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Synthesis of (5-azido-2-nitrobenzoyl)amido, (4-azido-2-nitrophenyl)amino, and (5-azido-2-nitro-3,4,6-trifluorophenyl)amino derivatives of 17α-methylamino-, 17α-ethylamino-, and 17α-propylamino-5α-dihydrotestosterone as reagents of different linker lengths for the photoaffinity labeling of sex hormone binding globulins and androgen receptors

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Abstract

The photoactivable aryl azide reagents, *N*-(5-azido-2-nitrobenzoyl)oxysuccinimide, 4-azido-1-fluoro-2-nitrobenzene, and 4-azido-1-nitro-2,4,5,6-tetrafluorobenzene have been condensed at the extremity of three 17α-aminomethyl, 17α-aminoethyl, and 17α-aminopropyl side-chains introduced on (17S)-spiro-(3,3-dimethoxy)-5α-androstan-17β,2′-oxirane either directly, by ammonolysis, in the first case, or by conversion to nitrile intermediates with cyano or cyanomethyl anions and subsequent reduction to amines with lithium aluminum hydride, in the two other cases. The 3,3-dimethoxy group of these photoreagents was cleaved by acidolysis to a 3-ketone, which was reduced with sodium borohydride to a 3β-alcohol. All of these compounds were characterized by ^{1}H- and ^{13}C-NMR as well as by ^{1}H, ^{13}C heteronuclear 2D NMR, which helped to resolve ambiguous assignments. Significant differences of substituent-induced effects on ^{13}C NMR signals were observed according to the 17α-side-chain length, the structure of the terminal aryl azide groups, and the solvent, showing a different behavior of *N*-5-azido-2-nitrobenzoyl derivatives as compared with 4-azido-2-nitrophenylamino and 5-azido-2-nitro-3,4,6-trifluorophenylamino derivatives. The *N*-5-azido-2-nitrobenzoyl conjugates of the three 17α-aminomethyl, aminoethyl, and aminopropyl derivatives of 5α-dihydrotestosterone were tested as ligands for purified human sex hormone-binding globulin and for the cytosolic androgen receptor of rat ventral prostate by competition experiments with tritiated 5α-dihydrotestosterone. The increasing lengths of the aminomethyl, aminoethyl, and aminopropyl spacer arms of *N*-5-azido-2-nitrobenzoyl conjugates were found to correspond to decreasing relative binding affinities for sex hormone-binding globulin (0.76, 0.47, and 0.10, respectively, versus 1.00 for 5α-dihydrotestosterone) while only the longer aminoethyl and aminopropyl conjugates interacted significantly with the androgen receptors (0.05 and 0.10, respectively).

Keywords: 17α-[(5-azido-2-nitrobenzoyl)amidomethyl/ethyl/propyl]-5α-androstane-3β,17β-diol; 17α-[(4-azido-2-nitrophenyl/5-azido-2-nitro-2,4,6-trifluorophenyl)/aminoalkyl]-5α-androstane-3β,17β-diol; Photoaffinity labeling reagents; Human sex hormone-binding globulin; Rat prostate cytosolic androgen receptor; ^{1}H/^{13}C NMR

1. Introduction

Several studies have demonstrated the usefulness of 17α-substituted derivatives of testosterone and 5α-dihydrotestosterone (DHT) as ligands for affinity chromatography of androgen-binding proteins such as sex hormone-binding globulins (SHBGs) and androgen receptors. Previous reports (reviewed in ref. 1) have shown that immobilized 17α-(2′-carboxyethynyl)-testosterone or 17α-(6′-hexanoic)-DHT both interact with SHBG, while only 17α-(2′,3′-epoxypropyl)-DHT, but not 17α-(carboxymethyl)-DHT, can be employed for the purification of the androgen receptor from rat ventral

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prostate cytosol, thus suggesting a differential recognition of these two proteins according to the structure of the 17α-substituent, especially at the level of the first three atoms of the side-chain. Moreover, 17α-alkyl substitution has been reported as a means to increase the oral activity of androgens, possibly as the result of modifications of binding characteristics with both receptor and transport proteins [2]. Previous studies from this laboratory have reported the synthesis and the ^{1}H NMR and ^{13}C NMR characterization of 17α-hexanoic derivatives of DHT and testosterone and of their 17α-hexynyl synthetic precursors [1], useful as ligands for affinity chromatography of both androgen receptors and SHBGs as well as the synthesis and characterization of the corresponding 17α-hemiglutaramidomethyl derivatives [3], useful for affinity chromatography of SHBGs only.

These affinity chromatography ligands may be readily converted to aryl azide photoaffinity labeling reagents, potentially able to interact with amino acids in the vicinity of the binding site of the steroid skeleton, provided that the addition of an aromatic photoactivable chromophore at the extremity of the 17α-aminoalkyl side-chain does not alter too much the binding properties or the degree of selectivity for androgen receptors and SHBGs. However, although photoaffinity labeling reagents have been claimed to have a theoretical ability to react with any interacting amino acid, numerous reports (reviewed in ref. 4) have often identified similar amino acid targets for a same structural class of photoreagents such as aryl azides. Therefore, optimization of the spacer arm structure might also favor an appropriate positioning of the photoactivable chromophore toward a more reactive amino acid, thus augmenting the probability of a successful labeling in a region vicinal to the steroid binding site where a much lesser degree of immobilization can be expected for the side-chain as compared with that of the steroid skeleton in the steroid binding site.

This work was undertaken with the view of preparing photoactivable derivatives of 17α-(aminoalkyl)-17β-hydroxy derivatives in both DHT and 5α-androstane-3β,17β-diol series as potential reagents for photoaffinity labeling of androgen binding proteins such as SHBG and androgen receptors. Three different 17α-aminomethyl, aminoethyl, and aminopropyl side-chains of increasing lengths were introduced on the steroid skeleton and each one was linked to three 5-azido-2-nitrobenzoyl, 4-azido-2-nitrophenyl, and 5-azido-2-nitro-3,4,6-trifluorophenyl chromophores. Preliminary experiments were undertaken with radioinert 5-azido-2-nitrobenzoyl derivatives in order to estimate the effects of the side-chain length on the binding affinities for SHBG and androgen receptors, using competitive displacement of tritiated DHT.

2. Experimental

2.1. General methods

5α-Androstane-3,17-dione and other chemicals were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Chromatographic and spectrometric methods were similar to those previously mentioned in a preceding article [3], except for IR spectra, which were recorded on a Bruker Vector 22 spectrometer. Liquid secondary ion mass spectrometry (LSIMS) experiments (positive mode) were performed on a ZAB-2-SEQ mass spectrometer (Micromass, Manchester, UK) equipped with a Cs^{+} gun operating at 40 keV. The accelerating voltage was 8 kV, and the instrumental resolution was 1000 or 5000 (10% valley) for low- or high-resolution measurements, respectively. Calibrations were performed with $(CsI)_nCs^{+}$ cluster ions. Steroid samples were dissolved in 2 μl of methanol and added to 2 μl of 1-thioglycerol matrix (except for 3,3-dimethoxy-17α-derivatives, which were added to a 3-nitrobenzyl alcohol matrix) introduced on a stainless-steel probe tip. Spectra were recorded in the mass range 100-1000. Data for exact mass measurements were acquired using data system control in the multichannel analyser mode (MCA), and 10 scans were summed to give the final spectrum. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 300 and DRX 500 spectrometers at 300.13 and 500.13 MHz, respectively, for ^{1}H, and at 75.47 and 125.75 MHz, for ^{13}C. ^{1}H chemical shifts were measured relative to tetramethylsilane. Samples were prepared by dissolving 10–25 mg of steroid in 0.75 ml of $CDCl_3$ (99.8%) or C_5D_5N (99.5%) solvents purchased from CEA (Saclay, France). The ^{1}H chemical shifts are estimated to be accurate to ±0.01 ppm and coupling constants to ±0.5 Hz. The ^{13}C chemical shifts are estimated to be accurate to ±0.05 ppm. DEPT experiments [5] were systematically performed in order to differentiate quaternary, methine, methylene, and methyl carbon atoms. The ^{1}H, ^{13}C heteronuclear single quantum correlation (HSQC) experiments, the HSQC-TOCSY (total correlation spectroscopy) experiments, and the ^{1}H, ^{13}C heteronuclear multiple bond correlation (HMBC) experiments were performed on the Bruker DRX 500 spectrometer, using the standard gradient pulse sequences from Bruker [6–8]. The relaxation delay was D1 = 1.5 s in all cases. The pulse sequences were optimized using coupling constants of 145 Hz for HSQC experiments, and 10 Hz for HMBC experiments, except for the $^{3}J_{CCNH}$ coupling of NH with the aromatic C-1″ carbon atom of compound 11, which could be observed only at 6 Hz, but not at 10 or 4 Hz. The mixing time for HSQC-TOCSY experiments was 15 ms. All 2D spectra were recorded at high resolution (td_1 = 1024) for carbon, in order to improve separation of carbon signals. ^{13}C chemical shift increments were considered to be significant only if they were observed in all three 3,3′dimethoxy-, 3-oxo-, and 3β-hydroxysteroid series and if the increment had a magnitude equal to or above 0.10 ppm for at least two of these three series.

*2.2. 3,3-(Dimethoxy)-5α-androstan-17-one **(1)***

A solution of 5α-androstane-3,17-dione (10 g, 34.7 mmol) in 250 ml of methanol was stirred for 1 h at 40°C in

the presence of *p*-toluenesulfonic acid monohydrate (100 mg, 0.53 mmol). The reaction mixture was neutralized with MeONa (60 mg, 1.1 mmol), and the solvent was evaporated under reduced pressure. Water (300 ml) was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water and evaporated under reduced pressure. The product was analyzed by TLC on silica gel (Rf 0.45, petroleum ether-ethyl acetate 4:1 v/v) and recrystallized from methanol to give the pure dimethoxy derivative (7.2 g, 21.5 mmol, 62%): m.p. 120–121°C, recrystallized from methanol; (reported [9] 128–129°C); $[\alpha]_D$ = +82.1° ($CHCl_3$) (reported [9] +100.2° in $CHCl_3$); νmax (CCl_4): 1740 (17 − C = 0), 1110–1055 cm^{-1} (3,3-dimethoxy); ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.86 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.73 (3H, s, 19-CH_3), 0.79 (3H, s, 18-CH_3), 3.19 and 3.22 (2 × 3H, 2s, dimethoxy).

2.3. (17S)-Spiro-3,3-(dimethoxy)-5α-androstan-17β,2′-oxirane ***(2)***

A solution of 17-oxosteroid **1** (6.9 g, 20.6 mmol) and of trimethylsulfonium iodide (6.8 g, 33.3 mmol) in 160 ml of DMF was stirred for 1 h at 22°C in the presence of potassium t-butoxide (5.1 g, 45.4 mmol). The solvent was concentrated to 20 ml under reduced pressure (<0.01 mm Hg, t < 37°C) and ice-cooled water (1 liter) was added. The aqueous suspension was extracted with ethyl acetate. The combined organic layers were washed with water and evaporated under reduced pressure. The product was analyzed by TLC on silica gel (Rf 0.60, petroleum ether/ethyl acetate 4:1 v/v) and purified by flash-chromatography on silica gel (petroleum ether/ethyl acetate 4:1 v/v) to give the oxirane derivative (6.5 g, 18.6 mmol, 90%) as a white crystalline solid: m.p. 80–82°C, recrystallized from diethyl ether; $[\alpha]_D$ = +8.0° ($CHCl_3$); ν_{max} (CCl_4): 1105–1055 (3,3-dimethoxy), 1085 cm^{-1} (OCH_2); ^1H NMR ($CDCl_3$) δ 0.87 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 2.60 and 2.89 (2H, d: J = 5.1 Hz, CH_2O), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.74 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3), 2.57 and 2.90 (2H, d: J = 5.3 Hz, CH_2O), 3.18 and 3.22 (2 × 3H, 2s, dimethoxy); MS (LSIMS$^+$) *m/z* (relative intensity %) 371 (MNa^+, 10), 317 (MH^+-32, 100). High-resolution MS calculated for $C_{22}H_{36}O_3Na$ (MNa^+): 371.2562; found: 371.2537.

2.4. 17α-Aminomethyl-3,3-(dimethoxy)-5α-androstan-17β-ol ***(3)***

A solution of oxirane **2** (1.0 g, 2.87 mmol) in 125 ml of ethanol 95% was stirred for 72 h at 40°C in the presence of 50 ml of NH_4OH (25%) and of 1.5 ml of acetic acid. The reaction mixture was evaporated under reduced pressure. The precipitate was dissolved in a mixture of petroleum ether/ethyl acetate 4:1 v/v and filtered on a column of silica gel 70–230 Mesh in the same solvent. The column was washed with 100 ml of petroleum ether/ethyl acetate 4:1 v/v mixture, and the pure 17α-aminomethyl derivative was then eluted with a chloroform/methanol/NH_4OH 100:20:5 v/v/v mixture. The white solid product (0.75 g, 2.05 mmol, 72%) was analyzed by TLC on silica gel (Rf 0.55, chloroform/methanol/NH_4OH 100:20:5 v/v/v): m.p. 177–179°C, recrystallized from diethyl ether; $[\alpha]_D$ = - 8° ($CHCl_3$); ν_{max} (CCl_4): 1105–1055 (3,3-dimethoxy), 1085 cm^{-1} (OCH_2); ^1H NMR ($CDCl_3$) δ 0.80 (3H, s, 19-CH_3), 0.88 (3H, s, 18-CH_3), 2.51 and 2.92 (2H, dd: J = 12.5 and 12.5 Hz, CH_2N), 3.13 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.77 (3H, s, 19-CH_3), 1.11 (3H, s, 18-CH_3), 2.77 and 3.15 (free amine [3]) (2H, 2d: J = 12.3 and 12.8 Hz, CH_aNH and CH_bNH), 3.20 and 3.23 (2 × 3H, 2s, dimethoxy); MS (LSIMS$^+$) *m/z* (relative intensity %) 366 (MH^+, 100), 334 (MH^+-32, 16). High-resolution MS calculated for $C_{22}H_{40}O_3N_1$ (MH^+): 366.3008; found: 366.3002.

2.5. 17α-Cyanomethyl-3,3-(dimethoxy)-5α-androstan-17β-ol ***(4)***

A solution of oxirane **2** (1.5 g, 4.3 mmol) in a mixture of 38 ml of ethanol 95% and 7.5 ml of water was stirred for 18 h at 25°C in the presence of KCN (3.9 g, 59.9 mmol). The reaction mixture was evaporated under reduced pressure. The precipitated product was extracted in dichloromethane, washed with water, and dried under reduced pressure to give the pure 17α-cyanomethyl derivative as a white solid (1.6 g, 4.26 mmol, 99%). The product was analyzed by TLC on silica gel (Rf 0.25, petroleum ether/ethyl acetate 4:1 v/v; Rf 0.35, chloroform/ethyl acetate 5:1 v/v): m.p. 148–151°C; $[\alpha]_D$ = - 2.8° ($CHCl_3$); ν_{max} (CCl_4): 3605 (OH), 2250 weak (C≡N), 1105–1055 cm^{-1} (dimethoxy); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3), 2.55 (2H, dd: J = 37.1 and 16.2 Hz, CH_2CN), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.75 (3H, s, 19-CH_3), 1.09 (3H, s, 18-CH_3), 2.92 (2H, dd: J = 16.4 Hz and 31.3 Hz, CH_2CN), 3.20 and 3.23 (2 × 3H, 2s, dimethoxy); MS (LSIMS$^+$) *m/z* (relative intensity %) 398 (MNa^+, 53), 344 (MH^+-32, 100). High-resolution MS calculated for $C_{23}H_{37}O_3N_1Na$ (MNa^+): 398.2671; found: 398.2661.

2.6. 17α-Aminoethyl-3,3-(dimethoxy)-5α-androstan-17β-ol ***(5)***

A solution of cyanomethyl derivative **4** (1.0 g, 2.66 mmol) in 25 ml of anhydrous tetrahydrofuran was added dropwise to a suspension of $LiAlH_4$ (1.8 g, 47.4 mmol) in 125 ml of anhydrous tetrahydrofuran, at 4°C, under a nitrogen atmosphere, and stirred overnight at 22°C. The excess of $LiAlH_4$ was destroyed by successive dropwise additions of water (2.0 ml), 15% aqueous NaOH (2.0 ml), and water (6.0 ml). The reaction mixture was filtered, and the solid fraction was rinsed with tetrahydrofuran (4 times, 50 ml). After evaporation of the solvent, the crude residue (1.02 g)

was purified by TLC on silica gel (Rf 0.35, chloroform/methanol/NH_4OH 60:10:2 v/v/v) to give 17α-aminoethyl derivative (0.61 g, 1.61 mmol, 60.5%) as a white solid: m.p. 138–141°C, recrystallized from diethyl ether; $[\alpha]_D$ = - 14 ° ($CHCl_3$); ν_{max} (CCl_4): 3605 (OH, NH_2), 1105–1055 cm^{-1} (dimethoxy); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3), 1.90 (2H, m, C$H_2$$CH_2NH_2$), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.78 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 2.0 (2H, m, C$H_2$$CH_2NH_2$), 3.20 and 3.23 (2 × 3H, 2s, dimethoxy); MS ($LSIMS^+$) *m/z* (relative intensity %) 380 (MH^+, 100), 362 (MH^+ -18, 68), 348 (MH^+-32, 15), 330 (MH^+-18–32, 7). High-resolution MS calculated for $C_{23}H_{42}O_3N_1$ (MH^+): 380.3165; found: 380.3163.

