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Synthesis of Libraries of 16β-Aminopropyl Estradiol Derivatives for Targeting Two Key Steroidogenic Enzymes

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Two libraries, each consisting of 48 16β-aminopropyl estradiol derivatives, phenols and sulfamates, respectively, were synthesized by solid-phase parallel chemistry through a seven-step reaction sequence. Following the attachment of a C18-steroid sulfamate precursor on a trityl chloride resin, diversity elements were first introduced on the 16β-aminopropyl chain of the steroid by acylation reactions with eight Fmoc-amino acids. After deprotection, the free amine function of the resulting compounds was reacted with six carboxylic acids for the introduction of a second diversity level. The two variants employed for the cleavage of compounds from the solid support, acidic and nucleophilic, allowed the corresponding libraries of sulfamate and phenol derivatives in yields of 8–50% and 13–58% to be obtained with an average HPLC purity of 94% and 91%, respectively. Potent steroid sulfatase inhibitors and interesting SAR results were generated from the screening of the sulfamate library. Furthermore, moderate inhibitors of type 1 17β-HSD resulted from the partial screening of phenol library. Thus, these two categories of compounds were synthesized to rapidly identify potential inhibitors of steroid biosynthesis for the hormonal therapy of estrogen-dependent diseases, and also to demonstrate the versatility and efficiency of the recently developed sulfamate linker.

Introduction

Estrogenic steroids estradiol (E_2) and androst-5-ene-3β,17β-diol (Δ^5-diol) stimulate the growth of breast cancer tumors. Steroidogenic enzymes have become important biological targets since it was discovered that estrogens can be synthesized inside malignant cells from sulfated precursors such as estrone sulfate (E_1S) and dehydroepiandrosterone sulfate (DHEAS) available in the blood circulation.[1–3] Indeed, breast tumors were found to possess the enzymes required for these transformations, among which are steroid sulfatase and 17β-hydroxysteroid dehydrogenases (17β-HSDs), and others such as P450-aromatase and 3β-hydroxysteroid dehydrogenase (3β-HSD).[4–6] Concentrations of estrogens and steroid sulfates are higher in malignant breast tissues than in plasma, and intense steroid sulfatase and 17β-HSD activities have been detected in tumors.[7–9] By reducing levels of steroid hormones in tumors, steroid sulfatase inhibitors and 17β-HSD inhibitors may serve as therapeutic agents in the treatment of estrogen-sensitive diseases such as breast cancer. Thus, several research groups have focused their work on the development of such inhibitors.[10–15]

Solid phase synthesis and combinatorial chemistry are powerful methodologies for the development and optimization of compounds with therapeutic potential, through generation and screening of large and diversified libraries.[16–25] To take advantage of these tools in the development of new steroidogenic enzyme inhibitors and other therapeutic agents,[26] we first explored the attachment of sulfamates generated from phenols on a trityl chloride polymeric support, thinking we could use this sulfatase inhibitory group as an anchor for solid-phase chemistry.[27] Interestingly, acidic and nucleophilic cleavage of compounds from this resin gave rise to sulfamate or phenol derivatives, respectively,[28,29] two broad families of biologically relevant therapeutic compounds. Thus, phenolic derivatives might inhibit type 1 17β-HSD activity as it was shown previously that position 16 or 15 of E_2 is a good location to introduce pharmacophores for the inhibition of this enzyme.[30–34] However, phenolic derivatives could be also used as selective estrogen receptor modulators and used to target numerous other biological targets.[35–37] On the other hand, sulfamate derivatives are well known for their ability to inhibit the steroid sulfatase activity,[10–14] and to possess other biological properties such as carbonic anhydrase inhibition, antibacterial, antitumoral, cytotoxic, and anticonvulsive activities.[38–43] The multidetachable sulfamate linker then appears to be a judicious chemical tool for generating libraries of both sulfamate and phenol derivatives. We now report the application of this methodology in the synthesis of two libraries of 16β-substituted E_2 derivatives, sulfamates and phenols (Figure 1), which were tested as inhibitors of steroid sulfatase and type 1 17β-HSD, respectively.

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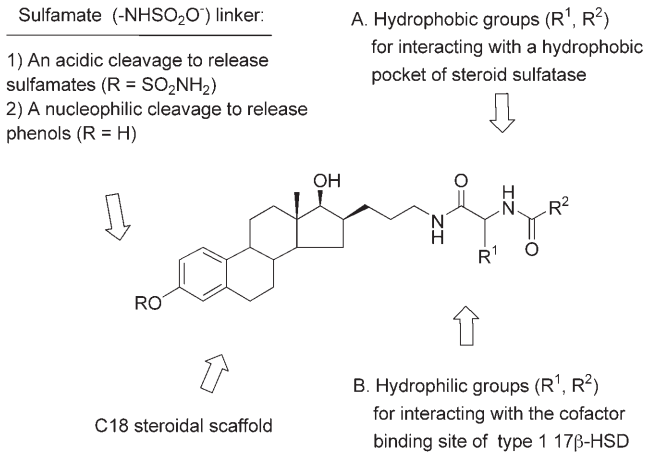
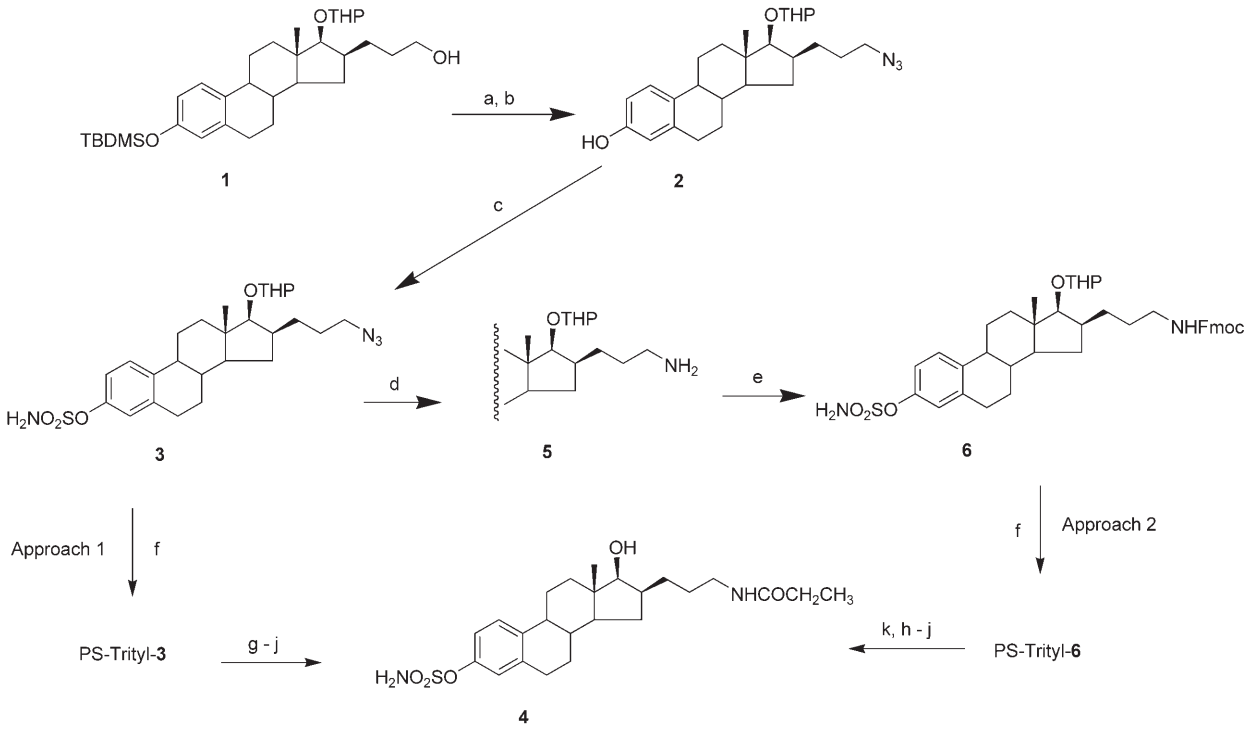


Figure 1. Model structure of potential inhibitors targeting the enzymes steroid sulfatase (A) and estrogenic17β-HSDs (B) for the treatment of estrogen-sensitive diseases.

