Brassinosteroids: Synthesis and Activity of Some Fluoro Analogues

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Three types of 5α-androstane and ergostane analogues of brassinolide, containing a fluorine atom in either the 3α or the 5α positions or in 3α and 5α positions, were prepared using standard operations (reaction of 3β-alcohols with (diethylamino)sulfur trifluoride, cleavage of epoxide with HF in py or $BF_3 \cdot Et_2O$). The 5α-fluorine was found to affect chemical reactivity (e.g., electrophilic addition to the Δ^2-double bond) as well as physical properties (e.g., NMR, chromatographic behavior) of the products. Cytotoxicity of the products was studied using human normal and cancer cell lines with 28-homocastasterone as positive control and their brassinolide type activity was established using the bean second-internode test with 24-epibrassinolide as standard. The equivalence of F and OH groups was observed in some of the active compounds. The anticancer and the brassinolide-type activity do not correlate with each other: ergostane derivatives were most active in the former test while androstane derivatives were best in the latter.

Introduction

Brassinosteroids are plant hormones with many potential applications in agriculture due to their ability to stimulate growth of plants even under unsuitable conditions (lack of irrigation, nutrients, inadequate temperature).[1,2] Steroids were several times found to act on other than expected sites.[3,4] Thus some brassinosteroids also exert surprising antiviral and cancerostatic activities even in human cells.[5,6] The effect of brassinolide on androgen-independent human prostate cancer PC-3 cell was established: brassinolide (**1**, see Figure 1) was found to induce a time and concentration-dependent cytotoxicity in PC-3 cells. The mode of cell death appeared to be predominately apoptosis. Western blot studies indicated that treatment with brassinolide triggered a time-dependent decrease in the expression of antiapoptotic protein Bcl-2. Some other members of the brassinolide family (24-epibrassinolide and 28-homocastasterone, compounds **2** and **3**) were found[7] to inhibit the growth, at micromolar concentrations, of several human cancer cell lines without affecting the growth of normal cells. In addition, antiviral activity against herpes simplex virus types I and II (selectivity index against HSV-1 > 78.5) was reported for brassinosteroid derivatives.[8,9]

The most active brassinosteroid, brassinolide (**1**), was isolated and later synthesized[10] in many complicated and hence uneconomic ways. More easily accessible analogues have been therefore sought to replace brassinolide: often used and studied 24-epibrassinolide (**2**) is easily available from ergosterol although its activity is slightly lower than that of brassinolide. Equally active 28-homobrassinolide (**4**) was produced from commercial sitosterol; it even retains the important (*S*) configuration at C-24. Besides many others, the brassinosteroid family[11] also includes active compounds that contain a 6-oxo group instead of the lactone ring (e.g., castasterone, **5**).

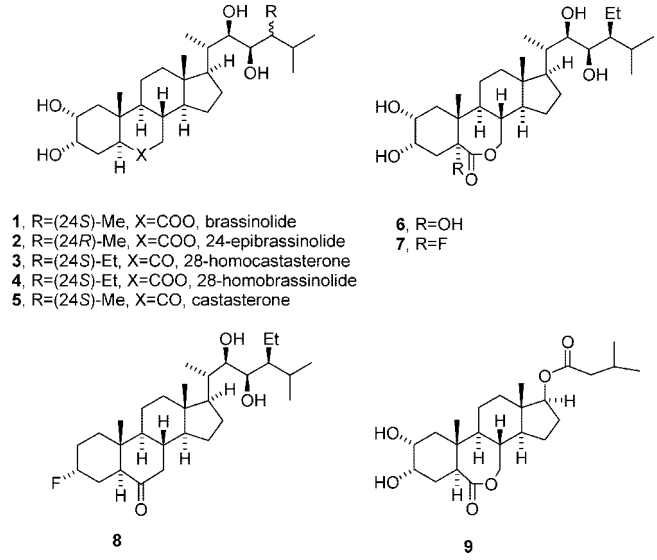

Figure 1

Electronegative substituents[12,13] (a hydroxyl group, fluorine) were also introduced to position 5α, leading to the active compounds such as **6** and **7**. Because the fluorine atom often[14] behaves as a hydroxyl isoster, and the metabolic stability of the C−F bond is much higher than that of the C−OH bond, analogues of brassinosteroids were sought in which the 3α hydroxyl was replaced with the 3α-fluorine (compound **8**). This would have its practical significance because good stability of products applied is invaluable and the brassinosteroid metabolism[15] is known to start with oxidation of the 3α hydroxyl, leading to inactive 3-ketones.

In another development, many structural changes were made to the side chain of brassinosteroids. Recently, compounds lacking the classical sterol side chain, e.g., compound **9**, which basically is ester of a 5α-androstane derivative,[16] were also found to have an activity comparable to natural brassinosteroids.

In this paper, we describe the preparation and properties of two types of epicastasterone analogues with the fluorine atom in positions 3α or 5α or both. The first type contains a 17β-

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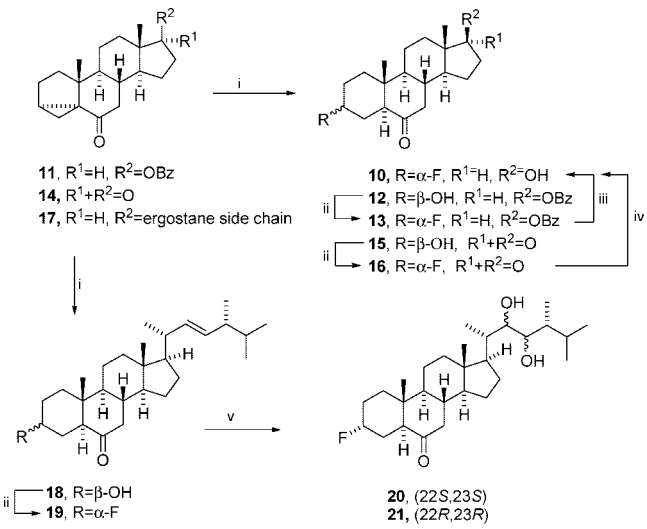
Scheme 1[a]



