Synthesis of High Affinity Fluorine-Substituted Ligands for the Androgen Receptor. Potential Agents for Imaging Prostatic Cancer by Positron Emission Tomography

Aijun Liu, Kathryn E. Carlson, and John A. Katzenellenbogen*

Department of Chemistry, University of Illinois, 1209 West California Street, Urbana, Illinois 61801.
Received September 17, 1991

We have prepared nine androgens substituted with fluorine at C-16 or C-20 to evaluate their potential, as positron emission tomographic (PET) imaging agents for prostatic cancer when labeled with the positron emitting radionuclide fluorine-18 ($t_{1/2}$ = 110 min). These compounds represent members from the following classes of androgens: testosterone (T), 5α-dihydrotestosterone (DHT), 7α-methyl-19-nortestosterone (MNT), mibolerone (Mib), and metribolone (R1881). All of these compounds were prepared by functionalization of suitable androgen precursors, and the synthetic routes were developed to allow the introduction of fluorine by a fluoride ion displacement reaction late in the synthesis, as is required for the preparation of these compounds in fluorine-18 labeled form. We have also prepared four androgens in which the C-3 carbonyl or 17β-hydroxyl groups are replaced by fluorine. Most of the fluorine-substituted androgens show high affinity for the androgen receptor (AR), although fluorine substitution lowers their affinity by a small factor. None of the androgens where fluorine replaces oxygen functions at C-3 or C-17 have substantial affinity for AR. Derivatives of the natural androgens (T and DHT) as well as MNT have little affinity for other steroid hormone receptors (progesterone and mineralocorticoid receptors), whereas the Mib and R1881 derivatives have somewhat greater heterologous binding. With sex steroid binding protein, a human serum binding protein, the pattern of binding affinities is nearly the reverse, with derivatives of Mib, R1881 and MNT having low affinity, and DHT and T, high affinity. From these fluorine-substituted compounds, we can select several whose preparation in fluorine-18 labeled form for further tissue distribution studies is merited.

Introduction

The effective treatment of prostatic cancer requires early detection and accurate staging of the disease.[1-4] Furthermore, the prospects for effective hormonal therapy, which involves castration or suppression of androgen production by treatment with estrogens or high doses of gonadotropin releasing hormone, can be assessed by the measurement of androgen receptor levels in the tumor.[5-11] An accurate measurement of these receptor levels, however, is difficult, since the tumor often contains both receptor-positive and receptor-negative cells,[12] and partial tissue sampling methods, such as needle biopsy and transurethral resection, may provide an assessment of the receptor status of only a portion of the tumor.[4,13]

Steroid hormone receptor-positive tumors have been imaged by positron emission tomography (PET), using receptor ligands labeled with fluorine-18, a positron emitting radionuclide with a short half life (110 min). In fact, both primary and metastatic estrogen receptor-positive breast tumors can be imaged.[14] Thus, this approach provides information on both the receptor content and the spread of cancer. The effectiveness of such imaging agents, however, depends critically on certain well-optimized physicochemical characteristics: high binding affinity for the intended receptor target coupled with low binding by other receptors and nonspecific binders and an appropriate chemical and metabolic stability.[15,16]

We have recently begun to investigate the feasibility of imaging the prostate by PET. In an expansion of earlier work on the uptake of androgens by the prostate,[17,18] we have recently demonstrated that androgens of several structural types (testosterone, dihydrotestosterone, 19-nortestosterone, mibolerone, and metribolone (R1881)) accumulate selectively in the prostate in a receptor-specific fashion.[19] We have also prepared some fluorine-substituted androgens and determined their binding affinity for the androgen receptor,[20,21] and we have prepared one of these, 20-fluoromibolerone, in fluorine-18-labeled form and demonstrated its selective uptake by the prostate in rats.[21]

In order to provide a more complete delineation of the

*Address correspondence to: John A. Katzenellenbogen, Department of Chemistry, University of Illinois, 1209 W. California Street, Urbana, IL 61801.

(1) Gittes, R. F. Carcinoma of the Prostate. *N. Engl. J. Med.* **1991**, *324*, 236-245.
(2) Williams, G. Pointers to Prognosis in Prostatic Cancer. *Rev. Endocr.-Relat. Cancer* **1987**, *26*, 23-28.
(3) Blute, M. L.; Nativ, O.; Zincke, H.; Farrow, G. M.; Therreau, T.; Lieber, M. M. Pattern of Failure After Radical Retropubic Prostatictomy for Clinically and Pathologically Localized Adrenocarcinoma of the Prostrate Influence of Tumor Deoxyribonucleic Acid Ploidy. *J. Urol.* **1989**, *142*, 1262-1265.
(4) Williams, G. New Prospects for Endocrine Therapy in Prostatic Cancer. *Rev. Endocr.-Relat. Cancer* **1985**, *22*, 5-10.
(5) Pertschuk, L. P.; Rosenthal, H. E.; Macchia, R. J.; Eisenberg, K. B.; Feldman, J. G.; Wax, S. H.; Kim, D. S.; Whitmore, W. F., Jr.; Abrahams, J. E.; Gaetjens, E.; Wise, G. J.; Hen, H. W.; Karr, J. P.; Murphy, G. P.; Sandberg, A. A. Correlation of Histochemical and Biochemical Analyses of Androgen Binding in Prostatic Cancer. *Cancer* **1982**, *49*, 984-993.
(6) Ekman, P.; Snochowski, M.; Dahlberg, E.; Gustafsson, J. Å. Steroid Receptors in Metastatic Carcinoma of the Human Prostate. *Eur. J. Cancer* **1979**, *15*, 257-262.
(7) Diamond, D. A.; Barrack, E. R. The Relationship of Androgen Receptor Levels to Androgen Responsiveness in the Dunning R3327 Rat Prostate Tumor Sublines. *J. Urol.* **1984**, *132*, 821-827.
(8) Müntzing, J.; Kirdani, R. Y.; Murphy, G. P.; Sandberg, A. A. A Rat Prostatic Adenocarcinoma as a Model for the Human Disease. *Invest. Urol.* **1979**, *17*, 37-41.
(9) Ekman, P.; Snochowski, M.; Zetterberg, A.; Högberg, B.; Gustafsson, J. Å. Steroid Receptor Content in Human Prostatic Carcinoma and Response to Endocrine Therapy. *Cancer* **1979**, *44*, 1173-1181.
(10) Trapma, J.; Stalpers, C. R.; van der Korput, J. A. G. M.; Kuiper, G. G. J. M.; Brinkmann, A. O. The Androgen Receptor: Functional Structure and Expression in Transplanted Human Prostate Tumors and Prostate Tumor Cell Lines. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 837-842.
(11) Concolino, G.; Maroechi, A.; Margiotta, G.; Conti, C.; Di Silverio, F.; Tenaglia, R.; Ferraro, F.; Bracci, U. Steroid Receptors and Hormone Responsiveness of Human Prostatic Carcinoma. *Prostate* **1982**, *3*, 475-482.
(12) Schulze, H.; Isaacs, J. T. Biology and Therapy of Prostatic Cancer. *Cancer Surveys* **1986**, *5*, 487-503.
(13) Sandberg, A. A.; Karr, J. P. Hormone and Receptor Assays in the Management of Prostatic Cancer. *Rev. Endocr.-Relat. Cancer* **1987**, *26*, 17-21.

0022-2623/92/1835-2113$03.00/0

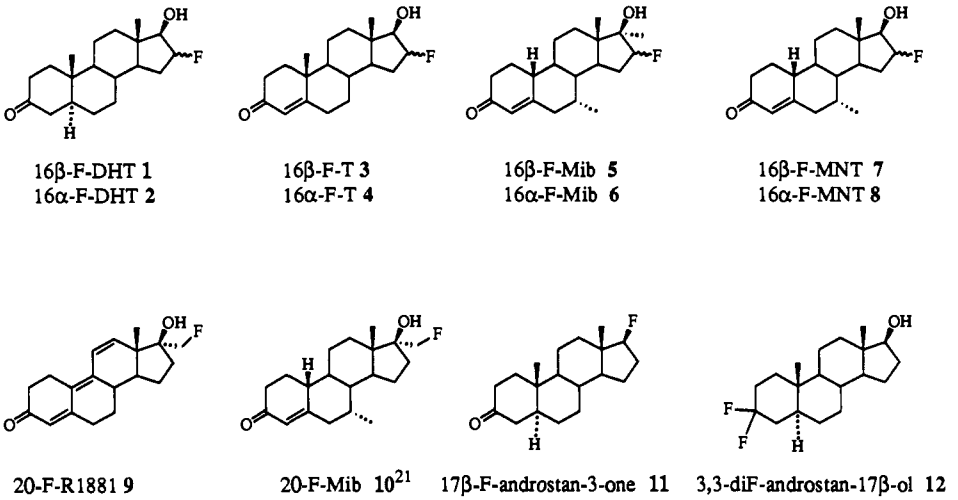


Figure 1. Fluorinated androgens synthesized.

androgen steroid skeletal type and site of fluorine substitution that will give the most appropriate binding and stability characteristics, we describe in this study the synthesis of several new fluorine substituted androgens shown in Figure 1, selected from various structural classes. In all cases, fluorine substitution has been made at the 16 or 20 position, a site where it can be introduced late in the synthesis by a fluoride ion displacement reaction, a prerequisite for labeling with fluorine-18. We also prepared 3,3-difluoro- and 17-fluoroandrostanes. We have measured the binding affinities of these fluorine-substituted androgens not only for the androgen receptor, but for other binders of androgens, namely progesterone and mineralocorticoid receptors and sex steroid binding protein. From this study emerges an impressive pattern of androgen structural types, binding affinities and binding selectivities from which one can select compounds on which further study for the development of androgen receptor based imaging agents is merited. We will describe elsewhere the preparation of these selected fluoroandrogens in fluorine-18-labeled form and their tissue distribution in rats in vivo.[22]

Results and Discussion

Design Considerations. High-affinity ligands for the androgen receptor come from several steroid structural types. The major circulating androgen, testosterone, has lower affinity for the androgen receptor than its metabolite, 5α-dihydrotestosterone, that is produced in target tissues by the action of the 5α-reductase.[23] A potential problem with the natural androgens, however, is their rapid metabolism in vivo[19,24,25] and even in in vitro assay procedures,[26] as well as their high-affinity binding to sex steroid binding protein,[19,27] a serum steroid carrier protein. Synthetic androgens of the mibolerone and metribolone classes were developed to reduce these problems, and they excel in their absence of blood binding and in their increased metabolic stability.[28,29] However, these synthetic androgens show considerable binding toward other steroid

(14) (a) Mintun, M. A.; Welch, M. J.; Siegel, B. A.; Mathias, C. J.; Brodack, J. M.; McGuire, A. H.; Katzenellenbogen, J. A. Positron Tomographic Imaging of Estrogen Receptors in Human Breast Tumors. *Radiology* **1988**, *169*, 45–48. (b) McGuire, A. H.; Dehdashti, F.; Siegel, B. A.; Lyss, A. P.; Brodack, J. W.; Mathias, C. J.; Mintun, M. A.; Katzenellenbogen, J. A.; Welch, M. J. Positron Tomographic Assessment of 16α-[^{18}F]Fluoro-17β-Estradiol Uptake in Metastatic Breast Carcinoma. *J. Nucl. Med.* **1991**, *32*, 1526–1531.

(15) Katzenellenbogen, J. A. The Development of Gamma-Emitting Hormone Analogs as Imaging Agents for Receptor-Positive Tumors. In *The Prostate Cell: Structure and Function, Part B*; Murphy, G. P., Sandberg, A. A., Eds.; A. Liss: New York, 1981; pp 313–327.

(16) Katzenellenbogen, J. A.; Liu, A.; Brandes, S. J.; Carlson, K. E.; Welch, M. J. Strategies for the Development of Androgen Receptor-Based Imaging Agents for Prostate Cancer. In *Radionuclides in the Prostate Gland*; Limouris, G. S., Shoukla, S. K., Eds.; Edizioni Associate: Rome, 1991; pp 91–103.

(17) Tveter, K. J.; Attramadal, A. Selective Uptake of Radioactivity in Rat Ventral Prostate Following Administration of Testosterone-1,2-^{3}H. *Acta Endocr.* **1968**, *59*, 218–226.

(18) Symes, E. K. Uptake and Retention of Androgens by the Rat Ventral Prostate and Consideration of Their Use as Site Directing Agents. *Biochem. Pharmacol.* **1982**, *31*, 3231–3236.

(19) Carlson, K. E.; Katzenellenbogen, J. A. A Comparative Study of the Selectivity and Efficiency of Target Tissue Uptake of Five Tritium Labeled Androgens in the Rat. *J. Steroid Biochem.* **1990**, *36*, 549–561.

(20) Brandes, S. J.; Katzenellenbogen, J. A. Fluorinated Androgens and Progestins: Molecular Probes for Androgen and Progesterone Receptors with Potential Use in Positron Emission Tomography. *Mol. Pharmacol.* **1987**, *32*, 391–403.

(21) Liu, A.; Katzenellenbogen, J. A.; VanBrocklin, H. F.; Mathias, C. J.; Welch, M. J. 20-[^{18}F]Fluoromibolerone, a Positron-Emitting Radiotracer for Androgen Receptors: Synthesis and Tissue Distribution Studies. *J. Nucl. Med.* **1991**, *32*, 81–88.

(22) Liu, A.; Dence, C. S.; Katzenellenbogen, J. A.; Welch, M. J. Fluorine-18 Labeled Androgens: Radiochemical Synthesis and Tissue Distribution Studies on Six Fluorine-Substituted Androgens. Potential Imaging Agents for Prostatic Cancer. *J. Nucl. Med.*, in press.

(23) Liao, S.; Witte, D.; Schilling, K.; Chang, C. The Use of a Hydroxylapatite-Filter Steroid Receptor Assay Method in the Study of the Modulation of Androgen Receptor Interaction. *J. Steroid Biochem.* **1984**, *20*, 11–17.

(24) Chamberlain, J.; Jagarinec, N.; Ofner, P. Catabolism of [4-^{14}C]Testosterone by Subcellular Fractions of Human Prostate. *Biochem. J.* **1966**, *99*, 610–616.

(25) Gore, M. B. R.; Baron, D. N. 3α-Hydroxysteroid Dihydrogenation in Subcellular Fractions of Rat Ventral Prostate and Other Tissues. *J. Endocrinol.* **1965**, *33*, 353–363.

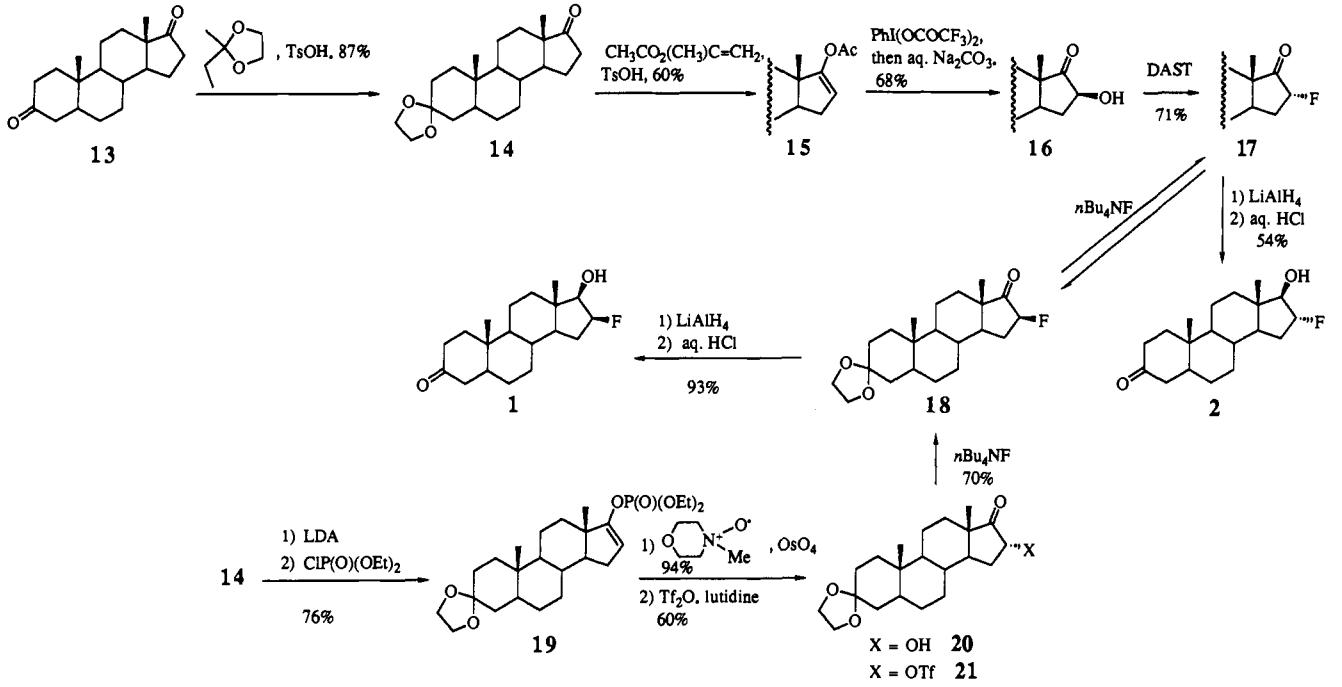
(26) Verhoeven, G.; Heyns, W.; Demoor, P. Testosterone Receptors in the Prostate and Other Tissues. *Vitam. Horm.* **1975**, *33*, 265–281.

(27) Zava, D. T.; Landrum, B.; Horwitz, K. B.; McGuire, W. L. Androgen Receptor Assay with [^{3}H]Methyltrienolone (R1881) in the Presence of Progesterone Receptors. *Endocrinology* **1979**, *104*, 1007–1012.

(28) Murthy, L. R.; Johnson, M. P.; Rowley, D. R.; Young, C. Y. F.; Scardino, P. T.; Tindall, D. J. Characterization of Steroid Receptors in Human Prostate Using Mibolerone. *Prostate* **1986**, *8*, 241–253.

(29) Bonne, C.; Raynaud, J. P. Assay of Androgen Binding Sites by Exchange with Methyltrienolone (R1881). *Steroids* **1976**, *27*, 497–505.

Scheme I



hormone receptors, notably the progesterone and the mineralocorticoid receptors,[19,28] which may compromise the selectivity of their uptake.

The sites of fluorine substitution that we have selected, at C-16 and C-20, are ones that are readily accessible by synthesis, and the compounds we have prepared represent a reasonably comprehensive survey of analogues at these sites. In addition, we have probed for the effect of hydrogen bonding at C-3 and C-17 by preparing certain fluoro–deoxy androgen analogues.

Chemical Synthesis. 16β- and 16α-Fluorodihydrotestosterone (1 and 2). The synthesis of 16β-fluorodihydrotestosterone (1) and 16α-fluorodihydrotestosterone (2), starting from 5α-androstanedione (13), has been achieved in high yields (Scheme I). 16α-Fluoroandrostanedione and 16α-fluoro-17α-hydroxyandrostan-3-one are produced as side products. While monoketal 14 has been prepared in low yield from 5α-androstanedione and ethylene glycol,[30,31] we improved the yield by employing 2-methyl-2-ethyl-1,3-dioxolane, a more selective ketal exchange reagent.[31] Despite the sensitivity of the ketal function to acids, we prepared enol acetate 15 by standard methods with little loss of the ketal group. Enol acetate 15 was converted to 16β-hydroxy ketone 16 by treatment with bis(trifluoroacetoxy)iodobenzene, followed by a mild hydrolysis that avoids isomerization to the more stable 17-hydroxy 16-one function.[32] Treatment of the 16β-hydroxy ketone 16 with (diethylamido)sulfur trifluoride (DAST)[33] gave the 16α-fluoro ketone 17 in high yield. nBu_4NF in THF effected epimerization of the 16α-fluoro substituent in 17 to give 16β-fluoro ketone 18, presumably through a process of enolization–ketonization with the fluoride salt acting as a base.[32] A $LiAlH_4$ reduction of the separated epimers, followed by acid deprotection, furnished the desired products 1 and 2, respectively. The modest yield obtained in the reduction of fluoro ketone 17 to 16α-fluorodihydrotestosterone (2) is ascribed to repulsion of the incoming hydride by the strongly electronegative fluorine substituent at the 16α-position; in this reaction, 15% of the 17α-hydroxy epimer was produced. The epimeric ketone with fluorine in the 16β-position does not encounter this repulsion and is reduced efficiently and stereoselectively.

