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Chemical synthesis of 16β-propylaminoacyl derivatives of estradiol and their inhibitory potency on type 1 17β-hydroxysteroid dehydrogenase and binding affinity on steroid receptors

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Abstract

The 17β-hydroxysteroid dehydrogenases (17β-HSDs) are members of a family of enzymes that catalyze the interconversion of weakly active sexual hormones (ketosteroids) and potent hormones (17β-hydroxysteroids). Among the known isoforms of 17β-HSD, the type 1 catalyzes the NAD(P)H-mediated reduction of estrone (E_1) to estradiol (E_2), a predominant mitogen for the breast cancer cells. Therefore, the inhibition of this particular enzyme is a logical approach to reduce the concentration of estradiol in breast tumors. To develop inhibitors of type 1 17β-HSD activity, we hypothesized that molecules containing both hydrophobic and hydrophilic components should be interesting candidates for interacting with both the steroid binding domain and some amino acid residues of the cofactor binding domain of the enzyme. Firstly, a conveniently protected 16β-(3-aminopropyl)-E_2 derivative was synthesized from commercially available E_1. Then, a representative of all class of NHBoc-protected amino acids (basic, acid, aromatic, aliphatic, hydroxylated) were coupled using standard procedures to the amino group of the precursor. Finally, cleavage of all protecting groups was performed in a single step to generate a series of 16β-propylaminoacyl derivatives of E_2. The enzymatic screening revealed that none of the novel compounds can inhibit the reductive activity of type 1 17β-HSD. On the other hand, all of these E_2 derivatives did not show any significant binding affinity on four steroid receptors including the estrogen receptor. Additional efforts aimed at improving the inhibitory potency of these steroidal derivatives on type 1 17β-HSD without providing estrogenic activities is under investigation using a combinatorial chemistry approach.

Keywords: Steroid; Peptidosteroid; Estradiol; Hydroxysteroid dehydrogenase; Chemical synthesis; NMR data

1. Introduction

Estrogens and peptide growth factors are known to promote the proliferation of an important proportion (46–77%) of breast tumor cells [1,2]. Estrogens exert their action via nuclear estrogen receptors (ERα and ERβ) giving transcriptional activator complexes that bind to specific regulating sequences of estrogen sensitive genes [3]. Based on this principle, antiestrogens were designed to interfere with the binding of estrogens to their receptors and/or the binding of the complex to DNA. Nonsteroidal antiestrogen such as tamoxifen [4,5], raloxifen [6,7], and EM-800 [8–13] have shown very promising effects in both in vitro and in vivo systems and are now used in clinical trials for the prevention and the treatment of breast cancers. The history of steroidal antiestrogens is shorter, but resulted in interesting activities displayed by ICI 164384 [14], ICI 182780 [15], and RU 58810 [16]. These studies have clearly demonstrated the importance of a long alkylamide side chain at position 7α or 11β of estradiol (E_2) for their antiestrogenic activities. On the other hand, our studies proved that similar side chains do not provide potent antiestrogenic activity when they substitute position 17α, 16α, 15α and 15β of E_2 [17,18]. Several other derivatives of E_2 and their relative ER binding activities were reported resulting in very interesting structure-activity relationships (SAR) summarized in a review article [19]. All these data are very important because the 3D structure of ER is only partially known [20,21].

In a complementary approach to treat estrogen-sensitive diseases such as breast cancer, we want to develop inhibi-

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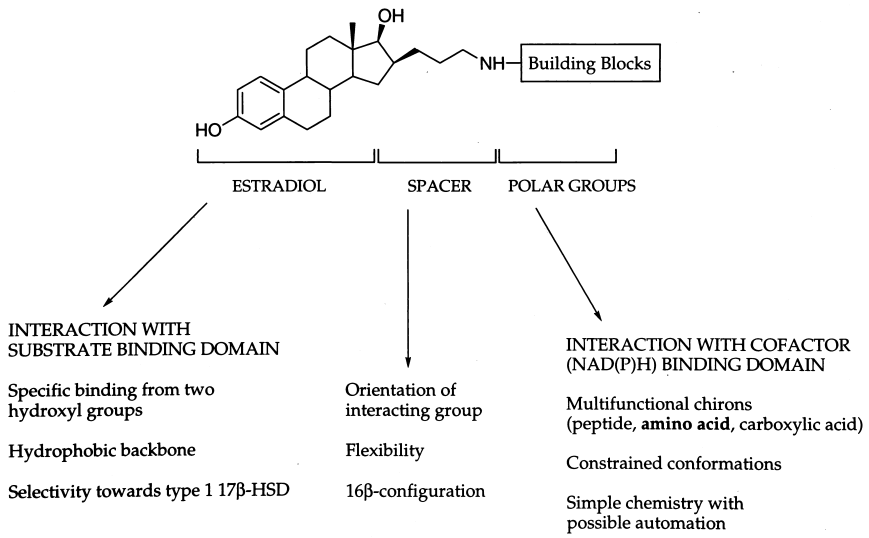


Fig. 1. Hypothesis for the design of prototype type 1 17β-HSD inhibitors.

tors of type 1 17β-hydroxysteroid dehydrogenase (17β-HSD), which is responsible for the last enzymatic step in the biosynthesis of E_2, the most potent estrogen. This enzyme catalyzes preferentially the reduction of estrone (E_1) to E_2 in intact cells [22–25] and is expressed in about 50% of the breast tumor specimens [25–27]. For their effectiveness, these inhibitors should be devoid of any estrogenic activity and, if possible, they may display antiestrogenic activity (dual-action blockers). Recently, the 3D structures of type 1 17β-HSD, alone and complexed with substrates E_2 and cofactor, have been elucidated [28–31]. It has been clearly indicated that the consensus sequence Tyr-X-X-X-Lys together with a serine are the most important amino acid residues involved in the catalytic reaction. Particularly, Tyr-155 and Ser-142 are involved in an interaction with either the ketone or the hydroxyl at the position 17. Moreover, residues that allows high specificity in binding of C18 steroid: His-221 and Glu-282 forms hydrogen bond with the phenolic hydroxyl group at position 3. As expected, several residues that interact with the substrate are hydrophobic (Val, Met, Leu, Pro, Phe), while most of the residues that bind to the cofactor are polar (Cys, Ser, Arg, Asp, Asn, Lys). Therefore, we hypothesized that an inhibitor of type 1 17β-HSD should possess both these structural elements, namely hydrophobic and hydrophilic components. In an exploratory study, we then decided to synthesize E_2 derivatives (compounds **12–20**) bearing various polar chemical groups (Fig. 1 and Scheme 1) as prototype inhibitors of type 1 17β-HSD.

