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THESIS

SYNTHESIS OF 16β-HALO CYPROTERONE ACETATE

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Cyproterone acetate is a progestin offers useful therapeutic treatment for androgen-mediated diseases such as proatate cancer, acne, androgenic alopecia, precocious puberty and benign prostatic hyperplasia. The substitution on the C-16 of cyproterone acetate affects the progestation and anti-androgenic activities. Therefore, introduction of substiuents on the C-16 could enhance biological activity.

16β-Bromo, 16β-chloro and 16β-iodo cyproterone acetate were synthesized from the same key intermediate, 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione. The key intermediate has been successfully synthesized by two different approaches. The first approach involved 6 steps starting from a commercially available 16-dehydropregnenolone acetate with an overall yield of 12.7%. The second approach starting from cyproterone acetate involved only 2 steps with an overall yield of 79%.

16β-Bromo and 16β-chloro cyproterone acetate were obtained from 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione by 3 steps with an overall yield of 60% and 65%, respectively. The key feature of epoxide ring opening at C-16, C-17 using 2 M HCl led to 16β-chloro cyproterone whilst using a mixture of bromine and triphenyl phosphine in tetrahydrofuran provided 16β-bromo cyproterone. Acetylation of 16β-chloro and 16β-bromo cyproterone gave 16β-chloro and 16β-bromo cyproterone acetate.

16β-Iodo cyproterone acetate was first successfully accomplished from 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione with an overall yield of 68%. The key strategy also involved epoxide ring opening at C-16, C-17 using 47% hydroiodic acid in dioxane.

Our successful synthesis of 16β-bromo, 16β-chloro and 16β-iodo cyproterone acetate will be very useful for the development of steroid drugs with progestational and anti-androgenic diseases.

Watcharaporn Thaharn
Student's signature

Boonsong Kongkathip
Thesis Advisor's signature

19 / 04 / 2006

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Watcharaporn Thaharn

March 2006

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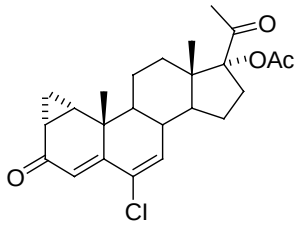
LIST OF ABBREVIATIONS

α	=	alpha
β	=	beta
δ	=	chemical shift (ppm)
ν_{max}	=	maximum absorption frequency
Calcd.	=	calculated
CI-MS	=	chemical ionization mass spectroscopy
COSY	=	Correlation Spectroscopy
cm^{-1}	=	reciprocal centimeter (wave number)
d	=	doublet
dd	=	doublet of doublet
EI-MS	=	electron impact mass spectroscopy
FTIR	=	Fourier Transform Infrared Spectroscopy
g	=	gram
h	=	hour
HRMS	=	High Resolution Mass Spectroscopy
Hz	=	Hertz
J	=	coupling constant
m	=	multiplet
mg	=	milligram
min	=	minutes
mL	=	milliliter
M^+	=	molecular ion
m.p.	=	melting point
m/z	=	a value of mass divided by charge
NMR	=	Nuclear Magnetic Resonance
2D-NMR	=	Two Dimention Nuclear Magnetic Resonance
NOE	=	Neclear Overhauser Effect
ppm	=	part per million
rt	=	room temperature

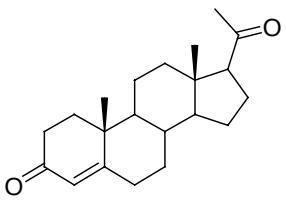
SYNTHESIS OF 16β-HALO CYPROTERONE ACETATE

INTRODUCTION

Cyproterone acetate (**1**), a progestin, offers useful therapeutic treatment for androgen-mediated diseases. It inhibits the effect of androgens on target tissues and reduces the production of testosterone. This drug is commonly used in conjunction with oral contraceptive or sometimes prescribed to women to treat female pattern baldness or hirsutism. When cyproterone acetate is given in combination with the pill, it is likely to reduce hair growth, lighten the hair color, and decrease the hair thickness in hirsute areas. In a study conducted by Peereboom-Wynia and co-workers (1989) showed that female test subjects who were treated with cyproterone acetate exhibited a statistically significant increase in anagen hairs as well as a statistically significant decrease in telogens hairs as compared to the control group. In men, cyproterone acetate competitively inhibits the effect of androgens from both the testis and the adrenal cortex. The sex drive and virility are also reduced and the function of the testis is inhibited. These changes are reversible after discontinuation of the therapy. This drug can have some serious effects on male's sex drive and is sometimes prescribed for people undergoing male-female sex change.



cyproterone acetate (**1**)



progesterone (**2**)

Figure 1 Structures of cyproterone acetate (**1**) and progesterone (**2**)

In 1998, Gruber and co-workers concluded that topically applied cyproterone acetate in combination with liposomes are as effective as oral anti-androgen

medication in acne treatment while reducing the risk of adverse effects and avoiding high serum cyproterone acetate concentrations.

Cyproterone acetate is a drug used in the treatment of prostate cancer (Schering Health Care Limited., 1995). It is the most common cancer in man in many western countries and the second leading cause of cancer deaths in men. Every year in the UK, approximately 21,000 men are diagnosed with prostate cancer and over 10,000 men die from the disease. Treatment of prostate cancer is a type of hormonal therapy which interfere with the production or action of particular hormones in the body. Hormones are substances produced naturally in the body, acting as chemical messengers and help to control the activity of cells and organs. Most prostate cancers need supplies of the male hormone testosterone to grow. This is produced by the testes and adrenal glands. On the surface of the prostate cancer cells are proteins called receptors and cyproterone acetate has a structure similar to testosterone. It works by attaching itself to the receptors on the surface of the prostate cancer cells to block and prevent the attachment of testosterone. Without testosterone, the cancer cells either grow more slowly or stop growing altogether. Cyproterone acetate may be given together with injections of another type of hormonal therapy drug such as goserelin, buserelin, triptorelin or leuprorelin. These drugs block the production of a hormone produced by the pituitary gland (leuteinising hormone), which stimulates the production of testosterone.

In 2002, the Van der Spuy and co-workers studied medical therapies for treatment of hirsutism. Hirsutism is a distressing and relatively common endocrine problem in women which may prove difficult to manage. Cyproterone acetate, an anti-androgen, is frequently used to treat hirsutism, usually in combination with ethylestradiol.

Peereboom-Wynia and co workers (1989) studied the effect of cyproterone acetate on hair root and hair shaft diameter in androgenetic alopecia in females. Twenty female patients suffering from androgenic alopecia were treated during one year with 50 micrograms ethinyl estradiol plus 2 mg of cyproterone acetate. The

therapeutic results warrant the conclusion that cyproterone acetate seems to be effective in androgenetic alopecia in women.

Doring and co-workers (1983) studied the treatment of moderately severe virilism of women with Diane and Androcur 10. They reported on 20 female patients suffering from acne, seborrhea, hirsutism, and androgenic alopecia during the years 1980-1982 who have been treated with 1 tablet of Androcur 10 (10 mg cyproterone acetate) daily, in the first 15 days applied together with Diane (0.05 mg ethinylestradiol and 2 mg of cyproterone acetate). The results achieved after 2 years show an improvement of acne in 80% of the cases, of seborrhea in 95%, and of both hirsutism and androgenic alopecia in 45% each.

Possible side effect of cyproterone acetate, each person's respond to any medicine is unique. Many people have very few side effects with cyproterone acetate, while others may experience more. When cyproterone acetate is taken for a few months, any side effects will only last for as long as taking the drug. In the use of cyproterone, some of the major side effect are as follow; (Tayside University Hospitals NHS Trust, 2000).

1) Liver abnormalities, including development of cancers have been reported in animals. Clinical experience to date has not indicated liver cancers induced in humans.

2) Tiredness is common in the first few weeks of treatment but becomes much less from the third month.

3) Breathlessness may occur.

4) About one patient in five develops transient, or perhaps in some cases, permanent breast enlargement. In rare cases milk production and tender benign nodules of the breast have been reported. Symptoms mostly subside after cessation of treatment or reduction of dosage.

5) During long term treatment, changes in body weight have been reported, chiefly weight gain.

6) Patchy loss of body hair, dry skin, increased growth of scalp hair and lightening of hair color can occur.

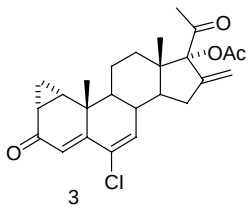
7) In rare instances, bones can become weakened (osteoporosis).

8) Anaemia has been reported in some patients on long term treatment.

9) Rarely adrenal gland failure can occur.

10) Cyproterone acetate inhibits sperm production in men and may lead to gynaecomastia.

The first synthesis of cyproterone acetate was reported by Wiechert in 1966. Cyproterone acetate is an active antiandrogen so far encountered, closely related analogs are also active. Furthemore the chemistry and the progestational potentiating effect of 16-methylene cyproterone acetate (**3**) have been described that a methylene group at C-16 position increases the progestational activity (Shapiro *et al.*, 1969). Therefore, introduction of substiuents on the 16-position to enhance biological activity such as methyl group is very interesting.



3

Figure 2 Structure of 16-methylene cyproterone acetate (**3**)

Otherwise, synthesis of ideal anti-androgenic molecule is still interesting because it can offer useful treatment for androgen-mediated diseases, such as prostate cancer, acne, seborrhea, androgenic alopecia, precocious puberty and benign prostatic hyperplasia. So modification of steroid structures represents a continuing effort on the part of the steroid chemist to alter biological activity. The alterations in activity, although often predictable, are in some case quite unexpected. A variety of steroids have been synthesized showing higher androgenic activity than testosterone concomitant with several new compounds with anti-androgenic activity. Examples of

steroidal molecules with high anti-androgenic effects are cyproterone acetate, which is an antagonist of androgen recepters, and finasteride, which has selectivity for 5-reductase isozyme type 2 inhibitor. The 16-methylene group could increase progestational activity and enhanced the anti-androgenic activity. The 16α- and 16β-methyl group have been described, but the assay data are not available and the synthetic development is still very attractive to steroid chemists.

The objectives of this study are:

1) To synthesize 16β-bromo, 16β-chloro and 16β-iodo cyproterone acetate starting from 16-dehydropregnenolone acetate.

2) To synthesize 16β-bromo, 16β-chloro and 16β-iodo cyproterone acetate starting from cyproterone acetate.

LITERATURE REVIEWS

Biological Activity of Cyproterone Acetate and Related Compounds

Studies on progestational activity

Shapiro *et al*. (1972) reported the progestational activities and the syntheses of 1α,2α-cyclomethylene-16-methylene compounds **3, 9, 10** and the precursor 1,4,6 trienes **5, 7, 8**. In all cases, the trienes exhibited higher progestational activity than the corresponding 1α,2α-cyclomethylene derivatives when were tested intramuscularly in the rabbits as shown in Table 1. Table 1 lists the intramuscular activities of three 1α,2α-cyclomethylene-16-methylene compounds and four corresponding 1,4,6-triene compounds. Included also are the oral progestational activities, except for compound **9** and **5**. The comparison of the activities of related 16-unsubstituted-6-chloro compounds **1** and **6** is also listed. The potentiating effect of the 16-methylene group is revealed in the intramuscular activities found for **7** and **3** as compared to **6** and **1**, respectively. Whereas in the 16-methylene series, the introduction of the 1α,2α-cyclomethylene moiety **(9**, **3**, **10**) decreases the intramuscular progestational activity compared to the corresponding 1,4,6-trienenes (**5**, **7**, **8**) the opposite effect can be observed for the 16-unsubstituted **1** and **6**.

Table1 Progestational activity

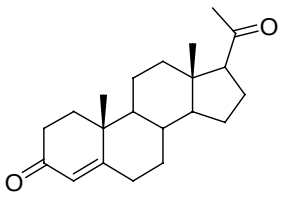
Compounds	Progestational activity	
	Im	Oral
 2	-	1

Table1 (Continued)

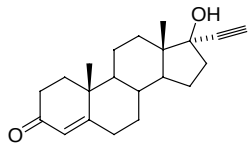
75 15.6

Compounds

Progestational activity

Im

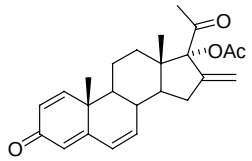
Oral



4

-

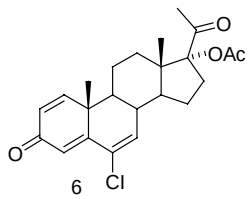
2.8



5

25.5

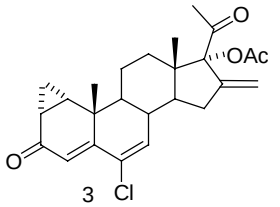
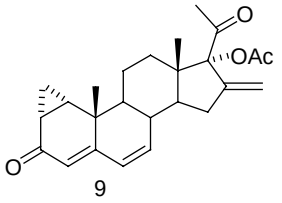
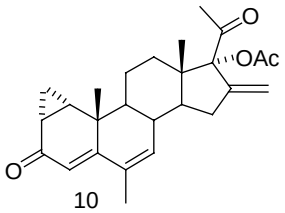
-



6

6.4

Table1 (Continued)

Compounds	Progestational activity	
	Im	Oral
 3	120	15.6
 9	6	-
 10	15.9	45.6

Im = Intramuscular administration

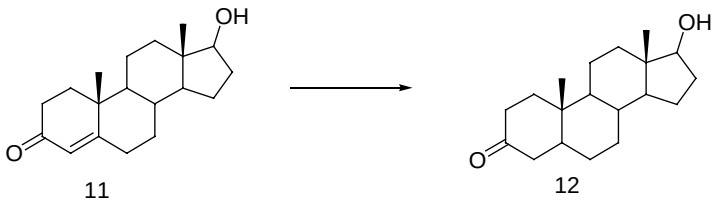
Oral = Oral administration

Studies on anti-androgenic activity

Cyproterone acetate (**1**) was tested for antiandrogenic activity in both normal male and orchiectomized rats. It inhibited the action of testosterone, which was given to orchiectomized rats in daily doses of 100 μg for 7 days. Cyproterone acetate, when

given to pregnant rats from the 16th to the 19th day of gestation, induced feminization of the male fetuses by preventing androgen-dependent differentiation of the male genitalia. Cyproterone acetate, 10 mg/kg orally, showed contraceptive action in 83% of 12 rats. The anti-androgenic activity of cyproterone acetate was further confirmed in testosterone-stimulated, orchiectomized, and adrenalectomized rats, thus indicating that its action is independent of the adrenals. (Popper *et al.*, 1967)

The androgen metabolism of human prostate was regulated by variety of androgen metabolizing enzymes. Among those, the 5α-reductase possesses the highest potential activity. It converts testosterone (**11**) into the more potent androgen dihydrotestosterone (**12**) as shown in Scheme 1. (Ramirez *et al.*, 2002)



11 12

Scheme 1

Thus 5α-reductase dictates the cellular availability of dehydrotestosterone (**12**) and consequently the androgen responsiveness of human prostate. As a result of this, several new clinically useful inhibitor for treatment of benign prostatic hyperplasia and male pattern baldness were developed shortly after the discovery of the enzyme 5α-reductase. Recently, several new inhibitors of 5α-reductase were described as potential clinical candidates for the treatment of benign prostatic hyperplasia. These compounds include finasteride (**13**), episteride (**14**), cyproterone acetate (**1**), BOMT (**15**) and FCE282607 (**16**). (Bratoeff *et al.*, 2000)

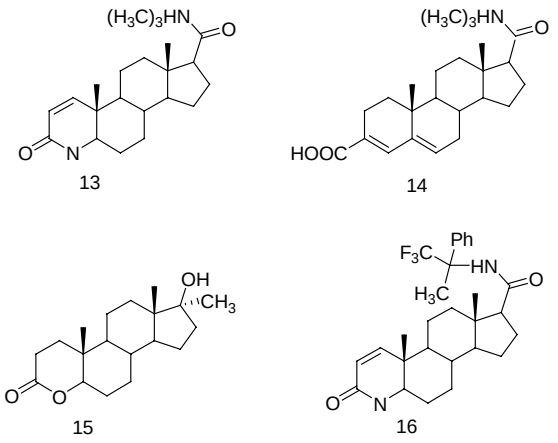


Figure 3 Structures of finasteride (**13**), episteride (**14**),BOMT (**15**) and FCE282607 (**16**)

Finasteride (**13**) inhibits the conversion of testosterone (**11**) to dehydrotestosterone (**12**) in epithelium and stroma of human benign prostatic hyperplasia. This steroid has greater affinity for 5α-reductase type 2 than type 1 enzyme, bout present in the human prostate. The type 2 isoenzyme is the dominant form in genital tissue, however, the exact physiological roles of these enzymes has yet to be fully elucidated.

Recently, the pharmacological evaluation of new steroidal compound as 5α-reductase inhibitors: **17-21** was reported by Ramirez and co-workers in 2002. The pharmacological evaluation was determined in flank organs, seminal vesicles and the effect of the new steroidal compounds on the in vitro metabolism of [3H] testosterone to [3H] dehydrotestosterone in seminal vesicle homogenates of gonadectomized male hamsters. Flank organs are pilosebaceous structures androgen dependent. In males, these glands measured 8 mm and shrink upon castration, until they look as those of females, however daily injuctions of testosterone (**11**) or dehydrotestoterone (**12**) restores their original size. The presence of 5α-reductase in flask organ as well, the inhibition of this enzyme by finasteride (**13**) has been demonstated several years ago. The new steroidal compounds **17-21** decreased the diameter of the pigmented spot as

compared to testosterone (**11**), thus suggesting an inhibitory effect on enzyme 5α-reductase and also the presence of an anti-androgenic effect (Table 2).

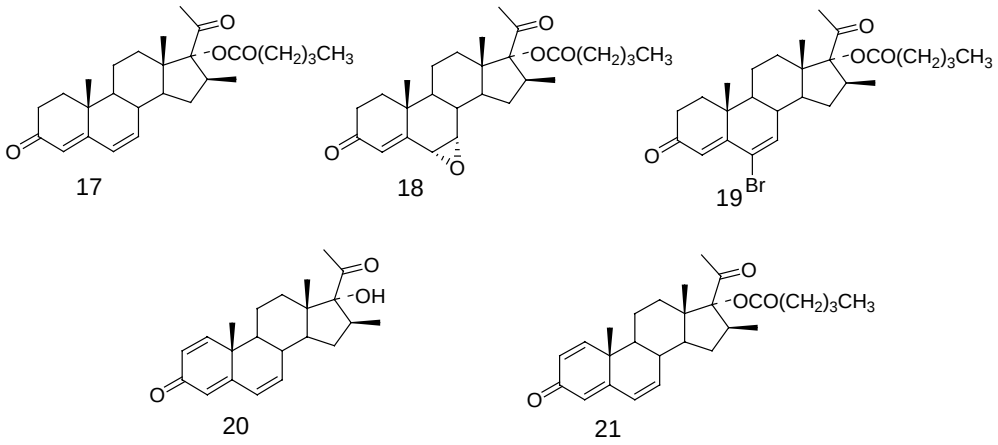


Figure 4 Structures of steroid compounds decreased the diameter of the pigmented spot.

Table 2 The diameter of flank organs and the weight of seminal vesicle

Compounds	Treatment (mg)	Diameter of the pigmented spot (mm)	Weight of seminal vesicle (mg)
Control	0.2	2.75	177
11	0.2	4.25	317
11+13	0.2	3.00	216
11+17	0.2	3.00	265
11+18	0.2	2.00	208
11+19	0.2	3.00	246
11+20	0.2	3.00	231
11+21	0.2	1.80	202

Studies involving cyproterone acetate

Namer *et al.* (1981) studied cyproterone acetate involving clinical applications of anti-androgens. Anti-androgens, substances that prevent androgens from expressing their activity at target cells, play an important role in the treatment of prostate cancer. The most frequently used substances have either a steroidal structure (cyproterone acetate) or a non-steroidal structure (flutamide or anandron). Anti-androgens have been tested both alone and in combination with treatments aimed at inhibiting testicular secretion (castration, LH-RH analogs), there by producing complete blockade of androgen secretion and action. Patients treated by such combination protocols have often shown an improvement in the percentage of remissions and, less often, improvement in survival. Administration of anti-androgens improves the clinical symptoms of patients with benign prostatic hypertrophy, but the exact mechanism of their action requires further investigation. Cutaneous manifestations due to hyperandrogenicity (hirsutism, alopecia, acne) have also been improved by cyproterone acetate, which is often given together with estrogens (reversed sequential regime), by spironolactone or topically applied products. Finally, anti-androgens have been successfully used to treat breast cancer in men, early puberty, hypersexuality and sexual deviations.

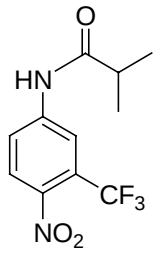
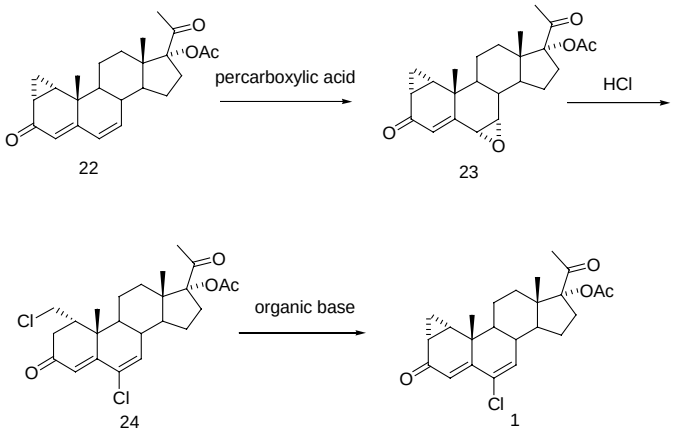


Figure 5 Structure of flutamide

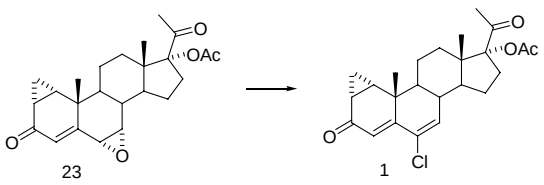
Synthesis of cyproterone acetate (1)

Wiechert *et al.* (1966) reported the first synthesis of cyproterone acetate (**1**) starting from 1α,2α-cyclomethylene-17α-acetoxy-4,6-pregnadiene-3,20-dione (**22**). The preparation of 6α,7α-epoxy-1α,2α-cyclomethylene-17α-acetoxy-4-pregnene-3,20-dione (**23**) from 1α,2α-cyclomethylene-17α-acetoxy-4,6-pregnadiene-3,20-dione (**22**) was accomplished by using m-CPBA. Treatment of 6α,7α-epoxy steroid (**23**) with hydrochloric acid in acetic acid provided cyclopropane ring opening product **24** which was subsequently ring closured to give the cyproterone acetate (**1**) by treatment with collidine as shown in Scheme 2.



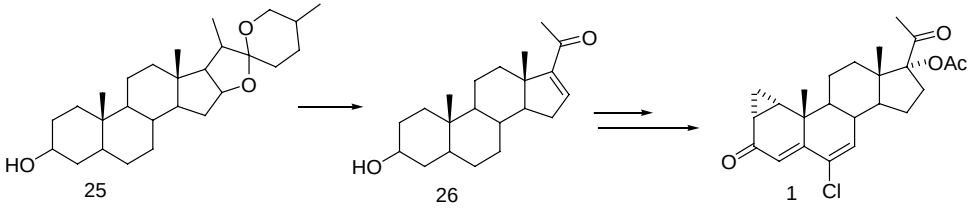
Scheme 2

In 1973, Harris and Miskowowicz reported the successful conversion of 6α,7α-epoxy-1α,2α-methylene-17α-acetoxy-4-pregnene-3,20-dione (**23**) to cyproterone acetate (**1**) without cyclopropane ring opening by using carbinoliminium salt chloride as shown in Scheme 3.



Scheme 3

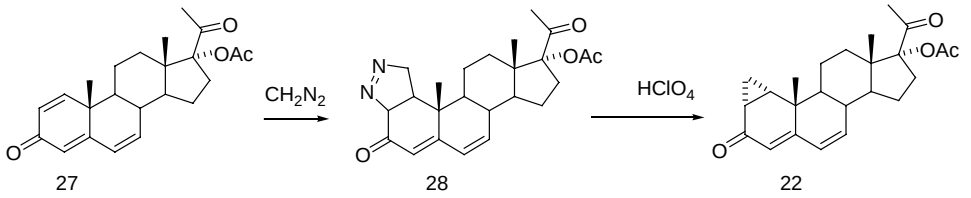
Recently in 2003, Kongkathip and co-workers reported the synthesis of cyproterone acetate (**1**) starting from tigogenin (**25**) obtained from the waste juice of *Agave sisalana* leaves as shown in Scheme 4.



Scheme 4

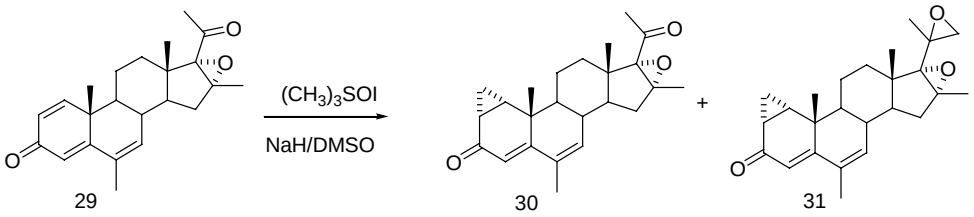
Introduction of the 1α,2α-cyclopropane moiety into 1,4,6-triene-3-one system

The introduction of the 1α,2α-cyclopropane moiety into 1,4,6-triene-3-one system has been accomplished by two methods. The first method utilized diazomethane to give 1α,2α-pyrazoline **28** (Wiechert *et al*., 1966) followed by treatment with strong acid provided 1α,2α-cyclomethylene-17α-acetoxy-4,6-pregnadiene-3,20-dione (**22**) (Krakower *et al*., 1966; Shapiro *et al*., 1969) as shown in Scheme 5.



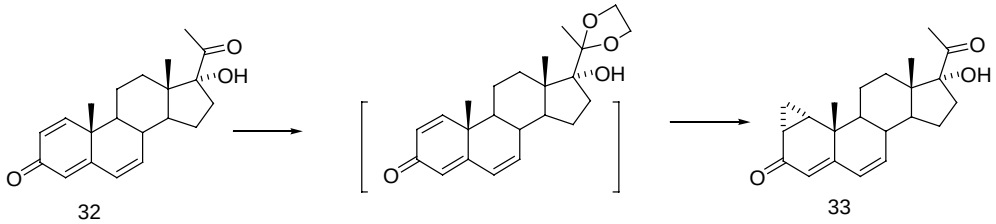
Scheme 5

The second method utilized trimethylsulfoxonium iodide (Corey *et al.,* 1965) to effect the formation of 1α-2α-cyclopropane moiety of unsaturated ketone **30** and epoxy steroid **31** as shown in Scheme 6.



Scheme 6

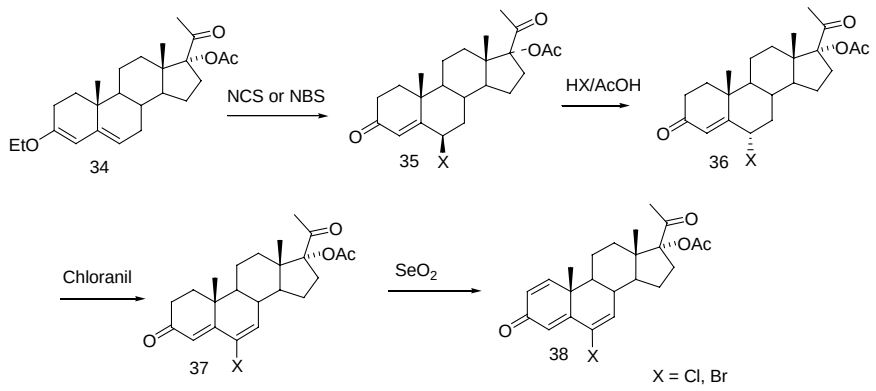
In 1986, Amold and co-workers reported a multisteps process for the production of 1α,2α-cyclomethylene-17α-hydroxy-4,6-pregnadiene-3,20-dione (**33**) involving ketalization of the 20-keto group of 17α-hydroxy-1,4,6-pregnatriene-3,20-dione (**32**). Then, cyclopropyl was introduced into the C-1,2 position using trimethylsulfoxonium iodide and potassium hydroxide followed by hydrolysis of the cyclic ketal group using H_2SO_4 to provide 1α,2α-cyclicmethylene-17α-hydroxy-4,6-pregnadiene-3,20-dione (**33**) as shown in Scheme 7.



Scheme 7

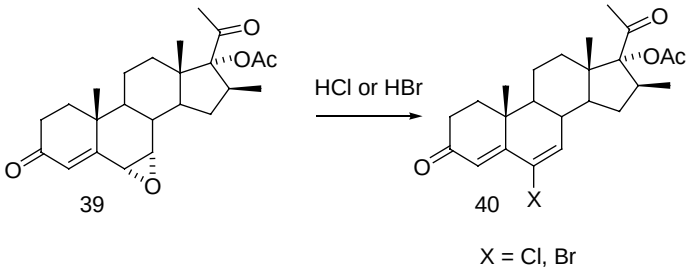
Synthesis of 6-halo progestational agents

17α-Acetoxyprogesterone-3-ethyl enol ether (**34)** was halogenated using *N*-halosuccinimide to afford 6β-halo-17α-acetoxy progesterone (**35**), then converted to 6α-halo-17α-acetoxy progesterone (**36**) by treatment with hydrogen halide in acetic acid. 6-Dehydro-halo-17α-acetoxy progesterone (**37**) was obtained by choranill oxidation of 6α-halo-17α-acetoxy progesterone (**36**) followed by oxidative dehydrogenation of **37** using selenium dioxide to furnish 6-halo-1,4,6-pregnatriene-3,20-dione (**38**) as shown in Scheme 8. (Ringold *et al*., 1959)



Scheme 8

In 1999, Cabeza and co workers reported the synthesis of halo vinyl compound **40** by treatmemt of 6α,7α-epoxy derivative **39** with hydrochloric or hydrobromic acid in acetic acid as shown in Scheme 9.

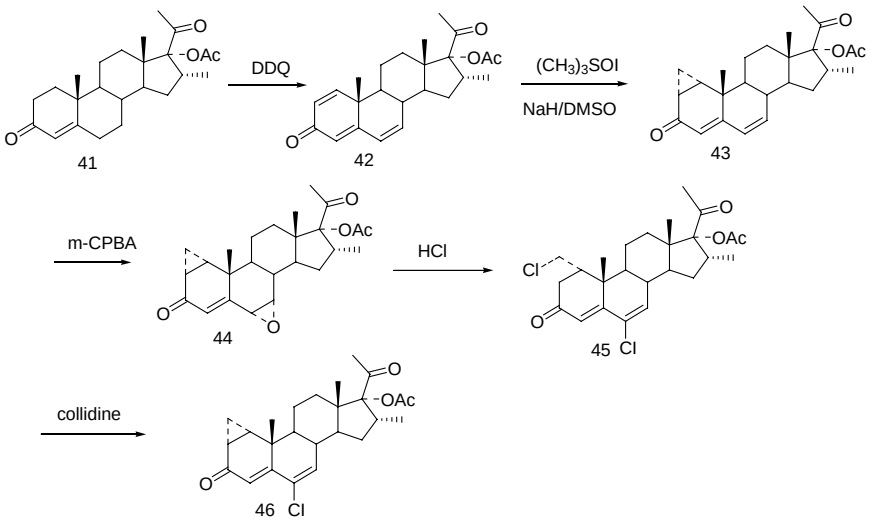


Scheme 9

Synthesis of 16α-methyl cyproterone acetate (46)

In 1973, Ursula Lachnit-Fixson reported the synthesis of 16α-methyl cyproterone acetate (**46**). Dehydrogenation of 16α-methyl-17α-acetoxy-progesterone (**41**) using 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) provided 16α-methyl-17α-acetoxy-1,4,6-pregnatriene-3,20-dione (**42**). A cyclopropyl was introduced into the C-1,2 position of 16α-methyl-17α-acetoxy-1,4,6-pregnatriene-3,20-dione (**42**) by using trimethyl sulfoxonium iodide to afford 1α,2α-methylene-16α-methyl-17α-acetoxy-4,6-pregnadiene-3,20-dione (**43**). Epoxidation of 1α,2α-methylene-16α-

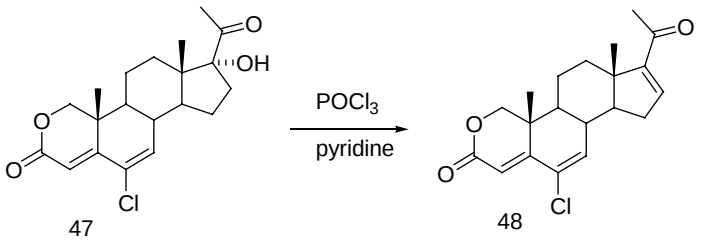
methyl-17α-acetoxy-4,6-pregnadiene-3,20-dione (**43**) using m-CPBA led to epoxy intermediate **44** which was then treated with hydrogen chloride to provide 1α-chloromethyl-6-chloro-16α-methyl-17α-acetoxy-4,6-pregnadiene-3,20-dione (**45**). 16α-Methyl cyproterone acetate (**46**) was obtained in high yield when 1α-chloromethyl-6-chloro-16α-methyl-17α-acetoxy-4,6-pregnadiene-3,20-dione (**45**) was treated with collidine as shown in Scheme 10.



Scheme 10

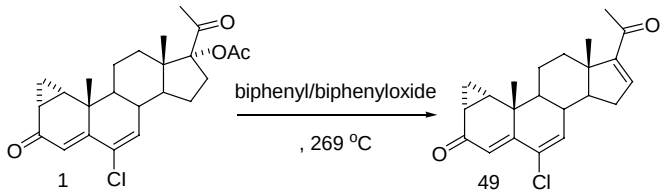
Synthesis of 16,17-olefinic steroid

Takegawa *et al*. (1993) reported a conversion of 17α-hydroxy steroid (**47**) to the corresponding 16,17-olefinic steroid (**48**) by treatment with phosphorus oxychloride in pyridine as shown in Scheme 11.



Scheme 11

In 1981, Kerb and co-workers reported a single step and high yield conversion of cyproterone acetate (**1**) to 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**49**) by heating in a mixture solution of biphenyl/biphenyloxide at 269 ^{o}C as shown in Scheme 12.

