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Synthesis of Estradiol Analogues Based on the A-CD Steroid Ring System

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Synthesis of estradiol analogues based on the A-CD steroid ring system

By Daria Karolina Klonowska

B.Sc., University of Ottawa, Canada, 2007

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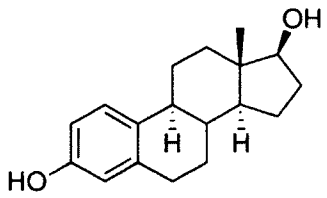
Abstract

This thesis describes the synthesis of a series of estrogen analogs related to estradiol but lacking the B ring of the natural hormone. Those compounds are prepared by methods developed previously in our lab and variations thereof. Typically this involved coupling of the enatinomerically pure CD-ring ketone **A** with the lithio derivatives **B** derived from several substituted suitable protected 4-bromophenols. The compounds thus obtained have been evaluated as potential estrogen receptor agonists.

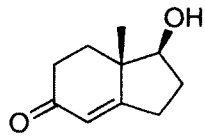
Molecular Mechanics calculations, carried out by Prof. Wright's group at Carleton University indicate that the preferred conformation of compounds such as **C** is one in which the plane of the aromatic ring is essentially perpendicular to that made by the carbon skeleton of the CD ring system. However their calculations strongly suggested that in the receptor this angle is reduced to 21 +/-5°. This compares with the approximately 10° difference in these planes in the natural hormone estradiol. In order to test whether compounds in which the dihedral angle between the aromatic ring and the CD ring system is fixed at positions other than 10 or 20 ° the A-CD, the spiro derivatives **D** were prepared. In the spiro-5 derivative (n = 1) the planes are essentially perpendicular (90°). When n = 6 as in compound **D** [spior-6] there is more flexibility and the dihedral angle can be as small as 60 °. The relative binding affinity [estradiol = 100] for the spiro-5 compound is RBAα = 2.5, RBAβ = 10.7. These data are comparable to the parent A-CD analogue **E** [RBAα = 1.5, RBAβ = 21]. The RBAs for the 6-spiro compound were somewhat smaller [RBAα = 0.3, RBAβ = 3.5]. These results indicate that either the estrogen receptor can accommodate almost equally well the two compounds with different shapes or that the calculations indicating the **E** rotates from its preferred

conformation in which the ring A is perpendicular to the plane of the CD rings to a conformation in which the two planes in the presence of the receptor is in error. The RBAs for the compounds in the families **C** and **F** [R = H, Cl and CF_3] are smaller than those observed for the saturated analogs **E**.

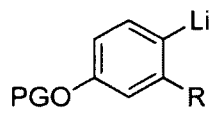
The eventual goal of the research carried out by the Durst/Wright groups was to prepare compounds which cannot be metabolized to form the reactive ortho-quinones which have been implicated as one of the triggers of estrogen related breast cancers. The A-CD compounds represented by the **C, E** and **F** families can in principle be metabolized to ortho-quinononones, perhaps more slowly in cases where the substituent is an electron withdrawing group. In contrast, no quinone formation is possible when the phenol is replaced by a pyrazole ring as in **G**. The compound **G** was synthesized; the relative binding assays were: RBAα = 0.04 and RBAβ = 0.06. Although the binding is quite low relative to estradiol, the result is considered quite encouraging since it shows that pryazole can be a surrogate for the phenol. The low RBAs observed for **G** is probably due to the much shorter distance (~ 9Å) between the NH and OH in **G**, as compared to the ideal 11 Å found in estradiol. It is predicted that inserting an atom, for example, CH_2 or O or S between the pyrazole and the CD ring in compound **G** will significantly increase the binding affinity to the estrogen receptors.



estradiol



A



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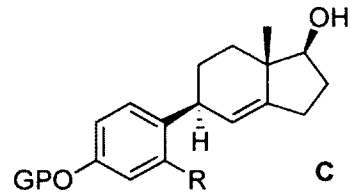
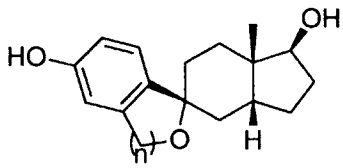
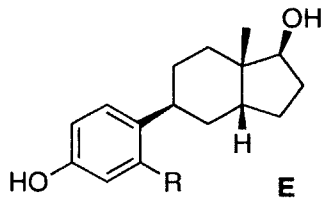


C



D

spiro 5 (n = 1),
spiro-6 (n = 2)



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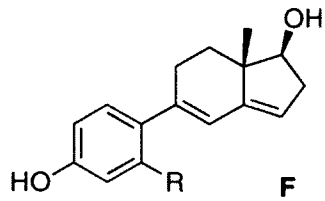

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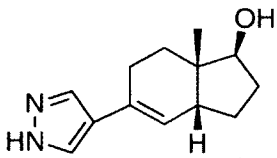

G

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Thank you all

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List of Abbreviations and symbols

°C	degrees Celsius
^{13}C NMR	carbon-13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
br	Broad
brine	saturated NaCl solution
CDCl3	deuterated chloroform
COSY	correlation spectroscopy
d	Doublet
DCM	Dichloromethane
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhancement polarization transfer
DIPEA	Diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
equiv.	Equivalents
ER	Estrogen Receptor
Et	Ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	Ethanol

HMQC	heteronuclear multiple quantum coherence
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
HRT	Hormone Replacement Therapy
Hz	Hertz
Ile	Isoleucine
IR	Infrared
J	coupling constant
Leu	Leucine
m	Multiplet
m/z	mass to charge ratio
Met	Methionine
mL	Milliliters
MS	low resolution mass spectroscopy
MW	molecular weight
NMR	nuclear magnetic resonance
Ph	Phenyl
ppm	parts per million
PTSA	p-toluenesulfonic acid
q	Quartet
rt	room temperature

s	Singlet
SERMS	Selective Estrogen Receptor Modulator
t	Triplet
TBDMS-Cl	*tert*-butyldimethylsilyl chloride
THF	Tetrahydrofuran
TLC	thin layer chromatography
δ	chemical shift
Δ	Heat

Chapter 1. Introduction

1.1. Menopause and Hot Flashes

Menopause in females is the gradual change in physiology characterized by loss of menses due to decreased estrogen production. The peri-menopause is the time just before and just after the menopause has established. **[1]** Menopause is accompanied by the changes in cardiovascular health, bone mineral density and cognitive function. Perimenopausal and postmenopausal women (75-85%) experience the symptom termed as 'hot flashes' which is the feeling of intermittent intense heat, **[2, 3]** which causes discomfort and affects the quality of life. The decrease in estrogen levels which triggers the vasomotor response resulting in peripheral vasodilatation, increased skin temperature and increased blood flow is often observed as redness of the upper face, neck and torso. **[3]** Hormone Replacement Therapy (HRT) has been a solution to the hot flashes symptoms but it carries with it an increased risk of breast and uterine cancer and a higher incidence of coronary heart disease and stroke. **[4]**

1.2. Osteoporosis

One of unfortunate results of menopause is a decrease of bone density which may lead to osteoporosis. Osteoporosis is caused by the imbalance between the osteoclastic and osteoblastic activity accompanied by an increased rate of bone turnover observed with menopause. Osteoclasts are the bone-resorbing cells which tightly adhere to the bone surface and then secrete acid which dissolves the hydroxyapatite mineral and proteolytic enzymes that degrade the organic matrix of bone. Osteoblasts are the bone-forming cells that synthesize a highly cross-linked, lamellar organic matrix (osteoid), which becomes

mineralized by extracellular processes. **[5]** During natural or surgically induced menopause declining levels of circulating 17β-estradiol and estrone have been associated with the rate of bone loss in women and animals. **[6]**

Osteoporosis, which often starts at menopause, may lead to many serious complications resulting from the acquired injuries due to broken bones. This results in a decrease in physical activity which can manifest itself in a weakened immune system. Together these can lead to serious medical complications or even death. The incidence of postmenopausal type I osteoporosis increases in women over 50 years of age where the risk of fractures rises to about 75% in elderly women. **[7, 8]**

O. Johnell et al **[9]** examined the incidence of hip fracture in seventeen European countries between 1983 and 1985 with categorized patient sex and age. They reported that women are at two times greater risk of osteoporotic fracture than men. The highest incidence of hip fracture was found in the northern part of Europe and the lowest in the Mediterranean area. People of Northern European ancestry had higher incidence of hip fracture than Africans or Asians. The incidence of hip fracture in women compared to men was 70-80%, except Yougoslavia and Turkey, where it was evenly distributed between men and women. **[9]**

The reason for the women to be at higher risk of osteoporotic fracture is due to the lower peak bone density achieved at adulthood, greater susceptibility to rapid bone loss associated with menopause, a greater predisposition to fall and a greater tendency than men to survive well into the age of vulnerability. **[10, 11]** For some of those women with rapidly progressing osteoporosis hormone replacement therapy is highly recommended.

receptor complex. The ligand-receptor complex binds to DNA and initiates gene transcription resulting in the production of specific proteins, which give rise to physiologic responses in the target tissue, **(figure 1.2.)**. **[15, 16]**

Two estrogen receptors, ERα and ERβ have been identified. In 1950's Jensen and Jacobsen discovered the estrogen receptor molecule **[13]**; it was cloned first in 1986 by Green et al. **[17]** The second receptor was discovered ten years later by Kuiper et al. **[18]** Estrogens (E_{1-3}) are known to show little selectivity for either receptor. **[19, 20]**

It has been reported that the estrogen receptor ERα binds freely diffusible estrogen, which leads to the formation of a high-affinity ligand-receptor complex in the nucleus. **[22, 23]** The estrogen-receptor complex binds to the estrogen response elements (ERE), which are specific DNA sequences, and then modulates the expression of a variety of estrogen responsive genes such as proto-oncogenes. **[24, 26]**

There are two classes of genes which mutation leads to cancer. In the first class called proto-oncogenes there is a gain-of-function leading to cancer and the overactive mutants are called oncogenes. The second class are tumor suppressor genes in which mutation results in a loss of function. **[27]**

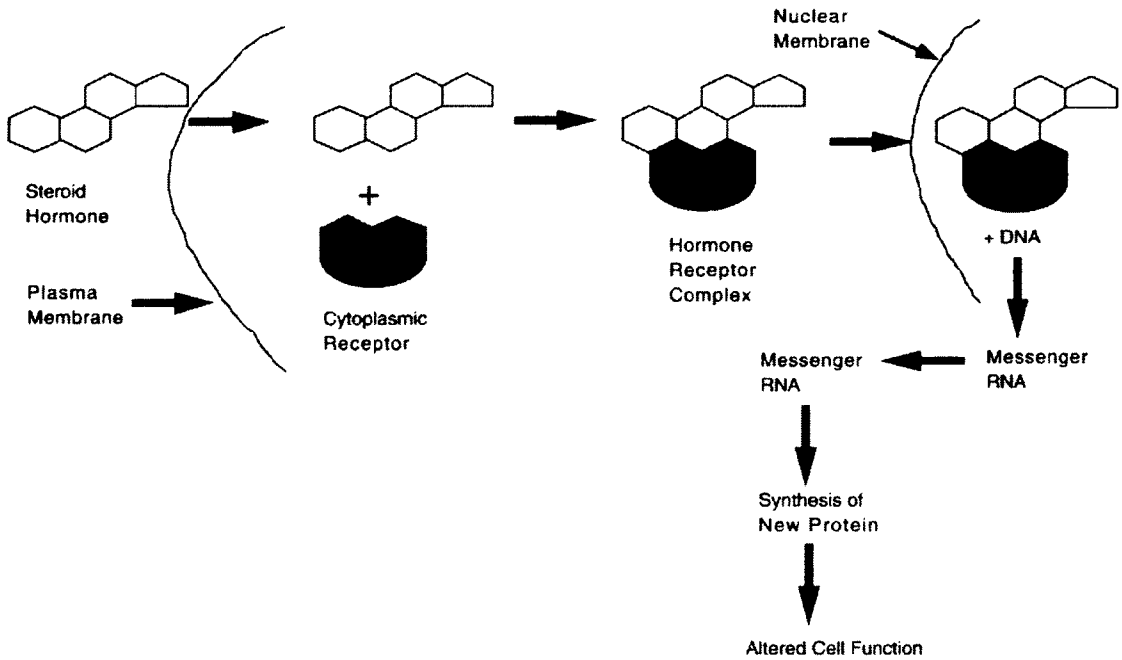
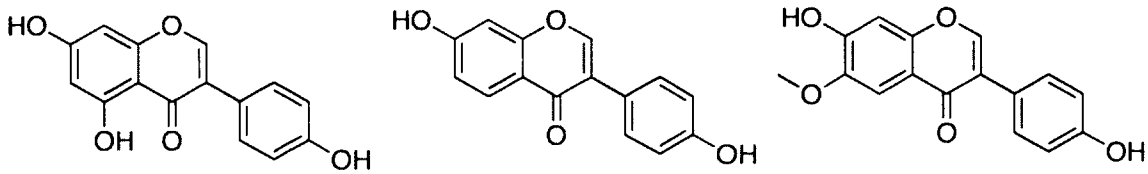


Figure 1.2. The mechanism of the steroid ligand showing diffusion through the cell and nuclear membranes into the nucleus and gene transcription leading to the altered cell function. This figure was taken directly from Figure 1, reference **[16]**.

There are also many endocrine disrupters present in the environment targeting ER, such as the phyto-estrogens produced by plants as bactericidal and fungicidal agents. The presence of these compounds, for example genistein, **5**, present in soy, daidzein **6**, and glycitein, **7**, in the human diet seems to have beneficial role in the reduction of hormone dependent breast and prostate cancer, heart disease and a reduction in the symptoms of menopause. **[28]** The phytoestrogens can interact directly with estrogen receptors or indirectly by modulating endogenous estrogens concentrations. **[29, 30]**



5, genistein **6**, daidzein **7**, glycitein

Figure 1.3. The structure of genistein, daidzein and glycitein. Obtained from ref. **[30]**.

1.4. Problems with existing hormone replacement therapy (HRT)

Prevention of menopausal symptoms, which are the result of low estrogens levels, is highly desirable since it can result in an improved quality of life by reducing the incidence of osteoporosis, atherosclerosis and 'hot flashes'. Most of the existing hormone replacement therapies try to restore hormone balance. **[32]** Estrogen therapy is a known and an effective treatment of symptoms associated with menopause but it carries with it many potential risks such as increased risk of breast cancer, endometrial cancer, stroke, venous thromboembolism, and coronary heart disease (CHD). **[32, 33]** The most commonly prescribed HRT over the past fifty years has been Premarin® marketed by Wyeth Ayerst. The components of this treatment are discussed later in this chapter. Between 1976 and 1986 the famous Nurses' Health Study was established in order to study estrogen replacement therapy and risk of breast cancer in postmenopausal women. The study included registered female nurses 30-55 years old, who completed and mailed the questionnaire related their menopausal status, suspected risk factors related to cardiovascular diseases and breast cancer. The follow-up questionnaires were mailed to the participants every two years in order to record any major medical events. **[34]** Among 480,665 persons participating in the Nurses' Study 1,050 breast cancer incidents were noted during 12 years follow-up. **[35]** Based on the study the past users of estrogen

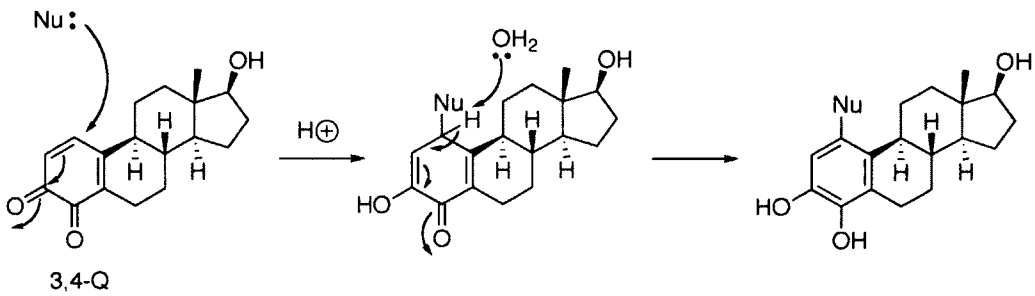
replacement were not at increased risk including those who used estrogen replacement for 10 years. Unfortunately, the risk was higher among the current users, especially with increasing age, but not with the duration of use. **[34]**

Problems associated with the existing hormone replacement therapy such as increased risk of breast cancer, endometrial cancer, stroke, venous thromboembolism, and coronary heart disease (CHD), **[32, 33]** encouraged people to look for different options. One of the alternatives to estrogen therapy is soy, which is rich in isoflavones. Soy products are part of Asian diets and have the potential of reducing risk of CHD **[36]**, osteoporosis **[37, 38]** and certain cancers **[39, 40]**. The three major isoflavons present in soy are genistein, **5**, daidzein **6**, and glycitein, **7**. **[41]** Genistein is an isoflavonoid phyto-estrogen has a strong preference for binding to ERβ. The relative binding affinity (RBA) of genistein to the ERα = 5 vs. 100 for estradiol and ERβ = 36 vs. 100 for the estradiol. **[42]** The Practice Bulletin issued by the American College of Obstetricians and Gynecologists **[43]** stated that soy and isoflavones may be helpful for treatment of vasomotor symptoms such as hot flashes for up to 2 years. However, there is lack of standardization of available products and difficulty in interpretation of the available data. **[43]**

1.5. Breast cancer and the quinone formation

The usage of estrogens for hormone replacement therapy was shown by the Nurses' Study to increase the risk of breast cancer. The involvement of estrogen in this process is not simple and probably due to several factors. A probable contributing mechanism involves metabolism of estradiol. Estradiol is transformed to catechols by enzymatic hydroxylation and then enzymatic oxidation to give carcinogenic ortho-quinones.

These quinones can react with DNA bases by acting as Michael Acceptors. Nucleophiles such as DNA bases or nucleophilic residues of proteins can readily add to o-quinones potentially leading to carcinogenicity. The 3,4-quinones have a longer half life (12 min) than 2,3 quinones (47s) **[44]** and have been shown to be the more dangerous isomers.



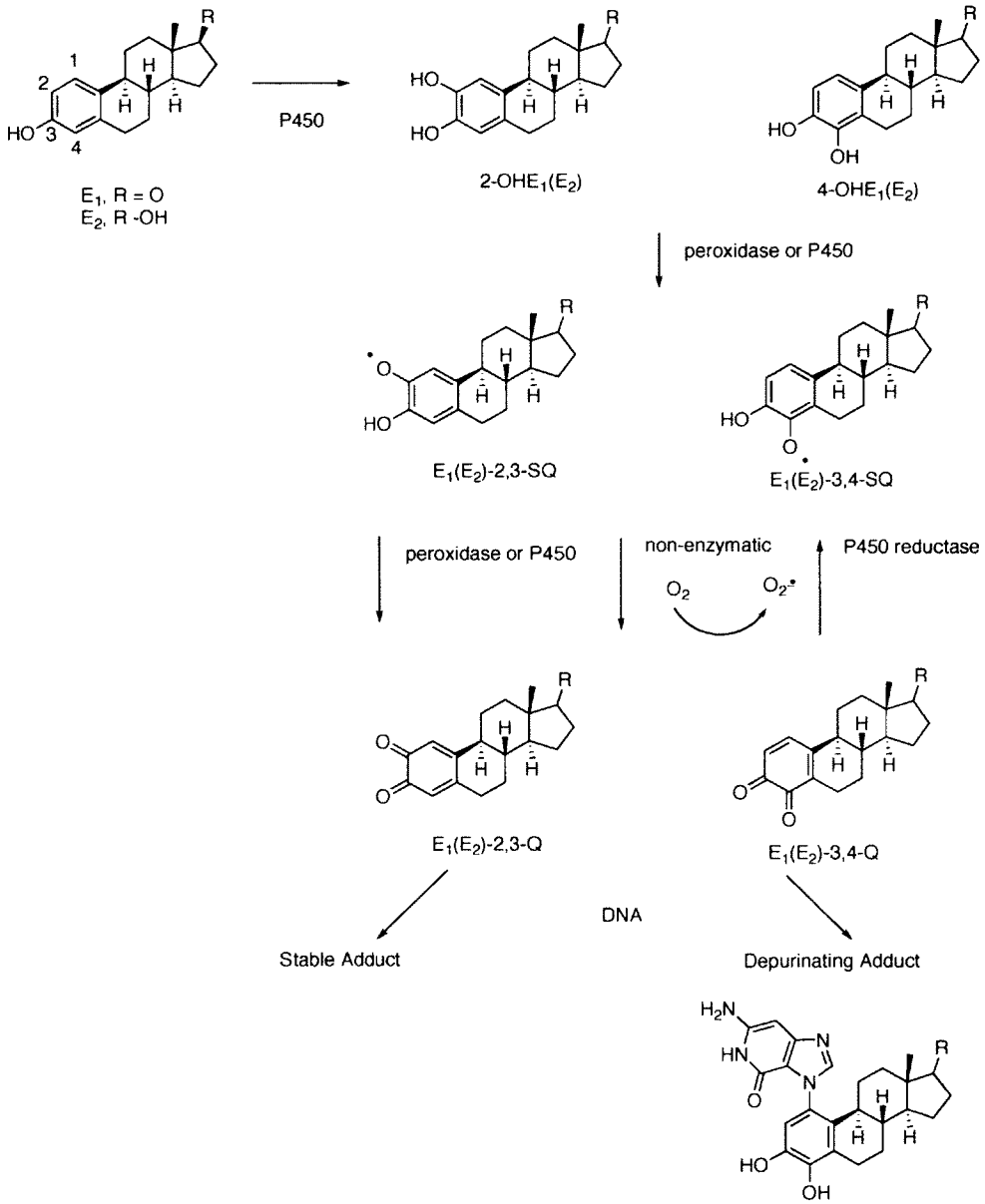
Scheme 1.1. The mechanism of nucleophilic addition of DNA or protein to the estrogen 3,4 quinone.

Cavalieri et al. demonstrated that catechol estrogen-3,4- quinones are endogenous tumor initiators. **[45]** The mutation of oncogenes and tumor suppressor genes leads to cancer. **[46]** The loss of depurinating adducts, leads to apurinic sites on the DNA, which need to be repaired. If they are not repaired, the mis-replication of DNA leads to carcinogenic mutation. Based on that theory the Cavalieri group proposed that carcinogenic catechol derivatives of estrone, must form electrophilic intermediates, ortho-quinones which then bind covalently to DNA to form depurinating adducts.

They carried out in *vitro* and in *vivo* tests, where they found that 4-OHE catechol activated by cytochrome P450 and peroxidases binds to DNA and forms depurinating adducts. **[46]**

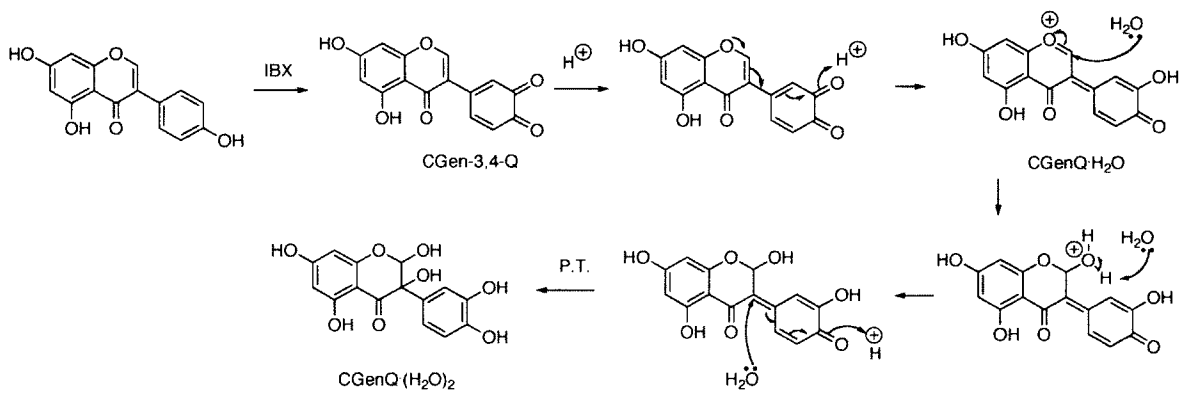
The pathway leading to the formation of carcinogenic estrogen metabolites starts with the enzymatic hydroxylation of 17β–estradiol (E_2) or estrone (E_1) to form both 2,3-catechols

and 3,4-catechols. These can be partially oxidized to semi-quinones $E_1(E_2)$-2,3-SQ and $E_1(E_2)$-3,4-SQ or to quinones $E_1(E_2)$-2,3-Q and $E_1(E_2)$-3,4-Q. The $E_1(E_2)$-3,4-Q, binds to DNA to form a depurinating adducts which are lost from the DNA by cleavage of the glycosidic bond leaving depurinated DNA. **[46]**



Scheme 1.2. Formation of quinones and DNA adducts. Adapted from Figure 1 of reference **[46]**.

M. L. Gross et al. found, using the glutathione trapping method proposed by Bolton **[47]** and co-workers, that catechol genistein quinone (CGenQ) is very short lived, with half life of 4+/-1 s at physiological conditions. The CGenQ is able to react with neighboring nucleophiles, such as water to form a dihydrate CGenQ·$(H_2O)_2$, which structure was identified by NMR, by M. L. Gross and co-workers. Based on this they proposed, that the short lived o-quinones, such as 2,3-Q, are not as dangerous as longer lived 3,4-Q because they react quickly with nearby nucleophiles, such as solvent especially water and thus are not able to survive to react with the DNA bases, which could lead to DNA depurination and possible carcinogenicity. M. L. Gross and co-workers have oxidized genistein to the CGen-3,4-Q using IBX, and proposed the following reaction scheme to account for the formation of the isolated dihydrate CGenQ·$(H_2O)_2$, **(scheme 1.3.). [48]**



Scheme 1.3. The mechanism leading to the formation of dihydrate CGenQ·$(H_2O)_2$. The key intermediates were proposed by M. L. Gross et al. on Scheme, reference **[48]**.

1.6. The existing drugs for hormone replacement therapy

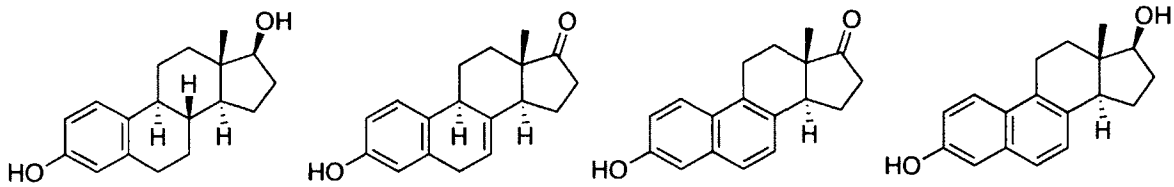
The benefits of estrogen therapy are tremendous, from greatly decreased ischemic heart disease, to improved HDL cholesterol levels and fibrinogen levels, and to lower risk of death due to coronary heart disease for women treated for up to 10 years compared to

women who were never taking estrogen. **[49, 50, 51-56]** The increased risk of breast and uterine cancer was noted in 2003 due to the long term usage of those drugs and since that time usage of estrogenic compounds for the prevention of menopausal symptoms dropped dramatically.

Unfortunately, none of the existing hormone replacement therapies are able to restore the bone loss. Many women wait too long with solving the problem, when the significant irreversible bone loss occurs. **[5]**

Prescription drugs for HRT include combination of the hormones estradiol and estrone and O-sulfate conjugated equine estrogens [CEEs], such as equilin and equilenin isolated from pregnant mares. These are produced by the Wyeth Corp. and marketed under the names Premarin and Prempro. The first class are ring B saturated steroids including natural sex hormones, such as estrone, 17β-estradiol and 17α-estradiol. The second group are ring B unsaturated estrogens such as equilin, **8**, equilenin, **9**, 17β-dihydroxyequilenin, **10,** 17β-dihydroequilin and 17α-dihydroequilenin. **[57]**

The conjugated equinine estrogens (CEE) are derived from the urine of pregnant mares. For women, who do not want to use medication derived from animal sources there is a plant source combination of estrogens developed such as Cenestin. Premarin is the most known CEE and on May 15, 2010 it will have been on the market for 68 years. **[5]**

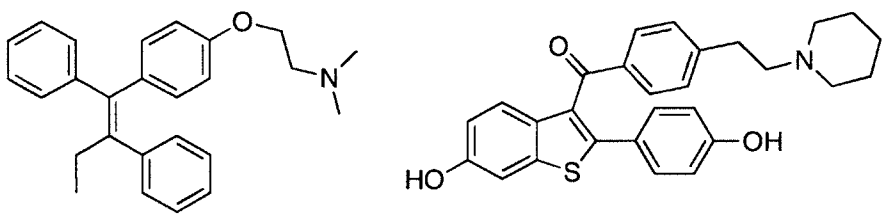


1, 17β-estradiol **8**, equilin **9**, equilenin **10**, 17β-dihydroxyequilenin

Figure 1.4. The structure of conjugated equine estrogens. **[5]**

Another group of compounds used for treatment of HRT are selective estrogen receptor modulators (SERMs). They are estrogen agonists in selective tissues such as bone or in the cardiovascular system, but have antagonist activity or no activity in the reproductive tissues. They have very large structural diversity and include triphenylethylene derivatives, such as Tamoxifen/Nolvadex, dihydronaphthalene derivatives such as Nafoxidine, benzopyrans derivatives such as Levormeloxifene, and benzothiophene derivatives such as Raloxifene/Evista. **[5]**

Tamoxifen, **11,** has been shown to prevent bone loss **[58, 59]**, and to have beneficial effects on post menopausal breast cancer patients cardiovascular system. **[60]** Unfortunately, Tamoxifen does not affect hot flashes and other lifestyle symptoms of menopause. Additionally it induces DNA adduct formation **[61, 62]** and causes liver cancer in rats. **[63]** Another SERM Raloxifene, **12**, belongs to the family of benzothiophenes and is highly effective in the prevention of bone loss in postmenopausal women. **[64]**



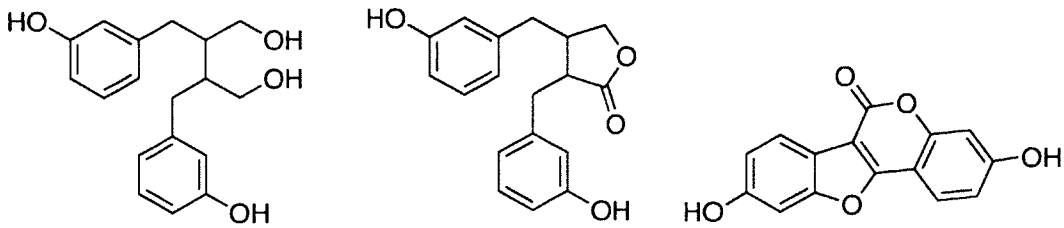
11, Tamoxifen **12**, Raloxifene

Figure 1.5. The structure of Tamoxifen and Raloxifene. **[5]**

The phytoestrogens are products derived from plants, which bind to the estrogen receptor. They are considered weaker estrogens because they bind less strongly to the estrogen receptor compared with estradiol. They are more selective towards ERβ than ERα

subtype. **[16]**

They are three main groups of phytoestrogens, the first are isoflavones, such as genistein and daidzein, which can be found in soy, lentils and other legumes. The second group, such as entrodiol, **13**, or enterolactone, **14**, are found in flaxseed and other seed oils, belong to the lignan family. The last final group are coumestans, such as coumestrol, **15** found in red clover, sun flower seeds and bean sprouts. These natural products are able to bind to the estrogen receptor. **[16]** Even though many women take phytoestrogens to combat the symptoms of menopause there is considerable question in the medical profession concerning the value of these mixtures.



13, enterodiol **14**, enterolactone **15**, coumestrol

Figure 1.6. The structure of phytoestrogens: coumestrol, **[65]** enterodiol and enterolactone. **[66]**

Phytoestrogens can act as agonists, when the endogenous estrogen levels are low or as antagonists, when the endogenous level of estrogens are high. **[67]** Phytoestrogens can alter the levels of sex hormone binding globulin (SHBG). **[30, 68]** The SHBG is a glycoprotein produced by hepatocytes and its levels in human blood are dependent on the steroidal and peptidic hormones, T_4, and dietary factors. **[69, 70-78]**

1.7. Estrogen receptor α and β

There are two types of estrogen receptors- ERα and ERβ – with the latter being discovered only recently. **[13, 18]** The amino acids composition between the two receptors is very close, approximately 97% in the DNA binding domain and approximately 56% in the ligand binding domain (LBD). **[79]**

The ERα and ERβ have different tissues distribution in the body. Both of the receptor subtypes my be present in the same organs but be expressed to various degree or they can be present in the same tissue but in different cell types. The ERα receptor is mainly expressed in uterus, prostate (stroma), ovary (theca cells), testes (Leydig cells), bone, breast, various regions of the brain, liver, and white adipose tissue. ERβ is expressed in colon, prostate (epithelium), testis, ovary (granulose cells), bone marrow, salivary gland, vascular endothelium and certain regions of the brain. **[16]**

The structural differences between ERα and ERβ and their different tissue distribution are important factors when designing Selective Estrogen Receptor Modulator (SERMS), because any structural changes can alter their agonist or antagonist activity. **[16]**

There are two critical amino acids differences in ERα and ERβ, which have crucial role on the ligand binding. In ERα Leu_{384} is changed for Met_{336} in ERβ and Met_{421} in ERα is replaced by Ile_{373} in ERβ. **[80]**

1.8. Estrogen agonist and antagonist

A compound is an estrogen agonist, if after binding to the estrogen receptor and forming ligand-estrogen receptor complex it stimulates and enhances the activity due to forming

the complex. The antagonist in contrast inhibits the activity, which would occur from the formation of activated ligand-estrogen receptor complex. **[16]**

The mode of binding of estrogen ligand in the ER was already established. There are two hydrogen bonding sites inside of the ER binding pocket, which are important for the agonist activity of ligand-ER complex. The first one involves Glu-Arg-water triad, where the A-ring phenolic hydroxyl group binds. The second site involves the His residue, which forms hydrogen bond with 17β hydroxyl group of estradiol. **[81]**

1.9. Design of novel estrogen agonists based on the ACD-ring system

The ideal estrogen agonist should decrease the symptoms of menopause, but do not increase the odds of developing breast or uterine cancer. The evidence that the metabolism of estrogens leads to o-quinone formation suggests strongly that compounds that have estrogenic activity but cannot be metabolized to o-quinones or form o-quinones reluctantly, or form relatively unstable o-quinones may have reduced breast cancer causing effects. Such compounds should not show pure ERβ selectivity, since such compounds tend not to relieve the typical menopausal symptoms.

The research program started with the natural ligand 17β-estradiol. In the ACD-analogues the skeleton of estradiol has been preserved, except removed ring-B. Removing ring-B adds to the structure flexibility, which allows for a great degree of rotation around ring A. This possibly allows the ligand to adjust itself to the preferred conformation inside of the estrogen receptor binding pocket. Different substituents can be introduced on the C5 position of the A-ring to improve the selectivity towards ERα or

ERβ; these may have an impact on binding to the ER receptors. **[82]**

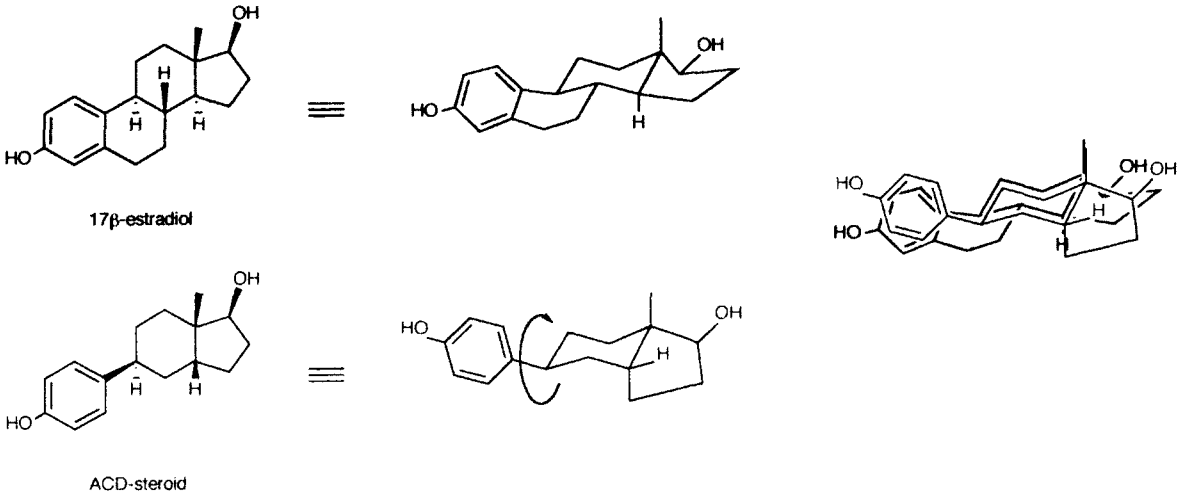


Figure 1.7. The comparison between 3-dimensional structure of estradiol and ACD-steroid.

The interatomic distance between C3 and C17 hydroxyl groups of 17β-estradiol is 11 Angstrom, optimum distance for hydrogen bonding is thought to be 11 +/- 0.5 Å and is important for binding to Arg_{394} and His_{524} amino acid residues inside of binding pocket. **[83, 84]**

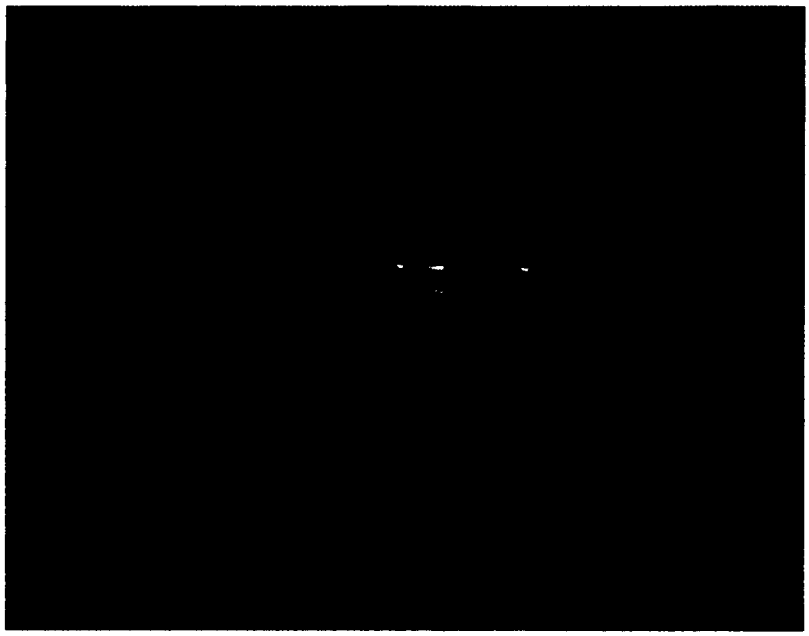


Figure 1.8. The 17β-estradiol (grey) and ACD-steroid, trans-CD-ring junction (green) inside of ERβ binding pocket.

In initial experiments, the parent A-CD structure and a number of derivatives bearing substituents in the A ring were synthesized. These were sent to Professor J. Katzenellenbogen's laboratory in the University of Illinois for bio-assays. The results showed that the parent compound showed significant binding to both ERα and ERβ with strong ERβ selectivity. The introduction of electron withdrawing substituents such as F and Cl at position 5 on the aromatic ring (for ease of comparison we continue to use the estrogen numbering system) increased the binding constants beyond that observed for estradiol. Additionally, the ERβ to ERα selectivity was reduced to less than 5. Theoretical calculations have shown that electron withdrawing group (EWG) as a

substitutent on the A-ring should retard ortho-quinone formation and thus make such compounds potentially safer than estradiol. **[84, 85]**

The goal of this thesis was to build on the initially encouraging results and generate other ACD analogs that might improve on the results already obtained. A more detailed description of the preliminary results and the goals are given in the Introduction to the next chapter of this thesis.

Chapter 2. Results and Discussion

2.1. Design of novel estrogen agonists based on the ACD-ring system

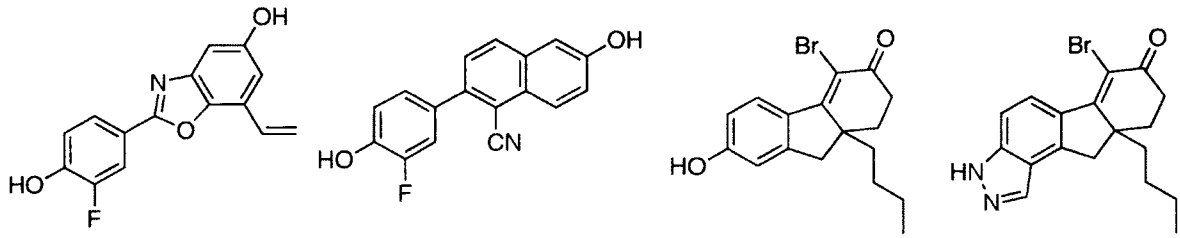
This project was initiated in 2006, with the grant application to the Canadian Breast Cancer Foundation, Ontario Division titled "Reduction of breast cancer risk factors by molecular engineering. The redesign of hormonal supplements". One serious side effect with the existing hormone replacement drug Premarin, as shown by the Women's Health Study is that it significantly increased the risk of estrogen related cancers. A key component of the carcinogenicity of estradiol is likely derived from its metabolism to the carcinogenic quinones, 2,3- quinones and 3,4-quinones. **[44, 45]** The 3,4-quinones are especially damaging. Due to their longer half life, they are more likely to act as Michael acceptors and bind to DNA bases to form depurinating adducts leading to possible DNA replication errors.

The ideal estrogen agonist should decrease the symptoms of menopause, but do not lead to the quinone formation thereby reducing significantly the carcinogenic side effects. It cannot be ERβ selective only, because compounds with only ERβ selectivity are deprived of estrogenic character.

The initial stage of the project involved the synthesis of a 'deconstructed estradiol'. The 17β-estradiol is an ABCD-steroid, which was deconstructed to the ACD-steroid by removing two of the CH_2 groups that form ring-B. This concept was inspired by the structure of genistein, a phytoestrogen found in soy products.

Many other research groups have reported structures that bind strongly and have high β-selectively to the estrogen receptors. Major contributions have come from Wyeth Ayerst researchers who described compounds such as ERB-041, **16**, and WAY 202196, **17**. **[86,**

87] These compounds are biaryl systems with two phenolic substituents located such that they give the approximate 11.5Å distance between them which is necessary for strong binding to the estrogen receptors. These compounds, which are highly ERβ selective as indicated above, show no estrogenic effects including relief of hot flashes; also they do not appear to protect against osteoporosis. **[86, 87]**



16, ERB-041 **17**, WAY 202196 **18** **19**

Figure 2.1. Structures of compounds synthesized by Wyeth Ayerst such as ERB-041, and WAY 202196.

Merck researchers have synthesized series of ERβ selective phenolic tetrahydrofluorenones, such as compound **18** **[88]** and a series of fused pyrazole tetrahydrofluorenones, such as compound **19**. They have shown that fused pyrazole is a suitable phenol group replacement. The analogues from the pyrazole series were selective ERβ agonists in a cell based transactivation assay and showed improved bioavailability compared to the benzene analogs. **[89]**

Compared to the above structures, especially the biaryl compounds, the use of the natural steroidal CD ring moiety should allow us to introduce more structural diversity and possibly tune both the level of activity and the β vs. α selectivity. Compared to estradiol the removal of ring-B allows us to introduce substituents in the A ring that will possibly prevent or at least retard ortho-quinone formation, especially long lived quinones.

