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Synthesis of C-6 fluoroandrogens: Evaluation of ligands for tumor receptor imaging

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Seven androgens, substituted with fluorine at C-6, were prepared as potential imaging agents for androgen receptor–positive prostate tumors and were evaluated in vitro in terms of their lipophilicity and their relative binding affinities (RBA, relative to R 1881 = 100) for the androgen receptor and for sex steroid binding protein. Introduction of a fluorine atom into the C-6 position of an androgen generally decreases binding affinity to the androgen receptor, except in the two cases: 6α-fluoro-19-nor-testosterone (RBA = 41.6 versus 30.6 for the unsubstituted steroid) and 6α-fluorotestosterone (RBA = 8.9 versus 6.6). Receptor binding of the C-6 fluoro-androgens is also stereospecific, showing higher binding affinities for the α-epimers compared to the corresponding β-epimers (4:1–15:1). Binding affinity to sex steroid binding protein is the lowest with 19-nor-testosterone, which is also the least lipophilic androgen studied. Based on the binding properties of compounds in this series, 6α-fluoro-19-nor-testosterone appears to have the most promise as a tumor imaging agent. (*Steroids* **60**:414–422, 1995)

Keywords: C-6-fluoroandrogens; fluorine substitution; relative binding affinity; 6α- and 6β-epimers; log $P_{o/w}$; prostate tumors

Introduction

Steroids labeled with ^{18}F can be used as imaging agents for receptor-positive tumors using positron-emission tomography (PET). We have successfully developed 16α-[^{18}F]fluoroestradiol as such an agent for imaging estrogen receptor-positive primary and metastatic breast tumors.[1–2] We have also developed ^{18}F labeled progestins and androgens that show promising target tissue selective uptake in rats and may prove to be suitable agents for imaging breast[3–5] and prostate tumors in humans, respectively.[6–8]

In examining the effect of fluorine substitution on the binding affinity of androgens to the androgen receptor, we have found several sites at which substitution results in markedly reduced binding affinity. For example, fluorine substitution at the 16α- or 16β-position in testosterone and 5α-dihydrotestosterone and at the 20 position in mibolerone results in a 2–20-fold reduction in the affinity of the derivative, compared to that of the parent steroid. Nevertheless, some of these agents may still prove useful for in vivo imaging.[6–9]

We are continuing to examine other sites of fluorine substitution. Substitution at the 11β-position, that can be effected by a halofluorination-reduction sequence, appears to be very well tolerated and may, in fact, increase receptor binding; studies on fluorine substitution at this site are the subject of another report.[10] The C-6 position is also a chemically accessible site for fluorine substitution, and although only a limited number of fluoroandrogens substituted at C-6 have been prepared, they appear to have substantial affinity for the androgen receptor.[11]

In this report, we present the synthesis and in vitro binding characterization of seven androgens with fluorine substitution at the C-6 position, in testosterone (T), 5α-dihydrotestosterone (DHT), 19-nor-testosterone (19-nor-T), and 5α-dihydro-19-nor-testosterone (19-nor-DHT) as potential tumor imaging agents. Furthermore, we examine and evaluate the feasibility of preparing these compounds by a direct fluoride ion substitution route, as a convenient and rapid method for labeling the steroids with ^{18}F at the high specific activity required for in vivo studies.

Experimental

Thin-layer chromatography (TLC) was performed using Merck silica gel plates with F-254 indicator (0.25 mm) and visualized by phosphomolybdic acid (PMA) or by UV illumination. Melting

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points were determined on a Thomas Hoover melting point apparatus and were uncorrected. Gas Chromatography (GC) was performed on a Hewlett Packard Model 5790A chromatograph using an Ultra 1 capillary column (12 m × 0.2 mm, 0.33 μm film thickness). High Performance Liquid Chromatography (HPLC) was performed isocratically on a Varian 5060 liquid chromatograph with a semipreparative C-18 column (Whatman Partisil 10 ODS, 0.9 × 50 cm) or on a Spectra-Physics Model 8700 with a semipreparative SiO_2 column (Whatman Partisil M-9, 0.9 × 50 cm). Proton magnetic resonance (^{1}H NMR) spectra were obtained on a General Electric QE-300 (300 MHz) or a Varian XL-200 (200 MHz) spectrometer, and the data are reported in ppm relative to $CDCl_3$ as an internal standard (δ 7.26). Low-resolution electron-impact (EI) mass spectra were obtained on a Finnigan MAT CH-5 spectrometer, and fast-atom bombardment (FAB) mass spectra, on a VG instrument (ZAB HF). High resolution EI exact mass spectra were recorded on a Varian MAT 731 spectrometer. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois.

A general procedure for product isolation involved an aqueous quench (or aqueous acid or base quench) and organic extraction, drying of the extract over Na_2SO_4, and evaporation of solvent in vacuo. Product purification was achieved by flash chromatography.[12] Samples for binding affinity assays were further purified by HPLC: 4-dehydro-androgens **1, 5,** and **6** were purified by semi-preparative normal phase HPLC using a solvent mixture of CH_2Cl_2 containing 5% *i*PrOH (A) and hexane (B); 4,5α-dihydro-androgens **3, 4, 7,** and **8** were purifed by semipreparative reverse-phase HPLC using a 1:1 v/v solvent mixture of water and acetonitrile. The eluant was monitored at 254 nm for the former androgens and at 210 or 290 nm for the latter, and the flow rate was 4 mL/min.

3,17β-Diacetoxyandrosta-3,5-diene (10a) and 3,17β-diacetoxy-estra-3,5-diene (10b)

To testosterone **9a** (200 mg, 0.69 mmol) were sequentially added acetic anhydride (0.17 mL, 1.78 mmol), pyridine (0.045 mL, 0.56 mmol), and acetyl chloride (0.38 mL, 5.36 mmol). The reaction mixture was refluxed at 120°C for 5 h. After evaporation of the solvents, the residue was triturated in ethanol (120 mg). The remaining mother liquor was purified (hexane/EtOAc 5:1, v/v) and combined with the triturated white solid **10a** (total 220 mg, 86%): ^{1}H NMR (300 MHz) δ 0.83 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 17β-$OCOCH_3$), 2.13 (s, 3 H, 3-$OCOCH_3$), 4.61 (m, 1 H, 17α-H), 5.39 (t, 1 H, J = 2.6 Hz, 6-H), 5.69 (d, 1 H, J = 1.9 Hz, 4-H); MS (70 eV) m/z (relative intensity) 372 (M^+, 7), 330 (100); HRMS calculated for $C_{23}H_{32}O_4$ 372.2301, found 372.2300.

Dienol acetate **10b** was obtained as above. A mixture of 19-nor-testosterone **9b** (500 mg, 1.83 mmol), acetic anhydride (0.44 mL, 4.7 mmol), pyridine (0.12 mL, 1.48 mmol), and acetyl chloride (1 mL, 14.1 mmol) was refluxed at 120°C for 5 h. Ethanol trituration (551 mg) and purification of the remaining mother liquor (42 mg) afforded a white solid **10b** (593 mg, 91%): ^{1}H NMR (300 MHz) δ 0.82 (s, 3 H, 18-CH_3), 2.04 (s, 3 H, 17β-$OCOCH_3$), 2.13 (s, 3 H, 3-$OCOCH_3$), 4.62 (m, 1 H, 17α-H), 5.47 (t, 1 H, J = 2.5 Hz, 6-H), 5.76 (d, 1 H, J = 2.0 Hz, 4-H); MS (70 eV) m/z (relative intensity) 358 (M^+, 3), 316 (100), 273 (2), 256 (2), 241 (2); HRMS calculated for $C_{22}H_{30}O_4$ 358.2144, found 358.2155.

