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Chemical synthesis of 2β-amino-5α-androstane-3α,17β-diol *N*-derivatives and their antiproliferative effect on HL-60 human leukemia cells

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Abstract—Even though few steroids are used for the treatment of leukemia, 2β-(4-methylpiperazinyl)-5α-androstane-3α,17β-diol (**1**) was recently reported for its ability to inhibit the proliferation of human leukemia HL-60 cells. With an efficient procedure that we had developed for the aminolysis of hindered steroidal epoxides, we synthesized a series of 2β-amino-5α-androstane-3α,17β-diol *N*-derivatives structurally similar to **1**. Hence, the opening of 2,3α-epoxy-5α-androstan-17β-diol with primary and secondary amines allowed the synthesis of aminosteroids with diverse length, ramification, and functionalization of the 2β-side chain. Sixty-four steroid derivatives were tested for their capacity to inhibit the proliferation of HL-60 cells; thus obtaining first structure–activity relationship results. Ten aminosteroids with long alkyl chains (7–16 carbons) or bulky groups (diphenyl or adamantyl) have shown antiproliferative activity over 78% at 10 μM and superior to that of the lead compound. The 3,3-diphenylpropylamino, 4-nonylpiperazinyl and octylamino derivatives of 5α-androstane-3α,17β-diol inhibited the HL-60 cell growth with IC_{50} of 3.1, 4.2 and 6.4 μM, respectively. They were also found to induce the HL-60 cell differentiation.

1. Introduction

Leukemia is a major type of cancer affecting a significant segment of the population, and especially children. In fact, leukemia is the most frequent childhood cancer, with 26% of all cases, and 30% mortality.[1] The American Cancer Society (ACS) estimated that 35,070 new cases of leukemia would be diagnosed in the United States in 2006, whereas about 22,280 adults and children would die of leukemia during 2006.[2] Although the incidence rate for this disease remains relatively unchanged, some success has fortunately been attained in its treatment. Since the early 1950s, mortality rates for childhood cancer have declined by more than 50%, with most of the improvement occurring after 1970.[3] Forty years ago,[4] nearly no one could survive acute lymphoid leukemia or 'childhood leukemia', but today, 86% of the children and teens affected are still alive 5 years after the diagnosis.[2] The five-year survival rate of all people with acute myeloid leukemia has also increased over time from 38% to 65%.[2] But even if the success of clinical trials in identifying new agents and treatment modalities has been significant, current treatments have many limitations related to their side effects and the development of acquired drug resistance.[5] The new therapeutic agents thus needed should be more active and produce less side effects and they also should act through a mechanism different from that of cytotoxic agents already used.

Up to now, few steroidal compounds have been investigated for potential antileukemic activity. Notably, corticosteroids like prednisone or prednisolone are well documented for the treatment of lymphoid leukemia.[6–10] Other steroid derivatives have been investigated for potential inhibitory effect on leukemic cells, such as 7-keto hybrid steroidal esters of nitrogen mustard.[11] Estrogen derivatives,[12] and 1,25-dihydroxyvitamin D_3[13] and its derivatives,[14,15] have also been studied and good results obtained but they do not always suffice for the purpose of cancer therapy. Recently, He and Jiang[16] working with human myeloid leukemia HL-60 cells reported the antiproliferative properties of 2β-(4-methylpiperazinyl)-5α-androstane-3α,17β-diol, the

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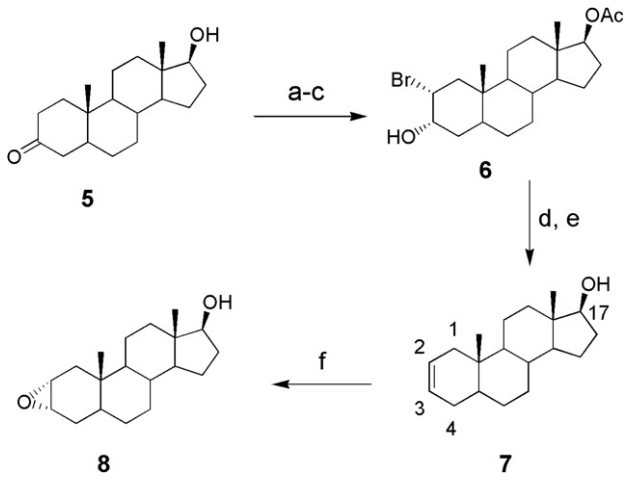
aminosteroid **1** illustrated in Figure 1. This compound and its 17-keto analogue **2** were also reported to induce differentiation of HL-60 cells.[17,18] No structure–activity relationship (SAR) study was however conducted in order to optimize the antileukemic potential of this newly reported lead compound.

In search of a novel family of anticancer agents with fewer side effects and/or a different mechanism of action, we first verified the antileukemic activity of compound **1** and, second, synthesized analogous products with the aim of improving potency following two strategies. We first generated libraries of aminosteroids **3** using parallel solid-phase organic synthesis,[19] while a solution-phase approach was used for the synthesis of compounds **4**. Since the synthesis of aminosteroids such as **4** presents certain limitations, we had previously developed a new method for the efficient aminolysis of 2,3α-steroidal epoxides,[20] which allowed us to obtain a larger series of 2β-aminosteroids and then extend our SAR study. In this paper, we report the chemical synthesis and characterization of more than 64 compounds of general structure **4** (Fig. 1) and their level of antiproliferative activity in HL-60 cells.

2. Results and discussion

2.1. Chemical synthesis

2.1.1. Synthesis of epoxide 8. The first step for generating all aminosteroids presented here was the synthesis of epoxide **8**. It was obtained from commercially available dihydrotestosterone (DHT; **5**) by a known sequence of reactions (Scheme 1).[19] Briefly, DHT was acetylated and brominated at C2, the ketone was reduced into a 3α-OH and bromoalcohol **6** was transformed into alkene **7**, which after hydrolysis of the acetate and double bond epoxidation afforded stereoselectively the 2,3α-epoxide **8**.



Scheme 1. Synthesis of epoxide **8**. Reagents and conditions: (a) Ac_2O, pyridine, DMAP cat., rt; (b) Br_2, AcOH, rt; (c) K-selectride, −78 °C, THF; (d) Zn dust, AcOH, reflux; (e) K_2CO_3/H_2O, MeOH, reflux; (f) *m*-CPBA, CH_2Cl_2, 0 °C.

Although the way reported above afforded epoxide **8** in acceptable yield and purity when carefully carried out, we also explored various other possibilities for the synthesis of **8** with fewer synthetic steps and potentially better purity.[21] Shapiro's reaction[22] was first tried out as a straightforward approach for obtaining **7** from the corresponding tosylhydrazone derivative of DHT (**5**). Various bases and conditions were then used in order to improve the regioselectivity of the elimination step, but a mixture of two alkenes (C-2,3 and C-3,4) was always observed and our attempts to separate the two isomers were unsuccessful. These elimination reactions never afforded less than 6% of C-3,4 alkene, which was found unsatisfactory for our purposes. A second strategy we put to test was the elimination of a good C-3 leaving group such as a tosylate or a bromide, easily obtained from epiandrosterone, but at least 10% of C-3,4 isomer was obtained in both cases. Since we were able to pro-

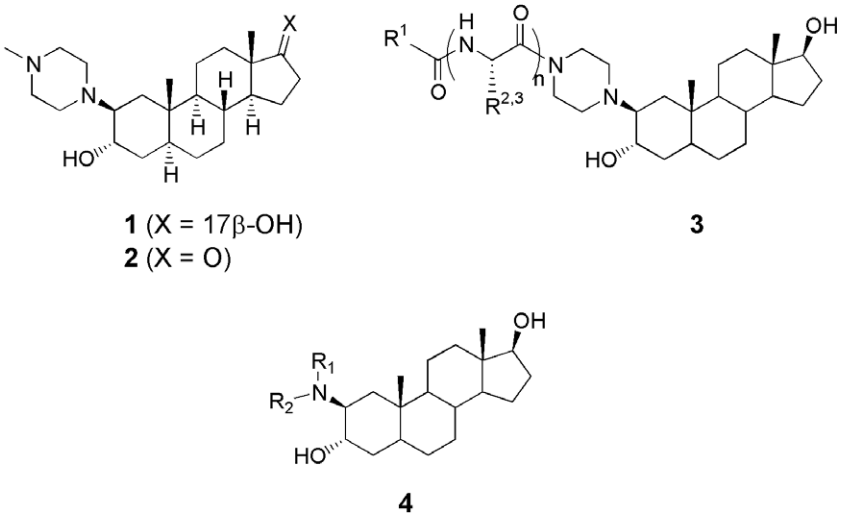
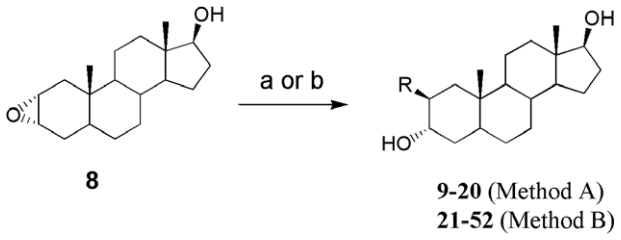


Figure 1. Lead compound **1**, its analogue **2**, and general structures of related aminosteroids **3** and **4**. Illustrated only for steroids **1** and **2**, the stereogenic centers are the same for all other aminosteroids reported in this paper. The compounds synthesized in our study and represented by the general structure **4** are given in Tables 1–4 and in Supplementary data.

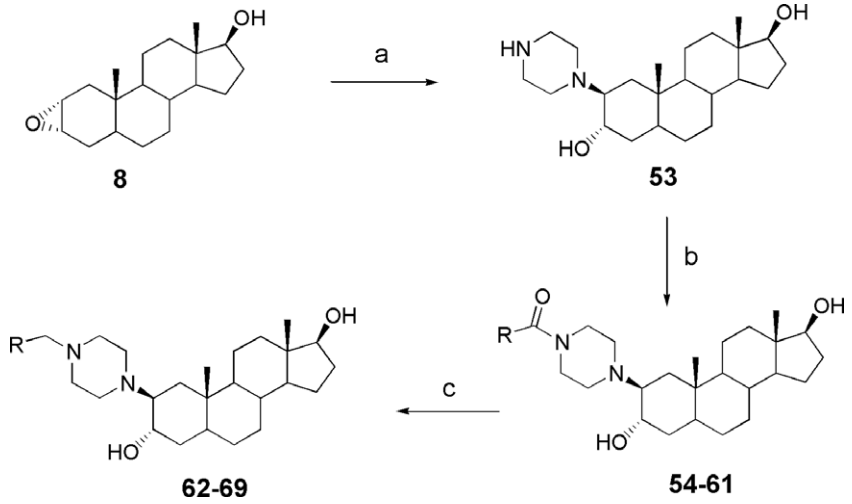
duce efficiently and rapidly a mixture of alkenes, we next considered the possibility of isomerizing the C-3,4 alkene into a C-2,3 alkene. Various catalysts known to facilitate a double bond isomerization[23,24] were thus tested with the substrate in refluxing toluene or DMF. Although an enrichment in C-2,3 isomer (compound **7**) was observed, much degradation occurred. The low rate of isomerization and the loss of products convinced us to generate alkene **7** with the method described above and using the C2-bromination pathway.

2.1.2. Synthesis of aminosteroids 9–53 by opening of epoxide 8. To start our SAR study, we first added a diversity of cyclic amines at the C2-carbon of **8** using Method A, an aminolysis procedure known in the literature.[25–27] An excess of amine (30 equiv) used as solvent and water (18 equiv) were refluxed with epoxide **8** to afford the desired products **9–20** with low to medium yields (10–88%) after tedious purification (Scheme 2). However, a number of amines did not react, thus limiting the possibility of diversification. We then applied a new procedure,[20] Method B, which we had previously developed for the aminolysis of a hindered steroidal epoxide and that allows introducing a larger diversity of amines; we thus obtained targeted aminosteroids **21–52** in medium to high yields (35–99%) with easier work-up procedure and purification than with Method A. Only 3 equiv of amine with 20% molar of $Gd(OTf)_3$ as catalyst was needed to provide the desired product by heating (150–190 °C) in a Schlenk tube the epoxide **8** for 2 h (primary amines) or overnight (secondary and cyclic amines). In addition to cyclic amines, various aliphatic amines were also used to generate aminosteroids and to extend our SAR study, which would not have been possible with the classical aminolysis procedure applied in Method A. The regio and stereoselectivity outcome of the aminolysis of epoxide **8** deserves some comments. In fact, in agreement with Barton's generalization,[28,29] ring opening of a steroidal epoxide gives trans-diaxially dispersed substituents. Thus, the aminolysis of 2,3α-epoxide **8** will generate a diaxial amino (2β-N) alcohol (3α-OH) derivative as represented by compounds **9–53**. This regio and stereochemical aminolysis of such steroidal epoxide was documented in the literature[25–27] and confirmed by the X-ray analysis of compound **53**.[19]

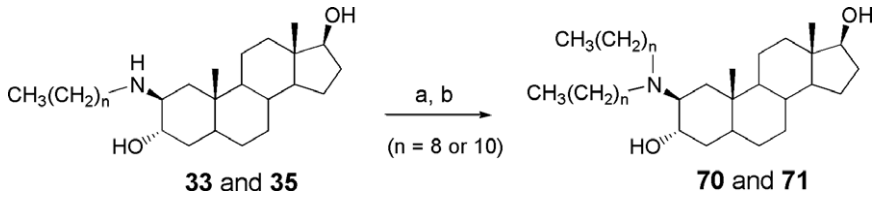


Scheme 2. Synthesis of aminosteroids **9–52** with methods A and B. Reagents and conditions: (a) Method A: amine (30 equiv), H_2O (18 equiv), reflux, 24 h; (b) Method B: amine (3 equiv), $Gd(OTf)_3$ (0.2 equiv), toluene, Schlenk tube, 150–190 °C, 2 h or overnight. The nature of R groups is given in Tables 1–4 and their structure in Supplementary data.