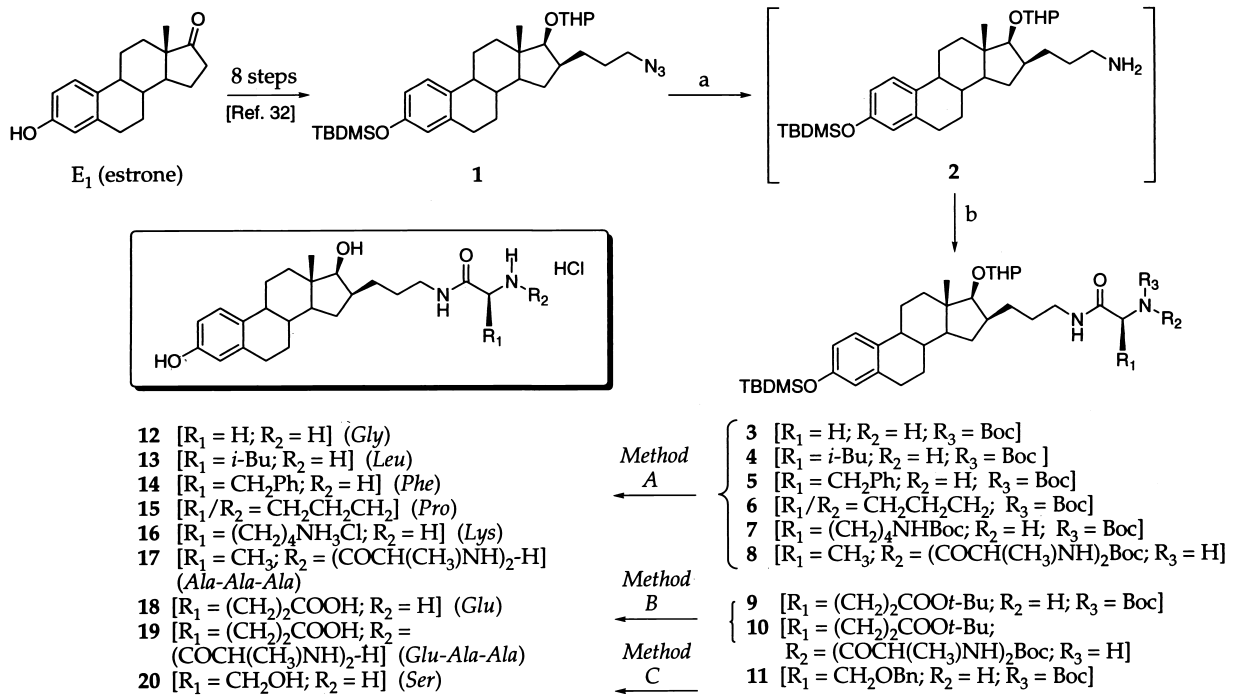
2. Experimental

2.1. General methods

Reagents were obtained from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Usual solvents were obtained from Fisher Scientific (Montreal, Canada) and were used as received. Anhydrous solvents were obtained from Aldrich in SureSeal bottles, which were conserved under positive argon pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. All anhydrous reactions were performed in oven-dried glassware under positive argon pressure. Flash chromatographies were performed on E. Merck 60 230-400 mesh silica gel. Thin-layer chromatographies were performed on 0.25 mm E. Merck silica gel 60 F_{254} plates and visualized by UV (254 nm) and/or cerium ammonium molybdate. Infrared (IR) spectra were obtained from a KBr pellet with the solid compound or from a thin film on NaCl pellet with the solubilized compound (usually in $CDCl_3$). They were recorded on a Pelkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only significant bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were reported in ppm and recorded at 300 MHz for ^1H and 75.5 MHz for ^{13}C on a Bruker AC/F300 spectrometer (Bruker, Billerica, MA, USA). Only significant signals were reported for ^1H NMR while all signals were listed for ^{13}C NMR. Low-resolution mass spectra were recorded with a PE Sciex API-150ex apparatus (Foster City, CA) equipped with a turbo ionspray source.

2.2. Synthesis of 16β-propylaminoacyl derivatives of E_2 (Scheme 1)

*Preparation of the intermediate amine **2**:* To a suspension of prereduced palladium 5% on activated carbon (300 mg) in EtOAc (20 ml) was added a solution of the azide **1** [32] (3.43 g; 6.20 mmol) in 30 ml of EtOAc. The resulting mixture was stirred under hydrogen atmosphere at 25°C for 16 h. Then, celite was added to the reaction mixture and the slurry was filtered through a celite pad eluting with EtOAc. The organic solvent was evaporated under reduced pressure



Scheme 1. Reagents and conditions: (a) H_2, Pd/C, EtOAc, rt, 1 atm; (b) NHBoc-*L*-AA-COOH, DCC, HOBt, CH_2Cl_2, 0°C (30–66%, two steps); *Method A*: 3 N HCl, MeOH, rt (47–90%); *Method B*: HCl (anh.), CH_2Cl_2, 0°C (33–92%); *Method C: i.* 3 N HCl, MeOH, rt, *ii.* H_2, Pd/C, EtOH, 60 psi (46%, two steps).

to give 3.4 g of the amine **2** in excellent purity. Therefore, this compound was used for the coupling reaction without further purification.

Typical procedure for amino acid coupling using DCC/HOBt method: The N-*t*-Boc amino acid or peptide obtained by a standard method [33] (1.0 eq.) was dissolved in dry CH_2Cl_2 under argon and the solution was cooled at 0°C before sequential addition of DCC and HOBt (1.0 eq., each). The resulting mixture was stirred for 1 h at 0°C. Then, the amine **2** (1.0 eq.), dissolved in dry CH_2Cl_2, was added dropwise and the reaction mixture was allowed to warm very slowly to room temperature. After 3 h, the reaction mixture was filtered and diluted in CH_2Cl_2. The organic phase was washed successively with a saturated aqueous solution of $NaHCO_3$, water, a 1N HCl solution, and brine. The organic layers were dried over $MgSO_4$, and the solvents were evaporated to dryness. The crude compound was purified by flash chromatography to give pure amino-acyl estradiol derivatives **3–11**.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(t-butoxycarbonylamino)-acetamide (***3***). White amorphous solid (54% yield); IR (NaCl, film): 3320 (NH), 1705 (C=O, carbamate), 1665 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.17 (s, 6H, $Si(CH_3)_2$), 0.77 and 0.82 (2s, 3H, 18-CH_3), 0.96 (s, 9H, $SiC(CH_3)_3$), 1.45 (s, 9H, $(CH_3)_3C$ of N-Boc), 2.78 (m, 2H, 6-CH_2), 3.24 (m, 2H, NCH_2 of 16β-propyl), 3.47 and 3.87 (2m, 2H, OCH_2 of THP), 3.71 (d, J = 9.7 Hz, H, 17α-CH), 3.76 (m, 2H, NCH_2 of glycyl), 4.59 and 4.66 (2m, 1H, CH of THP), 5.20 (m, 1H, NH), 6.20 (m, 1H, NH), 6.54 (s_{app}, 1H, 4-CH), 6.59 (d_{app}, J = 8.4 Hz, 1H, 2-CH), 7.11 (2d, J = 8.4 Hz, 1H, 1-CH); LRMS: calcd for $C_{39}H_{65}N_2O_6Si$ $[M+H]^+$ 685.5, found 685.6 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-isobutyl-2-(t-butoxycarbonylamino)-acetamide (***4***). White amorphous solid (40% yield); IR (NaCl, film): 3300 (NH), 1700 (C=O, carbamate), 1650 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.17 (s, 6H, $Si(CH_3)_2$), 0.77 and 0.82 (2s, 3H, 18-CH_3), 0.93 (d, J = 5.9 Hz, 6H, 2 × CH_3 of isobutyl), 0.97 (s, 9H, $SiC(CH_3)_3$), 1.43 (s, 9H, $(CH_3)_3C$ of N-Boc), 2.78 (m, 2H, 6-CH_2), 3.23 (m, 2H, NCH_2 of 16β-propyl), 3.49 and 3.92 (2m, 2H, OCH_2 of THP), 3.71 and 3.78 (2d, J = 9.6 Hz, 1H, 17α-CH), 4.10 (m, 1H, NCH of leucyl), 4.62 and 4.68 (2m, 1H, CH of THP), 5.00 (m, 1H, NH), 6.30 (m, 1H, NH), 6.54 (d, J = 2.4 Hz, 1H, 4-CH), 6.58 (dd, J_1 = 8.5 Hz and J_2 = 2.4 Hz, 1H, 2-CH), 7.11 (d, J = 8.5 Hz, 1H, 1-CH); LRMS: calcd for $C_{43}H_{71}N_2O_6Si$ $[M-H]^-$ 739.5, found 739.4 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-benzyl-2-(t-butoxycarbonylamino)-acetamide (***5***). White amorphous solid (49% yield); IR (NaCl, film): 3300 (NH), 1700 (C=O, carbamate), 1655 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.18 (s, 6H, $Si(CH_3)_2$), 0.76 and 0.81 (2s, 3H, 18-CH_3), 0.97 (s, 9H, $SiC(CH_3)_3$), 1.41 (s, 9H, $(CH_3)_3C$ of N-Boc), 2.79 (m, 2H, 6-CH_2), 3.04 (m, 2H, $C\underline{H}_2Ph$), 3.20 (m, 2H, NCH_2 of 16β-propyl), 3.48 and 3.92 (2m, 2H, OCH_2 of THP), 3.69 and 3.76 (2d, J = 9.8 Hz, 1H, 17α-CH), 4.26 (m, 1H, NCH of phenylalanyl), 4.57 and 4.67