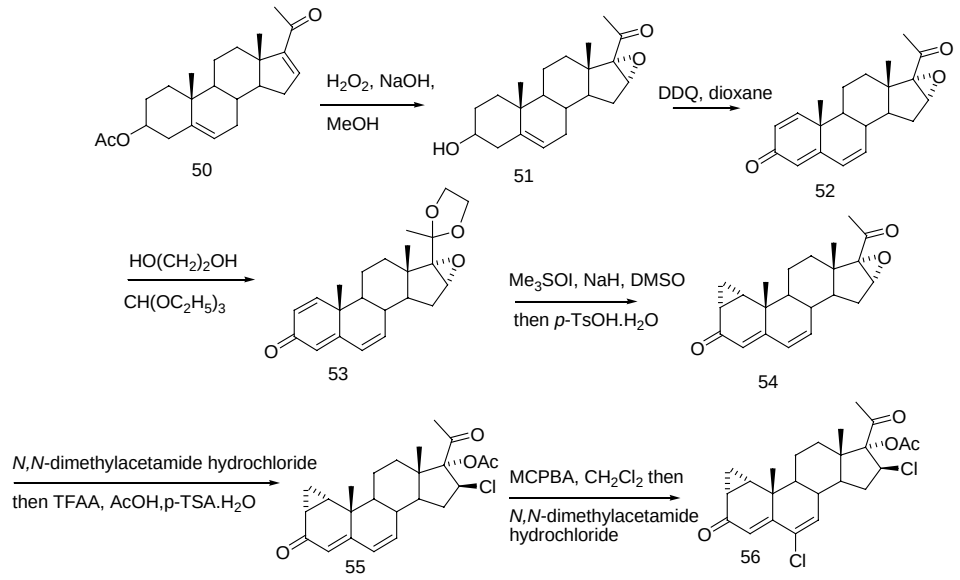


Scheme 12

Synthesis of 16β-bromo cyproterone acetate (59) and 16β-chloro cyproterone acetate (56)

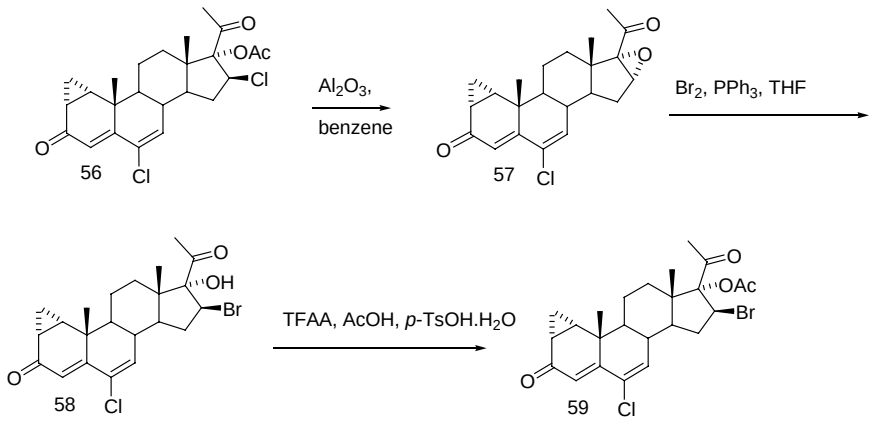
The first synthesis of 16β-bromo cyproterone acetate (**59**) and 16β-chloro cyproterone acetate (**56**) were reported by Kongkathip *et al.*(2003). In the synthesis of 16β-chloro cyproterone acetate (**56**), a commercially available 16-dehydropregnenolone acetate (**50**) was used as starting material. 1,4,6-Trienone epoxide **52**, a key intermediate, was prepared in 2 steps by epoxidation of 16-dehydropregnenolone acetate (**50**) using hydrogen peroxide in base to give epoxide steroid (**51**) followed by oxidative-dehydrogenation using 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ). Ketalization of the 20-keto group of 1,4,6-trienone epoxide **52** using ethylene glycol, ethyl orthoformate and *p*-toluenesulfonic acid provided 16α,17α-epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (**53**). A cyclopropyl ring was introduced into the C-1,2 position of compound **53** by using trimethyl sulfoxonium iodide then hydrolysis of 20-ketal group using *p*-toluenesulfonic acid afforded 1α,2α-cyclomethylene-16α-17α-epoxy-4,6-pregnadiene-3,20-dione (**54**). Epoxide ring opening of compound **54** using *N*,*N*-dimethylacetamide hydrochloride gave 16β-chloro steroid intermediate, which was then immediately acetylated using acetic acid, trifluoroacetic anhydride and *p*-toluenesulfoic acid to give the compound

55. 16β-Chloro cyproterone acetate (**56**) was obtained by treatment of compound **55** with m-chloroperbenzoic acid to generate 6α,7α-epoxy intermediate which was treated with *N*,*N*-dimethylacetamide hydrochloride in dimethylsulfoxide. (Scheme 13)



Scheme 13

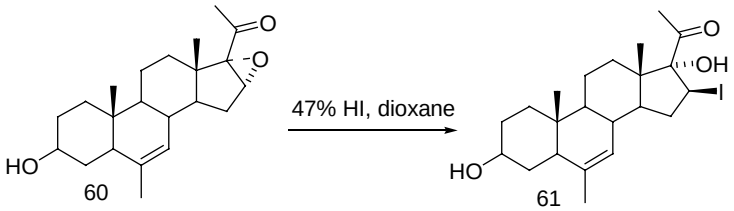
The synthesis of 16β-bromo cyproterone acetate (**59**) was accomplished in 3 steps from 16β-chloro cyproterone acetate (**56**). The first step was epoxide ring closure of compound **56** using basic Al_2O_3 in benzene to produce 1α,2α-cyclomethylene-16α-17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) followed by bromohydrin formation using a mixture of bromine and triphenyl phosphine in tetrahydrofuran. Finally, acetylation of bromohydrin intermediate **58** to give 16β-bromo cyproterone acetate (**59**) was achived in good yield by treatment with acetic acid, trifluoroacetic anhydride and *p*-toluenesulfoic acid as shown in Scheme 14. (Kongkathip *et al*., 2003)



Scheme 14

Synthesis of 16β-iodo pregnanes

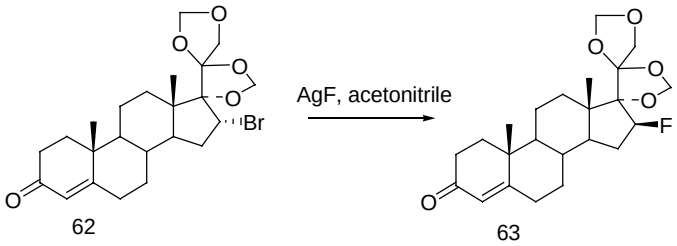
In 1965, Eeaton and co-workers reported the synthesis of 16β-iodo-3β,17α-dihydroxy-6-methyl-5-pregnen-20-one (**61**) by treatment of 6-methyl-16α,17α-oxidopregnenolone (**60**) with 47% aqueous hydroiodic acid in dioxane as shown in Scheme 15.



Scheme 15

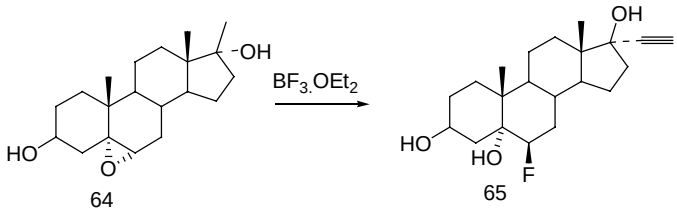
Introduction of the fluoro moiety into steroids molecules

Moreland *et al*. (1962) reported the preparation of 16β-fluoro-17α,20; 21-bis-methylenedioxy-(20*R*)-pregn-4-ene-3one (**63**) by replacement of 16α-bromo steroid (**62**) with silver fluoride in acetonitrile as shown in Scheme 16.



Scheme 16

6β-Fluoro-17α-ethynyl-androstane-3β,5α,17β-triol (**65**) was obtained by treatment of the 5α,6α-epoxide **64** with boron trifluoride in benzene as shown in Scheme 17. (Lawrence *et al*., 1959)



Scheme 17

MATERIALS AND METHODS

Materials

Instrumentation

Melting points (m.p.) were determined on a MEL-TEMP electrothermal apparatus at Department of Chemistry, Kasetsart University and are reported uncorrected in ^{o}C.

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

^{1}H NMR and ^{13}C NMR spectra, operated at 400, 300 and 100 MHz, respectively, were determined on a VARIAN ^{UNITY} INOVA 400 MHz spectrometer at the Department of Chemistry, Faculty of Science, Kasetsart University. Chemical shifts (δ) were recorded as δ values (ppm) with tetramethylsilane (TMS) as internal reference. Deuterochloroform were used as sovents. Coupling constants (*J*) are reported in Hertz, and multiplicity is defined as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, and m = multiplet.

Mass Spectra (MS) were measured on AGILENT 1100 series LC/MSB TRAP at the Kasetsart University.

Elemental analysis was obtained on LECO CHNS-932 at the Kasetsart University.

Chromatographic systems

1. Thin-layer chromatography (TLC)

Analytical Thin-Layer Chromatography (TLC) was carried out on aluminum sheets with 230-400 mesh silica gel (Merck) impregnated with a fluorescent indicator (254 nm). The chromatograms were visualized under ultraviolet light (UV nm) and by spraying with vanillin reagent, followed by heating in the oven.

2. Column chromatography (CC)

Column chromatography was performed on glass column using Merck silica gel 60 (60-230 mesh). The size of chromatographic column used depended on the amount (weight) of the sample. The ratio of sample and the adsorbent was about 1:40-80 by weigh.

3. Flash column chromatography

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

Chemical reagents

Solvents and reagents used for synthesis were obtained from commercial sources and used directly without purification unless noted.

Dry tetrahydrofuran (THF) was freshly distilled under N_2 from sodium with benzophenone ketyl as an indicator.

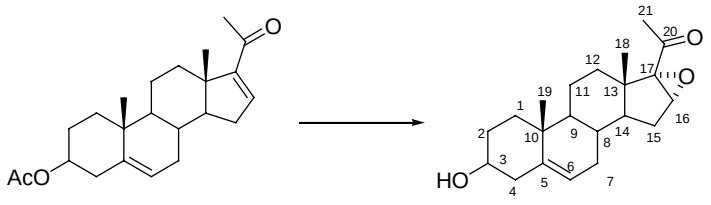
Dioxane was dried over fresh potassium hydroxide pellets for 24 h then refluxed over excess of sodium for 6-12 h and distilled.

Dichloromethane was dried over anhydrous calcium chloride and distilled from calcium hydride immediately before use.

Vanillin reagent was prepared from vanillin (6 g) in ethanol (200 mL) and then treated dropwise with *conc.* H_2SO_4 (6 mL) with stirring at 0 ^{o}C.

Methods

3β-Hydroxy-16α,17α-epoxy-5-pregnene-20-one (51)



50 51

To a cold (0 °C) solution of 16-dehydropregnenolone acetate (**50**) (100 mg, 0.28 mmol) in methanol (15 mL) was added dropwise, aqueous solution of sodium hydroxide (0.4 mL, 4 N) and 40% hydrogenperoxide (0.4 mL, 5.27 mmol). After being stirred at room temperature for 24 h, the reaction mixture was poured into saturated aqueous NaCl (12 mL) and extracted with dichloromethane (3x8 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford 3β-hydroxy-16α,17α-epoxy-5-pregnene-20-one (**51**) (83 mg, 90%) as a white solid which was directly engaged in the next step without further purification. Recrystallization of compound **51** from ethyl acetate gave a white plates with m.p. 175-177 °C.

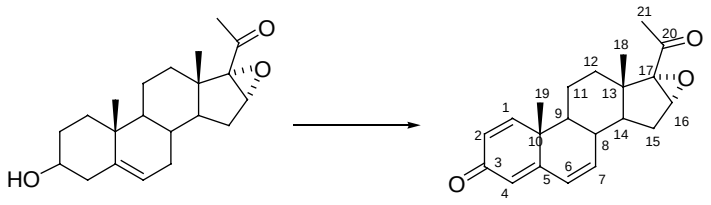
FTIR (KBr), ν_{max}, cm^{-1}: 3347 (O-H), 1635 (C=O), 1600 (C=C), 1324 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.3 (s, 1H, C-6), 3.65 (s, 1, C-16), 3.50-3.34 (m, 1H, C-3), 2.25-1.61 (m, 9H, C-2, C-4, C-7, C-8, C-15), 1.60-1.25 (m, 8H, C-1, C-9. C-11, C-12, C-14), 2.01 (s, 3H, C-21), 1.1 (s, 3H, C-18), 1.0 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 400 MHz), δ: 202.5 (C-20), 126.8 (C-5), 122.7 (C-6), 72.3 (C-17), 70.7 (C-3), 61.5 (C-16), 45.5 (C-9), 42.3 (C-14), 41.5 (C-13), 39.2 (C-15), 38.5 (C-11), 36.5 (C-4), 34.5 (C-10), 32.3 (C-1), 31.6 (C-2), 31.3 (C-8), 29.2 (C-21), 26.5 (C-7), 20.1 (C-12), 19.6 (C-18), 16.7 (C-19)

MS (EI), *m/z* (relative intensity): 330 (M^+, 8), 312 (100), 287 (32), 273 (21), 239 (15), 145 (32), 91 (12), 55 (12)

16α,17α-Epoxy-1,4,6-pregnatriene-3,20-dione (52)



51 52

To a solution of 3β-hydroxy-16α,17α-epoxy-5-pregnene-20-one (**51**) (600 mg, 1.82 mmol) in dry dioxane (20 mL) under nitrogen atmosphere was added, a solution of 2,3-dicyano-6,7-dichloro-p-benzoquinone (DDQ) (2.04 g, 8.96 mmol) in dry dioxane (20 mL). After being stirred and refluxed for 24 h, the resulting suspension was filtered and the filtrate was evaporated under reduced pressure. The residue was diluted with dichloromethane (20 mL) and filtered through a short column of neutral alumina. The filtrate was concentrated under reduced pressure and purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/5) to provide 16α,17α-epoxy-1,4,6-pregnatriene-3,20-dione (**52**) (383 mg, 65%) as a yellow solid. Recrystallization of compound **52** from ethyl acetate gave a colorless prism with m.p. 175-176 °C.

FTIR (KBr), ν_{max}, cm^{-1}: 1697 (C=O), 1649 (C=C), 1600 (C=C), 1342 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.08 (d, *J* = 10.1 Hz, 1H, C-1), 6.28 (d, *J* = 10.1 Hz, 1H, C-2), 6.27 (d, *J* = 9.8 Hz, 1H, C-7), 6.03 (d, *J* = 0.9 Hz, 1H, C-4), 5.96 (dd, *J* = 9.8, 1.9 Hz, 1H, C-6), 3.78 (s, 1H, C-16), 2.45-2.40 (m, 1H, C-15), 1.91-1.68 (m, 3H, C-9, C-8, C-14), 1.57-1.38 (m, 5H, C-11, C-12, C-15), 2.06 (s, 3H, C-21),1.24 (s, 3H, C-18), 1.19 (s, 3H, C-19)

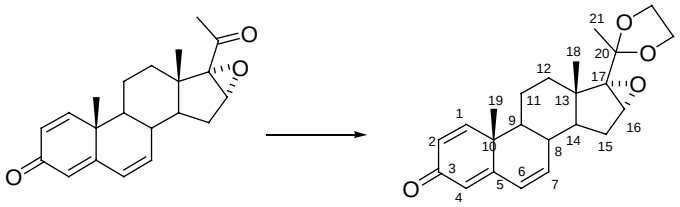
^{13}C NMR ($CDCl_3$, 75 MHz), δ: 204.7 (C-3), 186.4 (C-20), 162.3 (C-5), 152.8 (C-1), 137.2 (C-7), 128.4 (C-2), 124.3 (C-4,6), 70.5 (C-17), 60.3 (C-16), 48.9 (C-10), 43.2 (C-14), 42.4 (C-13), 41.4 (C-15), 35.8 (C-9), 31.4 (C-11), 27.1 (C-8), 26.0 (C-21), 21.6 (C-12), 21.0 (C-18), 15.3 (C-19)

MS (EI), *m/z* (relative intensity): 324 (M^+, 5), 281 (23), 264 (100), 248 (30), 170 (30), 128 (30), 91(12), 55 (25)

HRMS calcd. for $C_{21}H_{24}O_3$ [M^+]: 324.1725. Found: 324.1723**.**

Anal. calcd. for $C_{21}H_{24}O_3$: C, 77.75; H, 7.46. Found: C, 77.58; H, 7.50.

16α, 17α-Epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (53)



A solution of 16α,17α-epoxy-1,4,6-pregnatriene-3,20-dione (**52**) (220 mg, 0.68 mmol), ethylene glycol (0.23 mL, 4.11 mmol), triethylorthoformate (0.33 mL, 2.17 mmol) and *p*-toluene sulfonic acid (22.05 mg, 0.12 mmol) in dichloromethane (15 mL) was stirred at room temperature for 8 h. Triethylamine (0.3 mL) was added to the reaction mixture and diluted with water (5 mL). The aqueous layer was extracted with dichloromethane (3x10 mL) and the combined organic extracts were washed with water (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/5) to provide 16α,17α-epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (**53**) (179 mg, 72%). Recrystallization of compound **53** from ethyl acetate/hexane gave a white prism with m.p. 144-146 °C.

FTIR (KBr), ν_{max} , cm^{-1}: 1701 (C=O), 1658 (C=C), 1603 (C=C), 1043 (C-O)

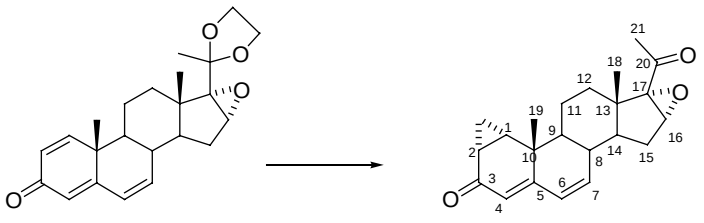
^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.06 (d, *J* = 10.1 Hz, 1H, C-1), 6.27 (dd, *J* = 10.1, 1.9 Hz, 1H, C-2), 6.26 (dd, *J* = 9.8, 2.9 Hz, 1H, C-7), 6.03 (d, *J* = 0.9 Hz, 1H, C-4), 5.99 (dd, *J* = 9.8 ,1.9 Hz, 1H, C-6), 3.92-4.04 (m, 4 H, -O-CH_2CH_2-O-), 3.46 (s, 1H, C-16), 2.45-2.39 (m, 1H, C-15), 2.07-1.83 (m, 3H, C-8, C-9, C-14), 1.74-1.21 (m, 5H, C-11, C-12, C-15), 1.44 (s, 3H, C-21), 1.23 (s, 3 H, C-18), 1.09 (s, 3 H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 186.5 (C-3), 162.6 (C-20), 152.9 (C-5), 137.8 (C-1), 128.3 (C-7), 128.1 (C-2), 124.1 (C-4), 1.09.4 (C-6), 70.5 (C-17), 65.3 (C-OH), 64.8 (C-OH), 58.4 (C-16), 48.9 (C-10), 43.7 (C-14), 42.6 (C-13), 41.5 (C-15), 35.9 (C-9), 32.8 (C-11), 26.6 (C-8), 23.7 (C-21), 21.7 (C-18), 21.0 (C-12), 15.8 (C-19)

MS (EI), *m/z* (relative intensity): 368 (M^+, 2), 350 (21), 320 (6), 293 (23), 87 (100)

HRMS calcd. for $C_{23}H_{28}O_4$: 368.1988 Found: 368.1978.

1α,2α-Cyclomethylene-16α,17α-epoxy-4,6-pregnadiene-3,20-dione (54)



53 54

To a suspension of sodium hydride (718 mg, 17.9 mmol) in dimethyl sulfoxide (10 mL) under nitrogen atmosphere was added dropwise, a solution of trimethyl sulfoxonium iodide (3.54 g, 15.5 mmol) in dimethyl sulfoxide (20 mL). After being stirred for 30 min at room temperature, a solution of 16α,17α-epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (**53**) (1.2 g, 3.30 mmol) in dimethyl

sulfoxide (24 mL) was added to the above reaction mixture and stirred at room temperature for 2 h. The reaction mixture was diluted with cold water (15 mL) and extracted with dichloromethane (3x10 mL). The combined organic layers were washed with water (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane (25 mL) then, *p*-toluenesulfonic acid (60 mg) was added and stirred at room temperature for 2 h. A cold aqueous solution of 20% K_2CO_3 (15 mL) was then added to the reaction mixture and extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with water (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/6) to provide 1α,2α-cyclomethylene-16α,17α-epoxy-4,6-pregnadiene-3,20-dione (**54**) (0.79 g, 72%). Recrystallization of compound **54** from ethyl acetate gave white crystal with m.p. 236-238 °C.

FTIR (KBr), ν_{max} , cm^{-1}: 1701 (C=O), 1657 (C=C), 1621 (C=C)

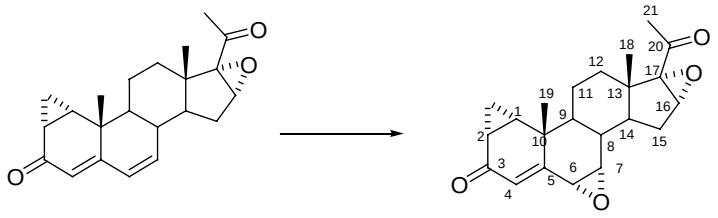
^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.09 (dd, *J* = 9.8, 2.8 Hz, 1H, C-7), 5.94 (dd, *J* = 9.8, 1.9 Hz, 1H, C-6), 5.54 (s, 1H, C-4), 3.78 (s, 1H, C-16), 2.35-2.32 (m, 1H, C-15), 2.21-1.85 (m, 4H, C-1, C-8, C-9, C-14), 1.98 (s, 3H, C-21), 1.80-1.34 (m, 5H, C-2, C-11, C-12, C-15), 1.45-1.37 (m, 1H, C-cyclopropane), 1.23 (s, 3 H, C-18), 1.17 (s, 3H, C-19), 0.83-0.80 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 205.0 (C-3), 199.0 (C-20), 147.0 (C-5), 138.2 (C-7), 128.8 (C-6), 120.6 (C-4), 60.4 (C-17), 49.2 (C-16), 43.2 (C-10), 35.4 (C-14), 31.4 (C-11), 27.1 (C-9), 26.0 (C-13), 25.5 (C-8), 25.8 (C-15), 25.4 (C-21), 22.9 (C-1), 20.9 (C-2), 16.1 (C-12), 15.2 (C-18), 14.7 (C-19), 12.8 (C-cyclopropane)

MS (EI), *m/z* (relative intensity): 338 (M^+, 8), 295 (80), 278 (100), 263 (27), 107 (5)

HRMS calcd. for $C_{23}H_{26}O_3$: 338.1882 Found: 338.1876

1α,2α-Cyclomethylene-6α,7α-epoxy-16α,17α-epoxy-4-pregnene-3,20-dione (66)



54 66

m-Chloroperbenzoic acid (225.2 mg, 1.31 mmol) was added to a solution of 1α,2α-cyclomethylene-16α,17α-epoxy-4,6-pregnadiene-3,20-dione (**54**) (200 mg, 0.59 mmol) in dichloromethane (20 mL). After being stirred at room temperature for 12 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (12 mL) and extracted with dichloromethane (3x9 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane: 2/5) to provide 1α,2α-cyclomethylene-6α,7α-epoxy-16α,17α-epoxy-4-pregnene-3,20-dione (**66**) (126 mg, 60%) as a white solid with m.p. 168-169 °C.

FTIR (KBr), ν_{max}, cm^{-1}: 1694 (C=O), 1657 (C=C), 1373 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.85 (d, *J* = 1.2 Hz, 1H, C-4), 3.71 (s, 1H, C-16), 3.34 (d, *J* = 3.6 Hz, 1H, C-6), 3.18 (d, *J* = 3.6 Hz, 1H, C-7), 2.15-2.12 (m, 1H, C-8), 2.04-2.00 (m, 1H, C-15), 1.97 (s, 3H, C-21), 1.92-1.70 (m, 5H, C-1, C-2, C-15, C-14) 1.53-1.17 (m, 5H, C-11, C-12, C-cyclopropane), 1.17 (s, 3H, C-18), 1.05 (s, 3H, C-19), 0.81-0.77 (m, 1H, C-cyclopropane)

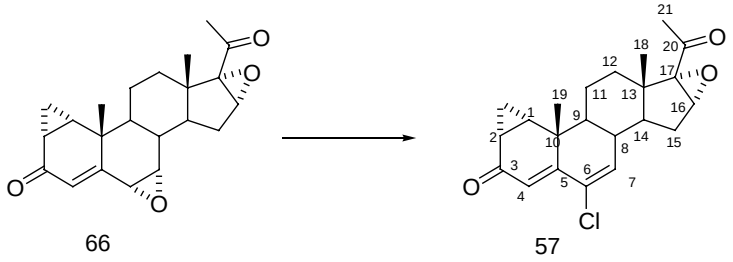
^{13}C NMR ($CDCl_3$, 100 MHz), δ: 204.3 (C-3), 196.8 (C-20), 154.6 (C-5), 127.4 (C-4), 70.0 (C-17), 59.9 (C-16), 53.7 (C-6), 52.2 (C-7), 41.9 (C-10), 40.8 (C-

14), 38.0 (C-9), 36.7 (C-13), 32.2 (C-1), 30.8 (C-15), 26.5 (C-11), 25.6 (C-8), 25.0 (C-2), 24.15 (C-21), 23.5 (C-12), 20.2 (C-18), 14.7 (C-19), 12.8 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 355 (M^++1, 100), 337 (16), 319 (4), 313 (3), 295 (11), 277 (3)

Anal. calcd**.** for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39 Found: C, 74.58; H, 7.35

<u>1α,2α-Cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (57)</u>



To a stirred solution of 1α,2α-cyclomethylene-6α,7α-epoxy-16α,17α-epoxy-4-pregnene-3,20-dione (**66**) (130 mg, 0.37 mmol) in dimethylsulfoxide (10 mL) under nitrogen atmosphere was added, a solution of *N,N*-dimethyl acetamide hydrochloride (138 mg, 1.12 mmol) in dimethylsulfoxide (5 mL). After being stirred at 65 °C for 30 h, the reaction mixture was cooled to room temperature and poured into water (8 mL) and extracted with ethyl acetate (3 x 6 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduce pressure. The residue was dissolved in benzene (10 mL) then added basic alumina oxide (600 mg) and stirred at room temperature for 24 h. The resulting suspension was filtered and washed with dichloromethane (3x10 mL). The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 2/5) to give 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) (95 mg, 70 %) as a colorless solid; m.p.189-190 °C.

FTIR (KBr), ν_{max}, cm^{-1}: 1703 (C=O), 1663 (C=C), 1234 (C-O)

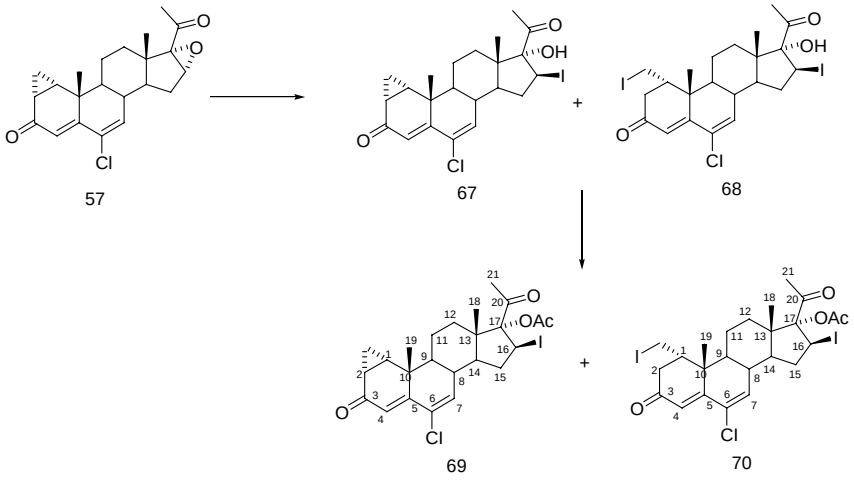
^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.10 (s, 1H, C-4), 6.06 (d, J = 2.2 Hz, 1H, C-7), 3.69 (s, 1H, C-16), 2.31-2.28 (m, 1H, C-15), 2.12-2.07 (m, 1H, C-8), 1.98 (s, 3H, C-21), 1.97-1.51 (m, 5H, C-1, C-2, C-15, C-14), 1.50-1.19 (m, 6H, C-12, C-11, C-9, C-cyclopropane) 1.16 (s, 3H, C-18), 1.06 (s, 3H, C-19), 0.80-0.75 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 205.0 (C-20), 198.8 (C-3), 152.9 (C-5), 136.9 (C-7), 130.9 (C-6), 121.1 (C-4), 70.9 (C-17), 60.7 (C-16), 49.1 (C-10), 43.5 (C-14), 42.9 (C-9), 39.5 (C-13), 36.6 (C-1), 31.7 (C-15), 27.4 (C-11), 26.7 (C-8), 26.5 (C-2), 25.9 (C-21), 23.4 (C-12), 21.3 (C-18), 15.6 (C-19), 13.0 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 374 (M^++2, 3), 372 (M^+, 10), 329 (24), 313 (24), 294(100), 279 (34), 109 (8), 91 (20)

Anal. calcd. for $C_{22}H_{25}ClO_3$**:** C, 70.86; H, 6.76 Found: C, 70.86; H, 6.67

<u>1α,2α-Cyclomethylene-16β-iodo-17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (69)</u>



To a solution of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) (50 mg, 0.13 mmol) in dioxane under nitrogen atmosphere was added, 47 % hydroiodic acid (0.2 mL, 1.12 mmol). After being stirred at room temperature for 24 h, the reaction mixture was poured into water (12 mL) and stirred for an additional 1 h. The yellow precipitate was filtered to afford a mixture of 1α,2α-cyclomethylene-16β-iodo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**67**) and 1α-iodomethyl-6-chloro-16β-iodo-17α-hydroxy-4,6-pregnadiene-3,20-dione (**68**) (90 mg) which was used in the next step without further purification.

Acetic acid (0.42 mL, 7.3 mmol) was slowly added to a solution of trifluoroacetic anhydride (1 mL, 7.2 mmol) in dichloromethane (2 mL). After being stirred under nitrogen at room temperature for 30 min, *p*-toluenesulfonic acid (60 mg, 0.32 mmol) was added to the resulting mixture solution and cooled to 0 ^{o}C. An ice-cooled solution of 1α,2α-cyclomethylene-16β-iodo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**67**) and 1α-iodomethyl-6-chloro-16β-iodo-17α-hydroxy-4,6-pregnadiene-3,20-dione (**68**) (90 mg) in dichloromethane (3 mL) was added to the above mixture at 0 ^{o}C. After 40 min, a cooled saturated aqueous $NaHCO_3$ (5 mL) was added carefully to the reaction mixture and extracted with dichloromethane (3x10 mL). The combined organic layers were washed with water (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/4) to give 1α,2α-cyclomethylene-16β-iodo-17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (**69**) (28 mg, 38%) and 1α-iodomethyl-6-chloro-16β-iodo-17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (**70**) (35 mg, 39%) both as light yellow solids. Recrystallization of compound **69** and **70** from hexane/ethyl acetate gave a colorless crystal with m.p. 141-143 °C and m.p. 150-152 °C, respectively.

69:

FTIR (KBr), ν_{max}, cm $^{-1}$: 1735 (C=O), 1650 (C=C), 1242 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.12 (s, 1H, C-4), 6.08 (d, *J* = 2.1 Hz, 1H, C-7), 4.10 (t, *J* = 8.4 Hz, 1H, C-16), 2.74-2.65 (m, 1H, C-15), 2.49-2.40 (m, 1H, C-8), 2.06 (s, 3H, OAc), 2.09 (s, 3H, C-21), 2.10-1.55 (m, 4H, C-1, C-2, C-15, C-14), 1.54-1.25 (m, 6H, C-12, C-11, C-9, C-cyclopropane),1.16 (s, 3H, C-18), 1.05 (s, 3H, C-19), 0.81-0.77 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 200.4 (C-3), 198.4 (C-20), 171.0 (C=O, OAc), 152.5 (C-5), 136.1 (C-7), 131.3 (C-6), 121.4 (C-4), 92.4 (C-17), 49.5 (C-16), 49.2 (C-10), 48.3 (C-14), 41.3 (C-9), 39.3 (C-13), 38.4 (C-1), 33.6 (C-15), 28.0 (C-11), 26.7 (C-OAc), 25.9 (C-8), 23.5 (C-2), 21.6 (C-21), 21.1 (C-12), 19.5 (C-18), 16.8 (C-19), 12.9 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 544 (M^++2, 7), 542 (M^+, 23), 507 (1), 482 (13), 477 (55), 447 (19),415 (100), 373 (71), 357 (37), 314 (9), 283 (20), 153 (13), 109 (8), 91 (20)

Anal. calcd. for $C_{24}H_{28}ClIO_4$: C, 53.10; H, 5.20. Found: C, 53.39; H, 4.935.

70:

FTIR (KBr), ν_{max}, cm $^{-1}$: 776 (C=O), 1687 (C=C), 1267 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.37 (s, 1H, C-4), 6.25 (d, *J* = 2.0 Hz, 1H, C-7), 4.25 (t, *J* = 8.6 Hz, 1H, C-16), 3.48-3.44 (m, 1H, C-I), 3.02-2.97 (m, 1H, C-I), 2.91-2.85 (m, 1H, C-2), 2.88-2.80 (m, 1H, C-2), 2.74-2.67 (m, 1H, C-15), 2.51-2.46 (m, 1H, C-8), 2.42-2.38 (m, 1H, C-1), 2.13 (s, 3H, OAc), 2.18 (s, 3H, C-21), 2.13-1.21 (m, 7H, C-9, C-11, C-12, C-14, C-15), 1.32 (s, 3H, C-18), 1.3 (s, 3H, C-19)

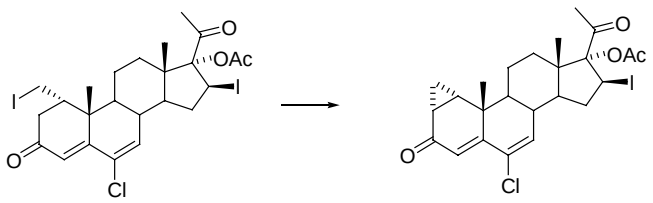
^{13}C NMR ($CDCl_3$, 100 MHz), δ: 200.4 (C-3), 197.5 (C-20), 171.2 (C=O, OAc), 154.9 (C-5), 137.8 (C-7), 131.6 (C-6), 124.1 (C-4), 92.1 (C-17), 49.5 (C-16),

49.3 (C-10), 45.4 (C-14), 45.4 (C-9), 42.9 (C-13), 41.3 (C-1), 40.0 (C-8), 38.4 (C-15), 33.4 (C-2), 27.9 (C-11), 21.7 (C-I), 20.6 (C-OAc), 20.3 (C-21), 19.1 (C-12), 16.8 (C-18), 6.1 (C-19)

MS (CI), *m/z* (relative intensity): 672 (M^++2, 32), 670 (M^+, 70), 655 (23), 610 (38), 569 (18), 543 (98), 416 (33), 415 (100), 357 (32), 313 (12), 150 (3), 109 (3)

Anal. calcd. for $C_{24}H_{29}ClI_2O_4$: C, 42.98; H 4.36. Found: C, 42.95; H, 4.06.

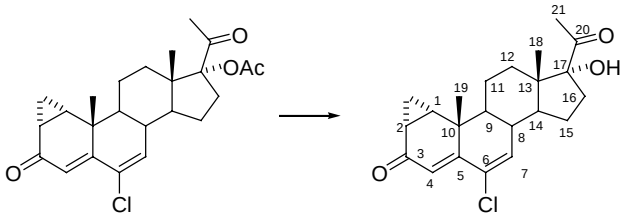
16β-Iodo cyproterone acetate (69)



70 69

1,8-Diazabicyclo[5.4.0.]undec-7-en (0.1 mL, 0.67 mmol) was added to a stirred solution of 1α-iodomethyl-6-chloro-16β-iodo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**70**) (40 mg, 0.06 mmol) in benzene (6 mL). After 2 h at room temperature, the reaction mixture was poured into water (6 mL) and extracted with dichloromethane (3x6 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to afford 16β-iodo cyproterone acetate (**69**) as a white solid (38 mg, 77%).

1α,2α-Cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (71)



1 71

Sodium hydroxide (38 mg, 0.95 mmol) was added to a solution of cyproterone acetate (**1**) (200 mg, 0.48 mmol) in methanol (10 mL). After being stirred at room temperature for 3 h, the reaction mixture was poured into water (10 mL) and extracted with dichloromethane (3x10 mL). The combined organic layers were washed with water (10 mL), saturated aqueous NaCl (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product of 1α,2α-cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (**71**) (170 mg, 95 %) was obtained as a white solid which was directly engaged in the next step without further purification. Recrystallization of compound **71** from ethyl acetate gave a colorless crystal with m.p. 238-240 °C.

FTIR (KBr), ν_{max}, cm $^{-1}$: 3443 (O-H), 1716 (C=O), 1641 (C=C), 1605 (C=C), 1372 (C=O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.17 (d, 1H, J = 2.15 Hz, C-7), 6.08 (s, 1H, C-4), 3.15 (s, 1H, O-H), 2.72-2.65 (m, 1H, C-16), 2.24-2.22 (m, 1H, C-8), 2.22 (s, 3H, C-21), 1.98-1.42 (m, 7H, C-1, C-2, C-15, C-14, C-11), 1.50-1.10 (m, 4H, C-9, C-12, C-cyclpropane),1.15 (s, 3H, C-18), 0.72 (s, 3H, C-19), 0.80-0.76 (m, 1H, C-cyclopropane)

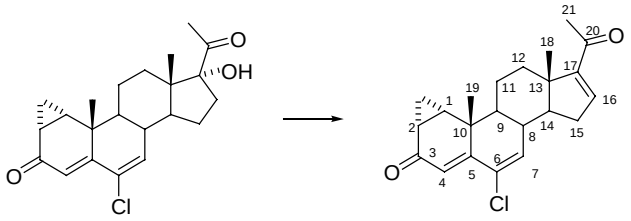
^{13}C NMR ($CDCl_3$, 100 MHz), δ: 212 (C-3), 199.0 (C-20), 153.2 (C-5), 137.8 (C-7), 130.6 (C-6), 120.9 (C-4), 90 (C-17), 49.3 (C-10), 48.4 (C-14), 48.4 (C-9), 39.4

(C-13), 38.8 (C-1), 34.1 (C-11), 30.7 (C-15), 28.5 (C-11), 26.8 (C-8), 25.9 (C-2), 23.9 (C-21), 23.5 (C-12), 21.3 (C-19), 15.8 (C-18), 13.1 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 376 (M^++2, 7), 374 (M^+, 22), 356 (24), 321 (12), 313 (100), 257 (43)

Dehydration of 1α,2α-cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (71)

Using thionyl chloride



71 72

Thionyl chloride (0.20 mL, 0.5 mmol) was added to a cooled solution of 1α,2α-cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (**71**) (60 mg, 0.16 mmol) in pyridine (5 mL). After being stirred under nitrogen atmosphere at room temperature for 3 h, the reaction mixture was poured into a cold water (8 mL) and extracted with dichloromethane (3x6 mL). The combined organic layers were washed with water (6 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/9) to give the 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**72**) (12 mg, 21%). Recrystallization of compound **72** from hexane provided a colorless crystal with m.p. 243-245 °C.

Using phosphorus oxychloride

To a cooled solution of 1α,2α-cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (**71**) (2 g, 5.35 mol) in pyridine (30 mL) under nitrogen atmosphere was added phosphorus oxychloride (20 mL, 0.22 mol). After being stirred at room temperature for 14 days, the reaction mixture was poured into a cold water (40 mL) and extracted with dichloromethane (3x30 mL). The combined organic layers were washed with water (35 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/9) to give the 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**72**) (120 mg, 6.3 %).

