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Fluorinated Androgens and Progestins: Molecular Probes for Androgen and Progesterone Receptors with Potential Use in Positron Emission Tomography

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SUMMARY

In order to develop imaging agents for receptor-positive tumors of the breast and prostate, we have investigated the binding affinity of several fluorine-substituted steroids in the testosterone and nortestosterone series for the androgen receptor and the progesterone receptor. The 6α- and 11β-fluoro-, and 16α-fluoroalkyl-substituted steroids were prepared by an olefin bromofluorination reaction followed by dehydrobromination or reductive debromination. The 17α-fluoromethyl derivatives were prepared by fluoride ion attack on the 17-spiroepoxide or 17-spiro sulfate and the 17α-fluoropropynyl derivative, by reaction of a propargyl alcohol precursor with diethylaminosulfur trifluoride. Of the compounds synthesized, 17α-(3-fluoro-1-propynyl)nortestosterone was found to possess the highest binding affinity and selectivity for the progesterone receptor, and 11β-fluoronordihydrotestosterone had the greatest affinity for the androgen receptor. Both receptor systems seem to tolerate reasonably well the substitution of fluorine for hydrogen.

The assay of progesterone receptor (PR) concentration in breast tumors (1) and androgen receptor (AR) concentration in prostate tumors (2) provides important prognostic information for distinguishing hormone-responsive neoplasms from those that are nonresponsive. In principle, a progestin or androgen labeled at high specific activity with an appropriate radionuclide and having a suitably high affinity and selectivity for PR or AR, respectively, might be used to image receptor-positive tumors and to quantify their receptor content *in vivo*.

Several workers have described the preparation of androgens and progestins labeled with single photon-emitting radionuclides of bromine or iodine (3–6). However, in order to obtain optimal resolution, efficiency, and speed for imaging receptors *in vivo*, it appears preferable to utilize positron-emitting radionuclides, such as fluorine-18, together with positron emission transaxial tomography. A few reports on the development of fluorine-labeled progestins and androgens have appeared (7–9), but the problem has not yet been approached in an organized fashion.

We have begun a systematic investigation of the development of fluorine-substituted androgens and progestins that might be prepared in F-18-labeled form as probes for AR and PR. This report describes the rationale behind our choice of certain candidate fluorinated androgens and progestins in the testosterone and the nor- and 5α-dihydrotestosterone series, and it presents their synthesis and the determination of their binding affinities for AR and PR.

Experimental Procedures

Chemical Materials and Methods

General. Melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were recorded in $CDCl_3$ on Varian EM-390 (90 MHz), XL-200 (200 MHz), HR-220 (220 MHz), or Nicolet NT-360 (360 MHz) spectrometers; chemical shifts are reported as parts per million (δ) downfield from tetramethylsilane. Fluorine magnetic resonance spectra were obtained from a Nicolet NT-360 (338.8 MHz) spectrometer; chemical shifts are reported as parts per million from $CFCl_3$. Infrared spectra were recorded on Perkin-Elmer 137B, 700, or 1320 or Beckman IR-12 spectrophotometers and are reported in units of frequency (cm^{-1}). Low resolution electron impact and high resolution electron impact exact mass determinations were obtained on Finnigan MAT CH-5 and MAT 731 instruments, respectively. Fast atom bombardment (FAB) mass spectra were obtained on a V. G. Instruments ZAB HF spectrometer. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois. Only major diagnostic NMR, IR, and mass spectra signals are reported; all spectra are consistent with the assigned structures.

Silica gel used in flash chromatographic purifications was 32–63 μm

ABBREVIATIONS: PR, progesterone receptor; AR, androgen receptor; FAB, fast atom bombardment; HPLC, high pressure liquid chromatography; THF, tetrahydrofuran; DBH, 5,5-dimethyl-1,3-dibromohydantoin; DMF, dimethylformamide; DAST, diethylaminosulfur trifluoride; EDTA, ethylenediaminetetraacetate; RBA, relative binding affinity; T, testosterone; nor-T, 19-nor-testosterone; DHT, 5α-dihydrotestosterone; nor-DHT, 19-nor-dihydrotestosterone.

(Woelm). Glass-backed plates coated with 0.25-mm silica gel and F-254 indicator (Merck) were used for analytical TLC unless otherwise noted. Preparative layer chromatography was performed on 20 × 20 cm glass plates with a 0.25-mm thickness of Merck Silica Gel 60PR-254. High pressure liquid chromatograph (HPLC) was performed on a Varian model 5060 ternary solvent (hexane/methylene chloride/isopropanol) system equipped with a 7125 Rheodyne loop injector and a Perkin-Elmer LC-75 Spectrophotometric Detector (254 and 275 nm). Altex Spherisorb (3.2 mm × 25 cm) or Varian SI-5 (4 mm × 30 cm) silica gel columns were used for analytical work, and Whatman M-9 10/50 and Alltech silica gel columns were used for preparative HPLC. All compounds involved in binding assays were shown to be >99% pure by HPLC.

Solvents and reagents were obtained from commercial sources (Burdick and Jackson, Baker, Fisher, Mallinckrodt, Aldrich, Sigma, Upjohn, Eastman, or Alfa) and either used directly or purified as deemed necessary by distillation from an appropriate drying agent. Estrone and nortestosterone were obtained as generous gifts from G. D. Searle Co.

Except where mentioned otherwise, a standard procedure was used for product isolations: the reaction mixture was extracted several times with ethyl acetate or dichloromethane, the combined organic extracts were dried over an anhydrous salt (normally sodium sulfate), and the solvent was removed under reduced pressure. All reactions were run under a nitrogen atmosphere unless otherwise noted.

17β-Hydroxy-11β-fluoro-4-androsten-3-one (1) (Ref. 10). A solution of $LiAl(tBuO)_3H$ (32 mg, 0.127 mmol) and dione 24 (30 mg, 0.098 mmol) in 1 ml of THF was stirred 45 min at 0°, then combined with water and acidified with 5% HCl (aqueous). The desired product was obtained in 60% yield following product isolation (ethyl acetate) and purification (30% hexanes in ethyl acetate). Melting point 167–169°; ^{1}H NMR δ 3.53–3.77 (*m*, 1H, 17-H); IR ($CHCl_3$) 3608 (OH), 1665 (C═O, α,β-unsaturated), 1616 (C═C); mass spectrum 306 (73, M^+), 123 (100). Analysis. Calculated for $C_{19}H_{27}O_2F$: C, 74.47; H, 8.88; F, 6.20. Found: C, 74.33; H, 8.91; F, 5.87. HRMS Calculated for $C_{19}H_{27}O_2F$: *m/z* 306.1994. Found: *m/z* 306.1997.

17β-Hydroxy-11β-fluoroandrostan-3-one (2). Ammonia (1.5 ml) was condensed into a flask, and tetrahydrofuran (1 ml) and 4 mg (0.571 mmol) of lithium metal were then added. Next, a solution, cooled to −42°, of testosterone **1** (52 mg, 0.170 mmol) in 1 ml of tetrahydrofuran was added. Excess lithium was added to maintain the initial deep blue color of the reaction. The mixture was stirred 10 min at −42° and ethylene dibromide was then added (blue color discharged). This was followed by the addition of 1 ml of a 4:1 solution of methanol in acetic acid and evaporation at room temperature of the ammonia. Product isolation [methylene chloride; combined organic extracts washed with 5% HCl (aqueous) to neutrality] and purification (30% hexanes in ethyl acetate) afforded dihydrotestosterone **2** in 51% yield. Melting point 199–202° ^{1}H NMR δ 0.80–2.60 (*m*, 20H, backbone, 17-OH); IR (KBr) 3406 (OH), 1707 (C═O); mass spectrum 308 (7, M^+), 124 (100). HRMS Calculated for $C_{19}H_{29}O_2F$: *m/z* 308.2152. Found: *m/z* 308.2152.

11β-Fluoroestran-17β-ol (3). A benzene solution of dihalo **31**, tributyltin hydride (2.5 eq), and a crystal of AIBN was refluxed for 6 hr, then concentrated and purified (40% hexane/60% ethyl acetate), yielding the desired product as a white solid with melting point 162–168°. ^{1}H NMR 0.65–2.60 (*m*, 22H, backbone, 17-OH), 4.98 (*dq*, *J* = 49, 3 Hz, 1H, 11-H); IR (neat) 3412 (OH), 1711 (C═O), mass spectrum 294 (22, M^+), 274 (100). HRMS Calculated for $C_{18}H_{27}O_2F$: *m/z* 294.1995. Found *m/z* 294.1996.

17α-Ethynyl-17β-hydroxy-11β-fluoro-4-androsten-3-one (4). Deprotection of diene **26** (36 mg, 0.105 mmol) was effected by dissolving the compound in 2 ml of methanol and adding 15 drops of 5% HCl (aqueous). The resulting heterogeneous mixture was stirred 1 hr at room temperature and then transferred to a conical test tube and centrifuged. The reaction solvent was decanted and a small portion of ethyl acetate was added to rinse the solid product. This mixture was also centrifuged, the ethyl acetate was decanted, and the product dried. Fluoroethisterone **4** was obtained in 70% yield (24 mg). Melting point 252–254°; ^{1}H NMR 2.60 (*s*, 1H, —C≡CH); IR (KBr) 3400 (OH), 1653 (C═O); mass spectrum 330 (60, M^+), 91 (100). Analysis. Calculated for $C_{21}H_{27}O_2F$: C, 76.32; H, 8.24; F, 5.75. Found: C, 76.39; H, 8.32; F, 5.94.

17β-Hydroxy-6α-fluoro-4-androsten-3-one (5) (Ref. 11). Dione **35** (284 mg, 0.93 mmol) was dissolved in tetrahydrofuran and $LiAl(tBuO)_3H$ (236 mg, 0.93 mmol) was added. After 45 min, water and 5% HCl (aqueous) were added to the reaction. Product isolation (ethyl acetate) and purification (20% hexane/80% ethyl acetate) afforded 160 mg (56%) of androstenone **5** as a white solid with melting point 168–172° (literature 164–166°). ^{1}H-NMR 0.73–2.60 (*m*, 18H, backbone, 17-OH), 3.67 (*bt*, *J* = 8 Hz, 1H, 17-H), IR(KBr) 3500 (OH), 1669 (C═O); mass spectrum 306 (13, M^+), 142 (100).

17α-Ethynyl-17β-hydroxy-6α-fluoro-4-androsten-3-one (6) (Ref. 12). The addition of $CDCl_3$ to diene **37**, a yellow oil, resulted in the oil turning a darker yellow and solidifying. The deuterated chloroform was removed under pressure and the residue obtained was purified (40% ethyl acetate in hexanes), affording acetylide **6** in low yield. Melting point 208–217° (literature 237–239°); ^{1}H NMR δ 2.60 (*s*, 1H, —C≡CH), 5.10 (*dddd*, *J* = 49, 12, 6, 2 Hz, 1H, 6-H), 6.10 (s,1H, 4-H); IR (KBr) 3410 (OH), 3255, 1656 (C═O); mass spectrum 330 (26, M^+), 41 (100).