As will be discussed later, the product 1 was found to have high affinity for AR. In order for its ultimate synthesis in F-18-labeled form, we developed a route in which fluorine is introduced by fluoride ion displacement of a triflate (Scheme I). The enol phosphate 19, produced by the reaction of ketone 14 with LDA followed by $ClP(O)(OEt)_2$, was converted to the 16α-hydroxy ketone 20 with *N*-methylmorpholine *N*-oxide/OsO_4. The triflate 21 was prepared without difficulty, and fluoride ion displacement with nBu_4NF to give fluoro ketone 18 proceeded rapidly and in good yield. Reduction and deprotection of the β-fluoro ketone 18 to give compound 1 are rapid and convenient.

16β- and 16α-Fluorotestosterone (3 and 4). As shown in Scheme II, ketalization of testosterone followed by PCC oxidation gave ketone 24. Conversion of this ketone to the enol silyl ether 25 required a large excess of reagents (10 equiv LDA and TMSCl). Bromination was done with the milder reagent, NBS rather than Br_2, that is normally used.[34] The 16α-bromo ketone intermediate was deketalized before being transformed into the 16α-hydroxy analogue 26 by exposure to aqueous KOH, in an epimerization–substitution process.[34] Treatment of hydroxy ketone 26 with triflic anhydride and 2,6-lutidine effected triflation of the 16α-hydroxy group and at the same time protected the 3-ketone function as the dienol triflate.

(30) Herzog, H. L.; Jevnik, M. A.; Tully, M. E.; Hershberg, E. B. Cyclic Ketals of 4-Androstene-3,17-dione. *J. Am. Chem. Soc.* **1953**, *75*, 4425–4427.

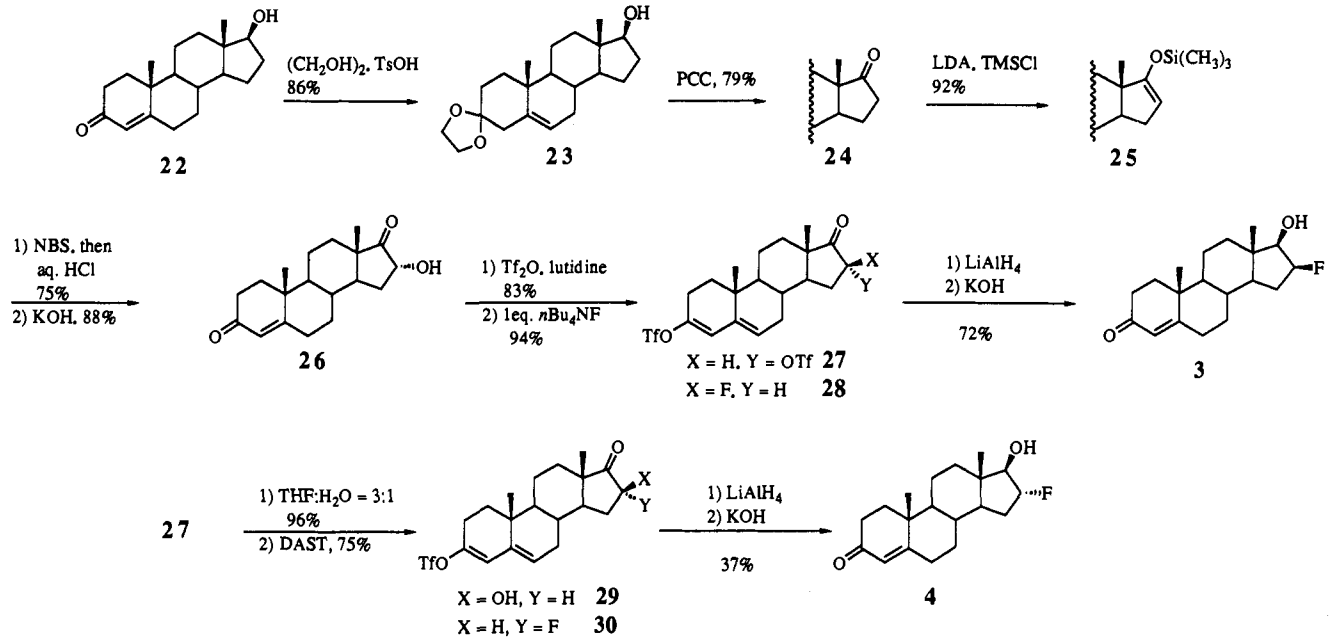
(31) Rosenkranz, G.; Velasco, M.; Sondheimener, F. Steroids. LVII. Cycloethylene Ketals of Androstane-3,6,17-trione. Synthesis of Androstan-3-one-6β,17β-diol. *J. Am. Chem. Soc.* **1954**, *76*, 5024–5026.

(32) Kiesewetter, D. O. Synthesis of Fluorine-18 Labeled Estrogens for Receptor Based Imaging of Mammary Tumors. Ph.D. Dissertation, University of Illinois, Urbana, IL, 1979; pp 27–35.

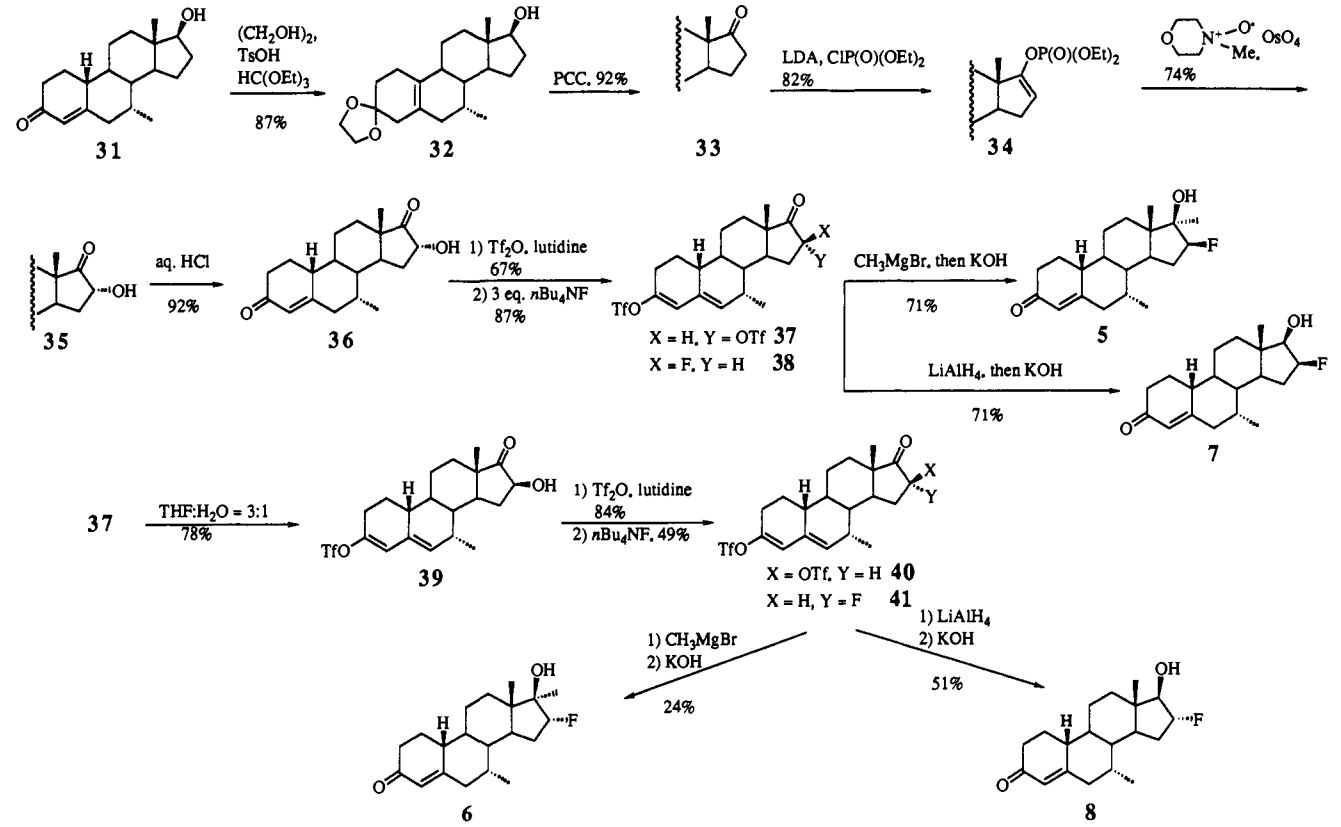
(33) Middleton, W. J. New Fluorinating Reagents. Dialkylaminosulfur Fluorides. *J. Org. Chem.* **1975**, *40*, 574–577.

(34) Nambara, T.; Fishman, J. Ring D α-Halo Ketones of 14β-Steroids. *Steroids* **1961**, *26*, 4569–4574.

Scheme II



Scheme III

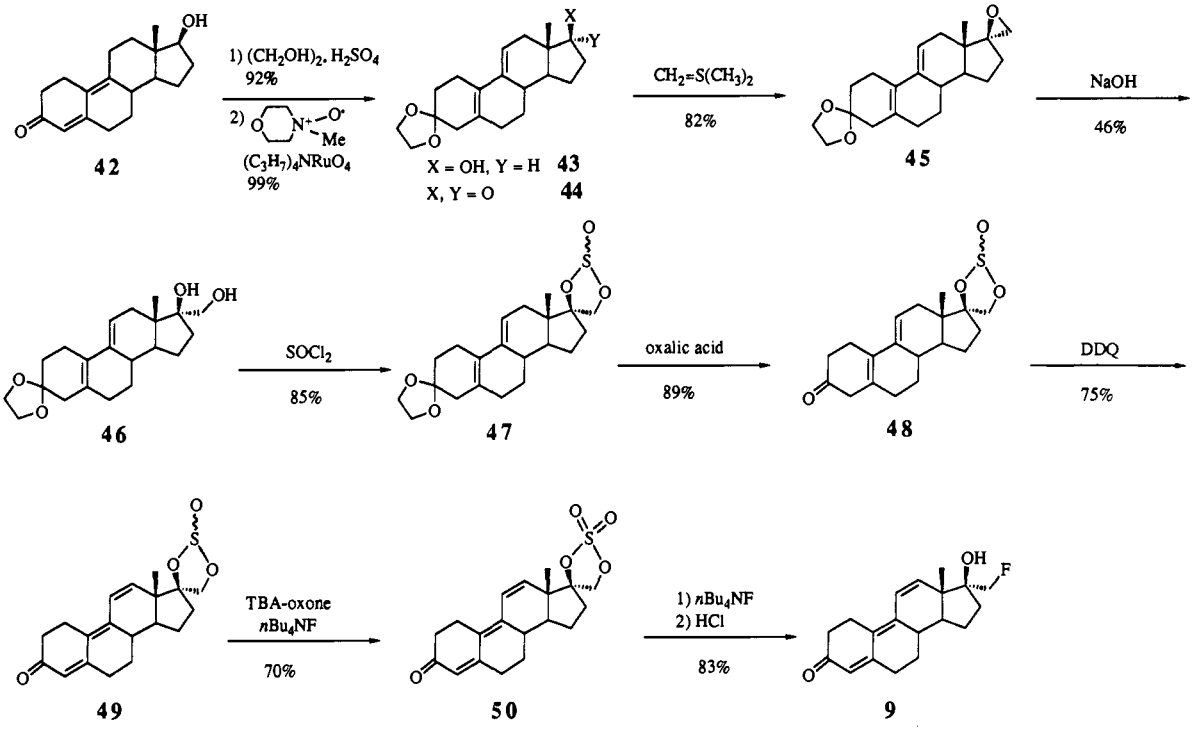


Fluoride displacement with nBu_4NF, followed by $LiAlH_4$ reduction and deprotection with base, provided 16β-fluorotestosterone (**3**).

Inversion of the 16-hydroxy function proved to be troublesome, as treatment of the 16α-triflate ketone **27** under aqueous basic conditions furnished the 16α-hydroxy compound, presumably by the same epimerization–substitution process involved in the transformation of the 16α-bromo ketone function to the 16α-hydroxy ketone, discussed above. We found, however, that a mixture of THF and water (3:1), warmed to ca. 55 °C, effected this transformation successfully, producing the 16β-hydroxy ketone **29**. This compound was fluorinated with DAST to give 16α-fluoro ketone **30**, which was reduced and deprotected to furnish 16α-fluorotestosterone (**4**). Again, with this stereoisomer, reduction stereoselectivity was low, and 13% of the 17α-hydroxy epimer was produced as a byproduct.

16β- and 16α-Fluoro-7α-methyl Androgens (5–8). The starting material for all fluorinated 7α-methyl androgens was 7α-methyl-19-nortestosterone (**33**), which was obtained from Upjohn. We have also prepared this material from 19-nortestosterone by the methods of Bucourt and co-workers (not shown).[35]

Scheme IV



The synthesis of both 16β-fluoromibolerone (16β-F-Mib) (**5**) and 16β-fluoro-7α-methyl-19-nortestosterone (16β-F-MNT) (**7**), outlined in Scheme III, is a combination of portions of the approaches given in Schemes I and II. However, it proved to be considerably more difficult to optimize the reaction conditions in this sequence (Scheme III). All the reactions seemed to require more vigorous conditions to go to completion, yet if they were too strong, reaction yields suffered because of side reactions. The reason for this difference is not clear. Perhaps the 7α-methyl group decreases the accessibility of the α side of the steroid that is usually sterically unincumbered, or it changes the solvation of the α side.

Ketalization of the 3-ketone function in **31** was carried out using TsOH as a catalyst and $HC(OEt)_3$ as a dehydration agent, instead of azeotropic distillation that is normally used. During this reaction, the double bond moved to the more stable 5(10)-position. Oxidation of the 17β-hydroxy group in **32** with PCC was followed by conversion of the ketone **33** to enol phosphate **34**; oxidation with *N*-methylmorpholine *N*-oxide/$(C_3H_7)_4NRuO_4$ was less efficient. This stable enol species was readily oxidized by *N*-methylmorpholine *N*-oxide/OsO_4 to provide 16α-hydroxy ketone **35**, and acid deketalization gave the hydroxy dione **36**. Conversion of this compound to the protected fluoro ketone **38** proceeded by the sequence used to prepare compound **28** (Scheme II). Reduction with $LiAlH_4$, followed by removal of 3-triflate function in base, gave 16β-F-MNT (**7**). Alternatively, reaction with CH_3MgBr, followed by deprotection, gave 16β-F-Mib (**5**).

The 16β-hydroxy ketone **39** is the key intermediate in the synthesis of the epimeric species 16α-F-Mib (**6**) and 16α-F-MNT (**8**) also shown in Scheme III. This intermediate was readily synthesized from the 16α-trifloxy ketone **37** by heating in a mixture of THF and water, as discussed before. Transformation of the 16β-hydroxy ketone **39** to 16α-fluoro ketone **41** could be achieved either by using DAST (not shown in Scheme III) or by way of triflation and then fluorination with nBu_4NF. The latter approach could be adapted for fluorine-18 labeling, while the former one offered a shorter alternative. Finally, $LiAlH_4$ reduction or CH_3MgBr addition, followed by deprotection with base, completed the synthesis of the targets **6** and **8**, respectively. In this series, the electronic repulsion from the 16α-fluorine substituent is amplified by the steric effect of the 7α-methyl group, so that the incoming hydride reagent and methyl anion have greater difficulty in reaching the α side of the ketone than in the testosterone or dihydrotestosterone series. The stereoselectivity of these two addition reactions was low, even though they were carried out in Et_2O, which usually gives much better stereoselectivity than THF;[36] in the CH_3MgBr reaction, the stereoselectivity was even reversed, with more 17α-hydroxy epimer produced than the desired 17β-hydroxy epimer.

20-Fluoro-R1881 (9). The synthesis of 20-fluoro-R1881, as outlined in Scheme IV, utilized the same spiro cyclic sulfate strategy to activate the C-20 position as we have reported previously for 20-fluoromibolerone.[21] A dienone compound, 17β-hydroxy-4,9(10)-estradien-3-one (**42**), was selected as the starting material, since the 3-ketone function needs to be protected during the sulfonium ylide reaction. Having the trienone system in place from the start would make such protection much more difficult.

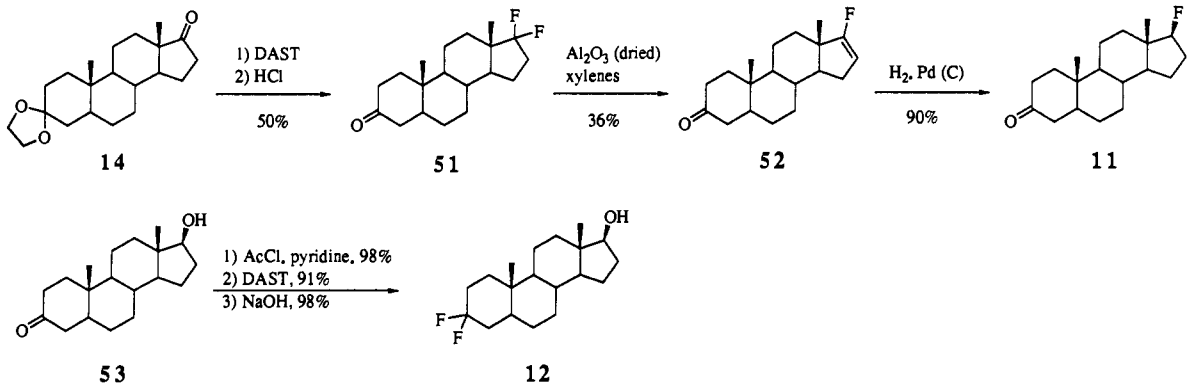
The dienone **42** was prepared from estradiol 3-methyl ether following a literature procedure.[37] Ketalization of

(35) Bucort, B.; Vignau, M.; Torelli, V.; Richard-Foy, H.; Geynet, C.; Secco-Millet, C.; Redeuilh, G.; Baulieu, E.-E. New Biospecific Adsorbents for the Purification of Estradiol Receptor. *J. Biol. Chem.* **1978**, *253*, 8221–8228.

(36) Kiesewetter, D. O.; Kilbourn, M. R.; Landvatter, S. W.; Heiman, D. F.; Katzenellenbogen, J. A.; Welch, M. J. Preparation of Four Fluorine-18 Labeled Estrogens and their Selective Uptake in Target Tissues of Immature Rats. *J. Nucl. Med.* **1984**, *25*, 1212–1221.

(37) (a) Wilds, A. L.; Nelson, N. A. The Facile Synthesis of 19-Nortestosterone and 19-Norandrostenedione from Estrone. *J. Am. Chem. Soc.* **1953**, *75*, 5366–5369. (b) Perelman, M.; Farkos, E.; Forefeld, E. J.; Kraal, R. J.; Rapala, R. T. A New Class of Active Steroids: The 19-Nor-$\Delta^{4,9}$-3-ketosteroids. *J. Am. Chem. Soc.* **1960**, *82*, 2402–2403.

