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J. Comb. Chem., **2003**, 5 (4), 429-440• DOI: 10.1021/cc020115u • Publication Date (Web): 29 April 2003

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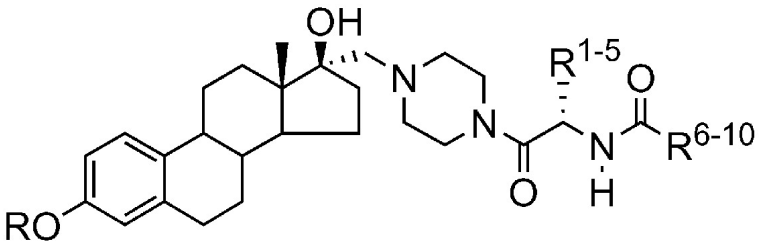

R = H or SO_2NH_2 (two model libraries)

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Solid-Phase Parallel Synthesis of 17α-Substituted Estradiol Sulfamate and Phenol Libraries Using the Multidetachable Sulfamate Linker

Liviu C. Ciobanu and Donald Poirier*

Medicinal Chemistry Division, Oncology and Molecular Endocrinology Research Center, Centre Hospitalier Universitaire de Québec (CHUQ), Pavillon CHUL, Sainte-Foy (Québec), G1V 4G2, Canada

Received December 12, 2002

We report an application of the multidetachable sulfamate linker in the synthesis of two model libraries of N-derivatized 17α-piperazinomethyl estradiols (phenols and sulfamates) by solid-phase parallel chemistry. The solid-phase precursor, a 3-sulfamoyl-17α-(*N*-trifluoroacetyl-piperazinomethyl) estradiol, was synthesized in solution from estrone and loaded efficiently onto trityl chloride resin as polymeric support. After cleavage of the trifluoroacetyl protecting group, sequential acylation reactions with five Fmoc-protected amino acids and five carboxylic acids were performed to introduce two levels of molecular diversity. Finally, the resins were split into two parts, and acidic (5% trifluoroacetic acid in dichloromethane) and nucleophilic (piperazine in tetrahydrofuran) cleavages were used to generate libraries A (5 × 5 sulfamates) and B (5 × 5 phenols) members in overall yields of 18−66% and high HPLC purities (87−96%) without purification steps. A preliminary screening test for inhibition of steroid sulfatase showed that the phenols were clearly weaker inhibitors, as compared to their sulfamate analogues. The most potent inhibitors were those with suitable hydrophobic amino acid and carboxylic acid substituents. Thus, compounds with a phenylalanine residue as the first element of diversity inhibited over 90% of steroid sulfatase activity at a concentration of 1 nM in homogenates of HEK-293 transfected cells, being as potent as the leading inhibitor 17α-*tert*-butylbenzyl estradiol 3-*O*-sulfamate previously reported. These results suggest that the steroid sulfatase inhibitory potency of estradiol derivatives, sulfamoylated or not, can be increased by the hydrophobic effect of a suitable substituent introduced in the proximity of the D ring of the steroid. The present work also demonstrated the efficiency and the cleavage versatility of the sulfamate linker to generate libraries of compounds with relevant biological importance, phenols and sulfamates.

Introduction

Solid-phase combinatorial chemistry is a valuable tool in the development and optimization of compounds with relevant biological applications in different fields of medicine.[1−6] Although the elaboration of steroid libraries should allow screening of a great variety of compounds and possibly facilitate the discovery of potential drug candidates, few examples of synthetic methodologies have been developed for the solid-phase chemistry of steroids,[6−8] a family of compounds with notable therapeutic importance. In the past several years, part of the work of our group has been focused on the development of a new linker and optimization of solid-phase coupling conditions for different hydroxy and keto steroids from estrane and androstane series.[7−10] Our aim was to apply in solid-phase a series of synthetic sequences of reactions for the generation of combinatorial libraries of potential inhibitors of key steroidogenic enzymes and receptor antagonists.[8,11]

During the work toward the development of steroid sulfatase inhibitors as therapeutic agents for estrogen-sensitive cancers, the sulfamate group came into attention as a good pharmacophore. Thus, estrone 3-*O*-sulfamate as well as other steroidal and nonsteroidal sulfamate derivatives were reported as very potent inactivators of the enzyme steroid sulfatase.[12,13] Further on, we demonstrated that the combination of a sulfamate group at C3 and a hydrophobic substituent at C17 within the same steroidal molecule resulted in significant improvement of steroid sulfatase inhibition when compared to that of compounds using only one of these two substituents.[14] Sulfamate derivatives from alcohols and phenols were also known to display interesting antitumoral,[15] cytotoxic,[16] and anticonvulsive[17] properties and recently, learning and spatial memory enhancement.[18] Thus, we were interested in the attachment of sulfamate derivatives on polymeric support that should open possibilities to synthesize a large number of such compounds by means of combinatorial chemistry. Since the triphenylmethyl (trityl) group was previously used in solution to protect sulfamates of primary alcohols,[19] we have chosen the trityl chloride resin to explore the coupling of sulfamate compounds on polymeric support and their convenient cleavage. We have then shown that the sulfamate group can be used as an anchoring group or a linker for the solid-phase synthesis of two families of compounds, sulfamates and phenols.[20,21] Following these preliminary results, we now report the full details of a direct

* To whom correspondence should be addressed. Phone: (418) 654-2296. Fax: (418) 654-2761. E-mail: donald.poirier@crchul.ulaval.ca.

10.1021/cc020115u CCC: $25.00 © 2003 American Chemical Society
Published on Web 04/29/2003

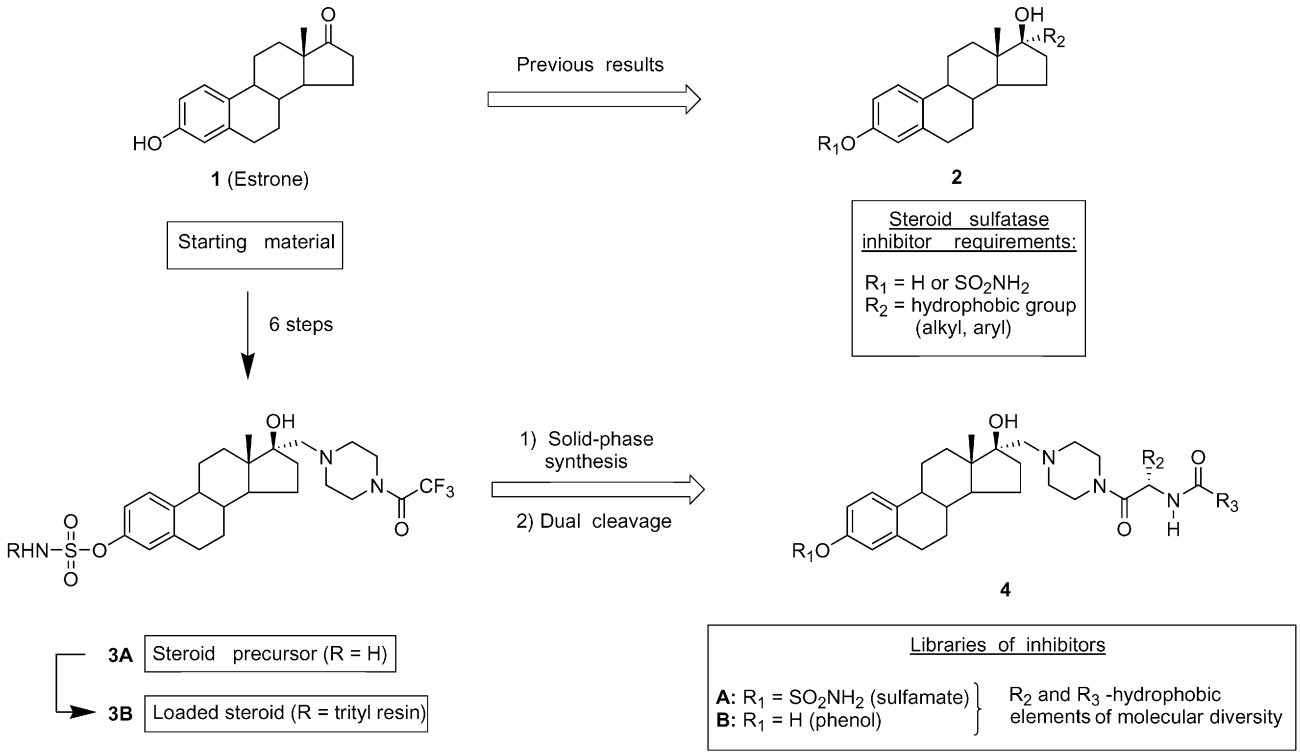
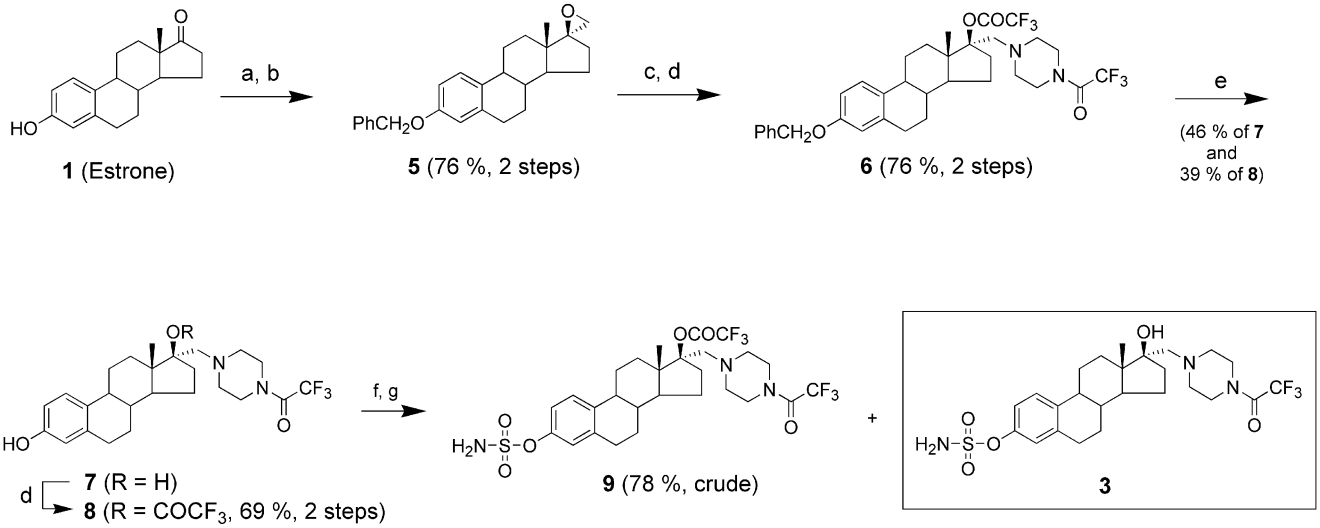


