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Effects on Bioactivity due to C-5 Heteroatom Substituents on Synthetic 28-Homobrassinosteroid Analogs

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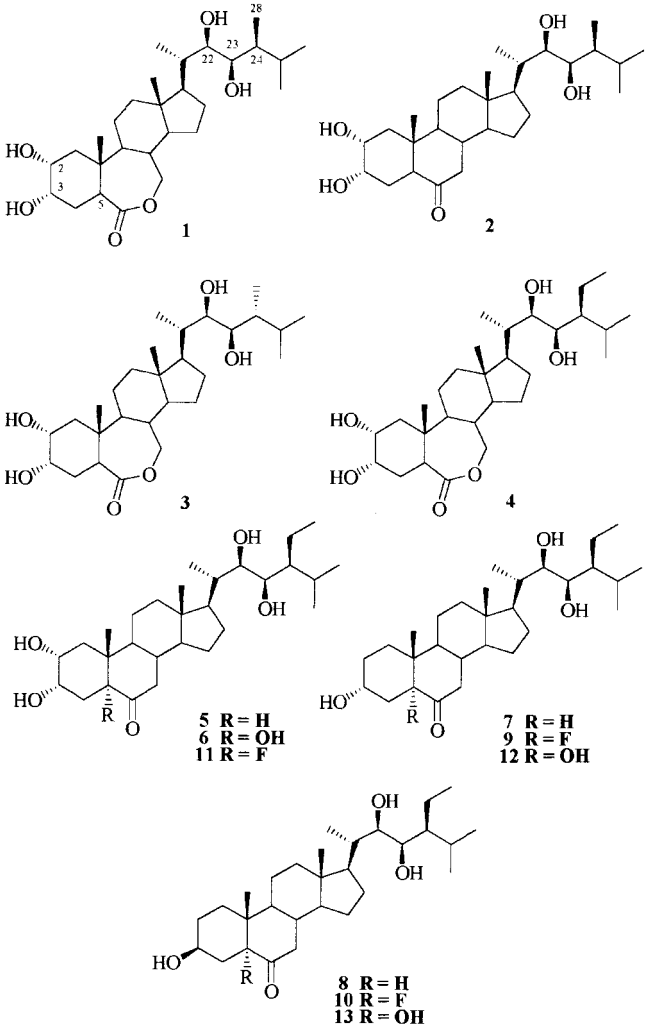
Abstract—Five new 28-homobrassinosteroids have been synthesized, namely, (22*R*,23*R*)-5-fluoro-3α,22,23-trihydroxy-5α-stigmastan-6-one, (22*R*,23*R*)-5-fluoro-3β,22,23-trihydroxy-5α-stigmastan-6-one, (22*R*,23*R*)-5-fluoro-2α,3α,22,23-tetrahydroxy-5α-stigmastan-6-one, (22*R*,23*R*)-3α,5,22,23-tetrahydroxy-5α-stigmastan-6-one and (22*R*,23*R*)-3β,5,22,23-tetrahydroxy-5α-stigmastan-6-one. Their bioactivities were evaluated by the rice lamina inclination test. C-5α Fluorinated analogs showed excellent in vitro bioactivity, also revealed at low doses, while C-5α hydroxylated analogs resulted in an important decrease in bioactivity. Previously given explanations to justify the decreasing effect due to C-5α electronegative groups should be revised.

Introduction

Brassinolide (**1**) is a powerful steroidal plant regulator that was discovered in 1979 by Grove, Mandava and co-workers[1] and displays activity at doses as low as 1 ng per individual plant in some species. This compound, as well as related brassinosteroids that were discovered subsequently, have therefore attracted considerable attention to their synthesis,[2,3] biosynthesis and metabolism,[2,4] bioactivity and field applications,[2,5–8] and molecular biology.[9–12]

Castasterone (**2**), the B-ring ketone analog of **1**, is also intrinsically bioactive in some plant species, although it serves as the biosynthetic precursor of **1** in others.[2]

Several variations of the side chain segment C-24 to C-28 have also been reported. Some of these, such as 24-epibrassinolide (**3**), 28-homobrassinolide (**4**) and 28-homocastasterone (**5**), possess substantial bioactivity and have been widely employed in field trials[2] because of their greater synthetic accessibility compared to **1**.



Keywords: brassinosteroids; bioactivity; C-5 fluorinated analogs; C-5 hydroxylated analogs.

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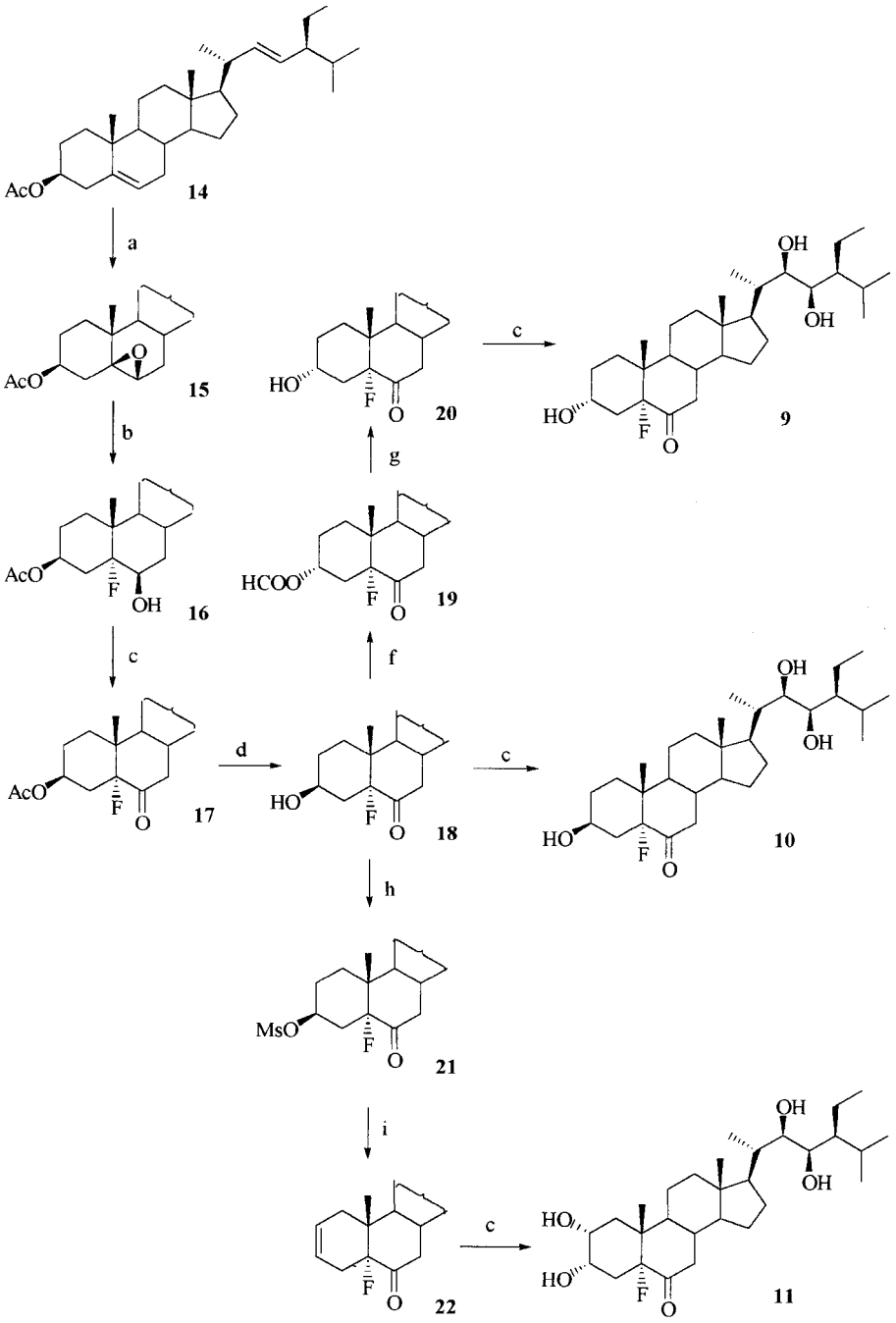


