Synthesis of Inhibitors of Steroid Sulfatase and Towards the Synthesis of a Chiral Electrophilic Fluorinating Reagent

By

Yong Liu

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Abstract

Steroid sulfatase (STS) catalyzes the desulfation of sulfated steroids such as estrone sulfate to the corresponding steroid such as estrone. Inhibitors of STS are believed to have potential for treating estrogen-dependent breast cancer.

A new class of potential irreversible suicide inhibitors of STS, based on aryl sulfates bearing a monofluoromethyl or difluoromethyl group ortho to the sulfate group, was synthesized. Key to the success of these syntheses was the use of new sulfation methodology recently developed in the Taylor group. A new and efficient route to 4-formyl estrone, a time-dependent, irreversible STS inhibitor, is also reported.

Several new classes of potential, reversible STS inhibitors were synthesized. These compounds are analogs of known STS substrates in which the sulfate group is replaced with an α,α-difluoromethylenesulfonamide group, a boronic acid group or a sulfinic acid group. We also report the synthesis of estrone sulfate analogs that bear a carboxylate moiety at the 17-position and a sulfate surrogate at the 3-position. It is anticipated that these compounds will inhibit STS by interacting with Arg98 which lies at the periphery of the active site. Key to the success of this synthesis was the use of the t-butyl group as a protecting group for the 2-position of estrone.

Finally, our preliminary investigations into the synthesis of a new class of chiral electrophilic fluorinating agents are presented. These reagents are based on a chiral binaphthyl sulfonimide scaffold and are expected to be capable of performing enantioselective electrophilic fluorinations. Such reagents may be useful in synthesizing organofluorines of biological significance including STS inhibitors.

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List of Abbreviations

2-FE1	2-formylestrone
4-FE1	4-formylestrone
Ac	acetyl
AIBN	2,2′-azo-bis(isobutyronitrile)
All	allyl
aq	aqueous
Ar	aryl
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ARS	aryl sulfatase
ARSA	aryl sulfatase A
BINOL	2,2′-dihydroxy-1,1′-binaphthalene
Bn	benzyl
br	broad
Bu	butyl
CA	cinchona alkaloids
conc	concentrated
cacld	calculated
cat	catalyst
CHO	Chinese Hamster Ovary
compd	compound
COUMATE	coumarin sulfamate
CTH	catalytic transfer hydrogenation
d	doublet
DAST	diethylaminosulfur trifluoride

DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIBAL	diisobutylaluminium hydride
DFMS	difluoromethylenesulfonamide
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMB	2,4-dimethoxybenzyl
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
E^+	electrophile
E1	estrone
E1S	estrone sulfate
E2	estradiol
EAS	electrophilic aromatic substitution
EMATE	estrone sulfamate
eq or equiv	equivalent(s)
ether	diethyl ether
EF	electrophilic fluorination
ESI	electrospray ionization
Et	ethyl
fGly	formyl glycine
Gln	glutamine
F-C	Friedel-Crafts
h	hour
His	histidine
HMPA	hexamethylphosphoric amide
HRMS	high resolution mass spectrometry
HMT	hexamethyltetramine

HTS	high throughput screening
Hz	Hertz
i	iso
IC_{50}	concentration of an inhibitor that is required for 50% inhibition of catalytic activity
IR	infrared spectroscopy
J	spin coupling constant
K_i	equilibrium dissociation constant of the enzyme-inhibitor complex
LRMS	low resolution mass spectrometry
Lys	lysine
m	multiplet
M	molar
*m*CPBA	*meta*-chloroperbenzoic acid
Me	methyl
mesylate	methanesulfonate
min	minute
mL	millilitre
Ms	methanesulfonyl
mmol	millimole
mol	mole
MOM	methoxymethyl
mp	melting point
MUS	4-methylumbelliferyl-6-*O*-sulfate
m/z	mass/charge
N	normal
NaHMDS	sodium hexamethyldisilazane
NBA	*N*-bromoacetamide

NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NFSi	*N*-fluorobenzenesulfonimide
NKR	Newman-Kwart rearrangement
nM	nanomolar
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
Nu	nucleophile
PCC	pyridinium chlorochromate
Ph	phenyl
pM	picomolar
PMB	*para*-methoxybenzyl
Pr	propyl
PTP1B	protein tyrosine phosphatase 1B
PTSA	*para*-toluenesulfonic acid
q	quartet
quint	quintet
R	alkyl group
rt.	room temperature
s	singlet
s	secondary
S_N2	substitution nucleophilic bimolecular
STS	steroid sulfatase
t	triplet
t	tertiary
TBATB	tetrabutylammonium tribromide
TBDMS/TBS	*t*-butyldimethylsilyl

TCE	2,2,2-trichloroethyl
THF	tetrahydrofuran
THP	tetrahydropyranyl
Thr	threonine
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TFAc	trifluoroacetyl
TLC	thin-layer chromatography
TMEDA	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine
TMS	trimethylsilyl
triflate	trifluoromethanesulfonate
XS	excess
αK_i	the K_i for binding to the STS-substrate complex
μM	micromolar

Chapter 1

Inhibitors of Steroid Sulfatase

1.1 Steroid sulfatase and breast cancer

Steroids and steroid analogs have been used as drugs for many years.[1] Perhaps the best known steroidal drug is norethindrone (Figure 1.1), also known as "the pill" a female oral contraceptive. Norethindrone, as well as most other steroid-based drugs used today, are not naturally occurring but instead are synthetic in that they are prepared in a laboratory by organic chemists in pharmaceutical companies. Consequently, new methods for constructing steroids and modifying existing steroids are very important. This thesis deals mainly with the synthesis of analogs of two very important natural human steroids, estrone (also known as E1) and estradiol (also known as E2) (Figure 1.1).

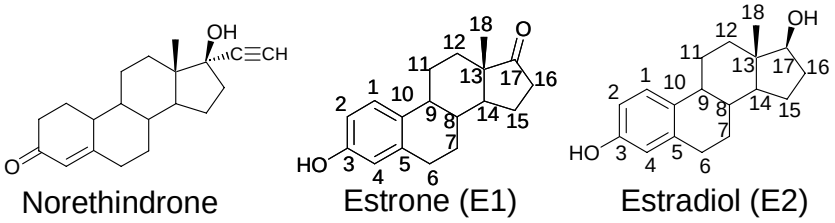
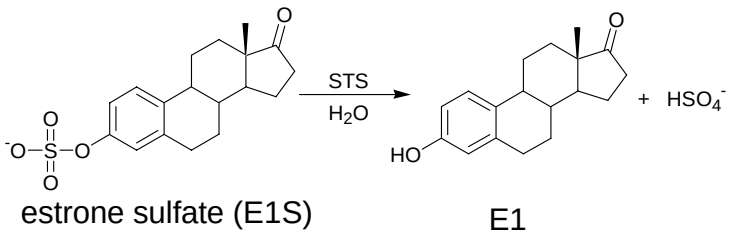


Figure 1.1. Structures of Norethindrone, estrone and estradiol

Our interest in synthesizing E1 and E2 analogs is a result of our interest in developing inhibitors of the enzyme steroid sulfatase (STS).[2] STS catalyzes the hydrolysis of the sulfate group from sulfated steroids, such as estrone sulfate (E1S), to give the corresponding desulfated steroids, such as E1 (Scheme 1.1). The sulfated steroids, such as E1S, are believed to be the storage forms of the corresponding steroids.



Scheme 1.1. A reaction catalyzed by STS

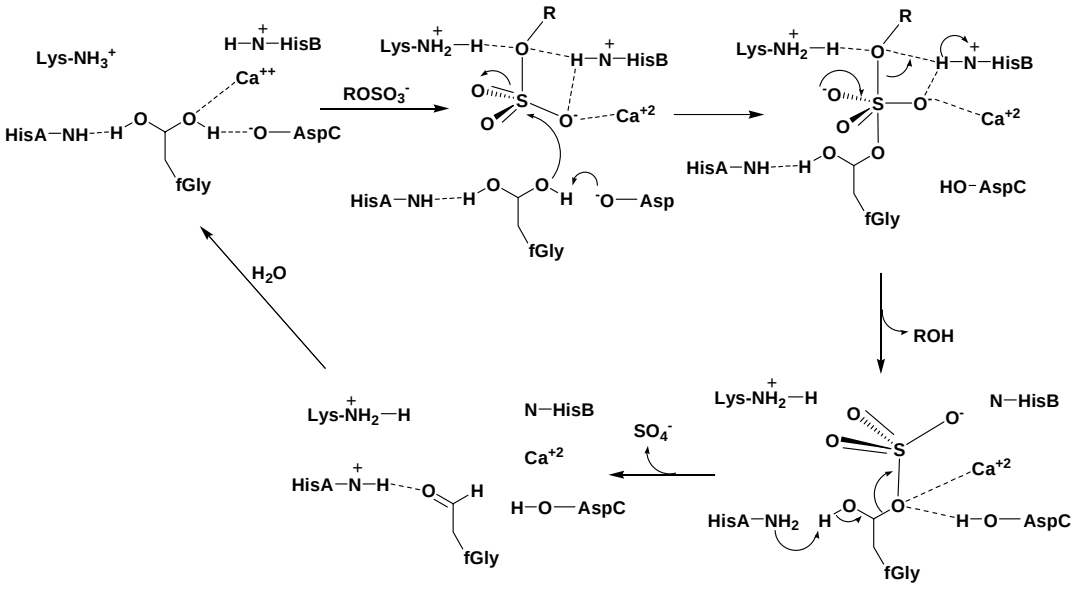
A high proportion (40%) of breast cancers in post-menopausal women are estrogen-dependent. In other words, the cancer cells require estrogens, especially E2, for survival. In the cell, E2 is obtained by reduction of the 17-keto group in E1 by 17β-hydroxysteroid dehydrogenase. E2 then binds to the estrogen receptor on the nuclear membrane where it, along with the receptor itself, becomes encapsulated and brought into the nucleus. Once in the nucleus, this complex (other proteins are believed to be involved) then binds to specific regions of DNA and acts as a transcription factor, controlling the expression of certain genes that are important for cell growth. Not surprisingly, significant STS activity have been detected in the majority of breast tumors.[3] Recently, a study correlated a poor prognosis for estrogen-dependent breast cancer in pre- and post-menopausal patients if a high expression of STS mRNA in their tumors was detected.[3]

Due to the important role of STS in the biosynthesis of E2, considerable interest has arisen in the last decade in developing inhibitors of STS in the expectation that such inhibitors could be used as therapeutics for treating estrogen-dependent breast cancer.[4]

1.2 Steroid sulfatase – structure and mechanism

STS is one of a class of enzymes known as aryl sulfatases (ARS's). Only STS is capable of catalyzing the hydrolysis of sulfated steroids efficiently. Most other ARS's act upon sulfated carbohydrates.[5] All ARS's undergo a unique enzymatic post-translational modification which

converts a cysteine (eukarytotes) or serine (prokaryotes) residue into an active site formyl glycine (fGly). Addition of water to the aldehyde yields a stable formylglycine hydrate.[6,7] The structure of STS[8] and several other ARS's[9] have been elucidated by x-ray crystallography. There is a very high degree of sequence and structural homology at the active site such that their active sites are almost superimposable hence it is believed that all ARS's function by a similar mechanism. Several mechanisms have been proposed for aryl sulfatases.[5] On the basis of crystal structures of another known ARS called aryl sulfatase A (ARSA) as well as on kinetic studies on the wild type and specific mutants of ARSA, a mechanism has been proposed by von Figura and coworkers that is now the most widely accepted mechanism for ARS's (Scheme 1.2).[10,11] One of the hydroxyls of the formylglycine hydrate attacks the sulfur atom of the substrate resulting in cleavage of the S-O bond, release of the hydroxyl or phenolic portion of the substrate and formation of a sulfated hydrate. The sulfate group is then eliminated from the hydrate to give inorganic sulfate and formyl glycine which is then



Scheme 1.2. Proposed mechanism for aryl sulfatases

rehydrated. Several other active site residues, including two conserved histidines, are believed to function as general acids and bases during the reaction.

Dr. Debashis Ghosh, at the Hauptmann-Woodward Institute in Buffalo, New York, reported the crystal structure of STS in 2003.[8] The enzyme is mushroom shaped. It has a hydrophobic domain which is comprised of two hydrophobic α-helices which make up the stem of the mushroom and are believed to anchor STS into the membrane of the endoplasmic reticulum (Figure 1.2).

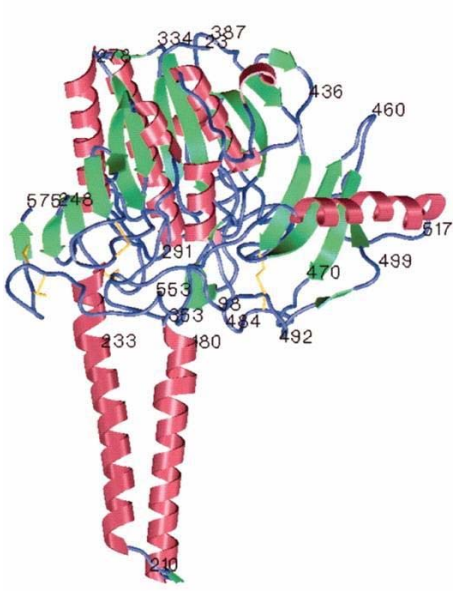


Figure 1.2. Ribbon structure of STS (courtesy of D. Ghosh)

The active site is buried in a polar globular domain (the head of the mushroom) that lies close to the lumenal side of the membrane (Figure 1.3). The fGly hydrate is residue 75. STS crystallized with the fGly hydrate sulfated. Whether this is the resting state of the enzyme in solution or if it is an artifact of the crystallization process due to the presence of sulfate in the crystallization buffer is still unknown. The active site contains a Ca^{2+} ion which is required for catalytic activity.[12] The side chains of Asp35, Asp136, Asp342, Gln343 and the sulfated fGly hydrate act as ligands to the Ca^{2+}

atom (Figure 1.3). Lys134, Lys368 and Arg79 are also believed to interact with the carboxylic side chains of the Asp residues. The amino groups of Lys134 and Lys368 side chains are also believed to interact, possible by salt bridges, with two of the sulfate oxygen atoms of the sulfated fGly hydrate. The imidazole of His136 is involved in a hydrogen-bond with the hydroxyl group of the hydrate while the Nε of His290 is 2.6 Å away from one of the sulfate oxygens of the sulfated hydrate. His136 and His290 are believed to act as general bases and general acids (see scheme 1.2) during the reaction. His346 side chain contacts Lys368 and Thr291 side chains through a bridging water molecule (not shown).

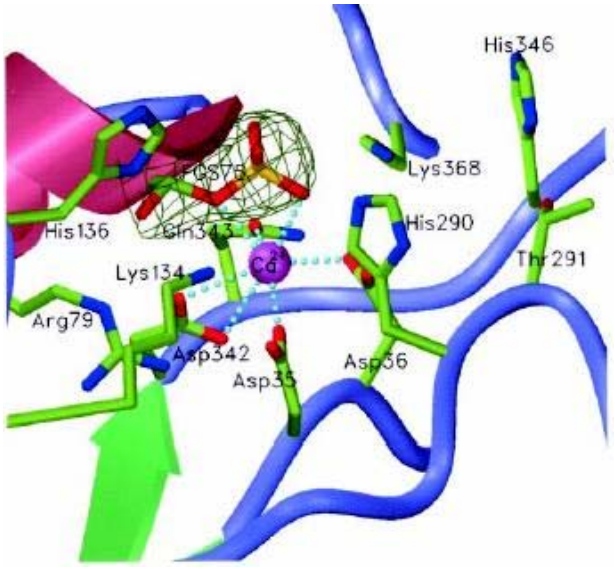


Figure 1.3. Active site of STS. The calcium ion is shown in purple. The sulfur of the sulfated fGly hydrate is in yellow (courtesy of D. Ghosh).

1.3 Inhibitors of STS

In general, STS inhibitors can be divided into two classes: Irreversible aryl sulfamate inhibitors and reversible non-sulfamate inhibitors.[2,4] The former category constitutes the vast majority

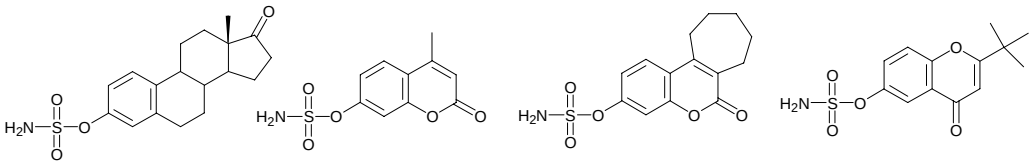


Figure 1.4. Structures of sulfamate inhibitors **1.1**-**1.4**

of STS inhibitors reported to date. Irreversible aryl sulfamate inhibitors are suicide inhibitors of STS in that the S-O bond is first hydrolyzed by the enzyme resulting in the release of the phenolic portion of theinhibitor and sulfamic acid which then irreversibly inhibits STS by a yet unknown mechanism.[2,4] The earliest examples of this class is EMATE (**1.1**, Figure 1.4).[13] It has been reported to have a K_i of 670 nM using crude microsomal preparations of STS at an unspecified pH.[13] However, EMATE is estrogenic due to the release of E1 during the inhibition process, and, consequently, cannot be used as a therapeutic.[14] Nevertheless, many studies have successfully addressed the estrogenicity issue and a considerable number of very potent aryl sulfamate-based, nonestrogenic STS inhibitors have been developed such as coumarins **1.2** (also known as COUMATE)[15] and **1.3** (also known as 667-COUMATE),[16] and chromenone **1.4**,[17] to name but a few.[4] All of these compounds are also very effective STS inhibitors in cellular assays. Although 667-COUMATE has successfully completed phase I clinical trials for treating breast cancer,[18] concerns have been raised about their poor stability in aqueous solution and potential side effects of sulfamate-based drugs when used over the long term.[4]

A variety of reversible, non-sulfamate STS inhibitors have been developed. Only the most relevant and/or potent ones will be discussed here. Early studies focused on replacing the sulfate group of estrone or estradiol with *O*-, *N*-, or *S*-linked sulfate surrogates though compounds of this type have not yet been shown to be potent STS inhibitors.[4] This class of STS inhibitors will be discussed in

greater detail in chapter 3. In addition to steroids bearing sulfate surrogates, other steroidal compounds have been examined as reversible STS inhibitors.[4] A variety of marketed drugs in the form of progestins have been examined for STS inhibition in intact breast tumor cells using labeled E1S.[4] In some instances, decreases in the levels of E1 and E2 were found. However, progestin acts upon a variety of cellular targets and therefore these results must be interpreted with caution. In the majority of these studies, it was never ascertained whether this decrease was due to STS inhibition or a decrease in levels of active STS. Poirier reported that certain 17α-benzyl- substituted estradiol derivatives, such as compounds **1.5** and **1.6** (Figure 1.5), are reversible inhibitors of STS and are the most potent reversible inhibitors to date some with K_i's in the low nM region.[19,20] Compounds that are highly hydrophobic are easily removed from the blood supply by absorption into fatty tissues which can lead to a variety of problems. Thus, the highly hydrophobic nature of these compounds raises serious concerns as to their potential for drug development.

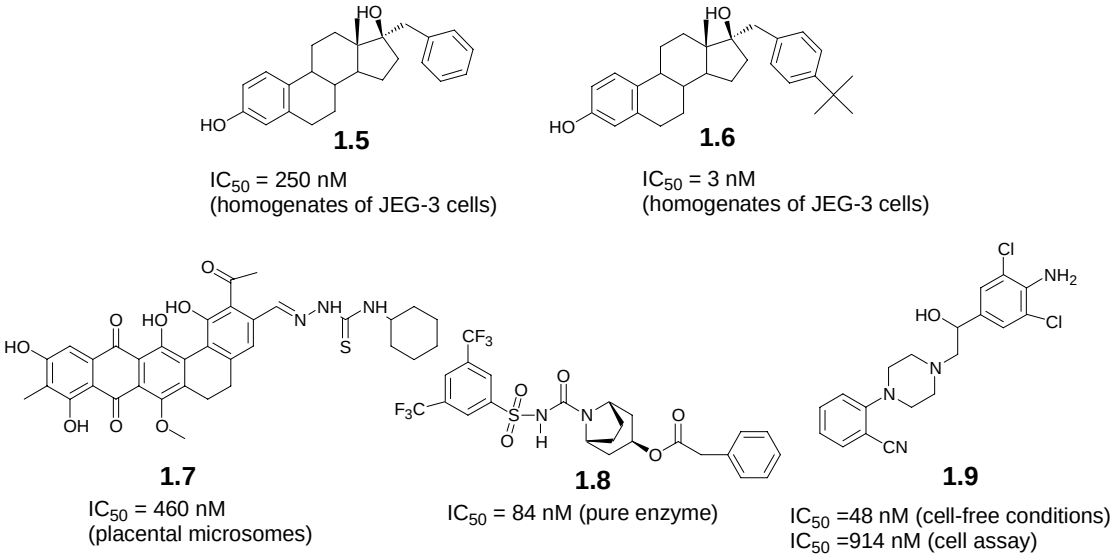


Figure 1.5. Structures of reversible inhibitors **1.5-1.9**

Potent, non-steroidal, non-sulfamate, reversible small molecule inhibitors of STS are very rare. High throughput screening (HTS) of compound libraries has been the most successful approach to this class of compounds to date. By screening a library of derivatives of the natural product madurahydroxylactone, Jutten et al. discovered thiosemicarbazones derivatives, such as compound **1.7** (K_i = 350 nM), were non-competitive STS inhibitors (Figure 1.5).[21] However, the relatively high molecular weight of this class of compounds, as well as the fact that this class of compounds acts upon a wide range of biological targets suggests that it is unlikely that it will be an effective scaffold on which to construct therapeutically useful STS inhibitors. HTS followed by SAR studies on an initial hit by researchers at Novartis yielded compound **1.8** as a reversible, competitive STS inhibitor with a IC_{50} of 84 nM with pure STS.[22] However, this compound was not very effective when tested in a CHO (Chinese Hamster Ovary) cell line overexpressing STS. HTS by researchers at Bayer yielded compound **1.9** as an STS inhibitor with an IC_{50} of 48 nM under cell-free conditions though only 914 nM in a cellular assay.[23] The modality of inhibition was not reported. No models have been proposed that rationalizes the binding modes of any of the above compounds.

1.4 Objective and overview

In this thesis we present several new avenues to the design of both irreversible and reversible STS inhibitors. These proposed inhibitors are mainly E1 and E2 analogs modified at the 2-, 3-, 4- and 17-positions. Other compounds include bicyclic and tricyclic coumarin and chromenone derivatives which are considered to be analogs of the A, B and/or C rings of the steroid scaffold. The main objective of the work presented in this thesis is to synthesize the proposed inhibitors. Although this thesis is concerned mainly with the synthesis of the proposed inhibitors, the global or long term

objectives are to develop potent STS inhibitors that could be used as therapeutics for treating breast cancer.

In chapter 2 we propose a new class of STS suicide inhibitors based on aryl sulfates which bear a monofluoromethyl or difluoromethyl group ortho to the sulfate group. These compounds were prepared using new sulfation methodology that was recently developed in the Taylor group. Inhibition studies on these compounds, by Vanessa Ahmed, a graduate student in the Taylor group, led to the discovery of an entirely new class of irreversible STS inhibitor and part of the work in this chapter is devoted to devising an efficient route to this new class of STS inhibitors.

In chapter 3, several new classes of potential reversible STS inhibitors are constructed. These compounds are analogs of known STS substrates in which the sulfate group is replaced with hydrolytically stable functional groups that may interact with specific residues in the active site by both reversible covalent and non-covalent interactions. Inhibition studies on these compounds, again by Vanessa Ahmed, allowed us to predict the presence of a second steroid binding site. In collaboration with Dr. Debashis Ghosh, a second steroid binding site was found by obtaining the structure of one of our inhibitors complexed with STS.

The work presented in chapter 4 is not directly related to the stated objective of this thesis, which is to construct inhibitors of STS. Of specific interest in the Taylor group is the synthesis of organofluorines by electrophilic fluorination (EF). These organofluorines are often designed to be inhibitors of therapeutically significant enzymes including STS. In chapter 4 we present our preliminary investigations into the synthesis of a new class of chiral electrophilic fluorinating agent. These new reagents are expected to be capable of performing enantioselective EF's. Such a reagent

may be useful in synthesizing organofluorines of biological significance including STS inhibitors.

1.5 References

1. Fullerton, D. S. "Textbook of Organic, Medicinal and Pharmaceutical Chemistry", Chapter 23, Delgado, J. N.; Remers, W. A. Ed., Lippincott Williams & Wilkins, New York, 1998.
2. For an excellent review of the biology and regulation of STS see: Reed, M. J., Purohit A., Woo, L.W., Newman, S.P., Potter, B.V. *Endocr. Rev.* **2005**, *26*, 171.
3. Miyoshi, Y.; Ando, A.; Hasegawa, S.; Ishitobi, M.; Taguchi, T.; Tamaki, Y. Noguchi, S. *Clin. Cancer Res.* **2003**, *9*, 2288.
4. For an excellent review on STS inhibitors see: Nussbaumer, P.; Billich, A. *Med. Res. Rev.* **2004**, *24*, 529.
5. For an excellent review on aryl sulfatases see: Hanson, S. R.; Best, M. D.; Wong, C. H. *Angew. Chem. Int. Ed.* **2004**, 43, 5736.
6. Schmidt, B.; Selmer, T.; Ingendoh, A.; von Figura, K. *Cell* **1995**, *82*, 271.
7. von Figura, K.; Schmidt, B.; Selmer, T.; Dierks, T. *Bioessays* **1998**, *20*, 505.
8. Hernandez-Guzman, F. G.; Higashiyama, T.; Pangborn, W.; Osawa, Y.; Ghosh, D. *J. Biol. Chem*. **2003**, *78*, 22989.
9. Ghosh, D. *Meth. Enzymol.* **2005**, *400*, 273.
10. Lukatela, G.; Kraub, N.; Theis, K.; Selmer, T.; Gieselmann, V.; von Figura, K.; Saenger, W. *Biochemistry* **1998**, *36*, 3654.
11. von Bulow, R.; Schmidt, B.; Dierks, T.; von Figura, K.; Uson, I. *J. Mol. Biol*. **2001**, *305*, 269.
12. Stevens, R. L.; Fluharty, M. H.; Skokut, H.; Kihara, H. *J. Biol. Chem*. **1975**, *250*, 2495.

13. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508.

14. Elger, W.; Schwarz, S.; Hedden, A.; Reddersen, G.; Schneider, B. *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 395.

15. Woo, L. W.; Howarth, N. M.; Purohit, A.; Hejaz, H. A. M.; Reed, M.; Potter, B.V. L. *J. Med. Chem*. **1998**, *41*, 1068.

16. Woo, L.W.L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. L. *Chem. Biol*. **2000**, *7*, 773.

17. Nussbaumer, P., Lehr, P., Billich, A. *J. Med. Chem.* **2002**, *45*, 4310.

18. Stanway, S. J.; Purohit, A.; Woo, L. W.; Sufi, S.; Vigushin, D.; Ward, R.; Wilson, R. H.; Stanczyk, F. Z.; Dobbs, N.; Kulinskaya, E.; Elliott, M.; Potter, B. V.; Reed, M. J.; Coombes, R. C. *Clin. Cancer Res.* **2006**, *12*, 1585.

19. Poirier, D.; Boivin, R. P. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1891.

20. Boivin, R. P.; Luu-The, V.; Lachance, R.; Labrie, F.; Poirier, D. *J. Med. Chem.* **2000**, *43*, 4465.

21. Jutten, P.; Schumann, W.; Härtl, A.; Heinisch; L., Grafe, U.; Werner, W.; Ulbricht, H. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1339.

22. Nussbaumer, P.; Horvath, A.; Lehr, P.; Wolff, B.; Geyl, D.; Billich, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3673.

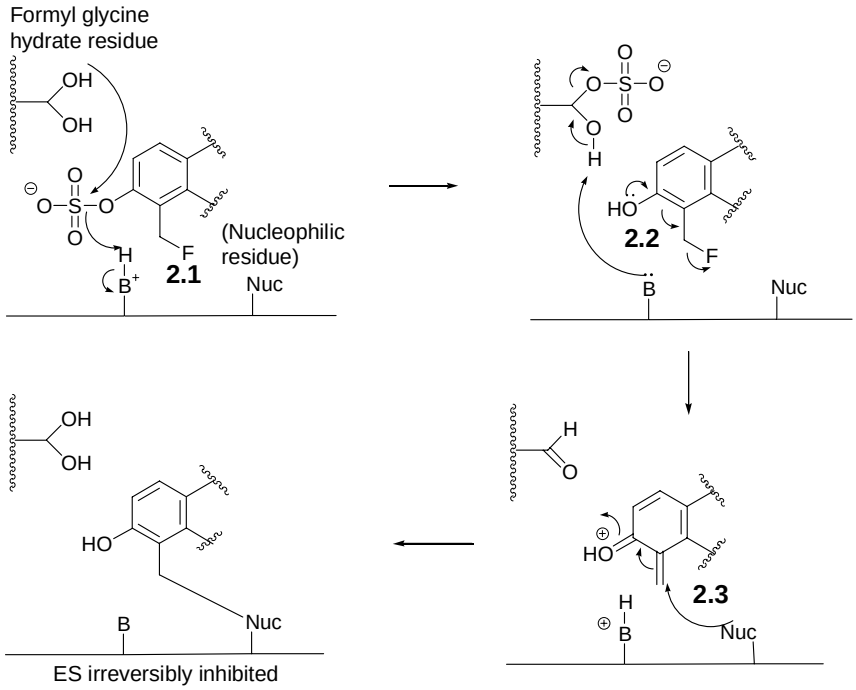
23. Lee, W.; DeRome, M.; DeBear, J.; Noell, S.; Epsatein, D.; Mahle, C.; DeCarr, L.; Woodruff, K.; Huang, Z.; Dumas, J. Abstract of papers, 226th ACS National Meeting, New York, NY, USA, Sept. 7-11, 2003, MEDI-301.

Chapter 2

Irreversible Inhibitors of STS: Synthesis of Estrogens Modified at the 2- and 4-Positions.

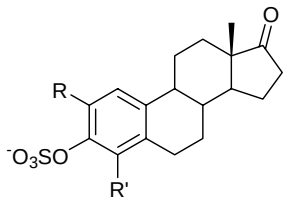
2.1 Introduction

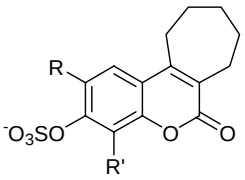
Irreversible inhibitors are often used as probes and tools for studying the biological function of enzymes and even some drugs are irreversible inhibitors.[1] We have already presented one class of irreversible suicide inhibitors of STS in the form of sulfamate-based inhibitors (section 1.3). We also wished to develop irreversible STS inhibitors but using a very different tactic to the sulfamate-based inhibitors. Our general approach is outlined in Scheme 2.1. Compounds of type **2.1**, bearing a mono- or difluoromethyl group ortho to the sulfate group of an aryl sulfate will be prepared (a monofluoromethyl compound is shown in Scheme 2.1 as an example). If these compounds are substrates for STS, the S-O bond will be cleaved forming estrone derivatives of type **2.2**. Compounds of type **2.2** are unstable and undergo elimination of fluoride ion and form quinone methides of type **2.3**. Quinone methides are very unstable and would rapidly react with any nucleophilic residues on STS thus forming a covalent linkage with STS. We knew from the crystal structure of STS that several residues in the active site, such as the formyl glycine hydrate, histidines 136, 296 and 346, lysines 368 and 134, aspartates 35 and 36, and arginine 79 could potentially react with the quinone methide. If this reaction occurs with a residue important for catalysis then activity will be irretrievably lost or diminished. This approach has been used by others for obtaining inhibitors of phosphatases,[2] proteases[3] and glycosidases.[4]



Scheme 2.1. Proposed mechanism for inhibition of STS by compounds of type **2.1**
B stands for amino acid basic residue and Nuc stands for nucleophilic residue

The specific suicide inhibitors that we wished to construct are compounds **2.4-2.11** (Figure 2.1). Four are steroid derivatives and four are coumarin derivatives. We chose to also prepare the coumarin derivatives since potent sulfamate-based STS inhibitors have been prepared based on this scaffold (see section 1.3).





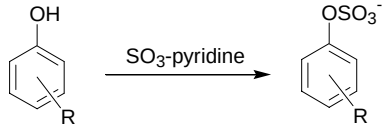
2.4, R = H, R' = CF_2H
2.5, R = CF_2H, R' = H
2.6, R = H, R' = CFH_2
2.7, R = CFH_2, R' = H

2.8, R = H, R' = CF_2H
2.9, R = CF_2H, R' = H
2.10, R = H, R' = CFH_2
2.11, R = CFH_2, R' = H

Figure 2.1. Proposed suicide inhibitors of STS.

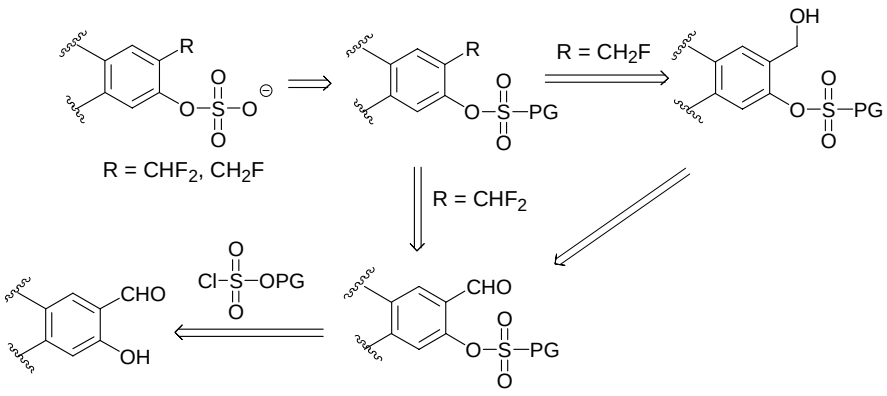
The synthesis of these compounds presented a certain challenge. The most common

approach to the construction of aryl sulfates is to sulfate the phenolic hydroxyl group at the end of the synthesis using sulfur trioxide-amine complexes or chlorosulfuric acid (Scheme 2.2). This step is performed as the last step in the synthesis due to the difficulties in purifying and carrying out further synthetic manipulations with compounds bearing anionic sulfates. However, this approach to aryl



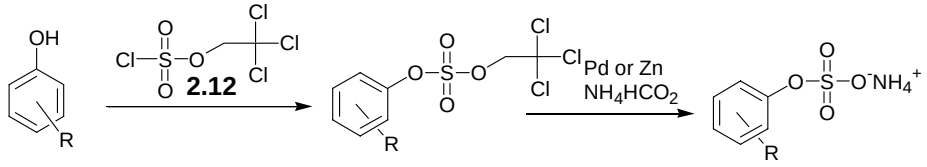
Scheme 2.2. The traditional approach to aryl sulfate synthesis

sulfate synthesis cannot be used for the construction of compounds **2.4-2.11** since ortho mono- or difluoromethylphenols and their derivatives (such as compounds of type **2.2**) are highly unstable. Therefore, a route to the potential suicide inhibitors was envisioned where the sulfate group would be introduced at an early stage in the synthesis as a protected sulfate diester. A retrosynthesis of the suicide inhibitors is outlined in Scheme 2.3. An ester of chlorosulfuric acid is reacted with the hydroxyl group of the ortho-formylated arene (ie. 2- or 4-formyl estrone). Conversion of the formyl group to the mono- or difluoromethyl group followed by removal of the sulfate protecting group would yield the desired product. The main difficulty with this approach was finding a protecting group that is



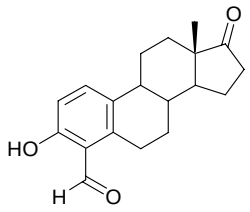
Scheme 2.3. Retrosynthesis of compounds **2.4-2.11**

compatible with aryl sulfate ester chemistry. Acid-labile protecting groups cannot be used due to the well-known instability of aryl sulfate monoesters to acids. Protecting groups removed by hydrogenolysis or photolysis are usually benzyl type moieties and benzylic sulfate diesters are highly unstable. The two protecting groups that have been reported for sulfate esters were developed for alkyl sulfates, namely carbohydrate sulfates, and are removed by base. Perlin and Penney protected carbohydrate sulfates as phenyl sulfate esters. Deprotection was achieved by hydrogenation of the phenyl group to a cyclohexyl group followed by treatment with base.[5.] Proud et al. used the 2,2,2-trifluoroethyl protecting group. Deprotection was achieved using strong base.[6.] These deprotection conditions are incompatible with aryl sulfate esters. The 2,2,2-trichloroethyl (TCE) moiety has been used extensively as a protecting group for carbon and phosphorus acids. It had never been seriously explored as a protecting group for sulfuric acid monoesters probably because it is usually removed using Zn/HOAc, which is not compatible with aryl sulfate monoesters. Nevertheless, Scott Ruttgaizer, a graduate student in the Taylor group found that TCE-protected aryl sulfates can be prepared in high yield by reacting phenolic derivatives with reagent **2.12** (Scheme 2.4) in the presence of triethylamine and DMAP in THF at rt. Most importantly, the TCE group was easily removed under almost neutral conditions using Pd/C or zinc in the presence of HCO_2NH_4 in MeOH. This represents the first protecting group ever developed for aryl sulfates. Although this was a potential route to the



Scheme 2.4. Synthesis of aryl sulfates using reagent **2.12**

synthesis of compounds **2.4-2.11**, it had only been used for preparing very simple aryl sulfates such as phenyl sulfate. This route had not yet been examined as a viable route to more complex sulfate esters such as **2.4-2.11**. The original objective of our work on the synthesis of STS suicide inhibitors was to develop straightforward syntheses of compounds **2.4-2.11** using the TCE protecting group strategy. As we will see, inhibition studies on these compounds led to the discovery of a new irreversible STS inhibitor in the form of 4-formyl estrone (**2.13**, Figure 2.2). 4-Formyl estrone is a known compound. However, its synthesis, as well as the synthesis of other estrone or estradiol derivatives modified at the 4-position, are challenging compounds to prepare. Consequently, an additional objective of the work presented in this chapter is to develop a method for synthesizing 4-formyl estrone and its derivatives.



2.13

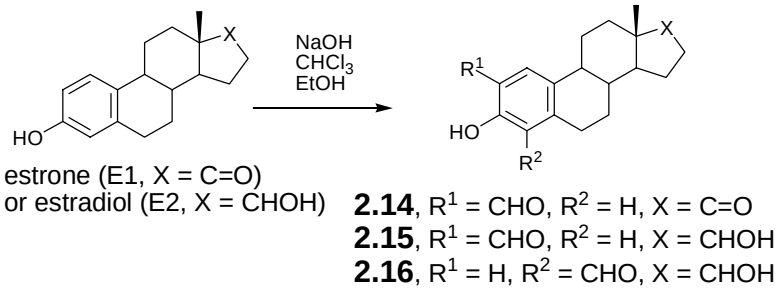
Figure 2.2. Structure of 4-formylestrone

2.2 Results and discussion

2.2.1 Synthesis of 2-formyl estrone

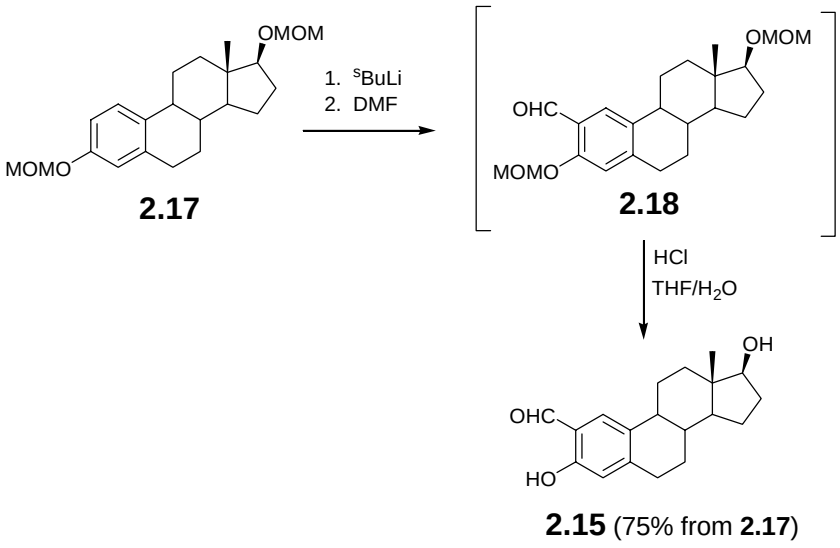
It can be seen from Scheme 2.3 that the synthesis of steroid derivatives **2.4-2.7** begins with the construction of 2-formyl estrone (**2.14**) and 4-formyl estrone (**2.13**). Steroid **2.14** as well as 2-formyl estradiol (**2.15**) were first reported in the patent literature by researchers at Organon.[7] Organon reported the preparation of these two steroids as well as **2.14** and 4-formyl estradiol (**2.16**) by reacting E1 or E2 with 1.5 M NaOH, $CHCl_3$ and EtOH (Reimer-Tiemann reaction, Scheme 2.5). The

reaction gave mixtures of the 2- and 4-isomeric aldehydes. No yields were reported.



Scheme 2.5. Organon's approach to the synthesis of formylated estrogens

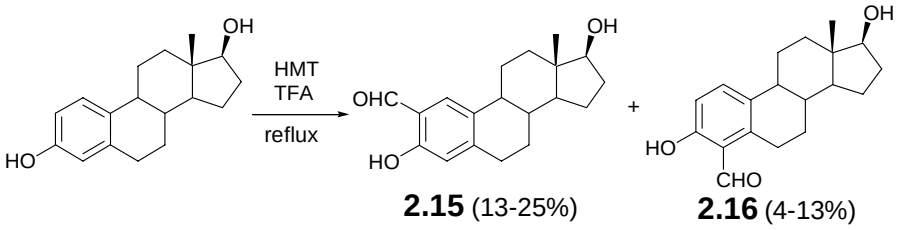
Later, Pert and Ridley reported that they were only able to obtain a 9% yield of **2.13** and **2.14**, using this procedure and E1 as substrate and were unable to obtain any **2.15** and **2.16** using E2 as substrate.[8] These workers attempted many other variations on the Reimer-Tiemann reaction but none were successful. These results prompted Pert and Ridley to examine alternative routes to these compounds. They reported that Vilsmeyer formylation of E1 using DMF/$POCl_3$ was unsuccessful. Another approach involving regiospecific lithiation of bis-MOM-protected E2 (**2.17**) at the 2-position and then reacting the lithiated species with dry DMF was attempted (Scheme 2.6).



Scheme 2.6. Pert and Ridley's synthesis of **2.15**

Although this gave the desired 2-formyl product **2.18** they found the compound to be unstable and could not purify it. However, reaction of crude **2.18** with THF-HCl gave **2.15** in a yield of 75% from the bis ether **2.17**. Later, Lovely et al. made minor modifications to this procedure and succeeded in preparing **2.15** in an overall yield of 83% starting from estradiol.[9] Although this was a potential route to 2-FE1 simply by oxidizing the hydroxyl group at the 17-position, we decided to determine if we could prepare 2-FE1 by direct formylation of estrone.

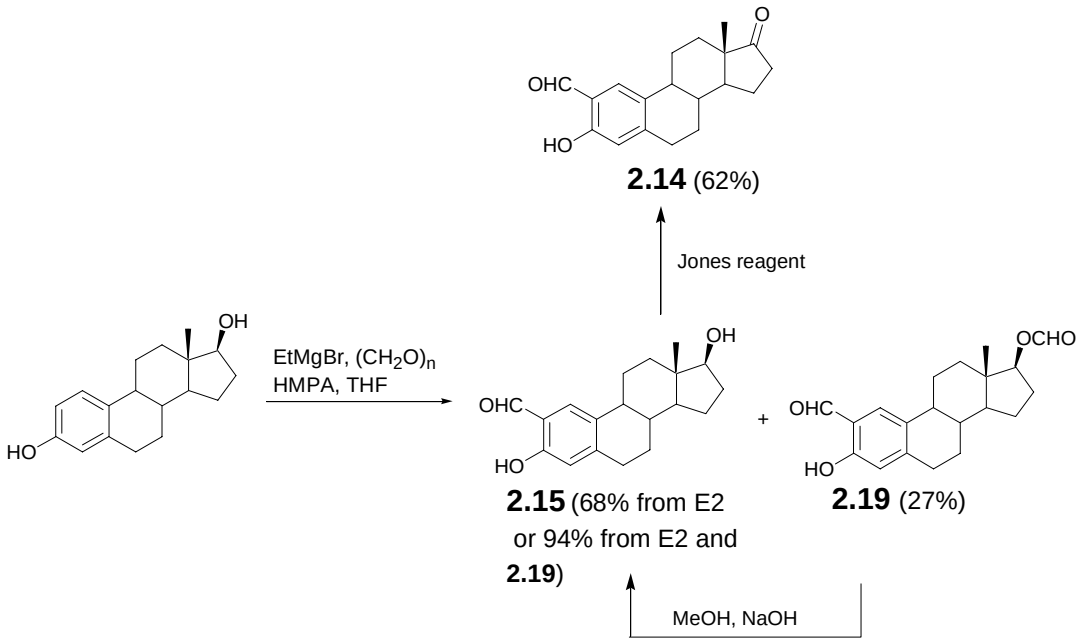
There are several reports in the primary scientific literature on the direct formylation of E2. Most involve subjecting E2 to TFA/HMT (hexamethylenetetramine) giving mixtures of the 2- and 4-isomers. Typical yields range from 13-25% for **2.15** and 4-13% for **2.16** (Scheme 2.7).[10,11]



Scheme 2.7. Synthesis of formylated estradiol using HMT-TFA

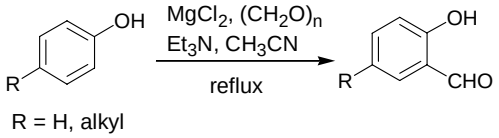
In 1991, Spyriounis et al. reported a one pot synthesis of **2.15** by reacting E2 with ethyl magnesium bromide, paraformaldehyde, triethylamine and HMPA.[12] They reported that this reaction produced a mixture of **2.15** (68%) and 2-formyl-estradiol-17β-formate (**2.19**, 27%). After basic hydrolysis of **2.19** the total yield of **2.15** was 94%. Peters et al also reported a 94% yield of **2.15** using this procedure.[13] These workers also reported that they were able to selectively oxidize the hydroxyl group at the 17-position using Jones reagent to give **2.14** in a 62% yield from **2.15** or in an overall yield of 58% from E2. We performed this reaction and found that the 4-isomer, **2.16**, was also formed in this reaction. However it could be removed by careful chromatography (very tough

separation) and **2.15** was obtained in a 75% yield. Even with the formation of the 4-isomer in this reaction, this is still a reasonable route to **2.15** and, therefore, to **2.14**. Nevertheless, we believed **2.14** could be prepared by direct formylation of E2.



Scheme 2.8. Spyriounis et al.'s synthesis of **2.15** and Peters et al.'s synthesis of **2.14**

In 1999, Hofsløkken and Skattebøl reported a convenient method for selective ortho formylation of phenols in good yield using 1.5 equiv $MgCl_2$, 6.5 equiv paraformaldehyde and 3.75 equiv triethylamine in refluxing acetonitrile or THF (Scheme 2.9).[14]

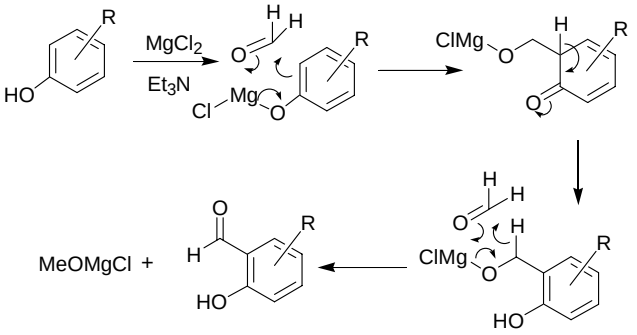


Scheme 2.9. Hofsløkken and Skattebøl's approach to the ortho-formylation of phenols

The proposed mechanism of this reaction is shown in Scheme 2.10. At least two equivalents of paraformaldehyde are required. The good ortho selectivity was attributed to the formation of 6-member ring complex. Paraformaldehyde acts as both electrophile (first step) and oxidant (last

step).

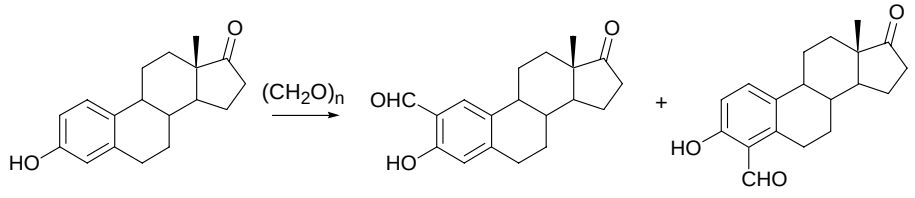
We applied this procedure to the formylation of E1 (1 mmol) using 2.6 equiv of $MgCl_2$, 7.0 equiv of triethylamine and 10 equiv of paraformaldehyde in acetonitrile at 80 ^oC (oil bath) for 15 h. To our delight, a mixture of **2.14** and **2.13** was obtained in about 60% yield in a ratio of 5:1. We



Scheme 2.10. Proposed mechanism for the formylation of phenols using $MgCl_2$, Et_3N and paraformaldehyde

attempted to try and increase the yield of the reaction and most importantly, improve upon the **2.14**:**2.13** ratio. Varying the amounts of $MgCl_2$, triethylamine and paraformaldehyde while maintaining the temperature at 80 ^oC, resulted in yields of **2.13** and **2.14** from 60-75%. However, the ratio of **2.14**:**2.13** did not change much from 5:1. Therefore, subsequent studies were performed using the amounts mentioned above. Decreasing the temperature to 42 ^oC and running the reaction for 2 days with or without HMPA (2.3 equiv) made little difference in yield and selectivity (entries 2 and 3 in Table 2.1). Performing the reaction in refluxing CH_3CN resulted in a decrease in yield (49%), however, the ratio of **2.14**:**2.13** changed to 13:1 (entry 4). Performing the reaction in THF at 65 ^oC gave similar results though decreasing the temperature to 40 ^oC using this solvent resulted in a decrease in yield (entries 5 and 6). The more favorable ratio was obtained at high temperature;

Table 2.1. Synthesis of **2.14** by direct formylation of E1



2.14 **2.13**

entry[a]	solvent	base	additive	T (^{o}C), time (h)	Yield/%[b]	Ratio[c]
1	CH_3CN	Et_3N	---	80, 15	60-75	5:1
2	CH_3CN[c]	Et_3N	---	42, 48	70	6:1
3	CH_3CN	Et_3N	HMPA[e]	42, 48	78	5:1
4	CH_3CN	Et_3N	---	125, 18	49	13:1
5	THF	Et_3N	---	65, 24	65	6:1
6	THF	Et_3N	---	42, 48	52	5:1
7	**CH_3CN[d]**	**Bu_3N**	**---**	**125, 18**	**66**	**14:1**
8	CH_3CN[d]	Bu_3N	HMPA[e]	125, 18	66	7:1
9	THF[d]	Bu_3N	---	100, 18	71	10:1
10	toluene	Bu_3N	---	120, 6	0	
11	DMF	Bu_3N	---	60, 6	0	

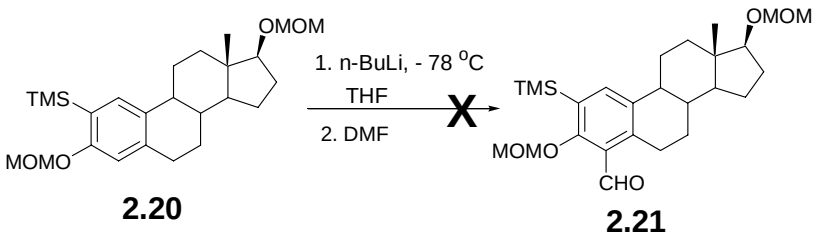
[a]All reactions were performed using 2.5 equiv $MgCl_2$, 10 equiv paraformaldehyde and 3 equiv base. [b]Purified yield (mixture of **2.13** and **2.14**) after column chromatography. [c]Ratio of **2.14** to **2.13** was determined by ^{1}H-NMR after column chromatography. [d]Performed in glass bomb [e]2.3 equiv.

however, the yield was lower. We reasoned that perhaps the yield was decreasing at the higher temperature due breakdown of the paraformaldehyde to volatile formaldehyde which then accumulates as solid paraformaldehyde along the walls of the condenser (a white solid is clearly evident on the walls of the condenser) or even escapes as formaldehyde through the condenser. We also reasoned that the ratio might be improved by increasing the steric bulk of the base since the 4-position of estrone is more sterically hindered. Therefore, we performed the reaction in a sealed glass bomb at

125 ^{o}C in CH_3CN using tributyl- amine (4.6 equiv) as base for 18 h. This gave **2.14** and **2.13** in a respectable yield of 66% in a 14:1 ratio (entry 7). Employing HMPA (2.3 equiv) as additive or by performing the reaction in a bomb using THF at a lower temperature resulted in similar yields but lower selectivity (entries 8 and 9). No reaction took place in toluene or DMF using tributylamine and no bomb (entries 10 and entry 11).

Using the best conditions from Table 2.1 (entry 7) we performed the formylation on 5.4 g (20 mmol) of E1 and obtained a 52% yield of **2.14** after chromatography. We believe that this is the best method to prepare **2.14**. It only requires one step starting from estrone, no toxic additives or expensive reagents are required and it is very easy to scale up and has a simple purification.

The preparation of 4-formyl estrone, **2.13,** is much more challenging than that of **2.14** due to the increased steric hindrance at the 4-position. Pert and Ridley attempted the synthesis of 4-formyl estradiol (**2.16**) by ortho lithiation of TMS derivative **2.20** (Scheme 2.11) followed by the addition of DMF.[8] However, no reaction occurred even when TMEDA was used as co-solvent. Quenching the reaction with D_2O did not result in the incorporation of deuterium. The authors attributed this to the MOM group having a preferred conformation that partially blocks C-4 which prevents lithiation even when using small bases such as MeLi.

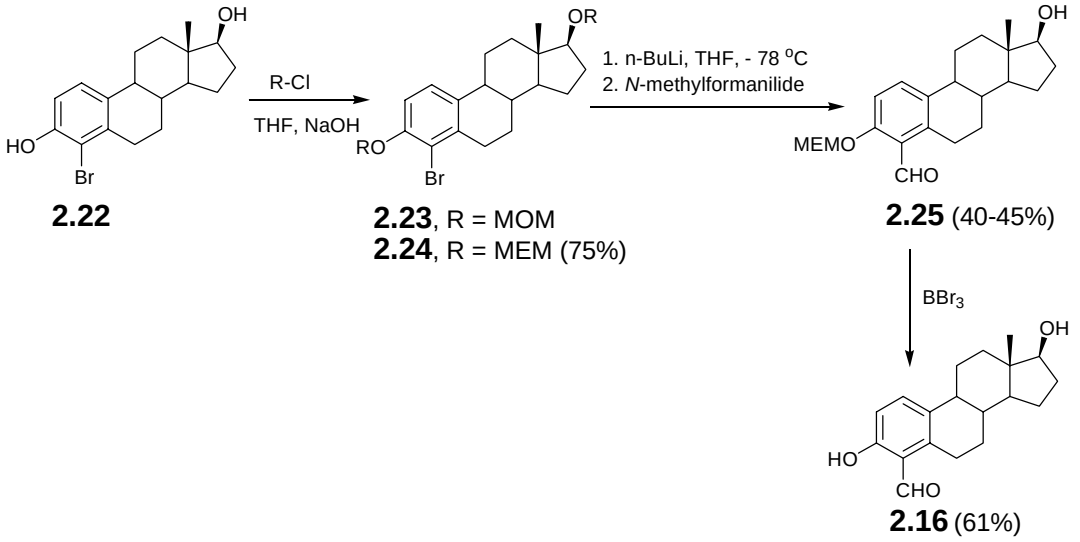


2.20 **2.21**

Scheme 2.11. Attempted ortholithiation and formylation of **2.20** by Pert and Ridley

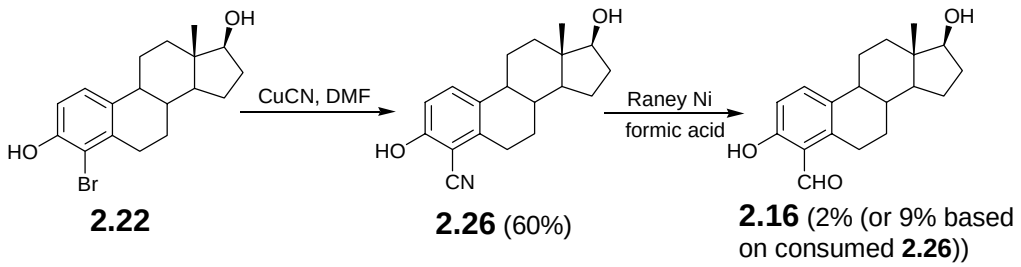
To obtain the desired lithated compound the authors turned their attention to a procedure

employing lithium-halogen exchange as the key step (Scheme 2.12). Compound **2.23** was prepared by reaction of 4-bromoestradiol (**2.22**) with MOMCl. However, the purification of **2.23** was very difficult and only 40-60% yields were obtained. However, the MEM derivative **2.24** was easily obtained in a 70-75% yield using MEMCl. Lithium-bromine exchange with *n*-BuLi followed by reaction with *N*-methylformanilide gave 4-formyl derivative **2.25** from **2.24** in 40-45% yield. Removal of the MEM group using BBr_3 gave **2.16** in 61% yield or in a 19% yield starting from **2.22**. Compound **2.22** was prepared by bromination of E2 though no yield was reported for this step and



Scheme 2.12. Pert and Ridley's synthesis of **2.16**.

so we could not calculate the overall yield of **2.16** from E2. These workers also prepared **2.16** by reacting **2.22** with cuprous cyanide to give cyano compound **2.26** and then reaction of **2.26** with Raney Ni-formic acid (Scheme 2.13). However, the best yield that they were able to obtain for the reduction was only 2% (or 9% based on consumed **2.26**). These workers also report that other common formylation procedures on E2, such as the Vilsmeier reaction, were unsuccessful.



2.22 **2.26** (60%) **2.16** (2% (or 9% based on consumed **2.26**))

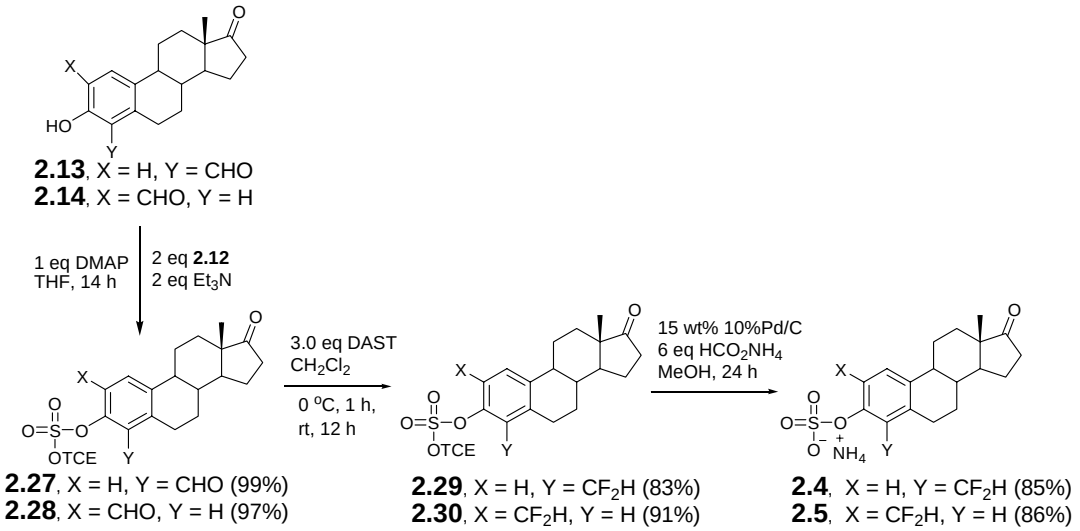
Scheme 2.13. Pert and Ridley's synthesis of **2.16** by reduction of cyano derivative **2.26.**

For the synthesis of **2.6** and **2.7** we decided not to devise a new synthesis of **2.13** or use Pert and Ridley's approach followed by oxidation of the ketone group. We mentioned earlier that **2.13** was obtained as a minor byproduct from the direct formylation of estrone using $MgCl_2$, paraformaldehyde and tributyl- or triethylamine in acetonitrile (Table 2.1). As a result of our optimization studies on this reaction, we were able to accumulate enough **2.13** to tackle the syntheses of compounds **2.6** and **2.7**. However, we will see later that **2.13** turns out to be an important compound for our inhibition studies and we will be revisiting the synthesis of this compound in section 2.2.3.

2.2.2 Synthesis of compounds 2.4-2.11

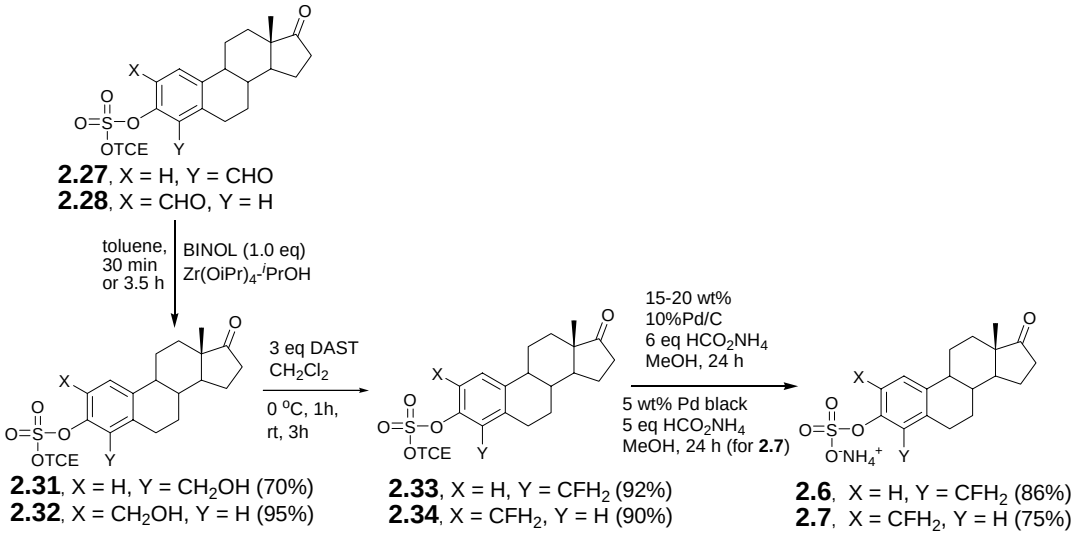
The synthesis of difluoromethyl derivatives **2.4** and **2.5** were begun by reacting **2.13** and **2.14** with $TCEOSO_2Cl$ (**2.12**) in the presence of 1 equiv of DMAP and 2 equiv of Et_3N (Scheme 2.14). This gave sulfate diesters **2.27** and **2.28** in 99% and 97% yields, respectively. These formylated species were converted into the difluoromethyl derivatives **2.29** and **2.30** in 91% and 83% yield respectively using 3.0 equiv of diethylaminosulfur trifluoride (DAST). No competing fluorination occurred at the 17-position nor did any loss of the TCE protecting group occur. Catalytic transfer hydrogenolysis of **2.29** and **2.30** using 10% Pd/C and ammonium formate gave compounds **2.4** and

2.5 in 86% and 85% yields, respectively.



Scheme 2.14. Synthesis of compounds **2.4** and **2.5**

To obtain the monofluoromethyl derivatives **2.6** and **2.7**, we had to selectively reduce the aldehyde groups in compounds **2.27** and **2.28** to the corresponding hydroxymethyl derivatives without reducing the keto group at the 17-position. Reaction of these aldehydes with $NaBH_4$ resulted in the reduction of both the aldehyde and keto group. Lorca et al have reported that aldehydes can be

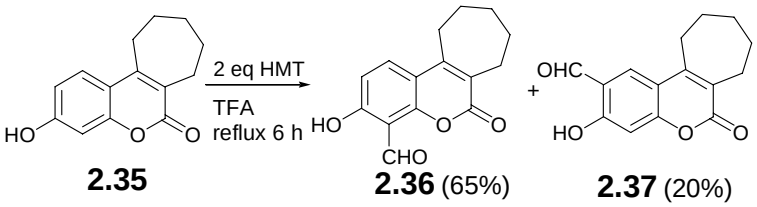


Scheme 2.15. Synthesis of compounds **2.6** and **2.7**.

selectively reduced over ketones using (±)-BINOL-Zr(O^iPr)$_4$-^{i}PrOH complex.[15] Subjecting 2-formyl derivative **2.28** to 1.0 equiv complex for 30 min resulted in a highly selective reduction of the aldehyde moiety and hydroxyl methyl derivative **2.32** was obtained in a 95% yield (Scheme 2.15). No concurrent reduction of the carbonyl at the 17-position was detected. However, subjecting 4-formyl derivative **2.27** with 1.0 equiv of this complex for 30 min resulted in partial reduction of the ketone moiety and a mixture of the desired compound **2.31**, the diol resulting from reduction of the aldehyde and ketone moiety and the monoalcohol resulting from reduction of just the ketone was obtained. Selectivity was lower with the 4-derivative due to the lower reactivity of the formyl group at the 4-position compared to the one at the 2-position. The mixture of the mono alcohols could not be separated. However, since the reduction of aldehyde was much faster than that of the keto group, use of a longer reaction time (3.5 h) provided desired alcohol **2.31** in 70% yield and the diol byproduct could be easily removed by chromatography. Monofluoromethyl compounds **2.33** and **2.34** were obtained in 92% and 90% yields, respectively, by subjecting **2.31** and **2.32** to 3 equiv of DAST. Removal of the TCE group from **2.34** using 15 wt. % of 10% Pd/C and 6 equiv ammonium formate proceeded well giving **2.6** in 86% yield. Surprisingly, removal of the TCE group in **2.5** using 20 wt % of 10% Pd/C and ammonium formate proceeded very slowly and, after 24 h, was far from complete. However, it was found that compound **2.7** could be obtained in a 75% yield by adding in 5 wt % of palladium black and stirring for an additional 24 h. Later we found that using Zn/HCO_2NH_4 in MeOH/THF (solubility in MeOH was so poor that THF had to be used as a co-solvent) the deprotected sulfate **2.7** could be obtained in 98% yield and the reaction was done in less than 15 min.

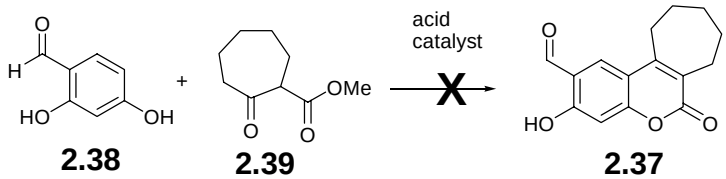
The synthesis of compounds **2.8**-**2.11** began with the synthesis of formyl coumarins **2.36** and

2.37.[16] To prepare **2.36** and **2.37** we first tried reacting coumarin **2.35**[17] with HMT (2 equiv) in refluxing TFA. This gave 8-formylcoumarin **2.36** in a 65% yield and 6-formylcoumarin **2.37** in a 20% yield (Scheme 2.16). These two isomers could be separated by chromatography. Other methods were examined. We tried the formylation reaction using $MgCl_2$, Et_3N and para-formaldehyde; however,



Scheme 2.16. Synthesis of compounds **2.36** and **2.37** using HMT-TFA

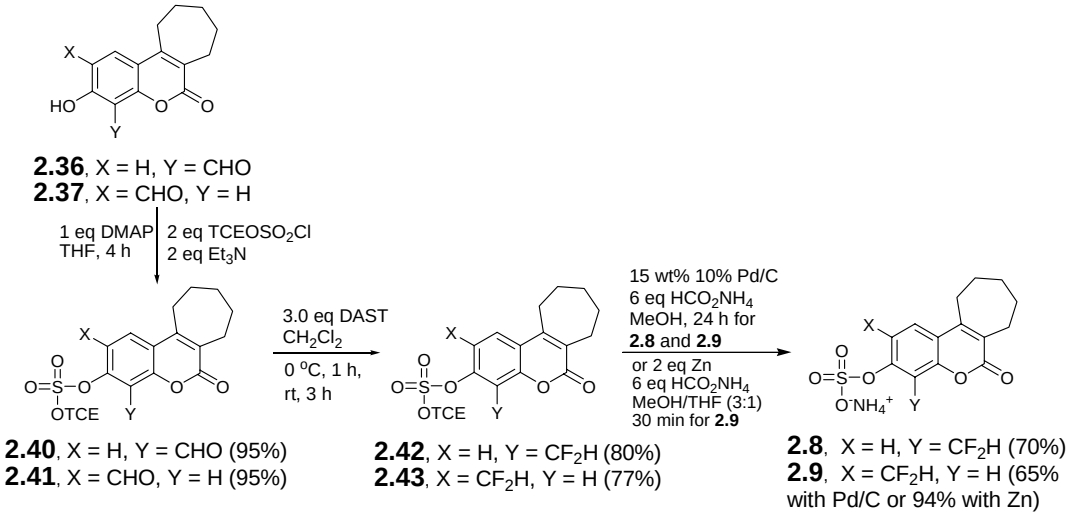
the yields of both isomers were lower than with HMT-TFA. Coumarin **2.35** is prepared by condensing resorcinol with 2-methoxycarbonylcycloheptanone in the presence of an acid catalyst (Pechmann condensation).[17] Therefore we thought that **2.37** could be obtained by condensing 4-fomylresorsinol (**2.38**) with 2-methoxycarbonyl cycloheptanone (**2.39**) (Scheme 2.17). However, this reaction was unsuccessful. Rather than spend more time trying to develop a method for preparing **2.37** in high yield, we decided to just stick with HMT-TFA approach. Even though the yield of **2.37** was low, the starting material, compound **2.35**, was inexpensive and easy to prepare and we were able to obtain quantities of **2.37** that were sufficient for our purposes.



Scheme 2.17. Attempted synthesis of **2.37** by a Pechmann condensation with **2.38**

Coumarins **2.8** and **2.9** were prepared as outlined in Scheme 2.18. The 4-formyl derivative

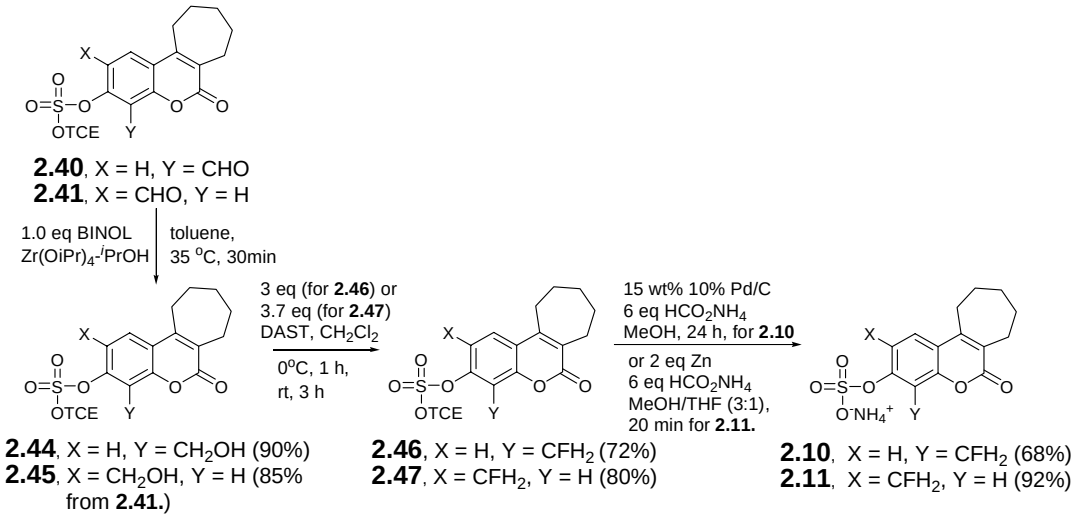
2.36 was sulfated to give sulfate ester **2.40** in a 95% yield using reagent **2.12**. Treatment of **2.40** with DAST and then removal of the TCE group using CTH gave the difluoromethyl compound **2.8** in 56% yield (2 steps). The 2-difluoromethyl compound **2.9** was prepared in good overall yield (48% over 3 steps) using the same approach employed for compounds **2.8**. Deprotection using Zn (2 equiv)/HCO_2NH_4 (6 equiv) in MeOH/THF gave **2.9** in a higher yield (94%) in 30 min and the overall yield (over 3 steps) for **2.9** went up to 69%.



Scheme 2.18. Synthesis of compounds **2.8** and **2.9**

To prepare the 4-monofluoro compound **2.10**, the aldehyde moiety in **2.40** had to be reduced to an alcohol. We first tried $NaBH_4$. Although this reaction appeared to go well by TLC, the product was very hard to purify by column chromatography and recrystallization. Therefore, we tried (±)-BINOL-Zr(O^iPr)$_4$-^{i}PrOH complex and it turned out to be an excellent reducing reagent at 35 ^oC giving the alcohol **2.44** in 90% yield. Treatment of **2.44** with DAST and then removal of the TCE group using CTH gave the difluoromethyl compound **2.10** in 49% yield (2 steps). 4-Monofluoro compound **2.11** was prepared in 63% overall yield (3 steps) using the same approach employed for

compounds **2.10**. It is interesting to note that deprotection of monofluoro derivative **2.47** using CTH (10% Pd/C, HCO_2NH_4) gave only defluorinated byproduct. Of all of the compounds prepared, this was the only one where defluorination was a serious problem. We do not have an explanation for this. Nevertheless, the deprotection was achieved using zinc (2 equiv) and ammonium formate (6 equiv) in MeOH/THF to give **2.11** in a 92% yield. No defluorinated products were detected using this procedure (Scheme 2.19).

