Synthesis of A-CD estrogens with potential for hormone replacement therapy.

Isolation of sesquiterpene lactones from *Neurolaena lobata* and diterpenoids from *Leretia cordata*.

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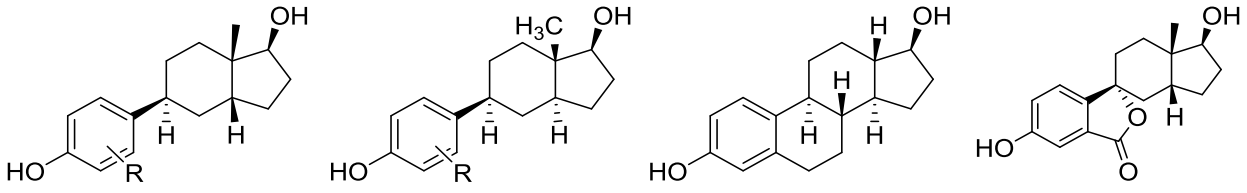
Dedication

To my mother and father

Nada and Khair Choueiri

Abstract

The use of estrogen supplements by postmenopausal women has been associated with increased risks of uterine and breast cancer. There are two possible mechanisms of toxicity: estradiol can promote breast cancer cell proliferation through estrogen receptor-α (ERα) and get metabolized to genotoxic *ortho*-quinones. The first part of this thesis describes the synthesis of a new series of non-carcinogenic A-CD estrogen agonists for hormone replacement therapy (HRT) with either the *cis* (**1**) or the *trans* (**2**) CD-ring junction. These compounds closely resemble estradiol (**3**) but lack the B-ring, allowing the ligands to adopt different conformation inside the binding pocket. One example of BC-spirolactone estrogen (**4**) is also described. Binding affinity and selectivity to both estrogen receptor subtypes ERα and ERβ were determined primarily by their Relative Binding Affinities (RBAs) and their Relative Transcription Activities (RTAs). Some ligands had binding affinities in the same order of magnitude as for estradiol itself and two were less toxic than estradiol. This work also shows that the receptors can accept almost equally well isomers that have very different molecular shapes.



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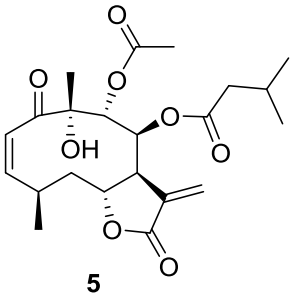
The second part of the thesis describes an ethno-pharmacological project on *Neurolaena lobata* (Asteraceae), a medicinal plant used in folk medicine in Central America to treat various symptoms such as headaches, fevers and skin diseases. Isolation and identification of five

sesquiterpene lactones (**5**) are described and bioassays results for inflammation are presented. All compounds isolated from the leaves of *N. lobata* were potent inhibitor of the pro-inflammatory cytokine TNFα, some even more than the positive control Parthenolide.



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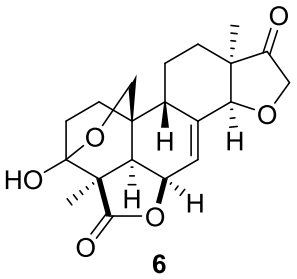
The final section of this work presents the first phytochemical study of the plant *Leretia cordata* (Icacinaceae). Characteristic secondary metabolites and the potential biological activities of the isolated compounds were identified. Four known complex polyoxygenated diterpenoids (**6**) with known anti-cancer properties have been isolated.



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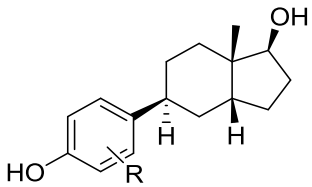
Résumé

La prise de suppléments d'estrogènes chez les femmes ménopausées est associée à des risques accrus de développer le cancer de l'utérus et du sein. Il y a deux mécanismes de toxicité possibles: l'estradiol induit la prolifération des cellules cancéreuses du sein via le récepteur d'œstrogènes-α (ERα) et l'estradiol peut être métabolisé en substances génotoxiques (*ortho*-quinones). La première partie de cette thèse décrit la synthèse d'une nouvelle série non-cancérigènes d'agonistes d'estrogènes A-CD pour le traitement hormonal substitutif (THS) avec soit la jonction *cis* (**1**) ou *trans* (**2**) de l'anneau CD. Alors que ces composés ressemblent à l'estradiol (**3**), ils n'ont pas d'anneau B, ce qui permet aux ligands d'adopter une conformation différente à l'intérieur du site actif. Un exemple de BC-spirolactone estrogène (**4**) est également décrit. L'affinité et la sélectivité des deux sous-types de récepteurs d'estrogènes ERα et ERβ ont été déterminées principalement par leur affinité de liaison relative et leur activité de transcription relative. Certains ligands ont des affinités de liaison dans le même ordre de grandeur que l'estradiol. De plus, deux composés ont été significativement moins toxiques que l'estradiol. Cette étude montre également que les récepteurs de l'estradiol peuvent lier aussi bien des isomères qui ont des formes moléculaires très différentes.



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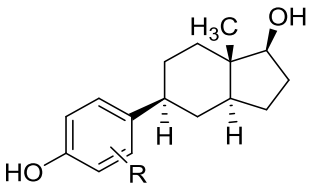

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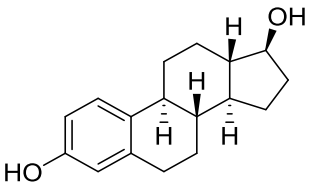

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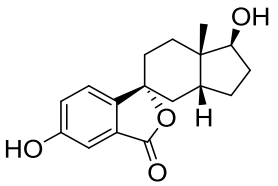

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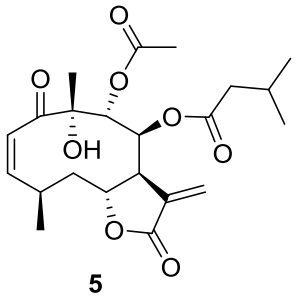
La deuxième partie de ce travail décrit un projet ethno-pharmacologique sur *Neurolaena lobata* (Asteraceae), une plante médicinale utilisée dans la médecine traditionnelle en Amérique centrale pour traiter diverses complications de la santé tels que les maux de tête, la fièvre et les maladies de la peau. L'isolement et l'identification de cinq lactones sesquiterpéniques (**5**) sont décrits et les résultats des essais biologiques pour l'inflammation sont présentés. Tous les composés isolés à partir des feuilles de *N. lobata* étaient de puissants inhibiteurs de la cytokine pro-inflammatoire TNFα, parfois même supérieure au contrôle positif utilisé, le parthénolide.



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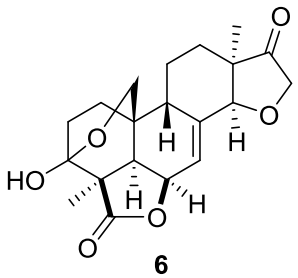
La dernière section présente la première étude phytochimique de la plante *Leretia cordata* (Icacinaceae). Les métabolites secondaires caractéristiques ainsi que l'activité biologiques des composés isolée sont identifiés. Quatre diterpénoïdes déjà connus ayant des structures complexes et polyoxygénés (**6**) et des propriétés anti-cancéreuses connues ont été isolés.



6

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Table of Contents

List of Figures

List of Schemes

List of Tables

Abbreviations

^{1}H	Proton NMR
^{13}C	Carbon 13 NMR
AF	Activation function
CDRD	Centre for drug research and development
CEE	Conjugated equine estrogens
CHD	Coronary heart disease
COMP.	Compound
COMT	Catechol-O-methyltransferase
COSY	Correlation spectroscopy
CVD	Cardiovascular disease
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DES	Diethylstilbestrol
DHP	Dihydropyran
DIPEA	*N*,*N*-diiospropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
E2	17β-Estradiol
ER	Estrogen receptor
ERE	Estrogen response elements
EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and drug administration
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
GSH	Glutathione
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HDL	High density lipoprotein
HPG	Hypothalamus-puitary adrenal
HRMS	High resolution mass spectrometry

HRT	Hormone replacement therapy
IARC	International Agency for Research on Cancer
IC_{50}	Half maximal inhibitory concentration
LBD	Ligand binding domain
LC_{50}	Median lethal concentration
LDL	Low density lipoprotein
LH	Luteinizing hormone
MHz	Megahertz
$MgSO_4$	Magnesium sulfate
MeOH	Methanol
MOM	Methyl methyl ether
NBS	*N*-bromosuccinimide
NH_4Cl	Ammonium chloride
NMR	Nuclear magnetic resonance
OICR	Ontario institute for cancer research
OC	Oral contraceptive
PEPI	Postmenopausal Estrogen/Progestin Interventions
PR	Progesterone receptor
QSAR	Quantitative structure-activity relationship
RBA	Relative Binding Affinity
RCT	Randomized clinical trial
rf	Retardation factor
rt	Room temperature
RTA	Relative Transcription Activation
SERM	Selective estrogen receptor modulator
SL	Sesquiterpene lactone
SHBG	Sex hormone binding globulin
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS (TBS)	*tert*-Butyldimethylsilyl ether
THF	Tetrahydrofuran
TLC	Thin layer chromatography

Chapter 1: Synthesis of A-CD estrogens as potential for hormone replacement therapy

1.1 Introduction

As women reach the age of 45 to 55 years, they generally undergo a series of physiological hormonal alterations mostly associated with a decrease in the sex hormones estrogen and progesterone. This stage in their life is called natural menopause and is defined by the permanent cessation of menses. It should be noted that menopause can also be induced earlier by removal or damage of the ovaries, otherwise known as surgical menopause [1]. Menopause is associated with osteoporosis and heart disease, as well as climacteric symptoms such as hot flashes, sleeping disturbance and mood swings, which can last for months or even years [2]. Doctors have prescribed estrogen supplements to postmenopausal women to reduce menopausal symptoms, however, these can lead to increased risk of breast and endometrial cancer [3]. Estrogen intake is known as hormone replacement therapy (HRT). In 1999, the International Agency for Research on Cancer (IARC) concluded that steroidal estrogens should be classified as human carcinogens based on evidence of epidemiological studies associating HRT in postmenopausal women with increased risk of endometrial cancer and a less consistent risk of breast cancer [4]. Ideally, a non-carcinogenic compound should be available to treat menopausal symptoms as an alternative to HRT.

Adolf Butenandt and Edward Adelbert Doisy isolated and determined the structure of estrone in the late 1920s [5]. The use of estrogens, commonly administrated as conjugated equine estrogens (CEE) to relieve menopausal symptoms, was approved in 1941 by the US Food and Drug Administration (FDA) [6]. Estrogen is a general term for the natural female hormones estriol (E1, **2**), estradiol (E2, **1**) and estrone (E3, **3**). Estradiol is the most potent endogenous estrogen, though it is not the primary estrogen found in women [7]. Estrone is the major estrogen

for non-pregnant women, whereas estriol is the predominant estrogen during pregnancy [8]. Under different physiological conditions, the metabolic conversion of E2 to E1 or E2 to E3 can provide differential activation for the estrogen receptors (ERs). Estrogens are a class of sex steroids derived from cholesterol, which regulate the development of the reproductive and cardiovascular system as well as bone density. They are primarily secreted by the ovaries, but they can also be secreted from the placenta, adipose tissues and adrenal glands [8]. Their skeleton contains 18 carbons arranged into an ABCD ring system, where the aromatic A-ring is hydroxylated at the C3 position [9].

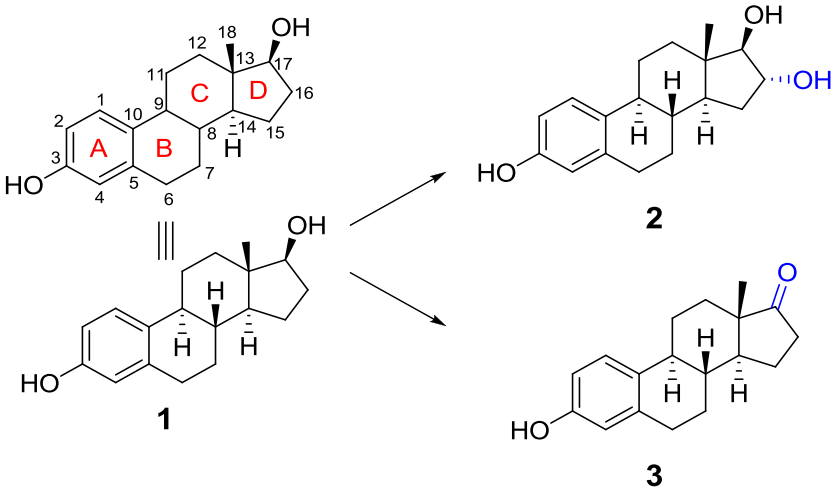


Figure 1.1.1 Chemical structures of estrogens. 18-C with a planar ABCD ring system, where the aromatic A-ring is hydroxylated at the 3 position: estradiol (**1**), estriol (**2**) and estrone (**3**).

The estrogen receptor (ER) was discovered in the late 1950s by Jensen and Jacobsen, who demonstrated that radiolabeled estradiol was only retained by estrogen tissues such as the uterus, vagina and pituitary gland in female rats [10]. During the following years, numerous studies were performed on the estrogen receptor and its ligand binding domain (LBD). However, for over 40 years, it was thought that 17β-estradiol uptake was mediated by one ER, now known as ERα. The second type of ER, ERβ, was found unexpectedly by Kuiper in 1996 while working

on the prostate of male rats to find novel androgen receptors cDNA library [10]. It is interesting to note that 17α-estradiol was found to have 5 times higher affinity to ERα than ERβ, whereas 17β-estradiol possesses approximately the same activity to both ERs [11]. Both ER subtypes share approximately 96% amino acid sequence identity in their DNA-binding domain (DBD) and 54% identity in their LBD but their N-terminus is poorly homologous (**Figure 1.1.2**) [9-11]. These findings allowed pharmaceutical companies to build selective ligands possessing different affinities for the ER subtypes, ERα and ERβ, other than natural estrogens or phytoestrogens.

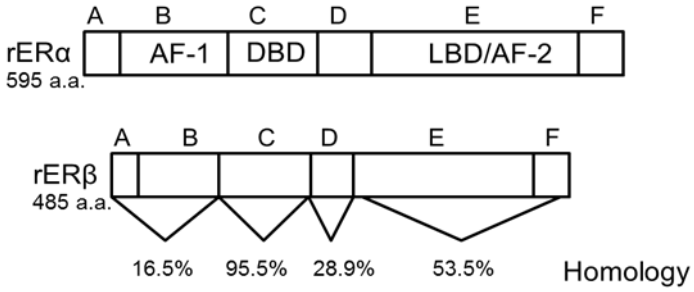


Figure 1.1.2 Comparison of the estrogen receptor subtypes. Adapted from [11].

1.1.1 Benefits of HRT

Estrogens are important for the regulation of many target tissues in the body. They stimulate the biosynthesis of proteins in the brain that may affect mood and emotions [14]. They are also involved in the distribution of body fat resulting in the female body contours [15]. Estrogens increase the synthesis of blood clotting factors and therefore enhance blood coagulation. They improve the cardiovascular system by decreasing the levels of low-density lipoprotein (LDL) cholesterol and lipoprotein (a) and by raising the levels of high-density lipoprotein (HDL) cholesterol [16]. In addition, they play an important role in preventing osteoporosis by inhibiting bone resorption [17]. For over 50 years, postmenopausal women have been using estrogen supplements to relieve climacteric symptoms such as sleeplessness, hot flashes, mood swings, headache, tachycardia and palpitations; furthermore, estrogens are used in pathology treatments

such as Alzheimer's disease, coronary heart disease (CHD) and osteoporosis [18], [19]. Oral intake of 0.625 mg or 1.25 mg CEE per day improved hot flashes and other climacteric symptoms [20]. Several studies have suggested estrogen intake reduces CHD by 30% to 50% [2], decreases the risk of hip fractures by 25% [17], and improves blood lipid levels [21].

Osteoporosis

Several clinical studies showed that a 10 year treatment of HRT can prevent bone loss, and consequently osteoporosis, in elderly women [22]. Osteoporosis is a skeletal disease which is defined by low bone mass and increases susceptibility to fractures, particularly in the hip, wrist and spine. Hip fractures carry a mortality rate of 20% among elderly women [23]. Bone loss and fractures occurs in both men and women with age, however the risk is higher in postmenopausal women because of their lower skeletal mass and higher life expectancy [22]. During menopause, bone loss is caused by changes in skeletal metabolism and architecture. In healthy adults, bone mass stays constant and bone remodelling occurs regularly [22]. The sequence of bone remodelling starts with osteoclasts that excavate a cavity on the bone surface. This cavity is replaced by osteoblasts that initiate the synthesis of a new matrix by filling the erosion cavity to form new bone, and the cycle starts over. Estrogen reduces bone remodelling turnover by regulating bone cell cytokines. At menopause, the decline in estrogen levels increases the activity of osteoclasts and, in particular, the reduction in progestin level causes a decrease in osteoblasts and therefore accelerates skeletal loss [22]. Following supplementation of estrogen by postmenopausal women, estrogen's action in the prevention of osteoporosis slows the rate of bone remodelling and reduces the overall bone loss.

Cardiovascular disease

Estrogen plays a significant role in cardiovascular functions and metabolism and its withdrawal is associated with high risk factors for cardiovascular disease (CVD) [24]. Estrogens provide antioxidant protection of LDL and HDL due to their phenolic A-ring [25]. CVD in women is independently associated with elevated levels of triglycerides, lipoprotein(a) and interleukin-6 (IL-6) [24], [26]. However, the relationship between menopause and CVD is still ambiguous because high risks of hypertension and hyperglycemia can be related to obesity as well. Weight gain in postmenopausal women and redistribution of body fat can be prevented by taking supplements of estrogen if started soon after menopause [24]. In addition to their effects on the lipid profile, estrogens act directly on vascular tissues and the heart [26]. They are associated with rapid vasodilation, induced nitric oxide regulating blood flow and a decrease of endothelin-1. Consequently, observational studies have concluded that HRT improves cardiovascular diseases in postmenopausal women [24].

1.1.2 Estrogens and menopause

Physical-chemical properties of estrogens

The general feature of endogenous estrogens is the presence of a steroidal skeleton containing 18 carbons arranged into planar ABCD ring systems, where the A-ring is an aromatic ring with a hydroxyl group at C3 (**Figure 1.1.1**). They differ in the D-ring, where 17β-estradiol is hydroxylated only at C17, estriol is hydroxylated at C16 and C17, and estrone contains a ketone at C17. Their physical and chemical properties are summarized in **Table 1.1.1**.

Table 1.1.1 Physical-Chemical Data of 17β-Estradiol, estriol and estrone.

Chemical Properties	**17β-Estradiol**	**Estriol**	**Estrone**
CAS #	50-28-2	50-27-1	53-16-7
Chemical formula	$C_{18}H_{24}O_2$	$C_{18}H_{24}O_3$	$C_{18}H_{22}O_2$
Molecular weight (g/mol)	272.38	288.38	270.37
Lipophilicity (Log P) [27]	4.01	2.45	3.13
pKa [28], [29]	10.71	10.38	10.34
H-bond donor/ H-bond acceptor	1 / 1	1 / 2	1 / 1
Solubility in water [4]	Practically insoluble	Practically insoluble	Practically insoluble
Octanol-air partition* (Log K_{OA})	12.8	12.7	10.9
Melting Point (^oC) [4]	173-179	282.0	254.5-256
Physical State	White to creamy white crystalline powder	White crystalline powder	White to creamy white crystalline powder

*Predicted data calculated using KOAWIN Software v1.10.

Steroidal estrogens are fat soluble and lipophilic (Log P between 2.45 and 4.01), indicating poor aqueous solubility [27], [30]. Their octanol-air coefficient are considerably high (Log K_{OA} >7), indicating that these compounds are not volatile. The natural estrogens estradiol, estrone, estrone sulfate (sodium salt) and estriol are available in different galenic preparations. Although estrogens are lipophilic, their water solubility is increased by binding to serum proteins albumin and sex hormone-binding globulin (SHBG). Metabolic transformation of estrogens leads to less active, more polar and water-soluble metabolites, which facilitate their systemic circulation [31]. In physiological environments, these molecules exist in their neutral form because their pKas are between 10.9 and 12.8. Estradiol (**E2**) is the most potent endogenous estrogen and is responsible

for most of the estrogenic activities in tissues [8]. **E2** is nonpolar and hydrophobic, with the exception of its hydroxyl groups at C3 and C17. Furthermore, **E2** acts as a hydrogen bond donor and acceptor with the amino acid residues in the ER. It is important to note that the distance between the hydroxyl groups at C3 and C17 of 17β-estradiol is approximately 11Å, which is the distance required for its binding affinity [32], [33]. As predicted by Lipinski's rule of 5 (MW <500; Log P <5; Hydrogen-bond donor <5 and Hydrogen-bond acceptor <10), steroidal estrogens are pharmacologically active [34].

Mechanism of action of estrogens

The actions of estrogens and other steroid hormones are mediated by the estrogen receptor (ER), a ligand-dependent nuclear transcription factor. The ER acts as an inducer or repressor of gene transcription, depending on the nature of the bound ligand and the coregulator proteins [35]. The mechanism of action of **E2** involves its interaction with the ER to form a complex of estrogen-ER, which leads to the activation of the ER by the binding of the complex to estrogen response elements (EREs) found in responsive genes (**Figure 1.1.3**, adapted from [36]). Once it crosses the cell membrane, estrogen binds to the ER at the ligand-binding domain (LBD) [37], which then induces a conformational change in the ER, where a helical section (H12) folds across the binding site. As a result, ERs dimerize through homodimerization (ERα–ERα or ERβ–ERβ or heterodimerization (ERα–ERβ), and this dimerization exposes a hydrophobic region, the activation function-2 (AF-2), that binds to coactivator proteins and finally completes the nuclear transcription factor [20]. Subsequently, this dimer ER–steroid complex binds to the EREs found on the promoters of target genes in the DNA and induces transcription, which leads to the formation of messenger RNA [38]. ERs can also mediate gene expression with coactivators and corepressors without directly binding to DNA through protein-protein interactions [39].

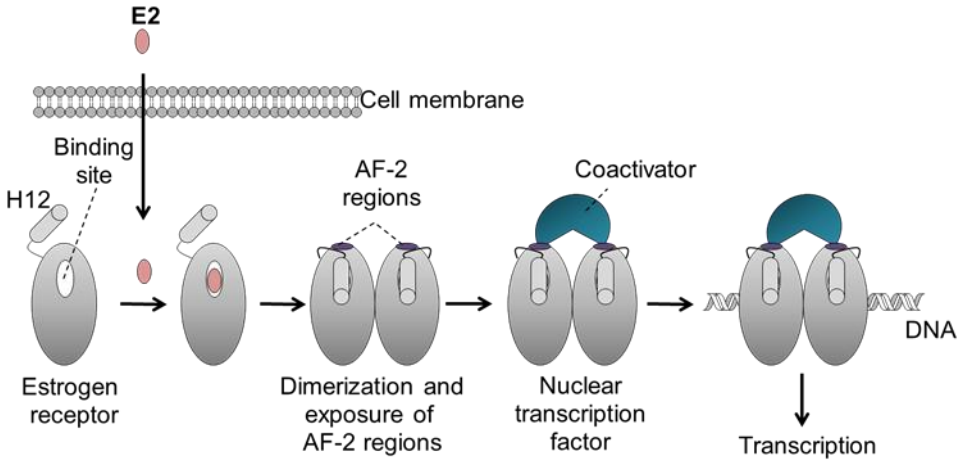


Figure 1.1.3 Transcription activation control by the estrogen receptor. Adapted from [36].

The LBD of the ER features a combination of hydrogen bonding and hydrophobic interactions as key interactions with steroidal estrogens and other ligands. The main skeletal features of a ligand include a phenolic hydroxyl group localized in the A-ring that forms a hydrogen bond triad with Glu 353, Arg 394 and a water molecule; and a hydroxyl group situated in the D-ring that interacts with His 524 **(Figure 1.1.4)** [37]. In addition, the distance between the two oxygen atoms should be between 11.0Å + 0.5Å for optimal hydrogen bonding, as in **E2** [32]. The hydrophobic backbone of the molecule forms van der Waals and non-covalent interactions with other regions of the binding pocket [37].

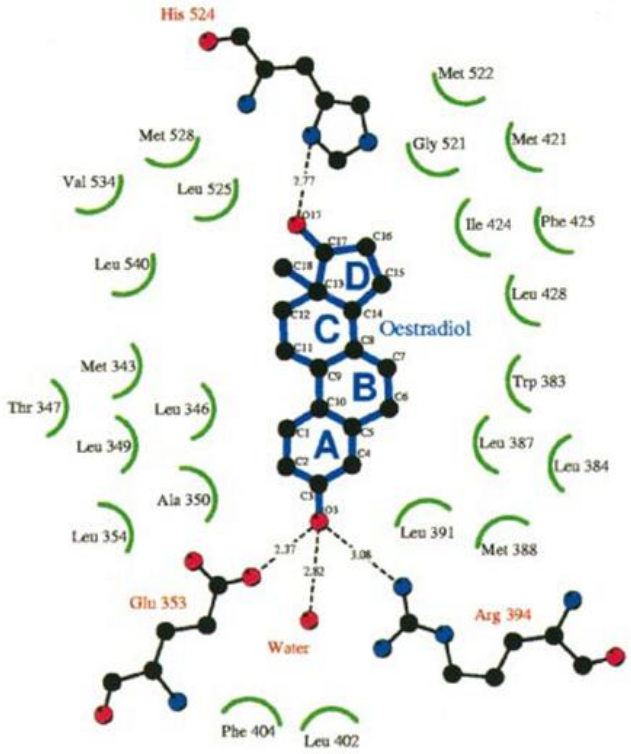


Figure 1.1.4 Representation of E2 in the LBD displaying key hydrogen bonding of E2 with the amino acid residues and a water molecule [37].

Current drugs used to treat the symptoms of menopause share features similar to the A-ring of estradiol since the flat and aromatic structures fit the narrow slot. On the other hand, the rest of the ER binding site is bigger than the size of the natural ligand; this allows various possibilities for the shape of the hydrophobic substituents [11]. There are two isoforms of the ER, ERα and ERβ [9-11]. Although they share approximately 60% of homology in their amino acid residues, the binding pocket only differs with two amino acid substitutions: Met421 and Leu384 in ERα are substituted by Ile373 and Met336 in ERβ, respectively (**Figure 1.1.5**) [35]. Consequently, these two substitutions result in the selectivity of ligands for the two ER isoforms [38].

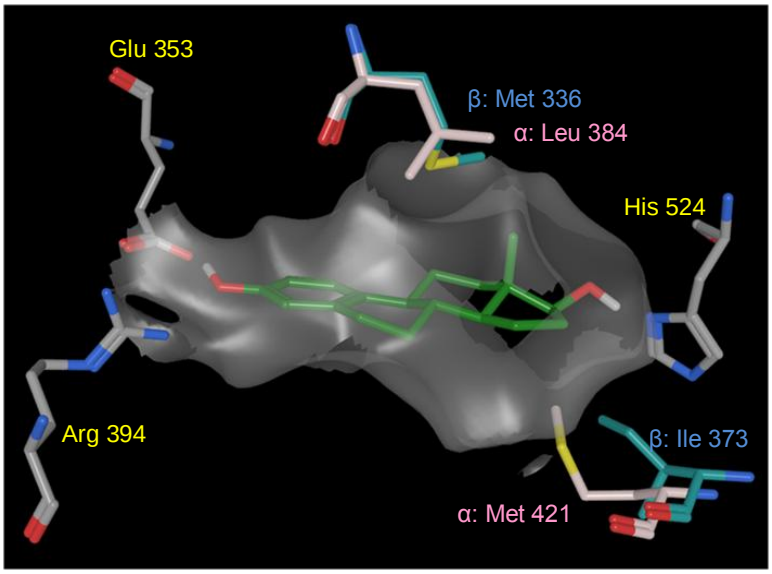


Figure 1.1.5 Computational model made by the Wright group at Carleton University showing **E2** inside the ERα/β active site where the main pharmacophores are indicated in yellow, ERα-specific residues in pink and ERβ-specific amino acids in blue.

The tissue distribution of both ER isoforms has been studied extensively. ERα is responsible for the development and maintenance of male and female organs and is the predominant isoform expressed in breast cancer. ERβ is the main subtype found in normal breast tissue and is also found in other organs such as ovarian granular cells, bone marrow, prostate epithelium, testes and brain [7]. Depending on their chemical structure, estrogenic ligands have different binding affinities to the two ER isoforms. **Table 1.1.2** summarizes the relative binding affinity (RBA) of some ligands for *in vitro* synthesized human ERα and rat ERβ as determined by competitive experiments [20]. Estradiol binds equally to both receptors while estrone and 17α-estradiol are α-selective and estriol and genistein are β-selective. Other parameters affecting the distribution of estrogen in the body include the binding to non-receptor proteins, vascular permeability, partition coefficient, hepatic blood flow and extrahepatic metabolism [40]. The difference in the tissue distribution of both ERs have led pharmaceutical companies to build specific agonist and

antagonist ligands to treat several pathologies and symptoms associated to menopause while maintaining antiproliferative functions in the breast and uterus [41].

Table 1.1.2 Relative Binding Affinity (RBA) of selected estrogenic compounds [20].

Estrogenic compounds	RBA for ERα	RBA for ERβ
17β-Estradiol	100	100
Estrone	60	37
Estriol	14	21
17α-Estradiol	58	11
4-Hydroxy-estradiol	13	7
2-Hydroxy-estradiol	7	11
Diethylstilbestrol	468	295
Coumestrol	94	185
Genistein	5	36

Physiology of menopause

The regularity of the menstrual cycle varies throughout the reproductive life of women. During menarche, estrogen and progesterone are produced primarily by the gonads of non-pregnant women and the placenta of pregnant women. Their formation depends on the secretions of both gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are mediated by gonadotropin-releasing hormone (GnRH) from the hypothalamic–pituitary–adrenal (HPG) axis. Before menopause, menstrual periods become irregular and less frequent. This phase, called perimenopause or climacteric, usually lasts from 2 to 8 years [42], [43]. It is associated with multiple endocrine, clinical, and biological changes resulting in several climacteric symptoms such as hot flashes, sleeping disturbances and mood swings. This period is defined by increased serum levels of FSH and LH and decreased secretions of inhibin and follistation, which are peptides that inhibit the release of FSH from the pituitary. It is also characterized by the decline in the supply of eggs and irregular ovulation [43–45]. When estrogen levels are no longer sufficient to surpass FSH secretion by the pituitary gland, FSH

levels rise. McKinlay and colleagues reported that 58% of women had hot flashes two years before the cessation of menses as a consequence of high concentration of gonadotropins and the inhibition of estrogen feedback [46]. According to the World Health Organisation (WHO) monograph "Research on the Menopause in the 1990s", natural menopause is defined by the permanent cessation of menstruation for 12 consecutive months [42]. In industrialized societies, the average age at menopause is 51 years [42]. Menopause is commonly diagnosed with an elevation of FSH levels over 40 IU/L and the decline of estradiol levels under 10 pg/mL, compared to women of childbearing age with FSH and estradiol levels of under 10 IU/L and over 60 pg/mL, respectively [44], [47].

Sex steroids are derived from cholesterol and their synthesis occurs in the ovarian and adrenal organs (**Figure 1.1.6**) [48]. Pregnenolone is the precursor to progesterone in the ovaries, and to dehydroepiandrosterone (DHEA) and androstenedione in adrenal glands. These adrenal and gonadal steroids are then converted to estradiol (**E2**) and estrone (**E1**) by aromatase, a membrane-bound enzyme containing a cytochrome P450 enzyme (CYP19A1) and a reductase enzyme [49]. Estriol is formed from estrone in non-pregnant women and from dehydroisoandrosterone in pregnant women [50]. After menopause, the secretion of estrogen by the ovaries ceases and all sex steroids are synthesized from DHEA in peripheral target endocrine tissues including the liver, adrenal glands, muscle, bone, adipose tissue and breast [31].

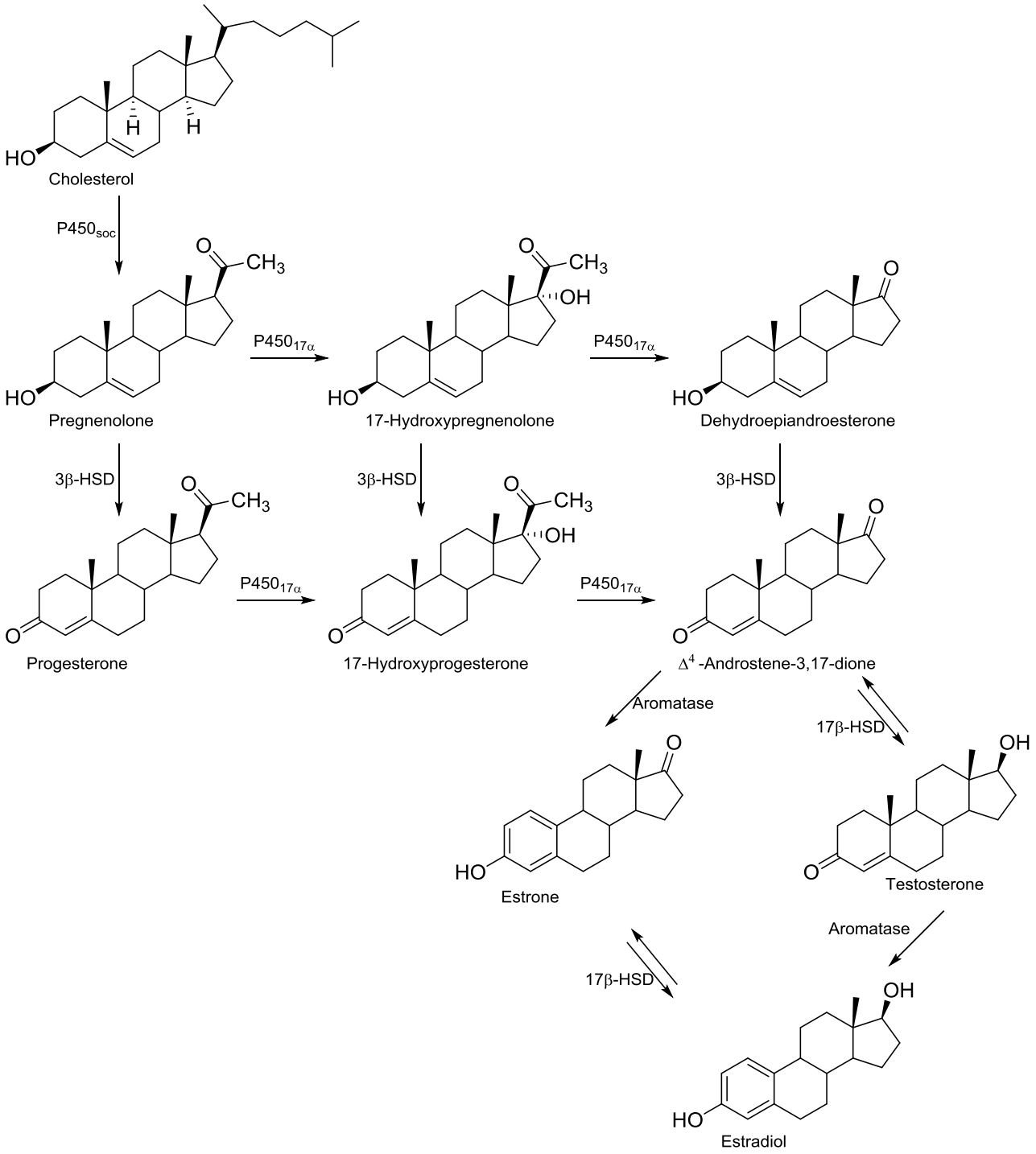


Figure 1.1.6 Biosynthesis of sex steroids [48].

1.1.3 Natural and synthetic estrogen supplements for HRT

Steroidal estrogens are mainly used by premenopausal women as oral contraceptives (OCs) and by postmenopausal women as HRT. Currently there are many alternatives to HRT on the market. Some of the most successful ones include selective estrogen receptor modulators (SERMs), tibolone and phytoestrogens.

Steroidal estrogens for HRT

The most common forms of HRT are conjugated equine estrogens (CCEs), obtained from pregnant mares. Estrone and its sulfate derivative are the most abundant compounds in estrogen formulations. The use of estrogen alone as HRT was prescribed in the 1960s to treat the climacteric symptoms of both natural and surgical menopause has been shown to increase the risks of endometrial cancer; however the addition of a progestogen reduces this risk [4]. These symptoms were associated with elevated concentrations of gonadotropins and feedback inhibition of estrogen. Premarin and Pempro, commercial formulations of HRT (**Figure 1.1.7**), are currently the most popular formulations in North America. Recently, standard-dose Premarin prescriptions (0.625 mg/day) have decreased by 33%, while sales of low-dose Premarin (0.45 mg/day) increased [52].

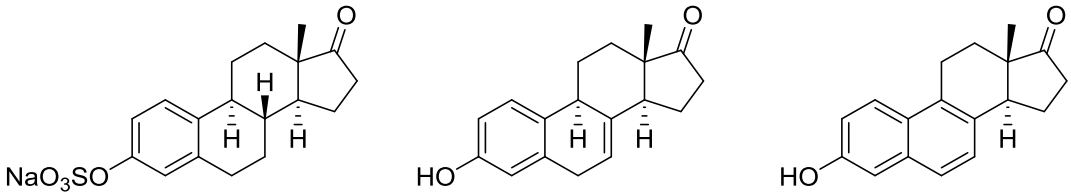


Figure 1.1.7 From left to right: estrone sulfate, and conjugated equine estrogens equilin and equilenin.

Synthetic non-steroidal SERMs

Selective estrogen receptor modulators (SERMs) have estrogenic activities with minimal side effects. Synthetic non-steroidal SERMs include tamoxifen (**4**), raloxifen (**5**) and lasofoxifene (**6**). Owing to their selectivity to one of the ER isoforms, phytoestrogens are often called natural SERMs. These ligands rely on differential interactions with ER and various proteins such as transcriptional coactivators or corepressors, the amount of which varies in different tissues [53]. Accordingly, the same ligand can act as an agonist or an antagonist in different organs, depending on the presence of coactivators or corepressors. SERMs maintain structural elements for binding to ERs and display weak agonist and antagonist activities.

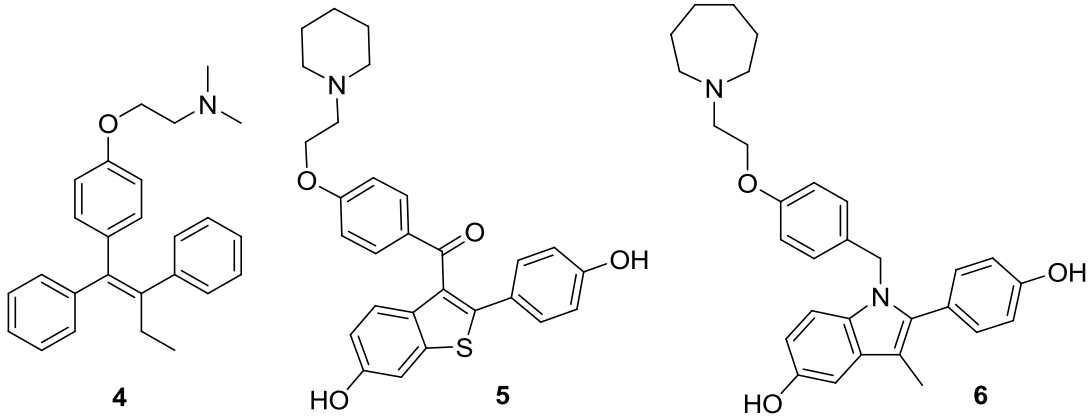


Figure 1.1.8 Chemical structures of tamoxifen (**4**), raloxifen (**5**) and lasofoxifene (**6**).

Tamoxifen is a prodrug of the active metabolite 4-hydroxytamoxifen and it was the first selective estrogen receptor modulator (SERM). It is an agonist in bone tissues used to prevent osteoporosis, an antagonist in the breast and partial agonist in the uterus used for the treatment of ERα-positive tumors as chemopreventive agent [38]. Raloxifene, a second generation SERM, acts as an agonist in bones and is used for treatment of postmenopausal osteoporosis. It is an antagonist in the breast and uterus and is used for treatment of hormone-dependent breast cancer [36]. Lasofoxifene is a third generation SERM that is being evaluated in phase III clinical trials for the

treatment of postmenopausal osteoporosis and has already been approved by the European Union [54]. Like other SERMs, it acts as a tissue-selective agonist on bone and lipid profile and as an antagonist in the uterus.

All three SERMs contain a protonated amino group side chain which interacts with Asp351 from the LBD and prevents the receptor from folding helix H12 [37], [55]. As a result, the helix is unable to seal the LBD and blocks transcription initiation (**Figure 1.1.9**) [34]. Clinical studies have shown that tamoxifen and raloxifen can lead to an increased risk of endometrial cancer owing to DNA mutations through the formation of carcinogenic *o*-quinone adducts [36]. A recent publication by Bolton and colleagues has shown that, like the other two SERMs, lasofoxifene was oxidized to *o*-quinone and was shown to form depurinated adducts. The authors concluded that, like estradiol, these adducts could contribute to the toxicity *in vitro* [56].

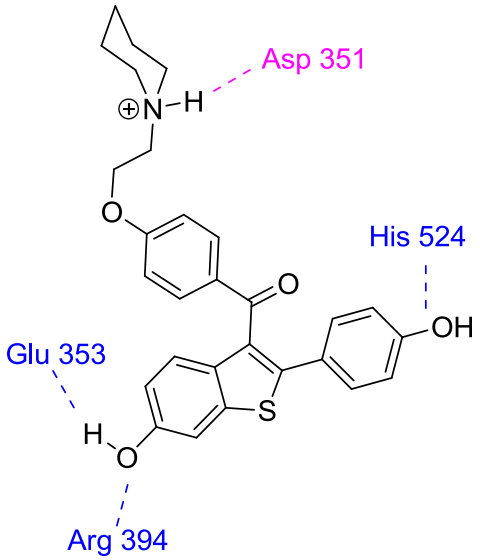


Figure 1.1.9 Interactions of raloxifen with the estrogen receptor [34].

Tibolone

Tibolone is a synthetic steroid used as an alternative to HRT to prevent postmenopausal osteoporosis and to relieve climacteric symptoms. Tibolone is a prodrug and rapidly converts into its metabolites 3α-hydroxytibolone, 3β-hydroxytibolone and Δ4-isomer which are responsible for its estrogenic, androgenic and progestagenic properties [57]. Like HRT, tibolone increases BMD and decreases HDL cholesterol, with fewer side effects than common HRT including the risks of endometrium and breast cancer risks; however, long term studies are still ongoing [58].

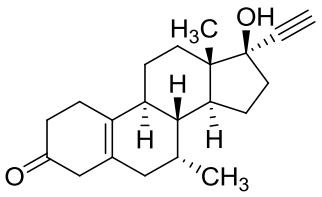


Figure 1.1.10 Chemical structure of tibolone.

Phytoestrogens

Several epidemiological studies have demonstrated that the Western communities have a higher incidence of heart diseases, osteoporosis, menopausal symptoms and breast cancer compared to Asian countries [59–63]. Among the main risk factors are unhealthy lifestyles and lack of phytoestrogens, which are mainly found in soy products in the Asian diet. Phytoestrogens are isoflavonoid derivatives and have been used as dietary supplements as an alternative to HRT. Some examples of plants containing phytoestrogens include *Pueraria montana* (kudzu root extract), *Trifolium pratense* (red clover extract), *Glycyrrhiza glabra* (licorice root), *Foeniculum vulgare* (fennel extract) and *Pimpinella anisum* (anise extract) [63], [64]. The herbs *Actaea racemosa* (black cohosh root), *Angelica sinensis* (dong quai), and *Dioscorea villosa* (wild yam

root or extract) are also used to treat menopausal symptoms, but they do not contain phytoestrogens [49], [65]. Phytoestrogens are classified in three different categories, isoflavones (**7**), coumestans (**8**) and lignans (**9, 10**). Soybean is the main source of isoflavones while coumestans and lignans are mostly found in flaxseeds. Common isoflavones are genistein, daidzein, formononetin and biochanin A. Coumestrol is the most common coumestan and the major lignans are enterolactone and enterodiol [60]. The metabolism of phytoestrogens results in the formation of heterocyclic phenols and stimulates the synthesis of SHBG, which subsequently reduces the level of endogenous estrogens [61]. Phytoestrogens bind to the ER and display weaker estrogenic activity than endogenous estrogens. Epidemiological studies correlate the high intake of soy in Japanese women with lower concentration of serum estrogen, as well as a reduced risk of breast cancer with respect to Western countries [62].

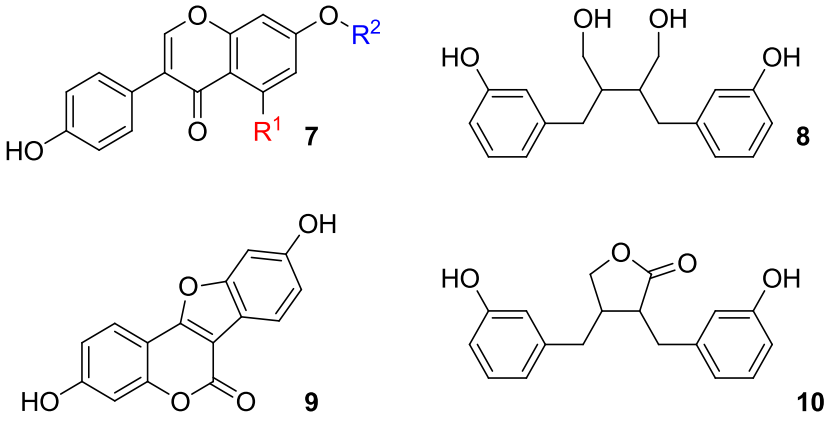


Figure 1.1.11 Chemical structures of main phytoestrogens: isoflavoids (**7**) such as genistein (R^1 = OH, R^2 = H), daidzein (R^1 = H, R^2 = H) and formononetin (R^1 = H, R^2 = CH_3), coumestrol (**8**) and the lignans enterodiol (**9**) and enterolactone (**10**).

Phytoestrogens possess similar skeletal features as estradiol, with a hydroxy-substituted aromatic ring that is distant of 12Å from a second planar hydroxyl group. Unlike estradiol which binds equally to both ER subtypes, genistein is more ERβ-selective by 18.5-time fold; however, its

bioavailability is limited [66]. The relative binding affinities (RBA) for genistein were 13% and 0.7% for ERβ and ERα, respectively, by radiolabeled competitive displacement assay with **E2** which binds at 100% for both ER isoforms. Due to its β-selectivity, genistein has been a source of inspiration to pharmaceutical companies as chemopreventive agent and for HRT. In addition to their estrogenic activity, phytoestrogens display antioxidant activities [67] and inhibit angiogenesis [68].

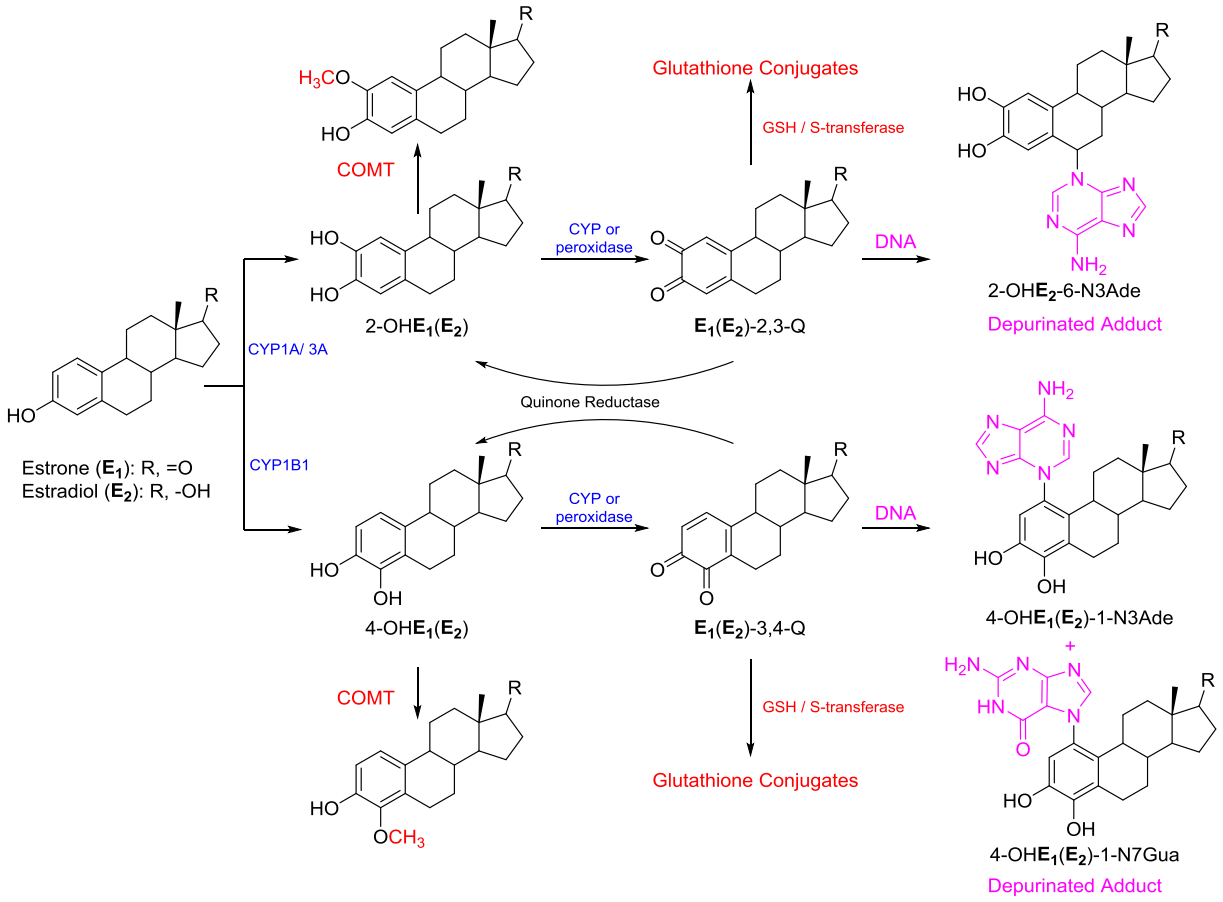
1.1.4 Pharmacokinetics of estrogens

ADME Summary

Estrogens have a large variability in the pharmacokinetic profile, depending on the route of administration and the expression of the estrogen receptors. Owing to the first by-pass metabolism, oral administration of estrogens provides a poor bioavailability of only 2 to 10% [69]. Estradiol and estrone have short half-lives of 20 to 30 minutes, while the sulfate derivative of estrone has a longer half-life of 10 to 12 hours [20]. Likewise, the clearance rate of equilin and dehydroequilin-17β are much greater than their corresponding sulfate metabolites. Estrogens are mostly absorbed from the GI tract and the majority of estrogens are bound to serum proteins in the systemic circulation. Approximately 98% of the circulating estrogens are bound to serum proteins, with high affinity to SHBG and low affinity to albumin [20]. They are then rapidly distributed into tissue-specific organs, depending on their distribution and expression of ERα or ERβ. Estrogens are easily metabolized to *o*-quinones in the liver and intestine. A major detoxification pathway is through methylation of the hydroxyl-catechols. Finally, estrogens and their metabolites are excreted in the urine, feces and bile and may undergo enterohepatic recirculation, thereby increasing serum concentrations of estrogens.

Estrogen metabolism

Estrogens are metabolized mainly in the liver and gut. The main metabolic pathway of estrogen involves phase I bioactivation and phase II detoxification (**Scheme 1.1.1)** [70]. There are two major pathways for estrogen metabolism. The first one is a hydroxylation to form the 2- and 4-catechol estrogens, and the second one is a hydroxylation at the 16α position [70]. In the catechol pathway, phase I metabolism involves the *o*-hydroxylation of estrone or estradiol by cytochrome P450 (CYP1A1, 1B1, 1A2 and 3A) to the corresponding 2-hydroxycatechol (2-OHE) or 4-hydroxycatechol (4-OHE) estrogen [71], [72]. 4-OHE has a longer half-life (12 min) than 2-OHE (47s) and appears to be significantly more carcinogenic. Higher levels of 4-OHE were found in breast cancer tissues compared to healthy breast tissues [52]. Both hydroxy-cathechols have significant binding affinity for the ERs (**Table 1.1.2**) and undergo further oxidative reactions to give *o*-quinone catechols. These Michael acceptors can alkylate nucleophiles such as adenine and guanine DNA bases. Conjugated equine estrogens are first hydrolyzed to their free form in the GI tract and are metabolized by CYP450 to give 4-hydroxy equilin (4-OHEN) and 4-hydroxyequilenin (4-OHEQ), and their corresponding oxidation *o*-quinones products [52]. 4-OHEQ-*o*-quinone isomerizes to 4-OHEN-*o*-quinone, which is the most stable form of quinone with a half life of 2.3 hours. The phase II detoxication pathway of estrogen plays a major role in helping to prevent the formation of depurinated adducts with adenine and guanine bases. Typical detoxification mechanisms include methylation of *o*-hydroxy catechol by the enzyme cathechol *o*-methyltransferase (COMT) and the formation of glucuronide, glutathione or sulfate conjugates, thereby increasing water solubility [39], [73].

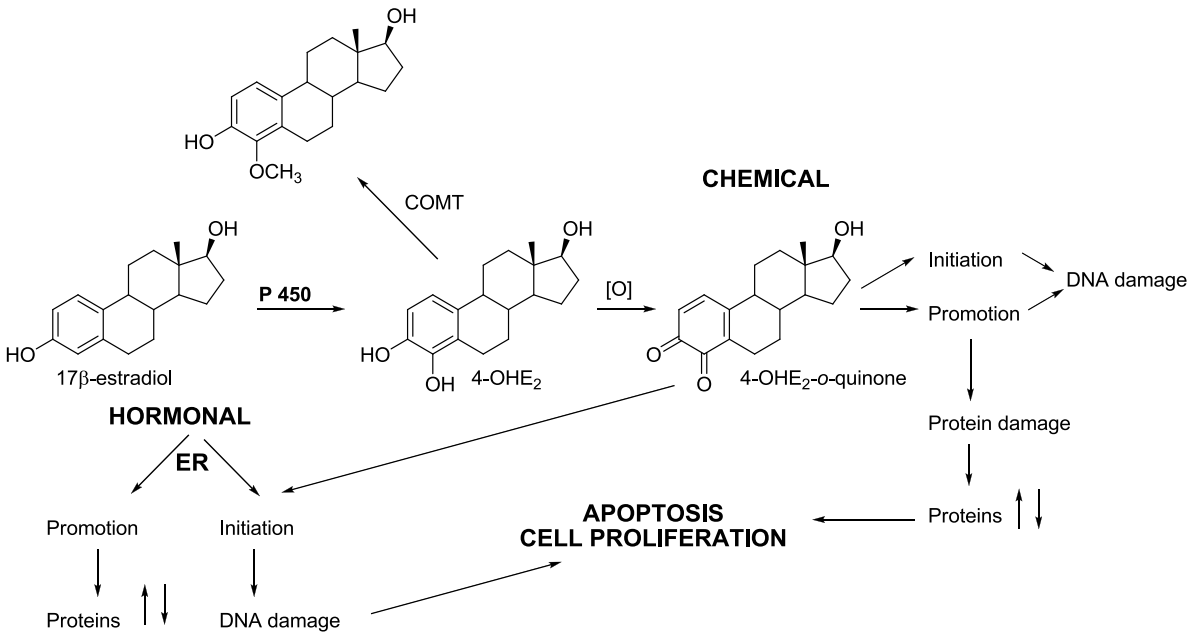


Scheme 1.1.1 Metabolism of estrogens. Phase I and phase II are indicated in blue and red labels respectively, whereas the DNA adducts are shown in purple [70].

1.1.5 Mechanism of estrogen toxicity

Breast cancers have been associated with hormones for over a century. In 1896, George Beatson demonstrated that oophorectomy, the surgical removal of the ovaries, of premenopausal women with advanced breast cancer could inhibit the growth of breast cancer [71]. Later, it was found that pharmaceutical doses of estrogen also inhibit the growth of breast cancer cells by the activation of apoptotic pathways [8]. Mechanisms of estrogen carcinogenesis are not yet fully understood, but the involvement of cellular control pathways that lead to cell proliferation and apoptosis is well documented [44]. To date, researchers have identified two main routes of estrogen carcinogenesis: (1) a hormonal proliferative pathway by which estrogen stimulates the

cell proliferation through ER signalling pathways, followed by genomic mutations throughout DNA replication and (2) a chemical pathway that generates genotoxic quinones (**Scheme 1.1.2**) [77]. For this reason, estrogen may promote and initiate carcinogenesis.



Scheme 1.1.2 Estrogen carcinogenesis hormonal and chemical pathways [77].

Hormonal pathway

The first route of estrogen carcinogenesis is mediated by the ER, and is known as the genomic pathway. It involves the stimulation of cell proliferation though gene transcription, which leads to an increased risk of genomic mutations during DNA replication [76]. There is also a non-genomic pathway involving ER coactivators that regulate post-translational modification of signalling proteins such as kinases, shortly after hormone exposure [76]. In addition, mitochondria have been implicated in the regulation of the proliferation of breast cells and the modulation of the nuclear gene expression [75], [76]. All three-ways (genomic, non-genomic and mitochondrial) modify gene expression by increasing cell proliferation and by decreasing apoptosis.

The classical hormonal carcinogenesis of breast cancer has been associated with ERα [39]. Several groups have demonstrated that β-selective ligands inhibit both the proliferation of breast cancer cells (MCF-7) and the stimulating effects associated with ERα [78], [79]. Chronic exposure to an elevated level of estrogens would result in cell proliferation signal transduction, mediated by ER, through spontaneous mutations [75]. Clinical studies have shown that oophorectomy before the age of 35 reduces the risk of developing breast cancer by 75% [71], while obesity and high endogenous estrogen levels contribute to an elevated risk (relative risk of 2.0-2.6) [39]. High levels of estradiol in postmenopausal women has been associated with a noticeable risk of mutations and tumorigenesis in uterus and breast tissues [39]. This is of particular concern for postmenopausal women as they cannot eliminate damaged cells in the uterine lining. Progesterone, through progesterone receptors (PRs), is also known to promote the proliferation and differentiation of normal mammary epithelium [39].

Genotoxicity

The second route of estrogen carcinogenesis is through its metabolism to form genotoxic species. In particular, the equine estrogen catechols present in Premarin®, 4-OHEN and 4-OHEQ, may act as carcinogen and tumor promoters by forming toxic estrogen quinones [52]. Cavalieri and colleagues demonstrated that the major DNA adducts produced from 4-OHEN-quinone *in vitro* and *in vivo* were the depurinated N7-guanine (**11**) and N3-adenine (**12**) adducts [80]. The authors found that the N3-adenine adduct is the only cause of mutations as it instantly depurinates, whereas the N7-guanine adduct takes hours to hydrolyze. Likewise, Bolton and colleagues have identified stable cyclic deoxyguanosine (**13**) and deoxyadenosine (**14**) adducts with the catechol 4-OHEN [81].

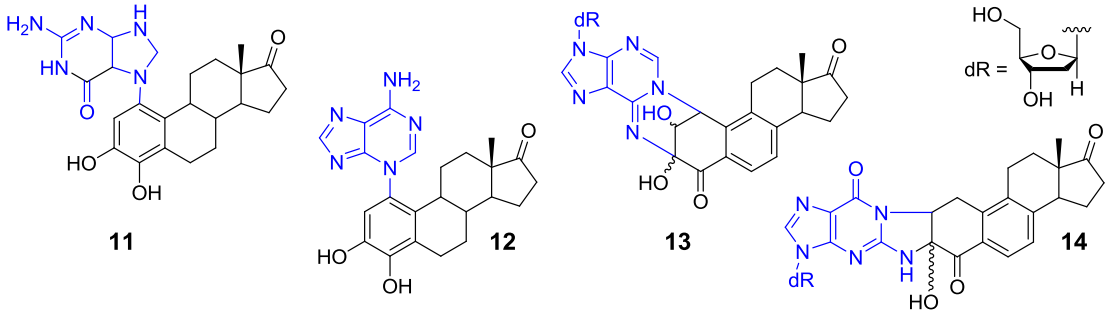
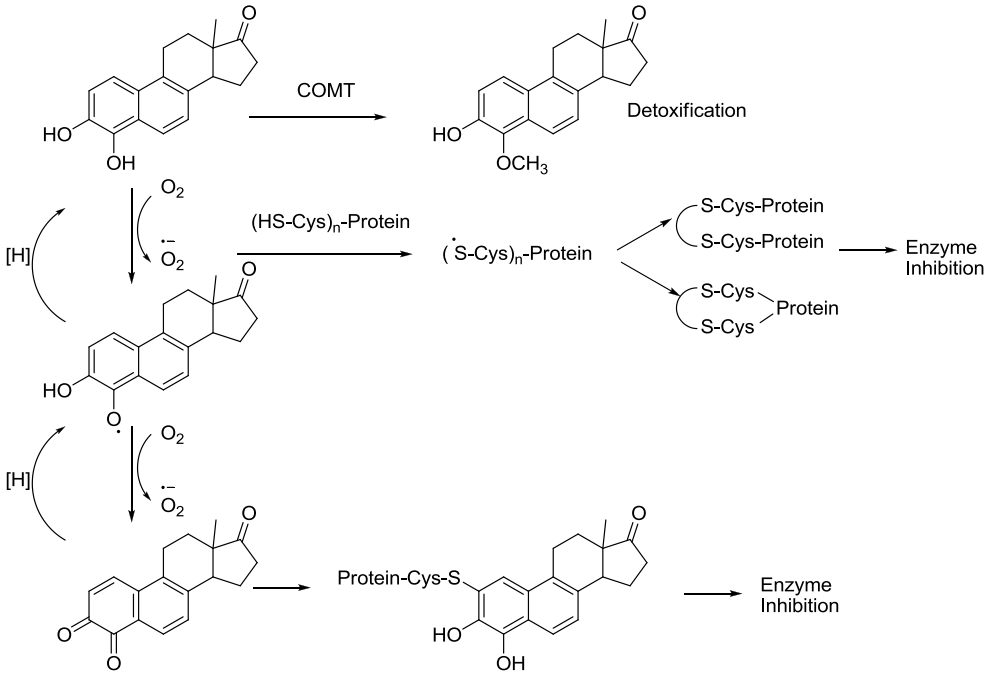


Figure 1.1.12 Structures of DNA adducts of 4-OHE_2. From left to right: 4-OHE_2 N7-guanine adduct, 4-OHE_2 N3-adenine adduct, 4-OHEN-deoxyguanosine cyclic adduct and 4-OHEN deoxyadenosine cyclic adduct.

Estrogen catechols have been linked directly to the initiation of breast tumors in rodents and humans [82]. Apurinic sites have been converted into tumor-initiating mutations by error-prone repair and cell transformation of MCF-10F cells to malignant cells [82], [83]. Human and animal studies have shown that breast and prostate cancers may be initiated by the formation of depurinated adducts [83]. Additionally, higher levels of estrogen-DNA adducts were found in women with breast cancer and men with prostate cancer compared to healthy subjects [70]. Initiation of the above cancers can be prevented by blocking the oxidation of estrogen metabolites with antioxidants, such as *N*-acetylcysteine and resveratrol [70]. Recently, Cavalieri and colleagues conducted a study on the ratio of serum estrogen-DNA adducts to their corresponding metabolites in women with early-onset breast cancer (n = 79) and those with an average (n = 63) or a high risk (n = 80) of developing breast cancer [84]. They found that the ratio was significantly lower in women with an average risk of developing breast cancer, compared to the other two groups (p values <0.0001). The authors concluded that estrogen-DNA adducts could potentially be used as biomarkers for risk of breast cancer.

Moreover, *o*-quinone catechols can cause damage within cells by alkylating proteins such as glutathione-*S*-transferase (GST), quinone reductase and thioredoxin reductase. Protein damage occurs via the formation of reactive oxygen species (ROS) through enzymatic one-electron redox cycling between the quinones and the semiquinone radicals (**Scheme 1.1.3**) [77]. Reduction of estrogen quinones back to hydroquinones can provide redox cycling to ROS.



Scheme 1.1.3 Mechanism by which 4-OHEN inactivates GST enzymes [77].

Bolton *et al.* have found that both 4-OHEN and 4-OHEQ are potent irreversible inhibitors of the glutathione *S*-transferase enzyme, which catalyze the conjugation of glutathione via a sulfhydryl group on a wide variety of substrates in order to increase their solubility. Both the quinone metabolism and ROS formation are causative agents of breast cancer and can induce radiation carcinogenesis if the toxic metabolites are not repaired by detoxifiying agents [52]. The formation of estrogen quinones is usually in homeostasis; however, when homeostasis is disrupted, the greater amount of apurinic sites can lead to the initiation of cancer [70].

1.1.6 Estrogen and health concerns in women

Health problems associated with estrogen supplement intake by women have been debated since their commercialisation in the 1930s [85]. Over the next 20 years, Premarin became a commercial success and was introduced increasingly to women, regardless of uncertainty about their safety. Between 1940 and 1970, diethylstilbestrol (DES), a synthetic estrogen, was widely used by pregnant women to prevent pregnancy complications; however, it was later discovered that DES increased vaginal tumor risk by 2.5 times in their prenatally exposed daughters, after they have reached the age of 40 [86]. In addition to the complaints about DES, the observation that an increased incidence of endometrial cancer has been associated with HRT resulted in a dramatic reduction of HRT prescriptions by 50% in the United States in the 1970s. In the 1980s, observational studies showed that the reported cancer risks could be prevented by antagonizing estrogen with progestin, and many combinations of estrogen and progestin were found to preserve the benefits of estrogen while decreasing these risks [85]. These results led to the widespread use of HRT for the next 20 years. However, HRT continued to be questioned by scientists, which led to several randomized clinical trials (RCTs) and epidemiological studies to examine the health risks associated with the use of exogenous estrogens especially for cardiovascular diseases and breast and uterine cancers.

To investigate the association between exogenous estrogens and health problems, many factors, such as age, obesity, heart problems, smoking and drinking habits, family history and the time of pregnancy, need to be taken into account. Most RCTs and epidemiological studies on HRT and cancer risk have examined the association of health problems with the use of Premarin (CCE) or Pempro (CCE plus progestin) [52]. Although initial epidemiological studies suggested that HRT would prevent the risk of cardiovascular disease, major clinical trials on HRT showed the

opposite, with a higher incidence of breast and uterine cancers [2], [3]. Evidence of these associations is mainly due to a positive association between high blood levels of estrogens and breast and uterine cancers. In a study done at the University of Oxford in 1998, data from 51 epidemiological studies were reanalyzed, which included more than 52,000 women with breast cancer and over 100,000 women without breast cancer. The study found a positive association between the duration of estrogen intake and breast cancer (2.3% increase per year of use) [87]. **Table 1.1.3** summarizes the results from major RCTs and epidemiological studies that have investigated the relationship between exogenous estrogens, cardiovascular diseases and breast and uterine cancers. The majority of studies have reported a small increase in breast and uterine cancer associated with HRT [18], [88]–[92]. However, some studies have not shown an association between estrogens and cancer risks [18], [93].

Table 1.1.3 Association of estrogens with CHD or cancer sites in RCTs/epidemiological studies.

Study	Year	Results	N (age; mean age)
NHS [88]	1976-1989	Positive trend with breast cancer HRT protective against CHD	121 700 (30-55; NA)
PEPI [93]	1995-1998	HRT protective against CHD	875 (45-65; 56)
HERS [18]	1998-2002	HRT increased CHD events	2763 (44-79; 67)
E3N [89], [90]	1990-2002	Positive trend with breast cancer	80,377 (40-66; 53)
MWS [91], [92]	1996-2001	Positive trend with breast cancer Small increase of ovarian cancer	1 million (50-64; 58)
WHI [18]	1997-2002	Positive trend with breast cancer HRT increased CHD events	16,608 (50-79; 63)

NHS – Nurses' Health Study
PEPI - Postmenopausal Estrogen/Progestin Interventions
HERS – Heart and Estrogen/progestin Replacement Study
E3N – Étude Epidémiologique auprès de femmes de l'Education Nationale
MWS – Million Women Study
WHI – Women's Health Initiative

The authors from the Million Women Study (MWS) which had enrolled more than one million women in the UK has concluded that women who use HRT have a higher risk of developing breast cancer than those not using HRT by 2-fold increase for the combined estrogen/progesterone supplements and by 1.4-fold increase for estrogen-only therapy [91]. They also found a slight increase in ovarian cancer by 1.2-fold for postmenopausal women using HRT, compared to non-users [92]. The Women's Health Initiative (WHI) trial was halted in 2002 after 5.2 years instead of 8.5 years by the Data and Safety Monitoring Board because the overall risk of using HRT exceeded the benefits [18]. This study was conducted on 16,608 women who received either one tablet of CEE (0.625 mg/day) and medroxyprogesterone acetate (2.5 mg/day) or a placebo. Participants in the study were aged between 50 and 79, although most women were over 60 years. This study found a statistically significant increase in the risks of breast cancer, stroke and heart attack, which contradicts previous findings [88], [93]. Further to the alarming results of the WHI study, a decrease in the use of HRT (66% for Pempro and 33% for Premarin) the following year was noticed in the United States and in several other countries [94]–[96]. Several studies from Western countries indicated that the dramatic decline in HRT has coincided with a lower incidence of breast cancer, with a risk reduction of 6.7% the next year, as revealed by the National Cancer Institute's Surveillance of the U.S. government [18]. A survey conducted by the Canadian Cancer Society (CCS) examined whether similar outcomes were found among Canadian women aged 50 to 69 [97]. Between 2002 and 2004, a decline of almost 10% in invasive breast cancer was associated with decreased use of HRT in women. In 2005, the National Toxicology Program NIEHS (National Institute of Environmental Health Sciences) classified the endogenous and exogenous estrogens as known human carcinogens due to evidence of elevated risks of endometrial cancer and breast cancer [52].

A recent randomized study in 2012 by Danish researchers reassessed the results from the WHI study to determine whether the risks identified were present if HRT was started early after menopause [98]. They conducted a randomized trial among 1006 women aged 45-58 years (mean age of 50 years) for over 10 years with an additional six year follow-up. Participants in this study were, on average, seven months after menopause as compared with the WHI trial, where most women were already 10 years past menopause. In this study, there was no association of HRT with increased risk of cancer, stroke, or coronary diseases. The authors concluded that the results of the WHI study were incorrectly generalized to the entire population, since the study focused mainly on HRT in women aged over 60 years.

Using the Bradford–Hill criteria for causation, it is possible to evaluate the association of exogenous estrogens and breast cancer [99]. In most studies, the strength of the association is weak because the published results do not show statistical significance (relative risk less than 2). Currently, the consistency between different studies is low, because of the 45 studies published between 1995 and 2000, 82% reported having no risk, 13% reported low risk, and 5% found a decreased risk [99]. There is a lack of the specificity criteria because most patients with breast cancer have never used HRT. The association is biologically plausible because the risk of breast cancer depends on hormone factors stimulating the growth of breast epithelial cells, such as early menarche and late menopause [100]. Furthermore, the presence of depurinated bases in breast cancer cells caused by *o*-quinones supports the biological plausibility [84]. Overall, there is a weak association between estrogen supplements and breast cancer by the standards of Bradford-Hills criteria.

Endogenous estrogens have also been associated with high risk of health problems. A recent study at the University of Oxford analyzed the combined results from 13 prospective studies with a population of 6,000 postmenopausal women and has established that the estrogen levels were strongly associated with several risk factors such as family history, smoking and drinking habits, and obesity. The authors concluded that estrogen levels may mediate the effects of these factors on the risk of breast cancer [101]. Observational studies have shown that late pregnancy and early menarche carry variable risks [87]. Moreover, an increased risk of breast cancer is associated with late pregnancies while early pregnancies are safer because the cell cycle is longer after the first pregnancy, allowing more time for DNA repair. Obesity and weight gain have been associated with increased risk factors in postmenopausal women with a relative risk of 1.99 for weight gain of more than 20 kg compared to women with unchanged weight [102], [103]. Weight gain was unrelated to breast cancer among premenopausal women. To summarize, both exogenous and endogenous estrogens have been associated with health risks, but most epidemiological studies are inconsistent in their results.

1.1.7 Design of non-carcinogenic estradiol analogs

Following the results of the WHI trial in 2002, many women turned to dietary supplements to relieve menopausal symptoms. However, studies funded by the National Institutes of Health (NIH) reported that botanical dietary supplements had a minimal effect or no effect in reducing climacteric symptoms [104], [105]. As mentioned in **Section 1.1.5**, studies have indicated that ERα is responsible for the proliferative effects of estrogens on breast cancer cells while ERβ has antiproliferative properties. These findings suggest that β-selective ligands would reduce the carcinogenic properties of current HRT drugs and may be helpful as a treatment for the

prevention of breast cancer. Accordingly, the ideal ligand would be β-selective, would prevent its oxidation to *o*-quinones, and would retain some estrogenic activity. For optimal binding, the primary requirement of the ligand structure is an aromatic hydroxyl group, and a second hydroxyl group located at approximately 11.0Å from the first one to form hydrogen bonds with the three primary pharmacophores Glu 353, Arg 394 and His 524 [106], [107]. Moreover, the ligand has to pass though a cell membrane, so its chemical structure should be essentially hydrophobic.

Previous efforts towards the synthesis of β-selective ligands for HRT

The discovery of ERβ triggered an intensive search for selective ligands by pharmaceutical companies. Recently, significant contributions from researchers at Wyeth Ayerst resulted in highly β-selective agonists such as ERB-041 (**15**) and WAY 202196 (**16**) [108]. These molecules are birayl systems containing two phenolic hydroxyl groups with O-O interatomic distance of approximately 11.5Å which is ideal to bind to the ERs. ERB-041 was found to bind to the ER with an IC_{50} of 5.4 nM with 200-fold β-selectivity relative to **E2** with an IC_{50} of 2-4 nM and equal selectivity to ERα and ERβ [109]. WAY 202196 had an IC_{50} of 2.7 nM and was 75 times more selective to ERβ than **E2** [110]. Despite evidence of activity in preclinical models of arthritis, these compounds did not show any estrogenic effect, such as the relief of hot flashes, and did not protect against postmenopausal osteoporosis in clinical trials [111].

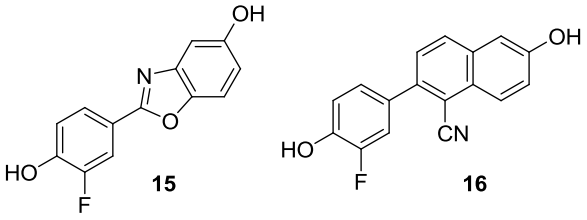


Figure 1.1.13 Chemical structures of ERB-041 (**15**) and WAY 202196 (**16**) designed by Wyeth Ayerst.

Design of new non-carcinogenic A-CD steroids for HRT

Since 2006, the Durst research group at the University of Ottawa has collaborated with the Wright group at Carleton University to use computer models to develop new SERMs that would not form *o*-quinones. A lead compound for this project was the β-selective phytoestrogen genistein which features an A-CD ring system. The objective of the research project was to design and synthesize several estrogen analogs which preserved the essential structure of estradiol to maintain similar pharmacophores and bioavailability. The hypothesis was that the removal of the B-ring would give more flexibility to the ligand, in contrast to **E2**, and that the ligand would have additional steric interactions in ERα with the methionine residue Met421. The free rotation around the rings A and C would allow the ligand to adopt the preferred conformation within the ER active site. In addition, different substituents could be introduced onto the A-ring to improve the β- selectivity. A target compound would be considered potentially useful at up to 10 times less active than estradiol at the nanomolar level.

The first attempt at preparing an A-CD estrogen agonist targeted 2-hydroxy-1,3 pyrimidine A-ring (**17**) as designed by Dr. Wright; but its synthesis was unsuccessful and this idea was abandoned [112]. Afterwards, the A-CD ligand was planned to have a simple phenolic A-ring as seen in compound **18**. It was designed to resemble estradiol, with the exception of two carbons in the B-ring, while retaining all the stereocenters identical. This compound was claimed to have been prepared by Dr. Muhammad Asim. Subsequently, the structure assignment was corrected as having the *cis* CD-ring and not the *trans* CD-ring junction as shown in the parent compound **19**. This ligand was then sent to Professor John Katzenellenbogen and Katheryn Carlson at the University of Illinois to determine its RBA values relative to estradiol. It was shown to bind at 21.5 ± 5% of the level of estradiol to ERβ and 1.5 ± 0.3% compared to estradiol to ERα.

Although this compound could in principle also form *o*-quinones and thus show similar toxicity to estradiol, the initial binding studies were encouraging and the Durst group began the synthesis of many analogs in order to improve potency by adding different substituents to A-ring. It was anticipated that some of these substitutions would reduce or eliminate the tendency to form the undesirable *o*-quinones. The numbering of the parent A-CD steroid was arbitrarily chosen to reflect its relationship to estradiol.

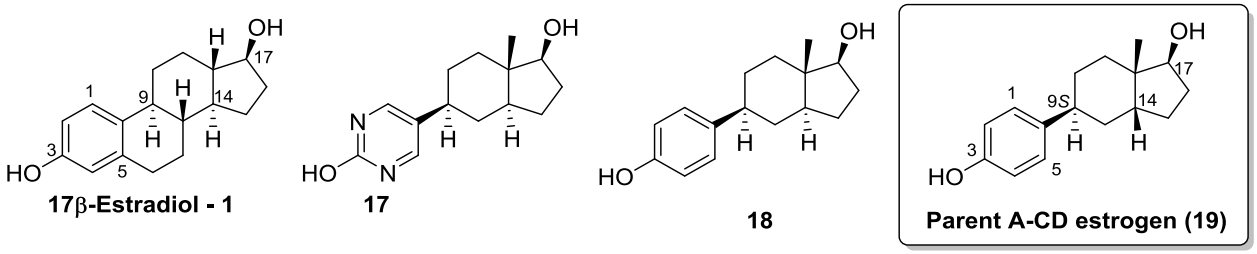


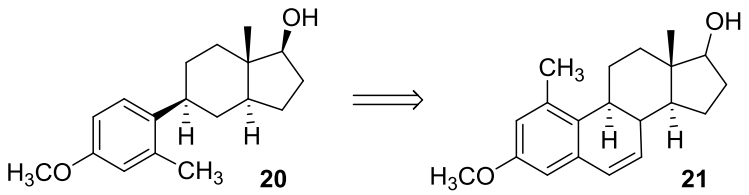
Figure 1.1.14 Chemical structures of 17-β-estradiol (**1**); A-CD analogs (**17** and **18**); and parent A-CD steroid **(19)**.

The results from preliminary studies have indicated that the "natural" 9(*S*) isomer was found to act as an agonist while the "non-natural" 9(*R*) isomer, with inversed configuration at C9, had a significantly reduced affinity. The addition of substituents at positions C2 and C4 of the A-ring appeared to decrease binding affinity for the two ER subtypes whereas the addition of substituents at position C5 has increased the binding affinity of the new compounds [32], [107]. A more detailed description of the preliminary results and the goals are given in the introduction to the next section of this thesis. The aim of this thesis was to build on the encouraging initial results and to generate additional A-CD estrogens that could improve these results.

1.2 Results and Discussion

A joint project between the Durst and the Wright groups was initiated in 2006 as a response to the mainly negative conclusions drawn by the authors of the Women's Health Initiative concerning the use of estrogen containing HRT by menopausal women. The successful grant application to the Canadian Breast Cancer Foundation, Ontario Division, was entitled "Reduction of breast cancer risk factors by molecular engineering. The redesign of hormonal supplements". The idea was to "deconstruct estradiol" by removing the B-ring while retaining its essential structure connectivity to bind to the key pharmacophores and increase its flexibility [107]. The remaining single bond between rings A and C enables free rotation of ring A with respect to ring C allowing the ligand to adjust itself to the preferred conformation inside of the ER binding site. The concern was that if the preferred conformation for binding to the receptor was a high energy conformation of the A-CD compounds, for example one in which ring A and ring C were essentially coplanar as in estradiol itself, then the binding of the A-CD compounds with respect to estradiol might be fairly low. In other words, the A-CD compounds might be significantly less active than estradiol. However, given that the natural hormone is active at the nanomolar level, a safe estrogen agonist could still be valuable even if it is 10 or more times less active than estradiol. The A-CD ring system was designed so that the A-ring could be functionalized with different substituents that might improve the selectivity towards ERβ and also reduce or eliminate the formation of *o*-quinones. The results of the initial studies presented in this Introduction include the contributions of Dr. Asim Muhammad, Daria Klownowska, Mohammed El Safiti, Yiming Qian and Samira Salari.

Prior to our research there have been several reports on A-CD compounds, also called B-ring seco-steroids or ring B seco-estradiols; these compounds have shown estrogenic and anti-fertility activities [113]–[117]. For example, Frank M. Hauser and coworkers reported the preparation of the 5-methyl derivative **20** starting with the unsaturated methylestradiol **21** [117].



Scheme 1.2.1 Synthesis of the seco-estrogen **20** starting from the unstaturated methyestradiol **21**.

Importantly, John A. Katzenellenbogen and coworkers have investigated many estrogenic compounds containing three rings (**22** and **23**) and examined factors that lead to differences in the selectivity of the ER isoforms [118]. Other research groups including those from Wyeth have studied additional variations of the 3-ring motif [108-111]. These factors include the steric interactions of the ligands with the different amino acid residues within the ER, and the size of the LBD. There are only two residues that are different in the active site between the two estrogen receptors: Met336 and Ile373 in ERβ are substituted with Leu384 and Met421 in ERα.

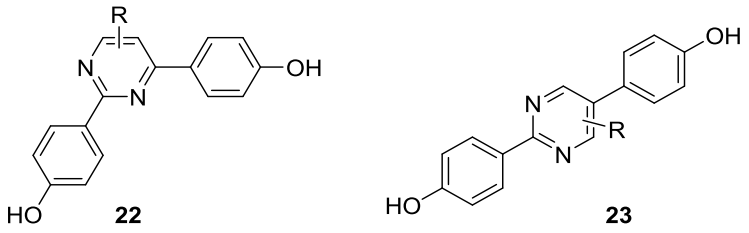


Figure 1.2.1 Estrogenic compounds containing three aromatic rings prepared by J.A. Katzenellenbogen and colleagues.

The D-ring of the *cis* CD-fused A-CD ligands was predicted to display unfavourable steric interactions with Met421 of ERα, which would presumably make these analogs ERβ selective. The ligand volume of **E2** is 282 $Å^3$ and that of the parent A-CDcompound **19** is 259 $Å^3$.

Therefore there is the potential for β- selectivity because ERβ is smaller than ERα (279 $Å^3$ compared to 379 $Å^3$, respectively) [32]. Finally, since the ligands are smaller than estradiol, the displacement of the helix (H12) should not be blocked as it is in the case of antiestrogens, and therefore the exterior parts of the receptor were not considered in the computational models. As mentioned above, removing the ring B in the A-CD compounds presents the opportunity to introduce different substituents in various positions in the A-ring. The docking studies suggested that some of these changes might have improved selectivity towards ERα or ERβ.

Previous studies have shown that halogenated estrogens maintained estrogenic activity similar to estradiol while preventing the formation of genotoxic species [119]–[122]. For example, Liehr and coworkers have demonstrated that unlike estradiol, 2-fluoroestradiol **(24)** did not induce renal cell carcinoma in male Syrian hamsters when the compounds were administered in hormonally equipotent doses, whereas 4-fluoroestradiol (**25**) induced kidney tumours, but at a slower rate than estradiol (**Figure 1.2.2**, adapted from [121]). Moreover, when 2-fluoroestradiol (**24**) was administrated to hamsters the metabolite 2-fluoro-4-hydroxyestradiol was rapidly conjugated to form methyl ethers, leading to low concentrations of catechol metabolites and hence its lack of carcinogenic activity. In contrast, the 4-fluoroestradiol (**25**) was rapidly converted to 2- and 4-hydroxy metabolites in the kidney and their methylation occurs less quickly [121]. Similarly, Bolton and coworkers have shown that incubation of 4-halogenated (fluoro, chloro or bromo) equilenin derivatives with tyrosinase in the presence of glutathione (GSH) formed less catechol-GSH conjugates compared to equilenin. They found that the 4-fluoroequilenin derivatives showed similar estrogenic effects than the conjugated equine estrogens found in HRT formulations but less overall toxicity [122]. Based on these combined findings, it was presumed that the addition of *o*-substituted halogens to A-CD ligands would not

significantly reduce binding affinity and thereby would likely result in ligands that would form lower levels of genotoxic species.

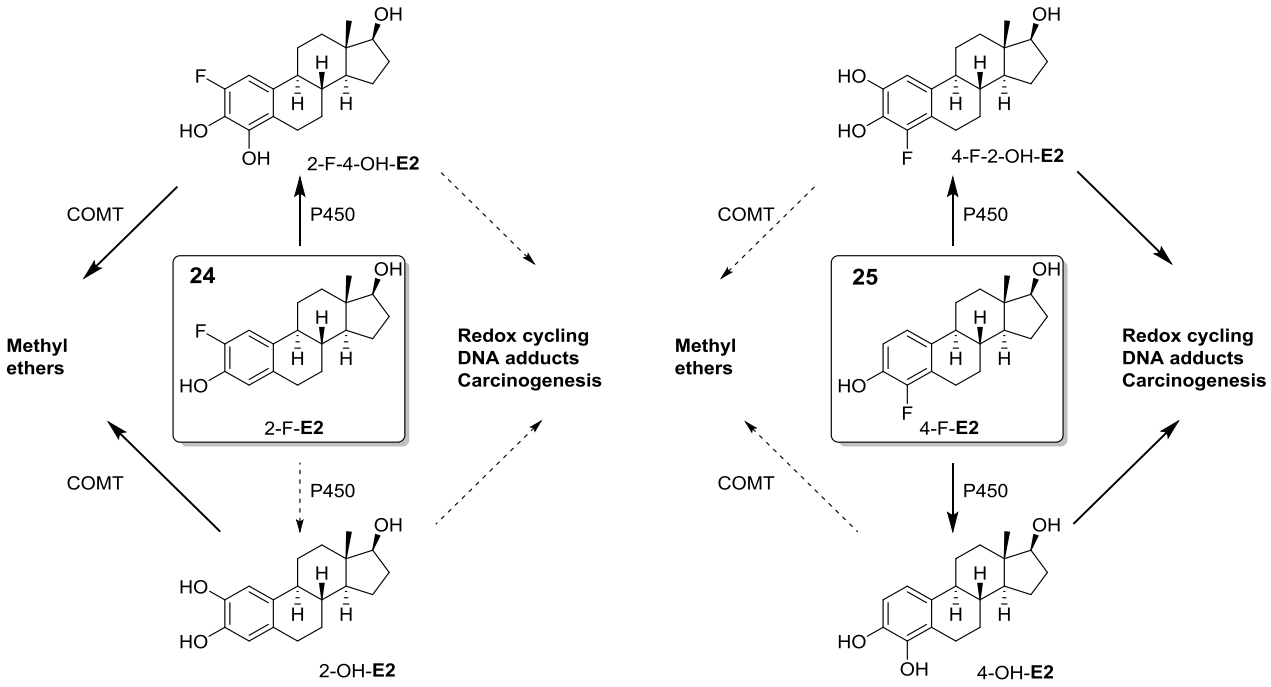
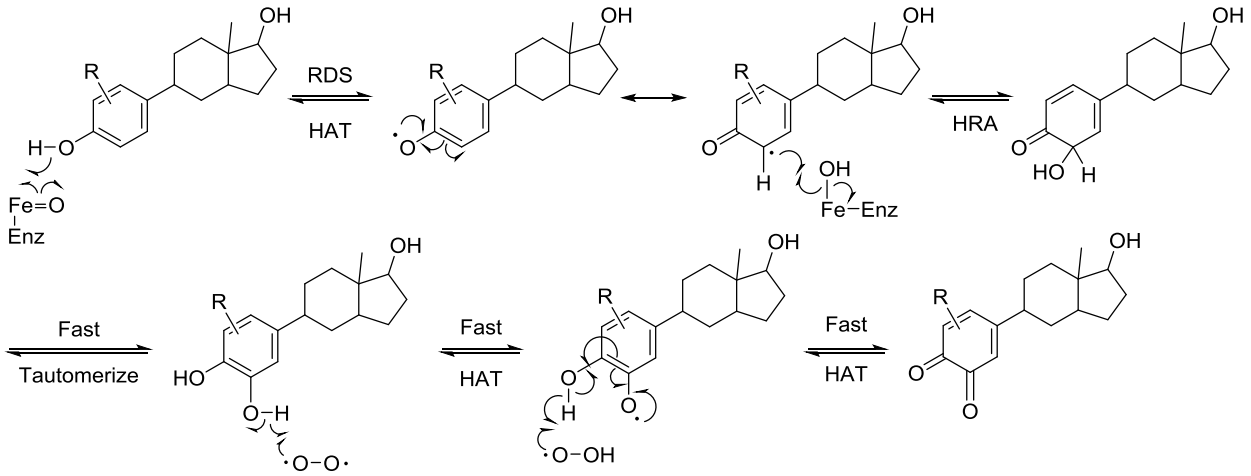


Figure 1.2.2 Proposed metabolic pathway of 2-fluoroestradiol (2-F-**E2**) and 4-fluoroestradiol (4-F-**E2**) by the group of Liehr. The hydroxy catechols of 4-F-**E2** are methylated by COMT at lower rates than those of 2-F-**E2**, which makes 4-F-**E2** catechols more available for further enzymatic transformation to genotoxic species. Adapted from [121].

The initial compounds prepared by the Durst group were designed with respect to two hypotheses: (1) fluorination at both ortho-positions on the A-ring would reduce quinone formation, and (2) the addition of other electron-withdrawing groups (EWGs) should also suppress the formation of these intermediates which have been implicated as causes of breast cancer [32], [123]. The latter hypothesis was rationalized as due to inductive and steric effects. The inductive effect from EWGs is understood to reduce the electron density in the A-ring, which increases the bond dissociation energy (BDE) of the aromatic O-H bond (**Scheme 1.2.2**). Homolytic cleavage of that bond to the phenoxyl radical is the rate determining step for

enzymatic hydroxylation to form hydroxy-catechols and subsequently *o*-quinones [123]. Therefore, increasing the BDE should result in the retardation or suppression of the catechol formation. **Section 1.2.9** explores these hypotheses.



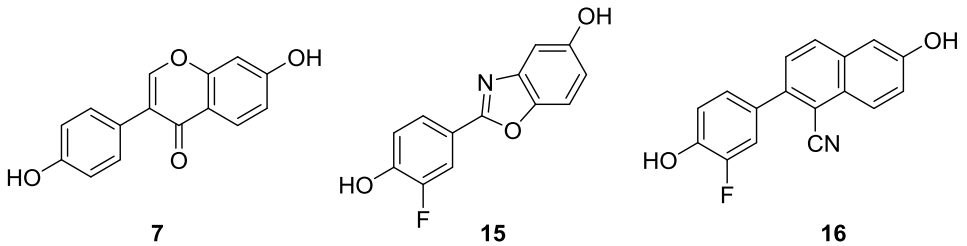
Scheme 1.2.2 Proposed enzymatic metabolism of A-CD estrogens where RDS= rate determining step, HAT = hydrogen atom transfer and HRA = hydroxyl radical addition. EWGs in the aromatic ring would decrease rate of phenoxyl radical formation, and thus may retard the formation of *o*-quinones.

The focus of this thesis project was to build a library of β-selective estrogen agonists with reduced potential for the formation of *o*-quinones as a potential alternative to current HRT or as chemopreventive agents. Ideally for a safe HRT, ERβ-selectivity is required to lower the incidence of hormonal carcinogenesis, but the compound should also have some ERα affinity as this receptor is predominantly responsible for the estrogenic activity that alleviate the effects of menopause. The range of estrogenic activity should be considered at up to 10 times less active than **E2** at the nanomolar level to be considered safe but still effective.

1.2.1 Preliminary results

General considerations

The initial concern was whether the A-CD estrogens would bind strongly to the estrogen receptors since these compounds have distinctly different molecular shapes than estradiol in their ground state. Based on the simple examination of molecular models, one can predict that the preferred conformation of the A-CD compounds having the 9*S* stereochemistry would be substantially different than that of estradiol. In the latter, the rigidity of the four fused rings results in an essentially planar model with the dihedral angle between the A-ring and the C-ring being at most 10^{o}. In contrast, in the A-CD compounds, a planar arrangement of rings A and C represents an energy maximum; therefore if the almost-planar arrangement was crucial for binding to the ERs, a strong binding would be unlikely since a significant amount of energy would need to be invested to attain such a conformation. Fortunately, an examination of the structure of genistein (**7**) and many of the synthetic estrogens reported by different research group including compounds such as ERB-041 (**15**) and WAY 202196 (**16**) reported by Wyeth researchers strongly suggested that the dihedral angle between the aromatic ring and the remaining part of the molecule could vary without seriously affecting the binding constants. In each of the above molecules, the planar conformation is not the favoured one, yet several of these compounds show binding affinities even stronger than estradiol itself [20], [108].



7 **15** **16**

Figure 1.2.3 Chemical structures of the natural phytoestrogen Genistein (**7**) and of the synthetic estrogenic compounds ERB-041 (**15**) and WAY-202196 (**16**) reported by Wyeth researchers.

As mentioned previously, the distance between the two OH groups in estradiol is 11.2 Å. This is considered an important parameter for binding to the ERs. Fortunately, in our A-CD compounds, the distance between these groups is essentially the same as that of estradiol. The Relative Binding Affinities (RBAs) to both estrogen receptors observed for the parent A-CD compound **19** showed that the initial concern that these compounds might not bind well due to the different shape of these compounds relative to estradiol was largely unfounded since the binding of **19** was only reduced by a factor of 5 for ERβ and about 65 for ERα. Recall that these compounds have the *cis* CD-ring fusion in contrast to the *trans* fusion found in the natural estrogens.

The initial synthesis yielded compound **26** in addition to **19**. Compound **19** has the "natural" (*S*) stereochemistry at C9 while in compound **26**, the stereochemistry at this center is inverted (**Figure 1.2.4**). This leads to a conformational change via a chair-to-chair interconversion of ring C such that the large aryl group (ring A) is equatorial resulting in structure **26**. In this structure, the relative positions of the two key OH groups are considerably changed relative to estradiol. It was therefore not surprising that this compound and subsequently all others with the 9(*R*) configuration showed significantly reduced binding affinities compared to estradiol and the 9(*S*) A-CD compounds.

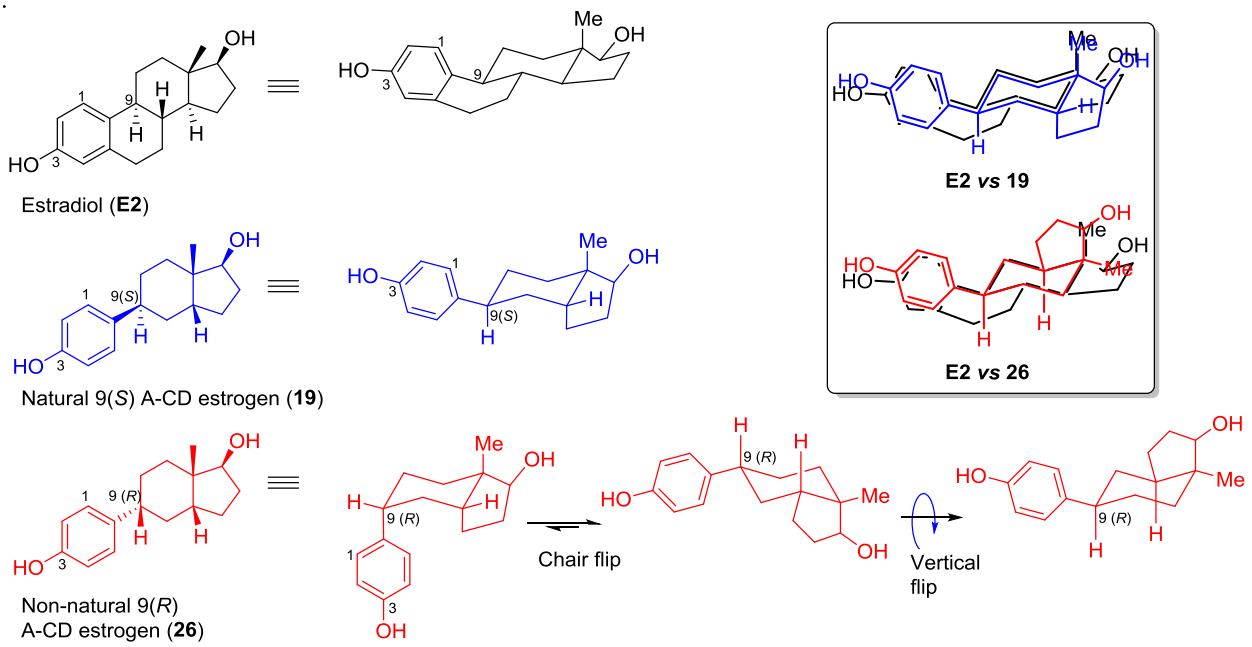


Figure 1.2.4 Conformational analysis of the natural and non-natural parent compounds, **19** and **26** respectively. By superimposing the A-CD structures with estradiol, one can observe that the natural 9(*S*) isomer **19** retains essentially the same O-O interatomic distance and spacing as estradiol, which is not the case for isomer **26**.

Bioassays of the preliminary A-CD steroids

In this thesis, all compounds were evaluated for their RBA to ERs by Professor John A. Katzenellenbogen and Katheryn E. Carlson at the University of Illinois. The RBAs were determined by a radiometric competitive binding assay using 10 nanomolars [^{3}H] estradiol and increasing concentration of the ligand being evaluated. Displacement of the radioactivity induced by the ligand represents binding to the receptor, which is quantified to give the RBA relative to estradiol. Binding affinities are expressed relative to the binding affinity of **E2**, which is set to 100% for both ER isoforms [32]. RBAs were calculated as the ratio of the half maximal inhibitory concentration (IC_{50}) of estradiol and IC_{50} of the test compound. The IC_{50} of estradiol to the estrogen receptors is at 1.2 nanomolars. Thus, a RBA of 50 for a new ligand indicates a binding level of 2.4 nanomolars. Other relationships are shown in the **Table 1.2.1** below.

Table 1.2.1 Comparison between the RBA values and the binding affinity in nanomolar levels of estrogenic ligands.

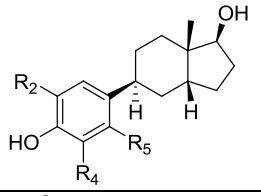
RBA value relative to estradiol = 100	Binding levels at nanomolar levels
200	0.6
100 (**E2**)	1.2
50	2.4
25	4.8
12.5	9.6
6.25	19.2
3.13	38.4
1.56	76.8

Some of these compounds were also tested in gene transcription assays to assess their relative transcription activation (RTA), which is a sensitive method that determines the hormonal activity of a ligand *in vitro*. These assays were conducted by Dr. Christine Pratt and colleagues at the University of Ottawa using HEK-293 cells transfected with the ERE-luciferase reporter plasmid and the ERα or ERβ expression vector. Transcription activation of the ligands at a concentration of 10 nM was compared to estradiol at the same concentration for the two different receptors, where estradiol is set at 100% for both ERs. To assess the degree of specificity for each ligand, RTA values were calculated as the ERβ / ERα activation. RTA results are an indication of the function of a ligand as an agonist or an antagonist for a specific ER subtype. A positive number indicates that it is able to activate the ER while a negative number indicates that it acts as an antagonist. For agonist ligands, a greater binding affinity causes a higher occurrence of gene transcription. RTA and RBA values are generally correlated but not in a linear relationship. As mentioned earlier, ERβ binding is preferred over ERα, however, ERα activity is still needed to relieve some of the undesirable menopausal symptoms. The challenge is to achieve the right proportions of activation without causing excessive cell proliferation, which increases the risk of breast cancer.

At the beginning of the project, several A-CD ligands with different substituents at positions C2, C4 and C5 of the A-ring were prepared by the Durst group. These compounds were designed to have the same absolute stereochemistry as estradiol at all centers except C14, which leads to a *cis* CD-ring junction instead of the *trans* CD-ring found in the natural hormone. The first analogs were important to draw conclusions about their trend of the binding activity.

One particularly important factor for binding is the (*S*) stereochemistry at C9. As mentioned previously, the parent compound **19**, with the natural (*S*) stereochemistry at C9, proved to be 14 times more β-selective in the RBA assays. In addition, the RTA results showed that it was a potent agonist for ERβ (164 %) and moderate agonist for ERα (4.2%). As predicted, compounds with inverted (*R*) stereochemistry at C9 (**26**) showed lower binding affinities by 30 times compared to the natural (*S*) isomers. Consequently, the investigation of non-natural isomers has not been pursued further. The preliminary findings are summarized in **Table 1.2.2**. This table contains (1) the compound identification number and substitution pattern at positions 2,4,5 on the A-ring, (2) relative binding affinity (RBA) values for binding of ligand to receptors ERα and ERβ and their β/α ratio, and (3) relative transcription activation (RTA) values for activation of the estrogen response element (ERE) by ligand-activated ERα or ERβ and their β/α ratio.

Table 1.2.2 Bioassays on the preliminary results of A-CD analogs prepared by the Durst group.



Comp.	Ring A (R_2, R_4, R_5)	RBA[b]			RTA[c]		
		ERα	**ERβ**	**β/α**	**ERα**	**ERβ**	**β/α**
Estradiol (E2)		100	100	1	100	100	1
Estrone (E1)		4.5 ± 0.7	3.8 ± 0.4	0.8	-	-	
19	H H H	1.5 ± 0.3	21.5 ± 5	15	4 ± 3	164 ± 2	39
26 [a]	H H H	0.06	0.61	10	-8.9	7.7	
27	H F H	1.0 ± 0.2	8.7 ± 1.7	8.3	-9 ± 9	196 ± 3	pure β
28	F F H	0.040 ± 0.002	0.277 ± 0.006	6.9	-	-	
29	Cl Cl H	0.004 ± 0.001	0.002 ± 0.001	0.5	-	-	
30	H H F	27.3 ± 0.7	136 ± 7	5.0	44 ± 12	158 ± 1	3.6
31	H F F	4.6 ± 0.9	43 ± 6	9.3	18 ± 4	151 ± 1	8.3
32	F F F	0.19 ± 0.01	1.7 ± 0.2	9.3	12.8 ± 0.4	95 ± 4	7.9

a- Parent compound with inverted (*R*) configuration at C9.

b- Relative Binding Affinity assay performed by the group of Dr. J. Katzenellenbogen at the University of Illinois.

c- Relative Transcription Activity performed by the group of Dr. C. Pratt at the University of Ottawa.

In order to reduce the formation of genotoxic species, compounds were prepared having *o*-substituents relative to the C3-hydroxy substituent. Ortho-fluorination (**27**) caused a reduction in RBA by a factor of ~2, whereas when both ortho-positions are substituted with a halogen atom (**28** and **29**), the overall binding affinity significantly decreased for both ERs, especially for the larger Cl substituents. The RTA results demonstrated that the ligands monosubstituted at C4, such as compound **27**, were shown to be slight antagonists for ERα and quite potent agonists for ERβ. Compound **27** showed surprisingly high transcription activity with higher β-selectivity than the parent compound **19** despite weaker binding levels. Substitution of F at C5 (**30**) significantly increased the binding affinity for ERα (27.3 ± 0.7%) and ERβ (136 ± 7%, which is more than estradiol), with a β-selectivity of 5.0. This compound is a strong agonist for both the

α- (44 ± 12%) and β- (158 ± 1%) receptor. This suggests that position C5 is important for obtaining a greater binding affinity to both ERs but it results in lower β/α selectivity both with respect to binding and transcription. Based on one example (compound **27**), substituent at C4 lower binding affinity but appear to be pure β-agonists.

As discussed earlier, the increase of the molecular volume by the addition of any substituents on the A-ring (**27** to **32**) resulted in a decrease of β-selectivity with respect to the parent compound **19** since ERβ has a smaller molecular volume than ERα. These results highlight the importance of the size and location of the functional groups on the A-ring. Lastly, the deactivating ortho position relative to the 3-hydroxy substituent was modulated by the additional substitution of the activating position C5 (**31** and **32**). Compound **32**, also called **L17**, was of particular interest as a potentially safe HRT because both ortho positions are blocked and it acted as a slightly selective β-agonist in the transcription activation assays.

These preliminary findings, when combined with results from other members of our group and predictions based on computational studies by Wright and coworkers, suggest that several additional changes need to be considered. These include:

i. Substituents at C5 in the aromatic ring of parent compound **19** such as EWGs (for example Cl, CF_3, and CHO groups) and EDGs (such as CH_3, OCH_3, OH groups). These compounds were expected to have a greater binding affinity compared to the parent compound **19**, based on the binding affinity observed for compound **30**.

Figure 1.2.5 Structures of *cis* A-CD estrogens with EWGs or EDGs substitutions at C5.

ii. Different substituents at C1, C2, C4 and C5 in the aromatic ring of the compound **19**. These compounds could be potentially safer alternatives to existing analogs.

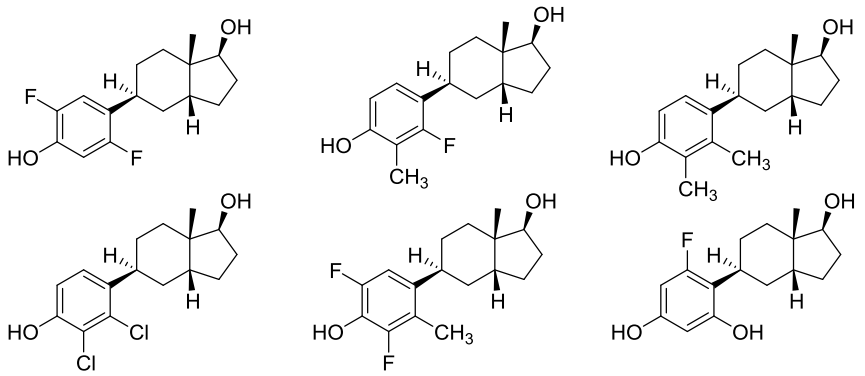


Figure 1.2.6 Structures of *cis* A-CD estrogens with polysubstituted A-ring.

iii. Variations in the C-ring, including C9-C11 and C9-C8 unsaturation and C9-OH substituents. Unsaturation in Ring C should result in a somewhat different preferred conformation of the ligand due to a flatting of the CD-ring. It was considered important to determine the relative binding affinity and β/α selectivity of such compounds. Since the binding pocket is large around the CD-ring area, C9-OH substituents may also be suitable even though this polar group reduces the LogP. Such compounds are synthetically attractive as they are the result of the first step in the coupling of the ring A and CD-ring moieties.

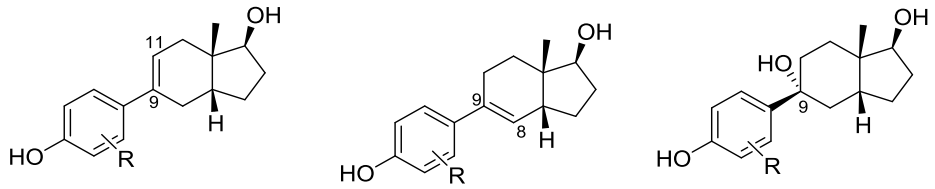


Figure 1.2.7 Structures of *cis* A-CD steroids with one double bond between C9-C11 or C9-C8 in the CD-ring, as well as the C9-hydroxy derivatives.

iv. *Trans* A-CD analogs of some representative compounds. Given that all stereocenters are identical to estradiol, *trans* A-CD analogs would likely have a higher binding affinity, but lower selectivity than the *cis* analogs.

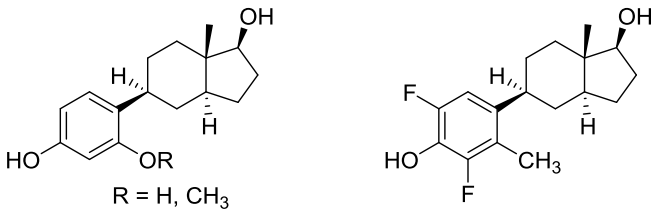


Figure 1.2.8 Structures of *trans* A-CD estrogens.

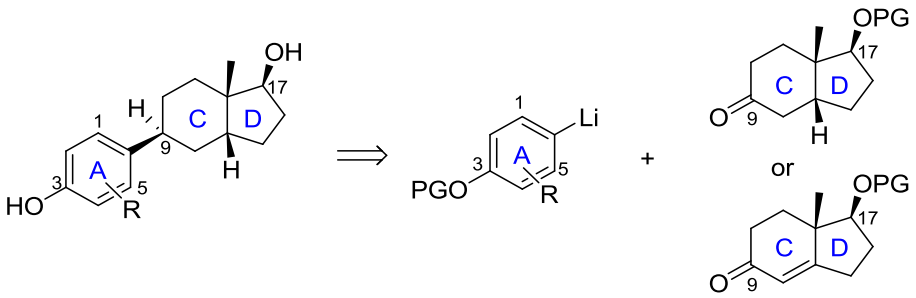
Additional rationalizations about the choice of these compounds as targets will be given at the beginning of each sub-section. The compounds described in this thesis are at the beginning stages of developing a quantitative structure-activity relationship (QSAR) and the main tool of analysis were based on RBA and RTA data, as used previously in the group. Analogs that showed improved activity and selectivity for ERβ were further investigated in **Section 1.2.9**.

1.2.2 Synthesis of new A-CD steroids with potential applications in HRT

General approach

A general approach to the synthesis of A-CD estrogens has been described by other members of our group; the retrosynthesis is shown in **Scheme 1.2.3**. This provides ready access to a library of compounds with different substituents in Ring A. The preparation of most of the A-rings required to make the desired analogs is shown as part of the synthesis of the final product. The chemistry involved is for the most part classic aromatic bromination and appropriate protection of the phenolic OH group. Whenever possible, the compounds required for the preparation of

the desired A-rings were purchased. As previously mentioned, the numbering system of our A-CD analogs has been kept the same as the endogenous estrogens.



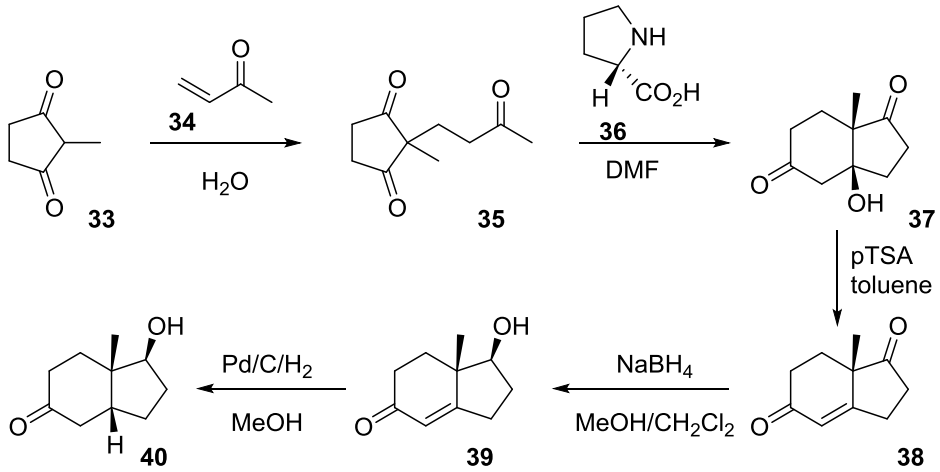
Scheme 1.2.3 Retrosynthesis of the A-CD compounds.

Briefly, this synthethic pathway involves the preparation of a suitably protected 4-bromophenol, converting it to its lithio derivative with *n*-BuLi in THF at -78^{o}C and coupling this with either the CD-ring ketone or enone, again with the OH group carrying an appropriate protecting group. This coupling results in a mixture of epimeric tertiary alcohol adducts at C9 position. Further adjustments of the functional groups via hydrogenolysis or dehydration/ hydrogenation and removal of the protecting groups yield the A-CD estrogens.

Enantioselective synthesis of the CD-ring moiety

It is important to note that the CD-ring enone **39** was prepared in essentially enantiomerically pure form following the well-known procedure described by Hajos and Parrish while they were working at Hoffmann-LaRoche in the early 1970's (**Scheme 1.2.4**) [124], [125]. It features a proline-catalyzed asymmetric intramolecular aldol reaction and represented an early example of highly enantioselective organocatalytic transformation. This reaction sequence has been carried out a number of times by different members of our group with the modification that the selective reduction of the enone was carried out with $NaBH_4$ in a mixture of methanol (MeOH) and

dichloromethane (DCM) at -78°C following the procedures described by Ward et al [126]. The structure of each intermediate of this sequence was confirmed by ^{1}H NMR data. Hydrogenation gave predominantly the *cis* fused CD-ring **40**.

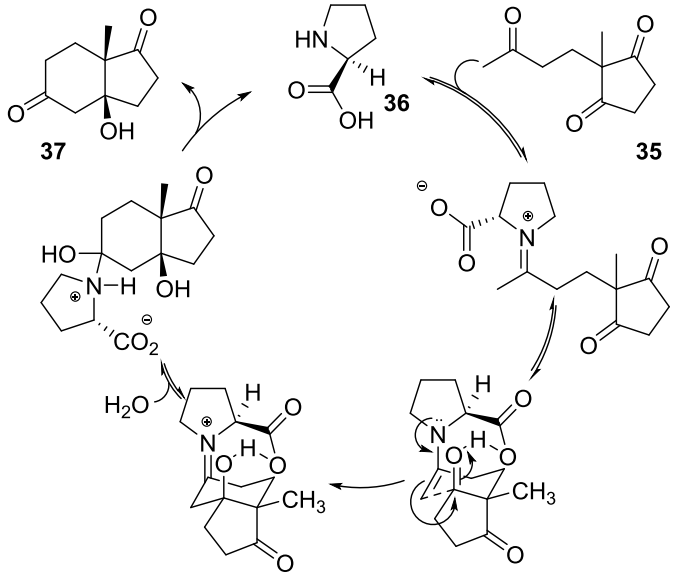


Scheme 1.2.4 Synthesis of the enantiomerically pure CD-ring moiety **40**.

Since significant amounts of the CD-ring moiety were required to achieve all the objectives listed above, the synthesis began with 100 g of the commercially available 2-methyl-cyclopentane-1,3-dione **33** and 125 g of methyl vinyl ketone **34**, suspended in water at room temperature for four days. The Michael adduct **35** was purified by column chromatography and recovered in a better yield (97%) than that reported in the literature (88%) [127].

The next step was the asymmetric Robinson annulation of the prochiral triketone **35**, developed by Hajos and Parrish, using 3% of L-proline **36** in *N*,*N*-dimethylformamide (DMF) aprotic solvent [124]. The most recent mechanism is that proposed by Houk and coworkers and involves a six-membered chair-like transition state (**Scheme 1.2.5**) [128], [129]. In this mechanism, L-proline **36** forms an enamine with the triketone **35** which then undergoes an asymmetric enamine aldol condensation, followed by hydrolysis of the iminium salt by water to give the *cis* hydrindanone ketol **37**. The *cis* conformation is rationalized by the favourable

electrostatic interactions between the carbonyl of the five-membered ring and the electron-rich π enamine bond, as well as the fundamental stability of *cis*-hydrindanone systems relative to *trans.* This mechanism is in contrast with the mechanistic experiments by Agami and coworkers, who proposed a transition state involving an enamine with two proline units in which the second one assists the $N-H\cdots O$ hydrogen transfer [130]. The ketol intermediate **37** was isolated accompanied by the dehydration product **38**. To ensure complete conversion, the crude mixture was dissolved in toluene in the presence of a catalytic amount of pTSA equipped with a Dean-Stark apparatus and refluxed until all of the water product had separated. The residue was purified by column chromatography to give the keto enone **38** as a light yellow oil in 91% yield over 2 steps.



Scheme 1.2.5 L-proline **36** catalyzed cyclization of triketone **35** to **37** as proposed by Houk *et al*.

The regio- and stereo-selective reduction of the C17 carbonyl to the corresponding 17β-hydroxy group was performed with sodium borohydride ($NaBH_4$) in a 1:1 mixture of MeOH and DCM at −78°C for one hour, as described by Ward and coworkers [126]. The observed regioselectivity comes from the more stable and less electrophilic 6-membered enone ring being more resistant to

reduction compared to the saturated 5-membered ring ketone. The 13-β-methyl group acts as a directing group for the reduction by blocking access to the reducing agent from the β-face of the CD-ring, resulting in hydride addition *trans* to the methyl group. The C17-ketone **38** was reduced to C17-β-hydroxy **39** in a 57% isolated yield using the Ward procedures [126].

The double bond of the CD-ring enone **39** was reduced using Pd/C under H_2 atmosphere, which gave the *cis*-fused hydrindan system **40** in a 77% isolated yield. The complete reduction of the olefin was confirmed by the loss of the alkene hydrogen and the two alkene carbon peaks in the ^{1}H NMR and ^{13}C NMR spectra, respectively. It is important to note that the hydrindan intermediate **40** has a *cis*-fused system, unlike the natural hormone with a *trans*-hydrindan junction. The *cis* hydrogenation is due to the concave shape of the enone which hinders approach of the hydrogen carrying catalyst from the bottom face (**Figure 1.2.9**).

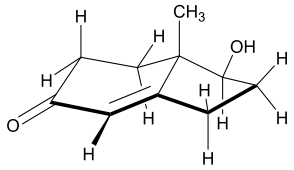
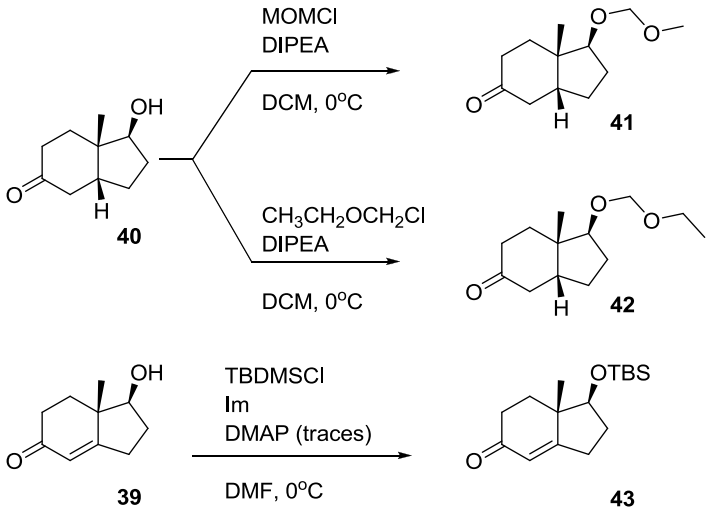


Figure 1.2.9 Structure of the enone **39** showing the concave structure.

Following the reduction of the enone alcohol **39** to the *cis*-keto alcohol **40**, the free hydroxy group was protected with methyl chloromethyl ether (MOMCl) in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane (DCM) to afford **41** in 74% isolated yield. Methyl chloroethyl ether was chosen as protecting group throughout the syntheses of the A-CD derivatives when the MOM-protecting group was discontinued from the manufacturer; the transformation to the desired compound **42** was produced in a 64% yield. For synthetic purposes, some of the CD-enone **39** was protected with *tert*-butyldimethyl silyl chloride

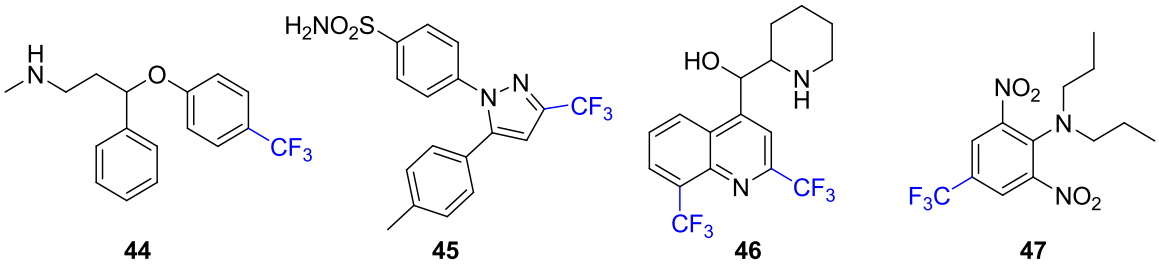
(TBDMSCl or TBSCl) in the presence of slight excess of imidazole and traces of DMAP to give the TBS-ether **43** in 72% yield.



Scheme 1.2.6 Protection of the *cis* CD-ring ketone **40** and enone **39**.

1.2.3 Synthesis and bioassays of C5-trifluoromethyl A-CD derivatives

The first objective was to prepare A-CD derivatives with EWGs such as a trifluoromethyl and chlorine groups at the C5 position of the A-ring. It was expected, based on the C5-fluoro A-CD analog **30,** that such substituents would increase the binding affinity compared to the parent compound **19** and likely even compared to **30**. Molecules bearing a trifluoromethyl group on the aromatic ring are numerous and important in the pharmaceutical and agrochemical industries mainly because of their improved metabolic stability due to the C-F bond strength, and increased lipophilicity compared to the non-fluorinated analogs [131]–[133]. The improved lipophilicity affects the partitioning of the drug into membranes and facilitates hydrophobic interactions of the drug molecule with specific binding sites within receptors. Some examples of compounds bearing a CF_3 substituent commonly used for pharmaceutical or agrochemical purposes include fluoxetine (**44**), celecoxib (**45**), mefloquine (**46**) and trifluraline (**47**).