17β-Hydroxy-16α-(2′-fluoropropyl)-4-estren-3-one (7) and (8). Liquid ammonia was added to a solution of estratriene **102** (280 mg, 0.809 mmol) in tetrahydrofuran at −40°; this was followed by the addition of 40–50 eq of lithium metal. The mixture was stirred for 2 hr and ethanol was added. The ammonia was evaporated and the presumed dienol ether intermediate was isolated (methylene chloride). Hydrolysis of the dienol ether was effected by warming it in methanol with 3 N HCl at 60° for 20 min. Product isolation and purification (30% hexanes in ethyl acetate) afforded product in 67% yield (182 mg) as a mixture of diastereomers separable by HPLC. ^{1}H NMR (mixture of diastereomers) 0.68–2.64 (*m*, 25H, backbone, 17-OH, —CH_2CHFCH_3), 5.81 (*bs*, 1H, 4-H). **7:** IR (neat) 3409 (OH), 1661 (C═O), 1616 (C═C); mass spectrum 334 (81, M^+), 110 (100). HRMS Calculated for $C_{21}H_{31}O_2F$: *m/z* 334.2308. Found: *m/z* 334.2309. **8:** IR (neat) 3418 (OH), 1661 (C═O), 1616 (C═C); mass spectrum 334 (77, M^+). HRMS Calculated for $C_{21}H_{31}O_2F$: *m/z* 334.2308. Found: *m/z* 334.2306.

17α-Fluoromethyl-17β-hydroxy-4-androsten-3-one (9). Dioxolane **49** (40 mg, 0.12 mmol) was dissolved in 2 ml of acetone and two drops of concentrated HCl were added. After 15 min, saturated sodium bicarbonate was added to the solution, and the acetone was evaporated. Product isolation (ethyl acetate) and purification (neutral alumina, 50% hexanes in ethyl acetate) gave 36 mg (94%) of fluoromethyl **9** as a white solid. Melting point 152–155°; ^{1}H NMR δ 0.72–2.60 (*m*, 20H, backbone, 17-OH), 5.72 (*bs*, 1H, 4-H); IR (KBr) 3385 (OH), 1653 (C═O), 1610 (C═C); mass spectrum 320 (38, M^+), 124 (100). Analysis. Calculated for $C_{20}H_{29}O_2F$: C, 74.96; H, 9.12; F, 5.90. Found: C, 75.29; H, 9.01; F, 6.12.

17α-Fluoromethyl-17β-hydroxy-4-estren-3-one (10). This compound was prepared in the same manner as nortestosterones **7** and **8**. A solution of estratriene **53** (80 mg, 0.25 mmol) and lithium (11.4 mmol) in 6 ml of tetrahydrofuran and 15–20 ml of ammonia was stirred for 1 hr before the addition of ethanol. The intermediate dienol ether isolated was stirred with 2.5 ml of concentrated HCl in a mixture of THF and water for 25 min at room temperature (HCl added to steroid solution at 0°). Product isolation (ethyl acetate; organic extracts washed with saturated sodium bicarbonate to neutrality) and purification (40% hexanes in ethyl acetate) afforded 31 mg (40%) of desired product. An analytical sample was prepared by recrystallization from ethyl acetate/hexanes. Melting point 160–162°; ^{1}H NMR δ 0.74–2.58 (m, 21H, backbone, 17-OH), 5.84 (*bs*, 1H, 4-H); IR 3418 (OH), 1663 (C═O); mass spectrum 306 (44, M^+), 91 (100). Analysis. Calculated for $C_{19}H_{27}O_2F$: C, 74.47; H, 8.88; F, 6.20. Found: C, 74.13; H, 8.77; F, 6.16.

17α-Fluoromethyl-17β-hydroxyestran-3-one (11). Fluoromethyl **11** was prepared using a procedure similar to that described for the synthesis of androstenone **2**. Ammonia (15–20 ml) was condensed

into a solution of 58 mg (0.190 mmol) of estrenone **10** in 8 ml of tetrahydrofuran at −42°. Lithium wire (40–50 mg) was then added and the reaction was stirred 45 min, when it was quenched with ethylene dibromide. After the addition of methanol/acetic acid (6 ml) and ammonia evaporation, dilute HCl was added to bring the pH of the solution to 6–7. The crude product was isolated and purified (50% hexanes in ethyl acetate) to afford 24 mg (41%) of ketone **11** as a white solid with melting point 144–147°. ^{1}H NMR δ 0.78–2.73 (*m*, 24H, backbone, 17-OH); IR (KBr) 3441 (OH), 1702 (C═O); mass spectrum 308 (14, M$^+$), 41 (100). Analysis. Calculated for $C_{19}H_{29}O_2F$: C, 73.99; H, 9.48; F, 6.16. Found: C, 73.81; H, 9.51; F, 6.02.

17α-(3′-Fluoropropynyl)-17β-hydroxy-4-estren-3-one (12) (Ref. 13). To a small volume of methanol containing a few drops of water was added 110 mg (0.296 mmol) of acetate **59** and 5 eq of potassium carbonate. The homogeneous mixture was stirred 6 hr at room temperature, and the product was then isolated (methylene chloride) and purified (40% hexanes in ethyl acetate) to afford 66 mg (68%) of fluoride **12** as an off-white solid. Melting point 159–162°; ^{1}H NMR δ 0.66–2.72 (*m*, 21H, backbone, 17-OH), IR (neat) 3375 (OH), 1659 (C═O); mass spectrum 330 (13, M$^+$), 91 (100). Analysis. Calculated for $C_{21}H_{27}O_2F$: C, 76.34; H, 8.24; F, 5.75. Found: C, 76.07; H, 8.10; F, 6.01.

11β-Fluoro-9α-bromo-4-androsten-3,17-dione (23) (Ref. 10). To a polyethylene container was added dimethyldibromohydantoin (DBH) (56 mg, 0.36 mmol) followed by CH_2Cl_2 (1 ml) and HF·pyridine (34.8 μl, 2.10 mmol HF). Diene **22** (102 mg, 0.36 mmol) was then added, and the solution was stirred for 1 hr and then quenched by slow addition of saturated $NaHCO_3$ (aqueous). Following product isolation (CH_2Cl_2), purification (40% hexanes in ethyl acetate) of the crude material gave 110 mg (82%) of dihalo **23**. Melting point 164–166°; ^{1}H NMR δ 1.04 (*d*, *J* = 3 Hz, 3H, 18-methyl), 1.66 (*d*, *J* = 4 Hz, 3H, 19-methyl), 5.25 (*dt*, *J* = 47, 3 Hz, 1H, 11-H); IR ($CHCl_3$) 1740 (C═O), 1669 (C═O, α,β-unsaturated) cm^{-1}; mass spectrum (FAB) *m/z* 385 (M$^+$ + 2), 383 (M$^+$).

11β-Fluoro-4-androsten-3,17-dione (24)(Ref. 10). Fluoroandrostenone **24** was synthesized following the procedure employed in the preparation of fluoroestranol **3**. Thus, from 0.89 mmol of dihalo **23** was obtained, after purification (35% hexanes in ethyl acetate), fluorodione **24** in 70% yield. ^{1}H NMR δ 0.82–2.63 (*m*, 17H, backbone), 5.17 (*dq*, *J* = 48, 3 Hz, 1H, 11-H); IR ($CHCl_3$) 1740 (C═O), 1667 (C═O, α,β-unsaturated); mass spectrum 304 (76, M$^+$), 41 (100). Analysis. Calculated for $C_{19}H_{25}O_2F$: C, 74.97; H, 8.28; F, 6.24. Found: C, 74.60; H, 8.23; F, 6.46. HRMS Calculated for $C_{19}H_{25}O_2F$: *m/z* 304.1842. Found: *m/z* 304.1840.

11β-Fluoro-3-methoxy-3,5-androstadien-17-one (25). Dione **24** (50 mg, 0.164 mmol) was dissolved in 250 μl of DMF and 250 μl of 2,2-dimethoxypropane. Methanol (10 μl) and *p*-toluenesulphonic acid (1.3 mg, 6.84 μmol) were added. The resulting mixture was refluxed 2.5 hr, then cooled and quenched with saturated sodium bicarbonate (aqueous). The product was isolated (ethyl acetate) and purified (25% ethyl acetate in hexane) to give 36 mg (68%) of the desired dienone. Melting point 149–154°; ^{1}H NMR δ 0.70–2.70 (*m*, 15H, backbone), 3.58 (*s*, 3H, 3-methoxy), 5.11 (*bs*, 1H, 4-H), 5.18 (*t*, *J* = 4 Hz, 1H, 6-H); IR (KBr) 1733 (C═O), 1623 and 1649 (C═C); 318 (100, M$^+$). HRMS Calculated for $C_{20}H_{27}O_2F$: *m/z* 318.1995. Found: *m/z* 318.1990.

17α-Ethynyl-11β-fluoro-3-methoxy-3,5-androstadien-17β-ol (26). Acetylide **26** was prepared in 99% yield from androstadienone **25** (34 mg, 0.107 mmol), following the procedure described for the synthesis of acetylide **37**; ^{1}H NMR δ 0.71–2.57 (*m*, 16H, backbone, 17-OH), 2.59 (*s*, 1H, —C≡CH); IR (neat) 3451 (OH), 1653 and 1630 (C═C); mass spectrum 344 (25, M$^+$), 43 (100). HRMS Calculated for $C_{22}H_{29}O_2F$: 344.2152. Found: 344.2155.

11β-Hydroxyestran-3,17-dione (28). Dione **27** (100 mg, 0.347 mmol) was dissolved in 4–5 ml of ethyl acetate, and 20 mg of 5% palladium on carbon were added. The mixture was stirred for 3 hr under approximately 1 atmosphere of hydrogen and then purified (30% hexanes in ethyl acetate) directly. The desired product was obtained in 91% yield as a 4:3 mixture of 5β- and 5α-epimers. Melting point 179–203°; ^{1}H NMR δ 0.70–2.66 (*m*, 22H, backbone, 11-OH); IR (KBr) 3490 (OH), 1730 (C═O, 5-membered ring), 1700 (C═O, 6-membered ring); mass spectrum 290 (63, M$^+$), 41 (100). HRMS Calculated for $C_{18}H_{26}O_3$: *m/z* 290.1882. Found: *m/z* 290.1883.

9(11)-Estren-3,17-dione (29). Pyridine (2 ml) and DMF (2 ml) were added to 71 mg (0.245 mmol) of dione **28**. Methanesulfonyl chloride (71 μl, 0.93 mmol) was added to this solution and the resulting mixture was heated at 80–85° for 1 hr. At this time, 3.8 eq more of MsCl were added and the reaction was heated another hour. The dark brown solution was cooled and diluted with water and aqueous $CuSO_4$. Product isolation (methylene chloride; combined organic extracts washed with water) and purification (40% hexanes in ethyl acetate) afforded the dione in 71% yield as a 4:3 mixture of 5β- and 5α-epimers. ^{1}H NMR δ 0.73–2.75 (*m*, 20H, backbone), 5.33–5.44 (*m*, 1H, 11-H of 5α-epimer), 5.55–5.65 (*m*, 1H, 11-H of 5β-epimer); IR (neat) 1736 (C═O, 5-membered ring), 1711 (C═O, 6-membered ring); mass spectrum 272 (50, M$^+$), 105 (100). HRMS Calculated for $C_{18}H_{24}O_2$: *m/z* 272.1776. Found: *m/z* 272.1780.

11β-Fluoro-9α-bromoestran-3,17-dione (30). Dihalo **30** was prepared in 90% yield from alkene **29** using the procedure described for the synthesis of androstenedione **23**. ^{1}H NMR δ 1.02 (*d*, *J* = 2 Hz, 3H, 18-CH_3), 5.13 (*dt*, *J* = 47, 3 Hz, 1H, 11-H); IR (CCl_4) 1748 (C═O, 5-membered ring), 1723 (C═O, 6-membered ring); mass spectrum (FAB$^+$) *m/z* 373 (M$^+$ + 2), 371 (M$^+$). HRMS Calculated for $C_{18}H_{25}O_2FBr$: *m/z* 371.1022. Found: *m/z* 371.1026.