Results and Discussion

Synthesis of the solid-phase precursors 3 and 6

Our precursors in the generation of sulfamate and phenol libraries of 16β-substituted E_2 derivatives were synthesized from alcohol **1**,[44] as reported in Scheme 1. This primary alcohol was directly converted to the corresponding azide derivative following a Mitsunobu reaction procedure.[45,46] Removal of the TBDMS protecting group was readily achieved upon treatment with TBAF to generate the phenolic compound **2**. Next, the sulfamate derivative **3** was obtained in over 95% yield from the phenol **2** by reaction with sulfamoyl chloride and DBMP as base.[40,47] Initially we intended to use compound **3** as our solid-phase precursor (approach I), because the reduction of an azide to the corresponding amine on solid phase is known in the literature.[48] Indeed, after successful coupling of compound **3** to trityl chloride resin, the reduction of the azide group with tin reagent was completed, and confirmed by IR analysis of a resin sample. However, TLC analysis from a mini-cleavage of resin samples after a reduction reaction indicated the presence of a minor side product having the same polarity as the azide **3**. In a model synthetic sequence on solid phase, the free amine was further acylated with propionic acid in the presence of DIPEA as base, and the THP protecting group of C17-OH was cleaved with a solution of 0.07 M of *p*-TSA in 1-butanol/1,2-dichloroethane (1:1). Next, the cleavage of the sulfamate compound from the resin was carried out by treatment with a solution of 30% hexafluoro-isopropanol (HFIP) in dichloromethane, a mild reagent that does not react with primary and secondary alcohols to give acylation products, like TFA does. As the HPLC purity of the sulfamate derivative **4** obtained from approach I was only 77%, we chose to investigate the reduction of the azide **3** in solution as an alternative strategy. Hydrogenation of **3** using 5% $Pd(OH)_2/C$ as catalyst gave the primary amine **5** that was used without purification in reaction with 9-fluorenylmethyl succinimidyl carbonate to obtain



Scheme 1. Synthesis of the solid-phase precursors **3** and **6**. a) Ph_3P, DIAD, $(C_6H_5O)_2P(O)N_3$, THF, RT, 2 h; b) TBAF, THF, 0 °C, 1 h; (c) H_2NSO_2Cl, DBMP, CH_2Cl_2, RT, 0.5 h; d) 5% (*w/w*) $Pd(OH)_2/C$, EtOAc-MeOH (1:9, *v/v*); e) $NaHCO_3$, FmocOSu, THF:H_2O (3:1, *v/v*), RT, 1 h; f) Polystyrene (PS) trityl chloride, DIPEA, CH_2Cl_2, RT, 6 h; g) 1.0 M Et_3N, 0.8 M PhSH, 0.2 M $SnCl_2$, THF, RT, 5 h; h) PyBOP, CH_3CH_2COOH, DIPEA, DMF, RT, 3 h; i) 0.07 M *p*-TSA in 1-butanol:$ClCH_2CH_2Cl$ (1:1), RT, 24 h; j) 30% HFIP/CH_2Cl_2, RT, 12 h; k) 20% piperidine/CH_2Cl_2, RT, 1 h.

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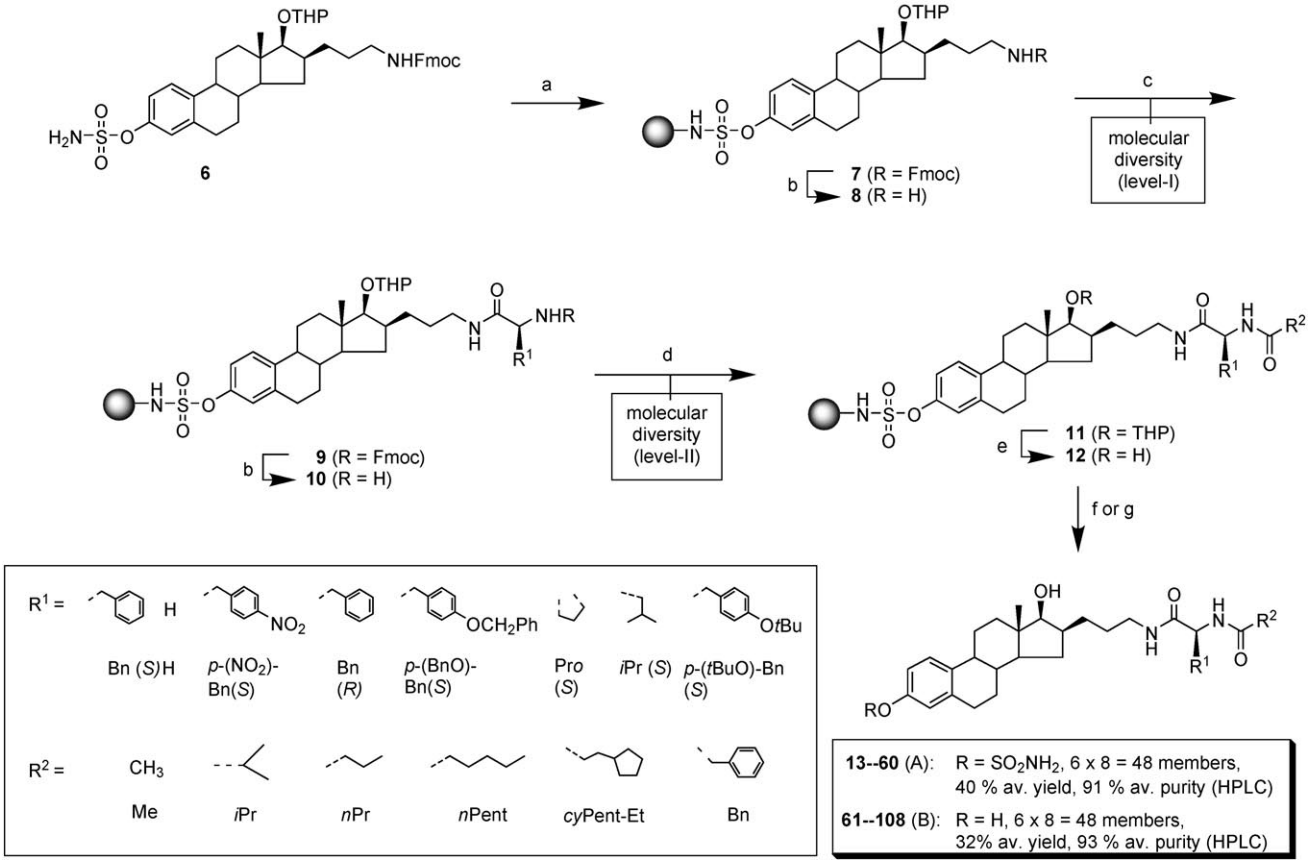
the Fmoc-protected derivative **6**, in 67% overall yield (2 steps). In the second approach (II), this latter compound was loaded on trityl chloride resin and the Fmoc protecting group was cleaved by treatment with a solution of 20% piperidine in dichloromethane to give the free primary amine. From this point, the model reaction sequence was continued as described above, and the purity of the sulfamate derivative **4** released from resin by acidic cleavage was 97%, as determined by HPLC. In the end, we chose to use the longer sequence of reactions in solution to gain on the purity of the products released from resin after solid-phase chemistry.

Introduction of molecular diversity

The synthesis of the two libraries of 16β-aminopropyl derivatives by solid-phase parallel chemistry was performed on a Labtech semi-automated synthesizer, starting from resin **8** containing a free amine group (Scheme 2). The coupling of sulfamate **6** to trityl chloride resin and the deprotection of the terminal amine were performed in a 25 mL peptide flask. The overall yield for this two-reaction step on solid phase was 70–75% as calculated by weight increase of the resin. Equal parts of the resin **8** (96 samples) were placed in the vessels of the reaction block of the synthesizer. The building blocks chosen for the introduction of molecular diversity in the first acylation reaction consisted of amino acids from the L series containing mostly hydrophobic substituents. Such groups might be well accommodated within a hydrophobic region of steroid sulfatase, thus, greatly inhibiting its activity.[49] Therefore, most amino acids serving as diversity elements (R^1) were from the phenylalanine (L or D) or the tyrosine series, but others such as glycine, L-leucine, and L-proline were used too, as less hydrophobic substituents might be better tolerated for inhibition of type 1 17β-HSD activity. The 96-vessel reaction block contains 8 rows of 12 vessels (columns). Each row was assigned an acylation reaction with one of the 8 amino acids used in the synthesis to give the resins **9**. After cleavage of the Fmoc protecting group, a second acylation was performed on resins **10** using 6 carboxylic acids to introduce the second level of diversity (R^2). A solution of each of the carboxylic acids, activated with DIPEA and PyBOP, was reacted with resins from two vessels of each row (column). The THP-protection at C17β-OH was removed by treatment with a solution of *p*-TSA in 1-butanol/1,2-dichloroethane (1:1, *v*/*v*) to give the final resins **12**. Compounds were then released from the polymeric support either by acidic or nucleophilic cleavage to obtain 48-member libraries of sulfamate and phenolic derivatives. A first cleavage was performed by vortexing the resins from columns 1, 3, 5, 7, 9, and 11 with a solution of 30% HFIP in dichloromethane for 12 h at room temperature. Compounds **13**–**60** (the sulfamate



Scheme 2. Solid-phase synthesis of sulfamates (A) and phenol (B) libraries. a) Polystyrene trityl chloride, DIPEA, CH_2Cl_2, RT, 12 h; b) 20% piperidine/CH_2Cl_2, RT, 1 h; c) PyBrOP, HOBt, $R^1CH(NHFmoc)COOH$, DIPEA, DMF, RT, 3 h; d) PyBOP, R^2COOH, DIPEA, DMF, RT, 3 h; e) 0.07 M *p*-TSA in 1-butanol:$ClCH_2CH_2Cl$ (1:1), RT, 24 h; f) 30% HFIP/CH_2Cl_2, RT, 12 h; g) piperazine, THF, 45–50 °C, 3 h.