17β-Acetoxy-6-fluoroandrost-4-ene (11a) and 17β-acetoxy-6-fluoroestr-4-ene (11b)

To dienol acetate **10a** (290 mg, 0.78 mmol) in acetonitrile (2 mL) was added 1-fluoropyridinium pyridine heptafluorodiborate (NFPy; 438 mg, 1.32 mmol).[13] The reaction mixture was allowed to stir at 38–40°C for one week. After addition of Et_2O, the reaction mixture was passed through an anhydrous $MgSO_4$ pad. Product purification (hexane/EtOAc 3:1, v/v) furnished a white solid **11a** (137 mg, 51%), as a 63:37 mixture of 6α- and 6β-fluoroepimers. Analytical samples were obtained by further chromatography.

17β-Acetoxy-6α-fluoro-4-androsten-3-one. m.p. 170–173°C, ^{1}H NMR (300 MHz) δ 0.83 (s, 3 H, 18-CH_3), 1.19 (s, 3 H, 19-CH_3), 2.05 (s, 3 H, 17β-$OCOCH_3$), 4.62 (m, 1 H, 17α-H), 5.10 (dddd, 1 H, J = 47.8, 12.3, 5.9, 1.9 Hz, 6β-H), 6.08 (s, 1 H, 4-H); MS (70 eV) m/z (relative intensity) 348 (M^+, 14), 328 (2), 306 (9), 288 (9); HRMS calculated for $C_{21}H_{29}O_3F$ 348.2101, found 348.2101. Analysis calculated for $C_{21}H_{29}O_3F$: C, 72.39; H, 8.39; F, 5.45. Found: C, 72.41; H, 8.42; F, 5.42.

17β-Acetoxy-6β-fluoro-4-androsten-3-one: m.p. 132–134°C; ^{1}H NMR (300 MHz) δ 0.86 (s, 3 H, 18-CH_3), 1.31 (d, 3 H, J = 1.3 Hz, 19-CH_3), 2.04 (s, 3 H, 17β-$OCOCH_3$), 4.61 (m, 1 H, 17α-H), 4.99 (dt, 1 H, J = 49.1, 2.6 Hz, 6α-H), 5.87 (d, 1 H, J = 5.0 Hz, 4-H); MS (70 eV) m/z (relative intensity) 348 (M^+, 40), 328 (10), 306 (58), 288 (69); HRMS calculated for $C_{21}H_{29}O_3F$ 348.2101, found 348.2101. Analysis calculated for $C_{21}H_{29}O_3F$: C, 72.39; H, 8.39; F, 5.45. Found: C, 72.39; H, 8.40; F, 5.43.

Electrophilic fluorination of **10b** by NFPy was carried out as above.[13] A reaction mixture of dienol acetate **10b** (160 mg, 0.45 mmol) and NFPy (240 mg, 0.72 mmol) in acetonitrile (1 mL) was stirred at 38–40°C for a week. The product purification (hexane/EtOAc 3:1, v/v) gave a **11b** as a 68:32 mixture of 6α- and 6β-fluoroepimers. Analytical samples were obtained by further chromatography.

17β-Acetoxy-6α-fluoro-4-estren-3-one. m.p. 123–125°C; ^{1}H NMR (300 MHz) δ 0.84 (s, 3 H, 18-CH_3), 2.05 (s, 3 H, 17β-$OCOCH_3$), 4.63 (m, 1 H, 17α-H), 4.98 (dddd, 1 H, J = 47.8, 11.5, 5.8, 1.3 Hz, 6β-H), 6.16 (s, 1 H, 4-H); MS (70 eV) m/z (rel intensity) 334 (M^+, 9), 314 (4), 292 (13), 274 (22); HRMS calculated for $C_{20}H_{27}O_3F$ 334.1944, found 334.1947. Analysis calculated for $C_{20}H_{27}O_3F$: C, 71.83; H, 8.14; F, 5.68. Found: C, 71.90; H, 8.16; F, 5.58.

17β-Acetoxy-6β-fluoro-4-estren-3-one. m.p. 132–134°C; ^{1}H NMR (300 MHz) δ 0.87 (s, 3 H, 18-CH_3), 2.05 (s, 3 H, 17β-$OCOCH_3$), 4.62 (m, 1 H, 17α-H), 5.05 (dt, 1 H, J = 49.0, 2.4 Hz, 6α-H), 5.96 (dd, 1 H, J = 4.7, 2.0 Hz, 4-H); MS (70 eV) m/z (relative intensity) 334 (M^+, 10), 314 (10), 292 (12), 274 (34); HRMS calculated for $C_{20}H_{27}O_3F$ 334.1944, found 334.1947. Analysis Calculated for $C_{20}H_{27}O_3F$: C, 71.83; H, 8.14; F, 5.68. Found: C, 71.61; H, 8.17; F, 5.44.

6-Fluoro-17β-hydroxy-4-androsten-3-ones (1 and 2) and 6-fluoro-17β-hydroxy-4-estren-3-ones (5 and 6)

Deprotection of 17β-acetyl group was carried out as described for androgen **7.** 17β-Acetoxy-6α-fluoro-4-androstene (59 mg, 0.17 mmol) was dissolved in a mixture of methanol and water (3 mL, 2:1 ratio, v/v), and K_2CO_3 (93 mg, 0.68 mmol) was added. Product isolation (CH_2Cl_2) and purification (hexane/EtOAc 5:4, v/v) afforded a white solid **1** (27.7 mg, 54%) and defluorination product (11 mg, 22%). Hydrolysis of 17β-acetyl group of 17β-acetoxy-6β-fluoro-4-androstene (10 mg, 0.03 mmol) afforded a white solid 6β-fluoro-17β-hydroxy-4-androsten-3-one **2** (3.1 mg, 36%). Hydrolysis of 17β-acetyl group of 17β-acetoxy-6α-fluoro-4-estrene (13.5 mg, 0.04 mmol) furnished a white solid 6α-fluoro-17β-hydroxy-4-estren-3-one **5** (6.8 mg, 58%), and hydrolysis of 17β-acetyl group of 17β-acetoxy-6β-fluoro-4-estrene (46 mg,

0.14 mmol) afforded a white solid 6β-fluoro-17β-hydroxy-4-estren-3-one **6** (13.9 mg, 34%).

6α-Fluoro-17β-hydroxy-4-androsten-3-one *1*. HPLC (A/B 45:55) t_R 33.5 min; ^{1}H NMR (300 MHz) δ 0.80 (s, 3 H, 18-CH_3), 1.20 (s, 3 H, 19-CH_3), 3.68 (m, 1 H, 17α-H), 5.10 (dddd, 1 H, J = 47.9, 12.2, 5.9, 1.8, Hz, 6β-H), 6.10 (s, 1 H, 4-H); MS (70 eV) m/z (relative intensity) 306 (M^+, 68), 286 (28); HRMS calculated for $C_{19}H_{27}O_2F$ 306.1995, found 306.1995.

