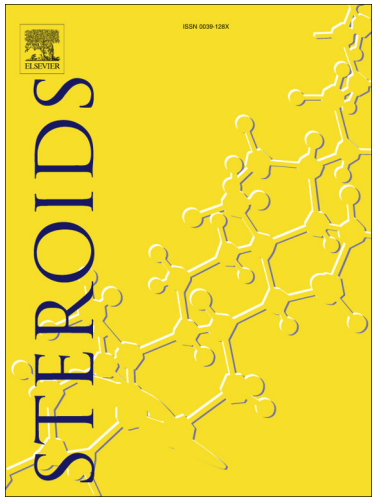

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Synthesis and Preliminary Evaluation of Dimeric-28-homobrassinosteroids for Plant Growth Regulators

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Abstract

Preparation of synthetic analogues of 28-homobrassinosteroids is reported. Also, the addition of the 28-homocastasterone at the C6 carbonyl group *via* allyl Gringard reagent followed by olefin cross metathesis resulted in dimeric analogues. Rice lamina inclination assay showed that the replacement of the C6 carbonyl group by 6α-allyl and 6β hydroxyl groups led to a decrease in bioactivity, whereas the dimeric analogues showed a reduced but significant bioactivity when compared to the 28-homocastasterone.

Keywords : Brassinosteroids, 28-homocastasterone, dimerization, 6α-allyl-6β hydroxyl homocastasterone, plant growth promoting activity, rice lamination inclination

1. Introduction

Brassinosteroids (BRs) are unusual steroids with a lactone B-ring structure and a dihydroxylate side chain [1]. These steroids have been isolated from many plant species and have been recognized as hormones potentially affecting a wide range of physiological responses in plants, including stem elongation, pollen tube growth, leaf bending and epinasty, root growth inhibition, induced synthesis of ethylene, activation of proton pump, xylem differentiation, synthesis of nucleic acids and proteins, activation of enzymes, and photosynthesis [2-4]. The brassinolide (**1**), a C28 brassinosteroid, has been reported to be the most biologically active of the BRs and widely studied for applications due to their potential agricultural utility [5]. 28-Homobrassinolide (**2**) and 28-homocastasterone (**3**), the most active C29 BRs [6] have also been intensively investigated for their potential agricultural uses. As BRs are present in plants only in very small quantities, chemical synthesis of BRs is the source for scientific and practical use. This results in numerous synthetic efforts and development of non-natural analogues of BRs that are easier to prepare and which have similar or even greater activity than the natural BRs [7-10].

