2β-(*N*-Substituted Piperazino)-5α-Androstane-3α,17β-Diols: Parallel Solid-Phase Synthesis and Antiproliferative Activity on Human Leukemia HL-60 Cells

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Leukemia is the most common cancer affecting children. A steroid possessing a methylpiperazine nucleus was recently reported to inhibit the proliferation of HL-60 leukemia cells. To speed up the development of this promising potential new drug, we generated libraries of analogues using parallel solid-phase organic synthesis (SPOS). A 6-step sequence of reactions, starting from dihydrotestosterone, afforded a steroidal 2,3α-epoxide, which was selectively opened to give, after *N*-Fmoc protection, a diol with suitable stereochemistry. The difference of reactivity between 3α-OH and 17β-OH was then used to allow the regioselective coupling of 17β-OH to chloro-activated butyldiethylsilane polystyrene. We next generated three libraries of 2β-piperazinyl-5α-androstane-3α,17β-diol *N*-derivatives with 1, 2, or 3 levels of molecular diversity in acceptable yields and purities for our biological screening assay. Several members of these libraries were more potent than the lead compound, especially five members with a proline as the first level of diversity and a cyclohexylcarbonyl, methylbutyryl, cyclohexylacetyl, cyclopentylpropionyl, or hexanoyl as the second level of diversity. They efficiently inhibited HL-60 cell proliferation with IC_{50} values of 0.58, 0.66, 1.78, 1.98, and 2.57 μM, respectively. The present work demonstrates the potential of our SPOS approach for the optimization of a new class of cytotoxic agents.

Introduction

Leukemia affects both sexes and all ages. Although often thought of as primarily a childhood disease, it is diagnosed 10 times more often in adults than in children. Despite its rarity, leukemia is the chief cause of death in children between 1 and 14 years old.[1]

Cancerous cells can be killed by irradiation, but this method is not selective and damages all cells in the body. Antibiotics and transfusions of blood components are used as supportive treatments. Under appropriate conditions, bone marrow transplantation may be useful in the treatment of certain leukemias. However, chemotherapy is the most effective method.[2] Various anticancer drugs are used, either in combination or alone. The anthracycline glycosides, especially doxorubicin (adriamycin) and daunorubicin, are potent chemotherapeutic agents, with clinical utility against a wide range of human malignancies.[3] However, their long-term effectiveness is often limited by a dose-related cumulative cardiotoxicity and the development of acquired drug resistance, mediated by overexpression of the ATP-dependent efflux proteins P-glycoprotein and multidrug-resistance protein in chemosensitive tumors of the multidrug-resistance phenotype.[4,5] For acute lymphoblastic leukemia, prednisone, a steroid hormone, combined with chemotherapeutic agents can bring remission in at least a third of children and half the adults struck by this disease.[6,7] Such an effect is rare in other forms of acute leukemia.

Moreover, a prominent phenotypic abnormality of human acute leukemia cells is the inability of the cells to differentiate to functional mature cells; instead, the cells are blocked at an early stage of development, remain in the proliferative pool, and rapidly accumulate.[8] Extensive studies on the differentiation of myeloid cells to monocytes/macrophages or neutrophils in response to retinoic acid,[9,10] dimethyl sulfoxide, phorbol-12-myristate-13-acetate,[10] vitamin D_3,[9] and dimethylformamide[11] have been described. Unfortunately, these compounds are rarely potent enough to induce differentiation in vivo when used at doses that do not cause serious clinical side effects. Thus, treatment options are still limited, and it is important to find more effective treatments that act differently and have fewer side effects. We focused our attention on the aminosteroid HY, 2β-(4′-methyl-1′-piperazinyl)-5α-androstane-3α,17β-diol (**1**), that He's research group[12,13] previously reported to exert an inhibitory activity on leukemic cells (Figure 1). More specifically, this molecule inhibits the proliferation of HL-60 cells, a human promyelotic leukemia cell line, and promotes cell differentiation. This 5α-androstane derivative also has some structural similarity with androsterone, a steroid that was reported to stimulate hematopoiesis both in mice and in humans.[14]

Solid-phase combinatorial chemistry is a valuable tool in the development and optimization of compounds with relevant biological applications in different fields of

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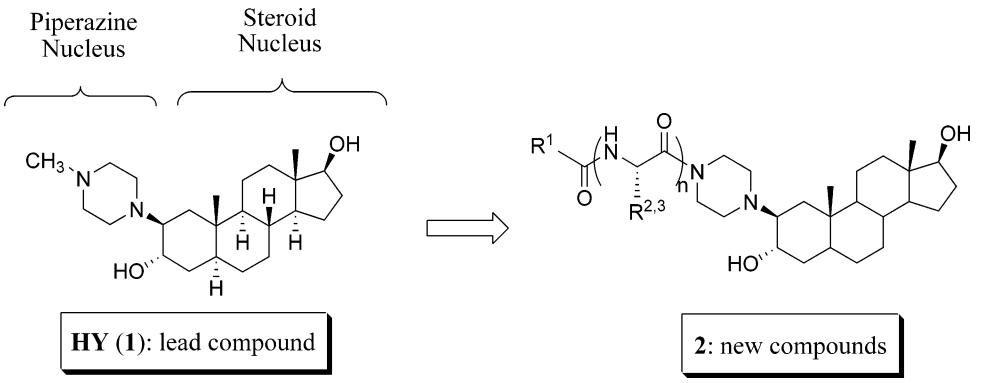
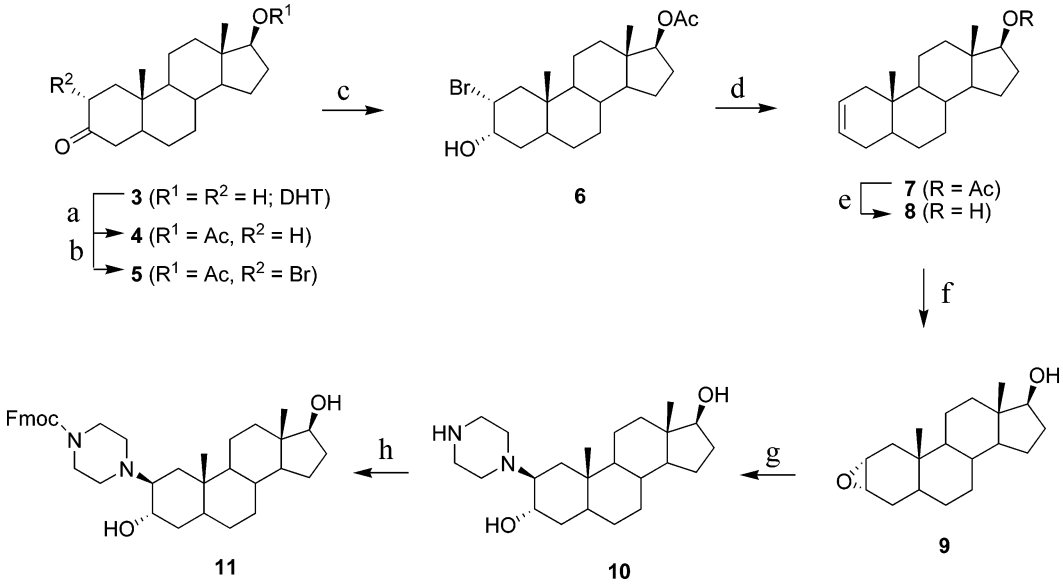


Figure 1. Chemical structures of 2β-(4′-methyl-1′-piperazinyl)-5α-androstane-3α,17β-diol, identified as HY (**1**), and of analogue compounds represented by the general structure **2** (n = 0, 1, or 2). The stereogenic centers are illustrated only for steroid **1**, but they are the same for all other steroid derivatives reported in this paper.

Scheme 1. Synthesis of Solid-Phase Precursor **9**[a]



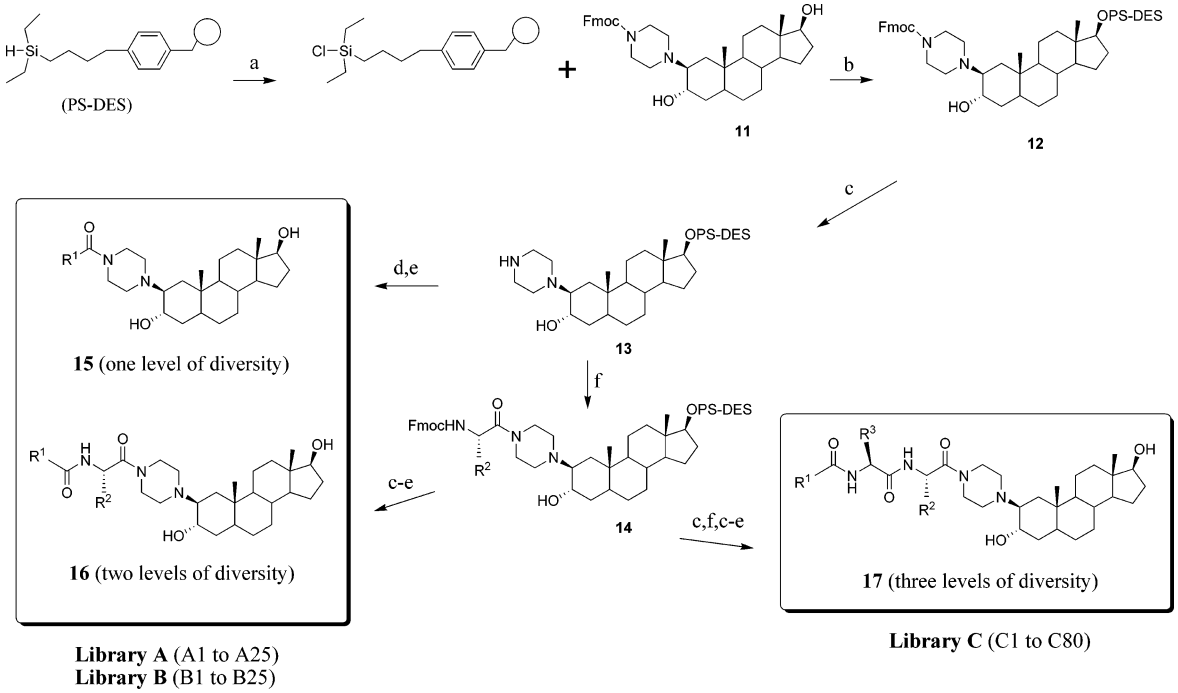
[a] Reagents and conditions: (a) Ac_2O, pyridine, DMAP, room temp (99%); (b) Br_2, AcOH, room temp (95%); (c) K-selectride, −78 °C, THF (71%); (d) Zn dust, AcOH, reflux (83%); (e) K_2CO_3/H_2O, MeOH, reflux (98%); (f) *m*-CPBA, CH_2Cl_2, 0 °C (85%); (g) piperazine, H_2O, reflux (69%); (h) Fmoc-OSu, $NaHCO_3$ (1M), H_2O/THF (5:1), room temp (70%).

medicine.[15−22] Given the high interest of our group in the solid-phase chemistry of steroid derivatives[23−27] and our previous work with a piperazine nucleus,[28] we decided to generate a series of HY derivatives using parallel solid-phase organic synthesis to optimize the biological activity of this lead compound. In this report, we describe the chemical synthesis of three libraries of aminosteroids **2** (Figure 1) and present data on their antiproliferative activity on HL-60 cells. We also give data on structure−activity relationships that will be useful in the optimization of the biological activity of this new family of 2β-substituted aminosteroids with potential for treatment of leukemia.