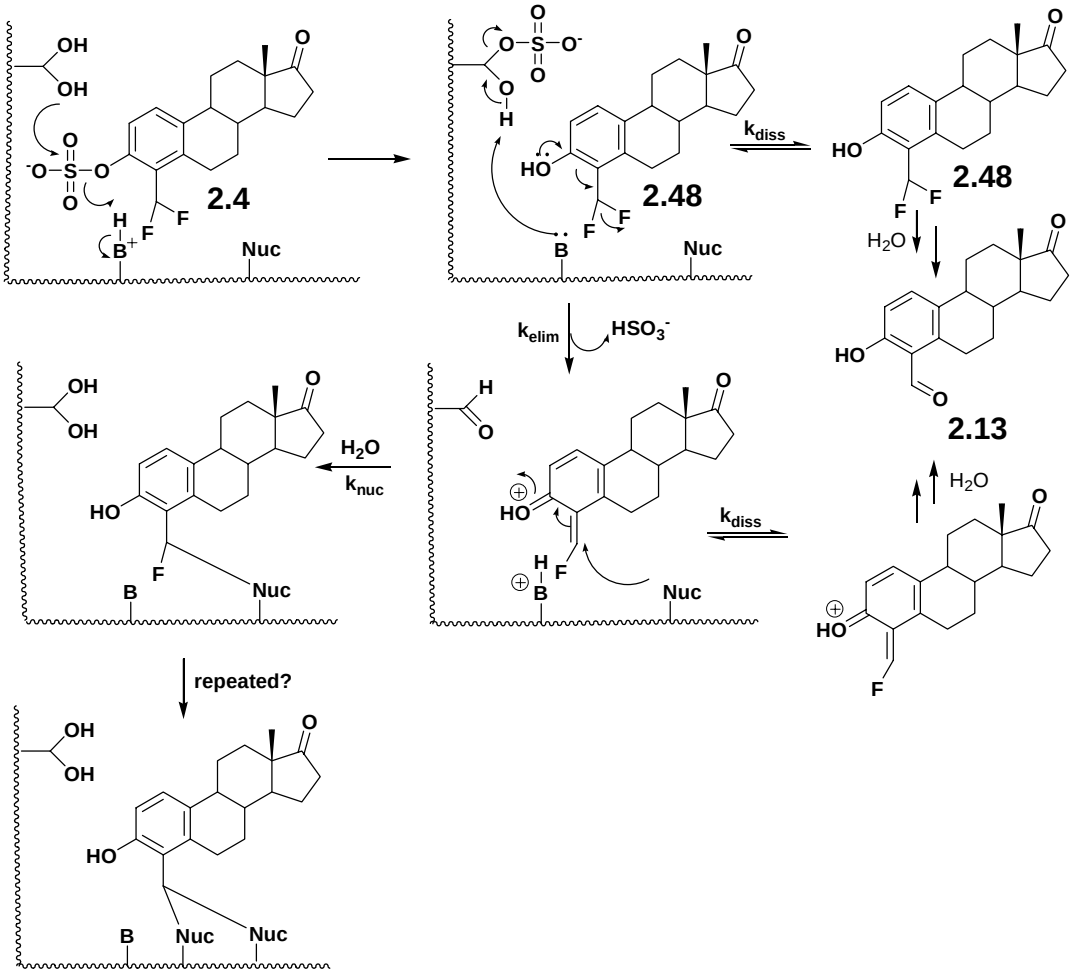


Scheme 2.19. Synthesis of compounds **2.10** and **2.11**

We have shown that the TCE protecting group strategy can be used for synthesizing compounds **2.4-2.11** and that using this strategy the synthesis of these compounds was relatively straightforward. These compounds were given to Vanessa Ahmed, a graduate student in the Taylor group, who then examined them as irreversible inhibitors of STS. A brief discussion of the results of these inhibition studies is necessary since these results had an important bearing on how we were to approach the design and synthesis of a new class of irreversible inhibitors of STS.

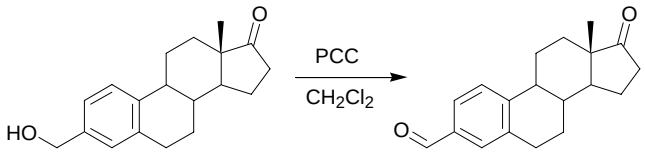
Of the eight compounds studied, compounds **2.4**, **2.7** and **2.11** exhibited irreversible,

time-dependent inhibition of STS. When STS was subjected to inhibitors **2.7** and **2.11**, a linear loss of STS activity occurred with time and the rate of inactivation was proportional to the concentration of inhibitor. We believe that these two compounds act as suicide inhibitors as outlined in Scheme 2.1. However, when STS was subjected to inhibitor **2.4**, loss of STS activity occurred in a nonlinear fashion and there was a significant lag phase at the beginning. Again, the rate of inactivation was proportional to the concentration of inhibitor. This suggested to us that an intermediate is being released from the active site, which then undergoes a non-enzymatic transformation and the resulting



Scheme 2.20. Proposed mechanism for the formation of **2.13** from **2.4**

species then irreversible binds to STS. Difluoromethyl phenols (such as compound **2.48** in Scheme 2.20) are more stable than monofluoromethyl phenols. Elimination of fluoride ion is slower in the difluoromethyl phenols and this is probably due to the build up of a slight positive charge on the carbon bearing the fluorine(s) at the transition state.[18] The additional fluorine destabilizes this positive charge and so elimination occurs more slowly. We reasoned that the difluoromethyl intermediate **2.48** is able to diffuse out of the active site into solution where it eliminates fluorine to form the quinone methide which reacts with water to give **2.13**. We reasoned that perhaps **2.13** is capable of binding in the STS active site and irreversibly inhibit STS. Indeed, subjecting STS to a 10 μM solution of **2.13** resulted in almost complete *irreversible* inactivation of STS within 60 minutes! So **2.13** is a potent, irreversible and time-dependent inhibitor of STS. Moreover, the 2-formyl isomer, **2.14,** was a much weaker irreversible inhibitor. We also prepared aldehyde **2.50** (Scheme 2.21) by PCC oxidation of **2.49**[19] (Scheme 2.21). Studies by Vanessa Ahmed in the Taylor group revealed that this was a much weaker irreversible STS inhibitor than **2.13**. So the inhibition with formylated estrones is greatest with the aldehyde group at the 4-position.



2.49 **2.50** (95%)

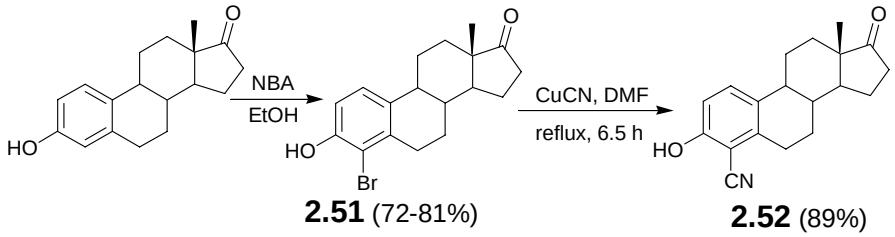
Scheme 2.21. Preparation of aldehyde **2.50**

How **2.13** is inhibiting STS is not yet known. One possibility is that it is forming a Schiff base with an active site lysine or arginine. Schiff base formation is usually reversible in water. However, perhaps water is excluded from the active site when the inhibitor is bound and so imine

formation is irreversible. In any case, these results are highly significant in that a new class of irreversible STS inhibitor was discovered. We wished to use this compound as a lead structure for the development of yet more potent STS inhibitors. However, as mentioned earlier, compound **2.13** is difficult to prepare in good yield. It was clear to us that if we wanted to use this as a lead structure then a much better synthesis of **2.13** and its derivatives would have to be devised. The majority of the remainder of this chapter focuses upon this issue.

2.2.3 New approaches to the synthesis of 2.13

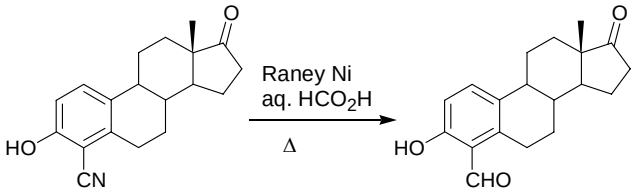
We initiated our studies focusing on the reduction of 4-cyanoestrone, an approach attempted by Pert and Ridley[8] for the synthesis of **2.16** (Scheme 2.13), since this appeared to be a fairly straightforward route to **2.13**. Although Pert and Ridley did not have much success with this approach, we anticipated that better yields for the cyano group reduction could be obtained by optimizing the Raney Ni reaction or by using reducing agents other than Raney Ni. The synthesis begins with 4-bromoestrone (**2.51**). Utne et al. reported that reaction of E1 with *N*-bromoacetamide (NBA) in EtOH gave **2.51** in an 83% yield after recrystallization.[20] In our hands, this reaction provided compound **2.51** in a 72-81% yield (Scheme 2.22). This level of selectivity is unusual for an electrophilic aromatic substitution (EAS) on E1. All other EAS reactions on E1 (not just bromination) always give mixtures of the 2- and 4- isomeric products (plus di-substituted product) and the 2-isomer usually dominates. Interestingly, for the bromination of E1, using a variety of brominating agents, the 4-isomer tends to predominate though not to the extent when NBA is used as brominating agent. For example, bromination of E1 with *N*-bromosuccinimide in DMF gives mixtures of **2.51,** 2-bromoestrone and the yield of **2.51** after purification is around 39%.[21] The high



Scheme 2.22. Synthesis of compound **2.52**

selectivity reported with NBA has not yet been explained. There are two major factors that affect the regioselectivity of an EAS reaction on E1. One is steric which clearly favors the 2-position. The other one is electronic which favors the 4-position. Perhaps the smaller size of NBA makes it less susceptible to steric factors and so the 4-isomer dominates. Compound **2.51** was then converted to corresponding cyano compound **2.52** in 89% yield using CuCN (2.5 equiv) in refluxing DMF for 6.5 h.[22] We initially attempted the reduction of the nitrile by heating it in formic acid/H_2O in the

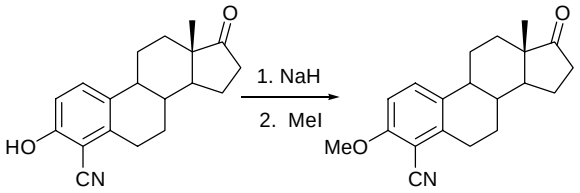
Table 2.2. Synthesis of **2.13** by reduction of the nitrile group in compound **2.52** using Raney Ni.



entry	% aq. HCO_2H	Temp/^oC	Time/h	Yield **2.13**	Recovered **2.52**
1	80%	140	24	18%	32%
2	75%	150-155	40	10%	32%
3	42%	150-155	24	12%	20%
4	60%	150-155	48	8%	0
5	60%	140	48	20%	32%
6	60%	130	36	12%	18%

presence of Raney Ni, conditions similar to those used by Pert and Ridley. Different temperatures and amounts of formic acid were examined. The reaction was very slow and at higher temperatures a considerable amount of decomposition products were formed. The best yield we obtained was only 20% (entry 5, Table 2.2) which means that the overall yield of **2.13** was only 12% starting from E1.

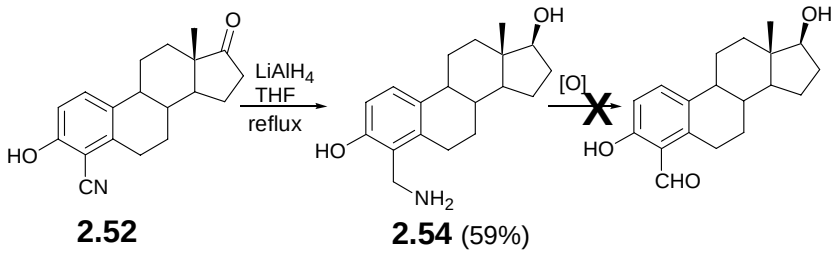
We also examined other reducing agents, such as DIBAL, PtO_2 in refluxing formic acid[23] and ($MeNHCH_2CH_2NHMe$)-$LiAlH_4$ complex.[24] However, these were even less successful in that only trace amounts of the aldehyde product was obtained. In most instances, the keto group at the 17-position was reduced to the alcohol though this was not considered to be a problem since it could be oxidized back to the ketone.[13] We also prepared 3-methoxy-4-cyanoestrone (**2.53**) and subjected it to DIBAL. However, only the ketone group was reduced.



2.52 **2.53** (79%)

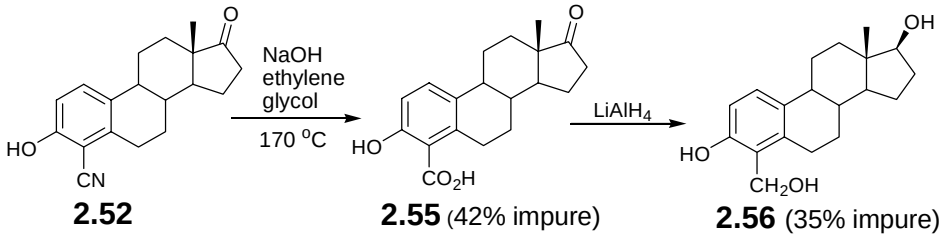
Scheme 2.23. Methylation of **2.52**

Reduction of **2.52** with $LiAlH_4$ in refluxing THF gave the corresponding primary amine **2.54** (Scheme 2.24) in 59% yield or in a 33% overall yield over 3 steps starting from E1. Although amine **2.54** is not our target compound, this synthesis of **2.54** is a considerable improvement over the literature synthesis which was accomplished in an 21% yield over 6 steps starting from estradiol.[25] Direct oxidation **2.54** using refluxing HMT[26] in HOAc failed to give the aldehyde.



Scheme 2.24. Reduction of **2.52** with $LiAlH_4$

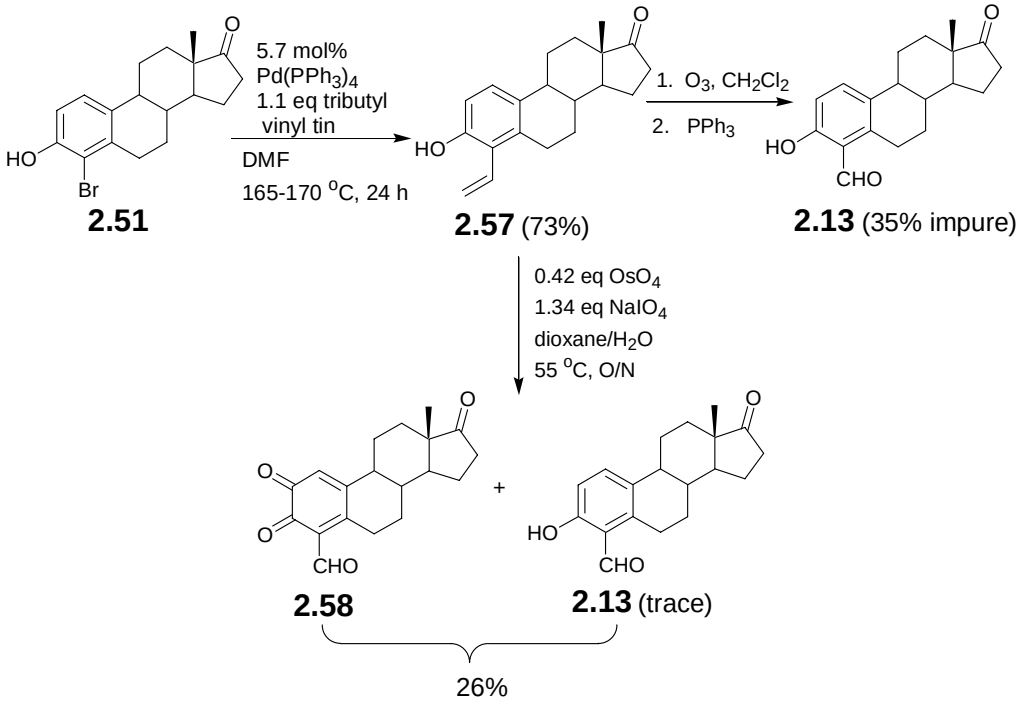
Nitrile **2.52** proved to be remarkably inert. Acidic hydrolysis in 70% sulfuric acid at 100 ^{o}C didn't go at all. Basic hydrolysis to acid **2.55** was achieved in about a 42% yield using NaOH in ethylene glycol at 170 ^{o}C (**Scheme 2.25**) though we were never able to completely purify the acid. Moreover, reduction of this impure acid to alcohol **2.56** using $LiAlH_4$ was very sluggish and only about a 35% yield of impure triol **2.56** was obtained.



Scheme 2.25. Attempted synthesis of **2.56**

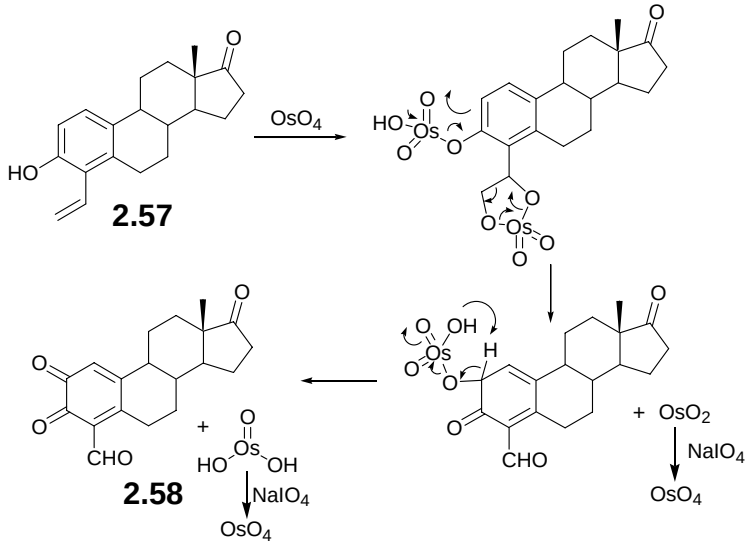
Since bromo compound **2.51** was readily obtained, we envisioned preparing **2.13** by converting **2.51** to a vinyl derivative followed by ozonolysis (Scheme 2.26). Stille coupling of **2.51** with 1.1 equiv of tributylvinyltin in degassed DMF in the presence of 5.7 mol% of $Pd(PPh_3)_4$ at 165-170 ^{o}C for 24 h gave 4-vinylestrone (**2.57**) in 73% yield.

Although ozonolysis of **2.57** afforded **2.13** the reaction was very messy (by ^{1}H NMR) and even after multiple columns an unidentified contaminant was present and the yield was low (35% impure). We also tried to prepare **2.13** by subjecting **2.57** to cat. $OsO_4/NaIO_4$ (Scheme 2.26).



Scheme 2.26. Attempted synthesis of **2.13** from alkene **2.57**

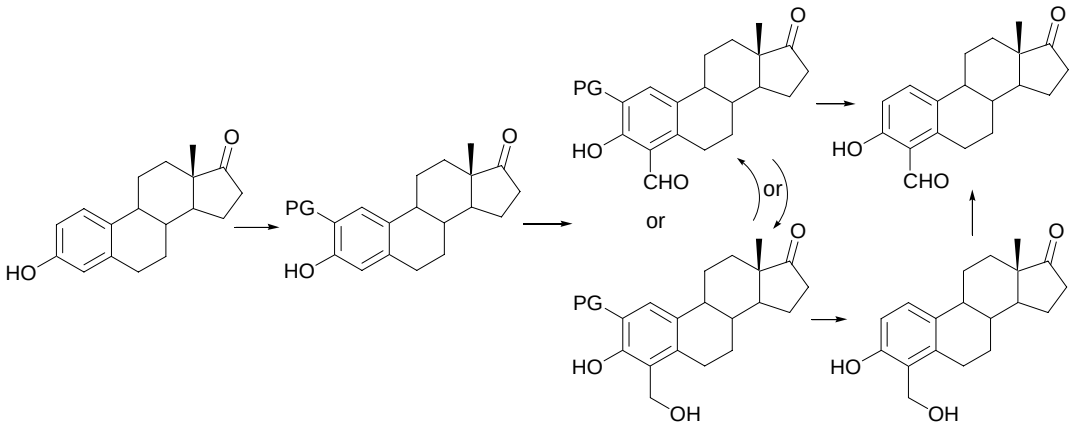
Interestingly, quinone compound **2.58** was isolated in a 26% yield and only of trace amounts of desired product **2.13**. The proposed mechanism for the formation of **2.58** is shown in Scheme 2.27.



Scheme 2.27. Proposed mechanism for the formation of **2.58**

To avoid the problems with direct transformation of alkene to aldehyde, our next approach was

to protect or block the 2-position first, formylate or hydroxymethylate the 4-position and then deprotect the 2-position (Scheme 2.28). The obvious difficulty with this approach is the selective

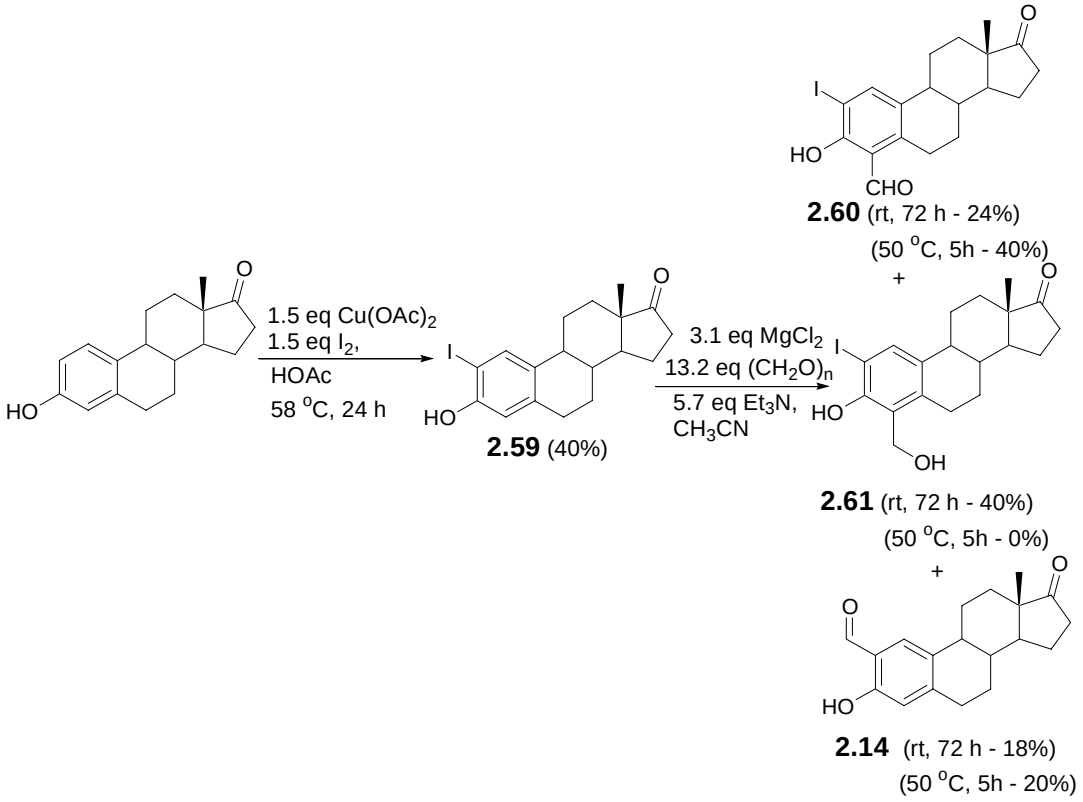


Scheme 2.28. Proposed synthesis of **2.13** by protection of the 2-position

introduction of an easily removed protecting group at the 2-position. We started with iodine since reports have appeared describing high yields of regioselective iodination of estrogens at the 2-position and iodine can be easily removed from aryl rings in high yield by hydrogenolysis. Even if the formyl group at the 4-position was reduced to the alcohol during the removal of the iodine, we knew that it could be selectively reoxidized to the aldehyde in good yield using Jones reagent.[13] In 1953, Hillmann-Elies et al. reported that 2-iodoestrone can be obtained in a 96% yield by reacting estrone with iodine and $Hg(OAc)_2$ in AcOH.[27] However, other groups have reported that they were unable to obtain this yield and degree of selectivity using this procedure and instead reported that isomeric mixtures were obtained and yields of 40-60% for 2-iodoestrone were achieved.[28] Horiuchi and Satoh reported that if $Cu(OAc)_2$ is used instead of $Hg(OAc)_2$ then the 17-enol acetate of 2-iodoestrone can be obtained in a 90% yield.[29] The enol acetate was reduced with $NaBH_4$ to 2-iodoestradiol in a 95% yield. Finally Page et al. reported that reaction of 3-acetoxyestrone with thallic trifluoroacetate in

TFA followed by treatment with copper iodide gave the 17-enol acetate of 2-iodoestrone.[30.] Hydrolysis of the enol acetate with potassium carbonate in methanol gave 2-iodoestrone in 87% yield starting from 3-acetoxyestrone.

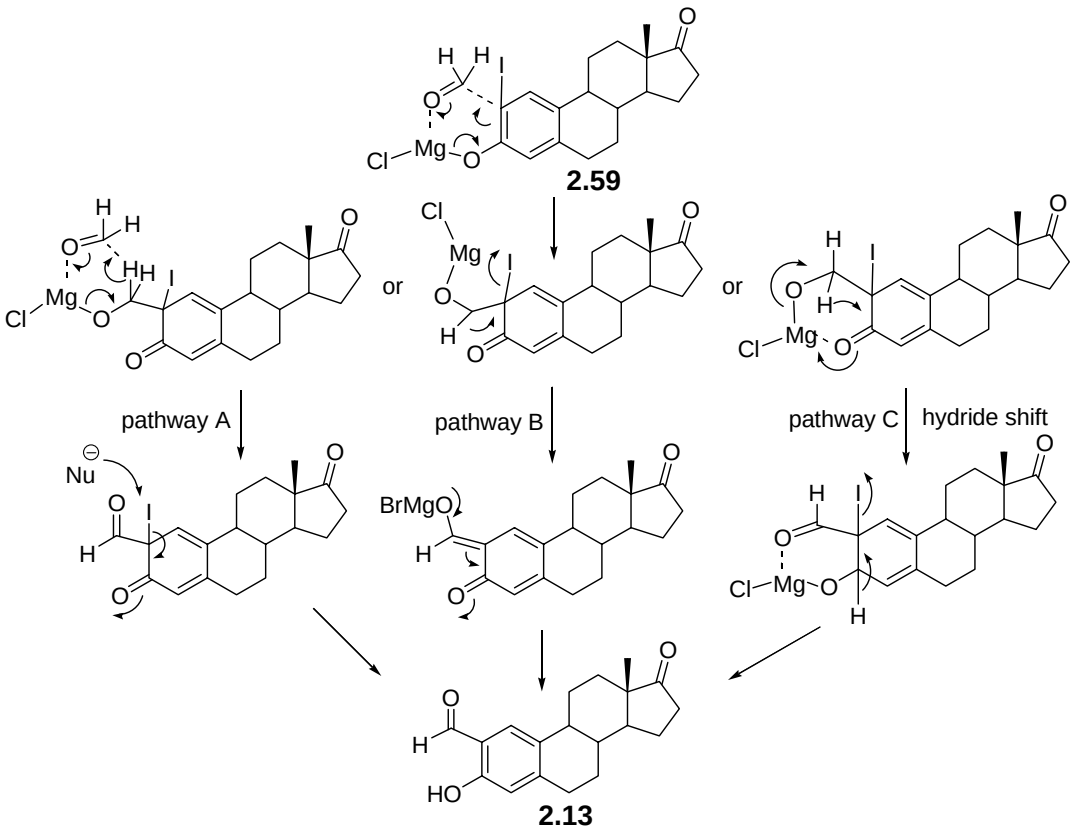
We wished to avoid the use of thallium salts due to their toxicity and it was clear from reports by other workers that the route using $Hg(OAc)_2$ was difficult to reproduce. Therefore we focused upon the route developed by Horiuchi and Satoh using 1.5 equiv of I_2 and 1.5 equiv of $Cu(OAc)_2$. In our hands we obtained only a 45% yield of 2-iodoestrone (**2.59**) using this procedure (Scheme 2.29). Moreover, we obtained **2.59** directly and none of the enol acetate was observed.



Scheme 2.29. Synthesis of **2.60**

Although the yield of **2.59** was disappointing, we subjected **2.59** to $MgCl_2$ (3.1 equiv), paraformaldehyde (13.2 equiv) and triethylamine (5.7 equiv) in acetonitrile at rt for 3 days which gave

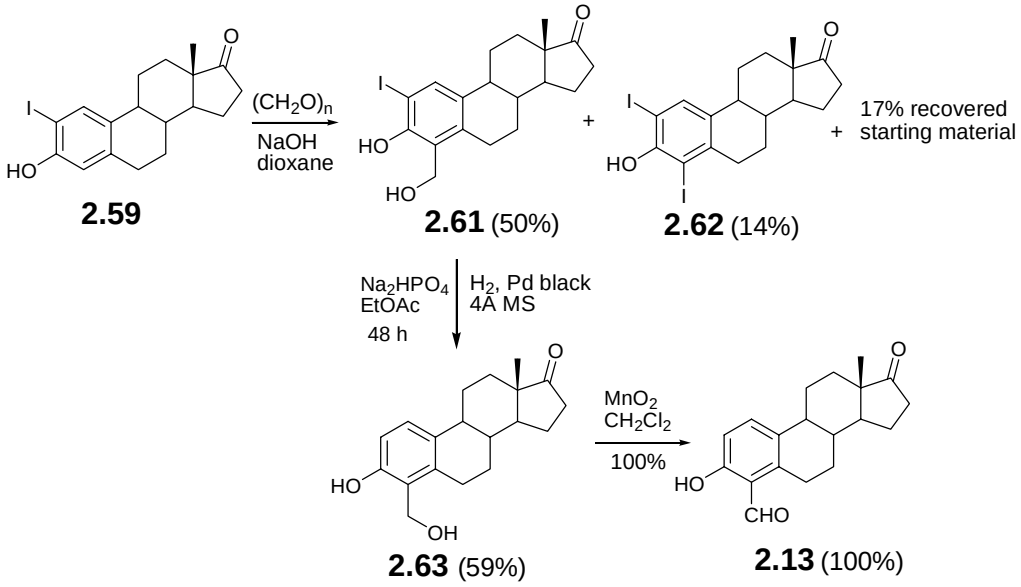
an easily separable mixture 4-formyl-2-iodoestrone (**2.60**, 24%) and 4-hydroxymethyl- 2-iodoestrone (**2.61**, 40%) and, surprisingly, **2.14** (Scheme 2.29). At higher temperature (50 ^{o}C, 5 h), no alcohol product was isolated and only **2.14** (20%) and the desired product **2.60** (40%) were obtained. To our knowledge, this is the first example of the substitution of an iodine for a formyl group mediated by the salt of a divalent metal. Several possible mechanisms for the formation of **2.14** are shown in Scheme 2.30 though we do not know which one (if any) is taking place.



Scheme 2.30. Possible mechanisms for the formation of **2.13** from **2.59**

The selective reduction of an aryl bromide in the presence of a formyl group has been accomplished using Zn-Ag, NaI, $NaHCO_3$ in DMSO.[31] However, subjecting **2.60** to these conditions did not result in any reaction. We then examined a variety of conditions (Pd/C or Pd black and H_2

with or without triethylamine in various solvents, AIBN/tin hydride) to remove the iodine; however, all of these reactions produced a complex mixture of products. ^{1}H NMR of some of these crude reaction mixtures suggested that polymerization was taking place.



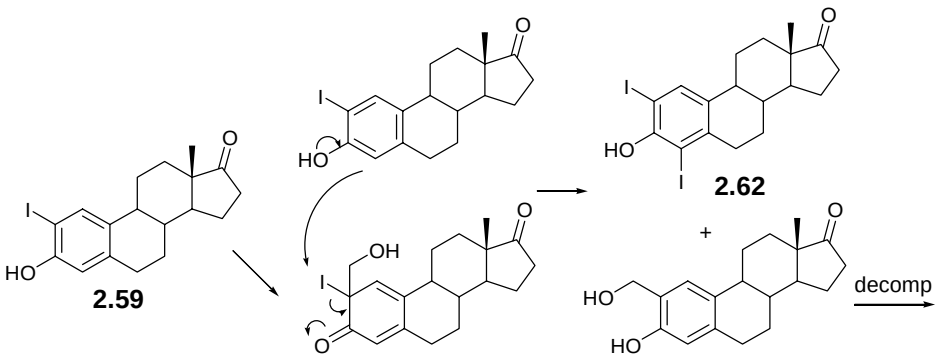
Scheme 2.31. Synthesis of **2.13** from **2.59**

We also performed a hydroxymethylation of 2-iodoestrone hoping that this would go in better yield than the formylation reaction (Scheme 2.31). After some experimentation it was found that a 50% yield of 4-hydroxymethyl-2-iodoestrone (**2.61**) could be obtained using 0.66 equiv of paraformaldehyde and 0.22 equiv NaOH in dioxane. A surprising byproduct of this reaction was 2,4-diiodoestone (**2.62**) which was formed in a 14% yield. Once again, removal of the iodine by hydrogenolysis proved to be difficult.

However, after considerable experimentation it was found that it could be removed using Pd black in the presence of Na_2HPO_4 which gave 4-hydroxymethylestrone **2.63** in 59% yield. Oxidation with MnO_2 in CH_2Cl_2 gave desired **2.13** in a 100% yield. The overall yield of **2.13** starting from

estrone was 12%.

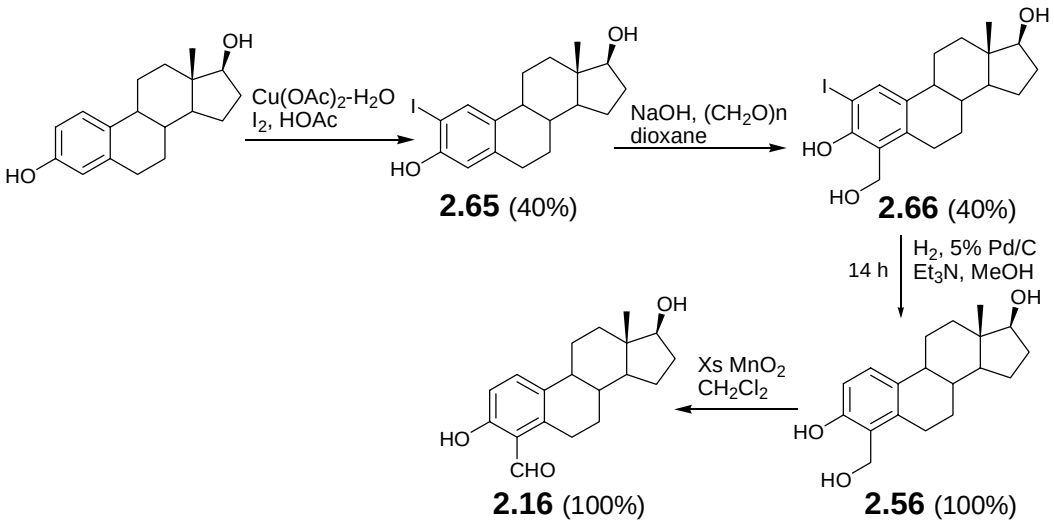
Formation of diiodo compound **2.62** during the hydroxymethylation of **2.59** was quite unexpected. A possible mechanism for its formation is shown in Scheme 2.32. Compound **2.59** reacts with formaldehyde at the 2-position to form intermediate **2.64** and another molecule of 2-iodoestrone attacks the iodine atom in **2.64** to give diiodoestrone. We believe that 2-hydroxyestrone decomposes under the reaction conditions via a quinone methide type intermediate and so it not detected.



Scheme 2.32. Proposed mechanism for the formation of **2.62** during the hydroxymethylation of **2.59**.

We also performed the iodination reaction on estradiol (E2) using the $Cu(OAc)_2$ procedure in the anticipation that this might give a better yield of the 2-isomer than the reaction with E1. Unfortunately, this gave 2-iodoestradiol (**2.65**) in only a 40% yield (Scheme 2.33). Hydroxymethylation of 2-iodoestradiol at 4-position with paraformaldehyde and NaOH gave corresponding triol **2.66** in a 40% yield. We were surprised to find that the iodine could be removed in **2.66** using 10% Pd/C and H_2 to give 4-hydromethylestradiol (**2.56**) in quantitative yield. Oxidation to 2-formyl estradiol (**2.16**) was achieved in quantitative yield using excess activated MnO_2

in CH_2Cl_2. The overall yield of 4-formylestradiol was 16% in 4 steps starting from estradiol. Oxidation of the alcohol at the 17-position in **2.16** was not performed since it was clear that this was not going to be an efficient route to **2.13**.

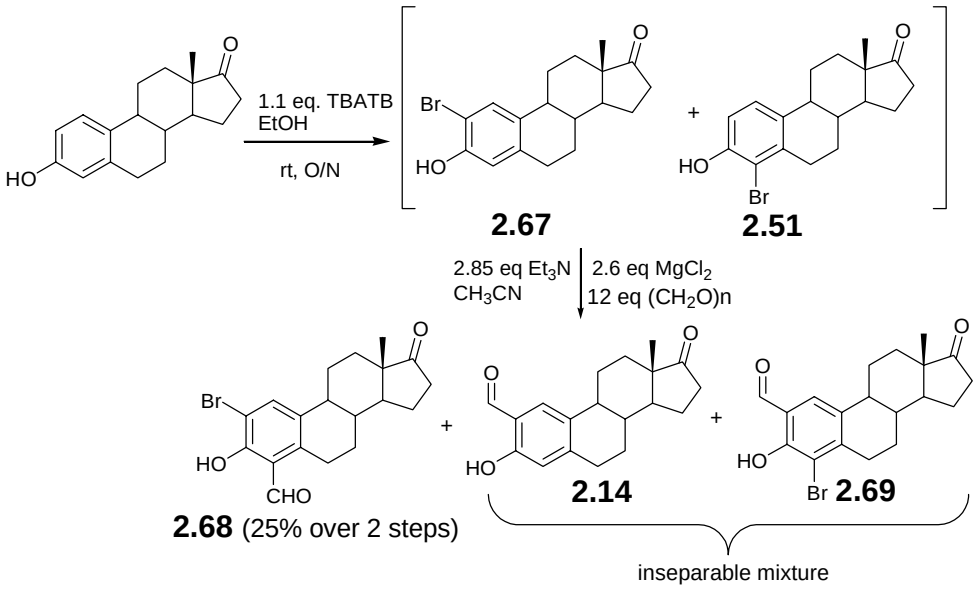


Scheme 2.33. Synthesis of **2.16** from E2

We decided to try the same approach to **2.13** except the iodine was replaced with bromine (Scheme 2.34). Bromination of estrone was performed using tetrabutylammonium tribromide (TBATB), a bulky brominating agent that we anticipated would preferably yield the 2-isomer. Using this reagent only 2-bromo- (**2.67**) and 4-bromoestrone (**2.51**) are formed and almost no estrone remained and no dibromide formed when 1.1 equiv of TBATB was used (when 1 equiv of TBABA was used, 10% of E1 was remained). Although the yield of the two isomers was almost quantitative, the ratio of 4-isomer **2.51** to 2-isomer **2.67** isomer was only about 1.2:1. These two isomers are not readily separated so we used the crude mixture to do the formylation reaction. Formylation was carried with using 2.6 equiv of $MgCl_2$, 12 equiv of paraformadehyde and 2.85 equiv of triethylamine in acetonitrile. A mixture of 2-bromo-4-formyl- estrone (**2.68**), 4-bromo-2-formylestrone (**2.69**) and

2.14 were obtained. The desired isomer **2.68** could be easily separated from the mixture; however the yield of **2.68** was only 25% yield starting from estrone.

It was clear that using an iodo or bromo group as a protecting group for the 2-position was not going to work. Bromination or iodination of E1 was not selective enough for the 2-position and the yields of the subsequent formylations or hydroxymethylations were too low. Ideally what we needed was a protecting group that could be introduced into the 2-positon in better yield, was stable to the formylation or hydroxymethylation conditions and could be removed in high yield.

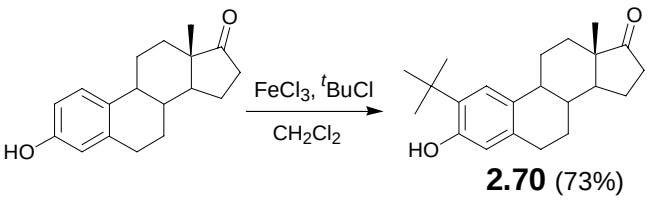


Scheme 2.34. Synthesis of **2.68** from E1

The *t*-butyl group has been used as a protecting group for the ortho position of substituted phenols for over 50 years.[32] It is usually introduced into phenols using a Friedel-Crafts (F-C) reaction and removed using Lewis acids such as $AlCl_3$ in an acceptor solvent such as benzene, toluene or nitromethane. 2-*t*-Butylestrone (**2.70**) is a known compound. It was first synthesized in 1968 by Lunn and Farkas by passing a slow stream of BF_3 over a solution of E1 and 6 equiv *t*-butyl alcohol in

n-pentane.[33] What was particularly significant about this was that the yield of the reaction was 89% and, due to the large size of the *t*-butyl group, no reaction occurred at the 4-position or the phenolic oxygen. Later, Goendoes et al. reported that **2.70** could be prepared in an 81% yield using Friedel-Crafts chemistry (*t*-butyl chloride as solvent and reactant, $FeCl_3$).[34] The high selectivity and yields of these reactions, coupled with the knowledge that the *t*-butyl group can be removed from phenolic derivatives in high yield using Lewis acids suggested to us that it could be used as a protecting group during the synthesis of **2.13**.

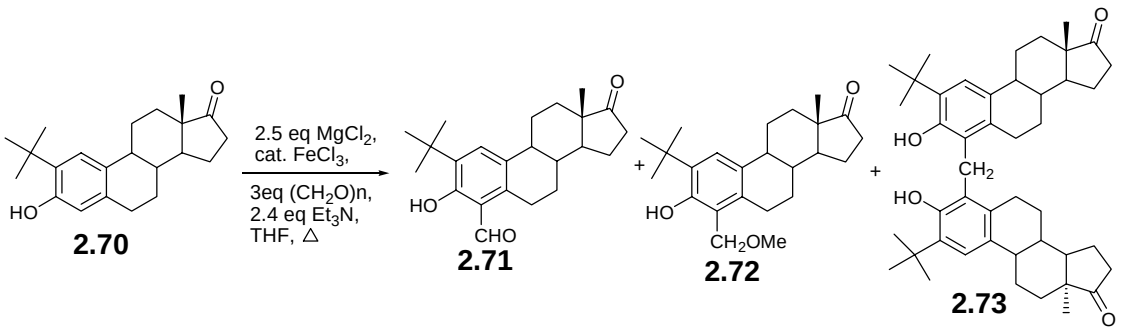
Although the reaction with BF_3/*t*-butyl alcohol gave higher yields of **2.70** we decided to use the F-C chemistry since we did not wish to work with gaseous BF_3 and all of the reagents for the F-C chemistry were already in the Taylor lab. We modified the reaction conditions of Goendoes et al. in that 30 equiv instead of 100 equiv of *t*-butyl chloride was used and methylene chloride instead of *t*-butyl chloride was used as solvent. Using these conditions, a 73% yield of **2.70** was obtained (Scheme 2.35).



2.70 (73%)

Scheme 2.35. Synthesis of **2.70** using an F-C reaction

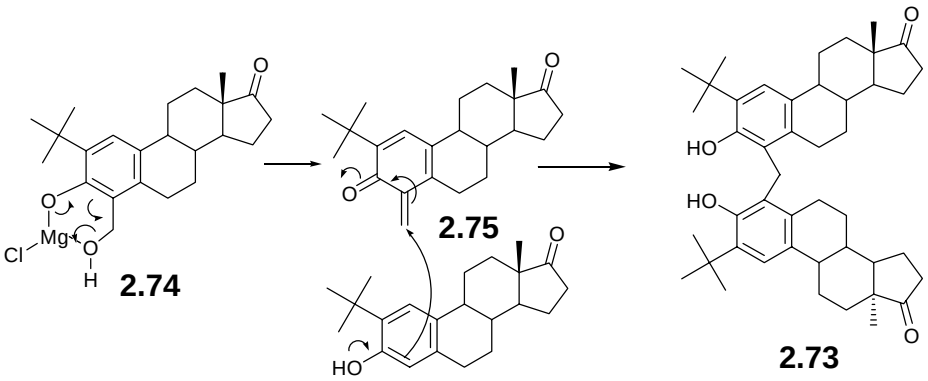
Our first attempt to formylate **2.70**, using 2.5 equiv $MgCl_2$, 12 equiv paraformaldehyde, 2.4 equiv Et_3N in THF (glass bomb, 70 ^oC, 3 h), gave aldehyde **2.71** and hydroxymethyl ether derivative **2.72**, which were surprisingly difficult to separate**,** in a yield of about 30% (Scheme 2.36). However, when we tried this reaction again, no reaction took place even after we played around with different



Scheme 2.36. Products resulting from the formylation of **2.70**

conditions such as temperature, ratio of reagents etc. This was initially very puzzling. However, we realized that when we performed this reaction the first time and got a modest yield of **2.71** and **2.72**, the compound **2.70** that we used had been purified only by chromatography. In the subsequent unsuccessful attempts, compound **2.70** had been further purified by recrystallization after chromatography. Clearly, there was an impurity in the non-recrystallized **2.70** that was promoting the reaction. We reasoned that this might be trace amounts of $FeCl_3$. To test this hypothesis, we took the recrystallized **2.70** and performed the formylation reaction in the presence of 50 mol% of anhydrous ferric chloride. This time, compounds **2.71** and **2.72** were obtained in a 40% yield in a ratio of 3:2 (Table 2.3, entry 1). It is possible that $FeCl_3$ enhances the electrophilic ability of carbonyl group of formaldehyde by coordination. With 10% ferric chloride and 3.3 equiv paraformaldehyde, the yield of **2.71** and **2.72** improved to 51% and the ratio of **2.71** to **2.72** increased to 5:1 (entry 2). However, a byproduct, which was identified as dimer **2.73**, was formed in a significant quantity (37%). Dimer formation has been noted before by Aldred et al. during the formylation of magnesium phenoxide with paraformaldehyde.[35] This was explained by the formation of a quinone methide **2.75** from loss of water of diol **2.74**, followed by nucleophilic attack

from another molecule of phenoxide (estrone). A similar mechanism can be invoked for the formation of dimer **2.73** (Scheme 2.37).



Scheme 2.37. Proposed mechanism for the formation of dimer **2.73**

Since ferric chloride is a strong, hygroscopic Lewis acid and difficult to handle especially in small amounts, we tried 10 mol% ferric acetate (non-hydroscopic) instead. To our delight, compound **2.71** was formed, however, once again compounds **2.72** and **2.73** were also obtained in significant quantities (Table 3, entry 3). We then attempted to optimize the yield of **2.71** using different amounts of catalyst, paraformaldehyde, reaction times and temperature. Performing the reaction with just 0.4 mol% catalyst at 50 ^{o}C resulted in an increase in the yield of **2.71** and **2.72** (69%) and the **2.71 to 2.72** ratio (11:1) and a decrease in the amount of dimer **2.73** (Table 3, entry 4). Using the same conditions and performing the react at rt for 72 h resulted in very poor conversion (entry 5). Performing the reaction with 0.4 mol% catalyst at 40 ^{o}C for 5.5 h resulted in a further increase in the yield of **2.71** and **2.72** (73%) and the **2.71** to **2.72** ratio (14:1) and a further decrease in the amount of dimer **2.73** (18%) (Table 3, entry 6). We could even reduce the amount of catalyst to 0.04% and react for 5.5 h at 40 ^{o}C and obtain a 67% yield of **2.71** and **2.72** in a 10:1 ratio and a 22% yield of dimer. Performing the reaction using a catalyst loading of 0.14 mol %, and 10 equiv

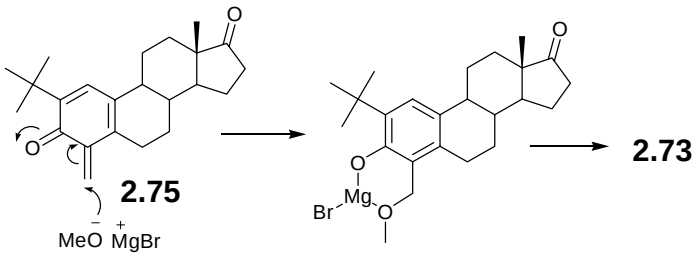
paraformaldehyde at 40 ^{o}C for 14 h gave a 91% yield of **2.71** and **2.72** in a ratio of 5:3 and no dimer was present.

Table 2.3. Formylation of **2.70** under different conditions.

entry[a]	Mol % catalyst	Equiv $(HCHO)_n$	Temp (^{o}C)	Time (h)	% yield **2.71** + **2.72** (ratio)[b]	% yield **2.73**
1	50 mol% $FeCl_3$	12	70	3	40 (3:2)	-
2	10 mol% $FeCl_3$	3.3	70	3	51 (5:1)	37
3	10 mol% $Fe(OAc)_3$	3.3	70	3	55 (3.5:1)	31
4	0.4 mol% $Fe(OAc)_3$	3	50	3	69 (11:1)	25
5	0.4 mol% $Fe(OAc)_3$	3	rt	72	<10%	-
6	**0.4 mol% $Fe(OAc)_3$**	**3**	**40**	**5.5**	**73 (14:1)**	**18**
7	0.04 mol% $Fe(OAc)_3$	3	40	5.5	67 (10: 1)	22
8	0.14 mol% $Fe(OAc)_3$	10	40	14h	91 (5:3)	-

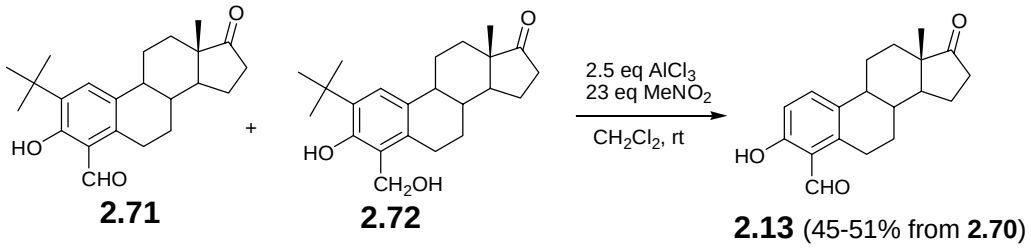
[a] All reactions were performed using using 2.5 equiv $MgCl_2$ and 2.4 equiv Et_3N in THF. [b]Ratio of **2.71** to **2.72** determined by ^{1}H NMR and after chromatography.

It appears that when a considerable excess of paraformaldehyde is used, dimer formation is suppressed. The reaction of **2.70** with formaldehyde might be faster than dimer formation and so in the presence of large quantities of paraformaldehyde all of **2.70** has reacted with paraformaldehyde before any free **2.70** can react with quinone methide. However, when excess paraformaldehyde, long reaction times and/or high catalyst loading are used the amount of methoxymethyl derivative **2.72** increases. This might be due to formation of methoxide which may attack quinone methide **2.75** (Scheme 2.38).



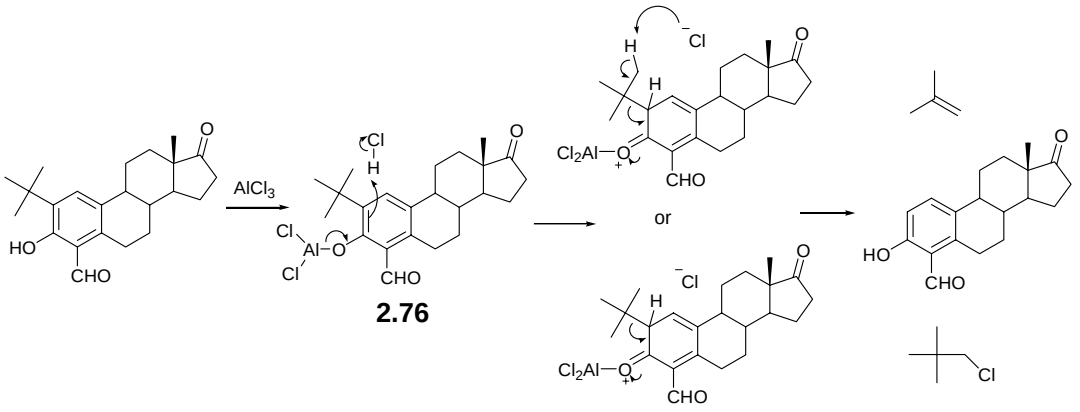
Scheme 2.38. Proposed mechanism for the formation of **2.72**.

Compounds **2.71** and **2.72** are difficult to separate. Therefore, we took the mixture (ratio 14:1) resulting from the reaction performed under the conditions in entry 6 in Table 2.3 (0.4 mol% $Fe(OAc)_3$, 3 equiv paraformaldehyde, 2.5 equiv $MgCl_2$, 2.4 equiv triethylamine, 5.5 h, 40 ^oC, sealed tube) and attempted a deprotection of this material using 2.5 equiv anhydrous aluminum chloride and 23 equiv nitromethane in CH_2Cl_2. The use of methylene chloride as co-solvent was necessary due to the poor solubility of the starting material in nitromethane. After stirring at rt for just 5 h, aldehyde **2.13** was the only material that was detected by TLC (Scheme 2.39). No 4-hydroxymethyl estrone was seen suggesting that this material somehow decomposed (most likely) or was converted into the aldehyde (unlikely) during the reaction. Typical yields (two steps involving formylation with 0.4 mol% $Fe(OAc)_3$, 3 equiv paraformaldehyde, 2.5 equiv $MgCl_2$, 2.4 equiv triethylamine, 5.5 h, 40 ^oC, sealed tube followed by deprotection) ranged from 45-51%. Thus, starting from E1, compound **2.13** was obtained in three steps in a respectable 35% yield.



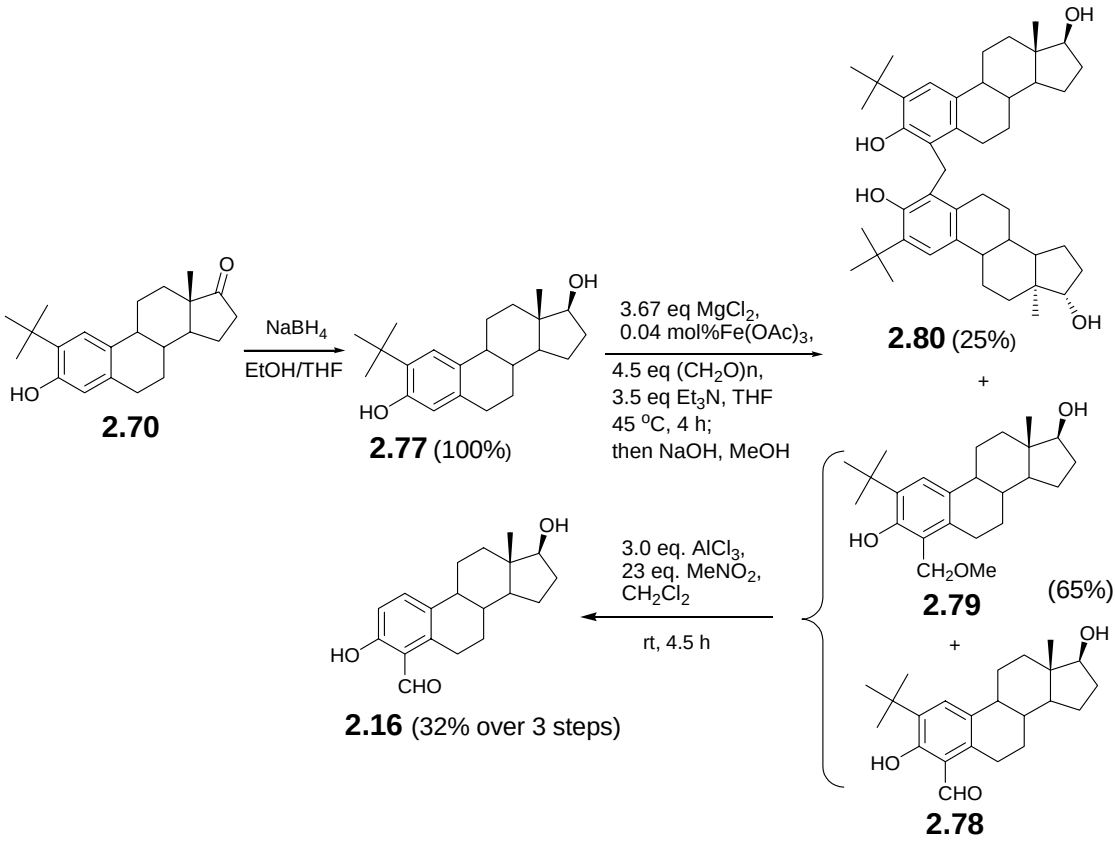
Scheme 2.39. De-*tert*-butylation of **2.71** and **2.72**.

A possible mechanism of the removal of the *tert*-butyl group is shown in Scheme 2.40. Nucleophilic attack of phenolate **2.76** to HCl, followed by loss of isobutene or 2,2-dimethyl-1-chloropropane, gives **2.13** as desired product.



Scheme 2.40. Proposed mechanism for the de-*tert*-butylation reaction

To try and further improve upon the overall yield of **2.13** we turned our attention back to the *t*-butylation reaction. Lunn and Farkas obtained **2.70** in an 89% yield using BF_3 and *t*-butyl alcohol. We reasoned that easy-to-handle $BF_3(OEt)_2$ could used instead of BF_3. It was found that by subjecting E1 to 3.0 equiv $BF_3(OEt)_2$, 2.0 equiv *t*-butyl alcohol in CH_2Cl_2 for 3 h, a 95% yield of **2.70** could be obtained. This brings the overall yield of **2.13** to 46% starting from E1. Further attempts to improve the yield of **2.13** are still in progress in the Taylor group. For example, we have shown that when performing the formylation reaction using 0.14% $Fe(OAc)_3$, 3 equiv paraformaldehyde, 2.5 equiv $MgCl_2$, 2.4 equiv triethylamine, 5.5 h, 40 ^oC, a 91% yield of **2.71** and **2.72** could be obtained in a ratio of 5:3 (entry 8, Table 2.3). Oxidation of the hydroxymethyl group in **2.72** to **2.71** in high yield should be feasible. This should enable us to obtain an even better overall yield of **2.13**.



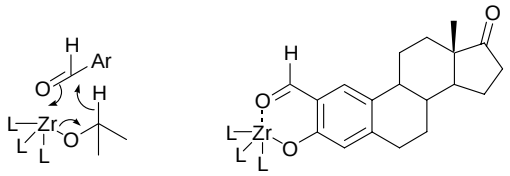
Scheme 2.41. Synthesis of **2.16** from **2.71**

We also investigated the preparation of 4-formylestradiol (**2.16**) using our *t*-butyl protecting group approach (Scheme 2.41). Compound **2.70** was reduced to 2-*tert*-butylestradiol (**2.77**) in 100% yield by using $NaBH_4$ in EtOH/THF. This reaction was very clean and no further purification was performed beyond a simple aqueous workup. Formylation using **3.67** equiv $MgCl_2$/0.04 mol% $Fe(OAc)_3$/3.5 equiv Et_3N/4.5 equiv $(HCHO)_n$, at 45 ^oC for 4 h followed by basic hydrolysis of the formate ester of the 17-hydroxyl group gave the corresponding aldehyde **2.78** and methyl ether **2.79** as an inseparable mixture in a 5:3 ratio in a 66% yield and dimer **2.80** in 25% yield. The *t*-butyl was removed using 3.0 equiv anhydrous $AlCl_3$ and 23 equiv nitromethane in CH_2Cl_2 at rt for 4.5 h which gave **2.16** in a 32% yield over 3 steps starting from **2.70** or 30% yield starting from E1. Again the

last step (deprotection) was very clean by TLC. Optimization of the formylation reaction is still in progress in the Taylor group.

2.2.4 Synthesis of other A-ring modified E1 and E2 derivatives.

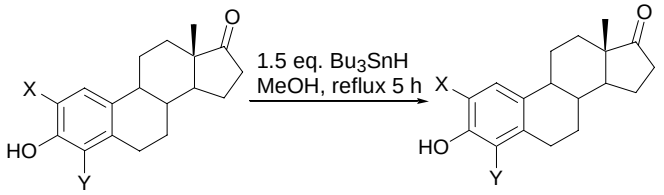
Our syntheses of **2.13** and **2.14** could be used for the rapid entry into other A-ring-modified estrone and estradiol derivatives. First we examined the synthesis of 4-hydroxymethyl estrone (**2.63**) and 2-hydroxymethyl estrone (**2.81**) from aldehydes **2.13** and **2.14**. We tried the selective reduction of the formyl group using (±)-BINOL-Zr(O^iPr)$_4$-^{i}PrOH complex but no reaction occurred. In order for this reaction to work, it must form a 6-membered ring intermediate so that hydride can be delivered from the isopropyl group to carbonyl group (Scheme 2.42). When there is a hydroxyl group at the ortho position of an aryl aldehyde, the zirconium most likely forms another 6-membered ring with both carbonyl and phenol which prevents the reaction from proceeding. $NaBH_4$ gave a complex mixture which was hard to separate.



Scheme 2.42. Mechanism for the reduction of an aldehyde using (±)-BINOL-Zr(O^iPr)$_4$-^{i}PrOH complex

There are many literature procedures for the selective reduction of aldehydes over ketones, but not many for salicylic aldehyde type compounds. Bu_3SnH in refluxing methanol has been used to achieve this transformation.[36] When **2.13** was treated with Bu_3SnH (1.5 equiv) in refluxing methanol for 5 h the desired alcohol **2.63** was obtained in a 42% yield (Scheme 2.43). Similarly,

2.81 can be prepared from **2.14** in a 52% yield (Scheme 2.43). Although the selectivity was excellent in that the 17-position ketone remained intact the yields were disappointing.

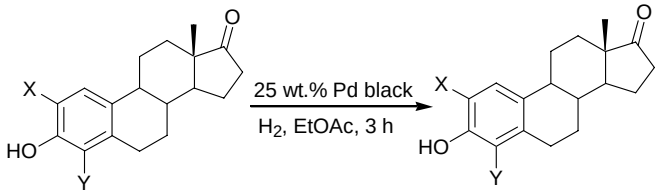


2.13, X = H, Y = CHO
2.14, X = CHO, Y = H

2.63, X = H, Y = CH_2OH (42%)
2.81, X = CH_2OH, Y = H (52%)

Scheme 2.43. Reduction of the formyl group in **2.13** and **2.14** using Bu_3SnH

Later we found that aldehydes **2.13** and **2.14** could be reduced to **2.63** and **2.81** in higher yields by hydrogenation using 25 wt.% Pd black/H_2 (balloon pressure) in EtOAc. The 4-isomer **2.63** was obtained in a 100% yield while the 2-isomer **2.81** was obtained in 65% yield (Scheme 2.44). Compounds **2.63** and **2.81** have been prepared by Singh et al. by hydroxymethylation of estrone protected at the 17-position with a 1,3-dioxolane ketal followed by removal of the ketal protecting group. The hydroxymethylation gave a 35% yield of the 2- and 4-isomers in a 5 to 1 ratio which could not be separated. Removal of the ketal protecting group was achieved in a 90% yield using 1 M HCl. This translates into an overall 6 % yield of **2.63** and a 25% yield of **2.81** from E1. We have prepared the same compounds in yields of 46% (for **2.63**) and 34% (for **2.81**) from E1.



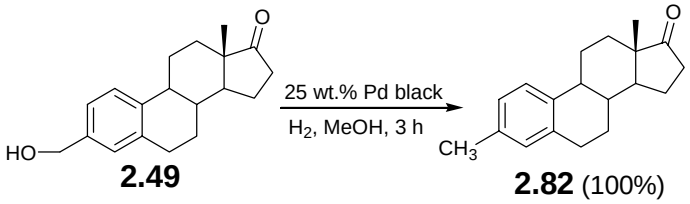
2.13, X = H, Y = CHO
2.14, X = CHO, Y = H

2.63, X = H, Y = CH_2OH (100%)
2.81, X = CH_2OH, Y = H (65%)

Scheme 2.44. Reduction of the formyl group in **2.13** and **2.14** using Pd black/H_2

The behavior of **2.13** and **2.14** to H_2/Pd black in EtOAc is very different to that of

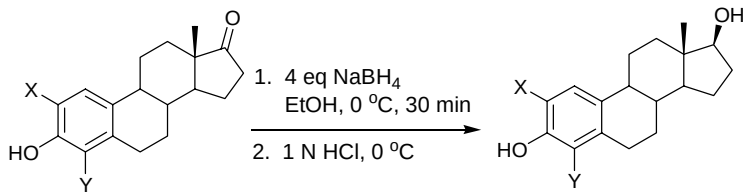
3-hydroxymethylestrone (**2.49**) which we found was easily reduced to 3-methylestrone (**2.82**) in quantitative yield using H_2/Pd black in MeOH (Scheme 2.45). We did not detect any 2- or 4-methylestrone during the reduction of **2.13** and **2.14**. This may be due to the intramolecular hydrogen bonding in **2.63** and **2.81** which somehow decreases their susceptibility to further reduction. This could also be due to a solvent effect; however, further studies will be necessary to determine this.



2.49 **2.82** (100%)

Scheme 2.45. Synthesis of **2.82** by reduction of **2.49** using Pd black/H_2

Reduction of **2.13** and **2.14** to the triols **2.56** and **2.83** was easily achieved using $NaBH_4$ in EtOH at 0 ^oC for 30 min followed by workup with 1 M HCl (Scheme 2.46). The 4-isomer, **2.56**, has been prepared by Lovely et al in 5 steps starting from E2 in an overall yield of 13%.[25] We have achieved its synthesis in 4 steps starting from E1 in 33% yield. Lovely et al. have also prepared the 2-isomer, **2.83**, by reduction of **2.15** which was prepared by the route outlined in Scheme 2.6.[9] This was a four step synthesis starting from E2 and gave **2.83** in an overall yield of 59%. We have achieved its synthesis in two steps starting from E1; however, the overall yield is 39%.

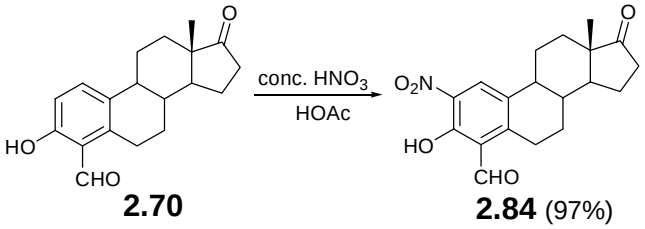


2.13, X = H, Y = CHO
2.14, X = CHO, Y = H

2.56, X = H, Y = CH_2OH (72%)
2.83, X = CH_2OH, Y = H (75%)

Scheme 2.46. Synthesis of triols **2.56** and **2.83** by reduction of **2.13** and **2.14**

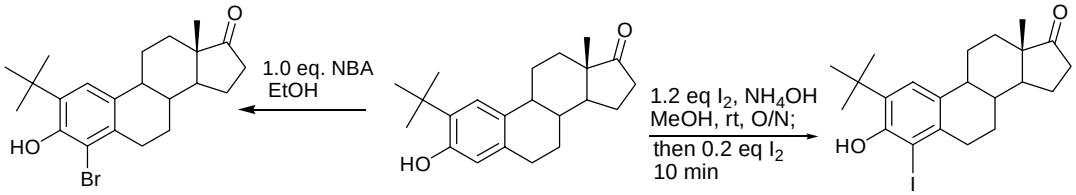
Now that we have an efficient synthesis of **2.13**, we can now easily prepare **2.13** derivatives modified at the 2-position. For example, we subjected **2.13** to HNO_3 in AcOH and obtained nitro derivative **2.84** in a 97% yield (Scheme 2.47). Additional modifications (halogenation, acylation, alkylation etc.) at the 2-position of **2.13** are in progress in the Taylor group.



2.70 **2.84** (97%)

Scheme 2.47. Synthesis of **2.84**

Our route to **2.13** can also be used for preparing other estrone derivatives modified at the 4-position. Although this has no connection to STS inhibitors, this is still important since (as we have shown throughout this chapter) the synthesis of estrone analogs bearing groups at the 4-position is challenging. This work has only just begun in the Taylor group. So far we have prepared 4-iodo- (**2.85**) and 4-bromo-2-*tert*-butylestrone (**2.86**) in 62% and 88% yields (neither optimized) (Scheme 2.48). Deprotections of these species are in progress. The synthesis of other estrone derivatives modified at the 4-position (NO_2, F, Cl, acyl, alkyl etc.) is in progress in the Taylor group.

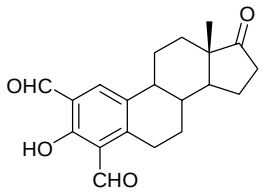


2.86 (88%) **2.71** **2.85** (62%)

Scheme 2.48. Iodination and bromination of **2.71**.

The 2-formyl isomer **2.14** did exhibit some inhibition with STS albeit considerably less than

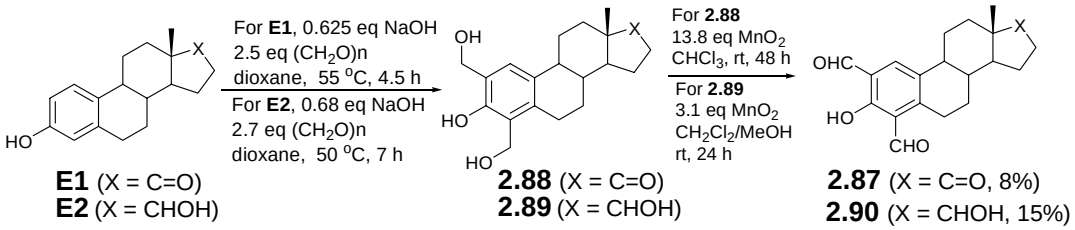
that of **2.13**. Therefore we reasoned that it might be possible to increase the potency of **2.13** by incorporating an additional aldehyde moiety at the 2-position (compound **2.87**, Figure 2.3).



2.87

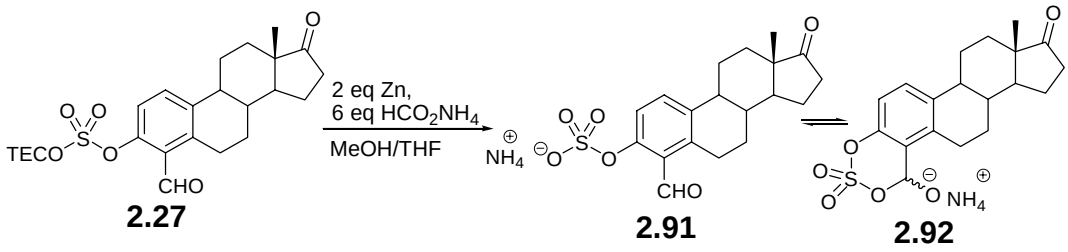
Figure 2.3. Structure of 2,4-diformyl estrone (**2.87**)

The synthesis of compound **2.87** was not easily accomplished and we have not yet succeeded in developing a good synthesis of this compound. Attempts to prepare **2.87** by direct bisformylation of E1 or by formylation of **2.13** using $MgCl_2$/paraformaldehyde was not successful. We also attempted a hydroxymethylation of **2.13** with paraformaldehyde and NaOH but this was also unsuccessful. Another route to **2.87** is by dihydroxymethylation of E1 followed by oxidation (Scheme 2.49). Dihydroxymethylation of E1 using paraformaldehyde and NaOH would not go to completion and the triol product **2.88** could not be purified by silica gel column chromatography even in the presence of triethylamine since it decomposed very quickly on the column. So crude **2.88** was subjected to MnO_2 in CH_2Cl_2; however, we could only isolate compound **2.87** in an 8% yield starting from estrone. We also tried E2 as substrate. Unfortunately, the overall yield of the bis-formylated product **2.90** was only slightly improved to 15% for the two steps. The inhibitory potency of dialdehydes **2.87** and **2.90** towards STS is currently under investigation.



Scheme 2.49. Synthesis of **2.87** and **2.90**.

We have also attempted to prepare 4-formylestrone sulfate (**2.91**) (Scheme 2.50) by deprotection of the sulfate in **2.27**. This is a particularly interesting compound since it could act either as a suicide inhibitor, an irreversible competitive inhibitor or a reversible competitive inhibitor. However, compound **2.91** was not stable. We even had to avoid heating when removing the solvent by rotary evaporation during workup. ^{1}H NMR and ^{13}C NMR of the reaction product revealed that it exists as an equilibrium mixture of aldehyde **2.91** and the hemiacetal **2.92.** Inhibition studies with this mixture and STS are in progress in the Taylor group.



Scheme 2.50. Synthesis of **2.91** and **2.92**

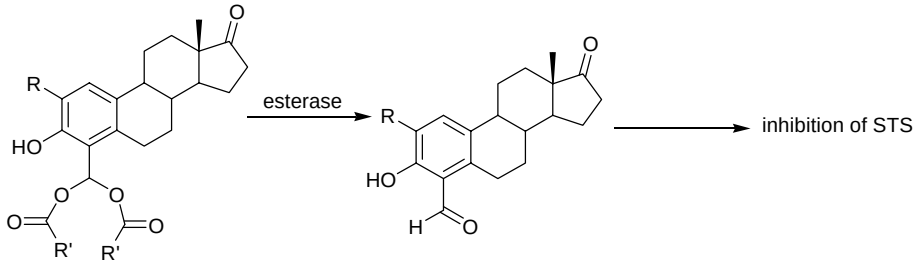
2.3 Summary and Future work

A series of compound (**2.4**-**2.11**) that were designed to act as suicide inhibitors of STS were synthesized. Some of these compounds (**2.6**, **2.7**, **2.10** and **2.11**) could not be prepared by traditional sulfation chemistry. Therefore, a new approach to the synthesis of aryl sulfates, recently developed in the Taylor group, was used. In this approach, the sulfate groups were introduced at the beginning

of syntheses as TCE-protected sulfodiesters using reagent **2.12**. Inhibition studies by Vanessa Ahmed in the Taylor group revealed that compounds **2.4**, **2.7** and **2.11** were indeed suicide inhibitors of STS. However, **2.4** was found to inhibit in a completely different manner from **2.7** and **2.11** in that 4-formyl estrone (**2.13**) a product resulting from the hydrolysis of an intermediate, was found to be the inhibitor. This result is highly significant since a new class of irreversible STS inhibitor was discovered. We wished to prepare analogs of **2.13** to see if we could improve upon its potency. However compound **2.13** is difficult to prepare in good yield using literature procedures. Therefore, a new, efficient synthesis of **2.13** compound was devised by formylating 2-*tert*-butyl estrone (**2.70**) followed by removal of the *t*-butyl group. In addition to being a route to **2.13**, this tactic of using a *t*-butyl protecting group at the 2-position has the potential of being a general method for the preparation of 4-substituted estrones and estradiols which are often very difficult to prepare.