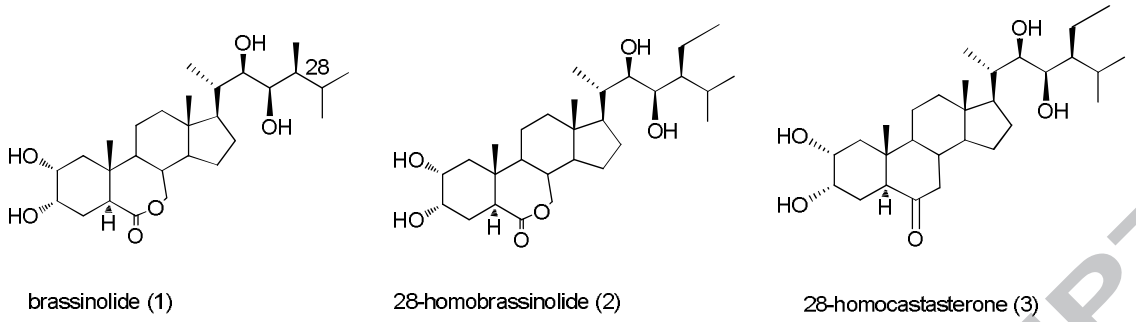
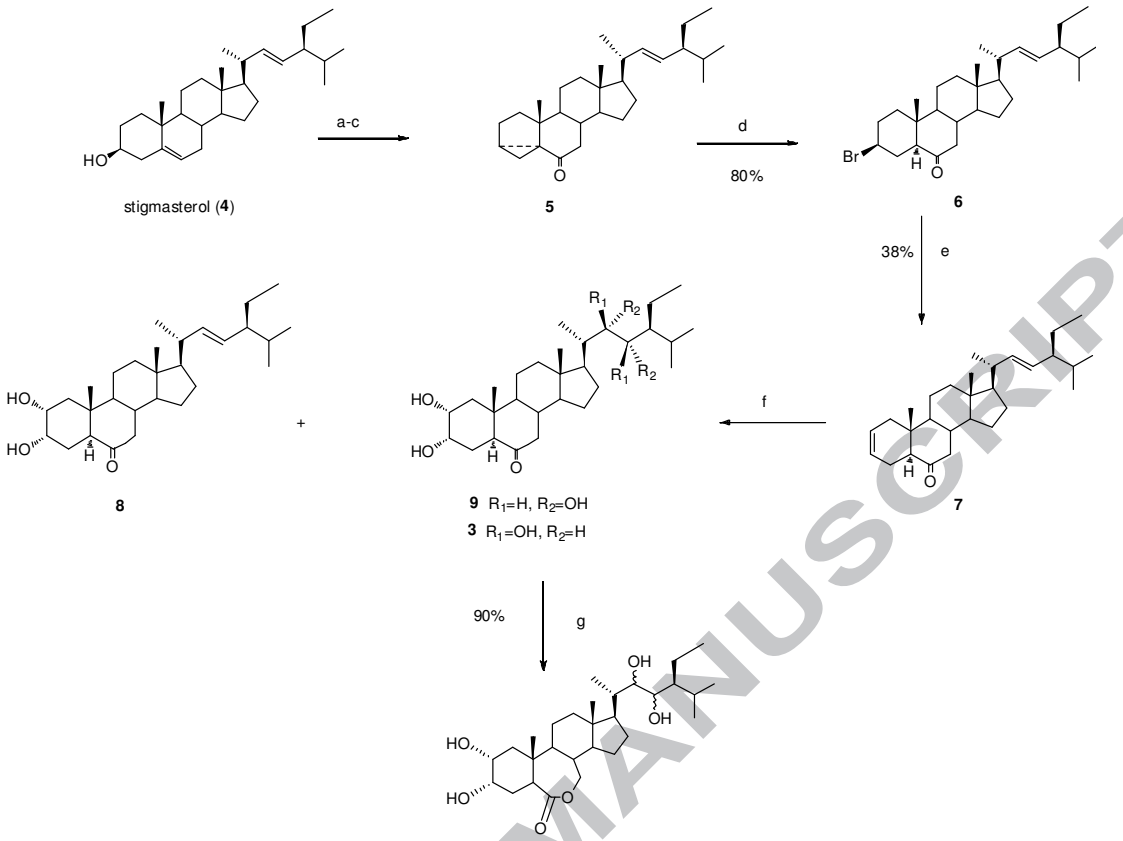


Figure 1. Structure of natural brassinolids

The studies on structure-activity relationship in brassinosteroids have shown the most active brassinosteroids have been shown to possess a *trans*-fused relationship between A and B-rings, with the substitutents at C-24 and C-25 also important for bioactivity [11]. The two vicinal diol moieties with attendant stereochemistry are required for optimum bioactivity [12]. The conversion of the four hydroxyl groups to the corresponding methyl ethers results in the loss of activity [13]. The transformation of B-ring lactone moiety suggests the polar functional groups are necessary for bioactivity [10, 14]. In this article, the preparation of the novel allyl alcohols and their dimeric-28-homobrassinosteroids are reported along with the improvement of the synthetic methodology. Also comparison of the bioactivities to 28-homocastasterone from rice leaf lamina inclination bioassay is shown.