Scheme V



the 3-ketone function in **42** was followed by oxidation of the 17β-hydroxy group in **43** to ketone **44** with *N*-methylmorpholine *N*-oxide/$(C_3H_7)_4NRuO_4$; PCC gave only low yield. Reaction of the ketone **44** with the sulfonium ylide $CH_2{=}S(CH_3)_2$ provided epoxide **45**, and treatment with NaOH under vigorous conditions gave diol **46** in modest yield, as expected. In the synthesis of the spiro cyclic sulfite **47**, it was important to keep the acidic reaction mixture cold and to remove the acid completely before product isolation, as acid residues caused ketal decomposition and reconjugation of the dienone.

Removal of the ketal in **47** without reconjugation using oxalic acid gave dienone **48**. The trienone system was introduced by reaction of the unconjugated dienone **48** with an excess amount of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[38] This dehydrogenation was effected at the cyclic sulfite stage, since the cyclic sulfite function in the D ring can tolerate both the acid deprotection and the DDQ oxidation process. Thereafter, oxidation of the spiro cyclic sulfite to the spiro cyclic sulfate by TBA-oxone in the presence of nBu_4NF,[21] and fluorine displacement, followed by acid hydrolysis, furnished the desired product 20-F-R1881 (**9**). The trienone compound **9** changes colors with pH of the media; in basic media, it was greenish blue, in acid, wine red, and at neutrality, very pale yellowish white.

Other Androgens. The synthesis of 17β-fluoroandrostan-3-one (**11**), as well as 3,3-difluoroandrostan-17β-ol (**12**), outlined in Scheme V, followed a literature procedure published by Bird and co-workers in 1979.[39] The geminal difluoro compound **12** was readily prepared from DHT (**53**) with DAST with acetate protection of the 17β-hydroxy function. To prepare the 17β-fluoro ketone **11**, we initially attempted to epimerize the 17β-hydroxy function in DHT and then activate it for displacement with fluoride ion. However, as observed by Bird et al. in their work,[39] competing elimination in the epimerization and elimination and Wagner–Meerwein rearrangement in the fluoride ion displacement made this approach impractical. Bird's route, shown in Scheme V, is simple, although quite lengthy and inefficient. The dehydrofluorination reaction of the 17,17-geminal difluoro ketone **51** in particular, requires vigorous conditions over dehydrated Al_2O_3, and is accompanied by extensive decomposition.

Assignment of Stereochemistry. The stereochemistry of 16- and 17-substitution was established by a combination of the known stereochemical specificity of reactions on the D ring of steroids, as well as by ^1H and ^{19}F NMR assisted by molecular mechanics structure modeling. For example, the incoming reagents in the electrophilic addition and oxidation at the C-16 position, as well as in the nucleophilic addition of hydride and Grignard reagent to the 17-ketone, prefer to attack the α-side of the D ring, because of the steric bulk of the β-oriented 18-CH_3 function.

As observed in 16-halogenated estrogens,[40] ^1H NMR of the 16-substituted ketones showed the following coupling pattern: the 16α-hydrogens of the 16β-substituted systems are triplets and the 16β-hydrogens of the 16α-substituted systems are doublets or small doublets of doublets. The substituents included F, Br, Cl, CH_3, and OH, as well as OTf.

^1H NMR of the 16-substituted 17-hydroxy compounds is also characteristic. The 17α-H is coupled with both the 16α- and 16β-H (or F) groups, while the 17β-H is only coupled with the 16β-H. In addition to the 17-H, the 16β-H is coupled with both of the 15-H's with a similar coupling constant, whereas the 16α-H is coupled with the two 15-H's with different coupling constants. This phenomenon is easy to understand when one observes the dihedral angles between the 16- and 17-groups, as well as between the 16- and 15-groups of the compounds with an sp^3 C-17 center or an sp^2 C-17 center. These dihedral angles are displayed in the X-ray structure[41] of testosterone and 7α-methyl-4-androstene-3,17-dione shown in Figure 2.

Interestingly, when a fluorine and a hydroxy function are oriented on the same side of the D ring, that is in 16β-F-17β-OH or 16α-F-17α-OH androgens, intramolecular hydrogen bonding is observed by both ^1H and ^{19}F NMR. The intramolecular H-bond could be through a trapped water molecule as a seven-membered ring or directly as a five-membered ring. In the five-membered ring case, the H-bond has to be bent, but nonlinear hydrogen bonds

(38) Bucourt, R.; Nedelec, L.; Torelli, V.; Gase, J. C.; Vignau, M. 19-nor heterocyclic steroids. III. New route to 2-oxa-9-ene and 9,11-diene-19-nor testosterones by condensation of a Reformatsky reagent with a δ-enolic lactone. *Bull. Soc. Chim. Fr.* **1975**, 9–10, 2043–2047.

(39) Bird, T. G. C.; Felsky, G.; Fredericks, P. M.; Jones, E. R. H.; Meakins, G. D. Studies in the Steroid Group. Part 86. Preparation of Oxygenated Monofluoro- and *gem*-Difluoro-5α-androstanes Using Diethylaminosulphur Trifluoride and Tetra-n-butylammonium Fluoride. *J. Chem. Res. (M)* **1979**, 4728–4755.

(40) Kiesewetter, D. O. Synthesis of Fluorine-18 Labeled Estrogens for Receptor Based Imaging of Mammary Tumors. Ph.D. Dissertation, University of Illinois, Urbana, IL, 1979; pp 43–45.

(41) (a) Roberts, P. J.; Petterson, R. C.; Sheldrick, G. M.; Isaacs, N. W.; Kennard, O. Crystal and Molecular Structure of 17β-Hydroxyandrost-4-en-3-one (Testosterone). *J. Chem. Soc., Perkin Trans. 2* **1973**, *14*, 1978–1984. (b) Cowe, H. J. de L.; Cox, P. J.; Sim, G. A. Structure of 7α-Methyl-4-androstene-3,17-dione. *Acta Crystallogr.* **1982**, *38*, 662–664.

Table I. Coupling Pattern of 17α-H in ^1H NMR with or without D_2O

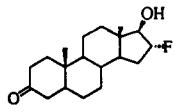
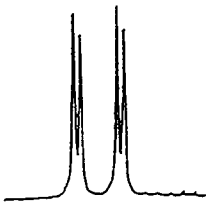
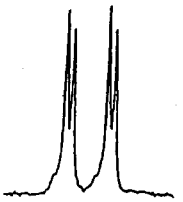
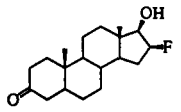
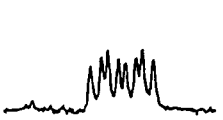
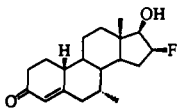
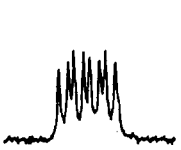
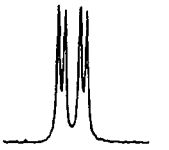
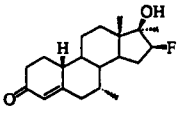
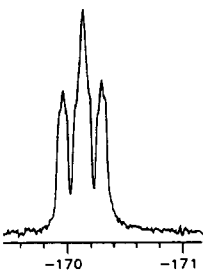
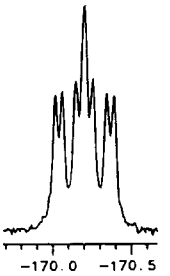
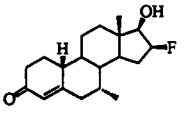
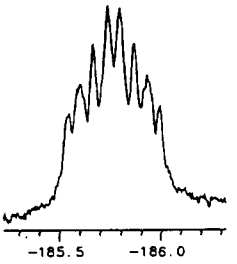
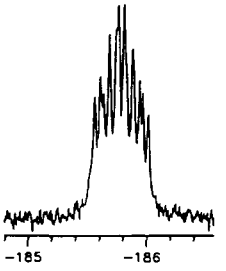
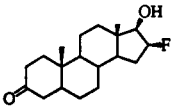
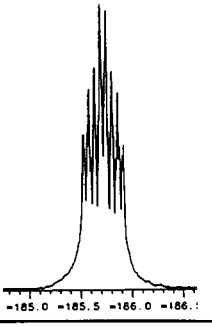
compounds	in $CDCl_3$	in $CDCl_3$ with D_2O added
 16α-F-DHT (200 MHz)		
 16β-F-DHT (200 MHz)		
 16β-F-Mib (300 MHz)		

Table II. ^{19}F NMR of Three 16β-Fluorinated Androgens with or without D_2O

compounds	in $CDCl_3$	in $CDCl_3$ with D_2O added
 16β-F-Mib (282 MHz)		
 16β-F-MNT (282 MHz)		
 16β-F-DHT (338 MHz)		

involving fluorine (O–H···F–C) have been reported,[42a] and the intramolecular H-bond between hydrogen and fluorine in a five-membered ring has been observed in fluoro alcohols.[42b] On the basis of MM2 calculations, the molecule with the fluorine and the hydroxy function on the same side of the D ring is more stable with an intramolecular H-bond than without it by about 2–4 kcal/mol, even though it is in a five-membered ring. Formation of this hydrogen bond does not cause the energy-minimized structure to undergo a visible change.

As shown in Table I, the 17α-H of the 16β-F-MNT as well as 16β-F-DHT, with both of the functional groups on the β-side, has a doublet of doublet of doublets coupling pattern that changed to a doublet of doublets with a D_2O shake, while the same H of the 16α-F-DHT with the two functional groups on opposite sides is a doublet of doublets that was not altered by the D_2O shake. It is reasonable to assume that the extra coupling in the 17α-H in 16β-F-MNT and 16β-F-DHT that disappeared with the D_2O shake is from the 17β-OH hydrogen that is engaged in an intramolecular H-bond with the syn-oriented 16β-fluorine substituent and thus not decoupled by rapid exchange. In ^{19}F NMR, the intramolecular H-bond was also observed. An extra small coupling was seen with the ^{19}F NMR obtained on a NT-360 spectrometer, and peaks were found to be broadened in the ^{19}F NMR obtained on a GE-300-WD. The extra coupling could be from the 17β-OH hydrogen which disappeared with D_2O shake, so that the coupling pattern sharpened, as shown in Table II.

Receptor Binding Assays and Structure–Activity Relationships. Relative binding affinities (RBA) of the fluorinated androgens we have prepared (as well as other androgens selected for comparison) for the androgen receptor (AR) were determined by a competitive radiometric binding assay, using as a source of AR, a cytosol preparation from the ventral prostate of 24 h orchidectomized mature male Holtzman rats. The incubation was at 0 °C for 18 h; [^3H]R1881 was used as a tracer, and dextran-coated charcoal as an absorbent for free ligands. In order to block any progesterone or corticosteroid receptors, the prostate cytosol was preincubated with triamicinolone acetonide.[43] For selected androgens, most of them having relatively high RBA values to AR, binding affinities to rat progesterone receptor (PgR), rat mineralocorticoid receptor (MR), and human sex steroid binding protein (SBP) have also been measured, using R5020, aldosterone, and estradiol as the standards, respectively. RBA values of the standards are 100 by definition, and details concerning RBA determination have been described elsewhere (See Experimental Section for references).

There are three proteins that bind androgens with high affinity: AR, SBP, and androgen binding protein (ABP). Even though SBP is very similar to ABP,[44] it is not present

(42) (a) Withers, S. G.; Street, I. P.; Rettig, S. The Preferred Conformation of 2-Fluoro-2-deoxy β-D-mannopyranosyl Fluoride. An X-ray Crystallographic and 2-Dimensional Proton Nuclear Magnetic Resonance Study. *Can. J. Chem.* **1986**, *64*, 232–236. (b) Murray-Rust, P.; Stallings, W. C.; Monti, C. T.; Preston, R. K.; Glusker, J. P. Intermolecular Interactions of the C-F Bond: The Crystallographic Environment of Fluorinated Carboxylic Acids and Related Structures. *J. Am. Chem. Soc.* **1983**, *105*, 3206–3214.

(43) Pinney, K. G.; Carlson, K. E.; Katzenellenbogen, J. A. [^3H]-DU41165: A High Affinity Ligand and Novel Photoaffinity Labeling Reagent for the Progesterone Receptor. *J. Steroid Biochem.* **1990**, *35*, 179–189.

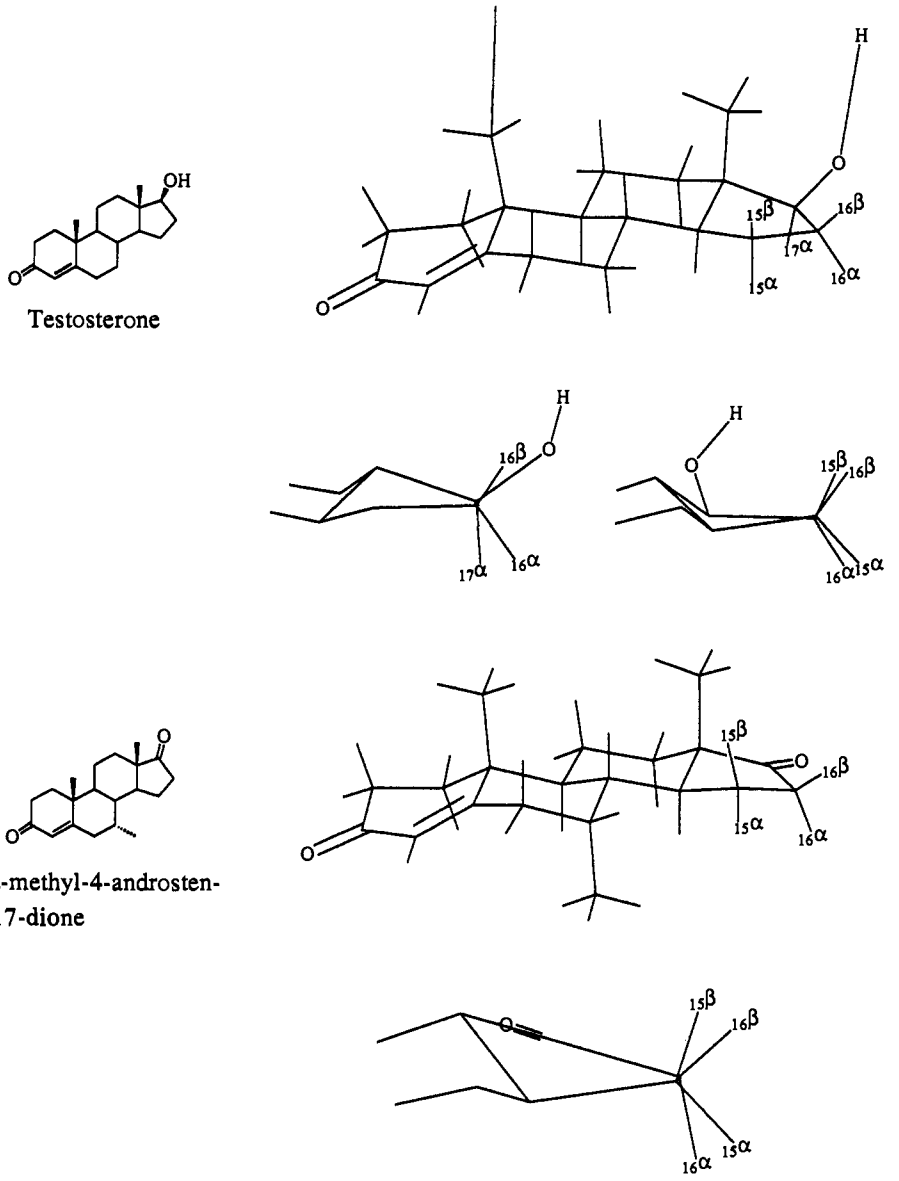


Figure 2. X-ray structure of androgens[41] and dihedral angles between D-ring H's.

in rats,[45] and thus should not interfere with our AR binding studies of androgens in rats either in vitro or in vivo. ABP is a protein, which in addition to AR, is present in rats and also binds tightly to androgens such as DHT (K_d = 0.8 nM) and testosterone (K_d = 2 nM).[46] However, when AR binding affinities of androgens are measured by our protocol, interference from ABP should be minimized because ABP is produced in rat testis and is found in testicular fluid and epididymis, but not in prostate.[47,48] Furthermore, DHT-AR complexes have a half life of longer than 4 days at 0 °C, whereas DHT-ABP complexes dissociate much more rapidly, with a half life of only 6 min.[46] Thus, they may be partially stripped during our charcoal treatment process (15 min at 0 °C).

RBA data of the fluorinated androgens and their protio analogues for AR, PgR, MR, and SBP are presented in Table III. Many of the fluorine-substituted androgens have high affinity for AR. In fact, seven fluorinated androgens, 16β-F-DHT (1), 16β-, 16α-, and 20-F-Mib (5, 6, and 10), 16β- and 16α-F-MNT (7 and 8), as well as 20-F-R1881 (9), have binding affinities for AR either close to or greater than 20% (relative to R1881), a level considered sufficient to merit their preparation in fluorine-18-labeled form for in vivo studies. The RBA value of 16β-F-T (3) is very low (2.1 vs 16β-F-DHT = 42.7), so that normally one might not expect this compound to be taken up or well

(44) Tindall, D. J.; Cunningham, G. R.; Means, A. R. Structural Requirements for Steroid Binding to Androgen Binding Proteins. *Int. J. Androl.* **1978**, Supplement 2, 434–447.

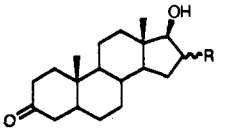
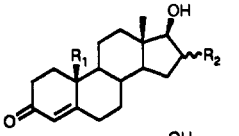
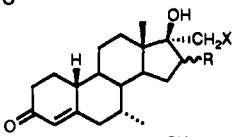
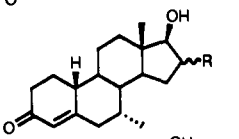
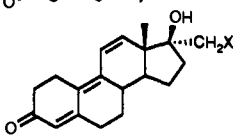
(45) (a) Murphy, B. E. P. Binding of Testosterone and Estradiol in Plasma. *Can. J. Biochem.* **1968**, *46*, 299–302. (b) Renoir, J. M.; Bodard, M. C.; Baulieu, E. E. Hormonal and Immunological Aspects of the Phylogeny of Sex Steroid Binding Plasma Protein. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 4578–4582.

(46) Tindall, D. J.; Cunningham, G. R.; Means, A. R. 5α-Dihydrotestosterone Binding to Androgen Binding Protein. *J. Biol. Chem.* **1978**, *253*, 166–169.

(47) Hansson, V.; Trygstad, O.; French, F. S.; McLean, W. S.; Smith, A. A.; Tindall, D. J.; Weddington, S. C.; Petrusz, P.; Nayfeh, S. N.; Ritzen, E. M. Androgen Transport and Receptor Mechanisms in Testis and Epididymis. *Nature* **1974**, *250*, 387–391 and references cited therein.

(48) Tindall, D. J.; Hansson, V.; McLean, W. S.; Ritzen, E. M.; Nayfeh, S. N.; French, F. S. Androgen-Binding Proteins in Rat Epididymis: Properties of a Cytoplasmic Receptor for Androgen Similar to the Androgen Receptor in Ventral Prostate and Different from Androgen-Binding Protein (ABP). *Mol. Cell. Endocrinol.* **1975**, *3*, 83–101.

