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Chemical synthesis and biological activities of 16α-derivatives of 5α-androstane-3α,17β-diol as antiandrogens

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Abstract—In our efforts to develop compounds with therapeutic potential as antiandrogens, we synthesized a series of 5α-androstane-3α,17β-diol derivatives with a fixed side-chain length of 3-methylenes at C-16α, but bearing a diversity of functional groups at the end. Among these, the chloride induced the best antiproliferative activity on androgen-sensitive Shionogi cells. Substituting the OH at C-3 by a methoxy group showed the importance of the OH. Moreover, its transformation into a ketone increased the androgen receptor (AR) binding but decreased the antiproliferative activity and induced a proliferative effect on Shionogi cells. These results confirm the importance of keeping a 5α-androstane-3α,17β-diol nucleus instead of a dihydrotestosterone nucleus. Variable side-chain lengths of 2-, 3-, 4-, and 6-methylenes at C-16α were investigated and the optimal length was found to be 3-methylenes. Although exhibiting a weak AR binding affinity, 16α-(3′-chloropropyl)-5α-androstane-3α,17β-diol (**15**) provided an antiproliferative activity on Shionogi cells similar to that of pure non-steroidal antiandrogen hydroxy-flutamide (77% and 67%, respectively, at 0.1 μM). The new steroidal compound, **15**, thus constitutes a good starting point for development of future antiandrogens with a therapeutic potential against prostate cancer.

1. Introduction

Prostate cancer is the most frequently diagnosed cancer in men in the United States, accounting for 33% of all cancers. It is estimated that 232,090 new cases of prostate cancer will be diagnosed and 30,350 men will die from this disease in 2005.[1] Androgens testosterone (T) and dihydrotestosterone (DHT) play an important role in the development, growth, and progression of prostate cancer.[2–5] Androgen receptor (AR) binds the male sex steroids, DHT and T, and regulates genes for male differentiation and development.[6] Therefore, mutations in the AR gene may lead to several diseases or conditions like prostate cancer or the androgen insensitivity syndrome.[7] Since an essential step in the action of androgens in target cells is binding to the receptor, a logical approach for neutralizing the action of androgens is the use of antiandrogens or compounds which prevent the interaction of T and DHT with the AR. Androgen ablation therapy has been shown to produce the most beneficial responses in multiple settings in prostate cancer patients.[8] Several studies have reported that a combination therapy of orchidectomy with an antiandrogen, to inhibit the action of adrenal androgens, significantly prolongs the survival.[7–13] Since prostate cancer is so highly sensitive to androgens, the antiandrogen used must be a compound having high specificity and affinity for the AR while not possessing any androgenic, estrogenic, progestational, glucocorticoid or any other hormonal and antihormonal activities.[14,15]

Since even the best treatment of advanced or metastatic prostate cancer can only prolong life with minimal or no possibility of cure,[9,16–20] it is important to increase the efficiency of known treatments. One of the strategies investigated is to develop a much more potent antiandrogen than flutamide, a compound known to have a weak AR binding affinity.[14] An interesting improvement was the development of selective androgen receptor modulators (SARMs), which required the synthesis of numerous non-steroidal compounds.[21,22] Several steroid scaffolds, such as cyproterone acetate,[23] spironolactone[24] and 4-aza-heterocycle steroid,[25] have also been

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reported as antiandrogens in the past, but their development was less impressive than non-steroidal antiandrogens.

Following a study of the inhibition of the steroidogenic enzyme type 3 17β-hydroxysteroid dehydrogenase,[26] we recently reported an interesting antiandrogenic profile for the synthetic 16α-androstane derivative **1** (16α-(3′-bromopropyl)-5α-androstane-3α, 17β-diol) (Fig. 1). Furthermore, its antiproliferative activity on androgen-sensitive (AR^+) Shionogi cells seems to be mediated through direct interaction with AR as supported by its AR binding affinity. Interestingly, it does not show significant affinities for estrogen, glucocorticoid, and progestin receptors.[26] With the aim of extending this exploratory work, a series of 16α-peptidosteroids represented by structure **2** was synthesized using solid-phase synthesis in parallel fashion.[27] The screening of the generated model libraries revealed interesting preliminary structure–activity relationship (SAR) related to their antiproliferative activities on Shionogi cells, but these activities were weak and not mediated by AR. We then decided to extend our SAR study by focusing more closely on the lead compound **1**. In this article, we report the synthesis of androstane derivatives **3**, which are variously substituted at the end of the C-16-α side-chain of 3-methylenes or differently modified at C-3 to modulate the biological activities. Focusing on two optimal substitutions, another series of 5α-androstane-3α,17β-diol derivatives bearing a bromoalkyl or chloroalkyl chain of variable length at C-16α (compounds **4**) was synthesized. In addition to the chemical synthesis, proliferative and antiproliferative activities on Shionogi cells as well as the binding affinities for AR were determined. A molecular modeling study was also performed in an attempt to analyze interactions between a representative compound and AR.

2. Results and discussion

2.1. Chemical synthesis

Dihydrotestosterone (**5**) was the starting material for the synthesis of all compounds. First, we synthesized steroid precursor **9** through a sequence of reactions described in Scheme 1. The carbonyl group of **5** was protected as ketal and the 17β-hydroxy group of **6** was oxidized with TPAP and NMO to obtain ketone **7**. An alkylation in alpha position of the carbonyl was then performed to give **8**, a mixture of 16α-allyl and 16β-allyl stereoisomers (90/10, evaluated by the ^1H NMR signal of CH_3-18). After purification by chromatography, a mixture of the major isomer α and minor isomer β (2%) was stereoselectively reduced using $LiAlH_4$ at low temperature to afford the secondary alcohol **9**. As previously observed for related compounds,[26,28,29] NMR signals at C-17 confirmed the C-16α and 17β orientation of the allyl and OH groups, respectively.

For the first part of our study, precursor **9** was submitted to an oxidative hydroboration yielding diol **10**. Subsequently, several derivatives having a different functionality at the end of a propyl side-chain were generated using strategies reported in Scheme 2. Chloride **11** was obtained from **10** after ketal hydrolysis and substitution of the hydroxy group using CCl_4 and PPh_3. Under these conditions, the hindered secondary alcohol at the C-17β position was not reactive. Diol **12** was easily obtained after deprotection of **10**. The primary alcohol of **12** was either substituted by an iodide giving **13** or reduced to give triol **14**. We selected potassium tri-*sec*-butyl-borohydride (K-Selectride) as the reducing agent, because this reagent[30] gave mainly the 3α-OH isomer, which was confirmed using ^1H and ^{13}C NMR data reported in the literature.[31,32] Alcohol **14** was substituted

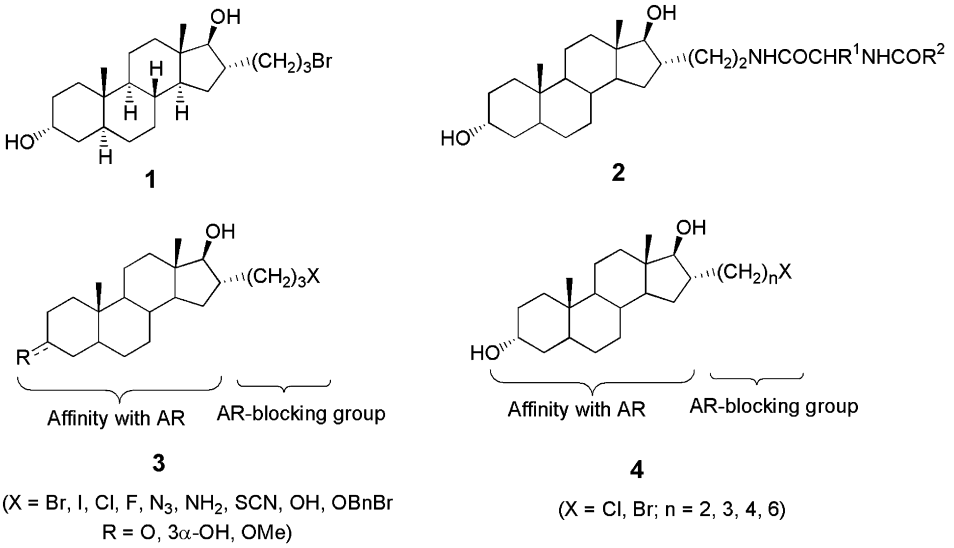
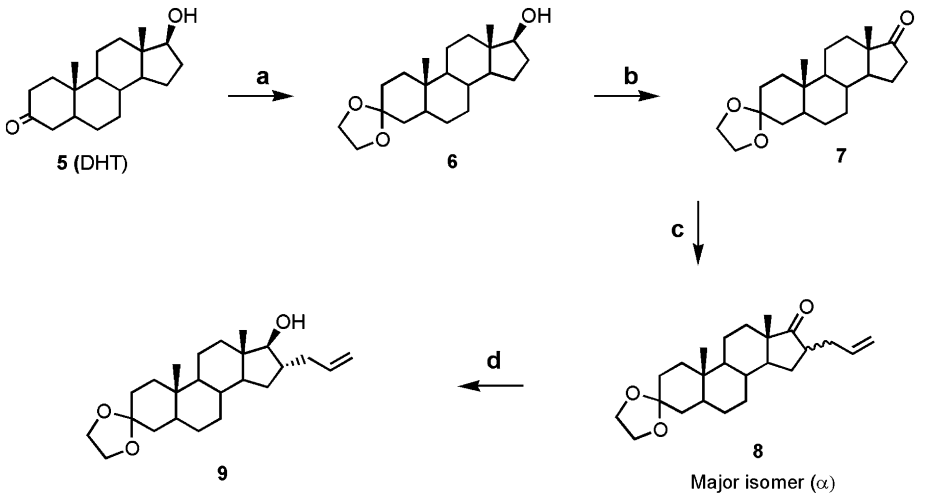
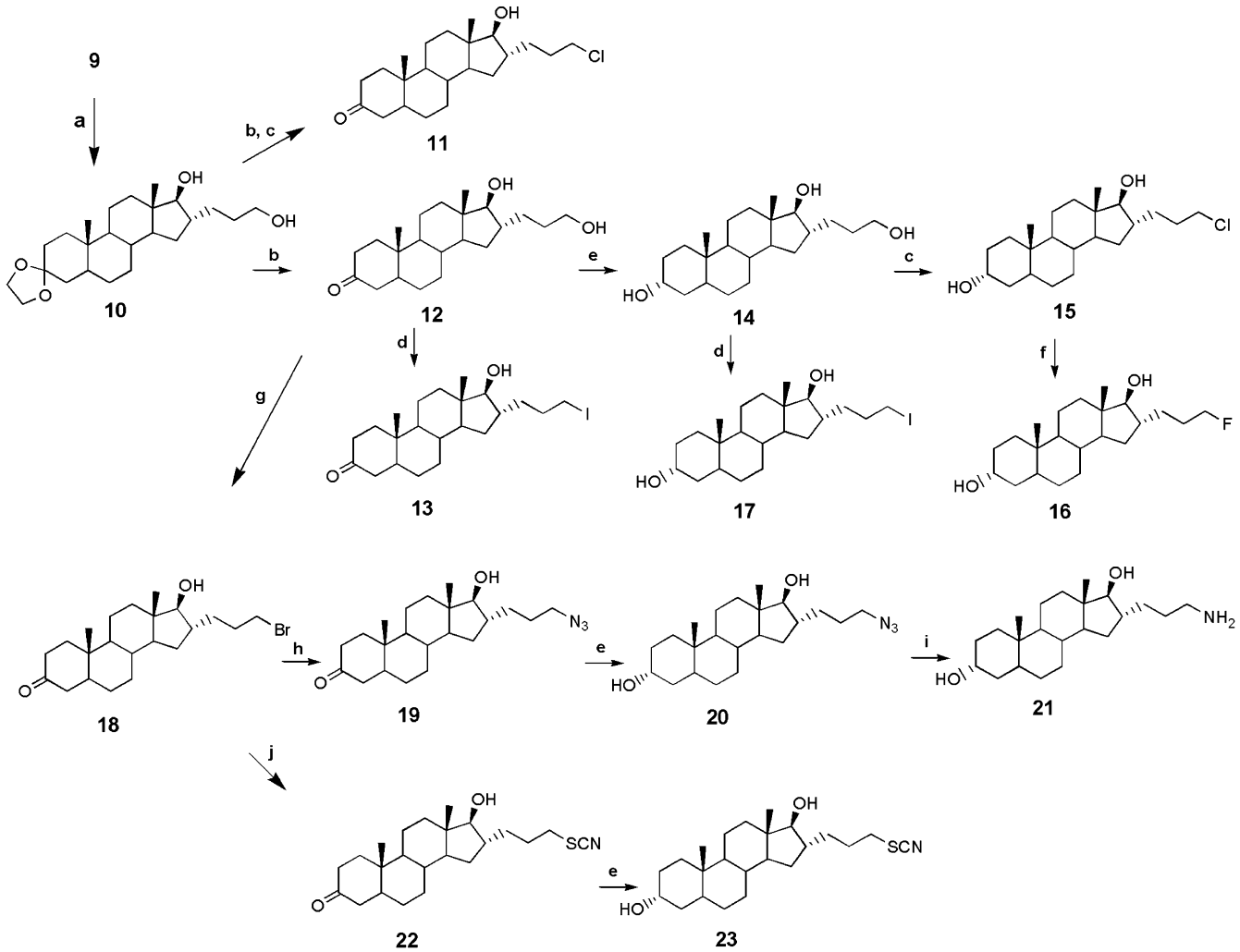


Figure 1. Chemical structures of 5α-androstane-3α,17β-diol derivatives **1** and **2** previously prepared as potential antiandrogens and general structures of analogues **3** and **4** synthesized to extend our SAR study. The stereogenic centers are illustrated only for steroid **1**, but they are the same for all other steroid derivatives reported in this paper.