Results and Discussion

1. Synthesis of compound 11. The libraries of aminosteroids **2** were obtained from a common precursor, the piperazino derivative **11**, which was loaded on a polymeric support for the purpose of solid-phase synthesis. The key step in the preparation of **11** is a diastereoselective opening of epoxide **9** generated from the corresponding alkene **7** (Scheme 1). Synthetic approaches for generating **7** from dihydrotestosterone (DHT) can be found in the literature,[29] but they lead to the formation of a mixture of regioisomers (2,3 and 3,4 double bonds). It was however possible to obtain the 2,3-alkene by rigorously controlling the bromation and reduction steps of the reaction sequence. Thus the acetylation of DHT (**3**), followed by a selective bromination of DHT acetate (**4**) at C-2 with Br_2 in acetic acid, afforded bromoketone **5**. The use of a stoichiometric equivalent of Br_2 is important to avoid a second bromination at C-4. K-selectride in THF, rather than the classical $NaBH_4$, was next used to obtain the bromohydrine **6**. Indeed, a better stereoselectivity for the reduction of bromoketone **5** was obtained with K-selectride, giving mainly the 3α-OH. This result was confirmed using ^{1}H NMR data of the well-known 3α-OH and 3β-OH 5α-androstanes reported in the literature.[30] The 3α-OH-androstane shows a fine signal at about 4.1 ppm, whereas the 3β-OH analogue gives a broad signal at 3.4 ppm. A flash chromatography is required at this step and a high purity of bromohydrine **6** is crucial for generation of the alkene **7** in excellent C-2,3 isomeric purity. In fact, the residual non-brominated compound **4** will be reduced to a

Scheme 2. Strategy for the Synthesis of Libraries of Aminosteroids[a]



[a] Reagents and conditions: (a) 1,3-dichloro-5,5-dimethylhydantoin, CH_2Cl_2, room temp; (b) imidazole, CH_2Cl_2, room temp (50−80%); (c) 20% piperidine in CH_2Cl_2, (v/v), room temp; (d) carboxylic acid (R^1COOH), PyBOP, HOBt, DIPEA, DMF, room temp; (e) (1) HF/pyridine, CH_2Cl_2, (2) $NaHCO_3$, (3) $MgSO_4$ anhydrous; (f) *N*-Fmoc-L-amino acid (FmocNHCH(R^2 or R^3)COOH), PyBOP, HOBt, DIPEA, DMF, room temp.

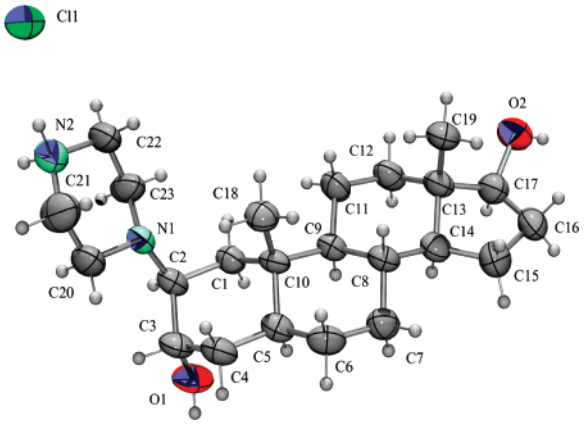


Figure 2. 3D crystal structure of **10** showing the right stereochemistry at the two newly generated stereocenters (2β-piperazine and 3α-OH).

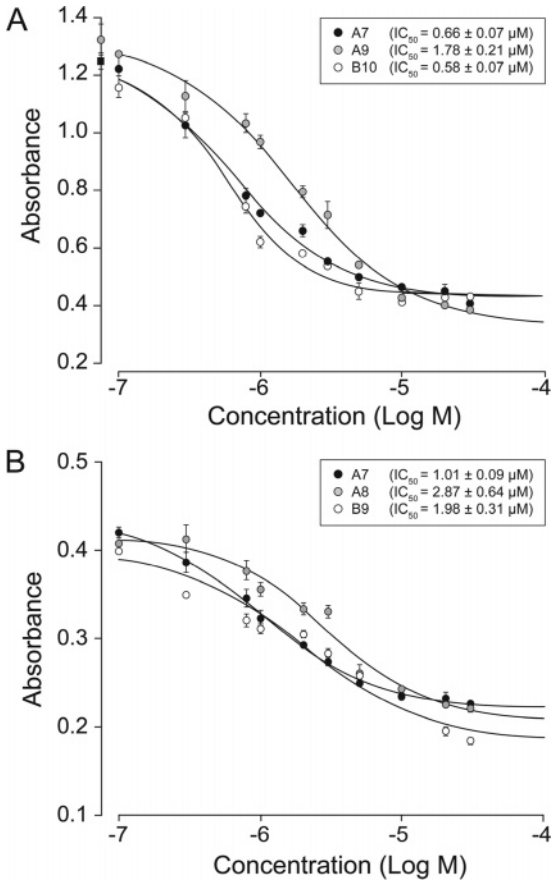
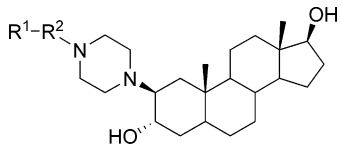


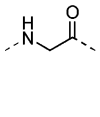
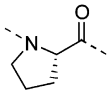
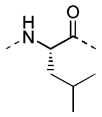
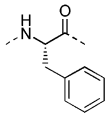
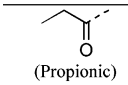
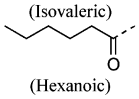
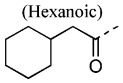
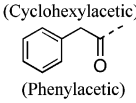
Figure 3. Effect of increasing concentrations of selected aminosteroids on HL-60 cell growth represented by the absorbance (see Experimental Section).

3α-OH compound (3α-hydroxy-17β-acetoxy-5α-androstane). However, the latter product must be totally absent during the next step because the elimination of this alcohol will give a mixture of 2,3- and 3,4-alkenes. Unfortunately, our attempt to purify this mixture by crystallization or chromatography was unsuccessful. However, the elimination of pure bromohydrine **6** in refluxing acetic acid with zinc powder gave the 2,3-alkene **7**. Hydrolysis of the acetate and epoxidation with *m*-CPBA provided the 2,3α-epoxide **9**. This was later regio- and stereoselectively opened to give,[31] after *N*-Fmoc protection of the piperazino nucleus of **10**, the solid-phase precursor **11**.

The C2β- and C3α-stereochemistry of **11** was determined by NMR analysis. After the key signals at C2 and C3 were identified, they were compared with ^1H and ^{13}C NMR data available in the literature for a steroid acting as a neuromuscular drug and possessing a 2β-morpholine and a 3α-OH group.[32] Our data for piperazino derivative **11** (2α-CH, 2.75 and 64.9 ppm; 3β-CH, 3.85 and 63.7 ppm) agree very well with reported data for the morpholino derivative (2α-

Table 1. Compound Number (in Bold) and Inhibition (%) of HL-60 Cell Growth at Two Concentrations (1 μM/10 μM) of Library A Members[a,b]



carboxylic acids (R^1)	amino acids (R^2)				
	 (Gly)	 (Pro)	 (Leu)	 (Phe)	No AA
 (Propionic)	**A1** 0 / 9	**A6** 0 / 72	**A11** 14 / 36	**A16** 0 / 76	**A21** 9 / 13
 (Isovaleric)	**A2** 9 / 13	**A7** [c] 44 / 93	**A12** ND	**A17** 7 / 87	**A22** 18 / 24
 (Hexanoic)	**A3** 0 / 44	**A8** [c] 49 / 91	**A13** 17 / 86	**A18** 8 / 98	**A23** 37 / 69
 (Cyclohexylacetic)	**A4** 6 / 61	**A9** [c] 40 / 96	**A14** 14 / 93	**A19** 12 / 87	**A24** 39 / 95
 (Phenylacetic)	**A5** 0 / 3	**A10** 34 / 79	**A15** 12 / 70	**A20** 16 / 96	**A25** 18 / 75

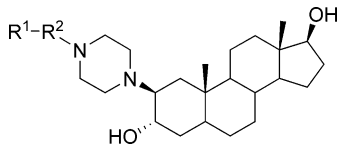
[a] HY inhibited 20% of cell proliferation at 10 μM. Potent cytotoxic agent doxorubicin inhibited 96 and 99% of cell proliferation at 1 and 10 μM, respectively. [b] Library members **A1**−**A20** (compound **16**) were generated from **14**, whereas **A21**−**A25** (compound **15**) were generated from **13** (see Scheme 2). [c] These compounds were purified and used for IC_{50} determination.

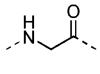
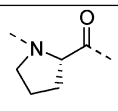
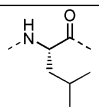
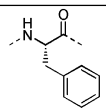
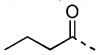
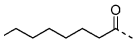
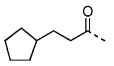
CH, 2.54 and 65.2 ppm; 3β-CH, 3.89 and 63.9 ppm). X-ray analysis of aminosteroid **10** (Figure 2), which is the direct precursor of **11**, unambiguously confirmed that the stereochemistry of all centers was the expected one.