2.1.3. Synthesis of aminosteroids 54–71 by amidation and reduction. The *N*-alkylpiperazine derivatives **54–69** were obtained from **53** (Scheme 3). The latter was first synthesized from epoxide **8** in 70% yield by the Method A of aminolysis given above, except that we utilized a Schlenk tube. The amidation of **53** with a carboxylic acid, HBTU as coupling agent, and DIPEA as base gave good yields (74–90%) of amides **54–61**. The carbonyl reduction with BH_3-THF afforded the alkylamine chelated with boron.[30] This complex was then broken in refluxing methanol overnight to give the desired products **62–69** in good yields after purification. In the formation of the last two compounds, **70** and **71** (Scheme 4), from the secondary amines **33** and **35**, no complexation occurred during the reduction step, probably due to high steric hindrance. A simple work-up followed by flash chromatography gave the tertiary amines **70** and **71**.



Scheme 3. Synthesis of aminosteroids **53–69**. Reagents and conditions: (a) Piperazine, H_2O, Schlenk tube (150–190 °C); (b) HBTU, carboxylic acid, DIPEA, DMF, 0 °C to rt; (c) BH_3-THF, THF, 0 °C to rt then reflux in MeOH overnight. The nature of the R groups is given in Tables 1, 2 and 4 and their structure in Supplementary data.



33 and 35 70 and 71

Scheme 4. Synthesis of *N*,*N*-dialkyl aminosteroids **70** and **71**. Reagents and conditions: (a) HBTU, carboxylic acid, DIPEA, DMF, 0 °C to rt; (b) BH_3-THF, THF, 0 °C to rt.

2.2. Biological results

HL-60 cells were incubated for 3 days at 37 °C with synthesized compounds in a humidified atmosphere of 95% air/5% CO_2. For screening purposes, the cell proliferation assay was performed at concentrations of 1 and 10 μM for all compounds and the results were expressed as the percentage of cell growth inhibition (Tables 1–4). Doxorubicin, a well-known cytotoxic agent used for the treatment of leukemia,[31] and lead compound **1** were used as references. The antiproliferative activity of **1** that we measured (16% at 10 μM) was however lower than expected based on published data.[16] The fact that our experimental conditions were not exactly the same probably explains this discrepancy. Regardless of this, we decided to begin a SAR study in order to generate 2β-aminosteroids with potentially better antiproliferative properties. Since we suspected that the methyl–piperazinyl moiety was not the optimal building block for cell growth inhibition, our strategy was to diversify the amino group at carbon 2. We first tested the effect of several cyclic amines and of a series piperazine derivatives on cell proliferation (Table 1). At a concentration of 1 μM, no significant antiproliferative activity was observed for any compound tested. Although most of the cyclic amines represented in Table 1 did not give good results, three compounds—2β-(4-benzylpiperidinyl)-5α-androstane-3α,17β-diol (**15**), 2β-{4-(4-nitrophenyl)-piperazinyl}-5α-androstane-3α,17β-diol (**18**), and 2β-(4-piperidinylpiperidinyl)-5α-androstane-3α,17β-diol (**52**)—inhibit significantly (over 50%) the cell growth at 10 μM.

Table 1. Percentage of inhibition of HL-60 cell growth by tertiary amines (cyclic amines and *N*-piperazine derivatives)[a]

#	R	Inhibition (%) at 1 μM	Inhibition (%) at 10 μM
1	4-Methylpiperazinyl	0.0 ± 4.7	16.2 ± 6.0
9	Pyrrolidinyl	0.0 ± 4.6	9.9 ± 4.8
10	Homopiperidinyl	0.0 ± 0.5	3.4 ± 9.8
11	4-Ethylpiperazinyl	0.0 ± 7.7	4.8 ± 6.3
12	4-Hydroxyethylpiperazinyl	0.8 ± 5.5	17.7 ± 3.4
13	Morpholinyl	14.9 ± 1.7	8.7 ± 5.7
14	Homopiperazinyl	1.0 ± 10.5	21.4 ± 4.3
15	4-Benzylpiperidinyl	9.9 ± 8.7	57.9 ± 1.7
16	1,2,3,4-Tetrahydroisoquinolinyl	3.4 ± 6.2	29.6 ± 4.8
17	1-Pyridin-2-yl-piperazinyl	0.0 ± 2.9	26.1 ± 4.6
18	1-(4-Nitrophenyl)-piperazinyl	12.5 ± 1.6	92.2 ± 1.6
19	4-Piperonylpiperazinyl	0.0 ± 2.2	29.1 ± 2.1
20	Pyrazolyl	8.4 ± 9.1	12.8 ± 4.9
49	4-Phenylpiperazinyl	7.0 ± 2.2	36.6 ± 5.4
50	4-Fluorophenylpiperazinyl	0.0 ± 5.8	10.1 ± 2.9
51	Piperidinyl	0.0 ± 9.9	1.5 ± 7.9
52	4-(4-Piperidinyl)piperidinyl	0.0 ± 1.3	77.8 ± 0.6
53	Piperazinyl	6.9 ± 7.9	5.9 ± 1.7
Doxo[b]	—	91.8–96.2	94.6–95.3

[a] The chemical structure of R group is provided in Supplementary data. Results are the means (±SD) of triplicates.
[b] Doxorubicin.

Table 2. Percentage of inhibition of HL-60 cell growth by tertiary amines (*N*-alkylamido or *N*-alkylamino derivatives of piperazine)[a]

#	R	Inhibition (%) at 1 μM	Inhibition (%) at 10 μM
1	4-Methylpiperazinyl	0.0 ± 4.7	16.2 ± 6.0
48	4-Acetylpiperazinyl	0.0 ± 6.0	0.0 ± 5.8
54	4-Propanoylpiperazinyl	0.0 ± 1.4	0.0 ± 7.1
55	4-Butanoylpiperazinyl	0.0 ± 2.9	4.6 ± 2.7
56	4-*iso*-Butanoylpiperazinyl	0.0 ± 7.6	1.1 ± 2.7
57	4-Pentanoylpiperazinyl	0.0 ± 3.8	7.4 ± 1.5
58	4-*iso*-Pentanoylpiperazinyl	0.0 ± 6.2	5.5 ± 2.4
59	4-Nonanoylpiperazinyl	8.1 ± 3.7	99.0 ± 0.7
60	4-Cyclohexylcarbonylpiperazinyl	0.0 ± 4.0	3.9 ± 3.7
61	4-Benzoylpiperazinyl	0.0 ± 5.5	11.4 ± 0.5
62	4-Propylpiperazinyl	0.0 ± 11.7	0.0 ± 11.3
63	4-Butylpiperazinyl	0.0 ± 4.2	7.6 ± 4.8
64	4-*iso*-Butylpiperazinyl	0.0 ± 5.2	5.0 ± 3.5
65	4-Pentylpiperazinyl	0.0 ± 10.8	7.7 ± 4.2
66	4-*iso*-Pentylpiperazinyl	0.0 ± 8.5	9.2 ± 2.2
67	4-Nonylpiperazinyl	9.1 ± 5.0	99.8 ± 0.2
68	4-Cyclohexylmethylpiperazinyl	0.0 ± 5.3	4.0 ± 0.6
69	4-Benzylpiperazinyl	0.0 ± 2.8	25.7 ± 2.8
Doxo[b]	—	91.8–96.2	94.6–95.3

[a] The chemical structure of R group is provided in Supplementary data. Results are the means (±SD) of triplicates.
[b] Doxorubicin.

The best results being obtained with the piperazine derivative **18**, we then extended our study by testing a series of compounds with various alkyl chains on the piperazine moiety. We first added different carboxylic acids on the piperazine moiety to generate amide derivatives **54**–**61**. The corresponding amine derivatives **62**–**69**, without a carbonyl group, were next synthesized and tested on HL-60 cells in the same conditions. Results reported in Table 2 are similar to those of Table 1, except for amide **59** and amine **67**. Indeed, at 10 μM the presence of a long alkyl side chain fully reduced (>99%) the cell growth. No antiproliferative effect was however obtained with shorter side chains.

We next elaborated and tested a third series of compounds, which were synthesized by the opening of

Table 3. Percentage of inhibition of HL-60 cell growth by secondary amines (normal and branched alkyls)[a]

#	R	Inhibition (%) at 1 μM	Inhibition (%) at 10 μM
1	4-Methylpiperazinyl	0.0 ± 4.7	16.2 ± 6.0
21	*n*-Propylamino	0.0 ± 3.9	0.4 ± 5.6
22	*i*-Propylamino	0.0 ± 7.4	0.0 ± 1.9
23	*n*-Butylamino	0.0 ± 3.4	3.1 ± 1.2
24	*i*-Butylamino	0.0 ± 2.9	0.0 ± 0.8
25	*tert*-Butylamino	0.0 ± 3.6	0.5 ± 2.2
26	*n*-Pentylamino	0.0 ± 0.8	0.0 ± 0.7
27	*i*-Pentylamino	0.0 ± 5.3	3.0 ± 5.6
28	*neo*-Pentylamino	0.0 ± 7.0	3.3 ± 7.9
29	*tert*-Pentylamino	0.0 ± 2.9	1.8 ± 4.1
30	*n*-Hexylamino	0.0 ± 5.4	6.9 ± 1.2
31	*n*-Heptylamino	10.1 ± 3.8	82.3 ± 3.3
32	*n*-Octylamino	0.0 ± 0.8	99.2 ± 2.9
33	*n*-Nonylamino	3.9 ± 4.5	89.9 ± 0.2
34	*n*-Decylamino	0.0 ± 4.9	96.9 ± 0.7
35	*n*-Undecylamino	0.0 ± 0.6	96.3 ± 0.3
36	*n*-Dodecylamino	0.0 ± 4.9	92.0 ± 0.3
37	*n*-Hexadecylamino	0.0 ± 5.2	94.8 ± 4.5
Doxo[b]	—	91.8–96.2	94.6–95.3

[a] The chemical structure of R group is provided in Supplementary data. Results are the means (±SD) of triplicates.
[b] Doxorubicin.

Table 4. Percentage of inhibition of HL-60 cell growth by secondary amines (cyclic and alkyl derivatives)[a]

#	R	Inhibition (%) at 1 μM	Inhibition (%) at 10 μM
1	4-Methylpiperazinyl	0.0 ± 4.7	16.2 ± 6.0
38	Cyclopentylamino	0.0 ± 5.7	0.0 ± 7.1
39	Cyclohexylamino	0.0 ± 3,5	0.0 ± 2.0
40	Cycloheptylamino	0.0 ± 8.8	0.0 ± 4.1
41	3-Ethoxypropylamino	0.0 ± 2.9	10.8 ± 1.2
42	3-Butoxypropylamino	4.8 ± 4.3	26.5 ± 1.9
43	2-Phenylethylamino	9.5 ± 1.7	42.8 ± 4.2
44	3-Phenylpropylamino	0.9 ± 3.8	37.6 ± 2.7
45	3,3-Diphenylpropylamino	12.9 ± 2.5	95.2 ± 0.5
46	Adamantylmethylamino	6.4 ± 3.7	85.8 ± 0.8
47	Diethylamino	0.0 ± 3.7	6.7 ± 3.0
70	Dinonylamino	9.8 ± 2.0	41.5 ± 1.5
71	Diundecylamino	11.7 ± 5.9	7.0 ± 1.9
Doxo[b]	—	91.8–96.2	94.6–95.3

[a] The chemical structure of R group is provided in Supplementary data. Results are the means (± SD) of triplicates.
[b] Doxorubicin.

epoxide **8** with normal or branched alkyl primary amines (Table 3). Although this series of secondary aminosteroids were not active at 1 μM, better results were attained with long *n*-alkylamines at 10 μM (compounds **31**–**37**). In fact, the levels of inhibition obtained with side chains longer (7 to 16 carbons) than hexyl (6 carbons) were all above 82%. Similarly as observed for the tertiary aminosteroids reported in Table 2, no interesting antiproliferative effect was obtained with the secondary aminosteroids having a short alkyl group (compounds **21**–**31**).

In the last series of aminosteroids (Table 4), we tested additional secondary amines such as cycloalkylamines, alkyl amines with an oxygen atom (ether function), and amines containing an aromatic ring at the end of the chain or a bulky group like adamantylmethyl. The only compounds that gave a good inhibition (86% and 95%) in this fourth screening were compounds **45** and **46**, with hindered and bulky building blocks 3,3-diphenylpropylamine and adamantylmethylamine, respectively. Dialkyl compounds **70** and **71** were synthesized because their corresponding monoalkyl chains (compounds **33** and **35**) afforded good results at 10 μM. But dialkyl compounds **70** and **71** did not afford any good inhibition. With their two long *n*-alkyl chains, it is possible that these tertiary aminosteroids remain in the phospholipid bilayer of the membrane cells.[32] On the other hand, compounds **45** and **46** are probably not retained in the phospholipid bilayer since their bulky groups are not structurally similar to the long alkyl chains of compounds **70** and **71**. Although less impressive than **45** and **46**, compounds **42**–**44** also produced some cell growth inhibition at 10 μM (27–43%). These compounds are however less potent than three aminosteroids (see **3** in Fig. 1) synthesized using solid-phase chemistry (IC_{50} = 0.58–2.87 μM)[19] and much less potent than doxorubicin (IC_{50} = 0.10 μM) used as a reference compound.

Figure 2 summarizes the biological screening data obtained with the more active aminosteroids synthesized in our study (Tables 1–4). They can be divided in two groups, the tertiary (**15**, **18**, **52**, **59**, and **67**) and secondary (**32**, **33**, **36**, **45**, and **46**) amines, but they roughly induce the same level of growth inhibition on HL-60 cells. Three representative candidates of each group were selected for IC_{50} determination, the *n*-octyl secondary amine **32**, the diphenyl–propyl secondary amine **45**, and the *n*-octyl–piperazinyl tertiary amine **67**. As reported in Figure 3, the IC_{50} values are similar for **45** and **67** (3.1 and 4.2 μM, respectively), but higher for **32** (6.4 μM).