The work described in this chapter is only the beginning of our work on the design of irreversible STS inhibitors. Studies to determine what amino acid residues are being modified by our inhibitors are in progress in the Taylor group by Vanessa Ahmed. It is known that STS can tolerate certain groups at the 2-position of estrone or estradiol derivatives which sometimes enhances their inhibitory potency and other biological properties. Consequently, future synthetic work will focus on the preparation of 2-substituted 4-formyl estrone derivatives. We anticipate that more potent STS inhibitors will be obtained from this series of compounds. Additional optimization studies of the formylation of **2.70** are in progress. We are aware that aldehyde-based inhibitors are often metabolically unstable in that aldehyde groups are readily oxidized to carboxylic acids *in vivo*. Thus, should highly potent inhibitors be obtained from these studies, they will be converted into acylal

prodrugs of type **2.93** for cellular studies. These prodrugs are designed to be activated by esterases[38] to release the aldehyde-bearing inhibitor (Scheme 2.51). Finally, as we have already mentioned, our tactic of using a *t*-butyl protecting group at the 2-position of E1 has the potential of being a general method for the preparation of 4-substituted estrones and estradiols. This is currently being examined in the Taylor group.



2.93

Scheme 2.51. Prodrug approach

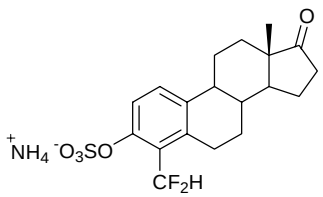
2.4 Experimental

2.4.1 General

All starting materials and reagents were obtained from Aldrich Chemical Company. Dichloromethane, acetonitrile and triethylamine were distilled from calcium hydride. DMF was distilled from calcium hydride under reduce pressure. Tetrahydrofuran (THF) and toluene were distilled from sodium/benzophenone. MnO_2 was activated by heating at 250 ^oC under high vacuum for 2 h. MOMCl (chloromethyl methyl ether) was prepared according to literature.[39] BuLi was titrated according to literature.[40.] Silica-gel chromatography was performed using silica gel 60 Å (230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Avance 300 spectrometer in $CDCl_3$ or CD_3OD at 300 MHz, 75 MHz and 282 MHz, respectively. NMR spectra are reported in parts per million (ppm) relative to internal

standards or solvent peaks. For NMR spectra run in $CDCl_3$, chemical shifts (δ) for ^1H NMR spectra are reported relative to internal Me_4Si (δ 0.0 ppm), chemical shifts (δ)for ^{13}C NMR spectra are relative to the solvent peak (δ 77.0 ppm, central peak), ^{19}F NMR relative to an external $CFCl_3$ (δ 0.0 ppm). For NMR spectra run in DMSO-d_6, chemical shifts (δ) for ^1H NMR spectra are reported relative to the residual solvent peak (δ 2.49 ppm), chemical shifts (δ) for ^{13}C NMR spectra are relative to the solvent peak (δ 39.5 ppm, central peak), ^{19}F NMR relative to an external $CFCl_3$ (δ 0.0 ppm). For NMR spectra run in CD_3OD, chemical shifts (δ) for ^1H NMR spectra are reported relative to the residual solvent peak (δ 3.31 ppm), chemical shifts (δ) for ^{13}C NMR spectra are relative to the solvent peak (δ 49.0 ppm, central peak), ^{19}F NMR relative to an external $CFCl_3$ (δ 0.0 ppm). Low-resolution (LRMS) and high-resolution (HRMS) electron impact (EI) mass spectra were recorded on a JEOL HX 110 double focusing mass spectrometer. Electrospray (ESI) mass spectra were obtained with a Waters/Micromass QTOF Ultima Global mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and uncorrected.

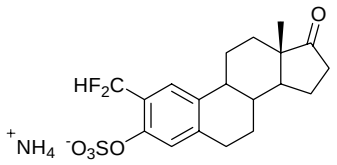
2.4.2 Syntheses



2.4

Ammonium 4-difluoromethyl-3-(sulfonatooxy)estra-1,3,5(10)-triene-17-one (2.4). To a solution of ester **2.29** (265 mg, 0.500 mmol) in HPLC grade MeOH (12 mL) was added ammonium formate (400 mg, 6.35 mmol, 12.7 equiv) and 10% Pd/C (53 mg of 20 wt%). The reaction mixture

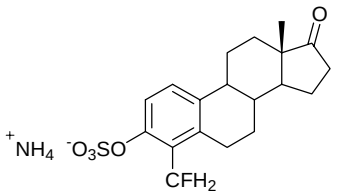
was stirred for 36 h. The Pd catalyst was removed by filtration and the solution was concentrated *in vacuo* to give a white residue. The residue was chromatographed on silica (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) as eluent. The residue was dissolved in water and lyophilized (three times) to afford **2.4** as a fluffy white powder (176 mg, 85%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.41 (d, J = 8.6 Hz, 1H, H-1), 7.25 (d, J = 8.9 Hz, 1H, H-2), 7.22 (t, J = 58.5 Hz, 1H, CHF_2), 3.30-3.19 (m, 1H, H-6), 3.10-2.98 (m, 1H, H-6), 2.52-1.99 (m, 6H), 1.89 (d, J = 9.2 Hz, 1H), 1.69-1.35 (m, 6H), 0.90 (s, 3H, CH_3, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 222.4 (C=O), 149.3 (t, J = 6.9 Hz, C-3), 138.2 (C-5), 137.5 (C-6), 128.4 (C-1), 124.2 (t, J = 21.5 Hz, C-4), 119.9 (C-2), 113.6 (t, J = 233 Hz, CF_2), 50.3 (C-14), 47.9 (C-13), 44.2 (C-7), 37.5 (C-8), 35.4 (C-16), 31.4 (CH_2), 25.8 (CH_2), 25.7 (CH_2), 21.9 (CH_2), 12.9 (CH_3, C-18); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ –114.51 (dd, J_{gem} = 311 Hz, $J_{H\text{-}F}$ = 53.6 Hz), -116.16 (dd, J_{gem} = 311 Hz, $J_{H\text{-}F.}$ = 53.6 Hz); LRMS (ESI) *m/z* (%) 399.1 (100); HRMS (ESI) calcd for $C_{19}H_{21}F_2O_5S^-$ 399.1083; found 399.1083.



2.5

Ammonium 2-difluoromethyl-3-(sulfonatooxy)estra-1,3,5(10)-triene-17-one (2.5). Prepared using the procedure described for **2.4** using ester **2.30** (498 mg, 0.94 mmol), ammonium formate (370 mg, 5.87 mmol, 6 equiv) and Pd/C (75 mg of 15 wt. %) in MeOH (12 mL) and stirred for 24 h. **2.5** was obtained as a fluffy white powder (337 mg, 86%) after chromatography using (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) and lyophilization. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.47 (s, 1H, H-1), 7.20 (s, 1H, H-4), 7.03 (t, J = 56 Hz, 1H, CHF_2), 4.86 (s, 4H, NH_4), 3.00-2.90 (m, 2H),

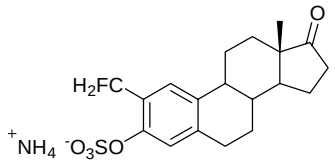
2.55-1.20 (m, 13H), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 222.5 (C=O), 148.0 (t, J = 7 Hz, C-3), 140.8 (t, J = 2 Hz, C-5), 137.2 (C-10), 124.5 (t, J = 23 Hz, C-2), 122.3 (t, J = 5 Hz, C-1), 122.2 (C-4), 112.0 (t, J = 233 Hz, CF_2), 50.2 (C-14), 47.9 (C-13), 43.9 (C-9), 38.0 (C-8), 35.4 (C-16), 31.3 (CH_2), 29.1(CH_2), 25.9(CH_2), 25.6(CH_2), 21.2(CH_2), 12.9(CH_3, C-18); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ -113.8 (d, J = 55.6 Hz); HRMS (ESI) calcd. for $C_{19}H_{21}F_2O_5S$ 399.1083, found 399.1096.



2.6

Ammonium 4-fluoromethyl-3-(sulfonatooxy)estra-1,3,5(10)-triene-17-one (2.6). Prepared using the procedure for **2.4** using TCE sulfate **2.33** (393 mg, 0.765 mmol), HCO_2NH_4 (600 mg, 9.52 mmol, 12.4 equiv), 10% Pd/C (80 mg, 20 wt%), MeOH (50 mL) and stirring for 24 h. **2.6** was obtained as a fluffy white powder (265 mg, 86%) after chromatography using (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) and lyophilization: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.34 (d, J = 8.6 Hz, 1H, H-1), 7.25 (d, J = 8.7 Hz, 1H, H-2), 5.63 (d, J = 48.2 Hz, 2H, CH_2F), 3.10 (dd, J = 17.4 Hz, J = 5.1 Hz, 1H, H-6), 2.98-2.87 (m, 1H, H-6), 2.52-2.00 (m, 6H), 1.88 (d, 1H, J = 10.0 Hz), 1.70-1.36 (m, 6H), 0.90 (s, 3H, CH_3); ^{13}C NMR (CD_3OD, 75 MHz) δ 222.3 (C=O), 149.2 (d, J = 4.8 Hz, C-3), 138.3 (d, J = 2.0 Hz, C-10), 137.2 (d, J = 3.2 Hz, C-5), 126.9 (d, J = 4.0 Hz, C-1), 126.6 (d, J = 14.2 Hz, C-4), 119.4 (d, J = 2.6 Hz, C-2), 77.12 (d, J = 158.6 Hz, CH_2F), 50.2 (C-14), 47.8 (C-13), 44.3 (C-9), 37.5 (C-8), 35.4 (C-16), 31.4 (CH_2), 26.0 (CH_2), 25.8 (CH_2), 21.1 (CH_2), 12.8 (CH_3, C-18); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ –134.0 (t, $J_{H\text{-}F}$ = 47.9 Hz); LRMS (ESI) *m/z* (%) 381.0960

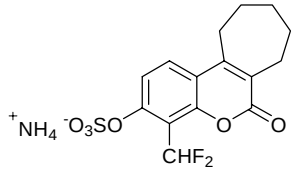
(100); HRMS (EI) calcd for $C_{19}H_{22}FO_5S$ 388.1172; found 388.1174.



2.7

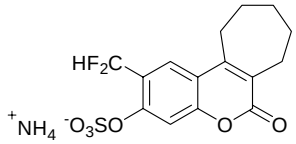
Ammonium 2-fluoromethyl-3-(sulfonatooxy)estra-1,3,5(10)-triene-17-one (2.7). ***Method A*** (CTH): To a solution of the ester **2.34** (395 mg, 0.77 mmol) in HPLC grade MeOH (20 mL) was added ammonium formate (291 mg, 4.62 mmol, 6 equiv) and 80 mg (20 wt. %) of 10% Pd/C. The reaction mixture was stirred for 24 h. After filtration and removal of MeOH by rotary evaporation, the residue was charged with ammonium formate (260 mg, 4.13 mmol), 20 mg of Pd black and MeOH (8 mL). The reaction mixture was stirred for 24 h. The Pd catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give a white residue. The residue was chromatographed on silica (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5). The residue was dissolved in water and lyophilized (three times) to afford the sulfate **2.7** as a fluffy powder (230 mg, 75 %). ***Method B*** (Zn/HCO_2NH_4): To a solution of ester **2.34** (400 mg, 0.779 mmol) in THF/MeOH (1:3, 16 mL) was added HCO_2NH_4 (300 mg, 4.76 mmol, 6 equiv) and Zn powder (102 mg, 1.57 mmol, 2 equiv) and stirred for 15 min. The reaction was filtered and the filtrate concentrated *in vacuo* to give a white residue. The residue was chromatographed on silica (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5). The residue was dissolved in water and lyophilized (three times) to afford **2.7** as a fluffy white powder (305 mg, 98%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.34 (s, 1H, H-1), 7.16 (s, 1H, H-4), 5.46 (d, J = 48 Hz, 2H, CH_2F), 4.87 (s, 4H, NH_4), 2.92-1.84 (m, 2H), 2.53-2.38 (m, 2H), 2.30-1.85 (m, 5H), 1.70-1.35 (m, 6H), 0.90 (s, 3H, CH_3, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 222.5 (C=O), 148.0 (d, J = 5 Hz, C-3), 138.4 (d, J = 3 Hz, C-5),

136.9 (d, J = 2 Hz, C-10), 126.6 (d, J = 17 Hz, C-2), 126.0 (d, J = 7 Hz, C-1), 121.9 (d, J = 1 Hz, C-4), 80.0 (d, J = 159 Hz, CH_2F), 50.2 (C-14), 47.9 (C-13), 44.0 (C-9), 38.1 (C-8), 35.5 (C-16), 31.4 (CH_2), 29.0 (CH_2), 26.1 (CH_2), 25.6 (CH_2), 21.2 (CH_2), 13.0 (CH_3, C-18); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ -134 (t, J = 52 Hz); HRMS (ESI) calcd. for $C_{19}H_{22}O_5FS$ 381.1177, found 381.1186; calcd. for $C_{19}H_{23}O_6S$ (F is hydrolysed to OH) 379.1220, found 379.1226; calcd. for $C_{19}H_{23}O_3$ (F is hydrolysed to OH, followed by loss of SO_3) 299.1652, found 299.1649.



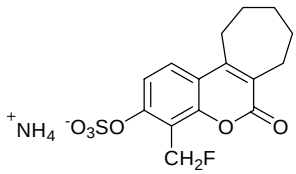
2.8

Ammonium 4-difluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*] chromen-3-yl sulfate (2.8). Prepared using the procedure for **2.4** using TCE sulfate **2.42** which gave ammonium sulfate **2.8** as fluffy white solid (70%) after chromatography (CH_2Cl_2/MeOH/NH_4OH = 10:2:0.5) and lyophilization. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.95 (d, J = 9.1 Hz, 1H, H-1), 7.53 (d, J = 9.0 Hz, 1H, H-2), 7.22 (t, J = 53.3 Hz, 1H, CHF_2), 3.02 (t, J = 5.3 Hz, 2H), 2.88 (t, J = 5.4 Hz, 2H), 1.92 (quint, J = 5.8 Hz, 2H), 1.72-1.55 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 162.3 (C=O), 155.7 (C_{Ar}), 154.2 (C_{Ar}), 152.3 (C_{Ar}), 128.9 (C_{Ar}), 128.7 (C_{Ar}), 119.5 (C_{Ar}), 118.2 (C_{Ar}), 115.2 (t, J = 22.7 Hz, C-4), 112.5 (t, J = 235 Hz, CHF_2), 32.9 (CH_2), 28.9 (CH_2), 27.5 (CH_2), 26.6 (CH_2), 26.1 (CH_2); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ -115.6 (t, J = 53 Hz); LRMS (ESI) *m/z* (%) 359 (100); HRMS (ESI) calcd. for $C_{15}H_{13}FO_6S$ 359.0406, found 359.0393.



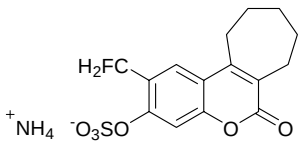
2.9

Ammonium 2-difluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*] chromen-3-yl sulfate (2.9). ***Method A*** (CTH): Prepared using the procedure for **2.4** using TCE sulfate **2.43** gave ammonium sulfate **2.9** as fluffy white solid (65%) after flash chromatography using (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) and lyophilization. ***Method B*** (Zn/HCO_2NH_4): Prepared using the same procedure for **2.7** using **2.43** (32 mg, 0.065 mmol), Zn (9.0 mg, 0.14 mmol, 2 equiv) and HCO_2NH_4 (25 mg, 0.40 mmol, 6 equiv) in THF (1 mL)/MeOH (3 ML) for 30 min. **2.9** (23 mg, 94%) was obtained as a white solid after flash chromatography. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.98 (s, 1H, H-1), 7.52 (s, 1H, H-4), 7.28 (t, J = 55.2 Hz, 2H, CH_2F), 3.04 (t, 2H, J = 5.2 Hz), 2.88 (t, J = 5.4 Hz, 2H), 1.93 (quint, J = 5.6 Hz, 2H), 1.70 (quint, J = 5.0 Hz, 2H), 1.59 (quint, J = 4.9 Hz, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ 161.9 (C=O, C-6), 154.1 (C_{Ar}, C-4a and C-11a overlapping), 152.3 (t, C-3), 127.6 (C-4), 123.3 (t, J = 21.8 Hz, C-2), 121.8 (t, J = 5.7 Hz, C-1), 116.3 (C-6a), 111.2 (t, J = 234 Hz, CHF_2), 109.2 (C-1a), 31.6 (CH_2), 27.6 (CH_2), 26.2 (CH_2), 25.2 (CH_2), 24.7 (CH_2); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ -114.8 (d, J = 55 Hz); LRMS (ESI) *m/z* (%) 359 (100); HRMS (ESI) calcd. for $C_{15}H_{13}FO_6S$ 359.0406, found 359.0393.



2.10

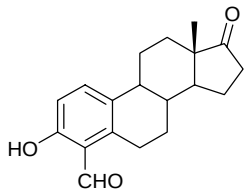
Ammonium 4-fluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*] chromen-3-yl sulfate (2.10). Prepared using the procedure for **2.4** using TCE sulfate **2.46** gave ammonium sulfate **2.8** (68%) as fluffy white solid after chromatography (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) and lyophilization. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.85 (d, J = 8.8 Hz, 1H, H-1), 7.53 (d, J = 8.9 Hz, 1H, H-2), 5.69 (d, J = 47.8 Hz, 2H, CH2F), 3.01-2.95 (m, 2H), 2.88-2.82 (m, 2H), 1.90 (pseudo s, 2H), 1.70-1.50 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 163.4 (C=O), 156.3 (C_{Ar}), 154.8 (C_{Ar}), 153.2 (C_{Ar}), 128.1 (C_{Ar}), 127.4 (d, J = 3.6 Hz, C-1), 119.1 (C_{Ar}), 117.9 (C_{Ar}), 117.6 (d, J = 15.3 Hz, C-4), 74.2 (d, J = 163 Hz, CH_2F), 33.0 (CH_2), 29.0 (CH_2), 27.5 (CH_2), 26.7 (CH_2), 26.1 (CH_2); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ -133.3 (t, J = 48 Hz); LRMS (ESI) *m/z* (%) 359 (51), 341 (100); HRMS (ESI) calcd. for $C_{15}H_{14}FO_6S$ 341.0500, found 341.0508.



2.11

Ammonium 2-fluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*] chromen-3-yl sulfate (2.11). To a stirred solution of **2.47** (195 mg, 0.412 mmol) in THF (3 mL) was added MeOH (9 mL), followed by ammonium formate (156 mg, 2.48 mmol, 6 equiv). Once the reaction mixture went clear, zinc dust (54 mg, 0.83 mmol, 2 equiv) was added slowly and stirring was continued for 20 min. The reaction was filtered and the filtrate concentrated and the residue chromatographed (CH_2Cl_2/MeOH/NH_4OH,10:2:0.5). The chromatographed material was dissolved in water and lyophilized (three times) to afford **2.11** as a fluffy white powder (136 mg, 92%). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.81 (s, 1H, H-1), 7.46 (s, 1H, H-4), 5.52 (d, J = 47.7 Hz, 2H, CH_2F), 3.00-2.97 (m, 2H),

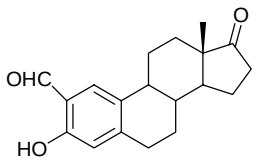
2.85-2.82 (m, 2H), 1.93-1.85 (m, 2H), 1.70-1.50 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 162.3 (C=O), 154.5 (C-11a), 152.9 (C_{Ar}), 152.2 (d, J = 3.8 Hz, C-3), 126.9 (C_{Ar}), 126.7 (d, J = 17.1 Hz, C-2), 124.6 (d, J = 8.0 Hz, C-1), 116.2 (C-6a), 108.7 (C-4), 79.2 (d, J = 164 Hz, CH_2F), 31.6 (CH_2), 27.5 (CH_2), 26.1 (CH_2), 25.3 (CH_2), 24.7 (CH_2); ^{19}F NMR (CD_3OD, 282 MHz, coupled) δ -136.4 (t, $J_{H\text{-}F}$ = 47.7 Hz); LRMS (ESI) *m/z* (%) 341.0495 (100); HRMS (ESI) calcd for $C_{15}H_{14}FO_6S$ 341.0495; found 341.0490.



2.13

4-Formylestra-1,3,5(10)-triene-17-one (2.13). ***Method A*** (reduction of nitrile **2.52** using Raney-Nickel): To a mixture of **2.52** (50 mg, 0.17 mmol) in 60% HCO_2H was added excess Raney-nickel (1.60 g). The resulting mixture was heated at 140 ^{o}C for 2 days. After cooling to rt, the mixture was treated with 10% HCl and extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated. The residue was subjected to chromatography (ethyl acetate/hexane, 1:2 to 100% ethyl acetate) to give pure **2.13** (10 mg, 20%) and unreacted **2.52** (16 mg, 32%). ***Method B*** (oxidation of diol **2.63** using MnO_2): To a solution of **2.63** (10.0 mg, 0.033 mmol) in CH_2Cl_2/MeOH (12 mL, 5:1) was added MnO_2 (180 mg, 2.07mmol, 62 equiv). The resulting mixture was stirred for 24 h at rt. After filtration and concentration, the residue was purified by flash chromatography (ethyl acetate/hexane 1:2) to give **2.13** as light yellow solid (9.9 mg, 100%). ***Method C*** (de-t-butyl of **2.78**): To a solution of **2.78** and **2.79** (14:1, 0.698 g, app. 2.14 mmol) in CH_2Cl_2 (10 mL) was added

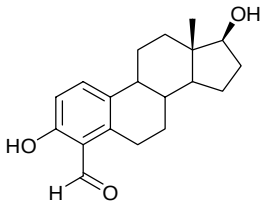
nitromethane (2.5 mL, app. 23 equiv). The resulting mixture was cooled to 0 ^{o}C and anhydrous $AlCl_3$ (700 mg, 5.24 mmol, app. 2.5 equiv) was added. After stirring 4 h at rt the reaction was quenched with ice water and 1N HCl. The mixture was extracted with CH_2Cl_2/EtOAc/Ether (3:5:5) and the combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by chromatography (methylene chloride) gave **2.13** as yellow crystalline solid (454 mg, 51% yield over 2 steps from **2.70**). ^{1}H NMR was identical to that reported in the literature.[11] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.96 (s, 1H, OH), 10.34 (s, 1H, CHO), 7.44 (d, J = 8.9 Hz, 1H, H-1), 6.76 (d, J = 9.0 Hz, 1H, H-2), 3.34 (dd, J = 17.1 Hz, J = 5.7 Hz, 1H, H-6), 3.19-3.07 (m, 1H), 2.48 (dd, J = 18.9 Hz, J = 9.3 Hz, 1H), 2.36-1.90 (m, 6H), 1.67-1.35 (m, 6H), 0.89 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.4 (C=O), 195.5 (CHO), 161.5 (C-3), 139.3 (C-5), 135.4 (C-1), 131.0 (C-10), 117.4 (C-1), 115.8 (C-2), 50.7 (C-14), 47.8 (C-13), 43.8 (C-9), 37.4 (C-4), 35.8 (C-16), 31.5 (C-12), 26.1 (CH_2), 26.0 (CH_2), 25.4 (CH_2), 21.4 (CH_2), 13.8 (CH_3, C-18).



2.14

2-Formylestra-1,3,5(10)-triene-17-one (2.14). To a mixture of E1 (1.35 g, 5.0 mmol), paraformaldehyde (1.50 g, 50.0 mmol, 10 equiv) and anhydrous magnesium chloride (1.25 g, 13.0 mmol, 2.6 equiv) in glass bomb was added acetonitrile (20 mL), followed by tributylamine (5.5 mL, 23 mmol, 4.6 equiv) at room temperature. After stirring 2 minutes, the tube was sealed and heated at 125 °C for 18 h. After cooling to rt, 6N HCl (15 mL) was added slowly and the resulting mixture was extracted with diethyl ether. The extracts were combined, washed with H_2O and brine then dried

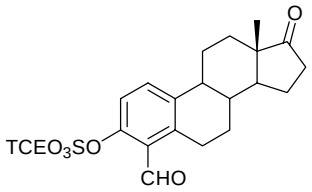
(Na_2SO_4) filtered and concentrated. Column chromatography (ethyl acetate/hexane, 1:2) gave **2.14** as a colorless solid (768 mg, 52% yield). When this reaction was performed using 5.4 g of E1 an identical yield was obtained. The ^{1}H NMR was identical to that reported in the literature.[11] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.65 (s, 1H, OH), 9.65 (s, 1H, CHO), 7.29 (s, 1H, H-1), 6.53 (s, 1H, H-4), 2.85-2.75 (m, 2H), 2.22-1.80 (m, 7H), 1.60-1.25 (m, 6H), 0.80 (s, 3H, CH_3, H-18).



2.16

4-Formyl-17*β*-hydroxylestra-1,3,5(10)-triene (2.16). ***Method A*** (by oxidation of triol **2.66** using MnO_2): To a solution of **2.66** (8 mg, 0.027mmol) in and CH_2Cl_2/MeOH (12 mL, 5:1) was added MnO_2 (180 mg, 2.07 mmol, 78 equiv). After stirring 24 h at rt, the insoluble solid was removed by filtration. The filtrate was washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane 1:2) to give of **2.16** as light yellow solid (8 mg, 100%). ***Method B*** (by de-t-butylation of **2.78** using $AlCl_3$): To a solution of the inseparable mixture of **2.78** and **2.79** (701 mg, app. 2.14 mmol) in CH_2Cl_2 (10 mL) at 0 ^oC was added nitromethane (2.5 mL, app. 23 equiv), followed by anhydrous $AlCl_3$ (850 mg, 6.37 mmol, 3 equiv). The resulting mixture was stirred for 4.5 h at rt before quenching with ice and 1 M HCl. 30 mL of CH_2Cl_2 was added and the mixture was extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (CH_2Cl_2) gave pure **2.16** as light yellow solid (290 mg, 32% over 3

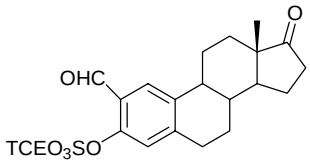
steps from **2.70**). ^{1}H NMR was identical to that reported in the literature.[11] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.94 (s, 1H, ArOH), 10.32 (s, 1H, CHO), 7.44 (d, J = 8.9 Hz, 1H, H-1), 6.74 (d, J = 8.8 Hz, 1H, H-2), 3.70 (t, J = 8.4 Hz, 1H), 3.18-2.98 (m, 2H), 2.28-1.15 (m, 13H), 0.75 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.7 (CHO), 161.4 (C-3), 139.6 (C-5), 135.5 (C-1), 131.7 (C-10), 117.5 (C-4), 115.6 (C-2), 81.7 (C-17), 49.7 (C-14), 43.8 (C-9), 43.1 (C-13), 38.0 (C-8), 36.6 (CH_2), 30.5 (CH_2), 26.7 (CH_2), 26.5 (CH_2), 25.5 (CH_2), 23.0 (CH_2), 11.1 (CH_3, C-18).



2.27

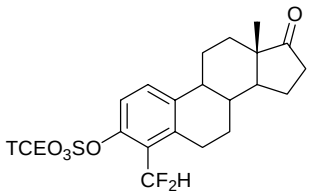
4-Formylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.27). To a solution of **2.13** (405 mg, 1.36 mmol), and DMAP (168 mg, 1.38 mmol, 1 equiv) in dry THF (40 mL) was added triethylamine (0.42 mL, 3 mmol, 2.2 equiv) followed by reagent **2.12** (0.40 mL, 3 mmol, 2.2 equiv). The mixture was then stirred overnight (14 h) at rt. The mixture was diluted with ethyl acetate and H_2O. After separation, the aqueous phase was extracted with ethyl acetate. The combined organics were washed with 0.5 N HCl, water, and brine, then dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue (ethyl acetate/hexane, 1:3 to 1:2.25) yielded pure **2.27** as a light yellow solid (690 mg, 99%). Mp: 104-105 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H, CHO), 7.53 (d, J = 8.8 Hz, 1H, H-1), 7.24 (d, J = 8.7 Hz, 1H, H-2), 4.83 (s, 2H, OCH_2CCl_3), 3.23 (dd, J = 18.9 Hz, J = 5.1 Hz, 1H, H-6), 3.11-2.99 (m, 1H), 2.44-2.20 (m, 3H), 2.09-1.93 (m, 3H), 1.86 (d, J = 12.4 Hz, 1H), 1.59-1.27 (m, 6H), 0.80 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.2 (C=O), 189.8 (CHO), 150.0 (C-3), 141.6 (C-5), 141.4 (C-10), 132.1 (C-1),

126.0 (C-4), 119.2 (C-2), 92.4 (CCl_3), 80.7 ($O\underline{C}H_2CCl_3$), 50.2 (C-14), 47.7 (C-13), 44.4 (C-9), 36.7 (C-8), 35.8 (C-16), 31.5 (CH_2), 27.5 (CH_2), 26.0 (CH_2), 26.0 (CH_2), 21.5 (CH_2), 13.8 (CH_3, C-18); LRMS (ESI) *m/z* (%) 528 ($M+2+NH_4^+$), 526 ($M+NH_4^+$), 511 ($M+2+H^+$), 509 ($M+H^+$); HRMS (EI) calcd for $[C_{21}H_{24}Cl_3O_6S+H]^+$ 509.0363; found 509.0353.



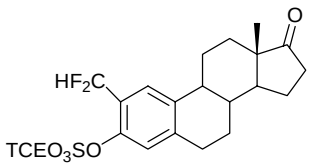
2.28

2-Formylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.28). Prepared using the procedure described for **2.27** using **2.14** (1.38 g, 4.63 mmol), DMAP (534 mg, 4.45 mmol), triethylamine (1.3 mL, 9.3 mmol, 2 equiv) and reagent **2.12** (1.2 mL, 9.0 mmol, 1.9 equiv) in THF (50 mL) and stirring for 14 h. Column chromatography of the residue (ethyl acetate/hexane, 1:2) yielded **2.28** as colorless oil (2.28 g, 97%) that solidified on standing. Mp:138-139 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.21 (s, 1H, OH), 7.85 (s, 1H, H-1), 7.24 (s, 1H, H-4), 4.92 (s, 2H, CH_2), 3.05-2.90 (m, 2H), 1.55-1.95 (m, 7H), 1.70-1.40 (m, 6H), 0.90 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.2 (C=O), 187.4 (CHO), 148.4 (C-3), 146.4 (C-5), 140.7 (C-10), 128.1 (C-1), 125.8 (C-2), 122.4 (C-4), 92.4 (CCl_3), 80.7 ($O\underline{C}H_2CCl_3$), 50.3 (C-14), 47.8 (C-13), 43.9 (C-9), 37.6 (C-8), 35.8 (C-16), 31.4 (CH_2), 29.9 (CH_2), 25.8 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 510 (M+2, 93), 508 (M^+, 94), 466 (74), 464 (70), 451 (44), 361 (56), 298 (100), 241 (89); HRMS (EI) calcd. for $C_{21}H_{23}Cl_3O_6S$ 508.0281, found 508.0291.



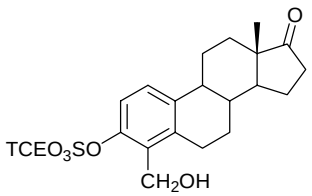
2.29

4-Difluoromethylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.29). To a solution of **2.27** (259 mg, 0.508 mmol) in dry methylene chloride (10 mL) at 0°C was added DAST (diethylaminosulfur trifluoride, 0.2 mL, 1.51 mmol, 3 equiv) via syringe. The pale orange mixture was stirred for 1 h at that temperature, then 12 h at room temperature. After quenching with saturated $NaHCO_3$, the reaction mixture was stirred for additional 10 min and extracted with ethyl acetate. The combined organic extracts were washed with water and brine then dried (Na_2SO_4) filtered and concentrated. Column chromatography of the residue (ethyl acetate/hexane, 1:3 to 1:1.25) yielded pure **2.29** as a white foam (225 mg, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45 (d, J = 8.7 Hz, 1H, H-1), 7.25 (d, J = 8.6 Hz, 1H, H-2), 7.01 (t, J = 53.4 Hz, 1H, CHF_2), 4.82 (s, 2H, OCH_2CCl_3), 3.24-2.97 (m, 2H), 2.50-1.90 (m, 7H), 1.63-1.35 (m, 6H), 0.86 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.3 (C=O), 146.6 (t, J = 5.2 Hz, C-3), 141.5 (C-5), 139.3 (C-10), 129.7 (C-1), 123.5 (t, J = 44.0 Hz, C-4), 118.6 (C-2), 111.9 (t, J = 273.5 Hz, CHF_2), 92.4 (CCl_3), 80.8 (OCH_2CCl_3), 50.4 (C-14), 47.8 (C-13), 44.3 (C-9), 37.0 (C-8), 35.9 (C-16), 31.5 (CH_2), 25.9 (CH_2), 25.8 (CH_2), 21.6 (CH_2), 13.9 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -112.83 (dd, J_{gem} = 316 Hz, $J_{H\text{-}F}$ = 53.6 Hz), -113.87 (dd, J_{gem} =316 Hz, $J_{H\text{-}F}$ = 53.6 Hz); LRMS (EI) *m/z* (%) 534 (M+4, 39), 532 (M+2, 100), 530 (M^+, 97), 490 (32), 488 (79), 486 (77), 475 (57), 473 (55), 301 (62), 275 (37), 263 (97), 97 (86); HRMS (EI) calcd for $C_{21}H_{23}Cl_3F_2O_5S$ 530.0299; found 530.0303.



2.30

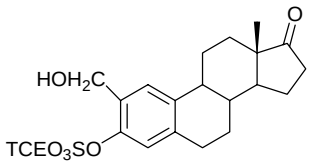
2-Difluoromethylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.30). Prepared using the procedure described for **2.29** using **2.28** (385 mg, 0.756 mmol), methylene chloride (10 mL) and DAST (diethylaminosulfur trifluoride, 0.300 mL, 2.27 mmol, 3 equiv). Column chromatography of the residue (ethyl acetate/hexane, 2:5) yielded **2.30** as a white foam (367 mg, 91%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (s, 1H, H-1), 7.20 (s, 1H, H-4), 6.82 (t, J = 55 Hz, 1H, CHF_2), 4.82 (s, 2H, CH_2), 2.95-2.85 (m, 2H), 2.50-1.90 (m, 7H), 1.65-1.35 (m, 6H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.3 (C=O), 145.4 (t, J = 5 Hz, C-3), 142.3 (t, J = 2 Hz, C-5), 140.3 (C-10), 124.5 (t, J = 6 Hz, C-1), 123.6 (t, J = 23 Hz, C-2), 121.3 (C-4), 111.3 (t, J = 237 Hz, CF_2), 92.4 (CCl_3), 80.6 ($O\underline{C}H_2CCl_3$), 50.3 (C-14), 47.8 (C-13), 44.0 (C-9), 37.6 (C-8), 35.8 (C-16), 31.4 (CH_2), 29.5 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz, decoupled) δ -112.2 (d, J_{gem} = 313 Hz), -112.4 (d, J_{gem} = 313 Hz); LRMS (EI) *m/z* (%) 532 (M+2, 60), 530 (M^+, 60), 488 (46), 486 (44), 475 (34), 473 (30), 301 (47), 263 (66), 97 (100); HRMS (EI) calcd. for $C_{21}H_{23}Cl_3F_2O_5S$ 530.0300, found 530.0308.



2.31

4-Hydroxymethylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.31). A

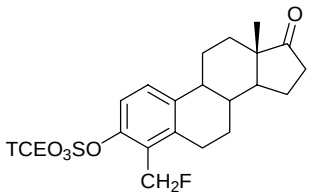
mixture of $Zr(O^iPr)_4 \cdot {}^iPrOH$ (194 mg, 0.500 mmol, 1.1 equiv) and (±)-BINOL (142 mg, 0.500 mmol, 1.1 equiv) in toluene (1 mL) was heated at 60 °C for 65 min. After cooling to room temperature, a solution of **2.27** (230 mg, 0.450 mmol, 1 equiv) in toluene (10 mL) was added. The reaction mixture was stirred for 3.5 h at room temperature. The reaction was quenched with saturated $NaHCO_3$ and extracted with ethyl acetate. The combined organic extracts were washed with water and brine then dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue (ethyl acetate/hexane, 1:2 to 1:1.5) yielded pure **2.31** as a white foam (161 mg, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, J = 8.8 Hz, 1H, H-1), 7.16 (d, J = 8.7 Hz, 1H, H-2), 4.86 (s, 2H, OCH_2CCl_3), 4.71 (t, J = 12.6 Hz, 2H, Ar$\underline{C}H_2$OH), 3.17 (dd, J = 17.4 Hz, 5.1 Hz, 1H, H-6), 3.02-2.90 (m, 1H, H-6), 2.52-2.00 (m, 6H), 1.93 (d, J = 8.5 Hz, 1H), 1.64-1.36 (m, 6H), 0.86 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.8 (C=O), 146.7 (C-3), 140.8 (C-5), 139.5 (C-10), 130.7 (C-4), 126.9 (C-1), 118.4 (C-2), 92.5 (CCl_3), 80.6 (O$\underline{C}H_2CCl_3$), 56.0 (Ar$\underline{C}H_2$OH), 50.4 (C-14), 47.9 (C-13), 44.5 (C-9), 37.2 (C-8), 35.9 (C-16), 31.6 (CH_2), 26.2 (CH_2), 26.1 (CH_2), 26.0 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 512 (M+2, 1), 510 (M^+, 2), 496 (25), 494 (62), 492 (60), 363 (23), 282 (47), 281 (100), 185 (36), 97 (67); HRMS (EI) calcd for $C_{21}H_{23}Cl_3O_5S$ (M-H_2O) 492.0335; found 492.0332.



2.32

2-Hydroxymethylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.32). Prepared using the procedure described for **2.31** using $Zr(O^iPr)_4 \cdot {}^iPrOH$ (1.34 g, 3.46 mmol) and (±)-BINOL (981 mg, 3.43 mmol, 1 equiv) in toluene (12 mL) and **2.28** (1.81 g, 3.55 mmol, 1 equiv) in

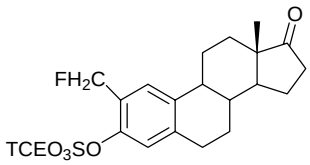
toluene (5 mL) and stirring for 30 min. Column chromatography of the residue (ethyl acetate/hexane, 2:3) yielded pure **2.32** as a white foam (1.72 g, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44 (s, 1H, H-1), 7.12 (s. 1H, H-4), 4.88 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 2.95-2.85 (m, 2H), 2.75-1.95 (m, 7H), 1.70-1.40 (m, 6H), 0.90 (3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 221.5 (C=O), 145.6 (C-3), 139.6 (C_{Ar}), 138.1 (C_{Ar}), 130.5 (C-1), 127.0 (C-2), 120.9 (C-4), 92.6 (CCl_3), 80.4 ($O\underline{C}H_2CCl_3$), 59.4 ($Ar\underline{C}H_2O$), 50.3 (C-14), 48.0 (C-13), 44.2 (C-9), 37.8 (C-8), 35.9 (C-16), 31.5 (CH_2), 29.2 (CH_2), 26.2 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 512 (M+2, 89), 510 (M^+, 85), 495 (22), 493 (22), 468 (23), 466 (23), 455 (22), 453 (23), 363 (55), 299 (95), 282 (100), 226 (55), 159 (57), 97 (71); HRMS (EI) calcd. for $C_{21}H_{25}Cl_3O_6S$ 510.0437, found 510.0440.



2.33

4-Fluoromethylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.33). This was prepared from **2.31** (130 mg, 0.254 mmol) and DAST (0.1 mL, 0.757 mmol, 3 equiv) using the procedure described for **2.29** except the reaction was stirred for 1 h at 0 ^{o}C and 4 h at room temperature. Column chromatography (ethyl acetate/hexane, 4:11) yielded pure **2.34** as a pale yellow oil (122 mg, 92%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41 (d, J = 8.8 Hz, 1H, H-1), 7.26 (d, J = 10.1 Hz, 1H, H-2), 5.52 (d, J = 47.7 Hz, 2H, CH_2F), 4.83 (s, 2H, OCH_2CCl_3), 3.15-2.90 (m, 2H, H-6), 2.63-2.24 (m, 3H), 2.18-2.01 (m, 3H), 1.95 (d, 1H, J = 9.4 Hz), 1.65-1.40 (m, 6H), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.4 (C=O), 147.3 (d, J = 4.5 Hz, C-3), 140.6 (d, J = 2.8 Hz,

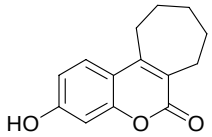
C-5), 140.0 (d, J = 1.8 Hz, C-10), 128.5 (d, J = 3.8 Hz, C-1), 125.7 (d, J = 14.9 Hz, C-4), 118.3 (d, J = 2.2 Hz, C-2), 92.5 (CCl_3), 75.7 (d, J = 165 Hz, one peak was hidden under $CDCl_3$, CH_2F), 80.6 ($O\underline{C}H_2CCl_3$), 50.4 (C-14), 47.8 (C-13), 44.4 (C-9), 37.2 (C-8), 35.9 (C-16), 31.5 (CH_2), 26.2 (CH_2), 26.1 (CH_2), 26.0 (CH_2), 21.6 (CH_2), 13.8 (CH_3); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -132.9 (t, $J_{H\text{-}F}$ = 47.7 Hz); LRMS (EI) *m/z* (%) 516 (M+4, 38), 514 (M+2, 100), 512 (M^+, 98), 472 (18), 470 (47), 468 (43), 457 (34), 455 (38), 395 (26), 283 (56), 245 (80), 97(93); HRMS (EI) calcd for $C_{21}H_{24}Cl_3FO_5S$ 512.0394; found 512.0391.



2.34

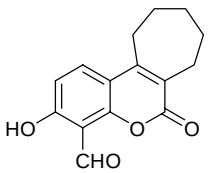
2-Fluoromethylestra-1,3,5(10)-triene-17-one-3-(2,2,2-trichloroethyl) sulfate (2.34). This was prepared from **2.32** (257 mg, 0.50 mmol) and DAST (0.20 mL, 1.5 mmol, 3 equiv) using the procedure described for **2.29** except the reaction was stirred for 1 h at 0 ^{o}C and 4 h at room temperature. Column chromatography (ethyl acetate/hexane, 4:9) yielded pure **2.34** as a white foam (231 mg, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39 (s, 1H, H-1), 7.18 (s, 1H, H-4), 5.43 (d, J = 48 Hz, 2H, CH_2F), 4.98 (s, 2H, OCH_2CCl_3), 3.00-2.85 (m, 2H), 2.55-1.90 (m, 7H), 1.65-1.35 (m, 6H), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.5 (C=O), 146.0 (d, J = 6 Hz, C-3), 140.1 (C-10), 140.1 (d, J = 1 Hz, C-5), 127.8 (d, J = 6 Hz, C-1), 125.6 (d, J = 14 Hz, C-2), 121.2 (d, J = 2 Hz, C-4), 92.5 (CCl_3), 80.5 (d, J = 2 Hz, $O\underline{C}H_2CCl_3$), 79.6 (d, J = 167 Hz, CH_2F), 50.4 (C-14), 47.9 (C-13), 44.1 (C-9), 37.8 (C-8), 35.9 (C-16), 31.5 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 25.8 (CH_2), 21.6

(CH_2), 13.9 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -132 (t, J = 47 Hz); LRMS (EI) *m/z* (%) 514 (M+2, 100), 512 (M^+, 98), 470 (42), 468 (37), 457 (35), 455 (38), 395 (30), 283 (58), 245 (93), 97 (85); HRMS (EI) calcd. for $C_{21}H_{24}FCl_3O_5S$ 512.0394, found 512.0396.

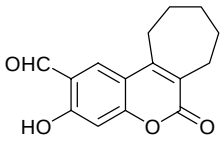


2.35

3-Hydroxy-6-oxo-8,9,10,11-tetrahydro-7*H*-cylohepta-[*c*][*1*]benzopyran or 3-hydroxy-8,9,10,11-tetrahydrocyclohepta[*c*]chromen-6(7*H*)-one (2.35). This was prepared according to the procedure of Woo et al.[17] A mixture of resorcinol (4.84 g, 44 mmol, 1.1 equiv) and 2-(methoxycarbonyl)cycloheptane (6.88 g, 40 mmol) was gently heated until the solution was clear. To this viscous solution at 0 ^oC was added TFA/conc. H_2SO_4 (6.80 mL/8.80 mL, premixed) dropwise over 1 h. The resulting mixture was stirred overnight before quenching with ice water. After stirring an additional 45 min, the precipitate was collected and washed thoroughly with H_2O then dried under high vacuum. This material was redissolved in acetone and purified by flash chromatography (acetone/hexane, 1:2 to 1:1) to give of coumarin **3.62** as white solid (8.76 g, 95%). ^1H NMR was identical to that reported in the literature.[17] ^1H NMR ($CDCl_3$, 300 MHz) δ 8.06 (brs, 1H, OH), 7.54 (d, J = 8.8 Hz, 1H, H-1), 7.12 (d, J = 1.6 Hz, 1H, H-4), 6.87 (dd, J = 8.8 Hz, J = 1.6 Hz, 1H, H-2), 2.95-2.85 (m, 4H), 1.88 (quint, J = 5.6 Hz, 2H), 1.70-1.50 (m, 4H).



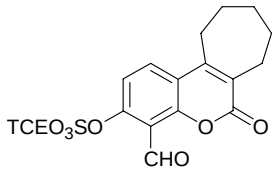
2.36



2.37

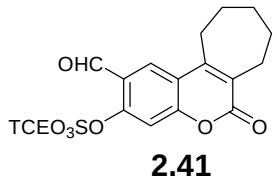
3-Hydroxy-4-formyl-8,9,10,11-tetrahydrocyclohepta[*c*]chromen-6(7*H*)-one (2.36) and 3-Hydroxy-2-formyl-8,9,10,11-tetrahydrocyclohepta[*c*]chromen -6(7*H*)-one (2.37). TFA (4.0 mL) was added to coumarin **2.35** (2.23 g, 9.7 mmol) at 0 ^{o}C. The resulting mixture was stirred until all of the coumarin was dissolved. HMT (2.73g, 19.4 mmol, 2 equiv) was added slowly. After addition the solution was heated under argon for 4 h at 88 ^{o}C. After cooling to rt, deionized water (2 mL) was added slowly then the mixture was heated at 88 ^{o}C for 2 h. After cooling, 2 M HCl (4 mL) was added and the mixture was extracted with Et_2O. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. Chromatography of the residue (ethyl acetate/hexane, 1:3 to 3:7) gave pure **2.36** as a light yellow solid (1.45 g, 65%) and **2.37** as an off-white solid (0.45 g, 20%). Characterization data for **2.36**: mp: 218-219 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.10 (s, 1H, ArO<u>H</u>), 10.62 (s, 1H, CHO), 7.79 (d, J = 9.1 Hz, 1H, H-1), 6.86 (d, J = 9.1 Hz, 1H, H-2), 2.92-2.85 (m, 4H), 1.90 (quint, J = 5.7 Hz, 2H), 1.70-1.58 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.0 (CHO), 163.8 (C=O), 160.1 (C_{Ar}), 154.5 (C_{Ar}), 153.6 (C_{Ar}), 132.3 (C_{Ar}), 125.4 (C_{Ar}), 113.5 (C_{Ar}), 111.5 (C_{Ar}), 108.2 (C_{Ar}), 31.5 (CH_2), 27.9 (CH_2), 26.3 (CH_2), 25.3 (CH_2), 24.6 (CH_2); LRMS (EI) *m/z* (%) 258 (M^+, 100), 243 (19), 230 (M-CO, 46), 215 (8), 202 (22), 201 (18); HRMS (EI) calcd for $C_{15}H_{14}O_4$ 258.0886; found 258.0892. Characterization data for **2.37**: mp: 123-124 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.26 (s, 1H, ArOH), 9.92 (s, 1H, CHO), 7.85 (s, 1H, H-1), 6.83 (s, 1H, H-4), 2.94-2.84 (m, 4H), 1.90 (quint, J = 5.8 Hz, 2H), 1.68 (quint, J = 5.3 Hz, 2H), 1.59 (quint, J = 5.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.3 (CHO), 163.4 (C=O), 161.1 (C_{Ar}), 158.5 (C_{Ar}), 152.8 (C_{Ar}), 131.1 (C_{Ar}), 126.7 (C_{Ar}), 118.0 (C_{Ar}), 113.8 (C_{Ar}), 105.0 (C_{Ar}), 31.9 (CH_2), 28.2 (CH_2), 26.7 (CH_2), 25.5 (CH_2), 25.0 (CH_2); LRMS (EI) *m/z* (%) 258 (M^+, 100), 243 (63), 230 (M-CO, 44), 229

(46), 215 (8), 201 (22); HRMS (EI) calcd for $C_{15}H_{14}O_4$ 258.0892; found 258.0892.



2.40

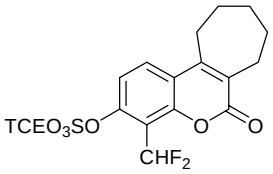
4-Formyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 2,2,2-tri-chloroethyl sulfate (2.40). Prepared using the procedure described for **2.27** using **2.36** (100 mg, 0.39 mmol), DMAP (47 mg, 0.39 mmol, 1 equiv), triethylamine (0.11 mL, 0.77 mmol, 2 equiv) and reagent **2.12** (0.11 mL, 0.77 mmol, 2 equiv) in THF (10 mL) and stirring for 4 h. Column chromatography of the residue (ethyl acetate/hexane, 3:7) yielded **2.40** (0.95 g, 95%) as colorless solid. Mp: 108-110 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.71 (s, 1H, CHO), 7.93 (d, J = 9.1 Hz, 1H, H-1), 7.48 (d, J = 9.2 Hz, 1H, H-2), 5.10 (s, 2H, CH_2CCl_3), 2.98-2.91 (m, 4H), 1.93 (quint, J = 5.9 Hz, 2H), 1.73-1.60 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.7 (CHO), 159.4 (C=O), 154.5, 152.4, 148.0, 130.1, 129.8, 119.9, 117.9, 116.0, 92.3 (CCl_3), 80.8 ($O\underline{C}H_2CCl_3$), 31.5 (CH_2), 28.2 (CH_2), 26.8 (CH_2), 25.1 (CH_2), 24.5 (CH_2); LRMS (EI) *m/z* (%) 472 (M+4, 6), 470 (M+2, 17), 468 (M^+, 17), 435 (5), 433 (7), 321 (15), 258 (69), 257 (100), 230 (41), 229 (28); HRMS (EI) calcd for $C_{17}H_{15}Cl_3O_7S$ 467.9604; found 467.9615.



2.41

2-Formyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.41). Prepared using the procedure described for **2.27** using **2.37** (0.390 g, 1.51 mmol), DMAP (0.190 g, 1.56 mmol, 1 equiv), triethylamine (0.44 mL, 3.1 mmol, 2 equiv) and reagent **2.12**

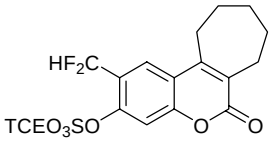
(0.40 mL, 3 mmol, 2 equiv) in THF (50 mL) and stirring for 6 h. Column chromatography of the residue (ethyl acetate/hexane, 1:4 to 1:2) yielded **2.41** as a colorless oil that solidified on standing (0.774 g, 100%). This contained a small amount of reagent **2.12** that could not be removed so this material was carried on to the next step. On a smaller scale, we obtained pure **2.12** (95%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.31 (s, 1H, CHO), 8.29 (s, 1H, H-1), 7.47 (s, 1H, H-4), 4.94 (s, 2H, CH_2CCl_3), 3.01-2.89 (m, 4H), 1.92 (quint, J = 5.2 Hz, 2H), 1.72-1.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.6 (CHO), 159.9 (C=O), 156.3 (C_{Ar}), 152.3 (C_{Ar}), 151.0 (C_{Ar}), 130.4 (C_{Ar}), 126.6 (C_{Ar}), 124.1 (C_{Ar}), 119.6 (C_{Ar}), 110.6 (C_{Ar}), 91.9 (CCl_3), 80.8 (O<u>C</u>H_2CCl_3), 31.5 (CH_2), 28.1 (CH_2), 26.8 (CH_2), 25.0 (CH_2), 24.5 (CH_2); LRMS (EI) *m/z* (%) 472 (M+4, 15), 470 (M+2, 39), 468 (M^+, 41), 435 (5), 433 (M-Cl, 7), 321 (19), 269 (21), 258 (100), 257 (76), 230 (43), 229 (47); HRMS (EI) calcd for $C_{17}H_{15}Cl_3O_7S$ 467.9604; found 467.9608.



2.42

4-Difluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.42). This was prepared using the procedure described for **2.29** using **2.40** (100 mg, 0.210 mmol), methylene chloride (20 mL) and DAST (diethylaminosulfur trifluoride, 0.084 mL, 0.64 mmol, 3 equiv), 0 ^{o}C, 1 h, then rt 3 h. Column chromatography of the residue (ethyl acetate/hexane, 1:3) yielded **2.42** as a white solid (80 mg, 80%). Mp: 81-83 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.85 (d, J = 9.0 Hz, 1H, H-1), 7.54 (d, J = 9.3 Hz, 1H, H-2), 7.35 (t, J = 52.9 Hz, 1H), 4.93 (s, 2H, CH_2CCl_3), 2.96-2.89 (m, 4H), 1.91 (quint, J = 5.7 Hz, 2H), 1.71-1.61 (m, 4H); ^{13}C NMR

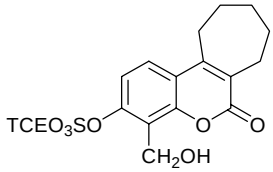
($CDCl_3$, 75 MHz) δ 159.6 (C=O), 152.5 (C_{Ar}), 150.8 (C_{Ar}), 148.7 (C_{Ar}), 129.7 (C_{Ar}), 127.8 (C_{Ar}), 119.2 (C_{Ar}), 116.5 (C_{Ar}), 113.7 (t, J = 23.1 Hz, C-4), 109.2 (t, J = 238 Hz, CHF_2), 92.0 (CCl_3), 80.7 ($O\underline{C}H_2CCl_3$), 31.5 (CH_2), 28.1 (CH_2), 26.7 (CH_2), 25.1 (CH_2), 24.5 (CH_2); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -114.0 (d, $J_{H\text{-}F}$ = 53 Hz); LRMS (ESI) *m/z* (%) 359 (100); HRMS (ESI) calcd for $C_{15}H_{13}F_2O_6S$ 359.0406; found 359.0393.



2.43

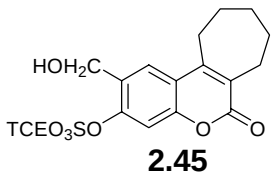
2-Difluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.43). This was prepared using the procedure described for **2.29** using **2.41** (100 mg, 0.21 mmol), methylene chloride (20 mL) and DAST (diethylaminosulfur trifluoride, 0.084 mL, 0.64 mmol, 3 equiv), 0 ^oC, 1 h, then rt for 3 h. Column chromatography of the residue (ethyl acetate/hexane, 1:3) yielded **2.42** as an off-white solid (80 mg, 80%). Mp: 84-86 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.96 (s, 1H, H-1), 7.52 (s, 1H, H-4), 6.96 (t, J = 54.7 Hz, 1H, CH_2F), 4.89 (s, 2H, OCH_2CCl_3), 2.97 (t, J = 5.0 Hz, 2H), 2.91 (t, J = 5.2 Hz, 2H), 1.97-1.85 (m, 2H), 1.75-1.55 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.6 (C=O), 154.2 (C_{Ar}), 152.2 (C_{Ar}), 148.0 (C_{Ar}), 130.4 (C_{Ar}), 123.3 (t, J = 6.4 Hz, C-1), 122.3 (t, J = 23.3 Hz, C-2), 119.3 (C_{Ar}), 110.4 (t, J = 239 Hz, CHF_2), 109.9 (C-4), 91.9 (CCl_3), 80.9 (CH_2CCl_3), 31.7 (CH_2), 28.2 (CH_2), 26.9 (CH_2), 25.2 (CH_2), 24.7 (CH_2); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -113.2 (d, $J_{H\text{-}F}$ = 54 Hz); LRMS (EI) *m/z* (%) 494 (M+4, 27), 492 (M+2, 66), 490 (M^+, 62), 479 (2), 477 (4), 475 (4), 466 (2), 464 (5), 462 (5), 439 (3), 437 (8), 435 (11), 373 (7), 360 (48), 345 (12), 332 (7), 280 (61), 279 (100), 265 (23), 251 (38); HRMS (EI) calcd for

$C_{17}H_{15}Cl_3F_2O_6S$ 489.9623; found 489.9629.



2.44

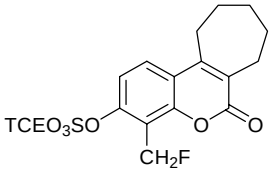
4-Hydroxymethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.44). Prepared using the procedure used for **2.31** using $Zr(O^iPr)_4$-^{i}PrOH (98 mg, 0.26 mmol, 1.2 equiv) and (±)BINOL (80 mg, 0.26 mmol, 1.2 equiv) in toluene (5 mL) and **2.40** (100 mg, 0.210 mmol) in toluene (2 mL), and stirring for 1 h at 35 ^{o}C. Column chromatography of the residue (acetone/hexane, 1:4 to 1:1) yielded pure **2.45** as a white solid (90 mg, 90%). Mp: 146-147 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (d, J = 9.0 Hz, 1H, H-1), 7.42 (d, J = 9.0 Hz, 1H, H-2), 4.98 (s, 2H, CH_2), 4.95 (s, 2H, CH_2), 2.96-2.89 (m, 4H), 2.67 (brs, 1H, OH), 1.91 (quint, J = 5.8 Hz, 2H), 1.67-1.61 (m, 4H, overlapping with H_2O); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.1 (C=O), 153.2 (C_{Ar}), 151.6 (C_{Ar}), 149.0 (C_{Ar}), 129.1 (C_{Ar}), 124.8 (C_{Ar}), 121.4 (C_{Ar}), 116.6 (C_{Ar}), 92.2 (CCl_3), 80.7 ($O\underline{C}H_2CCl_3$), 53.3 ($Ar\underline{C}H_2O$), 31.7 (CH_2), 28.2 (CH_2), 26.7 (CH_2), 25.3 (CH_2), 24.7 (CH_2); LRMS (EI) *m/z* (%) 474 (M+4, 6), 472 (M+2, 16), 470 (M^+, 16), 323 (25), 259 (9), 243 (18), 242 (66), 241 (100), 231 (10), 214 (32); HRMS (EI) calcd for $C_{17}H_{17}Cl_3O_7S$ 469.9761; found 469.9757.



2.45

2-Hydroxymethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.45). Prepared using the procedure used for **2.31** using $Zr(O^iPr)_4$-^{i}PrOH (700

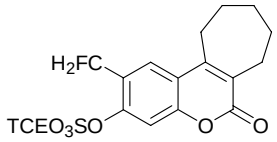
mg, 1.81 mmol, 1.1 equiv) and (±)BINOL (570 mg, 1.99 mmol, 1.2 equiv) in toluene (5 mL) and **2.41** (774 mg, 1.65 mmol) in toluene (40 mL), and stirring for 1 h at 35 ^{o}C. Column chromatography of the residue (ethyl acetate/hexane, 1:3 to 1:1) yielded pure **2.45** as a white solid (0.605g, 85% over two steps). Mp: 118-119 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88 (s, 1H, H-1), 7.40 (s, 1H, H-4), 1.90 (s, 2H), 4.85 (s, 2H), 2.97 (t, J = 5.2 Hz, 2H), 2.90 (t, J = 5.3 Hz, 2H), 1.91 (quint, J = 5.7 Hz, 2H), 1.71-1.56 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.4 (C_{Ar}), 153.0 (C_{Ar}), 151.8 (C_{Ar}), 129.4 (C_{Ar}), 129.4 (C_{Ar}), 125.0 (C_{Ar}), 119.3 (C_{Ar}), 109.5 (C_{Ar}), 92.1 (CCl_3), 80.7 ($O\underline{C}H_2CCl_3$), 59.1 ($Ar\underline{C}H_2O$), 31.7 (CH_2), 28.1 (CH_2), 26.8 (CH_2), 25.3 (CH_2), 24.7 (CH_2); LRMS (EI) *m/z* (%) 474 (M+4, 23), 472 (M+2, 52), 470 (M^+, 53), 444 (3), 442 (3), 323 (23), 260 (50), 259 (77), 243 (32), 242 (100), 231 (31), 214 (18); HRMS (EI) calcd for $C_{17}H_{17}Cl_3O_7S$ 469.9761; found 469.9756.



2.46

4-Fluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.46). Prepared from **2.44** using the procedure described for **2.29** using **2.44** (100 mg, 0.21 equiv), DAST (0.084 mL, 0.64 mmol, 3 equiv), 0 ^{o}C, 1h, then rt for 3 h. Column chromatography (ethyl acetate/hexane, 1:3) yielded pure **2.46** as an off-white solid (80 mg, 80%). Mp: 91-93 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (dd, J = 9.0 Hz, $J_{H\text{-}F}$ = 1.8 Hz, 1H, H-1), 7.48 (d, J = 9.0 Hz, 1H, H-2), 5.75 (d, J = 47.3 Hz, 2H, CH_2F), 4.90 (s, 2H, CH_2CCl_3), 2.96-2.90 (m, 4H), 1.91 (quint, 2H, J = 5.7 Hz), 1.69-1.55 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.2 (C=O), 152.6 (C_{Ar}),

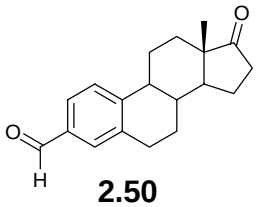
151.6 (C_{Ar}), 149.8 (C_{Ar}), 129.4 (C_{Ar}), 126.5 (d, J = 2.8 Hz), 119.3 (C_{Ar}), 116.2 (C_{Ar}), 116.1 (C_{Ar}), 92.0 (CCl_3), 80.6 ($O\underline{C}H_2CCl_3$), 72.4 (d, J = 167 Hz, CH_2F), 31.6 (CH_2), 28.0 (CH_2), 26.7 (CH_2), 25.2 (CH_2), 24.6 (CH_2); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -133.0 (t, $J_{H\text{-}F}$ = 47 Hz); LRMS (EI) *m/z* (%) 476 (M+4, 17), 474 (M+2, 47), 472 (M+, 43), 448 (1), 446 (3), 44 (3), 419 (3), 417 (5), 355 (23), 342 (33), 323 (13), 286 (9), 262 (52), 261 (100), 242 (71), 241 (54), 233 (27), 214 (28), 213 (25); HRMS (EI) calcd for $C_{17}H_{16}Cl_3FO_6S$ 471.9717; found 471.9714.



2.47

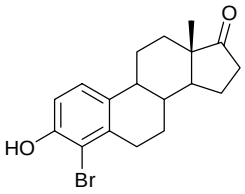
2-Fluoromethyl-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl 2,2,2-trichloroethyl sulfate (2.47). Prepared using the procedure described for **2.29** using **2.45** (380 mg, 0.806 mmol) and DAST (0.40 mL, 3.0 mmol, 3.7 equiv) in CH_2Cl_2 (30 mL), stir for 1 h at 0 ^oC and 3 h at rt. Flash chromatography of the residue (ethyl acetate/hexane 1:5 to 1:3) yielded **2.47** as pale yellow solid (0.30 g, 80%). Mp: 84-86 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.78 (s, 1H, H-1), 7.42 (s, 1H, H-4), 5.51 (t, J = 47.2 Hz, 2H, CH_2F), 4.87 (s, 2H, CH_2CCl_3), 2.94-2.85 (m, 4H), 1.90-1.83 (m, 2H), 1.70-1.50 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.9 (C=O), 153.0 (d, J = 2.2 Hz, C_{Ar}), 152.4 (C-11a), 148.2 (d, J = 3.7 Hz, C-3), 130.0 (C_{Ar}), 125.7 (d, J = 7.6 Hz, C-1), 124.6 (d, J = 17.9 Hz, C-2), 119.4 (C-6a), 109.8 (C-4), 92.1 (CCl_3), 80.8 ($O\underline{C}H_2CCl_3$), 79.0 (d, J = 168 Hz, CH_2F), 31.8 (CH_2), 28.2 (CH_2), 26.9 (CH_2), 25.3 (CH_2), 24.8 (CH_2); ^{19}F NMR ($CDCl_3$, 282 MHz, coupled) δ -134.5 (t, $J_{H\text{-}F}$ = 47.1 Hz); LRMS (EI) *m/z* (%) 476 (M+4, 16), 474 (M+2, 43), 472 (M^+, 41), 419 (4), 417 (6), 355 (14), 342 (43), 327 (13), 262 (49), 261 (100), 247 (13), 233 (35); HRMS (EI) calcd for

$C_{17}H_{16}Cl_3FO_6S$ 471.9717; found 471.9718.



2.50

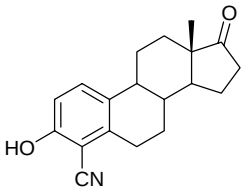
Estra-1,3,5(10)-triene-17-one-3-carbaldehyde (2.50). To a solution of 3-hydroxymethyl-estrone[19] (400 mg, 1.41 mmol) in CH_2Cl_2 (30 mL) at rt was added pyridinium chlorochromate (PCC, 600 mg, 2.80 mmol, 2 equiv). The reaction was stirred for 100 min then filtered through Celite. The filtrate was washed with H_2O and brine, then dried (Na_2SO_4), filtered and concentrated to give a brown solid. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:5, then ethyl acetate/hexane/CH_2Cl_2,1:5:3, then ethyl acetate/hexane, 1:1) gave pure **2.50** as a white solid (377 mg, 95%). Mp: 183-185 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.93 (s, 1H, CHO), 7.63 (d, J = 8.1 Hz, 1H, H-2), 7.59 (s, 1H, H-4), 7.44 (d, J = 8.0 Hz, 1H, H-1), 3.00-2.95 (m, 2H), 2.55-2.31 (m, 3H), 2.20-1.95 (m, 4H), 1.69-1.44 (m, 6H), 0.91 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.3 (C=O), 192.1 (CHO), 147.0 (C-10), 137.4 (C_{Ar}), 134.2 (C_{Ar}), 130.1 (C-4), 127.1 (CH_{Ar}), 126.0 (CH_{Ar}), 50.4 (C-14), 47.7 (C-13), 44.8 (C-9), 37.6 (C-8), 35.7 (C-16), 31.4 (CH_2), 29.1 (CH_2), 26.1 (CH_2), 25.5 (CH_2), 21.5 (CH_2), 13.7 (CH_3, C-18); LRMS (EI) *m/z* (%) 282 (M^+, 100), 264 (11), 253 (7), 238 (45), 225 (24); HRMS (EI) calcd for $C_{19}H_{22}O_2$ 282.1620; found 282.1618.



2.51

4-Bromoestra-1,3,5(10)-triene-17-one (2.51). Prepared according to the procedure of Utne

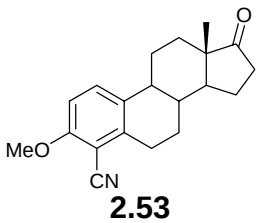
et al.[20] To a suspension of E1 (8.00 g, 29.6 mmol) in EtOH (1.0 L) (gentle heating of the suspension resulted in dissolution of E1, however, upon cooling to rt some E1 precipitated out of solution) was added *N*-bromoacetamide (NBA, 4.10 g, 2.97 mmol, 1.0 equiv) in portions. After addition, the resulting mixture was stirred for 24 h at rt. The reaction was filtered and the filtrate concentrated. The solid residue from the concentrated filtrate and the collected precipitate were combined and recrystallized from ethanol to give of **2.51** as a white solid (8.32 g, 81%). ^{1}H NMR was identical to that reported in the literature.[41] ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.82 (s, OH), 7.08 (d, J = 8.5 Hz, 1H, H-1), 6.73 (d, J = 8.4 Hz, 1H, H-2), 2.90-1.20 (m, 15H), 0.76 (s, 3H, CH_3, H-18).



2.52

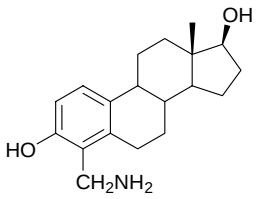
4-Cyanoestra-1,3,5(10)-triene-17-one (2.52). This was prepared according to the procedure of Labrie et al. with slight modifications.[22] A mixture of **2.51** (500 mg, 1.44 mmol) and CuCN (300 mg. 3.33 mmol, 2.3 equiv) in DMF (12 mL) was refluxed for 6.5 h. After cooling to rt, $FeCl_3$ (1 g) and conc. HCl (1 mL) were added and the mixture was heated at 55 ^{o}C for 30 min, cooled to rt and treated with H_2O (20 mL). The mixture was extracted with ethyl acetate and combined extracts were washed with H_2O and brine then dried (Na_2SO_4) filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane 1:2 to 1:1.5) to give **2.52** as white solid (375 mg, 89%). ^{1}H NMR and ^{13}C NMR were identical to that reported in the literature.[22] ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.68 (s, 1H, OH), 7.34 (d, J = 8.7 Hz, 1H, H-1), 6.74 (d, J = 8.6 Hz, 1H, H-2), 2.92-2.70 (m, 2H), 2.39 (dd, J = 18.6 Hz, J = 8.1 Hz, 1H), 2.27-2.25 (m, 1H), 2.13-1.85 (m, 4H),

1.69 (d, 1H, J = 8.4 Hz), 1.54-1.25 (m, 6H), 0.76 (s, 3H, CH_3, H-18).



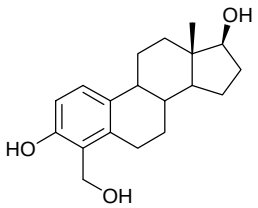
2.53

4-Cyanoestra-1,3,5(10)-triene-17-one-3-yl methylether (2.53).[42] To a solution of 4-cyanoestrone (200 mg, 0.68 mmol) in THF/DMF (50 mL/1 mL) at 0 ^oC was added NaH (60% dispersed in mineral oil, 40 mg, 1.5 equiv). After stirring for 15 min at rt, it was cooled to 0 ^oC and MeI (0.085 mL, 1.36 mmol, 2 equiv) was added. Reaction was stirred overnight before quenching with H_2O (10 mL) and extracted with ethyl acetate. The combined extracts were washed with brine the dried (Na_2SO_4), filtered and concentrated. The residue was subjected to chromatography (ethyl acetate/hexane, 1:2) to give light yellow solid. Treating the solid with hexane and then decanting off the hexane gave pure **2.53** as a white solid (163 mg, 79%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.41 (d, J = 8.8 Hz, 1H, H-1), 6.74 (d, J = 8.8 Hz, 1H, H-4), 3.87 (s, 3H, OCH_3), 3.12 (dd, J = 17.8 Hz, J = 5.8 Hz, 1H, H-10), 2.92 (ddd, J = 18.0 Hz, J = 11.5 Hz, J = 6.8 Hz, 1H, H-7), 2.49 (dd, J = 18.8 Hz, J = 9.2 Hz, 1H, H-6), 2.40-1.90 (m, 6H), 1.70-1.40 (m, 6H), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.4 (C=O), 159.9 (C-3), 141.9 (C-5), 132.9 (C-10), 130.9 (C-1), 115.6 (CN), 108.4 (C-2), 101.7 (C-4), 56.0 (OCH_3), 50.2 (C-14), 47.9 (C-13), 43.6 (C-9), 37.7 (C-8), 35.8 (C-16), 31.4 (CH_2), 28.4 (CH_2), 25.9 ($2CH_2$ overlapping), 21.5 (CH_2), 13.8 (CH_3, C-18).



2.54

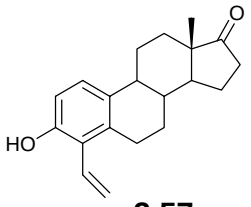
4-(Aminomethyl)-17*β*-hydroxylestra-1,3,5(10)-triene (2.54). To a suspension of $LiAlH_4$ (300 mg, 8.82 mmol, 13 equiv) in THF (20 mL) at 0 ^oC was added a solution of **2.52** (200 mg, 0.678 mmol) in THF (20 mL). After addition, the resulting mixture was stirred for 20 min at rt, then refluxed overnight (oil bath temp. 70 ^oC). The mixture was cooled to rt and poured onto ice water and the mixture was filtered through a pad of Celite. The filtrate was extracted with Et_2O and the combined extracts were washed with brine then dried (Na_2SO_4), filtered and concentrated. The residue was subjected to chromatography (ethyl acetate/methanol, 2:1) to give pure **2.54** as a yellow solid (120 mg, 59%). ^1H NMR and ^{13}C NMR were identical to that reported in the literature.[25] ^1H NMR (DMSO-d_6, 300 MHz) δ 6.94 (d, 1H, H-1), 6.43 (d, 1H, H-2), 5.00 (brs, 4H, NH_2 and 2 OH), 3.79 (s, 2H, $ArCH_2N$), 3.47 (s, 1H), 2.70-2.45 (m, 2H), 2.20-1.10 (m, 13H), 0.61 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.6 (C-3), 134.5 (C-5), 130.4 (C-10), 124.7 (C-1), 123.3 (C-4), 113.9 (C-2), 80.5 (C-17), 50.0 (C-14), 44.4 (C-9), 43.1 (C-13), 39.6 ($ArCH_2N$), 39.1 (C-8), 37.1 (CH_2), 30.4 (CH_2), 27.6 (CH_2), 26.9 (CH_2), 26.7 (CH_2), 23.2 (CH_2), 11.7 (CH_3, C-18).