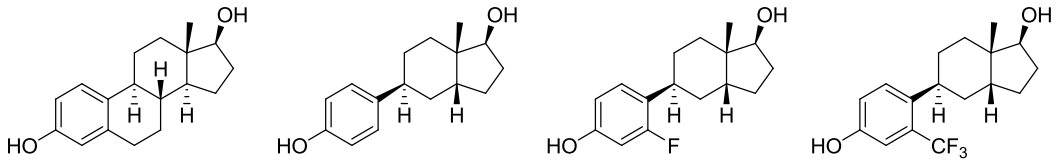


44 **45** **46** **47**

Figure 1.2.10 Example of molecules bearing a trifluoromethyl group in the aromatic ring used in the pharmaceutical (fluoxetine **44**, celecoxib **45** and mefloquine **46**) and agrochemical (trifluraline **47**) industries.

As seen in **Table 1.2.3**, the replacement of a hydrogen atom by a fluorine atom in the aromatic ring of our parent compound **19** resulted only in a modest increase in lipophilicity whereas the CF_3 group enhanced it greatly. Recall that the C5-fluoro compound **30** also showed an enhanced binding affinity to ERα and ERβ by factors of 18 and 6.5, respectively, compared to the parent compound **19**. Thus the C5-trifluoromethyl derivative **48** would likely display increased binding affinity compared to the C5-fluoro compound **30**. The preparation and biological characterization of five derivatives of **19** carrying the CF_3 group at C5 will be described in this section. The table below compares the lipophilicity and binding affinities of estradiol with several A-CD derivatives.

Table 1.2.3 Comparison of the lipophilicity profile and the binding affinity of 17β-Estradiol with the parent A-CD analog (**19**), and the derivatives bearing either a fluorine atom (**30**) or a trifluoromethyl group (**48**).



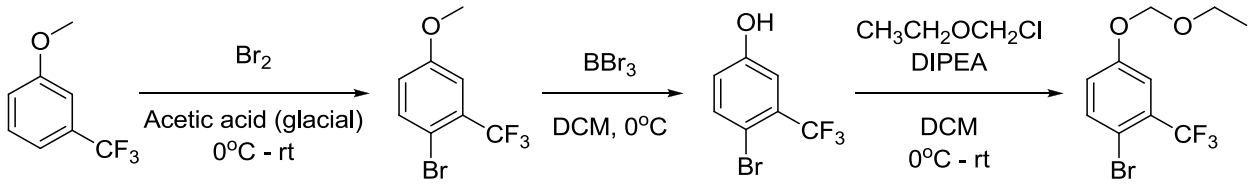
E2 **19** **30** **48**

Chemical Properties	**E2**	**19**	**30**	**48**
Chemical formula	$C_{18}H_{24}O_2$	$C_{16}H_{22}O_2$	$C_{16}H_{21}FO_2$	$C_{17}H_{21}F_3O_2$
Molecular weight	272.38	246.35	264.34	314.34
Lipohilicity (Log P)[a]	4.01[b]	3.77	3.98	4.75
RBA (ERα:ERβ)	100 : 100	1.5 : 21.5	27 : 136	**90 : 213** (see below)

a- Unless specified, the calculated data was predicted data using KOAWIN Software v1.10.
b-This is an experimental value obtained from the reference [27], calculated value = 3.94.

Synthesis of the C5-CF_3 A-CD derivatives

The synthesis of the trifluoromethyl A-CD derivatives followed the general procedures developed by our group. The preparation of the aromatic trifluoromethyl substituted ring A is depicted in **Scheme 1.2.7**. All intermediates in this sequence are known and their structure was confirmed based on comparison with the ^{1}H NMR data from literature.



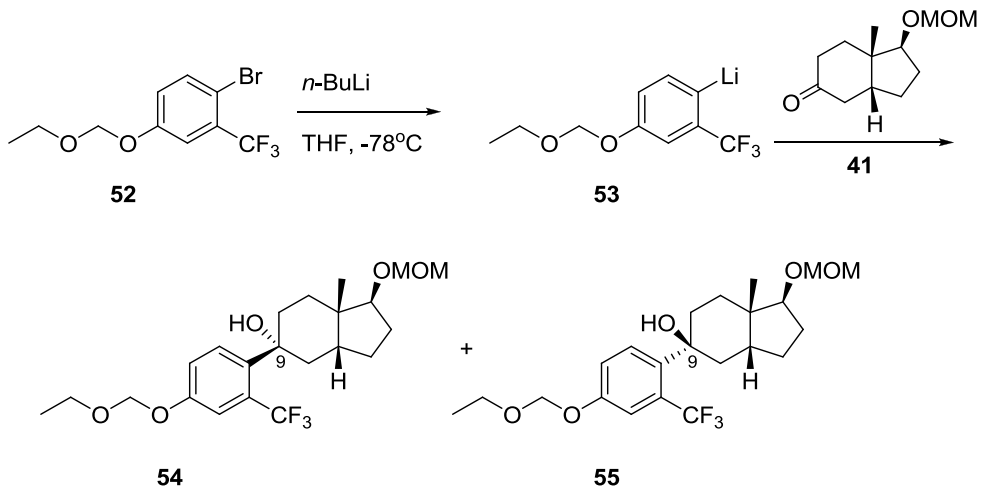
49 **50** **51** **52**

Scheme 1.2.7 Preparation of the protected bromo-trifluoromethylphenol A-ring **52**.

Previous studies in our group by Dr. M. Asim have shown that methoxybenzene substituents were better para-directing groups for bromination reactions than the corresponding phenols.

Accordingly, bromination of commercially available 1-methoxy-3-(trifluoro-methyl) benzene **49** was successfully achieved with bromine in glacial acetic acid. The desired product **50** was obtained in 59% yield. This compound was demethylated with boron tribromide in dry dichloromethane (DCM) at 0°C to give the bromophenol **51** and then re-protected as its methyl ethyl ether. This sequence was justified given the difficulty in demethylating the final A-CD adducts under normal conditions. The desired bromophenol **51** was obtained in 44% yield. The ^{1}H NMR spectrum of **51** showed the disappearance of the 3H singlet at 3.83 ppm belonging to the methoxy group. Methyl ethyl ether was preferred over TBS ether as protecting group for the bromophenol **51** since it could be more easily removed under acid conditions and thus minimized the number of overall steps in the final coupling sequence. Reaction of **51** with methyl chloroethyl ether in the presence of DIPEA in DCM gave the protected phenol **52** in 82% isolated yield. The ^{1}H NMR spectrum of **52** showed the appearance of one singlet at 5.16 ppm (2H), a quartet at 3.64 ppm (2H) and a triplet at 1.14 ppm (3H) belonging to the methyl ethyl group.

The protected A-ring **52** was reacted with an equimolar amount of *n*-butyllithium (*n*-BuLi) at -78^{o}C in THF under nitrogen atmosphere to form the arylanion intermediate **53** (**Scheme 1.2.8**). After about 15 minutes, MOM-protected CD-ring **41** was added and the cold solution was allowed to react for an additional 30 minutes after which the reaction quenched with a saturated NH_4Cl solution. An excess of the aryllithium (1.5 equivalents) relative to the protected CD-ring was necessary to maximize coupling efficiency. Column chromatography allowed us to remove unreacted starting materials and cleanly separate the epimeric mixture of tertiary alcohols at C9 **54** and **55** in 52% total overall yield as yellow gummy solids in an approximately 35:65 ratio, respectively.



Scheme 1.2.8 Coupling reaction **52** with **41** affording the stereoisomers **54** and **55**.

Previous studies in our laboratory have established the assignment of the stereochemistry at the C9 position based on the chemical shifts of the proton and carbon at C17 and the quaternary methyl group at C14 for the two A-CD epimers. These findings are applicable for stereoisomers where the sp^3 hybridized C9 position has either a hydroxy group or a hydrogen atom (**Figure 1.2.11**). The isomers with the "natural" stereochemistry show a doublet of doublets at 3.7-3.9 ppm; the same H in the "non-natural" isomer appeared as a triplet between 4.2-4.3 ppm in the ^{1}H NMR spectrum. The ^{13}C NMR signal for C17 in both the hydrogenated and the 9-hydroxy isomers is also diagnostic appearing at 80–82 ppm for the "natural" and at 72-74 ppm for the "**non-natural"** compounds.

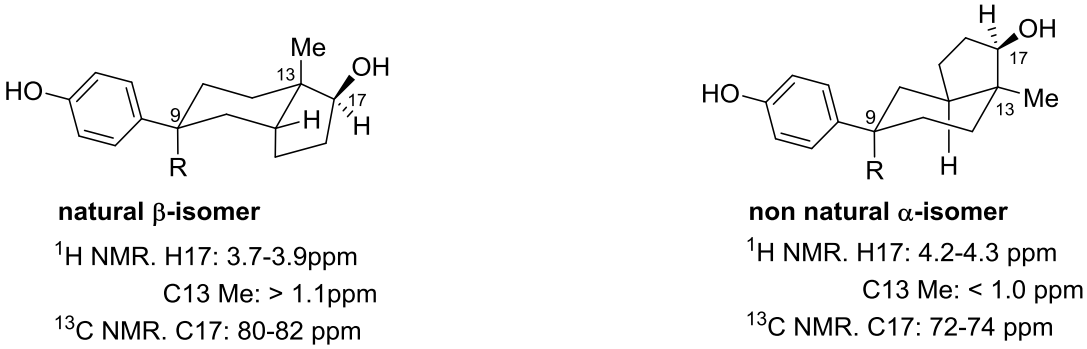


Figure 1.2.11 Assignment of the natural β- and non-natural α-stereoisomers at C9 (R= H or OH).

The ^{1}H NMR spectrum of **54** showed the C17-H proton as doublet of doublets at 3.70 ppm, and therefore this isomer was assigned the 9(*S*) "natural" A-CD stereochemistry (**Figure 1.2.12**). The more polar compound **55** has its C17-H proton as an apparent triplet at 4.20 ppm, which indicates the 9(*R*) "un-natural" stereochemistry. In addition, the quaternary methyl groups appeared as singlets at 1.09 ppm and 0.97ppm for the "natural" **54** and "un-natural" **55** isomers, respectively, following the trends observed in other A-CD epimers. It should be noted that this is important only for the purpose of assigning structures to **54** and **55**. It is not important for the preparation of other C5-CF_3 derivatives since the C9 stereocenter is eliminated in the next step.

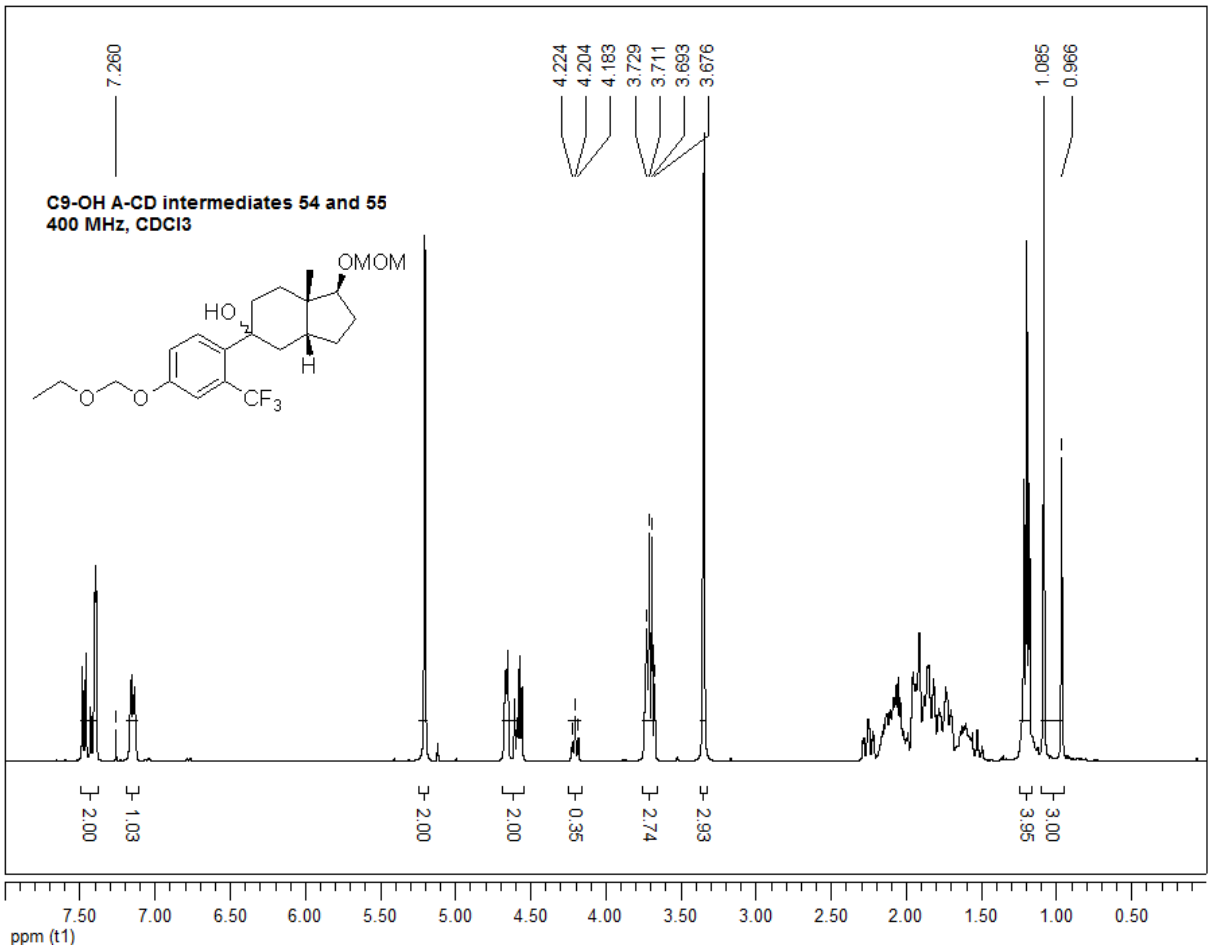
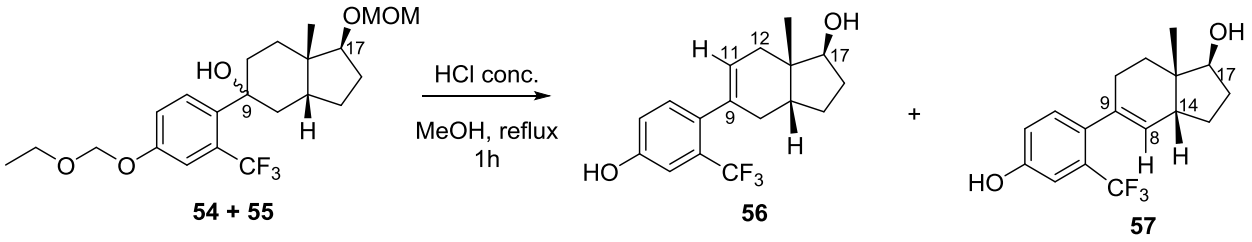


Figure 1.2.12 ^{1}H NMR spectrum of the C9-OH intermediates **54** and **55** in $CDCl_3$.

Acid catalyzed dehydration and deprotection (HCl in refluxing MeOH, 1 h) of the mixture of **54** and **55** gave a mixture of alkenes having a double bond between either C8−C9 or C9−C11 (**Scheme 1.2.9**). The alkene products **56** and **57** were isolated as a mixture in 83% combined yield by flash column chromatography.

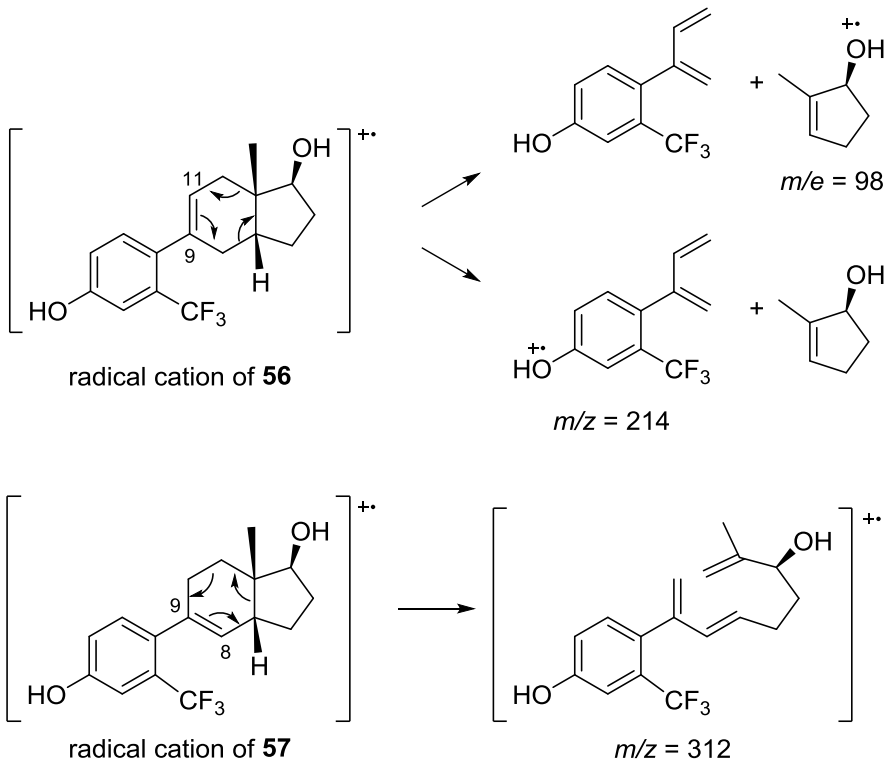


Scheme 1.2.9 One-pot acid-catalyzed dehydration/deprotection of **54**/ **55** yields the mixture of olefin isomers **56** and **57**.

The ^{1}H NMR of the mixture of two isomers showed peaks between 3.76 and 3.88 ppm for H17 and peaks between 5.45 and 5.50 ppm for the alkene protons. The ratio of the two isomers was approximately 35: 65 based on the integration of the two H17 peaks. These compounds were separated using preparative HPLC with a reverse phase C18 preparative HPLC column (10µm particle size, 21.2 x 250 mm).

The position of the double bond in the separated alkenes was assigned using 2D COSY and HMQC experiments and corroborated by their mass spectral fragmentation patterns. The ^{1}H NMR of the first HPLC peak assigned as the C9-C11 olefin **56** showed its H17 proton as a doublet of doublets at 3.76 ppm compared to an apparent triplet at 3.88 ppm for the same H in C8-C9 olefin **57**. The alkene protons were observed at 5.46 and 5.50 ppm for **56** and **57**, respectively. For the C9-C11 alkene **56**, the H11 coupled with signals integrating for two hydrogens at C12, while for the C8-C11 isomer **57**, the coupling of H8 was observed with the single proton at C14. The C17 signal appeared at 81.2 ppm for **56** compared to 79.4 ppm for **57**.

These structures were further confirmed by their MS fragmentation patterns. Importantly, the C9-C11 olefin **56** showed significant peaks at *m/z* = 98 and *m/z* = 214. These fragments corresponded to the reverse Diels-Alder reaction of the molecular ion (**Scheme 1.2.10**). Such fragmentation was not seen for **57** since the reverse Diels-Alder reaction would result in a linear triene of the same molecular weight as the molecular ion (*m/z* = 312). The ^{1}H and ^{13}C NMR spectra of compounds **56** and **57** are shown in **Figures 1.2.13** to **1.2.16**.



Scheme 1.2.10 Reverse Diels-Alder reactions in Mass Spectrometry of the C5-trifluoromethyl alkene derivatives.

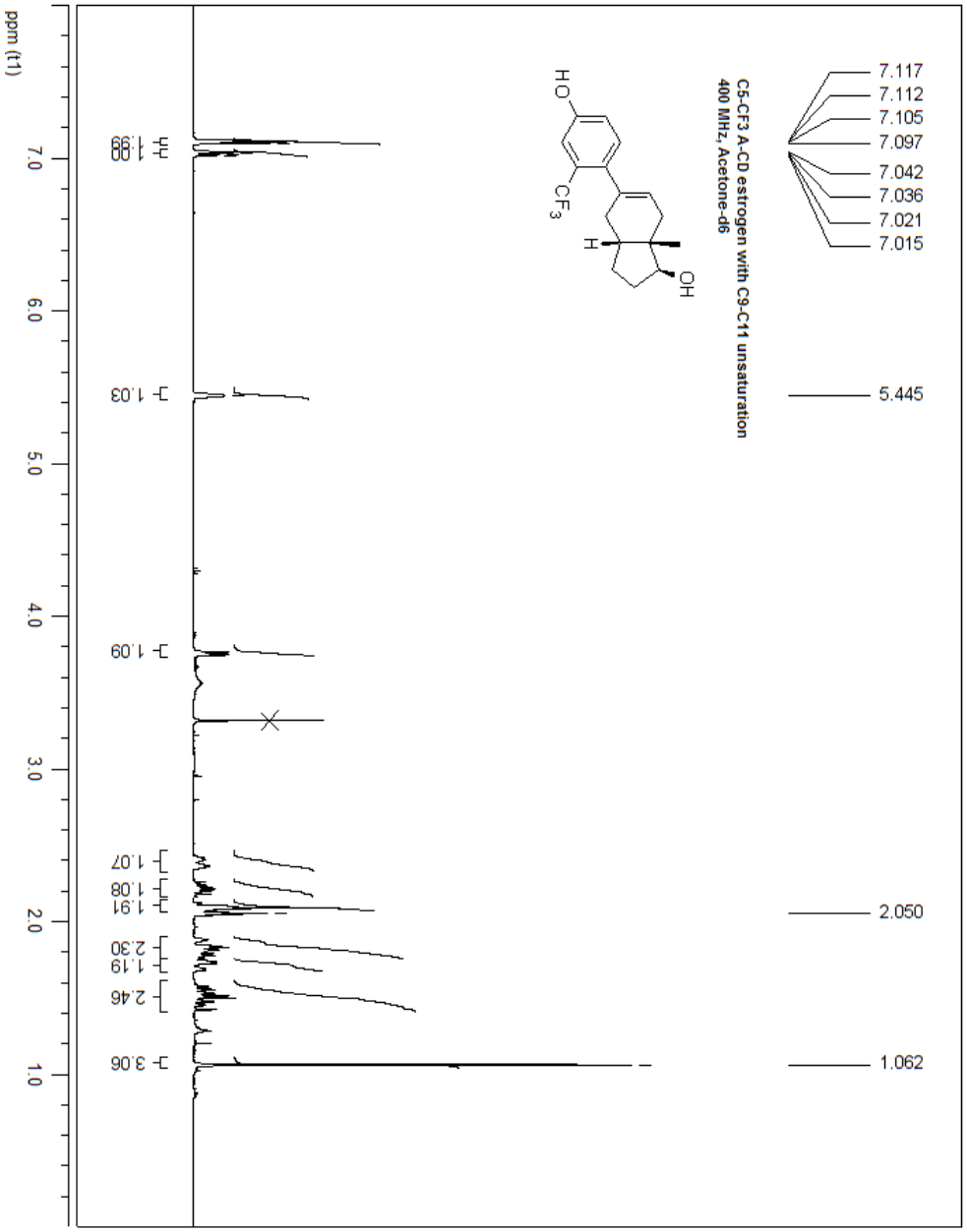


Figure 1.2.13 ^{1}H NMR spectrum of compound **56**.

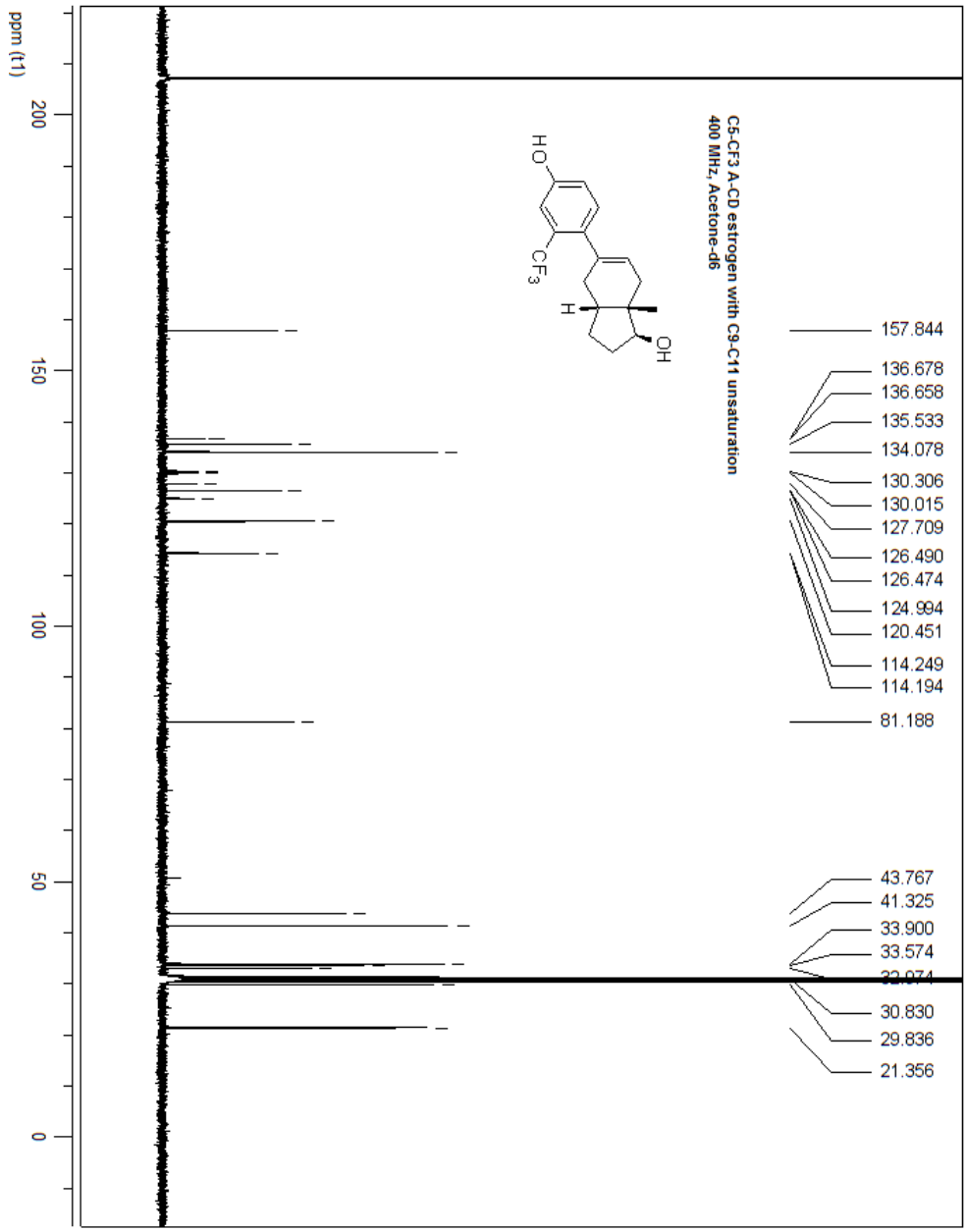


Figure 1.2.14 ^{13}C NMR spectrum of compound **56**.

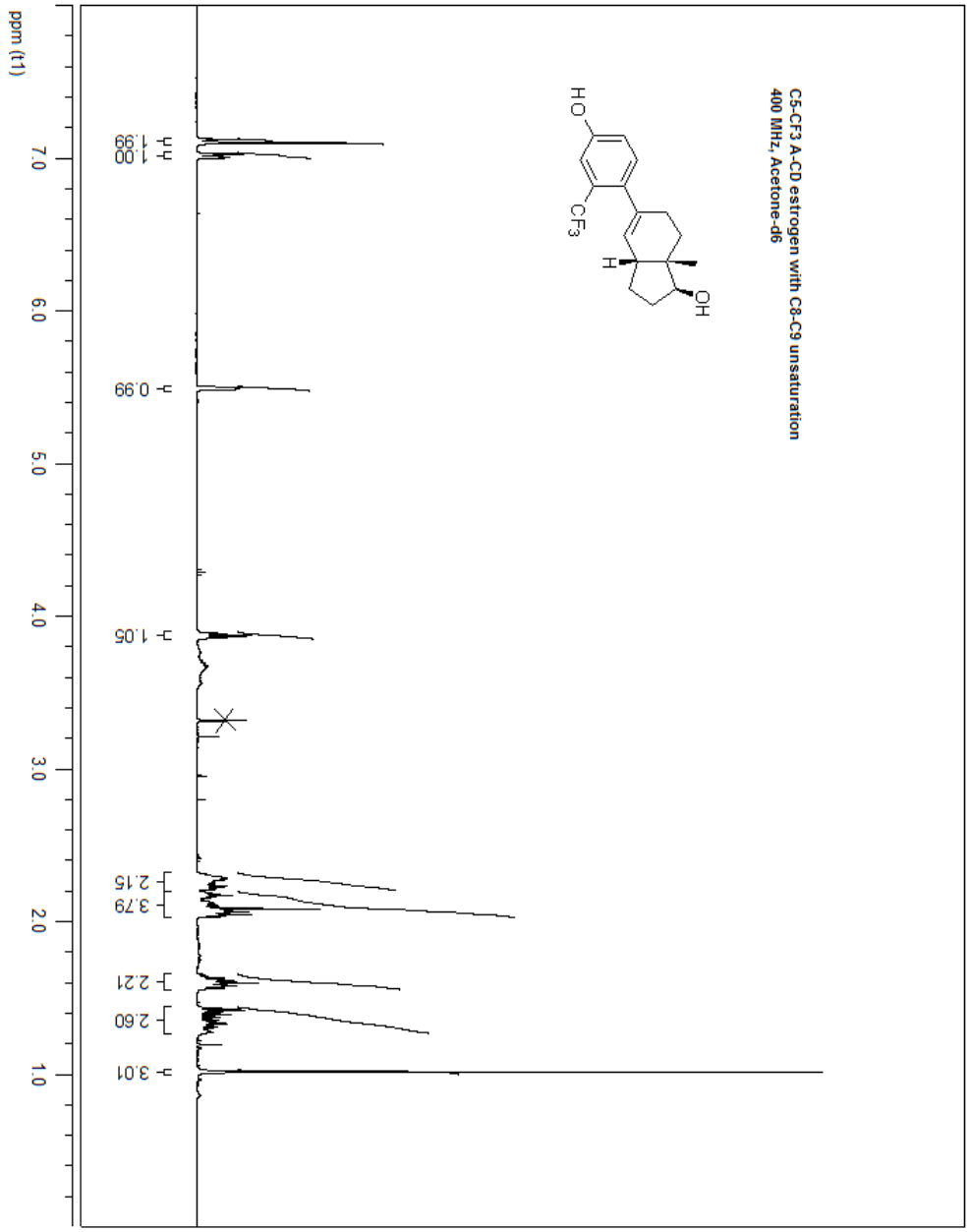


Figure 1.2.15 ^{1}H NMR spectrum of compound **57**.

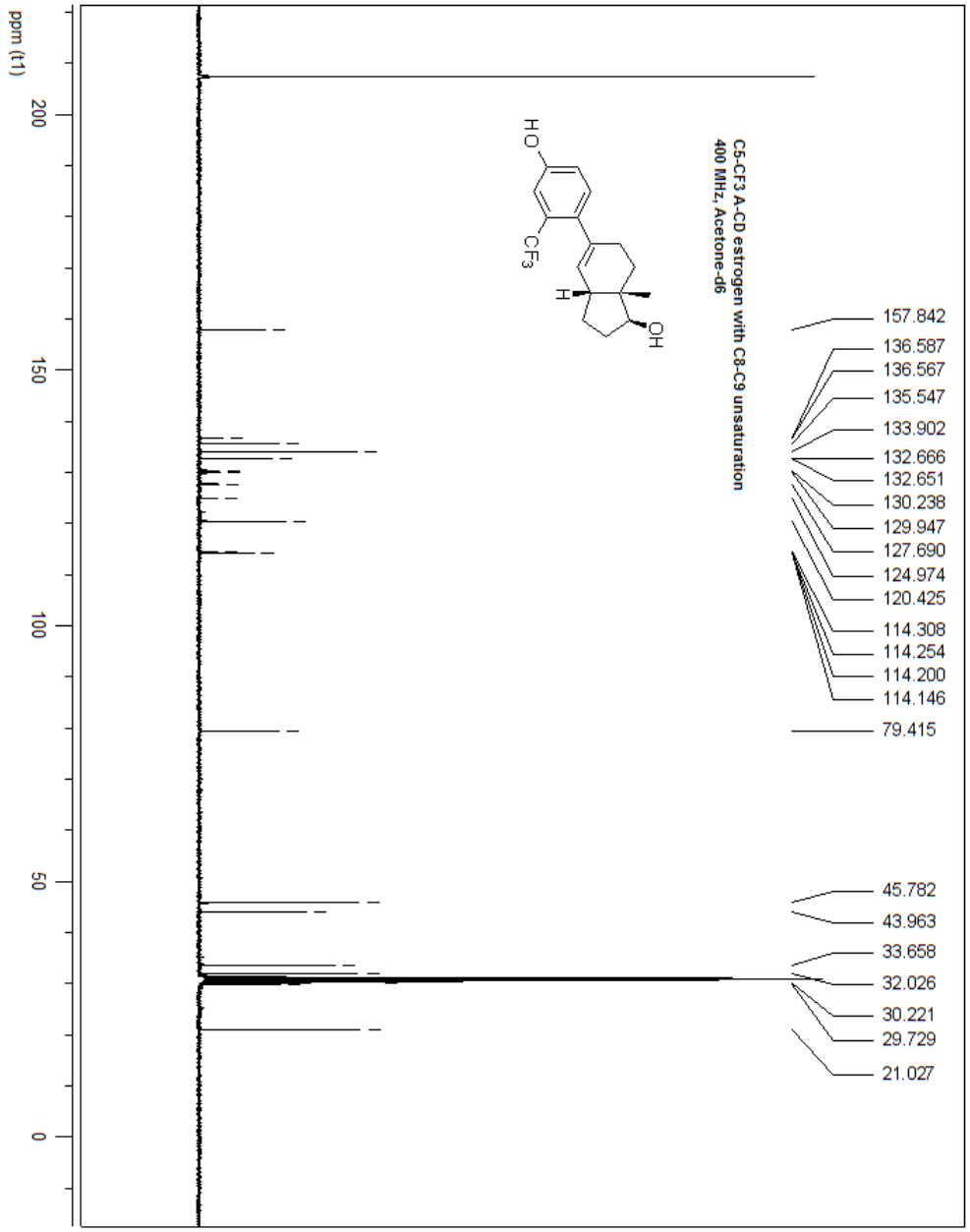
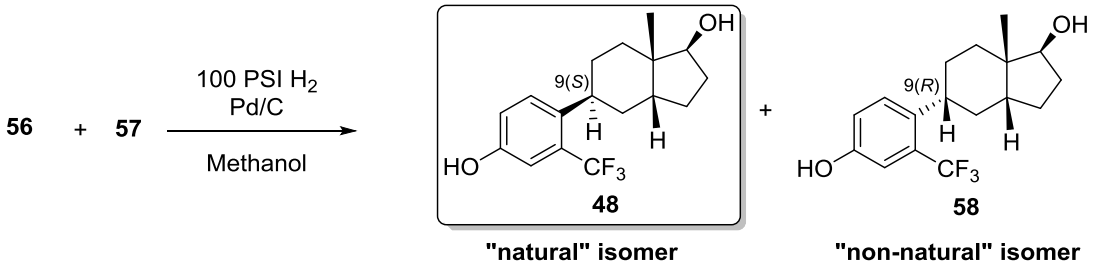


Figure 1.2.16 ^{13}C NMR spectrum of compound **57**.

Hydrogenation of the mixture of olefins **56** and **57** under high pressure of hydrogen with palladium on carbon afforded an epimeric mixture of the natural, 9(*S*) and non-natural 9(*R*) isomers in almost equal quantities (**Scheme 1.2.11**). The usual method of hydrogenation in our group, which consists of pressurizing two large balloons filled with hydrogen in a closed system containing the substrate in the presence of a catalytic amount of palladium on carbon, was unsuccessful in this case. The reduction of the mixture of alkenes **56** and **57** required a higher hydrogen pressure of 100 PSI. Removal of the catalyst and evaporation of the solvent gave a mixture of **48** and **58** isomers in 91% yield.



Scheme 1.2.11 Hydrogenation of the alkene mixture **56** and **57** yields 9(*S*) **48** and 9(*R*) **58**.

The ^{1}H NMR spectra of the crude mixture indicated that there was negligible stereoselectivity as shown by the appearance of two almost equally-sized peaks for both the C13 quaternary methyl groups and the H17 hydrogens. Silica gel column chromatography separated compounds **48** and **58**. The ^{1}H NMR spectra showed the same differences reported previously in our group with the less polar faster-eluting 9(*S*) isomer showing the H17 proton as a doublet at 3.65 compared to an apparent triplet at 4.46 for the 9(*R*) isomer. Another expected difference was the chemical shift of the tertiary methyl group which appeared at 1.17 ppm in the natural isomer **48** vs 0.91 ppm in the non-natural isomer **58**. Finally, the C17 carbon in **48** occurred as expected near 83 ppm compared to 74 ppm for **58**. The ^{1}H and ^{13}C NMR spectra of **48** and **58** are shown in **Figures 1.2.17** to **1.2.20**.

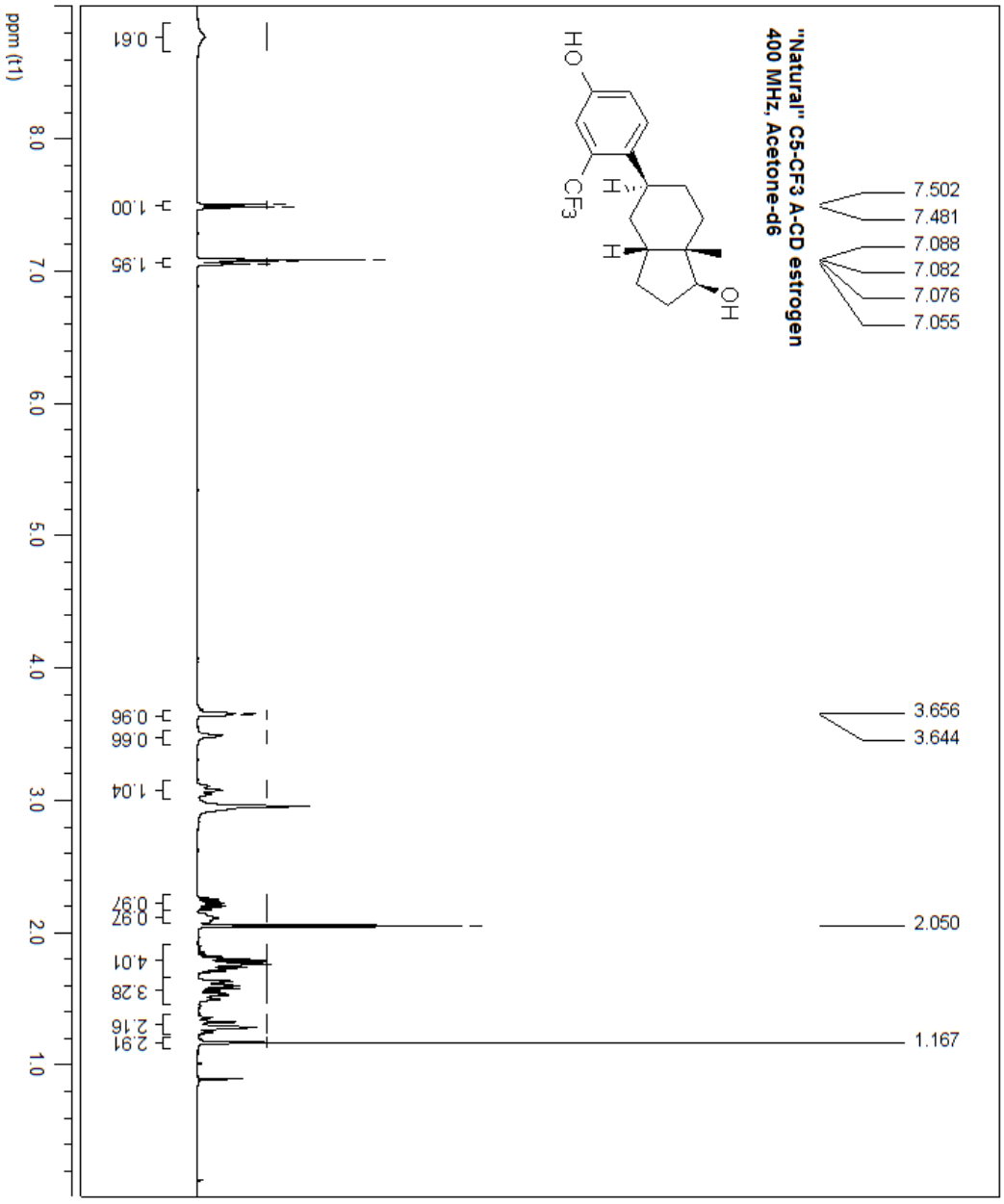


Figure 1.2.17 ^{1}H NMR spectrum of compound **48**.

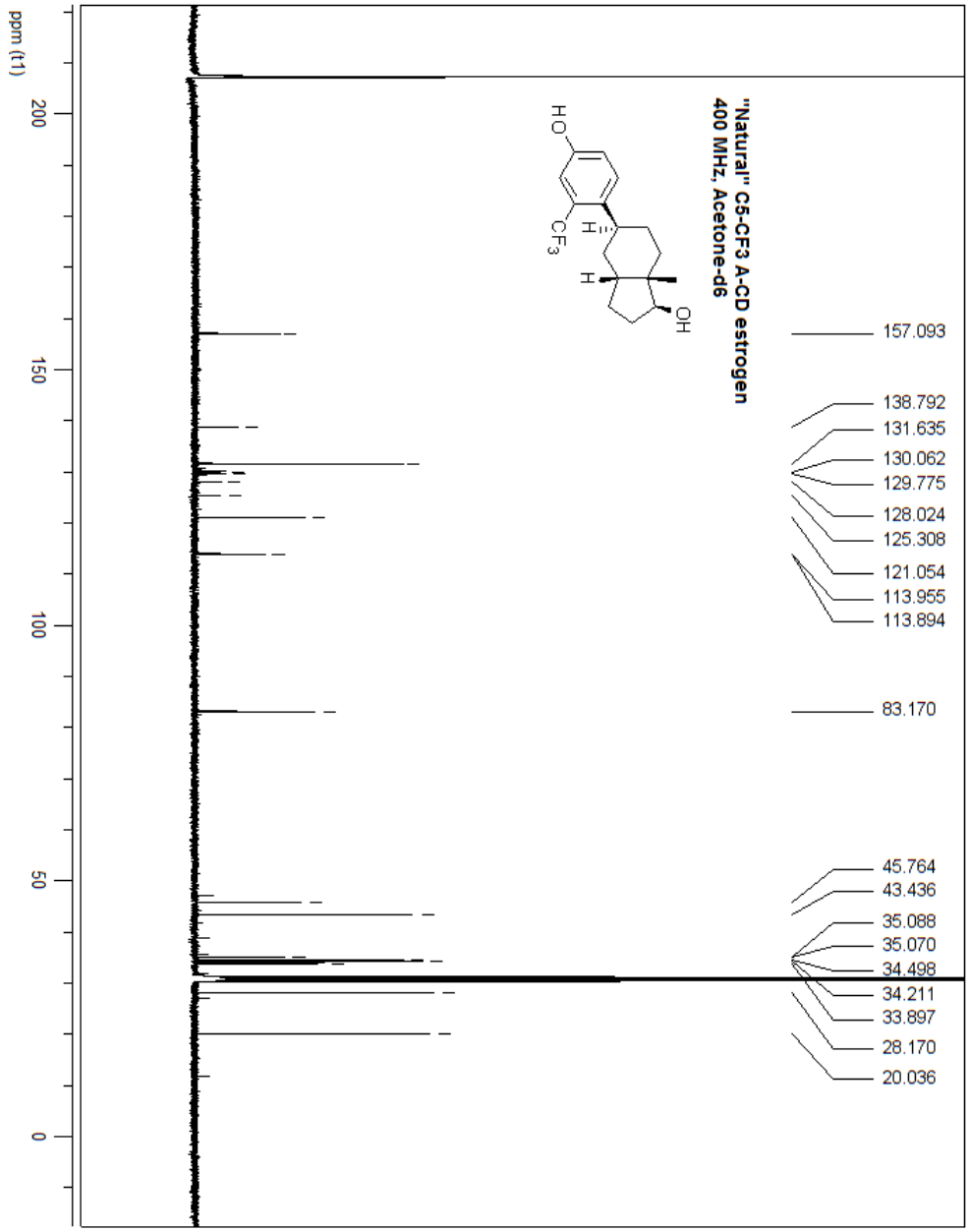


Figure 1.2.18 ^{13}C NMR spectrum of compound **48**.

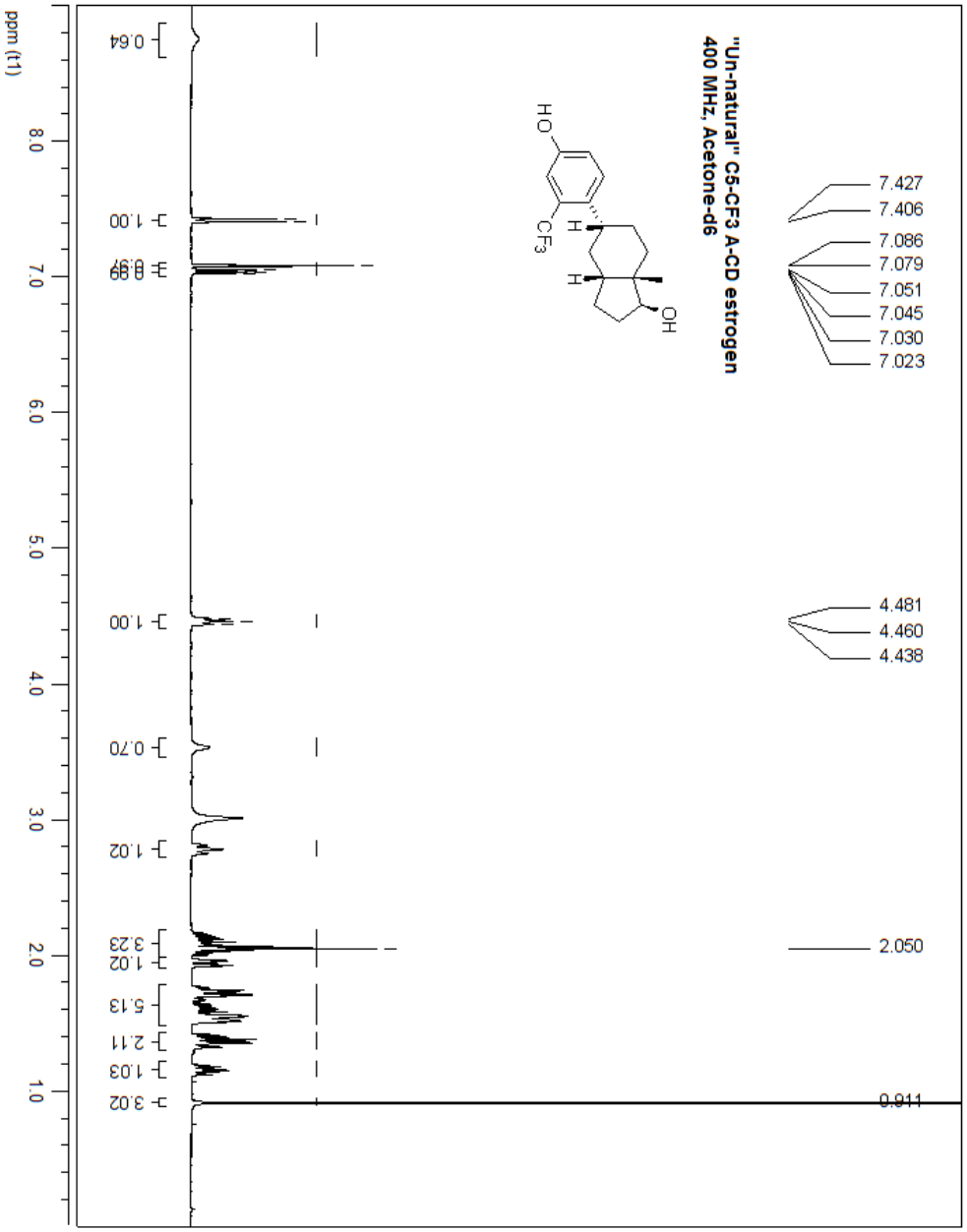


Figure 1.2.19 ^{1}H NMR spectrum of compound **58**.

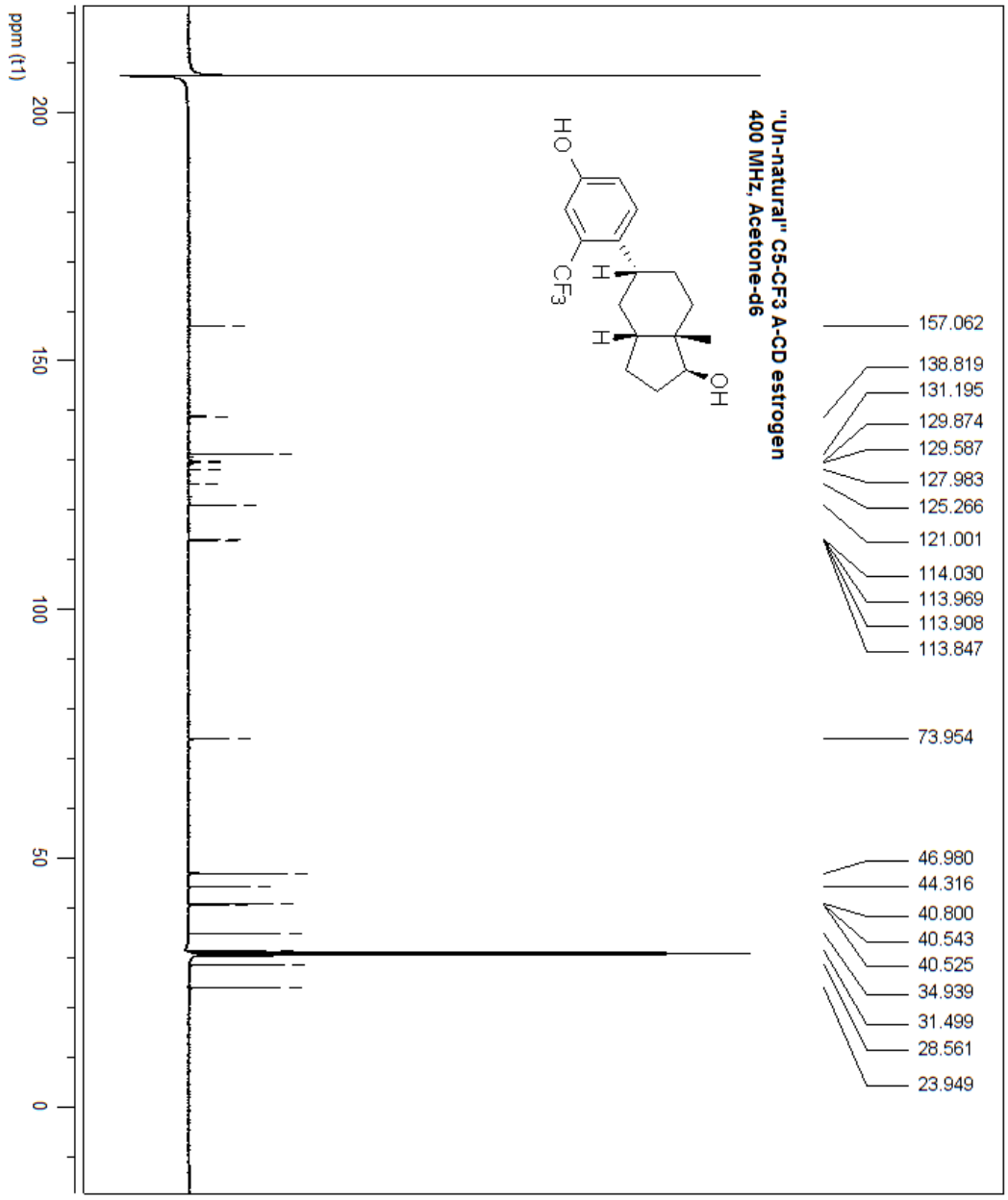


Figure 1.2.20 ^{13}C NMR spectrum of compound **58**.

The structural assignments of compounds **48** and **58** were confirmed by X-ray structure determinations performed by Dr. Ilia Korobkov at the University of Ottawa. The X-Ray structures in **Figure 1.2.21** verified not only the 9(*S*) and 9(*R*) configuration for **48** and **58,** respectively but also showed that in the free ligands the planes of ring A and ring C are essentially perpendicular to each other. For example, the C5C10 to C9C8 or C9C11 dihedral angle in both compounds **48** and **58** is close to 60°. Additionally, the X-ray of **58** showed the predicted inversion of Ring C resulting in a conformation in which the larger Ring A substituent is in an equatorial position.

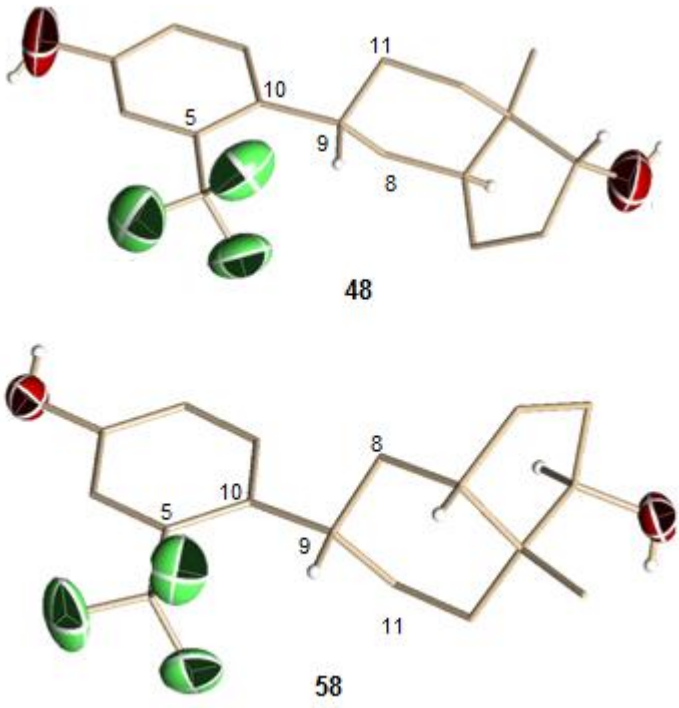


Figure 1.2.21 Structure of the natural **48** and non natural **58** C5-CF_3 isomers as determined by single crystal X-ray structure determination. This X-ray structure was performed by Dr. Ilia Korobkov at the University of Ottawa.

Incidentally, these crystal structures first alerted us that our initial *trans* CD-ring junction structure assignment as reported in our first publication was in error [107]. This error was

subsequently corrected via an Erratum to the first publication [134]. By good fortune, the unplanned synthesis of the A-CD estrogens with the *cis* CD-ring junction turned out to be highly fortuitous since these compounds show stronger binding affinities and higher β/α receptor and greater β/α estrogen agonist selectivity than the corresponding *trans* CD junctioned isomers. Compound **48** was found to bind more strongly than estradiol by almost a factor of 2 to the β-receptor and of similar potency based on the RTA data. Unfortunately, the β/α selectivity in both assays was low. Eventually, based on further evaluations by the laboratory of Dr. Christine Pratt we have designated this compound as a "super estrogen". The binding affinities for all of the trifluoromethyl derivatives are shown in **Table 1.2.4**.

Subsequently, Dr. Cristian Dabrota synthesized the *trans* fused CD-ring isomer **59** in an unambiguous manner. This compound has, as expected, substantially different ^{1}H and ^{13}C NMR spectra compared to either **48** or **58** (see **Table 1.2.4**).

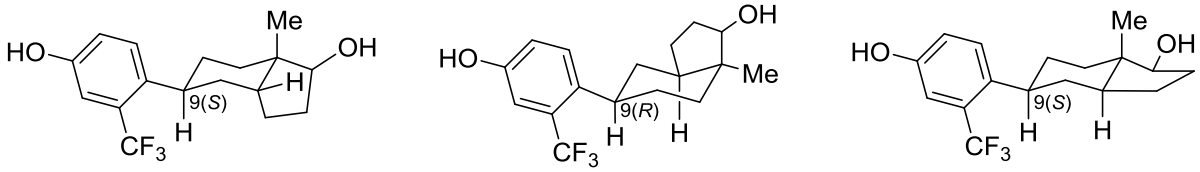


Figure 1.2.22 Chemical structures of C5-CF_3 A-CD analogs with the *cis* (**48** and **58**) and *trans* (**59**) CD-ring junctions.

Synthesis of the C5-CF_3 C9-OH A-CD estrogens

As mentioned above, we also prepared the C9-hydroxy derivatives of compounds **48** and **58** in order to test them for relative binding affinities. Computational studies indicated that, unlike the flat aromatic pocket around the aromatic ring, the region around the C-ring is wider and could allow more substituents. Earlier, other members of our group described the synthesis of several

derivatives having either the "natural" and "non-natural" stereochemistry at C9 (for example compounds **60-64**) [112].

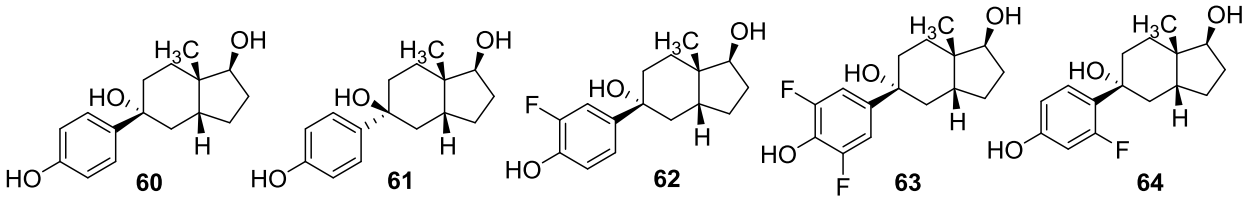
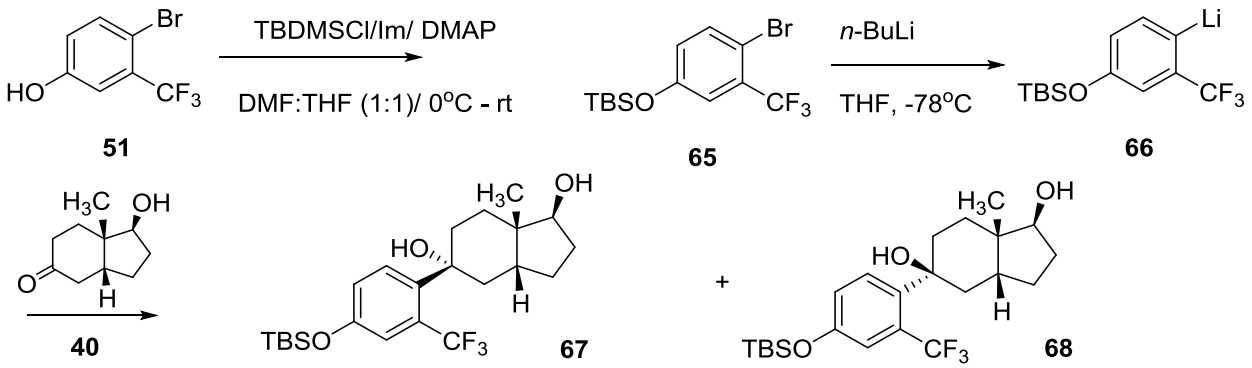


Figure 1.2.23 Previously prepared A-CD steroids with C9-hydroxy group [112].

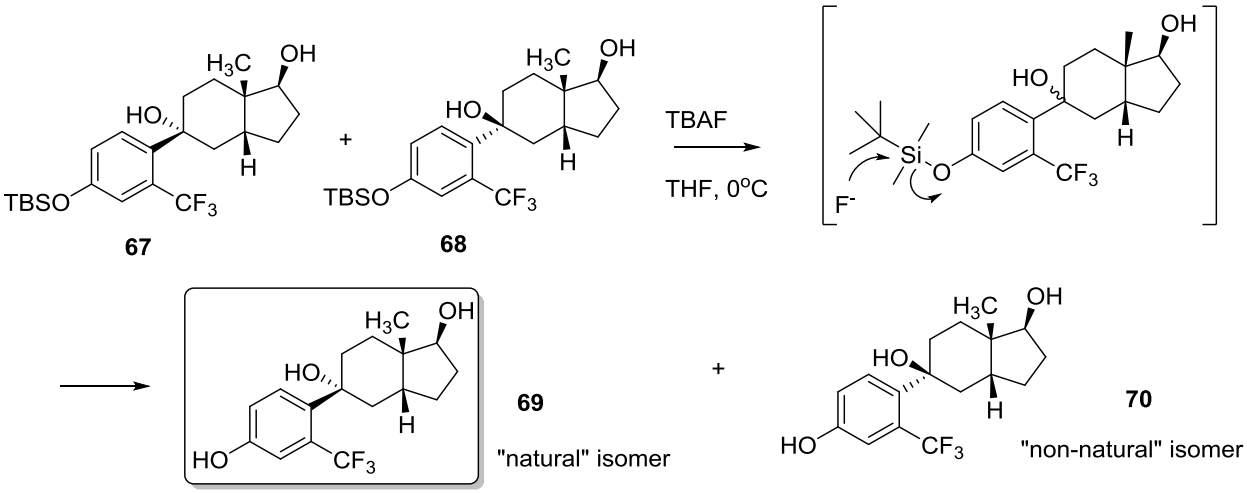
Most of these compounds (**60** to **64**) showed low binding affinity related to estradiol (≤0.01% for ERα and ≤0.12 for ERβ) possibly because of their low log P values. However compound **64** with a fluorine substituent at C5 binds significantly (0.17% for ERα and 3.6% for ERβ) and shows strong β-selectively (21-times fold). As expected the 9-OH compounds with the inverted conformation at C9 showed much lower binding affinities than those of the natural conformation.

For the preparation of the 5-CF_3 C9-hydroxy compound **68** it was necessary to use an OH protecting group that could be removed under non-acidic conditions such that the C9-OH group would be retained. The *tert*-butyl silyl (TBS) group served this purpose. The synthesis of the C3-O-TBS-protected C9-hydroxy intermediate is shown in **Scheme 1.2.12**. The protection of bromophenol **51** to **65** was successfully achieved in 82 % isolated yield with TBDMSCl in the presence of a slight excess of imidazole (1.3 equivalent) and traces of DMAP in a 1:1 mixture of DMF:THF. The ^{1}H NMR spectrum of compound **65** showed the appearance of two singlets at 0.98 (9H) and 0.21 (6H) ppm belonging to the TBS group.



Scheme 1.2.12 Coupling of the unprotected CD-ring **64** and TBS-protected A-ring to form the C9-hydroxy A-CD intermediates **67** and **68**.

Coupling of the phenol **65** gave a mixture of the C9 epimeric compounds **67** and **68** in 42% isolated yield using our normal coupling procedures with the exception that 2.5 equivalents of the aryllithium **66** were used per equivalent of the unprotected CD-ring. One equivalent was required to deprotonate the 17β-hydroxy group and a slight excess was used to ensure maximal coupling yield. The ^{1}H NMR spectrum of the epimeric mixture showed a ratio of approximately 1:2 based on the integrations of the C17-protons and the tertiary methyl groups for the natural and non-natural isomers. Treatment of the mixture of **67** and **68** with TBAF removed the TBS group and provided the C9-hydroxy diastereomers **69** and **70** in 75% isolated yield. These were separated by preparative HPLC. Once again the ^{1}H NMR and ^{13}C NMR spectra differences allowed us to assign the structures confidently. The ^{13}C NMR each showed two peaks associated with carbons carrying oxygen. The sample that had peaks at 76.3 and 82.7 ppm was assigned to natural isomer **69**, while that which showed absorptions at 74.1 and 75.3 ppm was designated as structure **70**.



Scheme 1.2.13 Deprotection of the C3-O-TBS-hydroxy group using TBAF affording the final compounds **69** and **70**.

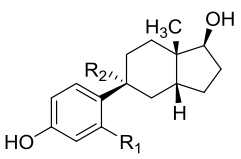
Bioassays for the 5-CF_3 compounds

The above 5-CF_3 compounds were sent for RBA assays. These were performed by Dr. Kathryn Carlson in the J. A. Katzenellenbogen group at the University of Illinois. RTA assays were determined by Prof. C. Pratt at the University of Ottawa. **Table 1.2.4** summarizes the binding affinity and transcription activities of the C5- trifluoromethyl compounds. For comparison, the results for the parent A-CD estrogens **19** are also included.

All compounds with a CF_3 substituent at C5 display enhanced binding affinity to the estrogen receptors relative to the parent A-CD analogs likely due to additional hydrophobic interactions within the receptor. Three of the derivatives, the 9(*S*) natural isomer and the two alkenes **56** and **57** bind to both the estrogen receptors as or more strongly than estradiol. However the high binding levels were accompanied by disappointingly low β/α binding and β/α transcription activity selectivity. The binding levels of the C9 hydroxy compound **69** also were substantial at almost 8% of the estradiol level but again the β/α ratio was essentially 1:1. Interestingly, the C5–CF_3 substituent enhanced not only the binding of the isomers with the natural

stereochemistry at C9 but also those with inverted stereochemistry especially towards the α-receptor. Thus for example the non-natural isomer **58** has RBA to ERα of 3.0 ± 0.9% in comparison to 0.06% for the parent compound **26** with that stereochemistry. Also, almost all compounds follow the trend with the "natural" sp^3 hybridized stereoisomer at C9 showing significantly a higher binding affinity compared to the non-natural isomers.

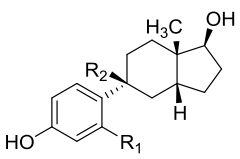
Table 1.2.4 Relative Binding Affinity (RBA) and Relative Transcription Activation (RTA) to ERα and ERβ of CF_3 derivatives with a *cis* CD-junction and their ratio of β/α selectivity.



"natural" analogues

19 R_1=H, R_2=H
48 R_1=CF_3, R_2=H

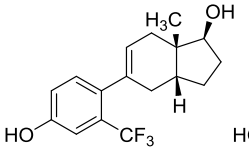
60 R_1=H, R_2=OH
69 R_1=CF_3, R_2=OH



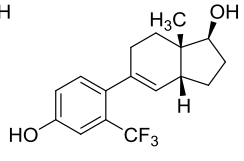
"non-natural" analogues

26 R_1=H, R_2=H
58 R_1=CF_3, R_2=H

61 R_1=H, R_2=OH
70 R_1=CF_3, R_2=OH



56



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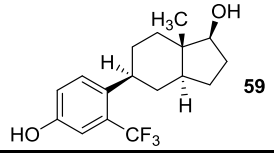


59

Comp.	RBA[a]			RTA[b]		
	ERα	ERβ	β/α	ERα	ERβ	β/α
2	100	100	1	100	100	1
19	1.5	21	14.0	4.4 ± 0.5	164 ± 5	37.7
26	0.06	0.6	10	-8.9	7.7	pure β
48	90 ± 10	213 ± 20	2.3	106	93	0.9
56	122 ± 11	174 ± 8	1.4	163	126	1.3
57	189 ± 1	201 ±18	1.1	138	213	1.5
58	3.1 ± 0.9	2.6 ± 0.6	0.8	-	-	-
59[c]	3.0 ± 0.4	1.9	0.6	-	-	-
60	0.011 ± 0.002	0.11 ± 0.04	9.5	-	-	-
61	0.013 ± 0.002	0.14 ± 0.03	11.1	-	-	-
69	7.7 ± 0.8	8 ± 1	1.0	11.9	105	8.8
70	9 ± 1	13.7 ± 0.4	1.5	-	-	-

a- Relative Binding Affinity assay prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.
b- Relative Transcription Activity prepared by the group of Dr. C. Pratt at the University of Ottawa.
c- This compound was prepared by Dr. Cristian Dobrata.

Specifically, compound **48** showed a binding affinity of 90 ± 10% to ERα and of 213 ± 20% (over two times stronger than estradiol) to ERβ, which is over 10 times stronger than that of the parent compound **19.** As anticipated the CF_3 group at C5 increased binding to the estrogen receptors more than a F group at the same position. Unfortunately the β to α selectivity was significantly reduced for **48** as compared to **19** (from 14 to 2.3 times fold) and thus these compounds are unlikely to have much potential in HRT. The alkenes **56** and **57** also displayed enhanced binding affinities to both estrogen receptors, again with negligible selectively. The RTA values of **48**, **56** and **57**, ranging from 100 to 200% of estradiol for both estrogen receptors, showed that these compounds are all strong ERα and ERβ agonists.

The binding affinity to the estrogen receptors of the non-natural isomers **58** and **70** is substantial with ERα and ERβ at approximately 3-7.7% and 2.6-14% of estradiol respectively. The comparable figures are $\leq$0.2% for the parent compounds.

The preferred conformations of the free ligands such a **48, 56** and **57** are ones in which the plane of the aromatic A-ring is roughly perpendicular to that of ring C. This conformation is at least 10 kcal/mol more stable than that in which these two rings are coplanar [135]. This suggests strongly that ligand compound **48** when bound in the estrogen receptor has a distinctly different shape than does estradiol. It can therefore be concluded, that the ER receptors can accommodate molecules with dihedral angles between rings A and C different than those in estradiol which is almost planar.

This conclusion was confirmed when we obtained by an X-ray of the bound non-natural C5-CF_3 ligand **58** inside the human ERα performed by Dr. Kendall W. Nettles at the Scripps Research Institute, Florida (**Figure 1.2.24**). Crystallization of many of the A-CD ligands with the estrogen

receptor was attempted. Ligand **58** and one other were the only ones that that co-crystallized with the protein. Both confirmed unequivocally that the conformation of these ligands inside the estrogen receptor is one in which the planes of the A and C rings are nearer to 70 to 90^{o} compared to the coplanar range in estradiol. It also demonstrates clearly that the CF_3 group of the A-ring in the bound ligand has the same arrangement that is opposite to the C17-hydroxy and the tertiary methyl groups of the *cis* CD-ring as is seen in the X-Ray structure of the free ligand.

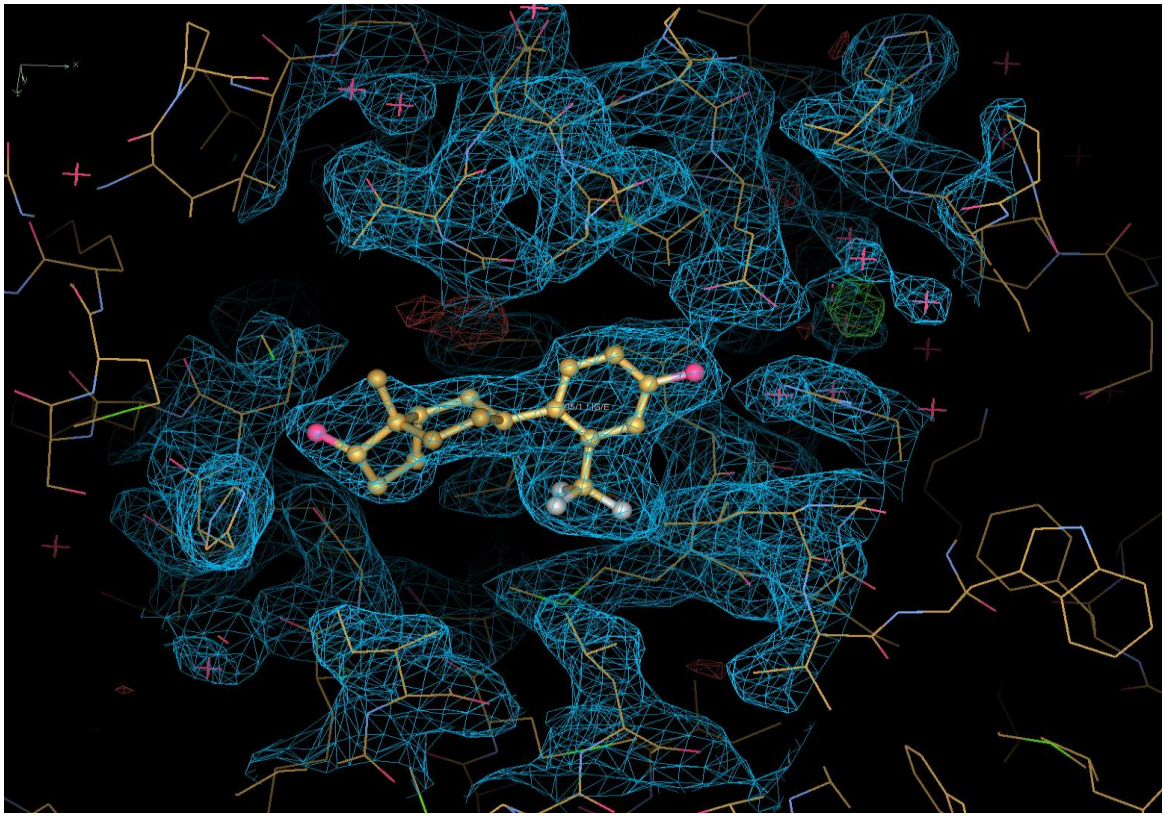


Figure 1.2.24 X-ray of the non-natural C5-CF_3 ligand **58** inside the human ERα performed by Dr. Kendall W. Nettles at the Scripps Research Institute, Florida.

1.2.4 Synthesis and bioassays of various C5 and/or C1 substituted *cis* A-CD estrogens

The bioassay results from the C5-F (**30**) and C5-CF_3 (**48**) were encouraging because they both had greater binding affinity to the estrogen receptors than the parent compound **19**. This section focuses on the synthesis and biological characterization of the several A-CD estrogens bearing EWG or EDG substitution at the C5 and/or C1 positions.

Synthesis of the C5-chloro A-CD derivatives

The C5-chloro A-CD ligand **71** has a calculated lipophilicity (Log P = 4.42) which is between that of the C5-F derivative **30** (Log P = 3.98) and the C5-CF_3 compound **48** (Log P = 4.75). The size of this substituent is also intermediate between F and CF_3. If Log P is a major determinant in the binding of ring A substituted A-CD compounds to the estrogen receptors, then we predict that **71** should have binding affinities intermediate between those observed for the C5-F and the C5-CF_3 derivatives.

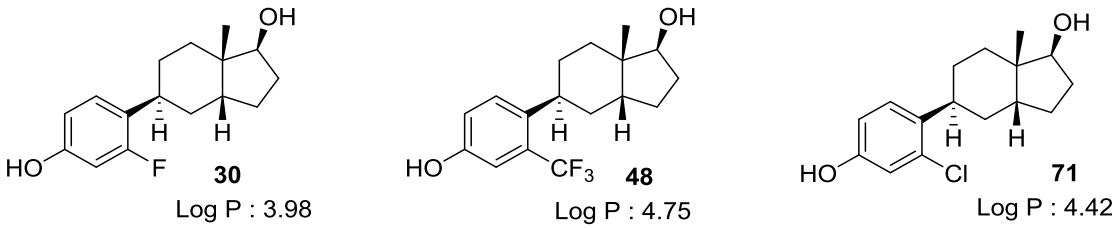
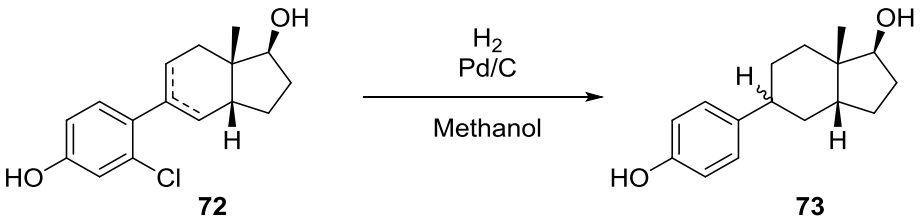


Figure 1.2.25 Chemical structures of C5-A-CD estrogens (F, CF_3, Cl) and their Log Ps.

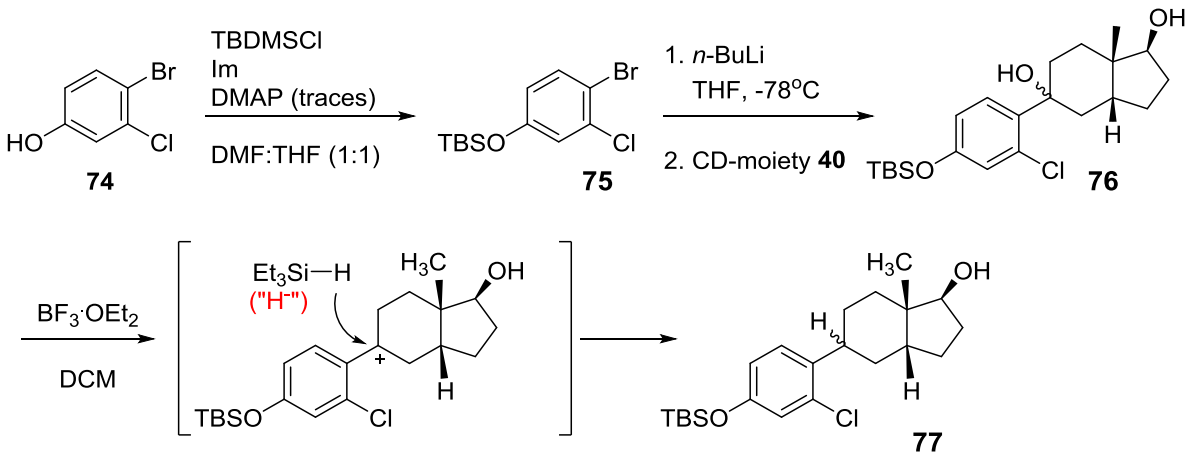
The synthesis of **71** was attempted following the method used for the preparation of **48**. However, the hydrogenation of the alkene mixture **72** resulted in the loss of the chlorine atom from the aromatic ring giving compound **73** (**Scheme 1.2.14**). To prevent the loss of the

aromatic Cl a Lewis acid–catalyzed hydrogenolysis of the C9-tertiary alcohol was chosen to avoid this problem.



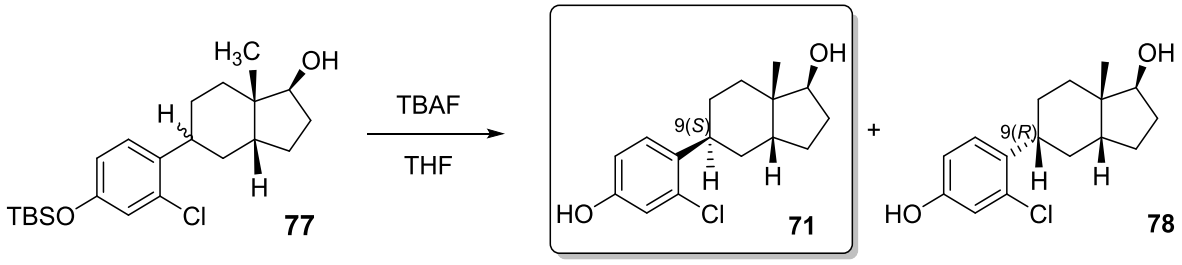
Scheme 1.2.14 Reduction of the C5-chloro alkenes **72** results in the loss of the chlorine atom.

The C9-tertiary alcohol adduct **76** was prepared in the usual manner starting with the TBS-ether **75,** [obtained in 82 % isolated yield from commercially available 4-bromo-3-chlorophenol,**74**] and unprotected CD-ring **40** (**Scheme 1.2.15**). The desired product was obtained in 6.5% isolated yield, an excess of both starting materials were recovered by flash column chromatography. Treatment of **76** with Et_3SiH and $BF_3.Et_2O$ at 0^oC in DCM for 1h [136] followed by quenching of the reaction mixture with NH_4Cl successfully reduced the C9-hydroxy group to afford the mixture of hydrogenated epimers **77** in quantitative yield. The proton NMR of the crude reaction product indicated almost no stereoselectivity had occurred in the reduction step.



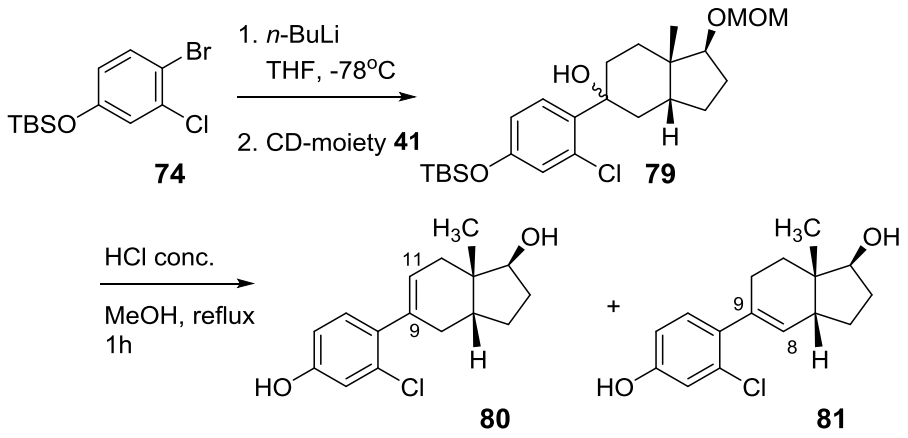
Scheme 1.2.15 Coupling of **74** with **40**, followed by hydrogenolysis of the C9-OH intermediate.

The TBS group was removed with tetra-*n*-butylammonium fluoride (TBAF) in THF at room temperature to give the final product as an approximately 1:1 mixture of diasteriomers in quantitative yield (**Scheme 1.2.16**). These isomers were separated by column chromatography and characterized by their ^{1}H and ^{13}C NMR spectra.



Scheme 1.2.16 Final step for the preparation of C5-chloro A-CD estrogens **71** and **78**.

The mixture of alkene isomers **80** and **81** was obtained in 47% yield from the C9-tertiary alcohol adducts **79** following the dehydration/deprotection sequence described for the trifluoromethyl analogs (**Scheme 1.2.17**). These compounds were purified by column chromatography and separated by preparative HPLC. The individual structural assignments were based on their ^{1}H and ^{13}C NMR spectra using the approaches previously described for the corresponding CF_3 compounds.



Scheme 1.2.17 Synthesis of **80** and **81** C5-Cl olefins.

The bioassay results for compound **71** will be described in **Table 1.2.5**. Our prediction that the lipophilicity of C5-substituted EWG A-CD estrogens is an important contributor to the binding affinity of these compounds to the estrogen receptors was tested. The RBA results for olefins **80** and **81** will be detailed in **Section 1.2.6**, along with other A-CD estrogens with unsaturation in the C-ring.

Synthesis of the C5-substituted EDGs A-CD estrogens

Initial calculations indicated that electron donating groups in the aromatic ring would increase the tendency to undergo metabolic ortho-hydroxylation eventually leading to the undesirable *o*-quinones [123]. While this could be the case for the C5-methyl, **82**, and C5-methoxy, **83**, the C5-hydroxy compound **84** was predicted to be a special case (see below). Based on the earlier results and their Log P values we anticipated strong binding for **82** but likely relatively weak binding for **83** due to the increase in the size of the C5 substituent and a lower Log P. The rather low Log P value for the C5 hydroxy compound **84** suggested potentially weak binding.

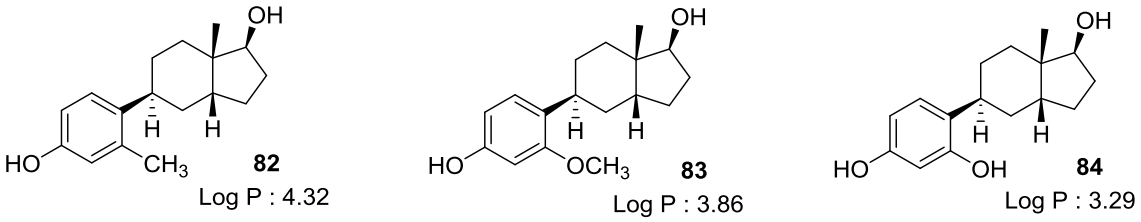
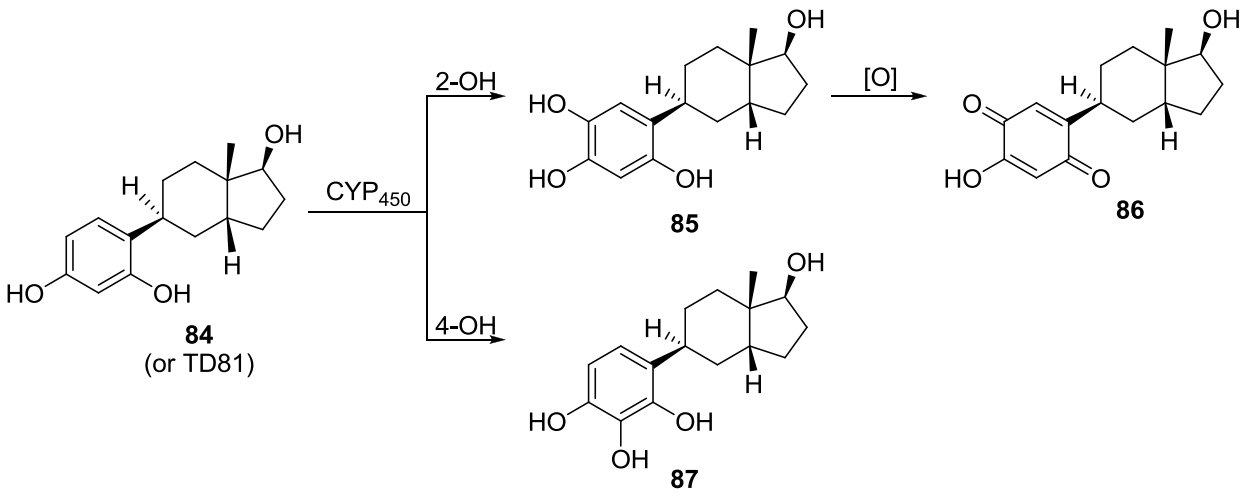


Figure 1.2.26 Structure of A-CD estrogens containing EDGs at C5. The Log P values were calculated with the KOAWIN Software v1.10.

In particular, we argued that compound **84** should have low toxicity. Enzymatic hydroxylation of **82** at the available two ortho positions can lead either to 2,4,5-trihydroxy **85** or 3,4,5-trihydroxy **87** metabolites. Further oxidation of **85,** the result of hydroxylation at position C2, will result in the preferential formation of the para-quinone **86**. *Para*-quinones, especially those carrying a strongly electron donating group such as OH, are considerably more stable and less reactive than

typical *ortho*-quinones and thus are not expected to induce oxidative stress.



Scheme 1.2.18 Potential metabolites of the C5-OH A-CD estrogen **84**.

Ortho hydroxylation of **84** at position C4 leads to the 3,4,5-trihydroxy derivative **87**. This structure is reminiscent of gallic acid **88** which is a trihydroxybenzoic acid secondary metabolite displaying strong anticancer and antioxidant activities relevant to the modulation of carcinogenesis [137]. The biological properties of compound **84** are detailed in **Section 1.2.9**. They show that this is the least toxic compound in our library. Fortunately, this compound was shown to bind reasonably strongly and with very good β/α selectivity to the estrogen receptors despite its low Log P. Also, it was shown to be a potent and highly β-selective agonist. The combination of these properties has made it an important compound for potential development in both HRT and breast cancer therapy. It will also be referred as **TD81** throughout this thesis since this is its designation within our group and with our collaborators.

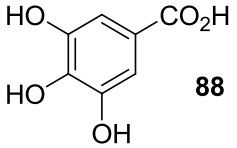
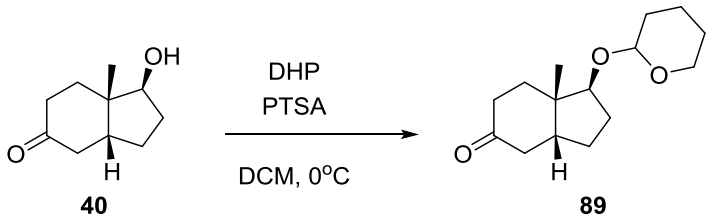


Figure 1.2.27 Gallic acid, **88**.

Preparation of the C5-methyl A-CD estrogen derivatives

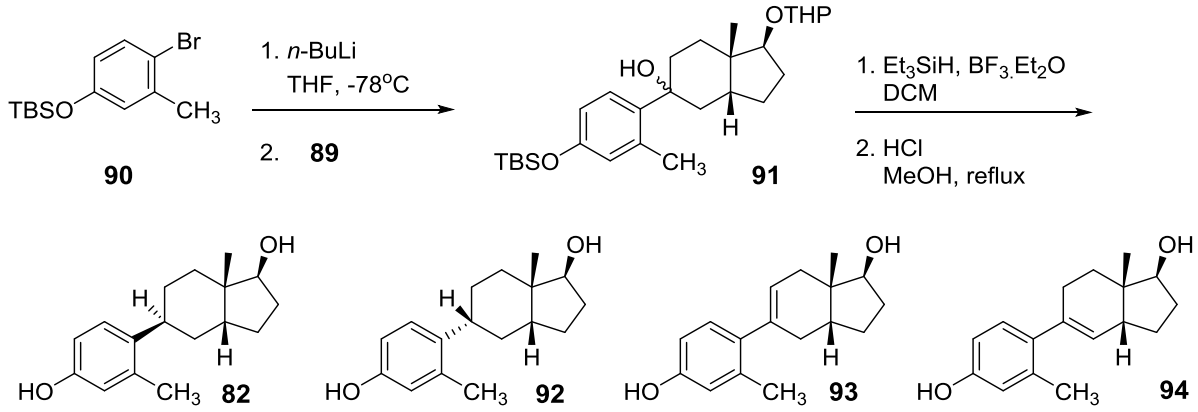
The protection of the CD-ring **40** with DHP in DCM catalyzed by PTSA afforded the protected CD-ring **89** in 90% yield (**Scheme 1.2.19**). The ^{1}H NMR indicated the presence of the tetrahydropyran moiety. This protecting group was not used for other coupling reactions because it makes the ^{1}H NMR spectrum more complex in the region between 1 and 3 ppm.



40 **89**

Scheme 1.2.19 Protection of CD-ring **40** with DHP affording the THP-protected CD-ring **89**.

Coupling of A-ring **90** [prepared from commercially available 4-bromo-3-methylphenol] with the THP-protected CD-ring **89** as shown in **Scheme 1.2.20** afforded the tertiary alcohol **91** in 65% yield. Treatment of **91** with Et_3SiH and $BF_3{\cdot}Et_2O$ afforded a complex mixture of unsaturated and saturated compounds as indicated by ^{1}H NMR spectrum. This crude mixture was deprotected using concentrated hydrochloric acid (2 – 3 drops) in MeOH at reflux giving a mixture of the 9(*S*) **82** and 9(*R*) **92**, as well as the olefins, **93** and **94**. These were separated by preparative-HPLC and characterized by their ^{1}H and ^{13}C NMR spectroscopy.

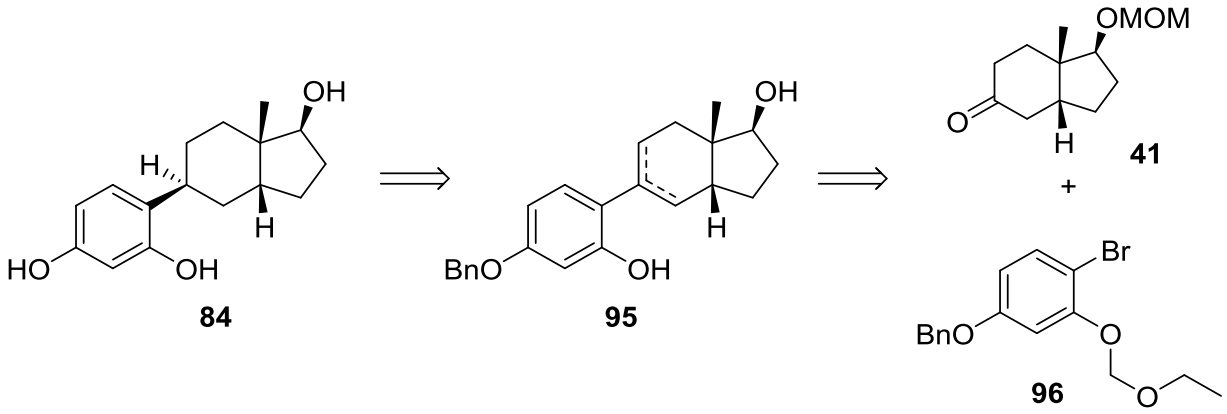


90 **91** **82** **92** **93** **94**

Scheme 1.2.20 Synthesis of the C5-Me A-CD estrogens.

C5-Hydroxy- estrogens and derivatives

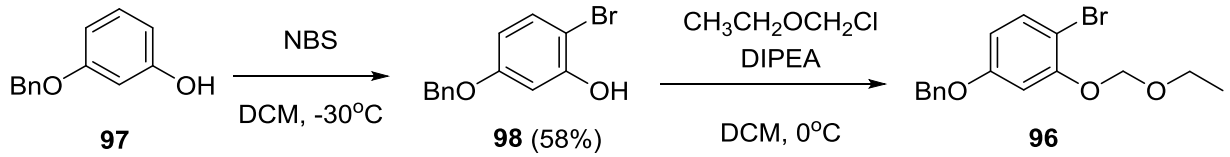
Because of the potential importance of **84** [TD81], the synthesis of this compound will be described in more detail; its retrosynthesis shown in **Scheme 1.2.21**. The approach was designed so that the key intermediate **95** would provide access to other C5 oxygenated derivatives by having the C3-hydroxy protected and C5-hydroxy group free. This intermediate would be prepared from the bromo-resorcinol **96**, bearing two different protecting groups, and the MOM-protected CD-ring **41**. Benzyl and methyl ethyl ethers were chosen as protecting groups because they can be selectively removed under different conditions.



Scheme 1.2.21 Retrosynthesis of **84** [TD81].

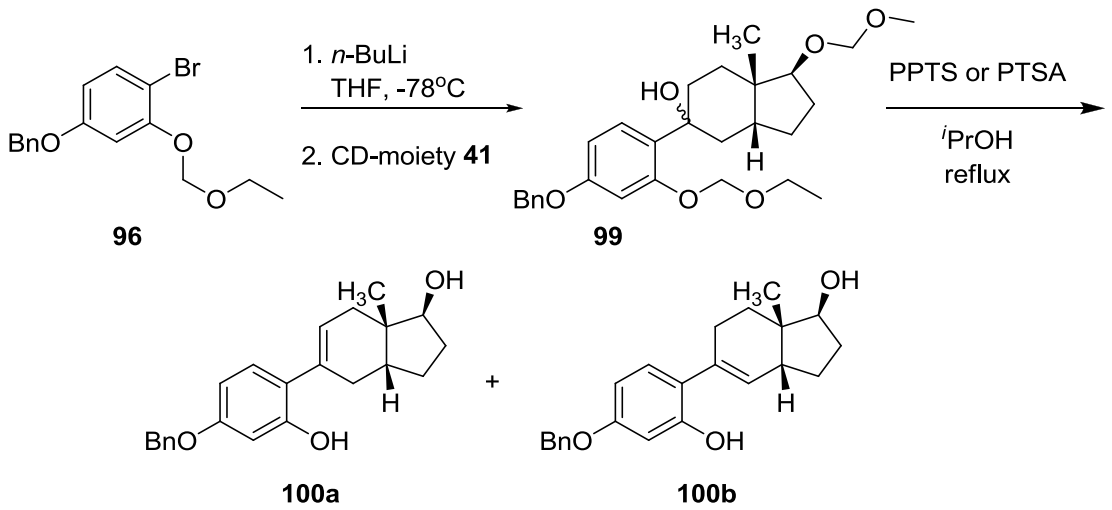
Bromo-resorcinol **96** was prepared in three steps from resorcinol [138]. Monoprotection of resorcinol **104** using 0.5 equivalent of benzyl bromide and 0.5 equivalent of potassium carbonate in acetone under refluxing conditions gave benzyloxy-3-hydroxybenzene **97** in 58% yield based on benzyl bromide. An excess of starting material as well as dibenzylated resorcinol were also recovered by flash column chromatography. Bromination of **97** when carried out using solid *N*-bromosuccinimide (NBS) under nitrogen atmosphere at -30^{o}C in dichloromethane gave **98** in 38% yield after column chromatography. The ^{1}H NMR spectrum of **98** was consistent with the literature data [138]. Reaction of **98** with methyl chloro ethyl ether under the conditions

previously described afforded **96** as a clear, colorless oil in a quantitative yield. The ^{1}H NMR spectrum showed the appearance of one singlet at 5.04 (2H) ppm due to the benzyl group, and one singlet at 5.27 (2H) ppm, one quartet at 3.78 (2H) ppm and one triplet at 1.24 (3H) ppm belonging to the methyl ethyl ether group.



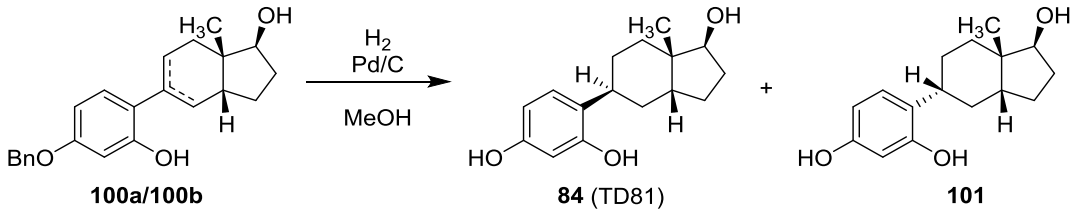
Scheme 1.2.22 Preparation of the diprotected bromo-resorcinol **96**.

Compound **99** was obtained in 72% isolated yield in a 45:55 ratio of C9-OH epimers via the usual coupling of the diether **96** and the MOM-protected CD-ring **41** (**Scheme 1.2.23**). Dehydration of **99** via the usual acid catalysis in solvents such as H_2O:THF, methanol, ethanol and propanol proved problematic affording an unidentifiable mixture of products. Finally, dehydration and deprotection of **99** to the alkenes **100a/100b** was successfully achieved in 56% isolated yield using PPTA or PTSA in isopropanol at 60-70°C.



Scheme 1.2.23 Preparation of the alkene mixture **100a** and **100b**.

Hydrogenation of the above isomeric alkenes occurred with concomitant hydrogenolysis of the benzyl protecting group, affording the desired compound **84** and its C9-epimer **102** (**Scheme 1.2.24**). The mixture of products **84** (or TD81) and **101** were separated by preparative HPLC and obtained in 90% overall yield in a 1.0:1.15 ratio. The ^{1}H and ^{13}C NMR spectra allowed us to confidently assign the structures of these compounds.



Scheme 1.2.24 Final step for the synthesis of **84** [TD81] and **101**.

The estrogen receptor binding affinities of TD81 were surprisingly good [RBAα = 0.26 ± 0.07; RBAβ = 4.0 ± 0.8; β/α = 15] given the low partition coefficient (LogP = 3.29) compared to **E2** (LogP = 4.01). The transcription assay showed that this compound was a potent and selective β agonist [RTAα = 3; RTAβ = 85; β/α = 28] (**Table 1.2.5**). These data coupled with the lack of toxicity towards hepatic cells suggested that this compound had considerable potential in estrogen related therapies.

Several other derivatives were prepared from the **100a/100b** mixture by selective alkylation of the aromatic hydroxy group C5. These were the C5-methoxy and the C5-O-$CH_2CH_2N(CH_3)_2$ compounds **83** and **102**, respectively. Both would address the question of whether substituents larger than OH, CH_3 and CF_3 could be tolerated at C5. The dimethylamino ethoxy side chain is the same side chain as found in 4-hydroxytamoxifen [active metabolite of tamoxifen] (**Figure 1.2.28**). We were interested in investigating whether this compound would act as an estrogen antagonist by preventing the displacement of helix H12, which is the mechanism of action of Tamoxifen [36].

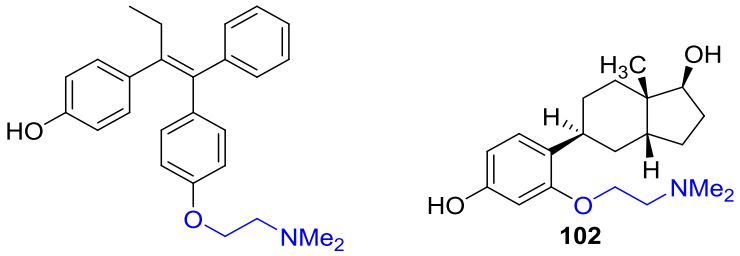
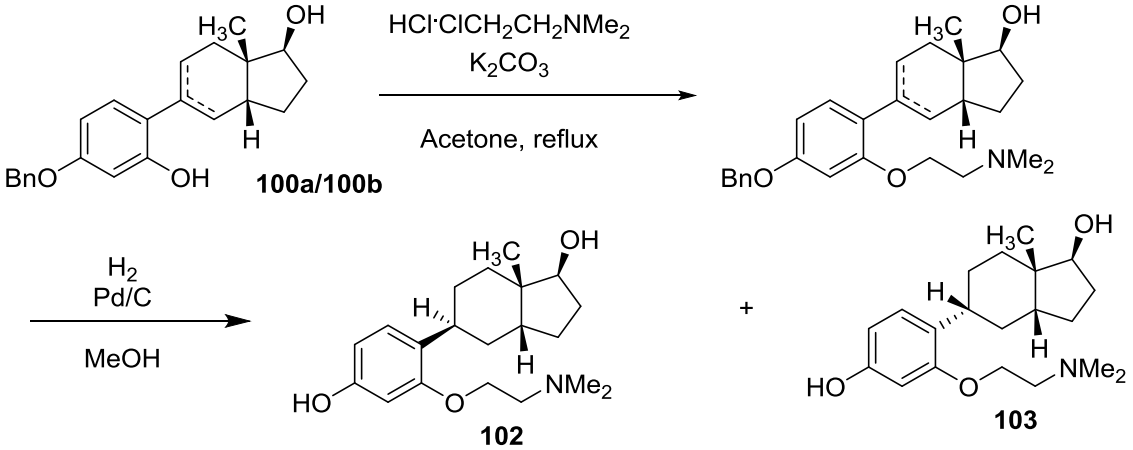


Figure 1.2.28 From left to right: structures of 4-hydroxytamoxifen and the C5-dimethyl amino-ethoxy A-CD estrogen **102**.

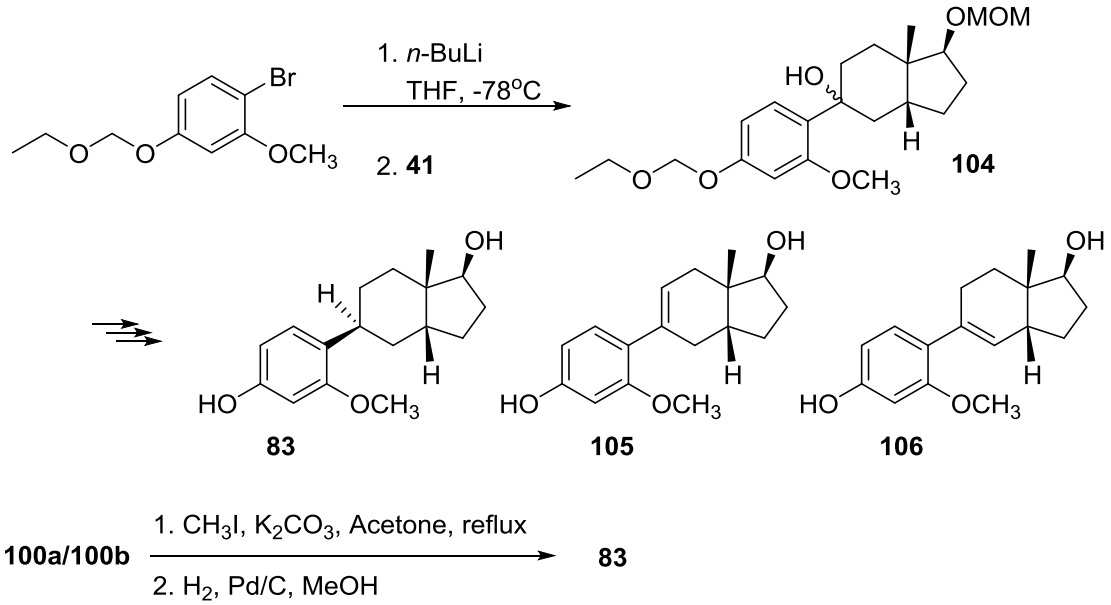
The conversion of the mixture of olefins **100a/100b** to **102** is depicted on **Scheme 1.2.25**. The aromatic hydroxy group at C5 was selectively alkylated in 89 % yield using two equivalents of 2-dimethyl aminoethyl chloride hydrochloride and five equivalents of potassium carbonate. The success of the alkylation was confirmed by ^{1}H NMR, which showed the expected ethyl as two triplets at 2.67 and 4.03 ppm and the dimethylamino signals as two singlets at 2.25 and 2.26 ppm. The hydrogenation step provided the mixture of natural and non natural aminoethyl A-CD estrogens **102** and **103**. These products were separated by preparative HPLC, characterized and sent for bioassays.



Scheme 1.2.25 Synthesis of the aminoethyl A-CD estrogens **102** and **103**.

The 5-methoxy A-CD estrogen **83** and its unsaturated analogs **105** and **106** were prepared via two different routes. The first route started with commercially available 4-bromo-3-

methoxyphenol and is shown in **Scheme 1.2.26**. The binding for this compound to both estrogen receptors was so low compared to the 5-OH and 5-CH_3 that we questioned our synthesis and wondered whether perchance we had prepared 3-methoxy-5-hydroxy isomer even though this did not seem reasonable based on the sequence followed. For that reason a second synthesis was carried out by methylating **100a/100b** followed by hydrogenation. This synthesis resulted in the formation of the same product as previously obtained. The product of this synthesis was also sent for binding studies which confirmed the original results.



Scheme 1.2.26 C5-methoxy derivatives.

Synthesis of TD81 analogs

Since compound **84** [**TD81**] had good RBA and excellent RTA data and no discernible hepatic toxicity (see **Section 1.2.9**), we decided to prepare analogs with higher Log Ps by adding either F, compound **107**, or Cl, compound **108**, at position C1 in the aromatic ring with the expectation that this would increase the levels of binding to the estrogen receptors and result in more potent and hopefully still β-selective agonists (**Figure 1.2.29**).

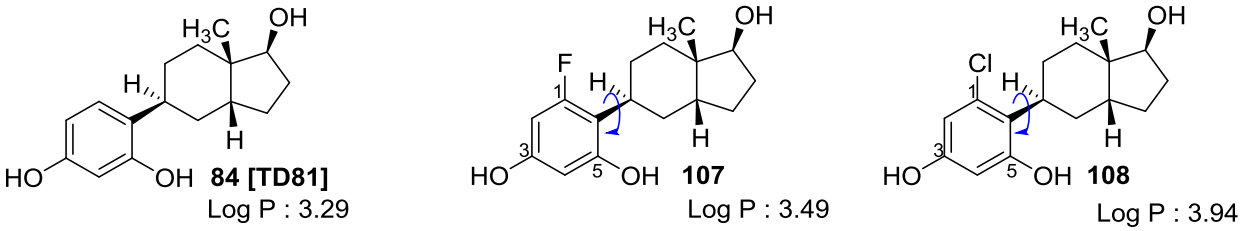
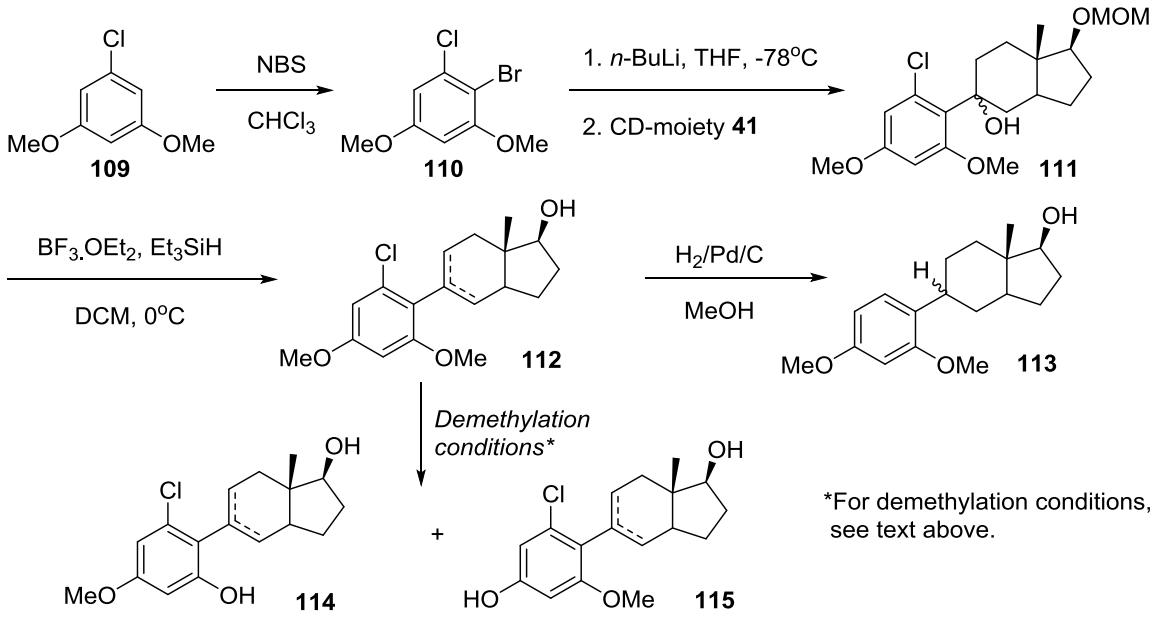


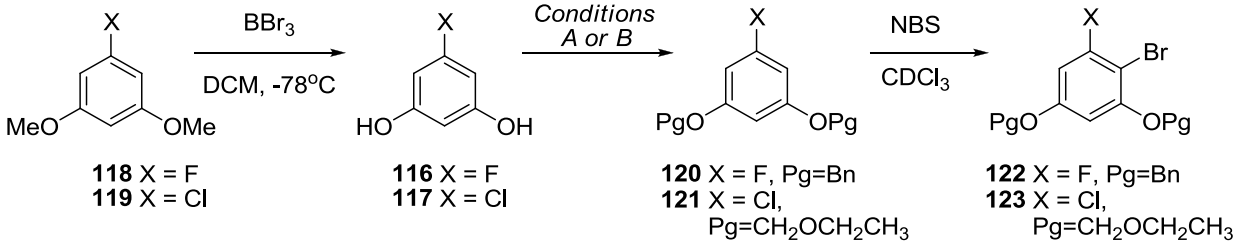
Figure 1.2.29 Structure of TD81 analogs. The Log P values were calculated with the KOAWIN Software v1.10.

The coupling of the dimethoxybromide **110**, obtained from bromination of **109** using NBS in chloroform, with protected CD ketone **41** gave **111**. Attempted reduction of the C9-OH group with triethylsilane yielded only the mixture of alkenes **115**. Hydrogenation of olefins **112** (with H_2/Pd/C in MeOH for 10-15 min) unfortunately resulted not only in reduction of the alkenes but also replacement of the aromatic Cl by H. We were also unable to demethylate **112** cleanly using standard demethylation procedures such as boron tribromide in DCM, iodocylohexane in DMF at reflux and sodium ethanethiolate in DMF at reflux [139]–[141]. These reaction conditions appeared to give a mixture of mono *O*-methyl compounds, presumably **114** and **115**.



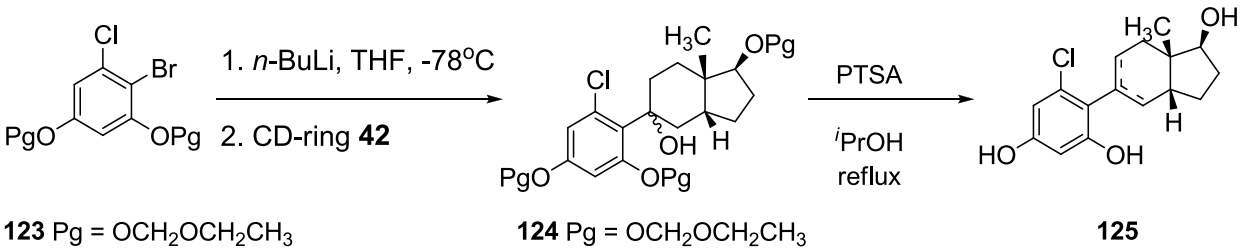
Scheme 1.2.27 First attempt to form the chloro analog of TD81.

For the second attempt we removed the two methoxy groups from ring A at the beginning of the synthesis and used protecting groups which could be easily removed at the end. Makriyannis and coworkers have described the syntheses of compounds **116** and **117** starting from **118** and **119** and using 2.3 equivalents of BBr_3 in dichloromethane at -78°C under nitrogen atmosphere [142]. In our hands, the use of 2.5 equivalents of BBr_3 with **119** provided a 1:1 mixture of the desired product **117** and 3-chloro-5-methoxyphenol. The use of 3 equivalents of BBr_3 cleanly afforded compounds both the 5-chloro-1,3-dihydroxybenzene, **117**, and the fluoro analog **116** in 100% and 98% yield, respectively. The ^{1}H NMR spectra of these compounds matched the literature data. The benzyl group was chosen as protecting group for compound **116** (affording compound **120** in 89%) because it would be removed in the final hydrogenation step; while the methyl ethyl ether **121**, obtained in 63% yield was chosen for **117**. Bromination of **120** with NBS afforded a mixture from which **122** was obtained in 79% isolated yield after purification via column chromatography and recrystalization. The ^{1}H NMR spectrum of **122** showed the required two distinct methylene groups belonging to the benzyloxy ether substituents at 5.11 ppm and 5.00 ppm. The other regioisomer has a plane of symmetry thus having only 1 methylene group at 5.14 ppm. Bromination of compound **121** with NBS providing the desired product **123** in 78% isolated yield.



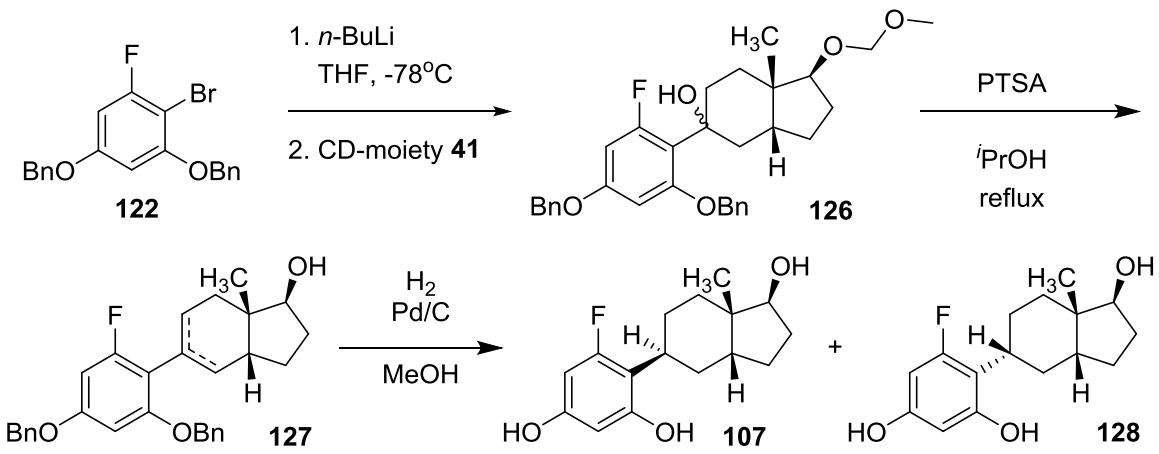
Scheme 1.2.28 Preparation of the brominated aromatics, **122** and **123**.

Coupling of the chlorinated-resorcinol **123** with **41** gave the expected C9-OH adducts **124** in modest yield mixed with unreacted starting materials. At this pointed it was decided to target the alkene mixture **125** since previous attempt at forming **108** had failed. Dehydration of **124** using PTSA and isopropanol, the procedure that had been successful in the TD81 series, gave a complex mixture including some of the expected alkenes in 8.8% yield over two steps. Resolution of the mixture via several silica gel chromatography columns followed by preparative HPLC provided a cleaner mixture of a small amount (11 mg) of the alkene products **125** based on proton NMR. Due to time constraints, low yields and the lengthy sequence the synthesis, the separation of these compounds was not completed.



Scheme 1.2.29 Attempted preparation of the alkenes **125**.

The synthesis of the C1-fluoro-C5-hydroxy A-CD estrogen **107** was not complicated by the loss of F during the final hydrogenation step. It followed the same sequence used in the preparation of **84** [**TD81**]. The tertiary alcohol **126**, obtained in 56% yield from the coupling of **122** with **41**, was deprotected and dehydrated to the mixture of **127** in 47% isolated yield. Hydrogenation of the mixture under standard conditions afforded the desired fluoro resorcinol analog **107** and its C9-epimer **128** in 96% yield.



Scheme 1.2.30 Synthesis of the fluoro-resorcinol analog **107**.

Bioassay results for the C5 and/or C1 A-CD estrogens

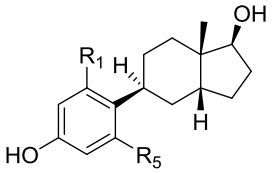
The biological characterizations for the "natural" 9(*S*) A-CD compounds with C5 and/or C1 substitutions in the A-ring are presented in **Table 1.2.5** (see **Section 1.2.6** for A-CD compounds with unsaturations in the C-ring). Preliminary results indicated that RBA values correlate qualitatively RTA results. Therefore, compounds in **Table 1.2.5** with low binding affinity (<0.001% of **E2**) were not sent for RTA. Generally, compounds with substitutions *meta* to the C3-OH group tend to enhance the binding affinity as compared to the parent compound **19 (**ERα = 1.5; ERβ =21 relative to **E2** = 100 for both ERs).

As predicted for C5 substituted A-CD compounds, a higher Log P normally results in higher binding affinity for both ER subtypes. For example, C5-EWG derivatives with the highest Log P (CF_3, Cl, F) have the highest affinity for ERα (89.7%, 52.3%, 27.3%) and for ERβ (205%, 168%, 136%), respectively. Binding levels to ERα appear to be more strongly influenced by this parameter than those of ERβ thereby reducing the β/α selectivity. This makes these compounds somewhat less interesting for HRT applications.

The RBAs for **82** (C5-CH_3) with ERα=2.82%, ERβ=33.6% showed that the effect of the C5-methyl group relative to C5-H was quite beneficial. Its β/α selectivity of 12 is quite similar to the 14 found for the parent **19**. The RTA values for **82** (RTAα = -10, RTAβ = 49) suggest that this compound is a reasonably potent and highly selective β-agonist, even showing some α-antagonism.

The non-toxic C5-OH compound **84** (**TD81**) has ERα = 0.26 ± 0.07% and ERβ = 4.0 ± 0.8%. The binding for this compound is surprisingly good considering that its Log P is considerably lower than that of estradiol and the parent A-CD, compound **19**. The β/α selectivity of 15 is the same as that found in **19**. The RTA data (ERα =3; ERβ = 85), resulting in a β/α selectivity of 28 for **TD81** is also highly promising. These data combined with its non-toxicity make it an interesting compound for potential applications in both the HRT and anti-cancer field.

Table 1.2.5 Relative Binding Affinity (RBA) and Relative Transcription Activation (RTA) of C5-substituted A-CD estrogens with the "natural" 9(*S*) configuration.



19, R_1,R_5=H
30, R_1=H, R_5=F
48, R_1=H, R_5=CF_3
71, R_1=H, R_5=Cl
82, R_1=H, R_5=Me
83, R_1=H, R_5=OMe
84, R_1=H, R_5=OH
103, R_1=H, R_5=$OCH_2CH_2NMe_2$
107, R_1=F, R_5=OH

Comp.	Log P[a]	RBA[b]			RTA[c]		
		ERα	ERβ	β/α	ERα	ERβ	β/α
2 (E2)	4.01	100	100	1	100	100	1
19	3.77	1.5	21	14.0	4.4 ± 0.5	164 ± 5	38
30	3.98	27.3 ± 0.7	136 ± 7	5.0	44 ± 12	158 ± 1	3.6
48	4.75	90 ± 14	205 ± 23	2.3	106	93	0.9
71	4.42	52 ± 12	168 ± 33	3.2	90 ± 13	189 ± 7	2.1
82	4.32	2.8 ± 0.5	34 ± 6	11.9	-9 ± 3	49 ± 10	pure β
83	3.86	0.016 ± 0.002	0.12 ± 0.03	7.7	-	-	-
84	3.29	0.26 ± 0.07	4.0 ± 0.8	15	3	85	28
103	3.55	0.009 ± 0.001	0.045 ± 0.006	5.0	-	-	-
107	3.49	10 ± 3	22 ± 6	2.2	75	115	1.5

a- The calculated data was predicted data using KOAWIN Software v1.10.

b- Relative Binding Affinity assay prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.

c- Relative Transcription Activity prepared by the group of Dr. C. Pratt at the University of Ottawa.

In an attempt to create a compound with binding affinity of higher than **TD81** we prepared compound **107** which has an additional F at position C1 (since our A-CD analogs have a free rotation between rings A and C, the positions C1 and C5 are interchangeable). The RBA results indicated that compound **107** had more than a four-fold increase in binding affinity to ERβ (22 ± 6%) compared to **84** (4.0 ± 0.8%). However its binding affinity for ERα increased even more from (0.26 ± 0.07) for **84** to (10 ± 3) for **107**, a 37 fold increase. As a result the β/α-selectivity ratio decreased to 2.2 for **107** compared to 15 for **84**.

