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Synthesis and bioactivity of C-29 brassinosteroid analogues with different functional groups at C-6

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Abstract

In this paper, we report the synthesis and bioactivity of four synthetic analogues of 28-homobrassinosteroids, in order to evaluate the influence in bioactivity when the C-6 keto group is replaced by different functional groups. The synthetic analogues are 6-deoxo-28-homocastasterone [(22*R*,23*R*)-stigmasta-2α,3α,22,23-tetraol], 6α-hydroxy-28-homocastasterone [(22*R*,23*R*)-stigmasta-2α,3α,6α,22,23-pentaol], 6β-hydroxy-28-homocastasterone [(22*R*,23*R*)-stigmasta-2α,3α,6β,22,23-pentaol], and [(22*R*,23*R*)-6α-fluorostigmasta-2α,3α,22,23-tetraol].

Results indicate that replacement of the 6-keto moiety by an β or α hydroxyl group led to a decrease in activity, whereas the 6-deoxo analogue showed a very low activity, confirming the importance of an electronegative moiety at C-6 to observe hormonal potency. The 6α-fluorinated analogue elicited a low activity, similar to that of the 6-deoxo analogue.

Keywords: *Oryza sativa*; Gramineae; Brassinosteroids; Synthesis of fluorinated analogues; Bioactivity

1. Introduction

Brassinosteroids (BRs) are recognized as a class of plant steroidal hormones that control many developmental and physiological processes including regulation of gene expression, cell division, differentiation, and homeostasis (Kripach et al., 1999; Sakurai, 1999a).

Since the discovery of brassinolide (BL) (**1a**) (Fig. 1) (Grove et al., 1979) as the growth-promoting factor responsible for the activity of *Brassica* pollen extracts, more than 40 related compounds have been isolated from a wide variety of plant species (Bajguz and Tretyn, 2003; Hayat and Ahmad, 2003). The exogenous application of BRs has shown a wide spectrum of responses, such as stem elongation, inhibition of root growth, leaf epinasty and xylem differentiation, brought about by changes in enzyme activity and gene expression (Cutler et al., 1991; Arteca, 1995; Mussig et al., 2002).

Brassinolide (**1a**) and castasterone (**2a**) are C_{28} brassinosteroids bearing an *S*-methyl group at C-24 in the side chain of its 5α-ergostane structure.

Though C_{28} brassinosteroids are the most ubiquitous in nature, other BRs with a different steroidal side-chain are known. Among them, 28-homobrassinolide (HBL) (**1b**) and 28-homocastasterone (HCS) (**2b**) are the most active C_{29} BRs with a 5α-stigmastane structure (Sakurai et al., 1999b) (Fig. 1); both compounds have been widely used in field trials.

Previous reports (Sakurai, 1999a; Sung et al., 2000) indicated that in the C_{28} series bioactivities decrease within the following order: C-6 lactone, C-6 ketone,

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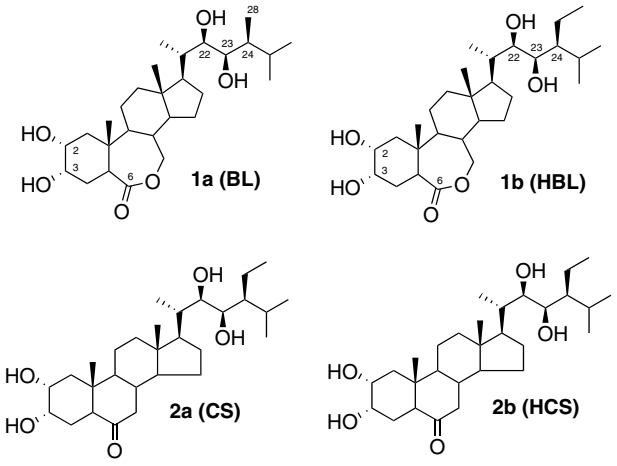


Fig. 1. Some C_{28} and C_{29} natural brassinosteroids.

C-6 α or β hydroxy, C-6 deoxo (compounds **1a**, **2a**, **4a**, **5a** and **3a**, respectively, Figs. 1 and 2).

In this paper, we report the synthesis and bioactivity of four new synthetic analogues of the C_{29} series, in order to evaluate the influence on bioactivity when the C-6 lactone or keto groups are replaced by different functionalities.

New synthetic analogues are 6-deoxo-28-homocastasterone [(22*R*,23*R*)-stigmasta-2α,3α,22,23-tetraol] (**3b**), 6α-hydroxy-28-homocastasterone [(22*R*,23*R*)-stigmasta-2α,3α,6α,22,23-pentaol] (**4b**), 6β-hydroxy-28-homocastasterone [(22*R*,23*R*)-stigmasta-2α,3α,6β,22,23-pentaol] (**5b**), and [(22*R*,23*R*)-6α-fluorostigmasta-2α,3α,22,23-tetraol] (**6**) (Fig. 2).