(2m, 1H, CH of THP), 5.10 (m, 1H, NH), 5.76 and 5.85 (2m, 1H, NH), 6.54 (d, J = 2.3 Hz, 1H, 4-CH), 6.60 (dd, J_1 = 8.4 Hz and J_2 = 2.3 Hz, 1H, 2-CH), 7.11 (2d, J = 8.4 Hz, 1H, 1-CH), 7.25 (m, 5H, Ph of phenylalanyl); LRMS: calcd for $C_{46}H_{71}N_2O_6Si$ $[M+H]^+$ 775.5, found 776.0 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(N-t-butoxycarbonylpyrrolidinyl)-acetamide (***6***). White amorphous solid (44% yield); IR (NaCl, film): 3325 (NH), 1692 (C=O, carbamate and amide); ^{1}H NMR ($CDCl_3$): 0.17 (s, 6H, $Si(CH_3)_2$), 0.77 and 0.82 (2s, 3H, 18-CH_3), 0.96 (s, 9H, $SiC(CH_3)_3$), 1.45 (s, 9H, $(CH_3)_3C$ of N-Boc), 2.78 (m, 2H, 6-CH_2), 3.20 (m, 2H, NCH_2 of 16β-propyl), 3.50 (m, 3H, NCH_2 of pyrrolidinyl and 0.5 × OCH_2 of THP), 3.90 (2m, 1H, 0.5 × OCH_2 of THP), 3.70 and 3.78 (2d, J = 9.8 Hz, 1H, 17α-CH), 4.20 (m, 1H, NCH of propyl), 4.58 and 4.69 (2m, 1H, CH of THP), 6.53 (d, J = 2.4 Hz, 1H, 4-CH), 6.59 (dd, J_1 = 8.3 Hz and J_2 = 2.4 Hz, 1H, 2-CH), 7.10 (2d, J = 8.3 Hz, 1H, 1-CH); LRMS: calcd for $C_{42}H_{69}N_2O_6Si$ $[M+H]^+$ 725.5, found 725.6 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-[4-(t-butoxycarbonylamino)-butyl]-2-(t-butoxycarbonyl-amino)-acetamide (***7***). White amorphous solid (66% yield); IR (NaCl, film): 3320 (NH), 1696 (C=O, carbamate), 1660 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.17 (s, 6H, $Si(CH_3)_2$), 0.77 and 0.82 (2s, 3H, 18-CH_3), 0.97 (s, 9H, $SiC(CH_3)_3$), 1.44 (s, 18H, $(CH_3)_3C$ of N-Boc), 2.78 (m, 2H, 6-CH_2), 3.10 (m, 2H, NCH_2 of lysyl), 3.25 (m, 2H, NCH_2 of 16β-propyl), 3.50 and 3.90 (2m, 2H, OCH_2 of THP), 3.70 and 3.77 (2d, J = 9.6 Hz, 1H, 17α-CH), 4.00 (m, 1H, NCH of lysyl), 4.58 and 4.69 (2m, 1H, CH of THP), 4.60 (m, 1H, NH), 5.10 (m, 1H, NH), 6.20 (m, 1H, NH), 6.54 (d, J = 2.3 Hz, 1H, 4-CH), 6.59 (dd, J_1 = 8.4 Hz and J_2 = 2.3 Hz, 1H, 2-CH), 7.10 (2d, J = 8.4 Hz, 1H, 1-CH); LRMS: calcd for $C_{48}H_{82}N_3O_8Si$ $[M+H]^+$ 856.6, found 856.5 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-methyl-2-{N^{α}-[N^{β}-(t-butoxycarbonylamino)-L-alanyl]-L-alanyl}-acetamide (***8***). White amorphous solid (26% yield); IR (NaCl, film): 3275 (NH), 1698 (C=O, carbamate), 1670 and 1630 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.17 (s, 6H, $Si(CH_3)_2$), 0.77 and 0.81 (2s, 3H, 18-CH_3), 0.97 (s, 9H, $SiC(CH_3)_3$), 1.44 (s, 9H, $(CH_3)_3C$ of N-Boc), 2.77 (m, 2H, 6-CH_2), 3.23 (m, 2H, NCH_2 of 16β-propyl), 3.50 and 3.90 (2m, 2H, OCH_2 of THP), 3.70 and 3.77 (2d, J = 9.6 Hz, 1H, 17α-CH), 4.20 (m, 1H, NCH of NHBoc-Ala), 4.42 (q, J = 6.8 Hz, 1H, NCH of alanyl), 4.54 (q, J = 7.5 Hz, 1H, NCH of alanyl), 4.58 and 4.72 (2m, 1H, CH of THP), 5.35 (m, 1H, NH), 6.54 (d, J = 2.4 Hz, 1H, 4-CH), 6.59 (dd, J_1 = 8.5 Hz and J_2 = 2.4 Hz, 1H, 2-CH), 6.90 (m, 1H, NH), 7.09 (m, 2H, 1-CH and NH), 7.22 (m, 1H, NH); LRMS: calcd for $C_{46}H_{77}N_4O_8Si$ $[M+H]^+$ 841.5, found 841.7 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-t-butoxycarbonylpropan-3-yl)-2-(t-butoxycarbonyl-amino)-acetamide (***9***). White amorphous solid (50% yield); IR (NaCl, film): 3330 (NH), 1730 (C=O, ester), 1690 (C=O, carbamate), 1658 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.18 (s, 6H, $Si(CH_3)_2$), 0.78 and 0.82 (2s, 3H, 18-CH_3), 0.97 (s, 9H, $SiC(CH_3)_3$), 1.43 and 1.45 (s, 18H, $(CH_3)_3C$ of N-Boc and *t*-butyl ester), 2.78 (m, 2H, 6-CH_2), 3.25 (m, 2H, NCH_2 of 16β-propyl), 3.49 and 3.93 (2m, 2H, OCH_2 of THP), 3.71 and 3.78 (2d, J = 9.9 Hz, 1H, 17α-CH), 4.08 (m, 1H, NCH of glutamyl), 4.59 and 4.69 (2m, 1H, CH of THP), 5.30 (m, 1H, NH), 6.33 (m, 1H, NH), 6.54 (d, J = 2.2 Hz, 1H, 4-CH), 6.59 (d_{app}, J = 8.4 Hz, 1H, 2-CH), 7.09 and 7.11 (2d, J = 8.4 Hz, 1H, 1-CH); LRMS: calcd for $C_{41}H_{69}N_2O_7Si$ $[M+H\text{-}THP]^+$ 729.5, found 729.0 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-t-butoxycarbonylpropan-3-yl)-2-{N^{α}-[N^{β}-(t-butoxy-carbonylamino)-L-alanyl]-L-alanyl}-acetamide (***10***). White amorphous solid (35% yield); IR (NaCl, film): 3280 (NH), 1731 (C=O, ester), 1700 (C=O, carbamate), 1667 and 1633 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.18 (s, 6H, $Si(CH_3)_2$), 0.77 and 0.81 (2s, 3H, 18-CH_3), 0.97 (s, 9H, $SiC(CH_3)_3$), 1.39 and 1.41 (2d, J = 6.4 Hz, 6H, 2 × CH_3 of alanyl), 1.43 and 1.44 (2s, 18H, $(CH_3)_3C$ of N-Boc and *t*-butyl ester), 2.78 (m, 2H, 6-CH_2), 3.22 (m, 2H, NCH_2 of 16β-propyl), 3.50 and 3.90 (2m, 2H, OCH_2 of THP), 3.71 and 3.78 (2d, J = 9.6 Hz, 1H, 17α-CH), 4.12 (m, 1H, NCH of NHBoc-Ala), 4.35 (m, 2H, NCH of alanyl and glutamyl), 4.59 and 4.67 (2m, 1H, CH of THP), 5.05 (m, 1H, NH), 6.54 (d, J = 2.4 Hz, 1H, 4-CH), 6.59 (dd, J_1 = 8.5 Hz and J_2 = 2.4 Hz, 1H, 2-CH), 6.78 (m, 2H, NH), 7.05 and 7.09 (2d, J = 8.5 Hz, 1H, 1-CH), 7.13 (m, 1H, NH); LRMS: calcd for $C_{52}H_{87}N_4O_{10}Si$ $[M+H]^+$ 955.6, found 955.8 m/z.

