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A Multidetachable Sulfamate Linker Successfully Used in a Solid-Phase Strategy to Generate Libraries of Sulfamate and Phenol Derivatives

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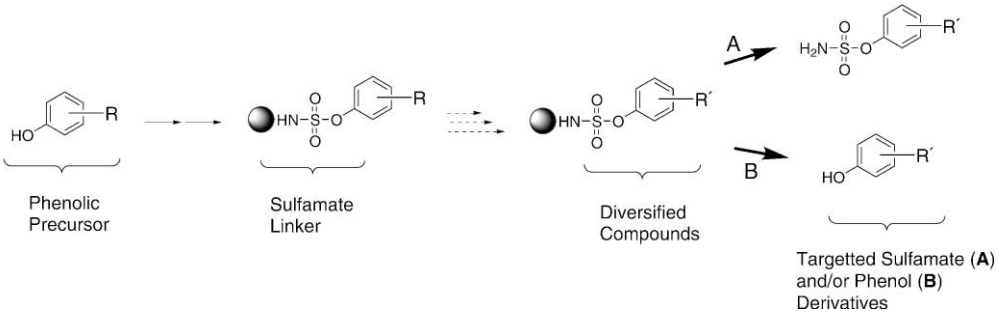
Abstract—The sulfamates and phenols constitute two families of compounds with numerous interesting biological properties. Using the ability of a new multidetachable sulfamate linker to generate these two families of compounds from the same resin, we designed and synthesized libraries of estradiol derivatives, sulfamoylated or not. A C-16β side chain was then judiciously diversified to target two key steroidogenic enzymes, the steroid sulfates and the type 1 17β-HSD. Four libraries of sulfamate and phenol derivatives were easily obtained by solid-phase parallel synthesis in good crude overall yields (13–62%) and HPLC purities (85–96%). Such strategy using the new two-in-line sulfamate linker could be also extended to other therapeutic targets than steroidogenic enzymes, thus adding to its potential.

Solid-phase synthesis and combinatorial chemistry emerged as powerful methodologies for accelerating the development of compounds with therapeutic potential through generation of libraries of small molecules.[1] The challenge now is to extend the ability of solid-phase chemistry to generate a large number of structurally diversified compounds by developing new tools. Among these are multidetachable linkers (two-in-line linkers),[2] linkers that can generate different compounds depending on the cleavage conditions used and, consequently, increase molecular diversity. Among more than 200 linkers developed,[2] only five examples of multidetachable linkers have been reported.[3] In this paper, we now report the use of a multidetachable sulfamate linker,[4] and a strategy that allow the synthesis of two different categories of biologically relevant compounds, sulfamates and phenols (Scheme 1).



Scheme 1. The multidetachable sulfamate linker generating diversified sulfamate/phenol derivatives depending on the cleavage conditions: acidic (**A**)/nucleophilic (**B**).

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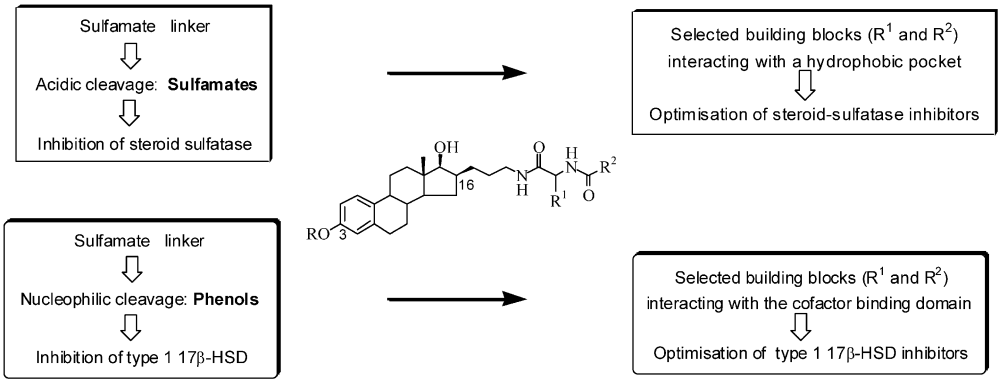