Table III. RBA Values of Fluorinated Androgens and Their Protio Analogues for AR, PgR, MR, and SBP

compound		relative binding affinity[a]			
		AR[b]	PgR[c]	MR[d]	SBP[e]
	R = H (DHT)	60.2 ± 16.0	0.23 ± 0.10	1.17 ± 0.14	2125 ± 999
	R = β-F (1)	42.7 ± 2.0	0.12 ± 0.04	0.06 ± 0.01	385 ± 83
	R = α-F (2)	3.7 ± 0.8	0.07 ± 0.01	0.05 ± 0.01	228 ± 54
	R_1 = H, R_2 = H (nor-T)	30.6 ± 1.5	3.10 ± 1.00	2.00 ± 1.20	29.9 ± 6.8
	R_1 = CH_3, R_2 = H (T)	5.9 ± 1.3	0.09 ± 0.02	4.19 ± 3.44	417 ± 88
	R_1 = CH_3, R_2 = β-F (3)	2.1 ± 1.0	–	–	–
	R_1 = CH_3, R_2 = α-F (4)	0.3 ± 0.1	–	–	–
	R = H, X = H (Mib)	118.0 ± 4.0	20.4 ± 4.2	5.70 ± 0.47	19.0 ± 8.3
	R = β-F, X = H (5)	30.8 ± 5.4	3.0 ± 0.6	0.08 ± 0.01	1.3 ± 0.7
	R = α-F, X = H (6)	37.3 ± 9.8	14.4 ± 1.5	0.37 ± 0.09	3.1 ± 0.8
	R = H, X = F (10)[21]	53.2 ± 3.1	10.3 ± 2.0	0.86 ± 0.07	3.0 ± 1.9
	R = H (MNT)	155.7 ± 14.4	3.7 ± 0.1	0.74 ± 0.32	28.7 ± 9.4
	R = β-F (7)	36.5 ± 4.4	1.6 ± 0.3	0.05 ± 0.01	3.75 ± 0.93
	R = α-F (8)	21.9 ± 3.3	5.7 ± 3.7	0.21 ± 0.10	4.01 ± 1.50
	X = H (R1881)	(100)	43.7 ± 9.1	25.4 ± 1.3	4.0 ± 0.9
	X = F (9)	18.8 ± 5.6	10.5 ± 1.2	4.5 ± 1.4	2.2 ± 0.3
	3,3-difluoro-17β-androstanol (12)	2.19			
	17β-fluoro-3-androstanone (11)	0.07			
	17-fluoro-16-androsten-3-one (52)	0.06			
	17,17-difluoro-3-androstanone (51)	0.15			

[a] Relative binding affinities were determined by a competitive radiometric binding assay; details are described in the Experimental Section. Values are the average of two or more determinations ± range (n = 2) or sd ($n \geq 3$) and are expressed on a percent scale relative to the affinity of the tritium-labeled tracer R1881. [b] The tracer (RBA = 100) was [^{3}H]R1881, K_d = 0.6 nM. [c] The tracer (RBA = 100) was [^{3}H]-R5020, K_d = 0.4 nM. [d] The tracer (RBA = 100) was [^{3}H]aldosterone, K_d = 0.42 nM. [e] The tracer (RBA = 100) was [^{3}H]estradiol, K_d = 1.6 nM.

retained by prostate target tissue. We have, however, done in vivo tissue distribution studies of this compound in fluorine-18-labeled form,[22] since in the prostate it may be reduced by the intracellular 5α-reductase, converting it from a ligand with relatively low affinity for AR (16β-F-T = 2.1) to one with higher affinity (16β-F-DHT = 42.7). As will be discussed elsewhere,[21,22] seven compounds including all the ones mentioned above, except the 16α-F-Mib (6), have been prepared in fluorine-18-labeled form, and in vivo studies have given very encouraging results.

Fluorine substitution at either the C-16 or C-20 position decreases binding affinities of the androgens for all the receptors, as well as for SBP, to a greater or a lesser extent. For AR binding, 16β-fluorination of MNT lowers the RBA by 4.3-fold and of DHT by 1.4-fold, while 16α-fluorination decreases the RBA to an even greater extent: by 21.9-fold in T to 3.2-fold in Mib. Fluorination at the C-20 position is tolerated better in Mib, where the RBA of 20-F-Mib (10) is about half of its protio analogue Mib (53.2 vs 118), but not so well in R1881, where 20-fluorine substitution diminishes the RBA by 5.3-fold. The reduction in binding affinity may be due to the larger size of the fluorine atom (vs the H atom) or its high electronegativity, and in the 16β- and 20-fluoro androgens, an intramolecular hydrogen bond may exist between the fluorine and the nearby 17β-OH group that causes the OH function to depart from an orientation which may be critical for receptor binding.[49]

Table IV. RBA Ratios of Androgens

compound	AR/PgR[a]	AR/MR[a]	AR/SBP[a]
DHT(16-H)	262	51.5	0.03
1(16β-F)	356	689	0.16
2(16α-F)	56.1	75.5	0.02
Mib(16-,20-H)	5.8	20.7	6.2
5(16β-F)	10.3	385	23.7
6(16α-F)	2.6	101	12.0
10(20-F)	5.2	61.9	17.7
MNT(16-H)	42.1	210	5.4
7(16β-F)	23.4	730	9.7
8(16α-F)	3.8	104	5.5
R1881(20-H)	2.3	3.9	25.0
9(20-F)	1.8	4.2	8.5

[a] Ratios were obtained by dividing RBA (AR) with RBA (PgR), or RBA (MR), or RBA (SBP).

The binding affinity of these androgens to other steroid receptors (heterologous binding to PgR and MR) and to the steroid binding protein in human serum SBP is shown in Table III. There are substantial levels of glucocorticoid receptor (GR) and PgR in human prostate, whereas MR levels are low. The binding of all of the classes of androgens to GR is low (data not shown), but binding of these analogues by the other steroid receptors needs to be considered.

(49) Schmit, J. P.; Quivy, J. I.; Rousseau, G. G. The Androgen Receptor Binding Site: A Conformational Study of Steroids in Relation with Their Affinity-I. Interaction with the D-Ring. *J. Steroid Biochem.* **1980**, *13*, 1387–1394.

(50) Pomper, M. G.; VanBrocklin, H. F.; Thieme, A. M.; Thomas, R. D.; Kiesewetter, D. O.; Carlson, K. E.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A. 11β-Methoxy-, 11β-ethyl-, and 17α-Ethynyl-substituted 16α-Fluoroestradiols: Receptor-Based Imaging Agents with Enhanced Uptake Efficiency and Selectivity. *J. Med. Chem.* **1990**, *33*, 3143–3155.

Table V. β Preference in 16-Fluoro Androgens vs α Preference in 16-Fluoro Estrogens

compound	RBA		
androgens	β	α	β/α[a]
16-F-DHT	42.7	3.7	11.5
16-F-T	2.1	0.27	7.8
16-F-MNT	36.5	21.9	1.7
16-F-Mib	30.8	37.3	0.83
estrogens			
16-F-E_2[50]	13	54	0.24
16-F-11β-OCH_3-E_2[50]	10	26	0.38
16-F-17α-ethynyl-E_2[50]	28	108	0.26
16-F-7α-CH_3-E_2[b]	45.4	39.3	1.16

[a] β/α was obtained by dividing RBA of the 16-β-fluoro compounds with RBA of the 16-α-fluoro compounds. [b] Liu, A.; Van Brocklin, H. F., unpublished.

Whereas the binding affinity of the natural androgens DHT and T and of MNT to PgR and MR is minimal, Mib and R1881 show substantial affinity for these receptors. In most cases, however, fluorine substitution reduces this heterologous binding to quite an acceptable level. Only compounds **6**, **9**, and **10** show affinities greater than 10% that of R5020, the tracer compound for PgR. This binding may be of sufficient magnitude to cause receptor-mediated uptake by PgR in human prostate. T and DHT have very high affinities for the serum binder SBP, although fluorine substitution reduces this considerably. The synthetic androgens as expected, show lower SBP binding; that of the fluorine-substituted analogues is considerably lower yet.

On the basis of their high binding selectivity (see Table IV for AR/PgR, AR/SBP, and AR/MR binding affinity ratios), it appears that 16β- and 20-F-Mib (**5** and **10**), as well as 16β- and 16α-MNT (**7** and **8**) are the most promising candidates as in vivo imaging agents. However, as will be reported in the in vivo uptake studies,[22] other factors also have to be taken into consideration.

It is interesting that in terms of receptor binding, the stereochemical preference for fluorine substitution at C-16 for the most part differs for androgens and estrogens. As shown in Table V, most of the 16β-fluoro androgens are found to have higher AR binding affinities than the 16α-fluoro epimers, whereas most of the 16α-fluoro estrogens are better ligands for the estrogen receptors (ER). The AR binding RBA ratios of 16β-F-DHT and 16β-F-T to the corresponding 16α-fluoro epimers are 11.5 and 7.8, respectively, whereas the ER binding β/α ratios are lower than 0.38 in the three pairs. The situation is changed when the 7α-methyl function is introduced into these molecules: The 16β- and 16α-fluorine epimers in the 7α-methyl series tend to bind equally well, as displayed by two pairs of the fluorinated androgens as well as one pair of estrogens.

The AR binding affinities of the four steroids in which an oxygen function is replaced by a fluorine (compound **11**, **12**, **51**, and **52**) are shown in Table III. All compounds have low affinity for AR, in particular those in which the 17β-hydroxyl function is replaced by fluorine. This indicates that in terms of AR binding, a single fluorine substituent is not a good mimic of a hydroxyl function, nor a geminal fluorine substituent of a carbonyl group.

Conclusions

We have prepared nine fluorine-substituted androgens and found that many of them have high binding affinity for the androgen receptor. In general, fluorine substitution lowers receptor binding by a modest factor, and while the natural androgens have lower affinity for other steroid hormone receptors (PgR and MR), the synthetic androgens have lower affinity for the human sex steroid binding protein. In all cases, fluorine substitution lowers this heterologous binding. From these compounds we have selected six for further study as prostate imaging agents. Their preparation in fluorine-18-labeled form and the results of tissue distribution studies in rats will be described elsewhere.[22]

Experimental Section

Chemical Synthesis. General. Solvents and reagents were purchased from the following commercial sources: Aldrich, Mallinckrodt, Sigma, Fisher, Baker, Eastman, or Alfa. THF and Et_2O were distilled from sodium benzophenone ketyl immediately prior to use. DMSO, CH_2Cl_2, MeOH, hexane, and xylenes were distilled from calcium hydride. Others were used as received, unless otherwise noted. Tetra-*n*-butylammonium monopersulfate (TBA-oxone) was prepared according to Trost's procedure.[51] 2-Methyl-2-ethyl-1,3-dioxolane was synthesized from methyl ethyl ketone and ethylene glycol, by following literature procedure.[52] 7α-Methyl-19-nortestosterone was a generous gift from Upjohn, and part was synthesized in our lab from 19-nortestosterone as discussed before.[35] Other steroidal compounds used were obtained from the following resources: Steraloids, Sigma, or Searle Laboratories.

Analytical thin-layer chromatography (TLC) was performed using 0.25-mm silica gel plastic- or glass-backed plates with or without F-254 indicator (Merck). Visualization was achieved by phosphomolybdic acid (PMA) or anisaldehyde spray reagents or by UV illumination. Flash chromatography was performed according to Still,[53] using Woelm silica gel (0.032–0.064 mm) and Merck silica gel (0.040–0.063 mm). Analytical gas–liquid chromatography (GLC) was performed in a Hewlett-Packard 5793A instrument equipped with a flame ionization detector. High-performance liquid chromatography (HPLC) was performed isocratically on a Spectra-Physics Model 8700 or a Varian 5060 liquid chromatograph with an analytical 5-μm SiO_2 column (4.6 mm × 30 cm, Varian Si-5 Micro Pak) or a preparative SiO_2 column (Whatman Partisil M-9, 0.9 cm × 50 cm).

Melting points were determined on either a Thomas Hoover, Fisher Johns, or Hacker melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1320 or an IBM IR/32 FT instrument. The data are given in cm^{-1} with only diagnostic bands reported. Proton magnetic resonance (^1H NMR) spectra were obtained on a Varian XL-200 (200 MHz) or a General Electric QE-300 (300 MHz) spectrometer using tetramethylsilane as an internal standard. The data are reported on the δ scale in the form: chemical shift (peak multiplicity, number of protons, coupling constants, assignment). ^{19}F NMR was obtained on a Nicolet NT-360 (at 338 MHz) and a General Electric GN-300 WB (at 282 MHz) spectrometer, using fluorotrichloromethane as an internal standard and deuterochloroform as a solvent. Low-resolution electron-impact mass spectra were obtained on a Finnigan MAT CH-5 spectrometer. High-resolution electron-impact exact mass determinations were obtained on a Varian MAT 731 spectrometer. Both low- and high-resolution fast atom bombardment (FAB) mass spectra were obtained on a VG instrument (ZAB HF), employing a dithiothreitol matrix. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois.

Unless stated otherwise, a general procedure for product isolation was used as follows: the reaction mixture was quenched with water (or aqueous acids or bases as specified), and extacted with Et_2O (or specified solvents) three times; the organic solution was then washed with saturated aqueous $NaHCO_3$ as well as brine

(51) Trost, B. M.; Braslau, R. Tetra-*n*-butylammonium Oxone. Oxidations under Anhydrous Conditions. *J. Org. Chem.* **1988**, *53*, 532–537.

(52) Dauben, H. J., Jr.; Lōken, B.; Ringold, H. J. Preparation of Ethylenedioxy Derivatives of Ketosteroids by Exchange Dioxolanation. An Improved Synthesis of Testosterone from Δ^4-Androstene-3,17-dione. *J. Am. Chem. Soc.* **1954**, *76*, 1359–1363.

(53) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(or water), dried over anhydrous Na_2SO_4 or $MgSO_4$ and the solvents were evaporated in vacuo.

3,3-(Ethylenedioxy)androstan-17-one (14). 5α-Androstane-3,17-dione (13, 3.5 g, 12.1 mmol) was dissolved in 20 mL of 2-methyl-2-ethyl-1,3-dioxolane and cooled to 0 °C in an ice-water bath. TsOH (0.13 g, 0.68 mmol) was added with stirring. After being stirred at 0 °C for 25 min, the reaction mixture, with a copious white precipitate formed, was diluted with hexane (20 mL) and filtered. The filtrate was washed with three 20-mL portions each of saturated $NaHCO_3$ solution and water twice and dried (Na_2SO_4). The solvents were evaporated and the residue as well as the solid (product) collected during filtration was purified by recrystallization, yielding 3.57 g (88.6%) of white crystalline monoketal 14: mp 153.5–154.5 °C (lit.[30] mp 155–156 °C); IR (KBr) 1750, 1745, 1102 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.86 (s, 3 H, 19-CH_3), 0.88 (s, 3 H, 18-CH_3), 3.92 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2); MS (70 eV) m/z (rel intensity) 332 (M^+, 5), 125 (20), 99 (100). Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.93; H, 9.88.

17-Acetoxy-3,3-(ethylenedioxy)androst-16-ene (15). Monoketal 14 (400 mg, 1.5 mmol) and TsOH (0.1 g, 0.52 mmol) were combined in isopropenyl acetate (25 mL). The solution was refluxed for 24 h. At this point, an additional 0.1 g of TsOH was added and the mixture was again refluxed for 24 h. The brown solution, diluted with Et_2O, was then sequentially washed with cold saturated $NaHCO_3$ and cold water and dried over Na_2SO_4. The solvents were removed in vacuo, and purification was achieved by flash chromatography (4:1 hexane–ethyl acetate) (silica gel was predried at 100 °C for 2 h). Enol acetate 15 (0.34 g, 60.3%) was yielded as a white crystalline solid: mp 144–145 °C; IR (KBr) 1769, 1758, 1216, 1098 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.86 (s, 3 H, 19-CH_3), 0.88 (s, 3 H, 18-CH_3), 2.14 (s, 3 H, 17-acetyl CH_3), 3.93 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.46 (dd, 1 H, J = 3.18 Hz, 2.27 Hz, 16-H); MS (70 eV) m/z (rel intensity) 374 (M^+, 10), 332 (89), 125 (9), 99 (100). Anal. Calcd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.80; H, 9.04.

16β-Hydroxy-3,3-(ethylenedioxy)androstan-17-one (16). To a solution of iodobenzene bis(trifluoroacetate) (0.5 g, 1.16 mmol) in CH_3CN (2 mL) was added at room temperature (RT) with stirring a solution of enol acetate 15 (245 mg, 0.66 mmol) in CH_2Cl_2 (1.5 mL), and the mixture was stirred at RT for 1.5 h and then cooled to −10 °C. To hydrolyze the formed 16β-trifluoroacetoxy intermediate, 4 mL of EtOH plus 4-mL solution of 1% $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ were added, and the mixture was stirred for 30 min at RT. After the product isolation, purification was achieved by flash chromatography (4:1 hexane–ethyl acetate). 16β-Hydroxy compound 16 (154 mg, 67.5%) was afforded as a white crystalline solid: mp 165–167 °C; IR (KBr) 3550, 1730, 1099 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.84 (s, 3 H, 19-CH_3), 0.94 (s, 3 H, 18-CH_3), 3.94 (s, 3 H, 3,3-ethylenedioxy CH_2CH_2), 2.34 (dd, 1 H, J = 7.2 Hz, 4.4 Hz, one of 15-H), 2.40 (dd, 1 H, J = 7.2 Hz, 4.4 Hz, another 15-H), 3.95 (t, 1 H, J = 9.09 Hz, 16α-H); MS (70 eV) m/z (rel intensity) 348 (M^+, 7), 125 (25), 99 (100). Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.22; H, 9.35.

16α-Fluoro-3,3-(ethylenedioxy)androstan-17-one (17) and 16α-Fluoro-androstane-3,17-dione (17a). A solution of 16β-hydroxy ketone 16 (1.0 g, 2.87 mmol) in CH_2Cl_2 (30 mL) was added to a solution of (diethylamido)sulfur trifluoride (DAST) (2.00 mL, 14.35 mmol) in CH_2Cl_2 (30 mL) contained in a polyethylene vial at −78 °C. The combined solution was stirred at −78 °C for 10 min and at RT for 1 h and refluxed for 35 min. The reaction mixture was then filtered through basic alumina, and the filtrate was washed with water, dried (Na_2SO_4), and concentrated in vacuo. Purification by flash chromatography (2:1 hexane–ethyl acetate) yielded 0.71 g (70.5%) of the white crystalline 16α-fluoro ketone 17: mp 186–187 °C; IR (KBr) 1768, 1111 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.82 (s, 3 H, 19-CH_3), 0.91 (s, 3 H, 18-CH_3), 3.93 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.10 (dd, 1 H, J_{HF} = 51 Hz, J_{HH} = 6.9 Hz, 16β-H); MS (70 eV) m/z (rel intensity) 350 (M^+, 10), 125 (28), 99 (100). Anal. Calcd for $C_{21}H_{31}O_3F$: C, 71.97; H, 8.92; F, 5.42. Found: C, 71.45; H, 8.89; F, 5.37.

16α-Fluoro ketone 17 (20 mg, 0.06 mmol) was dissolved in acetone (5 mL) and treated with 1 mL aqueous HCl (3 N). After it was stirred at RT for 1 h, product isolation gave a crude mixture that was purified by preparative TLC (4:1 hexane–ethyl acetate) to give 14.9 mg (85.1%) of the purified 16α-fluoro dione 17a as a white crystalline solid: mp 211–212 °C; ^1H NMR (200 MHz, $CDCl_3$) 0.94 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 5.10 (ddd, 1 H, J_{HF} = 50.2 Hz, J_{HH} = 8.0 Hz, 1.2 Hz, 16β-H); ^{19}F NMR (338.8 MHz, $CDCl_3$) −192.88 (dt, J_{HF} = 51.4 Hz, 28.6 Hz); MS (70 eV) m/z (rel intensity) 306 (M^+, 37), 232 (100), 217 (63), 124 (24), 109 (15). HRMS calcd for $C_{19}H_{27}O_2F$ 306.1995, found 306.1989.

