Synthesis and Optimization of a New Family of Type 3 17*β*-Hydroxysteroid Dehydrogenase Inhibitors by Parallel Liquid-Phase Chemistry

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Type 3 17*β*-hydroxysteroid dehydrogenase (17*β*-HSD) transforms 4-androstene-3,17-dione (Δ^4-dione) into the androgen testosterone. To produce potent inhibitors of this key steroidogenic enzyme, we performed parallel liquid-phase synthesis of 3*β*-substituted androsterone (ADT) libraries (A−D) in good yields and average high-performance liquid chromatography (HPLC) purities of 92−94%. The first library (A) of 3*β*-amidomethyl-ADT derivatives (168 members), including two levels of molecular diversity on the amide (R_1 and R_2), was synthesized with a parallel liquid-phase method (method I) in less time than with the classic chemistry method. The screening of library A revealed that relatively small hydrophobic chains at R_1 (5−8 carbons) and small hydrophobic substituents at R_2 (1−4 carbons) provided the most potent inhibitors. In accordance with these inhibition results, a second library (B) of 3*β*-amidomethyl-ADT derivatives (56 members) was generated in a very short time using an improved method based on scavenger resins and liquid-phase parallel chemistry. Library B produced more potent inhibitors than library A and provided useful structure−activity relationships that directed the design of a third library (C) of 49 members. Once again, very potent inhibitors were identified from library C and 3*β*-[(*N*-adamantylmethyl-*N*-butanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one (**C-7-3**) was identified as the most potent inhibitor of the three libraries with an inhibitory activity (IC_{50} = 35 nM) 18-fold higher than that of the natural substrate of the enzyme, Δ^4-dione, (IC_{50} = 650 nM) used itself as inhibitor. Finally, we designed a library (D) of 3-carbamate-ADT derivatives (25 members) using the efficient parallel liquid-phase method III, which allowed the synthesis of more rigid molecules with two levels of molecular diversity (R_1/R_2 and R_3) in the local area occupied by the adamantane group of **C-7-3**. Interestingly, one of the most potent inhibitors of library D, the 3*R*-spiro-{3′-[3″-*N*-morpholino-2″-(3‴-cyclopentylpropionyloxy)propyl]-2′-oxo-oxazolidin-5′-yl}-5α-androstan-17-one (**D-5-4**), showed an inhibitory activity on type 3 17*β*-HSD similar to that of compound **C-7-3**, while exhibiting a nonandrogenic profile.

Introduction

Type 3 17*β*-hydroxysteroid dehydrogenase (17*β*-HSD) is a steroidogenic enzyme that catalyzes the reduction of 4-androstene-3,17-one (Δ^4-dione) to testosterone (T) using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the cofactor.[1−3] This third member of the 17*β*-HSD enzyme family[4] is found almost exclusively in the testis and contributes to the production of approximately 60% of total active androgens in men.[5] The inhibition in the testis of type 3 17*β*-HSD and the inhibition in peripheral tissues of other key enzymes such as type 5 17*β*-HSD,[6,7] 5α-reductases,[8−10] and steroid sulfatase,[11] represent interesting strategies toward a complete blockade of the biosynthesis of androgens T and dihydrotestosterone (DHT) (Figure 1). In the case of hormone sensitive diseases such as prostate cancer, obtaining a complete androgen blockade is crucial to counter the proliferation effect of androgens on prostate cancer cells.[12−15] Many different endocrine treatments are now available to block either the testicular source of androgens (medical or surgical castration) or the effect of androgens T and DHT on the androgen receptor (AR) (antiandrogens) or both.[16,17] The optimal androgen blockade therapy presently used consists of the administration of an LHRH agonist (medical castration) in combination with a pure antiandrogen (flutamide).[12,18,19] However, recent studies have shown that the widely used chemical castration with depot LHRH agonist fails, in nearly 20% of cases, to achieve castrate levels of T in men (<20 ng/dL).[20,21] Also, the relatively weak affinity of the antiandrogen flutamide for the AR[22] leaves the residual androgens (from testis or adrenals), free to interact with the AR and to potentially activate the growth of prostate cancer cells, which is especially harmful in the case of androgen hypersensitive tumors.[23] Thus, to achieve an optimal blockade of androgen formation, we are interested in developing a potent inhibitor of type 3 17*β*-HSD, which will eventually be used as an adjuvant to an LHRH agonist and/or an antiandrogen for prostate cancer treatment.

Preliminary studies on the inhibition of type 3 17*β*-HSD identified androsterone (ADT) as a good lead compound (IC_{50} = 330 nM) (Figure 2).[24] The subsequent synthesis of ADT derivatives bearing various 3*β* hydrophobic chains gave more potent inhibitors than ADT.[25] In fact, with an IC_{50} value of 57 nM, 3*β*-phenylmethyl-

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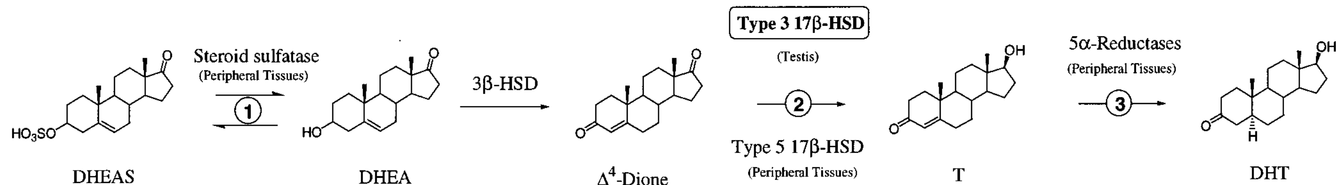


Figure 1. Role of type 3 17β-HSD in the transformation of the inactive androgen 4-androstene-3,17-dione (Δ^4-dione) into active androgens T and DHT. Various strategies (i–iii) to block their biosynthesis involve: (i) Inhibitors of steroid sulfatase (peripheral tissues); (ii) inhibitors of type 3 (testis) or type 5 17β-HSDs (peripheral tissues); and (iii) inhibitors of 5α-reductases (peripheral tissues). DHEAS, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone.

ADT (**1**) was the most potent type 3 17β-HSD inhibitor up to now. This molecule is six times more powerful than ADT and 13 times more powerful than Δ^4-dione (used as inhibitor) to inhibit the enzyme reduction of labeled Δ^4-dione (0.1 μM).[25] To optimize this inhibitor, we were interested in developing strategies for the rapid generation of a large number of diversified 3β-substituted-ADT derivatives. Our plan was essentially to use a combinatorial chemistry approach to generate libraries of steroid derivatives by either liquid-phase or solid-phase synthesis.[26,27] Recently, we have published a methodology for the solid-phase parallel synthesis of 3β-peptido-3α-hydroxy-5α-androstan-17-ones (**2**).[28,29] In addition to these 3β-peptido-steroids, we were also interested in obtaining compounds possessing a tertiary alkylamide chain at position 3β of ADT (compound **3**). Such compounds could be more biologically stable than peptido-steroids **2**, which are potentially sensitive to hydrolases (aminopeptidases, carboxypeptidases, and dipeptidases). While designing a combinatorial route to synthesize these desired 3β-amidomethyl-ADT derivatives of general structure **3**, we chose a 3β-oxirane functionality as precursor of molecular diversity. Following our preliminary results,[30] we now report a full account of the design, chemical synthesis, and biological evaluation of efficiency as type 3 17β-HSD inhibitors of 3β-amidomethyl-ADT libraries generated by efficient liquid-phase parallel synthesis.

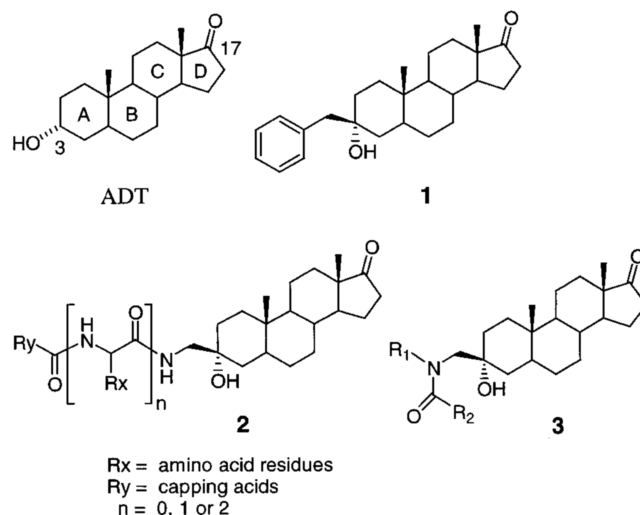


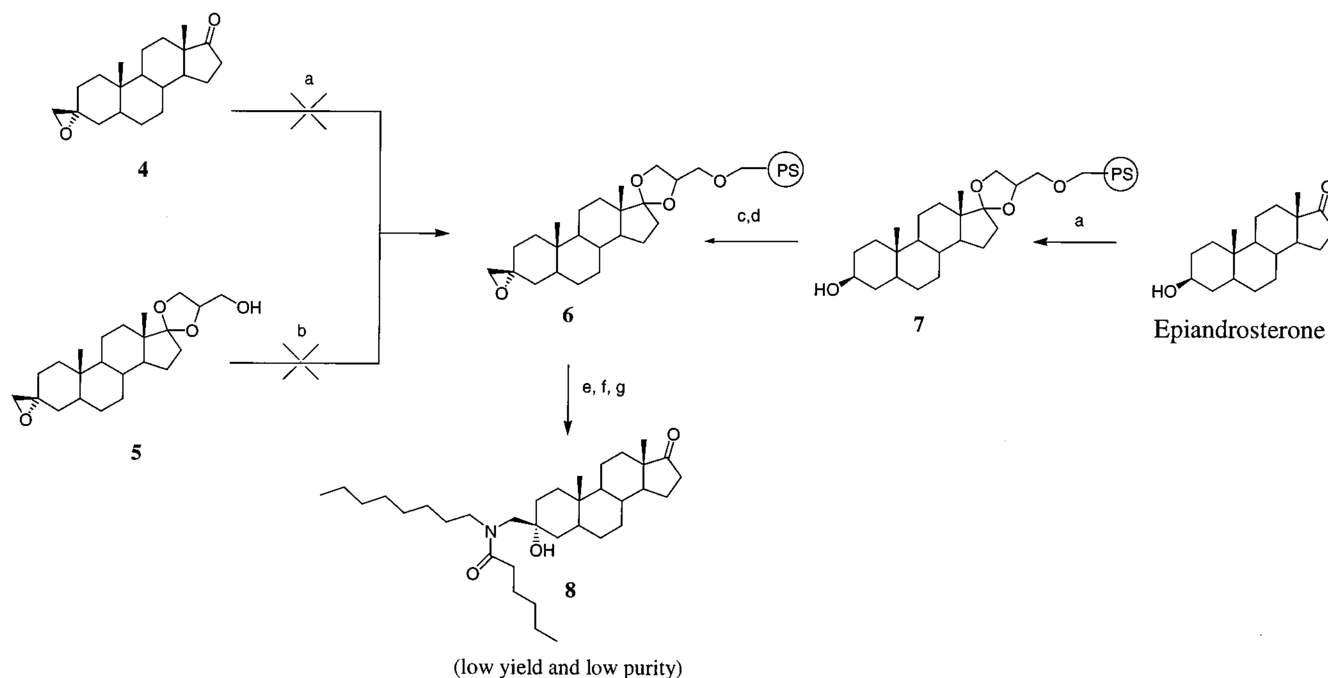
Figure 2. Steroidal inhibitors of type 3 17β-HSD derived from ADT and previously reported by us (compounds **1** and **2**) or proposed here (compound **3**).

Results and Discussion

Chemistry. Our choice to use the liquid-phase medium for the production of the desired libraries became clear after our disappointing attempts to link the key steroid precursors **4** and **5** on a solid support (Scheme 1). Indeed, the coupling of 3β-oxirane-androstan-17-one (**4**)[30] on polymer-bound glycerol, using the Leznoff acetal linker,[31,32] revealed the instability of the oxirane in these coupling conditions. Alternatively, we grafted the acetal linker on steroid **4** to generate **5** and tried coupling it with a Merrifield resin to obtain **6**. Once again, the oxirane was found instable in this coupling step. As a last attempt, we then generated oxirane **6** directly in the solid phase starting from the epiandrosterone linked to polymer-bound glycerol (resin **7**) and added two more steps to obtain the model compound **8**. As a result, we recovered at the end of the solid-phase sequence (oxidation, epoxidation, aminolysis, and acylation) a crude mixture (25% yield) containing the desired ADT derivative **8** but also impurities originating mainly from incomplete reactions. Consequently, we finally turned our attention to a liquid-phase combinatorial methodology to provide the library of desired 3β-alkylamide-3α-hydroxy-5α-androstan-17-ones.