N-{3-[3-(t-butyldimethylsilyloxy)-17β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-benzyloxymethyl)-2-(t-butoxycarbonylamino)-acetamide (***11***). White amorphous solid (33% yield); IR (NaCl, film): 3325 (NH), 1715 (C=O, carbamate), 1660 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.19 (s, 6H, $Si(CH_3)_2$), 0.78 and 0.83 (2s, 3H, 18-CH_3), 0.98 (s, 9H, $SiC(CH_3)_3$), 1.45 (s, 9H, $(CH_3)_3C$ of N-Boc), 2.79 (m, 2H, 6-CH_2), 3.30 (m, 2H, NCH_2 of 16β-propyl), 3.50 and 3.90 (2m, 4H, OCH_2 of THP and OCH_2 of seryl), 3.70 and 3.78 (2d, J = 9.8 Hz, 1H, 17α-CH), 4.25 (m, 1H, NCH of seryl), 4.55 (m, 2H, OCH_2Ph), 4.58 and 4.67 (2m, 1H, CH of THP), 5.40 (m, 1H, NH), 6.50 (m, 1H, NH), 6.55 (d, J = 2.1 Hz, 1H, 4-CH), 6.60 (dd, J_1 = 8.5 Hz and J_2 = 2.1 Hz, 1H, 2-CH), 7.05 and 7.09 (2d, J = 8.5 Hz, 1H, 1-CH), 7.31 (m, 5H, Ph); LRMS: calcd for $C_{47}H_{73}N_2O_7Si$ $[M+H]^+$ 805.5, found 805.4 m/z.

Typical procedure for the deprotection of acid labile protecting groups. *Method A (synthesis of compounds **12–17**):* To cooled solutions of protected compounds **3–8** in MeOH at 0°C was added concentrated hydrochloric acid to give a final concentration of 3 N. The mixture was stirred at 0°C for 2 h and the solvent was evaporated to dryness at room temperature under vacuum. Then, the crude com-

pounds were triturated in diethyl ether, filtered, and dried under vacuum. *Method B (synthesis of compounds **18** and **19**)*: To cooled solutions of the protected compounds **9** and **10** in dry CH_2Cl_2 at 0°C, anhydrous hydrogen chloride was gently bubbled twice for 15 min. Then, solvent was evaporated, the crude compounds were triturated with diethyl ether, washed with CH_2Cl_2, filtered, and dried under vacuum. *Method C (synthesis of compound **20**)*: To a cooled solution of the protected compound **11** in MeOH at 0°C was added concentrated hydrochloric acid to give a final concentration of 3 N. The mixture was stirred at 0°C for 2 h and the solvent was evaporated to dryness at room temperature under vacuum. The crude compound was then suspended in EtOH containing 10% palladium on activated carbon, and the resulting slurry was submitted to hydrogen pressure (60 psi) for 24 h at room temperature. Afterward, the suspension was filtered through celite and the solvent was evaporated under reduced pressure. The crude compound was triturated in diethyl ether, filtered, and dried under vacuum.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(ammonium hydrochloride)-acetamide (**12**)*. White solid (47% yield); IR (KBr): 3200 (br, OH and NH), 1693 (C=O, amide); ^{1}H NMR (CD_3OD): 0.76 (s, 3H, 18-CH_3), 2.75 (m, 2H, 6-CH_2), 3.25 (m, 2H, NCH_2 of 16β-propyl), 3.65 (s, 2H, NCH_2 of glycyl), 3.71 (d, J = 9.8 Hz, 1H, 17α-CH), 6.47 (d, J = 2.6 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.5 Hz and J_2 = 2.6 Hz, 1H, 2-CH), 7.06 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{23}H_{35}N_2O_3$ [M-HCl+H]$^+$ 387.3, found 387.4 m/z.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-isopropyl-2-(ammonium hydrochloride)-acetamide (**13**)*. White solid (81% yield); IR (KBr): 3300 (br, OH and NH), 1645 (C=O, amide); ^{1}H NMR (CD_3OD): 0.76 (s, 3H, 18-CH_3), 1.00 (AB system, J = 4.0 Hz, 6H, 2 × CH_3 of isopropyl), 2.75 (m, 2H, 6-CH_2), 3.25 (m, 2H, NCH_2 of 16β-propyl), 3.70 (d, J = 9.8 Hz, 1H, 17α-CH), 3.83 (AB system, J = 5.6 Hz, 1H, NCH of leucyl), 6.47 (d, J = 2.5 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.5 Hz and J_2 = 2.5 Hz, 1H, 2-CH), 7.05 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{27}H_{43}N_2O_3$ [M-HCl+H]$^+$ 443.3, found 443.5 m/z.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-benzyl-2-(ammonium hydrochloride)-acetamide (**14**)*. White solid (51% yield); IR (KBr): 3345 (br, OH and NH), 1654 (C=O, amide); ^{1}H NMR (CD_3OD): 0.75 (s, 3H, 18-CH_3), 2.76 (m, 2H, 6-CH_2), 3.08 (m, 4H, NCH_2 of 16β-propyl and C$\underline{H}_2$Ph), 3.68 (d, J = 9.8 Hz, 1H, 17α-CH), 4.04 (t, J = 7.4 Hz, 1H, NCH of phenylalanyl), 6.47 (d, J = 2.6 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.4 Hz and J_2 = 2.6 Hz, 1H, 2-CH), 7.05 (d, J = 8.4 Hz, 1H, 1-CH), 7.32 (m, 5H, Ph); ^{13}C NMR (Table 1); LRMS: calcd for $C_{30}H_{41}N_2O_3$ [M-HCl+H]$^+$ 477.3, found 477.5 m/z.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(pyrrolinylammonium hydrochloride)-acetamide (**15**)*. White solid (65% yield); IR (KBr): 3290 (br, OH and NH), 1654 (C=O amide); ^{1}H NMR (CD_3OD): 0.75 (s, 3H, 18-CH_3), 2.75 (m, 2H, 6-CH_2), 2.95 (m, 2H, NCH_2 of pyrolidinyl), 3.20 (m, 2H, NCH_2 of 16β-propyl), 3.61 (m, 1H, NCH of prolyl), 3.68 (d, J = 9.8 Hz, 1H, 17α-CH), 6.47 (d, J = 2.6 Hz, 1H, 4-CH), 6.52 (dd, J_1 = 8.4 Hz and J_2 = 2.6 Hz, 1H, 2-CH), 7.05 (d, J = 8.4 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{26}H_{39}N_2O_3$ [M-HCl+H]$^+$ 427.3, found 427.5 m/z.

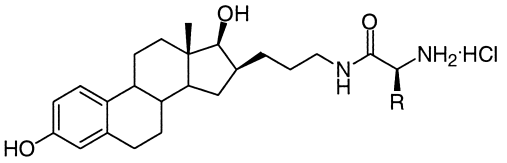
*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-ammonium hydrochlroride-but-4-yl)-2-(ammonium hydrochloride)-acetamide (**16**)*. White solid (82% yield); IR (KBr): 3300 (br, OH and NH), 1667 (C=O, amide); ^{1}H NMR (CD_3OD): 0.76 (s, 3H, 18-CH_3), 2.75 (m, 2H, 6-CH_2), 2.96 (t, J = 7.7 Hz, 2H, NCH_2 of lysyl), 3.26 (t, J = 6.4 Hz, 2H, NCH_2 of 16β-propyl), 3.70 (d, J = 9.7 Hz, 1H, 17α-CH), 3.89 (t, J = 6.7 Hz, 1H, NCH of lysyl), 6.47 (d, J = 2.6 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.4 Hz and J_2 = 2.6 Hz, 1H, 2-CH), 7.06 (d, J = 8.4 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{27}H_{44}N_3O_3$ [M-2HCl+H]$^+$ 458.3, found 458.5 m/z.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-methyl-2-{N^{α}-[N^{β}-(ammonium hydrochloride)-L-alanyl]-L-alanyl}-acetamide (**17**)*. White solid (81% yield); IR (KBr): 3300 (br, OH and NH), 1634 (C=O, amide); ^{1}H NMR (CD_3OD): 0.76 (s, 3H, 18-CH_3), 1.34 (d, J = 7.2 Hz, 3H, CH_3 of alanyl), 1.40 (d, J = 7.1 Hz, 3H, CH_3 of alanyl), 1.53 (d, J = 7.0 Hz, 3H, CH_3 of alanyl), 2.75 (m, 2H, 6-CH_2), 3.19 (m, 2H, NCH_2 of 16β-propyl), 3.69 (d, J = 9.8 Hz, 1H, 17α-CH), 3.95 (q, J = 7.0 Hz, 1H, NCH of alanyl), 4.29 (q, J = 7.1 Hz, 1H, NCH of alanyl), 4.38 (q, J = 7.1 Hz, 1H, NCH of alanyl), 6.46 (d, J = 2.3 Hz, 1H, 4-CH), 6.52 (dd, J_1 = 8.4 Hz and J_2 = 2.5 Hz, 1H, 2-CH), 7.06 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{30}H_{47}N_4O_5$ [M-HCl+H]$^+$ 543.4, found 543.6 m/z.