After we identified the representative aminosteroids **32**, **45**, and **67** from the antiproliferative screening assay, we tested their ability to induce the differentiation of HL-60 cells. In fact, an attractive alternative to the antiproliferative approach is to induce cell differentiation in order to convert malignant cells into mature resting cells.[33] Furthermore, the lead compound **1** was previously reported to induce the differentiation of HL-60 cells,[16] and the stimulation of cell differentiation by a therapeutic agent is particularly promising for the treatment of human leukemias. To do so, we evaluated expression of surface antigens CD11b (both granulocyte- and monocyte-like cell marker) and CD14 (monocyte-like cell marker) because in leukemia, the cell differentiation begins with an amplification of the expression of these two antigens. The markers were analyzed using flow cytometry after incubation for 72 h with medium supplemented with a 10 μM concentration of aminosteroids (Fig. 4). Vitamin D3 (VD3), a chemical agent well known to induce differentiation,[13–15] served as positive control. Treatment of HL-60 with VD3 increased CD11b and double-marked CD11b/CD14 expressing cells to 65% and 32%, respectively. A 3-day exposure to compound **1** induced a weak increase in the expression of CD11b but it did not modify the levels of expression of CD14

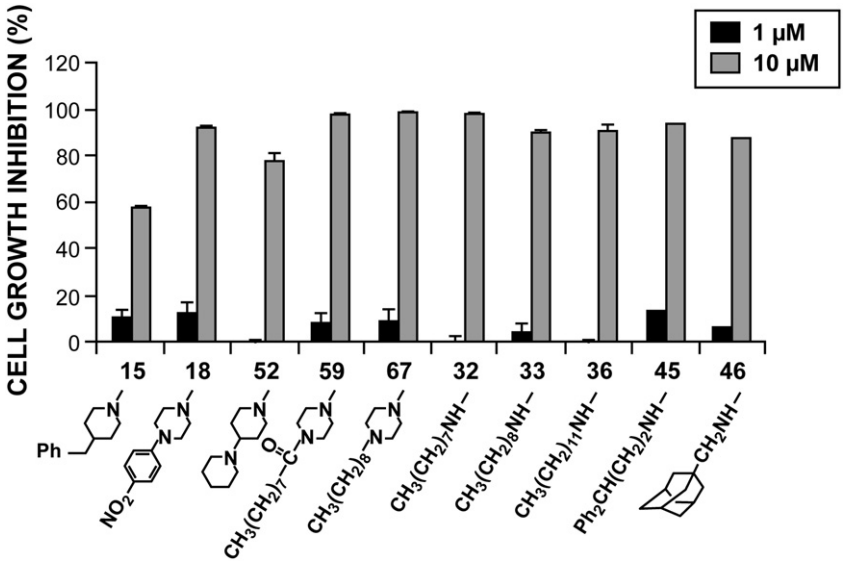


Figure 2. Percentage (%) of HL-60 cell growth inhibition produced by aminosteroids selected from our screening study and that produce over 50% of inhibition. Results are the means of triplicates (±SD). Only their 2β-groups are represented in the bottom of the histogram.

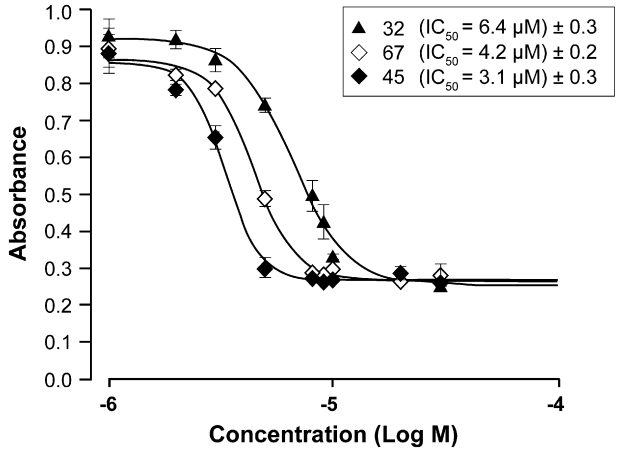


Figure 3. Effect of increasing concentrations of aminosteroids **32**, **45**, and **67** on HL-60 cell growth represented by the absorbance at 490 nm (see Section 4). Cells (1×10^4) were incubated with various concentrations of each compound for 3 days. IC_{50} represents the concentration that inhibits 50% of the cell growth. Results are the means (±SD) of triplicates.

or CD11b/CD14 (double-marked) cell surface markers as indicated by flow cytometry analysis. On the other hand, we observed a stronger differentiation of HL-60 cells following treatments with **32**, **45**, and **67**, as CD11b and double-marked CD11b/CD14 were significantly up-regulated during the treatment. The percentage of CD14 positive cells was however not affected. Compound **32** induced the expression of CD11b in 29% of cells and that of CD11b/CD14 in 10% of cells. It was however clearly less efficient than **45** and **67** in the induction of differentiation. In fact, after incubation with **45**, we measured 58% and 39% of cells expressing CD11b and double-marked CD11b/CD14, respectively, and these results were similar to those obtained after treatment with **67** (55% and 32%, respectively). Interestingly, these levels of differentiation are close to the effects obtained with VD3.

3. Conclusion

The chemical synthesis of a series of 2β-amino-5α-androstane-3α,17β-diol *N*-derivatives **9**–**71** was per-

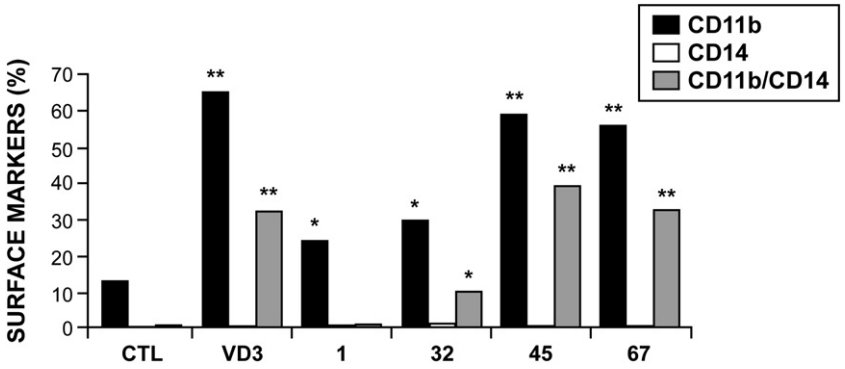


Figure 4. Expression (%) of CD11b, CD14, and CD11b/CD14 cell surface markers in HL-60 cells after a 72 h-treatment with vitamin D3 (VD3) or aminosteroid **1**, **32**, **45**, or **67**. Results are the means (±SEM) of triplicates. *,**Indicate a result significantly different from the control (CTL) (*$P < 0.05$; **$P < 0.01$).

formed in order to improve the antiproliferative properties on HL-60 leukemia cells of the lead compound **1**, the first representative of this family of aminosteroids. A large series of 2β-tertiary and 2β-secondary aminosteroids was thus obtained from the aminolysis of epoxide **8** using a classical or a new methodology. It appears that the 4-methylpiperazinyl group can be modified resulting in a better antiproliferative activity. The tertiary aminosteroid **67**, with an IC_{50} value of 4.2 μM, illustrates the inhibitory effect of a long nonyl side chain replacing the *N*-methyl group of **1**. This finding is in agreement with the results obtained with a series of piperazinyl derivatives generated by solid-phase organic synthesis, which clearly demonstrated that substituting the *N*-methyl piperazine ring with a more complex aminoacid building block modulates the antiproliferative activity.[19] The 4-methylpiperazinyl moiety can also be replaced by an appropriate secondary amine as exemplified by aminosteroids **32** and **45**. The diphenylpropyl amino moiety of **45** showed better antiproliferative potential than the octyl amino group of **32** (IC_{50} = 3.1 and 6.4 μM, respectively). In addition to their antiproliferative activity, the three aminosteroids tested induce the differentiation of HL-60 cells. Compounds **45** and **67** thus induce the same level of expression of cell markers as vitamin D3, a known differentiating agent.

We succeeded in optimizing the antiproliferative activity of lead compound **1** by a modification of the amino group at position 2β. This first SAR study identified some chemical groups more suitable for a better biological activity, but could be extended by modifying the stereochemistry at C2, the positioning of the amino moiety on the steroid nucleus, and the nature and stereochemistry of the substituent at C3. The steroid nucleus appears however to be a good scaffold because the biological activity was lost when the four-ring steroid nucleus was replaced by a single cyclohexyl nucleus (data not reported). It is however not clear yet if the tertiary and secondary aminosteroids identified in our study possess the same mechanism of action, and additional studies will be necessary to elucidate their mechanism of action. Identification of this mechanism could help us to rationalize our results and design other active compounds more efficiently.

4. Experimental

4.1. Chemistry

Dihydrotestosterone (DHT) was purchased from Steraloids (Wilton, NH, USA) or Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada), while chemical reagents were from Sigma–Aldrich Canada Ltd. The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. All anhydrous reactions were performed in oven-dried glassware under positive argon pressure. Thin-layer chromatography was performed on 0.20 mm silica gel 60 F254 plates (Fisher, Montréal, Canada) and compounds were visualized using ammonium heptamolybdate tetrahydrate. Flash column chromatography was performed on Silicycle R10030B 230-400 mesh silica gel (Québec, QC, Canada). Infrared spectra (IR) were obtained from a thin film obtained with compound usually solubilized in $CDCl_3$ and layered over a NaCl pellet. They were recorded with a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only significant bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC/F300 spectrometer (Billerica, MA, USA), a Bruker Avance 400 digital spectrometer or an Inova Varian AS 400 (Palo Alto, CA, USA). The chemical shifts (δ) were expressed in ppm and referenced to $CHCl_3$ (7.26 and 77.0 ppm) for ^1H and ^{13}C, respectively. Only characteristic signals were reported. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ionspray source. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Département de chimie de l'Université Laval (Québec, QC, Canada). The HPLC purity was determined with a Waters' apparatus (Waters Associates Milford, MA, USA) using a Nova Pak C18 reversed-phase column (150 mm × 3.9 mm id, 4 μm, 60 Å) and methanol and/or water containing 10 mM of ammonium acetate as eluent.

4.1.1. Classical method for aminolysis of epoxide 8 (synthesis of 1 and 9–20). Epoxide **8**[19] (1 mmol), the desired amine (30 mmol), and H_2O (18 equiv) were refluxed 24 h. The reaction mixture was then cooled, poured in water, and the precipitate was filtered. The solid was dissolved in CH_2Cl_2 and the solution dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was then purified by flash chromatography using an appropriate mixture of MeOH:CH_2Cl_2.

4.1.1.1. 2β-(4-Methylpiperazinyl)-5α-androstane-3α,17β-diol (1).[16] Yield: 88%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.08; IR (film): 3369 (OH); ^1H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70–2.10 (residual H), 2.32 (s, 4′-CH_3), 2.53 (m, 2× CH_2N and 2α-CH), 2.71 (m, 2× CH_2N), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.84 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 17.15, 20.78, 23.31, 28.16, 30.56, 31.09, 32.70, 34.64, 35.41, 35.72, 36.88, 38.43, 42.94, 45.58, 50.84, 55.22 (4×), 56.10, 63.60 (C3), 64.37 (C2), 81.26; LRMS for $C_{24}H_{43}N_2O_2$ $[M+H]^+$: 391.3 *m/z*; HRMS: calcd for $C_{24}H_{43}N_2O_2$ $[M+H]^+$ 391.33191, found 391.33211.

4.1.1.2. 2β-(Pyrrolidinyl)-5α-androstane-3α,17β-diol (9). Yield: 41%; TLC (hexanes/acetone, 6:4): R_f = 0.2; IR (film): 3325 (OH); ^1H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 1.04 (s, 19-CH_3), 0.70–2.10 (residual H), 2.38 (m, 2α-CH), 2.53 (m, 2× CH_2N), 3.62 (m, 17α-CH), 4.04 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.15, 13.23, 20.60, 23.43 (3×), 27.86, 30.53, 31.53, 32.57, 34.89, 35.07, 36.41, 36.78, 39.35, 43.01, 51.07, 51.47 (2x), 55.40, 65.98 (C3), 67.91 (C2), 81.98; LRMS for $C_{23}H_{40}NO_2$ $[M+H]^+$: 362.2 *m/z*; HRMS: calcd for $C_{23}H_{40}NO_2$ $[M+H]^+$ 362.30536, found 362.30593.

4.1.1.3. 2β-(Homopiperidinyl)-5α-androstane-3α,17β-diol (10). Yield: 18%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.26; IR (film): 3366 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70–2.20 (residual H), 2.51 (m, CH_2N), 2.75 (m, CH_2N and 2α-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.74 (m, 3β-CH); ^{13}C NMR (100.6 MHz, acetone-d_6): 11.81, 17.03, 21.74, 24.14, 27.63 (2×), 29.31, 30.43 (2×), 31.07, 32.26, 34.91, 35.85, 36.51, 36.58, 37.99, 39.57, 44.00, 52.03, 52.08 (2×), 57.29, 65.41 (C3), 66.80 (C2), 81.87; LRMS for $C_{25}H_{44}NO_2$ $[M+H]^+$: 390.0 *m*/*z*; HRMS: calcd for $C_{25}H_{44}NO_2$ $[M+H]^+$ 390.33666, found 390.33656.

4.1.1.4. 2β-(4-Ethylpiperazinyl)-5α-androstane-3α,17β-diol (11). Yield: 58%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.12; IR (film): 3396 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.84 (s, 19-CH_3), 1.10 (t, J = 7.1 Hz, 2″-CH_3), 0.60–2.20 (residual H), 2.43 (m, CH_2), 2.53 (m, 2 × CH_2N), 2.68 (3m, 2× CH_2N and 2α-CH), 3.61 (m, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.19, 11.87, 17.28, 20.79, 23.37, 28.26, 30.65, 31.18, 32.55, 34.66, 35.47, 35.72, 36.90, 38.43, 43.00, 50.87, 52.29, 53.32 (4×), 56.15, 63.53 (C3), 64.34 (C2), 81.41; LRMS for $C_{25}H_{45}N_2O_2$ $[M+H]^+$: 405.6 *m*/*z*; HRMS: calcd for $C_{25}H_{45}N_2O_2$ $[M+H]^+$ 405.34756, found 405.34813.