Scheme 1. Reagents and conditions: (a) $(CH_2OH)_2$, *p*-TSA, benzene, Dean-Stark apparatus, reflux, 24 h; (b) TPAP, NMO, molecular sieves, CH_2Cl_2, rt, 1 h; (c) i—LDA (DIPA, BuLi, THF, 0 °C), ii—$CH_2{=}CHCH_2Br$, THF, −78 to 0 °C, 24 h; (d) $LiAlH_4$, THF, −78 °C, 6 h.



Scheme 2. Reagents and conditions: (a) i—BH_3·THF, 0 °C, 5 h; ii—H_2O_2, NaOAc, H_2O, 0 °C to rt, 3 h; (b) HCl, acetone, rt, 1 or 2 h; (c) PPh_3, CCl_4, CH_2Cl_2, 0 °C to rt, 1 or 2 h; (d) PPh_3, I_2, CH_2Cl_2, imidazole, rt, 2 h; (e) K-Selectride, THF, −78 °C, 1 h; (f) TBAF, THF, reflux, 16 h; (g) PPh_3, CBr_4, CH_2Cl_2, 0 °C to rt, 2 h; (h) NaN_3, CH_3CN, 80 °C, 24 h; (i) Pd/C, MeOH, H_2, 3 h; (j) KSCN, EtOH, reflux, 16 h.

by a chloride to afford **15**, which was substituted by a fluoride to obtain **16**. Iodide **17** was generated directly from alcohol **14**, because it was not possible to reduce **13** into **17**. Indeed, this kind of iodoalkyl is sensitive to K-Selectride reducing conditions. The intermediate compound **12** was also substituted using CBr_4 and PPh_3 to produce bromide **18**. This compound was substituted by an azide to give **19**, which was reduced at the C-3 position giving **20**. Catalytic hydrogenation of azide group provided the free amine **21**. The bromide of **18** was also substituted by KSCN in ethanol to give **22** and this ketone reduced to generate alcohol **23**.

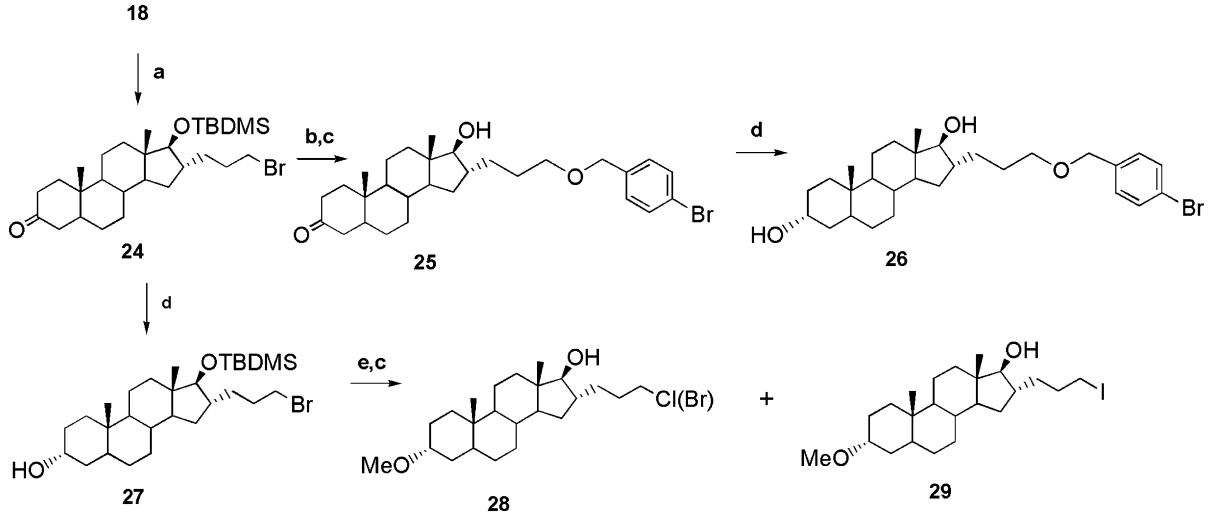
We next tried introducing an aromatic group at the end of the 16α side-chain by preparing compounds **25** and **26** (Scheme 3). The secondary alcohol of **18** was first protected as a *tert*-butyldimethylsilyl ether derivative **24** using TBDMS-OTf and 2,6-lutidine. The bromide of **24** was substituted by a 4-bromobenzyloxy group and the TBDMS intermediate was hydrolyzed to afford **25**, which, after carbonyl reduction with K-Selectride, afforded **26**. The methylation of the OH at position 3 of the steroid was also investigated. To obtain **28**, ketone **24** was reduced and the intermediate **27** was methylated using NaH and MeI. This S_N2 reaction also gives the iodo analogue. The mixture of bromide/chloride and iodo derivatives was then hydrolyzed to remove the TMDBS group, giving two new target compounds **28** and **29**.

For the second part of our study, we needed to prepare compounds with various side-chain lengths at C-16α. The synthesis of the compounds with the two longer side-chains required a Grubbs's metathesis between the key intermediate **9** and alcohols protected or not as benzyl esters (Scheme 4). Using a second-generation ruthenium catalyst[33–35] allowed us to generate in reasonable yields compounds **30** and **31** with 6- and 4-carbon chain, respectively. Chlorides **32** and **33** were generated by the following sequence of reactions: a double bond hydrogenation, a subsequent ester hydrolysis (for **31** only), a ketal hydrolysis, and the substitution of the primary alcohol using CCl_4 and PPh_3. At this point, part of ketones **32** and **33** was reduced at C-3 to give alcohols **34** and **35**. Compounds **38** and **39** bearing a bromoethyl chain were synthesized from **36**, which was available in our laboratory.[27] This precursor was acetylated and the TBDMS protecting group was thereafter removed in a THF solution of HF/pyridine leading to hydroxyl derivative **37**. This alcohol was oxidized using TPAP and NMO, and the acetyl group was removed with potassium carbonate to give **38**. To obtain compound **39**, the TBDMS of precursor **36** was hydrolyzed under acid conditions.

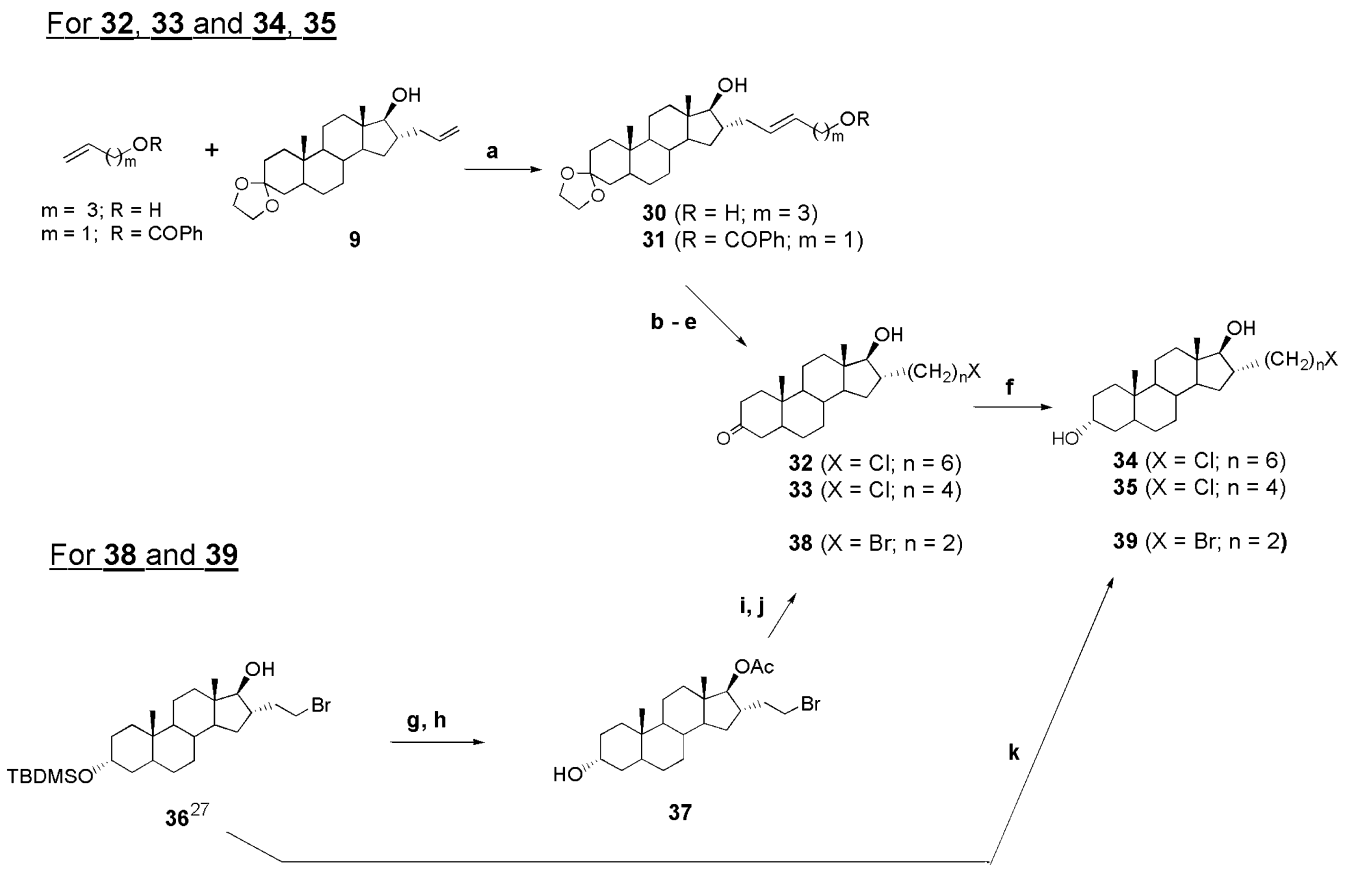
2.2. Biological activity

The antiproliferative activity of synthesized compounds on androgen-sensitive mammary carcinoma Shionogi cells is first reported as the percentage (%) of inhibition (Table 1). It corresponds to the ability of a compound to inhibit the proliferation induced by 0.3 nM of the natural potent androgen DHT. The proliferative activity is reported as the percentage (%) of cell stimulation induced by the tested compounds relative to stimulation (100%) induced by 0.3 nM DHT. Moreover, it is important to determine whether or not these compounds bind to androgen receptor (AR), in order to find out whether the antiproliferative activity is mediated by this receptor.