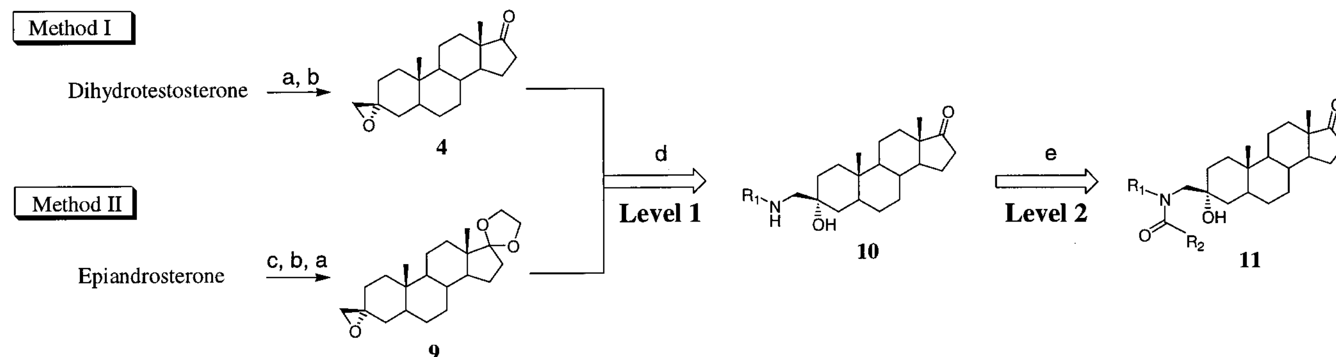
Method I. As a model, a library of 20 members was first synthesized to evaluate the versatility and limits of our proposed liquid-phase approach (method I, in Scheme 2).[30] The first level of diversity (R_1) was introduced by a regioselective opening of the oxirane precursor **4** by a series of four primary amines at reflux in the presence of lithium perchlorate.[33] This reaction was carried out in parallel using separate reactors (sealed vials), one for each different primary amine used ($4 \times R_1$). The resulting solution was diluted with ethyl acetate and washed with water to remove the perchlorate salt. The organic layer was then evaporated under reduced pressure, and the crude compound was quickly filtered in a silica gel syringe to remove the excess of primary amines and unreacted oxirane precursor **4** from the secondary amine **10**. The second level of molecular diversity (R_2) was then obtained by a regioselective reaction of the four secondary amines **10**, previously divided in 20 vials, with five acyl chlorides (R_2COCl) in the presence of pyridine to give the amides **11**. It was then necessary to perform silica gel filtration to obtain the 20 library members in a high average purity of 93% as determined by high-performance liquid chromatography (HPLC). Using the same method, a larger library of 168 members (library A) was generated in an average HPLC purity of 92% (Table 1) for a sampling of 12 members (using a root square random sampling).[34] However, after the synthesis of library A, we realized that some aspects of method I were not optimal, and it was found to be too laborious for an automated process. In fact, the repetitive silica gel filtration needed after

Scheme 1. Solid-Phase Assays[a]



[a] Reagents and conditions. (a) Steroid **4** or epiandrosterone (0.75 mmol), TMOF (0.75 mmol), $Sc(OTf)_3$, polymer-bound glycerol (0.25 mmol), toluene. (b) Steroid **5**, NaH, merrifield resin (1.0 mmol/g), DMF. (c) TPAP, NMO, molecular sieves, CH_2Cl_2. (d) NaH, Me_3SOI, DMSO. (e) Octylamine, $LiClO_4$, CH_3CN, 60 °C, room temperature. (f) Hexanoyl chloride, pyridine. (g) 2.0 N HCl in dioxane (containing 0.03% of H_2O), room temperature.

Scheme 2. Parallel Liquid-Phase Synthesis (Methods I and II)[a]



[a] Reagents and conditions. (a) NaH, Me_3SOI, DMSO. (b) TPAP, NMO, molecular sieves, CH_2Cl_2. (c) Ethylene glycol, *p*-TSA, toluene, reflux with Dean–Stark. Method I: (d) (i) R_1NH_2, $LiClO_4$, CH_3CN, 60 °C, room temperature; (ii) SiO_2 filtration. (e) (i) R_2COCl, pyridine; (ii) H_2O washing and then SiO_2 filtration. Method II: (d) (i) R_1NH_2, EtOH, 60 °C; (ii) acetone/HCl 3 M (1:1); (iii) SiO_2 filtration. (e) (i) R_2COCl, piperidinomethyl polystyrene (3.6 mmol/g); (ii) aminomethyl polystyrene (1.0 mmol/g), filtration.

the acylation step was identified as the most time-consuming step especially when the number of compounds generated is high. Thus, some modifications were necessary to avoid this limiting step and to improve this liquid-phase approach.

Method II. An unknown product (10–15%, by thin-layer chromatography (TLC) analysis), suspected to be a ketimine resulting from the reaction between the C17-ketone and the primary amine, was found to contaminate steroid **10** generated by method I. In fact, a silica gel filtration was necessary to remove this impurity and to obtain the library A members in good purity. To avoid the formation of undesirable byproducts, the carbonyl group of epiandrosterone was first protected as a dioxolane, an acid sensitive protective group, and then transformed into library precursor **9.** Steroid **9** was submitted to aminolysis using only an amine (R_1NH_2) in ethanol rather than an amine and $LiClO_4$ in acetonitrile, thus limiting the workup procedure to a simple evaporation of ethanol. The dioxolane protective group was then easily removed with a 3 N HCl aqueous solution in acetone, followed by a basic workup (10% $NaHCO_3$). A simple filtration on silica gel then afforded the desired steroid **10** in high purity. Thereafter, the second level of diversity (R_2) was added using an excess of the acyl chloride (2 equiv) in dichloromethane with an acid scavenger resin (piperidinomethyl polystyrene) and a nucleophilic resin (aminomethylated polystyrene),[35] based on CMR/R methodology both to mop up the proton released during acylation and to eliminate the excess of acid chloride, respectively. A simple filtration then removed the scavenger resins. The filtrate was then evaporated on a Speedvac apparatus giving the desired steroids of general structure **11**. Two

Table 1. Characterization of Sampled Members of Libraries A−D

library	compd	theoret mass	obsvd[a] mass	library size	average HPLC purity[b]	time[c] (days)	HPLC purity (%)[b]
			method I				
A	A-1-8	543.5	544.4				97
A	A-2-11	471.4	472.6				85
A	A-3-9	599.5	600.7				84
A	A-4-1	487.4	488.4				90
A	A-5-3	529.5	530.8				97
A	A-6-2	529.5	530.8				96
A	A-7-14	571.5	572.7	168	92	25	94
A	A-8-6	613.5	614.3				99
A	A-9-13	627.6	628.8				97
A	A-10-10	725.7	726.5				91
A	A-11-5	655.6	656.7				90
A	A-12-12	653.6	654.4				91
			method II				
B	B-2-4	479.2	480.4				94
B	B-2-7	429.3	430.6				97
B	B-3-6	455.3	456.6				82
B	B-4-1	419.3	420.3				97
B	B-4-4	497.2	498.3				95
B	B-5-1	447.3	448.5				97
B	B-5-6	473.4	474.6	56	94	4	94
B	B-6-1	475.4	476.3				90
B	B-6-6	501.4	502.4				82
B	B-6-7	503.4	504.6				93
B	B-7-1	441.3	442.6				99
B	B-8-2	469.2	470.6				92
B	B-8-4	479.2	480.4				91
			method II				
C	C-2-6	491.3	492.5				86
C	C-3-7	507.4	508.1				99
C	C-4-3	615.3	616.4				86
C	C-4-5	671.4	672.6	49	92	4	97
C	C-5-1	465.3	466.4				98
C	C-6-1	511.3	512.6				98
C	C-6-2	525.4	526.6				96
C	C-7-7	537.4	538.7				76
			method III				
D	D-1-1	542.4	543.5				>80[d]
D	D-2-2	612.5	613.2				>80[d]
D	D-3-3	576.4	577.4	25	>80[d]	4	>80[d]
D	D-4-4	610.4	611.1				>80[d]
D	D-5-5	648.4	649.5				>80[d]

[a] Low-resolution mass spectra (LRMS) in positive mode $(M + H)^+$. [b] HPLC purity of steroid derivatives released from resin (without purification steps). [c] Time required by one worker for the synthesis of the library. [d] Purity estimated to be higher than 80% by TLC and ^{1}H NMR analysis.

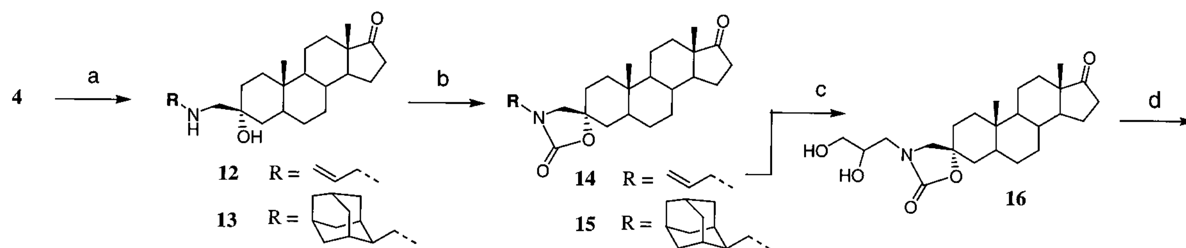
libraries (B and C) of 56 and 49 members were obtained with this method in high average purity (94 and 92%, respectively). Regarding the time required for preparing different libraries (Table 1), method II was found more advantageous than method I.

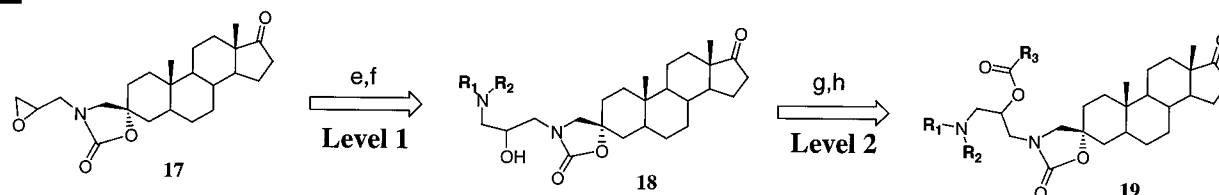
Method III. The parallel synthesis of the library D members of general structure **19** (Scheme 3) required a different methodology than that developed to generate libraries A−C (methods I and II). The synthesis of derivatives **19** first necessitates the synthesis of steroid precursor **17**. The previously described 3β-oxirane-5α-androstan-17-one (**4**)[30] was submitted to the aminolysis conditions of method II using allylamine in ethanol at 60 °C to generate olefin **12**. The cyclic carbamate of compound **14** was then obtained in treating the amino alcohol **12** with trisphosgene reagent[36] in the presence of diisopropylethylamine (DIPEA). Thereafter, the olefin of compound **14** was oxidized using *N*-methyl morpholine-*N*-oxide (NMO) with a catalytic amount of osmium tetroxide to give diol **16**, which was directly converted into terminal epoxide **17** by the one pot procedure reported by Kolb and Sharpless.[37]

The first level of diversity (R_1/R_2) was introduced by an aminolysis of the oxirane using a secondary amine (R_1R_2NH) and lithium perchlorate as reported above. The excess of secondary amine reagent was removed from **18** using a scavenger (methylisocyanate polystyrene), and the perchlorate salt was eliminated by a washing step with water. The diastereoisomeric ratio of the resulting β-amino-alcohol **18** was determined by the formation of a Mosher ester[38] and, as expected, was found to be 50:50. The choice to generate a racemic mixture rather than a pure chiral compound was herein advantageous considering that two compounds were screened at once. If however necessary, it is possible to perform a Jacobsen kinetic resolution,[39] in order to obtain a pure chiral epoxide **17**, as the precursor of library member in a pure diastereoisomeric form. From racemic secondary alcohol **18**, the second level of diversity (R_3) was obtained by using the optimized acylation conditions of method II. A 25-membered library of type D (compounds **19**) was thus generated to validate method III (Table 1) and to provide the targeted inhibitors.

Screening of Libraries A−C for Inhibitory Activity on Type 3 17β-HSD. Library A. Library A was designed to explore the hydrophobic region of the enzyme, a region previously identified by inhibition with 3β-alkyl-ADT derivatives. A library of 168 members (12 × 14), containing various alkylamines and aliphatic acyl chlorides building blocks, was generated by method I with the aim of finding the optimal carbon chain length at R_1 and R_2. A selection of 40 representative members of library A was chosen for a preliminary inhibition screening (Figure 3). The evaluation of the ability of selected members to inhibit the type 3 17β-HSD activity transfected in human embryonic kidney (HEK)-293 cells (homogenized) was done by measuring the amount of labeled T formed from the labeled natural substrate Δ^4-dione in the presence of NADPH as the cofactor. The results were expressed as inhibitory activity (percent) at a concentration of 3 μM. An obvious observation was that the long alkyl chains (nine carbons or more at R_1 and five carbons or more at R_2) were generally not well-tolerated by the enzyme. In fact, the compounds possessing a chain length smaller than eight carbons at R_1 and smaller than four carbons at R_2 (compounds **A-1-1**, **A-2-2**, **A-3-3**, and **A-4-4**) showed the strongest inhibition. Also, a short ramified chain at R_2 (*tert*-butyl, ethylhexyl, cyclobutyl, and cyclopropyl) seems to be well-tolerated by the enzyme (**A-1-14**, **A-2-13**, **A-3-12**, and **A-4-11**). Interestingly, the combination of a long chain at R_1 and of a short one at R_2 (compound **A-1-7**) was also found to give a good inhibition. Another important observation was that the compounds with a single level of diversity, without any R_2 chains (free secondary amine of general structure **10**), showed small inhibition values, bringing us to prefer the tertiary amide over the secondary amine (results not reported). Thus, this first library A provided useful structure−activity relationships (SAR) information about the dimension of the hypothetical hydrophobic pocket of the enzyme and

Scheme 3[a]





[a] Reagents and conditions. (a) Allylamine, EtOH, 60 °C. (b) Triphosgene, DIPEA, CH_2Cl_2. (c) OsO_4, NMO, *t*-BuOH, THF, H_2O, (7:3:1). (d) (i) $MeC(OMe)_3$, PPTS, CH_2Cl_2; (ii) AcBr, Et_3N, CH_2Cl_2; (iii) K_2CO_3, MeOH. Method III: (e) R_1R_2NH, $LiClO_4$, CH_3CN, 60 °C. (f) Methylisocyanate polystyrene (1.0 mmol/g), CH_2Cl_2, filtration, H_2O washing. (g) R_3COCl, piperidinomethyl polystyrene (3.6 mmol/g), dimethylaminopyridine polystyrene (1.0 mmol/g), EtOAc. (h) Aminomethyl polystyrene (1.0 mmol/g), CH_2Cl_2, filtration. (i) $NaHCO_3$ (10%), EtOAc, silica gel filtration.