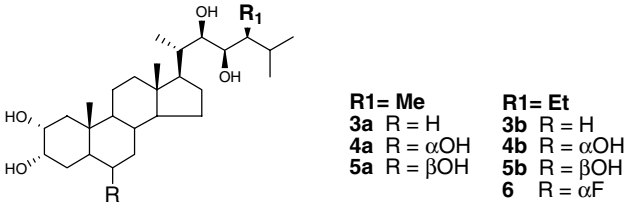


Fig. 2. Structures of brassinosteroids analogues.

2. Results and discussion

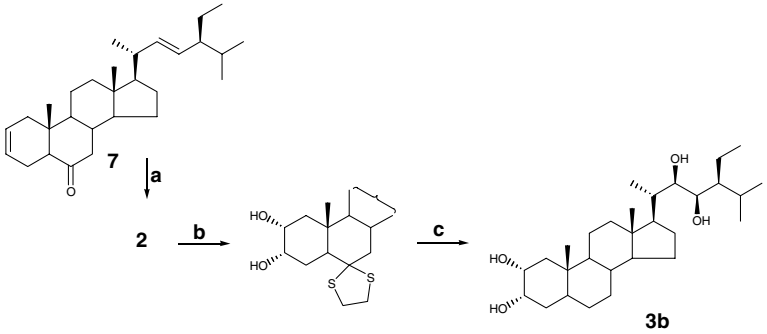
Synthesis of compound **3b** is shown in Scheme 1. Dienone **7** was obtained in four steps from stigmasterol following the procedure described by McMorris et al. (1994). 28-Homocastasterone (**2b**) was obtained from dienone **7** by osmium-catalyzed asymmetric dihydroxylation (CAD) (Sharpless et al., 1992) on double bonds at C-2 and C-22, using $K_3Fe(CN)_6$ as cooxidant and hydroquinidine-1,4-phtalazinediyl diether [$(DHQD)_2$-Phal] as chiral ligand. Subsequent treatment of compound **2b** with ethanedithiol and boron trifluoride etherate gave a dithiane intermediate, which was reduced by tri-*n*-butyltin hydride (Anastasia et al., 1985) to afford the desired compound **3b** in 68% yield.

Syntheses of compounds **4b** and **5b** are shown in Scheme 2. Reduction of **7** with sodium in ethanol, afforded the thermodynamically more stable equatorial 6α-alcohol (70% yield) (**8**), along with a 12% of the 6β-epimeric compound (**9**). Stereochemistry at C-6 of both isomers was deduced from examination of the multiplicity pattern of the H-6 in the ^1H NMR spectra.

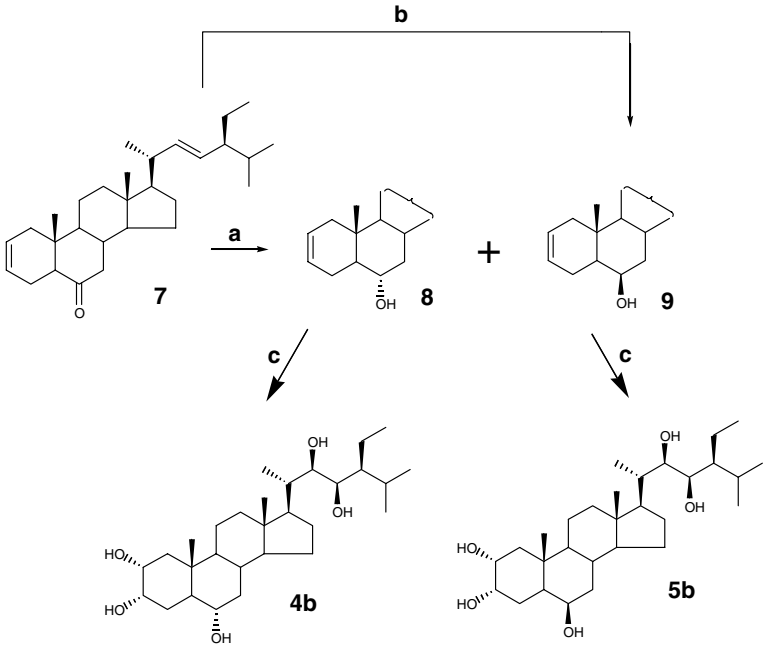
CAD of double bonds of compounds **8** and **9** gave compounds **4b** and **5b**, respectively, along with their corresponding 22*S*,23*S* diasteromers in a minor proportion (ratio 3:1), and with more than 30% of the starting material that could be recycled. Yields, after purification, were 32% and 30%, respectively.

In order to improve the yield of compound **9**, the dienone **7** was reduced using lithium tri-*t*-butoxyaluminium hydride in THF/methanol. The use of this bulky reducing reagent ensured hydride attack from the less hindered equatorial direction yielding only compound **9**.