Figure 1. Two libraries of 17α-substituted estradiols, sulfamates **4A** and phenols **4B**, targeted for the optimization of previously reported steroid sulfatase inhibitors.

Scheme 1. Synthesis of the Solid-Phase Precursor **3**[a]



[a] (a) $PhCH_2Br$, Cs_2CO_3, CH_3CN reflux; (b) NaH, $Me_3S^+I^-$, DMSO; (c) piperazine, EtOH, 55−60°C; (d) DMAP, $(CF_3CO)_2O$, CH_2Cl_2, 0 °C; (e) 20% $Pd(OH)_2/C$, H_2, MeOH/EtOAc (3:1, v/v); (f) H_2NSO_2Cl, DBMP, CH_2Cl_2; (g) silica gel flash chromatography, hexanes/acetone (70:30).

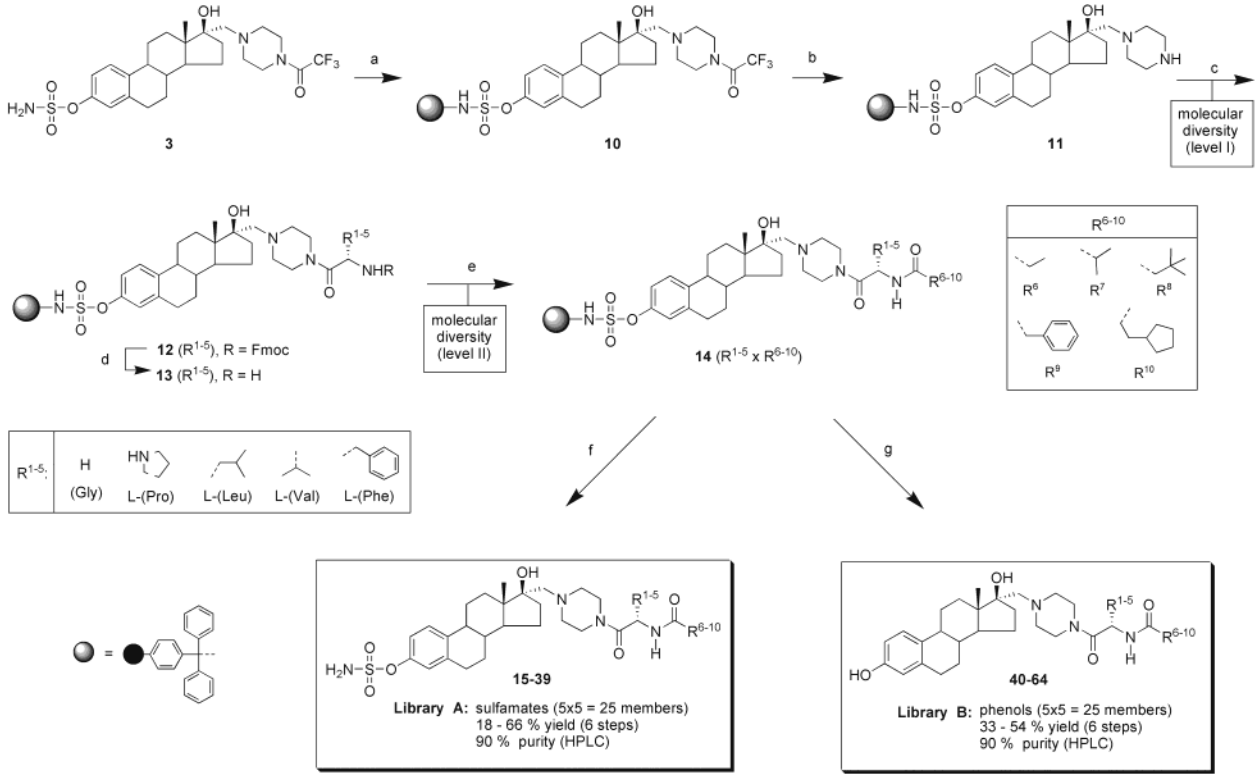
application of this new linking methodology by presenting the synthesis of two model libraries of N-derivatized 17α-piperazinomethyl estradiols as phenol and sulfamate compounds (Figure 1).

Results and Discussion

For the purpose of developing new inhibitors of steroid sulfatase, we explored the inhibitory effect of several alkyl, alkylamide, and aryl substituents introduced at position 17α of estradiol[22,23] or estradiol 3-*O*-sulfamates.[14] Since a screening test indicated that a 17α-piperazinomethyl estradiol 3-*O*-sulfamate was a promising scaffold to design steroid sulfatase inhibitors, we next synthesized libraries of N-substituted analogues using the sulfamate linker and solid-phase parallel chemistry. This first required the solution-phase synthesis of steroid **3** as our key intermediate for the solid-phase synthesis of libraries A and B.

1. Synthesis of the Solid-Phase Precursor 3 in Solution (Scheme 1). Our sequence of reactions for the synthesis of **3** started from estrone (**1**), which was first protected as the corresponding 3-*O*-benzyl ether. The protected estrone was next treated with dimethylsulfonium methylide, generated from trimethylsulfonium iodide and sodium hydride,[24] to provide the oxirane **5**. Aminolysis of the oxirane with an excess of piperazine in ethanol at 45−50 °C gave the free secondary amine and tertiary alcohol, which were both

Scheme 2. Solid-Phase Synthesis of Sulfamates **15**−**39** and Phenols **40**−**64** (Libraries A and B)[a]



[a]Reagents and conditions are: (a) DIPEA (6 equiv), CH_2Cl_2, rt, 12 h; (b) NaOH 3N (e equiv), THF, rt, 3 h; (c) PyBrOP, HOBt, $R^{1-5}CH(NHFmoc)COOH$ (e equiv), DIPEA (4 equiv), DMF, rt, 3 h; (d) 20% piperidine/CH_2Cl_2, rt, 1 h; (e) pyBOP (e equiv), $R^{6-10}COOH$ (3 equiv), DIPEA (6 equiv), DMF, rt, 3 h; (f) 5% TFA/CH_2Cl_2, rt, 4 h; (g) piperazine (10 equiv), THF, 45−50 °C, 3 h.

protected as trifluoroacetyl derivative **6**. Initially, the NH protection as a trifluoroacetamide by treatment with trifluoroacetic anhydride and 4-(dimethylamino)pyridine (DMAP) was accompanied by partial esterification of the C17β-alcohol. Further on, we found that prior protection of both the secondary amine and the C17β-alcohol was required to succeed sulfamoylation of phenol at C3. Otherwise, in the presence of the free C17β-tertiary alcohol, the sulfamoylation mainly gave side products. The use of a Fmoc protection strategy was inconvenient, since it led only to the amine protection product. Finally, an excess of trifluoroacetic anhydride gave the desired compound **6** as the major product. The release of phenol by hydrogenolysis of the benzyl ether **6** was, however, accompanied by partial hydrolysis of the C17-ester, requiring an additional step for reprotection of the mixture of compounds **7** and **8**. Thus, the phenol **8** was obtained in 69% overall yield from **6**. Treatment of **8** with sulfamoyl chloride[16] in the presence of nonnucleophilic base 2,6-di-*tert*-butyl-4-methylpyridine (DBMP)[25] gave the sulfamate **9** in 78% crude yield. Although the coupling assays on trityl chloride resin and model sequences of reactions with the sulfamate **9** worked well, the more stable compound **3** that resulted upon partial hydrolysis of the C17-ester during purification on silica gel column was then preferred for use in the solid-phase sequence of reactions.

