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Synthesis and evaluation of analogues of estrone-3-*O*-sulfamate as potent steroid sulfatase inhibitors

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ABSTRACT

Estrone sulfamate (EMATE) is a potent irreversible inhibitor of steroid sulfatase (STS). In order to further expand SAR, the compound was substituted at the 2- and/or 4-positions and its 17-carbonyl group was also removed. The following general order of potency against STS in two in vitro systems is observed for the derivatives: The 4-NO_2 > 2-halogens, 2-cyano > EMATE (unsubstituted) > 17-deoxyEMATE > 2-NO_2 > 4-bromo > 2-(2-propenyl), 2-*n*-propyl > 4-(2-propenyl), 4-*n*-propyl > 2,4-(2-propenyl) = 2,4-di-*n*-propyl. There is a clear advantage in potency to place an electron-withdrawing substituent on the A-ring with halogens preferred at the 2-position, but nitro at the 4-position. Substitution with 2-propenyl or *n*-propyl at the 2- and/or 4-position of EMATE, and also removal of the 17-carbonyl group are detrimental to potency. Three cyclic sulfamates designed are not STS inhibitors. This further confirms that a free or N-unsubstituted sulfamate group (H_2NSO_2O–) is a prerequisite for potent and irreversible inhibition of STS as shown by inhibitors like EMATE and Irosustat. The most potent derivative synthesized is 4-nitroEMATE (**2**), whose IC_{50}s in placental microsomes and MCF-7 cells are respectively 0.8 nM and 0.01 nM.

1. Introduction

It is reasoned that steroid sulfatase (STS) has a significant role in fueling the growth and development of hormone-dependent diseases, since the enzyme hydrolyses biologically inactive steroid sulfates back to their active unconjugated forms. In hormone-dependent breast cancer for example, STS is responsible for the conversion of estrone sulfate (E1S) to estrone (E1) and also dehydroepiandrosterone sulfate (DHEA-S) to DHEA. The formation of DHEA by the STS pathway constitutes the production of 90% of androstenediol which is an estrogen (ca. 100-fold weaker than estradiol), despite structurally being classified as an androgen.[1–4] On this basis, the inhibition of STS in this type of cancer might lead to estrogen deprivation and hence render therapeutic intervention in the malignancy.

Once STS had been identified as a target for delivering a potential new form of endocrine therapy, considerable attention was placed on designing inhibitors of this enzyme over the past two decades. One approach was to replace the sulfate group (OSO_3^-) of E1S with other sulfate surrogates or mimics that are not metabolically labile or hydrolysable by the enzyme in the course of competing with E1S for binding to the enzyme active site.[5–9] Despite the large number of compounds that have been explored in this category, none of these compounds was deemed to be potent enough or adequately attractive pharmaceutically for further development as an inhibitor of STS.

The breakthrough in the design of potent STS inhibitors came when the sulfate group of E1S was replaced by a sulfamate moiety (–OSO_2NH_2).[10] Estrone 3-*O*-sulfamate (EMATE) (Fig. 1) was found to be a highly potent inhibitor with an IC_{50} of 18 nM in a human placental microsomes preparation.[11] More exceptionally, EMATE inhibits STS in a time- and concentration-dependent manner, indicating that the mechanism of action is of an irreversible nature. EMATE is orally active in vivo. In one study,[12] administration at 10 mg/kg inhibited rat liver STS activity almost completely (99%) when given by the oral or subcutaneous route and the inhibition persisted (>95%) for up to 7 days after a single dosing. However, EMATE was unexpectedly shown to be more estrogenic than ethinylestradiol when administered orally in rats,[13] although it was revealed subsequently that estrogen sulfamates per se do not bind to the estrogen receptor.[14] A follow-up study demonstrated that STS has a crucial role in regulating the estrogenicity associated with EMATE as its estrogenicity is abolished when STS is inhibited by co-administration of the non-estrogenic STS inhibitor Irosustat (STX64, Fig. 1).[15] Nonetheless, this unwanted estrogenic property rendered the inhibitor undesirable for use as an anti-endocrine agent for hormone-dependent cancers. However, a congener of EMATE, estradiol

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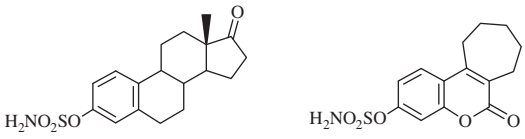


Figure 1. Structures of EMATE (estrone 3-*O*-sulfamate) and the non-steroidal STS inhibitor, Irosustat (STX64, BN83495).

3-*O*-sulfamate, entered human clinical trials in the late 90s as an orally active pro-drug for estradiol and good activity has also been reported in animal models of endometriosis, as an STS inhibitor.[16] It is currently in Phase IIa clinical trial for endometriosis.

As EMATE remains an interesting compound because of its diverse pharmacological properties, we further expand the SAR studies in this work. To this end, we substituted at the 2- and/or 4-position of A-ring with a nitro group, halogens, alkyl groups and a cyano group. The D-ring was modified by removal of the C17 carbonyl group. In addition, three compounds were prepared which can be loosely described as 'cyclic sulfamate' derivatives of EMATE as the N-atom of the sulfamate group of EMATE is bonded to either the 2- or 4-position of the steroid scaffold. The synthesized compounds were tested for STS inhibitory activity in a placental microsomes preparation and also in MCF-7cells.

2. Chemistry

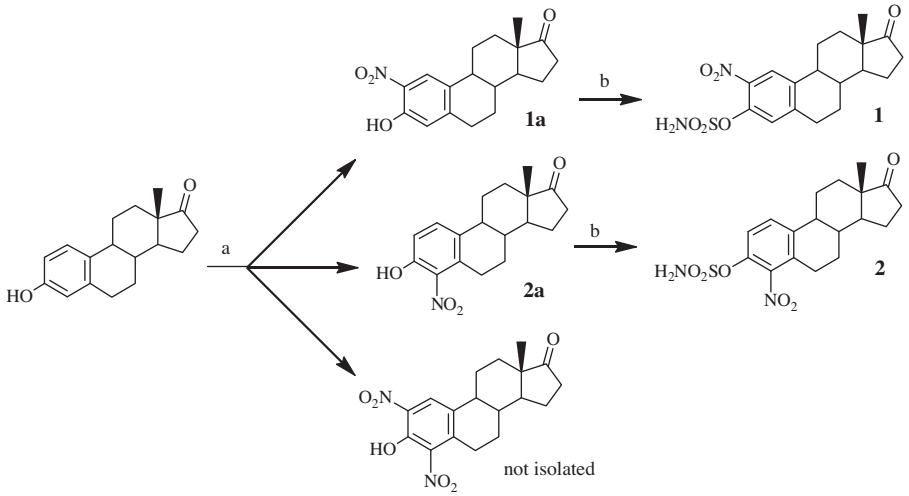
2-Nitroestrone (**1a**, Scheme 1) and 4-nitroestrone (**2a**, Scheme 1) were prepared by nitrating estrone according to the method of Tomson and Horwitz.[17] Compound **2a** came out of the reaction mixture overnight and was deposited as a yellow precipitate whereas **1a** was isolated from the filtrate and purified by several cycles of recrystallization. An earlier method was employed for the sulfamoylation of these nitrated estrones (Scheme 1). This involved treating a solution of **1a** or **2a** in anhydrous *N*,*N*-dimethylformamide (DMF) with sodium hydride (NaH) followed by addition of a freshly concentrated solution of sulfamoyl chloride in toluene, which was prepared according to the method of Woo et al.[18]

The synthesis of 2- and 4-alkylestrone was carried out as described by Patton.[19] A Claisen rearrangement of the intermediate 3-*O*-(2-propenyl)estrone (**3**, Scheme 2) to give 2- and 4-(2-propenyl)estrone was straightforward although the separation of the geometrical isomers obtained was particularly cumbersome. 2-(2-Propenyl)estrone (**4a**, Scheme 2) was only isolated in poor yield upon repeated and slow (over two weeks) fractional recrystallizations of the crude material in diethyl ether. Despite careful fractional recrystallization of the crude 4-(2-propenyl)estrone (**5a**, Scheme 2), this isomer could not be obtained in a pure state and the best sample in our hands contained approximately 25% of **4a** as shown by ^{1}H NMR. When a mixture of 2-(2-propenyl)- and 4-(2-propenyl)estrones in DMF was heated with 3-bromoprop-1-ene in the presence of NaH, a mixture of the 3-*O*-(2-propenyl) derivatives of **4a** and **5a** (**6** and **7**, respectively, Scheme 2) was obtained. When this mixture was subjected to Claisen rearrangement conditions 2,4-di-(2-propenyl)estrone (**8a**, Scheme 2) was obtained in good yield. Compounds **4a**, **5a** and **8a** were sulfamoylated in the same manner as **1a** to give sulfamates **4**, **5** and **8**, respectively.

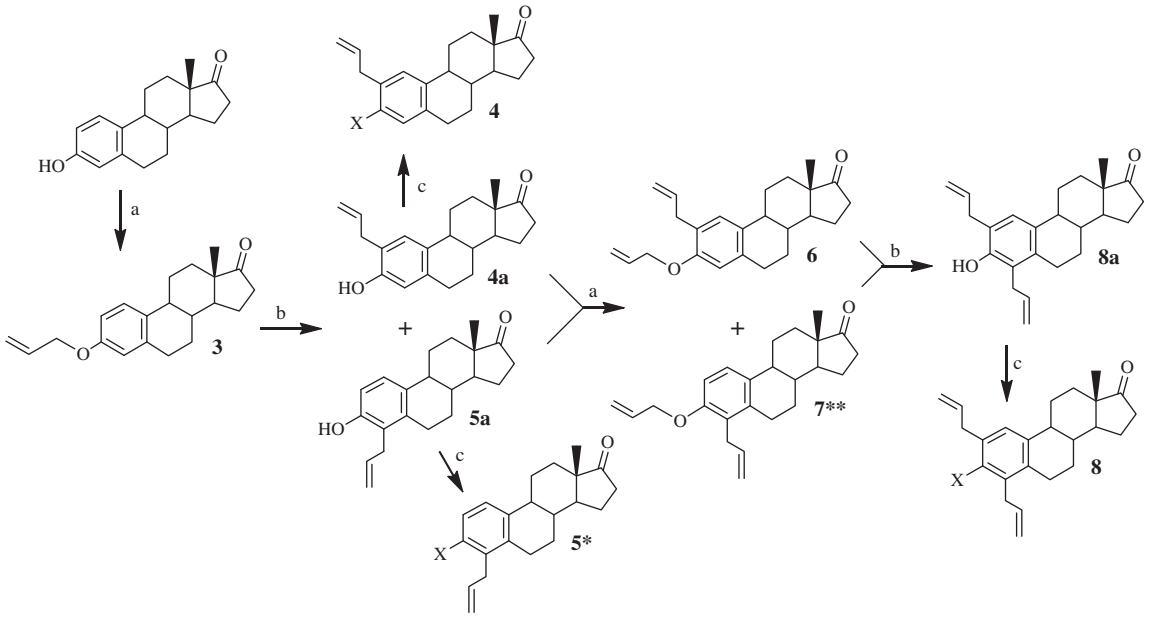
Initially, 2-*n*-propyl- (**9**), 4-*n*-propyl- (**10**) and 2,4-di-*n*-propyl- (**11**) EMATEs were obtained by sulfamoylating the corresponding parent phenols that were obtained upon hydrogenation of the (2-propenyl)estrones. However, it was found subsequently that the sulfamate group is not labile to the hydrogenation conditions employed and hence 2-(2-propenyl)-(**4**), 4-(2-propenyl)-(**5**) and 2,4-*n*-di-(2-propenyl)-(**8**) EMATEs were hydrogenated directly to give, respectively, 2-*n*-propyl-(**9**) and 4-*n*-propyl- (**10**) and 2,4-di-*n*-propyl-(**11**) EMATEs (Scheme 3). It is worth noting that while repeated attempts to recrystallize **5** had failed to afford a pure sample other than a batch that was co-recrystallized with **4**, pure **10** without contaminating by isomer **9** was obtained when the crude material was recrystallized from ethyl acetate/hexane.

The route to 2-fluoroestrone **12b** (Scheme 4) was described by Page et al.[20] and involved an electrophilic fluorination of estrone by Umemoto's reagent, *N*-fluoropyridinium triflate, followed by a direct acetylation using acetic anhydride in pyridine in order to facilitate purification of the fluorinated estrone derivative **12a**. In our hands, a low yield (12%, after recrystallization) of **12a** was obtained from estrone, primarily as a result of competing formation of other by-products (presumably 2- and 4-chloroestrones). Upon deacetylation of **12a** using potassium carbonate in methanol, 2-fluoroestrone (**12b**) was obtained which was purified by preparative TLC (45% yield). Sulfamoylation of **12b** with sulfamoyl chloride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) as base gave 2-fluoroEMATE (**12**) in 57 % yield.