2.56

17*β*-hydroxyl-4-(hydroxy methyl)estra-1,3,5(10)-triene (2.56). ***Method A*** (reduction of

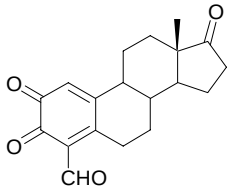
2.13 using $NaBH_4$): To a solution of **2.13** (100 mg, 0.336 mmol) in EtOH/THF (30 mL, 2:1, heated to make a solution then cooled) at 0 ^oC was added $NaBH_4$ (51 mg, 1.34 mmol, 4.0 equiv). The reaction was stirred 30 min at 0 ^oC. The solvent was removed *in vacuo* at 30 ^oC (water bath) and the residue was acidified with 1 N HCl at 0 ^oC and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:2 to 1:1) to give **2.56** as a white solid (72 mg, 72%). ***Method B*** (reduction of **2.66** using Pd/C and H_2): To a solution of **2.66** (12 mg, 0.028 mmol) in ethyl acetate (10 mL) was added 10% Pd/C (3 mg). The reaction mixture was evacuated, filled with H_2 and repeated this process 4 times, then stirred overnight. The reaction was filtered and concentrated and the residue purified by flash chromatography (ethyl acetate/hexane 1:2) to give of **2.56** as white solid (8 mg, 98%). The ^1H NMR was identical to that reported in the literature.[25] ^1H NMR (CD_3OD, 300 MHz) δ 7.05 (d, J = 8.4 Hz, 1H, H-1), 6.58 (d, J = 8.5 Hz, 1H, H-2), 4.58 (s, 2H, Ar$C\underline{H_2}$OH), 3.63 (t, J = 8.5 Hz, 1H, H-17), 3.00-2.93 (m, 1H, H-6), 3.00-2.72 (m, 1H), 2.28-1.10 (m, 13H), 0.74 (s, 3H, CH_3, H-18).



2.57

4-Vinylestra-1,3,5(10)-triene-17-one (2.57). To a solution of **2.51** (2.10 g, 6.05 mmol) and tributyl vinyltin (2.0 mL, 6.8 mmol, 1.1 equiv) in DMF (40 mL) was added $Pd(PPh_3)_4$ (400 mg, 0.347 mmol, 5.7 mol%). The resulting mixture was degassed 7 times using liquid nitrogen and high vacuum before heating at 165-170 ^oC for 24 h. After cooling to rt, the mixture was diluted with H_2O

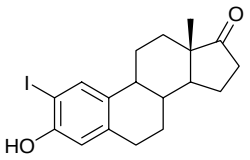
and extracted with ethyl acetate. The combined extracts were washed with H_2O, brine then dried (Na_2SO_4), filtered and concentrated. The residue was subjected to chromatography (ethyl acetate/hexane, 1:3 to 1:2.5) to give **2.57** as a white solid (1.31 g, 73%). Mp: 188-189 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.14 (dd, J = 8.7 Hz, J = 3.0 Hz, 1H, H-1), 6.79 (dd, J = 8.7 Hz, J = 3.3 Hz, 1H, H-2), 6.62 (ddd, 1H, J = 18.3 Hz, J = 12.7 Hz, J = 3.0 Hz, C<u>H</u>=CH_2), 5.70 (dd, J = 11.4 Hz, J = 1.8 Hz, 1H, CH=CH<u>H</u>$_{cis}$), 5.53 (t, J = 3.0 Hz, 1H, ArO<u>H</u>), 5.52 (dd, J = 18.0 Hz, J = 1.8 Hz, 1H, CH=C<u>H</u>$_{trans}$H, overlapping with ArOH), 2.82-2.59 (m, 2H), 2.54-2.33 (m, 2H), 2.30-1.89 (m, 5H), 1.66-1.32 (m, 6H), 0.67 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 221.2 (C=O), 150.8 (C-3), 135.3 (C-5), 132.4 (C-1), 131.5 (C-10) 125.6 (<u>C</u>H=CH_2), 123.4 (C-4), 120.5 (CH=<u>C</u>H_2), 113.0 (C-2), 50.4 (C-14), 47.9 (C-13), 44.2 (C-9), 37.7 (C-8), 35.9 (C-16), 31.6 (C-12), 27.9 (CH_2), 26.6 (CH_2), 26.1 (CH_2), 21.6 (CH_2), 12.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 296 (M^+, 100), 281 (2), 239 (8), 211 (12), 172 (10); HRMS (EI) calcd for $C_{20}H_{24}O_2$ 296.1776; found 296.1780.



2.58

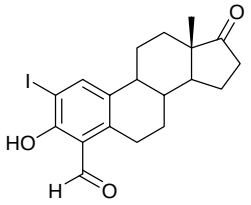
4-Formylestra-1,5(10)-diene-2,3,17-trione (2.58). To a solution of **2.57** (55 mg, 0.19 mmol) in dioxane/H_2O (6 mL, 5:1) at 55 ^oC was added OsO_4 solution (20 mg in 5 mL H_2O, 0.079 mmol, 0.42 equiv), then $NaIO_4$ (86 mg, 0.25 mmol, 1.3 equiv). The resulting mixture was stirred overnight at 55 ^oC then cooled to rt. 2 N HCl was added and extracted with ethyl acetate. The combined extracts were washed with H_2O then dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (ethyl acetate/hexane, 1:2) to give a mixture of **2.58** and trace **2.13** as yellow crystals

(15 mg, 26%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.23 (s, 1H, CHO), 7.91 (s, 1H, 1-H), 3.38-3.11 (m, 2H), 2.55-1.35 (m, 13H), 0.90 (s, 3H, CH_3, H-18).



2.59

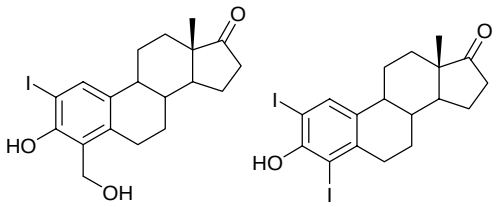
2-Iodoestra-1,3,5(10)-triene-17-one (2.59). **2.59** was prepared using a modified version of the procedure of Horiuchi et al.[29] A mixture of estrone (0.54 g, 2.0 mmol), $Cu(OAc)_2 \cdot H_2O$ (0.60 g, 60 mmol, 1.5 equiv) and iodine (0.76 g, 60 mmol, 1.5 equiv) in HOAc (60 mL) was heated at 58 ^oC for 24 h. The reaction was cooled to rt and concentrated. 5% $Na_2S_2O_3$ was added and the mixture was stirred for 10 min at rt before extracting with ethyl acetate. The combined extracts were dried (Na_2SO_4) filtered and concentrated. The residue was subjected to flash chromatography (ethyl acetate/hexane, 1:2.5) to give of **2.59** as a white solid (358 g, 45%). ^{1}H and ^{13}C NMR are identical to that reported in the literature.[43] ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.87 (s, 1H, ArO<u>H</u>), 7.43 (s, 1H, H-1), 6.55 (s, 1H, H-4), 2.75-2.65 (m, 2H), 2.39 (dd, J = 19.3 Hz, J = 8.6 Hz, 1H), 2.27-1.20 (m, 12H), 0.77 (s, 3H, CH_3, H-18).



2.60

4-Formyl-2-iodoestra-1,3,5(10)-triene-17-one (2.60). To a solution of **2.59** (100 mg, 0.25 mmol) in acetonitrile (10 mL) was added paraformaldehyde (100 mg, 3.33 mmol, 13.2 equiv), magnesium chloride (75 mg, 0.79 mmol, 3.1 equiv) and triethylamine (0.2 mL, 1.43 mmol, 5.7 equiv).

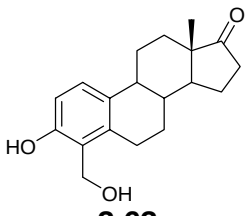
After addition, the reaction mixture was heated at 50 ^{o}C for 5 h. After cooling, the mixture was acidified with 2N HCl till clear. The mixture was extracted with ethyl acetate, washed with H_2O, 5% $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated. Purification by flash chromatography (ethyl acetate/hexane, 1:3 to 1:2.5) gave **2.14** as a light yellow solid (15 mg, 20%) and **2.60** as a yellow solid (43 mg, 40%). Characterization data for **2.60**: mp: 208-210 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.74 (s, 1H, OH), 9.67 (s, 1H, CHO), 7.44 (s, 1H, H-1), 3.01 (dd, J = 18.9 Hz, J = 5.7 Hz, 1H, H-6), 2.80-2.65 (m, 1H), 2.65-1.93 (m, 7H), 1.70-1.33 (m, 6H), 0.88 (m, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.2 (C=O), 195.5 (CHO), 158.3 (C-3), 149.6 (C_{Ar}), 133.9 (C_{Ar}), 130.5 (C-1), 118.3 (C_{Ar}), 94.0 (C-2), 50.2 (C-14), 47.4 (C-13), 43.8 (C-9), 37.7 (C-8), 37.4 (CH_2), 35.8 (CH_2), 31.3 (CH_2), 27.0 (CH_2), 26.1 (CH_2), 21.5 (CH_2), 13.7 (CH_3, C-18); LRMS (EI) *m/z* (%) 424 (M^+, 100), 380 (8), 367 (12), 326 (8), 298 (10); HRMS (EI) calcd for $C_{19}H_{21}IO_3$ 424.0535; found 424.0544.



2.61 **2.62**

4-(Hydroxymethyl)-2-iodoestra-1,3,5(10)-triene-17-one (2.61) and 2,4-Diiodoestra-1,3,5(10)-triene-17-one (2.62). To a mixture of **2.59** (180 mg, 0.455 mmol), paraformaldehyde (6.0 mg, 0.20 mmol, 0.44 equiv) in dioxane (3 mL) was added powderized NaOH (4.0 mg, 0.10 mmol, 0.22 equiv). The resulting mixture was heated in glass bomb at 50 ^{o}C for 4 h. The mixture was cooled and paraformaldehyde (3.0 mg, 0.10 mmol, 0.22 equiv) was added and heating was continued for an additional 2 h. After cooling to rt, it was acidified with 1 N HCl slowly and the extracted with

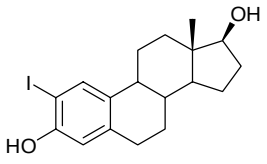
ethyl acetate, washed with H_2O and brine then dried (Na_2SO_4) and concentrated. The residue was subjected to flash chromatography (ethyl acetate/hexane 1:2 to 1:1) to give of **2.61** as a white solid (97 mg, 50%), **2.62** as a white solid (35 mg, 14%) and unreacted starting material (31 mg, 17%). Characterization data for **2.61:** mp 156-158 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.94 (s, 1H, ArOH), 7.16 (s, 1H, H-1), 5.41 (brs, 1H, $ArCH_2O\underline{H}$), 4.53 (s, 2H, $ArC\underline{H}_2OH$), 2.74 (dd, J = 17.4 Hz, J = 5.1 Hz, 1H, H-6), 2.65-2.23 (m, 3H), 2.23-1.85 (m, 4H), 1.80-1.63 (m, 1H), 1.60-1.18 (m, 6H), 0.76 (s, 3H, CH_3, H-18). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 220.0 (C=O), 152.0 (C-3), 137.9 (C_{Ar}), 133.2 (C_{Ar}), 126.3 (C_{Ar}), 125.1 (C-1), 96.5 (C-2), 60.7 ($Ar\underline{C}H_2O$), 49.9 (C-14), 47.7 (C-13), 44.2 (C-9), 37.6 (C-8), 37.0 (CH_2), 35.8 (CH_2), 31.7 (CH_2), 27.4 (CH_2), 26.3 (CH_2), 21.5 (CH_2), 13.9 (CH_3, C-18); LRMS (EI) *m/z* (%) 426 (M^+, 35), 408 (100), 326 (7), 298 (8), 281 (10); HRMS (EI) calcd for $C_{19}H_{23}O_3I$ 426.0692; found 426.0690. Characterization data for **2.62:** ^{1}H NMR was identical to that reported in the literature.[44] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (s, 1H, H-1), 5.76 (s, 1H, OH), 2.84 (dd, J = 17.7 Hz, J = 6.3 Hz, 1H, H-10), 2.71-2.59 (m, 1H, H-7), 2.49 (dd, J = 18.3 Hz, J = 9.0 Hz, 1H, H-6), 2.40-1.90 (m, 6H), 1.70-1.35 (m, 6H), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.4 (C=O), 151.5 (C-3), 140.6 (C-5), 135.9 (C-6), 135.8 (C-1), 92.0 (C-4), 78.4 (C-2), 50.1 (C-14), 47.8 (C-13), 43.8 (C-9), 37.3 (C-8), 35.8 (C-16), 31.4 (CH_2), 27.2 (CH_2), 26.2 (CH_2), 21.5 (CH_2), 13.7 (CH_3, C-18); LRMS (EI) *m/z* (%) 522 (M^+, 100), 465 (7), 424 (4), 412 (4).



2.63

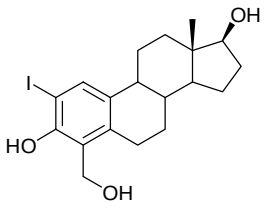
4-(Hydroxymethyl)estra-1,3,5(10)-triene-17-one (2.63). ***Method A*** (reduction of **2.61** using Pd black and H_2): To a solution of **2.61** (80 mg, 0.19 mmol) in ethyl acetate (50 mL) was added 4Å MS (50 mg), Na_2HPO_4 (30 mg) and the mixture was stirred for 30 min before Pd black (20 mg) was added. The reaction mixture was evacuated, filled with H_2 and this process was repeated 4 times. After stirring 2 days, the solids were removed by filtration. The filtrate was concentrated and the residue purified by flash chromatography (ethyl acetate/hexane, 1:2.5) to give of **2.63** as a white solid (33 mg, 59%). ***Method B*** (reduction of **2.13** using Bu_3SnH): To a suspension of **2.13** (298 mg, 1.0 mmol) in MeOH (40 mL) was added Bu_3SnH (438 mg, 1.50 mmol, 1.5 equiv). The mixture was refluxed for 7 h under argon during which the reaction mixture became clear. The mixture was concentrated and the residue was redissolved in CH_2Cl_2 by heating and purified by flash chromatography (ethyl acetate/hexane, 1:2, then CH_2Cl_2) to give **2.63** as a white solid (124 mg, 42%). ***Method C*** (hydrogenation of **2.13** using Pd black): To a solution of **2.13** (10.6 mg) in ethyl acetate (10 mL, dried from 4Å MS) was added Pd black (2.5 mg). The reaction mixture was evacuated, filled with H_2 and this process was repeated 4 times. After stirring 3 h, the catalyst was removed by filtration and the filtrate was concentrated to give **2.63** as a white solid (10.6 mg, 100%). Mp: 201-202 ^oC; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.03 (s, 1H, ArO<u>H</u>), 6.98 (d, J = 7.2 Hz, 1H, H-1), 6.50 (d, J = 7.2 Hz, 1H, H-2), 4.64 (s, 1H, $ArCH_2O$<u>H</u>), 4.48 (s, 2H, Ar<u>C</u>H_2OH), 2.99-2.70 (m, 2H), 2.47-1.92 (m, 6H), 1.73-1.70 (m, 1H), 1.55-1.27 (m, 6H), 0.78 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 220.2 (C=O), 153.8 (C-3), 137.1 (C_{Ar}), 130.7 (C_{Ar}), 125.4 (C-1), 125.1 (C-4), 113.3 (C-2), 55.0 (Ar<u>C</u>H_2OH), 50.8 (C-14), 47.7 (C-13), 44.3 (C-9), 37.8 (C-8), 35.9 (C-16), 31.9 (CH_2), 26.6 (CH_2), 26.3 (CH_2), 26.0 (CH_2), 21.6 (CH_2), 13.9 (CH_3, C-18); LRMS (EI) *m/z* (%) 300

(M^+, 63), 299 (43), 282 (M-H_2O, 100), 240 (14), 225 (18).



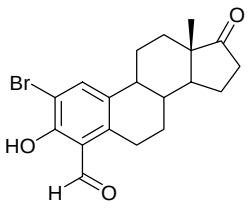
2.65

17*β*-Hydroxyl-2-iodoestra-1,3,5(10)-triene (2.65). ***Method A*** (direct iodination of E2): This was prepared using a modified version of the procedure of Horiuchi et al.[29] To a suspension of estradiol (1.0 g, 3.7 mmol) in acetic acid (120 mL) was added iodine (1.40 g, 5.5 mmol, 1.5 equiv) and $Cu(OAc)_2$ monohydrate (1.10 g, 5.50 mmol, 1.5 equiv). The resulting mixture was heated at 58 ^oC for 21 h. After cooling to rt, the insoluble solid was removed by filtration. The filtrate was concentrated *in vacuo* at 35 ^oC and the residue was treated with H_2O (50 mL) and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:2) gave **2.65** as a white solid (580 mg, 40%) ***Method B***: Reduction of **2.59** using $NaBH_4$ gave **2.65** in quantitative yield. ^1H NMR was identical to that reported in the literature.[45] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.50 (s, 1H, H-1), 6.70 (s, 1H, H-4), 5.06 (brs, 1H, ArOH), 3.71 (t, J = 8.4 Hz, 1H, H-17), 2.79-2.74 (m, 2H), 2.26-1.10 (m, 13H), 0.75 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.5 (C-3), 138.2 (C_{Ar}), 135.7 (C-1), 133.8 (C_{Ar}), 115.3 (C-4), 81.7 (C-2), 80.4 (C-17), 49.9 (C-14), 43.5 (C-9), 43.2 (C-13), 38.8 (C-8), 36.9 (CH_2), 30.3 (CH_2), 29.2 (CH_2), 27.1 (CH_2), 26.5 (CH_2), 23.2 (CH_2), 11.7 (CH_3, C-18).



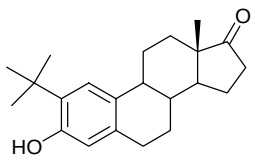
2.66

17*β*-Hydroxyl-4-(hydroxymethyl)-2-iodoestra-1,3,5(10)-triene (2.66). A mixture of **2.65** (180 mg, 0.455 mmol), paraformaldehyde (27 mg, 0.90 mmol, 2 equiv) and powderized NaOH (18 mg, 0.45 mmol, 1 equiv) in dioxane (3 mL) was heated at 52-55 ^{o}C for 3h before cooling to rt. After acidifying with 1 M HCl and treating with ice at 0 ^{o}C, the mixture was extracted with ethyl acetate. The combined extracts were washed with H_2O, brine then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane 1:2 to 1:1) to give **2.66** as white solid (79 mg, 41 %). Mp: 182 ^{o}C (dec.); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.59 (s, 1H, ArO<u>H</u>), 7.44 (s, 1H, H-1), 5.70 (s, OH, ArCH$_2$O<u>H</u>), 4.61 (s, 2H, ArC<u>H</u>$_2$OH), 4.45 (s, 1H, OH, CHO<u>H</u>), 3.47 (t, 1H, J = 7.7 Hz, H-17), 2.75-2.55 (m, 2H, H-10), 2.20-1.78 (m, 4H), 1.60-1.50 (m, 1H), 1.39-1.00 (m, 7H), 0.60 (s, CH$_3$, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 153.3 (C-3), 136.3 (C$_{Ar}$), 134.6 (C-1), 134.6 (C$_{Ar}$), 125.2 (C$_{Ar}$), 84.5 (C-17), 80.4 (C-2), 58.3 (Ar<u>C</u>H$_2$OH), 49.9 (C-14), 43.9 (C-9), 43.1 (C-13), 38.1 (C-8), 36.9 (CH$_2$), 30.4 (CH$_2$), 27.2 (CH$_2$), 26.8 (CH$_2$), 26.0 (CH$_2$), 23.2 (CH$_2$), 11.6 (CH$_3$, C-18); LRMS (EI) *m/z* (%) 428 (M$^+$, 22), 410 (100, M-H$_2$O), 396 (6); HRMS (EI) calcd for $C_{19}H_{25}IO_3$ 428.0848; found 428.0840.



2.68

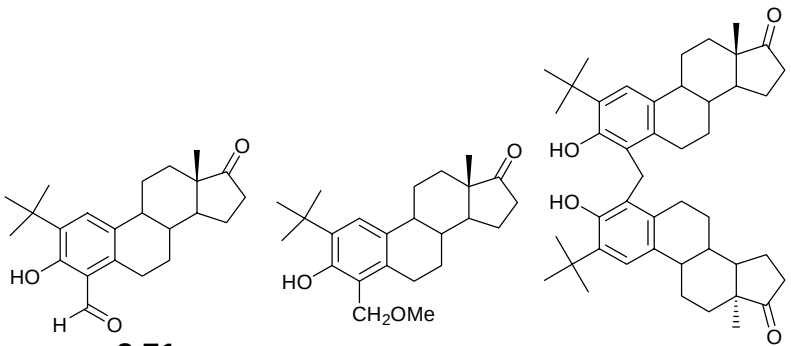
2-Bromo-4-formylestra-1,3,5(10)-triene-17-one (2.68). To a solution of E1 (270 mg, 1.00 mmol) in $CHCl_3$ (50 mL) was added a solution of tetrabutyl ammonium tribromide (TBATB, 540 mg, 1.1 mmol, 1.1 equiv) in $CHCl_3$ (10 mL) dropwise over 2 h. The resulting mixture was then stirred overnight. After washing with H_2O and brine, it was dried (Na_2SO_4) and concentrated (360 mg). To the crude residue was added $MgCl_2$ (250 mg, 2.6 mmol, 2.6 equiv), paraformaldhyde (360 mg, 120 mmol, 12 equiv), CH_3CN (15 mL), followed by triethylamine (0.40 mL, 2.9 mmol, 2.9 equiv). The resulting mixture was heated at 60 ^oC for 18 h. It was then cooled to rt, acidified with 2 M HCl till clear and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:2) to give an inseparable mixture (15:1) of **2.69** and **2.14** as light yellow solid (156 mg) and **2.68** as yellow solid (93 mg, 25%). Characterization data for **2.68**: ^1H NMR ($CDCl_3$, 300 MHz) δ 12.49 (s, 1H, CHO), 10.24 (s, 1H, OH), 7.64 (s, 1H, H-1), 3.35-3.00 (m, 2H), 2.45-1.40 (m, 13H), 0.84 (s, 3H, CH_3, H-18). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 220.1 (C=O), 195.2 (CHO), 157.8 (C-3), 139.1 (C-5), 138.2 (C-1), 132.8 (C-10), 118.1 (C-4), 109.1 (C-2), 50.2 (C-14), 47.7 (C-13), 44.1 (C-9), 37.3 (C-8), 34.7 (CH_2), 31.4 (CH_2), 26.1 (CH_2), 25.8 (CH_2), 25.2 (CH_2), 21.5 (CH_2), 14.1 (CH_3, C-18); LRMS (EI) *m/z* (%) 378 (M+2, 99), 376 (M^+, 100); HRMS (EI) calcd for $C_{19}H_{21}BrO_3$ 376.0674; found 376.0670.



2.70

2-*tert*-Butylestra-1,3,5(10)-triene-17-one (2.70). ***Method A*** ($FeCl_3$ and *t*-butyl chloride): This was prepared using a procedure adapted from Goendoes et al.[34.] To a vigorously stirred solution of E1 (10.8 g, 40.0 mmol) in CH_2Cl_2 (200 mL) was added *tert*-butyl chloride (100 mL) and anhydrous ferric chloride (6.50 g, 40.0 mmol, 1 equiv). After addition, the reaction mixture was stirred 2 days at rt before quenching with H_2O and 5% $NaHCO_3$. After separation and extraction of the aqueous layer with CH_2Cl_2, the combined organic extracts were washed with brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (methylene chloride) followed by treating the resulting solid with hexane gave of **2.70** as white solid (9.52 g, 73%) which could be recrystallized from chloroform. ***Method B*** (using $BF_3(OEt)_2$ and *t*-butyl alcohol): To a solution of estrone (7.00 g, 25.9 mmol) and t-butyl alcohol (4.95 mL, 51.8 mmol, 2.0 equiv) in dry methylene chloride (300 mL) was added $BF_3(OEt)_2$ (9.80 mL, 77.3 mmol, 3.0 equiv) over a period of one hour by syringe pump. After stirring for 2 h the reaction was quenched with sat. aq. $NaHCO_3$ and the layers separated. The organic layer was washed with water and brine then dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (methylene chloride) to give **2.70** as a white solid (8.1 g, 96%). ^{1}H NMR was identical to that reported in the literature.[34] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.19 (s, 1H, H-1), 6.44 (s, 1H, H-4), 5.09 (s, 1H, OH), 2.87-2.75 (m, 2H), 2.53-2.40 (m, 2H), 2.30-1.90 (m, 5H), 1.70-1.40 (m, 15H; 6H and $C(CH_3)_3$, s, 9H), 0.91 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 221.5 (C=O), 152.2 (C-3), 135.1 (C_{Ar}), 133.6 (C_{Ar}), 131.2 (C_{Ar}), 124.0 (C-1),

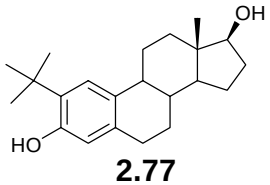
116.6 (C-4), 50.4 (C-14), 48.1 (C-13), 44.3 (C-9), 38.5 (C-8), 35.9 (C-16), 34.5 ($\underline{C}(CH_3)_3$), 31.6 (C-12), 29.7 (3C, $C(\underline{C}H_3)_3$), 28.8 (CH_2), 26.5 (CH_2), 26.0 (CH_2), 21.6 (CH_2), 13.9 (CH_3, C-18).



2.71 **2.72** **2.73**

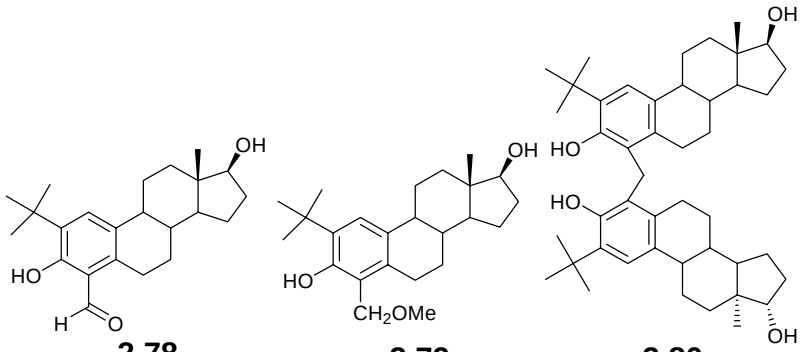
2-*tert*-Butyl-4-formylestra-1,3,5(10)-triene-17-one (**2.71**), **2-*tert*-Butyl-4-(methoxymethyl) estra-1,3,5(10)-triene-17-one (2.72) and Bis(2-*tert*-butylestra-1,3,5(10)–triene-17-one)-3-ylmethane (2.73).** To a mixture of 2-*tert*-butylestrone (984 mg, 3.00 mmol), para- formaldehyde (270 mg, 9.00 mmol, 3.0 equiv), $MgCl_2$ (700 mg, 7.37 mmol, 2.5 equiv) and $Fe(OAc)_3$ (2.7 mg, 0.4 mol%) in a glass bomb was added THF (50 mL), followed by triethylamine (1.0 mL, 7.20 mmol, 2.4 equiv). After purging with argon, the resulting mixture was heated at 40 ^oC for 5.5 h. It was then cooled to rt, diluted with ethyl acetate and acidified with 1N HCl and the resulting mixture was stirred 2 min and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane 1:3) gave **2.71** and **2.72** as an inseparable yellow solid mixture (749 mg, 73%), and dimer **2.73** as yellow solid (177 mg, 18%). Characteristic ^1H NMR assignments for **2.71:** ($CDCl_3$, 300 MHz) δ 12.80 (s, 1H, ArO$\underline{H}$), 10.34 (s, 1H, CHO), 7.47 (s, 1H, H-1). Characteristic ^1H NMR assignments for **2.72:** ($CDCl_3$, 300 MHz) δ 8.16 (s, 1H, ArO$\underline{H}$, exchangeable with D_2O), 7.20 (s, 1H,

H-1), 4.73 (d, J = 12.4 Hz, 1H, ArC<u>H</u>HOMe), 4.65 (d, J = 12.4 Hz, 1H, ArCH<u>H</u>OMe), 3.45 (s, 3H, OCH_3). Characterization data for **2.73**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.24 (s, 2H, H-1 x 2), 5.40 (s, 2H, ArO<u>H</u> x 2), 4.02 (s, 2H, ArC<u>H</u>$_2$Ar), 3.04 (dd, J = 16.8 Hz, J = 4.8 Hz, 2H, H-10 x 2), 2.90-2.79 (m, 2H), 2.55-2.29 (m, 6H), 2.21-1.97 (m, 8H), 1.67-1.23 (m, 12H, overlapping with s, 18H), 0.93 (s, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.9 (2C=O), 152.8 (2C, C-3), 134.7 (2C, C_{Ar}), 133.5 (2C, C_{Ar}), 132.2 (2C, C_{Ar}), 123.4 (2C, C-1), 121.6 (2C, C-4), 50.5 (2C, C-14), 47.9 (2C, C-13), 44.7 (2C, C-9), 37.6 (2C, C-8), 35.9 (2C, C-16), 34.8 (2<u>C</u>(CH$_3$)$_3$), 31.7 (2CH$_2$), 29.7 (2C(<u>C</u>H$_3$)$_3$), 27.6 (2CH$_2$) , 27.0 (2CH$_2$), 26.9 (2CH$_2$), 25.2 (Ar<u>C</u>H$_2$Ar), 21.6 (2CH$_2$), 13.9 (2CH$_3$, C-18); LRMS (EI) *m/z* (%) 664 (M^+, 38), 339 (57), 326 (100), 311 (50); HRMS (EI) calcd for $C_{45}H_{60}O_4$ 664.4492; found 664.4498



2.77

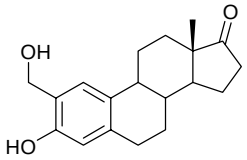
2-*tert*-Butyl-17*β*-hydroxyestra-1,3,5(10)-triene (2.77). To a solution of **2.70** (984 mg, 3.00 mmol) in ethanol/THF (36 mL, 5:1) at 0 ^{o}C was added $NaBH_4$ (228 mg, 6 mmol, 2 equiv). The resulting mixture was stirred for 1 h at 0 ^{o}C before quenching with 1 M HCl. After extracting with ethyl acetate, the combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane 1:2.5) gave **2.77** as a semi-solid (1.02 g, 100%). ^{1}H NMR was identical to that reported in the literature.[46] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.55 (s, 2H, H-1 and H-4), 3.90 (t, J = 8.2 Hz, 1H, H-17), 2.95-1.30 (m, 24H; m, 15 H and s, 9H, C(CH$_3$)$_3$ at 1.55 ppm), 0.92 (s, 3H, CH_3, H-18).



2.78 **2.79** **2.80**

2-*tert*-Butyl-4-formyl-17*β*-hydroxyestra-1,3,5(10)-triene (2.78), 2-*tert*-Butyl-17*β*-hydroxy-4-(methoxymethyl)estra-1,3,5(10)-triene(2.79) and Bis(17*β*-hydroxy-2-*tert*-Butylestra-1,3,5(10)-trien)-3-yl methane (2.80). To a mixture of **2.77** (1.02 g, 3.00 mmol), para-formaldehyde (405 mg, 13.5 mmol, 4.5 equiv), $MgCl_2$ (1.05 g, 11.0 mmol, 3.67 equiv) and $Fe(OAc)_3$ (0.3 mg, 0.04 mol%) in a glass bomb was added THF (40 mL), followed by triethylamine (1.50 mL, 10.7 mmol, 3.5 equiv). After purging with argon, the resulting mixture was heated at 45 ^oC for 4 h. After cooling to rt, it was diluted with ethyl acetate and acidified with 1 M HCl until it went clear. The mixture was extracted with ethyl acetate and washed with H_2O and brine, then dried (Na_2SO_4) filtered and concentrated. The residue obtained was dissolved in methanol (40 mL) and 1 M KOH (4 mL) was added to this solution. After stirring 5 min, it was extracted with ethyl acetate and combined extracts were washed with H_2O and brine then dried (Na_2SO_4) filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane 1:2.5) gave an inseparable mixture of **2.78** and **2.79** (701 mg, 66%, ratio 5:3) and dimer **2.80** as a yellow solid (280 mg, 28%) which can be recrystallized from $CHCl_3$/hexane. Characteristic ^1H NMR assignments for **2.78:** ($CDCl_3$, 300 MHz) δ 12.84 (s, 1H, ArO<u>H</u>), 10.29 (s, 1H, CHO), 7.52 (s, 1H, H-1). Characteristic ^1H NMR assignments for **2.79:** ($CDCl_3$, 300 MHz) δ 8.11 (s, 1H, ArO<u>H</u>), 7.24 (s, 1H, H-1), 4.73 (d, *J*

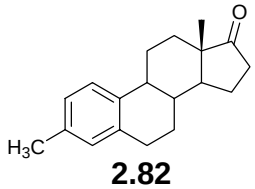
= 12.6 Hz, 1H, ArC<u>H</u>HO), 4.65 (d, *J* = 12.6 Hz, 1H, ArCH<u>H</u>O), 3.46 (s, 3H, OCH_3). Characterization data for **2.80**: mp: 206-208 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22 (s, 2H, H-1), 5.26 (s, 2H, ArO<u>H</u>, exchangeable with D_2O), 3.96 (s, 2H, ArC<u>H</u>$_2$Ar), 3.73 (t, *J* = 8.2 Hz, 2H, H-17 x 2), 2.97 (dd, *J* = 17.1 Hz , *J* = 5.1 Hz, 2H), 2.83-2.72 (m, 2H), 2.37-1.94 (m, 10H), 1.75-1.66 (m, 2H), 1.59-1.14 (m, 32H, C(C<u>H</u>$_3$)$_3$ 9H is overlapping in it)), 0.79 (s, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.7 (2C, C-3), 134.5 (2C, C_{Ar}), 133.6 (2C, C_{Ar}), 132.7 (2C, C_{Ar}), 123.5 (2C, C-1), 121.3 (2C, C-4), 81.9 (2C, C-17), 50.1 (2C, C-14), 44.6 (2C, C-9), 43.2 (2C, C-13), 38.0 (2C, C-8), 36.8 ($2CH_2$), 34.7 (2<u>C</u>$(CH_3)_3$), 30.7 ($2CH_2$), 29.7 (2C(<u>C</u>$H_3)_3$), 27.8 ($2CH_2$), 26.6 ($2CH_2$), 25.1 (Ar<u>C</u>H_2Ar), 23.1 ($2CH_2$), 11.1 ($2CH_3$, C-18); LRMS (EI) *m/z* (%) 668 (M^+, 44), 341 (43), 328 (100), 314 (47); HRMS (EI) calcd for $C_{45}H_{64}O_4$ 668.4805; found 668.4810.



2.81

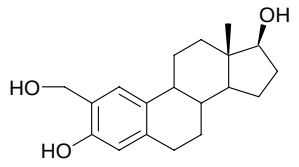
2-(Hydroxymethyl)estra-1,3,5(10)-triene-17-one (2.81). ***Method A*** (reduction of **2.14** using Bu_3SnH): Prepared using the procedure described above for **2.63** (method B) using **2.14** (596 mg, 2.00 mmol) and Bu_3SnH (873 mg, 3.00 mmol, 1.5 equiv) in MeOH (20 mL). Flash chromatography (ethyl acetate/hexane 1:2, then CH_2Cl_2, then ethyl acetate/hexane/CH_2Cl_2, 1:1:0.5) gave **2.81** as a white solid (311 mg, 52%). ***Method B*** (hydrogenation of **2.14** using Pd black): Prepared using the procedure described above for **2.63** (method C) using **2.14** (30 mg, 0.10 mmol) in ethyl acetate (10 mL) and Pd black (7.5 mg) and stirring 24 h followed by another portion of Pd black (2.5 mg) and stirred for 14 h. Flash chromatography (ethyl acetate/hexanes, 1:2 to 1:1.5) gave **2.81**

as a white solid (19 mg, 63%). Mp: 192 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.90 (s, 1H, ArOH), 7.13 (s, 1H, H-1), 6.42 (s, 1H, H-4), 4.77 (s, 1H, $ArCH_2O\underline{H}$), 4.39 (s, 2H, $ArC\underline{H}_2OH$), 2.70-2.28 (m, 2H), 2.08-1.89 (m, 4H), 1.72 (d, J = 6.3 Hz, 1H), 1.50-1.27 (m, 6H), 0.78 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 220.1 (C=O), 152.5 (C-3), 135.4 (C_{Ar}), 130.0 (C_{Ar}), 126.1 (C-2), 124.9 (C-1), 114.9 (C-4), 58.9 ($Ar\underline{C}H_2OH$), 50.1 (C-14), 47.8 (C-13), 44.0 (C-9), 38.5 (C-8), 35.8 (C-16), 31.8 (CH_2), 29.2 (CH_2), 26.7 (CH_2), 26.2 (CH_2), 21.6 (CH_2), 14.0 (CH_3, C-18); LRMS (EI) *m/z* (%) 300 (M^+, 67), 282 (M-H_2O, 100), 225 (11), 186 (13); HRMS (EI) calcd for $C_{19}H_{24}O_3$ 300.1725; found 300.1715.



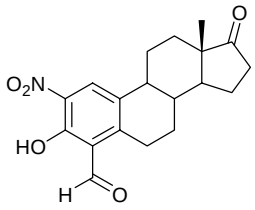
2.82

3-Methylestra-1,3,5(10)-triene-17-one (2.82). To a solution of 3-(hydroxymethyl)estrone[19.] (142 mg, 0.500 mmol) in MeOH (15 mL) was added palladium black (7 mg). The resulting mixture was stirred under H_2 (balloon pressure) overnight. After filtration, the filtrate was concentrated and purified by flash chromatography (ethyl acetate/hexane, 1:4) to give **2.83** as a white solid (136 mg, 93%). ^{1}H NMR was identical to that reported in the literature.[47] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.19 (d, J = 7.5 Hz, H-1), 6.98 (d, J = 8.0 Hz, H-2), 6.94 (s, H-4), 3.00-2.85 (m, 2H), 2.55-1.95 (10H; m, 7H and s, 3H, CH_3 at 2.30 ppm, overlapping), 1.75-1.35 (m, 6H), 0.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 220.9 (C=O), 136.7 (C_{Ar}), 136.3 (C_{Ar}), 135.3 (C_{Ar}), 129.7 (CH_{Ar}), 126.6 (CH_{Ar}), 125.3 (CH_{Ar}), 50.5 (C-14), 48.0 (C-13), 44.3 (C-9), 38.3 (C-8), 35.9 (CH_2), 31.6 (CH_2), 29.4 (CH_2), 26.6 (CH_2), 25.8 (CH_2), 21.6 (CH_2), 20.9 ($Ar\underline{C}H_3$), 13.9 (CH_3, C-18).



2.83

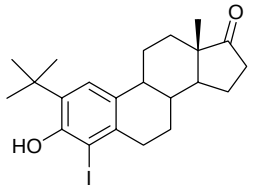
17*β*-Hydroxyl-2-(hydroxymethyl)estra-1,3,5(10)-triene (2.83). Prepared using the procedure described for the reduction of **2.13** to give **2.56** using **2.14** (100 mg, 0.336 mmol) and $NaBH_4$ (51 mg, 1.3 mmol, 4 equiv) in EtOH (20 mL). **2.83** was obtained as a white solid (75 mg, 75%). ^{1}H NMR was identical to that reported in the literature.[48] ^{1}H NMR (CD_3OD, 300 MHz) δ 7.12 (s, 1H, H-1), 6.44 (s, 1H, H-4), 4.57 (s, 2H, ArC$\underline{H}_2$O), 3.61 (t, J = 8.5 Hz, 1H, H-17), 2.76-2.64 (m, 2H), 2.26-2.07 (m, 1H), 2.03-1.10 (m, 12H), 0.73 (s, 3H, CH_3, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 152.5 (C-3), 136.5 (C_{Ar}), 131.0 (C_{Ar}), 125.1 (C-1), 124.3 (C-2), 114.6 (C-4), 81.1 (C-17), 59.9 (Ar$\underline{C}H_2$O), 49.9 (C-14), 44.0 (C-9), 43.0 (C-13), 39.1 (C-8), 36.6 (CH_2), 29.3 (CH_2), 27.1 (CH_2), 26.2 (CH_2), 22.6 (CH_2), 10.3 (CH_3, C-18).



2.84

4-Formyl-2-nitroestra-1,3,5(10)-triene-17-one (2.84). To a solution of **2.13** (90 mg, 0.30 mmol) in acetic acid (30 mL) at 45 ^{o}C was added conc. HNO_3 (0.20 mL, 2.8 mmol, 9.3 equiv). The reaction mixture was stirred overnight at rt. After removal of acetic acid *in vacuo*, the residue was purified by flash chromatography (ethyl acetate/hexane, 1:1) to give of **2.84** as a yellow solid (100 mg, 97%). Mp: 184-186 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.99 (brs, 1H, OH), 10.52 (s, 1H, CHO), 8.15 (s, 1H, H-1), 3.40 (dd, J = 18.6 Hz, J = 5.4 Hz, 1H), 3.16 (ddd, J = 18.3 Hz, J = 11.1 Hz, J = 6.9 Hz,

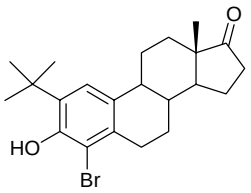
1H), 2.53-1.90 (m, 5H), 1.65-1.30 (m, 6H), 0.87 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.0 (C=O), 192.7 (CHO), 156.1 (C-3), 149.8 (C-5), 133.4 (C-2), 132.9 (C-10), 128.2 (C-1), 121.3 (C-4), 50.1 (C-14), 47.6 (C-13), 43.6 (C-9), 36.7 (C-8), 35.7 (C-16), 31.2 (CH_2), 27.4 (CH_2), 26.0 (CH_2), 25.6 (CH_2), 24.7 (CH_2), 21.4 (CH_2), 13.7 (CH_3, C-18); LRMS (EI) *m/z* (%) 343 (M^+, 75), 325 (M-CO, 100), 308 (7), 367 (10), 115 (11), 97 (12); HRMS (EI) calcd for $C_{19}H_{21}NO_2$ 343.1420; found 343.1419.



2.85

2-*tert*-Butyl-4-iodoestra-1,3,5(10)-triene-17-one (2.85). To a solution of **2.70** (100 mg, 0.307 mmol) in MeOH (10 mL) was added conc. NH_4OH (1 mL). The mixture turned yellow and was stirred 5 min before iodine (94 mg, 0.37 mmol, 1.2 equiv) was added. After stirring overnight, some starting material remained. Iodine (16 mg, 0.063 mmol, 0.2 equiv) was added and stirring was continued for 10 min before quenching with 10% $Na_2S_2O_3$. The ethanol was removed by rotatory evaporator and the resulting aqueous solution was extracted with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:6) to give **2.85** as a yellow oil (75 mg, 60%) which solidified upon standing. Mp: 157-158 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22 (s, 1H, H-1), 5.65 (brs, 1H, ArOH), 2.81 (dd, J = 17.4 Hz, J = 6.0 Hz, 1H), 2.71-1.93 (m, 9H), 1.65-1.42 (m, 5H), 1.38 (m, 9H, $C(CH_3)_3$), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.7(C=O), 150.6 (C-3), 136.7 (C_{Ar}), 133.6 (C_{Ar}), 132.8 (C_{Ar}),

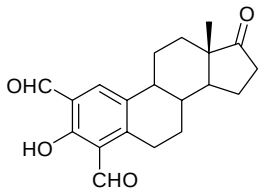
124.4 (C-1), 99.0 (C-4), 50.2 (C-14), 47.9 (C-13), 44.5 (C-9), 37.8 (C-8), 37.0 (C-16), 35.4 ($\underline{C}(CH_3)_3$), 31.6 (C-12), 29.5 ($C(\underline{C}H_3)_3$), 27.5 (CH_2), 26.3 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 452 (M^+, 66), 437 (M-CH_3, 100); HRMS (EI) calcd for $C_{22}H_{29}IO_2$ 452.1212; found 452.1208.



2.86

2-*tert*-Butyl-4-bromoestra-1,3,5(10)-triene-17-one (2.86). To a suspension of **2.70** (200 mg, 0.614 mmol) in absolute ethanol (10 mL) was added NBA (93 mg, 0.68 mmol, 1.1 equiv) and the reaction was stirred for 8 h. After a few hours of stirring the reaction became clear but then became cloudy again after the 8 h stirring period. Methylene chloride (20 mL) was added followed by a 5% aq. solution of $Na_2S_2O_3$ (20 mL) and the mixture was stirred for 20 min. The layers were separated and the aq. layer extracted with methylene chloride. The combined organics were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (methylene chloride) to give **2.86** as a white foam (219 mg, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.21 (s, 1H, H-1), 5.86 (brs, 1H, ArO$\underline{H}$), 2.92 (dd, J = 17.0 Hz, J = 7.0 Hz, 1H), 2.70 (m, 1H), 2.51 (dd, J = 19.0 Hz, J = 9.2 Hz, 1H), 2.43 (m, 1H), 2.28 (m, 1H), 2.13 (m, 3H), 1.97 (d, J = 7.3 Hz, 1H), 1.57 (m, 4H), 1.40 (s, 9H, $C(CH_3)_3$), 0.91 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.4 (C-3), 134.1 (C_{Ar}), 133.6 (C_{Ar}), 132.4 (C_{Ar}), 123.0 (C-1), 115.9 (C-4), 50.3 (C-14), 47.9 (C-13), 44.4 (C-9), 37.8 (C-8), 35.9 (C-16), 35.4 ($\underline{C}(CH_3)_3$), 31.6 (C-12), 29.5 ($C(\underline{C}H_3)_3$), 26.7 (CH_2), 26.2 (CH_2), 21.6 (CH_2), 13.9 (CH_3, C-18); LRMS (EI) *m/z* (%) 406 (M+2, 51), 404 (M^+, 51), 391 (M+2-Me, 100), 389 (M-Me, 99); HRMS (EI)

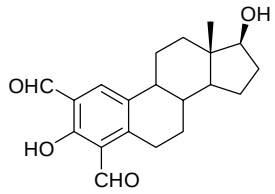
calcd for $C_{22}H_{29}BrO_2$ 404.1351; found 404.1360.



2.87

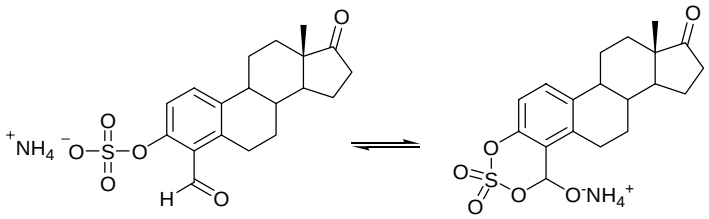
2,4-Diformylestra-1,3,5(10)-triene-17-one (2.87). A suspension of E1 (1.08, 4.00 mmol), paraformaldehyde (300 mg, 10.0 mmol, 2.5 equiv) and NaOH (fine powder, 100 mg, 2.50 mmol, 0.625 equiv) in dioxane (3 mL) was heated at 55 ^{o}C for 4.5 h before cooling to rt. After diluting with H_2O, the reaction mixture was acidified with 0.5 M HCl and then stirred for 2 min. The precipitate was collected by suction filtration and washed thoroughly with H_2O then dried under high vacuum to give 1.01 g of crude triol **2.87** as white solid. To a solution of **2.87** (1.0 g) in $CHCl_3$ (50 mL) was added activated MnO_2 (4.80 g, 55.2 mmol, 13.8 equiv) and the resulting mixture was stirred for 2 days at rt. After passing through a Celite pad, the filter cake was rinsed with $CHCl_3$ and the filtrate was washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:3 to 1:2.5 to 1:2) to give **2.87** as a yellow solid (88 mg, 7% over 2 steps starting from estrone). Mp: 212-213 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.27 (s, 1H, OH), 10.44 (s, 1H, CHO), 10.21 (s, 1H, CHO), 7.88 (s, 1H, H-1), 3.40 (dd, J = 18.3 Hz, J = 5.4 Hz, 1H, H-6), 3.22-3.10 (m, 1H), 2.61-2.37 (m, 2H), 2.25-1.89 (m, 5H), 1.63-1.38 (m, 6H), 0.87 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.0 (C=O), 194.3 (CHO), 190.3 (br, CHO), 163.6 (C-3), 148.4 (C-5), 134.1 (C-1), 132.2 (C-10), 121.5 (C_{Ar}), 119.1 (C_{Ar}), 50.1 (C-14), 47.7 (C-13), 43.6 (C-9), 37.0 (C-4), 35.7 (C-16), 31.3 (C-12), 26.7 (CH_2), 26.0 (CH_2), 25.7 (CH_2), 21.4 (CH_2), 13.7 (CH_3,

C-18); LRMS (EI) *m/z* (%) 326 (M^+, 100), 298 (99), HRMS (EI) calcd for $C_{20}H_{22}O_4$ 326.1518; found 326.1516.



2.90

2,4-Diformyl-17*β*-hydroxyestra-1,3,5(10)-triene (2.90). Same procedure as for **2.87** using E2 (1.0 g, 3.7 mmol), paraformaldehyde (300 mg, 10.0 mmol, 2.7 equiv), NaOH (fine powder, 100 mg, 2.50 mmol, 0.68 equiv) in dioxane (3 mL) heated at 50 ^oC for 7 h. 1.0 g of crude **2.89** was obtained as a white solid. Crude **2.89** (100 mg) was oxidized using activated MnO_2 (1.0 g, 11.5 mmol, 3.1 equiv) in CH_2Cl_2-MeOH (55 mL, 10:1), for 1 day at rt. Flash chromatography of the crude residue (ethyl acetate/hexane, 1:2.5) gave **2.90** as a yellow solid (17 mg, 15% over 2 steps starting from estradiol). Mp: 94-96 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 12.33 (s, 1H, OH), 10.46 (s, 1H, CHO), 10.28 (s, 1H, CHO), 7.94 (s, 1H, H-1), 3.72 (t, 1H, J = 8.4 Hz, H-17), 3.39 (dd, J = 18.8 Hz, J = 4.9 Hz, 1H), 3.20-3.05 (m, 1H), 2.40-2.30 (m, 1H), 2.20-1.65 (m, 6H), 1.55-1.15 (m, 7H), 0.76 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.5 (CHO), 190.1 (CHO), 163.6 (C-3), 148.6 (C-5), 134.1 (C-1), 132.8 (C-10), 121.5 (C_{Ar}), 119.0 (C_{Ar}), 81.6 (C-17), 49.8 (C-14), 43.6 (C-9), 43.1 (C-13), 37.6 (C-8), 36.4 (CH_2), 30.5 (CH_2), 26.8 (CH_2), 26.4 (2C, CH_2 x 2), 23.0 (CH_2), 11.0 (CH_3, C-18); LRMS (EI) *m/z* (%) 328 (M^+, 100), 300 (83); HRMS (EI) calcd for $C_{20}H_{24}O_4$ 328.1675; found 328.1674.



2.91 **2.92**

Ammonium 4-formyl-3-(sulfonatooxy)estra-1,3,5(10)-triene-17-one (2.91) and its cyclic tautomer (2.92). To a solution of 2.27 (165 mg, 0.324 mmol) in THF (5 mL) was added HCO_2NH_4 (122 mg, 1.94 mmol, 6 equiv). The mixture was stirred till the HCO_2NH_4 dissolved. The mixture was cooled to 0 ^oC and Zn dust (42 mg, 0.65 mmol, 2 equiv) was added slowly. After stirring for 1 h, another portion of Zn dust (21 mg, 0.33 mmol, 1 equiv) was added and stirring was continued for 3 h. After removal of solvents, the residue was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) to give a mixture of two tautomers **2.91** and **2.92** (acylic:cylic 2:3) as slightly yellow solids (75 mg, 59%). Characterization data for **2.91** (selected peaks): ^1H NMR (CD_3OD, 300 MHz) δ 10.32 (s, 1H), 7.57 (d, J = 8.7 Hz, 1H, H-1), 7.32 (d, J = 8.7 Hz, 1H, H-2), 0.88 (s, 3H, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 193 (CHO), 131.5 (C-1), 120.4 (C-2); Characterization data for **2.92** (selected peaks): ^1H NMR (CD_3OD, 300 MHz) δ 7.24 (s, 2H, H-1 and H-2), 5.84 (s, 1H, CHO (O)), 0.88 (s, 3H, H-18). ^{13}C NMR (CD_3OD, 75 MHz) δ 125.8 (C-1), 118.7 (C-2), 103.0 (CHO(O)).

2.5 References

1. Silverman, R. B. Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology, Vol. 1, CRC Press, 1988.

2. (a) Wang, Q.; Cechert, U.; Jirik, F.; Withers, S. G. *Biochem. Biophys. Res. Commun*. **1994**, *200*, 577. (b) Myers, J. K. Widlanski, T. S. *Science* **1993**, *262*, 1451.

3. Wakselman, M., *Nouv. J. Chim*. **1983**, *7*, 439.

4. Halazy, S.; berges, V.; Ehrhard, A.; Danzin, C. *Bioorg. Chem.* **1990**, *18*, 330.

5. Penney, C. L. Perlin, A. S. *Carbohydr. Res.* **1981**, *93*, 241.

6. Proud, A. D.; Prodger, J. C.; Flitsch, S. L. *Tetrahedron Lett*. **1997**, *41*, 7243

7. Organon, *Neth. Pat. Appl.* 6506542 (*Chem. Abstr.*, **1967**, *66*, 85950v).

8. Pert, D. J.; Ridley, D. D. *Aust. J. Chem*. **1989**, *42*, 405.

9. Lovely, C. J.; Gilbert, N. E.; Liberto, M. M.; Sharp, D. W.; Lin, Y. C.; Brueggemeier, R. W. *J. Med. Chem.* **1996,** *39,* 1917.

10. Cushman, M.; He, H.-M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1725.

11. Peters, R. H.; Chao, W.-R.; Sato, B.; Shigeno, K.; Zaveri, N. T.; Tanabe, M. *Steroids* **2003**, *68*, 97.

12. Spyriounis, D. M.; Rekka, E.; Demopoulos, V. J.; Kourounakis, P. N. *Arch. Pharm.* **1991**, *324*, 533.

13. Peters, R. H.; Chao, W.-R.; Sato, B.; Shigeno, K.; Zaveri, N. T.; Tanabe, M. *Steroids* **2003**, *68*, 97.

14. Hofsløkken, N. U.; Skattebol, L. *Acta Chim. Scand.* **1999**, *53*, 258.

15. Lorca, M.; Kuhn, D.; Kurosu, M. *Tetrahedron Lett.* **2001**, *42*, 6243.

16. The synthesis of **2.8-2.11** was performed in conjunction with Zena Qadoumi, an undergraduate working in the Taylor lab.

17. Woo, L. L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. *Chem. Biol.* **2000**, *7*, 773.

18. Chiang, Y.; Kresge, A. J.; Zhu, Y. *J. Am. Chem. Soc*. **2002**, *124*, 6349 and references therein.

19. Li, P.-K.; Pillai, R.; Dibbelt, L. *Steroids* **1995**, *60*, 299.

20. Utne, T.; Jobson, R. B.; Landgraf, F. W. *J. Org. Chem.* **1968**, *33*, 1654.

21. Numazawa, M.; Ogura, Y.; Kimura, K.; Nagaoka, M. *J. Chem. Res., Syn.* **1985**, *11*, 348.

22. Labrie, F.; Provencher, L.; Gauthier, S. *PCT Int. Appl.* **2004**, 53.

23. Xi, F.; Kamal, F.; Schenerman, M. A. *Tetrahedron Lett.* **2002**, *43*, 1395.

24. Cha, J. S.; Jang, S. H.; Kwon, S. Y. *Bull. Korean Chem. Soc.* **2002**, *23*, 1697.

25. Lovely, C. J.; Bhat, A. S.; Coughenour, H. D.; Gilbert, N. E.; Brueggemeier, R. W. *J. Med. Chem.* **1997**, *40,* 3756.

26. Tamura, K.; Kato, Y.; Ishikawa, A.; Kato, Y.; Himori, M.; Yoshida, M.; Takashima, Y.; Suzuki, T.; Kawabe, Y.; Cynshi, O.; Kodama, T.; Niki, E.; Shimizu, M. *J. Med. Chem.* **2003**, *46*, 3083.

27. Hillmann-Elies, A.; Hillmann, G.; Schiedt, U. *Naturforsch.* **1953**, *8B*, 436.

28. Ali, H.; Ghaffari, M. A.; Lier, E. V. *J. Steroid Biochem.* **1987**, *28*, 21.

29. Horiuchi, C. A.; Satoh, J. Y. *J. Chem. Soc., Chem. Commun.* **1982**, *12*, 671.

30. Page, P. C. B.; Hussain, F.; Maggs, J. L.; Morgan, P.; Park, B. K. *Tetrahedron* **1990**, *46*, 2059.

31. Chung, C. K.; Ho, M. S.; Lun, K. S.; Wong, M. O.; Wong, H. N. C.; Tam, S. W. *Syn. Commun.* **1988**, *18*, 507.

32. Kulka, M. *J. Am. Chem. Soc.*, **1954**, *76*, 5469.

33. Lunn, W. H. W.; Farkas, E. *Tetrahedron* **1968**, *24*, 6773.

34. Goendoes, G.; Dombi, G. *Monatsh. Chem.* **2002**, *133*, 1279.

35. Aldred, R.; Johnston, R.; Levin, D.; Neilan, J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1823.

36. Kamiura, K.; Wada, M. *Tetrahedron Lett.* **1999**, *40*, 9059.

37. Singh, V.; Lahiri, S.; Kane, V. V.; Stey, T.; Stalke, D. *Org. Lett.* **2003**, *5*, 2199.

38. (a) Watanabe, K.; Hayashi, H.; Mori, Y. *Pharmacol. Res*. **1993**, *28*, 59. (b) Angelis, Y. S.; Smonou, I. *Tetrahedron Lett.* **1997**, *38*, 8109.

39. Reggelin, M.; Doerr, S. *Synlett* **2004**, *6*, 1117.

40. Burchat, A. F.; Chong, J. M.; Nielsen, N. *J. Organomet. Chem.* **1997**, *542*, 281.

41. Page, P. C. B.; Hussain, F.; Bonham, N. M.; Morgan, P.; Maggs, J. L.; Park, B. K. *Tetrahedron* **1991**, *47*, 2871.

42. This compound has been prepared by Ponsold and Kasch using a different approach: Ponsold, K.; Kasch, H. *Tetrahedron Lett.* **1979**, *46*, 4465.

43. Leese, M. P.; Hejaz, H. A. M.; Mahon, M. F.; Newman, S. P.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2005**, *48*, 5243.

44. Numazawa, M.; Ogura, Y.; Kimura, K.; Nagaoka, M. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3701.

45. He, H.-M.; Fanwick, P. E.; Wood, K.; Cushman, M. *J. Org. Chem.* **1995**, *60*, 5905.

46. Miller, C. P.; Jirkovsky, I.; Hayhurst, D. A.; Adelman, S. J. *Steroids* **1996**, *61*, 305.

47. Uyanik, C.; Hanson, J. R.; Hitchcock, P. B. *J. Chem. Res., Syn.* **2003**, *12*, 795.

48. Edsall, A. B.; Mohanakrishnan, A. K.; Yang, D.; Fanwick, P. E.; Hamel, E.; Hanson, A. D.; Agoston, G. E.; Cushman, M. *J. Med. Chem.* **2004**, *47*, 5126.

Chapter 3

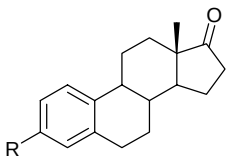
Synthesis of steroidal and non-steroidal compounds bearing sulfate surrogates

3.1 Introduction

3.1.1 Reversible inhibitors of STS bearing sulfate surrogates

Although most STS inhibitors are irreversible sulfamate inhibitors many reversible STS inhibitors have been reported. What has appeared in the literature on this class of inhibitors is

Table 3.1. STS inhibitors in which the sulfate group of estrone sulfate is replaced with an O-, N-, or S-linked sulfate surrogate.



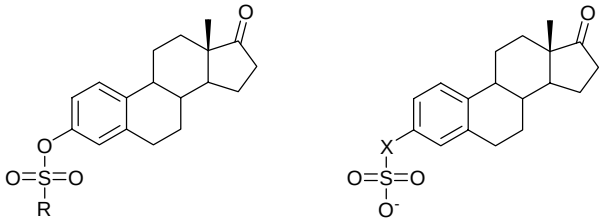
Cpd.	R	Inhibition	assay	Ref.
3.1	$-SO_2Cl$	65% at 60 μM	Placental microsomes	2
3.2	$-SO_3^-K^+$	K_i = 40 μM at pH 7	Purified STS	3
3.3	$-SO_2NH_2$	K_i = 140 μM at pH 7	Purified STS	3
3.4	$-SO_2F$	K_i = 35 μM at pH 7	Purified STS	3
3.5	$-SO_2CH_3$	K_i = 130 μM at pH 7.	Purified STS	3
3.6	-SH	10% at 10 μM	Placental microsomes	4
3.7	$-SSO_2NH_2$	12% at 50 μM	Placental microsomes	5
3.8	$-SSO_2N(CH_3)_2$	0% at 100 μM	Placental microsomes	5
3.9	$-SCON(CH_3)_2$	4% at 50 μM	Placental microsomes	5
3.10	$-NHSO_2NH_2$	53% at 50 μM	Placental microsomes	5
3.11	$-NHSO_2CF_3$	IC_{50} = 10 μM	Placental microsomes	6
3.12	$-N(SO_2CF_3)_2$	IC_{50} = 15 μM	Placental microsomes	6
3.13	$-NHCOCF_3$	IC_{50} = 9 μM	Placental microsomes	6
3.14	$-NHCONH_2$	IC_{50} = 13 μM	Placental microsomes	6
3.15	$-NH_2$	15% at 10 μM	Placental microsomes	4
3.16	$-OPO_3^{-2-}$	K_i = 5 μM at pH 7.0	Partially pure STS	7
3.17	$-OPO_2F$	K_i = 14 μM at pH 7.0	Partially pure STS	7
3.18	$-OSO_2NHCH_3$	93% at 10 μM	Intact MCF-7 cells	8
3.19	$-OSO_2N(CH_3)_2$	90% at 10 μM	Intact MCF-7 cells	8
3.20	$-OSO_2CH_3$	K_i = 23 μM	Purified STS	9

summarized in Table 3.1. The vast majority of these were never examined with pure enzyme and the modality of inhibition was not determined which makes it difficult to compare their potency with one another. Nevertheless, as can be seen from the Table, these have not proven to be highly effective inhibitors.

One the objectives of our work on STS inhibitors is to develop reversible STS inhibitors by replacing the sulfate group of known STS substrates with hydrolytically stable functional groups that may interact with specific residues in the active site by both reversible covalent and non-covalent interactions. Below we propose several such moieties.

3.1.2 The α,α-difluoromethylenesulfonamide group as a sulfate surrogate

Unlike sulfamates, primary sulfonamides have not been extensively examined as STS inhibitors. Sulfonamide **3.3** is a reversible STS inhibitor and exhibits a K_i of 140 μM when using ^{35}S-dehydroepiandosterone sulfate as substrate at pH 7.0.[3] This K_i is approximately 3.5 times greater than that of sulfonate **3.2** (40 μM) and both have K_i's that are much greater than that of E1S (0.9 μM) under the same conditions.[3] On the basis of these studies and on studies with other sulfonate analogs, it was concluded that an oxygen atom or an electronically similar link between the aryl moiety and the sulfur atom is essential for high affinity binding.[3]



1.1, X = O, R = NH_2 (EMATE)
E1S, X = O, R = O^-
3.21, X = CF_2
3.22, X = CH_2

Figure 3.1. Structures of EMATE, estrone sulfate (E1S) and selected analogs

In chapter 1, we discussed EMATE (compound **1**.**1,** section 1.3) as the prototypical sulfamate irreversible STS inhibitor. The S-O bond must be hydrolyzed by STS for irreversible inhibition to occur. Indeed, the S-O bond of EMATE is readily cleaved by STS, even though EMATE is an apparently neutral compound at physiological pH and the enzyme's natural substrate is an anionic sulfate ester. To account for STS's ability to readily bind and hydrolyze EMATE, as well as other sulfamates, it has been suggested that it is the conjugate base of EMATE, which is isoelectronic and isosteric to estrone sulfate (E1S, section 1.1), that binds to STS, even though the conjugate base is the minor species at physiological pH with the N-proton of EMATE having a pK_a of 9.5 in 70% aqueous methanol.[11-13] Nevertheless, such a hypothesis is consistent with the active site architecture of STS.[14] The active site contains a calcium ion as well as other cationic residues such as Lys134, Lys368, His290 and His136 some of which are probably involved in interacting with the anion of the sulfate substrate.[14]

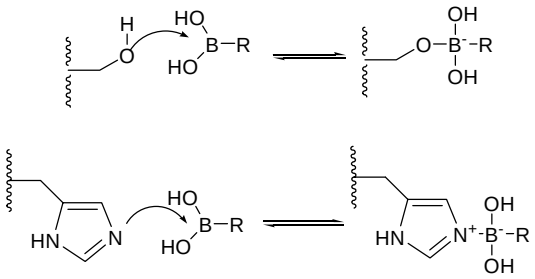
We recently demonstrated that the CF_2 group can be used as a stable replacement for the bridging oxygen in estrone sulfate in that compound **3.21** is a competitive STS inhibitor (K_i = 57 μM, pH 7.4, 0.1% Triton X-100) and was approximately 10-fold more potent than its non-fluorinated analogue **3.22**.[15,16] Since both **3.21** and **3.22** have pK_a's that are far below the pH at which the studies were performed (pH 7.4), we reasoned that their difference in potency was due to the fluorines interacting with residues in the active site perhaps by fluorine H-bonding with His290 which is believed to act as a general acid during the cleavage of the S-O bond.[14,16]

On the basis of our studies with compound **3.21**, and the possibility that it is the conjugate base of EMATE that binds to STS, we suggest that the α, α-difluoromethylenesulfonamide (DFMS,

R-$CF_2SO_2NH_2$) group might be an effective sulfate surrogate for obtaining STS inhibitors. The pK_a of the DFMS group should be approximately three pK_a units less than that of a primary non-fluorinated sulfonamide (pK_a ~10-11).[17] Therefore, a significant proportion of the conjugate base of DFMS-bearing compounds should be present at physiological pH and the fluorines will also be available for interacting with residues in the active site.

3.1.3 Boronic acids as STS inhibitors

Boronic acids have been used as inhibitors and probes of enzymes and proteins, such as serine proteases, for many years and recently, a highly potent and selective protease inhibitor in the form of a peptidyl boronic acid has recently been approved by the FDA for treatment of relapsed and refractory multiple myeloma.[18]



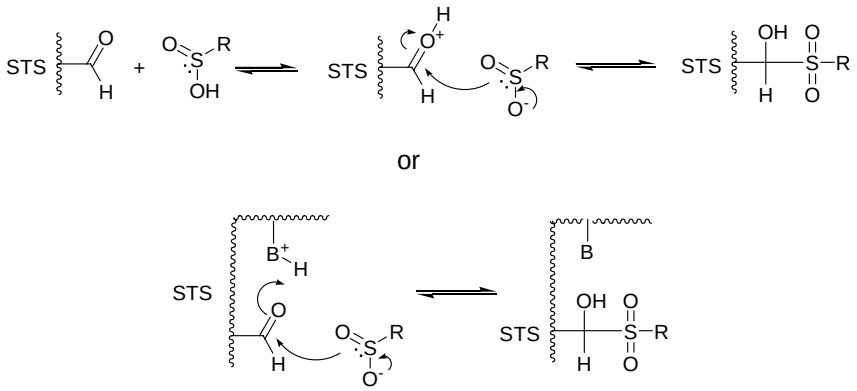
Scheme 3.1. Mechanism for the inhibition of serine protease with boronic acids

These inhibitors, some of which have K_i's in the subnanomolar range, function by forming reversible covalent adducts with active site residues, such as the crucial serine residue or an active site histidine residue as shown in Scheme 3.1.[18] As mentioned in Chapter 1, STS does not have an active site serine residue when in their catalytically active form. Instead, it has an active site formyl glycine hydrate which is a result of a post-translational enzymatic modification of a cysteine or serine residue. Addition of water to the aldehyde yields the stable formylglycine hydrate.[19,20] The hydrate attacks

the sulfur atom of the substrate resulting in cleavage of the S-O bond, release of the hydroxyl or phenolic portion of the substrate and formation of a sulfated hydrate (see Chapter 1, section 1.2, Scheme 1.2). The sulfate group is then eliminated from the hydrate to give inorganic sulfate and formyl glycine which is then rehydrated. Several other active site residues, including two conserved histidines (His136 and His290), are believed to function as general acids and bases during the reaction.[21] In light of this proposed mechanism, we reasoned that boronic acids might act as potent inhibitors of STS by forming reversible covalent adducts with the active site formyl glycine hydrate and/or histidine residues in a manner similar to serine proteases.

3.1.4 Sulfinic acids as STS Inhibitors

Sulfinic acids are known to react with aldehydes and the resulting covalent adducts (α-hydroxy sulfones) are relatively stable.[22] This prompted us to examine sulfinic acids as potential STS inhibitors. We anticipated that should the formylglycine hydrate be in equilibrium with its aldehyde form, then a sulfinic acid or its conjugate base might be able to form a covalent adduct with the aldehyde and inhibit STS (Scheme 3.2).



Scheme 3.2. Possible reaction of sulfinic acids with STS

3.1.5 Enhancing the potency of STS inhibitors by introducing anionic groups at the 17-position

In addition to the above mentioned approaches to STS inhibitors, the Taylor group has recently entered into a collaboration with the Ghosh group to develop rationally designed inhibitors of STS. The Ghosh group has modeled estrone into the active site of STS. Beyond the sulfate binding site, and moving towards the 17-position of STS, the vast majority of residues that are within van der Waals contact distance with rings B, C and D are (not surprisingly) hydrophobic residues, such as phenylalanine, leucine and valine. However, in relatively close proximity (about 5 Å) to the oxygen of the carbonyl at the 17-position is an arginine residue (Arg98). Modeling studies performed in the Ghosh group on estrone modified at the 17-position with a phosphate or carboxylate group suggest that such moieties might be capable of interacting with Arg98 by electrostatic or H-bonding interactions (Figure 3.2). These studies have prompted us to construct steroid derivatives bearing these anionic groups at the 17-position anticipating that this might be a means of increasing the potency of STS inhibitors.

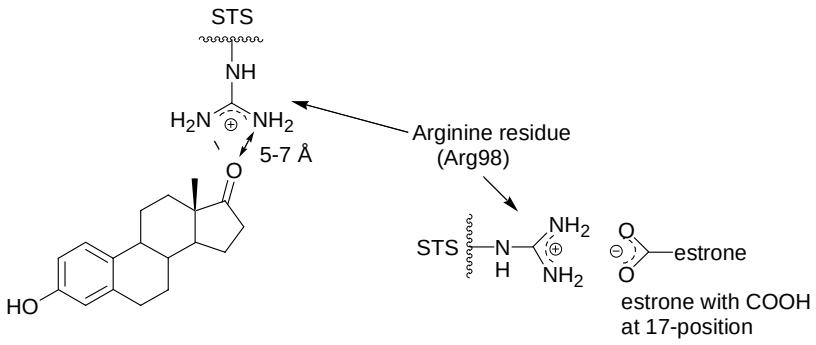


Figure 3.2. A potential approach to rationally designed STS inhibitors

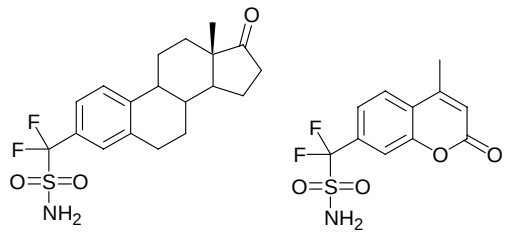
3.1.6 Objectives

The objective of the work presented in this chapter is to synthesize steroidal and non-steroidal compounds bearing the α, α-difluoromethylenesulfonamide group, the boronic acid group and the sulfinic acid group. It is anticipated that these compounds will be good competitive inhibitors of STS and so will be useful as lead structures for the development of highly potent STS inhibitors. Another objective is to modify known steroidal STS inhibitors such that they bear an anionic moiety, such as a carboxylate or phosphate group, at the 17-position. We anticipate that by adding such a group at this position that the resulting compounds will exhibit increased potency compared to their unmodified precursors.

3.2 Results and Discussion

3.2.1 Synthesis of α, α-difluoromethylenesulfonamides

To examine the DFMS group as a sulfate surrogate we decided to prepare compounds **3.23** and **3.24**. These compounds are the DFMS analogs of EMATE and Coumate which are potent irreversible suicide inhibitors of STS and are very good STS substrates (see Chapter 1, section 1.3).