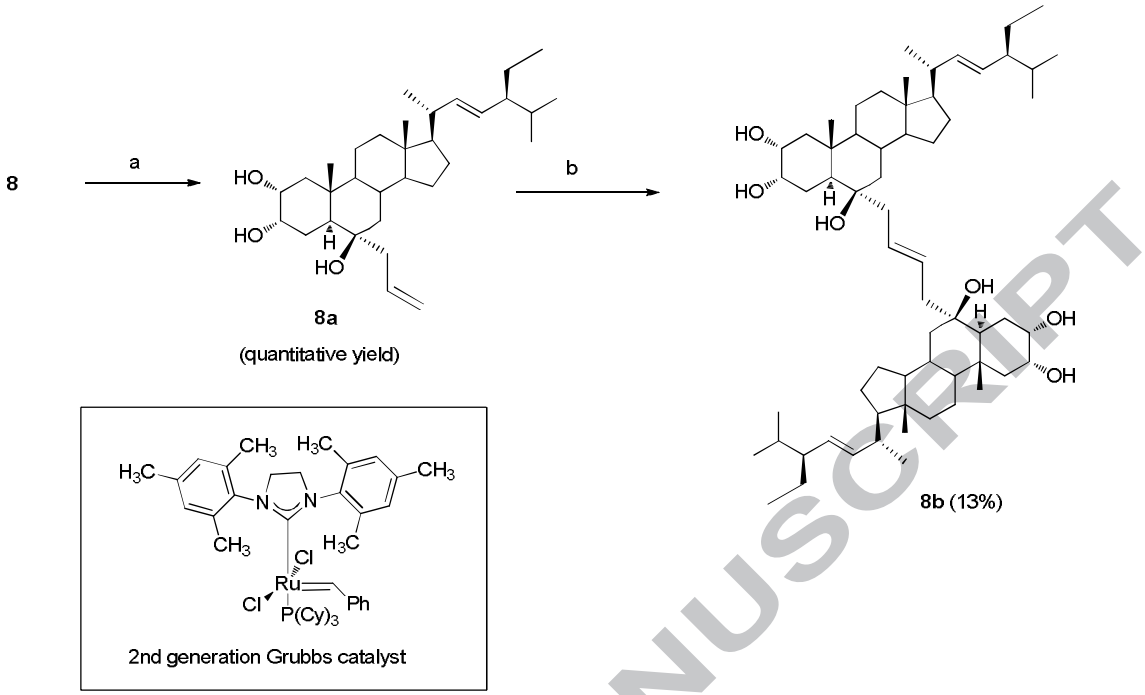
2. Results and Discussion

28-Homobrassinolide (**2**) and 28-homocastasterone (**3**) were prepared according to the synthesis pathway depicted in Scheme 1. The stigmasterol (**4**) was used as a starting material and then converted into the ketone (**5**) with modified protocol of McMorris et al. [5]. Acid-catalysed opening of the cycolprapane ring of the ketone (**5**) using HBr and AcOH gave the 3β-bromo-6-ketone (**6**) in 80% yield. Dehydrobromination using Li_2CO_3 in DMF dienone (**7**) in 38% yield. To form 28-homocastaterone, dienone (**7**) underwent osium-catalysed asymmetric dihydroxylation [15] using $K_3Fe(CN)_6$ as co-oxidant and dihydroquinidine 4-chlorobenzoate as chiral ligand. A mixture of the diol (**8**), the *22S,23S* product (**9**) and 28-homocastaterone (**3**) was obtained with yields of 16.1% , 17.1% and 12.8% respectively after purification by flash column chromatography. The configuration of the side chain diols of **3** and **9** was established by comparison with chemical shift and coupling constants of ^{1}H-NMR of known structures [16, 17]. Oxidation of the mixture of **3** and **9** *via* Baeyer-Villiger reaction using trifluoroacetic acid provided 28- homobrassinolide (**2**) in 90% yield [17].