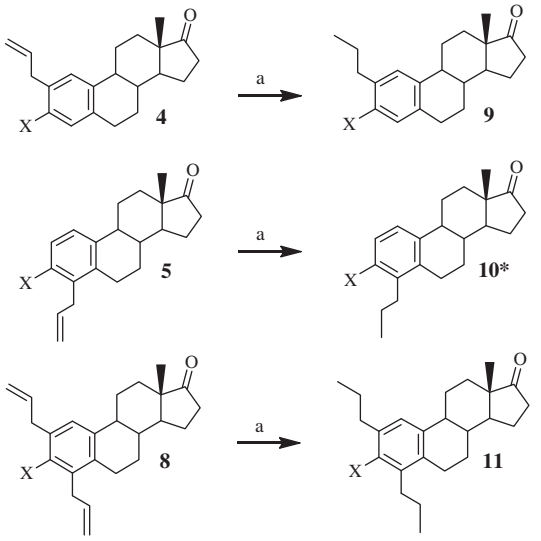
Several procedures for preparing the 2-chloro, 2-bromo, 2-iodo and 2-cyano derivatives of estrone are available but few of these



Scheme 1. Nitration of estrone to give predominately 2-nitroestrone (**1a**) and 4-nitroestrone (**2a**) which upon sulfamoylation gave their respective sulfamates (**1**) and (**2**). Reagents and conditions: (a) concd HNO_3/glacial acetic acid, 70–75 °C to rt; (b) NaH/DMF, H_2NSO_2Cl, 0 °C.



Scheme 2. Synthesis of 2-(2-propenyl)estrone 3-*O*-sulfamate (**4**), 4-(2-propenyl)estrone 3-*O*-sulfamate (**5**) and 2,4-di-(2-propenyl)estrone 3-*O*-sulfamate (**8**) via various intermediates (**3**), (**4a**), (**5a**), (**6**), (**7**) and (**8a**). Reagents and conditions: (a) NaH/DMF, 3-bromoprop-1-ene, 80 °C, 2 h; (b) *N*,*N*-diethylaniline, N_2, reflux, 6 h; (c) NaH/DMF, H_2NSO_2Cl, 0 °C. X = OSO_2NH_2 *contains ca. 25% of **4a** as shown by ^1H NMR; **impure, contains **6**.



Scheme 3. Hydrogenation of (2-propenyl)estrone sulfamates (**4**), (**5**) and (**8**) to give respectively 2-*n*-propylestrone 3-*O*-sulfamate (**9**), 4-*n*-propylestrone 3-*O*-sulfamate (**10**) and 2,4-di-*n*-propylestrone 3-*O*-sulfamate (**11**). Reagents: (a) Pd-C(10%)/absolute EtOH, H_2, 50 psi. X = OSO_2NH_2 *impure.

are regioselective, that is functionalizing predominantly at position 2. Compounds **15b**–**18b** (Scheme 4) were prepared as described by Page et al.[20] The direct arene thalliation with thallic trifluoroacetate (or triacetate) in trifluoroacetic acid and subsequent displacement of the estrogen–thallium(III) bis(trifluoroacetate) intermediate with copper(I) halides (halides = Cl, Br, I) and copper(I) cyanide gave a convenient regioselective method for the synthesis of 2-substituted estrones. Sulfamoylation of compounds **15b**–**18b** was carried out in a similar manner to **1a** to give the sulfamates **15**–**18**.

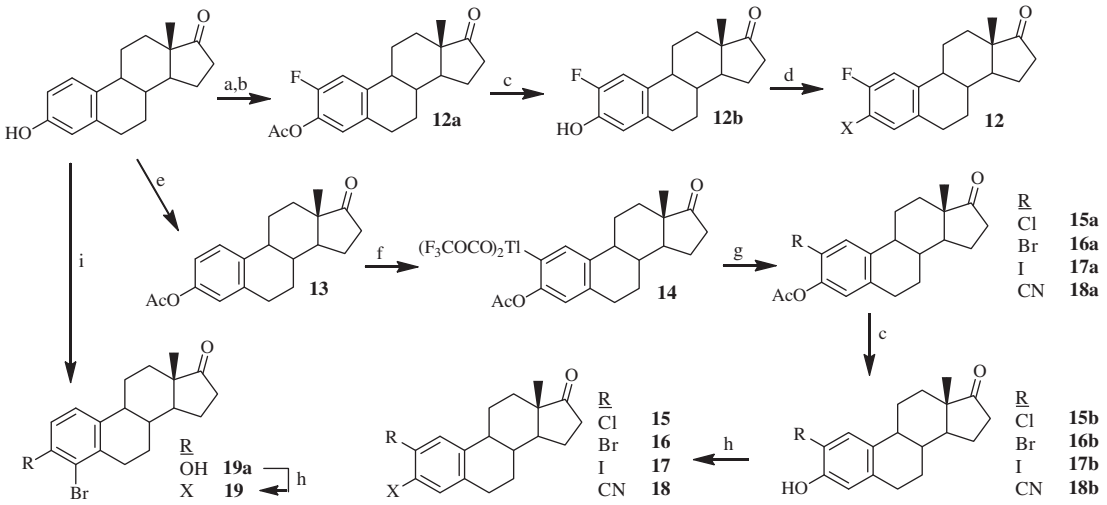
Slaunwhite and Neely[21] reported that 4-bromoestrone (**19a**) was obtained in high yield when a solution of estrone in aqueous acetic acid (contained 15–20% of water) was treated with 5% v/v of bromine in acetic acid in the presence of a small amount of powdered iron. In contrast, when aqueous acetic acid was replaced with glacial acetic acid, the authors reported that the bromination reaction yielded predominately 2-bromoestrone. However, in our hands, **19a** was obtained as the main product when a solution of estrone in glacial acetic acid (Scheme 4) was brominated under the conditions used. The sulfamoylation of **19a** to give sulfamate **19** was carried out in the same manner as **1a**.

The carbonyl group of estrone was reduced to a methylene group under Wolff–Kishner conditions for reduction of steroid ketones.[22] The product 3-hydroxy-1,3,5(10)-estratriene (**20a**, Scheme 5) was sulfamoylated in the same manner as **1a** to give 1,3,5(10)estratriene 3-*O*-sulfamate (**20**).

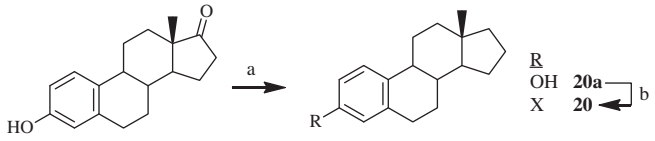
The chemistry developed by Andersen et al.[23,24] for the synthesis of 1,2,3-benzoxathiazole-2,2-dioxides was applied to the synthesis of 'cyclic sulfamates' (**21**), (**22**) and (**23**). The starting 2-amino (**21a**) and 4-aminoestrone (**23a**) were easily obtained by hydrogenating 2-nitro- (**1a**) and 4-nitroestrone (**2a**) respectively in the presence of palladium-charcoal. Whilst **23a** appears to be stable, decomposition of **21a** upon standing and exposure to air at room temperature was observed, as reported by Kraychy,[25] and hence **21a** was used without further purification. The selective N-tosylation of **21a** and **23a** with tosyl chloride to give 2- (**21b**) and 4-tosylamidoestrone (**23b**), respectively, was carried out in the presence of pyridine using conditions reported by Kurita[26] for the selective N-tosylation of *o*-aminophenol. Ring closure of **21b** and **23b** to give the respective cyclic compounds **21c** and **23c** was carried out with sulfuryl chloride in dichloromethane. Removal of the protecting tosyl group of **21c** and **23c** with an aqueous solution of potassium fluoride gave **21** and **23** in moderate to good yields. Deprotonation of **21** in DMF with NaH followed by refluxing with methyl iodide gave the N-methylated derivative **22**.

3. Biological results and discussion

The in vitro inhibitory activities of compounds against STS in a placental microsomes preparation are shown in Table 1. We reported the biological activity of **1**, **2**, **4**, **5**, **8**–**11** earlier[27] but herein



Scheme 4. Synthesis of 2- and 4-substituted derivatives of EMATE. Reagents and conditions: (a) *N*-fluoropyridinium triflate/1,2-trichloroethane, reflux, N_2, 24 h; (b) pyridine/acetic anhydride, reflux, 2 h; (c) K_2CO_3/MeOH, reflux, 3 h; (d) DBMP/DMF, H_2NSO_2Cl, 0 °C; (e) $(CH_3CO)_2O$/pyridine, reflux, 2 h; (f) $Tl(OCOCF_3)_3/CF_3COOH$, 0–5 °C, N_2, 24 h; (g) CuX/1,4-dioxane (X = Cl, Br, I, CN); (h) NaH/DMF, H_2NSO_2Cl, 0 °C; (i) Br_2/glacial acetic acid/powdered Fe, rt. X = OSO_2NH_2.



Scheme 5. Wolff–Kishner reduction of estrone and synthesis of sulfamate **20**. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, KOH/diethylene glycol, reflux; (b) NaH/DMF, H_2NSO_2Cl, 0 °C. X = OSO_2NH_2.

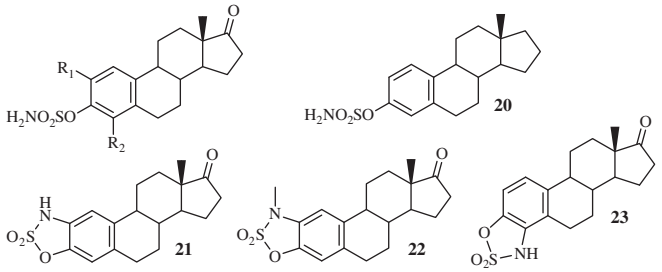
report full experimental data. Importantly, in order to assess the ability of compounds to cross the cell membrane and inhibit STS under conditions that closely resemble the tissue/physiological situation, we newly evaluate compounds (apart from **21**–**23**) in intact MCF-7 cells. The results are shown in Table 1.

We have demonstrated repeatedly in previous work that the STS inhibitory activity of an aryl sulfamate in general can be increased by either placing an electron-withdrawing group adjacent to the sulfamate group,[30–38] or by having the sulfamate group attached to an aryl ring system such as coumarin,[39–41] that renders the parent phenol a better leaving group by virtue of lowering its pK_a value. With the exception of **1**, the same effect is observed for **2**, **12**, **15**–**18** when tested in a placental microsome preparation since these sulfamates were found to be more potent than EMATE as STS inhibitors. A similar trend is observed for STS inhibition in MCF-7 cells with the exception of **17** and **18** which are comparable and slightly weaker inhibitors than EMATE, respectively. As shown in Table 2, the pK_a values of various substituted phenols, 2-nitroestrone (**1a**) and 4-nitroestrone (**2a**) are between 1 and 3 log units lower than that of an unsubstituted phenol such as estrone. We postulate in general that this lowering of pK_a value of the parent phenol improves its leaving group ability which in return facilitates the inactivation of the enzyme by its corresponding sulfamate through sulfamoylation of an essential amino acid residue in the active site.

Unexpectedly, 2-nitroEMATE (**1**) is less potent than EMATE as an STS inhibitor while its congener 4-nitroEMATE (**2**) is significantly more potent than EMATE despite their parent phenols sharing a similar calculated pK_a value of around 7 (Table 1). We reason that this result might be due to potential interaction(s) between the nitro group at the 2-position and the sulfamate group at the 3-position in **1**, rendering the sulfamate group either less stable chemically and/or less effective in inactivating the enzyme via sulfamoylation.[27] Due to steric hindrance presented by the neighbouring hydrogen atom(s) at C6, the nitro group at the 4-position of **2** might not be amenable to having potential interactions(s) with its sulfamate group at the 3-position, rendering it relatively more stable and effective in sulfamoylating the enzyme.[27] Another possible explanation for the higher STS inhibitory activity observed for **2** is that its 4-nitro group might interact with the enzyme active site favorably. A recent study carried out by Taylor and co-workers[42] has shown that 2-nitroestrone (**1a**, IC_{50} = 17 μM) was sevenfold less potent in a purified STS assay as an STS inhibitor than its 4-nitro isomer (**2a**, IC_{50} 2.4 μM). The authors attributed this finding to factors such as hydrogen bonding ability and/or other interactions of the nitro group placed at the 4-position of the steroid ring. Favorable interactions between a substituent and neighboring amino acid residue(s) in the active site have been observed before. For instance, 2-difluoromethylestrone 3-*O*-sulfamate was found to have an IC_{50} of 100 pM against STS in a placental microsomes preparation.[28] It has been reasoned that the electron-withdrawing effect of the 2-fluoromethyl group as well as the potential of the fluorine atoms in forming hydrogen bond with residues lining the catalytic active site of STS are contributive factors to the high potency observed.