First of all, substituting the bromide on the side-chain end with a diversity of functional groups leads to interesting SAR information. Clearly the substitution of the bromide atom of **1** by a more polar group such as OH, NH_2, and SCN (compounds **14**, **21**, and **23**) is detrimental to the antiproliferative activity. The same result is also observed with a very bulky group such as the bromobenzyloxy of **26**. Among the series of halogeno derivatives **1** and **15**–**17**, chloride **15** induced the higher antiproliferative activity at 0.1 μM. Although halogens are good leaving groups, the results obtained with **1**



Scheme 3. Reagents and conditions: (a) i—2,6-lutidine, TBDMS-OTf, CH_2Cl_2, −78 °C, 3 h; ii—HCl (10% aq); (b) AgOTf, 4-bromobenzyl alcohol, 2,6-di-*tert*-butyl-4-methyl-pyridine, CH_2Cl_2, 0 °C to rt, 24 h; (c) HCl, MeOH, CH_2Cl_2, rt, 2 h; (d) K-Selectride, THF, −78 °C, 1 or 2 h; (e) NaH, MeI, 15-crown-5, THF, 0 °C to rt, 24 h.



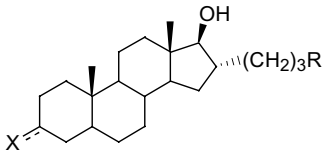
Scheme 4. Reagents and conditions: (a) Grubbs's catalyst, CH_2Cl_2, reflux, 24 h; (b) H_2, Pd/C, EtOAc, rt, 24 h; (c) NaOH (10% aq), MeOH, rt, 1.5 h; (d) HCl, acetone, rt, 1 or 2 h; (e) PPh_3, CCl_4, CH_2Cl_2, 0 °C to rt, 1 h; (f) K-Selectride, THF, −78 °C, 1 h; (g) Ac_2O, pyridine, DMAP, rt, 2 h; (h) HF/pyridine, THF, rt, 60 h; (i) TPAP, NMO, molecular sieves, CH_2Cl_2, rt, 2 h; (j) K_2CO_3, MeOH, reflux, 2 h; (k) HCl, MeOH, rt, 2 h.

and **15**–**17** suggest that these compounds do not work by formation of a covalent bond with AR or another target. Like chloride **15**, azide **20** also showed a good antiproliferative activity at 0.1 μM. Indeed, the mean result of three experiments with **15** (67 ± 5%) is not significantly different from that obtained with **20** (58 ± 9%). Among all these new 3α-OH-steroids, none produced proliferative activity at 0.1 μM, except compound **26**. The binding affinity of all these 3α-OH derivatives for AR was also evaluated but none showed an important affinity (1–17% at 0.1 μM and 4–53% at 1 μM).

In the light of these results, we decided to evaluate the importance of the 3α-OH group of these new derivatives by performing two modifications. First, substituting the 3α-alcohol by a methoxy group (**28** and **29**) induced a drop of antiproliferative activity and binding affinity for AR. Second, with the aim of increasing the antagonistic effect and AR binding affinity, we prepared and tested a series of ketones. As expected, the presence of a carbonyl at C3 greatly improved the AR binding. These better affinities were however associated with a proliferative activity at 0.1 μM ranging from 9% to 153% (100% for DHT at 0.3 nM). The increase of binding and proliferative activity was the least important for alcohol **12** but remarkable for 3-keto-steroid **25**. In fact, the proliferative activity of **25** (153% at 0.1 μM) was similar to that of **26** (163%), the 3α-OH analogue. Compared to that of **25** or DHT, the proliferative effects of ketones **11**, **13**, **19**, and **22** were weaker (21–36% at 0.1 μM), but are representative of compounds with both agonist and antagonist activities. Indeed, this conclusion is well-illustrated on Figure 2 where the antiproliferative and proliferative activities of ketone **11** and hydroxy-flutamide (OH-Flu),[36–38] expressed as cell DNA content, are reported at different concentrations. Thus, the pure antiandrogen OH-Flu inhibits the proliferation of Shionogi (AR^+) cells induced by DHT (OH-Flu + DHT) in a concentration-dependent fashion, up to 100% at the higher concentrations, but does not stimulate the cell proliferation when tested alone (OH-Flu) at any concentration. On the other hand, ketone **11** only partially inhibits the cell proliferation induced by DHT (**11** + DHT), down to ~40% at the highest concentration, but clearly stimulates cell proliferation when tested alone (up to ~52% at 1 μM). As a result, the two curves corresponding to antiproliferative (**11** + DHT) and proliferative (**11** only) activities converge to a same level of cell growth, indicating mixed activities. Thus, the presence of a carbonyl group at position 3 of the androstane derivatives leads to a compound with mixed androgenic and antiandrogenic activities, confirming the importance of keeping the 3α-OH.

After we determined that a 3α-OH and a Cl atom at the end of the 16α side-chain formed an interesting combination, we wanted to find the optimal side-chain length (2-, 3-, 4- or 6-methylenes), the one that induces the best

Table 1. Antiproliferative activity, proliferative activity, and AR binding affinity



Compound	X	R	Antiproliferative activity (%)[a]		Proliferative activity (%)[b] (0.1 μM)	AR binding[c]	
			0.1 μM	1 μM		0.1 μM	1 μM
1	3α-OH	Br	42 (45;51) [46 ± 3]	88 (68;83) [80 ± 6]	0	25	68
14	3α-OH	OH	21 (14;14) [16 ± 2]	45 (41;19) [35 ± 8]	0	3	4
15	3α-OH	Cl	77 (64;61) [67 ± 5]	97 (91;93) [94 ± 2]	0	5	36
16	3α-OH	F	43 (49;41) [44 ± 2]	76 (79;55) [70 ± 8]	0	1	12
17	3α-OH	I	39 (28;42) [36 ± 4]	96 (87;86) [90 ± 3]	0	17	53
20	3α-OH	N_3	60 (41;73) [58 ± 9]	93 (86;89) [89 ± 2]	0	8	33
21	3α-OH	NH_2	0	0	1	2	4
23	3α-OH	SCN	2	50	5	11	38
26	3α-OH	OCH_2PhBr	0	7	163	10	34
28	3α-OMe	Cl (Br)	18	99	0	5	11
29	3α-OMe	I	0	46	0	3	9
11	O	Cl	44	42	36	93	99
12	O	OH	54	46	9	17	66
13	O	I	41	76	22	95	99
19	O	N_3	73	58	33	92	99
22	O	SCN	0	45	21	90	98
25	O	OCH_2PhBr	0	0	153	95	99
OH-Flu[d]			67	100	0	5	33
R1881[e]			—	—	—	97	100
T[f]			—	—	—	72	95
DHT[g]			—	—	100	94	95

[a]Percentage of antiproliferative activity on Shionogi (AR^+) cells at 0.1 and 1 μM of tested compounds. The cells were stimulated with 0.3 nM DHT. SEM ⩽ 2.7% for the first value, representing an experiment performed in triplicate. Data in parentheses are from two other experiments performed 3 years later in triplicate (SEM ⩽ 3.3%). Data in [ ] represent means ± SEM of the three experiments.
[b]Percentage of proliferative activity on Shionogi (AR^+) cells at 0.1 μM of tested compounds. The stimulation induced by 0.3 nM of androgen DHT was set as 100%. SEM ⩽ 3.5% of one experiment in triplicate.
[c]Percentage of binding affinity on human androgen receptor at 0.1 and 1 μM of tested compounds. The binding of R1881 (1 μM) was set as 100%. SEM ⩽ 2.2% of one experiment in triplicate.
[d]Hydroxy-flutamide (pure antiandrogen).
[e]Methyltrienolone (synthetic potent androgen).
[f]Testosterone (natural androgen).
[g]Dihydrotestosterone (natural androgen).

antiproliferative activity (Fig. 3). Compound **15** with a side-chain length of 3-methylenes displayed the greater potency. In fact, at concentrations of 0.1 and 1 μM, this chloride derivative inhibited by 77% and 97%, respectively, the DHT stimulation. The antiproliferative activity was however lower with both a shorter and a longer side-chain. As reported in Figure 4, the pattern of response of the 3α-OH-steroid **15** is comparable to the one obtained with the non-steroidal pure antiandrogen OH-Flu. Thus, **15** inhibits the proliferation of Shionogi (AR^+) cells induced by DHT (**15** + DHT) in a concentration-dependent fashion, up to 100% at the higher concentrations, but does not stimulate the cell proliferation when tested alone (**15** only). Furthermore, these two compounds have a similar binding affinity for AR (33% and 36% at 1 μM for OH-Flu and **15**, respectively).

Since the AR binding affinity of 3α-OH-steroids is low and does not correlate well with the biological results, it is possible that the antiproliferative activity is not the result of an antiandrogenic activity, mediated by AR, but instead an effect not mediated by AR. We then tested ketone **11**, alcohol **15**, OH-Flu, and DHT in the non-androgen-sensitive (AR^-) PC-3 cells. These human prostatic carcinoma cells do not respond to androgens and glucocorticoids, and are useful in assessing their response to chemotherapeutic agents.[39] As shown in Figure 5, the cell growth was not reduced by a treatment with ketone **11**, alcohol **15**, androgen DHT or antiandrogen OH-Flu at concentrations tested. Thus, **11** and **15** are non-cytotoxic compounds in PC-3 (AR^-) cells suggesting that the antiproliferative effect observed in Shionogi (AR^+) cells by **15** is mediated by AR despite its low binding affinity.

2.3. Molecular modeling

In order to understand the antiproliferative activity demonstrated by 16α-(3′-chloropropyl)-5α-androstane-3α, 17β-diol (**15**), we tried to identify the preferred binding position of lead compound **1** within the AR ligand-binding domain (LBD) by modeling and energy

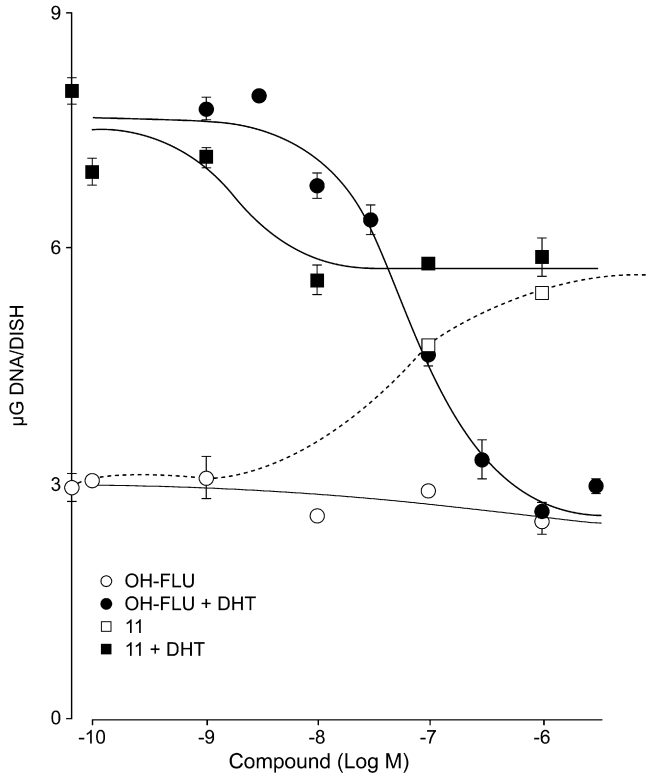


Figure 2. Antiproliferative and proliferative effects induced by the 3-keto-steroid **11** on Shionogi (AR^+) cells. The concentration of DHT is 0.3 nM. The pure antiandrogen OH-Flu was used as a reference compound. Data are expressed as means ± SEM of one experiment in triplicate.