Removing the ring-B, also gives the structure flexibility, because it allows for essentially free rotation around ring A to ring C bond. This allows the ligand to adjust itself to the preferred conformation inside of the estrogen receptor binding pocket. The concern existed that if the preferred conformation for binding to the receptor was a high energy conformation of the A-CD compounds, then the binding of the ACD compounds relative to estradiol might be quite low. In other words the A-CD compounds might be very much less active than estradiol. However since the natural hormone is active at the nanomolar level, a safe estrogen agonist even if 10 times less active might still be a worthwhile target.

Based on examination of simple molecular models, one would predict that the preferred conformation of the A-CD compounds having the 9(S) stereochemistry is one in which the plane of the A and the average of the CD ring are close to 90^{o}. Recent docking studies carried out by our collaborator, Prof. James S. Wright and his associate Dr. Hooman Shadnia indicated, that the ER bound A-CD ligand adopts a conformation, in which the A ring is in 21 +/-5 ^{o} angle to plane of the CD ring **[83]** and in the estradiol, this relationship is close to co-planar. **[82]** If so then due to possibility of free rotation of the A-ring, we expected that the A-CD structures should bind significantly to both the estrogen receptors. The two known estrogen receptors, estrogen α and β receptors share a lot of similarity but also have key differences. For example, the Met_{336} in ERβ has been switched for Leu_{386} in ERα, and Ile_{373} in ERβ has been switched for Met_{421} in ERα.

As mentioned above, removing the ring B in the A-CD compounds presents the opportunity of introducing different substituents in various positions in the A-ring. Substituents could also be introduced into ring C and thus a large library of analogues

could be generated. The docking studies suggested that some of these might have improved selectivity towards ERα or ERβ.

As proof-of–principle the parent A-CD compound, **20**, was prepared and its binding to the estrogen receptor (ERβ) was compared to the standard estradiol whose binding is set arbitrarily at 100. Estradiol shows essentially no ERα vs. ERβ selectivity. **[19, 20]** Compound **20** showed relative binding values of 1.5 for ERα and 21 for ERβ. This combination of both significant binding and selectivity in favor of the β-receptor indicated considerable potential for this family of compounds and within the next few months more than 20 analogs of one were prepared and evaluated for ER binding and β/α selectivity. Key results are given in **table 2.1.**

The synthesized compounds were evaluated in the laboratory of Prof. John Katzenellenbogen at the University of Illinois in the Relative Binding Affinity (RBA) assay by measuring the competition for binding between tritium-labeled estradiol and increasing concentration of ligand under investigation. **[42]** The relative binding affinity (RBA) is quantified as a measure of displacement in radioactivity of estradiol where value for the estradiol is set to 100% for both receptors. **[82]** The transcription activation assay was carried out using COS-7 cells, which were transfected with ERE-luciferase reporter plasmid and the ERα or ERβ. **[90]** The RTA of the ligand at concentration 10nM was compared to the one of estradiol at that concentration for each receptor. **[82]** The RTA results are important for the ligand assessment, because the positive value for the RTA, means that the ligand was not only able to bind to the ER, but that it was able to activate it. The ligand by activating transcription enhances the desired function and acts as an agonist.

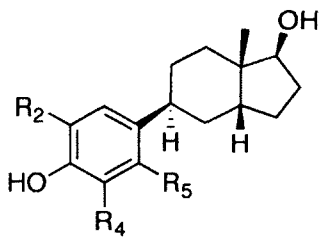


Table 2.1. Relative Binding Affinity (RBA) and Relative Transcription Activation (RTA) to ERα and ERβ and the ratio of selectivity of selected saturated CD-ring A-CD compounds.

Compound	Ring A			RBA			RTA		
	R_2	R_4	R_5	ERα	ERβ	ERβ/ ERα	ERα	ERβ	ERβ/ ERα
1				100	100	1	100	100	1
20	H	H	H	1.5	21.5	14	4.6	158	44
21 9(R)	H	H	H	0.061	0.608	10.0	-8.9	7.7	N/A
22	H	F	H	1.0	8.7	8.7	-5.3	8.2	N/A
23	H	CH_3	H	0.05	0.02	0.4	-	-	-
24	F	F	H	0.04	0.028	0.5	-	-	-
25	H	H	F	27	135	5.0	44.3	161	3.6
26	H	H	Cl	49	168	3.4	-	-	-
27	H	H	CH_3	2.8	34	12.1	-10	163	N/A
28	H	F	F	4.6	42.8	9.3	18	150	8.4

The key conclusions that were drawn from these results were: (1) the stereochemistry at C9 was very important since the stereoisomer **20** which has the same, 9(S) configuration as does estradiol was more than 30 times more active than **21** which had the inverted 9(R) configuration; (2) substituents at C2 and C4, even as small as F caused a significant decrease in the binding affinity; (3) substituents at C5 increased binding significantly to the point where the 5-F or a 5-Cl substituted 9(S) compounds **25** and **26**, respectively, showed stronger binding to ERβ than estradiol itself. Interestingly, the selectivity decreases as the binding affinity increases.

These results combined with predictions based on docking studies suggested that several additional variations not yet studied were worth pursuing. These are:

I. Additional substituents at C5 in the aromatic ring, in particular additional electron withdrawing groups such as CO_2Me and CHO; also substituents larger than a CH_3 group. Potential target compounds are **29-32**.

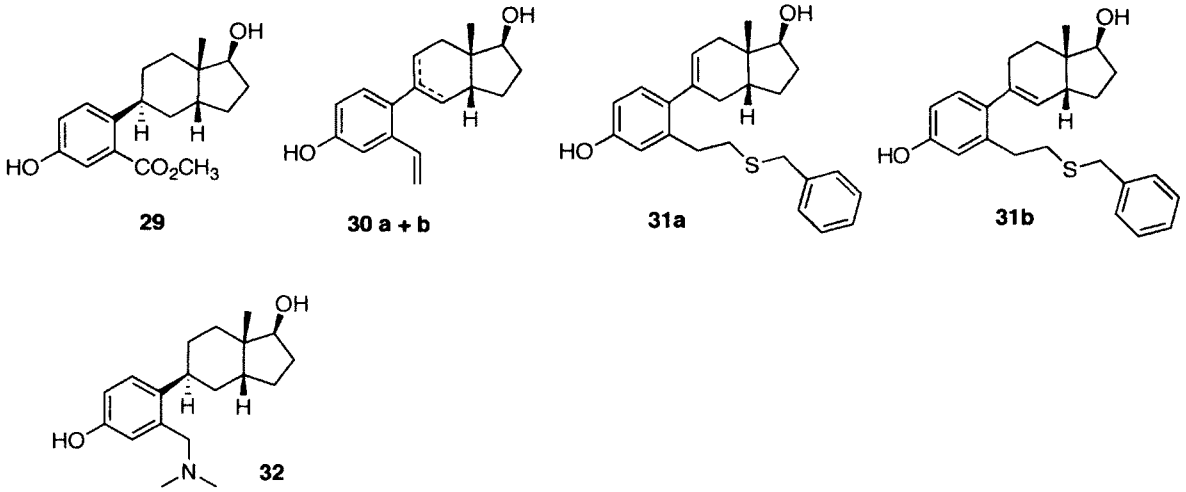


Figure 2.2. The structures of A-CD steroids with electron withdawing group **29** and larger substituents **30 (a+b)**, **31a** and **31b**, **32**.

II. Compounds with fixed conformations different than estradiol but with fixed or almost fixed dihedral angles between the ring A and CD rings planes. Possible target structures are shown below as compounds **33** to **36**.

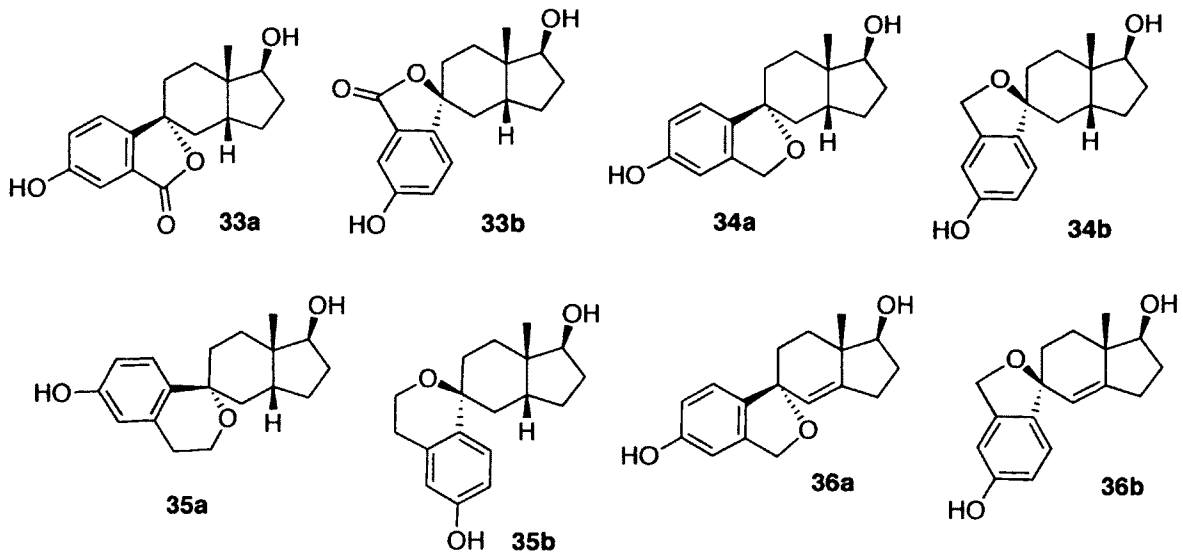


Figure 2.3. The structures of 5- and 6-spiro steroids with fixed dihedral angles between the ring A and CD rings planes.

III. Compounds with unsaturation in the C and or D rings for example compounds **36-44**.

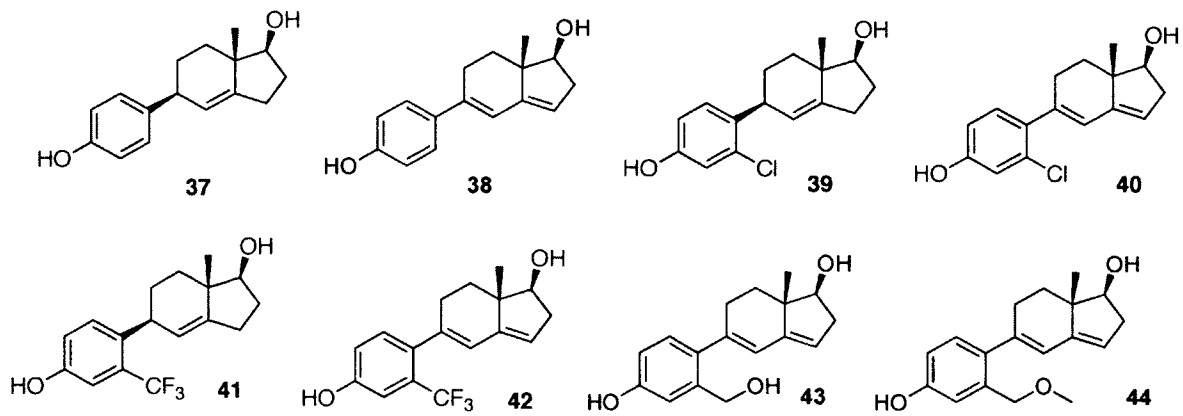
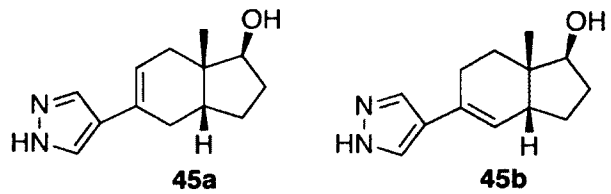


Figure 2.4. The structures of ACD-steroids with one double bond between C8 and C14, in **37, 39, 41** in the CD-ring and two double bonds between C8 and C9 and C14 and C15, in **38, 40, 42, 43, 44**.

IV. Compounds where the phenolic A ring was replaced by a 1,2-pyrazole for example **45a** and **45b**.



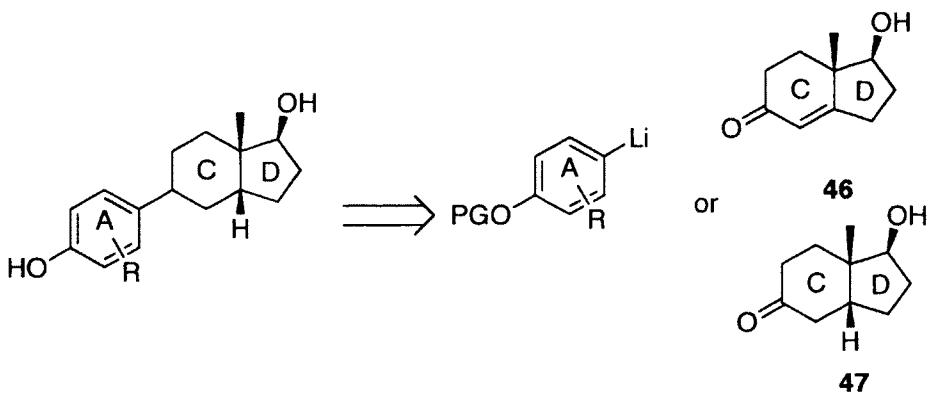
45a **45b**

Figure 2.5. The structures of A-CD steroids with one double bond between C9 and C11, in **45a** in the CD-ring and double bond between C8 and C9 in **45b.**

The rationale for each of these investigations is discussed at the beginning of each section.

2.2. Enantioselective synthesis of the CD-ring moiety

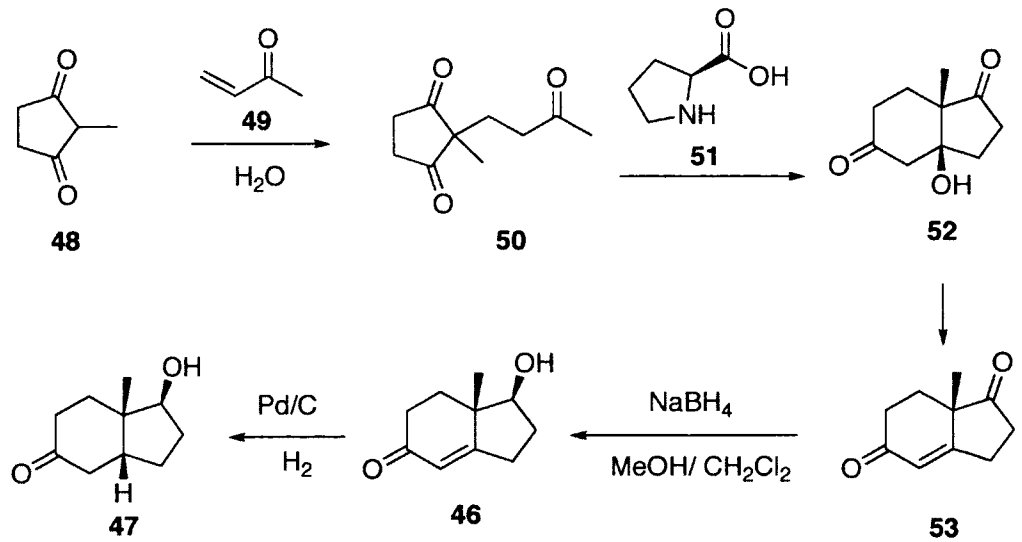
The synthesis of A-CD estradiol analogues involves coupling of the A–ring with CD-ring ketone. The A-ring precursors can either be purchased from the commercial source or it can be synthesized using common synthetic procedures. Brominated and O-protected A-ring partners were coupled with the CD-ring ketones using n-buthyllithium, (**scheme 2.1.**)



Scheme 2.1. The retrosynthesis of the A-CD steroid.

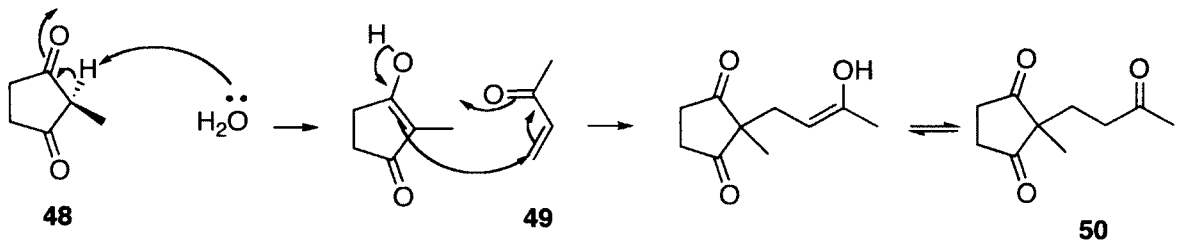
Our synthesis followed the well known highly stereoselective synthesis of the CD ring of steroids with the natural (S)-stereochemistry at C19 initially reported by Hajos and Parrish. This synthesis had also been previously carried out successfully in our group by Dr. M. Asim. It was repeated since we needed considerable amounts of this compound to accomplish all the above stated goals. The sequence is shown in **scheme 2.2.** .

The CD-ring was prepared by method developed by Hajos and Parish using L-proline as the organocatalyst. **[91-94]**



Scheme 2.2. The synthesis of CD-ring.

In the first step 12.5 g of commercially available 2-methylcyclopentane-1,3-dione, **48**, were reacted with methyl vinyl ketone, **49**, in the presence of water at room temperature for five days to give the adduct **50**, **(scheme 2.3.)**. The crude product mixture [27.2 g] consisting of **50** and excess of methyl vinyl ketone was used as such in the next step. The literature yield for **50** is reported as 88%. **[94]**



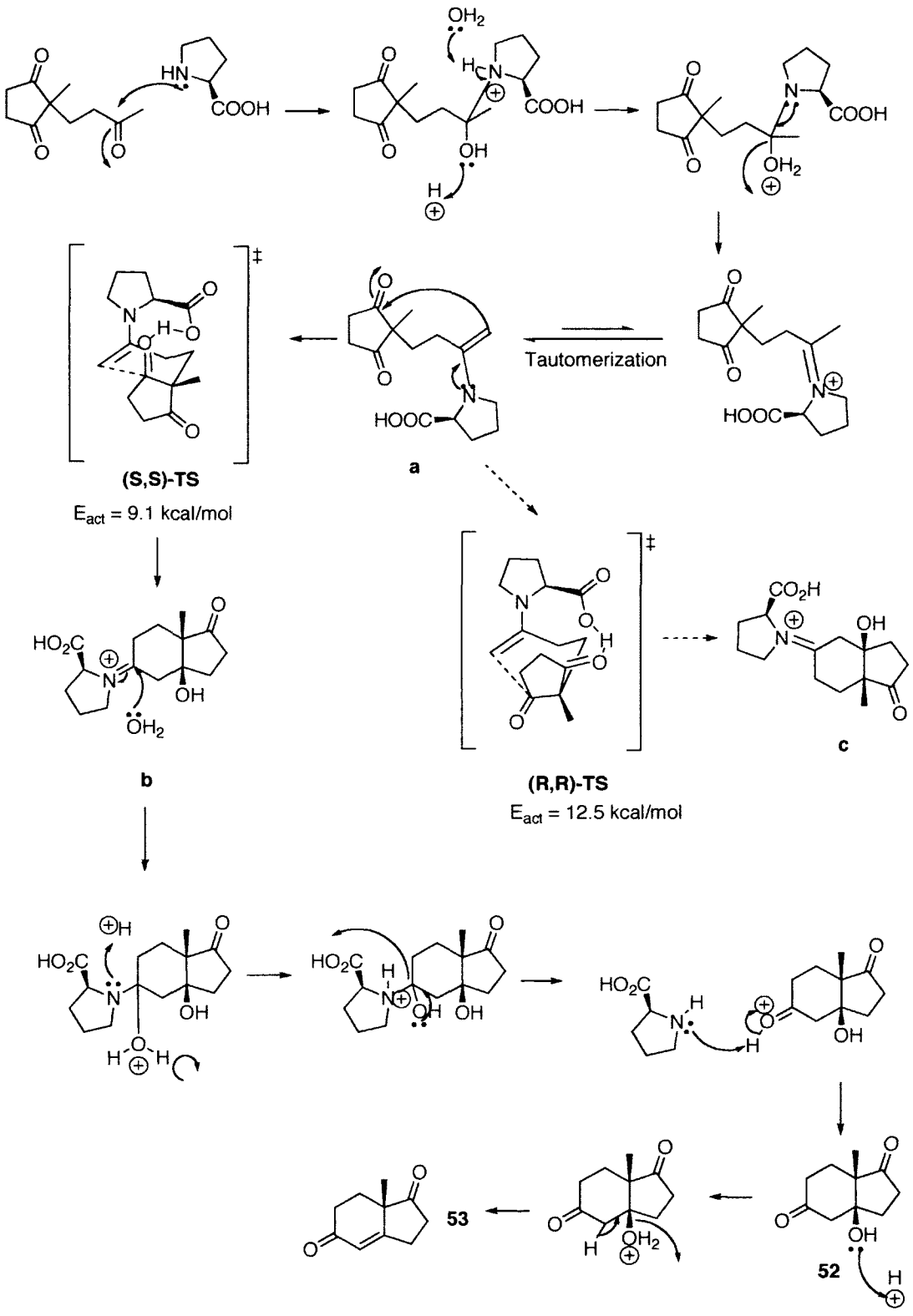
Scheme 2.3. Mechanism leading to the formation of **50**.

The second step, developed by Hajos and Parrish, involves catalysis using 3% molar equivalent of (S)-(-)-proline, which results in the enatioselective synthesis of the bicyclic ring system **52**. The conversion of **50** to **52** was performed in N,N-dimethylformamide (DMF) polar aprotic solvent. Recrystallization afforded optically pure **52**. **[93]**

The proposed transition state of the ring closure has been proposed by Houk et al in 2003, where they were able to show that only one molecule of proline is involved in the transition state of this intermolecular aldol reaction, with 6-membered chair and with enamine in the catalytic mechanism, **(scheme 2.4.)**. **[95]**

The cyclization product **52** has methyl and hydroxyl group in the *cis* conformation rather than *trans* because there are preferred electrostatic interactions between the carbonyl of the five-member ring and the electron-rich enamine π bond. **[96]** Additionally, there is as the inherent stability of *cis*-hydrindanone systems relative to *trans*. **[96-99]**

In the proline catalyzed mechanism intermediate **a** has a choice of attack onto two different carbonyl groups, which can lead to two different transition states **(S,S)-TS** giving intermediate **b** or **(R,R)-TS** intermediate **c**. Bahmanyar and Houk investigated the origin of stereoselectivity in that reaction and they proposed that the **(S,S)-TS** is preferred because in **(R,R)-TS** intramolecular hydrogen bonding forces the iminium double bond out of planarity. The energy of activation for the formation of the preferred transition state **(S,S)-TS** was calculated to be 9.1 kcal/mol, which is lower by 3.4 kcal/mol than that in **(R,R)-TS**. **[100]**

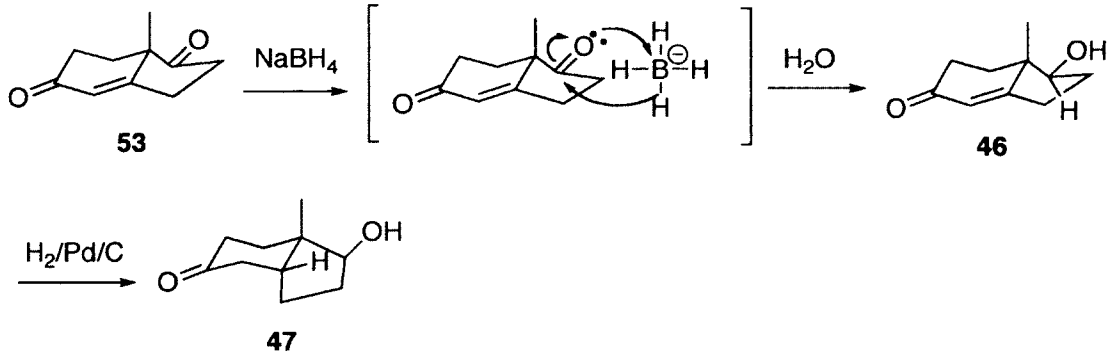


Scheme 2.4. The L-proline catalyzed cyclization of ring-B of the CD-ring, proposed by Houk et al. transition state **[95]** and dehydration of **52** to **53**.

In our hands the cyclization of **50** with L-proline for three days gave **52** accompanied by the dehydration product **53** due to catalysis by acidic L-proline. Thus, in order to ensure complete conversion to **53** the semi-purified material was subjected to dehydration with tosic acid. After purification compound **53**, was obtained as a brown oil in 94.6% yield over 2 steps. The reported literature yield of **53** over two steps was 90-94% yield. **[93]** The ^{1}H NMR spectrum of **53** showed a 3H singlet at 1.27 ppm and a 1H doublet at 5.92 ppm as expected for **53**. The complete proton NMR was essentially identical to one of compound **53** prepared earlier in the group by Dr. M. Asim.

The next step in the synthesis is a reduction with $NaBH_4$. The selectivity comes from the fact that because the 5-ring ketone is more electrophilic it is reduced preferentially compared to the less electrophilic 6-ring enone. The stereochemistry of the reduction of the 5-ring ketone is achieved due to the steric hindrance of the methyl group. The hydride adds selectively from the less hindered α-face, that is trans to the 13–β-methyl group (**scheme 2.5.**).

In our first synthetic attempt, reduction of compound **53** was carried out in methanol at 0°C. The result was over-reduction with both ketones being reduced. This was probably due to the fact that the amount of hydride used was not carefully controlled. In order to avoid this we turned to the general method developed by Ward et al. **[101]** for selective reduction of ketones in the presence of conjugated enones. These authors showed that saturated carbonyl groups could be reduced selectively in the presence of enones a series of compounds if the reduction was carried out in a mixture of DCM and methanol at -78 °C. Under these conditions compound **53** was reduced cleanly to the desired **46** in 57% isolated yield.



Scheme 2.5. $NaBH_4$ reduction and hydrogenation of the CD-ring.

The ^1H NMR spectrum of the product of **46** was consistent with the assigned structure. It showed the required 1H singlet at 5.76 ppm for the olefinic proton, a doublet of doublets at 3.82 ppm due to the newly introduced hydrogen at C17 in the 5-membered ring, and the 3H's, singlet at 1.12 ppm due to the quaternary methyl group. The spectrum was identical to that obtained earlier by Dr. Asim for compound **46**.

The final step in the synthesis is the hydrogenation with Pd/C and H_2. The reaction was quite efficient and the final CD-ring ketone **47** was obtained in 86% yield. The ^1H NMR spectrum of the final product **47** showed a 1H doublet of doublets at 3.84 ppm and the methyl group singlet at 1.17 ppm. Again, the spectrum of this compound was essentially identical to one obtained earlier in our group.

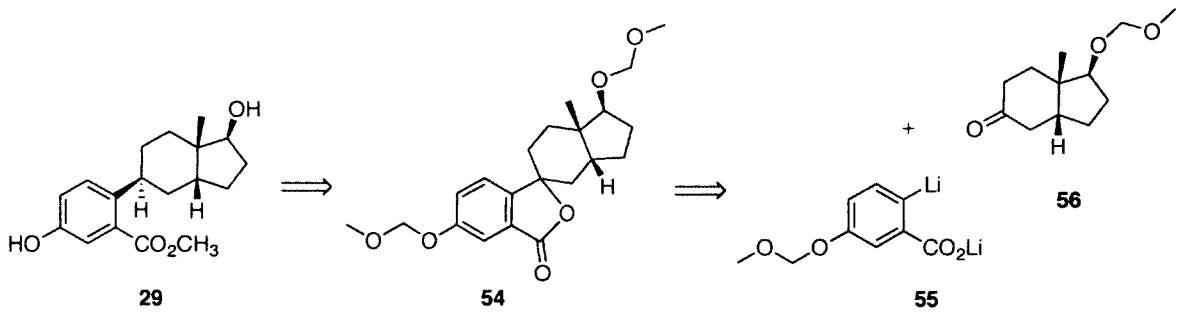
Our group discovered, after this thesis was submitted that the hydrogenation of **46** did not give the *trans* CD-ring, but instead the hydrogen was added on the same side of the CD-ring as the quaternary methyl group, giving the *cis* CD-ring product, which is speculated to be due to the concave shape of the molecule. The two methylene groups appear to have a greater effect on the stereochemistry of the hydrogenation than the quaternary methyl group. All the CD-ring structures in the current version of the thesis have been corrected.

I. New Substitutients at C5 in the A-ring

As mentioned above and illustrated in **table 2.1.**, substitution at C5 with F, Cl and CH_3 in ring-A increases significantly the binding of A-CD type compounds to the estrogen receptors. The order in the binding affinity is Cl > F > CH_3 > H. Docking studies indicated that substituents larger than CH_3 would interact sterically with residues in the binding pocket and thus show decreased binding affinity. Despite this indication we felt that it was necessary to prove the point experimentally. The target compound chosen was the methyl ester derivative **29**. This compound was of interest not only for the reason cited above but also due to its strong electron withdrawing character. Calculations of the OH bond dissociation energy which, we believe is a key indication of the tendency of a phenol to form a catechol and thus an ortho-quinone indicated that EWGs in the position meta to the phenol increased the BDE of the phenolic OH by several Kcal mol. Thus, ortho-quinone formation in **29** should be retarded significantly relative to **20**.

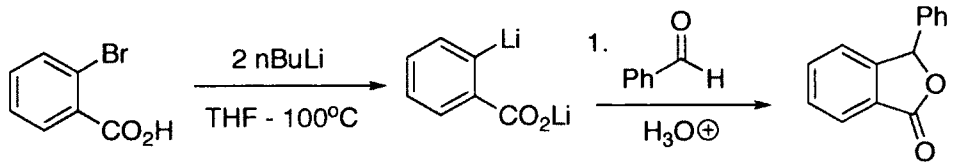
2.3. Efforts towards the synthesis of methyl ester 29

Our retrosynthesis of **29** is shown below.



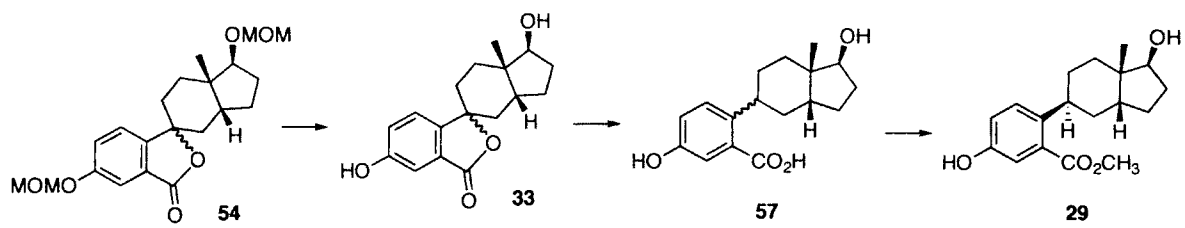
Scheme 2.6. Retrosynthesis of the methyl ester **29**.

The key synthetic step is the lithiation of the bromoacid to obtain **55** and its coupling to the protected CD ring ketone **56**, (**scheme 2.6.**). The bromine-lithium exchange in ortho bromo acids and subsequent trapping of the lithiated species with aldehydes had been reported by Parham et al. **[102, 103]** to result in the formation of phthalides, (**scheme 2.7.**). This result was verified in our lab about 30 years ago by Denise Leblanc. **[104]**



Scheme 2.7. Parham metalation of ortho bromo acids.

If successful in our case this should lead to the lactone **33**. This compound is interesting on its own as a spiro A-CD derivative. Hydrogenolysis of **33** would be expected to result in the formation of the mixture of acids **57** which could then be esterified and separated to yield the desired **29**, (**scheme 2.8.**).

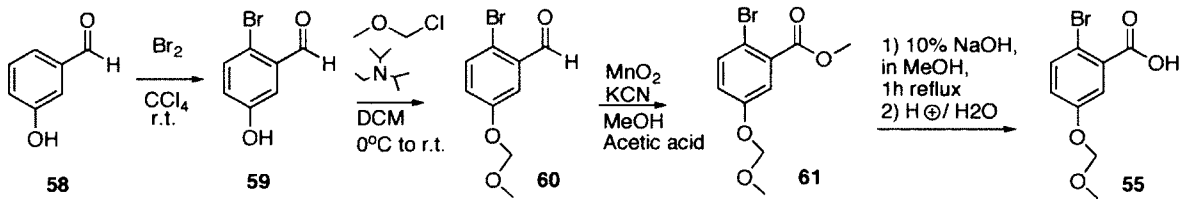


Scheme 2.8. Synthetic strategy leading towards the methyl ester **29**.

The synthesis started with bromination of 3-hydroxybenzaldehyde **58**. The hydroxy group is an ortho- para- directing substituent, but C. B. Koning et al. reported that bromination of **58** when performed in CCl_4 afforded only the para- product in 51% yield. **[105]** In our hands their procedure afforded a mixture of starting material and brominated

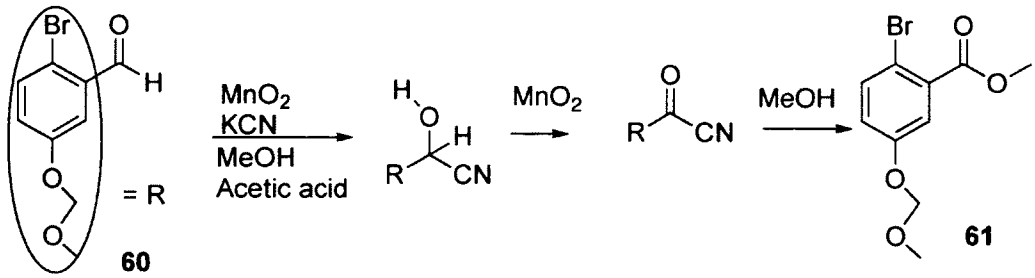
product. The desired product, obtained in 58% yield, can easily be separated from the unreacted starting material by recrystallization from dichloromethane and ethyl acetate. The ^{1}H NMR spectrum obtained was consistent with compound **59**.

In the next step, aldehyde **59**, was protected with chloromethyl methyl ether (MOM-Cl). The desired MOM protected compound was obtained as yellow oil in 97% yield. The ^{1}H NMR spectrum of **60** showed the appearance of two singlet at 5.15 (2H) and 3.42 (3H) ppm belonging to the MOM group.



Scheme 2.9. Synthesis of the acid **55**.

The aldehyde **60** was converted to methyl ester **61**, using the method developed E. J. Corey et al. which involves reacting **60** with NaCN in methanol in the presence of manganese dioxide. **[106]** In the first step the aldehyde is converted to its cyanohydrin. Since the cyanohydrin is also benzylic alcohol it is oxidized by manganese dioxide to a carbonyl group, in this case to an acyl cyanide. In the presence of alcohol the acyl cyanide is then transformed into its methyl ester, (**scheme 2.10.**). The methyl ester **61** was obtained in 93% yield. In ^{1}H NMR spectrum of the product the aldehyde singlet at 10.23 ppm was replaced by new singlet at 3.83 ppm (3H) due to the newly formed methyl ester.

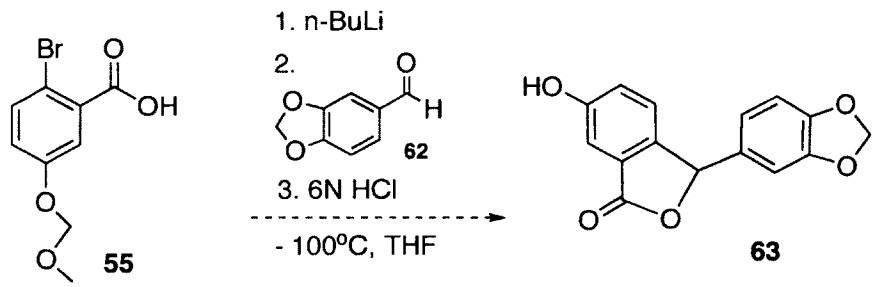


Scheme 2.10. Oxidation of aromatic aldehyde to an ester using MnO_2 and KCN in MeOH and acetic acid. **[106]**

Compound **61** was then hydrolyzed to the acid **55** in 86% yield by refluxing it with sodium hydroxide in methanol followed by acidic work up. The ^{1}H NMR spectrum of the product showed the disappearance of the methyl ester singlet and appearance of the broad at 11.47 ppm for the acidic hydrogen.

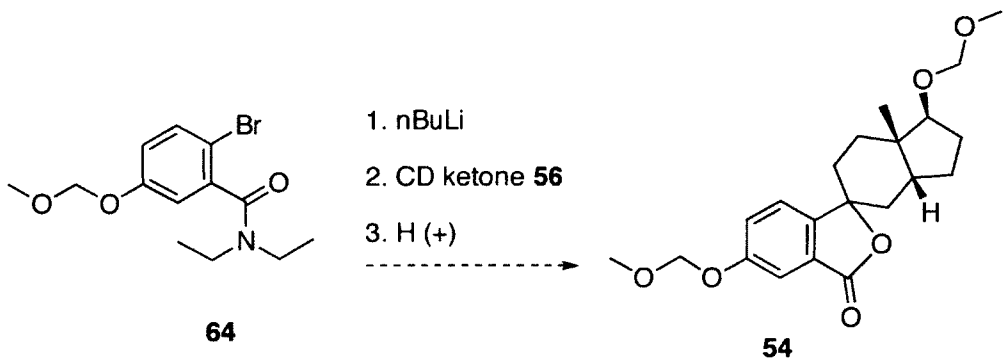
The key metalation of **55** and coupling to the CD ring was first attempted in a model study using piperonal **62** as the carbonyl component. The halogen-lithium exchange in **55** was attempted using n-BuLi at -100°C in methanol/ liquid nitrogen bath following the Parham procedure. Efforts were made to ensure dry glassware and solvents. The time between the nBuLi and piperonal addition was less than one minute to try to avoid self condensation of **55**. Unfortunately, after several attempts no evidence of successful coupling between dilithiated **55** and piperonal **62**, and formation of compound **63** was not obtained as judged by ^{1}H NMR spectrum of the crude reaction product. The TLC showed the disappearance of the piperonal starting material and the formation of the new spot. The newly formed spot was isolated by column chromatography. The ^{1}H NMR spectrum indicated a mixture of compounds, some of which had incorporated n-butyl

units from the nBuLi, probably via addition to piperonal and to the lithium carboxylate. One could speculate that the difficulty in carrying out the desired reaction on **55** as compared to ortho bromobenzoic acid is due to the electron donating OMOM group para to the Br.



Scheme 2.11. Coupling of acid **55** with piperonal **62** and expected product **63**.

One possible solution is to convert the acid **55** into a secondary amide such as the N,N diethyl amide. Such compounds have been metalated directly by the Victor Snieckus group and the lithiated derivatives are stable at low temperature. It is expected that halogen-lithium exchange in the bromo amide **64** should be successful and coupling with the CD ring ketone followed by acid treatment should also result in the formation of the lactone **54**. Due to a change in priorities the amide route was not pursued, but this approach is being investigated by another member of our group.



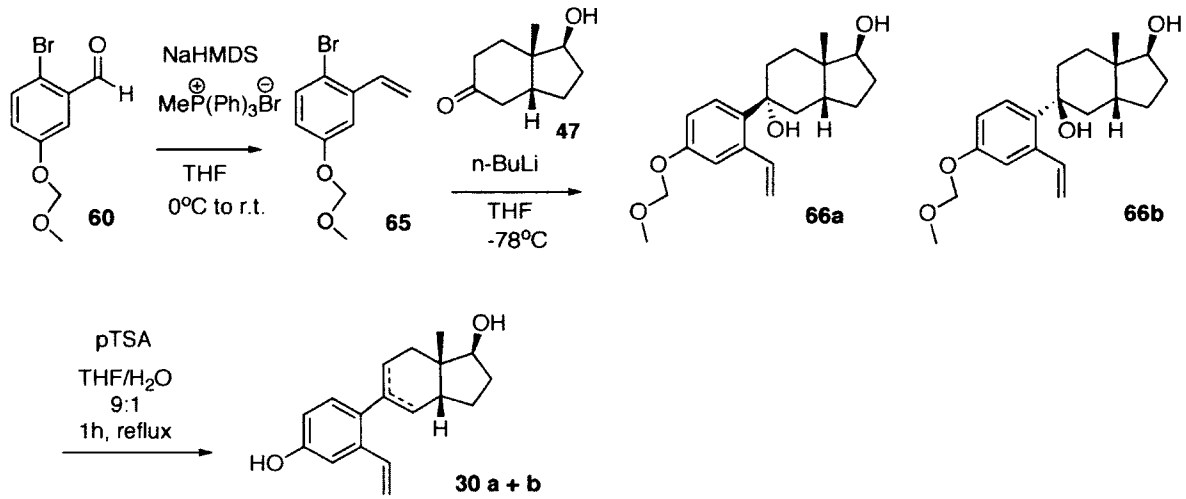
Scheme 2.12. Synthesis of **54** using A-ring with N,N diethyl amide **64.**

2.4. Synthesis of the 5-vinyl A-CD derivatives 30a and 30b

Since the 5-methyl A-CD compound showed significant binding to the estrogen receptors it was decided to test experimentally whether somewhat larger substituents at the 5 position of the A ring could be tolerated by these receptors. The question also arose whether larger substituents might show unusual and interesting binding selectivity for one of the two receptors. Replacement of the 5-methyl substituent with a 5-vinyl group represents one of the smallest possible structural changes.

The synthesis of the 5-vinyl compounds started with MOM-protected 3-hydroxybenzaldehyde **60.** This compound was converted to the corresponding vinyl compound **65** in 91% yield via a Wittig reaction using sodium bis(trimethylsilyl)amide and methyl triphenyl phosphonium bromide, (**scheme 2.13.**). The ^{1}H NMR spectrum of **65**, showed the appearance of three protons belonging to vinyl group at 7.02 ppm (dd, J = 17.6, 11.2, 1H), 5.71 ppm (dd, J = 17.6, 1.2, 1H) and at 5.37 ppm (dd, J = 10.8, 0.8 Hz, 1H), as required by compound **65**.

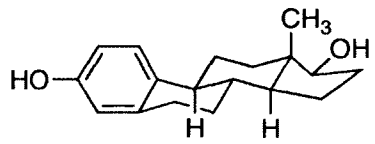
In the next step the vinyl compound **65** was reacted with nBuLi at -78 ^{o}C in THF under a nitrogen atmosphere. Since the hydroxyl group of the CD-ring **47** was not protected 3.0 equivalents of lithiated **65** were used per 1 equivalent of CD-ring. The first equivalent of lithiated A-ring is used to deprotonate the CD-ring hydroxyl group and an additional extra equivalent was used to maximize the yield of coupling to the CD-ring ketone. The enantiomeric mixture of tertiary alcohols **66a** and **66b** was obtained in a combined 85% yield. The isomers were separated by column chromatography and obtained as beige semi-solids: 0.134g (43.4% yield) **66a** and 0.128g (41.4% yield) **66b**.



Scheme 2.13. Synthesis of the vinyl compounds **30 a+b**.