2. Solid-Phase Synthesis of Libraries A and B (Scheme 2). Two model libraries of 25 phenols and 25 corresponding sulfamates were synthesized by solid-phase parallel chemistry starting with the steroid **3** loaded to trityl chloride resin. In the coupling reaction, the resin was swelled in dry dichloromethane and treated with diisopropylamine and steroid sulfamate **3**. The coupling yields of **10**, calculated either by the increase of the resin weight or by the difference between the initial amount of steroid and the amount of uncoupled steroid, were in the range of 75%. Next, the dried resin **10** was split into five equal portions, and each was treated with a solution of 3 N NaOH in THF to release the free secondary amine **11**. The hydrolysis of trifluoroacetamide was confirmed by the disappearance of the amide band in the IR spectra. Each of the five resins **11** was then split again into five equal portions, which were transferred into 25 fritted polystyrene columns equipped with a three-way stopcock. The introduction of the first level of molecular diversity ($\mathbf{R^{1-5}}$) was performed by an acylation of each group of five resins with one of five chosen Fmoc-protected amino acids. After the usual filtration workup, formation of the corresponding amides was confirmed by IR analysis of the resins **12**. Furthermore, a mini cleavage test easily provided step-by-step information about the course of the solid-phase reactions performed. After removal of the Fmoc protecting group with a solution of 20% piperidine in dichloromethane, the second level of molecular diversity ($\mathbf{R^{6-10}}$) was introduced to resins **13** by an amidation step using five chosen carboxylic acids. At the end of this second acylation step, each of the 25 resins **14** was split in two equal portions for the release of the free sulfamate and phenolic steroids by two different methods of cleavage. Consequently, a first group of resins **14** was treated with a solution of 5% TFA in dichloromethane to obtain the library A (sulfamates **15**−**39**) after filtration and evaporation in a Speedvac apparatus.

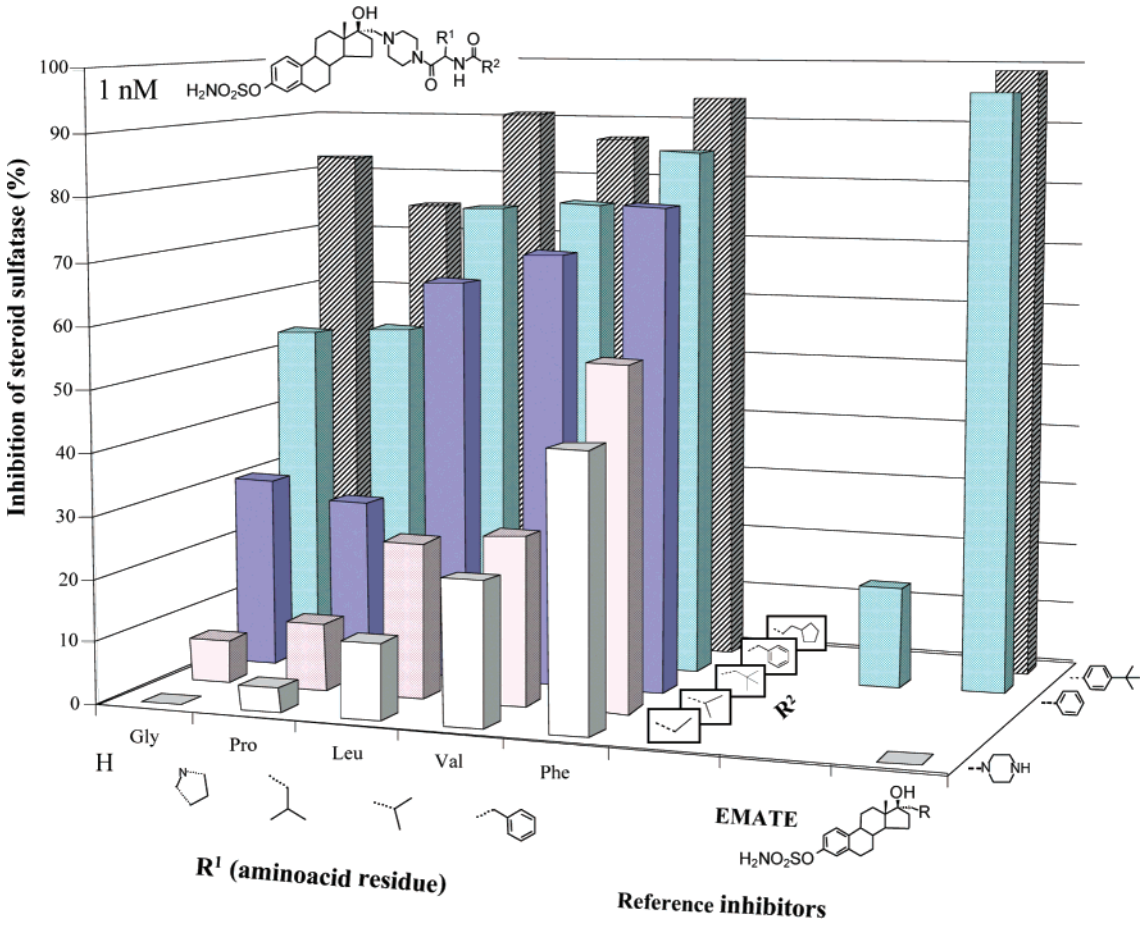


Figure 2. Inhibition of steroid sulfatase activity transfected in HEK-293 cells by sulfamates **15**−**39** of library A and reference inhibitors. Compounds were tested once at a concentration of 1 nM in homogenized cells, transforming [^{3}H]E_1S (100 μM) into [^{3}H]E_1. Experimental error ±5%. Additional data are reported in the Supporting Information.

The second group of resins **14** was transferred to small vials and reacted with 10 equiv of piperazine in THF at 45 °C to obtain the library B (phenols **40**−**64**). In this case, two additional steps, extraction in EtOAc and washing of the organic phase with water, respectivley, were required to remove the excess of piperazine prior to final evaporation of solvent.

3. Characterization of Libraries A and B. The sulfamate (A) and phenol (B) libraries were submitted to a random sampling, and the members were characterized by IR, ^{1}H NMR, and HRMS analysis. In addition, the purity of these members was determined by HPLC analysis. Expected masses were confirmed for all compounds, except for the fact that the sulfamate derivatives were obtained as TFA salts of the tertiary amine from the piperazinomethyl unit. The average yields for the solid-phase reaction sequences (6 steps) were 49 and 45% for the libraries A and B, respectively. The HPLC purity of the final compounds ranged between 87 and 96%, whereas the average purity was 91% in the sulfamate series and 90% in the phenol series (Tables 1 and 2). No purification was required at the end of the sequences, except for the extraction and washing steps needed in the case of phenols.

4. Inhibition of Steroid Sulfatase by Compounds from Libraries A and B. Compounds from libraries A and B, sulfamates and phenols, and reference inhibitors were tested for inhibitory activity in homogenates of HEK-293 cells transfected with steroid sulfatase according to a previously reported procedure.[14] Since we expected that sulfamate derivatives would be more potent inhibitors than their phenolic analogues, compounds were tested at concentrations in the range of 1 nM to 1 μM. For the sulfamate library (A), the rates of inhibition were high, and a distinction between the inhibitory potency of compounds was possible only based on the results from the test at 1 nM (Figure 2). These results indicated that phenylalanine and 3-cyclopentyl propionic acid residues were the best diversity elements for providing high inhibition of steroid sulfatase. Thus, compound **39**, which incorporated in its structure these two diversity elements, was the best inhibitor from the library A, with 94% inhibition of steroid sulfatase activity at 1 nM. This value is close to that obtained with 17α-*tert*-butylbenzyl estradiol 3-*O*-sulfamate (99%) and the benzyl analogue (96%), the most potent inhibitors of steroid sulfatase yet reported, and higher than that of the reference inhibitor estrone 3-*O*-sulfamate (16%). For comparison, the 17α-piperazinomethyl estradiol 3-*O*-sulfamate scaffold (without the N substituent) was a good inhibitor at concentrations of 100 and 10 nM (98 and 50%, respectively; unreported data), but no inhibition resulted at 1 nM. On the other hand, phenols from library B inhibited steroid sulfatase activity only at a concentration of 1 μM (Figure 3). Interestingly, compounds incorporating in their structure phenylalanine or 3-cyclopentyl propionic acid residues as first and second level of diversity elements, were