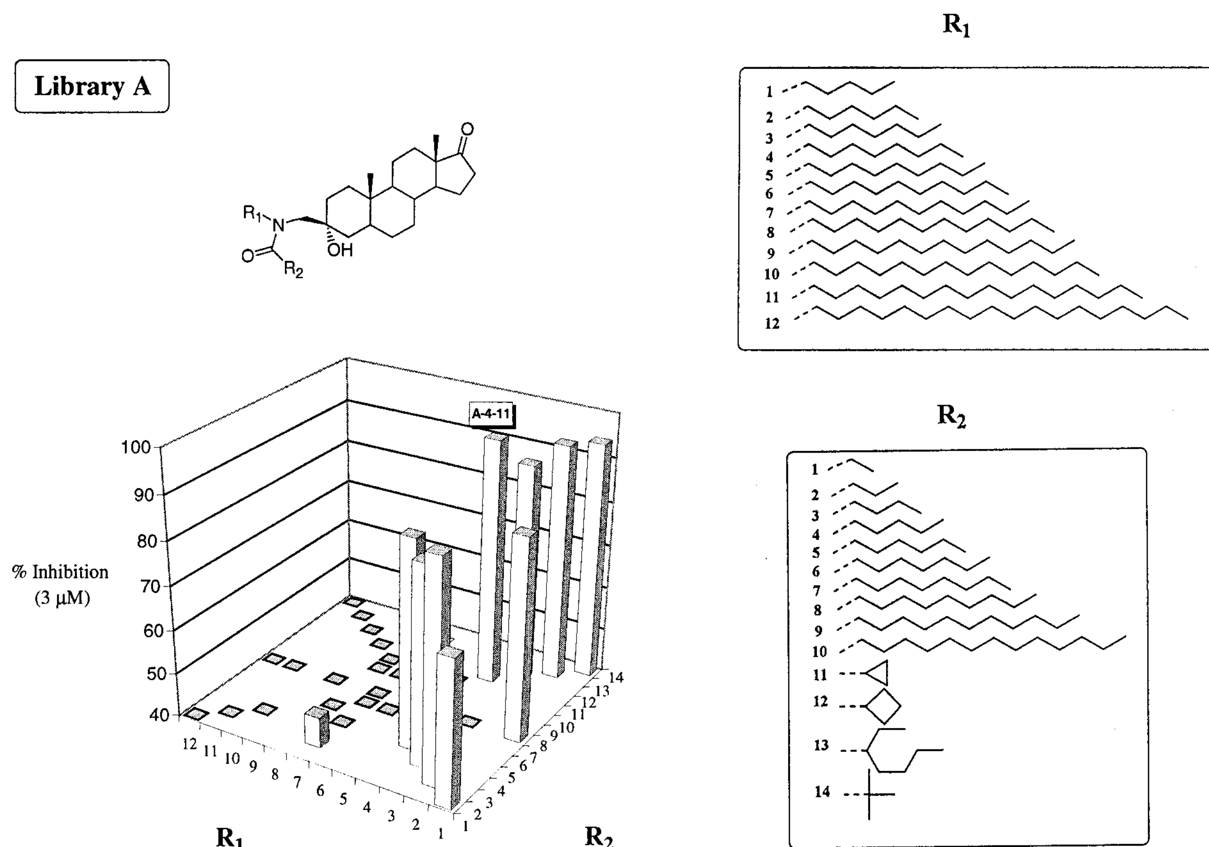


Figure 3. Inhibition of type 3 17β-HSD (percent) by a selection of 40 members of library A at a concentration of 3 μM.

provided us with useful guidelines for elaborating new libraries with novel building blocks on the tertiary amide.

Library B. On the basis of what we learned from library A, library B was synthesized with various types of building blocks with a maximum length of eight atoms at R_1 and of four atoms at R_2. Also, the library was designed as to determine the relative importance of certain parameters such as the polarity of the R_2 chain (halogen vs methylene) and/or the rigidity and density of the carbon chain at R_1 (cyclohexyl, cyclopentyl, cyclopropyl, and ether). The percentage of inhibition was measured at a concentration of 0.3 μM for all library members (Figure 4), yielding a wide range of inhibition values ranging from 17 to 93%. Important SAR information was acquired from this screening. It was obvious that a cyclohexylmethyl group at R_1 produced the highest inhibition for most R_2 groups (**B-1-7**, **B-1-6**, **B-1-**

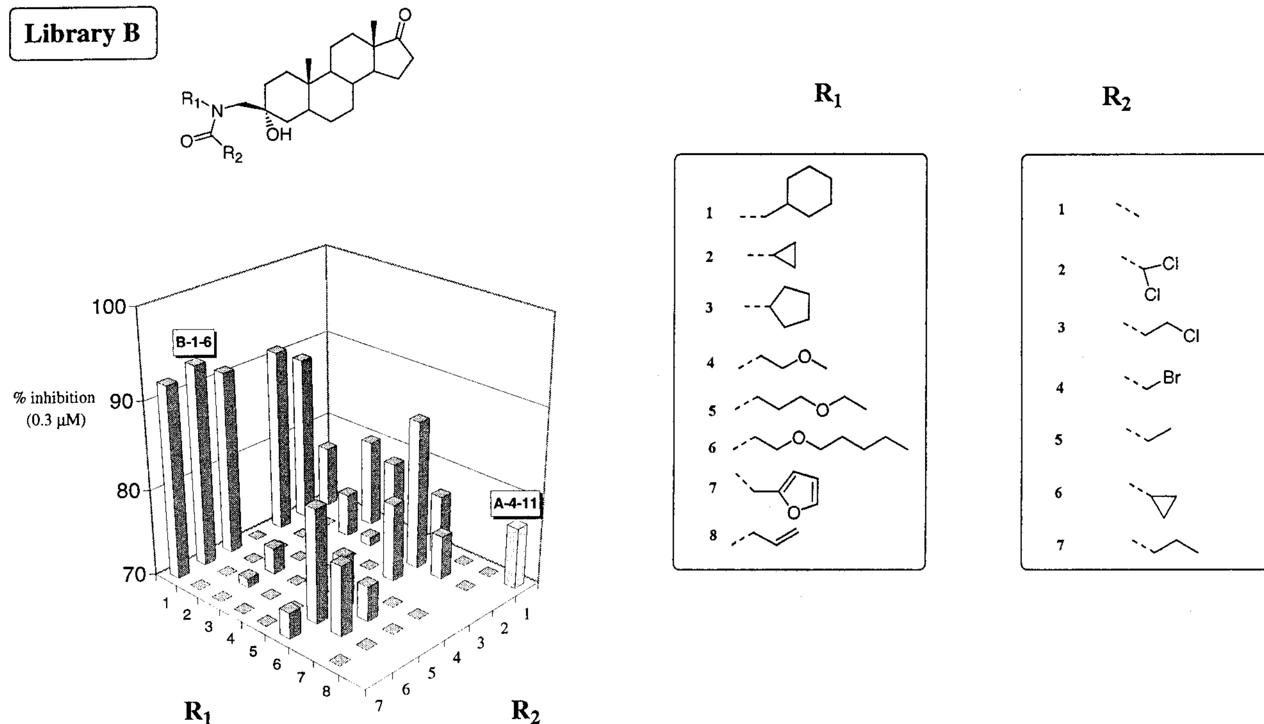


Figure 4. Inhibition of type 3 17β-HSD by 56 members of library B at a concentration of 0.3 μM.

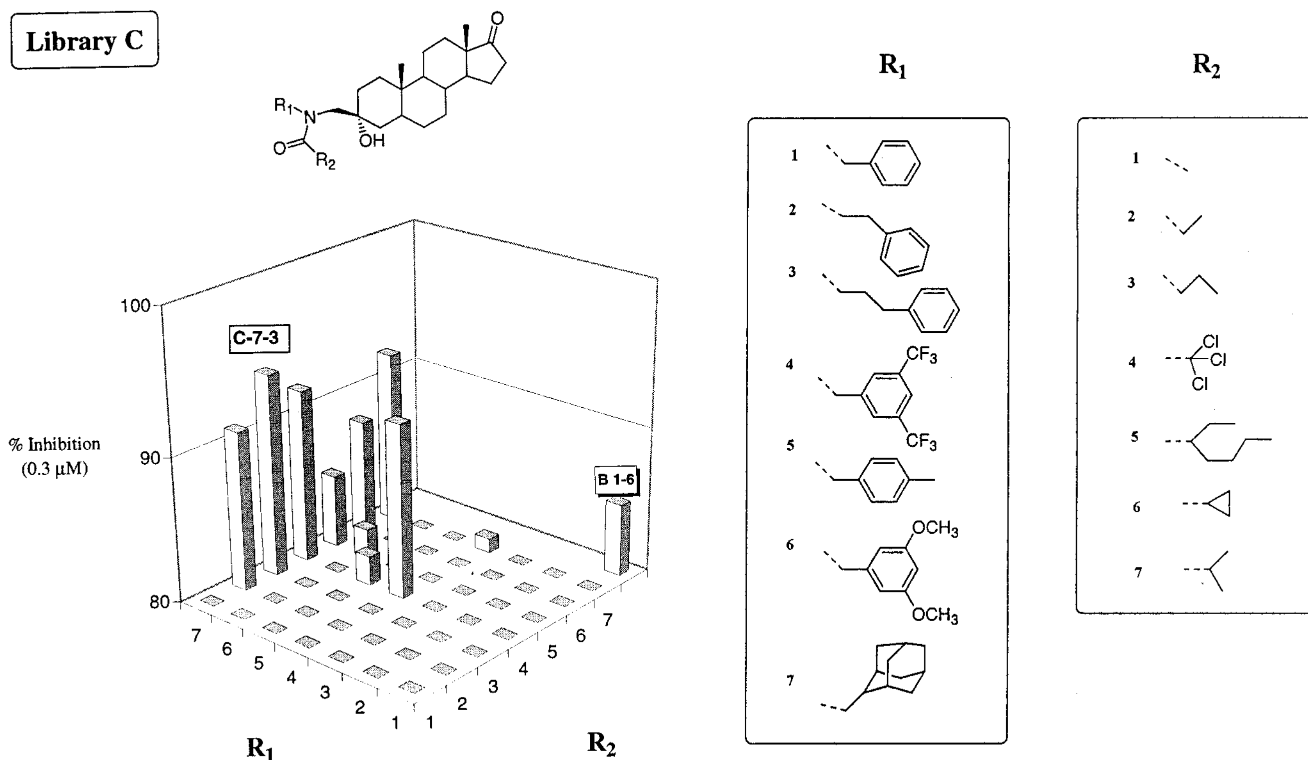
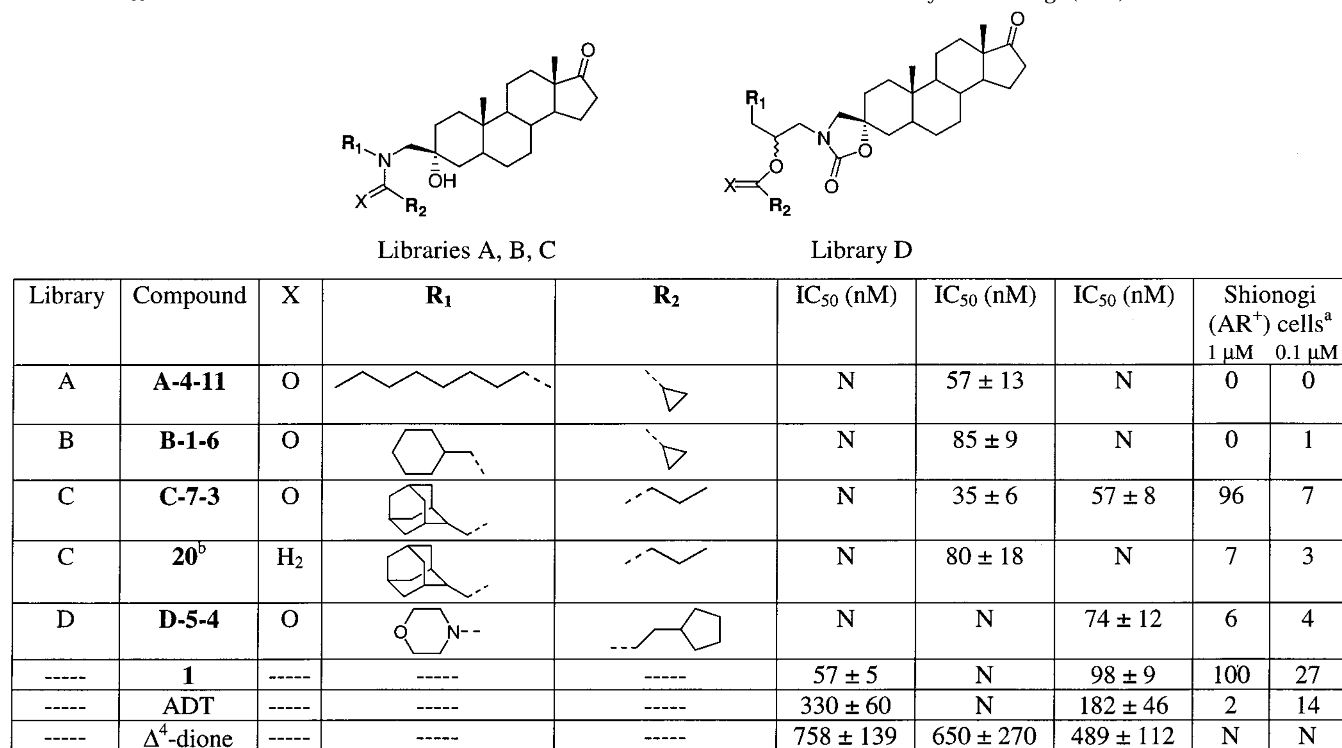


Figure 5. Inhibition of type 3 17β-HSD by 49 members of library C at a concentration of 0.3 μM.