Derivatives **12** and **15**–**17**, which are substituted with a halogen at the 2-position, are clearly more potent STS inhibitors than EMATE when tested in placental microsomes. The activities of these halogenated compounds are of the same order of magnitude, although the bromo congener is the most potent inhibitor of the series with an IC_{50} of 1.65 nM. When tested in MCF-7 cells, only **15** and **16** are significantly more potent than EMATE. The activities of **12** and **17** are considered to be similar to that of EMATE. Given the pK_a value of their respective parent phenol is similar at around 8.5 (Table 2), other factors such as the lipophilicity of compounds might influence the different in vitro activity observed. In general, a more lipophilic inhibitor has a higher affinity for the enzyme active site and hence exhibits better inhibitory activity than one that is less lipophilic. As fluoro derivative **12** is the least lipophilic, whereas iodo derivative **17** is the most lipophilic, one might expect the potency of these 2-halogenated derivatives of EMATE against STS to be in an ascending order from **12** to **17**. However, the fact that **17** ($IC_{50(P.M.)}$ = 6.1 nM and $IC_{50(MCF\text{-}7)}$ = 0.81 nM, Table 1) is a weaker STS inhibitor than its bromo congener **16** ($IC_{50(P.M.)}$ = 1.65 nM and

Table 1
Inhibition of STS in a placental microsomes (P.M.) preparation and in MCF-7 cells by derivatives of EMATE **1**, **2**, **4**, **5**, **8**–**12**, **15**–**18**, **19** and **20**, and 'cyclic sulfamates' **21**–**23**



Compd[d]	R_1	R_2	P.M. IC_{50}[a] (nM)	MCF-7 IC_{50}[a] (nM)/% inhibition[a]
EMATE	H	H	18[b]	0.83[d]
1	NO_2	H	70[c]	8.3
2	H	NO_2	0.8[c]	0.01
4	2-Propenyl	H	2500[c]	37
5[e]	H	2-Propenyl	9000[c]	153
8	2-Propenyl	2-Propenyl	>10,000[c]	<10%@10 μM
9	*n*-Propyl	H	2900[c]	236
10	H	*n*-Propyl	>10,000[c]	<10%@10 μM
11	*n*-Propyl	*n*-Propyl	>10,000[c]	<10%@10 μM
12	F	H	5.6	0.62
15	Cl	H	3.4	0.44
16	Br	H	1.65	0.11
17	I	H	6.1	0.81
18	CN	H	5.2	1.2
19	H	Br	1200	42
20	—		199	97%@0.01 μM
21	—		>10,000	nd
22	—		>10,000	nd
23	—		>10,000	nd

[a] Unless stated otherwise, errors are <5% of the reported value (from triplicate experiments).
[b] Ref. 11, cf. 0.4 nM[27], 9.1 nM[28].
[c] Ref. 27.
[d] IC_{50} 0.065 nM.[29]
[e] Contains 25% of 2-(2-propenyl)EMATE (**4**); — not applicable; nd: not determined.

Table 2
pK_a values of various phenols, 2-nitroestrone (**1a**) and 4-nitroestrone (**2a**) as determined by ACD/Labs Software v 11.02

Compound	pK_a
Phenol	9.86 ± 0.10
2-Fluorophenol	8.71 ± 0.10
2-Chlorophenol	8.50 ± 0.10
2-Bromophenol	8.43 ± 0.10
2-Iodophenol	8.52 ± 0.10
2-Cyanophenol	7.17 ± 0.10
2-Nitrophenol	7.14 ± 0.14
1a	7.43 ± 0.40
2a	7.10 ± 0.40

$IC_{50(MCF\text{-}7)}$ = 0.11 nM, Table 1) suggests that steric hindrance rendered by the bulkier iodine atom of **17** interferes with the binding of its sulfamate group to the catalytic site, preventing the inhibitor from inactivating the enzyme effectively. Hence, for derivatives of EMATE substituted at the 2-position with a halogen, it appears that having a bromine atom is most optimized for STS inhibition and binding of the inhibitor to STS active site. It is worth noting that a recent study carried out by Lu et al.[43] provides evidence for weak halogen-oxygen bonding between halogenated ligands and protein kinases, with the strength of the interactions calculated decreases in the order H ≈ I > Br > Cl. Although it is much weaker than hydrogen bonding, the authors argue that halogen-bonding may play an important role in inhibitor recognition and binding. With reference to our work here, it is not clear whether halogen-oxygen bonding plays a role in the binding of halogenated EMATEs to the STS active site.

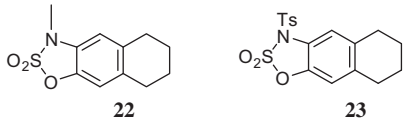
While brominating EMATE at the 2-position results in a highly potent STS inhibitor **16**, substitution of EMATE at the 4-position with a bromine atom is detrimental to activity. As shown in Table 2, the IC_{50} values of the isomer 4-bromoEMATE (**19**) against STS in placental microsomes (1200 nM) and MCF-7 cells (42 nM) are a few orders of magnitude lower than those of EMATE ($IC_{50(P.M.)}$ = 18 nM and $IC_{50(MCF\text{-}7)}$ = 0.83 nM) and **16** ($IC_{50(P.M.)}$ = 1.65 nM and $IC_{50(MCF\text{-}7)}$ = 0.11 nM). Given the electron-withdrawing effect of the bromine atom in **16** and **19** is expected to be similar, it is possible that any beneficial interaction provided by a bromine atom is better recognized by the enzyme active site when it is substituted at the 2-position of EMATE.

In placental microsomes, the IC_{50} value observed for **20** (17-deoxyEMATE, IC_{50} = 199 nM, Table 2) is 11-fold higher than that of EMATE (IC_{50} = 18 nM, Table 2). Hence, removal of the 17-carbonyl group of EMATE significantly reduces STS inhibitory activity, demonstrating an important role for hydrogen bonding between the D-ring of steroidal inhibitor and the active site. However, not all disruptions made to the D-ring of EMATE are detrimental. Previous work has shown that 3-sulfamoyloxy-16,17-*seco*-estra-1,3,5(10)-triene-16,17-imide, a steroid-like derivative of EMATE, is equipotent to EMATE as an STS inhibitor.[11]

As shown in Table 1, all derivatives of EMATE substituted at the 2- and/or 4-positions with an aliphatic group are weak STS inhibitors. However, substitution of 2-propenyl or *n*-propyl group at the 2-position is better tolerated by the enzyme than that at the

4-position as shown by the higher inhibitory activity observed for **4** ($IC_{50(P.M.)}$ = 2500 nM and $IC_{50(MCF\text{-}7)}$ = 37 nM) and **9** ($IC_{50(P.M.)}$ = 2900 nM and $IC_{50(MCF\text{-}7)}$ = 236 nM) than **5** ($IC_{50(P.M.)}$ = 9000 nM and $IC_{50(MCF\text{-}7)}$ = 153 nM) and **10** ($IC_{50(P.M.\ and\ MCF\text{-}7)}$s: >10,000 nM). One possible reason for this difference in activity between 2- and 4-alkylated derivatives is the steric hindrance exerted by the hydrogen atom(s) at the neighboring C6 atom on the substituent at the 4-position, restricting the rotation of alkyl groups and hence making them less tolerated by the enzyme. When considering substitution made at either the 2- or 4-positon alone, apparently there is no significant difference in the inhibitory activities observed between the more rigid 2-propenyl group and the more flexible *n*-propyl group (**4** vs **9**, and **5** vs **10**), suggesting that both groups are not well tolerated by the enzyme active site. Clearly, the steric hindrance posed by these groups is expected to be even more prominent when they are substituted at both the 2- and 4-positions of EMATE (**8** and **11**). The shielding of the sulfamate group from interacting effectively with the catalytic site of the enzyme by these aliphatic substituents might explain the overall weak STS inhibitory activity observed for compounds **4**, **5**, **8**, and **9**–**11**. It is interesting to note that 2-methylEMATE, a smaller chain relative of **9**, was found to be even a weaker STS inhibitor with an IC_{50} of over 10,000 nM in a placental microsomes preparation.[28] Despite a methyl group often being viewed to be isosteric and isolipophilic with the chlorine atom,[44] which means steric hindrance might not be the main detrimental factor, the hyperconjugative electron-donating effect of the methyl group of 2-methylEMATE on the A-ring, as reflected by the calculated pK_a of 10.31 for 2-methylphenol, might render the sulfamate group less effective in inactivating STS via sulfamoylation. The best 2-alkyl derivative of EMATE reported to date as an inhibitor of STS is 2-ethylEMATE. Its IC_{50} in a placental microsomes preparation was found to be 820 nM.[45]

We postulated in previous work several mechanisms through which an aryl sulfamate (e.g., EMATE) might inhibit STS.[7,40] Two of which are (i) nucleophilic attack on the sulfur atom of the sulfamoyl group by the hydrated form of formylglycine residue (*gem*-diol) in the STS active site; and (ii) specific or non-specific sulfamoylation by an aryl sulfamate of an essential nucleophilic amino acid residue in the STS active site. Andersen et al.[23,24] reported that some 1,2,3-benzoxathiazole 2,2-dioxides (e.g., **22** and **23**, Fig. 2) are reactive toward nucleophiles. When the candidate was 3-tosyl-1,2,3-benzoxathiazole 2,2-dioxides (**22**), hydroxide ion and amines attacked the endocyclic sulfur atom of cyclic sulfamate, resulting in ring opening. Given this observation, we prepared the cyclic sulfamate derivatives of EMATE, **19**, **20** and **21** (Scheme 6). The rationale is that if a nucleophilic residue in the enzyme active site attacks the sulfur atom of cyclic sulfamate, these cyclic sulfamates might exhibit STS inhibitory activities. In contrast to acyclic sulfamates like EMATE, where we expect the parent phenol to be released upon sulfamoylation of the enzyme by the sulfamate group, there should be no release of estrone expected for cyclic sulfamates **19**, **20** and **21** upon ring opening as their steroid skeleton should remain attached to the sulfamoyl group via its *N*-atom. Unfortunately, as shown in Table 2, none of these cyclic sulfamates was found to be active against STS. It is possible that the cyclic sulfamate moiety of **19**, **20** and **21** did not bind or align in a similar manner to the sulfamate group of EMATE in the catalytic site of STS for potential attack by a nucleophilic amino acid. Alternatively, the lack of an unsubstituted sulfamate group in **19**, **20** and **21** might be the culprit, as it has been shown earlier that a free sulfamate group (H_2NSO_2OAr) is prerequisite for potent irreversible inhibition of STS.[10,41,46]



22 23

Figure 2. Structures of 3-methyl-1,2,3-benzoxathiazole 2,2-dioxide (**22**) and 3-tosyl-1,2,3-benzoxathiazole 2,2-dioxide (**23**).

4. Conclusions

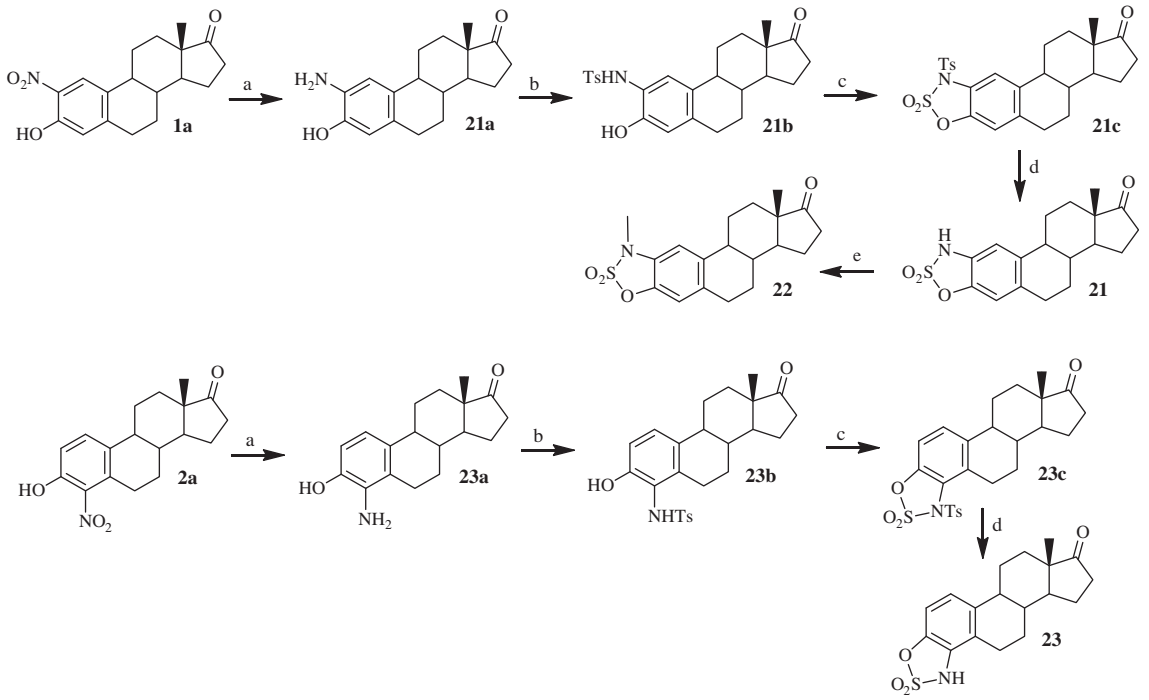
A series of 2- and/or 4-substituted derivatives of EMATE was prepared and its 17-carbonyl group removed. The following general order of potency against STS is observed: The 4-NO_2 > 2-halogens, 2-cyano > unsubstituted (EMATE) > 17-deoxyEMATE > 2-NO_2 > 4-bromo > 2-(2-propenyl), 2-*n*-propyl > 4-(2-propenyl), 4-*n*-propyl > 2,4-(2-propenyl) = 2,4-di-*n*-propyl. The general SAR derived from the studies indicates that EMATE derivatives that have electron-withdrawing substituents (4-nitro, 2-halogens and 2-cyano) on the A-ring, show a higher STS inhibition than EMATE in vitro, with the exception of having a nitro group at the 2-position which results in a significant lowering in potency. This reinforces the relationship between the pK_a value/leaving group ability of a parent phenol and the potential of an aryl sulfamate in the inactivation of STS, presumably through sulfamoylation of an essential amino acid residue. The introduction of a bromine at the 4-position of or removal of the 17-carbonyl group of EMATE is detrimental to activity. The derivatives resulted are significantly weaker STS inhibitors than EMATE. When the 2- and/or 4-positions of the A-ring are substituted with the bulkier 2-propenyl or 2-*n*-propyl groups, the potent STS inhibition shown by EMATE is significantly reduced or abolished. For an aryl sulfamate to be effective as an STS inhibitor, inter alia, its sulfamate group must be presented to the catalytic site of the enzyme unhindered and the required interactions with various key amino acid residues in the active site uninterrupted. Three cyclic sulfamates prepared are not STS inhibitors. This further confirms that a free or N-unsubstituted sulfamate group (H_2NSO_2O–) is a prerequisite for potent and irreversible inhibition of STS as shown by inhibitors like EMATE and Irosustat.