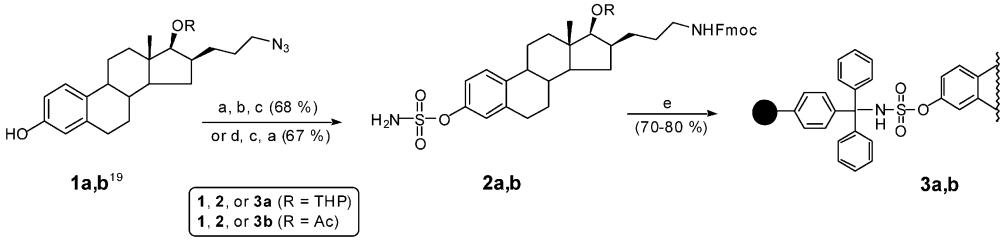
Figure 1. General structure of the enzyme inhibitors synthesized with our strategy using the sulfamate linker.

The sulfamate derivatives constitute orally active prodrugs,[5] potential antibacterial,[6] cytotoxic,[7] anticonvulsive,[8] antitumoral,[9] and antisulfatase compounds,[10] while phenol derivatives belong to a large family of compounds with equally interesting biological properties.[11–13] Because of its ability to generate these two families of compounds from the same resin, the sulfamate linker is an interesting tool for combinatorial chemistry. To illustrate this, we synthesized libraries of sulfamates and phenols with the aim of producing inhibitors of the key steroidogenic enzymes steroid sulfatase and type 1 17β-hydroxysteroid dehydrogenase (17β-HSD).

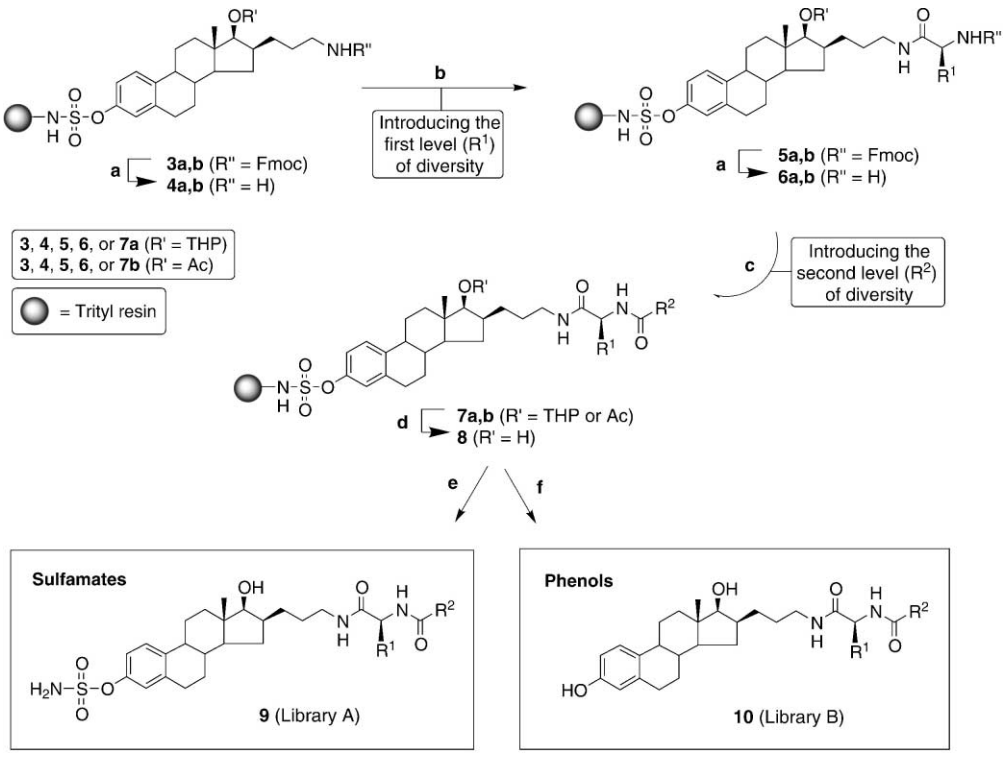
Controlling estrogenic and androgenic steroid concentrations by inhibiting steroidogenic enzymes is a good way to prevent and potentially cure breast and prostate cancers. The sulfamate group and especially the aryl sulfamate group is well recognized to strongly inhibit steroid sulfatase.[10] Additional studies also clearly suggest the presence in this enzyme of a hydrophobic region neighboring the D-ring of steroid substrates.[14] Based on this information, compounds that contain both sulfamate and hydrophobic groups were designed and found to inhibit steroid sulfatase.[15] As for our other enzymatic target, a phenol group is the main constituent of the natural substrate of type 1 17β-HSD,[16] and most of the known inhibitors have a phenolic group.[17] We have also recently reported that a hydrophobic side chain ending with a polar group and inserted at the C-16β position (D-ring) of estradiol can interact with the enzyme cofactor binding domain to inhibit type 1 17β-HSD.[18] For both enzymes discussed above, the inhibitors remain to be optimized and efficient combinatorial approaches are needed. Using the multidetachable sulfamate linker, we designed libraries of estradiol derivatives, sulfamoylated or not, with a C-16β side chain judiciously diversified to target our two key steroidogenic enzymes (Fig. 1).