2. Solid-Phase Synthesis of Libraries A, B, and C. *N*-Fmoc-2β-piperazino-5α-androstane-3α,17β-diol (**11**) was coupled to chlorosilyl resin, previously generated in situ from butyldiethylsilane polystyrene (PS-DES resin). In the coupling reaction,[24] the resin was swelled in dry dichloromethane and treated with imidazole and the steroid (Scheme 2). The coupling yields of **11** giving **12**, calculated either by the increase of the resin weight or by the difference between the initial amount of steroid **11** and the amount of uncoupled **11**, were in the range of 50−80%, depending on the batch of resin **12**. The IR spectra of resin **12** showed the presence of the characteristic carbamate band. The Fmoc protecting group was easily cleaved with a solution of 20% piperidine in dichloromethane to give resin **13**, which showed the characteristic IR and ^{13}C NMR signals of steroid **10** linked on PS-DES resin. Resin **13** was split and placed in the vessels of the reaction block of an ACT-Labtech semi-automated synthesizer for parallel synthesis. Library A (Table 1) contains two series of compounds **15** and **16** that were generated in parallel in the same experiment. Five compounds **15** with only one level of diversity were obtained by an acylation (PyBOP/HOBt) of five resin **13** samples using the selected carboxylic acids and after cleavage of the silyl bond by vortexing the resin with a solution of HF-pyridine in dichloromethane. On the other hand, twenty compounds **16** with two levels of diversity were generated by an acylation of twenty resin **13** samples using the selected Fmoc-protected aminoacids, followed by the Fmoc cleavage, an acylation with selected carboxylic acids, and final cleavage of the steroid from the solid support with a solution of HF-pyridine in dichloromethane. Library B (Table 2) containing two series of compounds **15** and **16** was generated as reported above for library A but with different carboxylic acids as building blocks. Library C contains eighty compounds **17** with three levels of diversity. They were obtained from the intermediate resin **14** following the sequence of reactions reported above for the synthesis of **16**. Briefly, cleavage of the Fmoc protecting group of **14**, introduction of a second level of diversity by coupling a Fmoc-protected aminoacid (R^3) activated with PyBOP and HOBt, cleavage of the Fmoc protecting group, introduction of the last level of diversity with a carboxylic acid (R^1), and release of the steroid from the solid support afforded **17**.

All members of the three libraries were analyzed by TLC, and the results confirmed the reactivity of all building blocks used in the elaboration of the libraries. The TLC analysis also confirmed the library uniformity and allowed us to perform a random sampling of each library. The selected members were then characterized by ^1H NMR and MS analyses, and the purity was determined by HPLC. The purity

Table 2. Compound Number (in Bold) and Inhibition (%) of HL-60 Cell Growth at Two Concentrations (1 μM/10 μM) of Library B Members[a,b]



carboxylic acids (R^1)	amino acids (R^2)				
	 (Gly)	 (Pro)	 (Leu)	 (Phe)	No AA
 (Isobutyric)	**B1** 0 / 0	**B6** 16 / 87	**B11** 22 / 65	**B16** 0 / 84	**B21** 0 / 0
 (Butyric)	**B2** 0 / 9	**B7** 24 / 88	**B12** 18 / 43	**B17** 0 / 68	**B22** 0 / 8
 (Octanoic)	**B3** 0 / 45	**B8** 15 / 91	**B13** 0 / 51	**B18** 1 / 11	**B23** 36 / 95
 (Cyclopentyl Propionic)	**B4** 5 / 55	**B9**[c] 49 / 95	**B14** 4 / 73	**B19** 2 / 54	**B24** 36 / 84
 (Cyclohexyl Carboxylic)	**B5** 0 / 16	**B10**[c] 58 / 93	**B15** 15 / 70	**B20** 0 / 82	**B25** 0 / 0

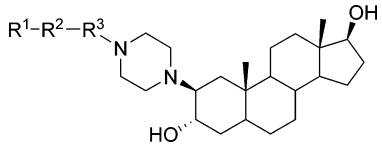
[a] HY inhibited 20% of cell proliferation at 10 μM. Potent cytotoxic agent doxorubicin inhibited 96 and 99% of cell proliferation at 1 and 10 μM, respectively. [b] Library members **B1**−**B20** (compound **16**) were generated from **14**, whereas **B21**−**B25** (compound **15**) were generated from **13** (see Scheme 2). [c] These compounds were purified and used for IC_{50} determination.

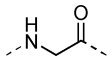
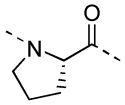
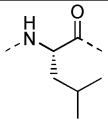
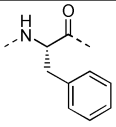
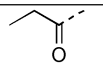
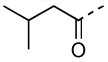
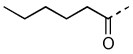
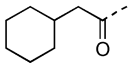
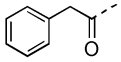
of aminosteroids released from the resin was found to be 80−88% (mean of 84%) for library A, 52−92% (mean of 77%) for library B, and 60−80% (mean of 71%) for library C. The overall mean crude yields for the solid-phase sequence of reactions were 75% for library A (6 steps), 94% for library B (6 steps), and 93% for library C (8 steps). To identify some hits, all members of libraries A−C were submitted to a preliminary antiproliferative assay in HL-60 cells.

3. Antiproliferative Effect of Aminosteroids on HL-60 Cells. Newly synthesized aminosteroids from libraries A−C were tested to evaluate their effect on human myeloid leukemia HL-60 cell growth. The assay was performed at concentrations of 1 and 10 μM for each compound, and the results are expressed as the percentage of cell growth inhibition (Tables 1−3). Interesting SAR results were obtained from the 130 aminosteroids. Thus, for compounds **A21**−**A25** and **B21**−**B25**, with only one level of diversity (R^1), the cyclohexylacetyl and octanoyl groups gave the best inhibition. In fact, compounds **A24** and **B23** inhibited >90% of the cell proliferation at 10 μM. For compounds **A1**−**A20** and **B1**−**B20**, with two levels of diversity (R^1 and R^2), the results also demonstrated the efficiency of a cyclohexylacetyl or an octanoyl as capping group, although similar groups, such as cyclopentylpropionyl and hexanoyl also gave good results. However, the antiproliferative effect is strongly modulated by the presence and nature of an aminoacid. Indeed, compounds bearing a proline at R^2 showed the highest antiproliferative activity, better than the effect obtained with a leucine or a phenylalanine. Only a weak activity was achieved by compounds bearing a glycine at the same position. Compounds **A7**−**A9**, **A14**, **A18**, **A20**, and **B8**−**B10** showed >90% cell growth inhibition at 10 μM, but at the lower concentration of 1 μM, only compounds with a proline as amino acid gave interesting results.

The aminosteroids from library C contain three levels of diversity: the first two levels (R^2 and R^3) consist of Gly, Pro, Leu, or Phe amino acids, and the last level (R^1) consists of one of the five carboxylic acids previously used for the elaboration of library A. Most of them were clearly less active than analogue compounds of library A bearing the same element of diversity but only one or two levels of diversity. The Gly−Gly arrangement gave practically inactive compounds (0−30% of inhibition). Only seven compounds gave over 90% of inhibition at 10 μM, the best combination of amino acids being Phe (as level R^2) and Pro (as level R^3) with 66−96% of growth inhibition and Phe−Gly with 37−94%. Five hits were obtained with these combinations of amino acids (**C63**, **C64**, **C65**, **C67**, and **C69**). The combination Leu−Pro, with a cyclohexylacetyl capping group (**C49**), and the combination Pro−Phe, with a phenylacetyl capping

Table 3. Compound Number (in Bold) and Inhibition (%) of HL-60 Cell Growth at Two Concentrations (1 μM/10 μM) of Library C Members[a,b]



carboxylic acids (R^1)	amino acids (R^3)															
	 (Gly)				 (Pro)				 (Leu)				 (Phe)			
	amino acids (R^2)															
	Gly	Pro	Leu	Phe	Gly	Pro	Leu	Phe	Gly	Pro	Leu	Phe	Gly	Pro	Leu	Phe
 (Propionic)	**C1** 0/6	**C6** 8/8	**C11** 5/15	**C16** 0/0	**C21** 0/17	**C26** 0/0	**C31** 13/22	**C36** 0/10	**C41** 0/12	**C46** 0/10	**C51** 8/43	**C56** 3/18	**C61** 5/76	**C66** 10/66	**71** 4/72	**C76** 0/41
 (Isovaleric)	**C2** 6/8	**C7** 0/28	**C12** 9/13	**C17** 0/0	**C22** 1/7	**C27** 0/60	**32** 0/29	**C37** 0/46	**C42** 8/4	**C47** 0/48	**C52** 19/58	**C57** 0/29	**62** 0/37	**C67**[c] 8/96	**C72** 10/82	**C77** 0/37
 (Hexanoic)	**C3** 0/8	**C8** 21/23	**C13** 0/0	**C18** 0/6	**C23** 0/17	**C28** 3/52	**C33** 1/31	**C38** 1/73	**C43** 0/44	**C48** 0/63	**C53** 20/55	**C58** 2/5	**C63** 0/92	**C68** 10/89	**C73** 7/56	**C78** 0/14
 (Cyclohexyl-acetic)	**C4** 2/12	**C9** 4/30	**C14** 0/2	**C19** 0/28	**C24** 3/13	**C29** 0/41	**C34** 4/46	**C39** 0/82	**C44** 0/71	**C49**[c] 0/98	**C54** 8/33	**C59** 2/8	**C64** 0/92	**C69** 9/95	**C74** 9/47	**C79** 0/1
 (Phenylacetic)	**C5** 0/6	**C10** 12/16	**C15** 5/4	**C20** 0/17	**C25** 0/22	**C30** 6/4	**C35** 5/51	**C40**[c] 10/90	**C45** 4/13	**C50** 2/27	**C55** 14/31	**C60** 8/30	**C65** 5/94	**C70** 11/86	**C75** 9/48	**C80** 2/34

[a] HY inhibited 20% of cell proliferation at 10 μM. Potent cytotoxic agent doxorubicin inhibited 96 and 99% of cell proliferation at 1 and 10 μM, respectively. [b] Library members **C1**−**C80** (compound **17**) were generated from **14** (see Scheme 2). [c] These compounds were purified and tested for purpose of comparison with the crude material.

group (**C40**), gave two more hits. At the lower concentration of 1 μM, however, their cell growth inhibition was negligible (0−10%). To ascertain that the lower efficiency of library C members was not the result of their purity (about 71%), three representative compounds (**C40**, **C49**, and **C67**) were selected and tested in HL-60 cells before and after purification. The results indicated no significant difference between the crude and purified library members (Supporting Information).