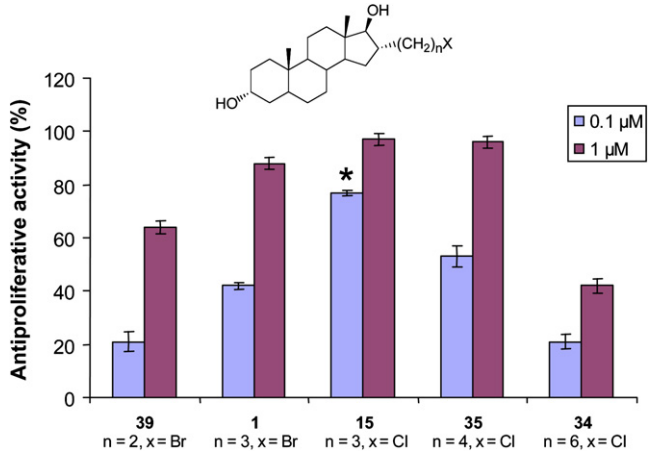


Figure 3. Antiproliferative activity on Shionogi (AR^+) cells as a function of the side-chain length at C-16α. The cells were simultaneously incubated with 0.3 nM DHT. SEM ⩽ 4% of one experiment in triplicate. *Alcohol **15** is significantly different from all other compounds at 0.1 µM ($p < 0.01$).

minimization (Fig. 6). The AR-LBD is mainly composed of α-helices arranged as a three-layered antiparallel α-helical sandwich, a fold common to all steroid receptors.[40] The ligand-binding pocket (LBP) is mainly composed of hydrophobic residues, the side-chains of which can easily adopt variable positions in order to better fit the hydrophobic core of the steroid and stabilize

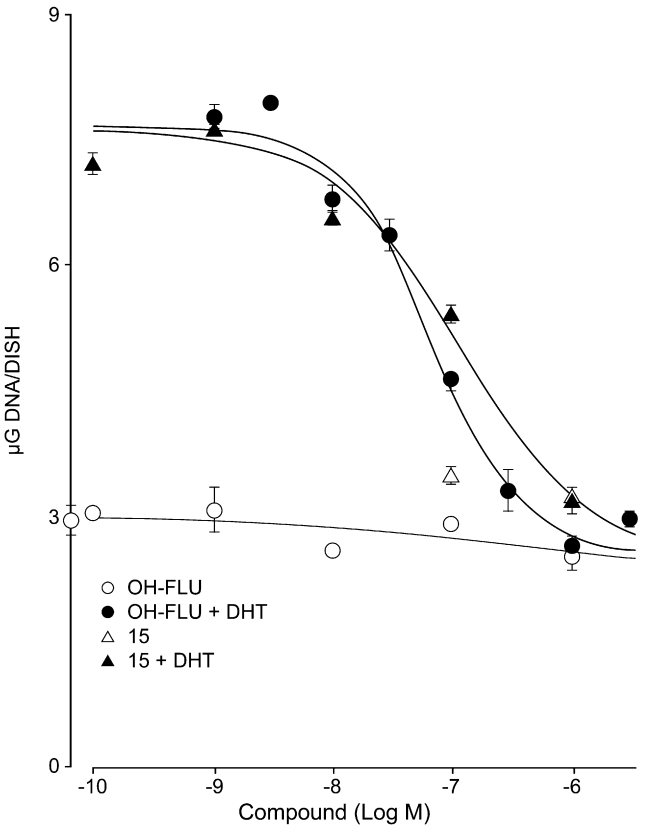


Figure 4. Antiproliferative and proliferative effects induced by the 3α-OH-steroid **15** on Shionogi (AR^+) cells. The concentration of DHT is 0.3 nM. The pure antiandrogen OH-Flu was used as a reference compound. Data are expressed as means ± SEM of one experiment in triplicate.

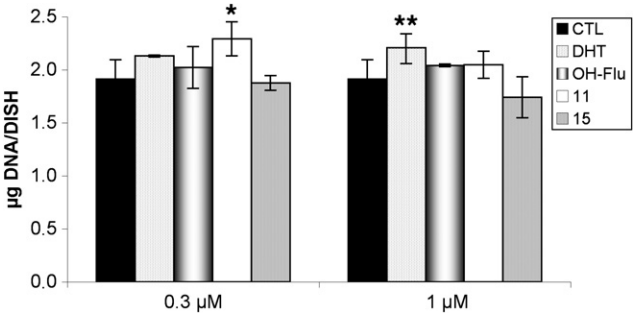


Figure 5. Proliferative effect induced by 3-keto-steroid **11**, 3α-OH-steroid **15**, OH-Flu, and DHT on PC-3 (AR^-) cells. Data are expressed as means ± SEM of one experiment in triplicate. Significantly different from control (CTL): *($p < 0.01$); **($p < 0.05$).

it. When binding an agonist, the top of the LBP is closed by an α-helix structure at the carboxyl-terminal end of the receptor, helix 12 (H12).[7,41–43] A precise positioning of this helix is required for activation of AR[41] and, as shown by crystallographic studies made on the human estrogen receptor, a different positioning of H12 could account for the functional differences between agonists and antagonists.[44] This position shift would prevent coactivators from binding to the receptor. Structural modeling of **1** bound inside the LBP of AR and comparison with crystal structures of AR-LBD in complex with

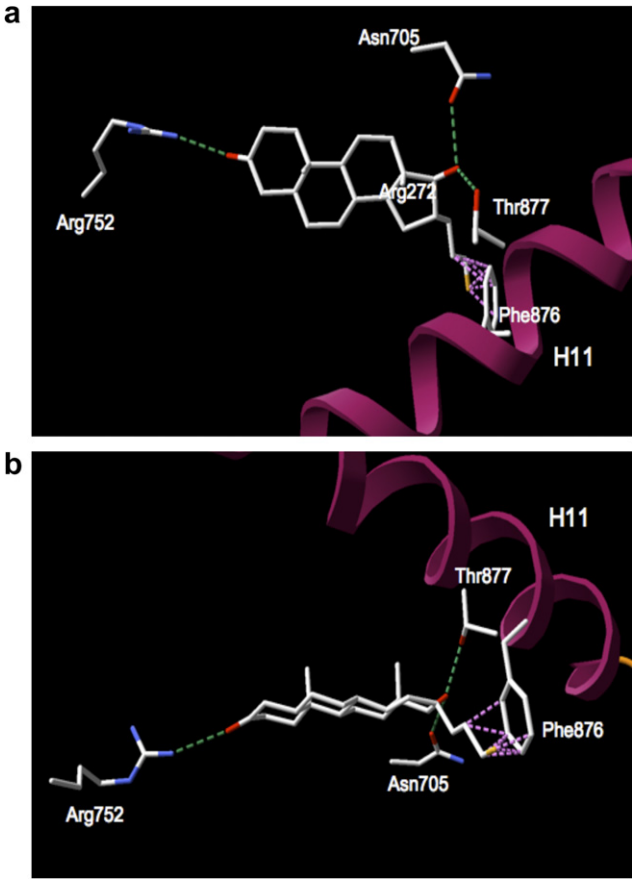


Figure 6. Hypothetical position of 3α-OH-steroid **1** in the human androgen receptor ligand binding site. Schematic views (β-face of the steroid nucleus (a) and about 90° rotated view (b)) of **1** in the optimal binding position. Putative hydrogen bonds established by the ligand with the receptor (residues Arg752, Asn705, and Thr877) are indicated by broken green lines. Possible contacts between the C16α-side-chain of the ligand and the Phe876 residue of helix 11 (see text) are indicated by broken pink lines. Carbon atoms are depicted in white, oxygen atoms in red, nitrogen atoms in blue, and bromine atom in orange. The figures were generated with Swiss-PdbViewer.[46]

agonist ligands, DHT and testosterone,[43] suggest that **1** is well stabilized through numerous van der Waals contacts with hydrophobic residues delineating the LBP and by hydrogen bonds established at both extremities of its steroidal nucleus. Its 16α-(3′-bromopropyl) side-chain is oriented toward the main chain of helix 11 and clashes with the side-chain of one of its residues, Phe876. However, Söderholm et al.[45] reported the presence of a small favored volume for acceptor interactions close to the solvent accessible surface, located near the amide group of residue Leu880 and the carbonyl oxygen of Phe876. The possibility thus exists that this interference with helix 11 has an impact on the positioning of the C-terminal end of the receptor and causes a misplacement of H12 leading to the generation of the antagonist-like conformation of AR. These interactions could explain the antagonistic effect of alcohols **1** and **15**. Moreover, the agonistic effect of the latter's 3-keto analogue, compound **11**, could be explained by a higher AR binding affinity due to the presence of a ketone function at C-3, like in DHT. Since the oxygen atom of this ketone group has a lone pair of electrons, it could act as a hydrogen bond acceptor able to establish a strong interaction with a charged amino acid, Arg752, like DHT does.[43] Thus, the specific interaction involving the C-3 ketone may orient the steroid core similarly to DHT and thus reduce the efficiency of the alkyl chain in repositioning H12.

3. Conclusions

We have synthesized a novel series of antiandrogens by adding a short side-chain at position 16α of a 5α-androstane-3α,17β-diol nucleus. Among the series of functional groups attached at the end of the alkyl side-chain, we found that an azide or halogen best improves the antiproliferative activity on androgen-sensitive (AR^+) Shionogi cells, especially when this element of diversity is a chloride. Moreover, the optimal side-chain length was found to be 3-methylenes. However, all of these 3α-OH-steroids display a weak binding affinity for the AR. We also showed the importance of a 3α-hydroxy group since its replacement by a methoxy decreases biological activity. Moreover, the presence of a ketone at position 3 brought a better binding to AR, while unfortunately inducing proliferation of Shionogi (AR^+) cells. These opposite effects certainly explain the poor antiandrogenic activity of these 3-keto-steroids at higher concentrations. Clearly, the 3α-OH-steroids and the 3-keto-steroids work differently. The latter have a good AR binding affinity, but work as mixed antagonist/agonist compounds, whereas the former have a low AR binding affinity and a profile of activity similar to that of the non-steroidal antiandrogen OH-Flu. Among the compounds tested, the 3α-OH-steroid **15** with a chloropropyl 16α-side-chain displays the highest antiandrogenic activity on Shionogi cells. Its inhibition of DHT-induced cell stimulation is comparable to that obtained with the non-steroidal antiandrogen OH-Flu. Furthermore, compound **15** shows no proliferative agonistic activity on Shionogi (AR^+) cells and does not reduce the proliferation of PC-3 (AR^-) cells.

Based on modeling studies we tried to explain how compound **1** antagonizes the AR. It was proposed that the bromide of the 16α chain of the steroid likely makes contacts with residues of helix 11, leading to a different positioning of helix 12 and thus preventing coactivators to bind to the receptor. Additional studies will however be necessary to better understand the mechanism of action of compounds **1** and **15**. Moreover, a better understanding of the key interactions between **15** and AR could contribute to the design of a second generation of this new family of antiandrogens with improved biological activity.

4. Experimental

4.1. Chemical synthesis

4.1.1. General. Dihydrotestosterone (DHT) was obtained from Steraloids (Newport, RI, USA) and

chemical reagents were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Nepean, ON, Canada) and VWR International (Montréal, Qc, Canada) and were used as received. Anhydrous solvents were purchased from Aldrich and VWR in SureSeal bottles, which were conserved under positive argon pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. Flash chromatography was performed on Silicycle 60 (Québec, Qc, Canada) 230–400 mesh silica gel. Thin-layer chromatography (TLC) was performed on 0.25 mm silica gel 60 F_{254} plates (Whatman, Madison, UK) and compounds were visualized by exposure to UV light (254 nm) or a heated solution of ammonium/sulfuric acid/water. Infrared (IR) spectra were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only the significant bands reported in cm^{-1}. ^1H and ^{13}C spectra were recorded with a Brucker AC/F300 spectrometer (Billerica, MA, USA) at 300 and 75.5 MHz, respectively, and a Bruker AVANCE 400 spectrometer at 400 (^1H) and 100.6 (^{13}C) MHz. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 and 77.16 ppm), acetone (2.05 and 206.26 ppm) or methanol (3.31 and 49.0 ppm) for ^1H and ^{13}C, respectively. Low-resolution mass spectra (LRMS) were recorded with an LCQ Finnigan apparatus (San Jose, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) source on positive mode. High-resolution mass spectra (HRMS) were provided by the Regional Laboratory for Instrumental Analysis (Université de Montréal, Montréal, Qc, Canada). High-performance liquid chromatography (HPLC) analyses were carried out using a Waters Associates System (Waters 600E Pump, Waters 717 plus Autosampler, Waters 996 Photodiode Array Detector: 200-400 nm) (Milford, MA, USA), a Nova-Pack C18 column (150 × 3.9 mm, 4 μm, 60 Å), and a solution of MeOH containing 20 mM $AcONH_4$ as eluent (1 mL/min flow rate). The analysis was only possible for a compound having a ketone, an iodide, an azide or a phenyl group.

