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3,17-Disubstituted 2-Alkylestra-1,3,5(10)-trien-3-ol Derivatives: Synthesis, In Vitro and In Vivo Anticancer Activity

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Estradiol-3,17-*O,O*-bis-sulfamates inhibit steroid sulfatase (STS), carbonic anhydrase (CA), and, when substituted at C-2, cancer cell proliferation and angiogenesis. C-2 Substitution and 17-sulfamate replacement of the estradiol-3,17-*O,O*-bis-sulfamates were explored with efficient and practical syntheses developed. Evaluation against human cancer cell lines revealed the 2-methyl derivative **27** (DU145 $GI_{50} = 0.38\ \mu M$) as the most active novel bis-sulfamate, while 2-ethyl-17-carbamate derivative **52** ($GI_{50} = 0.22\ \mu M$) proved most active of its series (cf. 2-ethylestradiol-3,17-*O,O*-bis-sulfamate **4** $GI_{50} = 0.21\ \mu M$). Larger C-2 substituents were deleterious to activity. 2-Methoxy-17-carbamate **50** was studied by X-ray crystallography and was surprisingly 13-fold weaker as an STS inhibitor compared to parent bis-sulfamate **3**. The potential of **4** as an orally dosed anti-tumor agent is confirmed using breast and prostate cancer xenografts. In the MDA-MB-231 model, dramatic reduction in tumor growth or regression was observed, with effects sustained after cessation of treatment. 3-*O*-Sulfamoylated 2-alkylestradiol-17-*O*-carbamates and sulfamates have considerable potential as anticancer agents.

Introduction

Sulfamoylated analogues of 2-methoxyestradiol (2-MeOE2, **1**, Figure 1), such as 2-methoxyestradiol-3,17-*O,O*-bis-sulfamate (2-MeOE2bisMATE,[a] **3**) and 2-ethylestradiol-3,17-*O,O*-bis-sulfamate (2-EtE2bisMATE, **4**), have previously been shown to be potent inhibitors of cancer cell growth[1−5] and of steroid sulfatase (STS, IC_{50}'s of **3** and **4** 38 nM and 1 μM, respectively),[2,5] a clinical target for the treatment of hormone-dependent breast cancer.[6,7] The inhibition of STS by phenolic sulfamates is irreversible and is thought to result from either the transfer of a sulfamate group to a residue in the active site of the enzyme or a nucleophilic attack of the sulfamate group on the conserved formylglycine residue of the active site, generating a dead-end product.[8] Metabolic processes similar to those that metabolize estradiol and other steroidal hormones, such as conjugation of the 3- or 17-hydroxyl groups to form glucuronide or sulfate derivatives, or oxidation of the 17-hydroxyl group by 17β-hydroxysteroid dehydrogenase type II, rapidly generate less active metabolites of 2-MeOE2 (**1**).[9−11] The sulfamate groups at the 3- and 17-positions are not however metabolized in the same way, while inhibition of STS prevents STS-mediated desulfamoylation of the 3-*O*-sulfamate groups of **3** and **4**. Estrogen 3-*O*-sulfamates have also been reported to inhibit carbonic anhydrase II (CAII), which is highly expressed in red blood cells, by coordination of the monoanionic form of the sulfamate to the zinc atom in the active site of CAII.[5,12−14] The uptake of **3** by red blood cells, as a result of its reversible interaction with CAII ($IC_{50} = 379$ nM),[15] and the rapid, almost complete inhibition of STS combined with the resistance to deactivating conjugation and metabolism all contribute to the observed high oral bioavailability (85%) of 2-MeOE2bisMATE (**3**), which is much higher than that observed for the parent 2-MeOE2 (**1**).[16,17]

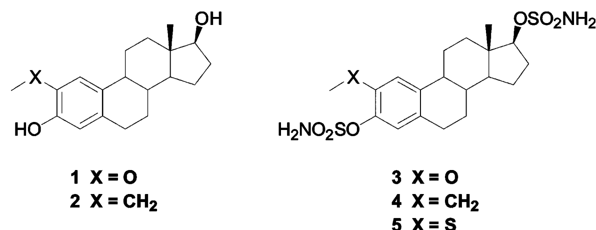

Figure 1. Structures of 2-methoxyestradiol (**1**), 2-ethylestradiol (**2**), 2-methoxyestradiol-3,17-*O,O*-bis-sulfamate (**3**), 2-ethylestradiol-3,17-*O,O*-bis-sulfamate (**4**), and 2-methylsulfanylestradiol-3,17-*O,O*-bis-sulfamate (**5**).

As a result of its inhibition of both cancer cell proliferation and angiogenesis, 2-MeOE2 (**1**) has generated significant interest as a potential anticancer agent, and a number of studies have highlighted possible mechanisms for its cytotoxicity.[18,19] The antiproliferative effects of **1** are independent of the estrogen receptor status of the cancer cell line and appear to be a result of its ability to disrupt tubulin polymerization by binding to the colchicine binding site on tubulin.[20] Several cellular responses, such as Bcl-2 phosphorylation, caspase-3 activation, down-regulation of hypoxia inducible factor-1, increased expression of the tumor suppressor protein p53, and G(2)/M cycle arrest have been reported.[20−29] Various analogues of 2MeOE2 (**1**) have been synthesized and tested for biological activities and several, such as 2-ethoxyestradiol, 2-((*E*)-1′-propenyl)-estradiol, and 2-methoxy-14,15-dehydroestradiol, for example,

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[a] Abbreviations: E2bisMATE, estradiol-3,17-*O*,*O*-bis-sulfamate; 2-MeOE2bisMATE, 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate; 2-EtE2bisMATE, 2-ethylestradiol-3,17-*O*,*O*-bis-sulfamate.

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were found to be more potent than 2-MeOE2 (**1**) as in vitro inhibitors of tubulin polymerization and cell proliferation.[30-34]

We previously demonstrated that 2-substituted estrogen derivatives that are sulfamoylated in the 3-position and in both the 3- and 17-positions (E2bisMATEs), such as **3** and **4**, inhibit cell cycle progression and cell proliferation of an array of normal and drug-resistant breast, prostate, and ovarian cancer cells in vitro.[1,2,4] The bis-sulfamates **3** and **4** exhibited mean GI_{50}'s of 87 and 18 nM, respectively, across the NCI 60 cell line screening panel (cf. 2-MeOE2, 1.3 μM).[5,30] The structure-activity relationship of 2-substituted estrogen 3-*O*-sulfamates and 2-substituted estrogen 3,17-*O,O*-bis-sulfamates has been investigated. The highest antiproliferative activities were observed for 3,17-*O,O*-bis-sulfamoylated estradiol derivatives with a methoxy (**3**), ethyl (**4**), or methylsulfanyl (**5**) group at the 2-position. The corresponding 3-*O*-mono-sulfamoylated estradiol derivatives were generally slightly less active in vitro than the bis-sulfamoylated compounds, though 6.5−150-fold more potent than their parent estradiol derivatives against the proliferation of MCF7 cells, while the 17-*O*-mono-sulfamoylated estradiol derivatives proved inactive. Analogous results were obtained across the NCI 60-cell line panel. Alkylation or acylation of the 3-, 17-, or 3,17-*O*-sulfamate-nitrogen(s) was not tolerated and caused a dramatic reduction in antiproliferative activity. These results indicate that 2-substituted estrogen 3-*O*-sulfamates, although exhibiting mechanistic similarities with the 2-substituted estradiol derivatives, are not prodrugs of the parent steroids and represent a novel class of anticancer agent. In addition, the antiangiogenic effects of **3** and **4** have been shown to be superior to those of 2-MeOE2 (**1**) with lower concentrations required to inhibit the growth of human vascular endothelial cells in vitro.[35] In vivo studies have also demonstrated the advantages of the bis-sulfamoylated compounds over the corresponding 2-substituted estradiol-3-*O*-mono-sulfamates and 2-substituted estradiol derivatives, presumably due to a combination of their enhanced antiproliferative activity and superior ADME properties (see above).

2MeOE2 (**1**) is a more potent inhibitor in vitro of MCF7 cell proliferation than 2-ethylestradiol (**2**) (respective GI_{50}'s are 2.35 and 10.5 μM). In contrast, in vitro results for the bis-sulfamate series show that the 2-MeO compound **3** ($GI_{50} = 0.25$ μM), though nearly 10-fold more active than **1**, is less potent than its 2-ethyl analogue **4**, which has a GI_{50} of 0.07 μM and is, in turn, 100 times more potent than the parent 2-ethylestradiol (**2**). In the present study, we set out to determine the effects of different small alkyl substitutions at the 2-position on antiproliferative activity and the effect of bioisosteric replacement of the 17-*O*-sulfamate group with a carbamate group. The syntheses of a series of novel 2-alkylated estradiol bis-sulfamates and a series of 2-alkylated 3-*O*-sulfamoylated estradiol 17-carbamates are described. The new compounds were then evaluated for in vitro antiproliferative activity, with the most active compound of the series being further evaluated in vivo in tumor xenograft models.

Chemistry

The syntheses of some 2-substituted estrogen 3,17-*O,O*-bis-sulfamates (E2bisMATEs) by sulfamoylation of the corresponding 2-substituted estradiol derivatives have been previously described.[5,13,36] Thus, in order to construct a sensible series of analogs for SAR purposes, a number of different 2-alkyl estradiol derivatives were prepared and converted into a series of 3- and 17-substituted derivatives (Scheme 1). 2-Methylestradiol **8** and 2-*tert*-butylestradiol **10** were obtained in two steps from estrone (**6**). 2-Methylestradiol (**8**) was accessed in 47% yield by reaction of **6** with *N,N,N′,N′*-tetramethyldiaminomethane and paraformaldehyde in a Mannich-type reaction as described by Patton to give the amine **7**,[37] which was then quaternized with iodomethane and the product reduced with $NaBH_4$ to give **8**. Friedel-Crafts alkylation of **6** using *tert*-butanol and $BF_3{\cdot}Et_2O$ in chloroform gave 2-*tert*-butylestrone **9** in high yield (93%),[38] which was then reduced with $NaBH_4$ to give 2-*tert*-butyl estradiol (**10**) in an overall yield of 79%.[39] Various methods for the syntheses of the 2-*n*-alkylestradiols **2**, **23**, and **24** have been reported in the literature, including the hydrogenation of the corresponding 2-alkenylestradiols that are available by Wittig reactions of 2-formylestradiol,[30] by palladium-catalyzed cross-couplings of 2-iodoestradiol,[40] by a Claisen rearrangement of estrone allyl ether[41] or by alkylation of ortho-lithiated MOM-protected derivatives of **6**.[42] Though all of these methods give good results, we decided to investigate a different approach that appeared amenable to a multigram scale, inspired by the synthesis of 2-ethyl-8α-estradiol described by González et al.[43] Friedel-Crafts acylation of the protected estradiol derivative **11**[44] with aluminum chloride and acetyl chloride, propionic anhydride, or butyric anhydride afforded the 2-acylated products **12**, **13**, and **14** in good yields. These were then demethylated using aluminum chloride and trimethylammonium chloride in DCM to give the desired phenols **15**, **16**, and **17** (Scheme 1).[45] Catalytic hydrogenation with palladium on charcoal afforded the 17-*O*-acetyl protected compounds **18**, **19**, and **20**, which were deprotected with sodium hydroxide in methanol/water to give the 2-alkylestradiols **2**, **23**, and **24**. In order to obtain 2-isopropylestradiol (**21**), the 2-acetyl compound **15** was reacted with 6 equivalents of methylmagnesium bromide to give the trihydroxy compound **22**. The benzylic hydroxyl group of **22** was removed by catalytic hydrogenation to afford the target 2-isopropyl estradiol (**21**) in good yield, as described by Pert and Ridley.[46]

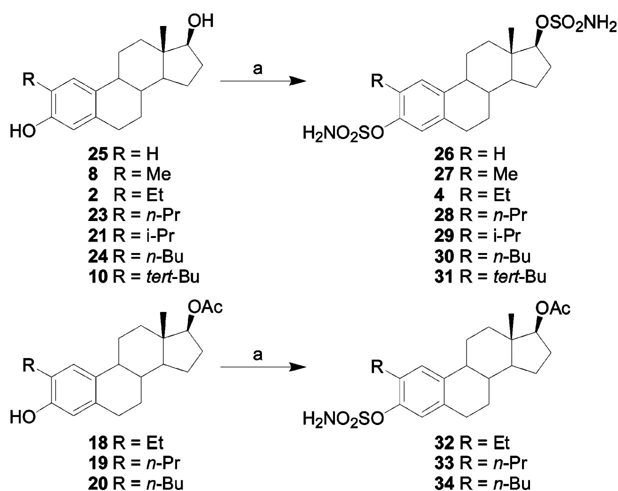
Estradiol (**25**), the series of 2-alkylestradiols **2**, **8**, **10**, **21**, **23**, **24** and acetates **18**, **19**, and **20** were sulfamoylated under standard conditions with sulfamoyl chloride in dimethyl acetamide to give the mono-sulfamate derivatives **32**−**34** and the bis-sulfamates **4**, **26**−**31** in good to excellent yields (Scheme 2).[47]