4.1.1.5. 2β-{4-(2-Hydroxyethyl)piperazinyl}-5α-androstane-3α,17β-diol (12). Yield: 67%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.07; IR (film): 3328 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70–2.20 (residual H), 2.45–2.75 (broad, 5 × CH_2N and 2α-CH), 3.62 (m, 17α-CH and CH_2OH), 3.84 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.19, 17.26, 20.90, 23.31, 28.21, 30.48, 31.13, 32.75, 34.65, 35.51, 35.77, 36.79, 38.44, 43.08, 45.69, 50.86, 53.35 (2×), 56.15, 57.60 (2×), 59.21, 63.66 (C3), 64.45 (C2), 81.83; LRMS for $C_{25}H_{45}N_2O_3$ $[M+H]^+$: 421.6 *m*/*z*; HRMS: calcd for $C_{25}H_{45}N_2O_3$ $[M+H]^+$ 421.34247, found 421.34279.

4.1.1.6. 2β-(Morpholinyl)-5α-androstane-3α,17β-diol (13). Yield: 42%; TLC (hexanes/acetone, 6:4): R_f = 0.57; IR (film): 3442 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.65–2.15 (residual H), 2.45 (m, CH_2N), 2.63 (m, CH_2N and 2α-CH), 3.73 (m, 17α-CH, 2× CH_2O), 3.86 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 16.94, 20.89, 23.33, 28.16, 30.53, 31.18, 32.58, 34.42, 35.50, 35.84, 36.80, 38.53, 43.10, 48.88 (2×), 50.90, 56.07, 63.65 (C3), 65.02 (C2), 67.48 (2×), 81.91; LRMS for $C_{23}H_{40}NO_3$ $[M+H]^+$: 378.3 *m*/*z*; HRMS: calcd for $C_{23}H_{40}NO_3$ $[M+H]^+$ 378.30027, found 378.30053.

4.1.1.7. 2β-(Homopiperazinyl)-5α-androstane-3α,17β-diol (14). Yield: 62%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.02; IR (film): 3372 (OH and NH); ^{1}H NMR (300 MHz, CD_3OD): 0.72 (s, 18-CH_3), 0.90 (s, 19-CH_3), 0.70–2.10 (residual H), 2.65–3.35 (m, 4× CH_2N and 2α-CH), 3.55 (t, J = 8.5 Hz, 17α-CH), 3.90 (m, 3β-CH); ^{13}C NMR (75 MHz, CD_3OD): 11.76, 17.15, 22.03, 24.28, 27.82, 29.40, 30.65, 32.41, 36.68, 36.87, 37.01, 38.12, 40.13, 43.53, 44.21, 46.05, 47.92, 48.88, 51.61, 52.23, 57.46, 66.97 (C2), 67.07 (C3), 82.47; LRMS for $C_{24}H_{43}N_2O_2$ $[M+H]^+$: 391.3 *m*/*z*; HRMS: calcd for $C_{24}H_{43}N_2O_2$ $[M+H]^+$ 391.33191, found 391.33195.

4.1.1.8. 2β-(4-Benzylpiperidinyl)-5α-androstane-3α,17β-diol (15). Yield: 18%; TLC (hexanes/acetone, 6:4): R_f = 0.45; IR (film): 3350 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.70–2.20 (residual H), 2.54 (d, J = 4.8 Hz, CH_2Ph), 2.32, 2.60 and 2.90 (3m, 2α-CH and 2× CH_2N), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.88 (m, 3β-CH), 7.12 (d, J = 7.1 Hz, 2H of Ar), 7.20 (t, J = 7.3 Hz, 1H of Ar), 7.29 (d, J = 7.1 Hz, 2H of Ar); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.15, 17.68, 20.97, 23.30, 28.17, 30.51, 31.00, 33.45 (2×), 35.40, 35.53, 35.88, 36.76, 37.72, 38.47, 42.73, 43.10, 44.94, 50.81, 52.32 (2×), 56.27, 63.73 (C3), 65.11 (C2), 81.78, 125.97, 128.23 (2×), 129.00 (2×), 140.06; LRMS for $C_{31}H_{48}NO_2$ $[M+H]^+$: 466.5 *m*/*z*; HRMS: calcd for $C_{31}H_{48}NO_2$ $[M+H]^+$ 466.36796, found 466.36801.

4.1.1.9. 2β-(1,2,3,4-Tetrahydroisoquinolinyl)-5α-androstane-3α,17β-diol (16). Yield: 10%; TLC (hexanes/acetone, 6:4): R_f = 0.69; IR (film): 3390 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.90 (s, 19-CH_3), 0.70–2.15 (residual H), 2.64 (m, 2α-CH), 2.93 (m, NCH_2–CH_2–Ar), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.74 and 3.90 (2d, J = 14.7 Hz, NCH_2–Ar), 3.96 (m, 3β-CH), 7.02 (m, 1H of Ar), 7.13 (m, 3H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 17.41, 20.93, 23.31, 28.22, 29.64, 30.50, 31.11, 32.74, 34.88, 35.54, 35.84, 36.78, 38.55, 43.07, 45.62, 50.84, 51.19, 56.23, 63.97 (C3), 64.35 (C2), 81.85, 125.69, 126.23, 126.52, 128.73, 134.30 (2×); LRMS for $C_{28}H_{42}NO_2$ $[M+H]^+$: 424.0 *m*/*z*; HRMS: calcd for $C_{28}H_{42}NO_2$ $[M+H]^+$ 424.32101, found 424.32141.

4.1.1.10. 2β-{4-(2-Pyridyl)piperazinyl}-5α-androstane-3α,17β-diol (17). TLC (hexanes/acetone, 6:4): R_f = 0.59; IR (film): 3401 (OH), 1596 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.65–2.15 (residual H), 2.56 (m, CH_2N), 2.73 (m, 3H, CH_2N and 2α-CH), 3.53 (m, 2× CH_2N), 3.62 (t, J = 8.6 Hz, 17α-CH), 3.88 (m, 3β-CH), 6.62 (m, 2H of Ar), 7.47 (m, 1H of Ar), 8.20 (m, 1H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.20, 17.26, 20.88, 23.32, 28.20, 30.48, 31.14, 32.66, 34.62, 35.49, 36.00, 36.76, 38.44, 43.10, 45.78 (2×), 48.05 (2×), 50.85, 56.11, 63.73 (C3), 64.72 (C2), 81.87, 107.09, 113.34, 137.46, 147.93, ~160.00; LRMS for $C_{28}H_{44}N_3O_2$ $[M+H]^+$: 454.1 *m*/*z*; HRMS: calcd for $C_{28}H_{44}N_3O_2$ $[M+H]^+$ 454.34280, found 454.34367.

4.1.1.11. 2β-{4-(4-Nitrophenyl)-piperazinyl}-5α-androstane-3α,17β-diol (18). Yield: 12%; TLC (hexanes/acetone, 6:4): R_f = 0.61; IR (film): 3406 (OH), 1597, 1327 (NO_2); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70–2.10 (residual H), 2.61 (m, CH_2N), 2.70 (m, 2α-CH), 2.79 (m, CH_2N), 3.42 (m, 2× CH_2N), 3.61 (t, J = 8.5 Hz, 17α-CH), 3.91 (m, 3β-CH), 6.81 (d, J = 9.4 Hz, 2H of Ar), 8.13 (d, J = 9.3 Hz, 2H of Ar); ^{13}C NMR (75 MHz, CD_3OD): 11.74, 14.28, 21.76, 24.30, 29.00, 30.62, 32.79, 33.90, 34.36, 36.53,

37.36, 38.11, 40.66, 44.15, 47.10, 48.21, 51.52 (2×), 52.42, 56.84, 66.57 (C2), 66.76 (C3), 82.50, 113.62 (2×), 126.73 (2×), 139.26, 156.59; LRMS for $C_{29}H_{44}N_3O_4$ $[M+H]^+$: 498.5 *m/z*; HRMS: calcd for $C_{29}H_{44}N_3O_4$ $[M+H]^+$ 498.33263, found 498.33251.

4.1.1.12. 2β-(4-Piperonylpiperazinyl)-5α-androstane-3α,17β-diol (19). Yield: 22%; TLC (hexanes/acetone, 6:4): $R_f = 0.39$; IR (film): 3378 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.70–2.15 (m, residual H), 2.46 (m, 2α-CH and 2× CH_2N), 2.66 (m, 2 × CH_2N), 3.40 (s broad, NCH_2Ar), 3.60 (t, $J = 8.5$ Hz, 17α-CH), 3.81 (m, 3β-CH), 5.93 (s, OCH_2O), 6.73 (m, 2H of Ar), 6.84 (s, 1H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.17, 17.23, 20.89, 23.33, 28.25, 30.54, 31.18, 32.86, 34.71, 35.55, 35.78, 36.85, 38.50, 43.11, 50.93, 53.44 (4×), 56.22, 62.70, 63.62 (C3), 64.46 (C2), 81.81, 100.84, 107.82, 109.49, 122.21, 131.94, 146.59, 147.61; LRMS for $C_{31}H_{47}N_2O_4$ $[M+H]^+$: 511.3 *m/z*; HRMS: calcd for $C_{31}H_{47}N_2O_4$ $[M+H]^+$ 511.35303, found 511.35339.

4.1.1.13. 2β-(Pyrazolyl)-5α-androstane-3α,17β-diol (20). Yield: 76%; TLC (hexanes/acetone, 6:4): $R_f = 0.57$; IR (film): 3358 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.76 (s, 19-CH_3), 0.70–2.20 (residual H), 3.63 (t, $J = 8.5$ Hz, 17α-CH), 4.53 (m, 2α-CH and 3β-CH); 6.36 (t, $J = 2.2$ Hz, 1H of Ar), 7.54 (d, $J = 2.3$ Hz, 1H of Ar), 7.63 (d, $J = 1.8$ Hz, 1H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.15, 14.82, 20.79, 23.28, 27.89, 30.38, 31.15, 34.17, 35.22, 35.76, 36.69, 39.27, 41.57, 43.01, 50.84, 55.41, 62.22 (C3), 67.38 (C2), 81.76, 105.23, 128.76, 138.83; LRMS for $C_{22}H_{35}N_2O_2$ $[M+H]^+$: 359.2 *m/z*; HRMS: calcd for $C_{22}H_{35}N_2O_2$ $[M+H]^+$ 359.26930, found 359.26927.

4.1.2. New method for aminolysis of epoxide 8 (synthesis of 1 and 21–52). In a Schlenk tube purged with argon were added epoxide **8** (1 mmol) dissolved in toluene (30 mL), the desired amine (3 mmol), and $Gd(OTf)_3$ (0.2 mmol). The reaction mixture was heated at 150–190 °C for 2 h (primary amines) or overnight (secondary and cyclic amines). The mixture was then cooled, poured on a silica gel column, and the chromatography performed with an appropriate mixture of MeOH:CH_2Cl_2.

4.1.2.1. 2β-(4-Methylpiperazinyl)-5α-androstane-3α,17β-diol (1).[16] This compound was obtained in 88% yield. The data were identical as reported in the previous section.

4.1.2.2. 2β-(*n*-Propylamino)-5α-androstane-3α,17β-diol (21). Yield: 84%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.12$; IR (film): 3428 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.92 (t, $J = 7.4$ Hz, 3′-CH_3), 0.94 (s, 19-CH_3), 0.70–2.40 (residual H), 2.58 and 2.68 (2m, 1′-CH_2), 2.77 (m, 2α-CH), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.81 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 11.70, 15.37, 20.63, 22.92, 23.31, 28.01, 30.46, 31.40, 33.19, 35.16, 36.07, 36.74, 39.22, 39.47, 43.02, 49.78, 50.90, 55.62, 59.63 (C2), 68.95 (C3), 81.87; LRMS for $C_{22}H_{40}NO_2$ $[M+H]^+$: 350.2 *m/z*; HRMS: calcd for $C_{22}H_{40}NO_2$ $[M+H]^+$ 350.30536, found 350.30647.

4.1.2.3. 2β-(*iso*-Propylamino)-5α-androstane-3α,17β-diol (22). Yield: 46%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.02$; IR (film): 3441 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.92 (s, 19-CH_3), 0.70-2.20 (residual H), 1.34 and 1.45 (2d, $J = 6.4$ Hz, 2 × 2′-CH_3), 3.31 (m, 1′-CH), 3.41 (m, 2α-CH), 3.63 (t, $J = 8.5$ Hz, 17α-CH), 4.10 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.14, 17.10, 17.77, 20.24, 21.06, 23.24, 27.87, 30.44, 30.63, 35.39, 35.91 (2×), 36.57, 38.89, 38.93, 43.04, 47.93, 50.61, 56.20, 56.76 (C2), 66.10 (C3), 81.76; LRMS for $C_{22}H_{40}NO_2$ $[M+H]^+$: 350.4 *m/z*; HRMS: calcd for $C_{22}H_{40}NO_2$ $[M+H]^+$ 350.30536, found 350.30588.

4.1.2.4. 2β-(*n*-Butylamino)-5α-androstane-3α,17β-diol (23). Yield: 55%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.18$; IR (film): 3358 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.91 (t, $J = 7.3$ Hz, 4′-CH_3), 0.95 (s, 19-CH_3), 0.70–2.20 (residual H), 2.56 and 2.68 (2m, 1′-CH_2), 2.74 (m, 2α-CH), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.81 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 13.99, 15.20, 20.44, 20.60, 23.31, 28.02, 30.47, 31.35, 32.19, 32.97, 35.14, 36.07, 36.75, 39.26, 39.49, 43.02, 47.90, 50.92, 55.56, 59.78 (C2), 69.13 (C3), 81.88; LRMS for $C_{23}H_{42}NO_2$ $[M + H]^+$: 364.4 *m/z*; HRMS: calcd for $C_{23}H_{42}NO_2$ $[M+H]^+$ 364.32101, found 364.32207.