5, **B-1-3**, and **B-1-2**). These compounds were also better inhibitors than the best library A member (**A-4-11**) when compared under the same conditions. Such results suggest that a more rigid carbon chain at R_1 increases the affinity for the enzyme. On the other hand, a more polar carbon chain bearing an ether function was not advantageous as compared to the hydrophobic chains. With these results in hand, we were interested to generate a new library exploring these benefic parameters (library C).

Library C. On the basis of the results obtained from library B, different, more rigid building blocks, such as adamantylmethyl, were used at R_1 to mimic the important hydrophobic effect of a cyclohexylmethyl group (Figure 5). However, small hydrophobic chains were used at R_2 but different ones than in libraries A and B.

Table 2. IC_{50} Values of Selected Inhibitors of Libraries A–D and Their Proliferative Activity on Shionogi (AR^+) Cells



Library	Compound	X	R_1	R_2	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	Shionogi (AR^+) cells[a] 1 µM	0.1 µM
A	**A-4-11**	O			N	57 ± 13	N	0	0
B	**B-1-6**	O			N	85 ± 9	N	0	1
C	**C-7-3**	O			N	35 ± 6	57 ± 8	96	7
C	**20**[b]	H_2			N	80 ± 18	N	7	3
D	**D-5-4**	O			N	N	74 ± 12	6	4
-----	**1**	-----	-----	-----	57 ± 5	N	98 ± 9	100	27
-----	ADT	-----	-----	-----	330 ± 60	N	182 ± 46	2	14
-----	Δ^4-dione	-----	-----	-----	758 ± 139	650 ± 270	489 ± 112	N	N

[a] Proliferative activity (%) induced by 1 and 0.1 µM of tested compound on Shionogi (AR^+) cells. Results are compared to the stimulation induced by 0.3 nM of androgen DHT (100%). N = not determined. [b] Synthesis of compound **20** was realized from compound **9** in a 4 step sequence of reactions (aminolysis, acylation, reduction of amide, and deprotection of acetal).

The results showed that the adamantylmethyl group clearly provides the highest inhibition at R_1, especially in the presence of small chains at R_2 (**C-7-2**, **C-7-3**, **C-7-4**, and **C-7-7**). Also, the compound **C-4-4** combining a 3,5-(ditrifluoromethyl)benzyl group at R_1 and a trichloro acetyl group at R_2 gave strong inhibition. All of these compounds were better inhibitors than the best library B member (**B-1-6**).

Determination of IC_{50} Values of the Best Inhibitors Identified from Libraries A–C. After the screening of libraries A–C, we were interested in determining the IC_{50} values of the best inhibitors of each library and to compare them with other known inhibitors (Table 2). One compound was chosen per library (**A-4-11**, **B-1-6**, and **C-7-3**), and their IC_{50} values were determined in the same enzymatic assay. The results showed that compound **C-7-3** bearing an adamantylmethyl group at R_1 was the most potent inhibitor of the three libraries (IC_{50} = 35 nM). Its IC_{50} value is approximately 18 times higher than the IC_{50} value of the natural substrate Δ^4-dione (IC_{50} = 650 nM) used itself as an inhibitor. Complementary to these results, the reduction of the amide function to an amine was done to verify the influence of the tertiary amide vs the tertiary amine on inhibition. The tertiary amine **20** (IC_{50} = 80 nM) was found to be only half as potent as the corresponding amide **C-7-3**. This suggests a positive effect of the carbonyl of the amide on inhibition. We therefore suspect the formation of a hydrogen bond between the carbonyl of amide derivatives and the 3α-alcohol that could favoring the affinity of the compound **C-7-3** for the enzyme.

Screening of Library D for Inhibitory Activity on Type 3 17*β*-HSD. Given the biological results of libraries A–C and the hypothesis of a hydrogen bond, we were curious about the effect of a rigidification of the 3*β*-amidomethyl-3α-hydroxy system. We thus elaborated a new strategy to quickly and easily generate a library (D) of cyclic carbamate derivatives of general structure **19** (Scheme 3). In addition to their rigid structure, these cyclic carbamate derivatives had the advantage of generating a local diversity in the same area that the adamantane group occupied in the library C. Such local diversity could generate additional benefical interactions in this enzyme area. From the inhibition results of library D (Figure 6), it can be seen that the morpholine (**D-5-2**, **D-5-4**, and **D-5-5**) and dipropylamine building blocks (**D-2-3**, **D-2-4**, and **D-2-5**) at R_1 and *tert*-butylphenyl at R_2 (**D-1-5**, **D-2-5**, **D-3-5**, **D-4-5**, and **D-5-5**) provide the strongest inhibition at 0.3 µM (80–90%), with values similar to the inhibition obtained with the best inhibitors of libraries A–C (**A-4-11**, **B-1-6**, and **C-7-3**). However, the rigidification of the 3α-OH and 3*β*-tertiary amide into a five-membered cyclic carbamate seems to slightly decrease the inhibition, as illustrated by a comparison between the compound **C-7-3** (noncyclic version) and the compound **15** (carbamate cyclic version). The IC_{50} of one of the best compounds of library D (**D-5-4**) was determined in the same assay as the best inhibitor of libraries A–C (**C-7-3**). The IC_{50} values of the two compounds (57 and 74 nM) are very close, and both are better than the IC_{50} of reference compound **1** (98 nM) (Table 2). Considering that the compounds of library D are mixtures of two diastereoisomers, the pure diastereoisomeric form of active compounds, like **D-5-4**, could possibly exert an even stronger inhibitory activity.

Androgenic Activity of Potent Inhibitors. A type 3 17*β*-HSD inhibitor to be used in prostate cancer treatment obviously must be nonandrogenic. Consider-

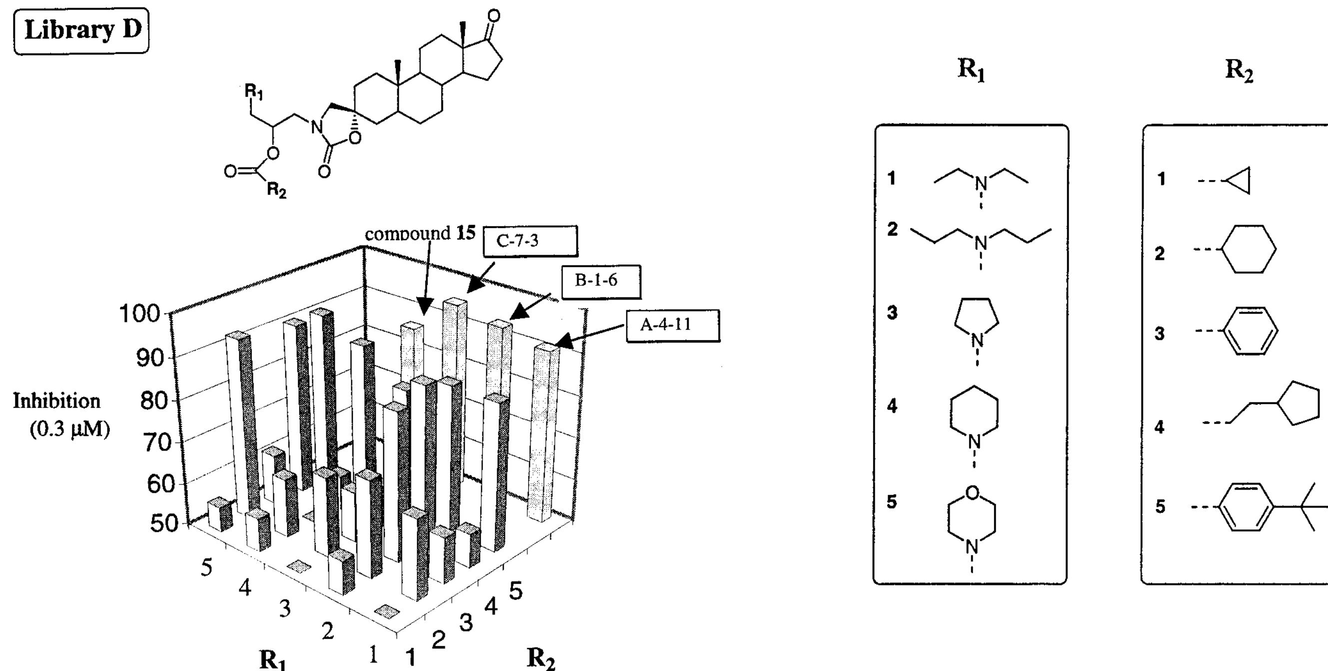


Figure 6. Inhibition of type 3 17β-HSD by 25 members of library D at a concentration of 0.3 μM.

ing their C19-steroid androgenic nucleus, we thus tested the two best inhibitors from our study (**C-7-3** and **D-5-4**) to evaluate their possible androgenic activity using the model of androgen sensitive (AR^+) Shionogi cells at two concentrations (1 and 0.1 μM). As shown in Table 2, compound **C-7-3** induced proliferation (96%) at 1 μM that was of the same order than compound **1** (100%), the best first generation inhibitor. On the other hand, the compound **D-5-4** displayed only a slight, not significant proliferative activity at 1 and 0.1 μM. Using this second criteria, inhibitor **D-5-4** was thus the best candidate from all generated libraries (A−D) showing both a good inhibitory effect on type 3 17β-HSD and a nonandrogenic profile.

Conclusion

To produce potent inhibitors of type 3 17β-HSD, we performed parallel liquid-phase synthesis of 3β-substituted-ADT libraries (A−D) in good yields and an average HPLC purity of 93%. The first library (A) of 3β-amidomethyl-ADT derivatives (**3**) (168 members), including two levels of molecular diversity on the amide (R_1 and R_2), was synthesized by a parallel liquid-phase method I faster (25 days) than with classic chemistry. The screening of library A revealed that relatively small hydrophobic chains at R_1 (5−8 carbons) and small hydrophobic substituents at R_2 (1−4 carbons) were two characteristics of the most potent inhibitors. According to these inhibition results, a second library (B) of 3β-amidomethyl-ADT derivatives (56 members) was generated in a very short time (4 days) using the improved method II based on scavenger resins and liquid-phase parallel chemistry. Library B generated more potent inhibitors than library A and provided useful information on SAR that was used for the design of a new library (C). Once again, library C (49 members) showed very potent inhibitors; compound **C-7-3** was identified as the most potent inhibitor of libraries A−C, with an inhibitory activity (IC_{50} = 35 nM) 18-fold higher than that of natural substrate Δ^4-dione (IC_{50} = 650 nM) used itself as an inhibitor. We finally designed a library (D) of 3-carbamate-ADT derivatives (25 members) using the efficient parallel liquid-phase method III, which allowed the preparation of more rigid molecules with two levels of molecular diversity (R_1/R_2 and R_3) in the local area occupied by the adamantane group in library C. One of the most potent inhibitors of library D, the compound **D-5-4**, showed an inhibitory activity similar to that of compound **C-7-3**, combined to a nonandrogenic profile.