Several efforts for introducing fluorine groups in the structure of brassinosteroids have been made (Jin et al., 1993; Back et al., 1999; Jiang et al. , 1993; Ramírez et al., 2000; Galagovsky et al., 2001); however, few stud-



Scheme 1. Synthesis of compound **3b** *Reagents and conditions:* (a) K_2OsO_4, $K_3Fe(CN)_6$, $(DHQD)_2Phal$, K_2CO_3, *t*-BuOH, H_2O, $CH_3SO_3NH_2$, r.t., 9 days; (b) $HSCH_2CH_2SH$, BF_3-Et_2O, CH_2Cl_2, r.t., 30 min; (c) *n*-Bu_3SnH, AIBN, benzene, reflux, 2 h.

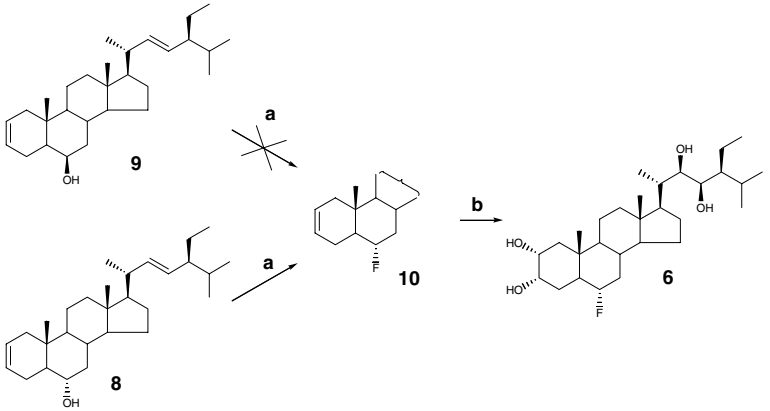


Scheme 2. Synthesis of compounds **4b** and **5b**. *Reagents and conditions:* (a) Na, EtOH, reflux, 3 h; (b) $(t\text{-BuO})_3AlH$, THF, MeOH, r.t., 1 day; (c) K_2OsO_4, $K_3Fe(CN)_6$, $(DHQD)_2Phal$, K_2CO_3, *t*-BuOH, H_2O, $CH_3SO_3NH_2$, r.t., 9 days.

ies on the effect on bioactivity have been reported. Synthesis of fluorinated analogue **6** is shown in Scheme 3. Stereoselective introduction of fluorine is usually achieved by bimolecular nucleophilic substitution (SN_2) of an hydroxyl group with diethylaminosulfur trifluoride (DAST) (Rozen et al., 1979). Treatment of **9** with DAST at −78 °C gave no reaction, whereas further attempts at higher temperatures led to decomposition of the substrate. This behaviour might be explained by the fact that the axial 19-methyl could block the SN_2 reaction on the axial 6β-hydroxyl group. A similar case was reported by Kumar et al. (1995) during the transformation of a hindered 17β-hydroxy steroid into a 17α-fluoro compound by DAST treatment. The desired compound was finally obtained starting from the corresponding 17α-hydroxy substrate, suggesting an SN_1 mechanism via a carbocationic intermediate (Shellhamer et al., 1996).

In fact, the treatment of the 6α-hydroxy steroid **8** with DAST yielded the expected compound (22*E*)-6α-fluoro-5α-stigmasta-2,22-diene (**10**).

The configuration at C-6 was established from analysis of the ^1H NMR spectra, as shown in Fig. 3. Fig. 3(a) shows the multiplet corresponding to the H-6 in the hydroxylated precursor **8**, consistent with two axial–axial couplings of 10.8 Hz ($J_{6\beta H-5\alpha H}$ and $J_{6\beta H-7\alpha H}$), and an axial–equatorial coupling of 4.6 Hz ($J_{6\beta H-7\beta H}$). Fig. 3(b) shows that, besides the large germinal J_{H-F}



Scheme 3. Synthesis of fluorinated analogue **6**. *Reagents and conditions:* (a) DAST, CH_2Cl_2, −78 °C, 20 min. (b) K_2OsO_4, $K_3Fe(CN)_6$, $(DHQD)_2Phal$, K_2CO_3, *t*-BuOH, H_2O, $CH_3SO_3NH_2$, r.t., 9 days.

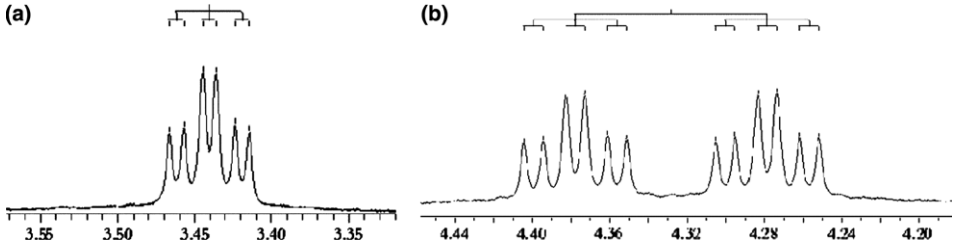


Fig. 3. (a) 6β-H signal for compound **8**. (b) 6β-H signal for compound **10** ($CDCl_3$, 500 MHz). The same J_{H-H} pattern is observed, confirming that fluorination proceeded with retention of the configuration.

coupling, the same pattern is observed for H-6 in compound **10**, confirming that fluorination occurred with retention of configuration.