17β-Hydroxy-11β-fluoro-9α-bromoestran-3-one (31). To 1 ml of a 0.04 M methanolic solution of $ErCl_3 \cdot 6H_2O$ was added 36 mg (0.097 mmol) of estranedione **30**, followed by 73.7 μl (0.679 mmol) of trimethyl orthoformate. The solution was stirred for 20 min at room temperature, and then $NaBH_4$ (7.2 mg, 0.189 mmol) was added, causing the mixture to effervesce. After the reaction had been stirred 15–30 min, a small quantity of 5% aqueous HCl was added and the solution was stirred another 30 min. Product isolation (methylene chloride) and purification (50% hexanes in ethyl acetate) afforded estranol **31** as an oil in 58% yield. ^{1}H NMR δ 0.70–2.60 (*m*, 22H, backbone, 17-OH), 3.77 (*bt*, *J* = 8 Hz, 1H, 17-H); IR (neat) 3331 (OH), 1709 (C═O); mass spectrum (FAB$^+$) *m/z* 375 (M$^+$ + 2), 373 (M$^+$). The molecular ion signal was too weak for HRMS.

5α-Bromo-6β-fluoro-3β,17β-androstanediol (33) and 5α-fluoro-6β-bromo-3β,17β-androstanediol (34). Dihalo **33** and **34** were synthesized in the same manner as dione **23**. Androstenediol (500 mg, 1.72 mmol) was stirred with DBH (271 mg, 0.946 mmol) and 240 μl (8.60 mmol) of HF·pyridine in methylene chloride for 30 min. Purification of the crude product (20% hexanes in ethyl acetate) resulted in the isolation of 410 mg (61%) of the isomeric dihalides **33** and **34**. ^{1}H NMR (mixture of isomers) δ 0.75 and 0.79 (2*s*, 3H, 18-CH_3), 1.24 (*d*, *J* = 6 Hz, 3H, 19-CH_3 of **33**), 1.32 (*s*, 3H, 19-CH_3 of **34**), 4.04–4.14 (*m*, 1H, 6-H of **34**), 4.82 (*dt*, *J* = 48, 3 Hz, 1H, 6-H of **33**); IR (KBr) 3410 (OH). The molecular ion signal was too weak for HRMS.

6α-Fluoro-4-androsten-3,17-dione (35) (Ref. 11). A solution of diols **33** and **34** in acetone was cooled to 0° and Jones reagent (H_2CrO_4/H_2SO_4) was added in excess as indicated by the orange color of the solution. The reaction was stirred 1 hr at room temperature and then aqueous sodium bisulfite and water were added. After the oxidation product was isolated (ethyl acetate) as a colorless foam, it was placed under high vacuum for 2–3 days, during which time it changed to a red-brown solid. The material was purified (40% hexanes in ethyl acetate) and dione **35** was isolated in 52% yield. Melting point 183–188° (literature 229–231°); ^{1}H NMR δ 0.92 (*s*, 3H, 18-CH_3), 1.21 (*s*, 3H, 19-CH_3), 0.84–2.62 (*m*, 17H, backbone), 5.13 (*dddd*, *J* = 48, 12, 6, 2 Hz, 1H, 6-H), 6.10 (*bs*, 1H, 4-H); IR (CCl_4) 1744 (C═O, 5-membered ring), 1690 (C═O, α-β-unsaturated); mass spectrum 304 (78, M$^+$), 41 (100).

6-Fluoro-3-methoxy-3,5-androstadien-17-one (36). Dienone **36** was prepared following the procedure for the synthesis of **25**.

Purification of the crude product (10:90:1 ethyl acetate/hexanes/triethylamine) afforded **36** as a white solid in 63% yield (135 mg). Melting point 160–168°; ^{1}H NMR δ 0.78–2.66 (*m*, 17H, backbone), 3.61 (*s*, 3H, 3-methoxy), 5.49 (*bs*, 1H, 4-H); IR (CCl_4), 1744 (C═O); mass spectrum 318 (100, M^+). Analysis. Calculated for $C_{20}H_{27}O_2F$: C, 75.44; H, 8.55; F, 5.97. Found: C, 75.55; H, 8.72; F, 5.98.

17α-Ethynyl-6-fluoro-3-methoxy-3,5-androstadien-17β-ol (37). To 1 ml of pentane cooled to 0° was added 178 μl (1.26 mmol) of (trimethylsilyl) acetylene. Butyllithium (422 μl, 0.629 mmol) was added dropwise, and a few drops of THF were subsequently added to dissolve the precipitated acetylide. A solution of diene **36** (50 mg, 0.157 mmol) in 500 μl of THF was added, and the mixture was stirred at 0° for 30 min. The reaction was then quenched with saturated aqueous NH_4Cl and the intermediate silyl acetylide was isolated (ethyl acetate). The resulting colorless oil was redissolved in 3 ml of methanol and 282 μl (1.41 mmol) of a 5 N KOH solution were added. The mixture was heated at 66–68° for 30 min, cooled, and quenched with saturated NH_4Cl. This product was isolated (ethyl acetate), and the residue was redissolved in ethyl acetate, filtered through neutral alumina, and concentrated to give 96% of acetylide **37** as an oil which solidified upon standing. Melting point 156–159°; ^{1}H NMR δ 0.72–2.52 (*m*, 18H, backbone, 17-OH), 2.57 (*s*, 1H, —C≡CH); IR (neat) 3478 (OH), 1634 (C═C); mass spectrum 344 (66, M^+), 43 (100). Analysis. Calculated for $C_{22}H_{29}O_2F$: C, 76.71; H, 8.49; F, 5.52. Found: C, 76.43; H, 8.59; F, 5.36.

16α-(2′-Propenyl)-3-methoxyestra-1,3,5(10)-trien-17-one (39). To 0.511 mmol of a freshly prepared LDA solution (1.6 eq of diisopropylamine in tetrahydrofuran added dropwise to 1 eq of butyllithium at −78°) at room temperature was added 150 mg (0.528 mmol) of estrone. The resulting mixture was stirred 40 min, then cooled to −45°. Allyl bromide (50 μl, 0.579 mmol) was then added and the solution was stirred for 9 hr, at which time another 25 μl of allyl bromide was added. After being stirred overnight at temperatures slowly rising from −45° to ambient, the reaction was quenched with saturated aqueous NH_4Cl and the product was isolated (ethyl acetate). Following purification (10% ether/90% hexanes), 126 mg (74%) of allylestrone methyl ether **39** were isolated as an inseparable 2:1 (NMR) mixture of C-16 epimers. Melting point 87.5–89.5°; ^{1}H-NMR δ 0.87 and 0.95 (2*s*, 3H, 18-CH_3), 1.08–2.72 (*m*, 14H, backbone, —CH_2CH═CH_2), 5.08–5.25 (m, 2H, —CH_2CH═CH_2), 5.65–5.92 (*m*, 1H, —CH_2C*H*═CH_2); IR (CCl_4) 1740 (C═O), 1614 (C═C); mass spectrum 324 (100, M^+). Analysis. Calculated for $C_{22}H_{28}O_2$: C, 81.44; H, 8.70. Found: C, 81.50; H, 8.75.

16α-(2′-Propenyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol (40). To a solution of estrone **39** (1.22 g, 3.77 mmol) in THF at 0° was added 215 mg (5.65 mmol) of lithium aluminum hydride. The resulting mixture was stirred for 1.5 hr and the reaction then was quenched by slow addition of water followed by 5% HCl (aqueous). Product isolation [ethyl acetate; organic layer washed with saturated $NaHCO_3$ (aqueous)] and purification (30% ethyl acetate in hexanes) afforded 794 mg (65%) of **40**, which could be recrystallized from hexane. Melting point 77.5–78.5°; ^{1}H NMR δ 1.00–3.10 (*m*, 17H, backbone, —CH_2CH═CH_2, 17-OH), 3.30 (*d*, J = 6 Hz, 1H, 17-H); IR (neat) 3383 (OH), 1610 (C═C); mass spectrum 326 (100, M^+). Analysis. Calculated for $C_{22}H_{30}O_2$: C, 80.94; H, 9.26. Found: C, 81.05; H, 9.46.

16α-(2′-Propenyl)-estra-1,3,5(10)-trien-3,17β-diol (41). Sodium thiophenol (2.29 g, 20.8 mmol) was slowly added to potassium *t*-butoxide (2.33, 20.8 mmol) in a flask cooled to 0°. The resulting slurry was dissolved upon the subsequent addition of diethylene glycol. Estratriene **40** (600 mg, 1.84 mmol) was then added and the solution refluxed for 24 hr. The reaction was stirred for several hours at room temperature, and the product was isolated (diethyl ether) and purified to give diol **41** in 80% yield. Melting point 80° (material melts at this temperature, then solidifies) and 164–167°. ^{1}H NMR δ 7.20 (*s*, 1H, 3-OH); IR (neat) 3333 (OH), 1610 (C═C); mass spectrum 312 (100, M^+). Analysis. Calculated for $C_{21}H_{28}O_2$: C, 80.73; H, 9.03. Found: C, 80.43; H, 8.84.

16α-(2′-Propenyl)-3-trifluoromethanesulfonyloxyestra-1,3,5(10)-trien-17β-ol (42). Diol **41** (265 mg, 0.85 mmol) was dissolved in methylene chloride and 688 μl (5.95 mmol) of 2,6-lutidine were added. The solution was cooled to 0° and triflic anhydride (200 μl, 1.19 mmol) was added. After 30 min at 0°, the mixture was quenched with water, and the product was isolated (methylene chloride; organic phase washed several times with aqueous $CuSO_4$ solution) and purified (35% ethyl acetate in hexanes) to give 308 mg (81%) of desired product as an oil which partially solidified upon standing. IR (neat) 3372 (OH), 1603 (C═C), 1140 (S═O), 920 (S—O—C); mass spectrum 444 (4, M^+), 43 (100). Analysis. Calculated for $C_{22}H_{27}O_4F_3S$: C, 59.44; H, 6.12; F, 12.82; S, 7.21. Found: C, 59.71; H, 6.18; F, 12.62; S, 7.22.

16α-(2′-Fluoro-3′-bromopropyl)-3-trifluoromethanesulfonyloxyestra-1,3,5(10)-trien-17β-ol (43). The bromofluorination of allylestratriene **42** was conducted in a manner similar to that employed in the preparation of dihalo **33** and **34**. Purification of the crude product (35% ethyl acetate in hexanes) afforded 44 mg (40%) of dihalo **43**, an oil, obtained as a mixture of diastereomers. ^{1}H NMR 3.55 (*dd*, J = 20, 5 Hz, 2H, —CH_2Br), 4.58–5.11 (*m*, 1H, —C*H*FCH_2Br); mass spectrum (FAB$^+$) *m/z* 543 (M^+). Analysis. Calculated for $C_{22}H_{27}O_4SF_4Br$: C, 48.63; H, 5.01; S, 5.90; F, 13.98; Br, 14.70. Found: C, 48.45; H, 5.05; S, 6.08; F, 13.67; Br, 14.83.