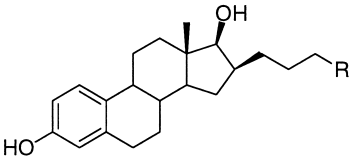
*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-hydroxycarbonylpropan-3-yl)-2-(ammonium hydrochloride)-acetamide (**18**)*. White solid (33% yield); IR (KBr): 3300 (br, OH and NH), 1715 (C=O, acid), 1670 (C=O, amide); ^{1}H NMR (CD_3OD): 0.77 (s, 3H, 18-CH_3), 2.47 (t, J = 7.9 Hz, 2H, C$\underline{H}_2$COOH), 2.76 (m, 2H, 6-CH_2), 3.26 (m, 2H, NCH_2 of 16β-propyl), 3.71 (d, J = 9.8 Hz, 1H, 17α-CH), 3.87 (t, J = 6.5 Hz, 1H, NCH of glutamyl), 6.47 (d, J = 2.5 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.5 Hz and J_2 = 2.5 Hz, 1H, 2-CH), 7.06 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{26}H_{39}N_2O_5$ [M-HCl+H]$^+$ 459.3, found 459.4 m/z.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-hydroxycarbonylpropan-3-yl)-2-{N^{α}-[N^{β}-(ammonium hydrochloride)-L-alanyl]-L-alanyl}-acetamide (**19**)*. White solid (90% yield); IR (KBr): 3320 (br, OH and NH), 1710 (C=O, acid), 1650 (C=O, amide); ^{1}H NMR (CD_3OD): 0.77 (s, 3H, 18-CH_3), 1.38 (d, J = 7.2 Hz, 3H, CH_3 of alanyl), 1.52 (d, J = 7.2 Hz, 3H, CH_3 of alanyl), 2.40 (q_{app}, J = 7.6 Hz, 2H, C$\underline{H}_2$COOH), 2.75 (m, 2H, 6-CH_2), 3.19 (m, 2H, NCH_2 of 16β-propyl), 3.70 (d, J = 10.1 Hz, 1H, 17α-CH), 3.93 (q, J = 7.1 Hz, 1H, NCH of alanyl), 4.37 (m, 2H, NCH of glutamyl and alanyl), 6.47 (d,

Table 1
^{13}C NMR chemical shifts (ppm) of peptidosteroids **12**–**20** and steroids **25** and **26***



12 - 20



25 and **26**

Carbons	**12** R = H	**13** R = $CH_2CH(CH_3)_2$	**14** R = CH_2Ph	**15** R = $CH_2CH_2CH_2$	**16** R = $(CH_2)_4NH_2$	**17** ** R = CH_3	**18** R = $(CH_2)_2COOH$	**19** ** R = $(CH_2)_2COOH$	**20** R = CH_2OH	**25** R = NH_2HCl	**26** R = H
1	127.19	127.17	127.16	127.16	127.18	127.17	127.18	127.17	127.17	127.20	126.48
2	113.73	113.72	113.71	113.74	113.73	113.74	113.73	113.71	113.75	113.75	112.68
3	155.90	155.88	155.88	155.91	155.90	155.91	155.93	155.91	155.91	155.95	153.43
4	116.03	116.02	116.01	116.05	116.03	116.05	116.02	116.02	116.04	116.03	115.23
5	138.74	138.72	138.72	138.76	138.73	138.76	138.76	138.77	138.76	138.73	138.21
6	30.72	30.72	30.71	30.72	30.72	30.73	30.73	30.74	30.73	30.74	29.63
7	28.74	28.73	28.74	28.73	28.74	28.75	28.76	28.76	28.75	28.77	27.39
8	39.98	39.97	39.96	39.98	39.98	40.00	40.02	40.01	40.01	39.99	38.33
9	45.39	45.36	45.35	45.40	45.37	45.41	45.43	45.42	45.41	45.39	43.98
10	132.65	132.61	132.60	132.65	132.62	132.64	132.65	132.67	132.67	132.58	132.70
11	27.55	27.55	27.54	27.57	27.55	27.57	27.58	27.57	27.57	27.56	26.30
12	39.00	38.97	38.97	39.02	38.99	39.04	39.02	39.04	39.02	38.95	37.70
13	45.22	45.21	45.20	45.18	45.21	45.20	45.25	45.20	45.24	45.28	44.12
14	49.93	49.91	49.90	49.97	49.92	49.99	49.98	49.99	49.97	49.94	48.55
15	33.51	33.53	33.49	33.58	33.48	33.57	33.56	33.57	33.57	33.53	32.33
16	41.29	41.28	41.27	41.30	41.23	41.34	41.32	41.35	41.28	41.20	39.68
17	83.11	83.07	83.06	83.12	83.11	83.18	83.12	83.18	83.13	82.89	82.57
18	13.26	13.28	13.25	13.23	13.32	13.26	13.24	13.26	13.25	13.22	12.36
1′	30.28	30.35	30.28	30.28	30.32	30.26	30.38	30.33	30.30	29.90	33.70
2′	29.57	29.57	29.47	29.73	29.43	29.61	29.63	29.62	29.57	28.58	21.70
3′	40.90	40.92	40.89	40.49	40.88	40.74	40.98	40.75	40.98	41.02	14.29
NHCO	167.02	170.45	169.27	176.89	169.83	170.84	169.58	170.84	168.01	–	–
NHCO′	–	–	–	–	–	174.10	–	174.42	–	–	–
NHCO″	–	–	–	–	–	174.64	–	174.97	–	–	–
CHR	50.04	53.14	55.84	61.70	54.24	50.14	54.02	54.10	56.34	–	–
CHR′	–	–	–	–	–	50.46	–	50.11	–	–	–
CHR″	–	–	–	–	–	50.55	–	50.68	–	–	–
R	–	41.83	38.76	32.15	32.18	18.52	28.05	28.88	61.82	–	–
″	–	25.51	135.69	26.99	23.03	–	30.87	31.02	–	–	–
″	–	22.32	130.52 (2x)	47.99	28.00	–	176.17	173.26	–	–	–
″	–	22.98	130.03 (2x)	–	40.30	–	–		–	–	–
″	–	–	128.80	–	–	–	–	–	–	–	–
R′	–	–	–	–	–	17.61	–	17.56	–	–	–
R″	–	–	–	–	–	17.95	–	17.80	–	–	–