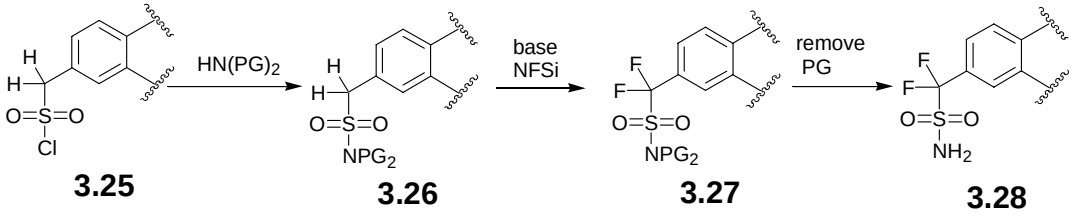


3.23 **3.24**

Figure 3.3. Structures of target sulfonamides

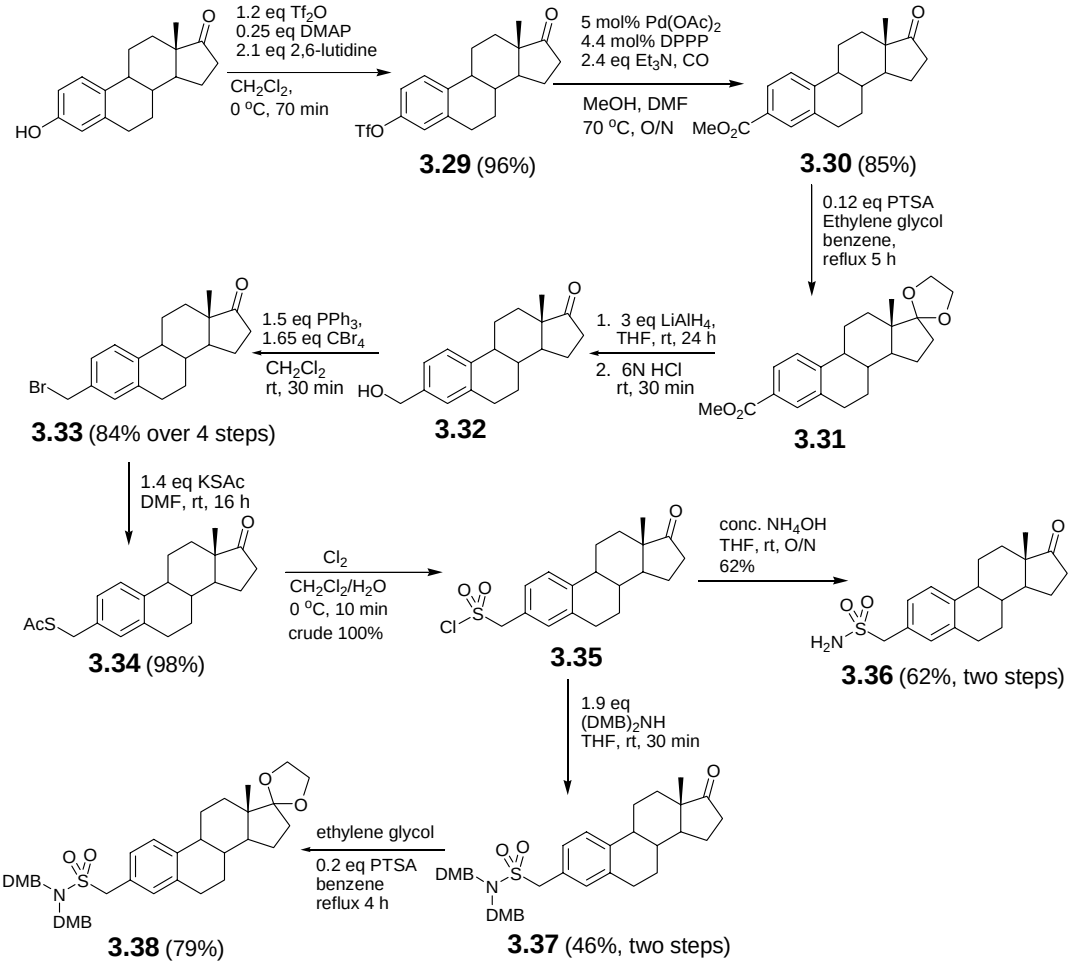
Bryan Hill, a post-doc in the Taylor group, had recently shown that α, α-difluorosulfonamides can be prepared by electrophilic fluorination of α-carbanions of protected sulfonamides using

N-fluorobenzenesulfonimide (NFSi).[23] To our knowledge, this is the only route to this class of compounds. Therefore, our general approach to compounds **3.23** and **3.24** was to prepare the sulfonamides of type **3.26** from sulfonyl chlorides of type **3.25** and then subject these to electrophilic fluorination followed by deprotection of the sulfonamide moiety (Scheme 3.3).



Scheme 3.3. Proposed route to difluoromethylenesulfonamides

The synthesis of the protected estrone sulfonamide is outlined in Scheme 3.4. We first prepared benzyl bromide **3.33** using a slightly modified version of the procedure of Li et al.[9.] Thus, estrone was converted to its triflate **3.29** in 96% yield by treating estrone with triflic anhydride, cat. DMAP in the presence of 2,6-lutidine. Use of triethylamine as base instead afforded a mixture of the desired triflate, ditriflate and unreacted estrone. Palladium catalyzed carboxylation of estrone trilfate **3.29** using $Pd(OAc)_2$/dppp gave the corresponding methyl ester **3.30** in 85% yield. The ketone at the 17-position was protected as ketal **3.31** using PTSA/ ethylene glycol in refluxing benzene. This reaction was subjected to an aqueous workup but the crude ketal was not chromatographed. Reduction of the ester moiety in **3.31** to the alcohol with $LiAlH_4$ followed by acidic hydrolysis of the ketal group at the 17-position gave alcohol **3.32**. Again, no chromatography of **3.32** was necessary. The crude alcohol **3.32** was brominated using triphenylphosphine and carbon tetrabromide to give bromide **3.33**. The overall yield from ester **3.30** to bromide **3.33** was 84% over 4 steps and only one column purification was required. Bromide **3.33** was converted to thioacetate **3.34** in 98% yield by

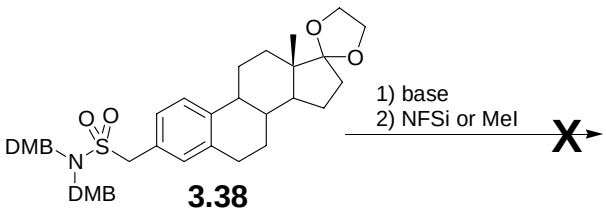


Scheme 3.4. Synthesis of sulfonamides **3.38** and **3.36**

treating **3.33** with potassium thioacetate (1.4 equiv) in DMF. Oxidative chlorination of **3.34** with Cl_2 in H_2O/CH_2Cl_2 gave the crude sulfonyl chloride **3.35**. Since it would be useful to compare the inhibitory potency of **3.23** with is non-fluorinated analogue (**3.36**), we prepared **3.36** in 62% yield by reacting crude sulfonyl chloride **3.35** with an excess amount of ammonium hydroxide. To prepare the fluorinated analog of **3.36**, we had to prepare **3.36** with the sulfonamide moiety protected. The Taylor group had recently developed a new protecting group for sulfonamides in the form of the bis(2,4-dimethoxybenzyl) (DMB) moiety.[23] This group is stable to a wide variety of conditions yet is removed using TFA in CH_2Cl_2. Reaction of crude **3.35** with bis(2,4-dimethoxybenzyl) amine

($(DMB)_2NH$) gave DMB-protected sulfonamide **3.37** in 46% yield. No additional base such as triethylamine was required for the reaction with $(DMB)_2NH$; otherwise a much lower yield or none of the desired product was obtained. The ketone at the 17-position was then protected as ketal **3.38** in 79% yield using PTSA (0.2 equiv) and ethylene glycol in refluxing benzene.

We then attempted the electrophilic fluorination reaction on sulfonamide **3.38** (Scheme 3.5). However, no product was formed and only starting material remaining. A variety of different conditions (different bases, temperatures, reaction times etc.) were tried; however, no reaction occurred. We also tried to methylate the benzylic position using MeI but still no reaction occurred. Quenching the reaction with D_2O or MeOD did not result in the incorporation of any deuterium at the α-position. The α-position may be too sterically hindered due to the large size of DMB group and the estrone backbone. We reasoned that perhaps this reaction would proceed if a smaller protecting group was used.

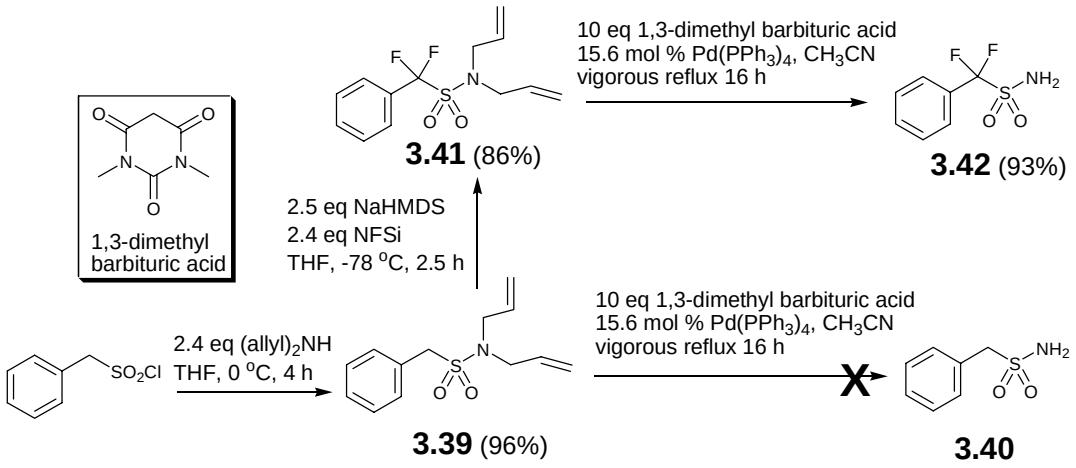


Scheme 3.5. Attempted fluorination of **3.38**

Only a small number of protecting groups have been developed for sulfonamides. Bryan Hill in the Taylor group had previously demonstrated that two of them, the benzyl and 4-methoxybenzyl groups, were very difficult to remove from α, α-difluorosulfonamides and so these groups were not considered as possible replacements for the DMB group in **3.38**.[23] The dimethylpyrrole group has been used as a protecting group for sulfonamides.[24] However, this group

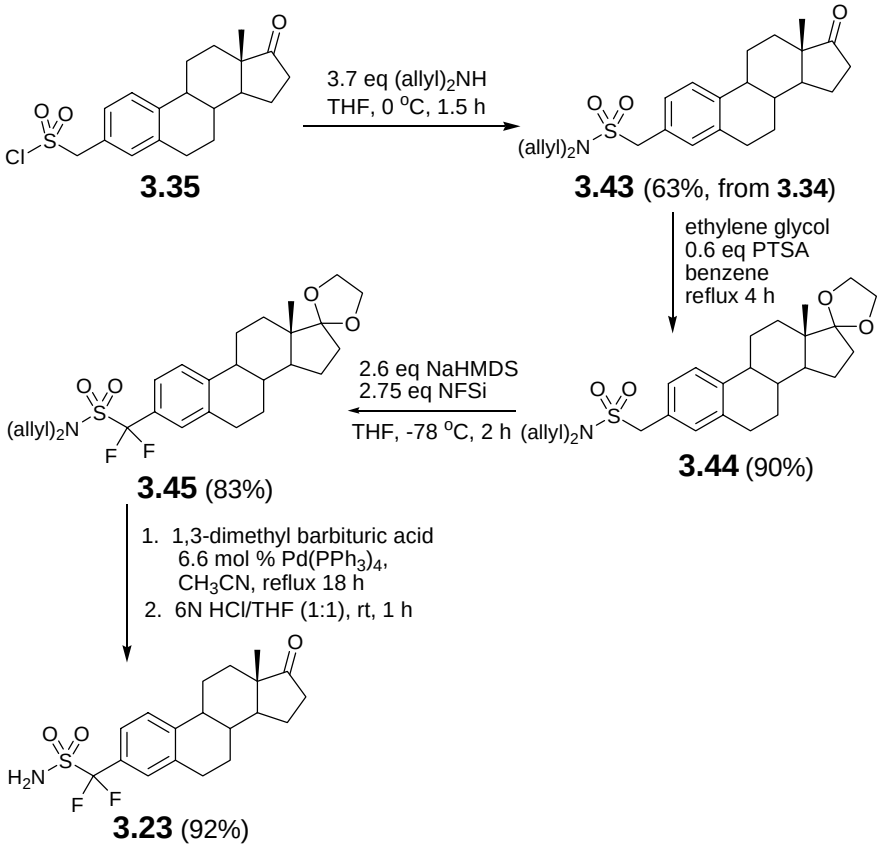
is removed under harsh conditions (refluxing concentrated TFA in H_2O) and we expected that the presence of fluorines would make this group even more difficult to remove as was the case with the 4-methoxybenzyl group.[23] Therefore, we turned to the allyl group which has been used extensively for the protection of amines and esters. Although diallyl sulfonamides are known compounds, the allyl group has never been used as a sulfonamide protecting group. Nevertheless, we anticipated that its relatively small size would enable us to fluorinate the corresponding steroidal sulfonamide and that it could be removed under conditions that have been developed for its removal from amines and esters.

Before we tried allyl deprotection, we tried a model fluorination reaction on benzene derivative **3.39** which was readily obtained by reacting α-toluenesulfonyl chloride with diallyl amine (Scheme 3.6). Electrophilic fluorination of **3.39** using NaHMDS/NFSi in THF at -78 ^oC gave difluorosulfonamide **3.41** in 86% yield. However, attempts to deprotect sulfonamide **3.41** using conditions commonly used to remove allyl groups from amines ($TolSO_2Na$, dimedone or 2-thiobenzoic acid as allyl scavengers in the presence of Rh or Pd catalysts)[25] were unsuccessful or proceeded very slowly giving low yields of monodeprotected products after prolonged reaction times. Garro-Helion et al. have reported that allyl groups can be removed from amines using 1,3-dimethylbarbituric acid as the allyl scavenger and $Pd(PPh_3)_4$ in CH_2Cl_2 at 30 ^oC.[26] These conditions resulted in a very slow monodeprotection of **3.41**. However, by performing the reaction in refluxing CH_3CN, the deprotected product **3.42** was obtained in 93 % yield. Worthy of note is that its non-fluoro analog **3.39** did not undergo similar allyl deprotection to give **3.40** which may explain why this group has never been used as a protecting group for sulfonamides.



Scheme 3.6. Fluorination of **3.39** and deprotection of **3.41**

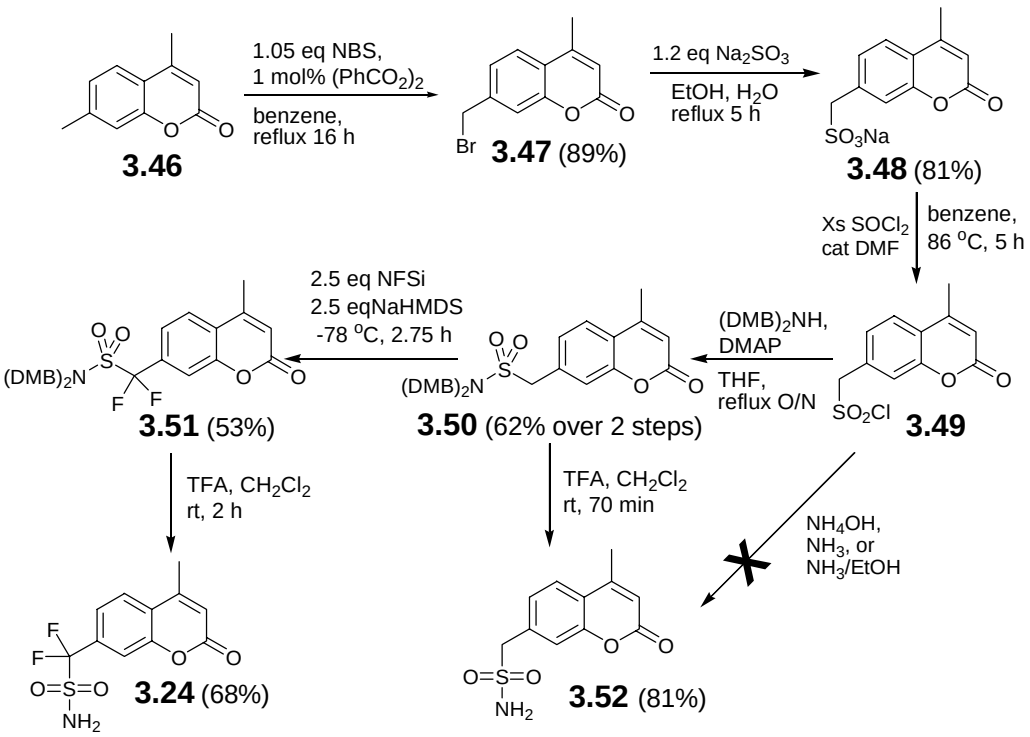
These results prompted us to apply allyl protection to the synthesis of **3.23** (Scheme 3.7). Oxidative chlorination of **3.35** followed by reaction of the sulfonyl chloride with diallylamine gave



Scheme 3.7. Synthesis of **3.23** using allyl protection

sulfonamide **3.43** in 63% yield. The ketone was protected as a ketal in 90% yield and the resulting compound, **3.44**, was subjected to NaHMDS/NFSi. This gave the fluorinated sulfonamide **3.45** in 83% yield which supported our hypothesis that steric factors played a key role in preventing the electrophilic fluorination of sulfonamide **3.38**. The allyl protecting groups in sulfonamide **3.45** were readily removed using 1,3-dimethylbarbituric acid in the presence of cat. $Pd(PPh_3)_4$ in refluxing CH_3CN. Treating the resulting deprotected product with dilute HCl in THF gave compound **3.23** in an outstanding 92% yield (two steps). The overall yield of compound **3.23** starting from estrone was 29% over 13 steps.

The synthesis of coumarin **3.24** is outlined in Scheme 3.8. 4,7-Dimethyl coumarin **3.46** was prepared from *m*-cresol and ethyl acetoacetate via a Pechmann condensation.[27] The brominated compound **3.47** was obtained in 89% yield by treating **3.46** with 1.05 equiv of NBS in refluxing benzene in the presence of 1 mol% of benzoyl peroxide.[28] It is interesting to note that this bromination occurs exclusively at the methyl group at the 7-position and no bromination occurs on the methyl group at the 4-position. The regiochemistry was confirmed by comparing the chemical shifts of 4- and 7-methyls on brominated product **3.47** and its precursor **3.46** from ^{13}C NMR (DMSO-d_6). In compound **3.46**, $\delta_{C\text{-}4'}$ = 18.4 ppm, $\delta_{C\text{-}7'}$ = 21.4 ppm.[27] In compound **3.47**, $\delta_{C\text{-}4'}$ = 18.5 ppm, $\delta_{C\text{-}7'}$ = 33.3 ppm. Reaction of bromide **3.47** with sodium sulfite (Na_2SO_3) in refluxing EtOH/H_2O (1:1) for 5 h gave sulfonate **3.48** in 81% yield after recrystallization from water. Treatment of sulfonate **3.48** with $POCl_3$ in refluxing CH_3CN did not result in any reaction, while the use of $SOCl_2$ and catalytic amount of DMF gave the desired sulfonyl chloride **3.49**. It was essential to remove all of the sodium bromide that resulted from the sulfonation reaction before performing the chlorination reaction. If



Scheme 3.8. Synthesis of compound **3.24** and **3.52**

sulfonate **3.48** is contaminated with sodium bromide during the chlorination reaction a significant amount of benzylic bromide forms possibly by nucleophilic attack of bromide ion on the benzylic carbon to release SO_2 and chloride. So repeated recystallization of **3.48** was required to remove NaBr. The crude sulfonyl chloride **3.49** was reacted with bis(2,4-dimethoxybenzyl)amine to give sulfonamide **3.50** in 62% yield over 2 steps. Electrophilic fluorination of **3.50** using NaHMDS (2.5 equiv) and NFSi (2.5 equiv) at -78 ^{o}C for 2.75 h gave desired difluorosulfonamide **3.51** in 53% yield. This reaction was slow to go to completion probably due to the resonance structure of its enolate which stabilizes the anion formed and lowers its reactivity. We also found that best yields were obtained when performing the reaction under relatively dilute conditions (1.0 mM) as higher concentrations gave lower yields. Deprotection of sulfonamide **3.51** went smoothly to give **3.24** in

68% yield using TFA/CH_2Cl_2. The overall yield of sulfonamide **3.24** from coumarin **3.46** was 16% (6 steps)

Since it would be useful to compare the inhibitory potency of difluorosulfonamide **3.24** with its non-fluorinated analog **3.52**, we attempted to prepare **3.52** by reacting sulfonyl chloride **3.49** with conc. ammonium hydroxide or ammonia in ethanol; however, none of the desired product was obtained even when heating in a glass bomb. Therefore, we attempted to prepare it by deprotection of **3.50**. Interestingly, when the deprotection was performed using TFA/THF only monodeprotected product was obtained. However, when we used TFA/CH_2Cl_2, it went to completion. This reaction was relatively clean from TLC. However, purifying the compound by silica gel chromatography was very difficult due to the very poor solubility of **3.52** in most organic solvents. Nevertheless, we found that we could purify this compound by first adding ether to the crude product and filtering. A non polar impurity was soluble in this solvent while **3.52** was not. This material contained what appeared to be an insoluble polymer of some type (pink colored impurity). Dissolving the filtered material in hot ethanol resulted in solublization of **3.52** but not the polymer which could be removed by filtration. Using this procedure, pure sulfonamide **3.52** was obtained in an 81% yield.

3.2.2 Synthesis of boronic acids

To examine the boronic acid group as a sulfate surrogate we decided to prepare compounds **3.53-3.56**. Compounds **3.53**, **3.55** and **3.56** were chosen since they are the boronic acid analogs of EMATE (**1.1**), 667-Coumate (**1.3**) and chromenone sulfamate inhibitor **1.4** (see Chapter 1, section 1.3) all of which are known to be readily hydrolyzed by STS. Compound **3.54** is the boronic acid analog compound **1.5**, a potent reversible inhibitor of STS (see Chapter 1, section 1.3). We anticipated that

the addition of a boronic acid group to **1.5** would further increase its potency.

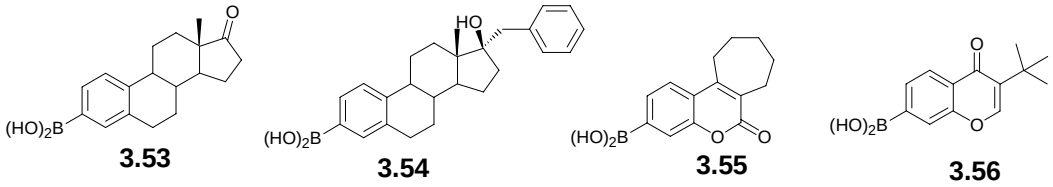
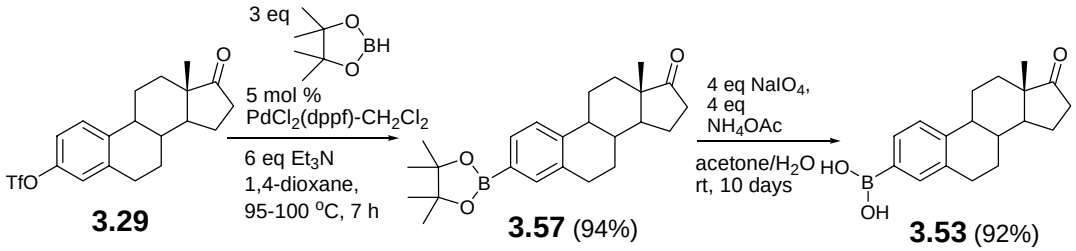


Figure 3.4. Structures of target boronic acids

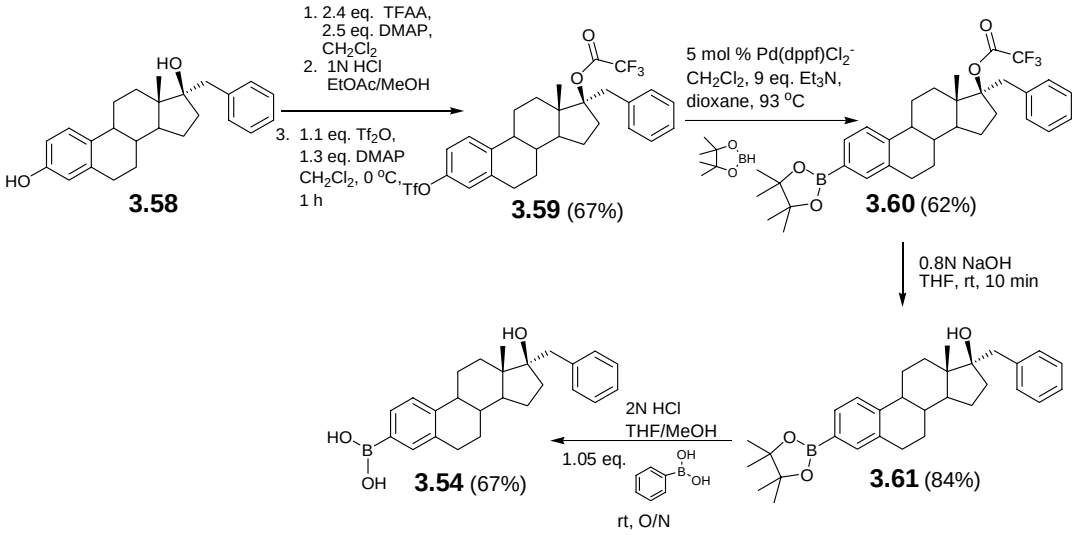
We began with the synthesis of steroid derivative **3.53** (Scheme 3.9). Recently, Masuda and co-workers reported palladium-catalyzed borylation of aryl triflates with dialkoxyborolanes.[29] Following their procedure with slight modification (higher temp, more borolane, Et_3N and longer reaction time), estrone triflate **3.29** was converted to its corresponding boronate **3.57** in 94% yield using 4,4,5,5-tetramethyl-1,3,2-dioxaborolane in dioxane at 95-100 ^oC in the presence of Pd(dppf)Cl_2-CH_2Cl_2 catalyst (5 mol%) and triethylamine. Simple acid or base hydrolysis of boronate **3.57** to the boronic acid yielded many byproducts and we were unable to obtain pure boronic acid. We also tried transesterifcation using HCl and phenyl boronic acid.[30] Although this reaction did go cleanly the product was hard to purify. We then tried a procedure developed by Yu et al. which involved subjecting **3.57** to $NaIO_4$ (4 equiv)/NH_4OAc (4 equiv) in acetone/H_2O. Although the reaction was slow and took 10 days to go to completion, it proceeded very cleanly and estrone boronic acid **3.53** was isolated in a 92% yield.



Scheme 3.9. Synthesis of compound **3.53**

To prepare compound **3.54,** we first tried a Grignard reaction using benzyl magnesium bromide on boronates **3.57** and **3.53** but this was unsuccessful. Therefore, we had to do the Grignard addition first and then install the boronate ester (Scheme 3.10). 17α-benzylestradiol **3.58** was prepared in 73% yield by Grignard addition of benzyl maganesium bromide to estrone according to Ciobanu et al.'s procedure.[32] To facilitate the separation of **3.58** from unreacted estrone, the mixture was subjected to $NaBH_4$ which reduced the ketone in estrone to give estradiol which could be easily separated from **3.58**. We attempted a Pd-catalyzed coupling of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane on the triflate of **3.58** but this gave a hard-to-identify product that was clearly not the desired product. Therefore, we decided to protect the 17-OH. Ciobanu et al. reported that the 17-position of an estradiol derivative can be protected with the trifluoroacetyl (TFAc) group using trifluoroacetic anhydride (TFAA) though they reported that the ester formed was quite labile and partially hydrolyzed during hydrogenation and silica gel column conditions.[33.] We anticipated that we should be able to selectively protect the 17-position of **3.58** with a TFAc group by simply subjecting it to excess trifluoroacetic anhydride (TFAA), which would give the TFAc esters of both the phenolic OH and the 17-OH, and then hydrolyzing the more hydrolytically labile phenolic ester. Reaction of **3.58** with excess TFAA in the presence of DMAP gave a mixture of diacetate and monoacetates. The TFAc group at the phenolic position was very labile as TLC always showed two spots (around 1:1 ratio of diacetate and monoacetate and starting material was gone). We were unable to drive this reaction to completion. Therefore, the resulting mixture was treated with 1N HCl to give the 17-TFAc ester together with some starting material. Since this TFAc ester was not particularly stable, the crude mixture was directly converted to its corresponding phenolic triflate **3.59**

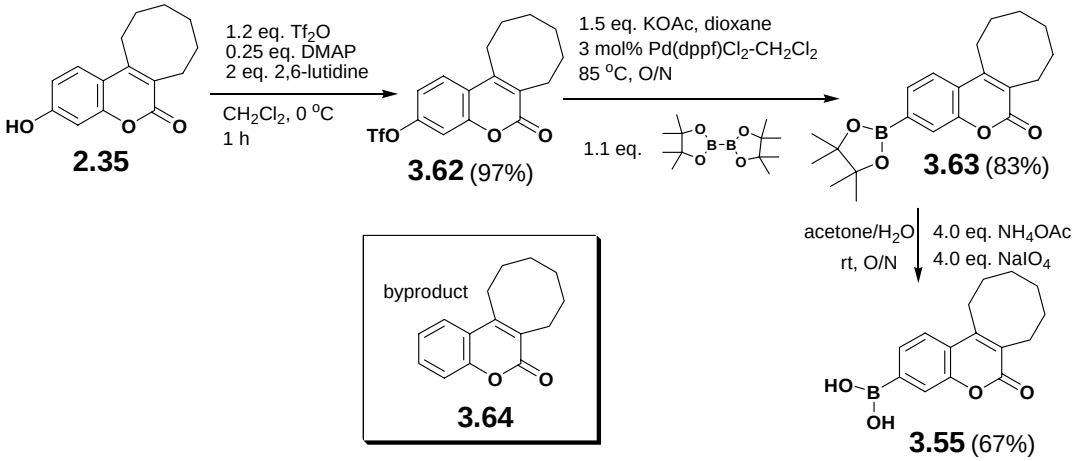
by using triflic anhydride and DMAP. Overall yield of triflate **3.59** was a respectable 67% over 3 steps starting from 17α-benzylestradiol. 4,4,5,5-tetramethyl-1,3,2-dioxaborolane was coupled to triflate **3.59** to give boronate ester **3.60** in 62% yield. During this reaction the TFAc ester moiety remained intact. Hydrolysis of the TFAc ester in **3.60** was accomplished by adding excess 0.8 N NaOH dropwise to a solution of **3.60** in THF which gave compound **3.61** in an 84% yield. Boronate **3.61** was readily deprotected by transesterification using phenylboronic acid and 2 N HCl which gave **3.54** in 67% yield and no difficulties were encountered in its purification. We do not yet have an explanation for their difference in reactivity under these conditions.



Scheme 3.10. Synthesis of compound **3.54**

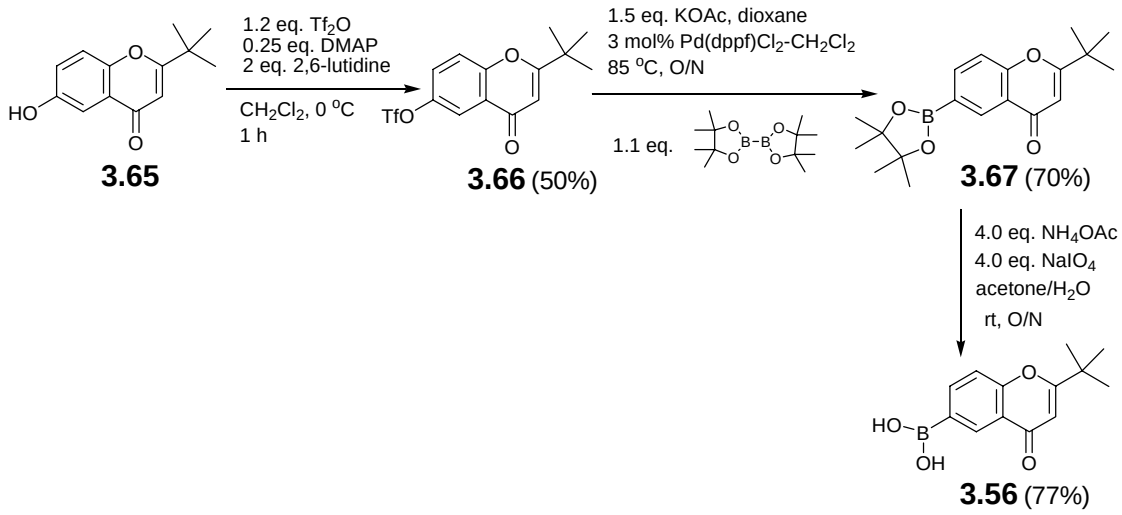
To obtain coumarin boronic acid **3.55**, we started by converting coumarin **2.35**[34] to its triflate **3.62** in 97% yield using triflic andydride (Scheme 3.11). For the conversion from triflate to boronate **3.63**, we first tried 4,4,5,5-tetramethyl-1,3,2-dioxaborolane. To our surprise, the only isolated byproduct was the reduced product **3.64** where the triflate was replaced by hydrogen. There are some literature precedents for this type of reductive byproduct.[29,35] However in these instances, they were

just minor byproducts, while in our case, it was predominant. We hypothesized that the hydrogen source may have been pinacolborane. Therefore, we tried pinacol diborolane instead and obtained the desired coumarin boronate **3.63** in 83% yield. Deprotection of boronate **3.63** with $NaIO_4/NH_4OAc$ afforded the desired boronic acid **3.55** in 67% yield. The faster rate may be due to its better solubility in acetone/H_2O than estrone boronate **3.57**.



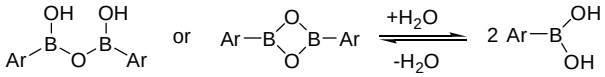
Scheme 3.11. Synthesis of coumarin boronic acid **3.55**

Chromenone boronic acid **3.56** was synthesized in a similar manner to **3.55** (Scheme 3.12). The triflation of chromenone **3.65**[36] didn't go to completion and only a 50% yield of triflate **3.66** was obtained together with 16% recovered starting material. The reaction was not optimized further. Again, coupling reaction of **3.66** with 4,4,5,5-tetramethyl-1,3,2-dioxaborolane didn't give the desired boronate. However, the desired product chromenone boronate **3.67** was obtained in 70% yield by coupling with the diborolane. Cleavage of pinacol moiety using $NaIO_4/NH_4OAc$ gave chromenone boronic acid **3.56** in 77% yield.



Scheme 3.12. Synthesis of chromenone boronic acid **3.56**

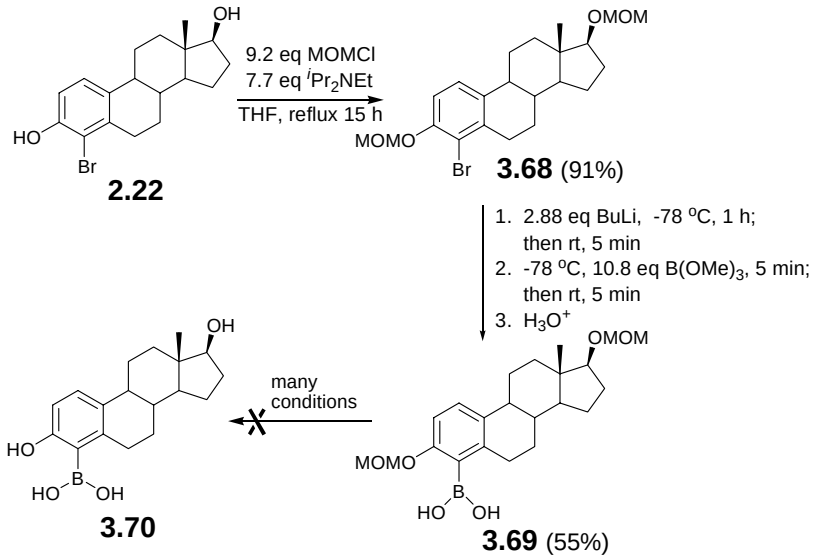
The coumarin and chromenone boronic acids can easily form boronic anhydrides (Scheme 3.13) and so sometimes the ^{1}H NMR will show another set of peaks in addition to the desired product though the compounds will show up as a single spot by TLC. The ratio of boronic acid to anhydride depends on how dry the NMR solvent is. For example, different DMSO-d_6 ampules have different amounts of water present and so the ratio changes from one ampule to another. In order to avoid this complexity, a drop of D_2O was added to the DMSO-d_6 every time an NMR was run. This resulted in the break down of the boronic anhydride to the free boronic acid.



Scheme 3.13. Formation of boronic anhydrides

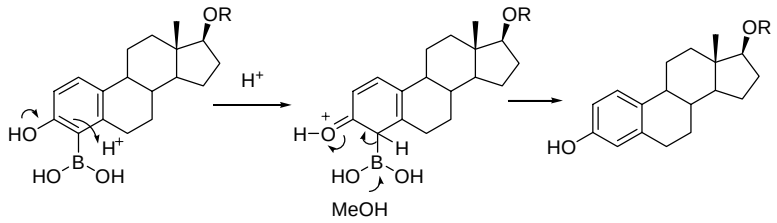
In Chapter 2 we found that 4-formyl estrone (**2.13**) is a good irreversible inhibitor of STS. We hypothesized that it inhibited STS by forming a Schiff base with an active site arginine or lysine. Since boronic acids can form adducts with lysines and arginines we thought that estrone or estradiol with a boronic acid group at the 4-postion (compound **3.70**, Scheme 3.14) might also be a good STS

inhibitor. Reaction of 4-bromoestradiol (**2.22**)[37] with MOMCl gave the desired diMOM protected steroid **3.68** in 91% yield (Scheme 3.14). Lithium-halogen exchange, followed by treating with trimethyl borate, gave boronic acid **3.69** in 55% yield along with debrominated byproduct (30%) and another byproduct (6%) where bromine was replaced by OH. Unfortunately, removal of the MOM



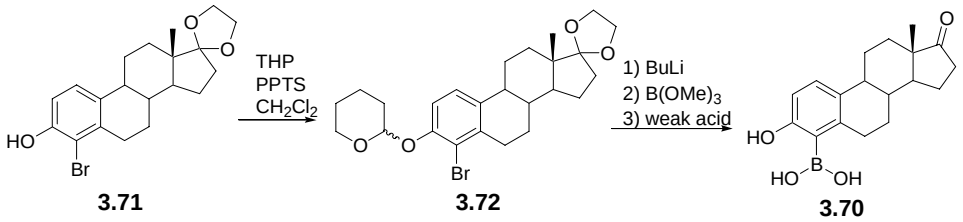
Scheme 3.14. Attempted synthesis of boronic acid **3.70**

group was very difficult. Several methods were tried such as BBr_3 in CH_2Cl_2 at -78 ^oC, conc. HCl in THF at rt, 1 M HCl at rt to reflux in THF/MeOH, amberlyst-15 in refluxing THF; however, none of the desired product was isolated. Deprotection of MOM using weak acid PPTS in refluxing methanol gave estradiol as the major product and the 17-MOM derivative as a minor product. A possible mechanism that accounts for the loss of the boronic acid moiety is shown in Scheme 3.15.



Scheme 3.15. Proposed mechanism for loss of the boronic acid moiety.

The synthesis of compound **3.70** is still in progress in the Taylor group but using a slightly different approach (Scheme 3.16). Removing the MOM group requires conditions that are too harsh. Therefore, we will use 4-bromoestrone protected at the 3-position with a tetrahydropyranyl (THP) group and the 17-position protected with a cyclic ketal (**3.71**). Ketal **3.71** will be treated with THP in the presence of PPTS to form acetal **3.72**. Compound **3.70** will be obtained by treating **3.72** with BuLi followed by boronation and treatment with mild acid. Another route using a Pd-catalyzed cross-coupling reaction with 4-bromoestrone or 3-THP protected 4-bromoestrone and pinacolboronane is also being investigated.



Scheme 3.16. Alternative route to boronic acid **3.70**

3.2.3 Synthesis of Sulfinic Acids

To examine the sulfinic acid group as a sulfate surrogate we decided to prepare compounds **3.73** and **3.74** both of which can be considered the sulfinic acid analogs of estrone sulfate.

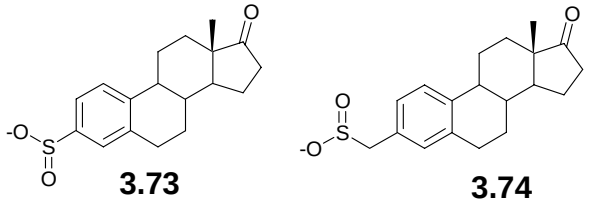
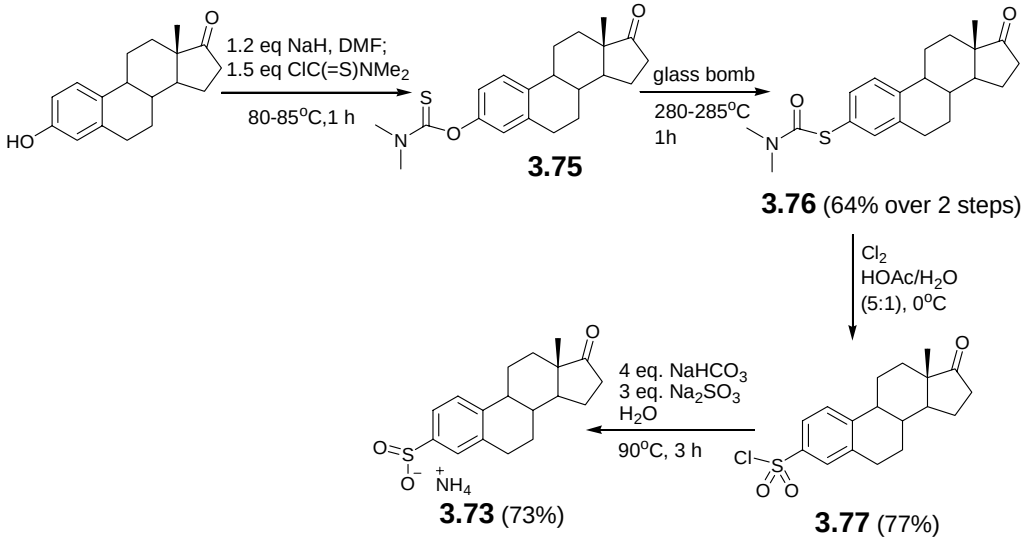


Figure 3.5. Structures of target sulfinic acids

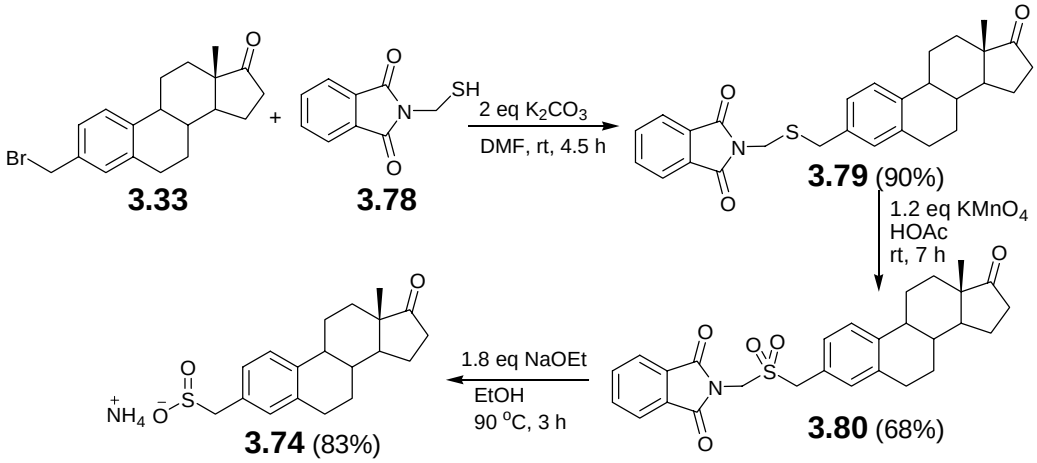
The syntheses of **3.73** and **3.74** were straightforward. For the synthesis of **3.73** we decided to use Binisti et al.'s route to phenyl sulfinic acids which involves heating a sulfonyl chloride with

Na_2SO_3 and $NaHCO_3$ (3 equiv) in H_2O at 90 ^oC.[38] Synthesis of estra-3-sulfonyl chloride **3.77** has been reported by Li et al. in 25% yield over 5 steps starting from estrone.[2] Reaction of estrone with *N,N*-dimethyl thiocarbamoyl chloride gave crude *O*-aryl thiocarbamate **3.75** (Scheme 3.17). Crude **3.75** underwent a Newman-Kwart rearrangement to *S*-aryl thiocarbamate **3.76** when heated to 280-285 ^oC for 1 h in a glass bomb. The yield for these two steps was 64% after recrystallization. Li et al. cleaved **3.76** to the mercaptan and then protected the mercaptan with a benzyl group. This benzyl thioether was then converted into the sulfonyl chloride **3.77** by oxidative chlorination. However, we anticipated that **3.76** could be converted into the acid chloride directly by oxidative chlorination. Indeed, subjecting **3.76** to chlorine gas in $HOAc/H_2O$ afforded desired chloride **3.77** in 77% yield after chromatography. The overall yield of estrone sulfonyl chloride **3.77** was 49% over 3 steps, a significant improvement over the literature procedure. Reduction of **3.77** with Na_2SO_3 and $NaHCO_3$ in H_2O at 90 ^oC for 3 h gave aryl sulfinic acid **3.73** in 73% yield as its ammonium salt after chromatography using $CH_2Cl_2/MeOH/NH_4OH$ (10:2:0.5).



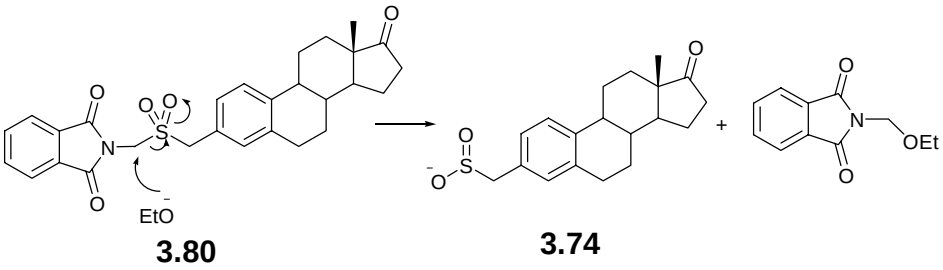
Scheme 3.17. Synthesis of compound **3.73**

The preparation of estra-3-methylsufinic acid (**3.74**) began with 3-(bromomethyl) estrone **3.33** (Scheme 3.18). Reaction of **3.33** with thiol **3.78** gave sulfide **3.79** in 90% yield. Oxidation of **3.79** with *m*CPBA (*m*-chloroperoxybenzoic acid) resulted in oxidation of the sulfide and a Bayer-Villiger reaction on the ketone at the 17-position to form the corresponding lactone. However, we found that the selective oxidation of sulfide **3.79** to the corresponding sulfone **3.80** could be achieved in 68% yield by using 1.2 equiv $KMnO_4$ in HOAc at rt. Sulfone **3.80** was reduced to sulfinic acid **3.74** using NaOEt in refluxing EtOH.[39] Chromatography of this material using CH_2Cl_2/MeOH/NH_4OH (10:2:0.5) gave the ammonium salt in 83% yield.



Scheme 3.18. Synthesis of sulfinic acid **3.74**

The mechanism for the reduction of the sulfone to the sulfinic acid is shown in Scheme 3.19. The pK_a's of phthalimide and phenyl sulfinic acid in H_2O is 8.3 and 2.1, respectively. Thus, the benzylic sulfinic acid has a much lower pK_a value than phthalimide and so the carbon-sulfur bond instead of carbon-nitrogen bond breaks to form the sulfinic acid.

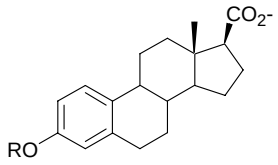


Scheme 3.19. Proposed mechanism for the formation of sulfinic acid **3.74** from sulfone **3.80**

Compound **3.74** is not very stable. Attempts to convert it from the ammonium salt to the sodium salt by ion-exchange chromatography resulted in complete decomposition. It decomposed quickly in DMSO or methanol and broke down slowly in D_2O. This is in contrast to sulfinate **3.73** which showed no significant decomposition after 3 days in D_2O at rt.

3.2.4 Synthesis of steroids modified at the 17-position with a carboxylic acid group

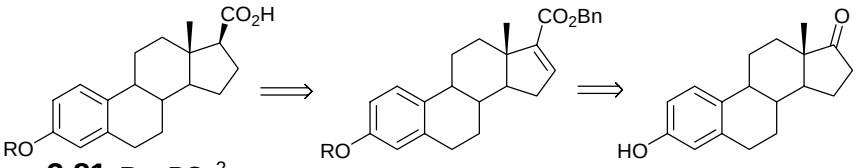
To examine whether the presence of an anionic moiety at the 17-position can increase the potency of both reversible and irreversible STS inhibitors we have designed compounds **3.81**-**3.83** as our initial targets. These compounds bear a carboxylate group at the 17-position which we anticipate will interact with Arg146. Compounds **3.81** and **3.82** bear a phosphate and a methanesulfonate group at the 3-position. Estrone-3-phosphate (**3.16**, Table 3.1) is a competitive STS inhibitor and at pH 7.0 exhibits a K_i of 5 μM.[7] Estrone-3-methanesulfonate (**3.20**, Table 3.1) has been reported to be a competitive STS inhibitor with a K_i of 23 μM at pH 7.0.[9] Therefore, we anticipate that compounds **3.81** and **3.82** will be reversible STS inhibitors. Compound **3.83** is EMATE with a carboxylate at the 17-position and should be an irreversible STS inhibitor.



3.81, R = PO_3^{2-}
3.82, R = SO_2CH_3
3.83, R = SO_2NH_2

Figure 3.6. Structures of target steroids modified at the 17-position

Our general route to the 17-COOH derivatives is outlined in Scheme 3.20. α,β-Unsaturated carboxylic acids **3.84-3.86** will be prepared starting from estrone. Hydrogenolysis of these species would yield the desired products.

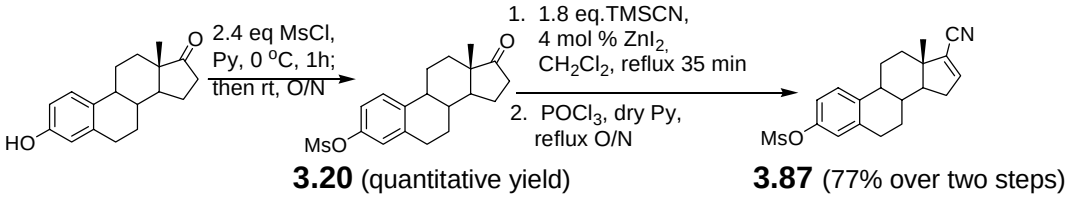


3.81, R = PO_3^{2-}
3.82, R = SO_2CH_3
3.83, R = SO_2NH_2

3.84, R = PO_3^{2-}
3.85, R = SO_2CH_3
3.86, R = SO_2NH_2

Scheme 3.20. Retrosynthesis of compounds **3.18-3.83**

The synthesis of compounds **3.81-3.83** began with the construction of nitrile **3.87** using a literature procedure (Scheme 3.21).[40] Thus, estrone was treated with MsCl and pyridine to give mesylate **3.20** in quantitative yield. Mesylate **3.20** was treated with trimethylsilyl cyanide (TMSCN) and catalytic zinc iodide in dichloromethane followed by acidic workup (heating in conc. HCl). The resulting cyanohydrin was dehydrated with phosphorus oxychloride ($POCl_3$) and dry pyridine to

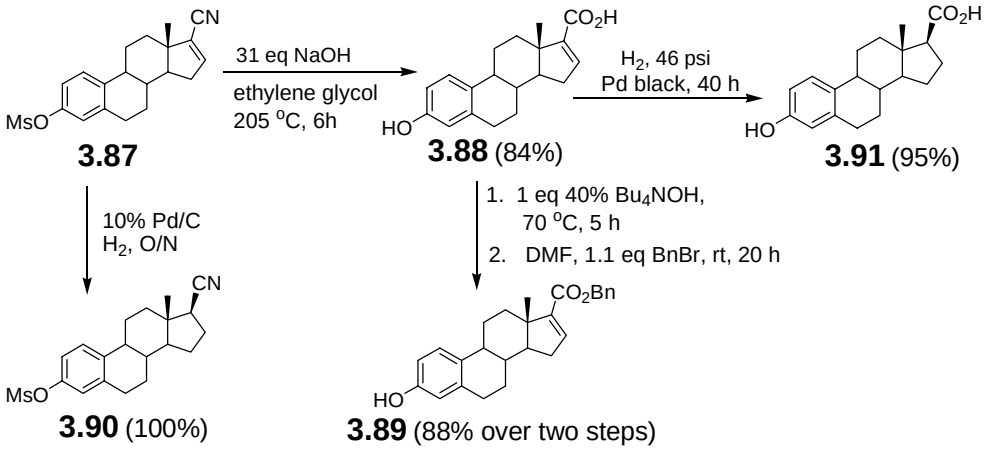


3.20 (quantitative yield) **3.87** (77% over two steps)

Scheme 3.21. Synthesis of nitrile **3.87**

afford unsaturated cyanide **3.87** in 77% yield over two steps.

Hydrolysis of nitrile **3.87** with excess solid sodium hydroxide in ethylene glycol at 205 ^{o}C for 6 h gave carboxylic acid **3.88** in 84% yield (Scheme 3.22). In a large scale, we got a 58% yield of acid **3.88** over 3 steps starting from mesylate **3.20** with a single column purification required at the last step. Benzylation of acid **3.88** was first performed by refluxing acid **3.88** with benzyl bromide in THF in the presence of one equivalent of weak base DBU (1,8-diazabicyclo[5.4.0]undec-7-ene). However, this conversion rate was low even after refluxing overnight. A two-step procedure was then investigated which involved selective deprotonation of **3.88** with 1 equiv of tetrabutylammonium hydroxide (Bu_4NOH, 40% aqueous solution) followed by treatment of the resulting ammonium salt

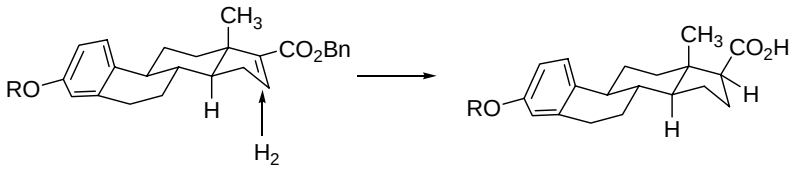


Scheme 3.22. Synthesis of compounds **3.89-3.90**.

with benzyl bromide in DMF. This worked well and gave the desired benzyl ester **3.89** in 81% yield. Before we attempted any modifications at the 3-position we first worked out conditions for hydrogenolysis of the double bond using **3.88** as a model compound. To our surprise, subjecting **3.88** to 10% Pd/C and H_2 did not result in hydrogenation of the double bond even under 45 psi of H_2, while the double bond in nitrile **3.87** was easily hydrogenated using 10% Pd/C under balloon pressure

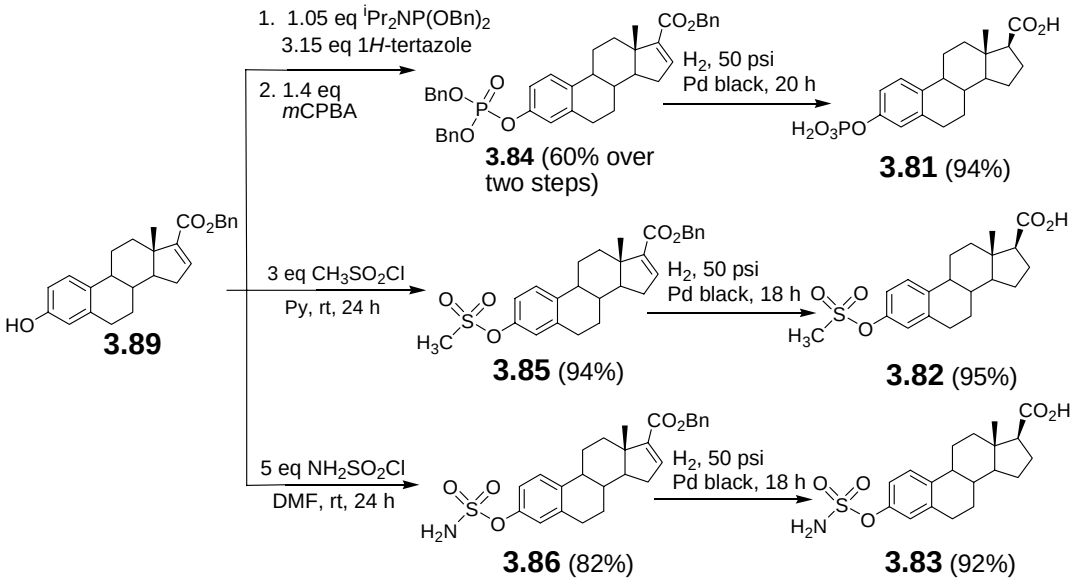
H_2 to give **3.90** in quantitative yield. However, when this reaction was performed with Pd black under 45 psi H_2 the desired carboxylic acid **3.91** was obtained in high yield.

Hydrogenation of **3.88** resulted in the exclusive formation of the β-isomer of **3.91** (COOH is coming out of the plane). As shown in Scheme 3.23, hydrogen can only attack from the backside of the methyl at 16-position due to steric hinderance, thus producing the β–carboxylic acid.



Scheme 3.23. Formation of the β-isomer from hydrogenation of **3.88**

With the conditions for the hydrogenation worked out we then modified the 3-position of **3.89** (Scheme 3.24). The phosphorylation reaction was accomplished by reacting compound **3.89** with dibenzyldiisopropylphosphoramidite ($^{i}Pr_2NP(OBn)_2$) in the presence of 1*H*-tetrazole followed by *in situ* oxidation of the phosphite intermediate with *m*CPBA (*meta*- chloroperoxybenzoic acid) to provide the phosphate **3.84** in 60 % yield. We anticipated that we could convert **3.84** to **3.81** in a single step by hydrogenolysis of the benzyl esters and hydrogenation of the double bond. Hydrogenation over Pd black resulted in reduction of double bond and global deprotection of benzyl groups to give phosphoric acid **3.81** in 94% yield. Mesylation of **3.89** with methanesulfonyl chloride in the presence of pyridine gave desired mesylate **3.85** in 94% yield. Hydrogenation of the double bond and hydrogenolysis of the benzyl group gave the desired product **3.82** in 95% yield. Similarly, sulfamate **3.86** was obtained in 82% yield by reacting **3.89** with sulfamoyl chloride in DMF. Hydrogenation of **3.86** over Pd black under 50 psi H_2 gave desired product **3.83** in 92% yield.



Scheme 3.24. Synthesis of compounds **3.81-3.83**

3.2.5 Preliminary Results from Inhibition Studies.

This chapter would not be complete without a brief discussion of the results from inhibition studies with these compounds. Boronic acids **3.53**-**3.56** and sulfonamides **3.23** and **3.24** have been examined as STS inhibitors by Vanessa Ahmed, a graduate student in the Taylor group. Studies with the steroids modified at the 17-position (compounds **3.81**-**3.83**) as well as the sulfinic acids (compounds **3.74** and **3.74**) are still in progress.

Inhibition studies with the boronic acids were carried out using purified STS in 0.1 M Tris buffer containing 5% DMSO with 4-methylumbelliferyl-6-*O*-sulfate (MUS) as substrate. Estrone-3-boronic acid (**3.53**) is a reversible, competitive STS inhibitor with a K_i of 2.8 μM at pH 7.0. Comparing the potency of **3.53** with the many other estrone derivatives bearing sulfate surrogates that have appeared in the literature is difficult since very few have been examined with pure enzyme and the modality of inhibition was rarely determined. Nevertheless, a comparison of **3.53** with other

estrone derivatives bearing sulfate surrogates using pure enzyme or placental microsomes (Table 3.1) reveals that compound **3.53** is one of the most potent inhibitors of this class. Estrone itself is a non-competitive STS inhibitor with a competitive K_i (the K_i for binding to free STS) of 63 μM and an αK_i (the K_i for binding to the STS-substrate complex) of 111 μM. Thus, replacing the 3-OH of estrone with a boronic acid moiety resulted in about 23-fold increase in potency and changes the modality of inhibition from noncompetitive to competitive Ms. Ahmed's inhibition studies did not allow us to determine whether a reversible covalent adduct is formed between **3.53** and STS. However, she was able to determine that **3.53** is not a slow-binding inhibitor which is a phenomenon often associated with boronic acid inhibitors of serine proteases.

Compound **3.54** is a 10-fold more potent inhibitor than compound **3.53** but is a non-competitive inhibitor with a K_i of 252 nM and an αKi of 300 nM. Surprisingly, compound **3.54**, which is identical to **1.5** (**3.54** but with an OH at the 3-position instead of a boronic acid group) was also a noncompetitive inhibitor and exhibited an almost identical K_i and αKi to that of **3.54** and so both inhibitors exhibit similar affinities for both the free and substrate bound form of the enzyme. These results suggest that both **3.54** and **1.5** preferably bind in a region outside the active site. Indeed our results with these compounds, as well as the fact that estrone is a non-competitive inhibitor, suggest the presence of a second steroid binding site. If **3.54** and **1.5** bind to STS in a similar manner then the boronic acid moiety in **3.54** may not be contributing very much to its affinity for STS.

IC_{50}'s were determined for coumarin and chromenone boronic acids **3.55** and **3.56** at pH 7.0. Both of these compounds were relatively poor STS inhibitors with the coumarin having an IC_{50} of 171 μM and the chromenone having an IC_{50} of 86 μM.

At pH 8.8, compound **3.53** was a competitive inhibitor with a K_i of 6.8 μM. STS is crystallized at pH 8.6[14] so we were pleased to see that compound **3.53** exhibited a good affinity for STS even at basic pH. Compounds **3.53** and **3.54** were sent to Dr. Debashis Ghosh for crystallographic studies and so far we have obtained the structure of the STS-**3.53** complex (Figure 3.7, unpublished results). Compound **3.53** was found to bind noncovalently in a hydrophobic site (site 2) at the top of the two α-helices and at the entrance to a tunnel that leads into the active site. The boronic acid group interacts with the side chain of Asn241 and with the side chain of Gln583 by a water-mediated H-bond. The steroid skeleton forms hydrophobic interactions with a variety of hydrophobic residues. Binding of **3.53** at site 2 blocks the entrance to the tunnel but does not occupy any part of the active site.

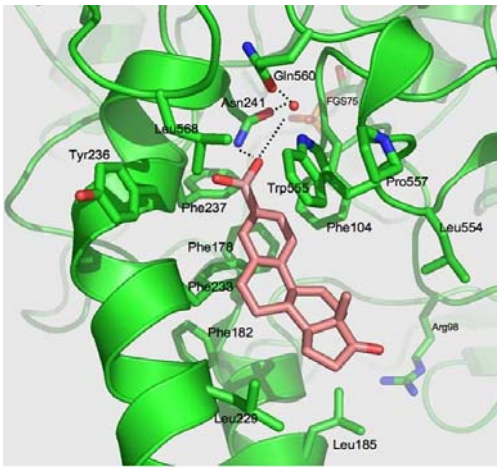


Figure 3.7. Crystal structure of compound **3.53** bound to STS. The inhibitor is shown in red (courtesy of D. Ghosh, unpublished)

The fGly hydrate is sulfated in the STS-**3.53** complex. We believe that hydrate sulfation prevents the binding of **3.53** in the active site, and so it occupies site 2 in the crystal structure but occupies the active site when STS is in solution where the hydrate is only transiently sulfated. Nevertheless, we believe that some inhibitors that we and others have prepared bind in site 2 *in solution*.

For example, compound **3.54** which is a *noncompetitive* inhibitor which indicates that it is binding in a site outside the active site. We suspect that this compound binds in the second site where the benzyl group would be able to project down into a channel between the hydrophobic helices. Compound **3.54** was based on Boivin et al's report that estradiol derivatives bearing benzylic groups at the 17-position, such as **1.5**, are potent inhibitors of STS.[41] We have found that **1.5** is a *noncompetitive* inhibitor of the pure enzyme with a K_i identical to **3.54** and we believe that **1.5** and similar compounds[41,42] bind in site 2.

Inhibition studies with the steroid sulfonamides were carried out using purified STS in tris buffer containing 10 % DMSO with 4-methylumbelliferyl-6-*O*-sulfate (MUS) as substrate at 25 ^{o}C. We were unable to do inhibition studies with the coumarin sulfonamide **3.24** due to its poor solubility in the assay mix. The fluorinated sulfonamide **3.23** exhibited mixed inhibition with STS with a competitive K_i of 82 μM at pH 7.0. Its nonfluorinated analog, **3.36**, also exhibited mixed inhibition with a competitive K_i of 350 μM. So the presence of the fluorines increases the potency by approximately 4.3-fold. At pH 8.8, compound **3.23** exhibited mixed inhibition with STS with a competitive K_i of 28 μM while compound **3.36** had a K_i of 503 μM, an 18-fold difference.

Professor Scott Taylor determined the pK_a of compound **3.23** to be 8.0 in 0.1 M bis-tris propane, 10% DMSO at 25 ^{o}C. Thus, at pH 7.0 10% of sulfonamide **3.23** exists as the conjugate base. The pK_a of sulfonamide **3.40** in aqueous solution has recently been determined by potentiometric titration to be 10.5.[43] Thus, under our conditions, the pK_a of sulfonamide **3.36** is probably about 10.5. Therefore, at pH 7.0, less than 0.02% of **3.36** exists as the conjugate base. At pH 7.0, the sulfonamide **3.23** has an approximately 4.3-fold greater affinity for STS than compound **3.36**, a modest difference considering the concentration of the conjugate base of inhibitor **3.23** at

physiological pH is at least 500 times greater than that of inhibitor **3.36**. This would suggest that, at pH 7.0, STS does not have a strong preference for binding to the conjugate base of **3.23** and has a modest affinity for the neutral form of **3.23**. The 4.3-fold difference could be due to a variety of factors such as the fluorines interacting with residues in the active site. The K_i for **3.23** does indeed decrease as the pH increases from 7.0 to 8.8 which is what one would expect if the anion exhibited a greater affinity for the enzyme. However, the decrease is not as large (3-fold) as one would expect based solely on the difference in the concentrations of the neutral and anionic forms of **3.23** (63-fold higher at pH 8.8 compared to pH 7.0) and if STS did not bind or had an extremely poor affinity for the neutral form. However, our studies with the nonfluorinated sulfonamide **3.36** indicate that STS will bind neutral sulfonamides though not very well. Thus, STS may have just a modest preference for binding the conjugate base of sulfonamides over their neutral form. The 18-fold difference in K_i's between compounds **3.23** and **3.36** at pH 8.8 could be due to differences in the concentrations of their respective conjugate bases as well as other factors such as the fluorines in **3.23** interacting with residues in the active site.

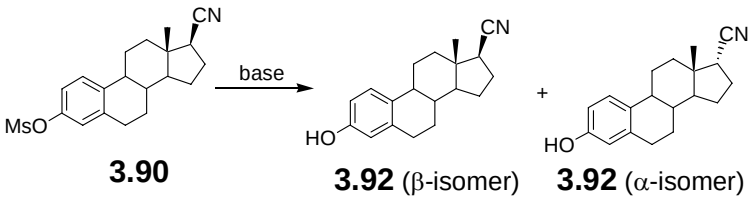
Although it appears that the ionization state of **3.23** is not a major factor in the binding of **3.23** to STS, this may not necessarily be the case with EMATE. The K_i of EMATE has been determined to be 670 nM using a radiometric assay and crude microsomal preparations of STS at an unspecified pH and so appears to have a considerably greater affinity for STS than compound **3.23**.[25] Although the pK_a of EMATE in purely aqueous solution is probably lower than that reported in 70% aqueous methanol (9.5), its pK_a is certainly greater than that of inhibitor **3.23**. If it is the conjugate base of EMATE that interacts with STS, then this would suggest that the anionic sulfamoyl group binds with

an affinity that is dramatically greater than the conjugate base of **3.23**. It is possible that the difluoromethylene linkage in **3.23** actually hinders the anionic nitrogen from interacting optimally with active site residues while this may not a problem with EMATE. However, comparing K_i's of irreversible inhibitors to K_i's of reversible inhibitors should be approached with caution due to the possibility that the inactivation step by the irreversible inhibitor is completely or partly rate limiting. Nevertheless, our results intimate that the possibility that EMATE may bind to STS as the neutral species cannot be entirely discounted.

3.2.6 Future work

Although an effective route to the synthesis of DFMS's was developed, the results for the inhibition studies with compound **3.23** reveals that the substitution of the sulfate group in estrone sulfate with a DFMS group did not yield a particularly effective inhibitor. Indeed, compound **3.23** exhibits a potency that is about the same as estrone at pH 7.0. This is in contrast to boronic acid inhibitor **3.53** which is a good competitive inhibitor of STS and is 23-fold more potent than estrone. Nevertheless, even compound **3.53** does not display a submicromolar potency. Perhaps the most significant result from the series of compounds synthesized in this chapter so far is the discovery of an alternative steroid binding site since this opens up a new paradigm for inhibitor design. We will use this structure as a starting point for the design of noncompetitive inhibitors. This will be a purely rational approach relying heavily on molecular modeling. It will be interesting to see if compound **3.54** binds in the second site as we have predicted. The Ghosh group has obtained diffraction quality crystals of the STS-**3.54** complex and we should have the STS-**3.54** complex structure soon. We are aware that should **3.54** be shown to occupy the same site as **3.53** in the crystal structure that this would

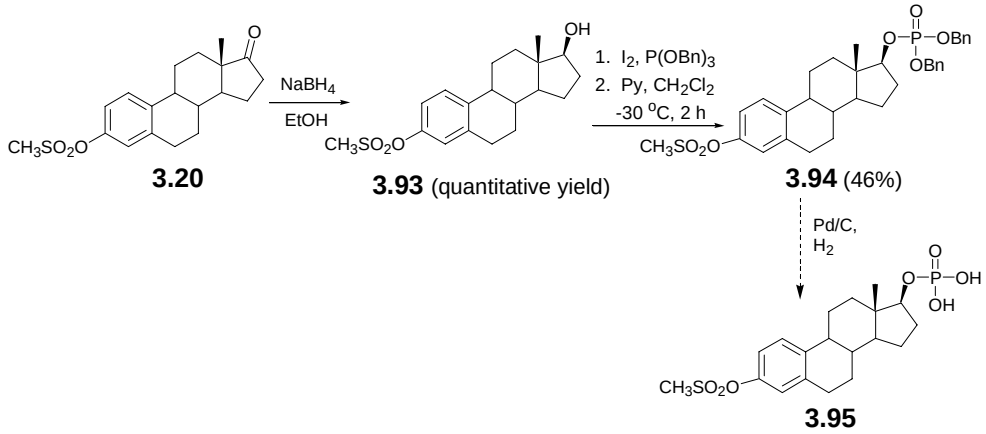
not necessarily mean that this is how it binds to STS in solution. However, the fact that **3.54** is a purely noncompetitive inhibitor clearly indicates that it is binding in a region outside the active site and that it would be very reasonable assumption that if it is binding to site 2 in the crystal structure then this would also be the case in solution.



3.90 **3.92** (β-isomer) **3.92** (α-isomer)

Scheme 3.25. Epimerization of compound **3.90**

The steroids that were modified with a carboxylic acid group at the 17-position have yet to be examined as STS inhibitors. Even if these compounds prove to be poor STS inhibitors, more work will be done on this class of compounds. So far, we have prepared only the β-isomers. We will attempt to prepare the α-isomers by subjecting **3.90** to base which we anticipate will epimerize the 17-position (Scheme 3.25). We may even be able to epimerize and convert the cyano group to the carboxylic acid group in a single step. The resulting epimers will be separated either at this stage or at a later stage of the synthesis which will proceed using an approach similar to that in Scheme 3.22.

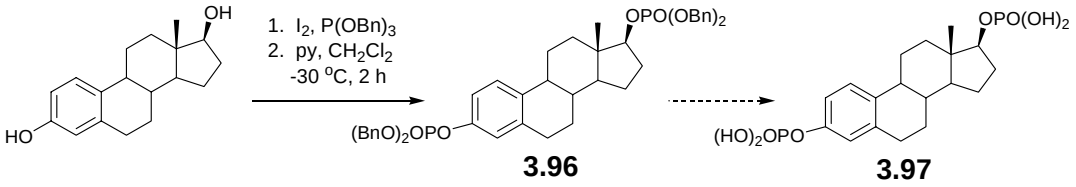


3.20 **3.93** (quantitative yield) **3.94** (46%) **3.95**

Scheme 3.26. Synthesis of compound **3.95**

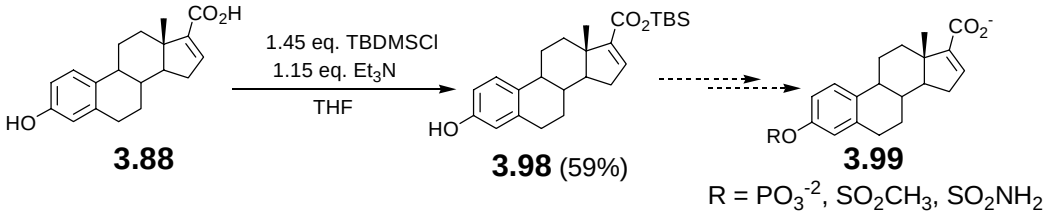
The 17-phosphate analogs of **3.81-3.83** (α- and β-isomers) will also be prepared and we have already made some progress on this class of compounds. For example, compound **3.94** has been prepared using the straightforward process outlined in Scheme 26. Hydrogenolysis will yield compound **3.95**. A similar approach will be used to obtain the 3-sulfamate analog of **3.95**.

We have also prepared diphosphate **3.96** as shown in Scheme 3.27, however, we are still in the process of purifying this compound. Hydrogenation of **3.96** will yield the desired diphosphate **3.97**.



Scheme 3.27. Route to diphosphate **3.97**

The α,β-unsaturated carboxylic acids of type **3.99** will also be synthesized (Scheme 3.28) from the silyl protected acid **3.98,** which we have recently prepared.



Scheme 3.28. Proposed synthesis of compounds of type **3.99**

As far as the sulfinic acid inhibitors are concerned, we will have to wait for the results for the inhibition studies before any future work on this class of compounds can proceed further.

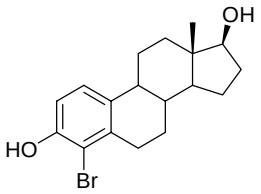
3.3 Experimental

3.3.1 General

THF, ether, 1,4-dioxane and benzene were distilled from sodium/benzophenone. Methylene chloride, triethyalmine, acetonitrile and 2,6-lutidine were distilled from CaH_2. Pyridine was distilled from KOH. DMF (*N,N*-dimethylformamide) and NMP (*N*-methylpyrrolidone) were distilled from CaH_2 under reduced pressure. $POCl_3$ and triethyl phosphite ($P(OEt)_3$) were distilled prior to use. Formic acid was distilled from phthalic anhydride. *N,N*-Dimethylthiocarbamoyl chloride was purified by vacuum distillation. NFSi was recrystallized from methanol prior to use. Potassium acetate was sublimed under vacuum and kept in a desiccator. Methanol was HPLC grade. Potassium thioacetate (KOAc) was prepared from thioacetic acid. Choromethyl methyl ether (MOMCl) was prepared according to literature.[44] Silica gel chromatography was performed using silica gel 60Å (230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^{1}H, ^{19}F, ^{31}P, ^{11}B and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer. Chemical shifts (δ) for ^{1}H NMR spectra run in $CDCl_3$, DMSO-d_6, CD_3OD, acetone-d_6, D_2O are reported in ppm relative to the internal standard tetramethylsilane (TMS). For ^{13}C NMR spectra run in $CDCl_3$, chemical shifts are reported in ppm relative to the $CDCl_3$ (δ = 77.0 for central peak), DMSO-d_6 (δ = 39.5 for central peak), CD_3OD (δ = 49.0 for central peak), acetone (δ = 77.0 for central peak), D_2O (δ 0.0 for external standard TMS). For the ^{19}F NMR spectra chemicals shifts are reported relative to an external $CFCl_3$ standard (δ 0.0 ppm). For the ^{31}P NMR spectra chemicals shifts are reported relative to an external 85% phosphoric acid standard (δ 0.0 ppm). For the ^{11}B NMR spectra chemicals shifts are reported relative to an external $B(OMe)_3$ standard (δ 0.0 ppm). Low-resolution (LRMS) and high-resolution (HRMS) electron impact

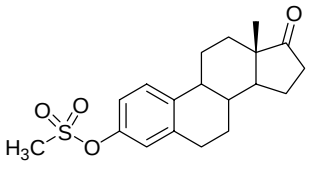
(EI) mass spectra were recorded on a JEOL HX 110 double focusing mass spectrometer. Electrospray (ESI) mass spectra were obtained with a Waters/Micromass QTOF Ultima Global mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.