16α-(2′-Fluoro-3′-bromopropyl)estra-1,3,5(10)-trien-3,17β-diol (44). To a solution of triflate **43** (246 mg, 0.453 mmol) in tetrahydrofuran was added 10 eq (172 mg) of lithium aluminum hydride. The reaction was stirred 1.3 hr at 0° and then quenched with water. Aqueous 5% HCl was added until the precipitated aluminum salts had dissolved, and the product was then isolated (ethyl acetate) and purified (40% ethyl acetate in 60% hexanes) to yield estradiol **44**, a colorless oil, in 79% yield. ^{1}H NMR 4.80 (*bs*, 1H, 3-OH); mass spectrum (FAB$^+$) 413 (M^++2), 412 (M^++1), 411 (M^+), 410 (M^+−1). Analysis. Calculated for $C_{21}H_{28}O_2FBr$: C, 61.32; H, 6.86; F, 4.62. Found: C, 61.24; H, 6.91; F, 4.66.

16α-(2′-Fluoropropyl)estra-1,3,5(10)-trien-3,17β-diol (45). Fluoroestradiol **45** was synthesized using the procedure described for the preparation of fluoroestranol **3**. The crude material obtained was flash chromatographed twice (40% ethyl acetate/60% hexane) in order to separate the products from residual Bu_3SnH. Fluoropropyl **45** was isolated in 82% yield as a mixture of diastereomers. ^{1}H NMR 1.10–2.40 (*m*, 18H, backbone, 17-OH, —CH_2CHFCH_3), 4.53–5.09 (*m*, 1H, —CH_2C*H*FCH_3); mass spectrum 332 (3, M^+), 43 (100). HRMS Calculated for $C_{21}H_{29}O_2F$ (one diastereomer): *m/z* 332.2152. Found: *m/z* 332.2156.

16α-(2′-Fluoropropyl)-3-methoxyestra-1,3,5(10)trien-3,17β-diol (46). To a solution of estradiol **45** (491 mg, 1.48 mmol) in DMF was added potassium carbonate (1.02 g, 7.40 mmol) and methyl iodide (184 μl, 2.96 mmol). The mixture was stirred at room temperature for 60 hr and, over the course of the reaction, several 184-μl portions of MeI were added. Following product isolation (methylene chloride and ethyl acetate; organic layer washed several times with water to remove residual DMF) and purification (30% ethyl acetate in hexanes), ether **46** was obtained in 60% yield (309 mg) as a light yellow oil. ^{1}H NMR δ 3.78 (s, 3H, 3-methoxy); mass spectrum 346 (2, M^+), 43 (100). HRMS Calculated for $C_{22}H_{31}O_2F$: *m/z* 346.2308. Found: *m/z* 346.2301.

3,3-Ethylenedioxy-5-androsten-17-one (47) (Ref. 14)**.** Ethylene glycol (110 mg, 1.77 mmol) was added to a solution of androstenedione (500 mg, 1.77 mmol) in 35 ml of benzene. *p*-Toluenesulphonic acid (1.5 mg) was added, and the resulting clear mixture refluxed for 2.5 hr, with water being removed via azeotropic distillation using a Dean-Stark trap. The reaction was quenched with water, and the organic phase was washed with saturated $NaHCO_3$ (aqueous), dried, and concentrated. Purification of the crude product (40% ethyl acetate in hexanes containing 0.1% triethylamine) afforded dioxolane **47** (229 mg, 60% yield based on recovered starting material) as a white solid. Melting point 196–198° (literature 185–192°); ^{1}H NMR δ 3.88–4.02 (m, 4H, —OCH_2CH_2O—), 5.35–5.45 (*m*, 1H, C-6); IR (KBr) 1740 (C═O); mass spectrum 330 (0.41, M^+), 99 (100).

17*S*-Spiro-2′-(1′-oxacyclopropane)-3,3-ethylenedioxy-5-androstene (48). Sodium hydride in 50% oil dispersion (86 mg, 1.81

mmol) was rinsed with hexane and the hexane was decanted. DMSO (3 ml) was added and the mixture was stirred at 75° for 45 min. The anion mixture was diluted with 3 ml of THF and cooled to 0°; then, a solution of trimethylsulfonium iodide (369 mg, 1.18 mmol) in 3 ml of DMSO was added dropwise. The reaction was stirred at 0° for 10 min. Next, dioxolane **47** (75 mg, 0.22 mmol) was dissolved in 10 ml of THF, and this solution was added to the reaction mixture, which was subsequently stirred at 0° for 2.5 hr. Water was added to quench the reaction, and the product was isolated (ethyl acetate) and purified (33% ethyl acetate in hexane containing 1% triethylamine), yielding epoxide **48** (73 mg, 93% yield) as a white solid with melting point 163–169°. ^{1}H NMR δ 2.60 (*d*, J = 5 Hz, 1H, H_a of epoxide methylene), 2.89 (*d*, J = 5 Hz, 1H, H_b of epoxide methylene); mass spectrum 344 (0.60, M^+), 99 (100). Analysis. Calculated for $C_{22}H_{32}O_2$: C, 76.70; H, 9.36. Found: C, 76.63; H, 9.40.

17α-Fluoromethyl-17β-hydroxy-3,3-ethylenedioxy-5-androstene (49). Potassium hydrogen bifluoride (198 mg, 2.54 mmol) was added to a solution of epoxide **48** (125 mg, 0.36 mmol) in 3 ml of diethylene glycol. The solution was stirred at 130° in a closed vessel for 4 hr and then quenched with water. Product isolation (ethyl acetate; organic phase washed with dilute NaCl) and purification (30% hexane in ether with 0.1% triethylamine) afforded fluoromethyl **49** as a white solid (10.4 mg, 8%). Melting point 146–149°; ^{1}H NMR δ 0.88–2.67 (m, 20H, backbone, 17-OH), 4.26 (*dd*, J = 60, 9 Hz, 1H, H_a of —CH_2F), 4.50 (*dd*, J = 60, 9 Hz, 1H, H_b of — CH_2F); IR (KBr) 3430 (OH); mass spectrum 364 (M^+) (offscale), 43 (100). HRMS Calculated for $C_{22}H_{33}O_3F$: m/z 364.2414. Found: m/z 364.2414.

17*S*-Spiro-3′-(1′-oxo-2′,5′-dioxa-1′-thiacyclopentane)-3-methoxyestra-1,3,5(10)-triene (51). Diol **50** (75 mg, 0.24 mmol) was dissolved in a small portion of THF and the resulting solution was cooled to 0°. Thionyl chloride (86.5 μl, 1.18 mmol) was added and the mixture was stirred at 0° for 3 hr. At this time, solvent and excess thionyl chloride were removed *in vacuo*, and the product was isolated [ethyl acetate; organic layer washed with saturated $NaHCO_3$ (aqueous)] and purified by filtration through neutral alumina. The desired product was obtained in 95% yield as a mixture of diastereomers. Melting point (one diastereomer) 177–179°; ^{1}H NMR (diastereomeric mixture) δ 0.99 and 1.07 (2*s*, 3H, 18-methyls), 1.20–3.05 (*m*, 15H, backbone), 4.00 (*d*, J = 9 Hz, 1H) and 4.77 (*d*, J = 9 Hz, 1H): H_a and H_b of cyclic sulfite methylene, diastereomer A, 4.30 (*d*, J = 9 Hz, 1H) and 4.59 (*d*, J = 9 Hz, 1H): H_a and H_b of cyclic sulfite methylene, diastereomer B; IR (Nujol) 1209 (S═O), 962 (S—O—C); mass spectrum 362 (100, M^+). Analysis. Calculated for $C_{20}H_{26}O_4S$: C, 66.27; H, 7.23; S, 8.84. Found: C, 66.13; H, 7.18; S, 8.90.

17*S*-Spiro-3′-(1′,1′-dioxo-2′,5′-dioxa-1′-thiacyclopentane)-3-methoxyestra-1,3,5(10)-triene (52). Cyclic sulfite **51** (60 mg, 0.44 mmol) was dissolved in 2 ml of CCl_4. Acetonitrile (2 ml) and 3 ml of H_2O were added to the solution, followed by 385 mg (1.80 mmol) of $NaIO_4$. The resulting mixture was cooled to 0° and $RuCl_3 \cdot 3H_2O$ (25 mg, 0.01 mmol) was added. The reaction mixture was stirred for 30 min at 0°, then diluted with water. The product isolated (methylene chloride) was filtered through silica gel with ethyl acetate and the material (126 mg, 76%) was further purified by recrystallization from ethyl acetate. Melting point 99–102° (decomposes); ^{1}H NMR 4.40 (*d*, J = 9 Hz, 1H, H_a of cyclic sulfate methylene), 4.77 (*d*, J = 9 Hz, 1H, H_b of cyclic sulfate methylene); mass spectrum 378 (M^+) (offscale), 278 (100). Analysis. Calculated for $C_{20}H_{26}O_5S$: C, 63.47; H, 6.92; S, 8.47. Found: C, 63.55; H, 7.13; S, 8.24. HRMS Calculated for $C_{20}H_{26}O_5S$: m/z 378.1467. Found: m/z 378.1484.

17α-Fluoromethyl-17β-hydroxy-3-methoxyestra-1,3,5(10)-triene (53). To a solution of sulfate **28** (1.00 g, 2.64 mmol) in THF at room temperature was added 13.2 ml of a solution of tetrabutylammonium fluoride in tetrahydrofuran (1 M). The resulting mixture was stirred for 1 hr at 50°, then cooled, and 2 N methanolic HCl was added. After 10 min at room temperature, the reaction was diluted with water and saturated $NaHCO_3$ (aqueous). The desired product was isolated (ethyl acetate) in 70% yield. Melting point 88–92°; ^{1}H NMR δ 1.10–3.02 (*m*, 16H, backbone, 17-OH), 4.43 (*dq*, J = 48, 9 Hz, 2H, —CH_2F); IR ($CHCl_3$) 3570 (OH); mass spectrum 318 (100, M^+). Analysis. Calculated for $C_{20}H_{27}O_2F$: C, 75.44; H, 8.55; F, 5.97. Found: C, 75.09; H, 8.60; F, 5.61. HRMS Calculated for $C_{20}H_{27}O_2F$: m/z 318.1992. Found: m/z 318.1992.

17α-Fluoromethyl-17β-hydroxy-5(10)-estren-3-one (54). Up through the preparation of the dienol ether intermediate, estrenone **54** was prepared in the same manner as nortestosterone **10**. The isolated dienol ether intermediate was dissolved in a mixture of THF and water so that the final concentration of steroid was very low. The solution resulting was cooled to 0° and HCl was added. The reaction was warmed to room temperature and stirred 1 hr, at which time the product was isolated [ethyl acetate; organic extracts washed with saturated $NaHCO_3$ (aqueous)] and purified (50% hexanes in ethyl acetate) to give estrenone **54** in 50% yield. ^{1}H NMR δ 1.01–2.92 (*m*, 22H, backbone, 17-OH); IR (neat) 1716 (C═O); mass spectrum (70 eV) m/z (relative intensity) 306 (83, M^+), 91 (100). Analysis. Calculated for $C_{19}H_{27}O_2F$: C, 74.47; H, 8.88; F, 6.20. Found: C, 74.27; H, 8.92; F, 6.06.

17α-(3′-(2-Tetrahydropyranyloxy)propynyl)-3-methoxyestra-3,5-dien-17β-ol (56). A pentane solution of propargyl tetrahydropyranoyl ether (1.89 ml, 14 mmol) was cooled to 0°. The subsequent addition of butyllithium (4.67 ml, 7 mmol) via syringe resulted in the formation of a white precipitate, which was dissolved with a minimum amount of tetrahydrofuran. A solution of dienone **55** (500 mg, 1.75 mmol) in THF was added to the anion mixture, and the reaction was then stirred 50 min at 0°. Saturated NH_4Cl (aqueous) was added to the solution, and the product was isolated (ethyl acetate) and purified (40% ethyl acetate in hexanes), to give 550 mg (74%) of diene **56**. ^{1}H-NMR δ 0.63–2.54 (*m*, 25H, backbone, 17-OH, THP methylenes), 3.45–3.96 (*m*, 2H, —CH_2O— of THP), 4.33 (*s*, 2H, —C≡CCH_2—), 4.79–4.89 (*m*, 1H, acetal); IR (neat) 3434 (OH); mass spectrum 426 (10, M^+), 85 (100). HRMS Calculated for $C_{27}H_{38}O_4$: m/z 426.2770. Found: m/z 426.2764.