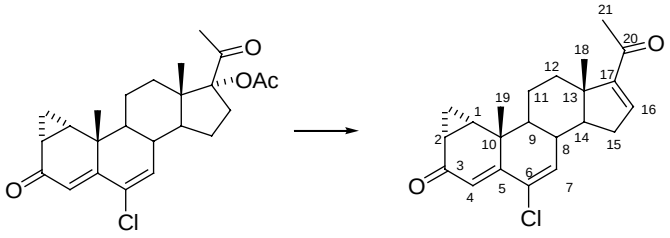
FTIR (KBr), ν_{max}, cm $^{-1}$: 1659 (C=O), 1583 (C=C), 1367 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.73 (dd, *J* = 3.6, 2.0 Hz, 1H, C-16), 6.24 (d, *J* = 1.8 Hz, 1H, C-7), 6.18 (s, 1H, C-4), 2.54-2.44 (m, 3H, C-15, C-8), 2.25-1.5 (m, 4H, C-1, C-2, C-14, C-9), 2.28 (s, 3H, C-21), 1.51-1.16 (m, 5H, C-11, C-12, C-cyclopropane), 1.23 (s, 3H, C-18), 0.98 (s, 3H, C-19), 0.86-0.82 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 198.8 (C-3), 197.0 (C-20), 155.5 (C-5), 153.1 (C-17), 144.0 (C-16), 137.3 (C-7), 130.9 (C-6), 121.1 (C-4), 54.1 (C-14), 49.2 (C-9), 47.3 (C-10), 39.6 (C-13), 37.4 (C-1), 34.9 (C-15), 32.2 (C-11), 27.7 (C-8), 26.6 (C-2), 25.9 (C-21), 23.4 (C-12), 21.6 (C-19), 16.3 (C-18), 13.0 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 358 (M^++, 26), 356 (M^+, 79), 313 (27), 283 (100), 278 (12), 187 (53)

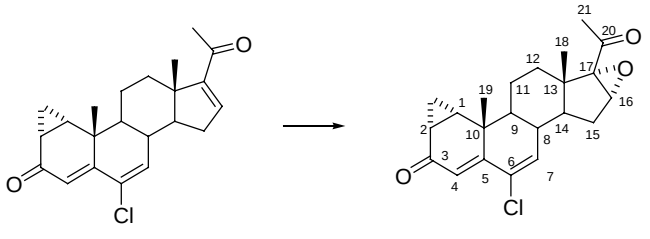
Deacetylation of cyproterone acetate (1)



1 72

A stirred solution of cyproterone acetate (**1**) (200 mg, 0.48 mmol) in a solution mixture of biphenyl/biphenyloxide (1:3) (4 mL) was heated at 269 ^{o}C under nitrogen atmosphere for 90 min. After cooling to room temperature, the reaction mixture was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/9) to give 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**72**) (160 mg, 94 %).

Epoxidation of 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (72)

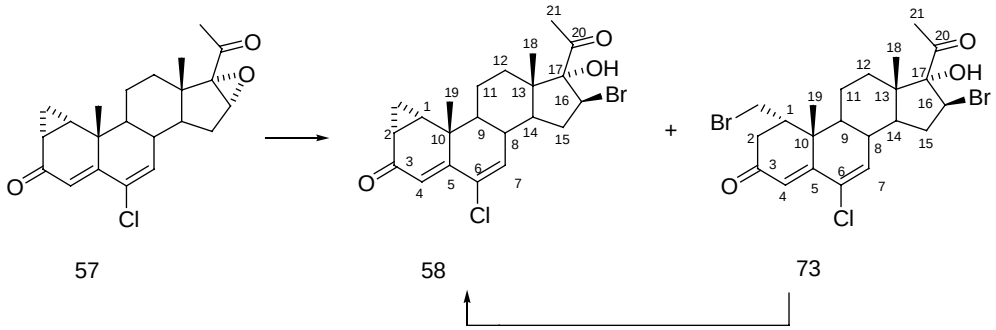


72 57

To a cooled (0 °C) solution of 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**72**) (50 mg, 0.13 mmol) in methanol (10 mL) was added dropwise, aqueous solution of 2.5 N sodium hydroxide (0.05 mL) and 40% hydrogenperoxide (0.18 mL, 2.4 mmol). After being stirred at room temperature for 1 h, the reaction mixture was poured into saturated aqueous NaCl (10 mL) and extracted with dichloromethane (3x6 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced

pressure to afford 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) (46 mg, 88%) as a white solid which was directly engaged in the next step without further purification.

16β-Bromo-1α,2α-cyclomethylene-6-chloro-17-hydroxy-4,6-pregnadiene-3,20-dione (58)



To a solution of bromine (0.1 mL, 1.9 mmol) in dry tetrahydrofuran (5 mL) was added dropwise, a solution of triphenylphosphine (125 mg, 0.48 mmol) in dry tetrahydrofuran (2 mL). After being stirred at room temperature for 30 min, a solution of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) (50 mg, 0.13 mmol) in dry tetrahydrofuran (2 mL) was added dropwise and stirred for an additional 30 min. The reaction mixture was poured into water (10 mL) and extracted with ethyl acetate (3x8 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was dissolved in dichloromethane (6 mL) and stirred at room temperature for 2 days. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/9) to provide 16β-bromo-1α,2α-cyclomethylene-6-chloro-17-hydroxy-4,6-pregnadiene-3,20-dione (**58**) (42 mg, 70%) and 1α-bromomethylene-16β-bromo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**73**) (11 mg, 15%) both as light yellow solids. Recrystallization of compound **58** and **73** from hexane/ethyl acetate gave a white crystal with m.p. 180-182 °C and 179-181 °C, respectively.

58:

FTIR (KBr), ν_{max}, cm $^{-1}$: 3443 (O-H), 1717 (C=O), 1636 (C=C), 1606 (C=C)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.19 (s, 1H, C-4), 6.17 (d, *J* = 2.0 Hz, 1H, C-7), 4.14 (dd, *J* = 7.9, 6.21 Hz, 1 H, C-16), 2.86-2.81 (m, 1H, C-15), 2.52-2.47 (m, 1H, C-8), 2.45 (s, 3H, C-21), 2.07-1.42 (m, 8H, C-1, C-2, C-9, C-11, C-12, C-14), 1.42-1.22 (m, 1H, C-cyclopropane), 1.40 (s, 3 H, C-18), 1.27 (s, 3 H, C-19), 0.87-84 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 205.3 (C-3), 198.7 (C-20), 153.1 (C-5), 137.5 (C-7), 130.4 (C-6), 120.7 (C-4), 89.0 (C-17), 51.6 (C-16), 48.8 (C-16), 48.2 (C-10), 47.1 (C-9), 39.3 (C-13), 38.1 (C-1), 38.0 (C-15), 31.5 (C-11), 28.3 (C-8), 26.6 (C-2), 25.8 (C-21), 23.4 (C-12), 20.6 (C-18), 15.6 (C-19), 13.0 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 456 (M^++4, 3), 454 (M^++2, 10), 452 (M^+, 6), 392 (38), 371 (100), 349 (12), 289 (24), 260 (11), 91 (3)

Anal. calcd for $C_{22}H_{26}BrClO_3$: C, 58.23; H, 5.77. Found: C, 58.24; H, 5.70.

73:

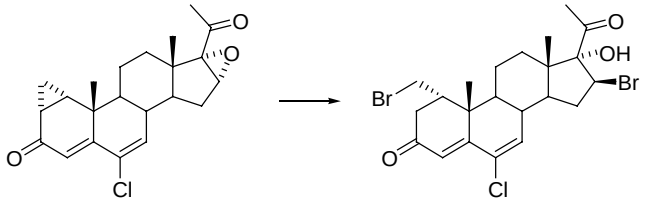
FTIR (KBr), ν_{max}, cm $^{-1}$: 3450 (O-H), 1735 (C=O), 1643 (C=C), 1612 (C=C)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.43 (s, 1H, C-4), 6.26 (d, 1H, *J* = 2.0 Hz, 1H, C-6), 4.16-4.13 (m, 1 H, C-16), 3.60-3.56 (m, 1H, C-Br), 3.10 (t, *J* = 11.2 Hz, 1H, C-Br), 3.02 (d, *J* = 17.6 Hz, 1H, C-2), 2.86-2.78 (m, 2H, C-2, C-15), 2.56-2.52 (m, 1H, C-8), 2.47-2.45 (m, 1H, C-1), 2.44 (s, 3H, C-21), 2.29-1.31 (m, 7H, C-9, C-11, C-12, C-14, C-15), 1.37 (s, 3H, C-18), 1.32 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 206.0 (C-3), 197.8 (C-20), 155.5 (C-5), 138.5 (C-7), 131.2 (C-6), 124.3 (C-4), 89.8 (C-17), 51.1 (C-16), 49.2 (C-10), 47.7 (C-14), 45.4 (C-9), 44.6 (C-1), 42.5 (C-13), 38.2 (C-15), 38.2 (C-2), 38.1 (C-11), 32.8 (C-8), 31.5 (C-Br), 29.6 (C-21), 20.6 (C-18), 19.7 (C-12), 16.5 (C-19)

MS (CI), *m/z* (relative intensity): 538 (M^++6, 0.2), 536 (M^++4, 1), 534 (M^++2, 2), 532 (M^+, 1.4), 499 (16), 453 (39), 455 (42), 417 (42), 373 (100), 313 (4)

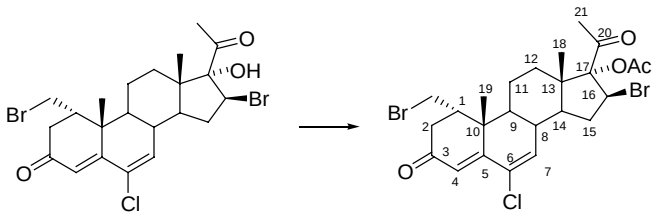
1α-Bromomethylene-16β-bromo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (73)



57 73

To a cooled (0 ^{o}C) solution of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadine-3,20-dione (**57**) (40 mg , 0.11 mmol) in glacial acetic acid (10 mL) was added dropwise, 48% hydrobromic acid (0.1 mL, 0.88 mmol). After being stirred at room temperature for 6 h, the reaction mixture was poured into water (10 mL). The white precipitate was filtered to afford 1α-bromomethylene-16β-bromo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**73**) (48 mg, 84%) which was used in the next step without further purification.

1α-Bromomethyl-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (74)



73 74

Acetic acid (0.2 mL, 3.50 mmol) was slowly added to a solution of trifluoroacetic anhydride (0.68, 4.80 mmol) in dichloromethane (2 mL). After being stirred at room temperature for 30 min, *p*-toluenesulfonic acid (38 mg, 0.2 mmol) was added to the reaction mixture and cooled to 0 ^{o}C. An ice-cooled solution of 1α-bromomethylene-16β-bromo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**73**) (145 mg, 0.27 mmol) in dichloromethane (6 mL) was then added to the reaction mixture at 0 ^{o}C. After stirring for 50 min, a cooled saturated aqueous $NaHCO_3$ (8 mL) was added carefully until the mixture was basic and extracted with dichloromethane (3x9 mL). The combined organic layers were washed with water (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to give 1α-bromomethyl-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**) as a yellow solid (120 mg, 77%); m.p. 165 ^{o}C. Recrystallization of compound **74** from hexane/ethyl acetate gave a colorless crystal.

FTIR (KBr), ν_{max}, cm $^{-1}$: 1738 (C=O), 1651 (C=C), 1239 (C-O), 1029 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.38 (s, 1H, C-4), 6.26 (d, *J* = 1.8 Hz, 1H, C-7), 4.25(dd, *J* = 7.2, 8.2 Hz, 1H, C-16), 3.59-3.55 (m, 1H, C-Br), 3.12 (t, *J* = 8.4 Hz, 1H, C-Br), 3.02-3.01 (m, 1H, C-2), 2.86-2.78 (m, 1H, C-15) 2.86-2.79 (m, 1H, C-2), 2.58-2.53 (m, 1H, C-8), 2.47-2.45 (m, 1H, C-1), 2.18 (s, 3H, AcO), 2.17 (s, 3 H, C-

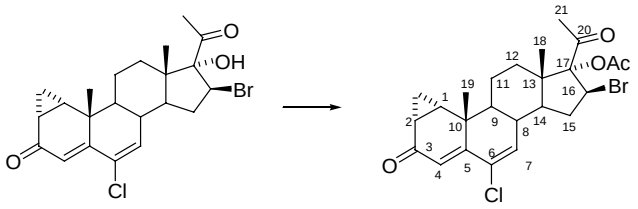
21), 2.06-1.97 (m, 1H, C-15), 1.88-1.78 (m, 2H, C-11, 14), 1.52-1.48 (m, 1H, C-11), 1.49-1.42 (m, 3H, C-9,12), 1.32 (s, 3H, C-18), 1.3 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 199.3 (C-3), 196.9 (C-20), 169.9 (C=O, AcO), 154.5 (C-5), 137.3 (C-7), 130.9 (C-6), 123.8 (C-4), 94.0 (C-17), 49.3 (C-10), 48.3 (C-16), 47.1 (C-14). 44.8 (C-9), 44.0 (C-1), 41.8 (C-13), 38.2 (C-15), 37.5 (C-2), 37.5 (C-11), 37.5 (C-8), 32.1 (C-Br), 28.1 (C -AcO), 21.2 (C-21), 19.9 (C-18), 19.3 (C-12), 15.5 (C-19)

MS (CI), *m/z* (relative intensity): 580 (M^++6, 1), 578 (M^++4, 7), 576 (M^++2, 10), 574 (M^+, 6), 514 (11), 471 (12), 414 (15), 390 (31), 331 (100), 91 (8)

Anal. calcd for $C_{24}H_{29}Br_2ClO_4$: C, 49.98; H, 5.07. Found: C, 49.70; H, 4.878.

<u>Acetylation of 16β-bromo cyproterone (58)</u>



58 59

Acetic acid (0.07 mL, 1.22 mmol) was slowly added to a stirred solution of trifluoroacetic anhydride (0.16 mL, 1.20 mmol) in dichloromethane (2 mL). After being stirred at room temperature for 30 min, *p*-toluenesulfonic acid (10 mg, 0.05 mmol) was added to the reaction mixture and cooled to 0 ^oC. An ice-cooled solution of 1α,2α-cyclomethylene-6-chloro-16β-bromo-17-hydroxy-4,6-pregnadiene-3,20-dione (**58**) (30 mg, 0.07 mmol) in dichloromethane (1 mL) was added to the above mixture at 0 ^oC. After being stirred for 20 min, a cooled saturated aqueous $NaHCO_3$ (5 mL) was added carefully to the reaction mixture and extracted with dichloromethane (3x5 mL). The combined organic layers were washed with water (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue

was purified by flash column chromatography on silica gel (ethyl acetate /hexane: 1/3) to provide 16β-bromo cyproterone acetate (**59**) as a white solid (26 mg, 78.8%); m.p. 165-166 ^{o}C.

FTIR (KBr), ν_{max}, cm $^{-1}$: 1738 (C=O), 1651 (C=C), 1239 (C-O), 1029 (C-O)

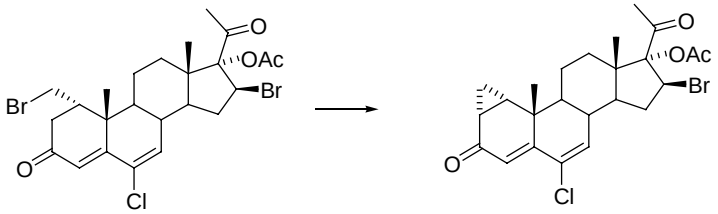
^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.11 (s, 1H, C-4), 6.08 (d, *J* = 2.01 Hz, 1H, C-7), 4.17 (dd, *J* = 7.2, 6.8 Hz, 1H, C-16), 2.76-2.69 (m, 1H, C-15), 2.43-2.37 (m, 1H, C-8), 2.17 (s, 3H, C-21), 2.10 (s, 3H, AcO), 1.95-1.42 (m, 7H, C-1, C-2, C-9, C-11, C-12, C-15), 1.38-1.20 (m, 1H, C-cyclopropane), 1.24 (s, 3 H, C-18), 1.17 (s, 3 H, C-19), 0.80-0.76 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 200.0 (C-3), 198.6 (C-20), 170.6 (C=O,-OAc), 152.5 (C-5), 136.3 (C-7), 131.3 (C-6), 121.4 (C-4), 94.8 (C-17), 49.8 (C-10), 49.1 (C-16), 48.4 (C-14), 47.7 (C-9), 39.3 (C-13), 38.7 (C-1), 38.1 (C-15), 34.2 (C-11), 28.8 (C-,OAc), 26.7 (C-8), 25.9 (C-2), 23.5 (C-21), 21.7 (C-12), 20.9 (C-18), 16.0 (C-19), 13.0 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 498 (M^++4, 2), 496 (M^++2, 10), 494 (M^+, 8), 434 (13), 413 (6), 391 (17), 312 (100), 105 (7), 91 (12)

Anal. calcd. for $C_{24}H_{28}BrClO_4$: C, 58.14; H, 5.69. Found: C, 58.14; H, 5.68

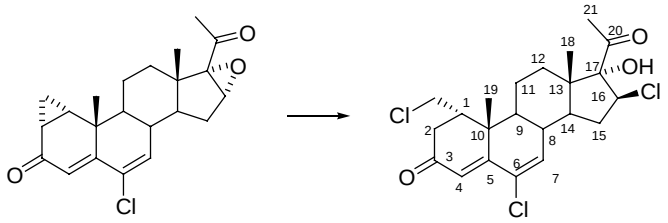
<u>16β-Bromo cyproterone acetate (59)</u>



74 59

1,8-Diazabicyclo[5.4.0.]undec-7-en (0.1 mL, 0.67 mmol) was added to a stirred solution of 1α-bromomethyl-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**) (40 mg, 0.07 mmol) in benzene (6 mL). After 2 h at room temperature, the reaction mixture was poured into water (6 mL) and extracted with dichloromethane (3x6 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to afford 16β-bromo cyproterone acetate (**59**) as a white solid (28 mg, 81%).

<u>1α-Chloromethylene-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (75)</u>



57 75

To a cooled (0 ^{o}C) solution of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadine-3,20-dione (**57**) (60 mg, 0.16 mmol) in glacial acetic acid (5 mL) was added dropwise, a solution of 2 M hydrochloric acid in diethyl ether (0.8 mL, 1.6 mmol). After being stirred at room temperature for 5 h, the reaction mixture was poured into water (10 mL). The white precipitate was filtered to give the crude product of 1α-chloromethylene-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**) (62 mg, 87%) which was used in the next step without further purification. Recrystallization of compound **75** from ethyl acetate/hexane provide a white crystal; m.p. 270-272 °C.

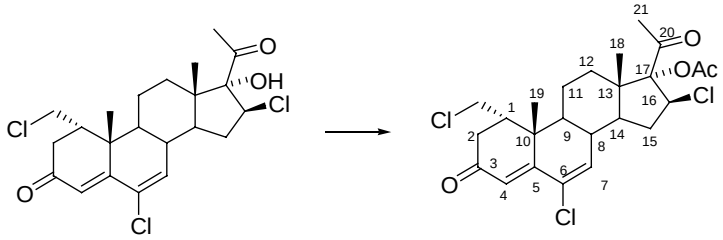
FTIR (KBr), ν_{max}, cm $^{-1}$: 3340 (O-H), 1712 (C=O), 1653 (C=C), 1600 (C=C), 1261 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.37 (s, 1H, C-4), 6.27 (d, *J* = 2.0 Hz, 1H, C-7), 4.18 (dd, *J* = 8.2, 6.4 Hz, 1H, C-16), 3.71-3.67 (m, 1H, C-Cl), 3.38 (1H, O-H), 3.28 (t, *J* = 11.2 Hz, 1H, C-Cl), 3.01 (dd, *J* = 18.4,0.8 Hz, 1H, C-2), 2.85-2.76 (m, 2H, C-2, C-15), 2.58-2.53 (m, 1H, C-8), 2.44 (s, 3H, C-21), 2.43-2.39 (m, 1H, C-1), 2.18-1.28 (m, 7H, C-9, C-11, C-12, C-14, C-15), 1.33 (s, 3H, C-18), 1.32 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 206.0 (C-3), 197.4 (C-20), 155.2 (C-5), 138.1 (C-7), 130.9 (C-6), 124.2 (C-4), 89.9 (C-17), 62.6 (C-16), 48.6 (C-10), 46.7 (C-14), 44.2 (C-9), 43.6 (C-1), 41.5 (C-13), 37.7 (C-8), 37.4 (C-15), 31.1 (C-11), 30.0 (C-Cl), 29.5 (C-21), 20.2 (C-2), 20.2 (C-18), 19.4 (C-12), 15.7 (C-19)

MS (CI), *m/z* (relative intensity): 448 (M^++4, 2), (446 (M^++2, 15), 444 (M^+, 24), 414 (58), 384 (20), 391 (61), 373 (16), 349 (24), 341 (9), 306 (5), 283 (100), 187 (51)

<u>1α-Chloromethyl-6-chloro-16β-chloro-17α-acetoxy-4,6-pregnadiene-3,20-dione (76)</u>



75 76

Acetic acid (0.14 mL, 2.45 mmol) was slowly added to a stirred a solution of trifluoroacetic anhydride (0.35, 2.52 mmol) in dichloromethane (2 mL). After being stirred at room temperature for 30 min, *p*-toluenesulfonic acid (15 mg, 0.08 mmol) was added to the reaction and cooled to 0 ^{o}C. An iced-cooled solution of 1α-chloro-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**) (50 mg, 0.11 mmol) in dichloromethane (6 mL) was then added to the above solution at 0 ^{o}C. After

being stirred for 45 min, a cooled saturated aqueous $NaHCO_3$ (7 mL) was added carefully until the mixture was basic and extracted with dichloromethane (3x9 mL). The combined organic layers were washed with water (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to give 1α-chloromethyl-6-chloro-16β-chloro-17α-acetoxy-4,6-pregnadiene-3,20-dione (**76**) (44 mg, 80%) as a white solid; m.p. 185-187 °C. Recrystallization of compound **76** from hexane/ethyl acetate gave a colorless crystal.

FTIR (KBr), ν_{max}, cm $^{-1}$: 1740 (C=O), 1723 (C=O), 1663 (C=C), 1600 (C=C), 1371 (C-O), 1243 (C-O)

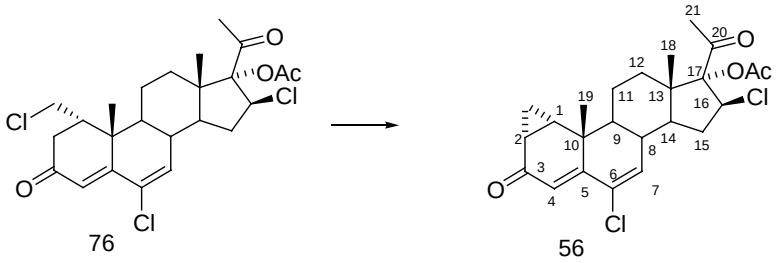
^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.38 (s, 1H, C-4), 6.26 (d, *J* = 3 Hz, 1H, C-7), 4.25 (dd, *J* = 12.0, 6.0 Hz, 1H, C-16), 3.70-3.66 (m, 1H, C-Cl), 3.28 (t, *J* = 11.2 Hz, 1H, C-Cl), 3.02-3.01 (m, 1H, C-2), 2.84-2.78 (m, 2H, C-2, C-15), 2.59-2.54 (m, 1H, C-8), 2.44-2.39 (m, 1H, C-1) 2.18 (s, 3H, AcO), 2.17 (s, 3 H, C-21), 2.05-1.26 (m. 7H, C-9, C-11, C-12, C-14, C-15), 1.32 (s, 3H, C-18), 1.3 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 199.9 (C-3), 197.4 (C-20), 170.4 (C=O, OAc), 155.2 (C-5), 137.9 (C-7), 131.5 (C-6), 124.7 (C-4), 95.3 (C-17), 61.5 (C-16), 49.8 (C-10), 47.0 (C-14), 45.3 (C-9), 44.5 (C-1), 43.9 (C-15), 41.8 (C-13), 38.1 (C-8), 38.1 (C-2), 37.2 (C-Cl), 33.8 (C-11), 29.2 (C, OAc), 21.7 (C-12), 20.5 (C-21), 19.9 (C-18), 15.6 (C-19)

MS (CI), *m/z* (relative intensity): 490 (M^++4, 6), 488 (M^++2, 57), 486 (M^+, 90), 451 (100), 433 (45), 414 (57), 391 (18), 349 (34), 313 (38), 283 (8), 91 (7)

Anal. calcd. for $C_{24}H_{29}Cl_3O_4$: C, 59.09; H, 5.99. Found: C, 58.79; H, 6.025.

16β-Chloro cyproterone acetate (56)



1,8-Diazabicyclo[5.4.0.]undec-7-en (DBU) (0.16 mL, 1.05 mmol) was added to a stirred solution of 1α-chloromethyl-6-chloro-16β-chloro-17α-acetoxy-4,6-pregnadiene-3,20-dione (**76**) (50 mg, 0.10 mmol) in benzene (10 mL). After 2 h at room temperature, the reaction mixture was poured into water (6 mL) and extracted with dichloromethane (3x8 mL). The combined organic layers were washed with water (12 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 2/5) to afford 16β-chloro cyproterone acetate (**56**) (40 mg, 86 %) as a light yellow solid; m.p. 190-192 °C. Recrystallization of compound **56** from hexane/ethyl acetate gave a colorless crystal.

FTIR (KBr), ν_{max}, cm $^{-1}$: 1737 (C=O), 1651 (C=O), 1240 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.19 (s, 1H, C-4), 6.16 (d, *J* = 1.5 Hz, 1H, C-7), 4.30 (dd, *J* = 8.4, 6.0 Hz, 1H, C-16), 2.82-2.76 (m, 1H, C-15), 2.51-2.46 (m, 1H, C-8), 2.21 (s, 3H, C-21), 2.14 (s, 3H, OAc), 2.14-1.41 (m, 8H, C-1, C-2, C-9, C-11, C-12, C-15), 1.40-1.22 (m, 1H, C-cyclopropane), 1.31 (s, 3 H, C-18), 1.24 (s, 3 H, C-19), 0.88-0.84 (m, 1H, C-cyclopropane)

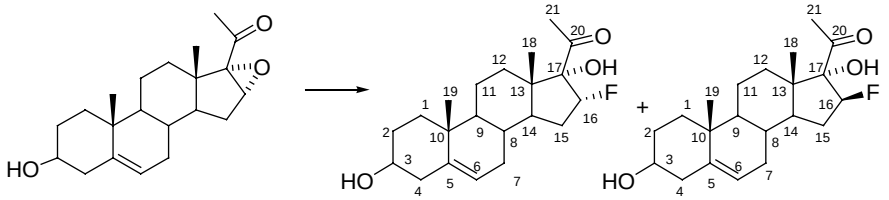
^{13}C NMR ($CDCl_3$, 100 MHz), δ: 199.8 (C-3), 198.2 (C-20), 170.0 (C=O, OAc), 152.1 (C-5), 135.9 (C-7), 130.8 (C-6), 120.9 (C-4), 94.9 (C-17), 61.2 (C-16), 49.1 (C-10), 48.0 (C-14), 46.5 (C-9), 38.9 (C-13), 37.7 (C-1), 37.7 (C-15), 33.6 (C-

11), 28.8 (C, OAc), 26.3 (C-8), 25.5 (C-2), 23.1 (C-21), 21.3 (C-12), 20.5 (C-18), 15.1 (C-19), 12.6 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 454 (M^++4, 1), 452 (M^++2, 6), 450 (M^+, 10), 415 (10), 390 (20), 355 (9), 378 (36), 330 (60), 313 (100), 288 (7), 277 (25), 91 (30), 77 (25), 55 (35)

HRMS calcd. for $C_{24}H_{28}Cl_2O_4$: 450.1365, Found 450.1366.

16α-Fluoro-3β-hydroxy-5-pregnene-20-one (77) and 16β-fluoro-3β-hydroxy-5-pregnene-20-one (78)



51 77 78

Boron trifluoride diethyl etherate (0.1 mL, 0.39 mmol) was added to a suspension of 3β-hydroxy-16α,17α-epoxy-5-pregnene-20-one (**51**) (50 mg, 0.15 mmol) and diisopropylamine trihydrochloride (37 mg, 0.23 mmol) in toluene (10 mL). After being stirred at room temperature for 48 h, a cold saturated aqueous $NaHCO_3$ (6 mL) was added to the reaction mixture and extracted with dichloromethane (3x8 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate /hexane: 2/5) to give 16α-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**77**) (13 mg, 25%) and 16β-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**78**) (8 mg, 15%) both as white solids. Recrystallization of compound **77** and **78** from hexane/ethyl acetate provided a white needle with m.p. 197-198 °C and m.p 195-196 °C, respectively.

77:

FTIR (KBr), ν_{max} , cm^{-1}: 3350 (O-H), 1690 (C=O), 1366 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.43-5.41 (m, 1H, C-6), 4.13 (t, *J* = 7.2 Hz, 1H, C-16), 3.56-3.53 (m, 1H, C-3), 2.59-2.52 (m, 1H, C-15), 2.44-2.40 (m, 1H, C-15), 2.30-1.82 (m, 6H, C-2, C-4, C-7, C-8), 1.78-1.10 (m, 9H, C-1, C-2, C-9, C-11, C-12, C-14), (s, 3H, C-21), 1.27 (s, 3H, C-18), 0.99 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 211.7 (C-20), 140.5 (C-5), 139.1 (C-17), 134.8 (C-13), 120.4 (C-6), 80.6 (C-16), 70.9 (C-3), 64.0 (C-10), 47.7 (C-14), 41.0 (C-15), 39.1 (C-11), 35.9 (C-4), 32.2 (C-9), 30.4 (C-1), 29.6 (C-2), 28.6 (C-8), 27.4 (C-7), 22.7 (C-21), 21.8 (C-12), 18.8 (C-18), 17.7 (C-19)

MS (EI), *m/z* (relative intensity): 312 (2), 287 (35), 269 (100), 251 (10), 157 (20), 131 (21), 91 (80), 57 (84), 55 (91)

78:

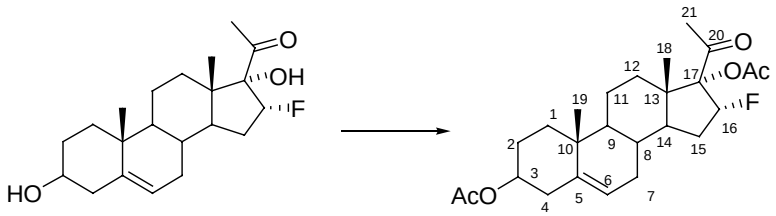
FTIR (KBr), ν_{max}, cm^{-1}: 3350 (O-H), 1701 (C=O), 1645 (C=C), 1351 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.42-5.41 (m, 1H, C-6), 4.51 (dd, *J* = 7.0, 5.3, Hz, 1H, C-16), 3.58-3.50 (m, 1H, C-3), 2.78-2.71 (m, 1H, C-15), 2.35-2.21 (m, 2H, C-7), 2.21-2.15 (m, 1H, C-15), 2.37-2.25 (m, 2H, C-4), 2.11 (s, 3H, C-21), 2.08-1.16 (m, 10H, C-1, C-2, C-8, C-9, C-11, C-12, C-14),1.18 (s, 3H, C-18) 0.99 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 210.6 (C-20), 140.5 (C-5), 137.3 (C-17), 135.0 (C-13), 120.3 (C-6), 74.5 (C-16), 70.9 (C-3), 65.5 (C-10), 47.6 (C-14), 41.1 (C-15), 38.8 (C-11), 35.9 (C-4), 30.9 (C-9), 30.4 (C-1), 30.0 (C-2), 28.3 (C-8), 25.48 (C-7), 22.7 (C-21), 21.68 (C-12), 17.7 (C-18), 12.5 (C-19)

MS (EI), *m/z* (relative intensity): 312 (5), 287 (34), 269 (100), 251 (29), 157 (50), 145 (48), 131 (23), 105 (67), 91 (75), 55 (54)

16α-Fluoro-3β,17α-diacetoxy-5-pregnene-20-one (79)



77 79

Acetic acid (0.07 mL, 1.22 mmol) was slowly added to a stirred mixture solution of trifluoroacetic anhydride (0.20 mL, 1.40 mmol) in dichloromethane (1 mL). After being stirred at room temperature for 30 min, *p*-toluenesulfonic acid (8.4 mg, 0.04 mmol) was added to the resulting mixture and cooled to 0 ^{o}C. An iced-cooled solution of 16α-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**77**) (20 mg, 0.06 mmol) in dichlorometane (2 mL) was added to the above mixture solution at 0 ^{o}C. After being stirred for 50 min, a cooled saturated aqueous $NaHCO_3$ (3 mL) was added and extracted with dichloromethane (3x5 mL). The combined organic layers were washed with water (8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to give 16α-fluoro-3β,17α-diacetoxy-5-pregnene-20-one (**79**) as a white solid (18 mg, 73%); m.p. 171-172 ^{o}C.

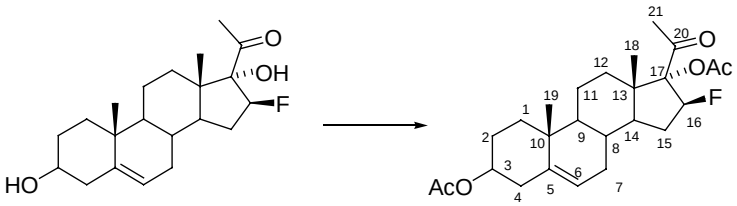
FTIR (KBr), ν_{max} , cm^{-1}: 1735 (C=O), 1701 (C=C), 1366 (C-O), 1234 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.39-5.38 (m, 1H, C-6), 5.11 (dd, *J* = 8.3, 6.0 Hz, 1H, C-16), 4.59-4.51 (m, 1H, C-3), 2.80-2.73 (m, 1H, C-15), 2.39-2.32 (m, 1H, C-15), 2.30-2.29 (m, 2H, C-7), 2.06 (s, 3H, C-21), 2.01-1.16 (m, 11H, C-1, C-2, C-8, C-9, C-11, C-12, C-14), 1.97, 1.94, (s, 6H, OAc), 1.15 (s, 3H, C-18), 0.94 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 208.9 (C-20), 170.5 (2C=O, OAc), 140.6 (C-5), 139.1 (C-17), 137.0 (C-13), 122.1 (C-6), 81.8 (C-16), 73.9 (C-3), 65.1 (C-10), 48.6 (C-14), 38.5 (C-5), 37.9 (C-11), 37.0 (C-4), 36.6 (C-9), 32.8 (C-1), 30.6 (C-2), 27.5 (C-8), 25.10 (C-21), 23.1 (C-7), 22.5 (C-12), 21.4 (C-OAc), 21.1 (C-OAc), 19.9 (C-18), 18.6 (C-19)

MS (CI), *m/z* (relative intensity): 435 (M^++1, 0.5), 389 (5), 374 (2), 371 (8), 354 (5), 349 (1), 331 (26), 313 (100), 295 (99), 157 (6), 91 (2), 77 (1)

16β-Fluoro-3β,17α-diacetoxy-5-pregnene-20-one (80)



78 80

Acetic acid (0.06 mL, 1.05 mmol) was slowly added to a stirred mixture of trifluoroacetic anhydride (0.16 mL, 1.15 mmol) in dichloromethane (1 mL). After being stirred at room temperature for 30 min, *p*-toluenesulfonic acid (8.4 mg, 0.04 mmol) was added to the resulting mixture and cooled to 0 ^{o}C. An ice-cooled solution of 16β-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**78**) (18 mg, 0.05 mmol) in dichloromethane (2 mL) was added to the above mixture solution at 0 ^{o}C. After stirring for 30 min, a cooled saturated aqueous $NaHCO_3$ (4 mL) was added and extracted with dichloromethane (3x5 mL). The combined organic layers were washed with water (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to give 16β-fluoro-3β,17α-diacetoxy-5-pregnene-20-one (**80**) as a white solid (16 mg, 72%).

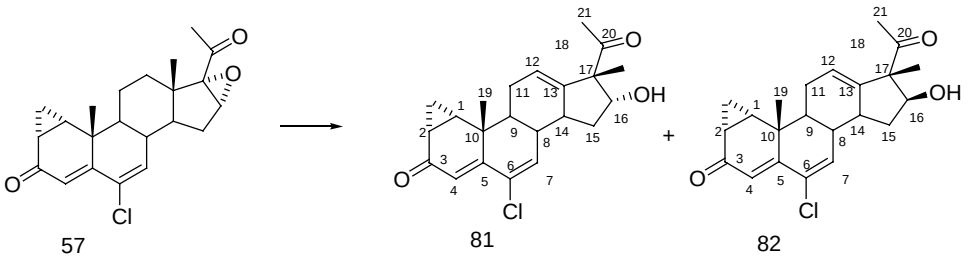
FTIR (KBr), ν_{max} , cm^{-1}: 1730 (C=O), 1610 (C=C), 1365 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.39-5.37 (m, 1H, C-6), 5.44 (dd, J = 7.8, 6.36, Hz, 1H, C-16), 4.58-4.52 (m, 1H, C-3), 2.77-2.70 (m, 1H, C-15), 2.35-2.01 (m, 7H, C-2, C-4, C-7, C-15), 2.09 (s, 3H, C-21), 1.95, 1.97, (s, 6H, OAc), 1.90-1.10 (m, 9H, C-1, C-8, C-9, C-11, C-12, C-14, C-15), 0.95 (s, 3H, C-18), 0.85 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 206.1 (C-20), 172.5, 170.1 (2C=O, OAc), 141.3 (C-5), 138.2 (C-17), 134.0 (C-13), 120.1 (C-6), 75.8 (C-16), 74.8 (C-3), 63.1 (C-10), 44.6 (C-14), 35.5 (C-5), 38.9 (C-11), 37.7 (C-4), 35.1 (C-9), 31.8 (C-1), 30.3 (C-2), 26.5 (C-8), 24.10 (C-21), 23.4 (C-7), 22.7 (C-12), 21.4 (C-OAc), 20.1 (C-OAc), 19.7 (C-18), 17.9 (C-19)

MS (CI), *m/z* (relative intensity): 435 (M^++1, 6), 389 (9), 374 (12), 371 (7), 354 (15), 349 (1), 331 (23), 313 (78), 295 (100), 157 (7), 91 (12), 77 (1)

16α-Hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (81) and 16β-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (82)



57 81 82

Boron trifluoride diethyl etherate (0.05 mL, 0.19 mmol) was slowly added to a suspension of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) (30 mg, 0.08 mmol) and diisopropylamine trihydrochloride (19.4 mg, 0.12 mmol) in toluene (5 mL). After being stirred at room temperature for 2 days, a cold saturated aqueous $NaHCO_3$ was added to the reaction mixture and extracted with dichloromethane (3x6 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure.