5. Experimental

5.1. General

All chemicals were purchased from either Aldrich Chemical Co. (Gillingham, UK) or Lancaster Synthesis (Morecombe, Lancashire, UK). All organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK). Anhydrous *N*,*N*-dimethylformamide (DMF), dichloromethane and pyridine were purchased from Aldrich. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[47] and was stored as a solution under N_2 in toluene as described by Woo et al.[48]

Thin layer chromatography (TLC) was performed on pre-coated plates (Merck TLC aluminium sheets Silica Gel 60 F_{254}, Art. No. 5554). Product(s) and starting material were detected by viewing under UV light and/or treating with a methanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed using gradient elution (solvents indicated in text) on wet-packed silica gel (Sorbsil C60). IR spectra were determined by a Perkin–Elmer 782 infrared spectrophotometer, and peak positions are expressed in cm^{-1}. ^1H, ^{13}C and ^{19}F NMR spectra were recorded with either a Jeol Delta 270 MHz or a Varian Mercury VX 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants *J* are given in Hz. Mass spectra were recorded at the Mass spectrometry Service Center,



Scheme 6. Synthesis of 'cyclic' derivatives of EMATE. Reagents and conditions: (a) Pd/C(10%)/CH_2Cl_2, H_2 (balloon), 20 h; (b) anhydrous pyridine, 0 °C, TsCl; (c) Et_3N/CH_2Cl_2, SO_2Cl_2/CH_2Cl_2 (0.5 M), −78 °C; (d) MeCN/aqueous KF, reflux, 3 h; (e) NaH/DMF, MeI, 90 °C, 3 h.

University of Bath. FAB mass spectra were measured using *m*-nitrobenzyl alcohol as the matrix. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected. HPLC was undertaken using a Waters 717 machine with Autosampler and PDA detector. The column used, conditions of elution and purity of sample are as indicated for each compound analysed.

5.2. Biology

Assays were performed essentially as described previously.[49] The ability of the compounds synthesized to inhibit STS activity was examined using placental microsomal preparations. Placental microsomes (100,000 g fraction) were prepared from a sulfatase-positive human placenta from a normal-term pregnancy.[50] To determine the IC_{50}s for the inhibition of STS, activity was measured in the presence of the inhibitor (0.1–10 μM) using [^{3}H]E1S (4×10^5 dpm) adjusted to 20 μM with unlabelled substrate. After incubation of the substrate-inhibitor with placental microsomes (125 μg of protein/mL) for 30 min, the product formed was isolated from the mixture by extraction with toluene (4 mL), using [4-^{14}C]E1 to monitor procedural losses.

Intact monolayers of MCF-7 breast cancer cells were incubated for 20 h at 37 °C with [^{3}H]E1S (5 pmol, 7×10^5 dpm, 60 Ci/mmol, NEN Du Pont, Boston, MA) in serum-free minimal essential medium (2.5 mL) with or without inhibitors (0.1–10 μM). After incubation, medium (1 mL) was removed and productestrone separated from E1S by solvent partition using toluene (4 mL). [^{14}C]Estrone (7×10^3 dpm, 52 mCi/mmol. Amersham International, UK) was used to correct for procedural losses. An aliquot of the organic phase was added to scintillation fluid and the ^{3}H and ^{14}C content measured by scintillation spectrometry. The mass of E1S hydrolyzed was calculated from the ^{3}H counts detected (corrected for the volume of medium and organic solvent used and for recovery of ^{14}C counts) and the specific activity of the substrate.

5.3. Syntheses

5.3.1. 2-Nitroestrone (1a) and 4-nitroestrone (2a)

These were prepared according to the method as described by Tomson and Horwitz.[17]

5.3.2. 2-Nitroestrone 3-*O*-sulfamate (1)

To a solution of **1a** (2.50 g, 7.94 mmol) in anhydrous DMF (100 mL) at ice/water temperature and under an atmosphere of nitrogen was added sodium hydride (60% dispersion in mineral oil, 350 mg, 8.73 mmol). After the evolution of hydrogen had ceased, sulfamoyl chloride in toluene (ca. 0.68 M, 18 mL) was introduced and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then concentrated in vacuo and the resulting residue in ethyl acetate (200 mL) was washed with hydrochloric acid (0.1 M, 150 mL) and then brine (4×100 mL). After drying ($MgSO_4$), filtration and evaporation of the organic layer (water bath temperature ⩽ 40 °C), the yellow residue (3.1 g) obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:2–1:1, gradient). The second fraction collected (yellow syrup, 730 mg, 1.85 mmol, 23%) was recrystallized from ethyl acetate/hexane (1:2) to give **1** as creamy needles (459 mg). Mp 158–162 °C (dec); R_f (ethyl acetate/hexane, 1:1) 0.28, cf. R_f 0.58 (**1a**); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.93 (3H, s, CH_3), 1.2–3.1 (15H, m), 5.25 (2H, br s, exchanged with D_2O, OSO_2NH_2), 7.29 (1H, s, C4H) and 7.98 (1H, s, C1H); LRMS (FAB^+) *m*/*z* (%) 548 [30, $(M+H+NBA)^+$], 395 [100, $(M+H)^+$], 378 [37, $(M-NH_2)^+$], 315 [30, $(M+H-H_2NSO_2)^+$], 298 [25, $(M-H_2NSO_2O)^+$]; LRMS (FAB^-) *m*/*z* (%) 546 [10, $(M-H+NBA)^-$], 393 [100, $(M-H)^-$], 314 [30, $(M-H_2NSO_2)^-$], 96 [50, $(H_2NSO_2O)^-$]. Found: C, 54.6; H, 5.58; N, 7.10. $C_{18}H_{22}N_2O_6S$ requires C, 54.81; H, 5.62; N, 7.10.

5.3.3. 4-Nitroestrone 3-*O*-sulfamate (2)

This was prepared from **2a** (1.0 g, 3.17 mmol) in a similar manner to the preparation of **1**. The crude product was fractionated by flash chromatography (chloroform/ethyl acetate, 4:1–2:1, gradient) and

the second fraction collected (pale yellow syrup, 300 mg, 761 μmol, 24%) was recrystallized from chloroform/hexane (1:1) to give **2** as pale yellow crystals (167 mg). Mp 176–178 °C; R_f (chloroform/ethyl acetate, 4:1) 0.20, cf. R_f 0.49 (**2a**); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.93 (3H, s, CH_3), 1.2–3.0 (15H, m), 5.1 (2H, br s, exchanged with D_2O, OSO_2NH_2), 7.43 (1H, d, $J \sim 9$ Hz, ArH) and 7.49 (1H, d, $J = 9$ Hz, ArH); LRMS (FAB^+) m/z (%) 395 [100, $(M+H)^+$]; LRMS (FAB^-) m/z (%) 393 [100, $(M-H)^-$], 314 [72, $(M-H_2NSO_2)^-$], 96 [38, $(H_2NSO_2O)^-$]. Found: C, 54.6; H, 5.57; N, 7.02. $C_{18}H_{22}N_2O_6S$ requires C, 54.81; H, 5.62; N, 7.10. HPLC: Spherisorb ODS2 (5 μm, 25 × 4.6 cm), methanol/water (75:25), 1 mL/min, λ_{max} 281 nm, t_R = 3.82 min.

5.3.4. 3-*O*-(2-Propenyl)estrone (3)

To a solution of estrone (10.0 g, 36.98 mmol) in DMF (70 mL) at ice/water temperature was treated cautiously with sodium hydride in portions (60% in mineral oil, 1.63 g, 40.68 mmol). The resulting mixture was heated with 3-bromoprop-1-ene (3.9 mL, 44.38 mmol) at 80 °C for 2 h and then stirred under an atmosphere of nitrogen at room temperature overnight. The reaction mixture was concentrated in vacuo and the resulting slurry diluted with ethyl acetate (300 mL). The organic layer was washed with brine (4 × 100 mL), dried ($MgSO_4$) and evaporated to give a yellow residue. Upon recrystallization of this crude product from hot absolute alcohol, **3** was obtained as pale yellow crystals (9.48 g, 30.54 mmol, 83%). Mp 106–108 °C [Lit.[19] (aq ethanol) 108–109 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (3H, s, CH_3), 1.3–2.6 (13H, m), 2.88 (2H, m), 4.51 (2H, m, CH_2O), 5.28 (1H, m, H_AH_BC=CH), 5.40 (1H, m, H_AH_BC=CH), 6.04 (1H, m, H_2C=C*H*), 6.66 (1H, d, $J = 2.7$ Hz, C4H), 6.73 (1H, dd, $J = 2.8$ and 8.5 Hz, C2H) and 7.19 (1H, d, $J = 8.5$ Hz, C1H). ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 13.84 (q), 21.56 (t), 25.79 (s), 25.88 (t), 26.54 (t), 29.65 (t), 31.57 (t), 35.84 (t), 38.31 (d), 43.95 (d), 47.99 (s), 50.37 (d), 68.75 (t), 112.29 (d), 114.78 (d), 117.45 (t), 126.28 (d), 132.17 (s), 133.50 (d), 137.71 (s) and 156.58 (s).

5.3.5. 2-(2-Propenyl)estrone (4a) and 4-(2-propenyl)estrone (5a)

These were prepared according to the method as described by Patton[19] via the Claisen rearrangement of **3**. A solution of **3** (7.68 g, 24.74 mmol) in diethylaniline (50 mL) was heated under an atmosphere of nitrogen at reflux temperature for 6 h. The cooled reaction mixture was diluted with ethyl acetate (300 mL), washed with hydrochloric acid (1 M, 4 × 100 mL) and then brine (4 × 100 mL), dried ($MgSO_4$) and evaporated to give a crude product (8.04 g). A 5.0 g portion of which was fractionated by flash chromatography (silica 400 g, ethyl acetate/hexane, 1:2). The second fraction collected gave a creamy residue (4.24 g) which was recrystallized from ether/hexane (3:2, 50 mL) to give **4a** as pale yellow crystals (721 mg, 2.32 mmol, 15%). Mp 175–182 °C [Lit.[19] 186–187 °C]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.2–2.6 (13H, m), 2.82 (2H, m, C6-H_2), 3.38 (2H, d, $J = 6.4$ Hz, C=CH–CH_2), 4.91 (1H, br s, exchanged with D_2O, OH), 5.16 (2H, m, H_2C=CH–), 5.99 (1H, m, H_2C=C*H*–), 6.57 (1H, s, ArH) and 7.02 (1H, s, ArH).

The mother liquor of **4a** was evaporated to give a creamy residue (3.45 g). A 2.45 g portion of which was recrystallized from ether/hexane (2:1, 42 mL) slowly in the refrigerator. After a week, a crop of yellow crystals (461 mg) was obtained which contained approximately 75% of **5a** and 25% of **4a** according to ^{1}H NMR.

5.3.6. 2-(2-Propenyl)estrone 3-*O*-sulfamate (4)

This was prepared from **4a** (600 mg, 1.93 mmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4–1:1 gradient) to give **4** as fluffy creamy residue (540 mg, 72%). Mp 153–155 °C; R_f (ethyl acetate/hexane, 1:1) 0.42, cf. R_f 0.53 (**4a**); IR ν_{max}/cm^{-1} (KBr) 3420, 3340, 3240, 3080, 2940, 2870, 1720, 1500, 1380, 1180; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.0–2.7 (13H, m), 2.91 (2H, m, C6-H_2), 3.45 (2H, d, $J = 6.4$ Hz, C=CH–CH_2), 4.99 (2H, br s, exchanged with D_2O, OSO_2NH_2), 5.08–5.20 (2H, m, H_2C=CH–), 5.97 (1H, m, H_2C=C*H*–), 7.14 (1H, s, ArH) and 7.18 (1H, s, ArH); LRMS (FAB^+) m/z (%) 389.1 [88, M^+], 147.1(60), 72.9(100); LRMS (FAB^-) m/z (%) 542.3 [20, $(M+NBA)^-$], 388.1 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 389.1674. $C_{21}H_{27}NO_4S$ requires 389.1661. Found: C, 64.9; H, 7.02; N, 3.49. $C_{21}H_{27}NO_4S$ requires C, 64.75; H, 6.99; N, 3.60.