17β-Acetoxy-17α-(3′-(2-tetrahydropyranyloxypropynyl)-3-methoxyestra-3,5-diene (57). Dienol ether **56** (424 mg, 0.995 mmol) was dissolved in 6 ml of pyridine and approximately 2 ml of acetic anhydride (21 mmol), and a few grains of 4-dimethylaminopyridine were added. The mixture was refluxed 4 hr and cooled, and aqueous copper sulfate and water were added. Product isolation (methylene chloride; organic extracts washed with water) and purification (20% ethyl acetate in hexanes) gave 345 mg (74%) of the desired material as an oil. ^{1}H NMR δ 0.70–2.80 (*m*, 24H, backbone, THP methylenes), 2.01 (*s*, 3H, 17-acetate); IR (neat) 1746 (C═O); mass spectrum 468 (13, M^+), 85 (100). Analysis. Calculated for $C_{29}H_{40}O_5$: C, 74.33, H, 8.60. Found: C, 74.59; H, 8.81.

17β-Acetoxy-17α-(3′-hydroxypropynyl)-4-estren-3-one (58) (Ref. 15). Dienol ether **57** (1.66 g, 3.55 mmol) was dissolved in 100 ml of an acetone/water (96:4) mixture. A few grains of *p*-toluenesulphonic acid were added, and the solution was refluxed for 1.5 hr, at which time the reaction was cooled and diluted with water, and the product was isolated (methylene chloride) and purified (30% hexanes in ethyl acetate) to yield 700 mg (54%) of the desired estrenone. Melting point 171–177°; ^{1}H NMR δ 0.79–2.84 (*m*, 21H, backbone, —≡CCH_2O*H*), 5.83 (*bs*, 1H, 4-H); IR (neat) 3440 (OH), 1742 (C═O, acetate), 1665 (C═O, α,β-unsaturated); mass spectrum 370 (5, M^+), 43 (100.)

17α-(3′-Fluoropropynyl)-17β-acetoxy-4-estren-3-one (59) (Ref. 15). A solution of 100 mg (0.270 mmol) of alcohol **58** in 8 ml of methylene chloride was cooled to −78° and 132 μl of DAST (diethylaminosulfur trifluoride) were added. The mixture was warmed to room temperature, stirred 65 min, and then quenched by slow addition of saturated aqueous bicarbonate solution and diluted with water. The crude product was isolated (CH_2Cl_2) and purified (45% ethyl acetate in hexanes) to yield 58 mg (58%) of propargyl fluoride **59** as an off-white solid. Melting point 134–144°; ^{1}H NMR δ 0.67–2.90 (*m*, 20H, backbone), 5.00 (*d*, J = 48 Hz, 2H, —CH_2F); mass spectrum 372 (12, M^+), 43 (100).

Biochemical Materials and Methods

Biochemicals were obtained from the following sources: [^{3}H]R5020 and [^{3}H]R1881, New England Nuclear; charcoal Norit A, leupeptin, triamcinolone acetonide, soybean trypsin inhibitor, and Tris, Sigma; dextran grade C, Schwarz/Mann; Triton X-114, Central Solvents and Chemical Co., Bedford Park, IL; EDTA, Baker.

Competitive radiometric binding assays. *Progesterone receptor (*Ref. 16*).* The PR levels in the uteri of immature rats were induced by estrogen treatment. Immature female Holtzman rats were given daily subcutaneous injections for 3 days of 0.1 ml of a solution of estradiol in sunflower seed oil (50 μg/ml, prepared by diluting 125 μl of 3.68 $\times$ 10^{-3} M estradiol in ethanol to a total volume of 2.5 ml with sunflower seed oil). The animals were sacrificed 24 hr after the last injection and the uteri were collected in PR buffer [0.01 M Tris, 0.0015 M EDTA, 0.02% NaN_3, 20 mM sodium molybdate, 0.021 M mercaptoethanol, 20% (v/v) glycerol; pH 7.4].

The uteri were rinsed with buffer, homogenized at two uteri per ml of buffer with an all-glass homogenizer, and centrifuged for 1 hr at 875 g. The supernatant (cytosol fraction, 11.6 nM in PR) was decanted and stored in 0.7-ml aliquots in liquid nitrogen. When required, an aliquot was thawed and then diluted with 5.3 ml of PR buffer, 0.75 ml of soybean trypsin inhibitor in buffer (50 mg/ml), 0.4 ml of leupeptin in buffer (10 mg/ml), and 0.075 ml of phenylmethylsulfonyl fluoride (PMSF) (0.1 M in isopropanol). Obtained was 7.3 ml of cytosol at approximately 1.1 nM in PR. Hydrocortisone (7.5 μl of a 1 $\times$ 10^{-3} M ethanolic solution) was added, and the mixture was incubated 30 min at 0°.

An accurately weighed sample of nonradioactive competitor (12–500 μg) was dissolved in 1:1 dimethylformamide/PR buffer to give a 7 $\times$ 10^{-4} nM stock solution. Serial dilutions with 1:1 dimethylformamide/PR buffer were prepared to give a series of concentrations ranging from 21 $\times$ 10^{-5} to 7 $\times$ 10^{-9} M. A dilution series ranging from 7 $\times$ 10^{-5} to 7 $\times$ 10^{-10} was prepared for the standard competitor progesterone.

Microtiter plates were cooled on ice, and 10 μl of a 7 $\times$ 10^{-8} M (87 Ci/mmol) [^{3}H]R5020 solution in PR buffer was added to each well, followed, in duplicate, by 10-μl aliquots of the competitor solutions (11 different concentrations plus a 1:1 dimethylformamide/PR buffer blank). The plates were vortexed gently and then 50 μl of rat cytosol were added to each well.

After addition of the cytosol, the plates were again vortexed, covered with adhesive film, and incubated at 0° for 18 hr. At this time, 10 μl of a dextran-Norit suspension were added to each well, and the plates were vortexed vigorously every 5 min for 15 min. The plates were again covered with adhesive film, centrifuged at 800 $\times$ *g* for 8 min, and 50-μl aliquots were withdrawn and counted in minivials in 4 ml of Triton-xylene scintillation fluid.

Competitive RBAs were determined by preparing a plot of bound [^{3}H]R5020 versus log competitor concentration. The midpoints of the curves correspond to the concentration of ligand (either cold R5020 or competitor) required to inhibit 50% of the high affinity [^{3}H]R5020 binding. The ratio of these concentrations is the RBA of the competitor.

*Androgen receptor (*Ref. 16*).* Assays to measure binding to AR were also run according to the method outlined above with the following modifications. Male Holtzman rats were orchidectomized and sacrificed 24 hr later. The ventral prostates were collected in AR buffer [0.01 M Tris, 0.0015 M EDTA, 0.02% NaN_3, 0.01 M thioglycerol, 20 mM sodium molybdate, 10% (v/v) glycerol; pH 7.4]. The supernatant (cytosol fraction) obtained upon homogenization was incubated for 30 min at 0° with 1 $\times$ 10^{-3} M (ethanol) triamcinolone (acetonide) before being used in the actual assays. Tritiated R1881 was employed as a tracer and dihydrotestosterone as a standard.

Results

Ligand Design

In designing fluorine-substituted ligands for AR and PR, we considered those features reported to enhance the binding affinity and selectivity of the ligands to their respective receptors and to decrease their affinity for other receptor systems (i.e., to maximize homologous and minimize heterologous binding).

Although many derivatives of progesterone bind with high affinity and selectivity to PR, it is generally agreed that certain androstanes and estranes appropriately substituted at C-17 (e.g., 17β-hydroxy-17α-ethynyl) have affinities for PR greater than or equal to that of progesterone. We thus deemed it possible to make, from a common androstane or estrane intermediate such as testosterone (T) or nortestosterone (nor-T) (or their 5α-dihydro analogs, DHT or nor-DHT), derivatives that would bind selectively to either AR or PR. Consequently, in this study we limited our investigation to these steroid classes (Fig. 1).

Elsewhere (17), we have reviewed in detail those alterations in the structures of T and nor-T which have been found either to enhance the binding of the parent ligand to AR or PR or to increase *in vivo* progestogenic or androgenic activity. One can surmise certain generalities from this review: bulky substituents on the back face of the D-ring (16α- and 17α-substituents) and the front face at the 17β-position enhance binding to PR but diminish binding to AR, and saturation of the 4,5-double bond usually enhances AR binding.

The effect of fluorine substitution on the affinities of androgens and progestins for their respective receptors has not been extensively investigated. Except for 11β-fluoroethisterone, the study of fluorinated ligands for PR has been confined to those containing a pregnane backbone (17). Of the few fluorinated androstanes synthesized, only 2α-fluorodihydrotestosterone (3) appears to have reasonable affinity for AR.

Thus, from what is known, it may be concluded that an androstane- or estrane-based ligand with high affinity and high selectivity for PR could have in the 17α-position a halomethyl group or an unsaturated alkyl group equal to or larger than ethynyl. An AR-selective ligand might best be in the nor-T or nor-DHT series, with only a hydrogen at 17α. Fluorine substitution would be well tolerated at the 6α- and 11β-positions although, since it is small, it might also be well tolerated elsewhere.

Based on these considerations we have prepared the fluorinated steroids **1–12** (Fig. 2) which contain fluorine at positions where fluorine-18 might ultimately be incorporated in a facile manner. We also prepared the non-fluorinated analogs **13–21** (Fig. 3) so that we could determine by direct comparison the

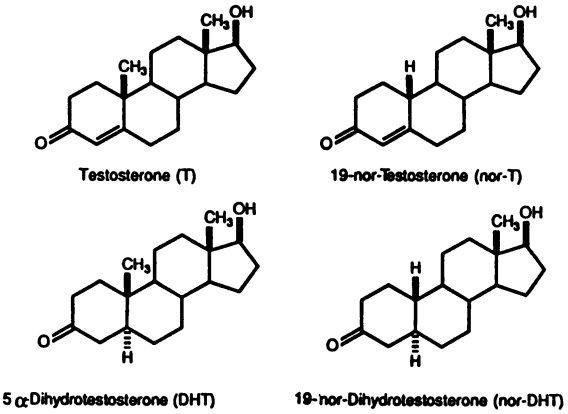


Fig. 1. Chemical structures of steroid classes investigated.