The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 2/5) to provide 16α-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**81**) (8 mg, 26.7 %) and 16β-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**82**) (7 mg, 23.3%) both as white solids.

81:

FTIR (KBr), ν_{max}, cm^{-1}: 3421 (O-H), 1694 (C=O), 1647 (C=C), 1071 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.28 (d, *J* = 2 Hz, 1H, C-7), 6.20 (s, 1H, C-4), 5.73 (d, *J* = 4 Hz, 1H, C-12), 4.60 (t, *J* = 6.4 Hz, 1H, C-16), 2.52-2.50 (m, 2H, C-15, C-11), 2.48-1.68 (m, 5H, C-1, C-2, C-8, C-11, C-14), 1.65-1.15 (m, 3H, C-9, C-15, C-cyclopropane), 2.18 (s, 3H, C-21), 1.26 (s, 3H, C-19), 1.11 (s, 3H, C-CH_3), 0.91-0.86 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 210.3 (C-3), 198.1 (C-20), 152.2 (C-5), 144.9 (C-13), 135.8 (C-7), 130.2 (C-6), 120.9 (C-12), 120.5 (C-5), 74.8 (C-16), 62.1 (C-17), 42.5 (C-8), 42.2 (C-9), 41.8 (C-14), 38.4 (C-10), 36.9 (C-15), 25.7 (C-1), 25.5 (C-21), 25.5 (C-11), 25.2 (C-2), 22.2 (C-CH_3), 17.8 (C-19), 12.3 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 374 (M^++2, 12), 372 (M^+, 34), 354 (90), 329 (21), 313 (16), 292 (2), 296 (16), 277 (100), 186 (16), 157 (6)

82:

FTIR (KBr), ν_{max}, cm^{-1}: 3415 (O-H), 1696 (C=O), 1645 (C=C), 1356 (C-O)

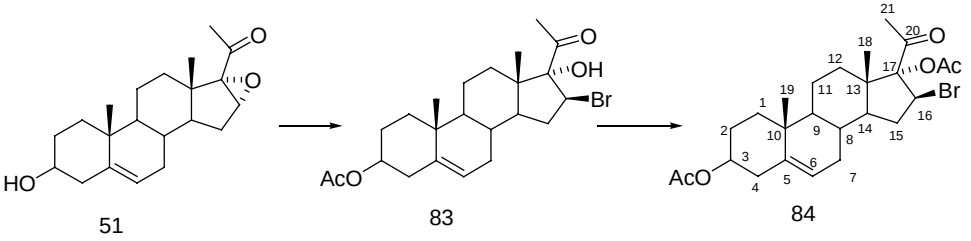
^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.18 (d, *J* = 2.15 Hz, 1H, C-7), 6.12 (s, 1H, C-4), 5.68-5.67 (m, 1H, C-12), 3.82 (t, *J* = 6.1 Hz, 1H, C-16), 2.42-2.37 (m, 1H, C-11), 2.27-2.19 (m, 1H, C-11), 2.06-2.03 (m, 1H, C-8), 2.09 (s, 3H, C-21), 2.08-1.10

(m, 6H, C-1, C-2, C-9, C-14, C-15, C-cyclopropane), 1.99-1.95 (m, 1H, C-15), 1.32 (s, 3H, C-19), 1.15 (s, 3H, CH_3), 0.86-0.78 (m, 1H, C-cyclopropane)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 213.4 (C-3), 198.0 (C-20), 152.1 (C-5), 143.8 (C-13), 135.9 (C-7), 130.1 (C-6), 120.9 (C-12), 120.5 (C-4), 80.1 (C-16), 62.0 (C-17), 60.4 (C-8), 42.5 (C-9), 42.4 (C-14), 41.8 (C-10), 36.6 (C-15), 27.3 (C-1), 25.4 (C-2), 25.3 (C-11), 22.2 (C-21), 20.6 (C-CH_3), 14.3 (C-19), 13.8 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 374 (M^++2, 15), 372 (M^+, 46), 355 (57) 329 (11), 313 (100), 292 (8), 296 (17), 277 (31), 186 (23), 157 (24)

16β-Bromo-3β,17α-diacetoxy-5-pregnene-20-one (84)



48% Hydrobromic acid (0.2 mL, 1.78 mmol) was added dropwise to a cooled solution of 3β-hydroxy-16α,17α-epoxy-5-pregnene-20-one (**51**) (100 mg, 0.30 mmol) in acetic acid (10 mL). After being stirred at room temperature for 6 h, the reaction mixture was poured into water (15 mL). The light yellow precipitate was filtered to afford the crude product of 16β-bromo-3β-acetoxy-17α-hydroxy-5-pregnene-20-one (**83**) (110 mg, 83%) which was used in the next step without further purification.

Acetic acid (0.30 mL, 5.24 mmol) was slowly added to a stirred solution of trifluoroacetic anhydride (0.74 mL, 5.32 mmol) in dichloromethane (3 mL). After being stirred under nitrogen atmosphere at room temperature for 30 min, *p*-toluenesulfonic acid (20 mg, 0.12 mmol) was added to the reaction mixture and cooled to 0 ^{o}C. An ice-cooled solution of 16β-bromo-3β-acetoxy-17α-hydroxy-5-

pregnene-20-one (**83**) (110 mg, 0.24 mmol) in dichloromethane (6 mL) was added to the above mixture at 0 ^{o}C. After stirring for 40 min, a cooled saturated aqueous $NaHCO_3$ (10 mL) was carefully added to the reaction mixture and extracted with dichloromethane (3x8 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate /hexane: 1/4) to give 16β-bromo-3β,17α-diacetoxy-5-pregnene-20-one (**84**) (100 mg, 83.3%). Recrystallization of compound **84** from ethyl acetate/hexane provided a light yellow crystal; m.p. 184-186 °C.

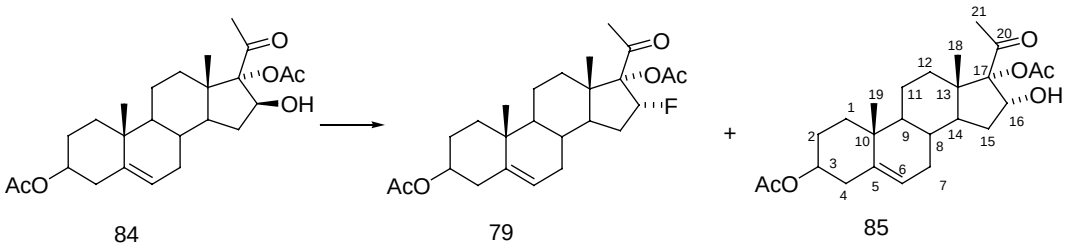
FTIR (KBr), ν_{max}, cm^{-1}: 1732 (C=O), 1500 (C=C), 1366 (C-O), 1241 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.29 (d, *J* = 5.1 Hz 1H, C-6), 4.56-4.48 (m, 1H, C-3), 4.11 (t, *J* = 8.2 Hz, 1H, C-16), 2.54-2.48 (m, 1H, C-15), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.28-1.22 (m, 16H, C-1, C-2, C-4, C-7, C-8, C-9, C-11, C-12, C-14, C-15, 1.97 (s, 3H, C-21), 1.12 (s, 3H, C-18), 0.96 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 119.9 (C-20), 170.5 (C=O, OAc), 170.0 (C=O, OAc), 139.8 (C-5), 121.7 (C-6), 94.5 (C-17), 73.7 (C-3), 50.2 (C-16), 49.5 (C-9), 49.3 (C-14), 48.5 (C-13), 39.2 (C-15), 38.0 (C-11), 36.8 (C-4), 36.5 (C-10), 33.3 (C-1), 31.7 (C-2), 31.3 (C-8), 28.2 (C-21), 27.6 (C-7), 21.4 (C-OAc), 21.0 (C-OAc), 20.1 (C-12), 19.2 (C-18), 15.6 (C-19)

MS (CI), *m/z* (relative intensity): 496 (M^++2, 1), 494 (M^+, 1), 434 (2), 415 (100), 373 (12), 355 (12), 313 (8), 295 (25), 277 (2)

16α-fluoro-3β,17α-diacetoxy-5-pregnene-20-one (79)



A suspension mixture of 16β-bromo-3β,17α-diacetoxy-5-pregnene-20-one (**84**) (100 mg, 0.20 mmol) and silver fluoride (50 mg, 0.39 mmol) in acetonitrile (12 mL) was stirred under nitrogen at 55 °C for 24 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in dicloromethane (15 mL) and washed with water. The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate /hexane: 2/5) to give 16α-fluoro-3β,17α-diacetoxy-5-pregnene-20-one (**79**) (12 mg, 13.6%) and 16α-hydroxy-3β,17α-diacetoxy-5-pregnene-20-one (**85**) (54 mg, 62.1%) both as white solids. Recrystallization of compound **85** from hexane/ethyl acetate gave a white crystal with m.p. 198-200 °C.

85:

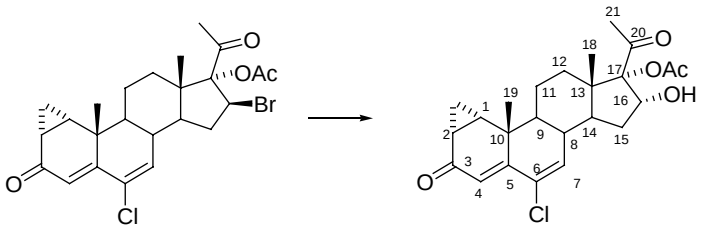
FTIR (KBr), ν_{max}, cm^{-1}: 3415 (O-H), 1696 (C=O), 1645 (C=C), 1356 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.65 (dd, *J* = 9.2, 2.9 Hz, 1H, C-16), 5.29 (d, *J* = 5.1 Hz, C-6), 4.57-4.49 (m, 1H, C-3), 3.17 (1H, OH), 2.20 (s, 3H, C-21), 1.99, 1.96 (s, 6H, OAc), 1.93-1.77 (m, 6H, C-4, C-7, C-15), 1.76-1.12 (m, 11H, C-1, C-2, C-8, C-9, C-11, C-12, C-14), 0.95 (s, 3H, C-18), 0.67 (s, 3H, C-19)

^{13}C NMR ($CDCl_3$, 100 MHz), δ: 209.1 (C-20), 170.5 (C=O, OAc), 169.7 (C=O, OAc), 139.7 (C-5), 122.0 (C-6), 89.9 (C-17), 75.8 (C-16), 73.7 (C-3), 49.5 (C-9), 49.4 (C-14), 47.5 (C-13), 37.9 (C-15), 36.8 (C-11), 36.5 (C-10), 32.1 (C-1), 31.6 (C-2), 31.3 (C-8), 30.3 (C-4), 27.6 (C-7), 27.1 (C-21), 21.4 (C-OAc), 20.9 (C-OAc), 20.0 (C-12), 19.2 (C-18), 15.0 (C-19)

MS (CI), *m/z* (relative intensity): 433 (M^++1, 46), 414 (21), 389 (28), 355 (100), 329 (25), 251 (46), 145 (23), 91 (45), 55 (34)

16α-Hydroxy cyproterone acetate (86)



59 86

Silver fluoride (20 mg, 0.16 mmol) was added to a solution of 16β-bromo cyproterone acetate (**59**) (50 mg, 0.10 mmol) in acetonitrile (7 mL). After stirring for 24 h at 55 °C under nitrogen atmosphere, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in dicloromethane (10 mL) and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 2/5) to give 16α-hydroxy cyproterone acetate (**86**) (36 mg, 82%)**.** Recrystallization of compound **86** from hexane/ethyl acetate gave a white crystal with m.p. 201-202 °C .

FTIR (KBr), ν_{max}, cm^{-1}: 3482 (O-H), 1736 (C=O), 1701 (C=O), 1646 (C=C), 1372 (C-O)

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.11 (s, 1H, C-7) 6.05 (s, 1H, C-4), 5.70 (dd, *J* = 9.4, 2.5 Hz, 1H, C-16), 3.18 (1H, OH), 2.28-1.65 (m, 6H, C-1, C-2, C-8, C-9, C-15), 2.22 (s, 3H, C-21), 2.00 (s, 6H, 2OAc), 1.66-1.12 (m, 5H, C-11, C-12, C-cyclopropane),1.14 (s, 3H, C-18), 0.75 (s, 3H, C-19), 0.80-0.76 (C-cyclopropane)

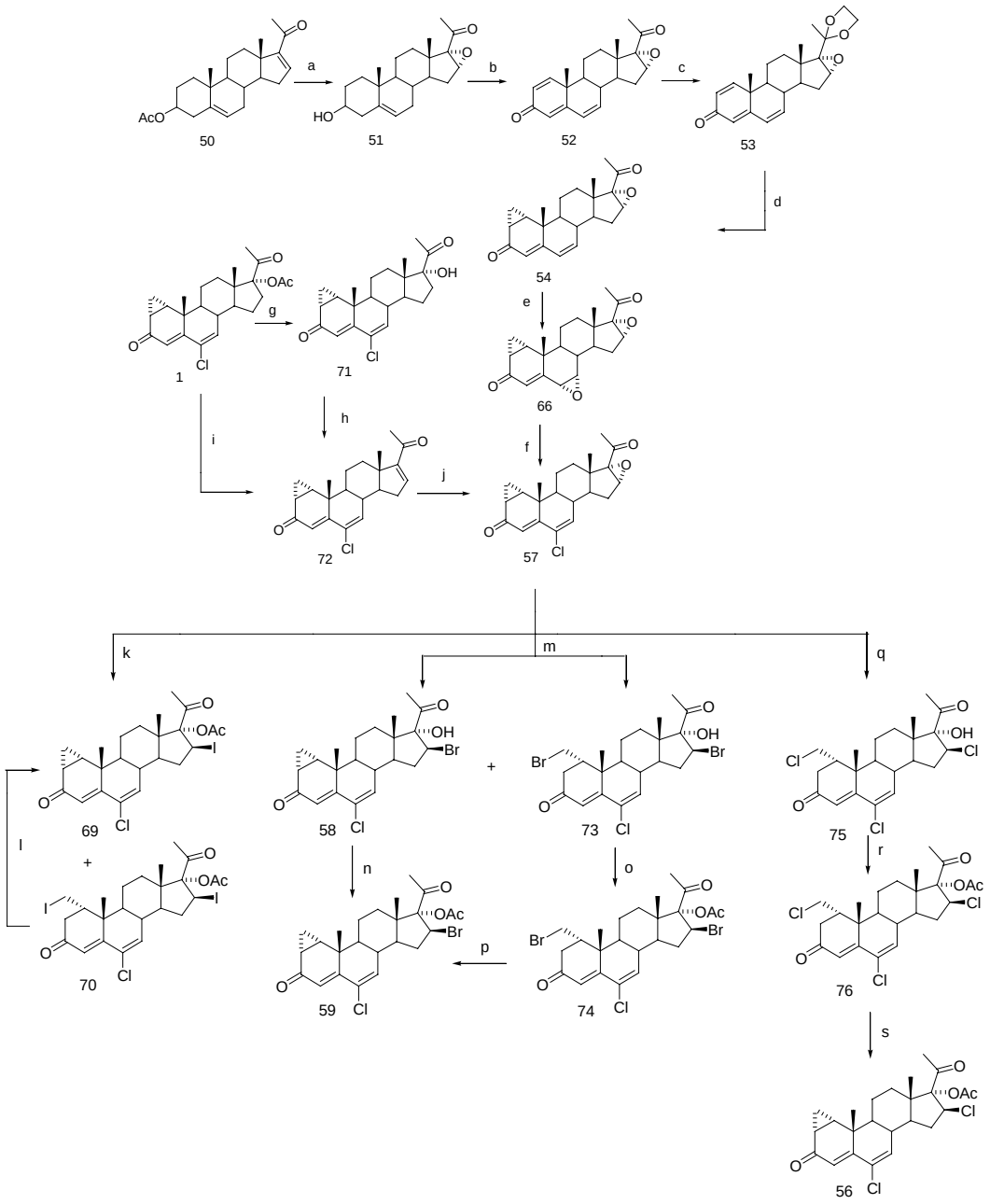
^{13}C NMR ($CDCl_3$, 100 MHz), δ: 208.6 (C-3), 198.0 (C-20), 169.4 (C-5), 152.1 (C=O, OAc), 136.1 (C-7), 130.4 (C-6), 120.6 (C-4), 88.5 (C-17), 75.3 (C-16), 48.0 (C-10), 47.6 (C-14), 46.8 (C-9), 38.7 (C-13), 37.8 (C-1), 31.5 (C-11), 30.3 (C-15), 27.2 (C-21), 25.9 (C-2), 25.2 (C-8), 22.8 (C-OAc), 20.9 (C-18), 20.3 (C-12), 14.9 (C-19), 12.3 (C-cyclopropane)

MS (CI), *m/z* (relative intensity): 434 (M^++2, 20), 432 (M^+, 61), 415 (34), 403 (6), 390 (12), 372 (29), 355 (5)

RESULTS

Synthesis of 16β-halo cyproterone acetate

16β-Halo cyproterone acetate was synthesized from 16-dehydro pregnenolone acetate (**50**) and cyproterone aceate (**1**) as shown in Scheme 18.



Scheme 18

Reagents conditions:

a) 40% H_2O_2, NaOH, MeOH, rt, 24 h, 90%

b) DDQ, dry dioxane, refux, 24 h, 65%

c) Ethylene glycol, triethylorthoformate, *p*- toluene sulfonic acid, CH_2Cl_2, rt, 8 h, 72%

d) Me_3SOI, NaH, DMSO, rt, 2h; then *p*-TsOH.H_2O, CH_2Cl_2, rt, 2 h, 72%

e) m-CPBA, CH_2Cl_2, rt, 12 h, 60%

f) *N,N*-dimethyl acetamide hydrochloride, DMSO, 65 ^oC, 30 h, then basic alumina oxide, rt, 24 h, 70%

g) NaOH, MeOH, rt, 3h, 95%

h) $SOCl_2$, pyridine, rt, 3 h, 21% or $POCl_3$, pyridine, rt, 14 days, 6.3%

i) Biphenyl/biphenyloxide (1:3), 269 ^oC, 90 min, 94%

j) 40% H_2O_2, NaOH, MeOH, 0 ^oC, 1 h, 88%

k) 47% HI, dioxane, rt, 24 h; TFAA, acetic acid, *p*-TsOH.H_2O, CH_2Cl_2, 0 ^oC, 40 min, **69** (38%), **70** (39%)

l) DBU, benzene, rt, 2h, 77 %

m) PPh_3, Br_2, THF, rt, 30 min; then CH_2Cl_2, rt, 2 days, **58** (70%), **73** (15%)

n) TFAA, acetic acid, *p*-TsOH.H_2O, CH_2Cl_2, 0 ^oC, 20 min, 79%

o) TFAA, acetic acid, *p*-TsOH.H_2O, CH_2Cl_2, 0 ^oC, 50 min, 77%

p) DBU, benzene, rt, 2 h, 81 %

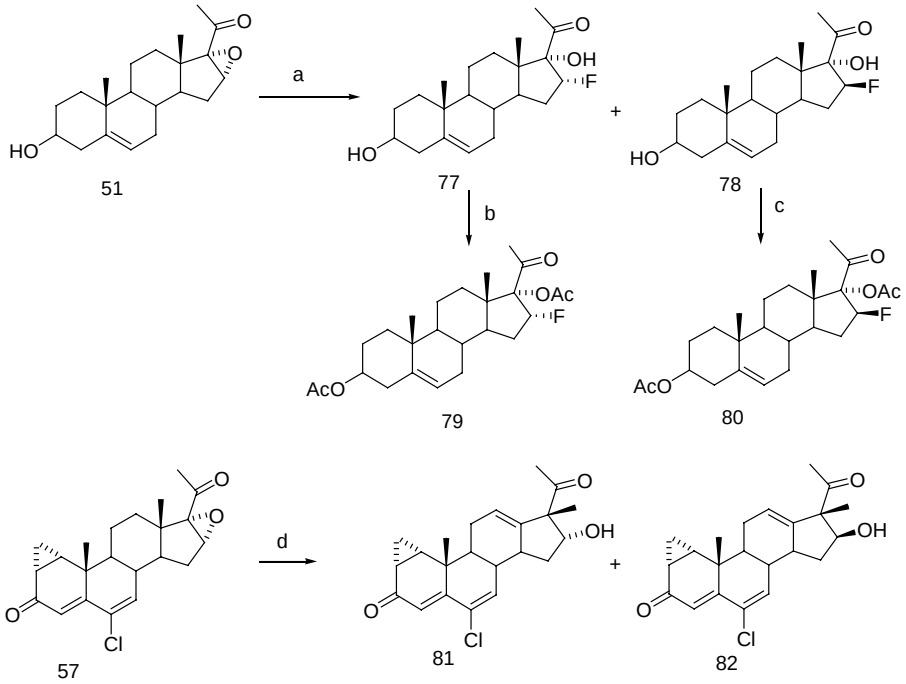
q) 2 M HCl, acetic acid, rt, 5 h, 87 %

r) TFAA, acetic acid, *p*-TsOH.H_2O, CH_2Cl_2, 0 ^oC, 45 min, 80%

s) DBU, benzene, rt, 2 h, 86%

An attempt to synthesis of 16β-fluoro-cyproterone acetate

An attempt to synthesize 16β-fluoro cyproterone acetate from 1α,2α-cyclomethylene-16α-17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) was studied by using diisopropylamine trihydrochloride and boron trifluoride diethyl etherate in toluene.



Scheme 19

Reagents conditions:

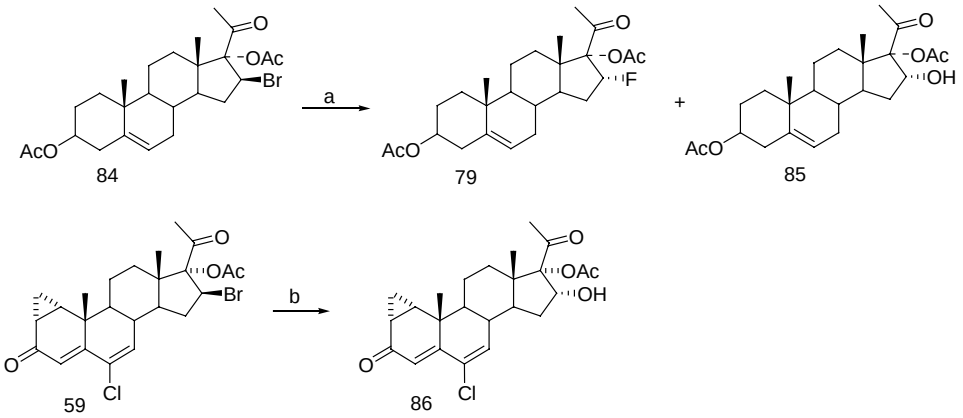
a) $BF_3.OEt_2$, diisopropylamine trihydrochloride,toluene, rt, 2 days, **77** (25%), **78** (15%)

b) TFAA, acetic acid, *p*-TsOH.H_2O, CH_2Cl_2, 0 ^oC, 50 min, 73%

c) TFAA, acetic acid, *p*-TsOH.H_2O, CH_2Cl_2, 0 ^oC, 30 min, 72%

d) $BF_3.OEt_2$, diisopropylamine trihydrochloride, toluene, rt, 2 days, **81** (27%), **82** (23%)

Direct displacement of 16β-bromo cyproterone acetate (**59**) to the corresponding 16α-fluoro compound was also studied by using silver fluoride in dry acetonitrile (Scheme 20).



Scheme 20

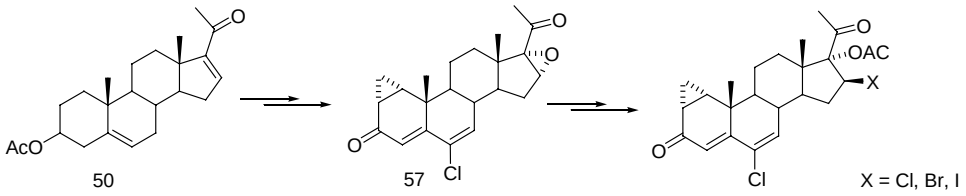
Reagents conditions:

a) AgF, acetonitrile, 55 ^{o}C, 24 h, **79** (13.6 %), **85** (62.1%)

b) AgF, acetonitrile, 55 ^{o}C, 24 h, 82 %

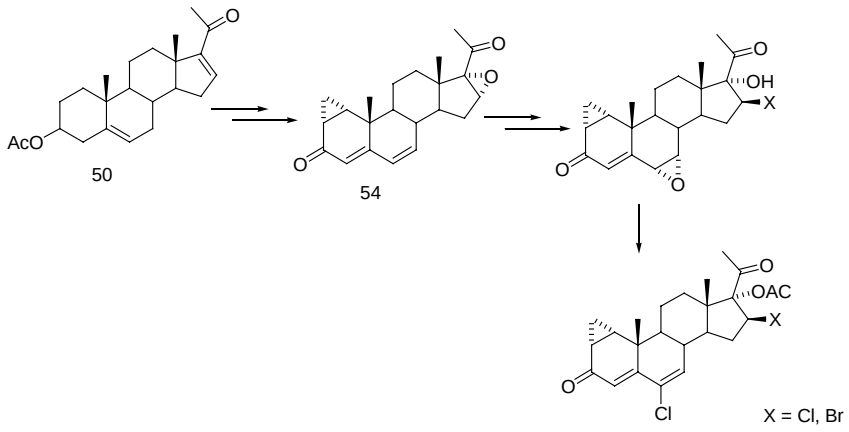
DISCUSSION

We reported herein the synthesis of 16β-bromo (**59**), 16β-iodo (**69**) and 16β-chloro (**56**) cyproterone acetate starting from a commercially available 16-dehydropregnenolone acetate (**50**) via the key intermediate 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) as shown in Scheme 21.



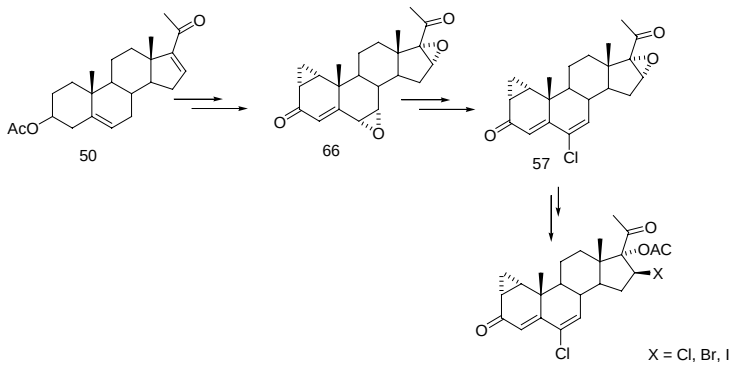
Scheme 21

Recently, Kongkathip and co-workers (2003) have reported the synthesis of 16β-bromo (**59**) and 16β-chloro (**56**) cyproterone acetate from 16-dehydropregnenolone acetate (**50**). Their synthetic approach was accomplished by first opening of the epoxide ring at C-16,17 of compound **54** with chloride and bromide anion then introduction of vinyl chloride functionality via epoxide at C-6,7 position was followed to afford 16β-chloro (**56**) and 16β-bromo (**59**) cyproterone acetate, respectively after acetylation as shown in Scheme 22.



Scheme 22

Our synthetic methodology was slightly different from the above report. Vinyl chloride was introduced via epoxide at C-6,7 of compound **66** to give the intermediate **57** before conversion of epoxide at C-16,17 position to halohydrin by nucleophilic attack at C-16. (Scheme 23)



Scheme 23

The intermediate **57** has been successfully synthesized in 6 steps from a commercially available 16-dehydropregnenolone acetate (**50**) (Scheme 18). Epoxidation of 16-dehydropregnenolone acetate (**50**) using hydrogen peroxide in aqueous solution of sodium hydroxide gave epoxide steroid (**51**) in 90% yield followed by oxidative-dehydrogenation using 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) afforded 1,4,6-trienone epoxide **52** in 65% yield. Protection of the C-20 keto group of 1,4,6-trienone epoxide **52** using ethylene glycol, ethyl orthoformate and *p*-toluenesulfonic acid provided 16α,17α-epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (**53**) in 72% yield. A cyclopropyl ring was introduced into C-1,2 position of **53** regio and stereospecifically by treated with trimethyl sulfoxonium ylide generated from dimetyl sulfoxonium iodide and sodium hydride. Hydrolysis of C-20 ketal group using *p*-toluenesulfonic acid afforded 1α,2α-cyclomethylene-16α-17α-epoxy-4,6-pregnadiene-3,20-dione (**54**) in 72% yield. Introduction of cyclopropyl ring at C-1,2 position of **53** afforded only the α-cyclopropyl isomer, due to the steric effect of the neighboring angular methyl group at C-19.

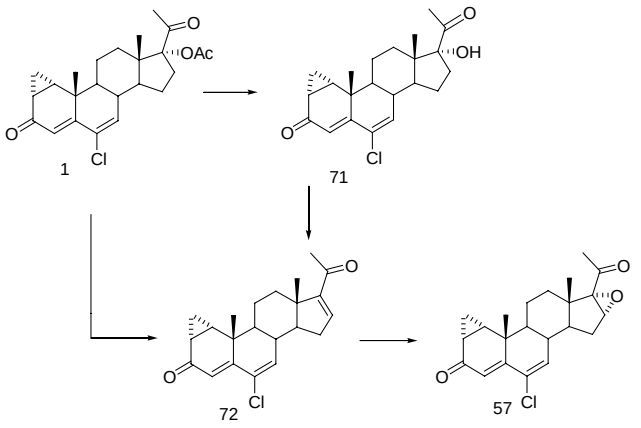
The ^{1}H NMR spectrum of **54** showed the multiplet signal at δ 0.83-0.80 and δ 1.45-1.37 indicated the presence of cyclopropane ring. Olefinic proton at C-4 appeared as a singlet at δ 5.54 whereas proton at C-6 and C-7 appeared at δ 5.94 (dd, J = 9.8, 1.9 Hz) and δ 6.09 (dd, J = 9.8, 2.8 Hz), respectively. These protons were assigned to be the patterns of cyclo-trieneone and confirmed by comparing with the ^{1}H NMR spectra 1α,2α-cyclomethylene-16-methylene progesterone derivatives reported by Shapiro *et al.* (1969). The IR spectrum of **54** showed absorption band at ν_{max} 1701 cm^{-1} for C=O stretching and absorption band at ν_{max} 1657, 1621 cm^{-1} for the C=C double stretching. The mass spectrum showed the molecular ion (M^+) at *m/z* 338.

Epoxidation of **54** using m-chloroperbenzoic acid afforded 1α,2α-cyclomethylene-6α,7α-epoxy-16α,17α-epoxy-4-pregnene-3,20-dione (**66**) in 60% yield. Selective conversion of 6α-7α-epoxy compound **66** to the corresponding vinylchloride **57** was achived by the reaction of **66** with *N,N*-dimethylacetamide hydrochloride in dimethylsulfoxide then treated with basic alumina oxide in benzene to produce 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) in 70% yield. (Scheme 23)

The structure of **57** was assigned based on ^{1}H NMR spectrum that showed the signal of C-16 proton at δ 3.69 as a singlet. Olefinic proton at C-4 appeared as a singlet at δ 6.10 whereas proton at C-7 appeared as a doublet at δ 6.06 (J = 2.2 Hz). The IR spectrum of **57** showed absorption band at ν_{max} 1703 cm^{-1} for C=O stretching and absorption band at ν_{max} 1663 cm^{-1} for the C=C stretching. Elemental analysis was confirmed for the molecular formula of compound **57** ($C_{22}H_{25}ClO_3$, found: C, 70.86; H, 6.67, calcd: C, 70.86; H, 6.76).

An alternative approach to synthesize the intermediate compound **57** was also accomplished from cyproterone acetate (**1**) via the formation of 16,17-unsaturated compound **72** . Two different routes have been tried. The first route, cyproterone acetate was hydrolysed to the corresponding cyproterone **71** using sodium hydroxide in methanol. Dehydration of **71** using phosphorus oxychloride in pyridine (Takegawa

et al, 1993) gave α,β unsaturated ketone **72** in only 6.5% yield. When Philouze's method (Philouze *et al*, 2003) (thionylchloride in pyridine) was used, the % yield of **72** was slightly increased (21%). The second route was a direct elimination of cyproterone acetate to α,β unsaturated ketone **72**. Ramesh's method (Ramesh *et al* 1996), direct elimination of cyproterone acetate (**1**) using potassium acetate in dimethyl formamide was unsuccessful due to the fact that potassium acetate attacked chloride at C-6 to generate cyclo-trieneone. A satisfactory yield was obtained when cyproterone acetate (**1**) was heated in a mixture solution of biphenyl/biphenyloxide (1:3) under refluxing condition (Kerb *et al*, 1981) as shown in Scheme 24.

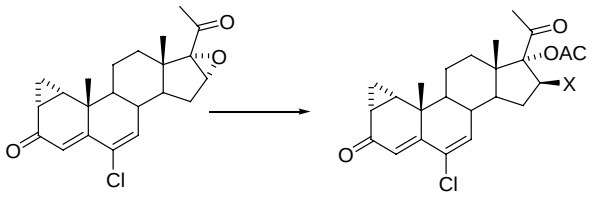


Scheme 24

The ^{1}H NMR spectrum of **72** showed signal of C-16 proton at δ 6.73-6.72 as multiplet. Olefinic proton of C-4 appeared as a singlet at δ 6.18 whereas proton at C-7 appeared as a doublet at δ 6.24 (J = 1.8 Hz). Compound **72** showed the similar ^{1}H NMR pattern which was reported by Kerb *et al*. (1981).The IR spectrum of **72** showed absorption band at ν_{max} 1659 cm^{-1} for C=O stretching and absorption band at ν_{max} 1583 cm^{-1} for the C=C stretching. The mass spectrum showed the molecular ion (M^+) at *m/z* 356.

The key strategy to synthesize 16-halogenated-cyproterone acetate was to open the epoxide ring at C-16,17 of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-

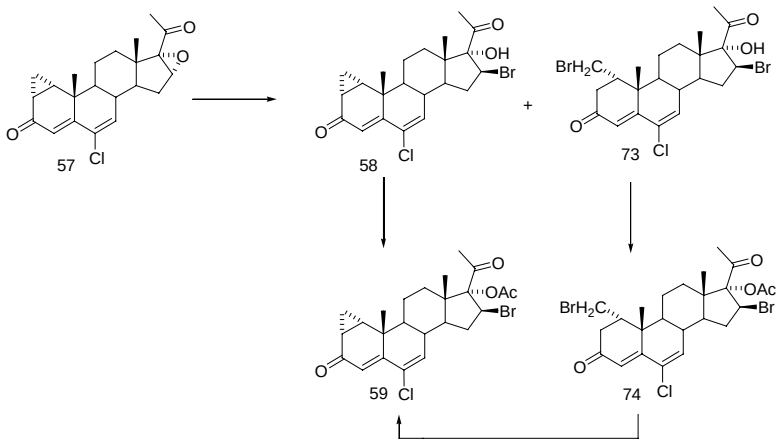
4,6-pregnadiene-3,20-dione (**57**) by halogen nucleophile (Scheme 25).



Scheme 25

16β-Bromo-cyproterone acetate (**59**) was synthesized from epoxide **57**. Bromohydrin functional group at C-16 and C-17 could be introduced by two methods. The first method, epoxide ring opening of compound **57** using a mixture of bromine and triphenyl phosphine in tetrahydrofuran afforded **58** and **73** in the ratio 2:3. Surprisingly, when **73** was left in dichloromethane solution, cyclopropane ring closuse to give **58** was detected. Therefore, the crude product of **58** and **73** was stirred in dichlomethane at room temperature for 2 days to give **58** and **73** in 70% and 15% yield respectively, however a small amount of starting compound **57** was also recovered. Acetylation of both bromohydrin intermediate **58** and **73** by treatment with acetic acid, trifluoroacetic anhydride and *p*-toluenesulfoic acid afforded the desired product 16β-bromo cyproterone acetate (**59**) in 79% yield and 1α-bromomethyl-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**) 77% yield, respectively. The second method epoxide **57** was treated with hydrobromic acid in acetic acid at 0 ^{o}C to afford only **73** in 81 % yield as shown in Scheme 26.

Many attempts to close the cyclopropane ring of **74** to **59** by various conditions have been tried. Treatment of **74** with pyridine at 100 ^{o}C for 20 minutes was unsuccessful, none of the expected product **59** was detected. Stirring **74** in dichloromethane provided unsatisfied result, only a small amount of **59** and starting compound **74** was recovered. However, the fascinating result was obtained by treatment of **74** with 1,8-diazabicyclo[5.4.0.]undec-7-en (DBU) in benzene (Baraldi *et al*., 1984).