As the multidetachable sulfamate linker was easily accessible from phenols,[4] we selected appropriate steroidal phenols **1a**,**b** (Scheme 2) as scaffolds for steroidogenic enzyme inhibitors, but numerous other phenol derivatives could also be used for targeting additional therapeutic targets. The key sulfamate intermediates **2a**,**b** were obtained from phenols **1a**,**b**[19] in good yields (67 and 68%) through a sequence of three steps: (1) sulfamoylation of phenol, (2) reduction of the azide, and (3) protection of the resulting amine as Fmoc derivative. Alternatively, the azide was reduced and the amine protected as Fmoc prior to the sulfamoylation. Sulfamates **2a**,**b** were then loaded (70–80% of weight increase) onto trityl chloride resin and spectra (IR and ^{13}C NMR) of the loaded resins **3a**,**b** confirmed the presence of the steroid backbone.

The parallel solid-phase synthesis of libraries A and B (Scheme 3) started with resins **3a**,**b**, which correspond to a phenolic steroid scaffold linked to a solid polystyrene support by a sulfamate group. During the solid-phase synthesis, the reactions were monitored by a mini-cleavage test and the formation of the desired resins confirmed by IR analysis. The Fmoc protecting group was first removed with a solution of 20% piperidine in DCM to free the amine for the next step. The resins **4a**,**b** were then split and placed in the vessels of the reaction block of an ACT-Labtech semi-automated synthesizer. The first level of diversity (R^1) was introduced by the



Scheme 2. Synthesis of sulfamate resins **3a**,**b** from phenolic precursors **1a**,**b** (see ref 20 for abbreviations). (a) NH_2SO_2Cl,[7] DBMP, CH_2Cl_2, rt, 1–24 h; (b) H_2, 20% $Pd(OH)_2/C$, AcOEt/MeOH (1:9), rt, 5 h; (c) Fmoc-OSu, $NaHCO_3$, THF/H_2O (3:1), 0 °C or rt, 1–2 h; (d) H_2, Pd/C, MeOH, rt, 2 h; (e) trityl-Cl resin, DIPEA, CH_2Cl_2, rt, 9–12 h.



Scheme 3. Solid-phase synthesis of diversified sulfamates **9** and phenols **10** (see ref 20 for abbreviations). (a) 20% piperidine in CH_2Cl_2, rt, 1 h; (b) PyBOP, HOBt, $R^1CH(NHFmoc)COOH$, DIPEA, DMF, rt, 3 h; (c) PyBrOP, HOBt, R^2COOH, DIPEA, DMF, rt, 3 h; (d) 0.07 M *p*-TSA in 1-butanol/$ClCH_2CH_2Cl$ (1:1), rt, 24 h (for THP) or 0.1 M MeONa/MeOH:THF (25/75) rt, 24 h (for Ac); (e) 30% HFIP in CH_2Cl_2, rt, 12 h; (f) piperazine, THF, 45–50 °C, 3 h or 30% DEA in THF, rt, 24 h.

acylation (PyBOP/HOBt) of each resin with one of the selected Fmoc-protected aminoacids as building blocks (Table 1) allowing the formation of resins **5a**,**b**. After cleavage of the Fmoc protecting group, the resins **6a**,**b** were split again and the second level of diversity (R^2) was introduced by coupling a selection of carboxylic acids activated with PyBrOP and HOBt. The C17-THP or Ac protective group of **7a**,**b** was removed by a treatment with *p*-TSA in DCE/1-butanol or MeONa in MeOH/THF to give the final resins **8**.