2.7. 17α-Cyanoethyl-3,3-(dimethoxy)-5α-androstan-17β-ol ***(6)***

A solution of anhydrous diisopropylamine (3.04 g, 30.04 mmol) in 60 ml of anhydrous tetrahydrofuran was stirred for 2 min at −76°C in the presence of n-butyl lithium (18.8 ml of a 1.6 M solution in hexane, 30.08 mmol) and let to react at −76°C with a solution of anhydrous acetonitrile (2 ml) in 6.0 ml of tetrahydrofuran. After a few minutes, an orange color was produced. A solution of oxirane **2** (2 g, 5.74 mmol) in 10 ml of anhydrous tetrahydrofuran was added and stirred for 2 h at 22°C. Water (5 ml) was added, and the reaction mixture was neutralized with HCl. After evaporation under reduced pressure, the product was extracted in ethyl acetate, washed with water, and dried under reduced pressure to give the 17α-cyanoethyl derivative as a white solid (2.1 g, 5.3 mmol, 92%) analyzed by TLC on silica gel (Rf 0.30, chloroform/ethyl acetate 10:1 v/v): m.p. 120–123°C, recrystallized from diethyl ether; $[\alpha]_D$ = −3.8° ($CHCl_3$); ν_{max} (CCl_4): 3630(OH), 2250 weak (C≡N), 1105–1055 cm^{-1} (dimethoxy); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.86 (3H, s, 18-CH_3), 2.38 and 2.43 (4H, m, CH_2CH_2CN), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.78 (3H, s, 19-CH_3), 1.05 (3H, s, 18-CH_3), 1.89 and 2.12 (2H, m, C$H_2$$CH_2CN$), 2.68 and 2.83 (2H, m, CH_2CH_2CN), 3.21 and 3.23 (2 × 3H, 2s, dimethoxy); MS ($LSIMS^+$) *m/z* (relative intensity %) 412 (MNa^+, 90), 358 (MH^+-32, 100). High-resolution MS calculated for $C_{24}H_{39}O_3N_1$ Na (MNa^+): 412.2827; found: 412.2818.

2.8. 17α-Aminopropyl-3,3-(dimethoxy)-5α-androstan-17β-ol ***(7)***

The solid cyanoethyl derivative **6** (500 mg, 1.29 mmol) was added to a suspension of $LiAlH_4$ (1.5 g, 4.08 mmol) in 40 ml of anhydrous tetrahydrofuran, at 4°C, under a nitrogen atmosphere, and stirred for 12 h at 22°C. The excess of $LiAlH_4$ was destroyed by successive dropwise additions of water (2 ml), 15% aqueous NaOH (2 ml), and water (6 ml). The reaction mixture was filtered, and the solid fraction was rinsed with tetrahydrofuran (4 times, 50 ml). After evaporation of the solvent, the crude residue (550 mg) was purified by TLC on silica gel (Rf 0.45, chloroform/methanol/NH_4OH 100:20:5 v/v/v) to give the 17α-aminopropyl derivative (0.35 g, 0.89 mmol, 69%) as a white solid: m.p. 66–69°C, recrystallized from diethyl ether; $[\alpha]_D$ = −2.4° ($CHCl_3$); ν_{max} ($CHCl_3$): 3620 (OH), 1105–1050 cm^{-1} (dimethoxy); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3), 2.82 and 2.65 (4H, m, C$H_2$$NH_2$), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy); ^1H NMR (C_5D_5N) δ 0.79 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 2.9 (2H, m, C$H_2$$NH_2$), 3.21 and 3.23 (2 × 3H, 2s, dimethoxy); MS ($LSIMS^+$) *m/z* (relative intensity %) 394 (MH^+, 100), 376 (MH^+-18, 25), 362 (MH^+-32, 21), 344 (MH^+-18–32, 63). High-resolution MS calculated for $C_{24}H_{44}O_3N_1$ (MH^+): 394.3321; found: 394.3330.

2.9. 17α-[(N-5-Azido-2-nitrobenzoyl)amidomethyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(8)***

A solution of 17α-aminomethyl derivative **3** (55 mg, 0.15 mmol), in a mixture of 1 ml of tetrahydrofuran and 0.25 ml of a $NaHCO_3$ 0.1 M solution, was acylated by progressive addition of a solution of *N*-(5-azido-2-nitrobenzoyloxy)succinimide (42 mg, 0.14 mmol, ANBNOS reagent, purchased from Pierce) in 1 ml of tetrahydrofuran. The reaction mixture was stirred for 24 h at 22°C in the dark and evaporated under a nitrogen stream. The crude product was analyzed by TLC on silica gel (Rf 0.4, petroleum ether/ethyl acetate 1:1 v/v; Rf 0.85, chloroform/methanol/NH_4OH 100:20:5 v/v/v) and purified by TLC on silica gel to give the 17α-(5-azido-2-nitrobenzoyl)amidomethyl derivative (60 mg, 0.108 mmol, 72%) as a white solid: m.p. 171–173°C, recrystallized from ethyl acetate; $[\alpha]_D$ = −23° ($CHCl_3$); ν_{max} (CCl_4): 3610–3470-3415 (OH and NH), 2120 (N_3), 1655 (CONH), 1520 (C-NO_2), 1105–1050 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 305 nm (ϵ = 14 200); ^1H NMR ($CDCl_3$) δ 0.88 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 3.15 and 3.20 (2 × 3H, 2s, dimethoxy), 3.35 and 3.77 (2H, d: J = 13.8 Hz and dd: J = 13.4 and 7.9 Hz, CH_a NH and CH_b NH), 6.33 (1H, m, NHCO), 7.09 (1H, d: J = 2.5 Hz, H-6′), 7.15 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 8.12 (1H, d: J = 8.8 Hz, H-3′); ^1H NMR (C_5D_5N) δ 0.77 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.24 and 3.25 (2 × 3H, s, dimethoxy), 3.78 and 4.32 (2H, d: J = 13.4 Hz and dd: J = 5.5 and 5.5 Hz, CH_aNH and CH_bNH), 7.18 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.52 (1H, d: J = 2.5 Hz, H-6′), 8.10 (1H, d: J = 8.8 Hz, H-3′), 9.38 (1H, m, NHCO); MS ($LSIMS^+$) *m/z* (relative intensity %) 578 (MNa^+, 15), 556 (MH^+, 62), 524 (MH^+-32, 100), 506 (MH^+-18–32, 71). High-resolution MS calculated for $C_{29}H_{42}O_6N_5$ (MH^+): 556.3135; found: 556.3162.

2.10. 17α-[(N-5-Azido-2-nitrobenzoyl)amidomethyl]-17β-hydroxy-5α-androstan-3-one ***(9)***

The 3,3-dimethoxy derivative **8** (30 mg, 0.053 mmol) was stirred at 22°C, for 4 h, in the dark, in 3 ml of a dioxane/H_2O/conc. HCl 90:10:1 v/v/v mixture. The reaction

mixture was neutralized by addition of solid $NaHCO_3$. The solvent was evaporated under reduced pressure, and the product was extracted in ethyl acetate, washed with water, and dried under reduced pressure to give the pure 17α-[(*N*-5-azido-2-nitrobenzoyl)amidomethyl]-3-oxo derivative (24 mg, 0.047 mmol, 89%), which was recrystallized from diethyl ether, and analyzed by TLC on silica gel (Rf 0.45, petroleum ether/ethyl acetate 1:1 v/v; Rf 0.5, toluene/ethyl acetate 1:2 v/v): m.p.170–172°C; $[\alpha]_D$ = +3.7° (EtOH); ν_{max} ($CHCl_3$): 3600–3430 (OH and NH), 2120 (N_3), 1705 (3-C = O), 1670 (CONH), 1525 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 306 nm (ϵ = 10 600); ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.36 and 3.76 (2H, d: J = 11.3 Hz and dd: J = 13.4 and 7.7 Hz, CH_aNH and CH_bNH), 6.49 (1H, m, NHCO), 7.09 (1H, d: J = 2.5 Hz, H-6′), 7.15 (1H, dd: J = 8.8 and 2.4 Hz, H-4′), 8.12 (1H, d: J = 8.8 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.93 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 3.76 and 4.30 (2H, d: J = 11.2 Hz and dd: J = 13.2 and 7.5 Hz, CH_aNH and CH_bNH), 7.15 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.52 (1H, d: J = 2.5 Hz, H-6′), 8.10 (1H, d: J = 8.8 Hz, H-3′), 9.44 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 510 (MH^+, 100), 492 (MH^+-18, 36). High-resolution MS calculated for $C_{27}H_{36}O_5N_5$ (MH^+): 510.2716; found: 510.2730.

2.11. 17α-[(N-5-Azido-2-nitrobenzoyl)amidomethyl]-5α-androstane-3β,17β-diol ***(10)***

A solution of the 3-oxo derivative **9** (35 mg, 0.07 mmol) in 8 ml of methanol was reduced for 5 min, at 4°C, in the dark, with a solution of $NaBH_4$ (5 mg, 0.13 mmol) in 250 μl of methanol. The reaction was stopped by addition of water (175 μl) and acetic acid (1.5 μl). The solution was evaporated under reduced pressure, and the product was purified by TLC on silica gel (Rf 0.40, chloroform/ethyl acetate 1:1 v/v) to give the 17α-[(*N*-5-azido-2-nitrobenzoyl)amidomethyl]-5α-androstane-3β,17β-diol derivative (15 mg, 0.029 mmol, 42%) as a white solid that was analyzed by TLC on silica gel (Rf 0.49, petroleum ether/ethyl acetate 1:1 v/v; Rf 0.34, chloroform/ethyl acetate 1:1 v/v): m.p. 114–115°C, recrystallized from ethyl acetate; $[\alpha]_D$ = −15.6° (EtOH); ν_{max} ($CHCl_3$): 3700–3610-3430 (OH and NH), 2120 (N_3), 1665 (CONH), 1530 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 306 nm (ϵ = 10 700); ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3), 3.36 and 3.78 (2H, d: J = 13.0 Hz and dd: J = 13.3 and 7.8 Hz, CH_aNH and CH_bNH), 3.61 (1H, m, H-3α), 6.38 (1H, m, NHCO), 7.09 (1H, d: J = 2.5 Hz, H-6′), 7.16 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 8.13 (1H, d: J = 8.8 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.86 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.76 and 4.33 (2H, d: J = 12.0 Hz and dd: J = 13.2 and 7.7 Hz, CH_aNH and CH_bNH), 3.93 (1H, m, H-3α), 7.14 (1H, dd: J = 8.7 and 2.4 Hz, H-4′), 7.52 (1H, d: J = 2.5 Hz, H-6′), 8.10 (1H, d: J = 8.7 Hz, H-3′), 9.43 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 512 (MH^+, 100), 494 (MH^+-18, 33). High-resolution MS calculated for $C_{27}H_{38}O_5N_5$ (MH^+): 512.2873; found: 512.2897.

2.12. 17α-[(N-5-Azido-2-nitrobenzoyl)amidoethyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(11)***

The 17α-aminoethyl steroid **5** (65 mg, 0.17 mmol) was condensed in the dark with *N*-(5-azido-2-nitrobenzoyloxy) succinimide (40 mg, 0.13 mmol), as described above, for the synthesis of the 17α-aminomethyl derivative **8**, and the product was purified by TLC on silica gel (Rf 0.50, petroleum ether/ethyl acetate 1:2 v/v) to give the 17α-(5-azido-2-nitrobenzoyl)amidoethyl derivative (45 mg, 0.086 mmol, 66%): m.p.151–152°C, recrystallized from ethyl acetate; $[\alpha]_D$ = +9.9° (EtOH); ν_{max} (CCl_4): 3510–3440 (OH and NH), 2120 (N_3), 1655 (CONH), 1520 (C-NO_2), 1105–1045 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 306 nm (ϵ = 12 300); ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.83 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy), 3.45 and 3.88 (2H, d: J = 3.8 Hz and dd: J = 13.8 and 7.2 Hz, CH_a NH and CH_bNH), 6.92 (1H, m, NHCO), 7.10 (1H, d: J = 2.3 Hz, H-6′), 7.12 (1H, dd: J = 8.4 and 2.3 Hz, H-4′, superimposed to H-6′ d at 7.10), 8.09 (1H, d: J = 8.4 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.77 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 3.22 and 3.23 (2 × 3H, 2s, dimethoxy), 4.22 (2H, dd, CH_2NH), 7.11 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.58 (1H, d: J = 2.5 Hz, H-6′), 8.06 (1H, d: J = 8.8 Hz, H-3′), 9.03 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 570 (MH^+, 25), 538 (MH^+-32, 60), 520 (MH^+-18–32, 100). High-resolution MS calculated for $C_{30}H_{44}O_6N_5$ (MH^+): 570.3292; found: 570.3264.

2.13. 17α-[(N-5-Azido-2-nitrobenzoyl)amidoethyl]-17β-hydroxy-5α-androstan-3-one ***(12)***

The 3,3-dimethoxy derivative **11** (45 mg, 0.086 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **9**, and the product was purified by TLC on silica gel (Rf 0.30, petroleum ether/ethyl acetate 1:2 v/v) to give the 17α-[(*N*-5-azido-2-nitrobenzoyl)amidoethyl]-3-oxo derivative (40 mg, 0.076 mmol, 95%): m.p. 98–99°C, recrystallized from ethyl acetate; $[\alpha]_D$ = +14.7° (EtOH); ν_{max} ($CHCl_3$): 3690–3420 (OH and NH), 2120 (N_3), 1705 (3-C = O), 1670 (CONH), 1525 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 305 nm (ϵ = 11 200); ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.46 and 3.89 (2H, m, and dd: J = 14.1 and 7.5 Hz, CH_aNH and CH_bNH), 6.83 (1H, m, NHCO), 7.10 (1H, d: J = 2 Hz, H-6′, 7.12 (1H, dd: J = 11.41 and 2.5 Hz, H-4′, superimposed to signal at 7.10), 8.10 (1H, d: J = 8.5 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.92 (3H, s, 19-CH_3), 1.11 (3H, s, 18-CH_3), 4.22 (2H, dd: J = 7.5 and 13.3 Hz, CH_aNH and CH_bNH), 7.12 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.57 (1H, d: J = 2.5 Hz, H-6′), 8.07 (1H, d: J = 8.8 Hz, H-3′), 9.56 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 524 (MH^+, 72), 506

(MH^+-18, 100). High-resolution MS calculated for $C_{28}H_{38}O_5N_5$ (MH^+): 524.2873; found: 524.2875.

2.14. 17α-[(N-5-Azido-2-nitrobenzoyl)amidoethyl]-hydroxy-5α-androstane-3β,17β-diol ***(13)***

The 3-oxo derivative **12** (28 mg, 0.053 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.40, chloroform/ethyl acetate 1:5 v/v) to give the 17α-[(*N*-5-azido-2-nitrobenzoyl)amidoethyl]-3β,17β-diol derivative (22 mg, 0.042 mmol, 79%): m.p. 162–164°C, recrystallized from ethyl acetate; $[\alpha]_D$ = −11.9° (EtOH); ν_{max} ($CHCl_3$): 3690–3620 (OH and NH), 2120 (N_3), 1670 (CONH), 1525 cm^{-1}(C-NO2); λ_{max} (EtOH) = 305 nm (ϵ = 11 750); ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, s, 18-CH_3), 0.82 (3H, s, 19-CH_3), 3.46 and 3.90 (2H, dd: J = 3.75 and 9.7 Hz, and dd: J = 7.2 and 13.8 Hz, CH_aNH and CH_bNH), 3.6 (1H, m, H-3α), 6.91 (1H, m, NHCO), 7.10 (1H, d: J = 2 Hz, H-6′, 7.12 (1H, dd: J = 11.41 and 2.5 Hz, H-4′, superimposed to H-6′ d at 7.10), 8.10 (1H, d: J = 8.5 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.85 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 3.90 (1H, m, H-3α), 4.22 (2H, dd: J = 7.5 and 13.0 Hz, CH_aNH and CH_bNH), 7.11 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.58 (1H, d: J = 2.1 Hz, H-6′, superimposed to signal of pyridine), 8.06 (1H, d: J = 8.8 Hz, H-3′), 9.54 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 548 (MNa^+, 27), 526 (MH^+, 53), 508 (MH^+-18, 100), 500 (MH^+-28, 32). High-resolution MS calculated for $C_{28}H_{40}O_5N_5$ (MH^+): 526.3029; found: 526.2998.

2.15. 17α-[(N-5-Azido-2-nitrobenzoyl)amidopropyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(14)***

The 17α-aminopropyl steroid **7** (60 mg, 0.153 mmol) was condensed in the dark with *N*-(5-azido-2-nitrobenzoyloxy)succinimide (40 mg, 0.13 mmol), as described above, for the synthesis of 17α-aminomethyl and 17α-aminoethyl derivatives **8** and **11**, and the product was purified by TLC on silica gel (Rf 0.35, petroleum ether/ethyl acetate 1:2 v/v) to give the 17α-(5-azido-2-nitrobenzoyl)amidopropyl derivative (50 mg, 0.086 mmol, 56%): m.p. 97–98°C, recrystallized from diethyl ether; $[\alpha]_D$ = −7.4° (EtOH); ν_{max} (CCl_4): 3630–3450-3330 (OH and NH), 2120 (N_3), 1685 (CONH), 1530 (C-NO_2), 1105–1055 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 305 nm (ϵ = 11 390); ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, s, 19-CH_3), 0.83 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy), 3.44 and 3.52 (2H, 2 m, CH_2NH), 6.42 (1H, m, NHCO), 7.11 (1H, d: J = 2.5 Hz, H-6′), 7.14 (1H, dd: J = 8.5 and 2.5 Hz, H-4′, superimposed to H-6′ d at 7.11), 8.10 (1H, d: J = 8.5 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.78 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 3.20 and 3.23 (2 × 3H, 2s, dimethoxy), 3.87 (2H, m, CH_2NH), 7.10 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.50 (1H, d: J = 2.5 Hz, H-6′), 8.04 (1H, d: J = 8.8 Hz, H-3′), 9.62 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 584 (MH^+, 11), 552 (MH^+-32, 49), 534 (MH^+-18–32, 100). High-resolution MS calculated for $C_{31}H_{46}O_6N_5$ (MH^+): 584.3448; found: 584.3417.