6β-Fluoro-17β-hydroxy-4-androsten-3-one *2*. ^{1}H NMR (300 MHz) δ 0.82 (s, 3 H, 18-CH_3), 1.31 (d, 3 H, J = 1.8 Hz, 19-CH_3), 3.70 (m, 1 H, 17α-H), 4.99 (dt, 1 H, J = 49.1, 2.7 Hz, 6α-H), 6.12 (d, 1 H, J = 4.8 Hz, 4-H).

6α-Fluoro-17β-hydroxy-4-estren-3-one *5*. HPLC (A/B 45:55) t_R 35 min; ^{1}H NMR (300 MHz) δ 0.80 (s, 3 H, 18-CH_3), 3.69 (t, 1 H, J = 8.5 Hz, 17α-H), 4.98 (dddd, 1 H, J = 47.9, 11.5, 5.8, 1.4 Hz, 6β-H), 6.16 (s, 1 H, 4-H); MS (70 eV) m/z (relative intensity) 292 (M^+, 100), 272 (65); HRMS calculated for $C_{18}H_{25}O_2F$ 292.1839, found 292.1838.

6β-Fluoro-17β-hydroxy-4-estren-3-one *6*. HPLC (A/B 35:65) t_R 30 min; ^{1}H NMR (300 MHz) δ 0.82 (s, 3 H, 18-CH_3), 3.67 (t, 1 H, J = 8.4 Hz, 17α-H), 5.05 (dt, 1 H, J = 49.2, 2.5 Hz, 6α-H), 5.95 (dd, 1 H, J = 4.6, 2.0 Hz, 4-H); MS (70 eV) m/z (relative intensity) 292 (M^+, 44), 272 (89); HRMS calculated for $C_{18}H_{25}O_2F$ 292.1839, found 292.1838.

6α-Fluoro-17β-hydroxy-5α-estran-3-one (7)

6α-Fluoroepimer of androgen **11b** (99 mg, 0.3 mmol) was dissolved in ethanol (30 mL), and 17 mg of 5% palladium on carbon were added. The reaction mixture was saturated with H_2 gas and stirred at room temperature for 1.5 h, filtered through a Celite pad, and concentrated. ^{1}H NMR revealed a 1:2:3 mixture of 17β-acetoxy-6α-fluoro-5α-, 17β-acetoxy-6α-fluoro-5β-androstane, and defluorinated products, based on the ratio of the peaks at δ 4.25 (5α-H, 6α-F), 4.72 (5β-H, 6α-F), 4.63 (17α-H), and 0.82 (18-CH_3). The mixture was used in subsequent reaction. Hydrolysis of 17β-acetyl group was accomplished with K_2CO_3 (170 mg, 1.23 mmol) in a mixture of methanol and water (7.8 mL, 1.6:1, v/v). The reaction mixture was allowed to stir at room temperature for 9 h. Product isolation (CH_2Cl_2) and purification (hexane/EtOAc 2:1, v/v) afforded a white solid **7** (6.4 mg, 7.4%): HPLC (water/CH_3CN 1:1 v/v, reversed phase) t_R 24 min; ^{1}H NMR (300 MHz) δ 0.77 (s, 3 H, 18-CH_3), 2.80 (dq, 1 H, J = 14.3, 3.6 Hz, one of 4-H), 3.68 (t, 1 H, J = 8.5 Hz, 17α-H), 4.25 (dtd, 1 H, J = 49.3, 10.3, 4.6 Hz, 6β-H); MS (70 eV) m/z (relative intensity) 294 (M^+, 14), 274 (100), 256 (11); HRMS calculated for $C_{18}H_{27}O_2F$ 294.1995, found 294.1995.

3,3-Ethylenedioxy-5-androsten-17β-ol (12a) and 3,3-ethylenedioxy-5-estren-17β-ol (12b)

Testosterone **9a** (20 g, 69.2 mmol) was dissolved in benzene (600 mL), and ethylene glycol (54 mL, 0.97 mol) and *p*-toluenesulfonic acid (TsOH, 0.25 g, 1.3 mmol) were added. The reaction mixture was refluxed for 24 h, and water was distilled from the mixture by a Dean-Stark trap. Recrystallization from EtOH and water afforded the white solid **12a** (19.9 g, 86%): m.p. 182–183°C; ^{1}H NMR (200 MHz) 0.76 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 2.58 (dm, 1 H, J = 14.4 Hz, one of 4-H), 3.65 (t, 1 H, J = 7.1 Hz, 17α-H), 3.95 (s, 4 H, 3-ketal), 5.36 (m, 1 H, 6-H), identical with the literature values[9]; MS (70 eV) m/z (relative intensity) 332 (M^+, 25), 99 (100).

Ketal **12b** was prepared as in the synthesis of **12a.** A benzene (200 mL) solution of 19-nor-testosterone (3 g, 10.9 mmol), ethylene glycol (18 mL, 328 mmol), and TsOH (30 mg, 0.16 mmol) was gently refluxed for 3 h.[14] Product isolation (Et_2O) and purification (EtOAc/hexane 1:1, v/v) afforded a mixture of C-5 alkene **12b** and C-5(10) regioisomer (1.84 g, 79%). Ratio of C-5 and C-5(10) isomers was determined to be 2:1 by ^{1}H NMR and GC (t_R = 8.21 min, Δ^5 and 8.78 min, $\Delta^{5(10)}$; 195°C, isothermal). The mixture was used in subsequent reaction. ^{1}H NMR (200 MHz) δ 0.75 (s, 3 H, 18-CH_3, $\Delta^{5(10)}$), 0.77 (s, 3 H, 18-CH_3, Δ^5, **12b**), 3.66 (m, 1 H, 17α-H), 3.96 (m, 4 H, 3-ketal), 5.46 (d, 1 H, J = 5.6 Hz, 6-H, Δ^5), identical with the literature values[4,15]; MS (70 eV) m/z (relative intensity) 318 (M^+, 13), 256 (4); HRMS calculated for $C_{20}H_{30}O_3$ 318.2195, found 318.2190.

3,3-Ethylenedioxy-17β-tetrahydropyranyloxy-5-androstene (13a) and 3,3-ethylenedioxy-17β-tetrahydropyranyloxy-5-estrene (13b)

Ketal **12a** (3 g, 9 mmol) was dissolved in CH_2Cl_2 (70 mL), and dihydropyran (1.64 mL, 18 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 22 mg, 0.09 mmol) were added.[16] The mixture was stirred at room temperature overnight, diluted with ether and extracted with half-saturated NaCl. Product isolation (Et_2O) and purification (hexane/EtOAc 5:1, v/v) afforded the product **13a** (2.87 g, 76%) as a diastereomeric mixture: ^{1}H NMR (200 MHz) δ 0.78; 0.80 (s, 3 H, 18-CH_3), 1.03; 1.04 (s, 3 H, 19-CH_3), 2.57 (dm, 1 H, J = 13.6 Hz, one of 4-H), 3.49 (m, 1 H, one of 5′-tetrahydropyranyl [THP] H), 3.64 (m, 1 H, 17α-H), 3.94 (m, 5 H, 3-ketal and another 5′-THP H), 4.63 (m, 1 H, 1′-THP H), 5.35 (m, 1 H, 6-H); MS (FAB) m/z (relative intensity) 417 (M + 1, 14), 333 (5). Analysis calculated for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 74.82; H, 9.71.