5.3.7. 4-(2-Propenyl)estrone 3-*O*-sulfamate (5) (contained ca. 25% of 4)

This was prepared from **5a** (contained ca. 25% of **4a** by ^{1}H NMR, 648 mg, 2.09 mmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4–1:1 gradient) and the second fraction collected corresponded to an inseparable mixture of ca. 75% of **5** and 25% of **4** as fluffy creamy residue (523 mg). Mp 75–80 °C; R_f (ethyl acetate/hexane, 2:1) 0.64, cf. R_f 0.73 (**4a** and **5a**); IR ν_{max}/cm^{-1} (KBr) 3480, 3280, 3080, 2940, 2870, 1730, 1480, 1380, 1185; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 0.89 (3H, s, CH_3), 1.2–3.0 (15H, m), 3.48 (2H, m, C=CH–CH_2), 5.06 (4H, m, 2H exchanged with D_2O, H_2C=CH– and OSO_2NH_2), 5.93 (1H, m, H_2C=C*H*–), 7.11 (~0.25H, s, ArH of 2-(2-propenyl) isomer **4**), 7.20 (~0.25H, ArH of 2-(2-propenyl) isomer **4**), 7.24 (~0.75H, d, $J = 8.9$ Hz, ArH of 4-(2-propenyl) isomer **5**), 7.28 (~0.75H, d, $J = 8.9$ Hz, ArH of 4-(2-propenyl) isomer **5**); LRMS (FAB^+) m/z (%) 389.1 [88, M^+], 310.2 [31, $(M-HNSO_2)^+$], 133.1(45), 72.9(64); LRMS (FAB^-) m/z (%) 542.3 [20, $(M+NBA)^-$], 388.2 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 389.1671. $C_{21}H_{27}NO_4S$ requires 389.1661. Found: C, 64.8; H, 7.08; N, 3.48. $C_{21}H_{27}NO_4S$ requires C, 64.75; H, 6.99; N, 3.60.

5.3.8. 2,4-Di-(2-propenyl)estrone (8a)

To a solution of a mixture of **4a** and **5a** (3.14 g, 10.11 mmol) in DMF (20 mL) at ice/water temperature was added cautiously sodium hydride (60% in mineral oil, 485 mg, 12.14 mmol). The resulting mixture was heated with 3-bromoprop-1-ene (1.05 mL, 12.14 mmol) at 80 °C for 2 h and then stirred under an atmosphere of nitrogen at room temperature overnight. The reaction mixture was diluted with ethyl acetate (300 mL) and the organic layer separated, washed with brine (300 mL, 4 × 100 mL), dried ($MgSO_4$) and evaporated to give a brown syrup (3.63 g). The Claisen rearrangement of this mixture of 2-(2-propenyl)estrone-3-*O*-(2-propenyl) ether (**6**) and 4-(2-propenyl)estrone-3-*O*-(2-propenyl) ether (**7**) was carried out according to the method as described by Patton.[19] The crude **8a** obtained was fractionated by flash chromatography (silica 200 g; ethyl acetate/hexane, 1:3–1:2 gradient). The third fraction collected gave a light brown residue which upon recrystallization from hot cyclohexane yielded **8a** as creamy bar-shaped crystals (2.64 g, 7.53 mmol, 75%). Mp 119–120 °C [Lit.[19] (ethanol) 121.5–122 °C]; R_f (ethyl acetate/hexane, 1:3) 0.43, cf. R_f 0.51 (**4a** and **4b**); ^{1}H NMR (270 MHz, CD_2Cl_2) δ 0.90 (3H, s, CH_3), 1.0–3.0 (15H, m), 3.40 (4H, m, 2 × C=CH–CH_2), 4.9–5.3 (5H, m, 1H exchanged with D_2O, 2 × H_2C=CH– and OH), 6.0 (2H, m, H_2C=C*H*–) and 6.98 (1H, s, C1H).

5.3.9. 2,4-Di-(2-propenyl)estrone 3-*O*-sulfamate (8)

This was prepared from **8a** (1.0 g, 2.85 mmol) in a similar manner to the sulfamoylaton of **1a**. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:2) to give **8** as fluffy pale yellow residue (722 mg, 1.68 mmol, 59%). Mp 75–80 °C; R_f (ethyl acetate/hexane, 2:1) 0.64, cf. R_f 0.72 (**8a**); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.1–3.0 (15H, m), 3.55 (4H, m, 2 × C=CH–CH_2), 4.9–5.3 (6H, m, 2H exchanged with D_2O, 2 × H_2C=CH– and OSO_2NH_2), 5.97 (2H, m, 2 × H_2C=C*H*–)

and 7.15 (1H, s, C1H); LRMS (EI, 70 eV) *m*/*z* (%) 429.1 [7, M^+], 350.2 [48, $(M-HN{=}SO_2)^+$] 149.0(40), 43.0[100, $(C_3H_7)^+$]; HRMS (EI) Found: 429.1999. $C_{24}H_{31}NO_4S$ requires 429.1974. Found: C, 66.8; H, 7.28; N, 3.22. $C_{24}H_{31}NO_4S$ requires C, 67.10; H, 7.28; N, 3.26.

5.3.10. 2-*n*-Propylestrone 3-*O*-sulfamate (9)

To a solution of **4** (250 mg, 642 μmol) in absolute ethanol (50 mL) was added a suspension of Pd–C (10%, 250 mg) in absolute ethanol (2 mL) and the resulting suspension was stirred at room temperature overnight under an atmosphere of hydrogen at 50 psi. After removal of the supported catalyst by filtration and evaporation of the filtrate, the crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4–2:1 gradient) to give **9** as fluffy light yellow residue (220 mg, 562 μmol, 88%). Mp 75–80 °C; R_f (ethyl acetate/hexane, 1:1) 0.53, cf. R_f 0.44 (**4**); IR ν_{max}/cm^{-1} (KBr) 3380, 3280, 3100, 2960, 2880, 1730, 1500, 1380, 1200; ^1H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, C18H_3), 0.96 (3H, t, J = 7.3 Hz, CH_3), 1.2–3.0 (19H, m), 4.98 (2H, br s, exchanged with D_2O, OSO_2NH_2), 7.12 (1H, s, Ar) and 7.18 (1H, s, Ar); LRMS (FAB^+) *m*/*z* (%) 390.1 [88, $(M-H)^+$], 147.1(55), 73.0(100); LRMS (FAB^-) *m*/*z* (%) 544.2 [17, $(M+NBA)^-$], 390.1 [100, $(M-H)^-$]. Found: C, 64.3; H, 7.61; N, 3.42. $C_{21}H_{29}NO_4S$ requires C, 64.42; H, 7.47; N, 3.58.

5.3.11. 4-*n*-Propylestrone 3-*O*-sulfamate (10)

This was prepared from **5** (250 mg, 481 μmol, contained ca. 25% of **4**) in a similar manner to the preparation of **9**. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4–1:1 gradient) and the second fraction isolated gave a mixture of **10** and **9** (ca. 3:1) as creamy residue (234 mg). A portion of this residue (145 mg) was recrystallized from ethyl acetate/hexane (1:3) to give pure **10** as soft white crystals (61 mg, 157 μmol, yield: 70% based on the amount of **5** present in the crude). Mp 194–198 °C; R_f (ethyl acetate/hexane, 1:1) 0.42, cf. R_f 0.40 (**5**); IR ν_{max}/cm^{-1} (KBr) 3400, 3320, 3240, 3000–2860, 1720, 1470, 1390, 1180; ^1H NMR (400 MHz, CD_2Cl_2) δ 0.89 (3H, s, C18-H_3), 1.00 (3H, t, J = 7.3 Hz, CH_3), 1.2–3.0 (19H, m), 5.11 (2H, br s, exchanged with D_2O, OSO_2NH_2) and 7.21 (2H, s, Ar); LRMS (FAB^+) *m*/*z* (%) 391.1 [100, M^+]; LRMS (FAB^-) *m*/*z* (%) 544.2 [20, $(M+NBA)^-$], 390.1 [100, $(M-H)^-$]. Found: C, 64.1; H, 7.45; N, 3.51. $C_{21}H_{29}NO_4S$ requires C, 64.42; H, 7.47; N, 3.58.

5.3.12. 2,4-Di-*n*-propylestrone 3-*O*-sulfamate (11)

This was prepared from **8** (200 mg, 466 μmol) in a similar manner to the preparation of **9**. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4–1:2 gradient) and the second fraction collected gave **11** as fluffy creamy residue (190 mg, 438 μmol, 94%). Mp 72–82 °C; R_f (ethyl acetate/hexane, 1:1) 0.64, cf. R_f 0.59 (**8**); IR ν_{max}/cm^{-1} (KBr) 3380, 3300, 3100, 2970, 2940, 2880, 1730, 1470, 1380, 1180; ^1H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, C18-H_3), 0.96 (3H, t, J = 7.3 Hz, CH_3), 1.00 (3H, t, J = 7.3 Hz, CH_3), 1.3–3.0 (23H, m), 5.02 (2H, br s, exchanged with D_2O, OSO_2NH_2) and 7.08 (1H, s, C1H); LRMS (FAB^+) *m*/*z* (%) 433.1 [100, M^+], 353.2 [43, $(M-H_2NSO_2)^+$], 255.2(50), 173.1(80); LRMS (FAB^-) *m*/*z* (%) 432.2 [100, $(M-H)^-$], 353.1 [8, $(M-H_2NSO_2)^-$]; HRMS (FAB^+) Found: 433.2311. $C_{24}H_{35}NO_4S$ requires 433.2287. Found: C, 66.6; H, 8.37; N, 2.97. $C_{24}H_{35}NO_4S$ requires C, 66.48; H, 8.14; N, 3.23.

5.3.13. 2-Fluoroestrone acetate (12a)

This was prepared according to the method as described by Page et al.[20] through heating a mixture of estrone (1.50 g, 5.55 mmol) and *N*-fluoropyridinium triflate (2.74 g, 11.0 mmol) in 1,1,2-trichloroethane (24 mL) at reflux temperature under nitrogen for 24 h. Mp 83–88 °C [Lit.[20] 88–90 °C]; ^1H NMR ($CDCl_3$, 270 MHz) δ 0.91 (3H, s, C18H_3), 1.38–1.75 (6H, m), 1.90–2.45 (6H, m), 2.32 (3H, s, CH_3) 2.46–2.57 (1H, m), 2.80–2.95 (2H, m, C6H), 6.83 (1H, d, J = 8.3 Hz, C4H) and 7.07 (1H, d, J = 13.2 Hz, C1H); LRMS (FAB^+) *m*/*z* (%) 331.1 (59, M^++1), 288.1 (100) and 270.1 (22); HRMS (FAB^+) Found: 331.1705. $C_{20}H_{23}FO_3$ (M^++1) requires 331.1709.

5.3.14. 2-Fluoroestrone (12b)

This was prepared according to the method as described by Page et al.[20] through heating a solution of **12a** (185 mg, 560 μmol) in methanol (5 mL) with K_2CO_3 (387 mg, 5 equiv) under reflux for 3 h. The crude residue obtained was recrystallized from petroleum ether (bp 60–80 °C)/absolute ethanol (2:8) to give **12b** (121 mg, 75% purity as estimated by ^1H NMR) as colorless crystals (mp 220–227 °C). Further purification by semi-preparative HPLC (Waters Radialpak C18 100 × 25 mm, MeOH/H_2O (60:40), 20 mL/min, λ_{max} 254 and 270 nm) gave **12b** as a white solid (72 mg, 45%). Mp 220–223 °C [Lit.[20] (petroleum ether/absolute ethanol) 220–222 °C]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.78 (3H, s, CH_3), 1.37–1.79 (6H, m), 1.91–2.40 (6H, m), 2.46–2.56 (1H, m), 2.80–2.90 (2H, m, C6H), 5.02 (1H, d, ex. with D_2O, J = 3.9 Hz, OH), 6.72 (1H, d, J = 9.4 Hz, C4H) and 6.98 (1H, d, J = 12.5 Hz, C1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ −144.50 (1F, t, J = 9.2 Hz); LRMS (FAB^+) *m*/*z* (%) 289.0 [100, $(M^+$+1)], 149.0 (33) and 120.0 (35); LRMS (FAB^-) *m*/*z* (%) 287.1.0 [100, $(M^-$−1)], 200.0 (25), 140.0 (25) and 123.0 (43); HRMS (FAB^+) Found 288.1520. $C_{18}H_{21}FO_2$ requires 288.1526. HPLC: Waters Radialpak C18 8 × 100 mm, MeOH/H_2O (60:40), 2 mL/min, λ_{max} 270 nm, t_R = 11.69 min, purity: 96.9.