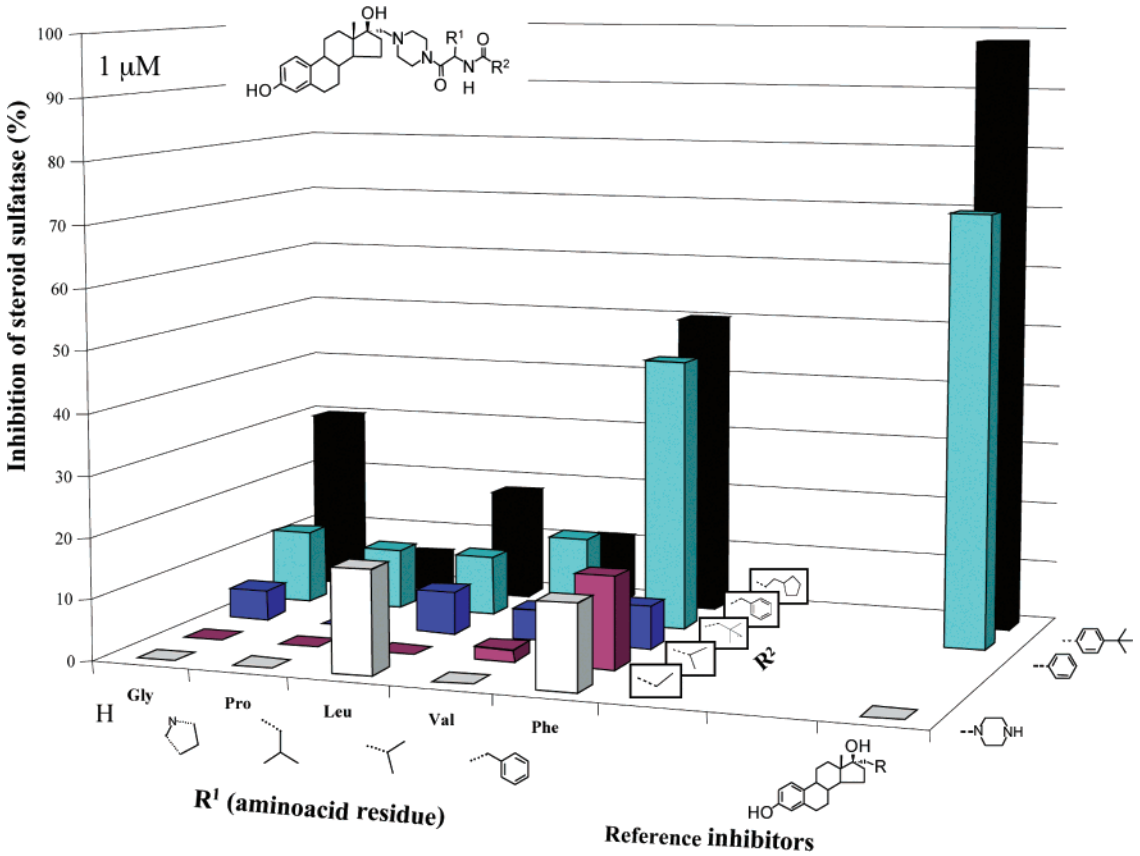


Figure 3. Inhibition of steroid sulfatase activity transfected in HEK-293 cells by phenols **40**−**64** of library B and reference inhibitors. Compounds were tested once at a concentration of 1 μM in homogenized cells, transforming [^{3}H]E_1S (100 μM) into [^{3}H]E_1. Experimental error ±5%. Additional data are provided in Supporting Information.

the best inhibitors in the phenol library. Correspondingly, the 3-hydroxy analogue of the best inhibitor from library A, compound **64**, was the most potent inhibitor from phenol library B, inhibiting 50% of the enzyme activity at 1 μM. In the same test, our previously reported 17α-benzyl- and 17α-*tert*-butylbenzyl estradiol inhibited 71 and 98% of steroid sulfatase activity, respectively.

Conclusion

We have successfully used the sulfamate linker for the attachment of a C18-steroidal compound to trityl chloride resin and to synthesize two libraries of N-derivatized 17α-piperazinomethyl estradiols as sulfamates (A) and phenols (B). The key intermediate to be loaded onto solid support was synthesized from estrone in a sequence of reactions (eight steps) in solution (15% yield). After its efficient coupling to trityl chloride resin, two levels of molecular diversity were introduced by subsequent acylation reactions with five Fmoc-protected amino acids and five carboxylic acids, using the parallel chemistry. Each of the resins was then split into two parts, and the desired compounds, sulfamates and phenols, were released by acidic treatment with 5% TFA in dichloromethane and by hydrolysis with an excess of piperazine in THF at 45 °C, respectively. The purity of compounds from both libraries was very good, exceeding 90% as determined by HPLC.

Within the members of the sulfamate and phenol libraries synthesized, those bearing a suitable hydrophobic substituents on the piperazinomethyl moiety are potent inhibitors of steroid sulfatase, an enzyme that plays a major role in intracrine synthesis of androgens and estrogens in prostate and breast tumors, respectively. Thus, in the sulfamate library, compounds from the 3-cyclopentylpropionyl series showed an inhibitory potency similar to the known inhibitor 17α-*tert*-butylbenzyl (or 17α-benzyl) estradiol 3-*O*-sulfamate. Furthermore, most of the library A compounds were more potent inhibitors than EMATE (without the 17α substituent). These results agree with the previous findings about the steroid sulfatase inhibitory effect of hydrophobic substituents introduced at position 17α of estradiol,[14,22,23] or surrounding position 17 of other steroidal nuclei,[26−28] but other factors, such as bulkiness of substituents, may also be involved. This work also illustrated the efficiency and versatility of the sulfamate linker for synthesizing compounds with relevant biological importance, such as sulfamate and phenol derivatives.

Experimental Section

General Methods. Estrone was purchased from Steraloids (Wilton, NH). Trityl chloride resin (200 mesh, 2.05 mmol/g theoretical loading), coupling reagents and Fmoc amino acids were supplied by Novabiochem (San Diego, CA). Other reagents and anhydrous solvents, such as methyl sulfoxide (DMSO), dichloromethane (CH_2Cl_2), and dimethylformamide (DMF) were obtained from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Fisher Scientific (Montréal, QC, Canada) provided the usual solvents. Prior to its use, tetrahydrofuran (THF) was distilled from sodium/benzophen-

one 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) or polystyrene PD-10 columns (Amersham Pharmacia Biotech AB; Uppsala, Sweden) coupled with a three-way stopcock (Bio-Rad Laboratories; Hercules, CA) were used for the solid-phase reactions on a Burrell wrist-action shaker model 75 (Burrell, Pittsburg, PA). 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 5% solution TFA in CH_2Cl_2 were used 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). Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC/F300 spectrometer (Billerica, MA) at 300 MHz (^{1}H) or 75 MHz (^{13}C), 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) equipped with a turbo ionspray source. The purity of compounds was determined by HPLC (Waters Associates, Milford, MA) using an ultraviolet detector (205−215 nm).

3-Benzyloxy-spiro-17(*S*)-oxirane-1,3,5(10)-estratriene (5). To a solution of estrone (**1**) (2.7 g; 10 mmol) in dry THF under argon were added successively cesium carbonate (4.88 g; 15 mmol) and benzyl bromide (3 mL; 25 mmol). After 3 h at reflux, the mixture was cooled to room temperature and filtered over a pad of silica gel, and the solvent was evaporated under reduced pressure. The benzyl ether of estrone was then used without purification in the next step. To a solution of trimethylsulfonium iodide (12.24 g; 60 mmol) in dry DMSO (600 mL) was added cautiously sodium hydride 60% in mineral oil (2.4 g; 60 mmol). After stirring for 2 h at room temperature, a solution of the crude benzyl ether in dry THF was added slowly, and the reaction was allowed to proceed at room temperature for 4 h. The mixture was cooled to 0 °C, cold water was added (500 mL), and the crude product was extracted with diethyl ether. The combined organic layer was washed with water (3 × 500 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography with hexanes/acetone/Et_3N (89:10:1) yielded 2.88 g (76%) of oxirane **5**. White solid; IR υ (film) no OH and C═O bands; ^{1}H NMR δ ($CDCl_3$) 0.93 (s, 3H, 18-CH_3), 1.26−2.27 (16H), 2.66 and 2.98 (2d of AB system, J = 5.0 Hz, CH_2 of oxirane), 2.87 (m, 2H, 6-CH_2), 5.04 (s, 2H, −OC*H*$_2$Ph), 6.74 (d, J = 2.2 Hz, 1H, 4-CH), 6.79 (dd, J_1 = 2.5 Hz and J_2 = 8.5 Hz, 1H, 2-CH), 7.21 (d, J = 8.4 Hz, 1H, 1-CH), 7.39 (m, 5H, OCH$_2$*Ph*); ^{13}C NMR δ ($CDCl_3$) 14.11, 22.69, 23.26, 25.99, 27.14, 29.07, 33.92, 38.88, 40.39, 43.87, 51.80, 53.63, 69.91, 70.50, 112.27, 114.82, 126.27, 127.40 (2×), 127.82, 128.51 (2×), 132.68, 137.28, 137.94, 156.75; LRMS for $C_{26}H_{31}O_2$ [MH^+] 375.2 *m*/*z*.