As part of a continuing SAR program, it was of interest to explore the replacement of the 17-sulfamate group. We decided to explore the use of a carbamate as a potential bioisostere for the sulfamate group, as it seemed likely that a carbamate could exploit some or all of the key interactions with the binding site obviously well satisfied by sulfamate. Since one of the reasons for the success of the bis-sulfamate series is blockade of metabolic conjugation[16] and the sulfamate group per se is unlikely to be the only, or perhaps optimal, motif at the 17-position that could imbue good activity in this class of compound, we undertook to design routes to mixed sulfamate−carbamate esters. The syntheses of the comprehensive series of 17β-carbamoylated estradiol derivatives **49**−**56** are thus illustrated in Scheme 3. The sulfamoylated estradiol and 2-methoxyestradiol mono-carbamates **49** and **50** were synthesized in four steps from the appropriate benzyl ethers **35** and **36**, which were first reacted with trichloroacetyl isocyanate to give the *N*-trichloroacetyl carbamates **37** and **38**.[48] The trichloroacetyl groups of **37** and **38** were then selectively hydrolyzed with K_2CO_3 in MeOH/THF/H_2O to yield the 3-*O*-benzyl-17β-carbamate derivatives **39** and **40**, respectively, which were then hydrogenated to give phenols **41** and **42**. Sulfamoylation of **41** and **42** was then carried out as described above to give **49** and **50**. A

Scheme 1. Syntheses of 2-Alkylestradiol Derivatives[a]

[a] Reagents and conditions: (a) *N,N,N′,N′*-tetramethyldiaminomethane, $(CHO)_n$, PhMe, EtOH, reflux; (b) (1) MeI, Et_2O; (2) $NaBH_4$, EtOH; (c) t-BuOH, BF_3·Et_2O, $CHCl_3$; (d) $NaBH_4$, MeOH, 0 °C; (e) AcCl or $(RC(O))_2O$, $AlCl_3$, DCM, 0 °C; (f) $AlCl_3$, NMe_3·HCl, DCM, reflux; (g) 10% Pd/C, H_2, MeOH, THF; (h) 6 equiv MeMgCl, THF, **15**; (i) NaOH, MeOH, H_2O.

Scheme 2. Synthesis of 2-Substituted Estradiol 3-*O*-Sulfamates and 3,17-*O,O*-Bis-sulfamates[a]



[a] Reagents and conditions: (a) $ClSO_2NH_2$, DMA, 0 °C.

second, more efficient approach to 2-alkylestradiol 17-carbamates was also developed, wherein reaction of the parent estradiol derivatives **2**, **8**, **10**, **21**, **23**, and **24** with excess trichloroacetyl isocyanate was followed by selective hydrolysis of the trichloroacetyl and phenolic carbamate groups in a one-pot procedure to give carbamates **43**−**48**. Sulfamoylation of phenols **43**−**48** gave the target sulfamates **51**−**56** in moderate to good yield.

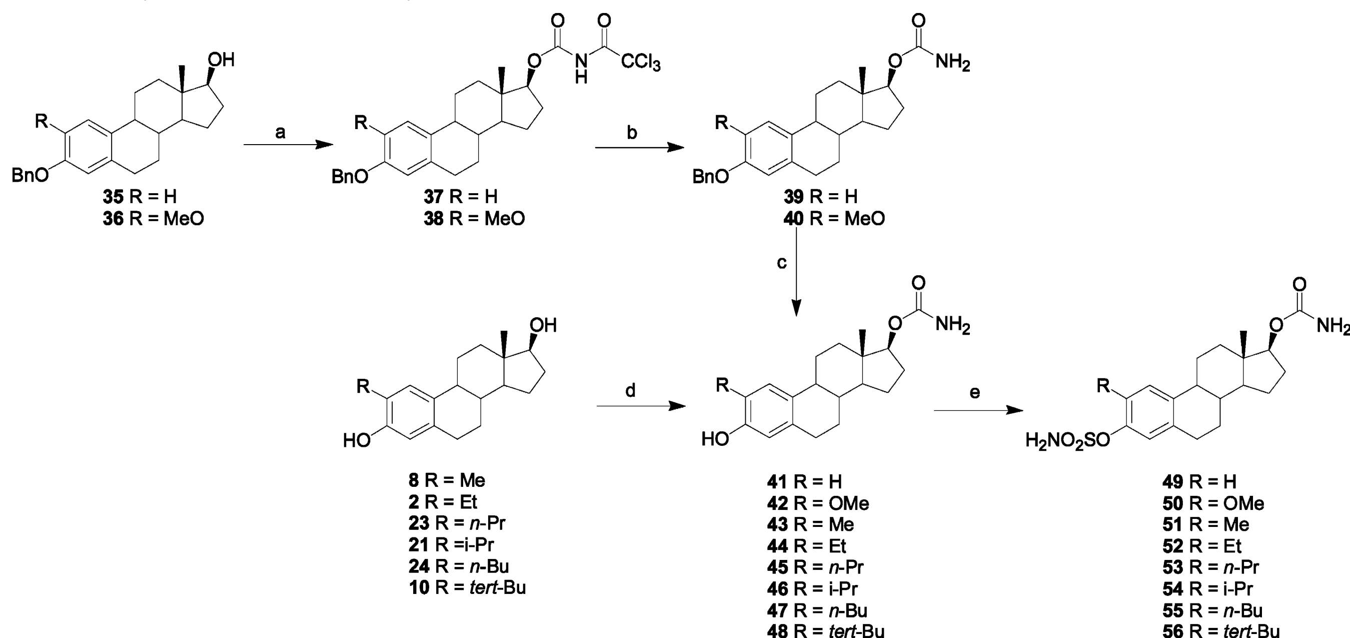
An X-ray crystal structure of **50** was obtained and is presented in Figure 2. This structure confirms that the integrity of the chiral centers of the estra-1,3,5(10)-triene core is maintained through the synthesis and also demonstrates the intramolecular hydrogen bonding between the sulfamate and carbamate NH_2-groups, with the respective carbamate and sulfamate oxygens in the crystallographically adjacent molecules of the unit cell (Figure 3).

Results and Discussion

The sulfamates and carbamates synthesized were evaluated for their ability to inhibit in vitro the proliferation of DU145 prostate cancer cells, MDA-MB-231 (ER−) human breast cancer cells, and, in some cases, MCF7 (ER+) breast cancer cells (Table 1). 2MeOE2 (**1**); the parent 2-alkyl estradiol derivatives **2**, **8**, **10**, **21**, **23**, **24**; and the bis-sulfamates **3** and **4** were tested side by side for comparison.

While in the 2-substituted estradiol series 2MeOE2 (**1**) is the most potent compound, in the bis-sulfamate series, 2-EtE2bisMATE (**4**) proved to be the most potent inhibitor of cancer cell proliferation in vitro in the tested cell lines. Of the novel bis-sulfamates **27**−**31**, only the 2-methyl substituted compound **27** displayed high in vitro activity with a GI_{50} of 0.25 μM in MDA-MB-231 cells, which is comparable to that obtained for 2-MeOE2bisMATE **3** (0.28 μM). Even a small increase in substituent size at the 2-position (e.g. 2-*n*-propyl **28**, 2-isopropyl **29**) was not well tolerated and resulted in a significant decrease in antiproliferative activity. The *n*-butyl and *tert*-butyl derivatives **30** and **31** showed only a very modest activity. An inspection of the 2-alkyl bisMATEs evaluated herein clearly shows that 2-ethyl substitution is optimal for antipro-

Scheme 3. Syntheses of 17-*O*-Carbamoylated Estradiol Derivatives and Their Sulfamates[a]



[a] Reagents and conditions: (a) $Cl_3C(O)NCO$, THF; (b) K_2CO_3, MeOH, THF, H_2O; (c) 10% Pd/C, H_2, MeOH, THF; (d) (1) $Cl_3C(O)NCO$, THF, 0 °C; (2) K_2CO_3, MeOH, THF, H_2O; (e) $ClSO_2NH_2$, DMA, 0 °C.

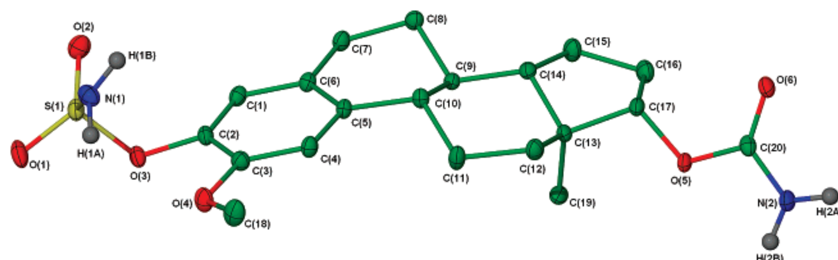


Figure 2. X-ray crystal structure of one of the molecules in the asymmetric unit of **50**. Ellipsoids are represented at 30% probability. Carbon-bound hydrogen atoms have been omitted for clarity.

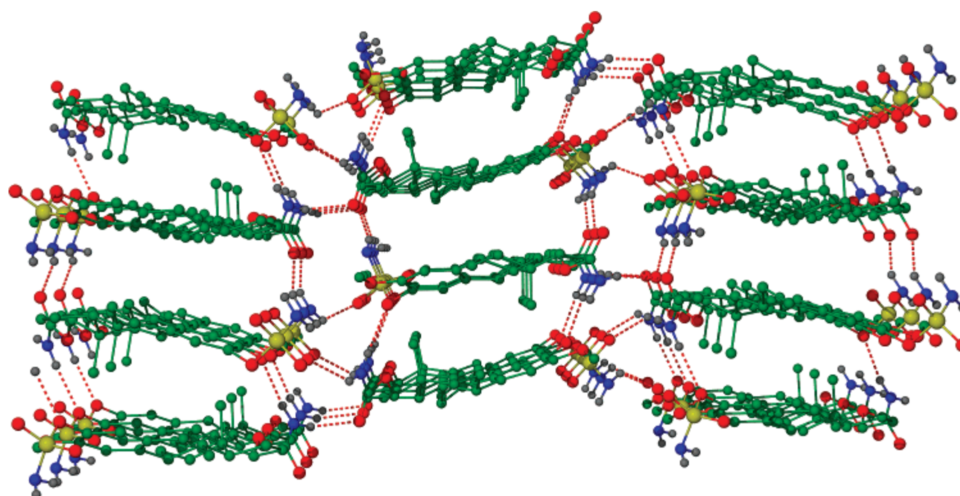


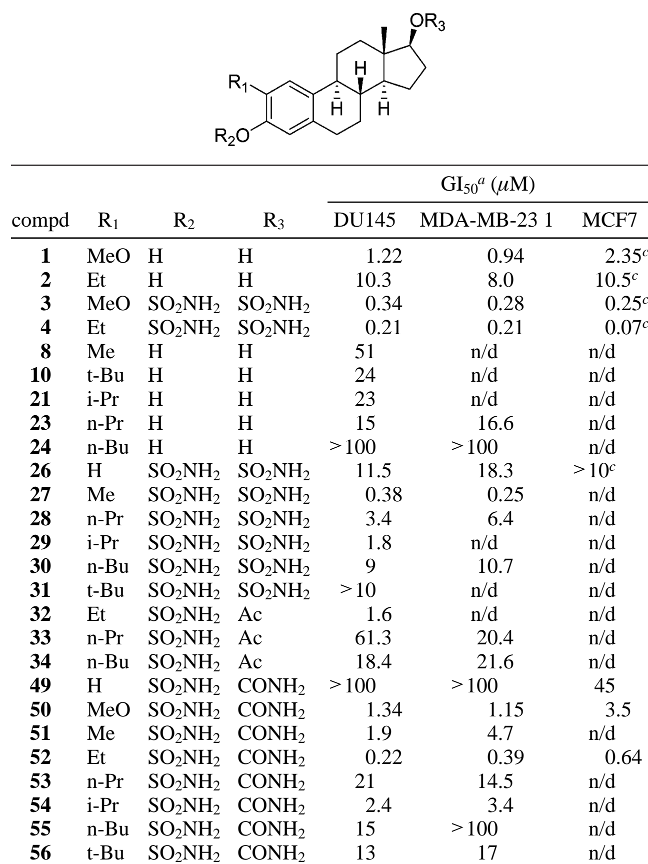
Figure 3. Intermolecular hydrogen bonding viewed perpendicular to the *bc* plane in the gross structure of **50**.

liferative activity and that the activity is proportional to the steric size of the 2-alkyl group.