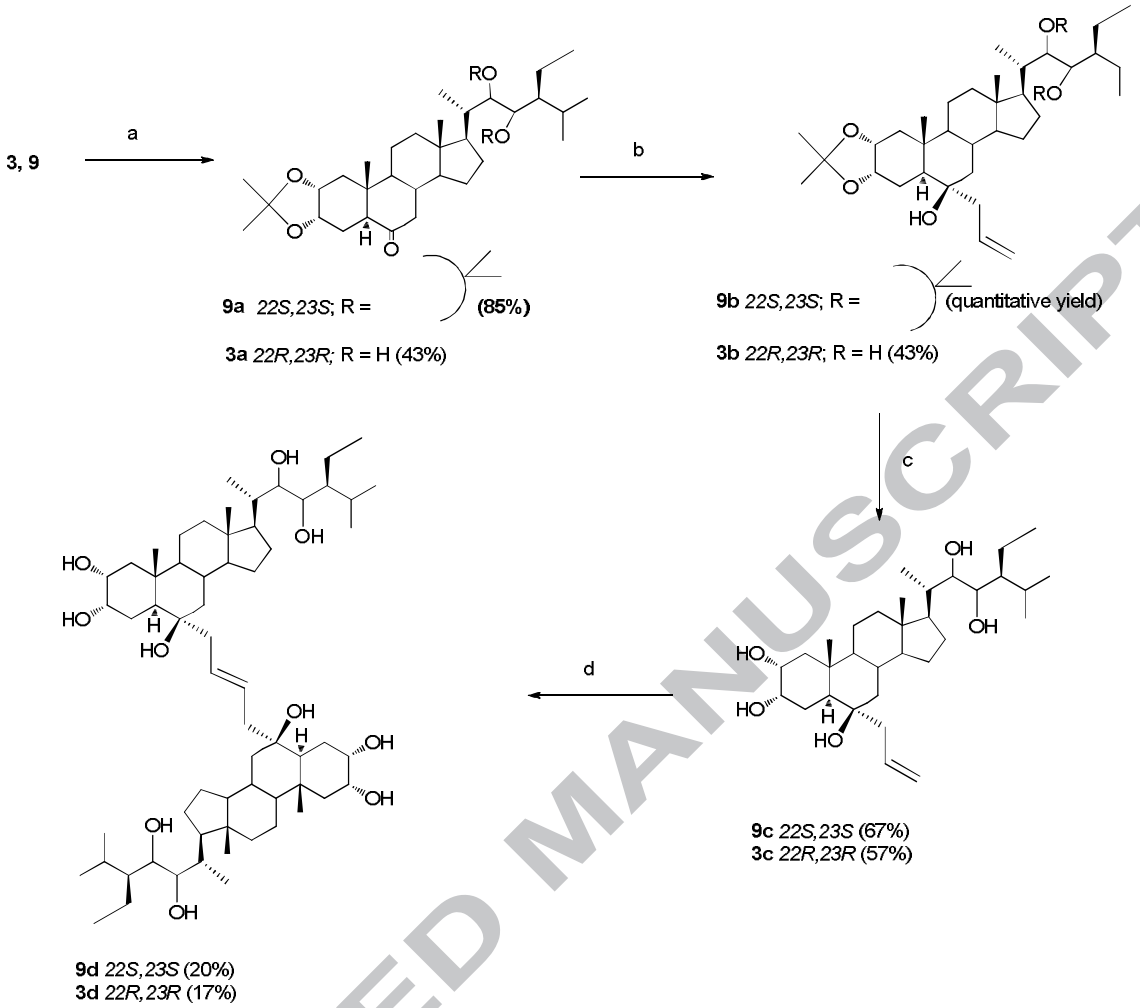


Scheme 1. Reagents and conditions:(a) MSCl, Pyr.-THF, 0°C; (b) $KHCO_3$, acetone-water, reflux; (c) CrO_3, AcOH-acetone, 0°C; (d) HBr, AcOH, rt..; (e) Li_2CO_3, DMF, reflux; (f) $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_3NH_2$, OsO_4, *t*-BuOH, rt..