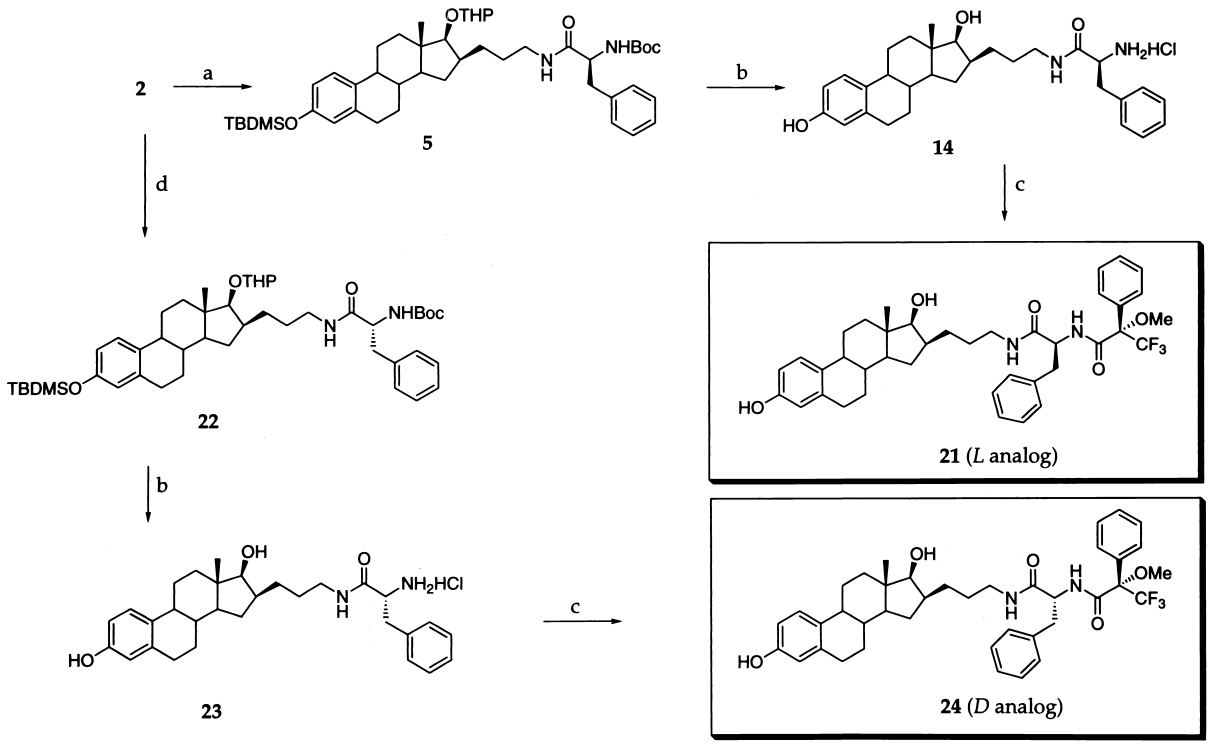
* NMR solvent: CD_3OD for all compounds except **26** in $CDCl_3$; (**) Val-Val ($COCH(CH_3)NHCOCH(CH_3)NH_2$) added at the terminal NH_2 group.

J = 2.3 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.4 Hz and J_2 = 2.3 Hz, 1H, 2-CH), 7.06 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{32}H_{49}N_4O_7$ $[M\text{-}HCl+H]^+$ 601.4, found 601.5 m/z.

*N-{3-[3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl]-propyl}-2-(S)-(1-hydroxymethyl)-2-(ammonium hydrochloride)-acetamide (**20**).* White solid (46%, two steps); IR (KBr): 3320 (br, OH and NH), 1672 (C=O, amide); ^{1}H NMR (CD_3OD): 0.77 (s, 3H, 18-CH_3), 2.76 (m, 2H, 6-CH_2), 3.25 (t, J = 7.1 Hz, 2H, NCH_2 of 16β-propyl), 3.70 (d, J = 10.4 Hz, 1H, 17α-CH), 3.80 and 3.90 (2m, 3H, NCH of seryl and CH_2OH), 6.47 (d, J = 2.6 Hz, 1H, 4-CH), 6.54 (dd, J_1 = 8.4 Hz and J_2 = 2.6 Hz, 1H, 2-CH), 7.06 (d, J = 8.4 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{24}H_{37}N_2O_4$ $[M\text{-}HCl+H]^+$ 417.3, found 417.4 m/z.

*2.3. Synthesis of Mosher amides **21** and **24** (Scheme 2)*

The compound **22** was obtained using the same coupling protocol as the compound **5**, but NHBoc-*D*-Phe-COOH was used instead of the corresponding *L*-isomer. At this step, spectral data of **22** were identical to those of compound **5**.



Scheme 2. Reagents and conditions: (a) NHBoc-*L*-Phe-COOH, DCC, HOBt, CH_2Cl_2, 0°C; (b) 3 N HCl, MeOH, rt; (c) (*S*)-MTPA, DCC, HOBt, $(i\text{-Pr})_2$EtN, DMF, 25°C; (d) NHBoc-*D*-Phe-COOH, DCC, HOBt, CH_2Cl_2, 0°C.

The deprotection was performed by the method A to give compound **23** whose spectral data were again identical to those of the compound **14**. Consequently, the amino group of each compound (**14** and **23**) was separately transformed to its corresponding Mosher amide following this typical procedure. To a cooled solution of (S)-(-)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) (3 mg; 0.015 mmol or 7 mg; 0.03 mmol) in dry DMF (300 μl or 500 μl) at 0°C was added dicyclohexylcarbodiimide (3 mg; 0.015 mmol or 6 mg; 0.03 mmol) and 1-hydroxybenzotriazole (2 mg; 0.015 mmol or 4 mg; 0.03 mmol) and the mixture was allowed to reach room temperature for 1 h. The reaction mixture was cooled at 0°C before the addition of diisopropylethylamine (2.5 μl; 0.015 mmol or 5 μl; 0.03 mmol) and compound **14** or **23** (7 mg; 0.015 mmol or 16 mg; 0.03 mmol) and then the reaction was stirred at 25°C for 2 h. Afterward, water following by 1 N hydrochloric acid solution were successively added and the crude compounds were extracted three times with EtOAc. The combined organic layers were washed with a saturated $NaHCO_3$ solution, water, brine, and dried over $MgSO_4$. After evaporation of the solvent, the crude compounds were purified by flash chromatography (hexanes:EtOAc, 1:1) to give respectively the amides **21** (10 mg, 98% yield) and **24** (12 mg; 55%). Only pertinent ^{1}H NMR ($CDCl_3$) spectral data are reported. For **21:** 4.66 (dd of CHα-amide), 3.05 (m of $C\underline{H}_2Ph$) and 3.29 (s of OCH_3). For **24:** 4.57 (dd of CHα-amide), 3.11 (m of $C\underline{H}_2Ph$) and 3.23 (s of OCH_3). All signals were significantly different when both compounds **21** and **24** were mixed together in the same NMR tube.

*2.4. Preparation of 16β-(aminopropyl)-E_2 (**25**) and 16β-(propyl)-E_2 (**26**) (Table 2)*

*3-(3, 17β-dihydroxy-estra-1, 3, 5 (10)-trien-16β-yl)-propylamine hydrochloride (**25**).* A solution of amine **2** (100 mg; 0.19 mmol) in 5 ml of MeOH containing 2% (v/v) of hydrochloric acid was stirred for 3 h at room temperature. Then, solvents were evaporated to dryness to give 68 mg (98% yield) of compound **25**. White solid; IR (KBr): 3250 (br, OH and NH_2); ^{1}H NMR (CD_3OD): 0.78 (s, 3H, 18-CH_3), 2.75 (m, 2H, 6-CH_2), 2.93 (m, 2H, NCH_2 of 16β-propyl), 3.73 (d, J = 9.8 Hz, 1H, 17α-CH), 6.46 (d, J = 2.3 Hz, 1H, 4-CH), 6.53 (dd, J_1 = 8.4 Hz and J_2 = 2.4 Hz, 1H, 2-CH), 7.06 (d, J = 8.5 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{21}H_{32}NO_2$ $[M\text{-}HCl+H]^+$ 330.2, found 330.4 m/z.