Scheme 1. Synthesis of 5α-fluorinated analogs. Reagents and conditions: (a) $KMnO_4/Fe(NO_3)_3/t$-BuOH/H_2O/CH_2Cl_2/rt; (b) BF_3 etherate/Et_2O; (c) PCC/$HPy^+CF_3COO^-/CH_2Cl_2$/rt; (d) K_2CO_3/MeOH/rt; (e) $K_2OsO_4/K_3Fe(CN)_6/(DHQD)_2Phal/K_2CO_3/t$-BuOH/$H_2O/CH_3SO_3NH_2$/rt; (f) HCOOH/$Ph_3P$/DEAD/Bz/rt; (g) $NaHCO_3$/MeOH/H_2O/rt; (h) MsCl/Py/rt (i) LiBr DMF/reflux.

Research on structure–activity relationships[5,7,13–16] in brassinosteroids has shown that 2α,3α and 22*R*,23*R* vicinal diol moieties are required for optimum bioactivity. The B-ring lactone moiety of **1** tolerates considerable variation in its structure, but the complete absence of a polar functional group results in the loss of all activity.[17] Other studies clearly indicate the existence of relationships between A and B-rings and the side chain functionalities with strong interdependence among requirements.[18] Bioactivity of extra-hydroxylated brassinosteroids in C-14 and in the side chain has also been recently studied with controversial results.[19]

Brosa et al.[20] reported a reduction of activity when a 5α-hydroxy moiety was introduced in compound **5**. It was then suggested that, if brassinosteroids act through a mechanism similar to that of animal steroidal hormones, the brassinosteroid receptor complex could involve hydrogen bonds between the protein residues and the steroid. Consequently, the fact that (22*R*,23*R*)-2α,3α,5,22,23-pentahydroxy-5α-stigmastan-6-one (**6**) showed 20% lower activity than the natural parent brassinosteroid (**5**) was explained by the decreasing ability of hydrogen bonding with the receptor, due to its capability to form an intramolecular hydrogen bond between the two hydroxy groups at C-3 and C-5.[21]

Table 1. Relevant ^{13}C NMR data (coupling constants (*J*) are in Hz)

	15	16	17	18	20	22	9	10	11
C3	71.2	70.4	69.6	66.9	65.3	121.6	65.3	66.9	66.8
C4	37.9	35.4 *J*=20.2	30.5 *J*=25.3	33.9 *J*=22.2	30.6 *J*=19.8	26.7 *J*=23.8	30.4 *J*=19.5	33.9 *J*=22.1	29.9 *J*=19.3
C5	62.4	98.6 *J*=166.9	100.8 *J*=176.0	101.3 *J*=175.4	102.5 *J*=172.4	98.3 *J*=176.0	102.5 *J*=172.0	101.3 *J*=176.9	98.2 *J*=176.9
C6	63.3	73.0 *J*=35.4	207.2 *J*=27.1	207.2 *J*=26.8	207.4 *J*=27.2	207.6 *J*=27.1	207.3 *J*=29.6	207.2 *J*=27.2	207.7 *J*=27.0
C10	35.0	38.4 *J*=19.3	43.2 *J*=19.8	43.2 *J*=15.3	43.7 *J*=19.0	42.4 *J*=20.8	43.7 *J*=19.3	43.2 *J*=14.8	42.8 *J*=24.8
C19	16.5	16.5 *J*=6.1	13.7 *J*=5.1	13.8 *J*=5.9	13.4 *J*=5.9	14.0 *J*=5.8	13.4 *J*=6.0	13.5 *J*=6.2	14.2 *J*=5.2
C22	138.1	137.9	137.9	137.9	137.9	137.9	74.2	74.1	74.1
C23	129.3	129.6	129.6	129.6	129.6	129.6	72.2	72.0	72.2

Our aim is to enlarge studies of the effects on bioactivity that could be elicited by introducing a hydrogen bond acceptor group at C-5 with α configuration on compounds bearing a 3α hydroxyl group, such as 28-homocastasterone (**5**), 28-homotyphasterol (**7**) and 28-homoteasterone (**8**). Hydroxyl and fluoro groups were chosen because of their stereo-electronic similarity.[22] In this paper we report the synthesis of five new 28-homobrassinosteroids: (22*R*,23*R*)-5-fluoro-3α,22,23-trihydroxy-5α-stigmastan-6-one (**9**), (22*R*,23*R*)-5-fluoro-3β,22,23-trihydroxy-5α-stigmastan-6-one (**10**), (22*R*,23*R*)-5-fluoro-2α,3α,22,23-tetrahydroxy-5α-stigmastan-6-one (**11**), (22*R*,23*R*)-3α,5,22,23-tetrahydroxy-5α-stigmastan-6-one (**12**), and (22*R*,23*R*)-3β,5,22,23-tetrahydroxy-5α-stigmastan-6-one (**13**). Bioactivities of the new analogs were evaluated using the rice lamina inclination test[23] and compared with those of the natural compounds **5**, **7**, and **8**. Unexpected high activity was detected for compound **9**.

Results and Discussion

Synthesis and structural elucidation

The synthesis of fluorinated compounds **9**, **10** and **11** is summarized in Scheme 1. All compounds in Scheme 1 are new, except **14** and **15** that have been previously described.[24]

Key compound **18** was obtained in four steps from stigmasteryl acetate (**14**). Thus, stereo and regioselective epoxidation of **14** with $KMnO_4/Fe(NO_3)_3$/*t*-BuOH, followed by nucleophylic opening of the 5β,6β epoxide with BF_3 etherate afforded compound **16**; the proper stereochemistry at C-5 was achieved by *trans*-diaxial acid catalyzed opening of the epoxide. Subsequent oxidation with PCC and hydrolysis with K_2CO_3 afforded compound **18.** Osmium-catalyzed asymmetric dihydroxylation[25] (CAD) of double bond of **18** using $K_3Fe(CN)_6$ as cooxidant and hydroquinidine-1,4-phthalazinediyl diether [$(DHQD)_2$-PHAL] as chiral ligand gave, after purification, 19% yield of compound **10**. In all cases CAD yields diasteromeric 22*S*,23*S* isomers in minor proportion (ratio 3:1) and more than 30% of starting material, that can be recycled.

Table 2. ^{19}F NMR data (coupling constants (*J*) are in Hz)

	δ (relative to $CFCl_3$)
16	−161.15 (*J*=39.5)
17	−159.73 (*J*=42.5)
18	−159.05 (*J*=39.5)
19	−157.00 (*J*=45.3)
20	−154.74 (*J*=48.6)
21	−159.61 (*J*=42.5)
22	−161.02 (*J*=45.0)
9	−154.74 (*J*=48.6)
10	−159.05 (*J*=42.5)
11	−155.20 (*J*=45.4)

Epimerization of the hydroxyl group of C-3 was achieved by treating **18** with DEAD/Ph_3P/HCOOH in benzene. Subsequent saponification of **19** with $NaHCO_3$ afforded compound **20**. CAD on **20** yielded **9** (21%).

Elimination of 3β-OH group to yield compound **22** was achieved by treating **18** with MsCl/Py and then, reflux with LiBr/DMF. CAD on diene **22** yielded **11** (15%).