The data for the binding affinity for the C5-OMe compound, **83**, which has a greater Log P than the C5-OH derivative (**84**), indicated that this substituent was not well tolerated by the estrogen receptors. This was even more evident for the larger C5-dimethylamino ethoxy ether **103** whose binding affinities decreased by a factor of 100 as compared to the parent compound **19**. These results suggest that substituents of the size of CH_3 and CF_3 or smaller at C5 in the A-CD estrogens increase the binding to the estrogen receptors relative to H. Typically an increase in Log P due to the substituent is beneficial especially in increasing the binding affinity to ERα. The results for the C5-OH and C5-O-CH_3 compounds do not fit these simple generalizations. Binding affinities to the estrogen receptors were higher than anticipated for the C5-OH compound but lower for the C5-O-alkyl compounds. A possible rationale is the formation of a novel hydrogen bond at the C5-OH group with residues of the receptor. This should be confirmed by an X-Ray of the ligand TD81 bound to the receptor.

Importantly, the RTA results shown in **Table 1.2.5** indicate that most of these C5-substituted A-CD estrogens are agonists to the respective ERs with several showing considerable selectivity. The data for compound **82** (C5-CH_3) suggests that it could be considered a pure agonist for ERβ, and a slight antagonist for the ERα. One can see that RTA results generally track RBA results.

Compounds with high biding affinities cause a significant increase in gene transcription. Those with high binding affinities but low selectivity show also low transcription selectivity.

1.2.5 Synthesis and bioassays of A-CD estrogens with various substitutions in the A-ring

Synthesis of L17 analogs

As mentioned earlier, compound **32** or [**L17**], designed to prevent enzymatic *ortho*-hydroxylation by replacing H2 and H4 with fluorine atoms, was shown to have low hepatic cell toxicity. It is both a moderately potent and selective β-agonist and, in contrast to **E2** or the C5-CF_3 derivative **48**, was shown not to cause uterine cell growth [143]. Combined, these properties suggested that this compound could be promising as a potential alternative to HRT. For this reason, the synthesis of analogs **129** and **130** was initiated as potential back-up candidates for **L17** (**Figure 1.2.30**). Since the lipophilic character of these molecules is linked positively to the binding affinity of our A-CD estrogens, we expect that these compounds would likely have a greater binding affinity than that of **L17**.

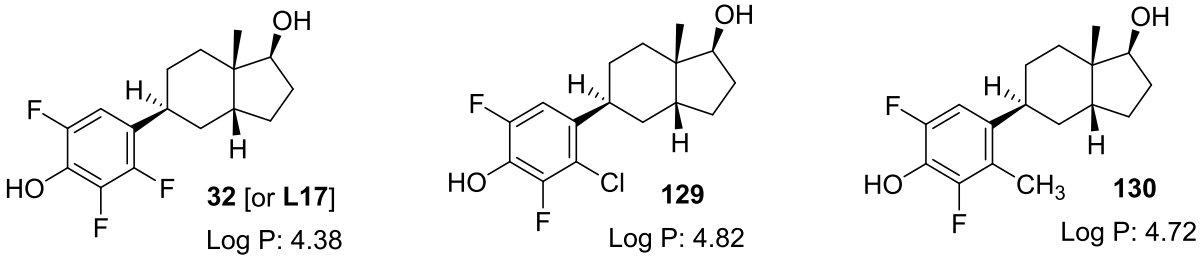
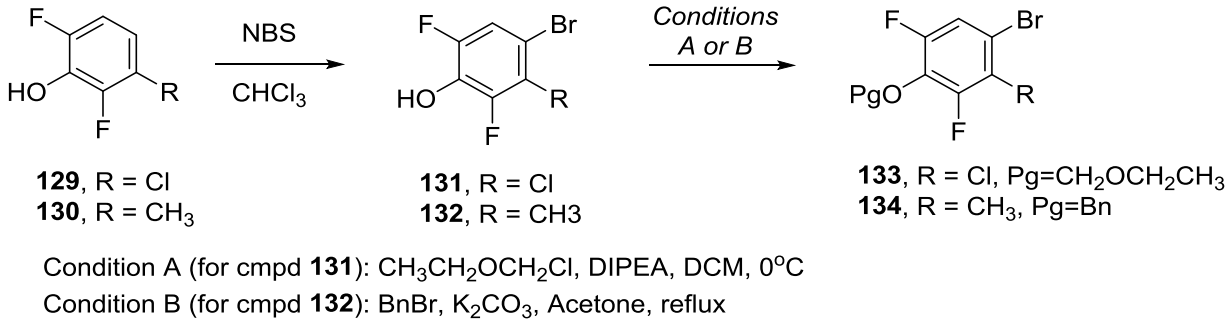


Figure 1.2.30 Structure of L17 analogs. The Log P values were calculated with the KOAWIN Software v1.10.

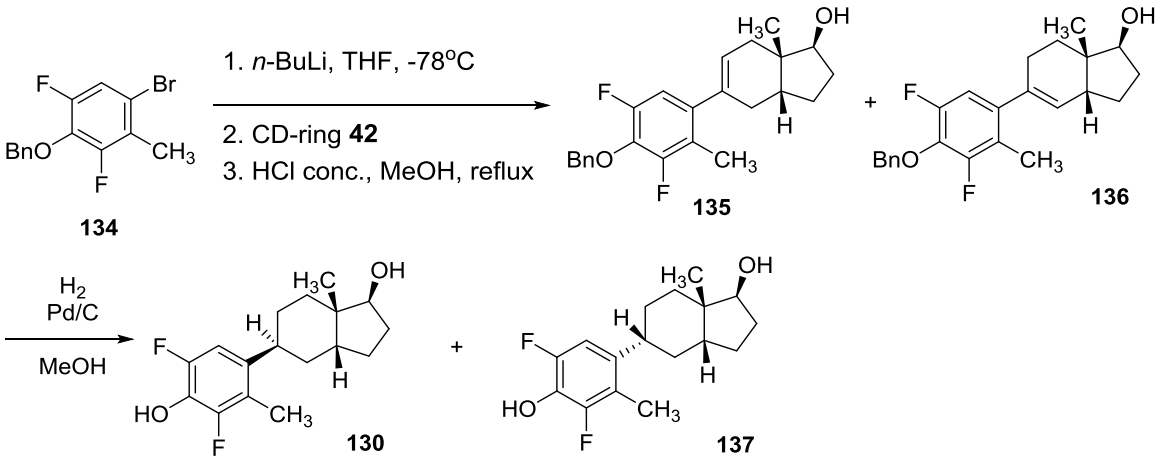
The A-rings for the compounds required for the preparation of **129** and **130** were obtained relatively routinely as shown in **Scheme 1.2.31**. The bromination procedure used was that reported by Marzi and coworkers [144] for the preparation of 4-bromo-2,3,6-trifluorophenol. The brominated compounds **131** and **132** were obtained in 54% and 78% isolated yield from the

commercially available **129** and **130**, respectively. The benzyl and $CH_2OCH_2CH_3$ groups were used as protecting groups. Standard reaction conditions gave the required protected A-rings **133** and **134** in 85% and 31.5% yields, respectively.



Scheme 1.2.31 Preparation of **133** and **134**.

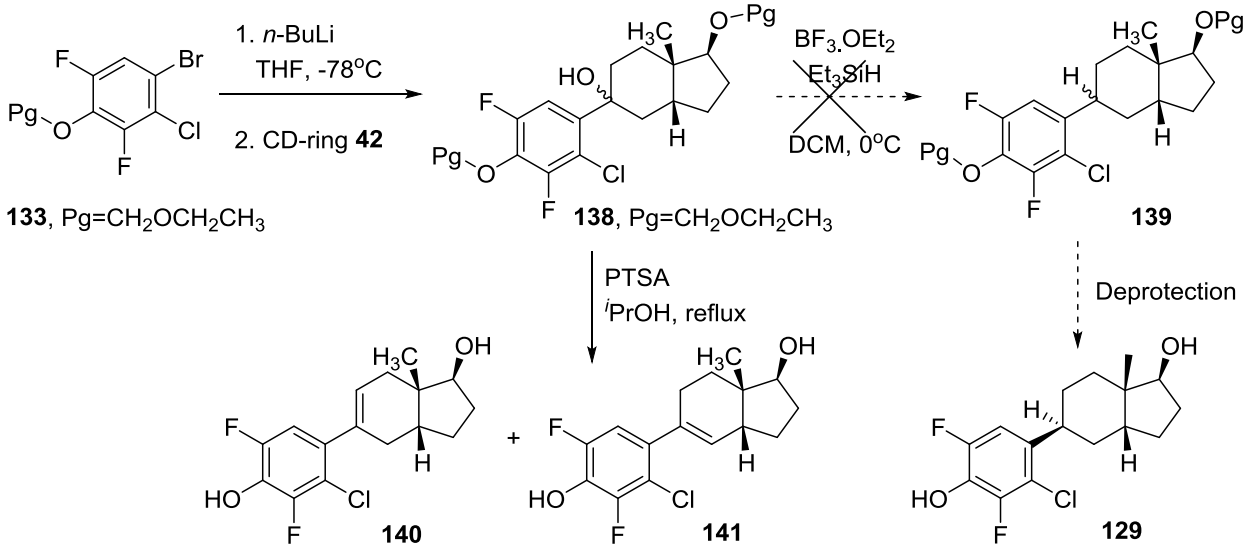
The synthesis of compound **130** is depicted on **Scheme 1.2.32**. The mixture of alkenes **135/136**, [obtained by coupling **134** and **42** followed by dehydration of the C9-OH adduct in 42% yield over 2 steps] was hydrogenated affording a mixture of C9-epimer **130** and **137**. These isomers were separated by preparative HPLC and their structure assignments were based on the ^{1}H and ^{13}C data as explained in the earlier sections.



Scheme 1.2.32 Synthesis of compound **130**.

Because compound **129** has a chlorine atom in the A-ring, its synthesis was attempted using the triethylsilane reduction which was successful in the case of the C5-Cl derivative. Treatment of

C9-OH intermediate **138** [obtained from coupling **133** with **42** in 43% yield] with Et_3SiH and $BF_3{\cdot}Et_2O$ did not provide the desired hydrogen delivery at C9, even as minor product. The ^1H NMR indicated the presence of unsaturated olefins (**Scheme 1.2.33**). Consequently the synthesis of **129** was abandoned and we prepared the alkenes **140** and **141** from the dehydration of **138** using PTSA and ^{i}PrOH. These were purified by silica gel chromatography and separated by preparative HPLC. The ^1H NMR and ^{13}C NMR spectra confirmed the structures of the alkenes.



Scheme 1.2.33 Attempted synthesis of compound **129**.

Preparation of other A-CD derivatives

At times in this project, it seemed appropriate to prepare specific A-CD analogs to address questions concerning the effect of additional substituents in the aromatic ring on binding to the estrogen receptor, their β/α receptor selectivity, or their toxicity relative to estradiol. Since the preparation of all of these compounds follows sequences and procedures already described it was decided to present these syntheses in the form of schemes with minimal comments. The structural assignments of the isomers are based on the ^1H and ^{13}C NMR data as explained in the earlier sections. The complete data is given in the Experimental section. Relevant references to

the preparation of intermediates are also given in the Experimental section. These five targeted A-CD structures are shown in **Figure 1.2.31**. Also included in the Schemes is the preparation of the related compounds with unsaturation in ring C.

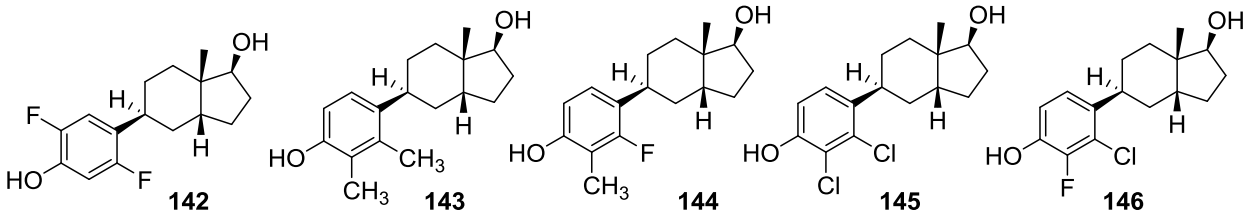
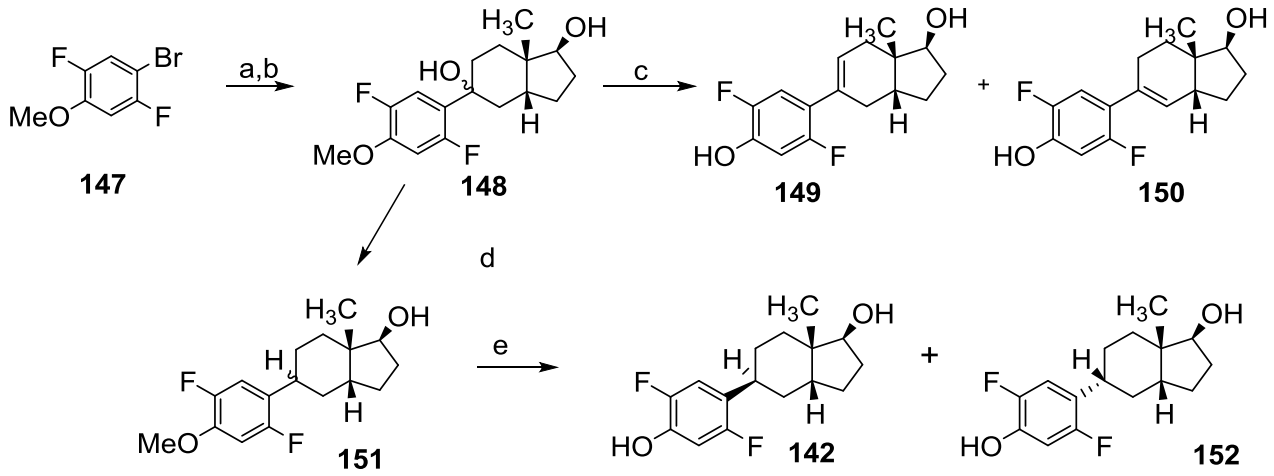


Figure 1.2.31 A-CD estrogens with disubstitution in the A-ring.

Compound 142 and its related alkenes

The use of unprotected CD-ring **40** (1 equiv.) with the 2,5-difluoroaryllithium (3 equiv.), derived from commercially available **147** and *n*-BuLi, afforded the tertiary alcohol adduct **148** in a rather poor yield of 35% (**Scheme 1.2.34**). Demethylation of **148** using 8 equiv. of sodium methanethiolate in DMF at 160^{o}C was accompanied, possibly during acidic workup, with dehydration to give a mixture of alkenes **149** and **150** in 38% overall yield. These isomers were also separated by preparative HPLC for the purpose of obtaining their binding affinities. The ^{1}H NMR and ^{13}C NMR spectra of these alkenes follow the trends described in **Section 1.2.6**. Reduction of **148** with Et_3SiH and $BF_3{\cdot}Et_2O$ afforded **151** in equal amounts of the C9-epimers in 46% yield. Removal of the methoxy group from the mixture of isomers was successfully achieved with BBr_3 in 90 % yield. Separation of these epimers by column chromatography afforded the desired products **142** and **152**.



Scheme 1.2.34 Synthesis of compounds **142**, **149**, **150** and **152**. a) *n*-BuLi, THF, -78°C; b) unprotected CD-ring **40**; c) NaSMe/DMF/160°C; d) $BF_3{\cdot}Et_2O$, Et_3SiH DCM, 0°C; e)BBr_3/ DCM, 0°C.

Compounds 143-146 and their related C-ring alkenes

The A-ring components required to prepare these compounds were obtained by regioselelective bromination of commercially available materials (using either NBS in acetonitrile or Br_2 in DCM, [145], [146]) followed by protection of the phenol with either TBDMSCl or methyl chloroethyl ether.

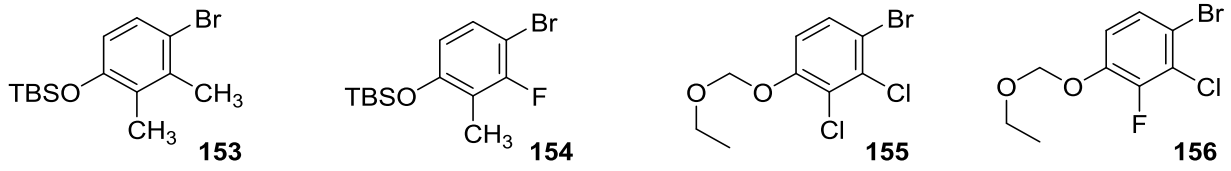
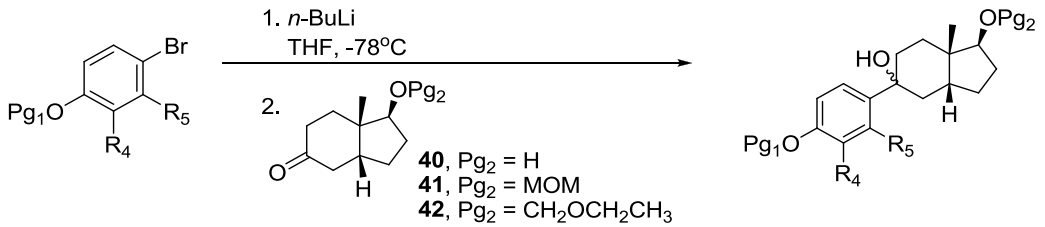


Figure 1.2.32 Structure of A-rings components required for compounds **143-146**.

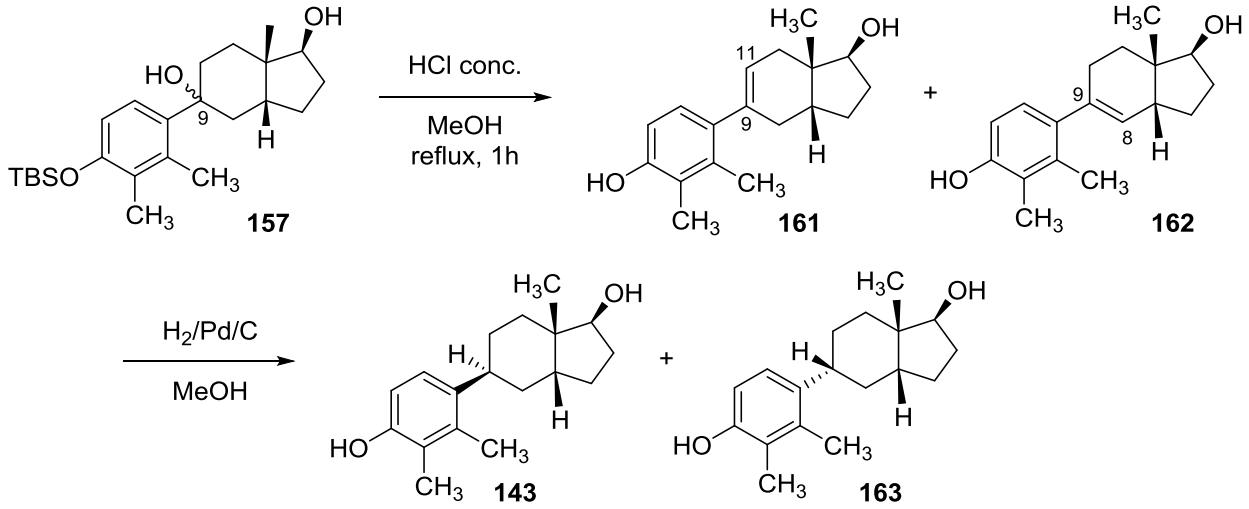
Coupling of all of the above A-ring components with the appropriate CD moiety **40**-**42** was straightforward (**Table 1.2.6**). The isolated coupling isolated yields ranged from 21% to 67% after purification by column chromatography.

Table 1.2.6 Coupling reaction of a brominated A-ring to the CD-ring moiety.



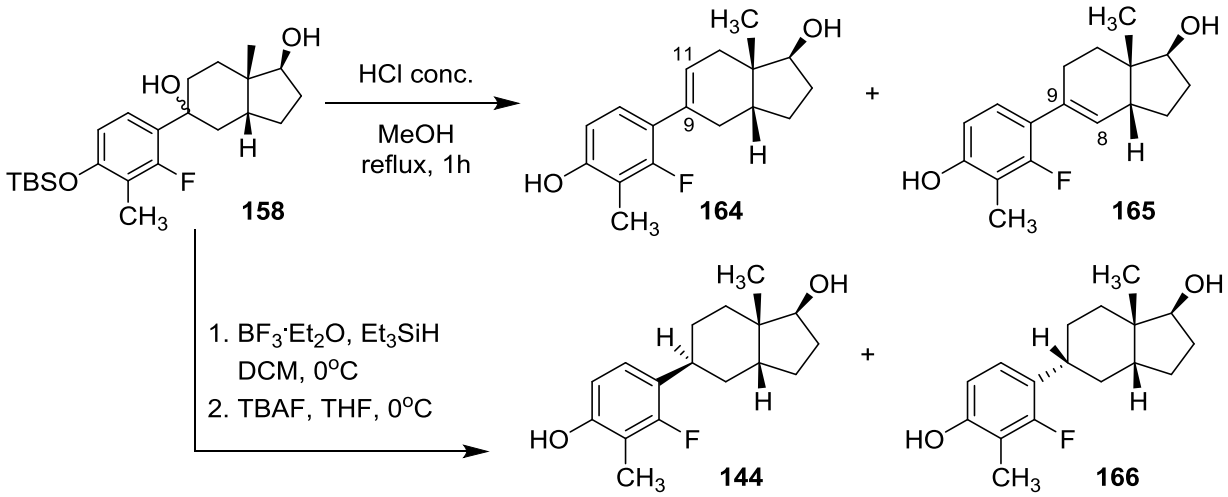
Comp.	A-ring (R_4, R_5, Pg_1)	CD-ring	Product	Yield
153	Me, Me, TBS	**40**	**157**	45%
154	Me, F, TBS	**40**	**158**	21%
155	Cl, Cl, $CH_2OCH_2CH_3$	**41**	**159**	66%
156	Cl, F, $CH_2OCH_2CH_3$	**42**	**160**	67%

Dehydration and deprotection of **157** was carried out in a one-pot reaction under acid-catalyzed conditions to give a mixture of isomeric alkenes, **161** and **162**, having a double bond in ring C (**Scheme 1.2.35**). These isomers were separated using preparative HPLC and assigned ^{1}H and ^{13}C NMR spectra (See **Section 1.2.6**). Finally, mixtures of these alkene isomers were hydrogenated under normal conditions to obtain the desired product **143** its C9-H epimers **163** in 79% yield.



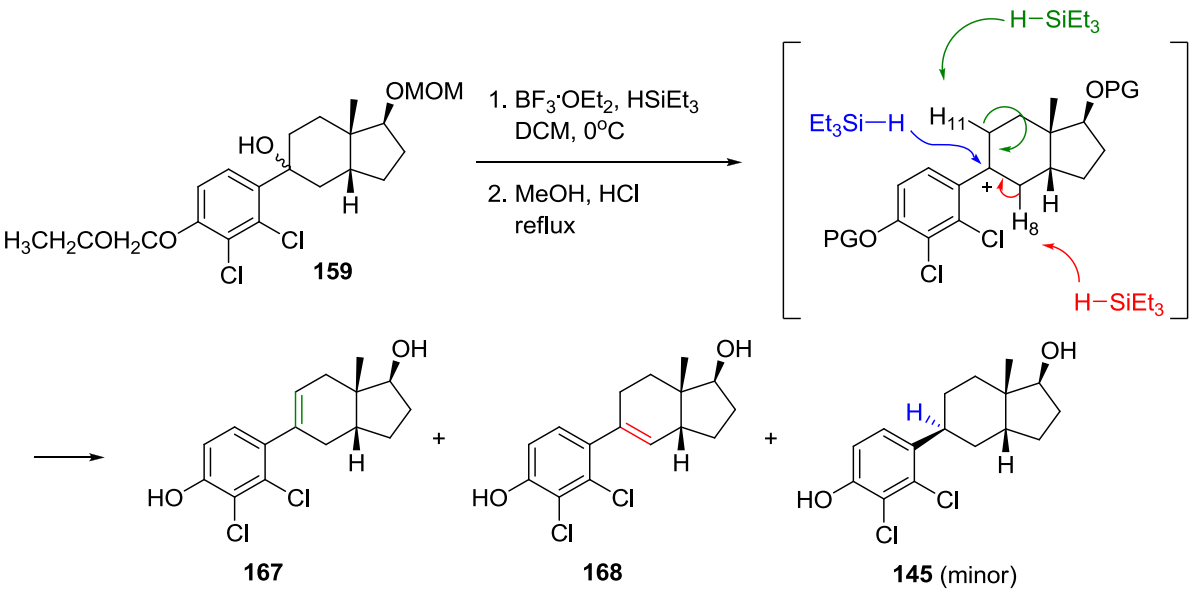
Scheme 1.2.35 Synthesis of the 4,5-dimethyl A-CD derivatives.

Treatment of **158** with concentrated HCl in MeOH at reflux afforded the mixture of olefin **164** and **165** in 96% yield (**Scheme 1.2.36**). Hydrogenolysis of **158** using Et_3SiH and $BF_3{\cdot}Et_2O$, followed by removal of the TBS group with TBAF in THF afforded the final 9(*S*) and 9(*R*) products, **144** and **166**, in 41% yield over two steps.



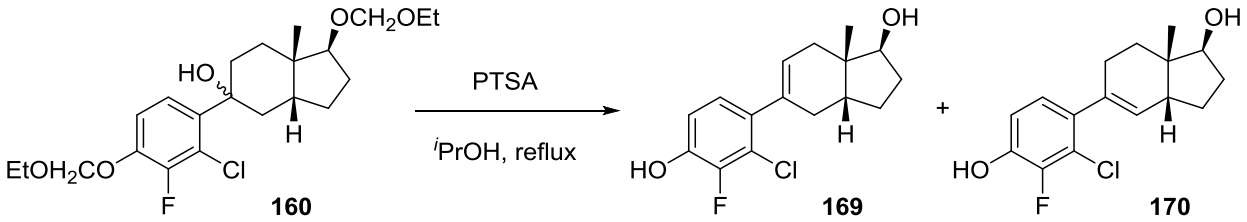
Scheme 1.2.36 Synthesis of the 4-CH_3-5-F A-CD derivatives.

Hydrgenolysis of the *o*-dichloro intermediate **159** using Et_3SiH and $BF_3{\cdot}Et_2O$ in DCM afforded a complex mixture containing small amounts of the desired reduction products at C9 but mainly dehydration to the C ring alkenes. Not all of the OH protecting groups were cleanly removed. To accomplish this, the above mixture was refluxed with HCl in MeOH (**Scheme 1.2.37**). The mixture was separated by preparative HPLC to give mainly the alkenes **167** and **168** and a small amount of the target **145**, identified by ^1H NMR. Because compound **145** was obtained in a small amount it was not further characterized or sent for RBA bioassays. Alternatively, compounds **167** and **168** were also obtained in one step from same the acidic treatment of **159**. Alkenes **167** and **168** were secured by their ^1H NMR and ^{13}C NMR spectra and sent for the RBA bioassays.



Scheme 1.2.37 $BF_3{\cdot}Et_2O$ catalyzed reaction of **159**.

In the case of the 4-fluoro-5-chloro A-CD hydroxy intermediate **160**, the reductive substitution did not give the desired hydrogen delivery at C9, even as minor product, but only provided the corresponding mixture of olefins (**Scheme 1.2.38**). As seen throughout this chapter the triethylsilane reduction was successful in some cases. It appears that when the A-rings are more substituted the hydrogen delivery from the triethylsilane is more difficult and elimination dominates. Dehydrating of **160** using PTSA and isopropanol at reflux afforded the alkenes **169** and **170** in 81% yield. The structure assignments via ^{1}H and ^{13}C NMR were once again consistent with data found on **Table 1.2.8**.

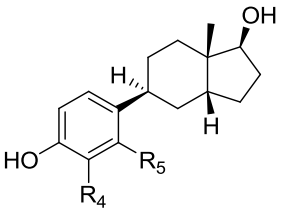


Scheme 1.2.38 Preparation of alkenes **169** and **170**.

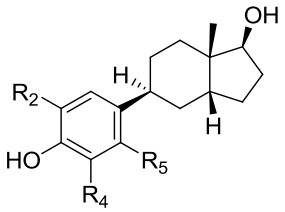
Bioassays of A-CD estrogens with various substitutions in the A-ring

Bioassays for the "natural" 9(*S*) A-CD compounds with various substitutions in the A-ring are shown in **Table 1.2.7**. When compared with **Table 1.2.5**, the binding affinities of polysubstituted compounds tend to have lower binding affinity than compounds with only one substition at position C5. This hypothesis was supported by computational studies performed by Dr. Wright and coworkers indicating that the C2-position of our A-CD estrogens interacts with two hydrogen bonding residues (Arg394, Glu353 in ERα) while the C4-position interacts only with one (Arg394) [30].

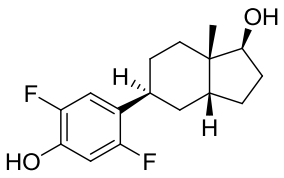
Table 1.2.7 Relative Binding Affinity (RBA) and Relative Transcription Activation (RTA) of A-CD estrogens with various substitutions in the A-ring.



31, R_4=F, R_5=F
143, R_4=CH_3, R_5=CH_3
144, R_4=CH_3, R_5=F



32 [L17], R_2=F, R_4=F, R_5=F
130, R_2=F, R_4=F, R_5=CH_3



142

Comp.	RBA[a]			RTA[b]		
	ERα	ERβ	β/α	ERα	ERβ	β/α
2 (E2)	100	100	1	100	100	1
19[c]	1.5	21	14.0	4.4 ± 0.5	164 ± 5	37.7
31[c]	4.6 ± 0.9	43 ± 6	9.3	18 ± 4	151 ± 1	8.3
32[c]	0.19 ± 0.01	1.7 ± 0.2	9.3	12.8 ± 0.4	95 ± 4	7.9
130	0.7 ± 0.2	5.3 ± 0.9	7.1	<5	75	15
142	0.4 ± 0.1	3.3 ± 0.7	8.7	<5	78	15.6
143	0.065 ± 0.001	0.18 ± 0.05	2.8	-	-	-
144	1.7 ± 0.5	1.8 ± 0.5	1.0	-	-	-

a- Relative Binding Affinity assay prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.
b- Relative Transcription Activity prepared by the group of Dr. C. Pratt at the University of Ottawa.
c- These compounds were prepared by Dr. Asim Muhammad.

As predicted by the higher Log Ps, the binding affinity for both receptor isoforms of the **L17** analog **130** (RBAα= 0.7 ± 0.2%, RBAβ= 5.3 ± 0.9%) was increased compared to **L17**, **32** (RBAα= 0.19 ± 0.01%, RBAβ= 1.7 ± 0.2%), in addition to similar β-selectivity (7.1 vs 9.3, respectively). The RTA value of **130** were ERα < 5 and ERβ = 75 with a β/α ratio of 15, which shows that this compound is a strong, selective, β-agonist. Therefore, based on these bioassays, it seems that compound **130** is a very plausible backup for **L17**. Further investigation will be undertaken, such as hepatic toxicity studies and cell proliferation studies. If these results are similar to **L17**, compound **130** might be a good candidate as a potential HRT or anti-cancer compound.

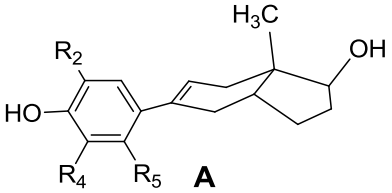
Substitution of the fluorine atoms with either one or two methyl groups of the 4,5-difluoro A-CD **31**, resulted in a significant decrease of binding affinity for both ER subtypes, as seen in compounds **143** and **144**, by over a factor of 20 for ERβ and their β-selectivity as observed by their β/α ratio. The RBA results for compound **142**, which has two fluorine atom at C2 and C5 has, were ERα= 0.4 ± 0.1% and ERβ= 3.3 ± 0.7%, which corresponds to β/α ratio of 8.7. Thus, compound **142** binds to the ER with decreased affinity compared with its regioisomer **31**, and with similar ERβ selectivity. The RTA values of **142** were <5% and 78%, for the ERα and ERβ, respectively, which indicates that this compound is a moderately strong, selective, ERβ agonist. As will be discussed in **Section 1.2.9**, compound **31** turned out to be toxic in hepatic human cell lines; however compound **142** was never sent for this bioassay. The question of weather this compound is also toxic to hepatic cell lines remains. These compounds may have significant differences in their metabolism and their toxicity, as observed with previous studies on 2-fluoroestradiol and 4-fluoroestradiol (**Figure 1.2.2**) [119–121].

1.2.6 Chemical and biological characterization of the C8/C9 and C9/C11 unsaturated *cis* CD-rings

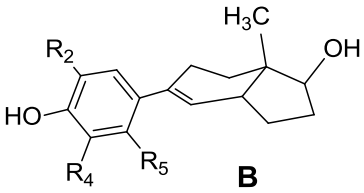
The individual structure assignment of the pairs of alkenes with double bonds either at C8-C9 or C9-C11 described in this chapter is shown in **Table 1.2.8**. These assignments are based on HOMCOR NMR experiments and on the observation of a retro Diels Alder fragmentation in the case of the C9-C11 isomer of the trifluoromethyl derivatives **56** and not in **57**.

The ^{1}H NMR spectra of the two pairs of alkenes shown below had consistent differences with the C9-C11 isomer showing H17 as a doublet of doublets in the 3.75 ppm range compared to a triplet at around 3.89 ppm for the C8-C9 isomer; the olefinic proton in the C8-C9 isomer was consistently at lower field than that in the C9-11 compounds. The ^{13}C spectra also showed consistent differences, with C17 occurring near 81 ppm for the C9-C11 isomer and near 79.5 ppm for the C8-C9 isomer.

Table 1.2.8 NMR characterization of some olefins reported in earlier sub-sections of this chapter with unsaturations between either C9-C11 or C8-C9 bonds.



A



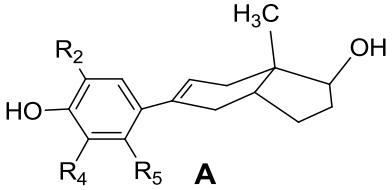
B

A-CD derivatives	**A- ^{1}H NMR**		**A- ^{13}C NMR**	**B- ^{1}H NMR**		**B-^{13}C NMR**
	H11	H17	C17	H8	H17	C17
5-Cl	5.50, bs	3.75, dd	81.2	5.55, bs	3.89, t	79.4
5-CF_3	5.45, bs	3.76, dd	81.2	5.50, bs	3.88, t	79.4
5-Me	5.42, bs	3.83, bs	80.6	5.43, bs	3.88, t	79.5
2,5-diF	5.82, bs	3.74, dd	81.0	5.87, bs	3.84, t	79.1
4-Me-5-F	5.70, bs	3,73, dd	81.2	5.75, bs	3.86, t	79.5
4,5-diCl	5.53, bs	3.76, dd	81.1	5.57, bs	3.88, t	79.4
4-F-5-Cl	5.56, bs	3.76, dd	81.1	5.61, bs	3.89, t	79.4

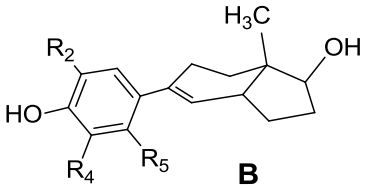
Bioassays of several A-CD estrogens with unsaturation in the C-ring are described in **Table 1.2.9**. The binding of these compounds to the estrogen receptors follow similar trends as observed for the corresponding 9(*S*) compounds, described in **Tables 1.2.5** and **1.2.7**. Substituents at C5 which increase the log P, for example, CF_3 and Cl, significantly increase the RBA as compared to the parent compounds **171/172**, especially for compounds which had the unsaturation between C8 and C9. In fact, the C5-CF_3 (**57)** and C5-Cl (**81**) derivatives have, at the moment, the highest binding affinities to the ERs within our library with RBAα= 189±1%, 195±25% and RBAβ=213%, 327±14%, respectively for the 5-CF_3 and 5-Cl compounds. However, these have negligible selectivity for the ERβ (with β/a ratio between 1.1 and 2.0 for both C8-C9 and C9-C11 alkenes).

Originally, the C5-Me olefins, **93** and **94**, were sent as a mixture and their RBA was 0.21% and 2% for ERα and ERβ, respectively (not shown in the below Table). Because of the low results, it was not considered important to test them once they were separated. The RBA results for the C5-OMe olefins were once again disappointing (< 0.05% of **E2**), indicating that this substituent at C5 is not tolerated in the binding site. The L17 analogs with a chlorine atom, **140** and **141**, had low to moderate affinities for ERα (3.35 ± 0.06% and 1.75 ± 0.09%) and for ERβ (6 ± 2% and 1.7 ± 0.3%) with disappointing β-selectivity (β/α ratio of ~1-2), as compared to L17 (β/α ratio of ~8). Compounds with at least one methyl substituent at C4 and C5, had low binding affinities as observed for the C8-C9 olefins **162** and **165**. By contrast, the C9-C11 4,5-dichloro analog **167** shows strong binding affinities for ERα and ERβ, 3±1% and 55±9% respectively, as well as high β-selectivity (β/α ratio of 18).

Table 1.2.9 Relative Binding Affinity (RBA) of A-CD estrogens with unsaturation in the C-ring.



A



B

Comp. (A,B)	**Ring A** (R2, R4, R5)	**RBA of A**[b]			**RBA of B**[c]		
		ERα	ERβ	β/α	ERα	ERβ	β/α
2	**E2**	100	100	1	100	100	1
171, 172[a]	H H H	0.39 ± 0.04	6.8	16	2.5 ± 0.2	4.3 ± 0.3	17
56, 57	H H CF_3	122 ± 11	174 ± 8	1.4	189 ± 1	213	1.1
80, 81	H H Cl	60 ± 8	118 ± 4	2.0	195 ± 25	327 ± 14	1.7
105, 106	H H OMe	0.0055 ± 0.0005	0.029 ± 0.002	4.8	0.006 ± 0.001	0.047 ± 0.002	6.7
140, 141	F F Cl	3.35 ± 0.06	6 ± 2	1.9	1.75 ± 0.09	1.7 ± 0.3	1.0
149, 150	F H F	0.44 ± 0.02	2.7 ± 0.5	5.9	0.48 ± 0.04	3.3 ± 0.6	8.7
161, 162	H Me Me	-	-	-	0.0645 ± 0.0005	0.18 ± 0.05	2.8
164, 165	H F Me	-	-	-	1.75	1.75	1.0
167, 168	H Cl Cl	3 ± 1	55 ± 9	18	0.39 ± 0.06	2.6 ± 0.5	6.6
169, 170	H F Cl	50 ± 6	149	3.0	28.7 ± 6.3	72.3 ± 1.7	2.5

a- This compound was prepared by Dr. Asim Muhammad.

b- Relative Binding Affinity assay of olefins **A** (C9-C11) prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.

c- Relative Binding Affinity assay of olefins **B** (C8-C9) prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.

Compound **150** with ERα= 0.48±0.04 and ERβ= 3.3±0.6 (β/α = 8.7) is worthy of additional comment. This compound was sent with many others to Prof. Kendall Nettles' group at at the Scripps Research Institute, Florida, and was co-crystallized with the human ERα (**Figure 1.2.33**). Although the crystal structure is as yet relatively unrefined it nevertheless shows that the dihedral angle of the A-ring vs that of ring C is almost perpendicular. This is consistent with the other conclusions we have made regarding the preferred conformation of the cis CD fused A-CD

steroids. It differs substantially from our earlier published work where docking studies suggested the two planes in question had a dihedral angle of less than 30 degrees [32]. See **Section 1.2.9** for more details about docking studies performed by our collaborator Dr. Wright.

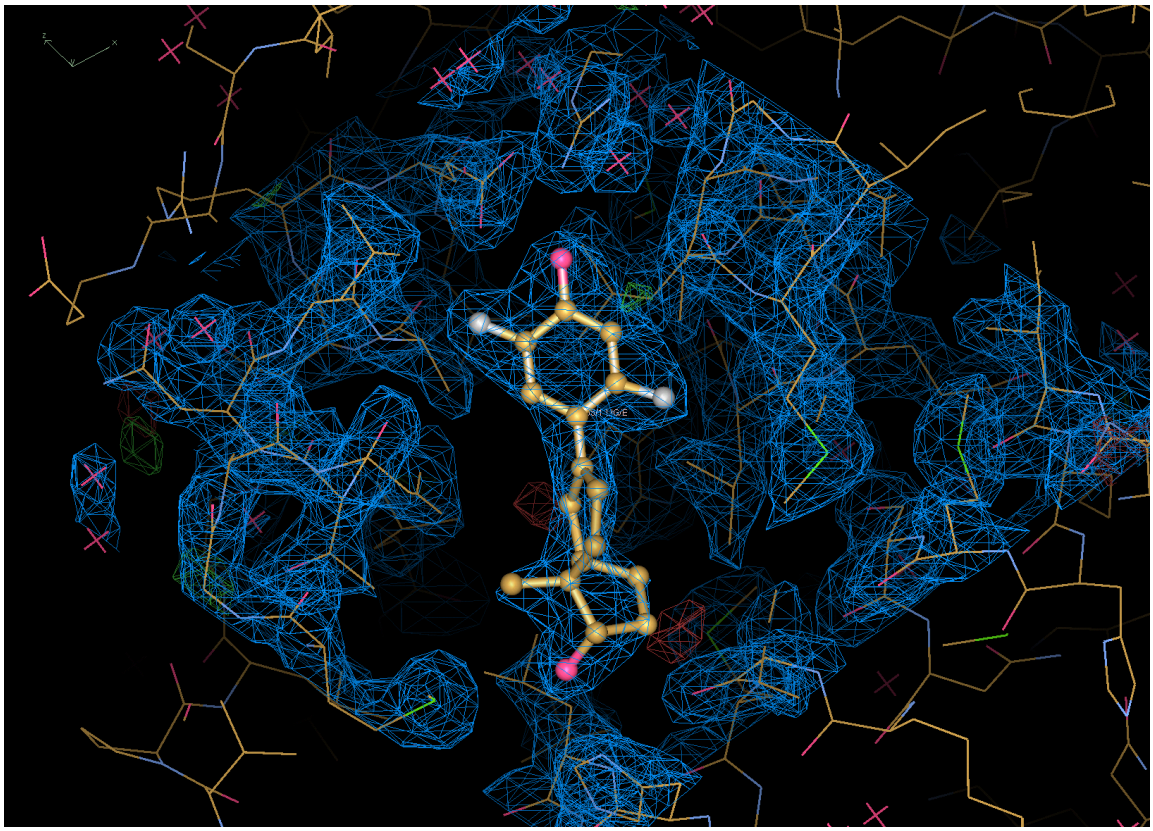


Figure 1.2.33 X-ray of the 2,5-diF ligand **150** inside the human ERα performed by Dr. Kendall W. Nettles at the Scripps Research Institute, Florida.

Unsaturation in the C-ring can be a desirable feature for an enhancement of estrogen receptor binding affinities. Unfortunately, as mentioned above, the increases in binding affinities were accompanied by large decreases in β/α receptor affinities and predicted low β/α translational selectivity. Based on additional evaluations of the C5-CF_3 derivatives, such compounds are expected to act in a similar way as estradiol. Currently, we see no potential applications for these compounds in either the HRT or anti-cancer areas.

1.2.7 Attempted synthesis of substituents at C5 bearing π-electron withdrawing groups

As mentioned earlier, one of the goals of this thesis was to prepare A-CD analogs which have EWGs positioned *meta* to the phenolic group since they may discourage the formation of *o*-quinones. The preparation of EWGs at the C5 position such as halide (fluoro, chloro) and trifluoromethyl groups was described earlier. Early in this project we became aware that these substituents at C5 increased binding to both estrogens receptors relative to the parent compound. The question arose of whether π-electron withdrawing substituents at this position at this position have a similar effect. The initial target compounds were the aldehyde **173**, carboxylic acid **174** and methyl ester **175**.

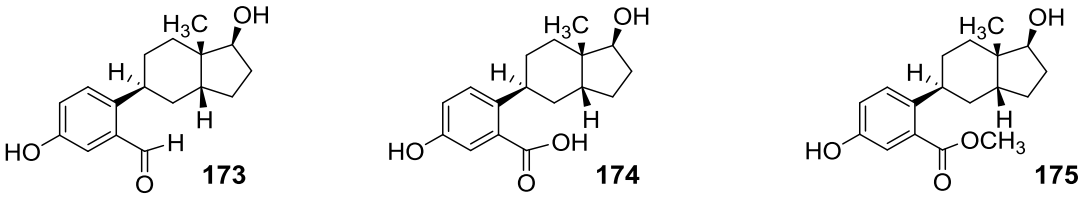
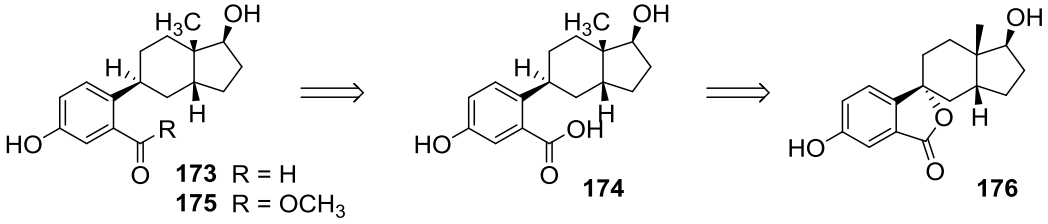


Figure 1.2.34 Structures of A-CD estrogens with p-electron withdrawing substituents at C5.

On paper the preparation of these three compounds appeared to be quite simple since many schemes could be readily proposed. In our hands, these very obvious routes failed for various reasons or, when partly successful, resulted in the formation of the interesting spiro lactone **176**. This compound was recognized as a potential intermediate to the acid **174** via hydrogenolysis of its benzylic ester function. The conversion of **174** to **173** and **175** would then be routine.



Scheme 1.2.39 Proposed retrosynthesis of A-CD estrogens with π-electron withdrawing substituents at C5, **173-175**, from the spiro lactone, **176**.

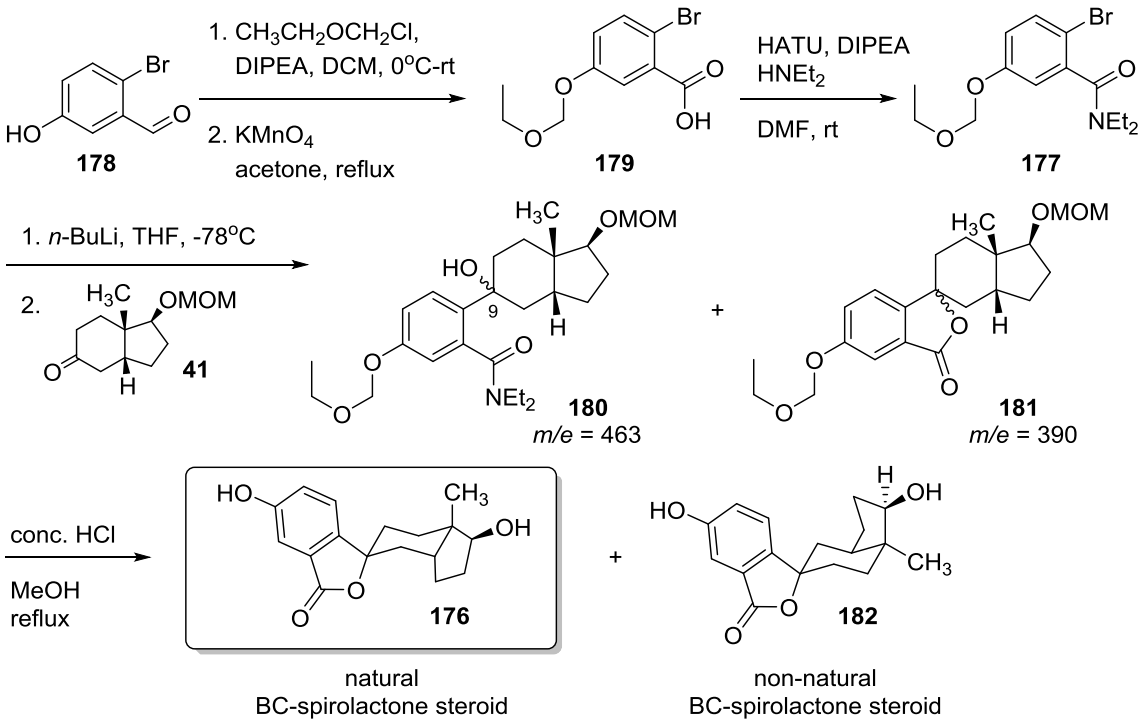
Synthesis of the spiro lactone 174

The most successful synthesis of the spiro lactone **176** involved lithium bromine exchange of the bromo diethylamide **177** and coupling of this compound with the usual protected CD-ring moiety, ketone **41**.[1] The amide was prepared in three steps from the commercially available 5-bromo-3-hydroxy-benzaldehyde **178**. Conversion of aldehyde **178** to acid **179** was achieved by the protection the phenol with $CH_3CH_2OCH_2Cl$, followed by oxidation of the aldehyde group using $KMnO_4$ in acetone at reflux in 89% yield over two steps. Activation the protected bromo acid **179** using HATU ((*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoro-phosphate) reagent in the presence of DIPEA and coupling it with diethylamine afforded amide **177** in 38% yield [147]. The halogen-metal exchange with *n*-BuLi in THF at -78^{o}C followed by the addition of **41** gave the expected coupling product **180** (*m/e* = 463) accompanied by **181** (*m/e*=390), as monitored by LC-MS, in 30% yield. The ^{1}H NMR spectrum of the mixture indicated the presence of a complex mixture of coupling products by the presence of the C17-H protons at 3.69, 3.75, 4.00, 4.20 ppm and the C14-quaternary methyl groups appearing at 0.88, 0.99, 1.13 and 1.14 ppm.

Treatment with strong acid converted **180/181** into a mixture of two spiro lactones. Compound **176**, which has the natural stereochemistry at C9, and its isomer **182** were obtained in 90% yield. The structural assignments of both structures were compared with the spiroethers prepared by Daria Klonowska in her MSc Thesis [148]. The ^{1}H NMR spectrum of **176** indicated the presence of the C17-H as a doublet of doublets at 3.67 ppm and the quaternary methyl group as a singlet at 1.18 ppm, while they appeared as a triplet at 4.44 ppm and a singlet at 0.97 ppm for

[1] This work was done in AstraZeneca R&D Montréal, where I had the opportunity to do an internship during the fall of 2010, and it was conducted under the co-supervision and helpful discussions of Dr. Vijayarathnam Santakumar.

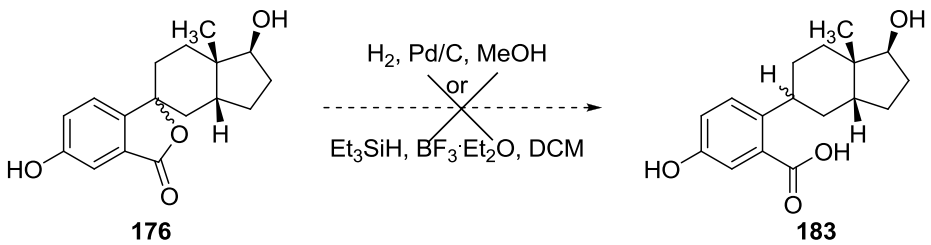
182, respectively. The ^{13}C NMR provided evidence of the spirolactone **176** showing peaks at 170.7, 87.5 and 79.9, belonging to the ester group attached to C5, and the carbon-oxygen bonds of C9 and C17. The HREIMS for **176** ($C_{17}H_{20}O_4$) was as expected (calculated = 288.1362, found = 288.1354).



Scheme 1.2.40 Preparation of spiro lactones **176** and **182**.

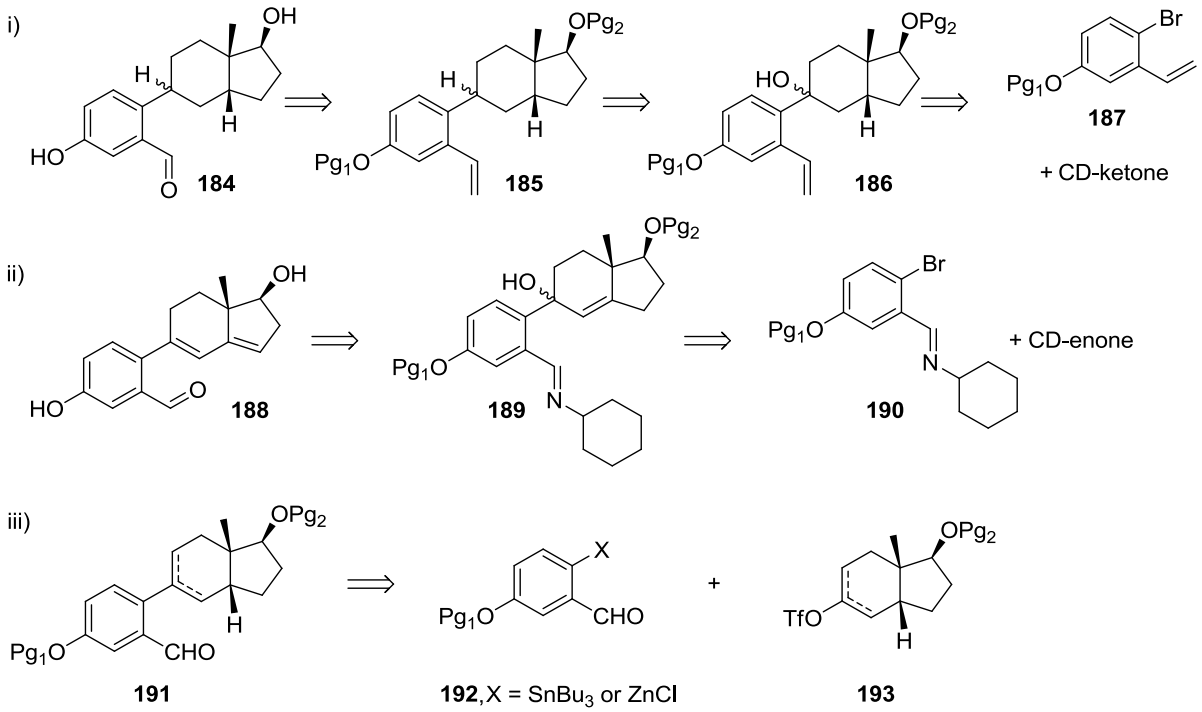
Attempted synthesis of the C5-CHO derivatives

Reduction of the spirolactone **176** was attempted using H_2 on Pd/C in MeOH and via a $BF_3{\cdot}Et_2O$ catalyzed reduction with triethylsilane (**Scheme 1.2.41**). However, after several trials no evidence of the expected acid **181** was observed.



Scheme 1.2.41 Attempted opening of the spirolactone **176** to obtain acid **183**.

Other approaches that we considered are shown below in **Scheme 1.2.42**. These include approach **i**: preparing the C5-vinyl derivative **185** and revealing the aldehyde **184** function via ozonolysis. This sequence resulted in the dehydration of the C9-OH **186** instead of the desired substitution product **185**. Approach **ii** involved the protection of the aldehyde as its cyclohexyl imine **190** and coupling with the CD-ring enone rather than the saturated ketone. The hypothesis was that dehydration of **189** to the diene **188** might take precedence over lactonization, and that the resultant diene **188** could then be hydrogenated to the desired aldehyde. However, the ^{1}H NMR spectrum indicated that the lactonization preceded the dehydration of the C9-OH, and therefore this sequence was abandoned. Approach **iii** was a sequence involving the Stille or Negishi coupling of a suitable A-ring **192** and CD-ring triflate **193**. After several trials, we did not observe the desired coupled product **191** using either cross-coupling sequence. In the end, preliminary investigations of each of these methods did not seem promising and the goal of preparing A-CD estrogens with π-electron withdrawing groups was not realized.

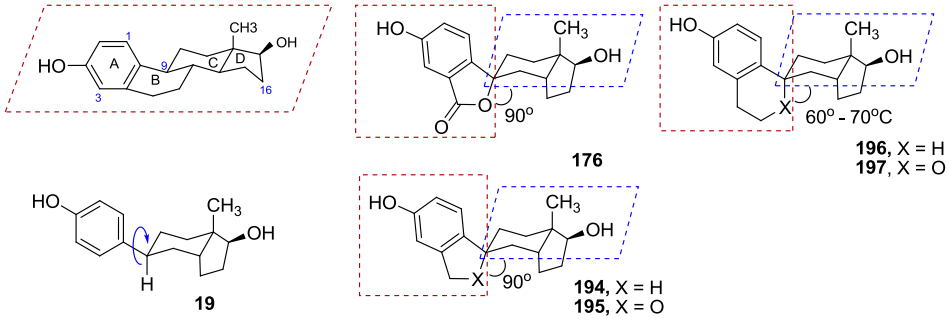


Scheme 1.2.42 Attempted approaches to prepare C5-CHO A-CD estrogens.

Bioassays of the spiro lactone 174

Because of its interesting shape and because this compound represented the only example of estrogen analogs bearing a π-electron withdrawing substituent at C5 in our library, the spiro lactone **176** was sent for the RBA assay. The results are shown in **Table 1.2.10**, along with other BC-spiro compounds prepared by previous members from the Durst group [148], [149]. The planes of the A-ring in these compounds are constrained to be either perpendicular or 60°-70° with respect to that of ring C. In contrast the A-CD estrogen compounds can adopt an infinite number of conformations as a result of rotation around the single bond joining rings A and C to give the strongest possible interaction with the estrogen receptors. By comparison, the native ligand estradiol is essentially flat. The combination of these results allowed us to examine how changes in the shape of ligands closely related to estradiol may affect their RBA results for ERα and ERβ.

Table 1.2.10 Relative Binding Affinity (RBA) of BC-spiro estrogens.



Comp.	A to C dihedral angle	RBA[a]		
		ERα	ERβ	β/α
2 (E2)	~10°	100	100	1
19[b]	Variable	1.5	21	14.0
176	90°	2.0 ± 0.4	6.1 ± 0.8	3.1
194[b]	90°	6 ± 2	41 ± 10	6.3
195[b]	90°	2.5 ± 0.1	9 ± 2	3.7
196[b]	~60-70°	4 ± 1	40 ± 1	10.7
197[b]	~60-70°	0.310 ± 0.002	3.5 ± 0.9	11.2

a- Relative Binding Affinity assay prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.

b- These compounds were prepared by Daria Klonowska or Dr. Asim Muhammad.

The spiro lactone **176** with a fixed dihedral angle of 90^{o} shows similar binding affinity as the parent compound **19** (with a flexible angle) to ERα (2.0±0.4% vs 1.5%) and lower binding affinity to the ERβ (6.1±0.8% vs 21%), with decreased ERβ vs. ERα selectivity. Other BC-spiro compounds with dihedral angles of 90^{o} or 60^{o}-70^{o} (**194** and **196**) have greater binding affinity to the ERs (RBAα = 6±2% and 4±1% and RBAβ = 41±10% and 40±1%) than the parent compound **19** as well as high ERβ selectivities of 6.3 and 10.7, respectively. These studies show that the estrogen receptors can accommodate ligands with distinctly different shapes than the endogenous hormone. These results are consistent with the ability of ERs to bind with ligands of various structural shapes and support the idea of the plasticity of the binding pocket [150], [151].

1.2.8 Synthesis and bioassays of the *trans* A-CD analogs

The crystal structures of the trifluoro- A-CD analogs **48** and **58** showed unambiguously that these A-CD estrogens had the *cis* CD-ring junction. At this point it became necessary to prepare examples of A-CD estrogens having the *trans* CD-junction. In these compounds all stereo-centers are potentially identical to those of the natural endogenous hormone. At this point, knowing the binding affinities for the *cis* A-CD compounds, we predicted that the *trans* A-CD compounds should have a greater affinity for estrogen receptors but would likely be less selective to the β vs α-receptors. In the end, the unintended synthesis of *cis* analogs was quite fortuitous because they bind with higher affinity and selectivity than the *trans* compounds. The first series of *trans* A-CD estrogens shown in **Figure 1.2.35** were prepared by Dr. C. Dabrota, Dr. M. Asim, Ana Gargaun and Ammara Butt [152].

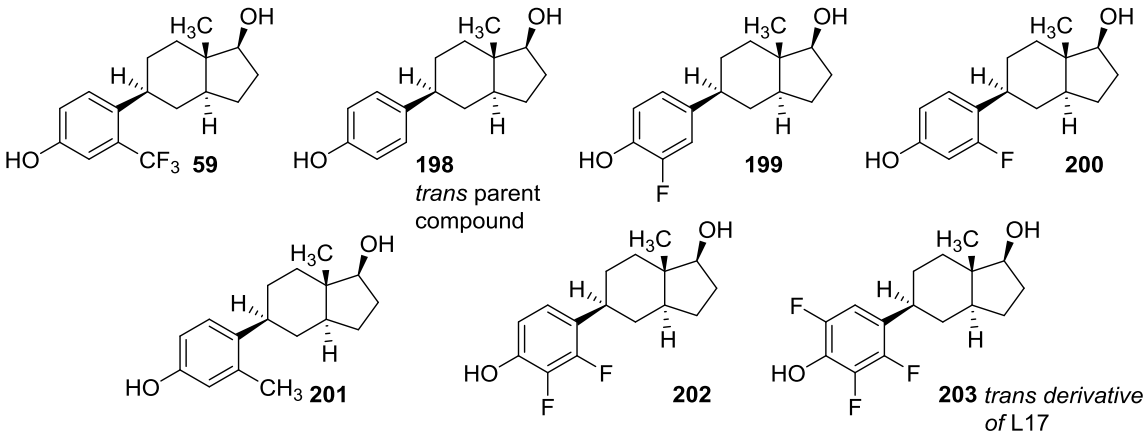


Figure 1.2.35 Series of *trans* A-CD estrogens prepared by other members from the Durst group.

Synthesis of the Trans A-CD analogs

The focus of this work was to prepare several CD *trans*-fused isomers of compounds of significant interest in the CD *cis*-fused series. This included compound **204**, the isomers of **TD81** and **207**, and the *trans* version of an analog of **L17**.

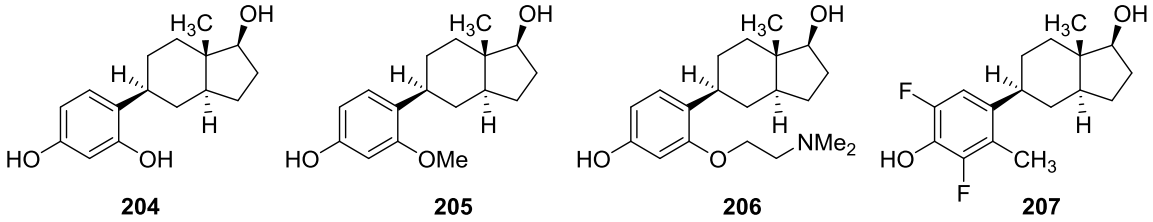
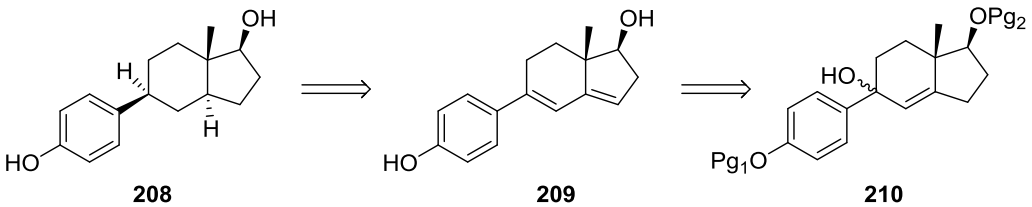


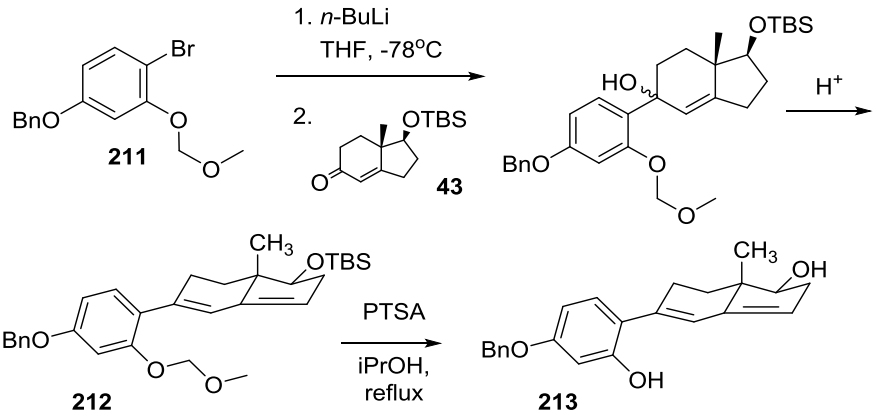
Figure 1.2.36 Structure of target *trans* A-CD estrogens.

Our approach to these *trans* analogs took advantage of the observation by Ana Gargaun [152] that the hydrogenation of the diene **209**, obtained from the dehydration of **210** led to the isomer **208** having the 9(*S*) configuration as major product.



Scheme 1.2.43 Approach to prepare the *trans* A-CD estrogens.

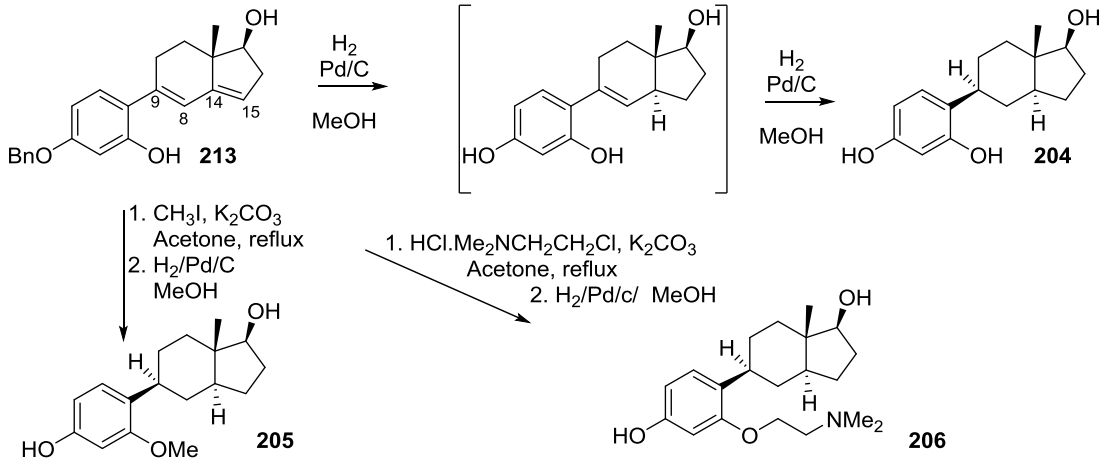
The synthesis of the necessary diene intermediates begins with the coupling of the resorcinol derivative **211** and TBS-protected CD-enone **43**. The initially-formed tertiary alcohol is readily dehydrated either on workup or by the acidic environment of silica gel to give the conjugated double bond system **212** in 40% yield. The ^{1}H NMR of **212** showed the presence of two allylic protons at 5.30 ppm and 6.25 ppm. The protecting groups of **212** were eliminated under the same acidic conditions used in the **TD81** series, affording **213** in 46% yield after flash column chromatography.



Scheme 1.2.44 Formation of diene **213**.

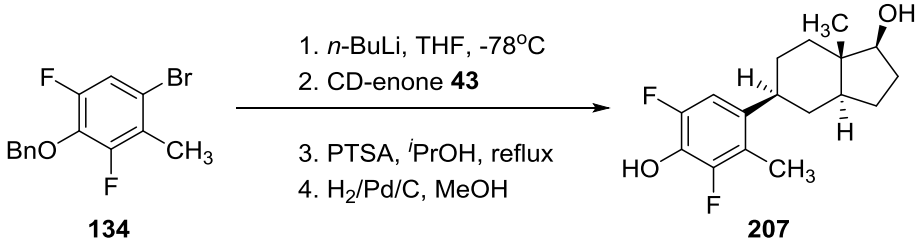
Although four stereoisomers could arise from the hydrogenation of diene **213**, the desired product **204** with the (*S*) configuration was obtained as a major product in 48% yield as anticipated based on the Gargaun example [152]. This is in contrast with the hydrogenation of the *cis* CD-junctioned alkene isomers **100a/100b** which gave a close to a 1:1 mixture of stereoisomers at C9. Alkylation of the free phenolic C5-OH in **213** followed by subsequent hydrogenation led predominantly to the final compounds **205** and **206** as the major stereoisomers. It seems reasonable to suggest that hydrogenation of these dienes occurs first at the C14-C15 double bond and preferentially from the side opposite the methyl group to install

the *trans* CD-ring junction. The second hydrogenation would also occur preferentially from the same side.



Scheme 1.2.45 Synthesis of the *trans* A-CD analogs **204** to **206**.

The preparation of the **L17** analog **207** is shown in **Scheme** 1.**2.46**. The NMR characteristics **207** vs its *trans* isomer showed the same variations as for the above compounds.



Scheme 1.2.46 Preparation of the *trans* A-CD estrogen **207**.

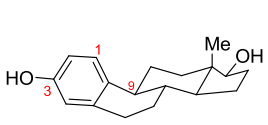
The ^{1}H and ^{13}C NMR spectra showed significant differences between these *trans* A-CD estrogens compared to the *cis* analogs. In the ^{1}H NMR spectrum, the C13 methyl group of the *trans* A-CD estrogens absorbed at 0.8 ppm compared with 1.1 ppm for the CD *cis*-fused derivatives. In the ^{13}C NMR spectrum, the C13 methyl carbon appeared at 12.0 for the *trans* compared to 20.2 ppm for the *cis* isomers. Additionally, the signal for C9 was consistently at upfield in the *cis* analogs (32.1 ppm) compared to the *trans* (~39 ppm) derivatives.

Bioassays of the Trans A-CD analogs

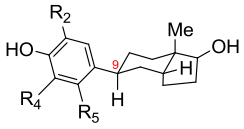
Table 1.2.11 shows the RBA results for the compounds prepared in this series, including the results from compounds prepared by other members from the Durst group. Our expectations were that the compounds with the *trans* CD-ring junction would have greater binding affinities for both ER subtypes but lower β-selectivity than the corresponding CD *cis* fused isomers because of their resemblance to estradiol was not realized with respect the bonding affinities but was correct concerning respect to β/α selectivity. In all cases, the binding affinity to the ERβ and the ERβ-selectivity was higher for the *cis* analogs vs *trans*. For example, the RBAβ results for C5-CF_3, C5-F and C5-CH_3 is 205±23%, 136±7%, 34±6% in the *cis* series, compared to 4.9±0.1%, 13.6±0.4% and 1.3±0.3% in the *trans* series. In the *cis* series, the average β/α ratio was about 9, while in the *trans* series, it was about 4.

The binding assays for the CD *trans* analogs of the compounds of highest interest, that are the **L17** and **TD81** were particularly disappointing with RBAα being = 0.03%, 0±0.03%, 0.086±0.007% and RBAβ = 0.10±0.03%, 0.2±0.4%, 0.23±0.04%, respectively for **203**, **204** and **207**. The corresponding numbers for the CD *cis*-fused isomers are 0.19±0.01, 0.26±0.07 and 0.7±0.2 for RBAα and 1.72±0.02, 4.0±0.8 and 5.3±0.9 for RBAβ. The difference is typically a factor of 3 to 5-fold in favor of the CD *cis* fused isomers with respect to RBAα and 17 to 25-fold for RBAβ illustrating the greater β vs α selectivity seen for the *cis* isomers.

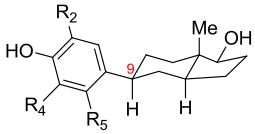
Table 1.2.11 Comparison of the RBA for the *cis* and *trans* A-CD estrogens.



E2



cis A-CD estrogens



trans A-CD estrogens

Comp. (*cis*, *trans*)	**Ring A** (R_2 R_4 R_5)	**RBA of *cis* A-CD[b]**			**RBA of *trans* A-CD[b]**		
		ERα	ERβ	β/α	ERα	ERβ	β/α
2	**E2**	100	100	1	100	100	1
19, 198[a]	H H H	1.5 ± 0.3	22 ± 5	15	2.4 ± 0.2	10 ± 1	4.2
48, 59[a]	H H CF_3	90 ± 14	205 ± 23	2.1	5 ± 1	4.9 ± 0.1	1.0
27, 199[a]	H F H	1.0 ± 0.1	8.7 ± 0.4	8.7	1.6 ± 0.2	6.8 ± 0.4	6.6
30, 200[a]	H H F	27.3 ± 0.7	136 ± 7	5.0	4.22 ± 0.06	13.6 ± 0.4	3.2
82, 201[a]	H H Me	2.8 ± 0.5	34 ± 6	12	0.5 ± 0.1	1.3 ± 0.3	2.9
31, 202[a]	H F F	4.6 ± 0.9	43 ± 6	9.2	0.9 ± 0.2	7 ± 2	7.8
32, 203[a]	F F F	0.19 ± 0.01	1.72 ± 0.02	9.2	0.03	0.10 ± 0.03	3.2
84, 204	H H OH	0.26 ± 0.07	4.0 ± 0.8	15	0 ± 0.3	0.2 ± 0.4	4.0
83, 205	H H OMe	0.016 ± 0.002	0.12 ± 0.03	7.7	0.010 ± 0.001	0.037 ± 0.008	3.7
110, 206	H H OR[c]	0.009 ± 0.001	0.045 ± 0.006	5.0	-	-	
135, 207	F F CH_3	0.7 ± 0.2	5.3 ± 0.9	7.1	0.086 ± 0.007	0.23 ± 0.04	2.6

a- These compounds were prepared by Dr. Asim Muhammad, Dr. Christian Dobrata or by Amara Butt.
b- Relative Binding Affinity assay prepared by the group of Dr. J. Katzenellenbogen at the University of Illinois.
c- R = $OCH_2CH_2NMe_2$

Conclusion

It was a misreading of the literature that led us to synthesize first the *cis* fused isomer. This "fortunate" error has led to the discovery of compounds that have significant potential in areas of medicine involving estrogen. Had we first prepared the *trans* fused isomers, it is highly doubtful that we would have carried out the analysis and made the decision to prepare the A-CD estrogens with the *cis* CD-ring fusion.

1.2.9 Conformational analysis and further biological evaluation of the A-CD estrogens

Computational studies

The docking studies on the A-CD estrogens were performed by the group of Dr. James Wright using the X-ray crystal structure of the protein found in the RCSB Protein Database (PDB entries 1GWR for ERα and 2J7X for ERβ) and using the H_PDB-Thaw program for energy minimization of the complex. The uDock 2.5 modeling software was used to determine the integration and energy minimization of a random conformation of each analog. The IF-E 6.0 software was used to evaluate the binding affinities between the ligand and important residues for binding in the active site [32]. As seen in **Figure 1.2.37**, the phenolic hydroxy group in the A-ring of our parent compound **19** forms a hydrogen bond triad with Glu353, Arg394 and water molecule. A second hydrogen bond is formed between the 17β-OH group and a histidine residue (His524). As mentioned in the Introduction, the two amino acid differences between the receptor active sites are that ERβ contains a Met336, which replaces Leu384 in ERα, and Ile373 in ERβ replaces Met421 in ERα. Steric interactions observed between the D-ring of an A-CD ligand and Met421 residue in ERα also advocated the ERβ selectivity observed in our A-CD estrogens.

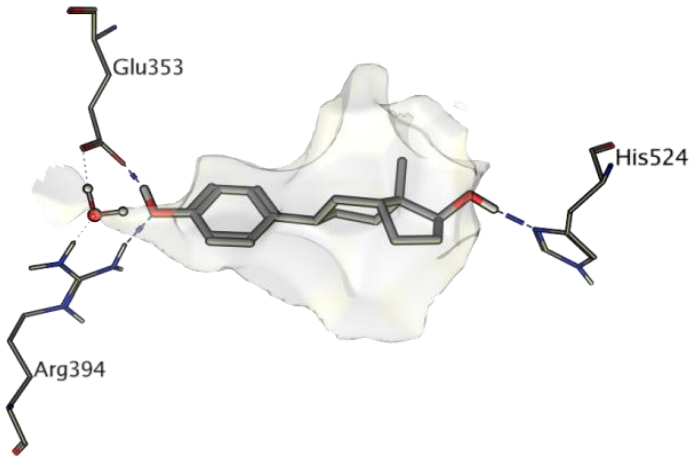


Figure 1.2.37 Computational model of our parent compound 19 inside the ER performed by Dr. J. Wright and colleagues showing the key interactions with the main pharmacophores.

When this project was initiated about six years ago, docking studies were carried out on compounds having the *trans* CD-ring fusion. These focused mainly on the interactions of the two hydroxy groups of the A-CD compounds with the estrogen receptors. Initial docking studies conducted by the Wright group indicated that these ligands adopt a conformation in which the dihedral angle between the A- and CD-rings is close to 30^o inside the ER, while in estradiol these rings are nearly coplanar, despite the fact that lowest energy conformation of the free ligands was one in which the plane of ring A was essentially perpendicular to that of ring C [32]. Subsequent experimental work led us to question the conclusions from the published docking studies. One important line of evidence came from the determination of the binding affinities of the spiro estrogens **194** and **196** in which the A-ring to C-ring dihedral angles are fixed at 90^o and about 70^o respectively. The spiro estrogen **194** (90^o dihedral angle) had binding values of 6.4 and 40.5 for ERα and ERβ, respectively (relative to estradiol's binding value of 100 to both receptors), while **196** with a dihedral angle of 70^o had a RBAα of 3.7 and RBAβ of 39.7. These data show that the spiro compounds bind comparably, indeed somewhat more strongly, than the parent compound A-CD compound **19** to both estrogen receptors, thereby showing clearly that the estrogen receptor can accommodate almost equally very similar molecules with completely different shapes ranging from the almost planar natural ligand estradiol to the spiro derivative **194** in which the ring A and ring C planes are at 90^o to each other. It thus seems quite reasonable that the A-CD estrogens should not be required to twist to a high energy, almost planar, conformation in order to bind strongly to the estrogen receptors.

In addition, two crystal structures obtained for the A-CD estrogen-ER bound ligands indicated a dihedral of close to 90^o for the ring A to ring C dihedral angle (See **Figures 1.2.29**). Very recently, Dr. Wright was able to model the ER-ligand interactions with greater confidence. The

new model now indicates that the dihedral angle between rings A and C ranges from 65° to 80° for most A-CD estrogens [135]. These improved parameters should allow the generation of additional compounds with more predictable and potentially stronger binding to the ERs.

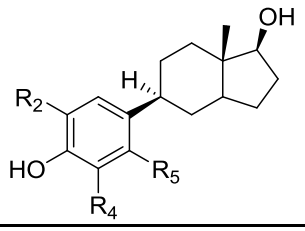
Cytotoxicity in rat liver cells

As mentioned earlier, one of the main causes of estrogen carcinogenesis involves quinones formation that can lead to mutations during cell replication. Our attempts at the prevention or reduction of *o*-quinone formation were based on three assumptions: 1) the presence of two ortho fluorine atoms of the phenolic OH group reduces the rate of the enzymatic ortho-hydroxylation; 2) the presence of one or more EWGs in the aromatic ring increases the BDE of the phenolic OH thereby hindering the formation of quinones; and 3) compound **84** [**TD81**] would not form toxic metabolites.

Cytotoxicity in rat hepatocytes was judged to be an indication of *o*-quinone formation. To this end the toxicity of several A-CD-estrogens relative to **E2** were determined (**Table 1.2.12**) [32]. In this assay, the median lethal concentration (LC_{50}) of **E2** was in the range 400−450 μM. The presence of quinone formation for **E2** was detected by adding dicoumerol, a quinone reductase inhibitor, and an increase of **E2** cytotoxicity was observed. The parent 9(*S*) isomer and its 9(*R*) epimer showed an enhanced toxicity compared to **E2** with an LC_{50} of ~400 μM to ~250 μM. As predicted, the trifluoro derivative **32 [L17]** showed no toxicity with an LC_{50} above the range of the assay (>600 μM). Compounds **30** and **31**, with one and two fluorine atoms, respectively, had a higher toxicity than **E2** which is in contradiction with our initial expectation that EWGs would lower the BDE, and thus make the compounds less likely to form quinones. It should be pointed out that the increased toxicity of the compounds carrying one or two fluorine atoms in the A-ring

might be due to an increased reactivity and hence possible increased toxicity of the quinones formed from these compounds. Such quinones would be more electrophilic due to the electronegativity of the fluorine substituents on the ring.

Table 1.2.12 Hepatocyte toxicity showing LC_{50} for 2h of exposure.



Comp.	A-ring (R_2, R_4, R_5)	Hepatic cell LC_{50} (μM)[b]
Estradiol (E2)		400-450
19 (9*S*)	H H H	320-400
26 (9*R*)[a]	H H H	350
30	H H F	250-280
31	H F F	155-200
32 [L17]	F F F	>600
84 [TD81]	H H OH	>600

a- "Non-natural" isomer with the inverted stereochemistry at C9
b- Hepatic toxicity assay performed by Dr. Peter J. O'Brien and colleagues from the University of Toronto

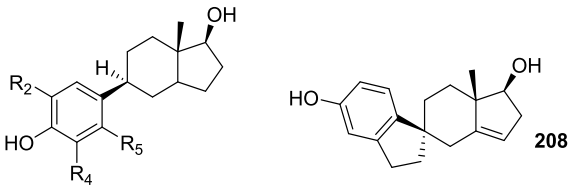
.

These data show that blocking both ortho positions in the phenolic group reduces toxicity, likely by reducing ortho quinone formation. In contrast, compounds with fluorine substituents (EWG) in the ortho position on the ring have greater toxicity than **E2**. Finally, we were pleased to observe that compound **84 [TD81]** showed no toxicity in this assay with an LC_{50} of over 600μM. This confirms our prediction that **TD81** would not form toxic quinones (see **Scheme 1.2.18**). Indeed, as is discussed below, **TD81** is metabolically very much more stable than **E2** or any of the A-CD compounds tested indicating that aromatic hydroxylation of this compound is very slow and that ortho quinone formation is unlikely. Based on a combination of RBA, RTA and toxicity studies, **L17** and **TD81** and their analogs described in the earlier sections of this chapter are being further investigated for both their potential in HRT and breast cancer applications.

Metabolic stability

The Center for Drug Research and Development (CDRD) in Vancouver, BC is committed to contribute to this project to the point where a pharmaceutical company would take interest and develop the compounds as potential HRT or as anti-cancer treatments. As part of their contribution, the Center is working on the development of analytical methods to measure the formation of *o*-quinones when these compounds are exposed to liver cell metabolism. The metabolic stability in human liver microsomes of estradiol **2**, our parent A-CD analogue **19**, compounds **32** and **84**, **L17** and **TD81**, and a BC-spiro-estrogen **208** were evaluated **(Table 1.2.13)** [153].

Table 1.2.13 Metabolic stability in human liver microsomes of selected A-CD estrogens.



Comp.	A-ring (R2, R4, R5)	Estimated half-lives (min)[a]
Estradiol (E2)		14.3 or 15.5
19 (9*S*)	H H H	28
32 [L17]	F F F	43
84 [TD81]	H H OH	378
208	-	28

a- The half-lives were determined Dr. Murray Webb and colleagues in the CDRD at the Univeristy of British Colombia.

In this assay, we found that **E2** had an estimated half-life of about 15 minutes, while the half-lives of compounds **19** and **208** were 28 minutes, compound **32 [L17]** was 43 minutes and compound **84 [TD81]** was 378 minutes (over 6h!). These results indicate that L17 is more stable than estradiol and therefore less prone to metabolism, while **TD81** is scarcely metabolized in

human liver microsomes, further supporting the idea that the latter compound would not form genotoxic species.

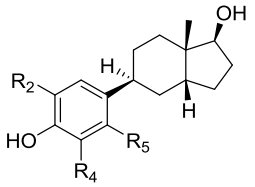
A second assay on metabolic stability of more than 20 A-CD ligands in mouse liver microsomes was performed at the Ontario Institute of Cancer Research (OICR) by Drs. Ahmed Aman and Barry Press in Toronto, ON [154]. Several of the analogs described in this thesis were included in this assay, where each compound's metabolic stability was classified based on the percent of material remaining after 30 minutes of incubation at 37^{o}C. They found that all compounds tested in this study had high levels of glucuronide conjugated and that most compounds had low percent remaining (less than 30%), with the exception of L17 (42%) and its alkene derivatives (77%). Data from this assay indicated that **L17** appears to reduce the Phase I (oxidation) metabolism within this chemical series.

Potential HRT or breast cancer treatments

The combination of low toxicity and selective β-RTA values obtained for TD81 and L17 led the group of Dr. Christine Pratt to assess the toxicity of these compounds via the hormonal pathway, where it was demonstrated that exposure of these Selective Estrogen Receptor Modulators (SERMs) reduced the proliferation of breast cancer cells MCF-7 [152]. Recently our interdisciplinary group (Pratt-Durst-CDRD) has received the proof of principle grant from the *Canadian Institutes of Health Research (*CIHR) to evaluate the application of these compounds for their potential utility in HRT. Compound **32 [L17]** is currently being investigated by the CDRD as a potential candidate for Phase 1 Clinical Trials and compound **84 [TD81]** has been patented for its anticancer properties.

To determine if these A-CD estrogens would be effective for HRT, hot flash control and uterine cell growth assays were performed by the group of Dr. Pratt (**Table 1.2.14**). The qualitative results are shown and are related to their RTA data. The potential for control of hot flashes was determined by the tail skin temperature (TST) test, which is an assay used to monitor temperature changes in ovariectomized rodents (to result in a drop of estrogen level stimulating menopause symptoms such as hot flashes) [155]. Compared to the solvent vehicle, the injection of estradiol decreased the temperature of the tail skin during the active phase of the rats at night. So far, we have obtained the results of these assays for estradiol **E2**, compounds **32 [L17]** and **84 [TD81]**.

Table 1.2.14 Hot flashes control, uterine cell growth and RTA of some estrogenic ligands.



Comp.	A-ring (R2, R4, R5)	Hot flash control[a]	Uterine cell growth[a]	RTA[a]		
				ERα	ERβ	β/α
Estradiol (E2)		Strong	Strong	100	100	1
30	H H F	Strong (Prediction)	Moderate (Prediction)	44.3	158	3.6
32 [L17]	F F F	Strong	Very little	13	95	8
84 [TD81]	H H OH	Slight	No	3	85	28

a-The hot flash control, uterine cell growth and RTA were performed by Dr. C. Pratt and colleagues at the University of Ottawa.

In the case of estradiol which has a β-selectivity of 1, there is strong control of hot flash and a large increase in uterine cell growth. **L17** strongly controlled hot flashes and produced a small amount of uterine cell growth. **TD81** only showed slight hot flash control, but no proliferation of

uterine cells was observed. These results suggest that **L17** and **TD81** would not be prone to the hormonal carcinogenesis pathway. They are consistent with the low transcription activity for the ERα (3%) which is responsible for the estrogenic effects, and high β-selectivity of 28 which contributes it to its anti-proliferative properties. As proof-of-principle, since compound **C5-F** (**30**) has a RTA β/α ratio of 3.6, which is midway between that of **E2** and **L17**, we predict that it will have a high effect on the hot flash control and a moderate increase of the uterine cell growth compared to estradiol.

1.3 General conclusions

Over 50 A-CD ligands which have either a *cis* or *trans* CD-ring junction and various substitution patterns in Ring A have been synthesized to be tested as HRT medications and characterized mainly by proton and carbon NMR. Additionally, these compounds have either a H or OH at C9 or double bonds at C8-C9 or C9-C11 in Ring C. One example of BC-spiro estrogen has also been described. In the case of the 5-CF_3 derivatives, additional structural information including X-ray structural determination of both the 9(*S*) and (*R*) isomers was obtained. The structure of the alkene isomers in this series was based on additional NMR evidence and mass spectral fragmentation patterns. The data obtained for the C5-CF_3 derivatives have allowed us to confidently assign structures in the other series.

The compounds described in this thesis were analysed based on RBA and RTA results, as preliminary stage of QSAR. The RBA results obtained had values from from 350% - 0.002% for both estrogen receptor subtypes with β-selectivity from 20 – 0.5x, while the RTA values ranged from 200% (strong agonists) down to slightly negative (possible antagonists). We noticed in these bioassays that as the binding affinity increased, the β-selectivity decreased. The *cis* CD-fused compounds generally bind more strongly and more selectively to ERβ than *trans* A-CD estrogen. Results obtained from the X-Ray structures inside the binding pocket and from the BC-spiro ligands indicated that the ERs can accommodate ligands with variable angles between the rings A and C (from 0^o to 90^o).

Analogs that showed improved activity and selectivity for ERβ were further investigated in **Section 1.2.9**. Findings from this section indicated that **L17** is a potential candidate for HRT since it controls hot flashes in the rat model and does not cause the proliferation of uterine and breast cancer cells. Ligand **TD81** has potential as anti-cancer agent against breast and uterine cancers. At the time of the submission of this thesis approximately ten of the compounds described herein have been submitted to the CDRD to test if they are cytostatic or cytotoxic towards MCF-7 cells.

1.4 Experimental Section

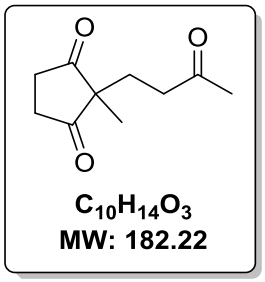
1.4.1 General Methods

All moisture-sensitive reactions were carried out under nitrogen atmosphere. Anhydrous solvents were obtained as follows: THF distilled from sodium and benzophenone and DCM distilled from calcium hydride. Reactions were monitored by TLC using aluminum-backed TLC plates (EMD Chemicals, TLC Silica gel 60 F_{254}), which were visualized by UV light (254 nm) then permanently stained with Hannessian's stain. Purification by flash chromatography was performed using SiliCycle SiliFlash ® F60 silica gel of 230-400 mesh and glass columns fitted with a cotton plug and a sand base or a fritted glass filter. Purification by preparative HPLC was performed using a reverse phase C18 preparative HPLC column (10µm particle size, 21.2 x 250 mm). The ^{1}H NMR and ^{13}C NMR were recorded on Bruker Avance 500, 400, and 300 spectrometers. Samples were dissolved in deuterated chloroform, methanol, or acetone as indicated. Chemical shifts are reported in parts per million (ppm) and are referenced relative to the deuterated solvent used. ^{1}H integration is provided in parentheses and coupling constants are reported in Hz. ^{1}H splitting patterns are reported as singlets (s), broad singlets (bs), doublets (d), triplets (t), quartets (q), multiplets (m), doublet of doublets (dd), triplet of triplets (tt) and doublet of doublet of doublets (ddd). NMR data was assigned based upon supplemental NMR experiments including COSY, DEPT, and HSQC, and by comparison with literature reports when available. Mass spectrometry (MS), using either electron impact (EI) or chemical ionization (CI), was performed on a V. G. Micromass 7070 HS mass spectrometer with an electron beam energy of 70 eV (for EI). High-resolution electron-ionization mass spectrometry (HREIMS) was performed on a Kratos Concept-11A mass spectrometer with an electron beam of 70 eV or a JEOL double focusing magnetic sector mass spectrometer JMS-AX505H. All spectral analyses were performed at facilities located at the University of Ottawa.

1.4.2 Preparation of the Hajos−Parrish Ketone

The CD-ring (Hajos-Parrish ketone) was prepared in enantiomerically pure form following published Hajos-Parrish ketone procedures [124], [125], [127], [156]. The ^{1}H NMR spectra of all intermediates of the Hajos-Parrish Ketone were consistent with the one obtained earlier in our group [32], [157].

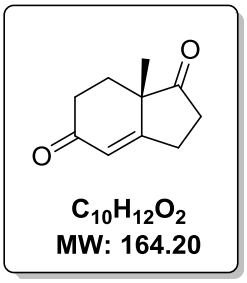
2-Methyl-2-(3-oxobutyl)cyclopentane-1,3-dione (35)



To a suspension of the 2-methylcyclopentane-1,3-dione **33** (100 g, 892 mmol) in distilled water (500 mL) was added at once methyl vinyl ketone **34** (125.05 g, 1784 mmol). The reaction was stirred for four days at room temperature, and then the reaction mixture was saturated with brine (100 mL). The reaction mixture was extracted with EtOAc (3 x 250 mL), the organic layers were combined and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50 % EtOAc in Hexanes) provided the triketone **35** as a yellowish oil (158.12 g, 97 %). The ^{1}H NMR of this compound was consistent with the one obtained earlier in our group [32], [157].

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

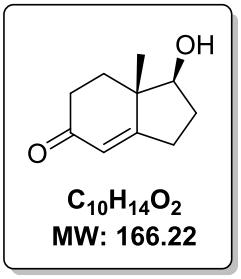
(+)-(7a*S*)-7,7a-Dihydro-7a-methyl-1,5(6*H*)-indandione (38)



To a suspension of (L)-proline (9.48 g, 85.56 mmol) in dimethylformamide (150 mL) was stirred at room temperature for 20 minutes. Triketone **35** (158 g, 877 mmol) was dissolved in dimethylformamide (500 mL) and added to a stirring solution of (L)-proline **36** and protected from light with aluminium foil. After three days the black reaction mixture was distilled excess dimethylformamide under reduced pressure. The reaction mixture was dehydrated without any purification. The crude reaction mixture was dissolved in toluene (150 mL), added p-toluenesulfonic acid (2.0 g), and equipped with Dean-stark apparatus. The reaction mixture was refluxed until the all water stopped coming. The cooled solution was washed with (saturated $NaHCO_3$, and brine solutions), the aqueous phases were extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and evaporated the solvent *in vacuo*, gave a brown oil. The residue was purified by flash chromatography (50 % EtOAc in hexanes), afforded CD-ring **38** as a light yellow oil (127.6 g, 90.8 %). The ^{1}H NMR of this compound was consistent with the one obtained earlier in our group [32], [157].

^{1}H NMR ($CDCl_3$, 400 MHz,) δ ppm 5.92 (d, *J* = 2.4 Hz, 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 Hz, 1H), 1.80 (ddd, *J* = 13.6, 13.6, 5.6 Hz, 1H), 1.27 (s, 3H)

Hajos-Parrish Enone: (+)-(1*S*,7a*S*)-7a-Methyl-7,7a-dihydro-1-hydroxy-5(6*H*)-indandione (39)

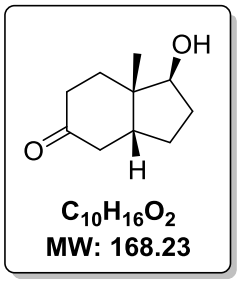


To a chilled (-78 °C) solution of enone **38** (88 g, 536 mmol) in a 1:1 mixture of DCM: MeOH (600 mL) was added $NaBH_4$ (7.1 g, 187 mmol) slowly. The reaction was stirred at -78^oC for 1h, quenched with acetone (60 mL) at -5^oC and stirred until the bubbling stopped. The solution was warmed to room temperature, the excess MeOH was evaporated *in vacuo* and the solution was extracted with DCM (3 x 200mL). The organic layers were combined and dried over $MgSO_4$ afforded the CD-enone **39** as a yellow oil (50g, 56%). The ^1H NMR of this compound was consistent with the one obtained earlier in our group [32], [157].

^1H NMR (400 MHz, $CDCl_3$) δ ppm 5.76 (s, 1H), 3.82 (dd, *J* = 10.4, 7.6 Hz, 1H), 2.70-2.61 (m, 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 (100 MHz, $CDCl_3$) δ ppm 199.4, 175.4, 123.3, 80.4, 45.1, 34.0, 33.2, 29.0, 26.4, 15.0

Hajos-Parrish Ketone: (+)-(1*S*,7a*S*)-7a-Methyl-hexahydro-1-hydroxy-5(6*H*)-indandione (40)

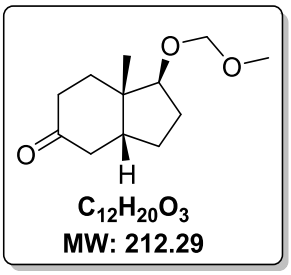


To a solution of CD enone **39** (5.02 g, 30.2 mmol) and Pd (10% on carbon) (4.0 g) in MeOH (400 mL) was added glacial acetic acid (160 mL) and 10% HCl (12 mL) and stirred under hydrogen atmosphere (used two big balloons of hydrogen gas) for one day. The resulting mixture was filtered through Celite pad and washed several times with EtOAc. The excess MeOH was evaporated *in vacuo*, brine was added and extracted with EtOAc. The organic layers were combined and dried over $MgSO_4$ gave as a colorless oil. The crude was purified by column chromatography eluting with 45 % EtOAc in hexanes afforded the saturated keto alcohol **40** as a white solid (3.9g, 77%). The ^{1}H NMR of this compound was consistent with the one obtained earlier in our group [32], [157].

^{1}H NMR (400 MHz, $CDCl_3$) 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 (100 MHz, $CDCl_3$) δ ppm 212.9, 79.9, 43.8, 43.2, 41.9, 36.8, 32.0, 32.0, 28.3, 19.3

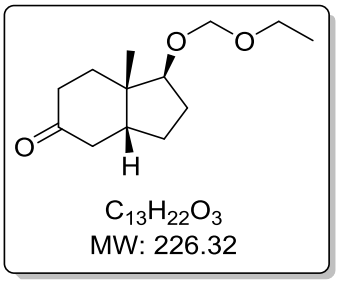
Protection of the Hajos−Parrish Ketone as its MOM ether (41)



To a chilled solution (0^oC) of the Hajos-Parrish Ketone **40** (2g, 11.89 mmol) in DCM (40 mL) under nitrogen was added diisopropylethylamine (DIPEA, 3.11 mL, 17.84 mmol) and chloromethyl methyl ether (1.35 mL, 17.84 mmol) dropwise. The solution was stirred for 30 min at 0^oC and was allowed to warm to room temperature and continued to stir for 3 days at room temperature. Brine (30 mL) was added to the solution and the solution was extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, concentrated *in vacuo* and subjected to column chromatography from 15% EtOAc:Hexanes to 40% EtOAc: Hexanes, providing the MOM-ether **41** as a white oil (1.86 g, 74%). The ^1H NMR of this compound was consistent with the one obtained earlier in our group [32], [157].

^1H NMR (300 MHz, $CDCl_3$) δ ppm 4.62 (d, *J* = 6.6 Hz, 1H), 4.56 (d, *J* = 6.6 Hz, 1H), 3.71 (t, *J* = 5.8, 5.8 Hz, 1H), 3.33 (s, 3H), 2.46 - 2.29 (m, 2H), 2.27 – 2.09 (m, 3H), 2.07 – 1.95 (m, 1H), 1.93-1.85 (m, 1H), 1.76 – 1.55 (m, 3H), 1.25 – 1.15 (m, 1H), 1.13 (s, 3H)

Protection of the Hajos−Parrish Ketone as its methyl ethyl ether (42)

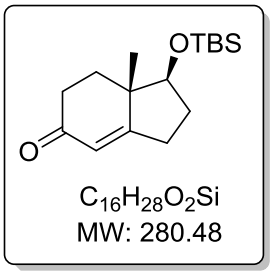


To a chilled solution (0^{o}C) of the Hajos-Parrish ketone **40** (1.6g, 9.52 mmol) in DCM (40 mL) under nitrogen was added diisopropylethylamine (DIPEA, 3.31 mL, 19.03 mmol) and chloromethyl methyl ether (1.77 mL, 19.03 mmol) dropwise. The solution was stirred for 30 min at 0^{o}C and was allowed to warm to room temperature and continued to stir for 3 days at room temperature. Brine (30 mL) was added to the solution and the solution was extracted with DCM (3 x 30 mL). The organic layer was dried over $MgSO_4$, concentrated *in vacuo*, and was subjected to column chromatography from 15% EtOAc:Hexanes to 40% EtOAc: Hexanes to give ether **42** as a white oil (1.37 g, 64%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.69 (*d*, *J* = 7.2 Hz, 1H), 4.63 (*d*, *J* = 7.2 Hz, 1H), 3.75 (t, *J* = 6.0, 6.0 Hz, 1H), 3.65-3.51 (m, 2H), 2.45 - 2.32 (m, 2H), 2.27 – 2.10 (m, 3H), 2.07 – 2.00 (m, 1H), 1.95-1.87 (m, 1H), 1.76 – 1.69 (m, 1H), 1.67 – 1.57 (m, 2H), 1.19 (t, *J* = 7.2 Hz, 3H), 1.12 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 212.7, 94.1, 83.9, 63.1, 43.9, 42.6, 42.4, 36.6, 32.4, 29.0, 28.3, 20.4, 15.0.

Protection of the Hajos-Parrish Enone as its TBS ether (43)



To a chilled (0^o C) solution of CD enone **39** (5.0 g, 30.1 mmol) and imidazole (2.66 g, 39.1 mmol) in a 1:1 mixture of DMF:THF (15 mL) was added *tert*-butyldimethylsilyl chloride (TBDMS-Cl, 5.0 g, 33.1 mmol) and and 4-dimethylaminopyridine (DMAP, traces). The solution was stirred overnight and allowed to warm to room temperature. Ether (35 mL) and water (45 mL) was used to dilute the solution followed by extraction with ether (2 x 25 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The solution was concentrated and subjected to flash chromatography (100 % Hexanes) to give the TBS-ether **43** as a clear oil of 5 (6.07 g, 72% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.75 (s, 1H), 3.73 (dd, *J* = 10.0, 8.0 Hz, 1H), 2.71-2.62 (m, 1H), 2.49 (ddd, *J* = 17.8, 14.4, 5.3 Hz, 1H), 2.41 – 2.32 (m, 2H), 2.04 – 1.93 (m, 2H), 1.84 – 1.66 (m, 2H), 1.08 (s, 3H), 0.88 (s, 9H), 0.04 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 199.3, 175.1, 123.2, 80.7, 45.6, 34.3, 33.4, 29.5, 26.5, 25.7 (TBS), 18.0, 15.2, -4.5, -4.9

1.4.3 General coupling procedures

The general procedures described for making the A-CD estrogens were as previously published in our group [32].

Protection of the bromophenols as TBS-ethers (Method A)

The appropriate 4-bromophenol (25 mmol) and imidazole (1.3 equiv.) were dissolved in a 1:1 DMF/THF solution (10 mL). TBDMSCl (1.1 equiv.) and DMAP (trace) were added and the reaction mixture was stirred overnight at room temperature. The mixture was then diluted with distilled water (35 mL) and ether (45 mL) and then extracted with EtOAc (3 x 25 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The crude product was purified on a flash column. Elution with hexane afforded of the desired product as a clear colorless oil in generally greater than 90 % yield.

Protection of the bromophenols as MOM-ethers (Method B)

N,*N*-Diisopropylethylamine (DIPEA, 36.95 mmol) and chloromethyl methyl ether (36.95 mmol) were added to a solution of 4-bromophenol (18.5 mmol) in 50 mL of dry DCM under nitrogen atmosphere at 0 °C. The resulting yellow mixture was stirred for 30 min at 0°C and then left overnight at room temperature. The organic mixture was diluted with brine (40 mL) and extracted with DCM (3 × 40 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product as clear colorless oil in generally greater than 90 % yield.

Coupling of the protected 4-bromophenols [Ring A] with the unprotected Hajos−Parrish Ketone (Method C)

Protected bromophenol derivative (8.92 mmol) was dissolved in dry THF (20 mL) under nitrogen. The solution was placed in a dry ice/acetone bath (−78 °C), and *n*-butyllithium (8.92 mmol) was added dropwise. The solution was stirred for 5 min, and a solution of unprotected Hajos−Parrish ketone **40** (2.97 mmol), dissolved in dry THF (2 mL), was added dropwise. The reaction mixture was quenched after 10 min with saturated NH_4Cl solution (10 mL) and water (10 mL). The solution was extracted with EtOAc (3 × 30 mL), dried over $MgSO_4$, filtered, and

evaporated under vacuo. Flash chromatography of the crude product starting the elution with 30% EtOAc/hexane to 50% EtOAc/hexane generally allowed the clean separation of both stereoisomers. The isomers having the A-ring in the equatorial position relative to the CD-ring eluted first.

Coupling of the protected 4-Bromophenols [Ring A] with O-protected Hajos−Parrish Ketone (Method D)

Suitably protected bromophenol (2.94 mmol) was dissolved in dry THF (20 mL) and placed in a dry ice/acetone bath (−78 °C). *n*-Butyllithium (2.94 mmol) was added dropwise, and the solution was left to stir for 5 min. A protected CD-ring (1.67 mmol) was dissolved in dry THF (2 mL) and added dropwise. After 10 min, the reaction mixture was quenched with saturated NH_4Cl solution (10 mL) and water (10 mL). The solution was extracted with EtOAc (3 × 30 mL), dried over $MgSO_4$, filtered, and evaporated under vacuo. The crude product was eluted with 5% EtOAc/hexane to 10% EtOAc/hexane on a silica gel column, affording a mixture of both isomers.

Dehydration and deprotection of the isomeric C9-OH-di-protected A-CD adducts (Method E)

Either the mixture of isomers obtained in the coupling reaction or the purified individual isomers can be used. A mixture of both adducts was refluxed in MeOH (5-10 mL) containing 3-5 drops of HCl concentrated. The progress of the reaction was monitored by TLC. At the end most of the MeOH was evaporated, EtOAc was added, and the mixture was washed with 5% $NaHCO_3$. Evaporation of the organic solvents after drying over $MgSO_4$ afforded a mixture of the C8−C9 and C9−C11 unsaturated 3-17-diols. Separation of these isomers was accomplished using reverse phase preparative recycling HPLC. All compounds produced by this route had ^{1}H and ^{13}C NMR spectra in agreement with the desired structures.

Dehydration and deprotection of the isomeric C9-OH-di-protected A-CD adducts (Method F)

The C9-OH intermediate was dissolved in 10 mL of isopropanol and either PPTS or PTSA was added to the mixture. The reaction was stirred at 60^{o}C to 80^{o}C for 1h. Once done, the solvent was partly evaporated and the reaction was quenched with a saturated solution of $NaHCO_3$ (2mL) and water (15 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL). The

organic layer was dried over $MgSO_4$, concentrated *in vacuo*, filtered and purified by column chromatography to give a the mixture of the C-ring unsaturated product.

Hydrogenation of the C-ring unsaturated derivatives (Method G)

The hydrogenations were carried out at room temperature in MeOH with Pd/C and hydrogen under balloon pressure. In the same cases, such as with the 5-CF_3 compounds, the hydrogenation was carried out under 100 PSI of hydrogen pressure. The products were isolated by filtering off the catalyst and evaporating the solvent. The yield of an approximately 1:1 mixture of 9-(*S*) and 9-(*R*) diastereomers was typically greater than 90%. The isomers were separated by either silica gel or preparative HPLC.

Hydrogenolysis of the C9-OH-3,17-di-MOM adducts (Method H)

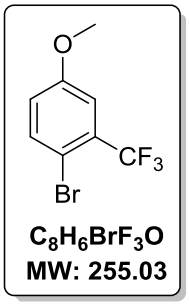
The mixture of adducts was dissolved in DCM (25 mL) at O°C and reacted with 8 equiv. of triethylsilane and 5 equiv of $BF_3.OEt_2$. The reaction mixture was stirred at low temperature for an hour, allowed to warm to room temperature. Then, the reaction was quenched with sat. NH_4Cl (10 mL) and the solution was extracted with DCM (3 x 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. Flash chromatography was used starting from 5% EtOAc:Hexanes to 30% EtOAc:Hexanes to give a mixture of both isomers.

Deprotection of the C9-OH-C3-TBS A-CD Adducts to form the 3,9,17-A-CD triols (Method I)

This reaction was carried out at room temperature with 1.3 equiv of TBAF in THF. The reaction progress was monitored by TLC; the typical reaction time was less than 15 min. The mixture was then diluted with brine and extracted with EtOAc. The organic layer was dried and the solvent evaporated to yield the mixture of isomers [3, 9-(*S*), 17-(*S*) and 3, 9-(*R*), 17-(*S*)] triols. These were separated on silica gel chromatography or HPLC.

1.4.4 Synthesis of the C5-CF_3 A-CD derivatives

1-bromo-4-methoxy-2-(trifluoromethyl)benzene (50)

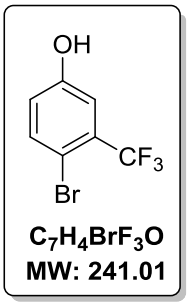


To a solution of 1-(trifluoromethyl)-3-methoxybenzene **49** (10.0 g, 56.8 mmol) in glacial acetic acid (10 mL) was added a solution of bromine (4.4 mL, 85.2 mmol) dissolved in 5 mL of glacial acetic acid dropwise with a bromine funnel. The solution mixture was stirred overnight. The reaction was quenched with 50 mL of water and the solution was extracted with EtOAc (3 x 25 mL). The organic phase was washed with 50 mL of water, the combined organic layers were dried over $MgSO_4$ and filtered and concentrated *in vacuo*, affording a yellow oil (8.38g, 59%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.57 (dd, *J* = 8.8, 0.5 Hz, 1H), 7.21 (d, *J* = 3.0 Hz, 1H), 6.91 (ddd, *J* = 8.8, 3.1, 0.5 Hz, 1H), 3.83 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 158.6, 135.7, 130.9 (q, *J* = 31.4 Hz), 122.7 (q, *J* = 273.5 Hz), 118.3 (q, *J* = 0.8 Hz), 113.9 (q, *J* = 5.6 Hz), 109.9(q, *J* = 1.9 Hz), 55.7

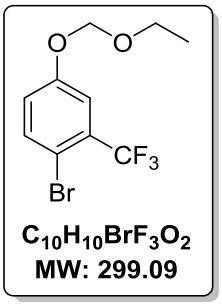
4-bromo-3-(trifluoromethyl)phenol (51)



To a solution of the methyl ether **50** (5g, 20.75 mmol) in DCM (120 mL) under nitrogen atmosphere was added a solution of boron tribromide in DCM (1M, 31.12 mL, 31.12 mmol). The solution was stirred overnight at room temperature. The reaction was quenched with water (100 mL) and the biphasic layer was saturated with brine (50 mL). The aqueous phase was extracted with DCM (100 mL x 3). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. Flash chromatography 100% Hexanes to 40% EtOAc:Hexanes was used to purify the compound, affording the bromophenol **51** (2.22g, 44.4%). The ^{1}H NMR spectrum agrees with the published data [158].

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.52 (d, *J* = 8.7, 1H), 7.18 (d, *J* = 3.0 Hz, 1H), 6.87 (dd, *J* = 8.7, 2.9 Hz, 1H), 5.32 (bs, 1H)

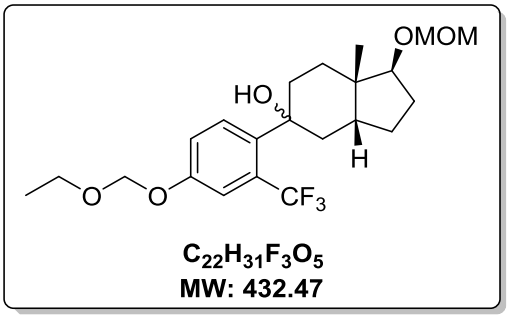
4-bromo-3-(trifluoromethyl)phenol (52)



This compound was prepared according to the general **Method B** using the bromophenol **51** (0.66 g, 2.32 mmol), DIPEA (0.81 mL, 4.63 mmol) and chloromethyl methyl ether (0.35 mL, 4.63 mmol) in 20 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired ether **52** as clear colorless oil (0.53 g, 80.1%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 ppm (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 2.9 Hz, 1H), 7.02 (dd, *J* = 8.8, 2.9 Hz, 1H), 5.16 (s, 2H), 3.64 (q, *J* = 6.8 Hz, 2H), 1.14 (t, *J* = 6.8 Hz, 3H)

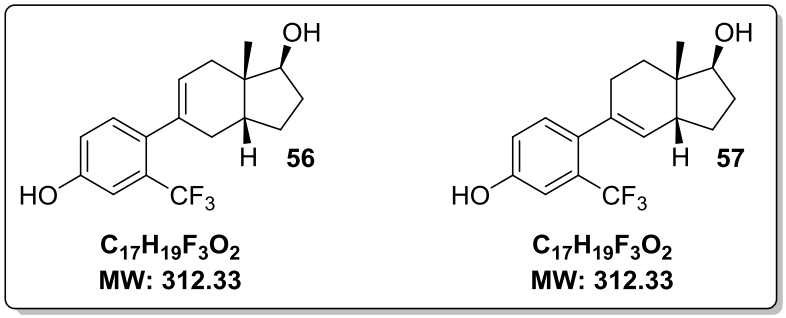
(1*S*,5*S*,7a*S*)-1-(methoxymethoxy)-5-(4-(methoxymethoxy)-2-(trifluoromethyl)phenyl)-7a-methyloctahydro-1*H*-inden-5-ol (54/55)



This compound was prepared according to the general **Method D** using ether **52** (0.53 g, 1.86 mmol) and *n*-BuLi (1.64 M, 1.13 mL, 1.86 mmol) in dry THF (20 mL), to which MOM-protected CD-ring **41** (263 mg, 1.24 mmol) in dry THF (4 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **54/55** (270 mg, 52%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (56)

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1H-inden-1-ol (57)



This compound was prepared according to the general **Method E** using the mixture of both isomers **54/55** (250 mg, 0.65 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers 168 mg, 83%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase to yield compounds **56** (49 mg, 24% yield) and **57** (88 mg, 43% yield), both as white solids.

Isomer 56

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.12 – 7.10 (m, 2H), 7.03 (dd, J = 8.4, 2.3 Hz, 1H), 5.45 (bs, 1H), 3.76 (dd, J = 6.4, 1.3 Hz, 1H), 2.43-2.34 (m, 1H), 2.27 – 2.17 (m, 1H), 2.13 – 2.03 (m, 2H), 1.89-1.75 (m, 2H), 1.73 – 1.67 (m, 1H), 1.57 – 1.42 (m, 2H), 1.06 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 81.2, 43.8, 41.3, 33.9, 33.6, 33.0, 29.8, 21.4. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum and no fluorine-decoupled ^{13}C NMR spectrum was taken; however the X-Ray structures (**Figure 1.2.21**) obtained were consistent with the presence of the desired aromatic ring.

HREIMS: calculated for $C_{17}H_{19}F_3O_2$ = 312.1337, found = 312.1325

Isomer 57

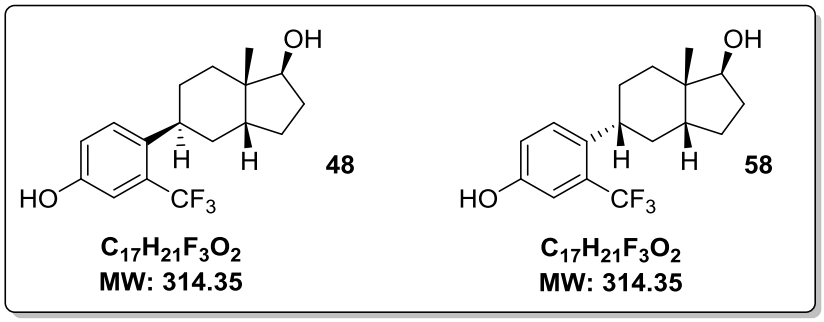
^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.13-7.11 (m, 2H), 7.02 (dd, J = 8.3, 2.3 Hz, 1H), 5.50 (bs, 1H), 3.88 (t, J = 5.7 Hz, 1H), 2.36-2.21 (m, 2H), 2.20-2.06 (m, 3H), 1.67-1.56 (m, 2H), 1.45-1.26 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 79.4, 45.8, 44.0, 33.7, 32.0, 30.2, 29.7, 21.0. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum and no fluorine-decoupled ^{13}C NMR spectrum was taken; however the X-Ray structures (**Figure 1.2.21**) obtained were consistent with the presence of the desired aromatic ring.

HREIMS: calculated for $C_{17}H_{19}F_3O_2$ = 312.1337, found = 312.1310

(1*S*,5*S*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyloctahydro-1*H*-inden-1-ol (48)

(1*S*,5*R*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyloctahydro-1*H*-inden-1-ol (58)



This compound was prepared according to the general **Method G** using the mixture of the unsaturated isomers **48** and **58** (88 mg, 0.28 mmol) under high pressure of H_2 (100 PSI) for 1h. Flash chromatography was performed to elute both isomers starting from 5% EtOAc:Hexanes to 40% EtOAc:Hexanes to yield compounds **48** (36 mg, 41%) and **58** (44 mg, 50%), both as white solids.

Isomer 48

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.76 (bs, 1H [OH]), 7.49 (d, J = 8.4 Hz, 1H), 7.10-7.03 (m, 2H), 3.65 (d, J = 5.1 Hz, 1H), 3.51-3.46 (m, 1H, [OH]), 3.15-3.00 (m, 1H), 2.28-2.06 (m, 2H), 1.88-1.67 (m, 4H), 1.65-1.46 (m, 3H), 1.37-1.22 (m, 2H), 1.17 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 83.2, 45.8, 43.4, 35.1, 35.1, 34.5, 34.2, 33.9, 28.2, 20.0. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum and no fluorine-decoupled ^{13}C NMR spectrum was taken; however the X-Ray structures (**Figure 1.2.21**) obtained were consistent with the presence of the desired aromatic ring.

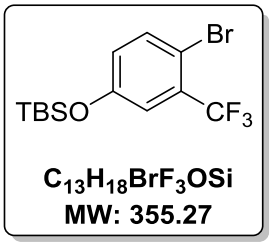
HREIMS: calculated for $C_{17}H_{21}F_3O_2$ = 314.1494, found = 314.1488

Isomer 58

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.75 (bs, 1H, [OH]), 7.42 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 2.6 Hz, 1H), 7.04 (dd, *J* = 8.5, 2.6 Hz, 1H), 4.46 (t, *J* = 8.4 Hz, 1H), 3.51 (bs, 1H, [OH]), 2.78 (dt, *J* = 12.1, 1.4 Hz, 1H), 2.17-1.99 (m, 2H), 1.98-1.90 (m, 1H), 1.80-1.47 (m, 5H), 1.42-1.30 (m, 2H), 1.16 (ddd, *J* = 13.9, 8.6, 4.4 Hz, 1H), 0.91 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 74.0, 47.0, 44.3, 40.8, 40.5, 40.5, 34.9, 31.5, 28.6, 23.9. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum and no fluorine-decoupled ^{13}C NMR spectrum was taken; however the X-Ray structures (**Figure 1.2.21**) obtained were consistent with the presence of the desired aromatic ring.

(4-bromo-3-(trifluoromethyl)phenoxy)(*tert*-butyl)dimethylsilane (51)

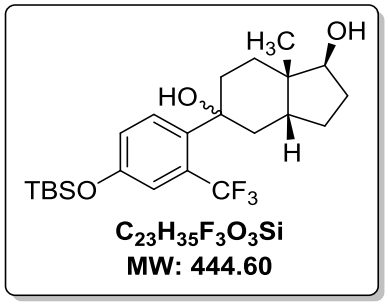


This compound was prepared according to the general **Method A** using the commercially available 4-bromo-3-(trifluoromethyl)phenol **51** (2.3 g, 9.54 mmol), imidazole (0.845 g, 12.41 mmol), TBDMSCl (1.58 g, 10.50 mmol) and DMAP (trace). The crude compound was concentrated and subjected to flash chromatography (100 % Hexanes) to give the TBS ether **51** as a clear colorless oil (2.17 g, 86.5 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.53 (d, *J* = 8.7 Hz, 1H), 7.16 (d, *J* = 2.9 Hz, 1H), 6.85 (dd, *J* = 8.7, 2.9 Hz, 1H), 0.99 (s, 9H), 0.22 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 155.0, 135.8, 131.0 (q, *J* = 31.3 Hz), 122.6 (q, *J* = 273.4 Hz), 124.4, 119.8 (q, *J* = 5.4 Hz), 110.7 (d, *J* = 1.9 Hz), 25.5, 18.2, -4.5

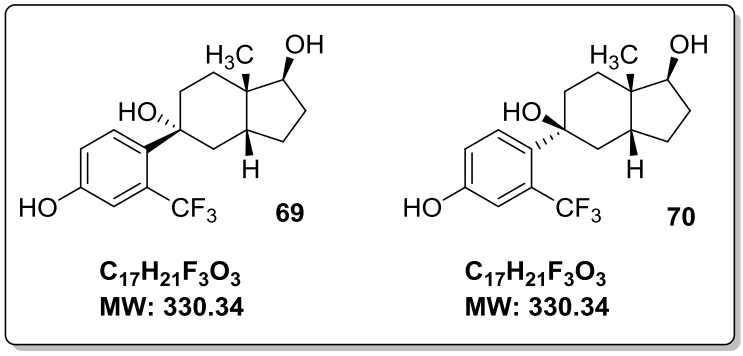
(1*S*,3a*R*,7a*S*)-5-(4-(*tert*-butyldimethylsilyloxy)-2-(trifluoromethyl)phenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol (67/68)



This compound was prepared according to the general **Method C** using the TBS ether **51** (1.59g, 4.46 mmol) and *n*-BuLi (2 M, 2.23 mL, 4.46 mmol) in dry THF (30 mL), to which unprotected CD-ring **40** (300 mg, 1.79 mmol) in dry THF (2 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers (340 mg, 17%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,5*R*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol (69)

(1*S*,3a*R*,5*S*,7a*S*)-5-(4-hydroxy-2-(trifluoromethyl)phenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol (70)



This compound was prepared according to the general **Method I** using the mixture of adducts **67/68** (340 mg, 0.77 mmol), and TBAF (1M, 0.84 mL, 0.84 mmol). The organic layer was dried and the solvent evaporated to yield the mixture of isomers [3, 9-(*S*), 17-(*S*) and 3, 9-(*R*), 17-(*S*)] triols (190 mg, 75%). These were separated on silica gel chromatography followed by preparative HPLC to yield compounds **69** (64 mg, 23%) and **70** (68 mg, 24.5%), both as white solids.

