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# Androsterone Derivatives Substituted at Position 16: Chemical Synthesis, Inhibition of Type 3 17β-hydroxysteroid Dehydrogenase, Binding Affinity for Steroid Receptors and Proliferative/antiproliferative Activity on Shionogi ($AR^+$) Cells

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**A series of androsterone (ADT) derivatives substituted at position 16 were efficiently synthesized in short reaction sequences; the ether analogues were also synthesized in the case of the methyl and allyl derivatives. The aim of this study was to develop inhibitors of the steroidogenic enzyme type 3 17β-hydroxysteroid dehydrogenase and then evaluate their ability to inhibit this activity in transfected HEK-293 cells. For each compound we measured the percentage of inhibition of the transformation of 4-androstene-3,17-dione, the natural substrate of this steroidogenic enzyme, into the active androgen testosterone. The synthesized compounds proved to be weak inhibitors of this enzyme, but interestingly, these ADT derivatives do not bind to androgen, estrogen, glucocorticoid, and progestin receptors, suggesting no unsuitable receptor-mediated effects. One exception, 16α-(3′-bromopropyl)-5α-androstane-3α,17β-diol (8), the only compound bearing a hydroxy group at position 17β instead of a ketone, showed a strong binding affinity for the androgen receptor (70% at 1 μM) and also exhibited an antiproliferative activity on Shionogi ($AR^+$) cells (86% at 1 μM), which was comparable to that of hydroxyflutamide, a pure antiandrogen (100% at 1 μM).**

*Keywords*: Enzyme; Inhibitors; Biosynthesis; Steroids; Androsterone; Hormones

## INTRODUCTION

Estrogens and androgens are more potent in their 17β-hydroxy than in their 17-keto configuration. The interconversion of the two forms is catalyzed by 17β-hydroxysteroid dehydrogenases (17β-HSDs), a family of steroidogenic enzymes widely distributed in human tissues that plays an important role in the transformation and regulation of intracellular steroid hormones.[1–5] They have individual cell-specific expression and substrate specificity. Type 3 17β-HSD,[6] also called testicular 17β-HSD or androgenic 17β-HSD, is principally found in the microsomal fraction of the testis. It catalyses the reduction of 4-androstene-3,17-dione ($\Delta^4$-dione) into testosterone (T) using NADPH as cofactor. Deficiency of type 3 17β-HSD in males has been associated with pseudohermaphroditism,[7] showing the importance of this enzyme in the production of male active hormones testosterone and dihydrotestosterone (DHT). The pathophysiological role of these active hormones in diseases like benign prostatic hyperplasia, prostate cancer,[8] acne,[9] hirsutism,[10] male-pattern baldness[11] is well known. In attempts to inactivate these hormones, inhibitors of 5α-reductases—which catalyse the transformation of testosterone into dihydrotestosterone (Figure 1)—have been developed.[12–14] A drawback of these inhibitors is that testosterone is still produced, and, although it is not as potent as DHT, the blockade of androgenic effect on the androgen receptor is far from complete. A more complete blockade would be achieved by type 3 17β-HSD inhibitors, as they

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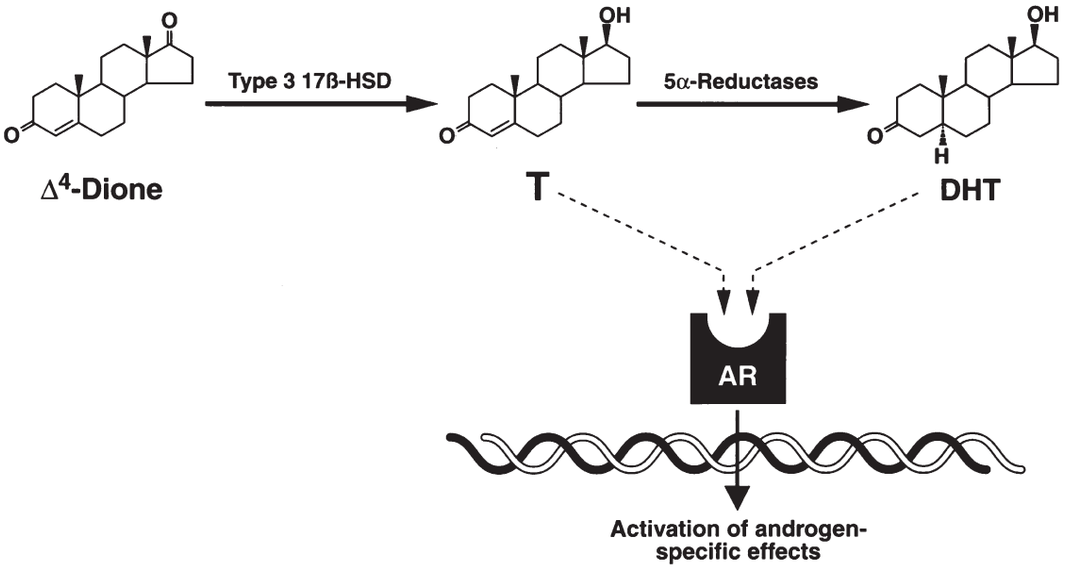



FIGURE 1 Enzymatic steps involved in biosynthesis of testosterone (T) and dihydrotestosterone (DHT) from 4-androstene-3,17-dione ($\Delta^4$-dione).



would block the formation of both T and DHT (Figure 1).[15–18] We thus undertook the development of type 3 17β-HSD inhibitors that do not have residual unsuitable androgenic activity. A first screening study,[19] with more than eighty steroidal compounds of different classes, allowed us to identify the C19-nucleus androsterone (ADT) as the best inhibitor of this series. This result agrees with the findings of Pittaway,[20] which suggested that a 17-keto group and a steroidal unaromatized A-ring were required for the inhibition of the type 3 17β-HSD activity in microsomal preparations of canine testis. On the other hand, our group demonstrated that the presence of a bromoalkyl side chain at position 16 of estradiol was favorable for the inhibition of type 1 17β-HSD.[21–23] Similarly, we designed ADT derivatives substituted at position 16 by a bromopropyl as potential inhibitors of type 3 17β-HSD (Figure 2). We also extended this exploratory work by synthesizing additional ADT derivatives substituted at C16. Herein, we report the chemical synthesis of the target compounds **6–8** and **14–20**, their ability to inhibit type 3 17β-HSD, their binding affinities for steroid receptors (AR, ER, GR, and PR), as well as their proliferative and antiproliferative activities on Shionogi ($AR^+$) cells.

## MATERIALS AND METHODS

### General

Androsterone (ADT), the starting material, was obtained from Steraloids (Wilton, NH, USA). Chemical reagents as well as dimethylformamide (DMF) and dichloromethane ($CH_2Cl_2$), 99.8% anhydrous grade, were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ont., Canada). Tetrahydrofuran (THF), used in anhydrous conditions, was distilled from sodium/benzophenone ketyl and kept under argon. Solvents for chromatography were purchased from BDH Chemicals (Montréal, Que., Canada) or Fisher Chemicals (Montréal, Que., Canada). Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 $F_{254}$ plates (E. Merck; Darmstadt, GE) and 230–400 mesh ASTM silica gel 60 (E. Merck) was used for flash chromatography. Infrared spectra (IR) are reported in $cm^{-1}$ and were obtained on a Perkin–Elmer 1600 (FT–IR series) spectrophotometer (Norwalk, CT, USA). Nuclear magnetic resonance spectra (NMR) were obtained at 300 MHz for $^1H$ and 75.5 MHz for $^{13}C$ with a Bruker AC/F300 spectrometer (Billerica, MA, USA). The chemical shifts ($\delta$) were referenced to chloroform (7.26 ppm for $^1H$ and 77.00 ppm for $^{13}C$) and are expressed in ppm. Only specific peaks are reported in $^1H$ NMR whereas all signals are reported in $^{13}C$ NMR. Low Resolution Mass Spectra (LRMS) were obtained on a PE Sciex API-150 ex. apparatus (Foster City, CA, USA) equipped with a turbo ionspray source. High Resolution Mass Spectra (HRMS) were provided by the Regional Center for Mass Spectroscopy (Université de Montréal, Montréal, Que., Canada).