3.3.1 Syntheses



2.22

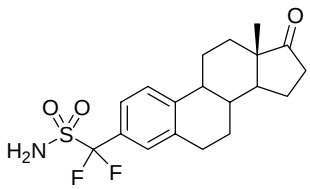
4-Bromo-17*β*-hydroxyestra-1,3,5(10)-triene (2.22). This was prepared according to the procedure of Lovely et al.[37] using NBA instead of NBS. A suspension of estradiol (8.00 g, 29.4 mmol) in ethanol (600 mL) was heated to make a clear solution then cooled to 0 ^{o}C before *N*-bromoacetamide (NBA, 4.10 g, 29.7 mmol) was added. The resulting mixture was stirred 2 h at 0 ^{o}C, then overnight at rt. After removal of solvent, the residue was recrystallized from ethanol to give 6.69 g (65%) of 4-bromoestradiol **2.22** as colorless crystals. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.78 (s, 1H, ArO<u>H</u>), 7.07 (d, J = 8.5 Hz, 1H, H-1), 6.71 (d, J = 8.4 Hz, 1H, H-2), 3.38 (t, J = 5.7 Hz, H-17), 2.90-1.20 (m, 15H), 0.61 (s, 3H, CH_3, H-18).



3.20

Estra-1,3,5(10)-trien-17-one-3-yl methanesulfonamide or 3-[(methanesulfonyl)oxy] estra-1,3,5-(10)-triene-17-one (3.20). This was prepared according to the procedure of Baldwin et al.[45] To a solution of estrone (10 g, 37 mmol) in pyridine (50 mL) at 0 ^{o}C was added methanesulfonyl

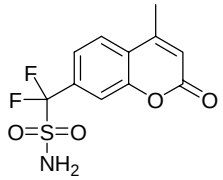
chloride (7.0 mL, 90 mmol, 2.4 equiv) slowly over 40 min. After addition, it was maintained at 0 ^{o}C for 1 h, and then stirred overnight at rt. The reaction mixture was cooled to 0 ^{o}C and 400 mL of 2 M HCl was added slowly. The mixture was stirred for 30 min and then filtered. The filter cake was washed with water and dried over high vacuum to give estrone mesylate **3.20** as yellow solid which was recrystallized from methanol to give light yellow or yellow prism (12.90 g, 100%). ^{1}H NMR was identical to that previously reported.[45] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29 (d, J = 8.3 Hz, 1H, H-1), 7.04-7.00 (s and d overlapping, 2H, H-2 and H-4), 3.11 (s, 3H, CH_3SO_3), 2.95-2.85 (m, 2H, H-6), 2.52-1.91 (m, 7H), 1.75-1.40 (m, 6H), 0.89 (s, 3H, CH_3, H-18).



3.23

Difluoro [estra-1,3,5(10)-trien-17-one]-3-methanesulfonamide (3.23). $Pd(PPh_3)_4$ (240 mg, 0.071 mmol, 6.6 mol %) was added to a solution of **3.45** (540 mg, 1.07 mmol) and 1,3-dimethylbarbituric acid (2.00 g, 14.1 mmol, 13 equiv) in dry CH_3CN (15 mL). The mixture was heated under reflux under an atmosphere of argon for 18 h, after which it was cooled and diluted with water (50 mL). The mixture was extracted with ether and the combined extracts washed with H_2O then dried (Na_2SO_4) and concentrated to leave a yellow oil. 6 M HCl (15 mL) was added to a solution of the residue in THF (50 mL). The mixture was stirred for 1 h at rt, after which it was diluted with of H_2O (50 mL) and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography

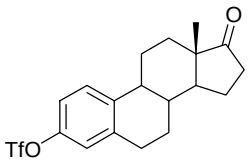
(ethyl acetate/hexane, 2:5) gave difluorosulfonamide **3.23** as a pale yellow solid which was recrystallized from CH_2Cl_2-hexane to give pure **3.23** as white crystalline solid (372 mg, 92%). Mp 165-167 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46-7.34 (m, 3H, H-1, H-2 and H-4), 5.27 (s, 2H, NH_2), 3.00-2.85 (m, 2H), 2.05-1.90 (m, 7H), 1.65-1.35 (m, 6H), 0.88 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 221.5 (C=O), 144.4 (C-6), 137.3 (C-5), 127.8 (t, J = 5.7 Hz, C-4), 125.8 (C-1), 125.1 (t, J = 25 Hz, C-3), 124.6 (t, J = 6.3 Hz, C-2), 120.7 (t, J = 281 Hz, CF_2), 50.5 (C-14), 48.1 (C-13), 44.5 (C-7), 37.8 (C-8), 35.9 (C-16), 31.5 (CH_2), 29.3 (CH_2), 26.2 (CH_2), 25.6 (CH_2), 21.7 (CH_2), 13.9 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ –102; LRMS (EI) *m/z* (%) 383 (M^+, 1), 303 (100); HRMS (EI) calcd for $C_{19}H_{23}F_2NO_3S$ 383.1367; found 383.1373.



3.24

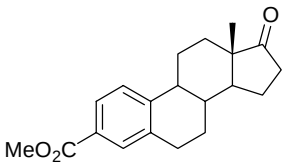
7-(4-methylcoumarin) difluoromethanesulfonamide (3.24). To a solution of **3.51** (311 mg, 0.530 mmol) in CH_2Cl_2 (12.5 mL) at rt was added TFA (5 mL) over 10 min. After stirring for 2 h at rt, the reaction was concentrated to give a pink residue. Acetone (40 mL) was added and the mixture was stirred for 15 min. After filtration and rinsing with acetone, the filtrate was subjected to flash column chromatography (acetone/hexane, 1:4 to 1:2) to afford **3.24** as a pale white solid (105 mg, 69%). ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.94 (d, J = 8.3 Hz, 1H, H-5), 7.61 (d, J = 8.1 Hz, 1H, H-6), 7.55 (s, 1H, H-8), 7.38 (s, 2H, NH_2), 6.44 (s, 1H, H-3, vinyl), 2.52 (s, 3H, CH_3); ^{13}C NMR (acetone-d_6, 75 MHz) δ 158.9, 153.0, 151.9, 132.2 (t, J = 23 Hz, C-7), 125.7, 122.6 (t, J = 6 Hz), 119.8 (t, J = 279 Hz, CF_2), 116.6, 115.6 (t, J = 6 Hz), 17.6 (CH_3); ^{19}F NMR (acetone-d_6, 282 MHz) δ –103.9; LRMS

(EI) *m/z* (%) 289 (M^+, 6), 209 (100), 181 (12); HRMS (EI) calcd for $C_{11}H_9F_2NO_4S$ 289.0220, found 289.0210.



3.29

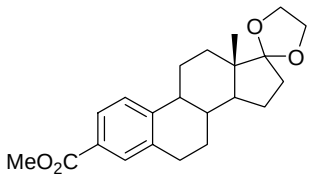
Estra-1,3,5(10)-trien-17-one-3-trifluoromethanesulfonate (3.29). Prepared according to the procedure of Li et al.[9] To a solution of estrone (10.0 g, 37.0 mmol), DMAP (1.13 g, 9.20 mmol, 0.25 equiv) and 2,6-lutidine (9.2 mL, 8.0 mmol, 2.1 equiv) in CH_2Cl_2 (300 mL) at 0 ^oC was added triflic anhydride (7.45 mL, 44.3 mmol, 1.2 equiv) slowly. After addition, the reaction mixture was stirred for 70 min at 0 ^oC before quenching with 2 M HCl (100 mL) at 0 ^oC. The organic layer was separated and washed with 2N HCl, 7.5% $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 1:8 to 1:5) gave **3.29** as a white solid, which was recrystallized from hexane to give estrone triflate **3.29** as white crystals (13.3 g, 90%). On a 30 g scale, a yield of 96% was obtained. ^1H NMR was identical to that reported in the literature.[9] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.32 (d, J = 7.5 Hz, 1H, H-1), 7.01 (d, J = 8.7 Hz, 1H, H-2), 6.98 (s, 1H, H-4), 2.93 (dd, J = 8.7 Hz, J = 4.2 Hz, 2H), 2.56-1.92 (m, 7H), 1.68-1.40 (m, 6H), 0.90 (s, 3H, CH_3, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -72.7.



3.30

3-Methoxycarbonylestra-1,3,5(10)-trien-17-one (3.30). Prepared according to according

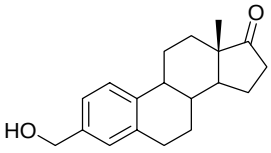
to the procedure of Li et al. except less Pd catalyst was used.[9] To a mixture of estrone triflate **3.29** (8.45 g, 27.1 mol), $Pd(OAc)_2$ (300 mg, mmol, 5.0 mol%) and 1,3-bis(diphenyl- phosphino) propane (DPPP, 485 mg, 1.18 mmol, 4.4 mol%) in a 250 mL round bottom flask under argon was added DMF (35 mL), MeOH (20 mL) and Et_3N (9.00 mL, 64.6 mmol, 2.4 equiv) successively. After purging with CO, the reaction mixture was heated at 70 ^oC under CO (balloon) overnight (16 h) and then cooled to rt. The mixture was diluted with brine and extracted with ether. The combined extracts were washed with 2 M HCl, sat. $NaHCO_3$, and brine then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 1:5 to 1:3) gave pure ester **3.30** as a white solid (5.55 g, 85%). ^1H NMR was identical to that reported in the literature.[9] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (d, J = 8.9 Hz, 1H, H-2), 7.76 (s, 1H, H-4), 7.33 (d, J = 8.0 Hz, 1H, H-1), 3.87 (s, 3H, CO_2CH_3), 3.00-2.85 (m, 2H), 2.55-1.95 (m, 7H), 1.70-1.40 (m, 6H), 0.90 (s, 3H, CH_3, H-18).



3.31

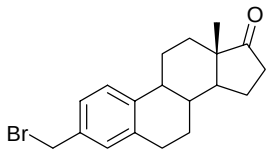
3-Methoxycarbonyl-17,17-ethylenedioxyestra-1,3,5(10)-triene (3.31). Prepared according to the procedure of Li et al.[9] A mixture of ester **3.30** (6.00 g, 19.3 mmol), *para*-toluene sulfonic acid (PTSA, 300 mg, 2.30 mmol, 0.12 equiv) and ethylene glycol (20 mL) in benzene (250 mL) was heated to reflux for 6 h using a Dean-Stark trap. The mixture was cooled to rt and the organic layer was separated and washed with H_2O and brine then dried (Na_2SO_4) and concentrated. After removal of solvent, ketal **3.31** was obtained as white solid (6.90 g, crude 100%). ^1H NMR was identical to that reported in the literature.[9] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, J = 8.3 Hz, 1H, H-2), 7.73 (s, 1H,

H-4), 7.32 (d, J = 8.1 Hz, 1H, H-1), 4.00-3.80 (m, 7H, OCH_2CH_2O and CO_2CH_3 overlapping), 3.00-2.85 (m, 2H), 2.42-2.20 (m, 2H)，2.05-1.27 (m, 11H), 0.87 (s, 3H, CH_3, H-18).



3.32

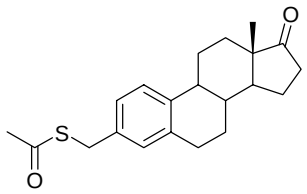
3-Hydroxymethylestra-1,3,5(10)-trien-17-one (3.32). Prepared according to the procedure of Li et al.[9] with some modifications. To a solution of crude **3.31** (6.90 g, about 19.3 mmol) in THF (150 mL) at 0 ^oC was added a suspension of $LiAlH_4$ (2.00 g, 59.0 mmol, 3 equiv) in THF (150 mL) and stirred 24 h at rt. The reaction mixture was quenched by the slow addition of ice. 6 M HCl (150 mL) was added and the mixture was stirred for 30 min at rt before extracting with Et_2O. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated to give alcohol **3.32** as a white solid (5.47 g, crude 100%). ^1H NMR was identical to that reported in the literature.[9] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.28 (d, J = 7.8 Hz, 1H, H-1), 7.18-7.07 (m, 2H, H-2 and H-4), 4.62 (s, 2H, Ar$C\underline{H}_2O$), 3.00-2.85 (m, 2H), 2.55-1.90 (m, 7H), 1.70-1.40 (m, 6H), 0.89 (s, 3H, CH_3, H-18).



3.33

3-Bromomethylestra-1,3,5(10)-trien-17-one (3.33).[9] To a solution of crude **3.32** (5.47 g, 19.3 mmol) and triphenylphosphine (7.57 g, 28.9 mmol, 1.5 equiv) in CH_2Cl_2 (200 mL) at 0 ^oC was added carbon tetrabromide (10.6 g, 31.8 mmol, 1.65 equiv). After stirring at rt for 30 min, the

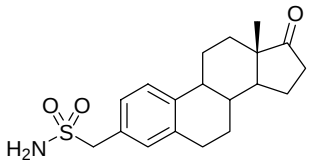
reaction was concentrated and the resulting residue was loaded onto a silica column and purified by flash chromatography (ethyl acetate/hexane/methylene chloride, 1:4:0.5 to 1:3:0.4) to give pure **3.33** as a white solid (5.70 g, 84% starting from ester **3.30**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, J = 8.4 Hz, 1H, H-1), 7.16 (d, J = 8.4 Hz, 1H, H-2), 7.12 (s, 1H, H-4), 4.44 (s, 2H, CH_2Br), 2.89 (dd, J = 8.7 Hz, J = 4.2 Hz, 2H), 2.54-1.93 (m, 7H), 1.65-1.35 (m, 6H), 0.89 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.7 (C=O), 140.3 (C_{Ar}), 137.0 (C_{Ar}), 135.2 (C_{Ar}), 129.6 (CH_{Ar}), 126.4 (CH_{Ar}), 125.9 (CH_{Ar}), 50.5 (C-14), 47.9 (C-13), 44.4 (C-9), 38.0 (C-8), 35.8 (CH_2), 33.7 (CH_2), 35.7 (CH_2), 31.6 (CH_2), 29.3 (CH_2), 26.4 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18).



3.34

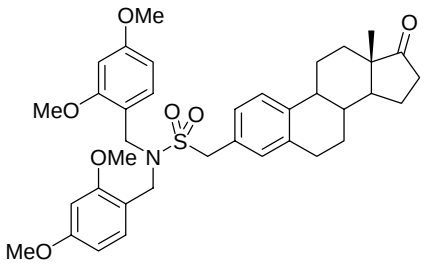
Thioacetic acid [3-methylestra-1,3,5(10)-trien-17-one] ester (3.34). Potassium thioacetate (3.80 g, 33.3 mmol, 1.4 equiv) was added to a solution of bromide **3.33** (8.30 g, 23.9 mmol) in DMF (250 mL) and the resulting mixture was stirred for 16 h. After removal of DMF, the residue was diluted with H_2O (80 mL) and EtOAc (120 mL). The layers were separated and the aqueous layer extracted with EtOAc. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 1:4) gave pure thioacetate **3.34** as a white solid (8.00 g, 98%). Mp: 78-79 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.20 (d, J = 8.0 Hz, 1H, H-1), 7.05 (d, J = 8.1 Hz, 1H, H-2), 7.00 (s, 1H, H-4), 4.04 (2H, s, Ar$C\underline{H}_2$S), 2.87 (dd, J = 8.4 Hz, J = 3.6 Hz, 2H), 2.53-1.92 (m, 7H and s, 3H, CH_3C(=O), overlapping), 1.65-1.33 (m, 6H), 0.89 (3H, s, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.9 (C=O), 195.3 ($CH_3(\underline{C}$=O)), 139.0 (C_{Ar}),

136.9 (C_{Ar}), 135.1 (C_{Ar}), 129.4 (C_{Ar}), 126.3 (C_{Ar}), 125.8 (C_{Ar}), 50.5 (C-14), 48.0 (C-13), 44.4 (C-7), 38.1 (C-8), 35.9 (C-16), 33.1 ($ArCH_2S$), 31.6 (CH_2), 30.5 ($\underline{C}H_3(\underline{C}{=}O)$), 29.4 ($CH_2$), 26.5 ($CH_2$), 25.8 ($CH_2$), 21.7 ($CH_2$), 13.9 ($CH_3$, C-18); LRMS (EI) *m/z* (%) 342 (M^+, 20), 299 (9), 267 (100); HRMS (EI) calcd for $C_{21}H_{26}O_2S$ 342.1654; found 342.1657.



3.36

Estra-1,3,5(10)-trien-17-one-3-methanesulfonamide (3.36). Concentrated NH_4OH (10 mL) was added over 10 min to a solution of crude **3.35** (prepared from thioacetate **3.34**, 600 mg, 1.64 mmol) in THF (100 mL) at 0 °C and the reaction was stirred overnight. H_2O (80 mL) was added and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 2:3) gave pure sulfonamide **3.36** as a white solid (353 mg, 62%). Mp: 189-191 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26 (d, J = 7.4 Hz, 1H, H-1), 7.16 (d, J = 6.4 Hz, 1H, H-2), 7.11 (s, 1H, H-4), 6.02 (s, 2H, NH_2) 4.22 (s, 2H, $ArC\underline{H}_2SO_2$), 2.90-2.78 (m, 2H), 2.50-2.30 (m, 3H), 2.10-1.95 (m, 3H), 1.85-1.75 (m, 1H), 1.70-1.35 (m, 6H), 0.87 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 218.6 (C=O), 139.8 (C_{Ar}), 136.5 (C_{Ar}), 131.4 (C_{Ar}), 128.2 (C_{Ar}), 125.4 (C_{Ar}), 60.2 ($Ar\underline{C}H_2SO_2$), 50.3 (C-14), 47.6 (C-13), 44.4 (C-7), 38.2 (C-8), 35.2 (C-16), 31.8 (CH_2), 29.1 (CH_2), 26.4 (CH_2), 25.6 (CH_2), 21.3 (CH_2), 13.3 (CH_3, C-18); LRMS (EI) *m/z* (%) 347 (M^+, 11), 342 (<1), 284 (1), 267 (100), 105 (10); HRMS (EI) calcd for $C_{19}H_{25}O_3NS$ 347.1555; found 347.1556.

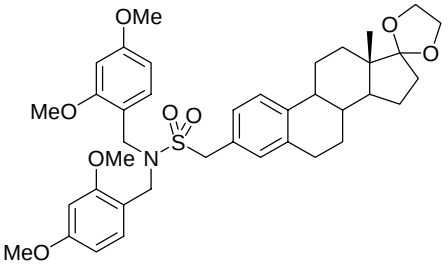


3.37

***N,N*-Bis-(2,4-dimethoxybenzyl)-[estra-1,3,5(10)-trien-17-one]-3-methanesulfonamide**

(3.37). H_2O (18 mL) was added to a solution of **3.34** (1.20 g, 3.51 mmol) in CH_2Cl_2 (40 mL). The mixture was cooled using an ice bath and Cl_2 was bubbled through the solution slowly. When TLC showed the reaction was complete, the solution was purged with N_2 for 10 min, then cold CH_2Cl_2 (50 mL) was added. After separation of the organic layer, the aqueous layer was extracted with cold CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated to give 1.30 g (crude which may contain sulfinyl chloride as a side product) of **3.35** as a white foam. ^{1}H NMR ($CDCl_3$) δ 7.34 (d, J = 8.1 Hz, 1H, H-1), 7.22 (d or dd, J = 9.2 Hz, 1H, H-2), 7.18 (1H, s, H-4), 4.80 (2H, s, CH_2S), 2.93 (dd, J = 8.7 Hz, J = 4.2 Hz, 2H), 2.54-1.93 (m, 7H), 1.70-1.60 (m, 6H), 0.89 (3H, s, CH_3, H-18). A solution of bis(2,4-dimethoxybenzyl)amine (1.50 g, 4.73 mmol, 1.88 equiv) in THF (10 mL) was added slowly to a solution of the crude sulfonyl chloride **3.35** (920 mg, 2.51 mmol) in THF (100 mL) at 0 °C. The mixture was stirred 0.5 h at rt then concentrated. The residue was purified by flash chromatography (ethyl acetate-hexane, 1:1) to give pure sulfonamide **3.37** as a white foam (751 mg, 46%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.20 (d, J = 8.7 Hz, 1H, ArH), 7.12 (d, J = 8.0 Hz, 1H, ArH), 6.76 (s, 1H, ArH), 6.35 (2s overlapping, 2H, ArH), 4.16 (s, 4H, CH_2N x 2), 3.96 (s, 2H, Ar$\underline{CH_2}SO_2$), 3.70 (s, 12H, OCH_3), 2.80-2.68 (m, 2H), 2.45-1.80 (m, 7H), 1.60-1.25 (m, 6H), 0.79 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.5 (C=O), 160.6 (2C), 158.4 (2C), 139.9 (C_{Ar}),

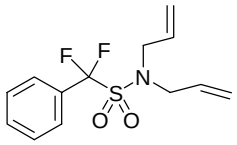
136.6 (C_{Ar}), 131.4 (C_{Ar}), 131.2 (2C), 128.1 (C_{Ar}), 127.0 (C_{Ar}), 125.5 (C_{Ar}), 117.2 (2C), 104.3 (2C), 98.3 (2C), 58.6 (Ar<u>C</u>H_2SO_2), 55.4 (2C), 55.2 (2C), 50.4 (C-14), 47.9 (C-13), 45.3 (C-7), 38.0 (C-8), 35.8 (C-16), 31.6 (CH_2), 29.3 (CH_2), 26.4 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 647 (M^+, 16), 582 (1), 497 (4), 432 (10), 404 (4), 316 (39), 267 (28), 178 (39), 151 (100); HRMS (EI) calcd for $C_{37}H_{45}O_7NS$ 647.2917; found 647.2917.



3.38

***N,N*-Bis (2,4-dimethoxybenzyl) 17,17-ethylenedioxyestra-1,3,5(10)-trien-3-methane-sulfonamide (3.38).** Ethylene glycol (3 mL) and PTSA (30 mg, 0.17 mmol, 0.2 equiv) was added to a solution of **3.37** (560 mg, 0.870 mmol) in benzene (60 mL). The mixture was heated under reflux for 4 h using a Dean-Stark trap. The reaction was allowed to cool and then extracted with Et_2O. The extract was washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 9:11) gave pure **3.38** as a white foam (472 mg, 79%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, J = 8.4 Hz, 2H, ArH), 7.19 (d, J = 8.4 Hz, 1H, ArH), 6.93 (d, J = 7.8 Hz, 1H, ArH), 6.77 (1H, s, ArH), 6.47-6.41 (d and s overlapping, 4H, ArH), 4.20 (s, 4H, CH_2N), 4.01 (s, 2H, ArC<u>H</u>$_2SO_2$), 3.96-3.84 (m, 4H, OCH_2CH_2O), 3.78 (s, 6H, OCH_3), 3.75 (s, 6H, OCH_3), 2.80-2.70 (m, 2H), 2.35-2.17 (m, 2H), 2.05-1.25 (m, 11H), 0.86 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.6, 158.4, 140.6, 136.9, 131.3, 127.9, 126.6, 125.6, 119.4, 117.4, 104.2, 93.4, 65.7 (O<u>C</u>H_2CH_2O), 65.3 (OCH_2<u>C</u>H_2O), 58.8 (Ar<u>C</u>H_2SO_2), 55.5 (2C, OCH_3), 55.2 (2C, OCH_3), 49.5

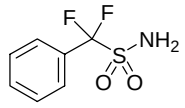
46.2, 45.4, 44.1, 38.8 (C-8), 34.3 (CH_2), 30.8 (CH_2), 29.4 (CH_2), 27.0 (CH_2), 26.0 (CH_2), 22.5 (CH_2), 14.4 (CH_3, C-18); LRMS (EI) *m/z* (%) 691 (M^+, 1), 476 (7), 316 (56), 151 (100), 99 (29); HRMS (EI) calcd for $C_{39}H_{49}O_8NS$ 691.3179; found 691.3176.



3.41

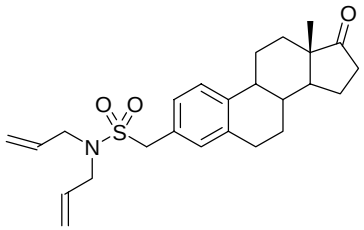
***N,N*-diallyl-1,1-difluoro-1-phenylmethanesulfonamide (3.41).** To a solution of diallylamine (1.50 mL, 12.2 mmol, 2.4 equiv) in THF (50 mL) at 0 °C was added a solution of α-toluenesulfonyl chloride (953 mg, 5 mmol) in THF (10 mL) over 10 min. After addition, it was stirred 4 h at 0 °C before quenching with H_2O (100 mL). The reaction mixture was extracted with ethyl acetate and combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3 to 1:2) gave 1.207 g (96%) of sulfonamide **3.39** as colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37-7.31 (m, 5H, H_{Ar}), 5.66-5.52 (m, 2H, C<u>H</u>=CH_2 x 2), 5.15 (d, J = 9.8 Hz, 2H, CH=C<u>H</u>cisH x 2), 5.13 (d, J = 16.5 Hz, 2H, CH=CH<u>H</u>trans x 2), 4.19 (s, 2H, PhC<u>H</u>$_2SO_2$), 3.61 (d, J = 6.4 Hz, 4H, NC<u>H</u>$_2$CH= x 2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 133.1 (2C, <u>C</u>H=CH_2 x 2), 130.7 (CH_{Ar}, 2C), 129.2 (C_{Ar}), 128.7 (CH_{Ar}, 2C), 128.7 (CH_{Ar}), 119.1 (2C, CH=<u>C</u>H_2 x 2), 59.1 (Ph<u>C</u>H_2S), 49.6 (2C, NCH_2 x 2). NaHMDS (1.0 M in THF, 20 mL, 20 mmol, 2.5 equiv) was added over 1 h to a solution of **3.39** (2.01 g, 8.00 mmol) and NFSi (6.00 g, 19.2 mmol, 2.4 equiv) in THF (30 m) at –78 °C. The mixture was stirred for 2.5 h at –78 °C, after which it was quenched with sat. NH_4Cl (10 mL) and extracted with EtOAc. The combined extracts were washed with H_2O and brine (30 mL) then dried (Na_2SO_4) and concentrated.

Purification of the residue by flash chromatography (ethyl acetate-hexane, 1:5) gave pure **3.41** as a colorless liquid (1.97 g, 86%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (d, J = 7.4 Hz, 2H, H-3 and H-5); 7.43-7.57 (m, 3H, H-2, H-4 and H-6), 5.70-5.86 (m, 2H, C<u>H</u>=CH_2), 5.27 (d, J = 8.8 Hz, 2H, CH=C<u>H</u>$_{cis}$H), 5.23 (d, J = 15.7 Hz, 2H, CH=CH<u>H</u>$_{trans}$), 3.93 (d, J = 6.5 Hz, 4H, NCH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 132.5 (2C, <u>C</u>H=CH_2), 132.0 (C-4), 129.1 (t, J = 22 Hz, C-1), 128.6 (2C, C-3 and C-5), 127.2 (t, J = 6.3 Hz, C-2 and C-6), 122.0 (t, J = 280 Hz, CF_2), 119.9 (2C, CH=<u>C</u>H_2), 50.0 (2C, NCH_2); ^{19}F NMR ($CDCl_3$, 282 MHz) δ –101; LRMS (EI) *m/z* (%) 287 (M^+, 1), 208 (7), 196 (7), 194 (8), 127 (100); HRMS (EI) calcd for $C_{13}H_{15}O_2NF_2S$ 287.0792; found 287.0800.



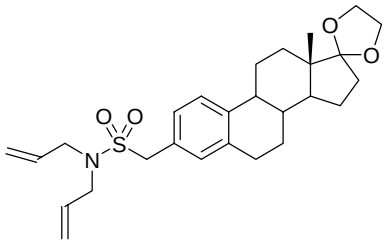
3.42

Difluoro(phenyl)methanesulfonamide (3.42). $Pd(PPh_3)_4$ (184 mg, 0.156 mmol, 15.6 mol %) and 1,3-dimethylbarbituric acid (1.42 g, 10.0 mmol, 10 equiv) were added to a solution of **3.41** (287 mg, 1.00 mmol) in dry CH_3CN (20 mL). The mixture was heated under vigorous reflux under an atmosphere of argon for 16 h, after which it was cooled and diluted with water (50 mL). The mixture was extracted with ether and the combined extracts were washed with H_2O then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3) gave pure difluorosulfonamide **3.42** as a white solid (194 mg, 93%). ^{1}H and ^{19}F NMR spectra were identical to that previously reported.[23.] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, J = 7.4 Hz, 2H), 7.54 (t, J = 7.3 Hz, 1H), 7.46 (t, J = 7.5 Hz, 2H), 5.34 (brs, 2H, NH_2); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -102.



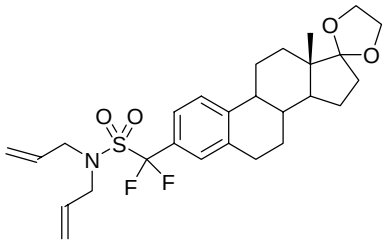
3.43

***N,N*-Diallyl-[estra-1,3,5(10)-trien-17-one]-3-methanesulfonamide (3.43).** A solution of **3.35** (prepared from thioacetate **3.34**, 1.50 g, 4.39 mmol) in THF (50 mL) was added over 20 min to a solution of diallylamine (2.0 mL, 17 mmol, 3.7 equiv) in THF (120 mL) at 0 °C. The mixture was stirred 1.5 h at rt, after which it was quenched with H_2O (100 mL) and extracted with EtOAc. The combined extracts were washed with H_2O and brine then (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3) gave pure sulfonamide **3.43** as a colorless oil (1.18 g, 63% from estrone thioaceate **3.34**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18 (d, J = 7.9 Hz, 1H, H-1), 7.10-7.00 (d and s overlapping, 2H, H-2 and H-4), 5.64-5.47 (m, 2H, C<u>H</u>=CH_2), 5.12 (s, 2H, CH=C<u>H</u>H), 5.07 (s, 2H, CH=CH<u>H</u>), 4.05 (s, 2H, Ar<u>C</u>H_2SO_2), 3.59 (d, J = 6.1 Hz, 4H, CH_2N), 2.85-2.77 (m, 2H), 2.45-1.80 (m, 7H), 1.60-1.30 (m, 6H), 0.80 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.5 (C=O), 140.3 (C_{Ar}), 136.9 (C_{Ar}), 133.2 (2C, <u>C</u>H=CH_2), 131.3 (C_{Ar}), 128.1 (C_{Ar}), 126.6 (C_{Ar}), 119.1 (2C, CH=<u>C</u>H_2), 125.7 (C_{Ar}), 58.6 (Ar<u>C</u>H_2SO_2), 50.4 (C-14), 49.6 (2C, NCH_2), 47.9 (C-13), 44.3 (C-7), 38.0 (C-8), 35.9 (C-16), 31.6 (CH_2), 29.3 (CH_2), 26.4 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.9 (CH_3, C-18); LRMS (EI) *m/z* (%) 427 (M^+, 1), 336 (6), 322 (3), 267 (100); HRMS (EI) calcd for $C_{25}H_{33}O_3NS$ 427.2181; found 427.2177.



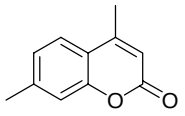
3.44

***N,N*-Diallyl 17,17-ethylenedioxyestra-1,3,5(10)-trien-3-methanesulfonamide (3.44).** Ethylene glycol (5 mL) and PTSA (280 mg, 1.47 mmol, 0.59 equiv) were added to a solution of **3.43** (1.18 mg, 2.50 mmol) in benzene (60 mL). The mixture was heated under reflux for 4h using a Dean-Stark trap. After cooling, the benzene layer was washed with H_2O and brine then dried (Na_2SO_4) and concentrated. The colorless oil residue was purified by flash chromatography (ethyl acetate/hexane, 1:3) to give pure **3.44** as a colorless oil (1.17 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, J = 8.0 Hz, 1H, H-1), 7.07 (d, J = 8.0 Hz, 1H, H-2), 7.03 (s, 1H, H-3), 5.67-5.50 (m, 2H, C$\underline{H}$=CH_2), 5.13 (d, J = 11.7 Hz, 2H, CH=C$\underline{H}_{trans}$H), 5.12 (d, J = 15.0 Hz, 2H, CH=CH$\underline{H}_{cis}$), 4.08 (s, 2H, ArC$\underline{H}_2SO_2$), 3.92-3.77 (m, 4H, OCH_2CH_2O), 3.62 (d, J = 6.1 Hz, 4H, NCH_2 x 2), 2.86 (pseudo s, 2H), 2.30-2.15 (m, 2H), 2.00-1.20 (m, 11H), 0.82 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.0 (C_{Ar}), 137.2 (C_{Ar}), 133.2 (2C, $\underline{C}$H=CH_2), 131.3 (C_{Ar}), 127.9 (C_{Ar}), 126.2 (C_{Ar}), 125.7 (C_{Ar}), 119.3 (C-17), 119.1 (2C, CH=$\underline{C}H_2$), 65.3 (O$\underline{C}H_2CH_2O$), 64.6 ($OCH_2\underline{C}H_2O$), 58.7 ($ArCH_2SO_2$), 49.6, 49.5 (2C, NCH_2), 46.1, 44.0, 38.8 (C-8), 34.3 (CH_2), 30.7 (CH_2), 29.5 (CH_2), 26.9 (CH_2), 26.0 (CH_2), 22.4 (CH_2), 14.4 (CH_3, C-18); LRMS (EI) *m/z* (%) (M^+, 13), 406 (6), 380 (2), 311 (100), 99 (88); HRMS (EI) calcd for $C_{27}H_{37}O_4NS$ 471.2443; found 471.2448.



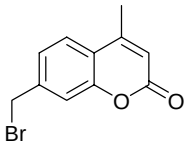
3.45

***N,N*-Diallyl difluoro [17,17-ethylenedioxyestra-1,3,5(10)-trien]-3-methanesulfonamide (3.45).** NaHMDS (1.0 M in THF, 4.8 mL, 4.8 mmol, 2.6 equiv) was added over 30 min to a solution of **3.44** (940 mg, 1.85 mmol) and NFSi (1.60 g, 5.08 mmol, 2.75 equiv) in THF (30 mL) at -78 ^{o}C. The mixture was stirred 2 h at -78 °C, after which it was quenched with sat. NH_4Cl (8 mL), diluted with H_2O (40 mL) and extracted with EtOAc. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate-hexane, 1:3) gave pure difluorosulfonamide **3.45** as white solid (844 mg, 83%). Mp: 109-110 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.32 (m, 3H, H-1, H-2 and H-4), 5.84-5.69 (m, 2H, C<u>H</u>=CH_2), 5.23 (d, J = 9.8 Hz, 2H, CH=C<u>H</u>$_{cis}$H), 5.21 (d, J = 17.6 Hz, 2H, CH=C<u>HH</u>$_{trans}$), 3.92 (d, J = 6.2 Hz, 4H, CH_2N), 3.89-3.77 (4H, m, OCH_2CH_2O), 2.86 (pseudo s, 2H), 2.35-2.20 (m, 2H), 2.05-1.25 (m, 11H), 0.84 (3H, s, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.7 (C-6), 137.3 (C-5), 132.7 (2C, <u>C</u>H=CH_2), 127.6 (t, J = 6.3 Hz, C-4), 126.0 (t, J = 23 Hz, C-3), 125.7 (C-1), 124.3 (t, J = 5.7 Hz, C-2), 122.3 (t, J = 281 Hz, CF_2), 119.9 (C-17), 119.2 (2C, CH=<u>C</u>H_2), 65.3 (OCH_2CH_2O), 64.6 (OCH_2CH_2O), 49.9, 49.5, 46.1 (2C, NCH_2), 44.3, 38.5 (C-8), 34.2 (CH_2), 30.7 (CH_2), 29.5 (CH_2), 26.8 (CH_2), 25.9 (CH_2), 22.4 (CH_2), 14.4 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ –101; LRMS (EI) *m/z* (%) 507 (M^+, 4), 433 (6), 381 (5), 347 (100), 99 (38); HRMS (EI) calcd for $C_{27}H_{35}O_4NF_2S$ 507.2255; found 507.2249.



3.46

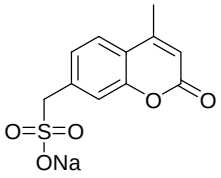
4,7-Dimethylcoumarin (3.46). This was prepared according to the procedure of Osborne.[27] To a stirred mixture of *m*-cresol (85 mL, 0.81 mol) and 75 % H_2SO_4 (120 mL) at 70 ^oC was added ethyl acetoacetate (104 g, 0.800 mol) dropwise over 1 h. Heating was continued for another hour before it was cooled down and poured onto ice water. The resulting slurry precipitate was collected by suction filtration and washed with 1 M NaOH and H_2O. Crystallization from acetone gave coumarin **3.46** as colorless prisms (37.3 g, 27 %). ^1H NMR was identical to that reported in the literature.[27] ^1H NMR (DMSO-d_6, 300 MHz) δ 7.53 (d, J = 8.5 Hz, 1H, H-5), 7.09 (d, J = 6.6 Hz, 1H, H-6), 7.08 (s, 1H, H-8), 6.22 (s, 1H, H-3), 2.34 (s, 3H, CH_3, H-7′), 2.32 (d, J = 0.9 Hz, 3H, CH_3, H-4′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.3 (C-2), 153.5 (C-4), 153.4 (C-9), 143.1 (C-7), 125.7 (C-6), 125.3 (C-5), 117.6 (C-10), 116.8 (C-8), 113.7 (C-3), 21.4 (C-7′, CH_3), 18.4 (C-4′, CH_3).



3.47

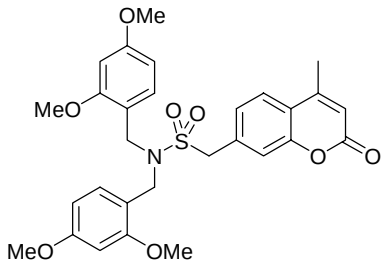
7-Bromomethyl-4-methylcoumarin (3.47). To a stirred suspension of 4,7-dimethyl coumarin **3.46** (8.70 g, 50.0 mmol) in 100 mL of dry benzene was *N*-bromosuccinimide (NBS, 9.35 g, 52.5 mmol) and benzoyl peroxide (115 mg, 0.500 mmol). The resulting mixture was heated to reflux overnight (16 h), cooled to room temperature, and filtered. Concentration of the filtrate provided a pale yellow solid, which was washed thoroughly with water. Recrystallization from ethanol gave **3.47** as white crystals (11.3 g, 89%). Mp: 205-206 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.56 (d, J = 8.0

Hz, 1H, H-5), 7.32 (s, 1H, H-8), 7.31 (d, J = 8.3 Hz, 1H, H-6), 6.28 (s, 1H, H-3, vinyl), 4.50 (s, 2H, $BrCH_2$), 2.41 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.1 (C-2), 153.3 (C-4), 153.2 (C-9), 142.8 (C-7), 126.3 (C-6), 125.8 (C-5), 119.9 (C-10), 117.3 (C-8), 115.1 (C-3), 33.3 (Ar$\underline{C}H_2$Br), 18.5 (C-4′, CH_3); LRMS (EI), *m/z* (relative intensity), 254 (M+2, 14), 252 (M^+, 14), 173 (M-Br, 100), 145 (19), 115 (11); HRMS (EI) calcd for $C_{11}H_9BrO_2$ 251.9786; found 251.9782.



3.48

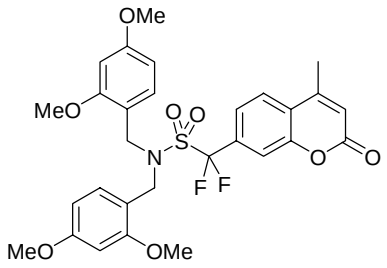
Sodium 7-(4-methylcoumarin) methane sulfonate (3.48). To a suspension of bromide **3.47** (6.075 g, 24 mmol) in 135 mL of ethanol was added a solution of Na_2SO_3 (3.600 g, 28.6 mmol) in H_2O (135 mL). The resulting mixture was refluxed for 5 h, cooled to room temperature and concentrated. The residue was recrystallized from water to afford **3.48** as colorless crystals (5.05 g, 76%). ^1H NMR (DMSO-d_6, 300 MHz) δ 7.56 (s, 1H, H-5), 7.24 (s, 2H, H-6 and H-8), 6.24 (s, 1H, H-3, vinyl), 3.89 (s, 2H, Ar$\underline{C}H_2SO_3$), 2.32 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.6 (C=O), 153.8, 152.9, 140.2, 127.2, 125.0, 118.4, 118.2, 114.0, 57.4 (Ar$\underline{C}H_2SO_3$), 18.6 (CH_3); HRMS (ESI) calcd for $C_{11}H_9O_5S$ 253.0176; found 253.0188.



3.50

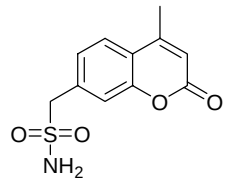
***N,N*-Bis(2,4-dimethoxybenzyl) 7-(4-methylcoumarin)methane sulfonamide(3.50).** To a

suspension of **3.48** (3.00 g, 10.9 mmol) in dry benzene (120 mL) was added DMF (0.40 mL) and thionyl chloride (5 mL). The resulting mixture was heated at 86 °C (oil bath temperature) for 5 h, cooled to room temperature and concentrated. The residue was washed with ice cold water (50 mL) and then transferred to a 250 mL of round bottom flask. Acetone was added to remove the water and the flask was dried under high vacuum for 1 h to give crude **3.49** as a light yellow solid. Dry THF (100 mL), DMAP (1.20 g, 10.0 mmol, 0.92 equiv), $(DMB)_2NH$ (3.0 g, 9.5 mmol, 0.87 equiv) were added. The resulting mixture was heated to reflux overnight then cooled to room temperature and concentrated. The residue was redissolved in 200 mL of CH_2Cl_2, washed with water, 10% citric acid, water again and brine then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (EtOAc/hexane, 1:1) gave **3.50** as a white solid (3.95 g, 66% over two steps from **3.48**) which could be recrystallized from ethyl acetate/hexane to give colorless needles. Mp: 107-108 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.47 (d, J = 8.1 Hz, 1H), 7.27-7.17 (m, 3H), 6.88 (s, 1H), 6.43-6.40 (m, 4H), 6.23 (s, 1H, H-3, vinyl), 4.20 (s, 4H, ArC$\underline{H}_2$N x 2), 4.06 (s, 2H, ArC$\underline{H}_2SO_2$), 3.77 (s, 6H, OCH_3 x 2), 3.77 (s, 6H, OCH_3 x 2), 2.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.8, 160.7, 158.5, 153.2, 152.3, 133.9, 131.5, 126.8, 124.7, 119.8, 119.2, 117.0, 115.3, 104.3, 98.4, 58.8 (Ar$\underline{C}H_2SO_2$), 55.5 (2C, OCH_3 x 2), 55.3 (2C, OCH_3 x 2), 45.9 (Ar$\underline{C}H_2N$), 18.7 (CH_3); LRMS (EI), *m/z* (relative intensity), 553 (M^+, 20), 338 (10), 316 (25), 178 (75), 151 (100), 121 (34); HRMS (EI) calcd for $C_{29}H_{31}NO_8S$ 553.1770; found 553.1769.



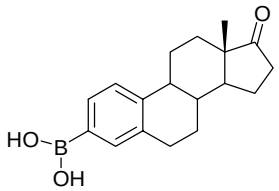
3.51

***N,N*-Bis(2,4-dimethoxybenzyl) 7-(4-methylcoumarin)difluoromethane sulfonamide (3.51).** To a solution of **3.50** (553 mg, 1.00 mmol) and NFSi (788 mg, 2.50 mmol, 2.5 equiv) in 60 mL of THF at -78 °C was added NaHMDS (1.0 M in THF, 2.5 mL, 2.5 mmol, 2.5 equiv) via a syringe pump over 50 min. After stirring 2.75 h at that temperature, the reaction was quenched with 5 mL of saturated aq. NH_4Cl at -78 °C. EtOAc (60 mL) and H_2O (20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organics were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. The residue was subjected to flash column chromatography (EtOAc/hexane, 1:2.5 to 1:1) which gave pure **3.51** as a white foam (311 mg, 53%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, J = 8.1 Hz, 1H, H-5), 7.52 (d, 1H, overlapping, H-6), 7.50 (s, 1H, H-8), 7.10 (d, J = 8.4 Hz, 2H), 6.31 (dd, J = 8.4 Hz, J = 1.8 Hz, 2H), 6.28 (s, 1H, H-3, vinyl), 6.24 (s, 2H), 4.46 (s, 4H, ArC$\underline{H}_2$N), 3.70 (s, 6H, OCH_3 x 2), 3.61 (s, 6H, OCH_3 x 2), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.5, 159.8, 158.2, 152.9, 151.5, 132.9 (t, J = 22 Hz), 130.5, 125.0, 122.8 (t, J = 5 Hz), 122.4, 121.4 (t, J = 282 Hz), 116.9, 116.4, 116.1 (t, J = 6 Hz), 104.0, 97.8, 55.3 (2C, OCH_3 x 2), 55.0(2C, OCH_3 x 2), 46.9 (Ar$\underline{C}H_2$N), 18.6 (CH_3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ –100.9; LRMS (EI), *m/z* (relative intensity), 589 (M^+, 38), 316 (12), 209 (40), 178 (72), 151 (100), 121 (30); HRMS (EI) calcd for $C_{29}H_{29}F_2NO_8S$ 589.1582; found 589.1591.



3.52

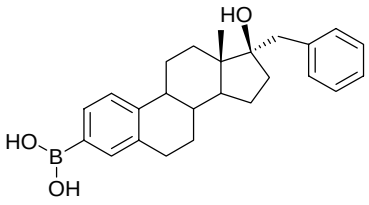
7-(4-Methylcoumarin) methane sulfonamide (3.52). To a solution of **3.50** (250 mg, 0.452 mmol) in CH_2Cl_2 (10 mL) at rt. was added TFA (1 mL) via syringe slowly. After addition, it was stirred for 70 min at rt then concentrated (without using a water bath) and a pink residue was obtained. The residue was dried over high vacuum. To this pink residue was added 15 mL of ether, stirred 5 min, and then filtered. The filter cake was transferred to an Erlenmeyer flask containing 75 mL of ethanol. The mixture was heated to reflux for 2 min, then filtered. The filtrate was concentrated to give **3.52** as a slightly yellow crystalline solid (93 mg, 81 %). Mp: 250-252 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.74 (d, J = 4.9 Hz, 1H, H-5), 7.35 (s, 2H, H-6 and H-8), 6.87 (s, 2H, NH_2), 4.38 (s, 2H, Ar$\underline{C}H_2SO_2$), 2.40 (s, 3H, C-4′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.1 (C-2), 153.5 (C-4), 153.1 (C-9), 135.7 (C-7), 127.4 (C-6), 125.7 (C-5), 119.6 (C-10), 118.9 (C-8), 114.8 (C-3), 60 (Ar$\underline{C}H_2SO_2$), 18.5 (C-4′, CH_3); LRMS (EI), *m/z* (relative intensity), 253 (M^+, 20), 173 (100), 145 (14), 115 (10), 91 (4); HRMS (EI) calcd for $C_{11}H_{11}NO_4S$ 253.0409; found 253.0406.



3.53

Estra-1,3,5(10)-trien-17-one-3-boronic acid (3.53). To a mixture of estrone boronate **3.57** (1.14 g, 3.00 mmol) in acetone (450 mL) was added a solution of ammonium acetate (924 mg, 12.0 mmol, 4 equiv) and sodium periodate (2.57 g, 12.0 mmol, 4 equiv) in H_2O (360 mL). The reaction

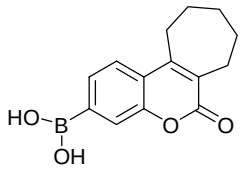
mixture was stirred for 10 days at room temperature. After removing the acetone by rotary evaporation the remaining aqueous solution was extracted with ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (acetone/hexane, 1:3) gave boronic acid **3.53** as a white solid (839 mg, 84%). Mp: 181-182 ^{o}C ; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.77 (s, 2H, $B(OH)_2$), 7.48 (d, J = 7.5 Hz, 1H, H-2), 7.45 (s, 1H, H-4), 7.18 (d, J = 7.5 Hz, 1H, H-1), 2.80 (br, s, 2H), 2.40-1.85 (m, 6H), 1.74 (br, s, 1H), 1.65-1.20 (m, 6H), 0.78 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 220.0 (C=O), 141.9 (C_{Ar}), 135.4 (C_{Ar}), 135.3 (C_{Ar}), 131.2 (C_{Ar}), 124.6 (C_{Ar}), 50.2 (C-14), 47.7 (C-13), 44.5 (C-7), 38.1 (C-8), 35.8 (C-16), 31.8 (CH_2), 29.3 (CH_2), 26.5 (CH_2), 25.7 (CH_2), 21.6 (CH_2), 13.9 (CH_3, C-18); LRMS (ESI) *m/z* (%) 299 (M+1, 100), 298 (M^+, 25), 281 (27), 231 (82); HRMS (ESI) calcd for $C_{18}H_{24}BO_3$ 298.1855; found 298.1862.



3.54

17*α*-Benzyl-17*β*-hydroxyestra-1,3,5(10)-trien-3-boronic acid (3.54). To a solution of **3.61** (47 mg, 0.1 mmol) and phenylboronic acid (13.0 mg, 0.105 mmmol, 1.05 equiv) in THF (8 mL)/MeOH (3 mL) was added 2 M HCl (2 mL). The resulting mixture was stirred overnight and then quenched with water. The mixture was extracted with ethyl acetate and the combined extracts were washed with water and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (acetone/hexane, 1:4 to 1:2) gave **3.54** as a white solid (26 mg, 67%). Mp:

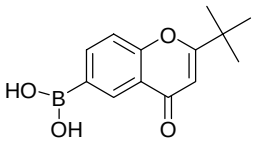
216-218 ^{o}C ; ^{1}H NMR (DMSO-d_6 with 1 drop of D_2O, 300 MHz) δ 7.46 (d, J = 7.4 Hz, 1H, H-2), 7.41 (s, 1H, H-1), 7.20-7.05 (m, 6H), 3.10 (s, 2H, $B(OH)_2$), 2.80-2.75 (m, 3H), 2.54 (d, J = 13.2 Hz, 1H), 2.35-2.25 (m, 1H), 2.25-2.15 (m, 1H), 1.85-1.75 (m, 1H), 1.70-1.20 (m, 10H), 0.78 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6 with 1 drop of D_2O, 75 MHz) δ 142.6 (C_{Ar}), 139.9 (C_{Ar}), 135.5 (C_{Ar}), 135.3 (C_{Ar}), 131.8 (C_{Ar}), 131.6 (C_{Ar}), 131.0 (br, C-3), 127.8 (C_{Ar}), 126.0 (C_{Ar}), 124.7 (C_{Ar}), 82.6 (C-17), 49.5, 47.2, 44.4 (C-7), 42.4 (Ph<u>C</u>H_2), 39.7 (C-8), 32.2 (CH_2), 31.3 (CH_2), 29.5 (CH_2), 27.6 (CH_2), 26.2 (CH_2), 23.3 (CH_3, C-18); LRMS (ESI, +LiOAc) *m/z* (%) 787 (2M+Li, 100), 397 (M+Li, 47); HRMS (ESI) calcd for $C_{25}H_{31}LiBO_3$ 396.2563; found 396.2549**.**



3.55

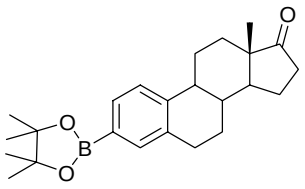
6-Oxo-8,9,10,11-tetrahydro-7*H*-cylohepta-[*c*][*1*]benzopyran-3-boronic acid (3.55) To a mixture of coumarin boronate **3.64** (342 mg, 1.00 mmol) in acetone (150 mL) was added a solution of ammonium acetate (308 mg, 4.00 mmol, 4 equiv) and sodium periodate (856 mg, 4.00 mmol, 4 equiv) in H_2O (120 mL). The reaction mixture was stirred overnight at room temperature. After removal of acetone by rotary evaporation, the residue was extracted with ethyl acetate and then concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:1 to 100% ethyl acetate) gave coumarin boronic acid **3.55** as a white solid (172 mg, 67%). Mp: >260 ^{o}C (dec.); ^{1}H NMR (DMSO-d_6 with 1 drop of D_2O, 75 MHz) δ 8.32 (s, 2H, $B(OH)_2$), 7.82 (d, J = 7.7 Hz, 1H), 7.70-7.60 (m, 2H), 3.00 (m, 2H), 2.78 (m, 2H), 1.80 (m, 2H), 1.56 (m, 2H), 1.47 (m, 4H); ^{13}C NMR (DMSO-d_6 with 1 drop of D_2O, 75 MHz) δ 161.4, 153.9, 151.8, 130.0, 128.8, 123.9, 121.9, 120.9, 31.7, 27.5,

26.6, 25.5, 25.0; ^{11}B NMR (DMSO-d_6 with 1 drop of D_2O, 96 MHz) δ 30.3; LRMS (ESI) *m/z* (%) 259 (M+1, 100), 258 (M+, 29); HRMS (ESI) calcd for $C_{14}H_{15}BO_4$ 258.1178; found 258.1177.



3.56

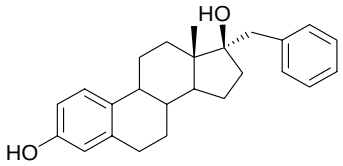
2-*tert*-Butyl-4*H*-1-benzopyran-4-one-6-boronic acid (3.56). To a mixture of chromenone boronate **3.67** (328 mg, 1.00 mmol) in acetone (150 mL) was added a solution of ammonium acetate (308 mg, 4.00 mmol) and sodium periodate (856 mg, 4.00 mmol) in H_2O (120 mL). The reaction mixture was stirred overnight at room temperature. After removal of acetone by rotary evaporation, the resulting aqueous solution was extracted with ethyl acetate and then concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:1 to 100% ethyl acetate) gave chromenone boronic acid **3.56** as a white solid (189 mg, 77%). Mp: 176-178 ^{o}C; ^{1}H NMR (DMSO-d_6 with 1 drop of D_2O, 300 MHz) δ 8.47 (s, 1H), 8.28 (s, 2H, partially exchanged with D_2O, $B(OH)_2$), 8.09 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 6.07 (s, 1H), 1.27 (s, 9H); ^{13}C NMR (DMSO-d_6 with 1 drop of D_2O, 75 MHz) δ 178.0, 176.0, 157.6, 140.0, 131.8, 122.3, 117.4, 106.7, 36.5, 27.8; ^{11}B NMR (DMSO-d_6 with 1 drop of D_2O, 96 MHz) δ 28.6; LRMS (ESI) *m/z* (%) 248 (M+1, 12), 247 (M^+, 100); HRMS (ESI) calcd for $C_{13}H_{15}BO_4$ 246.1178; found 246.1185.



3.57

3-Pinacolatoboroestra-1,3,5(10)-trien-17-one (3.57). To a mixture of estrone triflate **3.29**

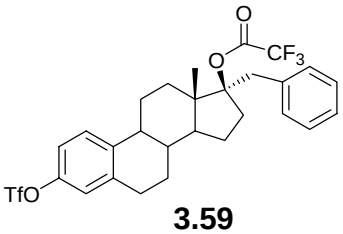
(404 mg, 1.00 mmol) and Pd(dppf)Cl_2-CH_2Cl_2 (44 mg, 0.050 mmol, 5 mol %) in dioxane (8 mL) under argon was added Et_3N (0.84 mL, 6.0 mmol, 6 equiv) and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.44 mL, 3.0 mmol, 3 equiv). The reaction mixture was heated at 95-100 ^oC for 7 h. The reaction was cooled to room temperature and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3) gave **3.57** as a white solid (356 mg, 94%). Mp: 193-194 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.58 (d, J = 8.0 Hz, 1H, H-2), 7.55 (s, 1H, H-4), 7.29 (d, J = 7.8 Hz, 1H, H-1), 2.95-2.88 (m, 2H), 2.65-2.40 (m, 2H), 2.40-2.25 (m, 1H), 2.20-1.90 (m, 4H), 1.70-1.35 (m, 6H), 1.32 (s, 12 H), 0.89 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.6 (C=O), 143.1, 135.8, 135.6, 132.2, 124.7, 83.6 (O$\underline{C}(CH_3)_2$), 50.6 (C-14), 47.9 (C-13), 44.7 (C-7), 38.0 (C-8), 35.8 (C-16), 31.6 (CH_2), 29.1 (CH_2), 26.5 (CH_2), 25.6 (CH_2), 24.8(OC($\underline{C}H_3)_2$), 21.6 (CH_2), 13.8 (CH_3, C-18); LRMS (EI) *m/z* (%) 381 (M+1, 25), 380 (M^+, 100), 379 (24), 323 (13), 294 (30), 281 (24); HRMS (EI) calcd for $C_{24}H_{33}BO_3$ 380.2523; found 380.2531.



3.58

17*α*-benzyl-17*β*-hydroxyestra-1,3,5(10)-triene (3.58). This is prepared according to the procedure of Ciobanu et al. with slight modifications.[32] Magnesium (22.0 g, 0.917 mol, 3.2 equiv) was activated by heating under argon and then suspended in Et_2O (250 mL). To this mixture was added benzyl bromide (34.0 mL, 48.9 g, 0.286 mol, 1 equiv) slowly over 3 h (syringe pump). The BnMgBr formed was added slowly to a solution of estrone (8.00 g, 29.6 mmol) in THF (800 mL) over 50 min. The resulting mixture was stirred overnight and quenched with sat. NH_4Cl. After removal of

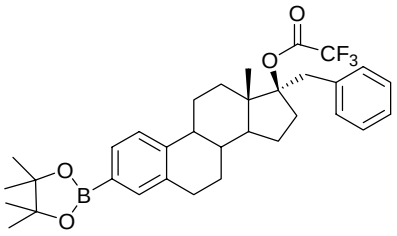
ether and THF *in vacuo*, ethyl acetate (300 mL) was added and the mixture was filtered to remove insoluble material. The filtrate was extracted with EtOAc and the combined extracts were dried (Na_2SO_4) and concentrated. The residue was redissolved in MeOH (500 mL). To this solution at 0 ^oC was added $NaBH_4$ (2.24 g) and the reaction was stirred 1 h at 0 ^oC. The reaction was concentrated and the residue diluted with water and extracted with ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated. The residue was subjected to flash chromatography (ethyl acetate/hexane, 1:4) and the resulting material recrystallized from ethyl acetate/hexane to give diol **3.58** as colorless crystals (7.14 g, 67%). ^1H NMR and ^{13}C NMR are identical to that reported in literature.[32] ^1H NMR (acetone-d_6, 300 MHz) δ 7.90 (s, 1H, ArOH), 7.33 (d, J = 7.4 Hz, 2H), 7.23 (t, J = 7.2 Hz, 2H), 7.18-7.13 (m, 1H), 7.08 (d, J = 8.4 Hz, 1H, H-1), 6.59 (dd, J = 8.3 Hz, J = 2.5 Hz, 1H, H-2), 6.52 (d, J = 2.3 Hz, 1H, H-4), 3.16 (s, 1H, OH), 2.90 (d, J = 13.3 Hz, 1H, PhCHH), 2.77-2.66 (m, 3H (1H from PhCHH)), 2.40-2.28 (m, 1H), 2.25-2.10 (m, 1H), 1.90-1.30 (m, 11H), 0.95 (s, 3H, CH_3, H-18).



3.59

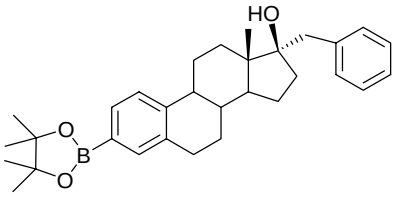
3-*O*-[(Trifluromethylsulfonyl)oxy]- 17*α*-benzyl-17*β*-trifluroacetyloxyestra-1,3,5(10)-triene (3.59). To a solution of estradiol **3.58** (2.89 g, 8.00 mmol) and DMAP (2.44 g, 20.0 mmol, 2.5 equiv) in dry CH_2Cl_2 (120 mL) at 0 ^oC was added trifluroacetic anhydride (TFAA, 2.70 mL, 19.4 mmol, 2.4 equiv) over 30 min. The mixture was stirred for 7 h at room temperature before quenching with water. The layers were separated and the aqueous phase was extracted with

methylene chloride and the combined organics were dried over Na_2SO_4. After removal of solvent, the residue was redissloved in ethyl acetate (150 mL) and methanol (30 mL). 1 M HCl (40 mL) was added and the resulting mixture was stirred 1 h at room temperature. Reaction was diluted with water and extracted with ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 1:8 to 1:6) afforded crude 17-trifluoro-acetoxyestrone as a white foam. This material was redissolved in methylene chloride (120 mL) and DMAP (1.22 g, 1.0 mmol, 1.25 equiv) was added. The resulting mixture was cooled to 0 ^oC and triflic anhydride (1.5 mL, 8.9 mmol, 1.1 equiv) was added over 10 min. After stirring for 1 h at 0 ^oC, the reaction was quenched with ice-cold water and extracted with methylene chloride. The combined extracts were washed with water and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:8) gave compound **3.59** as a white solid (3.13 g, 67%). Mp: 112-113 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.24 (m, 4H), 7.11 (d, J = 7.1 Hz, 2H), 7.02 (d, J = 8.8 Hz, 1H, H-2), 6.97 (s, 1H, H-4), 3.90 (d, J = 14.6 Hz, 1H, PhC<u>H</u>H), 2.96-2.85 (m, 2H), 2.77 (d, J = 14.6 Hz, 1H, PhCH<u>H</u>), 2.40-2.20 (m, 4H), 2.00-1.70 (m, 4H), 1.70-1.35 (m, 5H), 0.92 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.8 (q, J = 38 Hz, CF_3<u>C</u>=O), 147.6 (C-3), 140.3 (C_{Ar}), 139.2 (C_{Ar}), 135.8 (C_{Ar}), 130.4 (C_{Ar}), 128.4 (C_{Ar}), 127.2 (C_{Ar}), 126.9 (C_{Ar}), 121.2 (C_{Ar}), 118.8 (q, J = 333 Hz, CF_3), 118.2 (C_{Ar}), 114.6 (q, J = 285 Hz, CF_3), 99.9 (C-17), 50.5 (C-14), 48.2 (C-13), 43.6 (C-9), 38.9, 37.8, 33.0 (CH_2), 32.7 (CH_2), 29.4 (CH_2), 26.9 (CH_2), 26.1 (CH_2), 23.0 (CH_2), 14.2 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -72.7, -75.1; LRMS (CI) *m/z* (%) 608 (M+18, 25), 494 (40), 477 (87), 476 (100), 461 (29), 385 (70), 329 (41); HRMS (ESI) calcd for $[C_{28}H_{28}F_6O_5S+NH_4]^+$ 608.1905; found 608.1927.



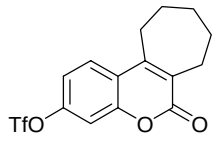
3.60

3-*O*-Pinacolatoboro-17*α*-benzyl-17*β*-trifluroacetyloxyestra-1,3,5(10)-triene (3.60). To a mixture of triflate **3.59** (1.60 g, 2.71 mmol) and Pd(dppf)Cl_2-CH_2Cl_2 (110 mg, 0.135 mol 5 mmol %) in dioxane (15 mL) under argon was added Et_3N (3.4 mL, 24 mmol, 9 equiv) and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.7 mL, 11.7 mmol, 4.3 equiv). The reaction mixture was heated at 92-96 ^oC for 3 h, cooled to room temperature, diluted with water and extracted with ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:10) gave compound **3.60** as a white solid (952 mg, 62%). Mp: 143-144 ^oC ; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.59 (d, J = 7.8 Hz, 1H, H-2), 7.54 (s, 1H, H-1), 7.36-7.27 (m, 4H), 7.11 (d, J = 6.5 Hz, 2H), 3.87 (d, J = 14.7 Hz, 1H, PhC<u>H</u>H), 2.92-2.88 (m, 2H), 2.78 (d, J = 14.7 Hz, 1H, PhCH<u>H</u>), 2.55-2.20 (m, 4H), 2.00-1.75 (m, 4H), 1.70-1.40 (m, 5H), 1.32 (s, 12H, $C(CH_3)_2$), 0.90 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.7 (q, J = 41 Hz, C=O), 143.2, 136.0, 135.7, 135.7, 132.3, 130.5, 128.4, 126.9, 126.2 (br, C-3), 124.8, 114.8 (q, J = 286 Hz, CF_3), 100.1 (C-17), 83.6 (O<u>C</u>$(CH_3)_2$), 50.7, 48.3, 44.2, 39.2, 38.0, 33.2, 32.8, 29.3, 27.4, 26.1, 24.9, 23.0, 14.3 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -75.1; ^{11}B NMR ($CDCl_3$, 96 MHz) δ 33.2; LRMS (EI) *m/z* (%) 568 (M^+, 8), 478 (9), 454 (100), 439 (28), 363 (62), 267 (32); HRMS (EI) calcd for $C_{33}H_{40}BF_3O_4$ 568.2972; found 568.2972.



3.61

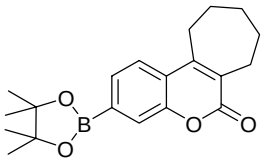
3-*O*-Pinacolatoboro-17*α*-benzyl-17*β*-hydroxyestra-1,3,5(10)-triene (3.61). To a solution of **3.60** (480 mg, 0.845 mmol) in THF (240 mL) at room temperature was added 0.8 M NaOH (24 mL) slowly over 10 min. After stirring for 10 min, water (60 mL) was added and the mixture was extracted with Et_2O. The combined extracts were washed with water and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:6 to 1:5) gave **3.61** as a white solid (335 mg, 84%). Mp: 211-213 ^oC ; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, J = 7.7 Hz, 1H, H-2), 7.59 (s, 1H, H-1), 7.37-7.24 (m, 6H), 3.00-2.90 (m, 3H), 2.95 (d, J = 12.8 Hz, 1H), 2.71-2.66 (m, 1H), 2.30-2.20 (m, 1H), 2.05-1.91 (m, 2H), 1.80-1.50 (m, 7H), 1.50-1.25 (m, 2H, overlapping), 1.36 (s, 12H, overlapping, $C(CH_3)_2$), 0.97 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.8, 138.5, 136.0, 135.7, 132.2, 131.1, 128.4, 126.3, 126.0 (br,C-3), 124.8, 83.6, 83.0, 49.8, 46.9, 44.7, 42.5, 39.4, 33.7, 31.5, 29.4, 27.6, 26.1, 25.0, 23.4, 14.6 (CH_3, C-18); ^{11}B NMR ($CDCl_3$, 96 MHz) δ 33.7; LRMS (EI) *m/z* (%) 472 (M^+, 8), 454 (7), 381 (96), 380 (100), 363 (79), 323 (18), 237 (22); HRMS (EI) calcd for $C_{31}H_{41}BO_3$ 472.3149; found 472.3151.



3.62

3-[(Trifluromethylsufonyl)oxy]-6-oxo-8,9,10,11-tetrahydro-7*H*-cylohepta-[*c*][*1*]benzopyran (3.62). To a solution of coumarin **2.35** (3.00 g, 13.0 mmol) and DMAP, (400 mg, 3.28 mmol,

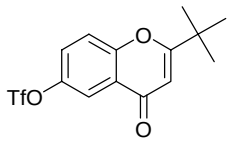
0.25 equiv) in methylene chloride (100 mL) at 0 °C was added 2,6-lutidine (3.05 mL, 26.0 mmol, 2 equiv) then triflic anhydride (2.65 mL, 15.6 mmol, 1.2 equiv) over 10 min. The reaction was stirred for 1 h at 0 °C then quenched with ice and 0.5 M HCl (30 mL). The layers were separated and the aqueous phase was extracted with methylene chloride. The combined organics were washed with 0.5 M HCl and 5% $NaHCO_3$ then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:4) gave triflate **3.62** as a white solid (4.56 g, 97%). Mp: 86-87 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.73 (d, J = 8.9 Hz, 1H), 7.21 (d, J = 2.0 Hz,1H), 7.18 (dd, J = 8.9 Hz, 2.0 Hz, 1H), 2.95-2.87 (m, 4H), 1.94-1.85 (m, 2H), 1.70-1.55 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.9, 152.9, 152.4, 149.8, 129.8, 125.8, 119.9, 118.6 (q, J = 315 Hz, CF$_3$), 117.0, 110.1, 31.7, 28.1, 26.8, 25.3, 24.7; ^{19}F NMR ($CDCl_3$, 282 MHz) δ -72.3; LRMS (EI) *m/z* (%) 362 (M^+, 60), 347 (7), 230 (15), 229 (100), 201 (18); HRMS (EI) calcd for $C_{15}H_{13}F_3O_5S$ 362.0436; found 362.0438.



3.63

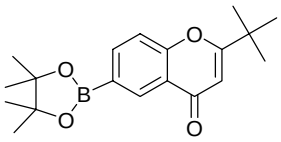
3-Pinacolatoboro-6-oxo-8,9,10,11-tetrahydro-7*H*-cylohepta-[*c*][*1*]benzopyran (3.63). To a mixture of coumarin triflate **3.62** (1.81 g, 5.00 mmol), bis(pinacolato)diboron (1.44 g, 5.65 mmol, 1.1 equiv), Pd(dppf)Cl_2-CH_2Cl_2 (123 mg, 0.150 mmol, 3 mol %) and dry KOAc (735 mg, 7.50 mmol, 1.5 equiv) under argon was added dioxane (30 mL). The resulting mixture was heated at 85-90 °C overnight. After cooling to room temperature, the reaction mixture was loaded directly onto a silica column and purified by flash chromatography (hexane then ethyl acetate/hexane, 1:4) to give boronate **3.63** as a white solid (1.41 g, 83 %). Mp: 119-120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (s, 1H),

7.59 (d, J = 8.6 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 2.90-2.82 (m, 4H), 1.83 (quint, J = 5.5 Hz, 2H), 1.62-1.49 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.8, 153.1, 151.9, 129.8, 129.7, 123.0, 122.8, 121.8, 84.2, 31.9, 27.8, 26.8, 25.5, 24.9, 24.8; ^{11}B NMR ($CDCl_3$, 96 MHz) δ 30.4; LRMS (EI) *m/z* (%) 340 (M^+, 100), 339 (30), 325 (19), 312 (17), 254 (10), 241 (18); HRMS (EI) calcd for $C_{20}H_{25}BO_4$ 340.1846; found 340.1838.



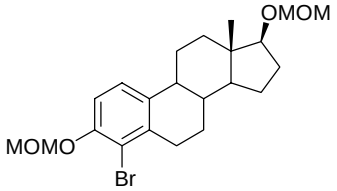
3.66

2-*tert*-Butyl-6-[(trifluromethylsulfonyl)oxy]-4*H*-1-benzopyran-4-one or 2-*tert*-butyl-4-oxo-4*H*-chromen-6-yl trifluoromethanesulfonate (3.66). To a solution of chromenone **3.65**[36] (2.16 g, 10.0 mmol) and DMAP (305 mg, 2.50 mmol, 0.25 equiv) in methylene chloride (80 mL) at 0 ^{o}C was added 2,6-lutidine (2.33 mL, 20.0 mmol, 2 equiv) then triflic anhydride (2.02 mL, 12.0 mmol, 1.2 equiv) over 10 min. After addition, the reaction mixture was stirred 1.5 h at 0 ^{o}C then quenched with ice and 0.5 M HCl (30 mL). The layers were separated and the aqueous phase was extracted with methylene chloride. The combined organics were washed with 0.5 M HCl and 5% $NaHCO_3$ then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:2 to 1:1) gave chromenone triflate **3.66** as a white solid (1.76 g, 50 %). Mp: 88-89 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (s, 1H), 7.52 (t, J = 9.3 Hz, 2H), 6.24 (s, 1H), 1.29 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.2, 176.8, 155.5, 145.8, 126.7, 124.5, 120.5, 118.6 (q, J = 315 Hz, CF_3), 117.9, 106.5, 36.5, 27.7; ^{19}F NMR ($CDCl_3$, 282 MHz) δ -72.5; MS (EI) *m/z* (%) 350 (M^+, 65), 217 (100); HRMS (EI) calcd for $C_{14}H_{13}F_3O_5S$ 350.0436; found 350.0442.



3.67

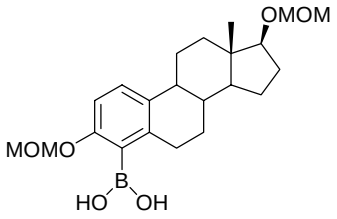
2-*tert*-Butyl-6-pinacolatoboro-4*H*-1-benzopyran-4-one (3.67). To a mixture of chromenone triflate **3.66** (1.04 g, 3.00 mmol), bis(pinacolato)diboron (861 g, 3.39 mmol, 1.1 equiv), Pd(dppf)Cl_2-CH_2Cl_2 (74 mg, 0.090 mmol, 3 mol %) and dry potassium acetate (441 mg, 4.50 mmol, 1.5 equiv) under argon was added dioxane (18 mL). The resulting mixture was heated at 85-90 ^oC for 16 h. After cooling to room temperature the reaction mixture was loaded onto a silica column and purified by flash chromatography (100% hexane, then ethyl acetate/hexane, 1:4) to give chromenone boronate **3.67** as a light yellow solid (725 mg, 75 %). Mp: 135-136 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.61 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 6.22 (s, 1H), 1.29 (s, 21H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.7, 175.8, 158.3, 139.1, 133.2, 122.6, 117.0, 107.0, 84.0, 36.4, 27.8, 24.8; ^{11}B NMR ($CDCl_3$, 96 MHz) δ 30.4; MS (EI) *m/z* (%) 328 (M, 100), 327 (95), 313 (75), 285(48), 271 (39), 229 (75); HRMS (EI) calcd for $C_{19}H_{25}BO_4$ 328.1846; found 328.1852.