5.3.15. 2-Fluoroestrone-3-*O*-sulfamate (12)

To a stirred solution of **12b** (65 mg, 230 μmol) in anhydrous DMF (7 mL) under an atmosphere of nitrogen was added at room temperature 2,6-di-*tert*-butyl-4-methylpyridine (139 mg, 0.68 mmol) then a solution of sulfamoyl chloride (0.68 M in toluene, 1.61 mL, 1.13 mmol) via a syringe. After stirring the reaction mixture at room temperature for 18 h, ethyl acetate (60 mL) was added and the organic layer separated was washed with brine (3 × 50 mL), dried ($MgSO_4$), filtered and evaporated. The crude product obtained was purified by flash chromatography (chloroform/acetone, 8:1) to afford **12** as a white solid, (47 mg, 57%). Mp 189–193 °C; R_f (chloroform/acetone, 8:1) 0.32; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.36–1.70 (6H, m), 1.92–2.36 (6H, m), 2.46–2.57 (1H, m), 2.80–2.95 (2H, m, C6H), 5.03 (2H, br s, ex. with D_2O, –OSO_2NH_2), 7.11 (1H, d, J = 9.7 Hz, C4H) and 7.13 (1H, d, J = 15.5 Hz, C1H); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −113.24 (1F, dd, J = 15.5 Hz and J = 9.7 Hz); LRMS (FAB^+) *m*/*z* (%) 367.0 (100, M^+), 350.0 (30), 288.1 (49), 258.0 (32), 243.1 (38), 178 (34), 133.0 (30) and 97.0 (35); LRMS (FAB^-) *m*/*z* (%) 366.0 [100, $(M-H)^-$] and 77.9 (21); HRMS (FAB^+) Found: 367.1267. $C_{18}H_{22}NO_4S$ requires 367.1254. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 150 mm); mobile phase MeCN/H_2O (90/10) isocratic; flow rate, 1 mL/min; t_R = 2.19 min; purity: 95%.

5.3.16. Estrone acetate (13)

This was prepared from estrone according to the method as described by Page et al.[20]

5.3.17. 2-Chloroestrone acetate (15a)

This was prepared according to the method as described by Page et al.[20] To a solution of **13** (500 mg, 1.60 mmol) in trifluoroacetic acid (TFA, 20 mL) was added thallic trifluoroacetate (1.74 g, 3.21 mmol) was and the resulting mixture was stirred at 0 °C under nitrogen for 24 h. Upon removal of TFA under reduced pressure at <40 °C, the crystalline estrone–thallium(III) complex (**14**) obtained was washed twice with 1,2-dichloroethane. 1,4-Dioxane (10 mL) and copper chloride (475 mg, 4.80 mmol) were

then added to this washed complex and the resulting mixture was heated under reflux for 3 h. Upon cooling and evaporation of solvent, the residue obtained was treated with water (30 mL) and the organic components were extracted into dichloromethane (3 × 50 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered and evaporated. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4) and the pale yellow solid obtained (526 mg) was further purified by recrystallization from methanol to give **15a** as white crystals (436 mg, 78%). Mp 195–197 °C; R_f (chloroform/acetone, 8:1) 0.78; IR ν_{max}/cm^{-1} (KBr) 1770, 1730 (C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (3H, s, C18H_3), 1.38–2.29 (12H, m), 2.33 (3H, s, CH_3CO), 2.5 (1H, m), 2.87 (2H, m, C6H), 6.85 (1H, s, C4H) and 7.34 (1H, s, C1H).

5.3.18. 2-Chloroestrone (15b)

This was prepared according to the method as described by Page et al.[20] A mixture of **15a** (400 mg, 1.15 mmol), K_2CO_3 (800 mg, 5.80 mmol) in MeOH (20 mL) was heated under reflux for 3 h. Upon cooling and evaporation of the solvent, water (20 mL) was added to the residue obtained and the crude product was extracted into dichloromethane (3 × 20 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered and evaporated to give a yellow crude product which was purified by recrystallization from methanol to give **15b** as white crystals (320 mg, 90%). Mp 221–223 °C [Lit.[20] 223–225 °C]; R_f (chloroform/acetone, 8:1) 0.70; IR ν_{max}/cm^{-1} (KBr) 3400 (OH), 1730 (C=O); ^1H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.4–2.54 (13H, m), 2.84 (2H, m, C6H), 5.27 (1H, s, ex, with D_2O, OH), 6.71 (1H, s, C4H) and 7.31 (1H, s, C1H).

5.3.19. 2-Chloroestrone 3-*O*-sulfamate (15)

This was prepared from **15b** (200 mg, 655 μmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (chloroform/acetone, 8:1) to give a beige residue (190 mg) which was further purified by recrystallization from acetone/hexane (1:2) to give **15** as white crystals (170 mg, 68%). Mp > 163 °C (dec); R_fs (chloroform/acetone, 8:1 and 4:1) 0.63 and 0.74 respectively; IR ν_{max}/cm^{-1} (KBr) 3500, 3200 (NH_2), 1720 (C=O), 1390 (SO_2); ^1H NMR (400 MHz, $CDCl_3$/DMSO-d_6: 10:1) δ 0.93 (3H, s, CH_3), 1.4–2.51 (13H, m), 2.86 (2H, m, C6H), 7.01 (2H, br s, ex. with D_2O, OSO_2NH_2), 7.25 (1H, s, C4H) and 7.35 (1H, s, C1H). LRMS (FAB^+) *m*/*z* (%) 537.2 (5), 383.2 (17), 206.2 (100), 97.1 (22); LRMS (FAB^-) *m*/*z* (%) 536.2 (45), 383.2 (100), 250.0 (52), 96.9 (49), 77.9 (34); HRMS (FAB^+) Found: 383.0956. $C_{18}H_{22}ClNO_4S$ requires 383.0958. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 150 mm); mobile phase MeCN/H_2O (96/4) isocratic; flow rate, 1 mL/min; t_R = 2.01 min; purity: 98%.

5.3.20. 2-Bromoestrone acetate (16a)

This was prepared from **13** (2.0 g, 6.40 mmol) in the same manner as the preparation of **15a** except that copper bromide (8.58 g, 38.41 mmol) was used. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:4) and the pale white solid isolated (2.05 g) was further purified by recrystallization from methanol to give **16a** as colorless crystals (1.79 g, 4.58 mmol, 72%). Mp 229–231 °C; R_fs (chloroform/acetone, 8:1 and ethyl acetate/hexane, 4:1) 0.89 and 0.62 respectively; IR ν_{max}/cm^{-1} (KBr) 1770, 1730 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, s, C18H_3), 1.25–2.3 (12H, m), 2.34 (3H, s, CH_3CO), 2.5 (1H, m), 2.86 (2H, t, *J* = 4.6 Hz, C6H), 6.85 (1H, s, C4H) and 7.49 (1H, s, C1H); LRMS *m*/*z* (FAB^+) 545.2 [90, $(M+H+NBA)^+$], 391.2 [100, $(M)^+$], 348.1 [90, $(M-CH_3CO)^+$] and 330.1 (10); HRMS (FAB^+) Found: 391.0856. $C_{20}H_{24}BrO_3$ requires 391.0909. Found: C, 61.2; H, 5.89. $C_{20}H_{23}BrO_3$ requires C, 61.39; H, 5.92.

5.3.21. 2-Bromoestrone (16b)

This was prepared from **16a** (1.5 g, 3.83 mmol) in the same manner as the preparation of its chloro analogue **15b**. The pale yellow crude product (1.3 g) obtained was purified by recrystallization from ethyl acetate/petroleum ether 60–80 °C (3:2) to give **16b** as pale yellow crystals (1.23 g, 3.52 mmol, 92%). Mp 190–193 °C [Lit.[20] 194–195 °C]; R_fs (chloroform/acetone, 8:1 and 4:1) 0.51 and 0.64 respectively; IR ν_{max}/cm^{-1} (KBr) 1710 (C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, s, C18H_3), 1.4–2.52 (13H, m), 2.84 (2H, m, C6H), 5.32 (1H, br s, ex. with D_2O, OH), 6.73 (1H, s, C4H) and 7.52 (1H, s, C1H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 13.81 (q, C18), 21.56 (t), 25.89 (t), 26.26 (t), 29.9 (t), 31.54 (t), 31.91 (t), 38.04 (d), 43.6 (d), 47.93 (s, C13), 50.38 (d), 114.94 (d, C4), 135.04 (d, C-1), 128.84 (s), 130.92 (s), 139.02 (s), 152.7 (s, C3) and 220 (s, C=O); LRMS (FAB^+) *m*/*z* (%) 502.1 [20, $(M+NBA)^+$], 349.1 [100, $(M)^+$] and 271.0 (20); LRMS (FAB^-) *m*/*z* (%) 502.2 [40, $(M+NBA)^-$] and 348.1 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 348.0753. $C_{18}H_{21}BrO_2$ requires 348.0725.

5.3.22. 2-Bromoestrone 3-*O*-sulfamate (16)

This was prepared from **16b** (500 mg, 1.43 mmol) in a similar manner to the sulfamoylation of **1a**. The crude product (620 mg) obtained was fractionated by flash chromatography (chloroform/acetone, 8:1). The beige residue obtained (510 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **16** as beige crystals (405 mg, 66%). Mp > 155 °C (dec); R_fs (chloroform/acetone, 8:1 and 4:1) 0.43 and 0.61 respectively; IR ν_{max}/cm^{-1} (KBr) 3500, 3300 (NH_2), 1730 (C=O), 1390 (SO_2); ^1H NMR (400 MHz, acetone-d_6) δ 0.92 (3H, s, CH_3), 1.41–2.49 (13H, m), 2.86 (2H, m, C6H), 7.27 (1H, s, C4H), 7.32 (2H, br s, ex. with D_2O, OSO_2NH_2) and 7.55 (1H, s, C1H); LRMS (FAB^+) *m*/*z* (%) 428.1 [100, $(M)^+$] and 349.2 [40, $(M+H\text{-}SO_2NH_2)^+$]; LRMS (FAB^-) *m*/*z* (%) 580.2 [M−H+NBA], 428.1 [100, $(M)^-$] and 349.1 [30, $(M+H\text{-}SO_2NH_2)^-$]; HRMS (FAB^+) Found: 428.0430. $C_{18}H_{23}BrNO_4S$ requires 428.0531. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 150 mm); mobile phase MeCN/H_2O (90/10) isocratic; flow rate, 1 mL/min; t_R = 2.19 min; purity: 98%.

5.3.23. 2-Iodoestrone acetate (17a)

This was prepared from **13** (5.0 g, 16.01 mmol) in the same manner as for the preparation of **15a** except that copper iodide (18.29 g, 96.03 mmol) was used. The crude product obtained was purified by flash chromatography with ethyl acetate/hexane (1:4) and the yellow solid obtained (6.25 g) was further purified by recrystallization from methanol to give **17a** as pale yellow crystals (5.89 g, 84%). Mp 170–172 °C (crystals turned brown at >145 °C); R_f (chloroform/acetone, 8:1) 0.8; IR ν_{max}/cm^{-1} (KBr) 1770, 1730 (C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, s, C18H_3), 1.4–2.31 (12H, m), 2.34 (3H, s, CH_3CO), 2.5 (1H, m), 2.87 (2H, t, *J* = 4.27 Hz, C6H), 6.82 (1H, s, C4H) and 7.69 (1H, s, C1H); LRMS (FAB^+) *m*/*z* (%) 592.1 [20, $(M+H+NBA)^+$], 439.1 [80, $(M+H)^+$], 396.1 [100, $(M+H\text{-}CH_3CO)^+$] and 270.2 (40); HRMS (FAB^+) Found: 439.0767. $C_{20}H_{24}IO_3$ requires 439.0770.

5.3.24. 2-Iodoestrone (17b)

This was prepared from **17a** (4.0 g, 9.13 mmol) in the same manner as for the preparation of its chloro analogue **15b**. The yellow crude product obtained was recrystallized from methanol to give **17b** as pale yellow crystals (3.29 g, 91%). Mp 207–209 °C (crystals turned brown at >169 °C) [Lit.[20] 167–168 °C]; R_f (chloroform/acetone, 8:1) 0.75; IR ν_{max}/cm^{-1} (KBr) 3400 (OH), 1730 (C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.4–2.56 (13H, m), 2.83 (2H, m, C6H), 5.24 (1H, s, ex. with D_2O, OH), 6.74 (1H, s, C4H) and 7.52 (1H, s, C1H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 13.82 (q, C-18), 21.57 (t), 25.89 (t), 26.26 (t), 29.98 (t), 31.57 (t), 31.81 (t), 39.0 (d), 43.9 (d), 48.3 (s, C13), 50.38 (d),

116.54 (d, C4), 138.14 (d, C1), 129.64 (s), 131.92 (s), 138.02 (s), 153.6 (s, C3) and 220 (s, C17, C=O); LRMS (FAB^+) *m/z* (%) 550.1 [20, $(M+H+NBA)^+$], 396.1 [100, $(M)^+$] and 271.2 (40); LRMS (FAB^-) *m/z* (%) 549.2 [20, $(M+NBA)^-$] and 395.1 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 397.0651. $C_{18}H_{22}IO_2$ requires 397.0665.

