*s*-trihydroxy-cholestan-$\Delta^{1,3,5(10)}$- estratriene (171)



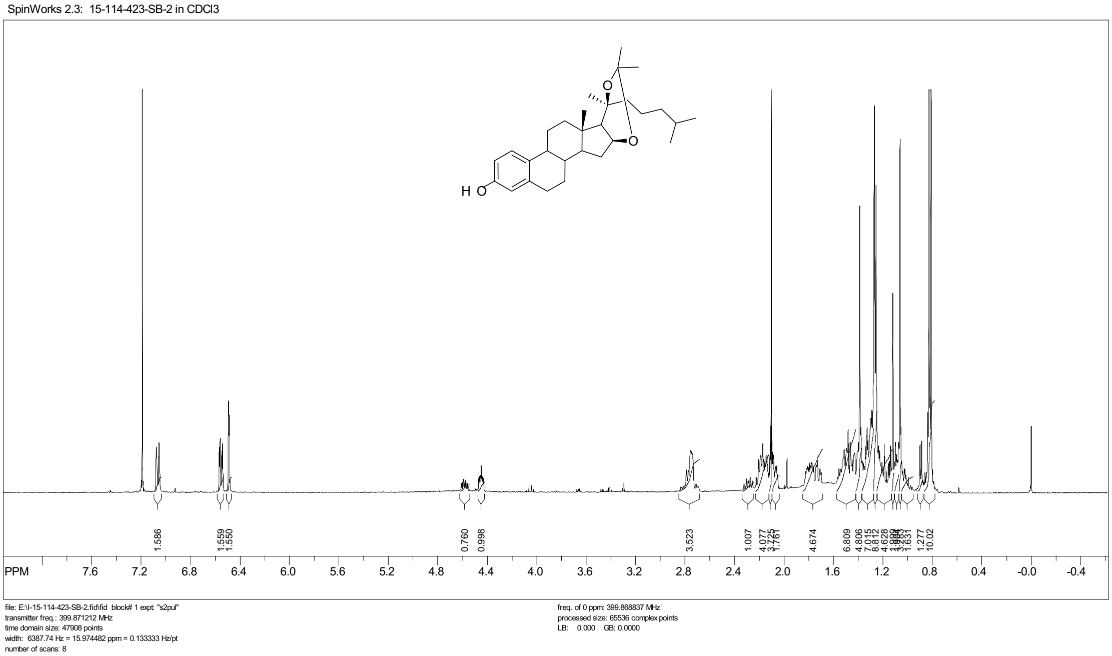
Apendix Figure 79 100 MHz ^{13}C NMR spectrum: 3, 16*S*, 20*S*-trihydroxy-cholestan-$\Delta^{1,3,5(10)}$-estratriene (171)



Apendix Figure 80 400 MHz ^{1}H NMR spectrum: 3-benzyloxy, 16*R*, 20*S*-trihydroxy-cholestane-$\Delta^{1,3,5(10)}$- estratriene (172)