[a](i) Sulfuric acid, dioxane; (ii) DAST, CH_2Cl_2; (iii) K_2CO_3, EtOH; (iv) $NaBH_4$, py; (v) OsO_4, N-methylmorpholine N-oxide, (*O*-(4-chlorobenzoyl)-hydroquinine), 2-methyl-2-propanol, water

hydroxy or 17β-acyloxy group, the second has the epibrassinolide side chain.

Results

Synthesis. The simplest fluoro analogue of this series, compound **10** (see Scheme 1), was prepared from the known[17] i-steroid **11**: the treatment with sulfuric acid, followed by the reaction of intermediate 3β-alcohol **12** with (diethylamino)sulfur trifluoride (DAST)[12] afforded the 3α-fluoro compound **13**, and on deprotection, the target compound **10**. Alternatively, i-steroid **14** was first converted into 3β-alcohol and 3α -fluoride **15** and **16**, respectively; the 17-oxo group was then selectively reduced with sodium borohydride in pyridine, yielding the above 17β-alcohol **10**.

The same sequence was repeated with 3α,5-cyclo-5α-ergost-22-en-6-one (**17**), which was converted into 3β-hydroxy ketone **18**. DAST was again used to produce the corresponding fluoride **19**. Osmium tetroxide catalyzed dihydroxylation of the Δ^{22}-double bond produced an unseparable mixture of (22*S*,23*S*)- and (22*R*,23*R*)-diols **20** and **21**, respectively. Surprisingly, both isomers had identical chromatographic properties on silica gel and a HPLC column. They could, however, be distinguished and characterized by NMR spectra (vide infra).

Our synthetic approach to 5α-fluoro analogues was based on Ramirez's experience[18] with a hydrogen fluoride−pyridine complex opening of 5β,6β-epoxides: thus compound **22** yielded fluoro alcohol **23** (ref 19, see Scheme 2); alternative reaction with boron trifluoride etherate afforded alcohol **23** in the same yield (91%).

The Jones oxidation of the fluorohydrin **23** yielded ketone **24** that was partially hydrolyzed with one equivalent of potassium carbonate to give monoacetate **25** (76%, the corresponding diol **26** was only isolated in 22%). The 3β-alcohol **25** was either treated with DAST to produce 3α-fluoride **27** or tosylated and solvolyzed with sodium nitrite in HMPA to yield the corresponding 3α-hydroxy derivative **29**. Hydrolysis of the protecting acetoxy group in compounds **27** and **29** led to analogues **28** and **30**.

The last analogue, 5-fluoro-2α,3α,17β-trihydroxy-5α-androstan-6-one (**31**) was obtained using OsO_4 dihydroxylation of the Δ^2-unsaturated acetate **32** and hydrolysis of the 2:3 mixture of acetates **33** and **34**. Alternatively, the triol **31** was prepared by dihydroxylation of alcohol **35** and separation of its mixture with compound **36**.

The structure of all products prepared was confirmed by IR and ^{1}H NMR spectra (see Experimental Section). The inversion of configuration at the fluorination of 3β-alcohols to 3α-fluorides is manifested by a multiplet pattern of the H-3 signal in their ^{1}H NMR spectra: instead of a broad multiplet of axial H-3 in 3β-alcohol ($W \sim 35$ Hz; a triplet of triplets with two large diaxial and two small axial−equatorial couplings), the 3α-fluorides display an equatorial H-3 proton as a doublet of multiplets at ∼4.90 ppm that arise from a large geminal coupling (J(F,H) ∼ 48 Hz) and four small vicinal interactions (J(H,H) = 2.5−4 Hz). The presence of fluorine at 5α-position in compounds **24**−**36** is manifested in ^{1}H NMR spectra by the additional splitting of H-7α and H-7β protons (∼2.60 and ∼2.25 ppm) with fluorine (J(F,H-7α) ∼ 7.5 Hz and J(F,H-7β) ∼ 3.5 Hz).

NMR Analysis. The presence of fluorine can be observed either directly in ^{19}F NMR spectra and/or indirectly as the additional splitting of signals in ^{1}H and ^{13}C NMR spectra. The identification of fluorine-coupled hydrogens and carbon atoms requires a complete structural assignment of ^{1}H and ^{13}C NMR signals. For such a detailed NMR analysis, we have chosen 3α-fluoro derivatives **10**, **20**, and **21**, 5α-fluoro derivatives **25**, **33**, and **34**, and 3α,5α-difluoro derivative **27**. The usual combination of homo- and heteronuclear 2D-NMR spectra (H,H-COSY, H,H-ROESY, H,C-HSQC, and H,C-HMBC) was used for structural assignment and the ^{1}H and ^{13}C NMR data are summarized in Supporting Information Tables 1 and 2.

Fluorine signals were observed as multiplets in ^{19}F proton coupled NMR spectra, and their chemical shifts are given in Supporting Information Table 3. There is a clear difference in chemical shifts between fluorine at the secondary position 3 (−177 to −184 ppm) and the tertiary position 5 (−154 to −160 ppm).