The 2-alkyl-3-hydroxy-17β-acetoxyestra-1,3,5(10)-trienes **18**−**20** proved to be devoid of antiproliferative activity (GI_{50} > 100 μM), while compound **32**, the sulfamoylated analogue of **18**, displays fairly good activity with a GI_{50} of 1.6 μM in the DU145 cell line (cf. 2-MeOE2, 1.22 μM). The 2-*n*-propyl and 2-*n*-butyl analogues **33** and **34** were only modestly active, once again emphasizing the poor tolerance of alkyl groups larger than ethyl for antiproliferative activity in the sulfamate series.

The findings in the 17β-carbamate series are similar to those obtained in the bis-sulfamate and 17β-acetate series. The nonsulfamoylated 17β-carbamates **41**−**48** displayed very little or no activity, while most of the corresponding sulfamates, with the exception of the 2-unsubstituted compound **49**, displayed enhanced antiproliferative activity. The 2-ethyl derivative **52**

Table 1. Antiproliferative Activities of Estradiol Derivatives against DU145, MDA MB-231, and MCF7 Cancer Cell Lines



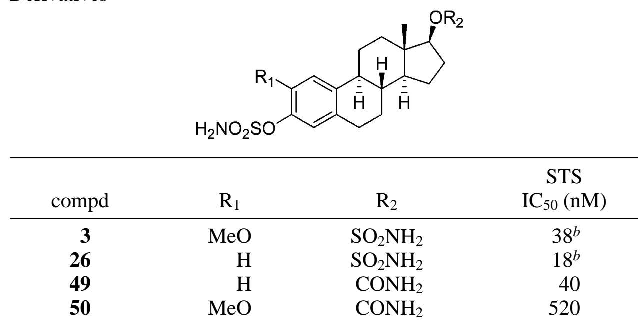
compd	R_1	R_2	R_3	GI_{50}[a] (μM) DU145	MDA-MB-23 1	MCF7
1	MeO	H	H	1.22	0.94	2.35[c]
2	Et	H	H	10.3	8.0	10.5[c]
3	MeO	SO_2NH_2	SO_2NH_2	0.34	0.28	0.25[c]
4	Et	SO_2NH_2	SO_2NH_2	0.21	0.21	0.07[c]
8	Me	H	H	51	n/d	n/d
10	t-Bu	H	H	24	n/d	n/d
21	i-Pr	H	H	23	n/d	n/d
23	n-Pr	H	H	15	16.6	n/d
24	n-Bu	H	H	>100	>100	n/d
26	H	SO_2NH_2	SO_2NH_2	11.5	18.3	>10[c]
27	Me	SO_2NH_2	SO_2NH_2	0.38	0.25	n/d
28	n-Pr	SO_2NH_2	SO_2NH_2	3.4	6.4	n/d
29	i-Pr	SO_2NH_2	SO_2NH_2	1.8	n/d	n/d
30	n-Bu	SO_2NH_2	SO_2NH_2	9	10.7	n/d
31	t-Bu	SO_2NH_2	SO_2NH_2	>10	n/d	n/d
32	Et	SO_2NH_2	Ac	1.6	n/d	n/d
33	n-Pr	SO_2NH_2	Ac	61.3	20.4	n/d
34	n-Bu	SO_2NH_2	Ac	18.4	21.6	n/d
49	H	SO_2NH_2	$CONH_2$	>100	>100	45
50	MeO	SO_2NH_2	$CONH_2$	1.34	1.15	3.5
51	Me	SO_2NH_2	$CONH_2$	1.9	4.7	n/d
52	Et	SO_2NH_2	$CONH_2$	0.22	0.39	0.64
53	n-Pr	SO_2NH_2	$CONH_2$	21	14.5	n/d
54	i-Pr	SO_2NH_2	$CONH_2$	2.4	3.4	n/d
55	n-Bu	SO_2NH_2	$CONH_2$	15	>100	n/d
56	t-Bu	SO_2NH_2	$CONH_2$	13	17	n/d

[a] GI_{50} figures are the mean values obtained from experiments performed in triplicate, SEM = ±10%. [b] n/d = not determined. [c] Taken from refs 5, 42.

was the most potent compound tested in this series, with GI_{50}'s of 0.22 and 0.39 μM in the DU145 and MDA-MB-231 cell lines, repectively. These results compare well with those obtained for **3** and **4**. However, while **4** displayed a GI_{50} of just 0.07 μM in the MCF7 cell line, a near 10-fold increase was observed for **52**. The carbamates with a methoxy, methyl, and isopropyl substituent in 2-position (**50**, **51**, and **54**) also showed good inhibitory effects against cancer cell growth but significantly less than **52**, while the 2-*n*-propyl, 2-*n*-butyl, and 2-*tert*-butyl substituted compounds **53**, **55**, and **56** displayed highly reduced activity in these assays. It would thus appear that replacement of the 17-*O*-sulfamate with a carbamate group is valid in the generation of 2-substituted estradiol-3-*O*-sulfamates with antiproliferative activity, and although such derivatives do not show uniformly high activity, their SAR mirrors that found in the estradiol-bis-sulfamate series.

To investigate the effect of carbamoylation of the 17-hydroxy group on the steroid sulfatase (STS) inhibitory activity of the estradiol-3-*O*-sulfamate compounds **49** and **50** were assayed using the radiometric assay developed by Purohit et al.,[49] wherein the STS-mediated conversion of tritiated E2-sulfate to E2 by placental microsomes is determined (Table 2). The bis-sulfamates **3** and **26** have been found in previous studies to be very potent inhibitors of STS, with IC_{50}'s of 38 and 18 nM, respectively. Replacement of the 17-*O*-sulfamate group of **26** with a carbamate group results in a slight decrease in sulfatase inhibitory activity. The IC_{50} for the carbamate **49** was determined as 40 nM, a 2-fold increase compared to the corresponding estradiol bis-sulfamate **26**. The IC_{50} for the 2-methoxy carbamate **50** is, however, surprisingly more than 13-times

Table 2. Inhibition of Placental STS by 3-*O*-Sulfamoylated Estradiol Derivatives



compd	R_1	R_2	STS IC_{50} (nM)
3	MeO	SO_2NH_2	38[b]
26	H	SO_2NH_2	18[b]
49	H	$CONH_2$	40
50	MeO	$CONH_2$	520

[a] IC_{50} figures are the mean values obtained from experiments performed in triplicate, SEM < ±7%. [b] Taken from ref 5.

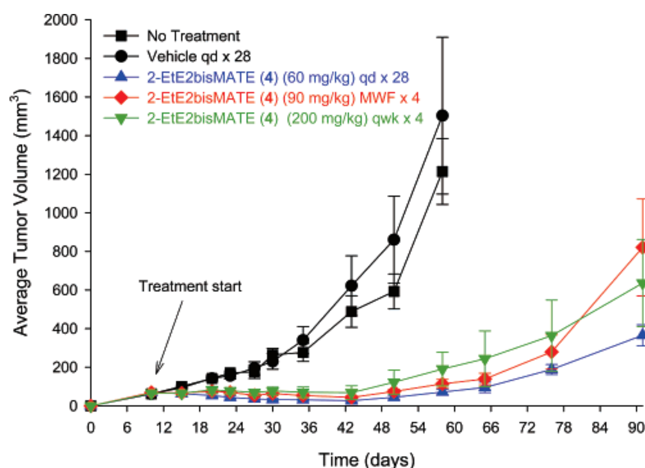


Figure 4. Comparison effect of 2EtE2bisMATE (**4**) using various po dosing schedules on the MDA-MB-231 xenograft model in female athymic nude mice. Dosing schedules: start of treatment on day 10; qd (daily) end of treatment on day 38; MWF (Monday, Wednesday, Friday) end of treatment on day 36; qwk (once a week) end of treatment on day 32.

higher than that observed for the corresponding parent bis-sulfamate **3**. We cannot rationalize this relative to the results for **26** vs **49** beyond supposing that differential binding of the carbamate motif presumably distorts binding of the group at the distal end relative to a sulfamate. The activity in the 2-alkyl series was not explored, as it had previously been determined that 2-EtE2bisMATE (**4**) is substantially less potent than 2-MeOE2bisMATE (**3**) as an irreversible inhibitor of STS.[5]

In Vivo Studies. 2-EtE2bisMATE (**4**) was selected for in vivo evaluation of its ability to inhibit tumor growth. These assessments were performed using xenografts derived from MDA-MB-231 (ER−) human breast cancer cells in female athymic nude mice and xenografts derived from DU145 human prostate cancer cells in male athymic nude mice using various po dosing schedules. The results for the MDA-MB-231 xenograft model are presented in Figure 4. All three different dosing schedules [i, 60 mg/kg, qd (once a day), 28d; ii, 90 mg/kg, MWF (Monday, Wednesday, Friday) × 4; iii, 200 mg/kg, qwk (once a week) × 4] using an unoptimized THF/PG vehicle system caused a dramatic reduction in tumor growth (qwk cohort) or tumor regression over the course of the dosing (qd and MWF cohorts); furthermore, these effects were sustained after the cessation of the treatment on day 38. No treatment-related deaths or significant weight loss were observed in any of the three cohorts.

The high efficacy demonstrated by **4** in this study underlines the potential of this agent as an orally dosed antitumor agent.

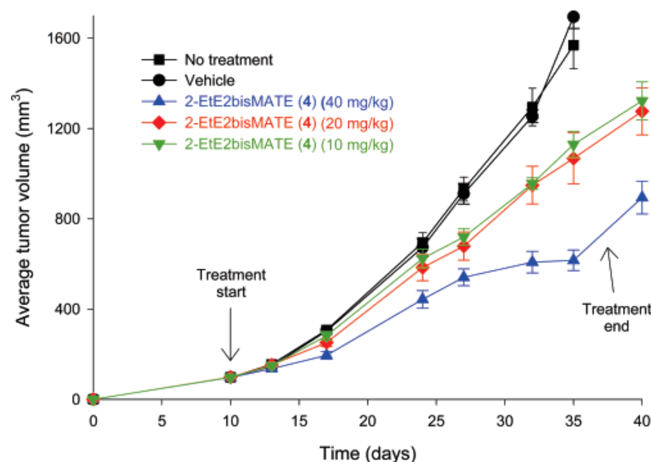


Figure 5. Comparison effect of various doses 2-EtE2bisMATE (**4**) using a qd × 28 schedule on the DU145 xenograft model in male athymic nude mice.

The observed reduction in tumor volume over the treatment period augers well for tumor suppression over extended dosing schedules.

The effects of different doses (10, 20, and 40 mg/kg, qd, 28d) of **4** in the highly aggressive DU145 human prostate cancer xenograft model in male athymic nude mice are shown in Figure 5. Here, after initiation of treatment, the various dosing cohorts show little activity over the first 7 days. After 25 days of treatment (day 35), however, the tumor growth in the 40 mg/kg treatment group appears to plateau with a 65% inhibition of tumor growth at this time point (control and vehicle group were euthanized at this point for ethical reasons). The 10 and 20 mg/kg groups effected more modest tumor growth, 24% and 27%, though there is no statistically significant difference between the inhibition effected by these groups. The activity of **4** in this highly aggressive xenograft model at nonoptimized doses was very encouraging.

Conclusion

In the present study, we have synthesized a series of novel 2-alkylestradiol-3,17-*O,O*-bis-sulfamates and sulfamoylated 2-alkylestradiol-17-*O*-carbamates. The compounds were evaluated as inhibitors of in vitro cancer cell growth in DU145, MDA-MB-231, and MCF7 human cancer cell cultures and compared to the parent estradiol derivatives. In the bis-sulfamate series the 2-methyl derivative **27** displayed antiproliferative activity similar to that of 2-ethylestradiol-3,17-*O,O*-bis-sulfamate (**4**), while introduction of larger alkyl-substituents at the C-2 position proved detrimental to antiproliferative activity.