*16β-propyl-estra-1, 3, 5 (10)-triene-3, 17β-diol (**26**).* A solution of 3-*t*-butyldimethylsilyloxy-17β-(tetrahydro-2H-pyran-2-yl-oxy)-16β-allyl-estra-1, 3, 5 (10)-triene [32] (5.3 g; 10.3 mmol) in 215 ml of MeOH/THF (93:7) containing 2% (v/v) of hydrochloric acid was stirred for 5 h at room temperature. Then, water was added and organic solvents were evaporated. The crude compound was extracted with EtOAc (3X) and the combined organic was washed with brine, dried over $MgSO_4$, and concentrated to dryness. To a solution of the crude alkene (1.2 g) in MeOH (100 ml) was

Table 2
Biological activities of 16β-propylaminoacyl derivatives of E_2 (**12**–**20**) and compounds **25** and **26** as revealed by the inhibition of type 1 reductive 17β-HSD activity and the binding affinity on four steroid receptors

Compounds	Amino acyl derivative	Inhibition (%) Type 1 17β-HSD (E_1 to E_2)		Binding affinity (%)							
				AR		PR		GR		ER	
		0.1 μM	1.0 μM	10 nM	1.0 μM	10 nM	1.0 μM	10 nM	1.0 μM	10 nM	1.0 μM
12	Gly	0	0	3 ± 2	6 ± 2	0 ± 2	0 ± 2	0 ± 4	0 ± 3	0 ± 2	5 ± 1
13	Leu	0	0	3 ± 2	6 ± 2	0 ± 2	0 ± 2	0 ± 3	1 ± 4	0 ± 1	6 ± 3
14	Phe	16	6	7 ± 1	8 ± 3	1 ± 2	0 ± 2	0 ± 4	1 ± 3	0 ± 2	6 ± 1
15	Pro	0	0	6 ± 2	5 ± 1	0 ± 3	1 ± 2	0 ± 3	0 ± 3	0 ± 2	5 ± 1
16	Lys	0	0	2 ± 2	0 ± 1	0 ± 3	0 ± 2	0 ± 3	0 ± 3	1 ± 1	5 ± 1
17	Ala-Ala-Ala	4	16	8 ± 1	7 ± 3	0 ± 2	0 ± 3	0 ± 2	0 ± 5	0 ± 2	6 ± 2
18	Glu	3	5	5 ± 1	9 ± 2	0 ± 4	1 ± 2	0 ± 4	5 ± 3	0 ± 1	12 ± 1
19	Glu-Ala-Ala	0	6	6 ± 2	6 ± 2	0 ± 2	0 ± 2	1 ± 4	0 ± 4	1 ± 4	5 ± 4
20	Ser	0	0	6 ± 1	3 ± 4	0 ± 2	0 ± 2	3 ± 3	0 ± 3	0 ± 2	5 ± 1
25*	-	1	22	0 ± 2	1 ± 2	0 ± 2	0 ± 1	0 ± 3	0 ± 3	0 ± 2	12 ± 1
26**	-	7	48	5 ± 1	4 ± 2	0 ± 1	6 ± 2	0 ± 3	0 ± 3	2 ± 1	69 ± 1
E_1	-	24	55	-	-	-	-	-	-	-	-
DHT	-	-	-	70 ± 1	100 ± 1	3 ± 2	40 ± 2	2 ± 2	6 ± 2	2 ± 2	4 ± 1
R5050	-	-	-	1 ± 4	28 ± 2	65 ± 2	99 ± 2	9 ± 2	85 ± 2	5 ± 2	5 ± 2
DEX	-	-	-	0 ± 1	2 ± 1	0 ± 3	1 ± 2	66 ± 2	99 ± 1	0 ± 3	0 ± 1
E_2	-	-	-	0 ± 2	34 ± 1	6 ± 3	25 ± 2	5 ± 2	12 ± 2	75 ± 1	100 ± 1

* Compound **25:** 16β-(aminopropyl)-E_2; **Compound **26**: 16β-(propyl)-E_2; Abbreviations of steroids used as standard: DHT: dihydrotestosterone; R5050: synthetic progestin; DEX: dexamethasone; E_2: estradiol; E_1: estrone.

added 5% palladium on activated carbon (200 mg). The resulting mixture was stirred under hydrogen atmosphere at 25°C for 20 h. The slurry was filtered through a celite pad eluting with MeOH and EtOAc. The organic solvent was evaporated under reduced pressure. Purification by flash chromatography (hexanes:EtOAc, 85:15) gave 1.0 g (31% yield, two steps) of the compound **26**. White solid; IR (KBr): 3466 (br, OH); ^{1}H NMR ($CDCl_3$): 0.78 (s, 3H, 18-CH_3), 0.93 (t, J = 7.1 Hz, 3H, CH_3 of propyl), 2.80 (m, 2H, 6-CH_2), 3.75 (d, J = 10 Hz, 1H, 17α-CH), 6.56 (d, J = 2.3 Hz, 1H, 4-CH), 6.62 (dd, J_1 = 8.4 Hz and J_2 = 2.5 Hz, 1H, 2-CH), 7.15 (d, J = 8.4 Hz, 1H, 1-CH); ^{13}C NMR (Table 1); LRMS: calcd for $C_{21}H_{34}NO_2$ $[M+NH_4]^+$ 332.3, found 332.4 m/z.

2.5. Inhibition of type 1 17β-HSD (Table 2)

This enzymatic assay on crude preparation of type 1 17β-HSD was performed as previously described [22]. Briefly, Human Embryonic Kidney (HEK)-293 cells transfected with cDNA encoding for type 1 17β-HSD were sonicated to liberate the crude enzyme that was used as the enzymatic pool without further purification. The enzymatic assay was performed as follows: a stock solution was first prepared containing the radiolabeled substrate [^{14}C]-E_1 (0.1 μM), NADH (1 mM) in a phosphate buffer (pH 7.4, 50 mM KH_2PO_4, EDTA 1 mM, 20% glycerol). For the assay, 890 μl of the stock solution and 10 μl of a solution of inhibitor dissolved in EtOH were added in a tube. The reaction was started by adding 100 μl of a solution of crude enzyme prepared as above. The mixture was incubated for 1 h at 37°C, and the reaction was stopped by adding an excess of unlabeled E_1 and E_2. Steroids were extracted with diethyl ether and solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2, spotted on a silica gel plate (TLC, 20 cm × 20 cm × 0.2 cm, Kieselgel 60 F254) and eluted with CH_2Cl_2/EtOAc (9:1). Less polar E_1 and more polar E_2 were identified on TLC as two rows of visible spots under UV light. Radioactivity signals associated to [^{14}C]-E_1 and [^{14}C]-E_2 were detected and quantified using a Phosphor Imager (Sunny Vale, CA). The percentage of transformation of [^{14}C]-E_1 into [^{14}C]-E_2 was calculated as follows: % trans. = 100 × [^{14}C]-E_2 (cpm) / ([^{14}C]-E_1 (cpm) + [^{14}C]-E_2 (cpm)). Subsequently, the percentage of inhibition = 100 × [(% trans. of control − % trans. of compound)/ (% trans. of control)].

2.6. Steroid receptor binding affinity screening (Table 2)

The affinity binding assays on estrogen and progestin receptors from rat uterine were carried out under the standard procedure established in our laboratory [34]. Assays for androgen receptor from rat ventral prostate were performed according to the procedure described by Luo and co-workers [35]. For binding assay on glucocorticoid receptor from rat liver, a slightly modified version of the procedure described by Asselin and co-workers was used [36]. In this case, separation of bound and free steroids was achieved with dextran-coated charcoal adsorption instead of protamine sulfate precipitation.

3. Results

Type 1 17β-HSD has a strong preference towards C18-steroid substrates compare to C19-steroids like androstenedione and dehydroepiandrosterone [22,37]. Steroidal inhibitors should obviously contain a C18-steroid nucleus as E_2 to bind to the substrate binding domain. Moreover, we wanted to attach the additional interacting polar group on the D-ring because the reaction site and the cofactor binding domain surround this part of E_2 molecule. Based on our previous studies [38–40], a 16β-propyl side-chain was used as a spacer group between the E_2 moiety and the amino acyl group (Fig. 1). The polar nature of NADH or NADPH cofactor motivates us to choose polar chemical groups. Because of their polarity, molecular diversity, chirality, structural complementary with protein, and accessibility, amino acids are excellent building blocks. Representatives of all groups of amino acid residues (aliphatic [Gly, Leu], aromatic [Phe], structural constrained [Pro], basic [Lys], acid [Glu], alcohol [Ser] as well as two tripeptides [Glu-Ala-Ala, Ala-Ala-Ala]) were thus added to the E_2 derivative (compounds **12–20**) to probe different areas of the cofactor binding domain. The natural *L*-series of amino acids were arbitrarily chosen. Two additional control molecules, without an amino acid residue, were used as control to evaluate the influence of amino acyl residues: 16β-aminopropyl-E_2 (**25**) and 16β-propyl-E_2 (**26**).