2.16. 17α-[(N-5-Azido-2-nitrobenzoyl)amidopropyl]-17β-hydroxy-5α-androstan-3-one ***(15)***

The 3,3-dimethoxy derivative **14** (25 mg, 0.043 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **9**, to give the 17α-[(*N*-5-azido-2-nitrobenzoyl)amidopropyl]-3-oxo derivative, which was purified by TLC on silica gel (Rf 0.35, petroleum ether/ethyl acetate 1:2 v/v) as a white solid (22 mg, 0.076 mmol, 95%): m.p. 81–83°C, recrystallized from ethyl acetate; $[\alpha]_D$ = −17.4° (EtOH); ν_{max} ($CHCl_3$): 3600–3455-3300 (OH and NH), 2120 (N_3), 1705 (3-C = O), 1670 (CONH), 1530 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 305 nm (ϵ = 11 000); ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 3.44 and 3.54 (2H, d: J = 13Hz, and dd: J = 6.2 and 6.6 Hz, CH_aNH and CH_bNH), 6.42 (1H, m, NHCO), 7.10 (1H, d: J = 2.3 Hz, H-6′), 7.13 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 8.10 (1H, d: J = 8.8 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.94 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.87 (2H, dd: J = 6.0 and 6.5 Hz CH_aNH and CH_bNH), 7.11 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.51 (1H, d: J = 2.5 Hz, H-6′), 8.06 (1H, d: J = 8.8 Hz, H-3′), 9.66 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 538 (MH^+, 34), 520 (MH^+-18, 100). High-resolution MS calculated for $C_{29}H_{40}O_5N_5$ (MH^+): 538.3029; found: 538.3047.

2.17. 17α-[(N-5-Azido-2-nitrobenzoyl)amidopropyl]-5α-androstane-3β,17β-diol ***(16)***

The 3-oxo derivative **15** (20 mg, 0.037 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.30, chloroform/ethyl acetate 1:1) to give the 17α-[(*N*-5-azido-2-nitrobenzoyl)amidopropyl]-3β,17β-diol derivative (18 mg, 0.033 mmol, 90%), which was analyzed by TLC on silica gel (Rf 0.40, chloroform/ethyl acetate 1:3 v/v): m.p. 166–167°C, recrystallized from diethyl ether; $[\alpha]_D$ = −23.8° (EtOH); ν_{max} ($CHCl_3$): 3690–3610-3530–3430-3310 (OH and NH), 2120 (N_3), 1670 (CONH), 1530 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 305 nm (ϵ = 11 700); ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.84 (3H, s, 18-CH_3), 3.45 and 3.55 (2H, 2 m, CH_2NH), 3.59 superimposed to CH_2NH m at 3.55 (1H, m, H-3α), 6.43 (1H, m, NHCO), 7.11 (1H, d: J = 2.4 Hz, H-6′), 7.13 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 8.10 (1H, d: J = 8.8 Hz, H-3′); ^{1}H NMR (C_5D_5N) δ 0.85 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.87 (3H, m, CH_2NH), 3.44 (1H, m, H-3α), 7.12 (1H, dd: J = 8.8 and 2.5 Hz, H-4′), 7.50 (1H, d: J = 2.4 Hz, H-6′), 8.07 (1H, d: J = 8.8 Hz, H-3′), 9.72 (1H, m, NHCO); MS (LSIMS$^+$) *m/z* (relative intensity %) 540 (MH^+, 55),

522 (MH^+-18, 100). High-resolution MS calculated for $C_{29}H_{42}O_5N_5$ (MH^+): 540.3186; found: 540.3174.

2.18. 17α-[(N-4-Azido-2-nitrophenyl)aminomethyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(17)***

A solution of 17α-aminomethyl steroid **3** (100 mg, 0.27 mmol) in 2 ml of tetrahydrofuran and 1 ml of an ethanol/ether/triethylamine 20:10:0.2 v/v/v mixture was stirred overnight in the dark, in the presence of 4-azido-1-fluoro-2-nitrobenzene (100 mg, 0.55 mmol) dissolved in 3 ml of tetrahydrofuran. The reaction mixture was evaporated under reduced pressure, in the dark. The product was purified with efficient separation from the reagent by TLC on silica gel (Rf 0.45, chloroform stabilized with 0.5% ethanol) to give the 17α-(*N*-4-azido-2-nitrophenyl)aminomethyl derivative (130 mg, 0.24 mmol, 91%), which was also analyzed by TLC on silica gel (Rf 0.70, chloroform/ethyl acetate 3:1 v/v): m.p.138–139°C, recrystallized from ethyl acetate; $[\alpha]_D$ = −7.9° (EtOH); ν_{max} ($CHCl_3$): 3690–3610-3380 (OH and NH), 2120 (N_3), 1520 (C-NO_2), 1105–1050 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 261 nm (ε = 21 900), 466 nm (ε = 5700); ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.94 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy), 3.25 and 3.43 (2H, 2 m, CH_2NH), 6.90 (1H, d: J = 9.2 Hz, H-6′), 7.12 (1H, dd: J = 9.2 and 2.6 Hz, H-5′), 7.88 (1H, d: J = 2.7 Hz, H-3′); 8.4 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.78 (3H, s, 19-CH_3), 1.17 (3H, s, 18-CH_3),), 3.21 and 3.24 (2 × 3H, 2s, dimethoxy), 3.60 and 3.74 (2H, dd: J = 12.2 and 5.6 Hz and d, J = 12.2 Hz, CH_aNH and CH_bNH), 7.35 (1H, dd: J = 9.2 and 2.6 Hz, H-5′), 7.44 (1H, d: J = 9.2 Hz, H-6′), 7.98 (1H, d: J = 2.6 Hz, H-3′); MS ($LSIMS^+$) *m/z* (relative intensity %) 528 (MH^+, 41), 496 (MH^+-32, 100). High-resolution MS calculated for $C_{28}H_{42}O_5N_5$ (MH^+): 528.3186; found: 528.3172.

2.19. 17α-[(N-4-Azido-2-nitrophenyl)aminomethyl]-17β-hydroxy-5α-androstan-3-one ***(18)***

The 3,3-dimethoxy derivative **17** (130 mg, 0.24 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **9**, and the product was purified by TLC on silica gel (Rf 0.35, chloroform stabilized with 0.5% ethanol) to give the 17α-[(*N*-4-azido-2-nitrophenyl)aminomethyl]-3-oxo derivative (60 mg, 0.124 mmol, 50%), which was also analyzed by TLC on silica gel (Rf 0.65, chloroform/ethyl acetate 3:1 v/v): m.p. 153–155°C, recrystallized from diethyl ether; $[\alpha]_D$ = −7.5° (EtOH); ν_{max} ($CHCl_3$): 3690–3610-3380 (OH and NH), 2120 (N_3), 1705 (3-C = O), 1520 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 260 nm (ε = 19 200), 464 nm (ε = 5000); ^1H NMR ($CDCl_3$) δ 0.97 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 3.27 and 3.43 (2H, dd: J = 3.6 and 6.0 Hz and d, J = 12.4 Hz, CH_aNH and CH_bNH), 6.90 (1H, d: J = 9.2 Hz, H-6′), 7.11 (1H, dd: J = 9.2 and 2.7 Hz, H-5′), 7.87 (1H, d: J = 2.7 Hz, H-3′); 8.4 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.95 (3H, s, 19-CH_3), 1.18 (3H, s, 18-CH_3), 3.59 and 3.69 (2H, dd: J = 3.1 and 5.5 Hz and d, J = 12.3 Hz, CH_aNH and CH_bNH), 7.32 (2H, m, H-5′ + H-6′), 7.97 (1H, d: J = 2.6 Hz, H-3′), 6.60 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %): 482 (MH^+, 100). High-resolution MS calculated for $C_{26}H_{36}O_4N_5$ (MH^+): 482.2767; found: 482.2772.

2.20. 17α-[(N-4-Azido-2-nitrophenyl)aminomethyl]-5α-androstane-3β,17β-diol ***(19)***

The 3-oxo derivative **18** (40 mg, 0.083 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.35, chloroform/ethyl acetate 3:1 v/v) to give the 17α-[(*N*-4-azido-2-nitrophenyl)aminomethyl]-3β,17β-diol derivative (26 mg, 0.054 mmol, 66%): m.p. 84–89°C, recrystallized from diethyl ether; $[\alpha]_D$ = −10.7° (EtOH); ν_{max} ($CHCl_3$): 3690–3610-3380 (OH and NH), 2120 (N_3), 1705 (3-C = O), 1520 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 260 nm (ε = 22 500), 459 nm (ε = 7400); ^1H NMR ($CDCl_3$) δ 0.84 (3H, s, 19-CH_3), 0.94 (3H, s, 18-CH_3), 3.26 and 3.41 (2H, dd: J = 3.3 and 5.9 Hz and d, J = 12.4 Hz, CH_aNH and CH_bNH), 3.58 (1H, m, H-3α), 6.90 (1H, d: J = 9.2 Hz, H-6′), 7.11 (1H, dd: J = 9.2 and 2.7 Hz, H-5′), 7.87 (1H, d: J = 2.8 Hz, H-3′); 8.39 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.86 (3H, s, 19-CH_3), 1.17 (3H, s, 18-CH_3), 3.57 and 3.72 (2H, dd: J = 3.3 and 5.7 Hz and d, J = 12.2 Hz, CH_aNH and CH_bNH), 3.89 (1H, m, H-3α), 6.60 (1H, m, NH), 7.33 (2H, m, H-5′+ H-6′), 7.97 (1H, d: J = 2.8 Hz, H-3′); MS ($LSIMS^+$) *m/z* (relative intensity %) 484 (MH^+, 100). High-resolution MS calculated for $C_{26}H_{38}O_4N_5$ (MH^+): 484.2924; found: 484.2916.

2.21. 17α-[(N-4-Azido-2-nitrophenyl)aminoethyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(20)***

The 17α-aminoethyl steroid **5** (65 mg, 0.171 mmol) was condensed with 4-azido-1-fluoro-2-nitrobenzene as described above for the 17α-aminoethyl derivative **17**, and the product was purified by TLC on silica gel (Rf 0.55, chloroform/ethyl acetate10:1 v/v) to give the 17α-(*N*-4-azido-2-nitrophenyl)aminoethyl derivative (30 mg, 0.055 mmol, 33%): m.p. 115–118°C, recrystallized from diethyl ether; $[\alpha]_D$ = −20.4° (EtOH); ν_{max} (CCl_4): 3690–3630-3380 (OH and NH), 2115 (N_3), 1520 (C-NO_2), 1105–1055 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 260 nm (ε = 20 800), 458 nm (ε = 5600); ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.88 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy), 3.50 and 3.55 (2H, 2 m, CH_2NH), 6.93 (1H, d: J = 9.2 Hz, H-6′), 7.14 (1H, dd: J = 9.2 and 2.7 Hz, H-5′), 7.87 (1H, d: J = 2.7 Hz, H-3′); 8.42 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.80 (3H, s, 19-CH_3), 1.11 (3H, s, 18-CH_3), 3.21 and 3.24 (2 × 3H, 2s, dimethoxy), 3.69 and 3.80 (2H, 2 m, CH_2NH), 7.11 (1H, d: J = 9.2 Hz, H-6′), 7.19 (1H, dd: J = 9.2 and 2.6 Hz, H-5′), 7.95 (1H, d: J = 2.7 Hz, H-3′), 9.07 (1H, m,

NH); MS (LSIMS$^+$) *m/z* (relative intensity %) 542 (MH^+, 53), 510 (MH^+-32, 100). High-resolution MS calculated for $C_{29}H_{44}O_5N_5$ (MH^+): 542.3342; found: 542.3336.

2.22. 17α-[(N-4-Azido-2-nitrophenyl)aminoethyl]-17β-hydroxy-5α-androstan-3-one ***(21)***

The 3,3-dimethoxy derivative **20** (13 mg, 0.024 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **18**, and the product was purified by TLC on silica gel (Rf 0.50, chloroform/ethyl acetate 10:1 v/v) to give the 17α-[(*N*-4-azido-2-nitrophenyl)aminoethyl]-3-oxo derivative (10 mg, 0.02 mmol, 84%): m.p. 129–131°C, recrystallized from ethyl acetate; $[\alpha]_D$ = +24.0° (EtOH); ν_{max} ($CHCl_3$): 3690–3630-3380 (OH and NH), 2120 (N_3), 1705 (3-C = O), 1520 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 260 nm (ϵ = 19 200), 463 nm (ϵ = 5200); ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 3.50 and 3.55 (2H, 2 m, CH_2NH), 6.94 (1H, d: J = 9.1 Hz, H-6′), 7.13 (1H, dd: J = 9.1 and 2.6 Hz, H-5′), 7.86 (1H, d: J = 2.6 Hz, H-3′), 8.40 (1H, m, NH); ^{1}H NMR (C_5D_5N) δ 0.96 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.68 and 3.78 (2H, 2 m, CH_2NH), 7.11 (1H, d: J = 9.3 Hz, H-6′), 7.18 (1H, dd: J = 9.3 and 2.6 Hz, H-5′), 7.95 (1H, d: J = 2.6 Hz, H-3′), 9.04 (1H, m, NH); MS (LSIMS$^+$) *m/z* (relative intensity %) 496 (MH^+,100). High-resolution MS calculated for $C_{27}H_{38}O_4N_5$ (MH^+): 496.2924; found: 496.2914.

2.23. 17α-[(N-4-Azido-2-nitrophenyl)aminoethyl]-5α-androstane-3β,17β-diol ***(22)***

The 3-oxo derivative **21** (22 mg, 0.044 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.30, chloroform/ethyl acetate 4:1 v/v) to give the 17α-[(*N*-4-azido-2-nitrophenyl)aminoethyl]-3β,17β-diol derivative (9 mg, 0.018 mmol, 42%): m.p. 136–137°C, recrystallized from diethyl ether; $[\alpha]_D$ = −28.3° (EtOH); ν_{max} ($CHCl_3$): 3695–3610- (OH and NH), 2120 (N_3), 1525 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 260 nm (ϵ = 18 800), 463 nm (ϵ = 5200); ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, s, 19-CH_3), 0.88 (3H, s, 18-CH_3), 3.49 (1H, m, H-3α), 3.50 and 3.54 (2H, 2 m, CH_2NH), 6.94 (1H, d: J = 9.1 Hz, H-6′), 7.13 (1H, dd: J = 9.1 and 2.6 Hz, H-5′), 7.86 (1H, d: J = 2.6 Hz, H-3′), 8.40 (1H, m, NH); ^{1}H NMR (C_5D_5N) δ 0.88 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.69 and 3.79 (2H, dd, J = 9.4 and 2.6 Hz and d: J = 12.5 Hz, CH_aNH and CH_bNH), 3.92 (1H, m, H-3α), 7.11 (1H, d: J = 9.2 Hz, H-6′), 7.18 (1H, dd: J = 5.0 and 7.5 Hz, and d: H-5′), 7.95 (1H, d: J = 2.6 Hz, H-3′), 9.07 (1H, m, NH); MS (LSIMS$^+$) *m/z* (relative intensity %) 498 (MH^+, 100). High-resolution MS calculated for $C_{27}H_{40}O_4N_5$ (MH^+): 498.3080; found: 498.3093.

2.24. 17α-[(N-4-Azido-2-nitrophenyl)aminopropyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(23)***

The 17α-aminopropyl steroid **7** (90 mg, 0.228 mmol) was condensed with 4-azido-1-fluoro-2-nitrobenzene as described above for the 17α-aminomethyl derivative **17**, and the product was purified by TLC on silica gel (Rf 0.40, chloroform/ethyl acetate 15:1 v/v) to give the 17α-(*N*-4-azido-2-nitrophenyl)aminopropyl derivative (60 mg, 0.108 mmol, 47%) which was also analyzed by TLC on silica gel (Rf 0.60, chloroform/ethyl acetate 4:1 v/v): m.p. 142–143°C, recrystallized from diethyl ether; $[\alpha]_D$ = −6.9° (EtOH); ν_{max} (CCl_4): 3695–3610 (OH and NH), 2120 (N_3), 1520 C-NO_2), 1105–1055 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 260 nm (ϵ = 22 400), 463 nm (ϵ = 5600); ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy), 3.36 (2H, m, CH_2NH), 6.90 (1H, d: J = 9.2 Hz, H-6′), 7.13 (1H, dd: J = 9.2 and 2.7 Hz, H-5′), 7.87 (1H, d: J = 2.7 Hz, H-3′), 8.12 (1H, m, NH); ^{1}H NMR (C_5D_5N) δ 0.80 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.21 and 3.24 (2 × 3H, 2s, dimethoxy), 3.47 (2H, m, CH_2NH), 7.07 (1H, d: J = 9.3 Hz, H-6′), 7.19 (1H, dd: J = 9.2 and 2.6 Hz, H-5′), 7.93(1H, d: J = 2.7 Hz, H-3′), 8.52 (1H, m, NH); MS (LSIMS$^+$) *m/z* (relative intensity %): 556 (MH^+, 41), 538 (MH^+-18, 5), 524 (MH^+-32, 100). High-resolution MS calculated for $C_{30}H_{46}O_5N_5$ (MH^+): 556.3499; found: 556.3502.