Carbamoylation of the 17β-hydroxy group of the 2-substituted estradiols afforded the 2-alkyl-17β-carbamoyloxyestra-1,3,5(10)-trien-3-ol series of compounds **41**−**48**, which did not substantially inhibit cancer cell proliferation. After sulfamoylation of the phenolic hydroxy group, a series of 2-alkyl-3-sulfamoyloxy-17β-carbamoyloxyestra-1,3,5(10)-trien-3-ols (compounds **49**−**56**) was obtained that showed much improved antiproliferative activity, with optimal activity obtained for the 2-ethyl-carbamate **52** in the three cell lines assayed. Introduction of an acetate group in the 17-position led to a reduction of cancer cell growth inhibition compared to the carbamate and bis-sulfamate analogs. Bioisosteric replacement of the 17-*O*-sulfamate group of **4** with a carbamate group (**52**) or, with less success, an acetate group (**32**) was thus achieved, albeit the bis-sulfamate compound remains the most active of the series. It was also confirmed that of the 2-alkyl substituents the 2-ethyl group is optimal in all three series, although the 2-methyl bis-sulfamate (**27**) also displayed promising activity.

In addition to these findings from in vitro assays, the potential of 2-EtE2bisMATE (**4**) as an antitumor agent was confirmed in MDA-MB-231 and DU145 xenograft models. In the MDA-MB-231 xenografts significant tumor regression was observed on p.o. dosing in two out of three schedules, while the remaining schedule still achieved significant growth inhibition. In the highly aggressive DU145 xenograft 65% growth inhibition was observed in the higher (40 mg/kg) dosed cohort with no signs of toxicity. Bis-substituted 2-alkylestradiol sulfamates show considerable potential as anticancer agents. Further studies directed toward clinical development, particularly of 2-EtE2bisMATE (**4**), are in progress.

Experimental Section

Biology. In Vitro Studies: Cell Lines. DU145 (brain metastasis carcinoma of the prostate), MCF7, and MDA-MB-231 (Metastatic pleural effusion of breast adenocarcinoma) established human cell lines were obtained from ATCC Global Bioresource Center. Cells were maintained in a 5% CO_2 humidified atmosphere at 37 °C in RPMI-1640 medium, supplemented with 10% fetal bovine serum, penicillin, and streptomycin.

Antiproliferative Assays. DU145 and MDA-MB-231 cells were seeded into 96-well microtiter plates (5000 cells/well) and treated with 10^{-9}−10^{-4} M of compounds or with vehicle control. At 96 h post-treatment, live cell counts were determined by WST-1 cell proliferation assay (Roche, Penzberg, Germany), as per the manufacturer's instructions. Viability results were expressed as a percentage of mean control values resulting in the calculation of the 50% growth inhibition (GI_{50}). All experiments were performed in triplicate. The MCF7 antiproliferative assay was carried out as reported previously.[42]

The inhibition of STS was assayed by the method of Purohit et al.[49]

In Vivo Studies. Studies were carried out following Institutional Guidelines that comply with the recommendations of the *Guide for Care and Use of Laboratory Animals* with respect to restraint, husbandry, surgical procedures, feed, and fluid regulation.

MDA-MB-231 Study. Mice: Female NCr-nude mice, 6−8 weeks of age, were fed water ad libitum (reverse osmosis, 0.17% Cl) and an autoclaved standard rodent (NIH31) diet consisting of 18% protein, 5% fat, 5% fiber, 8% ash, and 3% minerals. Mice were housed in microisolators on a 12-hour light cycle at 22 °C and 40%−60% humidity.

Tumors: Mice were implanted subcutaneously with 5 × 10^6 MDA-MB-231 cells in the flank. Tumors were monitored initially twice weekly, and then daily as the neoplasms reached the desired size, approximately 50−100 mm^3. Estimated tumor volume was calculated using the formula tumor volume (mm^3) = $(w^2 \times l)/2$, where w = width and l = length in mm of the breast carcinoma. Statistical significance calculated by one-way analysis of variance for all three treatment groups with respect to control gave $P < 0.001$.

DU145 Study. Mice: Male NCr-nude mice, 6−8 weeks of age, were fed water ad libitum (reverse osmosis, 0.17% Cl) and an autoclaved standard rodent (NIH31) diet consisting of 18% protein, 5% fat, 5% fiber, 8% ash, and 3% minerals. Mice were housed in microisolators on a 12-hour light cycle at 22 °C and 40%−60% humidity.

Tumors: Mice were implanted subcutaneously with 5 × 10^6 cells in the flank. Tumors were monitored initially twice weekly and then daily as the neoplasms reached the desired size, approximately 50−100 mm^3. Estimated tumor volume was calculated using the formula tumor volume (mm^3) = $(w^2 \times l)/2$, where w = width and l = length in mm of the carcinoma. Statistical significance calculated by one-way analysis of variance for all three treatment groups with respect to control gave $P < 0.001$.

Chemistry. All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK) or Lancaster Synthesis (Morecombe, UK). E1 (**6**) was purchased from Sequoia Research Products (Oxford, UK). All organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK). Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[50] and was stored in the refrigerator under positive pressure of N_2 as a solution in toluene as described by Woo et al.[51] The protected estradiol **11** was prepared according to the method of Bielman and Branlant.[52] Compound **35** was synthesized from estradiol (**25**),[53] and compound **36** was made according to a procedure from Rao and Cessac.[54] The bis-sulfamates **3**, **4**, and **26** have been described previously.[5,12,36] Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica gel 60 F_{254}, Art. No. 5554). Product(s) and starting materials(s) were detected by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed on silica gel (Matrex C60). IR spectra were determined on a Perkin-Elmer Spectrum RXI FT-IR as KBr disks, and peak positions are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury VX 400 NMR spectrometer at 400 and 100.6 MHz, respectively, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as an internal standard. The following abbreviations are used to describe resonances in ^1H NMR and ^{13}C NMR spectra: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multplet; hpt, heptet. Chemical shifts for AB systems were approximated by taking the middle of each doublet. HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector. A Waters Radialpack C_{18} reversed phase column (8 × 100 mm) was eluted with a methanol/water gradient at 2 mL/min. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath or at the EPSRC National Mass Spectrometry Service Centre, University of Wales Swansea. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Electrospray (ES) and atmospheric pressure chemical ionization (APCI) low-resolution mass spectra were obtained on a Waters Micromass ZQ. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Stanford Research Systems OptiMelt MPA100 and are uncorrected.

Crystal Data for 50. Empirical formula: $C_{20}H_{28}N_2O_6S$, M = 424.50, T = 150(2) K, λ = 0.710 73 Å, monoclinic, space group $P2_1$, a = 9.0350(1) Å, b = 25.5210(4) Å, c = 9.1370(1) Å, β = 100.288(1)°, U = 2072.96(5) Å^3, Z = 4, D_c = 1.360 g cm^{-3}, μ = 0.196 mm^{-1}; $F(000)$ = 904, crystal size 0.35 × 0.25 × 0.10 mm, unique reflections = 9299 [R(int) = 0.0528], observed $I > 2\sigma(I)$ = 6850, data/restraints/parameters: 9299/9/544, $R1$ = 0.0416 $wR2$ = 0.0780 (obsd data), $R1$ = 0.0737, wR2 = 0.0871 (all data), max peak/hole 0.385 and −0.376 e Å^{-3}, absolute structure parameter = 0.06(4). Data were collected on a Nonius kappaCCD diffractometer. The asymmetric unit was seen to consist of two independent molecules. Hydrogen atoms attached to N1 and N3 were located and refined at a distance of 0.89 Å from the parent atoms. The gross structure is dominated by extensive hydrogen bonding.

Crystallographic data for **50** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 632335. Copies of these data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax(+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

General Procedure 1. Preparation of Sulfamates. The sulfamates were prepared using the method of Okada et al.[47] The estradiol, 17β-carbamoyloxyestra-1,3,5(10)-trien-3-ol, or 17β-acetoxyestra-1,3,5(10)-trien-3-ol (0.35−1.0 mmol) was added to a stirred solution of sulfamoyl chloride (3 equiv per hydroxyl group) in anhydrous dimethylacetamide (5 mL) at 0 °C under an atmosphere of N_2. The reaction mixture was stirred for 2 h at 0 °C and then for another 14 h at room temperature. EtOAc (50 mL) and water (30 mL) were added. The organic layer was separated, washed with water (2 × 30 mL) and brine (30 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using mixtures of chloroform and acetone (typically between 20:1 and 5:1 by volume) to give the sulfamate or bis-sulfamate derivative.

General Procedure 2. Preparation of Carbamates. Trichloroacetyl isocyanate (8−9 equiv) was added to a solution of the estradiol (2 mmol) in THF at 0 °C. Stirring was continued for 30 min and water (5 mL) was then added carefully. MeOH (30 mL) and K_2CO_3 (2.76 g, 20.0 mmol) were then added, and the mixture was stirred vigorously at room temperature for 3 h. The mixture was poured into water (60 mL) and the products were extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL) and dried (Na_2SO_4). The solvents were removed under reduced pressure, and the residue was purified by flash chromatography (eluent $CHCl_3$/acetone typically between 20:1 and 5:1 by volume) to give the carbamate.

2-Ethylestradiol (2). To a suspension of **18** (1.03 g, 3.0 mmol) in MeOH (20 mL) was added 5 M NaOH (3 mL). The mixture was stirred for 2 h at room temperature before being poured into 2 M $KHSO_4$ (50 mL). The product was extracted with EtOAc (3 × 50 mL), and the combined organic layers were then washed with brine (50 mL) and dried (Na_2SO_4). The solvents were removed under reduced pressure, and the residue was purified by flash chromatography ($CHCl_3$/acetone 10:1 by volume) to give **2** (865 mg, 96%) as a white solid: mp 168−169 °C (lit.[30] mp 165−166 °C); ^1H NMR ($CDCl_3$) δ 0.77 (s, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.24−1.56 (m, 7H), 1.60−1.74 (m, 2H), 1.80−1.88 (m, 1H), 1.92−1.98 (m, 1H), 2.06−2.22 (m, 2H), 2.30−2.38 (m, 1H), 2.60 (q, J = 7.5 Hz, 2H), 2.72−2.86 (m, 2H), 6.49 (s, 1H), 7.05 (s, 1H); MS (APCI−) m/z 299.2 (100, [M − H]$^-$). Anal. ($C_{20}H_{30}O_2$) C, H.

2-(*N,N*-Dimethylamino)methylestrone (7). To a solution of estrone (**6**, 6.76 g, 25.0 mmol) in EtOH (30 mL) and toluene (30 mL) were added *N,N,N′,N′*-tetramethyldiaminomethane (5.0 g, 50 mmol) and paraformaldehyde (750 mg). The mixture was heated to reflux for 18 h and then cooled to room temperature before being concentrated under reduced pressure to a volume of ca. 10 mL. Water (50 mL) was added, and the products were extracted with Et_2O (3 × 60 mL). The combined organic layers were washed with brine (30 mL) and dried (Na_2SO_4). The solvents were removed under reduced pressure to give the crude product, which contained ca. 10% 4-(*N,N*-dimethylamino)methylestrone. Recrystallization from EtOH gave pure **7** (4.83 g, 59%) as a white crystalline solid: mp 174−175 °C (lit.[37] mp 169.5−171.5 °C); ^1H NMR ($CDCl_3$) δ 0.89 (s, 3H), 1.32−1.66 (m, 6H), 1.89−2.25 (m, 5H), 2.30 (s, 6H), 2.32−2.40 (m, 1H), 2.48 (dd, J = 18.8, 8.2 Hz, 1H), 2.80−2.88 (m, 2H), 3.53 (d, J = 13.7 Hz, 1H), 3.62 (d, J = 13.7 Hz, 1H), 6.56 (s, 1H), 6.86 (s, 1H), 11.92 (br s, 1H); MS (APCI+) m/z 328.1 (100, [M + H]$^+$). Anal. ($C_{21}H_{29}NO_2$) C, H, N.

2-Methylestradiol (8). To a solution of **7** (3.93 g, 12.0 mmol) in Et_2O (100 mL) was added methyl iodide (3.55 g, 25 mmol) and the mixture was stirred for 24 h at room temperature. The resulting white precipitate was collected by filtration, washed with Et_2O, and then suspended in EtOH (50 mL). $NaBH_4$ (1.0 g, 26.3 mmol) was added at 0 °C and the mixture was stirred for 18 h at room temperature. The reaction mixture was then poured into water (100 mL), and the products were extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/acetone 10:1) to give **8** as a white solid (2.72 g, 79%): mp 180−182 °C (EtOH, crystals contain 1 equiv EtOH) (lit.[30] mp 185−186 °C from ether); ^1H NMR ($CDCl_3$) δ 0.82 (s, 3H), 1.18−1.59 (m, 7H), 1.64−1.78 (m, 2H), 1.86−1.94 (m, 1H), 1.98−2.02 (m, 1H), 2.10−2.24 (m, 2H), 2.26 (s, 3H), 2.32−2.40 (m, 1H), 2.76−2.90 (m, 2H), 3.74−3.81 (m, 1H), 5.16 (s, 1H), 6.55 (s, 1H), 7.08 (s, 1H); MS (APCI+) m/z 287.3 (65, [M + H]$^+$), 269.3 (100). Anal. ($C_{19}H_{26}O_2 \cdot C_2H_5OH$) C, H.