While the J(F,C) couplings were obtained from the doublets observed for fluorine-coupled carbons in a ^{13}C NMR spectrum, the analysis of J(F,H) interactions from proton multiplets in a 1D ^{1}H NMR spectrum is difficult, particularly for protons in the crowded upfield region. Therefore, we used 2D-heteronuclear ^{19}F−^{1}H-COSY spectroscopy[20] for identification of fluorine coupled proton signals.

The typical J(F,H) observed in 3α-fluoro-, 5α-fluoro-, and 3α,5α-difluoro derivatives for the selected compounds **10**, **25**, and **27** are shown in Figure 2. The largest values were found for geminal ^2J(F-3,H-3) ∼ 48 Hz and the vicinal diaxial ^3J(Fax,Hax) = 43.8−45.6 Hz, while vicinal axial−equatorial ^3J(Fax,Heq) were in the range 7.5−14 Hz. Fluorine at position 5α showed additional long-range couplings ^4J(F,H-7α) ∼ 8 Hz, ^4J(F,H-7β) ∼ 3.5 Hz, and ^4J(F,H-19) ∼1.5 Hz.

The J(F,C) values in the selected compounds **10**, **25**, and **27** are shown in Figure 2. The largest values were found for one-bond interactions (^1J(C,F) ∼ 165−180 Hz), which are followed by geminal coupling values (^2J(F,C) = 19−27 Hz). Vicinal coupling values were found much smaller (^3J(F,C) = 0−5.8 Hz).

Biological Part. Anticancer Activity. Anticancer activity was determined by comparing human normal (fibroblast BJ) and cancer cell lines (T-lymphoblastic leukemia CEM, breast carcinoma MCF 7, and multiple myeloma RPMI 8226). Cells of all of these lines were exposed to six serial 4-fold dilutions of each drugs for 72 h, and the proportions of surviving cells were then estimated and IC_{50} values were calculated. The results, obtained

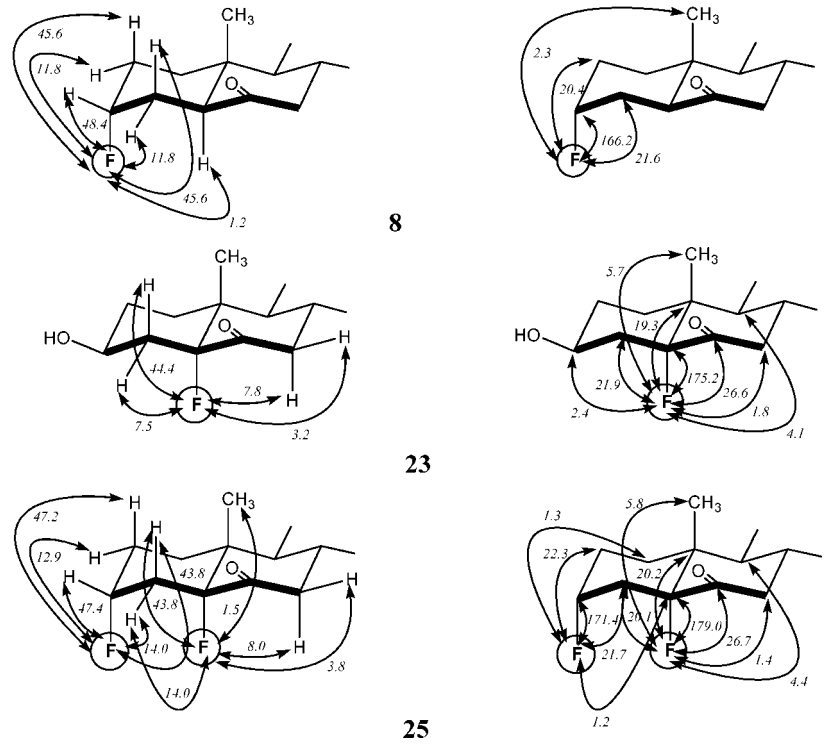


Figure 2. The observed *J*(F,H) and *J*(F,C) (indicated with arrows) are shown in partial structures of 3α-fluoro, 5α-fluoro, and 3α,5α-difluoro derivatives **8**, **23**, and **25**.

from calcein-AM assays, are presented in Table 1. 28-Homocastasterone was used as a positive control.

Brassinolide-Type Activity. Brassinolide-type activity was measured by the bean second-internode bioassay[21] modified by us[16] (see Table 2). Compounds causing maximal elongation above 20 mm are considered promising.

Discussion

Chemistry. All the reaction steps were carried out in a standard manner. Surprisingly, the presence of the strongly electronegative fluorine atom in position 5 exerted its effect on the reactivity of 3β-alcohols (e.g., **12**) with DAST: the yield of the 3α-fluorides sought (e.g., **10**) was lower than that of products of elimination, Δ^2-olefins (e.g., **32**), which lack the strong 1,3-diaxial interaction between substituents in positions 3α and 5α.

Another effect of the 5α-fluorine substitution was observed in the osmium catalyzed dihydroxylation of olefin **32**: while in nonfluorinated substrates the α-attack, producing 2α,3α-diols, markedly prevails, in the fluoro derivative **32**, the 2β,3β-diol, again lacking the strong 1,3-diaxial interactions, is the major product. A similar effect of an electronegative substituent (OH group) in position 5 on the ratio of substitution/elimination was already observed by Spanish authors.[13]

Biological Activity. The 3α-fluoro analogues of 24-epicastasterone (**20**, **21**) exhibit the same or better antiproliferative activity against the four tested cell lines (IC50 13.8−45.9 mM, see Table 1) as the 3α-hydroxyl containing 28-homocastasterone (**3**) and other natural brassinosteroids.[7] The 3α,5α-difluoride **27** is only slightly active against the leukemia cell line; other cell lines were not significantly affected.