Isomer 69

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.58 (d, J = 8. 8 Hz, 1H), 7.22 (d, J = 2.7 Hz, 1H), 7.01 (dd, J = 8.8, 2.7 Hz, 1H), (m, 1H), 3.76 (dd, J = 5.7, 2.7 Hz, 1H), 3.45 (d, J = 1.2 Hz, H [OH]), 3.40 (d, J = 4.0 Hz, 1H [OH]), 2.36 – 2.28 (m, 2H), 2.21 – 2.12 (m, 1H), 2.02-1.70 (m, 6H), 1.54-1.46 (m, 1H), 1.16 – 1.10 (m, 1H), 1.11 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 82.7, 76.3, 45.1, 43.7, 39.5, 39.4, 36.2, 34.0, 30.9, 29.7, 20.7. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum and no fluorine-decoupled ^{13}C NMR spectrum was taken; however the X-Ray structures (**Figure 1.2.21**) obtained were consistent with the presence of the desired aromatic ring.

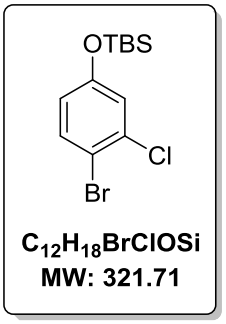
Isomer 70

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.53 (d, J = 8.7 Hz, 1H), 7.21 (d, J = 2.7 Hz, 1H), 6.99 (dd, J = 8.8, 2.7 Hz, 1H), 4.40 (dt, J = 8.8, 8.5, 5.7 Hz, 1H), 3.56 (bs, 1H [OH]), 3.49 (m, 1H [OH]), 2.18 – 1.99 (m, 4H), 1.91 – 1.53 (m, 6H), 1.17 – 1.09 (m, 1H), 0.92 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 75.3, 74.1, 44.9, 44.8, 43.6, 42.6, 35.8, 35.8, 31.5, 30.1, 28.3, 23.5. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum and no fluorine-decoupled 13C NMR spectrum was taken; however the X-Ray structures (**Figure 1.2.21**) obtained were consistent with the presence of the desired aromatic ring.

1.4.5 Synthesis of the C5-Cl A-CD derivatives

Preparation of the Protected Bromophenol: 4-bromo-3-chlorophenoxy)(*tert*-butyl)dimethylsilane (75)



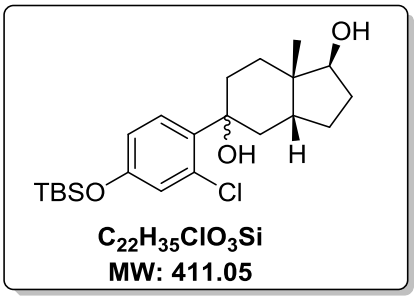
$C_{12}H_{18}BrClOSi$
MW: 321.71

This compound was prepared according to the general **Method A** using the commercially available 4-bromo-3-chlorophenol (4.97 g, 23.96 mmol), imidazole (2.12 g, 31.15 mmol), TBDMSCl (3.97 g, 26.35 mmol) and DMAP (trace). The crude compound was concentrated and subjected to flash chromatography (100 % Hexanes) to give ether **74** as a colorless oil (7.19 g, 93%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.43 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 2.8 Hz, 1H), 6.62 (dd, *J* = 8.7, 2.8 Hz, 1H), 0.98 (s, 9H), 0.21 (s, 6H)

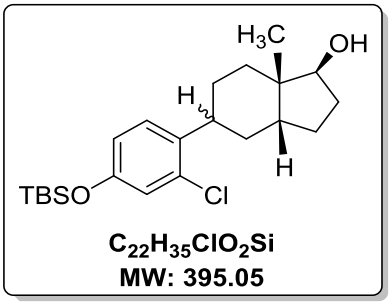
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 155.6, 134.6, 133.8, 122.2, 120.1, 113.7, 25.5, 18.1, -4.5

(1*S*,7a*S*)-hexahydro-1-hydroxy-7a-methyl-1*H*-inden-5(6*H*)-one (76)



This compound was prepared according to the general **Method C** using the TBS ether **75** (6.9g, 21.45 mmol) and *n*-BuLi (1.4 M, 15.32 mL, 21.45 mmol) in dry THF (40 mL), to which unprotected CD-ring **40** (1g, 5.95 mmol) in dry THF (5 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers (158.8 mg, 6.5%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,5*S*,7a*S*)-5-(4-(*tert*-butyldimethylsilyloxy)-2-chlorophenyl)-7a-methyloctahydro-1*H*-inden-1-ol (76)

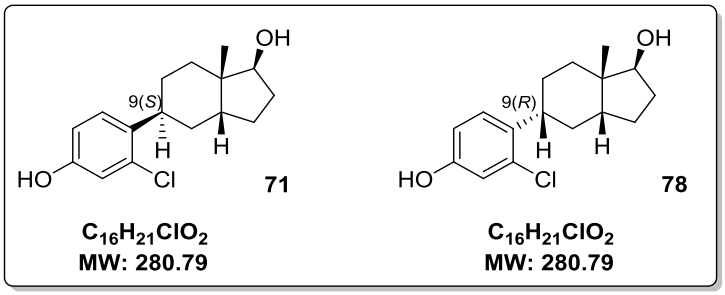


To a chilled (0^o C) solution of compound 6 (93 mg, 0.226 mmol) in dry DCM (25 mL) was added successively triethylsilane (116.28mg, 1.0 mmol) and $BF_3.OEt_2$ (86.58 mg, 0.61mmol). The solution was stirred for an hour at 0^oC and allowed to warm to room temperature. The reaction was quenched with sat. NH_4Cl (10 mL) and the solution was extracted with DCM (15 mL x 3). The combined organic layers were dried over $MgSO_4$ and filtered. The solution was evaporated *in vacuo*. Flash chromatography was used starting from 6% DCM: hexanes to 15% DCM:Hexanes to give the product with a quantitative yield (50 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.13 (d, *J* = 8.5 Hz, 1H), 7.05 (d, *J* = 8.6Hz, 1H), 6.84 (d, *J* = 2.5 Hz, 2H), 6.73 – 6.67 (m, 2H), 4.38 (t, *J* = 8.4 Hz, 1H), 3.74 (d, *J* = 5.4 Hz, 1H), 3.14 (tt, *J* = 12.3, 3.6 Hz, 1H), 2.86 (tt, *J* = 12.0, 2.7 Hz, 1H), 2.33 – 1.99 (m, 3H), 1.92 – 1.45 (m, 12H), 1.26 (s, 3H), 1.13 (s, 3H), 0.97 (s, 18 H), 0.19 (s, 12 H). Representative recorded peaks are doubled due to for the mixture of C9-H epimers.

(1*S*,3a*R*,5*S*,7a*S*)-5-(2-chloro-4-hydroxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (71)

(1*S*,3a*R*,5*R*,7a*S*)-5-(2-chloro-4-hydroxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (78)



The above hydrogenated mixture **75** (87 mg, 0.219 mmol) was dissolved in THF (2 mL), and TBAF (1M, 0.24 mL, 0.240 mmol) was added to the solution dropwise. The resulting mixture was left for 10 min, diluted with brine (1 mL) and water (1 mL), and extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was subjected to a silica gel column chromatography. Elution with 25% EtOAc/75% hexane afforded compounds **71** (35 mg, 57%) and **78** (29 mg, 47%), both as white solids.

Isomer 71

^{1}H NMR (400MHz, Acetone-d_6) δ ppm 8.59 (bs, 1H), 7.23 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 2.5 Hz, 1H), 6.77 (dd, J = 8.5, 2.6 Hz, 1H), 3.65 (t, J = 4.9 Hz, 1H), 3.47 (d, J = 4.3 Hz, 1H), 3.14 (tt, J = 12.2, 3.7 Hz, 1H), 2.28-2.06 (m, 2H), 1.88-1.77 (m, 2H), 1.73-1.50 (m, 5H), 1.35 (dt, J = 13.1, 3.9 Hz, 1H), 1.30-1.23 (m, 1H), 1.12 (s, 3H)

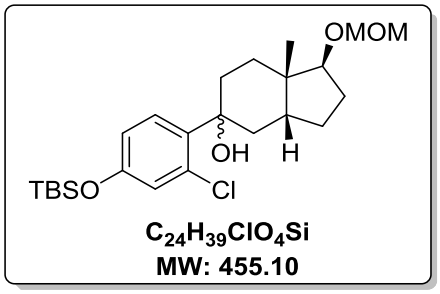
^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 157.9, 136.8, 135.1, 129.9, 117.8, 116.4, 83.2, 45.9, 43.5, 35.6, 34.3, 33.9, 33.2, 29.8, 28.2, 20.1

HREIMS: calculated for $C_{16}H_{21}ClO_2$= 280.1230, found = 280.1209

Isomer 78

^{1}H NMR (400MHz, Acetone-d_6) ppm 7.16 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 2.6 Hz, 1H), 6.76 (dd, J = 8.5, 2.6 Hz, 1H), 4.38 (t, J = 8.4 Hz, 1H), 2.26-0.13 (m, 11H), 0.91 (s, 3H)

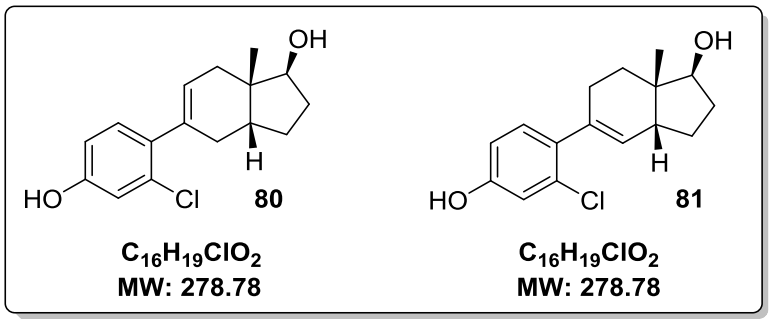
(1*S*,3a*R*,7a*S*)-5-(4-(*tert*-butyldimethylsilyloxy)-2-chlorophenyl)-1-(methoxymethoxy)-7a-methyloctahydro-1*H*-inden-5-ol (79)



This compound was prepared according to the general **Method D** using the TBS ether **74** (1.36 g, 4.24 mmol) and *n*-BuLi (1.8 M, 2.4 mL, 4.24 mmol) in dry THF (40 mL), to which MOM-protected CD-ring **41** (600 mg, 2.83 mmol) in dry THF (5 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers (808 mg, 63%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,7a*S*)-5-(2-chloro-4-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (80)

(1*S*,7a*S*)-5-(2-chloro-4-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (81)



This compound was prepared according to the general **Method E** using the mixture of both isomers of TBS ethers **79** (500 mg, 1.22 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (225 mg, 47%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 35% THF in water as mobile phase.

Isomer 80

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.01 (d, J = 8.3 Hz, 1H), 6.85 (d, J = 2.5 Hz, 1H), 6.74 (dd, J = 8.3, 2.5 Hz, 1H), 5.50 (bs, 1H), 3.75 (dd, J = 6.3, 1.9 Hz, 1H), 3.60 (bs, 1H [OH]), 2.43 - 2.36 (m, 1H), 2.23-2.07 (m, 3H), 1.89 (ddd, J = 18.0, 5.7, 2.8 Hz, 1H), 1.85-1.78 (m, 1H), 1.75-1.69 (m, 1H), 1.57-1.45 (m, 2H), 1.07 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 158.9, 136.8, 136.1, 134.1, 132.7, 126.6, 117.8, 116.0, 81.2, 44.0, 41.7, 34.2, 33.6, 31.6, 29.9, 21.7.

HREIMS: calculated for $C_{16}H_{19}ClO_2$= 278.1074, found = 278.1066

Isomer 81

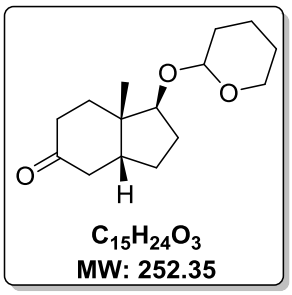
^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.03 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 2. 4 Hz, 1H), 6.75 (dd, J = 8.3, 2.5 Hz, 1H), 5.55 (bs, 1H), 3.89 (t, J = 5.8 Hz, 1H), 3.58 (bs, 1H [OH]), 2.92 (bs, 1H, [OH]), 2.33-2.02 (m, 5H), 1.65-1.57 (m, 2H), 1.44-1.29 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 158.7, 136.7, 136.1, 134.2, 132.9, 132.7, 117.8, 115.9, 79.4, 45.9, 44.0, 33.7, 32.1, 31.4, 30.5, 28.1.

HREIMS: calculated for $C_{16}H_{19}ClO_2$= 278.1074, found = 278.1065

1.4.6 Synthesis of the C5-CH_3 A-CD derivatives

(1*S*,3a*R*,7a*S*)-7a-methyl-1-(tetrahydro-2*H*-pyran-2-yloxy)hexahydro-1*H*-inden-5(6*H*)-one (89)

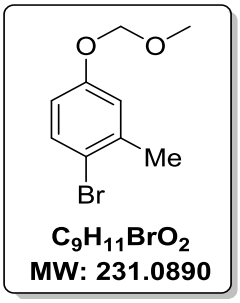


To a chilled solution (0°C) of the Hajos-Parrish ketone **40** (5 g, 29.72 mmol) in DCM (100 mL) under nitrogen was added dihydropyran (DHP, 5.4 mL, 50.44 mmol) dropwise and PTSA (few crystals). The solution was stirred for 30 min at 0°C and was allowed to warm to room temperature. Once the reaction was completed, sodium carbonate (20 mL) was added and the solution was extracted with DCM. The organic layer was dried over $MgSO_4$, concentrated *in vacuo*, and was subjected to column chromatography from 15% EtOAc:Hexanes to 40% EtOAc: Hexanes, affording the ether **89** as a colorless oil (6.8 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.79-4.42 (m, 1H), 3.83 (m, 2H), 3.56-3.41 (m, 1H), 2.15-1.46 (m, 12H), 2.53-2.18 (m, 4H), 1.33-0.96 (m, 4H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 213.2, 213.0, 100.3, 95.6, 85.6, 81.4, 62.8, 62.0, 44.1, 44.0, 43.0, 42.7, 42.4, 36.8, 32.8, 32.4, 31.0, 30.8, 30.6, 28.6, 28.4, 27.9, 25.6, 25.5, 20.5, 20.4, 19.8, 19.3. The doubling of recorded peaks is due to the presence of the stereocenter on the THP group.

1-bromo-4-(methoxymethoxy)-2-methylbenzene (90)

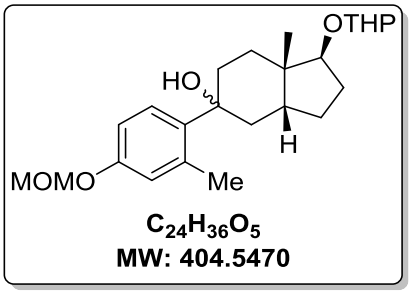


This compound was prepared according to the general **Method B** using the 4-bromo-3-methylphenol (2g, 10.69 mmol), DIPEA (3.7 mL, 21.39 mmol) and chloromethyl ethyl ether (1.60 mL, 21.39 mmol) in 20 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product as a clear colorless oil (2.3 g, 91%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.41 (d, *J* = 8.7 Hz, 1H), 6.94 (d, *J* = 2.9 Hz, 1H), 6.76 (dd, *J* = 8.7, 2.9 Hz, 1H), 5.14 (s, 2H), 3.47 (s, 3H), 2.37 (s, 3H)

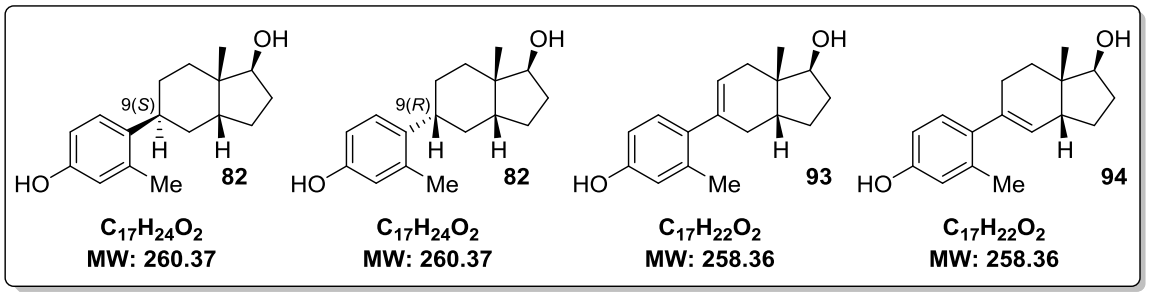
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 156.4, 138.9, 132.8, 118.7, 116.7, 115.3, 94.4, 55.9, 23.1

(1*S*,3a*R*,7a*S*)-5-(4-(methoxymethoxy)-2-methylphenyl)-7a-methyl-1-(tetrahydro-2*H*-pyran-2-yloxy)octahydro-1*H*-inden-5-ol (91)



This compound was prepared according to the general **Method D** using the MOM ether **90** (1.38g, 5.95 mmol) and *n*-BuLi (1.63 M, 3.65 mL, 5.95 mmol) in dry THF (40 mL), to which THP-protected CD-ring **89** (1 g, 3.97 mmol) in dry THF (5 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers (1.24 g, 65%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,5*S*,7a*S*)-5-(4-hydroxy-2-methylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (82) [9*S* isomer]
(1*S*,3a*R*,5*R*,7a*S*)-5-(4-hydroxy-2-methylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (82) [9*R* isomer]
(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-methylphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (93)
(1S,3aR,7aS)-5-(4-hydroxy-2-methylphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1H-inden-1-ol (94)



The following compounds were prepared in two steps according starting with the general **Method H** using the mixture of adducts **91** (1 g, 2.47 mmol), triethylsilane (3.3 mL, 20.52 mmol) and $BF_3.OEt_2$ (1.53 mL, 12.36 mmol). Flash chromatography was used starting from 5% EtOAc:Hexanes to 30% EtOAc:Hexanes to give a crude mixture of products which includes both hydrogenated isomers and dehydration isomers (531 mg). Then, the mixture of the four above adducts (531 mg) were deprotected according to the general **Method E**. The crude product was eluted with 5% EtOAc:hexanes to 30% EtOAc:hexanes on a silica gel column, affording a mixture of both hydrogenated and dehydrated final isomers. Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase to yield the "natural" isomer **82** (116 mg, 18 % over two steps), "un-natural" isomer **82** (20 mg, 3% over two steps), compound **93** (40 mg, 6% over two steps) and compound **94** (178 mg, 28%), all as white solids.

Isomer 82 (9*S*)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.11 (d, *J* = 8.2 Hz, 1H), 6.68-6.61 (m, 2H), 4.94 (bs, 1H [OH]), 3.75 (d, *J* = 5.7 Hz, 1H), 2.86 (tt, *J* = 11.8, 4.0 Hz, 1H), 2.27 (s, 3H), 2.33-2.22 (m, 1H), 2.15-2.06 (m, 1H), 1.96-1.75 (m, 2H), 1.71-1.51 (m, 6H), 1.35-1.27 (m, 2H), 1.15 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 153.2, 137.4, 136.8, 126.5, 117.0, 112.8, 82.7, 44.2, 41.7, 33.0, 32.5, 32.0, 31.7, 28.4, 26.5, 19.3, 18.4.

HREIMS: calculated for $C_{17}H_{24}O_2$= 260.1776, found = 260.1764

Isomer 82 (9*R*)

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.93 (bs, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 6.62-6.56 (m, 2H), 4.45-4.35 (m, 1H), 3.45 (d, *J* = 5.6 Hz, 1H, [OH]), 2.60 (tt, *J* = 11.9, 3.1 Hz, 1H), 2.22 (s, 3H), 2.16-1.98 (m, 2H), 1.93 (td, *J* = 13.8, 3.1 Hz, 1H), 1.75 (td, *J* = 12.5, 6.4 Hz, 1H), 1.66-1.46 (m, 4H), 1.38 (dt, *J* = 13.3, 4.7 Hz, 1H), 1.27-1.10 (m, 2H), 0.91 (s, 3H)

Isomer 93

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.91 (d, *J* = 8.1 Hz, 1H), 6.64 (d, *J* = 2.6 Hz, 1H), 6.60 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.42 (bs, 1H), 4.69-4.55 (m, 1H, [OH]), 3.86-3.79 (m, 1H), 2.40-2.24 (m, 2H), 2.23 (s, 3H), 2.12-2.00 (m, 2H), 1.92-1.81 (m, 2H), 1.80-1.71 (m, 1H), 1.62-1.37 (m, 3H), 1.09 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 154.0, 136.9, 136.7, 135.8, 129.5, 123.1, 116.6, 112.2, 80.6, 42.2, 39.7, 32.1, 31.9, 30.3, 28.3, 19.9, 19.8.

HREIMS: calculated for $C_{17}H_{22}O_2$= 258.1620, found = 258.1625

Isomer 94

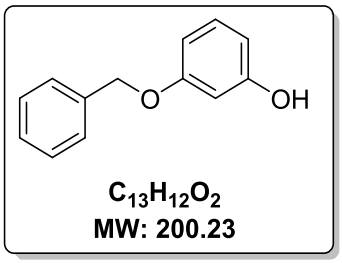
^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.12 (bs, 1H [OH]), 6.84 (d, *J* = 8.2 Hz, 1H), 6.63 (d, *J* = 2.6 Hz, 1H), 6.58 (dd, *J* = 8.2, 2.3 Hz, 1H), 5.43 (bs, 1H), 3.88 (t, *J* = 5.7 Hz, 1H), 3.66 (bs, 1H [OH]), 2.36-2.25 (m, 1H), 2.17 (s, 3H), 2.25-2.03 (m, 4H), 1.67-1.54 (m, 2H), 1.46-1.24 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 157.7, 138.0, 137.7, 137.4, 131.3, 130.9, 118.4, 114.2, 79.5, 45.9, 44.0, 33.7, 32.2, 30.8, 29.0, 21.2, 21.1

HREIMS: calculated for $C_{17}H_{22}O_2$= 258.1620, found = 258.1616

1.4.7 Synthesis of the C5-OH A-CD analogs [TD81]

3-(benzyloxy)phenol (97)

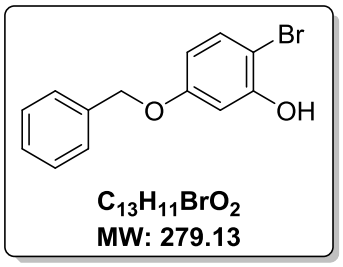


Benzyl bromide (2.7 mL, 22.70 mmol) was added dropwise to a stirred suspension of resorcinol (5 g, 45.41 mmol) and K_2CO_3 (3.14 g, 22.70 mmol) in acetone (40 mL) under nitrogen atmosphere [159]. The mixture was heated under reflux overnight and filtered to remove the solid potassium carbonate. The filtrate was concentrated and diluted in EtOAc (40 mL) and washed three times with water (20 mL) and 10% aqueous HCl solution (5 mL). The aqueous phase was extracted with EtOAc (3 x 40 mL). The combined organic solution was dried over $MgSO_4$, concentrated *in vacuo* and the crude product was purified column chromatography to give the pure product as color (2.87 g, 58%). The ^{1}H NMR spectrum agrees with the published data [159].

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.05-6.92 (m, 7H), 6.76-6.58 (m, 2H), 5.10 (s, 2H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 160.0, 136.9, 129.9, 128.5, 127.9, 127.4, 107.3, 102.2, 69.9

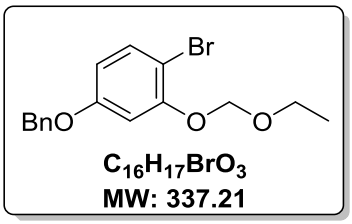
5-(benzyloxy)-2-bromophenol (98)



To a stirred solution of 3-(benzyloxy)phenol (2 g, 10.0 mmol) in DCM (30 mL) at -30°C was added solid *N*-bromosuccinimide (NBS, 1.78 g, 10.0 mmol) under nitrogen atmosphere [138]. The mixture was stirred at the same temperature for 30 minutes. After removal of the cooling bath, the mixture was kept at room temperature for 1h. Then, more DCM was added and the mixture was successively washed with H_2O (20 mL), brine (20 mL) and H_2O (20 mL). The organic solution was dried over $MgSO_4$, filtered, concentrated in vacuo and the crude product was purified by column chromatography heptanes 30%: DCM 70% to afford the product (1.05 g, 38%). The ^{1}H NMR spectrum agrees with the published data [138].

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.44 – 7.33 (m, 6H), 6.71 (d, *J* = 2.4 Hz, 1H), 6.51 (dd, *J* = 8.9, 2.8 Hz, 2H), 5.70 (bs, 2H), 5.02 (s, 2H)

4-(benzyloxy)-1-bromo-2-(ethoxymethoxy)benzene (96)

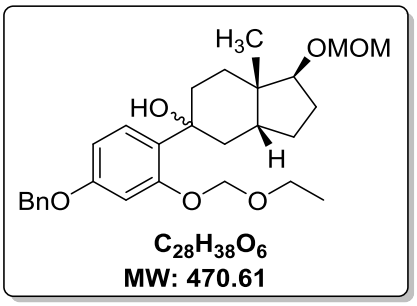


This compound was prepared according to the general **Method B** using the bromophenol **96** (964 mg, 3.24 mmol), DIPEA (1.3 mL, 7.52 mmol) and chloromethyl ethyl ether (0.7 mL, 7.52 mmol) in 20 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product as a clear colorless oil in quantitative yield (1.1g). The ^{1}H NMR spectrum agrees with the published data [138].

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.54-7.27 (m, 6H), 6.90 (d, *J* = 2.8 Hz, 1H), 6.55 (dd, *J* =8.8, 2.8 Hz, 1H), 5.28 (s, 2H), 5.04 (s, 2H), 3.78 (q, *J* = 7.1, 2H), 1.25 (t, *J* = 7.18 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 159.1, 154.7, 136.5, 133.1, 128.5, 128.0, 127.5, 108.9, 104.1, 103.6, 93.8, 70.2, 64.6, 15.0

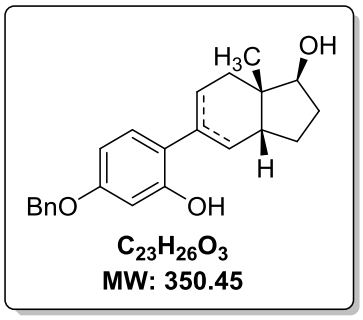
(1*S*,7a*S*)-5-(4-(benzyloxy)-2-((2-methoxyethoxy)methoxy)phenyl)-1-(methoxymethoxy)-7a-methyloctahydro-1*H*-inden-5-ol (99)



This compound was prepared according to the general **Method D** using the bromoresorcinol **96** (414 mg, 1.23 mmol) and *n*-BuLi (1.2 M, 1 mL, 1.23 mmol) in dry THF (10 mL), to which MOM-protected CD-ring **41** (200 mg, 0.94 mmol) in dry THF (4 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **99** (318 mg, 72%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,7a*S*)-5-(4-(benzyloxy)-2-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (100a)

(1*S*,7a*S*)-5-(4-(benzyloxy)-2-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (100b)



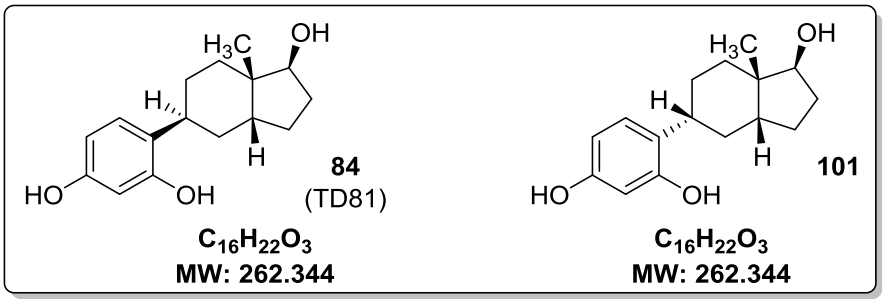
This compound was prepared according to the general **Method F** using the mixture of both isomers of triethers **99** (320 mg, 0.68 mmol) and PTSA (130 mg, 0.75 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers as white solids (82 mg, 34%).

^{1}H NMR (400 MHz, $CDCl_3$) ppm 7.44 – 7.31 (m, 5H), 6.98 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.58 (d, *J* = 2.5 Hz, 1H), 6.54 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.83 – 5.73 (m, 1H), 5.67 (s, 1H, [OH]), 5.04 (s, 2H), 3.93-3.82 (m, 1H), 2.46-2.05 (m, 4H), 1.99-1.75 (m, 1H), 5.86-5.71 (m, 1H), 5.69-5.65 (m, 1H), 1.72-1.30 (m, 4H), 1.09 and 1.05 (s, 3H [one singlet for both olefins])

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 158.8, 153.0, 136.9, 132.3, 130.7, 128.6, 128.6, 128.5, 127.9, 127.4, 124.6, 122.0, 107.1, 101.7, 101.6, 80.3, 79.2, 70.0, 43.8, 42.4, 42.1, 39.5, 32.2, 32.1, 31.8, 30.1, 30.0, 29.3, 28.3, 26.7, 20.0, 19.2. The doubling of recorded peaks is due to the presence of the two olefins **100a** and **100b**.

4-((1*S*,3a*R*,5*S*,7a*S*)-1-hydroxy-7a-methyloctahydro-1*H*-inden-5-yl)benzene-1,3-diol (84)

4-((1*S*,3a*R*,5*R*,7a*S*)-1-hydroxy-7a-methyloctahydro-1*H*-inden-5-yl)benzene-1,3-diol (101)



This compound was prepared according to the general **Method G** using the mixture of the 3-Benzyloxy unsaturated A-CD adducts **(100a/100b)** (60mg, 0.17 mmol) under high pressure of H_2 (used two big balloons of hydrogen gas) for one day. Flash chromatography was performed to elute both isomers starting from 5% EtOAc: Hexanes to 40% EtOAc: Hexanes afforded both the natural and unnatural triol isomers in one single spot (40 mg, 90%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase to yield the "natural" isomer **84** (17 mg, 38%) and "un-natural" isomer **101** (15 mg, 33%), both as pink solids.

Isomer 84

1H NMR (400 MHz, Acetone-d_6 [with few drops of MeOD]) δ ppm 6.93 (d, *J* = 8.3 Hz, 1H), 6.28 (d, *J* = 2.4 Hz, 1H), 6.24 (dd, *J* = 8.3, 2.4 Hz, 1H), 3.67-3.57 (m, 2H [C17-H and OH]), 3.04 (ddd, *J* = 16.0, 10.6, 5.1 Hz, 1H), 2.25-2.14 (m, 1H), 2.11-2.00 (m, 1H), 1.89-1.71 (m, 2H), 1.70-1.49 (m, 5H), 1.30 (dt, *J* = 12.9, 12.6, 4.5 Hz, 1H), 1.23-1.15 (m, 1H), 1.09 (s, 3H)

13C NMR (100 MHz, Acetone-d_6 [with few drops of MeOD]) δ ppm 157.9, 157.2, 129.0, 126.5, 108.2, 104.2, 83.5, 45.9, 43.6, 34.5, 33.7, 32.7, 32.1, 30.1, 28.3, 20.1

HREIMS: calculated for $C_{16}H_{22}O_3$= 262.1569, found = 262.1565

Isomer 101

1H NMR (400 MHz, Acetone-d_6) δ ppm 8.07 (s, 1H [OH]), 7.97 (s, 1H [OH]), 6.90 (d, *J* = 8.3 Hz, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 6.28 (dd, *J* = 8.3, 2.4 Hz, 1H), 4.41-4.36 (m, 1H), 3.49 (d, *J* = 5.6 Hz, 1H [OH]), 2.84-2.71 (m, 1H), 2.15-1.98 (m, 2H), 1.91 (td, *J* = 13.8, 3.2 Hz, 1H), 1.72 (td, *J* = 12.6, 6.4 Hz, 1H), 1.64-1.53 (m, 4H), 1.42-1.10 (m, 3H), 0.90 (s, 3H)

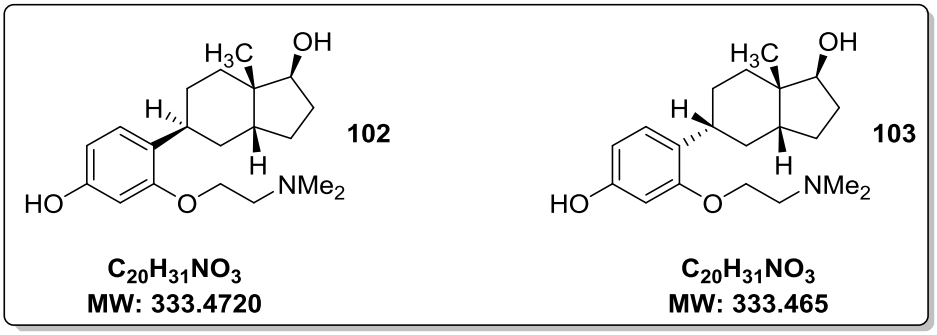
13C NMR (100 MHz, Acetone-d_6) δ ppm 157.9, 157.0, 128.6, 126.6, 108.3, 104.4, 74.0, 47.2, 44.5, 39.6, 37.7, 35.2, 31.3, 30.1, 28.7, 24.1

HREIMS: calculated for $C_{16}H_{22}O_3$= 262.1569, found = 262.1567

1.4.8 Synthesis of the C5-$OCH_2CH_2NMe_2$ analogs

(1*S*,3a*R*,5*S*,7a*S*)-5-(2-(2-(dimethylamino)ethoxy)-4-hydroxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (102)

(1*S*,3a*R*,5*R*,7a*S*)-5-(2-(2-(dimethylamino)ethoxy)-4-hydroxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (103)



2-Dimethyl aminoethyl chloride hydrochloride (66 mg, 0.46 mmol) was added at once to a stirred suspension of the benzyloxy A-CD adduct **75** (78 mg, 0.23 mmol) and K_2CO_3 (159 mg, 1.15 mmol) in acetone (7 mL) under nitrogen atmosphere. The mixture was heated under reflux for 2h and was allowed to cool at rt and stirring overnight. Once done, the reaction was filtered to remove the solid potassium carbonate, the filtrate was concentrated, diluted in EtOAc and washed with H_2O (2 x 15 mL). The combined organic phase was dried over $MgSO_4$, evaporated under reduced pressure to afford the crude product as a yellowish solid (86 mg, 56%).

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.48 - 7.29 (m, 5H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 6.53 (dd, *J* = 8.3, 2.4 Hz, 1H), 5.63 – 5.54 (m, 1H), 5.09 (s, 2H), 4.03 (t, *J* = 5.8 Hz, 2H), 3.88 – 3.73 (m, 1H), 2.50 – 1.25 (m, 2H), 2.72-0.81 (m, 7H), 1.05- 1.02 (m, 3H)

The above mixture (86 mg, 0.26 mmol) were hydrogenated according to the general **Method G** under high pressure of H_2 (used two big balloons of hydrogen gas) for 1 day. The crude was purified by column chromatography eluting with 45 % EtOAc in hexanes afforded a mixture of two isomers (60 mg, 69%). The separation needed to be carried out using a preparative reverse phase HPLC system (45% AcCN in H_2O) affording the two isomers the "natural" isomer **102** (16 mg, 18%) and "un-natural" isomer **103** (7 mg, 8%), both as white solids.

Isomer 102

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.00 (d, *J* = 8.2 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 6.37 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.02 (t, *J* = 5.7 Hz, 2H), 3.63 (d, *J* = 5.6 Hz, 1H), 3.09 (tt, *J* = 12.0, 4.0 Hz, 1H), 2.71 (t, *J* = 5.7 Hz, 3H), 2.29 (s, 6H), 2.22-2.15(m, 1H), 1.90-1.69 (m, 2H), 1.70-1.48 (5H), 1.38-1.20 (m, 2H), 1.00 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.0, 158.2, 128.7, 128.3, 108.7, 101.6, 83.3, 68.4, 60.1, 47.2, 45.9, 43.6, 34.6, 34.0, 33.1, 32.3, 29.8, 28.3, 20.2.

HREIMS: calculated for $C_{20}H_{31}NO_3$ = 333.2304, found = 333.2317

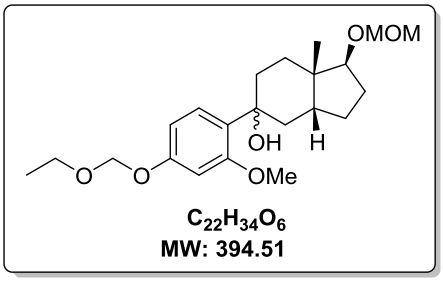
Isomer 103

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 6.93 (d, *J* = 8.2 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 6.34 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.38 (t, *J* = 8.5 Hz, 1H), 4.01 (t, *J* = 5.8 Hz, 2H), 2.77 (tt, *J* = 15.2, 2.8 Hz, 1H), 2.70 (t, *J* = 5.8 Hz, 2H), 2.29 (s, 6H), 2.05 (td, *J* = 4.41, 2.20 Hz, 8H), 2.14-1.98 (m, 2H), 1.90 (td, *J* = 13.7, 3.1 Hz, 1H), 1.73-1.50 (m, 5H), 1.37-1.05 (m, 3H), 0.89 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.0, 158.2, 128.5, 128.3, 108.6, 101.6, 74.0, 68.4, 60.1, 47.3, 47.2, 44.5, 39.6, 38.2, 35.6, 31.3, 30.1, 28.7, 24.1

1.4.9 Synthesis of the C5-OCH_3 A-CD derivatives

(1*S*,3a*R*,7a*S*)-5-(4-(ethoxymethoxy)-2-methoxyphenyl)-1-(methoxymethoxy)-7a-methyloctahydro-1*H*-inden-5-ol (104)



1-bromo-4-(ethoxymethoxy)-2-methoxybenzene [A-ring component] was prepared according to the general **Method B** using the commercially available 4-bromo-3-methoxyphenol (2g, 9.85 mmol), DIPEA (3.43 mL, 19.70 mmol) and chloromethyl ethyl ether (1.83 mL, 19.70 mmol) in 20 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product as clear colorless oil (2.5g, 97%).

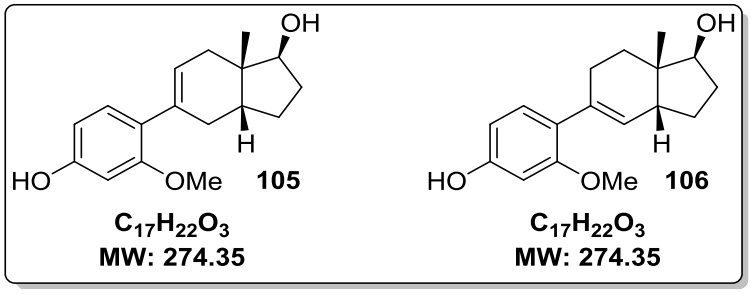
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.38 (d, *J* = 8.7, 1H), 6.62 (d, *J* = 2.6 Hz, 1H), 6.56 (dd, *J* = 8.7, 2.6 Hz, 1H), 5.19 (s, 2H), 3.85 (s, 3H), 3.71 (q, *J* = 7.1 Hz, 2H), 1.21 (t, *J* = 7.1 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 157.9, 156.4, 133.1, 108.8, 103.4, 101.5, 93.2, 64.3, 56.1, 15.0

The above A-ring (1.33 g, 4.5 mmol) was coupled according to the general **Method D** using *n*-BuLi (2.5 M, 1.8 mL, 4.45 mmol) in dry THF (15 mL), to which MOM-protected CD-ring **41** (640 mg, 3.0 mmol) in dry THF (3 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 20% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **41** (760 mg, 64%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-methoxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (105)

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2-methoxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (106)



This compound was prepared according to the general **Method F** using the mixture of both isomers of diprotected ethers **104** (380 mg, 0.96 mmol) and PTSA (125 mg, 0.73 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (170 mg, 65%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 42% AcCN in water as mobile phase to yield compounds **105** (36 mg, 14%) and **106** (83 mg, 32% yield), both as white solids.

Isomer 105

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.23 (bs, 1H [OH]), 6.89 (d, J = 8.1 Hz, 1H), 6.42 (d, J = 2.3 Hz, 1H), 6.35 (d, J = 8.1, 2.3 Hz, 1H), 5.59-5.47 (m, 1H), 3.82-3.66 (m, 1H), 3.73 (s, 3H), 3.48 (d, J = 4.6 Hz, 1H [OH]), 2.47-2.40 (m, 1H), 2.23-2.10 (m, 2H), 2.07 – 2.01 (m, 1H), 1.89 (ddd, J = 17.7, 5.5, 3.0 Hz, 1H), 1.80 – 1.68 (m, 2H), 1.55 – 1.37 (m, 2H), 1.03 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.9, 159.4, 137.1, 131.5, 126.9, 124.5, 108.6, 101.1, 81.3, 56.6, 44.1, 42.2, 34.7, 33.7, 31.4, 29.8, 22.1

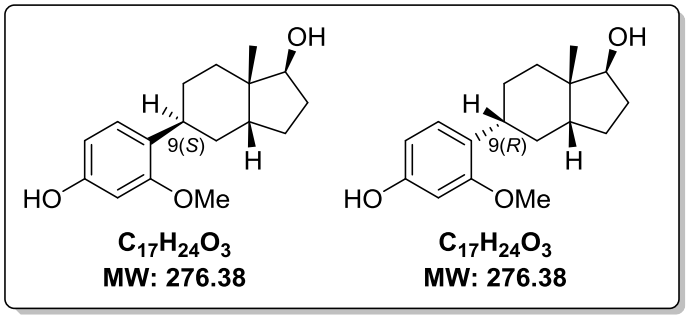
Isomer 106

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.24 (s, 1H), 6.88 (d, *J* = 8.1 Hz, 1H), 6.42 (d, *J* = 2.2 Hz, 1H), 6.35 (dd, *J* = 8.1, 2.3Hz, 1H), 5.57 (s, 1H), 3.87 (dd, *J* = 11.2, 5.8 Hz, 1H), 3.73 (s, 3H), 3.57 (d, *J* = 4. 78 Hz, 1H [OH]), 2.43-2.19 (m, 3H), 2.18-1.95 (m, 3H), 1.68-1.47 (m, 2H), 1.45-0.92 (m, 5H), 1.00 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.9, 159.4, 136.8, 131.5, 130.7, 126.6, 108.6, 101.0, 79.5, 56.6, 46.2, 44.0, 33.7, 32.5, 30.7, 27.6, 21.4

HREIMS: calculated for $C_{17}H_{22}O_3$ = 274.1569, found = 274.1583

9*S*: (1*S*,3a*R*,5*S*,7a*S*)-5-(4-hydroxy-2-methoxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (83)
9*R*: (1*S*,3a*R*,5*R*,7a*S*)-5-(4-hydroxy-2-methoxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol



This compound was prepared according to the general **Method G** using the mixture of the unsaturated isomers **105** and **106** (60mg, 0.22 mmol) under high pressure of H_2 (used two big balloons of hydrogen gas) for 1 day. Flash chromatography was performed to elute both isomers starting from 5% EtOAc: Hexanes to 40% EtOAc:Hexanes to give a mixture of products **83** and its 9(*R*) epimer (58mg, 95%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 42% AcCN in water as mobile phase to yield compounds **83** (21 mg, 35%) and its 9(*R*) epimer (7 mg, 12% yield), both as white solids.

For the natural 9(*S*) isomer

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.13 (b, 1H [OH]), 7.01 (d, *J* = 8.2 Hz, 1H), 6.42 (d, *J* = 2.4 Hz, 1H), 6.37 (dd, *J* = 8.2, 2.4 Hz, 1H), 3.76 (s, 3H), 3.64 (t, *J* = 4.7 Hz, 1H), 3.44 (d, *J* = 4.3 Hz, 1H [OH]), 3.07 (tt, *J* = 11.2, 4.4 Hz, 1H), 2.98-2.94 (m, 1H), 2.25 – 2.04 (m, 2H), 1.86-1.72 (m, 2H), 1.69 – 1.48 (m, 5H), 1.31 (dt, *J* = 13.1, 4.0 Hz, 1H), 1.24 – 1.19 (m, 1H), 1.11 (s, 3H).

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.8, 158.3, 128.7, 128.1, 108.6, 100.7, 83.3, 56.6, 45.9, 43.6, 34.5, 33.9, 32.9, 32.0, 30.1, 28.3, 20.1

HREIMS: calculated for $C_{17}H_{24}O_3$ = 276.1725, found = 276.1749

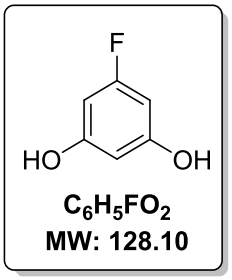
For the "un-natural" 9(*R*) isomer

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.12 (s, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.35 (dd, *J* = 8.2, 2.4Hz, 1H), 4.37 (dt, *J* = 8.7, 5.6 Hz, 1H), 3.75 (s, 3H), 3.47 (d, *J* = 5.6 Hz, 1H [OH]), 2.81-2.73 (m, 1H), 2.14 – 1.98 (m, 2H), 1.90 (td, *J* = 13.7, 3.1 Hz, 1H), 1.74-1.51 (m, 5H), 1.38-1.10 (m, 3H), 0.90 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.6, 158.3, 128.3, 128.2, 108.5, 100.6, 74.0, 56.5, 47.2, 44.5, 39.8, 37.5, 35.2, 31.3, 30.2, 28.7, 24.1

1.4.10 Synthesis of the C1-F TD-81 analog

5-fluorobenzene-1,3-diol (116)

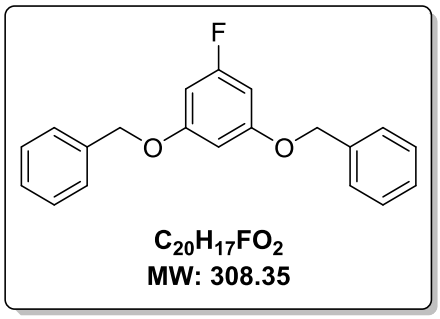


This compound was prepared following the procedures described by Makriyannis [142]. To a stirred solution of fluororesorcinol dimethyl ether **118** (1.5g, 9.6 mmol) in dry DCM (0.1M) at -78^{o}C under argon atmosphere was added boron tribromide (1M, 28 mL, 28.8 mmol). Following the addition, the reaction temperature was gradually raised over a period of 1h to r.t. and continued stirring until completion of the reaction. Unreacted boron tribromide was destroyed by adding MeOH and ice at 0^{o}C. The mixture was warmed to r.t., stirred for 40 min and concentrated *in vacuo*. The residue was diluted with EtOAc and washed with saturated sodium bicarbonate solution, water and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to yield the fluororesorcinol **116** (1.2g, 98%) as a white solid. The NMR spectra agree with the published data [142].

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 6.16 (d, *J* = 2.2 Hz, 1H), 6.15-6.13 (m, 2H)

^{13}C NMR (100MHz, $CDCl_3$) 166.1 (d, *J* = 239.9 Hz), 161.3 (d, *J* = 14.8 Hz), 100.5 (d, *J* = 2.8 Hz), 96.0 (d, *J* = 24.7 Hz)

(5-fluoro-1,3-phenylene)bis(oxy)bis(methylene)dibenzene (120)

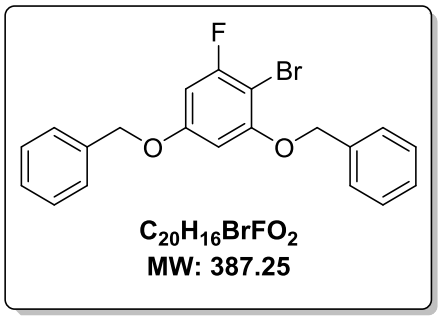


This compound was prepared following the procedures described by Frlan [159]. Benzyl bromide (2.45 mL, 20.6 mmol) was added dropwise to a stirred suspension of 5-fluorobenzene-1,3-diol **116** (1.12g, 8.7 mmol) and K_2CO_3 (2.85g, 20.6 mmol) in acetone (20 mL) under nitrogen atmosphere. The mixture was heated under reflux for 4h and filtered to remove the solid potassium carbonate. The filtrate was concentrated, diluted in EtOAc and washed with H_2O (2 x 15 mL). The combined organic phase was dried over $MgSO_4$, evaporated under reduced pressure and the crude product was purified by column chromatography using 20% EtOAc in Hexanes to give a white solid (2.4 g, 89%).

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.43 – 7.32 (m, 10H), 6.41 (dt, *J* = 2.2, 1.1 Hz, 1H), 6.35 (d, *J* = 2.2 Hz, 1H), 6.32 (d, *J* = 2.2 Hz, 1H), 5.01 (s, 4H)

^{13}C NMR (100MHz, $CDCl_3$) δ ppm 164.1 (d, *J* = 243.2 Hz), 160.6 (d, *J* = 13.9 Hz), 136.3, 128.6, 128.1, 127.5, 97.9 (d, *J* = 2.9 Hz), 95.4 (d, *J* = 25.6 Hz), 70.3

(4-bromo-5-fluoro-1,3-phenylene)bis(oxy)bis(methylene)dibenzene (122)

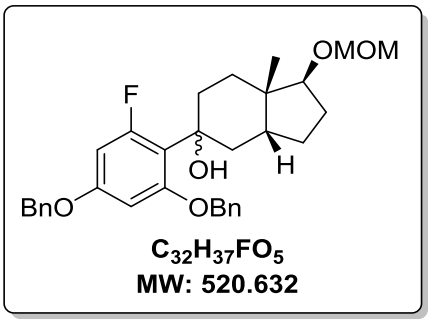


This compound was prepared following the procedures described by Simas [138]. To a stirred solution of diether **120** (1 g, 3.2 mmol) in DCM (20 mL) at –30°C was added solid NBS (577 mg, 3.2 mmol) under nitrogen atmosphere via a solid addition funnel. The mixture was stirred at the same temperature for 30 min. After removal of the cooling bath, the mixture was kept at r.t. for 3 days. Then, more DCM was added and the mixture was successively washed with H_2O (12 mL), brine (12 mL) and H_2O (12 mL). The organic layer was dried ($MgSO_4$) and concentrated. The crude material was purified by flash chromatography (hexanes/DCM, 30:70) and recrystallization affording the product **122** as a white solid (730 mg, 79% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.63-7.29 (m, 10H), 6.43 (dd, *J* = 11.2, 2.6 Hz, 1H), 6.42 (d, *J* = 2.4 Hz, 1H), 5.11 (s, 2H), 5.00 (s, 2H)

^{13}C NMR (100MHz, $CDCl_3$) δ ppm 160.5 (d, *J* = 233.8 Hz), 159.2 (d, *J* = 3.2 Hz), 156.7 (d, *J* = 6.6 Hz), 136.0 (d, *J* = 4.8 Hz), 128.708, 128.626, 128.311, 128.074, 127.510, 126.981, 97.8 (d, *J* = 2.7 Hz), 95.6 (d, *J* = 27.0 Hz), 91.3, 91.1, 71.0, 70.6

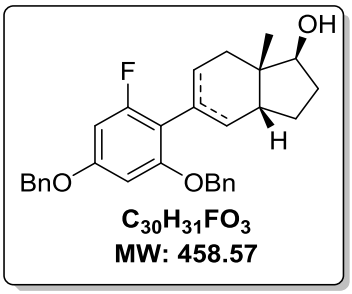
(1*S*,3a*R*,7a*S*)-5-(2,4-bis(benzyloxy)-6-fluorophenyl)-1-(ethoxymethoxy)-7a-ethyloctahydro-1*H*-inden-5-ol (126)



This compound was prepared according to the general **Method D** using the dibenzyl ether **122** (500 mg, 1.29 mmol) and *n*-BuLi (2.3M, 0.51mL, 1.19 mmol) in dry THF (10 mL), to which the protected CD-ring **41** (226.31 mg, 1.0 mmol) in dry THF (1 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **126** (300 mg, 45%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

[C9-C11 olefin]: (1*S*,3a*R*,7a*S*)-5-(2,4-bis(benzyloxy)-6-fluorophenyl)-7a-methyl-,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (127)

[C8-C9 olefin]: (1*S*,3a*R*,7a*S*)-5-(2,4-bis(benzyloxy)-6-fluorophenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (127)

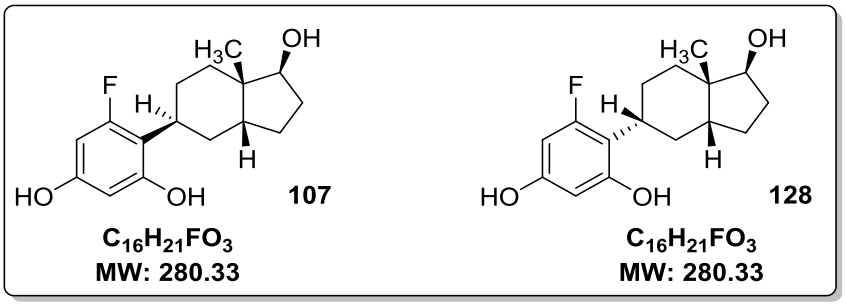


This compound was prepared according to the general **Method F** using the mixture of both isomers **126** (300 mg, 0.56 mmol) and PTSA (70mg, 0.41 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of unsaturated analogs **127** (120 mg, 47%).

H NMR (400MHz, $CDCl_3$) δ ppm 7.47-7.27 (m, 20H), 6.42-6.37 (m, 2H), 6.34 (dd, *J* = 10.9, 2.3 Hz, 2H), 5.67-5.60 (m, 1H), 5.57-5.51 (m, 1H), 5.01 (s, 4H), 4.99 (s, 4H), 3.89 (t, *J* = 11.5 Hz, 1H), 3.79 (dd, *J* = 6.31, 1.58 Hz, 1H), 2.52-1.98 (m, 8H), 1.93-1.67 (m, 3H), 1.65-1.34 (m, 7 H), 1.04 (s, 3H), 1.00 (s, 3H). The peaks are reported for the mixture of the two olefins.

5-fluoro-4-((1*S*,3a*R*,5*S*,7a*S*)-1-hydroxy-7a-methyloctahydro-1*H*-inden-5-yl)benzene-1,3-diol (107)

5-fluoro-4-((1*S*,3a*R*,5*R*,7a*S*)-1-hydroxy-7a-methyloctahydro-1*H*-inden-5-yl)benzene-1,3-diol (128)



This compound was prepared according to the general **Method G** using the mixture of the unsaturated isomers **127** (120 mg, 0.26 mmol) under high pressure of H_2 (used two big balloons of hydrogen gas) for 1 day. Flash chromatography was performed to elute both isomers starting from 5% EtOAc:Hexanes to 40% EtOAc:Hexanes to give a mixture of the products **107** and **128** as a yellowish gummy solid (70 mg, 96%). The two isomers needed be separated using a preparative reverse phase HPLC system eluting with 40% AcCN in water affording compounds **107** (28 mg, 38%) and **128** (17 mg, 23% yield), both as pink solids.

Isomer 107

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.58 (bs, 2H [OH]), 6.21 (dd, *J* = 2.3, 1.4 Hz, 1H), 6.05 (dd, *J* = 12.8, 2.4 Hz, 1H), 3.63 (d, *J* = 4.7 Hz, 1H), 3.54 (d, *J* = 3.6 Hz, 1H [OH]), 3.24 (tt, *J* = 12.8, 3.6 Hz, 1H), 2.25-2.04 (m, 4H), 1.86-1.71(m, 2H), 1.60-1.52 (m, 1H), 1.47-1.35 (m, 2H), 1.31-1.16 (m, 2H), 1.13 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 164.8 (d, *J* = 238.8 Hz), 158.8 (d, *J* = 12.9 Hz), 158.2 (d, *J* = 15.8 Hz), 113.4 (d, *J* = 17.0 Hz), 100.7 (d, *J* = 2.4 Hz), 96.5 (d, *J* = 27.6 Hz), 83.3, 45.8, 43.3, 34.3, 33.9, 30.0 (d, *J* = 2.4 Hz), 29.8, 28.0, 27.7 (d, *J* = 2.8 Hz), 20.0

HREIMS: calculated for $C_{16}H_{21}FO_3$ = 280.1475, found = 280.1462

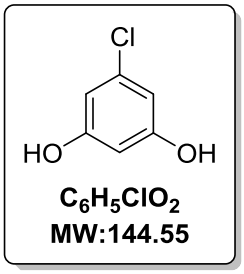
Isomer 128

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.55 (s, 1H [OH]), 8.47-8.38 (s, 1H [OH]), 6.19 (dd, *J* = 2.2, 1.4 Hz, 1H), 6.03 (dd, *J* = 12.8, 2.3 Hz, 1H), 4.41 (t, *J* = 8.2 Hz, 1H), 3.53 (bs, 1H [OH]), 2.94 (tt, *J* = 12.5, 3.2 Hz, 1H), 2.27-1.93 (m, 1H), 2.18-1.96 (m, 2H), 1.87 (td, J = 13.6, 3.2 Hz, 1H), 1.80-1.52 (m, 3H), 1.47-1.35 (m, 2H), 1.30 (dt, *J* = 13.5, 4.3 Hz, 1H), 1.16-1.08 (m, 1H), 0.88 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 164.5 (d, *J* = 238.9 Hz), 158.6 (d, *J* = 13.1 Hz), 158.2 (d, *J* = 15.7 Hz), 113.5 (d, *J* = 17.3 Hz), 100.7 (d, *J* = 2.6 Hz), 96.5 (d, *J* = 27.6 Hz), 74.0, 47.0, 44.4, 37.1 (d, *J* = 2.3 Hz), 35.5, 35.2, 31.3, 28.6, 27.9 (d, *J* = 2.7 Hz), 24.0

1.4.11 Synthesis of the C1-Cl TD-81 derivatives

5-chlorobenzene-1,3-diol (117)

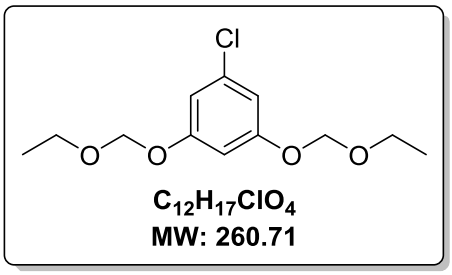


This compound was prepared following the procedures described by Makriyannis [142]. To a stirred solution of chlororesorcinol dimethyl ether (1.12g, 6.5 mmol) in dry DCM (0.1M) at -78^{o}C under argon atmosphere was added boron tribromide (1M, 19.5 mL, 19.5 mmol). Following the addition, the reaction temperature was gradually raised over a period of 1h to r.t. and continued stirring until completion of the reaction. Unreacted boron tribromide was destroyed by adding MeOH and ice at 0^{o}C. The mixture was warmed to r.t., stirred for 40 min and concentrated *in vacuo*. The residue was diluted with EtOAc and washed with saturated sodium bicarbonate solution, water and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to yield the chlororesorcinol **117** (0.95 g, 100%) as beige solid. The ^{1}H NMR spectrum agrees with the published data [142].

^{1}H NMR (400MHz, Acetone-d_6) δ ppm 8.74 (bs, 2H [OH]), 6.36 (d, J = 2.1 Hz, 1H), 6.29 (t, J = 2.1 Hz, 1H)

^{13}C NMR (400MHz, Acetone-d_6) δ ppm 161.2, 136.2, 108.9, 103.2

1-chloro-3,5-bis(ethoxymethoxy)benzene (121)

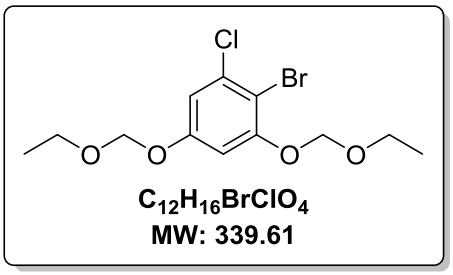


This compound was prepared according to the general **Method B** using the dihydroxybenzene **118** (1.0g, 7.5 mmol), DIPEA (5.95 mL, 26.1 mmol) and chloromethyl ethyl ether (2.43 mL, 26.1 mmol) in 40 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product as clear colorless oil (1.24 g, 63%).

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 6.72 (d, *J* = 2.2 Hz, 2H), 6.63 (t, *J* = 2.2 Hz, 1H), 5.17 (s, 4H), 3.71 (q, *J* = 7.1 Hz, 4H), 1.22 (t, *J* = 7.1 Hz, 6H)

^{13}C NMR (400MHz, $CDCl_3$) δ ppm 158.7, 135.0, 110.1, 103.4, 93.2, 64.4, 15.0

2-bromo-1-chloro-3,5-bis(ethoxymethoxy)benzene (123)



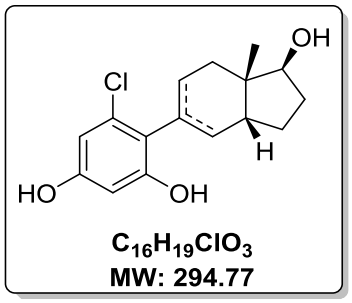
This compound was prepared following the procedures described by Simas [138]. To a stirred solution of diether **121** (1 g, 3.8 mmol) in DCM (15 mL) at –30°C was added solid NBS (682 mg, 3.8 mmol) under nitrogen atmosphere via a solid addition funnel. The mixture was stirred at the same temperature for 30 min. After removal of the cooling bath, the mixture was kept at r.t. for 3 days. Then, more DCM was added and the mixture was successively washed with H_2O (12 mL), brine (12 mL) and H_2O (12 mL). The organic layer was dried ($MgSO_4$) and concentrated. The crude material was purified by flash chromatography (hexanes/DCM, 30:70) affording the product **123** as white solid (740 mg, 78%).

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 6.89 (d, *J* = 2.6 Hz, 1H), 6.81 (d, *J* = 2.6 Hz, 1H), 5.26 (s, 2H), 5.17 (s, 2H), 3.76 (q, *J* = 7.2 Hz, 2H), 3.70 (q, *J* = 7.2 Hz, 2H) 1.22 (t, *J* = 7.1, 3H), 1.21 (t, *J* = 7.1, 3H)

^{13}C NMR (400MHz, $CDCl_3$) δ ppm 157.3, 155.7, 135.5, 111.1, 105.8, 103.5, 94.0, 93.3, 64.8, 64.5, 15.0, 15.0

[C9-C11 olefin]: 5-chloro-4-((1*S*,3a*R*,7a*S*)-1-hydroxy-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-5-yl)benzene-1,3-diol (125)

[C8-C9 olefin]: 5-chloro-4-((1*S*,3a*R*,7a*S*)-1-hydroxy-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-5-yl)benzene-1,3-diol (125)

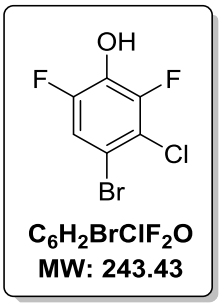


The coupling reaction was performed according to the general **Method D** using the diether **123** (662 mg, 1.95 mmol) and *n*-BuLi (2.3M, 0.75 mL, 1.73 mmol) in dry THF (15 mL), to the protected CD-ring **42** (340 mg, 1.5 mmol) in dry THF (2 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a complex mixture of both isomers **124** in addition to CD-ring starting material [ratio of approximately 1:2 product **124**: CD-ring **42**] (357 mg). The above mixture was deprotected and dehydrated according to the general **Method F** using PTSA (75mg, 0.43 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (39 mg, 8.8% over two steps). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase affording a cleaner mixture of the olefins C9-C11 (7 mg, 1.6% over two steps) and C8-C9 (11 mg, 3.7% over two steps), both as white solids. Due to the low coupling yield and difficulty to purify the final olefins, these compounds were not sent for bioassays.

For the C8-C9 olefin: ^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.51 (s, 1H [OH]), 7.85 (s, 1H [OH]), 6.41 (d, J = 2.3 Hz, 1H), 6.33 (d, J = 2.4 Hz, 1H), 5.59-5.41 (m, 1H), 4.05-3.70 (m, 1H), 3.56 (d, J = 4.8 Hz, 1H [OH]), 2.85-2.90 (m, 1H), 2.40-1.32 (m, 8H), 1.02 (s, 3H)

1.4.12 Synthesis of the C5-Cl L17 derivatives

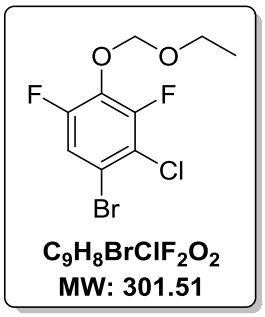
4-bromo-3-chloro-2,6-difluorophenol (131)



This compound was prepared following the procedures described by Marzi [144]. A solution containing 3-chloro-2,6-difluorophenol **129** (2 g, 13.51 mol) and *N*-bromo-succinimide (2.43 g, 13.51 mol) in chloroform (30 mL) was kept 2 h at 0^{o}C. After the addition of a saturated aqueous solution (15 mL) of sodium thiosulfate, the reaction mixture was extracted with DCM (3 x 20 mL). The organic layer was dried over $MgSO_4$, evaporated, purified by column chromatography 5% EtOAc in Hexanes to give the bromophenol **131** as a yellowish solid (1.77g, 54%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.22 (dd, *J* = 9.7, 2.3 Hz, 1H), 6.81-5.67 (m, 1H)

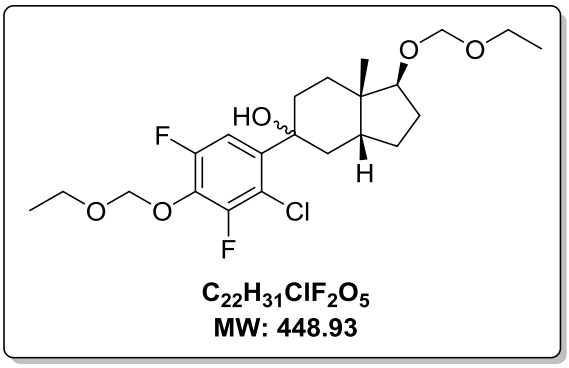
1-bromo-2-chloro-4-(ethoxymethoxy)-3,5-difluorobenzene (133)



This compound was prepared according to the general **Method B** using the bromophenol **131** (1.6 g, 6.57 mmol), DIPEA (2.29 mL, 13.15 mmol) and chloromethyl ethyl ether (1.22 mL, 13.15 mmol) in 30 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product **133** as clear colorless oil (1.69 g, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.26 (dd, *J* = 9.8, 2.3 Hz, 1H), 5.20 (s, 1H), 3.85 (q, *J* = 7.1 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 1H)

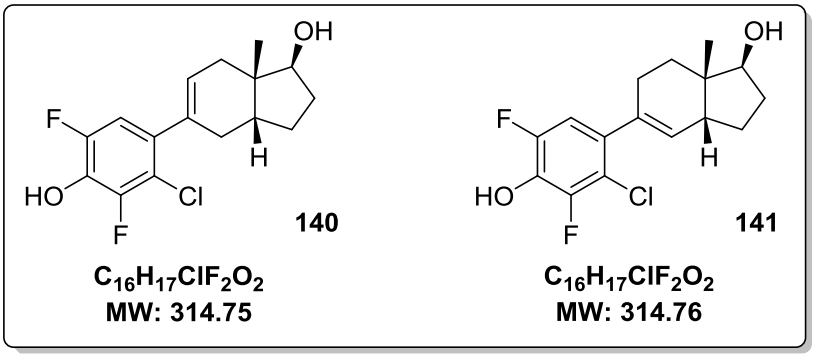
(1*S*,3a*R*,7a*S*)-5-(2-chloro-4-(ethoxymethoxy)-3,5-difluorophenyl)-1-(ethoxymethoxy)-7a-methyloctahydro-1*H*-inden-5-ol (138)



This compound was prepared according to the general **Method D** using the ether **133** (843 mg, 3.73 mmol) and *n*-BuLi (2.1 M, 2.8 mL, 5.59 mmol) in dry THF (25 mL), to which the protected CD-ring **42** (1.15 g, 5.08 mmol) in dry THF (4 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **138** (696 mg, 41.6%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(2-chloro-3,5-difluoro-4-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (140)

(1*S*,3a*R*,7a*S*)-5-(2-chloro-3,5-difluoro-4-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (141)



This compound was prepared according to the general **Method E** using the mixture of both isomers of diethers **138** (350 mg, 0.78 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (80 mg, 33%). Only 35 mg of these isomers were accomplished separated using reverse phase preparative recycling HPLC using 50% AcCN in water as mobile phase affording compounds **140** (8 mg, 3.3%) and **141** (3 mg, 1.2% yield), both as white solids.

Isomer 140

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 6.88 (dd, J = 11.1, 2.2 Hz, 1H), 5.62 (bs, 1H), 3.76(dd, J = 6.3, 1.9 Hz, 1H), 3.52 (bs, 1H [OH]), 2.48-2.37 (m, 1H), 2.25-2.07 (m, 3H), 1.91 (ddd, J = 8.1, 5.8, 2.9 Hz, 1H), 1.86-1.71 (m, 2H), 1.50 (m, 2H), 1.06 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 81.1, 44.0, 41.5, 34.1, 33.6, 31.2, 29.8, 21.6. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum assigned to the two fluorine atoms in the A-ring and no fluorine-decoupled ^{13}C NMR spectrum was taken.

HREIMS: calculated for $C_{16}H_{17}ClF_2O_2$ = 314.0885, found = 314.0915

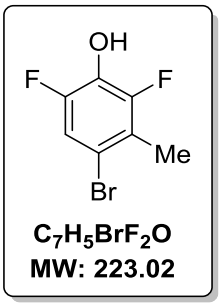
Isomer 141

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 6.88 (dd, *J* = 11.1, 2.2 Hz, 1H), 5.67 (bs, 1H), 3.88 (t, *J* = 5.8 Hz, 1H), 3.64 (bs, 1H, [OH], 2.45-2.07 (m, 5H), 1.70-1.53 (m, 2H), 1.48-1.32 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 79.4, 45.8, 44.0, 33.7, 31.9, 30.3, 27.7, 21.1. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum assigned to the two fluorine atoms in the A-ring and no fluorine-decoupled ^{13}C NMR spectrum was taken.

1.4.13 Synthesis of the C5-CH_3 L17 analogs

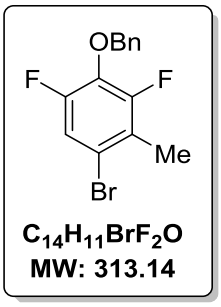
4-bromo-2,6-difluoro-3-methylphenol (132)



This compound was prepared following the procedures described by Marzi [144]. A solution containing 2,6-difluoro-3-methylphenol (5 g, 35.0 mol) and *N*-bromo-succinimide (6.23 g, 35.0 mol) in chloroform (30 mL) was kept 2 h at 0°C. After the addition of a saturated aqueous solution (15 mL) of sodium thiosulfate, the reaction mixture was extracted with DCM (3 x 20 mL). The organic layer was dried over $MgSO_4$, evaporated, purified by column chromatography 5% EtOAc in Hexanes to give **132** as a yellowish solid (6.1 g, 78%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.12 (dd, *J* = 9.7, 2.1 Hz, 1H), 5.77 (bs, 1H [OH]), 2.27 (s, 3H)

2-(benzyloxy)-5-bromo-1,3-difluoro-4-methylbenzene (134)



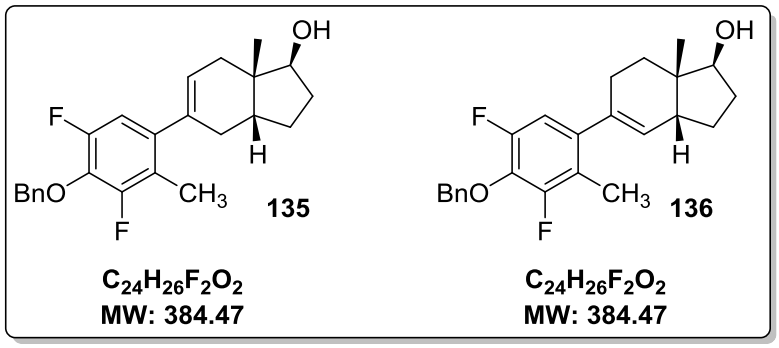
This compound was prepared following the procedures described by Chandrasekhar [160]. To a stirred solution of phenol **132** (4.3 g, 19.46 mmol) in acetone (40 mL) was added K_2CO_3 (5.38 g, 38.92 mmol), followed by benzyl bromide (1.85 mL, 15.57 mmol). The reaction mixture was refluxed for 30 min. After completion of the reaction, the reaction mixture was filtered, and washed with acetone. The filtrate was concentrated in vacuo and purified by silica gel column chromatography to yield the compound **134** (3.84 g, 31.5%) as colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.50-7.43 (m, 2H), 7.42-7.32 (m, 3H), 7.13 (dd, *J* = 10.0, 2.2 Hz, 1H), 5.16 (s, 2H), 2.28 (d, *J* = 2.8, 1.1 Hz, 3H)

^{13}C NMR (100MHz, $CDCl_3$) δ ppm 155.4 (dd, *J* = 67.8, 6.3 Hz), 152.9 (dd, *J* = 67.9, 6.3 Hz), 136.3, 134.4 (dd, *J* = 15.8, 14.2 Hz), 128.5, 128.2, 122.5 (dd, *J* = 17.6, 3.9 Hz), 117.3 (dd, *J* = 11.0, 6.5 Hz), 115.6 (dd, *J* = 22.3, 3.9 Hz), 76.0 (dd, *J* = 3.2, 3.2 Hz), 14.6 (d, *J* = 4.0 Hz)

(1*S*,3a*R*,7a*S*)-5-(4-(benzyloxy)-3,5-difluoro-2-methylphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (135)

(1*S*,3a*R*,7a*S*)-5-(4-(benzyloxy)-3,5-difluoro-2-methylphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (136)



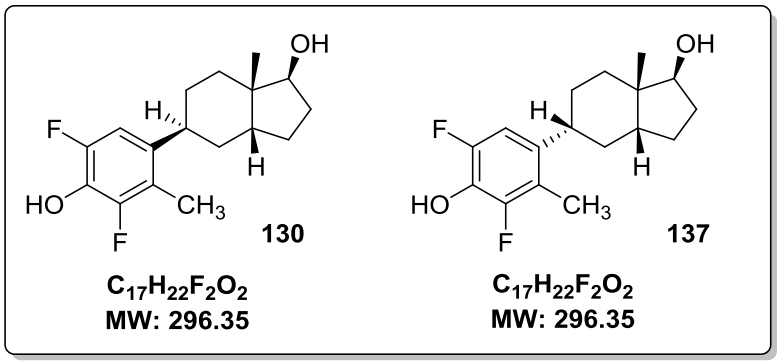
The C9-OH intermediate was prepared according to the general **Method D** using the benzyloxy ether **134** (2.08 g, 6.63 mmol) and *n*-BuLi (2 M, 3.31 mL, 6.63 mmol) in dry THF (20 mL), to which the-protected CD-ring **42** (741 mg, 4.42 mmol) in dry THF (2 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both C9-OH epimers (1.19 g, 58%). The ^{1}H spectrum was in agreement with the desired structure.

The mixture of C9-OH epimers (500 mg, 1.1 mmol) were then dehydrated and deprotected following to the general **Method E**. The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of products **135** and **136** isomers as yellow oil (310 mg, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.52-7.43 (m, 4H), 7.41-7.30 (m, 6H), 6.63 (dd, *J* = 4.1, 2.0 Hz, 1H), 6.60 (dd, *J* = 4.1, 2.0 Hz, 1H), 5.55 (bs, 1H), 5.46 (bs, 1H), 5.13 (s, 4H), 3.90 (t, *J* = 5.6 Hz, 1H), 3.82 (dd, *J* = 6.4, 1.8 Hz, 1H), 2.45-2.14 (m, 6H), 2.14-2.10 (m, 6H [C5-CH_3]), 2.10-1.99 (m, 3H), 1.94-1.71 (m, 2H), 1.69-1.30 (m, 9H), 1.08 (s, 3H), 1.04 (s, 3H). The recorded peaks are doubled due to the mixture of both olefins **135** and **136**.

(1*S*,3a*R*,5*S*,7a*S*)-5-(3,5-difluoro-4-hydroxy-2-methylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (130)

(1*S*,3a*R*,5*R*,7a*S*)-5-(3,5-difluoro-4-hydroxy-2-methylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (137)



This compound was prepared according to the general **Method G** using the mixture of the unsaturated isomers **135/136** (150 mg, 0.39 mmol) under high pressure of H_2 (used two big balloons of hydrogen gas) for one day. Flash chromatography was performed to elute both isomers starting from 5% EtOAc:Hexanes to 40% EtOAc:Hexanes to give a mixture of both olefins as a white solid (93 mg, 80%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase, to give compounds **130** (30 mg, 26%) and **137** (27 mg, 23%), both as white solids.

Isomer 130

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.98-8.16 (m, 1H), 6.87 (dd, *J* = 12.4, 2.1 Hz, 1H), 3.65 (d, *J* = 5.6 Hz, 1H), 3.60-3.48 (m, 1H), 2.99-2.88 (m, 1H), 2.28-2.19 (m, 1H), 2.16 (dd, *J* = 2.7, 1.0 Hz, 3H), 2.15-2.07 (m, 1H), 1.89-1.78 (m, 2H), 1.72-1.46 (m, 5H), 1.36 (dt, *J* = 13.2, 3.7 Hz, 1H), 1.26 (dtd, *J* = 6.9, 3.7, 1.1 Hz, 1H), 1.13 (s, 1H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 153.6 (dd, *J* = 25.7, 6.4 Hz), 151.3 (dd, *J* = 25.7, 6.4 Hz), 138.7 (dd, *J* = 6.5, 3.4 Hz), 132.9 (dd, *J* = 17.9, 16.2 Hz), 120.2 (dd, *J* = 13.3, 3.4 Hz), 109.9 (dd, *J* = 18.7, 3.2 Hz), 83.2, 45.9, 43.5, 35.0, 34.3, 33.9, 33.5, 30.0, 28.3, 20.1, 10.9 (d, *J* = 6.3 Hz)

HREIMS: calculated for $C_{17}H_{22}F_2O_2$ = 296.1588, found = 296.1594

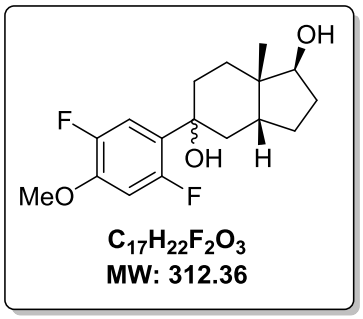
Isomer 137

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.87-8.18 (m, 1H [OH]), 6.81 (dd, *J* = 12.4, 2.1 Hz, 1H), 4.40 (t, *J* = 8.5 Hz, 1H), 3.71-3.30 (m, 1H [OH]), 2.74-2.56 (m, 1H), 2.16 (dd, *J* = 2.7, 1.1 Hz, 3H), 1.94 (td, *J* = 13.8, 3.2 Hz, 1H), 2.13-1.98 (m, 2H), 1.81-1.71 (m, 1H), 1.67-1.47 (m, 4H), 1.45-1.10 (m, 3H), 0.91 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 151.8 (dd, *J* = 31.7, 6.4 Hz), 149.4 (dd, *J* = 31.7, 6.4 Hz), 136.9 (dd, *J* = 6.5, 3.4 Hz), 131.1 (dd, *J* = 17.9, 16.1 Hz), 118.2 (dd, *J* = 13.4, 3.4 Hz), 107.8 (dd, *J* = 18.7, 3.2 Hz), 72.1, 45.1, 44.9, 42.6, 38.5, 37.9, 33.1, 28.6, 26.7, 22.1, 9.1 (d, *J* = 6.3 Hz)

1.4.14 Synthesis of the 2,5-difluoro A-CD derivatives

(1*S*,7a*S*)-5-(2,5-difluoro-4-methoxyphenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol (148)

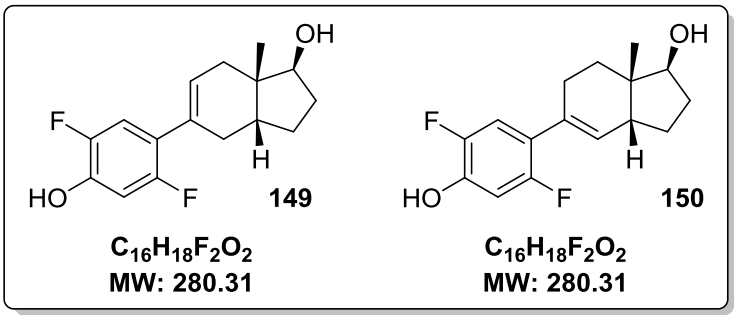


$C_{17}H_{22}F_2O_3$
MW: 312.36

This compound was prepared according to the general **Method C** using the commercially available 1-bromo-2,5-difluoro-4-methoxybenzene **147** (4.0 g, 17.85 mmol) and *n*-BuLi (1.6 M, 9.59 mL, 15.35 mmol) in dry THF (40 mL), to which unprotected CD-ring **40** (600 mg, 3.57 mmol) in dry THF (4 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers (221 mg, 20%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(2,5-difluoro-4-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (149)

(1*S*,3a*R*,7a*S*)-5-(2,5-difluoro-4-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (150)



To a solution of compound **53** (300 mg, 1.02 mmol) in DMF (2 mL) was added sodium methanethiolate (285 mg, 4.08 mmol) and the solution was heated to reflux for 3h. The reaction was quenched with crushed ice and the solution was extracted with EtOAc (3 x 5 mL). The organic phase was washed with NH_4Cl (5 mL) and re-extracted with 5 mL of EtOAc. The solution was dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography was used to purify the mixture starting from 25 % EtOAc:Hexanes to 40 % EtOAc:Hexanes and to give the mixture of products **130/137** as orange solids (100 mg, 37.9 %). These isomers were subjected to reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase, which affording compounds **130** and **137** cleanly, both as white solids.

Isomer 130

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.01 (dd, *J* = 11.8, 7.3 Hz, 1H), 6.72 (dd, *J* = 11.8, 7.5 Hz, 1H), 5.82 (bs, 1H), 3.74 (dd, *J* = 6.4, 2.6 Hz, 1H), 2.58-2.37 (m, 1H), 2.25-2.07 (m, 3H), 1.93 (ddd, *J* = 18.2, 5.9, 3.1 Hz, 1H), 1.85-1.72 (m, 2H), 1.58-1.32 (m, 2H), 1.03 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 132.0 (d, *J* = 1.5 Hz), 127.5 (d, *J* = 3.8 Hz), 124.0 (dd, *J* = 16.5, 6.0 Hz), 117.4 (dd, *J* = 20.6, 6.7 Hz), 107.0 (dd, *J* = 28.3, 3.0), 157.6 (dd, *J* = 241.5, 1.9 Hz), 149.5 (dd, *J* = 236.2, 2.8 Hz), 146.1 (dd, *J* = 15.1, 12.4 Hz), 81.0, 43.9, 41.8, 34.6, 33.5, 31.6, 29.9, 21.8.

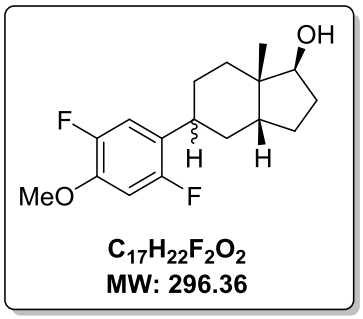
Isomer 137

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.00 (dd, J = 11.9, 7.3 Hz, 1H), 6.72 (dd, J = 11.8, 7.5 Hz, 1H), 5.84 (bs, 1H), 3.84 (t, J = 6.0 Hz, 1H), 2.40-2.24 (m, 2H), 2.19-2.06 (m, 3H), 1.68-1.49 (m, 2H), 1.49-1.25 (m, 2H), 1.00 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6 [data taken from the DEPT-135) δ ppm 79.1, 45.9, 33.4, 31.9, 30.2, 26.9, 26.8, 20.9. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum assigned to the two fluorine atoms in the A-ring and no fluorine-decoupled ^{13}C NMR spectrum was taken.

HREIMS: calculated for $C_{16}H_{18}F_2O_2$ = 280.1275, found = 280.1293

(1*S*,5*S*,7a*S*)-5-(2,5-difluoro-4-methoxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (151)

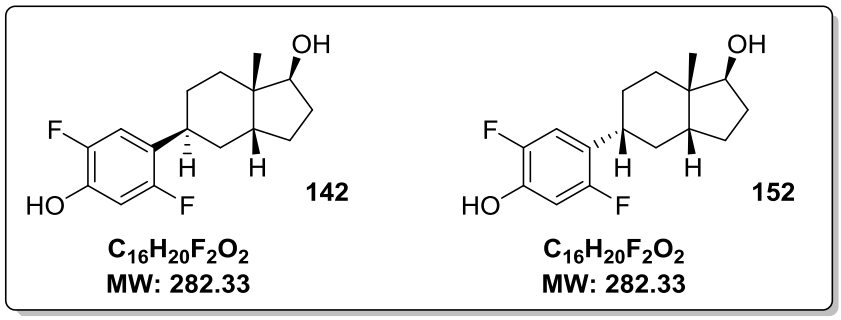


This compound was prepared according to the general **Method H** using the mixture of adducts **148** (171 mg, 0.55 mmol), triethylsilane (0.723 mL, 4.54 mmol) and $BF_3.OEt_2$ (0.34 mL, 2.75 mmol). Flash chromatography was used starting from 5% EtOAc:Hexanes to 30% EtOAc:Hexanes to give a mixture of compounds **151** (119 mg, 73%).

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.00-6.82 (m, 2H), 6.68-6.59 (m, 2H), 4.34 (t, *J* = 8.5 Hz, 1H), 3.83 (d, *J* = 1.6 Hz, 6H), 3.71 (d, *J* = 5.9 Hz, 1H), 3.12-2.60 (m, 1H), 2.42-2.05 (m, 2H), 1.98-1.34 (m, 20H), 1.11 (s, 3H), 0.91 (s, 3H). Recorded peaks are shown for the mixture of olefins.

(1*S*,3a*R*,5*S*,7a*S*)-5-(2,5-difluoro-4-hydroxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (142)

(1*S*,3a*R*,5*R*,7a*S*)-5-(2,5-difluoro-4-hydroxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (152)



To a chilled solution (0^oC) of compound **151** (66 mg, 0.226 mmol) DCM (4 mL) under nitrogen atmosphere was added boron tribromide (0.647 mL, 0.647 mmol) [161]. The reaction was quenched with water and sat. $NaHCO_3$ and the solution was extracted with DCM. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give a mixture of the products **142** and **152** (57 mg, 90%). These isomers were subjected to reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase, affording compounds **142** (27 mg, 42%) and **152** (30 mg, 47%) cleanly, both as white solids.

Isomer 142

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 9.00 (m, 1H), 7.06 (dd, *J* = 11.9, 7.0 Hz, 1H), 6.69 (dd, *J* = 11.2, 7.5 Hz, 1H), 3.87(bs, 1H [OH]), 3.68 (t, *J* = 8.4 Hz, 1H), 2.78-2.66 (m, 1H), 2.04-1.92 (m, 1H), 1.87 (td, *J* = 12.6, 3.1 Hz, 1H), 1.74-1.40 (m, 7H), 1.40-1.26 (m, 1H), 1.20 (dt, *J* = 12.7, 4.5 Hz, 1H), 0.84 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 82.6, 47.0, 44.2, 39.1, 38.7, 33.8, 31.670, 30.149, 27.064, 11.821. The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum assigned to the two fluorine atoms in the A-ring and no fluorine-decoupled ^{13}C NMR spectrum was taken.

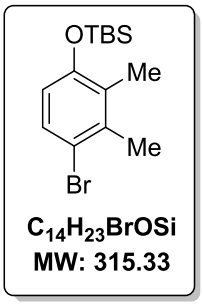
HREIMS: calculated for $C_{16}H_{20}F_2O_2$= 282.1431, found = 282.1424

Isomer 152

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.82 (bs, 1H [OH]), 7.04 (dd, *J* = 12.1, 7.0 Hz, 1H), 6.69 (dd, *J* = 11.1, 7.5 Hz, 1H), 4.39 (t, *J* = 8.3 Hz, 1H), 3.46 (d, *J* = 3.9 Hz, 1H [OH]), 2.69 (tt, *J* = 12.0, 2.8 Hz, 1H), 2.16-1.99 (m, 2H), 1.93 (td, *J* = 13.9, 3.2 Hz, 1H), 1.80-1.52 (m, 6H), 1.43-1.30 (m, 1H), 1.23-1.12 (m, 1H), 0.90 (s, 3H)

1.4.15 Synthesis of the 4,5-dimethyl A-CD derivatives

(4-bromo-2,3-dimethylphenoxy)(*tert*-butyl)dimethylsilane (153)



To a solution of 2,3-dimethylphenol (11g, 90 mmol) in acetonitrile (300 mL) was added *N*-bromosuccinimide (NBS, 15.218 g, 85.5 mmol) [145]. The reaction was stirred for 1.5h and NBS (3 x 800 mg) was added to the solution every 30 min. The solution was stirred over the week-end and was concentrated under reduced pressure and DCM (50 mL) was added. The succinimide was filtered and removed and the product was concentrated under reduced pressure. The residue was purified by silica gel chromatography 5% EtOAc:Hexanes to 20% EtOAc:Hexanes provided 4-bromo-2,3-dimethylphenol as a yellowish solid (10.0 g, 58%). The ^{1}H NMR spectrum agrees with the published data [145].

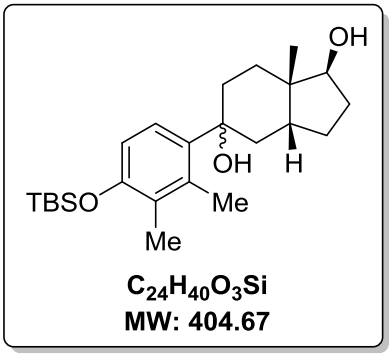
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.25 (d, *J* = 8.7 Hz, 1H), 6.52 (d, *J* = 8.6 Hz, 1H), 4.92 (s, 1H [OH]), 2.37 (s, 3H), 2.23 (s, 3H)

^{13}C NMR (100MHz, $CDCl_3$) δ ppm 152.5, 137.4, 129.8, 124.6, 116.3, 113.9, 19.8, 12.8

The above compound (4.98 g, 24.79 mmol) was prepared according to the general **Method A** using imidazole (2.19 g, 32.23 mmol), TBDMSCl (4.11 g, 27.27 mmol) and DMAP (trace). The crude compound was concentrated and subjected to flash chromatography (100 % Hexanes) to give the TBS **ether 153** as a colorless oil (6.70 g, 86 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.24 (d, *J* = 8.7 Hz, 1H), 6.43 (d, *J* = 8.6 Hz, 1H), 2.35 (s, 3H), 2.19 (s, 3H), 1.01 (s, 9H), 0.20 (s, 6H)

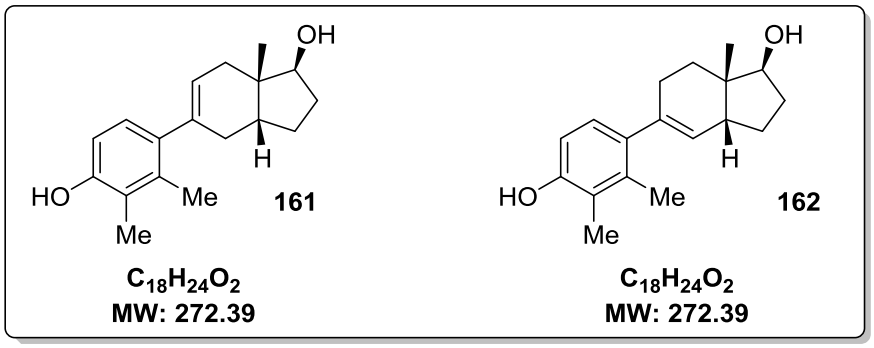
(1*S*,7a*S*)-5-(4-(*tert*-butyldimethylsilyloxy)-2,3-dimethylphenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol (157)



This compound was prepared according to the general **Method C** using the TBS ether **153** (6.57 g, 20.83 mmol) and *n*-BuLi (1.4 M, 14.9 mL, 20.83 mmol) in dry THF (20 mL), to which unprotected CD-ring **40** (1g, 5.95 mmol) in dry THF (3.5 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **157** (1.1g, 45%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2,3-dimethylphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (161)

(1*S*,3a*R*,7a*S*)-5-(4-hydroxy-2,3-dimethylphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (162)



This compound was prepared according to the general **Method E** using the mixture of both isomers of TBS ethers **157** (850 mg, 2.1 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (452 mg, 79%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 35% THF in water as mobile phase, affording compounds **161** (120 mg, 21%) and **162** (230 mg, 40%) cleanly, both as white solids.

Isomer 161

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.99 (s, 1H [OH]), 6.68 (d, J = 8.2 Hz, 1H), 6.63 (d, J = 8.2 Hz, 1H), 5.35 (bs, 1H), 3.79-3.69 (m, 1H), 3.56-3.50 (m, 1H, [OH]), 2.37-2.16 (m, 2H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11-1.96 (m, 2H), 1.89 (ddd, J = 18.0, 5.7, 2.7 Hz, 1H), 1.81-1.67 (m, 2H), 1.60-1.38 (m, 2H), 1.07 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 155.4, 139.2, 138.0, 136.4, 127.8, 124.6, 124.4, 113.7, 81.3, 44.0, 41.7, 34.1, 33.7, 33.0, 30.1, 21.7, 17.9, 13.1

HREIMS: calculated for $C_{18}H_{24}O_2$ = 272.1776, found = 272.1789

Isomer 162

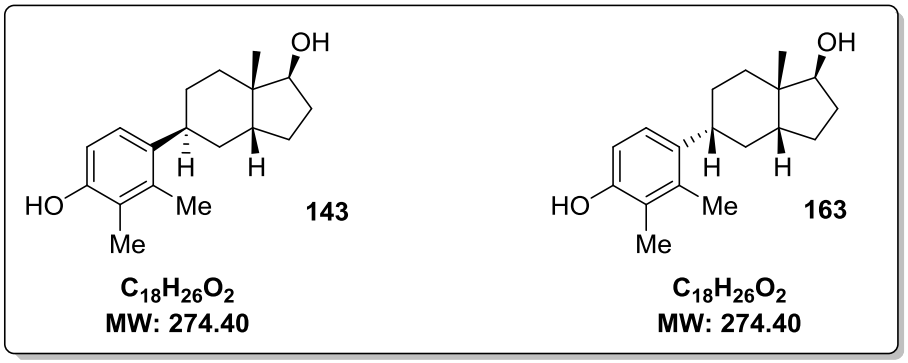
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.78 (d, *J* = 8.2 Hz, 1H), 6.59 (d, *J* = 8.1 Hz, 1H), 5.47 (bs, 1H), 4.93 (s, 1H, [OH]), 4.05-3.83 (m, 1H), 2.39-2.30 (m, 1H), 2.26-2.05 (m, 3H), 2.18 (s, 3H), 2.16 (s, 3H), 1.74-1.33 (m, 5H), 1.05 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 152.2, 137.2, 136.9, 135.2, 129.3, 126.2, 122.6, 111.9, 79.2, 43.8, 42.4, 32.1, 30.2, 29.1, 27.5, 19.4, 16.9, 11.9

HREIMS: calculated for $C_{18}H_{24}O_2$ = 272.1776, found = 272.1793

(1*S*,3a*R*,5*S*,7a*S*)-5-(4-hydroxy-2,3-dimethylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (143)

(1*S*,3a*R*,5*R*,7a*S*)-5-(4-hydroxy-2,3-dimethylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (163)



This compound was prepared according to the general **Method G** using the mixture of the unsaturated isomers **161/162** (225 mg, 0.826 mmol) under high pressure of H_2 for 1 day. Flash chromatography was performed to elute both isomers starting from 5% EtOAc:Hexanes to 40% EtOAc:Hexanes to give a mixture of both isomers as in one single spot (180 mg, 79%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 35% THF in water as mobile phase affording compounds **143** (72 mg, 32%) and **168** (80 mg, 35 %), both as white solids.

Isomer 143

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.00 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 8.4 Hz, 1H), 4.70-4.49 (m, 1H), 3.74 (d, *J* = 5.5 Hz, 1H), 2.92 (tt, *J* = 12.1, 3.8 Hz, 1H), 2.35-2.23 (m, 1H), 2.22 (s, 3H), 2.19 (s, 3H), 2.18-2.05 (m, 1H), 1.98-1.75 (m, 2H), 1.72-1.52 (m, 5H), 1.44-1.35 (bs, 1H [OH]), 1.31 (dd, *J* = 8.5, 3.7 Hz, 2H), 1.14 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 151.4, 137.4, 135.6, 123.3, 122.6, 112.3, 82.6, 44.3, 41.8, 33.7, 32.6, 32.1, 31.9, 28.5, 26.5, 18.5, 15.0, 12.2

HREIMS: calculated for $C_{18}H_{26}O_2$ = 274.1933, found = 274.1934

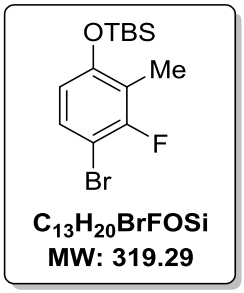
Isomer 163

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.91 (d, *J* = 8.4 Hz, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 4.66 (bs, 1H, [OH]), 4.39 (t, *J* = 8.2 Hz, 1H), 2.65 (tt, *J* = 12.4, 3.2 Hz, 1H), 2.29-2.18(m, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.15-1.97 (m, 1H), 1.90 (td, *J* = 13.8, 3.0 Hz, 1H), 1.85-1.75 (m, 1H), 1.65-1.39 (m, 3H), 1.35-1.05 (m, 3H), 0.94 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 151.4, 137.5, 135.5, 122.9, 122.7, 112.3, 73.8, 45.4, 42.7, 39.2, 38.5, 33.4, 30.0, 289, 26.9, 22.2, 15.0, 12.2

1.4.16 Synthesis of the 4-Me-5-F A-CD Adduct

(4-bromo-3-fluoro-2-methylphenoxy)(*tert*-butyl)dimethylsilane (154)



To a solution of 3-fluoro-2-methylphenol (6g, 47.57 mmol) in acetonitrile (150 mL) was added *N*-bromosuccinimide (8.043 g, 45.19 mmol) [145]. The reaction was stirred for 1.5h and *N*-bromosuccinimide (424 mg x 3) was added to the solution every 30 min. The solution was stirred for 1h and was concentrated under reduced pressure and DCM (25 mL) was added. The succinimide was filtered and removed and the product was concentrated under reduced pressure. The residue was purified by silica gel chromatography 5% EtOAc:Hexanes to 20% EtOAc:Hexanes provided 4-bromo-3-fluoro-2-methylphenol as a yellowish powder (7.68 g, 78.5%).

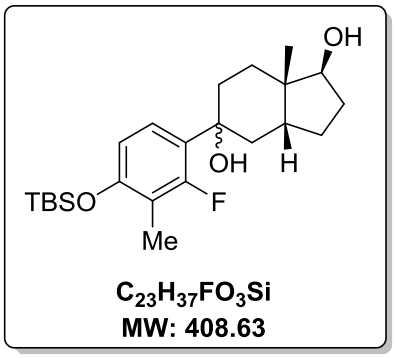
^{1}H NMR (300 MHz, Acetone-d_6) δ ppm 8.90 (bs, 1H [OH]), 7.23 (dd (apparent t), *J* = 8.4, 8.4 Hz, 1H), 6.67 (dd, *J* = 8.7, 1.3 Hz, 1H), 2.14 (d, *J* = 2.2 Hz, 1H)

The above compound (7.5 g, 36.58 mmol) was protected according to the general **Method A** using imidazole (3.23 g, 47.55 mmol), TBDMSCl (6.06 g, 40.24 mmol) and DMAP (trace). The crude compound was concentrated and subjected to flash chromatography (100 % Hexanes) to give the TBS ether **154** as a colorless oil (8.57 g, 98 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (dd (apparent t), *J* = 8.4, 8.4 Hz, 1H), 6.50 (dd, *J* = 8.7, 1.4 Hz, 1H), 2.16 (d, *J* = 2.3 Hz, 3H), 1.02 (s, 9H), 0.22 (s, 6H)

^{13}C NMR (100MHz, $CDCl_3$) 157.9 (d, *J* = 243.9 Hz), 154.4 (d, *J* = 7.4 Hz), 129.3 (d, *J* = 2.2 Hz), 118.2 (d, *J* = 18.8 Hz), 115.3 (d, *J* = 3.2 Hz), 100.2 (d, *J* = 22.7 Hz), 25.7, 18.3, 9.1 (d, *J* = 4.2 Hz), -4.3

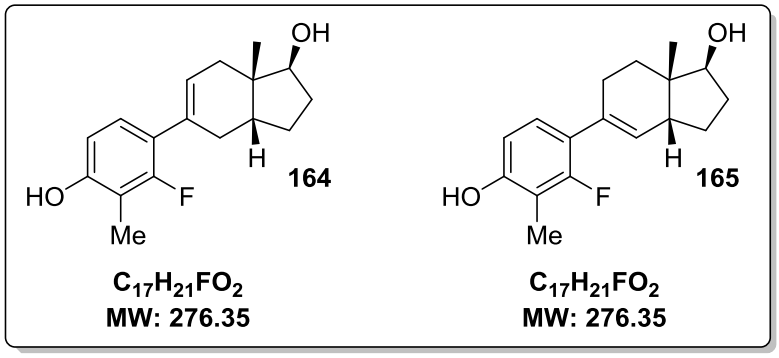
(1*S*,7a*S*)-5-(4-(*tert*-butyldimethylsilyloxy)-2-fluoro-3-methylphenyl)-7a-methyloctahydro-1*H*-indene-1,5-diol (158)



This compound was prepared according to the general **Method C** using the TBS ether **154** (6.65 g, 20.83 mmol) and *n*-BuLi (1.4 M, 14.9 mL, 20.83 mmol) in dry THF (40 mL), to which unprotected CD-ring **40** (1g, 5.95 mmol) in dry THF (4 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers (339 mg, 18%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(2-fluoro-4-hydroxy-3-methylphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (164)

(1*S*,3a*R*,7a*S*)-5-(2-fluoro-4-hydroxy-3-methylphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (165)



This compound was prepared according to the general **Method E** using the mixture of both isomers of TBS ethers **164**/**165** (317 mg, 0.776 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (202 mg, 96%). These isomers were subjected to reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase, which affording compounds **164** and **165** cleanly, both as white solids.

Isomer 164

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.61 (bs, 1H [OH], 6.89 (t, *J* = 8.7 Hz, 1H), 6.62 (dd, *J* = 8.4, 0.7 Hz, 1H), 5.71 (bs, 1H), 3.74 (dd, *J* = 6.0, 1.2 Hz, 1H), 3.55 (bs 1H, [OH]), 2.54-2.40 (m, 1H), 2.26-2.05 (m, 3H), 2.09 (d, *J* = 2.3 Hz, 3H), 1.92 (ddd, *J* = 18.9, 5.8, 3.0 Hz, 1H), 1.87-1.69 (m, 2H), 1.57-1.11 (m, 2H), 1.03 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 160.8 (d, *J* = 242.4 Hz), 157.2 (d, *J* = 8.6 Hz), 133.6 (d, *J* = 1.1 Hz), 128.0 (d, *J* = 7.0 Hz), 126.0 (d, *J* = 3.0 Hz), 124.4 (d, *J* = 16.1 Hz), 112.0 (d, *J* = 3.0 Hz), 113.4 (d, *J* = 20.9 Hz), 81.2, 44.0 (s), 42.0, 34.6, 33.6, 31.1, 29.9, 21.9, 8.9 (d, *J* = 6.3 Hz)

HREIMS: calculated for $C_{17}H_{21}FO_2$ = 276.1526, found = 276.1523

Isomer 165

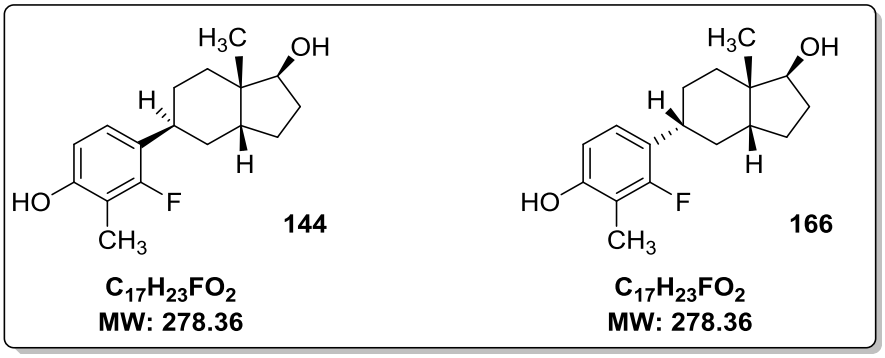
^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.59 (bs, 1H [OH]), 6.89 (t, *J* = 8.7 Hz, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 5.75 (bs, 1H), 3.86 (t, *J* = 5.9 Hz, 1H), 3.61 (bs, 1H [OH]), 2.42-2.22 (m, 3H), 2.17-1.99 (m, 1H), 2.09 (d, *J* = 2.3 Hz, 3H), 1.68-1.52 (m, 2H), 1.46-1.26 (m, 3H), 1.00 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 160.7 (d, *J* = 242.4 Hz), 157.2 (d, *J* = 8.6 Hz), 133.4 (d, *J* = 1.2 Hz), 132.2 (d, *J* = 2.8 Hz), 127.9 (d, *J* = 7.0 Hz), 124.2 (d, *J* = 16.0 Hz), 113.2 (d, *J* = 20.7 Hz), 112.0 (d, *J* = 2.8 Hz), 46.2, 44.0, 33.7, 32.3, 27.5, 27.5, 21.2, 8.9 (d, *J* = 6.4 Hz)

HREIMS: calculated for $C_{17}H_{21}FO_2$ = 276.1526, found = 276.1530

(1*S*,3a*R*,5*S*,7a*S*)-5-(2-fluoro-4-hydroxy-3-methylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (144)

(1*S*,3a*R*,5*R*,7a*S*)-5-(2-fluoro-4-hydroxy-3-methylphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (166)



The C9-OH epimers were prepared according to the general **Method H** using the mixture of adducts **158** (122 mg, 0.311 mmol), triethylsilane (0.41 mL, 2.58 mmol) and $BF_3.OEt_2$ (0.19 mL, 1.56 mmol). Flash chromatography was used starting from 5% EtOAc:Hexanes to 30% EtOAc:Hexanes providing the reduced C9-H epimers (70 mg, 55%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.81-6.94 (m, 2H), 6.52-6.55 (m, 2H), 4.38 (t, *J* = 8.5 Hz, 1H), 3.73 (d, *J* = 5.6 Hz, 1H), 3.06-2.67 (m, 2H), ~2.11 (two doublets, 6H [Me]), between 2.5-0.5 (m, 22H), 1.13 (3H, s), 1.01 (s, 18H), 0.93(s, 3H), 0.21 (s, 12H). Peaks are recorded for the two isomers.

The above C9-H epimers (62 mg, 0.154 mmol) were dissolved in THF (2 mL), and TBAF (0.17 mL, 0.170 mmol) was added to the solution dropwise. The resulting mixture was left for 10 min, diluted with brine (1 mL) and water (1 mL), and extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was subjected to a silica gel column chromatography. Elution with 25% EtOAc/75% hexane afforded a mixture of compounds **144** and **166** (20 mg, 47%), as well as the isolated compounds **144** (6mg, 27%) and **166** (6mg, 27%), both as white solids. Because only few mg (3-5 mg) are required for bioassays, the mixture was not further separated by preparative HPLC.

Isomer 144

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.94 (t, *J* = 8.4 Hz, 1H), 6.53 (d, *J* = 8.4 Hz, 1H), 4.8 (bs, 1H [OH]), 3.73 (d, *J* = 5.9 Hz, 1H), 3.06-2.90 (m, 1H), 2.26 (ddd, *J* = 18.3, 10.9, 5.5 Hz, 1H), 2.15 (d, *J* = 2.0 Hz, 1H), 2.13-2.03 (m, 1H), 1.94-1.76 (m, 2H), 1.73-1.58 (m, 5H), 1.42 -1.23 (m, 4H), 1.13 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 169.5, 153.3, 138.3, 129.4, 118.7, 112.7 (d, *J* = 5.9 Hz), 82.7, 44.0, 41.5, 33.1 (d, *J* = 1.9 Hz), 32.5, 32.1 (d, *J* = 2.31), 29.7, 29.3, 26.2, 18.4.

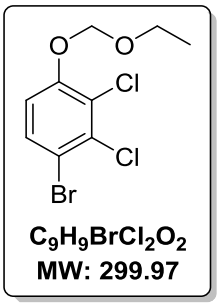
HREIMS: calculated for $C_{17}H_{23}FO_2$ = 278.1682, found = 278.1687

Isomer 166

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.86 (t, *J* = 8.4 Hz, 1H), 6.51 (dd, *J* = 8.4, 0.8Hz, 1H), 4.81 (bs, 1H, [OH]), 4.38 (t, *J* = 8.5 Hz, 1H), 2.71 (tt, *J* = 12.0, 4.0 Hz, 1H), 2.27-2.16 (m, 1H), 2.14 (d, *J* = 2.0 Hz, 3H), 2.13-2.00 (m, 1H), 1.88 (td, *J* = 14.2, 3.1 Hz, 1H), 1.83-1.75 (m, 1H), 1.72-1.61 (m, 2H), 1.55-1.37 (m, 3H), 1.35-1.07 (m, 2H), 0.93 (s, 3H)

1.4.17 Synthesis of the 4,5-dichloro A-CD analogs

1-bromo-2,3-dichloro-4-(ethoxymethoxy)benzene (155)



To a chilled (0°C) solution of 2,3-dichlorophenol (10 g, 61.35 mmol) in DCM (50 mL) was added bromine (3.68 mL, 71.43 mmol) from a dropping funnel within 40 minutes [146]. The red solution was stirred overnight at rt. The reaction mixture was slowly poured into 10% aqueous $NaHSO_3$ solution. The two phases were separated and the aqueous phase was extracted with DCM (3 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo.* The crude product was eluted with EtOAc: heptanes to EtOAc: heptanes on column chromatography afforded 4-bromo-2,3-dichlorophenol as white crystals (11.85 g, 80 %). The ^{1}H NMR spectrum agrees with the published data [146].

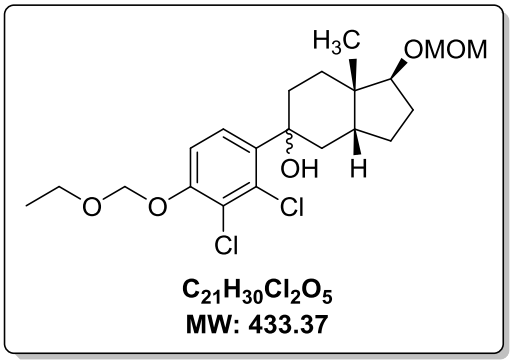
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.45 (d, *J* = 9.0 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 5.66 (s, 1H [OH])

The above compound (3.97 g, 9.85 mmol) was protected according to the general **Method B** using DIPEA (5.72 mL, 32.82 mmol) and chloromethyl ethyl ether (3.05 mL, 32.82 mmol) in 80 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product as a colorless oil (4.71g, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.45 (d, *J* = 9.0 Hz, 1H), 7.03 (d, *J* = 9.0 Hz, 1H), 5.29 (s, 2H), 3.75 (q, *J* = 7.1 Hz, 2H), 1.21 (t, *J* = 7.1 Hz, 3H)

^{13}C NMR (100MHz, $CDCl_3$) δ ppm 153.4, 134.0, 131.1, 124.0, 115.2, 114.9, 94.0, 64.9, 15.0

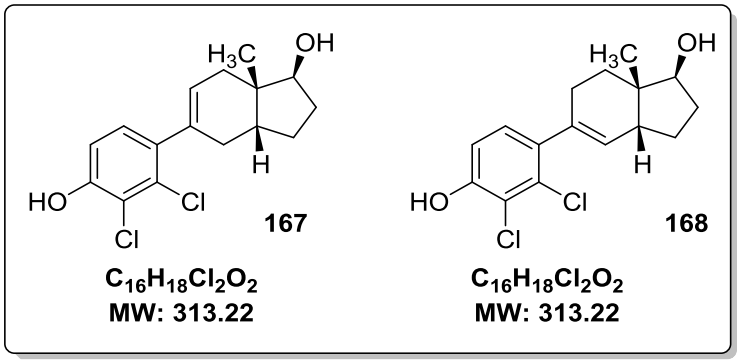
(1*S*,7a*S*)-5-(2,3-dichloro-4-((2-methoxyethoxy)methoxy)phenyl)-1-(methoxymethoxy)-7a-methyloctahydro-1*H*-inden-5-ol (159)



This compound was prepared according to the general **Method D** using ether **155** (2 g, 6.66 mmol) and *n*-BuLi (1.2 M, 3.32 mL, 3.98 mmol) in dry THF (25 mL), to which MOM-protected CD-ring **41** (940 mg, 4.43 mmol) in dry THF (1 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **159** (1.26 g, 40%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,7a*S*)-5-(2,3-dichloro-4-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (167)

(1*S*,7a*S*)-5-(2,3-dichloro-4-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (168)



This compound was prepared according to the general **Method E** using the mixture of both isomers of diethers **159** (477 mg, 1.1 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (162 mg, 47%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 40% AcCN in water as mobile phase, affording compounds **167** (50 mg, 14.5%) and **168** (39 mg, 11.3%), both as white solids.

Isomer 167

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 9.10 (bs, 1H, [OH]), 7.00 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 5.53 (bs, 1H), 3.76 (d, J = 5.9 Hz, 1H), 3.56 (d, J = 3.0 Hz, 1H [OH]), 2.48-2.34 (m, 1H), 2.25-2.06 (m, 3H), 1.90 (ddd, J = 18.0, 5.8, 2.8 Hz, 1H), 1.85-1.69 (m, 2H), 1.59-1.43 (m, 2H), 1.07 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 154.9, 138.1, 137.0, 132.9, 130.2, 127.0, 121.2, 116.4, 81.1, 44.0, 41.6, 34.1, 33.6, 31.4, 29.8, 21.7

HREIMS: calculated for $C_{16}H_{18}Cl_2O_2$ = 312.0684, found = 312.0672

Isomer 168

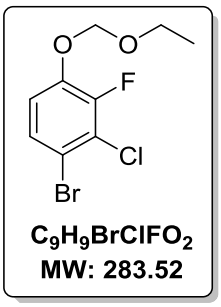
^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.00 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 5.57 (bs, 1H), 3.88 (t, J = 5.8 Hz, 1H), 3.68 (bs, 1H [OH]), 2.36-2.06 (m, 5H), 1.67-1.54 (m, 2H), 1.46-1.27 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 155.0, 138.0, 137.0, 133.2, 132.9, 130.1, 121.2, 116.4, 79.4, 45.8, 44.0, 33.7, 32.0, 30.4, 27.9, 21.2

HREIMS: calculated for $C_{16}H_{18}Cl_2O_2$ =312.0684, found = 312.0686

1.4.18 Synthesis of the 4-F-5-Cl A-CD Adduct

1-bromo-2-chloro-4-(ethoxymethoxy)-3-fluorobenzene (156)



To a solution of 3-chloro-2-fluorophenol (5.0 g, 34.12 mmol) in DCM (45 mL) was added bromine (4.10mL, 40.94 mmol) in 15 mL of DCM dropwise [146]. The reaction mixture was stirred overnight, and was slowly poured into a 10% aqueous sodium thiosulfate solution ($NaHSO_3$). The phases were separated, and the aqueous layer was extracted with DCM (3x 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was recrystallised with hot DCM and provided 4-bromo-3-chloro-2-fluorophenol as white solid (4.09g, 53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.30 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.85 (dd, *J* = 8.9, 8.4 Hz, 1H), 5.19 (bs, 1H [OH])

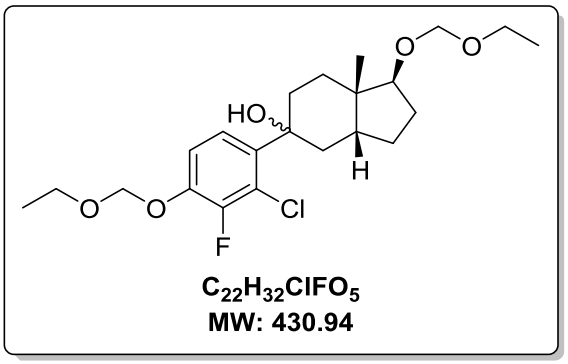
^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 147.9 (d, *J* = 241.6 Hz), 143.6 (d, *J* = 14.6 Hz), 128.3 (d, *J* = 4.2 Hz), 122.5 (d, *J* = 16.8 Hz), 116.4 (d, *J* = 2.1 Hz), 113.3 (d, *J* = 1.1 Hz)

The above compound (4.09g, 18.1 mmol) was protected according to the general **Method B** using DIPEA (3.36 mL, 36.3 mmol) and chloromethyl ethyl ether (6.32 mL, 36.3 mmol) in 60 mL of DCM. The crude product was purified on a silica column. Elution with 15% EtOAc in hexanes afforded the desired product **156** as clear colorless oil (3.86 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.32 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.05 (dd, *J* = 9.0, 7.9 Hz, 1H), 5.25 (s, 2H), 3.76 (q, *J* = 7.1 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 149.9 (d, *J* = 251.2 Hz), 145.4 (d, *J* = 11.1 Hz), 127.6 (d, *J* = 4.4 Hz), 123.2 (d, *J* = 17.1 Hz), 116.6 (d, *J* = 1.8 Hz), 115.0 (d, *J* = 1.1 Hz), 94.4, 64.9, 15.0

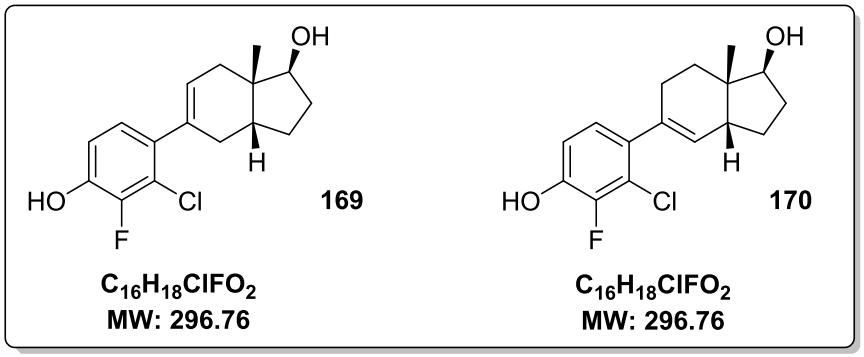
(1S,3aR,7aS)-5-(2-chloro-4-(ethoxymethoxy)-3-fluorophenyl)-1-(ethoxymethoxy)-7a-methyloctahydro-1H-inden-5-ol (160)



This compound was prepared according to the general **Method D** using the ether **156** (1.85 g, 6.53 mmol) and *n*-BuLi (2 M, 2.88 mL, 5.77 mmol) in dry THF (20 mL), to which the protected CD-ring **42** (1.15 g, 5.08 mmol) in dry THF (4 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both isomers **160** (1.06 g, 37.7%). The mixture of isomers appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

(1*S*,3a*R*,7a*S*)-5-(2-chloro-3-fluoro-4-hydroxyphenyl)-7a-methyl-2,3,3a,4,7,7a-hexahydro-1*H*-inden-1-ol (169)

(1*S*,3a*R*,7a*S*)-5-(2-chloro-3-fluoro-4-hydroxyphenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-ol (170)



This compound was prepared according to the general **Method F** using the mixture of both isomers of diethers **160** (400 mg, 0.93 mmol) and PTSA (100 mg, 0.58 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording a mixture of both dehydrated and deprotected isomers (225 mg, 82%). Separation of these isomers was accomplished using reverse phase preparative recycling HPLC using 42% AcCN in water as mobile phase affording compounds 169 (72 mg, 26%) and 170 (90 mg, 33%), both as white solids.