Experimental Section

General Information. DHT and epiandrosterone were purchased from Steraloids (Wilton, NH). Scavenger resins (methylisocyanate polystyrene, aminomethylated polystyrene, and piperidinomethyl polystyrene) were purchased from Calbiochem-Novabiochem Corp. (San Diego, CA), and the polymer-bound reagent ((dimethylamino)pyridine polystyrene, DMAP−PS) was purchased from Argonaut Technologies (SanCarlos, CA). Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), dimethyl sulfoxide (DMSO), toluene, and pyridine were obtained from Sigma-Aldrich. Anhydrous tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl and kept under argon. Libraries A−C were synthesized using sealed vials (10 mL), and library D was synthesized with an ACT LabTech manual synthesizer (Advanced ChemTech, Louisville, KY) using a 40 wells liquid-phase reaction block. Silica gel (SiO_2) filtration was performed using either 10 or 20 mL disposable polypropylene syringe (Sigma-Aldrich) using a cotton plug and sand at the end of the syringe. TLC and flash-column chromatography were performed on 0.20 mm silica gel 60 F254 plates with 230−400 mesh ASTM silica gel 60, respectively (E. Merck, Darmstadt, Germany). The purity of a random sampling of library was determined by HPLC (Waters Associates, Milford, MA) using a NovaPak C18 reversed phase column (150 mm × 3.9 mm id) and an ultraviolet detector (205 nM). Infrared spectra were recorded on a Perkin-Elmer series 1600 Fourier transform infrared spectrometer (Norwalk, CT), and only the significant bands were reported in cm^{-1}. Nuclear magnetic resonance spectra

(NMR) were recorded at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C on a Brucker AC/F300 spectrometer (Billerica, MA) and reported in parts per million. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA) equipped with a turbo ionspray source.

Preparation of Library A Members (Method I). 3β-Aminomethyl-3α-hydroxy-5α-androstan-17-ones (10). In 12 vials (15 mL) were added in parallel the spiro-3(*R*)-oxirane-5α-androstan-17-one (**4**)[30,40] (363 mg, 1.2 mmol), dry CH_3CN (10 mL), anhydrous lithium perchlorate (255 mg, 2.4 mmol), and the primary amines (1.2 mmol) individually. The mixtures were heated in sealed vials at 60 °C for 7 days under an atmosphere of argon, and the solutions were evaporated under vacuum. The crude products were dissolved in ethyl acetate (30 mL), washed with water (150 mL), and then purified by a fast filtration on silica gel (10 mL) in polypropylene syringes of 20 mL using EtOAc: hexanes (1:1) as the eluent. After the solvent was evaporated under reduced pressure, secondary amines **10** (see Figure 3 for R_1) were obtained in acceptable yields (60−80%).

3β-Amidomethyl-3α-hydroxy-5α-androstan-17-ones (11). Each of the secondary amines **10** obtained above was equally divided in 14 different portions for the acylation reaction. The appropriate acyl chlorides (0.05 mmol) were added individually to the secondary amine (0.05 mmol) in the presence of anhydrous pyridine (0.1 mmol) and CH_2Cl_2 (1 mL). After 3 h at room temperature, the CH_2Cl_2 was removed under vacuum (Speedvac apparatus, Winchester, VA) and ethyl acetate was added. The organic layer was washed with an aqueous NaOH solution (40%) and evaporated. The crude amides **11** were then purified by a fast silica gel filtration (EtOAc:hexanes, 3:7) using 10 g of silica gel in a 20 mL polypropylene syringe. The first 10 mL eluted was discarded, and the next 20 mL recovered was evaporated to give the 3β-amidomethyl-3α-hydroxy-5α-androstan-17-ones (168 members) in acceptable yield (40−70%) and a high average purity (92%) for a random sampling of 12 members: **A-1-8**, **A-2-11**, **A-3-9**, **A-4-1**, **A-5-3**, **A-6-2**, **A-7-14**, **A-8-6**, **A-9-13**, **A-10-10**, **A-11-5**, and **A-12-12** (Table 1). See Figure 3 for the chemical structure of compounds.

A-1-8. 3β-[(*N*-Pentyl-*N*-decanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3374 (OH), 1741 (C=O, ketone), 1620 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.89 and 0.92 (2t, 6H, J = 6.9 Hz, 2 × $CH_3$$CH_2$), 2.33 (t, 2H, J = 7.5 Hz, CH_2CO), 1.00−2.20 (42H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.31 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.64 (s, OH). LRMS for $C_{35}H_{62}NO_3$ [M + H]$^+$: 544.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:10:55): 97% of purity.

A-2-11. 3β-[(*N*-Hexyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3374 (OH), 1740 (C=O, ketone), 1610 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, J = 7.5 Hz, 3H, $CH_3$$CH_2$), 1.00−2.10 (34H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.35 (q_{app} of AB system, 2H, CH_2N-3β), 3.48 (m, 2H, $CH_2$$CH_2$N) 4.64 (s, OH). LRMS for $C_{30}H_{50}NO_3$ [M + H]$^+$: 472.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:20:45): 85% of purity.

A-3-9. 3β-[(*N*-Heptyl-*N*-dodecanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3306 (OH), 1742 (C=O, ketone), 1621 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.87 and 0.89 (2t, 6H, J = 6.6 Hz, 2 × $CH_3$$CH_2$), 1.00−2.20 (52H), 2.33 (t, J = 7.6 Hz, 2H, CH_2CO), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.31 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.65 (s, OH). LRMS for $C_{39}H_{70}NO_3$ [M + H]$^+$: 600.7 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 55:10:35): 84% of purity.

A-4-1. 3β-[(*N*-Octyl-*N*-propionyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3375 (OH), 1741 (C=O, ketone), 1620 (amide, C=O). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85, (s, 3H, CH_3-18), 0.86 (t, J = 7.5 Hz, 3H, CH_3CH_2), 1.15 (t, J = 7.3 Hz, 3H, CH_3CH_2), 1.00−2.20 (33H), 2.37 (m, 3H, CH_2CO and 1H of CH_2-16), 3.30 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.60 (s, OH). LRMS for $C_{31}H_{54}NO_3$ [M + H]$^+$: 488.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:20:45): 90% of purity.

A-5-3. 3β-[(*N*-Nonyl-*N*-pentanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (KBr): 3416 (OH), 1736 (C=O, ketone), 1611 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, J = 6.3 Hz, 3H, CH_3-CH_2), 0.92 (t, J = 7.3 Hz, 3H, $CH_3$$CH_2$), 1.00−2.20 (39H), 2.34 (t, J = 7.60 Hz, 2H, CH_2CO), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.30 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.65 (s, OH). LRMS for $C_{34}H_{60}NO_3$ [M + H]$^+$: 530.8 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:10:55): 97% of purity.

A-6-2. 3β-[(*N*-Decyl-*N*-butanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3306 (OH), 1739 (C=O, ketone), 1622 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, J = 6.4 Hz, 3H, $CH_3$$CH_2$), 0.96 (t, J = 7.3 Hz, 3H, $CH_3(CH_2)_2$CO), 1.00−2.10 (39H), 2.32 (t, J = 7.6 Hz, 2H, CH_2CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.31 (m, 4H, CH_2N-3β), 4.63 (s, OH). LRMS for $C_{34}H_{60}NO_3$ [M + H]$^+$: 530.8 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:10:55): 96% of purity.

A-7-14. 3β-[(*N*-Undecanyl-*N*-*tert*-butylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (KBr): 3432 (OH), 1743 (C=O, ketone), 1609 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-19), 0.88 (t, J = 6.3 Hz, 3H, $CH_3$$CH_2$), 1.07 (s, 9H, $(CH_3)_3$−C), 1.00−2.20 (40H), 2.24 (s, 2H, CH_2CO), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.32 (t, J = 7.7 Hz, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.76 (s, OH). LRMS for $C_{37}H_{66}NO_3$ [M + H]$^+$: 572.70 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:10:55): 94% of purity.

A-8-6. 3β-[(*N*-Dodecyl-*N*-octanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (KBr): 3422 (OH), 1739 (C=O, ketone), 1614 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-19), 0.87 (t, J = 8.0 Hz, 6H, 2 × $CH_3$$CH_2$), 1.00−2.47 (55H), 2.33 (t, J = 6.7 Hz, 2H, CH_2CO), 3.31 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.76 (s, OH). LRMS for $C_{40}H_{72}NO_3$ [M + H]$^+$: 614.3 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 25:30:45): 99% of purity.

A-9-13. 3β-[(*N*-Tridecyl-*N*-2′-ethylhexanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3386 (OH), 1741 (C=O, ketone), 1617 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, J = 6.7 Hz, 6H, 2 × $CH_3$$CH_2$), 0.90 (t, J = 6.8 Hz, 3H, $CH_3$$CH_2$), 1.00−2.20 (50H), 2.44 (m, 2H, CH_2CO and 1H of CH_2-16), 3.33 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.8 (s, OH). LRMS for $C_{41}H_{74}NO_3$ [M + H]$^+$: 628.8 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 55:10:35): 97% of purity.

A-10-10. 3β-[(*N*-Tetradecyl-*N*-tetradecanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3371 (OH), 1742 (C=O, ketone), 1620 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, J = 7.2 Hz, 6H, 2 × $CH_3$$CH_2$), 1.00−2.20 (67H), 2.33 (t, J = 7.6 Hz, 2H, CH_2CO), 2.42 (dd, J_1 = 8.5 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.32 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.76 (s, OH). LRMS for $C_{48}H_{88}NO_3$ [M + H]$^+$: 726.5 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 70:05:25): 91% of purity.

A-11-5. 3β-[(*N*-Hexadecyl-*N*-heptanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3331 (OH), 1742 (C=O, ketone), 1621 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, 6H, J = 5.7 Hz, 2 × $CH_3$$CH_2$), 1.00−2.20 (57H), 2.32 (t, J = 7.6 Hz, 2H, CH_2CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.30 (m, 4H, $CH_2$$CH_2$N and CH_2N-3β), 4.64 (s, OH). LRMS for $C_{43}H_{78}NO_3$ [M + H]$^+$: 656.7 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 55:10:35): 90% of purity.

A-12-12. 3β-[(*N*-Octadecyl-*N*-cyclobutylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3374 (OH), 1735 (C=O, ketone), 1618 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.88 (t, J = 6.3 Hz, 3H, $CH_3$$CH_2$), 1.00−2.50 (61H), 3.20 and 3.30 (2m, 4H, $CH_2$$CH_2$N and CH_2N-3β) 4.76 (s, OH). LRMS for $C_{43}H_{76}NO_3$ [M + H]$^+$: 654.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 55:10:35): 91% of purity.

Preparation of Libraries B and C Members (Method II). Synthesis of Spiro-3(*R*)-oxirane-5α-androstane-17-

dioxolane (9). A sequence of three steps was needed to obtain compound **9** from epiandrosterone.

(1) Protection of C17-Ketone as Dioxolane. To a solution of epiandrosterone (10.0 g, 34.4 mmol) in anhydrous toluene (500 mL) were added ethylene glycol (19.2 mL, 344 mmol) and *p*-toluenesulfonic acid (*p*-TSA) (654 mg, 3.4 mmol). The resulting solution was heated at reflux for 24 h under an atmosphere of argon using a Dean–Stark apparatus. The solution was poured in cold water (2 L) and extracted with EtOAc (2 × 500 mL) and washed with water (2 L). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to give 12.0 g of crude 3β-hydroxy-5α-androstane-17-dioxolane.

(2) Oxidation of 3β-OH to Ketone. To the crude dioxolane obtained above (12.0 g) in anhydrous dichloromethane (150 mL) were added molecular sieves (20 g), NMO (6.92 g, 39.4 mmol), and tetrapropylammonium perruthenate (TPAP) (692 mg, 1.96 mmol). The mixture was stirred for 2 h under an atmosphere of argon and then directly filtered on a silica gel plug (SiO_2, 100 g) to give 10.5 g of 3-oxo-5α-androstane-17-dioxolane.

(3) Formation of 3-Spiro-(*R*)-oxirane. To a solution of trimethylsulfoxonium iodide (13.9 g, 63.3 mmol) in dry DMSO (350 mL) was carefully added sodium hydride 60% in oil (2.5 g, 62.5 mmol) under an atmosphere of argon. The solution was stirred at room temperature for 1 h before adding the crude 3-oxo-5α-androstane-17-dioxolane (10.5 g, 31.6 mmol) dissolved in dry THF (200 mL). The mixture was stirred overnight, poured in ice/water (2 L), and extracted with EtOAc (2 × 300 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the resulting crude product (10.0 g) by flash chromatography (EtOAc:hexanes:triethylamine (TEA), 5:95:1) yielded 6.5 g (54% for 3 steps) of compound **9**. ^{1}H NMR ($CDCl_3$): 0.83 (s, 6H, CH_3-19 and CH_3-18), 2.59 (s, 2H, CH_2O-3β), 0.80–2.10 (22H), 3.88 (m, 4H, OCH_2CH_2O). ^{13}C NMR ($CDCl_3$): 11.2, 14.3, 20.3, 22.5, 28.3, 29.1, 30.6, 31.0, 34.1, 35.4, 35.6, 35.7, 35.9, 43.6, 45.8, 50.2, 53.4, 53.6, 58.4, 64.5 (CH_2O), 65.1 (CH_2O), 119.4 (C-17). LRMS for $C_{22}H_{35}O_3$ $[M + H]^+$: 347.4 *m/z*.

3β-Aminomethyl-3α-hydroxy-5α-androstan-17-ones (10). To the oxirane **9** (150 mg, 0.43 mmol) in ethanol (3 mL) were added in parallel the appropriate primary amines (1.3 mmol), and the mixtures were heated at 60 °C for 36 h. The resulting solutions were evaporated, and the crude products obtained were dissolved with acetone (5 mL). An aqueous HCl 3 N solution (5 mL) was then added to each reactor, and solutions were stirred for 2 h at room temperature. The acid mixtures were poured in a cold sodium bicarbonate solution (10%), and the aqueous layers were extracted twice with CH_2Cl_2. The organic phases were filtered on a cotton plug and evaporated under reduce pressure. The resulting crude products were then preadsorbed on silica gel (10 g) in a 20 mL polypropylene syringe, and elution was performed using EtOAc/hexanes, 1:9 (10 mL) to remove the unpolar byproducts. The desired more polar secondary amines were recovered using EtOAc (20 mL) in acceptable yields (50–70%) and directly used for the next step (second level of reaction).