2-*tert*-Butylestrone (9). To a stirred suspension of estrone (**6**, 5.41 g, 20.0 mmol) in $CHCl_3$ (100 mL) and *tert*-butanol (2 mL) was added $BF_3 \cdot (Et)_2O$ (10 mL) with a syringe at room temperature. The resulting orange solution was stirred for 30 min before water

(50 mL) was added carefully. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (30 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure, the solid residue was suspended in MeOH (50 mL), and the mixture was heated to reflux for 5 min. The product **9** (6.07 g, 93%), a white solid, was collected by filtration after cooling to room temperature, washed with MeOH, and dried under high vacuum: mp 242−245 °C (lit.[38] mp 242−245 °C); ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 3H), 1.40 (s, 9H), 1.42−1.70 (m, 6H), 1.93−2.30 (m, 5H), 2.40−2.55 (m, 2H), 2.75−2.88 (m, 2H), 4.94 (s, 1H, O*H*), 6.44 (s, 1H), 7.19 (s, 1H); MS (APCI−) *m/z* 325.4 (100, $[M - H]^-$). Anal. ($C_{22}H_{30}O_2$) C, H.

2-*tert*-Butylestradiol (10). To a solution of **9** (2.47 g, 7.56 mmol) in MeOH (25 mL) and THF (25 mL) cooled to 0 °C was added $NaBH_4$ (378 mg, 10.0 mmol). The resulting solution was stirred at this temperature for 2 h and then treated with water (50 mL) and EtOAc (100 mL). The organic layer was separated washed with water (50 mL) and brine (50 mL) and dried (Na_2SO_4). The solvents were removed under reduced pressure, and the residue was purified by flash chromatography ($CHCl_3$/acetone 10:1) to give **10** as a white solid (2.11 g, 85%): mp 174−176 °C (lit.[39] mp 174−176 °C); ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 1.14−1.36 (m, 4H), 1.38 (s, 9H), 1.41−1.58 (m, 4H), 1.65−1.72 (m, 1H), 1.82−1.87 (m, 1H), 1.92−1.98 (m, 1H), 2.06−2.22 (m, 2H), 2.30−2.38 (m, 1H), 2.68−2.84 (m, 2H), 3.74−3.77 (m, 1H), 4.86 (s, 1H, O*H*), 6.42 (s, 1H), 7.18 (s, 1H); MS (APCI−) *m/z* 327.5 (100, $[M - H]^-$); HRMS (ES+) *m/z* calcd for $C_{22}H_{33}O_2$ $[M + H]^+$ 329.2475, found 329.2473.

17*β*-Acetoxy-2-Acetyl-3-methoxyestra-1,3,5(10)-triene (12). Acetyl chloride (4.0 mL, 3.62 g, 46.1 mmol) was added dropwise to a cooled (0 °C) suspension of $AlCl_3$ (6.40 g, 48.0 mmol) in DCM (50 mL), and then a solution of **11** (7.90 g, 24.0 mmol) in DCM (25 mL) was added slowly and stirring was continued for 15 min at this temperature. The mixture was poured onto crushed ice, chloroform (100 mL) and water (100 mL) were added, and the resultant mixture was stirred vigorously for 10 min. The organic layer was separated, the aqueous layer was extracted with $CHCl_3$ (2 × 50 mL), and the combined organic layers were dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was crystallized from *i*-Pr_2O to give **12** (3.78 g, 85%) as a white crystalline solid: mp 187−189 °C (lit.[44] mp 192−194 °C); ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3H), 1.20−1.58 (m, 7H), 1.66−1.86 (m, 1H), 1.83−1.92 (m, 2H), 2.05 (s, 3H), 2.12−2.26 (m, 2H), 2.32−2.40 (m, 1H), 2.57 (s, 3H), 2.83−2.91 (m, 2H), 3.86 (3H, s), 4.66 (dd, *J* = 9.4, 7.8 Hz, 1H), 6.65 (s, 1H), 7.67 (s, 1H); MS (APCI+) *m/z* 371.4 (100, $[M + H]^+$). Anal. ($C_{23}H_{30}O_4$) C, H.

17*β*-Acetoxy-3-methoxy-2-propionylestra-1,3,5(10)-triene (13). Propionic anhydride (6.50 g, 50 mmol) was added dropwise to a cooled (0 °C) suspension of $AlCl_3$ (8.0 g, 60 mmol) in DCM (100 mL). To this was added over a period of 10 min a solution of **11** (8.21 g, 25 mmol) in DCM (50 mL) and stirring was continued for 30 min, at which time the reaction mixture was poured onto crushed ice and allowed to warm to room temperature. The organic layer was separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and the solvents were removed under reduced pressure. The resulting solid was recrystallized from MeOH to give **13** (7.306 g, 76%) as fine colorless needles: mp 160−161 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 1.13 (t, *J* = 7.4 Hz, 3H), 1.20−1.60 (m, 1H), 1.84−1.92 (m, 2H), 2.05 (s, 3H), 2.13−2.26 (m, 2H), 2.31−2.39 (m, 1H), 2.84 (m, 2H), 2.92−3.01 (m, 2H), 3.85 (s, 3H), 4.66 (dd, *J* = 9.0, 7.8 Hz, 1H), 6.64 (s, 1H), 7.64 (s, 1H); MS (APCI+) *m/z* 385.5 (100, $[M + H]^+$). Anal. ($C_{24}H_{32}O_4$) C, H.

17*β*-Acetoxy-2-butyryl-3-methoxyestra-1,3,5(10)-triene (14). Butyric anhydride (9.5 g, 60 mmol) was added dropwise to a cooled (0 °C) suspension of $AlCl_3$ (8.0 g 60 mmol) in DCM (100 mL). To this was added over a period of 10 min a solution of **11** (8.21 g, 25 mmol) in DCM (50 mL) and stirring was continued for 30 min, at which time the reaction mixture was poured onto crushed ice and allowed to warm to room temperature. The organic layer was separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was crystallized from MeOH to give the **14** (7.85 g, 78%) as fine colorless needles: mp 145−147 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 0.94 (t, *J* = 7.4 Hz, 3H), 1.20−1.58 (m, 7H), 1.62−1.76 (m, H), 1.84−1.91 (m, 1H), 2.05 (s, 3H), 2.12−2.25 (m, 2H), 2.30−2.39 (m, 1H), 2.83−2.98 (m, 4H), 3.84 (s, 3H), 4.66 (dd, *J* = 9.0, 7.8 Hz, 1H), 6.64 (s, 1H), 7.61 (s, 1H); MS (ES+) *m/z* 399.4 (100, $[M + H]^+$). Anal. ($C_{25}H_{34}O_4$) C, H.

17*β*-Acetoxy-2-acetylestra-1,3,5(10)-trien-3-ol (15). Trimethylammonium hydrochloride (15.77 g, 165 mmol) was added to a suspension of $AlCl_3$ (44.0 g, 0.33 mmol) in DCM (100 mL) at 0 °C. The resulting mixture was stirred for 30 min at room temperature before a solution of **12** (11.12 g, 30 mmol) in DCM (100 mL) was added slowly. The mixture was brought to reflux for 2 h and then, after cooling to room temperature, poured onto crushed ice. The organic layer was separated, washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was crystallized from EtOAc/hexane to give **15** (9.30 g, 87%) as light yellow needles: mp 198−200 °C (lit.[44] mp 202−204 °C); ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 3H), 1.20−1.60 (m, 7H), 1.70−1.79 (m, 1H), 1.84−1.94 (m, 2H), 2.05 (s, 3H), 2.13−2.31 (m, 3H), 2.58 (s, 3H), 2.76−2.92 (m, 2H), 4.68 (dd, *J* = 9.0, 7.8 Hz, 1H), 6.67 (s, 1H), 7.58 (2, 1H), 12.02 (s, 1H, O*H*); MS (ES+) *m/z* 357.5 (100, $[M + H]^+$). Anal. ($C_{22}H_{28}O_4$) C, H.

17*β*-Acetoxy-2-propionylestra-1,3,5(10)-trien-3-ol (16). Trimethylammonium hydrochloride (7.17 g, 75 mmol) was added to a suspension of $AlCl_3$ (20.0 g, 150 mmol) in DCM (100 mL) at 0 °C. The resulting mixture was stirred for 30 min at room temperature before a solution of **13** (5.77 g, 30 mmol) in DCM (100 mL) was added slowly. The mixture was brought to reflux for 2 h and then, after cooling to room temperature, poured onto crushed ice. The organic layer was separated, washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was crystallized from EtOAc/hexane to give **16** (4.39 g, 79%) as light yellow needles: mp 167−168 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (s, 3H), 1.27 (t, *J* = 7.4 Hz, 3H), 1.28−1.62 (m, 7H), 1.74−1.82 (m, 1H), 1.90−1.97 (m, 2H), 2.10 (s, 3H), 2.19−2.36 (m, 3H), 2.80−2.96 (m, 2H), 3.00−3.11 (m, 2H), 4.47 (dd, *J* = 9.0, 7.8 Hz, 1H), 6.72 (s, 1H), 7.68 (2, 1H), 12.19 (s, 1H, O*H*); MS (APCI+) *m/z* 371.6 (100, $[M + H]^+$). Anal. ($C_{23}H_{30}O_4$) C, H.

17*β*-Acetoxy-2-butyrylestra-1,3,5(10)-trien-3-ol (17). Trimethylammonium hydrochloride (8.60 g, 90 mmol) was added to a suspension of $AlCl_3$ (24.0 g, 180 mmol) in DCM (100 mL) at 0 °C. The resulting mixture was stirred for 30 min at room temperature before a solution of **14** (7.17 g, 18.0 mmol) in DCM (50 mL) was added slowly. The mixture was heated to reflux for 2 h and then, after cooling to room temperature, poured onto crushed ice. The organic layer was separated, washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was crystallized from MeOH to give **17** (5.81 g, 84%) as light yellow needles: mp 112−113 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H), 1.00 (t, *J* = 7.4 Hz, 3H), 1.20−1.58 (m, 7H), 1.70−1.81 (m, 3H), 1.84−1.92 (m, 2H), 2.05 (s, 3H), 2.12−2.32 (m, 3H), 2.79−2.95 (m, 2H), 4.68 (dd, *J* = 9.0, 8.2 Hz, 1H), 6.66 (s, 1H), 7.61 (2, 1H), 12.17 (s, 1H, O*H*); MS (ES−) *m/z* 383.3 (100, $[M - H]^-$). Anal. ($C_{24}H_{32}O_4$) C, H.

17*β*-Acetoxy-2-ethylestra-1,3,5(10)-trien-3-ol (18). Pd/C (250 mg, 10% Pd) was added to a solution of **15** (1.78 g, 5.0 mmol) in MeOH (20 mL) and THF (20 mL). The mixture was stirred under a H_2 atmosphere for 24 h before the catalyst was filtered off, and the solvents were removed under reduced pressure. The residue was crystallized from $CHCl_3$/hexane to give **18** (1.473 g, 86%) as fine colorless needles: mp 175−177 °C; ^{1}H NMR ($CDCl_3$) δ 0.82, 1.20 (t, *J* = 7.4 Hz, 3H), 1.22−1.60 (m, 7H), 1.68−1.78 (m, 1H), 1.82−1.90 (m, 2H), 2.07 (s, 3), 2.13−2.34 (m, 3H), 2.59 (q, *J* = 7.4 Hz, 2H), 2.70−2.86 (m, 2H), 4.68 (dd, *J* = 9.0, 7.8 Hz, 1H),

5.01 (s, 1H), 6.50 (s, 1H), 7.04 (s, 1H); MS (APCI−) *m/z* 341.5 (100, [M − H]⁻). Anal. ($C_{22}H_{30}O_3$) C, H.