The surprising finding of a lower activity in the 3β-hydroxy analogue **18** and complete inactivity of the 3α-fluoro analogue **19** may be ascribed to low solubility of compounds with a long and lipophilic side chain.

Similarly, the 5α-fluoro ketone **36** was only marginally active against multiple myeloma; this compound with a typical 2β,3β-dihydroxy grouping, however, is rather an analogue of ecdysone. This fact relates to the finding of ecdysteroids in men with severe pathological conditions, which was once suggested as a potential clinical marker.[22]

Table 1. Cytotoxic Effect (IC_{50}) of Brassinosteroids Determined by Calcein-AM Assays

compound	cell line[a]			
	CEM[b] (μM)	**MCF 7**[c] (μM)	**RPMI 8226**[d] (μM)	**BJ**[e] (μM)
3	13 ± 2.8	>50	26 ± 1.4	>50
10	>50	>50	>50	>50
12	>50	>50	>50	>50
13	>50	>50	>50	>50
16	>50	>50	>50	>50
18	26.4 ± 0.1	40.4 ± 3.9	27.0 ± 0.04	42 ± 2.3
19	>50	>50	>50	>50
20, **21**	13.8 ± 2.3	25.7 ± 4.3	23.5 ± 3.5	45.9 ± 0.3
25	>50	>50	>50	>50
26	>50	>50	>50	>50
27	42.9 ± 4.9	>50	>50	>50
28	>50	>50	>50	>50
29	>50	>50	>50	>50
30	>50	>50	>50	>50
31	>50	>50	>50	>50
32	>50	>50	>50	>50
33	>50	>50	>50	>50
34	>50	>50	>50	>50
35	>50	>50	>50	>50
36	>50	>50	45.4 ± 0.08	>50

[a] The IC_{50} values are expressed as mean ± SD values of three independent experiments performed in triplicate. [b] T-lymphoblastic leukemia cell line CEM. [c] Breast carcinoma cell lines MCF-7. [d] Multiple myeloma RPMI 8226. [e] Human fibroblast BJ.

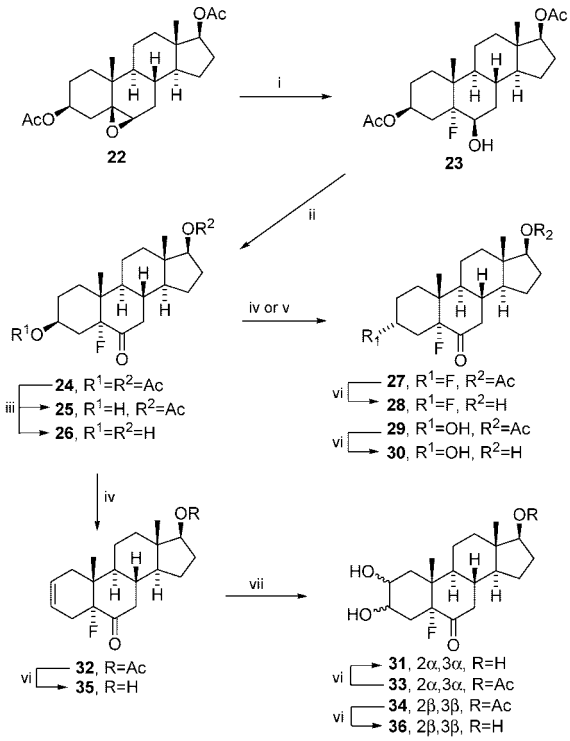
The others tested compounds had extremely weak or no detectable activity, even when tested in concentrations of up to 50 μM.

The Brassinolide-Type Activity. The above fluoro steroids exhibited surprisingly high activity (see Table 2), which negates the generally accepted essentiality of the 3α hydroxyl group

Table 2. Activity in the Bean Second-Internode Bioassay

compound	prolongation of the second internode SD (mm) at concentration 0^{-10} mol·L^{-1}	SD
10	5.9	±2.5
12	3.8	±1.9
13	0.8	±0.7
16	14.9	±1.2
18	9.4	±0.7
19	9.9	±4.3
20, 21	13.2	±5.1
25	17.1	±0.9
26	16.9	±6.7
27	19.8	±2.5
28	18.4	±2.3
29	21.4	±2.3
30	43.4	±0.9
31	19.5	±4.9
32	21.8	±3.0
33	12.3	±2.4
34	21.4	±4.1
35	13.6	±2.1
36	21.8	±2.9

Scheme 2[a]



[a](i) HF, py; (ii) Jones reagent, acetone; (iii) K_2CO_3, water, MeOH; (iv) DAST, CH_2Cl_2; (v) TosCl, py, $NaNO_2$, HMPA; (vi) HCl, MeOH; (vii) OsO_4, N-methylmorpholine N-oxide, 2-methyl-2-propanol, water

for this type of activity.[12] The most active compounds were 5α-fluoro ketones **30** and related compounds **27**−**32**, the effect of the additional 3α-fluorine being insignificant. The effect of the sole fluorine in the 3α position was lower than that in the 5α position (**10** < **30**).

The above results prove the equivalence of 3α-F and 3α-OH groups in this respect, which, combined with the known perfect metabolic stability of the C−F bond, shows that 3α-fluorinated analogues of brassinolide can lead to products useful for practical application. Both the activities studied, however, do not correlate with each other: the highest anticancer activity was found in the ergostane derivatives (compounds **20** and **21**), the highest brassinolide-type activity among the androstane[23] derivatives (**30**).