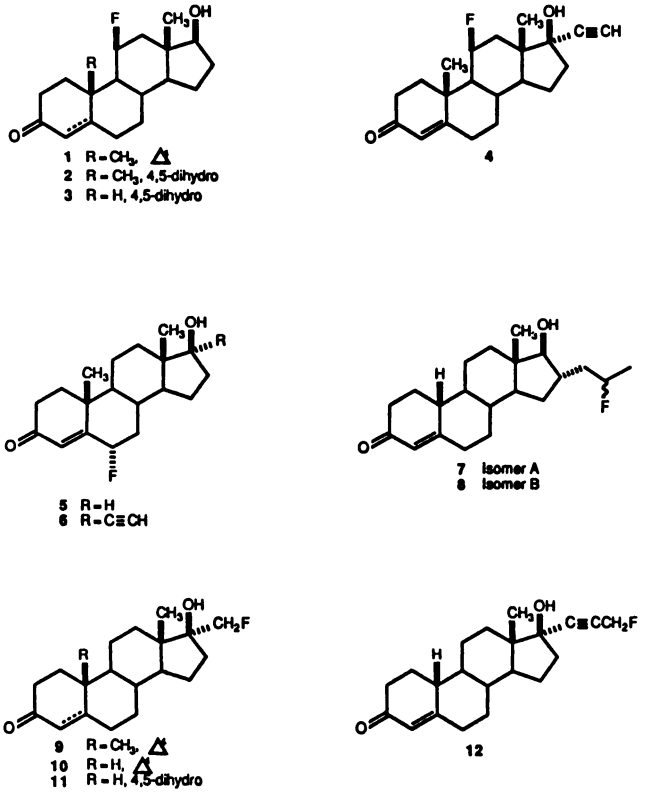
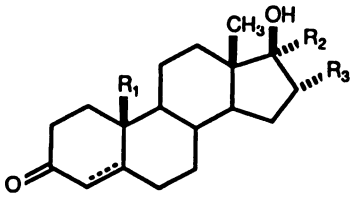


Fig. 2. Fluorinated steroids 1–12.



13 R_1 = CH_3, R_2 = H, R_3 = H, Δ^4

14 R_1 = CH_3, R_2 = H, R_3 = H, 4,5-dihydro

15 R_1 = CH_3, R_2 = C≡CH, R_3 = H, Δ^4

16 R_1 = H, R_2 = H, R_3 = H, Δ^4

17 R_1 = H, R_2 = H, R_3 = H, 4,5-dihydro

18 R_1 = H, R_2 = H, R_3 = $CH_2CH_2CH_3$, Δ^4

19 R_1 = H, R_2 = H, R_3 = $CH_2CH{=}CH_2$, Δ^4

20 R_1 = H, R_2 = CH_3, R_3 = H, Δ^4

21 R_1 = H, R_2 = C≡CCH_3, R_3 = H, Δ^4

Fig. 3. Non-fluorinated analogs of steroids 1–12.

effect fluorine substitution has on the binding of androgens and progestins to their respective receptors. Several of these compounds are known (**1, 5, 6, 12–15, 17–21**); however, in many cases, we have synthesized them by improved routes and, thus, in some cases we report their preparation and appropriate literature references under Experimental Procedures.

Chemical Synthesis

11β-Substituted steroids. The 11β-substituted T and nor-DHT derivatives were prepared from the corresponding 9(11)-olefins by a bromofluorination-debromination sequence. In the T series (Scheme 1), the desired bromofluorination precursor **22** was prepared in two steps from cortisol using known methods (18, 19). Bromofluorination of alkene **22** under controlled conditions (25°, 1 hr) gave the expected anti-Markownikow (9α-bromo-11β-fluoro) addition product (**23**). Bromination at C-16 also occurred as a result of extended reaction times (5 hr). Reductive debromination gave the key intermediate **24**.

Selective hydride reduction of **24** gave 11β-fluoro-T (**1**), and further dissolving reduction with lithium in ammonia gave 11β-fluoro-5α-dihydro-T (**2**). Selective protection of the A-ring enone system as the dienol ether (**25**), followed by ethynylation and deprotection under base and acid conditions, gave 11β-fluoroethisterone **4**.

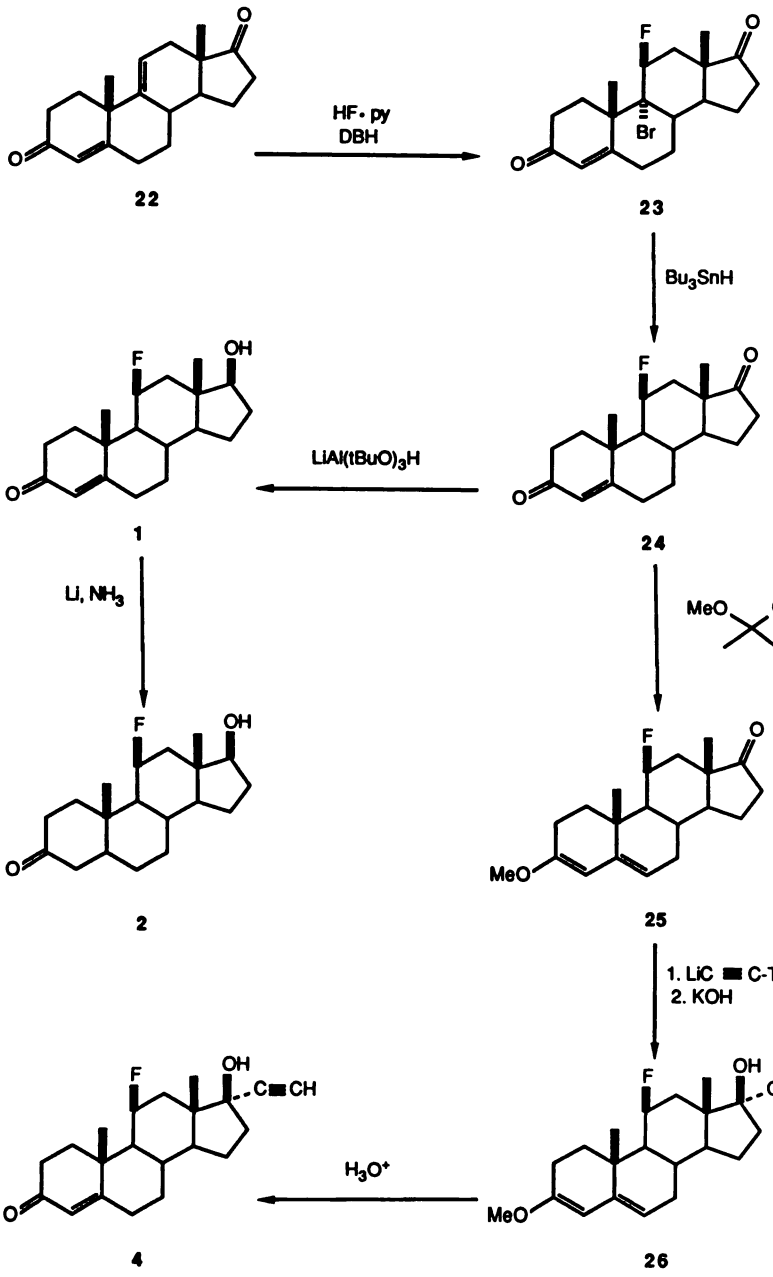
In the preparation of the 11β-fluoro nor-DHT (**3**) (Scheme 2), hydrogenation of the known 11β-hydroxy-19-norandrostenedione (**27**) (20) gave a mixture of 5α- and 5β-dihydro isomers (**28**) that could not be separated chromatographically. Dehydration to the 9(11)-olefin **29**, followed by bromofluorination and separation of the 5α- and 5β-isomers, gave dione **30** which could be reduced selectively at C-17 with borohydride in the presence of erbium chloride (21). Debromination furnished the desired 11β-fluoro-nor-5α-DHT (**3**).

Our attempts to adapt this route for the preparation of the corresponding 11β-nor-T were frustrated by double bond isomerizations that occurred upon attempted dehydration of 11β-hydroxy-19-norandrostenedione **27**.

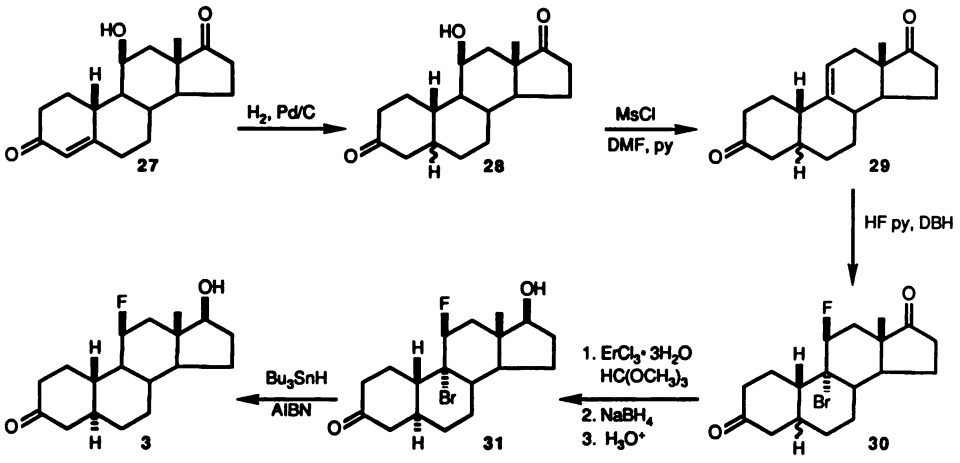
6α-Fluorosteroids. The 6α-fluorosteroids were prepared as outlined in Scheme 3. Bromofluorination of 5-androstene-3β,17β-diol (**32**) gave a mixture of the Markownikow/anti-Markownikow adducts **33** and **34**. Oxidation of this mixture was followed spontaneously by dehydration and epimerization to give the 6α-fluoroandrostenedione **35** as major product. Selective reduction gave the testosterone derivative **5**, and selective protection of the A-ring enone, followed by ethynylation and deprotection, gave the 17α-ethynyl derivative **6**.

16α-Substituted steroids. All 16α-substituted steroids were prepared from 16α-allyl estradiol 3-*O*-methyl ether **40**, as outlined in Scheme 4. This estradiol intermediate was synthesized from 3-methylestrone (**38**) by C-16 alkylation to give ketone **39** as a 2:1 mixture of 16α- to 16β-epimers. This was followed by hydride reduction and epimer separation. Birch reduction of the 3-*O*-methyl 16α-allyl estradiol **40** gave a mixture of the 16α-propyl and allyl nor-T derivatives **18** and **19**. Alternately, this compound was demethylated (**41**) and reprotected as its 3-*O*-trifluoromethanesulfonate (**42**). Bromofluorination of the allyl group in **42**, followed by deprotection and reductive debromination, afforded the fluoroalkylestradiol **45** as an epimeric mixture at C2′. Methylation of the 3-hydroxyl group to give **46**, followed by Birch reduction, gave 16α-fluoropropyl nor-T as a mixture of diastereomers (**7** and **8**), separable by HPLC.