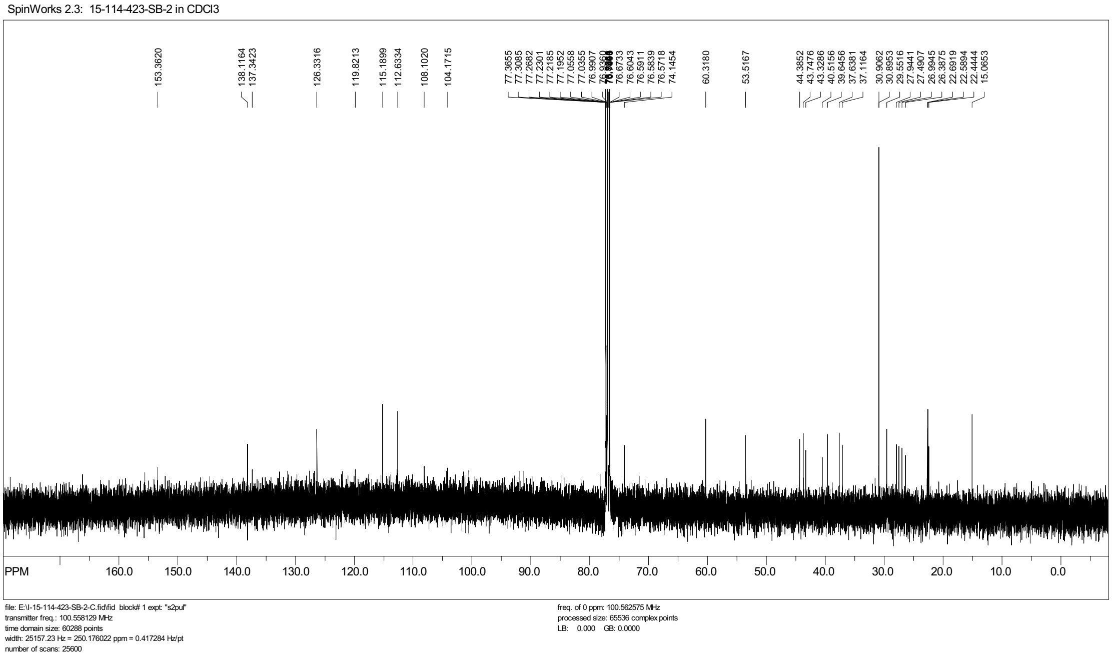


Apendix Figure 81 100 MHz ^{13}C NMR spectrum: 3-benzyloxy, 16*R*, 20*S*-trihydroxy-cholestane-$\Delta^{1,3,5(10)}$- estratriene (172)



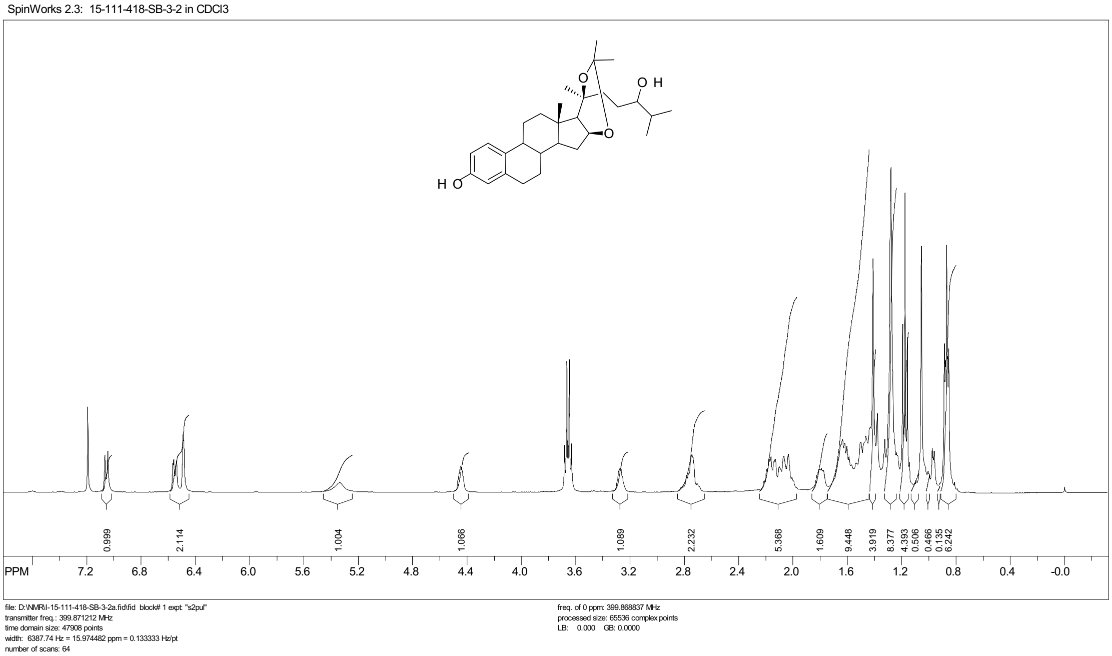
Apendix Figure 82 400 MHz ^{1}H NMR spectrum: 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$- estratriene (175)

Remark: ^{1}H NMR contained **175** and the compound that acetonide was removed in $CDCl_3$.