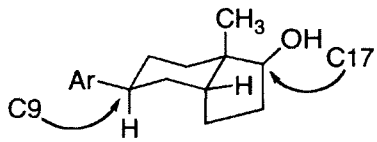
Earlier studies in our laboratory showed that either proton or carbon-13 NMR spectroscopy could be used to distinguish compounds with the 9(S) natural stereochemistry at C9 from those that have the un-natural 9(R) configuration. The chemical shifts of the proton and carbon at C17 were diagnostic. In the A-CD compounds with the natural 9(S) stereochemistry H17 appears near 3.9 ppm and the C17 near 80.9 ppm. These same nuclei resonated near 4.4 ppm and 73.7 ppm in the un-natural 9(R) derivative, (**table 2.2.**).

Estradiol



Stereochemistry

Natural (S) at C9



Non-natural (R) at C9

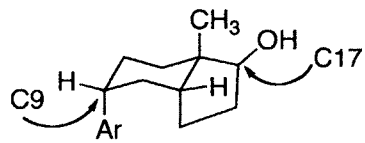


Table 2.2. Determination of stereochemistry at C9 based on CD-steroid.

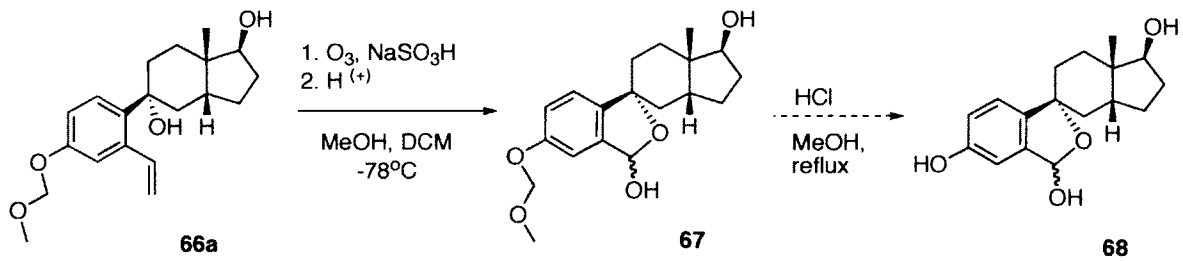
Parent A-CD steroid		natural stereochemistry at C9 δ(ppm)	un-natural 9(R) configuration δ(ppm)
^{1}H NMR	H17	doublet of doublets at 3.9	triplet at 4.4
^{13}C NMR	C17	80.9	73.7

Since we were able to separate the two isomers we assigned the stereochemistry. The ^{1}H NMR spectrum of **66a** showed H17 as doublet of doublets at 3.98 ppm, therefore this isomer was assigned the 9(S) "natural" A-CD stereochemistry. The ^{1}H NMR of compound **66b** has H17 as an apparent triplet at 4.21 ppm, which indicates the 9(R) "un-natural" stereochemistry. It should be realized that this is not important since the C9 stereocenter is eliminated in the next step.

Dehydration of **66b** in tetrahydrofuran and water (9:1), under reflux conditions using pTSA gave a mixture of isomers with the double bond at C9-C8 and C9-C11 in 40% isolated yield. The ^{1}H NMR of the mixture of two isomers showed peaks for H17 at 3.94 and 3.85 ppm. The ratio of the two isomers was 40 : 60 based on the integration of the two H17 peaks.

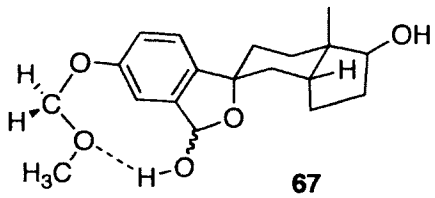
2.5. Ozonolysis of vinyl derivative 66a

Ozonolysis of the vinyl derivative **66a** was carried out in a mixture of methanol and dichloromethane. Compound **68** was a desirable target, because it could be used further for the synthesis of 5-spiro lactone **33** and the methyl ester **29**, (**scheme 2.14.**).



Scheme 2.14. The ozonolysis of the vinyl derivative **66a** to mixture of acetals **67** and attempted deprotection to **68**.

Workup of the reaction mixture followed by column chromatography gave in about 26 % yield a relatively pure compound whose NMR spectrum was consistent with an isomeric mixture of cyclic acetal **67**. The ^1H NMR showed two singlets at 5.99 and 5.97 ppm assigned to the acetal H. Additionally, the spectrum showed methoxy singlets of the MOM group at 3.47 and 3.44 ppm, H17 proton at 3.92-3.87 ppm and the quaternary methyl group as two singlets at 1.13 and 1.12 ppm. Interestingly, the $-CH_2$ of the MOM group appears as an AB quartet. This data is consistent with the structure **67**, in which the asymmetry due to the acetal ring is transferred to the $ROCH_2$-OCH_3 via internal hydrogen bonding. (**figure 2.6.**).



67

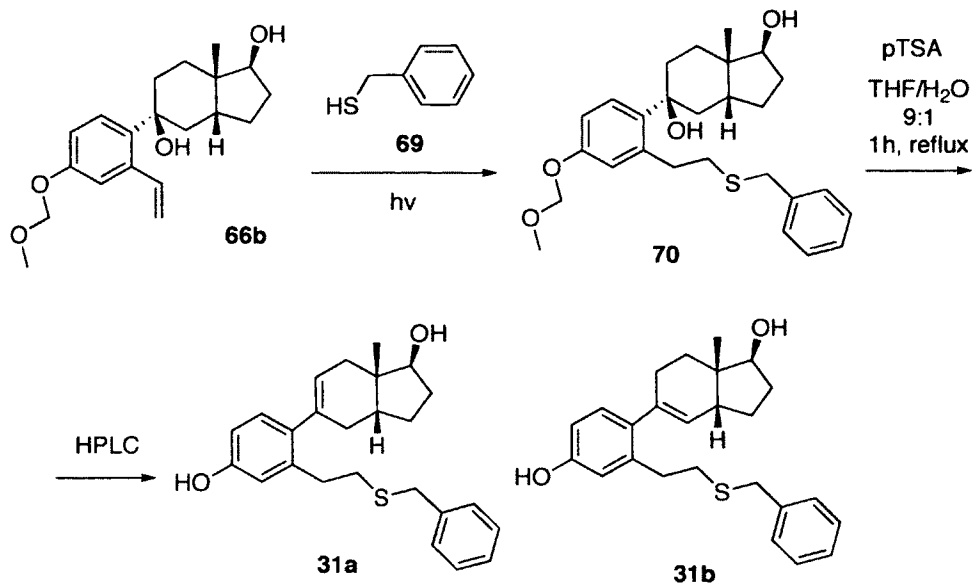
Figure 2.6. The hydrogen bonding between the MOM group and acetal hydroxyl in compound **67**.

Attempted MOM deprotection of **67** by refluxing it in methanol and HCl was expected to yield **68**. The TLC showed the formation of a single, less polar spot. Unfortunately, we were unable to isolate and characterize this compound and further work on this sequence was stopped.

2.6. Addition of benzyl mercaptan to 66b

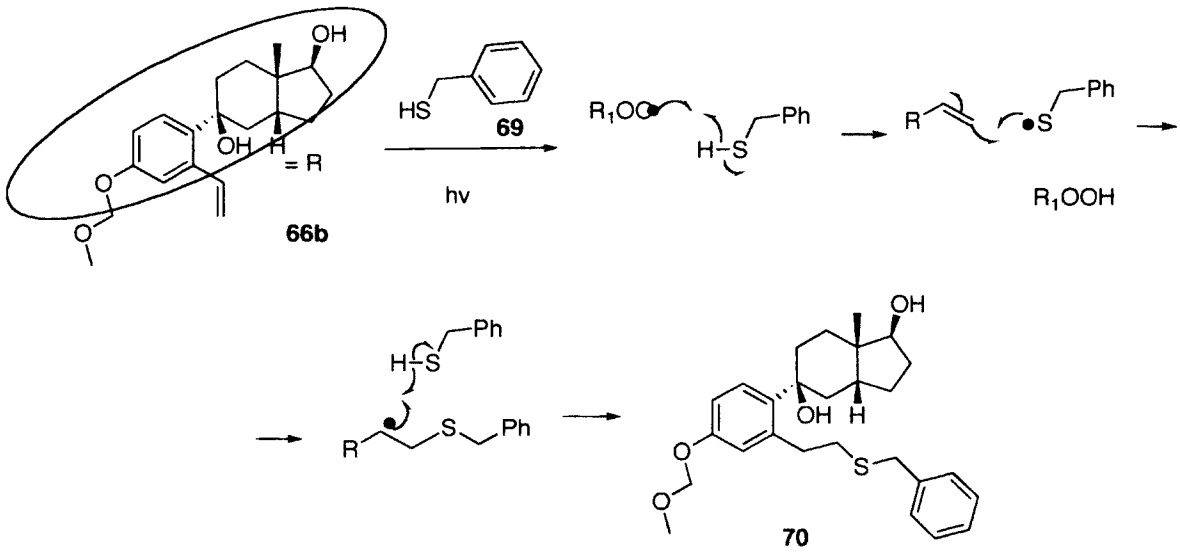
The availability of compound **66b** suggested the possibility of creating a large group at C5 by adding benzyl mercaptan in an anti-Markovnikov fashion. This would result after dehydration in structures such as **31a** and **31b**. Our collaborator at Carleton University Dr. Hooman Shadnia indicated that his modeling predicted that such compound would be ERα selective.

Thus compound **66b**, was dissolved in $CDCl_3$ and left exposed to daylight for two weeks in the presence of benzyl mercaptan **69**. The initial benzyl radical is created by abstraction of H from weak S-H bond in $PhCH_2S$-H to create a thiol radical. This radical reacts with the vinyl compound, to create preferentially the more stable benzyl radical intermediate and eventually lead to the formation of **70**, (**scheme 2.15.**). The final product was obtained in 69% after purification by column chromatography.



Scheme 2.15. The synthesis of sulfur compounds **31a** and **31b**.

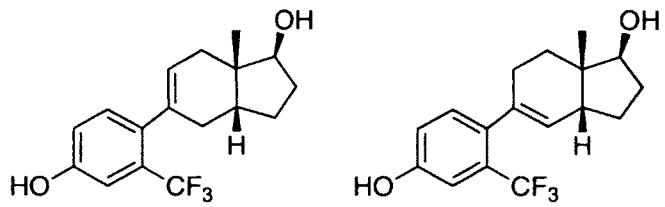
The ^{1}H NMR of the sulfur compound **70** shows aromatic protons as multiplets at 7.34-7.19 (m, 6H) and 6.81-6.78 (m, 2H) ppm. The MOM- protecting group is present as two singlets at 5.11 (s, 2H) and 3.44 (s, 3H) ppm. The C17 proton is a triplet at 4.25 ppm (dd, apparent t, J= 8.0, 8.0 Hz, 1H), which is an indication of the 9(R) "non-natural" steroidal stereochemistry. The three $-CH_2$ groups, which are part of C5 substituent occurred at 3.68 (s, 2H), 3.31-3.23 (m, 1H), 3.20-3.13 (m, 1H), and 2.69 (dd, apparent t, J= 8.0 Hz, 2H) ppm. The 11 protons of the CD-ring region are present in the multiplet 2.20-1.04 (m, 10H), 0.91-0.82 (m, 1H). The methyl group is present as singlet at 0.95 ppm (s, 3H).



Scheme 2.16. The radical mechanism leading to the formation of **70**.

Compound **70** was dehydrated and deprotected in tetrahydrofuran and water (9:1) by refluxing it with pTSA. The crude product was purified using column chromatography, from which a de-protected mixture of isomers **31a** and **31b** was obtained as yellow solid in 79% yield. The two isomers were separated by HPLC to give **31a** and **31b** in 19% and 36% yield, respectively.

The position of the double bond in **31a** and **31b** was assigned by comparing position of H17 proton in the ^{1}H NMR of compounds **71a** and **71b** synthesized by Christine Choueiri with -CF_3 at C5.



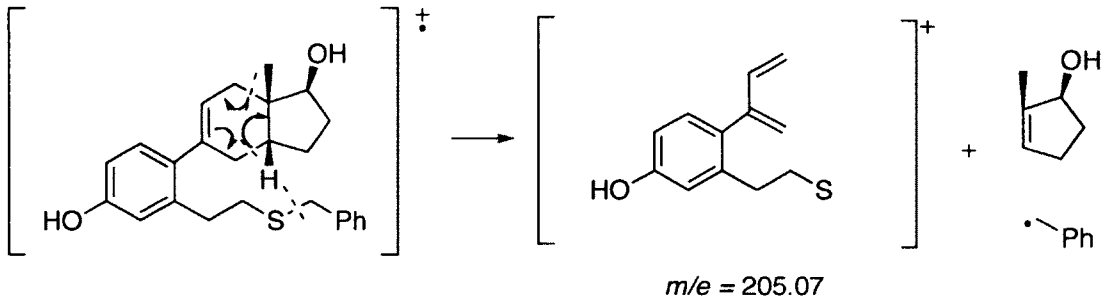
71a **71b**

Table 2.3. Determination of position of the double bond in **31a** and **31b** based on comparison of the ^{1}H NMR data of **71a** and **71b**.

Compound	H17 (ppm)	alkene H (ppm)
71a (C9-C11)	dd at 3.74	5.44
31a (C9-C11)	dd at 3.81	5.38
71b (C8-C9)	t at 3.88	5.49
31b (C8-C9)	t at 3.91	5.46

The chemical shifts of the alkene proton and H17 were used for determination of the position of the double bond. In the A-CD compounds with the double bond at C9-C11 as in **71a**, H17 appears near 3.74 ppm and the alkene proton 5.44 ppm. These same nuclei resonated near 3.88 ppm and 5.49 ppm in the derivative **71b** with the C8-C9, (**table 2.3.**). The ^{1}H NMR spectrum of **31a** showed H17 as doublet of doublets at 3.81 ppm, and the olefenic H at 5.38 ppm, therefore this isomer was assigned the position of the double bond at C9-C11. The ^{1}H NMR of compound **31b** has H17 as an apparent triplet at 3.91 ppm, and the olefenic H at 5.46 ppm, thus the double bond was assigned at C8-C9.

Compound **31a** showed additional evidence supporting the position of the double bond at C9-C11, which was peak of *m/e* = 205.1 present in the Mass Spectrometry (MS). It corresponds to the fragment after the reverse Diels-Alder reaction and after loss of benzyl group. (**scheme 2.17.**).



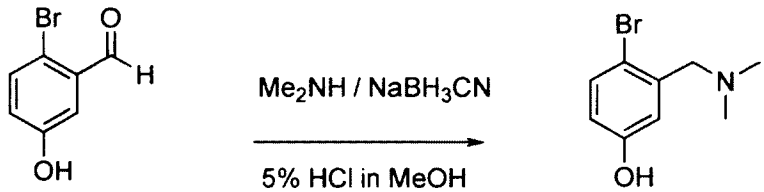
Scheme 2.17. The cleavage of reverse Diels-Alder reaction in Mass Spectrometry leading to a fragment *m/e* = 205.07.

The three $-CH_2$ groups appear at 3.71 (s, 2H), 2.80-2.76 (m, 2H) and 2.62-2.58 ppm (m, 2H) in **31a** and at 3.71 (s, 2H), 2.80-2.71 (m, 2H) and 2.62-2.56 ppm (m, 2H) in **31b**. The methyl group singlet is present at 1.07 ppm in **31a** and at 1.03 ppm in **31b**.

Compounds **31a** and **31b** were send for the binding assay.

2.7. Towards the synthesis of the 5-dimethylamino compound 32

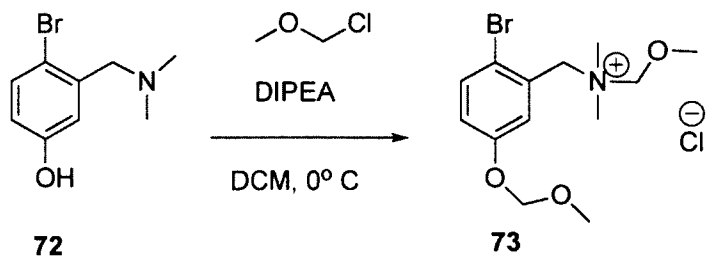
The synthesis of compound **32** with the basic dimethyl amine group at C5 was attempted because substitutions at C5 showed to improve binding to both estrogen receptors and no compounds in the ACD series with the basic properties had been prepared and evaluated. The bromoaldehyde **59** was reductively aminiated with dimethylamine and $NaBH_3CN$ in 55% isolated yield, (**scheme 2.18.**). The newly formed $-CH_2$ group in **72** is a singlet at 3.53 ppm (s, 2H) and the two new methyl groups also show as singlet at 2.32 ppm (s, 6H) in ^1H NMR spectrum.



59 **72**

Scheme 2.18. Reductive amination of **59** to **72**.

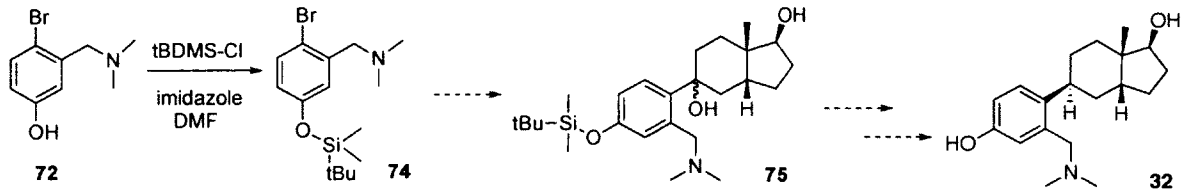
In order to prepare for the coupling of **72** to the CD ring ketone it was necessary to first protect the phenolic OH group. In the first attempt of protection 2 equivalents of MOM-Cl and diisopropylethyl amine were used. However, instead of desired monoprotected product, the doubly protected salt was obtained, where the second MOM group was added to the nitrogen of dimethyl amine. The nucleophilic lone pair of nitrogen was able to attack the MOM-Cl likely in an SN2 reaction leading to an unwanted salt **73**, (**scheme 2.19.**). The reaction was repeated with only 1 equivalent of MOM-Cl and diisopropylethyl amine, but unfortunately that also gave the same doubly MOM protected product **73**. The ^{1}H NMR spectrum showed three $-CH_2$ groups as singlets at 4.95 (2H), 4.91 (2H) and 4.69 (2H) ppm, where two of them belong to the MOM groups and the two singlets at 3.53 (3H), and 3.16 (3H) ppm, which also are indication that the two MOM groups were added.



72 **73**

Scheme 2.19. MOM-Cl protection of **72** to **73**.

The phenol was successfully protected with tBDMS to afford **74** in 79% isolated yield. This material was difficult to separate from excess tBDMS-Cl due to its very low polarity. At this point we received the results of the binding studies for the two sulfur compounds **31a** and **31b.** The data indicated very low binding relative to estradiol and the compounds with relatively small substituents at C5 as was predicted by the modeling studies. Thus the decision was made not to pursue the synthesis of the A-CD compound **32** via condensation of lithiated **74** with the CD ring ketone at this time. Nevertheless, about 1.4 g of compound **74** is available should the synthesis of **32** become desirable in the future.



Scheme 2.20. t-BDMS protection of **72** to **74.**

2.8. Biological data of the vinyl and sulfur compounds

Based on results presented below in **table 2.4** the mixture of 5-vinyl compounds **30(a + b)**, showed considerably lower binding affinity to both receptor subtypes than the corresponding 5-methyl derivatives thereby agreeing with the predictions. The binding affinity of the 5-vinyl mixture is comparable to that of a mixture of derivative parent unsaturated compounds **76(a + b)**.

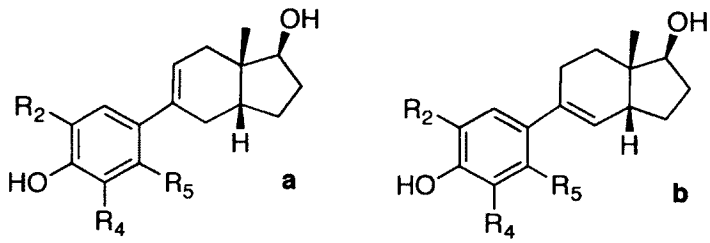


Table 2.4. Relative Binding Affinity (RBA) and Relative Transcription Activation (RTA).

Compound	Ring A			RBA			RTA		
	R_2	R_4	R_5	ERα	ERβ	ERβ/ ERα	ERα	ERβ	ERβ/ ERα
estradiol	H	H	H	100	100	1	100	100	1
30(a + b)	H	H	$CH{=}CH_2$	0.24	3.3	13.8	-	-	-
31a	H	H	CH_2SCH_2Ph	0.32	0.73	2.3	-	-	-
31b	H	H	CH_2SCH_2Ph	0.31	0.27	0.87	6.7	14.6	2.2
76(a + b)	H	H	H	0.21	2.0	9.5	-	-	-
77(a + b)	H	H	F	4.5	49	11	-	-	-
78a	H	H	Cl	60	118	2.0	-	-	-
78b	H	H	Cl	195	331	1.7	-	-	-
79(a + b)	H	H	CH_3	7.7	52.8	6.9	-	-	-

The objective of synthesizing sulfur compounds was to check if the ER binding pocket can accommodate very large substituents such as in **31a** and **31b**. Compound **31a** had RBA of ERα = 0.32, ERβ = 0.73, with the ERβ/ ERα = 2.3 and compound **31b** ERα = 0.31, ERβ = 0.27, and ERβ/ ERα = 0.87. Again, the results are consistent with the predictions including that compounds with large substituents are likely to show ERα selectivity.

II. Compounds with fixed conformations different than estradiol

Molecular mechanics calculations confirm the expectation based on examination of models that free A-CD ligands adopt the conformation in which the plane of the A ring is perpendicular to the plane of CD ring. The A-ring is able to rotate freely about the C-C bond linking ring A to ring C and the ligand can adjust itself within the ligand binding domain (LBD). Modeling studies by our collaborators, Drs. Wright and Shadnia indicated that the ER bound A-CD ligand adopts a conformation, in which the A ring is 21 +/-5 ^{o} angle to plane of the CD ring. **[83]** If this is indeed the case then rotation of the ligands to the take on the shape suggested to give optimum binding to the receptor, especially those that have relatively large substituents at C5, is energetically quite costly. It is therefore quite surprising that the 5-F, 5-Cl, 5-CH_3 and especially the 5-CF_3 A-CD compounds show binding to the estrogen receptor, especially the ERβ, which is comparable and even higher than that of estradiol. This would require that the above quoted 5-substituents must have significant positive interactions with the receptor to overcome the energy required to rotate from the preferred 90^{o} to the smaller, approximately, 20^{o} dihedral angle.

One way to test for the effect of changing the dihedral angle between the two planes is to synthesize compounds with fixed conformation and subsequently determine the binding constants. The natural hormone estradiol provides one example of such a system, one in which the dihedral angle between the two relevant planes is less than 10^{o}. The two compounds **34a** and **35a**, called 5-spiro ether and 6-spiro ether, respectively, provide two others with relatively fixed dihedral angles. The 5-spiro ether is locked in the conformation in which the A ring is essentially orthogonal to CD ring plane. The addition

of one more carbon to the spiro ring results in a compound that has more flexibility. Based on examination of models, the key dihedral angle in **35a** ranges between 60-70° without creating undue strain. Thus, it was felt that access to compounds **34a** and **35a** might provide insight into the preferred or possible A-ring to CD-rings dihedral angles that could be tolerated by the estrogen receptors.

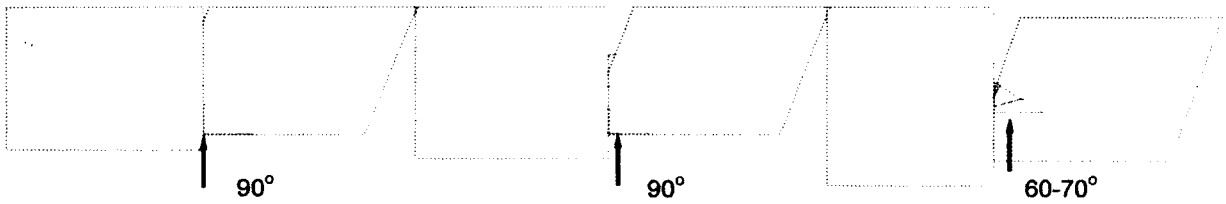


Figure 2.7. The angle between A- and CD-ring planes in parent A-CD compound, 5-spiro **34a** and 6-spiro **35a** analogues.

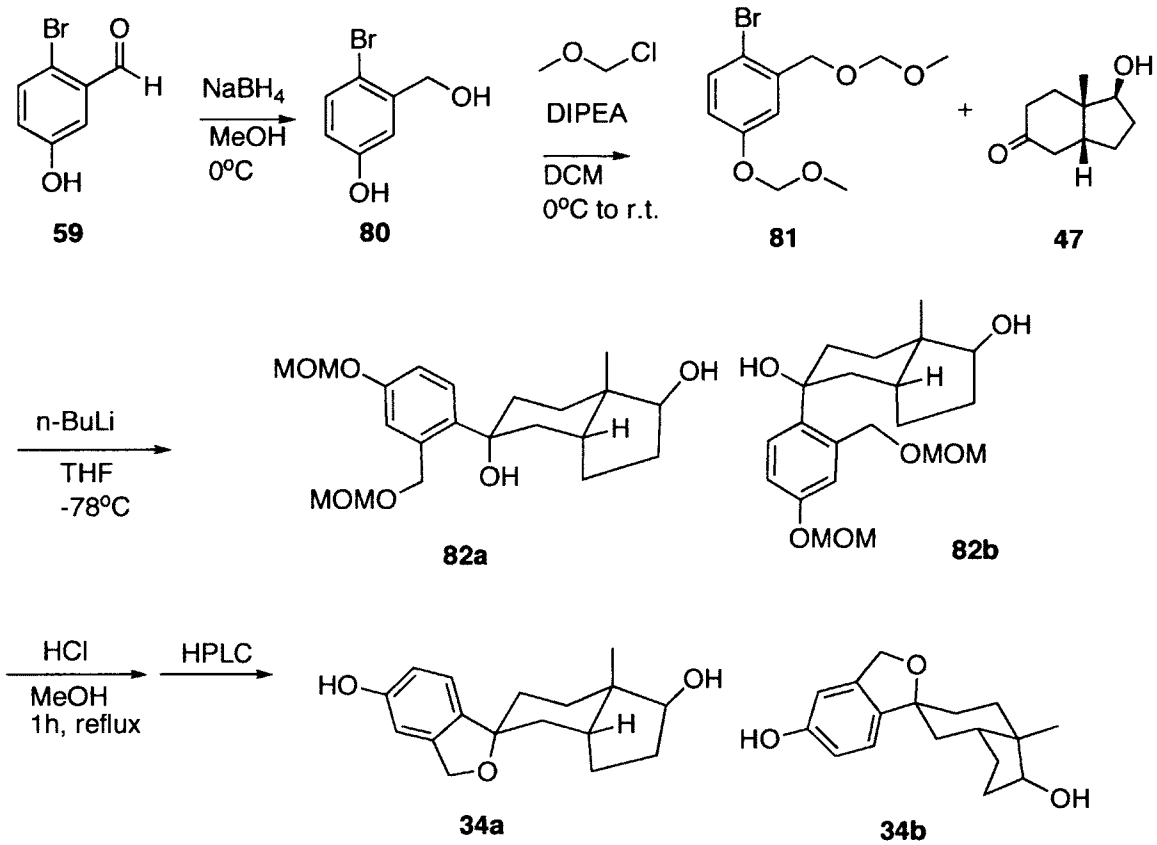
2.9. Synthesis of 5-spiro ether 34a and 34b

It was anticipated that the 5-spiro ether system would be formed by treating the condensation of product **82a** or **82b** with acid. This process should generate preferentially the benzylic tertiary cation, which would be trapped intramolecularly to give the spiro system. It was anticipated that both isomers, one having the natural 9(S) and the other the 9(R) configuration would be formed.

In the synthetic sequence bromoaldehyde **59** was reduced with $NaBH_4$ in 75% yield to the alcohol **80**. The ^1H NMR spectrum showed the disappearance of the aldehyde singlet at 10.29 ppm and the appearance of the required CH_2 group as a singlet at 4.6 ppm.

The alcohol **80**, was protected in 88% yield with chloromethyl methyl ether (MOM-Cl) to the desired doubly MOM protected compound **81**. The ^1H NMR spectrum of **81** showed

two singlets at 5.15 and 4.76 ppm belonging to the two $-CH_2$ protons and two singlets at 3.46 and 3.43 ppm integrating for 3H's are the MOMs' methyl groups, (**scheme 2.21.**).



Scheme 2.21. The synthesis of 5-spiroether.

In the next step the protected A-ring **81** was coupled with CD-ring using n-BuLi at -78°C. Since the hydroxyl group of the CD-ring was not protected, 2.5 equivalents of A-ring were used per 1 equivalent of CD-ring. A 2:1 mixture of diastereomeric tertiary alcohols **82a** and **82b** was obtained in a combined yield of about 42% after column chromatography, which removed the reduced excess A-ring byproduct. Extensive column chromatography enabled us to obtain a pure sample of **82a** as judged by its ^{1}HNMR. The doublet of doublets at 3.91 ppm of H17 and C17 at 80.7 ppm indicated the "natural" 9(S)

stereochemistry, which corresponds to A-ring carbon bonding to C9 in an equatorial position. In contrast in the "non natural" 9(R) isomer **82b** the H17 resonated as triplet at 4.32 ppm.

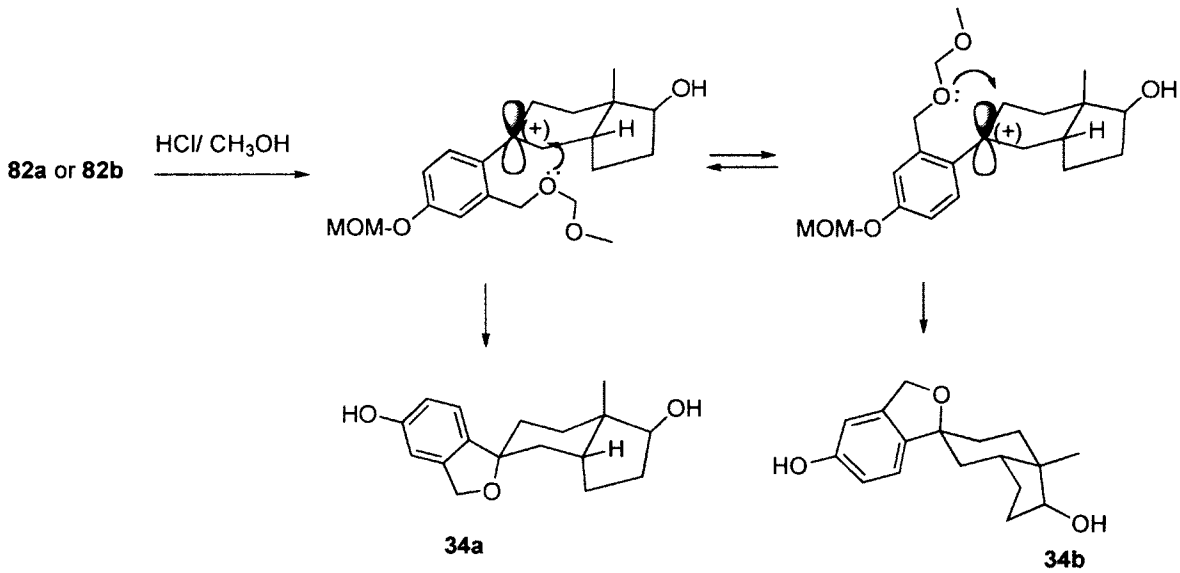
In the last step of the synthesis a mixture of **82a** and **82b** was reacted with HCl in methanol. This treatment caused both deprotection and cyclization and afforded a mixture of the 5-spiro ethers **34a** and **34b.** The sequence of reaction is not certain. Presumably the first step is dehydration of the tertiary benzylic alcohol at C9 to give a carbocation. This cation can be trapped by the benzylic oxygen of the MOM ether at C5 give the spiro structure in the form of an oxonium ion, which is eventually dealkylated. Another sequence involving first deprotection of the MOM groups and then trapping of either the benzylic tertiary cabocation or a benzylic secondary carbocation at C5 with the 9-OH group is considered less likely.

After extensive purification by HPLC and plate chromatography **34a** and **34b** were obtained in 15.2 and 12.0% yield, respectively. The ^{1}H NMR spectrum of the isomer assigned structure **34a** shows H17 as a doublet of doublets at 3.91 ppm and C17 at 80.8 ppm, which is again consistent with the aromatic A–ring being bonded equatorially relative to ring C. In second isomer **34b**, the H17 appears as a triplet at 4.40 ppm and C17 is found at 73.6 ppm indicative of the A–ring being axial with respect to ring C. The $-CH_2$ group of the 5-spiro-ring in **34a** shows as AB system at 4.97 (J = 12.8 Hz) and at 4.93 ppm (J = 12.8 Hz) but is found as singlet at 4.97 ppm in **34b**.

Compound **34a** shows six aromatic signals in ^{13}C NMR at 155.4, 140.9, 139.1, 121.7, 114.2 and 107.8 ppm. It has five required quaternaries present, the three aromatic

quaternaries at 155.4, 140.9 and 139.1 ppm and the spiro and CD-ring quaternaries at 86.9 and 43.3 ppm.

There are six $-CH_2$ groups, consistent with the **34a** structure; the spiro ether $-CH_2$ group is present at 70.2 ppm. The molecular ion in the HRMS occurred at m/z = 274.1564 which fits the required formula $C_{17}H_{22}O_3$.

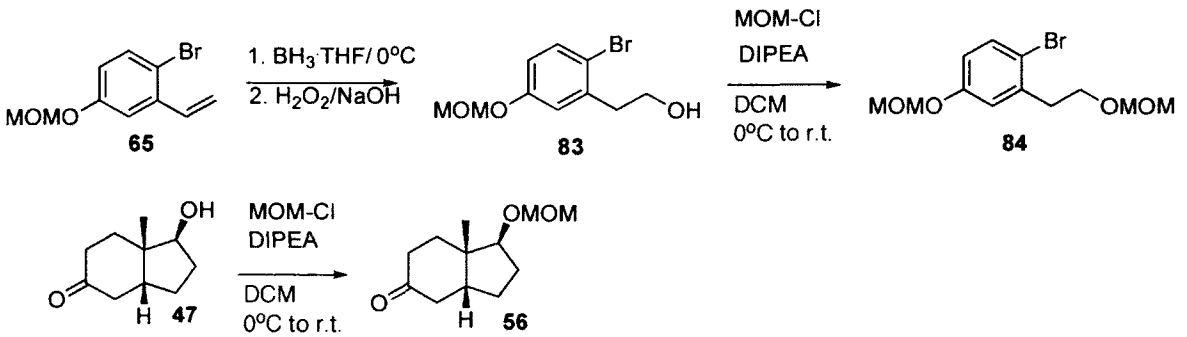


Scheme 2.22. The acid catalyzed cyclization and deprotection reaction in the formation of 5-spiro ether **34a** and **34b.**

2.10. Synthesis of 6-spiro ether

The synthesis of the 6-spiro ether **35a** was planned in similar manner as the successful 5-spiro ether synthesis, except both A and CD rings were protected with MOM group prior to coupling. The MOM protected vinyl derivative **65**, underwent regioselective hydroboration **[108]** to afford the alcohol **83** in 40% yield, (**scheme 2.23.**). The actual yield in this step was considerably higher since approximately half of the reaction material was lost due to a mechanical problem during oxidation step. The ^1H NMR

spectrum of **83**, showed the appearance of two new triplets at 3.80 ppm (t, J = 6.8 Hz, 2H) and at 2.92 ppm (t, J = 6.8, 2H), which belong to the newly formed $-CH_2$ groups and the new broad peak at 2.53 ppm (br, 1H) due to the newly formed –OH. Alcohol **83**, was protected with MOM group to give the doubly protected **84** in 96% yield. The newly added MOM group in ^1H NMR spectrum showed as singlet signals at 4.60 (2H) and 3.29 ppm (3H).



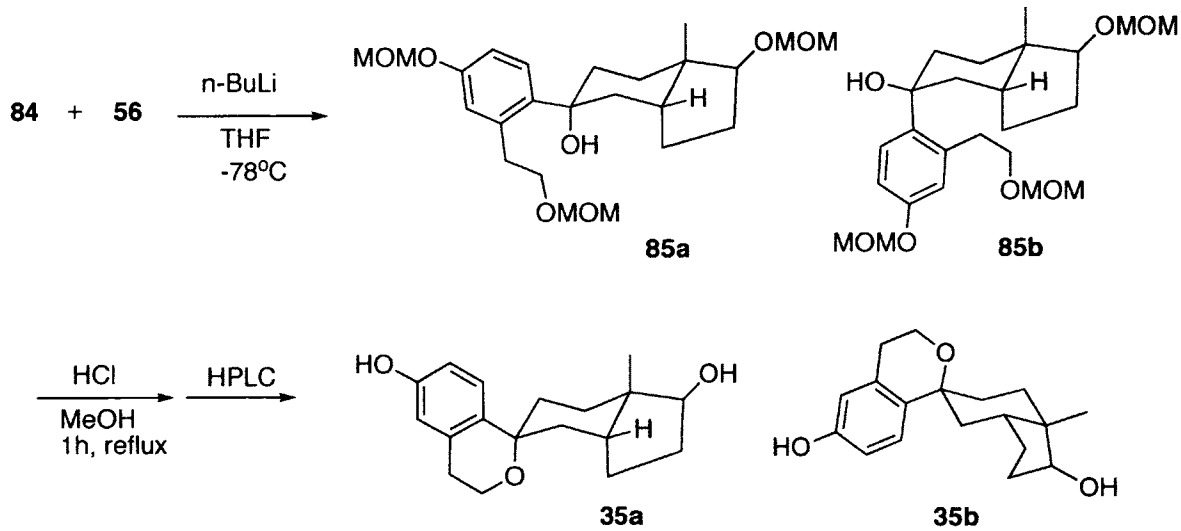
Scheme 2.23. Preparation of the two components required for synthesis of 6-spiroethers **35**.

Since only a limited quantity of the A-ring starting **84** was available, it was decided to protect the OH group in **47** with MOM group prior to coupling in order to use the A-ring moiety with maximum efficiency. The MOM protected CD-ring **56** was obtained in 70% isolated yield, 78% based on recovered starting material.

The coupling of the MOM-protected starting materials **84** and **56** was carried out in the usual manner. In the present case 1.5 equivalents each of **84** and n-BuLi were used per equivalent of MOM protected CD-ring, **56**. The coupling products **85a** and **85b** were obtained, however the complete separation of the isomers was not achieved; this was unnecessary for the next synthetic step. The estimated yield based on ^1H NMR was 42%

for more polar isomer **85a** and 30% for the less polar isomer **85b.** Some of the CD-ring starting material was also recovered likely due to competitive enolization of the six membered ketone, (**scheme 2.24.**).

The more polar isomer with the more upfield H17 at 3.84 ppm (dd, J = 6.0, 4.4 Hz, 1H) was assigned the "natural" at C9 and the less polar isomer having its H17 at 3.87 ppm (dd, J = 6.4, 4.4 Hz, 1H) was given the "non-natural" at C9 steroid stereochemistry as shown in **85b**. The mixture of **85a** (70%) and **85b** (30%) was treated with catalytic amount of acid in methanol under the reflux in order to accomplish both the cyclization and deprotection reactions. The estimated yield of product **35a** and **35b** based on ^{1}H NMR was 14.4% and 20.3%, respectively.



Scheme 2.24. The coupling and cyclization and deprotection reactions leading to 6-spiroether **35a** and **35b**.

The separation of the two isomers was quite challenging. Eventually success was achieved by a combination of silica gel plate chromatography and an HPLC separation using a system developed by Dr. Ammar Saleem in Professor Arnason's Laboratory in

the Department of Biology. This allowed us to isolate the desired pure **35a** in an overall yield of about 4%. Compound **35b** was not obtained pure. The best sample contained about 2% of **35a**. The overall yield of this mixture was almost 7%.

The overall features of the ^{1}H NMR spectrum of **35a** were consistent with the assigned structure. It showed the presence of three required 1,3,4 aromatic substitution with protons at 6.97 (d, J = 8.8 Hz, 1H), 6.66 (dd, J = 8.4, 2.4 Hz, 1H) and 6.53 (d, J = 2.8 Hz, 1H) ppm and the phenolic hydroxy proton at 6.02 (br, 1H) ppm. The multiplet at 3.88 – 3.77 (m, 3H), contained both H17 and the O–CH_2 protons of the spiro ring. The position of H17 proton at ~ 3.9 ppm is consistent with the 9(S) "natural" stereochemistry as in **35a**. The methyl group singlet shows at 1.18 ppm.

The ^{13}C NMR spectrum shows six aromatic signals at 153.7, 135.4, 135.3, 126.72, 114.9 and 113.5 ppm. The five required quaternary carbons, three aromatic quaternaries at 153.7, 135.4 and 135.3 ppm and two C-ring quaternaries were found at 75.9 and 43.6 ppm. The seven required –CH_2 groups show at 58.1, 33.6, 33.4, 32.3, 29.6, 27.8 and 27.3 ppm. The HRMS supports the molecular formula $C_{18}H_{24}O_3$.