### Synthesis of 16-bromopropyl-ADT Derivatives 6–8 (Scheme 1)

#### *Protection of Androsterone (Synthesis of 1)*

To a solution of androsterone (1.5 g, 5.17 mmol) in dry DMF (100 mL) was added imidazole (1.76 g, 5 eq)

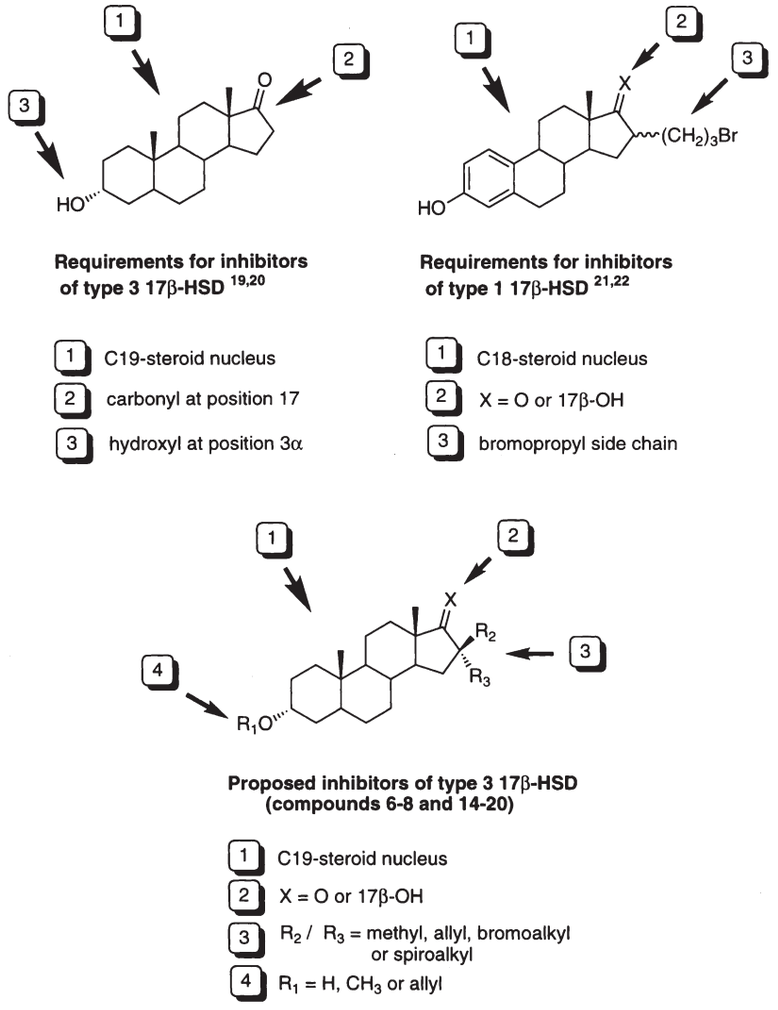



FIGURE 2 Rationale for the design of potential type 3 17β-HSD inhibitors (compounds **6**–**8** and **14**–**20**).

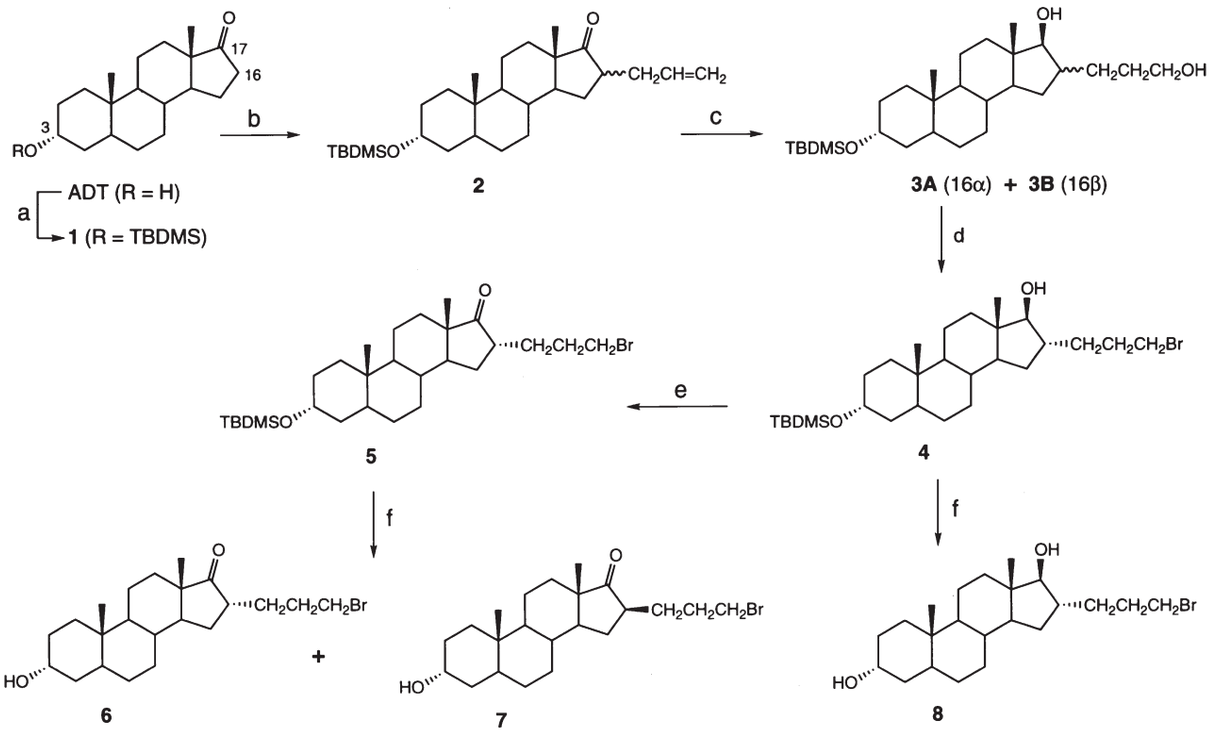
and *tert*-butyldimethylsilyl chloride (TBDMS-Cl) (2.34 g, 3 eq). The reaction was stirred at room temperature overnight, under an argon atmosphere. The mixture was then poured onto ice and filtered. The resulting solid (one spot on TLC) was washed with water and dried over phosphorus pentoxide under reduced pressure for 24 h to give the TBDMS-ADT (**1**).

3α-(*t*-Butyldimethylsilyloxy)-5α-androstan-17-one (**1**)

White solid; 94% yield; IR (KBr) 1741 (C = O, ketone); $^1$H NMR ($CDCl_3$) −0.02 (s, $Si(CH_3)_2$), 0.75 (s, $CH_3$-19), 0.82 (s, $CH_3$-18), 0.85 (s, $SiC(CH_3)_3$), 2.39 (dd, $J_1 = 8.5$ Hz, $J_2 = 14.4$ Hz, CH-16β), 3.93 ($t_{app.}$, $J = 2.6$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.91 ($Si(CH_3)_2$), 11.32 (C-19), 13.75 (C-18), 18.01 ($SiC(CH_3)_3$), 20.01, 21.69, 25.79 ($SiC(CH_3)_3$), 28.28, 29.62, 30.91, 31.52, 32.32, 35.02, 35.78, 36.04, 36.65, 38.98, 47.73, 51.43, 54.41, 66.68 (C-3), 221.29 (C-17); LRMS 405 $[M + H]^+$, 422 $[M + NH_4]^+$.