3-Benzyloxy-17α-(*N*-trifluoroacetylpiperazinomethyl-17*β*-trifluoroacetyloxy-1,3,5(10)-estratriene (6). In a 100-mL flask was solubilized the oxirane **5** (2.88 g; 7.7 mmol) in ethanol (50 mL). To the stirred solution was added piperazine (13.26 g; 154 mmol), and the reaction mixture was heated in an oil bath at reflux for 48 h. The bath was removed, and the reaction flask was allowed to come to room temperature. The mixture was then poured into water and extracted with EtOAc. The combined organic layer was intensively washed with water (5 × 400 mL) to remove the large excess of piperazine, then it was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The secondary amine was used without purification for the next step. To a solution of the amine (2.26 g; crude) and 4-(dimethyl amino)pyridine (DMAP, 2.26 g; 18.48 mmol) in dry CH_2Cl_2 (250 mL) at 0 °C was added trifluoroacetic anhydride (2.4 mL; 17 mmol), and the reaction mixture was stirred at room temperature for 5 h. After addition of water, the crude compound was extracted with CH_2Cl_2 (3×), and the organic layer dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the crude product by flash chromatography with hexanes/acetone (85:15) yielded 3.8 g of trifluoroacetate **6** (76%, two steps). White foam; IR υ (film) 1774 (C═O, ester), 1696 (C═O, amide); ^{1}H NMR δ ($CDCl_3$) 0.91 (s, 3H, 18-CH_3), 1.20−2.65 (18H), 2.87 (m, 2H, 6-CH_2), 3.41(d, J = 14.1 Hz, 1H of AB system of 17α-CH_2N), 3.57 and 3.66 (4H, $(CH_2)_2NCOCF_3$), 5.04 (s, 2H, OC*H*$_2$Ph), 6.73 (d, J = 2.4 Hz, 1H, 4-CH), 6.79 (dd, J_1 = 2.5 Hz and J_2 = 8.6 Hz, 1H, 2-CH), 7.19 (d, J = 8.6 Hz, 1H, 1-CH), 7.38 (m, 5H, OCH$_2$*Ph*); ^{13}C NMR δ ($CDCl_3$) 14.38, 23.45, 26.27, 27.40, 29.64, 32.98, 34.66, 39.36, 43.21, 43.35, 45.66, 48.14, 50.62, 53.30, 53.74, 58.65, 69.92, 98.59, 112.36, 114.82, 112.80, 114.48, 116.60 and 118.50 (four very weak signals of 2 × *C*F_3COO), 126.27, 127.39 (2×), 127.84, 128.51 (2×), 132.18, 137.21, 137.68, 155.20, 155.58, 156.16 and 156.80 (four very weak signals of 2 × CF_3*C*OO), 156.85; LRMS for $C_{34}H_{39}F_6N_2O_4$ [MH^+] 653.3 *m*/*z*.

3-Hydroxy-17α-(*N*-trifluoroacetylpiperazinomethyl)-17*β*-trifluoroacetyloxy-1,3,5(10)-estratriene (8). A solution of benzyl ether **6** (3.8 g; 5.82 mmol) and palladium hydroxide (20% w/w on activated carbon) (760 mg) in MeOH/EtOAc (3:1, v/v) was stirred under an atmospheric pressure of hydrogen for 8 h. After removal of the hydrogen source, the mixture was filtered over Celite, and the solvents were evaporated under reduced pressure. Purification by flash chromatography with hexanes/acetone (85:15) gave phenolic compounds **7** (1.25 g, 46% yield) and **8** (1.27 g, 39% yield). The former was reacted at 0 °C with trifluoroacetic anhydride (0.8 mL; 5 mmol) in the presence of DMAP (785 mg; 5.44 mmol) following the same procedure described above for the synthesis of compound **6**. Purification of the crude product by flash chromatography yielded an additional 935 mg of the phenolic compound **8**. White foam; IR υ (KBr) 3478 (OH), 1773 (C═O, ester), 1686 (C═O, amide); ^{1}H NMR δ ($CDCl_3$) 0.90 (s, 3H, 18-CH_3), 1.20−2.65 (18H), 2.82 (m, 2H, 6-CH_2), 3.41(d, J = 14.1 Hz, 1H of AB system of 17α-CH_2N), 3.57 and 3.66 (2m, 4H, $(CH_2)_2NCOCF_3$), 5.00 (m, OH), 6.57 (d, J = 2.3 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.2 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.13 (d, J = 8.4 Hz,

1H, 1-CH); ^{13}C NMR δ ($CDCl_3$) 14.39, 23.46, 26.27, 27.33, 29.46, 32.98, 34.69, 39.32, 43.28 (2×), 45.70, 48.13, 50.59, 53.24, 53.69, 58.61, 98.56, 112.77, 115.21, 112.70, 114.45, 116.58 and 118.25 (four very weak signals of 2 × *C*F_3COO), 126.46, 131.86, 137.91, 153.52, 155.14, 155.63, 156.17 and 156.72 (four very weak signals of 2 × CF_3*C*OO); LRMS for $C_{27}H_{33}F_6N_2O_4$ [MH^+] 563.2 *m*/*z*.

3-Sulfamoyloxy-17α-(*N*-trifluoroacetylpiperazinomethyl or piperazinomethyl)-17*β*-hydroxy-1,3,5(10)-estratriene (9 or 3). To a solution of phenol **8** (2.1 g; 3.73 mmol) in dry CH_2Cl_2 (300 mL) under argon were added successively 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) (2.3 g; 11.2 mmol) and sulfamoyl chloride[16] (2.6 g; 22.5 mmol). After 1 h at room temperature, the reaction was quenched with water (200 mL), and the crude product was extracted with CH_2Cl_2. The combined organic layer was washed with water (3×), dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography with hexanes/acetone (78:22) yielded 1.93 g (78%) of sulfamate **9**. During additional purification of **9** by flash chromatography on a silica gel column, the C17*β*-ester was partially hydrolyzed, with the formation of the corresponding 17*β*-alcohol **3**.

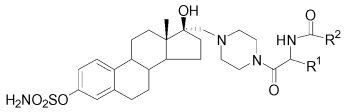
9. White foam; IR *υ* (KBr) 3381 (NH_2), 1778 (C═O, ester), 1690 (C═O, amide), 1383 and 1187 (S═O, sulfamate); ^{1}H NMR δ ($CDCl_3$) 0.90 (s, 3H, 18-CH_3), 1.20−2.65 (18H), 2.88 (m, 2H, 6-CH_2), 3.40 (d, J = 14.0 Hz, 1H of AB system of 17α-CH_2N), 3.56 and 3.65 (4H, $(CH_2)_2$-$NCOCF_3$), 4.92 (s, 2H, OSO_2NH_2), 7.04 (d, J = 2.2 Hz, 1H, 4-CH), 7.08 (d, J = 8.5 Hz, 1H, 2-CH), 7.30 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR δ (acetone-d_6) 14.81, 23.96, 26.94, 27.81, 29.58, 33.53, 34.82, 39.97, 43.90, 44.17, 46.41, 48.80, 50.76, 54.09, 54.66, 59.07, 63.45, 100.44, 120.19, 122.99, 127.36, 139.05, 139.20, 149.37 (the very weak signals of CF_3COO were not listed, because they were partially lost in background); LRMS for $C_{27}H_{34}F_6N_3O_6S$ [MH^+] 642.1 *m*/*z*.

3. White foam; IR *υ* (KBr) 3333 (OH and NH_2), 1684 (C═O, amide), 1376 and 1188 (S═O, sulfamate); ^{1}H NMR δ ($CDCl_3$) 0.92 (s, 3H, 18-CH_3), 1.20−2.40 (16H), 2.45 and 2.65 (2d, J = 13.4 Hz, 2H, AB-system of 17α-CH_2N), 2.73 (m, 4H, $CH_2N(CH_2)_2$), 2.88 (m, 2H, 6-CH_2), 3.62 and 3.71 (2m, 4H, $(CH_2)_2NCOCF_3$), 4.97 (s, 2H, OSO_2NH_2), 7.04 (d, J = 2.2 Hz, 1H, 4-CH), 7.08 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.30 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR δ (acetone-d_6) 14.81, 24.07, 26.97, 27.99, 30.17, 31.84, 34.25, 40.32, 44.23, 44.76, 46.74, 47.31, 50.36, 55.24, 55.83, 64.23, 83.73, 115.71 and 119.52 (two weak signals of *C*F_3-COO), 120.13, 122.95, 127.31, 139.22, 139.70, 149.34, 155.15 (very weak signal of CF_3*C*OO); LRMS for $C_{25}H_{35}F_3$-N_3O_5S [MH^+] 546.0 *m*/*z*.

Synthesis of Library Precursor Resin 11. Three coupling reactions were run in 25-mL peptide flasks equipped with three-way stopcocks using the same amount of resin and the following procedure. Trityl chloride resin (Novabiochem, 2.05 mmol/g theoretical loading) (1 g) was swollen under argon in dry CH_2Cl_2 (7 mL) and diisopropylethylamine (DIPEA) (3.10 mL). After 5 min, sulfamate **3** (1.34 g) was added as a solid in portions followed by an additional volume of dry CH_2Cl_2 (3 mL), and the mixture was shaken for 24 h at room temperature. The resin was filtered and washed three times with CH_2Cl_2, MeOH, and again with CH_2Cl_2, then dried overnight under vacuum to afford 1.95 g of resin **10**. IR (KBr) 1684 (C═O, amide), 1376 and 1188 (S═O, sulfamate) cm^{-1}. The coupling yield calculated by the means of the mass increase was 91%. The filtrate was collected and evaporated to dryness, then purified by flash chromatography on alumina with MeOH/CH_2Cl_2 (98:2) to isolate 345 mg of unreacted sulfamate **3**. The yield calculated by the recovered amount of compound **3** was 89%. The resin **10** was swollen in THF (10 mL), and aqueous 1.0 N NaOH solution (5.5 mL) was added slowly. The resin was shaken intensely at room temperature for 3 h, then filtered and washed with THF/H_2O (1:1, 2×), THF (2×) and CH_2Cl_2 (3×). After drying for 24 h, the resin **11** weighted 1.8 g. Acidic minicleavage (5% TFA/CH_2Cl_2, 4 h) of a sample from this resin and TLC analysis confirmed the complete deprotection of the secondary amine. Three batches of resin **11** were prepared as described above and mixed before the next step. Then, each of 25 reaction columns (PD-10) equipped with three-way stopcocks was loaded with 180 mg of resin **11**, which corresponded to 0.157 mmol of compound **3**. All reactions were run in parallel.