Finally, CAD on **10** gave, after purification, the desired compound **6** in 35% yield. All of the chemical structures were confirmed by HRMS and NMR spectroscopic analyses (DEPT-135, COSY-45 and HETCOR experiments).

Bioactivities were evaluated at four different doses for each compound using a modified RLIT (Takeno and Pharis, 1982) on local *Chuy* rice cultivar, using 28-homocastasterona (**2b**) as a control compound, and are shown in Fig. 4.

Results indicate that replacement of the 6-keto moiety by a β hydroxy group (**5b**) led to a similar bioactivity at the highest dose used, but to a decrease in lower doses. On the other hand, the replacement by an α hydroxy group (**4b**) led to a decrease in the activity at all doses. These results are in agreement with preliminar data reported by Brosa (1999) using the *Bahia* rice cultivar.

The 6-deoxo analogue (**3b**) showed very low activity, confirming the importance of an electronegative moiety at C-6 for hormonal potency (Sakurai, 1999a).

The 6α-fluorinated analogue (**6**) elicited a low activity, similar to that of the 6-deoxo analogue (**3b**). Many previous studies showed different effects on bioactivity due to the replacement of an hydrogen or an oxygenated functional group by fluorine. Two examples may be mentioned. On the one hand, Back et al. (1999) found that the 25-fluoro analogues of brassinolide and castasterone showed almost no activity while the corresponding 25-hydroxy analogues had potent biological activity. On the other hand, 5α-fluoro-28-homocastasterone and 5α-fluoro-28-homotyphasterol showed increased bioactivity compared with their related 5-hydroxy analogues (Ramírez et al., 2000; Schaefer et al., 2002).

The low bioactivity elicited by compound **6** indicates that, in this case, the presence of a 6-fluorine atom is not recognized in the biological system as an electronegative group like a 6-keto or a 6-hydroxy one, not withstanding the described isosteric and electronic similarities between an hydroxy and a fluoro group (Welch, 1987).

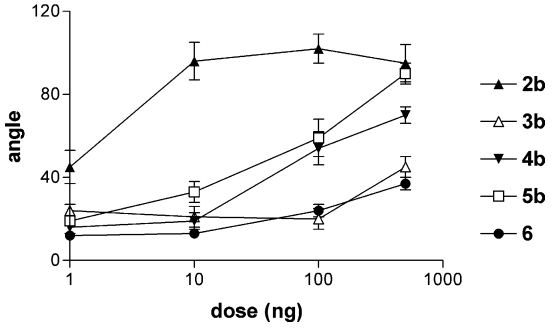


Fig. 4. Bioactivity in the rice lamina inclination test. Values are angles (degrees) between the lamina and the sheath, representing the means of 20 replicates ± standard error. Average angle of control: 13 ± 2.

3. Experimental

3.1. General experimental procedures

Melting points (m.p.) were determined on a Fisher Johns apparatus and are uncorrected. ^{1}H-NMR and ^{13}C NMR spectra were recorded on a Bruker AM-500 at 500 MHz and at 125.1 MHz, respectively; chemical shifts (δ) are given in ppm downfield from TMS as internal standard. Coupling constant *(J)* values are in Hz. ^{19}F-NMR spectra were recorded on a Brüker AM-500 at 470.4 MHz, and chemical shifts (δ) are given in ppm upfield from $CFCl_3$ as the internal standard. High-resolution mass spectra (EI and FAB) were obtained for all new compounds on a ZAB SEQ instrument (VG-Micromass). FAB were obtained using a DS (disulfide) matrix. Low resolution mass spectra were recorded on a Shimadzu QP-5050 at 70 eV. Unless otherwise indicated, all solvents and reagents used were of commercial grade. Reactions were monitored by TLC on precoated plates with silica gel F_{254} 0.2 mm (Merck). CC was carried out on silica gel 60, 0.04–0.063 mm (Merck).

3.2. Rice lamina inclination test

Rice seedlings *(Oryza sativa*, cv *Chuy)* were washed with EtOH (1 min) and water and were then left in water at 30 °C for two days (with a 16 h photoperiod). Germinated seeds were cultivated on agar under the same

growing conditions for four days. Intact seedlings (4–5 cm long) were inoculated with 0.5 μl of the test compound solution (in EtOH) just under the 2nd leaf joint. After 48 h in the dark (at 30 °C), the magnitude of the induced angle between the leaf and the sheath was measured.