### *Allylation of 1 (Synthesis of 2)*

A solution of diisopropylamine (0.82 mL, 1.2 eq) in dry THF was stirred under argon at 0°C and a 1.6 M solution of butyllithium in hexanes (3.52 mL, 1.15 eq) was added dropwise. After 30 min, the resulting lithium diisopropylamide (LDA) solution was cooled at −78°C and TBDMS-ADT (**1**) (1.98 g, 4.90 mmol) dissolved in dry THF was added dropwise. The mixture was allowed to stir for 1 h at 0°C, then cooled again to −78°C and 1.69 mL (4 eq) of allyl bromide was added dropwise. The reaction mixture was stirred overnight from −78°C to room temperature. Water was added to quench the reaction and the crude product was extracted with EtOAc. The organic phase was washed with a saturated NaCl solution, dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc: 9/1) afforded the expected 16-allylated product **2** as a mixture of 16α- and 16β-isomers.




SCHEME 1 Chemical synthesis of C16-bromopropyl ADT derivatives **6**–**8**. Reagents: (a) TBDMS-Cl, imidazole, DMF, r.t.; (b) i. LDA, THF, 0°C, ii. $CH_2=CHCH_2Br$, THF, −78°C to r.t.; (c) i. $BH_3$·THF, THF, 0°C, ii. $H_2O_2$, NaOH (3N); (d) $CBr_4$, $PPh_3$, $CH_2Cl_2$, 0°C; (e) PCC, $CH_2Cl_2$, r.t.; (f) MeOH–HCl (2%), r.t.

3α-(*t*-Butyldimethylsilyloxy)-16α,β-(allyl)-5α-androstan-17-one (**2**)

White solid; 70% yield; IR (KBr) 1737 (C = O, ketone), 1641 (C = C, alkene); $^1$H NMR ($CDCl_3$) 0.01 (s, $Si(CH_3)_2$), 0.77 (s, $CH_3$-19), 0.80 and 0.90 (2s, $CH_3$-18, 12/88: C16β-allyl/C16α-allyl, respectively), 0.88 (s, $SiC(CH_3)_3$), 2.00 (m, $CH_2$-1′), 3.95 ($t_{app.}$, $J = 2.6$ Hz, CH-3β), 5.02 (d, $J = 10.0$ Hz, 1H of $CH_2$-3′), 5.03 (d, $J = 17.3$ Hz, 1H of $CH_2$-3′), 5.7 (m, CH-2′); $^{13}$C NMR ($CDCl_3$) −4.86 ($Si(CH_3)_2$), 11.37 (C-19), 14.58 (C-18), 18.09 ($SiC(CH_3)_3$), 19.97, 25.87 ($SiC(CH_3)_3$), 26.75, 28.33, 29.67, 30.84, 31.65, 32.35, 35.00, 35.09, 36.15, 36.71, 39.04, 44.08, 48.51, 49.00, 54.52, 66.75 (C-3), 116.29 (C-3′), 136.53 (C-2′), 221.86 (C-17); LRMS 445 $[M + H]^+$, 462 $[M + NH_4]^+$.

### *Oxidative Hydroboration of 2 (Synthesis of 3A)*

To a stirred solution of 16-allyl-TBDMS-ADT (**2**) (1.37 g, 3.09 mmol) in dry THF (150 mL) at 0°C was added dropwise a 1 M borane solution in THF (14.2 mL, 4.6 eq). The mixture was allowed to react under argon for 3 h, then a 3N aqueous NaOH solution (5.1 mL) and $H_2O_2$ (30% w/v, 2.1 mL) were added respectively. The resulting mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of water and extracted with EtOAc. The organic phase was washed with water and a saturated NaCl solution, then dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography, using a mixture of hexanes and EtOAc (7/3) as eluent. Only the major 16α-isomer (alcohol **3A**) was recovered.

3α-(*t*-Butyldimethylsilyloxy)-16α-(3′-hydroxypropyl)-5α-androstan-17β-ol (**3A**)

White solid; 65% yield; IR (KBr) 3316 (OH, alcohol); $^1$H NMR ($CDCl_3$) 0.005 (s, $Si(CH_3)_2$), 0.75 (s, $CH_3$-19 and $CH_3$-18), 0.87 (s, $SiC(CH_3)_3$), 3.18 (d, $J = 7.4$ Hz, CH-17α), 3.64 (t, $J = 5.4$ Hz, $CH_2$-3′), 3.94 ($t_{app.}$, $J = 2.2$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.85 ($Si(CH_3)_2$), 11.42 (C-19), 12.06 (C-18), 18.09 ($SiC(CH_3)_3$), 20.26, 25.86 ($SiC(CH_3)_3$), 28.50, 29.73, 30.60, 31.13, 31.68, 32.09, 32.41, 35.30, 36.05, 36.75, 36.91, 39.10, 42.96, 43.98, 49.35, 54.58, 62.98 (C-3′), 66.83 (C-3), 88.11 (C-17); LRMS 465 $[M + H]^+$, 487 $[M + NH_4]^+$.

### *Bromination of 3A (Synthesis of 4)*

A solution of the alcohol **3A** (0.980 g, 2.11 mmol), $PPh_3$ (1.11 g, 2 eq) and $CBr_4$ (1.40 g, 2 eq) in dry $CH_2Cl_2$ (100 mL) was stirred at 0°C under argon. The reaction was monitored by TLC and was completed

after 3 h. The crude mixture was preadsorbed on silica gel and flash chromatography performed with hexanes/EtOAc: 95/5 as eluent to give the bromide **4**.

16α-(3′-Bromopropyl)-3α-(*t*-butyldimethylsilyloxy)-5α-androstan-17β-ol (**4**)

White solid; 70% yield; IR (KBr) 3360 (OH, alcohol); $^1$H NMR ($CDCl_3$) 0.005 (s, $Si(CH_3)_2$), 0.74 (s, $CH_3$-19), 0.75 (s, $CH_3$-18), 0.87 (s, $SiC(CH_3)_3$), 3.17 (d, $J = 6.7$ Hz, CH-17α), 3.41 (t, $J = 6.7$ Hz, $CH_2$-3′), 3.94 (m, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.86 ($Si(CH_3)_2$), 11.40 (C-19), 11.97 (C-18), 18.05 ($SiC(CH_3)_3$), 20.23, 25.85 ($SiC(CH_3)_3$), 28.46, 29.70, 30.37, 31.63 (2X), 32.39, 34.12, 34.28, 35.27, 36.02, 36.72, 36.81, 39.05, 42.91, 43.92, 49.36, 54.53, 66.78 (C-3), 88.11 (C-17); LRMS 526 and 528 $[M]^-$, 447 $[M - Br]^-$.

### *Oxidation of 4 (Synthesis of 5)*

A solution of alcohol **4** (0.300 g, 0.57 mmol) in dry $CH_2Cl_2$ (100 mL) was added dropwise at room temperature to a mixture of pyridinium chlorochromate (PCC) (0.184 g, 1.5 eq), NaOAc (0.140 g, 3 eq) and molecular sieves (4 Å) (0.050 g) in dry $CH_2Cl_2$ (60 mL). The resulting mixture was stirred at room temperature under argon for 3 h, then filtered on a silica gel column, using $CH_2Cl_2$ as eluent to give the ketone **5**.