Androgen **13b** was prepared as **13a.** A CH_2Cl_2 solution (150 mL) of ketal **12b** (3.3 g, 10 mmol), dihydropyran (2 mL, 21.9 mmol) and PPTS (28 mg, 0.11 mmol) was stirred at room temperature overnight.[16] A careful separation of alkene **13b** from C-5(10) isomer (hexane/EtOAc 5:1, v/v) afforded the product **13b** (2.1 g, 50%) as a diastereomeric mixture: ^{1}H NMR (300 MHz) δ 0.79; 0.80 (s, 3 H, 18-CH_3), 3.46 (m, 1 H, one of 5′-THP H), 3.64 (m, 1 H, 17α-H), 3.95 (m, 5 H, 3-ketal and another 5′-THP H), 4.63 (m, 1 H, 1′-THP H), 5.46 (d, 1 H, J = 5.6 Hz, 6-H); MS (70 eV) m/z (relative intensity) 402 (M^+, 39), 317 (12), 256 (7); HRMS calculated for $C_{25}H_{38}O_4$ 402.2770, found 402.2768. Analysis calculated for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51. Found: C, 74.56; H, 9.49.

3,3-Ethylenedioxy-17β-tetrahydropyranyloxy-5α-androstan-6α-ol (14a) and 3,3-ethylenedioxy-17β-tetrahydropyranyloxy-5α-estran-6α-ol (14b)

To alkene **13a** (0.7 g, 1.7 mmol) in THF (20 mL) was added borane (1 M in THF, 8.4 mL, 8.4 mmol). The reaction mixture was stirred at room temperature for 3 h before it was oxidized with 3N NaOH (2.8 mL) and 30% H_2O_2 (2.8 mL) at −10°C. The resulting mixture was allowed to stir at room temperature overnight. The product isolation (EtOAc) and purification (hexane/EtOAc 1:1, v/v) eluted the 5β-H,6β-alcohol (0.41 g, 56%), and further elution furnished the 6α-alcohol **14a** (0.15 g, 20%) as a diastereomeric mixture: ^{1}H NMR (200 MHz) δ 0.75; 0.77 (s, 3 H, 18-CH_3), 0.83 (s, 3 H, 19-CH_3), 3.39 (m, 1 H, 6β-H), 3.49 (m, 1 H, one of 5′-THP H), 3.63 (m, 1 H, 17α-H), 3.95 (m, 5 H, 3-ketal and another of 5′-THP H), 4.61 (m, 1 H, 1′-THP H); MS (70 eV) m/z (relative intensity) 434 (M^+, 3), 350 (3), 332 (3); HRMS calculated for $C_{26}H_{42}O_5$ 434.3032, found 434.3034. Analysis calculated for $C_{26}H_{42}O_5$: C, 71.85; H, 9.74. Found: C, 71.70; H, 9.78.

The 6α-alcohol **14b** was obtained as described above for **14a.**

A THF solution (50 mL) of alkene **13b** (1.17 g, 2.9 mmol) and borane (1 M in THF, 15 mL, 15 mmol) was stirred at room temperature for 4 h, and then the reaction mixture was oxidized with 3N NaOH (5 mL) and 30% H_2O_2 (5 mL) at −10°C. The product isolation (EtOAc) followed by purification (hexane/EtOAc 3:2, v/v) furnished the 5β-H,6β-alcohol (0.34 g, 28%) and the product **14b** (0.38 g, 31%) as a diastereomeric mixture: ^{1}H NMR (200 MHz) δ 0.78, 0.80 (s, 3 H, 18-CH_3), 3.24 (m, 1 H, 6β-H), 3.47 (m, 1 H, one of 5′-THP H), 3.63 (m, 1 H, 17α-H), 3.93 (m, 5 H, 3-ketal and another 5′-THP H), 4.62 (m, 1 H, 1′-THP H); MS (70 eV) m/z (relative intensity) 420 (M^+, 10), 318 (15), 257 (5); HRMS calculated for $C_{25}H_{40}O_5$ 420.2876, found 420.2870. Analysis calculated for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Found: C, 71.08; H, 9.52.

3,3-Ethylenedioxy-6β-fluoro-17β-tetrahydropyranyloxy-5α-androstane (15a) and 3,3-ethylenedioxy-6β-fluoro-17β-tetrahydropyranyloxy-5α-estrane (15b)

The 6α-alcohol **14a** (60 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (3 mL) at −78°C, and trifluoromethanesulfonic anhydride (44 μL, 0.26 mmol) and 2,6-lutidine (50 μL, 0.43 mmol) were added. The reaction mixture was stirred at −78°C for 10 min and concentrated before being purified (hexane/EtOAc 5:1, v/v, prechilled). The product (73 mg, 94%) was used for subsequent reaction without analysis. To the 6α-triflate (70 mg, 0.12 mmol) in THF (5 mL) was added nBu$_4$NF (1 M in THF, 0.37 μL, 0.37 mmol). The reaction mixture was refluxed for 1 h and then passed through a short silica plug. Product isolation (EtOAc) and purification (hexane/EtOAc 5:1, v/v) afforded white solid **15a** as a diasteromeric mixture (26 mg, 48%): ^{1}H NMR (300 MHz) δ 0.79; 0.81 (s, 3 H, 18-CH_3), 0.98; 0.99 (s, 3 H, 19-CH_3), 3.46 (m, 1 H, one of 5′-THP H), 3.63 (m, 1 H, 17α-H), 3.94 (m, 5 H, 3-ketal and another 5′-THP H), 4.53 (dq, 1 H, J = 48.5, 1.3 Hz, 6α-H), 4.63 (m, 1 H, 1′-THP H); MS (70 eV) m/z (relative intensity) 436 (M^+, 2), 416 (2), 352 (19).

The 6β-fluoro androgen **15b** was prepared as described for **15a.** A CH_2Cl_2 solution (2 mL) of the 6α alcohol **14b** (40 mg, 0.1 mmol), trifluoromethanesulfonic anhydride (29 μL, 0.17 mmol) and 2,6-lutidine (34 μL, 0.29 mmol) was stirred at −78°C for 10 min. Fluoride ion substitution on the 6α-triflate was achieved with nBu$_4$NF (1 M in THF, 0.29 μL, 0.29 mmol) at room temperature for 10 min. Product purification (hexane/EtOAc 4:1, v/v) afforded a white solid **15b** (20 mg, 50%) as a diastereomeric mixture: ^{1}H NMR (200 MHz) δ 0.79; 0.81 (s, 3 H, 18-CH_3), 3.46 (m, 1 H, one of 5′-THP H), 3.63 (m, 1 H, 17α-H), 3.94 (m, 5 H, 3-ketal and another 5′-THP H), 4.55 (dq, 1 H, J = 47.3, 1 Hz, 6α-H), 4.63 (m, 1 H, 1′-THP H); MS (70 eV) m/z (relative intensity) 422 (M^+, 4), 402 (2), 301 (16), 239 (13); HRMS calculated for $C_{25}H_{39}O_4F$ 422.2832, found 422.2832.

The 6β-fluoroandrogen **15b** can be obtained from the reaction of **14b** (30 mg, 0.07 mmol) with diethylaminosulfur trifluoride (DAST; 27 μL, 0.20 mmol).[17,18] The reaction mixture was stirred at −78°C for 10 min and passed through a short silica plug. Product purification (hexane/EtOAc 4:1, v/v) gave a white solid **15b** (14 mg, 47%).