16β-Fluoro-3,3-(ethylenedioxy)androstan-17-one (18). 16α-Fluoro ketone 17 (0.435 g, 1.2 mmol) and nBu_4NF (1 mL in THF, 1 mmol) were combined in THF (50 mL). The solution was stirred at RT for 40 min and then passed through silica gel. Flash chromatography (5:1 hexane–ethyl acetate) purification of the residue after concentration gave 0.173 g (39.8%) of the 16β-fluoro ketone 18 as a white crystalline solid and 0.193 g (44.4%) of the recovered starting material 17. 16β-Fluoro ketone 18 has a mp 191–192 °C: IR (KBr) 1755, 1106 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.85 (s, 3 H, 19-CH_3), 0.99 (s, 3 H, 18-CH_3), 2.48 (ddd, 1 H, J_{HF} = 12.75 Hz, J_{HH} = 7.90 Hz, 4.84 Hz, 15β-H), 3.94 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.70 (dt, 1 H, J_{HF} = 50.2 Hz, J_{HH} = 8.09 Hz, 16α-H); MS (70 eV) m/z (rel intensity) 350 (M^+, 7), 125 (21), 99 (100); HRMS calcd for $C_{21}H_{31}O_3F$ 350.2257, found 350.2261. Anal. Calcd for $C_{21}H_{31}O_3F$: C, 71.97; H, 8.92; F, 5.42. Found: C, 71.95; H, 8.94; F, 5.41.

16α-Fluorodihydrotestosterone (2) and 17α-Hydroxy-16α-fluoroandrostan-3-one (2a). To a suspension of $LiAlH_4$ (0.344 g, 8.62 mmol) in dry Et_2O (20 mL) cooled to −78 °C was added dropwise with stirring 16α-fluoro ketone 17 (0.275 g, 0.78 mmol) in Et_2O (35 mL). The mixture was stirred at −78 °C for 15 min, followed by 20 min at RT. It was then quenched with EtOAc. The product isolated was redissolved in 25 mL of acetone and treated with 15 mL of aqueous HCl (3 N) at RT for 2 h. The crude product was then purified by flash chromatography (5:4 hexane–ether, twice, very carefully), and 0.163 g (53.7%) of 17β-hydroxy epimer 2 and 45.3 mg (15.0%) of 17α-hydroxy epimer 2a were isolated. 17β-Hydroxy epimer 2 (white crystalline solid): mp 164–165 °C; IR (KBr) 3600–3040 (br), 1708, 1089, 1062, 1036 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.88 (s, 3 H, 18-CH_3), 1.02 (s, 3-H, 19-CH_3), 3.77 (dd, 1 H, J_{HF} = 28 Hz, J_{HH} = 5 Hz, 17α-H), 4.92 (ddt, 1 H, J_{HF} = 56 Hz, J_{HH} = 6.3 Hz, 5 Hz, 16β-H); ^1H NMR (300 MHz, $CDCl_3$ with D_2O shake) 0.78 (s, 3 H, 18-H), 1.01 (s, 3-H, 19-H), 3.76 (dd, 1 H, J_{HF} = 28.8 Hz, J_{HH} = 4.5 Hz, 17α-H), 4.82 (br s, HOD), 4.92 (ddt, 1 H, J_{HF} = 57 Hz, J_{HH} = 7.2 Hz, 4.2 Hz, 16β-H); ^{19}F NMR (338.8 MHz, $CDCl_3$) −180.56 (dq, J_{HF} = 55.2 Hz, 28.5 Hz); MS (70 eV) m/z (rel intensity) 308 (M^+, 30), 288 (79), 270 (19), 255 (15), 244 (44), 231 (100), 217 (37), 124 (17), 109 (13); HRMS calcd for $C_{19}H_{29}O_2F$ 308.2152, found 308.2149. Anal. Calcd for $C_{19}H_{29}O_2F$: C, 73.99; H, 9.48; F, 6.16. Found: C, 74.11; H, 9.50; F, 6.05.

The 17α-hydroxy epimer 2a (white crystalline solid): mp 195–196 °C; IR (KBr) 3550–3300 (br), 1689, 1110 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.69 (s, 3 H, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 3.78 (br d, 1 H, J = 5.57 Hz, 17β-H), 5.25 (dtd, 1 H, J_{HF} = 50 Hz, J_{HH} = 5.6 Hz, 2.4 Hz, 16β-H); ^1H NMR (200 MHz, DMSO dilute) 0.61 (s, 3 H, 18-CH_3), 0.94 (s, 3 H, 19-CH_3), 3.56 (br t, 1 H, J_{HF} = 5.0 Hz, J_{HH} = 5.0 Hz, 17β-H), 4.66 (dd, 1 H, J = 4.4 Hz, 1.6 Hz, 17β-OH), 5.13 (dm, 1 H, J_{HF} = 50.2 Hz, 16β-H); ^{19}F NMR (338.8 MHz, $CDCl_3$) −197.08 (dtd, J_{HF} = 53.9 Hz, 29.6 Hz, 9.1 Hz); MS (70 eV) m/z (rel intensity) 308 (M^+, 34), 288 (68), 270 (23), 255 (22), 244 (43), 231 (100), 217 (51), 124 (29), 110 (21); HRMS calcd for $C_{19}H_{29}O_2F$ 308.2151, found 308.2143.

16β-Fluorodihydrotestosterone (1). A suspension of $LiAlH_4$ (0.204 g, 5.00 mmol) in 15 mL dry Et_2O was cooled to −78 °C. Under protection of N_2, a solution of 16β-fluoro ketone 18 (0.110 g, 0.31 mmol) in 20 mL of Et_2O was added dropwise with stirring. Then the heterogenous mixture was treated in the same way as that described above in the synthesis of compound 2 and 2a. Purification by flash chromatography (5:4 hexane–ether) afforded 89 mg (92.7%) of the product 1 as a white crystalline solid: mp 180–181 °C; IR (KBr) 3525, 3389 (shoulder peak), 1705, 1079 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.94 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 3.39 (ddd, 1 H, J_{HF} = 25.5 Hz, J_{HH} = 9 Hz, 4.8 Hz, 17α-H), 4.95 (dddd, 1 H, J_{HF} = 55.2, Hz, J_{HH} = 5.2 Hz, 4.8 Hz, 2.4 Hz, 16α-H); ^1H NMR (200 MHz, $CDCl_3$ with D_2O shake) 0.83 (s, 3 H, 18-H), 1.03 (s, 3 H, 19-H), 3.36 (dd, 1 H, J_{HF} = 20.4 Hz, J_{HH} = 6.4 Hz, 17α-H), 4.77 (br s, HOD), 4.93 (dddd, 1 H, J_{HF} = 55.8 Hz, J_{HH} = 7.4 Hz, 6.4 Hz, 3.2 Hz, 16α-H); ^1H NMR (300 MHz, DMSO) 0.71 (s, 3 H, 18-H), 0.95 (s, 3 H, 19-H), 3.18 (dt, 1 H, J_{HF}

= 23.4 Hz, J_{HH} = 6.3 Hz, 17α-H), 4.71 (d, 1 H, J = 6.6 Hz, 17β-OH), 4.75 (dddd, 1 H, J_{HF} = 56.1 Hz, J_{HH} = 7.5 Hz, 6.3 Hz, 3.3 Hz, 16α-H); ^{19}F NMR (338.8 MHz, $CDCl_3$) −185.54 (dddd, J_{HF} = 55.7 Hz, 33.9 Hz, 23.4 Hz, 18.6 Hz, 5.1 Hz); MS (70 eV) m/z (rel intensity) 308 (M^+, 25), 288 (93), 270 (19), 255 (15), 244 (46), 231 (100), 217 (44), 124 (23), 110 (16); HRMS calcd for $C_{19}H_{29}O_2F$ 308.2151, found 308.2153. Anal. Calcd for $C_{19}H_{29}O_2F$: C, 73.99; H, 9.48. Found: C, 74.18; H, 9.54.

3,3-(Ethylenedioxy)-16-androsten-17-yl Diethyl Phosphate (19). A solution of LDA (3 mmol, freshly made at 0 °C) in THF (4.5 mL) was cooled to −78 °C, and ketone 14 (0.5 g, 1.5 mmol) was added as a solid in one batch. After 2 min, the cooling bath was removed and the solution was stirred at RT until a copious white precipitate formed (~6 min). It was cooled to −78 °C again, and $ClP(O)(OEt)_2$ (0.45 mL, 3.1 mmol) was added via a syringe. The mixture was then allowed to warm up until a homogenous solution was formed. It was cooled to −78 °C again to slow down the reaction before being warmed up gradually to RT (~1.5 h). The isolated product was subjected to flash chromatography (hexane–EtOAc = 1:1), yielding 0.53 g (75.7%) of the pure product 19 as a white flakelike crystalline solid: mp 95–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 0.84 (s, 3 H, 19-CH_3), 0.91 (s, 3 H, 18-CH_3), 1.35 (t, 6 H, J = 7.2 Hz, 2 ethoxy OCH_2CH_3), 3.94 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.17 (quintet, 4 H, J_{HP} = 7.2 Hz, J_{HH} = 7.2 Hz, 2 ethoxy OCH_2CH_3), 5.20 (t, 1 H, J = 1.2 Hz, 16-H); MS (70 eV) m/z (rel intensity) 468 (M^+, 12), 314 (7), 299 (10), 155 (8), 125 (29), 99 (100), 55 (8). Anal. Calcd for $C_{25}H_{41}O_6P$: C, 64.08; H, 8.82; P, 6.61. Found: C, 64.16; H, 8.88; P, 6.39.

16α-Hydroxy-3,3-(ethylenedioxy)androstan-17-one (20). A solution of enol phosphate 19 (0.46 g, 0.98 mmol) in 14 mL of acetone and *N*-methylmorpholine *N*-oxide (0.15 g, 1.27 mmol) in 3.7 mL of water were mixed together, and a solution of OsO_4 in tBuOH (2.5%, 30 μL, catalyst) was added. The mixture was stirred at RT for 2.5 h. Purification of the isolated product by flash chromatography (hexane–EtOAc = 1:1) gave 0.28 g (81%) of the pure 16α-hydroxy ketone 20 as white cottonlike crystals: mp 179–181 °C; IR (KBr) 3200–3600 (br), 1742, 1101, 1071 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 0.84 (s, 3 H, 19-CH_3), 0.96 (s, 3 H, 18-CH_3), 2.37 (s, less than 1 H, 16-O*H*), 3.94 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.38 (br d, 1 H, J = 8.2 Hz, 16β-H); MS (70 eV) m/z (rel intensity) 348 (M^+, 8), 125 (31), 99 (100). Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.39; H, 9.26. Found: C, 72.04; H, 9.28.

16α-[[(Trifluoromethyl)sulfonyl]oxy]-3,3-(ethylenedioxy)androstan-17-one (21). 16α-Hydroxy ketone 20 (0.1 g, 0.29 mmol) was dissolved in CH_2Cl_2 (7 mL) and cooled to 0 °C. Under N_2, 2,6-lutidine (100 μL, 0.86 mmol) and Tf_2O (87 μL, 0.52 mmol) were added sequentially with stirring. The reaction mixture was stirred at 0 °C for 20 min before it was diluted with EtOAc and passed through a short silica gel column. The product, very unstable on silica gel, was purified by a quick flash chromatography (hexane–EtOAc = 2:1, prechilled) and 0.13 g (93.5%) of white crystalline 21 was obtained: mp 125 °C with decomposition; ^{1}H NMR (200 MHz, $CDCl_3$) 0.82 (s, 3 H, 19-CH_3), 0.95 (s, 3 H, 18-CH_3), 2.11 (d, 1 H, J = 4.6 Hz, one of 15-H), 2.17 (d, 1 H, J = 4.6 Hz, another 15-H), 3.93 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.35 (t, 1 H, J = 4.6 Hz, 16β-H); MS (FAB) m/z (rel intensity) 481 (M + 1, 45); HRMS (FAB) calcd M + 1 for $C_{22}H_{31}O_6SF_3$ 481.1872, found 481.1874.

3,3-(Ethylenedioxy)-5-androsten-17β-ol (23). A flask equipped with a Dean–Stark trap was charged with testosterone 22 (20 g, 69.2 mmol), followed by addition of benzene (600 mL), ethylene glycol (54 mL), and TsOH (0.25 g, 1.3 mmol). The resulting two-phase mixture was refluxed for 24 h until no more water distilled from the reaction mixture. The isolated crude product was purified by recrystallization from EtOH and water, and 19.9 g (86%) of the white crystalline 23 was yielded: mp 182–183 °C (lit.[52] mp 183–184 °C); ^{1}H NMR (200 MHz, $CDCl_3$) 0.76 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 2.58 (dm, 1 H, J = 18 Hz, one of 4-H), 3.74 (t, 1 H, J = 7.12 Hz, 17α-H), 3.95 (br s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.37 (m, 1 H, 6-H); MS (70 eV) m/z (rel intensity) 332 (M^+, 3), 99 (100). Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.53; H, 9.95.

3,3-(Ethylenedioxy)-5-androsten-17-one (24). To a solution of 17β-hydroxy compound 23 (2 g, 6.0 mmol) in CH_2Cl_2 (150 mL) was added PCC (2.1 g, 9.7 mmol) in one portion. The heterogenous mixture was then stirred at RT for 2 h, during which time it turned from bright orange to dark brown. The reaction mixture was diluted with Et_2O and filtered through Celite. The filtrate was washed (saturated $NaHCO_3$ 2 × 30 mL and water 2 × 30 mL) and dried (Na_2SO_4). Flash chromatography (hexane–EtOAc = 2:1) gave 1.58 g (79.4%) of the pure ketone 24 as a white crystalline solid: mp 197–199 °C (lit.[54] mp 196–198 °C); ^{1}H NMR (200 MHz, $CDCl_3$) 0.89 (s, 3 H, 18-CH_3), 1.07 (s, 3 H, 19-CH_3), 2.40–2.68 (complex pattern, 3 H, 2 × 16-H, 1 × 4-H), 3.97 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.40 (br s, 1 H, 6-H); MS (70 eV offscale) m/z (rel intensity) 330 (M^+, 22), 131 (15), 105 (36), 99 (100), 91 (57), 55 (100). Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.41, H, 9.21.

3,3-(Ethylenedioxy)androst-5,16-dien-17-yl Trimethylsilyl Ether (25). A LDA solution (1.8 mmol) in THF (7 mL), freshly prepared in situ at 0 °C, was cooled to −10 °C. Under protection of N_2, a solution of ketone 24 (60 mg, 0.18 mmol) in 5 mL of dry THF was added dropwise via a syringe, and the mixture was stirred at −7 to −10 °C for 10 min before addition of TMSCl (0.31 mL, 2.4 mmol). It was stirred for another 10 min and then diluted with Et_2O. The formed precipitate was filtered, and the filtrate was washed (water 2 × 15 mL), dried ($MgSO_4$), and concentrated. The product purification by flash chromatography (hexane–EtOAc = 4:1) gave 67.1 mg (92%) of the pure enol silyl ether 25 as a white solid. A sample for identification was further purified by recrystallization from hexane to give a white crystalline solid: mp 171–172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 0.08–0.27 (m, 9 H, 17-$OSi(CH_3)_3$), 0.87 (s, 3 H, 18-CH_3), 1.05 (s, 3 H, 19-CH_3), 2.60 (dm, 1 H, J = 12.6 Hz, one of 4-H), 3.93 (m, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.48 (t, 1 H, J = 2 Hz, 16-H), 5.36 (m, 1 H, 6-H); MS (70 eV offscale) m/z (rel intensity) 402 (M^+, 100), 387 (68), 343 (24), 285 (14), 181 (62), 169 (99), 155 (45), 131 (59), 119 (98), 105 (100), 99 (100); (10 eV) 402 (M^+, 100), 99 (100); HRMS calcd for $C_{24}H_{38}O_3Si$ 402.2590; found 402.2585.

16α-Hydroxy-4-androstene-3,17-dione (26). Enol silyl ether 25 (100 mg, 0.25 mmol) was dissolved in CCl_4 (20 mL) and to this solution was added NBS (52 mg, 0.29 mmol) as a solid. The heterogenous mixture was stirred at RT for 2 h and then filtered. After concentration, the residue was redissolved in 10 mL of THF and treated with aqueous HCl (2 N, 5 mL) at RT for 2 h to remove the ketal protecting function. The isolated product was purified by flash chromatography (hexane–EtOAc = 1:1), giving 68 mg (75%) of pure 16α-bromo ketone intermediate as a white solid which has the same R_f as an authentic sample purchased from Steraloids. A white crystalline sample recrystallized from EtOH and water has a mp 166–168 °C (same as that of the authentic sample): ^{1}H NMR (200 MHz, $CDCl_3$) 0.96 (s, 3 H, 18-CH_3), 1.21 (s, 3 H, 19-CH_3), 4.54 (dd, 1 H, J = 4.8 Hz, 3.5 Hz, 16β-H), 5.74 (s, 1 H, 4-H).

The bromo ketone intermediate (68 mg, 0.19 mmol) was dissolved in 4 mL of THF and treated with aqueous KOH (1 mL × 5 N). The two-phase mixture was stirred vigorously at RT for ca. 5 h until TLC showed that most of the starting material was consumed. The product isolation and purification of the crude material by flash chromatography (hexane–EtOAc = 1:1) gave 37.6 mg (88.3% based on the consumed starting material) of the pure hydroxy ketone 26 as a white solid that has the same R_f on TLC as that of an authentic sample from Steraloids. A sample was recrystallized from EtOH and water, giving white crystals with a mp 185.5–187 °C (same as that of the authentic sample); ^{1}H NMR (200 MHz, $CDCl_3$) 1.01 (s, 3 H, 18-CH_3), 1.20 (s, 3 H, 19-CH_3), 4.38 (br d, 1 H, J = 7.4 Hz, 16β-H), 5.74 (s, 1 H, 4-H).

3,16α-Bis[[(trifluoromethyl)sulfonyl]oxy]androst-3,5-dien-17-one (27). 16α-Hydroxy compound 26 (50 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to 0 °C in ice–water. Under protection of N_2, 2,6-lutidine (25 μL, 0.2 mmol) and Tf_2O (30 μL, 0.18 mmol) were added, and the pinkish solution was stirred at 0 °C for 30 min. This process was repeated once more to complete the reaction. The reaction mixture was then diluted with Et_2O and passed through a short silica gel column. The solvents were evaporated in vacuo, and flash chromatography

(54) Brandes, S. J. Design, Synthesis and Biological Properties of Fluorinated Estrogens, Progestins and Androgens, Potential Imaging Agents for Receptor-Positive Mammary and Prostate Tumors. Ph.D. Dissertation, University of Illinois, Urbana, IL, 1986; p 172.

(hexane–EtOAc = 4:1) purification gave 77.9 mg of the pure bistriflate **27** as a white crystalline solid: mp 148 °C, started decomposing and turned to black tar; ^{1}H NMR (200 MHz, $CDCl_3$) 0.98 (s, 3 H, 18-CH_3), 1.03 (s, 3 H, 19-CH_3), 5.38 (t, 1 H, J = 4.7 Hz, 16β-H), 5.58 (br s, 1 H, 6-H), 6.01 (d, 1 H, J = 2.7 Hz, 4-H); MS (70 eV) m/z (rel intensity) 566 (M^+, 100), 433 (14), 391 (20), 291 (20), 283 (28), 265 (47), 255 (52), 213 (20), 131 (68), 105 (42), 91 (57), 68 (83), 55 (52); HRMS (EI) calcd for $C_{21}H_{24}O_7S_2F_6$ 566.0868; found 566.0869.