3.68

4-Bromo-3,17*β*-bis(methoxymethoxy)estra-1,3,5(10)-triene (3.68). This was prepared according to the procedure of Lovely et al.[37] To a solution of 4-bromoestradiol **2.22** (3.40 g, 9.7 mmol) in dry THF (100 mL) was added $^iPr_2NEt$ (21.3 mL, 89.3 mmol, 9.2 equiv). The resulting mixture was cooled to 0 ^oC, before MOMCl (5.7 mL, 75 mmol, 7.7 equiv) was added dropwise over 10 min.

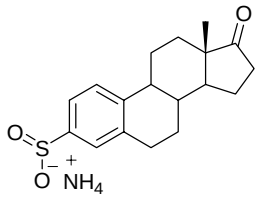
After addition, the reaction was warmed up to rt and stirred for 10 min at rt, then refluxed overnight (15 h, oil bath temp 86 ^{o}C). After cooling to rt and quenching with sat. NH_4Cl (50 mL), it was extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane 1:8) gave **3.68** as a white solid (3.85 g, 91%). ^{1}H NMR and ^{13}C NMR are identical to those reported in the literature.[37] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.19 (d, J = 8.7 Hz, 1H, H-1), 6.94 (d, J = 8.7 Hz, H-2), 5.20 (s, 2H, $ArOCH_2O$), 4.65 (d, J = 6.6 Hz, 1H, $C_{17}OC\underline{H}HO$), 4.62 (d, J = 6.6 Hz, 1H, $C_{17}OCH\underline{H}O$), 3.59 (t, J = 8.5 Hz, 1H, H-17), 3.50 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 2.97 (dd, J = 18.3 Hz, J = 6.0 Hz, 1H), 3.25-3.10 (m, 1H), 2.30-1.90 (m, 5H), 1.75-1.10 (m, 12H; m, 6H and s, 6H, CH_3 x 2 at 1.56 ppm), 0.78 (s, 3H, CH_3, H-18).



3.69

4-Bromo-3,17*β*-bis(methoxymethoxy)estra-1,3,5(10)-trien-4-boronic acid (3.69). To a solution of **3.68** (219 mg, 0.500 mmol) in dry THF (10 mL) at -78 ^{o}C was added ^{n}BuLi (1.6 M in hexane, 0.90 mL, 1.4 mmol, 2.9 equiv) dropwise over 3 min. After stirring 1 h at -78 ^{o}C, the cold bath was removed and reaction allowed to warm to rt gradually and stirred for 5 min at rt. The mixture was cooled back to -78 ^{o}C and trimethyl borate (0.60 mL, 5.4 mmol, 11 equiv) was added. The resulting mixture was stirred for 5 min at -78 ^{o}C and warmed up to rt and stirred 5 min at that temperature before cooling back to -78 ^{o}C. 1 M HCl (2 mL) was added slowly and the mixture

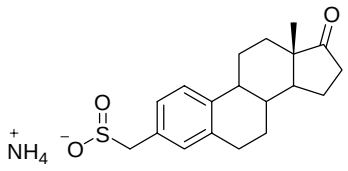
allowed to warm up to rt. The mixture was diluted with H_2O and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3 to 1:1) gave boronic acid **3.69** as a white solid (110 mg, 54%). Mp: 127-129 ^oC; ^1H NMR (CD_3OD, 300 MHz) δ 7.16 (d, J = 8.5 Hz, 1H, H-1), 6.81 (d, J = 8.3 Hz, 1H, H-2), 5.08 (s, 2H, $OC\underline{H}_2OCH_3$), 4.59 (s, 2H, $OC\underline{H}_2OCH_3$), 3.56 (t, J = 8.1 Hz, 1H, H-17), 3.40 (s, 3H, $OCH_2OC\underline{H}_3$), 3.31 (s, 3H, $OCH_2OC\underline{H}_3$), 2.80-2.60 (m, 2H), 2.30-1.15 (m, 13H), 0,77 (s, 3H, CH_3, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 156.5 (C-3), 139.3 (C-5), 133.4 (C-6), 126.5 (C-1), 110.5 (C-2), 95.7 ($O\underline{C}H_2OCH_3$), 94.1 ($O\underline{C}H_2OCH_3$), 86.7 (C-17), 54.8 ($OCH_2O\underline{C}H_3$), 54.1 ($OCH_2O\underline{C}H_3$), 49.8 (C-14), 44.0 (C-9), 42.7 (C-13), 38.5 (C-8), 37.1 (CH_2), 29.2 (CH_2), 27.7 (CH_2), 27.1 (CH_2), 26.2 (CH_2), 22.7 (CH_2), 10.9 (CH_3, C-18); LRMS (ESI) *m/z* (%) 826 (2M+NH_4, 30), 422 (M+NH_4, 90), 387 (100); HRMS (ESI) calcd for $[C_{28}H_{29}NO_3+NH_4]^+$ 422.2714; found 422.2687.



3.73

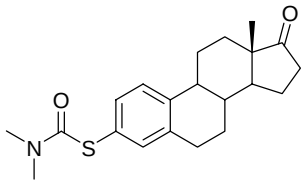
Ammonium estra-1,3,5(10)-trien-17-one-3-sulfinate (3.73). A mixture of estrone sulfonyl chloride **3.77** (380 mg, 1.08 mmol), $NaHCO_3$ (344 mg, 4.00 mmol, 4 equiv) and Na_2SO_3 (400 mg, 3.18 mmol, 3 equiv) in H_2O (20 mL) was heated at 90 ^oC for 3 h. The reaction was concentrated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5 to 16:4:1) to give sulfinate **3.73** as a white solid (264 mg, 73%). Mp: > 300 ^oC (dec.); ^1H NMR (CD_3OD, 300 MHz) δ 7.45-7.30 (m, 3H), 3.00-2.90 (m, 2H), 2.55-1.85 (m, 7H), 1.75-1.40 (m, 6H), 0.90 (s, 3H, CH_3,

H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 225.3 (C=O), 152.0 (C_{Ar}), 141.5 (C_{Ar}), 137.0 (C_{Ar}), 125.4 (CH_{Ar}), 124.2 (CH_{Ar}), 121.1 (CH_{Ar}), 50.2 (C-14), 48.4 (C-13), 44.2 (C-9), 37.9 (C-8), 35.7 (C-16), 31.2 (CH_2), 29.0 (CH_2), 26.1 (CH_2), 25.4 (CH_2), 21.1 (CH_2), 13.0 (CH_3, C-18); LRMS (ESI) *m/z* (%) 317 (M^+, 100); HRMS (ESI) calcd for $C_{18}H_{21}O_3S$ 317.1211; found 317.1216.



3.74

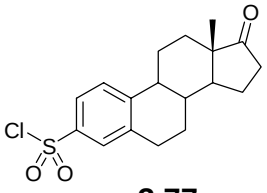
Ammonium estra-1,3,5(10)-trien-17-one-3-methanesulfinate (3.74). Sodium (15 mg, 0.65 mmol, 1.8 equiv) was added to dry EtOH (10 mL) and the resulting mixture was stirred at rt for 30 min to make a clear solution. This was added to a suspension of sulfone **3.80** (175 mg, 0.356 mmol) in EtOH (5 mL) and the mixture was refluxed at 90 ^{o}C for 3 h. After removal of solvent (water bath at 30 ^{o}C) the residue was purified by flash chromatography (methylene chloride/methanol/amommium hydroxide, 10:1:0.2 to 10:2:0.25 to 10:2.5:0.35) to give sulfinate **3.74** as a white solid (103 mg, 83%). Mp: 137 ^{o}C (dec.); ^{1}H NMR (D_2O, 300 MHz) δ 7.22 (d, J = 7.6 Hz, 1H, H-1), 7.04 (d, J = 8.8 Hz, 1H, H-2), 7.02 (s, 1H, H-4), 4.06 (CH_2SO_2), 2.80-2.70 (m, 2H), 2.50-1.25 (m, 13H), 0.77 (s, 3H, CH_3, H-18); ^{13}C NMR (D_2O, 75 MHz) δ 228.4 (C=O), 138.8, 137.0, 130.4, 129.6, 127.3, 125.5, 67.8 (Ar$\underline{C}H_2$S), 49.7 (C-14), 48.5 (C-13), 43.5 (C-9), 37.6 (C-8), 35.9 (CH_2), 30.9 (CH_2), 28.7 (CH_2), 25.9 (CH_2), 21.1 (CH_2), 13.3 (CH3, C-18); LRMS (ESI) *m/z* (%) 331 (100).



3.76

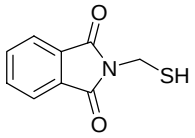
Estra-1,3,5(10)-trien-17-one-3-dimethylcarbamothioate (3.76). This was prepared according to the procedure of Li et al.[2] with some modifications. To a solution of estrone (15.0 g, 5.56 mmol) in DMF (225 mL) at 0 ^{o}C was added a suspension of NaH (60% dispersed in mineral oil, 2.64 g, 6.60 mmol, 1.2 equiv) in DMF (22 mL). After this addition, the resulting mixture was stirred 1 h at rt, then cooled to 0 ^{o}C before *N,N*-dimethylthiocarbamoyl chloride (10.6 g, 8.58 mmol, 1.5 equiv) was added. Subsequently, the reaction was heated at 80-85 ^{o}C for 1 h, and then it was cooled to rt and poured onto ice water (about 400 mL). Suction filtration followed by drying over high vacuum gave 21.76 g of crude *O*-aryl thiocarbamate **3.75** as pale yellow solid which was used directly for next step. Compound **3.75** (20.86 g) was added to a glass bomb and purged with argon then heated at 280-285 ^{o}C for 1 h before cooling to rt. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography (ethyl acetate/hexane, 1:2 to 1:3) and the material from the column was recrystallized from ethyl acetate/hexane to give **3.76** as light yellow crystals (12.71 g, 64% over 2 steps). ^{1}H NMR was identical to that reported in literature.[2] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28-7.19 (m, 3H), 3.01 (brs, 6H, $N(CH_3)_2$), 2.88 (dd, J = 8.7 Hz, J = 4.2 Hz, 2H), 2.46 (dd, J = 18.9 Hz, J = 9.0 Hz, 1H), 2.39-2.34 (m, 1H), 2.26 (dt, J = 4.2 Hz, J = 9.6 Hz, 1H), 2.15-1.90 (m, 4H), 1.63-1.36 (m, 6H), 0.86 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.7 (C=O), 167.2 (C(=O)S), 141.1 (C_{Ar}), 137.3, (C_{Ar}) 136.2 (CH_{Ar}), 133.0 (CH_{Ar}), 126.1 (CH_{Ar}), 125.5 (C_{Ar}), 50.4 (C-14), 47.9 (C-13), 44.3 (C-9), 37.8 (C-8), 36.8 (2C, $N(CH_3)_2$), 35.8 (C-16), 31.5 (CH_2), 29.2 (CH_2),

26.3 (CH_2), 25.5 (CH_2), 21.5 (CH_2), 13.8 (CH_3, C-18).



3.77

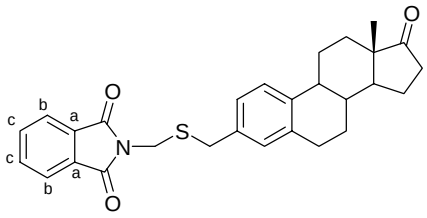
Estra-1,3,5(10)-trien-17-one-3-sulfonyl chloride (3.77).[2] To a solution of **3.76** (1.00 g, 3.70 mmol) in acetic acid (50 mL) was added H_2O (10 mL) and cooled to 0 ^oC. Chlorine gas was bubbled through the solution until a white precipitate formed. Bubbling was continued for an additional 2 min. The reaction was purged with nitrogen, diluted with ice-cold H_2O (50 mL) and extracted with ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated keeping the rotary evaporator bath at 35 ^oC. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography (ethyl acetate/hexane, 1:3 to 1:2) to give chloride **3.77** as a white solid (760 mg, 77%). ^1H NMR was identical to that reported in literature.[2] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.80-7.70 (s and d overlapping, 2H), 7.50 (d, J = 7.5 Hz, 1H, H-1), 3.10-2.90 (m, 2H), 2.60-2.35 (m, 3H), 2.25-1.95 (m, 4H), 1.75-1.45 (m, 6H), 0.91 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.0 (C=O), 148.4 (C_{Ar}), 141.6(C_{Ar}), 138.9(C_{Ar}), 127.1(CH_{Ar}), 126.8(CH_{Ar}), 124.1 (CH_{Ar}), 50.4(C-14), 47.7(C-13), 44.7(C-9), 37.4(C-8), 35.7 (CH_2), 31.4 (CH_2), 29.3 (CH_2), 25.8 (CH_2), 25.5 (CH_2), 21.5 (CH_2), 13.8 (CH_3, C-18).



3.78

***N*-Mecaptomethyl Phthalimide (3.78).** This was prepared according to the procedure of

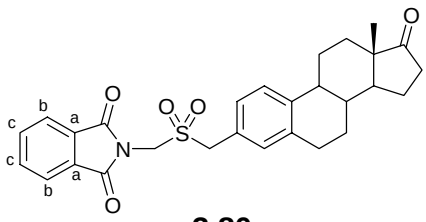
Forsch et al.[46] To a solution of thioacetic acid (2.30 mL, 32.0 mmol) and Et_3N (4.5 mL, 32 mmol, 1 equiv) in THF (100 mL) at 0 ^oC was added a solution of *N*-bromomethyl phthalimide (7.7 g, 32 mmol) in THF (90 mL) dropwise over 1 h. The resulting mixture was left in a fridge for 2 days. After removal of solvent, it was partitioned between ethyl acetate (150 mL) and H_2O (100 mL). The organic layer was separated and washed with 1% citric acid and brine then dried (Na_2SO_4) and concentrated. To the residue was added MeOH (240 mL) and then conc. HCl (90 mL) was added dropwise at 0 ^oC over 1.5 h. After stirring 17 h at rt, the precipitate was collected by suction filtration and washed with H_2O to give **3.78** as a crystalline white solid (4.64 g, 75% over 2 steps). ^1H NMR was identical to that reported in literature.[46] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.86 (dd, J = 5.1 Hz, J = 3.3 Hz, 2H), 7.72 (dd, J = 5.1 Hz, J = 3.0 Hz, 2H), 4.74 (d, J = 9.1 Hz, 2H, NCH_2S), 2.63 (t, J = 9.1 Hz, 1H, SH).



3.79

3-(Phthalimidyl methylthiomethyl) estra-1,3,5(10)-trien-17-one (3.79). To a solution of 3-bromomethyl estrone **3.33** (1.74 g, 5.00 mmol) and thiol **3.78** (1.00 g, 5.08 mmol, 1.01 equiv) in DMF (100 mL) was added potassium carbonate (1.38 g, 10.0 mmol, 2 equiv). The reaction mixture was stirred 4.5 h at rt. The reaction was concentrated and H_2O was added and the mixture was extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane,

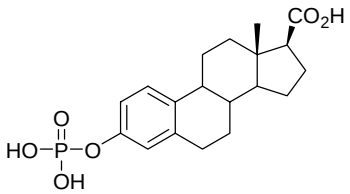
1:2 to 1:1) to give sulfide **3.79** as a white foam (2.07 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80-7.55 (m, 4H, H-b and H-c), 7.10-6.90 (m, 3H, H-1, H-2 and H-4), 4.63 (s, 2H, NCH_2S), 4.00 (s, 2H, $ArCH_2S$), 2.85-2.65 (m, 2H), 2.50-1.85 (m, 7H), 1.70-1.30 (m, 6H), 0.82 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.5 (C=O), 167.4 (2C, $(CO)_2N$), 138.4 (C-6), 136.5 (C-5), 135.2 (C-3), 134.0 (2CH, C-c), 131.9 (2C, C-a), 129.4 (C-4), 126.4 (C-1), 125.4 (C-2), 123.3 (2CH, C-b), 50.4 (C-14), 47.9 (C-13), 44.2 (C-9), 38.9 (NCH_2S), 38.0 (C-8), 36.2 ($Ar\underline{C}H_2S$), 35.8 (C-16), 31.6 (CH_2), 29.2 (CH_2), 26.4 (CH_2), 25.6 (CH_2), 21.6 (CH_2), 13.8 (CH_3, H-18); LRMS (EI) *m/z* (%) 459 (M^+, 9), 299 (100), 267 (10), 160 (21); HRMS (EI) calcd for $C_{28}H_{29}NO_3$ 459.1868; found 459.1876.



3.80

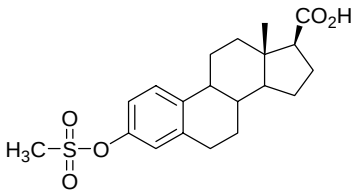
3-(Phthalimidy methylsulfonylmethyl) estra-1,3,5(10)-trien-17-one (3.80). To a solution of sulfide **3.79** (1.52 g, 3.31 mmol) in acetic acid (15 mL) was added $KMnO_4$ (grounded fine powder, 628 mg, 3.98 mmol, 1.2 equiv) slowly over 1 min (exothermic). The reaction mixture was stirred for 7 h before quenching with H_2O (150 mL) and extracting with ethyl acetate/Et_2O (1:1) then Et_2O. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated The residue was subjected to flash chromatography (ethyl acetate/hexane, 1:1 to 2:1) which gave pure sulfone **3.80** as a white solid (1.10 g, 68%). Mp: 207-209 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92-7.86 (m, 2H, H-b), 7.80-7.75 (m, 2H, H-c), 7.33-7.25 (m, 3H, H-1, H-2 and H-4), 4.86 (s, 2H, NCH_2SO_2), 4.29 (s, 2H, $ArC\underline{H}_2SO_2$), 2.93-2.87 (m, 2H), 2.51-1.90 (m, 7H), 1.67-1.33 (m, 6H), 0.87 (s,

3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.8 (C=O), 166.6 (2C, $(CO)_2N$), 141.1, 137.4, 135.0 (2C, C-c), 131.8, 131.5 (2C, C-a), 128.5, 126.1, 124.3, 124.3 (2C, C-b), 60.4 (NCH_2SO_2), 54.2 ($ArCH_2S$), 50.5 (C-14), 48.0 (C-13), 44.4 (C-9), 38.0 (C-8), 35.9 (C-16), 31.6 (CH_2), 29.3 (CH_2), 26.4 (CH_2), 25.7 (CH_2), 21.7 (CH_2), 13.9 (CH_3, C-18); LRMS (EI) *m/z* (%) 427 (M-SO_2, 40), 280 (100), 267 (78), 160 (76), 105 (21).



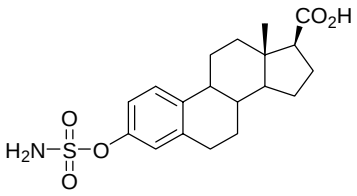
3.81

3-(Phosphonooxy)estra-1,3,5(10)-trien-17*β*-carboxylic acid (3.81). To a solution of **3.84** (324 mg, 0.5 mmol) in methanol was added Pd black (30 mg). It was then hydrogenated over 50 psi of H_2 for 18 h. After filtration and concentration of the filtrate, the residue was treated with ethyl acetate/hexane (1:5) to remove less polar impurities and acid **3.81** was obtained as a white solid (180 mg, 94 %). Mp: 211-213 ^oC; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.16 (d, J = 7.7 Hz, 1H, H-1), 6.83 (d, 1H, H-2, overlapping with s at 6.81), 6.81 (s, 1H, H-4), 0.61 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 175.2 (C=O), 149.8 (d, J = 6.5 Hz, C-3), 137.9 (C-5), 135.8 (C-10), 126.6 (C-1), 120.2 (d, J = 4.5 Hz, C-4), 117.8 (d, J = 4.5 Hz, C-2), 55.1 (C-17), 54.7 (C-14), 43.8 (C-13), 43.8 (C-9), 38.8 (C-8), 38.3 (C-12), 29.5 (CH_2), 27.6 (CH_2), 26.5 (CH_2), 24.2 (CH_2), 23.7 (CH_2), 13.6 (CH_3, C-18); ^{31}P NMR (DMSO-d_6, 121 MHz) δ -4.7; LRMS (ESI) *m/z* (%) 380 (M^+, 12), 379 (M-1, 50), 299 (100); HRMS (ESI) calcd for $C_{19}H_{24}O_6P$ (M-1) 379.1311; found 379.1321.



3.82

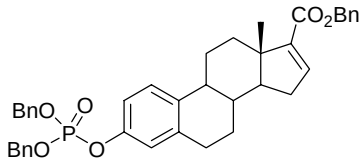
3-[(Methanesulfonyl)oxy]estra-1,3,5(10)-trien-17*β*-carboxylic acid (3.82). To a solution of mesylate **3.85** (520 mg, 1.12 mmol) in methanol (150 mL) was added Pd black (50 mg). It was then hydrogenated over 50 psi of H_2 for 20 h. The reaction was filtered and the filtrate was concentrated. The residue was subjected to flash chromatography (ethyl acetate/hexane, 1:5 to 1:1) to give pure carboxylic acid **3.82** as a white solid (332 mg, 77%). Mp: 183-185 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.92 (s, CO_2H), 7.32 (d, J = 8.5 Hz, 1H, H-1), 7.03 (d, J = 8.6 Hz, 1H, H-2), 6.99 (s, 1H, H-4), 3.29 (s, 3H, CH_3SO_2, overlapping with H_2O peak), 2.80 (s, 2H), 2.40-1.65 (m, 8H), 1.55-1.10 (m, 6H), 0.62 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 175.1 (C=O), 147.4 (C-3), 139.6 (C-5), 139.0 (C-10), 127.3 (C-1), 122.3 (C-4), 119.6 (C-2), 55.1 (C-17), 54.7 (C-14), 43.8 (C-9), 43.8 (C-13), 38.6 (C-8), 38.3 (C-12), 37.7 (CH_3SO_2), 29.4 (CH_2), 27.3 (CH_2), 26.3 (CH_2), 24.2 (CH_2), 23.7 (CH_2), 13.6 (CH_3, C-18); LRMS (ESI) *m/z* (%) 377 (M-1, 100); HRMS (ESI) calcd for $C_{20}H_{25}O_5S$ 377.1428; found 377.1418.



3.83

3-[(Aminosulfonyl)oxy]estra-1,3,5(10)-trien-17*β*-carboxylic acid (3.83). To a solution of **3.86** (430 mg, 0.921 mmol) in methanol (50 mL) was added Pd black (30 mg). The mixture was hydrogenated over 50 psi of H_2 for 18 h. The reaction was filtered and the filtrate concentrated.

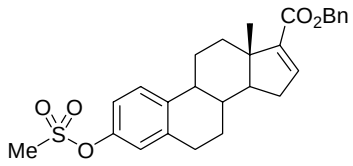
The residue was subjected to flash chromatography (acetone/hexane, 1:2.5 to 1:1) to give pure sulfamate **3.83** as a white solid (323 mg, 93%). Mp: > 250 ^{o}C (dec.); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.92 (s, CO_2H), 7.84 (s, 2H, NH_2), 7.30 (d, J = 8.5 Hz, 1H, H-1), 6.98 (d, J = 8.5 Hz, 1H, H-2), 6.93 (s, 1H, H-4), 2.80 (s, 2H), 2.35-1.73 (m, 8H), 1.55-1.15 (m, 6H), 0.62 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 175.1 (C=O), 148.4 (C-3), 138.7 (C-5), 138.4 (C-10), 127.0 (C-1), 122.3 (C-4), 119.7 (C-2), 55.1 (C-17), 54.7 (C-14), 43.9 (C-9), 43.8 (C-13), 38.6 (C-8), 38.3 (C-12), 29.5 (CH_2), 27.4 (CH_2), 26.4 (CH_2), 24.2 (CH_2), 23.7 (CH_2), 13.6 (CH_3, C-18); LRMS (ESI) *m/z* (%) 378 (M-1, 100); HRMS (ESI) calcd for $C_{19}H_{24}NO_5S$ 378.1380; found 378.1390.



3.84

3-[(Dibenzylphosphoryl)oxy]estra-1,3,5(10),16-tetraen-17-carboxylic acid benzylester (3.84). To a solution of **3.89** (610 mg, 1.57 mmol) and dibenzyl *N,N*-diisopropyl phosphoramidite (0.55 mL, 1.64 mmol, 1.05 equiv) in THF was added 1*H*-tetrazole (346 mg, 4.94 mmol, 3.15 equiv) in one portion. After stirring for 20 min at rt, it was cooled to -40 ^{o}C (acetonitrile in dry ice) and a solution of *m*CPBA (367 mg, 2.13 mmol, 1.35 equiv) in CH_2Cl_2 (5 mL) was added slowly over 20 min. It was stirred for 5 min at that temperature and then warmed to rt and stirred for 20 min. Ether (150 mL) and 10% aq. $Na_2S_2O_5$ (10 mL) were added. After separation, the organic phase was washed with 10% aq $Na_2S_2O_5$, 5% $NaHCO_3$, 0.5 M HCl, H_2O, and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3) gave compound **3.84** as a white solid (608 mg, 60%). Mp: 93-94 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.25 (m, 15H), 7.18

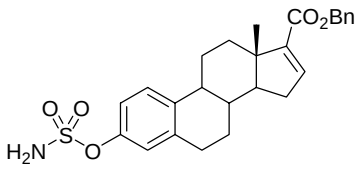
(d, J = 8.5 Hz, 1H, H-1), 6.92 (d, J = 8.3 Hz, 1H, H-2), 6.86 (s, 2H, H-4 overlapping with =C<u>H</u>CH_2), 5.20 (s, 2H, PhC<u>H_2</u>OC), 5.12 (d, J = 8.2 Hz, 4H, PhC<u>H_2</u>OP), 2.85-2.70 (m, 2H), 2.45-1.40 (m, 11H), 0.97 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.7 (C=O), 148.4 (d, J = 7.1 Hz, C-3), 146.8 (<u>C</u>=CH, C-17), 144.0 (C=<u>C</u>H, C-16), 138.3 (2C, P(OCH_2<u>C_6H_5</u>)$_2$), 137.3 (C-5), 136.4 (C, CO_2CH_2<u>C_6H_5</u>), 135.6 (d, J = 6.8 Hz, C-10), 128.6 (6CH, 3Ph), 128.0 (3CH, 3Ph), 128.0 (6CH, 3Ph), 126.3 (C-1), 120.0 (d, J = 4.6 Hz, C-4), 117.2 (d, J = 4.7 Hz, C-2), 69.8 (d, J = 5.8 Hz, 2CH_2, P(O<u>C</u>$H_2C_6H_5$)$_2$)), 65.7 (CO_2<u>C</u>$H_2C_6H_5$), 55.8 (C-14), 46.2 (C-13), 44.3 (C-9), 36.8 (C-8), 34.8 (CH_2), 31.7 (CH_2), 29.3 (CH_2), 27.5 (CH_2), 26.3 (CH_2), 16.2 (CH$_3$, C-18); ^{31}P NMR($CDCl_3$, 121 MHz) δ -4.7; LRMS (EI) m/z (%) 648 (M^+, 71), 557 (45), 91 (100); HRMS (EI) calcd for $C_{40}H_{41}O_6P$ 648.2641; found 648.2645.



3.85

3-[(Methanesulfonyl)oxy]estra-1,3,5(10),16-tetraen-17-carboxylic acid benzylester (3.85). To a solution of phenol **3.89** (500 mg, 1.29 mmol) in pyridine (6 mL) at 0 ^{o}C was added methanesulfonyl chloride (0.30 mL, 3.9 mmol, 3 equiv) via syringe over 5 min. The reaction was stirred for 24 h (during which the ice was allowed to melt). The brown mixture was poured onto ice water and extracted with ethyl acetate. The combined extracts were washed with water, 10% citric acid and water, then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3) gave mesylate **3.85** as a white foam (522 mg, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.20 (m, 6H, C_6H_5 and H-1), 7.10-6.95 (d, s overlapping, 2H, H-2

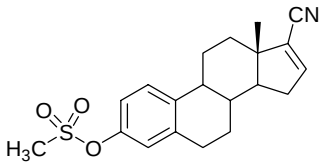
and H-4), 6.86 (s, 1H, C=CH, H-16), 5.20 (s, 2H, OC<u>H</u>$_2$C$_6$H$_5$), 3.10 (s, 3H, CH$_3$SO$_3$), 2.95-2.80 (m, 2H), 2.50-1.35 (m, 13H), 0.96 (s, 3H, CH$_3$, H-18); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 164.5 (C=O), 147.2 (C-3), 146.7 (<u>C</u>=CH, C-17), 144.0 (C=<u>C</u>H, C-16), 139.8 (C-5), 138.9 (C-10), 136.4 (C, CH$_2$<u>C</u>$_6$H$_5$), 128.6 (2CH, CH$_2$<u>C</u>$_6$H$_5$), 128.1 (CH, CH$_2$<u>C</u>$_6$H$_5$), 128.0 (2CH, CH$_2$<u>C</u>$_6$H$_5$), 126.7 (C-1), 121.9 (C-4), 118.9 (C-2), 65.7 (O<u>C</u>H$_2$C$_6$H$_5$), 55.8 (C-14), 46.1 (C-13), 44.4 (C-9), 37.3 (<u>C</u>H$_3$SO$_3$), 36.6 (C-8), 34.8 (CH$_2$), 31.7 (CH$_2$), 29.3 (CH$_2$), 27.3 (CH$_2$), 26.3 (CH$_2$), 16.2 (CH$_3$, C-18); LRMS (EI) *m/z* (%) 466 (M$^+$, 100), 451 (20), 375 (50), 331 (35), 235 (28), 91 (100); HRMS (EI) calcd for C$_{27}$H$_{30}$O$_5$S 466.1814; found 466.1815.



3.86

3-[(Aminosulfonyl)oxy]estra-1,3,5(10),16-tetraen-17-carboxylic acid benzylester (3.86). To a solution of **3.89** (500 mg, 1.29 mmol) in DMF (10 mL) was at 0 ^{o}C was added sulfamoyl chloride (750 mg, 6.49 mmol, 5 equiv). The mixture was stirred for 24 h at room temperature. The reaction was cooled to 0 ^{o}C and 10 mL of water was added and the reaction mixture was stirred for 30 min at 0 ^{o}C. More water was added and the mixture extracted with ethyl acetate. The combined extracts were washed with water and brine then dried (Na$_2$SO$_4$) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:3 to 1:2) to give sulfamate **3.86** as a white foam (493 mg, 82%). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.45-7.28 (m, 5H, C$_6$H$_5$), 7.26 (d, J = 8.7 Hz, 1H, H-1), 7.06 (d, J = 8.6 Hz, 1H, H-2), 7.02 (s, 1H, H-4), 6.86 (s, 1H, C=C<u>H</u>, H-16), 5.25 (s, 2H, NH$_2$), 5.18 (s, 2H, OC<u>H</u>$_2$C$_6$H$_5$), 2.95-2.80 (m, 2H), 2.45-1.98 (m, 6H), 1.95-1.80 (m, 1H), 1.70-1.25 (m, 6H), 0.93 (s,

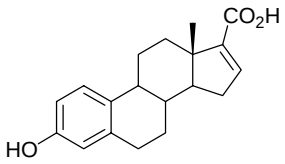
3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.9 (C=O), 147.9 (C-3), 146.6 (C=CH, C-17), 144.3(C=CH, C-16), 139.7 (C-5), 138.7 (C-10), 136.2 (C, $CH_2C_6H_5$), 128.6 (2CH, $CH_2C_6H_5$), 128.1 (CH, $CH_2C_6H_5$), 128.0 (2CH, $CH_2C_6H_5$), 126.5 (C-1), 122.0 (C-4), 119.0 (C-2), 65.8 ($OCH_2C_6H_5$), 55.8 (C-14), 46.1 (C-13), 44.4 (C-9), 36.6 (C-8), 34.8 (CH_2), 31.7 (CH_2), 29.3 (CH_2), 27.3 (CH_2), 26.3 (CH_2), 16.2 (CH_3, C-18); LRMS (EI) *m/z* (%) 467 (M^+, 21), 452 (10), 388 (59), 376 (20), 297 (27), 159 (30), 91 (100); HRMS (EI) calcd for $C_{26}H_{29}NO_5S$ 467.1766; found 467.1764.



3.87

17-Cyano-3-hydroxyestra-1,3,5(10),16-tetraen-3-methanesulfonate (3.87). This was prepared according to the procedure of Baldwin et al.[45,40b] To a mixture of estrone mesylate **3.20** (1.75 g, 5.00 mmol) and ZnI_2 (60 mg, 0.19 mmol, 4 mol%) in CH_2Cl_2 (12 mL) was added TMSCN (1.2 mL, 9.0 mmol, 1.8 equiv) via syringe over 15 min. After addition, the resulting mixture was refluxed 35 min and then cooled to rt and stirred 2.5 h. After adding conc. HCl (3 mL) to the reaction mixture, it was refluxed for 30 min. CH_2Cl_2 (6 mL) and H_2O (3 mL) were added and the mix was refluxed 10 min and cooled to rt. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 and the combined extracts were dried (Na_2SO_4) and concentrated and the residue dried over high vacuum for several hours. The resulting foam was dissolved in pyridine (9 mL) and $POCl_3$ (3 mL) was added dropwise. The reaction mixture was refluxed overnight. After cooling, it was poured slowly onto ice-cold 6N HCl (30 mL). The mixture was extracted with ethyl acetate and the combined extracts were washed with water and brine then dried (Na_2SO_4) and concentrated.

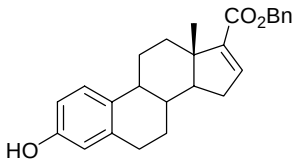
Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:3) gave compound **3.87** as a white solid (1.38 g, 77%). ^{1}H NMR was identical to that reported in literature.[45] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (d, J = 8.5 Hz, 1H, H-1), 7.02 (d, J = 8.7 Hz, 1H, H-2), 7.00 (s, 1H, H-4), 6.65 (s, 1H, C=C<u>H</u>, H-16), 3.11 (s, 3H, CH_3SO_3), 3.00-2.85 (m, 2H, H-6), 2.50-1.92 (m, 6H), 1.85-1.30 (m, 5H), 0.94 (s, 3H, CH_3, H-18).



3.88

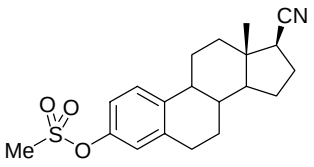
3-Hydroxyestra-1,3,5(10),16-tetraen-17-carboxylic acid (3.88). A suspension of **3.87** (1.00 g, 2.80 mmol) and NaOH (3.50 g, 87.5 mmol, 31 equiv) in ethylene glycol (20 mL) was refluxed for 6h. The mixture was cooled to rt and diluted and 75 mL of water and 35 mL of ether were added. The organic phase was discarded and the aq. phase was acidified with conc. HCl to a pH of about 1.5 and extracted with ethyl acetate. The combined organic extracts were washed with brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:1) gave carboxylic acid **3.88** as a white solid 702 mg (84%). Mp: 251-253 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.99 (brs, 1H, CO_2H), 8.97 (brs, 1H, ArO<u>H</u>), 6.98 (d, J = 8.2 Hz, 1H, H-1), 6.64 (s, 1H, =C<u>H</u>CH_2, H-16), 6.47 (d, J = 7.8 Hz, 1H, H-2), 6.41 (s, 1H, H-4), 2.80-2.60 (m, 2H), 2.45-1.20 (m, 11H), 0.83 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 166.1 (C=O), 155.4 (C-3), 147.7 (C-17), 143.1 (C-16), 137.5 (C-5), 130.9 (C-10), 126.2 (C-1), 115.4 (C-4), 113.2 (C-2), 55.8 (C-14), 45.9 (C-9), 44.1 (C-13), 37.3 (C-8), 35.0 (CH_2), 31.5 (CH_2), 29.4 (CH_2), 27.7 (CH_2), 26.6 (CH_2), 16.4 (CH_3, C-18); LRMS (EI) *m/z* (%) 298 (M^+, 100), 283 (14), 272 (20), 253 (10), 159 (31),

146 (23); HRMS (EI) calcd for $C_{19}H_{22}O_3$ 298.1569; found 298.1563.



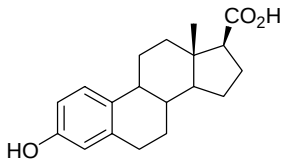
3.89

3-Hydroxyestra-1,3,5(10),16-tetraen-17-carboxylic acid benzylester (3.89). A mixture of acid **3.88** (2.96 g, 9.93 mmol) and Bu_4NOH (40% wt., 6.50 g, 10.0 mmol, 1 equiv) in H_20 (2 mL) was heated at 70 ^oC for 5h. The water was removed by rotary evaporation (water bath at 60 ^oC) and the residue was dried under high vacuum. The residue was dissolved in dry DMF (45 mL) and benzyl bromide (1.30 mL, 11.0 mmol, 1.1 equiv) was added dropwise. The resulting mixture was stirred for 20 h at rt. The reaction was diluted with H_2O and extracted with ethyl acetate. The combined organics were washed with water and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:5 to 1:3) gave benzyl ester **3.89** as a light yellow solid (3.38 g, 88%). Mp: 116-118 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.49-7.29 (m, 5H, $CH_2C_6\underline{H}_5$), 7.14 (d, J = 8.3 Hz, 1H, H-1), 6.91 (s, 1H, =C$\underline{H}CH_2$, H-16), 6..66 (d, J = 8.2 Hz, 1H, H-2), 6.60 (s, 1H, H-4), 5.66 (s, 1H, ArO$\underline{H}$), 5.23 (s, 2H, O$\underline{C}H_2$Ph), 3.00-2.85 (m, 2H), 2.50-1.35 (m, 11H), 0.98 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.3 (C=O), 153.6 (C-3), 146.8 ($\underline{C}$=CH, C-17), 144.7 (C=$\underline{C}$H, C-16), 138.0 (C-5), 136.2 (C, $CO_2\underline{C}_6H_5$), 132.6 (C-10), 128.6 (2CH, $CH_2\underline{C}_6H_5$), 128.1 (CH, $CH_2\underline{C}_6H_5$), 128.1 (2CH, $CH_2\underline{C}_6H_5$), 126.2 (C-1), 115.4 (C-4), 112.8 (C-2), 65.9 (O$\underline{C}H_2$Ph), 55.8 (C-14), 46.2 (C-13), 44.2 (C-9), 37.1 (C-8), 34.9 (CH_2), 31.8 (CH_2), 29.5 (CH_2), 27.7 (CH_2), 26.5 (CH_2), 16.2 (CH_3, C-18); LRMS (EI) *m/z* (%) 388 (M^+, 100), 373 (19), 297 (35), 281 (15), 159 (33), 91 (91); HRMS (EI) calcd for $C_{26}H_{28}O_3$ 388.2038; found 388.2039.



3.90

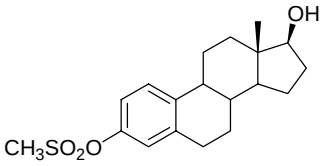
17*β*-Cyano-3-hydroxyestra-1,3,5(10)-trien-3-methanesulfonate (3.90). This was prepared according to the procedure of McGuire et al.[40b] using different solvent. A solution of **3.87** (320 mg, 0.896 mmol) in ethyl acetate (20 ml) and acetic acid (1.4 mL) was charged with 10% Pd/C (50 mg). The resulting mixture was hydrogenated under a balloon pressure of H_2 for 2 h. After filtration and concentration of the filtrate compound **3.90** was obtained as white crystals (321 mg, 100%). ^{1}H NMR was identical to that reported in literature.[45] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (d, J = 8.5 Hz, 1H, H-1), 7.02 (d, J = 8.6 Hz, 1H, H-2), 6.98 (s, 1H, H-4), 3.10 (s, 3H, CH_3SO_3), 2.90-2.80 (m, 2H), 2.40-1.82 (m, 8H), 1.60-1.16 (m, 6H), 0.84 (s, 3H, CH_3, H-18).



3.91

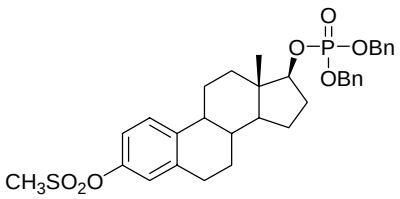
3-Hydroxyestra-1,3,5(10)-trien-17*β*-carboxylic acid (3.91). To a solution of **3.88** (100 mg, 0.336 mmol) in MeOH (20 mL) was added Pd black (30 mg) and the mixture was subject to 46 psi H_2 for 40 h. The reaction was filtered and the filtrate concentrated to give acid **3.91** as a white solid (95 mg, 95%). Mp: 281-282 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.70 (brs, 1H, $CO_2\underline{H}$), 8.95 (brs, 1H, Ar$O\underline{H}$), 6.99 (d, J = 8.1 Hz, 1H, H-1), 6.46 (d, J = 8.0 Hz, 1H, H-2), 6.39 (s, 1H, H-4), 2.75-2.55 (m, 2H), 2.40-1.15 (m, 14H), 0.60 (s, 3H, CH_3, H-18); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 175.2 (C=O), 155.4 (C-3), 137.5 (C-5), 130.7 (C-10), 126.4 (C-1), 115.4 (C-4), 113.2 (C-2), 55.2 (C-17), 54.7

(C-13), 43.9 (C-13), 43.7 (C-9), 39.1 (C-8), 38.4 (C-12), 29.6 (CH_2), 27.8 (CH_2), 26.6 (CH_2), 24.2 (CH_2), 23.7 (CH_2), 13.7 (CH_3, C-18); LRMS (EI) *m/z* (%) 300 (M^+, 100), 272 (5), 213 (10), 185 (24), 159 (17); HRMS (EI) calcd for $C_{19}H_{24}O_3$ 300.1725; found 300.1721.



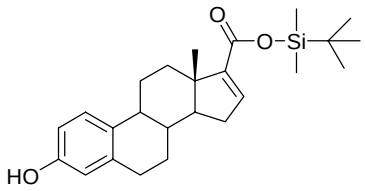
3.93

17*β*-Hydroxy-3-[(Methanesulfonyl)oxy]estra-1,3,5(10)-triene (3.93). To a solution of mesylate **3.20** (2.50 g, 7.18 mmol) in ethanol (120 mL) at 0 ^oC was added $NaBH_4$ (1.0 g, 26 mmol, 3.7 equiv). The reaction was stirred overnight at rt. EtOH was removed by rotary evaporation and the residue with treated with 2N HCl (60 ml) at 0 ^oC. After extraction with ethyl acetate, the combined extracts were washed with H_2O then dried (Na_2SO_4) and concentrated. Short column flash chromatography (acetate/hexane, 1:2) gave **3.93** as a white solid (2.5 g, 100%). Mp: 83-84 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.28 (d, J = 8.4 Hz, 1H, H-1), 7.00 (d, J = 8.4 Hz, 1H, H-2), 6.96 (s, 1H, H-4), 3.70 (t, J = 8.3 Hz, 1H, H-17), 3.09 (s, 3H, CH_3SO_2), 2.92-2.80 (m, 2H, H-6), 2.30-1.15 (m, 13H), 0.75 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.0 (C-3), 139.8 (C-5), 139.1 (C-10), 126.9 (C-1), 121.8 (C-4), 118.8 (C-2), 81.7 (C-17), 50.0 (C-14), 44.1 (C-9), 43.1 (C-13), 38.3 (C-8), 37.2 (CH_3SO_2), 36.6 (CH_2), 30.5 (CH_2), 29.5 (CH_2), 26.8 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 11.0 (CH_3, C-18); LRMS (EI) *m/z* (%) 350 (M^+, 100), 306 (5), 291 (52), 253 (13), 238 (21), 159 (13); HRMS (EI) calcd for $C_{19}H_{26}O_4S$ 350.1552; found 350.1557.



3.94

17*β*-(Dibenzylphosporyl)oxy-3-[(methanesulfonyl)oxy]estra-1,3,5(10)-triene (3.94). To a solution of tribenzyl phosphite (865 mg, 2.45 mmol, 4.3 equiv) in CH_2Cl_2 (10 mL) at 0 ^oC was added iodine (600 mg, 2.36 mmol, 4.1 equiv) in one portion. The mixture was stirred for 8 min at 0 ^oC before warming up to rt. The reaction turned brown. To this solution was added a solution of **3.93** (200 mg, 0.570 mmol) and pyridine (0.30 mL, 3.7 mmol, 6.5 equiv) in CH_2Cl_2 (10 mL) at -30 ^oC slowly and the resulting mixture was stirred for 2 h at that temperature. Ether was added and the mixture was washed with 0.3 M $KHSO_4$, sat. $NaHCO_3$, and brine then dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (acetone/hexane, 1:2.5 to 1:2) to give **3.94** as a colorless oil (162 mg, 46%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.24 (m, 11H), 7.00 (d, J = 8.4 Hz, 1H, H-2), 6.96 (s, 1H, H-4), 5.02 (t, J = 5.70 Hz, 4H, OC$\underline{H_2}$Ph x 2), 3.05 (s, 3H, CH_3SO_2), 2.88-2.76 (m or pseudo s, 2H, H-6), 2.25-1.80 (m, 7H), 1.58-1.10 (m, 6H), 0.77 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.1 (C-3), 139.5 (C-5), 138.9 (C-10), 136.0 (d, 2C, J = 7.0 Hz), 128.5 (4CH), 128.4 (2CH), 127.8 (4CH), 126.9 (C-1), 121.9 (C-4), 118.9 (C-2), 86.7 (d, J = 6.6 Hz, C-17), 69.1 (d, J = 2.0 Hz, OCH_2), 69.1 (d, J = 2.0 Hz, OCH_2), 49.1 (C-14), 43.8 (C-(), 43.3 (d, J = 5.7 Hz, C-13), 38.1 (C-8), 37.2 (CH_3SO_2), 36.3 (CH_2), 29.4 (CH_2), 28.4 (d, J = 2.2 Hz, C-16), 26.7 (CH_2), 25.9 (CH_2), 23.0 (CH_2), 11.5 (CH_3, C-18); ^{31}P NMR ($CDCl_3$, 121 MHz) δ -1.1; LRMS (EI) *m/z* (%) 610 (M^+, 10), 519 (32), 421 (19), 331 (70), 187 (72), 91 (100); HRMS (EI) calcd for $C_{33}H_{39}O_7PS$ 610.2154; found 610.2147.



3.98

3-Hydroxyestra-1,3,5(10),16-tetraen-17-carboxylic acid *tert*-buty(dimethyl) silylester (3.98). To a mixture of **3.88** (136 mg, 0.456 mmol) and triethylamine (53 mg, 0.53 mmol, 1.2 equiv) was added THF (4 mL). The resulting mixture was stirred for 5 min before TBDMSCl (76 mg, 0.661 mmol, 1.45 equiv) was added. After stirring 1 h, the reaction was quenched with H_2O then extracted with EtOAc. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:2) gave silyl ester **3.98** as a white solid (110 mg, 59%). Mp: 204-206 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.13 (d, 1H, J = 8.4 Hz, H-1), 6.79 (s, 1H, C=CH, H-16), 6.61 (d, J = 8.4 Hz, 1H, H-2), 6.56 (s, 1H, H-4), 5.10 (brs, 1H, ArO<u>H</u>), 2.90-2.80 (m, 2H, H-6), 2.35-1.30 (m, 11H), 0.95 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.92 (s, 3H, CH_3, H-18), 0.30 (s, 6H, Si(C<u>H</u>$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.1 (C=O), 153.4 (C-3), 148.5 (C-17), 144.6 (C-16), 138.0 (C-5), 132.7 (C-10), 126.2 (C-1), 115.2 (C-4), 112.6 (C-2), 55.8 (C-14), 45.8 (C-9), 44.2 (C-13), 37.1 (C-8), 34.8 (CH_2), 31.5 (CH_2), 29.4 (CH_2), 27.6 (CH_2), 26.4 (CH_2), 25.7 (3C, (<u>C</u>H$_3$)$_3$C), 17.8 (Si<u>C</u>(CH$_3$)$_3$), 16.0 (CH_3, C-18), -4.7 (2C, Si(CH$_3$)$_2$); LRMS (EI) *m/z* (%) 412 (M^+, 6), 397 (3), 356 (30), 355 (100), 298 (2); HRMS (EI) calcd for $C_{25}H_{36}O_3Si$ 412.2434; found 412.2425.

3.4 References

1. Nussbaumer, P.; Billich, A. *Med. Res. Rev.* **2004**, *24*, 529.

2. Li, P.-K.; Pillai, R.; Young, B. L.; Bender, W. H.; Martino, D. M.; Lin, F. T. *Steroids* **1993**, *58*,

106.

3. Dibbelt, L.; Li, P-K.; Pillai, R.; Knuppen, R. *J. Steroid Biochem. Mol. Biol.* **1994**, *50*, 261.

4. Selcer, K. W.; Li, P.-K.; *J. Steroid Biochem. Mol. Biol.* **1995**, *52*, 281

5. Woo, L. W. L.; Lightowler, M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Steroid Biochem. Mol. Biol.* **1996**, *57*, 79.

6. Selcer, K. W.; Jagannathan, S.; Rhodes, M. E.; Li, P.-K. *J. Steroid Biochem. Mol. Biol.* **1996**, *59*, 83.

7. Anderson, C.; Freeman, J.; Lucas, L. J. H.; Widlanski, T. S. *J. Am. Chem. Soc.*, **1995**, *117*, 3889-3890.

8. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem*. **1994**, *37*, 219.

9. Li, P-K.; Pillai, R.; Dibbelt, L. *Steroids* **1995**, 60, 299.

10. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Steroids* **1997**, 62, 346.

11. Sahm, U. G.; Williams, G. J.; Purohit, A.; Hidalgo-Afagones, M. I.; Parish, D.; Reed, M. J.; Potter, B. V. L.; Poulton, C. W. *Pharm. Sci.* **1996**, *2*, 17.

12. Chander, S. K.; Purohit, A.; Lawrence, L. W.; Woo, Potter, B. V. L.; Reed. M. J. *Biochem. Biophys. Res. Commun.* **2004**, *322*, 217.

13. Lawrence, L. W.; Woo, A.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. L. *Chem. Biol.* **2000**, *7*, 773.

14. Hernandez-Guzman, F. G.; Higashiyama, T.; Pangborn, W.; Osawa Y.; Ghosh, D. *J. Biol. Chem.* **2003**, *278*, 22989.

15. Chen M. J.; Taylor, S. D. *Tetrahedron Lett*. **1999**, *40*, 4149.

16. Lapierre, J.; Ahmed, V.; Chen, M-J.; Ispahany, M.; Guillemette, G.; Taylor, S. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 151.

17. Trepka, R. D.; Harrington, J. K.; Belisle, J. W. *J. Org. Chem.* **1974**, *39*, 1094.

18. Yang, W.; Gao, X.; Wang, B. *Med. Res. Rev*. **2003**, *23*, 346.

19. Schmidt, B.; Selmer, T.; Ingendoh, A.; von Figura, K. *Cell* 1995, *82*, 271-278.

20. von Figura, K.; Schmidt, B.; Selmer, T.; Dierks, T. *Bioassays* **1998**, *20*, 505.

21. Ghosh, D. *Meth. Enzymol.* **2005**, *400*, 273.

22. Stirling, C. J. M. in "The Chemistry of Sulfinic Acids, Esters and their Derivatives" S. Patai Ed, John Wiley and Sons Ltd., Chapter 1, pp 1-7 and references therein.

23. Hill, B.; Liu, Y.; Taylor, S. D. *Org. Lett.* **2004**, *6*, 4285.

24. Khanna, I. K.; Liu,Y. Y.; Huff, R. M.; Weier, R,. M.; Xu, X.; Koszyk, F. J.; Collins, P. W.; Cogburn, J. N.; Isakson, P. C.; Colboldt, C. M.; Massferrer, J. L.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Yuan, J.; Yang, D.-C.; Zhang, Y. Y. *J. Med. Chem.* **2000**, *43*, 3168.

25. Greene, T. W.; Wuts, P. G. "Protective Groups in Organic Synthesis" John Wiley and Sons, 1999, pp 574-575.

26. Garro-Helion, F.; Merzouk, A.; F. Guibe, F. *J. Org. Chem*. **1993**, *58*, 6109.

27. Osborne, A. G. *Tetrahedron,* **1981**, *37*, 2021.

28. Sanghvi, T. S.; Rao, A. S.; *Indian J. Chem. B*, **1980**, *19*, 952.

29. Murata, M.; Oyama, T.; Watanabe, S.; Masuda, Y. *J. Org. Chem.* **2000**, *65*, 164.

30. Decicco, C. P.; Song, Y.; Evans, D. A. *Org. Lett.* **2001**, *3*, 1029.

31. Yu, S.; Saenz, J.; Srirangam, J. K. *J. Org. Chem*. **2002**, *67*, 1699.

32. Ciobanu, L. C.; Boivin, R. P.; Luu-The, V.; Labrie, F.; Poirier, D. *J. Med. Chem.* **1999**, *42*, 2280.

33. Ciobanu, L. C.; Poirier, D. *J. Comb. Chem.* **2003**, *5*, 429.

34. Woo, L. L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. *Chem. Biol.,* **2000**, *7*, 773.

35. (a) Baudoin, O.; Guenard, D.; Gueritte, F. *J. Org. Chem.* **2000**, *65*, 9268. (b) Stadlwieser, J. F.; Dambaur, M. E. *Helv. Chim. Acta* **2006**, *89*, 936.

36. Nussbaumer, P.; Lehr, P.; Billich, A. *J. Med. Chem.* **2002**, *45*, 4310.

37. Lovely, C. J.; Bhat, A. S.; Coughenour, H. D.; Gilbert, N. E.; Brueggemeier, *J. Med. Chem.* **1997**, *40,* 3756.

38. Binisti, C.; Assogba, L.; Touboul, E.; Mounier, C.; Huet, J.; Ombetta, J.-E.; Dong, C. Z.; Redeuilh, C.; Heymans, F.; Godfroid, J. *J. Eur. J. Med. Chem.* **2001**, *36*, 809.

39. Uchino, M.; Suzuki, K.; Sekiya, M. *Chem. Pharm. Bull.* **1978**, *26*, 1837.

40. (a) Kuhl, A.; Kreiser, W. *Helv. Chim. Acta* **1998**, *81*, 2264. (b) McGuire, M. A.; Sorenson, E.; Owing, F. W.; Resnick, T. M.; Fox, M.; Baine, N. H. *J. Org. Chem.* **1994**, *45*, 6683.

41. Poirier, D.; Boivin, R. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1891.

42. Boivin, R. P.; Luu-The, V.; Lachance, R.; Labrie, F.; Poirier, D. *J. Med. Chem.,* **2000**, *43*, 4465.

43. Blackburn, G. M.; Turkmen, H. *Org. Biomol. Chem.* **2005**, *3*, 225.

44. Reggelin, M.; Doerr, S. *Synlett* **2004**, *6*, 1117.

45. Baldwin, J. E.; Barton, D. H. R.; Dainis, I.; Pereira, J. L. C. ***J.*** *Chem. Soc. (C)* **1968**, 2284.

46. Forsch, R. A.; Wright, J. E.; Rosowsky, A. *Hetercycles* **1999***, 51,* 1789.

Chapter 4

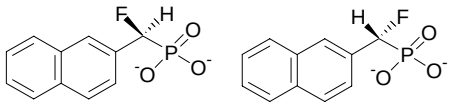
Towards the Synthesis of a Chiral Electrophilic Fluorinating Agent

4.1 Introduction and background

4.1.1 Enantioselective fluorination

As can be seen from the previous two chapters of this thesis, some of the compounds that we examined as STS inhibitors contained fluorine. Indeed, the Taylor group has been conducting research on the synthesis of organofluorines and their application as enzyme inhibitors for almost a decade. Of specific interest in the Taylor group is the synthesis of organofluorines by electrophilic fluorination (EF) and we have already shown how EF can be used to prepare inhibitors of STS (Chapter 3, section 2, compound **3.23**).

Due to the asymmetric nature of their binding/active sites, enzymes and other proteins are capable of discriminating between enantiomeric ligands. For example, the Taylor group has shown that the enzyme protein tyrosine phosphatase 1B (PTP1B) can distinguish between inhibitors **4.1** and **4.2** in that compound **4.1** is a 10-fold more potent inhibitor than its enantiomer **4.2**.[1] Compounds **4.1** and **4.2** (and similar compounds) were prepared by diastereoselective EF of diastereomeric phosphoramidate precursors which contained (-) ephedrine as a chiral auxiliary.[1] Although this was



4.1 **4.2**

Figure 4.1. Structures of chiral monofluoromethylenephosphonic acids **4.1** and **4.2**

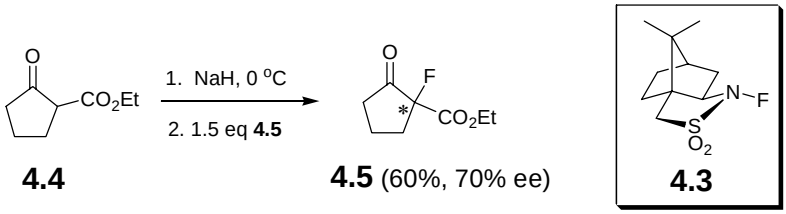
an effective route to this class of compounds, we would prefer a more direct approach and one that we could apply to the construction of other classes of chiral α-fluorinated compounds, such as chiral

α-monofluoromethylenesulfonic acids and α-monofluoromethylene- sulfonamides which we would like to examine as inhibitors of STS and other enzymes. One approach that is potentially more direct and general than diastereoselective EF is enantioselective EF. Recently, there has been considerable interest in developing methods for achieving enantioselective EF's.[2] This is not surprising considering that the occurrence of a fluorine substituent in commercial pharmaceutical compounds has increased from 2% in 1970 to estimates of more that 18% at present.[3]

In general, there are three tactics that have been employed for performing enantioselective EF's.[2] These involve the use of either 1) chiral *N*-fluoro reagents; 2) chiral transition-metal catalysts or 3) chiral organocatalysts.

4.1.2 Enantioselective fluorination using chiral electrophilic fluorinating agents.

Differding and Lang were the first to attempt this approach to chiral organofluorines.[4] In their pioneering study, camphor-derived *N*-fluoro sultam **4.3** was used to α-fluorinate a variety of enolates. For example, reaction of the sodium enolate of β-ketoester **4.4** with reagent **4.3** gave a 60% yield of **4.5** in 70% ee which represented the highest ee obtained in this study (Scheme 4.1).



4.4 **4.5** (60%, 70% ee) **4.3**

Scheme 4.1. Synthesis of **4.5** using Differding's chiral electrophilic fluorinating agent **4.3**

Later, Davis and coworkers prepared several derivatives of **4.3** (compounds of type **4.6**, Figure 4.2) and examined these as chiral electrophilic fluorinating agents using enolate **4.4** and enolates of similar carbonyl compounds as substrates. However, the ee's were not much better than

those obtained by Differding.[5] Takeuchi et al. later prepared a variety of chiral *N*-F compounds (**4.7-4.12**, Figure 4.2) and examined these as chiral electrophilic fluorinating agents using enolates of mainly cyclic ketones as substrates.[6] However, the ee's were generally quite poor.

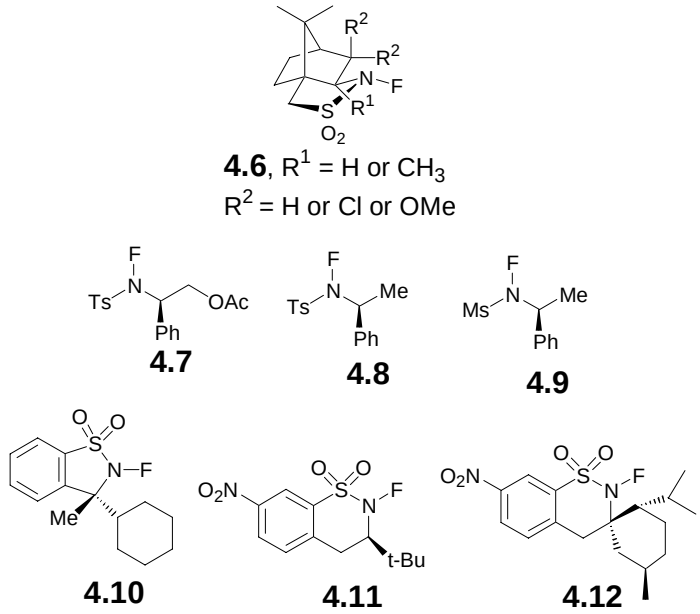
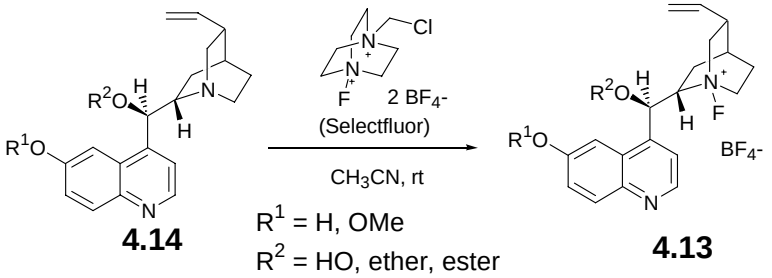


Figure 4.2. Chiral *N*-F reagents prepared by Davis et al and Takeuchi and coworkers

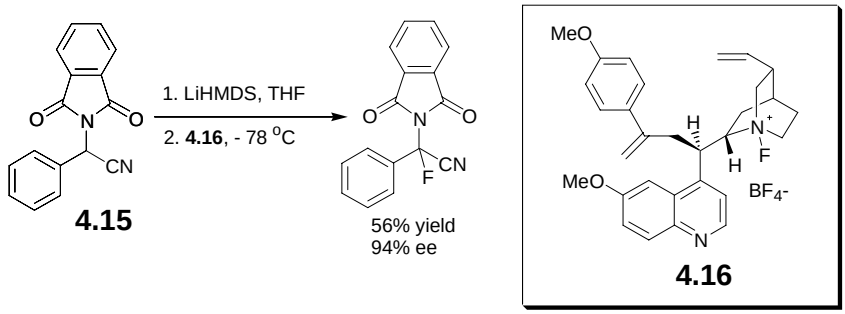
A major development in the area of asymmetric fluorination was the introduction of chiral *N*-fluoro ammonium salts of type **4.13** based on cinchona alkaloids (CA) as chiral electrophilic



Scheme 4.2. Synthesis of chiral Electrophilic fluorinating agents of type **4.13**

fluorinating agents.[7] These are readily prepared by reacting CA's of type **4.14** with Selectfluor, a commercially available electrophilic fluorinating agent (Scheme 4.2). These reagents were tested as chiral electrophilic fluorinating agents using mainly cyclic ketones, β-ketoesters, β-cyano esters and

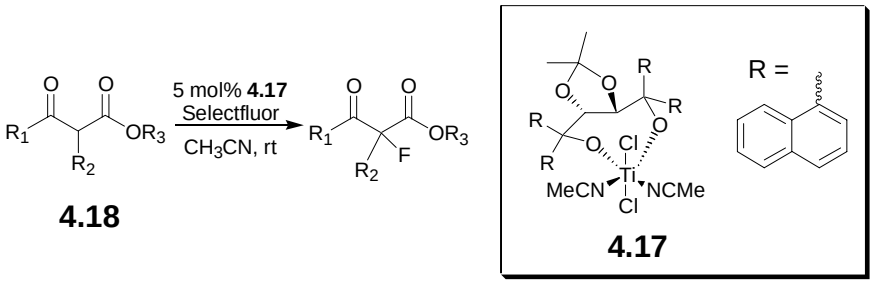
cyclic benzylic amides as substrates. The yield of fluorinated product was moderate to very good and the ee's were highly variable though in some instances, for example with compound **4.15** and reagent **4.16**, ee's up to 94% could be obtained (Scheme 4.3).



Scheme 4.3. Enantioselective EF of **4.15** using reagent **4.16**

4.1.3 Enantioselective fluorination using transition-metal catalysts

Table 4.1. Enantioselective EF of β-keto esters using Selectfluor and catalyst **4.17**



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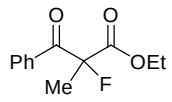
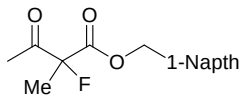
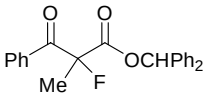
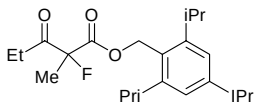
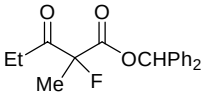
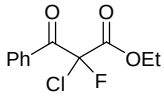
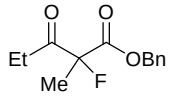
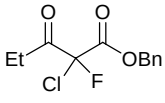
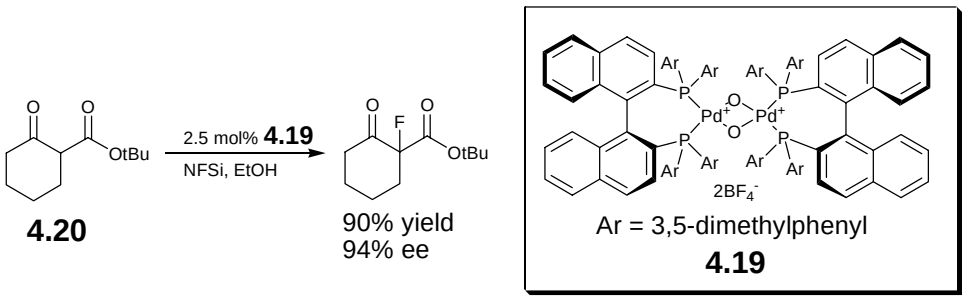
1		≥80	62	5		≥80	68
2		≥80	82	6		89	90
3		≥80	81	7		53	33
4		82	71	8		57	60

Enantioselective EF has been reported using chiral transition metal catalysts. So far this approach has been limited to the fluorination of readily enolizable substrates such as β-keto esters.

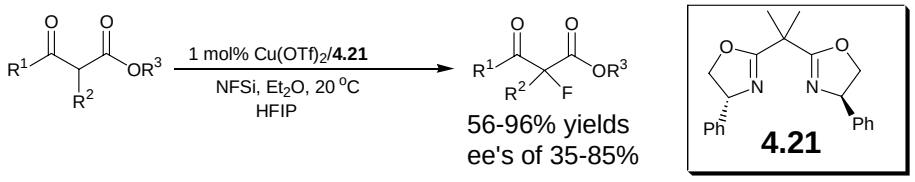
The first enantioselctive fluorination using transition metal catalysis was developed by Hintermann et al.[8] Using chiral titanium taddolate **4.17** and Selectfluor they achieved efficient fluorination of β-ketoesters of type **4.18**. The ee's were variable though in some instances ee's as great as 90% were obtained (Table 4.1).

Hamashima et al. developed dicationic palladium complexes, such as **4.19** (Scheme 4.4), for the efficient fluorination of β-ketoesters, β-ketophosphonates and oxindoles using NFSi. The highest ee obtained, 94%, was with cyclic substrate **4.20** (Scheme 4.4).[9]



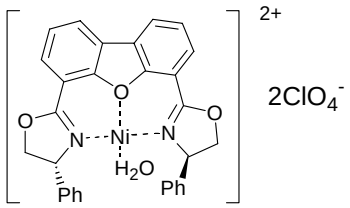
Scheme 4.4. An example of an enantioselective EF using NFSi and chiral Pd catalyst **4.19**

Cahard and coworkers have reported the enantioselective EF of β-ketoesters using NFSi and a chiral bis(oxazoline)-copper complex derived from ligand **4.21** and $Cu(OTf)_2$ (Scheme 4.5).[10] The ee's ranged from 35-85%.



Scheme 4.5. Enantioselective EF using NFSi and a chiral copper catalyst

In 2005, the Shibata and Toru groups disclosed highly enantioselective catalytic fluorinations (ee's from 83-99%) of β-ketoesters and cyclic benzylic tertiary amides using NFSi and catalytic amounts of $Ni(ClO_4)_2$-$6H_2O$/ligand **4.22** (Figure 4.3) in CH_2Cl_2.[11]

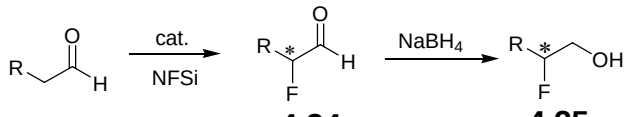


4.22

Figure 4.3. Structure of ligand **4.22**

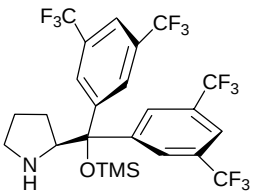
4.1.4 Enantioselective fluorination using chiral organocatalysts

Several reports[12] have appeared on the use of chiral organocatalysts to achieve enantioselective fluorinations of aldehydes and, to a much lesser extent, ketones. These reactions proceed via formation of a chiral enamine which then undergoes EF with either NFSi or Selectfluor.



4.24 **4.25**

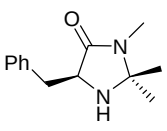
Jorgensen



4.23

Conditions:
catalyst **4.23** (1 mol%)
MTBE, rt
8 linear aldehydes
55-95% yield
91-97% ee (*S*)

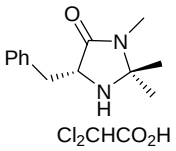
Barbas



4.26

Conditions:
catalyst **4.26** (30-100 mol%)
DMF, 4 deg.
6 linear aldehydes
40-90% yield
86-96% ee (*S*)

MacMillan



4.27

Conditions:
catalyst **4.27** (20 mol%)
THF/iPrOH, -10 deg.
9 linear aldehydes
54-96% yield
91-99% ee (*R*)

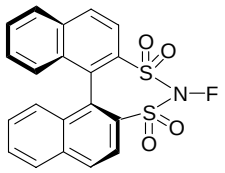
Scheme 4.6. Enantioselective α-fluorination of aldehydes using chiral organocatalysts

The first direct enantioselective fluorination using this approach was achieved using Selectfluor and (*S*)-proline as the chiral organocatalyst.[12a] However, the enantioselectivities were low. A much more efficient system was described by Jøgensen and co-workers (Scheme 4.6).[12b]

The silylated prolinol derivative **4.23**, acting as a catalyst through an enamine mechanism, showed high activity and selectivity for the fluorination of aldehydes. The obtained α-fluoro aldehydes **4.24** were reduced to more stable and less volatile fluoroalcohols **4.25**. Imidazolidinones **4.26** were used by Barbas and co-workers[12c] as organocatalysts for the enantioselective fluorination of branched aldehydes with moderate enantioselectivities or as stoichiometric chiral promoters for the fluorination of straight-chain aldehydes (up to 96% ee, Scheme 4.6). MacMillan et al. exploited the imidazolidinone catalyst **4.27** in the electrophilic fluorination of a range of aldehydes (Scheme 4.6).[12d] In most cases alcohols **4.25** were obtained in excellent enantioselectivities (up to 99% ee). These mild organocatalytic fluorination methods allow preparation of not only stable quaternary fluorinated carbon atoms in the form of α-fluoro, α-substituted carbonyl compounds but also much less stable α-fluoro aldehydes containing a tertiary C-F center.

4.1.5 Chiral halogenating agents based on chiral binaphthyl scaffolds

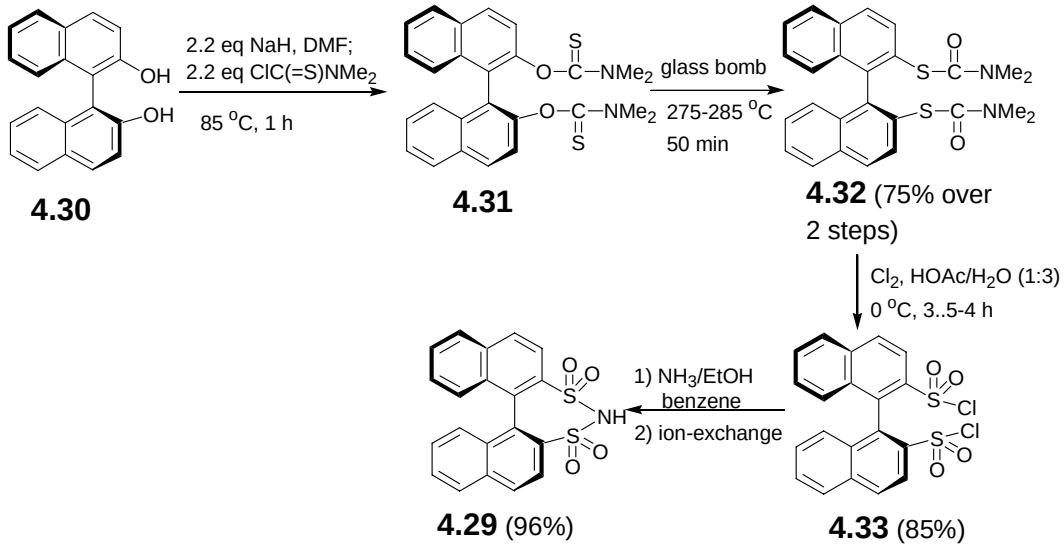
All of the above mentioned approaches to enantioselective EF have limitations. Enantioselective EF's using the cinchona alkaloid-based reagents exhibit highly variable ee's and the reactions must be done in acetone or acetonitrile (for solubility reasons) and low temperatures are required for obtaining good ee's. The transition metal catalysts only work well on highly enolizable compounds (ie. β-keto esters) and the organocatalysts only work on aldehydes and a few ketones. Ideally, we wished to develop a chiral electrophilic fluorinating agent that could be used to achieve



4.28

Figure 4.4. Structure of chiral N-F reagent **4.28**

highly enantioselective fluorinations in high yield on a wide variety of substrates including sulfonates and sulfonamides. Towards this end, we designed chiral binaphthyl *N*-fluorosulfonimide **4.28** as a chiral electrophilic fluorinating agent (Figure 4.4). This compound has a binaphthyl scaffold, which has been widely employed as a platform for reagents and catalysts in highly enantioselective transformations. The arylsulfonimide structure ensures that their fluorine transfer potential should be very high and exhibit electrophilic reactivity patterns, and be capable of fluorinating a variety of substrates including highly basic carbanions.[13] Dr. Rick Strickler and Dr. Chenguo Jia, two post-doctoral fellows in the Taylor group, designed a highly efficient synthesis of chiral sulfonimide **4.29**, the precursor to compound **4.28** (unpublished results, Scheme 4.7). A Newman-Kwart

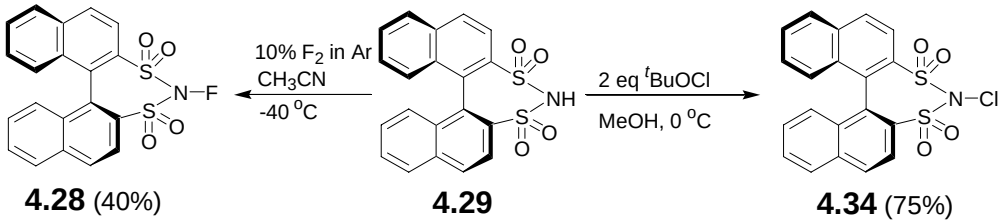


4.30 **4.31** **4.32** (75% over 2 steps) **4.33** (85%) **4.29** (96%)

Scheme 4.7. Synthesis of **4.29**

rearrangement (NKR) of *O*-aryl thiocarbamate **4.31** was used to construct *S*-aryl thiocarbamate **4.32**. Oxidative chlorination of **4.32** and cyclization of the resulting disulfonyl chloride **4.33** using ammonia in ethanol and benzene gave cyclic sulfonimide **4.29**.