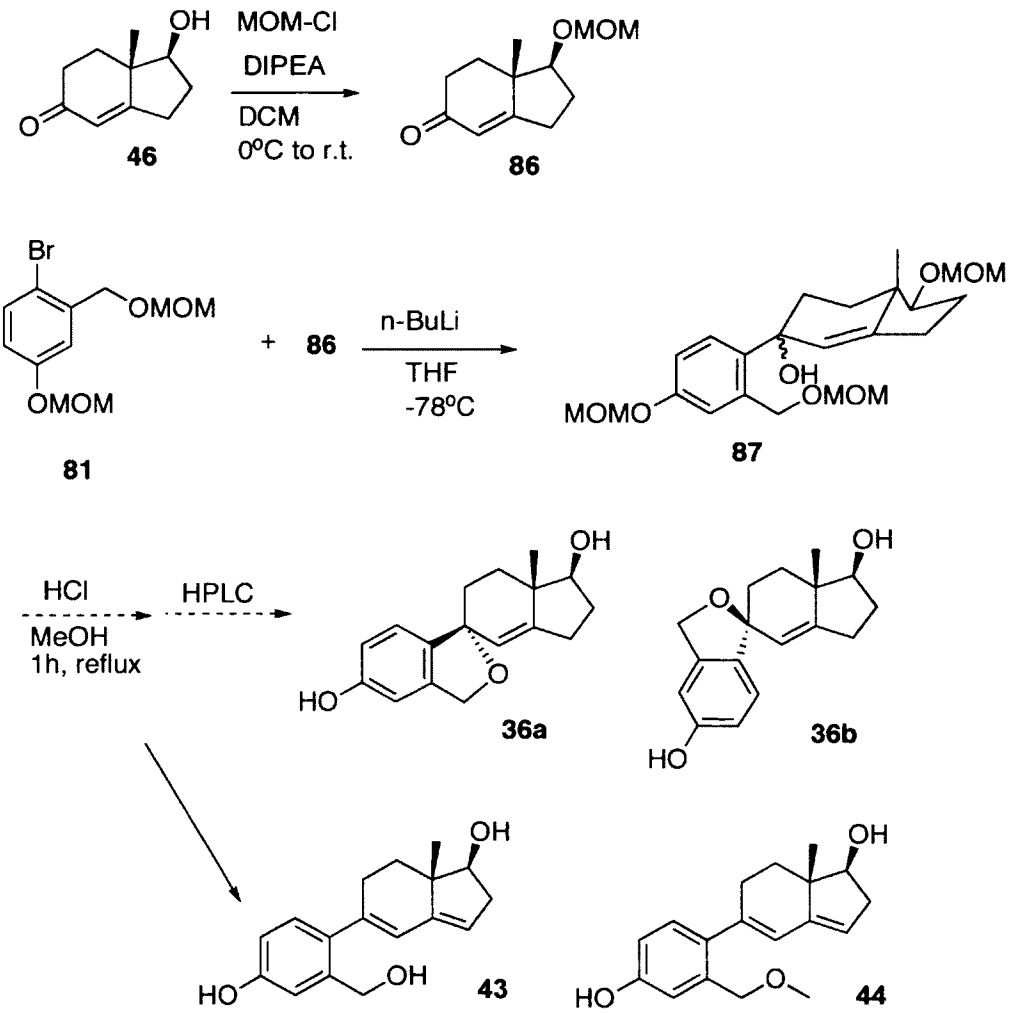
The NMR of compound **35b**, obtained from the previous synthesis, where it was obtained in a small quantity by separation by plate chromatography showed three aromatic protons at 6.91 (d, J = 8.4 Hz, 1H), 6.65 (dd, J = 8.4, 2.4 Hz, 1H), and 6.54 (d, J = 2.4 Hz, 1H) ppm and the phenolic hydroxy proton at 4.94 (s, 1H) ppm. The peak at 4.44 ppm (dd, J = 8.8, 8.8 Hz, 1H) is due to the H17. Its chemical shift indicates the 9(R) "non-natural" stereochemistry in which the spiro ether oxygen is axial to the six membered C ring. The O–CH_2-CH_2 protons of the 6-spiro ring system are present at multiplet centered at 3.85 ppm and 2.81 – 2.68 ppm.

2.11. Synthesis towards the 5-spiro ether with 8-14-unsaturation in ring C

Since the 5-spiro ether compound showed binding to the ERα and ERβ comparable to the parent A-CD derivative and the 6-spiro ether had good ERβ selectivity though weaker binding, the synthesis of another compound with locked conformation between the plane of A-ring and CD- ring was pursued. The synthesis of compound **36a** and **36b** with a double bond between C8 and C14 in the C-ring was expected to be quite straight forward following the above sequence but using the CD ring unsaturated ketone **86** in place of the saturated derivative **56**.

Thus, following the synthesis of the 6-spiro ether it was decided to protect the unsaturated CD-ring **46** with MOM group prior to coupling to A-ring **81.** The MOM protected CD-ring **86** was obtained in 72% yield. The ^{1}H NMR spectrum showed the newly formed $-CH_2$ group of MOM as an AB-system at 4.67 (d, J = 6.8 Hz, 1H) and 4.63 ppm (d, J = 6.4 Hz, 1H) and the MOM $-CH_3$ as singlet at 3.35 ppm and the double bond proton was present as singlet at 5.75 ppm.

The doubly MOM protected **81** was used for the coupling reaction with MOM- protected CD-ring **86**. In the reaction 1.5 equivalent of A-ring and n-BuLi were used per equivalent of **86**. Compound **87** was obtained in 23% yield after some challenging chromatography as an isomeric mixture at C9 as judged by the ^{1}H NMR. The stereochemistry at C9 of the major isomer was assigned as "natural" 9(S), since H17 is a triplet at 3.53 ppm. The minor isomer in the mixture had H17 as triplet at 3.93 ppm was assigned the "non-natural" stereochemistry at C9.



Scheme 2.25. Towards the synthesis of 5-spiro ether **36a** and **36b**.

In the final step, the isomeric mixture of **87** was treated with catalytic amount of acid in methanol under the reflux conditions in order to facilitate cyclization and deprotection reaction to give the expected 5-spiro ethers **36a** and **36b**. Unfortunately, the cyclization product was not obtained, but instead the reaction resulted in uncyclized, doubly conjugated compound **43** and **44**. Compound **44** resulted from the replacement of the benzylic alcohol by the solvent methanol. After extensive purification by column and plate chromatography and HPLC compounds **43** and **44** were obtained in 15.6 and 11.9%

yield, respectively. It could be speculated that the cyclization to the spiro compound did not occur because the additional double bond in ring C favors the loss of a proton to give the stabilized conjugated product **43** compared to formation of the somewhat strained spiro compound.

The ^{1}H NMR of compound **43** showed the two alkene protons at 6.00 (d, J = 2.4 Hz, 1H), and 5.29 ppm (s, 1H). The – CH_2 group at C5 appears as singlet at 4.54 ppm. The H17 is triplet at 3.99 ppm present together with two –OH protons (dd, apparent t, J = 8.4, 8.4 Hz, 2H, H17 &-OH) and 3.93 ppm (br, 1H). The six protons of the CD-ring region are present at 2.61-2.50 (m, 1H), 2.49-2.34 (m, 2H), 2.29 (dd, J = 18.4, 5.2 Hz, 1H), 1.95 (ddd, J = 12.8, 5.2, 1.2 Hz, 1H) and 1.45 ppm (ddd, apparent td, J = 12.4, 5.6, 5.6 Hz, 1H). The quaternary methyl group singlet is present at 1.01 ppm.

The ^{13}C NMR spectrum shows ten aromatic signals at 158.2, 148.2, 142.5, 141.4, 135.2, 130.7, 124.4, 120.3, 116.2 and 115.2 ppm. The six required quaternary carbons, five aromatic and double bond quaternaries at 158.2, 148.2, 142.5, 141.4, and 135.2 ppm and one C-ring quaternary is found at 46.4 ppm. The $–CH_2O$ shows at 63.4 ppm. The three required $–CH_2$ groups show at 39.6, 36.2 ppm and missing $-CH_2$ is hidden in Acetone-d6, based on dept 135 it is present at about 31.2 ppm. The HRMS supports the molecular formula of $C_{17}H_{20}O_3$.

The ^{1}H NMR of compound **44** showed the two alkene protons at 6.01 (d, J = 2.4 Hz, 1H), and 5.32 ppm (s, 1H). The – CH_2 group at C5 appears as singlet at 4.37 ppm. The H17 is a doublet of doublets at 4.09 ppm (J = 8.8, 7.6 Hz, 1H). The $–OCH_3$ group is a singlet at 3.37 ppm. The six protons of the CD-ring region are present at 2.62 (ddd, J = 16.0, 8.0, 3.6 Hz, 1H), 2.56-2.48 (m, 1H), 2.46-2.37 (m, 1H), 2.30 (dd, J = 18.8, 4.8 Hz, 1H), 1.97

(dd, J = 12.4, 4.4 Hz, 1H) and 1.52 ppm (ddd, apparent td, J = 12.4, 5.6, 5.6 Hz, 1H). The quaternary methyl group singlet is present at 1.04 ppm.

The ^{13}C NMR spectrum shows ten aromatic signals at 154.8, 145.7, 139.2, 136.4, 135.2, 129.4, 122.8, 118.7, 115.2 and 114.5 ppm. The six required quaternary carbons, five aromatic and double bond quaternaries at 154.8, 145.7, 139.2, 136.4 and 135.2 ppm and one C-ring quaternary is found at 44.5 ppm. The $-CH_2O$ shows at 72.2 ppm. The three required $-CH_2$ groups of the CD-ring show at 38.0, 34.1 and 29.2 ppm. The HRMS supports the molecular formula of $C_{18}H_{22}O_3$.

2.12. Biological data of the spiro derivatives

The preferred conformation of the free A-CD ligand is one in which the A ring is essentially orthogonal to the plane of CD ring. The objective of synthesizing 5-spiro ether analogue was to check the locked conformation at 90° and the effect which it had on binding to the ERs.

As described previously docking studies indicated that the A-CD ligands when bound inside of the ER binding pocket adopt a conformation in which the A ring is 21 +/-5 ° angle to plane of the CD ring. **[83]** The conformation of the 6-spiro ether **35a** is one in which the dihedral angle can vary between 90-60 °. Therefore one might have predicted that the 6-spiro ether should bind more strongly to the ERs than 5-spiro ether because it has more flexibility and is closer to the calculated preferred angle for binding of 21 +/-5 °.

However, the RBA results were opposite to this prediction, (**table 2.5.**).

Thus, compound **34a** with its orthogonal conformation between the A and CD ring planes binds to the ER with moderate affinity and ERβ selectivity and its RBA results are

comparable to the parent A-CD compound **20**, in which the A-ring due to free rotation can adopt the calculated "preferred" conformation of 21 +/-5 °. The RBA results for the 6-spiro ether **35a** were ERα = 0.31 and ERβ = 3.5, which corresponds to ERβ/ ERα = 11.3. Thus, compound **35a** binds to the ER with decreased affinity compared with its 5-spiro derivative **34a**, and somewhat increased ERβ vs. ERα selectivity. The RTA values of 6-spiro ether were ERα = 2.3 and ERβ = 56.5 with ERβ/ ERα = 24, which shows that this compound is a moderately strong, selective, ERβ agonist.

Based on these results it can be concluded, that the ER receptors can accommodate molecules with distinctly different shapes, for example estradiol (almost planar), the A-CD ligands (variable conformations), the 6-spiro ether with dihedral angle ranging from 90-60 ° and the 5-spiro ether (fixed at 90 °). This agrees with the conclusion by Robert W. Hsieh in an earlier study. **[109]**

Table 2.5. Relative Binding Affinity (RBA) and Relative Transcription Activation (RTA) to ERα and ERβ and the ratio of selectivity of 5 and 6- spiro ether derivatives.

Compound #	name	RBA			RTA		
		ERα	ERβ	ERβ/ ERα	ERα	ERβ	ERβ/ ERα
1	estradiol	100	100	1	100	100	1
20	Parent A-CD	1.5	21.5	14	4.6	158	44
34a	5-spiro ether	2.5	10.7	4.3	-	-	-
35a	6-spiro ether	0.31	3.5	11.3	2.3	56.5	24

III. Compounds with unsaturation in the C and or D rings for example compounds 37-44

The preferred conformation of the A-CD compounds such as **20** and derivatives carrying substituents at C5 of the aromatic ring is one in which the plane of the aromatic ring A and that made by the CD rings are essentially at 90° to each other. Docking studies indicate that binding to the estrogen receptors necessitates rotation around the bond joining the aromatic ring to ring C so that these planes take an angle of approximately 21 +/-5°. The change from the preferred 90° in the free ligand to about 20° in the bound state is energetically costly especially for compounds that have large substituents at C5 since there is considerable steric interaction between the C8 CH_2 group in ring C and the large C5 substituent.

Nevertheless, compounds such as the 5 -CF_3, 5 -Cl, and 5 -F bind more strongly to the ERβ receptor than does estradiol. The binding of these compounds to the ERα receptor is slightly less than that observed for estradiol.

It seemed reasonable that the barrier to rotation in compounds with an unsaturation between C9 and C8 should be lower than that of the saturated analogs since one of the offending hydrogens on C8 has been removed. Additionally the conjugation between the C9-C8 double bond and the aromatic ring A should help to reduce the energy of the more planar conformations. Thus, such compounds might be expected to bind more strongly to the estrogen receptors than the parent saturated compound.

Compounds such as **38, 40,** and **42-44** with an additional conjugated double bond were expected to show similar binding trends. Such compounds are readily accessible by coupling the available MOM or TBDMS protected A-ring components with the unsaturated MOM protected CD ring enone **86**. The coupling products would be expected to be converted into the doubly unsaturated derivative upon acid-catalyzed dehydration with concomitant deprotection.

Three compounds were chosen to determine the effect of introducing a second double bond into the CD rings. These were the parent compound, **38**, and the 5-Cl, **40**, and 5-CF_3, **42**, analogs. Also available in this series, from our attempts to prepare the unsaturated spiro compounds, were the derivatives **43** and **44**.

The other targets in this series were compounds **37**, **39** and **41**. These compounds have the natural (S) configuration at C9 but also have a double bond between C8 and C14. Again we chose to prepare the parent derivative **37** and 5-Cl, **39**, and 5- CF_3, **41**, analogs since these substituents at C5 greatly increase the RBAs relative to the parent compound. It was expected that these derivatives would have comparable binding to their parent saturated analogs.

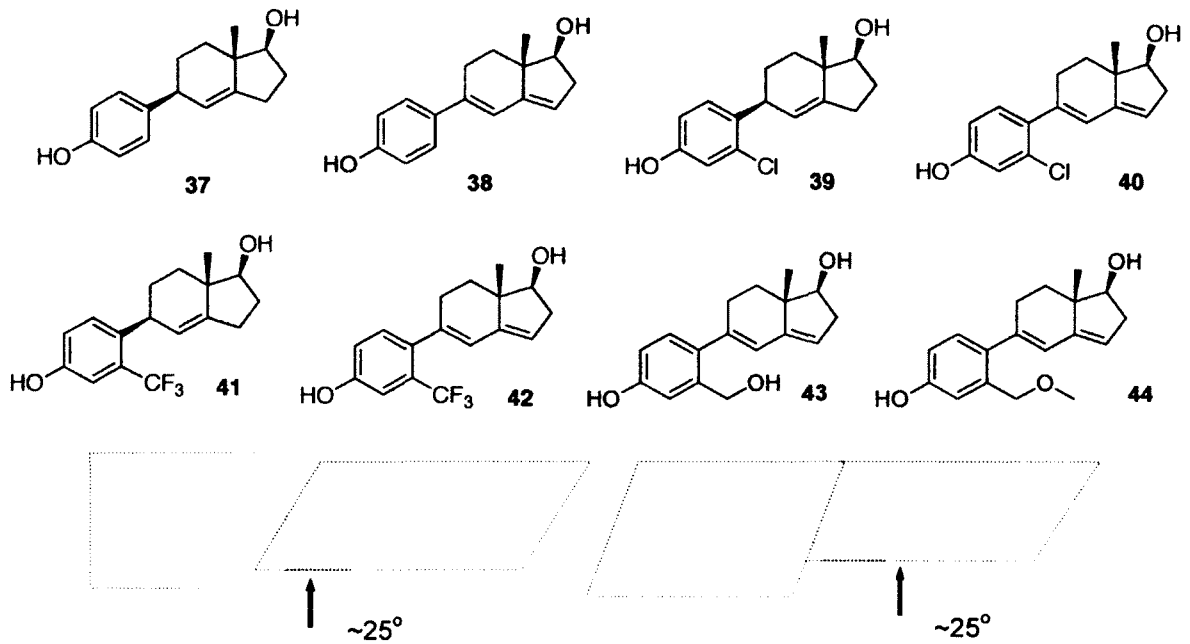


Figure 2.8. The angle between the A- and CD-ring planes in the unsaturated derivatives.

2.13. Synthesis of compound 37 and 38

The typical synthesis of these compounds is straightforward and involves coupling a suitably protected 4-bromophenol via its lithiated derivative to either the unsaturated CD ring **46** or protected CD-ring **86**. The product thus obtained is then reduced with triethyl silane (Et_3SiH) in the presence of $BF_3{\cdot}Et_2O$ to obtain **37** and treated with acid to obtain **38**.

The synthesis of compound **37**, the parent monounsaturated compound in the series, (**scheme 2.26.**) starts with protection of para-bromophenol **88** with TBDMS-Cl in the presence of imidazole in DMF at 0°C to obtain TBDMS protected A-ring **89** in 94% yield.

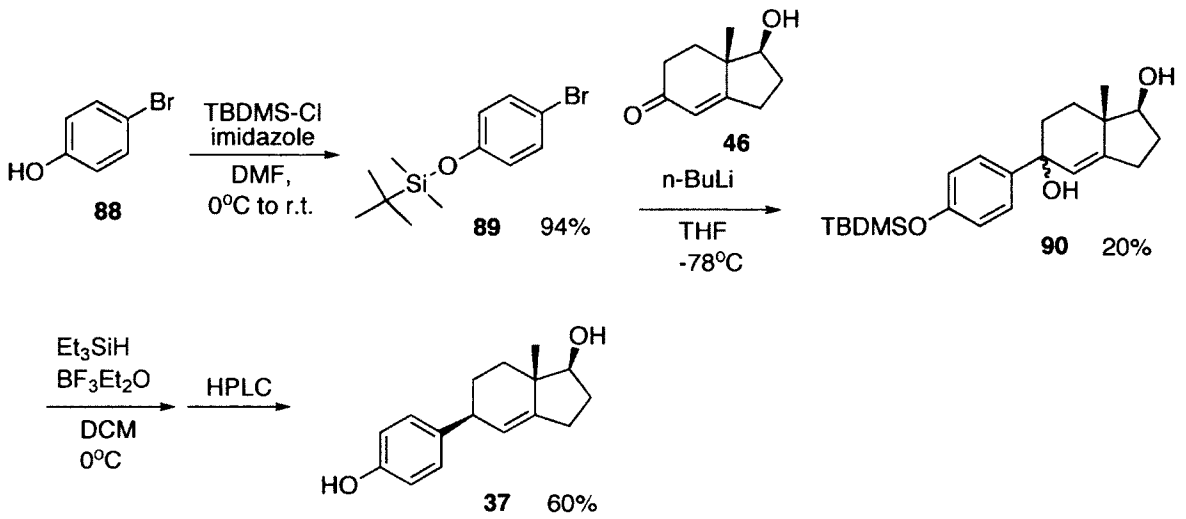
The coupling of the TBDMS-protected **89** and CD-ring **46** was carried out in the usual manner. In the present case, due to ready available of large quantity of compound **89**, 4 equivalents each of **89** and n-BuLi were used per equivalent of unsaturated CD-ring, **46**. The coupling product **90** was obtained as a mixture of isomers. The complete separation of the isomers was not achieved due to very low polarity of the material and it was not necessary for the next synthetic step. The estimated yield of product **90** was 20% based on recovered starting material **46**.

Finally, compound **90** was reduced with Et_3SiH in the presence of $BF_3.Et_2O$ to obtain after column chromatography and purification by HPLC the desired compound **37** in 60% yield. The ^1H NMR of **37** showed four aromatic protons at 7.00 (d, J = 8.4 Hz, 2H) and 6.75 ppm (d, J = 8.8, 2H). The alkene proton occurred as a singlet at 5.27 ppm and H17 as doublet of doublets at 3.64 ppm (dd, J= 10.0, 8.0 Hz, 1H). The benzylic allylic proton was identified by 2D NMR techniques as a multiplet at 3.26-3.21 ppm. The eight protons of the CD-ring region were present as upfield multiplets; the quaternary metyl group singlet resonated at 1.03 ppm.

The ^{13}C NMR spectrum shows six aromatic signals at 157.4, 147.6, 139.6, 130.0, 124.8 and 116.9 ppm. The alkene –CH carbon is present at 124.8 ppm. The four required $-CH_2$ groups of CD-ring region show at 37.2, 32.0, 30.8 and 27.4 ppm. The HRMS supports the molecular formula $C_{16}H_{20}O_2$.

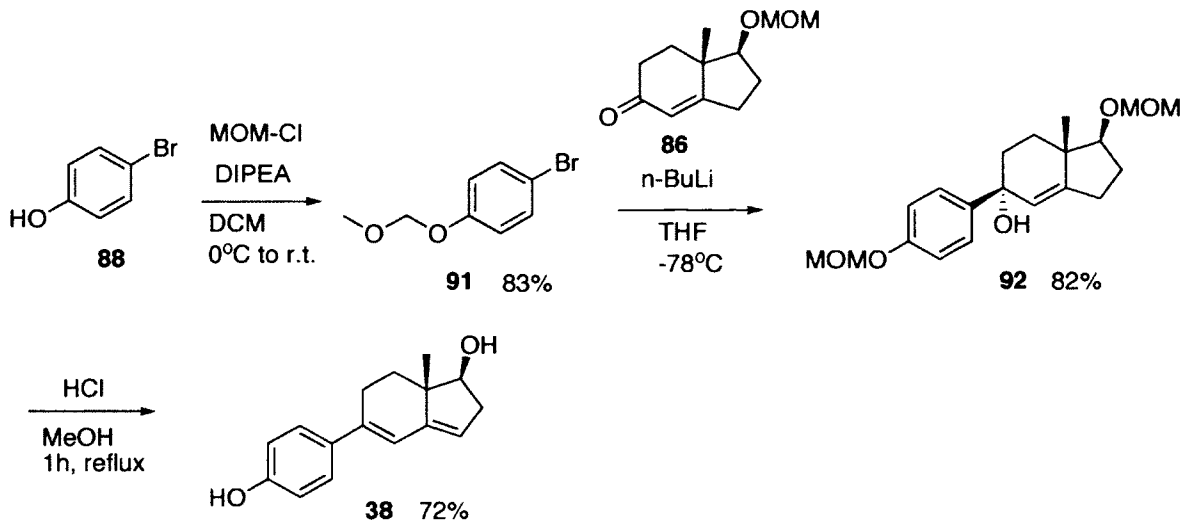
The equatorial position of the A-ring was determined by nOe measurement. Irradiation of the benzyllic allylic proton did not show an enhancement of the signal to the methyl group of the CD-ring, which is consistent with this proton being on the opposite side of the CD-ring plane and the A-ring being in the equatorial position. There was a positive

signal observed to the protons of the A-ring, CD-ring and to the alkene proton, which is consistent with the A-ring being in the equatorial position. The irradiation of the methyl group showed no significant enhancements of any signal in the NMR. The formation of **37** as the major reduction product is reasonable from a mechanistic point of view. The delivery of hydride from the triethylsilane to the intermediate carbocation formed from **90** via the action of the Lewis acid is, for steric reasons, is much more likely to occur from the side opposite to the methyl group thus leading to the observed stereochemistry. Similar stereochemical results were found for the other compounds generated in this series.



Scheme 2.26. Synthesis of compound **37**.

The doubly unsaturated compound **38** was obtained by a similar synthesis. The A-ring was protected with MOM and coupled to the MOM protected CD-ring. The crude coupling product **92** was dehydrated and deprotected in the presence of acid to yield **38**. (**scheme 2.27.**).



Scheme 2.27. Synthesis of compound **38**.

The NMR spectra of the intermediates were fully consistent with the assigned structures. The data is recorded in the experimental section. The structure of the diene **38** was supported by the HRMS and the NMR spectra. In particular, the ^{13}C NMR showed the required eight sp_2 carbons, four of which are quaternary. Key peaks in the proton spectrum were the two AB aromatic patterns, each one integrating for 2H and two 1H alkene Hs at 6.52 (d, J = 1.6 Hz, 1H) and 5.36 ppm (s, 1H). The required H17 absorbed at 3.98 ppm and the quaternary methyl group at 0.97 ppm.

2.14. Synthesis of compound 39 and 40

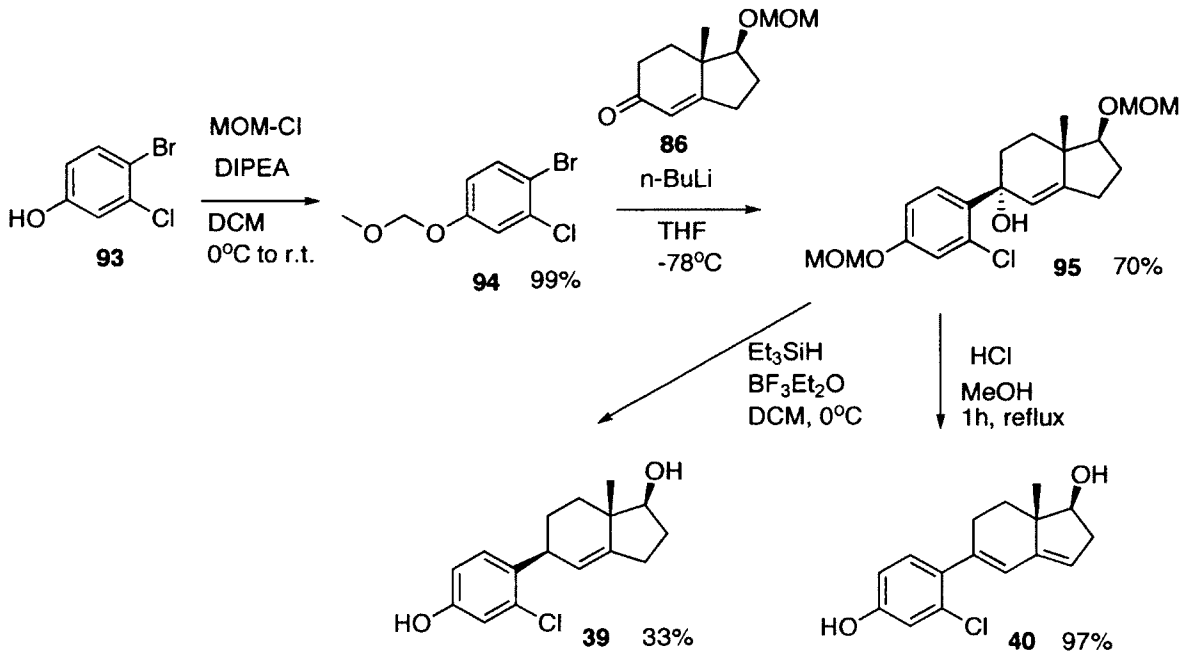
The synthesis of compound **39** and **40** was carried out in similar fashion, except that the process was simplified and the intermediate **95** was used for the synthesis of both products **39** and **40**, (**scheme 2.28.**).

MOM-protected **94** was coupled to the CD ring moiety **86**, in an estimated yield of 70% based on ^{1}H NMR of the crude reaction product.

Reduction of the crude product **95** with Et_3SiH in the presence of $BF_3{\cdot}Et_2O$ afforded **39** in 33% yield after column chromatography. The product was recrystallized and purified further by plate chromatography in order to obtain a white solid whose spectroscopic properties confirmed the expected structure. The ^{1}H NMR of **39** showed three aromatic protons at 7.05 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 2.8 Hz, 1H) and 6.77 (dd, J = 8.4, 2.8 Hz, 1H) ppm. The alkene proton occurred as a singlet at 5.22 ppm. The H17 appeared as triplet at 3.65 ppm. The peak at 3.73-3.68 ppm was identified by 2D NMR techniques as the benzylic allylic proton. The remaining eight protons of the CD-ring region were present as multiplets at 2.57-2.47 (m, 1H), 2.20-2.11 (m, 1H), 2.02-1.81 (m, 3H), 1.73-1.63 (m, 1H) and 1.47-1.28 (m, 2H) ppm. The quaternary methyl group occurred as a singlet at 1.02 ppm. The HRMS supports the molecular formula $C_{16}H_{19}ClO_2$.

The doubly unsaturated compound **40** was obtained from crude product **95** in 97% yield via treatment with acid, (**scheme 2.28.**).

The ^{1}H NMR spectrum of **40** showed the two alkene protons at 6.07 (d, J = 2.4 Hz, 1H) and 5.36 ppm (s, 1H). The H17 was a triplet at 4.00 ppm (t, J = 8.4, 1H), which overlapped with the broad of the –OH proton. The six protons of the CD-ring region were present at 2.69-2.58 (m, 1H), 2.50-2.32 (m, 3H), 1.98-1.94 (m, 1H) and 1.45 ppm (ddd, apparent td, J = 12.4, 12.4, 5.2 Hz, 1H). The quaternary methyl group showed as singlet at 1.02 ppm. The HRMS supports the molecular formula $C_{16}H_{17}ClO_2$.



Scheme 2.28. Synthesis of compound **39** and **40**.

2.15. Synthesis of compound 41 and 42

The synthesis of these compounds carrying the electron-withdrawing $-CF_3$ group at C5 was approached in the same simplified way as the synthesis of C5-Cl analog (**scheme 2.29**).

Commercially available compound **96** was brominated in glacial acetic acid to obtain compound **97** in 57% yield. The procedure was taken from Christine Choueiri who was the first one to obtain compound **97** in our lab. **[110]** The ^1H NMR showed the tri-substituted A-ring with the aromatic protons present at 7.57 (dd, J = 8.8, 0.4 Hz, 1H), 7.21 (d, J = 3.2 Hz, 1H) and 6.91 ppm (dd, J = 8.8, 2.8 Hz, 1H) and the methoxy group singlet at 3.83 ppm.

Compound **97** was de-methylated with BBr_3 in toluene to obtain compound **98** in 80% yield **[111]** and then protected with MOM-Cl to obtain **99** in 83% yield.

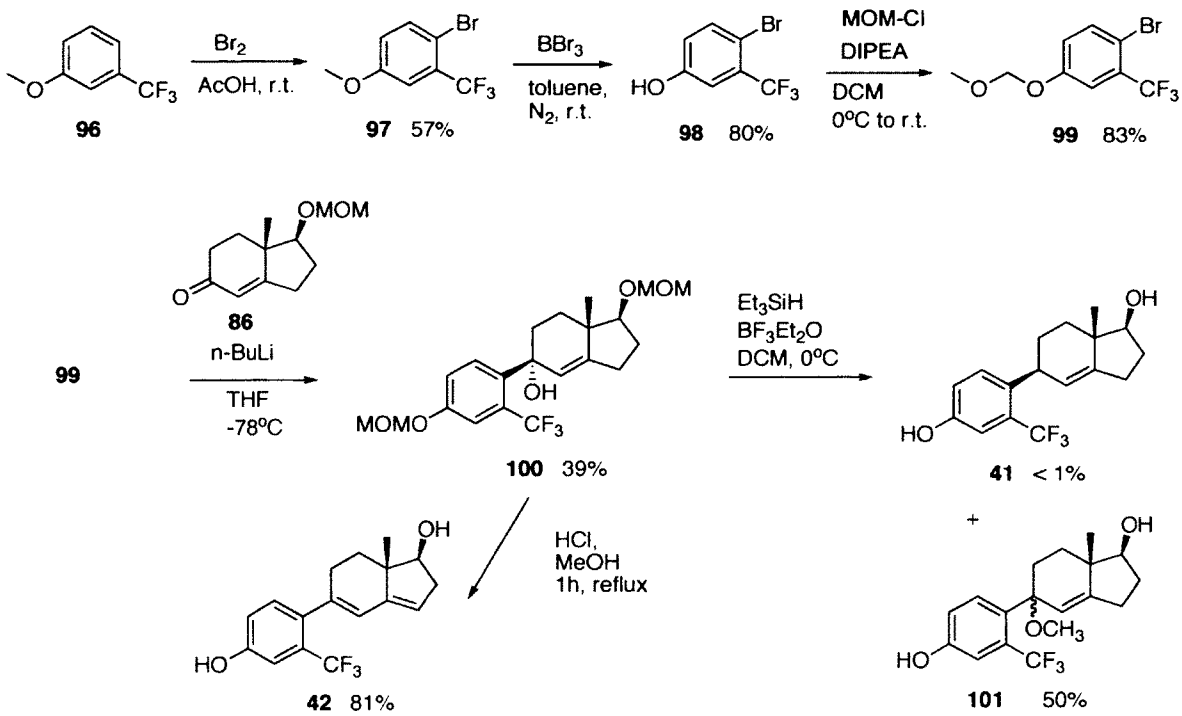
The MOM-protected **99** was coupled to the CD ring **86** to give the coupling product **100** in 39% yield, 60% based on recovered starting material, after purification by column chromatography.

Compound **100** was reduced with Et_3SiH in the presence of $BF_3{}^{\bullet}Et_2O$ to obtain **41** after column chromatography in less than 1% yield; the major isolated reaction product, obtained in 50% yield was the 9-methoxy derivative **101.**

The ^1H NMR of **41** showed three aromatic protons at 7.14 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H) and 7.03 (ddd, J = 8.4, 2.8, 0.4 Hz, 1H) ppm. The alkene proton showed as a doublet at 5.45 ppm (J = 0.8 Hz, 1H). The H17 appeared as triplet at 3.75 ppm (J = 7.6 Hz, 1H). The HRMS supports the molecular formula $C_{17}H_{19}F_3O_2$.

Treatment of **100** with acid in methanol led to the formation of the diene **42** in 81% isolated yield, (**scheme 2.29.**).

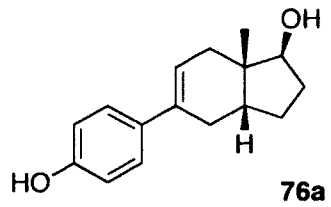
The ^1H NMR spectrum of **42** showed, in addition to the expected aromatic protons at 7.16 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 2.8 Hz, 1H) and 7.05 ppm (dd, J = 8.4, 2.8 Hz, 1H) and CD ring methylene protons at 2.62-2.37 (m, 3H), 2.25 (dd, J = 18.4, 5.6 Hz, 1H), 1.97 (dd, J = 12.4, 4.4 Hz, 1H) and 1.46 (ddd, J = 12.4, 12,4, 5.6 Hz, 1H) ppm. The two alkene protons were present at 6.00 (d, J = 2.0 Hz, 1H) and 5.34 (s, 1H) ppm. The H17 was a triplet at 4.02 (J = 8.0 Hz, 1H) ppm, and the quaternary methyl group showed as singlet at 1.02 (3H) ppm. The ^{13}C NMR also supported the assigned structure. The HRMS gave the molecular formula as the required $C_{17}H_{17}F_3O_2$.



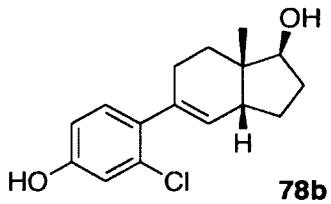
Scheme 2.29. Synthesis of compound **41** and **42**.

2.16. Biological data of unsaturated compounds

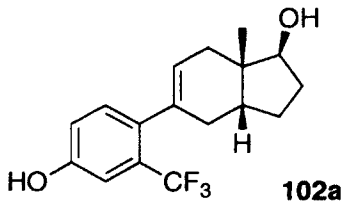
Table 2.5 shows the RBAs for the compounds prepared in this series in addition to some related structures. Generally, the results were disappointing. We had anticipated in particular that the dienes **38** and **42** might bind more strongly than the parent saturated compounds **20**, **26**, **103**. In each case the relative binding compared to the saturated analogs was reduced considerably. This was especially true for the 5- CF_3 compounds **41** and **42**. Also, the ERβ selectivity is lower in these compounds compared to the saturated derivatives; indeed the 5 CF_3 diene **42** is somewhat ERα selective.



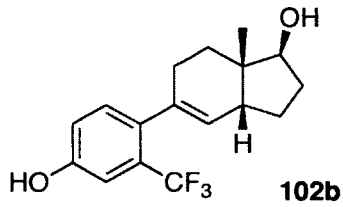
76a



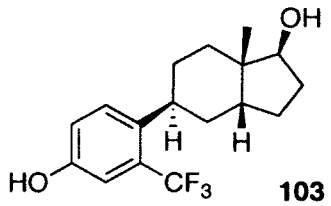
78b



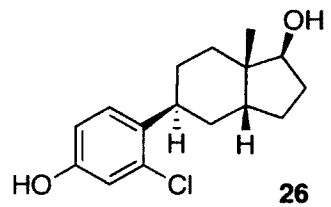
102a



102b



103



26

Table 2.6. Relative Binding Affinity (RBA) to ERα and ERβ and the ratio of selectivity of selected C5-substitutied saturated and unsaturated A-CD compounds.

Compound #	R_5	RBA		
		ERα	ERβ	ERβ/ ERα
1	estradiol	100	100	1
20	Parent A-CD	1.5	21.5	14
38	H	3.3	15.9	4.8
39	Cl	11.9	15.1	1.3
41	CF_3	43	49	1.1
42	CF_3	7.3	4.8	0.66
76a	H	60	120	2.0
78b	Cl	195	331	1.7
102a	CF_3	122	174	1.4
102b	CF_3	189	201	1.1
103	CF_3	90	203	2.3
26	Cl	49	168	3.4

We had expected better binding for the dienes because docking calculations performed by Drs. Wright and Shadina led us to believe that these compounds would more readily adopt the almost planar conformation than their saturated analogs. Both conjugation of the double bonds and removal of a hydrogen at C8 should lower the energy required to approach the desirable 20 degree dihedral angle for these compounds relative to their saturated analogs. The low binding affinity of the dienes is even more surprising

considering the results for the analogs **39** and **41** which have a single conjugated double bond in ring C.

Based on similar conjugation and steric interaction arguments we anticipated that the C8-C14 unsaturated compounds **39** and **41** might have stronger binding than their fully saturated analogs. Again this was not the case. At this time we are unable to suggest a plausible reason for the low estrogen receptor binding affinity of these compounds.

IV. Compounds where the phenolic A ring was replaced by a 1,2-pyrazole

Merck researchers have reported the use of the fused pyrazole as an isostere of phenol **[89]**. In A-CD analogues, the use of pyrazole would prevent carcinogenic quinone formation. Compounds **45a** and **45b** were synthesized even though we realized that distance between the C17 hydroxyl and the H of a pyrazole is shorter than the optimal distance for binding of 11 +/- 0.5 Å. Those compounds were relatively easy to synthesize and we wanted to check if they were able to bind to the ERs. If compounds **45a** and **45b** showed some activity, other analogues such as **104** with the longer interatomic distance between C17 hydroxyl and the pyrazole H could be synthesized. One example could include a $-CH_2$ or S or O as a spacer in between the A and the CD-ring (**figure 2.9.**)

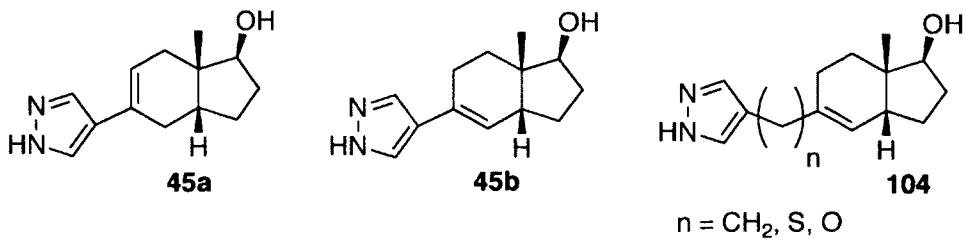


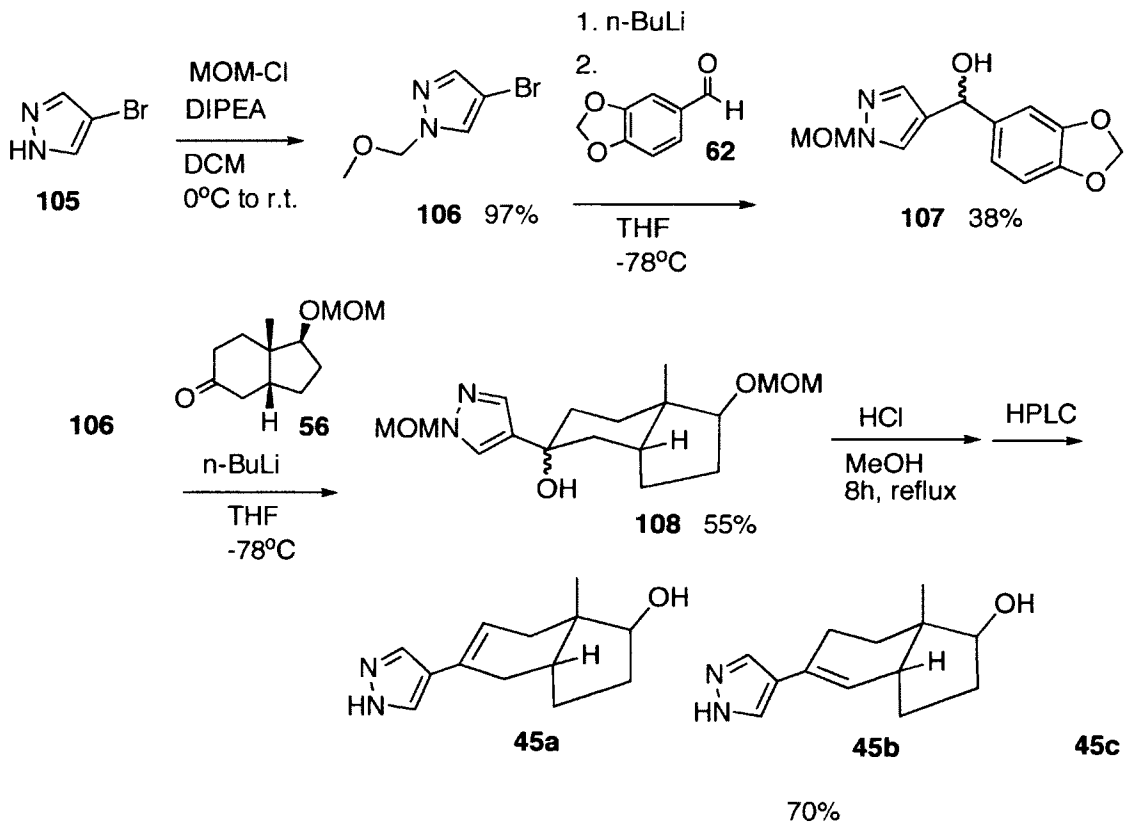
Figure 2.9. Compounds with pyrazole A-ring **45a** and **45b** and **104**.

2.17. Synthesis of compounds 45a and 45b

The synthesis of the pyrazole compound is straight forward and follows a similar strategy as the synthesis of previous unsaturated compounds (**scheme 2.30.**).

It involved protection with MOM group of the 4-bromo-1*H*-pyrazole **105** to obtain MOM-protected pyrazole **106** in 97% yield. The newly added MOM group showed in the ^{1}H NMR spectrum as two singlets at 5.26 (2H) and 3.23 (3H) ppm.

We decided that we would couple the A-ring **106** with piperonal **62** first prior to coupling it to the MOM-protected CD-ring in order to mitigate any difficulties in the lithiation of compound **106**. In the reaction, 1.5 equivalents each of **106** and n-BuLi were used per equivalent of piperonal **62**. The coupling product **107** was obtained in 38% yield and the ^{1}H NMR was consistent with the assigned structure.



Scheme 2.30. The synthesis of compounds **45a** and **45b**.

The MOM-protected **106** was coupled to the CD ring **56**. Because both A-ring and CD-ring were MOM protected, 1.5 equivalents each of **106** and n-BuLi were used per equivalent of CD-ring **56**. The coupling product **108** was obtained in 55% yield. The two isomers were separated by column chromatography to obtain **108a** in 37% and **108b** in 18% yield. The stereochemistry at C9 was not assigned because it was lost during the next synthetic step.

The mixture of isomers **108a** and **108b** was dehydrated and deprotected in the presence of acid to obtain three isomers **45a**, **45b** and **45c** in 5%, 12% and 27% yield, respectively. The position of the double bond could not be assigned with confidence based on the hetero Diels-Alder fragmentation in the MS. Key peaks in the ^{1}H NMR for the three isomers are summarized in **table 2.6.**. One of the differences in the ^{1}H NMR is the position of the methyl group in **45a** at 0.76 ppm, more upfield than in the other two isomers **45b** and **45c**, where it shows at 1.00 and 0.96 ppm respectively.