17β-Acetoxy-2-propylestra-1,3,5(10)-trien-3-ol (19). Pd/C (250 mg, 10%Pd) was added to a solution of **16** (4.11 g, 11.1 mmol) in MeOH (60 mL) and THF (20 mL). The mixture was stirred under a H_2 atmosphere for 24 h before the catalyst was filtered off, and the solvents were removed under reduced pressure. The residue was crystallized from $CHCl_3$/hexane to give **19** (3.54 g, 89%) as fine colorless needles: mp 167−169 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 1.20−1.60 (m, 10H) 1.80−1.90 (m, 2H), 2.06 (s, 3H), 2.13−2.33 (m, 3H), 2.48−2.60 (m, 2H), 2.70−2.86 (m, 2H), 4.68 (dd, *J* = 9.0, 8.2 Hz, 1H), 4.78 (s, 1H), 6.49 (s, 1H), 7.01 (s, 1H); MS (APCI+) *m/z* 357.6 (15, $[M + H]^+$), 297.5 (100, $[M − OAc]^+$; HRMS (ES+) calcd for $C_{23}H_{33}O_3$ $[M + H]^+$ 357.2435, found 357.2429.

17β-Acetoxy-2-butylestra-1,3,5(10)-trien-3-ol (20). Pd/C (300 mg, 10% Pd) was added to a solution of **17** (4.72 g, 12.2 mmol) in MeOH (30 mL) and THF (30 mL). The mixture was stirred under a H_2 atmosphere for 24 h before the catalyst was filtered off, and the solvents were removed under reduced pressure. The residue was crystallized from MeOH to give **20** (3.66 g, 81%) as fine colorless needles: mp 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H), 0.93 (t, *J* = 7.3 Hz, 3H), 1.20−1.60 (m, 11H), 1.69−1.78 (m, 1H), 1.81−1.90 (m, 2H), 2.06 (s, 3H), 2.14−2.31 (m, 3H), 2.38−2.60 (m, 2H), 2.70−2.83 (m, 2H), 4.68 (dd, *J* = 9.0, 7.8 Hz, 1H), 4.75 (s, 1H), 6.49 (s, 1H), 7.00 (s, 1H); MS (ES−) *m/z* 369.6 (100, $[M − H]^-$); HRMS (ES−) calcd for $C_{24}H_{33}O_3$ $[M − H]^-$ 369.2435, found 369.2428.

2-Isopropylestradiol (21). Pd/C (250 mg, 10% Pd) was added to a solution of **22** (2.63 g, 7.96 mmol) in MeOH. The mixture was stirred under a H_2 atmosphere for 24 h at room temperature before the catalyst was filtered off and the solvent was removed under reduced pressure. The solid residue was recrystallized from $CHCl_3$/hexane to give **21** as fine colorless needles (2.23 g, 89%): mp 149−152 °C (lit.[46] mp 103−105 °C); ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.25 (d, *J* = 6.7 Hz, 3H), 1.26−1.58 (m, 7H), 1.60 (s, 1H), 1.65−1.74 (m, 1H), 1.82−1.88 (m, 1H), 1.93−1.99 (m, 1H), 2.07−2.23 (m, 2H), 2.31−2.40 (m, 1H), 2.70−2.86 (m, 2H), 3.18 (hpt, *J* = 6.7 Hz, 1H), 3.72−3.80 (m, 1H), 5.04 (s, 1H), 6.48 (s, 1H), 7.12 (s, 1H); MS (APCI−) *m/z* 313.5 (100, $[M − H]^-$); HRMS (ES−) calcd for $C_{21}H_{29}O_2$ $[M − H]^-$ 313.2173, found 313.2182.

2-(1-Hydroxy-1-methylethyl)estradiol (22). A solution of **15** (3.56 g, 10.0 mmol) in THF (20 mL) was added slowly to a stirred solution of 3 M methylmagnesium chloride in THF (20 mL, 60 mmol). The mixture was stirred for 1 h at room temperature. Water (5 mL) was added carefully and the mixture was poured into saturated NH_4Cl solution (70 mL). The product was extracted with EtOAc (2 × 50 mL), and the combined organic layers were washed with water (50 mL) and brine (50 mL) and dried (Na_2SO_4). The solvents were removed under reduced pressure, and the solid white residue was recrystallized from EtOAc/hexane to give **22** as fine colorless needles (2.98 g, 90%): mp 155−158 °C (lit.[46] mp 160−162 °C); ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 1.12−1.51 (m, 7H), 1.66 (s, 3H), 1.68 (s, 3H), 1.69−1.73 (m, 1H), 1.80−1.89 (m, 1H), 1.90−1.98 (m, 1H), 2.05 2.19 (m, 2H), 2.23−2.32 (m, 1H), 2.50 (s, 1H), 2.75−2.85 (m, 2H), 3.66−3.75 (m, 1H), 6.60 (s, 1H), 6.98 (s, 1H), 8.70 (s, 1H); MS (APCI−) *m/z* 329.4 (100, $[M − H]^-$); HRMS (ES−) calcd for $C_{21}H_{29}O_3$ $[M − H]^-$ 329.2122, found 329.2129.

2-*n*-Propylestradiol (23). To a suspension of **19** (2.85 g, 8.0 mmol) in MeOH (40 mL) was added 5 M NaOH (5 mL). The mixture was stirred for 2 h at room temperature before being poured into 2 M $KHSO_4$ (50 mL). The product was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and the solvents were removed under reduced pressure. The residue was purified by flash chromatography ($CHCl_3$/acetone 10:1) to give **23** (2.29 g, 91%) as a white solid: mp 131−133 °C (lit.[30] mp 135−136 °C); ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 1.14−1.73 (m, 11H), 1.80−1.88 (m, 1H), 1.92−1.97 (m, 1H), 2.05−2.20 (m, 2H), 2.24−2.36 (m, 1H), 2.49−2.56 (m, 2H), 2.70−2.84 (m, 2H), 3.70−3.78 (m, 1H), 4.96 (s, 1H), 6.45 (s, 1H), 7.03 (s, 1H); MS (APCI−) *m/z* 313.6 (100, $[M − H]^-$); HRMS (ES+) calcd for $C_{21}H_{31}O_2$ $[M + H]^+$ 315.2330, found 315.2318.

2-*n*-Butylestradiol (24). To a suspension of **20** (2.96 g, 8.0 mmol) in MeOH (40 mL) was added 5 M NaOH (5 mL). The mixture was stirred for 2 h at room temperature before being poured into 2 M $KHSO_4$ (50 mL). The product was extracted with EtOAc (3 × 50 mL), the combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica ($CHCl_3$/acetone 10:1) to give **24** (2.33 g, 89%) as a white solid: mp 138−141 °C (lit.[30] mp 142−143 °C); ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.93 (t, *J* = 7.3 Hz, 3H), 1.14−1.73 (m, 13H), 1.80−1.84 (m, 1H), 1.91−1.97 (m, 1H), 2.04−2.21 (m, 2H), 2.29−2.36 (m, 1H), 2.48−2.60 (m, 2H), 2.70−2.85 (m, 2H), 3.68−3.78 (m, 1H), 4.70 (s, 1H), 6.49 (s, 1H), 7.02 (s, 1H); MS (ES−) *m/z* 327.5 (100, $[M − H]^-$). Anal. ($C_{22}H_{32}O_4$) C, H.

2-Methyl-3,17β-disulfamoyloxyestra-1,3,5(10)-triene (27). According to general procedure 1, compound **8** (143 mg, 0.50 mmol) was reacted with sulfamoyl chloride (6 equiv) in DMA (5 mL) to give **27** (196 mg, 88%) as a white solid. Recrystallization from acetone/hexane gave fine colorless needles: mp 155−158 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 1.16−1.44 (m, 6H), 1.63−1.75 (m, 2H), 1.79−1.86 (m, 1H), 1.90−2.00 (m, 1H), 2.10−2.21 (m, 2H), 4.34 (dd, *J* = 8.6, 8.2 Hz, 1H), 6.98 (s, 1H), 7.20 (s, 1H), 7.39 (s, 2H, OSO_2NH_2), 7.92 (s, 2H); MS (APCI−) *m/z* 443.5 (100, $[M − H]^-$), 212.2 (88%). Anal. ($C_{19}H_{28}N_2O_6S_2$) C, H, N.

2-*n*-Propyl-3,17β-disulfamoyloxyestra-1,3,5(10)-triene (28). According to general procedure 1, compound **23** (157 mg, 0.50 mmol) was reacted with sulfamoyl chloride (6 equiv) in DMA (5 mL) to give **28** (189 mg, 80%) as a white solid: mp 182−185 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 0.89 (t, *J* = 7.4 Hz, 3H), 1.20−1.45 (m, 6H), 1.48−1.60 (m, 2H), 1.63 (m, 2H), 1.80−1.86 (m, 1H), 1.88−2.00 (m, 1H, 2.10−2.26 (m, 2H), 2.29−2.40 (m, 1H), 2.53−2.62 (m, 2H), 2.74−2.82 (m, 2H), 4.34 (dd, *J* = 8.6, 8.1 Hz,), 7.00 (s, 1H), 7.19 (s, 1H), 7.41 (s, 2H), 7.95 (s, 2H); MS (APCI−) *m/z* 471.5 (100, $[M − H]^-$). Anal. ($C_{21}H_{32}N_2O_6S_2$) C, H, N.

2-Isopropyl-3,17β-disulfamoyloxyestra-1,3,5(10)-triene (29). According to general procedure 1, compound **21** (143 mg, 0.50 mmol) was reacted with sulfamoyl chloride (6 equiv) in DMA (5 mL) to give **29** (201 mg, 85%) as a white solid: mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 1.14 (d, *J* = 7.0 Hz, 3H), 1.16 (d, *J* = 7.0 Hz, 3H), 1.20−1.45 (m, 6H), 1.62−1.76 (m, 2H), 1.79−1.86 (m, 1H), 1.92−2.00 (m, 1H), 2.10−2.26 (m, 2H), 2.34−2.44 (m, 1H), 2.74−2.82 (m, 2H), 3.29 (hpt, *J* = 7.0 Hz, 1H), 4.34 (dd, *J* = 8.6, 8.2 Hz, 1H), 7.00 (s, 1H), 7.25 (s, 1H), 7.40 (s, 2H), 7.96 (s, 2H); MS (APCI−) *m/z* 471.6 (100, $[M − H]^-$). Anal. ($C_{21}H_{32}N_2O_6S_2$) C, H, N.

2-*n*-Butyl-3,17β-disulfamoyloxyestra-1,3,5(10)-triene (30). According to general procedure 1, compound **24** (143 mg, 0.50 mmol) was reacted with sulfamoyl chloride (6 equiv) in DMA (5 mL) to give **30** (189 mg, 78%) as a white solid: mp 148−150 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 0.89 (t, *J* = 7.4 Hz, 3H), 1.18−1.55 (m, 10H), 1.62−1.75 (m, 2H), 1.77−1.85 (m, 1H), 1.90−2.00 (m, 1H), 2.11−2.25 (m, 2H), 2.30−2.42 (m, 1H), 2.53−2.65 (m, 2H), 2.70−2.82 (m, 2H), 4.34 (dd, *J* = 8.6, 8.2 Hz,), 7.00 (s, 1H), 7.19 (s, 1H), 7.41 (s, 2H), 7.95 (s, 2H); MS (ES−) *m/z* 485.5 (100, $[M − H]^-$). Anal. ($C_{22}H_{34}N_2O_6S_2$) C, H, N.

2-*tert*-Butyl-3,17β-disulfamoyloxyestra-1,3,5(10)-triene (31). According to general procedure 1, compound **10** (329 mg, 1.00 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (3 mL) to give **31** (260 mg, 53%) as a white solid, which was then recrystallized from ethyl acetate/hexane: mp 218−219 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 1.32 (s, 9H), 1.15−2.40 (m, 13H), 2.72−2.84 (m, 2H), 4.33 (dd, *J* = 8.4, 8.2 Hz, 1H), 7.22 (s, 1H), 7.24 (s, 1H), 7.42 (s, 2H) and 8.15 (s, 2H); MS (APCI−) *m/z* 471.6 (100, $[M − H]^-$). Anal. ($C_{22}H_{34}N_2O_6S_2$) C, H, N.