6β-Fluoro-17β-hydroxy-androstan-3-one (4) and 6β-Fluoro-17β-hydroxy-estran-3-one (8)

To 6β-fluoro androgen **15a** (22 mg, 0.05 mmol) in acetone (5 mL) was added 3N HCl (aqueous, 0.8 mL). The reaction mixture was stirred at room temperature for 2 h. The product isolation (Et_2O) and purification (hexane/EtOAc 1:1,v/v) afforded 14.5 mg of the product **4** (94%): HPLC (reverse-phase) t_R 18.7 min; ^{1}H NMR (300 MHz) δ 0.80 (s, 3 H, 18-CH_3), 1.18 (d, 3 H, 19-CH_3), 2.80 (t, 1 H, J = 15.0 Hz, one of 4-H), 3.66 (t, 1 H, J = 8.5 Hz, 17α-H), 4.53 (dq, 1 H, J = 49.2, 2.1 Hz, 6α-H); MS (70 eV) m/z (relative intensity) 308 (M^+, 14), 288 (88); HRMS calculated for $C_{19}H_{29}O_2F$ 308.2152, found 308.2152.

An acetone solution (7.5 mL) of 6β-fluoro androgen **15b** (30 mg, 0.07 mmol) and 3N HCl (1.2 mL) was stirred at room temperature for 1 h. The product isolation (Et_2O) and product purification (hexane/EtOAc 3:2, v/v) afforded a white solid **8** (20 mg, 96%): m.p. 165–167°C; HPLC (reverse-phase) t_R 22.1 min; ^{1}H NMR (300 MHz) δ 0.80 (s, 3 H, 18-CH_3), 2.80 (t, 1 H, J = 14.9 Hz, one of 4-H), 3.66 (m, 1 H, 17α-H), 4.56 (dq, 1 H, J = 49.0, 1.7 Hz, 6α-H); MS (70 eV) m/z (relative intensity) 294 (M^+, 6), 274 (69), 256 (12); HRMS calculated for $C_{18}H_{27}O_2F$ 294.1995, found 294.1996. Analysis calculated for $C_{18}H_{27}O_2F$: C, 73.43; H, 9.24; F, 6.45. Found: C, 73.25; H, 9.31; F, 6.33.

3,3-Ethylenedioxy-6α-fluoro-17β-tetrahydropyranyloxy-5α-androstane (16a)

The 6α-alcohol **14a** (50 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (2.5 mL), and DAST (60 μL, 0.45 mmol) was added.[18] The reaction mixture was stirred at −78°C for 10 min. Product purification (hexane/EtOAc 4:1, v/v) gave a white solid **16a** (34 mg, 68%): ^{1}H NMR (300 MHz) δ 0.75; 0.77 (s, 3 H, 18-CH_3), 0.83 (s, 3 H, 19-CH_3), 3.47 (m, 1 H, one of 5′-THP H), 3.64 (m, 1 H, 17α-H), 3.95 (m, 5 H, 3-ketal and another 5′-THP H), 4.27 (dtd, 1 H, J = 49.9, 10.8, 4.9 Hz, 6β-H), 4.61 (d, 1 H, J = 3.6 Hz, 1′-THP H); MS (70 eV) m/z (relative intensity) 436 (M^+, 12), 416 (7), 315 (10), 253 (9); HRMS calculated for $C_{26}H_{41}O_4F$ 436.2989, found 436.2988. Analysis calculated for $C_{26}H_{41}O_4F$: C, 71.53; H, 9.47; F, 4.35. Found: C, 71.65; H, 9.55; F, 4.16.

6α-Fluoro-17β-hydroxyandrostan-3-one (3)

Deprotection of both 3-ketal and 17β-tetrahydropyranyl group was carried out as described for the preparation of **4** and **8.** To the 6α-fluoro androgen **16a** (34 mg, 0.078 mmol) in acetone (8 mL) was added 3N HCl (1.5 mL). The product isolation (Et_2O) and purification (hexane/EtOAc 1:1, v/v) afforded **3** as a white solid (23 mg, 95%): m.p. 162–164°C; HPLC (reverse-phase) t_R 17.9 min; ^{1}H NMR (300 MHz) δ 0.76 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 2.66 (dq, 1 H, J = 15.4, 3.9 Hz, one of 4-H), 3.67 (t, 1 H, J = 8.2 Hz, 17α-H), 4.34 (dtd, 1 H, J = 49.6, 10.8, 4.9 Hz, 6β-H); MS (70 eV) m/z (relative intensity) 308 (M^+, 5), 288 (81); HRMS calculated for $C_{19}H_{29}O_2F$ 308.2152, found 308.2155.

The configuration of the 6α- and 6β-fluoro isomers was determined by ^{1}H NMR spectroscopy and is consistent with the literature data. 6α-Fluoro-4-dehydro-androgens: 6-β-H (axial) is split into a dddd (J = 48, 12, 6, 2 Hz) by 6α-F, 7α-H, 7β-H and 4-H, respectively: 6β-fluoro-4-dehydro-androgens: 6α-H (equatorial) is split into a dt (J = 49, 2.6 Hz) by 6β-F, and 7β-H, respectively, and C-4-H and 19-CH_3 appears as a doublet (2 Hz) due to splitting by fluorine.[19,20] 6α-Fluoro-5α-dihydro-androgens: 6-β-H (axial) is split into a dtd (J = 49, 11, 5 Hz), by 6α-F, 5α-H, 7α-H, and 7β-H, respectively; 6β-fluoro-5α-dihydroandrogens: 6α-H (equatorial) is split into a dq (J = 49, 2 Hz) by 6β-F and 7β-H, respectively, and 19-CH_3 appears as a doublet due to splitting by fluorine.[19,20]

Biological methods

Binding assays. Relative binding affinities were determined as described previously: AR[11,21] and sex steroid binding protein.[22] The tritium-labeled tracer was R 1881 (Du Pont NEN) for androgen receptor and E_2 (Amersham) for steroid binding protein.

Androgen receptor cytosol and steroid binding protein were prepared and stored as previously reported.[11,22]

Log $P_{o/w}$ determinations. The log $P_{o/w}$ values were estimated as previously reported[23] from the log K'_w values that were determined by HPLC according to the recommendation of Minick et al.[24]