4.1.2. Synthesis of 3-dioxolane-5α-androstan-17β-ol (6).[47] To a solution of DHT (**5**) (5.1 g, 17.7 mmol) in dry benzene (80 mL) were added under an argon atmosphere ethylene glycol in excess (29.6 mL, 530 mmol) and *p*-TSA (33.6 mg, 0.177 mmol). The mixture was refluxed for 24 h using a Dean–Stark/water separator. The solution was quenched by the addition of saturated aqueous $NaHCO_3$ and concentrated under vacuum. The product was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and evaporated to dryness. Flash chromatography (hexanes/EtOAc, 95:5) afforded **6** as a white solid (5.2 g, 88%). IR (film) ν = 3384 (OH, alcohol); ^1H NMR (400 MHz, $CDCl_3$) δ = 0.73 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.85–2.15 (m, 23H), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.94 (s, OCH_2CH_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.3, 11.6, 20.9, 22,8, 23.5, 28.6, 30.7, 31.3, 31.6, 35.7, 36.2, 36.9, 38.1, 43.1, 43.9, 51.1, 54.3, 64.1 (2×), 82.0, 109.3; LRMS for $C_{21}H_{35}O_3$ $[MH]^+$: 335.2 *m*/*z*.

4.1.3. Synthesis of 3-dioxolane-5α-androstan-17-one (7). To a solution of alcohol **6** (5.2 g, 15.6 mmol) in dry CH_2Cl_2 (70mL) under argon were added molecular sieves (4 Å) (7.8 g) and 4-methylmorpholine-*N*-oxide (2.7 g, 23.3 mmol) and the mixture was stirred for 15 min at rt. Tetrapropylammonium perruthenate (273 mg, 0.8 mmol) was added and the solution was stirred for 1 h. The resulting mixture was filtered on silica gel column, using hexanes/acetone (80:20) as eluent to give 5.1 g (98%) of ketone **7**. IR (film) ν = 1740 (C=O, ketone); ^1H NMR (400 MHz, $CDCl_3$) δ = 0.83 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70–2.00 (m, 20 H), 2.07 (m, 16α-CH), 2.43 (dd, J_1 = 8.8 Hz, J_2 = 19.2, 16β-CH), 3.93 (s, OCH_2CH_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.5, 14.0, 20.6, 21.9, 28.4, 30.9, 31.2, 31.7, 35.2, 35.8, 36.0, 36.1, 38.1, 43.8, 47.9, 51.6, 54.3, 64.3 (2×), 109.4, 221.5; LRMS for $C_{21}H_{33}O_3$ $[MH]^+$: 333.1 *m*/*z*.

4.1.4. Synthesis of 3-dioxolane-16α-allyl-5α-androstan-17-one (8). A solution of diisopropylamine (2.35 mL, 16.8 mmol) in dry THF was stirred under argon at 0 °C and a 2.4-M solution of butyllithium in hexanes (7.0 mL, 16.8 mmol) was added dropwise. After 30 min, ketone **7** (5.1 g, 15.3 mmol) dissolved in dry THF was added dropwise in the resulting lithium diisopropylamine (LDA) solution. This mixture was allowed to stir for 30 min at 0 °C, then cooled at −78 °C and allyl bromide (1.45 mL, 16.8 mmol) was added dropwise. The reaction mixture was stirred for 24 h from −78 to 0 °C. Water was added to quench the reaction and the crude product was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 92:8) afforded the major 16α-isomer **8** containing only 1.5% of minor 16β-isomer (2.2 g, 38%) and starting material (60%). IR (film) ν = 1740 (C=O, ketone); ^1H NMR (400 MHz, $CDCl_3$) δ = 0.83 (s, 18-CH_3), 0.90 (s, 19-CH_3), 0.70–1.85 (m, 20H), 2.05 (m, 1 H of CH_2-1′), 2.50 (m, 16β-OH and 1 H of CH_2-1′), 3.93 (s, OCH_2-CH_2O), 5.03 (m, CH_2-3′), 5.77 (m, CH-2′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.5, 14.7, 20.5, 26.9, 28.4, 30.8, 31.2, 31.7, 35.1, 35.2, 35.8, 36.1, 38.0, 43.7, 44.2, 48.6, 49.1, 54.3, 64.3 (2×), 109.4, 116.5, 136.6, 222.0; LRMS for $C_{24}H_{37}O_3$ $[MH]^+$: 373.1 *m*/*z*.

4.1.5. Synthesis of 3-dioxolane-16α-allyl-5α-androstan-17β-ol (9). A solution of ketone **8** (2.2 g, 5.8 mmol) in dry THF (50 mL) and $LiAlH_4$ (329.4 mg, 8.7 mmol) was stirred under argon at −78 °C. After 10 h, the reaction was quenched by the addition of water and 15% aqueous NaOH solution. Rochelle salt (100 mL, 1 M solution in water) was added and the mixture extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 85:15) yielded 2.0 g (92%) of alcohol **9**. IR (film) ν = 3358 (OH); ^1H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.77 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.60–1.90 (m, 20H), 1.99 and 2.36 (2 m, CH_2-1′), 3.18 (dd, J_1 = 5.6 Hz, J_2 = 7.6 Hz, CH-17α), 3.60 (d, J = 5.4 Hz, 17β-OH), 3.87 (s, OCH_2CH_2O),

4.98 (m, CH_2-3′), 5.85 (m, CH-2′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.6, 12.1, 20.8, 28.6, 30.1, 31.3, 31.6, 35.4, 35.7, 36.1, 36.9, 38.1, 39.8, 43.4, 43.9, 44.0, 49.4, 54.3, 64.3 (2×), 87.6, 109.5, 115.8, 138.1; LRMS for $C_{24}H_{39}O_3$ $[MH]^+$: 375.1 *m/z*.

4.1.6. Synthesis of 3-dioxolane-16α-(3′-hydroxypropyl)-5α-androstan-17β-ol (10). To a stirred solution of alkene **9** (113 mg, 0.30 mmol) in dry THF (15 mL) at 0 °C was added dropwise a 1-M borane solution in THF (1.81 mL, 1.81 mmol). The mixture was allowed to react under argon for 5 h, then a 1-N aqueous NaOAc solution (1.81 mL) and H_2O_2 (33% w/v, 0.05 mL) were added and the resulting mixture was stirred at rt for 1 h. The reaction was quenched by addition of water, extracted with EtOAc, and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (70:30) as eluent to give diol **10** (86 mg, 73%). IR (film) ν = 3256 (OH); ^1H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.67–1.79 (m, 25H), 3.12 (dd, J_1 = 5.4 Hz, J_2 = 7.5 Hz, CH-17α), 3.41 (t, J = 5.2 Hz, CH_2-*OH*), 3.53 (dd, J_1 = 1.3 Hz, J_2 = 4.9 Hz, *CH_2*-OH), 3.58 (d, J = 5.3 Hz, 17β-OH), 3.86 (m, OCH_2CH_2O); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ = 11.9, 12.7, 21.6, ~30 (under solvent), 31.4, 32.1, 32.5, 32.8, 33.2, 36.3, 37.0, 38.0, 38.9, 44.0, 44.6, 44.9, 50.4, 55.6, 63.0, 64.8 (2×), 88.4, 109.6; LRMS for $C_{24}H_{41}O_4$ $[MH]^+$: 393.0 *m/z*.

4.1.7. Synthesis of 16α-(3′-chloropropyl)-17β-hydroxy-5α-androstan-3-one (11). Diol **10** (79 mg, 0.20 mmol) was dissolved in acetone (10 mL) and aqueous (10% v/v) HCl (7 mL) was slowly added with a syringe, and the reaction mixture was stirred for 2 h at rt. An aqueous saturated $NaHCO_3$ solution was added and the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and solvent evaporated under reduced pressure. This crude keto diol, PPh_3 (62 mg, 0.40 mmol), and CCl_4 (133 mg, 0.40 mmol) in dry CH_2Cl_2 (15 mL) were added at 0 °C and stirred at rt under argon for 2 h. The reaction mixture was quenched by addition of water, extracted with CH_2Cl_2, and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/acetone (80:20) as eluent to yield chloride **11** (36 mg, 49% for two steps). IR (film) ν = 3436 (OH), 1708 (C=O); ^1H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.70–2.60 (m, 25H), 3.16 (dd, J_1 = 5.8 Hz, J_2 = 7.3 Hz, CH-17α), 3.61 (t, J = 6.7 Hz, CH_2–Cl), 3.67 (d, J = 5.5 Hz, OH); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.7, 12.6, 21.7, 30.9, 31.3, 32.2, 32.5, 33.9, 36.2, 36.6, 37.8, 38.6, 39.4, 43.4, 44.9, 45.2, 46.2, 47.7, 50.2, 55.0, 88.2, 210.2; LRMS for $C_{22}H_{35}O_2$ $[M-Cl]^+$: 331.3 *m/z*; HRMS: calcd for $C_{22}H_{36}O_2Cl$ $[MH]^+$ 367.23983, found 367.23998; HPLC purity of 95% (*RT* = 31.7 min).

4.1.8. Synthesis of 16α-(3′-hydroxypropyl)-17β-hydroxy-5α-androstan-3-one (12). Diol **10** (994 mg, 2.5 mmol) was dissolved in acetone (100 mL). Aqueous (10% v/v) HCl (10 mL) was slowly added with a syringe and the reaction mixture was stirred for 2 h at rt. An aqueous saturated $NaHCO_3$ solution was then added and the reaction mixture was extracted with EtOAc. The organic phase was dried over $MgSO_4$ and solvent evaporated under reduced pressure. The solution was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the crude product was purified by flash chromatography with hexanes/EtOAc (80:20) as eluent to yield diol **12** (688 mg, 70%). IR (film) ν = 3396 and 3223 (OH), 1707 (C=O); ^1H NMR (400 MHz, CD_3OD) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.75–2.60 (m, 25H), 3.13 (d, J = 7.7 Hz, CH-17α), 3.55 (dd, J_1 = 5.6 Hz, J_2 = 6.6 Hz, *CH_2*-OH); ^{13}C NMR (75.5 MHz, CD_3OD) δ = 11.7, 12.6, 22.0, 30.0, 31.4, 32.5, 33.1, 36.6, 36.9, 38.1, 38.9, 39.8, 43.8, 45.1, 45.5, ~49 (under solvent peaks), 50.4, 55.4, 63.2, 88.8, 214.8; LRMS for $C_{22}H_{37}O_3$ $[MH]^+$: 349.1 *m/z*; HRMS: calcd for $C_{22}H_{36}O_3$ + Na $[M+Na]^+$ 371.25567, found 371.25554; HPLC purity of 99% (*RT* = 27.8 min).

4.1.9. Synthesis of 16α-(3′-iodopropyl)-17β-hydroxy-5α-androstan-3-one (13). Diol **12** (85 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and PPh_3 (95 mg, 0.36 mmol). I_2 (92 mg, 0.36 mmol) and imidazole (43 mg, 0.63 mmol) were added and the solution was stirred at rt under argon for 2 h. The mixture was quenched by addition of water, extracted with CH_2Cl_2, and the organic phase dried over $MgSO_4$. After evaporation of solvent under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (80:20) as eluent to yield iodide **13** (61 mg, 55%). IR (KBr) ν = 3350 (OH), 1709 (C=O); ^1H NMR (400 MHz, $CDCl_3$) δ = 0.78 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.75–2.50 (m, 26H), 3.20 (m, CH_2–I and CH-17α); LRMS for $C_{22}H_{36}O_2I$ $[MH]^+$: 459.1 *m/z*; HRMS: calcd for $C_{22}H_{35}O_2I$ + Na $[M+Na]^+$ 481.15739, found 481.15733; HPLC purity of 94% (*RT* = 33.1 min).