16α-(3′-Bromopropyl)-3α-(*t*-butyldimethylsilyloxy)-5α-androstan-17-one (**5**)

White solid; 86% yield; IR (KBr) 1734 (C = O, ketone); $^1$H NMR ($CDCl_3$) −0.009 (s, $Si(CH_3)_2$), 0.75 (s, $CH_3$-19), 0.86 (s, $SiC(CH_3)_3$), 0.87 (s, $CH_3$-18), 2.39 (m, CH-16β), 3.37 (m, $CH_2$-3′), 3.93 (m, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.90 ($Si(CH_3)_2$), 11.31 (C-19), 14.50 (C-18), 18.02 ($SiC(CH_3)_3$), 19.90, 25.81 ($SiC(CH_3)_3$), 27.70, 28.25, 29.61, 29.79, 30.81, 31.26, 31.64, 32.28, 33.28, 34.91, 36.05, 36.62, 38.95, 43.87, 48.41, 49.28, 54.43, 66.65 (C-3), 221.80 (C-17); LRMS 525 and 527 $[M + H]^+$, 542 and 544 $[M + NH_4]^+$.

### *Hydrolysis of Silylated Ether 5 (Synthesis of 6 and 7)*

The silylated ether **5** was dissolved in a methanolic solution of HCl (2%, v/v) and the resulting mixture was stirred at room temperature for 3 h. Water was added, the methanol evaporated under reduced pressure and the residue extracted with EtOAc. The organic phase was washed with a saturated NaCl solution and dried over $MgSO_4$. Flash chromatography using hexanes/EtOAc (8/2) gave the expected 16α-bromopropyl-ADT (**6**) and the 16β-epimer **7** in a 3 to 1 proportion (92% global yield).

16α-(3′-Bromopropyl)-3α-hydroxy-5α-androstan-17-one (**6**)

White solid; IR (film) 3591 and 3442 (OH, alcohol), 1734 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.77 (s, $CH_3$-19), 0.87 (s, $CH_3$-18), 2.38 (m, CH-16β), 3.37 (m, $CH_2$-3′), 4.02 ($t_{app.}$, $J = 2.3$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.10 (C-19), 14.50 (C-18), 19.85, 27.70, 28.15, 28.90, 29.79, 30.71, 31.29, 31.61, 32.04, 33.29, 34.88, 35.72, 36.17, 38.99, 43.87, 48.40, 49.26, 54.37, 66.24 (C-3), 221.82 (C-17); HPLC purity = 97.7% (C-18 NovaPak column, MeCN/$H_2O$/MeOH: 35/25/40); HRMS calcd for $C_{22}H_{36}O_2$ $^{79}$Br 411.1899 ($MH^+$), found 411.1888.

16β-(3′-Bromopropyl)-3α-hydroxy-5α-androstan-17-one (**7**)

White solid; IR (film) 3518 and 3256 (OH, alcohol), 1727 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.80 (s, $CH_3$-19), 0.82 (s, $CH_3$-18), 3.40 (m, $CH_2$-3′), 4.05 ($t_{app.}$, $J = 2.4$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.16 (C-19), 14.01 (C-18), 19.97, 28.24, 28.83, 28.99, 31.02, 31.08, 31.33, 31.90, 32.10, 33.31, 34.61, 35.78, 36.28, 39.13, 48.22, 48.36, 49.97, 54.56, 66.39 (C-3), 222.30 (C-17); HPLC purity = 98.9% (C-18 NovaPak column, MeCN/$H_2O$/MeOH: 30/30/40); HRMS calcd for $C_{22}H_{36}O_2$ $^{79}$Br 411.1899 ($MH^+$), found 411.1892.

### *Hydrolysis of Silylated Ether 4 (Synthesis of 8)*

With the same protocol as for the synthesis of **6** and **7**, the silylated ether **4** was hydrolyzed and purified to give the diol **8** in a 68% yield.

16α-(3′-Bromopropyl)-5α-androstane-3α,17β-diol (**8**)

White solid; IR (KBr) 3376 (OH, alcohols); $^1$H NMR ($CDCl_3$) 0.75 (s, $CH_3$-19), 0.78 (s, $CH_3$-18), 3.19 (d, $J = 7.1$ Hz, CH-17α), 3.42 (t, $J = 6.8$ Hz, $CH_2$-3′), 4.04 (m, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.17 (C-19), 11.95 (C-18), 20.19, 28.36, 28.93, 30.34, 31.51, 31.66, 32.14, 34.12, 34.26, 35.25, 35.82, 36.17, 36.75, 39.15, 42.92, 43.90, 49.36, 54.46, 66.52 (C-3), 88.06 (C-17); HPLC purity = 98.3% (C-18 NovaPak column, MeCN/$H_2O$/MeOH: 30/30/40); LRMS 430 and 432 $[M + NH_4]^+$.

## Synthesis of 16-disubstituted-ADT Derivatives 14–20 (Scheme 2)

### *Dialkylation at Position 16 (Synthesis of 9–13)*

TBDMS-ADT (**1**) was dissolved in dry THF and NaH (10 eq) was added. The mixture was stirred under argon at refluxing temperature for 1 h, followed by the addition of the appropriate iodide or bromide (8 eq): methyl iodide for **9**, allyl bromide for **10**, 1,5-dibromopentane for **11** and 1,6-dibromohexane for **12** and **13**. The reaction mixture was stirred at refluxing temperature overnight, then cooled at room temperature before addition of water. Extraction was performed with EtOAc, the organic phase was washed with a saturated NaCl solution, dried over $MgSO_4$ and evaporated to dryness under

reduced pressure. The yellow oil obtained was purified by flash chromatography using a mixture of hexanes and EtOAc as eluent. In the case of the alkylation with 1,6-dibromohexane, two products were recovered, **12** and **13**, in a 7 to 1 proportion.

3α-(*t*-Butyldimethylsilyloxy)-16,16-dimethyl-5α-androstan-17-one (**9**)

White solid; 89% yield; IR (film) 1739 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.01 (s, $Si(CH_3)_2$), 0.78 (s, $CH_3$-19), 0.87 (s, $CH_3$-18), 0.88 (s, $SiC(CH_3)_3$), 1.02 (s, $CH_3$ at C-16), 1.16 (s, $CH_3$ at C-16), 3.96 ($t_{app.}$, $J = 2.5$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.84 ($Si(CH_3)_2$), 11.39 (C-19), 14.47 (C-18), 18.11 ($SiC(CH_3)_3$), 19.97, 25.89 ($SiC(CH_3)_3$), 25.95, 27.29, 28.41, 29.71 (2X), 31.15, 32.36, 32.42, 34.61, 36.21, 36.75, 37.95, 39.13, 45.15, 48.21, 54.76, 66.79 (C-3), 225.69 (C-17); LRMS 433 $[M + H]^+$, 450 $[M + NH_4]^+$.

3α-(*t*-Butyldimethylsilyloxy)-16,16-diallyl-5α-androstan-17-one (**10**)

White solid; 89% yield; IR (film) 1735 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.01 (s, $Si(CH_3)_2$), 0.76 (s, $CH_3$-19), 0.86 (s, $CH_3$-18), 0.88 (s, $SiC(CH_3)_3$), 2.16 and 2.24 (2d, $J = 7.1$ Hz and 7.4 Hz, 2 × $CH_2$-1′), 3.96 ($t_{app.}$, $J = 2.1$ Hz, CH-3β), 5.03 (m, 2 × $CH_2$-3′), 5.74 (m, 2 × CH-2′); $^{13}$C NMR ($CDCl_3$) −4.85 ($Si(CH_3)_2$), 11.33 (C-19), 14.60 (C-18), 18.09 ($SiC(CH_3)_3$), 19.89, 25.86 ($SiC(CH_3)_3$), 28.38, 29.66, 31.05, 31.97 (2X), 32.26, 34.51, 36.19, 36.71, 39.10, 40.97, 42.47, 47.85, 48.57, 52.02, 54.69, 66.74 (C-3), 117.93 and 118.02 (C-3′), 134.21 and 134.29 (C-2′), 223.59 (C-17); LRMS 485 $[M + H]^+$, 502 $[M + NH_4]^+$.