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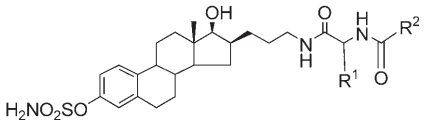
library A) were obtained in yields varying between 8 and 50% calculated for the solid phase reaction sequence from the coupling of the precursor **6** to trityl chloride resin. After a TLC analysis of each library member indicated the presence of only one compound, the average purity of 9 members randomly selected from the library was 94% as determined by HPLC analysis (Table 1). This result was satisfactory since no additional purification step was required after cleavage, the solvent and excess reagent (HFIP) were removed by evaporation in a Speed-vac apparatus. Nucleophilic cleavage was then used to

Table 1. Characterization and steroid sulfatase (STS) inhibitory activity of sulfamate library members **13**–**60**.

Compound			Overall yield (%)[a]	HPLC Purity (%)	LRMS $[MH]^+$ (*m/z*)	STS Inhibition (%)[b]
	R^1	R^2				1/10/100 nM
13		Me	39	–	–	14/80/96
14		*i*Pr	25	95.3	626.5	24/83/96
15		*n*Pr	21	–	–	42/91/97
16	Bn (*S*)	*n*Pent	33	–	–	88/96/97
17		*cy*Pent-Et	23	–	–	98/97/98
18		Bn	33	–	–	80/97/97
19		Me	8	–	–	2/46/94
20		*i*Pr	37	95.1	536.5	2/55/94
21		*n*Pr	40	–	–	5/60/94
22	H	*n*Pent	38	–	–	20/83/96
23		*cy*Pent-Et	47	–	–	48/91/97
24		Bn	46	–	–	22/88/96
25		Me	50	91.5	643.5	21/83/96
26		*i*Pr	41	–	–	40/88/97
27		*n*Pr	41	–	–	48/91/97
28	*p*-(NO_2)-Bn (*S*)	*n*Pent	41	–	–	79/96/97
29		*cy*Pent-Et	38	–	–	91/97/97
30		Bn	42	93.6	719.4	87/96/97
31		Me	45	–	–	12/76/95
32		*i*Pr	41	–	–	25/85/97
33		*n*Pr	41	–	–	40/89/97
34	Bn (*R*)	*n*Pent	42	–	–	78/96/97
35		*cy*Pent-Et	39	95.2	680.5	90/97/97
36		Bn	41	–	–	77/96/97
37		Me	41	–	–	84/97/98
38		*i*Pr	39	–	–	92/97/97
39		*n*Pr	40	94.5	732.7	92/97/97
40	*p*-(BnO)-Bn (*S*)	*n*Pent	39	–	–	95/97/97
41		*cy*Pent-Et	39	–	–	95/99/97
42		Bn	39	–	–	96/97/97
43		Me	49	–	–	2/31/89
44		*i*Pr	45	–	–	6/54/93
45		*n*Pr	46	–	–	7/61/94
46	*N*-Pro (*S*) (residue of proline)	*n*Pent	44	–	–	22/80/96
47		*cy*Pent-Et	45	95.8	630.6	44/88/97
48		Bn	48	–	–	23/82/96
49		Me	45	–	–	7/66/94
50		*i*Pr	44	–	–	19/78/94
51		*n*Pr	46	–	–	20/80/95
52	*i*Bu (*S*)	*n*Pent	45	94.2	620.5	55/92/97
53		*cy*Pent-Et	45	–	–	65/99/97
54		Bn	47	–	–	61/93/97
55		Me	45	–	–	38/90/96
56		*i*Pr	41	–	–	62/91/97
57		*n*Pr	43	–	–	68/93/97
58	*p*-(*t*BuO)-Bn (*S*)	*n*Pent	41	87.4	726.7	90/90/97
59		*cy*Pent-Et	42	–	–	96/94/97
60		Bn	41	–	–	89/96/97

[a] Yields calculated for the solid phase sequence (7 steps). [b] Data are expressed as the mean (spread $< \pm 5\%$) of duplicates in a single run. In this assay, the inhibitor EMATE[10] inhibited 79, 96 and 99% of the transformation of E_1S into E_1 at 1, 10 and 100 nM, respectively.

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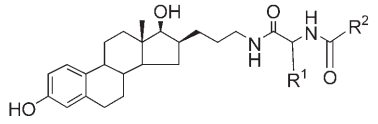
synthesize the 48 members of phenol library B (compounds **61**–**108**). Resins from the columns 2, 4, 6, 8, 10, and 12 of the reaction block were reacted with a solution of piperazine in THF at 45 °C, and then filtered. The reaction mixtures containing the desired products and piperazine were transferred from vials in preweighted tubes and concentrated by evaporation of the solvent in a Speed-vac apparatus at 40 °C. The crude products were then solubilized in dichloromethane and washed twice with water to remove the excess of reagent. Final evaporation of the solvent in the Speed-vac apparatus followed by

Table 2. Characterization and type 1 17β-HSD inhibitory activity of phenol library members.

Compound			Overall yield (%)[a]	HPLC Purity (%)	LRMS $[MH]^+$ (*m/z*)	17β-HSD1 Inhibition (%)[b]
	R^1	R^2				0.1/1/10 μM
61		Me	24	–	–	7/42/75
62		*i*Pr	30	–	–	–
63		*n*Pr	36	90.9	547.7	–
64	Bn (*S*)	*n*Pent	17	–	–	–
65		*cy*Pent-Et	25	–	–	–
66		Bn	26	–	–	–
67		Me	19	–	–	13/32/77
68		*i*Pr	49	–	–	–
69		*n*Pr	29	86.2	457.6	–
70	H	*n*Pent	47	–	–	–
71		*cy*Pent-Et	39	–	–	–
72		Bn	32	–	–	–
73		Me	29	–	–	12/46/76
74		*i*Pr	41	–	–	–
75		*n*Pr	42	–	–	–
76	*p*-(NO_2)-Bn (*S*)	*n*Pent	58	–	–	–
77		*cy*Pent-Et	25	91.5	646.6	–
78		Bn	31	–	–	–
79		Me	29	–	–	32/40/80
80		*i*Pr	36	–	–	–
81		*n*Pr	34	–	–	–
82	Bn (*R*)	*n*Pent	43	96.4	575.6	–
83		*cy*Pent-Et	31	–	–	–
84		Bn	28	–	–	–
85		Me	28	94.1	625.7	22/52/71
86		*i*Pr	33	–	–	–
87		*n*Pr	30	–	–	–
88	*p*-(BnO)-Bn (*S*)	*n*Pent	33	–	–	–
89		*cy*Pent-Et	33	–	–	–
90		Bn	20	–	–	–
91		Me	33	–	–	5/17/64
92		*i*Pr	37	88.0	497.5	–
93		*n*Pr	34	–	–	–
94	*N*-Pro (*S*) (residu of proline)	*n*Pent	33	–	–	–
95		*cy*Pent-Et	50	–	–	–
96		Bn	32	–	–	–
97		Me	13	–	–	12/27/71
98		*i*Pr	34	–	–	–
99		*n*Pr	44	–	–	–
100	*i*Bu (*S*)	*n*Pent	36	89.6	541.7	–
101		*cy*Pent-Et	32	–	–	–
102		Bn	25	–	–	–
103		Me	25	–	–	0/18/57
104		*i*Pr	31	–	–	–
105		*n*Pr	25	–	–	–
106	*p*-(*t*BuO)-Bn (*S*)	*n*Pent	26	–	–	–
107		*cy*Pent-Et	25	–	–	–
108		Bn	24	90.0	667.5	–

[a] Yields calculated for the solid phase sequence (7 steps). [b] Data are expressed as the mean (SD $< \pm 10\%$) of triplicates in a single run. In this assay, the inhibitor EM-251[30] inhibited 25, 70 and 90% of the transformation of E_1 into E_2 by type 1 17β-HSD at 0.1, 1 and 10 μM, respectively.

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additional drying on a vacuum pump provided the corresponding E_2 derivatives in yields ranging from 13 to 58%. After TLC analysis of each library member indicated the presence of only one compound, the average purity of 8 members of the library randomly selected was 91% as determined by HPLC (Table 2). Furthermore, analysis of compounds randomly selected from the two libraries by IR, NMR, and MS confirmed the expected chemical structures and masses.