Isomer 169

^{1}H NMR (400 MHz, Acetone-d_6) ppm 6.91 (dd, J = 8.4, 8.4 Hz, 1H), 6.85 (dd, J = 8.5, 1.6 Hz, 1H), 5.56 (bs, 1H), 3.76 (dd, J = 6.4, 1.9 Hz, 1H), 3.49 (bs, 1H [OH]), 2.46-2.34 (m, 1H), 2.26-2.06 (m, 3H), 1.90 (ddd, J = 18.1, 5.7, 2.8 Hz, 1H), 1.84-1.69 (m, 2H), 1.59-1.42 (m, 2H), 1.06 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) ppm 149.2 (d, J = 242.2 Hz), 146.3 (d, J = 13.2 Hz), 137.3 (d, J = 2.0 Hz), 135.9 (d, J = 1.9 Hz), 127.4, 126.4 (d, J = 3.8 Hz), 121.4 (d, J = 14.4 Hz), 117.7 (d, J = 3.0 Hz), 81.1, 43.9, 41.6, 34.1, 33.5, 31.4, 29.8, 21.6

HREIMS: calculated for $C_{16}H_{18}Cl_FO_2$ = 296.0979, found = 296.0979

Isomer 170

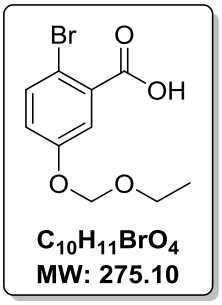
^{1}H NMR (400 MHz, Acetone-d_6) ppm 6.90 (dd, J = 8.5, 8.5 Hz, 1H), 6.85 (dd, J = 8.5, 1.3 Hz, 1H), 5.61 (bs, 1H), 3.89 (t, J = 5.8 Hz, 1H), 3.72 (bs, 1H [OH]), 2.37-2.06 (m, 5H), 1.67-1.55 (m, 2H), 1.47-1.27 (m, 2H), 1.02 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) ppm 149.3 (d, J = 242.1 Hz), 146.3 (d, J = 13.2 Hz), 137.3 (d, J = 2.0 Hz), 135.8 (d, J = 1.9 Hz), 133.6, 126.3 (d, J = 3.8 Hz), 121.4 (d, J = 14.4 Hz), 117.6 (d, J = 3.0 Hz), 79.4, 45.8, 44.0, 33.6, 32.0, 30.4, 27.9, 21.2

HREIMS: calculated for $C_{16}H_{18}Cl_FO_2$ = 296.0979, found = 296.0978

1.4.19 Synthesis of the spirolactone compound

2-bromo-5-(ethoxymethoxy)benzoic acid (179)



The 2-bromo-5-(ethoxymethoxy)benzaldehyde A-ring was prepared according to the general **Method B** using the commercially available 2-bromo-5-hydroxybenzaldehyde (2.00g, 9.95 mmol), DIPEA (3.47 mL, 19.90 mmol) and chloromethyl ethyl ether (1.85 mL, 19.90 mmol) in 40 mL of DCM. The crude product was purified on a silica column. Elution with 0.5% MeOH in hexanes afforded the desired product as clear colorless oil (2.28 g, 89%).

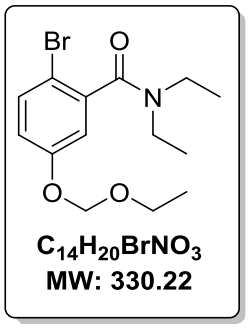
^{1}H NMR (400MHz, $CDCl_3$) δ ppm 10.31 (s, 1H), 7.56 (d, *J* = 3.2 Hz, 1H), 7.16 (dd, *J* = 8.8, 3.2 Hz, 1H), 2.24 (s, 2H), 3.71 (q, *J* = 7.2 Hz, 2H), 1.21 (t, *J* = 7.2 Hz, 3H)

The above aldehyde (1.69 g, 6.51 mmol) was dissolved in 5 mL of acetone and heated at reflux (70^{o}C) [162]. A hot solution of $KMnO_4$ (2.06g, 13.02 mmol) dissolved in 2 mL of water and 14 mL of acetone was added dropwise to the aldehyde solution over a period of 15 min. The solution was heated for 10 minutes. When completed, the mixture mixture was filtered hot to remove MnO_2. The precipitate was washed with acetone, the filtrate was combined and the solvent was evaporated, leaving a clear colorless oil, which was added to 10 mL of water. The mixture was cooled in a ice/water bath and acidified slowly by dropwise addition of conc. HCl. The water was evaporated *in vacuo* leaving a white solid (1.87 g, 100%).

^{1}H NMR (400MHz, Acetone-d_6) δ ppm 7.61 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 3.0 Hz, 1H), 7.13 (dd, *J* = 8.8, 3.1 Hz, 1H), 5.29 (s, 2H), 3.71 (q, *J* = 7.1 Hz, 1H), 1.16 (t, *J* = 7.1 Hz, 1H)

^{13}C NMR (100MHz, Acetone-d_6) δ ppm 166.2, 156.7, 135.0, 133.8, 120.7, 118.8, 112.0, 93.2, 64.2, 14.6

2-bromo-5-(ethoxymethoxy)-*N,N*-diethylbenzamide (177)

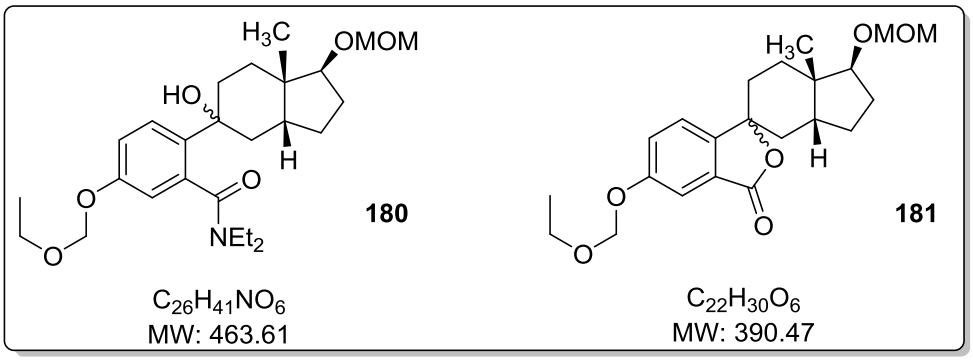


Diethylamine (0.94 mL, 9.10 mmol) was added to a stirring mixture of carboxylic acid **179** (500 mg, 1.82 mmol) HATU (2.073 g, 5.45 mmol) and DIPEA (0.97 mL, 5.45 mmol) in DMF (4 mL) under nitrogen atmosphere. After stirring for 24h, the reaction was concentrated and water (5 mL) and DCM (5 mL) were added to the reaction. The reaction was extracted with DCM (3 x 5 mL) and washed with a saturated solution of Na_2CO_3 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified using flash column chromatography with a 0.5% MeOH in hexanes elution solvent to afford the product as brownish oil (220 mg, 37%).

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.41 (d, *J*=8.6 Hz, 1 H), 6.94 - 6.87 (m, 2 H), 5.23 - 5.12 (m, 2 H), 3.86 - 3.64 (m, 3 H), 3.33 - 3.10 (m, 3 H), 1.28- 1.14 (m, 6 H), 1.06 (t, *J*=7.2 Hz, 3 H)

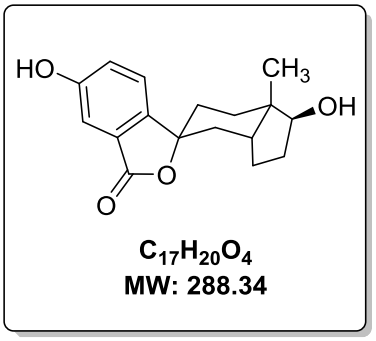
***N*,*N*-diethyl-2-((1*S*,7a*S*)-5-hydroxy-1-(methoxymethoxy)-7a-methyloctahydro-1*H*-inden-5-yl)-5-((2-methoxyethoxy)methoxy)benzamide (180)**

(1*S*,3a*R*,7a*S*)-5'-(ethoxymethoxy)-1-(methoxymethoxy)-7a-methyl-1,2,3,3a,4,6,7,7a-octahydro-3'*H*-spiro[indene-5,1'-isobenzofuran]-3'-one (181)



This compound was prepared according to the general **Method D** using the amide **177** (125 mg, 0.38 mmol) and *n*-BuLi (2M, 0.2 mL, 0.38 mmol) in dry THF (4 mL), to which MOM-protected CD-ring **41** (57 mg, 0.25 mmol) in dry THF (0.5 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column, affording a mixture of both compounds **180** and **181** (35 mg); which was monitored by LC-MS, and used as is for the next step.

(1*S*,1'*R*,3a*R*,7a*S*)-1,5'-dihydroxy-7a-methyl-1,2,3,3a,4,6,7,7a-octahydro-3'*H*-spiro[indene-5,1'-isobenzofuran]-3'-one (176)



This compound was prepared according to the general **Method E** using the mixture of both isomers of ethers **180/181** (35 mg). When completed, the reaction was concentrated *in vacuo*, purified first by column chromatography followed by preparative HPLC affording the spiro-lactone product **176** (22 mg, 30.5% over two steps) as a white solids.

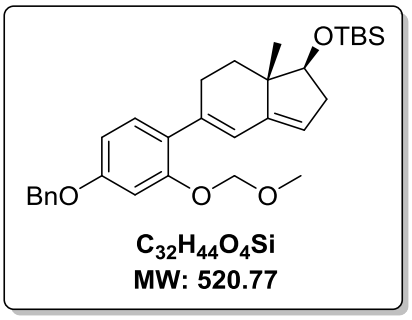
^{1}H NMR (400 MHz, MeOD) δ ppm 7.41 (d, *J* = 8.3 Hz, 1H), 7.16 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 3.82 (dd, *J* = 6.5, 2.9 Hz, 1H), 3.67 (bs, 1H, [OH]), 3.20 (bs, 1H), 2.40-2.18 (m, 1H), 2.16-2.00 (m, 4H), 1.97-1.81 (m, 1H), 1.78-1.51 (m, 2H), 1.39-1.31 (m, 2H), 1.18 (s, 3H)

^{13}C NMR (400MHz, MeOD) δ ppm 172.2, 160.1, 147.7, 127.7, 123.9, 123.8, 111.1, 89.0, 81.4, 44.4, 42.1, 36.4, 33.6, 32.3, 29.8, 28.4, 19.1

HREIMS calculated $C_{17}H_{20}O_4$= 288.1362, found = 288.1354

1.4.20 Synthesis of the *trans* A-CD analogs

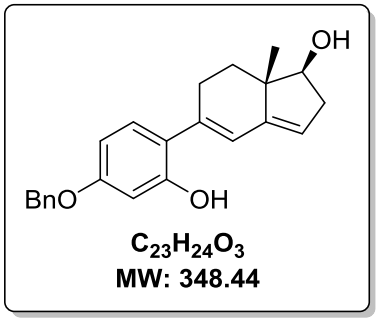
((1*S*,7a*S*)-5-(4-(benzyloxy)-2-(methoxymethoxy)phenyl)-7a-methyl-2,6,7,7a-tetrahydro-1*H*-inden-1-yloxy)(*tert*-butyl)dimethylsilane (212)



This compound was prepared according to the general **Method D** using 1-bromo-2-(methoxymethoxy)-4-(phenylmethoxy)-benzene **111** (1.3 g, 3.8 mmol) and *n*-BuLi (2.2 M, 1.6 mL, 3.5 mmol) in dry THF (20 mL), to which TBS-protected CD enone **43** (820 mg, 2.9 mmol) in dry THF (2 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column. The C9-OH intermediate obtained after coupling was eliminated under the acidic environment of the silica, affording the diene **212** (600 mg, 40%).

^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.50-7.29 (m, 5H), 7.11 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 6.60 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.25 (bs, 1H), 5.33 (bs, 1H), 5.13 (s, 2H), 5.05 (s, 2H), 4.01 (t, *J* = 8.1 Hz, 1H), 3.47 (s, 3H), 2.78-2.65 (m, 1H), 2.48 (dd, *J* = 18.3, 4.9 Hz, 1H), 2.42 (d, *J* = 8.3 Hz, 2H), 1.94-1.87 (m, 1H), 1.45 (dt, *J* = 12.4, 5.4 Hz, 1H), 1.01 (s, 3H), 0.93 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H)

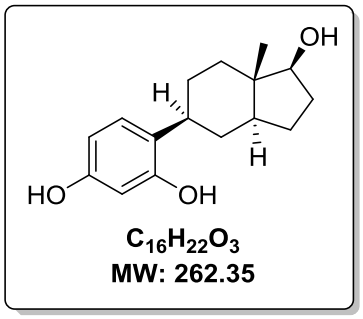
(1S,7aS)-5-(4-(benzyloxy)-2-hydroxyphenyl)-7a-methyl-2,6,7,7a-tetrahydro-1H-inden-1-ol (213)



This compound was prepared according to the general **Method F** using the mixture of both isomers of ethers **212** (600 mg, 1.15 mmol) and PTSA (59 mg, 0.34 mmol). The crude product was eluted with 5% EtOAc/hexanes to 40% EtOAc/hexanes on a silica gel column, affording the dehydrated product **213** as yellowish oil (185 mg, 46%).

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.32 (s, 1H, [OH]), 7.53-7.23 (m, 5H), 7.08 (d, J = 8.4 Hz, 1H), 6.53-6.49 (m, 2H), 6.34 (d, J = 2.4 Hz, 1H), 5.30 (bs, 1H), 5.06 (s, 2H), 4.01-3.92 (m, 2H), 2.95-2.84 (m, 1H, [OH]), 2.79-2.64 (m, 1H), 2.53 (dd, J = 18.2, 5.2 Hz, 1H), 2.48-2.33 (m, 2H), 1.98-1.92 (m, 1H), 1.46-1.36 (m, 1H), 1.01 (s, 3H)

4-((1S,3aS,5S,7aS)-1-hydroxy-7a-methyloctahydro-1H-inden-5-yl)benzene-1,3-diol (204)



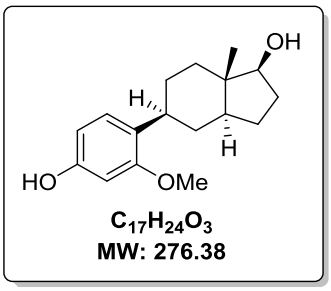
This compound was prepared according to the general **Method G** using the above unsaturated adduct **213** (60 mg, 0.17 mmol) under high pressure of H_2 (used two big balloons of hydrogen gas) for 1 day. The crude was purified by column chromatography eluting with 45 % EtOAc in hexanes afforded a mixture of two isomers as a yellowish oil (37 mg, 83%). The separation needed to be carried out using a preparative reverse phase HPLC system (45% AcCN in H_2O) affording compound **204** as white solid (10 mg, 22%).

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.11 (s, 1H, [OH]), 8.00 (s, 1H [OH]), 6.97 (d, *J* = 8.3 Hz, 1H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.29 (dd, *J* = 8.3, 2.4 Hz, 1H), 3.69-3.61 (m, 2H), 2.88-2.74 (m, 1H), 2.05-1.89 (m, 1H), 1.84 (td, *J* = 12.5, 3.2 Hz, 1H), 1.74-1.27 (m, 8H), 1.17 (dt, *J* = 12.6, 4.7 Hz, 1H), 0.84 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 157.9, 157.1, 129.3, 126.3, 108.3, 104.4, 82.8, 47.5, 44.4, 39.4, 39.1, 34.0, 31.9, 30.2, 27.3, 11.9.

HREIMS calculated $C_{16}H_{22}O_3$= 262.1569, found = 262.1570

(1*S*,3a*S*,5*S*,7a*S*)-5-(4-hydroxy-2-methoxyphenyl)-7a-methyloctahydro-1*H*-inden-1-ol (205)



Methyl iodide (0.02 mL, 0.344 mmol) was added at once to a stirred suspension of the benzyloxy A-CD adduct **213** (60 mg, 0.172 mmol) and K_2CO_3 (71 mg, 0.516 mmol) in acetone (5 mL) under nitrogen atmosphere. The mixture was heated under reflux for 2h and was allowed to cool at rt and stirring overnight. Once done, the reaction was filtered to remove the solid potassium carbonate, the filtrate was concentrated, diluted in EtOAc and washed with H_2O (2 x 15 mL). The combined organic phase was dried over $MgSO_4$, evaporated under reduced pressure to afford a crude diene product as a yellowish product (62 mg, 100%).

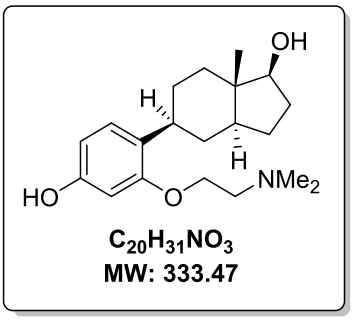
The above diene (62 mg, 0.171 mmol) was hydrogenated according to the general **Method G** under high pressure of H_2 (used two big balloons of hydrogen gas) for 1 day. The crude was purified by column chromatography eluting with 45 % EtOAc in hexanes followed by preparative reverse phase HPLC system (45% AcCN in H_2O). After HPLC, the desired product **205** was obtained as a white solid (19 mg, 40%, [ratio of 3.5: 1 *trans:cis* for the CD-junction]).

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 8.16 (s, 1H, [OH]), 7.01 (d, *J* = 8.2 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 6.36 (dd, *J* = 8.2, 2.4 Hz, 1H), 3.75 (s, 3H), 3.65-3.60 (m, 2H), 2.86-2.76 (m, 1H), 2.01-1.90 (m, 1H), 1.86-1.80 (m, 1H), 1.71-1.11 (m, 8H), 0.82 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm157.9, 156.5, 127.2, 126.8, 126.1, 106.8, 98.9, 80.9, 54.7, 45.6, 37.4, 37.3, 32.2, 30.1, 28.6, 25.4, 10.1

HREIMS calculated $C_{17}H_{24}O_3$= 262.1725, found = 262.1729

(1S,3aS,5S,7aS)-5-(2-(2-(dimethylamino)ethoxy)-4-hydroxyphenyl)-7a-methyloctahydro-1H-inden-1-ol (206)



2-Dimethyl aminoethyl chloride hydrochloride (46 mg, 0.32 mmol) was added at once to a stirred suspension of the benzyloxy A-CD adduct **213** (55 mg, 0.16 mmol) and K_2CO_3 (65.5mg, 0.474 mmol) in acetone (7 mL) under nitrogen atmosphere. The mixture was heated under reflux for 2h and was allowed to cool at rt and stirring overnight. Once done, the reaction was filtered to remove the solid potassium carbonate, the filtrate was concentrated, diluted in EtOAc and washed with H_2O (2 x 15 mL). The combined organic phase was dried over $MgSO_4$, evaporated *in vacuo* to afford the crude diene product as a yellowish product (50 mg, 75%).

^{1}H NMR (400 MHz, MeOD) δ ppm 7.46-7.25 (m, 5H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 1.9 Hz, 1H), 6.55 (td, *J* = 8.4, 2.3 Hz, 1H), 6.17 (bs, 1H), 5.28 (bs, 1H), 5.05 (d, *J* = 2.9 Hz, 2H), 4.05 (dt, *J* = 5.4, 2.5 Hz, 2H), 3.99-3.89 (m, 1H), 2.83-2.60 (m, 3H), 2.53-2.40 (m, 3H), 2.34 (s, 3H), 2.33 (s, 3H), 1.95 (dd, *J* = 12.4, 4.4 Hz, 1H), 1.41 (dt, *J* = 12.5, 5.4 Hz, 1H), 0.99 (s, 3H)

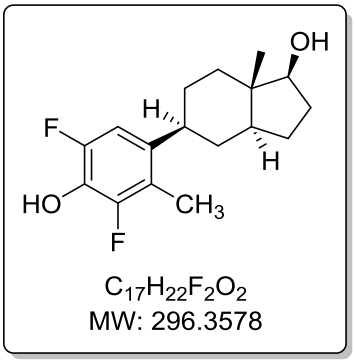
The above diene (50 mg, 0.12 mmol) was hydrogenated according to the general **Method G** under high pressure of H_2 (used two big balloons of hydrogen gas) for 4 h. The crude was purified by column chromatography eluting with 45 % EtOAc in hexanes followed by preparative HPLC (45% AcCN in water) affording compound **206** as a white solid (12mg, 30%, [ratio of 5: 1 *trans:cis* for the CD-junction]).

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 7.00 (d, J = 8.2 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 6.36 (dd, J = 8.2, 2.3 Hz, 1H), 4.02 (t, J = 5.8 Hz, 1H), 3.73-3.57 (m, 1H), 2.70 (t, J = 5.8 Hz, 1H), 2.28 (s, 6H), 2.03-1.81 (m, 2H), 1.73-1.24 (m, 8H), 1.21-1.10 (m, 1H), 0.84 (s, 3H)

^{13}C NMR (100 MHz, Acetone-d_6) δ ppm 159.0, 158.3, 129.2, 128.1, 108.7, 101.7, 82.7, 68.4, 60.1, 47.6, 47.3, 47.2, 44.5, 39.8, 39.2, 34.0, 31.9, 30.2, 27.3, 12.0

HREIMS calculated $C_{20}H_{31}NO_3$= 333.2304, found = 333.2291

(1S,3aS,5S,7aS)-5-(3,5-difluoro-4-hydroxy-2-methylphenyl)-7a-methyloctahydro-1H-inden-1-ol (207)



This compound was prepared according to the general **Method D** using the benzyloxy ether **134** (2g, 6.39 mmol) and *n*-BuLi (1.8 M, 3.27 mL, 5.89 mmol) in dry THF (30 mL), to which TBS-protected CD enone **43** (600mg, 2.14 mmol) in dry THF (2 mL) was added. The crude product was eluted with 5% EtOAc/hexanes to 10% EtOAc/hexanes on a silica gel column. The C9-OH intermediate dehydrated during purification which afforded the corresponding diene isomer (158 mg, 15 %) as a yellowish oil. The compound appeared to be consistent with the desired structure based on their ^{1}H NMR spectrum and were used directly without being fully characterized in the next step.

The above diene (62.5 mg, 0.13 mmol) was hydrogenated and reduced according to the general **Method G** under high pressure of H_2 (used two big balloons of hydrogen gas) for 4 h. The crude was purified by column chromatography eluting with 45 % EtOAc in hexanes. This step also resulted in the loss of C17-OTBS group. Further separation by preparative HPLC (45% AcCN in H_2O) provided a compound **207** as a white solid (9 mg, 23%, [ratio of 8: 1 *trans:cis* for the CD-junction]).

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm 6.88 (dd, J = 12.4, 2.0 Hz, 1H), 3.75 (bs, 1H, [OH]), 3.67 (t, J = 8.4 Hz, 1H), 2.78-2.66 (m, 1H), 2.16 (d, J = 1.9 Hz, 3H), 2.01-1.78 (m, 2H), 1.72-1.41 (m, 7H), 1.41-1.16 (m, 2H), 0.85 (s, 3H)

^{13}C NMR (125 MHz, Acetone-d_6) δ ppm 80.7, 45.3, 42.5, 39.7, 37.0, 32.4, 30.0, 29.7, 25.2, 10.0, 9.1 (d, J = 6.3 Hz). The "aromatic peaks" were not recorded due to the difficulty to interpret the fluorine-carbon coupling in the spectrum assigned to the two fluorine atoms in the A-ring and no fluorine-decoupled ^{13}C NMR spectrum was taken.

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Chapter 2: Isolation of potent anti-inflammatory sesquiterpene lactones from *Neurolaena lobata* (Asteraceae)

2.1 Introduction

Neurolaena lobata (L.) R. Br. Ex Cass. (Asteraceae), commonly known by the name Jackass bitters, is a medicinal plant widely used in Central America. It is found throughout Central America, southern Mexico and most of the Caribbean islands (**Figure 2.1.1**) [1], [2]. The indigenous traditional healers in these regions have used the leaves of this plant for various medicinal purposes such as treatment for stomach pains, headaches, diabetes, malaria, fevers, digestive disorders and skin diseases [2], [3]. Jackass Bitters is reported to possess anti-parasitic, anti-malarial, anti-ulcer and anti-bacterial and anti-fungal activities [2], [4–9]. Particularly relevant to our search for anti-inflammatory compounds from Central America was the report by De Las Heras and colleagues that the extract of *N. lobata* leaves was anti-inflammatory in carrageenan-induced inflammatory mouse paw edema in mice, reducing swelling by 19.5% [10]. Previous work on the leaves of this plant resulted in the isolation of several sesquiterpene lactones (SLs) [1], [11], [12], flavonoids [13] while the roots contained thymols [14].

Figure 2.1.1 Leaves and flowers of *N. lobata*.

On the basis of their use in folk medicine in Central America and their anti-inflammatory activities, we conducted an investigation of the leaves of this plant. The focus of this short chapter is based on the anti-inflammatory bioassays of five isolated sesquiterpene lactones. This ethnopharmacological project was carried out jointly with Brendan Walshe-Roussel, a PhD student from the Arnason group who performed the bioassays. The collection of plant material was made by him in collaboration with the Q'eqchi' Maya Healers in Belize.

2.1.1 Previous phytochemical reports

Previous phytochemical work on the leaves of this plant led to the isolation of several sesquiterpene lactones (SLs) [1], [11], [12] and flavonoids [13]. An earlier report on *N. lobata* was made by Manchand and Blount who isolated the germacranolide SLs neurolenins A (**1**) and B (**2**) as the main constituents from the leaves of plants collected in Trinidad [11]. Borges del Castillo and colleagues also isolated neurolenin B (**2**) and identified two SLs, the germacranolide lobatin **A** (**3**) and the furanoheliangolide lobatin B (**4**) from plants collected in Panama [12]. Later, Passreiter and colleagues identified seven additional SLs from the plant leaves collected in Guatemala [1].

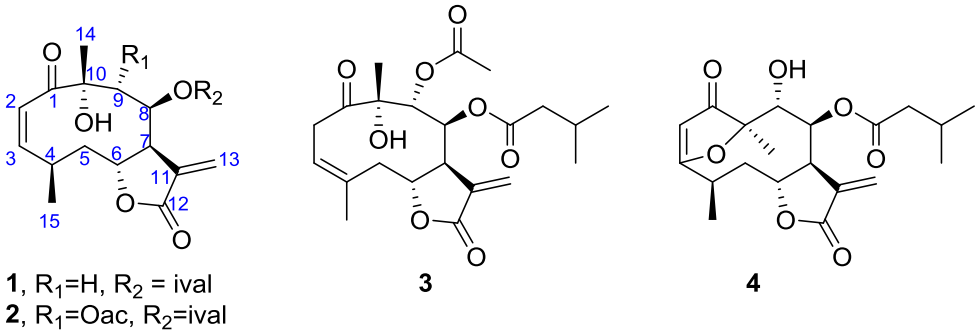


Figure 2.1.2 Chemical structure of neurolenin A and B (**1** and **2**) and lobatin A and B (**3** and **4**).

2.1.2 Biological activity of sesquiterpene lactones

A well-studied biological activity of many SLs is their anti-inflammatory action by activating the transcription factor nuclear factor kappa B (NF-κB), which is regulated by pro-inflammatory cytokines involved in the immune response, proliferation and apoptosis. Examples of these cytokines include tumor necrosis factor α (TNFα), interleukins and prostaglandins [15], [16]. When TNFα binds to its receptor, it causes the activation of the transcription factor nuclear factor kappa B (NF-κB) via the degradation of the inhibitor of NF-κB protein (IκB).

Structure-activity relationship (SAR) studies showed that their mode of action is ascribed to their exocyclic α-methylene-γ-lactone moiety that can serve as a site for covalent bonding via a Michael addition reaction [17]. In particular, Kwok and colleagues reported that the anti-inflammatory activity of the natural product parthenolide (**5**), a SL found in the anti-inflammatory medicinal herb feverfew (*Tanacetum parthenium* – Asteraceae), was mediated by the α-methylene γ-lactone moiety shared by other SLs [18]. Alkylation of parthenolide by a cysteine residue found in the activation loop of IκB results in the down-regulation of the TNFα-mediated inflammatory response [18]. Their work was further supported by the loss of activity of the reduced parthenolide (**6**).

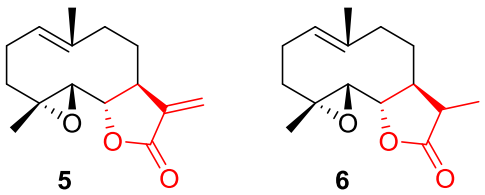


Figure 2.1.3 Structures of parthenolide (**5**) and reduced parthenolide (**6**).

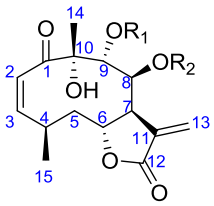
2.2 Results and Discussion

The combined findings from the phytochemical work and *in vivo* studies of the leaf extracts provided us an indication that the SLs present in *N. lobata* extracts could be responsible for anti-inflammatory activity. Moreover, feverfew and Jackass bitters share a similar history as traditional medicine for migraine headaches, arthritis and fever, and also share a similar biosynthetic class of active principles (SLs). Isolation and identification of the SLs present in the sample obtained from Belize was be the focus of this short chapter while their anti-inflammatory activity is described only briefly because it is provided in detail in the PhD thesis of Brendan Walshe-Roussel.

2.2.1 Plant extractions

The leaves of *N. lobata* (L.) were collected at the Itzamma Ethnobotanical Garden in Indian Creek, Toledo District, Belize and stored in 1L Nalgene bottles. The leaves were stored in a mixture of water and alcohol immediately after collection. Extraction methods are described in the experimental section. Wet leaves recovered from the packing liquid were ground into finely shredded pieces and extracted with 80% EtOH. Isolation of the sesquiterpene lactones was performed using several rounds of separation by column chromatography. Some compounds required further purification by preparative HPLC.

A total of five SLs were identified from the 80% EtOH leaf extract: neurolenin B (**2**), lobatin B (**4**), the isovaleryl ester isomers neurolenin C and D (**7**, **8**) and 9α-hydroxy-8β-isovalerianyloxy-calyculatolide (**9**). Structural determinations were carried out by the analysis of ^{1}H and ^{13}C NMR spectra and their MS traces [1], [2]. The yields of the isolated SLs are summarized in **Table 2.2.1**.



7, R_1 = ival, R_2= H
8, R_1 = H, R_2 = ival

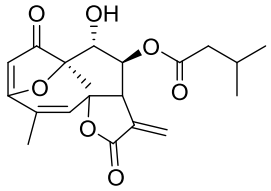


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Figure 2.2.1 Chemical structures of neurolenin **C** and **D**, **7** and **8**, and 9α-hydroxy-8β-isovalerianyloxy-calyculatolide, **9**.

Table 2.2.1 Yield of compounds isolated from *N. lobata*.

Compound	Mass (mg)	% of wet mass of plant[a]	% of dry ethanolic extract	% of dry plant material[b]
Neurolenin B (**2**)	69	0.014	0.17	0.075
Lobatin B (**4**)	100	0.021	0.24	0.11
Neurolenin C+D (**7,8**)	202	0.042	0.49	0.21
Compound **9**	50	0.010	0.12	0.054

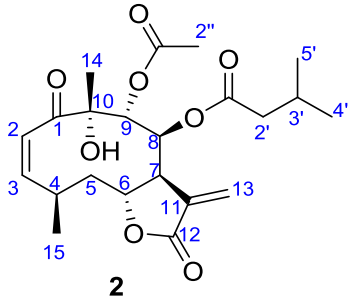
a- Percent yield in 80% EtOH crude extract.
b- Dry plant extract percentage was estimated by the mass of the compounds isolated divided by the combined dry ethanolic extract and residual plant material (93.3 g).

2.1.2 Sesquiterpene lactones isolated

Sesquiterpene lactones are naturally occurring phytochemicals that are formed from head-to-tail condensation of three isoprene units followed by cyclization and oxidative transformations [19]. They are classified based on their carbocyclic skeletons, and include several categories such as germacranolides, pseudoguainolides, eudesmanolides and heliangolides [20].

The compounds isolated belong to germacranolides (compounds **2**, **7** and **8**) and furanoheliangolildes (compounds **4** and **9**) families [1]. The NMR data observed for compounds **2**, **4** and **9** in this study were compared with literature values as seen in **Tables 2.2.2** and **2.2.3** [1]. Their MS fragmentation patterns further confirmed their structure.

Table 2.2.2 Comparison with literature for ^{1}H NMR data of compounds **2**, **4** and **9**.



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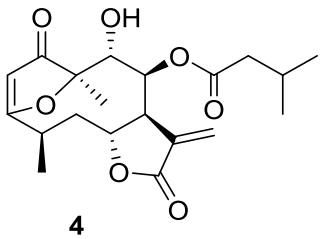

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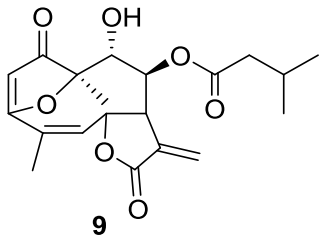

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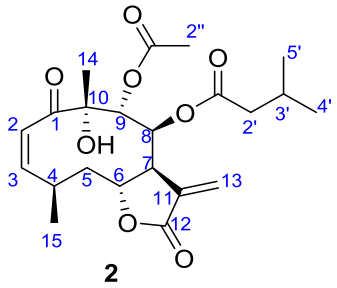
H	**Compound 2**		**Compound 4**		**Compound 9**	
	Observed δ[a]	Literature δ[b]	Observed δ[a]	Literature δ[b]	Observed δ[a,c]	Literature δ[b]
2	6.58 d	6.59 d	5.58 d	5.58 s	5.61 s	5.63 s
3	6.00 t	6.00 t	-	-	-	-
4	3.11 m	3.11 m	3.04 m	3.04 m	-	-
5a	1.83 ddd	1.83 ddd	2.60 ddd	2.60 m	5.95 m	5.98 m
5b	1.42 ddd	1.42 ddd	2.11-1.93 m	2.06 dd	-	-
6	4.54 dd	4.56 dd	4.48 dd	4.49 dd (br)	5.31 m	5.33 m
7	2.58 s (br)	2.58 s	3.63 m	3.63 m	3.86 m	3.83 m
8	5.55 s (br)	5.55 d	5.07 dd	5.08 d	5.05 d	5.10 dd
9	5.55 s (br)	5.55 d	4.10 d	4.12 d	4.02 d	4.04 d
13a	6.31s (br)	6.31 s (br)	6.33 d	6.34 d	6.32 d	6.36 d
13b	5.81 s (br)	5.81 s (br)	5.75 d	5.74 d	5.76 d	5.75 d
14	1.33 s	1.33 s	1.48 s	1.49 s	1.53 s	1.56 s
15	1.12 d	1.14 d	1.39 d	1.40 d	2.05 s	2.07 d
2'a	2.09 m	2.09 m	2.11-1.93 m	2.11 m	2.11 m	2.13 m
2'b	1.90-2.00 m	1.96 m	2.11-1.93 m	2.09 m	2.11 m	2.13 m
3'	1.90-2.00 m	1.95 m	2.11-1.93 m	1.98 m	0.96 m	2.00 m
4'	0.86 d	0.87 d	0.90 d	0.91 d	0.89 d	0.91 d
5'	0.86 d	0.87 d	0.89 d	0.90 d	0.87 d	0.89 d
2''	2.09 s	2.10 s	-	-	-	-

a- 400 MHz, $CDCl_3$, referenced by the solvent residual peak
b- 500 MHz, $CDCl_3$, TMS as internal standard
c- In general, the observed values are consistently lower than the literature value by approximately 0.02 ppm.

Table 2.2.3 Comparison with literature for ^{13}C NMR data of compounds **2**, **4** and **9**.



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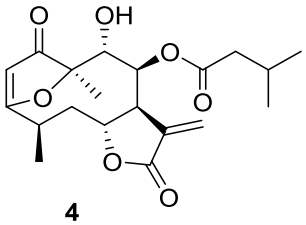

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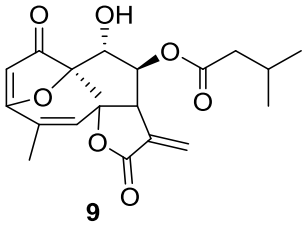

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C	Compound 2		Compound 4		Compound 9	
	Observed δ[a]	Literature δ[b]	Observed δ[c]	Literature δ[b]	Observed δ[a]	Literature δ[b]
1	204.6	204.7	204.4	204.3	204.4	203.9
2	125.3	125.4	105.4	103.7	104.2	104.2
3	148.1	148.2	194.3	193.1	186.0	185.7
4	28.2	28.3	32.9	31.4	131.4	131.4
5	40.2	40.3	42.6	40.9	134.0	134.3
6	76.3	76.4	74.6	72.9	73.1	73.4
7	41.2	41.3	47.5	45.8	43.8	44.1
8	73.9	74.0	79.8	77.4	77.8	77.2
9	73.8	73.9	76.1	74.6	75.3	75.2
10	79.3	79.4	92.7	91.0	90.2	89.8
11	134.8	134.9	143.5	139.9	139.3	139.1
12	168.8	168.7	169.9	168.8	169.0	168.6
13	126.5	126.5	123.4	123.9	124.7	124.6
14	23.7	23.7	19.6	18.9	19.5	19.6
15	19.7	19.7	17.4	16.2	17.8	17.7
1'	171.0	171.1	172.7	171.6	171.8	171.4
2'	42.5	42.6	44.2	42.8	42.8	42.8
3'	24.9	24.9	26.9	25.2	25.2	25.3
4'	22.2	22.3	23.5	22.3	22.2	22.2
5'	22.2	22.3	23.5	22.4	22.3	22.3
1''	170.2	170.3	-	-	-	-
2''	20.5	20.6	-	-	-	-

a- 100 MHz, $CDCl_3$, referenced by the solvent residual peak
b- 125 MHz, $CDCl_3$, TMS as internal standard
c- 100 MHz, Acetone-d_6, referenced by the solvent residual peak

It should be noted that compounds **7** and **8** were isolated as a mixture in a ratio of about 0.75:1, as determined by appropriate ^{1}H NMR signals after repeated flash chromatography experiments. The two isomers were separated by preparative HPLC, but upon re-injection of one of the single peak the mixture of isomers (2 peaks) would appear in the same ratio (**Figure 2.2.2**). This is due to the migration of isovaleric group from one to the neighbouring OH group (**Scheme 2.2.1**).

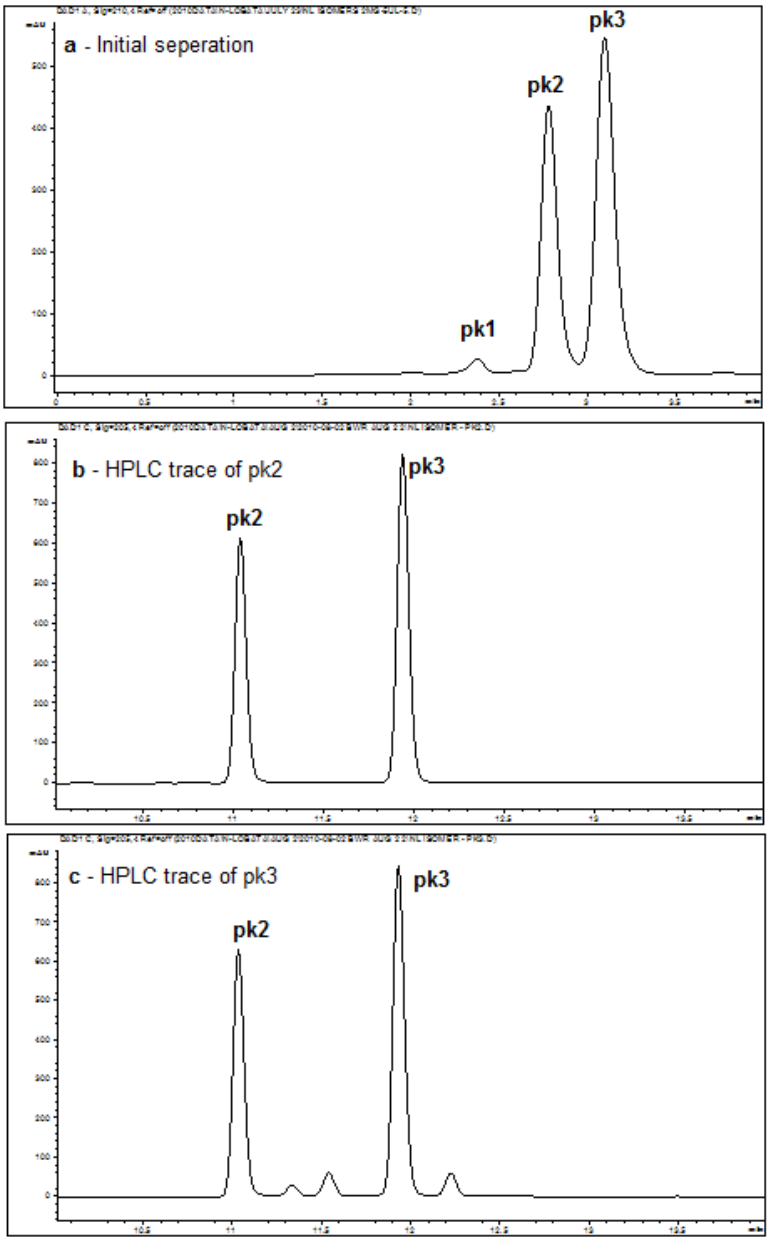
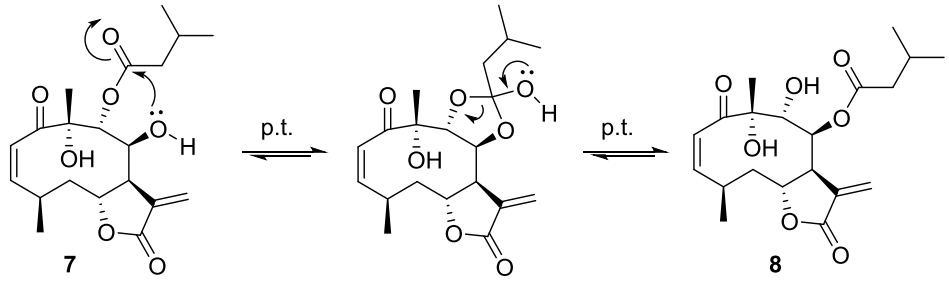


Figure 2.2.2 HPLC trace (UV signal at 210 nm) of the mixture of isovaleric isomers **7** and **8**, where **a** = initial HPLC trace of the isomers; **b** = HPLC trace of the second peak found in **a**; and **c** = HPLC trace of the third peak found in **a**.



Scheme 2.2.1 Mechanism of the migration of the isovaleric group from compounds **7** and **8**.

The identification of this mixture was facilitated by comparing it to neurolenin B (**2**), which differs in a loss of an acetate group (**Figure 2.2.3**). Once the mixture was found to share similar skeleton as the germacranolide **2**, we were able to assign these isomers confidently based on their ^{1}H and ^{13}C NMR spectra. Lastly, their MS fragmentation patterns confirmed their structure based on data reported in the literature [1]. Therefore, the bioassays were performed on the mixture of the isomers.

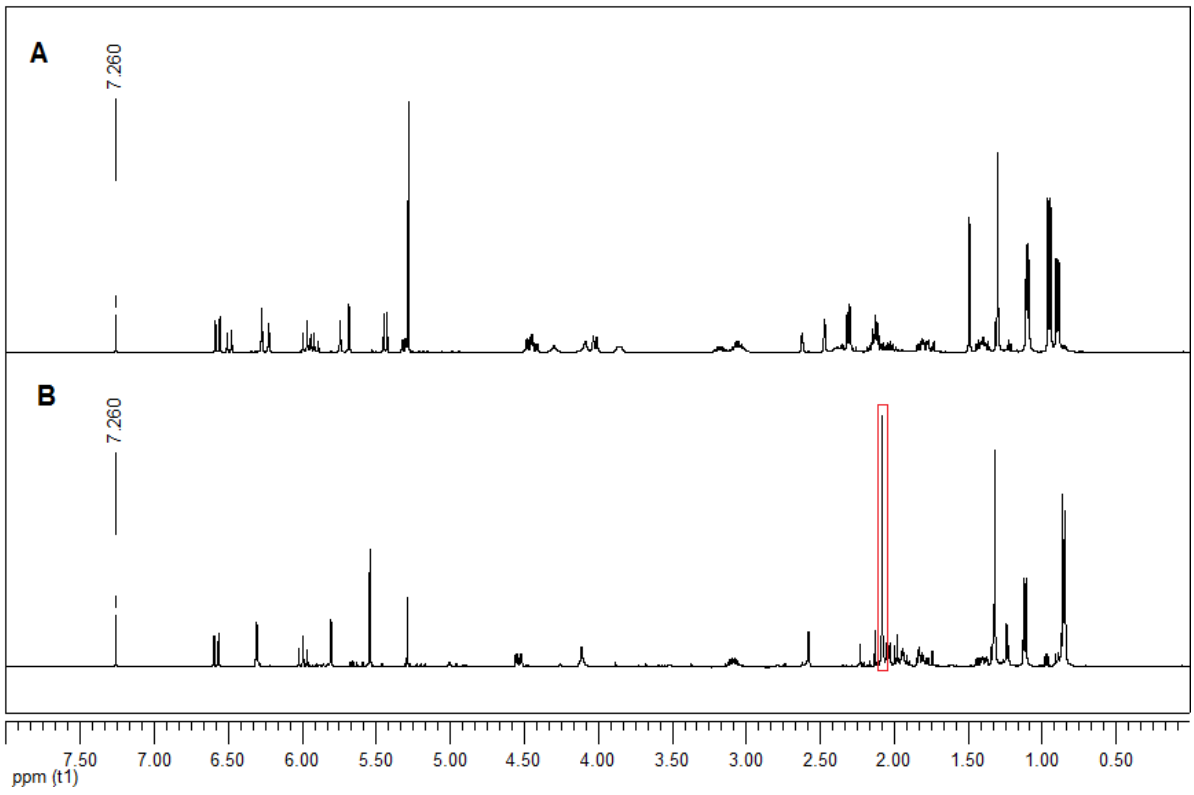


Figure 2.2.3 Comparison between NMR spectra of the mixture of sesquiterpene lactones (**7** and **8** - spectrum A) with neurolenin B (**2** - spectrum B) in $CDCl_3$.

2.1.3 Anti-inflammatory and cytotoxicity bioassay

The anti-inflammatory and cytotoxicity bioassays were carried out by Brendan Walshe-Roussel from the research group of Dr. Arnason. In the anti-inflammatory bioassay, parthenolide was the positive control for the inhibition of the inflammatory activity of lipopolysaccharide (LPS)-stimulated monocytes and ethanol was the vehicle (**Figure 2.2.4**). The five SLs all showed a concentration response in anti-inflammatory activity (**Table 2.2.4**). We noted that in all cases, the increase of the concentration from 0.4 μM to 40 μM of the isolates significantly reduced the production of TNFα, even greater than parthenolide.

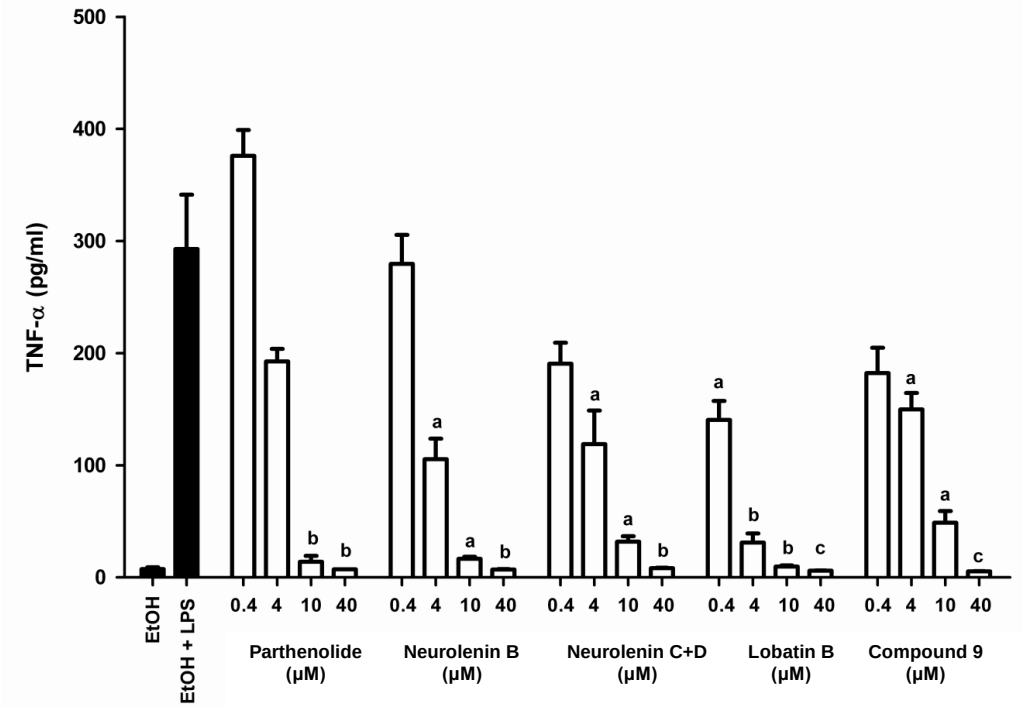


Figure 2.2.4 Anti-inflammatory activity of SLs isolated from *N. lobata* leaf extract in LPS-stimulated THP-1 with unstimulated and LPS-stimulated controls. Parthenolide was used as a positive control. Data obtained by Brendan Walshe-Roussel.

a- Statistically significant difference from EtOH + LPS control ($p \leq 0.05$).
b- No statistical difference from unstimulated EtOH control ($p \leq 0.05$).
c- Statistically lower than unstimulated EtOH control.

The five isolated SLs from *N. lobata* had IC_{50} values for TNFα that are lower than parthenolide in monocytes stimulated with LPS. Some compounds (**2,4,7** and **8**; IC_{50}= 0.17-2.5 µM) were even more potent than the positive control parthenolide (IC_{50}= 4.8 µM). The results provided a phytochemical and pharmacological basis for traditional anti-inflammatory use of the leaves. All SLs displayed moderate cytotoxic effects towards THP-1 monocytes at 40 µM, as seen in parthenolide (**Table 2.2.4**).

Table 2.2.4 – IC_{50}, cytotoxicity of parthenolide and compounds isolated from *N. lobata*.

Compound name	IC_{50}[a] (µM)	Cytotoxicity[b] ± S.E. (%)
Parthenolide	4.79	9.6±1.2
Neurolenin B	2.32	11.2±1.7
Neurolenin C+D	1.10	10.1±1.5
Lobatin B	0.17	12.4±2.0
Compound 5	1.30	10.6±1.9

[a] IC_{50} as calculated by linear regression of TNFα vs. log compound concentration in Graphpad Prism software.
[b] Cytotoxicity of compounds to THP-1 monocytes at 40 µM as assessed the release of lactate dehydrogenase.

Conclusion

The inhibitory effect of the five SLs isolated from the leaves of *N. lobata* on the production of the pro-inflammatory cytokine TNFα showed that our initial hypothesis was correct. Some compounds even displayed greater anti-inflammatory activity then parthenolide. The findings from this study provide a pharmacological basis for the anti-inflammatory effect observed in the leaves of *N lobata* as used in traditional medicine by the Q'eqchi' Maya healers of Belize and throughout the Central America and the Caribbean.

2.2 Experimental Section

2.2.1 General methods

Open column chromatography was carried out on silica gel 60 (70-230 mesh, Merck). Purifications were monitored by TLC (EMD Chemicals, TLC Silica gel 60 F_{254}), visualized by UV light (254 nm), then stained with Hannessian's stain. The preparative scale HPLC purification of isolates was undertaken on a reverse phase Gemini Axia column 250 mm × 21.2 mm I.D., particle size 10 microns (Phenomenex Inc., Torrance, CA, USA). The Agilent 1200 Series preparative HPLC system (Agilent Technologies, Montreal, QC, Canada) consisted of a binary pump (flow rate range 5–100 ml/min), an autosampler with a 2 ml loop, a diode array detector with a flow cell (path length 3 mm and maximum pressure limit 120 bars) and a fraction collector (40 ml collection tubes). NMR spectra were recorded on a Bruker Avance 400 spectrometer in either $CDCl_3$ or Acetone-d_6, at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Mass spectral analyses were conducted with an Electrospray mass spectrometer. All spectral analyses were performed at facilities located at the University of Ottawa.

2.2.2 Ethnobotanical interviews and sample collection

The ethnobotanical interviews were performed by Brendan Walshe-Roussel and they are described in his PhD thesis. *Neurolaena lobata* (L.) R. Br. Ex Cass was collected at the Itzamma Ethnobotanical Garden, a medicinal plant garden managed by the QMHA and BITI in Indian Creek, Toledo District, Belize. Collecting and export permits were obtained from the Belize Forest Department Ref. No. CD/60/3/08(33). The garden is at an elevation of approximately 30m above sea level. *N. lobata* leaves were collected at flowering stage and immediately preserved in 80% ethanol in H_2O. Authenticated voucher specimens are deposited at the University of Ottawa herbarium (OTT#17233) and the herbarium of the Universidad Nacional de Costa Rica (#13500).

2.2.3 Sample preparation, isolation and structural elucidation

Wet green leaves (478 g) were ground and then extracted with 80% EtOH in water (3L) overnight (**Scheme 2.3.1**). The alcohol was separated from the residue and the solvent was evaporated. The recovered leaf grounds were re-extracted twice using 80% EtOH and extracted overnight. The combined leaf extract (41g) was chromatographed on a glass column packed with silica gel using hexanes – EtOAc (1:0 → 0:1) and EtOAc – MeOH (1:0 →0:0.5), affording 14 fractions based on their analytical TLCs (fractions NL1 to NL14). Based on an examination of the ^{1}H NMR spectra, the presence of sesquiterpene lactones were found predominantly in fractions NL6 (980 mg) and NL7 (920 mg), which eluted between 40 to 60% EtOAc in hexanes. These fractions required further purification using repeated rounds of silica gel column chromatography and final purification with preparative HPLC to yield the SLs. These fractions were re-chromatographed in order to yield the known compounds **2, 4**, **7**, **8** and **9**, as identified by comparison of MS, ^{1}H and ^{13}C NMR data with those reported in the literature.

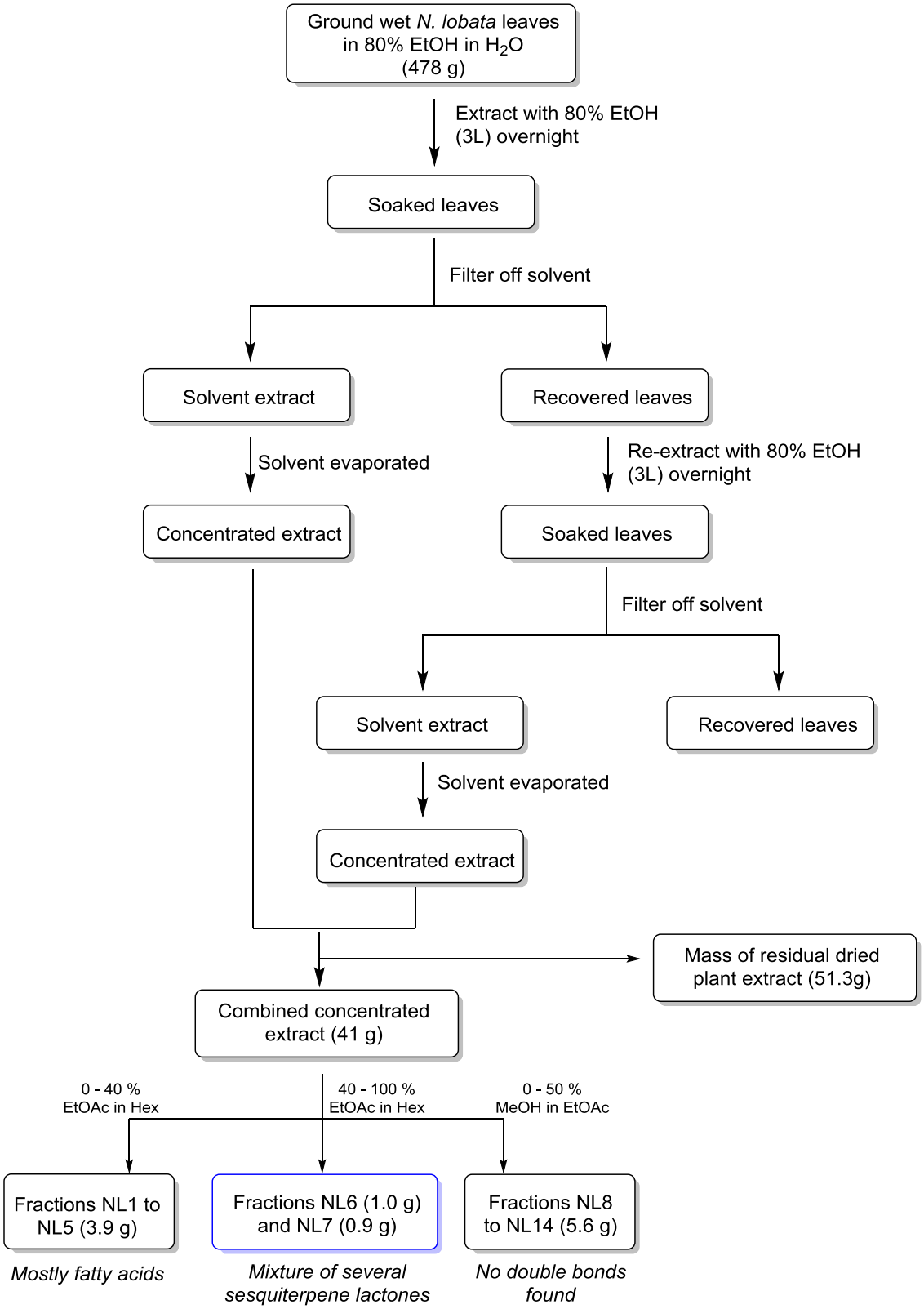


Figure 2.3.1 Extraction and first column chromatography of *N. lobata* leaves.

Fraction NL6 resulting from primary fractionation was rechromatographed in a glass column packed with silica gel eluting with hexanes – EtOAc (1:0 → 0:1) to obtain 7 secondary fractions (NL6-1 to NL6-7). Fraction NL6-4 (150 mg), which eluted with hexanes – EtOAc (6:4), was repurified with silica gel eluting with DCM – MeOH (1:0 → 0:0.1) to give 7 tertiary fractions (NL6-4-1 to NL6-4-7). Fractions NL6-4-1 and NL6-4-2 contained compound **2**, which eluted at 1.5% MeOH in DCM. Fractions NL6-4-3 contained the isomeric mixtures of compounds **4** and **7**, which eluted at 2% MeOH in DCM. Fractions NL6-4-4 contained compound **8**, which eluted at 3% MeOH in DCM. Fraction NL7 resulting from primary fractionation was rechromatographed in a glass column packed with silica gel eluting with hexanes – EtOAc (1:0 → 0:1) to obtain 10 secondary fractions (NL7-1 to NL7-10). Fraction NL7-5 (277 mg), which eluted with hexanes – EtOAc (1:1), was repurified with silica gel eluting with DCM – MeOH (1:0 →0:0.1) to give 12 tertiary fractions (NL7-5-1 to NL7-5-12). Fractions NL7-5-3 and NL7-5-4 contained the same isomeric mixture of compounds **2** and **4**, which eluted at 2% MeOH in DCM. Fractions NL7-5-6 and NL7-5-7 contained compound **9**, which eluted at 3% MeOH in DCM. Final purification was performed with preparative scale HPLC using a 40 min linear gradient of 40-45% of acetonitrile in water + 0.1% TFA at the flow rate of 31.5 ml/min, at the monitoring wavelength of 210 nm, band width 4, reference off.

2.2.4 Bioassays and statistics

Anti-inflammatory and cytotoxicity bioassays, as well as statistical and IC_{50} analyses were performed by Brendan Walshe-Roussel. The anti-inflammatory activity of the plant extract was evaluated by measuring TNFα reduction in LPS stimulated THP-1 monocyte assay with parthenolide as a positive control and ethanol as a vehicle control. Pure compounds were measured at 0.4, 4, 10, and 40 µM.

2.3 References

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Chapter 3: Isolation and characterization of diterpenoids from *Leretia cordata* (Icacinaceae)

3.1 Introduction

Leretia cordata Vell. Conc. (Icacinaceae), (**Figure 3.1.1**) also known as Curarina in Costa Rica, is a plant distributed in neotropical forests in Peru, Colombia, Guyana, Brazil and Costa Rica [1], [2]. The roots of the plants have been used for medicinal purposes in these regions as purgatives, emetics, and for treatments for snakebite. To date, there are no phytochemical reports on *L. cordata;* this short chapter is the first such for this species.

Figure 3.1.1 Leaves and flowers of *L. cordata.*

The Icacinaceae is a poorly studied family despite the fact that some members are reported to have anti-malarial and anti-cancer activities [3], [4]. Previous studies on this family revealed the presence of several oxidized species within monoterpenoids (iridoids, **1**), sesquiterpenoids (emmotins, **2**), diterpenoids (humirianthone, **3**) and quinoline alkaloids, the most well known being the anticancer drug camptothecin, **4** (**Figure 3.1.2**) [5].

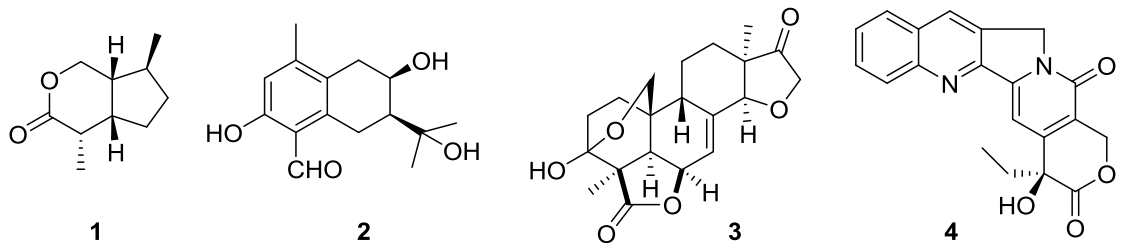


Figure 3.1.2 Examples of structures of secondary metabolites found in plants from the Icacinaceae family.

As part of our ongoing investigations of rare medicinal plants from Costa Rica, a search strategy used in order to find novel and characteristics secondary metabolites, we have undertaken an investigation of the leaf and bark extracts of *Leretia cordata*. This project was carried out jointly in collaboration with botanists from Universidad Autonoma de Costa Rica UNA, who carried out the collection and have kept voucher samples, and with Dr. Jose Antonio Guerrero, from the Arnason group at the University of Ottawa, who contributed to the purification of several compounds by HPLC. The technical help of undergraduate students Sherif Kaldas and Abdullah Akbar is also acknowledged.

3.2 Results and Discussion

3.2.1 Plant extractions

The initial phytochemical study of *Leretia cordata* was performed on the bark and the leaves of the plant to identify the characteristics secondary metabolites and to identify possible biological activities for the isolated compounds.

A total of two batches of *L. cordata* bark and leaves were collected from the Tirimbina Botanical reserve in Sarapiqui, Costa Rica; one collection occurred during the dry season and one during the wet season. The plant materials were stored in 1L Nalgene bottles in a 3:1 mixture of water and isopropanol in order to preserve them. Prior to beginning isolation, the plant materials were filtered from the packing liquid and dried in the open air for two days. The packing liquid was combined with the ethanolic extracts.

Dry ground plant materials were either 1) extracted with 95% ethanol to generate only one ethanolic extract or 2) partitioned with solvents of increasing polarity to generate hexane, ethyl acetate and ethanolic extracts. The structure of major constituents of these extracts were

determined after separation by column chromatography. Some compounds required further purification using either recrystallization or preparative-HPLC prior to investigation by NMR.

The phytochemical study of the leaf and bark of *L. cordata* resulted in the isolation of four known natural diterpenoids: humanrianthone **3**, humanrianthol **5**, annonalide **6** and its oxidation product **7** (**Figure 3.2.1**) [4], [6], [7]. The structure of these compounds were determined using their HRMS, ^{1}H and ^{13}C NMR spectra and by comparison of the data with that of known compounds. While the compounds are previously reported, this is the first report of their occurrence in *L. cordata*.

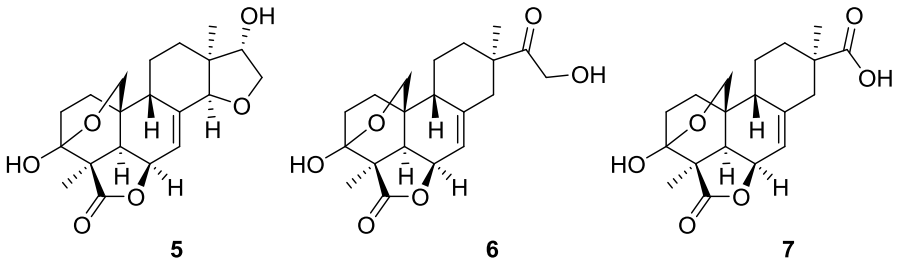


Figure 3.2.1 Structures of humanrianthol **5**, annonalide **6** and oxidation product of annonalide **7**.

The yield of these compounds based upon the dry weight of the plant material is summarized in the table below.

Table 3.2.1 Yield of compounds isolated from *L. cordata* based upon dry mass.

Compound	Leaf (wet season)		Bark (wet season)		Bark (dry season)	
	Mass (mg)	Yield [a] (%)	Mass (mg)	Yield [a] (%)	Mass (mg)	Yield [a] (%)
3	111.1	0.13				
5			4	0.005	105.1	0.03
6			17.5	0.021	16	0.005
7	32.4	0.045				

a- Percent of dry mass of plant.

3.2.2 Diterpenoids isolated

Humirianthone (compound **3**) was isolated from the ethanolic leaf extract of *L. cordata* collected during the wet season as a white solid after purification with silica gel chromatography and recrystallization from methanol. The formula was determined to be $C_{20}H_{24}O_6$ (HRMS found = 360.1549), indicating 9 degrees of unsaturation.

The most distinctive signals in the ^{1}H NMR spectrum were those of two diastereotopic methylene protons [δ: 3.89 (d, *J* = 17.1 Hz, 1H) and 4.41 (d, *J* = 17.1 Hz, 1H)] and [δ: 3.78 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.48 (dd, *J* = 8.9, 2.0 Hz, 1H)], two methines [4.97 (dd, *J* = 6.9, 4.8 Hz, 1H)] and [δ: 4.19 (s, 1H)] and one alkene hydrogen [δ: 6.04 (dd, *J* = 4.8, 0.8 Hz, 1H)] (**Figure 3.2.2**). In addition, 2 methyl groups were visible in the upfield region of the spectrum [δ: 0.89 (s, 3H), 1.27 (s, 3H)]. The ^{13}C NMR spectrum confirmed the presence of all 20 carbons in the molecular formula. It had one ketone [δ: 216.8], one lactone [δ: 178.5] and one double bond [δ: 143.7 and 119.2] (**Figure 3.2.3**). The DEPT spectrum indicated the carbon skeleton was composed of two methyls, six methylenes, five methines and seven quaternary carbons. Combining these findings, we performed a literature survey based on molecular formula and found that compound **3** matched the spectroscopical data of natural product humirianthone. This compound has been reported only by Kingston and colleagues who conducted a phytochemical study on *Casimirella ampla* (Icacinaceae) from Suriname and Madagascar and identified a total of 10 diterpenoids [4].

Table 3.2.2 compares the NMR data of the humirianthone isolated in this study with literature data.

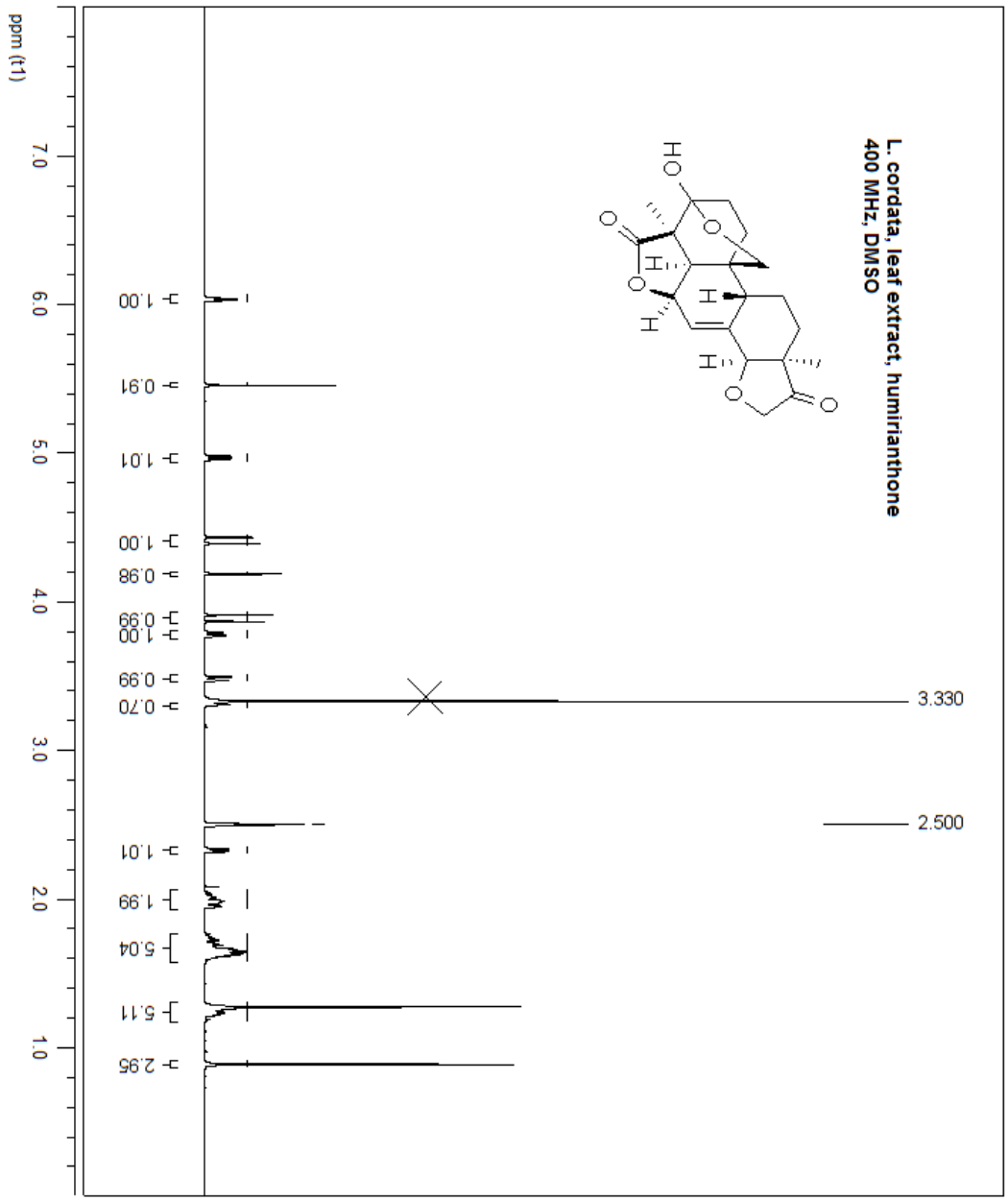


Figure 3.2.2 ^{1}H NMR spectrum of humirianthone (**3**) in DMSO-d_6.

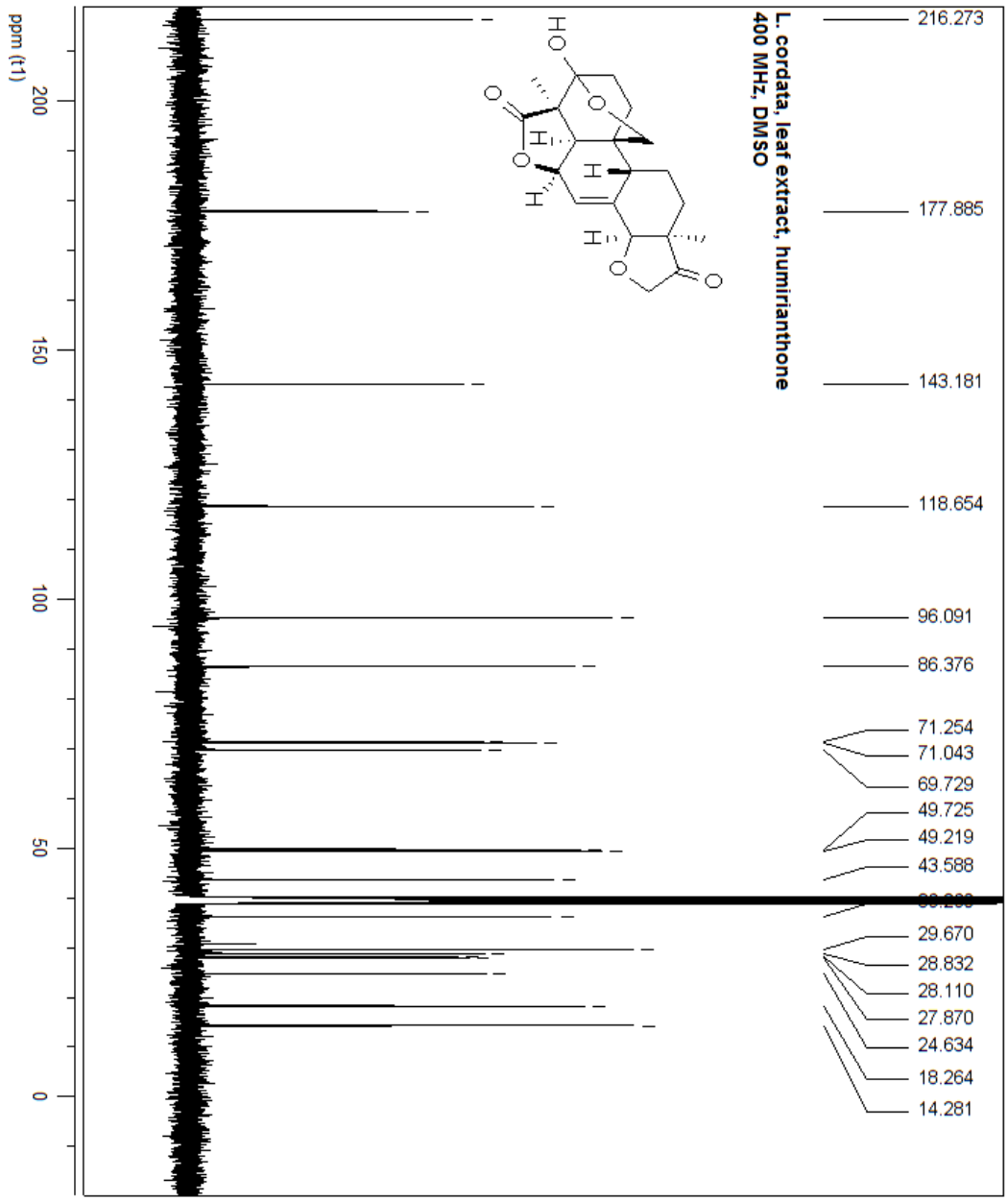
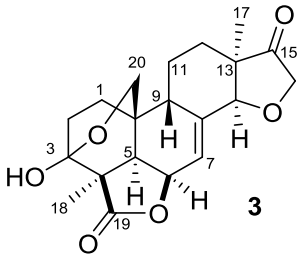


Figure 3.2.3 ^{13}C NMR spectrum of humirianthone **(3)** in DMSO-d_6.

Table 3.2.2 ^{1}H and ^{13}C NMR data observed for humirianthone with published data [4].



3

Position	Observed δ_H[a]	Literature δ_H[b]	Observed δ_C[a]	Literature δ_C[b]
1	1.60-1.76 m	1.62 m, 1.68 m	28.1	29.2
2	1.95-2.04 m	2.22 m, 2.25 m	27.9	28.5
3			96.1	97.2
4			49.7	50.8
5	2.33 dd (6.9, 1.9)	2.34 dd (2.6, 6.9)	43.6	45.1
6	4.97 dd (6.9, 4.8)	4.98 dd (2.3, 4.8)	71.3	72.5
7	6.04 dd (4.8, 0.8)	6.10 d (4.9)	118.7	119.2
8			143.2	144.3
9	1.60-1.76 m	1.68 dd (12.1, 2.9)	36.3	37.1
10			29.7	30.7
11	1.19-1.29 m; 1.60-1.76 m	1.18 m, 1.58 m	24.6	25.5
12	1.19-1.29 m	1.32 m	28.8	29.7
13			49.2	50.1
14	4.19 s	4.15 s	86.4	87.8
15			216.3	216.2
16	3.89 d (17.1) 4.41 d (17.1)	3.97 d (17.2) 4.53 d (17.2)	69.7	70.7
17	0.89 s	1.00 s	14.3	14.8
18	1.27 s	1.56 s	18.3	19.2
19			177.9	179.2
20	3.78 dd (8.8, 2.4) 3.48 dd (8.9, 1.9)	3.68 dd (3.4, 5.9) 4.31 dd (3.4, 5.9)	71.0	72.1
3-OH	5.46 s	7.02 s		

a- solvent: DMSO-d_6
b- solvent: Pyridine-d_5

The ^{1}H NMR spectrum showed slight variation in the chemical shifts due to the use of a different deuterated solvent. Moreover, there was a significant discrepancy in the coupling constant for the C20 methylene protons. We observed that the C20 methylene protons displayed a coupling constant (J = 8.8 Hz) which is consistent with a geminal relationship if the CH_2 group is bonded to an oxygen. In addition, one proton possessed long-range coupling to the C5-H proton as evidenced by their shared coupling constant (J = 1.9 Hz) and the other one a (J = 2.4 Hz) long range coupling. The coupling constant reported for this system [4] are 5.9 and 3.4 Hz. We are convinced that this data is in error, probably being incorrectly recorded since both J values are too small for a typical germinal coupling to in a $–CH_AH_B$-OR system. Additionally, Graebner and On'okoko and their colleagues reported J values of approximately 9 Hz for other compounds in this series [7], [8]. We have also observed a similar value for compounds **5**, **6** and **7**. The observed ^{13}C NMR spectrum for humirianthone (**3**) was consistent with the reported data.

The four diterpenoids isolated in this study from *L. cordata* were also found in another plant from the same Icacinaceae family [4]. Compounds **5**, **6** and **7** were identified as humanrianthol, annonalide and oxidation product of annonalide [4], [6], [7]. Their structural identification was made based upon key signals in the ^{1}H and ^{13}C NMR spectra and comparison with literature data [4], [6], [7]. Compounds **5** and **6** were isolated as a mixture from the bark extracts (during both the wet and dry seasons) after purification by column chromatography. The mixture was separated by preparative HPLC using a Gemini AXIA-250x21.2 MM preparative HPLC column using a 15 min linear gradient of 0-95% acetonitrile in water at a flow rate of 31.5 ml/min, at the monitoring wavelength of 210 nm.

Humirianthol (**5**) was isolated as white solid. Its ^{1}H NMR spectrum indicated the presence of the two methylene protons C16 and C20, appearing at [δ: 3.52 (d, *J* = 10.1 Hz, 1H) and 4.24 (dd , *J* = 9.7, 4.7 Hz, 1H)] and [δ: 3.73 (dd, *J* = 8.8, 2.8 Hz, 1H) and 3.41 (dd, *J* = 8.0, 1.7 Hz, 1H)] respectively (**Figure 3.2.4**). The ^{13}C NMR spectra indicated the presence of 20 carbons including a lactone [δ: 178.3] (**Figure 3.2.5)**. The DEPT spectrum revealed that the carbon skeleton consisted of two methyls, six methylenes, five methines and seven quaternary carbons. Its ^{1}H and ^{13}C NMR spectra were identical with the reported data in DMSO-d_6 (**Table 3.2.3**) [7].

Annonalide (**6**) was isolated as a white solid. The ^{1}H and ^{13}C NMR spectra were consistent with the absence of the tetrahydrofuran ring, compared to compounds **3** and **5 (Figures 3.2.6** and **3.2.7**, **Table 3.2.4)**. The main difference in the ^{1}H NMR was the C16 methylene protons which appeared at [δ: 4.30 (d, *J* = 5.75, 2H] in compound **6**; while it appeared at [δ: 3.89 (d, *J* = 17.1 Hz, 1H) and 4.41 (d, J = 17.1 Hz, 1H)] and at [δ: 3.52 (d, *J* = 10.1 Hz, 1H) and 4.24 (dd, *J* = 9.7, 4.7 Hz, 1H)] in compounds **3** and **5**, respectively. The ^{13}C NMR spectrum indicated the presence of 20 carbons, including ketone at [δ: 216.7], a lactone at [δ: 181.4]. The loss of C14 peak at [δ: 86.4 or 85.3] from compounds **3** and **5** and the presence of one at [δ: 66.5] confirmed the absence of a tetrahydrofuran moiety. The DEPT spectrum revealed that the carbon skeleton consisted of two methyls, eight methylenes, three methines and seven quaternary carbons. This compound is known but there was no published NMR data [6].

Compound **7**, identified as the oxidation product of annonalide, was isolated as a yellowish solid from the leaf extract during the wet season collection from the silica gel flash chromatography and recrystallization from methanol. The ^{1}H NMR spectrum showed little variations compared

to the other structures, with the major difference of the loss of C16 methylene protons (**Figure 3.2.8, Table 3.2.4**). The ^{13}C NMR spectrum indicated the presence of 19 carbons, with two carbonyl peaks at [δ: 181.7] and [δ: 181.4] and only two C-O peaks at [δ: 74.8] and [δ: 73.8] (**Figure 3.2.9**). The DEPT spectrum indicated that the carbon skeleton consisted of two methyls, six methylenes, four methines and seven quaternary carbons. This compound was previously obtained from the oxidation of annonalide with periodic acid and isolated as a natural product from a *C. ampla* (Icacinaceae) [4], [9].

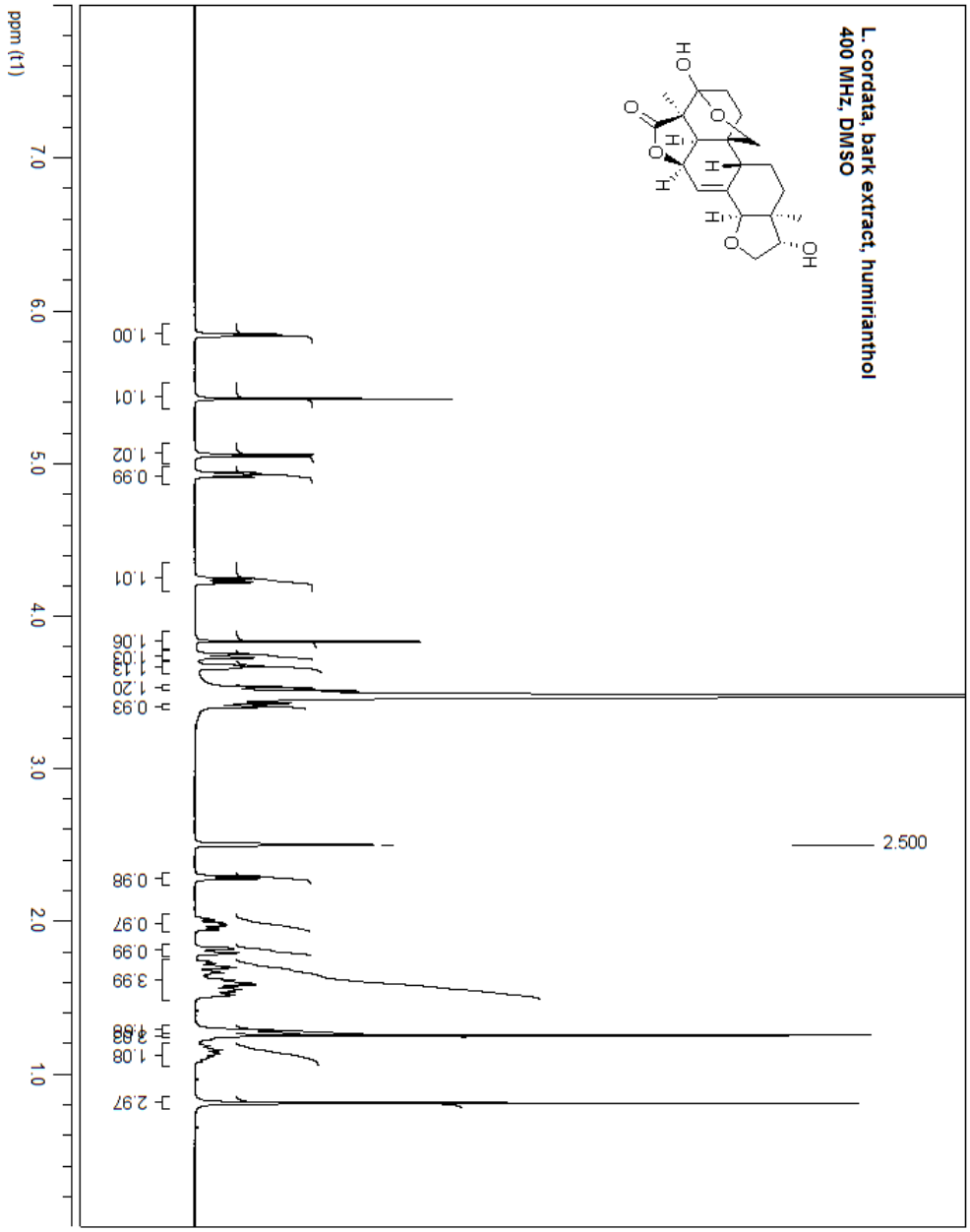


Figure 3.2.4 ^{1}H NMR spectrum of humirianthol (**5**) in DMSO-d_6.

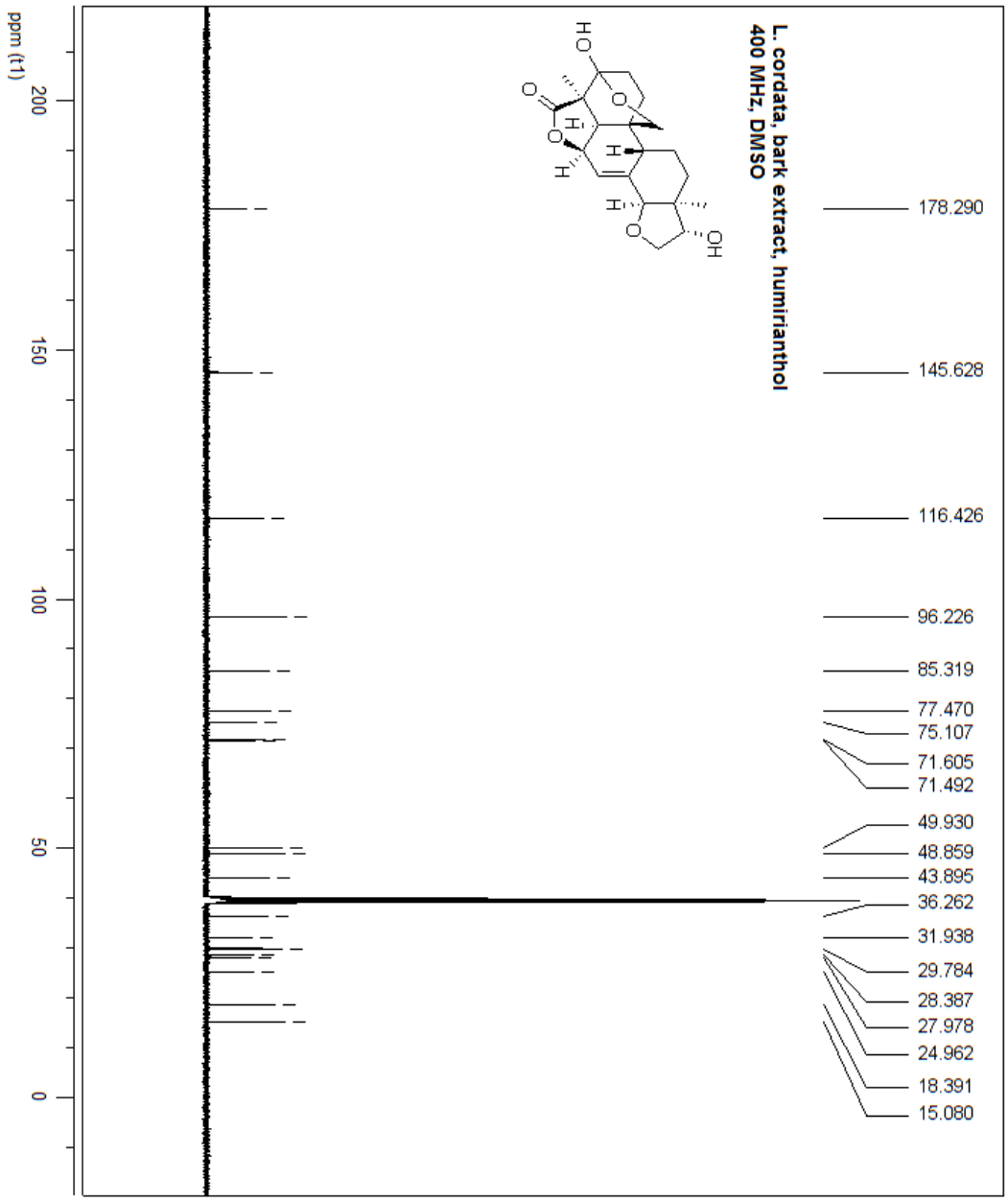
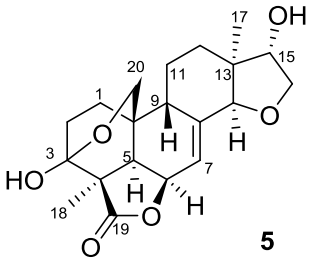


Figure 3.2.5 ^{13}C NMR spectrum of humirianthol (**5**) in DMSO-d_6.

Table 3.2.3 ^{1}H and ^{13}C NMR data observed for humirianthol with published data (DMSO-d_6, 400/100 MHz) [7].



5

Position	Observed δ_H	Literature δ_H	Observed δ_C	Literature δ_C
1	1.64-1.46 m	1.60 m; 1.65 m	28.4	28.3
2	1.75-1.65 m; 1.98 ddd (13.4, 11.3, 5.4)	1.70 m; 1.98 m	28.0	28.0
3			96.2	96.2
4			49.9	49.8
5	2.28 dd (6.8, 1.2)	2.27 dd (2.5, 7.0)	43.9	43.8
6	4.93 dd (6.8, 4.7)	4.92 dd (5.0, 7.0)	71.6	71.1
7	5.84 d (4.4)	5.84 d (5.0)	116.4	116.4
8			145.6	145.6
9	1.81 dd (12.3, 2.5)	1.80 dd (12.3, 2.8)	36.3	36.2
10			29.8	29.8
11	1.22-1.03 m	1.15 m, 1.52 m	25.0	24.9
12	1.30-1.26 m	1.28 m	31.9	31.9
13			48.9	48.1
14	3.84 (s)	3.83 s	85.3	85.3
15	3.66 t (4.2)	3.65 dd (4.0, 4.0)	77.5	77.4
16	3.52 d (10.1) 4.24 dd(9.7, 4.7)	3.52 d (9.5) 4.23	75.1	75.1
17	0.81 s	0.82 s	15.1	15.0
18	1.25 s	1.25 s	18.4	18.3
19			178.3	178.2
20	3.73 dd (8.8, 2.8) 3.41 dd (8.9, 1.7)	3.73 dd (2.5, 9.0) 3.41 dd (2.0, 9.0)	71.5	71.0
3-OH	5.42 s	5.35 s		
15-OH	5.05 d (4.3)	4.99 d (4.0)		

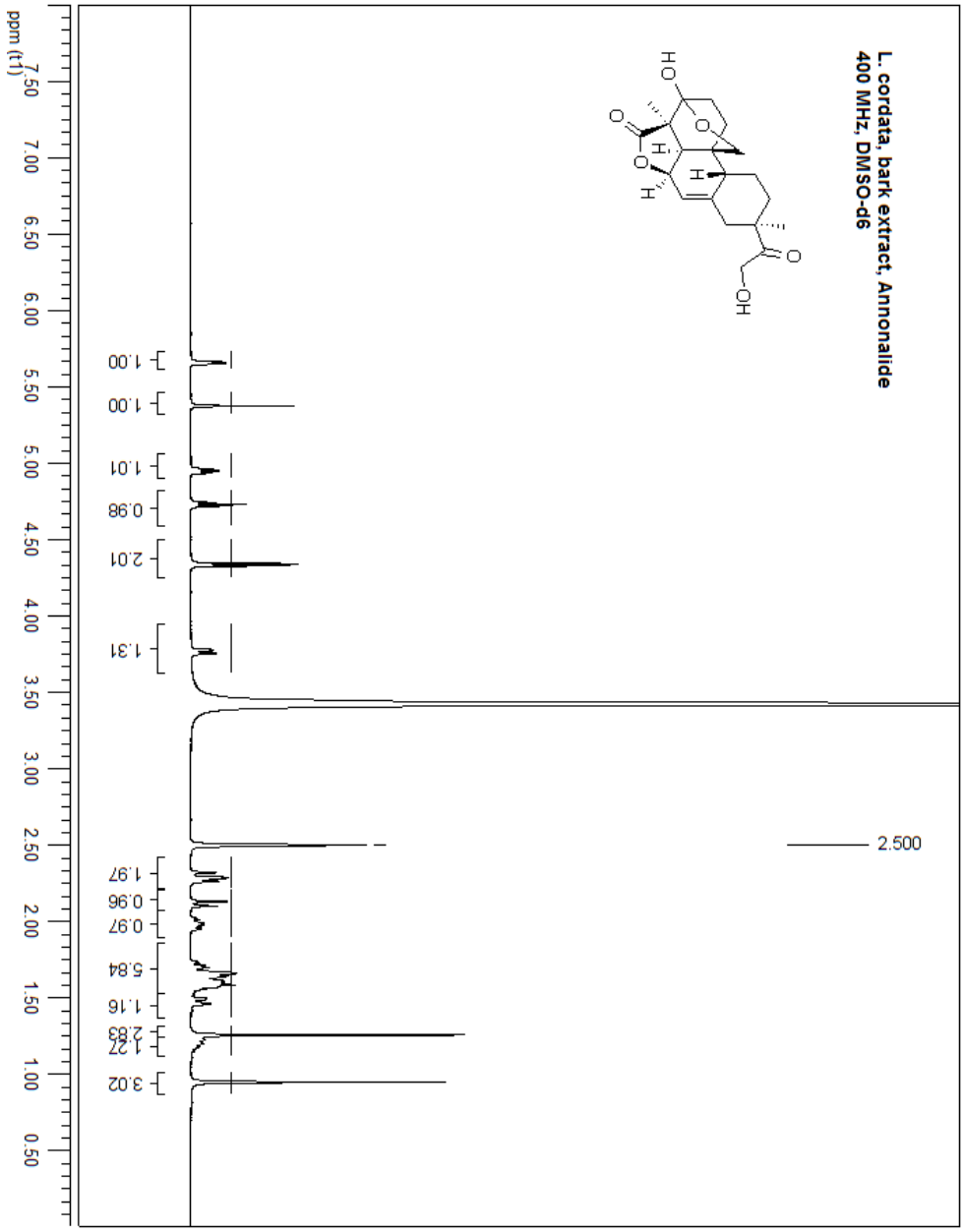


Figure 3.2.6 ^{1}H NMR spectrum of annonalide (**6**) in DMSO-d_6.

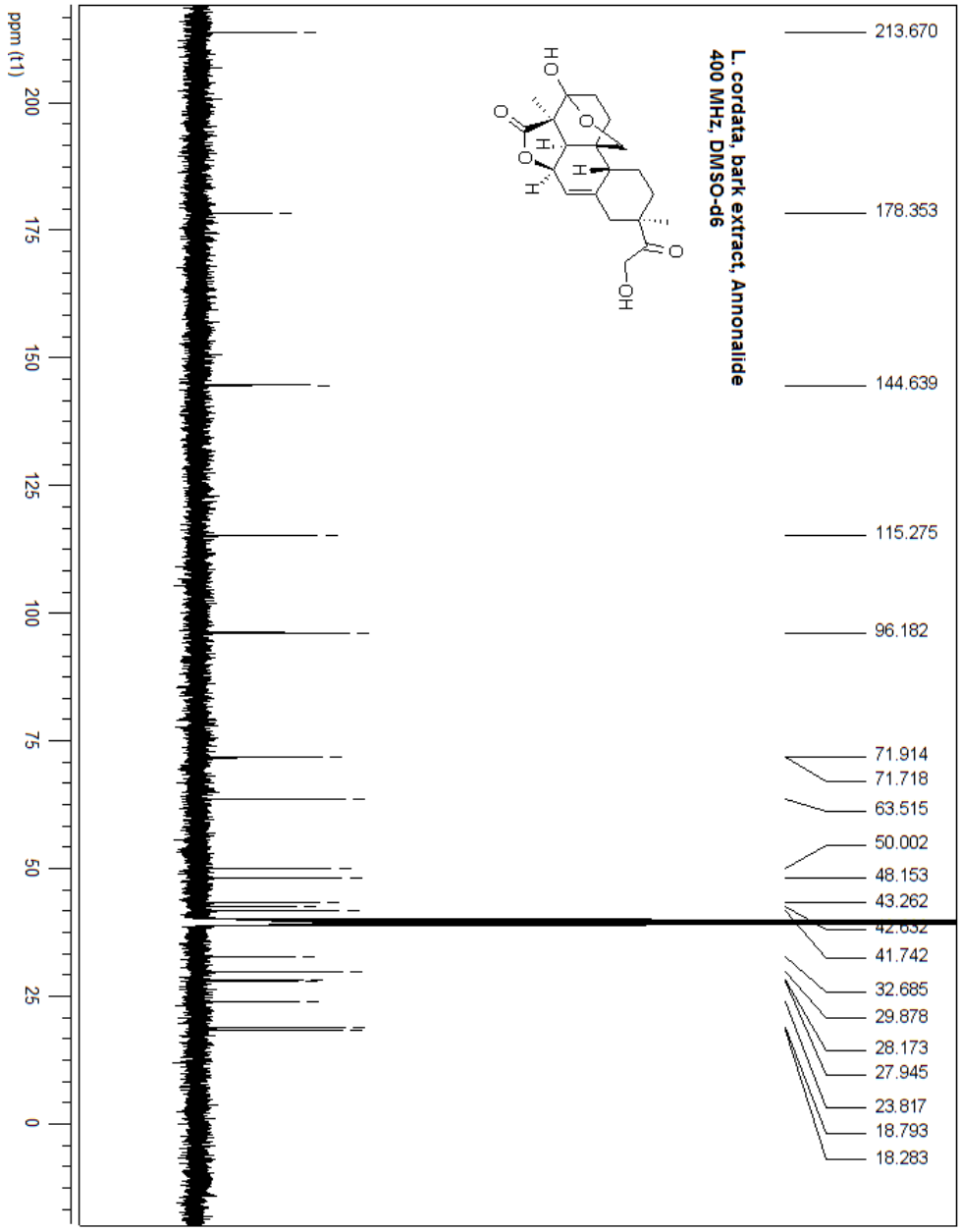


Figure 3.2.7 ^{13}C NMR spectrum of annonalide (**6**) in DMSO-d_6.

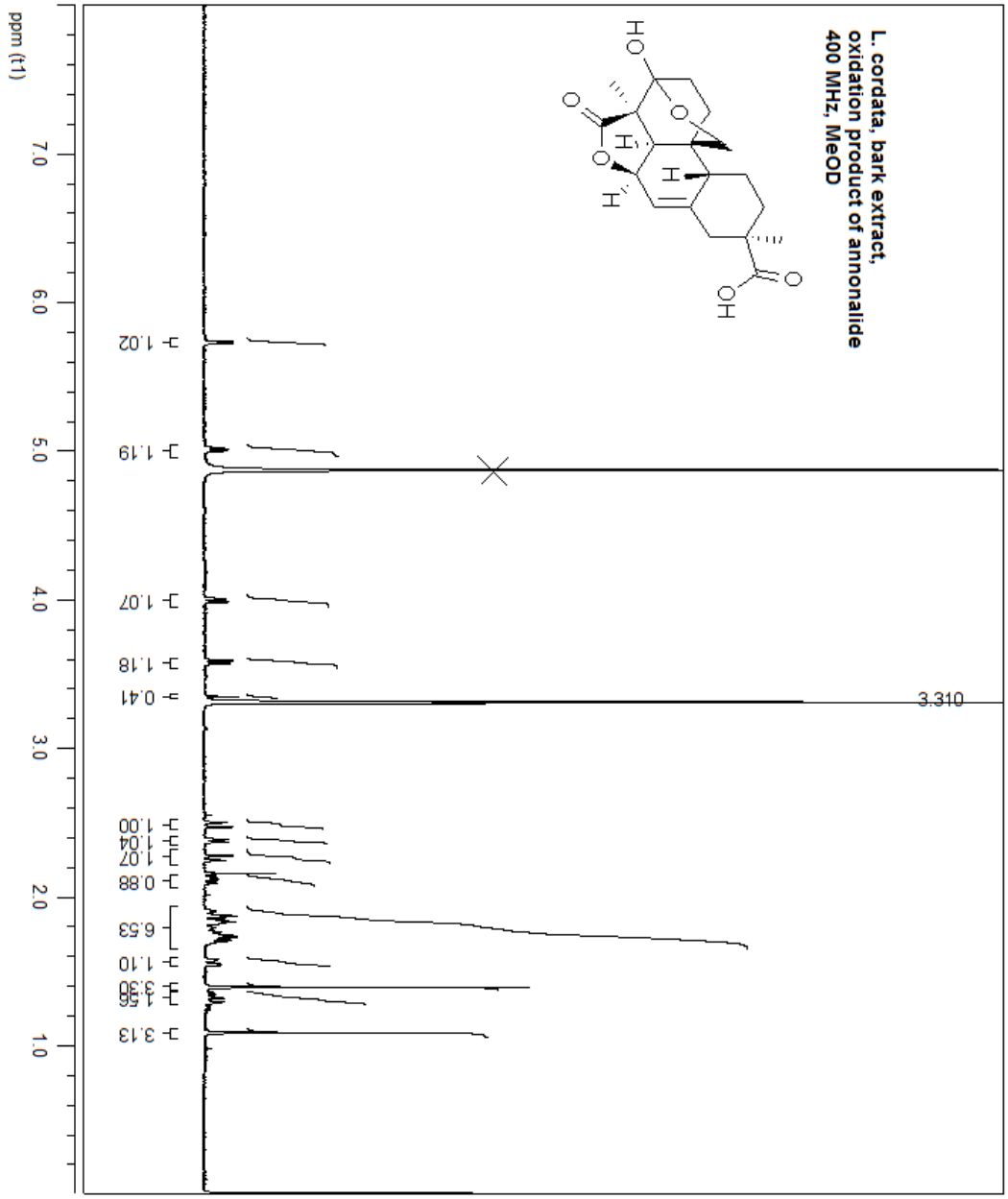


Figure 3.2.8 ^{1}H NMR spectrum of the oxidation product of annonalide (**7**) in MeOD.

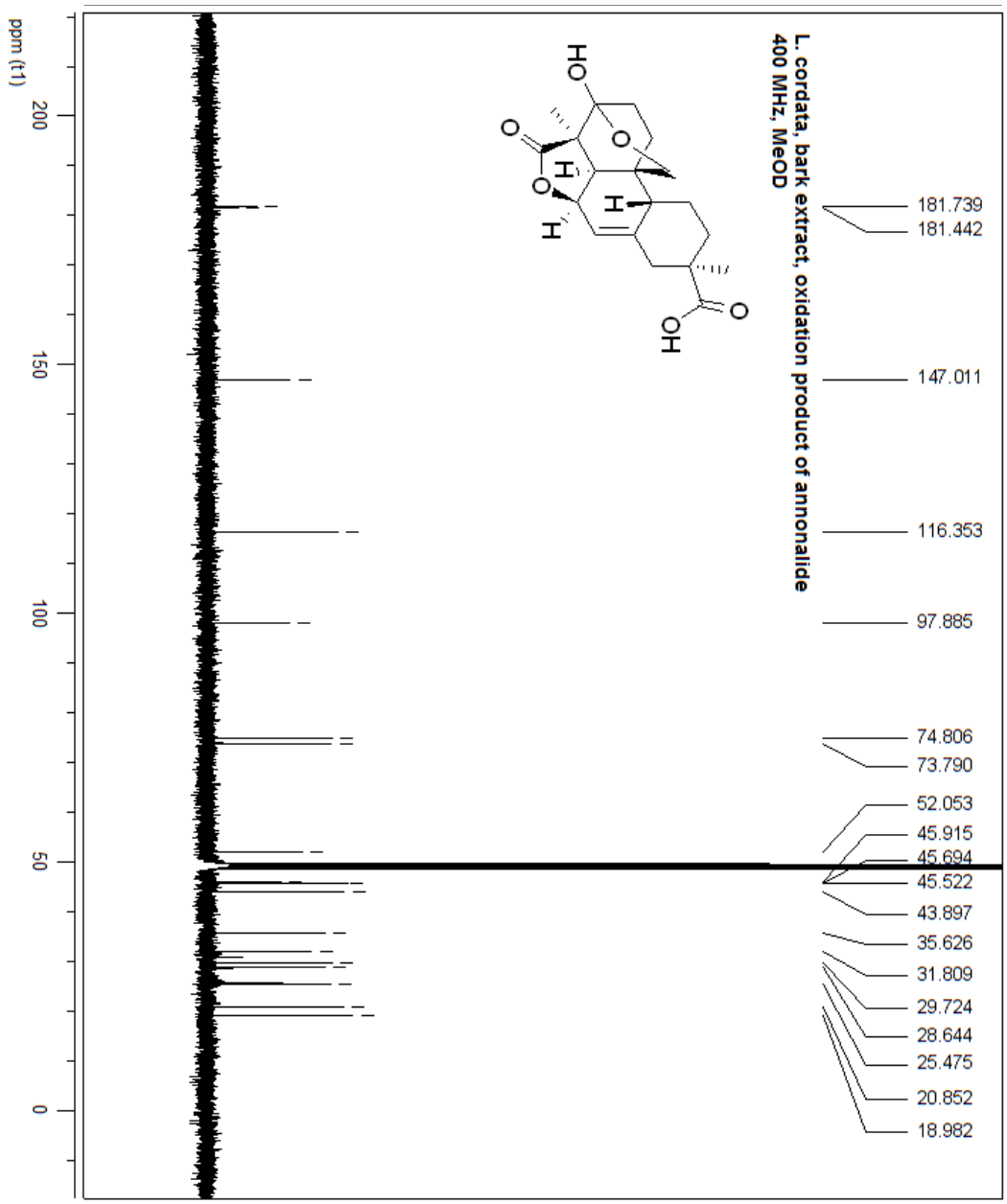
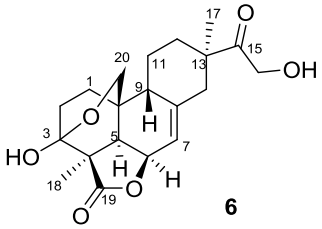
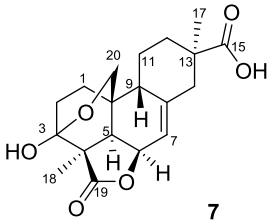


Figure 3.2.9 ^{13}C NMR spectrum of the oxidation product of annonalide (**7**) in MeOD.

Table 3.2.4 ^{1}H and ^{13}C NMR data observed for annonalide (**6**) and its oxidation product (**7**).



6



7

Position	Annonalide (6)[a]		Oxidation product of annonalide (7)[b]	
	Observed δ_H	Observed δ_C	Observed δ_H	Observed δ_C
1	1.76-1.52	28.1	1.94-1.68	29.7
2	1.76-1.52 2.03-1.93	27.9	1.94-1.68 2.13-2.06	28.6
3		96.2		97.9
4		50.0		52.1
5	2.27 dd (6.9, 1.4)	43.3	2.38 dd (6.9, 2.0)	45.9
6	4.95 dd (6.0, 6.0)	71.9	5.00 dd (6.0, 6.0)	74.8
7	5.66 d (4.6)	115.3	5.73 d (4.7)	116.4
8		144.6		147.0
9	1.48 dd (12.4, 2.5)	41.7	1.56 dd (12.8, 3.1)	43.9
10		29.9		31.8
11	1.25-1.07 m 1.76-1.52	23.8	1.37-1.26 1.94-1.68	25.5
12	1.76-1.52	32.7	1.94-1.68	35.6
13		48.2		45.7
14	2.12 d(12.0) 2.30 d (12.2)	42.6	2.49 d (12.2) 2.26 dd (12.3, 1.9)	45.5
15		213.7		181.7
16	4.34 d (5.7)	63.5	-	-
17	0.95 s	18.3	1.09 s	18.9
18	1.26 s	18.8	1.39 s	20.9
19		178.4		181.4
20	3.77 dd(8.8, 2.7) -	71.7	3.99 dd (9.0, 3.1) 3.58 dd (9.1, 2.0)	73.8
3-OH	5.37 s			
16-OH	4.73 t (5.8)			

a- Solvent: DMSO-d_6
b- Solvent: MeOD

3.2.3 Biological activities

A literature survey for biological activity of the isolated diterpenoids showed that compounds **3** and **6** exhibited cytotoxicity activity against the A2780 human ovarian cell lines with IC_{50} values of 6.1 and 3.9 μM while compound **7** was weakly active with an IC_{50} of 33 μM [4]. In addition, compounds **3** and **6** showed moderate to good activity against phytopathogenic fungi *Phytophthora infestans* with IC_{50} of 0.93 and 25 μM respectively, while it showed no significant activity against *Leptosphaeria nodurum, Pyricularia oryzae, Septoria tritici*, *Ustilago maydis* and *Saccharomyces cerevisiae* [4]. Because of the traditional use of *L. cordata* as a purgative, emetic and snakebite remedy, the isolated compounds might have activity against insects or other cancer cell lines. As preliminary bioassays, compounds **5** to **7** were tested as topical applications on the thoracic terga of *Ostrinia nubilalis* larvae [at a concentration of 1mg/ml and 0.1mg/ml] by a Suqi Liu, a PhD student from the group of Dr. Arnason at the Univeristy of Ottawa, to assess their mortality. The compounds were not active above the vehicle (acetone), therefore further investigation on these larvae was not pursued [10]. The isolated compounds could be examined in other appropriate bioassays. For example, the isolated compounds could be tested as anti-malarial agents. The crude plant extract of *Icacina senegalensis* (Icacinaceae), which contains several diterpenoids related to the compounds isolated in this study, inhibited *in vitro* *Plasmodium flalcirum* growth with an IC_{50} <5μM without host cell toxicity [3], [11].

Conclusion

This study represents the first phytochemical analysis of *L. cordata*. Four structurally interesting diterpenoids were isolated, which seem to be marker secondary metabolites in the Icacinaceae family. Disappointingly, none were new structures, several having been reported on only one previous occasion.

3.3 Experimental Section

3.3.1 General experimental procedures

Open column chromatography was carried out on silica gel 60 (70-230 mesh, Merck). Purifications were monitored by TLC (EMD Chemicals, TLC Silica gel 60 F_{254}), visualized by UV light (254 nm), then stained with Hannessian's stain. The preparative scale HPLC purification of isolates was undertaken on a reverse phase Gemini Axia column 250 mm × 21.2 mm I.D., particle size 10 microns (Phenomenex Inc., Torrance, CA, USA). The Agilent 1200 Series preparative HPLC system (Agilent Technologies, Montreal, QC, Canada) consisted of a binary pump (flow rate range 5–100 ml/min), an autosampler with a 2 ml loop, a diode array detector with a flow cell (path length 3 mm and maximum pressure limit 120 bars) and a fraction collector (40 ml collection tubes). NMR spectra were recorded on a Bruker Avance 400 spectrometer in either $CDCl_3$ or Acetone-d_6, at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Mass spectral analyses were conducted with an Electrospray mass spectrometer. All spectral analyses were performed at facilities located at the University of Ottawa.

3.3.2 Plant material

The leaves and the bark of *Leretia cordata* were collected in the Tirimbina Botanical reserve in Sarapiqui, Costa Rica in August 2010 and again in April 2012. In order to preserve the plant materials, they were packed in Nalgene bottles of 1L in a 3:1 mixture of isopropanol in water. Authenticated voucher specimens are deposited at the herbarium of the Universidad Nacional de Costa Rica. Prior to beginning isolation, the plant materials were filtered from the packing liquid and dried in the open air over the period of for two days.

3.3.3 Extraction and isolation

Plant materials were ground in Wiley mill to pass through a 1mm mesh and either A) extracted with 95% ethanol to generate only one ethanolic extract (August 2010 collection) or B) partitioned with solvents of increasing polarity to general hexane, ethyl acetate and ethanolic extracts (April 2012 collection) (**Figure 3.3.1**). Dry ground leaves (71g) and bark (86g) were extracted according to procedure **A** affording two ethanolic extracts of 1.90 g and 2.46 g for the leaves and the bark extract, respectively. Both extracts were purified by column chromatography. Dry ground bark (340g) was also extracted according to procedure **B**, yielding three fractions: hexanes extract (1.14 g), EtOAc extract (1.49 g) and EtOH extract (2.42 g). Only the EtOAc and EtOH fractions were chromatographed, as the hexanes fraction only showed the presence of fatty acids.

All the extracts were evaporated to dryness and subjected to multiple purifications by column chromatography using gradient solvent systems of hexanes – EtOAc (1:0 → 0:1) and EtOAc – MeOH (1:0 →0:0.5), affording several fractions based on their analytical TLCs. Compounds **3** and **7** were recrystallized using minimal amount of methanol while compounds **5** and **6** were separated by preparative HPLC using a Gemini AXIA-250x21.2 MM preparative HPLC column using a 15 min linear gradient of 0-95% acetonitrile in water at a flow rate of 31.5 ml/min, at the monitoring wavelength of 210 nm. The yields of the extractions are shown in **Table 3.2.1**.

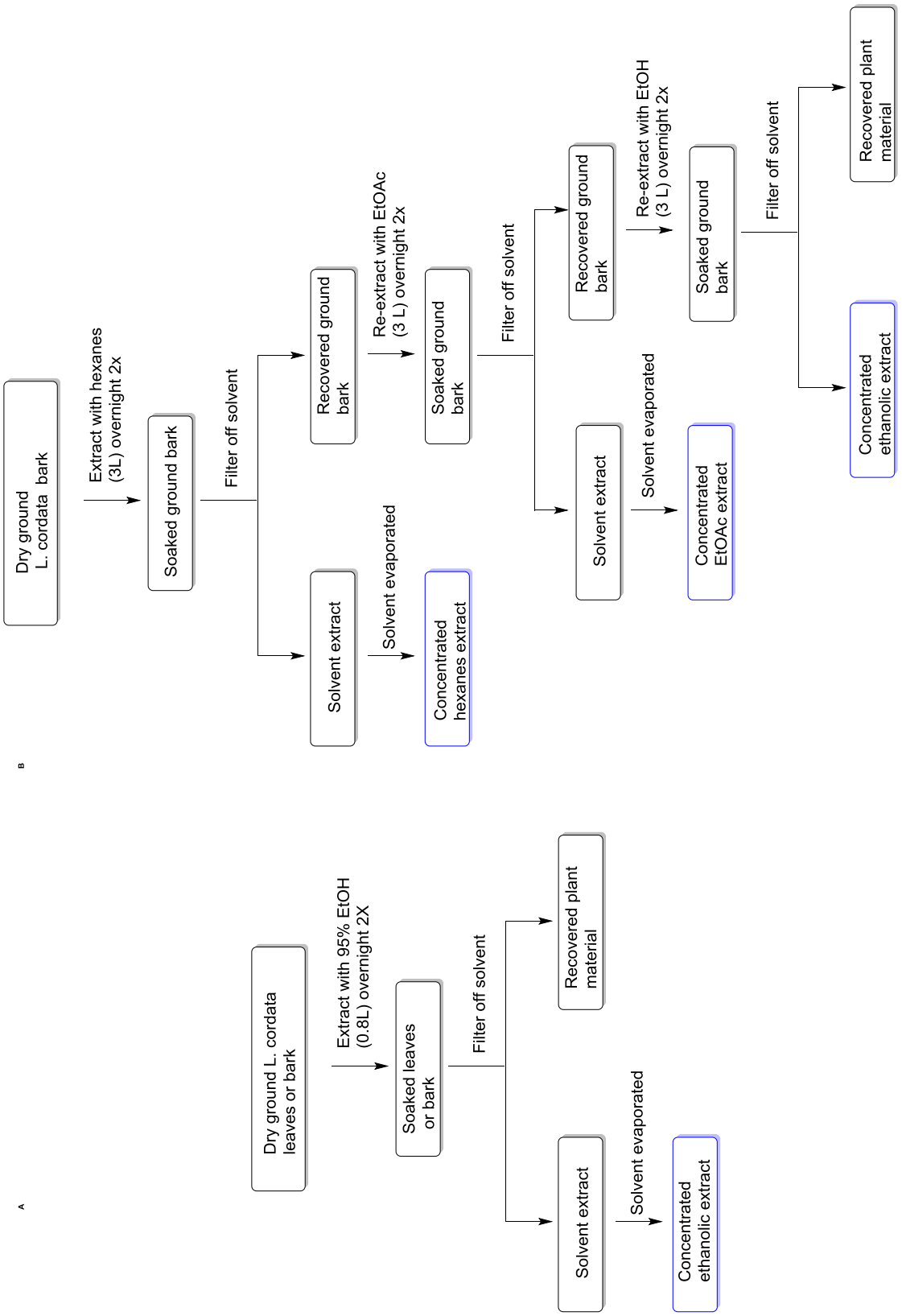


Figure 3.3.1 Extraction scheme for the A) ethanolic extraction (August 2010 collection) and B) fractional extraction with hexanes, EtOAc and ethanol (April 2012).

3.4 References

[1] G. Bourdy, S. J. DeWalt, L. R. Chávez de Michel, a Roca, E. Deharo, V. Muñoz, L. Balderrama, C. Quenevo, and a Gimenez, "Medicinal plants uses of the Tacana, an Amazonian Bolivian ethnic group.," *Journal of ethnopharmacology*, vol. 70, no. 2, pp. 87–109, May 2000.

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[10] "Personal communication." .

[11] V. C. Mbatchou and S. Dawda, "Phytochemical and pharmacological profile of genus Icacina," *Phytopharmacology*, vol. 2, no. 2, pp. 135–143, 2012.

Appendix 1 – NMR spectra for Chapter 1

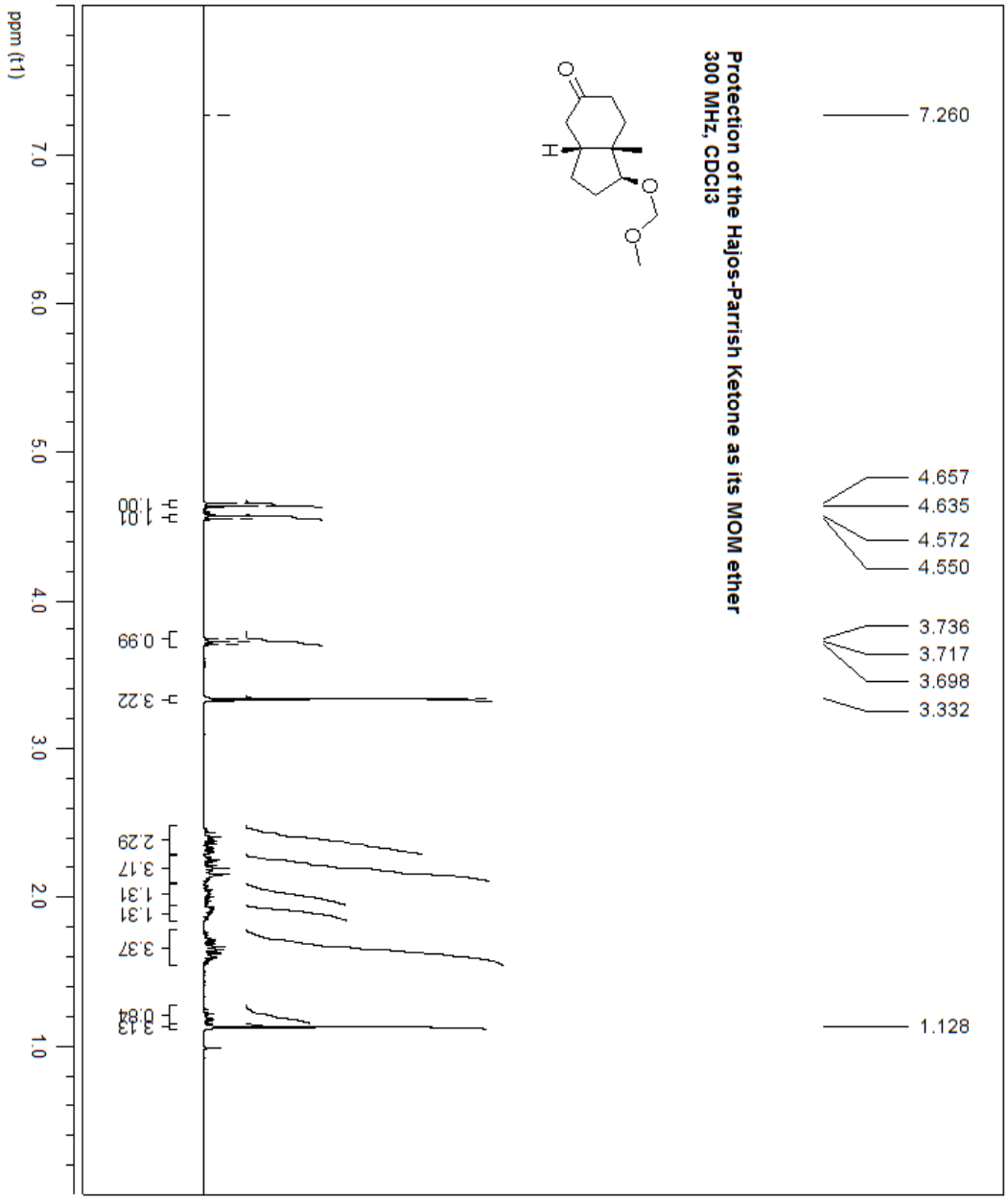

Figure a.1 ^{1}H NMR spectrum of the protected of the Hajos−Parrish Ketone as its MOM ether (**41**) in $CDCl_3$.