3α-(*t*-Butyldimethylsilyloxy)-16-spirocyclohexyl-5α-androstan-17-one (**11**)

White solid; 86% yield; IR (film) 1734 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.01 (s, $Si(CH_3)_2$), 0.78 (s, $CH_3$-19), 0.86 (s, $CH_3$-18), 0.88 (s, $SiC(CH_3)_3$), 3.96 ($t_{app.}$, $J = 2.5$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.85 ($Si(CH_3)_2$), 11.38 (C-19), 14.40 (C-18), 18.09 ($SiC(CH_3)_3$), 19.93, 22.43, 22.58, 25.47, 25.85 ($SiC(CH_3)_3$), 28.39, 29.67, 31.12, 31.81, 32.31 (2X), 32.95, 34.59, 36.16, 36.61, 36.70, 39.06, 48.43, 48.88, 50.38, 54.73, 66.74 (C-3), 225.31 (C-17); LRMS 473 $[M + H]^+$, 490 $[M + NH_4]^+$.

3α-(*t*-Butyldimethylsilyloxy)-16-spirocycloheptyl-5α-androstan-17-one (**12**)

White solid; 76% yield; IR (film) 1732 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.01 (s, $Si(CH_3)_2$), 0.77 (s, $CH_3$-19), 0.88 (s, $SiC(CH_3)_3$), 0.89 (s, $CH_3$-18), 3.95 ($t_{app.}$, $J = 2.6$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.81 ($Si(CH_3)_2$), 11.39 (C-19), 14.84 (C-18), 18.09 ($SiC(CH_3)_3$), 19.93, 23.84, 24.06, 25.86 ($SiC(CH_3)_3$), 28.39, 29.49, 29.54, 29.68, 31.16, 32.33, 32.48, 34.54, 35.83, 36.17, 36.48, 36.71, 38.80, 39.09, 47.99, 48.81, 52.39, 54.73, 66.74 (C-3), 224.16 (C-17); LRMS 487 $[M + H]^+$, 504 $[M + NH_4]^+$.

16,16-bis(6′-Bromohexyl)-3α-(*t*-butyldimethylsilyloxy)-5α-androstan-17-one (**13**)

White solid; 10% yield; IR (film) 1731 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.01 (s, $Si(CH_3)_2$), 0.77 (s, $CH_3$-19), 0.87 (s, $CH_3$-18), 0.88 (s, $SiC(CH_3)_3$), 3.40 (m, 2 × $CH_2$-6′), 3.96 (m, CH-3β); $^{13}$C NMR ($CDCl_3$) −4.86 ($Si(CH_3)_2$), 11.35 (C-19), 14.91 (C-18), 18.08 ($SiC(CH_3)_3$), 19.91, 24.25, 24.38, 25.39, 25.86 ($SiC(CH_3)_3$), 28.01, 28.39, 29.38, 29.53, 29.65, 31.14, 32.18, 32.25, 32.72, 32.79, 33.96 (2X), 34.10, 34.59, 35.82, 36.21, 36.69, 38.04, 39.10, 48.25, 48.82, 51.97, 54.66, 66.73 (C-3), 226.00 (C-17); LRMS 729, 731 and 733 $[M + H]^+$, 746, 748 and 750 $[M + NH_4]^+$ (according to isotopic combination of $^{79}$Br and $^{81}$Br).

### *Hydrolysis of Silylated Ethers 9–13 (Synthesis of 14–18)*

The silylated ethers **9**–**13** were hydrolysed with a methanolic solution of HCl (2%, v/v), following the procedure described above. A 90–96% yield of compounds **14**–**18** was generally obtained after purification by flash chromatography using a mixture of hexanes and EtOAc as eluent.

3α-Hydroxy-16,16-dimethyl-5α-androstan-17-one (**14**)

White solid; IR (film) 3435 (OH, alcohol), 1732 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.80 (s, $CH_3$-19), 0.88 (s, $CH_3$-18), 1.02 (s, $CH_3$ at C-16), 1.16 (s, $CH_3$ at C-16), 4.04 (m, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.15 (C-19), 14.42 (C-18), 19.91, 25.93, 27.27, 28.27, 28.98, 31.00, 32.08, 32.34, 34.52, 35.79, 36.29, 37.85, 39.13, 45.12, 48.17, 48.85, 54.64, 66.38 (C-3), 225.54 (C-17); HRMS calcd for $C_{25}H_{38}O_2$ ($M^+$) 318.2556, found 318.2565.

3α-Hydroxy-16,16-diallyl-5α-androstan-17-one (**15**)

White solid; IR (film) 3425 (OH, alcohol), 1733 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.79 (s, $CH_3$-19), 0.87 (s, $CH_3$-18), 2.20 (m, 2 × $CH_2$-1′), 4.05 (m, CH-3β), 5.05 (m, 2 × $CH_2$-3′), 5.71 (m, 2 × CH-2′); $^{13}$C NMR ($CDCl_3$) 11.15 (C-19), 14.63 (C-18), 19.90, 28.26, 28.98, 30.90, 31.99 (2X), 32.05, 34.49, 35.82, 36.32, 39.18, 41.12, 42.62, 47.92, 48.53, 52.03, 54.76, 66.43 (C-3), 117.98 (2X) (C-3′), 134.25 (2X) (C-2′), 223.54 (C-17); HPLC purity = 98.9% (C-18 Symmetry column, MeCN/$H_2O$/MeOH: 35/25/40); HRMS calcd for $C_{25}H_{38}O_2$ ($M^+$) 370.2872, found 370.2851.

3α-Hydroxy-16-spirocyclohexyl-5α-androstan-17-one (**16**)

White solid; IR (film) 3424 (OH, alcohol), 1727 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.80 (s, $CH_3$-19), 0.86 (s, $CH_3$-18), 4.04 ($t_{app.}$, $J = 2.5$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.17 (C-19), 14.41 (C-18), 19.91, 22.48, 22.60, 25.49, 28.32, 29.02, 31.05, 31.86, 32.10, 32.28, 33.04, 34.60, 35.81, 36.33, 36.67, 39.17, 48.46,

48.87, 50.36, 54.68, 66.44 (C-3), 225.05 (C-17); HRMS calcd for $C_{24}H_{38}O_2$ ($M^+$) 358.2872, found 358.2892.

3α-Hydroxy-16-spirocycloheptyl-5α-androstan-17-one (**17**)

White solid; IR (film) 3425 (OH, alcohol), 1725 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.78 (s, $CH_3$-19), 0.87 (s, $CH_3$-18), 4.03 ($t_{app.}$, $J = 2.4$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.12 (C-19), 14.78 (C-18), 19.84, 23.76, 24.03, 28.24, 28.93, 29.48, 29.52, 31.00, 32.01, 32.38, 34.44, 35.72, 35.85, 36.24, 36.47, 38.76, 39.08, 47.92, 48.72, 52.29, 54.58, 66.34 (C-3), 224.22 (C-17); HPLC purity = 98.3% (C-18 Symmetry column, MeCN/$H_2O$/MeOH: 30/15/55); HRMS calcd for $C_{25}H_{40}O_2$ ($M^+$) 372.3028, found 372.3018.