The final diversified compounds **9** (library A_1 or A_2) and **10** (library B_1 or B_2) were released from the solid support either by an acidic or a nucleophilic treatment. The acidic cleavage was performed by vortexing half of each of the resins **8** with a solution of 30% HFIP in DCM to allow the formation of sulfamate derivatives **9**. It also would have been possible to use 1–5% of TFA in DCM to generate sulfamate derivatives, but HFIP was found more suitable in the presence of a secondary alcohol by avoiding esterification with TFA. The nucleophilic cleavage was done using a solution of piperazine in THF at 45–50 °C or 30% DEA in THF at room temperature generating the phenol derivatives **10**. The later condition is however more efficient by avoiding the need of heating and the washing step to release residual piperazine.

Sulfamate libraries A_1 and A_2 were obtained in average crude overall yields of 40 and 46% for the solid-phase reactions while the average HPLC purity were 91 and 89%, respectively (Table 1). The average crude yields of phenol libraries B_1 and B_2 were 32 and 27% with a HPLC purity higher than 90%. These results were found satisfactory since no purification step was required after the final cleavage, the excess of reagent (HFIP or DEA) and solvents being removed simply by evaporation. Analysis of sulfamates **9** and phenols **10** randomly selected from libraries A_1, A_2, B_1, and B_2 by ^1H NMR, IR, and MS confirmed the expected chemical structures.[21]

After successfully obtaining two sulfamate libraries and two phenol libraries, we were interested in determining the inhibitory activity of all these compounds in order to validate our strategy. Focusing on the steroid sulfatase, we tested the sulfamate derivatives **9** (libraries A_1 and A_2) as enzyme inhibitors. A homogenate of human embryonic kidney (HEK)-293 cells transfected with a sulfatase expression vector was used as source of steroid sulfatase activity.[15] We measured the hydrolysis of [^{3}H]estrone sulfate to [^{3}H]estrone and calculated the inhibitory activity at an inhibitor concentration of 1 nM. Results are reported in Figure 2 with a cut-off value fixed at 78%, which is the value corresponding to the inhibitory activity obtained with estrone-3-*O*-sulfamate (EMATE), a well known and potent steroid sulfatase inhibitor.[22] Sixteen hits originate from library A_1 while only one hit originates from library A_2. These results can be explained by the fact that a good steroid sulfatase inhibitor needs a hydrophobic group neighboring the steroidal D-ring.[14] This requirement is well fulfilled by the members of library A_1 which were designed mainly with hydrophobic building blocks (R^1

Table 1. Characteristics of libraries **A** and **B**

Library	Compd	Average yield (%)	Average HPLC purity (%)	Building blocks	
				Amino acids (R^1)	Carboxylic acids (R^2)
A_1	48 Sulfamates	40 (21–50)	91 (87–96)	Gly L-Pro L-Leu D-Phe L-Phe L-Phe(p-NO_2) L-Tyr(But) L-Tyr(Bzl)	Acetic *i*-Butyric Butyric Hexanoic Phenylacetic 3-Cyclopentylpropionic
B_1	48 Phenols	32 (13–58)	93 (86–96)	Gly L-Pro L-Leu D-Phe L-Phe L-Phe(p-NO_2) L-Tyr(But) L-Tyr(Bzl)	Acetic *i*-Butyric Butyric Hexanoic Phenylacetic 3-Cyclopentylpropionic
A_2	30 Sulfamates	46 (35–53)	89 (88–91)	Gly L-Ala L-Pro L-Ile L-Phe	4-Pyridineacetic 2-Pyrazinoic 4-Aminophenylacetic 3-Aminopyrazine-2-carboxylic Benzoic Indole-2-carboxylic
B_2	30 Phenols	27 (16–62)	90 (85–95)	Gly L-Ala L-Pro L-Ile L-Phe	4-Pyridineacetic 2-Pyrazinoic 4-Aminophenylacetic 3-Aminopyrazine-2-carboxylic Benzoic Indole-2-carboxylic