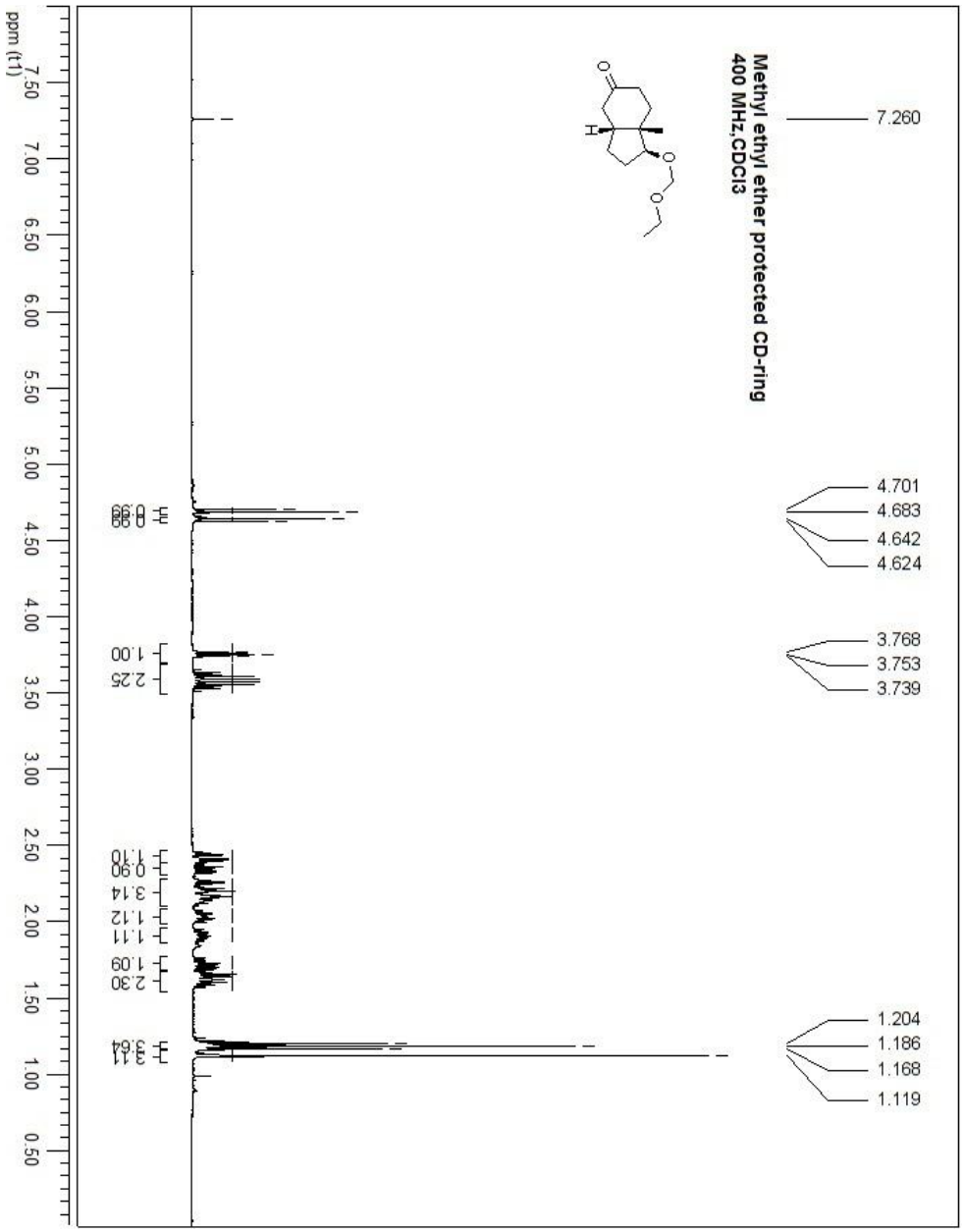

Figure a.2 ^{1}H NMR spectrum of the protected of the Hajos−Parrish Ketone as its methyl ethyl ether (**42**) in $CDCl_3$.

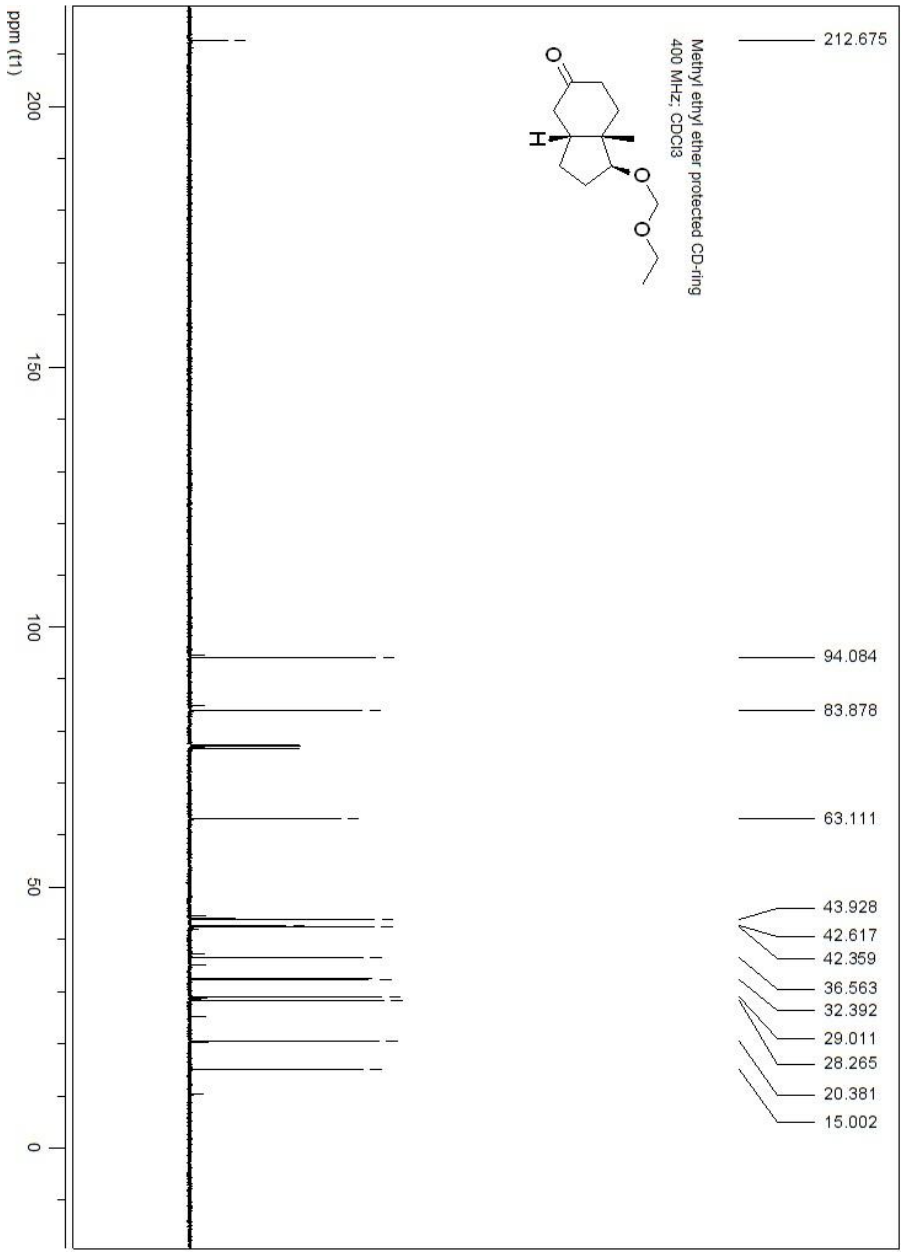

Figure a.3 ^{13}C NMR spectrum of the protected of the Hajos−Parrish Ketone as its methyl ethyl ether (**42**) in $CDCl_3$.

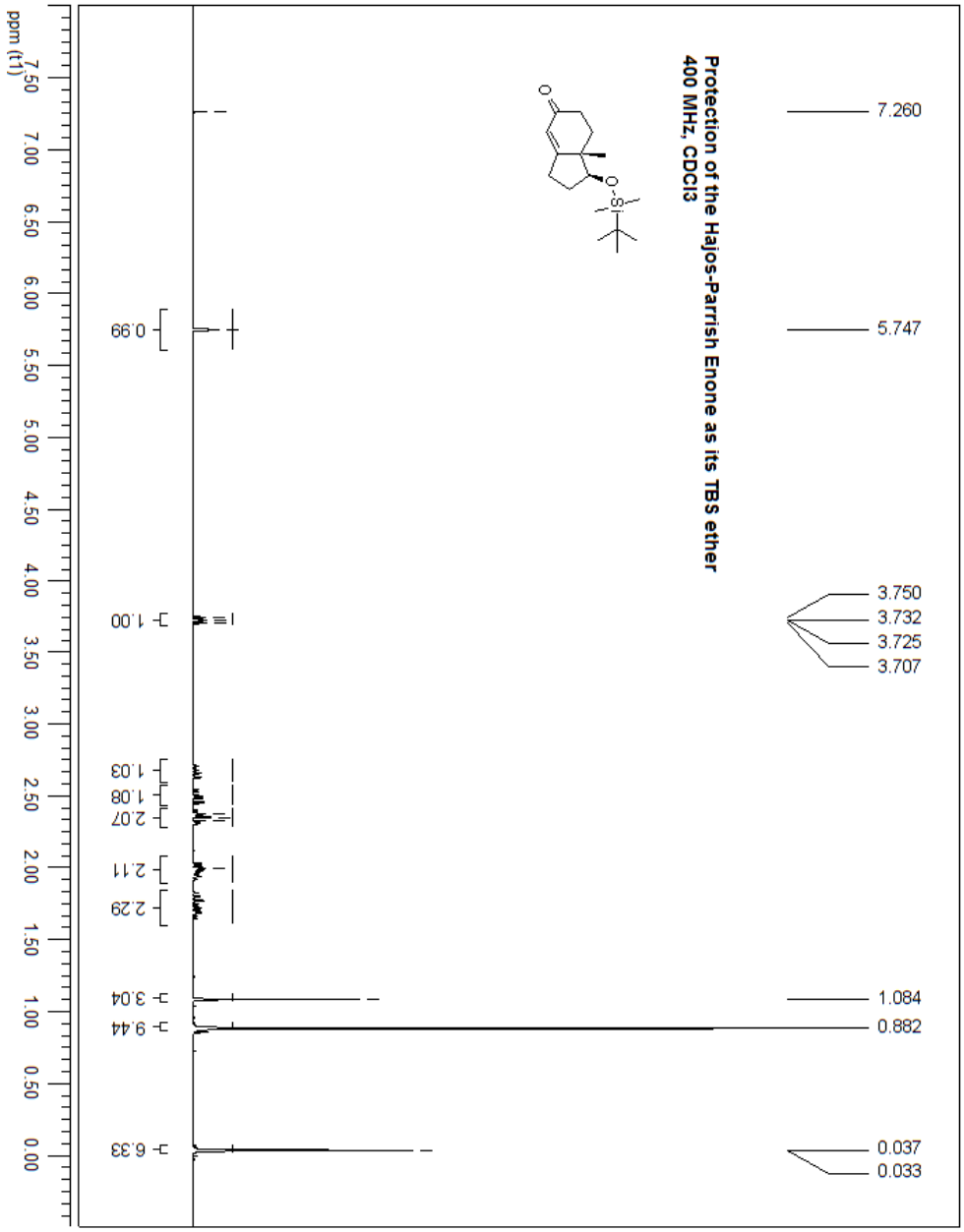

Figure a.4 ^{1}H NMR spectrum of the protected of the Hajos−Parrish enone as its TBS ether (**43**) in $CDCl_3$.

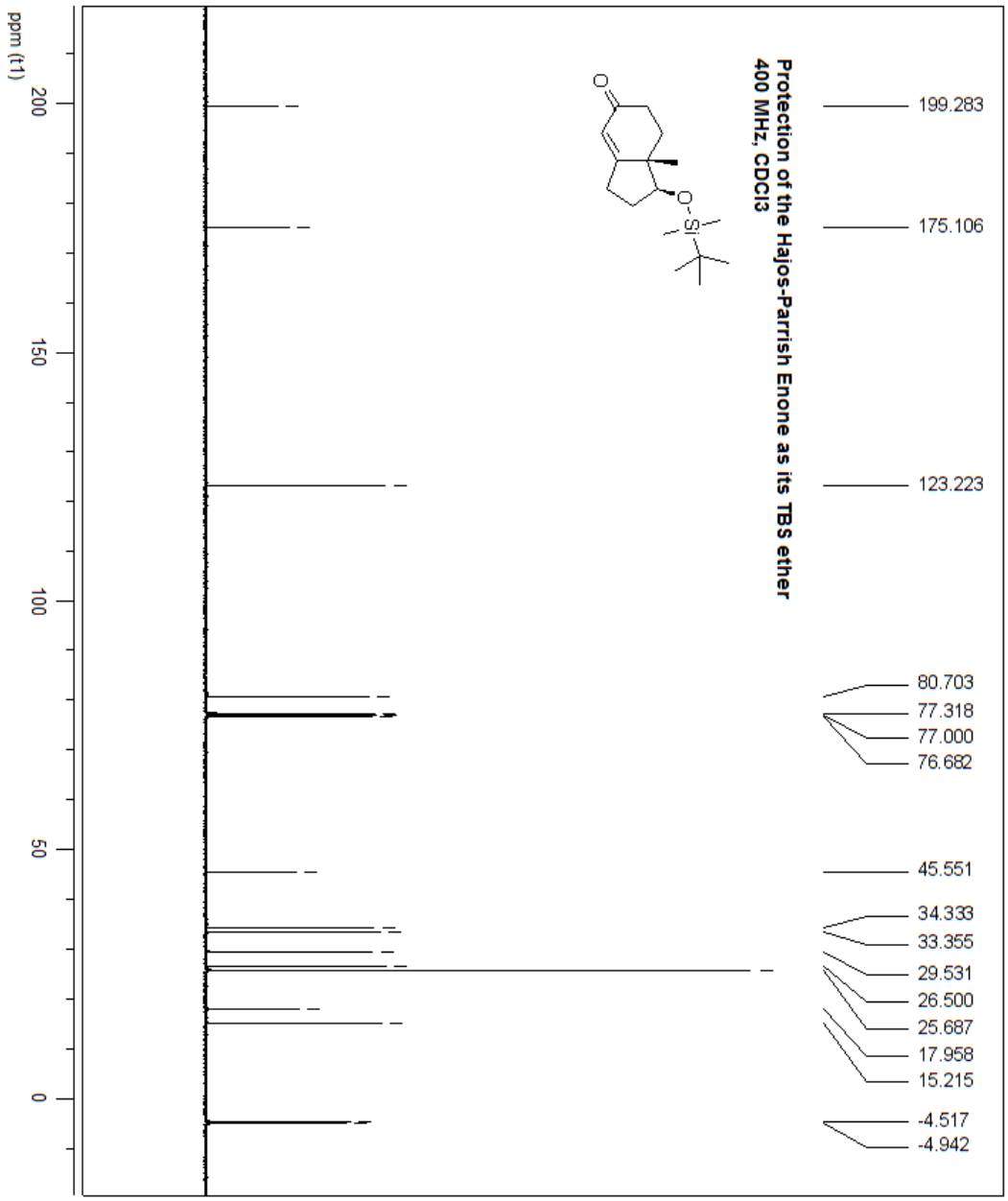

Figure a.5 ^{13}C NMR spectrum of the protected of the Hajos−Parrish enone as its TBS ether (**43**) in $CDCl_3$.

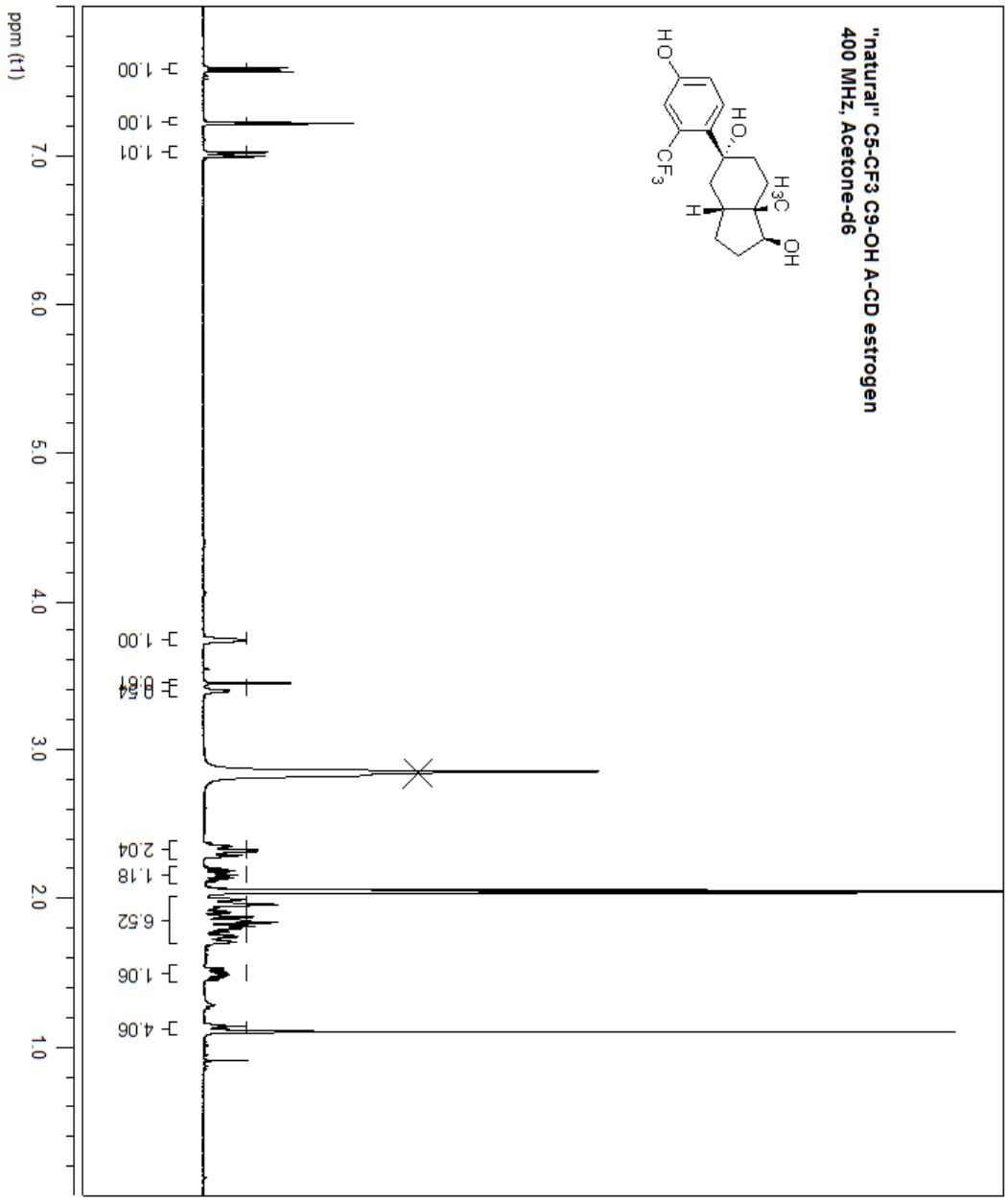

Figure a.6 ^{1}H NMR spectrum of the "natural" C5-CF_3 C9-OH A-CD estrogen (**69**) in Acetone-d_6.

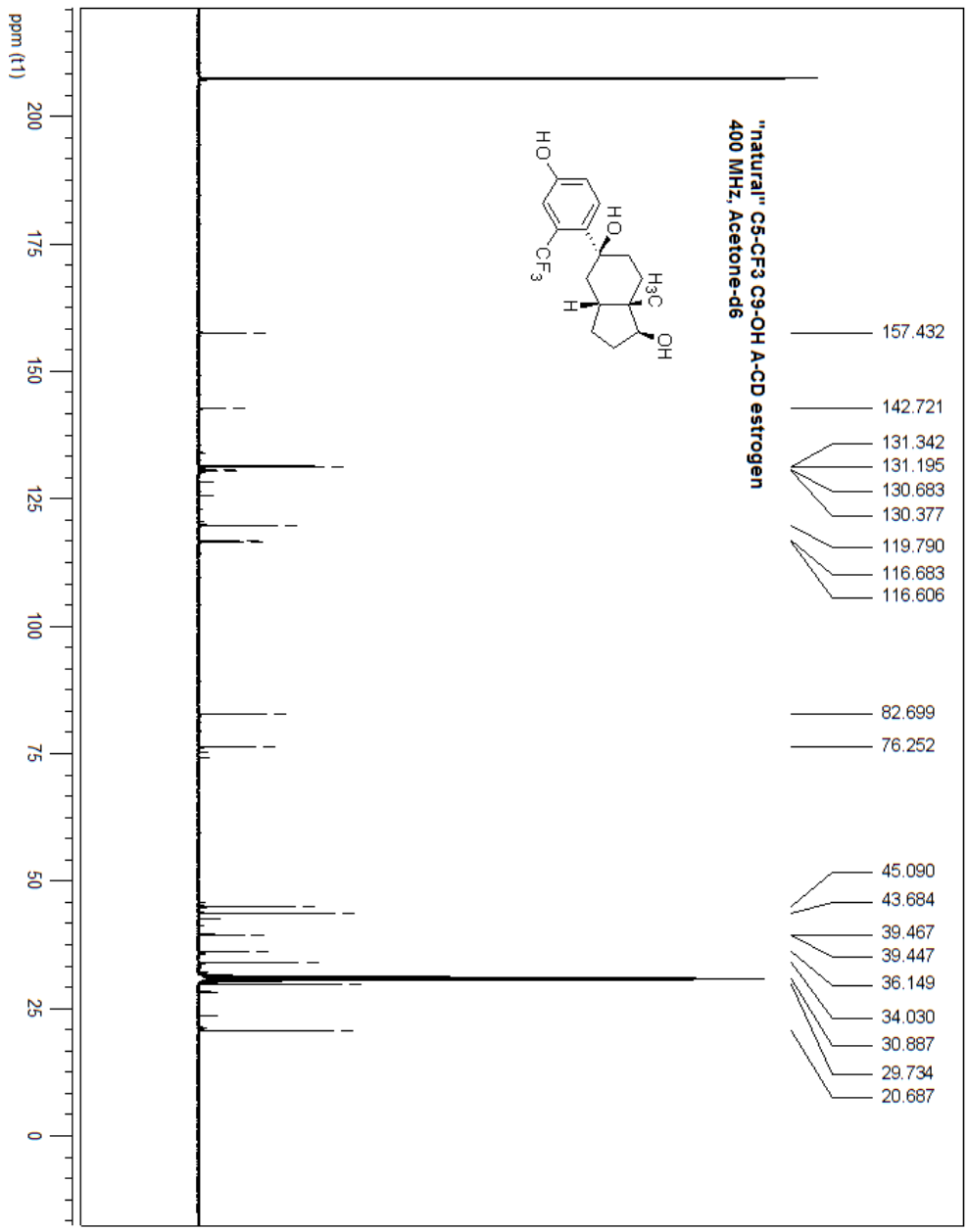

Figure a.7 ^{13}C NMR spectrum of the "natural" C5-CF_3 C9-OH A-CD estrogen (**69**) in Acetone-d_6.

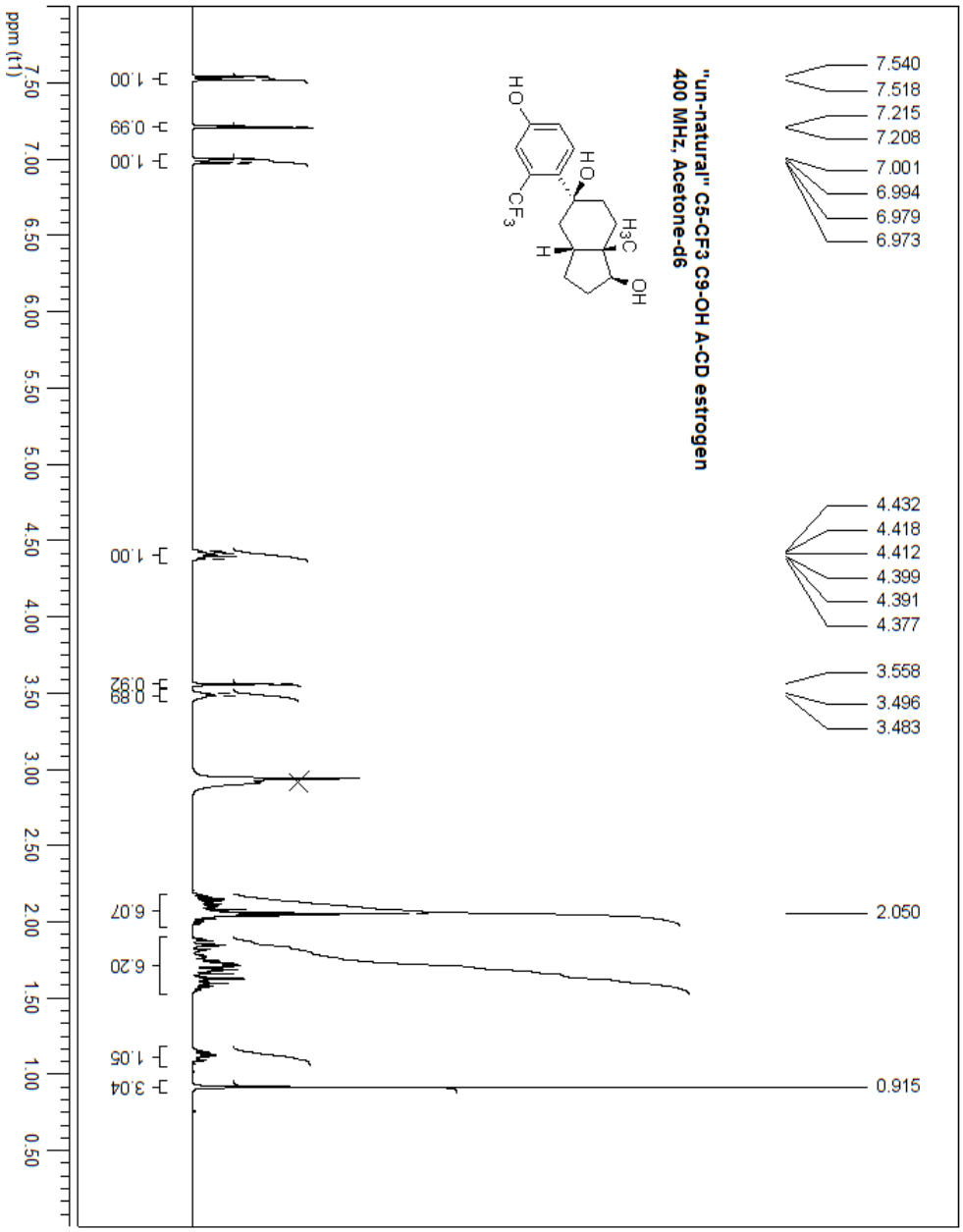

Figure a.8 ^{1}H NMR spectrum of the "un-natural" C5-CF_3 C9-OH A-CD estrogen (**70**) in Acetone-d_6.

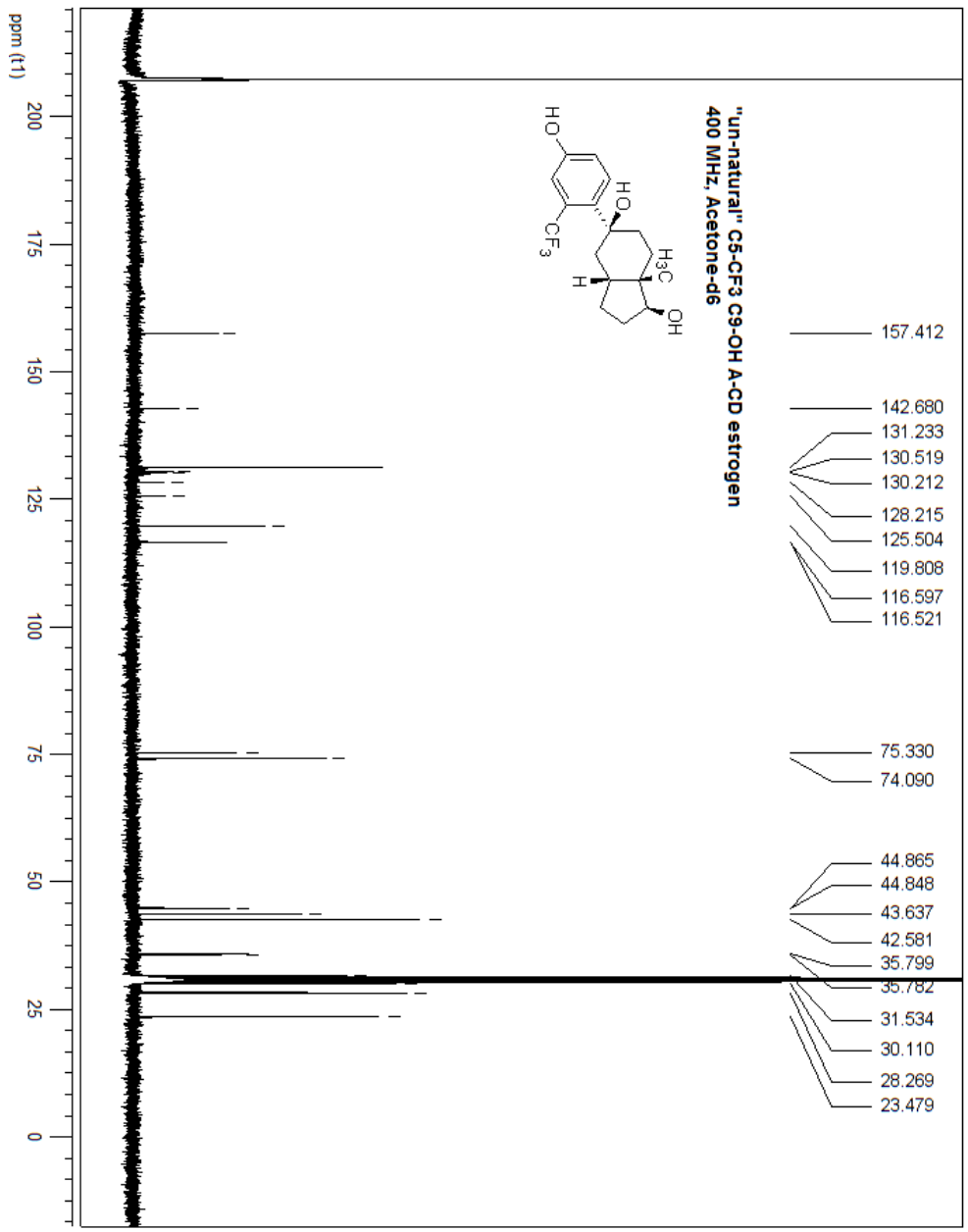

Figure a.9 ^{13}C NMR spectrum of the "un-natural" C5-CF_3 C9-OH A-CD estrogen (**70**) in Acetone-d_6.

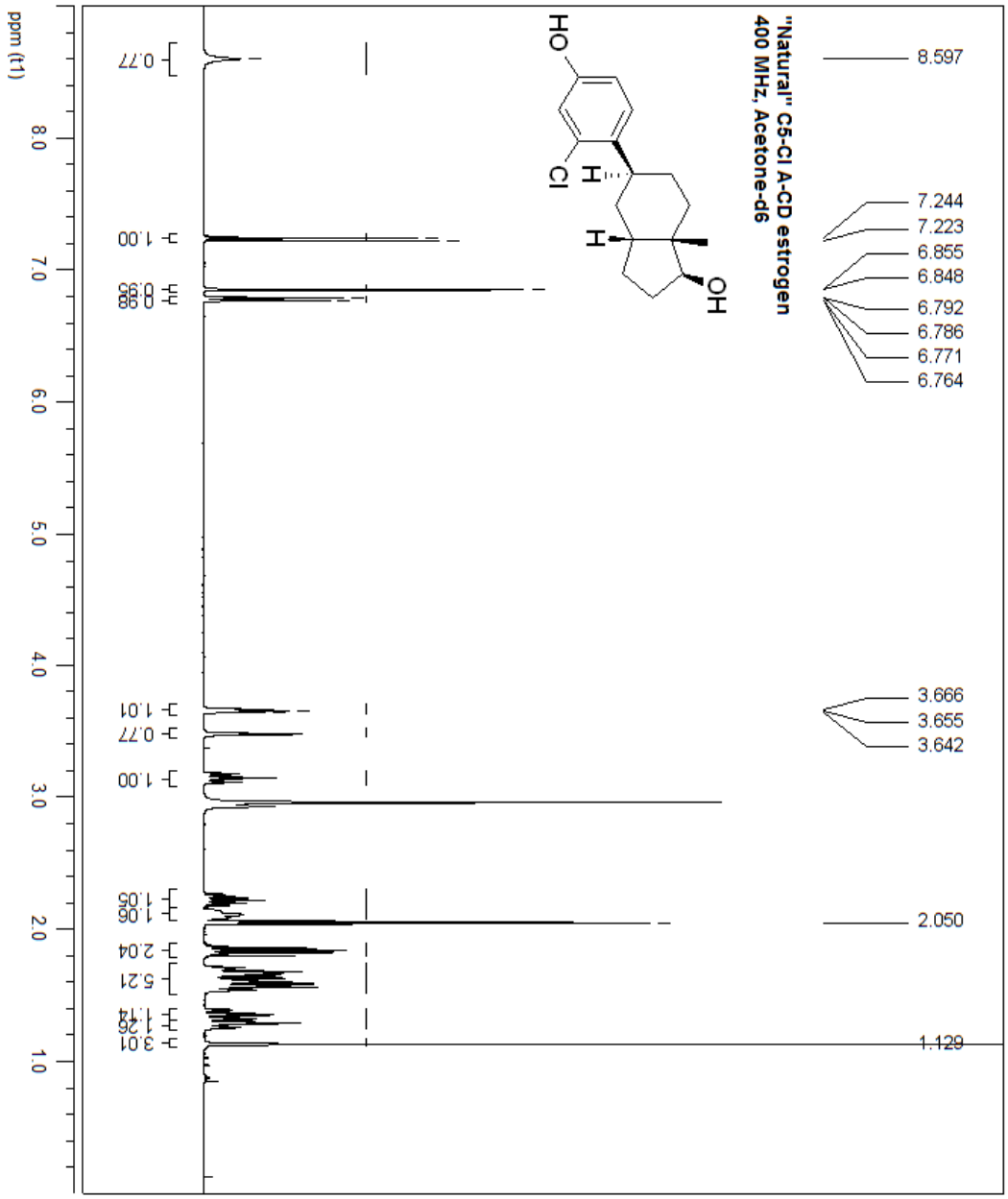

Figure a.10 ^{1}H NMR spectrum of the "natural" C5-Cl A-CD estrogen (**71**) in Acetone-d_6.

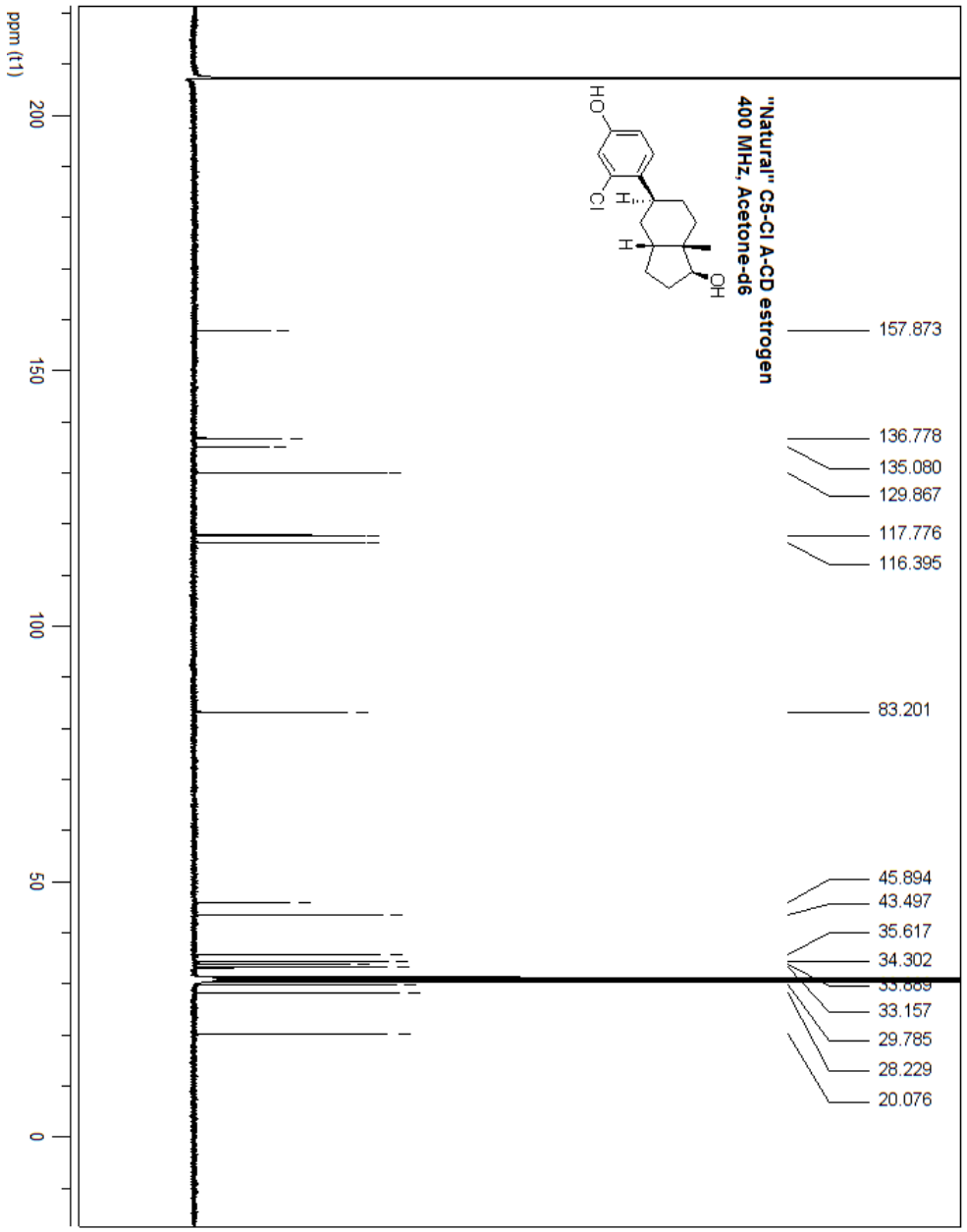

Figure a.11 ^{13}C NMR spectrum of the "natural" C5-Cl A-CD estrogen (**71**) in Acetone-d_6.

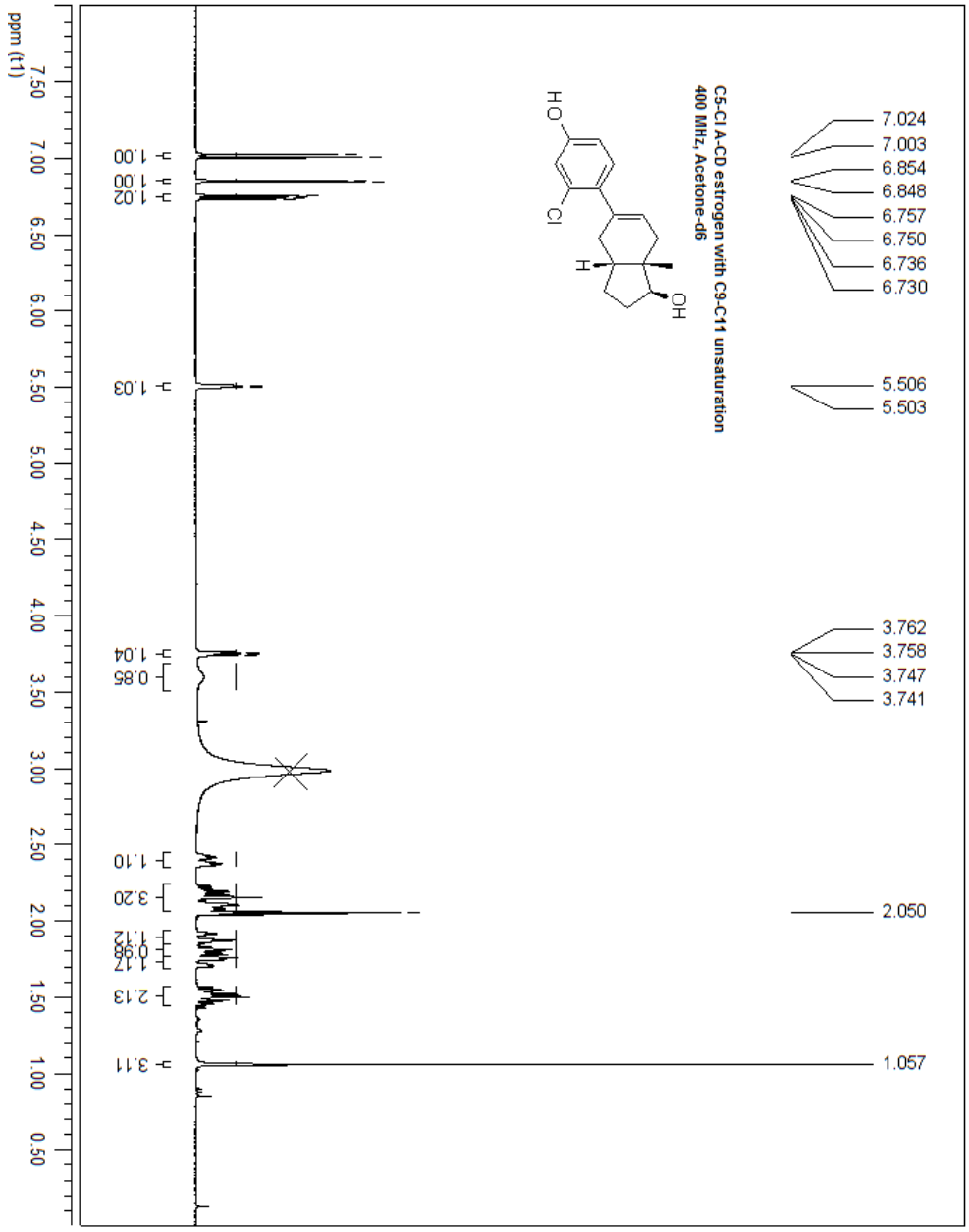

Figure a.12 ^{1}H NMR spectrum of C5-Cl A-CD estrogen with C9-C11 unsaturation (**80**) in Acetone-d_6.

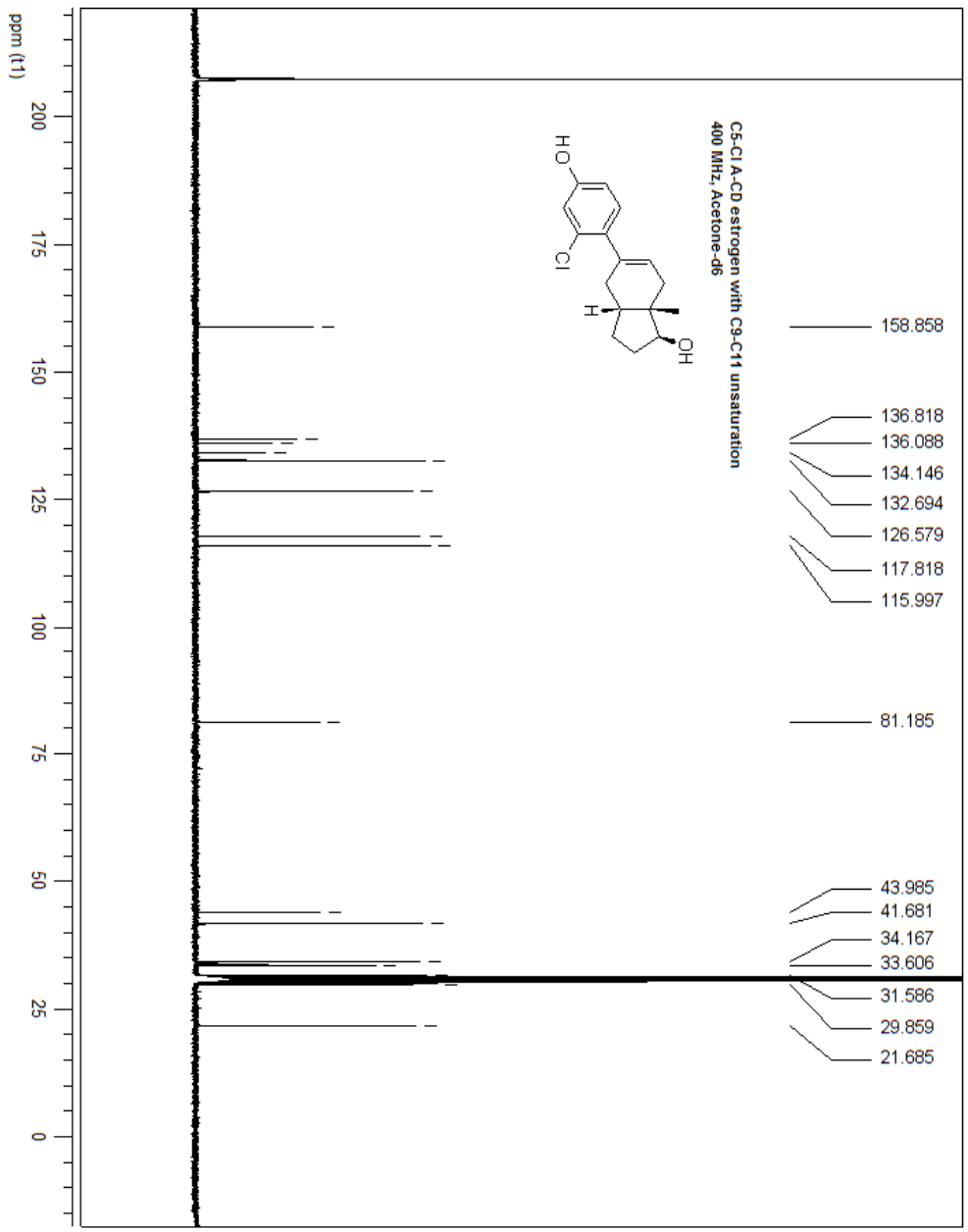

Figure a.13 ^{13}C NMR spectrum of C5-Cl A-CD estrogen with C9-C11 unsaturation (**80**) in Acetone-d_6.

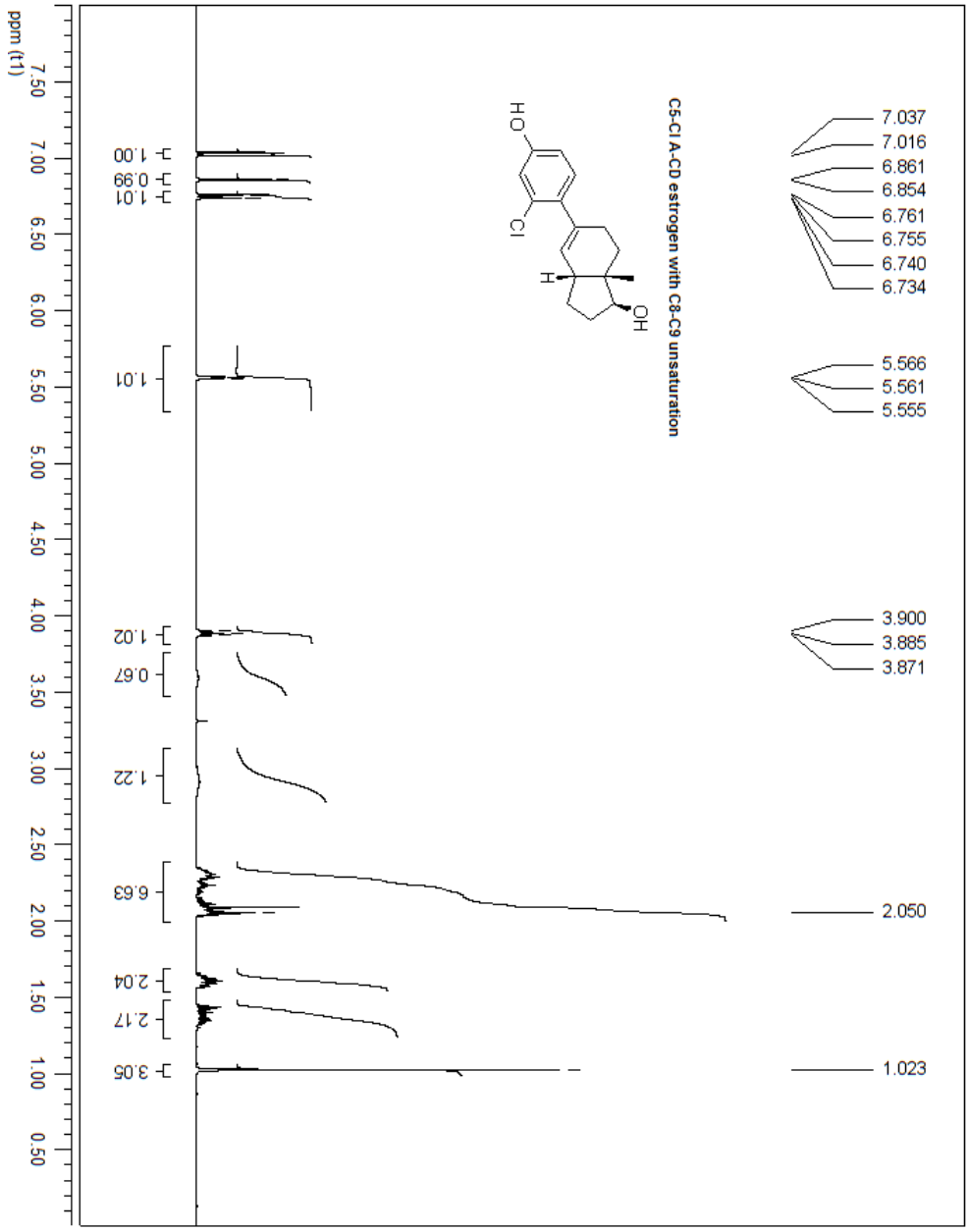

Figure a.14 ^{1}H NMR spectrum of C5-Cl A-CD estrogen with C8-C9 unsaturation (**81**) in Acetone-d_6.

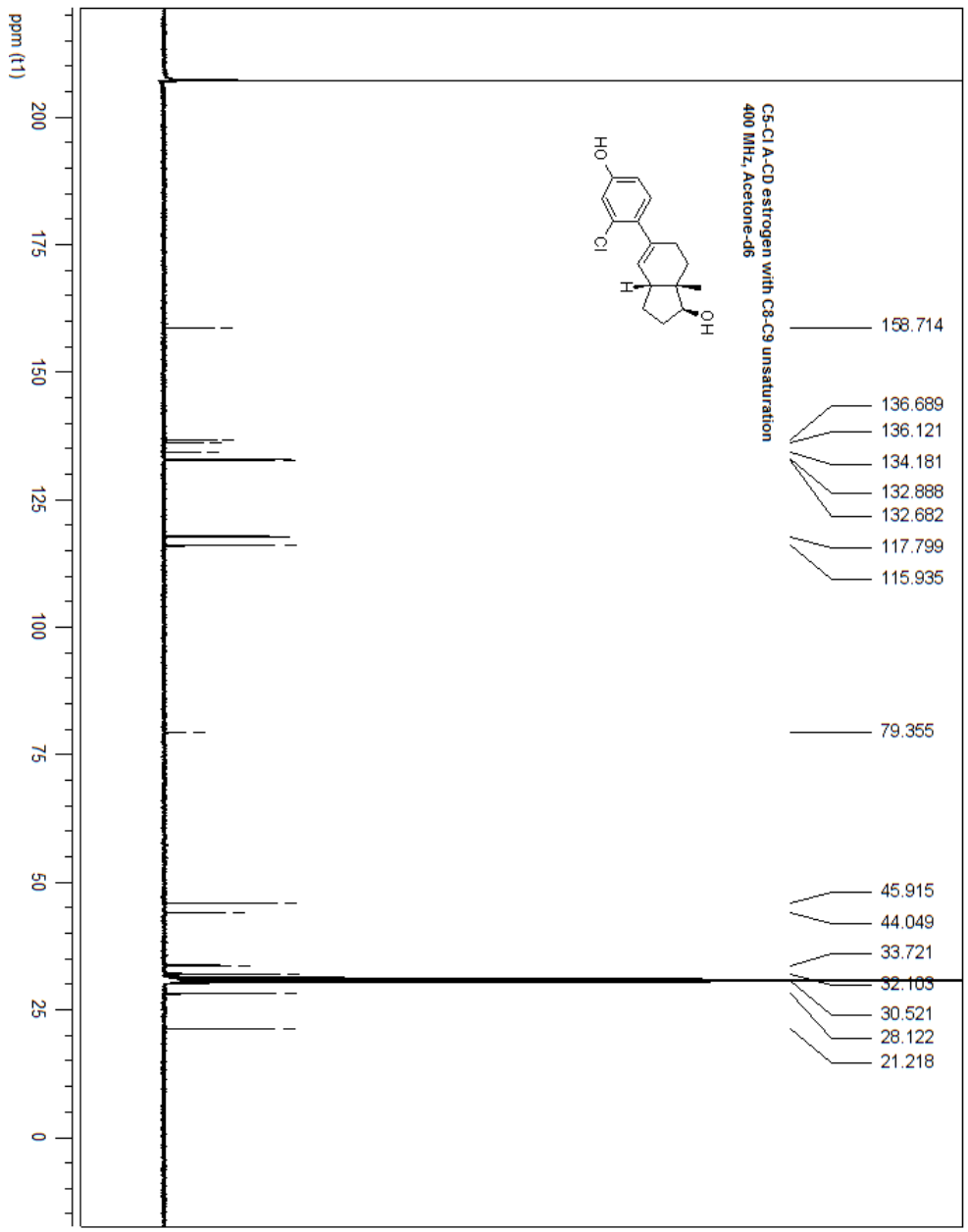

Figure a.15 ^{13}CNMR spectrum of C5-Cl A-CD estrogen with C8-C9 unsaturation (**81**) in Acetone-d_6.

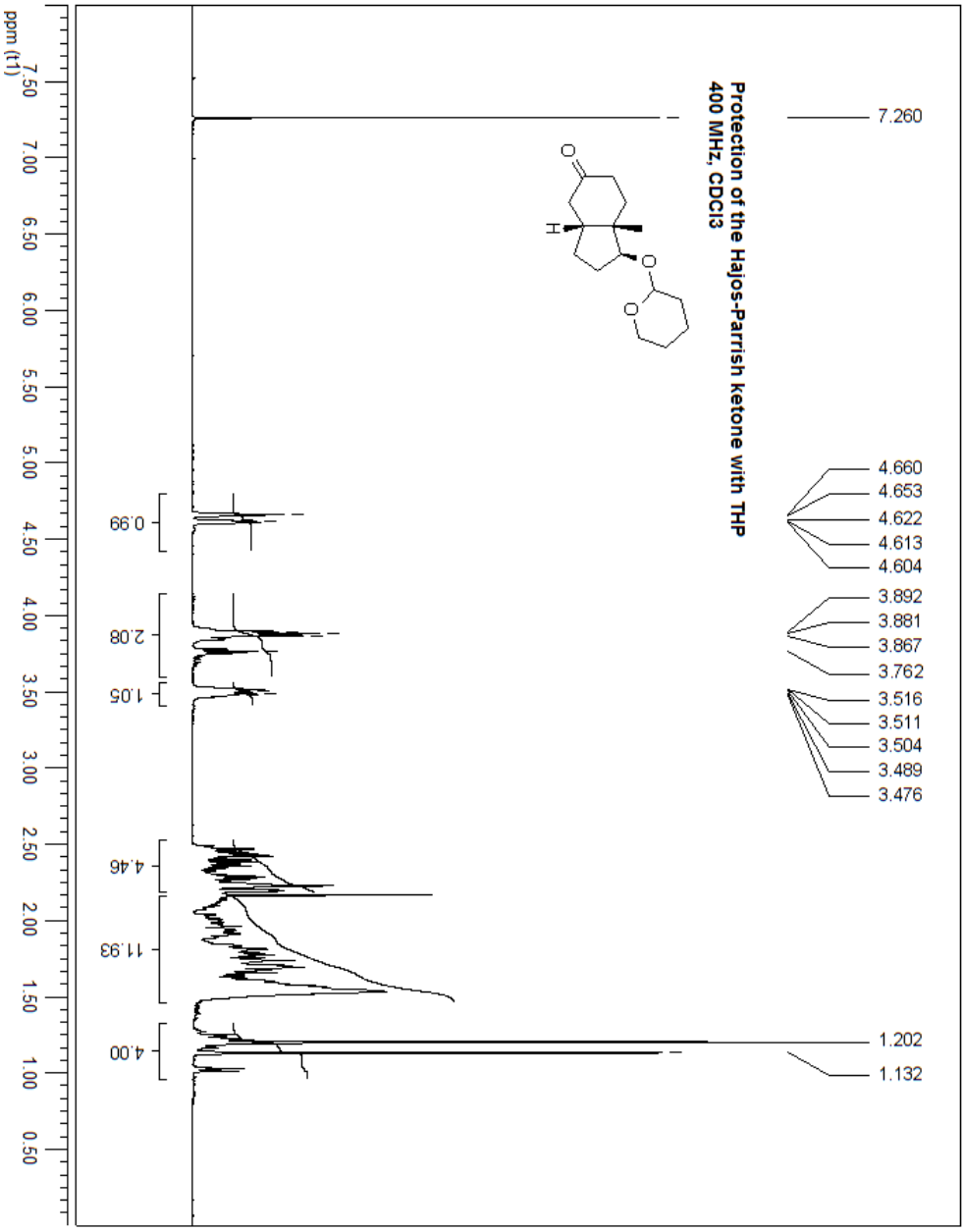

Figure a.16 ^{1}H NMR spectrum of the protection of the Hajos-Parrish ketone with THP (**89**) in $CDCl_3$.

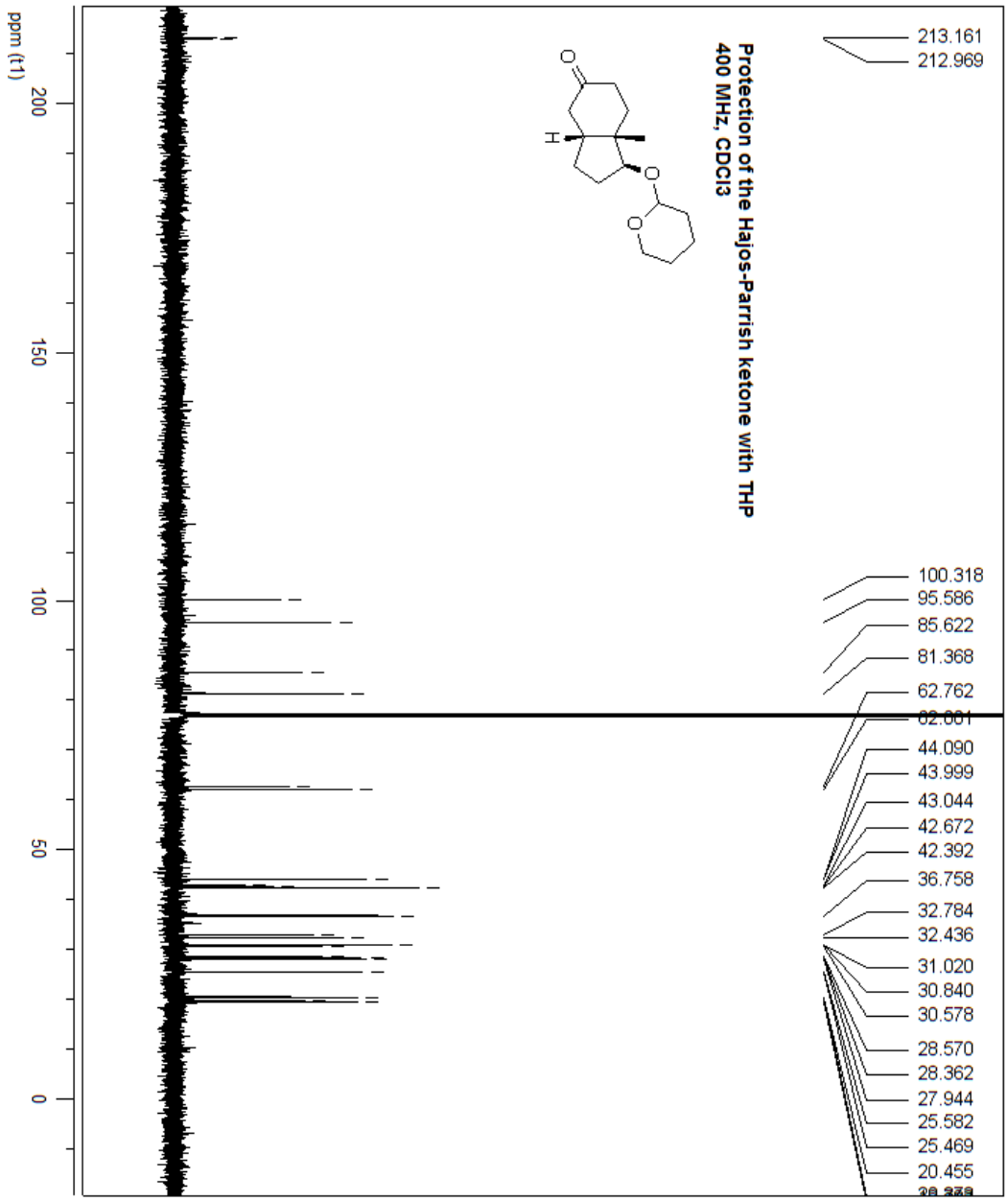

Figure a.17 ^{13}C NMR spectrum of the protection of the Hajos-Parrish ketone with THP (**89**) in $CDCl_3$.

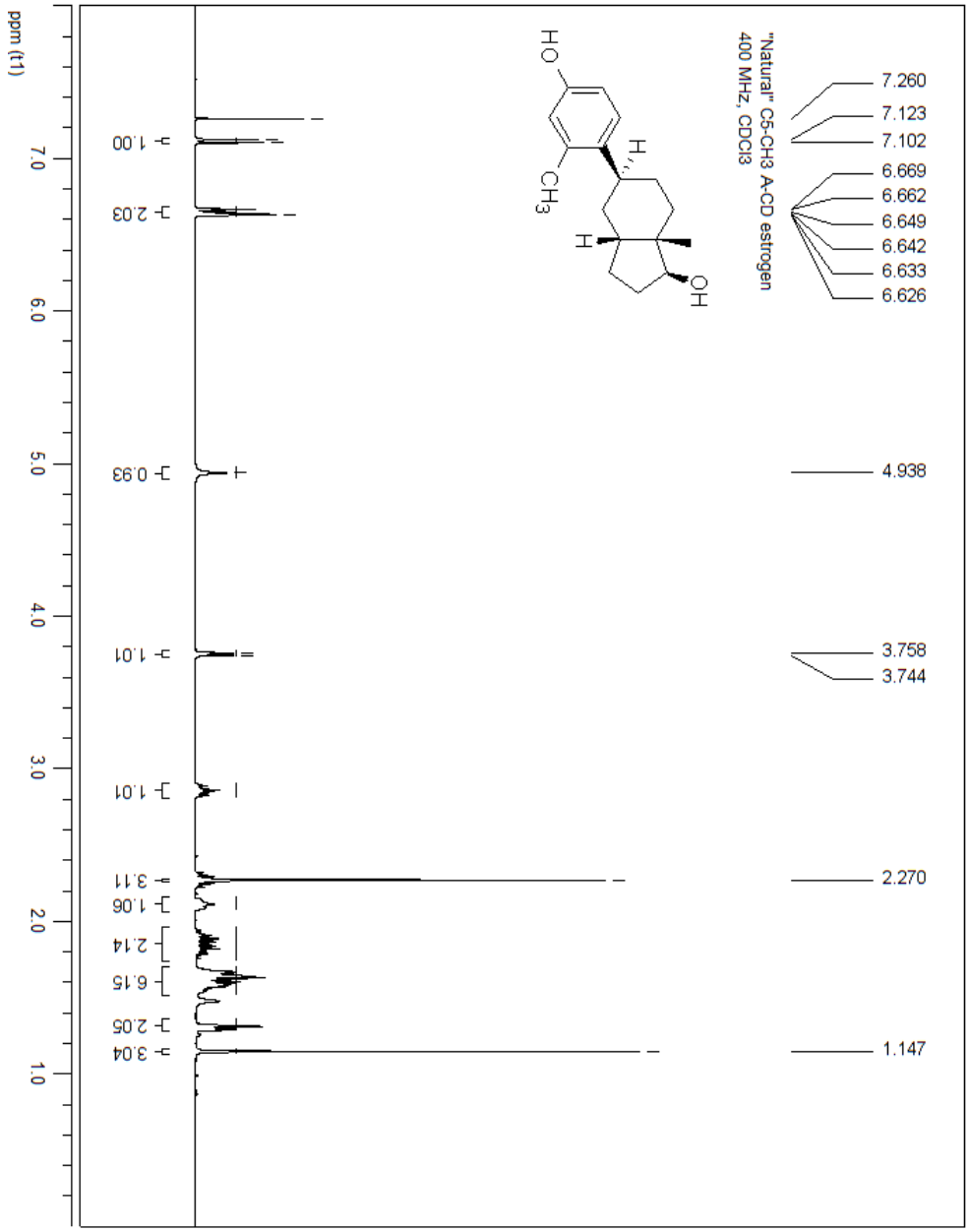

Figure a.18 ^{1}H NMR spectrum of "natural" C5-CH_3 A-CD estrogen (**82**) in Acetone-d_6.

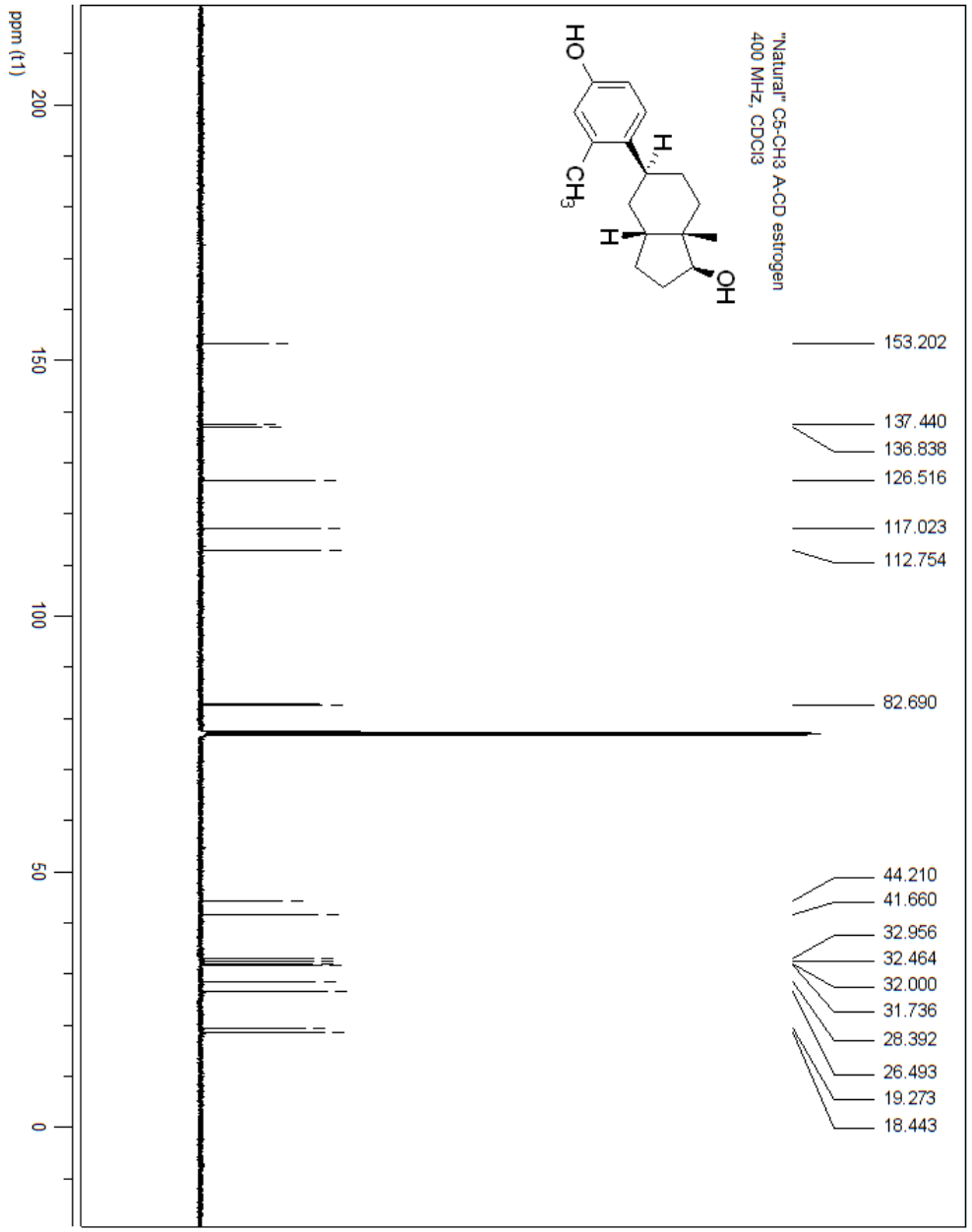

Figure a.19 ^{13}CNMR spectrum of "natural" C5-CH_3 A-CD estrogen (**82**) in Acetone-d_6.

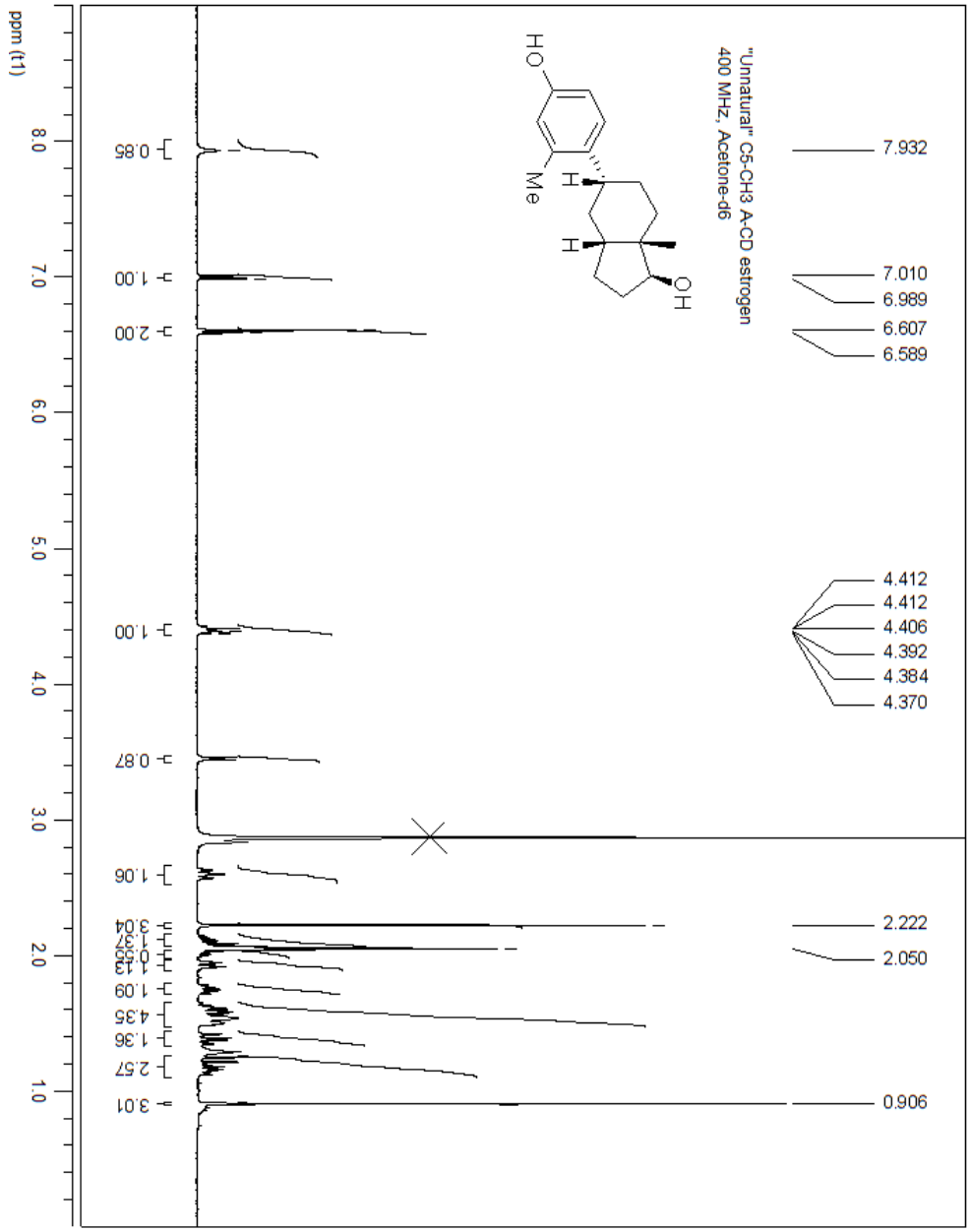

Figure a.20 ^{1}H NMR spectrum of "un-natural" C5-CH_3 A-CD estrogen (**82**) in Acetone-d_6.

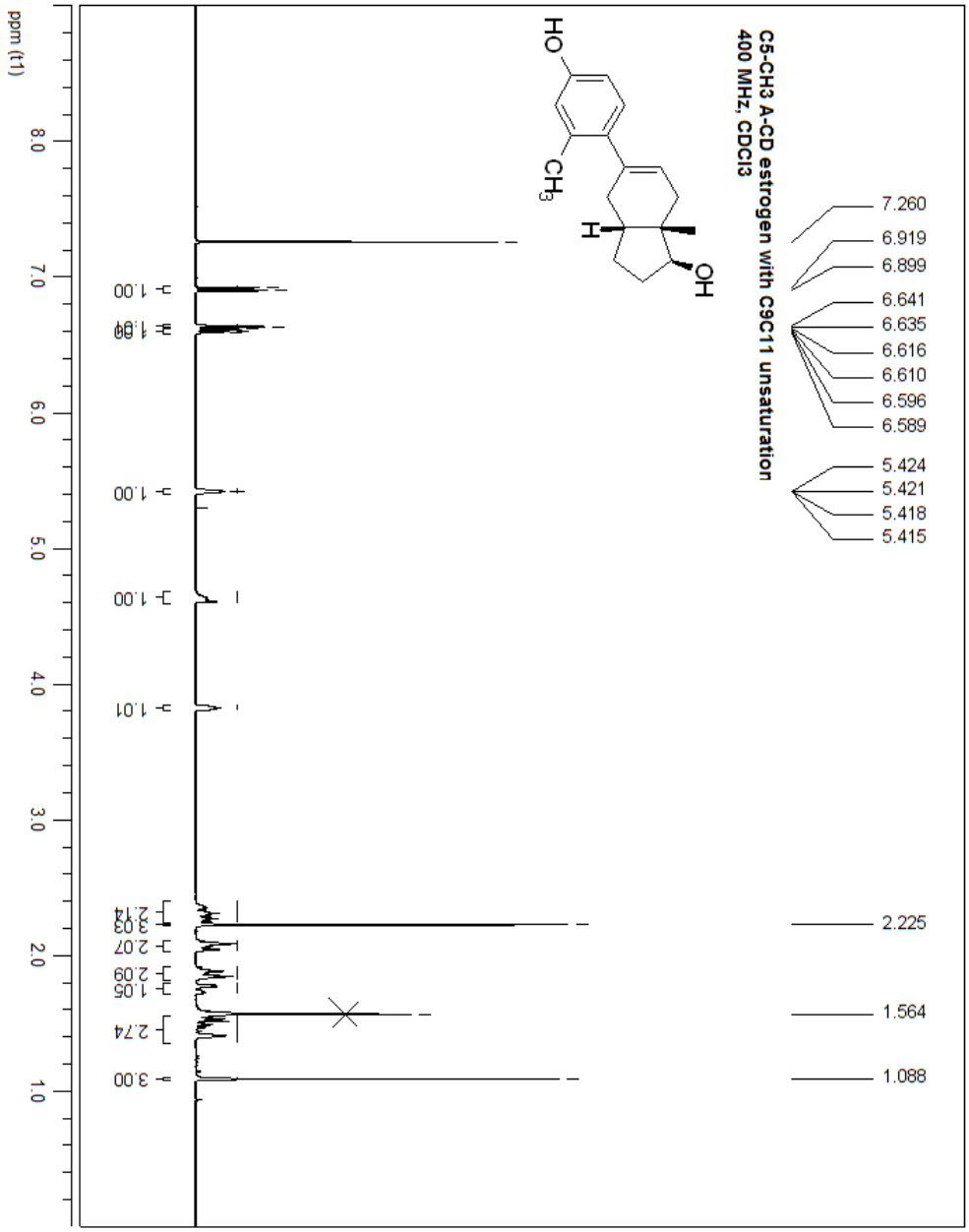

Figure a.21 ^{1}H NMR spectrum of C5-CH_3 A-CD estrogen with C9-C11 unsaturation (**93**) in Acetone-d_6.

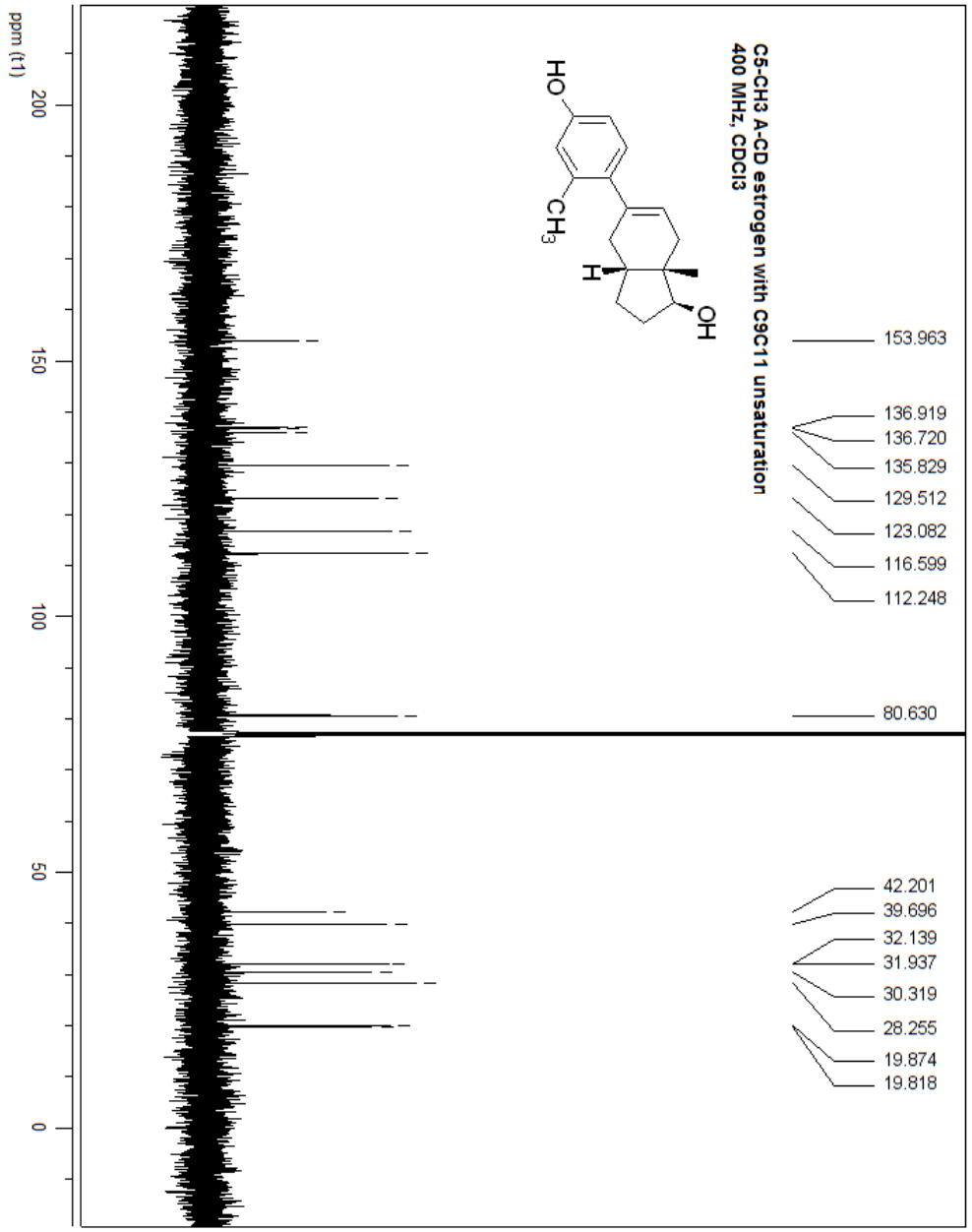

Figure a.22 ^{13}CNMR spectrum of C5-CH_3 A-CD estrogen with C9-C11 unsaturation (**93**) in Acetone-d_6.

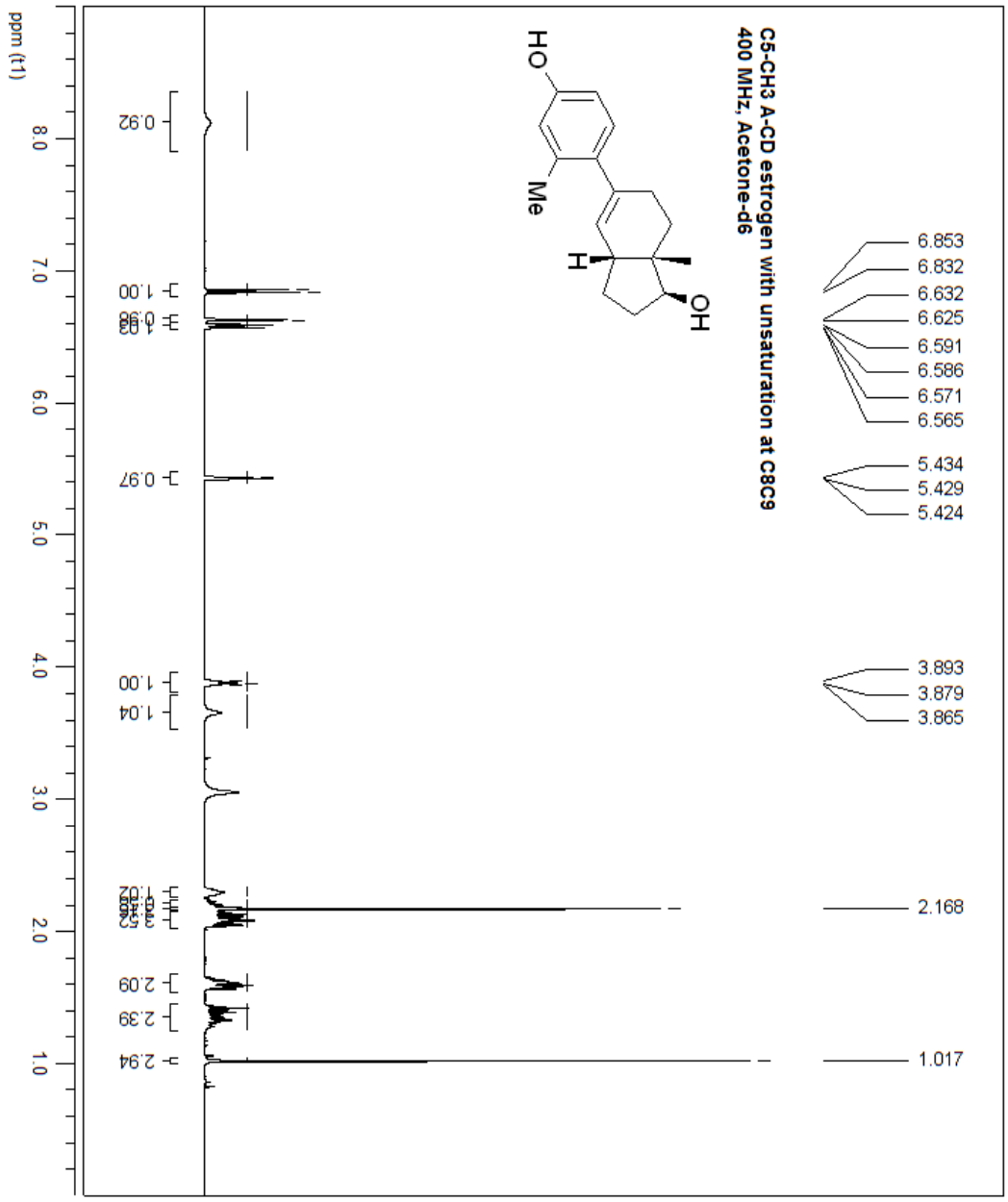

Figure a.23 ^{1}H NMR spectrum of C5-CH_3 A-CD estrogen with C8-C9 unsaturation (**94**) in Acetone-d_6.

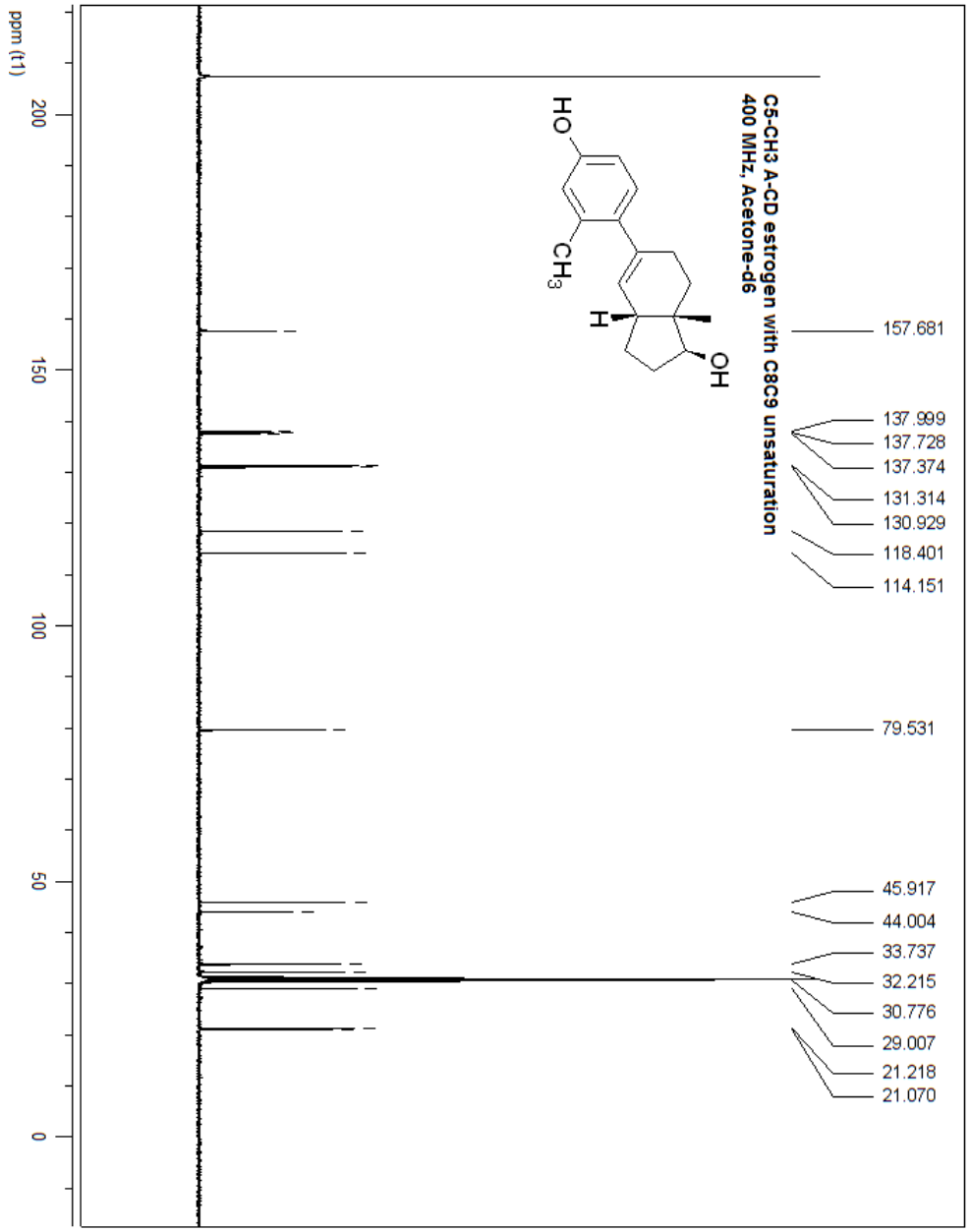

Figure a.24 ^{13}CNMR spectrum of C5-CH_3 A-CD estrogen with C8-C9 unsaturation (**94**) in Acetone-d_6.

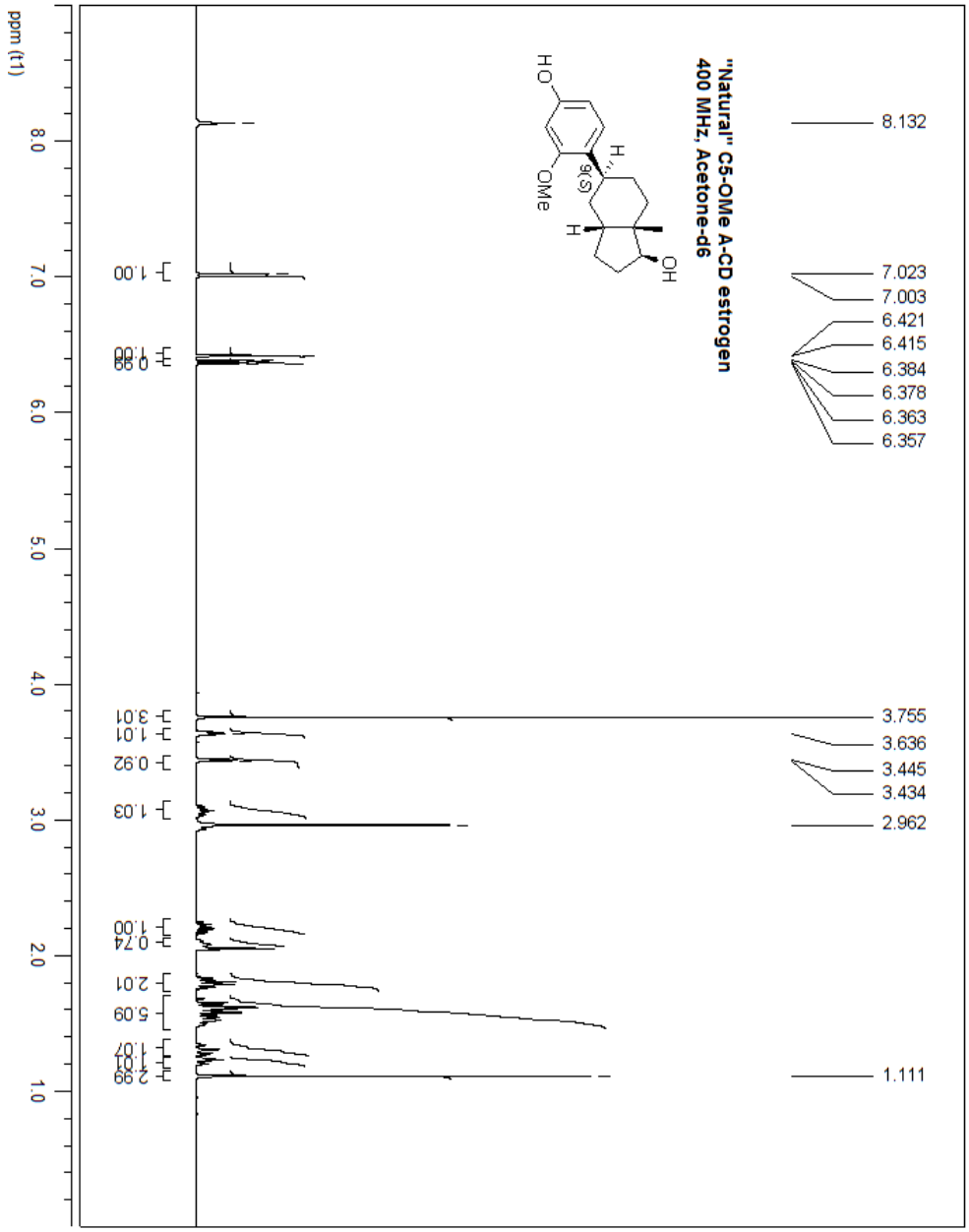

Figure a.25 ^{1}H NMR spectrum of the "natural" C5-OCH_3 A-CD estrogen (**83**) in Acetone-d_6.

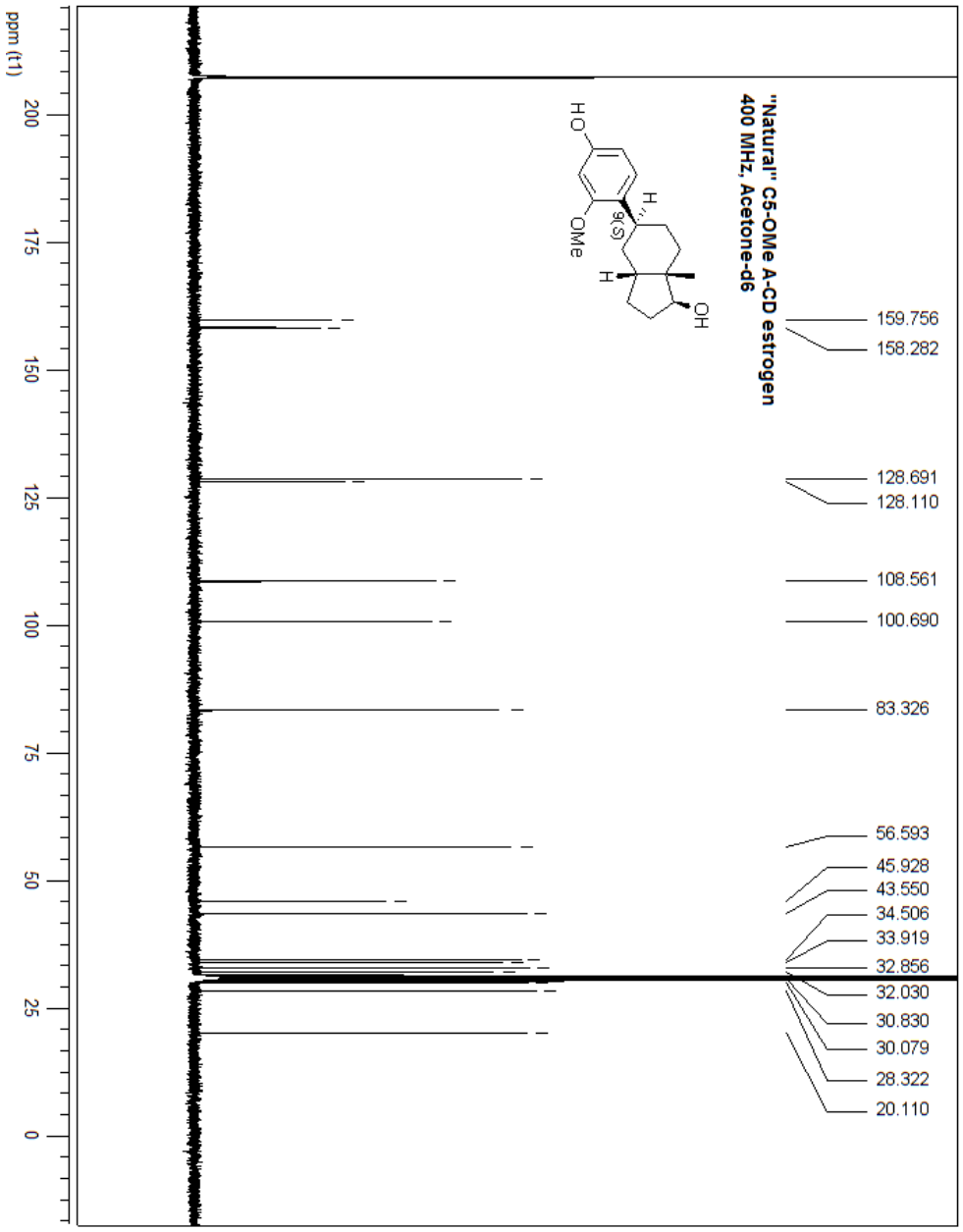

Figure a.26 ^{13}CNMR spectrum of the "natural" C5-OCH_3 A-CD estrogen (**83**) in Acetone-d_6.

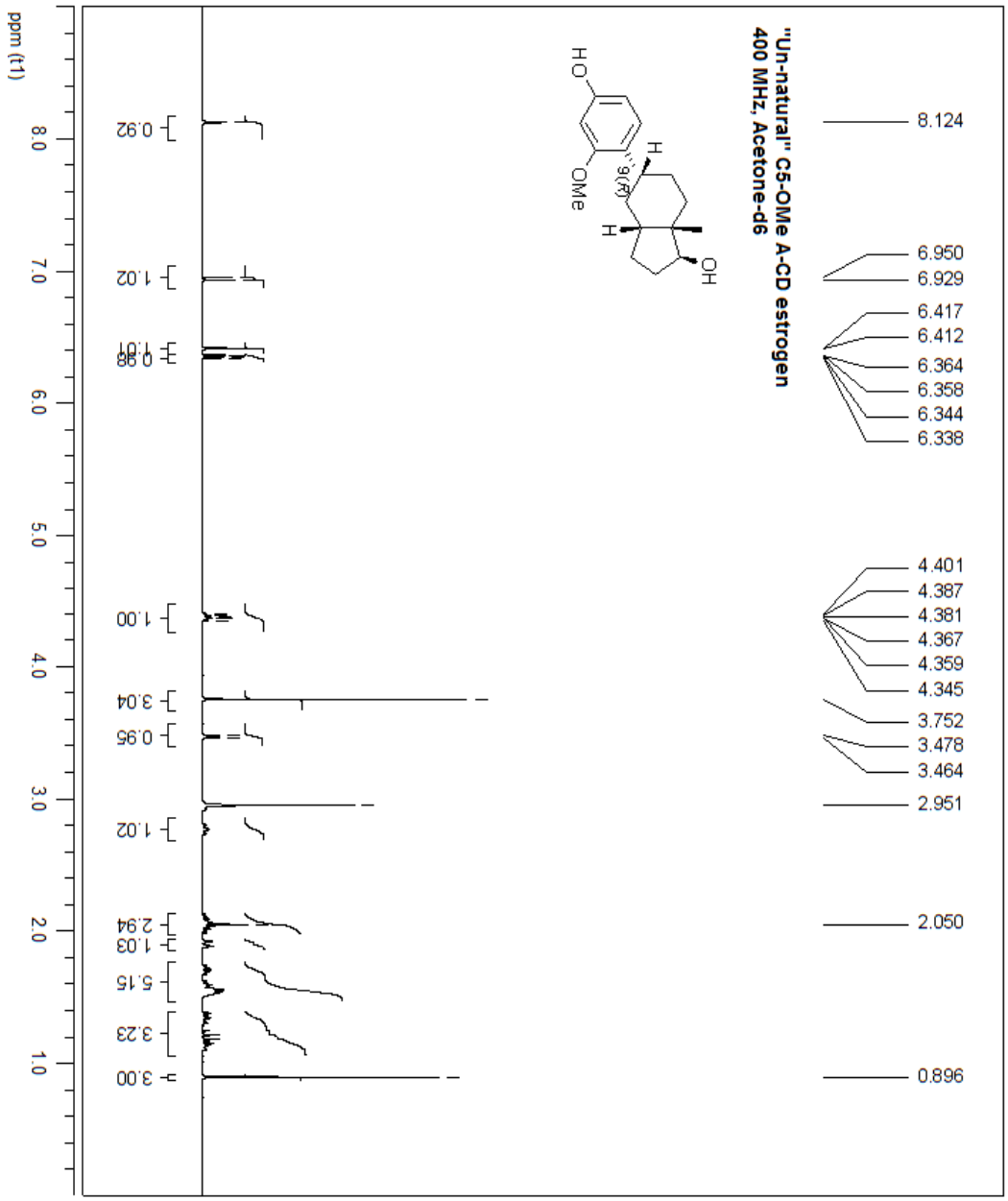

Figure a.27 ^{1}H NMR spectrum of the "un-natural" C5-OCH_3 A-CD estrogen (**83**) in Acetone-d_6.

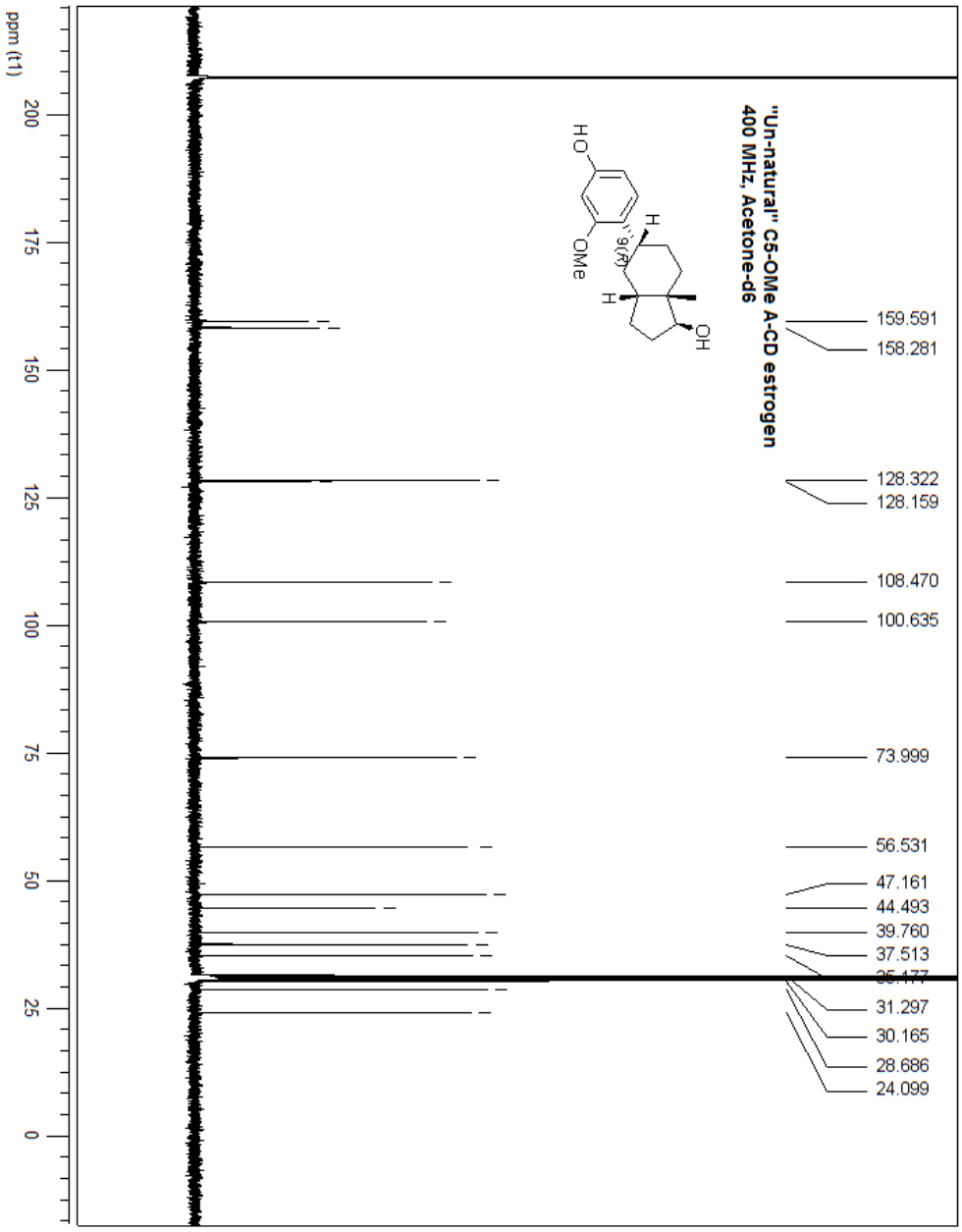

Figure a.28 ^{13}CNMR spectrum of the "un-natural" C5-OCH_3 A-CD estrogen (**83**) in Acetone-d_6.

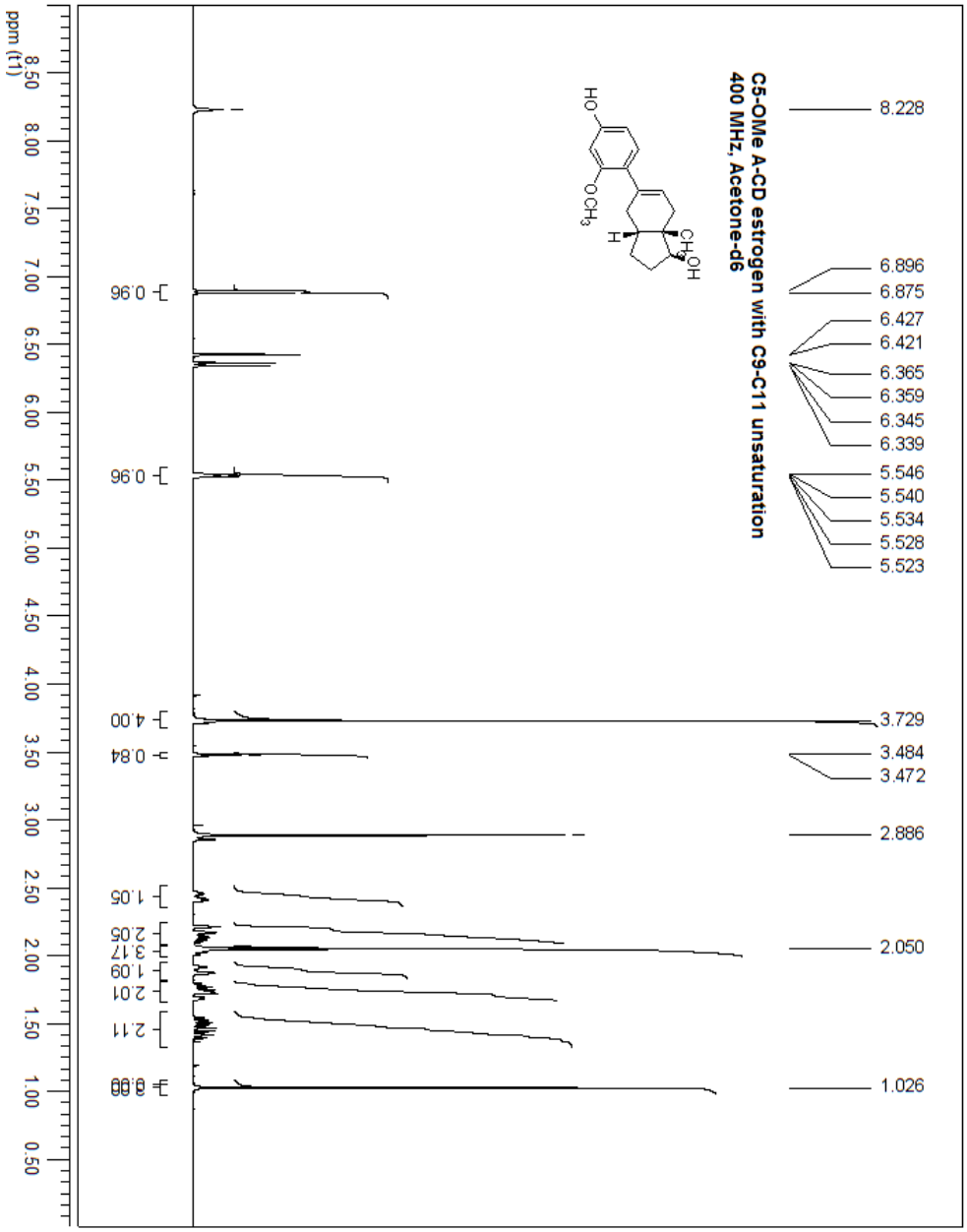

Figure a.29 ^{1}H NMR spectrum of C5-OCH_3 A-CD estrogen with C9-C11 unsaturation (**105**) in Acetone-d_6.

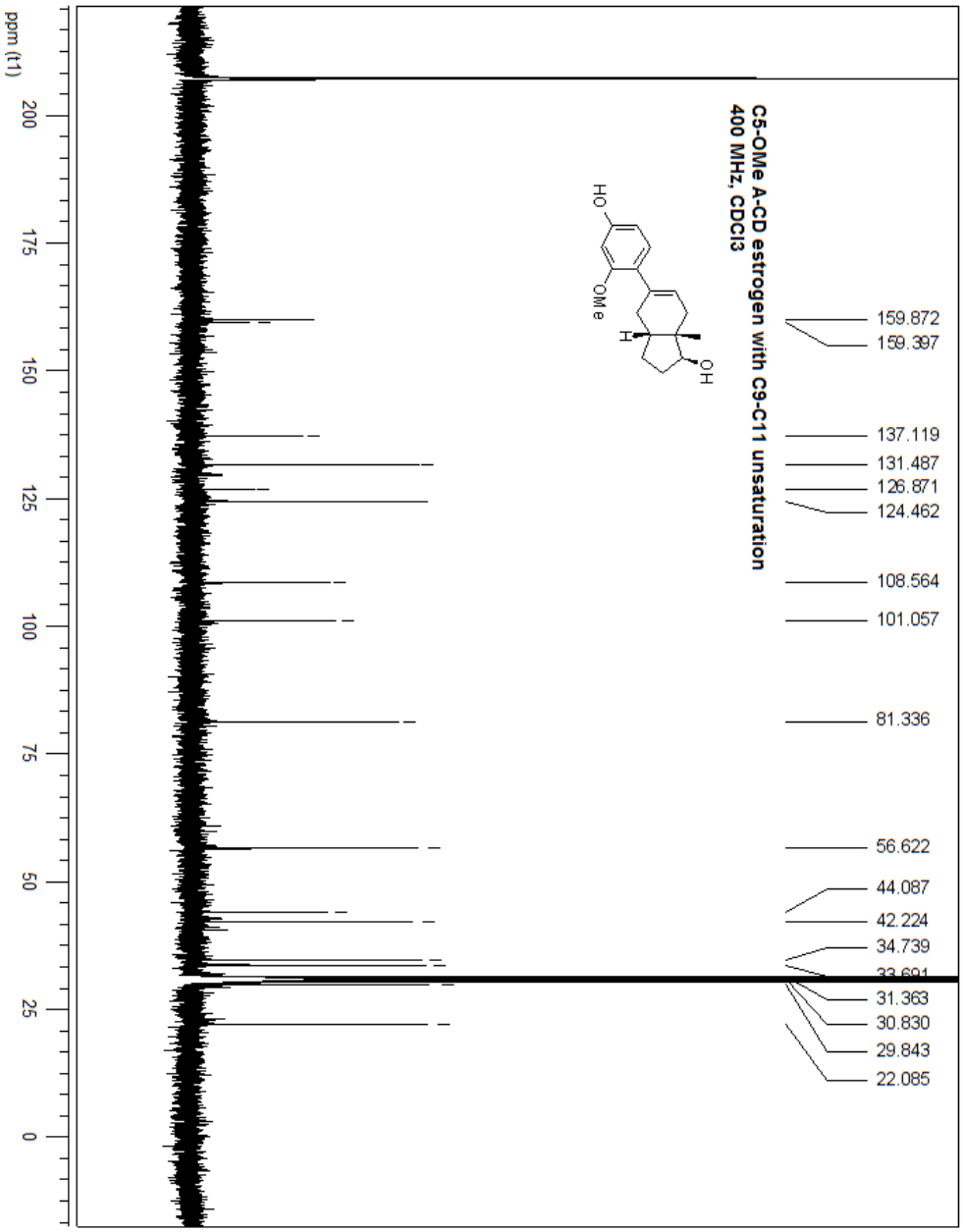

Figure a.30 ^{13}C NMR spectrum of C5-OCH_3 A-CD estrogen with C9-C11 unsaturation (**105**) in Acetone-d_6.

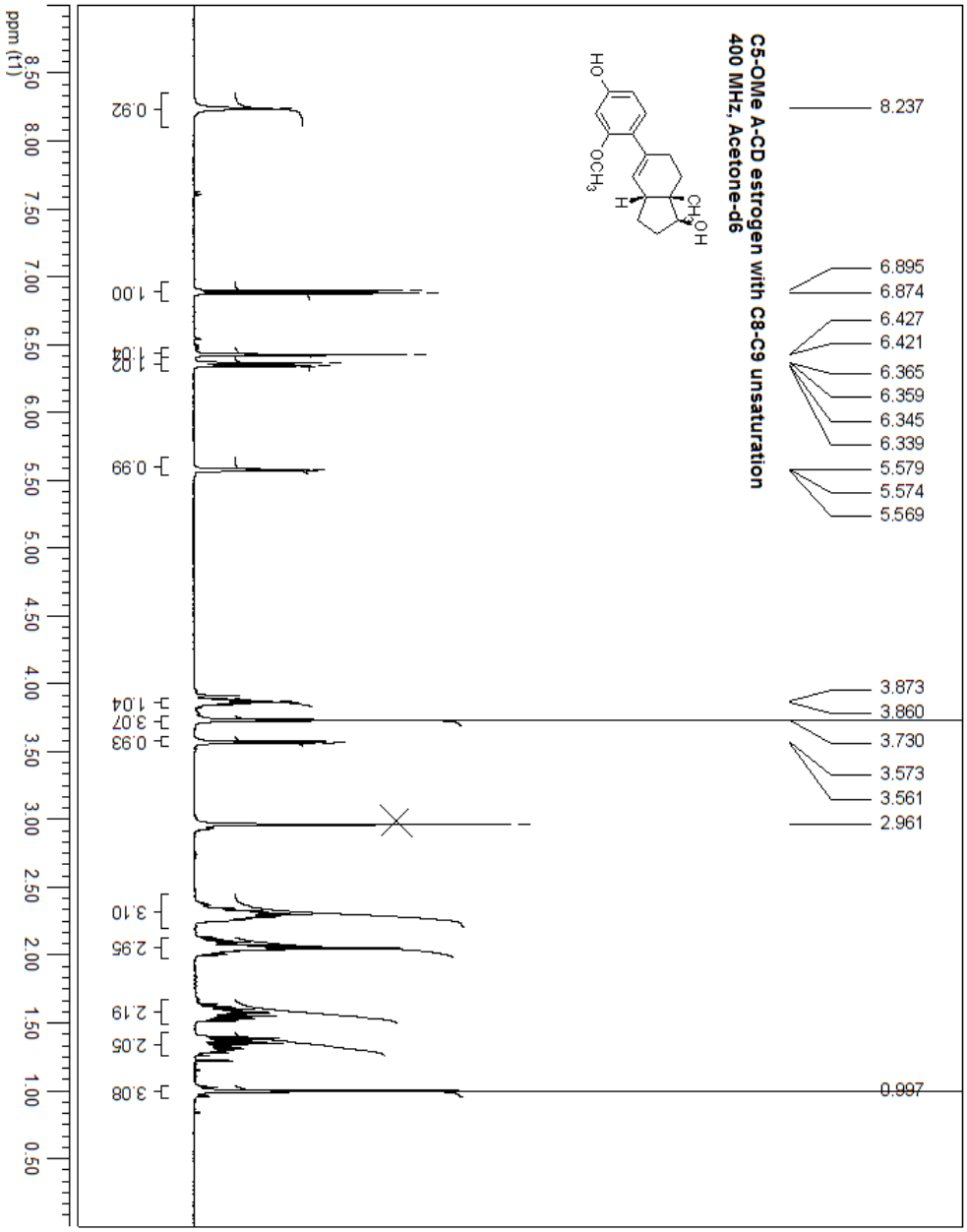

Figure a.31 ^{1}H NMR spectrum of C5-OCH_3 A-CD estrogen with C8-C9 unsaturation (**106**) in Acetone-d_6.

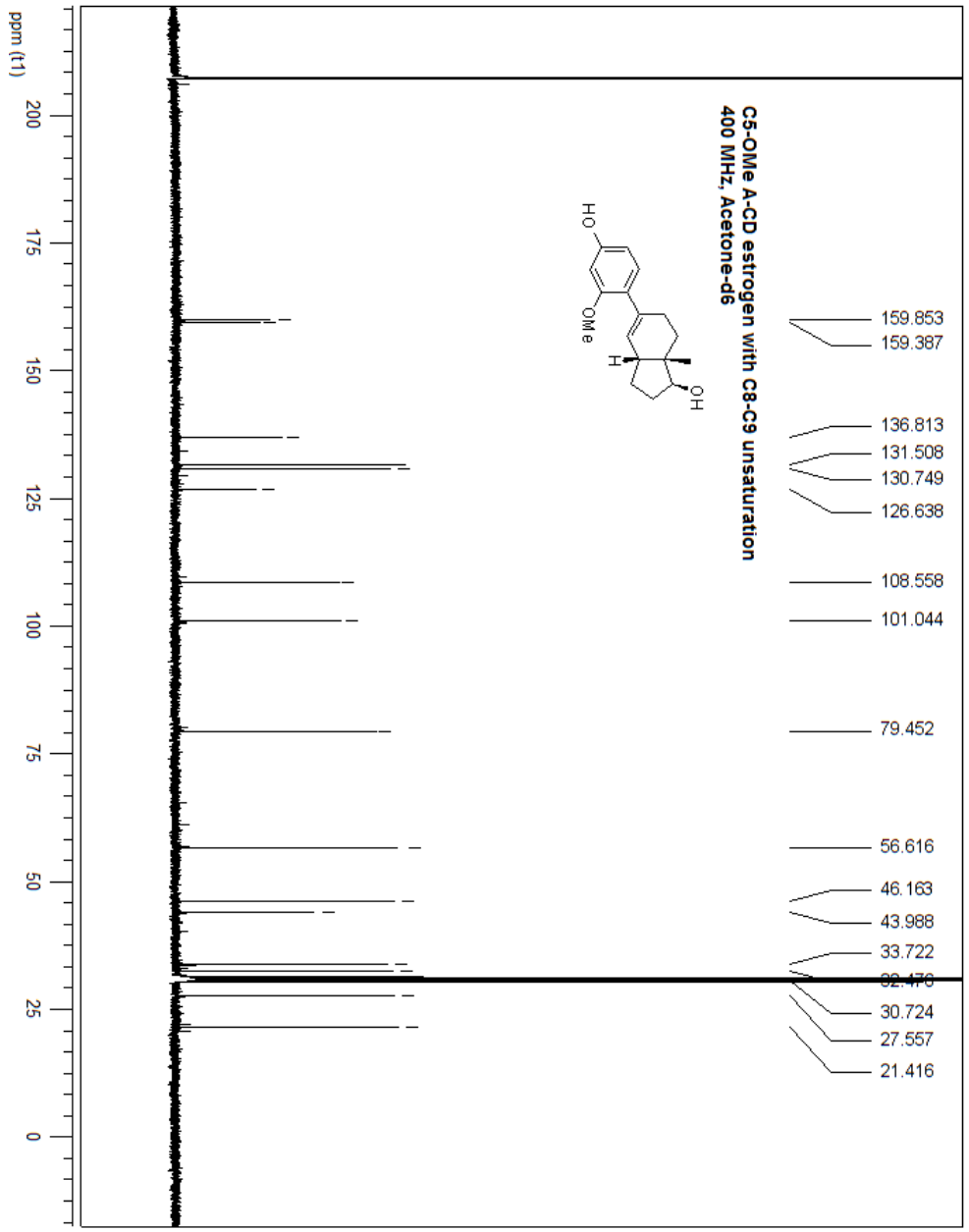

Figure a.32 ^{13}C NMR spectrum of C5-OCH_3 A-CD estrogen with C8-C9 unsaturation (**106**) in Acetone-d_6.

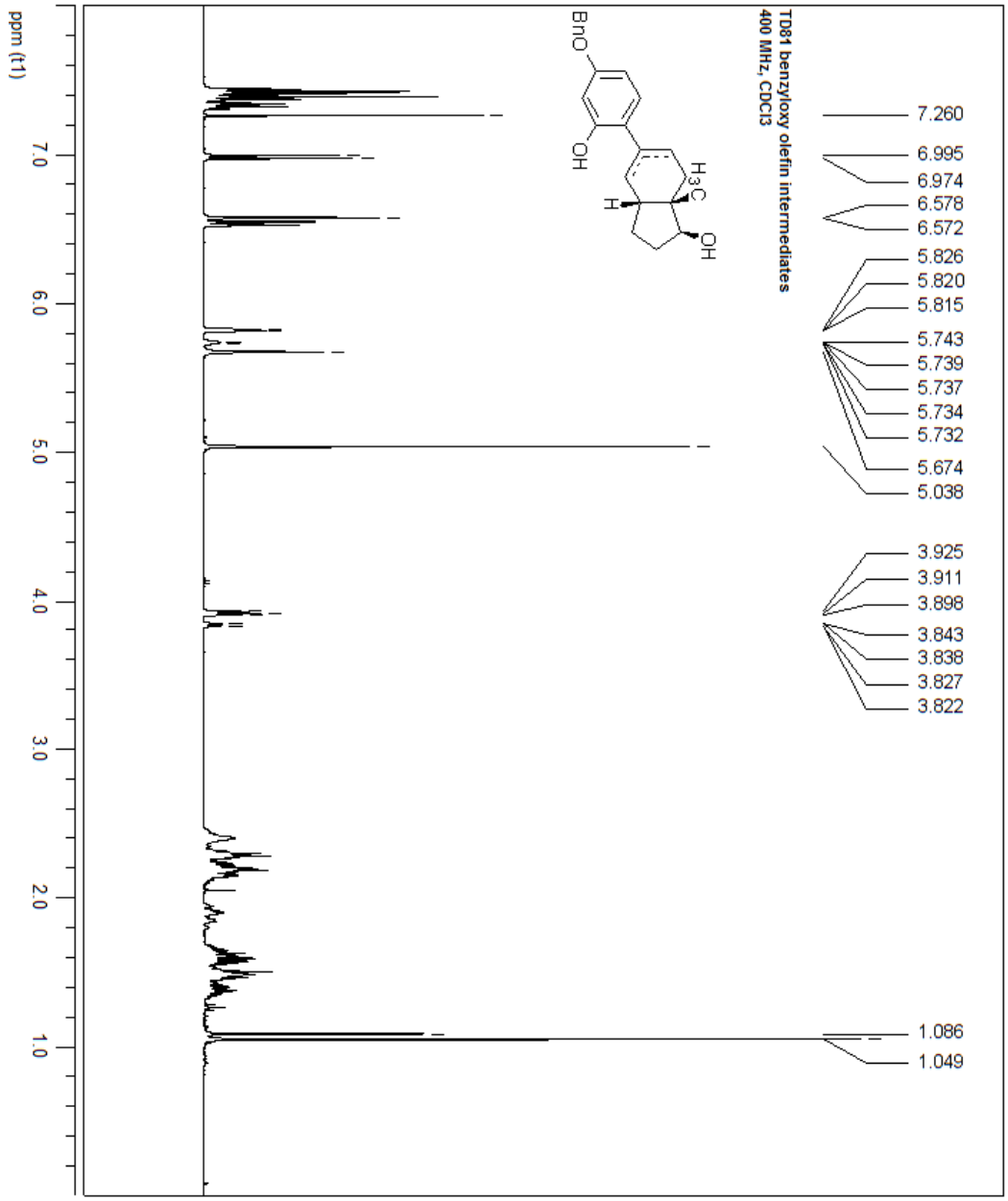

Figure a.33 ^{1}H NMR spectrum of the olefin benzyloxy intermediates of TD81 (**100**) in $CDCl_3$.