4.1.2.5. 2β-(*iso*-Butylamino)-5α-androstane-3α,17β-diol (24). Yield: 47%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.28$; IR (film): 3384 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.96 (m, 19-CH_3 and 2 × 3′-CH_3), 0.70–2.20 (residual H), 2.48 and 2.63 (2m, 1′-CH_2), 2.89 (m, 2α-CH), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.92 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 15.81, 20.35, 20.52, 20.74, 23.29, 27.64, 27.99, 30.45, 31.16, 33.81, 35.22, 36.09, 36.71, 39.10, 39.19, 43.03, 50.84, 55.02, 55.72, 59.99 (C2), 67.95 (C3), 81.86; LRMS for $C_{23}H_{42}NO_2$ $[M+H]^+$: 364.3 *m/z*; HRMS: calcd for $C_{23}H_{42}NO_2$ $[M+H]^+$ 364.32101, found 364.32141.

4.1.2.6. 2β-(*tert*-Butylamino)-5α-androstane-3α,17β-diol (25). Yield: 89%; TLC (CH_2Cl_2/MeOH, (9:1): $R_f = 0.20$; IR (film): 3427 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.75 (s, 18-CH_3), 0.95 (s, 19-CH_3), 1.47 (s, 3 × 2′-CH_3), 0.60–2.20 (residual H), 3.26 (m, 2α-CH), 3.64 (t, $J = 8.5$ Hz, 17α-CH), 4.12 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.15, 16.86, 20.85, 23.28, 26.86 (3×), 27.99, 30.39, 30.82, 35.40, 35.49, 36.43, 36.64, 38.96, 41.59, 43.07, 50.70, 56.25, 56.82 (C2), 59.18, 65.22 (C3), 81.81; LRMS for $C_{23}H_{42}NO_2$ $[M+H]^+$: 364.5 *m/z*; HRMS: calcd for $C_{23}H_{42}NO_2$ $[M+H]^+$ 364.32101, found 364.32143.

4.1.2.7. 2β-(*n*-Pentylamino)-5α-androstane-3α,17β-diol (26). Yield: 48%; TLC (CH_2Cl_2/MeOH, (9:1): $R_f = 0.24$; IR (film): 3337 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.90 (t, $J = 6.9$ Hz, 5′-CH_3), 0.95 (s, 19-CH_3), 0.70–2.20 (residual H), 2.60 and 2.74

(2m, 1′-CH_2), 2.83 (m, 2α-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.87 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 14.01, 15.53, 20.69, 22.49, 23.32, 28.01, 29.08, 29.34, 30.49, 31.26, 33.43, 35.20, 36.07, 36.74, 39.23, 39.28, 43.04, 48.78, 50.89, 55.66, 59.80 (C2), 68.60 (C3), 81.89; LRMS for $C_{24}H_{44}NO_2$ $[M+H]^+$: 378.4 *m/z*; HRMS: calcd for $C_{24}H_{44}NO_2$ $[M+H]^+$ 378.33666, found 378.33998.

4.1.2.8. 2β-(*iso*-Pentylamino)-5α-androstane-3α,17β-diol (27). Yield: 99%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.27; IR (film): 3416 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.94 (m, 19-$CH_3$and $2\times$4′-CH_3), 0.70–2.20 (residual H), 2.88 and 3.08 (2m, 1′-CH_2), 3.25 (m, 2α-CH), 3.63 (t, J = 8.5 Hz, 17α-CH), 4.18 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.16, 17.06, 21.04, 21.99, 22.37, 23.27, 25.95, 27.93, 30.46, 30.75, 34.48, 35.41, 53.63, 35.97, 36.61, 38.19, 39.00, 43.07, 44.24, 50.67, 56.00, 59.84 (C2), 65.89 (C3), 81.78; LRMS for $C_{24}H_{44}NO_2$ $[M+H]^+$: 378.4 *m/z*; HRMS: calcd for $C_{24}H_{44}NO_2$ $[M+H]^+$ 378.33666, found 378.33690.

4.1.2.9. 2β-(*neo*-Pentylamino)-5α-androstane-3α,17β-diol (28). Yield: 85%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.50; IR (film): 3363 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): 0.69 (s, 18-CH_3), 0.87 (s, $3\times CH_3$), 0.93 (s, 19-CH_3), 0.65–2.10 (residual H), 2.17 and 2.48 (2d, J = 11.4 Hz, 1′-CH_2), 2.64 (m, 2α-CH), 3.57 (t, J = 8.5 Hz, 17α-CH), 3.75 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$ + CD_3OD): 11.02, 15.07, 20.49, 23.16, 27.52 (3×), 27.95, 29.92, 31.23, 31.28, 32.65, 35.03, 35.91, 36.64, 39.14, 39.63, 42.87, 50.82, 55.47, 59.82 (C2), 60.25, 68.60 (C3), 81.48; LRMS for $C_{24}H_{44}NO_2$ $[M+H]^+$: 378.3 *m/z*; HRMS: calcd for $C_{24}H_{44}NO_2$ $[M+H]^+$ 378.33666, found 378.33729.

4.1.2.10. 2β-(*tert*-Pentylamino)-5α-androstane-3α,17β-diol (29). Yield: 35%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.30; IR (film): 3426 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.94 (s, 19-CH_3), 1.01 (t, J = 7.5 Hz, 4′-CH_3), 1.39 (s, 2′-CH_3), 1.40 (s, 2′-CH_3), 0.70–2.20 (residual H), 3.26 (m, 2α-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 4.17 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 7.92, 11.15, 16.91, 20.86, 23.25, 23.73, 24.06, 28.00, 30.31, 30.78, 31.84, 35.35, 35.45, 36.46, 36.60, 38.92, 41.72, 43.03, 50.63, 56.30, 56.30 (C2), 62.17, 65.02 (C3), 81.76; LRMS for $C_{24}H_{44}NO_2$ $[M+H]^+$: 378.2 *m/z*; HRMS: calcd for $C_{24}H_{44}NO_2$ $[M+H]^+$ 378.33666, found 378.33727.

4.1.2.11. 2β-(*n*-Hexylamino)-5α-androstane-3α,17β-diol (30). Yield: 74%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.32; IR (film): 3382 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.88 (t, J = 6.8 Hz, 6′-CH_3), 0.93 (s, 19-CH_3), 0.70–2.20 (residual H), 2.72 and 2.85 (2m, 1′-CH_2), 3.00 (m, 2α-CH) 3.62 (t, J = 8.5 Hz, 17α-CH), 3.96 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 13.98, 16.21, 20.84, 22.46, 23.29, 26.54, 27.81, 27.96, 30.45, 31.01, 31.40, 34.44, 35.29, 36.01, 36.68, 38.81, 39.09, 43.05, 46.84, 50.78, 55.82, 59.71 (C2), 67.41 (C3), 81.82; LRMS for $C_{25}H_{46}NO_2$ $[M+H]^+$: 392.4 *m/z*; HRMS: calcd for $C_{25}H_{46}NO_2$ $[M+H]^+$ 392.35231, found 392.35533.

4.1.2.12. 2β-(*n*-Heptylamino)-5α-androstane-3α,17β-diol (31). Yield: 77%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.31; IR (film): 3376 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.88 (t, J = 6.8 Hz, 7′-CH_3), 0.94 (s, 19-CH_3), 0.70–2.20 (residual H), 2.68 and 2.81 (2m, 1′-CH_2), 2.94 (m, 2α-CH), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.95 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.16, 14.06, 16.03, 20.79, 22.66, 23.29, 26.92, 27.96, 28.28, 28.96, 30.46, 31.08, 31.65, 34.17, 35.25, 36.02, 36.69, 38.96, 39.13, 43.04, 47.10, 50.81, 55.77, 59.75 (C2), 67.73 (C3), 81.84; LRMS for $C_{26}H_{48}NO_2$ $[M+H]^+$: 406.4 *m/z*; HRMS: calcd for $C_{26}H_{48}NO_2$ $[M+H]^+$ 406.36796, found 406.36919.

4.1.2.13. 2β-(*n*-Octylamino)-5α-androstane-3α,17β-diol (32). Yield: 93%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.38; IR (film): 3430 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.88 (t, J = 6.8 Hz, 8′-CH_3), 0.91 (s, 19-CH_3), 0.60–2.10 (residual H), 2.86 and 3.02 (2m, 1′-CH_2), 3.25 (m, 2α-CH), 3.62 (t, J = 8.1 Hz, 17α-CH), 4.08 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.16, 14.05, 16.89, 21.13, 22.58, 23.39, 26.38, 26.49, 28.07, 28.94, 28.98, 30.44, 30.74, 31.66, 35.38, 35.53, 35.95, 36.61, 38.42, 38.95, 43.05, 45.90, 50.68, 56.00, 59.69 (C2), 66.29 (C3), 81.77; LRMS for $C_{27}H_{50}NO_2$ $[M+H]^+$: 420.4 *m/z*; HRMS: calcd for $C_{27}H_{50}NO_2$ $[M+H]^+$ 420.38361, found 420.38386.

4.1.2.14. 2β-(*n*-Nonylamino)-5α-androstane-3α,17β-diol (33). Yield: 85%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.38; IR (film): 3383 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.87 (t, J = 6.4 Hz, 9′-CH_3), 0.93 (s, 19-CH_3), 0.60–2.10 (residual H), 2.66 and 2.79 (2m, 1′-CH_2), 2.93 (m, 2α-CH), 3.61 (t, J = 8.4 Hz, 17α-CH), 3.91 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.18, 14.10, 15.86, 20.76, 22.64, 23.30, 27.00, 27.98, 28.56, 29.22, 29.33, 29.43, 30.44, 31.12, 31.84, 33.98, 35.24, 36.03, 36.73, 39.04, 39.14, 43.04, 47.32, 50.84, 55.74, 59.72 (C2), 68.00 (C3), 81.83; LRMS for $C_{28}H_{52}NO_2$ $[M+H]^+$: 434.4 *m/z*; HRMS: calcd for $C_{28}H_{52}NO_2$ $[M+H]^+$ 434.39926, found 434.40196.

4.1.2.15. 2β-(*n*-Decylamino)-5α-androstane-3α,17β-diol (34). Yield: 88%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.42; IR (film): 3423 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.88 (t, J = 6.8 Hz, 10′-CH_3), 0.92 (s, 19-CH_3), 0.60–2.10 (residual H), 2.81 and 2.95 (2m, 1′-CH_2), 3.17 (m, 2α-CH), 3.62 (t, J = 8.4 Hz, 17α-CH), 4.04 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.16, 14.09, 16.70, 20.95, 22.65, 23.27, 26.63, 26.70, 27.93, 29.11, 29.27, 29.36, 29.48, 30.43, 30.82, 31.85, 35.22, 35.35, 35.97, 36.63, 38.49, 38.99, 43.05, 46.15, 50.71, 55.94, 59.67 (C2), 66.58 (C3), 81.77; $C_{29}H_{54}NO_2$ $[M+H]^+$: 448.5 *m/z*; HRMS: calcd for $C_{29}H_{54}NO_2$ $[M+H]^+$ 448.41491, found 448.41755.

4.1.2.16. 2β-(*n*-Undecylamino)-5α-androstane-3α,17β-diol (35). Yield: 83%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.42; IR (film): 3420 (OH and NH); ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.87 (t,

J = 6.4 Hz, 11′-CH_3), 0.92 (s, 19-CH_3), 0.70–2.20 (residual H), 2.77 and 2.88 (2m, 1′-CH_2), 3.08 (m, 2α-CH), 3.62 (t, J = 8.3 Hz, 17α-CH), 4.00 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.15, 14.11, 16.43, 20.86, 22.66, 23.25, 26.73, 27.25, 27.92, 29.19, 29.31, 29.40, 29.55 (2×), 30.38, 30.88, 31.87, 34.82, 35.29, 35.94, 36.62, 38.60, 38.99, 43.03, 46.46, 50.70, 55.84, 59.62 (C2), 66.96 (C3), 81.76; LRMS for $C_{30}H_{56}NO_2$ $[M+H]^+$: 462.4 *m/z*; HRMS: calcd for $C_{30}H_{56}NO_2$ $[M+H]^+$ 462.43056, found 462.43256.

4.1.2.17. 2β-(*n*-Dodecylamino)-5α-androstane-3α,17β-diol (36). Yield: 78%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.40; IR (film): 3421 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.87 (t, J = 6.6 Hz, 12′-CH_3), 0.92 (s, 19-CH_3), 0.60–2.10 (residual H), 2.71 and 2.84 (2m, 1′-CH_2), 3.00 (m, 2α-CH), 3.61 (t, J = 8.1 Hz, 17α-CH), 3.94 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.18, 14.11, 16.13, 20.82, 22.67, 23.29, 26.88, 27.94, 27.97, 29.27, 29.34, 29.45, 29.56, 29.63, 29.64, 30.41, 31.02, 31.90, 34.39, 35.28, 36.00, 36.70, 38.82, 39.07, 43.04, 46.92, 50.79, 55.80, 59.67 (C2), 67.50 (C3), 81.79; LRMS for $C_{31}H_{58}NO_2$ $[M+H]^+$: 476.4 *m/z*; HRMS: calcd for $C_{31}H_{58}NO_2$ $[M+H]^+$ 476.44621, found 476.44798.

4.1.2.18. 2β-(*n*-Hexadecylamino)-5α-androstane-3α,17β-diol (37). Yield: 82%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.44; IR (film): 3426 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.87 (t broad, 16′-CH_3), 0.92 (s, 19-CH_3), 0.60–2.10 (residual H), 2.76 and 2.90 (2m, 1′-CH_2), 3.08 (m, 2α-CH), 3.62 (t, J = 8.3 Hz, 17α-CH), 3.99 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 14.17, 16.41, 20.88, 22.68, 23.28, 26.76, 27.37, 27.95, 29.21, 29.35, 29.42, 29.57, 29.65, 29.68, 29.70 (4×), 30.44, 30.94, 31.96, 34.79, 35.35, 36.01, 36.67, 38.69, 39.04, 43.04, 46.58, 50.78, 55.89, 59.69 (C2), 67.13 (C3), 81.82; LRMS for $C_{35}H_{66}NO_2$ $[M+H]^+$: 532.5 *m/z*; HRMS: calcd for $C_{35}H_{66}NO_2$ $[M+H]^+$ 532.50881, found 532.50954.