3.1. Chemical synthesis

A well-known synthetic sequence described by our laboratory was used to generate the azide intermediate **1** (Scheme 1) [32,41]. Thereafter, the reduction of the azide using palladium-catalyzed hydrogenation yielded the amine precursor **2** that was used for N-Boc protected amino acid coupling according to the König and Geiger procedure [33], which gave the amides **3–11**. For the synthesis of the peptidosteroids **8** and **10**, the tripeptide building blocks NH-Boc-Ala-Ala-Ala-COOH and NHBoc-Glu-Ala-Ala-COOH were obtained using standard synthetic methods [42,43]. Modest yields obtained in the coupling reaction could be attributed to steric hindrance, partial deprotection of the phenolic TBDMS group under the reaction conditions, and difficulty during the purification step. The *t*-butoxy carbamate (*t*-Boc)-protected amino acids were chosen because the precursor **2** had already two other acid-labile protecting groups (THP, TBDMS).

According to the side-chain amino acid residue, three kinds of acidic conditions were used to yield the target compounds **12–20**. It is noteworthy that usual conditions for *t*-Boc cleavage with trifluoroacetic acid led to several side-products such as 17β-O-trifluoroacetate derivatives. Thus, protected compounds **3–8** bearing alkyl (Gly, Leu, Pro, Ala-Ala-Ala), benzyl (Phe), or basic (Lys) were submitted to 3 N HCl in MeOH to produce fully deprotected compounds **12–17**. Because of transesterification, the latter procedure was not appropriate when the side-chain contained a *t*-butyl ester (compounds **9** and **10**). We then tried 3 N HCl in EtOAc, but again deprotected compound was contaminated with about 15% of 17β-OAc from transesterification of solvent. Consequently, we used anhydrous HCl in CH_2Cl_2 to produce glutamyl derivatives of estradiol (compounds **18** and **19**). Finally, we obtained the seryl derivative **20** from **11** by a two-step deprotection procedure using first 3 N HCl in MeOH, and then hydrogenolysis of the benzyl ether group under pressure.

All compounds were purified by trituration in diethyl ether to yield pure solid characterized by IR, ^{1}H NMR, ^{13}C NMR (Table 1) and mass spectrometry. Furthermore, optical purity of the α-carbon center was proved by the synthesis of one representative of *D*-series amino acid as well as the Mosher amides of *L*-Phe and *D*-Phe derivatives (Scheme 2). Comparison of ^{1}H NMR spectra established that, as expected, no racemization occurred during the coupling reaction and the subsequent deprotection. Finally, 16β-(aminopropyl)-E_2 (**25**) was obtained from compound **2** after hydrolysis of TBDMS and THP protecting groups while 16β-(propyl)-E_2 (**26**) was synthesized from an allyl intermediate [32] after removal of TBDMS and THP protecting groups and reduction of double bond. Both compounds were used as references to evaluate the biological importance of the amino acid moiety.

3.2. Biological evaluation

The 16β-propylaminoacyl derivatives of E_2 (**12–20**) were tested on two systems to evaluate either their ability to inhibit the formation of E_2 from E_1 by type 1 17β-HSD or their affinities on steroid receptors according to established procedures (Table 2) [22,34–36]. An enzymatic screening test was performed using homogenated HEK-293 cells transfected with cDNA encoding for type 1 17β-HSD. It revealed that none of the added amino acids on the E_2 nucleus provided a good inhibitory potency on the reductive activity of type 1 17β-HSD. In fact, the 16β-propyl-E_2 (**26**) displayed a better inhibitory effect than the 16β-aminopropyl-E_2 (**25**), which was also better than all the 16β-propylaminoacyl derivatives of E_2 (**12–20**). The ammonium group seems to interact unfavorably with the enzyme and none of the amino acid residue can overcome this effect. Affinities toward steroid receptors of all 16β-(propylaminoacyl) derivatives of E_2 (**12–20**) were also evaluated. This biological data is very valuable to detect eventual compounds lacking selectivity, which can interact with other proteins that bind to steroids and provoke undesired effects in more complex biological systems. All compounds did not bind significantly to androgen, progestin, and glucocorticoid receptors. No significant binding affinities were also observed for all tested compounds with the estrogen receptor, except for the 16β-propyl-E_2 (**26**), which displayed a better, but unsuitable, affinity for this receptor. As an interesting consequence, the addition of an amino acid residue to es-

tradiol drastically reduced its affinity for the estrogen receptor.

4. Discussion

We have described the preparation of a series of 16β-propylaminoacyl derivatives of E_2 (**12–20**) containing several kinds of functional groups such as alkyl, aryl, carboxylic acid, alcohol, amine, and amides. All the newly synthesized compounds showed very low inhibitory potencies on type 1 17β-HSD activity. This observation means that whatever the substitution made on the starting molecule, no specific binding on the enzyme was created by this substitution. Moreover, all the substitutions led to clearly unfavorable interactions with the enzyme as witnessed by the decreasing inhibitory effect of the 16β-propyl-E_2 (**26**) $>$ 16β-(aminopropyl)-E_2 (**25**) $>>$ 16β-(propylaminoacyl)-E_2 (**12–20**). Two major factors can probably explain these results. The ammonium group, which is present in all inactive molecules, might surround that of the Lys-159 of the enzyme. If so, none of the amino acid residues introduced in α of this ammonium succeeded in changing the conformation of the molecule to provide any point of contact into the enzyme. Particularly, the carboxylate groups of compounds **18** and **19** were initially thought to interact with the ammonium group of the Lys-159, but it seems that the conformation of the molecule into the enzyme did not allow this salt bridge. This same situation was observed with the seryl group whose hydroxy group could have been the mimic of the hydroxy group of the cofactor ribose moiety, which is known to form a hydrogen bond with the Lys-159 [28–31]. The other factor that may contribute to the observed unfavorable binding properties of these compounds is the amide bond(s). Conformationally pseudo-rigid structures that can be introduced by amide bond might expose the functional groups of the molecule at the wrong place in the enzyme pocket. In addition, the presence of one and more amide bonds can also direct the ammonium group of the molecule toward that of the Lys-159 in the enzyme. Therefore, the combination of these two negative effects may be the explanation of why these compounds were inactive in inhibiting type 1 17β-HSD.

These 16β-propylaminoacyl derivatives of E_2 (**12–20**) did not show any affinity toward androgen, progestin, and glucocorticoid receptors. Interestingly, the addition of the amino acyl group led to E_2 derivatives possessing greatly reduced binding properties to estrogen receptor. While the binding affinities of 16α-substituted E_2 derivatives were extensively studied [19], the properties of 16β-substituted E_2 derivatives remained unknown. Our results demonstrated that polar and moderate size substituents are not tolerated by the estrogen receptor. These results are in agreement with the overall observation that E_2 derivatives bearing polar substituents generally show low binding affinity on the estrogen receptor [19].

In the light of this study, the prototype chemical structure can be modified to ultimately obtain a lead structure. Firstly, the amino group should be changed for a non positively charged polar group. This approach is under current investigation in our laboratory. Secondly, the 16β-propyl sidechain could be further lengthened to prevent the postulated negative interaction of functional groups with the Lys-159, which seems to surround the extremity of the propyl sidechain. The functional groups at the end of a longer sidechain could hopefully interact with other areas of the cofactor binding domain. However, this exploratory study has revealed the complexity of maximizing interactions with the cofactor binding domain. Consequently, we are currently developing a combinatorial chemistry program that should allow us to investigate the hybrid-molecule prototype with much more power [32,41,44,45]. The present study has revealed that, although amino acids are interesting building blocks, they may not be ideally suited for our biological system. Therefore, the present study help us to re-orientate the future design of libraries of such hybrids as type 1 17β-HSD inhibitors without estrogenic properties.

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