Addition of allylmagnesium bromide to the 6-ketone of **8** proceeded through the *si* face to give the triol (**8a**) as a single product in quantitative yield. The 6α-allyl group in **8a** was determioned by NOESY correlating the 5α-proton and CH_2 of 6α-allyl group. This stereochemistry was also supported by the known 6α-allyl steroid generated by the attack to the less hindered alpha face of the 6-ketosteroid [18]. Homodimerization of the triol (**8a**) with 2nd generation Grubbs catalyst in CH_2Cl_2 at 90 °C for 20 h generated the dimeric compound **8b** in 55% yield (Scheme 2). However, the addition of **3** and **9** under the same conditions were unsuccessful due to their solubility in the reaction conditions. Thus, protections of the hydroxyl groups of **3** and **11** with acetone and *p*-TsOH were investigated. Compound **9** was converted to diacetals (**9a**) in 85% yield. On the other hand, compound **3** with *22R, 23R*-dihydroxyl groups could not converted to diacetals, instead the acetal **3a** was obtained in 43% yield. Then, the addition of allylmagnesium bromide gave the allyl compounds **3b** and **9b** in 43% and quantitative yield, respectively. Deprotection followed by homodimerization using 2nd generation Grubbs catalyst gave **3d** and **9d** in 17% and 20% yield, respectively. (Scheme 3)



Scheme 2. Reagents and conditions: (a) allylmagnesium bromide (0.2 M in Et_2O) in Et_2O or CH_2Cl_2 at rt. 40 min; (b) 2nd generation Grubbs catalyst in CH_2Cl_2, 90 °C 20 h.



Scheme 3. Reagent and conditions: (a) acetone and *p*-TsOH, rt. 40 h. ; (b) allylmagnesium bromide (0.2 M in Et_2O) in Et_2O or CH_2Cl_2 rt. 40 min; (c) 70% AcOH, reflux 16h.; (d) 2nd generation Grubbs catalyst in CH_2Cl_2, 90 °C 20 h.

The plant growth promoting activities of the synthetic compounds were evaluated using rice lamina inclination bioassay with distilled water as a control and co-evaluated with 28-homocastasterone. Table 1 and graphically Figure 2 are shown the rice lamina inclination of the new synthetic compounds at a 10 ng/plant dose with the best result from 28-homocastasterone (**3**). It can be clearly noted that the 2α, 3α-OH groups and *22R* and *23R*-OH groups have significant importance for bioactivity (compounds **3a**, **10**, and **11**). The 6α-allylated, 6β-OH analogue (**3c**) did not show high sensitivity to the rice lamina inclination assay, confirming the carbonyl group is essential for activity [6, 17]. This might be due to the absence of the hydrogen bond formation between the 6β-OH group and the amino acids in brassinosteroid receptors [18]. The dimeric analogue (**3d**) increased slightly in bioactivity compared to the 6-allyl steroid (**3c**) but this compound was only 9.2% less active than that of the 28-homocastasterone (**3**). On the other hand, the dimeric analogues of **8** and **9** showed the increase in bioactivity than their monomers. These might be due to the binding sites of the dimeric molecules to other amino acids in the brassinosteroid receptors.

Table 1. Rice lamina inclination assay at a concentration of 10 ng/plant of 28-homocastasterone and the new analogs. Mean values from 15-20 replicates ± standard error.

Compounds	Average angle (°)
distilled water	15 ± 3
3	130 ± 8
3a	78 ± 9
3b	68 ± 5
3c	105 ± 5
3d	118 ± 6
8	17 ± 3
8a	23 ± 5
8b	20 ± 6
9	42 ± 7
9a	15 ± 2
9b	17 ± 2
9c	35 ± 3
9d	32 ± 4