17β-Carbamoyloxy-2-ethylestra-1,3,5(10)-trien-3-ol (32). According to general procedure 1, compound **18** (171 mg, 0.50 mmol)

was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **32** (196 mg, 93%) as a white solid, which was recrystallized from $CHCl_3$/hexane to give fine needles: mp 212−213 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 1.20 (t, J = 7.4 Hz, 3H), 1.22−1.60 (m, 7H), 1.68−1.79 (m, 1H), 1.84−1.92 (m, 2H), 2.05 (s, 3H), 2.15−2.33 (m, 3H), 2.68 (q, J = 7.3 Hz, 2H), 2.79−2.86 (m, 2H), 4.68 (dd, J = 8.7, 7.8 Hz, 1H), 4.98 (s, 2H), 7.07 (s, 1H), 7.17 (s, 1H); MS (APCI−) *m/z* 420.5 (100, [M − H]$^-$). Anal. ($C_{22}H_{31}NO_5$) C, H, N.

17*β*-Carbamoyloxy-2-*n*-propylestra-1,3,5(10)-trien-3-ol (33). According to general procedure 1, compound **19** (178 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **33** (194 mg, 89%) as a white solid, which was recrystallized from $CHCl_3$/hexane: mp 202−206 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 0.95 (t, J = 7.4 Hz, 3H), 1.20−1.66 (m, 9H), 1.69−1.78 (m, 1H), 1.84−1.91 (m, 2H), 2.05 (s, 3H), 2.14−2.34 (m, 3H), 2.56−2.64 (m, 2H), 2.78−2.86 (m, 2H), 4.68 (dd, J = 9.1, 7.8 Hz, 1H), 4.96 (br s, 2H), 7.07 (s, 1H), 7.15 (s, 1H); MS (ES−) *m/z* 434.5 (100, [M − H]$^-$). Anal. ($C_{23}H_{33}NO_5S$) C, H, N.

17*β*-Acetoxy-2-*n*-butyl-3-sulfamoyloxyestra-1,3,5(10)-triene (34). According to general procedure 1, compound **20** (185 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **34** (201 mg, 89%) as a white solid: mp 175−177 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 0.92 (t, J = 7.4 Hz, 3H), 1.20−1.61 (m, 11H), 1.68−1.77 (m, 1H), 1.83−1.91 (m, 2H), 2.05 (s, 3H), 2.15−2.32 (m, 3H), 2.61−2.68 (m, 2H), 2.79−2.85 (m, 2H), 4.68 (dd, J = 9.0, 7.8 Hz, 1H), 5.09 (br s, 2H), 7.06 (s, 1H), 7.14 (s, 1H); MS (ES−) *m/z* 448.6 (100, [M − H]$^-$). Anal. ($C_{24}H_{35}NO_5S$) C, H, N.

3-Benzyloxy-17*β*-[*N*-(2,2,2-trichloroacetyl)]carbamoyloxyestra-1,3,5(10)-triene (37). Trichloroacetyl isocyanate (0.50 mL, 4.20 mmol) was added to a solution of **35** (906 mg, 2.50 mmol) in THF (20 mL). The solution was stirred for 15 min at room temperature and then treated with water (0.5 mL) to destroy the excess of trichloroacetyl isocyanate. EtOAc (50 mL) and more water (30 mL) were added, the organic layer was separated and dried (Na_2SO_4), and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (EtOAc/hexane 1:3) followed by precipitation from a solution in DCM by addition of hexane to give **37** (1.32 g, 96%) as a white solid: mp 177−179 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H), 1.29−2.00 (m, 10H), 2.20−2.38 (m, 3H), 2.79−2.90 (m, 2H), 4.81 (dd, J = 9.0, 7.8 Hz, 1H), 5.31 (s, 2H), 6.73 (d, J = 2.3 Hz, 1H), 6.79 (dd, J = 8.6, 2.3 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H), 7.30−7.45 (m, 5H), 8.32 (s, 1H, N*H*); MS (FAB+) *m/z* 549.1 (15, [M]$^+$), 345.0 (25), 90.9 (100); HRMS (FAB+) calcd for $C_{28}H_{30}Cl_3NO_4$ [M]$^+$ 549.1240, found 549.1207.

3-Benzyloxy-17*β*-[*N*-(2,2,2-trichloroacetyl)]carbamoyloxy-2-methoxyestra-1,3,5(10)-triene (38). Trichloroacetyl isocyanate (0.20 mL, 1.68 mmol) was reacted with **36** (393 mg, 1.00 mmol) in THF (20 mL), as described for the synthesis of **37**. The crude product was purified by flash chromatography (EtOAc/hexane 1:5) to give **38** (534 mg, 92%) as a white solid: mp 193−195 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H), 1.26−1.28 (m, 6H), 1.65−1.91 (m, 3H), 1.95−2.02 (m, 1H), 2.20−2.40 (m, 3H), 2.68−2.84 (m, 2H), 3.88 (s, 3H, OC*H*$_3$), 4.82 (dd, J = 9.0, 7,8 Hz, 1H), 5.11 (s, 2H), 6.63 (s, 1H), 6.84 (s, 1H), 7.27−7.47 (m, 5H), 8.31 (s, 1H); MS (FAB+) *m/z* 579.1 (100, [M]$^+$), 375.2 (66), 73 (58); HRMS (FAB+) calcd for $C_{29}H_{32}Cl_3NO_5$ [M]$^+$ 579.1346, found 579.1323. Anal. ($C_{29}H_{32}Cl_3NO_5$) C, H, N.

3-Benzyloxy-17*β*-carbamoyloxyestra-1,3,5(10)-triene (39). A solution of K_2CO_3 (414 mg, 3.0 mmol) in water (10 mL) was added to a solution of **37** (1.10 g, 2.0 mmol) in THF (20 mL) and MeOH (20 mL). The mixture was stirred for 3 h at room temperature, EtOAc (60 mL) and water (60 mL) were added, the organic layer was separated, dried (Na_2SO_4), and the solvents were removed under reduced pressure. The residue was crystallized from DCM/hexane to give **39** (745 mg, 92%) as colorless needles: mp 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H), 1.26−1.86 (m, 7H), 1.73−1.82 (m, 1H), 1.88−1.98 (m, 2H), 2.22−2.38 (m, 3H), 2.82−2.96 (m, 2H), 4.67 (dd, J = 9.1, 7.7 Hz, 1H), 4.73 (bs, 2H), 5.07 (s, 2H), 6.76 (d, J = 2.7 Hz, 1H), 6.82 (dd, J = 8.6, 2.7 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 7.34−7.48 (m, 5H); MS (FAB+) *m/z* 405.2.0 (35, [M]$^+$), 91.0 (100); HRMS (FAB+) cald for $C_{26}H_{31}NO_3$ [M]$^+$ 405.2304, found 405.2296.

3-Benzyloxy-17*β*-carbamoyloxy-2-methoxyestra-1,3,5(10)-triene (40). A solution of K_2CO_3 (414 mg, 3.0 mmol) in water (10 mL) was added to a solution of **38** (407 mg, 0.70 mmol) in THF (10 mL) and MeOH (10 mL) as described for the synthesis of **39**. The crude product was crystallized from DCM/hexane to give **40** (262 mg, 86%) as colorless plates: mp 180−181 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H, H-18), 1.25−1.64 (m, 7H), 1.68−1.78 (m, 1H), 1.82−1.96 (m, 2H) 2.16−2.32 (m, 3H), 2.64−2.81 (m, 2H), 3.87 (s, 3H), 4.58 (br s, 2H) 4.63 (dd, J = 9.4, 8.2 Hz, 1H), 5.11 (s, 2H), 6.62 (s, 1H), 6.84 (s, 1H), 7.27−7.46 (m, 5H); MS (FAB+) *m/z* 435.0 (90, [M]$^+$), 90.9 (100); HRMS (FAB+) calcd for $C_{27}H_{33}NO_4$ [M]$^+$ 435.2410, found 435.2404.

17*β*-Carbamoyloxyestra-1,3,5(10)-trien-3-ol (41). Pd/C (50 mg, 10% Pd) was added to a solution of **39** (405 mg, 1.0 mmol) in MeOH (10 mL) and THF (10 mL). The mixture was stirred under a H_2 atmosphere for 18 h at room temperature, the catalyst was filtered off, and the solvents were removed under reduced pressure. The residue was crystallized from EtOAc/hexane to give **41** (271 mg, 86%) as fine needles: mp 280−284 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.76 (s, 3H), 1.18−21.52 (m, 7H), 1.60−1.82 (m, 3H), 2.00−2.15 (m, 2H), 2.18−2.30 (m, 1H), 2.64−2.78 (m, 2H), 4.56 (dd, J = 9.0, 8.2 Hz, 1H), 6.37 (br s, 2H), 6.42 (d, J = 2.7 Hz, 1H), 6.49 (dd, J = 8.6, 2.7 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 8.99 (s, 1H); MS (FAB+) *m/z* 315.0 (100, [M]$^+$), 255.0 (50); HRMS (FAB+) calcd for $C_{19}H_{25}NO_3$ [M]$^+$ 315.1834, found 315.1830. Anal. ($C_{19}H_{25}NO_3$) C, H, N.

17*β*-Carbamoyloxy-2-methoxyestra-1,3,5(10)-trien-3-ol (42). Pd/C (50 mg, 10%) was added to a solution of **40** (218 mg, 0.50 mmol) in MeOH (10 mL) and THF (10 mL). The mixture was stirred under a H_2 atmosphere for 18 h at room temperature, the catalyst was filtered off, and the solvents were removed under reduced pressure. The residue was precipitated from EtOAc with hexane to give **42** (173 mg, 100%) as a white powder: mp 235−238 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 1.28−1.50 (m, 7H), 1.59−1.70 (m,1H), 1.72−1.81 (m, 2H), 1.99−2.16 (m, 2H), 2.22−2.31 (m, 1H), 2.55−2.68 (m, 2H), 3.70 (s, 3H), 4.45 (dd, J = 9.0, 7.8 Hz, 1H), 6.40 (bs, 2H), 6.43 (s, 1H), 6.75 (s, 1H), 8.60 (s, 1H); MS (FAB+) *m/z* 345.2 (100, [M]$^+$); HRMS (FAB+) calcd for $C_{20}H_{27}NO_4$ [M]$^+$ 345.1940, found 345.1943. Anal. ($C_{20}H_{27}NO_4$) C, H, N.

17*β*-Carbamoyloxy-2-methylestra-1,3,5(10)-trien-3-ol (43). According to general procedure 2, compound **8** (573 mg, 2.0 mmol) was reacted with trichloroacetyl isocyanate (1.0 mL, 8.4 mmol) in THF (10 mL). After partial hydrolysis, workup, and purification, **43** (455 mg, 69%) was obtained as a white solid: mp >230 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.75 (s, 3H), 1.15−1.38 (m, 6H), 1.40−1.51 (m, 1H), 1.58−1.68 (m, 1H), 1.72−1.80 (m, 2H), 1.98−2.12 (m, 5H), 2.18−2.26 (m, 1H), 2.58−2.73 (m, 2H), 4.45 (dd, J = 8.9, 8.1 Hz, 1H), 6.40 (br s, 2H), 6.42 (s, 1H), 6.92 (s, 1H), 8.87 (s, 1H); MS (APCI+) *m/z* 330.5 (17, [M + H]$^+$), 269.4 (100). Anal. ($C_{20}H_{27}NO_3$) C, H, N.

17*β*-Carbamoyloxy-2-ethylestra-1,3,5(10)-trien-3-ol (44). According to general procedure 2, compound **2** (601 mg, 2.0 mmol) was reacted with trichloroacetyl isocyanate (1.0 mL, 8.4 mmol) in THF (10 mL). After partial hydrolysis, workup, and purification, **44** (577 mg, 84%) was obtained as a white solid: mp 243−245 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3H), 1.20 (t, J = 7.4 Hz, 3H), 1.21−1.74 (m, 9H), 1.80−1.95 (m, 2H), 2.10−2.36 (m, 2H), 2.68 (q, J = 7.4 Hz, 2H), 2.60−2.72 (m, 2H), 4.49−4.62 (m, 3H), 4.90 (s, 2H), 7.07 (s, 1H), 7.17 (s, 1H); MS (FAB+) *m/z* 343.1 (100, [M]$^+$); HRMS (FAB+) *m/z* calcd for $C_{21}H_{29}NO_3$ [M]$^+$ 343.2147, found 343.2147. Anal. ($C_{21}H_{29}NO_3$) C, H, N.