We also tested aminosteroid HY (**1**) and used doxorubicin, a well-known potent cytotoxic agent,[33] as a positive control (Table 1). Contrary to data previously reported in the literature,[12] the results indicated that the lead compound HY only has a weak effect on cell growth. Indeed, it inhibited about 20% of cell growth after 3 days of treatment at 10 μM. The aminosteroids from libraries A and B produced a much better inhibition of cell growth as represented by compounds **A7**, **A8**, **A9**, **B9**, and **B10**, which inhibited 40−58% and 93−96% of cell proliferation at 1 and 10 μM, respectively. At these two concentrations, their percentages of growth inhibition are just slightly lower than those of doxorubicin (96 and 99%).

The five aminosteroids exhibiting the best cytotoxic effect in our screening assay were selected for purification, characterization, and validation of biological activity. **A7**, **A9**, and **B10** were synthesized using the same approach presented above in larger amounts and were purified by flash chromatography, whereas **A8** and **B9** were purified by TLC. The five compounds have a proline as amino acid element of diversity (R^2), suggesting its important contribution to the cytotoxic activity. The yields (~65% for 5 steps after final purification) and purity obtained after this second synthesis were representative of the previously synthesized compounds. With pure compounds in our hands, we determined their IC_{50} values against HL-60 cells (Figure 3). Compounds **B10** and **A7** showed a better antiproliferative activity than **A9** since they displayed IC_{50} values of 0.58 ± 0.07 and 0.66 ± 0.07 μM, respectively, compared to 1.78 ± 0.21 μM for **A9**. Under the same conditions, compounds **B9** and **A8** (IC_{50} = 1.98 ± 0.31 and 2.87 ± 0.64 μM) showed a lower

antiproliferative activity than **A7** ($IC_{50} = 1.01 \pm 0.09\ \mu M$). Interestingly, these two experiments gave very similar IC_{50} values for **A7**, indicating the good reproducibility of our cell proliferation assay.

Conclusion

To speed up the development of aminosteroids with structural similarity to HY (Figure 1), we carried out a sequence of reactions taking advantage of our expertise in the solid-phase parallel synthesis of steroid derivatives. The key intermediate **11** (Scheme 1) was efficiently synthesized from DHT in 8 steps with an overall yield of 40%. Three libraries were then rapidly generated following a parallel approach (Scheme 2) giving individual compounds pure enough to be screened in our proliferative assay in HL-60 leukemia cells. Compounds **16** with two levels of diversity gave the best antiproliferative activity, especially for those bearing a proline at R^2 and an appropriate hydrophobic carboxylic acid as an *N*-capping group at R^1. In addition to generating SAR data, our results suggest that it is possible to modulate the antiproliferative activity by addition on a 3β-piperazinyl-5α-androstane-3β,17β-diol steroidal core of an appropriate combination of an amino acid and a carboxylic acid. We thus obtained several compounds with more potent cytotoxic activity than the lead compound HY. We also confirmed our results with pure aminosteroids **A7**, **A8**, **A9**, **B9**, and **B10**, thus establishing the usefulness of our solid-phase strategy.

The next step for us is to extend our SAR study by synthesizing additional libraries of aminosteroids **16** taking advantage of the large diversity of amino acids (natural and non-natural) and carboxylic acids commercially available. Although our preliminary results seem to indicate the efficiency of hydrophobic building blocks, our selection for the elaboration of libraries A−C was limited to only four aminoacids, mainly for the purpose of developing our chemical strategy. We must now extend this selection to a wider variety of groups. Thus the model libraries reported herein constitute a basic study for the preparation of future more voluminous libraries of aminosteroids exhibiting antiproliferative effects against leukemia cells.

Experimental Section

General Methods. Dihydrotestosterone (DHT) was purchased from Steraloids (Wilton, NH). The butyldiethylsilane polystyrene (PS-DES resin) with a loading of 1.58 mmol g^{-1} was supplied by Argonaut Technologies (San Carlos, CA). Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and Calbiochem-Novabiochem Corp. (San Diego, CA). The usual solvents were obtained from Fisher Scientific (Montreal, QC, Canada) and were used as received. Anhydrous dichloromethane (CH_2Cl_2), dimethylformamide (DMF), and pyridine were obtained from Sigma-Aldrich. The loading of steroid **11** on resin was performed in peptide synthesis vessels (25 mL) with frit equipped for vacuum filtration (ChemGlass Inc., Vineland, NJ). The reaction vessels were shaken with a Burrell wrist-action shaker model 75 (Pittsburgh, PA); the libraries of steroid derivatives were produced with an ACT LabTech manual synthesizer (Advanced ChemTech, Louisville, KY) using either 40 or 96 solid-phase reaction blocks. The completion of solid-phase reactions were monitored after a microcleavage by thin-layer chromatography (TLC). TLC and flash-column chromatography were performed on 0.20 mm silica gel 60 F254 plates and with 230−400 mesh ASTM silica gel 60, respectively (E. Merck, Darmstadt, Germany). The purity of a random sampling of final compounds released from solid support was determined by HPLC (Waters Associates Milford, MA) using a Nova Pak C18 reversed-phase column (150 mm × 3.9 mm i.d., 4 μm, 60 Å) and methanol containing 10 mM sodium acetate as eluent. The wave length of the UV detector was selected between 207 and 216 nM. Infrared spectra (IR) were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT), and the significant bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz for ^1H and 75.5 MHz for ^{13}C on a Bruker AC/F300 spectrometer (Billerica, MA) or 400 MHz for ^1H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer. The chemical shifts (δ) were expressed in parts per million and referenced to chloroform (7.26 and 77.0 ppm) or methanol (3.30 and 49.0 ppm) for ^1H and ^{13}C NMR, respectively. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA) equipped with a turbo ion-spray source. High-resolution mass spectra (HRMS) were provided by The Mass Spectrometry Unit (McGill University, Montréal QC, Canada). The X-ray analysis was performed by Marc Drouin at the Laboratoire de diffraction des rayons-X de l'Université de Sherbrooke (Sherbrooke QC, Canada). Elemental analyses were provided by the Regional Laboratory for Instrumental Analysis (Université de Montréal, Montréal QC, Canada).

17β-Acetoxy-5α-androstan-3-one (4). Acetic anhydride (68 mL, 723 mmol) was added under argon at room temperature to a solution of dihydrotestosterone (**3**) (21.02 g, 72.4 mmol) and dimethylaminopyridine (DMAP) (177 mg, 1.45 mmol) in dry pyridine (50 mL). The resulting mixture was stirred for 3 h and concentrated under vacuum, and the residue was treated with HCl 20% (800 mL). The product was extracted with EtOAc, and the organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 800 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc/hexanes, 1:5) yielded 23.9 g (99%) of **4** as a white solid. IR (film): ν 1736 (C═O, ester), 1719 (C═O, ketone). ^1H NMR ($CDCl_3$): δ 0.80 (s, 18-CH_3), 1.01 (s, 19-CH_3), 0.75−2.40 (residual CH and CH_2), 2.04 (s, CH_3CO), 4.59 (dd, $J = 9.0$ Hz, $J = 8.0$ Hz, 17α-CH). ^{13}C NMR ($CDCl_3$): δ 11.42, 12.08, 20.85, 21.16, 23.47, 27.48, 28.71, 31.16, 35.10, 35.66, 36.76, 38.08, 38.43, 42.57, 44.61, 46.55, 50.50, 53.64, 82.66, 171.16, 211.88. LRMS for $C_{21}H_{33}O_3$ $[M + H]^+$: 333.4 *m*/*z*.

2α-Bromo-17β-acetoxy-5α-androstan-3-one (5). A 1 M solution of bromine (2.4 mL of Br_2 in 47 mL of AcOH) was added dropwise to a solution of **4** (15.34 g, 46.2 mmol) in glacial acetic acid (460 mL) at room temperature. The resulting mixture was stirred for 1.5 h and concentrated under reduced pressure. The resulting red solution was dissolved in EtOAc (500 mL) and washed with a saturated aqueous

solution of $NaHCO_3$ (2 × 500 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc/hexanes, 1:5) yielded 18.0 g (95%) of **5** as a white solid. IR (film): ν 1729 (C═O, ketone and ester), 732 (C−Br). ^{1}H NMR ($CDCl_3$): δ 0.80 (s, 18-CH_3), 1.09 (s, 19-CH_3), 0.80−2.45 (residual CH and CH_2), 2.04 (s, CH_3CO), 2.63 (dd, J = 13.4 Hz, J = 6.3 Hz, 1H), 4.58 (dd, J = 9.0 Hz, J = 7.9 Hz, 17α-CH), 4.74 (J = 13.4 Hz, J = 6.3 Hz, 2β-CH). ^{13}C NMR ($CDCl_3$): δ 12.10 (2×), 20.96, 21.18, 23.47, 27.46, 28.18, 30.95, 34.65, 36.58, 38.96, 42.53, 43.82, 47.37, 50.33, 51.56, 53.45, 54.39, 82.52, 171.18, 201.01. LRMS for $C_{21}H_{32}{}^{79}BrO_3$ [M + H]$^+$: 411.3 *m*/*z*. HRMS Calcd for $C_{21}H_{31}{}^{79}BrO_3Na$ [M + Na]$^+$: 433.13488. Found: 433.13463.