16β-Fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]androst-3,5-dien-17-one (28). 16α-Triflate **27** (24 mg, 0.042 mmol) was dissolved in dry THF (24 mL) and treated with nBu_4NF (45 μL of 1 M solution in THF, 0.045 mmol). After being stirred at RT for 7 min, the reaction mixture was diluted with Et_2O and passed through silica gel. It was concentrated in vacuo, and 17.2 mg (93.5%) of the pure 16β-fluoro ketone **28** was isolated as a white crystalline solid from the crude material by flash chromatography (hexane–EtOAc = 4:1): mp 145–146.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.99 (s, 3 H, 18-CH_3), 1.05 (s, 3 H, 19-CH_3), 4.73 (dt, 1 H, J_{HF} = 50.2 Hz, J_{HH} = 8.4 Hz, 16α-H), 5.58–5.62 (very br s, 1 H, 6-H), 6.01 (br s, 1 H, 4-H); MS (70 eV) m/z (rel intensity) 436 (M^+, 100), 303 (63), 285 (32), 261 (45), 179 (35), 161 (29), 131 (42), 115 (34), 105 (75), 91 (81), 69 (84), 55 (56); HRMS (EI) calcd for $C_{20}H_{24}O_4F_4S$ 436.1331; found 436.1331. Anal. Calcd for $C_{20}H_{24}O_4F_4S$: C, 55.04; H, 5.54. Found: C, 55.26; H, 5.65.

16β-Fluorotestosterone (3). Fluoro ketone **28** (8 mg, 0.018 mmol) was dissolved in 2.5 mL of dry THF and added to a suspension of large excess $LiAlH_4$ in another 2.5 mL of dry THF cooled to −78 °C prior to the addition. The heterogenous mixture was stirred at −78 °C for 10 min. The isolated product was then redissolved in a mixture of solvents (MeOH–CH_2Cl_2 = 1:1, 1 mL) and treated with 0.12 mL of KOH solution in MeOH (5 N). It was stirred at 60 °C for ca. 10 min until TLC showed complete consumption of the starting material before being quenched with aqueous NH_4Cl (20%) solution. Product isolation and purification by flash chromatography (hexane–EtOAc = 1:1) gave 4 mg (72%) of the pure product **3** as a white crystalline solid. It was further purified by normal phase HPLC (50% hexane–50% CH_2Cl_2 (5% iPrOH), 5 mL/min, t_R = 11 min) for identification and biological tests: mp 143–145.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.87 (s, 3 H, 18-CH_3), 1.21 (s, 3 H, 19-CH_3), 3.39 (ddd, 1 H, J_{HF} = 21.3 Hz, J_{HH} = 11.1 Hz, 6.2 Hz, 17α-H), 4.96 (dddd, 1 H, J_{HF} = 55.2 Hz, J_{HH} = 11.1 Hz, 8.4 Hz, 3.8 Hz, 16α-H), 5.74 (br s, 1 H, 4-H); ^{19}F NMR (338.8 MHz, $CDCl_3$) −185.70 (ddddd, J_{HF} = 54.2 Hz, 37.6 Hz, 19.6 Hz, 13.9 Hz, 4.7 Hz); MS (70 eV) m/z (rel intensity) 306 (M^+, 10), 286 (2), 264 (13), 167 (11), 149 (100), 124 (19), 85 (23), 71 (46), 57 (61); HRMS (EI) calcd for $C_{19}H_{27}O_2F$ 306.1995, found 306.1989.

16β-Hydroxy-3-[[(trifluoromethyl)sulfonyl]oxy]-androst-3,5-dien-17-one (29). 16α-Triflate **27** (50 mg, 0.088 mmol) was dissolved in a mixture of solvents (THF–H_2O = 3:1, 8 mL) and stirred at 56 °C for 25 min. The isolated product was purified by flash chromatography (hexane–EtOAc = 2:1) and 36.9 mg (96.3%) of the pure 16β-hydroxy ketone **29** was obtained as a white solid: mp 110 °C, with decomposition; ^{1}H NMR (300 MHz, $CDCl_3$) 1.00 (s, 6 H, 18-CH_3, 19-CH_3), 3.98 (t, 1 H, J = 8.7 Hz, 16α-H), 5.61 (dd, 1 H, J = 3.0 Hz, 1.8 Hz, 6-H), 6.01 (d, 1 H, J = 1.8 Hz, 4-H); MS (70 eV) m/z (rel intensity) 434 (M^+, 77), 301 (56), 283 (33), 273 (44), 259 (61), 229 (49), 201 (38), 187 (31), 173 (30), 159 (62), 147 (31), 131 (47), 105 (92), 91 (100), 79 (77), 55 (73), 41 (96); HRMS (EI) calcd for $C_{20}H_{25}O_5F_3S$ 434.1375, found 434.1370.

16α-Fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]androst-3,5-dien-17-one (30). 16β-Hydroxy ketone **29** (14.4 mg, 0.033 mmol) was dissolved in 0.5 mL of CH_2Cl_2 in a polyethylene vial and to this solution was added slowly a solution of DAST (14.4 μL, 0.099 mmol) in 0.2 mL of CH_2Cl_2. The mixture was then capped with a Teflon-lined cap tightly and heated at 40 °C for 50 min before the product isolation (CH_2Cl_2). Flash chromatography (hexane–EtOAc = 4:1) purification gave 10.8 mg (74.7%) of the pure 16α-fluoro ketone **30** as a white crystalline solid: mp 187.5–189 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 0.97 (s, 3 H, 18-CH_3), 0.99 (s, 3 H, 19-CH_3), 5.12 (dd, 1 H, J_{HF} = 50.4 Hz, J_{HH} = 7.2 Hz, 16β-H), 5.60 (dd, 1 H, J = 3.0 Hz, 1.8 Hz, 6-H), 6.01 (d, 1 H, J = 1.8 Hz, 4-H); MS (70 eV) m/z (rel intensity) 436 (M^+, 78), 303 (65), 285 (34), 261 (48), 179 (44), 161 (36), 147 (47), 131 (52), 115 (51), 105 (92), 91 (100), 79 (85), 69 (85), 55 (66); HRMS calcd for $C_{20}H_{24}O_4F_4S$ 436.1331, found 436.1329.

16α-Fluorotestosterone (4). A suspension of $LiAlH_4$ (large excess) in 0.5 mL of dry Et_2O was cooled to −78 °C and to it was added with vigorous stirring a solution of 16α-fluoro ketone **30** (10.8 mg, 0.025 mmol) in 1.5 mL of dry Et_2O. The heterogenous mixture was stirred at −78 °C for 30 min and allowed to warm twice for 4 min without the cooling bath before it was recooled and quenched by the sequential addition of EtOAc and water. The isolated intermediate was redissolved in 3 mL of MeOH and treated with KOH–MeOH solution (0.5 mL, 5 N) at 65 °C for 15 min to remove the triflate protecting function. The product isolation and purification by flash chromatography (hexane–EtOAc = 1:1) yielded 2.8 mg (36.6%) of the 16α-fluorotestosterone (4) as a white crystalline solid and 1 mg (13.1%) of the 17α-hydroxy epimer. Both compounds were further purified by normal-phase HPLC (55% hexane–45% CH_2Cl_2 (5% iPrOH), 4 mL/min, t_R = 45 min for the 4 and 28 min for the 17α-hydroxy epimer) for identification and biological tests. The 16α-fluorotestosterone (4) has a mp 172–174 °C: ^{1}H NMR (300 MHz, $CDCl_3$) 0.82 (s, 3 H, 18-CH_3), 1.19 (s, 3 H, 19-CH_3), 3.78 (dd, J_{HF} = 28.5 Hz, J_{HH} = 4.5 Hz, 17α-H), 4.95 (dq, 1 H, J_{HF} = 54.6 Hz, J_{HH} = 4.5 Hz, 16β-H), 5.75 (s, 1 H, 4-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −180.55 (dq, J_{HF} = 54.2 Hz, 27.7 Hz); MS (70 eV) m/z (rel intensity) 306 (M^+, 73), 264 (93), 221 (37), 162 (15), 145 (24), 133 (19), 124 (100), 109 (53), 91 (50), 77 (31), 67 (29), 55 (39), 41 (46); HRMS calcd for $C_{19}H_{27}O_2F$ 306.1995, found 306.1992.

The 17α-hydroxy epimer has a mp 170–172 °C: ^{1}H NMR (300 MHz, $CDCl_3$) 0.72 (s, 3 H, 18-CH_3), 1.17 (s, 3 H, 19-CH_3), 3.80 (br d, J = 5.1 Hz, 17β-H), 5.25 (dtd, 1 H, J_{HF} = 49.8 Hz, J_{HH} = 5.1 Hz, 3.8 Hz, 16β-H), 5.72 (s, 1 H, 4-H); ^{19}F NMR (338.8 MHz, $CDCl_3$) −197.08 (dtd, J_{HF} = 53.9 Hz, 24.7 Hz, 10.2 Hz); MS (70 eV) m/z (rel intensity) 306 (M^+, 48), 264 (58), 221 (25), 163 (18), 145 (22), 124 (100), 105 (32), 91 (47), 79 (47), 55 (35); HRMS calcd for $C_{19}H_{27}O_2F$ 306.1995, found 306.1998.

7α-Methyl-3,3-(ethylenedioxy)-5(10)-estren-7-ol (32). The starting material enone **31** (0.54 g, 1.88 mmol) was dissolved in benzene (20 mL), followed by addition of ethylene glycol (5 mL), $H(OEt)_3$ (2 mL) as well as TsOH (0.1 g, catalyst) in order. The two-phase mixture was heated to 72 °C for 1.5 h and then isolated. Product purification by flash chromatography (hexane–EtOAc = 1:1) gave 0.54 g (86.4%) of the pure **32** as a white sticky solid, including, like the other 5(10)-ene compounds that will be described later in this series, a little 5(6)-ene isomer which gave the same final product eventually and therefore was not necessary to separate. Part (41 mg) of starting material was recovered. A sample for identification was further purified first by another flash chromatography and then recrystallization from EtOAc and pentane, forming cottonlike crystals: mp 145–147 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.72 (s, 3 H, 18-CH_3), 0.75 (d, 3 H, J = 7.6 Hz, 7α-CH_3), 3.68 (br t, 1 H, J = 8.4 Hz, 17α-H), 3.98 (m, 4 H, 3,3-ethylenedioxy CH_2CH_2); MS (70 eV) m/z (rel intensity) 332 (M^+, 54), 270 (15), 105 (14), 99 (57), 86 (100); HRMS calcd for $C_{21}H_{32}O_3$ 332.2352; found 332.2370. Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.08; H, 9.81.

7α-Methyl-3,3-(ethylenedioxy)-5(10)-estren-17-one (33). 17β-Hydroxy compound **32** (1.1 g, 3.31 mmol) was dissolved in 80 mL of CH_2Cl_2 and treated with PCC (1.46 g, 6.79 mmol) in the same procedure as that described in the synthesis of compound **24**. The crude product was purified by flash chromatography (hexane–EtOAc = 2:1) and 1.01 g (92.1%) of the pure ketone **33** was isolated as a white sticky solid. A sample for identification was further purified by recrystallization from EtOAc and pentane to give white crystals: mp 142–143 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.81 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 0.86 (s, 3 H, 18-CH_3), 3.96 (m, 4 H, 3,3-ethylenedioxy CH_2CH_2); MS (70 eV) m/z (rel intensity) 330 (M^+, 22), 268 (13), 159 (9), 99 (30), 86 (100); HRMS calcd for $C_{21}H_{30}O_3$ 330.2195, found 330.2201. Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 76.32; H, 9.20.

7α-Methyl-3,3-(ethylenedioxy)-5(10),16-estradien-17-yl Diethyl Phosphate (34). Ketone **33** (0.17 g, 0.50 mmol) was treated with LDA (1.5 mmol in 2 mL THF) and $ClP(O)(Et)_2$ (0.21 mL, 1.5 mmol) in the procedure described in the synthesis of enol phosphate **19**. The isolated product was purified by flash chromatography (hexane–EtOAc = 1:1) to give 0.19 g (82.4%) of the pure enol phosphate **34** as a colorless thick oil: ^{1}H NMR (200

MHz, $CDCl_3$) 0.79 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 0.90 (s, 3 H, 18-CH_3), 1.35 (t, 6 H, J = 7.0 Hz, 2 ethoxy OCH_2CH_3), 3.96 (m, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.16 (quintet, 4 H, J_{HP}, J_{HH} = 7.2 Hz, 2 ethoxy OCH_2CH_3), 5.20 (br s, 1 H, 16-H); MS (70 eV) m/z (rel intensity) 466 (M^+, 10), 211 (12), 180 (13), 155 (13), 99 (100), 86 (22); HRMS calcd for $C_{25}H_{39}O_6P$ 466.2480, found 466.2485.

16α-Hydroxy-7α-methyl-3,3-(ethylenedioxy)-5(10)-estren-17-one (35). Enol phosphate **34** (1.45 g, 3.11 mmol) was dissolved in acetone (39 mL) and added to a solution of *N*-methylmorpholine *N*-oxide (0.98 g, 8.09 mmol) in water (10 mL). A catalytic amount of OsO_4 in tBuOH (0.16 mL, 2.5%) was added to the solution and it was stirred at RT for 1.5 h. At this point, only about 50% of the starting material was consumed. To complete the reaction, another batch of OsO_4 (0.16 mL) was added, and after another 1.5 h, it was diluted with Et_2O and isolated. Product purification by flash chromatography (hexane–EtOAc = 1:1) gave 0.79 g (73.7%) of the pure 16α-hydroxy ketone **35** as a white foamy solid. A sample was further purified for identification by recrystallization from EtOH to give white crystals: mp 186.5–188.5 °C; IR (KBr) 3620–3140 (br), 1734 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.81 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 0.95 (s, 3 H, 18-CH_3), 3.97 (m, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.37 (br d, 1 H, J = 7.2 Hz, 16β-H); MS (70 eV) m/z (rel intensity) 346 (M^+, 15), 284 (9), 99 (45), 86 (100); HRMS calcd for $C_{21}H_{30}O_4$ 346.2120, found 346.2132.

16α-Hydroxy-7α-methyl-4-estrene-3,17-dione (36). Ketal **35** (55.1 mg, 0.16 mmol) was dissolved in MeOH (10 mL) and treated with aqueous HCl (3 N, 2 mL). The mixture was heated at 50 °C for 14 min. Most of the MeOH solvent was then removed in vacuo and the rest of the content was diluted with Et_2O. The product isolation and flash chromatography (hexane–EtOAc = 1:2) purification gave 44 mg (91.5%) of the white crystalline ketone **36**. Recrystallization from EtOH further purified the sample for identification, resulting in cottonlike crystals: mp 204–205 °C; ^1H NMR (200 MHz, $CDCl_3$) 0.72 (d, 3 H, J = 7.5 Hz, 7α-CH_3), 1.03 (s, 3 H, 18-CH_3), 4.39 (br d, 1 H, J = 8.4 Hz, 16β-H), 5.86 (br s, 1 H, 4-H); MS (70 eV) m/z (rel intensity) 302 (M^+, 59), 230 (100), 212 (17), 197 (18), 173 (19), 136 (31), 121 (43), 105 (38), 91 (49), 79 (49), 55 (42); HRMS calcd for $C_{19}H_{26}O_3$ 302.1882, found 302.1886. Anal. Calcd for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.25; H, 8.70.

7α-Methyl-3,16α-bis[[(trifluoromethyl)sulfonyl]oxy]-3,5-(6)-estradien-17-one (37). Hydroxy enone **36** (0.10 g, 0.33 mmol) was dissolved in CH_2Cl_2 (12 mL) and cooled to −40 °C. Under protection of N_2, 2,6-lutidine (0.31 mL, 2.25 mmol) as well as Tf_2O (0.37 mL, 2.15 mmol) were added in order, and the pinkish mixture was stirred at −40 to −30 °C for about 2 h until all the starting material was consumed. It was then diluted with Et_2O while kept at −30 °C and passed through a short silica gel column rapidly. The solvents were evaporated in vacuo, and the residue was purified by a quick flash chromatography (hexane–EtOAc = 2:1, prechilled) to give 0.13 g (67%) of the pure bistriflate **37** as a white cottonlike crystalline solid (including very little 2,5(6)-diene isomer): mp 137 °C, with decomposition; ^1H NMR (200 MHz, $CDCl_3$) 0.92 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 1.00 (s, 3 H, 18-CH_3), 5.38 (dd, 1 H, J = 5.8 Hz, 3.4 Hz, 16β-H), 5.69 (br d, 1 H, J = 6.8 Hz, 6-H), 6.05 (d, 1 H, J = 2.4 Hz, 4-H); MS (70 eV) m/z (rel intensity) 566 (M^+, 36), 433 (31), 283 (57), 265 (53), 255 (52), 213 (22), 187 (21), 171 (24), 161 (26), 157 (21), 147 (82), 131 (43), 119 (66), 107 (80), 91 (77), 79 (59), 69 (100), 55 (66); HRMS calcd for $C_{21}H_{24}O_7F_6S_2$ 566.0868, found 566.0863.

16β-Fluoro-7α-methyl-3-[[(trifluoromethyl)sulfonyl]oxy]-3,5(6)-estradien-17-one (38). Triflate **37** (92 mg, 0.18 mmol) was dissolved in dry THF (9 mL), cooled to 0 °C, and treated with nBu_4NF (0.51 mL, 1 M in THF, 0.51 mmol). The reaction mixture was stirred at 0 °C for 10 min before being diluted with Et_2O and passed through silica gel to remove the excess amount of the reagent. Purification of the product was achieved by flash chromatography (hexane–EtOAc = 4:1), yielding 61.8 mg (87.2%) of the pure fluoro ketone **38** as a white solid. Recrystallization from Et_2O gave further purified product as white fine crystals: mp 157 °C, with decomposition; ^1H NMR (300 MHz, $CDCl_3$) 0.91 (d, 3 H, J = 7.2 Hz, 7α-CH_3), 1.04 (s, 3 H, 18-CH_3), 4.75 (dt, 1 H, J_{HF} = 50.4 Hz, J_{HH} = 8.1 Hz, 16α-H), 5.70 (br d, 1 H, J = 5.1 Hz, 6-H), 6.06 (d, 1 H, J = 1.8 Hz, 4-H); MS (70 eV) m/z (rel intensity) 436 (M^+, 73), 421 (21), 303 (99), 285 (26), 261 (27), 175 (32), 167 (81), 155 (33), 149 (60), 131 (37), 119 (51), 105 (74), 91 (100), 79 (91), 69 (73), 55 (58); HRMS calcd for $C_{20}H_{24}O_4F_4S$ 436.1331, found 436.1333.

16β-Fluoromibolerone (5). To a solution of 16β-fluoro ketone **38** (23 mg, 0.055 mmol) in 4.2 mL of dry THF cooled to −55 °C was added dropwise with stirring CH_3MgBr (0.6 mL, 3 M, in Et_2O, 1.8 mmol), during which some white precipitate formed. The heterogenous mixture was stirred at −50 °C for 8 min and without the cooling bath for 4 min before being quenched with MeOH (5 mL, also as a solvent for the next reaction). KOH–MeOH solution (1.2 mL, 5 N) was then added to the reaction mixture that was full of white precipitate and it was stirred at 60 °C for 25 min before being quenched with aqueous HCl (3 N). The isolated crude product was purified by flash chromatography (hexane–EtOAc = 1:1), yielding 12.4 mg (70.5%) of the pure 16β-fluoromibolerone **5** as a white solid. It was further purified by normal phase HPLC (65% hexane–35% CH_2Cl_2 (5% iPrOH), 5 mL/min, t_R = 12.5 min) for identification: mp 128.5–130.5 °C; ^1H NMR (200 MHz, $CDCl_3$) 0.75 (d, 3 H, J = 7.4 Hz, 7α-CH_3), 0.95 (s, 3 H, 18-CH_3), 1.16 (d, 3 H, J = 2.8 Hz, 17α-CH_3), 4.55 (ddd, 1 H, J_{HF} = 54.5 Hz, J_{HH} = 7.5 Hz, 3.5 Hz, 16α-H), 5.83 (br s, 1 H, 4-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −170.07 (br dd, J_{HF} = 54.8 Hz, 39.0 Hz); ^{19}F NMR (282.3 MHz, $CDCl_3$) −170.07 (br dd, J_{HF} = 54.8 Hz, 39.0 Hz); ^{19}F NMR (282.3 MHz, $CDCl_3$ with D_2O added) −170.21 (ddd, J_{HF} = 55.1 Hz, 37.3 Hz, 13.3 Hz); MS (70 eV) m/z (rel intensity) 320 (M^+, 28), 300 (22), 229 (27), 187 (14), 174 (20), 161 (15), 147 (16), 133 (20), 119 (27), 105 (34), 91 (40), 79 (38), 71 (45), 55 (39), 43 (100); HRMS calcd for $C_{20}H_{29}O_2F$ 320.2152, found 320.2153.