4.1.10. Synthesis of 16α-(3′-hydroxypropyl)-5α-androstane-3α,17β-diol (14). Ketone **12** (957 mg, 2.75 mmol) was dissolved in dry THF (92 mL) and the solution was cooled at −78 °C. A 1 M solution of K-Selectride in THF (3.29 mL, 3.29 mmol) was then added dropwise under argon. After 1 h, the reaction was quenched by addition of a saturated aqueous NH_4Cl solution (20 mL) and allowed to warm up to rt. The solution was concentrated under reduced pressure and the mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the crude product was purified by flash chromatography (hexanes/acetone, 80:20) to afford **14** (481 mg, 50%). IR (KBr) ν = 3364 (OH); ^1H NMR (400 MHz, CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70–1.85 (m, 25H), 3.12 (d, J = 7.8 Hz, CH-17α), 3.55 (t, J = 6.0 Hz, *CH_2*–OH), 3.95 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, CD_3OD) δ = 11.7, 12.6, 21.4, 29.6, 29.7, 31.4, 32.5, 32.9, 33.1, 33.5, 36.7, 37.3, 38.2, 40.4, 43.8, 45.1, ~49 (under solvent peaks), 50.7, 56.1, 63.2, 67.2, 88.9; LRMS for $C_{22}H_{39}O_3$ $[MH]^+$: 351.0 *m/z*; HRMS: calcd for $C_{22}H_{38}O_3$ + Na $[M+Na]^+$ 373.27132, found 373.27102.

4.1.11. Synthesis of 16α-(3′-chloropropyl)-5α-androstane-3α,17β-diol (15). To a solution of triol **14** (117 mg, 0.33 mmol) in dry CH_2Cl_2 (18 mL) were added PPh_3 (350 mg, 1.34 mmol) and CCl_4 (205 mg, 1.34 mmol). After 2 h at rt under argon, the reaction mixture was quenched by addition of water, extracted with CH_2Cl_2, and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (80:20) as eluent to yield **15** (92 mg, 75%). IR (film) ν = 3356 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.75 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.70–2.00 (m, 25H), 3.19 (d, J = 7.1 Hz, CH-17α), 3.55 (d, J = 6.6 Hz, CH_2–Cl), 4.04 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.4, 12.1, 20.4, 28.5, 29.1, 30.5, 31.7 (2×), 32.3, 33.1, 35.4, 36.0, 36.3, 36.9, 39.3, 43.2, 44.1, 45.5, 49.5, 54.6, 66.7, 88.3; LRMS for $C_{22}H_{38}O_2Cl$ - H_2O $[MH-H_2O]^+$: 351.1 *m/z*; HRMS: calcd for $C_{22}H_{37}O_2Cl$ + Na $[M+Na]^+$ 391.23743, found 391.23768.

4.1.12. Synthesis of 16α-(3′-fluoropropyl)-5α-androstane-3α,17β-diol (16). Chloride **15** (23 mg, 0.06 mmol) was dissolved in dry THF (18 mL) and the solution was cooled at 0 °C and treated with tetrabutylammonium fluoride (1 M in THF, 150 μL). This mixture was refluxed overnight and then quenched by addition of water. The product was extracted with EtOAc and dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography with hexanes/acetone (95:5) to give **16** (16 mg, 73%). IR (film) ν = 3358 (OH); ^{1}H NMR (400 MHz, CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70–1.90 (m, 25H), 3.12 (d, J = 7.6 Hz, CH-17α), 3.96 (narrow m, CH-3β), 4.36 (t, J = 5.9 Hz, 1H of CH_2–F), 4.48 (t, J = 6.0 Hz, 1H of CH_2–F); ^{13}C NMR (75.5 MHz, CD_3OD) δ = 11.7, 12.6, 21.4, 29.6, 29.7, 30.4, 31.3, 32.3, 32.4, 32.9, 33.5, 36.7, 37.2, 38.2, 40.4, 43.7, 45.1, 50.7, 56.1, 67.2, 83.9, 88.9; LRMS for $C_{22}H_{38}O_2F\cdot H_2O$ $[MH-H_2O]^+$: 335.2 *m/z*; HRMS: calcd for $C_{22}H_{37}O_2F$ + Na $[M+Na]^+$ 375.26698, found 375.26678.

4.1.13. Synthesis of 16α-(3′-iodopropyl)-5α-androstane-3α,17β-diol (17). Using the same protocol as for the synthesis of **13**, the primary alcohol of **14** was substituted and the compound purified by chromatography with hexanes/EtOAc (92:8) as eluent to yield **17** (61 mg, 30%). IR (film) ν = 3354 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.75 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.75–2.00 (m, 25H), 3.20 (m, CH_2–I and CH-17α), 4.15 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 7.5, 11.2, 12.0, 20.2, 28.4, 29.0, 30.4, 31.5, 32.1, 32.4, 35.2, 35.8, 36.2, 36.6, 36.7, 39.2, 42.8, 43.9, 49.3, 54.4, 66.5, 88.1; LRMS for $C_{22}H_{37}O_2I$ + NH_4 $[M+NH_4]^+$: 477.9 *m/z*; HRMS: calcd for $C_{22}H_{37}O_2I$ + Na $[M+Na]^+$ 483.17304, found 483.17418; HPLC purity of 99% (*RT* = 33.0 min).

4.1.14. Synthesis of 16α-(3′-bromopropyl)-17β-hydroxy-5α-androstan-3-one (18). To a solution of diol **12** (947 mg, 2.72 mmol) in dry CH_2Cl_2 (18 mL) were added PPh_3 (1.42 g, 5.43 mmol) and CBr_4 (1.80 g, 5.43 mmol) at 0 °C. After 2 h of stirring at rt under argon, the reaction mixture was quenched by addition of water, extracted with CH_2Cl_2, and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/acetone (80:20) as eluent to yield **18** (862 mg, 77%). IR (film) ν = 3426 (OH), 1705 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.70–2.50 (m, 31H), 3.17 (dd, J_1 = 5.9 Hz, J_2 = 7.3 Hz, CH-17α), 3.51 (t, J = 6.8 Hz, CH_2-Br), 3.68 (d, J = 5.5 Hz, OH); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8, 12.6, 21.8, ∼29 (under solvent peaks), 30.9, 31.3, 32.2, 32.8, 35.3, 36.2, 37.9, 38.7, 39.4, 39.4, 43.3, 45.3, 47.7, 50.2, 55.1, 88.3, 210.2.

4.1.15. Synthesis of 16α-(3′-azidopropyl)-17β-hydroxy-5α-androstan-3-one (19). To a solution of bromide **18** (243 mg, 0.59 mmol) in dry CH_3CN (20 mL) was added NaN_3 (154 mg, 2.37 mmol) under an argon atmosphere. The mixture was stirred at 80 °C for 24 h, and the reaction quenched by addition of water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 80:20) yielded 212 mg (95%) of azide **19**. IR (film) ν = 3436 (OH), 2094 (N_3), 1711 (C=O); ^{1}H NMR (400 MHz, $(CDCl_3)$ δ = 0.79 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70–2.50 (m, 26H), 3.20 (d, J = 7.5 Hz, CH-17α), 3.29 (t, J = 6.6 Hz, CH_2–N_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.6, 12.1, 21.0, 27.9, 28.9, 30.5, 31.3, 32.9, 35.3, 35.9, 36.8, 38.3, 38.7, 43.4, 44.1, 44.8, 46.9, 49.3, 51.8, 54.1, 88.2, 212.2; LRMS for $C_{22}H_{36}O_2N_3$ $[MH]^+$: 374.2 *m/z*; HRMS: calcd for $C_{22}H_{35}O_2N_3$ + Na $[M+Na]^+$ 396.26215, found 396.26185; HPLC purity of 95% (*RT* = 31.6 min).

4.1.16. Synthesis of 16α-(3′-azidopropyl)-5α-androstane-3α,17β-diol (20). Using the same protocol as for the synthesis of **14**, ketone **19** was reduced into alcohol **20** and purified by flash chromatography with hexanes/EtOAc/CH_2Cl_2 (80:19:1) to give 63 mg (44%) of **20**. IR (KBr) ν = 3450 (OH), 2084 (N_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.75–1.80 (m, 25 H), 3.19 (d, J = 7.4 Hz, CH-17α), 3.29 (t, J = 6.6 Hz, CH_2–N_3), 4.04 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.4, 12.1, 20.3, 27.9, 28.5, 29.1, 30.5, 31.7, 32.3, 32.9, 5.4, 36.0, 36.3, 36.9, 39.3, 43.4, 44.1, 49.5, 51.8, 54.6, 66.7, 88.3; LRMS for $C_{22}H_{38}O_2N_3$ $[MH]^+$: 376.0 *m/z*; HRMS: calcd for $C_{22}H_{37}O_2N_3$ + Na $[M+Na]^+$ 398.27780, found 398.27757; HPLC purity of 99% (*RT* = 31.9 min).

4.1.17. Synthesis of 16α-(3′-aminopropyl)-5α-androstane-3α,17β-diol (21). To a solution of azide **20** (104 mg, 0.28 mmol) in MeOH (16 mL) was added 10% Pd-C (20 mg) and the mixture was stirred for 3 h under one atmosphere of H_2. After filtration through Celite, the solvent was removed under reduced pressure. Purification by flash chromatography (acetone/MeOH, 90:10) afforded 87 mg (90%) of amine **21**. IR (KBr) ν = 3348 (OH and NH_2); ^{1}H NMR (400 MHz, CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70–1.90 (m, 27H), 2.66 (t, J = 6.0 Hz, CH_2–NH_2), 3.12 (d, J = 7.6 Hz, CH-17α), 3.96 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, CD_3OD) δ = 11.7, 12.6, 21.4, 29.6,

29.7, 30.9, 31.3, 32.0, 32.9, 33.5, 33.9, 36.7, 37.2, 38.2, 40.4, 42.5, 43.8, 45.0, 50.7, 56.1, 67.2, 88.8; LRMS for $C_{22}H_{40}O_2N$ $[MH]^+$: 350.3 *m/z*; HRMS: calcd for $C_{22}H_{40}O_2N$ $[MH]^+$ 350.30536, found 350.30518.

4.1.18. Synthesis of 16α-(3′-thiocyanatopropyl)-17β-hydroxy-5α-androstan-3-one (22). A mixture of bromide **18** (72 mg, 0.18 mmol) and KSCN (34 mg, 0.36 mmol) in EtOH (15 mL) was refluxed overnight. The reaction mixture was quenched by addition of water and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 70:30) yielded 56 mg (82%) of **22**. IR (KBr) $\nu = 3519$ (OH), 2145 (SC≡N), 1705 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 0.79$ (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70–2.50 (m, 25H), 2.97 (t, $J = 7.2$ Hz, CH_2-SCN), 3.21 (d, $J = 7.4$ Hz, CH-17α); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 11.6$, 12.1, 21.0, 28.9 (2×), 30.5, 31.3, 34.1, 34.2, 35.3, 35.9, 36.7, 38.2, 38.7, 43.0, 44.1, 44.8, 46.9, 49.3, 54.1, 88.1, ~112 (vw), 212.2; LRMS for $C_{23}H_{36}O_2NS$ $[MH]^+$: 390.0 *m/z*; HRMS: calcd for $C_{23}H_{35}O_2NS + Na$ $[M+Na]^+$ 412.22807, found 412.22830; HPLC purity of 98% ($RT = 30.0$ min).

4.1.19. Synthesis of 16α-(3′-thiocyanatopropyl)-5α-androstane-3α,17β-diol (23). Using the same protocol as for the synthesis of **14**, the ketone of **22** (26 mg, 0.066 mmol) was reduced and purified using hexanes/EtOAc (70:30) as eluent to yield **23** (16.2 mg, 62%). IR (KBr) $\nu = 3475$ (OH), 2160 (SC≡N); ^{1}H NMR (400 MHz, CD_3OD) $\delta = 0.76$ (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70–2.00 (m, 25H), 3.04 (t, $J = 7.2$ Hz, CH_2–SCN), 3.14 (d, $J = 7.4$ Hz, CH-17α), 3.96 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, CD_3OD) $\delta = 11.7$, 12.6, 21.4, 29.6, 29.7, 30.1, 31.4, 32.9, 33.5, 35.1, 36.7, 37.2, 38.1, 40.4, 43.4, 45.2, ~49 (under solvent peaks), 50.7, 56.1, 67.2, 88.8, 113.7; LRMS for $C_{23}H_{37}O_2NS + NH_4$ $[M+NH_4]^+$: 409.1 *m/z*; HRMS: calcd for $C_{23}H_{37}O_2NS + Na$ $[M+Na]^+$ 414.24372, found 414.24366.