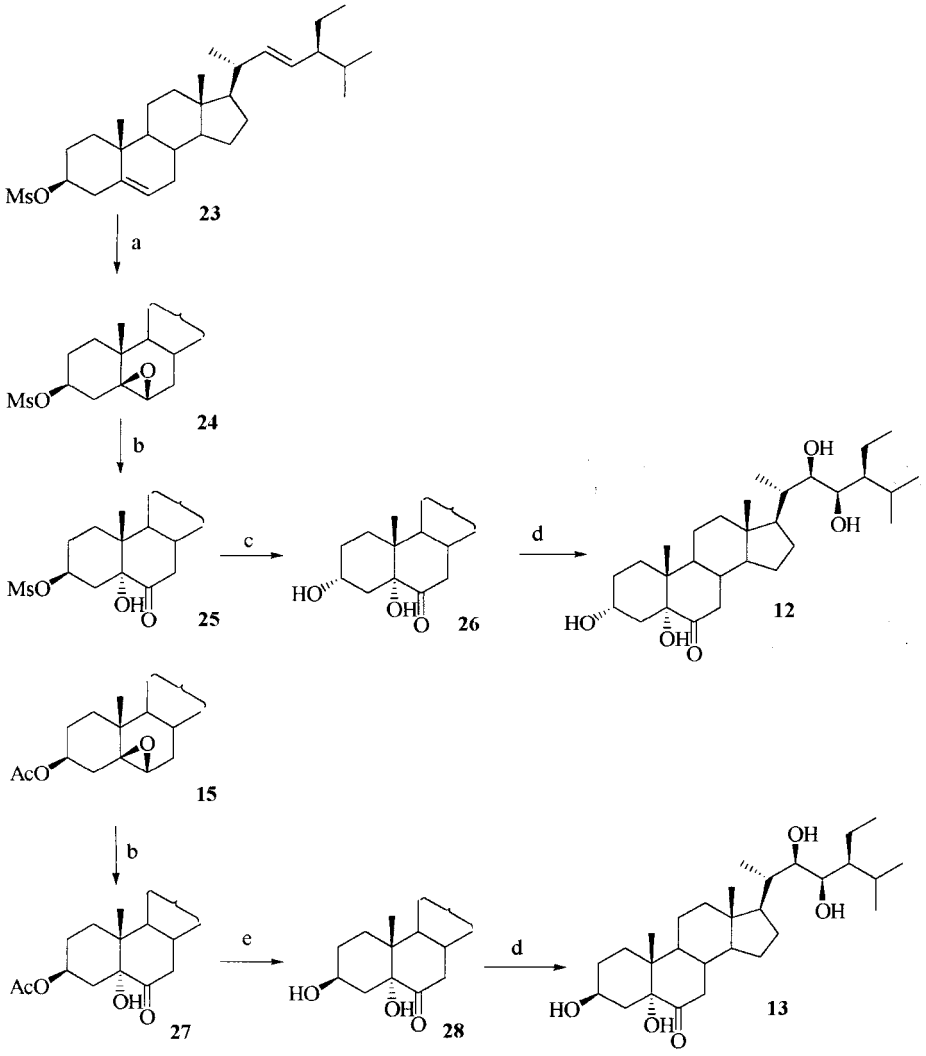
NMR and HRMS experiments were performed on all new compounds allowing the unequivocal assignment of the structures.

The position and configuration of the 5α-fluoro moiety was deduced mainly from its effect on the ^{13}C NMR spectra (Table 1). The acidic condition involved in the 5β,6β-epoxide opening favoured the attack of the fluoride at the most substituted position (C-5) with inversion of configuration at this centre. A DEPT experiment on compound **16** reveals that the resonance at 98.6 ppm (carbon bearing the fluorine) corresponds to a trisubstituted carbon. Other easily assigned carbons, such as C-6, C-10 and C-19, appear as doublets with $^{19}F-^{13}C$ coupling constants that are consistent with the proposed structure. Furthermore, the coupling pattern of the H-3 and H-6 multiplets in the ^1H NMR spectra agrees with an A/B *trans* junction. The fluorine chemical shifts (Table 2) are consistent with those expected for structurally related tertiary alkyl fluorides.[26] Spectral resolution allowed signals to appear as doublets ($J \approx 40-45$ Hz.) due to coupling with the vicinal H-4β.

Synthesis of compounds **12** and **13** is summarized in Scheme 2. All compounds in the Scheme 2 are new, except **27** that has been previously described.[24]

Compound **12** was obtained in four steps from stigmasteryl mesylate (**23**). Thus, stereo and regioselective epoxidation of **23** with $KMnO_4/Fe(NO_3)_3$/*t*-BuOH, followed by oxidative opening of the 5β,6β epoxide with Jones reagent afforded compound **25**. Subsequent epimerization of C-3 was achieved in two steps without isolation by treating **25** with LiBr/DMF/80°C/24 h, and then, reflux with water. CAD on compound **26** gave **12** in 17% yield.

Compound **13** was achieved in three steps from **15**.



Scheme 2. Synthesis of 5α-hydroxylated analogs. Reagents and conditions: (a) $KMnO_4/Fe(NO_3)_3/t$-BuOH/H_2O/CH_2Cl_2/rt; (b) Jones; (c) 1. LiBr/DMF/80°C. 2. H_2O/reflux; (d) $K_2OsO_4/K_3Fe(CN)_6/(DHQD)_2Phal/K_2CO_3/t$-BuOH/$H_2O/CH_3SO_3NH_2$/rt; (e) K_2CO_3/MeOH/rt

Intermediate compound **27**[24] was achieved in one step from **15** with Jones reagent. Hydrolysis of **27** with K_2CO_3/MeOH gave **28**. CAD on compound **28** afforded compound **13** (17% yield). Unequivocal assignments of the structures were verified by NMR and HRMS experiments.

Configurations at C-2, C-3, C-22 and C-23 in the new analogs were established by comparison with chemical shifts and coupling constants of known closely related structures.

Bioactivity

Bioactivity was evaluated using the highly sensitive modified rice lamina inclination test (*Oryza sativa*, *Chui* cultivar) based on the procedure developed by Takeno and Pharis.[23]

Table 3 shows the mean angle induced by single increasing doses of each compound. Best results came out from natural brassinosteroid **5** and its biosynthetic precursor **7** as well as from synthetic fluorinated analog **9**. Excellent in vitro bioactivity of compound **9** was also revealed at low doses. It is clearly noted that the introduction of an 5α-OH group resulted in a significant decrease in bioactivity (see compounds **6** and **12**, respectively). Compound **8** is an earlier biosynthetic precursor of **7** and the rice lamina inclination test did not show high sensitivity either to it or its 5α-hydroxylated or fluorinated analogs (see compounds **13** and **10**).