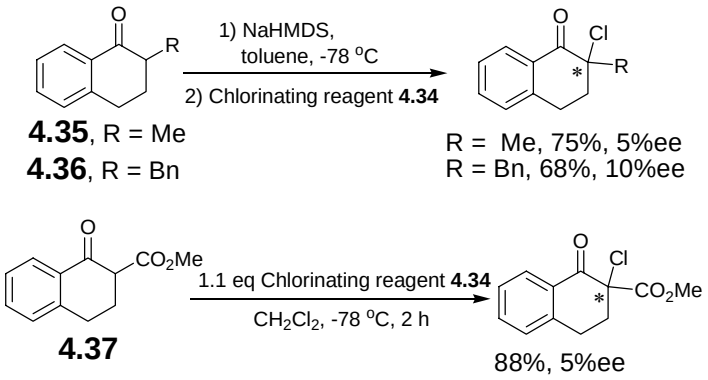
Subjecting sulfonimide **4.29** to a stream of fluorine gas in acetonitrile at -40 ^{o}C gave *N*-F compound **4.28** in about a 40% yield although we were unable to remove small amounts of byproducts resulting from ring fluorination (Scheme 4.8). It was also found that the *N*-chloro devivative **4.34** could be very easily prepared in 75 % yield by reacting **4.29** with *t*-butyl hypochlorite in methanol (Scheme 4.8).



Scheme 4.8. Synthesis of **4.28** and **4.34**

Since the *N*-chloro derivative **4.34** was much easier (and safer) to obtain in pure form and in good yield than the *N*-F derivative, **4.34** was used to ascertain the potential of this chiral sulfonimide scaffold to affect enantioselective transformations.Although **4.34** was a good chlorinating agent (Scheme 4.9) in that the α-chlorination of compounds **4.35-4.37** preceded in good yield, the best ee's we could obtain for these transformations ranged from 5-10%. We anticipated that similar results would be obtained with the *N*-F analog **4.28**.

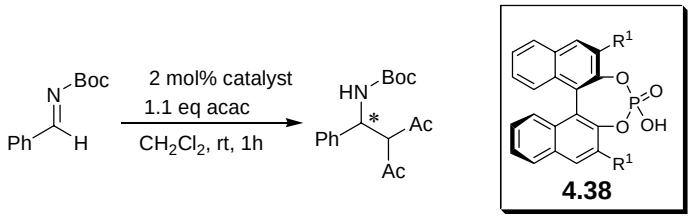
It was clear that reagents **4.28** and **4.34** were going to have to be modified if we wished to obtain highly enantioselective fluorinating or chlorinating agents. One potential approach to achieving this is to modify the naphthyl rings at the 3 and 3′ positions. This is a common approach



Scheme 4.9. Chlorination of **4.35-4.37** with reagent **4.34**

to increasing the ee's of enantioselective tranformations when using chiral reagents or catalysts based on the binaphthyl platform. For example, Uraguchi et al.[14a] as well as Akiyama et al.[14b] recentlyreported chiral Brønsted acid-catalyzed Mannich reactions via electrophilic activation using chiral BINOL based phosphoric acids of type **4.38** (Table 4.2).[12a,b] It is worthy of note is that the ee's of these transformations increased dramatically as the size of the aryl group at the 3 and 3′ positions increased. An ee of only 12% was obtained when the using 3,3′-unsubstituted compound **4.38a**

Table 4.2. An enantioselective Mannich reaction catalyzed by chiral Brønsted acids **4.38a-d**



catalyst	R^1	Yield (%)	Ee (%)
4.38a	H	92	12
4.38b	Ph	95	56
4.38c	4-biph	88	90
4.38d	4-(β-naphth)-C_6H_4	99	95

while an ee of 95% was obtained when the 3 and 3′ position were modified with 4-(β-naphth)C_6H_4 (compound **4.38d**, table 4.2). It was hypothesized that the aryl group as at the 3 and 3′ position shielded the phosphate moiety which led to efficient asymmetric induction.[14b]

4.1.6 Objectives

The objective of this project is to develop an efficient synthesis of fluorinating and chlorinating agents of type **4.40** and **4.41** and to ascertain their ability to perform enantioselective fluorinations and chlorinations. Key to the success of this work is the development of an efficient synthesis of cyclic sulfonimide precursors of type **4.39**. The work presented in this chapter represents our preliminary studies on the synthesis of these cyclic sulfonimides.

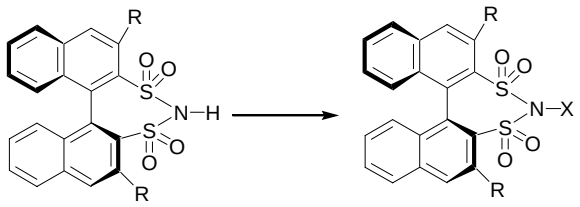
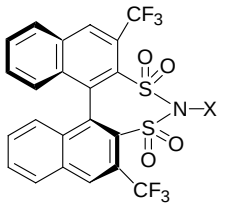


Figure 4.5. Structures of compounds of type **4.40** and **4.41**.

4.2 Results and discussion

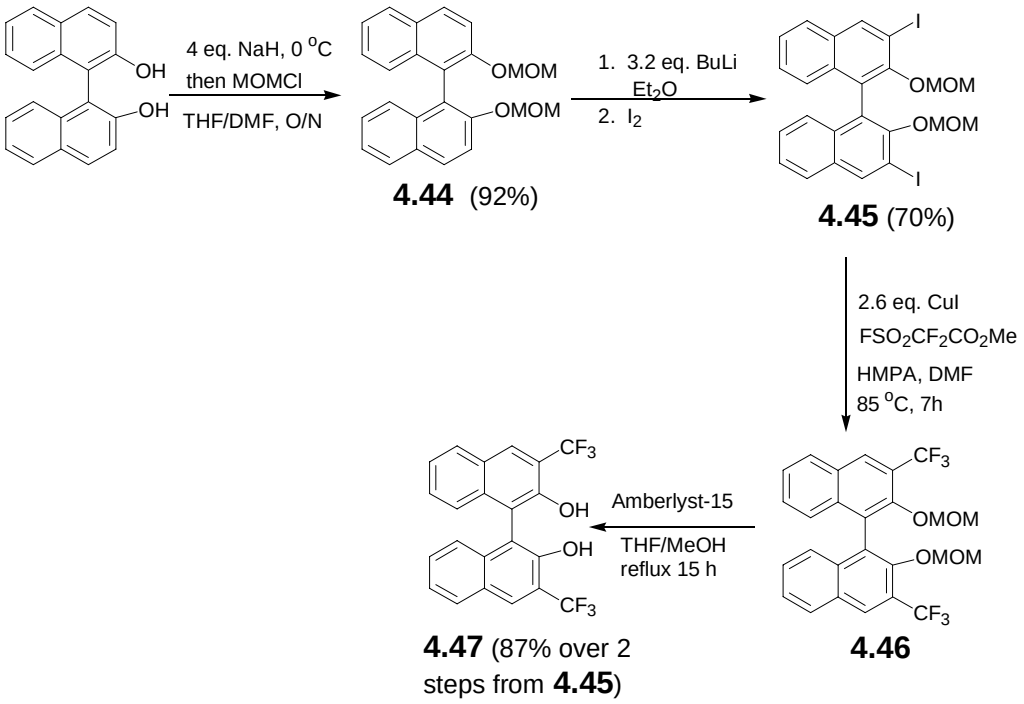
Our initial targets were compounds **4.42** and **4.43** which bear CF_3 groups at the 3 and 3′ positions (Figure 4.6). We anticipated that the electron withdrawing CF_3 groups would lower the electron density on the aromatic ring which would help prevent perfluorination of the naphthyl rings. Moreover, we were anticipating that its size, which is similar to an isopropyl group, would provide the necessary steric shielding to provide the desired enantioselectivity for the electrophilic halogenation reactions.



4.42, X = F
4.43 X = Cl

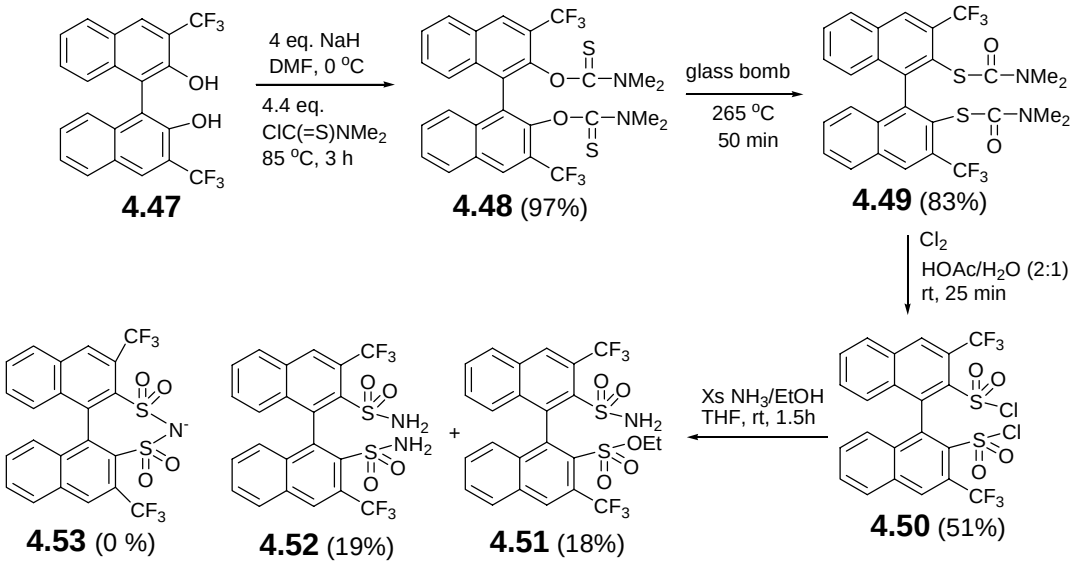
Figure 4.6. Structures of compounds **4.42** and **4.43**

We first prepared the bis-CF_3 BINOL derivative **4.47** using literature procedures (Scheme 4.10). We used racemic BINOL to work out the conditions before attempting the chiral version. MOM protection of BINOL using MOMCl is usually performed using large amounts of THF as solvent.[15] To reduce the amount of THF we used DMF as a co-solvent and this worked well giving product MOM-protected BINOL **4.44** in 92% yield. Lithiation followed by treating with iodine gave 3,3′-diiodo MOM protected BINOL **4.45** in 70% yield after column and recrystallization.[15] Reaction



Scheme 4.10. Synthesis of compound **4.47**

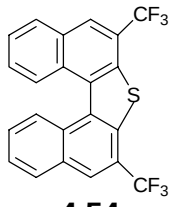
of iodo compound **4.45** with $FSO_2CF_2CO_2Me$, HMPA and CuI in DMF at 85 ^oC for 7 h gave crude CF_3 substituted product **4.46**.[16] Crude **4.46** was treated with amberlyst-15 in THF/MeOH to give **4.47** in 87% yield over 2 steps from **4.45**.[16]



Scheme 4.11. Synthesis and attempted cyclization of compound **4.50**

The CF_3 substitued BINOL **4.47** was then converted to corresponding *O*-aryl thiocarbamate **4.48** in 97% yield by reacting with NaH, *N,N*-dimethylthiocarbamoyl chloride in DMF at 85 ^oC for 3 h (Scheme 4.11). The NKR was carried out neat in a glass bomb at about 265 ^oC for 50 min to give desired *S*-aryl thiocarbamate **4.49** in 83% yield. The solid starting material has to melt first in order to have the reaction go. Once it melted the reaction proceeded quickly at 265 ^oC. The temperature is very important for this reaction. At temperatures lower than 265 ^oC the reaction was slow and the conversion rate was low. At higher temperature (280 ^oC) a considerable amount of cyclic sulfide byproduct **4.54** (Figure 4.7) was obtained. A similar byproduct was also found to form during the rearrangement of **4.31** to **4.32**. During the reaction, some solid starting material accumulates on the

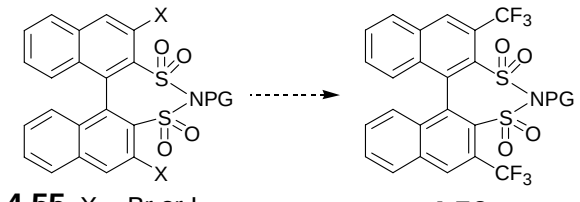
side of the glass bomb due to stirring. So we did the reaction at about 265 ^{o}C for 45 min, cooled it to rt, then scratched down the solid unreacted starting material on the sides and then heated it at 265 ^{o}C for another 5 min. Rearranged *S*-aryl thiocarbamate **4.49** underwent oxidative chlorination using chlorine in HOAc/H_2O (3:1) to give sulfonyl chloride **4.50** in 51 % yield.



4.54

Figure 4.7. Structure of byproduct **4.54**

The last step was the reaction of **4.50** with alcoholic ammonia to cyclize the disulfonyl groups of **4.50** to form sulfonamide **4.53**. Use of benzene as solvent gave a very messy mixture as ascertained by ^{19}F NMR. Then we tried THF as solvent; however, only byproducts **4.51** (18%) and disulfonamide **4.52** (19%) were formed but no cyclized sufonimide **4.53** was isolated. When we used CH_2Cl_2 and 2% DMF as co-solvent and added ammonia/EtOH slowly at 0 ^{o}C, more disulfonamide **4.52** (34%) was obtained. Reaction of **4.50** with allylamine only gave corresponding disulfonamide. We thought that perhaps the CF_3 group is too bulky so that the two naphthyl rings couldn't approach close enough to cyclize.



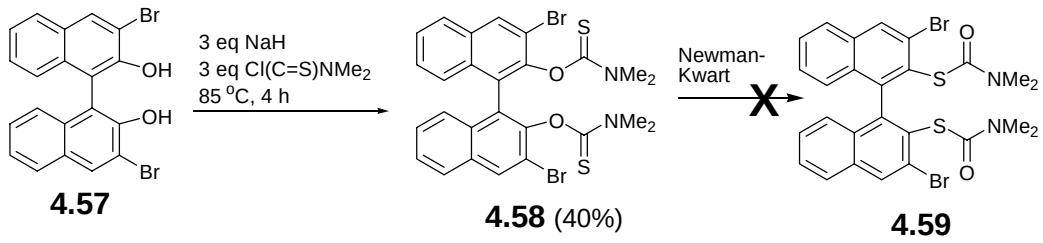
4.55, X = Br or I **4.56**

Scheme 4.12. Alternative route to CF_3 sulfonimide **4.56**

Due to the difficulty in cyclizing **4.50**, we then attempted to prepare dibromo or diiodo

sulfonimides of type **4.55** first and then install the CF_3 groups (Scheme 4.12).

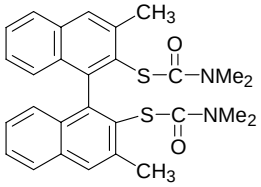
We treated 3,3′-dibromo BINOL, **4.57**[16] with *N,N*-dimethyl thiocarbamoyl chloride to give *O*-aryl thiocarbamate **4.58** (Scheme 4.13). We attempted an NKR of **4.58** by heating neat **4.58** at 265 ^{o}C; however, this gave a black tar instead of the desired product **4.59**. Compound **4.58** did not appear to be stable under these conditions. We also attempted this with the 3,3′-diiodo analogue of **4.58** but obtained a similar result.



4.57 **4.58** (40%) **4.59**

Scheme 4.13. Attempted NKR of compound **4.58**.

Due to the difficulties in cyclizing compound **4.50** we decided to see if this cyclization problem was a general phenomenon and would even be an issue with smaller substituents at the 3 and 3′ positions, such as methyl and phenyl. Racemic *S*-aryl thiocarbamate **4.60** (Figure 4.8) has been prepared by Cossu et al. starting from racemic BINOL and via an NKR of the corresponding *O*-aryl thiocarbamate.

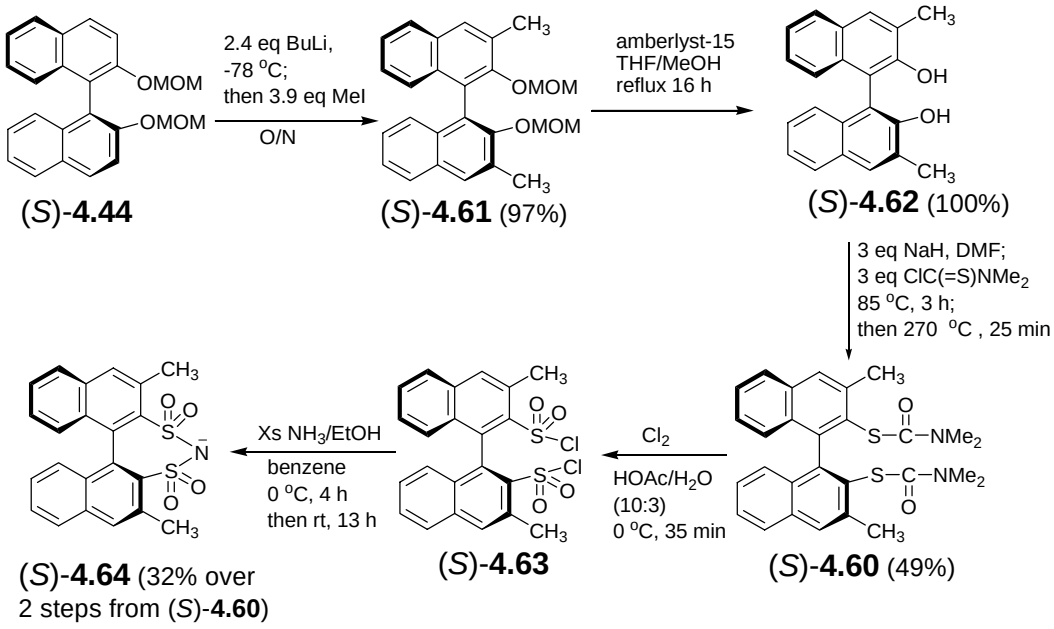


4.60

Figure 4.8. Racemic *S*-aryl thiocarbamate **4.60**

We constructed the (*S*)-enantiomer of **4.60** by a similar route (Scheme 4.14). Ortho lithiation

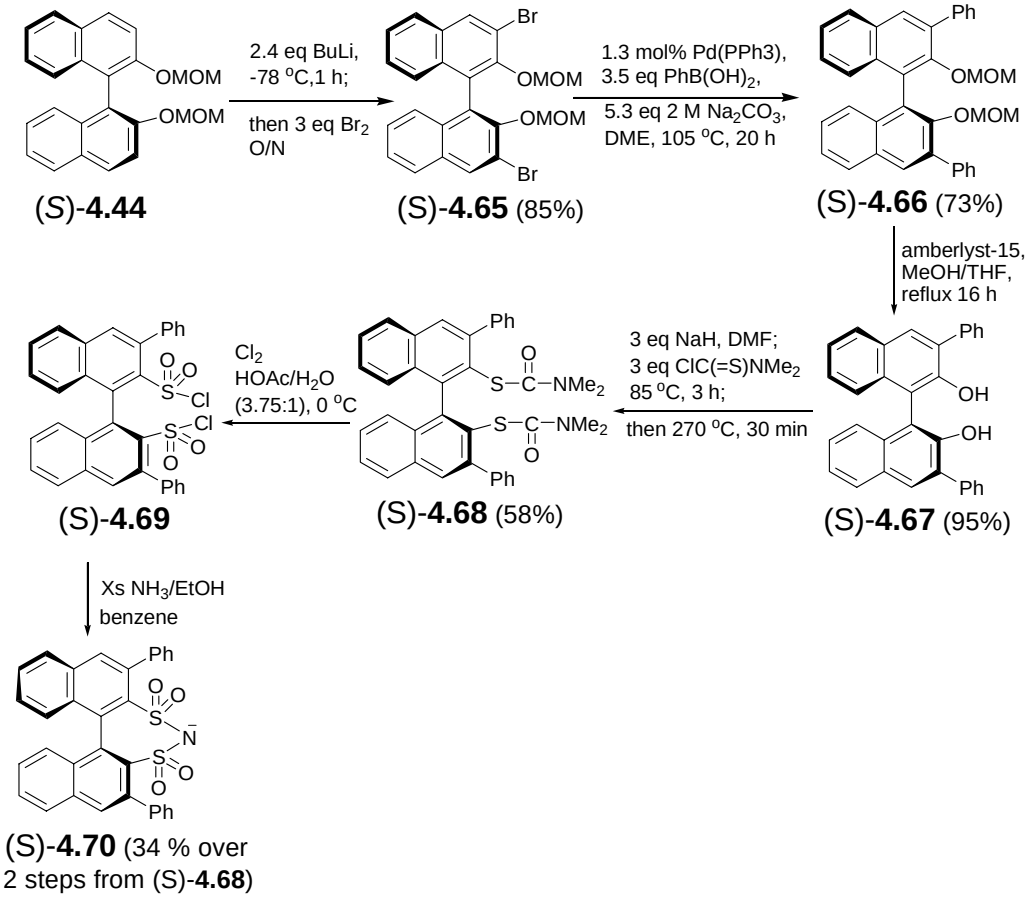
of (*S*)-**4.44** followed by methylation with MeI[17] and removal of the MOM protecting group using Amberlyst-15 resin[16] gave 3,3′-dimethyl substituted BINOL (*S*)-**4.62** in 97% yield (two steps). Compound (*S*)-**4.62** was reacted with thiocarbamyl chloride to give to *O*-aryl thiocarbamate which was rearranged to its *S*-aryl thiocarbamate isomer (*S*)-**4.60** by an NKR. Due to the complex ^{1}H NMR



Scheme 4.14. Synthesis of (*S*)-**4.64**

and hard purification of *O*-aryl thiocarbamate, these two steps were done together to give *S*-aryl thiocarbamate (*S*)-**4.60** in 49% yield.[18] Oxidative chlorination of (*S*)-**4.60** in HOAc/H_2O at 0 °C gave disulfonyl chloride (*S*)-**4.63** which could be purified by flash chromatography. However, some decomposition occurred on the column and a much lower yield was obtained so crude (*S*)-**4.63** was used for next step. Cyclization of (*S*)-**4.63** with excess NH_3/EtOH in benzene gave desired sulfonamide (*S*)-**4.64** in 32% yield over 2 steps from *S*-aryl thiocarbamate (*S*)-**4.64**. ^{1}H NMR indicated that the oxidative chlorination reaction proceeded very well and quite cleanly which suggested that the low yield for these two steps was the cyclization reaction. The overall yield of

(*S*)-**4.64** from BINOL was 15%. We have assumed that no racemization had taken place during this synthesis, however, this has yet to be confirmed.

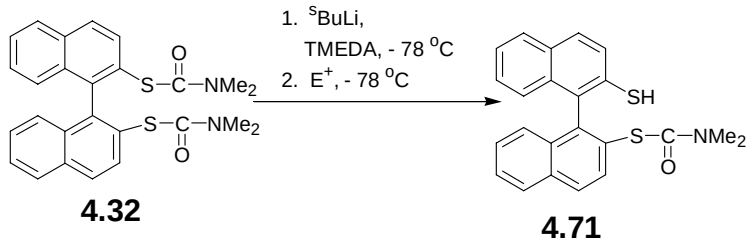


Scheme 4.15. Synthesis of (S)-**4.70**

(*S*)-3,3′-diphenyl BINOL ((*S*)-**4.67**) was prepared according to the procedure of Cox et al. (Scheme 4.15).[15] Ortholithiation of (*S*)-**4.44** followed by bromination gave (*S*)-**4.65** in 85% yield.[17] Suzuki coupling between dibromide (*S*)-**4.65** and phenyl boronic acid in the presence of 1.3 mol% $Pd(PPh_3)_4$ gave phenyl substituted BINOL derivative **4.66** in 73 % yield.[15] Deprotection of (*S*)-**4.66** using amberlyst-15 resin gave (*S*)-**4.67** in 95% yield.[16] Reaction of (*S*)-**4.67** with thiocarbamoyl chloride, followed by an NKR gave (*S*)-**4.68** in a 58% yield over 2 steps. Oxidative chlorination of

(*S*)-**4.68** was done at 0 ^{o}C in HOAc/H_2O (3.75:1). ^{1}H NMR revealed that this reaction proceeds quite cleanly, however, we could not obtain (*S*)-**4.69** in pure form due to its instability. Therefore, crude (*S*)-**4.69** was cyclized using XS ammonia in EtOH and benzene to give (*S*)-**4.70** in 34% yield over 2 steps. Again, the low yield appeared to be due to the cyclization reaction. The overall yield of (*S*)-**4.70** from compound (*S*)-**4.44** was 12%. We have again assumed that no racemization had taken place during this synthesis, however, this has yet to be confirmed.

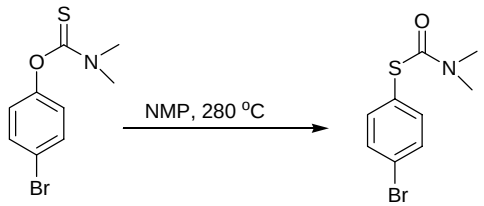
The overall low yields that we obtained for (*S*)-**4.64** and (*S*)-**4.70** prompted us to reevaluate this approach to the synthesis of this class of compounds. The main difficulty is the low yields obtained for the cyclization reactions. Ideally the route outlined in Scheme **4.12** would be best where the bromo or iodo derivatives of type **4.55** are prepared and then the alkyl or aryl subtituents at the 3 and 3′ positions are installed (for example by a Suzuki reaction) after cyclization. However, the NKR of the bromo and iodo precursors such as **4.58** did not work. An alternative is to introduce the bromine or iodine by ortholithiaton of *S*-aryl thiocarbamate **4.32**. However, when we subjected **4.32** to *sec*-BuLi in the absence or presence of TMEDA followed by the addition of electrophiles at -78 ^{o}C, compound **4.71** was obtained (Scheme 4.16). TLC analysis of the reaction mixture revealed that **4.71** was formed before the addition of the electrophile.



Scheme 4.16. Ortholithiation of compound **4.32**

4.3 Summary and future work

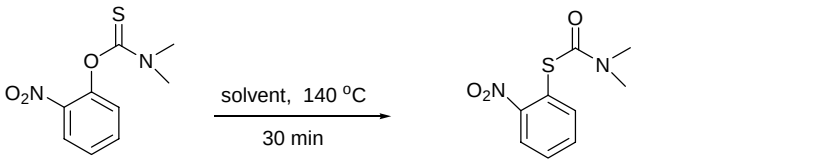
We have shown that CF_3 substituted BINOL sulfonimide **4.50** is difficult to cyclize. However, two chiral (*S*)-sulfonimides, methyl and phenyl substituted at the 3 and 3' positions, could be prepared via the cyclization of their disulfonyl chloride precursors. However, their overall yields were quite low. A more efficient route to sulfonimides of type **4.39** are required if this approach to enantioselective fluorination is to have some practical utility. The chlorination and fluorination of (*S*)-**4.64** and (*S*)-**4.70** and their evaluation as chiral fluorinating and chlorinating agents is in progress in the Taylor group.



4.72 **4.73** (98%)

Scheme 4.17. NKR of **4.72**

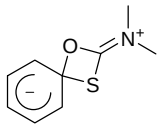
Very recently, Moseley et al have reported a detailed study of the NKR using simple substituted *O*-aryl thiocarbamates.[19] Several interesting results were obtained for these studies. First, they reported that compound **4.72** underwent an NKR to give *S*-aryl thiocarbamate **4.73** in 98% yield when the reaction was performed in 10 volumes of *N*-methylpyrrolidinone at 280 °C for 20 minutes (Scheme 4.17). No decomposition of the starting material or product occurred. They also found, using compound **4.74** as a model substrate (Scheme 4.18), that the NKR reaction proceeded much faster when performed in 10 volumes of formic acid in a sealed tube at 140 °C.



4.74 **4.75** (78% when done in formic acid, 23% when done in NMP)

Scheme 4.18. NKR of **4**.**74** in NMP and formic acid

For example, in NMP after 30 minutes at 140 °C, only 23% conversion of **4.74** to **4.75** occurred (Scheme 4.18). However, they achieved 78% conversion when the reaction was performed in formic acid at 140 °C for 30 minutes. This solvent effect is believed to be due to the stabilization of the proposed polar 4-center transition state which resembles intermediate **4.76** for NKR's (Figure 4.9).[20] We are optimistic that these conditions will allow us to obtain *S*-aryl thiocarbamate **4.59** via NKR of compound *O*-aryl thiocarbamate **4.58**.



4.76

Figure 4.9. Structure of the proposed four-center transition state/intermediate for the NKR

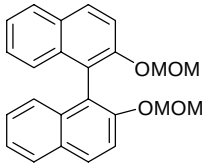
4.4 Experimental

4.4.1 General

All starting materials and reagents were obtained from Aldrich Chemical Company. Dichloromethane, acetonitrile and triethylamine were distilled from calcium hydride. DMF and HMPA were distilled from calcium hydride under reduce pressure. Tetrahydrofuran (THF) and benzene were distilled from sodium/benzophenone. Pentane was dried over Na_2SO_4 prior to use. *N,N*-dimethyl thiocarbamoyl chloride was distilled. Ethanol was distilled from phthalic anhydride.

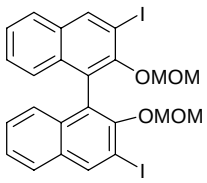
Ammonia/EtOH solution was prepared by bubbling ammonia (cylinder tank) through dry ethanol. MOMCl (chloromethyl methyl ether) was prepared according to literature.[21] BuLi was titrated according to literature.[22] Silica-gel chromatography was performed using silica gel 60 Å (230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Avance 300 spectrometer in $CDCl_3$ or CD_3OD at 300 MHz, 75 MHz and 282 MHz, respectively. NMR spectra are reported in parts per million (ppm) relative to internal standards or solvent peaks. For NMR spectra run in $CDCl_3$, chemical shifts (δ) for ^{1}H NMR spectra are reported relative to internal Me_4Si (δ 0.0 ppm), chemical shifts (δ) for ^{13}C NMR spectra are relative to the solvent peak (δ 77.0 ppm, central peak), ^{19}F NMR relative to an external $CFCl_3$ (δ 0.0 ppm). For NMR spectra run in DMSO-d_6, chemical shifts (δ) for ^{1}H NMR spectra are reported relative to the residual solvent peak (δ 2.49 ppm), chemical shifts (δ)for ^{13}C NMR spectra are relative to the solvent peak (δ 39.5 ppm, central peak), ^{19}F NMR relative to an external $CFCl_3$ (δ 0.0 ppm). For NMR spectra run in CD_3OD, chemical shifts (δ) for ^{1}H NMR spectra are reported relative to the residual solvent peak (δ 3.31 ppm), chemical shifts (δ) for ^{13}C NMR spectra are relative to the solvent peak (δ 49.0 ppm, central peak), ^{19}F NMR relative to an external $CFCl_3$ (δ 0.0 ppm). Low-resolution (LRMS) and high-resolution (HRMS) electron impact (EI) mass spectra were recorded on a JEOL HX 110 double focusing mass spectrometer. Electrospray (ESI) mass spectra were obtained with a Waters/Micromass QTOF Ultima Global mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.

4.4.2 Syntheses



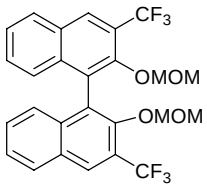
4.44

2,2′-Bis(methoxymethoxy)-1,1′-binaphthalene (4.44). This was prepared according to the procedure of Cox et al.[15] ***Racemic***: BINOL (49.7 g, 0.174 mol) was dissolved in DMF (150 ml) by heating and THF (50 ml) was then added. To a suspension of NaH (60% dispersed in mineral oil, 30 g, 0.75 mol, 4 equiv) in THF (200 ml) at 0 ^{o}C was added the BINOL solution over a period of one hour. The ice bath was removed and the reaction stirred for 20 min. The mixture was cooled to 0 ^{o}C and MOMCl (30 ml) was added and the mixture stirring overnight. The reaction was quenched with ice then poured onto ice/H_2O (500 ml) and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated. Triturating the oily residue with CH_2Cl_2 followed by filtration gave 29.6 g of **4.44** as pale yellow crystals. The filtrate was concentrated *in vacuo* and CH_2Cl_2 was added. This gave an additional 30.3 g of **4.44** (total = 59.9 g, 92%). ***Chiral*** ((*S*)-**4.44**): Prepared as a white solid in 96% yield using the same procedure except (*S*)-BINOL was used. ^{1}H NMR was identical to that reported in the literature.[16] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.94 (d, J = 9.0 Hz, 2H), 7.86 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 9.0 Hz, 2H), 7.22 (d, J = 11.3 Hz, 2H), 7.15 (t, J = 9.0 Hz, 2H), 5.07 (d, J = 6.8 Hz, 2H, OC<u>H</u>HO x 2), 4.96 (d, J = 6.7 Hz, 2H, OCH<u>H</u>O x 2), 3.13 (s, 6H, CH_3 x 2).



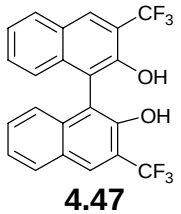
4.45

2,2′-Bis(methoxymethoxy)-3,3′-diiodo-1,1′-binaphthalene (4.45) This is prepared according to the procedure of Cox et al.[15] To a stirred solution of MOM-BINOL **4.44** (14.96 g, 40 mmol) in Et_2O (680 ml) at rt was added BuLi (1.6 M in hexane, 80.0 ml, 128 mmol, 3.2 equiv) over 30 min. After stirring 30 min at rt, it was cooled to 0 ^oC before THF (400 ml) was added. After stirring 1 h at 0 ^oC, iodine (32 g, 126 mmol, 3.15 equiv) was added and the resulting mixture was stirred 10 min at 0 ^oC and then the ice bath was removed and stirring was continued for 1h. Reaction was quenched with 10% $Na_2S_2O_3$ (300 ml) at 0 ^oC and stirred 10 min. After separation and extraction with Et_2O the combined extracts were washed with 10% $Na_2S_2O_3$ and brine (60 ml) then dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (ethyl acetate/hexane, 1:10) gave 14.46 g of **4.45** as pale yellow crystals which were recrystallized from CH_2Cl_2/hexane. The filtrate was subjected to flash chromatography (elution with ethyl acetate/hexane1:10) to give additional 3.02 g of **4.45** as yellow solid which was recrystallized to give pale yellow crystals (total = 17.48 g, 70%). ^1H NMR was identical to that reported in the literature.[16] ^1H NMR ($CDCl_3$, 300 MHz) δ 8.52 (s, 2H, H-4 and H-4′), 7.76 (d, J = 8.1 Hz, 2H), 7.41 (t, J = 7.1 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.16 (d, J = 8.5 Hz, 2H), 4.79 (d, J = 5.6 Hz, 2H, OC<u>H</u>HO x 2), 4.68 (d, J = 5.6 Hz, 2H, OCH<u>H</u>O x 2), 2.59 (s, 6H, CH_3 x 2).



4.46

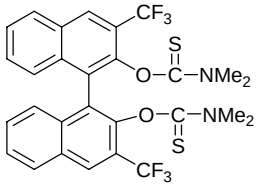
2,2′-Bis(methoxymethoxy)-3,3′-bis(trifluoromethyl)-1,1′- binaphthalene (4.46) This was prepared according to the procedure of Wu et al.[16.] with slight modifications. To a mixture of **4.45** (8.56 g, 13.7 mmol) and CuI (6.75 g, 35.3 mmol, 2.6 equiv) in DMF (120 ml) was added HMPA (10 ml) and $FSO_2CF_2CO_2Me$ (8.1 ml 63.5 mmol, 4.6 equiv). The resulting mixture was heated at 85 ^oC for 7 h before cooling to rt and diluting with Et_2O (150 ml). H_2O (200 mL) was added, the layers were separated and the aq. layer was extracted with Et_2O. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4) and concentrated to give crude **4.46** as an oil. This material was used for the next step. An analytic sample of **4.46** was obtained by flash chromatography of the residue (ethyl acetate/hexane, 1:8). ^1H, ^{13}C and ^{19}F NMR were identical to those reported in the literature.[16] ^1H NMR ($CDCl_3$, 300 MHz) δ 8.37 (s, 2H, H-4 and H-4′), 8.00 (d, J = 8.0 Hz, 2H), 7.53 (t, J = 7.4 Hz, 2H), 7.43 (t, J = 7.3 Hz, 2H), 7.26 (d, J = 8.5 Hz, 2H), 4.79 (dd, J = 5.4 Hz, J = 2.6 Hz, 2H), 4.55 (dd, J = 5.4 Hz, J = 2.6 Hz, 2H), 2.73 (s, 6H); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -60.5.



4.47

3,3′-Bis(trifluoromethyl)-1,1′-binaphthyl-2,2′-diol (4.47) This is prepared according to the procedure of Wu et al.[16] To crude **4.46** was added THF (170ml), MeOH (170 ml) and Amberlyst-15 (6.0 g). The resulting mixture was refluxed for 15 h. After filtration and

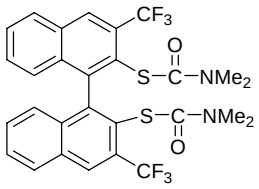
concentration, the residue was subjected to flash chromatography (ethyl acetate/hexane, 1:6) and the **4.47** obtained for the column was recrystallized from ethyl acetate/hexane to give **4.47** as colorless crystals (5.22 g, 87% from **4.45**). ^{1}H and ^{13}C NMR were identical to that reported in the literature.[16] ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.77 (s, 2H, OH x 2), 8.44 (s, 2H, H-4 and H-4′), 8.12 (d, J = 7.4 Hz, 2H), 7.47-7.35 (m, 4H), 7.02 (d, J = 8.3 Hz, 2H).



4.48

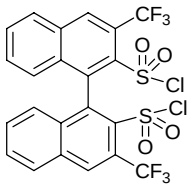
2,2′-Bis(*N*,*N*-dimethylthiocarbamoyloxy)-3,3′-bis(trifluoromethyl)-1,1′-binaphthalene (4.48) To a solution of CF_3-BINOL **4.47** (2.00 g, 4.74 mmol) in DMF (20 ml) at 0 ^{o}C was added NaH (60% dispersed in mineral oil, 760 mg, 19.0 mmol, 4 equiv) in one portion. The reaction mixture was stirred 20 min at 0 ^{o}C, before thiocarbamoyl chloride (2.34 g, 21.0 mmol, 4.4 equiv) was added and then heated at 85 ^{o}C for 3 h. After cooling to rt, the mixture was poured into cold 1 M NaOH (80 ml) slowly. The resulting precipitate was collected and washed with cold H_2O and cold hexane then redissolved in CH_2Cl_2 and washed with brine then dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane/methylene chloride, 2:2:1) to give *O*-aryl thiocarbamate **4.48** as a white solid (2.74 g, 97%). Mp: 260-262 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.33 (s, 2H, H-4 and H-4′), 7.95 (d, J = 8.0 Hz, 2H), 7.51 (t, J = 7.5 Hz, 2H, overlapping with d at 7.49), 7.49 (d, J = 9.4 Hz, 2H, overlapping with t at 7.51), 7.35 (t, J = 8.0 Hz, 2H), 3.17 (s, 6H, CH_3 x 2), 2.97 (s, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.5 (C=S), 145.7 (C-2 and C-2′), 134.6 (C_{Ar}), 130.3 (C_{Ar}), 129.2 (q, J = 4.0 Hz, C-4 and C-4′), 128.7 (C_{Ar}), 128.7 (C_{Ar}), 128.2 (C_{Ar}),

127.1 (C_{Ar}), 127.0 (C_{Ar}), 123.2 (q, J = 271 Hz, CF_3), 121.9 (q, J = 31.1 Hz, C-3 and C-3′), 43.1 (CH_3), 38.1 (CH_3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -60.3; LRMS (EI) *m/z* (%) 596 (M^+, 42), 577 (M-19, 7), 524 (9), 492 (4), 420 (33), 176 (21), 88 (100); HRMS (EI) calcd for $C_{28}H_{22}F_6N_2O_2S_2$ 596.1027; found 596.1025.



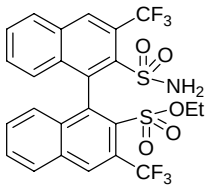
4.49

2,2′-Bis(*N,N*-dimethylcarbamoylthio)-3,3′-bis(trifluoromethyl)-1,1′-binaphthalene (4.49). *O*-Aryl thiocarbamate **4.48** (1.20 g, 2.01 mmol) in a glass bomb was purged with argon and heated at 260-268 ^{o}C for 45 min. After cooling to rt and scratching down some solids on the sides of the glass bomb, it was heated at 265 ^{o}C for another 5 min. Purification by flash chromatography (ethyl acetate/hexane, 1:4 to 1:2 to 1:1) gave *S*-aryl thiocarbamate **4.49** as a white solid (999 mg, 83%). Mp: 245 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (s, 2H, H-4 and H-4′), 7.98 (d, J = 8.0 Hz, 2H), 7.56 (t, J = 7.3 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.24-7.20 (m, 1H), 2.55 (s, 12H, CH_3 x 4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.0 (m, C=O x 2), 146.7 (C-2 and C-2′), 134.1, 132.2, 130.9 (q, J = 29.3 Hz, C-3 and C-3′), 128.6 (C_{Ar}), 128.5 (C_{Ar}), 128.4 (C_{Ar}), 128.3 (C_{Ar}), 124.5 (C_{Ar}), 123.3 (q, J = 272 Hz, CF_3), 36.6 (m); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -59.3; LRMS (EI) *m/z* (%) 596 (M^+, 8), 577 (M-19, 7), 524 (4), 420 (78), 351 (22), 350 (25), 176 (57), 72 (100); HRMS (EI) calcd for $C_{28}H_{22}F_6N_2O_2S_2$ 596.1027; found 596.1025.

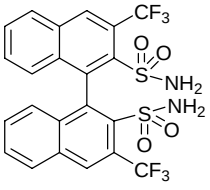


4.50

2,2′-Bis(chlorosulfonyl)-3,3′-bis(trifluoromethyl)-1,1′-binaphthalene (4.50). To a solution of **4.49** (500 mg, 0.839 mmol) in acetic acid (40ml) was added H_2O (20 ml). Cl_2 was bubbled through (2 bubbles per sec) at rt for 25 min before purging with N_2 for 5 min. After filtration, 482 mg of a pale yellow solid was obtained. ^{1}H NMR of this crude material showed mainly the desired product **4.50**. This crude material was used for the next step. An analytically pure sample was obtained by short column flash chromatography (CH_2Cl_2) to give **4.50** as a white solid (253 mg, 51%). Mp: > 265 ^{o}C (dec.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.70 (s, 2H, H-4 and H-4′), 8.15 (d, J = 7.5 Hz, 2H), 7.81 (t, J = 7.2 Hz, 2H), 7.54 (t, J = 7.8 Hz, 2H), 7.06 (d, J = 9.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 139.3 (C_{Ar}), 137.1 (C_{Ar}), 134.8 (C_{Ar}), 131.6 (C_{Ar}), 129.6 (t, J = 7.3 Hz, C-4 and C-4′), 129.2 (CH_{Ar}), 129.1 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 124.8 (t, J = 30.9 Hz, C-3 and C-3′), 124.5 (t, J = 272 Hz, CF_3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -54.6; LRMS (EI) *m/z* (%) 589 (M+4, 2), 587 (M+2, 10), 585 (M^+, 12), 538 (6), 536 (8), 487 (60), 452 (53), 440 (51), 404 (95), 389 (90), 388 (100), 350 (50), 320 (61); HRMS (EI) calcd for $C_{22}H_{10}Cl_2F_6O_4S_2$ 585.9302; found 585.9304.



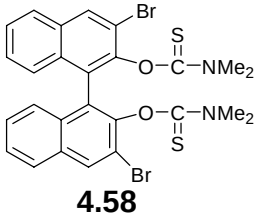
4.51



4.52

2-Ethoxysulfonyl-2′-sulfamoyl-3,3′-bis(trifluoromethyl)-1,1′-binaphthalene (4.51) and 2,2′-Bis(sulfamoyl)-3,3′-bis(trifluoromethyl)-1,1′-binaphthalene (4.52). To a solution of **4.50**

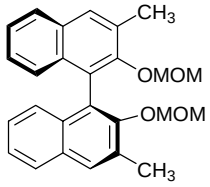
(850 mg, 1.45 mmol) in THF (40 ml) was added NH_3/EtOH solution (2.6 M, 30 ml, 78 mmol, 54 equiv) via syringe in 2 min at rt. After addition, the resulting mixture was stirred for 1.5 h t rt. The reaction was concentrated and the residue was purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/EtOAc 1:10 to 1:1) to give **4.51** as a pale yellow solid (145 mg, 18%) and **4.52** as a white solid (150 mg, 19%). Characterization data for **4.51**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.62 (s, 1H), 8.57 (s, 1H), 8.05 (t, J = 6.9 Hz, 2H), 7.70-7.60 (m, 2H), 7.48-7.38 (m, 2H), 6.98 (d, J = 8.7 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 5.45 (s, 2H, NH_2), 3.99 (q, J = 6.9 Hz, 2H, $OC\underline{H}_2CH_3$), 1.13 (t, J = 7.0 Hz, 3H, $OCH_2C\underline{H}_3$); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -55.16, -55.22; LRMS (EI) *m/z* (%) 577 (M^+, 5), 497 (15), 468 (95), 449 (100), 385 (64). Characterization data for **4.52**: mp: 248-250 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.79 (s, 2H, H-4 and H-4′), 8.25 (d, J = 7.9 Hz, 2H), 7.66 (t, J = 7.3 Hz, 2H), 7.44 (t, J = 7.5 Hz, 2H), 7.16 (s, 4H, NH_2 x 2), 6.72 (d, J = 8.5 Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 139.4 (C_{Ar}), 135.9 (C_{Ar}), 134.4 (C_{Ar}), 132.2 (C_{Ar}), 131.3 (q, J = 7.1 Hz, C-4 and C-4′), 130.6 (C_{Ar}), 129.7 (C_{Ar}), 127.4 (C_{Ar}), 124.2 (q, J = 269 Hz, CF_3), 123.6 (q, J = 31.1 Hz, C-3 and C-3′); ^{19}F NMR (DMSO-d_6, 282 MHz) δ -52.9; LRMS (EI) *m/z* (%) 548 (M^+, 3), 468 (100), 450 (10), 404 (20), 384 (12); HRMS (EI) calcd for $C_{22}H_{14}F_6N_2O_4S_2$ 548.0299; found 548.0313.



4.58

2,2′-Bis(*N*,*N*-dimethylthiocarbamoyloxy)-3,3′-bis(trifluoromethyl)-1,1′-binaphthalene (4.58). **4.58** was prepared using the same procedure for the preparation of **4.48** using **4.57**[17] (2.22 g, 5 mmol), DMF (45 ml), NaH (60% in mineral oil, 600 mg, 15 mmol, 3 equiv),

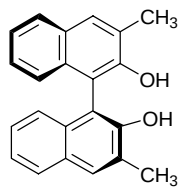
N,N-dimethylthiocarbamoyl chloride (1.853 g, 15 mmol, 3 equiv), 85 ^{o}C, 4 h. Flash chromatography (EtOAc/CH_2Cl_2/hexane, 1:1:10) followed by recrystallization from EtOH gave **4.58** as colorless crystals (1.25 g, 40%). ^{1}H NMR (DMSO-d_6,, 300 MHz) δ 8.46 (s, 2H), 7.94 (d, J = 8.1 Hz, 2H), 7.46 (t, J = 7.2 Hz, 2H), 7.26 (t, J = 7.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 3.13 (s, 6H), 2.96 (s, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 184.4 (C=S x 2), 146.6 (C_{Ar}), 133.1 (C_{Ar}), 132.3 (C_{Ar}), 131.8 (C_{Ar}), 127.9 (C_{Ar}), 127.6 (C_{Ar}), 127.1 (C_{Ar}), 126.9 (C_{Ar}), 126.5 (C_{Ar}), 116.6 (C_{Ar}), 43.0 (CH_3 x 2), 38.5 (CH_3 x 2).



(*S*)-**4.61**

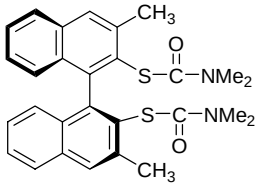
(*S*)-2,2′-Bis(*N,N*-dimethylthiocarbamoyloxy)-3,3′-dimethyl-1,1′-binaphthalene ((*S*)-4.61). This is prepared according to the procedure of Ooi et al.[17] and Cox et al.[15] To a solution of (*S*)-**4.44** (11.2 g, 30.0 mmol), in THF (60 ml) at -78 ^{o}C was added ^{n}BuLi (1.0 M in hexane, 72 ml, 72 mmol, 2.4 equiv) dropwise over 5 min. After addition, the resulting mixture was stirred for 1 h at 0 ^{o}C, before cooling to -78 ^{o}C again. Iodomethane (6.0 ml, 118 mmol, 3.9 equiv) was added over 10 min and reaction mixture was stirred overnight and quenched with sat. NH_4Cl (30 ml) and H_2O (10 ml). The mixture was extracted with ethyl acetate and the combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:2) to give (*S*)-**4.61** as white solid (11.72 g, 97%). ^{1}H NMR and ^{13}C NMR were identical to that reported in the literature.[16] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (s, 2H, overlapping with d at 7.83), 7.82 (d, J = 5.8 Hz, 2H, overlapping with s at 7.83), 7.38 (ddd, J = 10.4 Hz, J = 5.0 Hz, J = 2.6 Hz, 2H), 7.24-7.18 (m,

4H), 4.64 (dd, J = 5.8 Hz, J = 2.8 Hz, 2H, OC<u>H</u>HO x 2), 4.52 (dd, J = 5.8 Hz, J = 2.8 Hz, 2H, OCH<u>H</u>O x 2), 2.87 (s, 6H, CH_3), 2.62 (s, 6H, CH_3).



(*S*)-**4.62**

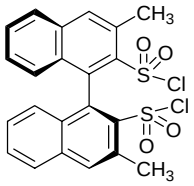
(*S*)-3,3′-Dimethyl-1,1′-binaphthyl-2,2′-diol ((*S*)-4.62). This is prepared according to the procedure of Cox et al.[15] A solution of (*S*)-**4.61** (6.06 g, 15 mmol), and Amberlyst-15 (2.50 g) in MeOH-THF (1:1, 240 mL) was refluxed for 16 h. The solution was cooled, filtered and concentrated to give (*S*)-**4.62** as a white solid (4.81 g, 100%). ^{1}H NMR was identical to that reported in the literature.[16] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (d, 2H, J = 7.1 Hz, overlapping with s at 7.79), 7.79 (s, 2H, overlapping with d at 7.80), 7.32 (t, J = 7.4 Hz, 2H), 7.21 (t, J = 7.4 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 5.08 (s, 2H, OH x 2), 2.49 (s, 6H, CH_3 x 2).



(*S*)-**4.60**

(*S*)-2,2′-Bis(*N,N*-dimethylcarbamoylthio)-3,3′-dimethyl-1,1′-binaphthalene ((*S*)-4.60).[18] This was prepared using the same procedure for the preparation of **4.48** and **4.49.** Compound (*S*)-**4.62** (3.10 g, 9.9 mmol), in DMF (40 ml), NaH (60% dispersion in mineral oil, 1.2g 30 mmol, 3.0 equiv), *N,N*-dimethylthiocarbamoyl chloride (3.71 g, 30.0 mmol, 3 equiv), 85 ^{o}C, 3 h, gave 4.09 g of crude *O*-aryl thiocarbamate as white solid. The crude *O*-aryl thiocarbamate was then heated in glass bomb at 270 ^{o}C for 25 min and the residue was purified flash chromatography (CH_2Cl_2/hexane, 1:1

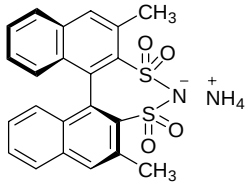
then ethyl acetate/hexane, 1:1) to give *S*-aryl thiocarbamate (*S*)-**4.60** as a white solid (2.35 g, 49% over 2 steps). Mp: 178-179 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.86 (s, 2H, H-4 and H-4′), 7.78 (d, J = 8.1 Hz, 2H), 7.37 (t, J = 7.4 Hz, 2H), 7.11 (t, J = 7.6 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 2.66 (s, 12H, CH_3 x 4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.8 (C=S x 2), 144.3 (C-2 and C-2′), 139.7 (C_{Ar}), 133.9 (C_{Ar}), 132.1 (C_{Ar}), 128.7 (C_{Ar}), 128.5 (C_{Ar}), 127.5 (C_{Ar}), 126.9 (C_{Ar}), 126.8 (C_{Ar}), 125.2 (C_{Ar}), 36.8 (4C, m, N($\underline{C}H_3$)$_2$ x 2), 22.1 (2C, Ar$\underline{C}H_3$ x 2). LRMS (EI) *m/z* (%) 488 (M^+, 28), 416 (2), 384 (5), 383 (5), 312 (100), 296 (15), 282 (5), 72 (41); HRMS (EI) calcd for $C_{28}H_{28}N_2O_2S_2$ 488.1592; found 488.1588.



(*S*)-**4.63**

(*S*)-2,2′-Bis(chlorosulfonyl)-3,3′-dimethyl-1,1′-binaphthalene ((*S*)-4.63). To a solution of (*S*)-**4.60** (1.30 g, 26.6 mmol) in acetic acid (100 ml) was added H_2O (30 ml) slowly by pipette. The resulting mixture was cooled to 0 ^{o}C before Cl_2 was bubbled through (2 bubbles per sec). When bubbling ceased, the flow rate of Cl_2 was adjusted to 1 bubble per 3-5 sec. The Cl_2 was stopped when the flow rate increased (35 min in total). After purging with N_2 for 3 min, it was filtered by suction filtration (ice in filter flask) to give 660 mg of crude (*S*)-**4.63** as yellow powder. The filtrate was extracted with CH_2Cl_2 and the combined extracts were dried (Na_2SO_4) and concentrated. The water bath in the rotary evaporators (low vacuum was used to remove CH_2Cl_2 while high vacuum was used to remove HOAc) was maintained at 30 ^{o}C. The residue was passed thru a short silica pad using CH_2Cl_2 as eluent to give additional 52 mg of (*S*)-**4.63** (total: 712 mg). This crude material was

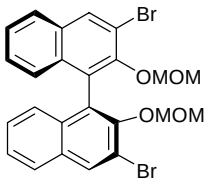
used directly for the next step. An analytically pure sample of (*S*)-**4.63** was obtained as a white solid by flash chromatography (CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.02 (s, 2H, H-4 and H-4′), 7.89 (d, J = 8.0 Hz, 2H), 7.60 (t, J = 7.4 Hz, 2H), 7.28 (t, J = 7.7 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 3.07 (s, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.2 (C_{Ar}), 137.5 (C_{Ar}), 135.1 (C_{Ar}), 133.4 (C_{Ar}), 132.7 (C_{Ar}), 131.0 (C_{Ar}), 130.2 (C_{Ar}), 127.8 (C_{Ar}), 127.6 (C_{Ar}), 127.5 (C_{Ar}), 22.3 (CH_3 x 2); LRMS (EI) *m/z* (%) 482 (M+2, 9), 480 (M+2, 39), 478 (M^+, 50), 344 (42), 312 (15), 296 (100), 280 (41); HRMS (EI) calcd for $C_{22}H_{16}Cl_2O_4S_2$ 477.9867; found 477.9858.



(*S*)-**4.64**

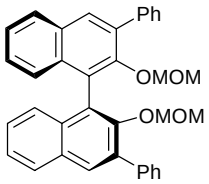
(*S*)-Ammonium 3,3′-dimethyl-1,1′-binaphthyl-2,2′-disulfonimide ((*S*)-4.64). To a solution of crude disulfonyl chloride (*S*)-**4.63** (710 mg) in benzene (60 ml) at 0 ^{o}C was added NH_3/EtOH (2.6 M, 15 ml) via syringe pump over 1.5 h. After addition, the resulting mixture was stirred for 4 h at 0 ^{o}C, then O/N (13 h) at rt. The mixture was filtered and filter cake was rinsed with dry benzene. The filtrate was concentrated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) to give (*S*)-**4.64** as a white solid (370 mg, 32% over 2 steps from (*S*)-**4.60**). Mp: 216-218 ^{o}C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.87 (d, J = 8.5 Hz, 2H), 7.85 (s, 2H), 7.42 (t, J = 7.3 Hz, 2H), 7.09 (t, J = 7.4 Hz, 2H, overlapping), 7.09 (brs, 4H, NH_4, overlapping), 6.63 (d, J = 8.0 Hz, 2H), 2.78 (s, 6H, CH_3 x 2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 139.6 (C_{Ar}), 136.8 (C_{Ar}), 133.6 (C_{Ar}), 131.8 (C_{Ar}, 4C), 128.0 (CH_{Ar}), 127.6 (CH_{Ar}), 127.4 (CH_{Ar}), 126.1 (CH_{Ar}), 22.8 (CH_3 x 2); LRMS (ESI) 422.1111 (100); HRMS (ESI) calcd for $C_{22}H_{16}NO_4S_2$ 422.0521; found

422.0511.



(*S*)-**4.65**

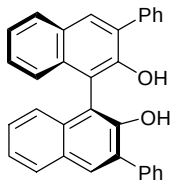
(*S*)-2,2′-Bis(methoxymethoxy)-3,3′-dibromo-1,1′-binaphthalene ((*S*)-4.65). This is prepared according to the procedure of Ooi et al.[17] To a solution (*S*)-**4.44** (14.98 g, 40 mmol) in THF (120 ml) at -78 ^{o}C was added BuLi (1.6 M in hexane, 60 ml, 96 mmol, 2.4 equiv) via syringe pump over 20 min. After stirring 1 h at 0 ^{o}C, it was cooled to -78 ^{o}C before a solution of Br_2 (6.15 ml, 19.2 g, 120 mmol, 3 equiv) in dry pentane (30 ml) was added over 30 min. The resulting mixture was stirred O/N, poured on to sat. Na_2SO_3 (150 ml) and stirred 15 min before extracting with EtOAc. Removal of solvent gave a yellow oil, which was purified flash chromatography (ethyl acetate/CH_2Cl_2/hexane, 1:1:15) which gave (*S*)-**4.65** as pale yellow oil (18.0 g, 85%) which solidified upon standing. ^{1}H NMR was identical to that reported in the literature.[17] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.39 (s, 2H, H-4 and H-4′), 7.78 (d, J = 8.1 Hz, 2H), 7.42 (t, J = 8.8 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.16 (d, J = 8.5 Hz, 2H), 4.80 (t, J = 6.5 Hz, 4H, OCH_2O x 2), 2.54 (s, 6H, CH_3 x 2).



(*S*)-**4.66**

(*S*)-2,2′-Bis(methoxymethoxy)-3,3′-diphenyl-1,1′-binaphthalene ((*S*)-4.66). This was prepared according to the procedure of Cox et al.[15] with some modifications. To a solution (*S*)-**4.65** (18.02 g, 33.75 mmol) in dimethoxyethane (DME, 210 ml) under argon was added $Pd(PPh_3)_4$ (500 mg,

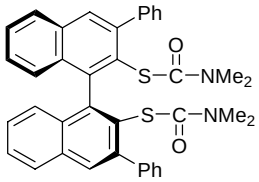
0.43 mmol, 1.3 mol %) and the resulting mixture was stirred 10 min before phenyl boronic acid (14.4 g, 118 mmol, 3.5 equiv) and 2 M aq. Na_2CO_3 (90 ml, 180 mmol, 5.3 equiv) were added successively. The mixture was heated at 105 ^oC for 34 h then cooled to rt. Insoluble solids were removed by suction filtration. The filtrate was concentrated to remove the organic solvent and the residue was extracted with CH_2Cl_2. The combined extracts were washed with sat. NH_4Cl and brine then dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:20 to 1:10) to give (*S*)-**4.66** as a white solid (12.79 g, 73%). ^1H NMR and ^{13}C NMR was identical to that reported in the literature.[16] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.94 (s, 2H, H-4 and H-4′), 7.87 (d, J = 8.1 Hz, 2H), 7.74 (d, J = 7.2 Hz, 4H), 7.48-7.25 (m, 6H), 4.39 (d, J= 5.7 Hz, 2H, OCHHO x 2), 4.35 (d, J= 5.7 Hz, 2H, OCHHO x 2), 2.33 (s, 6H, CH_3 x 2).



(*S*)-**4.67**

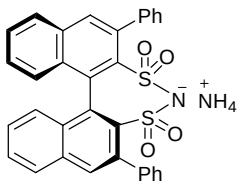
(*S*)-3,3′-Diphenyl-1,1′-binaphthyl-2,2′-diol ((*S*)-4.67). This was prepared according to the procedure of Wu et al.[16] To a solution of (*S*)-**4.66** (12.5 g, 23.95 mmol) in MeOH (150 mL) and THF (170 mL) was added Amberlyst-15 (10 g). The resulting mixture was refluxed for 16 h. The cooled solution was filtered and the filtrate concentrated. The residue was subjected to flash chromatography (ethyl acetate/hexane 1:10) to give (*S*)-**4.67** as a yellow solid which was recrystallized from ethyl acetate/hexane to give (*S*)-**4.67** as a white solid (9.88 g, 95%). ^1H and ^{13}C NMR was identical to that reported in the literature.[16] ^1H NMR ($CDCl_3$, 300 MHz) δ 8.04 (s, 2H, H-4 and H-4′), 7.94 (d, J = 7.9 Hz, 2H), 7.76 (d, J = 7.2 Hz, 2H), 7.51 (t, J = 7.4 Hz, 4H), 7.45-7.31

(m, 6H), 7.26 (d, J = 9.4 Hz, 2H), 5.37 (s, 2H, OH x 2).



(*S*)-**4.68**

2,2′-Bis(*N,N*-dimethylcarbamoylthio)-3,3′-diphenyl-1,1′-binaphthalene ((*S*)-4.68). This was prepared using the same procedure used for the preparation of **4.48** and **4.49.** Compound (*S*)-**4.67** (8.68 g, 19.8 mmol), DMF (20 ml), NaH (60% dispersion in mineral oil, 2.4 g, 60 mmol, 3 equiv), *N,N*-dimethylthiocarbamoyl chloride (7.42 g, 60 mmol, 3 equiv), 85-90 ^{o}C, 2 h. This gave 13.5 g of crude *O*-aryl thiocarbamate as a white solid. Crude *O*-aryl thiocarbamate (13.5 g), glass bomb, 270 ^{o}C, 30 min. *S*-aryl thiocarbamate (*S*)-**4.68** was obtained as a white solid after chromatography (7.08 g, 58%). Mp: 244-245 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 2H, H-4 and H-4′), 7.87 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 7.3 Hz, 2H), 7.47-7.21 (m, 6H), 2.40 (s, 12H, CH_3 x 4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.5 (C=S x 2), 144.4 (C-2 and C-2′), 144.2 (C_{Ar}), 142.0 (C_{Ar}), 133.5 (C_{Ar}), 132.4 (C_{Ar}), 130.3 (C_{Ar}), 129.4 (C_{Ar}), 128.1 (C_{Ar}), 128.0 (C_{Ar}), 127.6 (C_{Ar}), 127.2 (C_{Ar}), 127.1 (C_{Ar}), 126.8 (C_{Ar}), 126.2 (C_{Ar}), 36.7 (m, CH_3 x 4); LRMS (EI) *m/z* (%) 612 (M^+, 18), 596 (1), 540 (2), 508 (7), 436 (100), 358 (7), 83 (41), 72 (29); HRMS (EI) calcd for $C_{38}H_{32}N_2O_2S_2$ 612.1905; found 612.1921.

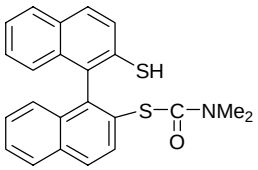


(*S*)-**4.70**

Ammonium 3,3′-diphenyl-1,1′-binaphthyl-2,2′-disulfonimide ((*S*)-4.70). (*S*)-**4.68** (1.00 g,

1.63 mmol) was dissolved in HOAc (75 ml) by heating. The solution was cooled to rt and H_2O (20 ml) was added. Cl_2 was bubbled thru at 2-3 bubbles per second for 20 min. After purging with N_2, the solid was collected by filtration to give crude (*S*)-**4.69** as a light yellow solid (714 mg). LRMS (EI) *m/z* (%) 606 (M+4, 20), 604 (M+2, 66), 602 (M^+, 85), 504 (28), 468 (92), 436 (38), 420 (94), 404 (100), 403 (59), 326 (31); HRMS (EI) calcd for $C_{32}H_{20}Cl_2O_4S_2$ 602.0180; found 602.0174. This material was used for the next step without any further purification.

(*S*)-**4.70** was prepared using the same procedure described for the preparation of (*S*)-**4.64**. Crude (*S*)-**4.69** (490 mg), benzene (100 ml), NH_3/EtOH (2.6M, 10 ml), O/N at rt. Additional NH_3/EtOH (2.6M, 10 ml), then 6 h at rt Flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 10:2:0.5) gave (*S*)-**4.70** as a white solid (215 mg, 34% over 2 steps from (*S*)-**4.68**). Mp: 309-311 ^oC; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.04-7.89 (m, 4H), 7.55-6.89 (m, 20H, 16 H_{Ar} and NH_4); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 142.2 (C_{Ar}), 138.9 (C_{Ar}), 136.1 (C_{Ar}), 133.1 (C_{Ar}), 132.3 (C_{Ar}), 132.1 (CH_{Ar}), 128.6 (CH_{Ar}), 128.0 (CH_{Ar}), 127.9 (CH_{Ar}), 127.2 (CH_{Ar}), 126.8 (CH_{Ar}); LRMS (ESI) *m/z* (%) 546.1510 (100); HRMS (ESI) calcd for $C_{32}H_{20}NO_4S_2$ 546.0834; found 546.0751.



4.71

2-(*N*,*N*-Dimethylcarbamoylthio)-2′-thio-1,1′-binaphthalene (4.71). To a solution of **4.32**[18] (115 mg, 0.250 mmol) and TMEDA (0.18 ml) in dry THF (12 ml) at -78 ^oC was added *sec*-BuLi (0.85 ml, 1.3 M in hexane, 1.1 mmol, 4.4 equiv) slowly over 15 min. After stirring for 1 h at -78 ^oC, TMSCl (0.5 ml) was added and reaction was stirred for 1 h at -78 ^oC, then 1 h at rt before quenching

with sat. NH_4Cl. The mixture was extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (EtOAc/hexane, 1:2) to give **4.71** as a white solid (70 mg, 72%). Mp: 168-170 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 7.84-7.81 (m, 3H), 7.52 (d, J = 8.7 Hz, 2H), 7.48 (t, J = 7.6 Hz, 1H), 7.37 (t, J = 7.4 Hz, 1H), 7.26 (t, J = 7.9 Hz, 1H), 7.21 (t, J = 7.3 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 3.42 (s, 1H, SH), 2.76 (s, 3H, CH_3), 2.62 (s, 3H, CH_3); LRMS (EI) *m/z* (%) 389 (M^+, 10), 316 (4), 284 (100), 282 (38), 72 (26); HRMS (EI) calcd for $C_{23}H_{19}NOS_2$ 389.0908; found 389.0904.

4.5 Reference

1. Kotoris, C.; Wen, W.; Taylor, S.D. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1271.

2. For recent reviews see: (a) Hamashima, Y.; Sodeoka, M. *Synlett* **2006**, 1467. (b) Ibrahim, H.; Togni, A. *Chem. Commun.* **2004**, *10*, 1147. (c) Prakash, G. K. S.; Beier, P. *Angew. Chem. Int. Ed.* **2006**, *45*, 2172.

3. Isanbor, C.; O'Hagan, D. *J. Fluorine Chem.* **2006**, *127*, 303.

4. Differding E.; Lang, R. W. *Tetrahedron Lett.* **1988**, *29*, 6087.

5. (a) Davis, F. A.; Zhou P.; Murphy, C. K. *Tetrahedron Lett.* **1993**, *34*, 3971. (b) Davis, F. A.; Zhau, P.; Murphy, C. K.; Sundarababu, G.; Qi, H.; Han, W.; Przeslawski, R. M.; Chen, B.-C.; Carroll, P. J. *J. Org. Chem.* **1998**, *63*, 2273.

6. (a) Takeuchi, Y.; Satoh, A.; Suzuki, T.; Kameda, A.; Dohrin, M.; Satoh, T.; Koizumi T.; Kirk, K. L. *Chem. Pharm. Bull.* **1997**, *45*, 1085. (b) Takeuchi, Y.; Suzuki, T.; Satoh, A.; Shiragami, T.; Shibata, N. *J. Org. Chem.* **1999**, *64*, 5708.(c) Shibata, N.; Liu, Z.; Takeuchi, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1954. (d) Liu, Z.; Shibata, N.; Takeuchi, Y. *J. Org. Chem.* **2000**,

65, 7583.

7. (a) Cahard, D.; Audouard, C.; Plaquevent, J.-C.; Roques, N. *Org. Lett.* **2000**, *2*, 3699; (b) Cahard, D.; Audouard, C.; Plaquevent, J.-C.; Toupet, L.; Roques, N. *Tetrahedron Lett.* **2001**, ***42***, 1867; (c) Baudequin, C.; Loubassou, J.-F.; Plaquevent, J.-C.; Cahard, D. *J. Fluorine Chem.* **2003**, ***122***, 189. (a) Shibata, N. Suzuki, E.; Takeuchi, Y. *J. Am. Chem. Soc.* **2000**, ***122***, 10728; (b) Shibata, N.; Suzuki, E.; Asahi, T.; Shiro, M. *J. Am. Chem. Soc.* **2001**, *123*, 7001; (c) Takahashi, T.; Fukuishima, A.; Tananka, Y.; Takeuchi, Y.; Kabuto, K.; Kabuto, C. *Chem. Commun.* **2000**, 788; (d) Mohar, B.; Baudoux, J.; Plaquevent, J.-C.; Cahard, D. *Angew. Chem. Int. Ed.* **2001**, *40*, 4214.

8. (a) Hintermann, L., Togni, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 4359. (b) Piana, S.; Devillers, I.; Togni, A.; Rothlisberger, U. *Angew. Chem. Int. Ed.* **2002**, *41*, 979.

9. (a) Hamashima, Y.; Yagi, K.; Takano, H.; TamOs, L.; Sodeoka, M. *J. Am. Chem. Soc.* **2002**, *124*, 14530. (b) Hamashima, Y.; Suzuki, T.; Shimura, Y.; Shimizu, T.; Umebayashi, N.; Tamura, T.; Sasamoto, N.; Sodeoka, M. *Tetrahedron Lett.* **2005**, *46*, 1447. (c) Hamashima, Y.; Suzuki, T.; Takano, H.; Shimura, Y.; Sodeoka, M.; *J. Am. Chem. Soc.* **2005**, *127*, 10164. (d) Hamashima, Y.; Toshiaki Suzuki, T.; Hisashi Takano, H.; Shimura, Y.; Tsuchiya, Y.; Moriya, K.; Goto, T.; Sodeoka, M. *Tetrahedron* **2006**, *62*, 7168.

10. Ma, J-A.; Cahard, D. *Tetrahedron Asymmetry* **2004**, *15*, 1007.

11. Shibata, N.; Kohno, J.; Takai, K.; Ishimaru, T.; Nakamura, S.; Toru, T.; Kanemasa, S. *Angew. Chem. Int. Ed.* **2005**, *44*, 4204.

12. (a) Enders, D.; Hüttl, M. R. M. *Synlett* **2005**, 991. (b) Marigo, M.; Fielenbach, D.; Braunton,

A.; Kjoersgaard, A.; Jøgensen, K. A. *Angew. Chem. Int. Ed.* **2005**, *44*, 3703. (c) Steiner, D. D.; Mase, N.; Barbas Ш, C. F. *Angew. Chem. Int. Ed.* **2005**, *44*, 3706. (d) Beeson, T. D.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2005**, *127*, 8826.

13. Taylor, S. D.; Kotoris, C.; Hum. G. H. *Tetrahedron*, **1999**, *55*, 12431.

14. (a) Uraguchi, D.; Terada, M. *J. Am. Chem. Soc*. **2004**, *126*, 5356. (b) Akiyama, T.; Itoh, J.; Yokayta, K.; Fuchibe, K. *Angew. Chem. Int. Ed*. **2004**, *43*, 1566.

15. Cox, P. J.; Wang, W.; Snieckus, V. *Tetrahedron Lett.* **1992**, *33*, 2253.

16. Wu, T., R.; Shen, L.; Chong, J. M. *Org. Lett.* **2004**, *6*, 2701.

17. Ooi, T.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **2003**, *125*, 5139.

18. Cossu, S.; De Lucchi, O.; Fabbri, D.; Valle, G.; Painter, G. F.; Smith, R. A. J. *Tetrahedron* **1997**, *53*, 6073.

19. Moseley, J. D.; Sankey, R. F.; Tang, O. N.; Gilday, J. P. *Tetrahedron*, **2006**, *62*, 4685.

20. (a) Relles, H. M.; Pizzolato, G. *J. Org. Chem.* **1968**, *33*, 2249. (b) Miyazaki, K.; *Tetrahedron Lett.* **1968**, *33*, 2758. (c) Kaji, A.; Araki, Y.; Miyazaki, K. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1393.

21. Reggelin, M.; Doerr, S. *Synlett* **2004**, *6*, 1117.

22. Burchat, A. F.; Chong, J. M.; Nielsen, N. *J. Organomet. Chem.* **1997**, *542*, 281.