Introduction of Two Levels of Molecular Diversity. Five stock solutions, each containing 2 mmol of Fmoc-protected amino acid from *L* series (Gly, Pro, Leu, Val and Phe), bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBrOP) as activating reagent and *N*-hydroxybenzotriazole (HOBt) as additive, were prepared in DMF (7 mL) and reacted with DIPEA (0.7 mL; 4 mmol) prior to addition to the resin. Shortly after the addition of DIPEA, the five stock solutions were divided into five equal volumes (1.6 mL), which were added to each of the reaction columns (5 × 5) containing the resin **11** (180 mg) swollen in DMF (0.6 mL). The resins were shaken under argon for 2 h, then filtered, washed with DMF (3×), and CH_2Cl_2 (3×), and dried under vacuum to afford five groups of five different resins **12** (with the first level of molecular diversity). IR band at 1718 cm^{-1} confirmed the presence of a Fmoc group of amino acid reacted with the resin. In addition, TLC analysis after a mini cleavage test with samples of resins **12** (5% TFA/CH_2Cl_2, 3 h) confirmed the completion of the coupling reaction. The resins **12** were then reacted 1 h with 2.0 mL of a solution of piperidine in CH_2Cl_2 (20%) for the cleavage of Fmoc group. After filtration, washing with CH_2Cl_2 (5×) and drying, the resins **13** (5 × 5) with distinct amino acid diversity were reacted with the same volume from a solution of carboxylic acid (propionic, isobutyric, *tert*-butyl acetic, phenyl acetic, and 3-cyclopentyl propionic) (2.4 mmol) activated with benzotriazole-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) (1.25 g, 2.4 mmol) and DIPEA (0.85 mL) in DMF (7 mL). The resins (5 × 5) were shaken for 3 h at room temperature, then filtered and washed with DMF and CH_2Cl_2 (4×) to afford 25 different resins **14** (with the second level of molecular diversity). The completion of the acylation was proven by mini cleavage as described above. Each of the 25 resins **14** was weighed, then divided in two portions (3:2 w/w) to perform the acidic and nucleophilic cleavages.

Table 1. Characterization of Members from Sulfamate Library (A)



Compound	R^1	R^2	Overall yield (%)[a]	HPLC Purity (%)	LRMS [MH^+] (m/z)
15	H		42	--	--
16	H		32	90	577.6
17	H		45	--	--
18	H		56	92	625.6
19	H		37	--	--
20	$(CH_2)_3$ of Proline		60	94	603.6
21	$(CH_2)_3$ of Proline		30	--	--
22	$(CH_2)_3$ of Proline		64	--	--
23	$(CH_2)_3$ of Proline		58	96	665.5
24	$(CH_2)_3$ of Proline		58	--	--
25			53	--	--
26			51	93	633.7
27			56	--	--
28			66	--	--
29			18	93	687.5
30			53	93	605.5
31			58	--	--
32			53	91	647.6
33			60	--	--
34			30	--	--
35			45	--	--
36			66	--	--
37			59	94	695.6
38			52	--	--
39			41	95	721.6

[a] Yields calculated for the solid-phase sequence (6 steps).

Generation of Sulfamate Library A by Acidic Cleavage. To each of the 25 resins **14** (95−126 mg) in PS-10 reaction columns and under argon was added a solution of 5% TFA in CH_2Cl_2 (1.5 mL). The mixtures were shaken for 4 h at room temperature, then filtered, and the organic layer was collected in preweighed tubes. The solvent was evaporated in a Speedvac apparatus, and the products (in the form of TFA salts) were dried under vacuum pump. The sulfamate derivatives **15**−**39** (18−65 mg; 18−66% from **11**) (Table 1) were obtained in high-average HPLC purity (90%) according to a random sampling of ten library A members, compounds **16**, **18**, **20**, **23**, **26**, **29**, **30**, **32**, **37**, and **39**.

16 (TFA Salt). White foam; IR υ (KBr) 3406 (OH, NH and NH_2), 1671 (C═O, amides), 1374 and 1183 (S═O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.97 (s, 3H, 18-CH_3), 1.09 (d, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 1.20−2.45 (13H), 2.54 (sept, J = 7.0 Hz, 1H, COC*H*(CH_3)$_2$), 2.85 (m, 2H, 6-CH_2), 3.40−4.20 (broad, 12H, 5 × CH_2N and COC*H*$_2$NH), 7.04 (m, 4H, 4-CH, 2-CH and SO_2NH_2), 7.33 (d, J = 8.5 Hz, 1H, 1-CH); LRMS for $C_{29}H_{44}N_4O_6S$, 577.6 [MH]$^+$ and 689.3 [M + CF_3COO]$^-$; HPLC purity = 90% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

18 (TFA Salt). White foam; IR υ (KBr) 3398 (OH, NH and NH_2), 1671 (C═O, amides), 1375 and 1183 (S═O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.97 (s, 3H, 18-CH_3), 1.20−2.40 (13H), 2.85 (m, 2H, 6-CH_2), 3.40−4.20 (broad, 12H, 5 × CH_2N and COC*H*$_2$NH), 3.60 (s, 2H, C*H*$_2$Ph), 7.01 (s, 1H, 4-CH), 7.06 (m, 3H, 2-CH and SO_2NH_2), 7.18 (m, NH), 7.30 (m, 6H, CH_2*Ph* and 1-CH); LRMS for $C_{33}H_{44}N_4O_6S$, 625.6 [MH]$^+$ and 737.3 [M + CF_3COO]$^-$; HPLC purity = 92% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

20 (TFA Salt). White foam; IR υ (KBr) 3422 (OH, NH and NH_2), 1675 and 1630 (C═O, amides), 1376 and 1184 (S═O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.97 (s, 3H, 18-CH_3), 1.04 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.20−2.40 (19H), 2.88 (m, 2H, 6-CH_2), 3.20−4.40 (broad, 12H, 6 × CH_2N), 4.90 (m, 1H, COCHN), 7.02 (s, 1H, 4-CH), 7.05 (m, 3H, 2-CH and SO_2NH_2), 7.35 (d, J = 8.5 Hz, 1H, 1-CH); LRMS for $C_{31}H_{46}N_4O_6S$, 603.6 [MH]$^+$ and 715.3 [M + CF_3COO]$^-$;

HPLC purity = 94% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4-OAc).

23 (TFA Salt). White foam; IR υ (KBr) 3422 (OH, NH and NH_2), 1671 and 1637 (C=O, amides), 1376 and 1185 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.97 (s, 3H, 18-CH_3), 1.20−2.40 (17H), 2.85 (m, 2H, 6-CH_2), 3.00−4.40 (broad, 12H, 6 × CH_2N), 3.70 (s, 2H, CH_2Ph), 4.93 (m, 1H, COCHN), 7.01 (s, 1H, 4-CH), 7.05 (m, 3H, 2-CH and SO_2-NH_2), 7.30 (m, 6H, CH_2*Ph* and 1-CH); LRMS for $C_{36}H_{48}N_4$-O_6S, 665.5 $[MH]^+$ and 777.3 $[M + CF_3COO]^-$; HPLC purity = 96% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

26 (TFA Salt). White foam; IR υ (KBr) 3410 (OH, NH, and NH_2), 1671 and 1647 (C=O, amides), 1376 and 1183 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.91 and 0.93 (2d, J = 6.7 Hz, $CH_2CH(CH_3)_2$), 0.97 (s, 3H, 18-CH_3), 1.06 and 1.09 (2d, J = 6.7 Hz, 6H, $COCH(CH_3)_2$), 1.20−2.40 (16H), 2.50 (sept, J = 6.9 Hz, 1H, C$H(CH_3)_2$), 2.86 (m, 2H, 6-CH_2), 3.00−4.40 (broad, 10H, 5 × CH_2N), 4.93 (dt, J_1 = 5.7 Hz, J_2 = 8.5 Hz, 1H, COC*H*NH), 7.02 (s, 1H, 4-CH), 7.06 (m, 3H, 2-CH, and SO_2NH_2), 7.17 (d, J = 8.5 Hz, NH), 7.33 (d, J = 8.6 Hz, 1H, 1-CH); LRMS for $C_{33}H_{52}N_4O_6S$, 633.7 $[MH]^+$ and 745.0 $[M + CF_3COO]^-$; HPLC purity = 93% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

29 (TFA Salt). White foam; IR υ (KBr) 3421 (OH, NH, and NH_2), 1671 and 1630 (C=O, amides), 1376 and 1184 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.91 and 0.93 (2d, J = 6.4 Hz, 6H, $CH_2CH(CH_3)_2$), 0.97 (s, 3H, 18-CH_3), 1.09−2.40 (29H), 2.85 (m, 2H, 6-CH_2), 2.80−4.20 (broad, 10H, 5 × CH_2N), 4.94 (dt, J_1 = 5.8 Hz, J_2 = 8.4 Hz, 1H, COC*H*NH), 7.02 (s, 1H, 4-CH), 7.06 (m, 3H, 2-CH and SO_2-NH_2), 7.25 (d, J = 8.4 Hz, NH), 7.34 (d, J = 8.5 Hz, 1H, 1-CH); LRMS for $C_{37}H_{58}N_4O_6S$, 687.5 $[MH]^+$ and 764.5 $[M + CF_3COO]^-$; HPLC purity = 93% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