16β-Fluoro-7α-methyl-19-nortestosterone (7). 16β-Fluoro ketone **38** (24 mg, 0.055 mmol) was dissolved in dry THF (4.2 mL) and added to a suspension of $LiAlH_4$ (large excess) in 0.4 mL of dry THF cooled to −78 °C prior to the addition. The heterogenous mixture was stirred at −78 °C for 10 min, then quenched with 0.4 mL of EtOAc while kept at −78 °C, and followed by warming and further quenching with 5 mL of MeOH. To this mixture, full of gray precipitate, was added 0.8 mL of KOH–MeOH (5 N) solution and it was stirred at 60 °C for 15 min before being neutralized with aqueous HCl (3 N). The product isolation and purification by flash chromatography (hexane–EtOAc = 1:1) gave 12 mg (71.4%) of pure **7** as a white glassy solid. It was further purified by normal-phase HPLC (63% hexane–37% CH_2Cl_2 (5% iPrOH), 5 mL/min, t_R = 13 min) for purpose of identification and biological tests: ^1H NMR (200 MHz, $CDCl_3$) 0.75 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 0.88 (s, 3 H, 18-CH_3), 3.41 (ddd, 1 H, J_{HF} = 20.6 Hz, J_{HH} = 10.0 Hz, 6.4 Hz, 17α-H), 4.96 (dtd, 1 H, J_{HF} = 55.4 Hz, J_{HH} = 6.4 Hz, 3.0 Hz, 16α-H), 5.83 (br s, 1 H, 4-H); ^1H NMR (300 MHz, $CDCl_3$ with D_2O added) 0.75 (d, 3 H, J = 6.9 Hz, 7α-CH_3), 0.88 (s, 3 H, 18-H), 3.41 (dd, 1 H, J_{HF} = 20.7 Hz, J_{HH} = 6.3 Hz, 17α-H), 4.82 (br s, HOD), 4.97 (dtd, 1 H, J_{HF} = 55.5 Hz, J_{HH} = 6.6 Hz, 3.3 Hz, 16α-H), 5.84 (br s, 1 H, 4-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −185.77 (br dddd, J_{HF} = 56.5 Hz, 37.6 Hz, 18.9 Hz, 14.1 Hz); ^{19}F NMR (282.3 MHz, $CDCl_3$ with D_2O added) −185.79 (dddd, J_{HF} = 56.1 Hz, 37.1 Hz, 18.1 Hz, 12.4 Hz); MS (70 eV) m/z (rel intensity) 306 (M^+, 37), 286 (39), 229 (36), 187 (21), 159 (25), 147 (21), 133 (29), 119 (43), 105 (52), 91 (70), 79 (68), 67 (47), 55 (99), 41 (100); HRMS calcd for $C_{19}H_{27}O_2F$ 306.1995, found 306.1995.

16β-Hydroxy-7α-methyl-3-[[(trifluoromethyl)sulfonyl]oxy]-3,5(6)-estradien-17-one (39). Bistriflate **37** (76 mg, 0.13 mmol) was dissolved in a mixed solvent (THF–H_2O = 3:1, 8 mL) and stirred at 65 °C for 15 min. It was then extracted with Et_2O three times and dried ($MgSO_4$). Flash chromatography (hexane–EtOAc = 1:1) purificatioin of the residue after concentration gave 45.6 mg (78.2%) of the pure 16β-hydroxy ketone **39** as a white glass and a small amount of the 17-hydroxy 16-one isomer. The compound **39**: ^1H NMR (300 MHz, $CDCl_3$) 0.92 (d, 3 H, J = 7.2 Hz, 7α-Ch_3), 0.99 (s, 3 H, 18-CH_3), 4.0 (t, 1 H, J = 8.7 Hz, 16α-H), 5.71 (br d, 1 H, J = 5.7 Hz, 6-H), 6.06 (d, 1 H, J = 2.1 Hz, 4-H); MS (70 eV) m/z (rel intensity) 434 (M^+, 54), 419 (15), 301 (70), 283 (19), 273 (44), 259 (41), 229 (38), 201 (40), 173 (41), 159 (30), 147 (30), 135 (35), 119 (44), 105 (50), 91 (83), 79 (70), 55 (66), 41 (100); HRMS calcd for $C_{20}H_{25}O_5F_3S$ 434.1375, found 434.1379.

7α-Methyl-3,16β-bis[[(trifluoromethyl)sulfonyl]oxy]-3,5-(6)-estradien-17-one (40). 16β-Hydroxy ketone **39** (42 mg, 0.097

mmol) was dissolved in CH_2Cl_2 (8 mL), cooled to −40 °C, and treated with Tf_2O (2 × 50.8 μL, 0.59 mmol) and 2,6-lutadine (2 × 42.4 μL, 0.61 mmol) in the same way as that for the compound **37** (higher temperature, −10 to 0 °C). A quick flash chromatography (hexane–EtOAc = 4:1, prechilled) gave 46 mg (84.1%) of the pure bistriflate **40** as a white crystalline solid: mp 110 °C, with decomposition; ^{1}H NMR (300 MHz, $CDCl_3$) 0.92 (d, 3 H, J = 7.2 Hz, 7α-CH_3), 1.04 (s, 3 H, 18-CH_3), 4.97 (t, 1 H, J = 8.7 Hz, 16α-H), 5.70 (br d, 1 H, J = 4.8 Hz, 6-H), 6.06 (d, 1 H, J = 2.1 Hz, 4-H); MS (70 eV) m/z (rel intensity) 566 (M^+, 36), 433 (42), 391 (19), 283 (60), 255 (27), 213 (25), 187 (26), 173 (35), 161 (29), 147 (70), 131 (35), 119 (53), 105 (55), 91 (78), 79 (59), 69 (100), 55 (71); HRMS calcd for $C_{21}H_{24}O_7F_6S_2$ 566.0868, found 566.0870.

16α-Fluoro-7α-methyl-3-[[(trifluoromethyl)sulfonyl]-oxy]-3,5(6)-estradien-17-one (41). Method A. 16β-Hydroxy ketone **39** (64.9 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (6.5 mL) and treated at RT with a solution of DAST (65 μL, 0.49 mmol) in 1.3 mL of CH_2Cl_2, freshly made prior to the addition. The resulted solutioin was heated at 43 °C for 45 min, and the crude product was isolated (CH_2Cl_2). It was purified by flash chromatography (hexane–EtOAc = 4:1) to give 27.9 mg (42.8%) of the pure 16α-fluoro ketone **41** as a white solid. Further purification by recrystallization from CH_2Cl_2 and hexane gave white needlelike crystals: mp 125.5–127.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.92 (d, 3 H, J = 7.2 Hz, 7α-CH_3), 0.94 (s, 3 H, 18-CH_3), 5.11 (dd, 1 H, J_{HF} = 51.6 Hz, J_{HH} = 7.0 Hz, 16β-H), 5.70 (br d, 1 H, J = 4.0 Hz, 6-H), 6.05 (d, 1 H, J = 1.4 Hz, 4-H); MS (70 eV) m/z (rel intensity) 436 (M^+, 62), 421 (20), 303 (100), 285 (21), 261 (26), 189 (20), 175 (32), 167 (62), 155 (24), 149 (50), 131 (33), 119 (37), 105 (61), 91 (75), 79 (64), 55 (55), 41 (78); HRMS calcd for $C_{20}H_{24}O_4F_4S$ 436.1331, found 436.1335.

Method B. Bistriflate **40** (15 mg, 0.027 mmol) was dissolved in 1 mL of THF and treated with nBu_4NF (53 μL, 1 M in THF, 0.053 mmol) at RT for 15 min. The isolated product was purified by flash chromatography (hexane–EtOAc = 4:1) to give 5.7 mg (49.1%) of the pure 16α-fluoro ketone **41** that has the same spectral characteristics as that made by the DAST reaction.

16α-Fluoromibolerone (6). To a solution of 16α-fluoro ketone **44** (14.5 mg, 0.033 mmol) in dry Et_2O (4 mL) cooled to −45 °C was added dropwise via a syring CH_3MgBr (0.9 mL, 3 M, in Et_2O, 2.7 mmol). The reaction mixture was stirred at −40 °C for 8 min and without the cooling bath for 4 min. To complete the reaction the mixture was cooled again and stirred longer (8 min plus 4 min) before it was quenched with water. The isolated crude intermediate was redissolved in MeOH (5 mL) and treated with KOH–MeOH solution (5 N, 1.1 mL). It was then heated at 57–59 °C for 22 min and isolated. Flash chromatography (hexane–EtOAc = 1:1) purification yielded 2.5 mg (23.6%) of the pure 16α-fluoromibolerone (**6**) as a white solid and 3 mg (28.3%) of the 17α-hydroxy epimer also as a white solid. The 16α-fluoromibolerone (**6**) was further purified by normal phase HPLC (60% hexane–40% CH_2Cl_2 (5% iPrOH), 4 mL/min, t_R = 24 min) for identification and biological tests and white crystals was obtained: mp 196 °C, with decomposition; ^{1}H NMR (200 MHz, $CDCl_3$) 0.76 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 0.92 (s, 3 H, 18-CH_3), 1.24 (d, 3 H, J = 5.0 Hz, 17α-CH_3), 5.02 (ddd, 1 H, J_{HF} = 53.8 Hz, J_{HH} = 8.6 Hz, 2.6 Hz, 16β-H), 5.83 (s, 1 H, 4-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −190.69 (dt, J_{HF} = 55.1 Hz, 23.7Hz); MS (70 eV) m/z (rel intensity) 320 (M^+, 26), 300 (20), 229 (26), 174 (21), 133 (20), 119 (26), 105 (33), 91 (38), 79 (33), 71 (41), 55 (35), 43 (100); HRMS calcd for $C_{20}H_{29}O_2F$ 320.2152, found 320.2153.

^{1}H NMR of the 17α-hydroxy epimer: (200 MHz, $CDCl_3$) 0.75 (s, 3 H, 18-CH_3), 0.81 (d, 3 H, J = 7.5 Hz, 7α-CH_3), 1.25 (s, 3 H, 17β-CH_3), 4.93 (ddd, 1 H, J_{HF} = 56.0 Hz, J_{HH} = 7.0 Hz, 4.4 Hz, 16β-H), 5.83 (s, 1 H, 4-H).

16α-Fluoro-7α-methyl-19-nortestosterone (8). To a suspension of large excess of $LiAlH_4$ in dry Et_2O (0.6 mL) cooled to −78 °C was added with stirring a solutioin of 16α-fluoro ketone **41** (10 mg, 0.023 mmol) in 2 mL of dry Et_2O. The resulted heterogenous mixture was stirred at −78 °C for 15 min before it was quenched with EtOAc first, while kept at −78 °C, and then with water. The isolated crude intermediate was redissolved in MeOH (3 mL) and treated with KOH–MeOH (0.75 mL, 5 N) at 60 °C for 12 min. The product isolation and purification by flash chromatography (hexane–ETOAc = 1:1) gave 3.6 mg (51.4%) of the pure **8** as a white solid and a small amount of the 17α-hydroxy epimer. The compound **8** was further purified by normal-phase HPLC (55% hexane–45% CH_2Cl_2 (5% iPrOH), 4 mL/min, t_R = 22 min), giving white crystals for both identification and biological studies: mp 60–62 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.79 (d, 3 H, J = 7.0 Hz, 7α-CH_3), 0.82 (s, 3 H, 18-CH_3), 3.80 (dd, 1 H, J_{HF} = 28.6 Hz, J_{HH} = 4.4 Hz, 17α-H), 4.92 (dq, 1 H, J_{HF} = 54.8 Hz, J_{HH} = 4.8 Hz, 16β-H), 5.84 (t, 1 H, J = 2.0 Hz, 4-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −180.60 (dq, J_{HF} = 54.5 Hz, 27.1 Hz); MS (70 eV) m/z (rel intensity) 306 (M^+, 57), 286 (25), 229 (35), 187 (19), 174 (20), 159 (26), 147 (20), 133 (27), 121 (30), 105 (48), 91 (65), 81 (44), 67 (45), 55 (62), 41 (100); HRMS calcd for $C_{19}H_{27}O_2F$ 306.1995, found 306.1992.

^{1}H NMR of the 17α-hydroxy epimer: (200 MHz, $CDCl_3$) 0.75 (s, 3 H, 18-CH_3), 0.82 (d, 3 H, J = 7.5 Hz, 7α-CH_3), 3.83 (br d, 1 H, J = 5.3 Hz, 17β-H), 5.25 (ddd, 1 H, J_{HF} = 50.0 Hz, J_{HH} = 11.0 Hz, 5.3 Hz, 16β-H), 5.84 (br s, 1 H, 4-H).

3,3-(Ethylenedioxy)-5(10),9(11)-estradien-17β-ol (43). Dienone **42** (1.72 g, 6.32 mmol) was dissolved in benzene (100 mL) and ethylene glycol (30 mL) with help of CH_2Cl_2 (6 mL) and heat (70 °C). To this solution was added about 100 μL of concentrated H_2SO_4. It was equipped with a Dean–Stark trap and refluxed for 2.5 h until the reaction completed, during which time 10 mL more of fresh benzene was added and water was collected. It was then cooled to 0 °C and quenched with cold saturated aqueous $NaHCO_3$ solution. The isolation and purification of the crude product by flash chromatography (hexane–EtOAc = 1:1) gave 1.84 g (92.0%) of the pure ketal **43** as a white glassy solid: ^{1}H NMR (300 MHz, $CDCl_3$) 0.74 (s, 3 H, 18-CH_3), 3.77 (q, 1 H, J = 7.9 Hz, 17α-H), 4.00 (s, 4 H, 3,3-ethylenedioxy (CH_2CH_2), 5.57 (t, 1 H, J = 2.4 Hz, 11-H); MS (70 eV) m/z (rel intensity) 316 (M^+, 100), 288 (75), 243 (100), 197 (37), 185 (19), 171 (31), 157 (27), 149 (73), 143 (33), 129 (38), 117 (32), 105 (36), 99 (57), 91 (59), 86 (69), 55 (48); HRMS calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2040. Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.31; H, 9.01.

3,3-(Ethylenedioxy)-5(10),9(11)-estradien-17-one (44). To a solution of 17β-hydroxy compound **43** (1.47 g, 4.80 mmol) and N-methylmorpholine N-oxide (1.03 g, 8.64 mmol) in 44 mL of CH_2Cl_2, was added $(nC_3H_7)_4NRuO_4$ (0.18 g, 0.43 mmol) in one portion as a solid. The resulted dark brown solution was stirred at RT for 15 min before it was quenched with aqueous $Na_2S_2O_3$ and filtered through Celite. The filtrate was washed (water twice) and dried ($MgSO_4$). The residue after concentration was purified by flash chromatography (hexane–EtOAc = 1:1) to give 1.45 g (99.3%) of the pure ketone **44** as a white crystalline solid: mp 155–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.87 (s, 3 H, 18-CH_3), 3.98 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.56 (t, 1 H, J = 2.4 Hz, 11-H); MS (70 eV) m/z (rel intensity) 314 (M^+, 70), 286 (49), 241 (47), 227 (21), 170 (25), 155 (24), 41 (21), 129 (28), 115 (23), 105 (22), 99 (22), 91 (37), 86 (100), 77 (27). Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.33. Found: C, 76.46; H, 8.34.

Spiro-2′-(1′-oxacyclopropane)-17(*S*)-[3,3-(ethylenedioxy)-5(10),9(11)-estradiene] (45). Ketone **44** (0.79 g, 2.51 mmol) was dissolved in dry THF (44 mL) and reacted with the $CH_2{=}S(CH_3)_2$ solution (12.55 mmol in 30 mL of dry DMSO and 20 mL of THF, prepared in situ prior to use) in the same way as that described in the synthesis of 20-F-Mib.[21] The isolated reaction mixture was separated by flash chromatography (hexane–EtOAc = 4:1) and 0.41 g (82.5% based on the consumed starting material) of the epoxide **45** as a white glassy solid as well as 0.31 g (38.8%) of the recovered starting material were obtained. The epoxide **45**: ^{1}H NMR (200 MHz, $CDCl_3$) 0.86 (s, 3 H, 18-CH_3), 2.79 (AB q, 2 H, $\Delta\nu$ = 0.27 ppm, J = 5.0 Hz, 17 spiro epoxide CH_2), 3.98 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 5.53 (br d, 1 H, J = 5.6 Hz, 11-H); MS (70 eV) m/z (rel intensity) 328 (M^+, 100), 300 (66), 255 (91), 211 (33), 169 (23), 155 (29), 143 (28), 129 (36), 117 (30), 105 (34), 99 (41), 91 (65), 86 (93), 77 (33), 55 (33), 41 (52); HRMS calcd for $C_{21}H_{28}O_3$ 328.2038, found 328.2041. Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.82; H, 8.60. Found: C, 76.80; H, 8.42.

17α-(Hydroxymethyl)-3,3-(ethylenedioxy)-5(10),9(11)-estradien-17β-ol (46). Epoxide **45** (0.52 g, 1.62 mmol) was dissolved in 1-methyl-2-pyrrolidinone (27 mL) and treated with aqueous NaOH solution (18 mL, 2 N) at 105 °C for 2 h. It was then cooled to RT, diluted with water, and extracted (Et_2O, 3 × 50 mL, EtOAc, 2 × 10 mL). After drying ($MgSO_4$), it was concentrated, and the residue was purified by flash chromatography (hexane–EtOAc = 1:1), giving 0.26 g (46.3%) of the pure diol **46** as a white solid:

mp 162–163 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.86 (s, 3 H, 18-CH_3), 3.54 (AB q, 2 H, $\Delta\nu$ = 0.31 ppm, J = 10.8 Hz, 17α CH_2OH), 3.97 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2) 5.55 (br d, 1 H, J = 1.8 Hz, 11-H); MS (70 eV) m/z (rel intensity) 346 (M^+, 20), 328 (13), 297 (100), 269 (21), 225 (23), 211 (81), 171 (18), 143 (18), 129 (21), 105 (17), 99 (30), 91 (29), 86 (36), 77 (15), 43 (48). Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.80; H, 8.51.