3β-Amidomethyl-3α-hydroxy-5α-androstan-17-one (11). Each of the secondary amines obtained above (eight for library B and seven for library C) was split in 7 parts (0.05 mmol) and dissolved in dry CH_2Cl_2 (1 mL). Piperidinomethyl polystyrene (40 mg, 0.15 mmol) and the appropriate acyl chloride (0.06 mmol) were added to the appropriate reactors and stirred for 2 h at room temperature. After the reaction was completed (TLC monitoring), aminomethyl polystyrene (0.15 mmol) was added to each reactor to scavenge the excess of unreacted acyl chloride. After 1 h, the suspensions were filtered to remove the resins and the solutions recovered were evaporated to give the desired library B (56 members) and library C (49 members) in good yields for both libraries (70–90%) and high average purity for a random sampling of library B members, **B-2-4**, **B-2-7**, **B-3-6**, **B-4-1**, **B-4-4**, **B-5-1**, **B-5-6**, **B-6-1**, **B-6-6**, **B-6-7**, **B-7-1**, **B-8-2**, and **B-8-4**, and library C members, **C-2-6**, **C-3-7**, **C-4-3**, **C-4-5**, **C-5-1**, **C-6-1**, **C-6-2**, and **C-7-7** (Table 1). See Figures 4 and 5 for the chemical structures of the compounds.

B-2-4. 3β-[(*N*-Cyclopropyl-*N*-bromoacetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3432 (OH), 1738 (C=O, ketone), 1645 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.79 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80–2.20 (25H), 2.43 (dd, J_1 = 8.8 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.00 (m, 1H, CHN), 3.16 (s, OH), 3.44 (s, 2H, CH_2N-3β), 4.18 (s, 2H, CH_2-Br). LRMS for $C_{25}H_{39}BrNO_3$ $[M + H]^+$: 480.4 *m/z* (^{79}Br) and 482.4 *m/z* (^{81}Br). HPLC (CH_3CN:H_2O:CH_3OH, 30:40:30): 94% of purity.

B-2-7. 3β-[(*N*-Cyclopropyl-*N*-butanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3421 (OH), 1740 (C=O, ketone), 1629 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.97 (t, *J* = 7.3 Hz, 3H, *CH_3*CH_2), 0.80–2.20 (27H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 2.56 (t, *J* = 7.5 Hz, 2H, CH_2CO), 2.78 (m, 1H, CHN), 3.40 (s, 2H, CH_2N-3β), 4.12 (s, OH). LRMS for $C_{27}H_{44}NO_3$ $[M + H]^+$: 430.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:35:30): 97% of purity.

B-3-6. 3β-[(*N*-Cyclopentyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3322 (OH), 1739 (C=O, ketone), 1610 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80–2.15 (34H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.24 (s, 2H, CH_2N-3β), 4.43 (m, 1H, CHN), 5.56 (s, OH). LRMS for $C_{29}H_{46}NO_3$ $[M + H]^+$: 456.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 40:25:35): 82% of purity.

B-4-1. 3β-[(*N*-2-Methoxymethyl-*N*-acetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3364 (OH), 1735 (C=O, ketone), 1618 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 and 0.79 (2s of conformers A and B, 3H, CH_3-19), 0.85 and 0.86 (2s of conformers A and B, 3H, CH_3-18), 0.80–2.10 (22H), 2.13 and 2.16 (2s of conformers A and B, 3H, CH_3CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.35 (s, 3H, CH_3O), 3.20–3.80 (m, 6H, OCH_2 and CH_2CH_2N and CH_2N-3β). LRMS for $C_{25}H_{42}NO_4$ $[M + H]^+$: 420.3 *m/z*. HPLC (CH_3-CN:H_2O:CH_3OH, 30:40:30): 97% of purity.

B-4-4. 3β-[(*N*-2′-Methoxyethyl-*N*-bromoacetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3416 (OH), 1734 (C=O, ketone), 1636 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.79 (2s of conformers A and B, 3H, CH_3-19), 0.85 and 0.86 (2s of conformers A and B, 3H, CH_3-18), 0.80–2.12 (24H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.35 and 3.36 (2s, conformers A and B, 3H), 3.20–3.80 (m, 6H OCH_2 and CH_2N and CH_2N-3β), 3.97 and 4.02 (2s, conformers A and B, 2H, CH_2-Br). LRMS for $C_{25}H_{42}NO_4$ $[M + H]^+$: 498.3 *m/z*. HPLC (CH_3-CN:H_2O:CH_3OH, 30:40:30): 95% of purity.

B-5-1. 3β-[(*N*-3′-Ethoxypropyl-*N*-acetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3375 (OH), 1739 (C=O, ketone), 1623 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 1.20 (t, *J* = 7.0 Hz, 3H, *CH_3*CH_2), 0.90–2.15 (24H), 2.16 (s, 3H, CH_3CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.33 (dapp of AB system, 2H, CH_2N-3β), 3.45 (m, 6H, CH_2N and 2 × CH_2O). LRMS for $C_{27}H_{46}NO_4$ $[M + H]^+$: 448.5 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 30:40:30): 97% of purity.

B-5-6. 3β-[(*N*-3′-Ethoxypropyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3380 (OH), 1739 (C=O, ketone), 1614 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 1.19 (t, *J* = 7.0 Hz, 3H, *CH_3*CH_2), 0.80–2.15 (28H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.35 (q_{app} of AB system, 2H, CH_2N-3β), 3.46 (m, 4H, 2 x CH_2O), 3.65 (m, 2H, CH_2N), 4.68 (s, OH). LRMS for $C_{29}H_{48}NO_4$ $[M + H]^+$: 474.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:35:30): 94% of purity.

B-6-1. 3β-[(*N*-2′-Pentoxyethyl-*N*-acetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3378 (OH), 1740 (C=O, ketone), 1624 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.92 (t, *J* = 7.3 Hz, 3H, *CH_3*CH_2), 0.80–2.15 (27H), 2.15 (s, 3H, CH_3CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.30–3.50 (m, 8H, 2 × CH_2O and CH_2N), 4.62 (OH). LRMS for $C_{29}H_{50}NO_4$ $[M + H]^+$: 476.3 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:30:35): 90% of purity.

B-6-6. 3*β*-[(*N*-2′-Pentoxyethyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α−androstan-17-one. IR (film): 3361 (OH), 1739 (C=O, ketone), 1612 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.92 (t, J = 7.3 Hz, 3H, CH_3CH_2), 0.80−2.15 (33H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.34 (q_{app} of AB system, 2H, CH_2N-3*β*), 3.42 (m, 4H, 2 × CH_2O), 3.64 (m, 2H, CH_2N). LRMS for $C_{31}H_{52}NO_4$ [M + H]$^+$: 502.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:30:35): 82% of purity.

B-6-7. 3*β*-[(*N*-2′-Pentoxyethyl-*N*-butanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (KBr): 3357 (OH), 1740 (C=O, ketone), 1618 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.93 and 0.96 (2t, J = 7.3 Hz, 6H, 2 × CH_3CH_2), 0.80−2.15 (30H), 2.36 (t, J = 7.5 Hz, 2H, CH_2CO), 2.42 (dd, J_1 = 8.8 Hz, J_2 = 19.5 Hz, 1H of CH_2-16), 3.30−3.50 (m, 8H, 2 × CH_2O and CH_2NCH_2). LRMS for $C_{31}H_{54}NO_4$ [M + H]$^+$: 504.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 45:20:35): 93% of purity.

B-7-1. 3*β*-[(*N*-Furan-2-ylmethyl-*N*-acetyl)-aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3395 (OH), 1737 (C=O, ketone), 1631 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (22H), 2.27 (s, 3H, CH_3CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.37 (q_{app} of AB system, 2H, CH_2N-3*β*), 4.02 (s, OH), 4.53 (s, 2H, CH_2N), 6.22 (d, J = 3.2 Hz, 1H, O−CH=*CH*), 6.34 (dd, J_1 =1.0, J_2 = 3.0 Hz, 1H, *CH*=CH−O), 7.38 (d, J = 1.1 Hz, 1H, O-*CH*=C). LRMS for $C_{27}H_{40}NO_4$ [M + H]$^+$: 442.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 30:40:30): 99% of purity.

B-8-2. 3*β*-[(*N*-Allyl-*N*-dichloroacetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3456 (OH), 1737 (C=O, ketone), 1666 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (22H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.42 (s, 2H, CH_2N-3*β*), 4.27 (d, J = 2.0 Hz, 2H, CH_2N), 5.17 (d, J = 17.0 Hz, 1H of *CH*$_2$=CH), 5.30 (d, J = 10.5 Hz, 1H of *CH*$_2$=CH), 5.81 (m, 1H, *CH*$_2$=*CH*), 6.22 (s, 1H, $CHCl_2$). LRMS for $C_{25}H_{38}Cl_2NO_3$ [M + H]$^+$: 470.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:35:30): 92% of purity.

B-8-4. 3*β*-[(*N*-Allyl-*N*-dibromoacetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3396 (OH), 1735 (C=O, ketone), 1654 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (22H), 2.43 (dd, J_1 = 8.8 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 3.37 (s, 2H, CH_2N-3*β*), 3.84 (s, 2H, CH_2Br), 4.17 (d, J = 2.3 Hz, 2H, CH_2N), 5.14 (d, 1H, J = 17.2 Hz, 1H of *CH*$_2$=CH), 5.26 (d, J = 10.3 Hz, 1H of *CH*$_2$=CH), 5.82 (m, 1H, CH_2=*CH*). LRMS for $C_{25}H_{39}BrNO_3$ [M + H]$^+$: 480.4 *m/z* (^{79}Br) and 482.5 *m/z* (^{81}Br). HPLC (CH_3CN:H_2O:CH_3OH, 30:40:30): 91% of purity.

C-2-6. 3*β*-[(*N*-2′-Phenylethyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3390 (OH), 1738 (C=O, ketone), 1614 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (25H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 2.94 (t, J = 7.6 Hz, 2H, *CH*$_2$Ph), 3.31 (q_{app} of AB system, 2H, CH_2N-3*β*), 3.76 (m, 2H, CH_2N), 7.25 (m, 5H, CH aromatic). LRMS for $C_{32}H_{46}NO_3$ [M + H]$^+$: 492.5 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:30:35): 99% of purity.

C-3-7. 3*β*-[(*N*-3′-Phenylpropyl-*N*-*i*-propylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (KBr): 3441 (OH), 1744 (C=O, ketone), 1608 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.74 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 1.09 (d, J = 6.7 Hz, 6H, (*CH*$_3$)$_2$CH), 0.80−2.15 (24H), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.1 Hz, 1H of CH_2-16), 2.62 (t, 2H, J = 7.2 Hz, CH_2Ph), 2.60 (m, 1H, CHCO), 3.28 (s, 2H, CH_2N), 3.33 (q_{app} of AB system, 2H, CH_2N-3*β*), 7.25 (m, 5H, CH aromatic). LRMS for $C_{33}H_{50}NO_3$ [M + H]$^+$: 508.5 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 40:25:35): 99% of purity.

C-4-3. 3*β*-[(*N*-3′,5′-Bis(trifluoromethyl)benzyl-*N*-butanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (KBr): 3440 (OH), 1739 (C=O, ketone), 1683 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.76 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.91 (t, J = 7.3 Hz, 3H, CH_3CH_2), 0.80−2.15 (24H), 2.27 (t, 2H, J = 7.3 Hz, CH_2CO), 2.43 (dd, J_1 = 9.0 Hz, J_2 = 19.4 Hz, 1H of CH_2-16), 3.42 (m, 2H, CH_2N-3*β*), 4.86 (s, 2H, CH_2N), 7.57 (s, 2H, CH aromatic), 7.64 (s, 1H, CH aromatic). LRMS for $C_{33}H_{44}F_6NO_3$ [M + H]$^+$: 616.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 40:20:40): 86% of purity.

C-4-5. 3*β*-[(*N*-3′,5′-Bis(trifluoromethyl)benzyl-*N*-2′-ethylhexanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3340 (OH), 1738 (C=O, ketone), 1630 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.76 (s, 3H, CH_3-19), 0.83 (m, 6H, 2 × CH_3CH_2), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (30H), 2.32 (m, 1H, CHCO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.34 and 3.55 (2m, 2H, CH_2N-3*β*), 4.88 (AB system, 2H, CH_2N), 7.60 (s, 2H, CH aromatic), 7.83 (s, 1H, CH aromatic). LRMS for $C_{37}H_{52}F_6NO_3$ [M + H]$^+$: 672.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 45:15:40): 97% of purity.

C-5-1. 3*β*-[(*N*-4′-Methylbenzyl-*N*-acetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3405 (OH), 1738 (C=O, ketone), 1632 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.75 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.47 (22H), 2.15 (s, 3H, CH_3CO), 2.35 (s, 3H, *CH*$_3$−Ph), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.0 Hz, 1H of CH_2-16), 3.37 (q_{app} of AB system, 2H, CH_2N-3*β*), 4.62 (s, 2H, CH_2N), 7.02 (d, J = 7.9 Hz, 2H, CH aromatic), 7.18 (d, J = 7.9 Hz, 2H, CH aromatic). LRMS for $C_{30}H_{44}NO_3$ [M + H]$^+$: 466.4 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:35:30): 98% of purity.