30 (TFA Salt). White foam; IR υ (KBr) 3378 (OH, NH, and NH_2), 1676 (C=O, amides), 1374 and 1183 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.91 (d, J = 6.7 Hz, 6H, $CH(CH_3)_2$), 0.97 (s, 3H, 18-CH_3), 1.08 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.20−2.40 (16H), 2.88 (m, 2H, 6-CH_2), 3.00−4.40 (broad, 10H, 5 × CH_2N), 4.70 (t_{app}, J = 8.1 Hz, 1H, COC*H*NH), 7.02 (s, 1H, 4-CH), 7.05 (m, 3H, 2-CH and SO_2-NH_2), 7.14 (d, J = 8.5 Hz, NH); 7.34 (d, J = 8.5 Hz, 1H, 1-CH); LRMS for $C_{31}H_{48}N_4O_6S$, 605.5 $[MH]^+$ and 717.5 $[M + CF_3COO]^-$; HPLC purity = 93% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

32 (TFA Salt). White foam; IR υ (KBr) 3422 (OH, NH and NH_2), 1671 and 1638 (C=O, amides), 1370 and 1184 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.92 and 0.93 (2d, J = 6.7 Hz, 6H, $CH(CH_3)_2$), 0.97 (s, 3H, 18-CH_3), 1.02 (s, 9H, $C(CH_3)_3$), 1.20−2.40 (16H), 2.85 (m, 2H, 6-CH_2), 3.20−4.40 (broad, 10H, 5 × CH_2N), 4.68 (t_{app}, J = 8.0 Hz, 1H, COC*H*NH), 7.02 (s, 1H, 4-CH), 7.05 (m, 3H, 2-CH and SO_2NH_2), 7.16 (d, J = 8.3 Hz, NH), 7.34 (d, J = 8.5 Hz, 1H, 1-CH); LRMS for $C_{34}H_{54}N_4O_6S$, 647.6 $[MH]^+$ and 759.4 $[M + CF_3COO]^-$; HPLC purity = 91% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

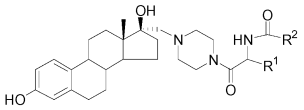
37 (TFA Salt). White foam; IR υ (KBr 3422 (OH, NH and NH_2), 1671 and 1637 (C=O, amides), 1369 and 1184 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.94 (s, 9H, $C(CH_3)_3$), 0.95 (s, 3H, 18-CH_3), 1.20−2.45 (15H), 2.80−4.20 (broad, 14H, 6-CH_2, CH_2Ph and 5 × CH_2N), 5.12 (q, J = 7.6 Hz, 1H, COC*H*NH), 7.01 (s, 1H, 4-CH), 7.06 (m, 3H, 2-CH and SO_2NH_2), 7.32 (m, 7H, 1-CH, CH_2*Ph* and NH); LRMS for $C_{38}H_{54}N_4O_6S$, 695.6 $[MH]^+$ and 807.5 $[M + CF_3COO]^-$; HPLC purity = 94% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

39 (TFA Salt). White foam; IR υ (KBr) 3422 (OH, NH and NH_2), 1670 and 1647 (C=O, amides), 1376 and 1184 (S=O, sulfamate); ^{1}H NMR δ (acetone-d_6) 0.95 (s, 3H, 18-CH_3), 1.00−2.40 (25H), 2.18 (t, J = 8.0 Hz, 2H, $COCH_2$-CH_2), 2.60−4.20 (broad, 14 H, 6-CH_2, CH_2Ph and 5 × CH_2N), 5.11 (q, J = 7.6 Hz, 1H, COC*H*NH), 7.01 (s, 1H, 4-CH), 7.06 (m, 3H, 2-CH and SO_2NH_2), 7.31 (m, 7H, 1-CH, CH_2*Ph* and NH); LRMS for $C_{40}H_{56}N_4O_6S$, 721.6 $[MH]^+$ and 833.0 $[M + CF_3COO]^-$; HPLC purity = 95% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

Generation of Phenol Library B by Nucleophilic Cleavage. Reactions were run in parallel in 5-mL vials with Teflon caps and small magnetic stirring bars. Into each vial were introduced the resin **14** (60−82 mg) and piperazine (40 mg; 0.5 mmol). After addition of freshly distilled THF (0.5 mL), the vials were sealed with Teflon caps and heated for 3 h in an oil bath at 45 °C, with gentle stirring. The oil bath was then removed, and the caps were opened, once the mixtures had cooled to room temperature. The resins were filtered over a cotton pad in small Pasteur pipets and washed with EtOAc (3 × 2 mL). Each filtrate was collected in a 20-mL vial, then water (5 mL) and EtOAc (5 mL) were added. After extraction with EtOAc, the organic layers were correspondingly transferred into preweighed tubes and washed with water (2×). The water was then removed with a pipet, and the organic layers were evaporated to dryness in a Speedvac apparatus at 40 °C and additionally dried for 48 h under vacuum pump. The phenol derivatives **40**−**64** (15−27 mg; 33−54% from **11**) (Table 2) were obtained in high average HPLC purity (90%) according to a random sampling of eight library B members, compounds **40**, **43**, **48**, **52**, **56**, **58**, **60**, and **64**.

40. White solid; IR υ (KBr) 3397 (OH and NH), 1637 (C=O, amides); ^{1}H NMR δ (acetone-d_6) 0.92 (s, 3H, 18-CH_3), 1.08 (t, J = 7.6 Hz, 3H, CH_2CH_3), 1.20−2.40 (13H), 2.24 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.52, 2.75 and 2.82 (3m, 8H, 3 × CH_2N and 6-CH_2), 3.40 (s, OH), 3.49 and 3.55 (2m, 4H, $(CH_2)_2NCO$), 3.99 (d, 2H, CCH_2NH−), 6.53 (d, J = 2.6 Hz, 1H, 4-CH), 6.60 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 6.95 (s, NH), 7.09 (d, J = 8.4 Hz, 1H, 1-CH), 7.97 (s, OH); LRMS for $C_{28}H_{42}N_3O_5$ $[MH^+]$, 484.4 m/z. HPLC purity = 88% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4-OAc).

Table 2. Characterization of Members from Phenol Library (B)



Compound	R^1	R^2	Overall yield (%)[a]	HPLC Purity (%)	LRMS [MH^+] (m/z)
40	H		41	88	484.4
41	H		48	--	--
42	H		40	--	--
43	H		48	88	546.5
44	H		49	--	--
45	$(CH_2)_3$ of Proline		35	--	--
46	$(CH_2)_3$ of Proline		49	--	--
47	$(CH_2)_3$ of Proline		33	--	--
48	$(CH_2)_3$ of Proline		44	90	586.5
49	$(CH_2)_3$ of Proline		49	--	--
50			44	--	--
51			46	--	--
52			44	87	582.5
53			49	--	--
54			54	--	--
55			53	--	--
56			46	91	540.9
57			43	--	--
58			47	90	588.4
59			50	--	--
60			44	94	574.5
61			51	--	--
62			46	--	--
63			37	--	--
64			52	94	642.5

[a] Yields calculated for the solid-phase sequence (6 steps).

43. White solid; IR υ (KBr) 3382 (OH and NH), 1638 (C═O, amides); ^{1}H NMR δ (acetone-d_6) 0.92 (s, 3H, 18-CH_3), 1.20−2.40 (13H), 2.50, 2.75 and 2.82 (3m, 8H, 3 × CH_2N and 6-CH_2), 3.40 (s, OH), 3.46 and 3.54 (2m, 4H, $(CH_2)_2$NCO), 3.59 (s, 2H, C*H*$_2$Ph), 3.99 (d, J = 4.4 Hz, 2H, COC*H*$_2$NH), 6.52 (d, J = 2.5 Hz, 1H, 4-CH), 6.60 (dd, J_1 = 2.5 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.08 (s, NH), 7.09 (d, J = 8.4 Hz, 1H, 1-CH), 7.30 (m, 5H, CH_2*Ph*), 7.97 (s, OH); LRMS for $C_{33}H_{44}N_3O_4$ [MH^+], 546.5 *m*/*z*. HPLC purity = 88% (C-18 NovaPak column, MeOH/H_2O (90:10) containing 20 mM NH_4OAc).