Inhibitory activity on steroid sulfatase

Sulfamate and phenol derivatives from libraries A and B were synthesized with the aim of targeting the inhibition of two steroidogenic enzymes involved in proliferation of estrogen-sensitive tumors, namely steroid sulfatase and type 1 17β-HSD. However, only the sulfamate derivatives were tested in the steroid sulfatase inhibition assay, as we expected, from our previous studies[50] that their phenol analogues might have only moderate inhibitory activity on this enzyme. Moreover, any beneficial contribution of the D-ring diversity elements on steroid sulfatase inhibition should be readily observed when comparing the inhibitory activity of sulfamate derivatives **13**–**61** to that of EMATE, a 3-*O*-sulfamate derivative of estrone (E_1) with no D-ring substituent.[10] For the inhibition assay,[51] a homogenate of human embryonic kidney (HEK)-293 cells transfected with an expression vector encoding steroid sulfatase was used as a source of enzyme activity. All sulfamate derivatives were tested at concentrations of 1, 10, and 100 nM and the hydrolysis of [^{3}H]-E_1S (100 μM) into [^{3}H]-E_1 was measured to calculate the % inhibitory activity (Table 1). Although most compounds fully inhibited the steroid sulfatase activity at 100 nM, a SAR-based study of their inhibitory activity is possible when comparing results obtained at the lower concentrations of 1 and 10 nM. An analysis of these results is presented in Figures 2 and 3, respectively, illustrating the influence of amino acid residues and that of the carboxylic acid N-capping groups on steroid sulfatase inhibition. Thus, in a series of compounds with the same carboxylic acid N-capping group (for example acetic acid, R^2 = Me, Figure 2), hydrophobic amino acid residues such as benzyl and *tert*-butyl ethers of (*S*)-tyrosine provide the best inhibition of steroid sulfatase activity (inhibition of 84% for **37** and 38% for **55** at 1 nM), while glycine and proline residues give lower inhibition (2% for **19** and 2% for **43**). On the other hand, in a series of compounds with the same amino acid residue (for example glycine, R^1 = H, Figure 3), higher inhibition at 1 nM (48%) was obtained with a bulky cyclopentyl carboxylic acid used as N-capping group. Overall, these results clearly support previous reports about the existence of a hydrophobic region in proximity to the D-ring of the natural substrate when bound to the enzyme. Since such hydrophobic interactions improve inhibition of steroid sulfatase, several sulfamate library members containing the amino acid and carboxylic acid residues mentioned above seem to be more potent inhibitors than EMATE. EMATE inhibited 79% of steroid sulfatase while fifteen sulfamates from library A provided 80 to 96% inhibition, when tested at 1 nM. Furthermore, such compounds bearing a 16β-alkylamide or 16β-peptide chain are expected to have reduced estrogenicity, just as required for any potential therapeutic agent to be used in the treatment of ER^+ breast cancer.

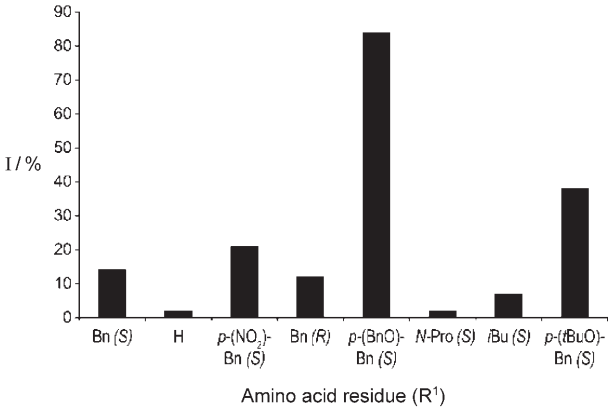

Figure 2. Influence of the amimo acid residue (R^1) on steroid sulfatase inhibition (I). Compounds **13**, **19**, **25**, **31**, **37**, **43**, **49**, and **55** (R^2 = Methyl, Table 1) were tested at 1 nM for the transformation of E_1S into E_1. The amino acid residues are: Bn = benzyl, Pro = three CH_2 of proline, and Bu = butyl.

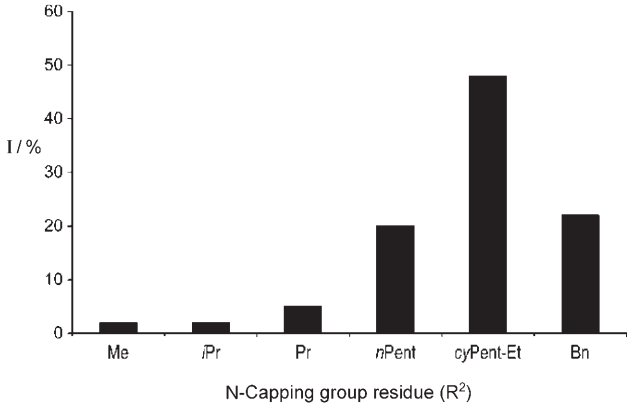

Figure 3. Influence of the N-capping group (R^2) on steroid sulfatase inhibition (I). Compounds **19**–**24** (R^1 = H, Table 1) were tested at 1 nM for the transformation of E_1S into E_1. The N-capping group residues are: Me = methyl, Pr = propyl, Pent = pentyl, Et = ethyl, and Bn = benzyl.

Inhibitory activity on type 1 17β-HSD

A series of phenol derivatives bearing a methyl group as the second level of molecular diversity (R^2) were tested for the inhibition of type 1 17β-HSD activity (Table 2). As the building blocks used in the elaboration of libraries were mostly hydrophobic (a steroid sulfatase requirement), only the phenols with lower hydrophobicity (**61**, **67**, **73**, **79**, **85**, **91**, **97**, and **103**) were selected to be tested on type 1 17β-HSD. For the assay,[52] human embryonic kidney (HEK)-293 cells transfected with cDNA encoding type 1 17β-HSD were sonicated to release the crude enzyme that was used directly without purification. The transformation of radiolabeled substrate [^{14}C]-E_1 (0.1 μM) into [^{14}C]-E_2 by type 1 17β-HSD in the presence of the cofactor NADH in excess, allowed determination of the ability of phe-

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nolic compounds to inhibit the enzyme activity (in %). Generally, inhibition percentages for the tested compounds are below or near that of the reference inhibitor EM-251, an E_2 derivative with a short bromopropyl side chain in C16α.[30] While the inhibitory activity is low, indications exist that residues such as Bn (*R*) and *p*-(BnO)-Bn (*S*) are well tolerated in this region of the enzymatic site of type 1 17β-HSD. Furthermore, the percentages of inhibition of E_2 derivatives are higher than that of unlabeled E_2, suggesting a beneficial inhibitory effect of the side chain at C16β. Thus, we are confident that optimization of type 1 17β-HSD inhibitors might be achieved using as molecular diversity elements a broader and better-targeted selection of building blocks, having the ability to interact strongly with key components of the enzymatic reaction. Such additional interactions with amino acids of the catalytic triad, amino acids involved in cofactor binding, or the cofactor itself will result in a better inhibitory effect on type 1 17β-HSD.

Conclusions

The attachment of a C18 steroid on a polymeric support using a multidetachable sulfamate linker allows us to synthesize libraries of sulfamate and phenol derivatives, depending on the chosen cleavage method, acidic or nucleophilic, respectively. Herein, we report the synthesis of two libraries of 16β-aminopropyl E_2 derivatives designed to target the enzyme steroid sulfatase and also the enzyme type 1 17β-HSD. These steroidogenic enzymes control important reactions involved in the biosynthesis of estrogenic steroids, which are known to be involved in the proliferation of ER^+ breast cancer tumors and in the stimulation of estrogen-sensitive diseases.

Coupling of steroidal sulfamate **6** to trityl chloride resin was achieved in satisfactory yields (70–80%) and required only a small excess of steroid (0.2 equiv) over the theoretical loading of the resin. A series of diversity elements were then introduced by a first acylation reaction at the terminal amine of the 16β-side chain with 8 Fmoc-amino acids, followed by amine deprotection and a second acylation with 6 carboxylic acids (8×6 members). Interestingly, the sulfamate linker has shown good resistance during 24 h treatment with a 0.07 M solution of *p*-TSA in 1-butanol/1,2-dichloroethane (1:1, *v*/*v*) for the cleavage of a C17-tetrahydropyranyl protecting group, prior to the last decoupling step. Thus, it appears to be more resistant to acids than dihydropyranyl (DHP) or silyl linkers that are normally cleaved in such reaction conditions. After deprotection, compounds loaded on the resin incorporated a C17β-secondary alcohol that was reactive toward esterification with TFA. Alternatively, a solution of 30% HFIP in dichloromethane was used for acidic cleavage to prevent this side reaction. At the end of a seven-step solid phase reaction sequence, the sulfamate compounds **13**–**60** were obtained in 8–50% yields and with a high average HPLC purity of 94%. The phenolic derivatives **61**–**108** resulted in 13–58% yields and an average HPLC purity of 91% after nucleophilic cleavage with piperazine in THF at 45 °C.

We found that hydrophobic residues of amino acids and of carboxylic acids used to sequentially introduce the molecular diversity elements on a 16β-aminopropyl side chain from E_2 are well suited for the inhibition of steroid sulfatase. Their inhibitory effect enhances the one that is achieved with a sulfamate group at position C3 of E_2. Thus, steroid sulfatase inhibitors have been assessed as comparable or better inhibitors than the reference compound EMATE and reduced estrogenicity was generated in this way, as expected. Moreover, the dual cleavage property of the sulfamate linker can be used in the synthesis of phenolic derivatives by means of solid phase and combinatorial chemistry. Herein, selected members of a library of 16β-aminopropyl E_2 derivatives synthesized using this methodology was tested for the inhibition of type 1 17β-HSD. While hits from this series were less potent than a reference inhibitor known from the literature, inhibitory activity was however detected and further optimization of results by broadening the selection of building blocks is possible.