2α-Bromo-17β-acetoxy-5α-androstan-3α-ol (6). A 1 M solution of K-selectride (21 mL, 21 mmol) was added dropwise under argon at −78 °C to a solution of **5** (4.3 g, 10.5 mmol) in dry THF (100 mL). The resulting mixture was stirred for 1 h, and the temperature was increased to 0 °C; a saturated aqueous solution of NH_4Cl (400 mL) was then added. The resulting mixture was extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting crude bromohydrin was purified by flash chromatography (EtOAc/hexanes, 10:90) to afford 3.07 g (71%) of **6** as a white solid. IR (film): ν 3447 (OH), 1717 (C═O), 735 (C−Br). ^{1}H NMR ($CDCl_3$): δ 0.77 (s, 19-CH_3), 0.83 (s, 18-CH_3), 2.03 (s, CH_3CO), 0.60−2.20 (residual CH and CH_2), 4.05 (s, 3β-CH), 4.46 (m, 2β-CH), 4.58 (t, J = 8.4 Hz, 17α-CH). ^{13}C NMR ($CDCl_3$): δ 11.79, 12.03, 20.20, 21.11, 23.37, 27.29, 27.38, 31.06, 34.57, 34.76, 36.59, 37.40, 39.56, 42.44, 43.42, 50.47, 53.80, 58.33, 69.57, 82.60, 171.10. LRMS for $C_{21}H_{37}{}^{79}BrO_3N$ [M + NH_4]$^+$: 429.9 *m*/*z*. HRMS Calcd for $C_{21}H_{33}{}^{79}BrO_3Na$ [M + Na]$^+$: 435.15053. Found: 435.15008.

17β-Acetoxy-5α-androst-2-ene (7). Zinc dust (3.77 g, 57.7 mmol) was added to a solution of bromohydrin **6** (3.4 g, 8.2 mmol) in acetic acid (100 mL), and the mixture was refluxed for 2 h. The solution was filtered, concentrated under vacuum, and diluted in water, and the product was extracted with EtOAc. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ until neutralization, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc/hexanes, 5:95) yielded 2.15 g (83%) of alkene **7** as a white solid. IR (film): ν 3018 (C═C), 1737 (C═O). ^{1}H NMR ($CDCl_3$): δ 0.75 (s, 19-CH_3), 0.78 (s, 18-CH_3), 0.75−2.20 (residual CH and CH_2), 2.04 (s, CH_3CO), 4.58 (dd, J = 9.0 Hz, J = 7.9 Hz, 17α-CH), 5.58 (m, 2H alkene). ^{13}C NMR ($CDCl_3$): δ 11.66, 12.04, 20.34, 21.21, 23.48, 27.49, 28.55, 30.23, 31.32, 34.66, 35.33, 36.89, 39.72, 41.39, 42.47, 50.67, 53.94, 82.90, 125.80 (2×), 171.31. LRMS for $C_{21}H_{33}O_2$ [M + H]$^+$: 317.3 *m*/*z*. HRMS Calcd for $C_{21}H_{32}O_2Na$ [M + Na]$^+$: 339.22945. Found: 339.22922.

5α-Androst-2-en-17β-ol (8). A solution of K_2CO_3 (3.15 g, 22.8 mmol) in water (20 mL) was added to a solution of **7** (1.80 g, 5.7 mmol) in methanol (100 mL), and the mixture was refluxed for 1.5 h. The reaction mixture was diluted in water and extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc/hexanes, 1:5) yielded 1.55 g (98%) of **8** as a white solid. IR (film): ν 3252 (OH), 3017 (C═C). ^{1}H NMR ($CDCl_3$): ν 0.74 (s, 19-CH_3), 0.76 (s, 18-CH_3), 0.70−2.10 (residual CH and CH_2), 3.63 (t, J = 8.5 Hz, 17α-CH), 5.58 (m, 2H alkene). ^{13}C NMR ($CDCl_3$): δ 11.05, 11.71, 20.48, 23.37, 28.60, 30.28, 30.50, 31.37, 34.70, 35.63, 36.75, 39.79, 41.51, 42.86, 51.00, 54.17, 82.01, 125.85 (2×). LRMS for $C_{19}H_{34}ON$ [M + NH_4]$^+$: 292.3 *m*/*z*. HRMS Calcd for $C_{19}H_{30}OAg$ [M + Ag]$^+$: 381.13412. Found: 381.13421.

2α,3α-Epoxy-5α-androstan-17β-ol (9). *m*-Chloroperbenzoic acid (*m*-CPBA, 77% pure, 1.23 g, 5.46 mmol) was added in six portions to a solution of **8** (1.02 g, 3.7 mmol) in dry CH_2Cl_2 (35 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C; then it was allowed to warm to room temperature and was stirred overnight. The mixture was concentrated under reduced pressure; the residue was diluted in EtOAc, and the solution was washed successively with a saturated aqueous solution of $Na_2S_2O_3$ (2 × 100 mL) and a saturated aqueous solution of Na_2CO_3 (2 × 100 mL). It was then dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc/hexanes, 1:5) yielded 909 mg (85%) of **9** as a white solid. IR (film): ν 3262 (OH). ^{1}H NMR ($CDCl_3$): δ 0.72 (s, 19-CH_3), 0.76 (s, 18-CH_3), 0.55−2.10 (residual CH and CH_2), 3.12 (m, 2H epoxide), 3.63 (t, J = 8.5 Hz, 17α-CH). ^{13}C NMR ($CDCl_3$): δ 11.02, 12.95, 20.45, 23.34, 28.26, 29.00, 30.44, 31.20, 33.70, 35.67, 36.28, 36.60, 38.27, 42.78, 50.78, 51.04, 52.42, 53.79, 81.88. LRMS for $C_{19}H_{31}O_2$ [M + H]$^+$: 291.3 *m*/*z*;. HRMS Calcd for $C_{19}H_{30}O_2Na$ [M + Na]$^+$: 313.21380. Found: 313.21362.

2β-Piperazino-5α-androstane-3α,17β-diol (10). A solution of **9** (6.46 g, 22.3 mmol) in piperazine (50 g, 582 mmol) and water (6.3 mL) was refluxed (160 °C) for 24 h. The mixture was poured in water (500 mL), and the precipitate was filtered. The solid was dissolved in CH_2Cl_2, and the solution was dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (MeOH/Et_3N/CH_2Cl_2, 14:1:85) yielded 5.76 g (69%) of **10** as a white solid. IR (NaCl film): ν 3370 (OH, alcohols and NH, amine). ^{1}H NMR (CD_3OD): δ 0.74 (s, 18-CH_3), 0.99 (s, 19-CH_3), 0.70−2.25 (residual CH and CH_2), 2.33, 2.48, 2.55 and 2.82 (4 m, 4 × CH_2N and 2α-CH), 3.54 (t, J = 8.6 Hz, 17α-CH), 4.05 (m, 3β-CH). ^{1}H NMR ($CDCl_3$): δ 0.71 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.65−2.15 (residual CH and CH_2), 2.42, 2.58 and 2.90 (3 m, 4 × CH_2N and 2α-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.84 (m, 3β-CH). ^{13}C NMR (CD_3OD): δ 11.7, 14.6, 21.8, 24.3, 29.1, 30.6, 32.7, 34.3, 36.2, 36.6, 37.3, 38.1, 40.5, 44.2, 46.8 (2×), 52.0 (2×), 52.4, 57.0, 66.4, 66.9, 82.5. ^{13}C NMR ($CDCl_3$): δ 11.2, 17.3, 20.9, 23.3, 28.2, 30.5, 31.1, 32.6, 34.7, 35.5, 35.7, 36.9, 38.4, 43.0, 46.7 (2×), 49.4 (2×), 50.9, 56.2, 63.3, 65.0, 81.6. LRMS for $C_{23}H_{41}O_2N_2$ [M + H]$^+$: 377.3 *m*/*z*. HRMS Calcd for $C_{23}H_{41}O_2N_2$ [M + H]$^+$: 377.31625. Found: 377.31596.

2β-[(*N*-(9-Fluorenylmethoxycarbonyl)-piperazino]-5α-androstane-3α,17β-diol (11). Aqueous $NaHCO_3$ (1 M, 37 mL) and *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu) in six portions were added successively to a solution of **10** (5.76 g, 15.3 mmol) in a mixture of THF/

water (5:1, 275 mL). The mixture was stirred for 3 h then diluted in water and extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc/hexanes, 1:1), and crystallization from a mixture of CH_2Cl_2 and hexanes yielded 6.1 g (70%) of **11** as a white solid. IR (NaCl film): ν 3423 (OH, alcohol), 1690 (C═O, carbamate), 1448 (aromatic ring), 1243 (C−O−C, carbamate). ^{1}H NMR ($CDCl_3$): δ 0.74 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70−2.20 (residual CH and CH_2), 2.4−2.9 (broad, 2 × CH_2N and 2α-CH), 3.4−3.7 (broad, 2 × CH_2NCO), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.85 (m, 3β-CH), 4.24 (t, J = 6.6 Hz, C*H*CH_2 of Fmoc), 4.45 (d, J = 6.3 Hz, CH_2O of Fmoc), 7.34 (t, J = 7.4 Hz, 2H of Fmoc), 7.40 (t, J = 7.4 Hz, 2H of Fmoc), 7.57 (d, J = 7.4 Hz, 2H of Fmoc), 7.77 (d, J = 7.5 Hz, 2H of Fmoc). ^{13}C NMR ($CDCl_3$): δ 11.2, 17.3, 20.9, 23.3, 28.1, 30.5, 31.1, 32.9, 34.7, 35.5, 35.8, 36.8, 38.4, 43.1, 44.1, 47.3, 48.0, 50.8, 56.1, 63.7, 64.9, 67.2, 81.8, 119.9 (2×), 124.9 (2×), 127.0 (2×), 127.7 (2×), 141.3, 143.9, 155.0. LRMS for $C_{38}H_{51}O_4N_2$ $[M + H]^+$: 599.3 *m*/*z*. HRMS Calcd for $C_{38}H_{51}O_4N_2$ $[M + H]^+$: 599.38433. Found: 599.38400.