Compound **45b** was sent for RBA and RTA testing and if it showed good binding to the ERs and agonist activity we would invest more time in complete structure determination. Unfortunately, but not surprisingly because of the < 11 Å distance between C17 hydroxyl and the pyrazole H, the RBA values were low: ERα = 0.04 and ERβ = 0.06. The RTA values of **45b** were ERα =16.4 and ERβ = 2.6 which showed that this compound a stronger ERα agonist than ERβ agonist. However, because the RBA values were very low, no more time was invested in the structure determination of **45a**, **45b** and **45c** and the synthesis of analogues such as in **104** was pursued by other group members.

Table 2.7. Comparison of the ^{1}H NMR data of three isomers obtained **45a**, **45b** and **45c.**

Compound	Pyrazole Hs (ppm)	alkene H (ppm)	H17 (ppm)	Methyl group (ppm)
45a	7.61 (s)	5.97-5.96 (m)	3.74 (t, J = 8.8 Hz)	0.76 (s)
45b	7.61 (s)	5.92 (d, J = 0.8 Hz)	3.71 (dd, J = 6.8, 2.4 Hz)	1.00 (s)
45c	7.60 (s)	5.95 (d, J = 4.0 Hz)	3.80 (t, J = 6.0 Hz)	0.96 (s)

Claims to original work

The synthesis of the following A-CD compounds was completed

- C5-vinyl A-CD unsaturated
- two A-CD unsaturated analogues with sulfur resulting from the benzyl mercaptan addition to the C5-vinyl derivative
- 5-spiro ether and 6-spiroether with their isomers
- C5- H, Cl, CF_3 with 8-14-unsaturation in ring C
- C5- H, Cl, CF_3 with unsaturation in the C and D rings
- A-CD analogue with the pyrazole A-ring

The synthesis of the following A-CD compounds was attempted

- 5-dimethylamino
- 5-spiro lactone leading to the C5 methyl ester
- 5-spiro ether with 8-14-unsaturation in ring C

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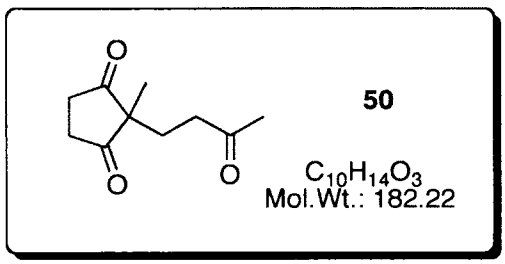
Chapter 3. Experimental

The CD-ring (Hajos-Parrish ketone) was prepared in enantiomerically pure form following published Hajos-Parrish ketone procedures. **[91-94]**

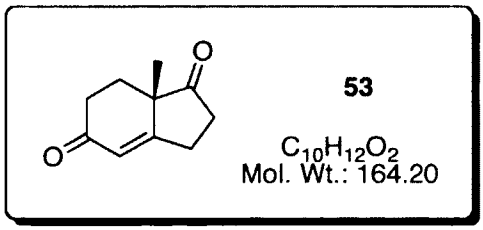
2-Methyl-2-(3-oxobutyl)cyclopentane-1,3-dione



A suspension of the 2-methylcyclopentane-1,3-dione **48** (12.5 g, 0.111 mol) and methyl vinyl ketone **49** (15.6 g, 0.223 mol) was stirred in 150 mL of distilled water for 5 days. The reaction mixture was saturated with NaCl and extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered through filter paper and concentrated *in vacuo*. The crude product (27.2 g) was used directly in the next step. The pure product obtained upon chromatography from the crude reaction product of an earlier synthesis gave an NMR spectrum consistent with that obtained earlier in our group by Dr. Muhammad Asim.

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 2.89-2.67 (m, 4H), 2.44 (t, J = 7.2, 2H), 2.08 (s, 3H), 1.87 (t, J = 7.2, 2H), 1.09 (s, 3H)

(*S*)-7a-Methyl-2,3,7,7a-tetrahydro-1*H*-indene-1,5(6*H*)-dione

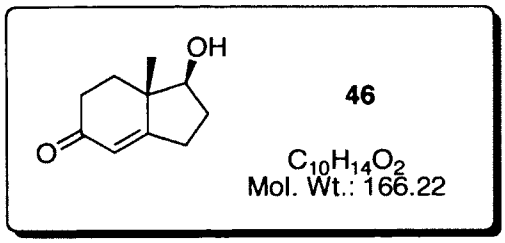


A suspension of S-(-) proline (1.39 g, 13.0 mmol) in DMF (25mL) was stirred under a nitrogen atmosphere at room temperature for 10 minutes, after which time the crude product **50** (27.2 g) from the previous experiment dissolved in DMF (125 mL) was added. The reaction mixture was then protected from the light with aluminum foil and stirred for 3 days. The DMF was distilled off under high vacuum and the crude product, a black oil, was passed through a pad of silica gel to obtain a brown-yellow oil. The NMR of this material indicated a mixture of the initially formed β-hydroxy ketone and compound **53**.

In order to ensure the completion of the dehydration, the semi-purified material was dissolved in toluene (100 mL) containing a catalytic amount of *p*-toluenesulfonic acid (pTSA). The reaction was stirred at room temperature for two days, after which the solvent was evaporated. The crude product was purified by column chromatography to obtain 17.31 g of the pure product, brown oil, 95% yield over 2 steps. The ^{1}H NMR of this material was consistent with the one obtained earlier in our group by Dr. Muhammad Asim.

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 5.92 (d, J = 2.4, 1H), 2.94-2.87 (m, 1H), 2.79-2.67 (m, 2H), 2.53-2.34 (m, 3H), 2.10 (ddd, J = 13.6, 4.8, 2.4, 1H), 1.80 (ddd, J = 13.6, 13.6, 5.6 Hz, 1H), 1.27 (s, 3H)

(1*S*,7a*S*)-1-Hydroxy-7a-methyl-2,3,7,7a-tetrahydro-1*H*-inden-5(6*H*)-one



46

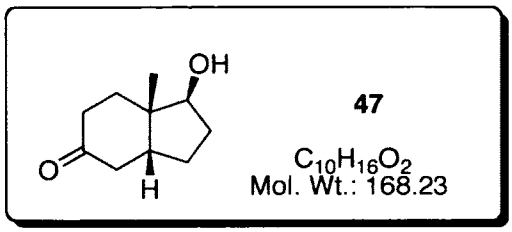
$C_{10}H_{14}O_2$
Mol. Wt.: 166.22

This procedure was adapted from Dale E. Ward **[101]**. Compound **53** (1.0 g, 6.1 mmol) was dissolved in a 50:50 solvent mixture of methanol and DCM. The reaction mixture was cooled in a dry ice/acetone bath to -78° C and then $NaBH_4$ (80 mg, 2.1 mmol) was added. The reaction mixture was stirred at -78° C for one hour, quenched with acetone (2 mL), and was allowed to warm to the room temperature. The reaction mixture was diluted with 1M NaOH (30 mL) and then extracted with DCM (3 x 30 mL). The organic extracts were combined, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by recrystallization or by rapid column chromatography. The final product (0.58 g, 57% yield), was obtained as beige semi-solid, which solidified upon standing.

^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) = 5.76 (s, 1H), 3.82 (dd, J = 10.4, 7.6 Hz, 1H), 2.68 (dddd, J = 19.6, 11.6, 2.0, 2.0 Hz, 1H), 2.55-2.32 (m, 4H), 2.15-2.06 (m, 2H), 1.86-1.72 (m, 2H), 1.12 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 199.4, 175.4, 123.3, 80.4, 45.1, 34.0, 33.2, 29.0, 26.4, 15.0

(1*S*,3a*R*,7a*S*)-1-hydroxy-7a-methylhexahydro-1*H*-inden-5(6*H*)-one



47

$C_{10}H_{16}O_2$
Mol. Wt.: 168.23

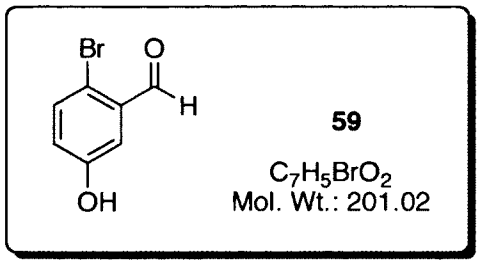
Compound **46** (0.580 g, 3.49 mmol) was dissolved in a mixture of methanol (10 mL), 10% HCl (0.16 mL) and acetic acid (3 mL), to which a pinch of 10% Pd/C was added. The reaction was stirred overnight under an atmosphere of hydrogen gas at room temperature and then filtered through a small column of celite with several EtOAc washings. The EtOAc filtrate was diluted with brine and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried with $MgSO_4$, filtered through a filter paper and concentrated *in vacuo*. The product was a beige semi-solid (0.506 g, 86% yield).

The NMR of material purified by column chromatography matched the one obtained earlier in our group by Dr. Muhammad Asim.

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 3.84 (dd, J = 6.4, 4.4 Hz, 1H), 2.48-2.37 (m, 2H), 2.29-2.21 (m, 3H), 2.17-2.08 (m, 1H), 2.98-2.89 (m, 1H), 2.65-2.82 (m, 3H), 1.29-1.20 (m, 1H), 1.17 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 212.9, 79.9, 43.8, 43.2, 41.9, 36.8, 32.0, 32.0, 28.3, 19.3

2-Bromo-5-hydroxy-benzaldehyde

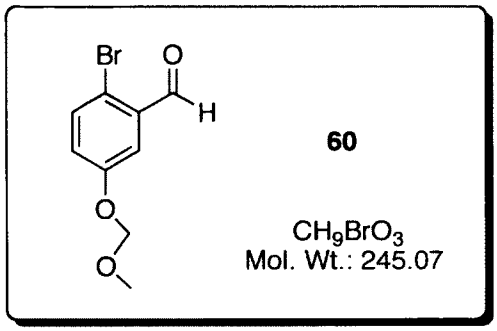


Bromine (8.42 mL, 26.2 g, 163.9 mmol) was added to a solution of 3-hydroxybenzaldehyde (20.0 g, 163.9 mmol) in CCl_4 (200 mL) under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. After the reaction was completed water (250 mL) and DCM (250 mL) were added. The aqueous layer was washed with DCM (100 mL) and then with EtOAc (3 x 100 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product, a white-pink solid, was purified by recrystallization with EtOAc and DCM to give white crystals of **59** (18.9 g, 58% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 10.29 (s, 1H), 7.52 (d, J= 8.4 Hz, 1H), 7.41 (d, J= 3.2 Hz, 1H), 7.01 (dd, J= 8.4, 3.2 Hz, 1H), 5.35 (s, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 191.8, 155.4, 134.9, 134.0, 123.2, 117.7, 115.6

2-Bromo-5-(methoxymethoxy)benzaldehyde

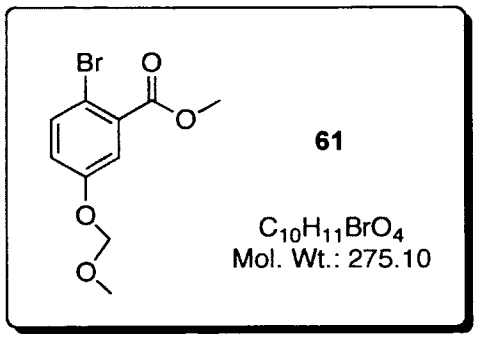


N,N-diisopropylethylamine (8.66 mL, 49.7 mmol) and chloromethyl methyl ether (3.77 mL, 49.7 mmol,) were added to a solution of compound **59** (5.00 g, 24.9 mmol) in 30 mL of dry DCM under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then left at room temperature overnight. The organic mixture was diluted with 10% aqueous NaOH (30 mL) and extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product **60** was yellow oil, 5.93 g, (97%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 10.23 (s, 1H), 7.51 (d, J= 3.2 Hz, 1H), 7.49 (d, J= 8.8 Hz, 1H), 7.11 (dd, J= 8.8, 3.2, 1H), 5.15 (s, 2H), 3.42 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 191.3, 156.7, 134.4, 133.9, 123.8, 118.6, 116. 2, 94.2, 56.0

Methyl 2-bromo-5-(methoxymethoxy)benzoate

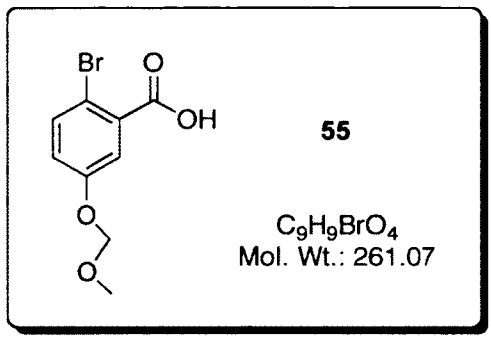


Compound **60** (1.00 g, 4.08 mmol) was dissolved in methanol, to which acetic acid (0.374 mL, 6.53 mmol), KCN (1.41 g, 21.6 mmol) and MnO_2 (7.45 g, 85.7 mmol) were added. The reaction was stirred overnight at room temperature and after completion the methanol was removed by rotary evaporation, then water (30 mL) and ether (30 mL) were added to the reaction mixture. The resulting mixture was then extracted with ether (3 x 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. **[106]** The product **61** was a brown oil (1.04 g, 92.6% yield).

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.44 (d, J = 8.8 Hz, 1H), 7.38 (d, J = 3.2 Hz, 1H), 6.94 (dd, J = 8.8, 2.8 Hz, 1H), 5.08 (s, 2H), 3.83 (s, 3H), 3.37 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 165.9, 155.9, 134.7, 132.5, 120.5, 118.6, 112.8, 94.1, 55.8, 52.1

2-Bromo-5-(methoxymethoxy)benzoic acid

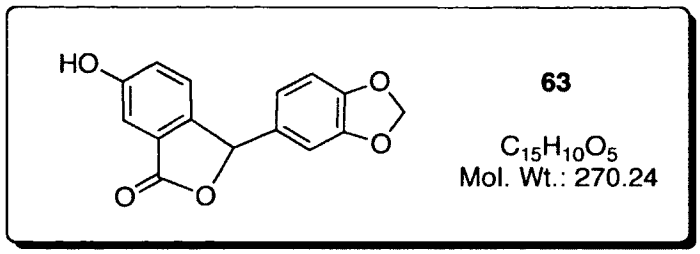


Compound **61** (4.73 g, 17.2 mmol) was dissolved in a mixture of methanol (10 mL) and water (10 mL), to which NaOH was added (1.00 g, 25.0 mmol). The reaction mixture was refluxed for one hour, after which it was cooled to room temperature and extracted with water (25 mL) and EtOAc (25 mL). The aqueous layer was acidified with concentrated HCl (2 mL) and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. The product **55** was a white-beige solid (4.49 g, 85.6% yield).

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 11.47 (br, 1H), 7.67 (d, J = 3.2 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.08 (dd, J = 8.8, 2.8 Hz, 1H), 5.20 (s, 2H), 3.48 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 170.7, 156.1, 135.5, 131.0, 121.8, 119.9, 114.1, 94.4, 56.1

3-(benzo[*d*][1,3]dioxol-5-yl)-6-hydroxyisobenzofuran-1(3*H*)-one

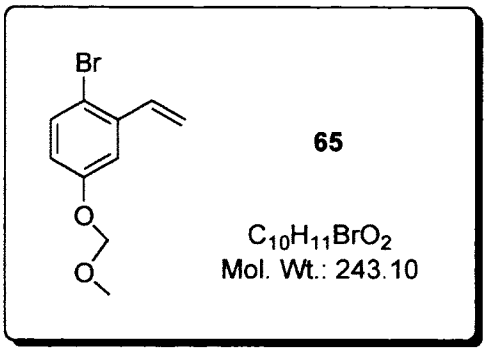


63

$C_{15}H_{10}O_5$
Mol. Wt.: 270.24

The protected A-ring, compound **55** (0.261 g, 0.999 mmol) was dissolved in dry THF (15 mL) and cooled to -100°C using liquid nitrogen/ methanol bath. The solution was kept under nitrogen atmosphere. n-buthyllithium (0.141 g, 2.18 mmol, 1.90 M) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes. Benzo[*d*][1,3]dioxole-5-carbaldehyde **62** (0.150 g, 0.999 mmol) was dissolved in dry THF (2 mL), and then added dropwise to the reaction mixture. The reaction was stirred for 10 minutes before 6 M HCl solution (20 mL) was added. The reaction was allowed to warm up to room temperature and was left stirring for 15 minutes. The reaction mixture was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product was purified using column chromatography to obtain a white solid (88.0 mg). The ^{1}H NMR spectrum of the isolated product indicated a mixture of compounds, some of which had incorporated n-butyl units from the nBuLi.

1-Bromo-4-(methoxymethoxy)-2-vinylbenzene



65

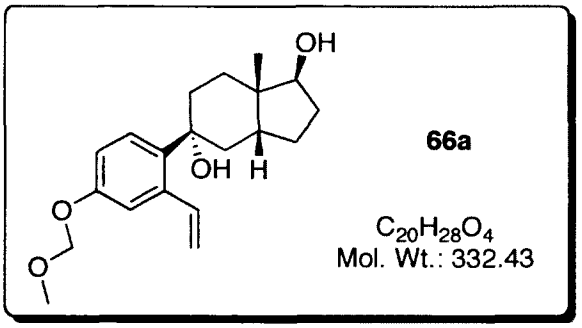
$C_{10}H_{11}BrO_2$
Mol. Wt.: 243.10

A THF solution of NaHMDS (7.10 g, 31.5 mmol, 31.5 mL) was added dropwise to methyl-triphenylphosphonium bromide (14.7 g, 41.1 mmol) suspended in dry THF (120 mL) under nitrogen atmosphere for 15 minutes at 0°C. The reaction mixture was stirred at 0°C for 30 minutes. Compound **60** (5.93g, 24.2 mmol) dissolved in dry THF (10 mL) was added dropwise and the reaction mixture was stirred for 2 hours while allowing the temperature to rise to room temperature. The reaction mixture was quenched with NH_4Cl (30 mL) and extracted with ether (30 mL). The organic phase was re-extracted with water (30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography to give the desired alkene as a yellow oil (5.34 g, 91% yield).

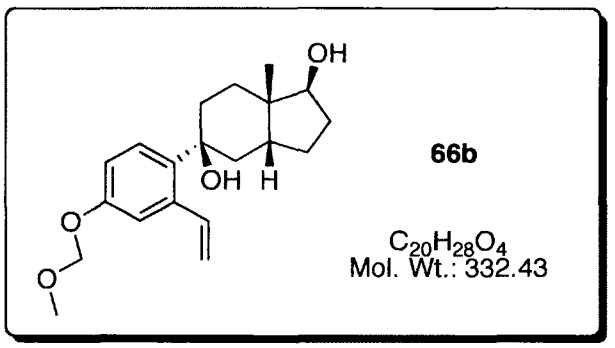
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.44 (d, J = 8.8 Hz, 1H), 7.24 (d, J = 3.2, 1H), 7.02 (dd, J = 17.6, 11.2, 1H), 6.85 (dd, J = 8.8, 3.2 Hz, 1H), 5.71 (dd, J = 17.6, 1.2, 1H), 5.37 (dd, J = 10.8, 0.8 Hz, 1H), 5.17 (s, 2H), 3.48 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): (δ ppm) = 156.6, 138.2, 135.6, 133.4, 117.3, 116.8, 115.5, 114.3, 94.4, 55.9

(1*S*,3a*R*,5*R*,7a*S*)-5-(4-(methoxymethoxy)-2-vinylphenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol



(1*S*,3a*R*,5*S*,7a*S*)-5-(4-(methoxymethoxy)-2-vinylphenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol



The protected A-ring, compound **65** (0.70g, 2.88 mmol), was dissolved in dry THF (20 mL) and cooled to -78°C using dry ice/ acetone bath. The solution was kept under nitrogen atmosphere. n-buthyllithium (0.184g, 2.88 mmol) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes.

The CD-ring, compound **47**, (0.156g, 0.930 mmol), was dissolved in dry THF (2 mL), and then added dropwise to the reaction mixture. The reaction was stirred for 10 minutes before it was quenched with saturated NH_4Cl (10 mL) solution and water (10 mL). The mixture was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product was purified using

column chromatography, from which two isomers were obtained. Both were beige semi-solids (0.134g, 43.4% yield of **66a** and 0.128g, 41.4% yield of **66b)**.

Compound 66a

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.69 (dd, J= 17.2, 10.8 Hz, 1H), 7.34 (d, J= 8.8 Hz, 1H), 7.14 (d, J= 2.8 Hz, 1H), 6.90 (dd, J= 8.8, 2.8 Hz, 1H), 5.51 (dd, J= 17.6, 1.6 Hz, 1H), 5.25 (dd, J= 10.8, 1.6 Hz, 1H), 5.18 (s, 2H), 3.98 (dd, J= 6.8, 4.4 Hz, 1H), 3.48 (s, 3H), 2.30-2.20 (m, 2H), 2.01-1.87 (m, 5H), 1.84-1.72 (m, 2H), 1.57-1.48 (m, 2H), 1.21-1.15 (m, 2H), 0.97 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 156.0, 139.5, 138.0, 137.5, 126.6, 116.6, 114.9, 114.1, 94.2, 79.3, 74.8, 55.9, 42.9, 41.9, 38.6, 33.8, 31.5, 29.0, 27.6, 19.6

Mass (EI): *m/z* (%) = 270.2 (M+, 24.4), 252.1 (3.5), 211.1 (17.1), 197.1 (10.7), 158.1 (36.5), 145.1 (16.9), 131.0 (100.0), 97.1 (11.4), 77.0 (5.1), 41.0 (6.3)

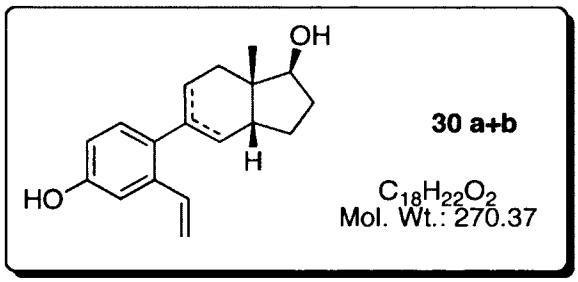
HRMS: calcd 270.1619, found 270.1620

Compound 66b

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.66 (dd, J= 17.6, 10.8 Hz, 1H), 7.24 (d, J= 8.8 Hz, 1H), 7.13 (d, J= 2.8 Hz, 1H), 6.87 (dd, J= 8.8, 2.8 Hz, 1H), 5.49 (dd, J= 17.2, 1.2 Hz, 1H), 5.23 (dd, J= 10.8, 1.6 Hz, 1H), 5.13 (s, 2H), 4.21 (dd, apparent t, J= 8.8, 8.8 Hz, 1H), 3.45 (s, 3H), 2.17-2.03 (m, 3H), 2.00-1.74 (m, 5H), 1.65-1.69 (m, 1H), 1.58-1.48 (m, 1H), 1.16-1.10 (m, 1H), 0.92 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 156.0, 139.2, 138.6, 137.7, 126.0, 116.2, 115.0, 114.3, 94.2, 73.8, 73.5, 55.8, 41.8, 41.7, 40.5, 33.0, 30.0, 28.0, 26.3, 21.4

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-vinylphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol and (1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-vinylphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol



Compound **66b** (0.096g, 0.289 mmol) was dissolved in THF (1.8 mL) and water (0.2 mL), to which a catalytic amount of pTSA was added. The reaction mixture was stirred under reflux conditions for 10 hours. After the reaction was completed according to a TLC, saturated sodium bicarbonate solution (10 mL) and DCM (10 mL) was added. The reaction mixture was extracted two more times with DCM (10 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product was purified using column chromatography, from which a mixture of isomers was obtained as yellow solid (31.0 mg, 40% yield).

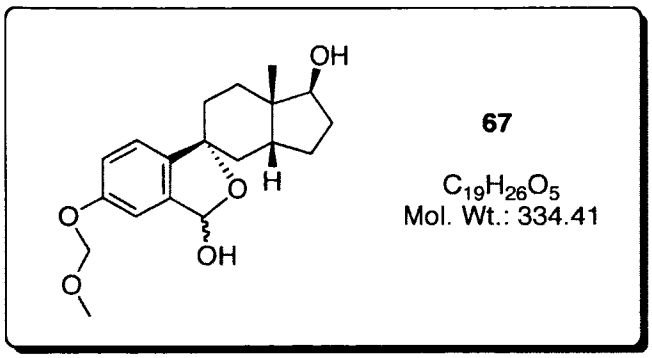
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.00 (dd, apparent t, J= 2.8, 2.8 Hz, 1H), 6.96 (dd, J= 8.0, 1.2, 1H), 6.83-6.75 (m, 1H), 6.72-6.69 (m, 1H), 5.61 (dd, J= 17.6, 1.2 Hz, 1H), 5.29-5.51 (m, 0.47H) 5.46-5.44 (m, 0.83H), 5.20 (dd, J= 10.8, 1.2 Hz, 1H), 3.94 (dd, apparent t, J= 5.6, 5.6, 0.4 H), 3.85 (dd, J= 6.4, 1.6 Hz, 0.6 H), 2.39-2.08 (m, 4H), 1.91-1.83 (m, 1H), 1.81-1.19 (m, 7H), 1.09 (s, 1.8H), 1.05 (s, 1.2H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 154.4, 136.6, 136.5, 136.1, 135.9, 135.4, 135.3, 134.8, 134.7, 130.8, 129.8, 129.7, 124.5, 114.7, 114.6, 114.2, 114.1, 111.6, 111.5, 80.6, 79.2, 43.9, 42.3, 42.1, 39.7, 32.2, 32.1, 31.8, 30.7, 29.0, 28.2, 27.4, 19.9, 19.3

Mass (EI): *m/z* (%) = 270.2 (M+, 24.4), 252.1 (3.5), 211.1 (17.1), 158.1 (36.5), 145.1 (16.9), 131.0 (100.0), 97.1 (111.4), 77.0 (5.1), 41.0 (6.3)

HRMS: calcd 270.1620, found 270.1620

(1*S*,1'*R*,3a*R*,7a*S*)-5'-(methoxymethoxy)-7a-methyl-1,2,3,3a,4,6,7,7a-octahydro-3'*H*-spiro[indene-5,1'-isobenzofuran]-1,3'-diol



67

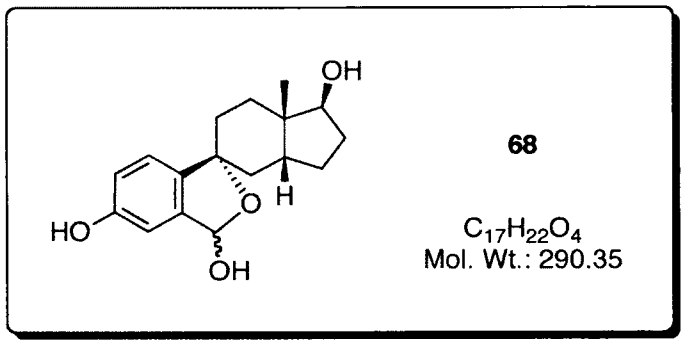
$C_{19}H_{26}O_5$
Mol. Wt.: 334.41

A solution of **66a** (0.060 g, 0.180 mmol) in mixture of DCM (3.5 mL) and methanol (0.5 mL) was cooled in dry ice/ acetone. Ozone was bubbled into the reaction mixture until the blue color persisted. The excess ozone was purged with argon gas until the solution turned clear. After 5 minutes, a 95% aqueous solution of sodium bisulfate was added dropwise. The reaction mixture was allowed to warm up to room temperature to decompose the ozonide. The reaction mixture was washed with 5% aqueous solution of $NaHCO_3$ and then water. The organic extract was dried over $MgSO_4$, filtered and concentrated. The crude product was purified using column chromatography, to obtain a mixture of isomers as white solid (16.0 mg, 26.5% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.09-7.06 (m, 1H), 7.03-6.99 (m, 2H), 5.99 (s, 0.6 H), 5.97 (s, 0.4 H), 5.20 (dd, J = 6.8, 1.2 Hz, 1H), 5.13 (d, J = 6.8 Hz, 1H), 3.92-3.87 (m, 1H), 3.47 (s, 5H), 3.44 (s, 1H), 2.35-1.94 (m, 4H), 1.92-1.75 (m, 5H), 1.72-1.49 (m, 5H), 1.41-1.34 (m, 1H), 1.29- 1.17 (m, 3H), 1.13 (s, 1.7 H), 1.12 (s, 1.5 H)

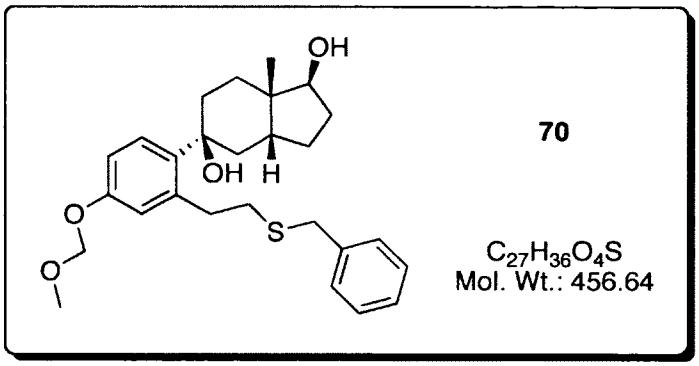
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 157.3, 141.3, 139.1, 138.9, 121.7, 121.7, 117.9, 117.7, 110.3, 110.3, 105.7, 105.3, 94.7, 87.0, 80.9, 80.6, 56.0, 54.8, 54.3, 43.2, 43.2, 41.1, 41.1, 37.5, 36.6, 34.8, 33.7, 32.0, 31.9, 29.6, 28.7, 28.2, 27.4, 27.2, 18.6, 18.4

(1*S*,1'*R*,3a*R*,7a*S*)-7a-methyl-1,2,3,3a,4,6,7,7a-octahydro-3'*H*-spiro[indene-5,1'-isobenzofuran]-1,3',5'-triol



The isomeric mixture of **67** (0.016 g, 0.047 mmol) was diluted with methanol (2 mL), to which 5 drops of concentrated HCl was added. The reaction mixture was refluxed for 1 hour then cooled to room temperature and diluted with water (5 mL). To the reaction mixture EtOAc was added and the reaction mixture was washed with and $NaHCO_3$ (3 x 15 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. Unfortunately, we were unable to isolate and characterize this compound.

(1*S*,3a*R*,5*S*,7a*S*)-5-(2-(2-(benzylthio)ethyl)-4-(methoxymethoxy)phenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol

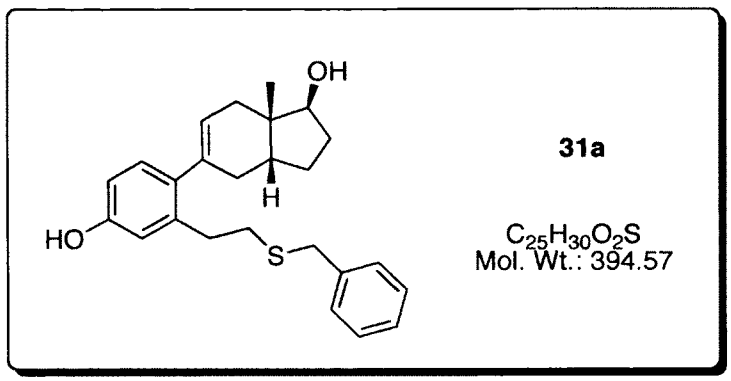


Compound **66b** (0.100g, 0.301 mmol), was dissolved in $CDCl_3$ and left exposed to the light for two weeks in the presence of benzyl mercaptan **69** (56.0 mg, 0.451 mmol). The crude material was purified by column chromatography to give the product **70**, (94.0 mg, 69.0% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.34-7.19 (m, 6H), 6.81-6.78 (m, 2H), 5.11 (s, 2H), 4.25 (dd, apparent t, J= 8.0, 8.0 Hz, 1H), 3.68 (s, 2H), 3.44 (s, 3H), 3.31-3.23 (m, 1H), 3.20-3.13 (m, 1H), 2.69 (dd, apparent t, J = 8.0 Hz, 2H), 2.20-1.04 (m, 11H), 0.95 (s, 3H), 0.91-0.82 (m, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 155.9, 141.4, 139.5, 138.4, 128.8, 128.3, 126.8, 126.3, 119.5, 113.2, 94.2, 73.9, 73.6, 55.9, 42.5, 41.8, 40.6, 36.4, 34.2, 33.7, 33.6, 30.1, 28.1, 26.4, 21.5

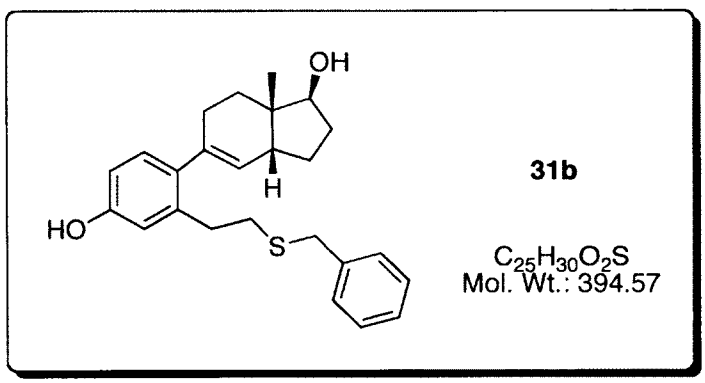
(1*S*,3a*R*,7a*S*)-5-(2-(2-(benzylthio)ethyl)-4-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol



31a

$C_{25}H_{30}O_2S$
Mol. Wt.: 394.57

(1*S*,3a*R*,7a*S*)-5-(2-(2-(benzylthio)ethyl)-4-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol



31b

$C_{25}H_{30}O_2S$
Mol. Wt.: 394.57

Compound **66b** (0.094g, 0.206 mmol) was dissolved in THF (1.8 mL) and water (0.2 mL), to which a catalytic amount of pTSA was added. The reaction mixture was stirred under reflux conditions for 24 hours. After the reaction was completed saturated sodium bicarbonate solution (10 mL) and DCM (10 mL) were added. The reaction mixture was extracted two more times with DCM (10 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product was purified using column chromatography, from which a de-protected mixture of isomers was obtained as yellow

solid (64 mg, 79% yield). The two isomers were separated by HPLC (15 mg, 19% yield of **31a** and 29 mg, 36% yield of **31b**).

Compound 31a

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.33-7.28 (m, 4H), 7.26-7.21 (m, 1H), 6.89 (d, J= 8.0 Hz, 1H), 6.62 (dd, J= 8.0, 2.4 Hz, 1H), 6.58 (d, J= 2.8 Hz), 5.38 (s, 1H), 4.98 (br, 1H), 3.81 (dd, J= 6.8, 1.6 Hz, 1H), 3.71 (s, 2H), 2.80-2.76 (m, 2H), 2.62-2.58 (m, 2H), 2.33-2.22 (m, 2H), 2.09-2.00 (m, 2H), 1.89-1.69 (m, 3H), 1.60-1.54 (m, 1H), 1.51-1.41 (m, 2H), 1.07 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 154.1, 139.2, 138.4, 136.6, 136.6, 135.3, 130.0, 128.8, 128.4, 126.9, 123.5, 115.6, 113.0, 80.5, 42.1, 39.6, 36.5, 33.2, 32.7, 32.0, 31.9, 31.1, 28.2, 19.9

Mass (EI): *m/z* (%) = 394.2 (M+, 1.4), 303.1 (100.0), 285.1 (6.5), 243.1 (3.9), 205.1 (3.3), 162.0 (14.2), 91.1 (35.4), 69.0 (6.7), 32.0 (10.8)

HRMS: calcd 394.1961, found 394.1961

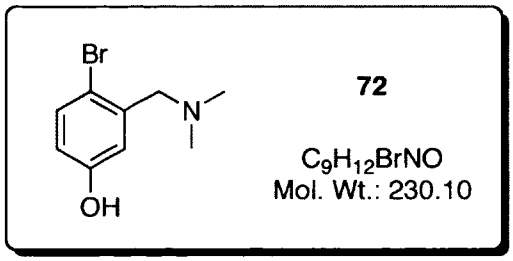
Compound 31b

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.33 (m, 4H), 7.26-7.21 (m, 1H), 6.89 (d, J= 8.0 Hz), 6.62 (dd, J= 8.0, 2.8 Hz, 1H), 6.59 (d, J= 2.4 Hz, 1H), 5.46-5.45 (m, 1H), 5.21 (br, 1H), 3.91 (dd apparent t, J= 5.2, 5.2, 1H), 3.71 (s, 2H), 2.80-2.71 (m, 2H), 2.62-2.56 (m, 2H), 2.33-2.28 (m, 1H), 2.25-2.04 (m, 4H), 1.66-1.51 (m, 3H), 1.45-1.30 (m, 2H), 1.03 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 154.3, 139.1, 138.3, 136.4, 135.2, 130.0, 129.8, 128.8, 128.5, 126.9, 115.7, 113.0, 79.1, 43.8, 42.3, 36.5, 33.3, 32.7, 32.1, 30.1, 28.9, 21.9, 19.3

Mass (EI): *m/z* (%) = 394.1 (M+, 0.3), 303.1 (87.4), 285.1 (6.8), 243.1 (6.6), 165.0 (21.9), 143.0 (14.2), 91.1 (100.0), 65.0 (9.1), 28.0 (32.8)

4-Bromo-3-((dimethylamino)methyl)phenol

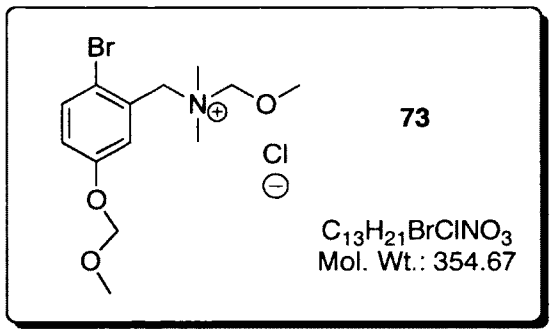


To a solution of dimethylamine (0.897 g, 19.9 mmol) in methanol (25 mL) and 5N HCl-methanol (2 mL, 9.99 mmol), compound **59** (1.00 g, 4.98 mmol) and $NaBH_3CN$ (0.219 g, 3.48 mmol) were added. The resulting solution was stirred for 4 days. Concentrated HCl was added until the pH < 2. The methanol was removed by rotary evaporation, then diluted with water (10 mL). The aqueous phase was extracted with ether (3 x 20 mL), then brought to pH ~ 8 by the addition of solid $NaHCO_3$. The basified solution was saturated with NaCl and extracted with ether (6 x 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified by column chromatography to give **72** (0.630 g, 55.0% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 8.19 (br, 1H), 7.32 (d, J = 8.8 Hz, 1H), 6.72 (d, J = 2.8 Hz, 1H), 6.59 (dd, J = 8.4, 2.8 Hz, 1H), 3.53 (s, 2H), 2.32 (s, 6H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 156.4, 137.1, 133.6, 118.7, 117.5, 114.3, 62.4, 44.9

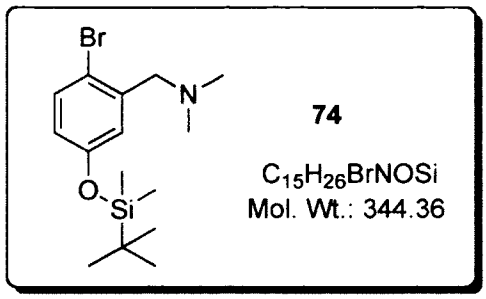
***N*-(2-bromo-5-(methoxymethoxy)benzyl)-1-methoxy-*N*,*N*-dimethylmethanaminium chloride**



N,N-diisopropylethylamine (0.953 mL, 5.47 mmol) and chloromethyl methyl ether (0.416 mL, 5.47 mmol) were added to a solution of compound **72** (0.630 g, 2.74 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then left at room temperature for 2 hours. The organic mixture was diluted with 10% aqueous NaOH (30 mL) and extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was a yellow solid (0.293 g, 30%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.28 (d, J = 8.8 Hz, 1H), 7.28 (d, J = 3.2 Hz, 1H), 6.78 (dd, J = 8.8, 2.8 Hz, 1H), 4.95 (s, 2H), 4.91 (s, 2H), 4.69 (s, 2H), 3.53 (s, 3H), 3.16 (s, 3H), 3.05 (s, 6H)

1-(2-Bromo-5-(tert-butyldimethylsilyloxy)phenyl)-N,N-dimethylmethanamine



74

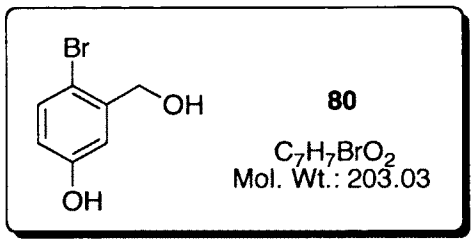
$C_{15}H_{26}BrNOSi$
Mol. Wt.: 344.36

Compound **72** (1.19 g, 5.15 mmol) was dissolved in a minimum amount of DMF (15 mL) and stirred in an ice bath at 0°C. Imidazole (0.701 g, 7.72 mmol) was added and the reaction was stirred for 5 minutes, after which *tert*-butyldimethylsilyl chloride (TBDMS-Cl) (1.55 g, 7.72 mmol) was added. The reaction mixture was stirred overnight at room temperature. After the reaction was completed according to TLC, the reaction mixture was diluted with brine (30 mL) extracted with ether (3 x 30 mL). The ether extracts were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The obtained crude product was purified with column chromatography to give a clear oil (1.43 g, 80.9% yield). The ^1H NMR showed that the product contained 3% of the TBDMS-Cl, which due to its low polarity was extremely difficult to remove. The yield of product based on the ^1H NMR was 1.39 g (78.5%).

^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.35 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 3.2 Hz, 1H), 6.60 (d, J = 8.8, 3.2 Hz, 1H), 3.44 (s, 2H), 2.28 (s, 6H), 0.97 (s, 9H), 0.18 (s, 6H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 154.9, 139.0, 133.0, 122.5, 120.1, 115.7, 63.2, 45.4, 25.6, 18.1, -4.5

4-Bromo-3-(hydroxymethyl)phenol

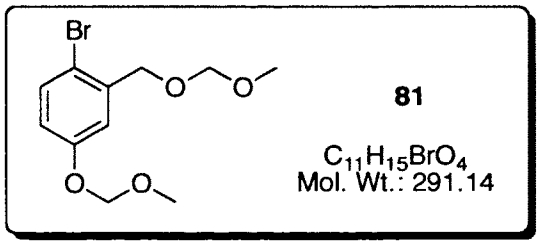


2-Bromo-5-hydroxybenzaldehyde **59** (6.84 g, 34.1 mmol) was dissolved in methanol (20 mL) and cooled to 0°C. Solid $NaBH_4$ (1.67 g, 44.3 mmol) was added in small portions to the reaction mixture. The reaction mixture was stirred overnight at room temperature, after which saturated NH_4Cl (10 mL) was added and the methanol was evaporated *in vacuo*. The reaction mixture was extracted with water (30 mL) and EtOAc (3 x 30 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography. The alcohol **80** was a white solid (5.16 g, 74.7% yield).