4.1.2.19. 2β-(Cyclopentylamino)-5α-androstane-3α,17β-diol (38). Yield: 98%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.36; IR (film): 3383 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.93 (s, 19-CH_3), 0.70–2.20 (residual H), 2.98 (m, 2α-CH), 3.36 (m, 1′-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.94 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 16.19, 20.81, 23.31, 23.79 (2×), 28.01, 30.50, 31.12, 31.78, 32.68, 34.00, 35.28, 36.10, 36.72, 39.11, 39.98, 43.07, 50.84, 55.85, 57.37, 58.10 (C2), 68.47 (C3), 81.88; LRMS for $C_{24}H_{42}NO_2$ $[M+H]^+$: 376.4 *m/z*; HRMS: calcd for $C_{24}H_{42}NO_2$ $[M+H]^+$ 376.32101, found 376.32138.

4.1.2.20. 2β-(Cyclohexylamino)-5α-androstane-3α,17β-diol (39). Yield: 91%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.43; IR (film): 3386 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.93 (s, 19-CH_3), 0.70–2.20 (residual H), 2.81 (m, 1′-CH), 3.11 (m, 2α-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.96 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 16.49, 20.88, 23.28, 24.63, 24.85, 25.33, 27.99, 30.46, 30.97, 31.99 (2×), 34.58, 35.31, 36.06, 36.66, 39.06, 39.64, 43.05, 50.75, 54.78, 55.91, 56.16 (C2), 67.51 (C3), 81.83; LRMS for $C_{25}H_{44}NO_2$ $[M+H]^+$: 390.3 *m/z*; HRMS: calcd for $C_{25}H_{44}NO_2$ $[M+H]^+$ 390.33666, found 390.33716.

4.1.2.21. 2β-(Cycloheptylamino)-5α-androstane-3α,17β-diol (40). Yield: 56%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.49; IR (film): 3396 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.70 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.70–2.20 (residual H) 2.96 (m, 2α-CH and 1′-CH), 3.58 (t, J = 8.5 Hz, 17α-CH), 3.91 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$ + CD_3OD): 11.08, 16.20, 20.77, 23.19, 23.71, 24.03, 27.60, 27.64, 27.94, 30.12, 30.95, 31.71, 33.65, 34.23, 35.22, 35.94, 36.60, 38.84, 38.93, 42.95, 50.70, 55.78, 56.40 (C2), 56.97, 66.97 (C3), 81.55; LRMS for $C_{26}H_{46}NO_2$ $[M+H]^+$: 404.4 *m/z*; HRMS: calcd for $C_{26}H_{46}NO_2$ $[M+H]^+$ 404.35231, found 404.35264.

4.1.2.22. 2β-(3-Ethoxypropylamino)-5α-androstane-3α,17β-diol (41). Yield: 39%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.38; IR (film): 3426 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.93 (s, 19-CH_3), 1.21 (t, J = 8.0 Hz, 6′-CH_3), 0.60–2.10 (residual H), 3.06 (m, 1′-CH_2), 3.21 (m, 2α-CH), 3.35 (m, 1′-CH_2), 3.54 (m, CH_2O), 3.63 (m, 17α-CH and CH_2O), 4.10 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.21, 15.00, 16.46, 20.88, 23.28, 25.43, 27.93, 30.41, 30.89, 34.63, 35.36, 35.82, 36.64, 37.72, 38.87, 43.08, 46.46, 50.75, 55.84, 59.79 (C2), 65.37 (C3), 67.33, 69.89, 81.78; LRMS for $C_{24}H_{44}NO_3$ $[M+H]^+$: 394.4 *m/z*; HRMS: calcd for $C_{24}H_{44}NO_3$ $[M+H]^+$ 394.33157, found 394.33273.

4.1.2.23. 2β-(3-Butoxypropylamino)-5α-androstane-3α,17β-diol (42). Yield: 82%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.43; IR (film): 3420 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.90 (s and t, 19-CH_3 and 8′-CH_3), 0.60–2.10 (residual H), 3.08 (m, 1′-CH_2), 3.21 (m, 2α-CH), 3.33 (m, 1′-CH_2), 3.45 (m, 2H, CH_2O), 3.62 (m, 17α-CH and CH_2O), 4.08 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.22, 13.87, 16.38, 19.22, 20.84, 23.31, 25.49, 27.86, 30.38, 30.89, 31.40, 34.51, 35.30, 35.77, 36.65, 37.77, 38.79, 43.07, 46.40, 50.73, 55.75, 59.79 (C2), 65.37 (C3), 69.93, 71.79, 81.74; LRMS for $C_{26}H_{48}NO_3$ $[M+H]^+$: 422.3 *m/z*; HRMS: calcd for $C_{26}H_{48}NO_3$ $[M+H]^+$ 422.36287, found 422.36324.

4.1.2.24. 2β-(2-Phenylethylamino)-5α-androstane-3α,17β-diol (43). Yield: 81%; TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.60; IR (film): 3370 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.68 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.60–2.10 (residual H), 2.83 (m, 1′-CH_2 and 2′-CH_2), 2.98 (m, 2α-CH), 3.58 (t, J = 8.7 Hz, 17α-CH), 3.81 (m, 3β-CH), 7.12-7.38 (m, 5H of Ar); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 15.45, 20.67, 23.33, 27.99, 30.55, 31.21, 33.42, 35.18, 35.55, 36.03, 36.61, 39.13, 39.19, 43.04, 48.97, 50.92, 55.65, 59.72 (C2), 68.56 (C3), 81.88, 126.51, 128.61 (2×), 128.74 (2×), 138.55; LRMS for $C_{27}H_{42}NO_2$ $[M+H]^+$: 412.3 *m/z*; HRMS: calcd for $C_{27}H_{42}NO_2$ $[M+H]^+$ 412.32101, found 412.32178.

4.1.2.25. 2β-(3-Phenylpropylamino)-5α-androstane-3α,17β-diol (44). Yield: 83%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.51$; IR (film): 3392 (OH and NH); ^1H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.60–2.10 (residual H), 2.68 (m, 1H of 1′-CH_2 and 3′-CH_2), 2.85 (m, 1H of 1′-CH_2), 2.93 (m, 2α-CH), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.90 (m, 3β-CH), 7.15–7.23 (m, 3H of Ar), 7.25–7.33 (m, 2H of Ar); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.19, 15.93, 20.77, 23.30, 27.93, 29.70, 30.46, 31.10, 33.04, 34.12, 35.25, 36.00, 36.70, 39.01, 39.12, 43.05, 46.50, 50.86, 55.76, 59.74 (C2), 67.75 (C3), 81.85, 126.14, 128.44 (2×), 128.52 (2×), 140.94. LRMS for $C_{28}H_{44}NO_2$ $[M+H]^+$: 426.3 *m/z*; HRMS: calcd for $C_{28}H_{44}NO_2$ $[M+H]^+$ 426.33666, found 426.33731.

4.1.2.26. 2β-(3,3-Diphenylpropylamino)-5α-androstane-3α,17β-diol (45). Yield: 82%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.66$; IR (film): 3360 (OH and NH); ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.60–2.10 (residual H), 2.24 (m, 2′-CH_2), 2.52 (m, 1H of 1′-CH_2), 2.67 (m, 2α-CH and 1H of 1′-CH_2), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.71 (m, 3β-CH), 4.04 (t, $J = 7.8$ Hz, 3′-CH), 7.15–7.30 (m, 10H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 15.19, 20.54, 23.31, 27.98, 30.46, 31.31, 32.89, 35.10, 35.64, 36.00, 36.72, 39.20, 39.59, 43.00, 46.46, 48.94, 50.89, 55.47, 59.69 (C2), 68.76 (C3), 81.86, 126.24 (2×), 127.76 (4×), 128.48 (4×), 144.53 (2×); LRMS for $C_{34}H_{48}NO_2$ $[M+H]^+$: 502.3 *m/z*; HRMS: calcd for $C_{34}H_{48}NO_2$ $[M+H]^+$ 502.36796, found 502.36818.

4.1.2.27. 2β-(Adamantylmethylamino)-5α-androstane-3α,17β-diol (46). Yield: 72%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.67$; IR (film): 3356 (OH and NH); ^1H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.96 (s, 19-CH_3), 0.60–2.00 (residual H), 2.09 (d, $J = 11.6$ Hz, 1H of 1′-CH_2), 2.39 (d, $J = 11.2$ Hz, 1H of 1′-CH_2), 2.63 (m, 2α-CH), 3.62 (t, $J = 8.7$ Hz, 17α-CH), 3.74 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.18, 15.23, 20.62, 23.34, 28.07, 28.44 (3×), 30.51, 31.42, 32.82, 33.36, 35.17, 36.18, 36.80, 37.24 (3×), 39.37, 39.95, 40.84 (3×), 43.05, 51.02, 55.59, 60.31, 61.09 (C2), 69.27 (C3), 81.96; LRMS for $C_{30}H_{50}NO_2$ $[M+H]^+$: 456.4 *m/z*; HRMS: calcd for $C_{30}H_{50}NO_2$ $[M+H]^+$ 456.38361, found 456.38410.

4.1.2.28. 2β-(Diethylamino)-5α-androstane-3α,17β-diol (47). Yield: 67%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.38$; IR (film): 3444 (OH); ^1H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.93 (s, 19-CH_3), 1.47 (t, $J = 7.1$ Hz, 2 × 2′-CH_3), 0.70–2.20 (residual H), 2.83 (m, 2α-CH), 3.05 and 3.39 (2m, 2 × 1′-CH_2), 3.64 (t, $J = 8.5$ Hz, 17α-CH), 4.10 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 10.23, 11.18, 11.36, 17.90, 21.08, 23.24, 27.89, 30.43, 30.57, 34.92, 35.44, 36.17, 36.31, 36.51, 38.77, 43.03, 44.27, 48.07, 50.51, 56.28, 63.70 and 63.75 (C2 and C3), 81.69; LRMS for $C_{23}H_{42}NO_2$ $[M+H]^+$: 364.3 *m/z*; HRMS: calcd for $C_{23}H_{42}NO_2$ $[M+H]^+$ 364.32101, found 364.32285.

4.1.2.29. 2β-(4-Acetylpiperazino)-5α-androstane-3α,17β-diol (48). Yield: 83%; TLC (hexanes/acetone, 6:4): $R_f = 0.06$; IR (film): 3384 (OH), 1630 (C═O); ^1H NMR (300 MHz, $CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 2.10 (s, 2″-CH_3), 0.65–2.15 (residual H), 2.50 (m, CH_2N), 2.70 (m, 2α-CH and CH_2N), 3.56 (m, 17α-CH and 2 × CH_2NCO), 3.87 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.18, 17.33, 20.94, 21.27, 23.29, 28.07, 30.47, 31.02, 33.28, 34.94, 35.48, 35.87, 36.71, 38.51, 41.31, 43.07, 46.18, 47.90, 48.52, 50.80, 56.09, 63.88 (C3), 64.99 (C2), 81.80, 168.86; LRMS for $C_{25}H_{43}N_2O_3$ $[M+H]^+$: 419.3 *m/z*; HRMS: calcd for $C_{25}H_{43}N_2O_3$ $[M+H]^+$ 419.32682, found 419.32700.

4.1.2.30. 2β-(4-Phenylpiperazino)-5α-androstane-3α,17β-diol (49). Yield: 80%; TLC (hexanes/acetone, 6:4): $R_f = 0.67$; IR (film): 3364 (OH), 1600 (CH aromatic); ^1H NMR (400 MHz, $CDCl_3$): 0.74 (s, 19-CH_3), 0.89 (s, 19-CH_3), 0.70–2.20 (residual H), 2.75 (m, CH_2N), 2.93 (m, 2α-CH and CH_2N), 3.28 (m, 2 × CH_2NCO), 3.63 (t, $J = 8.5$ Hz, 17α-CH), 3.94 (m, 3β-CH), 6.91 (m, 3H of Ar), 7.28 (m, 2H of Ar); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.18, 17.43, 20.95, 23.29, 28.09, 30.48, 31.03, 33.32, 35.12, 35.48, 35.91, 36.72, 38.55, 43.07, 48.04 (2×), 49.23 (2×), 50.78, 56.16, 63.97 (C3), 64.68 (C2), 81.82, 116.44 (2×), 120.29, 129.16 (2×), 151.17; LRMS for $C_{29}H_{45}N_2O_2$ $[M+H]^+$: 453.3 *m/z*; HRMS: calcd for $C_{29}H_{45}N_2O_2$ $[M+H]^+$ 453.34756, found 453.34788.

4.1.2.31. 2β-{4-(4-Fluorophenyl)-piperazino}-5α-androstane-3α,17β-diol (50). Yield: 97%; TLC (hexanes/acetone, 6:4): $R_f = 0.67$; IR (film): 3368 (OH); ^1H NMR (400 MHz, $CDCl_3$): 0.74 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.70–2.20 (residual H), 2.66 (m, CH_2N), 2.84 (m, 2α-CH and CH_2N), 3.13 (m, 2 × CH_2NCO), 3.62 (m, 17α-CH), 3.91 (m, 3β-CH), 6.87 (m, 2H of Ar), 6.96 (m, 2H of Ar); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.21, 17.26, 20.91, 23.31, 28.16, 30.50, 31.12, 32.92, 34.74, 35.50, 35.85, 36.77, 38.52, 43.08, 48.18 (2×), 50.62 (2×), 50.84, 56.13, 63.85 (C3), 64.60 (C2), 81.86, 115.42, 115.64, 118.00, 118.07, 147.80, 156.08 and 158.46 (C–F coupling); LRMS for $C_{29}H_{44}FN_2O_2$ $[M+H]^+$: 471.3 *m/z*; HRMS: calcd for $C_{29}H_{44}FN_2O_2$ $[M+H]^+$ 471.33813, found 471.33799.