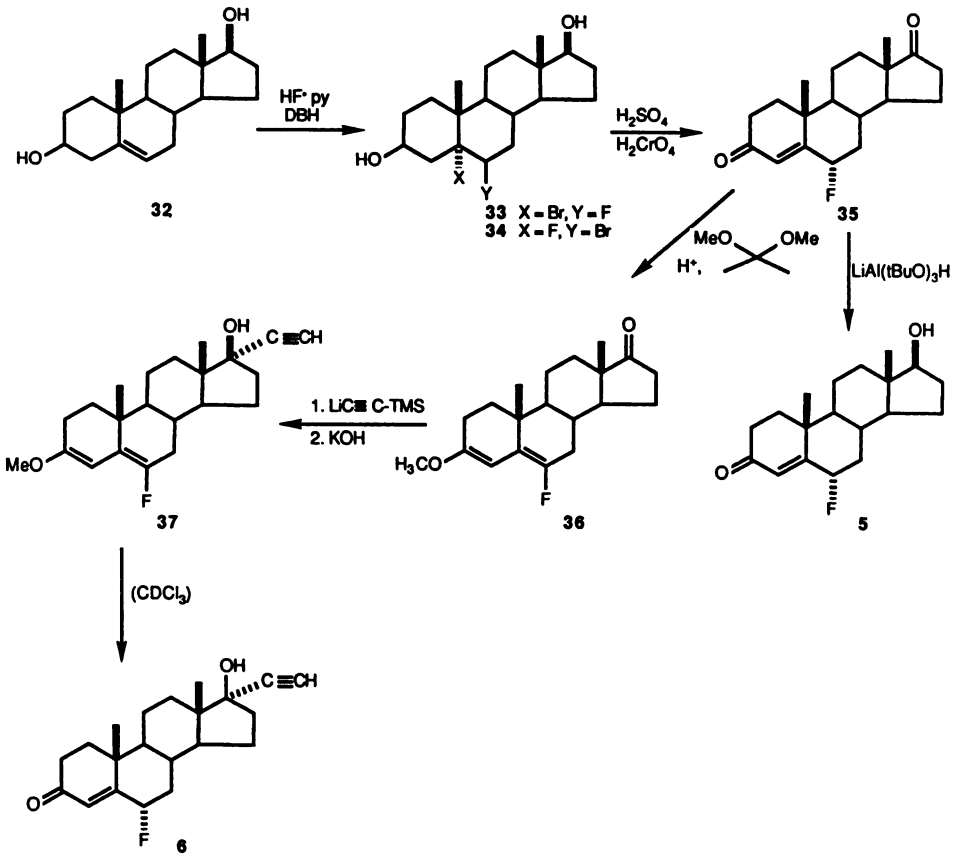
17α-Substituted steroids. The 17α-fluoromethyl T derivative was prepared as shown in Scheme 5. The A-ring enone of androstenedione was selectively protected as the ethylene ketal and the 17-ketone and then methylenated to the 17-spiroepox-



Scheme 1. Preparation of 11β-fluoro T and DHT derivatives.



Scheme 2. Preparation of 11β-fluoro nor-DHT.



Scheme 3. Preparation of the 6α-fluoro steroids.

ide (**48**). Treatment with KHF_2 at high temperatures followed by A-ring deprotection gave the 17α-fluoromethyl T derivative **9**, albeit in low yield.

As shown in Scheme 6, the nor-T derivatives were prepared from the known estratriol 3-*O*-methyl ether **50** (22). Reaction of this compound with thionyl chloride gave a diastereomeric mixture of cyclic sulfites **51**, which was oxidized to the corresponding cyclic sulfate **52** using ruthenium trichloride trihydrate. Fluoride ion displacement of the sulfate to yield 17α-fluoromethyl estrone 3-*O*-methyl ether **53** was followed by Birch reduction and hydrolysis under strongly acidic conditions to afford fluoromethyl nor-T (**10**). Hydrolysis of the dienol ether intermediate from the Birch reduction under mildly acidic conditions gave the unconjugated 5(10)-estren-3-one **54**, which was readily isomerized to the desired nor-T derivative (**10**). Nortestosterone **10** was further reduced under dissolving metal conditions to the 5α-dihydro analog **11**.

(3′-Fluoro-1′-propynyl)-nor-T **12** was prepared from 19-nor-T by the sequence outlined in Scheme 7. Ethynylation of the dienol ether of 19-norandrostenedione (**55**) with the anion of propargyl tetrahydropyranyl ether gave the intermediate adduct **56**. Protection of the 17-alcohol as the acetate, followed by acid deprotection, gave the propargyl alcohol (**58**) which was fluorinated using DAST, giving, after deprotection, the desired nor-T derivative **12**.

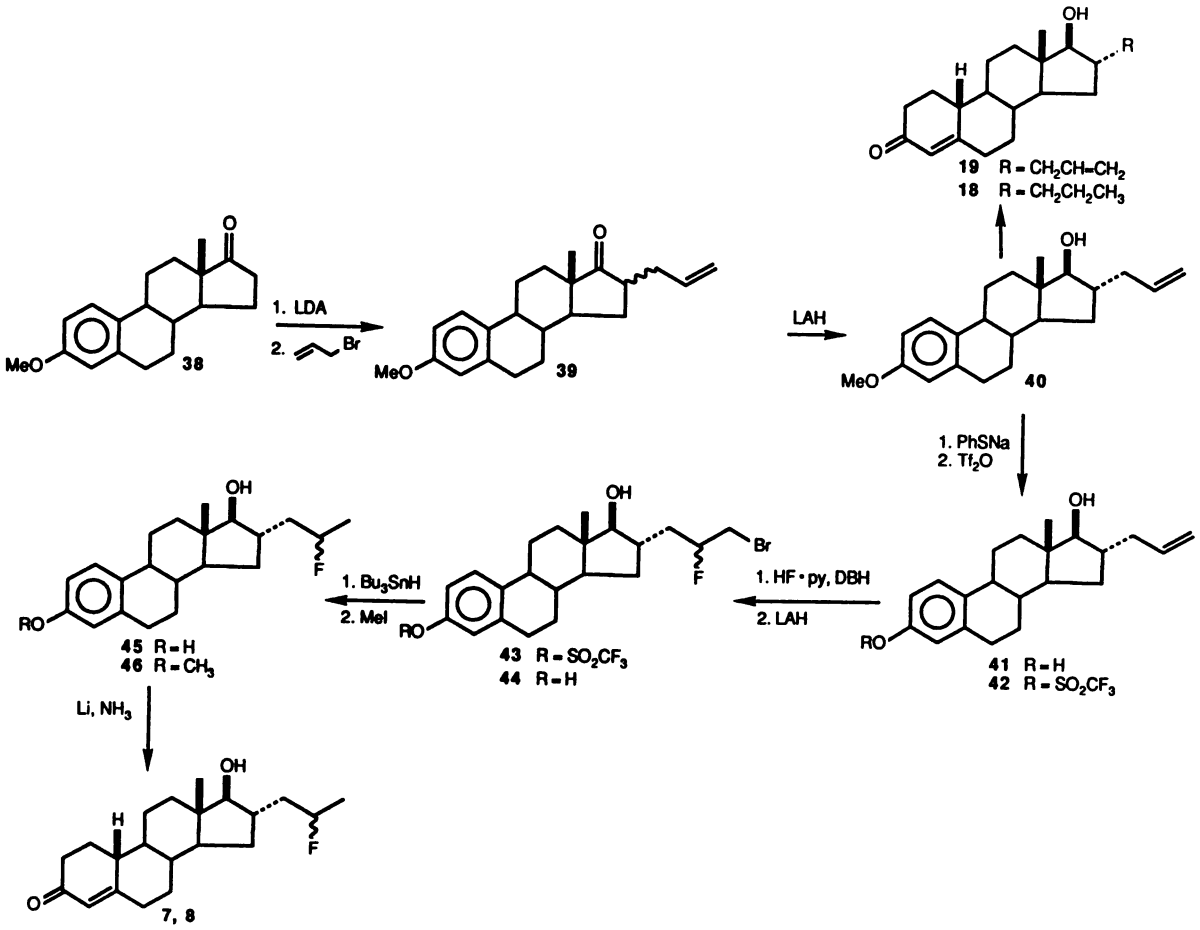
Binding Affinity for PR and AR

General. The AR binding affinities of the fluorine-substituted T and nor-T derivatives (and, in some cases, their 5α-dihydro analogs), together with some protio compounds, were measured *in vitro* by a competitive binding assay involving tritiated R1881 as a tracer and DHT as standard. (Relative to R1881, DHT has an affinity of 61%.) AR was obtained from the ventral prostate of orchidectomized adult male rats and was incubated with increasing concentrations of steroid competitor and [^{3}H]R1881 for 18 hr at 0°. Free ligand was removed using charcoal/dextran. The binding affinities of the compounds for PR were determined in a similar fashion, using PR obtained from the uteri of estrogen-primed immature female rats. R5020 was employed as tracer and progesterone as standard. (Relative to R5020, progesterone has an affinity of 12%).

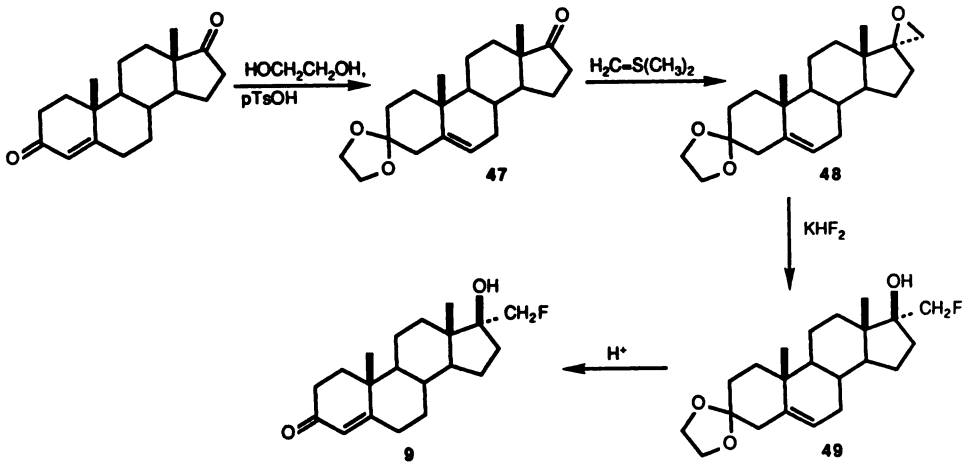
The results of these assays are shown in Table 1. (The values given are the average of duplicate determinations that are reproducible to within 30%). The data in this table are organized to facilitate comparisons: The T and the nor-T derivatives are placed at the head of two major columns, under which are shown the binding affinities for both PR and AR; the data given are RBA values, where the standard for PR is progesterone (RBA = 100), and for AR, DHT (RBA = 100); in the five cases where we have prepared 5α-dihydro derivatives, binding data are given in parentheses.

Although our structural studies do not systematically fill this table, a number of trends in the binding data are evident, with regard to both the site and nature of the substituent and the presence of fluorine.

Binding selectivity for PR versus AR. Our binding data confirm the general notion that, in the T and nor-T series, introduction of a substituent on the back face of the D-ring increases affinity for PR and, in most cases, decreases affinity for AR. Thus, compounds with high affinity for AR are found primarily in the 17α-H series (Table 1, lines 1–3): here is found the highest affinity compound, 11β-fluoro-5α-dihydro-19-nortestosterone (**3**; Table 1, line 2, RBA = 130). Outside of the



Scheme 4. Preparation of 16α-substituted steroids.



Scheme 5. Preparation of the 17α-fluoromethyl T.

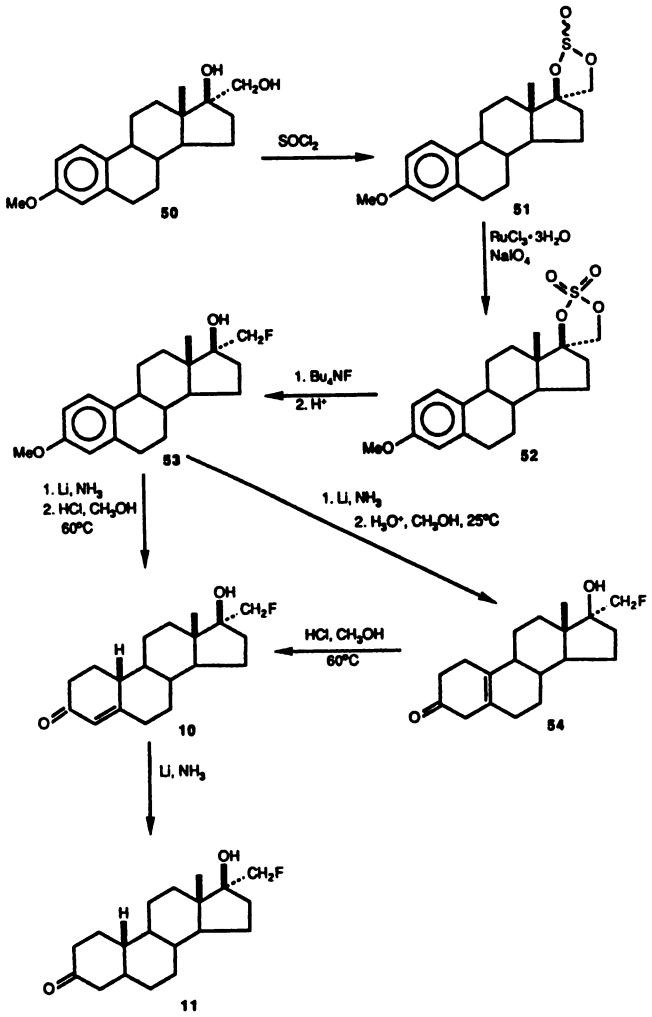
17α-H series, the next highest binding androgens are in the series with the smallest 17α-substituent, 17α-CH_3 (Table 1, line 9); those with larger 17α- and 16α-substituents (Table 1, lines 4–8 and 11–14, respectively) are lower affinity androgens.