3.3. Syntheses

3.3.1. (22R,23R)-stigmasta-2α,3α,22,23-tetraol (**3b**)

Treatment of 28-homocastasterone (**2b**) (15 mg) (McMorris et al., 1994) with ethanedithiol (50 μl) and boron trifluoride etherate (50 μl) for 30 min afforded the corresponding dithiane as a glass, which was dissolved, without purification, in dry benzene (5 ml), and treated with tri-*n*-butyltin hydride (0.1 ml) in the presence of 2,2′-azobis(isobutironitrile) (3 mg). After work-up, the obtained product was purified by silica gel TLC (CH_2Cl_2/CH_3CN 9:1) to afford **3b** (10 mg), m.p.: 223–224 °C. ^{1}H NMR ($CDCl_3$:CD_3OD, 95:5): 0.70 (H-18, 3H, *s*), 0.81 (H-19, 3H, *s*), 0.89 (H-21, 3H, *d*, *J* = 6.2 Hz), 0.92–0.99 (H-26, H-27 and H-29, 9H, *m*), 3.59 (H-22, 1H, *dd*, *J* = 8.4 Hz, 1.3 Hz), 3.69 (H-23, 1H, *dd*, *J* = 8.4 Hz, 1.3 Hz), 3.72 (H-2β, 1H, *m*), 4.05 (H-3β, 1H, *dd*, *J* = 6.0 Hz, 3.0 Hz). ^{13}C NMR ($CDCl_3$:CD_3OD, 95:5): 11.6 (*q*, C-18), 11.7 (*q*, C-21), 13.0 (*q*, C-19), 13.5 (*q*, C-29), 19.0 (*t*, C-28), 19.3 (*q*, C-26), 20.8 (*t*, C-11), 20.8 (*q*, C-27), 23.8 (*t*, C-15), 27.7 (*t*, C-6), 28.5 (*t*, C-16), 29.0 (*d*, C-25), 32.7 (*t*, C-7), 34.5 (*t*, C-4), 36.0 (*d*, C-8), 36.5 (*d*, C-20), 37.6 (*s*, C-10), 38.5 (*s*, C-5), 39.8 (*t*, C-1), 40.9 (*t*, C-12), 42.2 (*s*, C-13), 46.4 (*d*, C-24), 52.3 (*d*, C-17), 53.6 (*t*, C-9), 55.5 (*d*, C-14), 68.1 (*d*, C-3), 68.7 (*d*, C-2), 72.2 (*d*, C-23), 74.3 (*d*, C-22). MS (FAB) *m/z* (rel. int.): 487 [$(M + Na)^+$, 10], 447 (8), 429 (12), 231 (30), 136 (100). HRMS (FAB): Calculated for $C_{29}H_{52}O_4Na$ $(M + Na)^+$: 487.3763, found: 487.3770.

3.3.2. (22E)-stigmasta-2,22-dien-6α-ol (**8**)

Sodium metal (53 mg, 2.3 mmol) was cut into small pieces and added to a refluxing solution of (22*E*)-stigmasta-2,22-dien-6-one **7** (100 mg, 0.25 mmol) in anhydrous EtOH (20 ml) (McMorris et al., 1994). After cooling, the mixture was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed, dried and evaporated in vacuo. Chromathographic purification of the crude product (hexane:EtOAc, 95:5) gave **9** (12 mg), m.p.: 107–109 °C. ^{1}H NMR ($CDCl_3$): 0.72 (H-18, 3H, *s*), 0.80 (H-27, 3H, *d*, *J* = 6.5 Hz), 0.81 (H-29, 3H, *t*, *J* = 7.4 Hz), 0.85 (H-26, 3H, *d*, *J* = 6.5 Hz), 0.97 (H-19, 3H, *s*), 1.02 (H-21, 3H, *d*, *J* = 6.7 Hz), 2.30 (H-4β, 1H, *m*), 3.85 (H-6α, 1H, *m*), 5.02 (H-23, 1H, *dd*, *J* = 15.1 Hz, 8.5 Hz), 5.16 (H-22, 1H, *dd*, *J* = 15.1 Hz, 8.6 Hz), 5.56–5.71 (H-2 and H-3, 2H, *m*).

^{13}C NMR ($CDCl_3$): 12.2 (*q*, C-18), 12.2 (*q*, C-29), 15.2 (*q*, C-19), 19.0 (*q*, C-26), 20.8 (*t*, C-11), 21.0 (*q*, C-21), 21.2 (*q*, C-27), 24.3 (*t*, C-15), 25.4 (*t*, C-28), 26.4 (*t*, C-4), 28.9 (*t*, C-16), 31.9 (*d*, C-25), 34.2 (*d*, C-8), 34.6 (*s*, C-10), 39.7 (*t*, C-12), 39.9 (*t*, C-1), 40.5 (*d*, C-20), 41.7 (*t*, C-7), 42.5 (*s*, C-13), 44.5 (*d*, C-5), 51.2 (*d*, C-24), 54.2 (*t*, C-9), 56.1 (*d*, C-14), 56.3 (*d*, C-17), 70.5 (*d*, C-6), 125.6 (*d*, C-3), 125.7 (*d*, C-2), 129.3 (*d*, C-23), 138.3 (*d*, C-22). MS (EI) *m/z* (rel. int.): 412 (M^+, 2), 351 (1), 271 (3), 253 (3), 199 (2), 55 (100).