C-6-1. 3*β*-[(*N*-3′,5′-Bis(methoxy)benzyl-*N*-acetyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3405 (OH), 1737 (C=O, ketone), 1627 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.75 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 0.80−2.15 (21H), 2.14 (s, 3H, CH_3CO), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 3.39 (q_{app}, 2H, CH_2N-3*β*), 3.78 (s, 6H, 2 × OCH_3), 4.17 (s, OH), 4.59 (s, 2H, CH_2N), 6.25 (d, J = 2.0 Hz, 2H, CH aromatic), 6.36 (s, 1H, CH aromatic). LRMS for $C_{31}H_{46}NO_5$ [M + H]$^+$: 512.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:35:30): 98% of purity.

C-6-2. 3*β*-[(*N*-3′,5′-Bis(methoxy)benzyl-*N*-propionyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3416 (OH), 1738 (C=O, ketone), 1620 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.75 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 1.13 (t, J = 7.4 Hz, 3H, CH_3CH_2), 0.80−2.15 (21H), 2.38 (m, 3H, CH_3*CH*$_2$CO and 1H of CH_2-16), 3.40 (q_{app} of AB system, 2H, CH_2N-3*β*), 3.78 (s, 6H, 2 × OCH_3), 4.18 (s, OH), 4.60 (s, 2H, CH_2N), 6.24 (d, J = 2.0 Hz, 2H, CH aromatic), 6.36 (d, J = 1.9 Hz, 1H, CH aromatic). LRMS for $C_{32}H_{48}NO_5$ [M + H]$^+$: 526.6 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 35:35:30): 96% of purity.

C-7-7. 3*β*-[(*N*-Adamantylmethyl-*N*-*i*-propylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3394 (OH), 1738 (C=O, ketone), 1615 (C=O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 1.13 (d, J = 6.5 Hz, 6H, (*CH*$_3$)$_2$CH), 0.80−2.09 (36H), 2.42 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H of CH_2-16), 2.95 (m, 1H, CHCO), 3.13 (s, 2H, CH_2N-3*β*), 3.44 (s, 2H, CH_2N), 4.71 (OH); LRMS for $C_{35}H_{56}NO_3$ [M + H]$^+$: 538.7 *m/z*. HPLC (CH_3CN:H_2O:CH_3OH, 45:15:40): 76% of purity.

Preparation of Library D Members (Method III). 3*β*-Allylaminomethyl-3α-hydroxy-5α-androstan-17-one (12). To a solution of spiro-3(*R*)-oxirane-5α-androstan-17-one (**4**)[30,46] (10.0 g, 34.4 mmol) in anhydrous ethanol was added allylamine (4.5 mL, 68.8 mmol), and the solution was stirred overnight at 60 °C. The resulting solution was evaporated under reduced pressure and purified by flash chromatography using EtOAc/hexanes (1:1) as the eluent to give compound **12** (9.8 g, 82%). IR (KBr): 3500−3330 (NH and OH), 1737 (C=O, ketone). ^{1}H NMR ($CDCl_3$): 0.71 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 0.90−2.10 (24H), 2.36 (dd, J_1 = 8.6 Hz, J_2 = 18.9 Hz, 1H of CH_2-16), 2.42 (s, 2H, CH_2N-3*β*), 3.21 (d, J = 5.9 Hz, 2H, $CHCH_2N$), 5.03 (d, J = 10.3 Hz, 1H, CH_2=CH), 5.10 (dd, J_1 = 1.3 Hz, J_2 = 17.0 Hz, 1H, *CH*$_2$=CH), 5.82 (m, 1H, *CH*=CH_2). ^{13}C NMR ($CDCl_3$): 11.1, 13.7, 20.1, 21.6, 28.1, 30.7, 31.4, 31.6, 33.5, 34.9, 35.7, 35.9, 38.5, 40.5, 47.6, 51.3, 52.9, 54.1, 60.0, 69.8, 115.7, 136.7, 221.2. LRMS for $C_{23}H_{38}NO_2$ [M + H]$^+$: 360.2 *m/z*.

3*R*-Spiro-(3′-allyl-2′-oxo-oxazolidin-5′-yl)-5α-androstan-17-one (14). To a solution of compound **12** (2.6 g, 7.2 mmol) in anhydrous CH_2Cl_2 (200 mL) at 0 °C under an atmosphere of argon were added DIPEA (2.5 mL, 14.4 mmol) and

triphosgene (1.07 g, 3.6 mmol). The solution was stirred at room temperature for 3 h. The resulting solution was diluted with CH_2Cl_2 (300 mL) and washed with a water/ice mixture (1 L). The organic layer was filtered on a cotton plug and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography using EtOAc/hexanes (4:6) as the eluent to give compound **14** (2.52 g, 91%). IR (KBr): 1734 (C=O, ketone and carbamate). ^1H NMR ($CDCl_3$): 0.81 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.90−2.15 (21H), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.2$ Hz, 1H of CH_2-16), 3.16 (s, 2H, CH_2N-3β), 3.84 (d, $J = 5.5$ Hz, 2H, CH_2N), 5.20 (d, $J = 14.8$ Hz, 1H, CH_2=CH), 5.21 (d, $J = 12.2$ Hz, 1H, CH_2=CH), 5.73 (m, 1H, CH_2=*CH*). ^{13}C NMR ($CDCl_3$): 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.4, 32.8, 33.8, 35.0, 35.3, 35.8, 39.4, 40.8, 46.6, 47.7, 51.3, 53.9, 55.9, 78.8, 118.4, 132.1, 157.2, 221.2. LRMS for $C_{24}H_{36}NO_3$ $[M + H]^+$: 386.1 *m/z*.

3*R*-Spiro-(3′-adamantylmethyl-2′-oxo-oxazolidin-5′-yl)-5α-androstan-17-one (15). To a solution spiro-3(*R*)-oxirane-5α-androstan-17-one (**4**)[30,40] (1.0 g, 3.44 mmol) in ethanol (99% in benzene) was added 1-adamantane-methylamine (1.2 mL, 6.88 mmol), and the solution was stirred overnight at 60 °C. The resulting solution was evaporated under reduced pressure and purified by flash chromatography using EtOAc/hexanes (1:1) as the eluent to give compound **13** (1.1 g, 70%). To a solution of compound **13** (1.0 g, 2.1 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0 °C under an atmosphere of argon were added DIPEA (458 μL, 2.6 mmol) and triphosgene (227 mg, 0.75 mmol). The solution was stirred at room temperature for 30 min. The resulting solution was diluted with CH_2Cl_2 (150 mL) and washed with a water/ice mixture (1 L). The organic layer was then filtered on a cotton plug and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography using CH_2Cl_2/MeOH (95:5) as the eluent to give compound **15** (0.82 g, 79%). IR (film): 1739 (C=O, ketone and carbamate). ^1H NMR ($CDCl_3$): 0.81 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (37H), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.0$ Hz, 1H of CH_2-16), 2.85 (s, 2H, CH_2N), 3.30 (s, 2H, CH_2N-3β). ^{13}C NMR ($CDCl_3$): 11.4, 13.8, 20.2, 21.7, 27.9, 28.2 (3×), 30.6, 31.5, 32.7, 33.9, 35.0, 35.4, 35.8, 36.8 (5×), 39.3, 40.8 (3×), 47.7, 51.3, 53.9, 57.1, 60.4, 78.3, 158.6, 221.1. LRMS for $C_{32}H_{48}NO_3$ $[M + H]^+$: 494.6 *m/z*. HPLC (MeOH: H_2O: 72: 28 + $AcNH_4$ (20 mM)): 98% of purity.

3*R*-Spiro-(3′-(2″,3″-dihydroxypropyl)-2′-oxo-oxazolidin-5′-yl)-5α-androstan-17-one (16). To a solution of compound **14** (2.49 g, 6.5 mmol) in *tert*-butyl alcohol/THF/H_2O (10:3:1) (70 mL) were added NMO (908 mg, 7.8 mmol) and osmium tetroxide (50 mg, 0.2 mmol), and the solution was stirred for 2 h at room temperature. The mixture was then diluted with EtOAc (250 mL) and poured in a 10% (w/v) sodium bisulfite solution (750 mL). The organic layer was washed with water (750 mL) and brine (300 mL), dried with $MgSO_4$, filtered, and evaporated under reduce pressure. The resulting crude product was purified by flash chromatography using CH_2Cl_2/MeOH (9:1) as the eluent to give compound **16** (2.43 g, 90%). IR (KBr): 3404 (OH), 1734 (C=O, ketone and carbamate). ^1H NMR ($CDCl_3$): 0.79 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 0.80−2.15 (23H), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.2$ Hz, 1H of CH_2-16), 3.33 (m, 4H, CH_2N-3β and CH_2N), 3.57 (m, 2H, CH_2OH), 3.84 (m, 1H, *CH*OH). ^{13}C NMR ($CDCl_3$): 11.3, 13.7, 20.2, 21.7, 27.7, 30.5, 31.4, 32.7, 33.8, 34.9, 35.3, 35.8, 39.2, 40.8, 46.4, 47.7, 51.2, 53.8, 58.0, 63.7, 70.2, 79.8, 158.6, 221.3. LRMS for $C_{24}H_{38}NO_5$ $[M + H]^+$: 420.4 *m/z*.

3*R*-Spiro-(3′-(2″,3″-epoxypropyl)-2′-oxo-oxazolidin-5′-yl)-5α-androstan-17-one (17). To a solution of diol **16** (2.21 g, 5.3 mmol) in anhydrous CH_2Cl_2 (40 mL) under an atmosphere of argon were added trimethylorthoacetate (808 μL, 6.3 mmol) and pyridinium *p*-toluenesulfonate (PPTS) (67 mg, 0.3 mmol). The solution was stirred for 15 min at room temperature and then immediately evaporated under reduced pressure, followed by 1 min under a mechanical pump. The resulting crude mixture was dissolved in anhydrous CH_2Cl_2 (10 mL), TEA (53 mg, 0.5 mmol, 66 μL) and acetyl bromide (780 mg, 63 mmol) were then added, and the mixture was stirred for 30 min under an atmosphere of argon. The solution was evaporated to dryness under reduced pressure and immediately dissolved in methanol (170 mL) containing K_2CO_3 (2.19 g) and then vigorously stirred for 3 h. The resulting solution was poured in a 10% ammonium chloride solution (500 mL) and extracted twice with EtOAc (2 × 150 mL). The resulting crude product was purified by flash chromatography using EtOAc/hexanes (1:1) as the eluent to give compound **17** (1.38 g, 65%). IR (film): 1734 (C=O, ketone and carbamate). ^1H NMR ($CDCl_3$): 0.80 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 0.90−2.15 (21H), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.1$ Hz, 1H of CH_2-16), 2.54 (d, $J = 2.1$ Hz, 1H, *CH_2*(O)CH), 2.78 (t, 1H, *CH_2*-(O)CH), 3.05 (m, 2H, CH_2N), 3.22 and 3.29 (2d of AB system, 2H, CH_2N-3β), 3.80 (m, 1H, CH_2(O)*CH*). ^{13}C NMR ($CDCl_3$): 11.3, 13.7, 20.1, 21.7, 27.8, 30.5, 31.4, 32.6, 33.8, 34.9, 35.3, 35.8, 39.2, 40.8, 44.2, 46.0, 47.7, 50.3, 51.2, 53.8, 57.4, 79.8, 157.4, 221.0. LRMS for $C_{24}H_{36}NO_4$ $[M + H]^+$: 402.2 *m/z*.

Synthesis of Intermediate Carbamate 18. To compound **17** (5 × 200 mg, 0.50 mmol) in dry CH_3CN (1 mL) were added the appropriate secondary amine (1.0 mmol) and anhydrous lithium perchlorate (64 mg, 0.60 mmol). The solutions were heated at 60 °C overnight in sealed vials. Methylisocyanate polystyrene (600 mg, 1.0 mmol/g) and CH_2Cl_2 (2 mL) were added to each reaction vessel and stirred for 2 h at room temperature. The five resins were removed by filtration, and the resulting filtrates were diluted with CH_2Cl_2 (15 mL) and washed with water (25 mL). The resulting solutions were filtered on a cotton plug and then evaporated under reduced pressure to give the five desired β-amino-alcohols **18** in good yields (60−80%) and high TLC purities.

Synthesis of Compound 19. Each of the five β-amino alcohols **18** obtained above were split in 5 parts (0.05 mmol) and dissolved with EtOAc (1 mL). Piperidinomethyl polystyrene (40 mg, 0.15 mmol) and DMAP−PS (15 mg, 0.02 mmol) were added to each of the 25 reactors. The acyl chlorides (0.50 mmol) were added to the appropriate reactors and shaken for 48 h at 72 °C under an atmosphere of argon. After the reaction was completed (TLC monitoring), aminomethyl polystyrene resin (0.55 mmol, 200 mg) and CH_2Cl_2 (1 mL) were added to each reactor to scavenge the excess of unreacted acyl chloride. After 1 h, the suspensions were filtered and the recovered solutions were evaporated to give the crude products containing a trace of carboxylic acid, a byproduct coming from the hydrolysis of the acyl chloride during the reaction. Therefore, the library members were dissolved with EtOAc (5 mL) and washed with a 10% $NaHCO_3$ solution. Finally, the products were filtered on sillica gel (3 g) and eluted first with hexanes (10 mL) and then EtOAc (20−30 mL) to recover the desired library members in moderate yields (20−40%) and purity higher than 80% (by TLC and ^1H NMR analysis) for a sampling of library D members **D-1-1**, **D-2-2**, **D-3-3**, **D-4-4**, and **D-5-5** (Table 1). See Figure 6 for the chemical structures of compounds.