In the T and nor-T series, affinity for the PR appears to be increased by 17α- and 16α-substituents (Table 1, lines 4–10 and 11–14 versus 1–3). The fluorine-substituted compound with the highest affinity for PR is the 17α-fluoropropynyl nor-T (**12**; Table 1, line 7, RBA = 66).

Where we have studied it (Table 1, lines 1, 2, and 8), reduction of the 4,5-olefin appears to have a relatively small effect on PR binding; binding to AR is increased in the T series (Table 1, lines 1 and 2) but relatively unaffected in the nor-T series (Table 1, lines 1 and 10).

Effect of Fluorine Substitution

11β-Fluorosteroids (Table 1, lines 2 and 5). Fluorine in the 11β-position is not well tolerated by PR but appears to enhance binding of certain androgens to AR. The best androgens are in this series: 11β-fluoro-5α-DHT (**2**; Table 1, line 2, RBA = 62) and 11β-fluoro-5α-dihydro-nor-T (**3**; Table 1, line 2, RBA = 130). The latter compound has the highest affinity



Scheme 6. Preparation of 17α-fluoromethyl nor-T and nor-DHT.

for AR of any of the compounds tested: both the 19-nor character and the 11β-fluoro substituent appear to be important contributors to the affinity of this compound.

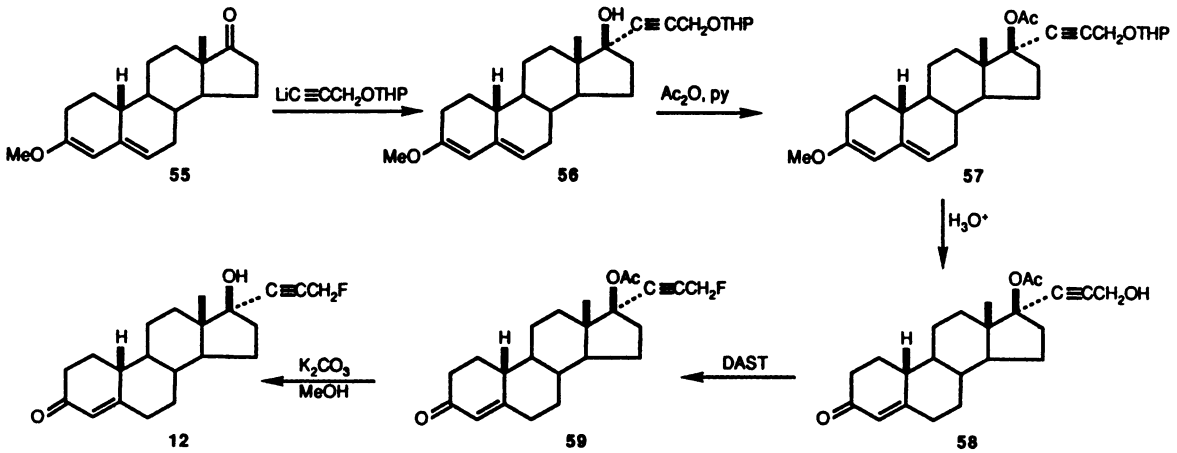
Interestingly, although the binding of T (**13**), 5α-DHT (**14**), and ethisterone (**15**) to AR and PR is decreased when fluorine is introduced (Table 1, lines 1 and 2; lines 4 and 5), the affinity of dihydro nor-T (**17**) for AR is increased upon incorporation of fluorine (Table 1, lines 1 and 2: RBA of **17** = 40; RBA of **3** = 130). A parallel observation has been made by Gilbert *et al.* (23): addition of an 11β-chloro group significantly enhanced the progestational activity of 19-norsteroids only; the potency of chlorinated 19-methyl progestins such as 11β-chloroprogesterone was either diminished or unchanged from that of the unsubstituted parent ligand. These workers have suggested that the increase in activity as a result of 11β-halogenation in the 19-norsteroids might result from a combination of electronic effects and conformational distortion resulting from 1,3-diaxial interaction between the 11β-substituent and the C-18 methyl group. The additional 1,3-diaxial interaction with a C-19 methyl group may result in a greater and disadvantageous conformational change in the steroid, which would counteract any useful electronic effects (23).

Saturation of the 4,5-double bond enhances the affinity of the 11β-fluoroandrogen for AR (Table 1, line 2) with only a slight increase in PR binding: conversely, the addition of a 17α-ethynyl group increased the affinity of the ligand for PR (Table 2, line 2 versus line 5: RBA of **1** = 0.4 versus RBA of **4** = 3), and decreased AR binding. Removal of the C-19 methyl group increased the affinity of the parent ligand (11β-fluoro DHT) for both AR and PR (Table 1, line 2); fortunately, the binding of **3**, the highest affinity androgen, to PR is still low.

6α-Fluoro steroids. No change in the affinity of T for AR or PR is noted upon the introduction of 6α-fluorine (Table 1, lines 1 and 3: **5** versus **13**), but 6α-fluoro-17α-ethynyl-T exhibits a greater affinity for PR than does 17α-ethynyl-T (Table 1, lines 4 and 6: RBA of **15** versus RBA of **6** = 32).

16α-(2-Fluoropropyl) steroids. The 16α-alkylated nortestosterones **18** and **19** bind with much higher affinity to PR and with much lower affinity to AR than does the parent nortestosterone (**16**). When fluorine is included in the alkyl side chain, though, PR binding decreases; AR binding is unaffected. We were not surprised at the drastic drop in affinity for AR upon 16α-alkylation (**7**, **9**, **18**, or **19** versus **16**), since Hochberg and colleagues (4) have noted that 16α-bromo- and iodo-substituted dihydrotestosterones bind only minimally to AR. In addition, Goto and co-workers (24–26) have observed a reduction in androgenic activity upon the introduction of 16-alkyl substituents into 19-nortestosterone.

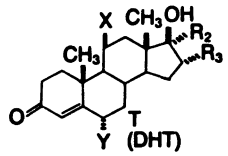
17α-(Fluoroalkyl) steroids. The substitution of fluorine for a 17α-methyl hydrogen in 17α-methyl nor-T (**20** versus

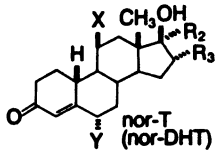


Scheme 7. Preparation of (3′-fluoro-1′-propynyl)-nor-T.

TABLE 1

Relative binding affinities of progestins and androgens 1–21 for progesterone and androgen receptors[a]





Line No.	R_2 (17α)	R_3 (16α)	X (11β)	Y (6α)	Compound number	RBA PR	RBA AR	Compound number	RBA PR	RBA AR
1.	H	—	H	H	**13 (14)**[b]	1 (0.8)	10 (100)	**16 (17)**	16 (10)	50 (40)
2.	H	—	F	H	**1 (2)**	0.4 (2)	5 (62)	**(3)**	— (6)	— (130)
3.	H	—	H	F	**5**	1	10	—	—	—
4.	—C≡CH	—	H	H	**15**	6	1	—	—	—
5.	—C≡CH	—	F	H	**4**	3	1	—	—	—
6.	—C≡CH	—	H	F	**6**	32	2	—	—	—
7.	—C≡CCH_3	—	—	—		—	—	**21**	55	0.2
8.	—C≡CCH_2F	—	—	—		—	—	**12**	66	0.1
9.	—CH_3	—	—	—		—	—	**20**	83	57
10.	—CH_2F	—	—	—	**9**	1	2	**10 (11)**	43 (16)	8 (5)
11.	H	$CH_2CH{=}CH_2$	—	—	—	—	—	**19**	84	1
12.	H	$CH_2CH_2CH_3$	—	—	—	—	—	**18**	120	3
13.	H	CH_2CHFCH_3	—	—	—	—	—	**7**	18	1
14.	H	CH_2CFHCH_3	—	—	—	—	—	**8**	37	2

[a] Average of duplicate determinations. Values are generally reproducible ±30%.
[b] Numbers in parentheses = 5α-dihydro.

10) effects a decrease in binding to both PR and AR; this decrease is much larger (7-fold) for binding to AR than that observed for PR binding (2-fold). On the other hand, 17α-fluoropropynyl nor-T binds to PR with a greater affinity than 17α-propynyl nor-T.

Conclusion

In this study, we have sought to find fluorine-substituted steroid derivatives with high binding affinity and selectivity towards the AR and PR; the compounds with the most favorable properties in this regard are, for the AR, 11β-fluoronordihydrotestosterone (**3**): RBA (AR) = 130, RBA (PR) = 6, and for the PR, 17α-(3-fluoro-1-propynyl)-19-nortestosterone (**12**): RBA (PR) = 66, RBA (AR) = 0.1. We are presently investigating the preparation of these two compounds and some closely related analogs in fluorine-18-labeled form, and are studying their use as imaging agents for AR- and PR-positive tumors of the prostate and breast, respectively.

A number of interesting trends have become apparent in our studies. (1) Where direct comparisons can be made between the fluoro- and the protio-substituted derivatives, fluorine substitution appears well tolerated and results in relatively small changes in receptor affinity [11β: RBA(F)/RBA(H) is 1.4 for AR and 0.55 for PR; 6α: RBA(F)/RBA(H) is 1.5 for AR and 3.2 for PR; 17α: RBA(F)/RBA(H) is 0.32 for AR and 0.86 for PR]. (2) In the T and nor-T series, we have confirmed the known effects of substitution at 16α and 17α, enhancing affinity for PR and reducing affinity for AR, and we have found that removal of the 19-methyl group increases the affinity of T derivatives for both AR and PR, while having a variable effect on dihydro T analogs. Saturation of the 4,5-double bond enhances the binding to AR, while usually reducing affinity for PR.

As our first study in the area of developing fluorine-substituted steroids optimized in terms of their affinity and selectivity towards the AR and PR, we have chosen to limit the scope of our structural investigations to steroids in the T and nor-T series and their dihydro analogs. There are a number of very high affinity ligands for the PR based on retroprogesterone (27), some having cyclopropyl substituents in the A-ring, and many steroidal 4,9(10)-dien-3-one and 4,9(10),11-trien-3-one systems have high affinity for both AR and PR, although achieving selectivity for one or the other of these receptors may be more difficult using these dienone and trienone-based ligands (16). In addition, these fluorine-substituted steroids are to be used as imaging agents *in vivo*; thus, the pattern and rate of their metabolism will ultimately also be important. These are issues that we will be considering in future investigations.

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