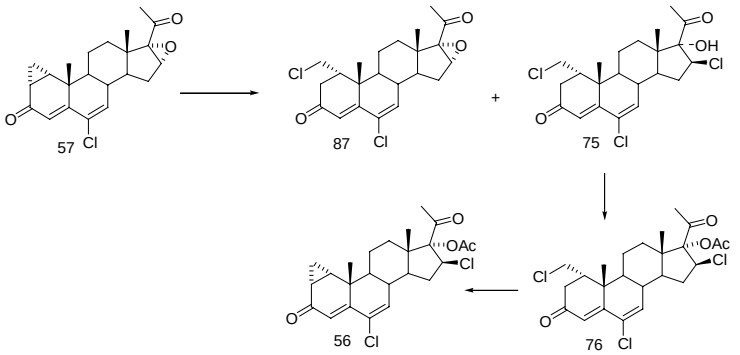
Scheme 26

The ^{1}H NMR spectrum of **59** showed the signal of C-16 at δ 4.17 (dd, J = 6.77, 7.21 Hz). Olefinic proton at C-4 appeared as a singlet at δ 6.11 whereas proton at C-7 appeared as a doublet at δ 6.08 (J = 2.01 Hz).These protons showed similar ^{1}H NMR pattern of 16β-bromo cyproterone acetate reported by Kongkathip *et al*. (2003). The IR spectrum of **59** showed absorption band at ν_{max} 1738 cm^{-1} for C=O stretching and absorption band at ν_{max} 1651 cm^{-1} for the C=C stretching. Elemental analysis was confirmed molecular formula of compound **59** ($C_{24}H_{28}BrClO_4$, found: C, 58.14; H, 5.68, calcd.: C, 58.14; H, 5.69).

16β-Chloro-cyproterone acetate (**56**) was also synthesized from the key intermediate epoxide (**57**). Epoxide ring opening of **57** using *N,N*-dimethylacetamide hydrochloride afforded 1α-chloromethylene-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**) (34%) and 1α-chloromethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**87**) (24%). Acetylation of 1α-chloromethylene-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**) by treatment with acetic acid, trifluoroacetic anhydride and *p*-toluenesulfonic acid in dichloromethane obtained 1α-chloromethylene-16β-chloro-17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (**76**) in 80% yield. Finally, cyclopropane ring closure of compound **76**

using 1,8-diaza bicycle [5.4.0.]undec-7-en (DBU) in benzene gave 16β-chloro cyproterone acetate (**56**) in 86% yield (Sheme 27).

Moreover, we also persevered to increase the yield of chlorohydrin **75** by opening epoxide ring **57** using 2 M hydrochloric acid. The method produced only **75** in good yield (87%).



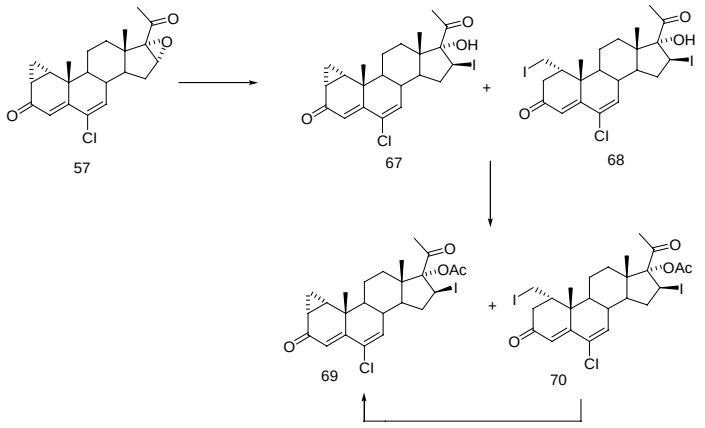
Scheme 27

The ^{1}H NMR spectrum of **56** showed the signal of C-16 at δ 4.30 (dd, J = 6.39, 4.5 Hz). Olefinic proton at C-4 appeared as a singlet at δ 6.19 whereas proton at C-7 appeared as a doublet at δ 6.16 (J = 1.5 Hz). These protons showed the similar ^{1}H NMR pattern of 16β-chloro cyproterone acetate which was reported by Kongkathip *et al*. (2003). The IR spectrum of **56** showed absorption band at ν_{max} 1737 cm^{-1} for C=O stretching and absorption band at ν_{max} 1651 cm^{-1} for the C=C stretching. The chemical ionization mass spectrum showed the molecular fragment ion (M^++1) at *m/z* 451. Compound **56** had a molecular formula of $C_{24}H_{28}Cl_2O_4$**,** as indicated by the high resolution mass spectrometry (HRMS) (found: 450.1366, calcd.: 450.1365).

The synthesis of 16β-iodo cyproterone acetate was accomplished in 3 steps from **57**. Epoxide ring opening of **57** using 47% hydriodic acid in dioxane afforded 16β-iodo-cyproterone (**67**) and 1α-iodomethyl-6-chloro-16β-iodo-17α-hydroxy-4,6-pregnadiene-3,20-dione (**68**). An attempt to purify the crude reaction by using flash

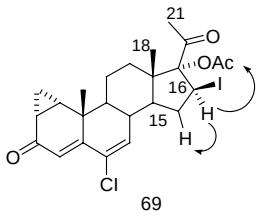
column chromatography was failed, only epoxide ring closure products was observed. As mention above, the crude product of the mixture iodohydrin **67** and **68** was acetylated to afford 16β-iodo-cyproterone acetate (**69**) in 38% yield and by product 17α-acetoxy-6-chloro-16β-iodo-1α-iodomethyl-4,6-pregnadiene-3,20-dione (**70**) in 39% yield as shown in Scheme 28.

Furthermore, to increase the yield of the desired product (**69**), cyclopropane ring closure of **70** was performed by using diazabicyclo[5.4.0.]undec-7-en (DBU) to provided **69** in good yield.



Scheme 28

The structure of 16β-iodo-cyproterone acetate (**69**) was assigned based on ^{1}H NMR spectrum that showed the proton signals at C-16 as a triplet at 4.10 ppm (J = 8.38 Hz). This proton was assigned to be the α-configuration and determined by comparing the ^{1} H NMR spectra with 16β-iodo steroid (Eeaton *et al*., 1975). Elemental analysis was confirmed molecular formula of compound **69** ($C_{24}H_{28}ClIO_4$, found: C, 53.39; H, 4.935, calcd: C, 53.10; H, 5.20).

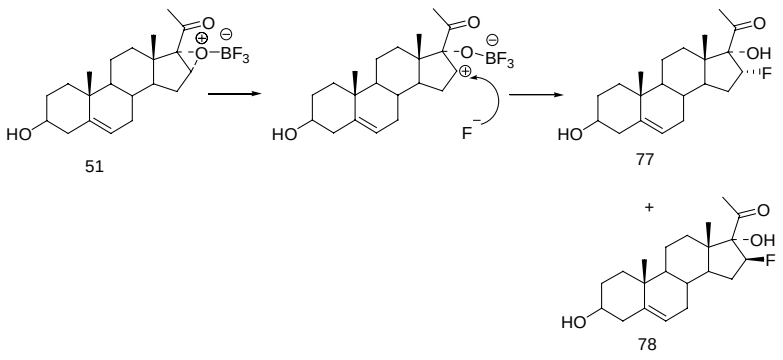


69

Figure 6 NOE experiment of 16β-iodo-cyproterone acetate (**69**)

Furthermore, the configuration 16-iodo of **69** was confirmed from NOE experiments. Irradiation at 16-H (δ 4.10) of **69** shows 1.07% enhancement of 17α-acetoxy protons and 3.93% enhancement of 15β-H whereas shows no enhancement of 18-methyl protons. Irradiation of 18-methyl protons (δ 1.16) shows no enhancement of 16-H indicate that the 16-iodo group should be aligned on β phase.

An attempt to synthesize 16β-fluoro cyproterone acetate using from intermediate **57** by opening 16α,17α-epoxy with fluoride anion nucleophile to fluorohydrin was studied. Treatment of **51** with boron trifluoride diethyl etherate and diisopropylamine trihydrochloride provided the mixture of **77** in 25% yield and **78** in 15% yield. The plausible mechanism of this conversion involves activating of epoxide ring with boron trifluoride to generate carbocation and then fluoride anion attack on both phase to give **77** and **78** as shown in Scheme 29.



Scheme 29

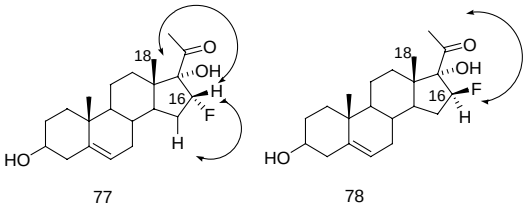
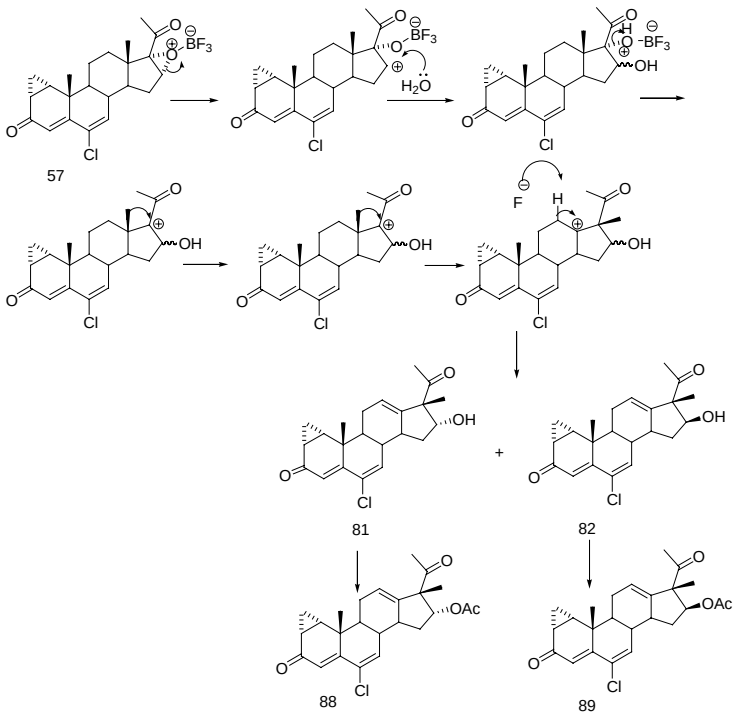


Figure 7 NOE experiment of 16β-fluoro of **77** and 16α-fluoro of **78**

The stereochemistry of 16-fluoro group of **77** and **78** was determined from NOE experiments. Irradiation at 16-H (δ 4.13) of **77** shows 1.98% and 1.22% enhancement of 18-methyl protons and 15β-H, respectively moreover, irradiation of 18-methyl protons (δ 1.27) reveals 1.13% enhancement of 16-H. As the results, indicating these protons are on the same side, so it is obviously show that 16-fluoro group of **77** aligned on α phase. Similaly, 16-H (δ 4.51) of **78** was irradiated, the NOE results reveals 1.02% enchancement of 21-methyl protons whereas irradiation of 18-methyl protons (δ 1.18) shows no enhancement of 16-H indicating it should be assigned the 16-fluoro group of **78** aligned on β phase.

Unfortunately, treatment of intermediate **57** with boron trifluoride diethyl etherate and diisopropylamine trihydrochloride in toluene provided 16α-hydroxy-1α,2α-cyclomethylene-17β-methyl-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**81**) (27%) and 16β-hydroxy-1α,2α-cyclomethylene-17β-methyl-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**82**) (23%) instead of the desired 16-fluoro cyproterone. Shapiro *et al.* has reported that the nature of the group at C-20 in 21-carbon-17-oxygenated steroids undergoing Wagner-Meerwein rearrangement (Hoff *et at*., 1970). Therefore, we presumed that the mechanism of this conversion involves sequentially, activating of epoxide ring with boron trifluoride to generate carbocation at C-16 then water attacked on both phase to generate 16-hydroxy intermediate, methyl at C-19 migrated to give carbocation at C-13 and finally double bond formation by elimination of adjacent proton let to compound **81** and **82**. (Scheme 30)



Scheme 30

The structure of 16-hydroxy **81** and **82** was also confirmed by acetylation to afforded **88** and **89**, respectively. The configuration of 16-acetoxy group of **88** and **89** was determined by NOE experiments. Irradiation of 16-CH (δ 5.15)of **88** shows 2.68% and 1.44% enhancement of 17-methyl protons and 16-acetoxy protons. Moreover, irradiation at 17-methyl proton (δ 1.23) reveals 2.89% and 3.17% enhancement of 16-H and 12-H, indicating these protons are on the same side. As a result 16-acetoxy group of **88** should be aligned on α phase. Similarly, 16-H (δ 5.42) of **89** was irradiated, the NOE results reveals 3.36% enhancement of 16-acetoxy protons whereas shows no enhancement of 17-metyl protons. Irradiation of 17-methyl proton (δ 1.18) reveals 0.4% enhancement of 12-H, whilst no enhancement of 16-H indicating it should be assigned the 16-acetoxy group of **89** aligned on β phase.

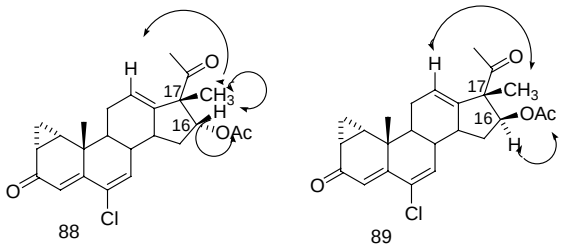
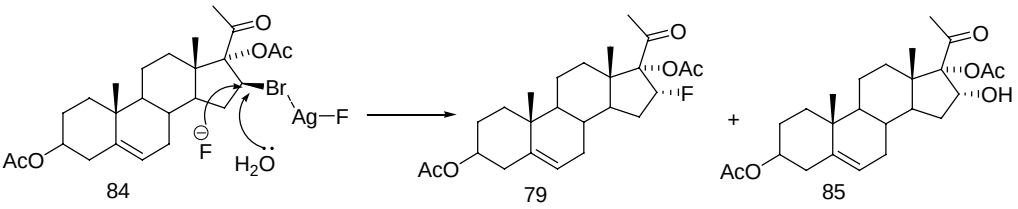


Figure 8 NOE experiment of 16α-acetoxy group of **88** and 16β-acetoxy of **89**.

Alternatively, direct displacement of 16β-bromo to 16α-fluoro was also investigated. The reaction of **84** with silver fluoride in dry acetonitrile provided the corresponding 16α-hydroxy compound **85** as major product (61.2%) together with 16α-fluoro compound **79** as a minor product (13.6%). The plausible mechanism of this conversion involves coordination of 16β-bromo and silver atom followed by nucleophile attack either by fluoride or water to give **79** and **85**, respectively. (Scheme 31)

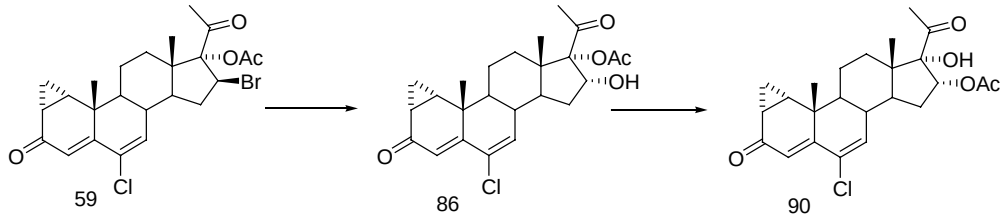


Scheme 31

The ^{1}H NMR signal of proton at C-16 of **85** appeared as a doublet of doublet at 5.81 ppm (J = 9.4, 2.4 Hz). This proton was assigned to be the β-configuration and confirmed by comparing with the ^{1}H NMR spectrum of 16α-hydroxy steroid which was reported by Takegawa *et al.*(1993).

However, when 16β-bromo cyproterone acetate (**59**) was treated with silver fluoride in dry acetonitrile, unfortunately, no 16-fluoro compound was found only

16α-hydroxy cyproterone acetate (**86**) was obtained. Changing the solvent from to toluene or cyclohexane also provided the same result (Scheme 32).



Scheme 32

The formation of 16α-hydroxy was confirmed by acetylation of **86**. Surprisingly**,** transacetylation product **90** was obtained when **86** was reacted with trifluoroacetic anhydride, acetic acid, *p*-toluenesulfonic acid in dichloromethane. In comparison, the ^{1}H NMR signal proton at C-16 of **86** appeared as doublet of doublet at δ 5.70 (J = 2.5, 9.4 Hz) and present of hydroxyl group at δ 3.18 whereas, **90** appeared as a doublet of doublet at δ 6.25 (J = 1.9, 9.0 Hz). Moreover, **86** was confirmed by IR spectrum showed absorption band at ν_{max} 3402 cm^{-1} for O-H stretching and also confirmed D_2O exchange experiment.

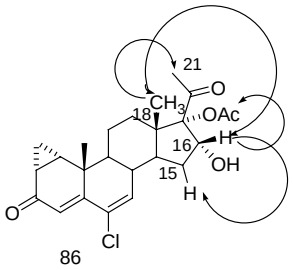


Figure 9 NOE experiment of 16α-hydroxy cyproterone aceate (**86**)

The configuration of 16-hydroxy group of **86** was determined from NOE experiments. Irradiation at proton at 16-CH (δ 5.70) of **86** shows 1.72%, 3.20% and

0.66% enhancement of 18-methyl protons, 15β-H and 17α-acetoxy protons, respectively. Moreover, irradiation at 18-methyl proton (δ 1.14) reveals 1.17% and 2.07% enhancement of 16-H and 21-methyl protons indicating these protons are on the same side. As a result 16-hydroxy group of **86** should be aligned on α phase.

CONCLUSION

The synthesis of 16β-iodo (**69**), 16β-bromo (**59**) and 16β-chloro (**56**) cyproterone acetate was accomplished from the key intermediate 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**). The key strategy of this synthesis is epoxide ring opening at C-16, C-17 of the key intermediate (**57**) to produce the 16β-halogenated-cyproterone. The intermediate **57** has been successfully synthesized in 6 steps from a commercially available 16-dehydropregnenolone acetate (**50**) with an overall yield of 12.7%. Epoxidation of 16-dehydropregnenolone acetate **(50**) using hydrogen peroxide in aqueous solution of sodium hydroxide gave epoxy steroid (**51**) followed by oxidative-dehydrogenation using 2,3-dichloro-5,6,-dicyano-p-benzoquinone (DDQ) provided 1,4,6-trienone epoxide (**52**). Protection of the C-20 keto group of 1,4,6-trienone epoxide (**52**) using ethylene glycol, ethyl orthoformate and *p*-toluenesulfonic acid provided 16α,17α-epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (**53**). A cyclopropyl ring was introduced into C-1,2-position of compound **53** using trimethyl sulfoxonium ylide then hydrolysis of C- 20 ketal group using *p*-toluenesulfonic acid afforded 1α,2α-cyclomethylene-16α,17α-epoxy-4,6-pregnadiene-3,20-dione (**54**). Epoxidation of **54** using m-chloroperbenzoic acid (m-CPBA) let to 6α,7α epoxy intermediate (**66**) which was subsequently undergone formation of vinyl chloride when treated with *N,N*-dimethylacetamide hydrochloride in dimethyl sulfoxide followed by basic alumina oxide in benzene to produce 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**).

An another approach to synthesize the key intermediate **57** was achieved in 2 steps starting from cyproterone acetate (**1**) with an overall yield of 79%. A solution of **1** in the mixture of biphenyl/biphenyloxide (1:3) was heated under refluxing condition provided α,β unsaturated ketone (**72**) in excellent yield. Epoxidation of compound **72** using hydrogen peroxide in aqueous solution of sodium hydroxide afforded the intermediate (**57**).

The synthesis of 16β-iodo cyproterone acetate (**69**) has been accomplished for the first time in 3 steps reaction from 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) with an overall yield of 68%. Treatment of the key epoxide intermediate (**57**) with 47% hydroiodic acid in dioxane followed by acetylation using acetic acid, trifluoroacetic anhydride and *p*-toluenesulfonic acid in dichloromethane provided 16β-iodo cyproterone acetate (**69**) and by product 1α-iodomethyl-6-chloro-16β-iodo-17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (**70**). Treatment of compound **70** with 1,8-diazabicyclo[5.4.0.]undec-7-en (DBU) afforded 16β-iodo cyproterone acetate (**69**).

The synthesis of 16β-bromo cyproterone acetate (**59**) has been accomplished in 3 steps from 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**) with an overall yield of 65%. Epoxide ring opening of the intermediate **57** using a mixture solution of bromine and triphenyl phosphine in tetrahydrofuran afforded 16β-bromo cyproterone (**58**) and by product 1α-bromomethylene-16β-bromo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**73**). Acetylation of compound **58** and **73** using acetic acid, trifluoroacetic anhydride and *p*-toluenesulfoic acid afforded the desired product 16β-bromo cyproterone acetate (**59**) and 1α-bromomethyl-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**). Treatment of **74** with 1,8-diazabicyclo[5.4.0.]undec-7-en (DBU) in benzene produced 16β-bromo cyproterone acetate (**59**).

The synthesis of 16β-chloro-cyproterone acetate (**56**) was also accomplished in 3 steps from the intermediate (**57**) with an overall yield of 60 %. Epoxide ring opening of **57** using 2 M hydrochloric acid provided only cyclopropane ring opening product, 1α-chloromethylene-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**). Acetylation of **75** by treatment with acetic acid, trifluoroacetic anhydride and *p*-toluenesulfonic acid in dichloromethane gave 1α-chloromethylene-16β-chloro-17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (**76**). Cyclopropane ring closure of compound **76** to provide 16β-chloro cyproterone acetate (**56**) was achieved by treated with 1,8-diaza bicycle [5.4.0.]undec-7-en (DBU) in benzene.

Attempt to open the epoxide ring of the intermediate **57** to 16β-fluoro cyproterone acetate using boron trifluoride diethyl etherate and diisopropylamine trihydrochloride in toluene was unsuccessful. Alternative, direct displacement of 16β-bromo cyproterone acetate (**59**) to the corresponding 16α-fluoro compound using silver fluoride in dry acetonitrile failed to give 16α-fluoro cyproterone acetate, 16α-hydroxy cyproterone acetate was obtained instead.

Our successful synthesise of 16β-iodo (**69**), 16β-bromo (**59**) and16β-chloro (**56**) cyproterone acetate will be very useful for the development of steroid drugs with progestational and anti-androgenic activities.

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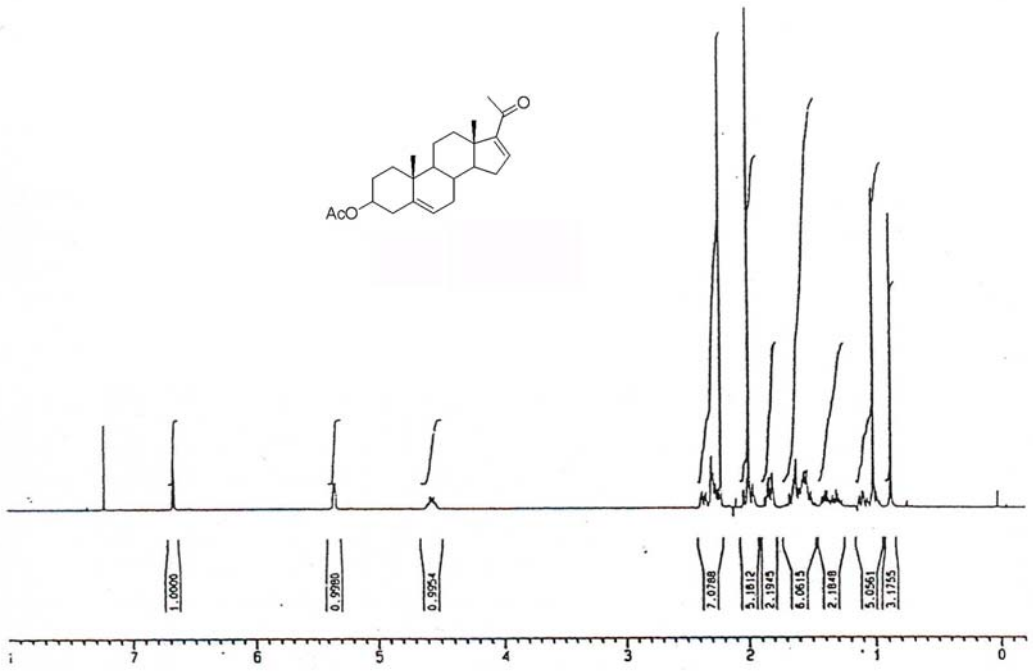
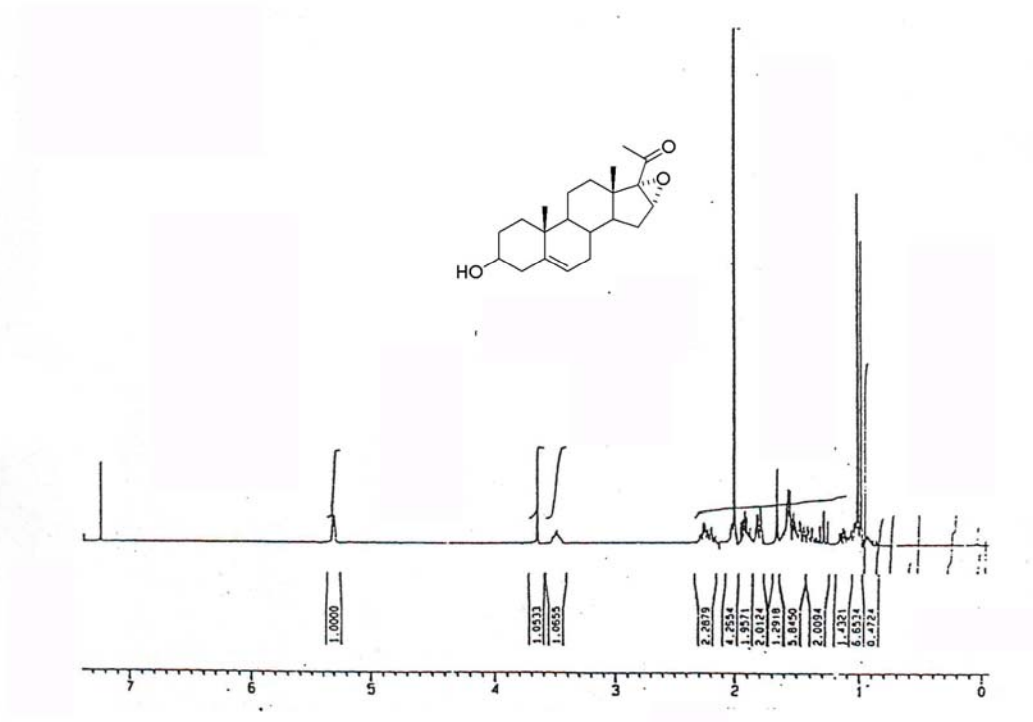
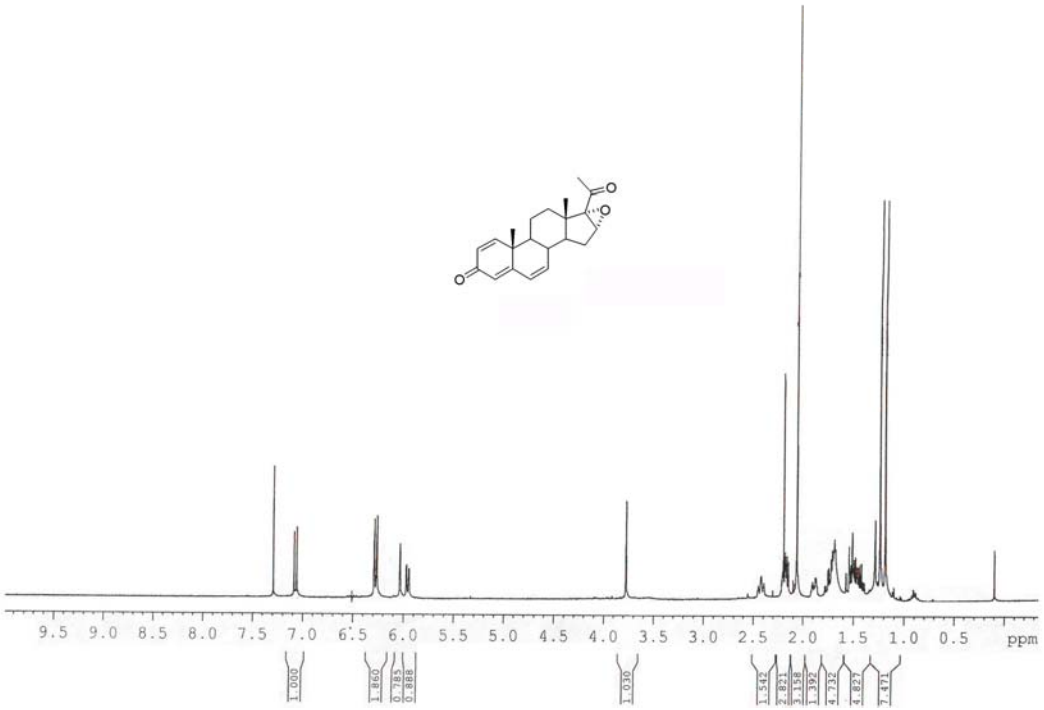
APPENDIX



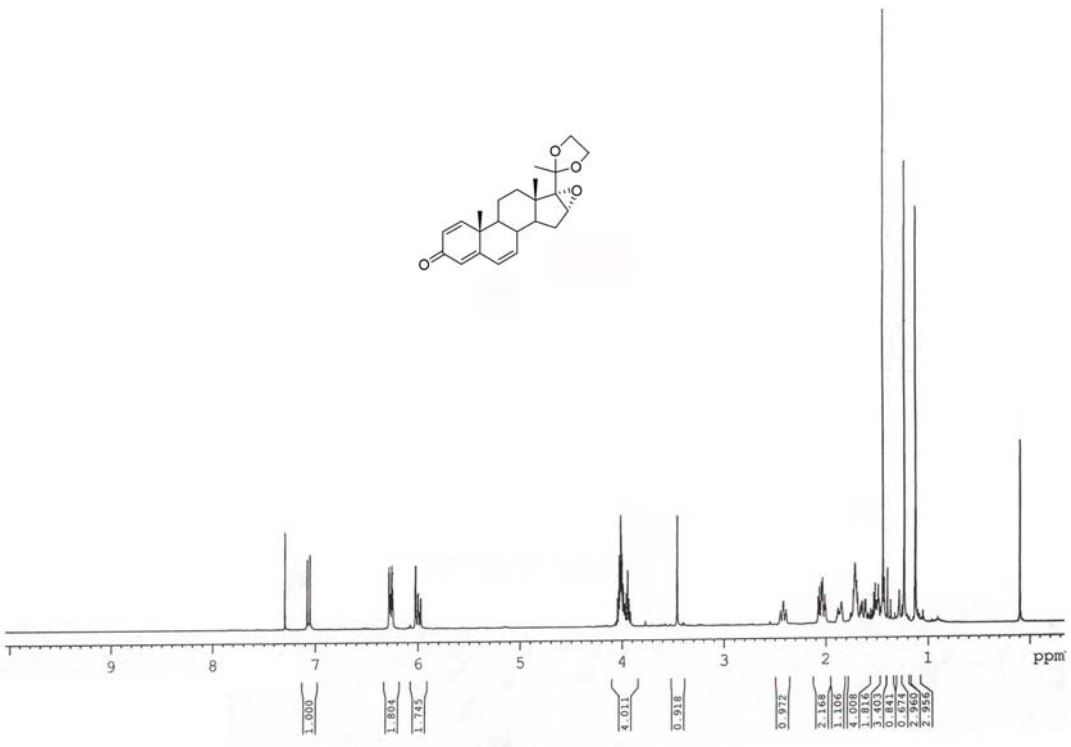
Appendix Figure 1 400 MHz ^{1}H NMR spectrum of 16-dehydropregnenolone acetate (**50**)



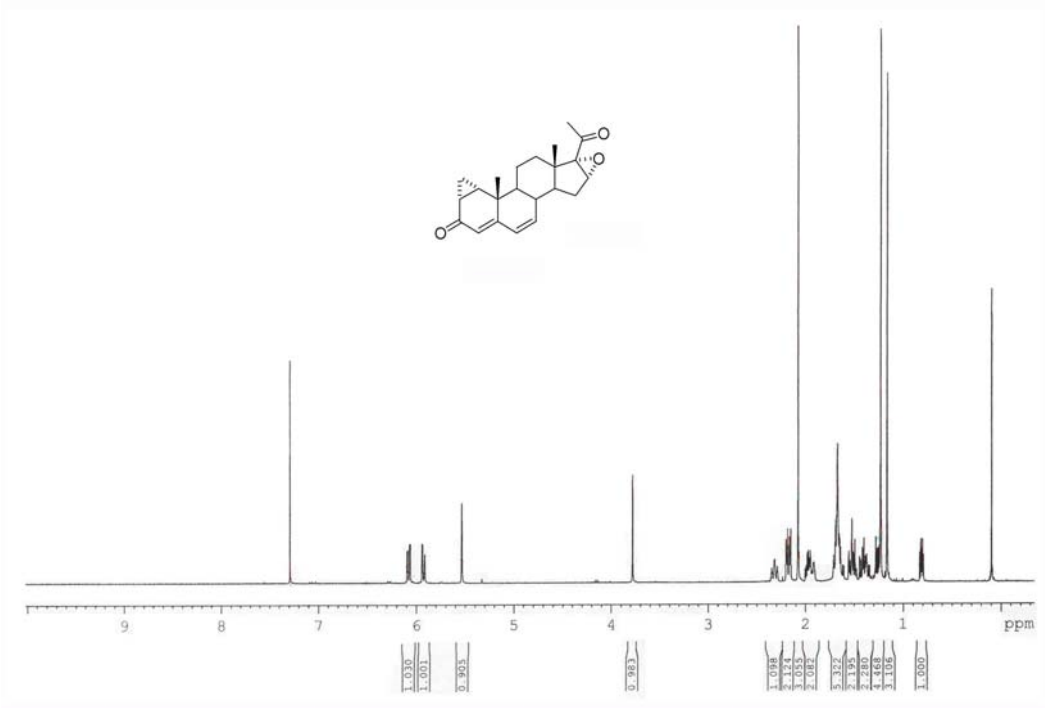
Appendix Figure 2 400 MHz ^{1}H NMR spectrum of 3β-hydroxy-16α,17α-epoxy-5-pregnene-20-one (**51**)



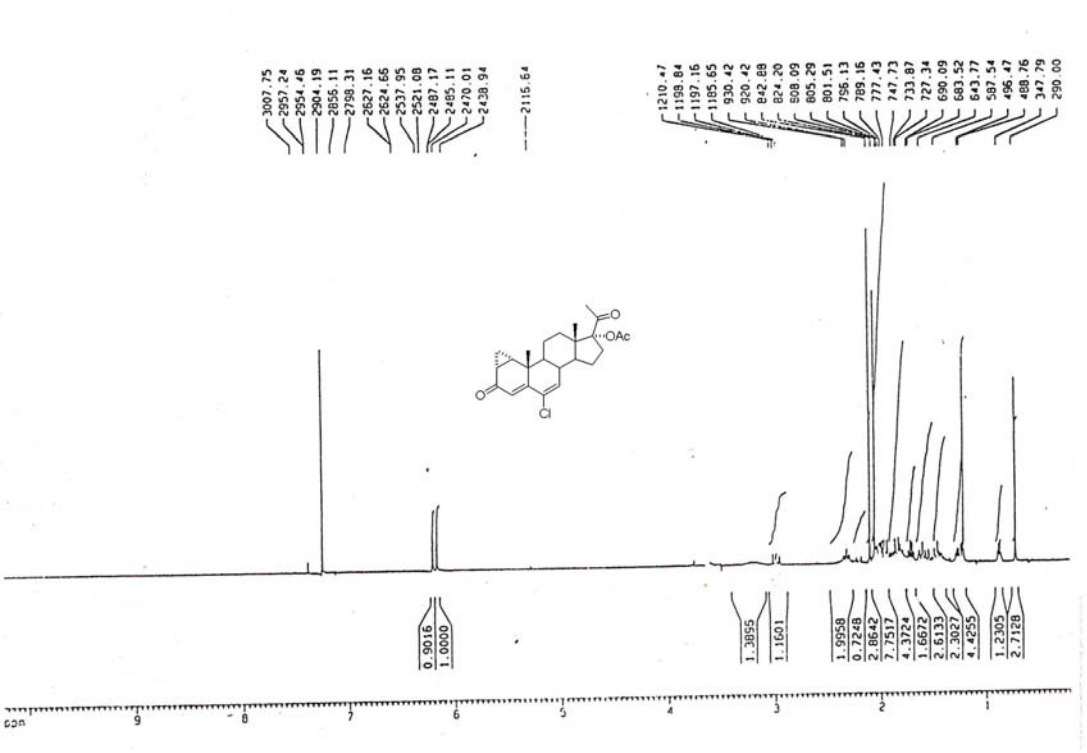
Appendix Figure 3 400 MHz ^{1}H NMR spectrum of 16α,17α-epoxy-1,4,6-pregnatri ene-3,20-dione (**52**)