2.25. 17α-[(N-4-Azido-2-nitrophenyl)aminopropyl]-17β-hydroxy-5α-androstan-3-one ***(24)***

The 3,3-dimethoxy derivative **23** (35 mg, 0.63 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **12**, and the product was purified by TLC on silica gel (Rf 0.35, chloroform/ethyl acetate 15:1 v/v) to give the 17α-[(*N*-4-azido-2-nitrophenyl)aminopropyl]-3-oxo derivative (31 mg, 0.061 mmol, 97%), which was also analyzed by TLC on silica gel (Rf 0.55, chloroform/ethyl acetate 4:1 v/v): m.p. 132–134°C, recrystallized from diethyl ether; $[\alpha]_D$ = +8.7° (EtOH); ν_{max} ($CHCl_3$): 3740–3640-3370 (OH and NH), 2120 (N_3), 1715 (3-C = O), 1520 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 261 nm (ϵ = 21 300), 460 nm (ϵ = 5300); ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.35 (2H, dd: J = 5.8 and 6.0 Hz and d, J = 12.2 Hz, CH_aNH and CH_bNH), 6.89 (1H, d: J = 9.2 Hz, H-6′), 7.13 (1H, dd: J = 9.2 and 2.6 Hz, H-5′), 7.86 (1H, d: J = 2.6 Hz, H-3′), 8.11 (1H, m, NH); ^{1}H NMR (C_5D_5N) δ 0.95 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 3.46 (2H, dd: J = 5.8 and 6.6 Hz and d, J = 12.1 Hz, CH_aNH and CH_bNH), 7.06 (1H, d: J = 9.3 Hz, H-6′), 7.18 (1H, dd: J = 9.3 and 2.6 Hz, H-5′), 7.92 (1H, d: J = 2.6 Hz, H-3′), 8.51 (1H, m, NH); MS (LSIMS$^+$) *m/z* (relative intensity %) 510 (MH^+, 100). High-resolution MS calculated for $C_{28}H_{40}O_4N_5$ (MH^+): 510.3080; found: 510.3086.

2.26. 17α-[(N-4-Azido-2-nitrophenyl)aminopropyl]-5α-androstane-3β,17β-diol ***(25)***

The 3-oxo derivative **24** (30 mg, 0.059 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.30, chloroform/ethyl acetate 4:1 v/v) to give the 17α-[(*N*-4-azido-2-nitrophenyl)aminopropyl]-3β,17β-diol derivative (12 mg, 0.024 mmol, 41%): m.p. 118–120°C, recrystallized from diethyl ether; $[\alpha]_D$ = −15.3° (EtOH); ν_{max} ($CHCl_3$): 3690–3600-3390- (OH and NH), 2120 (N_3), 1520 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 260 nm (ϵ = 20 500), 463 nm (ϵ = 5000); ^1H NMR ($CDCl_3$) δ 0.83 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3), 3.36 (2H, m, CH_2NH), 3.59 (1H, m, H-3α), 6.86 (1H, m, NHCO), 6.88 (1H, d: J = 9.3 Hz, H-6′), 7.12 (1H, dd: J = 9.2 and 2.8 Hz, H-5′), 7.87 (1H, d: J = 2.6 Hz, H-3′); 8.12 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.88 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.46 (2H, dd: J = 6.7 and 13 Hz, CH_aNH and CH_b NH), 3.91 (1H, m, H-3α), 7.07 (1H, d: J = 9.2 Hz, H-6′), 7.19 (1H, dd: J = 9.2 and 2.7 Hz, H-5′), 7.93 (1H, d: J = 2.7 Hz, H-3′), 8.53 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 512 (MH^+, 100). High-resolution MS calculated for $C_{28}H_{42}O_4N_5$ (MH^+): 512.3236; found: 512.3255.

2.27. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminomethyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(26)***

A solution of 17α-aminomethyl steroid **3** (50 mg, 0.137 mmol) in 3 ml of tetrahydrofuran was stirred for 8 days in the dark, in the presence of a solution of 4-azido-1-nitro-2,3,5,6-tetrafluorobenzene [10] (50 mg, 0.212 mmol) in 1 ml of tetrahydrofuran and 1 ml of an ethanol/diethyl ether/triethylamine 20:10:0.2 mixture. The reaction mixture was evaporated under reduced pressure, and the product was purified by TLC on silica gel (Rf 0.30, petroleum ether/ethyl acetate 5:1 v/v) to give the 17α-(5-azido-2-nitro-3,4,6-trifluorophenyl)aminomethyl derivative (37 mg, 0.064 mmol, 47%): m.p. 126.5–127.5°C, recrystallized from diethyl ether; $[\alpha]_D$ = −15.7° (EtOH); ν_{max} (CCl_4): 3630–3380 (OH and NH), 2125 (N_3), 1505 (C-NO_2), 1105–1055 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 237 nm (ϵ = 19 400), 301 nm (ϵ = 8500), 420 nm (ϵ = 3800); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3), 3.13 and 3.19 (2 × 3H, 2s, dimethoxy), 3.33 and 3.55 (2H, 2 m, CH_2NH), 6.90 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.77 (3H, s, 19-CH_3), 1.12 (3H, s, 18-CH_3), 3.21 and 3.23 (2 × 3H, 2s, dimethoxy), 3.71 and 3.93 (2H, 2 m, CH_2NH), 7.72 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 582 (MH^+, 31), 550 (MH^+-32, 100). High-resolution MS calculated for $C_{28}H_{39}O_5N_5F_3$ (MH^+): 582.2903; found: 582.2938.

2.28. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminomethyl]-17β-hydroxy-5α-androstan-3-one ***(27)***

The 3,3-dimethoxy derivative **26** (121 mg, 0.21 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **9**, and the product was purified by TLC on silica gel (Rf 0.40, chloroform/ethyl acetate 2:1 v/v) to give the 17α-[(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)aminomethyl]-3-oxo derivative (100 mg, 0.187 mmol, 89%): m.p. 111–114°C, recrystallized from diethyl ether; $[\alpha]_D$ = −3.9° (EtOH); ν_{max} ($CHCl_3$): 3690–3615-3370 (OH and NH), 2130 (N_3), 1705 (3-C = O), 1505 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 235 nm (ϵ = 22 500), 298 nm (ϵ = 9500), 420 nm (ϵ = 4400); ^1H NMR ($CDCl_3$) δ 0.92 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.37 and 3.54 (2H, 2 m, CH_2NH), 6.87 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.94 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 3.67 and 3.89 (2H, dd: J = 3.0 and 4.0 Hz and d, J = 12.2 Hz, CH_aNH and CH_bNH), 7.67 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 536 (MH^+, 100), 518 (MH^+-18, 9). High-resolution MS calculated for $C_{26}H_{33}O_4N_5F_3$ (MH^+): 536.2485; found: 536.2507.

2.29. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminomethyl]-5α-androstane-3β,17β-diol ***(28)***

The 3-oxo derivative **27** (35 mg, 0.065 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.30, petroleum ether/ethyl acetate 2:1) to give the 17α-[(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)aminomethyl]-3β,17β-diol (19 mg, 0.035 mmol, 54%): m.p. 156–157°C, recrystallized from ethyl acetate; $[\alpha]_D$ = −17.9° (EtOH); ν_{max} ($CHCl_3$): 3690–3615 (OH and NH), 2130 (N_3), 1505 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 236 nm (ϵ = 20 300), 300 nm (ϵ = 8600), 421 nm (ϵ = 3900); ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3), 3.39 and 3.55 (2H, 2 m, CH_2NH), 3.59 superimposed to CH_2NH m at 3.55 (1H, m, H-3), 6.85 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.85 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 3.69 and 3.90 (2H, 2 m, CH_2NH), 3.90 superimposed to CH_2NH m at 3.90 (1H, m, H-3α), 7.68 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 538 (MH^+, 100), 520 (MH^+-18, 16). High-resolution MS calculated for $C_{26}H_{35}O_4N_5F_3$ (MH^+): 538.2641; found: 538.2662.

2.30. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminoethyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(29)***

The 17α-aminoethyl steroid **5** (100 mg, 0.264 mmol) was condensed with 4-azido-1-nitro-2,3,5,6-tetrafluorobenzene, as described above for the synthesis of the 17α-aminomethyl derivative **26**, and the product was purified by TLC on silica gel (Rf 0.60 chloroform/ethyl acetate 20:1 v/v) to give the 17α-(5-azido-2-nitro-3,4,6-trifluorophenyl)aminoethyl derivative (70 mg, 0.118 mmol, 45%): m.p.

66–69°C, recrystallized from diethyl ether; $[\alpha]_D = +34.2°$ (CCl_4); ν_{max} ($CHCl_3$): 3620–3345 (OH and NH), 2130 (N_3), 1505 (C-NO_2); 1105–1050 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 237 nm (ϵ = 21 300), 298 nm (ϵ = 8500), 419 nm (ϵ = 4000); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.85 (3H, s, 18-CH_3), 3.14 and 3.19 (2 × 3H, 2s, dimethoxy), 3.63 (2H, 2 m, CH_2NH), 6.94 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.78 (3H, s, 19-CH_3), 1.08 (3H, s, 18-CH_3), 3.21 and 3.23 (2 × 3H, 2s, dimethoxy), 3.79 and 3.90 (2H, 2 m, CH_2NH), 7.75 (1H, m, NH); ^{19}F NMR ($CDCl_3$) δ 144.31 (1F, s broad, F-6), 146.83 (1F, dd: J = 22.8 and 11.3 Hz, F-2), 163.06 (1F, d: J = 24.0 Hz, F-3); MS ($LSIMS^+$) *m/z* (relative intensity %) 596 (MH^+, 36), 578 (MH^+-18, 6), 564 (MH^+-32, 100), 546 (MH^+-32–18, 25). High-resolution MS calculated for $C_{29}H_{41}O_5N_5F_3$ (MH^+): 596.3060; found: 596.3069.

2.31. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminoethyl]-17β-hydroxy-5α-androstan-3-one ***(30)***

The 3,3-dimethoxy derivative **29** (45 mg, 0.076 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **9**, and the product was purified by TLC on silica gel (Rf 0.35, chloroform/ethyl acetate 20:1 v/v) to give the 17α-[(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)aminoethyl]-3-oxo derivative (40 mg, 0.073 mmol, 96%): m.p. 125–127°C, recrystallized from diethyl ether; $[\alpha]_D = +23.8°$ (EtOH); ν_{max} ($CHCl_3$): 3680–3610-3345 (OH and NH), 2130 (N_3), 1705 (3-C = O), 1505 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 238 nm (ϵ = 19 200), 299 nm (ϵ = 7200), 420 nm (ϵ = 3300); ^1H NMR ($CDCl_3$) δ 0.89 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.63 (2H, m, CH_2NH), 6.95 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.94 (3H, s, 19-CH_3), 1.08 (3H, s, 18-CH_3), 3.80 and 3.90 (2H, 2 m, CH_2NH), 7.74 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 550 (MH^+, 100), 532 (MH^+-18, 28). High-resolution MS calculated for $C_{27}H_{35}O_4N_5F_3$ (MH^+): 550.2641; found: 550.2649.

2.32. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminoethyl]-5α-androstane-3β,17β-diol ***(31)***

The 3-oxo derivative **30** (22 mg, 0.04 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.35, chloroform/ethyl acetate 4:1 v/v) to give the 17α-[(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)aminoethyl]-3β,17β-diol (13 mg, 0.024 mmol, 59%): m.p. 97–100°C, recrystallized from diethyl ether; $[\alpha]_D = +47°$ (EtOH); ν_{max} (CCl_4): 3625–3340 (OH and NH), 2125 (N_3), 1505 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 238 nm (ϵ = 18 200), 302 nm (ϵ = 6900), 431 nm (ϵ = 7900); ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.85 (3H, s, 18-CH_3), 3.64 (2H, m, CH_2NH), 3.59 superimposed to CH_2NH m at 3.64 (1H, m, H-3α), 6.95 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.87 (3H, s, 19-CH_3), 1.08 (3H, s, 18-CH_3), 3.79 and 3.91 (2H, 2 m, CH_2NH), 3.91 superimposed to CH_2NH m at 3.91 (1H, m, H-3α), 7.77 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 552 (MH^+, 100), 534 (MH^+-18, 26). High-resolution MS calculated for $C_{27}H_{37}O_4N_5F_3$ (MH^+): 552.2797; found: 552.2811.

2.33. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminopropyl]-3,3-dimethoxy-5α-androstan-17β-ol ***(32)***

The 17α-aminopropyl steroid **7** (100 mg, 0.254 mmol) was condensed with 4-azido-1-nitro-2,3,5,6-tetrafluorobenzene, as described above for the synthesis of 17α-aminomethyl and 17α-aminoethyl derivatives **26** and **29**, and the product was purified by TLC on silica gel (Rf 0.50, chloroform/ethyl acetate 20:1 v/v) to give the 17α-(5-azido-2-nitro-3,4,6-trifluorophenyl)aminopropyl derivative (80 mg, 0.131 mmol, 52%): m.p. 127–129°C, recrystallized from diethyl ether; $[\alpha]_D = -8.3°$($CHCl_3$); ν_{max} (CCl_4): 3625–3340 (OH and NH), 2125 (N_3), 1505 (C-NO_2), 1105–1055 cm^{-1} (dimethoxy); λ_{max} (EtOH) = 237 nm (ϵ = 18 700), 301 nm (ϵ = 8300), 417 nm (ϵ = 3500); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 19-CH_3), 0.85 (3H, s, 18-CH_3), 3.14 and 3.19 (2×3H, 2s, dimethoxy), 3.44 (2H, m, CH_2NH), 6.57 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.79 (3H, s, 19-CH_3), 1.09 (3H, s, 18-CH_3), 3.20 and 3.23 (2 × 3H, 2s, dimethoxy), 3.58 (2H, m, CH_2NH), 7.68 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 610 (MH^+, 25), 592 (MH^+-18, 4), 578 (MH^+-32, 100), 560 (MH^+-18–32, 39). High-resolution MS calculated for $C_{30}H_{43}O_5N_5F_3$ (MH^+): 610.3193; found: 610.3216.

2.34. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminopropyl]-17β-hydroxy-5α-androstan-3-one ***(33)***

The 3,3-dimethoxy derivative **32** (54 mg, 0.088 mmol) was acidolyzed, as described above for the synthesis of the 3-oxo derivative **12**, and the product was purified by TLC on silica gel (Rf 0.40, chloroform/ethyl acetate 20:1 v/v) to give the 17α-[(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)-aminopropyl]-3-oxo derivative (48 mg, 0.085 mmol, 97%): m.p. 53–54°C, recrystallized from diethyl ether; $[\alpha]_D = +16.6°$ (EtOH); ν_{max} ($CHCl_3$): 3690–3610-3390 (OH and NH), 2130 (N_3), 1720 (C = O), 1505 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 237 nm (ϵ = 17 000), 302 nm (ϵ = 7300), 413 nm (ϵ = 2850); ^1H NMR ($CDCl_3$) δ 0.88 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.45 (2H, m, CH_2NH), 6.57 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.95 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 3.58 (2H, m, CH_2NH), 7.28 (1H, m, NH); MS ($LSIMS^+$) *m/z* (relative intensity %) 564 (MH^+, 100), 546 (MH^+-18, 27). High-resolution MS calculated for $C_{28}H_{37}O_4N_5F_3$ (MH^+): 564.2797; found: 564.2784.

2.35. 17α-[(N-5-Azido-2-nitro-3,4,6-trifluorophenyl)aminopropyl]-5α-androstane-3β,17β-diol ***(34)***

The 3-oxo derivative **33** (36 mg, 0.064 mmol) was reduced with $NaBH_4$, as described above for the synthesis of the diol derivative **10**, and the product was purified by TLC on silica gel (Rf 0.35, chloroform/ethyl acetate 4:1 v/v) to give the

17α-[(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)aminopropyl]-3β,17β-diol (20 mg, 0.035 mmol, 55%): m.p. 110–111°C, recrystallized from diethyl ether; $[\alpha]_D = +6.0°$ (EtOH); ν_{max} ($CHCl_3$): 3690–3610-3380 (OH and NH), 2130 (N_3), 1510 cm^{-1} (C-NO_2); λ_{max} (EtOH) = 237 nm (ϵ = 17 900), 302 nm (ϵ = 7400), 417 nm (ϵ = 3400); ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 19-CH_3), 0.85 (3H, s, 18-CH_3), 3.45 (2H, m, CH_2NH), 3.59 (1H, m, H-3α), 6.59 (1H, m, NH); ^1H NMR (C_5D_5N) δ 0.87 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 3.58 (2H, m, CH_2NH), 3.91 (1H, m, H-3α), 7.30 (1H, m, NH); MS (LSIMS$^+$) *m/z* (relative intensity %) 566 (MH^+, 100), 548 (MH^+-18, 19). High-resolution MS calculated for $C_{28}H_{39}O_4N_5F_3$ (MH^+): 566.2954; found: 566.2960.