Results

Synthesis of fluorine-substituted androgens 1, 2, and 5–7

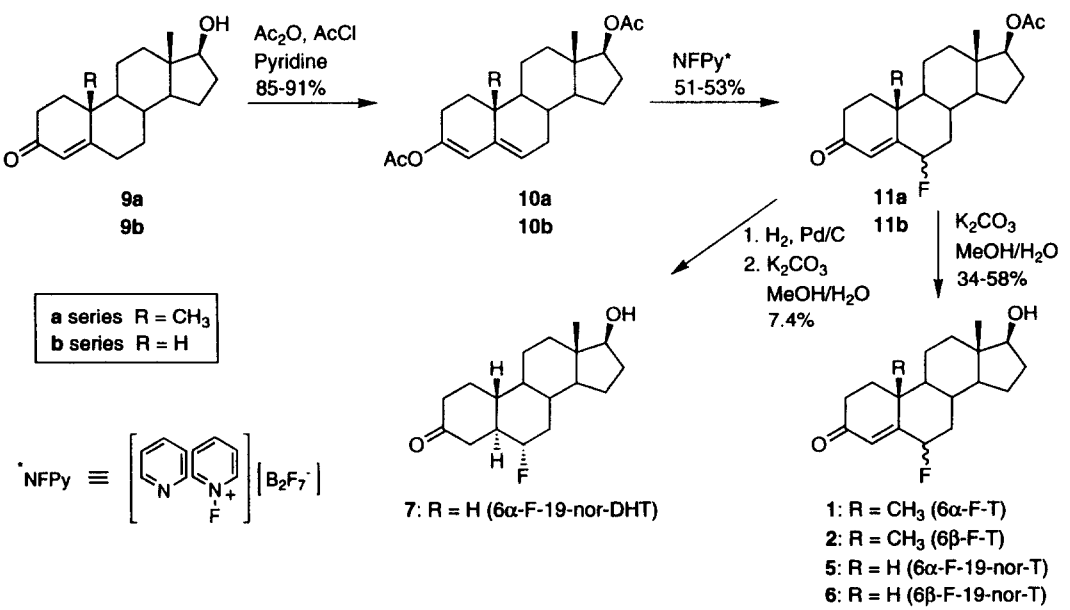
Androgens **1, 2,** and **5–7** were prepared from androgen **9,** and their syntheses are shown in Scheme 1. Acetylation of enone **9** afforded the dienol acetate and at the same time protected the 17β-alcohol. The electrophilic fluorinating agent 1-fluoropyridinium pyridine heptafluorodiborate (NFPy) was used to introduce a fluorine atom at the C-6 position.[13] While axial attack is expected to produce the 6β-isomers under conditions of kinetic control, epimerization to the equatorial 6α-isomers is quite rapid. Thus, the ratio of the 6α- to the 6β-isomer varies, depending on the reaction temperature and time, but the 6α-epimer predominates under the conditions we have utilized. Hydrolysis of the 17β-acetyl group with K_2CO_3 was rather troublesome: elimination of HF from C-6,7 (32–37% of product) was unavoidable both from the 6α- and 6β-isomers, and the formation of another by-product, the 6α-methoxy androgen, was observed from the 6β-isomer. Nevertheless, electrophilic fluorination by NFPy was successful and afforded fluorine-substituted testosterones and 19-nor-testosterones **1, 2, 5,** and **6.** 6β-F-Testosterone **2,** however, was obtained in very low yield.

The other androgen (**7**) was obtained by catalytic hydrogenation of the C-4 double bond in the 6α-F isomer of **11b,** which furnished not only the 5α- and 5β-dihydroepimers of **11b,** but also their corresponding defluorination products. Hydrolysis of the 17β-acetyl group gave 6α-fluoro-19-nor-5α-dihydrotestosterone **7.** Although dissolving-metal reduction of the 6α-F-isomer of **11b** afforded only the 5α-dihydroepimer, it resulted in elimination of hydrogen fluoride and therefore was not useful.

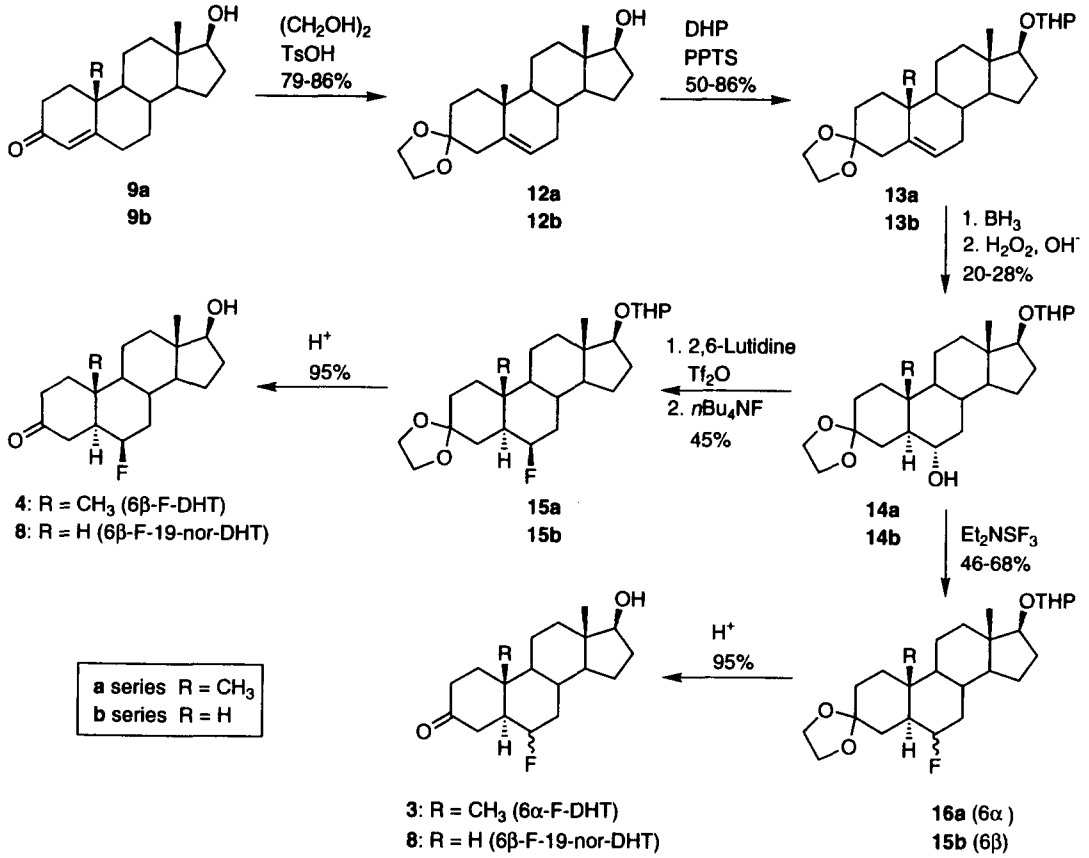
Synthesis of fluorine-substituted androgens 3, 4 and 8

The common precursor **14** of androgens **3, 4,** and **8** was prepared from androgen **9** in three steps (Scheme 2). Testosterone **(9a)** and 19-nor-testosterone **(9b)** are structurally analogous, and therefore similar reaction conditions were employed in these three steps. The 3-ketone group was protected as the ketal under acidic conditions,[25] during which the double bond in **9a** moved from C-4 to C-5 to give ketal **12a;** in the 19-nor series **(9b),** that lacks the CH_3 group at the C-10 position, the double bond moves to both C-5 and C-5(10). The ratio of the C-5 to C-5(10) alkenes in **12b** is dependent on reaction time, temperature, and acid catalyst concentration,[15] such that use of a limited amount of toluenesulfonic acid (0.015 Eq), a short reaction time (3 h) and a gentle reflux afforded a 1.9:1 ratio of the desired alkene **12b** in 79% overall yield; under more vigorous conditions, this ratio was reversed, giving a 1:2 mixture favoring the undesired C-5(10) isomer.[15] Protection of the 17β-OH group of ketal **12** to give tetrahydropyranyl ether **13** was carried out using a mild acid catalyst, pyridinium *p*-toluenesulfonate, to avoid deprotection of the ketal function at the C-3 position.[16] In the 19-nor-testosterone series, a mixture of C-5 and C-5(10) alkenes was carefully separated at this stage, and the desired isomer **13b** was used for the next reaction.