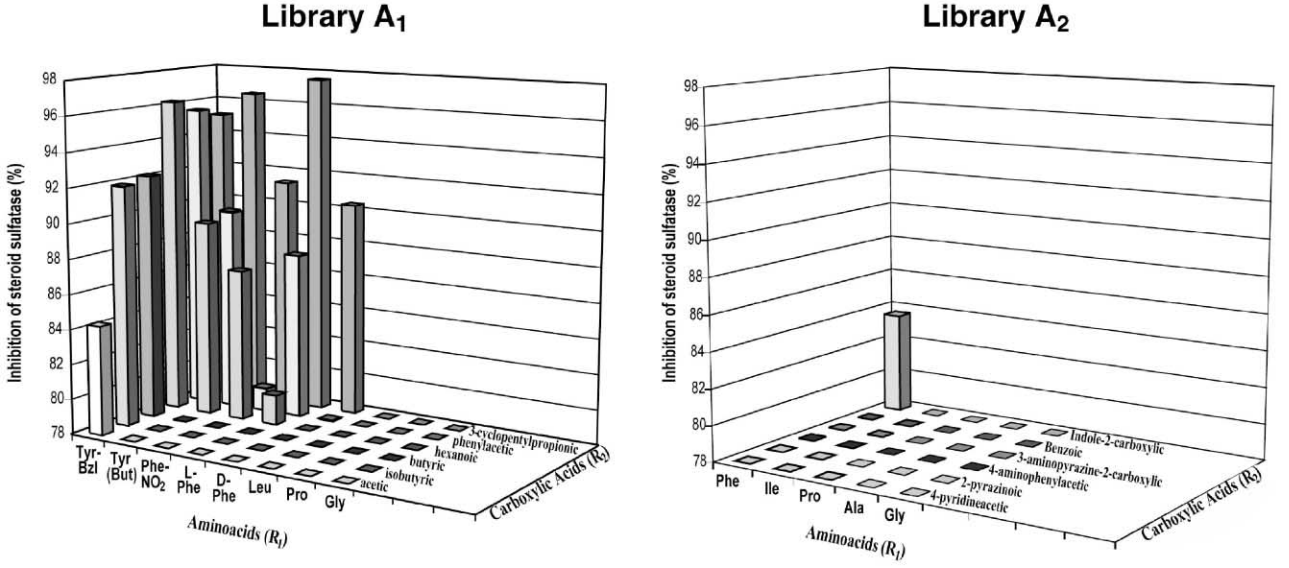


Figure 2. Inhibition (%) of steroid sulfatase activity ([^{3}H]estrone sulfate to [^{3}H]estrone) by libraries A_1 and A_2 members. HEK-293 cells transfected with a sulfatase expression vector and homogenized were used as enzyme source. All compounds were tested at a concentration of 1 nM. At this concentration, the potent inhibitor EMATE[22] inhibits 78% of the enzyme activity. The assay was performed as described in ref 15, except that the concentration of enzyme substrate ([^{3}H]estrone sulfate) was 50 μM.

and R^2) that better interact with the hydrophobic enzyme pocket. On the opposite, the members of library A_2 were synthesized with polar building blocks (R^2) which are less favorable for steroid sulfatase inhibition. In fact the members of this library were initially designed to interact with the cofactor binding domain of type 1 17β-HSD.

In conclusion, the sulfamate linker appears to be a versatile tool for the simultaneous solid-phase synthesis of

biologically-relevant sulfamate and phenol derivatives, simply by varying the cleavage conditions. This new multidetachable linker was thus used for the rapid synthesis of libraries of inhibitors targeting two key steroidogenic enzymes. More importantly, our strategy using the new two-in-line sulfamate linker could be extended to other therapeutic targets, thus adding to its potential. As a preliminary result, potent steroid sulfatase inhibitors were obtained thus demonstrating that the reported strategy works well for the optimization of enzyme inhibitors. The full scope of this strategy and full details of the experimental will be reported in due course.