HRMS (EI): Calculated for $C_{29}H_{48}O$: 412.3705, found: 412.3712.

Further elution with the same solvent gave **8** (70 mg), m.p.: 119–120 °C. ^{1} H NMR ($CDCl_3$): 0.68 (H-18, 3H, *s*), 0.77 (H-19, 3H, *s*), 0.80 (H-27, 3H, *d*, *J* = 6.5 Hz), 0.81 (H-29, 3H, *t*, *J* = 7.4 Hz), 0.85 (H-26, 3H, *d*, *J* = 6.4 Hz), 1.02 (H-21, 3H, *d*, *J* = 6.7 Hz), 2.40 (H-4β, 1H, *m*), 3.44 (H-6β, 1H, *ddd*, *J* = 10.7 Hz, 10.7 Hz, 4.6 Hz), 5,02 (H-23, 1H, *dd*, *J* = 15, 1 Hz, 8.5 Hz), 5.16 (H-22, 1H, *dd*, *J* = 15.1 Hz, 8.6 Hz), 5.56–5.71 (H-2 and H-3, 2H, *m*).

^{13}C NMR ($CDCl_3$): 12.1 (*q*, C-29), 12.2 (*q*, C-18), 13.0 (*q*, C-19), 18.9 (*q*, C-26), 20.8 (*t*, C-11), 21.0 (*q*, C-21), 21.0 (*q*, C-27), 24.2 (*t*, C-15), 25.4 (*t*, C-28), 25.8 (*t*, C-4), 28.8 (*t*, C-16), 31.9 (*d*, C-25), 34.2 (*d*, C-8), 35.4 (*s*, C-10), 39.7 (*t*, C-12), 40.0 (*t*, C-1), 40.4 (*d*, C-20), 41.4 (*t*, C-7), 42.4 (*s*, C-13), 48.7 (*d*, C-5), 51.2 (*d*, C-24), 53.6 (*t*, C-9), 56.0 (*d*, C-17), 56.2 (*d*, C-14), 71.6 (*d*, C-6), 125.2 (*d*, C-3), 125.5 (*d*, C-2), 129.4 (*d*, C-23), 138.2 (*d*, C-22). MS (EI) *m/z* (rel. int.): 412 (M^+, 2), 351 (1), 314 (1), 271 (4), 229 (3), 55 (100).

HRMS (EI): Calculated for $C_{29}H_{48}O$: 412.3705, found: 412.3699.

3.3.3. (22E)-stigmasta-2,22-dien-6β-ol (**9**)

Compound **7** (150 mg, 0.36 mmol) was dissolved in 15 ml of a 9:1 mixture of THF and MeOH under an inert atmosphere. To this stirred solution, lithium tri-*t*-butoxy-aluminium hydride (280 mg, 1.1 mmol) was added. After 24 h, stirring the solution was poured into 5% HCl and the whole was extracted with CH_2Cl_2. The crude product was purified by Si gel CC (hexane:EtOAc, 95:5) to afford **9** (140 mg, 92%).

3.3.4. (22R,23R)-stigmasta-2α,3α,6α,22,23-pentaol (**4b**)

A mixture of **8** (60 mg, 0.14 mmol), *t*-BuOH:water (1:1, 10 ml), $(DHQD)_2$-Phal (60 mg, 0.08 mmol), methansulfonamide (45 mg, 0.26 mmol), potassium ferricyanide (150 mg, 0.46 mmol), potassium carbonate (62 mg, 0.45 mmol), and potassium osmate dihydrate (7 mg, 0.02 mmol) was stirred at room temperature for 9 days. An excess of $NaHSO_3$ was added until no evolution of bubbles was observed. Layers were separated and the aqueous phase was thoroughly extracted with CH_2Cl_2:MeOH (95:5).

Combined organic layers were sequentially washed with 0.25 M H_2SO_4 and 2% NaOH. Purification by column chromatography (CH_2Cl_2/acetonitrile gradient)