Synthesis of Resin 12. 1,3-Dichloro-5,5-dimethylhydantoin (1.86 g, 9.47 mmol) in dry CH_2Cl_2 (10 mL) was added to PS-DES resin (2.00 g, 1.58 mmol g^{-1} theoretical loading) that had been dried under vacuum during 2 days, then put into a 50 mL peptide flask under argon, and swollen in dry CH_2Cl_2 (10 mL). After 1 h, the resulting chlorosilyl resin was washed under argon with dry CH_2Cl_2 (3 × 20 mL). The disappearance of the SiH band at 2100 cm^{-1} was confirmed in the IR spectrum, and the resin was next used for the loading step. Under argon, the resin was swollen in dry CH_2Cl_2 (10 mL), and a solution of imidazole (645 mg, 9.46 mmol) and hydroxysteroid **11** (5.66 g, 9.47 mmol) in CH_2Cl_2 (10 mL) was added. The mixture was vortexed with a Burrell wrist-action shaker for 4 h at room temperature. The resin was washed with CH_2Cl_2 (5 × 20 mL) and MeOH (3 × 20 mL) and was dried overnight under vacuum to give 3.24 g of **12** with a loading of 0.64 mmol g^{-1}. IR (KBr): ν 3465 (OH, alcohol), 1702 (C═O, carbamate). The free steroid **11** (4.26 g) was easily recovered after a flash chromatography with EtOAc/hexanes (1:1).

Synthesis of resin 13. A solution of piperidine in CH_2Cl_2 (1:5, v/v) (30 mL) was added to the resin **12** (3.24 g, 2.07 mmol), and the resulting solution was vortexed for 1 h at room temperature. The resin was then washed with CH_2Cl_2 (3 × 30 mL) and MeOH (3 × 30 mL) and dried overnight under vacuum to give 2.75 g of **13**. IR (KBr): ν 3435 (OH, alcohol and NH, amine), no C═O band of carbamate at 1702 cm^{-1}. ^{13}C NMR ($CDCl_3$, using the conditions reported in reference 24): δ 4.40, 5.33, 6.24, 6.76, 6.95, 11.46, 12.25, 13.09, 14.06, 17.25, 20.95, 22.41, 22.97, 23.45, 28.27, 31.19, 32.65, 34.64, 35.53, 35.73, 37.21, 38.44, 40.27, 43.41, 46.54, 49.38, 50.62, 56.29, 63.44, 65.08, 81.62, 127.90.

Synthesis of Library A. The library members (Table 1) represented by compounds **15** and **16** were synthesized in parallel from 25 samples of resin **13**.

Synthesis of 15. A solution of benzotriazole-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) (33.4 mg, 0.064 mmol), *N*-hydroxybenzotriazole (HOBt) (8.7 mg, 0.064 mmol), carboxylic acid (0.064 mmol) (propionic acid, isovaleric acid, hexanoic acid, cyclohexylacetic acid, or phenylacetic acid), and diisopropylethylamine (DIPEA) (22.4 μL, 0.128 mmol) in dry DMF (0.5 mL) was added to five samples of resin **13** (5 × 50 mg, 0.032 mmol) under argon. The suspension was vortexed for 2 h at room temperature. The resin was washed with CH_2Cl_2 (5 × 2 mL) and MeOH (2 × 2 mL) and dried under vacuum. To release the diversified steroid **15**, each resin was treated with a mixture of CH_2Cl_2/THF (10:1) (0.5 mL) and a solution of HF/pyridine (20 μL) for 45 min. CH_2Cl_2 (0.1 mL) and $NaHCO_3$ (100 mg) were added to the resulting solution, and the mixture was vortexed for 30 min. CH_2Cl_2 (0.2 mL) and anhydrous $MgSO_4$ (100 mg) were added, and the resin was vortexed for 2 min before it was filtered. The filtrate was washed with a saturated solution of $NaHCO_3$ (5 mL) and finally with water (5 mL). The organic layer was evaporated under reduced pressure to give **15** (see Table 1, compounds **A21**−**A25**).

Synthesis of 16. A solution of PyBOP (33.4 mg, 0.064 mmol), HOBt (8.7 mg, 0.064 mmol), *N*-Fmoc-protected L-amino acid (0.064 mmol) (glycine, proline, leucine, or phenylalanine), and DIPEA (22.4 μL, 0.128 mmol) in dry DMF (0.5 mL) was added to twenty samples of resin **13** (20 × 50 mg, 0.032 mmol) under argon. The suspension was vortexed for 2 h at room temperature. The resin was washed with CH_2Cl_2 (5 × 2 mL) and MeOH (2 × 2 mL) and dried under vacuum to give **14**. A solution of piperidine (20%, v/v) in CH_2Cl_2 (0.5 mL) was added to these resins. The mixture was vortexed for 1 h at room temperature, washed with CH_2Cl_2 (5 × 2 mL) and MeOH (2 × 2 mL), and dried under vacuum overnight to give the resins with a free secondary amine. A solution of PyBOP (33.4 mg, 0.064 mmol), HOBt (8.7 mg, 0.064 mmol), a carboxylic acid (0.064 mmol) (propionic acid, isovaleric acid, hexanoic acid, cyclohexylacetic acid, or phenylacetic acid), and DIPEA (22.4 μL, 0.128 mmol) in dry DMF (0.5 mL) was added to each resin under argon. The suspension was vortexed for 2 h at room temperature. The resin was washed with CH_2Cl_2 (5 × 2 mL) and MeOH (2 × 2 mL) and dried under vacuum to give resin. The procedure reported above for the synthesis of **15** was used to release the free steroids **16** (see Table 1, compounds **A1**−**A20**). After a TLC analysis of each member of library A that confirmed the presence of a major compound, the twenty-five compounds with one and two levels of molecular diversity were submitted to a random sampling that selected five compounds (**A5**, **A8**, **A14**, **A16**, and **A22**), which were characterized by ^{1}H NMR and LRMS.

A5. Yield: 43%. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.70−2.20 (residual CH and CH_2), 2.3−2.8 (broad, 4 × CH_2N and 2α-CH), 3.2−3.8 (broad, 17α-CH and 2 × CH_2NCO), 3.63 (s, *CH*$_2$Ph), 4.00 (m, 3β-CH and NH*CH*$_2$CO), 6.55 (NH), 7.30 and 7.37 (2 m, 5H, CH_2*Ph*). LRMS for $C_{33}H_{50}O_4N_3$ $[M + H]^+$: 552.3 *m*/*z*. HPLC purity: 80% (t_R = 28.4 min).

A8. Yield: 56%. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.89 (t, J = 6.8 Hz, *CH*$_3$CH$_2$), 0.70−2.30 (residual CH and CH_2), 2.25 (q, J = 7.6 Hz, CH_2CO), 2.4−3.0 (broad, 2 × CH_2N and 2α-CH), 3.54 and 3.65 (2 m, 17α-CH, 2 × CH_2NCO and CH_2N of proline), 3.95 (m, 3β-

CH), 4.82 (m, NCHCO). LRMS for $C_{34}H_{58}O_4N_3$ $[M + H]^+$: 572.3 *m/z*. HPLC purity: 88% (t_R = 30.2 min).

A14. Yield: 65%. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.91 (d, J = 6.6 Hz, $(CH_3)_2CH$), 0.70−2.10 (residual CH and CH_2), 2.06 (d, J = 6.8 Hz, NHCOCH_2), 2.3−3.0 (broad, 2 × CH_2N and 2α-CH), 3.3−3.9 (broad, 2 × CH_2NCO), 3.63 (q, J = 6.1 Hz, 17α-CH), 3.95 (m, 3β-CH), 4.95 (m, NHCHCO), 6.20 (NH). LRMS for $C_{37}H_{64}O_4N_3$ $[M + H]^+$: 614.3 *m/z*. HPLC purity: 85% (t_R = 32.5 min).

A16. Yield: 69%. ^{1}H NMR ($CDCl_3$): δ 0.75 (s, 18-CH_3), 0.83 (s, 19-CH_3), 1.14 (t, J = 7.6 Hz CH_2CH_3), 0.70−2.20 (residual CH and CH_2), 2.22 (q, J = 7.6 Hz, NHCOCH_2), 2.4−3.1 (broad, 2 × CH_2N, 2α-CH, and CH_2-Ph), 3.2−3.6 (broad, 2 × CH_2NCO), 3.64 (q, J = 6.4 Hz, 17α-CH), 3.82 (m, 3β-CH), 5.17 (m, NHCHCO), 6.38 (NH), 7.20 (m, 2H of Ph), 7.30 (m, 3H of Ph). LRMS for $C_{35}H_{54}O_4N_3$ $[M + H]^+$: 580.3 *m/z*. HPLC purity: 84% (t_R = 29.7 min).

A22. Yield: 64%. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.97 (d, J = 6.5 Hz, $(CH_3)_2CH$), 0.70−2.20 (residual CH and CH_2), 2.22 (d, J = 6.8 Hz, $COCH_2$), 2.4−2.9 (broad, 2 × CH_2N and 2α-CH), 3.4−3.8 (broad, 2 × CH_2NCO), 3.63 (q, J = 6.4 Hz, 17α-CH), 3.91 (m, 3β-CH). LRMS for $C_{28}H_{49}O_3N_2$ $[M + H]^+$: 461.2 *m/z*. HPLC purity: 83% (t_R = 30.0 min).

Synthesis of Library B. For the preparation of this library (Table 2), we used the same procedure as for library A (see above), but the carboxylic acids (R^1 level) were changed to isobutyric acid, butyric acid, octanoic acid, cyclopentylpropionic acid, and cyclohexylcarboxylic acid. The loading of resin **12** was 0.55 mmol g^{-1}, and we used 48 mg of resin for the synthesis of each library member. For the cleavage step that gives **15** and **16**, we performed the reaction only in CH_2Cl_2 instead of CH_2Cl_2/THF (10:1). TLC analysis of each member confirmed the presence of a major compound, and the sampling selected five compounds (**B5**, **B8**, **B14**, **B16**, and **B22**), which were characterized by ^{1}H NMR and LRMS.

B5. Yield: 95%. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.70−2.30 (residual CH and CH_2), 2.6−3.3 (broad, 2 × CH_2N and 2α-CH), 3.5−4.2 (broad, 2 × CH_2NCO, NHCH_2CO, and 3β-CH), 3.63 (q, J = 6.1 Hz, 17α-CH), 6.50 (NH). LRMS for $C_{32}H_{54}O_4N_3$ $[M + H]^+$: 544.3 *m/z*. HPLC purity: 77% (t_R = 29.3 min).