17*β*-Carbamoyloxy-2-*n*-propylestra-1,3,5(10)-trien-3-ol (45). According to general procedure 2, compound **23** (629 mg, 2.0 mmol) was reacted with trichloroacetyl isocyanate (1.0 mL, 8.4 mmol) in THF (10 mL). After partial hydrolysis, workup, and purification, **45** (579 mg, 81%) was obtained as a white solid: mp 225−229 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3H), 0.97 (t, J = 7.4

Hz, 3H), 1.20−1.48 (m, 6H), 1.54−1.64 (m, 3H), 1.68−1.74 (m, 1H), 1.82−1.86 (m, 1H), 1.88−1.92 (m, 1H), 2.14−2.23 (m, 2H), 2.26−2.31 (m, 1H), 2.49−2.56 (m, 2H), 2.75−2.83 (m, 2H), 4.59−4.63 (m, 3H), 4.67 (s, 1H), 6.49 (s, 1H), 7.01 (s, 1H); MS (ES−) *m/z* 356.6 (100, [M − H]$^-$); HRMS (EI) calcd for $C_{22}H_{31}NO_3$ [M]$^+$ 357.2298, found 357.2293. Anal. ($C_{22}H_{31}NO_3$) C, H, N.

17β-Carbamoyloxy-2-isopropylestra-1,3,5(10)-trien-3-ol (46). According to general procedure 2, compound **21** (629 mg, 2.0 mmol) was reacted with trichloroacetyl isocyanate (1.0 mL, 8.4 mmol) in THF (10 mL). After partial hydrolysis, workup, and purification, **46** (509 mg, 71%) was obtained as a white solid: mp 221−222 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.25 (d, *J* = 6.7 Hz, 3H), 1.28−1.63 (m, 7H), 1.67−1.76 (m, 1H), 1.80−1.92 (m, 2H), 2.14−2.26 (m, 2H), 2.28−2.36 (m, 1H), 2.70−2.84 (m, 2H), 3.18 (hpt, *J* = 6.7 Hz, 1H), 4.63 (dd, *J* = 8.7, 8.1 Hz, 1H), 4.74 (s, 2H), 5.27 (s, 1H), 6.50 (s, 1H), 7.10 (s, 1H); MS (APCI−) *m/z* 356.5 (12, [M − H]$^-$), 313.4 (50), 295.4 (100), 187.1 (95). Anal. ($C_{22}H_{31}NO_3$) C, H, N.

2-*n*-Butyl-17β-carbamoyloxyestra-1,3,5(10)-trien-3-ol (47). According to general procedure 2, compound **24** (657 mg, 2.0 mmol) was reacted with trichloroacetyl isocyanate (1.0 mL, 8.4 mmol) in THF (10 mL). After partial hydrolysis, workup, and purification, **47** (587 mg, 79%) was obtained as a white solid: mp 171−174 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3H), 0.93 (t, *J* = 7.4 Hz, 3H), 1.20−1.62 (m, 11H), 1.66−176 (m, H), 1.80−1.92 (m, 2H), 2.11−2.32 (m, 3H), 2.50−2.61 (m, 2H), 2.70−2.83 (m, 2H), 4.62 (dd, *J* = 9.0 Hz, 7.8 Hz, 1H), 4.66 (br s, 2H), 4.92 (s, 1H), 6.50 (s, 1H), 7.01 (s, 1H); MS (ES−) *m/z* 370.7 (100, [M − H]$^-$); HRMS (EI) calcd for $C_{23}H_{33}NO_3$ [M]$^+$ 371.2455, found 371.2452. Anal. ($C_{23}H_{33}NO_3$) C, H, N.

2-*tert*-Butyl-17β-carbamoyloxyestra-1,3,5(10)-trien-3-ol (48). According to general procedure 2, compound **10** (657 mg, 2.0 mmol) was reacted with trichloroacetyl isocyanate (1.0 mL, 8.4 mmol) in THF (10 mL). After partial hydrolysis, workup, and purification, **48** (539 mg, 80%) was obtained as a white solid: mp >250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.76 (s, 3H), 1.14−1.51 (m, 16H), 1.58−1.70 (m, 1H), 1.72−1.82 (m, 2H), 1.99−2.15 (m, 1H),2.18−2.25 (m, 1H), 2.56−2.74 (m, 2H), 4.45 (dd, *J* = 8.6 Hz, 8.2 Hz, 1H), 6.40 (br s, 2H), 6.43 (s, 1H), 7.00 (s, 1H), 8.93 (s, 1H); MS (APCI−) *m/z* 370.0 (100, [M − H]$^-$). Anal. ($C_{23}H_{33}NO_3$) C, H, N.

17β-Carbamoyloxy-3-sulfamoyloxyestra-1,3,5(10)-triene (49). According to general procedure 1, compound **41** (157 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **49** (193 mg, 98%) as a white solid: mp 206−210 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 1.20−1.55 (m, 7H), 1.62−1.71 (m, 1H), 1.75−1.88 (m, 2H), 2.00−2.12 (m, 1H), 2.16−2.36 (m, 2H), 2.78−2.86 (m, 2H), 4.47 (dd, *J* = 9.0, 7.8 Hz, 1H), 6.40 (bs, 2H), 6.96 (d, *J* = 2.3 Hz, 1H), 7.00 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.90 (s, 1H); MS (FAB-) *m/z* 393.2 (100, [M − H]$^-$); HRMS (FAB+) calcd for $C_{19}H_{26}N_2O_5S$ [M]$^+$ 394.1562, found 394.1555.

17β-Carbamoyloxy-2-methoxy-3-sulfamoyloxyestra-1,3,5(10)-triene (50). According to general procedure 1, compound **42** (120 mg, 0.35 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **50** (130 mg, 88%) as a white solid, which was recrystallized from acetone/cyclohexane to give colorless monoclinic crystals: mp 201−204 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 1.30−1.52 (m, 7H), 1.61−1.70 (m, 1H), 1.76−1.84 (m, 2H), 2.00−2.12 (m, 1H), 2.16−2.24 (m, 1H), 2.33−2.40 (m, 1H), 2.70−2.76 (m, 2H), 3.76 (s, 3H), 4.47 (dd, *J* = 9.0, 7.8 Hz, 1H), 6.40 (bs, 2H), 6.98 (s, 2H), 7.83 (s, 2H); MS (FAB+) *m/z* 424.1 (100, [M]$^+$); HRMS (FAB+) calcd for $C_{20}H_{28}N_2O_6S$ [M]$^+$ 424.1668, found 424.1666. Anal. ($C_{20}H_{28}N_2O_6S$) C, H, N.

17β-Carbamoyloxy-2-methyl-3-sulfamoyloxyestra-1,3,5(10)-triene (51). According to general procedure 1, compound **43** (165 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **53** (114 mg, 56%) as a white solid: mp 209−211 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.20−1.53 (m, 8H), 1.60-.170 (m, 1H), 1.72−1.85 (m, 2H), 2.00−2.11 (m, 1H), 2.12−2.20 (m, 1H), 2.22 (s, 3H), 2.26−2.35 (m, 1H), 2.72−2.81 (m, 2H), 4.47 (dd, *J* = 8.6, 8.2 Hz, 1H), 6.40 (br s, 2H), 6.98 (s, 1H), 7.19 (s, 1H), 7.93 (s, 2H); MS (ES−) *m/z* 407.7 (100, [M − H]$^-$); HRMS (ES−) *m/z* calcd for $C_{20}H_{27}N_2O_5S$ [M − H]$^-$ 407.1646, found 407.1643. Anal. ($C_{20}H_{28}N_2O_5S$) C, H, N.

17β-Carbamoyloxy-2-ethyl-3-sulfamoyloxyestra-1,3,5(10)-triene (52). According to general procedure 1, compound **44** (344 mg, 1.0 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **52** (254 mg, 60%) as a white solid: mp 220−223 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 1.12 (t, *J* = 7.4 Hz, 3H), 1.20−1.54 (m, 7H), 1.60−1.72 (m, 1H), 1.74−1.85 (m, 2H), 2.00−2.12 (m, 1H), 2.16−2.23 (m, 1H), 2.26−2.38 (m, 1H), 2.61 (q, *J* = 7.4 Hz, 2H), 2.74−2.82 m, 2H), 4.47 (dd, *J* = 9.0, 8.2 Hz, 1H), 6.43 (br s, 2H), 7.00 (s, 1H), 7.20 (s, 1H), 8.32 (s, 2H); MS (APCI−) *m/z* 421.5 (100, [M − H]$^-$); HRMS (FAB+) *m/z* calcd for $C_{21}H_{30}N_2O_5S$ [M]$^+$ 422.1875, found 422.1872. Anal. ($C_{21}H_{30}N_2O_5S$) C, H, N.

17β-Carbamoyloxy-2-*n*-propyl-3-sulfamoyloxyestra-1,3,5(10)-triene (53). According to general procedure 1, compound **45** (179 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **53** (129 mg, 59%) as a white solid: mp 171−173 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H), 1.20−1.78 (m, 10H), 1.82−1.94 (m, 2H), 2.13−2.32 (m, 3H), 2.58−2.64 (m, 2H), 2.79−2.85 (m, 2H), 4.56−4.68 (m, 3H), 5.12 (s, 2H), 7.08 (s, 1H), 7.15 (s, 1H); HRMS (ES−) *m/z* calcd for $C_{22}H_{31}N_2O_5S$ [M − H]$^-$ 435.1959, found 435.1959. Anal. ($C_{22}H_{32}N_2O_5S$) C, H, N.

17β-Carbamoyloxy-2-isopropyl-3-sulfamoyloxyestra-1,3,5-(10)-triene (54). According to general procedure 1, compound **46** (179 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **54** (142 mg, 65%) as a white solid: mp 202−205 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.16 (d, *J* = 6.5 Hz, 3H) 1.20−1.51 (m, 7H), 1.61-1.72 (m, 1H), 1.74−1.86 (m, 2H), 2.01−2.13 (m, 1H), 2.15−2.25 (m, 1H), 2.31−2.42 (m, 1H),2.70−2.84 (m, 2H), 3.29 (hpt, *J* = 6.5 Hz, 1H), 4.47 (dd, *J* = 8.6, 8.2 Hz, 1H), 6.40 (br s, 2H), 6.99 (s, 1H), 7.24 (s, 1H), 7.96 (s, 2H); MS (ES−) *m/z* 435.5 (100, [M − H]$^-$); HRMS (ES−) *m/z* calcd for $C_{22}H_{31}N_2O_5S$ [M − H]$^-$ 435.1959, found 435.1959. Anal. ($C_{22}H_{32}N_2O_5S$) C, H, N.

2-*n*-Butyl-17β-carbamoyloxy-3-sulfamoyloxyestra-1,3,5(10)-triene (55). According to general procedure 1, compound **47** (186 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **55** (137 mg, 61%) as a white solid: mp 162−165 °C; ^{1}H NMR ($CDCl_3$) δ 0.79 (s, 3H), 0.92 (t, *J* = 7.4 Hz, 3H), 1.20−1.62 (m, 11H), 1.66−1.75 (m, 1H), 1.82−1.94 (m, 2H), 2.12−2.32 (m, 3H), 2.60−2.67 (m, 2H), 2.78−2.84 (m, 2H), 4.60 (dd, *J* = 8.6, 8.2 Hz, 1H), 4.73 (br s, 2H), 5.35 (s, 2H), 7.07 (s, 1H), 7.14 (s, 1H); HRMS (ES+) *m/z* calcd for $C_{23}H_{38}N_3O_5S$ [M+NH_4]$^+$ 468.2527, found 468.2527. Anal. ($C_{23}H_{34}N_2O_5S$) C, H, N.

2-*tert*-Butyl-17β-carbamoyloxy-3-sulfamoyloxyestra-1,3,5(10)-triene (56). According to general procedure 1, compound **48** (186 mg, 0.50 mmol) was reacted with sulfamoyl chloride (3 equiv) in DMA (5 mL) to give **56** (178 mg, 79%) as a white solid: mp 217−220 °C; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 1.20−1.52 (m, 16H), 1.60−1.70 (m, 1H), 1.75−1.86 (m, 2H), 2.00−2.12 (m, 1H), 2.14−2.22 (m, 1H), 2.26−2.36 (m, 1H), 2.72−2.80 (m, 1H), 4.47 (dd, *J* = 8.6, 8.2 Hz, 1H), 6.40 (br s, 2H), 7.22 (s, 1H), 7.23 (s, 1H), 8.14 (s, 2H); MS (APCI−) *m/z* 449.6 (100, [M − H]$^-$). Anal. ($C_{23}H_{34}N_2O_5S$) C, H, N.

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Supporting Information Available: Microanalysis data for **2**, **7**−**9**, **12**−**18**, **24**, **27**−**34**, **38**, **41**−**48**, and **50**−**56**; ^{13}C NMR data (all compounds); IR data for **37**−**42**, **49**, and **50;** and crystallographic data for compound **50**. This material is available free of charge via the Internet at http://pubs.acs.org.

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