D-1-1. 3*R*-Spiro-{3′-[3″-diethylamino-2″-(cyclopropylcarbonyl)propyl]-2′-oxo-oxazolidin-5′-yl}-5α-androstan-17-one. IR (film): 1742 (C=O, ketone, ester and carbamate). ^1H NMR ($CDCl_3$): 0.81 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (25H), 1.02 (t, $J = 6.7$ Hz, 6H, 2 × *CH_3*CH_2), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.2$ Hz, 1H of CH_2-16), 2.57 (m, 7H, 3x CH_2N and CHCO), 3.18−3.37 and 3.47 (3m, 4H, CH_2-3β and CH_2-NCOO), 5.15 (broad s, 1H, CHOCO). LRMS for $C_{32}H_{51}N_2O_5$ $[M + H]^+$: 543.5 *m/z*.

D-2-2. 3*R*-Spiro-{3′-[3″-dipropylamino-2″-(cyclohexylcarbonyl)propyl]-2′-oxo-oxazolidin-5′-yl}-5α-androstan-17-one. IR (film): 1742 (C=O, ketone, ester and carbamate). ^1H NMR ($CDCl_3$): 0.81 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.86 (t, $J = 7.3$ Hz, 6H, 2 × *CH_3*CH_2), 0.80−2.15 (35H), 2.40 (m, 8H, 3 × CH_2N, CHCO and 1H of CH_2-16), 3.13, 3.35 and 3.60 (3m, 4H, CH_2N-3β and CH_2NCOO), 5.15 (broad s, 1H, CHOCO). LRMS for $C_{37}H_{61}N_2O_5$ $[M + H]^+$: 613.2 *m/z*.

D-3-3. 3*R*-Spiro-{3′-[3″-*N*-pyrolidine -2″-(3‴-benzoyloxy)-propyl]-2′-oxo-oxazolidin-5′-yl}-5α-androstan-17-one. IR (film): 1738 (C=O, ketone, ester and carbamate). ^1H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 0.80−2.15 (25H), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.2$ Hz, 1H of CH_2-16), 2.69 (broad

s, 4H, 2 × CH_2N), 2.88 (m, 2H, N*CH*$_2$−CHO), 3.25 and 3.38 (2d of AB system, 2H, CH_2N-3β), 3.50 and 3.70 (2m, 2H, CH_2-NCOO), 5.47 (broad s, 1H, CHOCO), 7.45 (m, 2H, CH aromatic), 7.58 (m, 1H, CH aromatic), 8.03 (d, $J = 7.5$ Hz, 2H, CH aromatic). LRMS for $C_{35}H_{49}N_2O_5$ [M + H]$^+$: 577.4 *m*/*z*.

D-4-4. 3*R*-Spiro-{3′-[3′′-*N*-piperidine- 2′′-(3′′′-cyclopentylpropionyloxy)propyl]-2′-oxo-oxazolidin-5′-yl) }-5α-androstan-17-one. IR (film): 1739 (C═O, ketone, ester and carbamate). ^{1}H NMR ($CDCl_3$): 0.81 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.15 (38H), 2.32 (t, $J = 7.7$ Hz, 2H, CH_2CO), 2.42 (m, 7H, 3 × CH_2N and 1H of CH_2-16), 3.17, 3.35 and 3.55 (3m, 4H, CH_2N-3β and CH_2NCOO), 5.20 (broad s, 1H, CHOCO). LRMS for $C_{37}H_{59}N_2O_5$ [M + H]$^+$: 611.1 *m*/*z*.

D-5-5. 3*R*-Spiro-{3′-[3′′-*N*-morpholino-2′′-(4′′′-*tert*-butyl-benzoyloxy)propyl]-2′-oxo-oxazolidin-5′-yl)}-5α-androstan-17-one. IR (film): 1738 (C═O, ketone, ester and carbamate). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 1.33 (s, 9H, $(CH_3)_3C$), 0.80−2.15 (21H), 2.42 (dd, $J_1 = 8.7$ Hz, $J_2 = 19.2$ Hz, 1H of CH_2-16), 2.55 (m, 6H, 3 × CH_2N), 3.20, 3.40 and 3.70 (3m, 4H, CH_2N-3β and CH_2NCOO), 3.66 (broad s, 4H, 2 × CH_2O), 5.41 (broad s, 1H, CHOCO), 7.46 (d, $J = 7.7$ Hz, 2H, CH aromatic), 7.93 (d, $J = 8.3$ Hz, 2H, CH aromatic). LRMS for $C_{39}H_{57}N_2O_6$ [M + H]$^+$: 649.5 *m*/*z*.

Purification of Selected Type 3 17β-HSD Inhibitors for Libraries A−D. Before, to be used for IC_{50} determination and cell proliferative assay (Table 2), the inhibitors **A-4-11**, **B-1-6**, **C-7-3**, and **D-5-4** were synthesized again, purified by flash chromatography (SiO_2), and characterized.

A-4-11. 3β-[(*N*-Octyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3364 (OH), 1734 (C═O, ketone), 1608 (C═O, amide). ^{1}H NMR ($CDCl_3$): 0.77 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 0.88 (t, $J = 6.2$ Hz, 3H, *CH*$_3$$CH_2$), 0.95−2.20 (39H), 2.42 (dd, $J_1 = 8.5$ Hz, $J_2 = 18.8$ Hz, 1H of CH_2-16), 3.32 (q_{app} of AB system, 2H, CH_2N-3β), 3.47 (m, 2H, CH_2CH_2N), LRMS for $C_{32}H_{54}NO_3$ [M + H]$^+$: 500.7 *m*/*z*. HRMS calcd for $C_{32}H_{54}NO_3$ [M + H]$^+$, 500.4103; found, 500.4090. HPLC (CH_3CN:H_2O:CH_3OH, 35:20:45): 98% of purity.

B-1-6. 3β-[(*N*-Cyclohexylmethyl-*N*-cyclopropylcarbonyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3420 (OH), 1740 (C═O, ketone), 1616 (C═O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 0.80−2.20 (37H), 2.42 (dd, $J_1 = 8.6$ Hz, $J_2 = 19.2$ Hz, 1H of CH_2-16), 3.35 (m, 4H, CH_2N-3β and CH_2N), 4.65 (s, OH). LRMS for $C_{31}H_{50}NO_3$ [M + H]$^+$: 484.7 *m*/*z*. HRMS calcd for $C_{31}H_{50}NO_3$ [M + H]$^+$, 484.3791; found, 484.3781. HPLC (CH_3CN:H_2O:CH_3OH, 40:20:40): 99% of purity.

C-7-3. 3β-[(*N*-Adamantylmethyl-*N*-butanoyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one. IR (film): 3392 (OH), 1734 (C═O, ketone), 1616 (C═O, amide). ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.96 (t, $J = 7.3$ Hz, 3H, *CH*$_3$$CH_2$), 1.00−2.20 (38H), 2.40 (m, 3H, CH_2*CH*$_2$CO and 1H of CH_2-16), 3.11 (s, 2H, CH_2N), 3.47 (s, 2H, CH_2N-3β), 4.82 (s, OH). LRMS for $C_{35}H_{56}NO_3$ [M + H]$^+$: 538.6 *m*/*z*. HRMS calcd for $C_{35}H_{56}NO_3$ [M + H]$^+$, 538.4260; found, 484.4265. HPLC (H_2O containing 0.1% TFA): 95% of purity.

D-5-4. 3*R*-Spiro-{3′-[3′′-*N*-morpholino-2′′-(3′′′-cyclopentylpropionyloxy)propyl]-2′-oxo-oxazolidin-5′-yl)}-5α-androstan-17-one. IR (film): 1738 (C═O, ketone, ester and carbamate). ^{1}H NMR ($CDCl_3$): 0.81 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 0.80−2.20 (33H), 2.33 (t, $J = 7.5$ Hz, 2H, CH_2CO), 2.46 (m, 7H, 3 × CH_2N and 1H of CH_2-16), 3.18, 3.35 and 3.56 (3m, 4H, CH_2N-3β and $CHCH_2N$), 3.66 (broad s, 4H, CH_2O), 5.19 (m, 1H, CHOCO). LRMS for $C_{36}H_{57}N_2O_6$ [M + H]$^+$: 613.2.4 *m*/*z*. HPLC (CH_3CN:H_2O:CH_3OH, 55:25:20): 90% of purity. HRMS calcd for $C_{36}H_{58}N_2O_6$ [M + H]$^+$, 613.4217; found, 613.4246.

Synthesis of Compound 20 (Reported in Table 2). Compound **9** was submitted to aminolysis using 1-adamantylmethylamine and then to an acylation reaction with valeryl chloride under the conditions reported above for method II, except that the dioxolane group was not hydrolyzed (Scheme 2). The tertiary amide **11** with a C17-dioxolane group was then reduced using a solution of BH_3 (1.0 M) in THF followed by the removal of the dioxolane group (acetone/HCl 3M, 1:1) to give the desired 3β-[*N*-1-adamantylmethyl-*N*-butyl)aminomethyl]-3α-hydroxy-5α-androstan-17-one (**20**). IR (film): 3523 (OH), 1737 (C═O, ketone). ^{1}H NMR ($CDCl_3$): 0.74 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 0.89 (t, $J = 7.2$ Hz, 3H, *CH*$_3$$CH_2$), 0.80−2.15 (41H), 2.17 (s, 2H, CH_2N), 2.34 (s, 2H, CH_2N), 2.42 (m, 3H, CH_2N and 1H of CH_2-16), 4.09 (s, OH). ^{13}C NMR ($CDCl_3$): 11.2, 13.8, 14.1, 20.2, 20.5, 21.7, 28.4 (4×), 29.8, 30.7, 31.6, 34.3 (2×), 34.7, 35.0, 35.6, 35.8, 37.0 (2×), 41.1, 41.2, 41.7- (4×), 47.8, 51.4, 54.3, 59.3, 69.0, 69.7, 71.2, 221.4. LRMS for $C_{35}H_{58}NO_2$ [M + H]$^+$: 524.4 *m*/*z*. HRMS calcd for $C_{35}H_{58}NO_2$ [M + H]$^+$, 524.4468; found, 524.4473.

Inhibition of Type 3 17β-HSD (Screening of Libraries A−D) and IC_{50} Determination. Preparation of the Enzymatic Source. The expression vectors encoding for type 3 17β-HSD were transfected into HEK-293 cells using the calcium phosphate procedure.[2] Cells were then sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM of ethylenediaminetetraacetic acid (EDTA), and centrifugated at 10 000*g* for 1 h to remove the mitochondria, plasma membranes, and cell fragments. The supernatant was further centrifugated at 100 000*g* to separate the microsomal fraction, which was used as the source of type 3 17β-HSD activity for the enzymatic assay.

Enzymatic Assay. The inhibition test was carried out at 37 °C in 1 mL of 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM of EDTA, 5 mM of cofactor (NADPH), 0.05 μM [4-^{14}C] 4-androstene-3-17-dione (New England Nuclear, Boston, MA), and the indicated concentration of compounds to be tested. The reaction was stopped after 2 h by adding 2 mL of diethyl ether containing 10 μM of unlabeled 4-androstene-3-17-dione (Δ^4-dione) and T. The metabolites were extracted twice with 2 mL of diethyl ether, evaporated, and then dissolved in CH_2Cl_2 before being applied on silica gel 60 TLCs. TLC plates were developed in a mixture of toluene and acetone (4:1). Substrate [^{14}C] Δ^4-dione and metabolite [^{14}C] T were identified by comparison with reference steroids and revealed by autoradiography and then quantified using the PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The percentage of transformation and the percentage of inhibition were calculated from eqs 1 and 2, respectively.

$$\%\ \text{transformation} = ([^{14}\text{C}]\text{-T}/([^{14}\text{C}]\text{-T} + [^{14}\text{C}]\text{-}\Delta^4\text{-dione})) \times 100 \quad (1)$$

$$\%\ \text{inhibition} = [(\%\ \text{transformation without inhibitor} - \%\ \text{transformation with inhibitor})/\%\ \text{transformation without inhibitor}] \times 100 \quad (2)$$

The IC_{50} value, the concentration of inhibitor that gives 50% of enzymatic inhibition, was calculated by a computer (DE_{50} program, CHUL Research Center, Québec, Canada).

Proliferative Activity on Shionogi (AR$^+$) Mammary Cells. Assays on the proliferation of AR$^+$ Shionogi mammary carcinoma cells were carried out according to the procedure previously described by Sam et al.[41] Two concentrations of tested compound (0.1 and 1 μM) were used in the assay. The results were expressed as the percentage (%) of proliferation, as compared to the stimulation induced by 0.3 nM of the androgen DHT (100%).

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