16,16-Bis(6′-bromohexyl)-3α-hydroxy-5α-androstan-17-one (**18**)

White solid; IR (film) 3426 (OH, alcohol), 1728 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.80 (s, $CH_3$-19), 0.88 (s, $CH_3$-18), 3.40 (2t, $J = 6.9$ Hz, 2X $CH_2$-6′), 4.05 (m, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.15 (C-19), 14.95 (C-18), 19.90, 24.24, 24.50, 28.04 (2X), 28.28, 28.99, 29.39, 29.53, 31.03, 32.05, 32.16, 32.77 (2X), 33.98 (2X), 34.09, 34.58, 35.78, 35.90, 36.31, 38.23, 39.18, 48.35, 48.77, 52.00, 54.70, 66.43 (C-3), 225.08 (C-17); HRMS calcd for $C_{31}H_{52}O_2\,{}^{79}Br_2$ ($M^+$) 614.2334, found 614.2317.

### *Synthesis of Trialkylated Compounds 19 and 20*

Androsterone (ADT) was submitted to an excess of NaH (15 eq) and methyl iodide (10 eq) (for compound **19**) or allyl bromide (10 eq) (for compound **20**) following the same procedure described above for the dialkylation. In the case of the alkylation with allyl bromide, 10% of dialkylated product (compound **15**) was also obtained. The crude mixtures were purified by flash chromatography using a mixture of hexanes and EtOAc as eluent.

16,16-Dimethyl-3α-methoxy-5α-androstan-17-one (**19**)

White solid; 66% yield; IR (film) 1735 (C = O, ketone); $^1$H NMR ($CDCl_3$) 0.79 (s, $CH_3$-19), 0.86 (s, $CH_3$-18), 1.00 and 1.14 (2s, 2 × $CH_3$ at C-16), 3.27 (s, $CH_3O$), 3.41 ($t_{app.}$, $J = 2.3$ Hz, CH-3β); $^{13}$C NMR ($CDCl_3$) 11.34 (C-19), 14.40 (C-18), 19.88, 25.02, 25.92, 27.25, 28.33, 30.93, 32.35, 32.47, 32.76, 34.50, 36.06, 37.85, 39.49, 45.07, 48.17, 48.82, 54.58, 55.56 ($CH_3O$), 75.33 (C-3), 225.12 (C-17); HPLC purity = 95.9% (C-18 NovaPak column, $H_2O$/MeOH: 15/85); HRMS calcd for $C_{22}H_{36}O_2$ ($M^+$) 3332.2715, found 3332.2733.

3α-(Allyloxy)-16,16-diallyl-5α-androstan-17-one (**20**)

White foam; 60% yield; IR (film) 1734 (C = O, ketone), 1638 (C = C, alkene); $^1$H NMR ($CDCl_3$) 0.79 (s, $CH_3$-19), 0.86 (s, $CH_3$-18), 2.20 (m, 2 × $CH_2$-1′), 3.57 (m, CH-3β), 3.92 (d, $J = 4.6$ Hz, $CH_2O$), 5.04 (m, 2 × $CH_2$-3′ at position 16), 5.13 and 5.26 (2d, $J = 17.0$ Hz 10.3 Hz, respectively, $CH_2$-3′ at position 3α), 5.72 (m, 2 × CH-2′ at position 16), 5.90 (m, CH-2′ at position 3α); $^{13}$C NMR ($CDCl_3$) 11.39, 14.64, 19.90, 25.60, 28.30, 30.84, 31.99 (2X), 32.58, 33.15, 34.48, 36.10, 39.56, 41.12, 42.66, 47.92, 48.54, 52.03, 54.67, 68.80 (C-3), 73.18 ($CH_2O$), 116.06, 117.96 (2X), 134.27 (2X), 135.79, 223.61 (C-17); HPLC purity = 96.0% (C-18 NovaPak column, MeCN/$H_2O$/MeOH: 45/15/40); HRMS calcd for $C_{28}H_{42}O_2$ ($M^+$) 410.3185, found 410.3167.

### Inhibition of Type 3 17β-HSD (Table I)

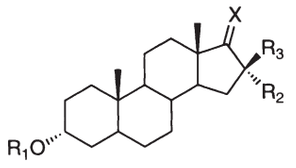
The expression vectors encoding type 3 17β-HSD were transfected into human embryonic kidney (HEK)-293 cells using the calcium phosphate procedure.[24,25] Cells were then sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM of ethylenediaminetetraacetic acid (EDTA), and centrifugated at 10,000*g* for 1 h to remove the mitochondria, plasma membranes, and cells fragments. The supernatant was further centrifugated at 100,000*g* to separate the microsomal fraction which was used as source of type 3 17β-HSD activity for the enzymatic assays.

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 EDTA, 2 mM cofactor NADPH, 0.1 μM [4-$^{14}$C] 4-androstene-3,17-dione ([$^{14}$C] $\Delta^4$-dione; New England Nuclear, Boston, MA, USA) and the indicated concentration of compounds to be tested. The reaction was stopped after 1 h by adding 2 mL of diethyl ether containing 10 μM of unlabeled 4-androstene-3,17-dione ($\Delta^4$-dione) and testosterone (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 TLC plates. TLC plates were developed in a mixture of toluene and acetone (4:1). Substrates ([$^{14}$C] $\Delta^4$-dione) and metabolites ([$^{14}$C] T) were identified by comparison with reference steroids and revealed by autoradiography, then quantified using a PhosphorImager (Molecular Dynamics, SunnyVale, CA, USA). The percentage of transformation and the percentage of inhibition were calculated from Equations (1) and (2), respectively:

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

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

TABLE I Inhibition of type 3 17β-HSD by ADT derivatives **6**–**8** and **14**–**20**




| Compounds | X | $R_1$ | $R_2$ | $R_3$ | Inhibition (%)* 0.3 μM | 3 μM |
|---|---|---|---|---|---|---|
| ADT | O | H | H | H | 50 | 88 |
| **6** | O | H | $(CH_2)_3Br$ | H | 17 | 47 |
| **7** | O | H | H | $(CH_2)_3Br$ | 14 | 39 |
| **8** | 17β-OH | H | $(CH_2)_3Br$ | H | 15 | 49 |
| **18** | O | H | $(CH_2)_6Br$ | $(CH_2)_6Br$ | 2 | 28 |
| **14** | O | H | $CH_3$ | $CH_3$ | 23 | 56 |
| **15** | O | H | $CH_2CH=CH_2$ | $CH_2CH=CH_2$ | 13 | 59 |
| **16** | O | H | $-(CH_2)_5-$ | | 23 | 65 |
| **17** | O | H | $-(CH_2)_6-$ | | 31 | 75 |
| **19** | O | $CH_3$ | $CH_3$ | $CH_3$ | 5 | 46 |
| **20** | O | $CH_2CH=CH_2$ | $CH_2CH=CH_2$ | $CH_2CH=CH_2$ | 16 | 44 |
| $\Delta^4$-dione† | O | 3-keto-4-ene | H | H | 24 | 78 |

* For the transformation of [$^{14}$C]$\Delta^4$-dione into [$^{14}$C]T; error ± 10%. † Unlabelled enzyme substrate used as inhibitor.



### Steroid Receptors Binding Affinity Assays (Table II)

The binding affinity assays on estrogen and progestin receptors from rat uterus were carried out under the standard procedure established in our laboratory.[26] The assay on androgen receptor from rat ventral prostate was performed according to the procedure described by Luo *et al.*[27] In the case of glucocorticoid receptor from rat liver, the affinity binding assay was done using a procedure described by Asselin and co-workers that we modified slightly.[28] Herein, a dextran-coated charcoal adsorption, instead of a protamine sulfate precipitation, was used to achieve the separation of bound and free steroids.