Acknowledgements

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20. Abbreviations used: DBMP = 2,6-di-*tert*-butyl-4-methylpyridine; DCE = dichloroethane; DEA = diethylamine, DIPEA = diisopropylethylamine; Fmoc = 9-fluorenylmethyloxycarbonyl; FmocOSu = *N*-(9-fluorenylmethyloxy-carbonyloxy) succinimide; HFIP = 1,1,1,3,3,3-hexafluoro-2-propanol; HOBt = *N*-hydroxybenzotriazole; PyBOP = benzotriazole-1-yl-oxy-*tris*-pyrrolidino-phosphonium hexafluorophosphate; PyBrOP = bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate; TFA = trifluoroacetic acid; THP = tetrahydropyranyl.
21. Characterization of one member of each library. **Library A_1**: *3*-O-*sulfamate-16β-(*N-*phenylacetyl*-L-p-*nitrophenyl-alanine-aminopropyl)-estradiol*. White foam; IR ν (KBr): 3378, 3296, 3080 (OH, NH, NH_2), 1648 (C=O, amides), 1520 and 1346 (NO_2), 1376 and 1186 (S=O, sulfamate); ^{1}H NMR δ (CD_3OD): 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_2Ph), 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_2*Ph*), 7.32 (d, J = 8.5 Hz, 1-CH), 7.37 and 8.03 (2d, J = 8.7 Hz, 2×2H, CH_2*Ph-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). **Library B_1**: *16β*-(N-*butyryl-glycine-aminopropyl*)-*estradiol*. Light yellow gum; IR ν (KBr): 3397, 3298 (OH, NH), 1654 and 1630 (C=O, amides); ^{1}H NMR δ (CD_3OD): 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 (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). **Library A_2**: *3*-O-*sulfamate-16β-(N-2-*

pyrazinoyl-L-*isoleucine-aminopropyl)-estradiol*. Light yellow solid; IR ν (film): 3301 (OH, NH), 1653 (C=O, amides), 1374 and 1186 (S=O, sulfamate); ^{1}H NMR δ (CD_3OD): 0.75 (s, 18-CH_3), 0.96 (t, $J=7.4$ Hz, CH_2CH_3), 1.01 (d, $J=6.8$ Hz, $CHCH_3$), 1.05–2.40 (19H), 2.87 (m, 6-CH_2), 3.23 (m, CH_2NH), 3.70 (d, $J=9.9$ Hz, 17α-CH), 4.47 (d, $J=8.0$ Hz, $CHNH$), 7.03 (d, $J=2.4$ Hz, 4-CH), 7.06 (dd, $J_1=2.3$ Hz, $J_2=9.7$ Hz, 2-CH), 7.31 (d, $J=8.6$ Hz, 1-CH), 8.70 (dd, $J_1=1.5$ Hz, $J_2=2.4$ Hz, 5′-CH), 8.78 (d, $J=2.5$ Hz, 6′-CH), 9.25 (d, $J=1.4$ Hz, 3′-CH); LRMS for $C_{32}H_{46}N_5O_6S$ [MH^+]: 628.4 *m*/*z*; HPLC purity = 90.4% (C-18 NovaPak column, 50% of CH_3OH:H_2O (90:10) and 50% of H_2O, both containing 20 mM of NH_4OAc). **Library B_2**: *16β-(*N-*benzoyl*-L-*alanine-aminopropyl)-estradiol*. Light yellow solid; IR ν (film): 3300 (OH, NH), 1636 (C=O, amides). ^{1}H NMR δ (CD_3OD): 0.77 (s, 18-CH_3), 0.90–2.40 (16H), 1.47 (d, $J=7.2$ Hz, $CHCH_3$), 2.78 (m, 6-CH_2), 3.23 (m, CH_2NH), 3.70 (d, $J=9.9$ Hz, 17α-CH), 4.55 (q, $J=7.2$ Hz, $CHCH_3$), 6.49 (d, $J=2.5$ Hz, 4-CH), 6.54 (dd, $J_1=2.4$ Hz, $J_2=8.5$ Hz, 2-CH), 7.08 (d, $J=8.4$ Hz, 1-CH), 7.48 (m, 3′ and 5′-CH), 7.55 (m, 4′-CH), 7.89 (dd, $J_1=1.5$ Hz, $J_2=8.5$ Hz, 2′ and 6′-CH). LRMS for $C_{31}H_{41}N_2O_4$ [MH^+]: 505.5 *m*/*z*. HPLC purity = 90.3% (C-18 NovaPak column, 50% of CH_3OH/H_2O (90:10) and 50% of H_2O, both containing 20 mM of NH_4OAc).

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