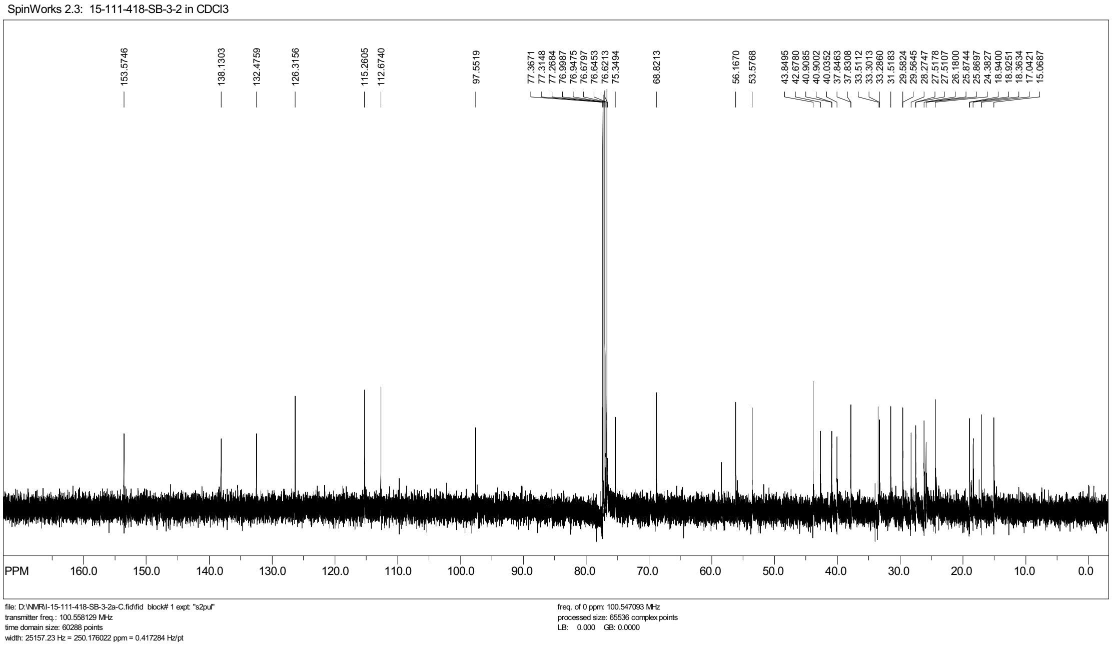


Apendix Figure 83 100 MHz ^{13}C NMR spectrum: 3-hydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$- estratriene (175)

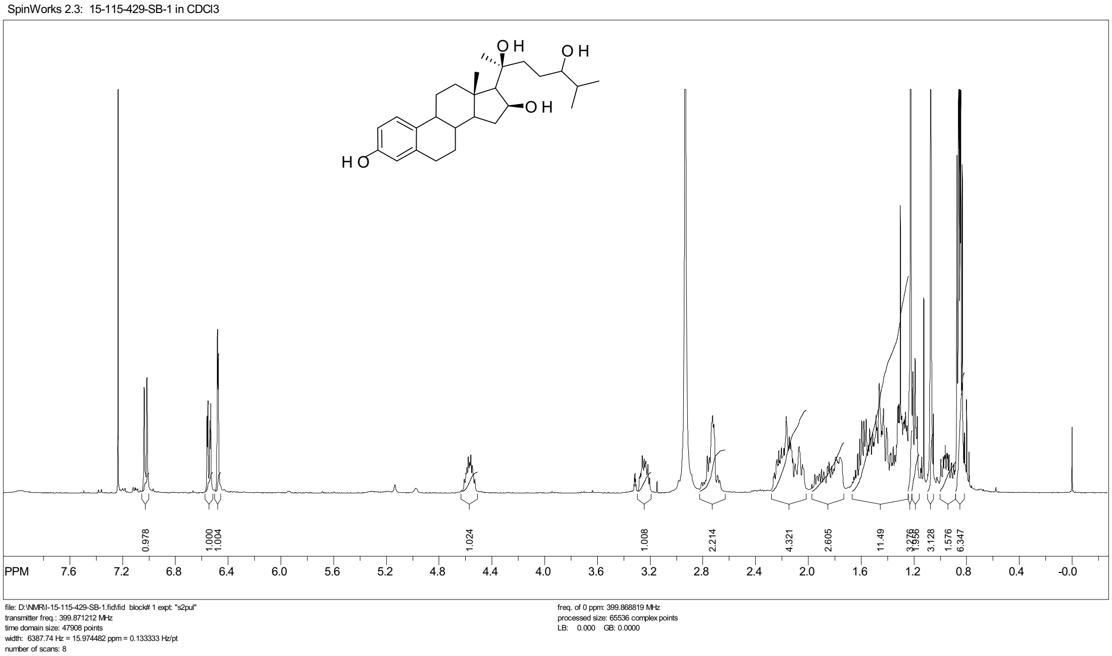
Remark: **175** was completely converted to **171** during the period of ^{13}C experiment.