Table 3. Rice lamina inclination test (values are angles (degrees) between the lamina and sheath, representing the means of 15–25 replicates ±standard error. Average angle of control: 15±2)

Compound	Dosage (ng)			
	2	20	200	1000
5	30±2	84±5	95±5	108±5
6	11±2	17±2	75±5	72±5
11	30±3	68±6	95±4	93±4
7	15±2	66±5	112±3	121±2
12	14±2	40±3	73±7	71±5
9	24±2	101±5	107±5	137±4
8	15±1	30±2	34±5	25±4
13	14±1	12±1	31±4	41±3
10	15±1	17±2	46±3	29±4

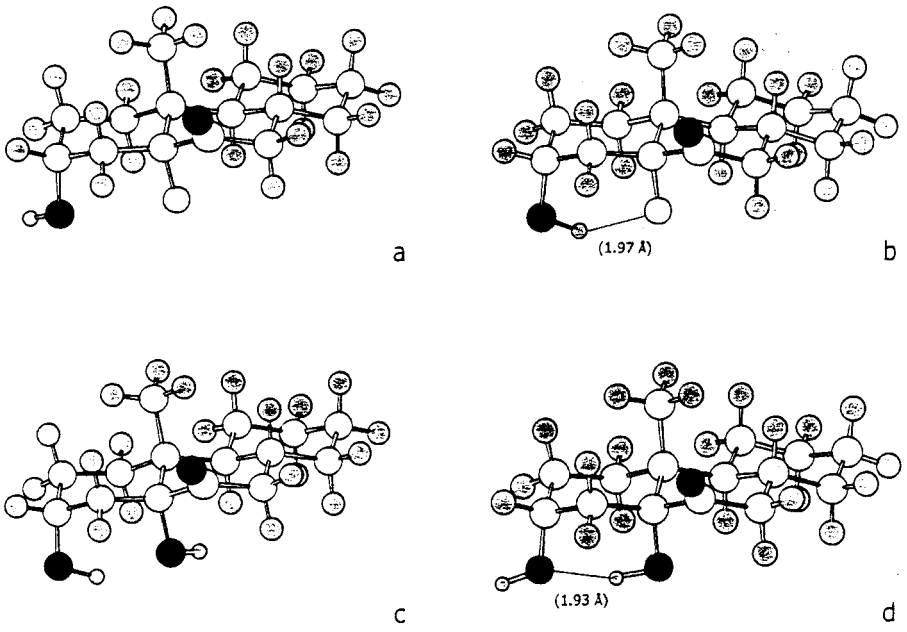


Figure 1.

Molecular modeling

In order to examine the intramolecular hydrogen bond formation between a hydrogen bond acceptor in C-5 and the hydroxy group of C-3, a theoretical study was carried out. Because of the computational expense of a full conformational search for each compound, substructures concerning rings A, B and C properly functionalized were evaluated.

The semiempirical PM3 Hamiltonian, as implemented in the MOPAC program, was utilized to find the possible minima for each structure in a systematic conformational search. Those conformations that were verified as minima via normal mode analysis were optimized at the B3LYP/6-31G** level using JAGUAR v 3.0.[27]

Both fluorinated compounds **9** and **11** may form an intramolecular hydrogen bond between the hydroxyl in C-3 and the fluorine in C-5. To evaluate this hypothesis, a substructure of compound **9** was used as a model. Only two conformations proved to be minima (Fig. 1a and b). Examination of conformation b reveals that the hydrogen in the hydroxyl group forms a very close contact of 1.97 Å with the fluorine, forming a favorable six-membered ring. In addition, conformation b is more stable than conformation a in about 3.7 kcal mol^{-1}. These results are consistent with a typical hydrogen bond involving fluorine.[28,29]

A similar study on the hydroxylated analog **12** showed that both C-3 and C-5 hydroxyls act, alternatively, as acceptor or donor in intramolecular hydrogen bond formation. All minima conformations involved distances lower than 2 Å between hydrogen and oxygen, i.e. conformations c and d in Fig. 1. These results and a slightly difference of 0.3 kcal mol^{-1} between calculated minima conformations of compounds **6** and **12** suggest an intramolecular bonded state with favorable six-membered ring involving 3α and 5α hydroxyls under physiological conditions.

Conclusions

The reported[20] decrease in bioactivity on the rice lamina inclination test due to the introduction of a 5α-OH group on natural brassinosteroid **5** was extended in this work to compound **7**, another natural brassinosteroid. However, the proposed explanation to justify this decreasing effect[20] should be revised. This proposal postulates interference on the complex receptor-substrate formation due to the presence of an intramolecular hydrogen bond between substituents of C-3 and C-5 of the brassinosteroid structure.

Our results provide clear evidence that the introduction of a 5α-fluoro group does not affect bioactivity values compared with those of 28-homocastasterona (**5**) and 28-homotyphasterol (**7**) at doses of 200–1000 ng, and that this fluorinated group may enhance response sensitivity at a 20 ng/plant dose as well. This fact, associated with the molecular modeling prediction of the existence of an intramolecular hydrogen bonding between 3α-hydroxyl group and the 5α-fluor group requires a revision of the previously described hypothesis. Further studies should be done to generalize the effect of a 5α-fluor group in brassinosteroids. This is required since the rice lamina inclination bioassay used in this work did not provide significant results for all the tested compounds. We suggest that this bioassay involves a biological system incapable of testing bioactivity of early precursors of brassinosteroids such as 28-homoteasterone (**8**).

Experimental

Melting points (mp) were determined on a Fisher Johns apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-500 at 500 MHz; ^{13}C NMR spectra were recorded on a Bruker AC-200 at 50.3 MHz. Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. ^{19}F NMR spectra were recorded on

a Bruker AM-500 at 470.4 MHz, chemical shifts (δ) are given in ppm upfield from $CFCl_3$ as the internal standard. Coupling constant (J) values are in Hz. High-resolution mass spectra (EI) were obtained for all new compounds on a ZAB BEqQ instrument (VG-Micromass). Low-resolution mass spectra were recorded on a Shimadzu QP-5000 at 70 eV. Unless otherwise indicated, all solvents and reagents used were of commercial grade. Reactions were monitored by TLC on precoated plates with silicagel F_{254} 0.2 mm (Merck). Column chromatography was carried out on silica gel 60, 0.04–0.063 mm (Merck).

(22*E*)-3β-Acetoxy-5-fluoro-6β-hydroxy-5α-stigmast-22-ene (16). 3β-Acetoxy-5β,6β-epoxystigmast-22-ene (**15**)[24] (650 mg, 1.38 mmol), dissolved in diethyl ether (20 ml), was treated with $BF_3\cdots Et_2O$ (0.7 ml) for 1 h in an ice bath. The reaction mixture was poured into sodium bicarbonate (sat. solution) and then extracted with ethyl acetate. The organic layer was dried (sodium sulfate) and evaporated. The crude product was purified by chromatography (hexane/ethyl acetate 9:1) to afford compound **16** in 77% yield. Mp: 215–216°C. ^{1}H NMR ($CDCl_3$): 0.70 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 1.16 (19-H_3, 3H, s), 2.02 (CH_3CO–, 3H, s), 3.73 (6α-H, 1H, dt, J_{FH}=4.5 Hz, J=2.3 Hz), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.07 (3α-H, 1H, m), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 12.2 12.3 (C29 and C18), 19.0 (C26), 21.0 (C11), 21.1 (*C*H_3CO), 21.2 (C21 and C27), 16.5 (C19, J_{CF}=6.1 Hz), 24.2 (C15), 25.4 (C28), 26.5 (C2), 28.8 (C16), 29.7 (C1), 31.9 (C25), 32.0 (C8), 35.0 (C7), 39.7 (C12), 40.4 (C20), 42.6 (C13), 45.9 (C9), 51.2 (C24), 35.4 (C4, J_{CF}=20.2), 55.8 56.0 (C17 and C14), 38.4 (C10, J_{CF}=19.3 Hz,), 70.4 (C3, J_{CF}=3.8 Hz), 73.0 (C6, J_{CF}=35.4 Hz), 98.6 (C5, J=166.9 Hz),129.4 (C23), 138.2 (C22), 170.4 (CH$_3$*C*O). ^{19}F NMR ($CDCl_3$): −161.2 (d, J_{FH}=39.5 Hz). MS (EI): *m*/*z* (%): 490 (9), 475 (1), 447 (3), 349 (21), 43 (100). HRMS (EI): Calculated for $C_{31}H_{51}O_3F$: 490.3822, found 490.3823.