2.36. Competitive binding assays with SHBG

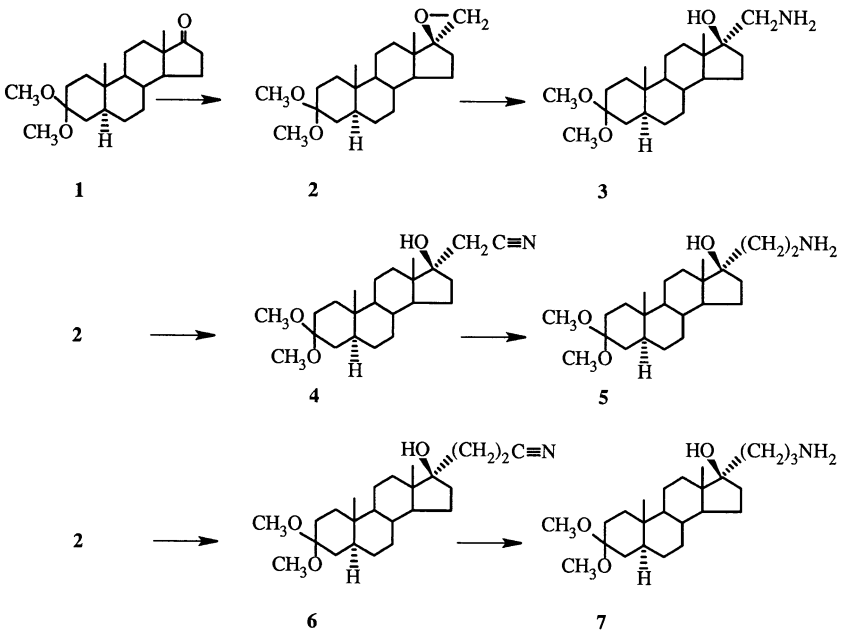
Aliquots (100 μl) of purified human SHBG (5.5 nM in 10 mM phosphate buffer pH 7.4, 0.15 M NaCl, 0.1% gelatin and 0.1% NaN_3) in triplicate tubes were incubated in the dark with [1,2,4,5,6,7-$^3H_6$]DHT (100 μl, 100 000 dpm, 1 nM, s.a. 114 Ci/mmol, purchased from Amersham) and seven concentrations (ranging from 8.6 to 172 nM) of radioinert steroid competitors (DHT and 17α-aminomethyl-, 17α-aminoethyl-, and 17α-aminopropyl-ANB derivatives of DHT) in 300 μl of the same buffer for 1 h at 22°C. Free and bound steroid fractions were separated by dextran-coated charcoal (prepared from 5 g of Norit A and 0.5 g of Dextran T-70 in 1 liter of 10 mM phosphate buffer, pH 7.4, 0.15 M NaCl). For each competitor concentration, the radioactivity of bound tracer (B) was expressed as percent of radioactivity bound in the absence of competitor (B_0).

2.37. Competitive binding assays with androgen receptors of rat ventral prostate

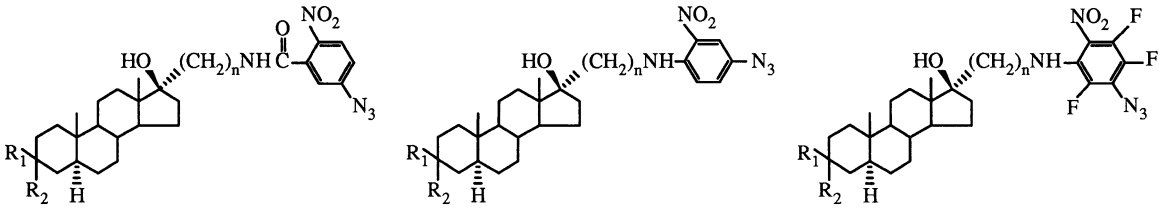
Aliquots of cytosol of prostates from rats castrated 24 h before being sacrificed (200 μl in Tris·HCl 10 mM, pH 7.4, 1.5 mM EDTA, 10% glycerol, and 20 mM sodium molybdate, containing 0.1 mM leupeptin and 0.1 mM bacitracin) in triplicate tubes were incubated in the dark with [1,2,4,5,6,7-$^3H_6$]DHT (100 μl, 30 000 dpm, 0.3 nM) and seven concentrations (ranging from 1.25 to 125 nM) of radioinert steroid competitors (DHT and 17α-aminomethyl-, 17α-aminoethyl-, and 17α-aminopropyl-ANB derivatives of DHT) in 300 μl of the same buffer for 4 h at 4°C. Nonspecific binding was estimated by incubation in the same conditions in the presence of 625 nM of each of the radioinert competitors. Free and bound steroid fractions were separated by dextran-coated charcoal. For each competitor concentration, the radioactivity of bound tracer (B) was expressed as percent of radioactivity bound in the absence of competitor (B_0), after substraction of nonspecifically bound radioactivity.

3. Results and discussion

In this study, the synthetic route chosen for the access to 17α-aminoalkyl derivatives of 5α-dihydrotestosterone (Scheme 1) was established from 5α-androstane-3,17-dione, which was converted to a 3,3-(dimethoxy)-5α-androstan-17-one precursor **1**. The choice of this derivative rather than that of the corresponding 3,3-ethylenedioxy analog, employed in a previous synthesis of 17α-hexanoic derivatives [1], was made with the view of preparing radioactive



Scheme 1.



n=1
8 R_1= R_2= OCH_3
9 R_1,R_2= O
10 R_1= H, R_2= OH

n=2
11 R_1= R_2= OCH_3
12 R_1,R_2= O
13 R_1= H, R_2= OH

n=3
14 R_1= R_2= OCH_3
15 R_1,R_2= O
16 R_1= H, R_2= OH

n=1
17 R_1= R_2= OCH_3
18 R_1,R_2= O
19 R_1= H, R_2= OH

n=2
20 R_1= R_2= OCH_3
21 R_1,R_2= O
22 R_1= H, R_2= OH

n=3
23 R_1= R_2= OCH_3
24 R_1,R_2= O
25 R_1= H, R_2= OH

n=1
26 R_1= R_2= OCH_3
27 R_1,R_2= O
28 R_1= H, R_2= OH

n=2
29 R_1= R_2= OCH_3
30 R_1,R_2= O
31 R_1= H, R_2= OH

n=3
32 R_1= R_2= OCH_3
33 R_1,R_2= O
34 R_1= H, R_2= OH

Scheme 2.

analogs from commercially available radiolabeled 3-oxosteroid precursors without the difficulty of performing a microscale dioxolanation reaction that requires a device for removing water formed in the reaction. The formation of a dimethoxyacetal can be easily performed with high selectivity for the 3-oxo group of 5α-androstane-3,17-dione by refluxing in methanol in the presence of p-toluenesulfonic acid catalyst [11]. Reduction of the 17-ketone with sodium borohydride led to the 3,3-(dimethoxy)-5α-androstan-17β-ol derivative prepared also directly from DHT [9] (data not shown).

Condensation of the 17-ketone with the dimethylsulfonium methylide reagent [12] generated from trimethylsulfonium iodide and potassium tert-butoxide in dimethylformamide [13] led to the formation of the 17β-spirooxirane **2** as the major product [14], in 90% yield, while only traces of the 17α-isomer [15] could be detected.

The 17β-spirooxirane **2** was transformed by nucleophilic opening into either the 17α-aminomethyl-17β-hydroxy intermediate **3**, obtained in 72% yield after treatment with concentrated ammonium hydroxide in the presence of a low amount of acetic acid [16], or the 17α-cyanomethyl-17β-hydroxy intermediate **4**, obtained in nearly quantitative yield after reaction with potassium cyanide in ethanol [16]. The 17α-cyanomethyl-17β-hydroxy compound **4** was converted into the 17α-aminoethyl-17β-hydroxy intermediate **5** in 60% yield, by reduction with lithium-aluminum hydride in tetrahydrofuran, at room temperature. No noticeable formation of unsubstituted 17β-hydroxy side-product could be detected in this reaction despite the thermal unstability of 17α-cyanomethyl-17β-hydroxy derivatives, which may regenerate the 17-ketone in basic conditions [10,16]. The 17β-spirooxirane **2** was also transformed into the 17α-cyanoethyl-17β-hydroxy intermediate **6** in 92% yield, by direct opening with the cyanoethyl anion generated from acetonitrile at −76°C in the presence of butyllithium and diisopropylamine. The 17α-cyanoethyl-17β-hydroxy compound **6** was then reduced with lithium-aluminum hydride in tetrahydrofuran, at room temperature, to the 17α-aminopropyl-17β-hydroxy intermediate **7** in 69% yield. This synthetic scheme provides a more straightforward access to 17α-aminomethyl, 17α-aminoethyl, and 17α-aminopropyl spacer arms than other methods previously reported for estradiol derivatives, using transformations of 17α-cyano and 17α-ethynyl precursors [17]. The reduction of 17α-cyano intermediates had also been employed in this laboratory as an alternative method for an earlier synthesis of 17α-aminomethyl derivatives of both DHT and testosterone series [3].

The three 17α-aminomethyl-, 17α-aminoethyl-, and 17α-aminopropyl-17β-hydroxy intermediates **3**, **5**, and **7** (Scheme 1) were either acylated with the commercially available *N*-(5-azido-2-nitrobenzoyloxy)succinimide reagent to give 17α-(5-azido-2-nitrobenzoyl)amidoalkyl (ANB) derivatives **8**, **11**, and **14**, in 72, 66, and 56% yields, respectively, or condensed with 4-azido-1-fluoro-2-nitrobenzene to give 17α-(4-azido-2-nitrophenyl)aminoalkyl (ANP) derivatives **17**, **20**, and **23**, in 91, 33, and 47% yields, respectively, while a similar condensation with 4-azido-1-nitro-2,3,5,6-tetrafluorobenzene [10] led to the corresponding 17α-(5-azido-2-nitro-3,4,6-trifluorophenyl)aminoalkyl (ANF) derivatives **26**, **29**, and **32**, in 89, 45, and 52% yields, respectively. In all cases, higher yields were obtained for 17α-aminomethyl derivatives than for the aminoethyl and aminopropyl analogs. The 3,3-dimethoxy protecting groups of each of these compounds was then removed by mild acidolysis (1% HCl in 10% aqueous dioxane), monitored by thin-layer chromatography, to regenerate the corresponding 3-oxosteroids, **9**, **12**, and **15**, for ANB derivatives, **18**, **21**, and **24**, for ANP derivatives, and **27**, **30**, and **33**, for ANF

Table 1
^{13}C NMR data ($CDCl_3$) for 5α-androstane-3β,17β-diol (A-diol) and 17β-hydroxy (3-MeO-DHT), 17-oxo (**1**), 17β-spirooxirane (**2**), 17α-cyanomethyl (**4**) and 17α-cyanoethyl (**6**), 17α-aminomethyl (**3**), 17α-aminoethyl (**5**), and 17α-aminopropyl (**7**) derivatives of 3,3-dimethoxy-5α-androstane

	A-diol	**3-MeO-DHT**	**1**	**2**	**4**	**6**	**3**	**5**	**7**
Carbon	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$
1	37.04	35.06	34.97	35.04	35.01	35.04	35.07	35.09	35.08
2	31.52	28.33	28.27	28.30	28.30	28.32[a]	28.35	28.35	28.34
3	71.31	100.38	100.25	100.33	100.28	100.33	100.37	100.41	100.40
4	38.19	35.55	35.49	35.50	35.45	35.50	35.50	35.52	35.51
5	44.92	42.50	42.43	42.47	42.42	42.44	42.49	42.54	42.51
6	28.58	28.33	28.14	28.30	28.19	28.27[a]	28.35	28.41	28.39
7	31.63	31.54	30.81	31.42	31.61	31.62[a]	31.76	31.82	31.64
8	35.56	35.53	35.04	35.62	36.28	36.36	36.12	36.24	36.34
9	54.49	54.20	54.14	54.11	53.87	53.92	54.10	54.22	54.14
10	35.58	35.83	35.89	35.83	35.79	35.81	35.81	35.83	35.81
11	20.84	20.78	20.44	20.55	20.70	20.79	20.82	20.86	20.89
12	36.75	36.76	31.55	29.05	31.90	31.66[a]	32.61	31.82	31.83
13	43.00	43.01	47.81	40.16	46.36	46.57	45.66	46.47	46.53
14	51.02	51.02	51.42	52.82	51.03	50.55	51.00	50.27	50.53
15	23.39	23.40	21.78	23.53	23.40	23.55	23.54	23.88	23.86
16	30.55	30.53	35.85	33.98	37.19	34.78	34.80[b]	35.01	34.50
17	81.98	81.95	221.38	70.54	81.86	82.68	81.65[b]	84.16[b]	82.22
18	11.14	11.15	13.82	14.38	14.09	14.26	14.52	14.00	14.62
19	12.36	11.66	11.62	11.62	11.63	11.64	11.63	11.65	11.65
CH_3O		47.50	47.51	47.50	47.52	47.52	47.49	47.50	47.49
CH_3O		47.46	47.45	47.45	47.46	47.47	47.44	47.45	47.44
$C\underline{C}H_2O$				53.63					
C≡N					118.78	121.02			
$\underline{C}H_2CN$					28.14	12.08			
$\underline{C}H_2CH_2CN$						32.69			
$\underline{C}H_2NH$							47.44[b]	38.18[b]	42.26[b]
$\underline{C}H_2CH_2NH$								36.17[b]	34.88[b]
$CH_2\underline{C}H_2CH_2$									26.83[b]

[a] Assignments may be exchanged.
[b] Chemical shifts corresponding to the free amine [3].

derivatives, all obtained in high yields (84–97%) except for the 17α-aminomethyl ANP derivative **18** recovered only in 50% yield owing to losses during chromatographic purifications. These 3-oxosteroids were then reduced in mild conditions using approximately a two-molar excess of a 2 mM solution of sodium borohydride in methanol (prepared extemporaneously) to generate the corresponding 3β-hydroxy steroids, **10**, **13**, and **16**, for ANB derivatives, **19**, **22**, and **25**, for ANP derivatives, and **28**, **31**, and **34**, for ANF derivatives, most of them isolated in moderate yields (42–66%) except for 17α-aminoethyl and 17α-aminopropyl ANB derivatives **13** and **16**, obtained in higher yields (79 and 90%, respectively). Using these mild conditions and careful monitoring by thin-layer chromatography, this reduction step did not alter the structures of the three aryl azide chromophores, as confirmed by spectrometric characterizations and by comparison with reference samples obtained without reduction step, via direct coupling of the aryl azide chromophores to 17α-aminoalkyl derivatives of 5α-androstane-3β,17β-diol (data not shown).

Attempts were made to synthesize the 17α-aminoalkyl analogs deuterated at the 16 position, useful as reference compounds for ^{13}C NMR characterizations, but remained unsuccessful. Despite several modifications aimed at optimizing experimental conditions, the formation of the 17β-spirooxirane from the 3,3-(dimethoxy)-5α-[16-$^2H_2$]-androstan-17-one precursor obtained from the nondeuterated analog **1** by exchange in the presence of deuterated sodium methylate, as previously reported for 3,3-(ethylenedioxy)-5α-androstan-17-one [3], failed to maintain a significant amount of deuterium at the 16-position (data not shown).

The stereoselectivity of the conversion of a 17-oxo steroid to a 17β-2′-spirooxirane has been verified by comparing the ^1H and ^{13}C NMR spectra of 17α-aminomethyl derivatives, obtained in this study by direct ammonolysis, with those of 17α-aminomethyl derivatives previously synthesized by reduction of 17α-cyanohydrins of established configurations [3]. Moreover, nucleophilic opening of a 17β-2′-spirooxirane with either cyanide or cyanoethyl anions and subsequent reduction to ethyl and propylamine derivatives cannot be expected to generate 17β-aminoalkyl-17α-hydroxy intermediates.

The assignments of the ^1H NMR signals for 18- and 19-methyl protons of 17α-substituted derivatives of the three 3,3-dimethoxy-, 3-oxo-, and 3β-hydroxy-5α-andro-

Table 2
^{13}C NMR data (C_5D_5N) for 5α-androstane-3β,17β-diol (A-diol) and 17β-hydroxy (3-MeO-DHT), 17-oxo (**1**), 17β-spirooxirane (**2**), 17α-cyanomethyl (**4**) and 17α-cyanoethyl (**6**), 17α-aminomethyl (**3**), 17α-aminoethyl (**5**), and 17α-aminopropyl (**7**) derivatives of 3,3-dimethoxy-5α-androstane

	A-diol	**3-MeO-DHT**	**1**	**2**	**4**	**6**	**3**	**5**	**7**
Carbon	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N
1	37.61[a]	35.41	35.26	35.35	35.32	35.40	35.36	35.39	35.42
2	32.49	28.88	28.81	28.85	28.87	28.91	28.90	28.90	28.92
3	70.61	100.49	100.40	100.44	100.47	100.52	100.53	100.54	100.55
4	39.34	35.94	35.90	35.90	35.88	35.93	35.92	35.94	35.94
5	45.33	42.73	42.59	42.67	42.64	42.69	42.65	42.69	42.70
6	29.14	28.68	28.41	28.57	28.59	28.66	28.70	28.72	28.73
7	32.04	32.02	31.08[a]	31.72	32.05	32.17	32.17	32.23	32.26
8	35.88	35.84	35.11	35.75	36.54	36.58	36.45	36.58	36.66
9	54.90	54.56	54.30	54.27	53.99	54.12	54.22	54.29	54.32
10	35.93	36.06	36.06	36.03	36.00	36.03	36.04	36.05	36.05
11	21.30	21.25	20.73	20.85	21.15	21.26	21.21	21.27	21.35
12	37.58[a]	37.57	32.18[a]	29.26	32.35	32.17	32.55	32.28	32.26
13	43.60	43.61	47.83	40.40	47.01	47.14	46.16	47.08	47.17
14	51.42	51.43	51.48	52.80	50.97	50.96	51.26	50.68	51.01
15	23.85	23.85	21.94	23.73	23.78	24.00	24.05	24.23	24.27
16	31.00	31.02	35.87	34.19	36.89	34.27	34.28[b]	35.31	34.75
17	81.41	81.40	219.71	70.37	81.47	81.93	82.40[b]	83.57[b]	82.30
18	11.94	11.93	13.85	14.69	14.90	15.19	15.25	14.87	15.47
19	12.57	11.83	11.68	11.73	11.76	11.81	11.80	11.82	11.85
CH_3O		47.32	47.33	47.33	47.35	47.35	47.34	47.34	47.34
CH_3O		47.27	47.27	47.27	47.28	47.29	47.28	47.28	47.28
CCH_2O				53.24					
C≡N					120.45	122.12			
$\underline{C}H_2CN$					28.87	12.42			
$\underline{C}H_2CH_2CN$						33.97			
CH_2NH							48.24[b]	38.40[b]	43.45[b]
$\underline{C}H_2CH_2NH$								37.20[b]	35.31[b]
$CH_2\underline{C}H_2CH_2$									28.20[b]

[a] Assignments may be exchanged.
[b] Chemical shifts corresponding to the free amine [3].

stan-17β-ol series (see Section 2), were made on the assumption that modifications of 3- or 17-substituents should influence predominantly the chemical shifts of 19- or 18-methyl protons, respectively, and were confirmed by correlation with those previously established for 17α-aminomethyl derivatives of 3-oxo-5α-androstan-17β-ol, 3,3-(ethylenedioxy)androstan-17β-ol, and 3-oxoandrost-4-en-17β-ol [3].