An attractive approach for reducing levels of estrogens in breast cancer tumors is to use therapeutic agents capable of blocking both steroid sulfatase and type 1 17β-HSD activities. While sulfamate derivatives of E_2 are known as potent inhibitors of steroid sulfatase,[10–13] the phenolic compound released after inactivation of the enzyme might be designed to be a non-estrogenic type 1 17β-HSD inhibitor. Thus, the introduction of an appropriate molecular diversity on the D-ring of EMATE and E_2 will enhance the inhibition of both steroid sulfatase and type 1 17β-HSD inhibition, and will reduce the estrogenic activity of the E_2 nucleus. When careful optimization of the molecular diversity introduced on the D-ring of E_2 is performed, the sulfamate linker is certainly a useful tool for the synthesis of such inhibitors targeting two key steroidogenic enzymes. Indeed, the sulfamate linker that we developed[27–29] was recently used in the synthesis of focused libraries of 16-substituted E_1 derivatives as inhibitors of type 1 17β-HSD.[53] In summary, the multidetachable sulfamate linker can be used profitably in the solid-phase combinatorial synthesis of E_2 and E_1 derivatives, sulfamoylated or not, to potentially accelerate the discovery of compounds with therapeutic action against hormone-sensitive diseases and eventually for the synthesis of other types of phenolic compounds with important biological applications.

Experimental Section

General: Trityl chloride resin (200 mesh, 2.05 mmol g^{-1} theoretical loading), coupling reagents, and Fmoc amino acids were supplied by Novabiochem (San Diego, CA). Other reagents and anhydrous solvents such as dichloromethane (CH_2Cl_2), and dimethylformamide (DMF) were obtained from Sigma–Aldrich (Oakville, ON, Canada). Fisher Scientific (Montréal, QC, Canada) provided the usual solvents. Prior to its use, tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. Solution phase reactions were performed in oven-dried glassware with magnetic stirring bars, under argon. Fritted peptide synthesis vessels (25 mL) equipped for vacuum filtration (ChemGlass Inc, Vineland, NJ) were used for the solid phase coupling reaction and synthesis of the resin intermediate **8** on a Burrell wrist-action shaker Model 75 (Burrell, Pittsburg, PA, USA). Additionally, parallel synthesis of sulfamate and phenol libraries from resin **8** were performed on a Labtech semi-

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automated synthesizer from Advanced ChemTech (Louisville, KY, USA). Analytical thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F_{254} plates (E. Merck; Darmstadt, Germany), and compounds were visualized using UV light or ammonium molybdate/sulfuric acid/water (with heating). Flash column chromatography was performed with 230–400/mesh ASTM silica gel 60 (E. Merck). Mini-cleavage assays on 2–3 mg sample resins with 0.1 mL of a 30% solution 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in CH_2Cl_2 were performed to monitor by TLC the completion of solid-phase reactions. Infrared spectra (IR) were expressed in cm^{-1} and recorded on a Perkin–Elmer series 1600 (FT-IR) spectrometer (Norwalk, CT, USA). Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC/F300 spectrometer (Billerica, MA, USA) at 300 MHz (^1H), and the chemical shifts (δ) were expressed in ppm. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ionspray source.

3-[3-Hydroxy-17β-(tetrahydro-2*H*-pyran-2-yl-oxy)-estra-1,3,5(10)-triene-16β-yl]-azidopropane (2): Diethyl azodicarboxylate (DIAD) (6.4 mL, 32.3 mmol) was added dropwise to a concentrated solution of triphenylphosphine (8.47 g, 32.3 mmol) in dry THF and this mixture was stirred at 0 °C for 30 min. A solution of alcohol **1**[44] (8.47 g, 16.15 mmol) in dry THF (100 mL) and diphenylphosphoryl azide (7 mL, 32.3 mmol) were added successively to the resulting ylide precipitate. The reaction mixture was stirred for 30 min at 0 °C followed by another 2 h at room temperature, then filtered over a pad of silica gel using hexanes/acetone (80:20) as eluent. The product was concentrated by solvent evaporation under reduced pressure, then purified by flash chromatography with hexanes/EtOAc (95:5) to give the azide intermediate as an amorphous white solid (16.4 g, 90% yield). To a cooled solution of the latter (16.4 g, 28.9 mmol) in dry THF (500 mL) tetrabutylammonium fluoride (35 mL, 1.0 M solution in THF) was added dropwise, and the reaction mixture was stirred at 0 °C for 1 h. A saturated solution of $NaHCO_3$ was added and the crude compound was extracted with EtOAc (3×). The combined organic layers were washed with brine, filtered over $MgSO_4$, and evaporated under reduced pressure. Purification by flash chromatography yielded the corresponding phenolic steroid **2** (11.9 g, 94% yield). Spectral data were identical to those previously reported.[44]

3-[3-Sulfamoyloxy-17β-(tetrahydro-2*H*-pyran-2-yl-oxy)-estra-1,3,5(10)-triene-16β-yl]-azidopropane (3): In a 1000 mL flask equipped with a magnetic stirrer the phenolic steroid **2** (6.3 g, 14.35 mmol) in dry CH_2Cl_2 (400 mL) was solubilized. 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) (5.9 g, 28.7 mmol) and sulfamoyl chloride[40] (2.5 g, 21.53 mmol) were added successively to the solution cooled at 0 °C. The reaction was monitored on TLC and stopped after 30 min by the addition of water (200 mL). After extraction with CH_2Cl_2 the mixture was filtered over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography with hexanes/acetone (80:20) to yield the sulfamate **3** (14.3 g, 96%). White foam; IR (KBr): $\tilde{\nu}$ = 3373 (NH), 2097 (N_3, azide), 1384 and 1188 cm^{-1} (S=O, sulfamate); ^1H NMR ($CDCl_3$): δ = 0.82 and 0.86 (2 s, 18-CH_3), 1.00–2.40 (22H), 2.90 (m, 6-CH_2), 3.30 (m, CH_2N), 3.54 and 3.95 (2m, CH_2O of THP), 3.76 and 3.84 (2d, J = 10.0 Hz, 17α-H), 4.63 and 4.73 (2 m, CHO of THP), 4.89 (s, SO_2NH_2), 7.05 (d, J = 2.3 Hz, 4-CH), 7.10 (dd, J^1 = 2.3 Hz and J^2 = 8.3 Hz, 2-CH), 7.32 ppm (d, J = 8.3 Hz, 1-CH).

3-[3-Sulfamoyloxy-17β-(tetrahydro-2*H*-pyran-2-yl-oxy)-estra-1,3,5(10)-triene-16β-yl]-(*N*-fluorenyl-methoxycarbonyl)-amino-propane (6): A solution of azide **3** (3.6 g, 7.02 mmol) and palladium hydroxide (5% *w/w* on activated carbon) (730 mg) in MeOH/EtOAc (9:1, *v/v*) was stirred under an atmospheric pressure of hydrogen for 5 h. After removal of the hydrogen source, the mixture was filtered over Celite and the solvents evaporated under reduced pressure to give the amine intermediate **5**, which was used without purification in the next step. IR (KBr): $\tilde{\nu}$ = 3391 and 3243 cm^{-1} (NH_2 and SO_2NH_2, disappearance of the azide band at 2097 cm^{-1}). A 1.0 N solution of $NaHCO_3$ (7.02 mmol) and 9-fluorenylmethyl succinimidyl carbonate (2.85 g, 8.43 mmol) were added successively to a stirring solution of the amine **5** in THF/H_2O (3:1, *v/v*) (200 mL) at room temperature. After 2 h, water was added and the crude product extracted with EtOAc (1×) and CH_2Cl_2 (2×). The combined organic layer was filtered over $MgSO_4$ and the crude product was concentrated under reduced pressure. Purification by flash chromatography with hexanes/acetone/Et_3N (74:25:1) gave the *N*-Fmoc derivative **6** (3.4 g, 68% yield for 2 steps). White foam; IR (KBr): $\tilde{\nu}$ = 3391 and 3067 (NH_2 and SO_2NH_2), 1702 (C=O, carbamate), 1382 and 1188 cm^{-1} (S=O, sulfamate); ^1H NMR ($CDCl_3$): δ = 0.78 and 0.82 (2 s, 18-CH_3), 1.00–2.40 (22H), 2.85 (m, 6-CH_2), 3.14 (m, CH_2N), 3.48 and 3.95 (2 m, CH_2O of THP), 3.73 and 3.78 (2d, J = 10.0 Hz, 17α-H), 4.22 (t, J = 6.8 Hz, CH_2CH of Fmoc), 4.39 (d, J = 6.9 Hz, OCH_2 of Fmoc), 4.60 and 4.67 (2 m, CHO of THP), 4.79 and 4.97 (2 m, NH), 5.04 (s, SO_2NH_2), 7.03 (d, J = 2.4 Hz, 4-CH), 7.08 (dd, J^1 = 2.4 Hz and J^2 = 8.5 Hz, 2-CH), 7.30 (m, 1-CH and 2H of Fmoc), 7.40 (t_{app}, J = 7.4 Hz, 2H of Fmoc), 7.59 and 7.77 ppm (2d, J = 7.3 Hz, 4H of Fmoc). LRMS calculated for $C_{41}H_{51}N_2O_7S$ [MH^+]: 715.5 *m/z*; HPLC purity = 46.4 and 51.1%, respectively for the two diastereoisomers (C-18 NovaPak Column, CH_3OH: H_2O containing 20 mM of NH_4OAc).