separated **4b** from its 22*S*, 23*S* diasteromer, with 32% yield, m.p.: 227–225 °C. ^{1}H NMR ($CDCl_3$:CD_3OD, 95:5): 0.67 (H-18, 3H, *s*), 0.82 (H-19, 3H, *s*), 0.89 (H-21, 3H, *d*, J = 6.2 Hz), 0.92 0.99 (H-26, H-27 and H-29, 9H, *m*), 3.29 (H-6β, *ddd*, J = 10.8 Hz, 10.7 Hz, 4.7 Hz), 3.56 (H-22, 1H, *dd*, J = 8.4 Hz, 1.3 Hz), 3.67 (H-23, 1H, *dd*, J = 8.4 Hz, 1.3 Hz), 3.71 (H-2β, 1H, *m*), 3.98 (H-3β, 1H, *dd*, J = 6.0 Hz, 3.0 Hz). ^{13}C NMR ($CDCl_3$:CD_3OD, 95:5): 11.7 (*q*, C-21), 11.8 (*q*, C-18), 13.1 (*q*, C-19), 13.4 (*q*, C-29), 18.7 (*t*, C-28), 19.2 (*q*, C-26), 20.7 (*t*, C-11), 20.9 (*q*, C-27), 23.8 (*t*, C-15), 27.5 (*t*, C-4), 28.4 (*t*, C-16), 28.9 (*d*, C-25), 34.1 (*d*, C-8), 36.9 (*d*, C-20), 37.9 (*s*, C-10), 39.7 (*t*, C-1), 40.7 (*t*, C-12), 41.3 (*t*, C-7), 42.2 (*s*, C-13), 46.4 (*d*, C-24), 48.9 (*d*, C-5), 52.4 (*d*, C-17), 53.6 (*t*, C-9), 55.6 (*d*, C-14), 68.4 (*d*, C-3), 68.5 (*d*, C-2), 69.1 (*d*, C-6), 72.3 (*d*, C-23), 74.2 (*d*, C-22). MS (FAB) *m/z* (rel. int.): 503 [$(M + Na)^+$, 25], 305 (10), 231 (22), 154 (31), 55 (100). HRMS (FAB): Calculated for $C_{29}H_{52}O_5Na$ $(M + Na)^+$: 503.3712, found: 503.3720.

*3.3.5. (22R,23R)-stigmasta-2α,3α,6β,22,23-pentaol (**5b**)*

Compound **9** (75 mg, 0.18 mmol) was tetrahydroxylated as described above for compound **8**. Chromatographic purification (CH_2Cl_2:CH_3CN gradient) afforded **5b** in 30% yield, m.p.: 234–235 °C. ^{1}H NMR ($CDCl_3$:CD_3OD, 95:5): 0.70 (H-18, 3H, *s*), 0.89 (H-21, 3H, *d*, J = 6.2 Hz), 0.92–0.99 (H-26, H-27 and H-29, 9H, *m*), 1.02 (H-19, 3H, *s*), 3.57 (H-22, 1H, *dd*, J = 8.4 Hz, 1.3 Hz), 3.68 (H-23, 1 H, *dd*, J = 8.4 Hz, 1.3 Hz), 3.74 (H-2β, 1H, *m*), 3.79 (H-6α, 1H, *m*), 4.01 (H-3β, 1H, *dd*, J = 6.0 Hz, 3.0 Hz). ^{13}C NMR ($CDCl_3$:CD_3OD, 95:5): 11.7 (*q*, C-18), 11.7 (*q*, C-21), 13.5 (*q*, C-29), 15.6 (*q*, C-19), 18.7 (*t*, C-28), 19.3 (*q*, C-26), 20.7 (*t*, C-11), 21.1 (*q*, C-27), 23.9 (*t*, C-15), 28.1 (*t*, C-4), 28.5 (*t*, C-16), 28.9 (*d*, C-25), 34.1 (*d*, C-8), 37.0 (*d*, C-20), 37.1 (*s*, C-10), 39.7 (*t*, C-1), 40.7 (*t*, C-12), 41.9 (*t*, C-7), 42.3 (*s*, C-13), 46.4 (*d*, C-24), 50.5 (*d*, C-5), 52.7 (*d*, C-17), 54.2 (*t*, C-9), 55.5 (*d*, C-14), 68.5 (*d*, C-3), 69.1 (*d*, C-2), 70.9 (*d*, C-6), 72.3 (*d*, C-23), 74.3 (*d*, C-22). MS (FAB) *m/z* (rel. int.): 503 [$(M + Na)^+$, 62], 317 (9), 231 (31), 154 (92), 55 (100). HRMS (FAB): Calculated for $C_{29}H_{52}O_5Na$ $(M + Na)^+$: 503.3712, found: 503.3730.