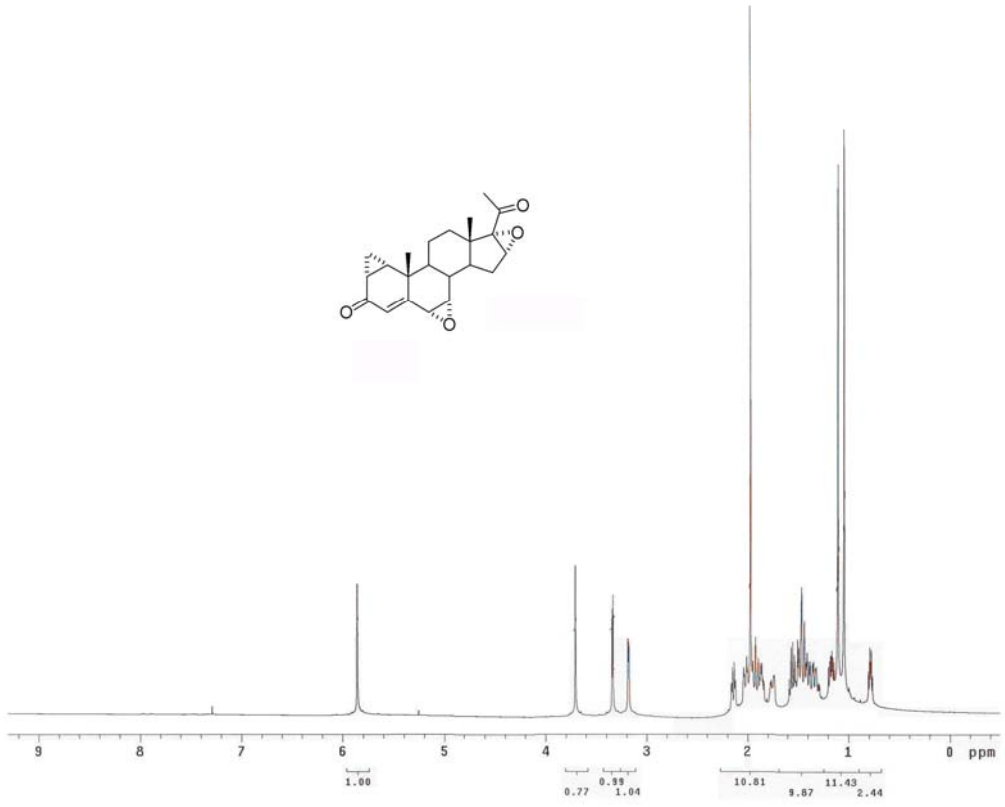
Appendix Figure 4 400 MHz ^{1}H NMR spectrum of 16α,17α-epoxy-1,4,6-pregnatriene-20-ethylene ketal-3-one (**53**)



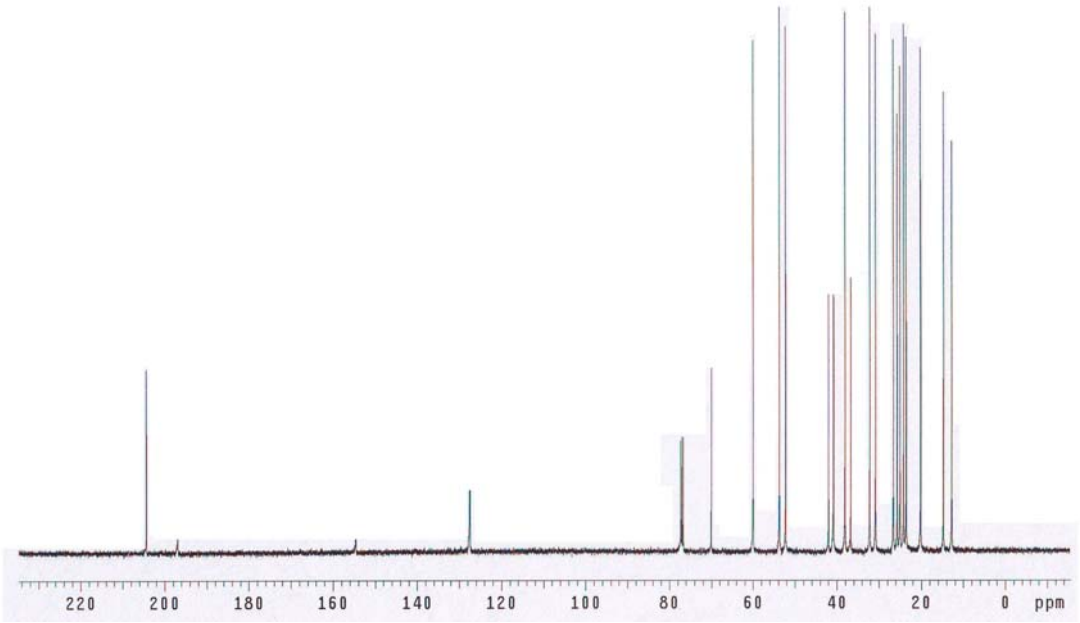
Appendix Figure 5 400 MHz ^{1}H NMR spectrum of 1α,2α-cyclomethylene-16α,17α-epoxy-4,6-pregnadiene-3,20-dione (**54**)



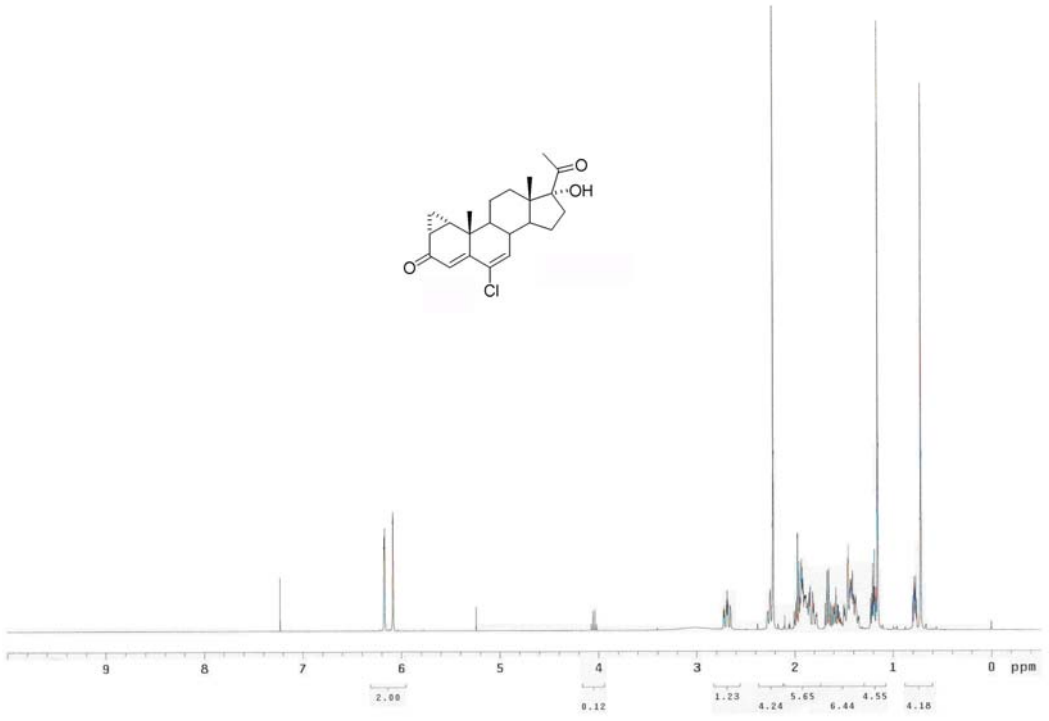
Appendix Figure 6 400 MHz ^{1}H NMR spectrum of cyproterone acetate (**1**)



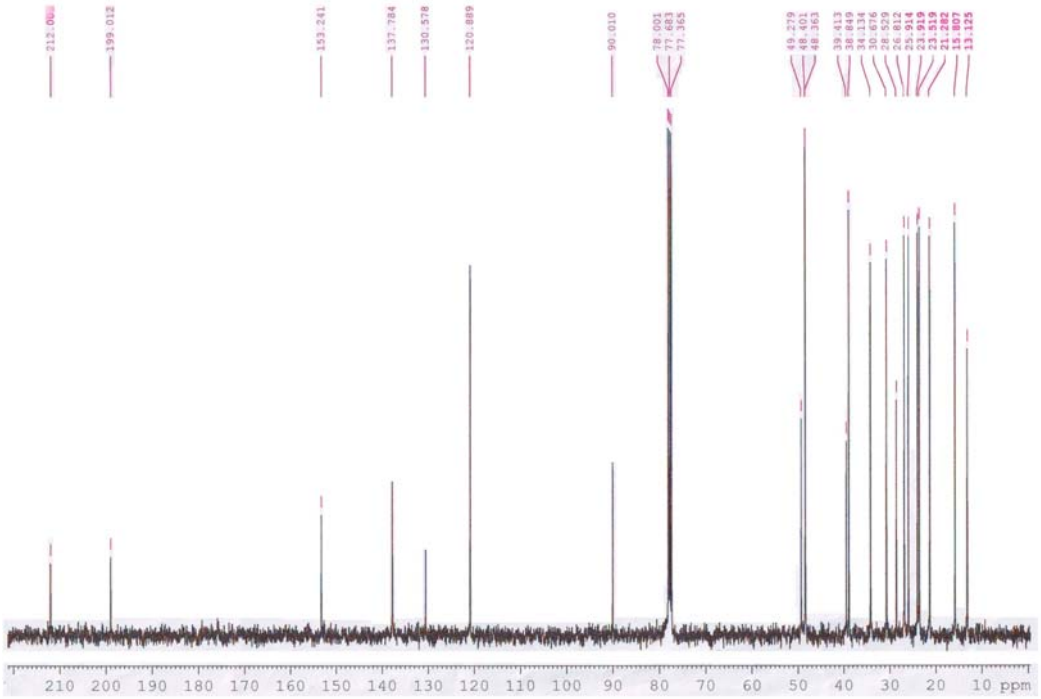
Appendix Figure 7 400 MHz ^{1}H NMR spectrum of 1α,2α-cyclomethylene-6α,7α-epoxy-16α,17α-epoxy-4-pregnene-3,20-dione (**66**)



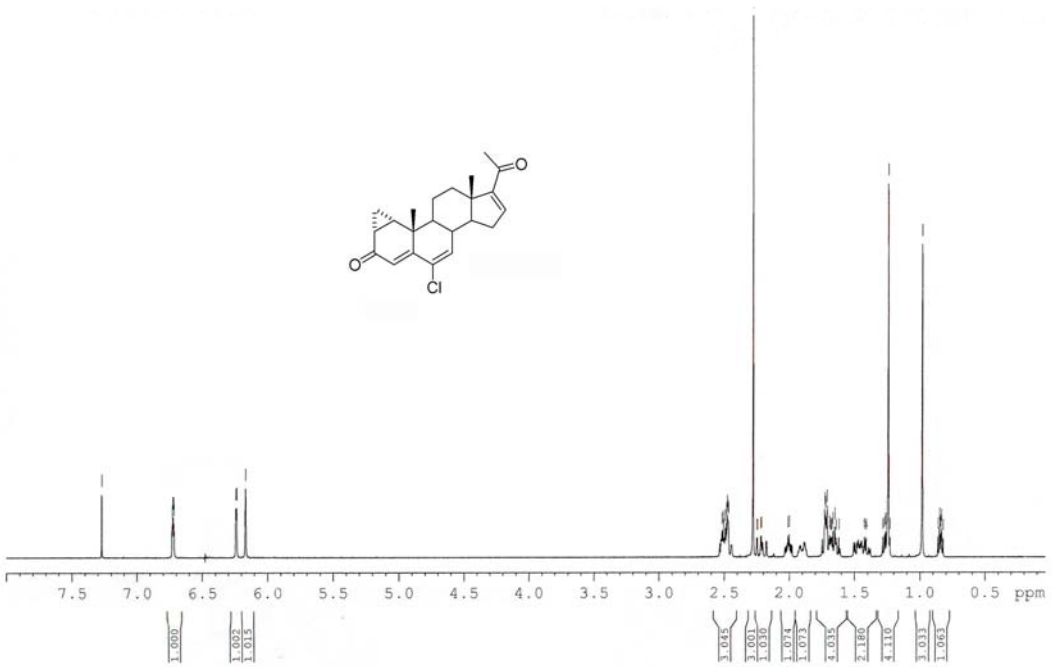
Appendix Figure 8 100 MHz ^{13}C NMR spectrum of 1α,2α-cyclomethylene-6α,7α-epoxy-16α,17α-epoxy-4-pregnene-3,20-dione (**66**)



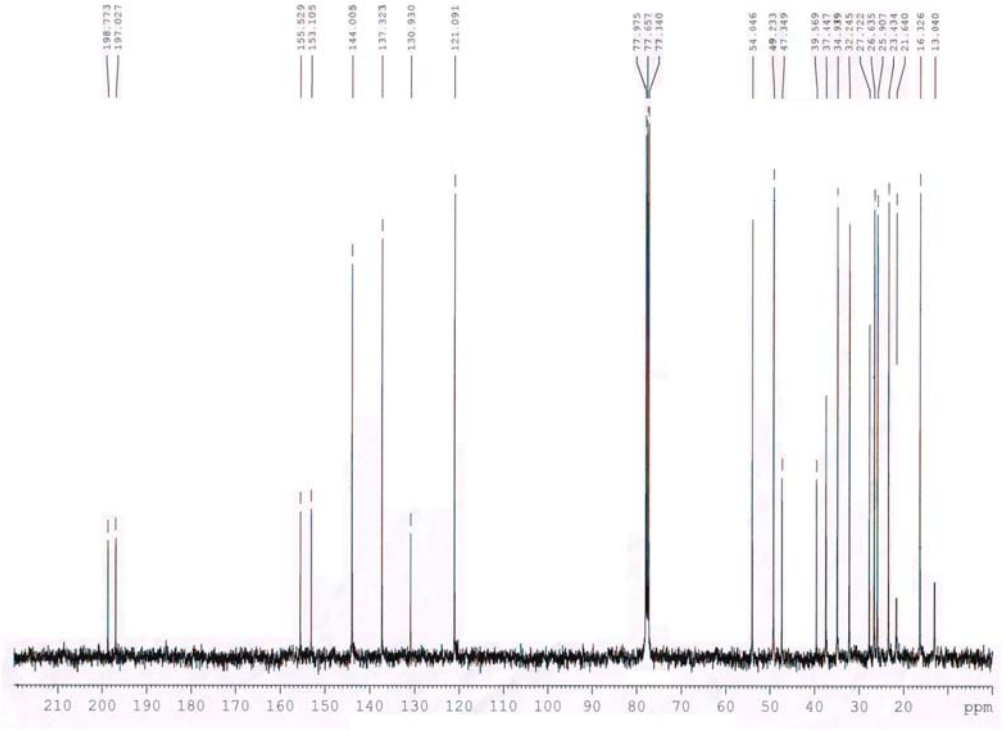
Appendix Figure 9 400 MHz ^{1}H NMR spectrum of 1α,2α-cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (**71**)



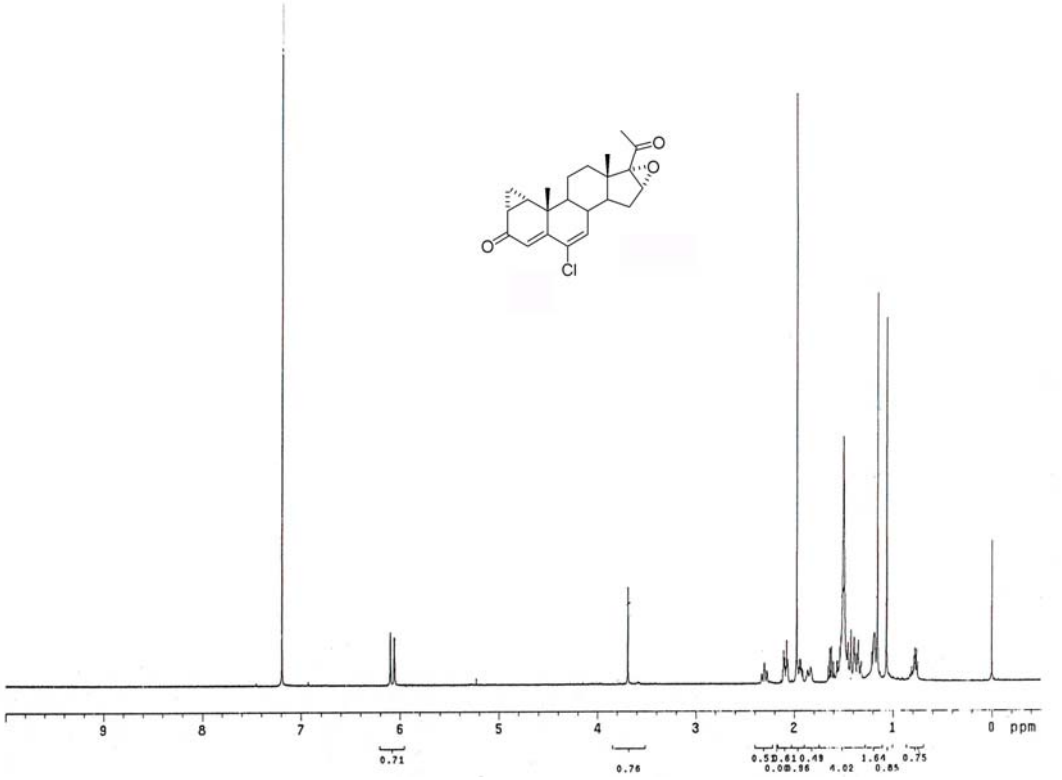
Appendix Figure 10 100 MHz ^{13}C NMR spectrum of 1α,2α-cyclomethylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (**71**)



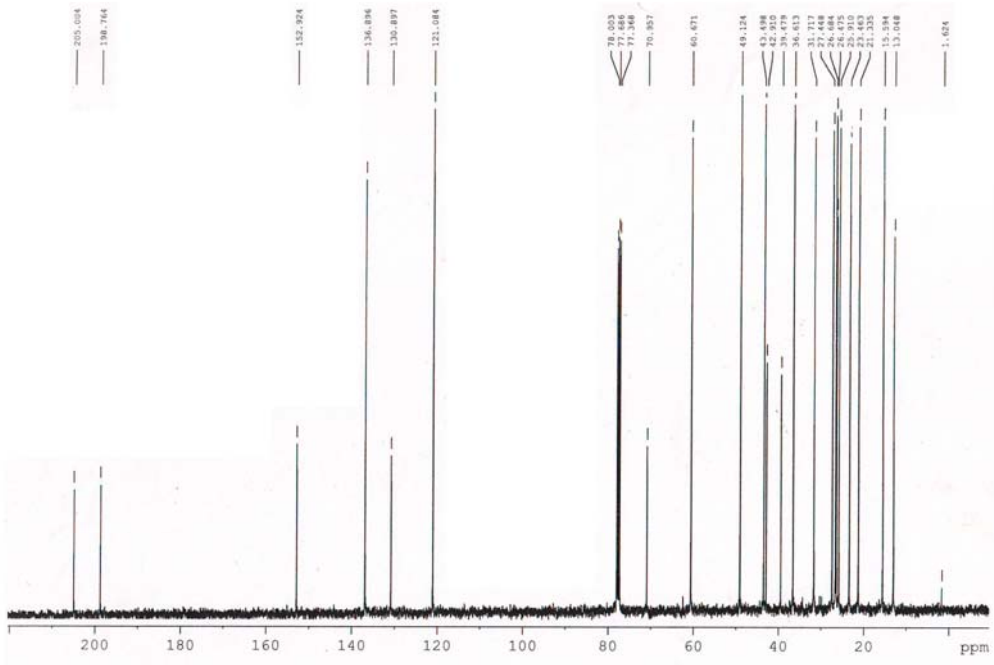
Appendix Figure 11 400 MHz ^{1}H NMR spectrum of 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**72**)



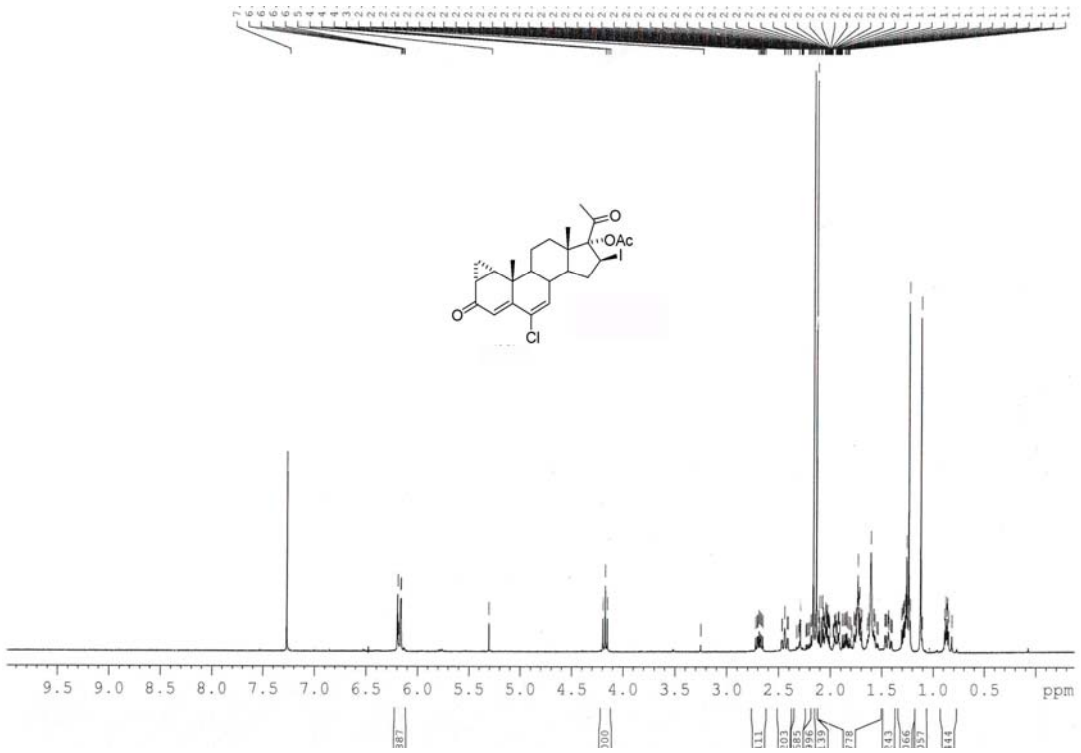
Appendix Figure 12 100 MHz ^{13}C NMR spectrum of 1α,2α-cyclomethylene-6-chloro-4,6,16-pregnatriene-3,20-dione (**72**)



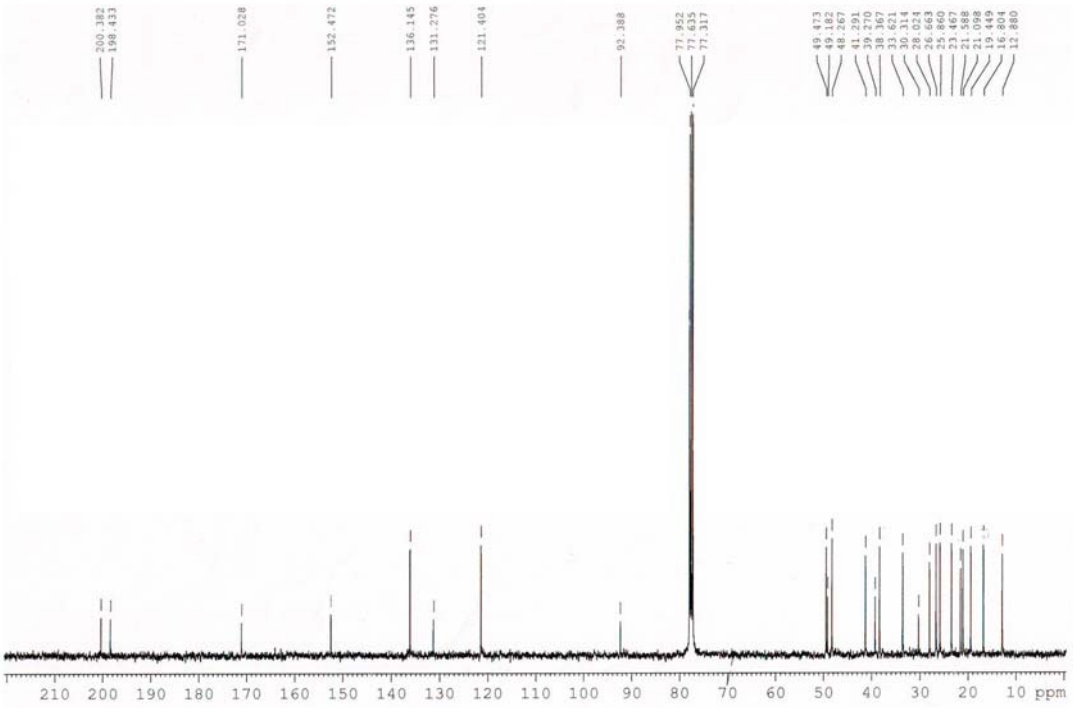
Appendix Figure 13 400 MHz ^{1}H NMR spectrum of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**)



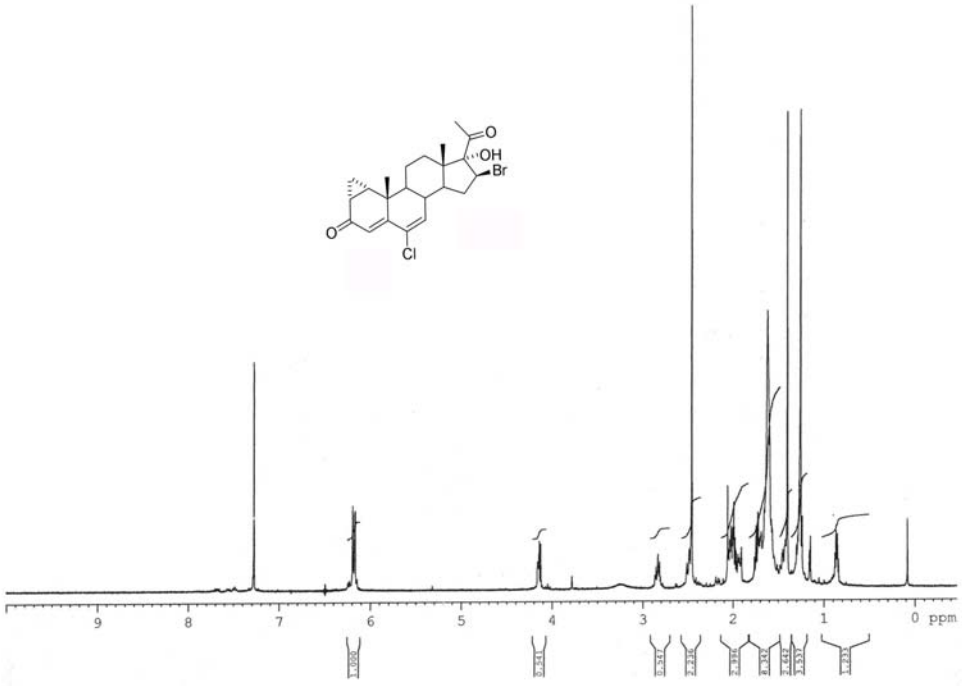
Appendix Figure 14 100 MHz ^{13}C NMR spectrum of 1α,2α-cyclomethylene-16α,17α-epoxy-6-chloro-4,6-pregnadiene-3,20-dione (**57**)



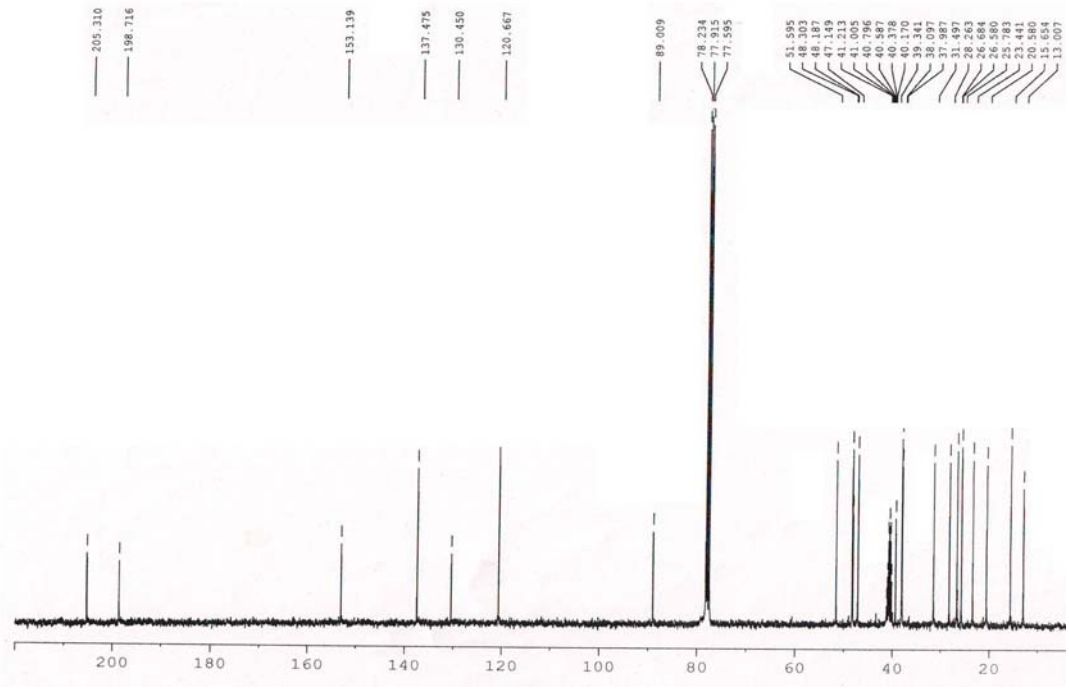
Appendix Figure 15 400 MHz ^{1}H NMR spectrum of 16β-iodo cyproterone acetate (**69**)



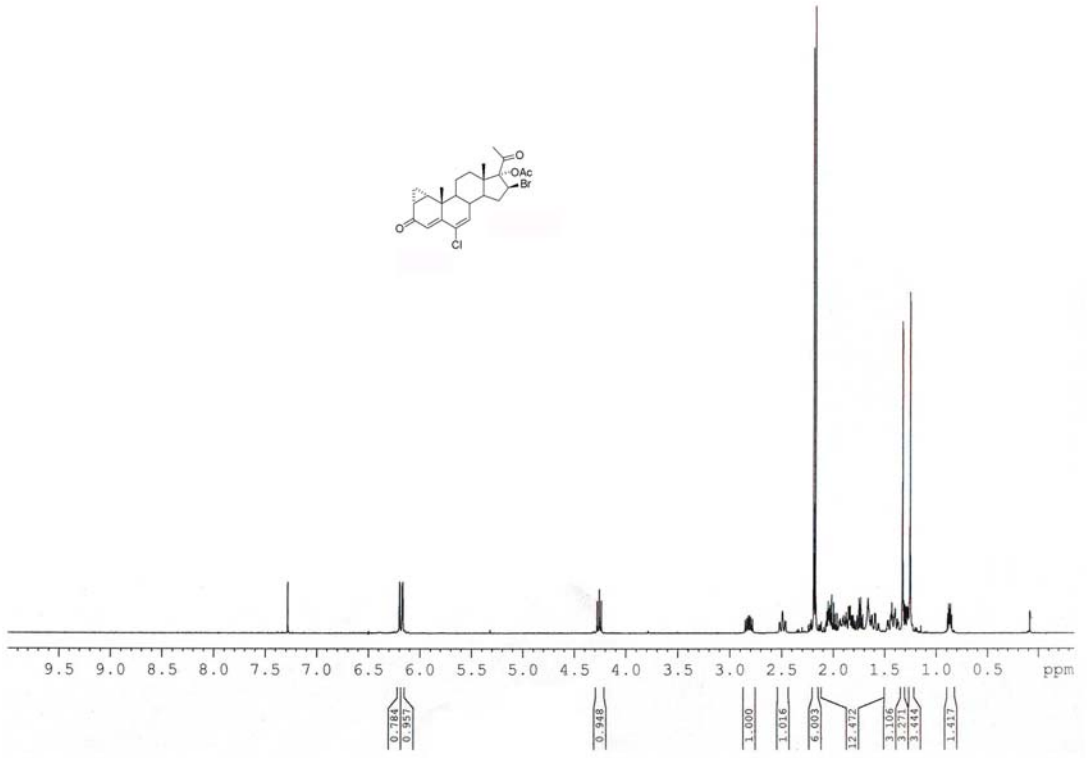
Appendix Figure 16 100 MHz ^{13}C NMR spectrum of 16β-iodo cyproterone acetate (**69**)



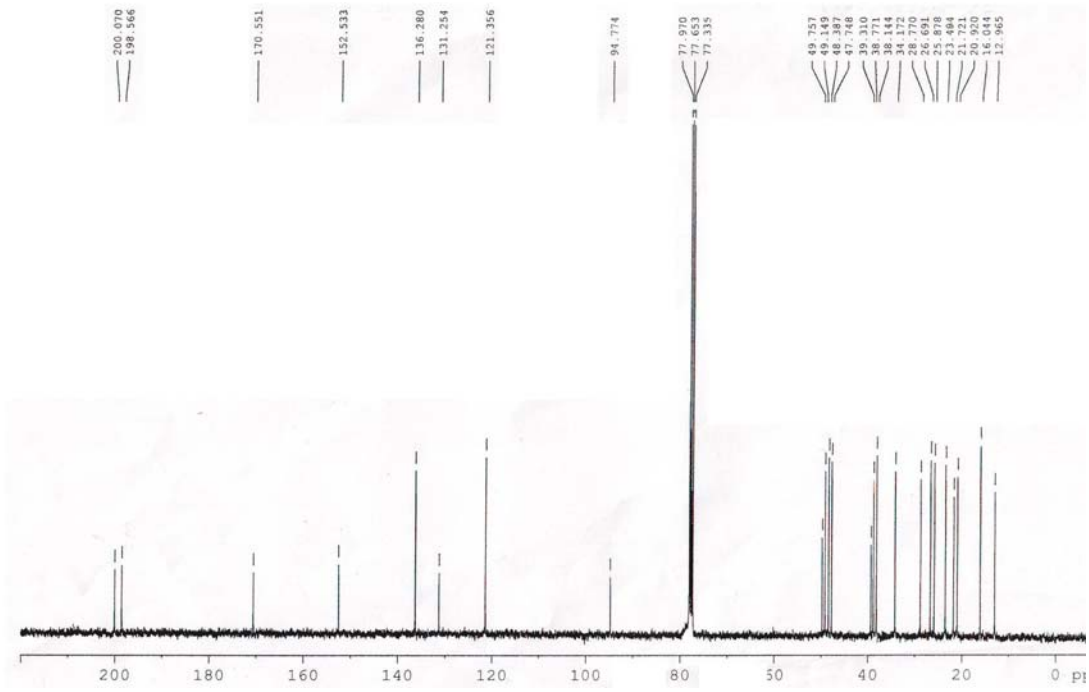
Appendix Figure 19 400 MHz ^{1}H NMR spectrum of 1α,2α-cyclomethylene-6-chloro-16β-bromo-17-hydroxy-4,6-pregnadiene-3,20-dione (**58**)



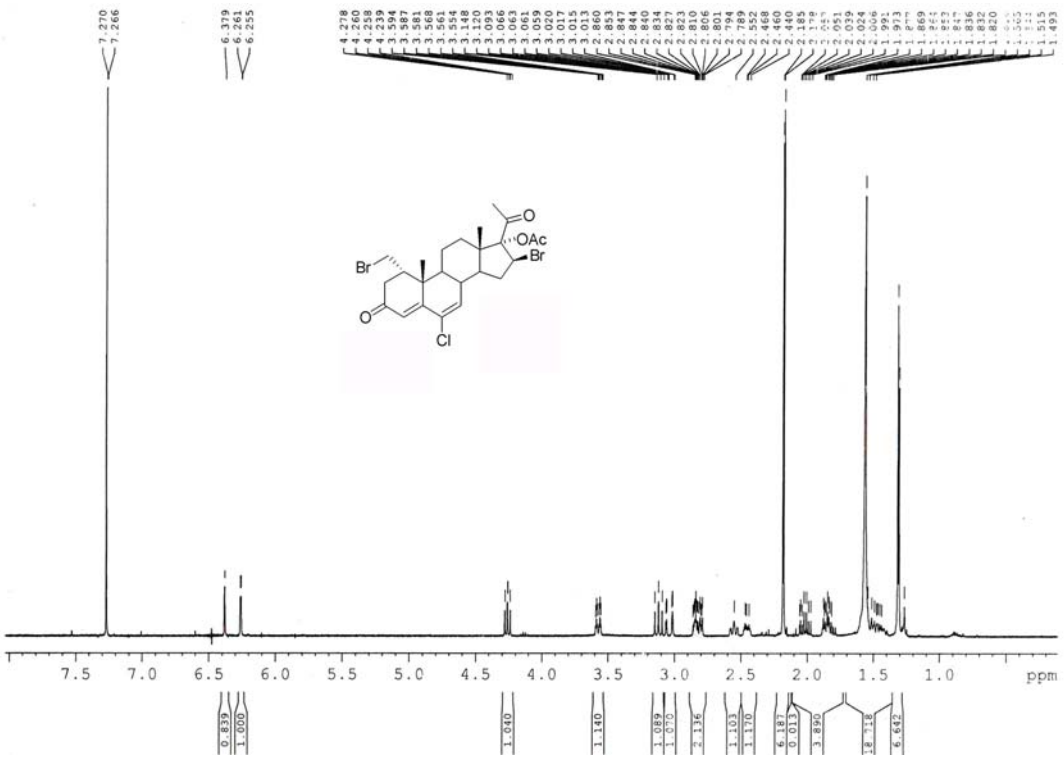
Appendix Figure 20 100 MHz ^{13}C NMR spectrum of 1α,2α-cyclomethylene-6-chloro-16β-bromo-17-hydroxy-4,6-pregnadiene-3,20-dione (**58**)