Coupling of Fmoc-derivative 6 on trityl chloride resin: Trityl chloride resin (Novabiochem, 2.05 mmol g^{-1} theoretical loading) (1 g) was swollen under argon in dry CH_2Cl_2 (5 mL) and diisopropylethylamine (DIPEA) (3.10 mL). After 5 min, sulfamate derivative **6** (1.76 g, 2.46 mmol) was added gradually as a solid and an additional volume of dry CH_2Cl_2 (2 mL). The mixture was then vortexed for 9 h at room temperature. The resin was filtered and washed three times with CH_2Cl_2, CH_3OH, and again with CH_2Cl_2, then dried overnight under vacuum to afford 2.09 g of resin **7**. IR (KBr): $\tilde{\nu}$ = 1700 cm^{-1} (C=O, Fmoc). The coupling yield calculated as the mean of the weight increase was 80%. The filtrate was collected and evaporated to dryness, then purified by flash chromatography on alumina with hexanes:acetone (70:30) to isolate 585 mg of unreacted sulfamate **6**. The yield, calculated as the recovered amount of compound **6**, was 78%.

Cleavage of the Fmoc protecting group (synthesis of the amine resin 8): For the cleavage of the Fmoc protecting group, resin **7** was swollen in a 10 mL mixture of CH_2Cl_2:piperidine (4:1, *v/v*) and agitated within the 25 mL peptide-flask for 1 h at room temperature. The resulting amine resin **8** was filtered and washed three times successively with CH_2Cl_2, CH_3OH, and CH_2Cl_2 (10 mL each). Another cycle of deprotection was then performed using the same conditions described above. After drying for one day under vacuum pump, the resulting resin **8** weighed 1.73 g. This weight corresponds to a 77% yield for the successive reactions of coupling and Fmoc deprotection on solid-support starting from the precursor **6**. Note: Solid-phase coupling of sulfamate **6** and Fmoc cleavage were done as described above with an additional 3 g of trityl chloride resin to provide the amount of precursor **8** needed for the synthesis of two libraries of 48 members (sulfamates **13**–**60** and phenols **61**–**108**).

Solid-phase synthesis of resins 12: Reactions were run in parallel on the ACT-LabTech synthesizer starting with resin **8** (80 mg, 0.071 mmol) in each of the 96 vessels of the reaction block. The block was installed on the variable-speed orbital shaker of the synthesizer and closed with the top clamp plate. To prevent the loss of reagent through the fritted vessels a positive flow of argon 10 cc min^{-1} was applied below the reaction block. The inside face

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of the top clamp plate was covered with a thin film of Teflon that was much more resistant in the conditions of various reactions than the original Sheet Septa. This minor modification helped to prevent contamination of the products with septum residues. An orbital speed of 675 rpm was used for each reaction.

Eight stock solutions each containing a Fmoc protected amino acid (1.7 mmol), bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) (795 mg, 1.7 mmol) and *N*-hydroxybenzotriazole (HOBt) (230 mg, 1.7 mmol), were prepared in DMF (7–8 mL). The solutions were vortexed with *N*,*N*-diisopropylethylamine (DIPEA) (0.6 mL, 3.4 mmol) and shortly after the activation of the carboxylic group (2 min), 0.9 mL of the solution from the same vials were added to the resins **8** (80 mg, 71 μmol) in their assigned reaction vessels (1 row of 12 vessels). The 96 resins were vortexed under argon at room temperature for 2 h, then filtered, washed with DMF (3×) and CH_2Cl_2 (3×), and dried under vacuum to afford the resins **9** (with the first level of molecular diversity). An IR spectrum confirmed the formation of the amide bond. In addition, TLC analysis after a mini-cleavage test (30% HFIP/CH_2Cl_2, 1 h) with 8 samples of resins **9** confirmed the completion of the coupling reaction. As reported above, the resins **9** were reacted with 0.9 mL solution of CH_2Cl_2: piperidine (4:1, *v*/*v*) for the cleavage of Fmoc group, giving the amines **10**. Carboxylic acids (acetic, isobutyric, butyric, 3-cyclopentyl propionic, and phenyl acetic) (3.4 mmol each) were preactivated for the last coupling reaction by mixing for 2 min with benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (1.77 g, 3.4 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (1.2 mL, 6.8 mmol), and DMF (12 mL) in a 20 mL vial. The appropriate carboxylic acid solution (0.9 mL, 0.14 mmol) was added to resins **10** (each type assigned to a group consisting of 2 columns×8 vessels). The mixtures were shaken for 3 h at room temperature under argon, then filtered and washed with DMF and CH_2Cl_2 (4×) to afford two series of 48 different resins **11**. Completion of the acylation reaction was proven again by mini-cleavage as described above. Resins **11** were then vortexed 24 h with a solution of 0.07 M of *p*-toluenesulfonic acid monohydrate (*p*-TSA) in 1-butanol/1,2-dichloroethane (1:1 *v*/*v*) for the cleavage of the C17-tetrahydropyranyl ether. Prior to the decoupling of steroid derivatives from polymeric support by acidic or nucleophilic cleavage, the resulting resins **12** were washed intensively with CH_2Cl_2 (4×), MeOH (4×), and CH_2Cl_2 (2×) to remove traces of *p*-TSA.

Generation of sulfamate library A by acidic cleavage: On the ACT-Labtech synthesizer the vials block was installed on the variable-speed orbital shaker, under the reaction vessel block. To each of the 48 resins **12** (columns 1, 3, 5, 7, 9, and 11) was added 1.5 mL solution of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (30%) in CH_2Cl_2. The system was closed with the top clamp plate and mixtures were vortexed at 675 rpm under a weak flow of argon directed as positive pressure. After 12 h at room temperature, the resins were filtered under vacuum, washed with CH_2Cl_2 (2×6 mL), CH_3OH/CH_2Cl_2 (1:2), then filtered again. The organic layers were transferred from the vials to the corresponding preweighted tubes (15 mL volume each) and the solvent was evaporated in a Speed-vac apparatus at 40 °C. Products were dried further over 48 h on vacuum pump. Sulfamate derivatives **13**–**60** (Table 1) were obtained in yields ranging from 8 to 50% and a high average purity of 94% as determined by HPLC analysis of 9 members randomly selected from the library.

14: White foam; IR (KBr): $\tilde{\nu}$ = 3387 and 3329 (OH, NH, and NH_2), 1648 and 1618 (C=O, amides), 1385 and 1185 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.77 (s, 18-CH_3), 0.94 and 1.04 (2d, *J* = 6.9 Hz, $CH(CH_3)_2$), 1.00–2.40 (16H), 2.43 (sept, *J* = 6.9 Hz, $CH(CH_3)_2$, 2.87 (m, 6-CH_2 and 1H of CH_2Ph), 3.11 (m, CH_2N and 1H of CH_2Ph), 3.70 (d, *J* = 9.7 Hz, 17α-H), 4.56 (dd, J^1 = 6.4 Hz, J^2 = 8.7 Hz, CHN), 7.01 (d, *J* = 2.4 Hz, 4-CH), 7.04 (dd, J^1 = 2.4 Hz and J^2 = 8.6 Hz, 2-CH), 7.23 (m, CH_2Ph), 7.32 ppm (d, *J* = 8.6 Hz, 1-CH); LRMS for $C_{34}H_{48}N_3O_6S$ [MH^+]: 626.5 *m*/*z*; HPLC purity = 95.3% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

20: White foam; IR (KBr): $\tilde{\nu}$ = 3405 and 3310 (OH, NH, and NH_2), 1654 (C=O, amides), 1377 and 1187 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.77 (s, 18-CH_3), 1.13 (2d, *J* = 6.8 Hz, $CH(CH_3)_2$), 1.00–2.30 (16H), 2.51 (sept, *J* = 6.9 Hz, $CH(CH_3)_2$, 2.87 (m, 6-CH_2), 3.20 (t, *J* = 6.7 Hz, CH_2N), 3.71 (d, *J* = 9.7 Hz, 17α-H), 3.79 (s, $COCH_2$), 7.00 (d, *J* = 2.4 Hz, 4-CH), 7.04 (dd, J^1 = 2.4 Hz and J^2 = 8.6 Hz, 2-CH), 7.32 ppm (d, *J* = 8.6 Hz, 1-CH); LRMS for $C_{27}H_{42}N_3O_6S$ [MH^+]: 536.5 *m*/*z*; HPLC purity = 95.1% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