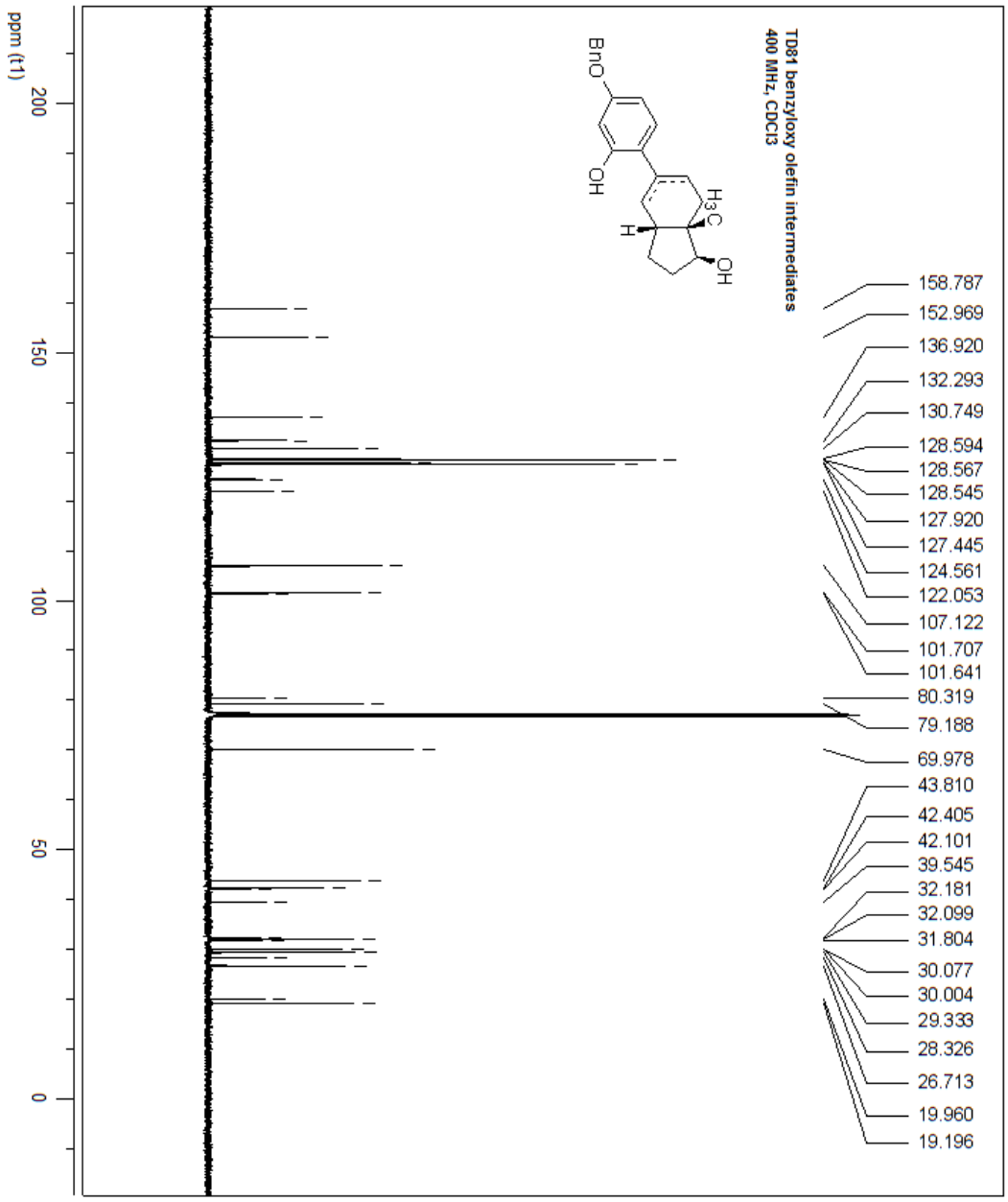

Figure a.34 ^{13}C NMR spectrum of the olefin benzyloxy intermediates of TD81 (**100**) in $CDCl_3$.

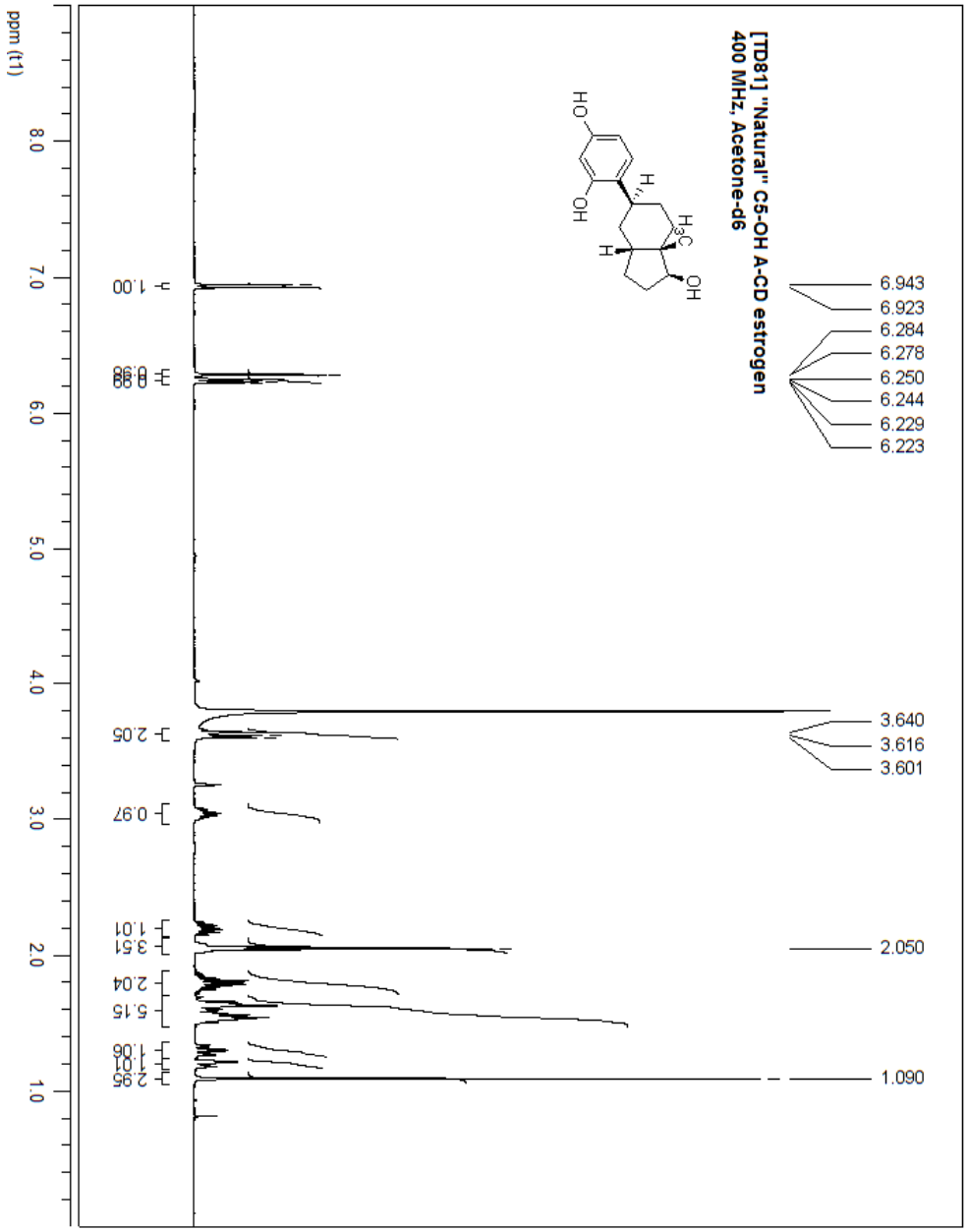

Figure a.35 ^{1}H NMR spectrum of the "natural" C5-OH A-CD estrogen (**84**) in Acetone-d_6.

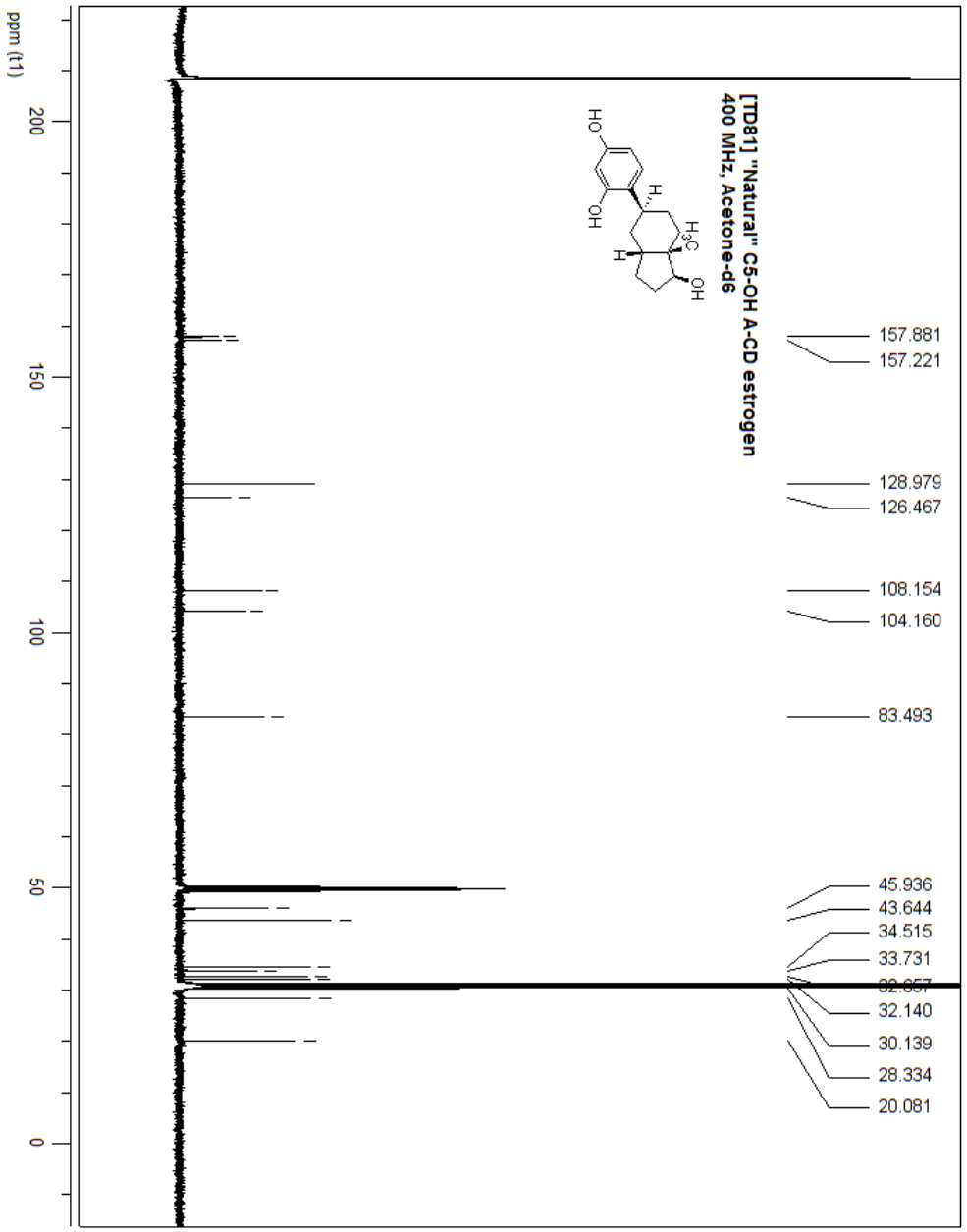

Figure a.36 ^{13}C NMR spectrum of the "natural" C5-OH A-CD estrogen (**84**) in Acetone-d_6.

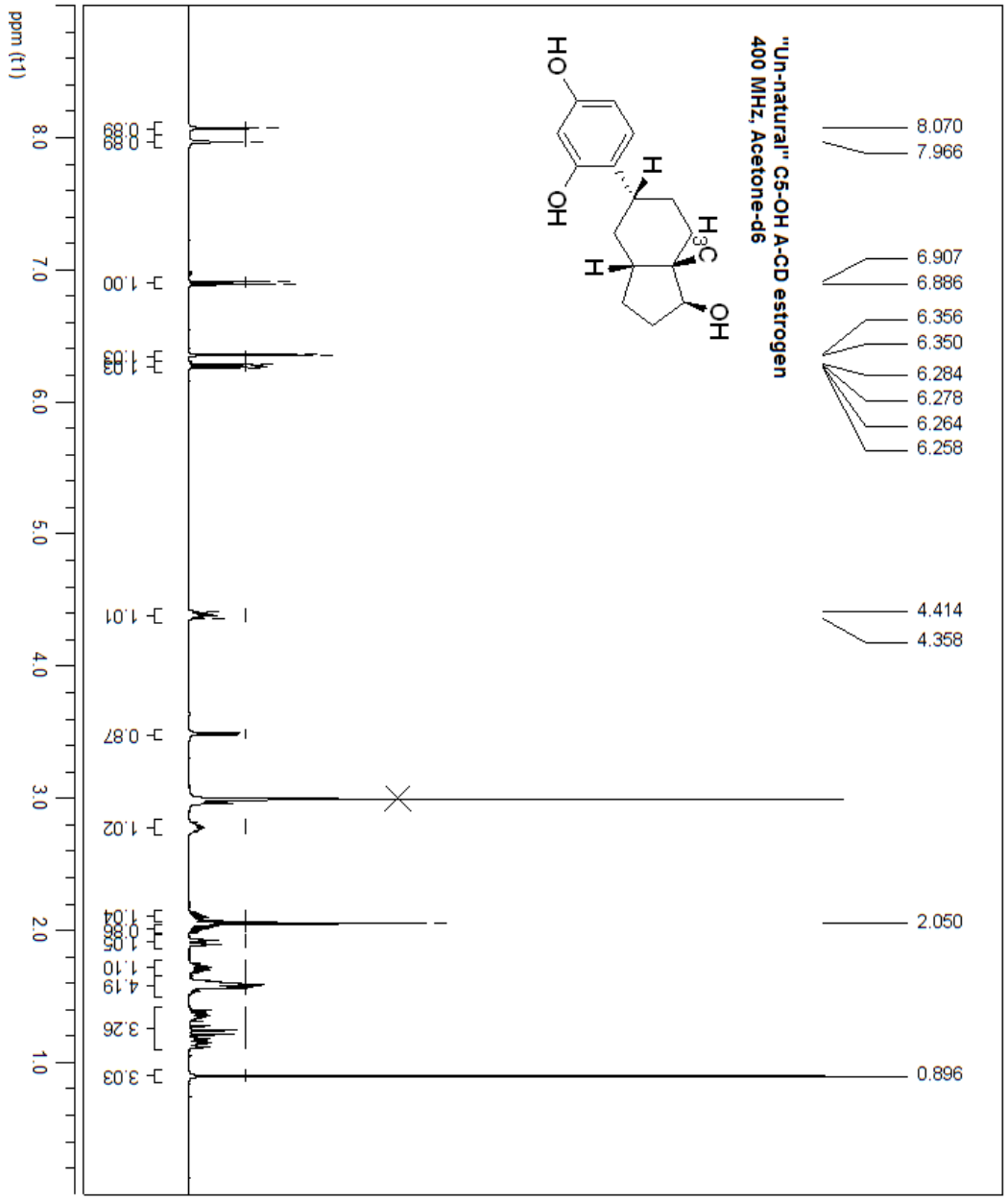

Figure a.37 ^{1}H NMR spectrum of the "un-natural" C5-OH A-CD estrogen (**101**) in Acetone-d_6.

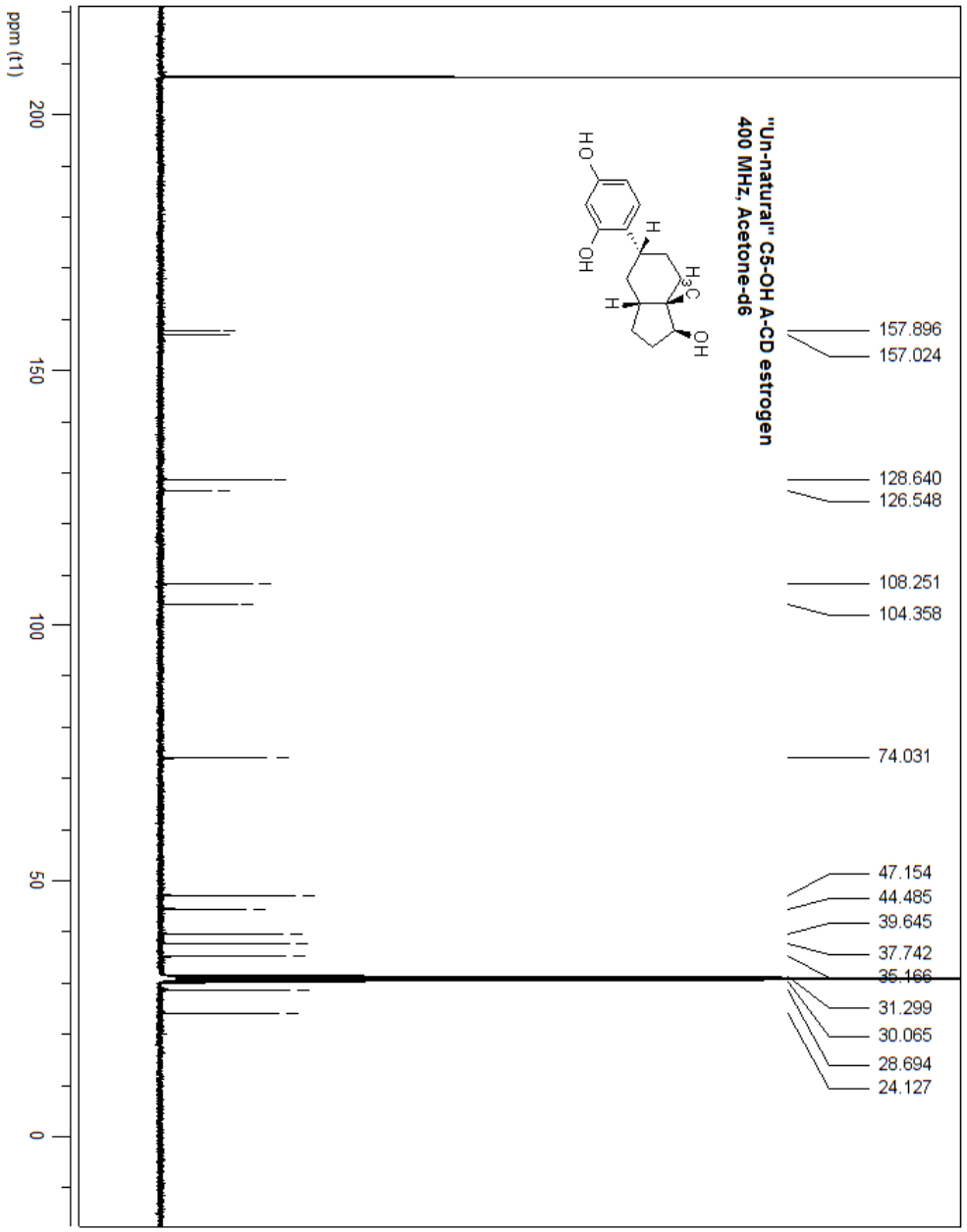

Figure a.38 ^{13}C NMR spectrum of the "un-natural" C5-OH A-CD estrogen (**101**) in Acetone-d_6.

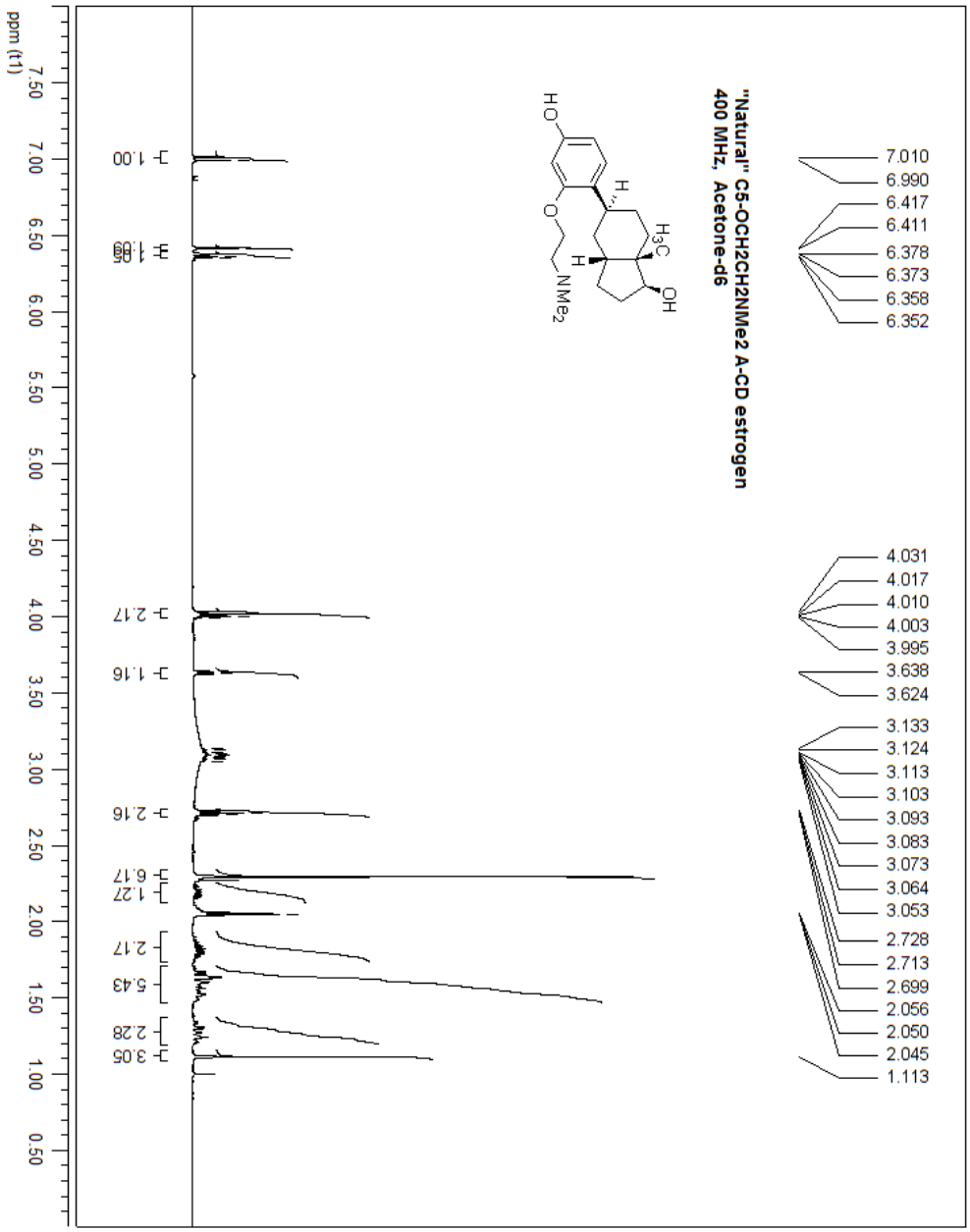

Figure a.39 ^{1}H NMR spectrum of the "natural" C5-$OCH_2CH_2NMe_2$ A-CD estrogen (**102**) in Acetone-d_6.

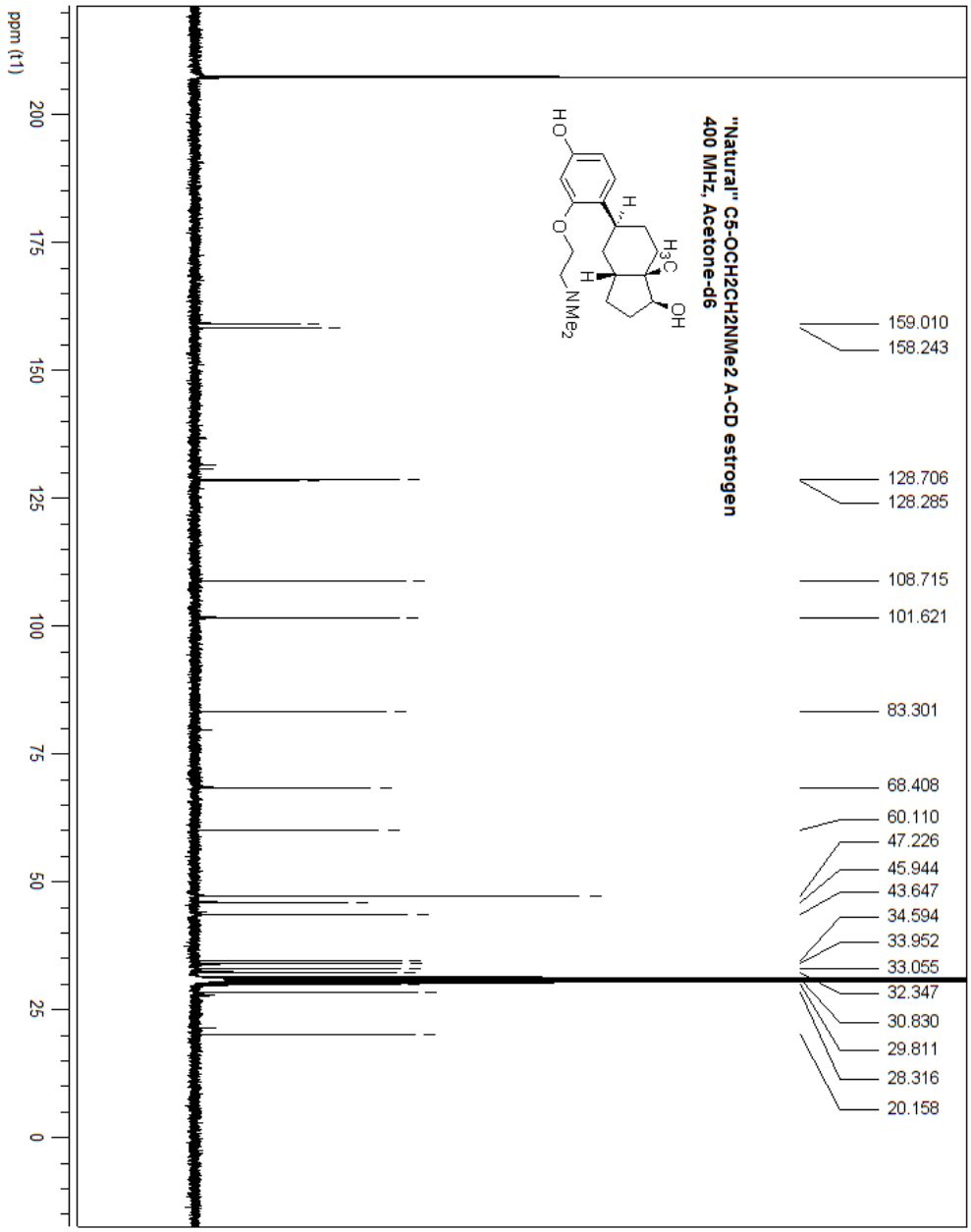

Figure a.40 ^{13}C NMR spectrum of the "natural" C5-$OCH_2CH_2NMe_2$ A-CD estrogen (**102**) in Acetone-d_6.

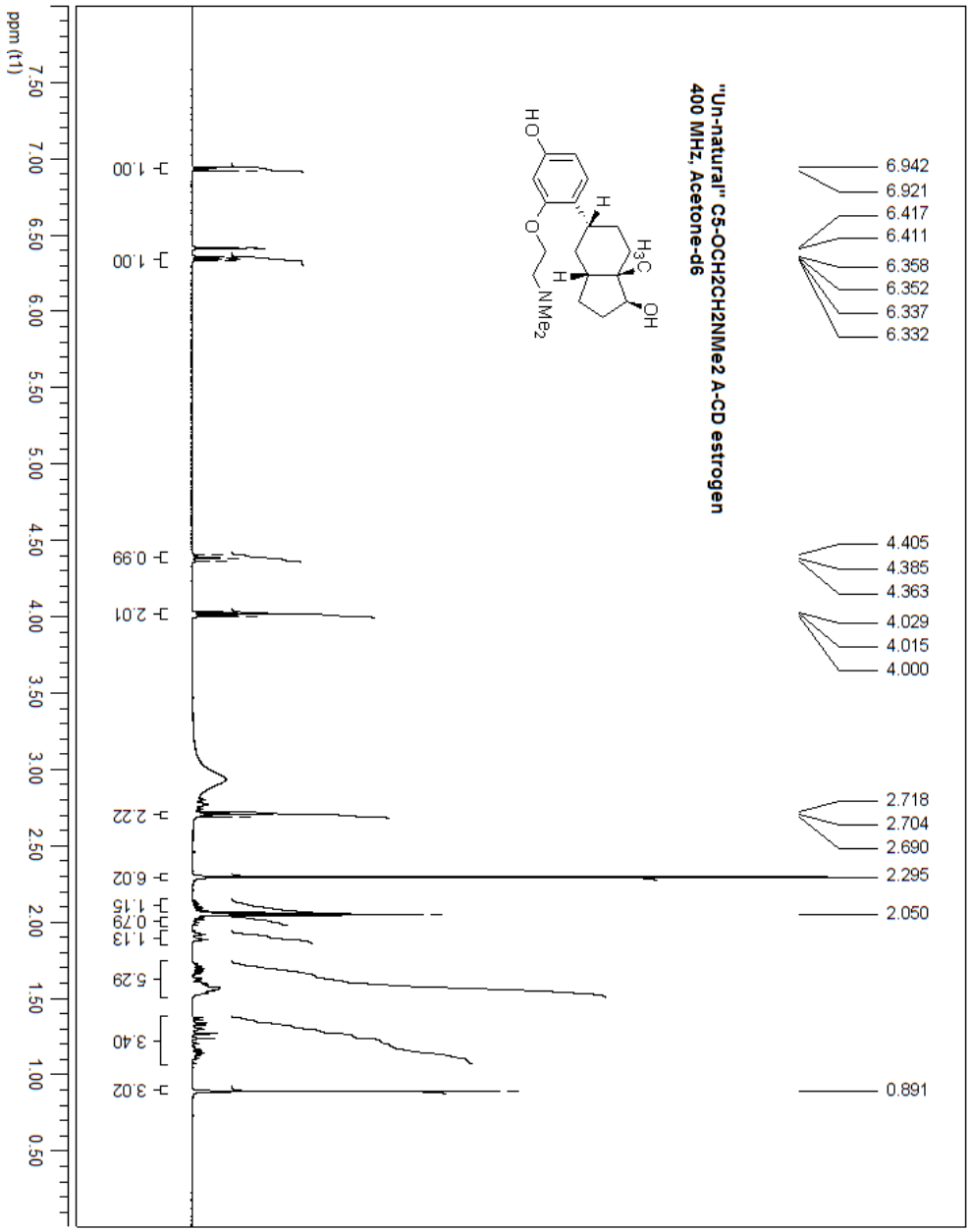

Figure a.41 ^{1}H NMR spectrum of the "un-natural" C5-$OCH_2CH_2NMe_2$ A-CD estrogen (**103**) in Acetone-d_6.

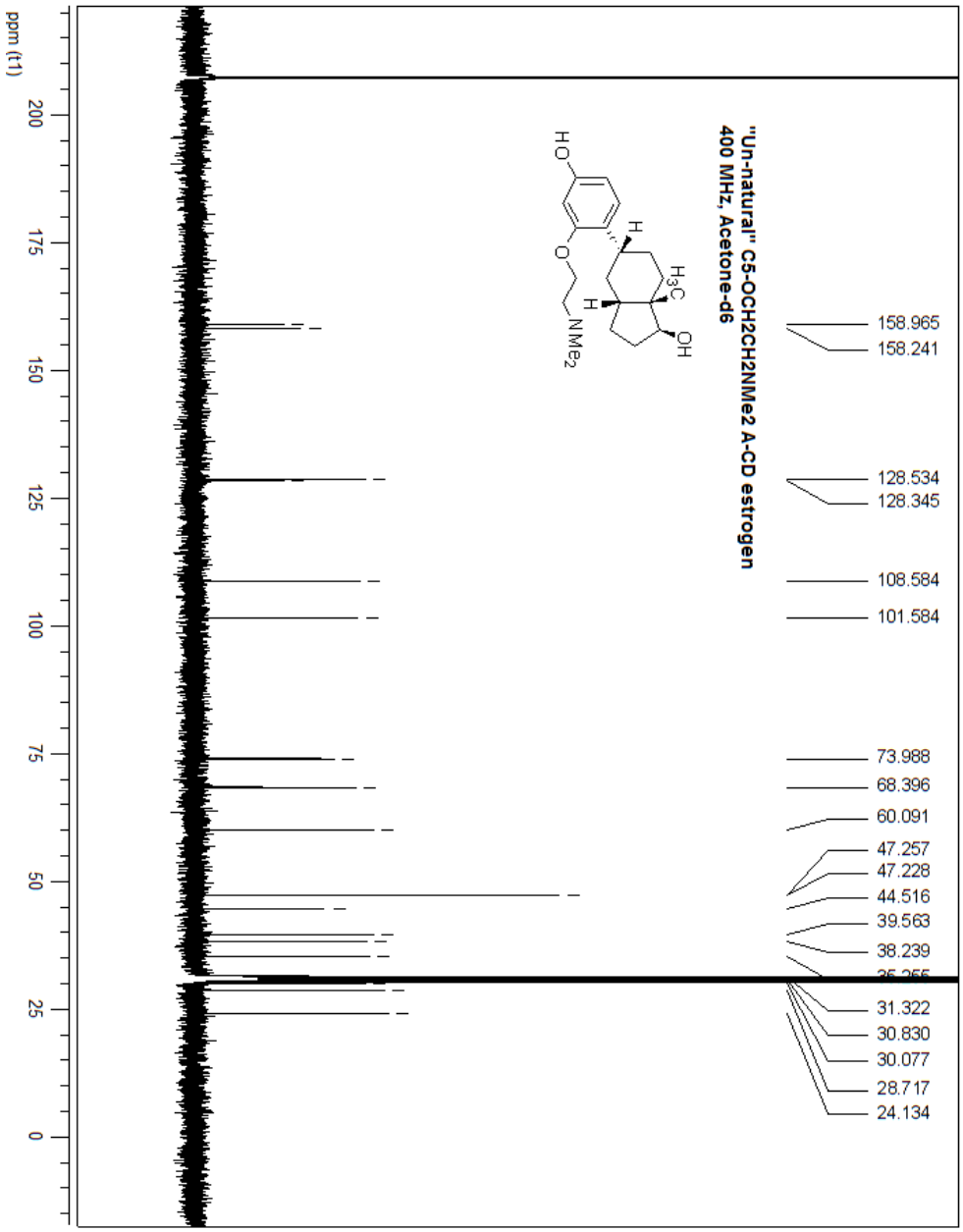

Figure a.42 ^{13}C NMR spectrum of the "un-natural" C5-$OCH_2CH_2NMe_2$ A-CD estrogen (**103**) in Acetone-d_6.

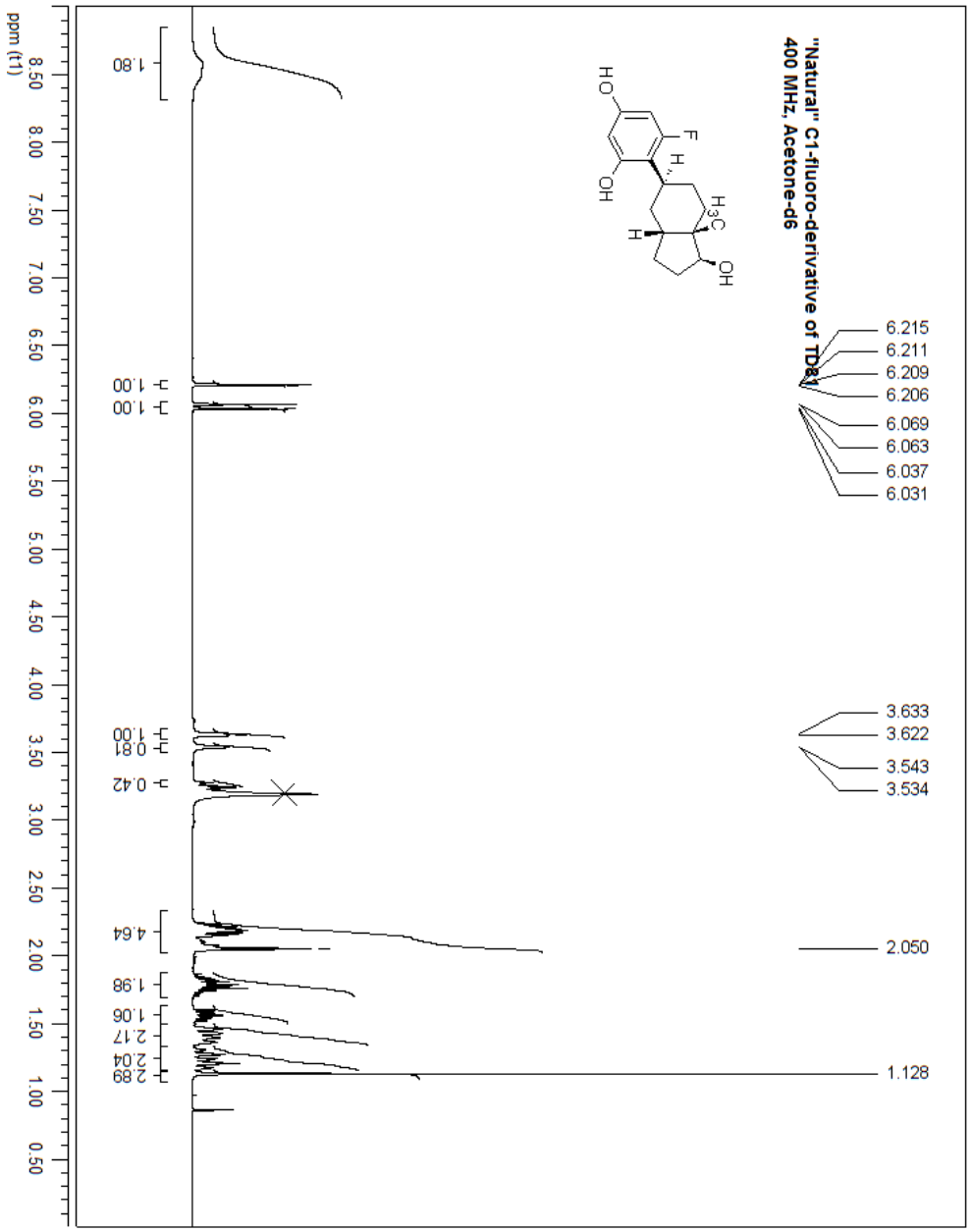

Figure a.43 ^{1}H NMR spectrum of the "natural" C1-fluoro-derivative of TD81 (**107**) in Acetone-d_6.

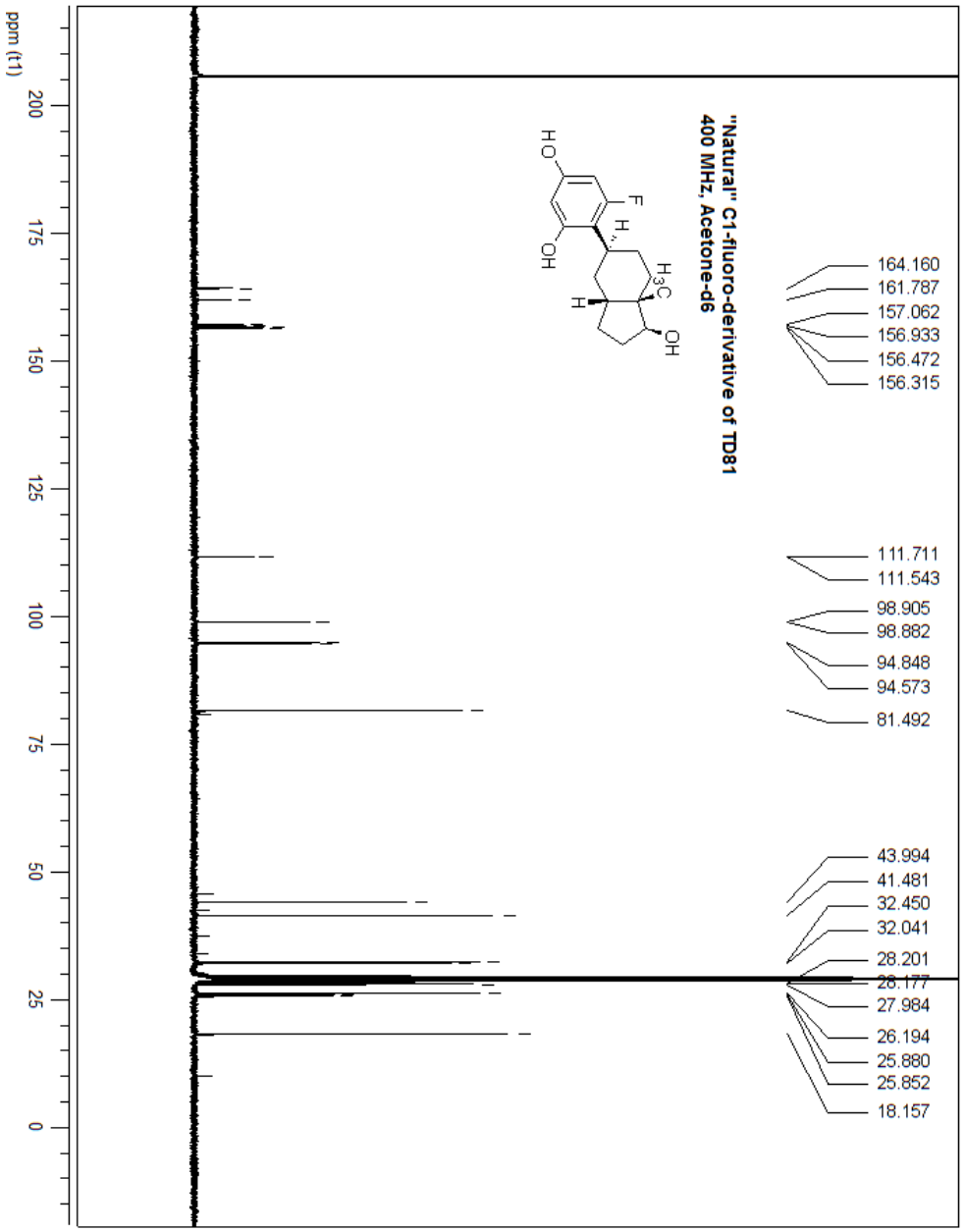

Figure a.44 ^{13}C NMR spectrum of the "natural" C1-fluoro-derivative of TD81 (**107**) in Acetone-d_6.

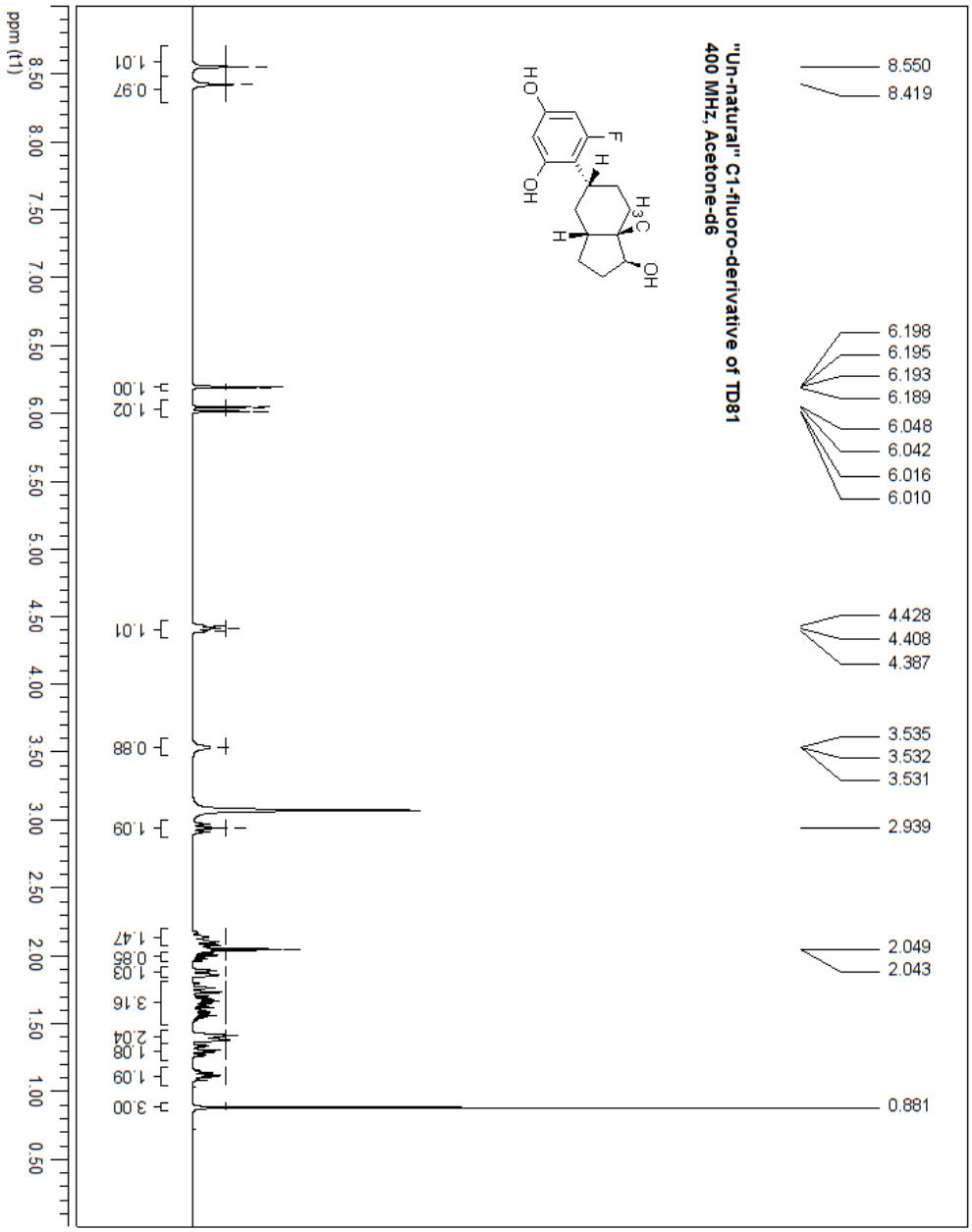

Figure a.45 ^{1}H NMR spectrum of the "un-natural" C1-fluoro-derivative of TD81 (**128**) in Acetone-d_6.

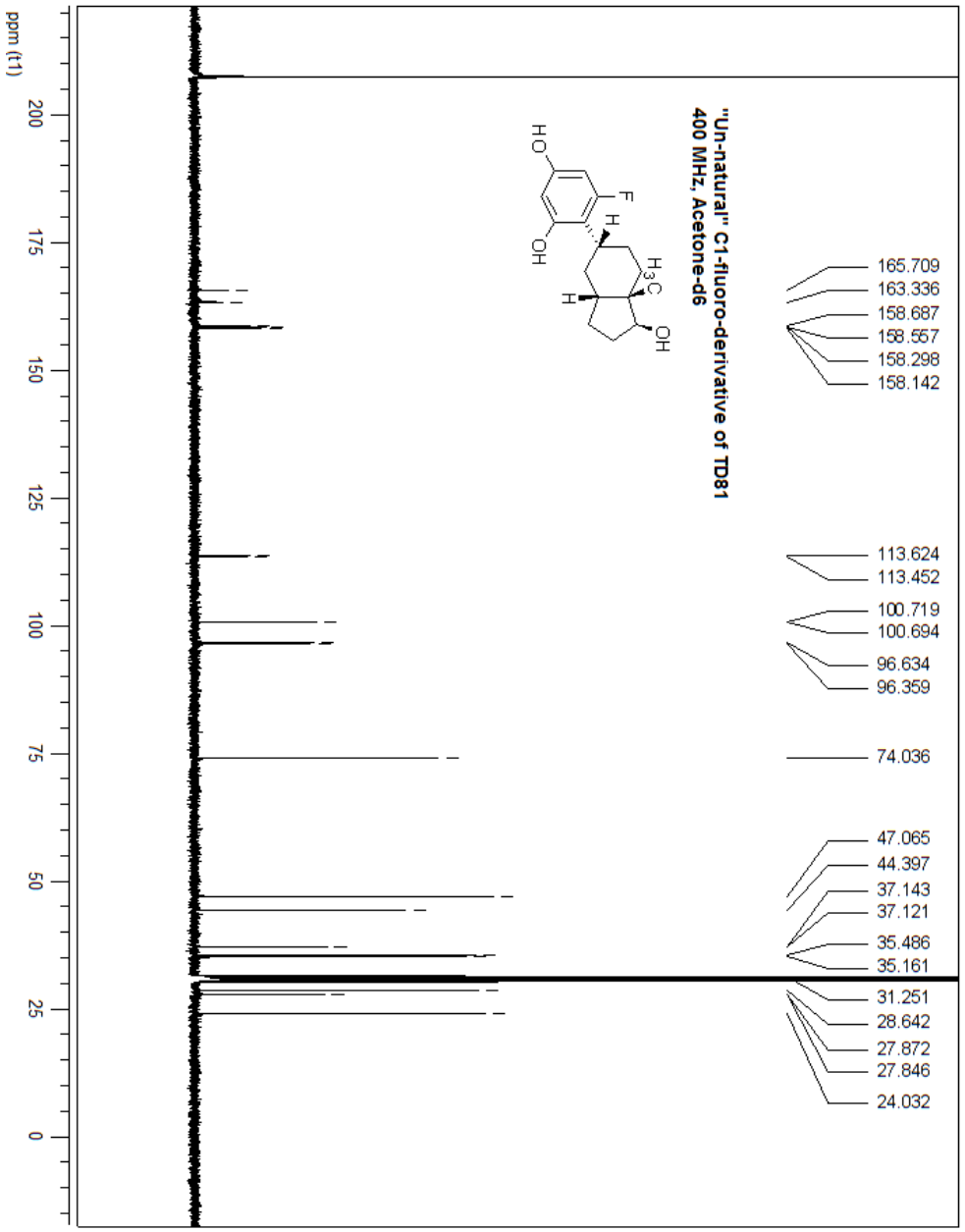

Figure a.46 ^{13}C NMR spectrum of the "un-natural" C1-fluoro-derivative of TD81 (**128**) in Acetone-d_6.

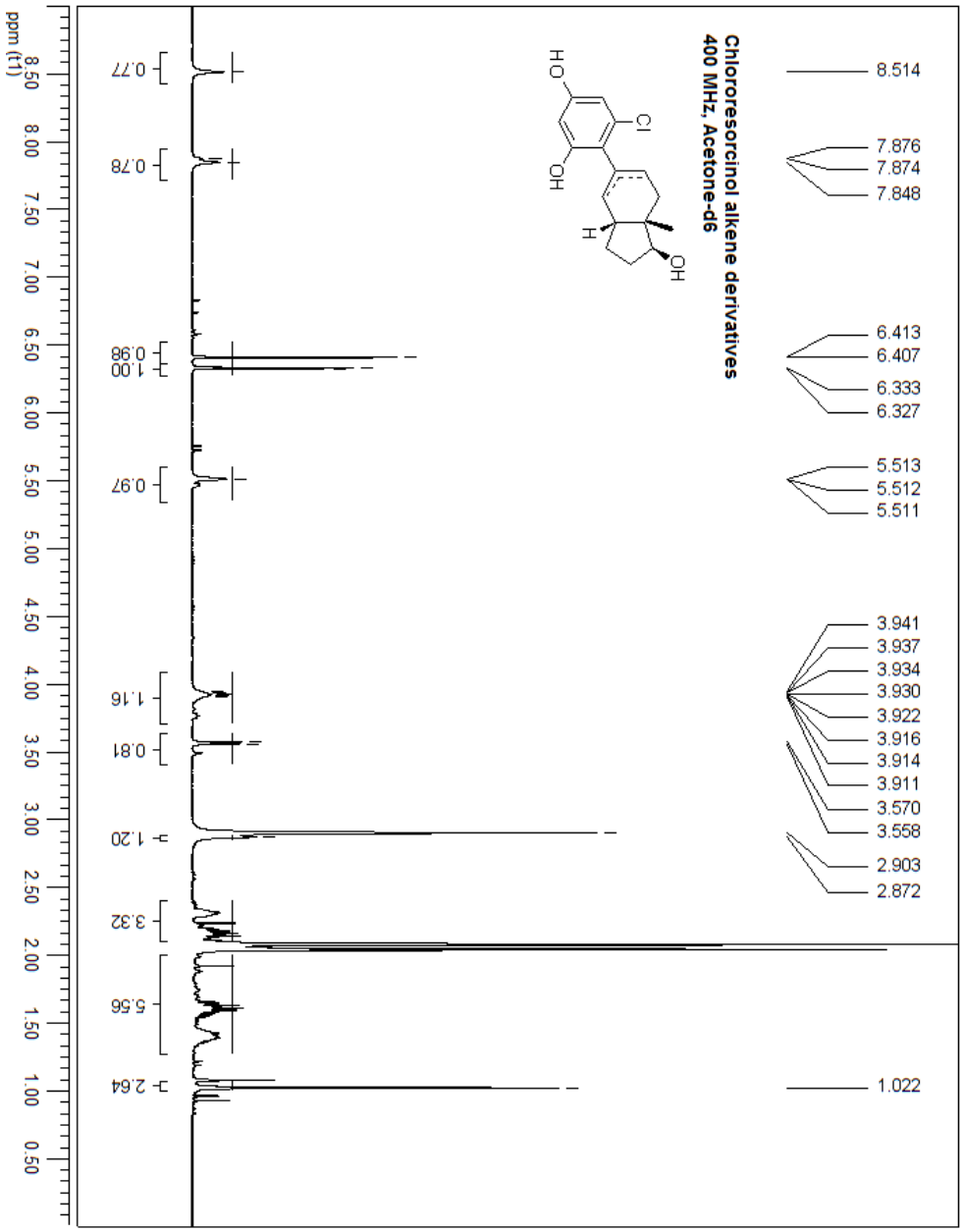

Figure a.47 ^{1}H NMR spectrum of the C1-chloro-alkene derivatives of TD81 (**125**) in Acetone-d_6.

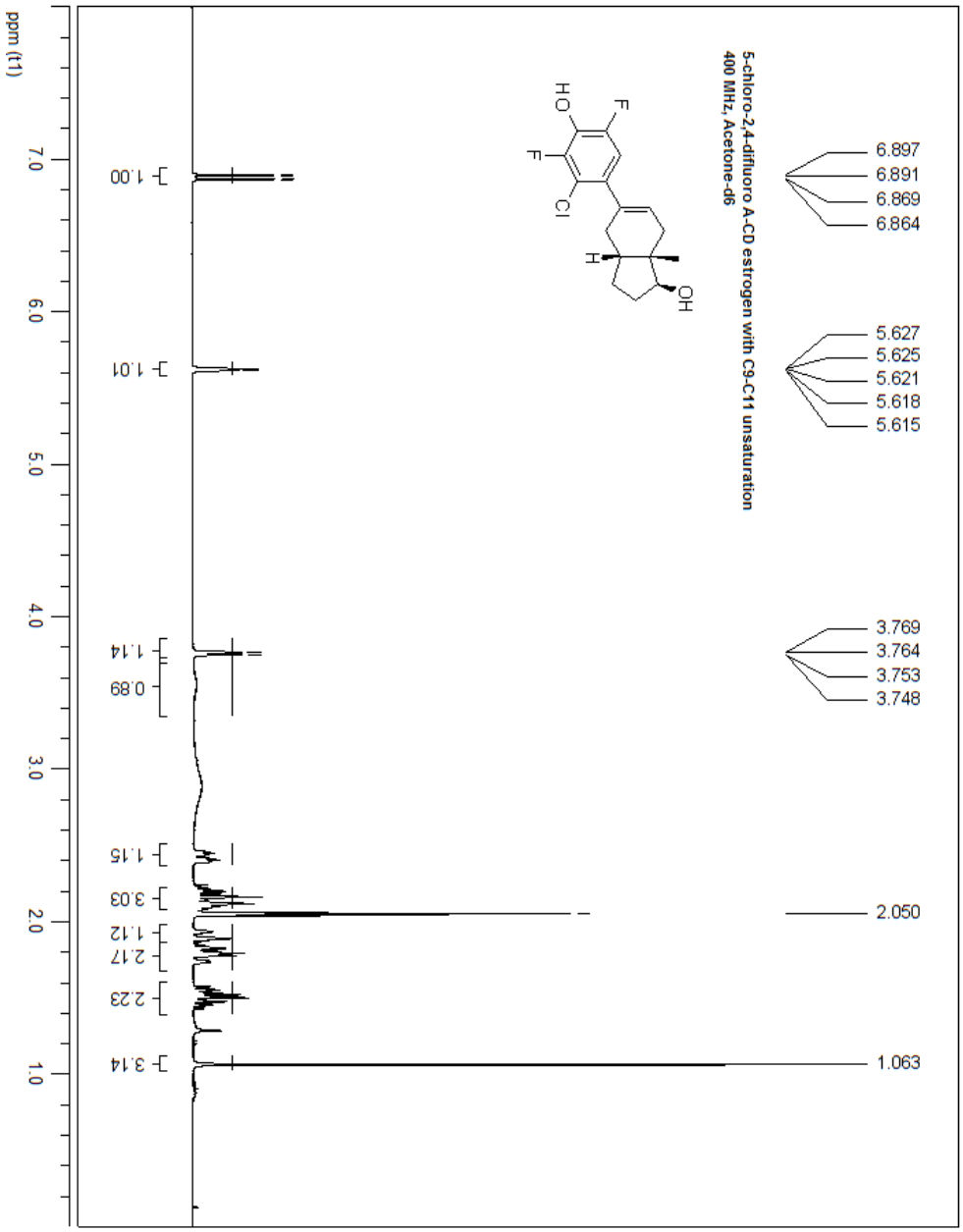

Figure a.48 ^{1}H NMR spectrum of 5-Cl-2,4-diF A-CD estrogen with C9-C11 unsaturation (**140**) in Acetone-d_6.

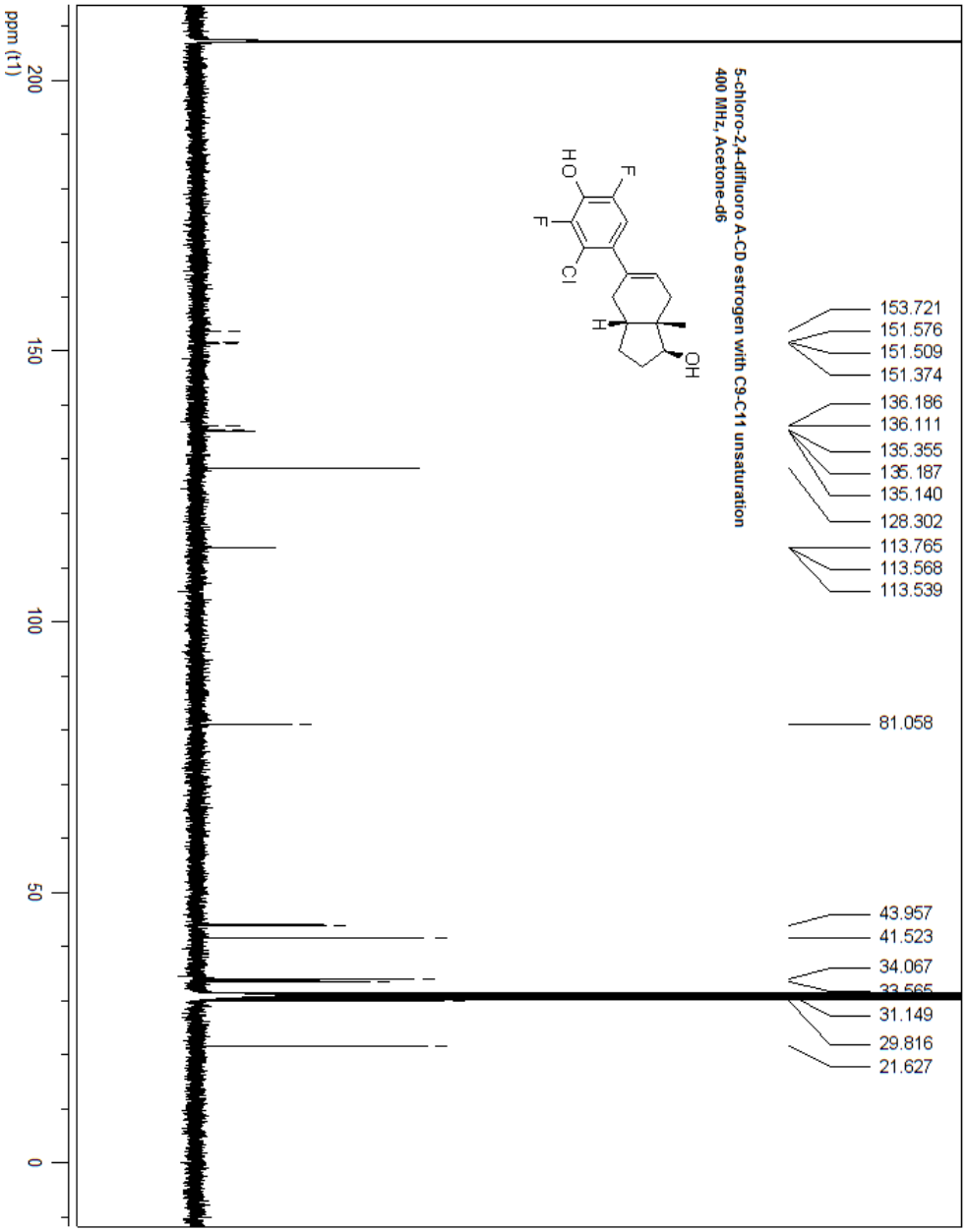

Figure a.49 ^{13}C NMR spectrum of 5-Cl-2,4-diF A-CD estrogen with C9-C11 unsaturation (**140**) in Acetone-d_6.

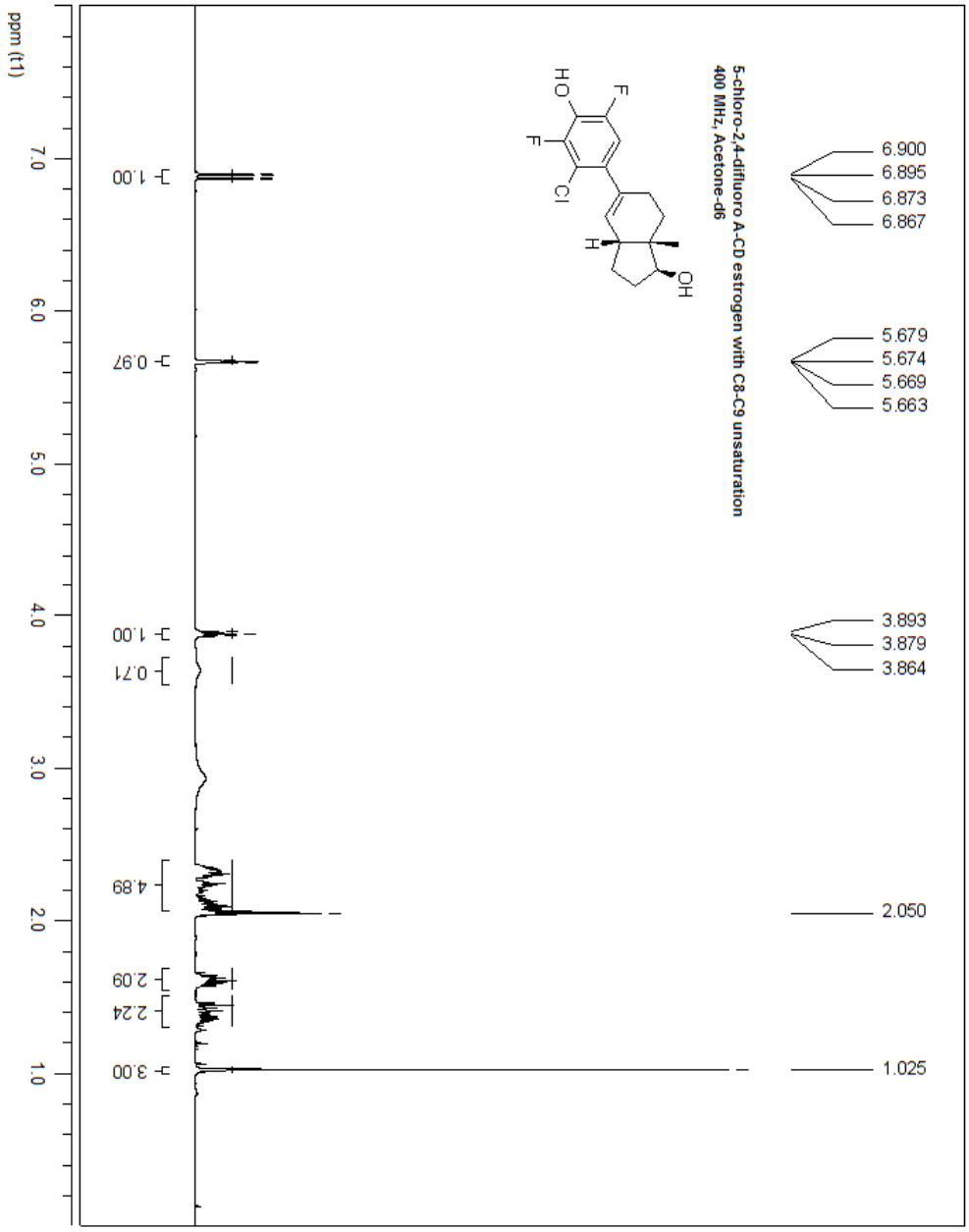

Figure a.50 ^{1}H NMR spectrum of 5-Cl-2,4-diF A-CD estrogen with C8-C9 unsaturation (**141**) in Acetone-d_6.

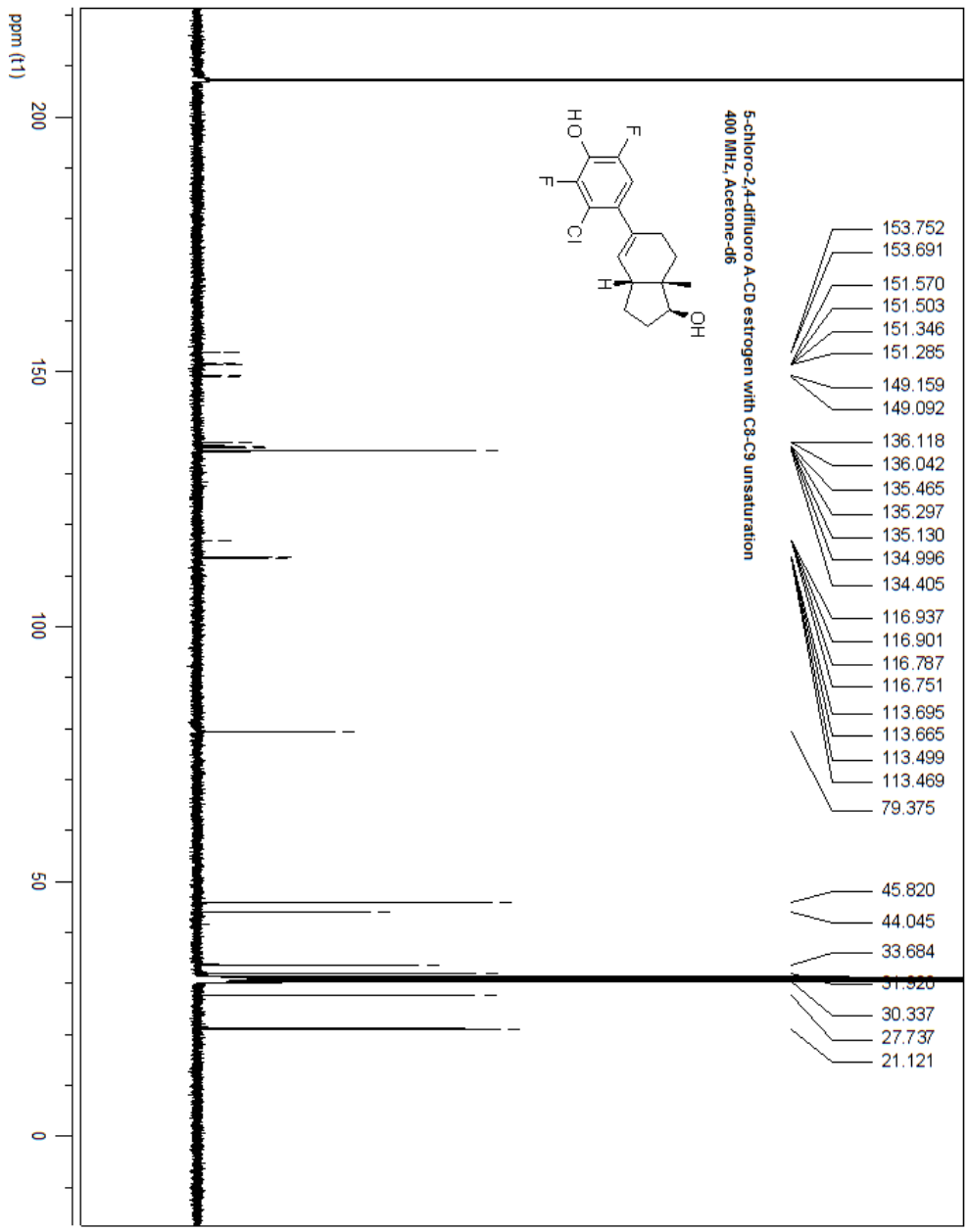

Figure a.51 ^{13}C NMR spectrum of 5-Cl-2,4-diF A-CD estrogen with C8-C9 unsaturation (**141**) in Acetone-d_6.

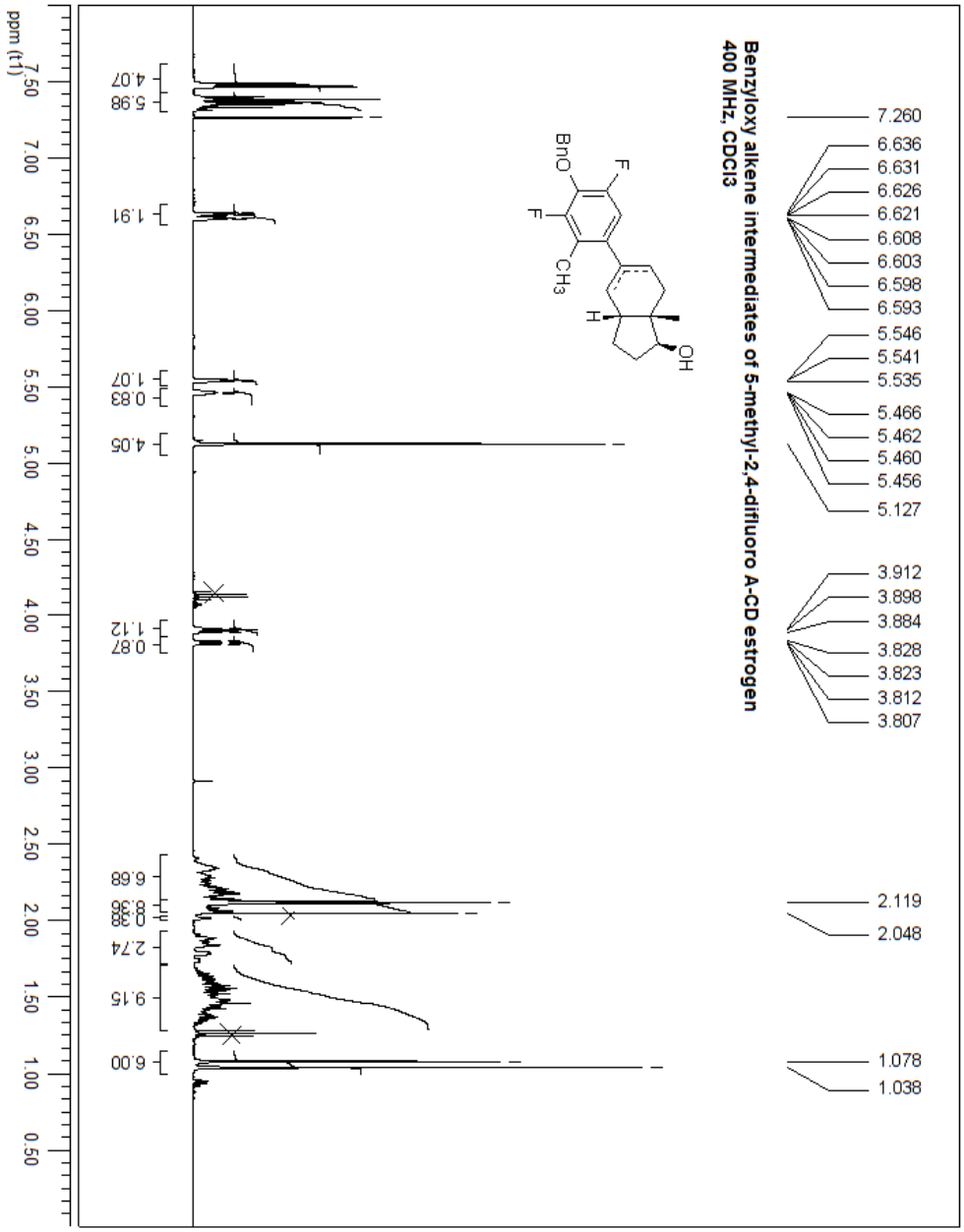

Figure a.52 ^{1}H NMR spectrum of benzyloxy alkene intermediates of 5-methyl-2,4-difluoro A-CD estrogen (**135/136**) in Acetone-d_6.

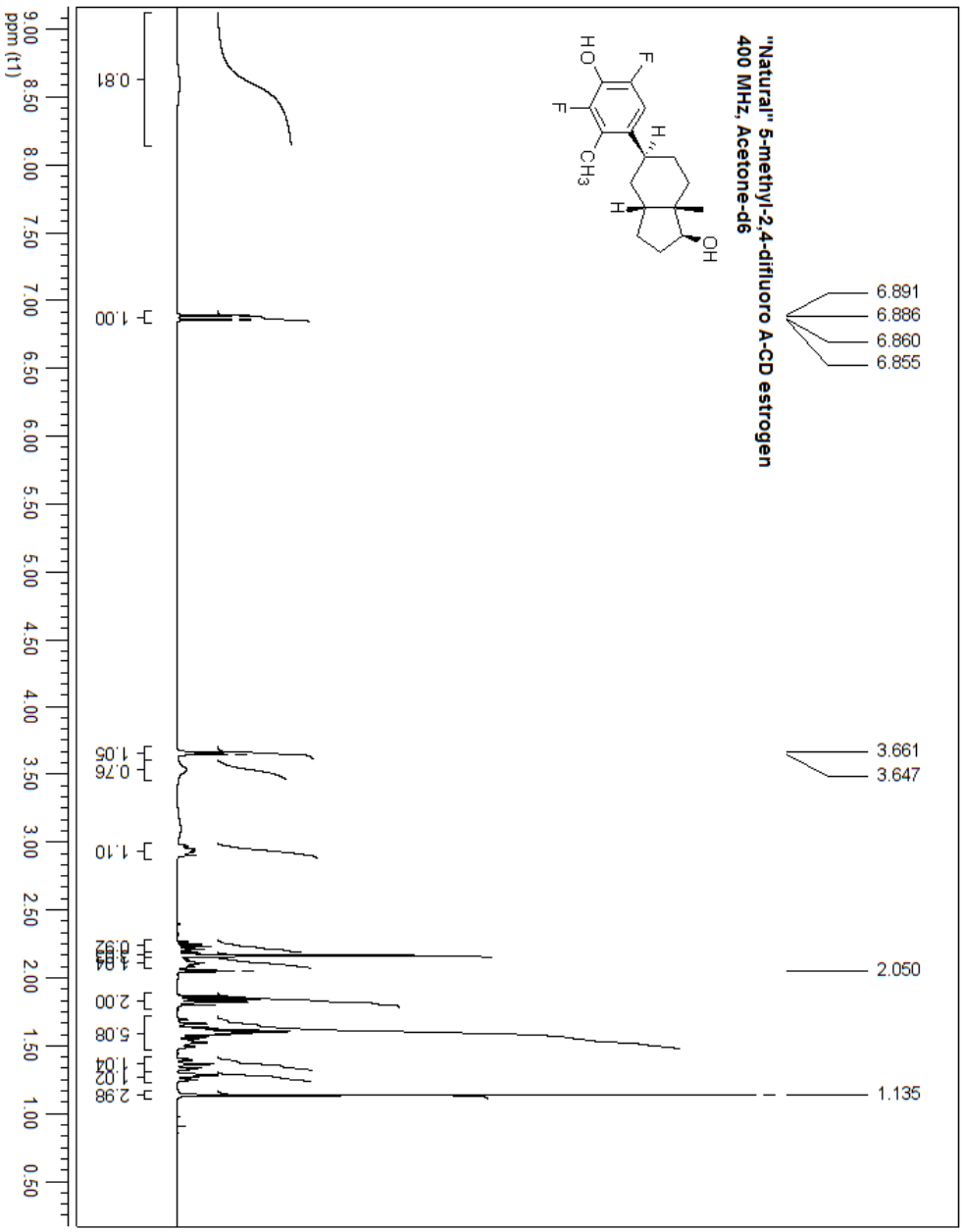

Figure a.53 ^{1}H NMR spectrum of "natural" 5-methyl-2,4-difluoro A-CD estrogen (**130**) in Acetone-d_6.

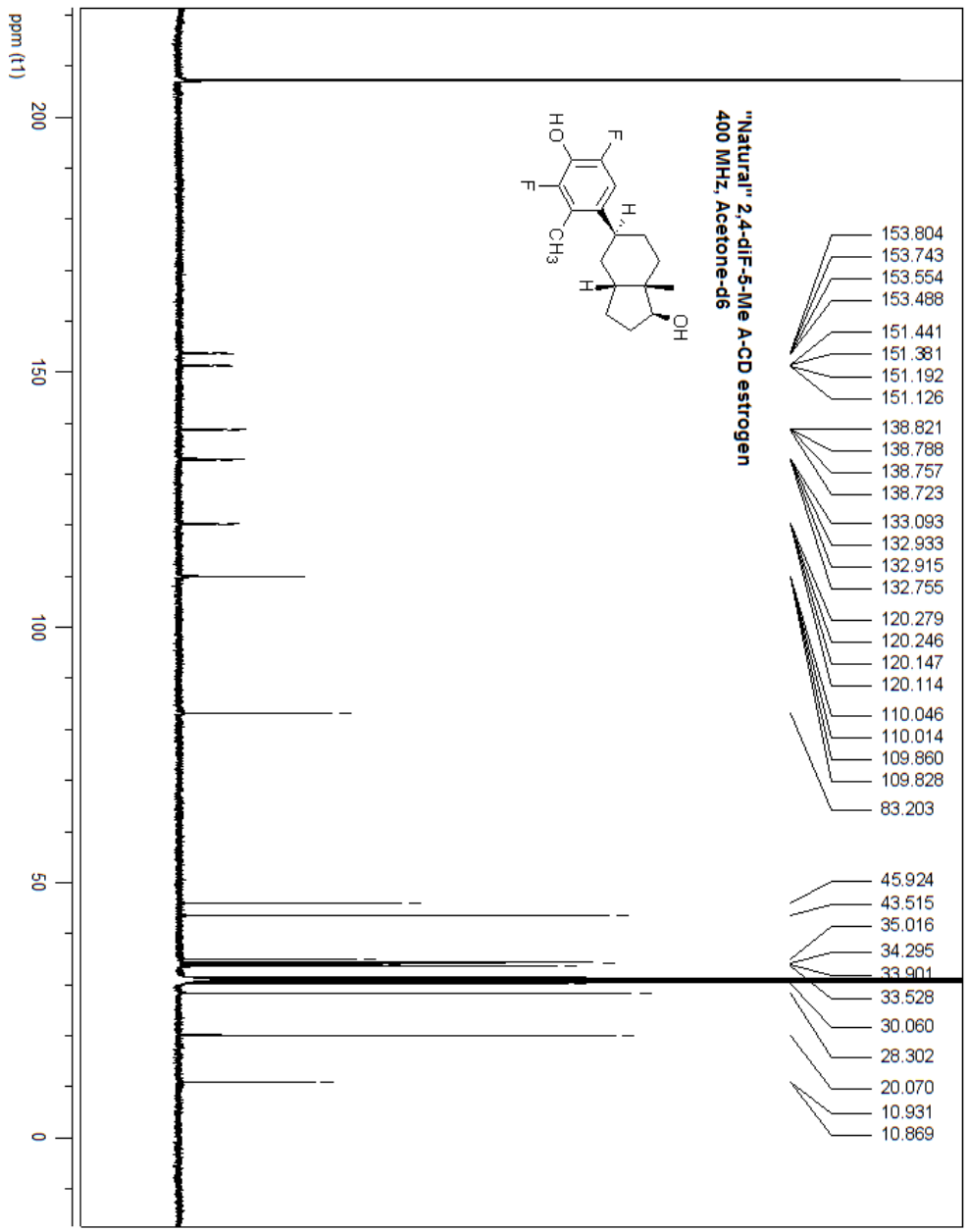

Figure a.54 ^{13}C NMR spectrum of "natural" 5-methyl-2,4-difluoro A-CD estrogen (**130**) in Acetone-d_6.

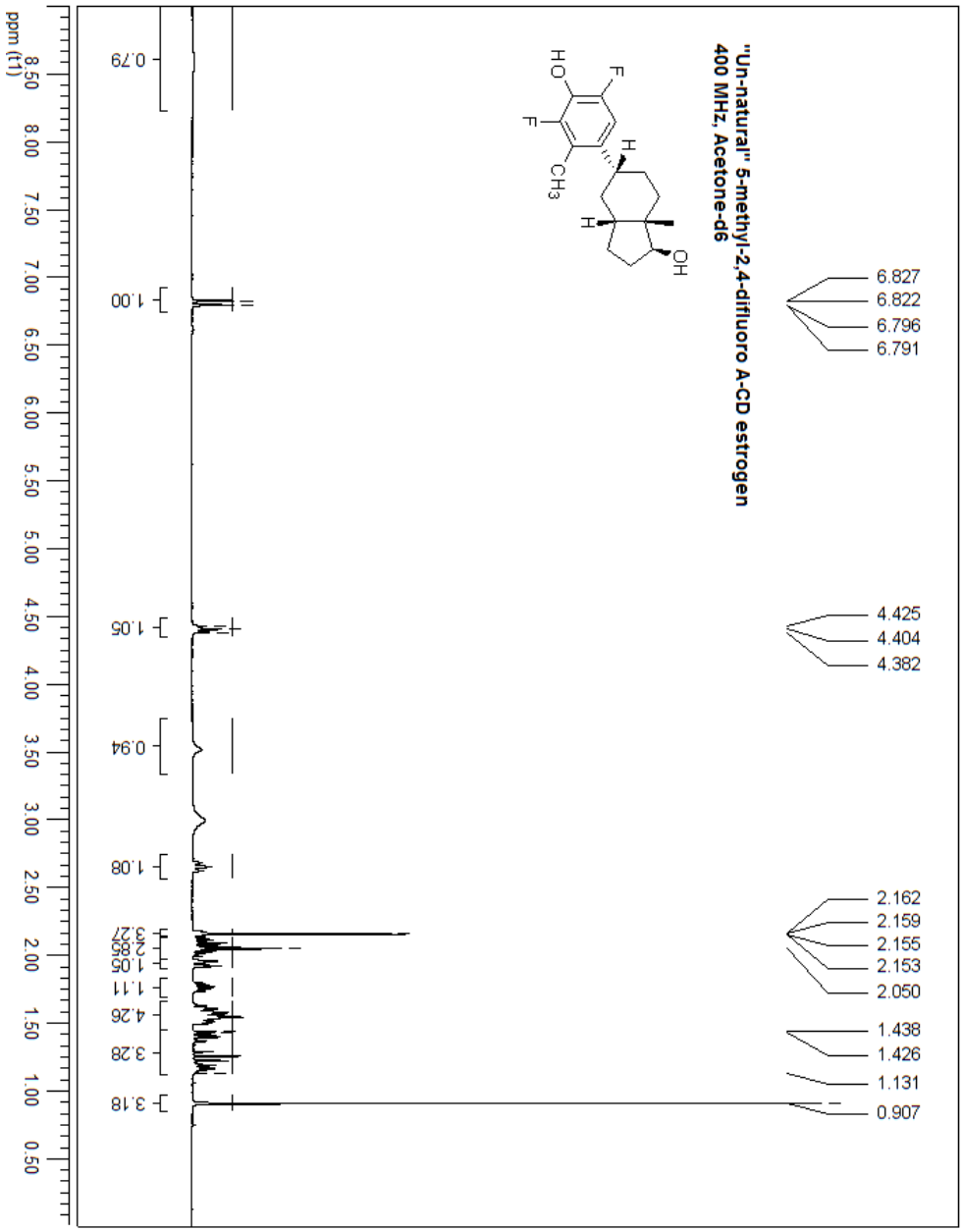

Figure a.55 ^{1}H NMR spectrum of "un-natural" 5-methyl-2,4-difluoro A-CD estrogen (**137**) in Acetone-d_6.

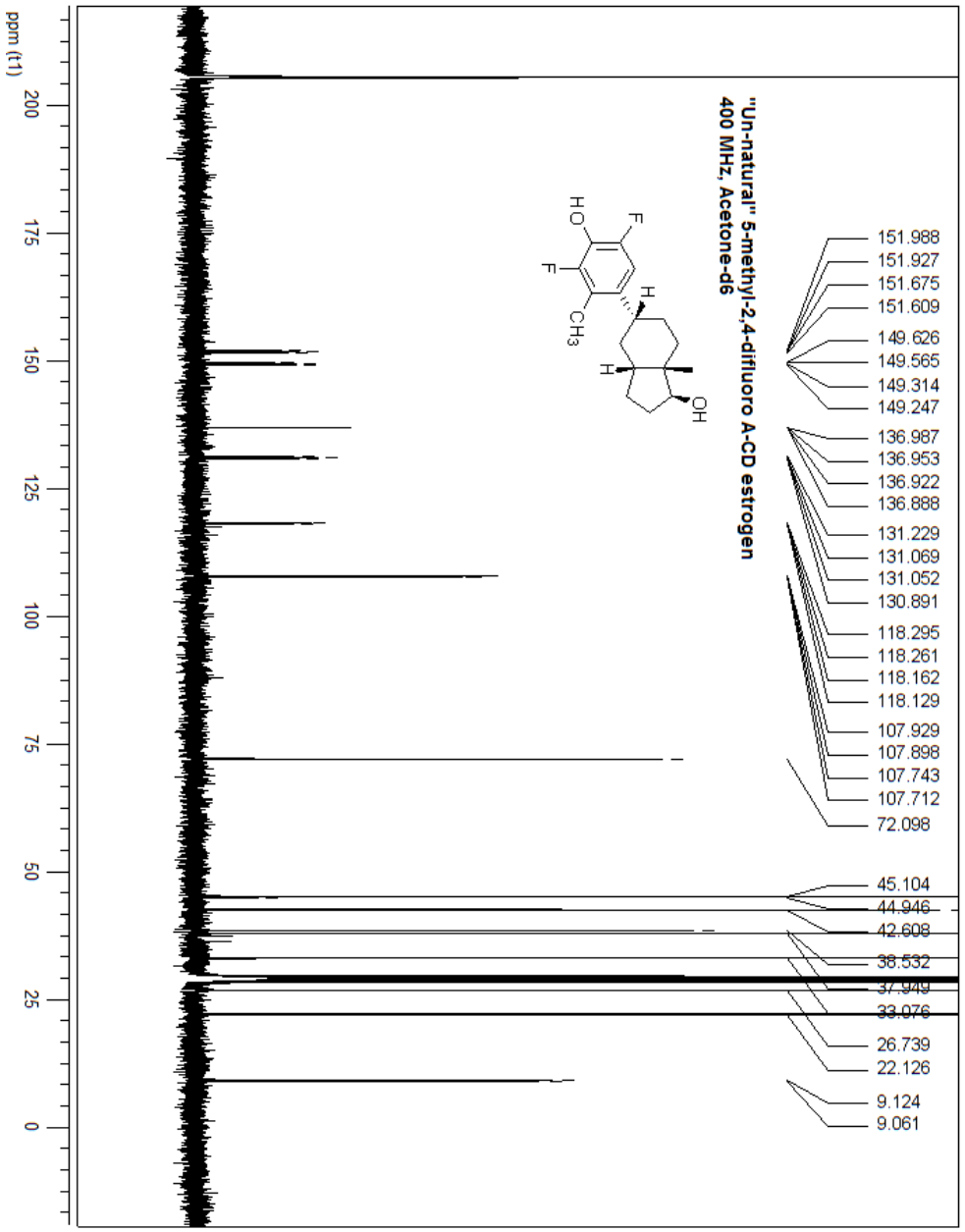

Figure a.56 ^{13}C NMR spectrum of "un-natural" 5-methyl-2,4-difluoro A-CD estrogen (**137**) in Acetone-d_6.

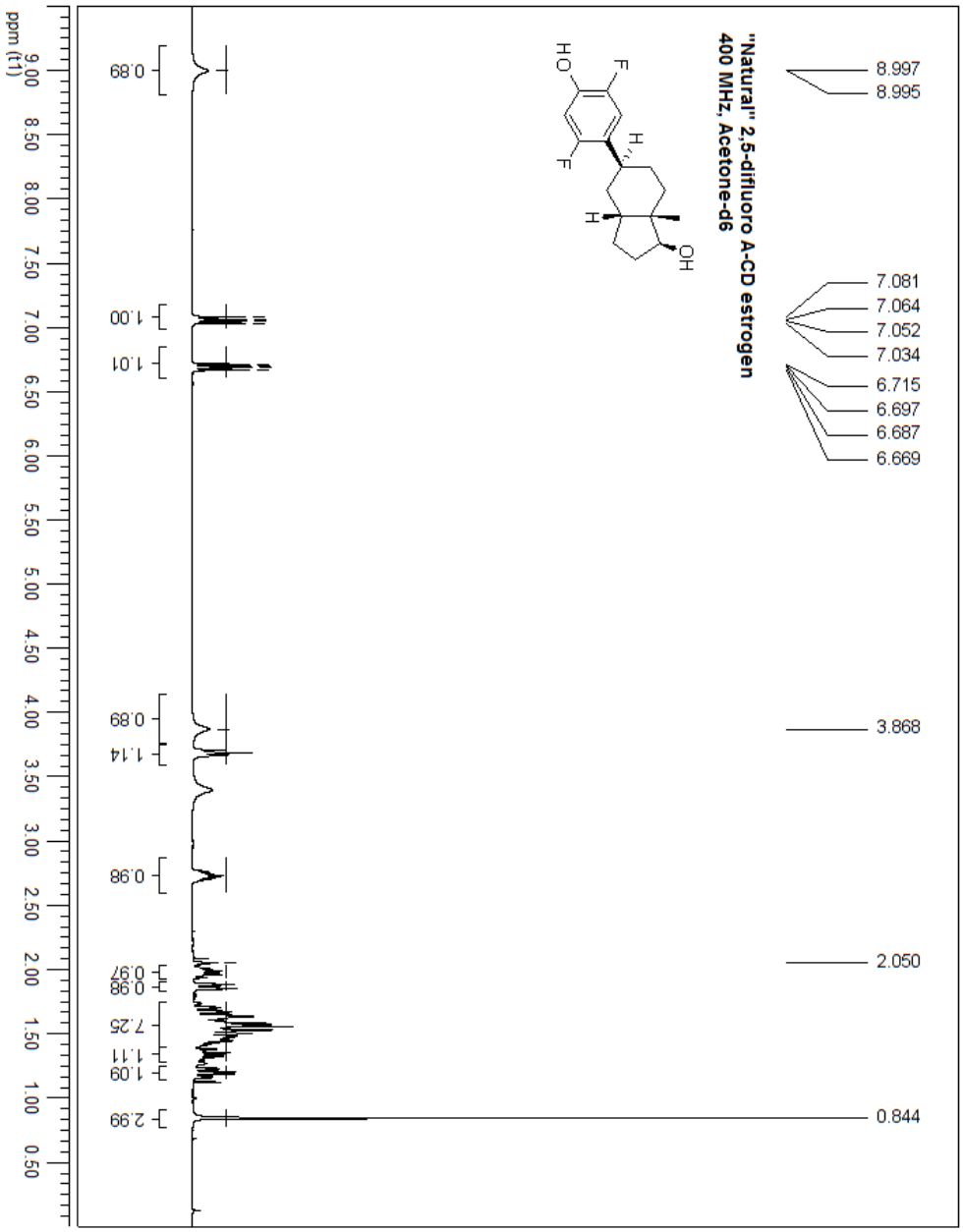

Figure a.57 ^{1}H NMR spectrum of the "natural" 2,5-difluoro A-CD estrogen (**142**) in Acetone-d_6.

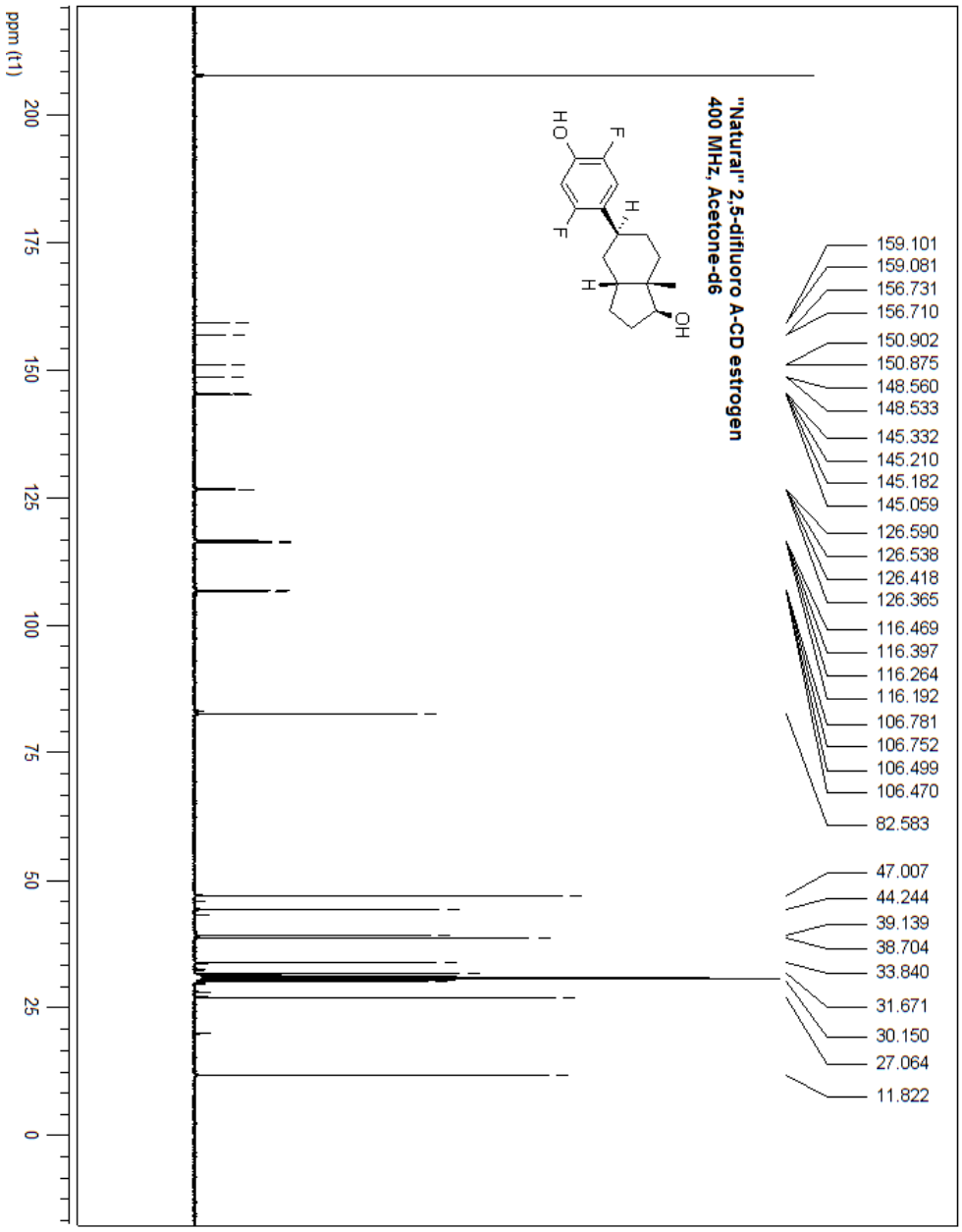

Figure a.58 ^{13}C NMR spectrum of the "natural" 2,5-difluoro A-CD estrogen (**142**) in Acetone-d_6.

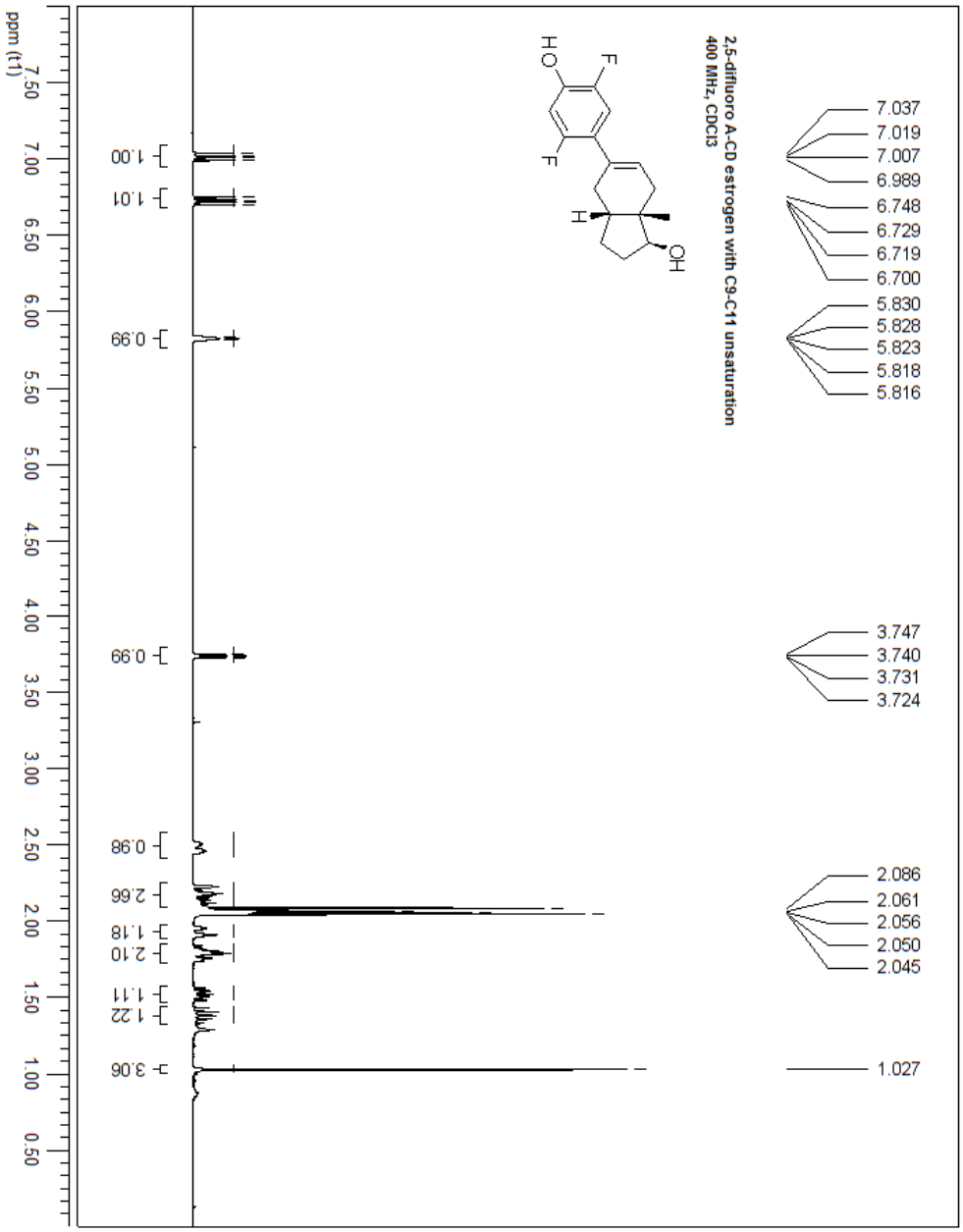

Figure a.59 ^{1}H NMR spectrum of 2,5-difluoro A-CD estrogen with C9-C11 unsaturation (**149**) in Acetone-d_6.

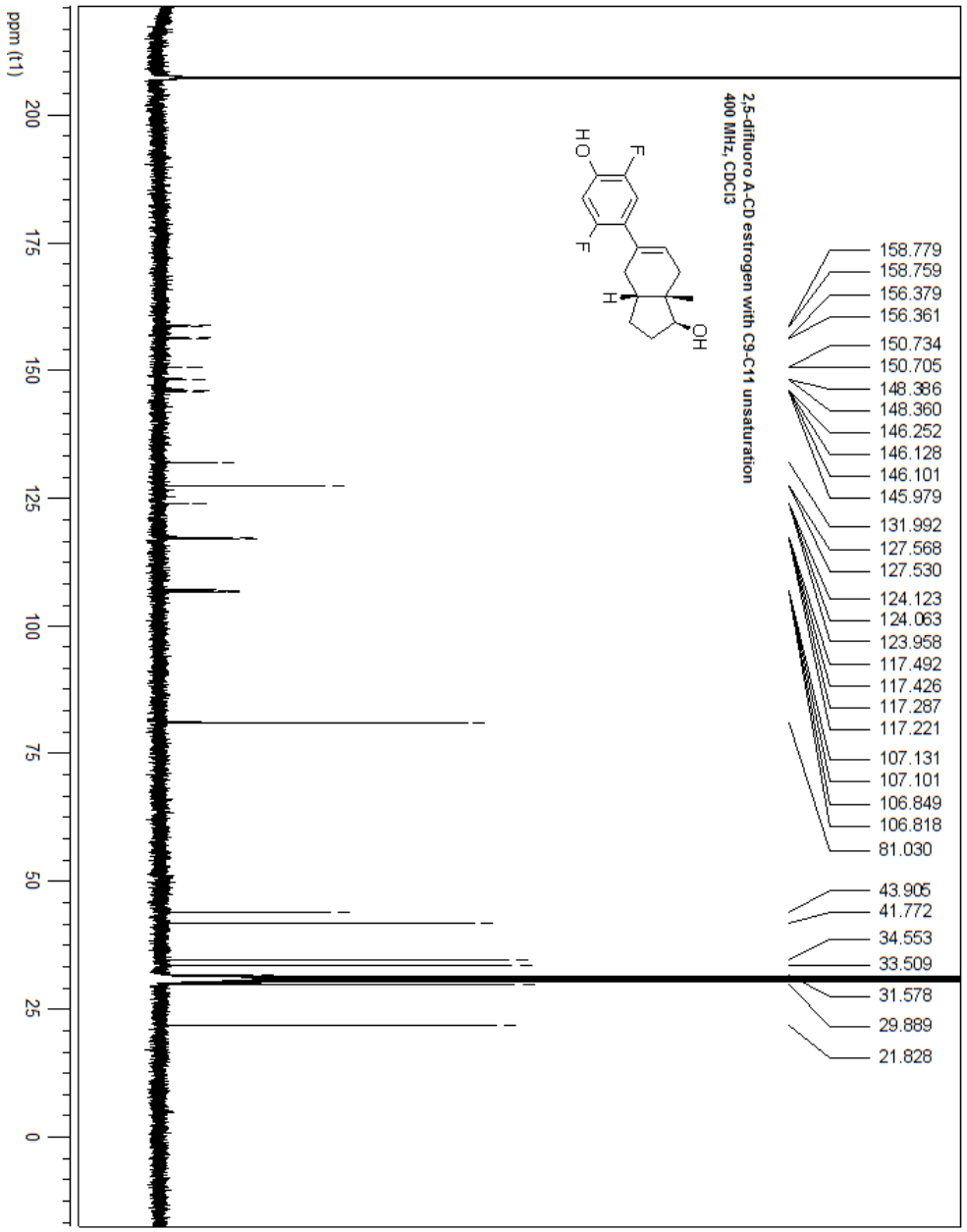

Figure a.60 ^{13}C NMR spectrum of 2,5-difluoro A-CD estrogen with C9-C11 unsaturation (**149**) in Acetone-d_6.

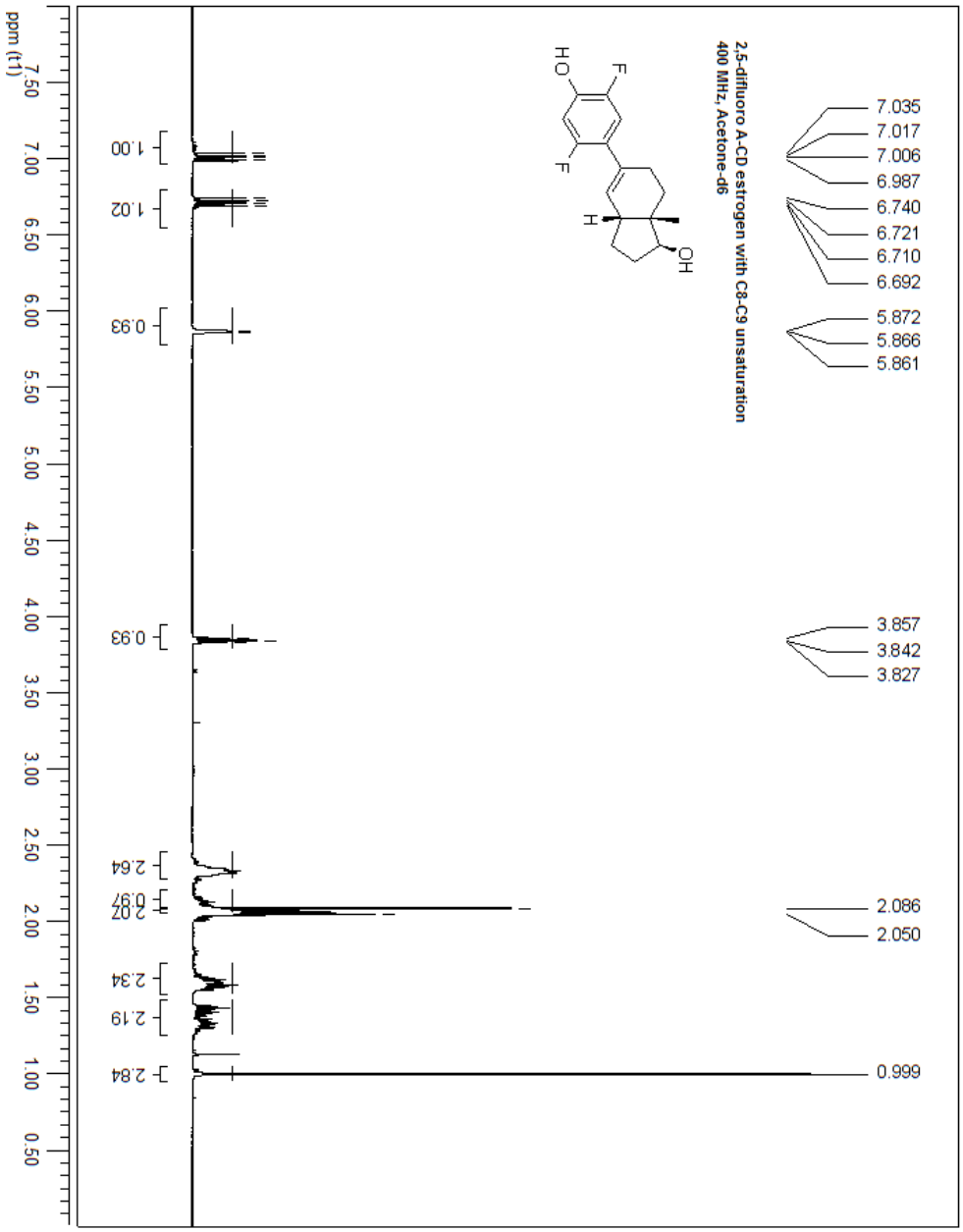

Figure a.61 ^{1}H NMR spectrum of 2,5-difluoro A-CD estrogen with C8-C9 unsaturation (**150**) in Acetone-d_6.

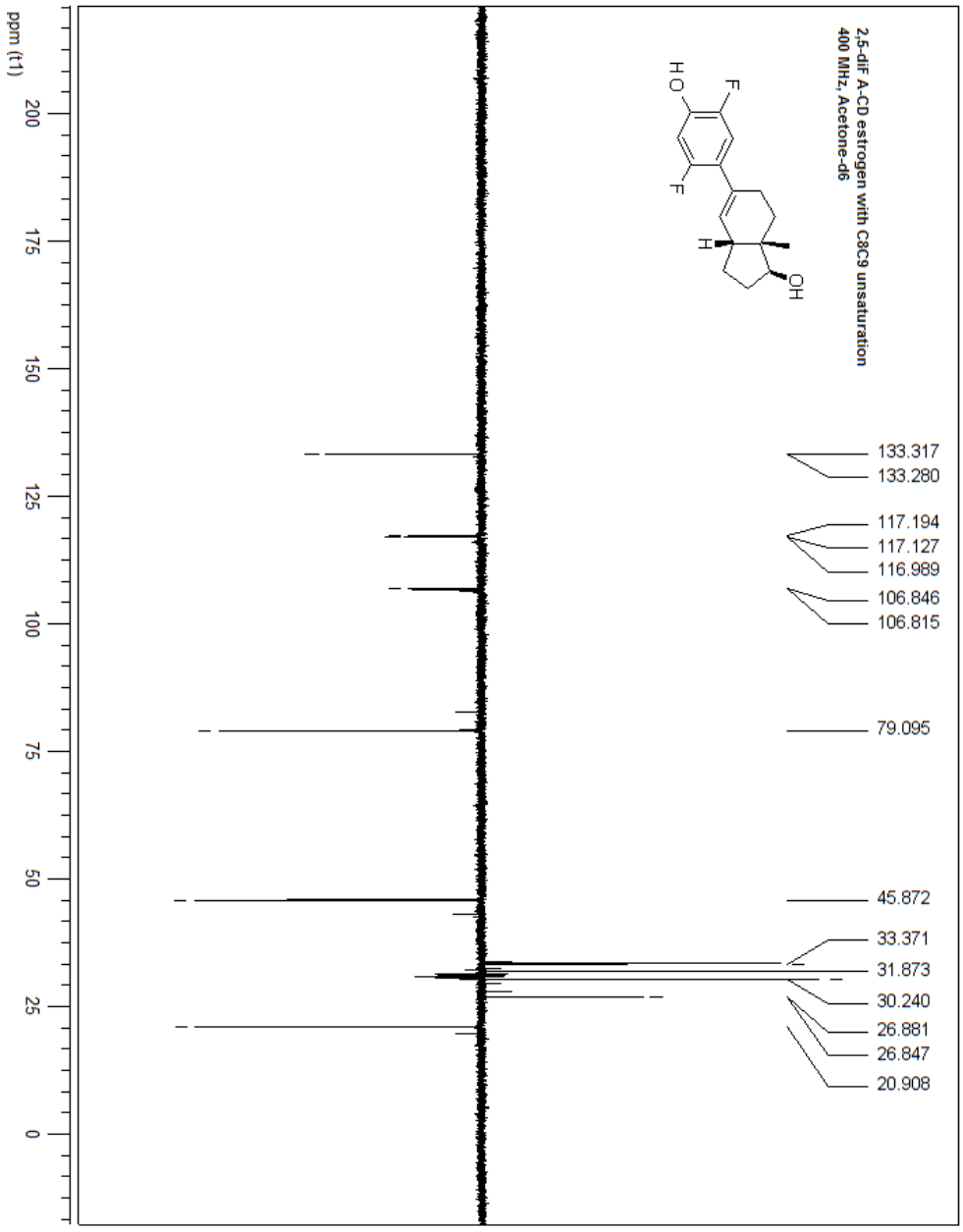

Figure a.62 DEPT-135 NMR spectrum of 2,5-difluoro A-CD estrogen with C8-C9 unsaturation (**150**) in Acetone-d_6.

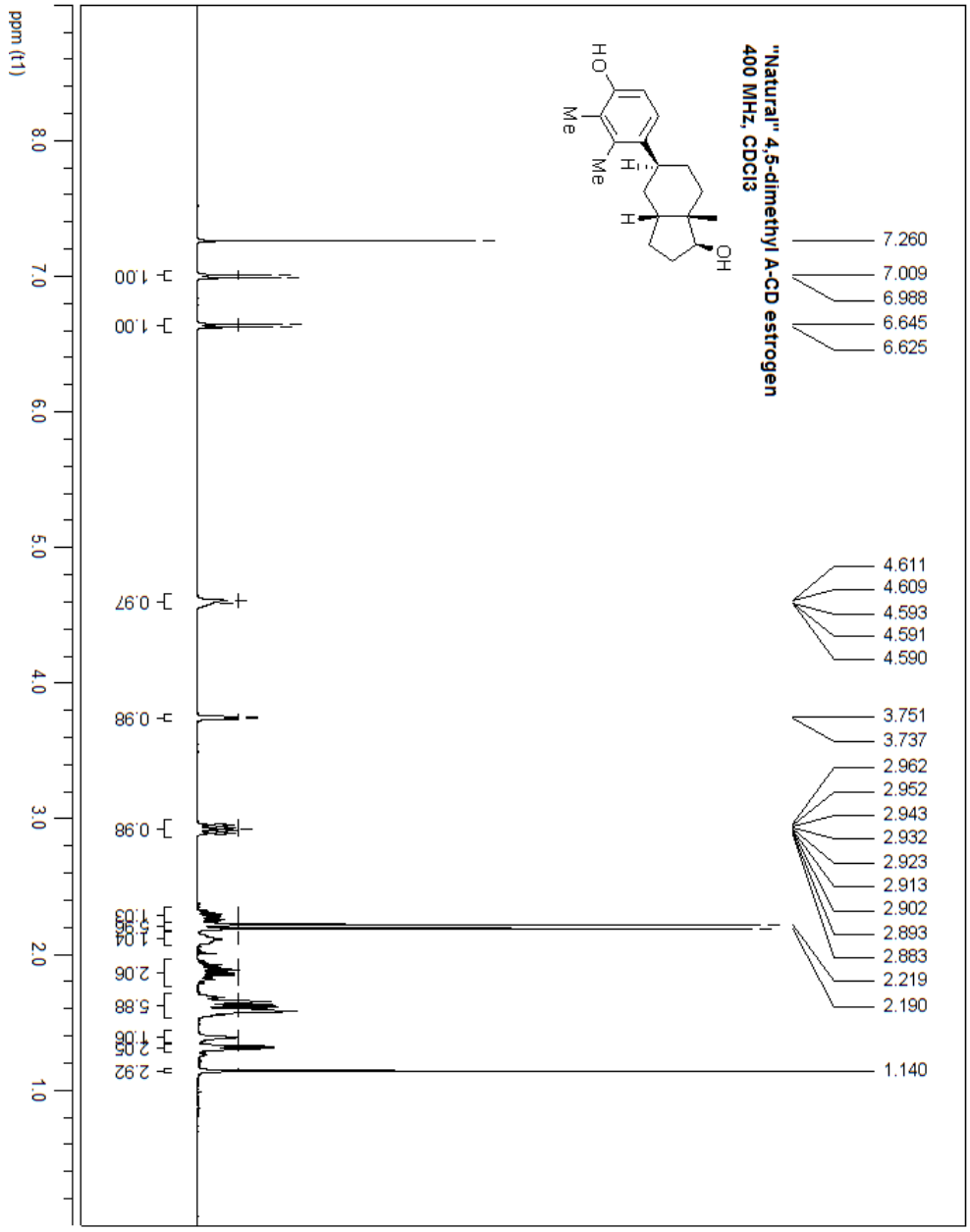

Figure a.63 ^{1}H NMR spectrum of the "natural" 2,5-dimethyl A-CD estrogen (**143**) in Acetone-d_6.

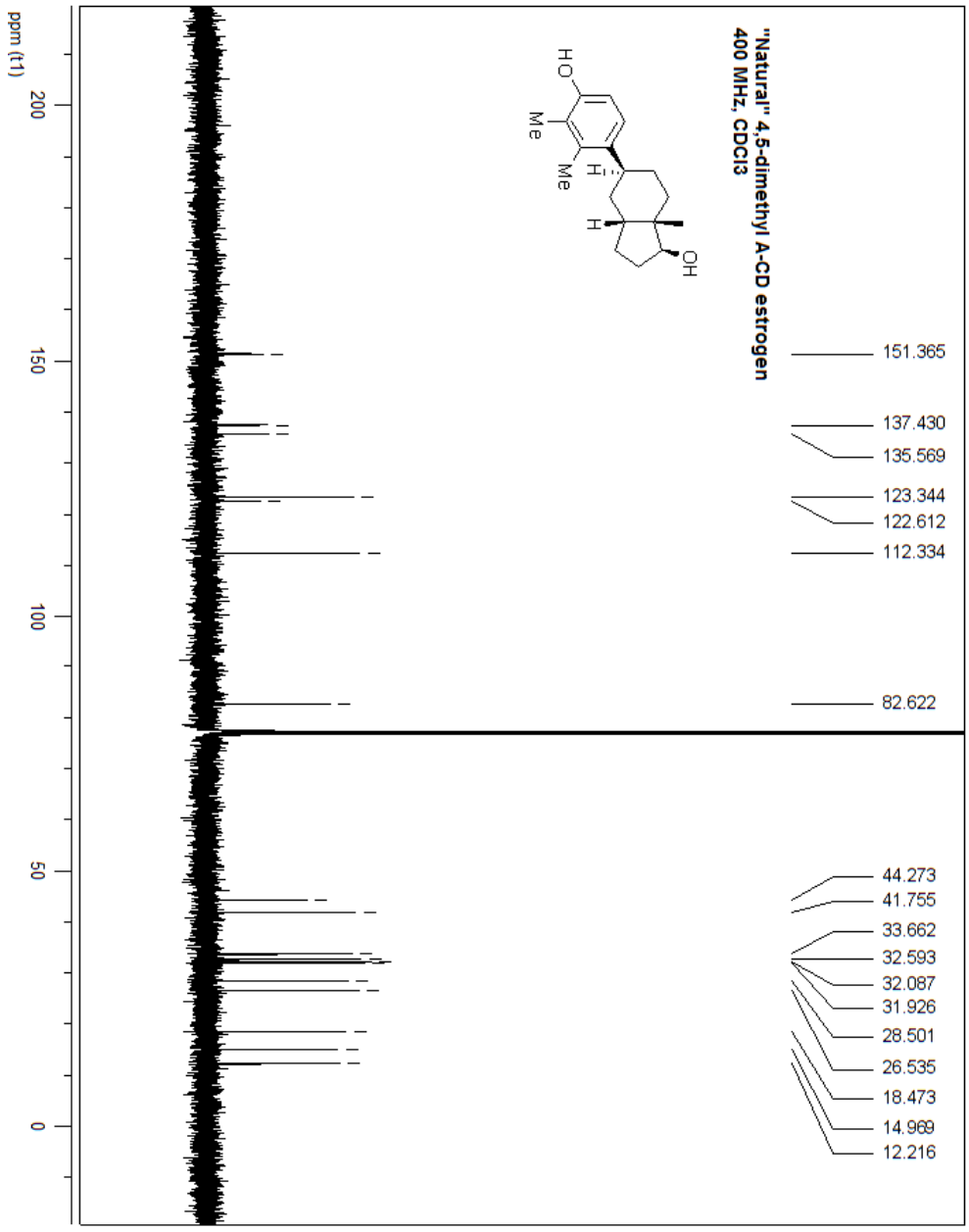

Figure a.64 ^{13}C NMR spectrum of the "natural" 2,5-dimethyl A-CD estrogen (**143**) in Acetone-d_6.