48. White solid; IR υ (KBr) 3422 (OH), 1637 (C═O, amides); ^{1}H NMR δ (acetone-d_6) 0.92 (s, 3H, 18-CH_3), 1.20−2.40 (18H), 2.51, 2.75 and 2.80 (3m, 8H, 3 × CH_2N and 6-CH_2), 3.40−3.70 (m, 8H, $(CH_2)_2$NCO, C*H*$_2$Ph and NCH_2 of proline), 4.92 (m, 1H, COCHN), 6.52 (d, J = 2.6 Hz, 1H, 4-CH), 6.59 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.09 (d, J = 8.4 Hz, 1H, 1-CH), 7.26 (m, 5H, CH_2*Ph*), 7.93 (s, OH); LRMS for $C_{36}H_{48}N_3O_4$ [MH^+], 586.5 *m*/*z*. HPLC purity = 90% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

52. White solid; IR υ (KBr) 3320 (OH and NH), 1629 (C═O, amides); ^{1}H NMR δ (acetone-d_6) 0.91 and 0.95 (2d, J = 6.6 Hz, 6H, CH(C*H*$_3$)$_2$), 0.92 (s, 3H, 18-CH_3), 1.02 (s, 9H, C(C*H*$_3$)$_3$), 1.20−2.35 (16H), 2.10 (s, 2H, COC*H*$_2$C-(C*H*$_3$)$_3$), 2.52, 2.75 and 2.81 (3m, 8H, 3 × CH_2N and 6-CH_2), 3.40 (m, OH), 3.57 (m, 4H, $(CH_2)_2$NCO), 4.96 (q, J = 7.6 Hz, 1H, COC*H*NH), 6.52 (d, J = 2.6 Hz, 1H, 4-CH), 6.59 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.00 (d, J = 8.5 Hz, NH), 7.09 (d, J = 8.4 Hz, 1H, 1-CH), 7.93 (s, OH); LRMS for $C_{35}H_{56}N_3O_4$ [MH^+], 582.5 *m*/*z*. HPLC purity = 87% (C-18 NovaPak column, 70% MeOH/H_2O (90:10) and 30% H_2O, both containing 20 mM NH_4OAc).

56. White solid; IR υ (KBr) 3405 (OH and NH), 1625 (C═O, amides); ^{1}H NMR δ (acetone-d_6) 0.87 and 0.90 (2d, 6H, J = 6.8 Hz, CH(C*H*$_3$)$_2$), 0.92 (s, 3H, 18-CH_3), 1.06 and 1.11 (2d, J = 6.7 Hz and J = 7.0 Hz, 6H, CH(C*H*$_3$)$_2$), 1.29−2.40 (15H), 2.51, 2.75 and 2.82 (3m, 8H, 3 × CH_2N and

6-CH_2), 3.40 (s, OH), 3.57 and 3.63 (2m, 4H, $(CH_2)_2NCO$), 4.73 (dd, $J_1 = 6.7$ Hz, $J_2 = 9.1$ Hz, 1H, COC*H*NH), 6.52 (d, $J = 2.6$ Hz, 1H, 4-CH), 6.60 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.4$ Hz, 1H, 2-CH), 6.95 (d, $J = 9.1$ Hz, NH), 7.09 (d, $J = 8.4$ Hz, 1H, 1-CH), 7.97 (s, OH); LRMS for $C_{32}H_{50}N_3O_4$ [MH^+], 540.9 *m*/*z*. HPLC purity = 91% (C-18 NovaPak column, 50% MeOH/H_2O (90:10) and 50% H_2O, both containing 20 mM NH_4OAc).

58. White solid; IR υ (KBr) 3396 (OH and NH), 1624 (C═O, amides); ^1H NMR δ (acetone-d_6) 0.82 and 0.87 (2d, $J = 6.7$ Hz, 6H, $CH(CH_3)_2$), 0.92 (s, 3H, 18-CH_3), 1.20−2.35 (14H), 2.47, 2.75 and 2.82 (3m, 8H, 3 × CH_2N and 6-CH_2), 3.40 (s, OH), 3.57 and 3.58 (2m, 4H, $(CH_2)_2NCO$), 3.58 (s, 2H, CH_2Ph), 4.72 (dd, $J_1 = 6.7$ Hz, $J_2 = 9.0$ Hz, 1H, COC*H*NH), 6.52 (d, $J = 2.5$ Hz, 1H, 4-CH), 6.59 (dd, $J_1 = 2.5$ Hz and $J_2 = 8.4$ Hz, 1H, 2-CH), 7.09 (d, $J = 8.4$ Hz, 1H, 1-CH), 7.16 (d, $J = 9.0$ Hz, NH), 7.33 (m, 5H, CH_2*Ph*), 7.94 (s, OH); LRMS for $C_{36}H_{50}N_3O_4$ [MH^+], 588.4 *m*/*z*. HPLC purity = 90% (C-18 NovaPak column, 70% MeOH/H_2O (90:10) and 30% H_2O, both containing 20 mM NH_4OAc).

60. White solid; IR υ (KBr) 3405 (OH and NH), 1628 (C═O, amides); ^1H NMR δ (acetone-d_6) 0.91 (s, 3H, 18-CH_3), 1.02 (t, $J = 7.6$ Hz, 3H, CH_2CH_3), 1.20−2.30 (15H), 2.18 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 2.45, 2.64, 2.75 and 2.81 (4m, 8H, 3 × CH_2N and 6-CH_2), 2.93 (m 2H, CH_2Ph), 3.35 and 3.50 (2m, 5H, $(CH_2)_2NCO$ and OH), 5.10 (q, $J = 7.5$ Hz, 1H, COC*H*NH), 6.53 (d, $J = 2.3$ Hz, 1H, 4-CH), 6.60 (dd, $J_1 = 2.5$ Hz and $J_2 = 8.4$ Hz, 1H, 2-CH), 7.09 (d, $J = 8.4$ Hz, 1H, 1-CH), 7.16 (m, NH), 7.24 (m, 5H, CH_2*Ph*), 7.97 (s, OH); LRMS for $C_{35}H_{48}N_3O_4$ [MH^+]: 574.5 *m*/*z*. HPLC purity = 94% (C-18 NovaPak column, 70% MeOH/H_2O (90:10) and 30% H_2O, both containing 20 mM NH_4OAc).

64. White solid; IR υ (KBr) 3314 and 3396 (OH and NH), 1634 (C═O, amides); ^1H NMR δ (acetone-d_6) 0.91 (s, 3H, 18-CH_3), 1.00−2.30 (23H), 2.19 (t, $J = 7.4$ Hz, 2H, $COCH_2CH_2$), 2.45, 2.65, 2.75 and 2.81 (4m, 8H, 3 × CH_2N and 6-CH_2), 2.93 (m, 2H, CH_2Ph), 3.30−3.60 (m, 5H, $(CH_2)_2NCO$ and OH), 5.12 (q, $J = 7.6$ Hz, 1H, COC*H*NH), 6.53 (d, $J = 2.3$ Hz, 1H, 4-CH), 6.60 (dd, $J_1 = 2.5$ Hz and $J_2 = 8.4$ Hz, 1H, 2-CH), 7.09 (d, $J = 8.4$ Hz, 1H, 1-CH), 7.25 (m, 6H, CH_2*Ph* and NH), 7.97 (s, OH); LRMS for $C_{40}H_{55}N_3O_4$ [MH^+], 642.5 *m*/*z*. HPLC purity = 94% (C-18 NovaPak column, 70% MeOH/H_2O (90:10) and 30% H_2O, both containing 20 mM NH_4OAc).

Steroid Sulfatase Assay. Human embryonic kidney (HEK)-293 cells (American Type Culture Collection, Rockville, MD), transiently transfected with a sulfatase expression vector (pCMV-sulfa), were used as a source of steroid sulfatase activity as previously reported.[14] For the assay, the HEK-293 cell homogenate was prepared by repeated freezing (−80 °C) and thawing (5 times), and homogenization using a Dounce homogenizer. The reaction was carried out at 37 °C in 1.25 mL of 100 mM Tris−acetate buffer (pH 7.4) containing 5 mM EDTA, 10% glycerol, 100 μM [^{3}H]E_1S ([6,7-^{3}H]estrone sulfate ammonium salt; 43.10 Ci/mmol; New England Nuclear; Boston, MA) as substrate and an ethanolic solution of the compound to be tested (at appropriate concentrations). After 2 h of incubation, the reaction was stopped by adding 1.25 mL of xylene. The tubes were then shaken and centrifuged at 2000*g* for 10 min to separate the organic and aqueous phases. Radioactivity in 750 μL of each phase (organic/free steroids; aqueous/sulfated steroids) was determined by liquid scintillation counting with a Beckman LS3801 (Irvine, CA). The distribution of steroids in the organic and aqueous phases, respectively, was first determined using radiolabeled E_1 and E_1S, and we found that the contaminated distribution was <5%. Background contamination was determined using the same incubation conditions, and without an enzyme source, it was found to be <5% and was subtracted in the calculation of the enzymatic activity. The results were expressed as percentage (%) of [^{3}H]E_1 produced (100% for the control without inhibitor) and the percentage (%) of inhibition then calculated.

Acknowledgment. This work was supported by the Medical Research Council of Canada (now the Canadian Institutes of Health Research), the Fonds de la Recherche en Santé du Québec and the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche. Authors also thank the Division of Medicinal Chemistry (Laboratory of Molecular Endocrinology) for providing chemical facilities and Dr. Van Luu-The for performing the biological assays. Helpful discussions with Ioan Radu and René Maltais were kindly appreciated.

Supporting Information Available. HPLC chromatograms of random sampling members from libraries A and B (compounds **16**, **18**, **20**, **23**, **26**, **29**, **30**, **32**, **37**, **39**, **40**, **43**, **48**, **52**, **56**, **58**, **60**, and **64**), detailed listing of ^{13}C NMR chemical shifts (Table 3) for two typical analogous sulfamate and phenol derivatives (compounds **30** and **55**, respectively), and inhibition (%) of steroid sulfatase activity by compounds **15**−**64** at four concentrations of 1, 10, 100, and 1000 nM (Tables 4 and 5). This material is available free of charge via the Internet at http://pubs.acs.org.

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CC020115U