B8. Yield: 92%. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.87 (t, J = 7.0 Hz, CH_3CH_2), 0.88 (s, 19-CH_3), 0.70−2.20 (residual CH and CH_2), 2.30 (m, CH_2CON), 2.5−3.2 (broad, 2 × CH_2N and 2α-CH), 3.55 and 3.66 (2 m, (m, 17α-CH and CH_2N of proline), 3.3−4.0 (broad, 2 × CH_2NCO), 4.02 (m, 3β-CH), 4.80 (m, NCHCO). LRMS for $C_{36}H_{62}O_4N_3$ $[M + H]^+$: 600.3 *m/z*. HPLC purity: 87% (t_R = 31.9 min).

B14. Yield: 96%. ^{1}H NMR ($CDCl_3$): δ 0.74 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.92 and 0.96 (2d, J = 6.5 Hz, $(CH_3)_2$-CH), 0.70−2.15 (residual CH and CH_2), 2.21 (dd, J_1 = 7.0 Hz and J_2 = 8.6 Hz, CH_2CONH), 2.6−3.1 (broad, 2 × CH_2N and 2α-CH), 3.63 (q, J = 6.3 Hz, 17α-CH), 3.4−4.2 (broad, 2 × CH_2NCO), 4.02 (m, 3β-CH), 4.94 (m, NHCHCO), 6.10 (NH). LRMS for $C_{37}H_{64}O_4N_3$ $[M + H]^+$: 614.2 *m/z*. HPLC purity: 79% (t_R = 32.7 min).

B16. Yield: 95%. ^{1}H NMR ($CDCl_3$): δ 0.75 (s, 18-CH_3), 0.88 (s, 19-CH_3), 1.13 and 1.14 (2d, J = 6.8 Hz, $(CH_3)_2$-CH), 0.70−2.2 (residual CH and CH_2), 2.37 (quintet, J = 6.9 Hz, $(CH_3)_2CH$), 2.7−3.2 (broad, 2 × CH_2N and 2α-CH), 3.65 (m, 17α-CH), 3.3−4.2 (broad, 2 × CH_2NCO, CH_2Ph and 3β-CH), 5.05 (m, NHCHCO), 7.32 (m, CH_2Ph), 6.25 (NH). LRMS for $C_{36}H_{56}O_4N_3$ $[M + H]^+$: 594.2 *m/z*. HPLC purity: 52% (t_R = 30.2 min).

B22. Yield: 95%. ^{1}H NMR ($CDCl_3$): δ 0.74 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.97 (t, J = 7.4 Hz, CH_3CH_2), 0.70−2.20 (residual CH and CH_2), 2.29 (t, J = 7.5 Hz, CH_2CO), 2.5−3.2 (broad, 2 x CH_2N and 2α-CH), 3.63 (q, J = 6.3 Hz, 17α-CH), 3.4−4.1 (broad, 2 × CH_2NCO), 4.00 (m, 3β-CH). LRMS for $C_{27}H_{47}O_3N_2$ $[M + H]^+$: 447.4 *m/z*. HPLC purity: 92% (t_R = 29.0 min).

Synthesis of Library C. For the synthesis of library C (Table 3), we used the same building blocks and procedure that were for library A (see above) with only 27 mg of resin **13** (80 × 0.0134 mmol). Furthermore, two levels of amino acids (R^2 and R^3) were successively introduced before the addition of the final carboxylic acids (R^1). To release **17**, we used pure CH_2Cl_2 (0.3 mL) instead of a mixture of CH_2Cl_2/THF (10:1). TLC analysis of each member of library C confirmed the presence of a major compound. From the eighty compounds with three levels of molecular diversity, a sampling selected ten compounds (**C9**, **C20**, **C21**, **C32**, **C42**, **C53**, **C56**, **C64**, **C70**, and **C78**), which were characterized by ^{1}H NMR and LRMS.

C9. Yield: 98%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 1.01 (s, 19-CH_3), 0.70−2.2 (residual CH and CH_2), 2.26 (t, J = 6.8 Hz, CH_2CO), 2.4−2.8 (broad, 2 × CH_2N and 2α-CH), 3.3−3.8 (m, 2 × CH_2NCO, NHCH_2CO, 17α-CH and CH_2N of proline), 4.08 (m sharp, 3β-CH), 4.50 (m, NCHCO). LRMS for $C_{38}H_{63}O_5N_4$ $[M + H]^+$: 655.6 *m/z*. HPLC purity: 70% (t_R = 32.2 min).

C20. Yield: 98%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70−2.1 (residual CH and CH_2), 2.4−2.7 (broad, 2 × CH_2N and 2α-CH), 3.21 (dd, J_1 = 5.0 Hz and J_2 = 14.0 Hz, CHCH_2Ph), 3.47 (d, J = 6.6 Hz COCH_2-Ph), 3.55 (m, 2 × CH_2NCO and 17α-CH), 4.05 (m sharp, 3β-CH and NHCH_2CO), 4.70 (dd, J_1 = 5.0 Hz and J_2 = 9.7 Hz, NHCHCO), 7.08 (d, J = 7.8 Hz, 2H of Ph), 7.20 (m, 8H of 2 × Ph). LRMS for $C_{42}H_{59}O_5N_4$ $[M + H]^+$: 699.5 *m/z*. HPLC purity: 60% (t_R = 31.6 min).

C21. Yield: 98%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 1.01 (s, 19-CH_3), 1.13 (t, J = 7.6 Hz, CH_3CH_2), 0.7−2.1 (residual CH and CH_2), 2.28 (q, J = 7.6 Hz, CH_3CH_2CO), 2.4−2.8 (broad, 2 × CH_2N and 2α-CH), 3.5−3.8 (m, 2 × CH_2NCO, 17α-CH, and CH_2N of proline), 3.9−4.2 (m, 3β-CH and NHCH_2CO), 4.95 (m, NCHCO). LRMS for $C_{33}H_{55}O_5N_4$ $[M + H]^+$: 587.3 *m/z*. HPLC purity: 71% (t_R = 28.0 min).

C32. Yield: 98%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 0.92−0.99 (8s, 2 × $(CH_3)_2CH$), 1.01 (s, 19-CH_3), 0.7−2.3 (residual CH and CH_2), 2.5−2.9 (broad, 2 × CH_2N and 2α-CH), 3.4−3.8 (broad, 2 × CH_2NCO and 17α-CH), 4.09 (m, 3β-CH), 4.70 (m, NHCHCO), 4.90 (m, NCHCO). LRMS for $C_{39}H_{67}O_5N_4$ $[M + H]^+$: 671.5 *m/z*. HPLC purity: 80% (t_R = 32.1 min).

C42. Yield: 91%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 0.95 (m, 2 × (CH_3)$_2$CH), 1.01 (s, 19-CH_3), 0.7−2.1 (residual CH and CH_2), 2.13 (d, J = 6.3 Hz, CHCH_2CO), 2.5−2.9 (broad, 2 × CH_2N and 2α-CH), 3.4−3.7 (broad, 2 × CH_2NCO), 3.55 (t, J = 8.7 Hz, 17α-CH), 3.80 (m, NHCHCO), 3.85 (d, J = 7.2 Hz, NHCH_2CO), 4.10 (m, 3β-CH). LRMS for $C_{36}H_{63}O_5N_4$ [M + H]$^+$: 631.6 *m*/*z*. HPLC purity: 71% (t_R = 32.6 min).

C53. Yield: 95%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 0.90−0.97 (7s, 2 × (CH_3)$_2$)CH and CH_3CH_2), 1.01 (s, 19-CH_3), 0.7−2.1 (residual CH and CH_2), 2.22 (t, J = 7.6 Hz, CH_2CO), 2.5−2.9 (broad, CH_2N and 2α-CH), 3.4−3.9 (broad, 2 × CH_2NCO), 3.55 (t, J = 8.6 Hz, 17α-CH), 4.09 (3β-CH), 4.39 (t, J = 7.6 Hz, NHCHCO), 4.95 (m, NHCHCO). LRMS for $C_{41}H_{73}O_5N_4$ [M + H]$^+$: 701.4 *m*/*z*. HPLC purity: 72% (t_R = 35.5 min).

C56. Yield: 97%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 0.94 (d, J = 6.4 Hz, (CH_3)$_2$CH), 1.01 (s, 19-CH_3), 1.01 (t, J = 7.6 Hz, CH_3CH_2), 0.7−2.1 (residual CH and CH_2), 2.15 (q, J = 7.6 Hz, CH_2CO), 2.5−2.9 (broad, 2 × CH_2N and 2α-CH), 2.85 and 3.15 (2 dd, CH_2Ph), 3.55 (t, J = 8.6 Hz, 17α-CH), 3.4−3.9 (broad, 2 × CH_2NCO), 4.10 (3β-CH), 4.64 (m, NHCHCO), 3.90 (m, NHCHCO). LRMS for $C_{41}H_{65}O_5N_4$ [M + H]$^+$: 693.4 *m*/*z*. HPLC purity: 70% (t_R = 33.3 min).

C64. Yield: 96%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 0.95 (s, 19-CH_3), 0.7−2.1 (residual CH and CH_2), 2.12 (d, J = 6.9 Hz, CHCH_2CO), 2.3−2.7 (m, 2 × CH_2N and 2α-CH), 2.95 (m, CH_2Ph), 3.1−3.5 (broad, 2 × CH_2NCO), 3.56 (t, J = 8.6 Hz, 17α-CH), 3.82 (s, NHCH_2CO), 4.00 (3β-CH), 5.07 (t, J = 7.0 Hz, NHCHCO), 7.23 (d, J = 6.5 Hz, 2H of Ph), 7.29 (m, 3H of Ph). LRMS for $C_{42}H_{65}O_5N_4$ [M + H]$^+$: 705.5 *m*/*z*. HPLC purity: 72% (t_R = 33.8 min).