Functionalization of the 6α-position of C-5 alkene **13** was obtained by hydroboration-oxidation. Despite the presence of the axial 19β-CH_3 (H) group, the reaction proceeds with cis addition of the elements of water to the C-5 alkene from both the α- and β-side of the steroid backbone: the ratio of 5α-H,6α-OH to 5β-H,6β-OH product isomers is



Scheme 1



Scheme 2

approximately 1:2.7 in the testosterone series **14a,** compared to 1:1.1 in the 19-nor-testosterone series **14b.** Predominant cis addition from the β-side is expected in alkene **13,** due to the presence of the 3α-axial oxygen. This has also been observed by another group[26]; they also showed that the ratio of 6α- to 6β-alcohol increases to 3.5:1 when the C-3 position is occupied by the 3β-hydroxyl group. However, it is surprising to see a higher ratio of 6α- to 6β-alcohol when an H atom rather than a CH_3 group occupies the 10β position. This phenomenon was also seen in the epoxidation of the C-5 alkene **13** with *m*-chloroperoxybenzoic acid, in which the ratio of 5α,6α- to 5β,6β-epoxide increases from 1:1 to 3:1 as one goes from the 19-CH_3 to the 19-H series (Choe and Katzenellenbogen, unpublished results).[15] After hydrolysis of the 3-ketal and 17β-tetrahydropyranyl protecting groups, the configurations of the resulting 6α-alcohol (**14**) and 6β-alcohol were confirmed by ^1H NMR comparison to those of known[27] or similar structure.[28] The undesired 5β-H,6β-alcohol can be recycled to the 5α-H,6α-alcohol (**14**) by oxidation of the 6β-hydroxyl group to the 6-ketone, followed by epimerization at the C-5 center using potassium hydride and reduction of the ketone to the 6α-alcohol by lithium aluminum hydride.

The 6α-alcohol group of the precursor **14** was fluorinated by diethylaminosulfur trifluoride (DAST) to yield **16a** or **15b,** or by trifluoromethanesulfonylation followed by displacement with fluoride ion (tetrabutylammonium fluoride, nBu$_4$NF) to give androgen **15.** Since the reaction with DAST goes via S_N1 displacement,[17,18] the stereochemistry of the C-6 center is retained in **16a,** but inverted in **15b,** the stereochemical outcome being a function of whether the C-10β position is substituted with a CH_3 group or an H atom.

Receptor binding affinities

The binding affinities of these androgens to the androgen receptor (AR)[11,21] and to sex steroid binding protein (SBP), a carrier protein present in the blood of primates,[22] were measured by competitive radiometric assays relative to the synthetic androgen, R 1881 and estradiol, respectively; the results expressed as relative binding affinity (RBA) values are given in Table 1. Introduction of a fluorine atom at the C-6–position of the androgen lowers its binding affinity, except at the 6α-position of 19-nor-testosterone and testosterone. Moreover, the AR binding affinities of the 6-fluoroandrogens depend on the configuration at the C-6 center: the ratio of RBA values of 6α-F-DHT (**3**), 6α-F-19-nor-T (**5**) and 6α-F-19-nor-DHT (**7**) to the corresponding 6β-fluoro epimers (**4, 6,** and **8**) are 15:1, 15:1, and 4:1, respectively. The RBA of DHT to AR is 10 times higher than that of testosterone. The C-6 fluorine-substituted DHT derivatives, likewise, have higher affinities than their testosterone counterparts. On the other hand, the affinities in the 19-nor series are higher for testosterone-based compounds than for the DHT-based compounds. The highest RBA to the AR of the C-6 fluoroandrogens is obtained with 6α-fluoro-19-nor-testosterone (**5**).

Table 1 Relative binding affinities, lipophilic properties, and binding selectivity indices of C-6 fluoroandrogens

Compound	RBA[a] AR (R 1881 = 100)	RBA[a] SBP (estradiol = 100)	log $P_{o/w}$[b]	NSB[c]	BSI[d]
T	6.6 ± 1.4	417.0 ± 88	3.45	1.18	5.6
6α-F-T (**1**)	8.9 ± 0.7	268.0 ± 122	3.15	0.87	10.2
DHT	60.9 ± 17.2	1808.0 ± 699	3.90[e]	1.87	32.6
6α-F-DHT (**3**)	29.1 ± 16.1	691.0 ± 146	3.24[e]	0.95	30.6
6β-F-DHT (**4**)	1.9 ± 0.7	585.0 ± 85	3.31[e]	1.02	1.9
19-nor-T	30.6 ± 1.5	29.9 ± 6.8	3.17	0.88	34.8
6α-F-19-nor-T (**5**)	41.6 ± 5.7	14.8 ± 9	2.90	0.67	62.1
6β-F-19-nor-T (**6**)	2.8 ± 1.7	1.9 ± 0.5	2.93	0.69	4.1
19-nor-DHT	24.3 ± 0.4	213.0 ± 97	3.85[e]	1.78	13.7
6α-F-19-nor-DHT (**7**)	13.0 ± 1.6	40.8 ± 1.3	3.02[e]	0.76	17.1
6β-F-19-nor-DHT (**8**)	3.1 ± 1.7	32.8 ± 7	3.07[e]	0.80	3.9
R 1881	100	4.0 ± 0.9	3.29	1.00	100

[a]Relative binding affinities (RBA) were determined in a competitive radiometric assay; details of assay are described in the Experimental section. Values are expressed as percentages relative to the affinity of the indicated tritium-labeled tracer and are the average of two or more determinations ± standard deviation. [b]Log $P_{o/w}$ (log of octanol/water partition coefficient) was measured according to Minick et al.[24] [c]Non-specific Binding (NSB) was calculated according to the equation: log NSB = 0.447 (log $P_{o/w}^{x}$ − log $P_{o/w}^{R\ 1881}$). Thus for R 1881, NSB = 1.[29] [d]Binding Selectivity Index (BSI) = RBA/NSB. Thus for R 1881, BSI = 100.[29] [e]HPLC traces of androgens were monitored at 290 nm, and standard at 254 nm.

The RBA values of androgens and C-6 fluorine-substituted androgens to SBP are also shown in Table 1. Natural androgens bind to SBP with high binding affinity. DHT has an affinity 4 times higher than testosterone, while the 19-nor-androgens have lower binding affinity to SBP than their corresponding 19-CH_3 counterparts. Fluorine substitution at the C-6 position of testosterone and DHT decreases binding affinity but maintains the same relationships seen with the unsubstituted ligands DHT > T > 19-nor-DHT > 19-nor-T. The effect of fluorine stereochemistry on SBP binding is only observed in 19-nor-testosterone: the 6α-epimer binds 8 times tighter than the 6β-epimer. However, when the A-ring of the androgen is saturated (Table 1, **3, 4, 7,** and **8**), there is no preference between the α- and β-epimers for SBP binding.

Octanol-water partition coefficient, non-specific binding, and binding selectivity index

The lipophilicity of substituted estrogens and progestins has been estimated from octanol-water partition coefficients and used to predict their binding to low-affinity, nonspecific sites.[29] Octanol-water partition coefficients (log $P_{o/w}$) of androgens were measured using a reverse-phase HPLC method[23,24] and are given in Table 1. Introduction of a fluorine atom at the C-6 position diminishes log $P_{o/w}$ values, indicating that the fluorine-substituted androgens are less lipophilic than their unsubstituted androgens. An interesting observation is that the lipophilicity of the 6β-epimers is slightly higher than that of the 6α-epimers in all cases (Table 1, **1** and **3–8**). The most non-lipophilic ligand based on log $P_{o/w}$ values is 6α-fluoro-19-nor-testosterone (**5**), which also is the ligand that has the highest RBA among the C-6 fluorine-substituted androgens studied.