(22*E*)-3β-Acetoxy-5-fluoro-5α-stigmast-22-en-6-one (17). A solution of compound **16** (300 mg, 0.61 mmol) in methylene chloride (60 ml) was treated with pyridinium chlorochromate (500 mg, 1.32 mmol) and pyridinium trifluoroacetate (100 mg, 0.52 mmol). The mixture was stirred for 3 h at room temperature and then filtered through Celite, and the solution evaporated under reduced pressure. After chromatographic purification of the crude product (hexane/ethyl acetate 95:5), compound **17** was isolated in 84% yield. Mp: 162–163°C. ^{1}H NMR ($CDCl_3$): 0.67 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.83 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.05 (CH_3CO–, 3H, s), 2.70 (7α-H, dd, J=12.6 Hz, 12.3 Hz), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.04 (3α-H, 1H, m), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 12.1 12.2 (C29 and C18), 13.7 (C19, J_{CF}=5.1 Hz), 18.9 (C26), 21.1 (C11), 21.1 21.3 (C27 and C21), 21.2 (*C*H_3CO), 23.9 (C15), 25.4 (C28), 26.0 (C2), 28.7 (C16), 30.3 (C1), 30.5 (C4, J_{CF}=25.3 Hz), 31.8 (C25), 37.8 (C8), 39.2 (C12), 40.4 (C20), 42.5 (C7), 42.9 (C13), 45.4 (C9, J_{CF}=3.9 Hz), 51.2 (C24), 55.8 56.3 (C17 and C14), 43.2 (C10, J_{CF}=19.8 Hz), 69.6 (C3), 100.8 (C5, J_{CF}=176.0 Hz), 129.6 (C23), 137.9 (C22), 170.4 (CH$_3$*C*O), 207.2 (C6, J_{CF}=27.1 Hz). ^{19}F NMR ($CDCl_3$): −159.7 (d, J_{FH}=42.5 Hz). MS (EI) *m*/*z* (%): 488 (0.3), 445 (0.2), 410 (1), 347 (2), 43 (100). HRMS (EI): Calculated for $C_{31}H_{49}O_3F$: 488.3666, found 488.3663.

(22*E*)-5-Fluoro-3β-hydroxy-5α-stigmast-22-en-6-one (18). To a solution of compound **17** (540 mg, 1.10 mmol) in methanol (60 ml) potassium carbonate (100 mg, 0.72 mmol) was added. The reaction mixture was stirred for 4 h at room temperature and then ammonium chloride (saturated solution) was added. The methanol was evaporated and the aqueous phase extracted with methylene chloride. The organic layer was dried and evaporated. The residue was purified by chromatography (hexane/ethyl acetate 9:1) to afford compound **18** in 96% yield. Mp: 149–151°C. ^{1}H NMR ($CDCl_3$): 0.68 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 0.90 (19-H_3, 3H, s), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.70 (7α-H, dd, J=12.6 Hz, 12.3 Hz), 4.05 (3α-H, 1H, m), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 12.2 (C18 and C29), 13.8 (C19, J_{CF}=5.9 Hz), 19.0 (C26), 21.1 21.2 (C21 and C27), 21.2 (C11), 24.0 (C15), 25.4 (C28), 28.6 (C16), 30.0 30.8 (C1 and C2), 31.9 (C25), 33.9 (C4, J_{CF}=22.2 Hz), 37.8 (C8), 39.4 (C12), 40.3 (C20), 42.7 (C7), 43.2 (C10, J_{CF}=15.3 Hz), 42.9 (C13), 45.6 (C9), 51.2 (C24), 56.0 56.5 (C17 and C14), 66.9 (C3), 101.3 (C5, J_{CF}=175.4 Hz), 129.7 (C23), 137.9 (C22), 207.2 (C6, J_{CF}=26.8 Hz). ^{19}F NMR ($CDCl_3$): −159.1 (d, J_{FH}=42.5 Hz). MS (EI) *m*/*z* (%): 446 (14), 426 (4), 403 (9), 333 (23), 305 (33), 55 (100). HRMS (EI): Calculated for $C_{29}H_{47}O_2F$: 446.3560, found 446.3560.

(22*R*,23*R*)-5-Fluoro-3β,22,23-trihydroxy-5α-stigmastan-6-one (10). A mixture of **18** (198 mg, 0.44 mmol), THF (8 ml), *t*-butanol/water (1:1) (32 ml), $(DHQD)_2$-PHAL (252 mg, 0.32 mmol), methanesulfonamide (308 mg, 2.77 mmol), potassium ferricyanide (1.60 g, 4.80 mmol), potassium carbonate (672 mg, 2.63 mmol), and potassium osmate dihydrate (29 mg, 0.08 mmol) was stirred at room temperature for 9 days. An excess of sodium bisulfite ($NaHSO_3$) was added until no evolution of bubbles was observed. Layers were separated and the aqueous phase was thoroughly extracted with methylene chloride/methanol (5%). Combined organic layers were washed with 0.25 M sulfuric acid and 2% sodium hydroxide, dried and evaporated. The crude solid was purified by column chromatography (hexane/ethyl acetate) to give compound **10** (19% yield). Mp: 207°C. ^{1}H NMR ($CDCl_3$/MeOD 95:5): 0.68 (18-H_3, 3H, s), 0.90 (19-H_3, 3H, s), 0.91 (21-H_3, 3H, d, J=6.8 Hz), 0.92–0.99 (26-H_3, 27-H_3 and 29-H_3, 9H, m), 2.58 (7α-H, 1H, dd, J=12.5 Hz, 12.5 Hz), 3.58 (22-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.71 (23-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.88 (3α-H, 1H, m). ^{13}C NMR ($CDCl_3$/MeOD 95:5): 11.6 (C21), 12.2 (C18), 13.3 (C29), 13.8 (C19, J_{CF}=6.2 Hz), 18.7 (C28), 19.1 (C26), 21.2 (C27), 21.2 (C11), 24.0 (C15), 28.6 (C16), 28.7 (C25), 30.0 30.8 (C1 and C2), 33.9 (C4, J_{CF}=22.1 Hz), 36.8 (C20), 37.8 (C8), 39.4 (C12), 42.7 (C7), 42.9 (C13), 43.2 (C10, J_{CF}=14.8 Hz), 45.6 (C9), 46.5 (C24), 56.0 56.5 (C17 and C14),

66.9 (C3), 72.0 (C23), 74.1 (C22), 101.3 (C5, J_{CF}= 176.9 Hz), 207.2 (C6, J_{CF}=27.2 Hz). ^{19}F NMR ($CDCl_3$/MeOD 95:5): −159.1 (d, J_{FH}=42.5 Hz). MS (FAB): 481 $(M+H)^+$, 479 $(M-H)^+$, 463 $(M+H-H_2O)^+$, 445 $(M+H-2H_2O)^+$, 425. HRMS (FAB): calculated for $C_{29}H_{48}O_4F$ $(M-H)^+$: 479.3537, found 479.3529.

(22*E*)-5-Fluoro-3α-formyloxy-5α-stigmast-22-en-6-one (19). To a solution of compound **18** (228 mg, 0.50 mmol), formic acid (0.035 ml), triphenylphosphine (212 mg, 0.83 mmol) in dry benzene (10 ml) was dropped diethyl azodicarboxylate (DEAD) (170 mg, 0.98 mmol) in benzene (2 ml) with stirring and allowed to stand for 24 h. After evaporation of solvents the crude product was purified by column chromatography (hexane) to give compound **19** in 77% yield. Mp: 151–152°C. ^{1}H NMR ($CDCl_3$): 0.68 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.80 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d, J= 6.3 Hz), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.62 (7α-H, dd, J=12.6 Hz, 12.3 Hz), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.27 (3β-H, 1H, m), 8.02 (1H, s, *H*COO-). ^{13}C NMR ($CDCl_3$): 12.2 (C18 and C29), 13.5 (C19, J_{CF}=6.0 Hz), 19.0 (C26), 21.0 21.1 (C21 and C27), 21.1 (C11), 23.9 (C15), 24.8 26.4 (C1 and C2), 25.3 (C28), 25.9 (C4, J_{CF}=22.1 Hz), 28.6 (C16), 31.8 (C25), 37.9 (C8), 39.3 (C12), 40.4 (C20), 42.3 (C7), 43.4 (C10, J_{CF}=19.8 Hz), 43.0 (C13), 45.4 (C9, J_{CF}=5.0 Hz), 51.2 (C24), 55.8 56.4 (C17 and C14), 67.2 (C3), 99.0 (C5, J_{CF}=178.8 Hz), 129.7 (C23), 137.9 (C22), 160.5 (H*C*OO-), 207.8 (C6, J_{CF}=27.0 Hz). ^{19}F NMR ($CDCl_3$): −157.0 (d, J_{HF}=45.3 Hz). MS (EI) *m*/*z* (%): 474 (11), 431 (6), 362 (18), 333 (30), 55 (100). HRMS (EI): calculated for $C_{30}H_{47}O_3F$: 474.3509, found 474.3509.