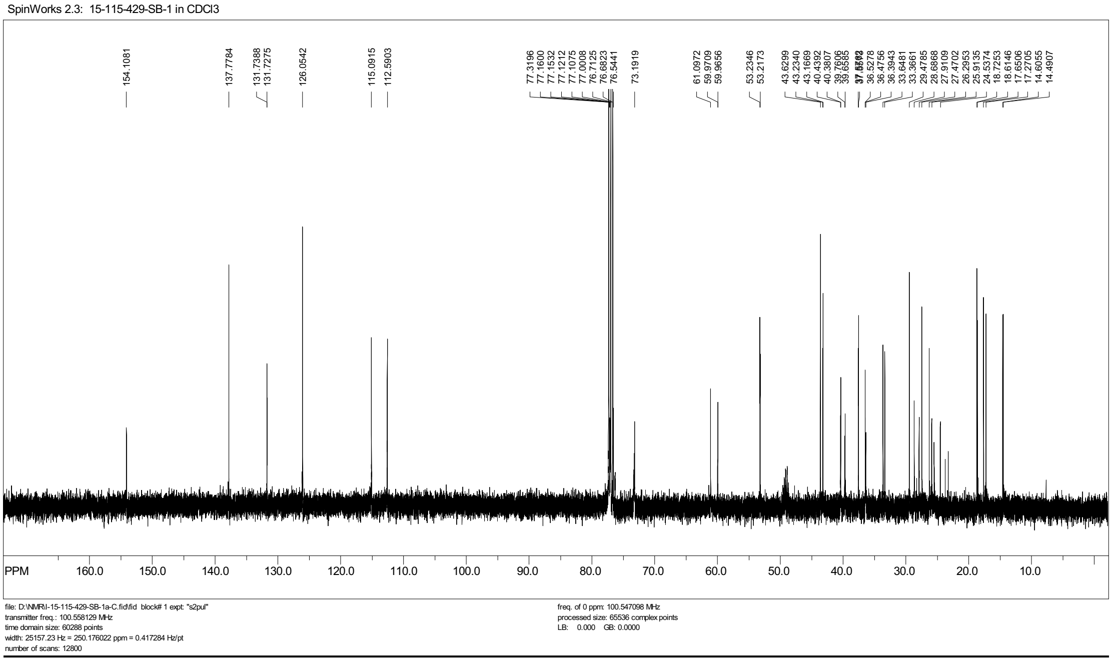
Apendix Figure 84 400 MHz ^{1}H NMR spectrum: 3,24-dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$- estratriene (173)



Apendix Figure 85 100 MHz ^{13}C NMR spectrum: 3,24-dihydroxy-(16*S*, 20*S*)-16, 20-acetonide-cholestan-$\Delta^{1,3,5(10)}$- estratriene (173)



Apendix Figure 86 400 MHz ^{1}H NMR spectrum: 3, 16*S*, 20*S*, 24-tetrahydroxy-24-cholestan-$\Delta^{1,3,5(10)}$- estratriene (174)



Apendix Figure 87 100 MHz ^{13}C NMR spectrum: 3, 16*S*, 20*S*, 24-tetrahydroxy-24-cholestan-$\Delta^{1,3,5(10)}$- estratriene (174)

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OUTPUT

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1. **First synthesis of 3,16,20-polyoxygenated cholestanes, new cytotoxic steroids from the gorgonian *Leptogorgia sarmentosa***

Suthinee Boonananwong, Boonsong Kongkathip, Ngampong Kongkathip,* ***Steroids***, 2008, *73, 1123-1127.*

2. **Synthesis and Biological Evaluation of 3,16,20-Polyoxygenated Steroids of Marine Origin and their Analogs**

Suthinee Boonananwong, Boonsong Kongkathip, Ngampong Kongkathip,* **Kasetsart J. (Nat. Sci.)** *42 : 531-542* (2008)