5.3.25. 2-Iodoestrone 3-*O*-sulfamate (17)

This was prepared from **17b** (500 mg, 1.26 mmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (chloroform/acetone, 8:1) and the beige residue collected (450 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **17** as beige crystals (342 mg, 57%). Mp > 145 °C (dec); R_fs (chloroform/acetone, 8:1 and 4:1) 0.61 and 0.73 respectively; IR ν_{max}/cm^{-1} (KBr) 3500, 3200 (NH_2), 1720 (C=O), 1390 (SO_2); ^1H NMR (400 MHz, acetone-d_6) δ 0.92 (3H, s, CH_3), 1.4–2.53 (13H, m), 2.83 (2H, m, C6H), 7.25 (1H, s, C4H), 7.32 (2H, br s, ex. with D_2O, OSO_2NH_2) and 7.76 (1H, s, C1H); LRMS (FAB^+) *m/z* (%) 629.1 [40, $(M+H+NBA)^+$], 476.1 [100, $(M+H)^+$] and 396.1 [40, $(M+H-SO_2NH_2)^+$]; LRMS (FAB^-) *m/z* (%) 628.1 [35, $(M+NBA)^-$], 474.1 [100, $(M-H)^-$] and 395.1 [20, $(M-SO_2NH_2)^-$]; HRMS (FAB^+) Found: 476.0387. $C_{18}H_{23}INO_4S$ requires 476.0393. Found: C, 45.8; H; 4.74; N, 2.85. $C_{18}H_{22}INO_4S$ requires C, 45.48; H, 4.66; N, 2.95.

5.3.26. 2-Cyanoestrone (18b)

2-Cyanoestrone acetate (**18a**) was prepared from **13** (500 mg, 1.60 mmol) in the same manner as for the preparation of **15a** except that the estrone–thallium (III) complex was heated with copper cyanide in pyridine instead. Without the isolation of product, crude **18a** obtained was directly deacetylated in the same manner as the preparation of its chloro analogue **15b**. Upon fractionation of crude **18b** by flash chromatography (ethyl acetate/hexane, 1:4), the pale yellow solid obtained (286 mg) was further purified by recrystallization from methanol to give **18b** as white crystals (260 mg, 80%). Mp 266–268 °C [Lit.[20] 265–266 °C]; R_f (chloroform/acetone, 8:1) 0.74; IR ν_{max}/cm^{-1} (KBr) 1770, 1730 (C=O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, s, CH_3), 1.39–2.56 (13H, m), 2.86 (2H, m, C6CH_2), 5.97 (1H, br s, ex. with D_2O, C3OH), 6.71 (1H, s, C4H) and 7.52 (1H, s, C1H).

5.3.27. 2-Cyanoestrone 3-*O*-sulfamate (18)

This was prepared from **18b** (200 mg, 677 μmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (chloroform/acetone, 8:1) and the pale yellow residue collected (217 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **12** as beige crystals (181 mg, 70%); Mp > 205 °C (dec); R_fs (chloroform/acetone, 8:1 and 4:1) 0.60 and 0.72 respectively; IR ν_{max}/cm^{-1} (KBr) 3500, 3200 (NH_2), 1720 (C=O), 1390 (SO_2); ^1H NMR (400 MHz, $CDCl_3$/DMSO-d_6; 5:1) δ 0.91 (3H, s, CH_3), 1.4–2.49 (13H, m), 2.99 (2H, m, C6H), 7.33 (1H, s, C4H), 7.57 (1H, s, C1H) and 8.0 (2H, br s, ex. with D_2O, OSO_2NH_2); LRMS (FAB^+) *m/z* (%) 528.2 [24, $(M+H+NBA)^+$], 375,1 [65, $(M+H)^+$], 206.2 (100); LRMS (FAB^-) *m/z* (%) 527.2 [24, $(M+NBA)^-$], 373,2 [65, $(M-H)^-$], 294.2 [43, $(M-H_2NSO_2)^-$]; HRMS (FAB^+) Found: 375.1375. $C_{19}H_{23}N_2O_4S$ requires 375.1379. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 150 mm); mobile phase MeCN/H_2O (70/30) isocratic; flow rate, 1 mL/min; t_R = 1.12 min; purity: 94%.

5.3.28. 4-Bromoestrone (19a)

To 300 mL of glacial acetic acid was added estrone (1.0 g, 3.70 mmol) and the suspension was gently heated with stirring until it became a solution. Upon cooling to ice-water temperature, powdered iron (10–15 mg) was added to the stirred solution, followed by a dropwise addition of a 5% v/v solution of bromine in glacial acetic acid until the reaction mixture no longer decolorized the added bromine. After stirring for an additional 5 min, the reaction mixture was poured into ice and water. The precipitate that formed was collected by filtration, washed with water and recrystallized from 98% ethanol to give **19a** as white powder (300 mg, 858 μmol, 23%). Mp 260–263 °C (Lit.[21] 264–265 °C); R_f (chloroform/acetone, 4:1) 0.76; ^1H NMR (270 MHz, $CDCl_3$) δ 0.90 (3H, s, C18H_3), 1.30–3.06 (15H, m), 5.55 (1H, s, ex. with D_2O, OH), 6.88 (1H, d, *J* = 8.6 Hz, C2H) and 7.19 (1H, d, *J* = 8.6 Hz, C1H); LRMS (FAB^+) *m/z* (%) 349.1 [84, $(M+1)^+$], 348.1 (100); LRMS (FAB^-) *m/z* (%) 502.1 [64, $(M-H+NBA)^-$], 500.1 (55), 349.1 [100, $(M-H)^-$], 347.1 (88). HRMS (FAB^+) Found: 348.0716. $C_{18}H_{21}BrO_2$ requires 348.0725.

5.3.29. 4-Bromoestrone sulfamate (19)

This was prepared from **19a** (286 mg, 816 μmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (acetone/hexane, 1:4) The residue collected was further purified by recrystallization from ethyl acetate/hexane to give **19** as white solid (90 mg, 26%). Mp 200–201 °C; R_f (chloroform/acetone, 4:1) 0.47, cf. R_f 0.76 (**19a**); IR ν_{max}/cm^{-1} (KBr) 3361, 3228 (NH_2), 1719 (C=O), 1390 (SO_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, s, CH_3), 1.36–1.72 (6H, m), 1.92–2.22 (4H, m), 2.25–2.42 (2H, m), 2.47–2.58 (1H, m), 2.71–2.84 (1H, m), 3.00–3.10 (1H, m), 4.99 (2H, br s, ex. with D_2O, OSO_2NH_2) and 7.29 (2H, d, *J* = 7.3 Hz, C1H and C2H); LRMS (FAB^+) *m/z* (%) 428.1 [100, $(M+H)^+$], 349.1 [27, $(M-SO_2NH_2)^+$] 348.1 (30), 476.1 [100, $(M+H)^+$] and 396.1 [40, $(M+H-SO_2NH_2)^+$]; LRMS (FAB^-) *m/z* (%) 580.1 [45, $(M-H+NBA)^-$], 428.1 [98, $(M)^-$], 349.1 (10), 347.1 [8, $(M+H-SO_2NH_2)^-$], 77.9 (50); HRMS (FAB^+) Found: 428.0460. $C_{18}H_{23}BrNO_4S$ requires 428.0531. Found: C, 50.6; H; 5.21; N, 3.18. $C_{18}H_{22}BrNO_4S$ requires C, 50.47; H, 5.18; N, 3.27.

5.3.30. 3-Hydroxy-1,3,5(10)-estratriene (20a)

This was prepared by heating a mixture of estrone (10.0 g, 36.98 mmol), hydrazine monohydrate (15 mL, mmol), potassium hydroxide (15 g, mmol) in ethanol (5 mL) and diethylene glycol (120 ml) under the conditions for a Wolff–Kishner reduction of steroid ketones. The crude product obtained was purified by flash chromatography (ethyl acetate/hexane, 1:4) to give **20a** (7.0 g, 65%). Mp 132–135 °C [Lit.[22] (aq ethanol) 134–134.5 °C]; IR ν_{max}/cm^{-1} (KBr) 3371 (OH), 2926 (ArH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.74 (3H, s), 1.08–1.94 (13H, m), 2.14–2.30 (2H, m), 2.74–2.90 (2H, m, C6H), 4.47 (1H, s, ex. with D_2O, OH), 6.56 (1H, d, *J* = 2.7 Hz, C4H), 6.62 (1H, dd, *J* = 2.7 and 8.6 Hz, C2H) and 7.17 (1H, d, *J* = 8.6 Hz, C1H); LRMS (FAB^+) *m/z* (%) 256.1 (100, M^+), 159.1 (17), 145.0 (8) and 133.0 (11); LRMS (FAB^-) *m/z* (%) 408.2 [51, $(M-H+NBA)^-$], 255.2 [100, $(M-H)^-$], 195.1 (21) and 139.0 (9); HRMS (FAB^+) Found: 256.1826. $C_{18}H_{24}O$ requires 256.1827.

5.3.31. 1,3,5(10)-Estratriene 3-*O*-sulfamate (20)

This was prepared from **20a** (1.0 g, 3.90 mmol) in a similar manner to the sulfamoylation of **1a**. The crude product obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:3) and the second fraction collected (911 mg, 69%) was recrystallized from chloroform/hexane (6:7) to give a crop of **20** as white crystals (280 mg); Mp 124–125 °C; R_f (ethyl acetate/hexane, 1:2) 0.40, cf. R_f 0.50 (**20a**); IR ν_{max}/cm^{-1} (KBr) 3410 and 3100 (NH_2), 3000–2800, 1380, 1360; ^1H NMR (270 MHz, $CDCl_3$) δ 0.74 (3H, s, CH_3), 1.0–3.0 (17H, m), 4.91 (2H, s, ex. with D_2O, OSO_2NH_2), 7.04 (1H, d, *J* = 2.3 Hz, C4H), 7.07 (1H, dd, *J* = 2.3 and 8.4 Hz, C2H) and 7.32 (1H, d, *J* = 8.6 Hz, C1H); LRMS (CI) *m/z* (%) 353.1 [100, $(M+NH_4)^+$], 257 (11), 256 (19), 255 (11); HRMS (NH_4^+) Found: 353.1897. $C_{18}H_{29}N_2O_3S$ requires 353.1899. Found: C, 64.3; H, 7.55; N, 4.16. $C_{18}H_{25}NO_3S$ requires C, 64.45; H, 7.51; N, 4.18.

5.3.32. 2-Aminoestrone (21a)

To a solution of **1a** (1.0 g, 3.17 mmol) in dichloromethane (50 mL) was added a suspension of Pd-C (10%, 200 mg) in dichloromethane (3 mL). The resulting suspension was stirred at room temperature under an atmosphere of hydrogen (balloon) for 20 h at which time the reduction was complete according to TLC. After removal of the supported catalyst by filtration and evaporation of filtrate, the crude product obtained (940 mg) [R_f (chloroform/acetone, 4:1) 0.31, cf. R_f 0.76 (**1a**)] was used for the next reaction without further purification.

5.3.33. 2-Tosylamidoestrone (21b)

To a stirred suspension of the freshly prepared crude **21a** (900 mg, 3.15 mmol) in anhydrous dichloromethane (30 mL) at ice/water temperature was added anhydrous pyridine (0.5 mL) followed by tosyl chloride (605 mg, 3.17 mmol). After stirring at room temperature for 3 h, the reaction mixture was filtered and the filtrate thus obtained was diluted with ethyl acetate (50 mL). The organic layer was washed with hydrochloric acid (1 M, 2 × 50 mL) followed by brine (4 × 50 mL), dried ($MgSO_4$), filtered and evaporated to give a yellow residue (1.23 g). This was recrystallized from ethyl acetate/hexane (4:3) to give a crop of slightly impure **21b** as fine bright yellow crystals (390 mg). The yellow residue (720 mg) obtained from the evaporation of the mother liquor was fractionated by flash chromatography (ethyl acetate/hexane, 1:2 to 4:1 gradient). The third fraction obtained gave a white residue (568 mg) which was recrystallized from ethyl acetate/hexane (2:3) to give **21b** as soft creamy crystals (365 mg, 830 μmol, overall yield ca. 45%). Mp 217–220 °C; R_f (ethyl acetate/hexane, 2:1) 0.45, cf. R_f 0.58 (**1a**); IR ν_{max}/cm^{-1} (KBr) 3380, 3240, 3000–2800, 1710, 1340, 1160; ^1H NMR (400 MHz, DMSO-d_6) δ 0.80 (3H, s, C18H_3), 1.1–2.5 (16H, m), 2.65 (2H, m, C6H_2), 6.42 (1H, s, C4-H), 6.87 (1H, s, C1H), 7.31 (2H, AA′BB′, Tosyl), 7.61 (2H, AA′BB′, Tosyl), 9.01 (1H, s, ex. with D_2O, NH or OH) and 9.18 (1H, s, ex. with D_2O, NH or OH); LRMS (FAB^+) *m*/*z* (%) 439.1 (74, M^+), 284.1 [100, $(M-Tosyl)^+$]; LRMS (FAB^-) *m*/*z* (%) 592.2 [11, $(M+NBA)^-$], 438.1 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 439.1817. $C_{25}H_{29}NO_4S$ requires 439.1817. Found: C, 68.1; H, 6.82; N, 3.13. $C_{25}H_{29}NO_4S$ requires C, 68.31; H, 6.65; N, 3.19.