25: White foam; IR (KBr): $\tilde{\nu}$ = 3385 (OH, NH, and NH_2), 1654 (C=O, amides), 1522 and 1346 (NO_2), 1376 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.75 (s, 18-CH_3), 1.91 (s, CH_3CO), 0.90–2.40 (16H), 2.88 (m, 6-CH_2), 3.10 (m, CH_2N and 2H of CH_2Ph), 3.68 (d, *J* = 9.6 Hz, 17α-H), 4.62 (t, *J* = 7.6 Hz, COCH), 7.01 (d, *J* = 2.6 Hz, 4-CH), 7.04 (dd, J^1 = 2.6 Hz, J^2 = 8.5 Hz, 2-CH), 7.32 (d, *J* = 8.5 Hz, 1-CH), 7.48 and 8.17 ppm (2d, *J* = 8.7 Hz, 2×2H, CH_2Ph-*p*-NO_2); LRMS for $C_{32}H_{43}N_4O_8S$ [MH^+]: 643.5 *m*/*z*; HPLC purity = 91.5% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

30: White foam; IR (KBr): $\tilde{\nu}$ = 3378, 3296 and 3080 (OH, NH, and NH_2), 1648 (C=O, amides), 1520 and 1346 (NO_2), 1376 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.76 (s, 18-CH_3), 0.90–2.40 (16H), 2.88 (m, 6-CH_2), 2.99 (dd, J^1 = 8.2 Hz, J^2 = 13.7 Hz, 1H of CH_2PhNO_2), 3.14 (m, CH_2N), 3.36 (dd, J^1 = 6.1 Hz, J^2 = 13.7 Hz, 1H of CH_2PhNO_2), 3.43 and 3.49 (2d, *J* = 14.1 Hz, $COCH_2$Ph), 3.69 (d, *J* = 9.6 Hz, 17α-H), 4.66 (dd, J^1 = 6.1 Hz, J^2 = 9.2 Hz, COCH), 7.00 (d, *J* = 2.4 Hz, 4-CH), 7.04 (dd, J^1 = 2.4 Hz, J^2 = 8.5 Hz, 2-CH), 7.20 (m, CH_2Ph), 7.32 (d, *J* = 8.5 Hz, 1-CH), 7.37 and 8.03 ppm (2d, *J* = 8.7 Hz, 2×2H, CH_2Ph-*p*-NO_2); LRMS for $C_{38}H_{47}N_4O_8S$ [MH^+]: 719.4 *m*/*z*; HPLC purity = 93.6% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

35: White foam; IR (KBr): $\tilde{\nu}$ = 3380, 3310 and 3085 (OH, NH, and NH_2), 1648 (C=O, amide), 1376 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.76 (s, 18-CH_3), 0.90–2.40 (29H), 2.86 (m, 6-CH_2 and 1H of CH_2Ph), 3.11 (m, CH_2N and 1H of CH_2Ph), 3.70 (d, *J* = 9.7 Hz, 17α-H), 4.57 (dd, J^1 = 6.3 Hz, J^2 = 9.0 Hz, COCH), 7.00 (d, *J* = 2.4 Hz, 4-CH), 7.04 (dd, J^1 = 2.4 Hz and J^2 = 8.5 Hz, 2-CH), 7.23 (m, CH_2Ph), 7.32 ppm (d, *J* = 8.5 Hz, 1-CH); LRMS for $C_{38}H_{54}N_3O_6S$ [MH^+]: 680.5 *m*/*z*; HPLC purity = 95.2% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

39: White foam; IR (KBr): $\tilde{\nu}$ = 3380 and 3066 (OH, NH, and NH_2), 1648 (C=O, amides), 1377 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.75 (s, 18-CH_3), 0.83 (t, *J* = 7.4 Hz, CH_2CH_3), 0.90–2.40 (20H), 2.82 (m, 6-CH_2 and 1H of CH_2PhO), 3.08 (m, CH_2N and 1H of CH_2PhO), 3.68 (d, *J* = 9.6 Hz, 17α-H), 4.50 (t_{app}, *J* = 7.6 Hz, COCH), 5.04 (s, OCH_2Ph), 6.91 and 7.14 (2d, *J* = 8.6 Hz, 2×2H, CH_2PhO), 6.99 (d, *J* = 2.5 Hz, 4-CH), 7.04 (dd, J^1 = 2.5 Hz and J^2 = 8.6 Hz, 2-CH), 7.34 ppm (m, OCH_2Ph and 1-CH); LRMS for $C_{41}H_{54}N_3O_7S$ [MH^+]: 732.7 *m*/*z*; HPLC purity = 94.5% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

47: White foam; IR (KBr): $\tilde{\nu}$ = 3397 and 3074 (OH, NH, and NH_2), 1648 and 1630 (C=O, amides), 1376 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.78 (s, 18-CH_3), 0.90–2.40 (33H), 2.88 (m, 6-CH_2), 3.18 (t, *J* = 6.8 Hz, CH_2N), 3.54 and 3.65 (m, CH_2N-proline), 3.71 (d, *J* = 9.6 Hz, 17α-CH), 4.34 (m, COCH), 7.00 (d, *J* = 2.2 Hz, 4-CH), 7.04 (dd, J^1 = 2.2 Hz and J^2 = 8.5 Hz, 2-CH), 7.32 ppm (d, *J* = 8.5 Hz, 1-CH); LRMS for $C_{34}H_{52}N_3O_6S$ [MH^+]: 630.6 *m*/*z*; HPLC

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purity = 95.8% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

52: White foam; IR (KBr): $\tilde{\nu}$ = 3386 and 3074 (OH, NH, and NH_2), 1648 and 1630 (C=O, amides), 1376 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.77 (s, 18-CH_3), 0.91 (t, J = 6.9 Hz, CH_2CH_3), 0.92 and 0.95 (2d, J = 6.5 Hz, $CH(CH_3)_2$), 0.95–2.40 (27H), 2.87 (m, 6-CH_2), 3.18 (t, J = 6.8 Hz, CH_2N), 3.71 (d, J = 9.7 Hz, 17α-CH), 4.35 (dd, J^1 = 6.3 Hz, J^2 = 8.8 Hz, COCH), 7.00 (d, J = 2.5 Hz, 4-CH), 7.04 (dd, J^1 = 2.5 Hz, J^2 = 8.4 Hz, 2-CH), 7.32 ppm (d, J = 8.4 Hz, 1-CH); LRMS for $C_{33}H_{54}N_3O_6S$ [MH^+]: 620.5 m/z; HPLC purity = 94.2% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

58: White foam; IR (KBr): $\tilde{\nu}$ = 3386 and 3082 (OH, NH, and NH_2), 1647 (C=O, amides), 1367 and 1186 cm^{-1} (S=O, sulfamate); ^1H NMR (CD_3OD): δ = 0.77 (s, 18-CH_3), 0.88 (t, J = 7.1 Hz, CH_2CH_3), 1.31 (s, $(CH_3)_3C$), 1.00–2.40 (24H), 2.84 (m, 6-CH_2 and 1H of CH_2Ph), 3.03 (dd, J^1 = 6.8 Hz, J^2 = 13.5 Hz, 1H of CH_2Ph), 3.11 (m, CH_2N), 3.71 (d, J = 9.6 Hz, 17α-CH), 4.52 (t_{app}, J = 7.6 Hz, COCH), 6.90 and 7.15 (2d, J = 8.5 Hz, 2×2H of O*Ph*CH_2), 7.00 (d, J = 2.4 Hz, 4-CH), 7.06 (dd, J^1 = 2.4 Hz and J^2 = 8.5 Hz, 2-CH), 7.32 ppm (d, J = 8.5 Hz, 1-CH); LRMS for $C_{40}H_{60}N_3O_7S$ [MH^+]: 726.7 m/z; HPLC purity = 87.4% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

Generation of phenol library B by nucleophilic cleavage: On the ACT-Labtech synthesizer the heat-cool module was properly attached on the orbital shaker, under the reaction vessel block. 0.9 mL (0.063 mmol) from a stock solution of piperazine in THF (0.7 M) was added to each reaction vessel. The top clamp plate was installed properly and the reaction block was rotated at 675 rpm for 3 h at 45 °C. At the end of the cleavage reaction the heat-cool module was replaced carefully with the 96 vial cleavage block. The resins were filtered under vacuum, washed with EtOAc (2×4 mL) and CH_3OH (1×1.5 mL), then filtered again. Each filtrate solution was transferred into a 15 mL preweighted tube. The tubes were placed in the Speed-vac apparatus and the solvents were evaporated under reduced pressure at 40 °C. Products were then resolubilized in CH_2Cl_2 and washed with water (2×). Water was removed with a Pasteur pipette and the organic layers evaporated to dryness in a Speed-vac apparatus at 40 °C and additionally dried for 48 h under vacuum pump. The phenol derivatives **61**–**108** (Table 2) were obtained in yields ranging from 13 to 49% and a high average purity of 91% was determined by HPLC analysis of 8 members randomly selected from the library.