(22*E*)-5-Fluoro-3α-hydroxy-5α-stigmast-22-en-6-one (20). Compound **19** (300 mg, 0.63 mmol) was hydrolyzed with sodium bicarbonate (100 mg, 0.94 mmol) in a mixture of 30 ml of methanol and 2 ml of water at room temperature for 6 h under stirring. The reaction mixture was neutralized with 2 M HCl, the solvent evaporated and the aqueous layer extracted with ethyl acetate. The organic layer was washed, dried and evaporated to give a residue that was purified by column chromatography to give compound **20** in 88% yield. Mp: 149–150°C. ^{1}H NMR ($CDCl_3$): 0.68 (18-H_3, 3H, s), 0.79 (19-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.61 (7α-H, dd, J= 12.6 Hz, 12.3 Hz), 4.07 (3β-H, 1H, m), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 12.2 (C18 and C29), 13.4 (C19, J_{CF}=5.9 Hz), 19.0 (C26), 21.0 21.1 (C21 and C27), 21.1 (C11), 23.9 (C15), 25.8 27.8 (C1 and C2), 25.3 (C28), 30.6 (C4, J_{CF}=19.8 Hz), 28.6 (C16), 31.8 (C25), 37.9 (C8), 39.3 (C12), 40.3 (C20), 42.3 (C7, J_{CF}=2.2 Hz), 43.7 (C10, J_{CF}=19.0 Hz), 43.0 (C13), 45.6 (C9, J_{CF}=4.9 Hz), 51.2 (C24), 55.8 56.4 (C17 and C14), 65.3 (C3), 102.5 (C5, J_{CF}=172.4 Hz), 129.7 (C23), 137.9 (C22), 207.4 (C6, J_{CF}=27.2 Hz). ^{19}F NMR ($CDCl_3$): −154.7 (d, J_{HF}= 48.6 Hz). MS (EI) *m*/*z* (%): 446 (5), 426 (1), 403 (2), 365 (4), 334(12), 305 (15), 55 (100). HRMS (EI): Calculated for $C_{29}H_{47}O_2F$: 446.3560, found 446.3560.

(22*R*,23*R*)-5-Fluoro-3α,22,23-trihydroxy-5α-stigmastan-6-one (9). Compound **20** (220 mg, 0.49 mmol) was treated in a similar way as described for compound **18**. Crude solid was purified by column chromatography (hexane/ethyl acetate) to give compound **9** (21% yield). Mp: 152–153. ^{1}H NMR ($CDCl_3$/MeOD 95:5): 0.68 (18-H_3, 3H, s), 0.86 (19-H_3, 3H, s), 0.91 (21-H_3, 3H, d, J=6.8 Hz), 0.92–0.99 (26-H_3, 27-H_3 and 29-H_3, 9H, m), 3.58 (22-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.71 (23-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 4.07 (3β-H, 1H, m). ^{13}C NMR ($CDCl_3$/MeOD 95:5): 11.6 (C21), 12.1 (C18), 13.3 (C29), 13.4 (C19, J_{CF}=6.0 Hz), 18.6 (C28), 19.2 (C26), 20.8 (C27), 21.1 (C11), 23.9 (C15), 25.9 27.5 (C1 and C2), 28.6 (C16), 28.8 (C25), 30.4 (C4, J_{CF}=19.5 Hz), 36.8 (C20), 37.7 (C8), 39.3 (C12), 42.3 (C7, J_{CF}=1.9 Hz), 43.7 (C10, J_{CF}=19.3 Hz), 43.2 (C13), 45.6 (C9, J_{CF}=4.9 Hz), 46.4 (C24), 55.8 56.4 (C17 and C14), 65.3 (C3), 72.1 (C23), 74.1 (C22), 102.5 (C5, J_{CF}=172.0 Hz), 207.4 (C6, J_{CF}= 26.9 Hz). ^{19}F NMR ($CDCl_3$/MeOD 95:5): −154.7 (d, J_{HF}=48.6 Hz). MS (FAB): 481 $(M+H)^+$, 479 $(M-H)^+$, 463 $(M+H-H_2O)^+$, 445 $(M+H-2H_2O)^+$, 425. HRMS (FAB): Calculated for $C_{29}H_{48}O_4F$ $(M-H)^+$: 479.3537, found 479.3540.

(22*E*)-5-Fluoro-3β-mesyloxy-5α-stigmast-22-en-6-one (21). To a stirred solution of compound **18** (250 mg, 0.51 mmol) in pyridine (20 ml), methanesulfonyl chloride (1.5 ml) was added. After 1 h at room temperature, the reaction mixture was acidified with 5% aq. HCl in an ice bath and extracted with methylene chloride. The organic layer was washed with water, dried and evaporated. The crude product was purified by column chromatography (hexane/ethyl acetate 9:1) to give **21** in 89% yield. Mp: 175–177°C. ^{1}H NMR ($CDCl_3$): 0.68 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.82 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 0.85 (19-H_3, 3H, s), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.71 (7α-H, 1H, dd, J=12.6 Hz, 12.5 Hz), 3.01 (C*H*$_3$SO$_3$-, 3H, s), 4.90 (3α-H, 1H, m), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 12.2 (C18 and C29), 13.7 (C19, J_{CF}=4.9 Hz), 19.0 (C26), 21.1 (C11), 21.1 21.2 (C27 and C21), 23.9 (C15), 25.3 (C28), 27.5 (C2), 28.6 (C16), 30.4 (C1), 31.8 (C25), 37.7 (C8), 38.7 (*C*H$_3$SO$_3$), 39.2 (C12), 40.3 (C20), 42.5 (C13), 43.0 (C7), 45.5 (C9, J_{CF}=3.0 Hz), 51.2 (C24), 31.4 (C4, J_{CF}=21.1 Hz), 56.3 (C14 and C17), 43.5 (C10, J_{CF}=16.8 Hz), 77.9 (C3), 129.7 (C23), 137.8 (C22), 206.9 (C6, J_{CF}=26.2 Hz), 100.9 (C5, J_{CF}=176.0 Hz). ^{19}F NMR ($CDCl_3$): −159.6 (d, J_{FH}=42.5 Hz). MS (EI): *m*/*z* (%), 524 (1), 481 (1), 383 (7), 55 (100). HRMS (EI): calculated for $C_{30}H_{49}O_4SF$ 524.3335, found 524.3330.