Experimental Section

3α-Fluoro-17*β*-hydroxy-5α-androstan-6-one (10). (1) From Benzoate 13. Compound **13** (500 mg, 1.21 mmol) was added to a solution of potassium carbonate in ethanol (5%, 50 mL) and the reaction mixture was heated to reflux for 2 h. The solution was concentrated to a quarter of its original volume and diluted with brine. The product was extracted with chloroform and worked up as usual (see Supporting Information), yielding Compound **10** as white solid (240 mg, 64%); mp 189−193 °C (acetone/heptane), $[\alpha]_D$ −15.6 (c 0.26). IR: 3614, 1066 (OH), 1706 (CO), 991 (C−F). For ^{1}H, ^{13}C, and ^{19}F NMR data, see Supporting Information Tables 1−3. Anal. ($C_{19}H_{29}FO_2$) C, H.

(2) From Diketone 16. A solution of sodium borohydride in pyridine (1 mL, 0.65 mmol) was added to a solution of diketone **16** (120 mg, 0.39 mmol) in pyridine (2 mL) at −10 °C. After standing at rt for 72 h, the reaction mixture was poured into dilute hydrochloric acid, the product was extracted with chloroform, and then worked up as usual. PLC chromatography yielded the product **10** (26 mg, 21%), identical with the sample prepared above.

3*β*-Hydroxy-6-oxo-5α-androstan-17*β*-yl Benzoate (12). Following the method A (see Supporting Information), i-steroid **11**[17] (3.31 g, 8.46 mmol) yielded compound **12** as white crystals (3.10 g, 90%); mp 232−236 °C (acetone), $[\alpha]_D$ +9.5 (c 0.23). IR: 3609, 1054 (OH), 1710 (CO), 1280, 978 (C−O), 1603, 1451 (arom.). ^{1}H NMR δ: 0.79 (s, 3H, H-18), 0.94 (s, 3H, H-19), 3.58 (m, 1H, $W \sim$ 35 Hz, H-3), 4.90 (t, 1H, J = 8.5 Hz, H-17), 7.48 (m, 3H, aromatic protons), and (8.05 m, 2H, aromatic protons). Anal. ($C_{26}H_{34}O_4$) C, H.

3α-Fluoro-6-oxo-5α-androstan-17*β*-yl Benzoate (13). Alcohol **12** (100 mg, 0.24 mmol) was treated with DAST according to the method B. A little of 6-oxo-androst-2-ene-17β-yl benzoate was found in a NMR spectrum of the product, therefore, the mixture was treated with 3-chloroperoxybenzoic acid in dichloromethane and the admixture was oxidized to a more polar derivative. White crystals of compound **13** (37 mg, 37%) were obtained by PLC on three plates (toluene/ethyl acetate, 7:3); mp 196−198 °C (acetone/heptane), $[\alpha]_D$ +9.7 (c 0.30). IR: 1710 (CO), 1279 (C−O), 1602, 1452 (arom), 991 (C−F). ^{1}H NMR δ: 0.77 (s, 3H, H-18), 0.94 (s, 3H, H-19), 4.90 (t, 1H, J = 7.8 Hz, H-17), 4.92 (dm, 1H, J(F,H-3) = 48 Hz, H-3), 7.48 (m, 3H, aromatic protons), and 8.05 (m, 2H, aromatic protons). Anal. ($C_{26}H_{33}FO_3$) C, H.

3*β*-Hydroxy-5α-androstane-6,17-dione (15). i-Steroid **14**[24,25] (3.6 g, 12.57 mmol) was converted into a white solid **15** (2.9 g, 76%) according to the method A; mp 176−178 °C (acetone/heptane, ref 25, records the same value), $[\alpha]_D$ +57.7 (c 0.5, ref 25, gives +58). ^{1}H NMR δ: 0.79 (s, 3H, H-18), 0.88 (s, 3H, H-19), 3.59 (tt, 1H, J = 11.2 and 4.7 Hz, H-3).

3α-Fluoro-5α-androstane-6,17-dione (16). The 3β-alcohol **15** (100 mg, 0.3 mmol) was converted into 3α-fluoro steroid **16** according to the method B. The product was treated with 3-chloroperoxybenzoic acid as above and purified by PLC (3 plates, toluene/ethyl acetate, 7:3) to yield compound **16** as a white solid (44 mg, 44%); mp 211−213 °C (methanol), $[\alpha]_D$ +50.3 (c 0.11). IR: 1735, 1710 (CO), 989 (C−F). ^{1}H NMR δ: 0.77 (s, 3H, H-18), 0.88 (s, 3H, H-19), 4.92 (dm, 1H, J(F,H-3) = 48 Hz, H-3). Anal. ($C_{19}H_{27}FO_2$) C, H.

(22*E*,24*R*)-3*β*-Hydroxy-5α-ergost-22-en-6-one (18). i-Steroid **17**[26] (200 mg, 0.5 mmol) was converted into 3β-alcohol **18**[27,28] according to the method A. A white precipitate of compound **18** (200 mg, 95%) crystallized from methanol; mp 186−187 °C, (refs 27, 28 records the same value), $[\alpha]_D$ −33.1 (c 0.5). ^{1}H NMR δ: 0.68 (s, 3H, H-18), 0.76 (s, 3H, H-19), 0.82 (d, 3H, J = 6.5 Hz, H-26), 0.83 (d, 3H, J = 7.0 Hz, H-27), 0.91 (d, 3H, J = 6.4 Hz, H-21), 1.01 (d, 3H, J = 6.2 Hz, H-28), 3.58 (m, 1H, $W \sim$ 35 Hz, H-3), 5.18 (m, 2H, H-23 and H-24).