4.1.2.32. 2β-(Piperidinyl)-5α-androstane-3α,17β-diol (51). Yield: 86%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.19$; IR (film): 3424 (OH); ^1H NMR (300 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.70–2.20 (residual H), 2.34 (m, CH_2N), 2.60 (m, CH_2N and 2α-CH), 3.62 (t, $J = 7.8$ Hz, 17α-CH), 3.80 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.21, 17.98, 21.05, 22.32, 23.22, 23.56 (2×), 27.86, 30.36, 30.59, 34.94, 35.41, 36.16, 36.26, 36.53, 38.59, 43.03, 50.53, 51.80 (2x), 56.13, 63.78 (C3), 66.12 (C2), 81.63; LRMS for $C_{24}H_{42}NO_2$ $[M+H]^+$: 376.4 *m/z*; HRMS: calcd for $C_{24}H_{42}NO_2$ $[M+H]^+$ 376.32101, found 376.32177.

4.1.2.33. 2β-(4-Piperidinylpiperidinyl)-5α-androstane-3α,17β-diol (52). Yield: 73%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.08$; IR (film): 3423 (OH); ^1H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.70–2.20 (residual H), 2.59 (m, CHN), 2.73 (m, 2α-CH), 2.90 (m, 4 × CH_2N), 3.62 (t, $J = 8.4$ Hz, 17α-CH), 3.81 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.15, 17.33, 20.91, 23.12, 23.29, 24.17 (2×), 27.03, 27.44,

28.18, 30.48, 31.08, 33.05, 34.86, 35.53, 35.76, 36.77, 38.44, 43.09, 50.20 (4×), 50.87, 56.19, 63.90, 63.90 (C3), 64.55 (C2), 81.80; LRMS for $C_{29}H_{51}N_2O_2$ $[M+H]^+$: 459.4 *m/z*; HRMS: calcd for $C_{29}H_{51}N_2O_2$ $[M+H]^+$ 459.39451, found 459.39454.

4.1.3. 2β-Piperazinyl-5α-androstane-3α,17β-diol (53).[19] A solution of epoxide **8** (6.46 g, 19.4 mmol) in piperazine (33.3 g, 388 mmol) and H_2O (5 mL) was heated in a Schlenk tube at 150–190 °C overnight, then poured in water (500 mL) and the precipitate was filtered. The solid was dissolved in CH_2Cl_2 and the solution dried over $MgSO_4$, filtered and evaporated to dryness. Purification of the crude product by flash chromatography (MeOH:Et_3N:CH_2Cl_2, 14:1:85) yielded 5.76 g (70%) of compound **53** as a white solid. TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.02; IR (film): 3370 (OH and NH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.71 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.65–2.15 (residual H), 2.42, 2.58 and 2.90 (3m, 2α-CH and 4 × CH_2N), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.84 (m, 3β-CH); ^{13}C NMR, 75 MHz ($CDCl_3$): 11.18, 17.31, 20.86, 23.31, 28.23, 30.52, 31.15, 32.58, 34.67, 35.50, 35.72, 36.87, 38.40, 43.04, 46.67 (2×), 49.38 (2×), 50.87, 56.17, 63.35 (C3), 65.03 (C2), 81.60; LRMS for $C_{23}H_{41}N_2O_2$ $[M+H]^+$: 377.3 *m/z*; HRMS: calcd for $C_{23}H_{41}N_2O_2$ $[M+H]^+$ 377.31625, found 377.31624.

4.1.4. Synthesis of *N*-amide derivatives of 53 (general procedure for the synthesis of 54–61). HBTU (*O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) (1 equiv) and carboxylic acid (1.1 equiv) were dissolved in DMF at 0 °C. Then, diisopropylethylamine (DIPEA) was added to the mixture and allowed to react for 5 min. Thereafter, compound **53** (1 equiv) dissolved in DMF was added to the solution and the temperature was raised to room temperature for 2–3 h. The resulting mixture was diluted in ethyl acetate and washed with water (4×). The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to dryness. Purification of the crude product by flash chromatography (1–4% MeOH in CH_2Cl_2) yielded the desired amide as a white solid.

4.1.4.1. 2β-(4-Propanoylpiperazinyl)-5α-androstane-3α,17β-diol (54). Yield: 85%; TLC (hexanes/acetone, 6:4): R_f = 0.19; IR (film): 3419 (OH), 1629 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 1.13 (t, J = 7.4 Hz, 3″-CH_3), 0.60–2.20 (residual H), 2.32 (q, J = 7.5 Hz, 2″-CH_2), 2.48 (m, CH_2N), 2.69 (m, 2α-CH and CH_2N), 3.49 (m, CH_2NCO), 3.63 (m, 17α-CH and CH_2NCO), 3.88 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 9.45, 11.17, 17.22, 20.89, 23.28, 26.39, 28.10, 30.44, 31.05, 32.97, 34.72, 35.45, 35.80, 36.72, 38.44, 41.70, 43.06, 45.58, 48.04, 48.57, 50.81, 56.04, 63.75 (C3), 64.90 (C2), 81.79, 172.19; LRMS for $C_{26}H_{45}N_2O_3$ $[M+H]^+$: 433.3 *m/z*; HRMS: calcd for $C_{26}H_{45}N_2O_3$ $[M+H]^+$ 433.34247, found 433.34278.

4.1.4.2. 2β-(4-Butanoylpiperazinyl)-5α-androstane-3α,17β-diol (55). Yield: 82%; TLC (hexanes/acetone, 6:4): R_f = 0.24; IR (film): 3390 (OH), 1634 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.96 (t, J = 7.4 Hz, 4″-CH_3), 0.60–2.20 (residual H), 2.29 (t, J = 7.5 Hz, 2″-CH_2), 2.55 (m broad, CH_2N), 2.75 (m broad, 2α-CH and CH_2N), 3.65 (m broad, 17α-CH and 2 × CH_2NCO), 3.90 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 13.98, 17.41, 18.66, 20.95, 23.28, 28.04, 30.46, 30.96, 33.43, 35.08 (2×), 35.46, 35.91, 36.68, 38.50, 41.03, 43.06, 45.16, 47.93, 48.58, 50.75, 56.06, 63.88 (C3), 65.13 (C2), 81.79, 171.43; LRMS for $C_{27}H_{47}N_2O_3$ $[M+H]^+$: 447.3 *m/z*; HRMS: calcd for $C_{27}H_{47}N_2O_3$ $[M+H]^+$ 447.35812, found 447.35804.

4.1.4.3. 2β-(4-*iso*-Butanoylpiperazinyl)-5α-androstane-3α,17β-diol (56). Yield: 75%; TLC (hexanes/acetone, 6:4): R_f = 0.30; IR (film): 3402 (OH), 1624 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 1.11 (d, J = 6.6 Hz, 2 × 3″-CH_3), 0.60–2.20 (residual H), 2.50 (m, CH_2N), 2.75 (m, 2α-CH, 2″-CH and CH_2N), 3.60 (m broad, 17α-CH and 2 × CH_2NCO), 3.88 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 17.18, 19.38 (2×), 20.89, 23.28, 28.11, 29.94, 30.45, 31.07, 32.87, 34.62, 35.46, 35.79, 36.72, 38.43, 41.90, 43.06, 45.73, 48.15, 48.81, 50.81, 56.04, 63.75 (C3), 64.81 (C2), 81.79, 175.30; LRMS for $C_{27}H_{47}N_2O_3$ $[M+H]^+$: 447.3 *m/z*; HRMS: calcd for $C_{27}H_{47}N_2O_3$ $[M+H]^+$ 447.35812, found 447.35798.

4.1.4.4. 2β-(4-Pentanoylpiperazinyl)-5α-androstane-3α,17β-diol (57). Yield: 80%; TLC (hexanes/acetone, 6:4): R_f = 0.31; IR (film): 3398 (OH), 1624 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.92 (t, J = 7.3 Hz, 5″-CH_3), 0.60–2.20 (residual H), 2.31 (t, J = 7.7 Hz, 2″-CH_2), 2.60 (m broad, CH_2N), 2.80 (m broad, 2α-CH and CH_2N), 3.60 (m broad, 17α-CH and 2 × CH_2NCO), 3.91 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 13.86, 17.40, 20.94, 22.53, 23.28, 27.33, 28.04, 30.44, 30.96, 32.90, 33.46, 35.11, 35.45, 35.91, 36.68, 38.49, 40.99, 43.06, 45.14, 47.97, 48.57, 50.75, 56.04, 63.87 (C3), 65.14 (C2), 81.76, 171.62, LRMS for $C_{28}H_{49}N_2O_3$ $[M+H]^+$: 461.3 *m/z*; HRMS: calcd for $C_{28}H_{49}N_2O_3$ $[M+H]^+$ 461.37377, found 461.37345.

4.1.4.5. 2β-(4-*iso*-Pentanoylpiperazinyl)-5α-androstane-3α,17β-diol (58). Yield: 79%; TLC (hexanes/acetone, 6:4): R_f = 0.31; IR (film): 3402 (OH), 1626 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.96 (d, J = 6.6 Hz, 2× 3″-CH_3), 0.60–2.10 (residual H), 2.19 (d, J = 8.2 Hz, 2′-CH_2), 2.43 (m, CH_2N), 2.63 (m, CH_2N and 2α-CH), 3.46 (m, CH_2NCO), 3.61 (m, CH_2NCO and 17α-CH), 3.86 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.20, 17.21, 20.91, 22.77 (2×), 23.32, 25.80, 28.17, 30.52, 31.12, 32.80, 34.59, 35.51, 35.81, 36.77, 38.46, 41.95, 42.03, 43.09, 46.31, 48.20, 48.73, 50.87, 56.10, 63.75 (C3), 64.81 (C2), 81.88, 170.97; LRMS for $C_{28}H_{49}N_2O_3$ $[M+H]^+$: 461.3 *m/z*; HRMS: calcd for $C_{28}H_{49}N_2O_3$ $[M+H]^+$ 461.37377, found 461.37384.

4.1.4.6. 2β-(4-Nonanoylpiperazinyl)-5α-androstane-3α,17β-diol (59). Yield: 70%; TLC (hexanes/acetone, 6:4): R_f = 0.43; IR (film): 3394 (OH), 1628 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s,

19-CH_3), 0.87 (t, J = 7.0 Hz, 9″-CH_3), 0.65–2.15 (residual H), 2.30 (t, J = 7.7 Hz, 2″-CH_2), 2.41 (m, CH_2N), 2.64 (m, 2α-CH and CH_2N), 3.46 (m, CH_2NCO), 3.62 (m, 17α-CH and CH_2NCO), 3.88 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.17, 14.08, 17.18, 20.88, 22.63, 23.28, 25.34, 28.13, 29.15, 29.35, 29.47, 30.46, 31.09, 31.79, 32.80, 33.30, 34.57, 35.47, 35.76, 36.73, 38.43, 41.91, 43.06, 46.07, 48.09, 48.63, 50.83, 56.07, 63.73 (C3), 64.76 (C2), 81.82, 171.68; LRMS for $C_{32}H_{57}N_2O_3$ $[M+H]^+$: 517.5 *m/z*; HRMS: calcd for $C_{32}H_{57}N_2O_3$ $[M+H]^+$ 517.43637, found 517.43622.

4.1.4.7. 2β-(4-Cyclohexylcarbonylpiperazinyl)-5α-androstane-3α,17β-diol (60). Yield: 84%; TLC (hexanes/acetone, 6:4): R_f = 0.37; IR (film): 3382 (OH), 1628 (C=O); ^1H NMR (300 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.60–2.10 (residual H), 2.43 (m, 2″-CH), 2.40–3.20 (m broad, 2α-CH and 2 × CH_2N), 3.65 (m broad, 17α-CH and 2 × CH_2NCO), 3.93 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 17.56, 18.59, 21.01, 23.26, 25.71 (2×), 27.98, 29.33 (2×), 30.45, 30.85, 33.92, 34.69, 35.45, 36.03, 36.63, 38.52, 40.23 (2×), 42.64, 43.06, 44.59, 48.85 (2×), 50.68, 54.51, 56.02, 63.96 (C3), 65.45 (C2), 81.75, 174.52; LRMS for $C_{30}H_{51}N_2O_3$ $[M+H]^+$: 487.5 *m/z*; HRMS: calcd for $C_{30}H_{51}N_2O_3$ $[M+H]^+$ 487.38942, found 487.38903.

4.1.4.8. 2β-(4-Benzoylpiperazinyl)-5α-androstane-3α,17β-diol (61). Yield: 90%; TLC (hexanes/acetone, 6:4): R_f = 0.29; IR (film): 3392 (OH), 1622 (C=O); ^1H NMR (300 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.60–2.20 (residual H), 2.40–2.90 (m broad, 2α-CH and 2 × CH_2N), 3.55 (m broad, 17α-CH and 2 × CH_2NCO), 3.87 (m, 3β-CH), 7.40 (s, 5H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 17.28, 20.92, 23.28, 28.08, 30.45, 31.02, 33.15, 34.81, 35.47, 35.84, 36.72, 38.45, 42.18 (2×), 43.06, 48.07, 48.66, 50.79, 56.06, 63.78 (C3), 65.01 (C2), 81.79, 127.08 (2×), 128.51 (2×), 129.85, 135.37, 170.29; LRMS for $C_{30}H_{45}N_2O_2$ $[M+H]^+$: 481.5 *m/z*; HRMS: calcd for $C_{30}H_{45}N_2O_3$ $[M+H]^+$ 481.34247, found 481.34230.

4.1.5. Synthesis of *N*-alkylpiperazino derivatives 62–69 (general procedure). To an amidopiperazino derivative selected from **54** to **61** (1 equiv) dissolved in dry THF was added at 0 °C a 1.0 M solution of BH_3-THF (10 equiv) and the mixture was stirred at room temperature for 90 min. Water was then added and the mixture was extracted with CH_2Cl_2, the organic layer dried over $MgSO_4$, filtered and evaporated to dryness. The crude residue was dissolved in MeOH and heated at reflux overnight to break the boron–nitrogen complex. The solvent was evaporated and the amine was purified by flash chromatography (2–7% MeOH in CH_2Cl_2) to yield the desired free amine as a white solid.