*3.3.6. (22E)-6α-fluorostigmasta-2,22-diene (**10**)*

A solution of **8** (78 mg, 0.19 mmol) in CH_2Cl_2 (10 ml) was slowly added to a solution of DAST (0.1 ml) in CH_2Cl_2 (3 ml) cooled to −78 °C. After 20 min the mixture was warmed to room temperature and poured into water, washed with $NaHCO_3$ dried and evaporated. Purification of the crude product by Si gel cc graphy (hexane:EtOAc, 98:2) gave **10** (63 mg), m.p.: 95–97 °C. ^{1} H NMR ($CDCl_3$): 0.68 (H-18, 3H, *s*), 0.77 (H-19, 3H, *s*), 0.80 (H-27, 3H, *d*, J = 6.5 Hz), 0.81 (H-29, 3H, *t*, J = 7.4 Hz), 0.85 (H-26, 3H, *d*, J = 6.5 Hz), 1.02 (H-21, 3H, *d*, J = 6.7 Hz), 2.38 (H-4β, 1H, *m*), 4.33 (H-6β, 1H, *double m*, $J_{H,F}$ = 49.7 Hz), 5.02 (H-23, 1H, *dd*, J = 15.1 Hz, 8.5 Hz), 5.16 (H-22, 1H, *dd*, J = 15.1 Hz, 8.6 Hz), 5.56–5.71 (H-2 and H-3, 2H, *m*). ^{13}C NMR ($CDCl_3$): 12.1 (*q*, C-29), 12.2 (*q*, C-18), 13.0 (*q*, C-19), 19.0 (*q*, C-26), 20.8 (*t*, C-11), 21.0 (*q*, C-21), 21.0 (*q*, C-27), 24.2 (*t*, C-15), 25.3 (*t*, C-28), 25.4 (*dt*, C-4, J_{CF} = 3.8 Hz), 28.7 (*t*, C-16), 31.9 (*d*, C-25), 33.9 (*dd*, C-8, J_{CF} = 11.4 Hz), 35.7 (*d*, C-10, J_{CF} = 9.4 Hz), 38.0 (*dt*, C-7, J_{CF} = 16.3 Hz), 39.6 (*t*, C-12), 39.8 (*t*, C-1), 40.4 (*d*, C-20), 42.5 (*s*, C-13), 46.6 (*dd*, C-5, J_{CF} = 15.1 Hz), 51.2 (*d*, C-24), 53.4 (*t*, C-9), 56.0 (*d*, C-17), 56.1 (*d*, C-14), 94.3 (*dd*, C-6, J_{CF} = 169.1 Hz), 124.9 (*d*, C-3), 125.3 (*d*, C-2), 129.4 (*d*, C-23), 138.1 (*d*, C-22). MS (EI) *m/z* (rel. int.): 414 (M^+, 1), 371 (1), 302 (3), 273 (5), 260 (3), 55 (100). HRMS (EI): Calculated for $C_{29}H_{47}F$: 414.3662, found: 414.3656

*3.3.7. (22R,23R)-6-fluorostigmasta-2,3,22,23-tetraol (**6**)*

Compound **10** (60 mg, 0.14 mmol) was tetrahydroxylated as described for compound **8**. Chromatographic purification (CH_2Cl_2:acetonitrile gradient) afforded **6** (24 mg, 35% yield), m.p.: 221–222 °C. ^{1}H NMR ($CDCl_3$:CD_3OD, 95:5): 0.67 (H-18, 3H, *s*), 0.82 (H-19, 3H, *s*), 0.89 (H-21, 3H, *d*, J = 6.3 Hz), 0.92–0.99 (H-26, H-27 and H-29, 9H, *m*), 3.56 (H-22, 1H, *d*, J = 8.9 Hz), 3.68 (H-23, 1H, *d*, J = 8.9 Hz), 3.71 (H-2β, 1H, *m*), 3.99 (H-3β, 1H, *d*, J = 3.0 Hz), 4.27 (H-6β, *double m*, $J_{H,F}$ = 50.0 Hz). ^{13}C NMR ($CDCl_3$:CD_3OD, 95:5): 11.7 (*q*, C-18), 11.7 (*q*, C-21), 13.2 (*q*, C-19), 13.4 (*q*, C-29), 18.8 (*t*, C-28), 19.3 (*q*, C-26), 20.7 (*t*, C-11), 21.0 (*q*, C-27), 23.9 (*t*, C-15), 27.5 (*t*, C-16), 28.3 (*dt*, C-4, J_{CF} = 3.6 Hz), 28.9 (*d*, C-25), 33.9 (*dd*, C-8, J_{CF} = 10.5 Hz), 36.9 (*d*, C-20), 37.9 (*dt*, C-7, J_{CF} = 17.7 Hz), 38.3 (*d*, C-10, J_{CF} = 8.8 Hz), 39.6 (*t*, C-1), 40.6 (*t*, C-12), 42.4 (*s*, C-13), 43.3 (*dd*, C-5, J_{CF} = 14.6 Hz), 46.4 (*d*, C-24), 52.5 (*d*, C-17), 53.3 (*t*, C-9), 55.9 (*d*, C-14), 68.1 (*d*, C-3), 68.3 (*d*, C-2), 72.3 (*d*, C-23), 74.3 (*d*, C-22), 92.4 (*dd*, C-6, J_{CF} = 168.6 Hz). MS (FAB) *m/z* (rel. int.): 505 [$(M + Na)^+$, 12], 465 (10), 447 (9), 307 (15), 231 (27), 154 (100), 137 (88). HRMS (FAB): Calculated for $C_{29}H_{51}O_4FNa$ $(M + Na)^+$: 505.3669, found: 505.3649.

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