### Proliferative and Antiproliferative Activities on Shionogi ($AR^+$) Cells (Table III)

Assays of the proliferation of Shionogi mammary carcinoma cells as well as of the inhibition of 0.3 nM DHT-induced proliferation were carried out according to the procedure described previously.[27,29]

## RESULTS AND DISCUSSION

### Chemical Synthesis

Androsterone (ADT) was used as starting material for the synthesis of compounds **6**–**8** and **14**–**20**. The first two targeted bromopropyl derivatives **6** and **7** were obtained through the sequence of reactions illustrated in Scheme 1. The 3α-hydroxy group of ADT was first protected as a *tert*-butyldimethylsilyl ether using TBDMS-Cl and imidazole in DMF followed by the alkylation in alpha position of the carbonyl group of 3-TBDMS-ADT (**1**). The enolate generated with LDA from **1** was trapped with allyl bromide, leading to a mixture of the two stereoisomers 16α-allyl and 16β-allyl (88/12, evaluated by $^1$H NMR signal of $CH_3$-18) along with 10% of diallylated product. The mixture of the two stereoisomers (compound **2**) was submitted to an oxidative hydroboration ($BH_3$ in THF followed by $H_2O_2$ and NaOH) yielding a mixture of the corresponding primary alcohols **3A** and **3B**. Stereoselective reduction of the carbonyl at position 17 to secondary alcohol also occurred during this process. The stereoisomers were separated by flash chromatography after this step, as the separation was easier to perform at this point than with the allyl epimeric mixture **2**. Only the 16α-isomer **3A** was used for the next steps. Substitution of the primary alcohol with $CBr_4$ and $PPh_3$ in $CH_2Cl_2$ lead to bromide **4**. Under these conditions, the hindered secondary alcohol at C17β was not reactive. This hydroxy group was then oxidized with PCC to yield ketone **5**. A methanolic solution of HCl (2%, v/v) was used for the hydrolysis of the silylated ether of compound **5**. A TLC revealed however the presence of two products, and NMR analysis ($CH_3$-18 signal)[30] confirmed that epimerisation at position 16 occurred. The two C16 stereoisomers **6** and **7** were thus obtained after chromatography, the 16α-isomer being the major product. Other hydrolysis conditions: HF/$CH_3CN$, room temperature and HF-Py/THF, 0°C, also led to epimerisation at position 16; no hydrolysis occurred with TBAF/THF at 0°C or at room temperature. Hydrolysis of the

TABLE II Binding affinities (%) of compounds **6**–**8** and **14**–**20** for steroid receptors

| | Androgen receptor (%) | | Estrogen receptor (%) | | Glucocorticoid receptor (%) | | Progestin receptor (%) | |
|---|---|---|---|---|---|---|---|---|
| Compounds | 10 nM | 1 μM | 10 nM | 1 μM | 10 nM | 1 μM | 10 nM | 1 μM |
| ADT | 0 ± 2 | 2 ± 2 | 0 ± 1 | 0 ± 2 | 1 ± 1 | 4 ± 1 | 0 ± 3 | 0 ± 4 |
| **6*** | 1 ± 1 | 14 ± 1 | 0 ± 2 | 0 ± 2 | 3 ± 1 | 0 ± 1 | 4 ± 1 | 8 ± 1 |
| **7*** | 1 ± 1 | 1 ± 1 | 0 ± 3 | 1 ± 2 | 6 ± 2 | 5 ± 2 | 4 ± 2 | 4 ± 2 |
| **8*** | 4 ± 1 | 70 ± 1 | 0 ± 2 | 3 ± 3 | 5 ± 1 | 3 ± 2 | 4 ± 1 | 11 ± 3 |
| **14**† | – | – | – | – | – | – | – | – |
| **15** | 1 ± 1 | 1 ± 2 | 5 ± 2 | 0 ± 3 | 0 ± 1 | 1 ± 2 | 0 ± 2 | 0 ± 2 |
| **16**† | – | – | – | – | – | – | – | – |
| **17** | 1 ± 1 | 1 ± 1 | 2 ± 1 | 4 ± 1 | 0 ± 3 | 0 ± 1 | 0 ± 2 | 0 ± 1 |
| **18**† | – | – | – | – | – | – | – | – |
| **19** | 0 ± 2 | 0 ± 2 | 0 ± 1 | 0 ± 2 | 0 ± 3 | 0 ± 1 | 0 ± 2 | 0 ± 1 |
| **20**† | – | – | – | – | – | – | – | – |
| DHT‡ | 70 ± 1 | 100 ± 1 | 2 ± 2 | 4 ± 1 | 2 ± 2 | 6 ± 2 | 3 ± 2 | 40 ± 2 |
| $E_2$‡ | 0 ± 2 | 34 ± 1 | 75 ± 1 | 100 ± 1 | 5 ± 2 | 12 ± 2 | 6 ± 3 | 25 ± 2 |
| DEX‡ | 0 ± 1 | 2 ± 1 | 0 ± 3 | 0 ± 1 | 66 ± 2 | 99 ± 1 | 0 ± 3 | 1 ± 2 |
| R5050‡ | 1 ± 4 | 28 ± 2 | 5 ± 2 | 4 ± 1 | 9 ± 2 | 85 ± 2 | 65 ± 2 | 99 ± 2 |

*Concentrations are 0.1 μM and 10 μM instead of 10 nM and 1 μM. †Data not available. ‡DHT: dihydrotestosterone; $E_2$: estradiol; DEX: dexamethasone; R5050: synthetic progestin.

intermediate silylated ether **4** under the same acid conditions led to the formation of compound **8**.

The dialkylation at position 16 was performed from TBDMS-ADT (**1**), using NaH in excess and the corresponding halide or dihalide compound in refluxing THF,[31] to afford compounds **9**–**13** (Scheme 2). The TBDMS protecting group was thereafter removed in a methanolic HCl (2%, v/v) solution leading to compounds **14**–**18**. A similar alkylation was performed directly on ADT to obtain its trialkyl derivatives **19** and **20**.

## Inhibition of Type 3 17β-HSD

The evaluation of the ability of the described compounds to inhibit type 3 17β-HSD activity was done with transfected HEK-293 cells. Using cell homogenate microsomal fraction, we measured the amount of testosterone formed from labeled natural substrate $\Delta^4$-dione in the presence of NADPH as cofactor. The results of enzymatic assays are summarized in Table I. ADT which gave the best inhibition in a previous screening study was used as a reference compound.[19] Unfortunately, substituting the ADT nucleus at position 16 with a bromopropyl side chain (compounds **6**–**8**) led to a lower inhibitory activity. The orientation (α or β) of the side chain made no difference. Similarly, compound **18**, obtained during the synthesis of **17** and bearing two bromohexyl side chains at position 16, also gave poor results. So even though adding a bromoalkyl side chain at position 16 of an estradiol nucleus led to the synthesis of potent irreversible inhibitors of type 1 17β-HSD,[21–23] adding such a chain on an ADT nucleus did not yield good type 3 17β-HSD inhibitors. We then wanted to see the effect of a hydrophobic chain at C16-position of an ADT nucleus, in order to investigate possible hydrophobic interactions in the active site of the enzyme. Compounds **14**–**17** were synthesized and tested. Again, the percentages of inhibition were lower than that of ADT. While exploring the position 16 of the ADT nucleus, we decided to evaluate the C3-ether analogs of **14** and **15**. Compounds **19** and **20** were thus synthesized and tested, but their inhibitory activity was not higher than that of **14** and **15**.