(22*E*,24*R*)-3α-Fluoro-5α-ergost-22-en-6-one (19). The 3β-alcohol **18** (210 g, 0.5 mmol) was treated with DAST according to the

method B. The product was purified using PLC to yield 3α-fluoride **19** (81 mg, 38%) as a white solid; mp 168−169 °C (methanol), $[\alpha]_D$ −24.7 (c 0.24). IR: 1705 (CO), 986 (C−F). ^{1}H NMR δ: 0.68 (s, 3H, H-18), 0.74 (s, 3H, H-19), 0.82 (d, 3H, J = 6.6 Hz, H-26), 0.83 (d, 3H, J = 6.6 Hz, H-27), 0.91 (d, 3H, J = 6.8 Hz, H-21), 1.01 (d, 3H, J = 6.6 Hz, H-28), 4.91 (dm, 1H, J(F,H-3) = 48 Hz, H-3), 5.18 (m, 2H, H-23 and H-24). Anal. ($C_{28}H_{45}FO$) C, H. Olefin (22*E*,24*R*)-5α-ergosta-2,22-dien-6-one was isolated as a more polar side product.

(22*S*,23*S*,24*R*)-3α-Fluoro-22,23-dihydroxy-5α-ergostan-6-one (20) and (22*R*,23*R*,24*R*)-3α-Fluoro-22,23-dihydroxy-5α-ergostan-6-one (21). The 3α-fluoro derivative (**19**) (170 mg, 0.41 mmol) was hydroxylated according to the method C1. Evaporation of the solvent gave a white solid of a mixture (**20** and **21**, 115 mg, 63%), which was inseparable using silica gel chromatography (column or HPLC); mp 168−176 °C (methanol). IR: 3628, 3551 (OH), 1382, 1369 (CH_3), 1082 (C-OH), 1704 (CO), 987 (C−F). For ^{1}H, ^{13}C, and ^{19}F NMR data, see Supporting Information Tables 1−3. Anal. ($C_{28}H_{47}FO_3$) C, H.

5-Fluoro-6-oxo-5α-androstane-3*β*,17*β*-diyl Acetate (24). Alcohol **23**[19] (230 mg, 0.56 mmol) was oxidized in acetone (4 mL) with the Jones reagent at 0 °C. After 5 min, the excess of reagent was reduced with solution sodium metabisulfite (5 mL), and the product was extracted with chloroform. The extract was washed with brine, dried, and concentrated in vacuo. Crystallization yielded white crystals of ketone **24** (210 mg, 91%); mp 167.5−168 °C (acetone/heptane), $[\alpha]_D$ −21.9 (c 0.3), (ref 19 gives 185−186 °C and $[\alpha]_D$ −27). IR: 1727, 1367, 1260, 1247, 1048 (acetate), 1017 (C−F). ^{1}H NMR δ: 0.78 (s, 3H, H-18), 0.84 (s, 3H, H-19), 2.03 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.25 (dt, 1H, J = 12.5 and 3.7 Hz, H-7β), 2.60 (dt, 1H, J = 12.5 and 7.7 Hz, H-7α), 4.64 (t, 1H, J = 8.6 Hz, H-17), 5.00 (m, 1H, W ∼ 35 Hz, H-3).

5-Fluoro-3*β*-hydroxy-6-oxo-5α-androstan-17*β*-yl Acetate (25). A solution of potassium carbonate (670 mg, 4.85 mmol) in water (12 mL) and methanol (24 mL) was added to a solution of diacetate **24** (1.83 g, 4.48 mmol) in methanol (180 mL). After 1 h at rt, acetic acid (0.6 mL) was added and the solution was concentrated in vacuo. Brine precipitated a white solid, which was extracted with ethyl acetate. The extract was washed with brine, dried, and concentrated in vacuo. Chromatography of the remainder on a column of silica gel (150 mL) yielded alcohol **25** (1.25 g, 76%); mp 165−167 °C (1.18 g, acetone/heptane), $[\alpha]_D$ −16.4 (c 0.18). IR: 3500, 3439, 3611, 1048 (OH), 1725, 1257 ($COCH_3$). For ^{1}H, ^{13}C, and ^{19}F NMR data, see Supporting Information Tables 1−3. Anal. ($C_{21}H_{31}FO_4$) C, H.

5-Fluoro-3*β*,17*β*-dihydroxy-5α-androstan-6-one (26). The more polar component of the above chromatography, diol **26** (317 mg, 22%) crystallized from chloroform; mp 217−219 °C, $[\alpha]_D$ −23.2 (c 0.23). IR: 3610, 3444, 1058 (OH), 1726 (CO). ^{1}H NMR δ: 0.73 (s, 3H, H-18), 0.82 (s, 3H, H-19), 2.23 (ddd, 1H, J_{gem} = 12.8, J(7) = 3.4 and J(7,5αF) = 4.2 Hz, H-7β), 2.59 (dt, 1H, J_{gem} = 12.8, J(7,8) = 12.2 and J(7α,F) = 7.9 Hz, H-7α), 3.66 (t, 1H, J = 8.7 Hz, H-17), 3.89 (m, 1H, W ∼ 35 Hz, H-3). Anal. ($C_{19}H_{29}FO_3$) C, H.

3α,5-Difluoro-6-oxo-5α-androstan-17*β*-yl Acetate (27). Following the method B, alcohol **25** (300 g, 0.8 mmol) was treated with DAST. The residue was separated by PLC (6 plates, a mixture of light petroleum/ether, 7:3). The polar zone afforded a white solid (47 mg, 16%) of 3α-fluoride **27**; mp 168−170 °C (acetone/heptane), $[\alpha]_D$ −23.2 (c 0.12). IR: 1725 (CO), 1256 (C−O), 1018, 993 (C−F). For ^{1}H, ^{13}C, and ^{19}F NMR data, see Supporting Information Tables 1−3. Anal. ($C_{21}H_{30}F_2O_3$) C, H.