4.1.20. Synthesis of 16α-(3′-bromopropyl)-17β-(*t*-butyldimethylsilyloxy)-5α-androstan-3-one (24). To a solution of **18** (118 mg, 0.29 mmol) in dry CH_2Cl_2 (30 mL), cooled at −78 °C, were added 2,6-lutidine (0.07 mL, 0.63 mmol) and TBDMS-OTf (0.06 mL, 0.26 mmol). The reaction mixture was stirred for 3 h under argon, then quenched by addition of water and 10% HCl. The acidification step was performed to recover the ketone in position 3. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude compound by flash chromatography (hexanes/acetone, 95:5) afforded **24** (128 mg, 85%). IR (film) $\nu = 1715$ (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) $\delta = 0.08$ and 0.10 (2s, $Si(CH_3)_2$), 0.80 (s, 18-CH_3), 0.91 (s, $SiC(CH_3)_3$), 1.07 (s, 19-CH_3), 0.70–2.50 (m, 40H), 3.26 (d, $J = 7.3$ Hz, CH-17α), 3.51 (t, $J = 6.4$ Hz, CH_2–Br); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) $\delta = -3.7$ and −3.6, 11.7, 12.8, 18.8, 21.8, 26.5, ~30 (under solvent peaks), 32.2, 32.8, 34.1, 35.3, 36.2, 36.6, 38.3, 38.6, 39.4, 44.2, 45.2, 47.7, 49.9, 55.0, 88.8, 210.2; LRMS for $C_{28}H_{49}BrO_2Si$ $[M-Br]^+$: 445.2 *m/z*.

4.1.21. Synthesis of 16α-(3′-(4″-bromobenzyloxy)propyl)-17β-hydroxy-5α-androstan-3-one (25). AgOTf (57 mg, 0.22 mmol), 4-bromobenzyl alcohol (56 mg, 0.30 mmol), and 2,6-di-*tert*-butyl-4-methyl pyridine (91 mg, 0.44 mmol) were dissolved in dry CH_2Cl_2 (30 mL) and cooled at 0 °C. To this solution was added bromide **24** (78 mg, 0.15 mmol) and the mixture was stirred for 24 h from 0 °C to rt. After filtration and evaporation of solvent under reduced pressure, the crude product was purified by flash chromatography with hexanes/EtOAc (98:2) to afford the arylether derivative in 76% yield (71 mg). The TBDMS group of this compound was hydrolyzed with HCl (2% v/v) in $MeOH/CH_2Cl_2$ (1:1). After 2 h at rt, water was added; the methanol was evaporated under reduced pressure, the aqueous phase extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. Flash chromatography using hexanes/acetone (90:10) yielded **25** (48 mg, 83%). IR (KBr) $\nu = 3423$ (OH), 1700 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) $\delta = 0.79$ (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.75–2.55 (m, 25H), 3.15 (dd, $J_1 = 5.6$ Hz, $J_2 = 7.5$ Hz, CH-17α), 3.48 (t, $J = 6.1$ Hz CH_2O), 3.62 (d, $J = 5.4$ Hz, OH), 4.46 (s, OCH_2Ph), 7.26 (d, $J = 8.5$ Hz, 2H of Ph), 7.47 (d, $J = 8.4$ Hz, 2H of Ph); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) $\delta = 11.7$, 12.6, 21.7, ~30 (under solvent peaks), 31.2, 32.2, 33.2, 36.2, 36.6, 37.9, 38.6, 39.4, 43.9, 44.8, 45.2, 47.7, 50.2, 55.1, 71.5, 72.4, 88.3, 121.4, 130.3 (2×), 132.1 (2×), 139.7, 210.2; LRMS for $C_{29}H_{42}O_3Br$ $[MH]^+$: 517.0 and 518.9 *m/z*; HRMS: calcd for $C_{29}H_{42}O_3Br + Na$ $[M+Na]^+$ 539.21313, found 539.21295; HPLC purity of 94% ($RT = 34.5$ min).

4.1.22. Synthesis of 16α-(3′-(4″-bromobenzyloxy)propyl)-5α-androstane-3α,17β-diol (26). Using the same protocol as for the synthesis of **14**, ketone **25** (45 mg, 0.087 mmol) was reduced into alcohol **26** in 2 h. The alcohol was recovered in the organic layer and purified by flash chromatography (hexanes/acetone, 95:5) to afford **26** (27 mg, 60%). IR (KBr) $\nu = 3384$ (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) $\delta = 0.76$ (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.70–1.85 (m, 25H), 3.14 (dd, $J_1 = 5.7$ Hz, $J_2 = 7.3$ Hz, CH-17α), 3.29 (d, $J = 3.1$ Hz, OH), 3.48 (t, $J = 6.1$ Hz, CH_2O), 3.59 (d, $J = 5.4$ Hz, OH), 3.93 (m, CH-3β), 4.47 (s, OCH_2Ph), 7.31 (d, $J = 8.4$ Hz, 2H of Ph), 7.52 (d, $J = 8.4$ Hz, 2H of Ph); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) $\delta = 11.8$, 12.7, 21.2, ~30 (under solvent peaks), 30.8, 31.3, 32.8, 33.3, 36.4, 37.1, 38.0, 40.0, 44.0, 44.9, 50.5, 55.9, 66.1, 71.6, 72.4, 88.4, 121.5, 130.4 (2×), 132.2 (2×), 139.8; LRMS for $C_{29}H_{44}O_3Br$ $[MH^+]$: 518.9 and 521.0 *m/z*; HRMS: calcd for $C_{29}H_{44}O_3Br$ $[MH]^+$ 519.24683, found 519.24662; HPLC purity of 96% ($RT = 36.3$ min).

4.1.23. Synthesis of 16α-(3′-bromopropyl)-17β-(t-butyldimethylsilyloxy)-5α-androstan-3α-ol (27). Using the same protocol as for the synthesis of **14**, ketone **24** (69 mg) was reduced into alcohol **27**. The alcohol was recovered in the organic layer and purified by flash chromatography (hexanes/acetone, 95:5) to afford **27** (40 mg, 58%). IR (film) $\nu = 3352$ (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) $\delta = 0.07$ and 0.10 (2s, $Si(CH_3)_2$), 0.77 (s, 18-CH_3), 0.80 (s, 19-CH_3), 0.91 (s, $SiC(CH_3)_3$), 0.70–

2.00 (m, 40H), 3.23 (m, CH-17α), 3.52 (t, $J = 6.6$ Hz, CH_2–Br), 3.80 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) $\delta = -3.7$ and -3.6 ($Si(CH_3)_2$), 8.4, 11.8, 18.8, 21.2, 26.5, ~30 (under solvent peaks), 32.1, 32.6, 32.8, 33.2, 33.5, 34.1, 35.3, 36.4, 37.0, 38.4, 38.6, 40.0, 44.1, 44.9, 46.2, 50.2, 55.7, 66.1, 88.9; LRMS for $C_{28}H_{51}O_2Br$ $[MH]^+$: 526.1 and 528.2 *m/z*.

4.1.24. Synthesis of 16α-(3′-bromo/chloropropyl)-3α-methoxy-5α-androstan-17β-ol (28) and 16α-(3′-iodopropyl)-3α-methoxy-5α-androstan-17β-ol (29). Bromide **27** (40 mg, 0.08 mmol) was dissolved in dry THF (18 mL) and the solution was cooled at 0 °C. NaH (18 mg, 0.45 mmol) was added and the mixture was stirred for 30 min before the addition of MeI (43 μL, 0.68 mmol) and 15-crown-5 ether. The reaction mixture was stirred at rt for 24 h and then quenched by addition of water. The product was extracted with CH_2Cl_2 and dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography with hexanes/EtOAc (98:2) giving a mixture of two methoxy derivatives (38 mg, 90%). The TBDMS group of these compounds was removed with a solution of concentrated HCl in $MeOH/CH_2Cl_2$ (1:1), after 2 h, the reaction was quenched by addition of a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The crude compound was purified by flash chromatography using hexanes/EtOAc (95:5) to give **28** (21 mg, 70%) and **29**. Compound **28:** IR (film) $\nu = 3436$ (OH); ^1H NMR (400 MHz, $CDCl_3$) $\delta = 0.75$ (s, 18-CH_3), 0.79 (s, 19-CH_3), 0.70–2.00 (m, 25H), 3.18 (d, $J = 7.2$ Hz, CH-17α), 3.29 (s, OCH_3), 3.48 (m, 1H of CH_2–Cl and CH-3β), 3.55 (t, $J = 6.6$ Hz, 1H of CH_2Cl); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 11.6$, 12.1, 20.3, 25.2, 28.6, 30.5, 31.6, 31.9, 32.7, 32.9, 34.4, 35.4, 36.1, 36.9, 39.7, 43.1, 43.2, 44.1, 49.5, 54.5, 55.8, 75.7, 88.3; LRMS for $C_{23}H_{39}O_2Cl$–CH_3O $[M–OCH_3]^+$: 351.2 *m/z*. Compound **29**: IR (NaCl) $\nu = 3474$ (OH); ^1H NMR (400 MHz, $(CDCl_3)$ $\delta = 0.74$ (s, 18-CH_3), 0.79 (s, 19-CH_3), 0.70–2.00 (m, 25H), 3.20 (m, CH-17α and CH_2–I), 3.29 (s, OCH_3), 3.42 (m, CH-3β); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 7.6$, 11.6, 12.1, 20.3, 25.2, 28.6, 30.5, 31.6, 32.6, 32.7, 32.9, 35.4, 36.1, 36.8, 36.9, 39.7, 43.0, 44.1, 49.5, 54.5, 55.8, 75.7, 88.2; LRMS for $C_{23}H_{39}O_2I$–CH_3O $[M–OCH_3]^+$: 443.0 *m/z*; HRMS: calcd for $C_{23}H_{39}O_2I + Na$ $[M+Na]^+$ 497.18869, found 497.18845.

4.1.25. 6′-(3″-Dioxolane-17″β-hydroxy-5″α-androstane-16″α-yl)-hex-2′-enol (30). This compound was prepared as reported below for the synthesis of **31** in 36% yield (630 mg). ^1H NMR (400 MHz, $CDCl_3$) $\delta = 0.75$ (s, 18″-CH_3), 0.81 (s, 19″-CH_3), 0.60–2.50 (m, 33H), 3.20 (d, $J = 7.5$ Hz, CH-17″α), 3.65 (m, CH_2-OH and OH), 3.93 (s, OCH_2CH_2O), 5.48 (m, CH-2′ and CH-3′); LRMS for $C_{27}H_{44}O_4$ $[MH^+]$: 433.2 *m/z*.

4.1.26. Synthesis of 4′-(3″-dioxolane-17″β-hydroxy-5″α-androstan-16″α-yl)-but-2′-enyl-benzoate (31). To a solution of alkene **9** (300 mg, 0.80 mmol) in dry CH_2Cl_2 (12 mL) were added benzoic acid allyl ester (390 mg, 2.4 mmol) and 2nd-generation Grubbs catalyst (tricyclohexylphosphine[1,3-bis(2,4,6-tri-methylphenyl)-4,5-dihydroimidazol-2-ylidene][benzylidine]ruthenium(IV) dichloride) (102 mg, 0.12 mmol). This mixture was refluxed overnight and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (hexanes/EtOAc, 95:5) to yield 190 mg (47%) of **31**. IR (film) $\nu = 3423$ (OH), 1713 (C=O, ester); ^1H NMR (400 MHz, $(CD_3)_2CO$) $\delta = 0.76$ (s, 18″-CH_3), 0.82 (s, 19″-CH_3), 0.60–2.50 (m, 23H), 3.21 (d, $J = 7.7$ Hz, CH-17″α), 3.88 (s, OCH_2-CH_2O), 4.77 (d, $J = 6.2$ Hz, CH_2-4′), 5.76 and 5.95 (2m, CH-2′ and CH-3′), 7.52 (m, 2H of Ph), 7.64 (m, 1H of Ph), 8.04 (m, 2H of Ph); LRMS for $C_{32}H_{45}O_5$ $[MH]^+$: 508.9 *m/z*.