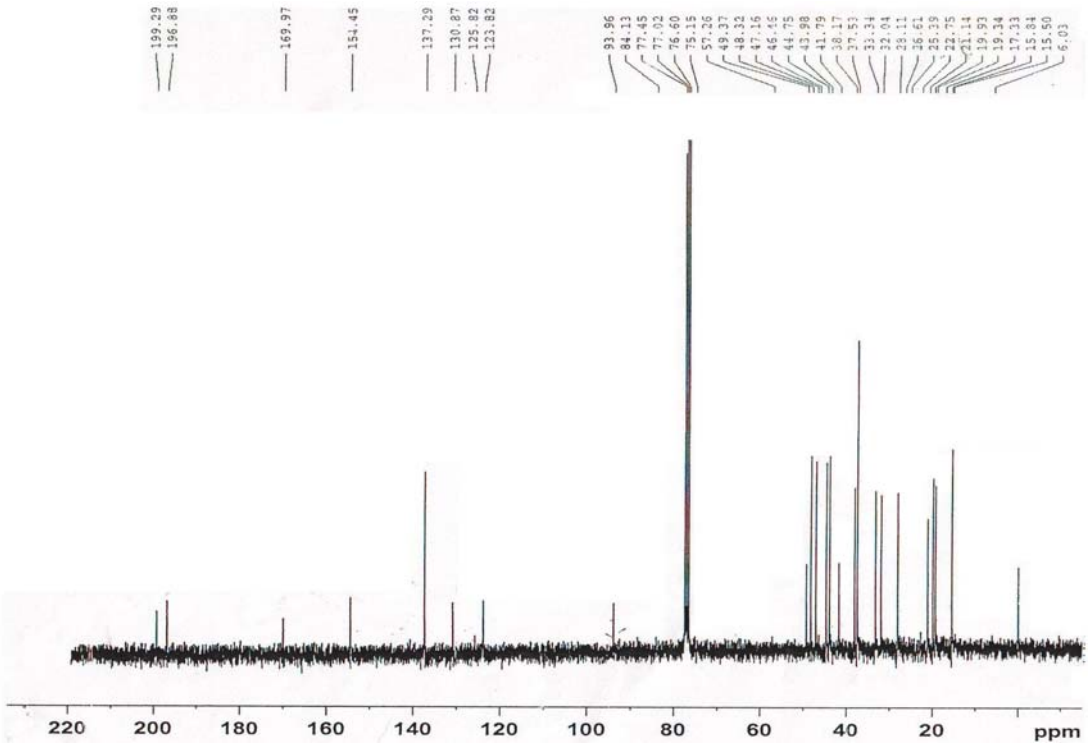
Appendix Figure 21 400 MHz ^{1}H NMR spectrum of 16β-bromo cyproterone acetate (**59**)



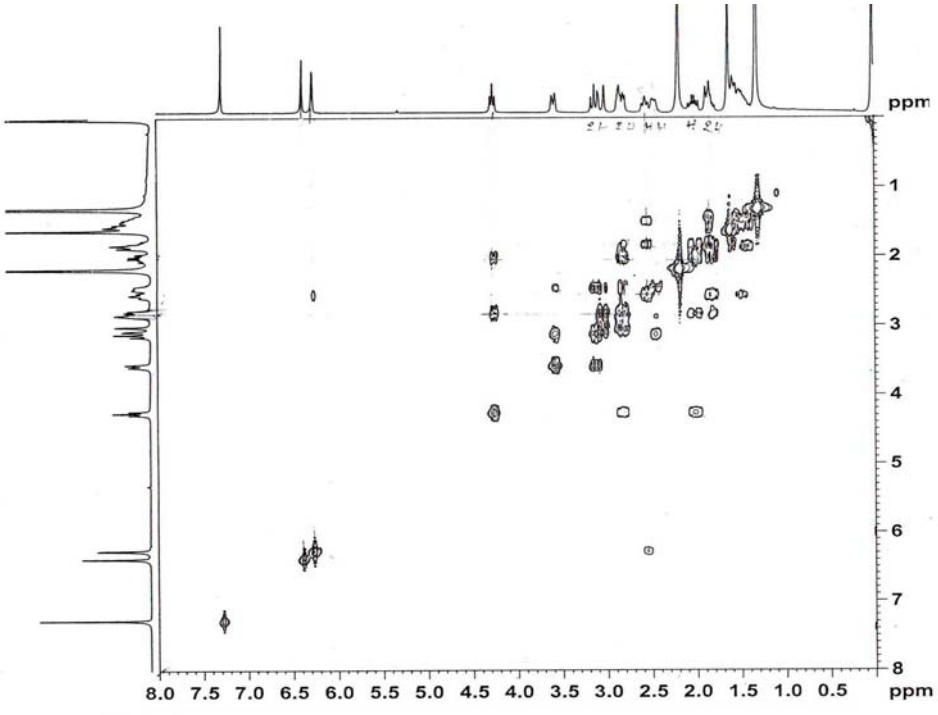
Appendix Figure 22 100 MHz ^{13}C NMR spectrum of 16β-bromo cyproterone acetate (**59**)



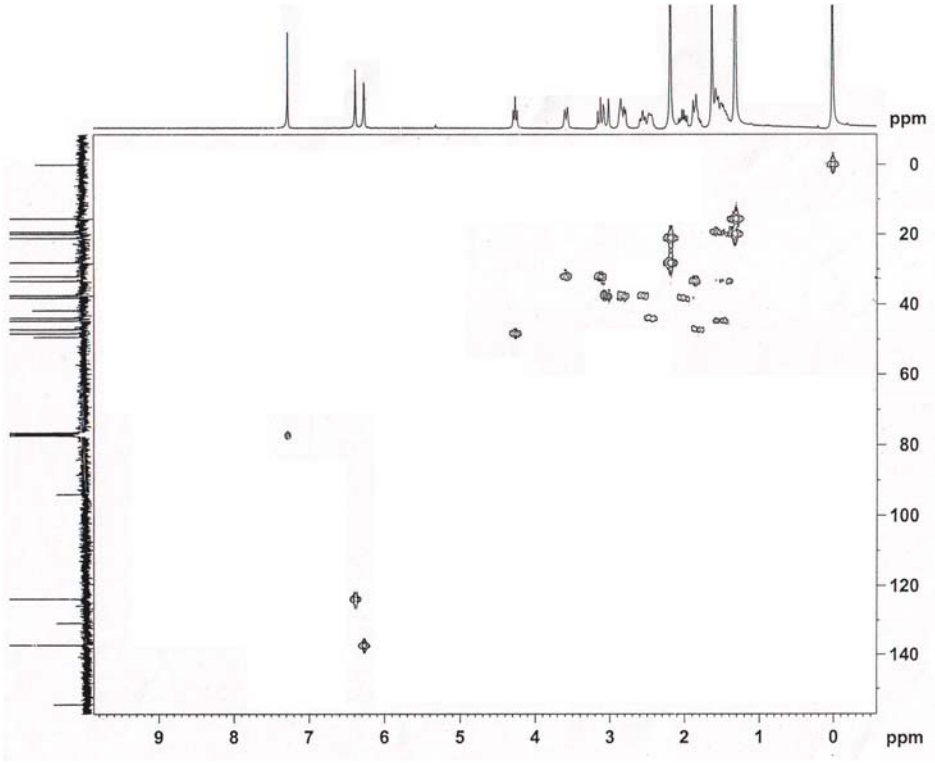
Appendix Figure 23 400 MHz ^{1}H NMR spectrum of 1α-bromomethy-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**)



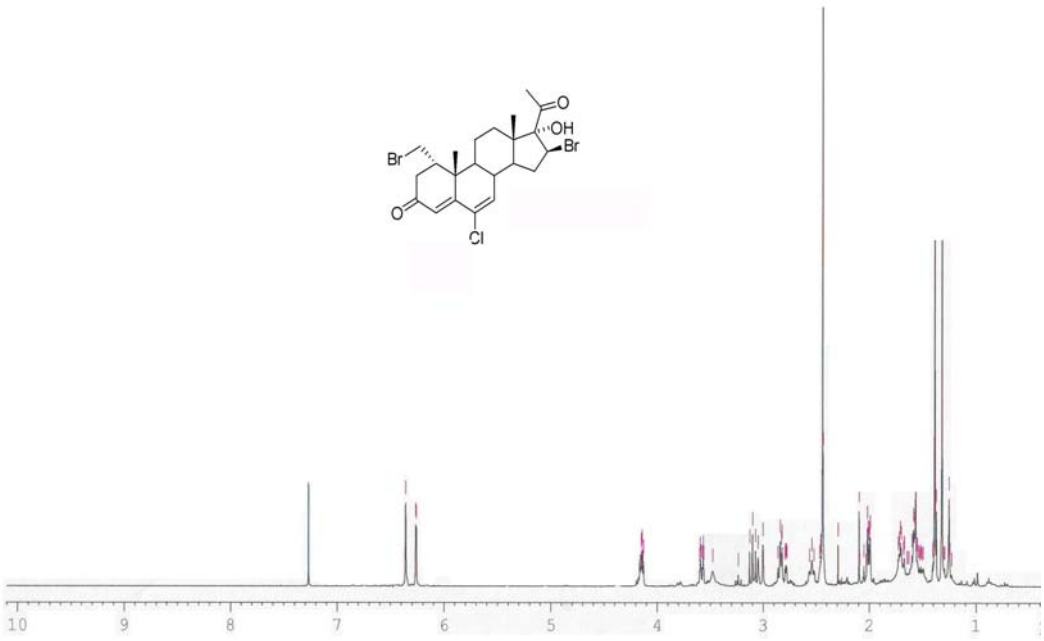
Appendix Figure 24 100 MHz ^{13}C NMR spectrum of 1α-bromomethyl-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione



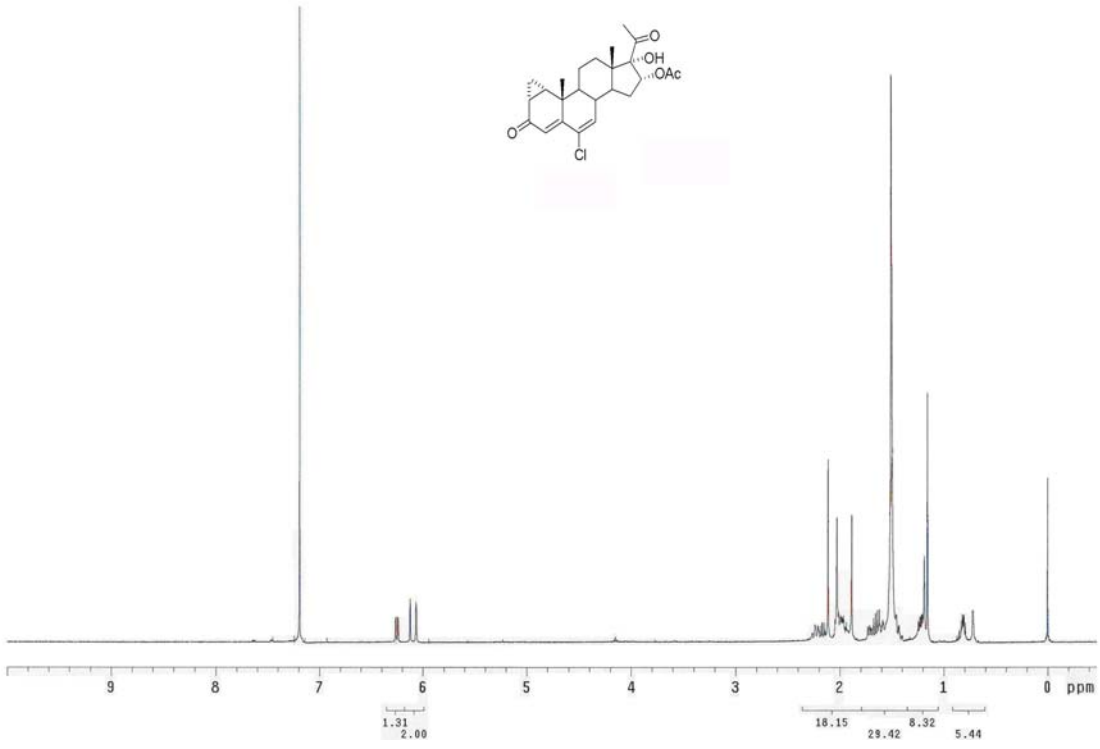
Appendix Figure 25 2D-COSY NMR spectrum of 1α-bromomethy-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**)



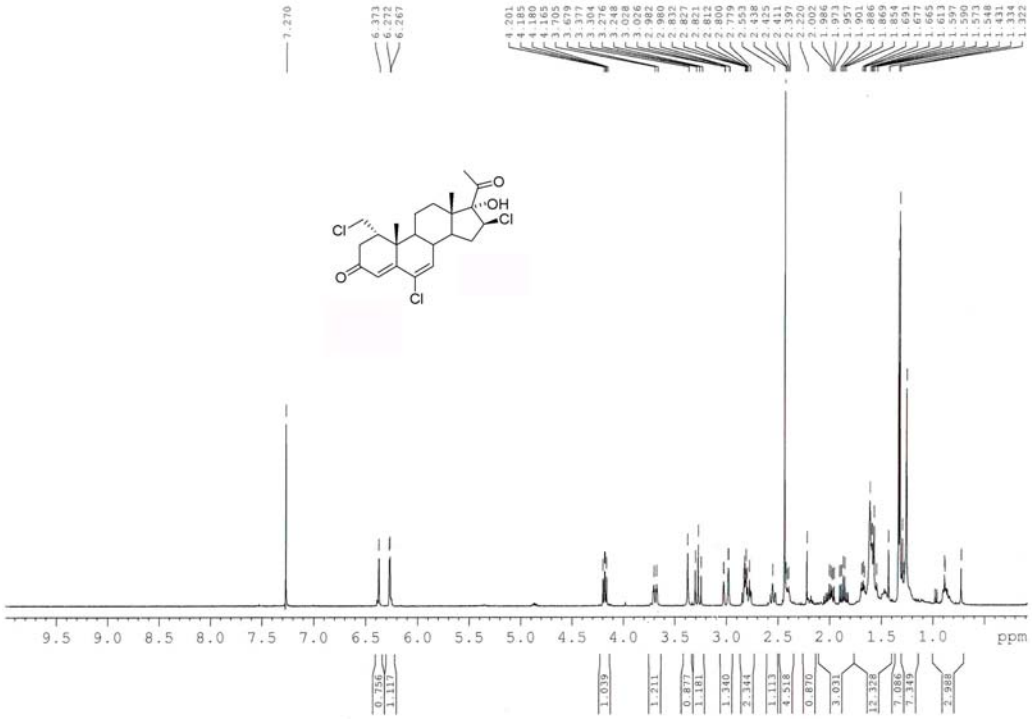
Appendix Figure 26 2D-HMQC NMR spectrum of 1α-bromomethy-6-chloro-16β-bromo-17α-acetoxy-4,6-pregnadiene-3,20-dione (**74**)



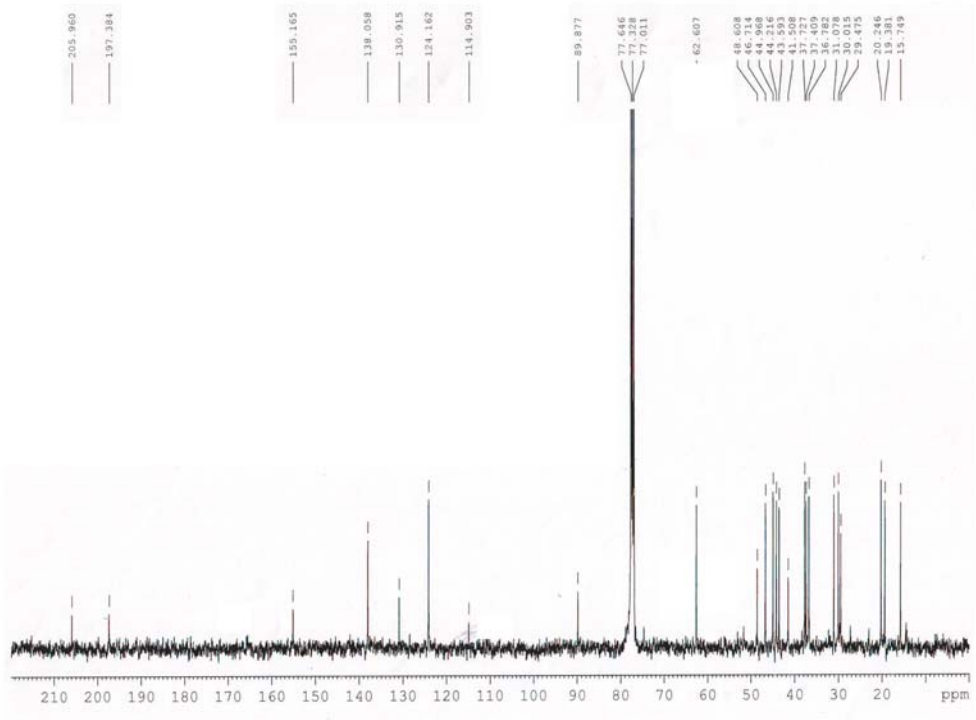
Appendix Figure 27 400 MHz ^{1}H NMR spectrum of 1α-bromomethyl-16β-bromo-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione(**73**)



Appendix Figure 28 400 MHz ^{1}H NMR spectrum of 16α-acetoxy-1α,2α-cyclo methylene-6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (**90**)

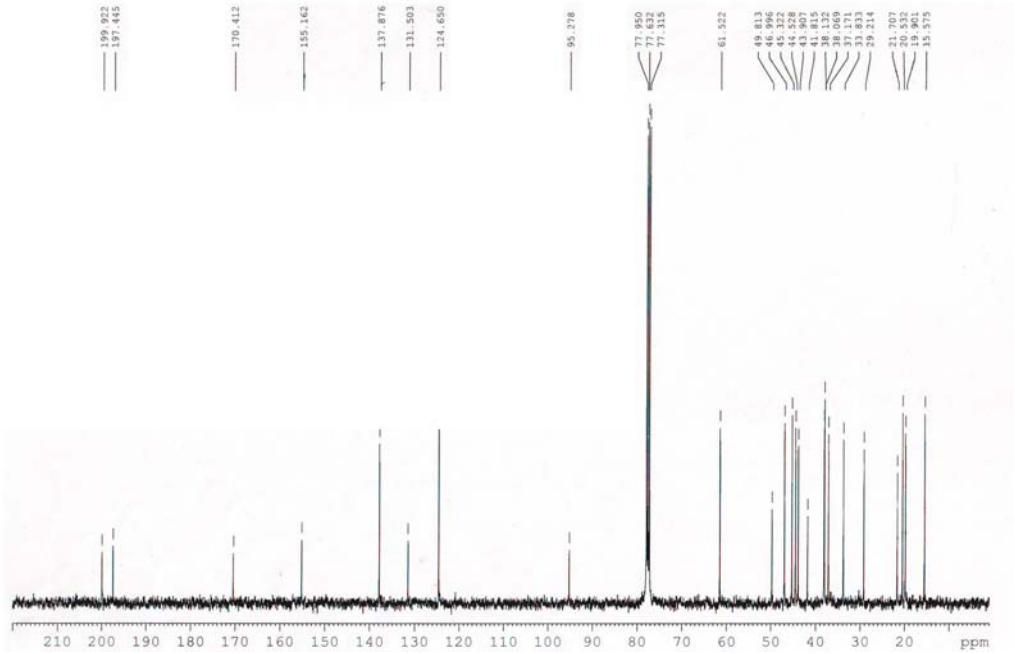


Appendix Figure 29 400 MHz ^{1}H NMR spectrum of 1α-chloro-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**)

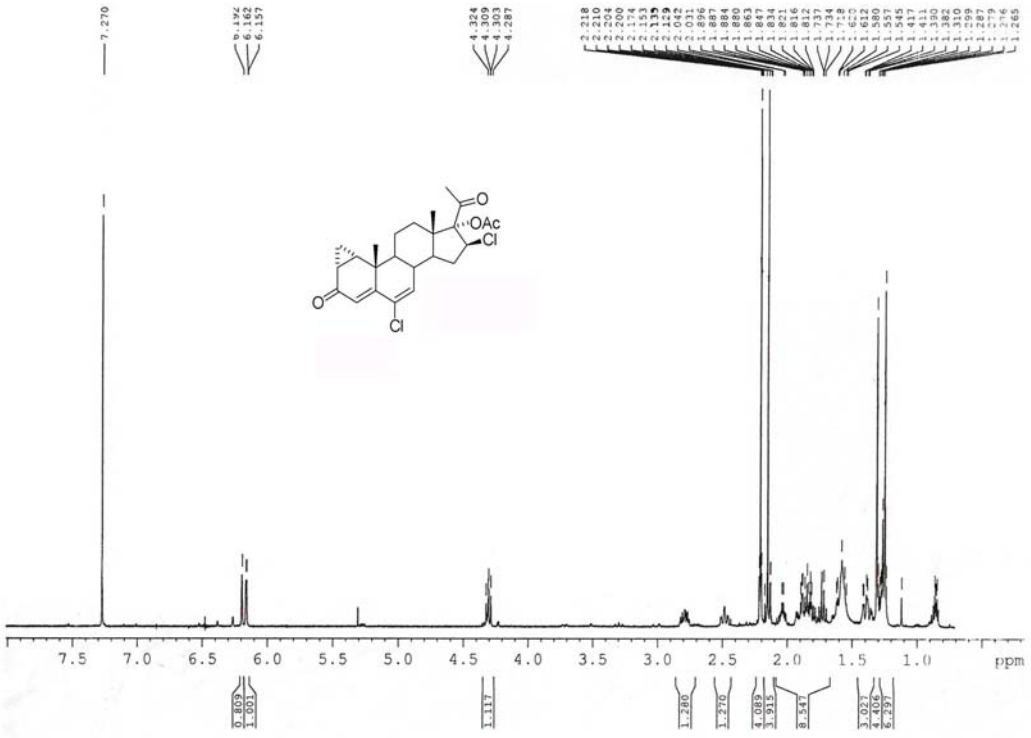


Appendix Figure 30 100 MHz ^{13}C NMR spectrum of 1α-chloro-16β-chloro-17α-hydroxy-6-chloro-4,6-pregnadiene-3,20-dione (**75**)

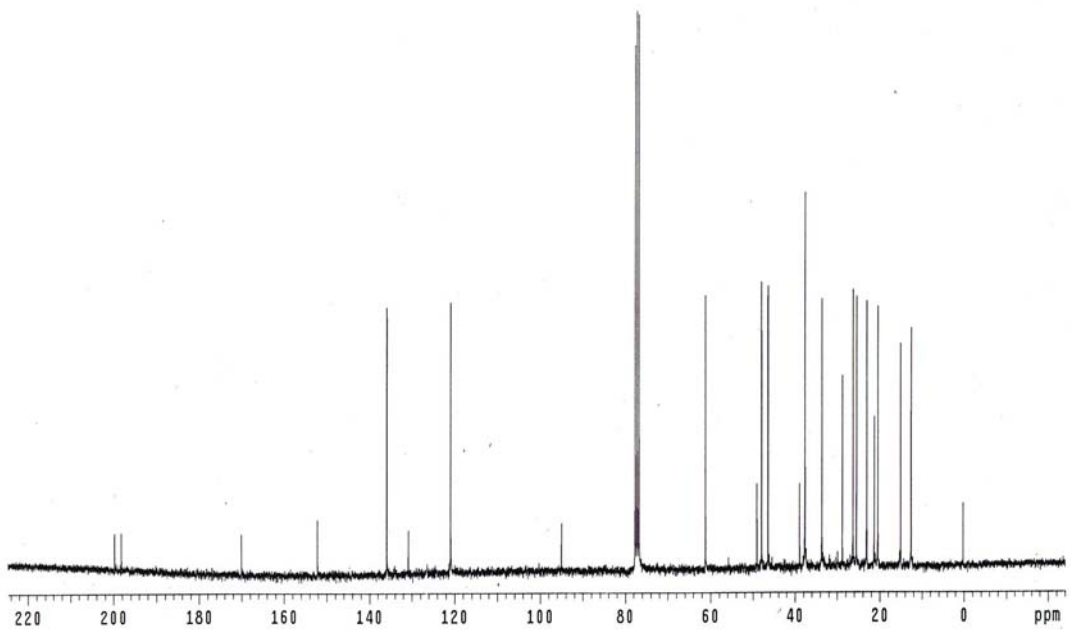
Appendix Figure 31 400 MHz ^{1}H NMR spectrum of 1α-chloromethyl-6-chloro-16β-chloro-17α-acetoxy-4,6-pregnadiene-3,20-dione (**76**)



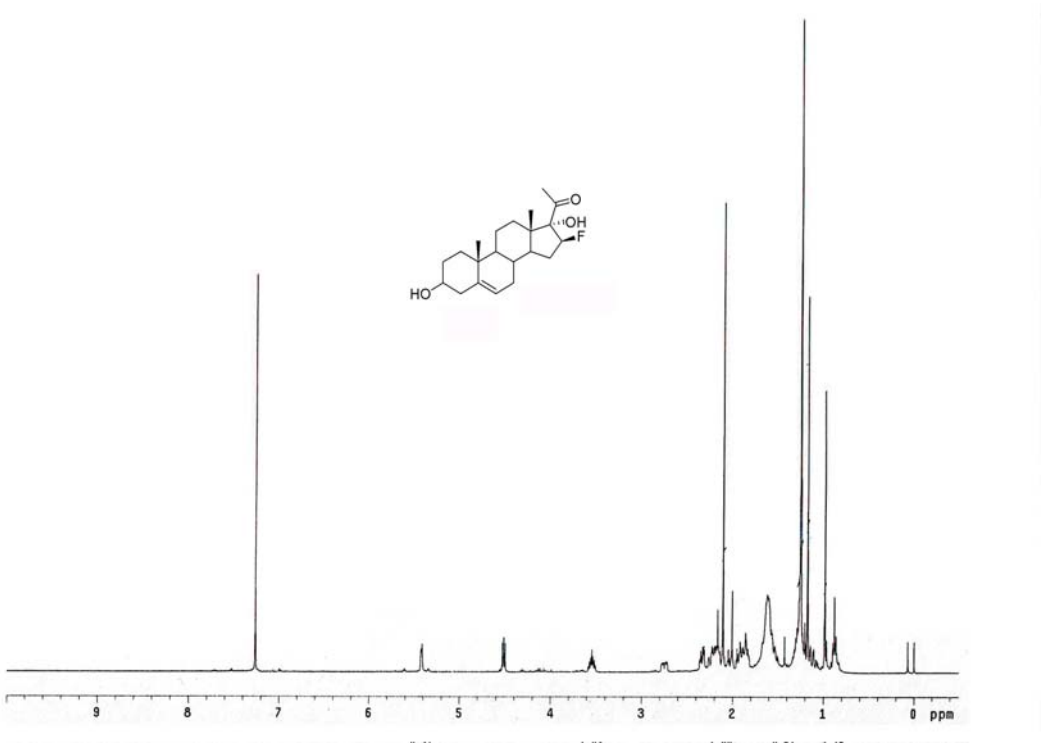
Appendix Figure 32 100 MHz ^{13}C NMR spectrum of 1α-chloromethyl-6-chloro-16β-chloro-17α-acetoxy-4,6-pregnadiene-3,20-dione (**76**)



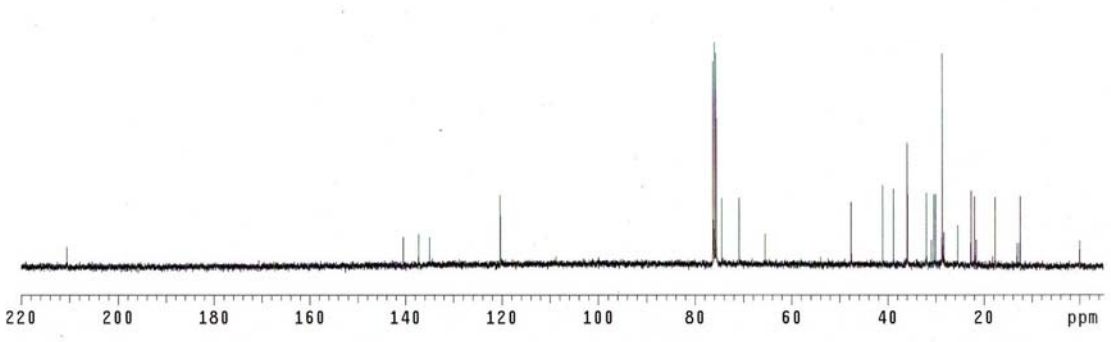
Appendix Figure 33 400 MHz ^{1}H NMR spectrum of 16β-chloro cyproterone acetate (**56**)



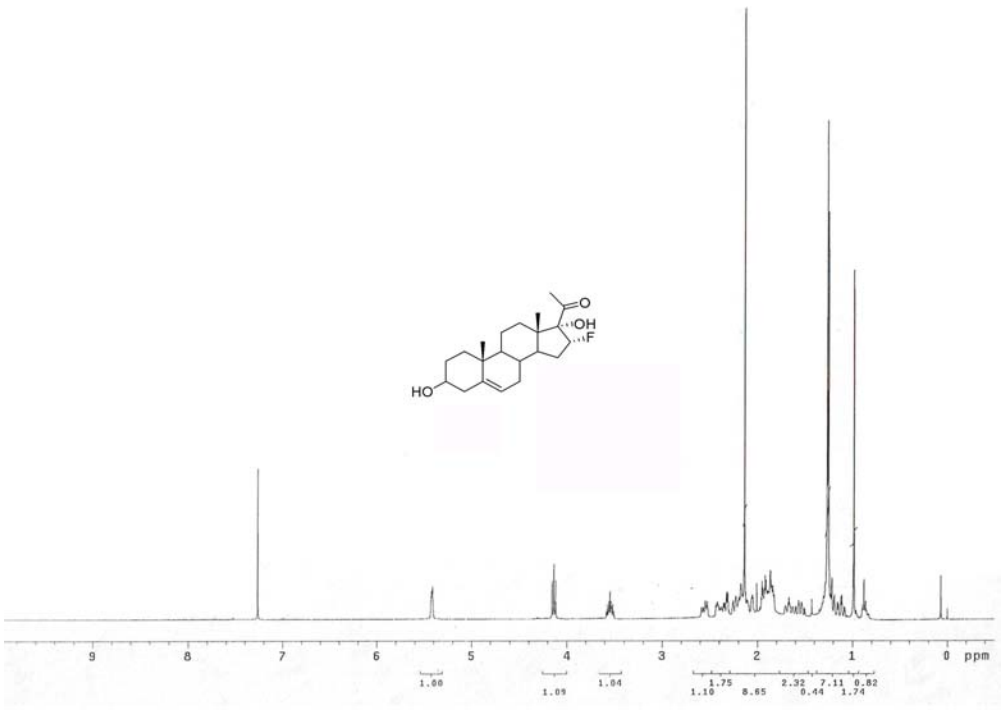
Appendix Figure 34 100 MHz ^{13}C NMR spectrum of 16β-chloro cyproterone acetate (**56**)



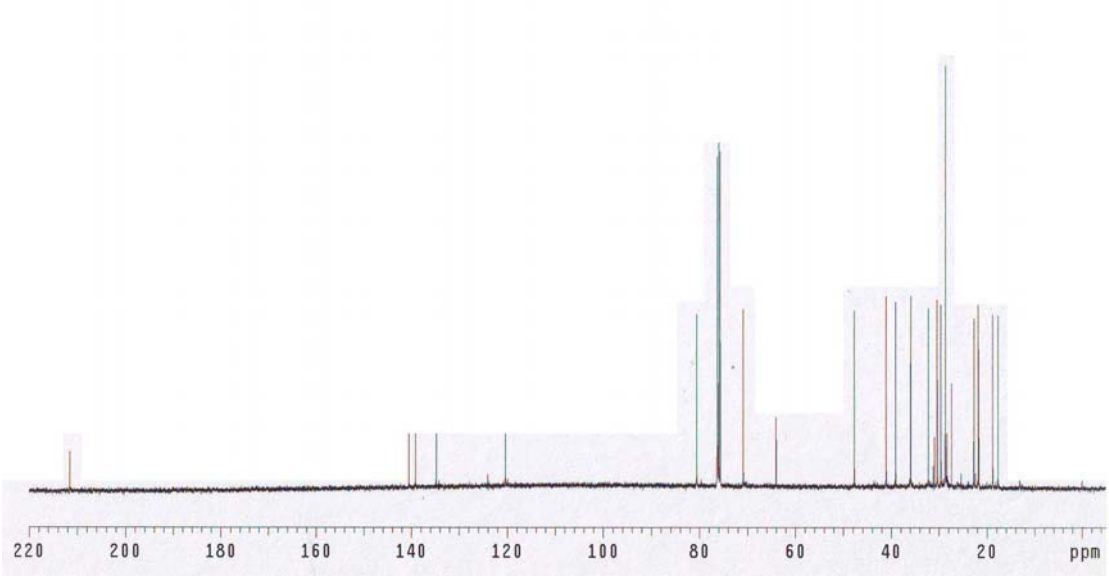
Appendix Figure 35 400 MHz ^{1}H NMR spectrum of 16β-fluoro-3β,17α-dihydroxy -5-pregnene-20-one (**78**)



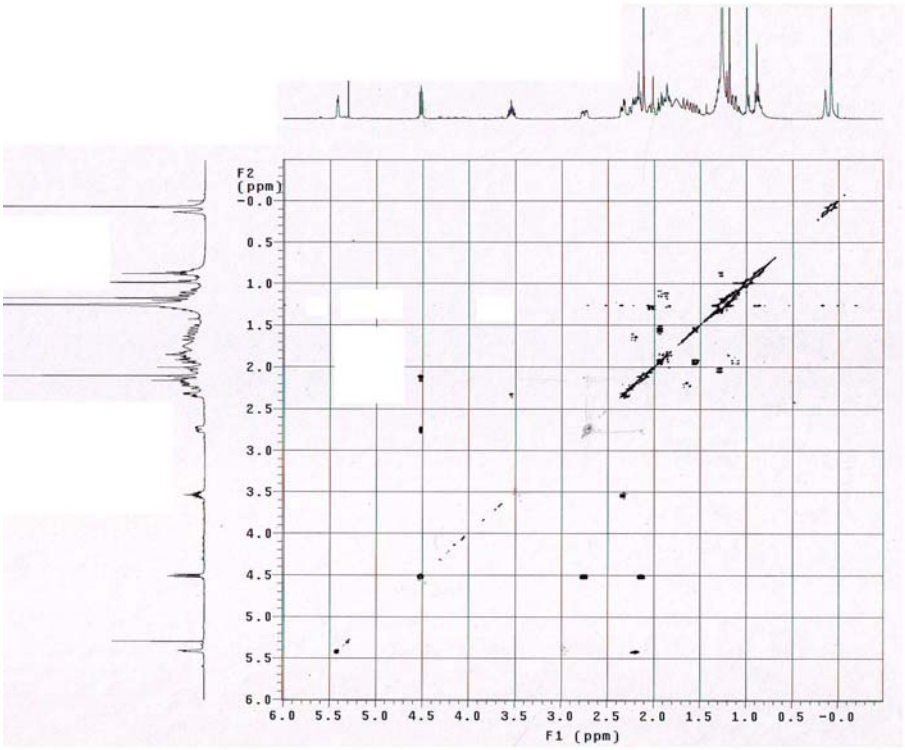
Appendix Figure 36 100 MHz ^{13}C NMR spectrum of 16β-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**78**)



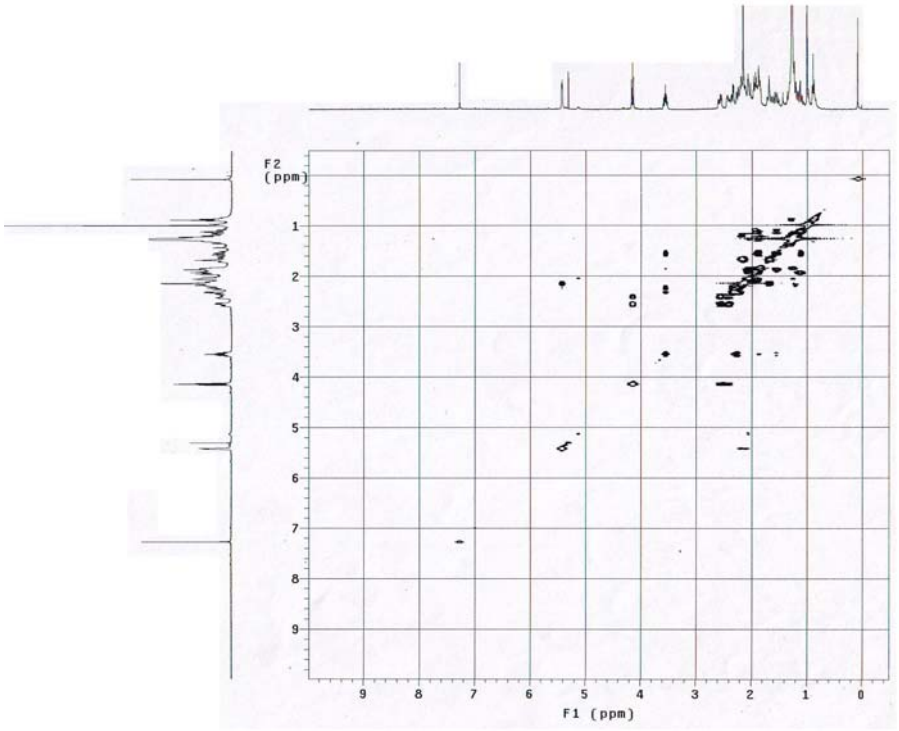
Appendix Figure 37 400 MHz ^{1}H NMR spectrum of 16α-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**77**)