^{1}H NMR (acetone-d6, 400 MHz,): δ (ppm) = 8.63- 8.42 (br, 1H), 7.32 (d, J= 8.4 Hz, 1H), 7.15 (d, J= 3.2 Hz, 1H), 6.68 (dd, J= 8.4, 3.2 Hz, 1H), 4.60 (s, 2H), 4.53-4.44 (br, 1H)

^{13}C NMR (acetone-d6, 100 MHz,): δ (ppm) = 159.0, 144.1, 134.5, 117.2, 117.0, 111.6, 65.1

1-Bromo-4-(methoxymethoxy)-2-((methoxymethoxy)methyl)benzene



81

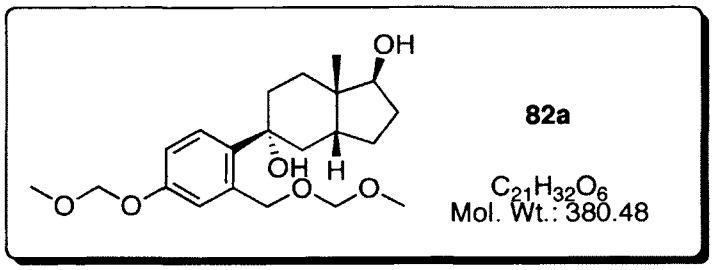
$C_{11}H_{15}BrO_4$
Mol. Wt.: 291.14

N,N-diisopropylethylamine (13.3 mL, 76.2 mmol) and chloromethyl methyl ether (5.79 mL, 76.2 mmol) were added to a solution of 4-bromo-3-(hydroxymethyl)phenol, compound **80** (5.16 g, 25.4 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then was left at room temperature overnight. Next, 10% NaOH (30 mL) was added. The mixture was extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was purified by column chromatography to obtain the desired compound as a yellow oil (6.49 g, 88%).

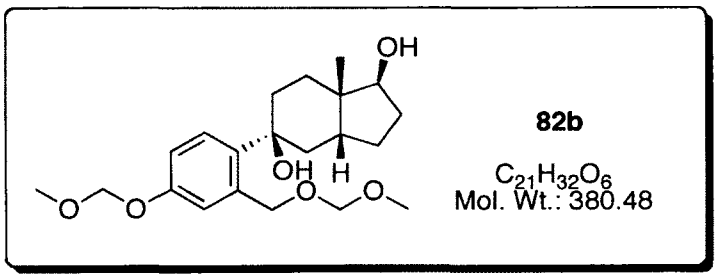
^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.42 (d, J= 8.8 Hz, 1H), 7.19 (d, J= 3.2 Hz, 1H), 6.85 (dd, J= 8.8, 3.2 Hz, 1H), 5.15 (s, 2H), 4.76 (s, 2H), 4.61 (s, 2H), 3.46 (s, 3H), 3.43 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 156.6, 138.4, 133.0, 117.0, 116.7, 114.1, 96.1, 94.3, 68.6, 55.9, 55.4

(1*S*,3a*R*,5*R*,7a*S*)-5-(4-(methoxymethoxy)-2-((methoxymethoxy)methyl)phenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol



(1*S*,3a*R*,5*S*,7a*S*)-5-(4-(methoxymethoxy)-2-((methoxymethoxy)methyl)phenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol



The protected compound **81** (4.33g, 14.9 mmol) was dissolved in dry THF (20 mL) under a nitrogen atmosphere. The mixture was cooled to -78°C using a dry ice/ acetone bath. n-Buthyllithium (9.14 mL, 1.63 M) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes. Compound **47** (1.00 g, 59.5 mmol) was dissolved in dry THF (2 mL) and was added dropwise to the reaction mixture. The reaction mixture was stirred for 10 min at -78 °C, quenched with saturated NH_4Cl solution (10 mL) and water (10 mL), and extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated to yield a crude beige semi-solid (3.92 g), which was purified by column chromatography. The ^{1}HNMR of the different fractions obtained indicated a complex mixture including the desired compounds, **82a** and **82b** and some unreacted starting materials. Based on ^{1}H NMR, the mixture was

estimated to contain as **82a** (0.641g, 28%) and **82b** (0.318g, 14%). After extensive column chromatography, pure **82a** was isolated (0.129 g, 6%) as judged by its ^{1}HNMR.

Compound 82a

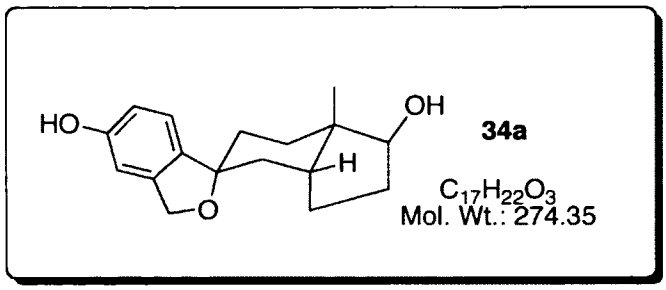
^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.32 (d, J = 8.8 Hz, 1H), 7.06 (d, J = 2.8 Hz, 1H), 6.94 (dd, J = 8.4, 2.8 Hz, 1H), 5.17 (s, 2H), 4.94 (d, J = 11.6 Hz, 1H), 4.89 (d, J = 11.6 Hz, 1H), 4.71 (s, 2H), 3.91 (dd, J= 6.0, 3.6 Hz, 1H), 3.47 (s, 3H), 3.40 (s, 3H), 2.97 (s, 1H), 2.31- 2.04 (m, 3H), 1.99-1.87 (m, 4H), 1.84-1.74 (m, 2H), 1.56-1.49 (m, 1H), 1.21-1.15 (m, 1H), 1.04 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 155.7, 140.2, 137.1, 127.6, 119.9, 114.9, 95.7, 94.3, 80.7, 75.3, 69.0, 56.0, 55.6, 43.3, 41.8, 38.5, 34.6, 32.0, 28.8, 27.8, 19.2

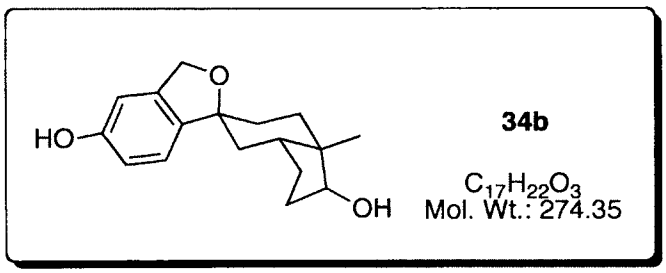
Compound 82b (was obtained as mixture with compound **47**, CD-ring, only the major characteristic peaks of compound **82b** are listed)

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.24 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 2.4 Hz, 1H), 6.93 (dd, J = 8.8, 2.8 Hz, 1H), 5.16 (s, 2H), 4.93 (d, J = 11.6 Hz, 1H), 4.88 (d, J = 11.6 Hz, 1H), 4.71 (s, 2H), 4.32 (apparent t, dd, J = 8.4, 8.4 Hz, 1H), 3.46 (s, 3H), 3.39 (s, 3H), 2.86 (br, 1H), 0.96 (s, 3H)

5-Spiro-ether 34a



5-Spiro-ether 34b



Compound **82a** (0.129 g, 0.339 mmol) and 0.141 g of mixture of **82a: 82b**: EtOAc (~86: 11: 3, approximately 0.266 g, 0.698 mmol for both isomers combined), were diluted with 5 mL of methanol. 3-5 drops of concentrated HCl was added. The reaction mixture was refluxed for 1 hour then cooled to room temperature and diluted with water (5 mL). The methanol was removed by rotary evaporation and the reaction mixture was extracted with EtOAc (3 x 15 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product (0.239 g), a beige semi-solid, was purified by column chromatography to yield a mixture of the two isomers (0.140 g). Further purification by HPLC and plate chromatography gave **34a** (29.0 mg, 15%) and **34b** (23.0 mg, 12%), both as white solids.

Compound 34a

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 6.97 (d, J = 8.4 Hz, 1H), 6.71 (dd, J = 8.0, 2.4 Hz, 1H), 6.65 (d, J = 2.0 Hz, 1H), 5.87 (br, 1H), 4.97 (d, J = 12.8 Hz, 1H), 4.93 (d, J = 12.8 Hz, 1H), 3.91 (dd, J = 6.4, 2.8 Hz, 1H), 2.33 - 2.22 (m, 1H), 2.13 - 1.99 (m, 2H), 1.91-1.63 (m, 6H), 1.57 - 1.49 (m, 1H), 1.27-1.22 (m, 1H), 1.12 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 155.4, 140.9, 139.1, 121.7, 114.2, 107.8, 86.9, 80.8, 70.2, 43.3, 41.1, 35.7, 32.7, 31.7, 28.3, 27.3, 18.5

HRMS: calcd 274.1564, found 274.1564

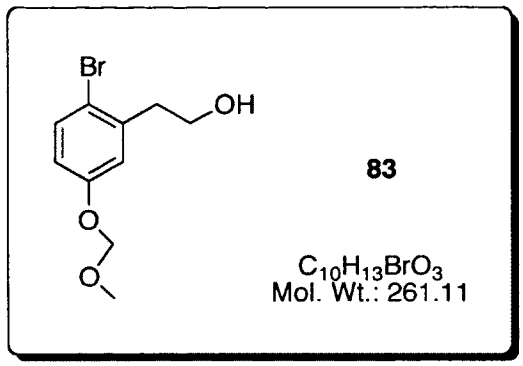
Compound 34b

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 6.88 (d, J = 8.0 Hz, 1H), 6.71 (dd, J = 8.0, 2.0 Hz, 1H), 6.65 (d, J = 1.6 Hz, 1H), 5.41 (br, 1H), 4.97 (s, 2H), 4.40 (apparent t, dd J = 8.4, 8.4 Hz), 2.29 – 2.17 (m, 1H), 2.13-2.01 (m, 2H), 1.79 – 1.55 (m, 6H), 1.37 - 1.31 (m, 1H), 1.19 - 1.11 (m, 1H), 0.95 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 155.3, 140.6, 138.9, 121.3, 114.3, 107.9, 86.7, 73.6, 70.4, 42.0, 41.1, 40.7, 32.0, 30.2, 28.4, 26.5, 21.5

Mass (EI): *m/z* (%) = 274.2 (M+, 18.9) 187.1 (37.3), 161.1 (100), 135.0 (26.0)

2-(2-Bromo-5-(methoxymethoxy)phenyl)ethanol

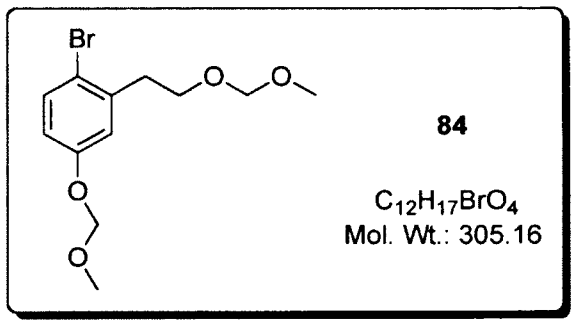


Compound **65, (**5.00 g, 20.6 mmol) was dissolved in dry THF (20 mL) at 0°C and kept under nitrogen. A 1M solution of borane (2.65 g, 30.8 mmol) in THF was added dropwise and the reaction was stirred at room temperature overnight. The reaction mixture was transferred to the separatory funnel and a 30% solution of H_2O_2 (30 mL) was added. The reaction mixture was shaken and left for 5 minutes, after which 3M NaOH solution (30 mL) was added dropwise. The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic extracts were combined dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography to give **83 (**2.17 g, 40% yield) as a clear oil.

^{1}H NMR ($CDCl_3$, 400 MHz,): (δ ppm) = 7.39 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 3.2 Hz, 1H), 6.77 (dd, J = 8.8, 3.2 Hz, 1H), 5.10 (s, 2H), 3.80 (t, J = 6.8 Hz, 2H), 3.42 (s, 3H), 2.92 (t, J = 6.8, 2H), 2.53 (br, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz,): (δ ppm) = 156.2, 138.8, 133.2, 119.0, 116.2, 115.8, 94.2, 61.6, 55.8, 39.2

1-bromo-4-(methoxymethoxy)-2-(2-(methoxymethoxy)ethyl)benzene

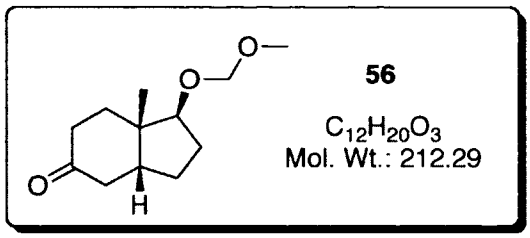


N,N-diisopropylethylamine (2.17 mL, 12.4 mmol) and chloromethyl methyl ether (0.946 mL, 12.4 mmol) were added to a solution of compound **83** (2.17 g, 8.31 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then was left at room temperature overnight, after which 10% NaOH (30 mL) was added to the reaction mixture and it was extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude product was obtained as a yellow oil (2.43 g, 95% yield).

^{1}H NMR ($CDCl_3$, 400 MHz,): (δ ppm) = 7.38 (d, J = 8.8 Hz, 1H), 6.96 (d, J = 2.8 Hz, 1H), 6.77 (dd, J = 8.8, 3.2 Hz, 1H), 5.11 (s, 2H), 4.60 (s, 2H), 3.73 (t, J = 7.2 Hz, 2H), 3.43 (s, 3H), 3.29 (s, 3H), 2.98 (t, J = 7.2, 2H)

^{13}C NMR ($CDCl_3$, 100 MHz,): (δ ppm) = 156.3, 139.0, 133.1, 118.8, 116.2, 115.8, 96.1, 94.3, 66.4, 55.8, 54.9, 36.4

(1*S*,3a*R*,7a*S*)-1-(methoxymethoxy)-7a-methylhexahydro-1*H*-inden-5(6*H*)-one



56

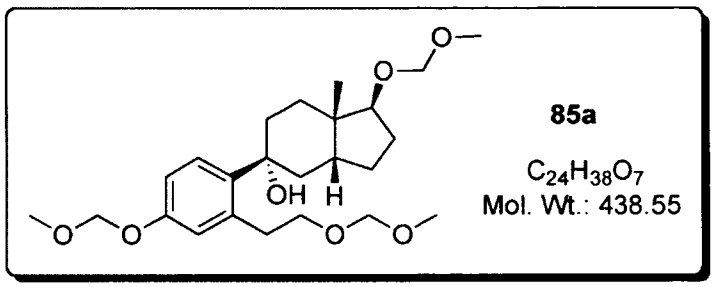
$C_{12}H_{20}O_3$
Mol. Wt.: 212.29

N,N-diisopropylethylamine (2.49 mL, 14.3 mmol) and chloromethyl methyl ether (1.36 mL, 17.8 mmol) were added to a solution of compound **47** (2.00 g, 11.9 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then was left at room temperature overnight. The reaction mixture was diluted with 10% aqueous NaOH (30mL) and extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was purified by column chromatography to obtain compound **56** as a yellow oil, (1.77 g, 70% yield) and recovered starting material, compound **47** (0.2 g). The yield based on recovery of the starting material was 78%.

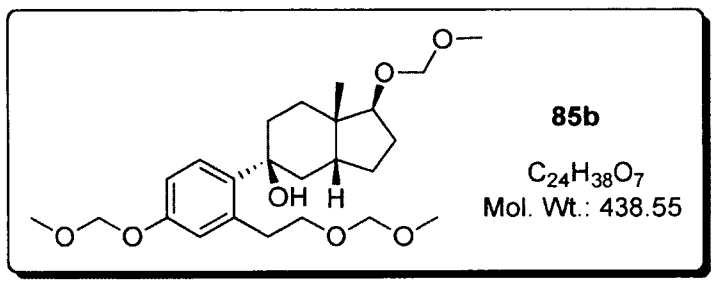
^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 4.61 (d, J = 6.8 Hz, 1H), 4.52 (d, J = 6.8, 1H), 3.68 (t, J = 6.0, 6.0 Hz, 1H), 3.29 (s, 3H), 2.41 - 2.25 (m, 2H), 2.24 – 2.07 (m, 3H), 2.02 – 1.95 (m, 1H), 1.91-1.82 (m, 1H), 1.72 – 1.53 (m, 3H), 1.20 – 1.12 (m, 1H), 1.09 (m, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 212.5, 95.3, 83.8, 55.1, 43.8, 42.5, 42.1, 36.4, 32.2, 28.9, 28.1, 20.2

(1*S*,3a*R*,5*R*,7a*S*)-1-(methoxymethoxy)-5-(4-(methoxymethoxy)-2-(2-(methoxymethoxy)ethyl)phenyl)-7a-methyloctahydro-1*H*-inden-5-ol



(1*S*,3a*R*,5*S*,7a*S*)-1-(methoxymethoxy)-5-(4-(methoxymethoxy)-2-(2-(methoxymethoxy)ethyl)phenyl)-7a-methyloctahydro-1*H*-inden-5-ol



The diprotected compound **84** (2.68 g, 8.79 mmol) was dissolved in dry THF (20 mL) under nitrogen. The solution was cooled to -78°C using a dry ice/acetone bath. n-Buthyllithium (4.62 mL, 8.79 mmol, 1.90 M) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes. The protected CD-ring, **56** (1.24 g, 5.86 mmol), was dissolved in dry THF (2 mL), and then added dropwise to the reaction mixture. The mixture was stirred for 10 minutes and then quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The mixture was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product (3.28 g) was a beige semi-solid. Careful column chromatography of the crude product yielded a number of fractions which were identified by their proton NMR

spectra. These included in order of increasing polarity: i) Reduced **84** (0.880 g, 3.88 mmol), ii) a mixture of the first compound **84** and recovered compound **56** (0.181g), iii) a complex fraction containing the less polar isomer **85b**, compound **56**, and the more polar isomer **85a** (0.710 g), iv) a mixture consisting mainly of **85b** with some **85a** (0.345g), and v) almost pure **85a** (0.962 g). The estimated yield based on ^{1}H NMR and calculated percent composition of the mixture was: more polar isomer **85a** (1.08 g, 42%) and less polar isomer **14b**, (0.782 g, 30.4%).

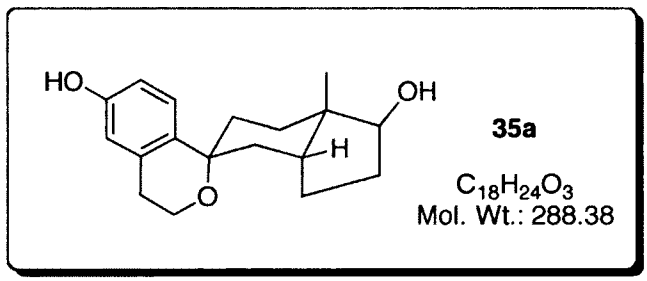
Compound 85a

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.26 (d, J = 8.8 Hz, 1H), 6.87 (d, J = 2.8 Hz, 1H), 6.78 (dd, J = 8.4, 2.8 Hz, 1H), 5.10 (s, 2H), 4.62 (d, J = 6.8 Hz, 1H), 4.54 (d, J = 6.4 Hz, 1H), 4.48 (s, 2H), 3.84 (dd, J = 6.0, 4.4 Hz, 1H), 3.73 (t, J = 6.0 Hz, 2H), 3.41 (s, 3H), 3.39-3.21 (m, 2H), 3.31 (s, 3H), 3.14 (br, 1H), 3.11 (s, 3H), 2.19 - 2.04 (m, 2H), 1.96-1.76 (m, 7H), 1.61 – 1.44 (m, 1H), 1.61-1.48 (m, 1H), 0.97 (s, 3H)

Compound 85b

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.29 (d, J = 8.8 Hz, 1H), 6.90 (d, J = 2.8 Hz, 1H), 6.82 (dd, J = 8.8, 2.8 Hz, 1H), 5.14 (s, 2H), 4.66 (d, J = 6.8 Hz, 1H), 4.58 (d, J = 6.8 Hz, 1H), 4.52 (s, 2H), 3.87 (dd, J = 6.4, 4.4 Hz, 1H), 3.77 (t, J = 6.4 Hz, 2H), 3.45 (s, 3H), 3.35 (s, 3H), 3.42-3.23 (m, 2H), 3.13 (s, 3H), 3.11 (br, 1H), 2.21-2.11 (m, 2H), 1.99-1.77 (m, 7H), 1.64-1.56 (m, 1H), 1.24-1.16 (m, 1H), 1.00 (s, 3H)

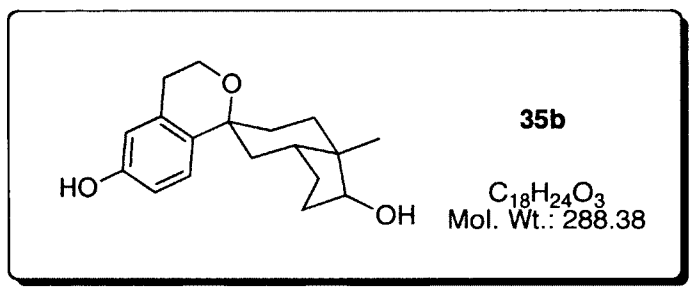
6-Spiro-ether 35a



35a

$C_{18}H_{24}O_3$
Mol. Wt.: 288.38

6-Spiro-ether 35b



35b

$C_{18}H_{24}O_3$
Mol. Wt.: 288.38

A mixture of both isomers of **85** (1.287 g, 2.934 mmol; approximately 7:3 **a**:**b**) was diluted with 5mL of methanol, and 10 drops of concentrated HCl was added. The reaction mixture was stirred under reflux conditions for 1 hour, after which it was cooled to room temperature, then diluted with water (5 mL). The methanol was removed by rotary evaporation and the reaction mixture was extracted with EtOAc (3 x 15 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The obtained crude product (0.820 g), a beige semi-solid, was purified by column chromatography to obtain the mixture of two isomers **35a** (0.122 g, 14%) and **35b** (0.172 g, 20%) with methanol as minor impurity.

The two isomers were purified by HPLC with the assistance of Dr. Ammar Saleem followed by plate chromatography to give pure **35a** (35.0 mg, 4% yield) and a mixture of **35a** and **35b** as a white solid (69.0 mg total, 1:5 ratio of **35a** to **35b**, 8%). The NMR of **35b** was obtained from a previous synthesis and was purified by plate chromatography.

Compound 35a

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 6.97 (d, J = 8.8 Hz, 1H), 6.66 (dd, J = 8.4, 2.4 Hz, 1H), 6.53 (d, J = 2.8 Hz, 1H), 6.02 (br, 1H), 3.88 – 3.77 (m, 3H), 2.85 – 2.78 (m, 1H), 2.65 (ddd, apparent dt, J = 16.0, 4.0, 4.0 Hz, 1H), 2.31 – 2.17 (m, 2H), 2.05 – 1.63 (m, 8H), 1.55 – 1.49 (m, 1H), 1.18 (s, 3H), 1.09 (ddd, apparent dt, J = 13.2, 2.4, 2.4 Hz, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 153.7, 135.3, 135.3, 126.7, 114.8, 113.4, 82.5, 75.9, 58.0, 43.5, 41.1, 33.6, 33.4, 32.3, 29.6, 27.7, 27.3, 18.3

Mass (EI): *m/z* (%) = 288.2 (M+, 9.6), 201.1 (22.6), 175.1 (100), 162.1 (36.4), 149.1 (48.5)

HRMS: calcd 288.1729, found 288.1729

Compound 35b

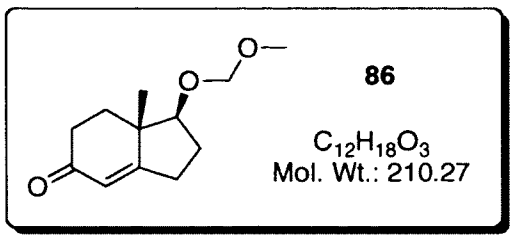
^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 6.91 (d, J = 8.4 Hz, 1H), 6.65 (dd, J = 8.4, 2.4 Hz, 1H), 6.54 (d, J = 2.4 Hz, 1H), 4.94 (s, 1H), 4.44 (dd, apparent t, J = 8.8, 8.8 Hz, 1H), 3.85 (ddd, apparent td, J = 5.6, 5.6, 1.2 Hz, 2H), 2.81 – 2.68 (m, 2H), 2.29 – 2.20 (m, 1H), 2.10 – 2.98 (m, 2H), 1.81 – 1.67 (m, 4H), 1.62 – 1.54 (m, 4H), 1.17 – 1.11 (m, 1H), 0.93 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 153.4, 135.2, 135.0, 126.3, 114.9, 113.4, 75.1, 73.8, 58.5, 41.8, 41.1, 40.3, 31.9, 30.1, 29.6, 27.8, 26.4, 21.5

Mass (EI): *m/z* (%) = 288.2 (M+, 26.7), 201.1 (63.8), 175.1 (100.0), 162.1 (57.6), 149.1 (67.0)

HRMS: calcd 288.1743, found 288.1744

(1*S*,7a*S*)-1-(Methoxymethoxy)-7a-methyl-2,3,7,7a-tetrahydro-1*H*-inden-5(6*H*)-one

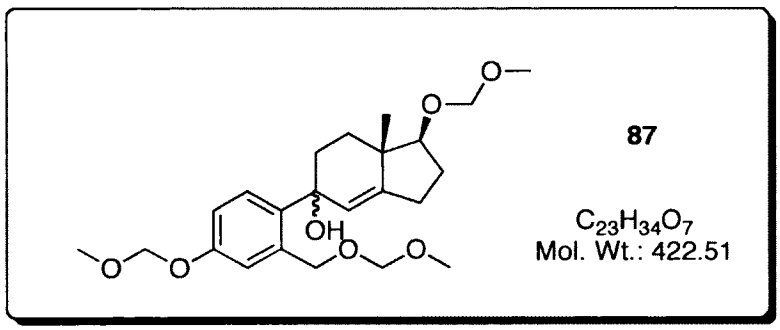


N,N-diisopropylethylamine (4.71 mL, 27.1 mmol) and chloromethyl methyl ether (2.06 mL, 27.1 mmol) were added to a solution of compound **46** (3.00 g, 18.0 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then was left at room temperature overnight. The reaction was not completed after 24 hours, so more of the N,N-diisopropylethylamine (1.57 mL, 9.02 mmol) and chloromethyl methyl ether (0.685 mL, 9.02 mmol) were added and the reaction mixture was stirred for one day at room temperature. The reaction mixture was diluted with 10% aqueous NaOH (30 mL) and extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was purified by column chromatography to obtain compound **86** as a yellow oil (2.74 g, 72% yield).

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 5.75 (s, 1H), 4.67 (d, J = 6.8 Hz, 1H), 4.63 (d, J = 6.4 Hz, 1H), 3.67 (dd, J = 10.0, 7.2 Hz, 1H), 3.35 (s, 3H), 2.70 (dddd, apparent qt, J = 19.6, 11.6, 2.0, 2.0 Hz, 1H), 2.55-2.46 (m, 1H), 2.42-2.31 (m, 2H), 2.18-2.09 (m, 2H), 1.88-1.76 (m, 2H), 1.14 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 199.0, 174.3, 123.2, 96.0, 85.3, 55.3, 44.8, 34.3, 33.2, 26.8, 26.4, 15.8

(1*S*,5*R*,7a*S*)-1-(Methoxymethoxy)-5-(4-(methoxymethoxy)-2-((methoxymethoxy)methyl)phenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-ol



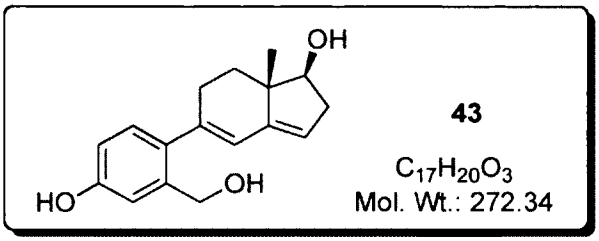
The protected A-ring, compound **81** (1.95 g, 6.70 mmol) was dissolved in dry THF (20 mL) and cooled to -78°C using a dry ice/ acetone bath. The solution was kept under nitrogen atmosphere. n-buthyllithium (6.70 mmol, 4.11 mL, 1.63 M) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes. The protected unsaturated CD-ring, compound **86** (0.939 g, 4.47 mmol) was dissolved in dry THF (2 mL), and then added dropwise to the reaction mixture. The reaction was stirred for 10 minutes and then was quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The mixture was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The obtained crude product (2.25 g) was a beige semi-solid. The crude product was purified using column chromatography, from which an isomeric mixture was obtained (0.438 g, 23.2% yield, 1:10 **a:b**) as a yellow oil. The debrominated A-ring (0.661 g, 3.11 mmol) and unreacted CD-ring (0.120 g, 0.570 mmol) was also obtained.

Compound 87 Only the peaks of the major isomer are listed

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.18 (d, J = 2.8 Hz, 1H), 7.07 (d, J = 8.8 Hz, 1H), 6.82 (dd, J = 8.8, 2.8 Hz, 1H), 5.31 (br, 1H), 5.13 (s, 2H), 4.96 (d, J = 12.4 Hz, 1H), 4.89 (d, J = 12.4 Hz, 1H), 4.71 (s, 2H), 4.61 (d, J = 6.8 Hz, 1H), 4.56 (d, J = 6.8 Hz, 1H), 3.53 (dd, apparent t, J = 8.4, 8.4 Hz, 1H), 3.43 (s, 3H), 3.38 (s, 3H), 3.29 (s, 3H), 2.91 (br, 1H), 2.57 (dd, J = 15.6, 13.6 Hz, 1H), 2.28–2.20 (m, 1H), 2.17-2.06 (m, 3H), 1.78-1.62 (m, 2H), 1.17 (ddd, J = 13.2, 13.2, 3.6 Hz, 1H), 1.06 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 156.1, 146.9, 137.7, 137.5, 129.3, 125.9, 118.6, 113.5, 95.8, 95.6, 94.1, 85.6, 75.4, 67.7, 55.8, 55.3, 55.0, 43.2, 35.2, 32.8, 26.8, 25.4, 16.4

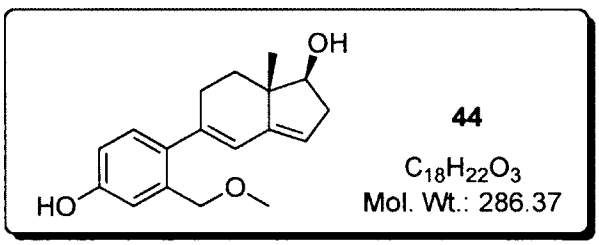
(1S,7aS)-5-(4-Hydroxy-2-(hydroxymethyl)phenyl)-7a-methyl-2,6,7,7a-tetrahydro-1H-inden-1-ol



43

$C_{17}H_{20}O_3$
Mol. Wt.: 272.34

(1S,7aS)-5-(4-hydroxy-2-(methoxymethyl)phenyl)-7a-methyl-2,6,7,7a-tetrahydro-1H-inden-1-ol



44

$C_{18}H_{22}O_3$
Mol. Wt.: 286.37

Compound **87** (0.383 g, 0.906 mmol) was diluted with 5mL of methanol, and 3-5 drops of concentrated HCl were added. The reaction mixture was refluxed for 1 hour then cooled to room temperature and diluted with water (5 mL). The methanol was removed by rotary evaporation and the reaction mixture was extracted with EtOAc (3 x 15 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The obtained crude mixture was purified by column and plate chromatography and HPLC to yield compounds **43** (38 mg, 15% yield) and **44** (31 mg, 12% yield), both as white solids.

Compound 43

^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 8.22 (br, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.68 (dd, J = 8.4, 2.8 Hz, 1H), 6.00 (d, J = 2.4 Hz, 1H), 5.29 (s, 1H), 4.54 (s, 2H), 3.99 (dd, apparent t, J = 8.4, 8.4 Hz, 1H), 3.93 (br, 1H), 3.93 (br, 1H), 2.61-

2.50 (m, 1H), 2.49-2.34 (m, 2H), 2.29 (dd, J = 18.4, 5.2 Hz, 1H), 1.95 (ddd, J = 12.8, 5.2, 1.2 Hz, 1H), 1.45 (ddd, apparent td, J = 12.4, 5.6, 5.6 Hz, 1H), 1.01 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) = 158.2, 148.2, 142.5, 141.4, 135.2, 130.7, 124.4, 120.3, 116.2, 115.2, 83.2, 63.4, 46.4, 39.6, 36.2, 16.6 (missing -CH2 hidden in Acetone-d6, based on dept 135)

Mass (EI): *m/z* (%) = 272.1 (M+, 91.1), 254.1 (64.4), 239.1 (32.9), 211.1 (45.0), 184.1 (100.0), 165.1 (36.9), 131.1 (20.3), 115.1 (30.5), 77.0 (31.7), 65.0 (18.5), 39.0 (22.4), 27.0 (12.8)

HRMS: calcd 272.1425, found 272.1425

Compound 44

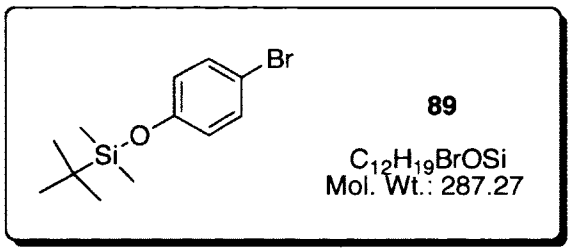
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.01 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 2.8 Hz, 1H), 6.72 (dd, J = 8.4, 2.8 Hz, 1H), 6.01 (d, J = 2.4 Hz, 1H), 5.74 (br, 1H), 5.32 (s, 1H), 4.37 (s, 2H), 4.09 (dd, J = 8.8, 7.6 Hz, 1H), 3.37 (s, 3H), 2.62 (ddd, J = 16.0, 8.0, 3.6 Hz, 1H), 2.56-2.48 (m, 1H), 2.46-2.37 (m, 1H), 2.30 (dd, J = 18.8, 4.8 Hz, 1H), 1.97 (dd, J = 12.4, 4.4 Hz, 1H), 1.52 (ddd, apparent td, J = 12.4, 5.6, 5.6 Hz, 1H), 1.04 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) = 154.8, 145.7, 139.2, 136.4, 135.2, 129.4, 122.8, 118.7, 115.2, 114.5, 82.0, 72.2, 58.2, 44.5, 38.0, 34.1, 29.2, 14.8

Mass (EI): *m/z* (%) = 286.2 (M+, 15.6), 254.1 (20.0), 184.1 (18.6), 149.0 (69.1), 124.1 (28.1), 84.0 (6.9), 43.0 (100.0)

HRMS: calcd 286.1558, found 286.1558

(4-Bromophenoxy)(tert-butyl)dimethylsilane

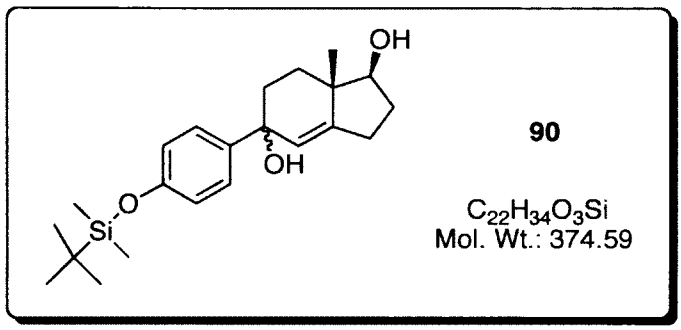


4-bromophenol **88** (10 g, 57.8 mmol) was dissolved in the minimum amount of DMF (15 mL) and stirred in an ice bath at 0°C. Imidazole (5.9 g, 86.7 mmol) was added and the reaction was stirred for 5 minutes, after which the TBDMS-Cl (13.07 g, 86.7 mmol) was added. The reaction mixture was stirred overnight at room temperature. After the reaction was completed the reaction mixture was diluted with brine (30 mL) extracted with ether (3 x 30 mL). The ether extracts were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified with column chromatography to give clear oil (15.64 g, 94% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.33 (d, J= 8.4 Hz, 2H), 6.73 (d, J= 9.2, 2H), 0.99 (s, 9H), 0.20 (s, 6H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 154.8, 132.2, 121.8, 113.6, 25.6, 18.1, -4.5

(1S,7aS)-5-(4-(Tert-butyldimethylsilyloxy)phenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1H-indene-1,5-diol



90

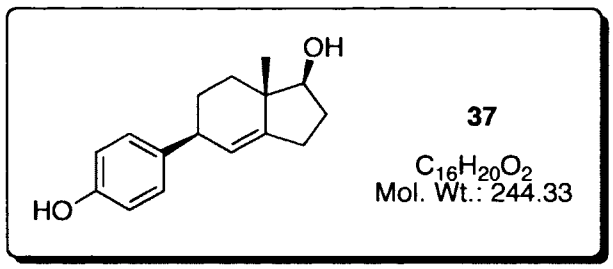
$C_{22}H_{34}O_3Si$
Mol. Wt.: 374.59

The protected A-ring, compound **89** (10 g, 34.8 mmol), was dissolved in dry THF (20 mL) and cooled to -78°C using a dry ice/ acetone bath. The solution was kept under nitrogen atmosphere. n-buthyllithium (2.23 g, 34.8 mmol) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes.

The CD-ring, compound **46** (1.45 g, 8.7 mmol), was dissolved in dry THF (2 mL), and then added dropwise to the reaction mixture. The reaction was stirred for 10 minutes and then was quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The mixture was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product was purified using column chromatography, from which a mixture of isomers was obtained as a white solid (0.278 g, 8.5% yield). Other fractions included debrominated **89** (5.7 g), CD-ring with butylated CD-ring (0.331 g) and recovered CD-ring starting material (0.838 g). The yield estimated based on recovery of starting material was 20%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.32 (d, J= 8.8 Hz, 2H), 6.77 (d, J= 8.8 Hz, 2H), 6.488 (br, 1H), 5.35 (s, 1H), 4.02 (dd, J= 8.8, 7.6, 1H), 2.56-2-48 (m, 1H), 2.25-1.91 (m, 5H), 1.78-1.57 (m, 2H), 1.23-1.56 (m, 1H), 0.97 (s, 3H), 0.96 (s, 9H), 0.17 (s, 6H)

(1S,7aS)-5-(4-Hydroxyphenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1H-inden-1-ol



37

$C_{16}H_{20}O_2$
Mol. Wt.: 244.33

Triethylsilane (0.902 g, 7.8 mmol) and $BF_3.Et_2O$ (0.663 g, 4.7 mmol) were added over a solution of compound **90** (0.142 g, 0.379 mmol) in anhydrous DCM (10 ml) at 0°C. The reacting mixture was stirred from 0°C to room temperature for 2 hours. After the reaction was completed a saturated solution of NH_4Cl (10 ml) was added. The reaction mixture was extracted with DCM (3 x 15 ml). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography and by HPLC to give pure compound **37** as white solid (56 mg, 60% yield).

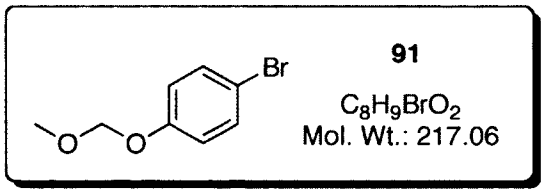
^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 8.10 (br, 1H), 7.00 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 8.8, 2H), 5.27 (s, 1H), 3.92 (br, 1H), 3.64 (dd, J= 10.0, 8.0 Hz, 1H), 3.26-3.21 (m, 1H), 3.07 (br, 1H), 2.55-2.45 (m, 1H), 2.17-2.08 (m, 1H), 1.98-1.83 (m, 3H), 1.73-1.63 (m, 1H), 1.60-1.49 (m, 1H), 1.37-1.30 (m, 1H), 1.03 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz): δ (ppm) = 157. 4, 147.6, 139.6, 130.0, 124.8, 116.9, 83.0, 44.9, 44.5, 37.2, 32.0, 30.8, 27.4, 18.6

Mass (EI): *m/z* (%) = 244.1 (M+, 23.8), 226.1 (3.5), 185.1 (8.2), 134.1 (8.5), 120.1 (100.0), 107.1 (30.7), 91.1 (14.2), 65.0 (8.6), 39.0 (14.9), 27.0 (7.7)

HRMS: calcd 244.1457, found 244.1457

1-bromo-4-(methoxymethoxy)benzene

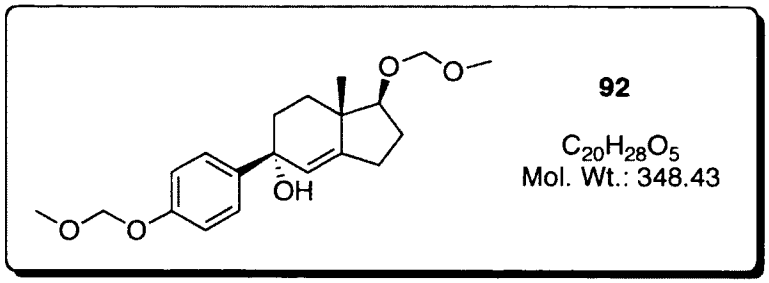


N,N-diisopropylethylamine (13.3 mL, 76.3 mmol) and chloromethyl methyl ether (5.79 mL, 76.3 mmol) were added to a solution of 4-bromo-phenol (10.2 g, 58.7 mmol) in dry DCM (50 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then left at room temperature for two hours. The organic mixture was diluted with 10% aqueous NaOH (50 mL) and extracted with DCM (3 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was a yellow oil (10.5 g, 83% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.38 (d, J = 9.2 Hz, 2H), 6.92 (d, J = 9.2 Hz, 2H), 5.14 (s, 2H), 3.46 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 156.2, 132.2, 118.0, 114.1, 94.4, 55.9

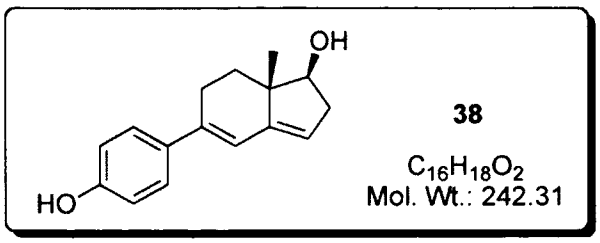
(1*S*,5*R*,7a*S*)-1-(methoxymethoxy)-5-(4-(methoxymethoxy)phenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-ol



The protected A-ring, compound **91** (1.66 g, 7.67 mmol) was dissolved in dry THF (20 mL) and placed in a dry ice/ acetone bath (-78°C). n-Butyllithium (0.491 g, 7.67 mmol) was added dropwise and the solution was left to stir for 5 minutes. The protected unsaturated CD ring **86** (1.24 g, 5.89 mmol) was dissolved in dry THF (2 mL) and added dropwise. After 10 minutes, the reaction mixture was quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The solution was extracted with EtOAc (3 x 30 mL), dried over $MgSO_4$, filtered and evaporated under vacuum. The crude reaction product was obtained as a yellow oil (2.21 g). This mixture consisted of compound **92** (1.68 g, 82%), debrominated A-ring, MOM-protected CD-ring and EtOAc.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.35 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 8.8 Hz, 2H), 5.35 (s, 1H), 5.15 (s, 2H), 4.64 (d, J = 6.4 Hz, 1H), 4.60 (d, J = 6.8 Hz, 1H), 3.57 (dd, J = 9.6, 8.4 Hz, 1H), 3.46 (s, 3H), 3.32 (s, 3H), 2.62-2.54 (m, 1H), 2.29-2.20 (m, 1H), 2.16-2.08 (m, 2H), 1.99-1.90 (m, 1H), 1.86-1.72 (m, 2H), 1.70-1.65 (m, 1H), 1.31 (dd, J = 13.6, 2.8 Hz, 1H), 1.09 (s, 3H)

(1S,7aS)-5-(4-Hydroxyphenyl)-7a-methyl-2,6,7,7a-tetrahydro-1H-inden-1-ol



Impure **92** from the previous reaction (1.00g, estimated to contain 0.759g, 2.18 mmol of **92**) was dissolved in methanol (5 mL) and concentrated HCl (5 drops) was added. The solution was refluxed for 2 hours. The mixture was concentrated *in vacuo* and subjected to silica gel column chromatography to afford a compound **38** as a yellow solid (0.379 g, 72% yield).