63: White-yellow gum; IR (KBr): $\tilde{\nu}$ = 3318 (OH and NH), 1648 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.76 (s, 18-CH_3), 0.81 (t, J = 7.4 Hz, CH_2CH_3), 0.90–2.30 (20H), 2.76 (m, 6-CH_2), 2.87 (dd, J^1 = 8.8 Hz, J^2 = 13.6 Hz, 1H of CH_2Ph), 3.09 (m, CH_2N and 1H of CH_2Ph), 3.69 (d, J = 9.6 Hz, 17α-H), 4.57 (dd, J^1 = 6.5 Hz, J^2 = 8.7 Hz, COCH), 6.47 (d, J = 2.6 Hz, 4-CH), 6.53 (dd, J^1 = 2.6 Hz, J^2 = 8.4 Hz, 2-CH), 7.07 (d, J = 8.4 Hz, 1-CH), 7.24 ppm (m, CH_2*Ph*); LRMS for $C_{34}H_{47}N_2O_4$ [MH^+]: 547.7 m/z; HPLC purity = 90.9% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

69: White-yellow gum; IR (KBr): $\tilde{\nu}$ = 3397 and 3298 (OH and NH), 1654 and 1630 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.77 (s, 18-CH_3), 0.95 (t, J = 7.4 Hz, CH_2CH_3), 0.90–2.30 (20H), 2.76 (m, 6-CH_2), 3.20 (t, broad, CH_2N), 3.70 (d, J = 9.7 Hz, 17α-H), 3.80 (s, CH_2CO), 6.47 (d, J = 2.6 Hz, 4-CH), 6.52 (dd, J^1 = 2.6 Hz, J^2 = 8.5 Hz, 2-CH), 7.07 ppm (d, J = 8.5 Hz, 1-CH); LRMS for $C_{27}H_{41}N_2O_4$ [MH^+]: 457.6 m/z; HPLC purity = 86.2% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

77: White foam; IR (KBr): $\tilde{\nu}$ = 3386 and 3304 (OH and NH), 1647 (C=O, amides), 1522 and 1345 cm^{-1} (NO_2); ^1H NMR (CD_3OD): δ = 0.75 (s, 18-CH_3), 0.90–2.30 (29H), 2.76 (m, 6-CH_2), 3.00 (dd, J^1 = 9.1 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 3.14 (t, J = 7.0 Hz, CH_2N), 3.22 (dd, J^1 = 6.3 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 3.67 (d, J = 9.6 Hz, 17α-H), 4.68 (dd, J^1 = 6.4 Hz, J^2 = 9.1 Hz, COCH), 6.47 (d, J = 2.6 Hz, 4-CH), 6.52 (dd, J^1 = 2.6 Hz, J^2 = 8.4 Hz, 2-CH), 7.06 (d, J = 8.4 Hz, 1-CH), 7.48 and 8.15 ppm (2d, J = 8.7 Hz, 2×2H, CH_2*Ph*-*p*-NO_2); LRMS for $C_{38}H_{52}N_3O_6$ [MH^+]: 646.6 m/z; HPLC purity = 91.5% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

82: White-yellow gum; IR (KBr): $\tilde{\nu}$ = 3306 (OH and NH), 1647 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.75 (s, 18-CH_3), 0.86 (t, J = 7.1 Hz, CH_2CH_3), 0.95–2.30 (24H), 2.76 (m, 6-CH_2), 2.86 (dd, J^1 = 8.9 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 3.12 (m, CH_2N and 1H of CH_2Ph), 3.68 (d, J = 9.7 Hz, 17α-H), 4.57 (dd, J^1 = 6.3 Hz, J^2 = 8.9 Hz, COCH), 6.47 (d, J = 2.6 Hz, 4-CH), 6.53 (dd, J^1 = 2.6 Hz, J^2 = 8.4 Hz, 2-CH), 7.06 (d, J = 8.4 Hz, 1-CH), 7.24 ppm (m, CH_2*Ph*); LRMS for $C_{36}H_{51}N_2O_4$ [MH^+]: 575.6 m/z; HPLC purity = 96.4% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

85: White-yellow gum; IR (KBr): $\tilde{\nu}$ = 3298 (OH and NH), 1648 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.74 (s, 18-CH_3), 0.90–2.30 (24H), 2.74 (m, 6-CH_2), 2.83 (dd, J^1 = 7.9 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 2.98 (dd, J^1 = 7.3 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 3.07 (m, CH_2N), 3.66 (d, J = 9.7 Hz, 17α-H), 4.49 (t_{app}, J = 7.6 Hz, COC*H*CH_2), 5.03 (s, OCH_2Ph), 6.46 (d, J = 2.6 Hz, 4-CH), 6.53 (dd, J^1 = 2.6 Hz, J^2 = 8.4 Hz, 2-CH), 6.91 and 7.14 (2d, J = 8.6 Hz, CH_2*Ph*O), 7.06 (d, J = 8.4 Hz, 1-CH), 7.34 ppm (m, OCH_2*Ph*); LRMS for $C_{43}H_{57}N_2O_5$ [MH^+]: 625.7 m/z; HPLC purity = 94.1% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

92: White foam; IR (KBr): $\tilde{\nu}$ = 3422 (OH and NH), 1647 and 1624 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.77 (s, 18-CH_3), 0.95–2.30 (16H), 2.77 (m, 6-CH_2 and $CH(CH_3)_2$), 3.18 (m, CH_2N), 3.64 (m, CH_2N-proline), 3.69 (d, J = 9.6 Hz, 17α-CH), 4.42 (m, CHN-proline), 6.47 (d, J = 2.6 Hz, 4-CH), 6.53 (dd, J^1 = 2.6 Hz, J^2 = 8.4 Hz, 2-CH), 7.07 ppm (d, J = 8.4 Hz, 1-CH); LRMS for $C_{30}H_{45}N_2O_4$ [MH^+]: 497.5 m/z; HPLC purity = 88.0% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

100: White solid; IR (KBr): $\tilde{\nu}$ = 3306 (OH and NH), 1647 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.76 (s, 18-CH_3), 0.90 (t, J = 7.1 Hz, CH_2CH_3), 0.92 and 0.95 (2d, J = 6.3 Hz, $CH(CH_3)_2$), 0.95–2.30 (27H), 2.76 (m, 6-CH_2), 3.18 (t, J = 6.9 Hz, CH_2N), 3.69 (d, J = 9.7 Hz, 17α-CH), 4.35 (dd, J^1 = 6.3 Hz, J^2 = 8.7 Hz, COCH), 6.47 (d, J = 2.6 Hz, 4-CH), 6.53 (dd, J^1 = 2.6 Hz, J^2 = 8.4 Hz, 2-CH), 7.06 ppm (d, J = 8.4 Hz, 1-CH); LRMS for $C_{33}H_{53}N_2O_4$ [MH^+]: 541.7 m/z; HPLC purity = 89.6% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

108: White solid; IR (KBr): $\tilde{\nu}$ = 3397 and 3315 (OH and NH), 1648 cm^{-1} (C=O, amides); ^1H NMR (CD_3OD): δ = 0.75 (s, 18-CH_3) 0.90–2.40 (16H), 2.76 (m, 6-CH_2), 2.86 (dd, J^1 = 8.2 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 3.02 (dd, J^1 = 6.7 Hz, J^2 = 13.7 Hz, 1H of CH_2Ph), 3.12 (m, CH_2N), 3.49 and 3.52 (2d, J = 14.4 Hz, COC*H*$_2$Ph), 3.69 (d, J = 9.7 Hz, 17α-CH), 4.52 (dd, J^1 = 6.8 Hz, J^2 = 8.1 Hz, COCH), 6.47 (d, J = 2.6 Hz, 4-CH), 6.53 (dd, J^1 = 2.6 Hz and J^2 = 8.4 Hz, 2-CH), 6.86 and 7.08 (2d, J = 8.4 Hz, 2×2H of O*Ph*CH_2), 7.07 (d, J = 8.4 Hz, 1-CH), 7.20 ppm (m, $COCH_2$*Ph*); LRMS for $C_{42}H_{55}N_2O_5$ [MH^+]: 667.5 m/z; HPLC purity = 90.0% (C-18 NovaPak column, 50% of CH_3OH: H_2O (90: 10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

Steroid sulfatase assay: This enzymatic assay was carried out according to a procedure previously described for the transformation of natural substrate [^3H]-E_1S (100 μM) into [^3H]-E_1 by homogenated HEK-293 cells overexpressing the enzyme activity.[51]

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Type 1 17β-HSD assay: This enzymatic assay was carried out according to a procedure previously described for the transformation of natural substrate [^{14}C]-E_1 (0.1 μM) into [^{14}C]-E_2 by homogenated HEK-293 cells overexpressing the enzyme activity.[52]

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