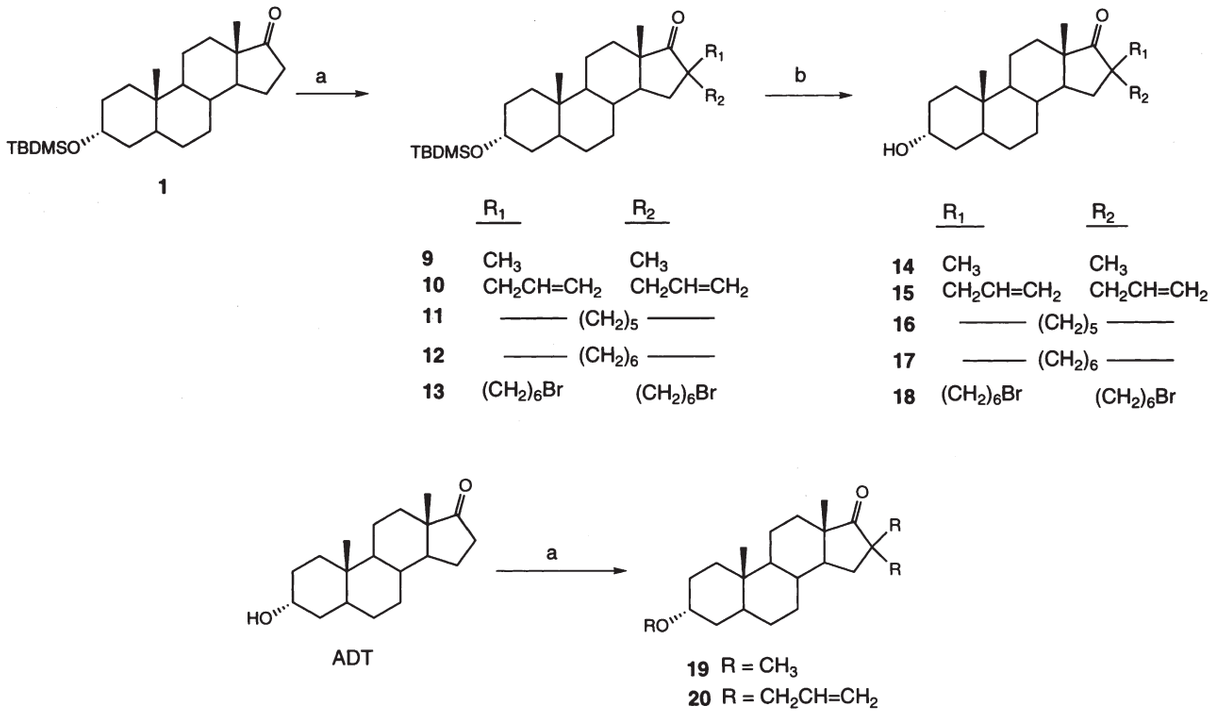
TABLE III Proliferative and antiproliferative activities of compounds **6**–**8** and **14**–**20** on Shionogi ($AR^+$) cells

| | Proliferative activity (%) | | Antiproliferative activity (%) | |
|---|---|---|---|---|
| Compounds | 0.1 μM | 1 μM | 0.1 μM | 1 μM |
| ADT | 14 | 0 | 0 | 20 |
| **6** | 0 | 0 | 6 | 49 |
| **7** | 32 | 52 | 13 | 0 |
| **8** | 0 | 0 | 29 | 86 |
| **14** | 12 | 0 | 29 | 43 |
| **15** | 0 | 0 | 0 | 18 |
| **16** | 0 | 16 | 2 | 35 |
| **17** | 5 | 0 | 0 | 36 |
| **18** | 0 | 7 | 11 | 16 |
| **19** | 5 | 0 | 2 | 22 |
| **20** | 0 | 0 | 0 | 16 |
| OH–Flu* | 0 | 0 | 56 | 100 |

*OH–Flu: hydroxyflutamide (pure antiandrogen).[32,33]

## Binding Affinity for Steroid Receptors

When designing an inhibitor for a steroidogenic enzyme, it is important to see whether or not it binds to steroid receptors, in order to avoid unsuitable side effects in the case of a therapeutic use of the product. Thus, the binding affinities of our compounds for four steroidal receptors: androgen, estrogen, glucocorticoid and progestin receptors were evaluated (Table II). As expected, they showed no affinity at all for the estrogen receptor since the chemical




| | $R_1$ | $R_2$ |
|---|---|---|
| **9** | $CH_3$ | $CH_3$ |
| **10** | $CH_2CH{=}CH_2$ | $CH_2CH{=}CH_2$ |
| **11** | $(CH_2)_5$ | |
| **12** | $(CH_2)_6$ | |
| **13** | $(CH_2)_6Br$ | $(CH_2)_6Br$ |

| | $R_1$ | $R_2$ |
|---|---|---|
| **14** | $CH_3$ | $CH_3$ |
| **15** | $CH_2CH{=}CH_2$ | $CH_2CH{=}CH_2$ |
| **16** | $(CH_2)_5$ | |
| **17** | $(CH_2)_6$ | |
| **18** | $(CH_2)_6Br$ | $(CH_2)_6Br$ |

SCHEME 2 Chemical synthesis of C16-disubstituted ADT derivatives **14**–**20**. Reagents: (a) NaH, RI(Br), THF, Δ; (b) MeOH–HCl (2%), r.t.



structures of our compounds (C19 steroids) are very different from that of the natural estrogen estradiol (a C18 steroid). Interestingly, they did not show significant affinities for glucocorticoid and progestin receptors either. These compounds, however, were expected to show some affinity for the androgen receptor, because they are C19 steroids like DHT, the natural substrate. But the percentages of binding affinities obtained were not significant, except for compound **8**, the only one with a 17β-hydroxy group instead of a carbonyl. In fact, active androgens T and DHT possess a hydroxyl at position 17β, confirming its importance for the binding affinity on the androgen receptor.

### Proliferative and Antiproliferative Assays on Shionogi Cells

Shionogi cells are known to be androgen receptor positive. The proliferative and antiproliferative properties of the described compounds on this cell line were evaluated and compared to those of hydroxyflutamide, which is a potent *in vitro* antiandrogen (Table III).[32,33] None of the C19 steroid derivatives substituted at position 16 stimulates the proliferation of Shionogi cells, except compound **7**, which shows a proliferative activity (32% at 0.1 μM and 52% at 1 μM). This proliferative activity, however, is not mediated by the androgen receptor, since no binding affinity is associated with compound **7**. The antiproliferative activity was measured by the inhibition of 0.3 nM DHT-induced proliferation on Shionogi cells. At 1 μM, almost all the compounds show slight antiproliferative activities, but compound **8**, which binds to the androgen receptor, shows the greatest effect (29% at 0.1 μM and 86% at 1 μM), comparable to that of hydroxyflutamide (100% at 1 μM), the active metabolite of the well known pure antiandrogen flutamide.

In conclusion, a series of ADT derivatives substituted at position 16 have been synthesized in short sequences of reactions. These compounds are poor inhibitors of the transformation of the natural substrate $\Delta^4$-dione into the androgenic metabolite testosterone by type 3 17β-HSD. However, they do not bind to estrogen, glucocorticoid and progestin receptors, suggesting they do not have unsuitable residual agonist activity on these three steroid receptors. Furthermore, these androsterone derivatives did not show unsuitable proliferative activity on Shionogi cells. Interestingly, 16α-(3′-bromopropyl)-5α-androstane-3α,17β-diol (**8**) was the only one to bind to the androgen receptor and to show an antiproliferative activity on Shionogi cells, an activity being comparable to that of the pure antiandrogen hydroxyflutamide at the concentrations studied. Although this exploratory work at C16-position of ADT did not provide good inhibitors of type 3 17β-HSD, it allowed us to identify compound **8**, thus paving the way to a new family of antiandrogens.

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