C70. Yield: 98%. ^{1}H NMR (CD_3OD): δ 0.72 (s, 18-CH_3), 0.94 (s, 19-CH_3), 0.7−2.2 (residual CH and CH_2), 2.25−2.75 (broad, 2 × CH_2N and 2α-CH), 3.00 (d, J = 7.6 Hz, CH_2Ph), 3.4−3.7 (broad, 2 × CH_2NCO and CH_2N of proline), 3.56 (t, J = 8.4 Hz, 17α-CH), 3.75 (s, CH_2Ph), 3.97 (m, 3β-CH), 4.42 (m, NCHCO), 5.04 (t, J = 7.7 Hz, NHCHCO), 7.25 (m, 2 × Ph). LRMS for $C_{45}H_{63}O_5N_4$ [M + H]$^+$: 739.3 *m*/*z*. HPLC purity: 72% (t_R = 33.3 min).

C78. Yield: 84%. ^{1}H NMR (CD_3OD): δ 0.74 (s, 18-CH_3), 0.86 (t, J = 7.4 Hz, CH_3CH_2), 0.95 (s, 19-CH_3), 0.70−2.10 (residual CH and CH_2), 2.13 (t, J = 7.5 Hz, CH_2CO), 2.5−3.7 (broad, 2 × CH_2N, 2 × CH_2NCO and 2α-CH), 3.58 (m, 17α-CH), 4.07 (3β-CH), 4.60 (m, NHCHCO), 5.02 (m, NHCHCO), 7.25 (m, 2 × Ph). LRMS for $C_{47}H_{69}O_5N_4$ [M + H]$^+$: 769.6 *m*/*z*. HPLC purity: 70% (t_R = 34.7 min).

Synthesis, Purification, and Characterization of A7, A9, and B10. These three compounds were generated from resin **12** (256 mg, 0.52 mmol g^{-1}) following the sequence of reactions reported in Scheme 2 for the synthesis of **16**. The final purification by flash chromatography with a mixture of CH_2Cl_2 and MeOH (10:1) yielded 44 mg of **A7**, 54 mg of **A9**, and 66 mg of **B10**.

A7. 2β-{4-[1-(3-Methyl-butyryl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17β-diol. Amorphous white solid. Yield: 59%. IR (KBr): ν 3420 (OH), 1636 (C═O, amides). ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.96 and 0.97 (2d, J = 6.1 Hz, (CH_3)$_2$-CH), 0.70−2.30 (residual CH and CH_2), 2.4−3.0 (broad, 2 × CH_2N and 2α-CH), 3.54, 3.63, 3.68 and 3.85 (4 m, 17α-CH, 2 × CH_2NCO and CH_2N of proline), 3.90 (m, 3β-CH), 4.84 (m, NCHCO). ^{13}C NMR ($CDCl_3$): δ 11.2, 17.9, 21.1, 22.7 (2×), 23.3, 25.0, 25.4, 28.0, 29.2, 30.5, 30.9, 35.5 (2×), 36.0, 36.7 (2×), 38.7, 43.1, 43.4, 44.2 weak (2×), 47.4, 49.8 weak (2×), 50.7, 55.4, 56.2, 64.1, 66.4 weak, 81.8, 171.4 (2×). Anal. Calcd for $C_{33}H_{55}O_4N_3$·0.5H_2O: C, 69.93; H, 9.96; N, 7.41. Found: C, 69.62; H, 10.15; N, 7.37. LRMS for $C_{33}H_{56}O_4N_3$ [M + H]$^+$: 558.4 *m*/*z*. HRMS Calcd for $C_{33}H_{56}O_4N_3$ [M + H]$^+$: 558.42653. Found: 558.42593.

A9. 2β-{4-[1-(2-Cyclohexyl-acetyl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17β-diol. Amorphous white solid. Yield: 67%. IR (KBr): ν 3442 (OH), 1631 (C═O, amides). ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70−2.30 (residual CH and CH_2), 2.4−3.0 (broad, 2 × CH_2N and 2α-CH), 3.54, 3.60, 3.68 and 3.80 (4 m, 17α-CH, 2 × CH_2NCO and CH_2N of proline), 3.89 (m, 3β-CH), 4.85 (m, NCHCO). ^{13}C NMR ($CDCl_3$): δ 11.2, 17.3, 21.0, 23.3, 24.8, 26.1, 26.2, 26.3, 28.1, 29.2, 30.4, 31.0, 33.3 (3×), 34.6, 35.5 (2×), 35.9, 36.7, 38.6, 42.1, 43.1, 45.5 weak (2×), 47.4, 48.8 weak (2×), 50.8, 55.6, 56.1, 63.9, 65.1 weak, 81.8, 171.3 (2×). Anal. Calcd for $C_{36}H_{59}O_4N_3$·H_2O: C, 70.21; H, 9.98; N, 6.82. Found: C, 69.19; H, 10.00; N, 6.72. LRMS for $C_{36}H_{60}O_4N_3$ [M + H]$^+$: 598.4 *m*/*z*. HRMS Calcd for $C_{36}H_{60}O_4N_3$ [M + H]$^+$: 598.45783. Found: 598.45731.

B10. 2β-{4-[1-(Cyclohexyl-carbonyl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17β-diol. Amorphous white solid. Yield: 88%. IR (KBr): ν 3427 (OH), 1632 (C═O, amides). ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70−2.30 (residual CH and CH_2), 2.37 (td, J_1 = 3.0 Hz, J_2 = 11.4 Hz, CHCON), 2.4−3.1 (broad, 2 × CH_2N and 2α-CH), 3.60, 3.72 and 3.95 (3 m, 17α-CH, 3β-CH, 2 × CH_2NCO and CH_2N of proline), 4.80 (m, NCHCO). ^{13}C NMR ($CDCl_3$): δ 11.2, 17.8, 21.2, 23.3, 25.2, 25.7 (2×), 25.8, 27.9, 28.7, 28.9, 29.1, 30.5, 30.8, 35.5 (2×), 36.1, 36.7 (2×), 38.9, 42.5, 43.1, 44.2 weak (2×), 47.1, 49.3 weak (2×), 50.6, 55.1, 56.3, 64.4, 66.0 weak, 81.7, 174.9 (2×). Anal. Calcd for $C_{35}H_{57}O_4N_3$·H_2O: C, 69.65; H, 9.99; N, 6.82. Found: C, 69.85; H, 9.88. N, 6.98. LRMS for $C_{35}H_{58}O_4N_3$ [M + H]$^+$: 584.5 *m*/*z*. HRMS Calcd for $C_{35}H_{58}O_3N_3$ [M + H]$^+$: 584.44218. Found: 584.44208.

Purification and Characterization of A8 and B9. These two compounds were purified by preparative TLC (Partisil PKSF silica gel 150 Å, 20 cm × 20 cm × 1 mm plate). The chromatography was performed with a mixture of CH_2Cl_2 and CH_3OH (97:3) as eluent.

A8. 2β-{4-[1-(Hexanoyl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17β-diol. Amorphous white solid. IR (film): ν 3412 (OH), 1635 (C═O, amides). ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.89 (t, J = 6.8 Hz, CH_3CH_2), 0.70−2.30 (residual CH and CH_2), 2.30 (q, J = 7.4 Hz, CH_2CO), 2.4−3.0 (broad, 2 × CH_2N and 2α-CH), 3.53 and 3.65 (2m, 17α-CH, 2 × CH_2NCO and CH_2N of proline), 3.90 (m, 3β-CH), 4.84 (m, NCHCO). ^{13}C NMR ($CDCl_3$): δ 11.2, 14.0, 17.4, 21.0, 22.5, 23.3, 24.3, 24.9, 28.0, 29.2, 30.5, 31.0, 31.6, 31.7, 34.0, 34.5, 35.5, 36.0,

36.7, 38.7, 43.1, 45.0 weak (2×), 47.2, 49.0 weak (2×), 50.8, 55.5, 56.2, 64.1, 65.0 weak, 81.8, 171.9 (2×). LRMS for $C_{34}H_{58}O_4N_3$ $[M + H]^+$: 572.5 *m/z*. HRMS Calcd for $C_{34}H_{58}O_4N_3$ $[M + H]^+$: 572.44218. Found: 572.44204.

B9. 2*β*-{4-[1-(Cyclopentyl-propionyl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17*β*-diol. Amorphous white solid. IR (film): ν 3406 (OH), 1637 (C═O, amides). ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70−2.30 (residual CH and CH_2), 2.31 (q, $J = 7.8$ Hz, CH_2CO), 2.4−3.0 (broad, 2 × CH_2N and 2α-CH), 3.53, 3.63, 3.69 and 3.78 (4m, 17α-CH, 2 × CH_2NCO and CH_2N of proline), 3.89 (m, 3*β*-CH), 4.84 (m, NCHCO). ^{13}C NMR ($CDCl_3$): δ 11.2, 17.4, 21.0, 23.3, 24.9, 25.1 (2×), 28.1, 29.2, 30.5, 30.8, 31.0, 32.5 (2×), 32.6, 33.8, 35.5, 35.9, 36.7, 38.6, 39.8, 42.1, 43.1, 45.7 weak (2×), 47.2, 48.9 weak (2×), 50.8, 55.6, 56.2, 64.0, 65.1, 81.8, 172.0 (2×). LRMS for $C_{36}H_{60}O_4N_3$ $[M + H]^+$: 598.3 *m/z*. HRMS Calcd for $C_{36}H_{60}O_4N_3$ $[M + H]^+$: 598.45783. Found: 598.45761.

Cell culture. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, MO) containing L-glutamine (2 nM) and antibiotics (100 IU penicillin/mL, 100 μg streptomycin/mL), supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37 °C. Cells were currently maintained in continuous exponential growth by twice a week by dilution of the cells in culture medium.

Cell Proliferation Assay. The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 1×10^4 cells in a total of 100 μL medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ) were incubated at 37 °C and 5% CO_2. Compounds were dissolved in ethanol to prepare the stock solution of 1×10^{-2} M. These compounds and doxorubicin (Novapharm, Toronto, Canada) were diluted at multiple concentrations with culture media, added to each well, and incubated for 3 days. Following each treatment, MTS (20 μL) was added to each well, and the culture was incubated for 4 h. MTS is converted to water-soluble colored formazan by a dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

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Supporting Information Available. ^{1}H NMR, COSY, ^{13}C NMR (APT), HSQC, and NOESY spectra (Figures 1−11) of key intermediate compound **10** and the representative final aminosteroid **A9**, the effect on HL-60 cell growth of three library C members (Figure 12), and crystallographic data (Tables 1−7) for the X-ray analysis of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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