The ^1H NMR chemical shifts, in $CDCl_3$, of 19-methyl protons of 3-oxo derivatives (1.02/1.04 ppm) were significantly deshielded as compared with those of 3,3-dimethoxy and 3β-hydroxy derivatives (0.80/0.82 and 0.82/0.84 ppm, respectively), while a small deshielding effect of +0.03/+0.04 ppm was also exerted on the 18-methyl signals as compared to the chemical shifts observed for 3β-hydroxysteroid derivatives, in agreement with reported substituent-induced effects for these two groups [18]. In C_5D_5N, similar but weaker effects were also often found. The ANB conjugates of both 17α-aminoethyl and 17α-aminopropyl side-chains of 3,3-dimethoxy- and 3β-hydroxy-5α-androstan-17β-ol derivatives as well as the corresponding ANF analogs, showed very close 18- and 19-methyl signals in $CDCl_3$, separated only by 0.01–0.04 ppm. However, a more significant difference was found for the corresponding ANP derivatives, thus leading to an unambiguous assignment of the more shielded signals for the 19-methyl groups of these compounds, which could then be extended to ANB and ANF analogs. On the other hand, 18- and 19-methyl signals in C_5D_5N were easily differentiated, owing to the strong deshielding effect on the 18-methyl signals at ~ 1.1 ppm, resulting from the presence of a vicinal 17α-hydroxy group. A more detailed inspection of these results reveals that 17α-aminomethyl side-chains exert stronger deshielding effects on 18-methyl signals, in both $CDCl_3$ and C_5D_5N solvents, than the two other aminoethyl and aminopropyl derivatives, while the magnitude of these effects is slightly larger for ANP derivatives as compared with the two other ANB and ANF chromophores. The more pronounced deshielding effect on the 18-methyl signal of the shorter 17α-aminomethyl link despite insignificant or very slight downfield shifts increments observed when changing a 17α-methyl substituent for larger 17α-ethyl or 17α-[5′-(methoxycarbonyl)pentyl] groups [1] points out to the hypothesis of a predominant anisotropic effect of the NH group,

Table 3
^{13}C NMR data ($CDCl_3$) for 17α-(*N*-5-azido-2-nitrobenzoyl)amidomethyl, ethyl, and propyl derivatives of 3,3-dimethoxy- (**8**, **11**, **14**), 3-oxo- (**9**, **12**, **15**), and 3β-hydroxy-5α-androstan-17β-ol (**10**, **13**, **16**)

	8	**11**	**14**	**9**	**12**	**15**	**10**	**13**	**16**
Carbon	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$
1	35.03	35.03	35.03	38.45	38.51	38.53	36.99	36.98	36.99
2	28.34	28.31[a]	28.33	38.10	38.13	38.16	31.43	31.46	31.50[a]
3	100.31	100.34	100.37	211.96	211.96	212.12	71.17	71.24	71.26
4	35.50	35.51	35.52	44.61	44.64	44.67	38.09	38.11	38.13
5	42.40	42.44	42.46	46.53	46.65	46.67	44.80	44.85	44.86
6	28.34	28.30[a]	28.33	28.76	28.79	28.83	28.53	28.55	28.60
7	31.57	31.66	31.70	31.28	31.39	31.42	31.63	31.73	31.78
8	36.35	36.42	36.41	36.22	36.33	36.29	36.34	36.42	36.41
9	53.79	53.92	53.96	53.52	53.64	53.64	54.09	54.18	54.21
10	35.81	35.81	35.81	35.69	35.73	35.72	35.54	35.55	35.55
11	20.76	20.78	20.82	21.03	21.05	21.09	20.83	20.83	20.87
12	31.73	31.47	31.52	31.64	31.39	31.42	31.72	31.46	31.49[a]
13	45.88	46.70	46.52	45.87	46.65	46.51	45.85	46.68	46.50
14	50.65	50.29	50.44	50.48	50.13	50.22	50.63	50.26	50.40
15	23.59	23.60	23.64	23.60	23.61	23.64	23.58	23.59	23.64
16	34.61	34.61	34.73	34.48	34.63	34.63	34.60	34.65	34.74
17	83.44	84.81	83.64	83.30	84.73	83.56	83.39	84.90	83.62
18	14.08	13.98	14.34	14.12	14.00	14.37	14.09	13.96	14.34
19	11.63	11.64	11.65	11.46	11.49	11.48	12.31	12.33	12.33
CH_3O	47.50	47.51	47.51						
CH_3O	47.50	47.47	47.46						
$\underline{C}H_2NH$	46.12	36.84	40.95	46.18	36.82	40.90	46.12	36.84	40.92
$\underline{C}H_2CH_2$		33.96	33.32		34.00	33.30		33.88	33.29
$CH_2\underline{C}H_2CH_2$			23.43			23.51			23.47
NH$\underline{C}$O	166.14	165.40	165.70	166.22	165.40	165.74	166.14	165.40	165.71
1′	135.53	135.78	135.62	135.48	135.78	135.59	135.53	135.79	135.63
2′	142.20	142.30	142.31	142.16	142.30	142.25	142.19	142.27	142.28
3′	126.91	126.80	126.82	126.89	126.83	126.83	126.92	126.82	126.83
4′	119.03	119.07	119.10	119.03	119.08	119.09	119.00	119.05	119.09
5′	146.28	146.22	146.22	146.28	146.22	146.25	146.28	146.23	146.23
6′	119.99	119.87	119.96	119.98	119.92	119.99	119.98	119.88	119.97

[a] Assignments may be exchanged.

although other factors, such as hydrogen bonding, hydrophobicity, and conformational effects, cannot be ruled out [19].

The assignments of ^{13}C NMR resonances for carbon atoms of the two unsubstituted 17β-hydroxy reference compounds, 5α-androstane-3β,17β-diol (A-diol) and 3,3-(dimethoxy)-5α-androstan-17β-diol (3-MeO-DHT), and of the 3,3-(dimethoxy)-5α-androstan-17-one precursor **1**, in both $CDCl_3$ and C_5D_5N solvents (Tables 1 and 2), were based on data reported in previous studies [1,3,20] for 5α-androstane-3β,17β-diol, DHT, 3,3-(ethylenedioxy)-5α-androstan-17β-ol, 3,3-(ethylenedioxy)-5α-androstan-17-one, and 3β-hydroxy-5α-androstan-17-one. The rather close resonances of C2 and C7 carbon atoms of 5α-androstane-3β,17β-diol, especially in $CDCl_3$, could be assigned on the assumption that changes in the structure of 17α-side-chains should affect more strongly the signals of C7 carbon atoms than those of C2 carbon atoms, as confirmed by similar effects observed for 17α-aminoalkyl-3,3-dimethoxy-5α-androstane derivatives, unambiguously characterized in this study by ^{1}H, ^{13}C heteronuclear 2D NMR HSQC-TOCSY experiments (vide infra). Therefore, the more deshielded of these two signals was assigned to C7, in $CDCl_3$, and to C2, in C_5D_5N. The rather close resonances of the two pairs of carbon atoms, C1/C4 of 3,3-dimethoxy-5α-androstan-17β-ol, in both $CDCl_3$ and C_5D_5N solvents, and C2/C6, in C_5D_5N, could be differentiated only by correlation with those established by ^{1}H, ^{13}C 2D NMR for 17α-aminoalkyl-3,3-dimethoxy-5α-androstan-17β-ol derivatives (vide infra).

The assignments for the resonances of the C1 to C10, and C19 carbon atoms of rings A and B of the steroid skeletons of 17α-substituted 3,3-dimethoxy-, 3-oxo-, and 3β-hydroxy-5α-androstan-17β-ol derivatives in both $CDCl_3$ and C_5D_5N solvents (Tables 1–8), were based on data mentioned above for the corresponding unsubstituted 17β-hydroxy derivatives, while assignments for close signals of methylene carbon atoms (i.e. C2/C6 or C7/C12) were corroborated by ^{1}H, ^{13}C 2D NMR. The assignments for the resonances of the carbon atoms of rings C and D were made by correlation with those previously established for both unsubstituted and 17α-substituted derivatives of the 3,3-

Table 4
^{13}C NMR data (C_5D_5N) for 17α-(*N*-5-azido-2-nitrobenzoyl)amidomethyl, ethyl, and propyl derivatives of 3,3-dimethoxy- (**8**, **11**, **14**), 3-oxo- (**9**, **12**, **15**), and 3β-hydroxy-5α-androstan-17β-ol (**10**, **13**, **16**)

	8	**11**	**14**	**9**	**12**	**15**	**10**	**13**	**16**
Carbon	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N
1	35.37	35.33	35.41	38.70	38.73	38.81	37.57	37.57	37.65
2	28.95	28.91	28.91	38.37	38.38	38.41	32.58	32.58	32.59
3	100.56	100.54	100.55	210.40	210.41	210.45	70.65	70.66	70.66
4	35.90	35.91	35.95	44.90	44.92	44.94	39.39	39.41	39.42
5	42.50	42.58	42.71	46.76	46.81	46.92	45.22	45.26	45.38
6	28.65	28.69	28.73	29.08	29.10	29.15	29.16	29.18	29.24
7	31.95	32.11	32.25	31.57	31.71	31.82	32.06	32.19	32.29
8	36.57	36.64	36.68	36.43	36.48	36.53	36.66	36.71	36.77
9	53.96	54.05	54.25	53.57	53.79	53.89	54.30	54.48	54.61
10	36.00	36.02	36.05	35.93	35.92	35.96	35.94	35.93	35.98
11	21.24	21.29	21.34	21.40	21.47	21.51	21.29	21.36	21.40
12	32.16	32.40	32.25	32.03	32.33	32.15	32.17	32.44	32.29
13	46.71	47.34	47.28	46.66	47.30	47.18	46.70	47.33	47.22
14	51.45	51.04	50.97	51.20	50.87	50.72	51.46	51.10	50.98
15	24.16	24.22	24.20	24.18	24.22	24.21	24.21	24.26	24.24
16	34.01	34.85	34.59	33.97	34.83	34.52	34.01	34.86	34.57
17	83.04	82.54	82.66	83.03	82.51	82.63	83.06	82.57	82.67
18	15.05	15.18	15.42	15.02	15.16	15.40	15.07	15.20	15.43
19	11.79	11.80	11.84	11.34	11.36	11.39	12.56	12.57	12.61
CH_3O	47.35	47.34	47.35						
CH_3O	47.32	47.30	47.28						
$\underline{C}H_2NH$	47.67	37.44	41.49	47.61	37.42	41.46	47.65	37.45	41.48
$\underline{C}H_2CH_2$		37.02	35.07		36.98	35.00		37.00	35.03
$CH_2\underline{C}H_2CH_2$			24.75			24.76			24.76
$NH\underline{C}O$	166.93	166.09	166.02	166.95	166.06	166.05	166.94	166.06	166.04
1′	137.01	136.91	136.89	136.99	136.87	136.89	137.03	136.90	136.93
2′	143.58	143.65	143.65	143.56	143.65	143.65	143.56	143.67	143.61
3′	126.87	126.86	126.81	126.88	126.87	126.83	126.88	126.87	126.81
4′	120.16	120.04	120.06	120.17	120.02	120.05	120.14	120.03	120.06
5′	145.95	146.03	145.99	145.96	146.03	145.96	145.96	146.03	145.95
6′	120.16	120.19	120.15	120.17	120.21	120.18	120.17	120.20	120.16

(ethylenedioxy)-, 3-oxo-, and 3β-hydroxy-5α-androstan-17β-ol series [1,3]. This correlation method led to the identification of carbon atoms of the steroid skeletons of the 17β-spirooxirane **2**, of 17α-cyanoalkyl derivatives **4** and **6**, and of aminoalkyl precursors **3**, **5**, and **7** (Tables 1 and 2), while the remaining signals were assigned to carbon atoms of the 17α-side-chain and differentiated by ^{1}H, ^{13}C heteronuclear 2D NMR HSQC and HMBC techniques for the 17α-cyanoethyl derivative **6** and the 17α-aminoethyl precursor **5**, and by comparing with ANB, ANP, and ANF analogs for the 17α-aminoethyl and 17α-aminopropyl precursors **5** and **7** (vide infra). Assignments for the carbon atoms of 17α-n-propylamine side-chain of compound **7** were also confirmed by correlation with those previously established by 2D NMR for 17α-n-propyl and 17α-n-propyl alcohol derivatives of estradiol [21], using known substituent-induced effects of free amine groups on n-alkanes [22].

The assignments for the carbon atoms of the ANB derivative of 17α-aminomethyl-3-oxo-5α-androstan-17β-ol **9** (Tables 3 and 4) were made more precisely by correlation with those previously reported for the corresponding acetamido or hemiglutaramido (either as acid or methyl ester) derivatives [3]. This correlation could then be extended to confirm assignments for carbon atoms of rings C and D of compounds **8** and **10** in the two other 3,3-dimethoxy and 3β-hydroxy series, as well as for those of the aminomethyl analogs in the ANP and ANF series **17**, **18**, **19** (Tables 5 and 6), and **26**, **27**, **28** (Tables 7 and 8), respectively. However, the extension of these correlation methods for identifying carbon atom signals of the steroid skeletons of the ANB, ANP, and ANF derivatives in the two homologous 17α-aminoethyl and 17α-aminopropyl series (Tables 3–8) led to several ambiguities that could be resolved only by use of ^{1}H, ^{13}C heteronuclear 2D NMR correlation techniques. Two-dimensional NMR experiments using in parallel HSQC and HSQC-TOCSY techniques were performed in both $CDCl_3$ and C_5D_5N solvents for the ANB and ANP derivatives of 17α-aminoethyl- and 17α-aminopropyl-3,3-dimethoxy-5α-androstan-17β-ol **11**, **20**, and **14**, **23**, respectively. For HSQC spectra, assignments for singlet signals of the carbon atoms of C18, C19, and 3,3-dimethoxy methyl groups were easily deduced from the corresponding ^{1}H NMR spectra, while those for the singlet signals of methine C5, C8, C9, and C14 carbon atoms were made by a com-

Table 5
^{13}C NMR data ($CDCl_3$) for 17α-(*N*-4-azido-2-nitrophenyl)aminomethyl, ethyl, and propyl derivatives of 3,3-dimethoxy- (**17**, **20**, **23**), 3-oxo- (**18**, **21**, **24**), and 3β-hydroxy-5α-androstan-17β-ol (**19**, **22**, **25**)

	17	**20**	**23**	**18**	**21**	**24**	**19**	**22**	**25**
Carbon	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$
1	35.03	35.03	35.05	38.54	38.54	38.55	37.01	37.00	37.01
2	28.24	28.29[a]	28.33	38.11	38.12	38.14	31.46	31.50	31.48
3	100.32	100.32	100.36	211.90	211.90	212.01	71.24	71.23	71.26
4	35.47	35.49	35.50	44.61	44.64	44.65	38.08	38.10	38.13
5	42.47	42.45	42.47	46.72	46.69	46.71	44.91	44.87	44.88
6	28.24	28.28[a]	28.33	28.72	28.77	28.80	28.50	28.54	28.57
7	31.71	31.69	31.73	31.41	31.43	31.44	31.79	31.78	31.80
8	36.31	36.40	36.40	36.20	36.32	36.28	36.33	36.41	36.39
9	53.99	53.97	54.03	53.67	53.71	53.72	54.25	54.25	54.27
10	35.82	35.80	35.82	35.77	35.74	35.73	35.58	35.56	35.56
11	20.80	20.79	20.85	21.05	21.06	21.10	20.86	20.85	20.89
12	32.34	31.49	31.68	32.26	31.43	31.59	32.33	31.46	31.67
13	46.09	46.69	46.54	46.07	46.69	46.52	46.07	46.67	46.51
14	51.32	50.39	50.60	51.11	50.23	50.39	51.30	50.38	50.56
15	23.53	23.61	23.67	23.54	23.63	23.66	23.54	23.61	23.66
16	35.82	34.78	35.05	35.77	34.77	34.99	35.77	34.78	35.03
17	83.18	83.48	83.42	83.10	83.43	83.36	83.17	83.43	83.40
18	14.13	14.02	14.37	14.15	14.03	14.37	14.14	14.02	14.36
19	11.64	11.65	11.65	11.48	11.50	11.48	12.33	12.33	12.34
CH_3O	47.53	47.52	47.52						
CH_3O	47.46	47.47	47.46						
$\underline{C}H_2NH$	49.35	39.59	44.14	49.34	39.56	44.08	49.35	39.57	44.11
$\underline{C}H_2CH_2$		34.99	33.81		35.02	33.79		35.03	33.79
$CH_2\underline{C}H_2CH_2$			23.54			23.48			23.48
1′	143.66	143.41	143.41	143.61	143.45	143.37	143.68	143.41	143.40
2′	131.68	131.37	131.39	131.67	131.37	131.41	131.67	131.36	131.34
3′	115.61	115.52	115.60	115.61	115.50	115.57	115.62	115.53	115.59
4′	127.52	127.33	127.59	127.58	127.34	127.61	127.51	127.35	127.58
5′	128.12	128.19	128.23	128.12	128.19	128.23	128.10	128.20	128.25
6′	116.06	115.96	115.97	116.03	115.98	115.94	116.05	115.95	115.94

[a] Assignments may be exchanged.

bination of results of DEPT experiments and of correlations with the corresponding ^{13}C NMR signals previously reported for similar 3,3-(ethylenedioxy)-5α-androstan-17β-ol reference structures [1]. The resonances for the remaining methylene groups of the steroid skeleton carbon atoms C1, C2, C4, C6, C7, C11, C12, C15, and C16 which are shared by all 17 substituted derivatives, and thus easily differentiated from those of the 17α-aminoethyl and aminopropyl side-chains, were assigned using the ^{1}H, ^{13}C heteronuclear HSQC-TOCSY correlation technique via analysis of the hydrogen-relayed transfer of magnetization through coupled spins along vicinal carbon atom sequences. The signals for C4, C6, C7, C11, and C15 carbon atoms were readily identified from their coupling with unequivocally characterized vicinal methine groups, while those for C12 and C16 carbon atoms were established from their coupling with previously assigned CH_2-11 and CH_2-15 methylene carbon atoms. Assignments for the signals of C1 and C2 carbon atoms were mainly supported by correlation with the corresponding ^{13}C chemical shifts of 3,3-(ethylenedioxy)-5α-androstan-17β-ol reference structure [1] and confirmed by a weak coupling between C2 and C4 signals in C_5D_5N.