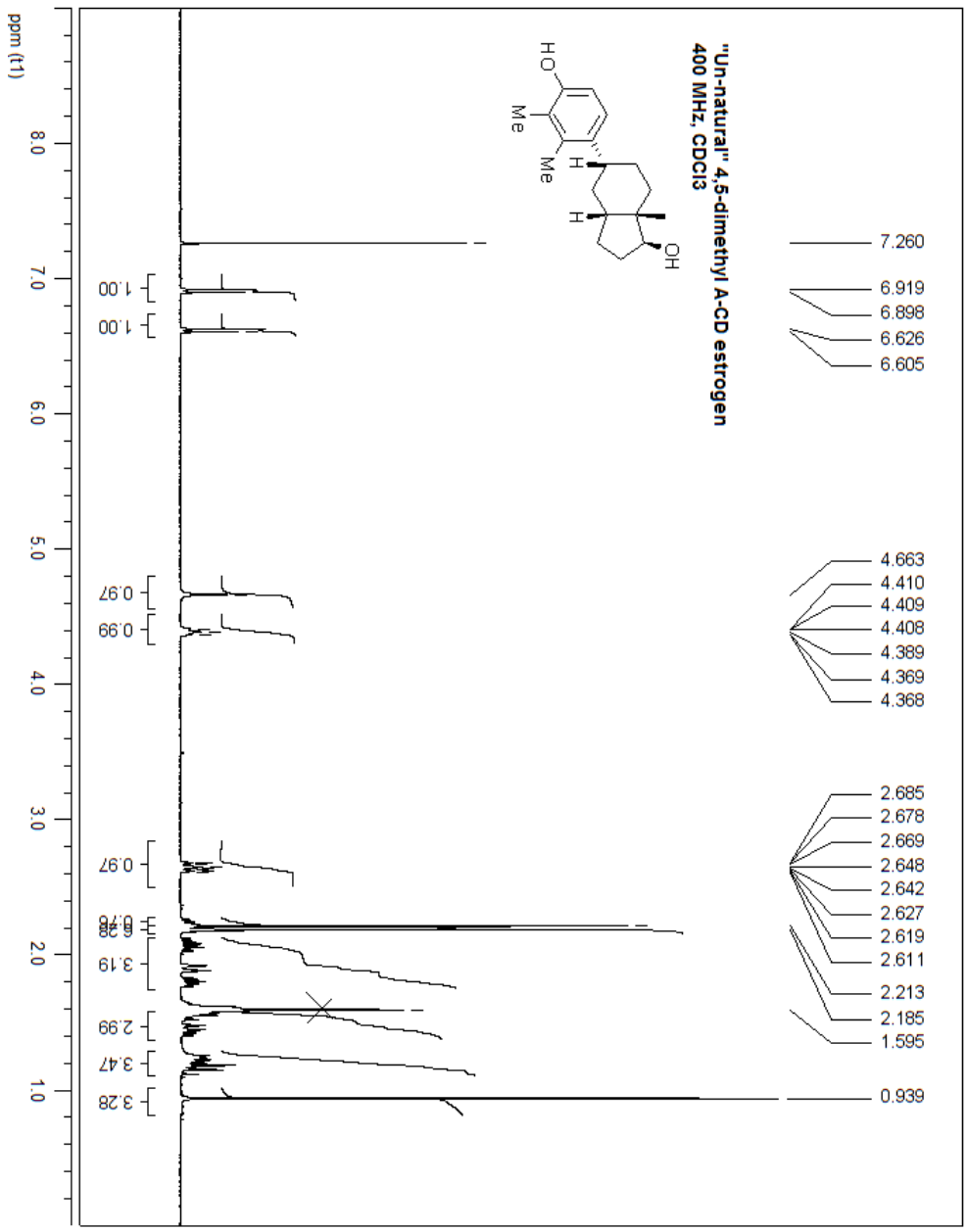

Figure a.65 ^{1}H NMR spectrum of the "un-natural" 2,5-dimethyl A-CD estrogen (**163**) in Acetone-d_6.

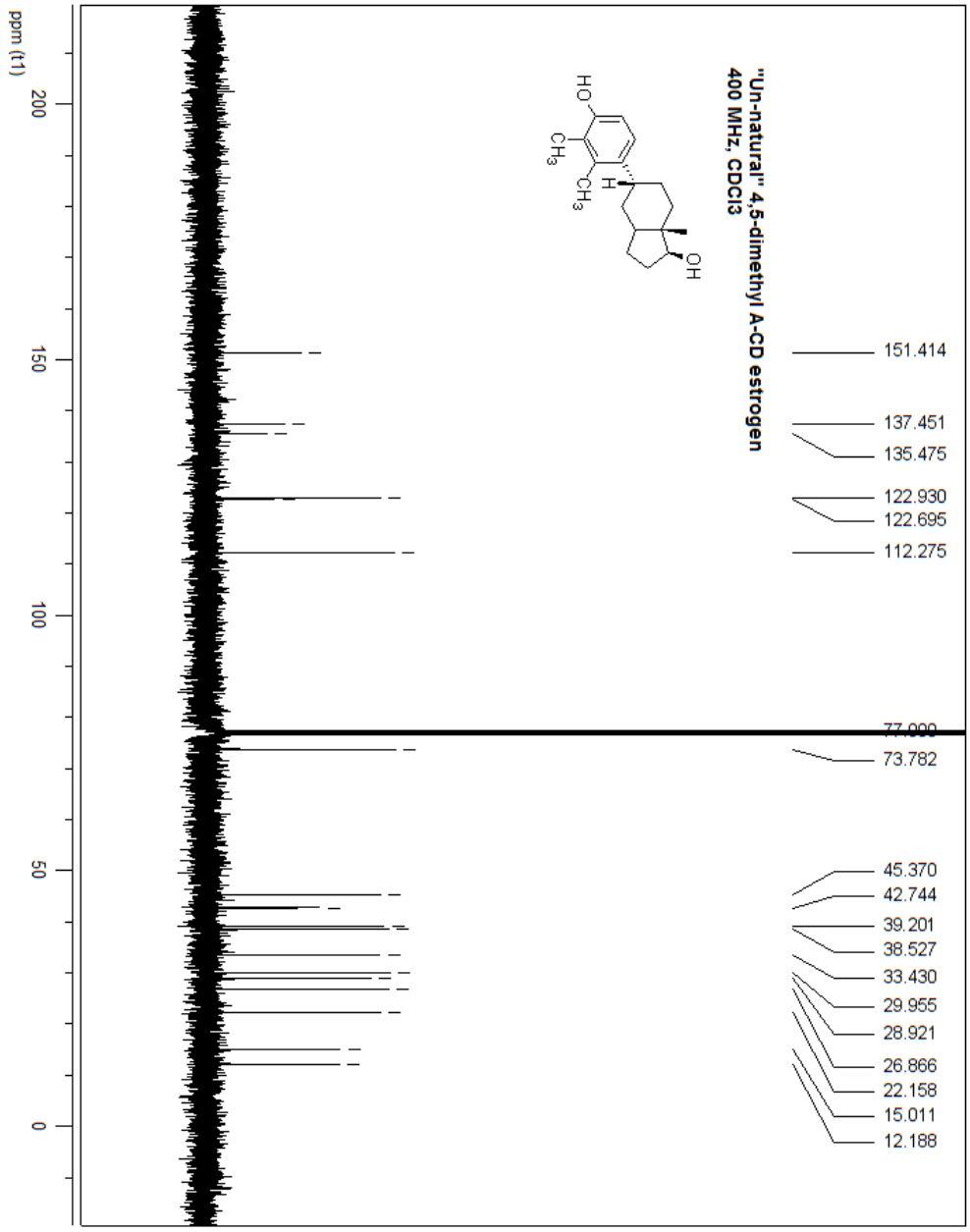

Figure a.66 ^{13}C NMR spectrum of the "un-natural" 2,5-dimethyl A-CD estrogen (**163**) in Acetone-d_6.

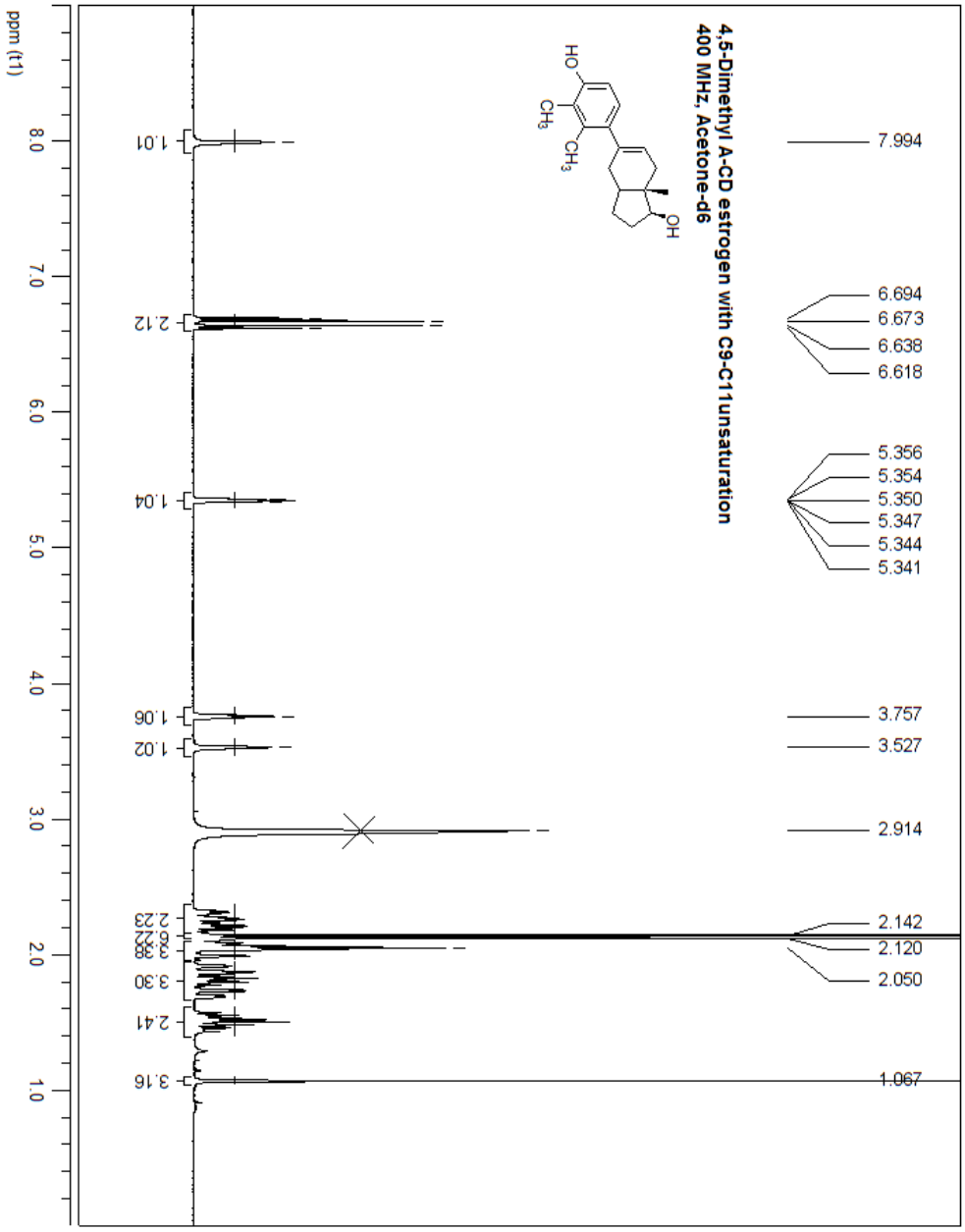

Figure a.67 ^{1}H NMR spectrum of 4,5-dimethyl A-CD estrogen with C9-C11 unsaturation (**161**) in Acetone-d_6.

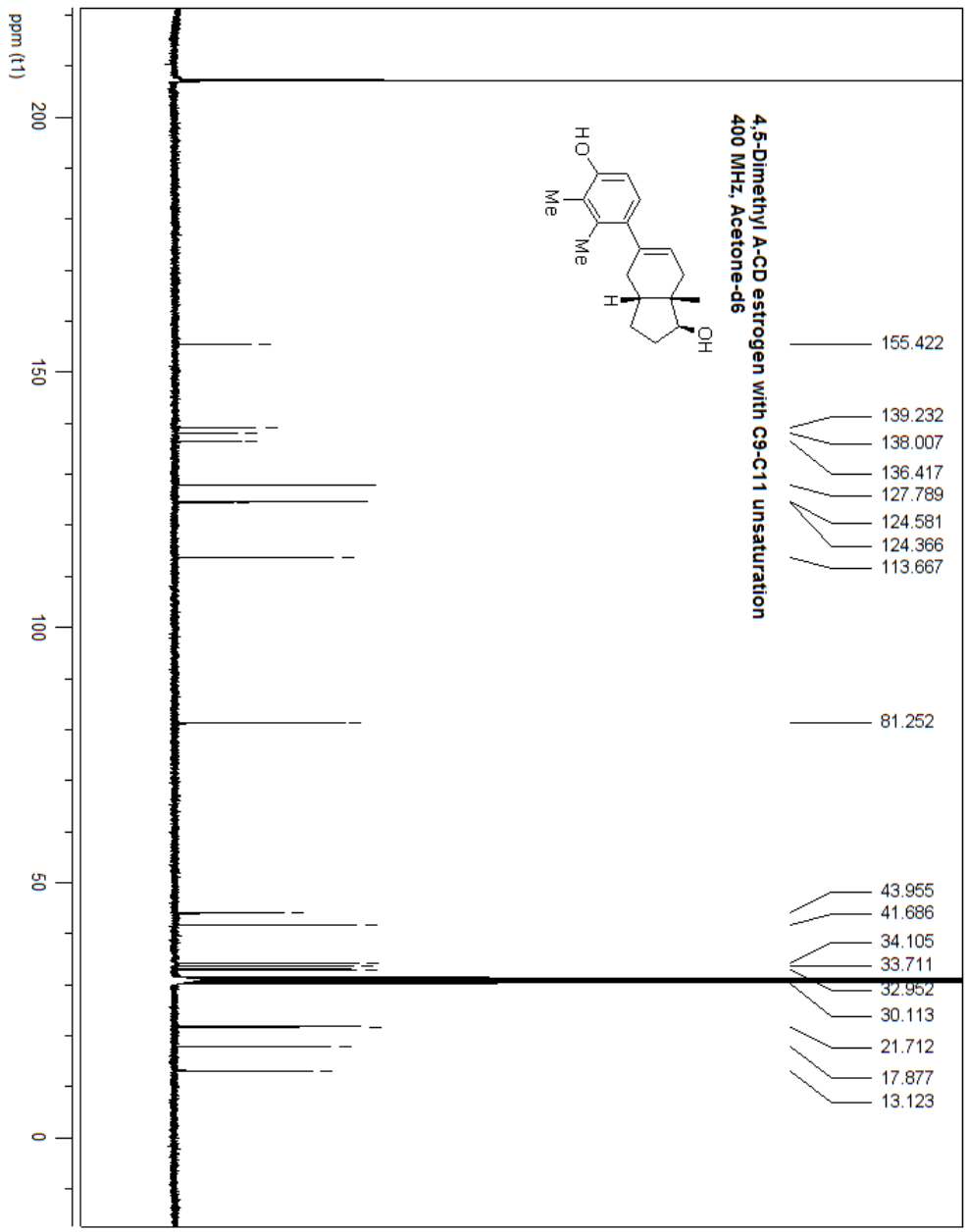

Figure a.68 ^{13}C NMR spectrum of 4,5-dimethyl A-CD estrogen with C9-C11 unsaturation (**161**) in Acetone-d_6.

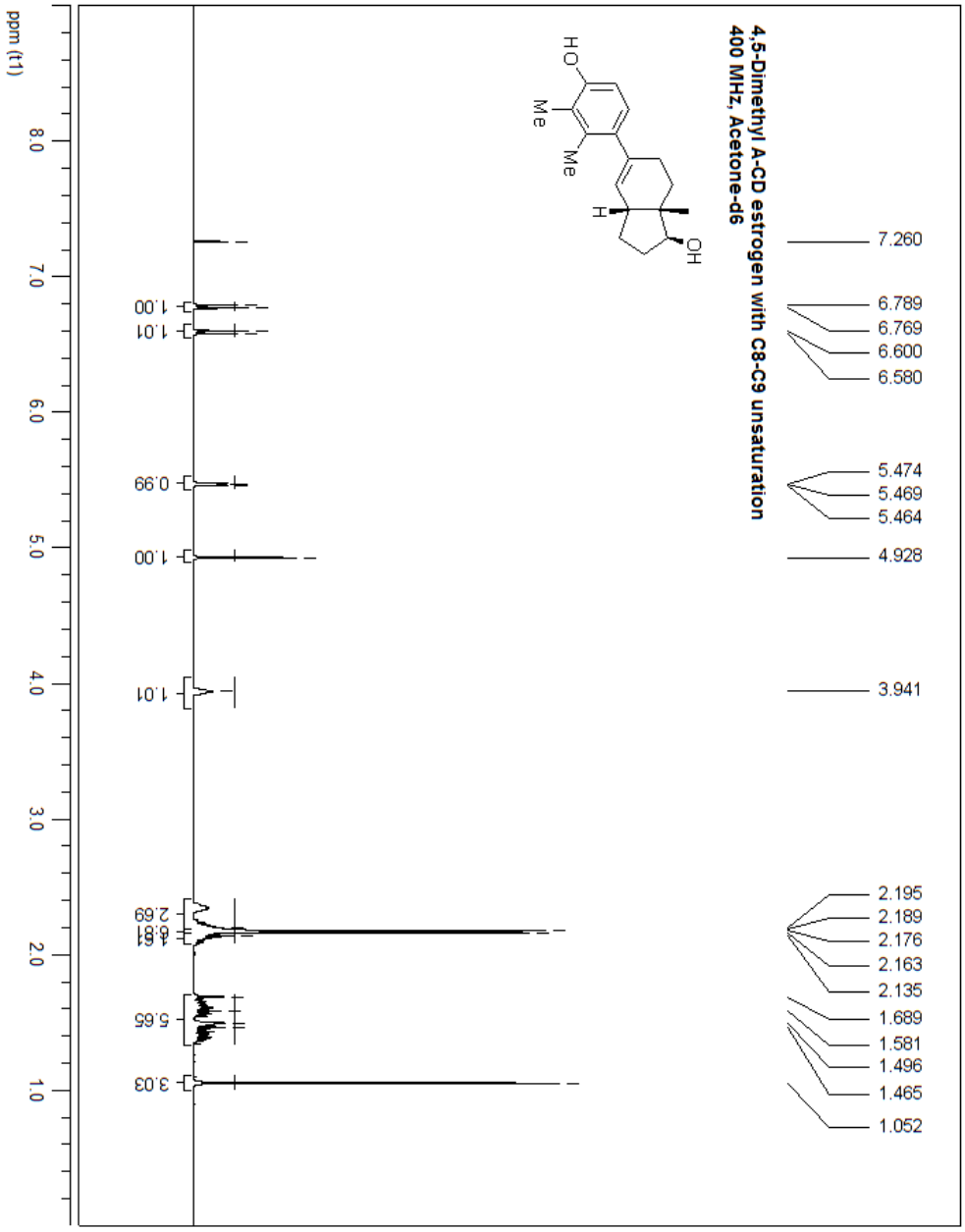

Figure a.69 ^{1}H NMR spectrum of 4,5-dimethyl A-CD estrogen with C8-C9 unsaturation (**162**) in $CDCl_3$.

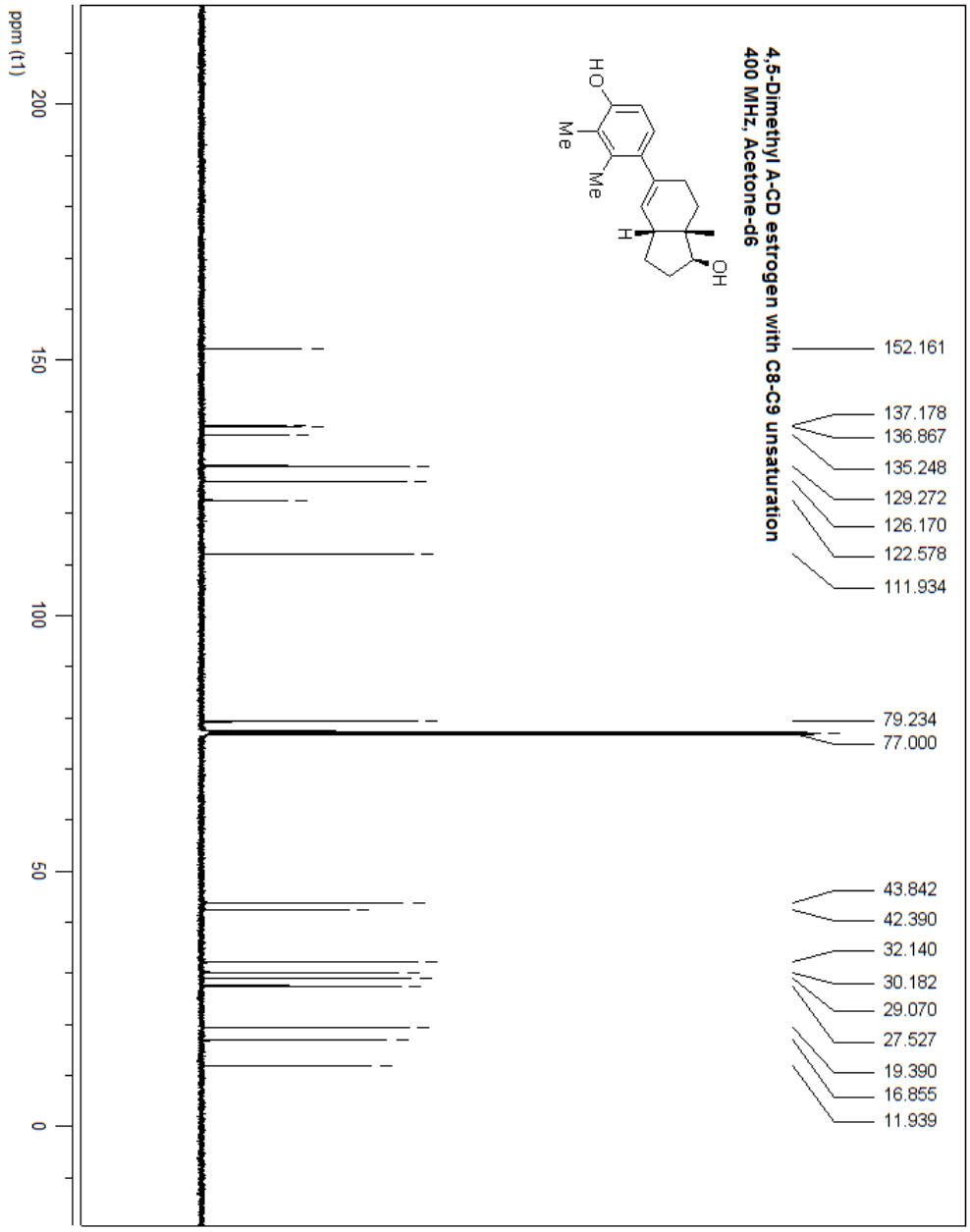

Figure a.70 ^{13}C NMR spectrum of 4,5-dimethyl A-CD estrogen with C8-C9 unsaturation (**162**) in $CDCl_3$.

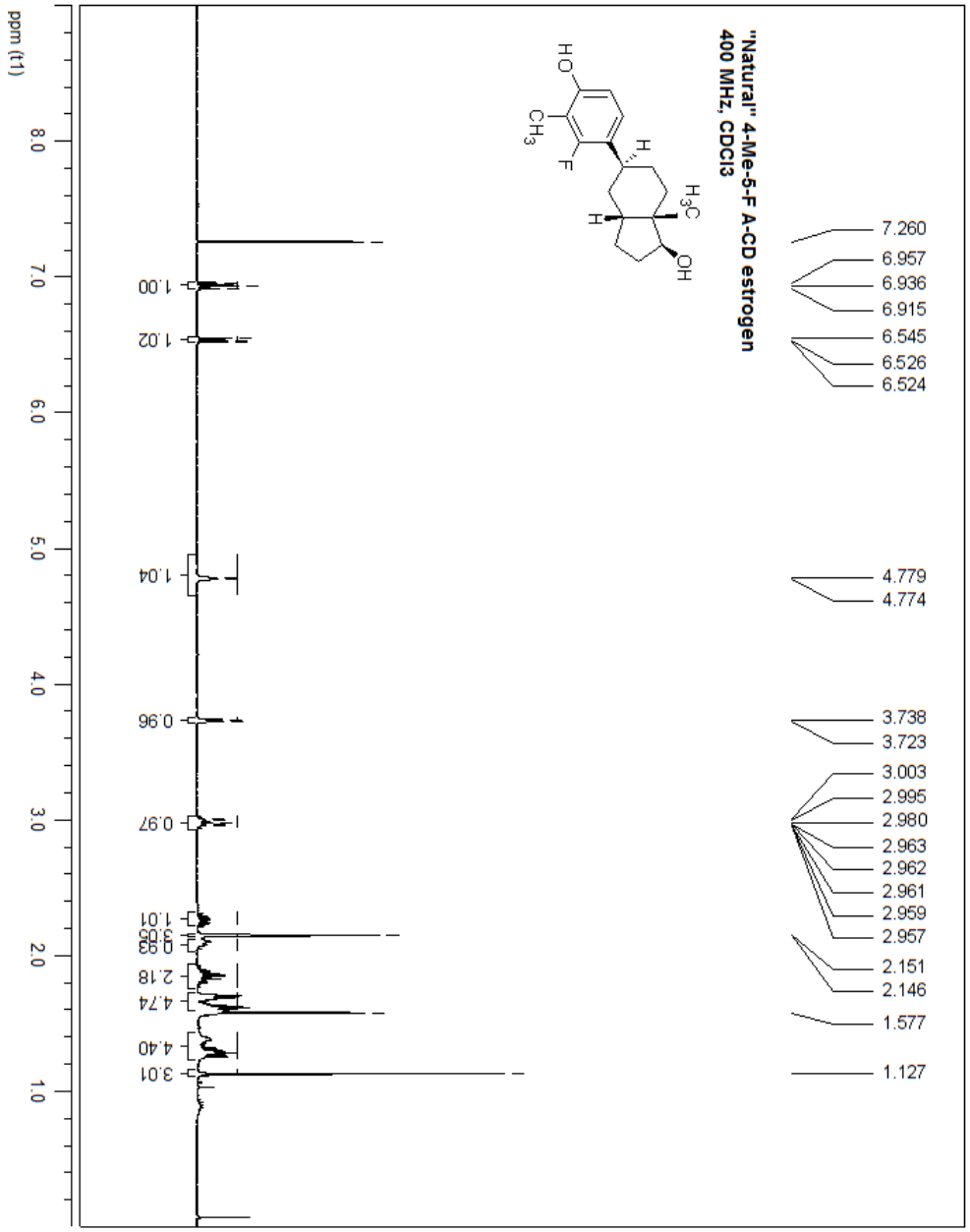

Figure a.71 ^{1}H NMR spectrum of "natural" 4-Me-5-F A-CD estrogen (**144**) in $CDCl_3$.

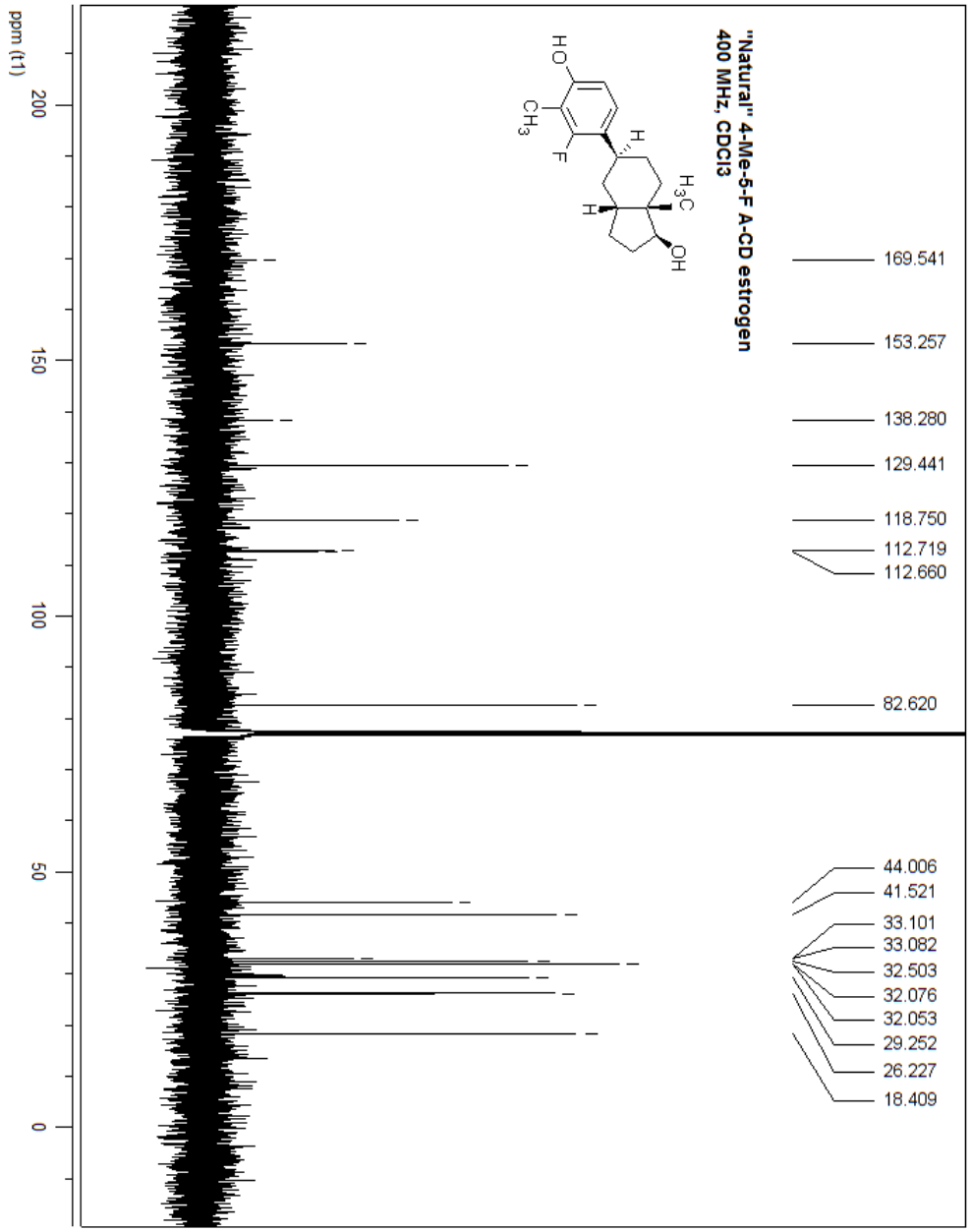

Figure a.72 ^{13}C NMR spectrum of "natural" 4-Me-5-F A-CD estrogen (**144**) in $CDCl_3$.

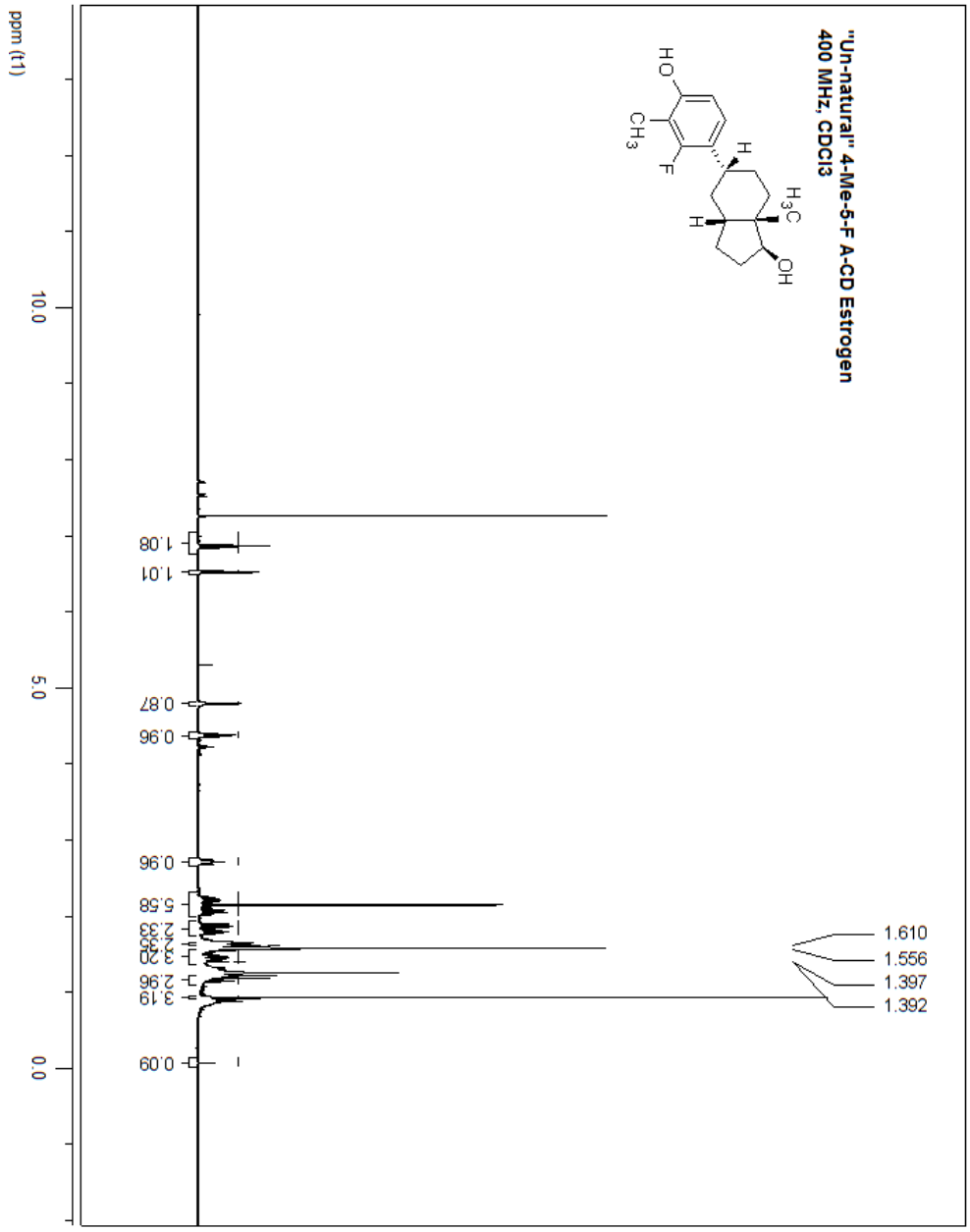

Figure a.73 ^{1}H NMR spectrum of "un-natural" 4-Me-5-F A-CD estrogen (**166**) in $CDCl_3$.

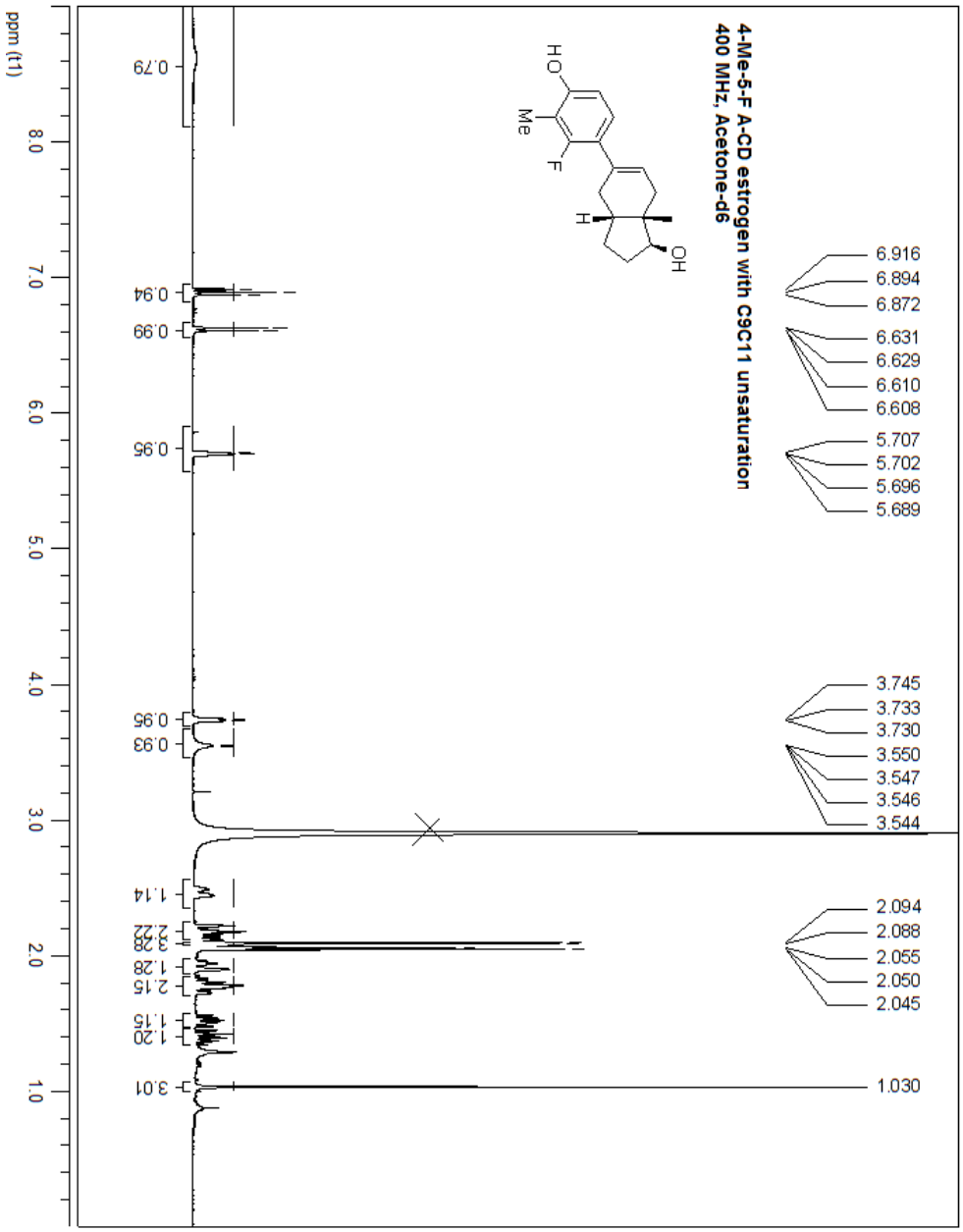

Figure a.74 ^{1}H NMR spectrum of 4-Me-5-F A-CD estrogen with C9-C11 unsaturation (**164**) in Acetone-d_6.

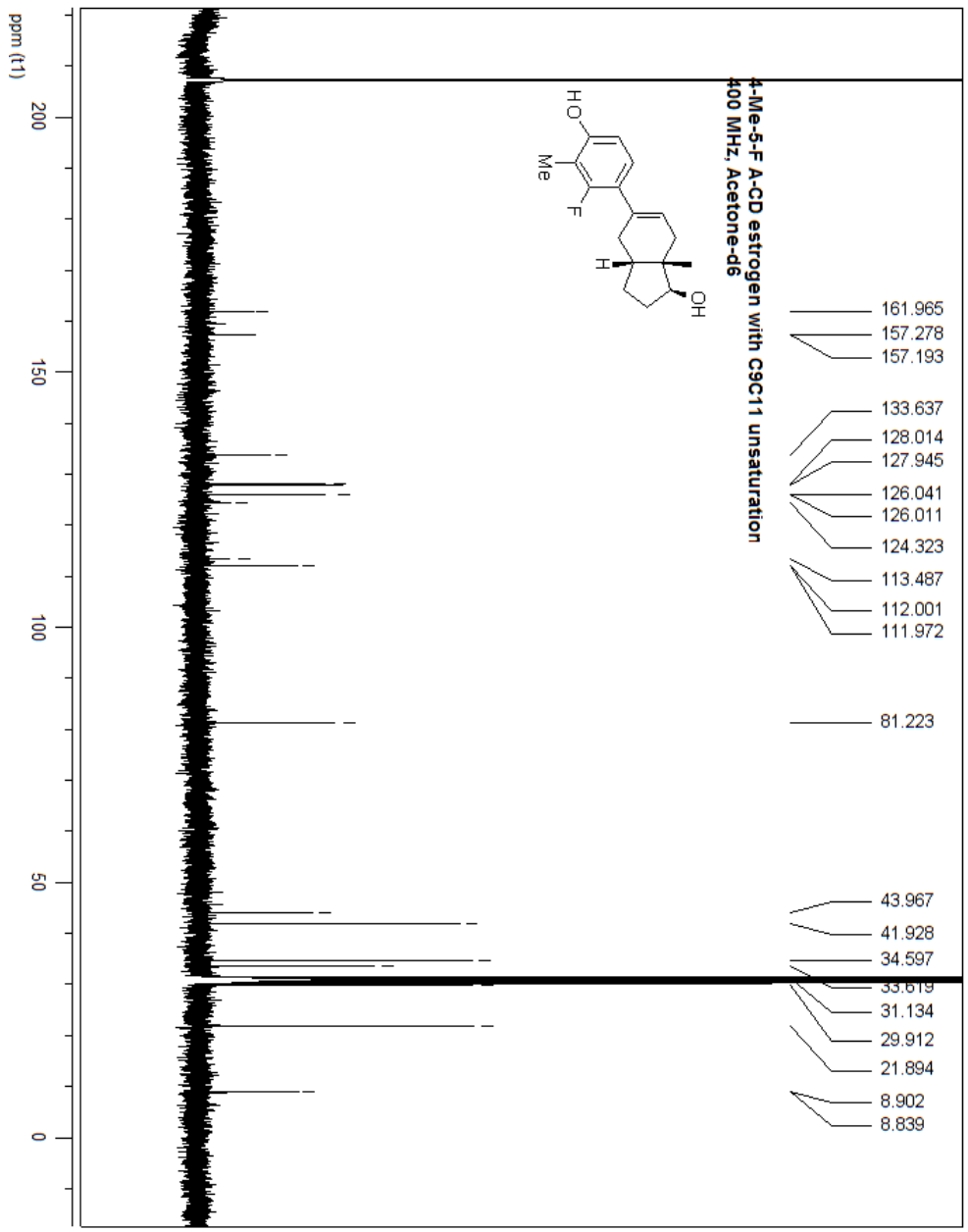

Figure a.75 ^{13}C NMR spectrum of 4-Me-5-F A-CD estrogen with C9-C11 unsaturation (**164**) in Acetone-d_6.

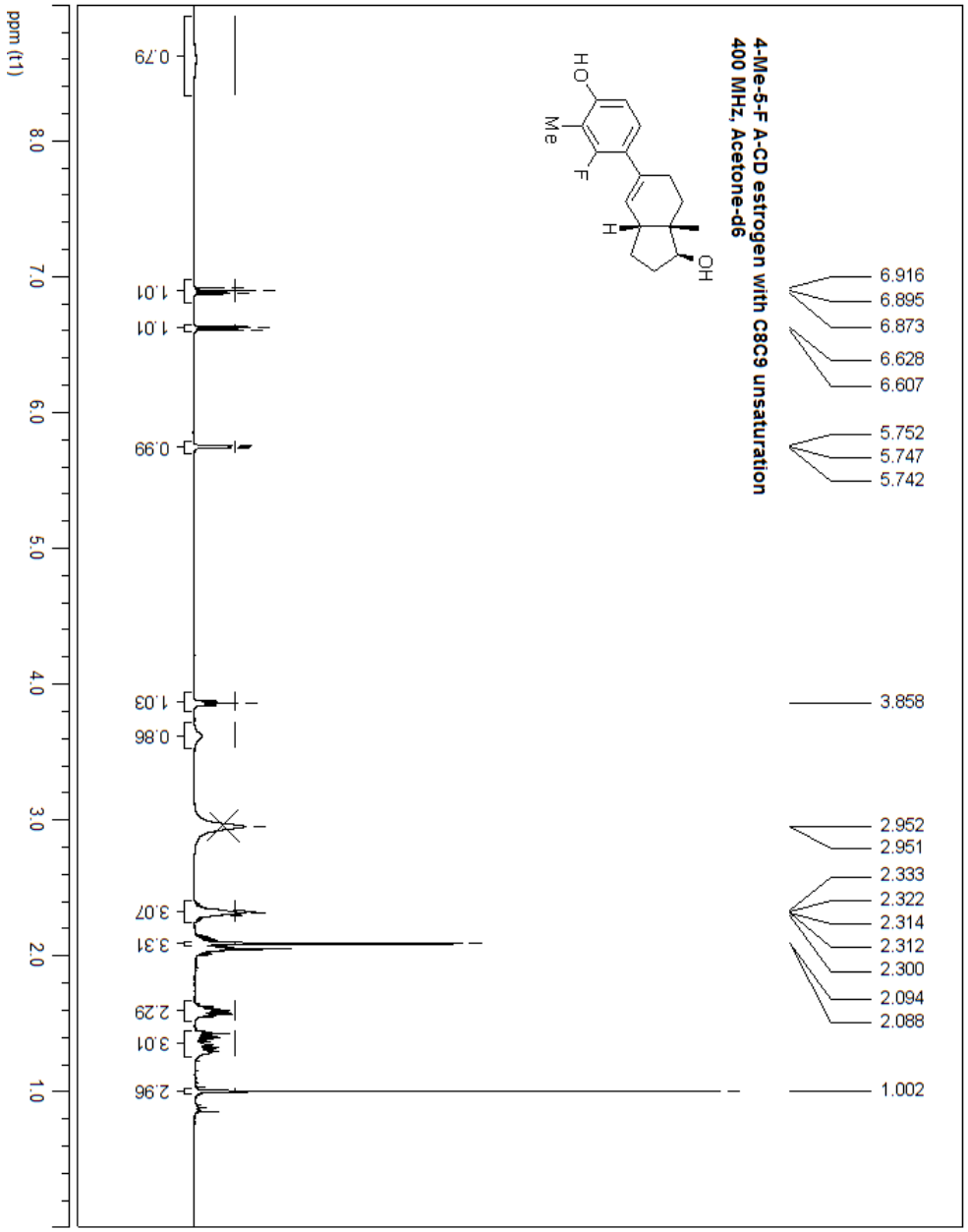

Figure a.76 ^{1}H NMR spectrum of 4-Me-5-F A-CD estrogen with C8-C9 unsaturation (**165**) in Acetone-d_6.

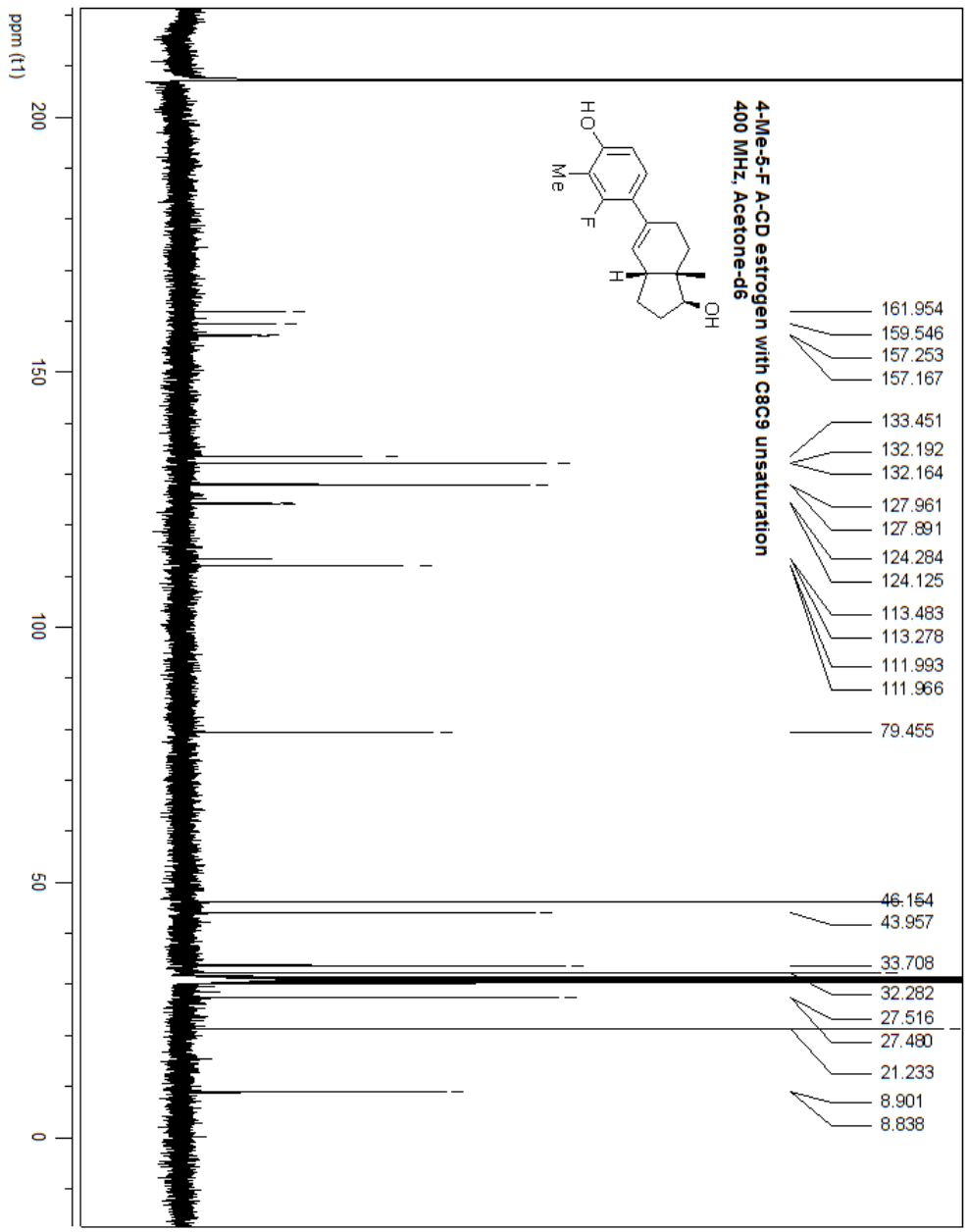

Figure a.77 ^{13}C NMR spectrum of 4-Me-5-F A-CD estrogen with C8-C9 unsaturation (**165**) in Acetone-d_6.

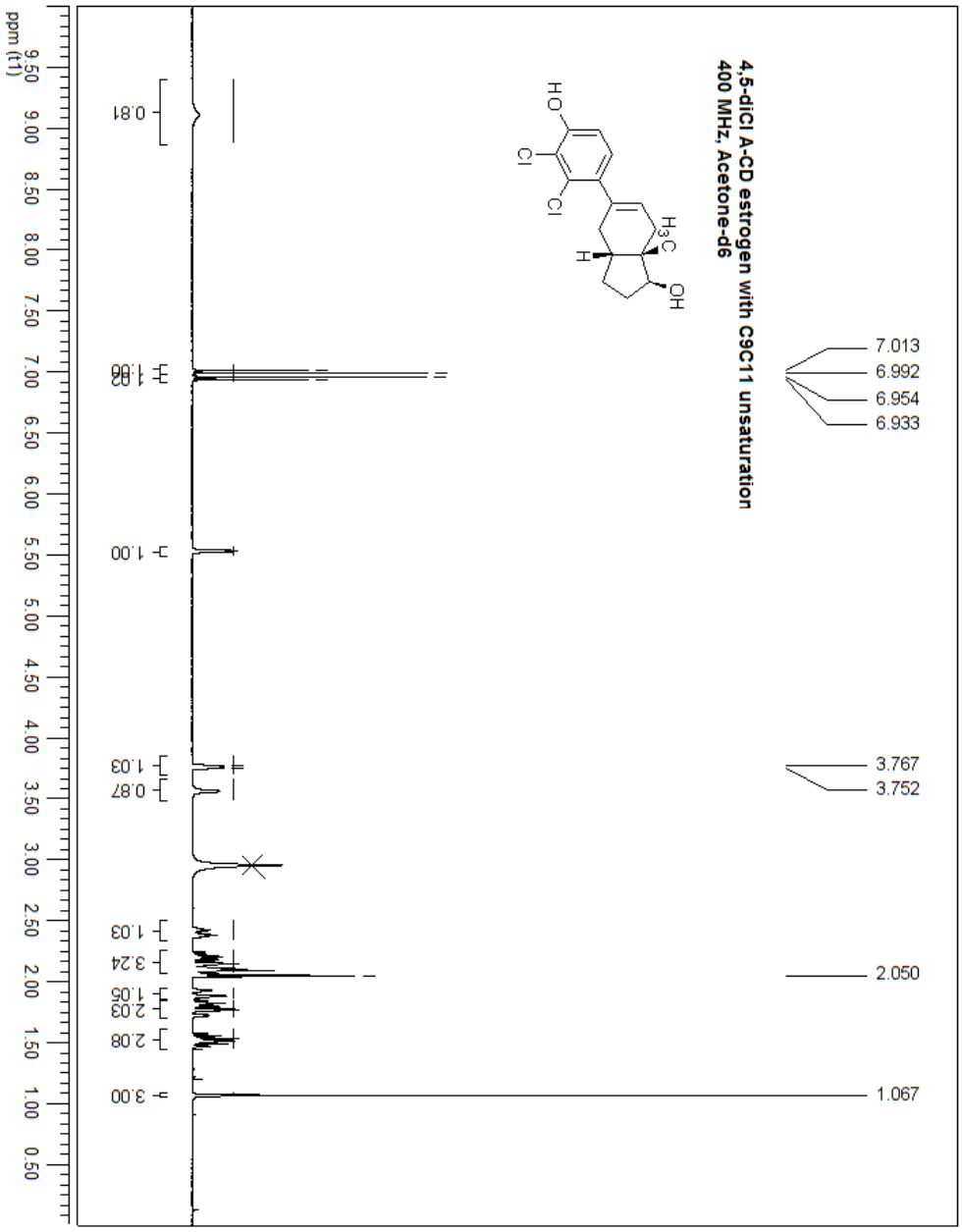

Figure a.78 ^{1}H NMR spectrum of 4,5-diCl A-CD estrogen with C9-C11 unsaturation (**167**) in Acetone-d_6.

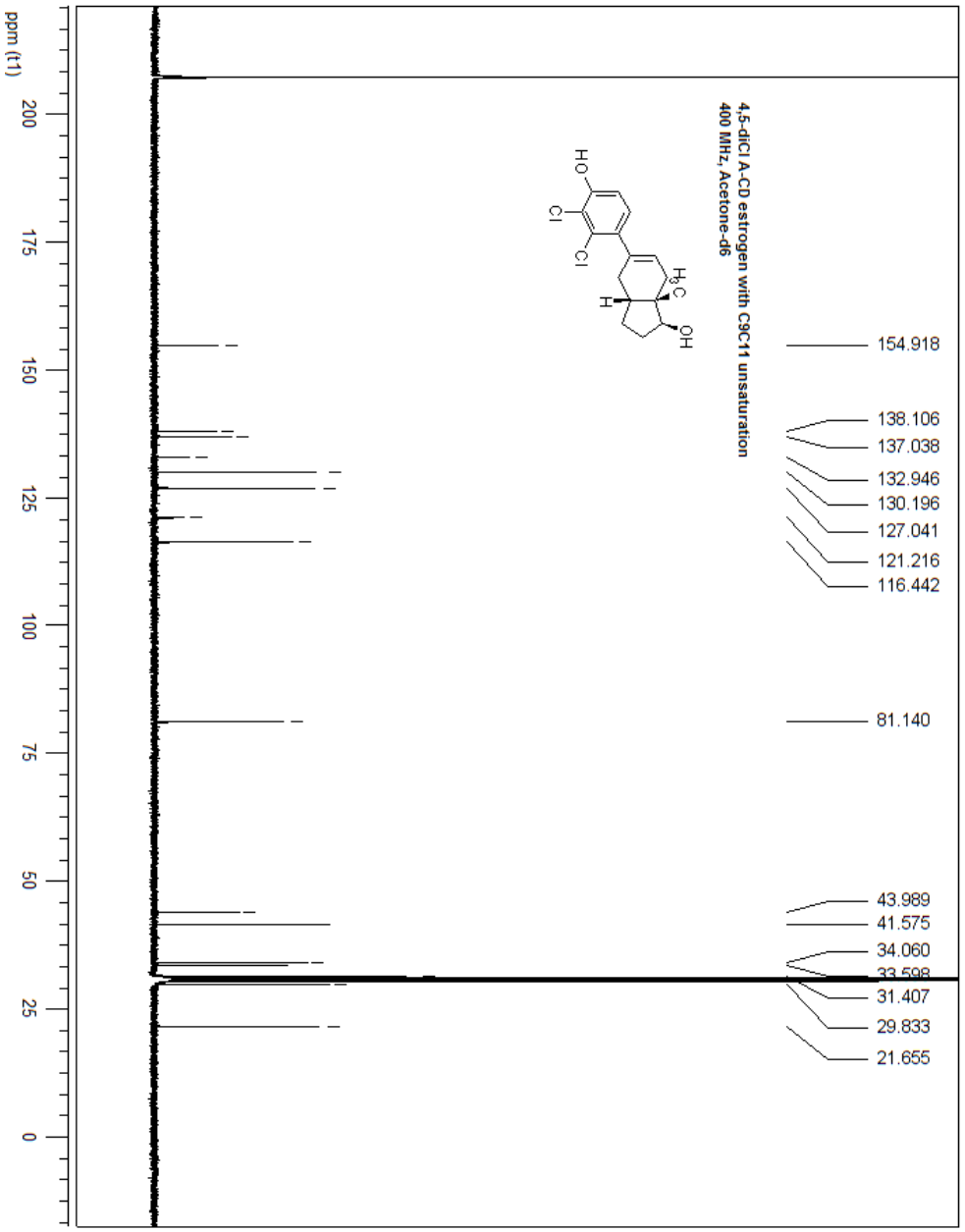

Figure a.79 ^{13}C NMR spectrum of 4,5-diCl A-CD estrogen with C9-C11 unsaturation (**167**) in Acetone-d_6.

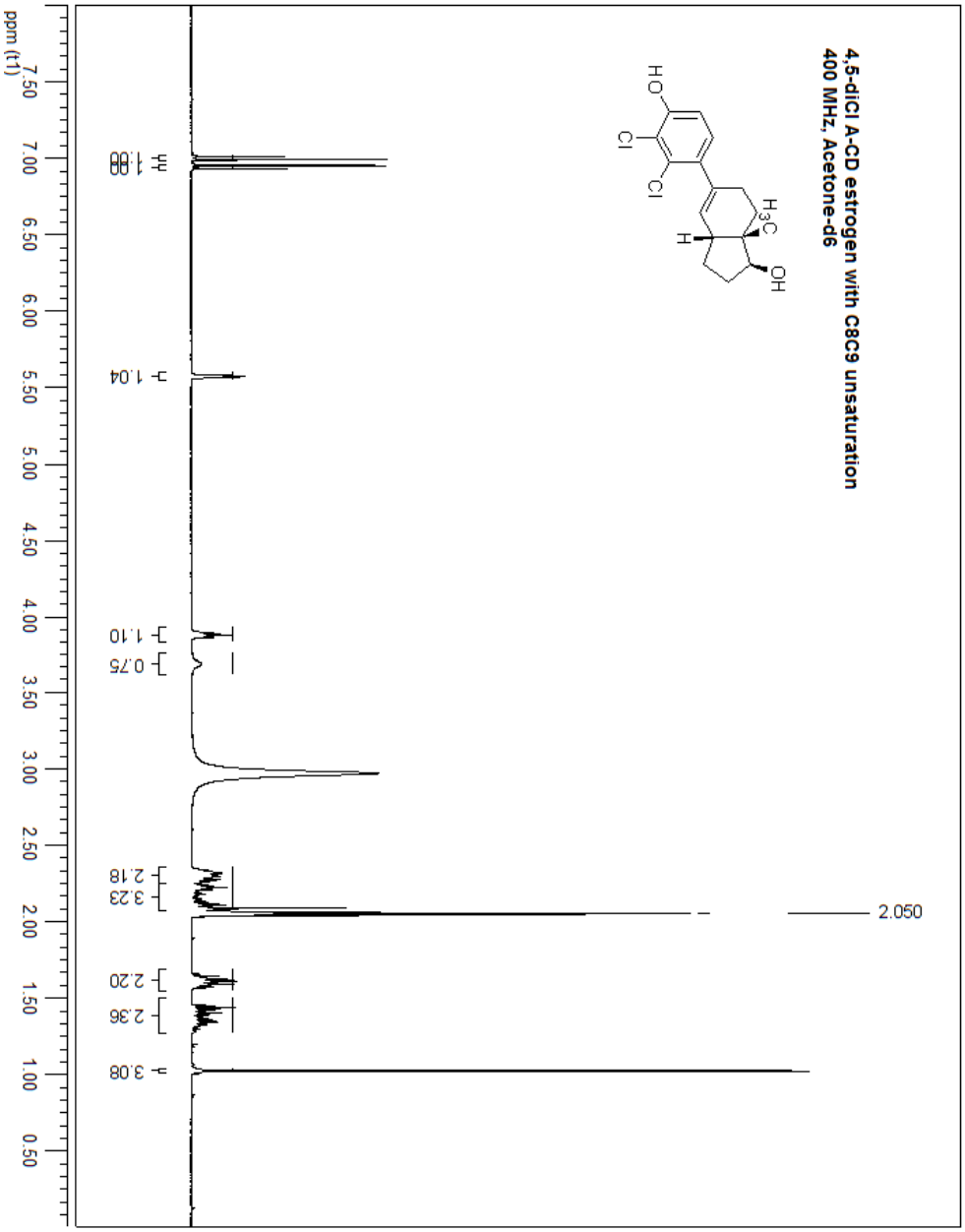

Figure a.80 ^{1}H NMR spectrum of 4,5-diCl A-CD estrogen with C8-C9 unsaturation (**168**) in Acetone-d_6.

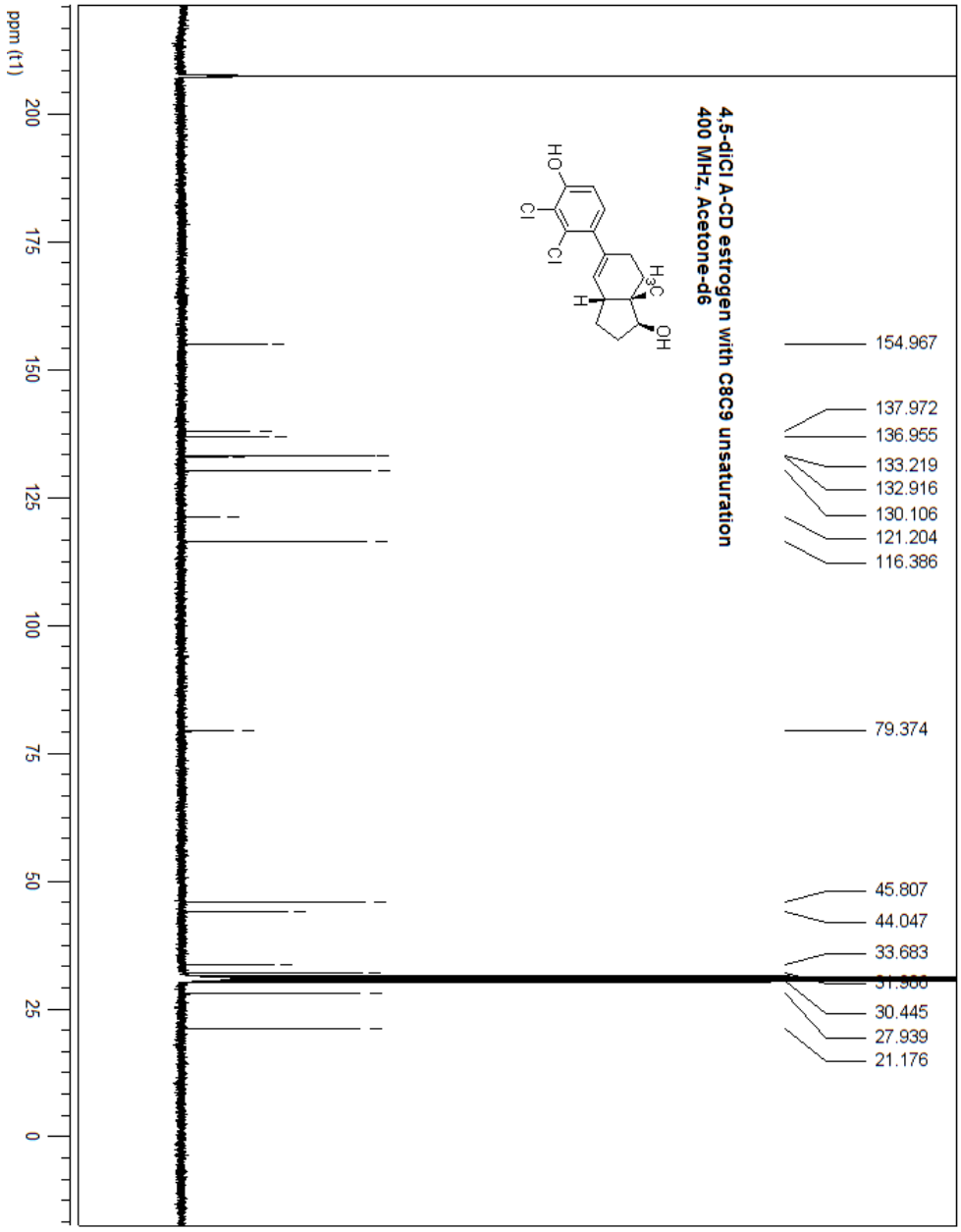

Figure a.81 ^{13}C NMR spectrum of 4,5-diCl A-CD estrogen with C8-C9 unsaturation (**168**) in Acetone-d_6.

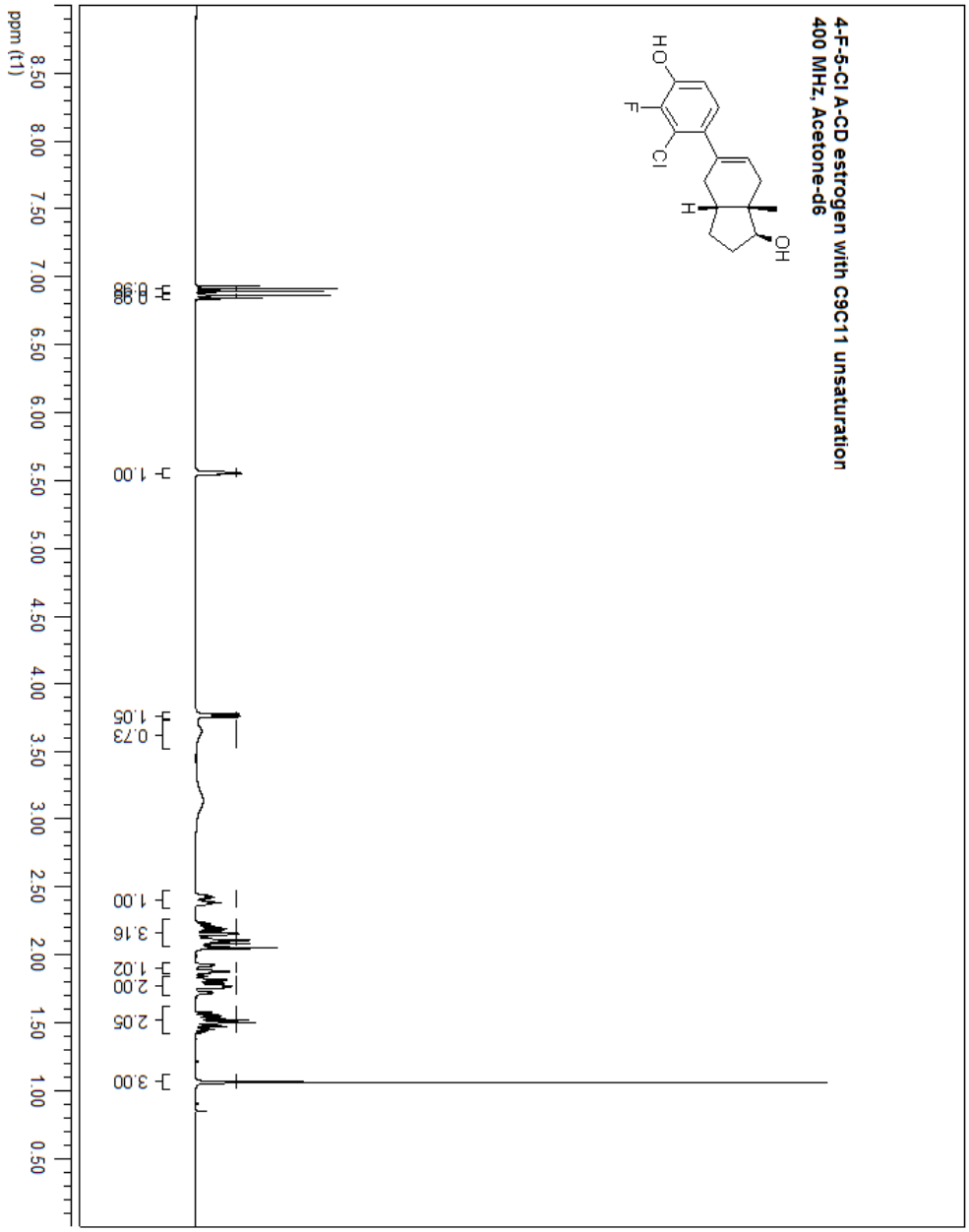

Figure a.82 ^{1}H NMR spectrum of 4-F-5-Cl A-CD estrogen with C9-C11 unsaturation (**169**) in Acetone-d_6.

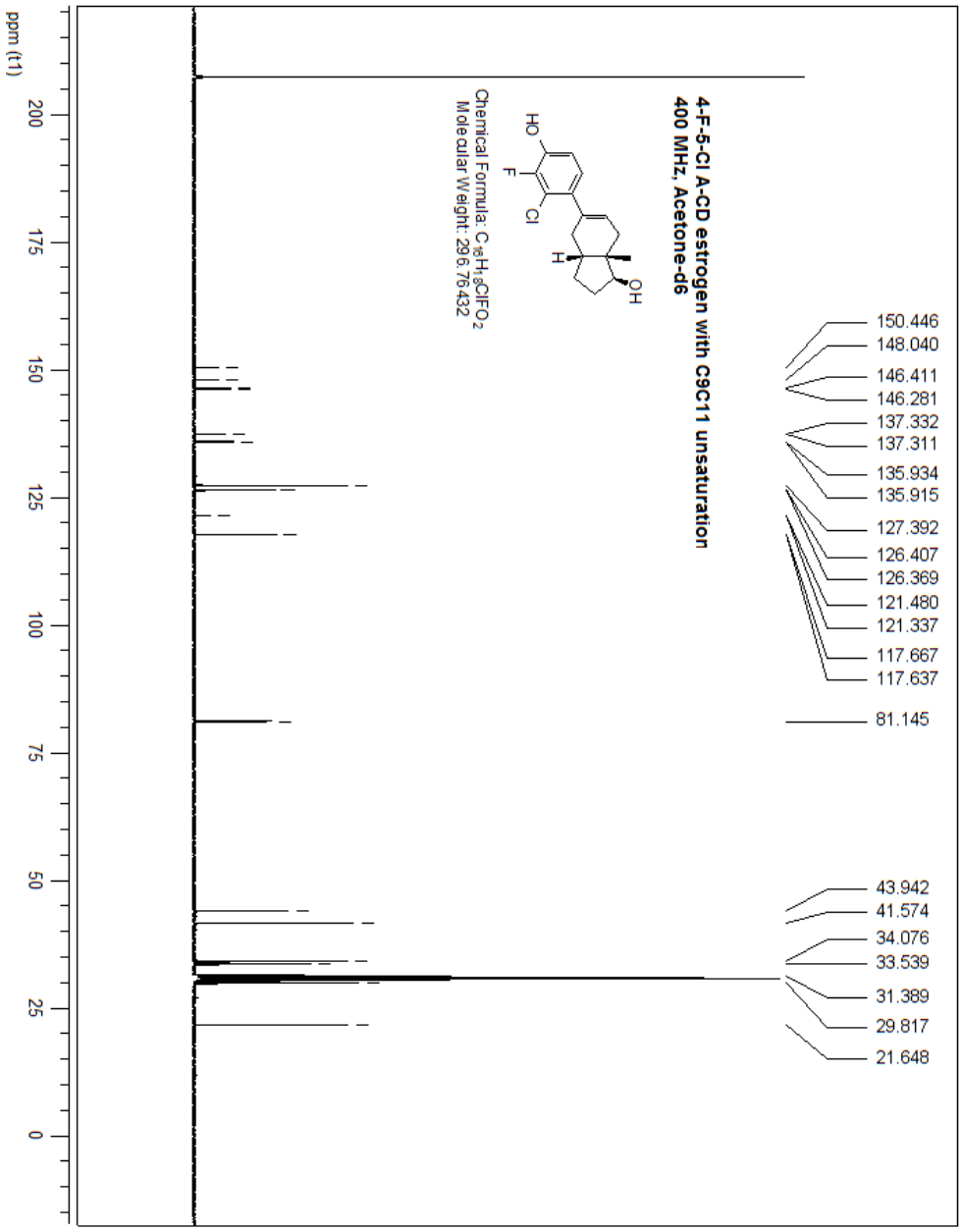

Figure a.83 ^{13}C NMR spectrum of 4-F-5-Cl A-CD estrogen with C9-C11 unsaturation (**169**) in Acetone-d_6.

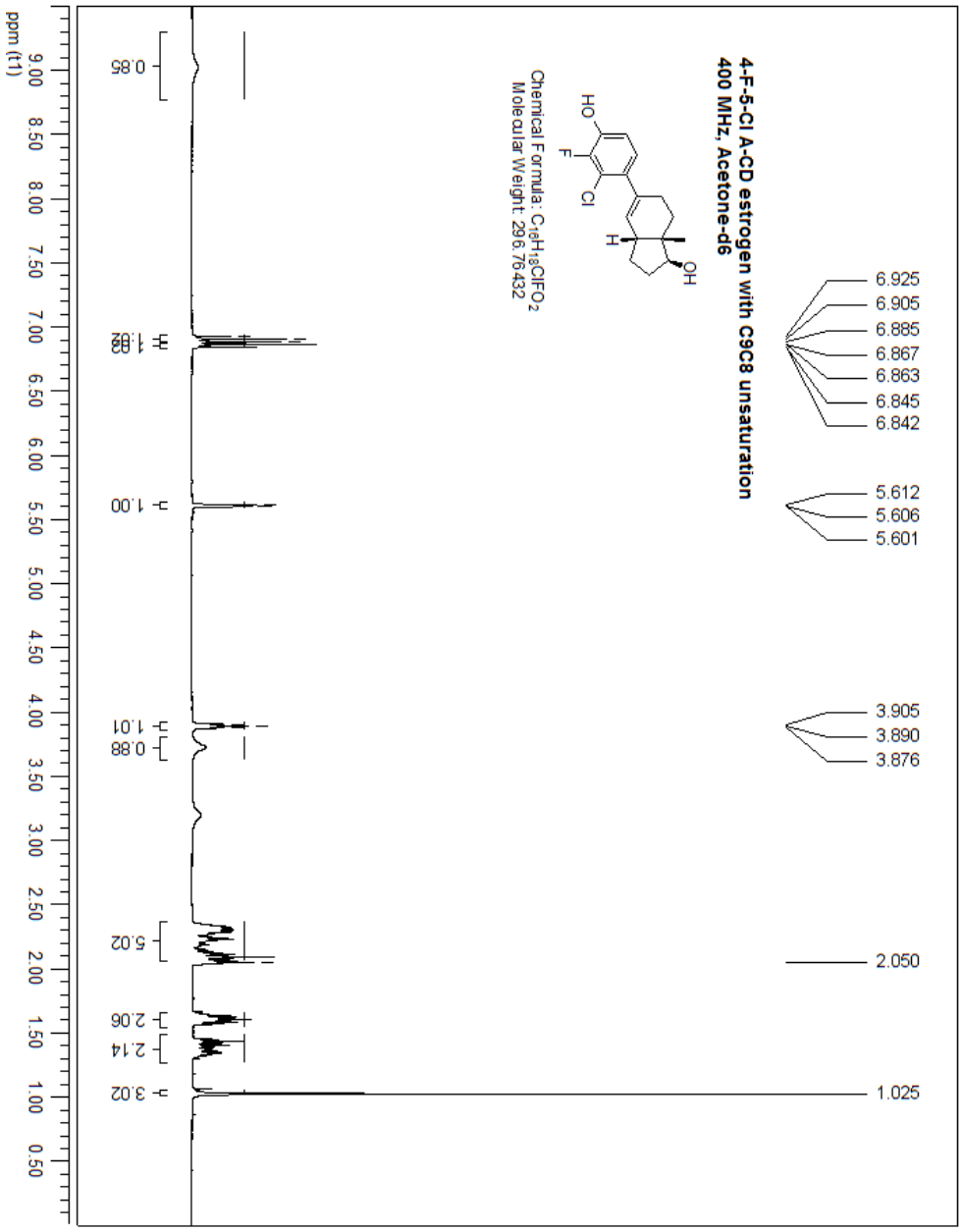

Figure a.84 ^{1}H NMR spectrum of 4-F-5-Cl A-CD estrogen with C8-C9 unsaturation (**170**) in Acetone-d_6.

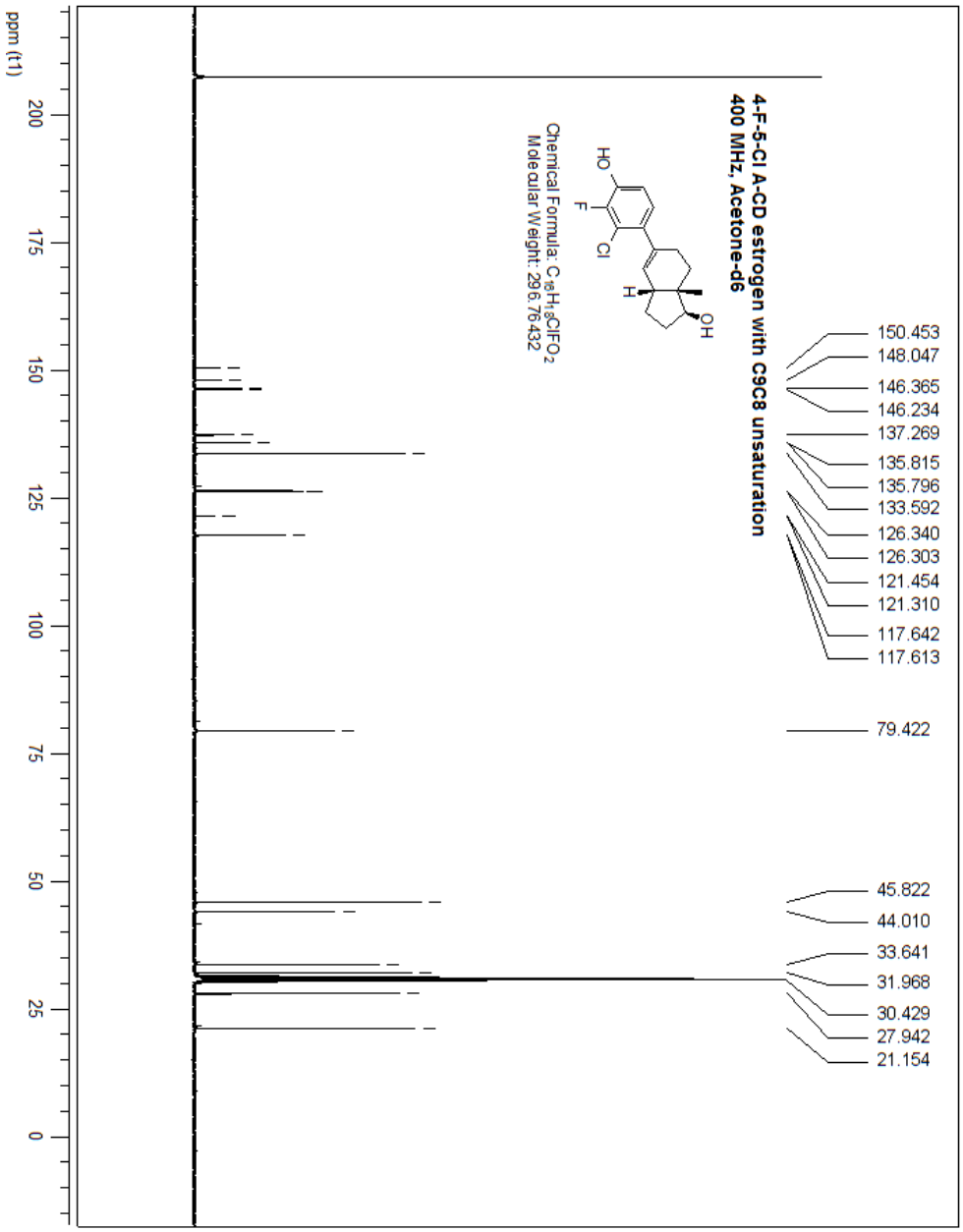

Figure a.85 ^{13}C NMR spectrum of 4-F-5-Cl A-CD estrogen with C8-C9 unsaturation (**170**) in Acetone-d_6.

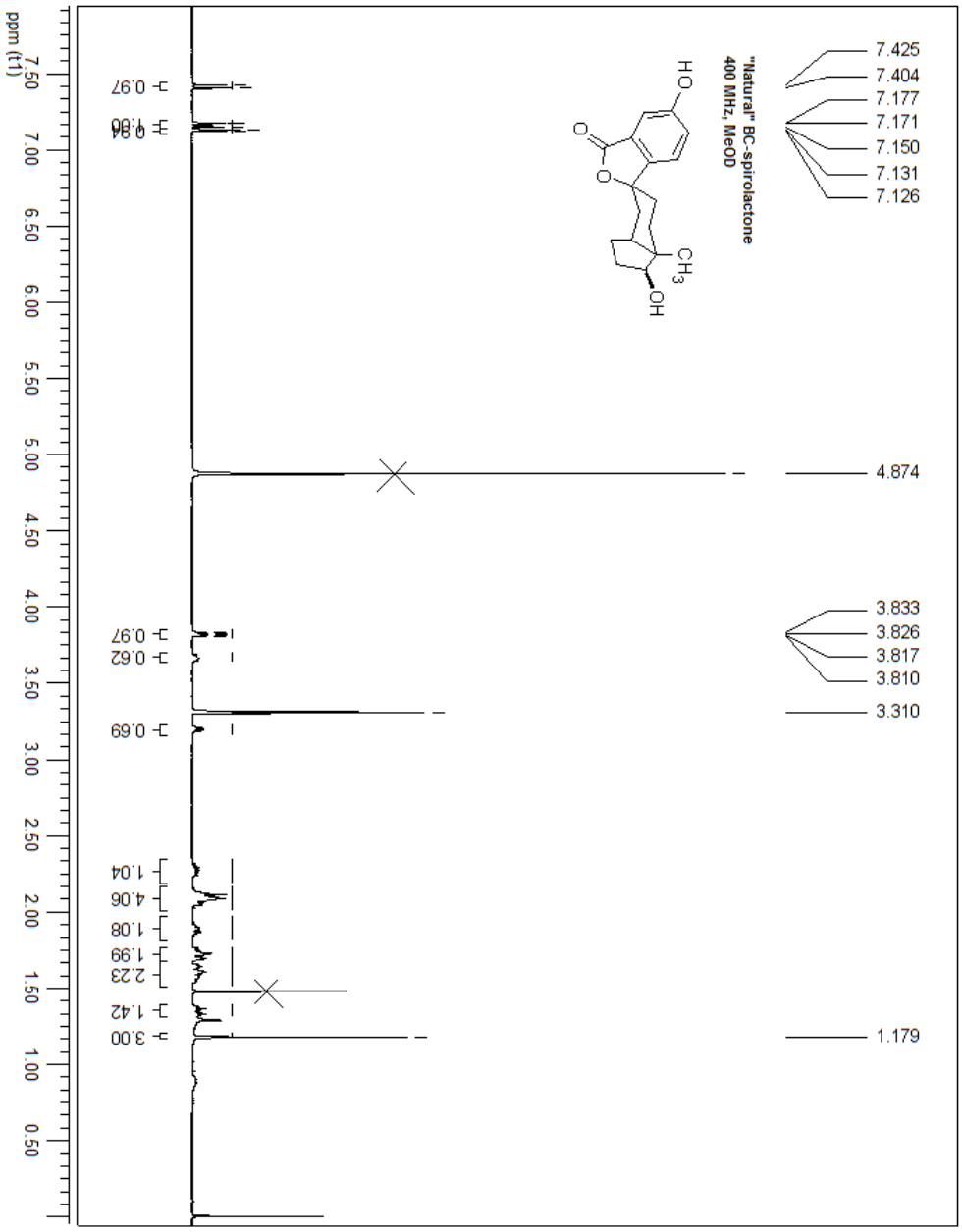

Figure a.86 [1]H NMR spectrum of BC-spirolactone (**176**) in MeOD.

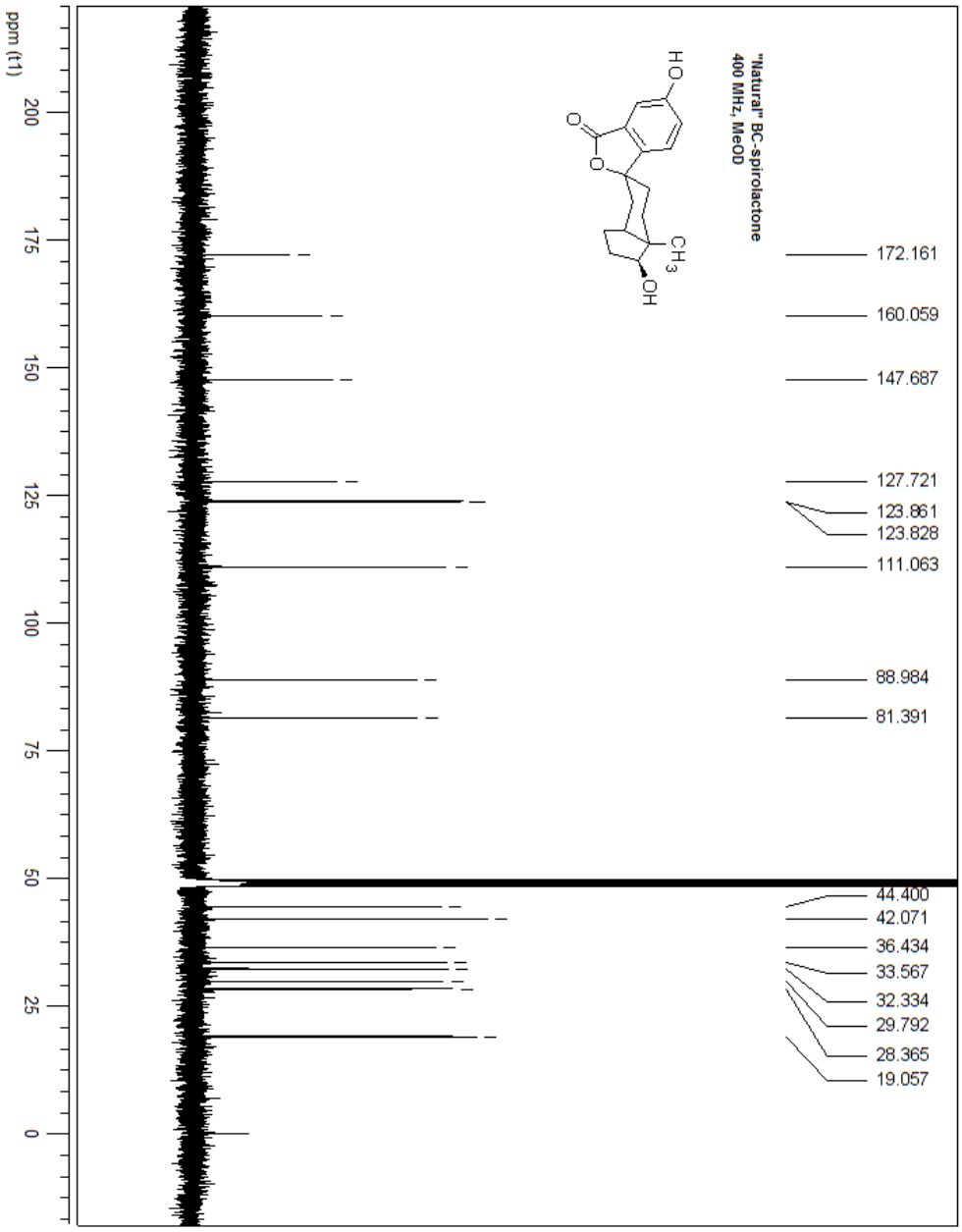

Figure a.87 ^{13}C NMR spectrum of of BC-spirolactone (**176**) in MeOD.

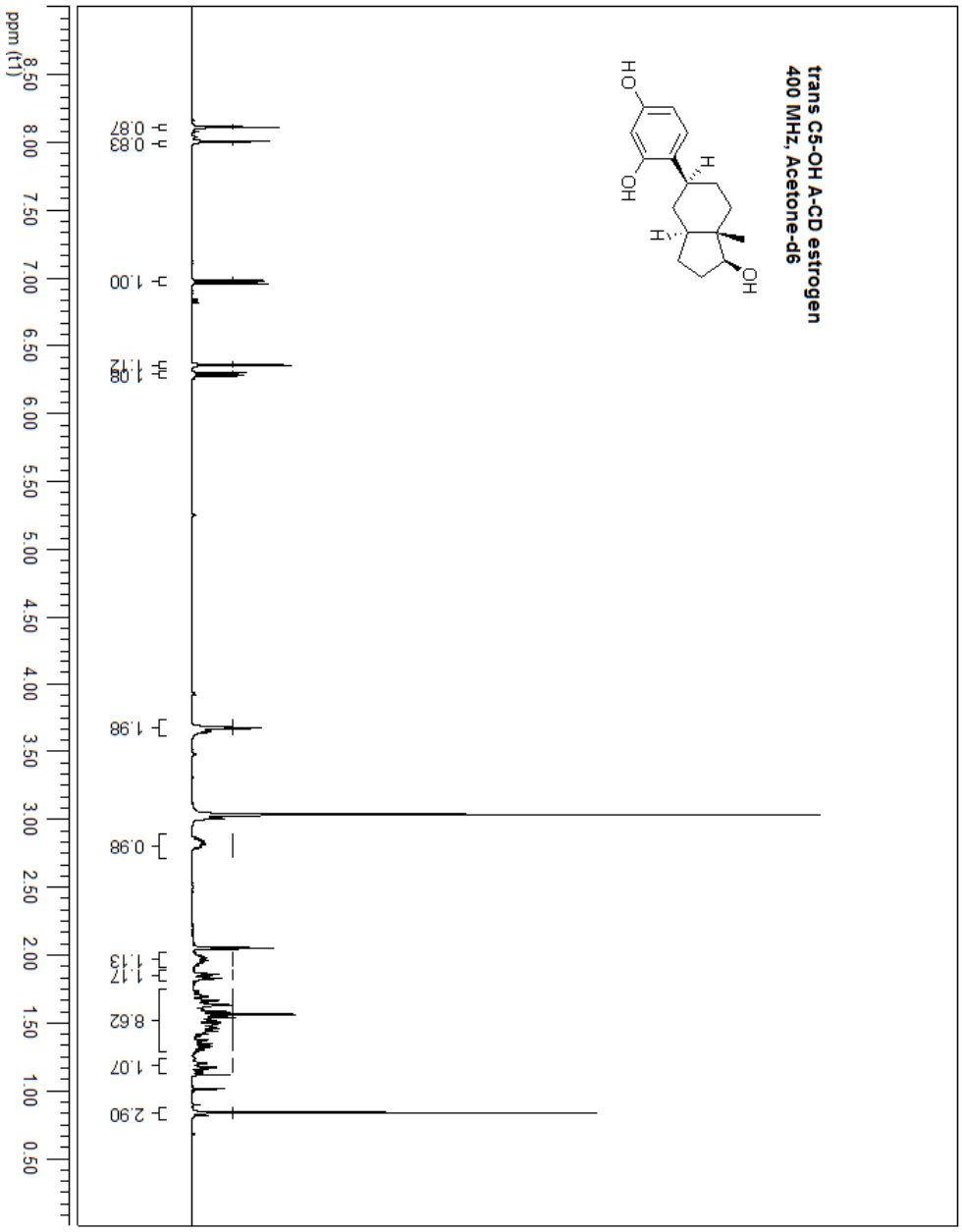

Figure a.88 ^{1}H NMR spectrum of *trans* C5-OH A-CD estrogen (**204**) in Acetone-d_6.

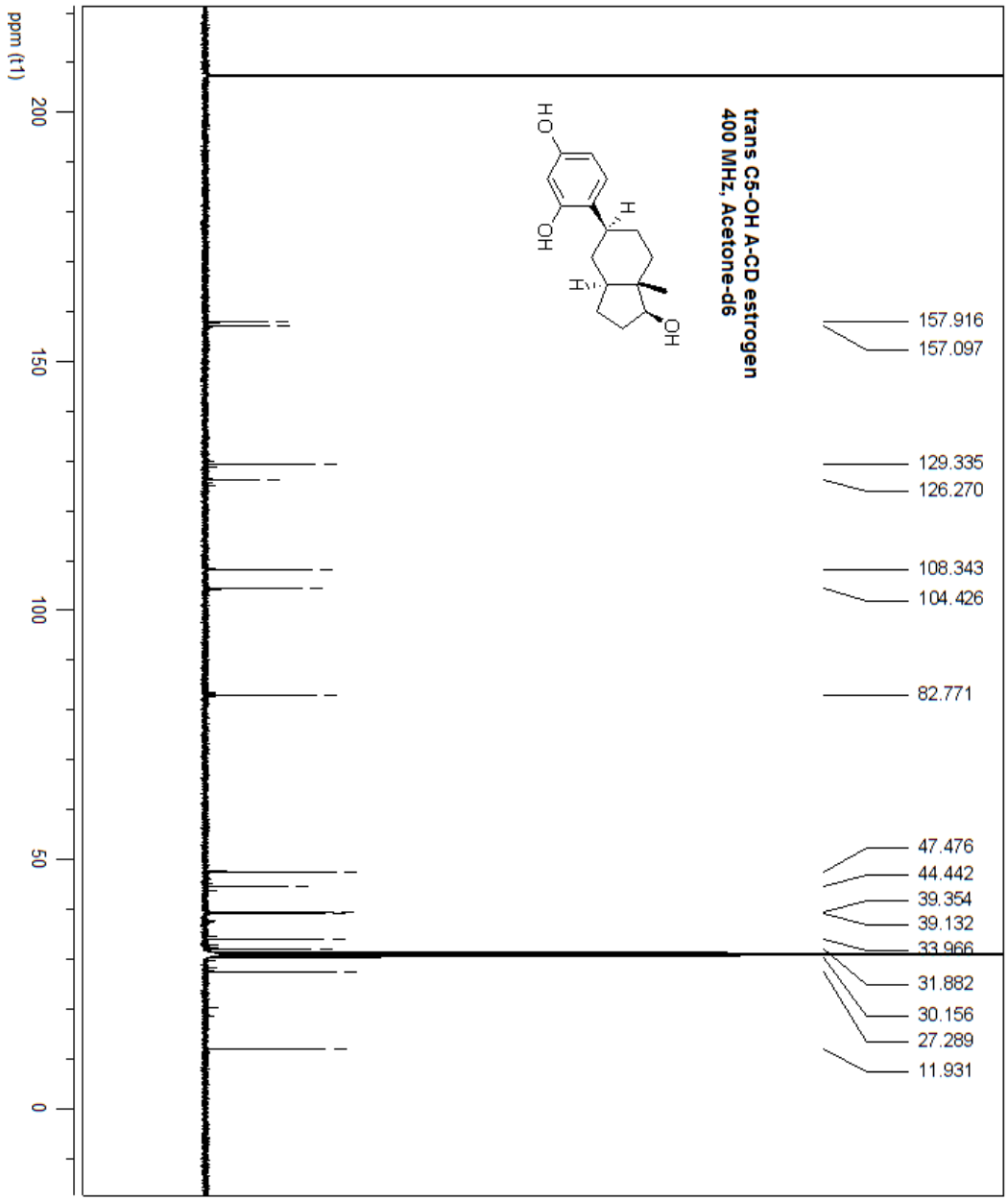

Figure a.89 ^{13}C NMR spectrum of *trans* C5-OH A-CD estrogen (**204**) in Acetone-d_6.

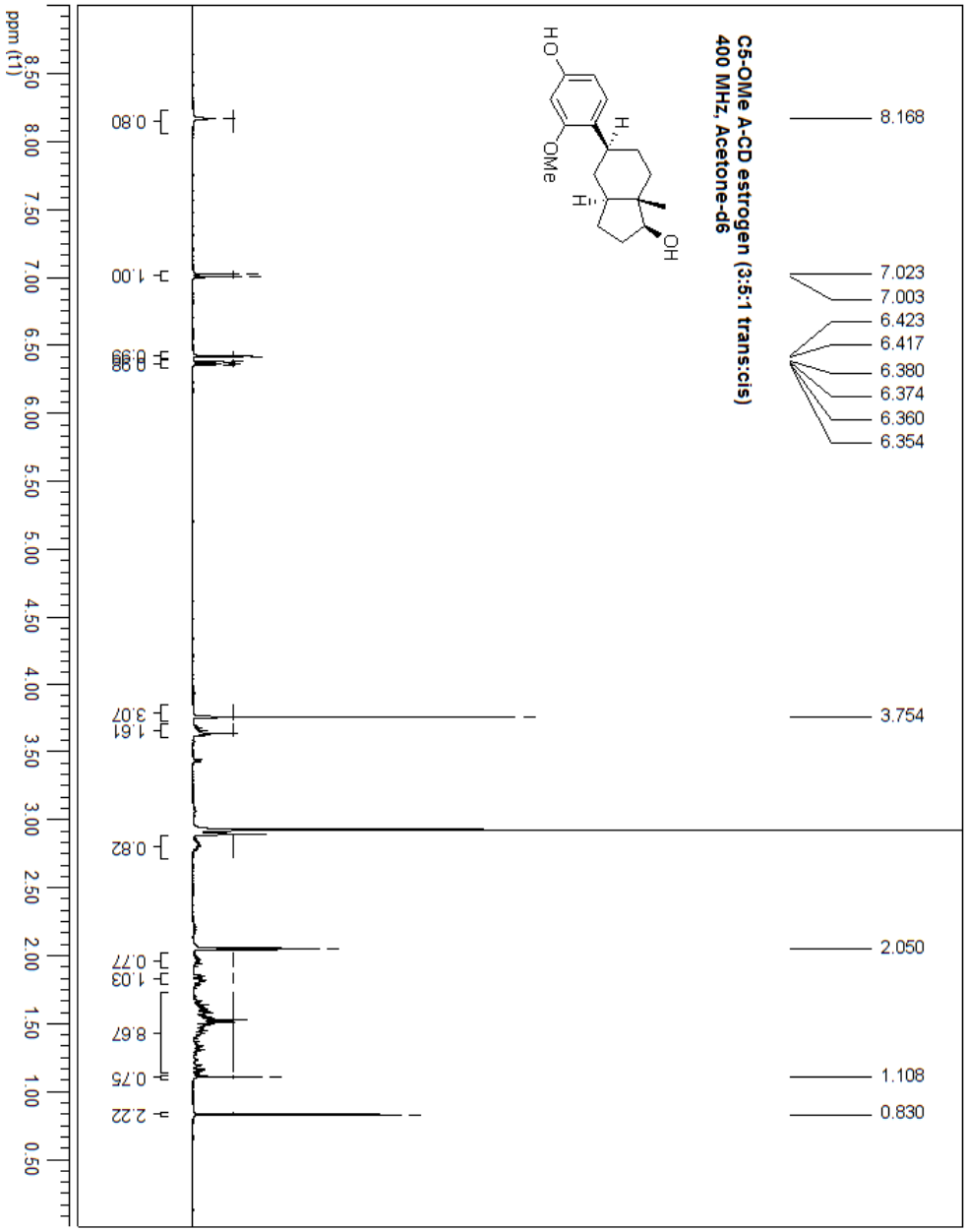

Figure a.90 ^{1}H NMR spectrum of *trans* C5-OMe A-CD estrogen (**205**) in Acetone-d_6.

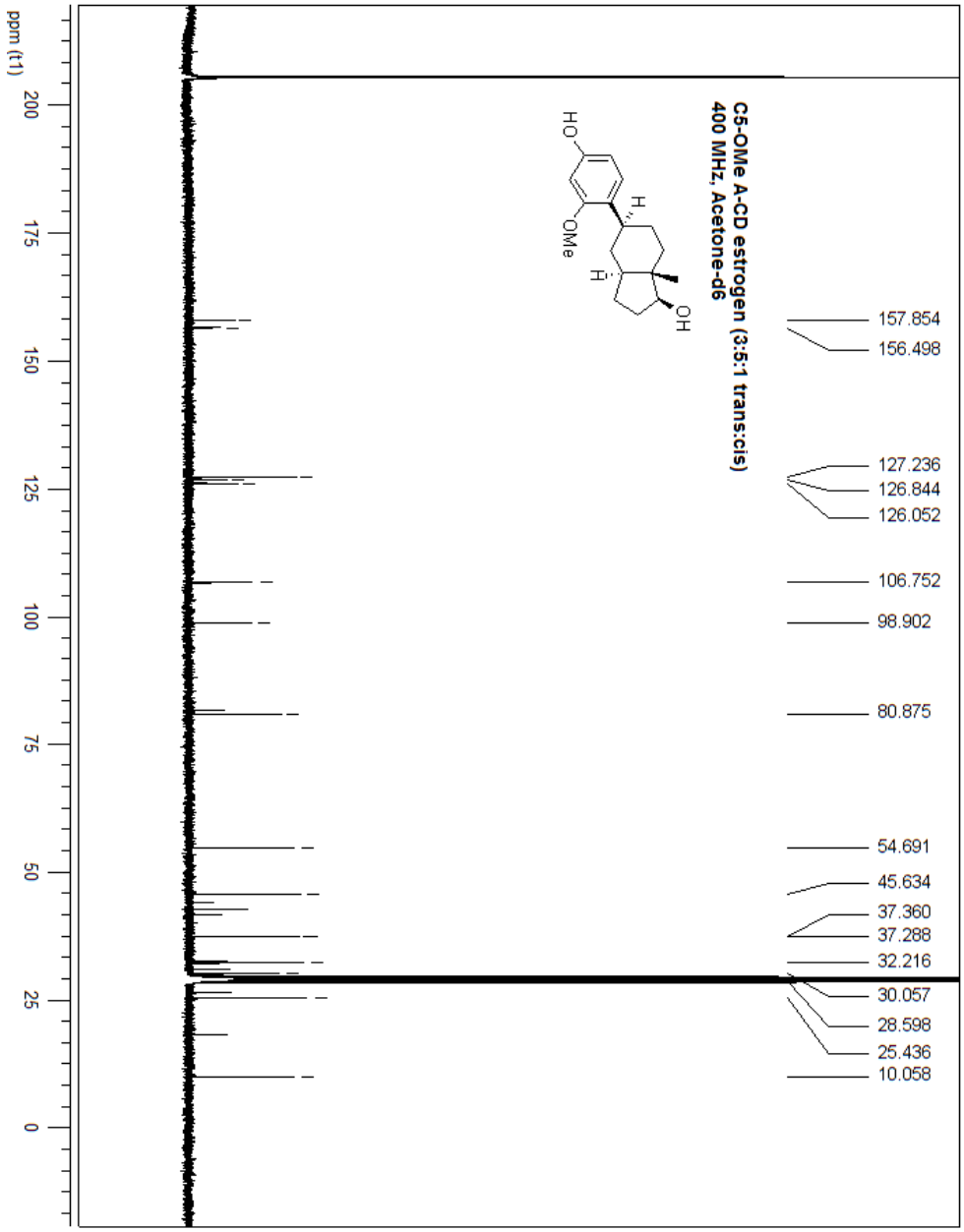

Figure a.91 ^{13}C NMR spectrum of *trans* C5-OMe A-CD estrogen (**205**) in Acetone-d_6.

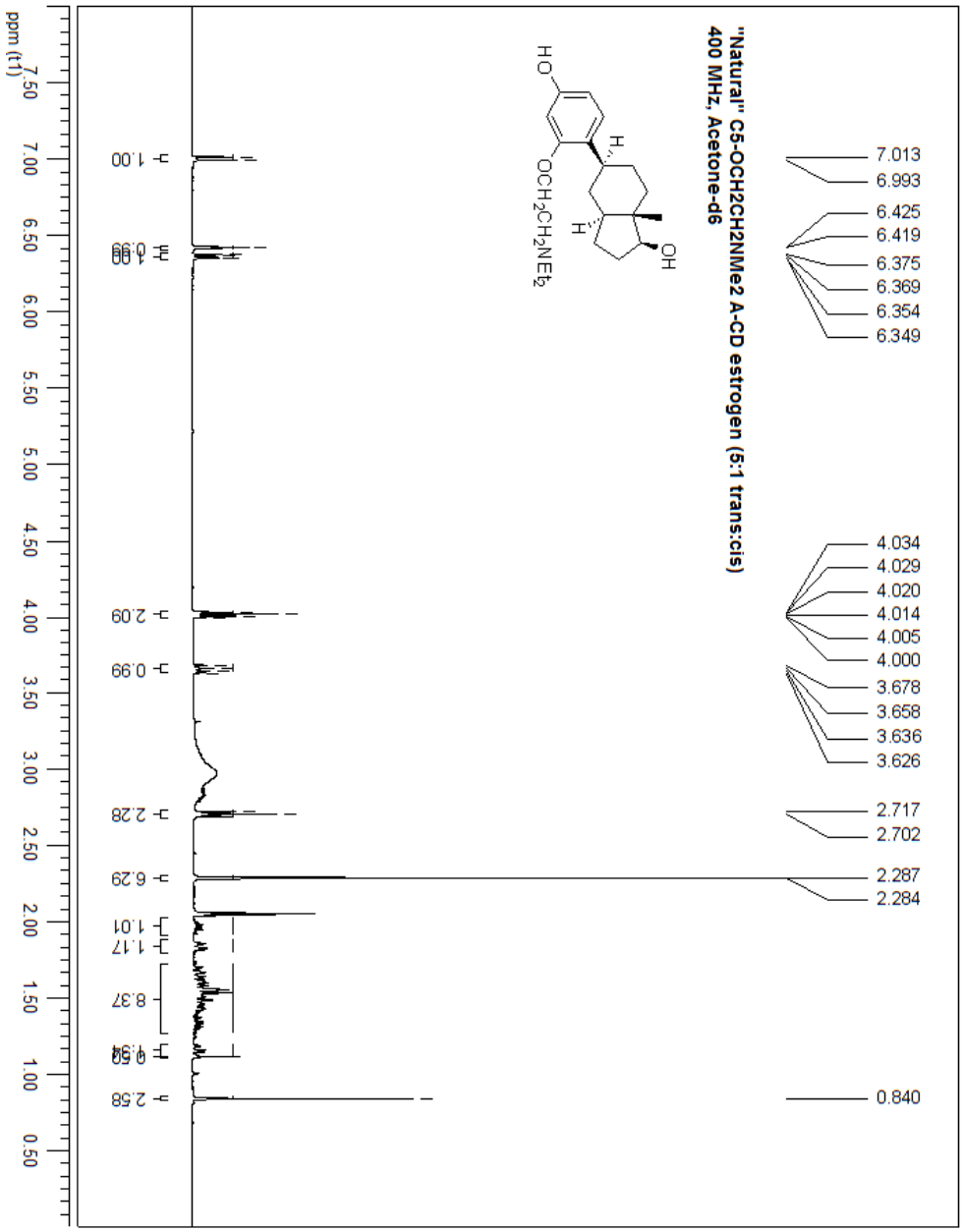

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Figure a.92 ^{1}H NMR spectrum of *trans* C5-$OCH_2CH_2NMe_2$ A-CD estrogen (**206**) in Acetone-d_6.

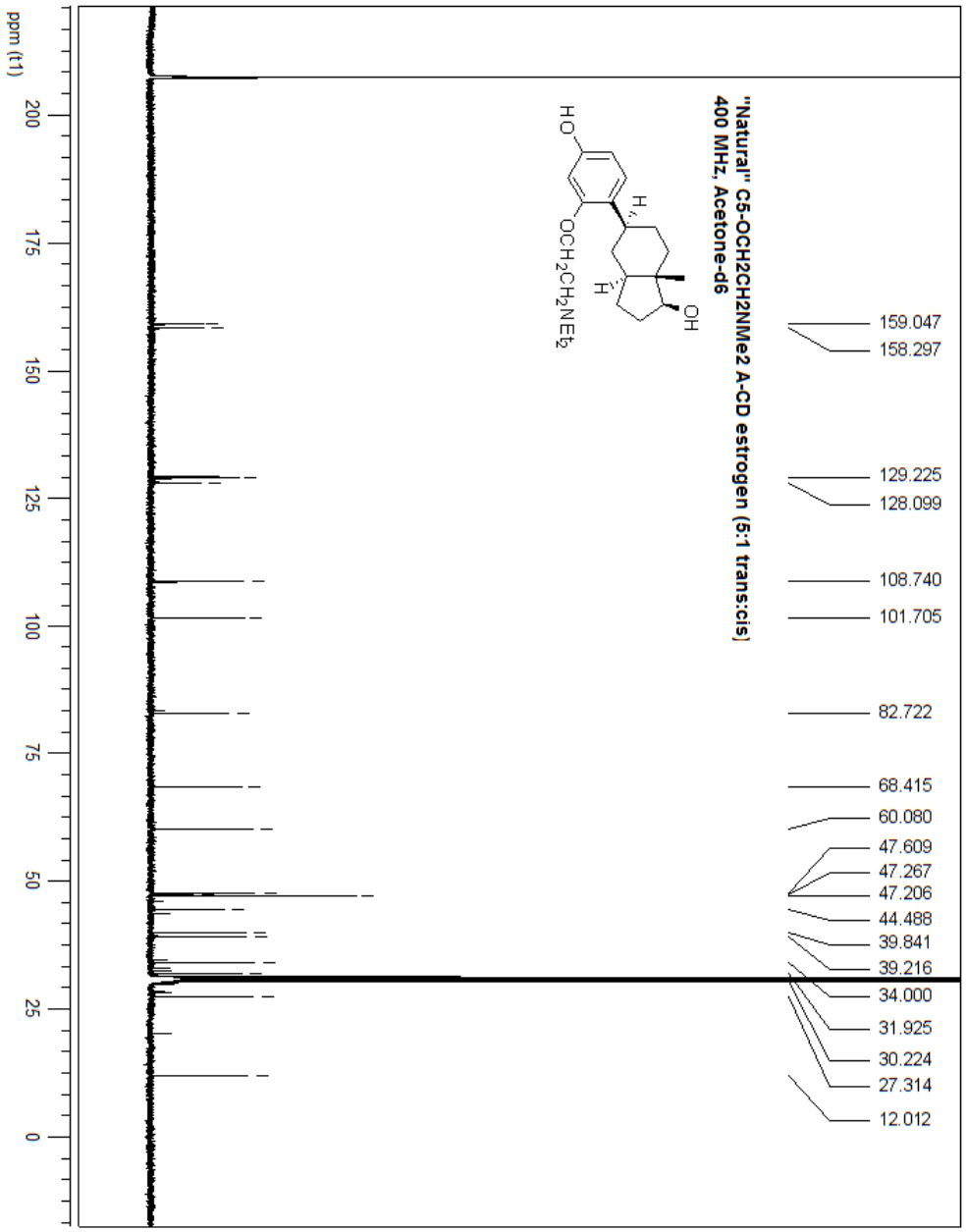

Figure a.93 ^{13}C NMR spectrum of *trans* C5-$OCH_2CH_2NMe_2$ A-CD estrogen (**206**) in Acetone-d_6.

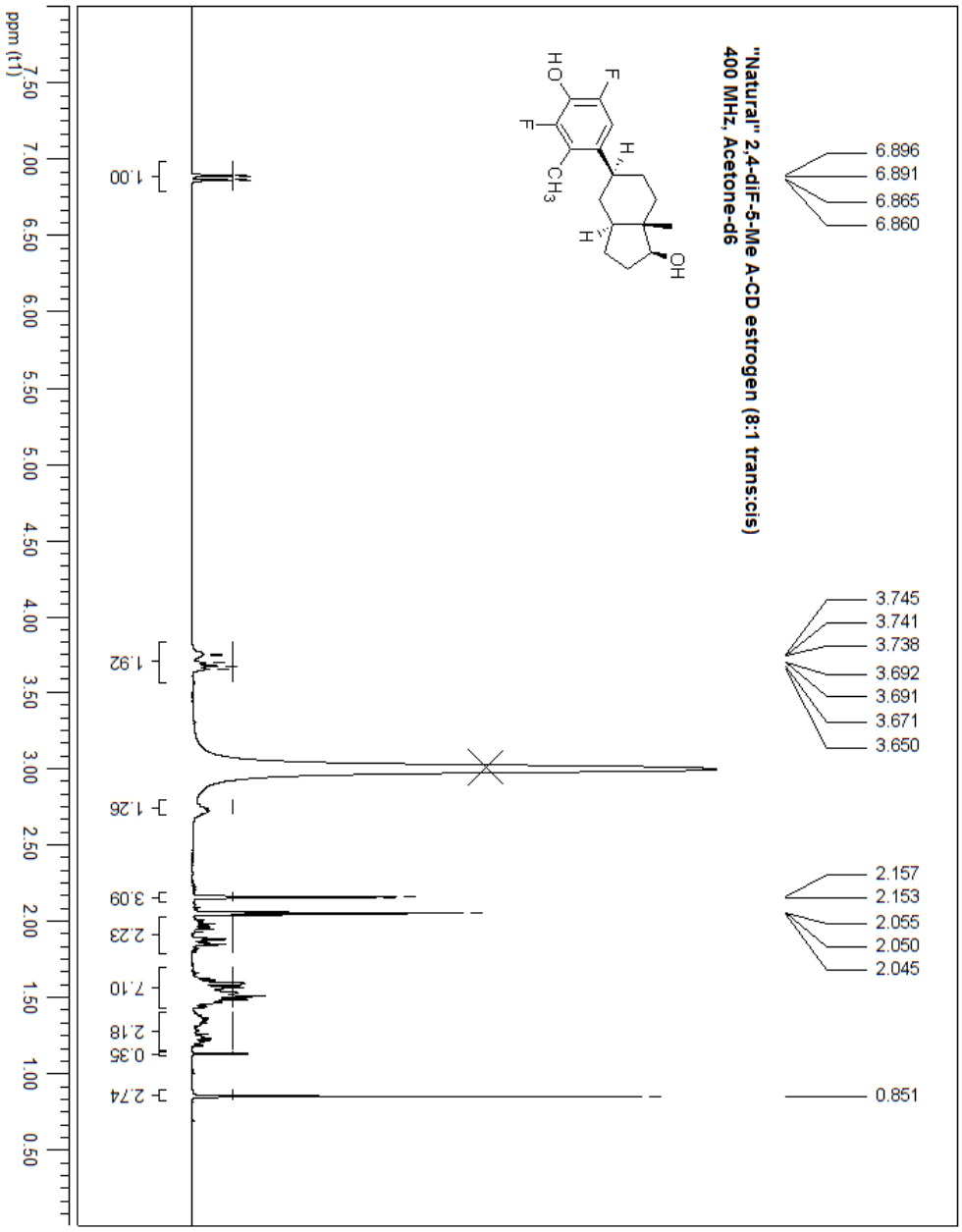

Figure a.94 ^{1}H NMR spectrum of *trans* 2,4-diF-5-Me A-CD estrogen (**207**) in Acetone-d_6.

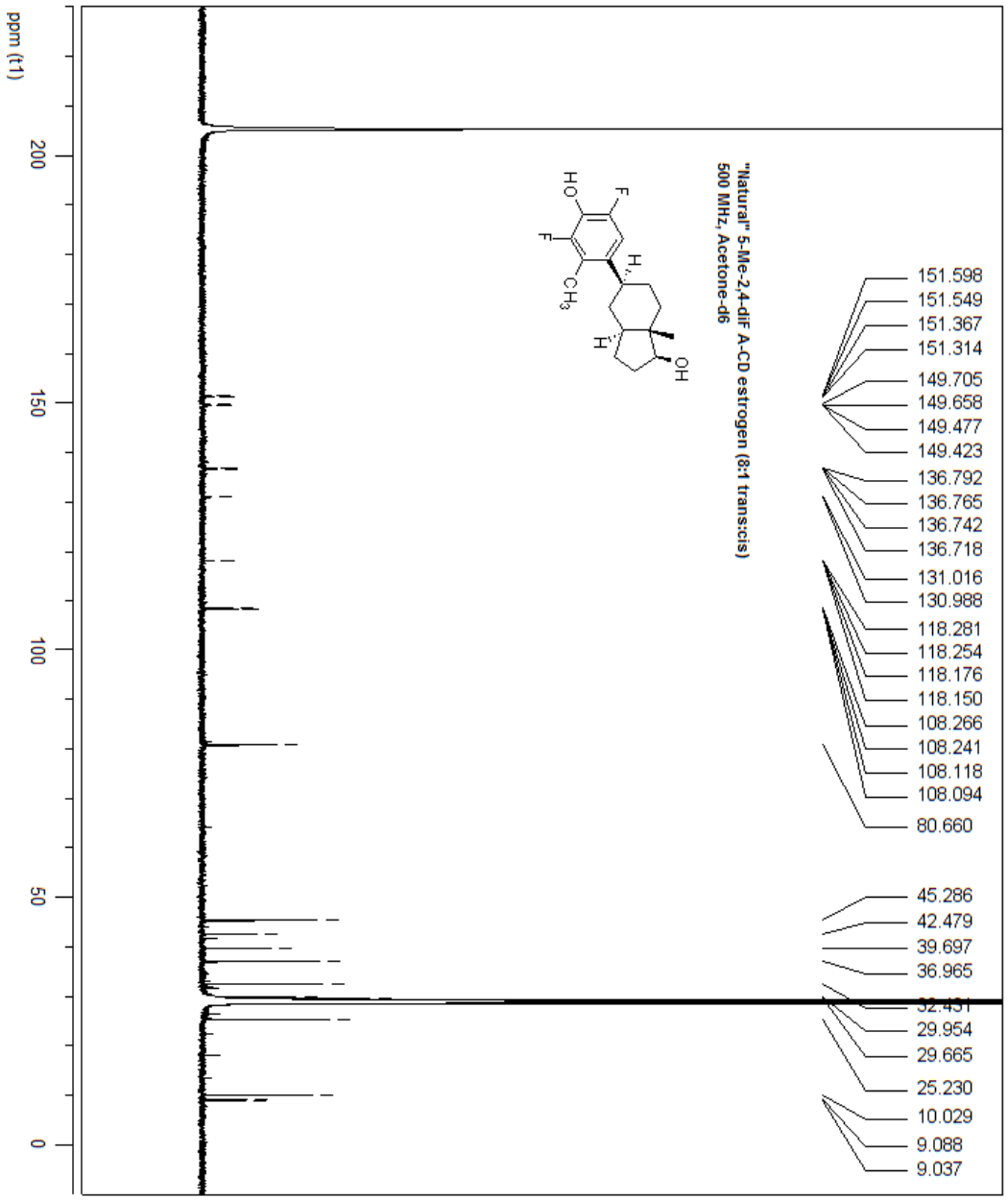

Figure a.95 ^{13}C NMR spectrum of *trans* 2,4-diF-5-Me A-CD estrogen (**207**) in Acetone-d_6.

Claims to original research

1. The synthesis of more than fifty A-CD estrogens with a *cis* CD-ring junction and the evaluation of their RBA and RTA data.

2. The preparation of one example of a BC-spirolactone estrogen with a fixed 90^{o} dihedral angle between rings A and C and the evaluation of the binding affinity of a series of BC-spiro estrogens.

3. The synthesis of four A-CD estrogens with a *trans* CD-ring junction and the comparison of their potency and selectivity to ERβ with the *cis* A-CD estrogens.

4. The preparation of **TD81** and its analog designed to prevent the formation of carcinogenic *ortho*-quinones, and validation of its safety and effectiveness with potential use for the treatment of breast cancer.

5. The preparation of **L17** analog and the evaluation of **L17** and its analog with potential use for hormone replacement therapy.

6. The isolation and identification of five sesquiterpene lactones from the leaves of *Neurolaena lobata* (Asteraceae) and evaluation of their anti-inflammatory effects.

7. The isolation and characterization of four complex polycyclic diterpenoids from the bark and the leaves of *Leretia cordata* (Icacinaceae) with anti-cancer properties.

Publications

(1) Wright, J. S.; Shadnia, H.; Anderson, J. M.; Durst, T.; Asim, M.; El-Salfiti, M.; Choueiri, C.; Pratt, M. A. C.; Ruddy, S. C.; Lau, R.; Carlson, K. E.; Katzenellenbogen, J. A.; O'Brien, P. J.; Wan, L. *Journal of medicinal chemistry* **2011**, *54*, 433–48.

(2) Walshe-Roussel, B.; Choueiri, C.; Saleem, A.; Asim, M.; Caal, F.; Cal, V.; Rojas, M. O.; Pesek, T.; Durst, T.; Arnason, J. T. *Phytochemistry* **2013**, *92*, 122–7.

(3) Asim, M.; El Salfiti, M.; Qian, Y.; Choueiri, C.; Salari, S.; Cheng, J.; Shadnia, H.; Bal, M.; Christine Pratt, M. A.; Carlson, K. E.; Katzenellenbogen, J. A.; Wright, J. S.; Durst, T. *Bioorganic & medicinal chemistry letters* **2009**, *19*, 1250–1253.

(4) Asim, M.; Klonowska, D.; Choueiri, C.; Korobkov, I.; Carlson, K. E.; Katzenellenbogen, J. A.; Durst, T. *Bioorganic & medicinal chemistry letters* **2012**, *22*, 3713–7.