Non-specific binding affinity (NSB) of substituted estrogens and progestins has been estimated from the difference in log $P_{o/w}$ values between the binding standards estradiol or R 5020 and the substituted analogs, respectively.[29] When the NSB of the C-6 fluoroandrogens is estimated relative to their corresponding unsubstituted androgens, it decreases by ~50% in the 4,5α-dihydro-androgens, but only by ~25% in the 4-dehydro-androgens. If R 1881 is used as standard for NSB (NSB = 1) and the ratio of RBA to NSB is calculated,[29] 6α-fluoro-19-nor-testosterone (**5**) has the highest ratio, 62 relative to that of R 1881 (100). This ratio has been termed the binding selectivity index (BSI) and has been useful in making correlations between the in vitro binding properties of steroids and their in vivo uptake efficiency and selectivity.[29]

Discussion

Fluorine-substituted androgens appear to be promising as in vivo imaging ligands for AR-positive prostate cancer. Several androgens have been labeled with ^{18}F and have shown selective prostate uptake in rats[6,7]; some of these have even been used to image the prostate in baboons.[8] Ligands for this purpose should have high binding affinity to the AR, high binding selectivity (i.e., a high BSI value), and appropriate metabolic features.[30] Although the last property can be investigated only by tissue distribution studies using animals, the first two properties can be evaluated through structure-affinity studies. In this regard, the effect of fluorine substitution at a number of sites in the androgen structure has been studied: 2α, C-3, 6α, 11β, C-16, 17β or 17α-CH_3.[9,11,31] In most cases, the RBA of androgens to the AR decreases with fluorine substitution. Therefore, it is important to study the effect of fluorine substitution on androgens carefully, in terms of receptor binding affinity and non-specific binding, in order to optimize the behavior of ligands for imaging AR-positive prostate cancer.

In this report, a fluorine atom was introduced at the C-6 position of testosterone, DHT, 19-nor-testosterone, and 19-nor-DHT. The C-6 position is a site where chemical manipulation is feasible, and it is known that fluorine substitution at the C-6 position of progesterone, whose structure is related to these androgens, has little effect on the binding affinity of the derivatives to the progesterone receptor.[32]

For radiochemical syntheses of ^{18}F-labeled steroids, S_N2 displacement using fluoride ion (nBu$_4$NF) is the most commonly used method. However, introduction of a fluorine atom into the C-6 position by S_N2 displacement is limited to the 6β-position of DHT (Scheme 2, **4** and **8**). Unsuccessful attempts were made to obtain the 6α-fluoroandrogens by S_N2 displacement: the 5α-H,6β-alcohol obtained from opening the 5β,6β-epoxide by LAH was activated to its 6β-trifluoromethanesulfonate ester or 6β-methanesulfonate ester; however, both esters had a great tendency toward the elimination under fluoride ion displacement conditions.

Therefore, other synthetic methods were used to prepare the 4,5α-dihydro-6α-epimer and 4-dehydro-6-fluoro-androgens (Scheme 1). The C-6 alcohol was converted to the C-6 fluoroandrogen by DAST: 6α-F-DHT was obtained from the 6α-alcohol **14a,**[18] but the 6β-F rather than the 6α-F epimer was obtained from **14b,** due to the S_N1 character of the reaction with DAST.[17] For the synthesis of 6-fluoro-testosterone and DHT, electrophilic fluorination was carried out on dienol acetate **10** (Scheme 1). Because of the greater thermodynamic stability of the equatorial 6α-epimer, 6β-F-testosterone **2** was obtained in such low yield after fluorination and hydrolysis that it was not available for further studies.

Fluorine substitution at the C-6 position generally lowers the AR binding affinity but has less effect than when the substitution is at C-16 or C-20.[6–9] In fact, two derivatives, 6α-F-T and 6α-F-19-nor-T, have affinities slightly greater than that of the parent compounds. In the testosterone/DHT series (19-CH_3), the RBA of the DHT-based compounds are higher than those of the testosterone-based compounds, whether substituted with C-6 fluorine or not. However, in the 19-nor series, this trend is reversed, and the testosterone-based compounds have higher affinity than their DHT counterparts. This has also been seen in two synthetic androgen series: 5α-dihydro-7α,17α-dimethyl-19-nor-testosterone (5α-dihydro-mibolerone) and 5α-dihydro-7α-methyl-19-nor-testosterone (5α-dihydro-MNT) have lower binding affinities to AR than do their corresponding 19-nor-T counterparts.[33]

For all C-6 fluoroandrogens studied, the α-epimer has the higher RBA to the AR than the β-epimer. This result might be explained by putative hydrogen bonding between the electronegative fluorine atom at the C-6 position and a proton donor of the receptor,[32] this hydrogen bond being stronger when the fluorine atom is in an equatorial position. Alternatively, the slightly larger atomic radius of fluorine versus hydrogen may create steric interference when the fluorine atom is on the β- rather than the α-side of the ligand. In the 4-dehydro-androgens, **1, 2, 5,** and **6,** this result might also be explained by an inductive effect of the fluorine atom at the C-6 position,[34,35] that would decrease the dipole moment of the α,β-unsaturated ketone at the C-3. This effect, which would be stronger with the 6β-fluorine than with the 6α-fluorine atom, would diminish binding affinity if a high charge density on the 3-carbonyl oxygen were required for hydrogen bonding to the receptor.

Sex steroid binding protein recognizes different structural components of the androgen ligand for binding than does AR. Introduction of a fluorine atom at the C-6 position decreases the binding affinities to SBP, but trends seen with the unsubstituted androgens are maintained with the fluorine-substituted ones: DHT-based ligands always have higher affinity than testosterone ligands, and 19-nor compounds always have considerably (average of 15 times) lower affinity than the 19-CH_3 compounds.

The C-6 fluoroandrogens are less lipophilic than their corresponding unsubstituted androgens, and the C-6α epimers are slightly, but consistently, less lipophilic than the C-6β epimers (Table 1). The low AR binding affinity of the β-epimers compared to the α-epimers might be related to their greater lipophilic properties, in addition to their steric/inductive effects on the androgen structure.

All of these indicators suggest that 6α-F-19-nor-testosterone **(5)** is the best candidate as a potential imaging ligand for AR-positive prostate cancer among the C-6 fluorine-substituted androgens studied. It has good AR affinity and low SBP affinity and lipophilicity. However, a new synthesis of this compound that utilizes fluoride ion is needed for it to be available in ^{18}F-labeled form at sufficiently high specific activity for tissue distribution and PET imaging studies.

We have prepared seven androgens substituted with fluorine at C-6, either by nucleophilic substitution or electrophilic fluorination. The effect of a fluorine atom on the C-6 position of the androgen was investigated using their RBA to AR and to SBP and their log $P_{o/w}$ values. Among all C-6 fluorine-substituted androgens studied, 6α-fluoro-19-nor-testosterone **5** shows the most promising properties, high binding affinity for the AR, low binding affinity to SBP, and low lipophilicity.

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