(22*E*)-5-Fluoro-5α-stigmasta-2,22-dien-6-one (22). A solution of compound **21** (354 mg, 0.67 mmol) in 10 ml of DMF (dried over barium oxide) was treated with LiBr (217 mg, 2.49 mmol) under nitrogen and refluxed for 1 h. The solvent was evaporated under reduced pressure, the residue suspended in water and extracted with methylene chloride. The organic layer was dried (sodium sulfate) and evaporated. Purification by column chromatography (hexane) of the crude product afforded compound **22** in 76% yield. Mp: 121°C. ^{1}H NMR ($CDCl_3$): 0.75 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.82 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d,

J=6.3 Hz), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.72 (7α-H, 1H, dd, J=12.6 Hz, 7.9 Hz), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.56–5.71 (2-H and 3-H, 2H, m). ^{13}C-NMR ($CDCl_3$): 12.2 and 12.9 (C18 and C 29), 14.0 (C19, J_{CF}=5.8 Hz), 19.0 (C26), 21.0 and 21.1 (C27 and C21), 21.2 (C11), 24.0 (C15), 25.3 (C28), 26.7 (C4, J_{CF}=23.8 Hz), 28.6 (C16), 31.8 (C25), 34.7 (C1), 37.9 (C8), 39.3 (C12), 40.4 (C20), 42.4 (C10, J_{CF}=20.8 Hz), 42.8 (C13), 43.0 (C7, J_{CF}=1.9 Hz), 46.0 (C9, J_{CF}=2.0 Hz), 51.2 (C24), 55.9 and 56.4 (C14 and C17), 98.3 (C5, J_{CF}=176.0 Hz), 121.6 (C3), 124.5 (C2), 129.7 (C23), 137.9 (C22), 207.6 (C6, J_{CF}=27.1 Hz). ^{19}F NMR ($CDCl_3$): −161.0. (d, J_{FH}=45.0 Hz). MS (EI) *m/z* (%): 428 (12), 408 (2), 385 (7), 316 (18), 287 (25), 55 (100). HRMS (EI): calculated for $C_{29}H_{45}OF$ 428.3454, found 428.3454.

(22*R*,23*R*)-5-Fluoro-2α,3α,22,23-tetrahydroxy-5α-stigmastan-6-one (11). Compound **22** (760 mg, 1.61 mmol) was treated in a similar way as described for compound **18**. Purification by column chromatography (methylene chloride/ethyl acetate gradient) afforded **11** in 15% yield. Mp: 245°C. ^{1}H NMR: 0.68 (18-H_3, 3H, s), 0.91 (21-H_3, 3H, d, J=6.8 Hz), 0.92–0.99 (26-H_3, 27-H_3 and 29-H_3, 9H, m), 0.96 (19-H_3, 3H, s), 2.61 (7α-H, 1H, dd, J=12.5 Hz, 12.5 Hz), 3.58 (22-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.71 (23-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.76 (2β-H, 1H, m), 4.05 (3β-H, 1H, dd, J=6.0 Hz, 3.0 Hz). ^{13}C-NMR: 11.6 (C21), 11.7 (C18), 13.4 (C29), 14.2 (C19, J_{CF}=5.2 Hz), 18.7 (C28), 19.2 and 20.9 (C26 and C27), 20.9 (C11), 23.6 (C15), 27.3 (C16), 28.8 (C25), 29.9 (C4, J_{CF}= 19.3 Hz), 34.3 (C1), 36.8 (C20), 37.4 (C8), 39.9 (C12), 42.0 (C13), 42.8 (C7), 42.8 (C10, J_{CF}=24.8 Hz), 45.2 (C9, J_{CF}=3.9 Hz), 46.4 (C24), 52.3 and 55.9 (C14 and C17), 66.8 (C3), 67.6 (C2), 74.1 (C22), 72.2 (C23), 98.2 (C5, J_{CF}= 176.9 Hz), 207.7 (C6, J_{CF}=27.0 Hz). ^{19}F NMR ($CDCl_3$): −155.2 (J=45.4 Hz). MS (FAB): 497 $(M+H)^+$, 495 $(M-H)^+$, 479 $(M+H-H_2O)^+$, 461 $(M+H\text{-}2H_2O)^+$, 441. HRMS (FAB): Calculated for $C_{29}H_{48}O_5F$ $(M-H)^+$: 495.3486, found 495.3470.

(22*E*)-5β,6β-Epoxy-3β-mesyloxystigmast-22-ene (24). A mixture of potassium permanganate (2 g, 12.65 mmol) and ferric nitrate nonahydrate (1 g, 2.47 mmol) was ground to a fine powder and water (100 μl) was added. To a stirred suspension of this mixture in methylene chloride (20 ml), (22*E*)-3β-mesyloxystigmast-22-ene (**23**) (2 g, 4.08 mmol) and *t*-butyl alcohol (2 ml) were added. After 1h at room temperature the reaction was completed and the product was separated from the inorganic residue by adding ether (50 ml), stirring for 5 min. and filtering through Celite. After evaporation of the solvent, the crude product was purified by column chromatography (hexane/ethyl acetate 97:3) yielding **24** (73%). Mp: 115–166°C. ^{1}H NMR ($CDCl_3$): 0.67 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J= 6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.84 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d, J=6.3 Hz),), 1.09 (19-H_3, 3H, s), 2.20–2.40 (7α-H and 4α-H, 2H, m), 2.99 (CH_3SO_3-, 3H, s), 3.07 (6αH, 1H, d, J=2.5 Hz), 4.70 (3α-H, 1H, m), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 11.6 (C18 and C29), 16.5 (C19), 19.3 (C26), 20.8, 20.9 (C21 and C27), 21.8 (C11), 24.4 (C15), 25.3 (C28), 29.1 (C16), 29.2 (C2), 29.6 (C8), 32.2 (C7), 32.4 (C25), 35.0 (C10), 36.5 (C1), 38.3 (C4), 38.4 (CH_3SO_3), 39.6 (C12), 40.4 (C20), 42.6 (C13), 50.5 (C9), 51.0 (C24), 55.3 (C14 and C17), 62.3 (C5), 62.3 (C6), 79.6 (C3), 129.3 (C23), 138.1 (C22). MS (EI): *m/z* (%), 506 (0.2), 410 (10), 392 (16), 367 (4), 55 (100). HRMS (EI): calculated for $C_{30}H_{50}O_4S$: 506.3430, found 506.3428.

(22*E*)-5-hydroxy-3β-mesyloxy-5α-stigmast-22-en-6-one (25). Jones reagent (0.3 ml) was added dropwise to a stirred solution of compound **24** (230 mg, 0.45 mmol) in acetone (20 ml). After 1 h, isopropyl alcohol was added to stop the reaction. The mixture was neutralized with solid sodium bicarbonate, filtered, and the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography (hexane/ethyl acetate 85:15) to afford compound **25** in 85% yield. Mp: 150°C (d). ^{1}H NMR ($CDCl_3$): 0.68 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.84 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d, J=6.3 Hz),), 2.71 (7α-H, 1H, dd, J=12.6 Hz, 12.5 Hz), 3.02 (CH_3SO_3, 3H, s), 4.95–5.07 (3α-H and 23-H, 2H, m), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 11.9 12.1 (C29 and C18), 13.7 (C19), 18.9 (C26), 21.0 21.3 (C27 and C21), 21.1 (C11), 23.9 (C15), 25.3 (C28), 27.5 (C2), 28.6 (C16), 29.6 (C1), 31.7 (C25), 33.5 (C4), 37.2 (C8), 38.5 (CH_3SO_3–), 39.3 (C12), 40.3 (C20), 41.6 (C7), 42.3 (C13), 42.9 (C10), 44.1 (C9), 51.1 (C24), 55.8 56.3 (C17 and C14), 79.5 (C3), 80.3 (C5), 129.5 (C23), 137.9 (C22), 212.4 (C6). MS (EI) *m/z* (%): 522 (3), 504 (1), 426 (8), 408 (11), 365 (19), 269 (24), 55 (100). HRMS (EI): Calculated for $C_{30}H_{50}O_5S$: 522.337896, found 522.337861.