3α,5-Difluoro-17*β*-hydroxy-5α-androstan-6-one (28). Acetate **27** (70 mg, 0.19 mmol) was hydrolyzed according to the method D. Chromatography of the remainder by PLC on two plates yielded compound **28** as white crystals (46 mg, 74%); mp 180−182 °C (acetone/heptane), $[\alpha]_D$ −15.6 (c 0.24). IR: 3614 (OH), 1725 (CO), 1027, 993 (C−F). ^{1}H NMR δ: 0.74 (s, 3H, H-18), 0.79 (s, 3H, H-19), 3.70 (dt, 1H (J(17,16α) = 8.5, J(17,16β) = 8.5 and J(17,OH) = 6.0 Hz, H-17), 4.92 (dm, 1H, J = 47 Hz, H-3). Anal. ($C_{19}H_{28}F_2O_2$) C, H.

5-Fluoro-3α-hydroxy-6-oxo-5α-androstan-17*β*-yl Acetate (29). 4-Toluenesulfonyl chloride (200 mg, 1.04 mmol) was added to a solution of 3β-alcohol (**25**, 200 mg, 0.55 mmol) in pyridine (2 mL). After 48 h, the mixture was diluted with brine, the white precipitate was extracted with chloroform, the extract was washed with water, dilute hydrochloric acid, then potassium hydrogen carbonate solution, and then dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo to dryness afforded tosylate, which was solvolyzed with sodium nitrite (480 mg, 0.70 mmol) in HMPA (25 mL) at 90 °C for 2 h under nitrogen atmosphere. The mixture was poured into water and the product was extracted with chloroform. The extract was subsequently washed with water, dilute hydrochloric acid, water, and then potassium hydrogen carbonate solution and water. Evaporation of the solvent and PLC (5 plates, benzene/ethyl acetate, 7:3) yielded the 3α-alcohol **29** (83 mg, 41%) as white crystals; mp 139−142 °C (acetone/heptane), $[\alpha]_D$ −13.6 (c 0.21). IR: 3617, 1017 (OH), 1725 (AcO). ^{1}H NMR δ: 0.78 (s, 3H, H-18), 0.80 (s, 3H, H-19), 2.04 (s, 3H, CH_3CO), 4.08 (m, 1H, W ∼ 16 Hz, H-3), 4.64 (t, 1H, J = 8.5 Hz, H-17). Anal. ($C_{21}H_{31}FO_4$) C, H.

5-Fluoro-3α,17*β*-dihydroxy-5α-androstan-6-one (30). Compound **29** (70 mg, 0.19 mmol) was hydrolyzed according to the method D to afford compound **30** (50 mg, 81%) as white crystals; mp 260−262 °C (acetone/heptane), $[\alpha]_D$ −12.4 (c 0.31). IR: 3617, 1070 (OH), 1725 (CO), 1020 (C−F). ^{1}H NMR δ: 0.74 (s, 3H, H-18), 0.80 (s, 3H, H-19), 3.70 (t, 1H, J = 8.5 Hz, H-17), 4.08 (m, 1H, W ∼ 16 Hz, H-3). Anal. ($C_{19}H_{29}FO_3$) C, H.

5-Fluoro-2α,3α,17*β*-trihydroxy-5α-androstan-6-one (31). (1) By Hydrolysis. Acetate **33** (50 mg, 0.13 mmol) was hydrolyzed according to the method D, yielding triol **31** as white crystals (32 mg, 72%); mp 187−189 °C (methanol), $[\alpha]_D$ −5.7 (c 0.19). IR: 3608, 3561 (OH), 1726 (CO), 1074, 1046, 999 (C-OH), 1015 (C−F).^{1}H NMR δ: 0.74 (s, 3H, H-18), 0.83 (s, 3H, H-19), 3.71 (dt, 1H, J(17,16α) = 8.4 Hz, J(17,16β) = 8.5 Hz and J(17,OH) = 6.0 Hz, H-17), 3.75 (m, 1H, W = 33 Hz, H-2), 4.04 (m, 1H, W = 26 Hz, H-3). Anal. ($C_{19}H_{29}FO_4$) C, H.

(2) By Dihydroxylation. The olefin **35** (61 mg, 0.20 mmol) was hydroxylated according to the method C2. PLC chromatography (2 plates, elution toluene/ethyl acetate, 1:1) afforded the above triol **31** (21 mg, 31%) as the more polar product.

5-Fluoro-6-oxo-5α-androst-2-en-17*β*-yl Acetate (32). The olefin **32** (170 mg, 60%) was isolated as the less polar product in the PLC preparation of compound **27**; mp 156−158 °C (acetone/heptane), $[\alpha]_D$ −3.0 (c 0.21). IR: 1724 (CO), 1661 (CC), 1257 (C−O), 1018 (C−F). ^{1}H NMR δ: 0.74 (s, 3H, H-18), 0.79 (s, 3H, H-19), 2.05 (s, 3H, $COCH_3$),4.64 (t, 1H, J = 8.0 Hz, H-17), 5.63 (m, 2H, H-2 and H-3). Anal. ($C_{21}H_{29}FO_3$) C, H.

5-Fluoro-2α,3α-dihydroxy-6-oxo-5α-androstan-17*β*-yl Acetate (33). The olefin **32** (70 mg, 0.20 mmol) was hydroxylated according to the method C2. PLC chromatography (3 plates, toluene/ethyl acetate, 6:4) yielded diol **33** (25 mg, 33%) as a white solid; mp 172−174 °C (methanol), $[\alpha]_D$ −7.3 (c 0.22). IR: 3604, 3560 (OH), 1726 (CO), 1255, 1034 (C−O), 1047, 996 (C-OH), 1019 (C−F). For ^{1}H, ^{13}C, and ^{19}F NMR data, see Supporting Information Tables 1−3. Anal. ($C_{21}H_{31}FO_5$) C, H.