The assignments for the carbon atoms of the 17α-aminomethyl, 17α-aminoethyl, and 17α-aminopropyl side-chains of ANB, ANP, and ANF derivatives were straigthtforward for the CH_2N carbon atom, which is either the only methylene carbon atom of aminomethyl side-chains, or was also readily identified as the more deshielded signal, in the two other cases, as confirmed by ^{1}H, ^{13}C 2D NMR correlation with the corresponding deshielded NH proton signal of ANB and ANP derivatives. Identification of the CH_2-1′ methylene groups of aminoethyl side-chains and of the CH_2-2′ methylene groups of aminopropyl side-chains was established by HSQC-TOCSY from their coupling with the vicinal CH_2N methylene carbon atom, while the residual CH_2-1′ methylene groups of aminopropyl side-chains were identified from their strong coupling with the vicinal CH_2-2′ methylene group and their weak coupling with the CH_2N methylene carbon atom. The signals for these side-chain carbon atoms appeared as doublets, or, in some instances, as broad singlets, as found for the 2-nitro-4-azido-phenylaminopropyl derivative **23**, in both $CDCl_3$ and C_5D_5N solvents, and for the two other CH_2-1′ and CH_2-2′ groups of the 2-nitro-5-azidobenzoylamidopropyl derivative **14**, in $CDCl_3$ only.

Table 6
^{13}C NMR data (C_5D_5N) for 17α-(*N*-4-azido-2-nitrophenyl)aminomethyl, ethyl, and propyl derivatives of 3,3-dimethoxy- (**17**, **20**, **23**), 3-oxo- (**18**, **21**, **24**), and 3β-hydroxy-5α-androstan-17β-ol (**19**, **22**, **25**)

	17	**20**	**23**	**18**	**21**	**24**	**19**	**22**	**25**
Carbon	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N
1	35.36	35.40	35.43	38.78	38.78	38.81	37.60	37.63	37.67
2	28.90	28.91	28.92	38.36	38.38	38.39	32.52	32.59	32.59
3	100.53	100.54	100.55	210.38	210.35	210.43	70.64	70.64	70.64
4	35.89	35.93	35.93	44.89	44.92	44.92	39.32	39.40	39.41
5	42.62	42.69	42.72	46.90	46.89	46.89	45.34	45.37	45.38
6	28.61	28.69	28.72	29.04	29.11	29.11	29.12	29.19	29.21
7	32.10	32.20	32.27	31.70	31.79	31.85	32.17	32.26	32.33
8	36.49	36.64	36.67	36.37	36.51	36.52	36.60	36.73	36.75
9	54.06	54.18	54.31	53.74	53.86	54.00	54.44	54.56	54.71
10	36.01	36.06	36.07	35.95	35.97	35.96	35.95	35.98	35.98
11	21.28	21.28	21.35	21.47	21.47	21.52	21.35	21.35	21.41
12	32.86	32.06	32.34	32.79	31.97	32.26	32.89	32.08	32.38
13	46.71	47.36	47.24	46.68	47.33	47.19	46.71	47.36	47.23
14	51.39	50.87	51.13	51.14	50.64	50.93	51.40	50.87	51.17
15	24.03	24.12	24.21	24.06	24.13	24.21	24.08	24.16	24.25
16	35.52	34.22	34.87	35.55	34.20	34.84	35.53	34.22	34.88
17	82.41	82.94	82.67	82.40	82.93	82.64	82.43	82.96	82.67
18	15.07	15.06	15.40	15.04	15.02	15.35	15.07	15.07	15.41
19	11.78	11.83	11.84	11.35	11.39	11.39	12.54	12.60	12.60
CH_3O	47.36	47.36	47.36						
CH_3O	47.29	47.29	47.30						
$\underline{C}H_2NH$	50.51	40.52	44.50	50.45	40.50	44.43	50.48	40.52	44.46
$\underline{C}H_2CH_2$		35.81	34.73		35.76	34.68		35.78	34.72
$CH_2\underline{C}H_2CH_2$			24.35			24.30			24.32
1′	144.54	143.97	143.89	144.49	143.94	143.86	144.51	143.96	143.88
2′	131.66	131.63	131.61	131.62	131.64	131.61	131.60	131.62	131.60
3′	116.18	116.15	116.13	116.15	116.16	116.12	116.15	116.15	116.12
4′	127.18	127.01	127.32	127.17	127.02	127.32	127.14	127.03	127.30
5′	128.70	128.40	128.48	128.66	128.39	128.47	128.66	128.40	128.47
6′	117.10	116.50	116.62	117.05	116.48	116.60	117.05	116.50	116.61

The assignments for the ^{13}C chemical shifts of aromatic carbon atoms of 5-azido-2-nitro benzoylamido and of 4-azido-2-nitrophenylamino groups (Tables 3–6) were made by using the ^{1}H, ^{13}C heteronuclear HMBC correlation technique performed, in C_5D_5N, on 17α-aminoethyl ANB and ANP derivatives of 3,3-dimethoxy-5α-androstan-17β-ol **11** and **20**, respectively. On the other hand, no ^{13}C signals could be detected for the 5-azido-2-nitro-3,4,6-trifluorophenylamino chromophore, as expected for polyfluoroaromatic compounds. For the 17α-(5-azido-2-nitrobenzoyl)amidoethyl analog **11**, the doublet signals of C3″, C4″, and C6″ methine carbon atoms were identified by correlation with their corresponding ^{1}H NMR signals. Assignment for the resonance of the C2″ quaternary carbon atom was made easily from the strongest $^3J_{CCCH}$ couplings of H4″ with C2″ and C6″ carbon atoms and of H6″ with C2″ and C4″ carbon atoms, as expected for aromatic rings, since the resonances for C4″ and C6″ carbon atoms were previously established through correlation with signals of attached protons. A strong coupling of H3″ with the two remaining C1″ and C5″ quaternary carbon atoms was observed but did not afford useful information for differentiating these two signals. However, in optimized conditions (see Section 2.1), a weak $^3J_{CCNH}$ coupling of the NH proton was detected with only one of these two carbon atoms, postulated, therefore, as the C1″ carbon atom. An HMBC experiment was also performed in $CDCl_3$, which confirmed that the order of the very close C4″ and C6″ signals assigned for compound **11**, in C_5D_5N, was conserved in $CDCl_3$. For the 17α-(4-azido-2-nitrophenyl)aminoethyl derivative **20**, the signals of the C3″, C5″, and C6″ methine carbon atoms were readily identified by correlation with their ^{1}H NMR signals. The strongest couplings were found to occur for $^3J_{CCCH}$ interactions between H3″ and C1″ or C5″ carbon atoms, H5″ and C1″ or C3″ carbon atoms, and H6″ and C2″ or C4″ carbon atoms, while a strong $^3J_{CCNH}$ coupling was also observed between NH and both C2″ or C6″ carbon atoms. These observations led to an unequivocal identification of the resonances for C1″, C2″, and C3″ carbon atoms, since for H3″ and H5,″ one of the two coupled carbon atoms, C5″ and C3,″ respectively, was already identified, whereas for H6,″ the C2″ carbon atom could be differentiated from C4″ by its coupling with NH, which was otherwise coupled with the already identified C6″ carbon atom. The carbon signals of the polyfluoroaromatic ring of ANF derivatives could not be detected but the corresponding CH_2N signals appeared as narrow dou-

Table 7
^{13}C NMR data ($CDCl_3$) for 17α-(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)-aminomethyl, ethyl, and propyl derivatives of 3,3-dimethoxy- (**26**, **29**, **32**), 3-oxo- (**27**, **30**, **33**), and 3β-hydroxy-5α-androstan-17β-ol (**28**, **31**, **34**)

	26	**29**	**32**	**27**	**30**	**33**	**28**	**31**	**34**
Carbon	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$	δ-$CDCl_3$
1	34.98	35.04	35.06	38.48	38.53	38.56	36.96	37.00	37.01
2	28.22[a]	28.32	28.34	38.10	38.13	38.15	31.45	31.46	31.48
3	100.30	100.34	100.37	211.98	211.95	211.97	71.24	71.24	71.25
4	35.46	35.50	35.51	44.60	44.64	44.67	38.08	38.10	38.13
5	42.40	42.47	42.48	46.64	46.68	46.72	44.84	44.87	44.88
6	28.29[a]	28.32	28.34	28.73	28.76	28.82	28.50	28.53	28.58
7	31.62	31.69	31.73	31.33	31.40	31.44	31.70	31.76	31.79
8	36.26	36.41	36.40	36.18	36.29	36.30	36.29	36.40	36.38
9	53.79	53.99	54.03	53.49	53.68	53.74	54.07	54.24	54.27
10	35.79	35.81	35.82	35.74	35.86	35.75	35.56	35.55	35.55
11	20.75	20.78	20.84	21.02	21.03	21.10	20.82	20.82	20.88
12	32.04	31.43	31.62	31.98	31.34	31.54	32.05	31.41	31.60
13	45.88	46.69	46.52	45.89	46.66	46.50	45.88	46.66	46.48
14	51.06	50.34	50.56	50.89	50.13	50.38	51.07	50.31	50.53
15	23.48	23.56	23.64	23.51	23.56	23.64	23.50	23.55	23.63
16	35.46	34.68	34.97	35.41	34.61	34.96	35.46	34.65	34.92
17	83.32	83.88	83.32	83.28	83.80	83.27	83.34	83.85	83.29
	83.35*			83.31*					
18	14.07	13.91	14.33	14.09	13.92	14.34	14.07	13.91	14.33
19	11.62	11.65	11.65	11.46	11.49	11.50	12.30	12.33	12.33
CH_3O	47.53	47.52	47.51						
CH_3O	47.46	47.47	47.46						
$\underline{C}H_2NH$	52.15	42.84	47.08	52.11	42.84	47.04	52.11	42.95	47.08
	52.23*	42.99*	47.22*	52.25*	42.93*	47.19*	52.25*	42.86*	47.17*
$\underline{C}H_2CH_2$		35.86	33.40		35.86	33.39		35.86	33.38
$CH_2\underline{C}H_2CH_2$			25.11			25.07			25.07

[a] Assignments may be exchanged.
* Signal corresponding to the second peak of a narrow doublet.

blets, as found also for C17 carbon atoms of the shorter aminomethyl side-chains. The hypothesis of a contamination with an o-azido-nitrophenyl isomer was ruled out by ^{19}F NMR of the 4-azido-1-nitro-2,4,5,6-tetrafluorobenzene reagent (two symmetrical multiplets at 149.7 and 146.3 ppm), as previously reported [10,23], and of the aminoethyl ANF derivative **29** (see Section 2).

Substituent-induced effects for the shorter 17α-aminomethyl ANB, ANP, and ANF side-chains in all three 3,3-dimethoxy-, 3-oxo-, and 3β-hydroxy-5α-androstane series were calculated by comparing with the ^{13}C NMR spectra of the three corresponding unsubstituted 17β-hydroxy analogs, **2**, DHT [1], and **1**, respectively. The effects of increasing the linker length from aminomethyl ($n = 1$) to aminoethyl ($n = 2$) and aminopropyl ($n = 3$) derivatives with a same terminal group were estimated using $\Delta\delta_{2-1}$, $\Delta\delta_{3-2}$, and $\Delta\delta_{3-1}$ increments calculated for each carbon atom of the steroid skeleton.

Modification of the length of the 17α-side-chain had either insignificant or very weak effects on the ^{13}C NMR resonances for carbon atoms C1 to C11, and C19, remote from the 17-substituent. These weak effects were observed mainly for C5, C7, C8, and C9 carbon atoms (data not shown) and differed slightly according to the structures of ANB, ANP, and ANF chromophores. In most of these cases, an increase of the side-chain length was found to produce weak $\Delta\delta_{2-1}$ deshielding effects and, most often, weaker $\Delta\delta_{3-2}$ deshielding effects, which resulted in larger $\Delta\delta_{3-1}$ deshielding effects, culminating at +0.35/+0.46 ppm (for C9 of ANF derivatives, in C_5D_5N).

The effects of lengthening the aminomethyl spacer to an aminoethyl substituent were, in most cases, much greater on carbon atoms C12 to C18 (Table 9), as expected from their vicinity from the modified 17α-side-chain, than on more remote carbon atoms C1 to C10, and C19, although only very slight effects ($\leqslant$ 0.18 ppm) were observed for C15 (mostly downfield shifts). In $CDCl_3$, C12, C14, and C18 carbon atoms of the three ANB, ANP, and ANF derivatives and the C16 carbon atom of ANP and ANF derivatives underwent shielding effects. The strongest shielding effects were found for C12, C14, and C16 of ANP derivatives, all slightly larger than those found for ANF derivatives, while the weakest shielding effects were observed for C18 in all three series and for C12 and C14 of ANB derivatives. On the other hand, in the same $CDCl_3$ solvent, C13 and C17 carbon atoms in all series and the C16 carbon atom of ANB derivatives underwent deshielding effects. The deshielding effects on C13 were rather similar for the three ANB, ANP, and ANF derivatives, while those on C17 were much stron-

Table 8
^{13}C NMR data (C_5D_5N) for 17α-(*N*-5-azido-2-nitro-3,4,6-trifluorophenyl)-aminomethyl, ethyl, and propyl derivatives of 3,3-dimethoxy- (**26**, **29**, **32**), 3-oxo- (**27**, **30**, **33**), and 3β-hydroxy-5α-androstan-17β-ol (**28**, **31**, **34**)

	26	29	32	27	30	33	28	31	34
Carbon	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N	δ-C_5D_5N
1	35.25	35.37	35.44	38.69	38.76	38.80	37.52	37.61	37.66
2	28.87	28.90	28.92	38.34	38.38	38.40	32.54	32.57	32.58
3	100.52	100.53	100.53	210.32	210.34	210.38	70.64	70.63	70.64
4	35.88	35.93	35.94	44.87	44.91	44.93	39.35	39.39	39.40
5	42.50	42.68	42.72	46.77	46.86	46.90	45.23	45.34	45.38
6	28.60	28.68	28.71	29.02	29.09	29.12	29.10	29.17	29.21
7	32.03	32.18	32.29	31.64	31.77	31.84	32.12	32.24	32.33
8	36.47	36.64	36.67	36.33	36.49	36.52	36.57	36.71	36.74
9	53.87	54.16	54.33	53.62	53.86	53.99	54.35	54.56	54.70
10	36.01	36.05	36.06	35.92	35.94	35.96	35.93	35.96	35.97
11	21.25	21.25	21.35	21.45	21.43	21.52	21.34	21.32	21.41
12	32.72	32.01	32.29	32.67	31.93	32.21	32.80	32.03	32.33
13	46.58	47.36	47.19	46.56	47.32	47.16	46.60	47.35	47.20
14	51.48	50.77	51.10	51.30	50.56	50.87	51.57	50.79	51.11
15	24.05	24.07	24.17	24.05	24.06	24.18	24.08	24.09	24.22
16	35.08	34.14	34.87	35.11	34.10	34.83	35.13	34.10	34.86
17	82.67	83.34	82.59	82.65	83.34	82.57	82.72	83.38	82.60
	82.70*								
18	15.08	14.95	15.34	15.05	14.91	15.33	15.08	14.95	15.38
19	11.75	11.81	11.83	11.32	11.37	11.39	12.52	12.58	12.60
CH_3O	47.36	47.36	47.34						
CH_3O	47.29	47.29	47.28						
$\underline{C}H_2NH$	53.38	43.53	47.34	53.33	43.49	47.29	53.38	43.53	47.30
	53.51*	43.66*	47.46*	53.47*	43.36*	47.42*	53.51*	43.62*	47.40*
$\underline{C}H_2CH_2$		36.64	34.51		36.54	34.44		36.56	34.48
$CH_2\underline{C}H_2CH_2$			25.92			25.87			25.90

* Signal corresponding to the second peak of a narrow doublet.

ger for ANB derivatives than for ANP and ANF derivatives. The C16 carbon atom of ANB derivatives exhibited a very slight deshielding effect, while the C15 carbon atom remained almost unaffected in all series. When changing the $CDCl_3$ solvent for C_5D_5N, the $\Delta\delta_{2-1}$ increments of ANB derivatives were significantly modified for carbon atoms C12, C16, C17 and C18, as shown by a change of sign but not of magnitude for C12 and C18, a much stronger deshielding effect for C16, and a change of sign leading to a much weaker shielding effect for C17. Otherwise, in C_5D_5N solvent, $\Delta\delta_{2-1}$ increments of all C12 to C18 carbon atoms of ANP and ANF derivatives were found to be rather close to those observed in $CDCl_3$ solvent.