Spiro-3′-(1′-oxo-2′,5′-dioxa-1′-thiacyclopentane)-17(*S*)-[3,3-(ethylenedioxy)-5(10),9(11)-estradiene] (47). A flame-dried flask was charged with diol **46** (0.22 g, 0.64 mmol) and 16 mL of THF was then cooled to −10 °C in an ice–salt bath. $SOCl_2$ (0.25 mL, 2.56 mmol) was added dropwise to the solution, and the resulting mixture was stirred at −10 to 0 °C for about 1 h until TLC showed that the reaction was complete. It was quenched in the cold with saturated aqueous $NaHCO_3$. Purification was achieved by flash chromatography (hexane–EtOAc = 2:1) to give 0.21 g (85.3%) of a pure glassy mixture of two epimers of the cyclic sulfite **47**: ^{1}H NMR (200 MHz, $CDCl_3$) 0.89, 0.96 (two s, total 3 H, 18-CH_3 of the two epimers), 3.98 (s, 4 H, 3,3-ethylenedioxy CH_2CH_2), 4.29 (AB q, 1 H, $\Delta\nu$ = 0.71 ppm, J = 9.4 Hz, spiro cyclic sulfite CH_2 of one epimer), 4.37 (AB q, 1 H, $\Delta\nu$ = 0.14 ppm, J = 9.2 Hz, spiro cyclic sulfite CH_2 of another epimer), 5.53–5.60 (br m, 1 H, 11-H); MS (70 eV) m/z (rel intensity) 328 (M^+, 100), 300 (66), 255 (91), 211 (33), 169 (23), 155 (29), 143 (28), 129 (36), 117 (30), 105 (34), 99 (41), 91 (65), 86 (93), 77 (33), 55 (33), 41 (52); HRMS calcd for $C_{21}H_{28}O_3$ 328.2038, found 328.2041. Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.82; H, 8.60. Found: C, 76.80; H, 8.42.

Spiro-3′-(1′-oxo-2′,5′-dioxa-1′-thiacyclopentane)-17(*S*)-(5(10),9(11)-estradien-3-one) (48). Ketal **47** (69 mg, 0.18 mmol) was dissolved in 18 mL of MeOH with the help of CH_2Cl_2 (1.2 mL) and heating, and to this solution was added aqueous oxalic acid (9.7 mL, 11%). The mixture was stirred at RT for 5.5 h before it was quenched with saturated aqueous $NaHCO_3$. To assure a good yield, the MeOH solvent was removed in vacuo before the product isolation. Quick flash chromatography (hexane–EtOAc = 4:1) purification gave 54.5 mg (88.9%) of a pure mixture of two epimers of the deprotected unconjugated cyclic sulfite **48** as a white glassy solid, which is not stable at RT nor on silica gel: ^{1}H NMR (300 MHz, $CDCl_3$) 0.93, 0.99 (two s, total 3 H, 18-CH_3 of the two epimers), 2.88 (br s, 2 H, 4-H), 4.32 (AB q, 1 H, $\Delta\nu$ = 0.71 ppm, J = 9.0 Hz, spiro cyclic sulfite CH_2 of one epimer), 4.40 (AB q, 1 H, $\Delta\nu$ = 0.15 ppm, J = 9.3 Hz, spiro cyclic sulfite CH_2 of another epimer), 5.60, 5.65 (two br s, total 1 H, 11-H of the two epimers); MS (70 eV) m/z (rel intensity) 348 (M^+, 15), 284 (12), 253 (100), 211 (14), 169 (12), 155 (14), 129 (15), 105 (13), 91 (25), 77 (14), 55 (16), 41 (20); HRMS calcd for $C_{19}H_{24}O_4S$ 348.1395, found 348.1397.

Spiro-3′-(1′-oxo-2′,5′-dioxa-1′-thiacyclopentane)-17(*S*)-(4,9(10),11-estradien-3-one) (49). A solution of unconjugated dienone **48** (0.14 g, 0.40 mmol) in benzene (14 mL) and an orange solution of DDQ (0.36 g, 1.59 mmol) also in benzene (14 mL) were combined together. Wrapped with aluminum foil, the reaction mixture was stirred at RT for 18 h. It was then diluted with Et_2O, washed (2 × aqueous $NaHCO_3$, 2 × H_2O), and dried ($MgSO_4$). The residue after concentration was purified by flash chromatography (hexane–EtOAc = 2:1) and 0.10 g (74.8%) of a white glassy mixture of two epimers of the trienone **49** was obtained: ^{1}H NMR (300 MHz, $CDCl_3$) 1.06, 1.12 (two s, total 3 H, 18-CH_3 of the two epimers), 4.30 (AB q, $\Delta\nu$ = 0.39 ppm, J = 8.4 Hz, spiro cyclic sulfite CH_2 of one epimer), 4.35 (AB q, $\Delta\nu$ = 0.082 ppm, J = 8.7 Hz, spiro cyclic sulfite CH_2 of another epimer), 4 peaks total 2 H, 5.81 (s, 1 H, 4-H), 6.37 (AB q, $\Delta\nu$ = 0.40 ppm, J = 10.2 Hz, 11- and 12-H of one epimer), 6.60 (AB q, $\Delta\nu$ = 0.049 ppm, J = 10.2 Hz, 11- and 12-H of another epimer), 4 peaks total 2 H; MS (10 eV) m/z (rel intensity) 346 (M^+, 64), 293 (39), 282 (49), 224 (35), 167 (24), 149 (100), 71 (25); HRMS calcd for $C_{19}H_{22}O_4S$ 346.1239, found 346.1240. Anal. Calcd for $C_{19}H_{22}O_4S$: C, 65.87; H, 6.40; S, 9.26. Found: C, 65.79; H, 6.45; S, 9.23.

Spiro-3′-(1′,1′-dioxo-2′,5′-dioxa-1′-thiacyclopentane)-17-(*S*)-(4,9(10),11-estratrien-3-one) (50). To a solution of cyclic sulfite **49** (23 mg, 0.066 mmol) in CH_2Cl_2 (2 mL) was added TBA-oxone (0.3 g × 37.5%, 0.31 mmol) and the mixture was cooled to 0 °C. A nBu_4NF solution in THF (2 mL, 1 M, 2 mmol) was then added dropwise slowly, and it was stirred at 0 °C for 45 min. While the solution was kept cold, it was diluted with Et_2O, quenched with water, and neutralized with aqueous HCl (1 N). The product isolated was purified by a quick flash chromatography (hexane–EtOAc = 1:1, prechilled) and 16.8 mg (69.8%) of the pure white crystalline cyclic sulfate **50** was obtained: mp 120 °C, with decomposition; ^{1}H NMR (200 MHz, $CDCl_3$) 1.14 (s, 3 H, 18-CH_3), 4.49 (AB q, 2 H, $\Delta\nu$ = 0.11 ppm, J = 8.8 Hz, cyclic sulfate CH_2), 5.82 (s, 1 H, 4-H), 6.55 (AB q, 2 H, $\Delta\nu$ = 0.13 ppm, J = 10.2 Hz, 11- and 12-H); MS (70 eV) m/z (rel intensity) 362 (M^+, 24), 264 (36), 251 (12), 222 (19), 207 (15), 149 (25), 91 (18), 83 (14), 71 (24), 57 (41), 43 (100); HRMS calcd for $C_{19}H_{22}O_5S$ 362.1188, found 362.1190; MS (FAB) m/e (rel intensity) 363 (M+1, 33); HRMS (FAB) calcd for $C_{19}H_{22}O_5S$+H 363.1266, found 363.1268.

20-Fluoro-R1881 (9). Cyclic sulfate **50** (5.6 mg, 0.016 mmol) was dissolved in 1.6 mL of dry THF and treated with nBu_4NF (62 μL × 1 M in THF, 0.062 mmol) at RT for 35 min. At this point, HCl–MeOH solution (0.14 mL, 2 N) was added, and it was stirred again at RT for 10 min to hydrolyze the formed fluoro bisulfate intermediate. It was noticed that upon addition of nBu_4NF, the solution turned to green and when acidified, it changed to a wine red color which disappeared after neutralization. The product isolation and purification by flash chromatography (hexane–EtOAc = 1:1) gave 3.9 mg (82.5%) of the pure 20-fluoro-R1881 (**9**) as a yellowish crysatalline solid. It was further purified by normal-phase HPLC (60% hexane–40% CH_2Cl_2 (5% iPrOH), 4 mL/min, ^{t}R = 25 min) for identification and biological tests: mp 60–62 °C, with decomposition; ^{1}H NMR (300 MHz, $CDCl_3$) 1.05 (s, 3 H, 18-CH_3), 4.34 (d, AB q, 2 H, J_{HF} = 47.4 Hz, $\Delta\nu$ = 0.24 ppm, J = 9.3 Hz, 17α-CH_2F), 5.79 (s, 1 H, 4-H), 6.43 (AB q, 2 H, $\Delta\nu$ = 0.089 ppm, J = 9.9 Hz, the two upper field peaks doubled, J = 2.1 Hz, 11- and 12-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −225.27 (t, J_{HF} = 47.7 Hz); MS (70 eV) m/z (rel intensity) 302 (M^+, 100), 284 (16), 269 (24), 251 (39), 226 (65), 211 (69), 197 (34), 183 (26), 167 (21), 155 (39), 141 (39), 128 (35), 115 (34), 105 (29), 91 (44), 77 (29); HRMS calcd for $C_{19}H_{23}O_2F$: 302.1682; found: 302.1689.

17,17-Difluoroandrostan-3-one (51). A polyethylene vial was charged with ketone **14** (155 mg, 0.47 mmol) as well as 0.45 mL of DAST that served as both a reagent and a solvent. It was capped tightly and heated with stirring at 80 °C for 3.5 h. It was then diluted with EtOAc, quenched with silica gel, and filtered. The filtrate was washed with water and concentrated. The residue was redissolved in acetone (5 mL) and treated with aqueous HCl (2 mL, 3 N) at RT for 5 min. The isolated product was purified by flash chromatography (hexane–EtOAc = 4:1) and 71.6 mg (49.5%) of the pure geminal difluoro ketone **51** was obtained as a white solid. Further purificatioin by recrystallization from CH_2Cl_2 and hexane gave white crystals that have a mp 112–114 °C: ^{1}H NMR (200 Mhz, $CDCl_3$) 0.89 (d, 3 H, J = 2.0 Hz, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), ^{19}F NMR (338.8 MHz, $CDCl_3$) −102.59 (dt, J_{FF} = 217.6 Hz, J_{HF} = 25.4 Hz, 17α-F), −115.11 (dd, J_{FF} = 218.2 Hz, J_{HF} = 11.5 Hz, 17β-F), agreed with the ^{1}H NMR reported in literature;[39] MS (70 eV) m/z (rel intensity) 310 (M^+, 30), 238 (100), 223 (17), 119 (13), 107 (25), 91 (28), 81 (49), 67 (41), 55 (53), 41 (60); HRMS calcd for $C_{19}H_{28}OF_2$ 310.2108, found 310.2111.

17-Fluoro-16-androsten-3-one (52). In a flame-dried flask, geminal difluoro ketone **51** (50 mg, 0.16 mmol) was dissolved in dry xylenes (4 mL) and to this solution was added 1.5 g of Al_2O_3 (baked at >300 °C for 3 days and cooled under N_2 atmosphere). The heterogeneous mixture, under N_2, was vigorously stirred at 138–145 °C for 2 h before the Al_2O_3 was filtered and rinsed with EtOAc. The combined filtrate was concentrated in vacuo and then under a stream of N_2. Flash chromatography (hexane–EtOAc = 4:1) purificatioin of the residue gave 17 mg (36.3%) of the vinyl fluoro ketone **52** as a white solid. A sample was recrystallized from hexane to give white crystals for identification: mp 129–130 °C (lit.[39] mp 132–134 °C); ^{1}H NMR (200 MHz, $CDCl_3$) 0.97 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 4.87 (dt, 1 H, J = 3.2 Hz, 1.6 Hz, 16-H); ^{19}F NMR (282 3 MHz, $CDCl_3$) −132.28 (dd, J_{HF} = 6.8 Hz, 2.0 Hz), agreed with the ^{1}H NMR reported in literature;[37] MS (70 eV) m/z (rel intensity) 290 (M^+, 42), 275 (100), 255 (26), 235 (35), 198 (19), 165 (63), 145 (66), 131 (28), 123 (36), 109 (55), 91 (52), 79 (50), 67 (49), 55 (62), 41 (75); HRMS calcd for $C_{19}H_{27}OF$ 290.2046, found 290.2048.

17β-Fluoroandrostan-3-one (11). Vinyl fluoro ketone **53** (7.5 mg, 0.026 mmol) was dissolved in 1.5 mL of AcOH, and to the solution was added Pd–C catalyst (8 mg, 5%). The mixture was stirred under H_2 atmosphere at RT for 3 h before it was filtered

through Celite. The residue after concentration of the filtrate was purified by flash chromatography (hexane–EtOAc = 4:1) to give 7.2 mg (95%) of the 17β-fluoro ketone 11 as a white solid. It was further purified by recrystallization from hexane to give white cottonlike crystals for identification and biological tests: mp 130–131 °C (lit.[39] mp 129–131 °C); ^{1}H NMR (300 MHz, $CDCl_3$) 0.84 (d, 3 H, J = 2.4 Hz, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 4.49 (ddd, 1 H, J_{HF} = 56.1 Hz, J_{HH} = 9.0 Hz, 7.2 Hz, 17α-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −195.10 (dd, J_{HF} = 57.3 Hz, 26.3 Hz), agreed with the ^{1}H NMR reported in literature;[39] MS (70 eV) m/z (rel intensity) 292 (M^+, 32), 220 (100), 201 (13), 121 (20), 107 (36), 93 (39), 81 (51), 67 (47), 55 (50), 41 (58); HRMS calcd for $C_{19}H_{29}OF$ 292.2202, found 292.2203.

3,3-Difluoroandrostan-17β-ol (12). DHT **53** (0.1 g, 0.34 mmol) was dissolved in CH_2Cl_2 (2 mL) and treated with pyridine (31 μL, 0.38 mmol) as well as CH_3COCl (0.1 mL, 1.4 mmol) at RT for 2 h. The isolated crude product was purified by recrystallization from EtOH and water, giving 0.11 g (98.3%) of the pure DHT acetate as a white needlelike crystalline solid: mp 155–156 °C (Steraloids catalog reported 156–157 °C).

The acetate (50 mg, 0.15 mmol) was treated with DAST (~150 μL) by the same procedure as that described in the synthesis of the compound **51**. Flash chromatography (hexane–EtOAc = 4:1) purification of the isolated product gave 48.5 mg (91%) of the pure geminal difluoro intermediate. It was recrystallized from EtOH and water to give white crystals: mp 126–128 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.77 (s, 3 H, 18-CH_3), 0.82 (s, 3 H, 19-CH_3), 2.02 (s, 3 H, acetyl CH_3), 2.89–3.00 (m, 2 H, 2 or 4-H), 4.57 (dd, 1 H, J = 9.6 Hz, 7.6 Hz, 17α-H), agreed with the ^{1}H NMR reported in literature;[37] MS (10 eV) m/z (rel intensity) 354 (M^+, 13), 294 (100), 279 (60), 149 (81), 94 (45); HRMS calcd for $C_{21}H_{32}O_2F_2$ 354.2370, found 354.2381.

The intermediate (20.9 mg, 0.59 mmol) was treated with NaOH–MeOH solution (5 mL, 0.5 N) at RT for 1.5 h. The isolated crude material was purified by flash chromatography (hexane–EtOAc = 2:1) and 18 mg (97.8%) of the pure **12** was obtained as a white solid, which was further purified by flash chromatography twice more to give white crystals for identification and biological tests: mp 149–151 °C (lit.[55] mp 154–156 °C); ^{1}H NMR (200 MHz, $CDCl_3$) 0.72 (s, 3 H, 18-CH_3), 0.82 (s, 3 H, 19-CH_3), 3.63 (br t, 1 H, J = 8.0 Hz, 17α-H), 2.98–3.08 (br s, <1 H, 17β-O*H*); ^{19}F NMR (338.8 MHz, $CDCl_3$) −89.59 (dbr d, J_{FF} = 233.4 Hz, J_{HF} = 2.8 Hz, one of the 3-F), −99.41 (dtt, J_{FF} = 233.7 Hz, J_{HF} = 34.1 Hz, 13.6 Hz, another 3-F); MS (70 eV) m/z (rel intensity) 312 (M^+, 51), 268 (16), 253 (100), 185 (24), 145 (11), 123 (33), 107 (22), 95 (29), 81 (35), 67 (40), 55 (42), 41 (41); HRMS calcd for $C_{19}H_{30}OF_2$ 312.2265, found 312.2270.

Biological Methods. Relative Binding Affinity (RBA). Relative binding affinities of androgens were determined in several receptor and binding protein systems as described in previous publications: androgen receptor (AR),[20,56] progesterone receptor (PgR),[20,27] mineralocorticoid receptor (MR),[43] and sex steroid binding protein (SBP).[57] The standard of the RBA measurement was tritium-labeled R1881 (K_d = 0.6 nM), R5020 (K_d = 0.4 nM), aldosterone (K_d = 3.9 nM), and estradiol (K_d = 1.6 nM) for AR, PgR, MR, and SBP, respectively. RBA values of the standards are 100 by definition.

Acknowledgment. We are grateful for support of this research through a grant from the Department of Energy (DE FG02 86ER60401). We thank Dr. V. VanRheenen of the Upjohn Co. and John Baran of G. D. Searle for supplying certain steroidal precursors.

(55) Upjohn Co. Steroids Brit 966491. *Chem. Abstr.* **1964**, *61*, 13385f.
(56) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. Photoaffinity Labels for Estrogen Binding Proteins of Rat Uterus. *Biochemistry* **1973**, *12*, 4085–4092.
(57) McElvany, K. D.; Carlson, K. E.; Katzenellenbogen, J. A.; Welch, M. J. Factors Affecting the Target Site Uptake Selectivity of Estrogen Radiopharmaceuticals: Serum Binding and Endogenous Estrogens. *J. Steroid Biochem.* **1983**, *18*, 635–641.

Bisquinolines. 1. *N,N*-Bis(7-chloroquinolin-4-yl)alkanediamines with Potential against Chloroquine-Resistant Malaria

Jonathan L. Vennerstrom,*,† William Y. Ellis,‡ Arba L. Ager, Jr.,§ Steven L. Andersen,‡ Lucia Gerena,‡ and Wilbur K. Milhous‡

College of Pharmacy, University of Nebraska Medical Center, 600 South 42nd Street, Omaha, Nebraska 68198-6025, and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, and Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida 33177.
Received December 26, 1991

On the basis of observations that several bisquinolines such as piperaquine possess notable activity against chloroquine-resistant malaria, 13 *N,N*-bis-(7-chloroquinolin-4-yl)alkanediamines were synthesized and screened against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo. Twelve of the thirteen bisquinolines had a significantly lower resistance index than did chloroquine; the resistance index was apparently unrelated to either in vitro or in vivo activity. Except for two compounds, there was a reasonable correlation between in vitro and in vivo activities. Seven of the thirteen bisquinolines had IC_{50}'s of less than 6 nM against both chloroquine-sensitive (D-6) and -resistant (W-2) clones of *P. falciparum* and were curative against *P. berghei* at doses of 640 mg/kg. In contrast to chloroquine, these bisquinolines did not show any toxic deaths at curative dose levels. Four bisquinolines, however, caused skin lesions at the site of injection. Maximum activity was seen in bisquinolines with a connecting bridge of two carbon atoms where decreased conformational mobility seemed to increase activity. Bisquinoline **3** ((±)-*trans*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine was not only the most potent bisquinoline in vitro, but was clearly unique in its in vivo activity—80% and 100% cure rates were achieved at doses of 160 and 320 mg/kg, respectively. In summary, these preliminary results support the premise that bisquinolines may be useful agents against chloroquine-resistant malaria.

By a large margin, malaria is the most prevalent disease in the world. It is estimated for the year 1986 that some 489 million people contracted malaria, 2.3 million of whom died from the disease.[1] Whereas effective antimalarial drugs exist, drug resistance, particularly resistance to

† University of Nebraska Medical Center.
‡ Walter Reed Army Institute of Research.
§ University of Miami.

(1) Stürchler, D. How much Malaria is there Worldwide? *Parasitol. Today* **1989**, *5*, 39–40.

0022-2623/92/1835-2129$03.00/0