5.3.34. Estra-1,3,5(10)-trien-17-oxo[3,2*d*]-1,2,3-oxathiazole-3-tosyl-2,2-dioxide (21c)

Sulfuryl chloride (1.40 mL of a 0.5 M solution in dichloromethane, 700 μmol) was added dropwise over a 5 min period to a stirred mixture of **21b** (280 mg, 637 μmol) and triethylamine (0.27 mL, 1.911 mmol) in dichloromethane (10 mL) at −78 °C under an atmosphere of nitrogen. After an additional 15 min, the reaction mixture was allowed to warm to room temperature, concentrated and the yellow residue obtained was dissolved in ethyl acetate (50 mL). The organic layer was washed with hydrochloric acid (1 M, 50 mL) followed by brine (4 × 50 mL), dried ($MgSO_4$), filtered and evaporated to give a yellow residue (390 mg) which was fractionated by flash chromatography (chloroform/acetone, 16:1). The first fraction isolated upon evaporation gave the product (192 mg, 383 μmol, 60%) which was recrystallized from ethyl acetate/hexane to give **21c** as white crystals (90 mg). Mp 245–247 °C (dec); IR ν_{max}/cm^{-1} (KBr) 3000–2850, 1745, 1400, 1390, 1220; ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, s, C18H_3), 1.40–1.75 (6H, m), 2.0–2.6 (10H, m), 2.87 (2H, m, C6H_2), 6.79 (1H, s, C4H), 7.31 (2H, AA′BB′, Tosyl), 7.57 (1H, s, C1H) and 7.82 (2H, AA′BB′, Tosyl); LRMS (FAB^+) *m*/*z* (%) 655.0 [25, $(M+H+NBA)^+$], 501.0 (100, M^+), 420.2 (45), 346.0 [70, $(M-Tosyl)^+$]; LRMS (FAB^-) *m*/*z* (%) 346.2 [100, $(M-Tosyl)^-$]; HRMS (FAB^+) Found: 501.1299. $C_{25}H_{27}NO_6S_2$ requires 501.1280. Found: C, 59.6; H, 5.46; N, 2.80. $C_{25}H_{27}NO_6S_2$ requires C, 59.86; H, 5.43; N, 2.79.

5.3.35. Estra-1,3,5(10)-trien-17-oxo[3,2*d*]-1,2,3-oxathiazole-2,2-dioxide (21)

To a solution of **21c** (148 mg, 295 μmol) in acetonitrile (25 mL) was added a solution of potassium fluoride (171 mg, 2.95 mmol) in water (4 mL). The resulting white suspension/emulsion was refluxed for 3 h, cooled and evaporated. The white residue obtained was dissolved in ethyl acetate (50 mL) and the organic layer washed with hydrochloric acid (1 M, 50 mL) followed by brine (4 × 50 mL). After drying ($MgSO_4$) and filtration, the filtrate on evaporation gave a light purple residue (141 mg) which was recrystallized from ethyl acetate/hexane (1:3, 8 mL) to give **21** as light purple powder (68 mg, 196 μmol, 66%). Mp 130–137 °C; IR ν_{max}/cm^{-1} (KBr) 3700–2500, 2920, 2860, 1720, 1490, 1370, 1190; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, s, C18H_3), 1.40–1.70 (6H, m), 1.90–2.40 (6H, m), 2.52 (1H, dd, *J* 8.8 and ~19 Hz), 2.90 (2H, m, C6H_2), 6.73 (1H, s, ex. with D_2O, NH), 6.86 (1H, s, Ar) and 6.97 (1H, s, Ar); LRMS (FAB^+) *m*/*z* (%) 500.9 [7, $(M+H+NBA)^+$], 346.9 (100, M^+); LRMS (FAB^-) *m*/*z* (%) 693.4[10, $(2\,M-H)^-$], 346.2 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 347.1196. $C_{18}H_{21}NO_4S$ requires 347.1191. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 100 mm); mobile phase MeCN/H_2O (96/4), gradient elution: 50:50 MeCN/H_2O to 95:5 MeCN/H_2O over 5 min; flow rate, 1 mL/min; t_R = 2.01 min; purity: 98%.

5.3.36. Estra-1,3,5(10)-trien-17-oxo[3,2*d*]-1,2,3-oxathiazole-3-methyl-2,2-dioxide (22)

To a solution of **21** (32 mg, 92 μmol) in anhydrous DMF (5 mL) at room temperature was added sodium hydride (60% dispersion in mineral oil, 4 mg, 100 μmol). After 10 min, the resulting yellow mixture was heated in the presence of methyl iodide (0.2 mL, 3.21 mmol) at 90 °C for 3 h. Upon cooling and diluting with ethyl acetate (20 mL), the organic layer that separated was washed with brine (30 mL, 4 × 10 mL), dried ($MgSO_4$), filtered and evaporated to give a light brown residue (37 mg) which was recrystallized from ethyl acetate/hexane (3:4 , 7 mL) to give **22** as light yellow/brown crystals (12 mg, 33 μmol, 36%). Mp turned brown at ca. 230 °C and melted at >250 °C to form a dark brown syrup; R_f (chloroform/acetone, 1:1) 0.80, cf. R_f 0.51 (**21**); ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, s, C18H_3), 1.40–2.60 (~13H), 2.88 (2H, m, C6H_2), 3.26 (3H, s, NCH_3), 6.73 (1H, s, Ar) and 6.84 (1H, s, Ar); LRMS (FAB^+) *m*/*z* (%) 361.2 (100, M^+), 330.2 (8), 298.3 (17), 173.2 (17), 97.1 (15); LRMS (FAB^-) *m*/*z* (%) no peaks were observed; HRMS (FAB^+) Found: 361.1359. $C_{19}H_{23}NO_4S$ requires 361.1348. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 150 mm); mobile phase MeCN/H_2O (96/4) isocratic; flow rate, 1 mL/min; t_R = 2.39 min; purity: >99%.

5.3.37. 4-Aminoestrone (23a)

To a solution of **2a** (1.49 g, 4.73 mmol) in tetrahydrofuran (THF, 80 mL) was added a suspension of Pd-C (10%, 300 mg) in THF (2 mL) and the resulting suspension was stirred at room temperature under an atmosphere of hydrogen (balloon) for 20 h. After removal of the supported catalyst by filtration and evaporation of filtrate, the crude product obtained (1.42 g) [R_f chloroform/acetone, 4:1) 0.53, cf. R_f 0.68 (**2a**)] was used for the next reaction without further purification.

5.3.38. 4-Tosylamidoestrone (23b)

To a stirred suspension of the freshly prepared crude **23a** (500 mg, 1.75 mmol) in anhydrous dichloromethane (15 mL) at ice/water temperature was added anhydrous pyridine (0.28 mL) followed by tosyl chloride (334 mg, 1.75 mmol). After stirring at room temperature for 3 h, the reaction mixture was diluted with ethyl acetate (100 mL). The organic layer was washed with hydrochloric acid (1 M, 50 mL) followed by brine (4 × 50 mL), dried ($MgSO_4$), filtered and evaporated to give a yellow residue (790 mg) which was fractionated by flash chromatography (ethyl

acetate/hexane, 1:1 to 2:1 gradient). The major fractions isolated gave a light yellow residue (670 mg) and this was recrystallized from acetone/diethyl ether (1:25, 52 mL) in the refrigerator to give **23b** as light yellow crystals (185 mg, 421 μmol, 24%), mp 206–209 °C; TLC (chloroform/acetone, 4:1) R_f 0.64, cf. R_f 0.49 (**23a**); IR ν_{max}/cm^{-1} (KBr) 3420, 3300, 3000–2850, 1730, 1600, 1500, 1160; ^1H NMR (400 MHz, DMSO-d_6) δ 0.83 (3H, s, C18H_3), 1.0–2.5 (18H, m), 6.53 (1H, d, J = 8.6 Hz, C1H), 7.00 (1H, d, J = 8.5 Hz, C2H), 7.30 (2H, AA′BB′, Tosyl), 7.60 (2H, AA′BB′, Tosyl), and 8.87 (2H, br s, ex. with D_2O, NH and OH); LRMS (FAB^+) m/z (%) 440.1 [45, $(M+H)^+$], 284.1 [100, $(M-Tosyl)^+$]; LRMS (FAB^-) m/z (%) 592.2 [10, $(M+NBA)^-$], 438.2 [100, $(M-H)^-$]; HRMS (FAB^+) Found: 440.1881. $C_{25}H_{30}NO_4S$ requires 440.1896. Found: C, 68.2; H, 6.74; N, 3.24. $C_{25}H_{29}NO_4S$ requires C, 68.31; H, 6.65; N, 3.19.

5.3.39. Estra-1,3,5(10)-trien-17-oxo[4,3*d*]-1,2,3-oxathiazole-3-tosyl-2,2-dioxide (23c)

Sulfuryl chloride (2.0 mL of a 0.5 M solution in dichloromethane, 1.00 mmol) was added dropwise with stirring over a 5 min period to **23b** (400 mg, 910 μmol) and triethylamine (0.39 mL, 2.73 mmol) in dichloromethane (20 mL) at ice–water temperature under an atmosphere of nitrogen. After an additional 15 min, the reaction mixture was allowed to warm to room temperature, concentrated and diluted with ethyl acetate (50 mL). The organic layer was washed with hydrochloric acid (1 M, 100 mL) followed by brine (4 × 50 mL), dried ($MgSO_4$), filtered and evaporated to give a light yellow residue/syrup (550 mg) which was fractionated by flash chromatography (chloroform/acetone, 16:1). The first fraction isolated upon evaporation gave **23c** as a creamy residue (312 mg). Mp 105–115 °C; R_f (chloroform/acetone, 8:1) 0.69, cf. R_f 0.48 (**23b**); ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, s, C18H_3), 1.4–2.4 (m), 2.45 (3H, s, ArCH_3), 2.5–2.6 (~H, m), 3.20 (1H, m), 3.35 (1H, m), 6.86 (1H, d, J = 8.5 Hz, ArH), 7.24 (2H, AA′BB′, Tosyl), 7.34 (1H, d, J = 8.5 Hz, ArH), 7.51 (2H, AA′BB′, Tosyl); LRMS (FAB^+) m/z (%) 879.6 (20), 599.4 (40), 337.2 (15), 91.0 (100); LRMS (FAB^-) m/z (%) 346.1 [100, $(M-Tosyl)^-$]. This fraction was used for the next reaction without further purification.

5.3.40. Estra-1,3,5(10)-trien-17-oxo[4,3*d*]-1,2,3-oxathiazole-2,2-dioxide (23)

To a solution of crude **23c** (150 mg, 299 μmol) in acetonitrile (20 mL) was added a solution of potassium fluoride (56 mg, 964 μmol) in water (5 mL). The resulting mixture was refluxed for 4 h, cooled, concentrated and diluted with ethyl acetate (50 mL). The organic layer was washed with hydrochloric acid (1 M, 50 mL) followed by brine (4 × 50 mL). After drying ($MgSO_4$) and filtration, the filtrate on evaporation gave a yellow-green syrup (170 mg) which was fractionated by flash chromatography (ethyl acetate/hexane, 1:1 to 4:1 gradient) to give a green residue (95 mg). A solution of this fraction in absolute ethanol was heated with activated charcoal and upon filtration and evaporation gave **23** as a creamy powder (85 mg, 245 μmol, 82%); IR ν_{max}/cm^{-1} (KBr) 3300–3000, 3000–2850, 1710, 1450, 1370, 1190; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, s, C18H_3), 1.2–2.8 (~15H, m), 6.73 (1H, br s, NH), 6.94 (1H, d, J = 8.5 Hz, ArH) and 7.07 (1H, d, J = 8.5 Hz, ArH); LRMS (FAB^+) m/z (%) 879.6 (50), 599.3 (100), 530.3 (20), 337.2 (30), 263.2 (24), 219.1 (20), 97.0 (55); LRMS (FAB^-) m/z (%) 1086.0 (10), 346.1 [100, $(M-H)^-$], 297.2 (25); HRMS (FAB^-) Found: 346.1128. $C_{18}H_{20}NO_4S$ requires 346.1113. HPLC: Waters 'Symmetry' C18 (packing: 3.5 μm, 4.6 × 150 mm); mobile phase MeCN/H_2O (96/4) isocratic; flow rate, 1 mL/min; t_R = 1.20 min; purity: >98%.

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