Changing the aminoethyl spacer for an aminopropyl substituent led also to characteristic upfield or downfield shifts of carbon atoms C12 to C18 (Table 9). The corresponding $\Delta\delta_{3-2}$ increments for all three ANB, ANP, and ANF derivatives, in both $CDCl_3$ and C_5D_5N solvents, were found to exhibit in most cases a sign opposite to that of the corresponding $\Delta\delta_{2-1}$ increments and very often a much lower magnitude, thus leading to $\Delta\delta_{3-1}$ increments having the same sign as $\Delta\delta_{2-1}$ increments. The largest $\Delta\delta_{3-2}$ increments were found for C17 carbon atom of ANB derivatives, in $CDCl_3$, while rather strong effects were also observed on C17 (upfield shifts) and C18 (downfield shifts) carbon atoms of ANF derivatives in both $CDCl_3$ and C_5D_5N solvents and on C16 carbon atoms (downfield shift) of ANP and ANF derivatives, in C_5D_5N only. Exceptions to the general rule of change of sign were observed in the case of ANB derivatives, for $\Delta\delta_{3-2}$ increments of C15 and C16 in $CDCl_3$, and of C14 and C18 in C_5D_5N, and, in the case of ANP and ANF derivatives, for $\Delta\delta_{3-2}$ increments of C15, in both $CDCl_3$ and C_5D_5N solvents, which, in all cases, contributed to an increase of the magnitude of the corresponding $\Delta\delta_{3-1}$ increment of unchanged sign as compared with that of the $\Delta\delta_{2-1}$ increment. Exceptions to the general rule of lower magnitude leading to a change of sign for the corresponding $\Delta\delta_{3-1}$ increments, as compared with the $\Delta\delta_{2-1}$ increments, were observed for $\Delta\delta_{3-2}$ increments of C17 of ANF derivatives and of C18 of ANP and ANF derivatives, in both $CDCl_3$ and C_5D_5N solvents, and of C18 of ANB derivatives, in $CDCl_3$ only. Changing the $CDCl_3$ solvent for C_5D_5N was found to modify the sign of $\Delta\delta_{3-2}$ increments only for C12, C14, C15, C16, and C17 carbon atoms of ANB derivatives and also resulted in significant modifications of magnitudes for C12, C16, and C17 carbon atoms of these derivatives. Significant solvent effects were also found for C12, C14, C16, and C17 carbon atoms of ANP and ANF derivatives, leading to larger magnitudes of the corresponding $\Delta\delta_{3-2}$ increments in C_5D_5N solvent than in $CDCl_3$.

Lengthening the 17α-side-chain from methyl to ethyl and

Table 9
^{13}C NMR chemical shift increments for 17α-aminomethyl ANB, ANP, and ANF substituents versus unsubstituted 17β-OH steroid analogs ($\Delta\delta$ 17α-CH_2NHR-17β-OH) and for lengthening 17α-aminoalkyl side chains from aminomethyl to aminoethyl and aminopropyl substitutents and ($\Delta\delta$ 2-1, $\Delta\delta$ 3-2, and $\Delta\delta$ 3-1)

C atom	δ-$CDCl_3$				δ-C_5D_5N			
	$\Delta\delta$ 17α-CH_2NHR -17β-OH	$\Delta\delta$ 2-1	$\Delta\delta$ 3-2	$\Delta\delta$ 3-1	$\Delta\delta$ 17α-CH_2NHR -17β-OH	$\Delta\delta$ 2-1	$\Delta\delta$ 3-2	$\Delta\delta$ 3-1
12								
ANB	−5.01/−5.03	−0.25/−0.26	+0.03/+0.05	−0.21/−0.23	−5.39/−5.41	+0.24/+0.30	−0.15/−0.18	+0.09/+0.12
ANP	−4.39/−4.42	−0.83/−0.87	+0.16/+0.21	−0.66/−0.67	−4.63/−4.71	−0.80/−0.82	+0.28/+0.30	−0.51/−0.53
ANF	−4.67/−4.72	−0.61/−0.64	+0.19/+0.20	−0.42/−0.45	−4.75/−4.85	−0.71/−0.77	+0.28/+0.30	−0.43/−0.47
13								
ANB	+2.85/+2.89	+0.78/+0.83	−0.14/−0.18	+0.64/+0.65	+3.10/+3.12	+0.63/+0.64	−0.06/−0.12	+0.52/+0.57
ANP	+3.07/+3.09	+0.60/+0.62	−0.15/−0.17	+0.44/+0.45	+3.10/+3.14	+0.65.	−0.12/−0.14	+0.51/+0.53
ANF	+2.87/+2.91	+0.77/+0.81	−0.16/−0.18	+0.60/+0.64	+2.97/+3.02	+0.75/+0.78	−0.15/−0.17	+0.60/+0.61
14								
ANB	−0.34/−0.39	−0.35/−0.37	+0.09/+0.15	−0.21/−0.26	+0.02/+0.05	−0.33/−0.41	−0.07/−0.15	−0.48
ANP	+0.28/+0.30	−0.88/−0.93	+0.16/+0.21	−0.72/−0.74	−0.01/+0.04	−0.50/−0.53	+0.26/+0.30	−0.21/−0.26
ANF	+0.04/+0.07	−0.72/−0.76	+0.22/+0.25	−0.50/−0.54	+0.05/+0.15	−0.71/−0.78	+0.31/+0.33	−0.38/−0.46
15								
ANB	+0.19/+0.22	+0.01	+0.03/+0.05	+0.04/+0.06	+0.31/+0.38	+0.04/+0.06	−0.01/−0.02	+0.03/+0.04
ANP	+0.13/+0.16	+0.07/+0.09	+0.03/+0.06	+0.12/+0.14	+0.18/+0.26	+0.07/+0.09	+0.08/+0.09	+0.15/+0.18
ANF	+0.08/+0.13	+0.05/+0.08	+0.08	+0.13/+0.16	+0.20/+0.25	+0.01/+0.02	+0.10/+0.13	+0.12/+0.14
16								
ANB	+4.05/+4.08	0.00/+0.15	0.00/+0.12	+0.12/+0.15	+2.99/+3.01	+0.84/+0.86	−0.26/−0.31	+0.55/+0.58
ANP	+5.22/+5.36	−0.99/−1.04	+0.22/+0.27	−0.74/−0.78	+4.50/+4.58	−1.30/−1.35	+0.64/+0.66	−0.65/−0.71
ANF	+4.91/+5.00	−0.78/−0.81	+0.27/+0.35	−0.45/−0.54	+4.06/+4.14	−0.94/−1.03	+0.73/+0.76	−0.21/−0.28
17								
ANB	+1.41/+1.59	+1.37/+1.51	−1.17/−1.28	+0.20/+0.26	+1.64/+1.75	−0.49/−0.52	+0.10/+0.12	−0.38/−0.40
ANP	+1.19/+1.39	+0.26/+0.33	−0.03/−0.07	+0.23/+0.26	+1.01/+1.12	+0.53	−0.27/−0.29	+0.24/+0.26
ANF	+1.36/+1.60	+0.49/+0.53	−0.53/−0.56	−0.03/−0.05	+1.27/+1.37	+0.64/+0.69	−0.75/−0.78	−0.08/−0.12
18								
ANB	+2.93/+2.96	−0.10/−0.13	+0.36/+0.38	+0.25/+0.26	+3.12/+3.14	+0.13/+0.14	+0.23/+0.24	+0.36/+0.38
ANP	+2.98/+3.00	−0.11/−0.12	+0.34/+0.35	+0.22/+0.24	+3.13/+3.16	0.00/−0.02	+0.33/+0.34	+0.31/+0.34
ANF	+2.92/+2.93	−0.16/−0.17	+0.42	+0.25/+0.26	+3.14/+3.17	−0.13/−0.14	+0.39/+0.43	+0.26/+0.30

propyl linkers was found to exert parallel effects on ^{13}C NMR resonances of the CH_2N carbon atoms in all three ANB, ANP, and ANF series, thus suggesting the absence of any significant influence of the size of the linker on the two possible *cis-trans* conformations of alkylamido NH-CO bonds of ANB derivatives as compared with the corresponding alkylamino bonds of ANP and ANF analogs. This observation points out to the hypothesis that a same conformation of the amide bond of ANB derivatives is present for the three different linkers, as confirmed by the absence of splitted ^{13}C NMR resonances of carbon atoms, which surround the amide group even after lowering temperature down to −60°C in $CDCl_3$ solvent. This presumed unique conformation corresponds probably to the *trans* rotamer (with the NH proton *anti* to the carbonyl group), reported as the predominant one for N-monosubstituted amides [24–26]. On the other hand, both *cis* and *trans* isomers are often observed for N-disubstituted amides as reported for 16-alkylamido steroidal side-chains [27].

The general trend observed in this study is that the effects of the 17α-aminoalkyl-ANB conjugates on ^{13}C NMR resonances of steroidal carbon atoms are most often very different from the rather similar effects observed for ANP and ANF groups. This difference probably results mainly from the carbonyl group of the amide function of ANB derivatives. The influence of the conjugated phenyl ring of ANB derivatives could be estimated for the shorter 17α-aminomethyl derivatives only, by comparing the effects of the ANB terminal group with those reported for aliphatic acetylamido and hemiglutaramido methyl ester terminal groups [3]. While almost no significant differences were found between 17α-acetylamidomethyl and 17α-hemiglutaramidomethyl derivatives of 3-oxo-androstan-17β-ol, the presence of a 17α-(5-azido-2-nitrobenzoyl)amidomethyl substituent led to small but significant differences, as compared with the acetylamido derivative, for the resonances of C12 to C17 carbon atoms. Deshielding effects ranging from +0.11 to +0.19 ppm were observed either in $CDCl_3$, for C16, or in C_5D_5N, for C13 and C14, and in both solvents, for C12 and C15, while a weak shielding effect was also found for C17, in C_5D_5N only. On the other hand, the direct coupling of the terminal amino group of the side-chain to the nitroaryl azide rings of ANP and ANF derivatives led to similar behaviors, even when changing the side-chain length, thus indicating that the presence of fluorine atoms on the ANF derivative has a rather limited influence. Increments were also calculated for

changing the 17α-cyanomethyl substituent of compound **4** for the 17α-cyanoethyl group of compound **6**, which revealed extremely large $\Delta\delta_{2-1}$ shielding effects on C16 (−2.41 and −2.62 ppm in $CDCl_3$ and in C_5D_5N, respectively) much higher than the deshielding effects on C17 (+0.82 and +0.46 ppm in $CDCl_3$ and in C_5D_5N, respectively). Increments for the increasing lengths of 17α-aminomethyl, 17α-aminoethyl, and 17α-aminopropyl substituents of unsubstituted amino precursors **3**, **5**, and **7**, showed the highest values for C17 ($\Delta\delta_{2-1}$ shielding effects of −2.51 and −1.17 ppm in $CDCl_3$ and in C_5D_5N, respectively; $\Delta\delta_{3-2}$ deshielding effects of +1.94 and +1.27 ppm in $CDCl_3$ and in C_5D_5N, respectively), while large effects, often different in sign or magnitude from those found for ANB, ANP, or ANF derivatives, were also observed on C16, as well as on C12, C13, C14, C15, and C18 carbon atoms. These results, established for intramolecular interactions, suggest that improvements of the sensitivity of NMR equipment, which could be expected in the future for developing biologic studies, may lead to more subtle investigations of intermolecular protein-ligand interactions in a binding site.

Experiments have been made in order to estimate the effects of increasing the side-chain length of 17α-amidoalkyl-ANB derivatives of DHT on the binding properties with SHBG. Competition experiments with the three radioinert 17α-aminomethyl-, 17α-aminoethyl-, and 17α-aminopropyl-ANB conjugates of DHT **9**, **12**, and **15**, and unlabeled DHT were performed on purified human SHBG [28], using tritiated DHT as tracer (Fig. 1, upper panel). Relative binding affinities (RBAs) for SHBG were calculated as the ratio of the concentration of radioinert unsubstituted DHT that gave 50% displacement of the binding of the radioactive tracer to the concentration of 17α-aminoalkyl competitors giving the same inhibition. Increasing lengths of amidomethyl-, amidoethyl-, and amidopropyl-ANB derivatives were found to correspond to decreasing RBA values for SHBG (0.76, 0.47, and 0.10, respectively versus 1.00 reference value for DHT). The association constants of these three amidoalkyl-ANB competitors ($1.6 \times 10^9\ M^{-1}$, $1.0 \times 10^9\ M^{-1}$, and $0.2 \times 10^9\ M^{-1}$, respectively) were estimated indirectly from RBA values using the equation $K_{a\ (competitor)} = K_{a\ (DHT)} / [(1/RBA)(1 + R) - R]$, where $K_{a\ (DHT)}$ is the association constant of DHT for SHBG ($2.2 \times 10^9\ M^{-1}$ at 22°C) [28] and R the bound to free ratio of tritiated DHT tracer at 50% displacement (R = 0.036) [29]. Similar preliminary binding assays were also performed on cytosolic androgen receptors of rat ventral prostate employing a reported protocol [30], modified by the use of a tritiated DHT tracer more similar to the structures of 17α-aminoalkyl competitors than the usual tritiated 17α-methyltrienolone (R1881) tracer and a shorter incubation time, which may limit uncontrolled degradation effects of proteases. Competition experiments with the three 17α-amidoalkyl-ANB derivatives of DHT (Fig. 1, lower panel) showed that only amidoethyl and amidopropyl derivatives interacted significantly with the androgen receptors (RBA values of 0.05 and 0.10, respectively). These RBA values are only estimates

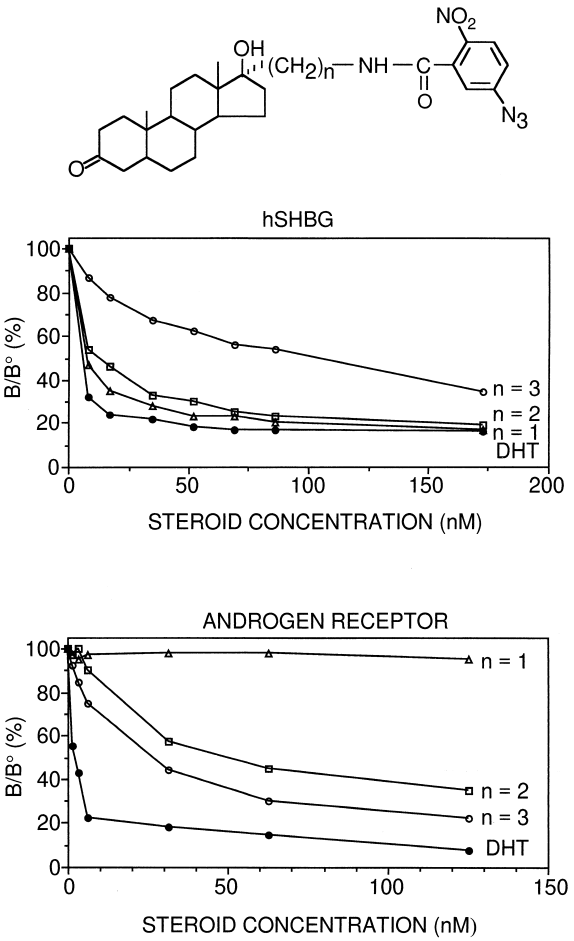


Fig. 1. Displacement of tritiated DHT from purified human SHBG (upper panel) and from cytosolic androgen receptors of rat ventral prostate (lower panel) by radioinert 17α-aminomethyl- ($n = 1$), 17α-aminoethyl- ($n = 2$), and 17α-aminopropyl- ($n = 3$) ANB conjugates of DHT **9**, **12**, and **15**, and unlabeled DHT. Incubations were carried out as indicated in Section 2, at 22°C for SHBG and at 4°C for androgen receptors.

established from rough measurements providing merely qualitative data, not suitable for calculations of the corresponding assosiation constants [31]. Nevertheless, these results are consistent with the increasing relative efficiencies reported for similar methyl, ethyl, and propyl linkers on the capacity of 17α-haloacetamidoalkyl estrogenic derivatives to inhibit the specific binding of estradiol with the estrogen receptor [17]. Similarly, 17α-carboxymethylamide derivatives of DHT were unable to bind to androgen receptors [32] whereas 17α-iodovinyl derivatives showed much higher binding affinities [33]. Successful affinity labeling experiments of purified human SHBG with 17α-aminoalkyl-ANB derivatives of [3α-^{3}H]5α-androstane-3β,17β-diol have already shown that two different peptides are specifically labeled according to the length of the 17α-amidoalkyl linker. Identification of these peptides as well as parallel studies with 17α-acetamidoalkyl ANP and ANF analogs, in order to reveal possible similar effects of the linker size, are

in progress in our laboratory, and the results of these studies will be published in a future article.

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