(22*E*)-3α,5-Dihydroxy-5α-stigmast-22-en-6-one (26). A solution of compound **25** (250 mg, 0.48 mmol) in DMF (20 ml) was treated with lithium bromide (80 mg, 0.82 mmol) and stirred under nitrogen for 24 h at 80°C. Then, water (1 ml) was added and the mixture was refluxed for 2 h. The solvent was evaporated under reduced pressure and the residue was extracted with methylene chloride. The organic layer was dried and evaporated to yield a crude product that was purified by column chromatography to afford compound **26** in 71%. Mp: 209–211°C. ^{1}H NMR ($CDCl_3$): 0.67 (18-H_3, 3H, s), 0.75 (19-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 2.71 (7α-H, 1H, dd, J=12.6 Hz, 12.6 Hz), 4.30 (3β-H, 1H, m), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 12.2 (C18 and C29), 13.7 (C19), 19.0 (C26), 21.1 (C11), 21.1 and 21.2 (C21 and C27), 24.0 (C15), 25.4 (C2), 25.4 (C28), 28.3 (C1), 28.8 (C16), 31.4 (C4), 31.9 (C25), 37.7 (C8), 39.6 (C12), 40.5 (C20), 41.7 (C7), 43.0 (C13), 43.8 (C10), 44.8 (C9), 51.2 (C24), 56.0 and 56.5 (C17 and C14), 67.5 (C3), 80.3 (C5), 129.5 (C23), 138.1 (C22), 211.9 (C6). MS (EI) *m/z* (%): 444 (11), 426 (12), 408 (5), 365 (21), 269 (26), 55 (100). HRMS (EI): Calculated for $C_{29}H_{48}O_3$: 444.3603, found 444.3604.

(22*R*,23*R*)-3α,5,22,23-Tetrahydroxy-5α-stigmastan-6-one (12). Compound **26** (150 mg, 0.34 mmol) was treated in a similar way as described for compound **18**. The crude solid was purified by column chromatography (methylene

chloride/isopropanol) to give compound **12** (17% yield). Mp: 238°C. ^{1}H NMR ($CDCl_3$/MeOD 95:5): 0.67 (18-H_3, 3H, s), 0.75 (19-H_3, 3H, s), 0.91 (21-H_3, 3H, d, J= 6.8 Hz), 0.92–0.99 (26-H_3, 27-H_3 and 29-H_3, 9H, m), 2.71 (7α-H, 1H, dd, J=12.6 Hz, 12.5 Hz), 3.58 (22-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.71 (23-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.87 (3β-H, 1H, m). ^{13}C NMR ($CDCl_3$/MeOD 95:5): 11.6 (C21), 12.2 (C18), 13.3 (C29), 13.7 (C19), 19.0 (C28), 19.2 (C26), 20.9 (C27), 21.1 (C11), 24.0 (C15), 25.4 (C2), 28.3 (C1), 28.8 (C16), 31.4 (C4), 28.8 (C25), 36.7 (C20),37.7 (C8), 39.6 (C12), 41.7 (C7), 43.0 (C13), 43.8 (C10), 44.8 (C9), 46.4 (C24), 56.0 and 56.5 (C17 and C14), 67.5 (C3), 72.2 (C23), 74.2 (C22), 80.3 (C5), 211.9 (C6). MS (FAB): 479 $(M+H)^+$, 477 $(M-H)^+$, 461 $(M+H-H_2O)^+$, 443 $(M+H\text{-}2H_2O)^+$, 425. HRMS (FAB): Calculated for $C_{29}H_{49}O_5$ $(M-H)^+$: 477.3580, found 479.3567.

(22*E*)-3β,5-Dihydroxy-5α-stigmast-22-en-6-one (28). To a solution of compound **27**[24] (325 mg, 0.62 mmol) in methanol (100 ml) potassium carbonate (100 mg, 0.72 mmol) was added. The reaction mixture was stirred for 6 h at room temperature and then ammonium chloride (saturated solution) was added. The methanol was evaporated and the aqueous phase extracted with methylene chloride. The organic layer was dried and evaporated. The residue was purified by column chromatography (hexane/ethyl acetate 1:1) to afford compound **28** in 94% yield. Mp: 250°C. ^{1}H NMR ($CDCl_3$): 0.67 (18-H_3, 3H, s), 0.79 (27-H_3, 3H, d, J=6.0 Hz), 0.80 (29-H_3, 3H, t, J=7.2 Hz), 0.82 (19-H_3, 3H, s), 0.85 (26-H_3, 3H, d, J=6.3 Hz), 1.01 (21-H_3, 3H, d, J=6.6 Hz), 2.58 (7α-H, 1H, dd, J=12.6 Hz, 12.5 Hz), 3.95 (3α-H, 1H, m), 5.02 (23-H, 1H, dd, J=15.1 Hz, 8.6 Hz), 5.15 (22-H, 1H, dd, J=15.1 Hz, 8.6 Hz). ^{13}C NMR ($CDCl_3$): 11.8 (C18 and C29), 13.6 (C19), 18.6 (C26), 20.7 (C11), 20.7 21.0 (C27 and C 21), 23.6 (C15), 24.9 (C28), 28.3 (C16), 29.5 (C2), 30.0 (C1), 31.4 (C25), 35.7 (C4), 37.0 (C8), 39.1 (C12), 40.0 (C20), 41.5 (C7), 42.0 (C13), 42.6 (C10), 43.9 (C9), 50.8 (C24), 55.5 (C17), 56.0 (C14), 66.3 (C3), 79.7 (C5), 129.0 (C23), 137.7 (C22), 212.9 (C6). MS (EI) *m/z* (%): 444 (0.6), 426 (0.2), 383 (0.4), 365 (0.7), 303 (1), 269 (2), 42 (100). HRMS (EI): Calculated for $C_{29}H_{48}O_3$: 444.3603, found 444.3604.

(22*R*,23*R*)-3β,5,22,23-Tetrahydroxy-5α-stigmastan-6-one (13). Compound **28** (252.0 mg, 0.57 mmol) was treated in a similar way as described for compound **18**. The crude solid was purified by column chromatography (methylene chloride/isopropanol) to give compound **13** (17% yield). Mp: 238°C. ^{1}H NMR ($CDCl_3$/MeOD 95:5): 0.67 (18-H_3, 3H, s), 0.82 (19-H_3, 3H, s), 0.91 (21-H_3, 3H, d, J= 6.8 Hz), 0.92–0.99 (26-H_3, 27-H_3 and 29-H_3, 9H, m), 2.58 (7α-H, 1H, dd, J=12.6 Hz, 12.5 Hz), 3.58 (22-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.71 (23-H, 1H, dd, J=8.4 Hz, 1.3 Hz), 3.95 (3α-H, 1H, m). ^{13}C NMR ($CDCl_3$/MeOD 95:5): 11.6 (C21), 11.8 (C18), 13.2 (C29), 13.6 (C19), 19.0 (C28), 19.2 (C26), 20.7 (C11), 20.9 (C27), 23.6 (C15), 28.3 (C16), 28.8 (C25), 29.5 (C2), 30.1 (C1), 35.7 (C4), 36.8 (C20), 37.0 (C8), 39.1 (C12), 41.6 (C7), 42.0 (C13), 42.6 (C10), 43.9 (C9), 46.4 (C24), 55.5 (C17), 55.8 (C14), 66.1 (C3), 72.2 (C23), 74.1 (C22), 79.7 (C5), 212.8 (C6). MS (FAB): 479 $(M+H)^+$, 461 $(M+H-H_2O)^+$, 443 $(M+H\text{-}2H_2O)^+$, 425. HRMS (FAB): Calculated for $C_{29}H_{51}O_5$ $(M+H)^+$: 479.3737, found 479.3733.

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