4.1.5.1. 2β-(4-Propylpiperazinyl)-5α-androstane-3α,17β-diol (62). Yield: 66%; TLC (hexanes/acetone, 6:4): R_f = 0.08; IR (film): 3380 (OH); ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.88 (t, J = 7.2 Hz, 3″-CH_3), 0.60–2.10 (residual H), 2.30, 2.50 and 2.70 (3m broad, 2α-CH, 1″-CH_2 and 4 × CH_2N), 3.58 (m, 17α-CH), 3.82 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.21, 11.90, 17.25, 19.71, 20.80, 23.36, 28.24, 30.63, 31.15, 32.64, 34.67, 35.47, 35.73, 36.90, 38.43, 43.00, 50.86, 53.60 (4×), 56.13, 60.57, 63.56 (C3), 64.34 (C2), 81.36; LRMS for $C_{26}H_{47}N_2O_2$ $[M+H]^+$: 419.3 *m/z*; HRMS: calcd for $C_{26}H_{47}N_2O_2$ $[M+H]^+$ 419.36321, found 419.36326.

4.1.5.2. 2β-(4-Butylpiperazinyl)-5α-androstane-3α,17β-diol (63). Yield: 47%; TLC (hexanes/acetone, 6:4): R_f = 0.12; IR (film): 3380 (OH); ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.93 (t, J = 7.3 Hz, 4″-CH_3), 0.60–2.20 (residual H), 2.55, 2.70 and 2.85 (3m broad, 2α-CH, 1″-CH_2 and 4 × CH_2N), 3.61 (m, 17α-CH), 3.85 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.21, 13.98, 17.25, 20.70, 20.79, 23.36, 28.23, 28.60, 30.62, 31.15, 32.65, 34.67, 35.47, 35.74, 36.89, 38.43, 43.00, 50.85, 53.60 (4×), 56.13, 58.38, 63.57 (C3), 64.34 (C2), 81.37; LRMS for $C_{27}H_{49}N_2O_2$ $[M+H]^+$: 433.4 *m/z*; HRMS: calcd for $C_{27}H_{49}N_2O_2$ $[M+H]^+$ 433.37886, found 433.37909.

4.1.5.3. 2β-(4-*iso*-Butylpiperazinyl)-5α-androstane-3α,17β-diol (64). Yield: 35%; TLC (hexanes/acetone, 6:4): R_f = 0.43; IR (film): 3370 (OH); ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.90 (d, J = 6.5 Hz, 2 × 3″-CH_3), 0.60–2.15 (residual H), 2.20, 2.60 and 2.80 (3m broad, 2α-CH, 1″-CH_2 and 4 × CH_2N), 3.62 (m, 17α-CH), 3.84 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.19, 17.41, 20.92 (3×), 23.28, 25.20, 28.13, 30.46, 31.05, 33.20, 35.03, 35.48, 35.82, 36.74, 38.49, 43.07, 50.80, 53.33 (4×), 56.18, 63.81 (C3), 64.41 (C2), 66.44, 81.79; LRMS for $C_{27}H_{49}N_2O_2$ $[M+H]^+$: 433.4 *m/z*; HRMS: calcd for $C_{27}H_{49}N_2O_2$ $[M+H]^+$ 433.37886, found 433.37931.

4.1.5.4. 2β-(4-Pentylpiperazinyl)-5α-androstane-3α,17β-diol (65). Yield: 62%; TLC (hexanes/acetone, 6:4): R_f = 0.38; IR (film): 3385 (OH); ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.88 (t, J = 6.9 Hz, 5″-CH_3), 0.60–2.15 (residual H), 2.40, 2.60 and 2.75 (3m broad, 2α-CH, 1″-CH_2 and 4 × CH_2N), 3.60, (t, J = 8.5 Hz, 17α-CH), 3.84 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.19, 13.98, 17.21, 20.83, 22.47, 23.32, 25.84, 28.19, 29.56, 30.53, 31.12, 32.84, 34.70, 35.47, 35.77, 36.81, 38.46, 43.03, 50.84, 53.34 (4×), 56.10, 58.46, 63.71 (C3), 64.42 (C2), 81.59; LRMS for $C_{28}H_{51}N_2O_2$ $[M+H]^+$: 447.4 *m/z*; HRMS: calcd for $C_{28}H_{51}N_2O_2$ $[M+H]^+$ 447.39451, found 447.39473.

4.1.5.5. 2β-(4-*iso*-Pentylpiperazinyl)-5α-androstane-3α,17β-diol (66). Yield: 70%; TLC (hexanes/acetone, 6:4): R_f = 0.18; IR (film): 3382 (OH); ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.88 (d, J = 6.4 Hz, 2 × 4″-CH_3), 0.65–2.15 (residual H), 2.40, 2.55 and 2.70 (3m broad, 2α-CH, 1″-CH_2 and 4 × CH_2N), 3.58 (t, J = 8,5 Hz, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.21, 17.22, 20.82, 22.63 (2×), 23.35, 26.64, 28.22, 30.61, 31.14, 32.74, 34.69, 35.15, 35.47, 35.76, 36.87, 38.46, 43.00, 50.85, 53.51 (4×), 56.12, 56.84, 63.64 (C3), 64.37 (C2), 81.42. LRMS for $C_{28}H_{51}N_2O_2$ $[M+H]^+$: 447.5 *m/z*;

HRMS: calcd for $C_{28}H_{51}N_2O_2$ $[M+H]^+$ 447.39451, found 447.39431.

4.1.5.6. 2β-(4-Nonylpiperazinyl)-5α-androstane-3α,17β-diol (67). Yield: 44%; TLC (hexanes/acetone, 6:4): $R_f = 0.31$; IR (film): 3386 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.88 (t, $J = 6.9$ Hz, 9″-CH_3), 0.65–2.10 (residual H), 2.33, 2.48 and 2.68 (3m broad, 2α-CH, 1″-CH_2 and $4 \times CH_2N$), 3.61 (t, $J = 8.5$ Hz, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.20, 14.13, 17.31, 20.84, 22.68, 23.35, 26.81, 27.59, 28.27, 29.28, 29.55 (2×), 30.56, 31.18, 31.87, 32.65, 34.69, 35.51, 35.76, 36.84, 38.46, 43.07, 50.87, 53.79 (4×), 56.16, 58.81, 63.59 (C3), 64.39 (C2), 81.75; LRMS for $C_{32}H_{59}N_2O_2$ $[M+H]^+$: 503.5 *m/z*; HRMS: calcd for $C_{32}H_{59}N_2O_2$ $[M+H]^+$ 503.45765, found 503.45720.

4.1.5.7. 2β-(4-Cyclohexylmethylpiperazinyl)-5α-androstane-3α,17β-diol (68). Yield: 78%; TLC (hexanes/acetone, 6:4): $R_f = 0.47$; IR (film): 3380 (OH); ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.60–2.10 (residual H), 2.16 (d, $J = 6.6$ Hz, 1″-CH_2), 2.50 and 2.75 (2m broad, 2α-CH and $4 \times CH_2N$), 3.61 (m, 17α-CH), 3.82 (m, 3β-CH); ^{13}C NMR (75 MHz, $CDCl_3$): 11.19, 17.36, 20.90, 23.30, 26.04 (2×), 26.64, 28.18, 30.48, 31.10, 31.87 (2×), 32.99, 34.81, 35.49 (2×), 35.78, 36.76, 38.45, 43.08, 50.82, 53.77 (4×), 56.17, 63.70 (C3), 64.38 (C2), 65.38, 81.80; LRMS for $C_{30}H_{53}N_2O_2$ $[M+H]^+$: 473.5 *m/z*; HRMS: calcd for $C_{30}H_{53}N_2O_2$ $[M+H]^+$ 473.41016, found 473.40999.

4.1.5.8. 2β-(4-Benzylpiperazinyl)-5α-androstane-3α,17β-diol (69). Yield: 46%; TLC (hexanes/acetone, 6:4): $R_f = 0.37$; IR (film): 3372 (OH); ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.65–2.20 (residual H), 2.55 and 2.75 (2m broad, 2α-CH and $4 \times CH_2N$), 3.53 (s, PhC$\underline{H}_2$N), 3.61 (t, $J = 8.5$ Hz, 17α-CH), 3.82 (m, 3β-CH), 7.30 (s, 5H of Ar); ^{13}C NMR (75 MHz, $CDCl_3$): 11.18, 17.40, 20.91, 23.29, 28.14, 30.47, 31.06, 33.12, 34.97, 35.48, 35.81, 36.75, 38.46, 43.06, 50.80, 52.98 (4×), 56.16, 62.78, 63.75 (C3), 64.41 (C2), 81.76, 127.29, 128.29 (2×), 129.29 (2×), 137.34; LRMS for $C_{30}H_{47}N_2O_2$ $[M+H]^+$: 467.5 *m/z*; HRMS: calcd for $C_{30}H_{47}N_2O_2$ $[M+H]^+$ 467.36321, found 467.36408.

4.1.6. Synthesis of *N,N*-dialkylamino derivatives 70 and 71. HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) (1 equiv) and carboxylic acid (1.1 equiv) were dissolved in DMF at 0 °C. Then, diisopropylethylamine (DIPEA) was added in the mixture and allowed to react for 5 min. Thereafter, compound **33** or **35** (1 equiv) dissolved in DMF was added to the solution and the temperature was raised to room temperature for 2–3 h. The resulting mixture was diluted in ethyl acetate and washed with water (4×). The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to dryness. Purification of the crude product by flash chromatography (1–2% MeOH in CH_2Cl_2) yielded the amide as a pale yellow oil. This, compound was dissolved in THF at 0 °C, BH_3-THF (10 equiv) was added to the solution and temperature raised to room temperature for 90 min. Water was then added and mixture was extracted with CH_2Cl_2, the organic layer dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography (8% acetone in hexanes) gave the desired amine as slight yellow oil.

4.1.6.1. 2β-(Dinonylamino)-5α-androstane-3α,17β-diol (70). Yield: 47%; TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.71$; IR: 3397 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.88 (t, $J = 6.4$ Hz, $2 \times 9'$ CH_3), 0.70–2.10 (residual H), 2.29 and 2.42 (2m, $2 \times 1'$-CH_2), 2.75 (m, 2α-CH), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.73 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.16, 14.08 (2×), 17.60, 20.93, 22.65 (2×), 23.35, 27.43 (2×), 28.47, 29.20 (2×), 29.36 (2×), 29.60 (4×), 30.59, 31.17, 31.88 (2×), 33.31, 34.92, 35.64, 35.72, 36.84, 38.49, 43.12, 50.22 (2×), 50.91, 56.43, 60.89 (C2), 63.96 (C3), 81.93; LRMS for $C_{37}H_{70}NO_2$ $[M+H]^+$: 560.5 *m/z*; HRMS: calcd for $C_{37}H_{70}NO_2$ $[M+H]^+$ 560.54011, found 560.54026.

4.1.6.2. 2β-(Diundecylamino)-5α-androstane-3α,17β-diol (71). Yield: 59%. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.73$; IR (film): 3407 (OH); ^{1}H NMR (400 MHz, $CDCl_3$): 0.73 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.87 (t, $J = 6.8$ Hz, $2 \times 11'$-CH_3), 0.70-2.10 (residual H), 2.29 and 2.42 (2m, $2 \times 1'$-CH_2), 2.78 (m, 2α-CH), 3.62 (t, $J = 8.5$ Hz, 17α-CH), 3.73 (m, 3β-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.17, 14.09 (2×), 17.61, 20.94, 22.67 (2×), 23.34, 27.42 (2×), 28.46, 29.27 (2×), 29.33 (2×), 29.43 (2×), 29.59 (2×), 29.64 (4×), 30.57, 31.16, 31.91 (2×), 33.33, 34.95, 35.64, 35.72, 36.87, 38.49, 43.11, 50.21 (2×), 50.90, 56.43, 60.88 (C2), 63.96 (C3), 81.91; LRMS for $C_{41}H_{78}NO_2$ $[M+H]^+$: 616.6 *m/z*; HRMS: calcd for $C_{41}H_{78}NO_2$ $[M+H]^+$ 616.60271, found 616.60234.

4.2. Biological evaluation of synthesized compounds

4.2.1. Cell culture. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, MO, USA) 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 with twice a week dilution of the cells in culture medium.

4.2.2. 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, Madison, WI, USA), 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 Company, Lincoln Park, NJ, USA) were incubated at 37 °C, 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 incubated for 4 h. MTS is converted to water-soluble colored formazan by dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data were reported as the percentage of cell proliferation inhibition compared to the control (basal cell proliferation) or IC_{50} value (50% of cell growth inhibition).

4.2.3. Assay of the expression of CD11b and CD14 cell surface antigens. For analysis of cellular differentiation, expression of cell surface antigens was measured using immunofluorescence staining. Cells were seeded in RPMI-1640 medium in 25-cm^2 tissue culture flasks (VWR, Montréal, QC, Canada), containing 10 μM of an aminosteroid or vitamin D3 (VD3) (from Sigma–Aldrich Canada Ltd., Oakville, ON, Canada) and incubated for 72 h. After treatment, cells were harvested, counted, and washed with cold phosphate-buffer saline (PBS). They were stained using simultaneously mouse anti-human CD11b antibody conjugated with *R*-phycoerythrin (RPE) and mouse anti-human CD14 antibody conjugated with fluorescein isothiocyanate (FITC) (both from Becton Dickinson Biosciences, San Jose, CA, USA). Controls were stained with mouse RPE-conjugated IgG_1 and FITC-conjugated IgG_2. Following incubation for 45 min, cells were washed twice with cold PBS, resuspended in 500 μL of PBS, and analyzed on a Coulter EPICS XL (Beckman–Coulter, Miami, FL, USA) giving the percentage of cells marked with CD11b only, CD14 only, and CD11b/CD14 (double marked).

4.3. Statistical analysis

Statistical significance was determined according to the Duncan–Kramer multiple range test.[34]

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Supplementary data

The HPLC purity and chemical structures of aminosteroids tested in our study. The ^{13}C NMR spectra of non UV-detectable aminosteroids. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.03.031.

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