5-Fluoro-2*β*,3*β*-dihydroxy-6-oxo-5α-androstan-17*β*-yl Acetate (34). The lipophilic zone from the above chromatography afforded diol **34** (36 mg, 47%) as a white solid; mp 237−239 °C (methanol), $[\alpha]_D$ −21.3 (c 0.25). IR: 3604, 3560 (OH), 1726 (CO), 1257, 1035 (C−O), 1046, 993 (C-OH), 1018 (C−F). For ^{1}H, ^{13}C, and ^{19}F NMR data, see Supporting Information Tables 1−3. Anal. ($C_{21}H_{31}FO_5$) C, H.

5-Fluoro-17*β*-hydroxy-5α-androst-2-en-6-one (35). The 17β-acetoxy derivative **32** (330 mg, 0.95 mmol) was hydrolyzed according to the method D, yielding the 17β alcohol **35** (206 mg, 71%) as a white solid; mp 151−153 °C (acetone/heptane), $[\alpha]_D$ −5.0 (c 0.27). IR: 3614, 1060 (OH), 1724 (CO), 1661 (CC), 1023 (C−F). ^{1}H NMR δ: 0.74 (s, 3H, H-18), 0.75 (s, 3H, H-19), 3.70 (dt, 1 H, J(17,16α) = 8.5, J(17,16β) = 8.5 and J(17,OH) = 6.0 Hz, H-17), 5.64 (m, 2H, W ∼ 47 Hz, H-2 and H-3). Anal. ($C_{19}H_{27}FO_2$) C, H.

5-Fluoro-2β,3β,17β-trihydroxy-5α-androstan-6-one (36). (1) By Hydrolysis. Following the method D, the acetate **34** (50 mg, 0.13 mmol) was hydrolyzed to yield white crystals of the 17β-alcohol **36** (33 mg, 75%); mp 213−215 °C (methanol), $[\alpha]_D$ −15.0 (c 0.22). IR: 3625, 3612 (OH), 1726 (CO), 1074, 1045, 998 (C-OH), 1016 (C−F). ^{1}H NMR δ: 0.73 (s, 3H, H-18), 1.03 (s, 3H, H-19), 3.64 (dt, 1H, J(17,16α) = 8.5, J(17,16β) = 8.5 and J(17,OH) = 6.0 Hz, H-17), 3.85 (m, 1 H, $W \sim 24.5$ Hz, H-3), 3.98 (m, 1H, $W \sim 14.0$ Hz, H-2). Anal. ($C_{19}H_{29}FO_4$) C, H.

(2) By Dihydroxylation. A less polar zone of the PLC plates used in the above preparation of diol **31** from olefin **35** was eluted to yield diol **36** (31 mg, 46%) identical with the above sample.

Biological Evaluation. Calcein-AM Cytotoxicity Assay. The cell lines (T-lymphoblastic leukemia cell line CEM, breast carcinoma cell lines MCF-7, multiple myeloma RPMI 8226, and human fibroblast BJ) were cultured in DMEM medium (Gibco BRL) supplemented with 10% of fetal calf serum, 4 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin, at 37 °C in a fully humidified atmosphere containing 5% CO_2. The cell suspension of approximate density of 1.25×10^5 cells/mL was redistributed into 96-well microtiter plates and after 3 h of stabilization the tested steroids were added in different concentrations. Tested compounds were dissolved in DMSO before addition to cultures. Control cultures were treated with DMSO alone. The final concentration of DMSO in the reaction mixture never exceeded 0.6%. Four-fold dilutions of the intended test concentration were added at time zero in 20 μL aliquots to the microtiter plate wells. Each tested compound was evaluated at six 4-fold dilutions. In routine testing, the highest well concentration was 50 μM, but it can be the matter of change dependent on the agent. After 72 h of cultivation, the cells were incubated with Calcein AM solution (Molecular Probes) for 1 h. Fluorescence of viable cells was quantified with Fluoroscan Ascent (Microsystems). The percentage of surviving cells in each well was calculated from the equation $IC_{50} = (OD_{\text{drug exposed well}}/\text{mean } OD_{\text{control wells}}) \times 100\%$; each compound was tested in triplicate, and the entire test was repeated at least three times. The IC_{50} value, the concentration lethal to 50% of the cells, of each tested substance, was calculated from the obtained dose response curves.[7]

The Bean Second-Internode Bioassay. Seeds of bean (*Phaseolus vulgaris* L., cv. Pinto) germinated for 2 days were selected for uniformity from a large population of seedlings and then transferred into pots containing perlite and 1/10 diluted Hoagland solution (half concentration, pH 5.7).[21] Seedlings were grown in a light-controlled cultivation room (25−27 °C, light 48 W·m^{-2}, light/dark period 16/8 h). Seven-day-old plants with second internodes 2 mm long were treated with different amounts of tested compounds in 5 μL fractionated lanolin. The substances were applied in microdrops to the scar left after the removal of bract from the base of the second internode. The control plants were treated with lanolin alone. At least seven plants were used for each experiment, and the assays were repeated at least three times. The length of the second internodes was measured after 5 days, and the difference in length between treated and control plants provided a measure of activity (see Table 2).

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Supporting Information Available: Supplementary data on routine experimental procedures, combustion analysis of products, and their NMR spectra are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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