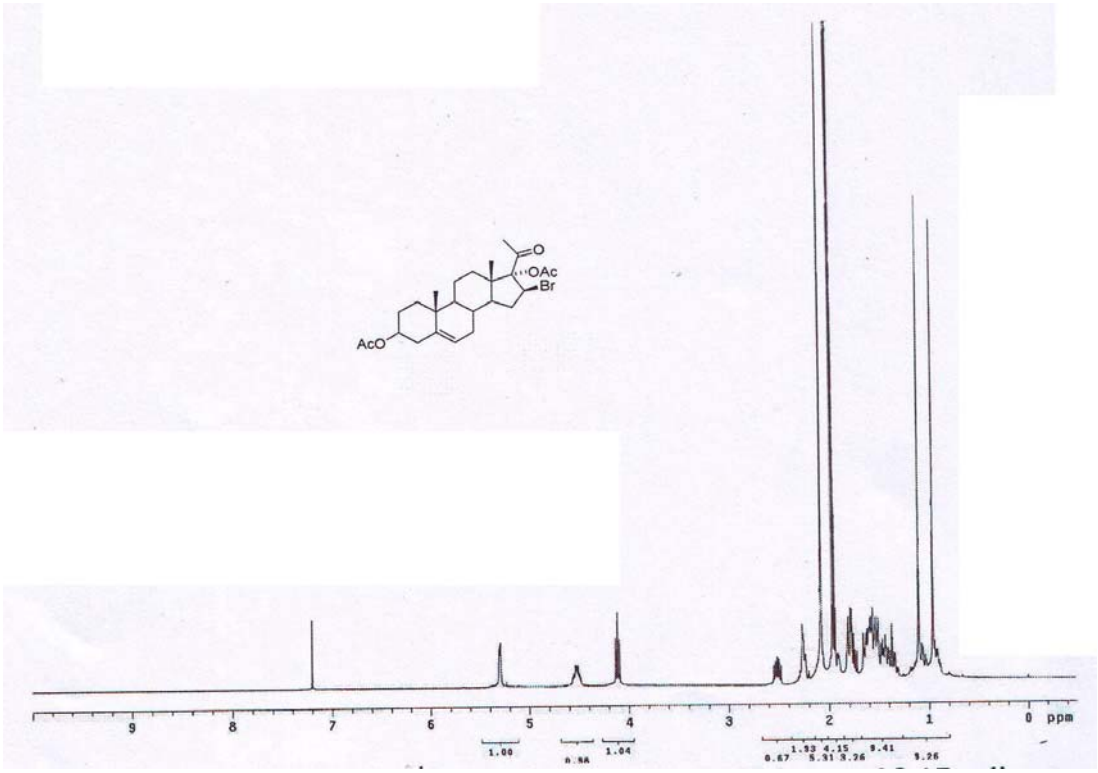
Appendix Figure 38 100 MHz ^{13}C NMR spectrum of 16α-fluoro-3β,17α-dihydroxy -5-pregnene-20-one (**77**)



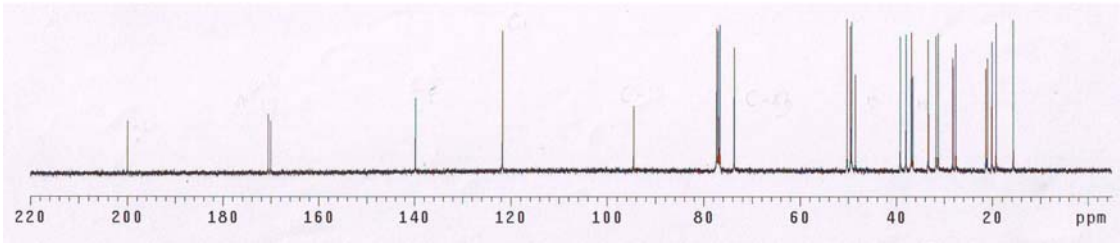
Appendix Figure 39 2D-COSY NMR spectrum of 16β-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**78**)



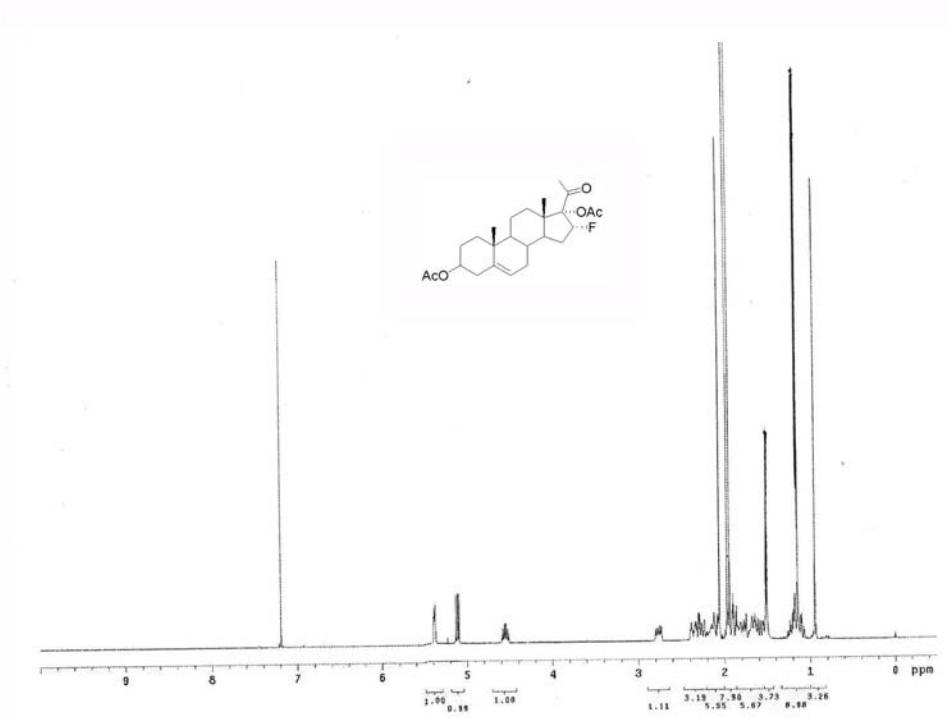
Appendix Figure 40 2D-COSY NMR spectrum of 16α-fluoro-3β,17α-dihydroxy-5-pregnene-20-one (**77**)



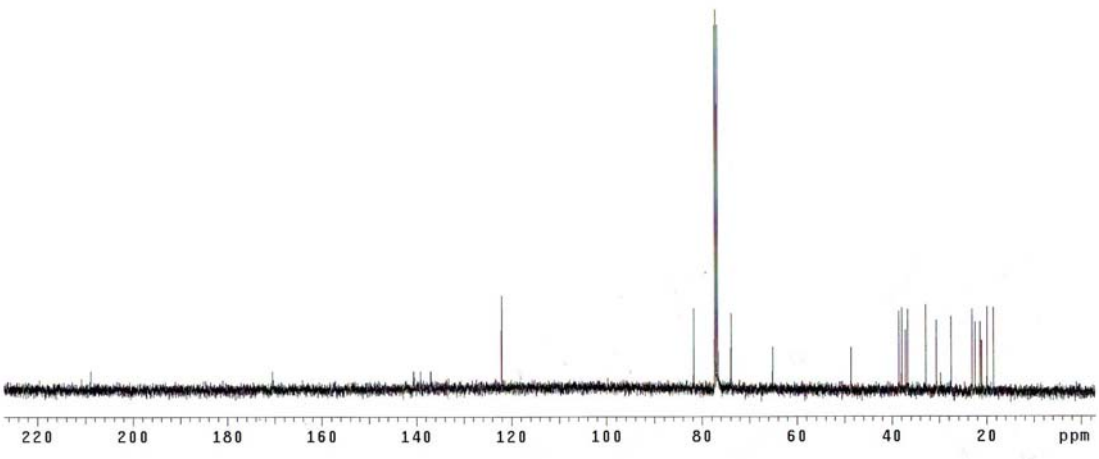
Appendix Figure 41 400 MHz ^{1}H NMR spectrum of 16β-bromo-3β,17α-diacetoxy--5-pregnene-20-one (**84**)



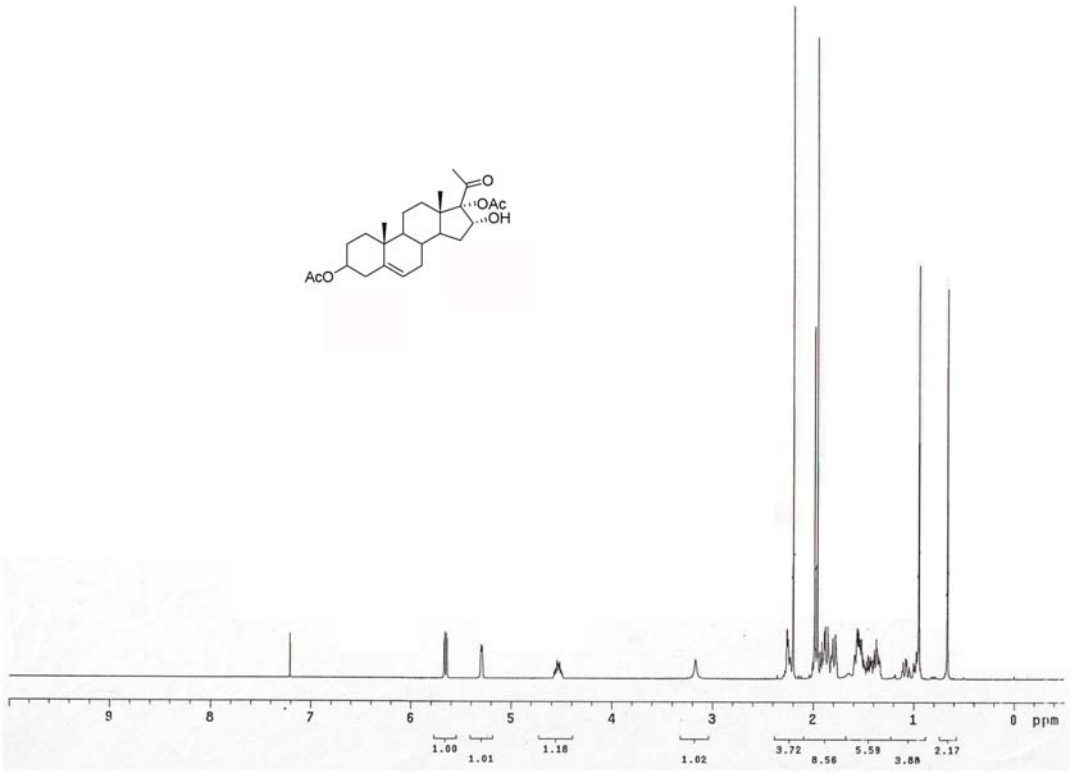
Appendix Figure 42 100 MHz ^{13}C NMR spectrum of 16β-bromo-3β,17α-diacetoxy -5 -pregnene-20-one (**84**)



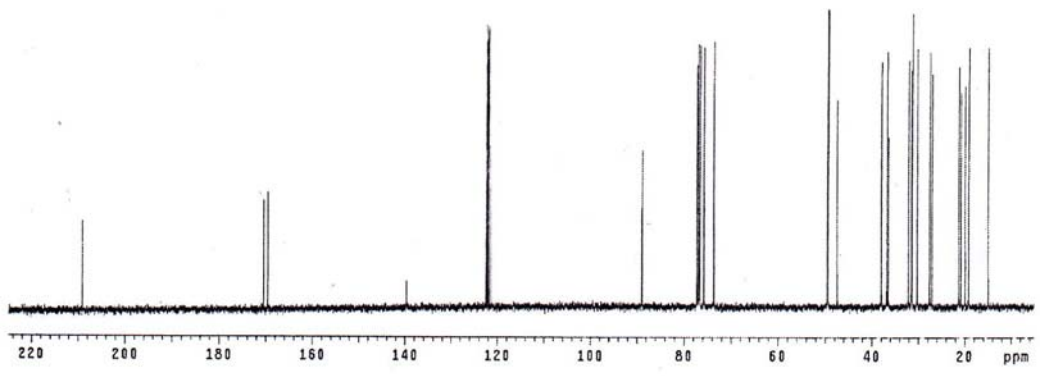
Appendix Figure 43 400 MHz ^{1}H NMR spectrum of 16α-fluoro-3β,17α-diacetoxy-5-pregnene-20-one (**79**)



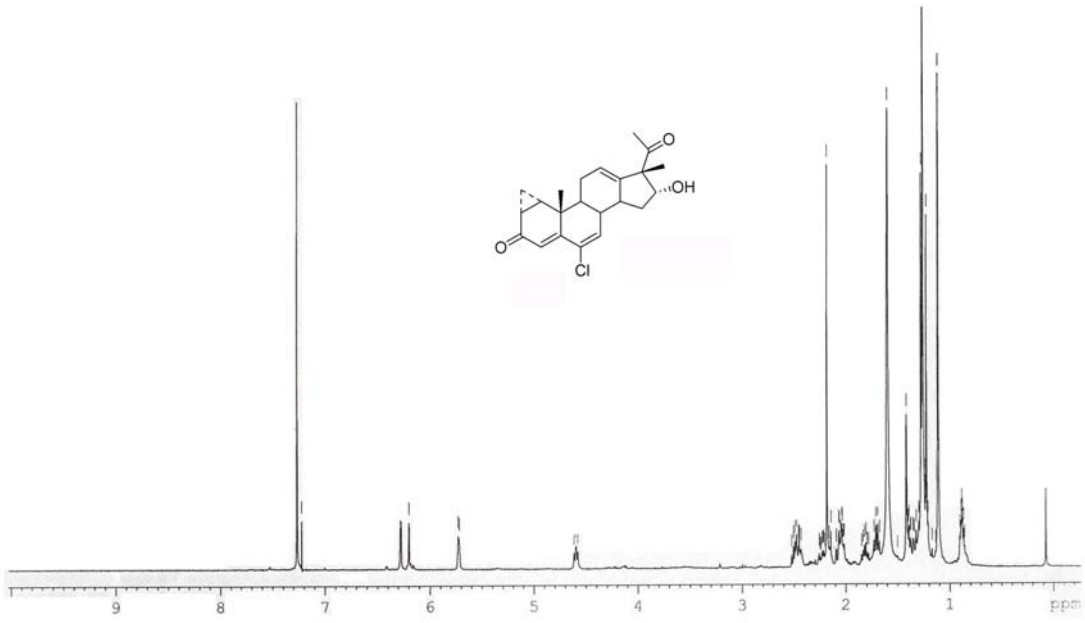
Appendix Figure 44 100 MHz ^{13}C NMR spectrum of 16α-fluoro-3β,17α-diacetoxy-5-pregnene-20-one (**79**)



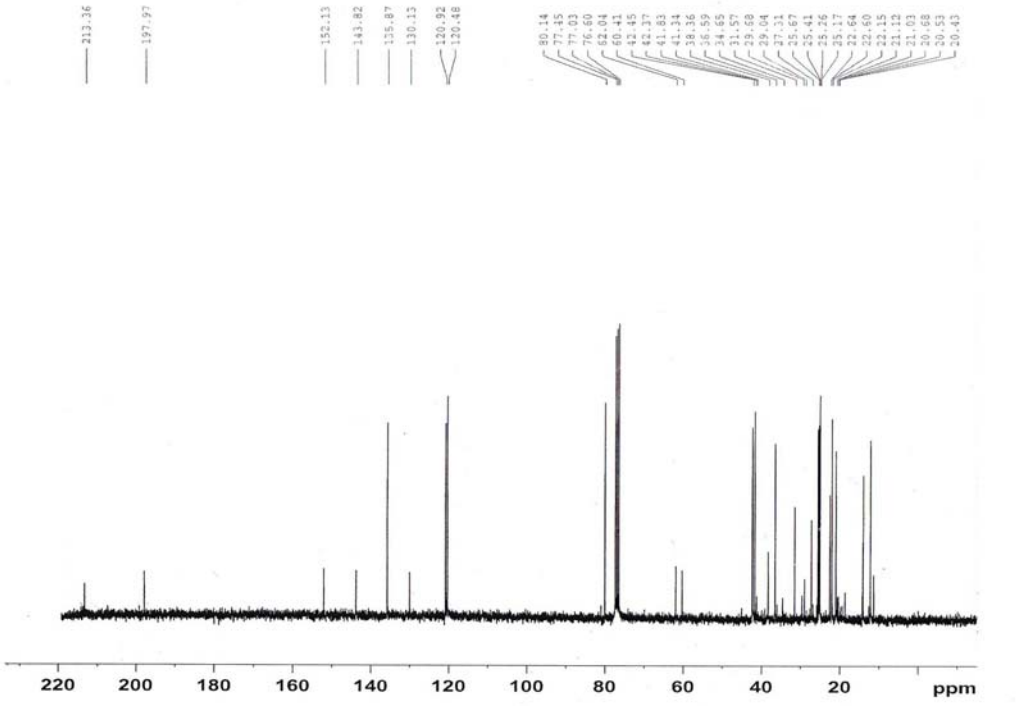
Appendix Figure 45 400 MHz ^{1}H NMR spectrum of 16α-hydroxy-3β,17α-diacetoxy-5-pregnene-20-one (**85**)



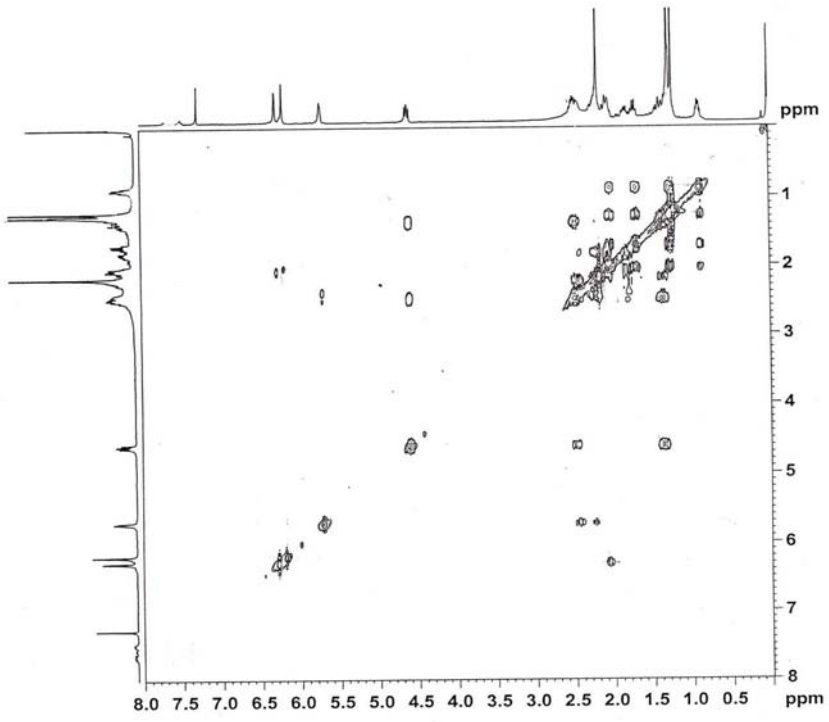
Appendix Figure 46 100 MHz ^{13}C NMR spectrum of 16α-hydroxy-3β,17α-diacetoxy-5-pregnene-20-one (**85**)



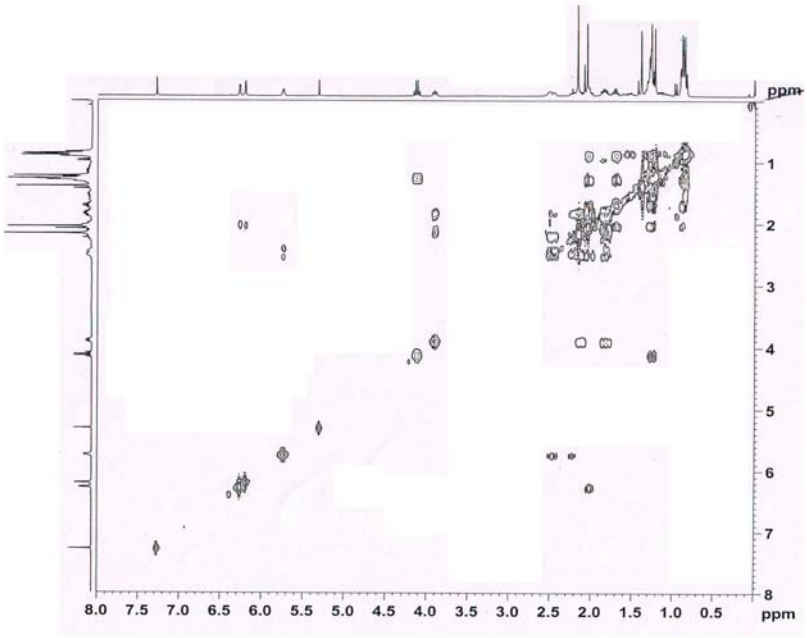
Appendix Figure 47 400 MHz ^{1}H NMR spectrum of 16α-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**81**)



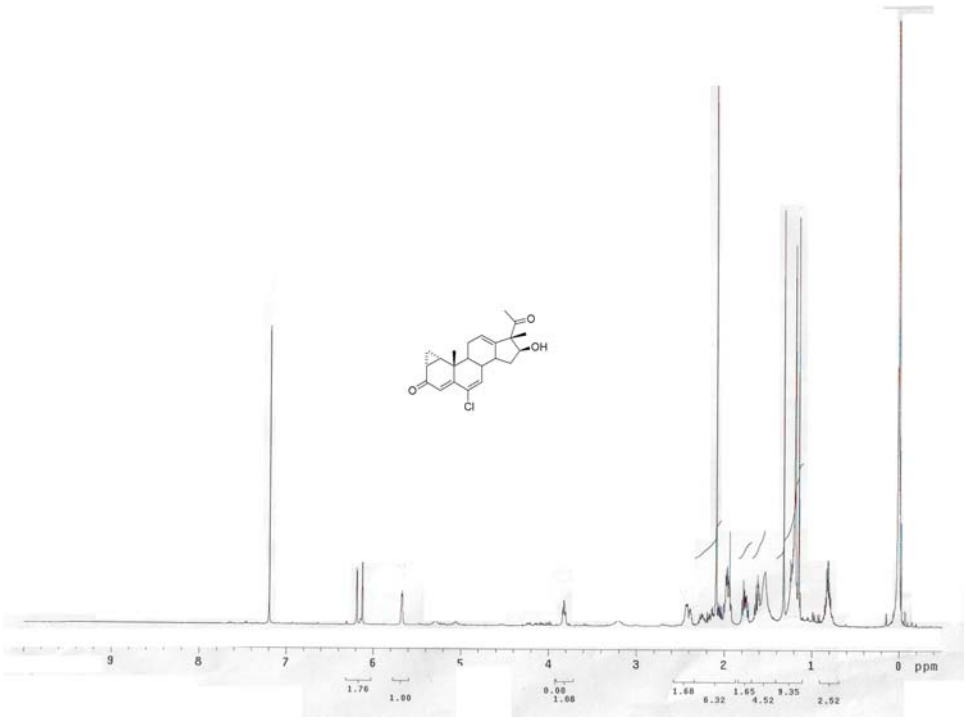
Appendix Figure 48 100 MHz ^{13}C NMR spectrum of 16α-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**81**)



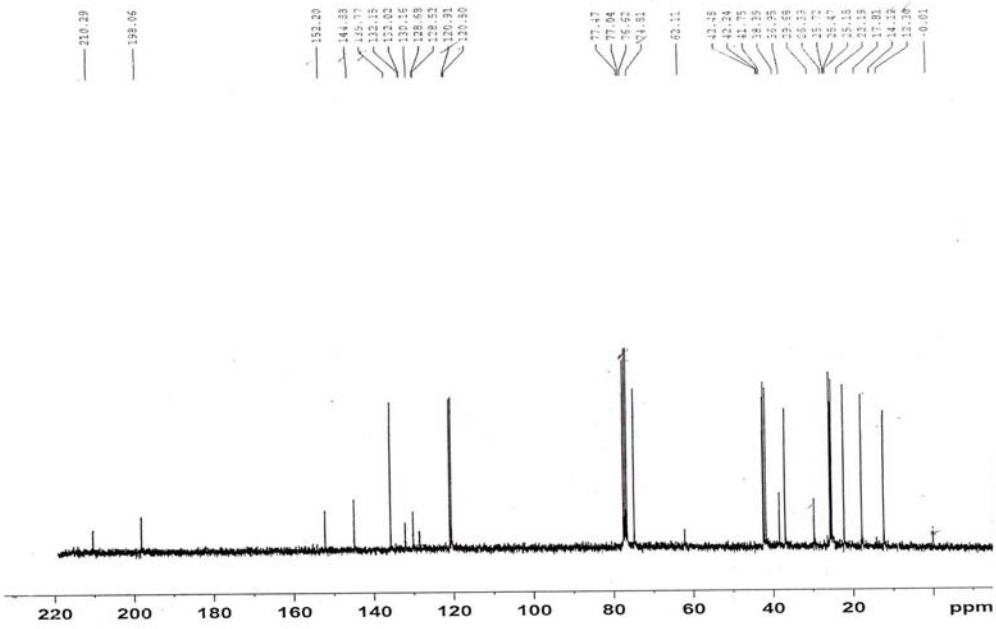
Appendix Figure 49 2D-COSY spectrum of 16α-hydroxy-17β-methyl- 1α,2α-cyclo methylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**81**)



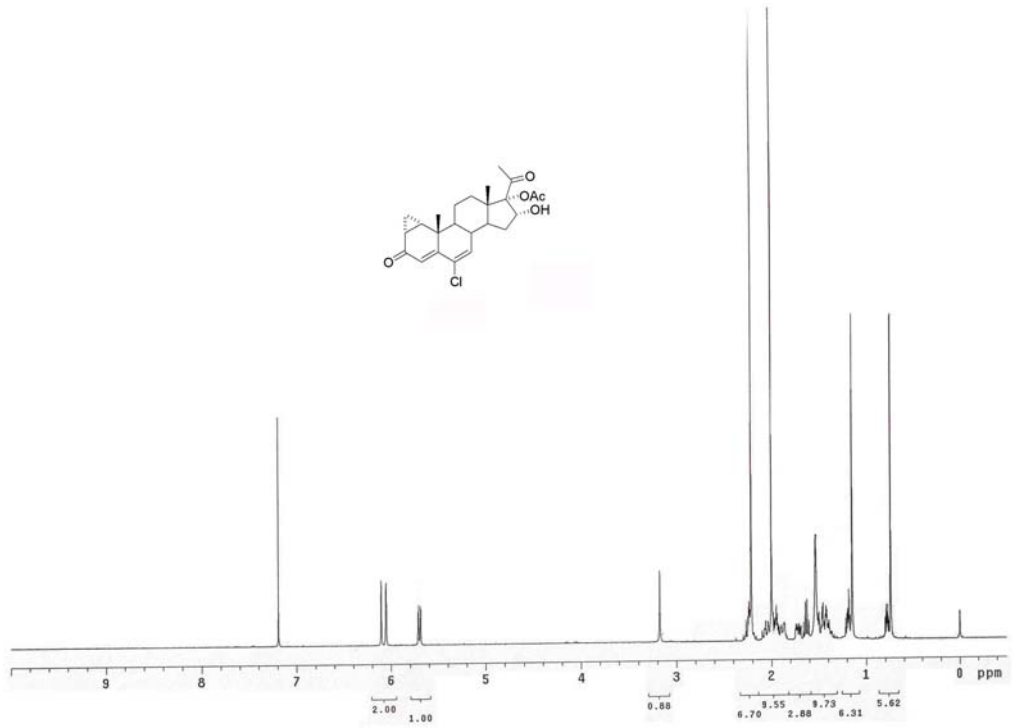
Appendix Figure 50 2D-COSY spectrum of 16β-hydroxy-17β-methyl-1α,2α-cyclo methylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**82**)



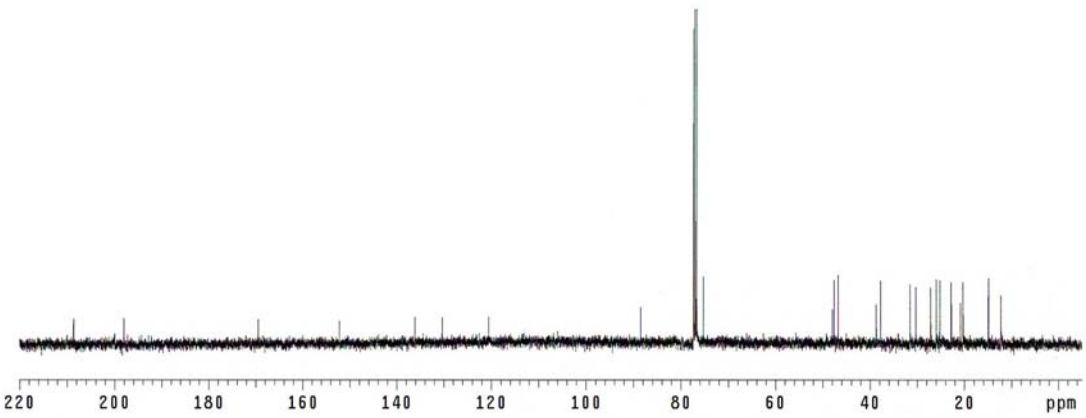
Appendix Figure 51 400 MHz ^{1}H NMR spectrum of 16β-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**82**)



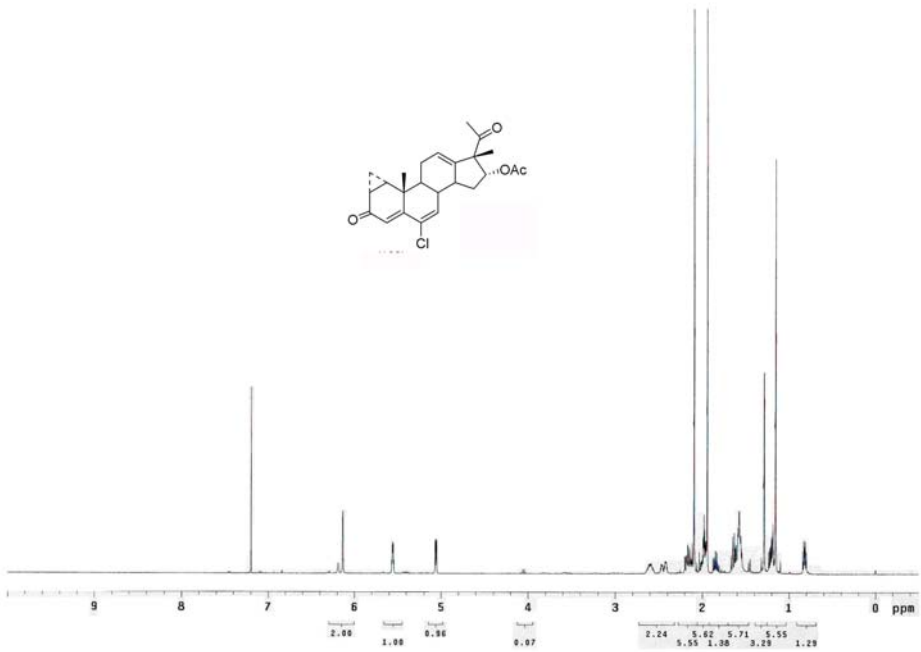
Appendix Figure 52 100 MHz ^{13}C NMR spectrum of 16β-hydroxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (82)



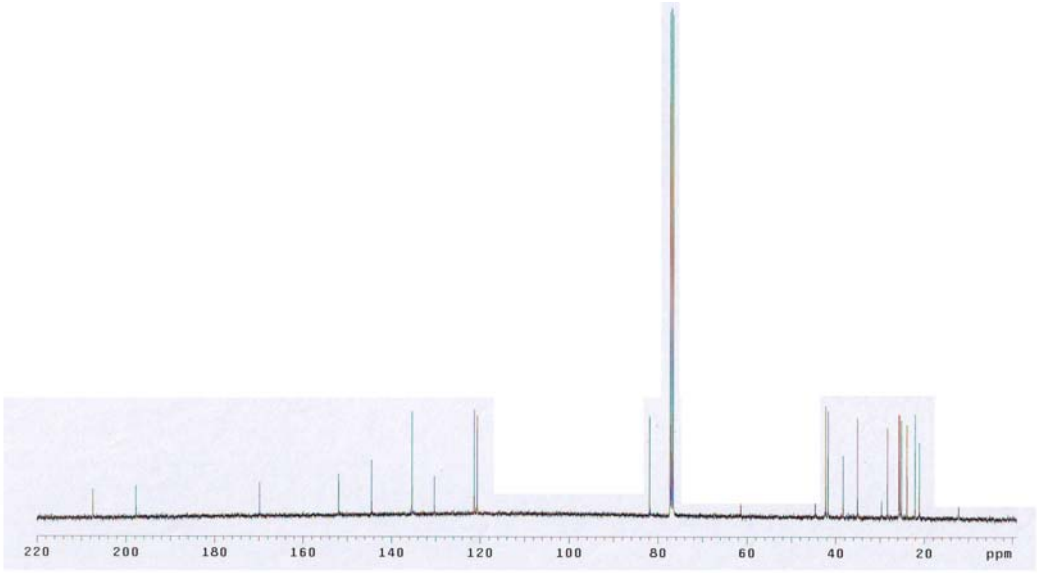
Appendix Figure 53 400 MHz ^{1}H NMR spectrum of 16α-hydroxy cyproterone acetate (**86**)



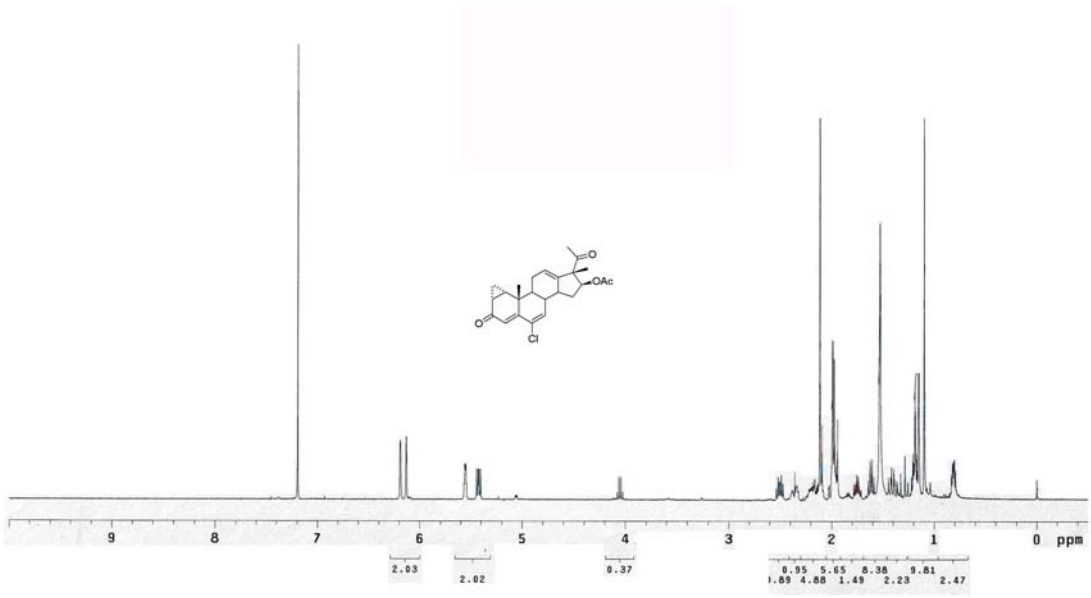
Appendix Figure 54 100 MHz ^{13}C NMR spectrum of 16α-hydroxy cyproterone acetate (**86**)



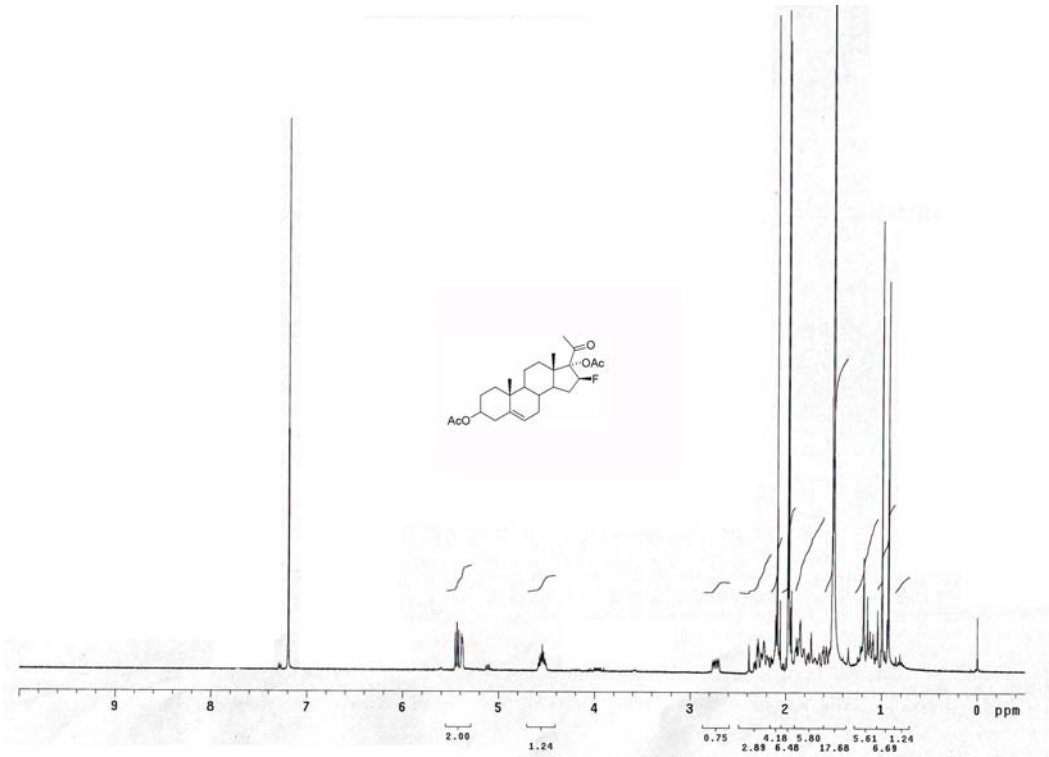
Appendix Figure 55 400 MHz ^{1}H NMR spectrum of 16α-acetoxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**88**)



Appendix Figure 56 400 MHz ^{13}C NMR spectrum of 16α-acetoxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**88**)



Appendix Figure 57 400 MHz ^{1}H NMR spectrum of 16β-acetoxy-17β-methyl-1α,2α-cyclomethylene-18-nor-6-chloro-4,6,12-pregnatriene-3,20-dione (**89**)



Appendix Figure 58 400 MHz ^{1}H NMR spectrum of 16β-fluoro-3β,17α-diacetoxydroxy-5-pregnene-20-one (**80**)