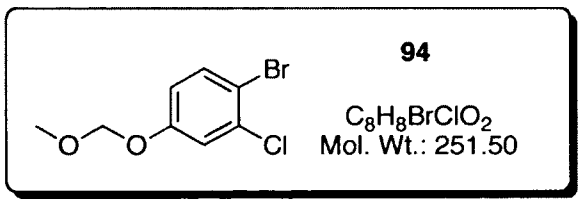
^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 8.38 (br, 1H), 7.38 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.52 (d, J = 1.6 Hz, 1H), 5.36 (s, 1H), 3.98 (dd, apparent t, J = 8.8, 8.8 Hz, 1H), 3.98 (br, 1H), 2.94 (br, 1H), 2.67-2.36 (m, 4H), 2.02 (ddd, J = 12.8, 5.2, 1.6 Hz, 1H), 1.41 (ddd, apparent td, J = 12.4, 12.4, 6.4 Hz, 1H), 0.97 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) = 158.8, 148.8, 139.0, 134.3, 128.1, 120.5, 119.8, 117.0, 83.2, 46.5, 39.7, 36.0, 27.1, 16.5

Mass (EI): *m/z* (%) = 242.1 (M+, 100.0), 199.1 (16.9), 162.0 (18.4), 107.0 (42.9), 91.1 (8.9), 57.1 (19.8), 43.0 (29.9), 27.0 (11.2)

HRMS: calcd 242.1316, found 242.1316

1-Bromo-2-chloro-4-(methoxymethoxy)benzene

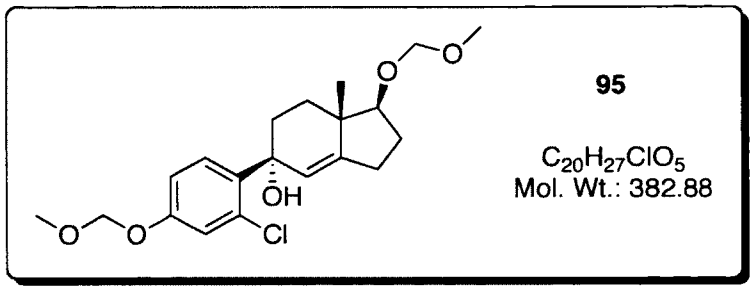


N,N-diisopropylethylamine (6.29 mL, 36.2 mmol) and chloromethyl methyl ether (2.75mL, 36.2 mmol) were added to a solution of 4-bromo-3-chlorophenol **93** (5.00 g, 24.1 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then left at room temperature for one hour. The organic mixture was diluted with 10% aqueous NaOH (30 mL) and extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was a yellow oil (5.97 g, 99% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.46 9 (d, J = 8.8 Hz, 1H), 7.16 (d, J = 2.8 Hz, 1H), 6.81 (dd, J = 8.8, 2.8 Hz, 1H), 5.13 (s, 2H), 3. 45 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 156.8, 134.7, 133.8, 118.2, 116.3, 114.1, 94.4, 56.0

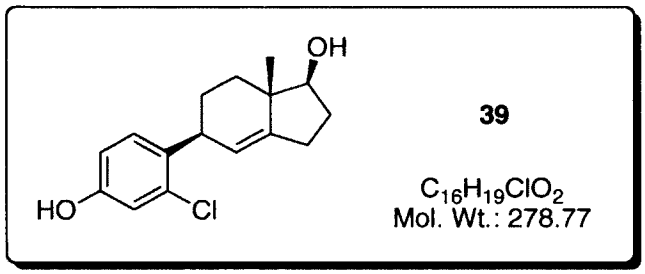
(1*S*,5*R*,7a*S*)-5-(2-chloro-4-(methoxymethoxy)phenyl)-1-(methoxymethoxy)-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-ol



The protected A-ring, compound **94** (2.33 g, 9.27 mmol) was dissolved in dry THF (20 mL) and placed in a dry ice/ acetone bath (-78 °C). n-Butyllithium (0.594 g, 9.27 mmol) was added dropwise and the solution was left to stir for 5 minutes. The protected unsaturated CD ring **86** (1.50 g 7.13 mmol) was dissolved in dry THF (2 mL) and added dropwise. After 10 minutes, the reaction mixture was quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The solution was extracted with EtOAc (3 x 30 mL), dried over $MgSO_4$, filtered and evaporated under vacuum. The crude product was obtained as a yellow oil (3.03 g) consisting of a mixture of compound **95** (1.91 g, 70%), debrominated A-ring, MOM-protected CD-ring and EtOAc.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.20 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 2.8 Hz, 1H), 6.84 (dd, J = 8.8, 2.8 Hz, 1H), 5.46 (s, 1H), 5.14 (s, 2H), 4.62 (d, J = 6.4 Hz, 1H), 4.58 (d, J = 6.8 Hz, 1H), 3.51 (dd, J = 9.6, 8.4 Hz, 1H), 3.44 (s, 3H), 3.31 (s, 3H), 2.66-2.46 (m, 2H), 2.32-2.20 (m, 1H), 2.16-2.02 (m, 2H), 1.89-1.66 (m, 2H), 1.12 (dd, J = 8.8, 2.4 Hz, 1H), 1.09 (s, 3H)

(1*S*,5*S*,7a*S*)-5-(2-chloro-4-hydroxyphenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-ol



39

$C_{16}H_{19}ClO_2$
Mol. Wt.: 278.77

Triethylsilane (2.52 g, 21.7 mmol) and $BF_3.Et_2O$ (1.85 g, 13.1 mmol) were added to a solution of crude **95** (1.0 g, estimated to contain 0.7 g, 1.82 mmol of **95**) in anhydrous DCM (10 mL) at 0°C. The reaction mixture was stirred from 0°C to room temperature for 2 hours. After this time, the reaction was not finished as judged by the TLC, thus an additional portion of $BF_3.Et_2O$ was added (0.741 g, 5.22 mmol) and the reaction was left overnight. After the reaction was completed a saturated solution of NH_4Cl (10 mL) was added. The reaction mixture was extracted with DCM (3 x 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography and to give yellow semi solid with small amount of EtOAc (0.165 g, 32% yield). The product was recrystallized and purified by plate chromatography to obtain a white solid (17 mg, 3% yield).

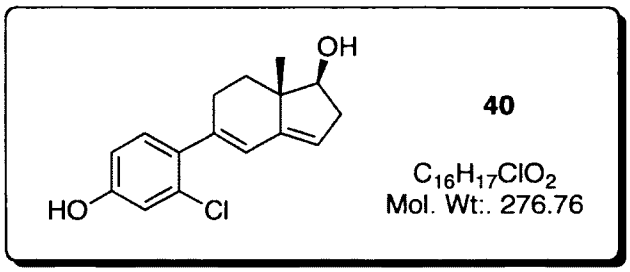
^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 8.54 (br. 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 2.8 Hz, 1H), 6.77 (dd, J = 8.4, 2.8 Hz, 1H), 5.22 (s, 1H), 3.88 (br, 1H), 3.73-3.68 (m, 1H), 3.65 (dd, apparent t, J = 8.8, 8.8 Hz, 1H), 2.57-2.47 (m, 1H), 2.20-2.11 (m, 1H), 2.02-1.81 (m, 3H), 1.73-1.63 (m, 1H), 1.47-1.28 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) = 158.1, 148.8, 136.4, 135.1, 130.9, 123.5, 117.6, 116.6, 82.9, 45.0, 40.9, 37.1, 30.8 (-CH_2 hidden in Acetone-d6, taken from DEPT 135) 29.7, 27.4, 18.5

Mass (EI): *m/z* (%) = 278.1 (M+, 20.8), 260.1 (6.6), 234.1 (11.7), 154.0 (100.0), 107.1 (84.5), 77.0 (8.1), 29.0 (9.6)

HRMS: calcd 278.1083, found 278.1083

(1*S*,7a*S*)-5-(2-chloro-4-hydroxyphenyl)-7a-methyl-2,6,7,7a-tetrahydro-1*H*-inden-1-ol



40

$C_{16}H_{17}ClO_2$
Mol. Wt:. 276.76

The impure coupling product containing compound **95** (0.210 g, estimated to contain 0.147 g, 0.384 mmol of **95**) was dissolved in methanol (5 mL) and concentrated HCl (5 drops) was added. The solution was refluxed for 2 hours. The mixture was concentrated *in vacuo* and subjected to silica gel column chromatography to afford compound **40** as a yellow solid (0.103 g, 97% yield).

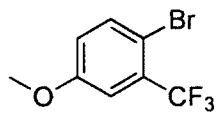
^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 8.76 (br, 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 2.4 Hz, 1H), 6.78 (dd, J = 8.4, 2.4 Hz, 1H), 6.07 (d, J = 2.4 Hz, 1H), 5.36 (s, 1H), 4.00 (dd, apparent t, J = 8.4, 8.4, 1H) 4.00 (br, 1H), 2.98 (br, 1H), 2.69-2.58 (m, 1H), 2.50-2.32 (m, 3H), 1.98-1.94 (m, 1H), 1.45 (ddd, apparent td, J = 12.4, 12.4, 5.2 Hz, 1H), 1.02 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) = 159.0, 147.9, 140.5, 135.3, 134.0, 132.5, 125.4, 121.4, 118.0, 116.0, 83.1, 46.4, 39.6, 35.9, 29.7, 16.6

Mass (EI): *m/z* (%) = 276.1 (M+, 100.0), 247.1 (52.3), 233.1 (27.9), 205.0 (14.4), 181.1 (17.5), 143.0 (21.5), 115.1 (18.4), 77.0 (21.1), 43.0 (37.4)

HRMS: calcd 276.0925, found 276.0925

1-Bromo-4-methoxy-2-(trifluoromethyl)benzene



97

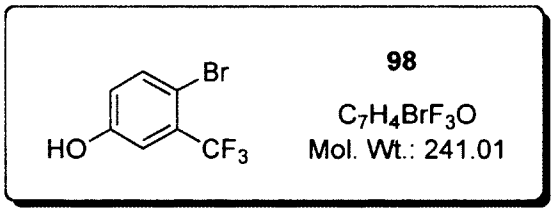
$C_8H_6BrF_3O$
Mol. Wt.: 255.03

Bromine (1.07 mL, 20.7 mmol) dissolved in glacial acetic acid (5 mL) was added dropwise using a dropping funnel, to a solution of 1-methoxy-3-(trifluoromethyl)benzene **96** (2.81 g, 15.9 mmol) in glacial acetic acid (10 mL) under nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The reaction was not complete after this time according to TLC, so more bromine (0.25 mL, 4.8 mmol) was added. After the reaction was completed brine (50 mL) and EtOAc (50 mL) were added. The aqueous layer was washed with EtOAc (3 x 50 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography to give a clear oil (2.31 g, 57% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.57 (dd, J = 8.8, 0.4 Hz, 1H), 7.21 (d, J = 3.2 Hz, 1H), 6.91 (dd, J = 8.8, 2.8 Hz, 1H), 3.83 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 158.6, 135.6, 130.7 (q, J = 31 Hz, 1C), 122.6 (q, J = 271.7 Hz, 1C), 118.2, 113.8 (q, J = 5.6 Hz, 1C), 109.8 (q, J = 1.8 Hz, 1C), 55.6

4-Bromo-3-(trifluoromethyl)phenol



98

$C_7H_4BrF_3O$

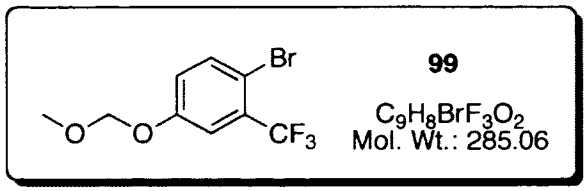
Mol. Wt.: 241.01

BBr_3 (1.28 mL, 13.6 mmol) was added dropwise to a solution of **97** (2.31 g, 9.06 mmol) in toluene (10 mL) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for one hour. After the reaction was completed crushed ice (~30 g) and EtOAc (30 mL) were added. The aqueous layer was washed with EtOAc (3 x 30 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography to give a brown oil (1.70 g, 80% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.52 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 2.8 Hz, 1H), 6.86 (ddd, J = 8.8, 3.2, 0.4 Hz, 1H), 5.45 (br, 1H),

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 154.5, 135.9, 131.0 (q, J = 31.3 Hz, 1C) 122.4 (q, J = 271.7 Hz, 1C), 119.9, 115.2 (q, J = 5.5 Hz, 1C), 110.1 (q, J = 1.8 Hz, 1C)

1-Bromo-4-(methoxymethoxy)-2-(trifluoromethyl)benzene



99

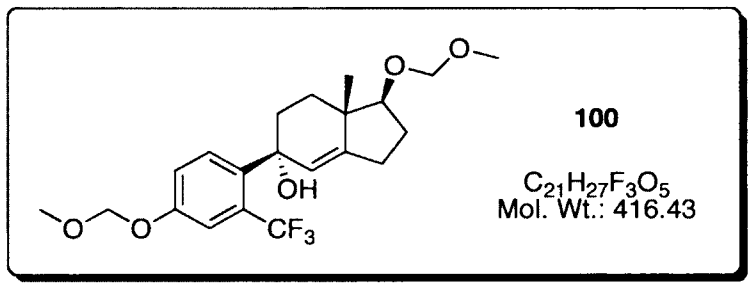
$C_9H_8BrF_3O_2$
Mol. Wt.: 285.06

N,N-diisopropylethylamine (1.77 mL, 10.6 mmol) and chloromethyl methyl ether (0.804 mL, 10.6 mmol) were added to a solution of 4-Bromo-3-(trifluoromethyl)phenol, compound **98** (1.70 g, 7.05 mmol) in dry DCM (15 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then was left at room temperature for 1 hour, after which 10% aqueous NaOH (20 mL) was added. The mixture was extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product was purified by column chromatography to obtain the desired compound **99** as a clear oil (1.67 g, 83% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.58 (dd, J = 8.8, 0.4 Hz, 1H), 7.36 (d, J = 2.8 Hz, 1H), 7.07 (ddd, J = 8.8, 3.2, 0.4 Hz, 1H), 5.18 (s, 2H), 3.47 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 156.2, 135.7, 130.9 (q, J = 31.2 Hz, 1C), 122.5 (q, J = 271.8 Hz, 1C), 120.4, 116.2 (q, J = 5.5 Hz, 1C), 111.2 (q, J = 1.9 Hz), 94.5, 56.2

(1*S*,5*R*,7a*S*)-1-(methoxymethoxy)-5-(4-(methoxymethoxy)-2-(trifluoromethyl)phenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-ol

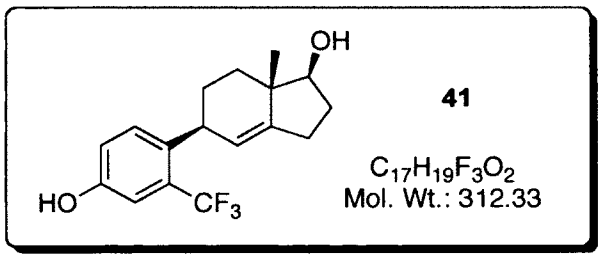


Compound **99** (0.835 g, 2.93 mmol) was dissolved in dry THF (10 mL) under a nitrogen atmosphere. The mixture was cooled to -78°C using a dry ice/ acetone bath. n-Buthyllithium (1.32 mL, 2.23 M) was added dropwise to the reaction mixture and was stirred for 5 minutes. A solution of compound **86** (0.616 g, 2.93 mmol) dissolved in dry THF (2 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred for 10 min at -78°C, quenched with saturated NH_4Cl solution (10 mL) and water (10 mL) and extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated to yield a crude product (1.14 g) which was purified by column chromatography to yield **100** as a clear oil (0.470 g, 39% yield). The yield based on the recovery of the starting material **86** (0.220 g) was 60%.

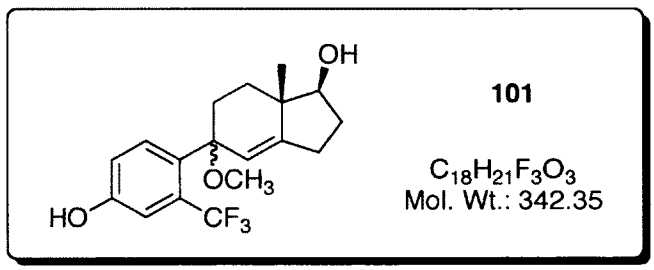
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.44 (d, J = 2.8 Hz, 1H), 7.24 (d, J = 8.8 Hz, 1H), 7.09 (dd, J = 8.8, 2.8 Hz, 1H), 5.31 (s, 1H), 5.18 (s, 2H), 4.67 (d, J = 6.8 Hz, 1H), 4.62 (d, J = 6.8 Hz, 1H), 3.61 (dd, J = 9.6, 8.0 Hz, 1H), 3.47 (s, 3H), 3.34 (s, 3H), 2.67-2.58 (m, 1H), 2.33-2.24 (m, 2H), 2.19-2.04 (m, 2H), 1.84-1.76 (m, 1H), 1.71 (ddd, apparent dt, J = 13.6, 3.6, 3.6 Hz, 1H), 1.30-1.22 (m, 1H), 1.10 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 155.8, 145.1, 138.02 132.1, 131.5, 123.3, 119.6, 118.8, 114.1, 96.2, 94.5, 86.7, 55.1, 55.2, 44.3, 36.6, 35.7, 34.6, 29.6, 27.7, 15.4

(1*S*,5*S*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-ol



(1*S*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-5-methoxy-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-ol



Triethylsilane (0.544 g, 4.68 mmol) and $BF_3.Et_2O$ (0.561 g, 3.95 mmol) were added to a solution of compound **100** (0.194 g, 0.466 mmol) in anhydrous DCM (10 mL) at 0°C. The reaction mixture was stirred, and allowed to warm to room temperature over 2 hours. After the reaction was completed a saturated solution of NH_4Cl (10 mL) was added. The reaction mixture was extracted with DCM (3 x 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography and to give yellow semi solid compound **41** (9.5 mg, 0.065%) yield. The side product **101** (80 mg, 50% yield) was also formed.

Compound 41

^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 8.86 (br, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.03 (ddd, J = 8.4, 2.8, 0.4 Hz, 1H), 5.45 (d, J = 0.8 Hz, 1H), 3.75 (t, J = 7.6 Hz, 1H), 3.75 (br, 1H), 2.41-2.24 (m, 2H), 2.22-2.06 (m, 3H), 1.92 (ddd, J = 12.4, 6.0, 2.0 Hz, 1H), 1.69-1.54 (m, 2H), 1.51-1.37 (m, 2H), 0.88 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) =157.8, 137.4, 136.0, 134.0, 130.3, 130.1, 126.2 (q, J = 271.3 Hz, 1C), 120.41, 114.23 (q, J = 5.5 Hz, 1C), 81.0, 45.9, 43.9, 35.8, 32.4, 32.0, 25.8, 11.8

Mass (EI): *m/z* (%) = 312.1 (M+, 26.8), 294.1 (32.8), 268.1 (53.4), 253.0 (49.3), 188.0 (100.0), 110.0 (41.7), 109.0 (54.3), 107.0 (74.3), 97.0 (39.2), 43.0 (19.0)

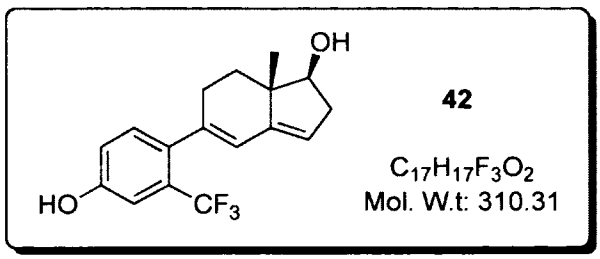
HRMS: calcd 312.1318, found 312.1318

Compound 101 (Only the peaks of the major isomer are listed)

^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 9.10-8.55 (br, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.02 (dd, J = 8.4, 2.0 Hz, 1H), 3.36 (dd, J = 8.8, 6.8 Hz, 1H), 3.31 (s, 3H), 3.11-2.89 (br, 1H), 2.41-2.29 (m, 1H), 2.27-2.16 (m, 1H), 2.16-2.06 (m, 1H), 1.99 (ddd, J = 12.4, 6.4, 1.6 Hz, 1H), 1.71-1.52 (m, 3H), 1.49-1.28 (m, 1H), 0.88 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) =157.9, 157.8, 142.6, 137.5, 135.6, 134.0, 133.9, 132.5, 132.5, 129.7, 129.6, 127.6, 124.9, 120.3, 114.2, 114.1, 90.5, 89.0, 58.8, 58.5, 46.3, 45.9, 43.8, 43.7, 36.8, 32.6, 31.9, 29.8, 25.7, 21.2, 12.1

(1*S*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyl-2,6,7,7a-tetrahydro-1*H*-inden-1-ol



42

$C_{17}H_{17}F_3O_2$
Mol. W.t: 310.31

Compound **100** (0.276 g, 0.662 mmol) was diluted with 5 mL of methanol, to which 3-5 drops of concentrated HCl were added. The reaction mixture was refluxed for 1 hour, then cooled to room temperature and diluted with water (5 mL). The methanol was removed by rotary evaporation and the reaction mixture was purified by column chromatography to yield compound **42** as a yellow solid, with acetone as a minor impurity, (0.166 g, 81% yield).

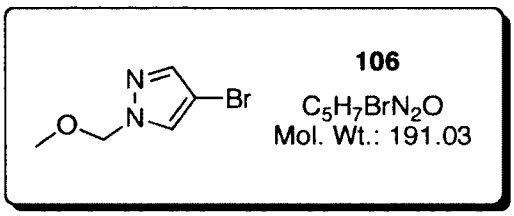
^{1}H NMR (Acetone-d6, 400 MHz): δ (ppm) = 9.12-8.76 (br, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 2.8 Hz, 1H), 7.05 (dd, J = 8.4, 2.8 Hz, 1H), 6.00 (d, J = 2.0 Hz, 1H), 5.34 (s, 1H), 4.29-4.09 (br, 1H), 4.02 (t, J = 8.0 Hz, 1H), 2.62-2.37 (m, 3H), 2.25 (dd, J = 18.4, 5.6 Hz, 1H), 1.97 (dd, J = 12.4, 4.4 Hz, 1H), 1.46 (ddd, J = 12.4, 12,4, 5.6 Hz, 1H), 1.02 (s, 3H)

^{13}C NMR (Acetone-d6, 100 MHz,): δ (ppm) = 158.1, 147.6, 139.8, 135.6 (q, J = 2.1 Hz, 1C), 133.7, 130.0 (q, J = 29.3, 1C), 126.2 (q, J = 271.5 Hz, 1C), 125.34 (q, J = 1.8 Hz, 1C), 121.4, 120.5, 114.3 (q, J = 5.3 Hz, 1C), 83.1, 46.3, 39.5, 36.0, 31.4, 16.4

Mass (EI): *m/z* (%) = 310.1 (M+, 100.0), 281.1 (87.6), 270.1 (57.0), 254.1 (43.6), 213.1 (33.3), 167.0 (18.6), 162.0 (17.5), 152.0 (12.0), 151.0 (14.2), 143.0 (13.5), 115.1 (13.9), 91.1 (14.1), 77.0 (18.8), 63.0 (22.1), 43.0 (55.8), 39.0 (28.5), 29.0 (20.1), 27.0 (25.9)

HRMS: calcd 310.1176, found 310.1176

4-Bromo-1-(methoxymethyl)-1*H*-pyrazole



106

$C_5H_7BrN_2O$

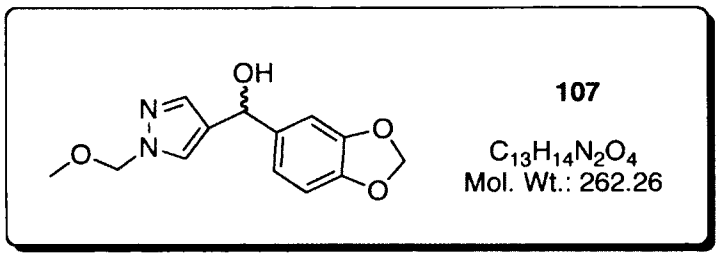
Mol. Wt.: 191.03

N,N-diisopropylethylamine (7.11 mL, 40.8 mmol) and chloromethyl methyl ether (3.10 mL, 40.8 mmol) were added to a solution of 4-bromo-1*H*-pyrazole **105** (3.00 g, 20.4 mmol) in dry DCM (30 mL) under nitrogen atmosphere at 0°C. The resulting yellow mixture was stirred for 30 minutes at 0°C, then left at room temperature for 2 hours. The organic mixture was diluted with 10% aqueous NaOH (30 mL) and extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting product **106** was yellow oil (3.78 g, 97% yield).

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.53 (s, 1H), 7.43 (s, 1H), 5.26 (s, 2H), 3.23 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 140.3, 129.4, 94.4, 82.1, 56.5

Benzo[*d*][1,3]dioxol-5-yl(1-(methoxymethyl)-1*H*-pyrazol-4-yl)methanol

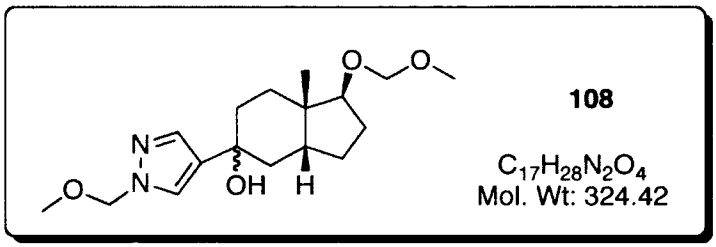


The protected A-ring, compound **106** (0.225 g, 1.18 mmol), was dissolved in dry THF (20 mL) and cooled to -78°C using dry ice/ acetone bath. The solution was kept under nitrogen atmosphere. n-buthyllithium (0.471 g, 1.18 mmol) was added dropwise to the reaction mixture and the solution was stirred for 5 minutes. Benzo[*d*][1,3]dioxole-5-carbaldehyde **62** (0.118 g, 0.785 mmol), was dissolved in dry THF (2 mL), and then added dropwise to the reaction mixture. The reaction was stirred for 10 minutes and then was quenched with saturated NH_4Cl (10 mL) solution and water (10 mL). The mixture was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated. The crude product (0.218 g) was purified using column chromatography to obtain a beige semi-solid (78.0 mg, 37.9% yield).

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.34 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 1.6 Hz, 1H), 6.82 (ddd, J = 8.0, 1.6, 0.8 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.09 (d, J = 1.6 Hz, 1H), 5.93 (s, 2H), 5.86 (s, 1H), 5.36 (d, J = 11.2 Hz, 1H), 5.27 (d, J = 10.8 Hz, 1H), 3.94 (br, 1H), 3.24 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 147.7, 147.3, 145.4, 138.6, 134.7, 119.8, 108.0, 107.3, 107.0, 101.0, 80.0, 67.5, 56.3

(1*S*,3a*R*,7a*S*)-1-(methoxymethoxy)-5-(1-(methoxymethyl)-1*H*-pyrazol-4-yl)-7a-methyloctahydro-1*H*-inden-5-ol



Protected A-ring, compound **106** (0.675 g, 3.53 mmol) was dissolved in dry THF (20 mL) and placed in a dry ice/ acetone bath (-78°C). n-Butyllithium (0.226 g, 3.53 mmol) was added dropwise and the solution was left to stir for 5 minutes. The protected CD ring **56** (0.500 g, 2.36 mmol) was dissolved in dry THF (2 mL) and added dropwise. After 10 minutes, the reaction mixture was quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The solution was extracted with EtOAc (3 x 30 mL), dried over $MgSO_4$, filtered and evaporated under vacuum. The crude product (0.795 g) was purified by column chromatography to afford a mixture of both isomers as a yellow oil (0.420 g, 55% yield). The isomers were separated by column chromatography to obtain **108a** in 0.280 g (37%) and **108b** in 0.140 g in (18%).

Compound 108a

^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.43 (d, J = 0.8 Hz, 1H), 7.41 (d, J = 0.8 Hz, 1H), 5.21 (s, 2H), 4.55 (d, J = 6.8 Hz, 1H), 4.47 (d, J = 6.4 Hz, 1H), 3.96 (dd, apparent t, J = 6.8, 6.8 Hz, 1H), 3.24 (s, 3H), 3.19 (s, 3H), 2.80 (br, 1H), 2.10-2.00 (m, 1H), 1.92-1.82 (m, 3H), 1.79-1.58 (m, 3H), 1.55-1.40 (m, 3H), 1.16-1.08 (m, 1H), 0.77 (s, 3H)

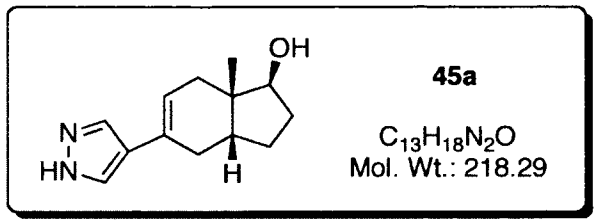
^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 137.7, 129.3, 126.9, 95.6, 81.6, 80.4, 69.1, 56.3, 54.8, 42.4, 41.9, 41.1, 34.3, 29.7, 27.4, 27.0, 20.9

Compound 108b

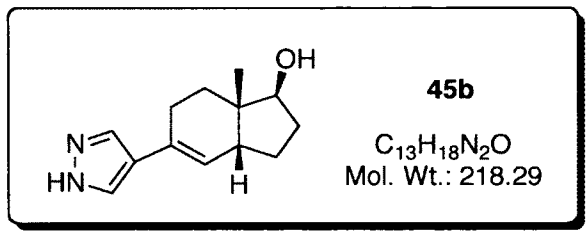
^{1}H NMR ($CDCl_3$, 400 MHz,): δ (ppm) = 7.46 (s, 1H), 7.44 (s, 1H), 5.26 (s, 2H), 4.61 (d, J = 6.8 Hz, 1H), 4.55 (d, J = 6.8 Hz, 1H), 4.10 (dd, apparent t, J = 8.0, 8.0 Hz, 1H), 3.31 (s, 3H), 3.26 (s, 3H), 2.14-1.92 (m, 4H), 1.86-1.55 (m, 5H), 1.37 (dd, apparent t, J = 12.8, 13.6 Hz, 1H), 1.17-1.10 (m, 1H), 0.93 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz,): δ (ppm) = 137.0, 132.9, 125.9, 96.0, 81.8, 77.8, 68.7, 56.6, 55.0, 42.7, 42.0, 40.0, 34.0, 27.8, 27.2, 26.5, 22.3

(1*S*,3a*R*,7a*S*)-7a-methyl-5-(1*H*-pyrazol-4-yl)-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol



(1*S*,3a*R*,7a*S*)-7a-methyl-5-(1*H*-pyrazol-4-yl)-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol



A mixture of both isomers of compound **108** (0.420 g, 1.29 mmol) was dissolved in methanol (5 mL) and conc. HCl (5 drops) was added. The solution was refluxed for 2 hours. The mixture was concentrated *in vacuo* and subjected to column chromatography to afford a mixture of both isomers as a beige semi–solid (0.198 g, 70% yield). The two isomers were separated by HPLC to afford three isomers **45a** (14 mg, 5%), **45b** (33 mg, 12% yield) and **45c** (77 mg, 27% yield) as white solids.

Compound 45a

^{1}H NMR (Methanol-d4, 400 MHz): δ (ppm) = 7.61 (s, 2H), 5.97-5.96 (m, 1H), 3.74 (t, J = 8.8 Hz, 1H), 2.43-2.38 (m, 1H), 2.21 (ddd, J = 17.2, 5.6, 1.6 Hz, 1H), 2.12-1.99 (m, 3H), 1.79-1.65 (m, 2H), 1.60-1.37 (m, 2H), 0.76 (s, 3H)

^{13}C NMR (Methanol-d4, 100 MHz,): δ (ppm) = 129.2, 125.1, 121.1, 82.3, 42.8, 42.1, 39.7, 31.7, 30.7, 26.5, 10.7

Mass (EI): *m/z* (%) = 218.1 (M+, 80.1), 173.1 (14.2), 159.0 (100.0), 145.0 (26.2), 119.0 (16.9), 108.0 (37.7), 41.0 (25.1), 39.0 (24.2), 29.0 (12.7)

HRMS: calcd 218.1425, found 218.1425

Compound 45b

^{1}H NMR (Methanol-d4, 400 MHz): δ (ppm) = 7.61 (s, 2H), 5.92 (d, J = 0.8 Hz, 1H), 3.71 (dd, J = 6.8, 2.4 Hz, 1H), 2.44-2.37 (m, 1H), 2.25-2.16 (m, 2H), 2.11-2.15 (m, 1H), 1.92-1.78 (m, 2H), 1.73 (dd, J = 18.0, 4.8 Hz, 1H), 1.56-1.48 (m, 1H), 1.43-1.32 (m, 1H), 1.00 (s, 3H)

^{13}C NMR (Methanol-d4, 100 MHz,): δ (ppm) = 127.2, 125.3, 119.4, 81.1, 43.7, 40.8, 33.2, 32.4, 29.4, 28.6, 20.6

Mass (EI): *m/z* (%) = 218.1 (M+, 99.1), 203.1 (6.5), 185.1 (20.2), 174.1 (11.9), 159.1 (70.9), 145.1 (26.1), 120.1 (100.0), 109.1, 97.1 (27.4), 81.0 (48.0), 53.0 (13.5), 39.0 (26.6), 27.0 (12.5)

HRMS: calcd 218.1411, found 218.1411

Compound 45c

^{1}H NMR (Methanol-d4, 400 MHz): δ (ppm) = 7.60 (s, 2H), 5.95 (d, J = 4.0 Hz, 1H), 3.80 (t, J = 6.0 Hz, 1H), 2.34 - 2.25 (m, 3H), 2.13 - 2.03 (m, 2H), 1.64 – 1.54 (m, 2H), 1.45 – 1.39 (m, 1H), 1.36 – 1.27 (m, 1H), 0.96 (s, 3H)

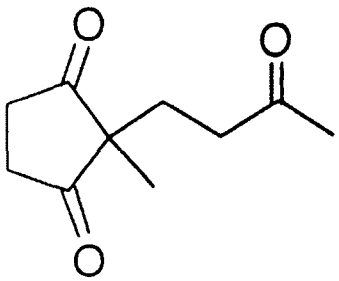
^{13}C NMR (Methanol-d4, 100 MHz,): δ (ppm) = 127.3, 125.6, 125.0, 79.3, 45.3, 43.6, 32.5, 31.0, 29.9, 25.3, 20.1

Mass (EI): *m/z* (%) = 218.1 (M+, 96.3), 200.1 (17.6), 159.1 (100.0), 145.1 (57.3), 120.1 (65.5), 109.1 (78.2), 94.1 (63.6), 81.0 (85.0), 57.0 (23.8), 39.0 (52.8), 28.0 (27.3)