4.1.27. 16α-(6′-chlorohexyl)-17β-hydroxy-5α-androstan-3-one (32). This compound was prepared in three steps (16 mg, 18%) as reported below for the synthesis of **33** except that there was no benzoic acid ester to be hydrolyzed. IR (film) $\nu = 3444$ (OH), 1712 (C=O); ^1H NMR (400 MHz, $(CD_3)_2CO/D_2O$) $\delta = 0.78$ (s, 18-CH_3), 1.06 (s, 19-CH_3), 0.70–2.60 (m, 31H), 3.12 (d, $J = 7.6$ Hz, CH-17α), 3.59 (t, $J = 6.7$ Hz CH_2-Cl); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) $\delta = 11.7$, 12.6, 21.8, 27.7, ~30 (under solvent peaks), 31.2, 32.2, 33.5, 36.2, 36.6, 37.9, 38.6, 39.4, 44.1, 44.8, 45.2, 45.9, 47.7, 50.2, 55.1, 88.3, 210.2; LRMS for $C_{25}H_{41}O_2$ $[M-Cl]^+$: 373.3 *m/z*.

4.1.28. Synthesis of 16α-(4′-chlorobutyl)-17β-hydroxy-5α-androstan-3-one (33). A suspension of alkene **31** (190 mg, 0.37 mmol) and 10% Pd–C (28 mg) in EtOAc was hydrogenated at one atmosphere for 24 h. After filtration through Celite, the solvent was removed under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 90:10) afforded 157 mg (83%) of alkane derivative. An aqueous 10% NaOH solution (8 mL) was added to this intermediate (157 mg, 0.31 mmol) dissolved in methanol (15 mL) and the reaction mixture was stirred for 1.5 h at rt. The product was extracted with CH_2Cl_2, the organic phase dried over $MgSO_4$, and the solvent was removed under reduced pressure to give the crude diol (124 mg). This diol was dissolved in acetone (15 mL) and 10% aqueous HCl (8 mL) was slowly added with a syringe. The reaction mixture was stirred for 1 h at rt. An aqueous saturated $NaHCO_3$ solution was then added and the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure. This alcohol (70 mg, 0.17 mmol), PPh_3 (101 mg, 0.39 mmol) and CCl_4, (62 mg, 0.40 mmol) in dry CH_2Cl_2 (10 mL) were then stirred at 0 °C under argon for 1 h. The reaction was quenched by addition of water, extracted with CH_2Cl_2, and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (80:20) as eluent to yield chloride **33** (34 mg, 24% for four steps). IR (film) $\nu = 3420$ (OH), 1710 (C=O); ^1H NMR (400 MHz, $(CD_3)_2CO/D_2O$) $\delta = 0.78$ (s, 18-CH_3), 1.06 (s, 19-CH_3), 0.70–2.60 (m, 27H), 3.13 (d, $J = 7.6$ Hz, CH-17α), 3.59 (t, $J = 6.7$ Hz, CH_2–Cl); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) $\delta = 11.7$, 12.6, 21.7, 26.4, 30.6, 31.1, 32.2,

33.7, 35.8, 36.1, 36.6, 37.8, 38.5, 39.3, 43.9, 44.7, 45.2, 45.8, 47.6, 50.2, 55.0, 88.2, 210.2; LRMS for $C_{23}H_{39}O_2Cl$ $[M]^+$: 380.5 *m*/*z*.

4.1.29. 16α-(6′-chlorohexyl)-5α-androstane-3α,17β-diol (34). This compound was prepared in 75% yield (15 mg) as reported below for **35**. IR (film) ν = 3358 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.70–1.90 (m, 31H), 3.12 (dd, J_1 = 5.6 Hz, J_2 = 7.5 Hz, CH-17α), 3.28 (d, J = 3.1 Hz, OH), 3.55 (m, CH_2-Cl and OH), 3.94 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8, 12.7, 21.1, 27.7, 28.9, ~30 (under solvent peaks), 31.2, 32.7, 33.2, 33.5, 36.4, 36.7, 37.0, 38.0, 39.9, 44.1, 44.8, 45.9, 50.5, 55.8, 66.0, 88.4; LRMS for $C_{25}H_{43}O_2$ $[M-Cl]^+$: 375.3 *m*/*z*.

4.1.30. Synthesis of 16α-(4′-chlorobutyl)-5α-androstane-3α,17β-diol (35). Using the same protocol as for the synthesis of **14,** chloride **33** (26 mg, 0.06 mmol) was dissolved in dry THF (2 mL) and the solution cooled at −78 °C. A 1-M solution of K-Selectride in THF (60 μL, 0.06 mmol) was added dropwise under argon. After 1 h, the reaction was quenched by the addition of a saturated aqueous NH_4Cl solution (1 mL). The mixture was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the crude product was purified by flash chromatography with hexanes/EtOAc/CH_2Cl_2 (80:19:1) as eluent to afford **35** (17 mg, 72%). IR (film) ν = 3355 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.70–1.90 (m, 27H), 3.28 (dd, J_1 = 5.6 Hz, J_2 = 7.5 Hz, CH-17α), 3.28 (d, J = 3.1 Hz, OH), 3.60 (m, CH_2–Cl and OH), 3.93 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8, 12.6, 21.1, 26.5, ~30 (under solvent peaks), 31.1, 32.7, 33.2, 33.8, 35.8, 36.3, 37.0, 38.0, 39.9, 44.0, 44.8, 45.9, 50.5, 55.8, 66.0, 88.3; LRMS for $C_{23}H_{39}O_2$ $[M-Cl]^+$: 347.3 *m*/*z*.

4.1.31. Synthesis of 16α-(2′-bromoethyl)-17β-acetoxy-5α-androstan-3α-ol (37). To a solution of bromide **36** (180 mg, 0.35 mmol) in pyridine (5 mL) were added Ac_2O (357 mg, 0.35 mmol) and DMAP (1.2 mg, 0.01 mmol) under an argon atmosphere. After 2 h at rt, the reaction was quenched by addition of a saturated aqueous $NaHCO_3$ solution and the mixture extracted with EtOAc. The organic phase was washed with a saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated under reduced pressure yielding 190 mg of crude intermediate. The TBDMS group of this intermediate (180 mg) was removed with HF/pyridine (276 μL, 6.48 mmol) in THF at rt for 60 h and quenched by addition of a saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted with EtOAc and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 85:15) yielded 71 mg (49% for two steps) of alcohol **37**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 18-CH_3), 0.78 (s, 19-CH_3), 3.33 (s, $COCH_3$), 0.70–2.30 (m, 32H), 3.35 (m, CH_2–Br), 4.03 (narrow m, CH-3β), 4.53 (d, J = 7.6 Hz, CH-17α).

4.1.32. Synthesis of 16α-(2′-bromoethyl)-17β-hydroxy-5α-androstan-3-one (38). To a solution of alcohol **37** (30 mg, 0.07 mmol) in dry CH_2Cl_2 (2 mL) under argon were added molecular sieves (4 Å) (50 mg) and 4-methylmorpholine-*N*-oxide (12 mg, 0.10 mmol) and the mixture was stirred for 15 min at rt. Tetrapropylammonium perruthenate (2.4 mg, 0.07 mmol) was added and the solution was stirred for 2 h. The resulting mixture was filtered on a silica gel column, using hexanes/acetone (85:15) as eluent to give 29 mg (96%) of the corresponding ketone. Subsequently, a solution of K_2CO_3 (36 mg, 0.26 mmol) in MeOH (1 mL) was added to a solution of ketone (29 mg, 0.06 mmol) in MeOH (1 mL) and the mixture was refluxed for 2 h. The MeOH was evaporated under reduced pressure, and the residue dissolved in EtOAc, and washed with a saturated aqueous NH_4Cl solution. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 85:15) yielded 9 mg (35%) of ketone **38**. IR (film) ν = 3422 (OH), 1707 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.80 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70–2.50 (m, 23H), 3.22 (d, J = 7.1 Hz, CH-17α), 3.48 (m, CH_2-Br); LRMS for $C_{21}H_{34}O_2Br$ $[MH]^+$: 397.0 and 399.0 *m*/*z*.

4.1.33. 16α-(2′-bromoethyl)-5α-androstane-3α,17β-diol (39). The TBDMS group of ketone **36** (30 mg, 0.06 mmol) was hydrolyzed with concentrated HCl (3% v/v) in MeOH. After 2 h at rt, water was added, the methanol was evaporated under reduced pressure, the aqueous phase extracted with EtOAc, and the organic phase dried over $MgSO_4$. Flash chromatography using hexanes/EtOAc (85:15) yielded **39** (19 mg, 82%). IR (film) ν = 3374 (OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.70–2.20 (m, 23H), 3.22 (d, J = 7.1 Hz, CH-17α), 3.55 (m, CH_2–Br), 4.05 (narrow m, CH-3β); LRMS for $C_{21}H_{35}O_2Br{\cdot}2H_2O$ $[MH-2H_2O]^+$: 363.1 and 365.1 *m*/*z*.

4.2. Assessment of proliferative and antiproliferative activities on Shionogi (AR^+) cells

These cell culture assays were performed as reported in the literature.[27] After a 10-day treatment, the cell growth was assessed by quantifying DNA content using a modified Fiszer-Szafarz method,[48] as previously described,[49] and the results were reported as μg of DNA (Figs. 2 and 4) or percentages (Fig. 3 and Table 1). The antiproliferative assay on androgen-sensitive (AR^+) Shionogi mammary carcinoma cells was carried out at indicated concentrations of the synthesized compounds and the results are reported as cell DNA content (μg) or the percentage (%) of inhibition relative to the proliferation induced by 0.3 nM DHT. The antiproliferative activity (%) = 100 × [DNA (DHT) − DNA (DHT + Compound)/DNA (DHT) − DNA (Control)]. The proliferation assay was carried out at indicated concentrations and results are reported as cell DNA content (μg) or the percentage (%) of stimulation, taking the stimulation of androgen DHT (0.3 nM) as 100%. The proliferative activity (%) = 100 × [DNA (Compound) − DNA (Control)/DNA (DHT) − DNA (Control)].

4.3. Assessment of proliferative activity on PC-3 (AR^-) cells

4.3.1. Cell culture. PC-3 cells were received from ATCC and were routinely grown in MEM supplemented with 5% (v/v) fetal bovine serum (FBS), 100 IU penicillin/mL, 50 μg streptomycin sulfate/mL, and 1% (v/v) non-essential amino acids. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. They were subcultured at near-confluence after gentle digestion in a solution of 0.1% trypsin (Wisent Inc.) in HEPES buffer containing 3 mM ethylenediaminetetraacetic acid (pH 7.2). Cells were pelleted by centrifugation, resuspended in culture medium, and replated.

4.3.2. Measurement of cell proliferation. Cells at passage 28 were plated in 24-well plates at a density of 7000 cells/well and allowed to adhere to the surface of the plates for 24 h. Thereafter, the medium was replaced with fresh medium containing 2% (v/v) charcoal-stripped FBS and the indicated concentrations of compounds diluted from stock solutions prepared in 99% redistilled ethanol (a 1000-fold concentrated solution). Control cells received only the ethanolic vehicle (0.1% EtOH, v/v). Such a concentration of ethanol does not affect cell growth. The indicated concentrations of compounds were added to triplicate dishes, and cells were grown for 9 days with changes of medium every 2–3 days. Cell proliferation was determined by measurement of DNA content as previously described.[49]

4.4. Steroid receptor affinity assay

The binding affinity assay was performed in human embryonic kidney (HEK-293) cells stably transfected with human androgen receptor according to the procedure described by Labrie et al. [50] except that the results were expressed as the percentage of binding affinity at 0.1 and 1 μM of tested compound (the binding affinity of R1881 (1 μM) was set as 100%).

4.5. Molecular modeling

To predict the optimal position of ligand in the steroid binding site of human androgen receptor (hAR), we have used a computational strategy similar to that proposed by Zhorov and Lin.[51] The receptor was represented by a double-shell model based on our hAR crystallographic structures.[43] The inner flexible shell was composed of hAR ligand binding domain amino acids having at least one atom within 8 Å of the ligand. Residue internal coordinates of the flexible shell were allowed to move during minimization steps. The other amino acids of the model were included in the outer rigid shell, in which they were not allowed to vary during energy minimization.

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