HRMS: calcd 218.1419, found 218.1419

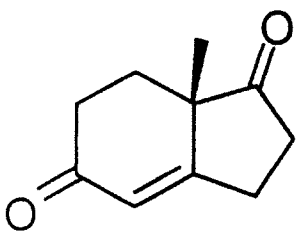
Appendix

CDCl3, NMR 300 MHz



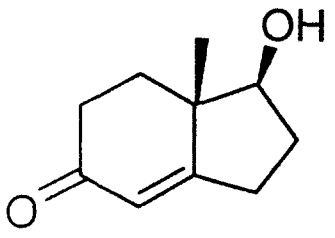
50

CDCl3, NMR 400 MHz



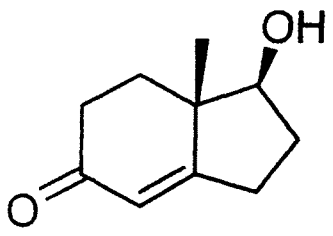
53

CDCl3, NMR 400 MHz



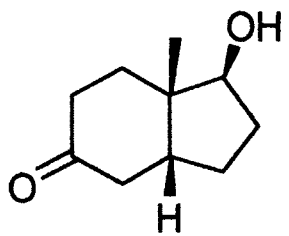
46

CDCl3, NMR 100 MHz



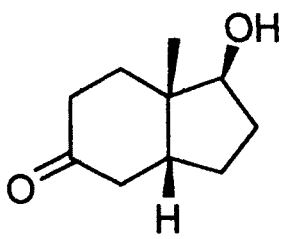
46

CDCl3, NMR 400 MHz

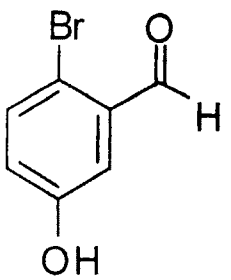


47

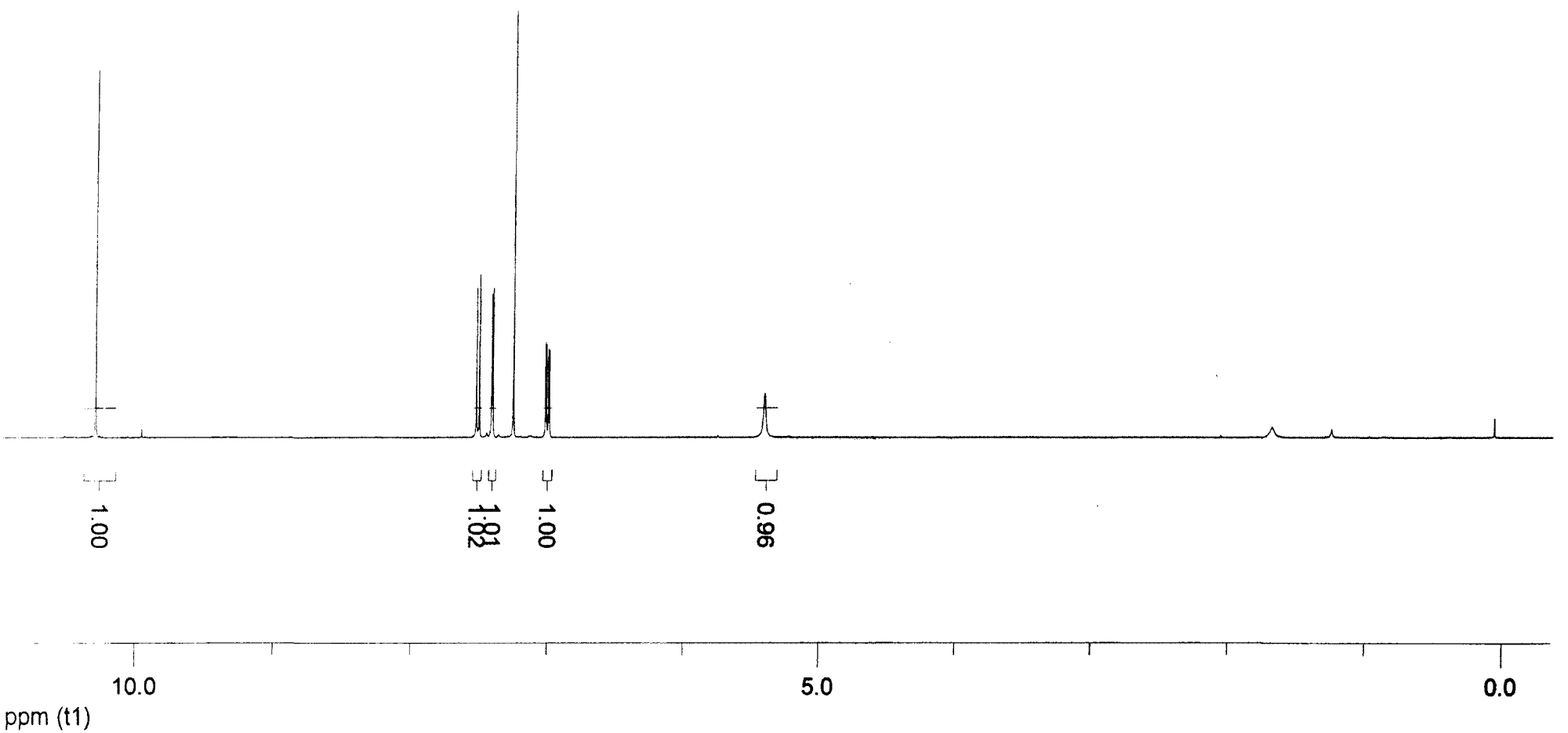
CDCl3, NMR 100 MHz



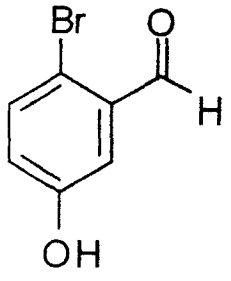
CDCl3, NMR 400 MHz



59

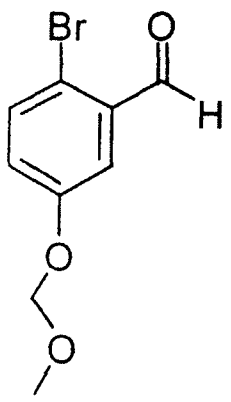


CDCl3, NMR 100 MHz



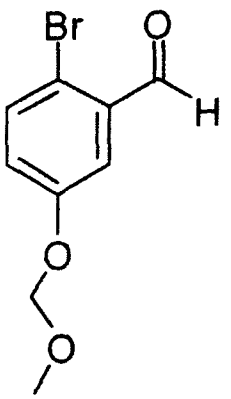
59

CDCl3, NMR 400 MHz



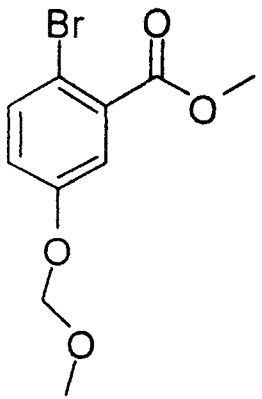
60

CDCl3, NMR 100 MHz



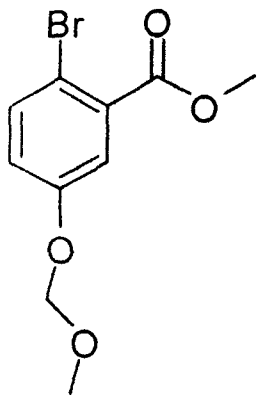
60

CDCl3, NMR 400 MHz



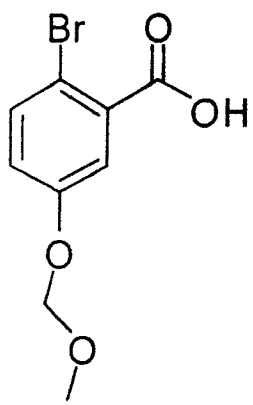
61

CDCl3, NMR 100 MHz



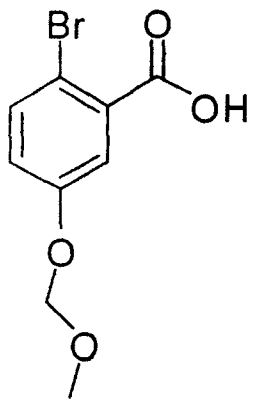
61

CDCl3, NMR 400 MHz



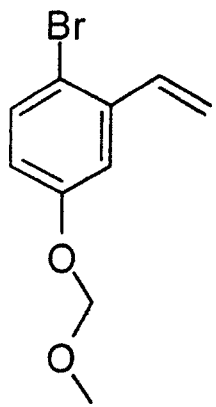
55

CDCl3, NMR 100 MHz



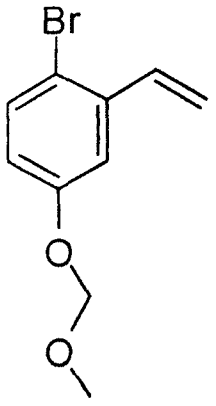
55

CDCl3, NMR 400 MHz



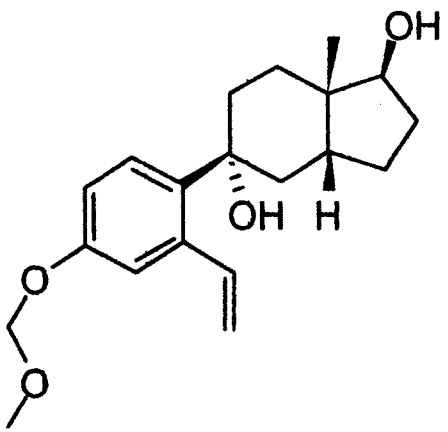
65

CDCl3, NMR 100 MHz



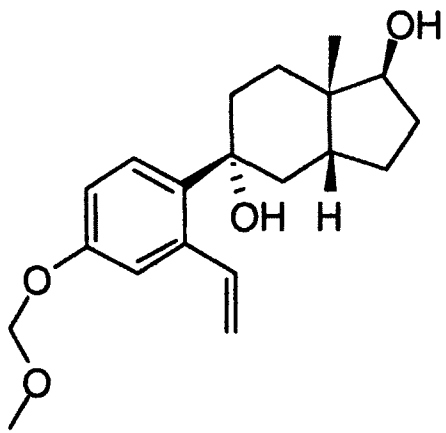
65

CDCl3, NMR 400 MHz



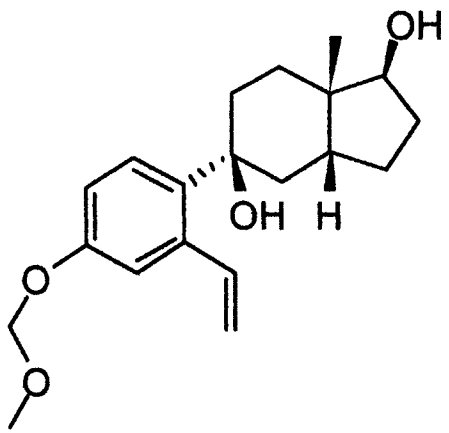
66a

CDCl3, NMR 100 MHz



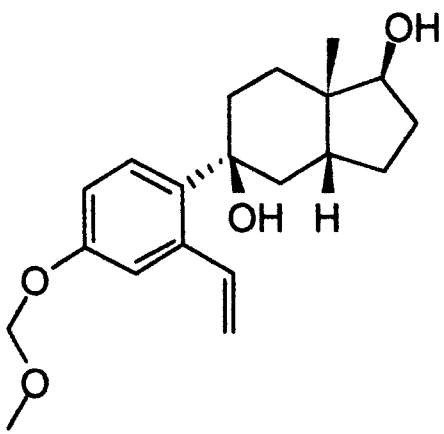
66a

CDCl3, NMR 400 MHz



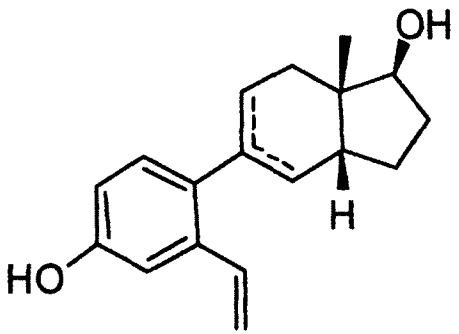
66b

CDCl3, NMR 100 MHz



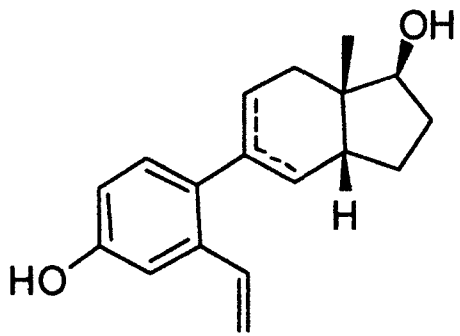
66b

CDCl3, NMR 400 MHz



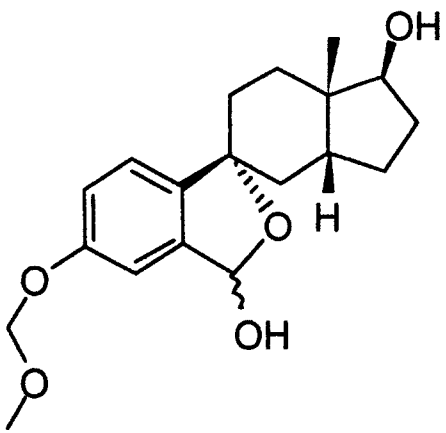
30 a + b

CDCl3, NMR 100 MHz



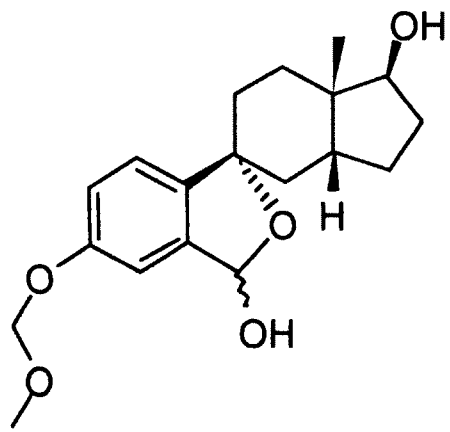
30 a + b

CDCl3, NMR 400 MHz



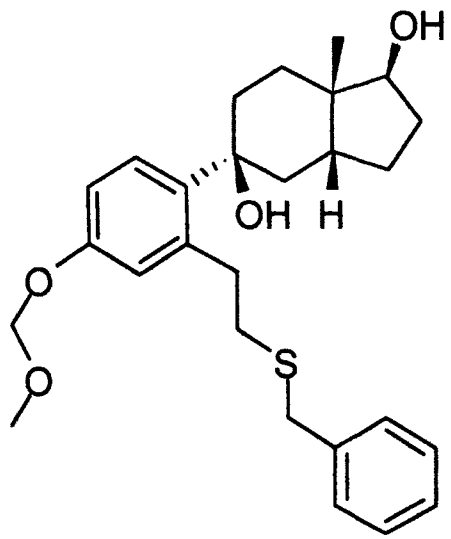
67

CDCl3, NMR 100 MHz



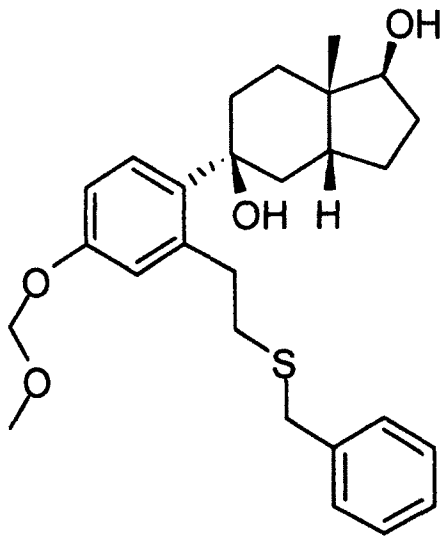
67

CDCl3, NMR 400 MHz



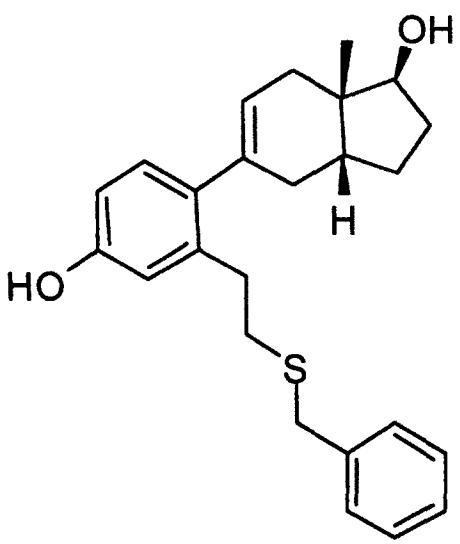
70

CDCl3, NMR 100 MHz



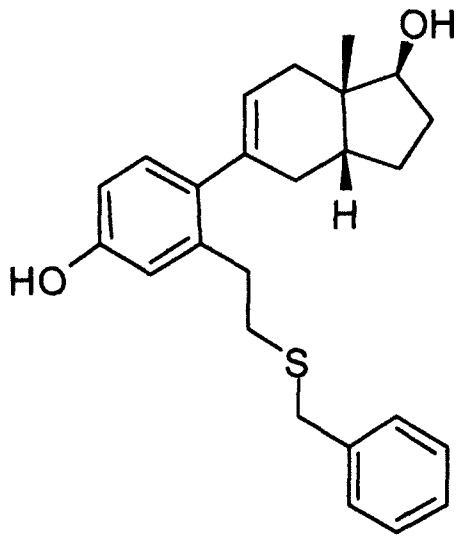
70

CDCl3, NMR 400 MHz



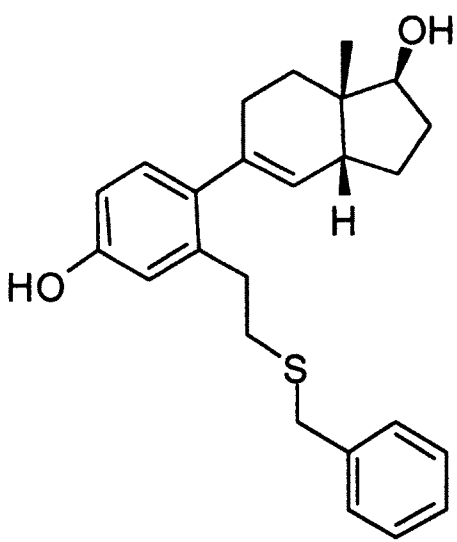
31a

CDCl3, NMR 100 MHz



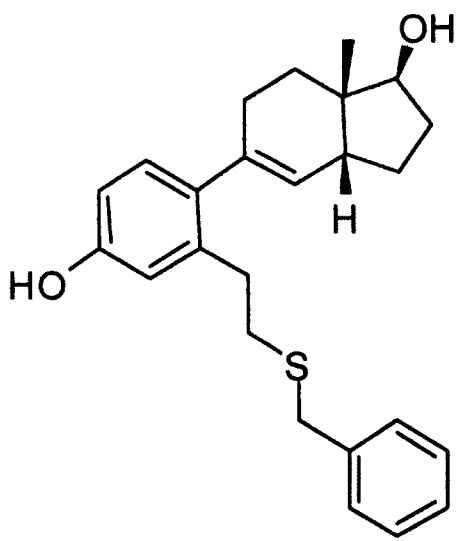
31a

CDCl3, NMR 400 MHz



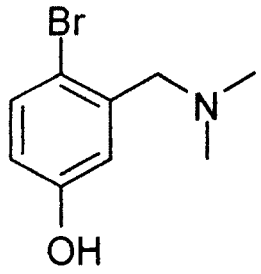
31b

CDCl3, NMR 100 MHz



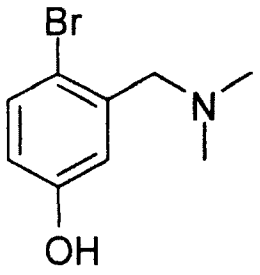
31b

CDCl3, NMR 400 MHz



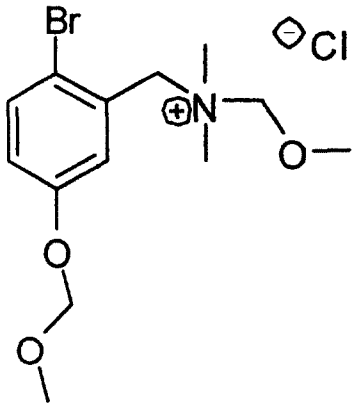
72

CDCl3, NMR 100 MHz



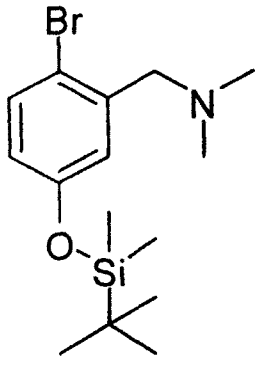
72

NMR, 400 MHz



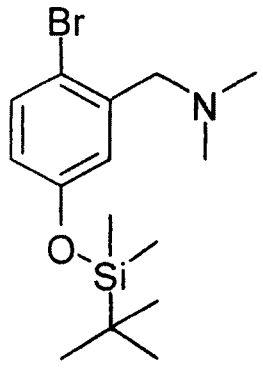
73

CDCl3, NMR 400 MHz



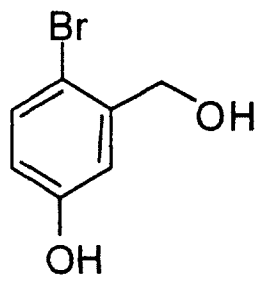
74

CDCl3, NMR 100 MHz



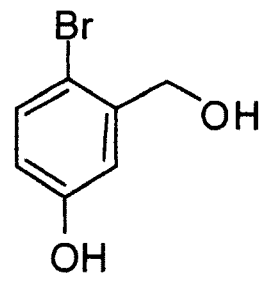
74

Acetone-d6, NMR 400 MHz



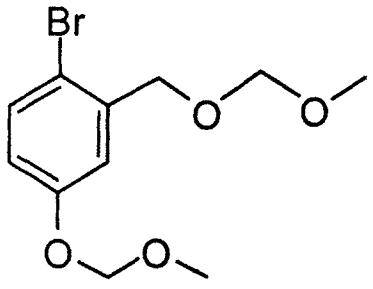
80

Acetone-d6, NMR 100 MHz



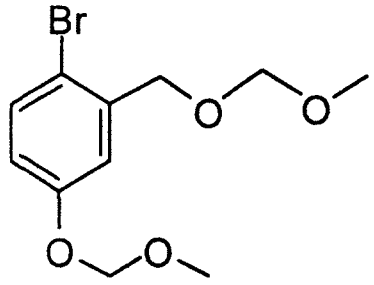
80

CDCl3, NMR 400 MHz



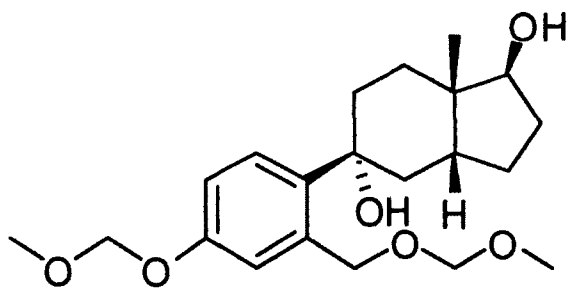
81

CDCl3, NMR 100 MHz



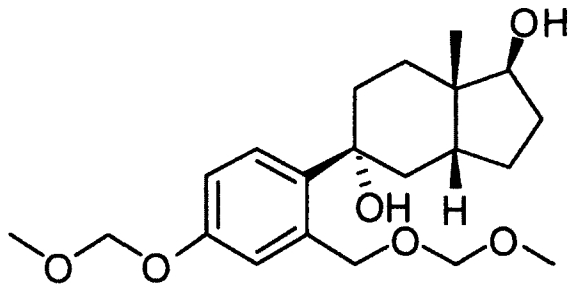
81

CDCl3, NMR 400 MHz



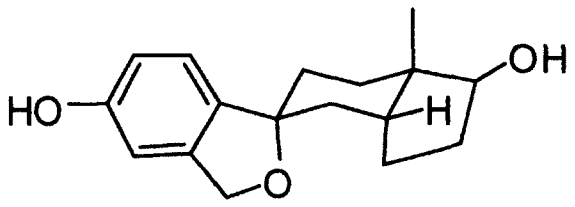
82a

CDCl3, NMR 100 MHz



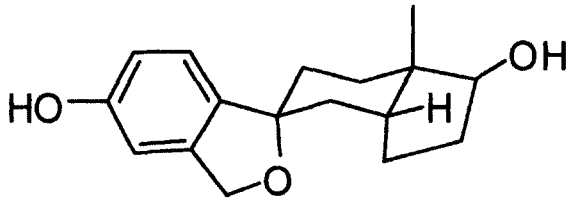
82a

CDCl3, NMR 400 MHz



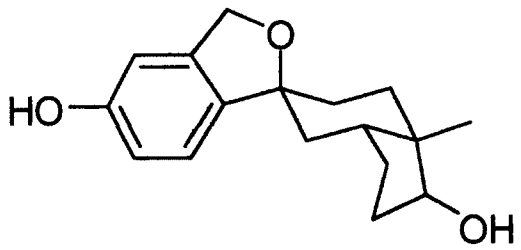
34a

CDCl3, NMR 100 MHz



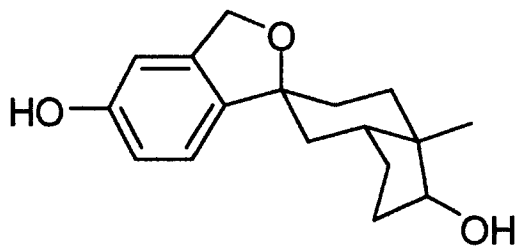
34a

CDCl3, NMR 400 MHz



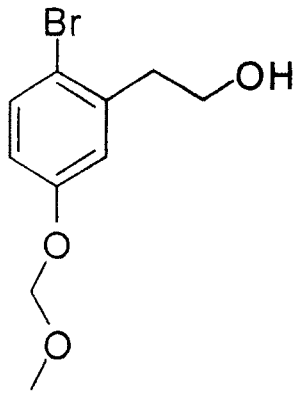
34b

CDCl3, NMR 100 MHz



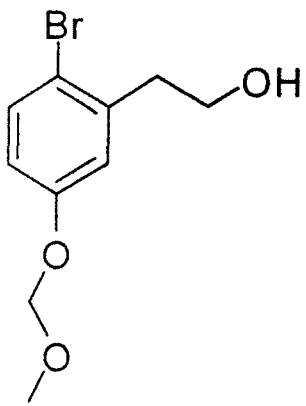
34b

CDCl3, NMR 400 MHz



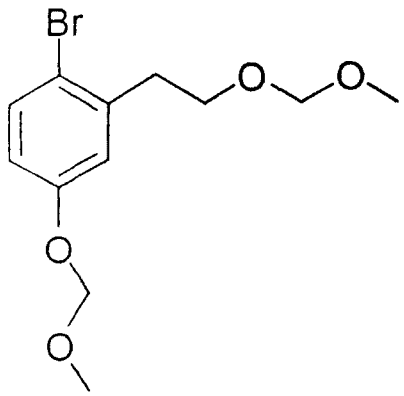
83

CDCl3, **NMR 100 MHz**



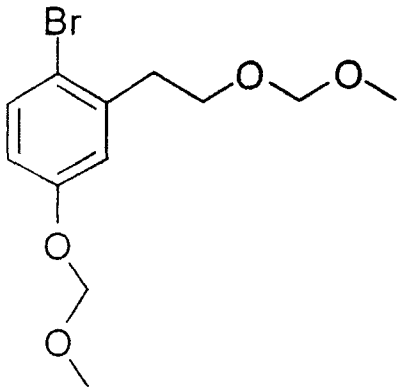
83

CDCl3, NMR 400 MHz



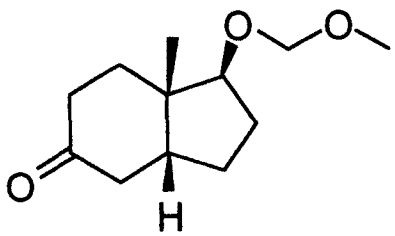
84

CDCl3, NMR 100 MHz



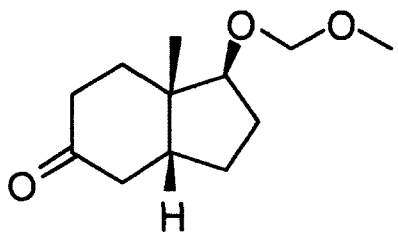
84

CDCl3, NMR 400 MHz



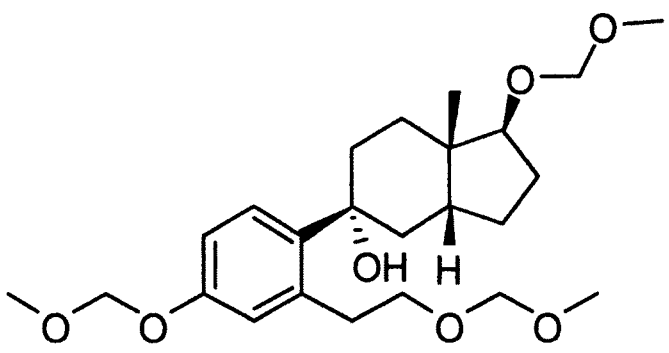
56

CDCl3, NMR 100 MHz



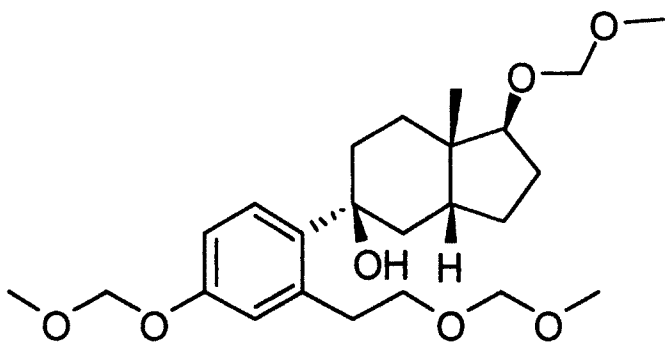
56

CDCl3, NMR 400 MHz



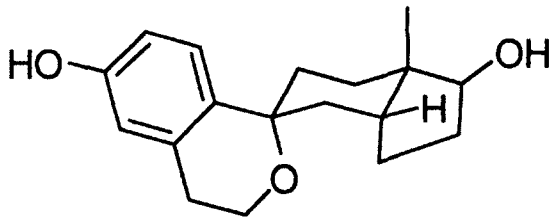
85a

CDCl3, NMR 400 MHz



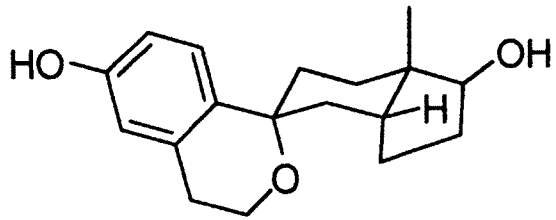
85b

CDCl3, NMR 400 MHz



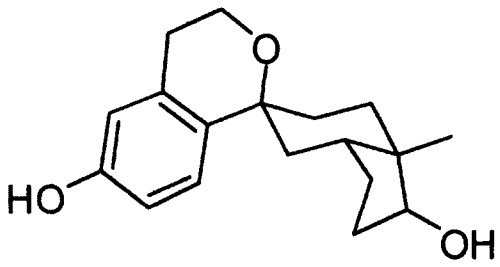
35a

CDCl3, NMR 100 MHz



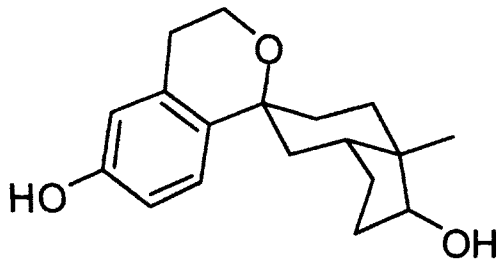
35a

CDCl3, NMR 400 MHz



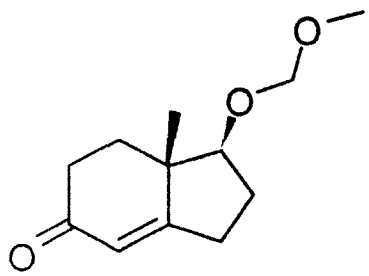
35b

CDCl3, NMR 100 MHz



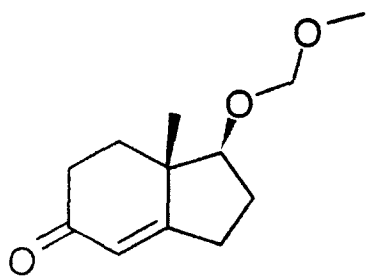
35b

CDCl3, NMR 400 MHz



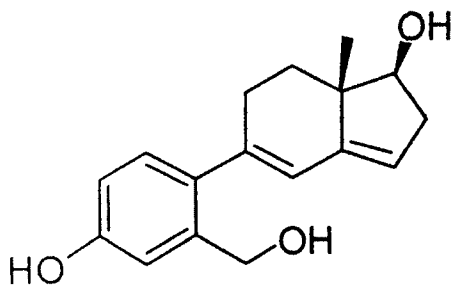
86

CDCl3, NMR 100 MHz



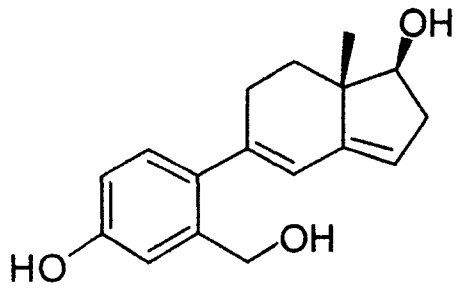
86

Acetone-d6, NMR 400 MHz



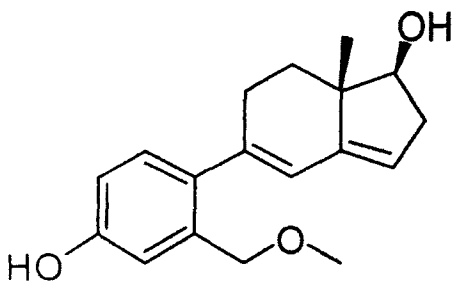
43

Acetone-d6, NMR 100 MHz



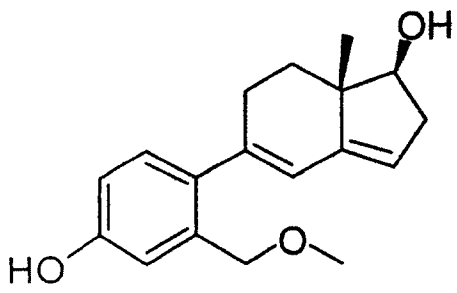
43

CDCl3, **NMR 400 MHz**



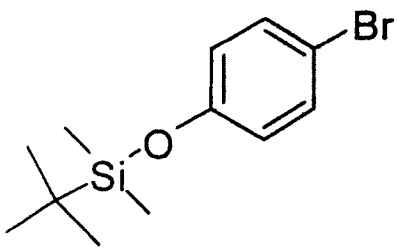
44

CDCl3, NMR 100 MHz



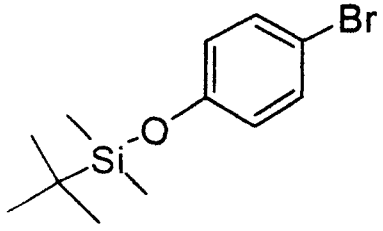
44

CDCl3, **NMR 400 MHz**



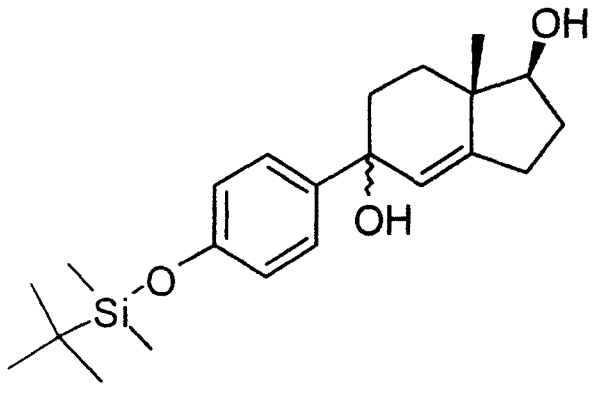
89

CDCl3, NMR 100 MHz



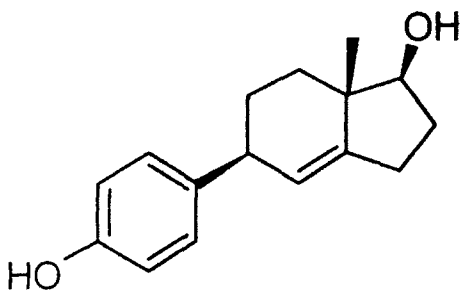
89

CDCl3, **NMR 400 MHz**



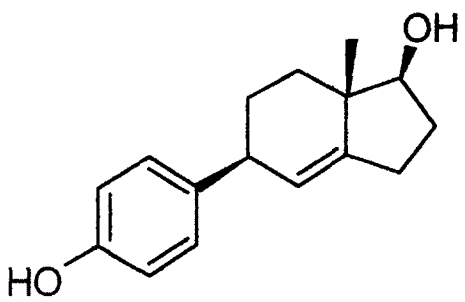
90

Acetone-d6, NMR 400 MHz



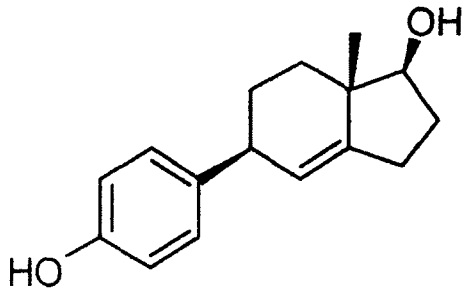
37

Acetone-d6, NMR 100 MHz

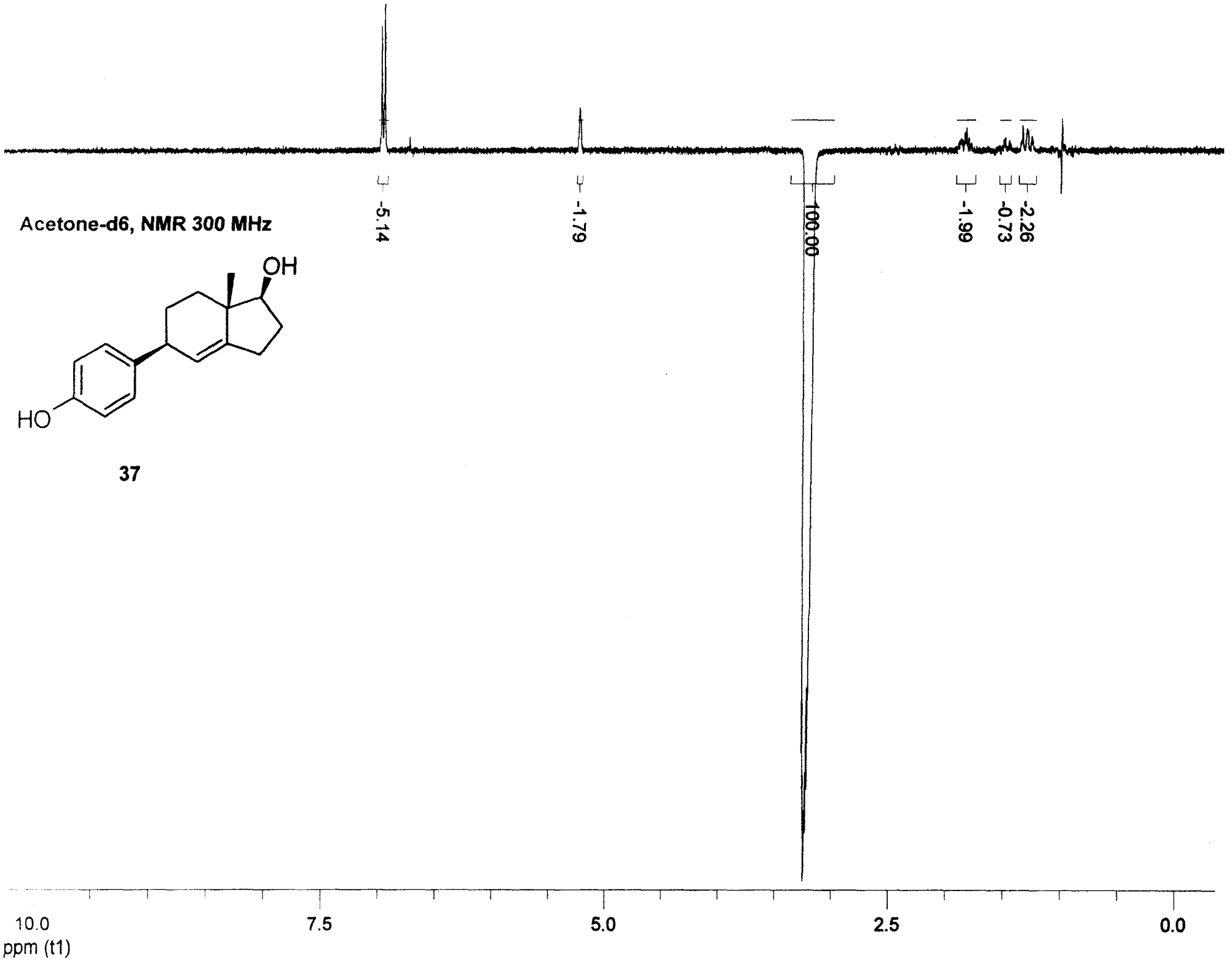


37

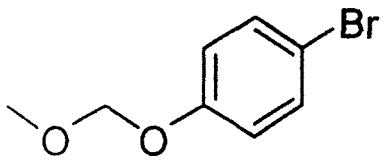
Acetone-d6, NMR 300 MHz



37

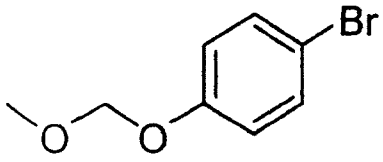

Acetone-d6, NMR 300 MHz
OH
HO
37
-5.14
-1.79
100.00
-1.99
-0.73
-2.26
10.0
7.5
5.0
2.5
0.0
ppm (t1)

CDCl3, **NMR 400 HMz**



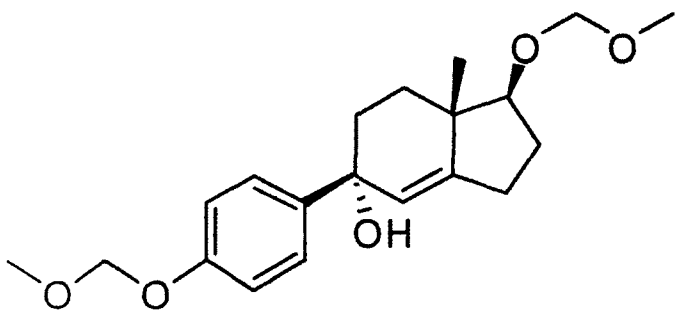
91

CDCl3, NMR 100 MHz



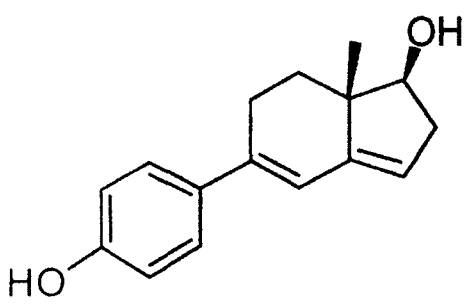
91

CDCl3, **NMR 400 MHz**



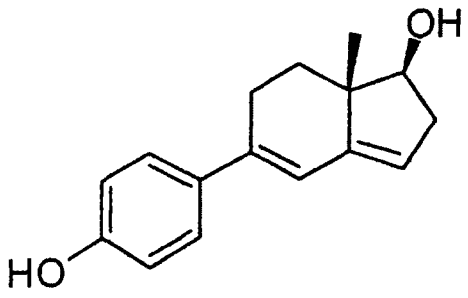
92

Acetone-d6, NMR 400 MHz



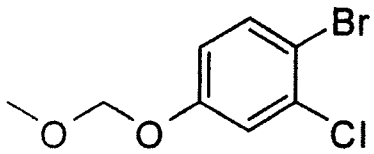
38

Acetone-d6, NMR 100 MHz



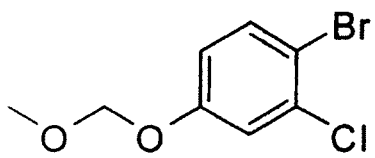
38

CDCl3, NMR 400 MHz



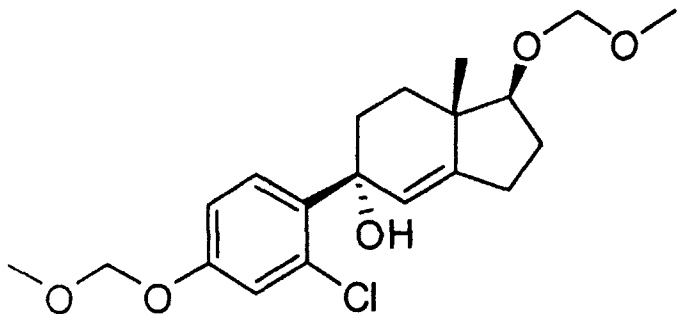
94

CDCl3, NMR 100 MHz



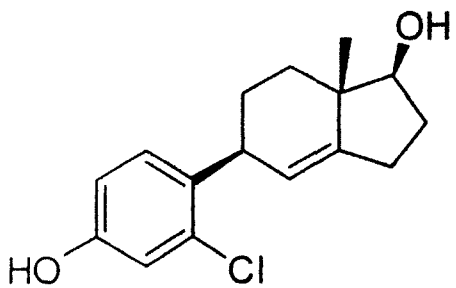
94

CDCl3, NMR 400 MHz



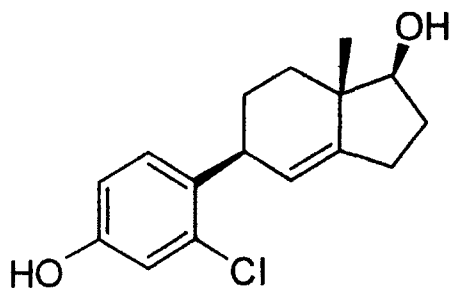
95

Acetone-d6, NMR 400 MHz



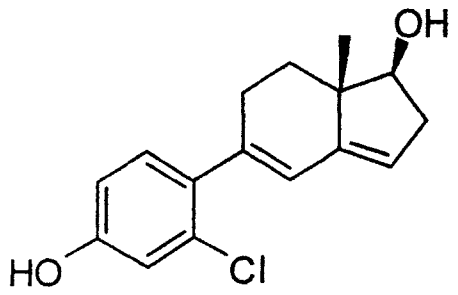
39

Acetone-d6, NMR 100 MHz

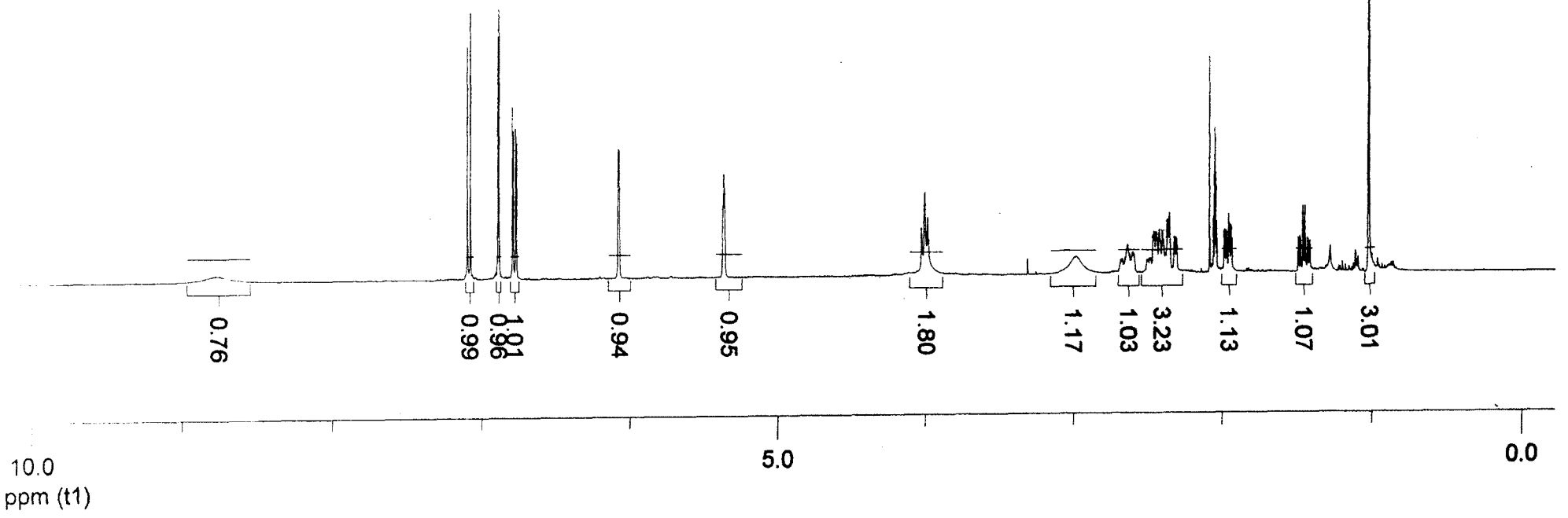


39

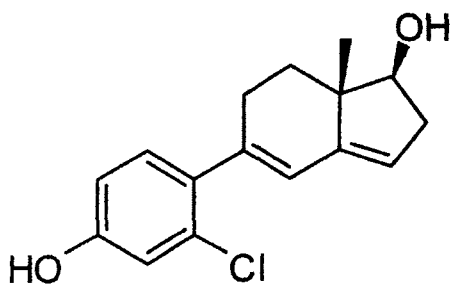
Acetone-d6, NMR 400 MHz



40

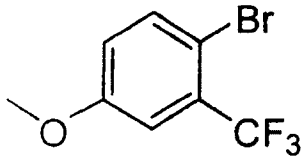


Acetone-d6, NMR 100 MHz



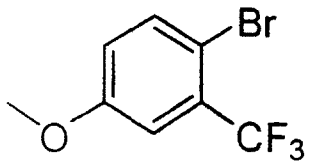
40

CDCl3, NMR 400 MHz



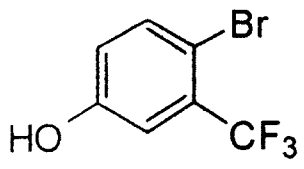
97

CDCl3, NMR 100 MHz



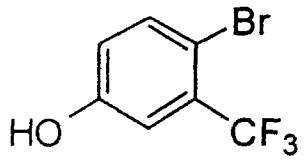
97

CDCl3, NMR 400 MHz



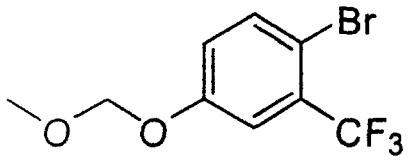
98

CDCl3, NMR 100 MHz



98

CDCl3, NMR 400 MHz



99