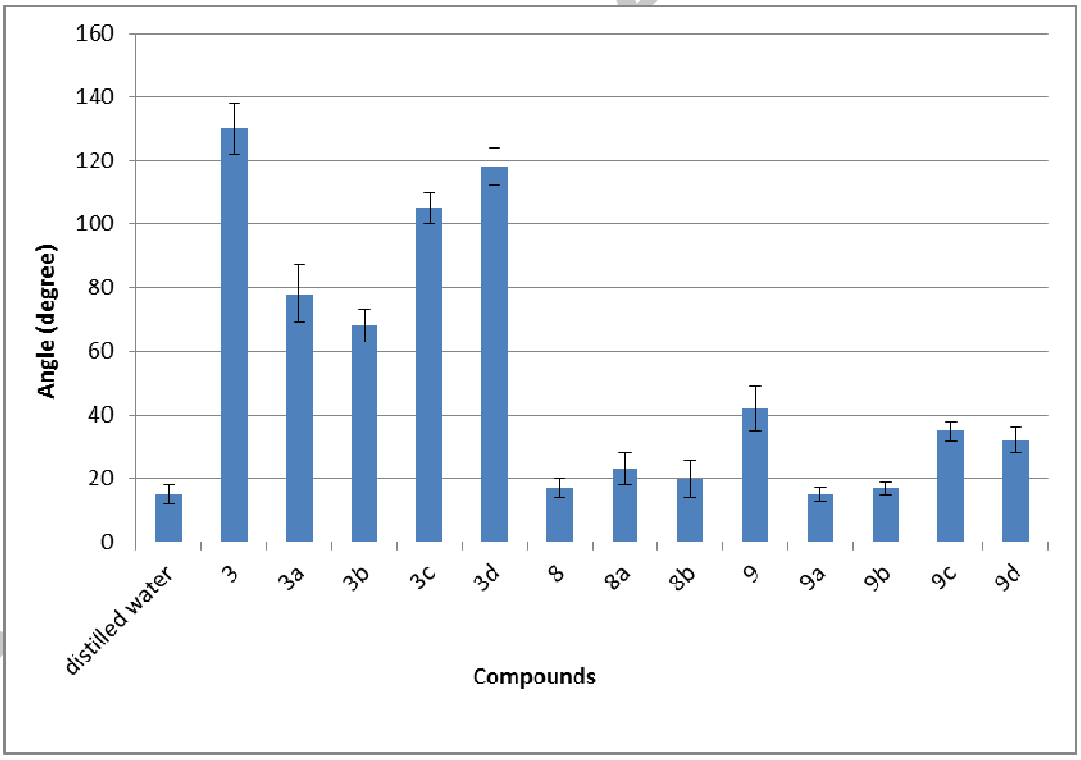


Figure 2. Rice lamina inclination assay of the new synthetic compounds (values are angles in degrees) between the lamina and sheath, representing the means of 15-20 replicates±standard error.

3. Conclusion

The 28-homocastasterone (**3**), and analogues including their dimeric compounds were prepared from stigmasterol (**4**) *via* a key intermediate olefin (**7**). Evaluation of the rice lamina inclination bioassay of the synthesized compounds showed they have lower bioactivity than 28-homocastasterone. However, the novel molecules can be further investigated for other properties, such as persistence, and the results give design considerations for molecules with structures recognized by brassinosteroid receptors.

4. Experimental

4.1 General Methods:

General: Melting points were determined with a Stuart Scientific SMP 2 melting point apparatus and are uncorrected. Infrared spectra were recorded on CH_2Cl_2-film with a Perkin Elmer Spectrum GX FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with a Bruker Avance 300 spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C) using TMS as an internal standard. Optical rotations were measured on JASCO P-1010 digital polarimeter. Mass spectra were recorded with a POLARIS Q or HEWLETT PACKARD 5973 mass spectrometer. Reactions were monitored by TLC using aluminium or plastic sheets precoated with silica gel 60 F_{254}. Column chromatography was performed with Kieselgel 60. (*22E,24S*)-3α,5-cyclo-stigmast-22-en-6-one (**5**) was prepared in 3 steps from commercial stigmasterol (**4**).[5]

4.2 Rice lamina inclination assay

Rice seedlings were washed in distilled water and then left in water at 30 ^{o}C for 24 hrs. Germinated seeds were cultivated on agar under the same growing conditions for 7-8 days before each of the tested compounds was applied to the top portion of the lamina with a micropipette (1 µL/plant). Distilled water was used as a control. After incubation at the same growth condition for 2 more days the rice lamination were obtained and the angle of lamina inclination between the leaf and the sheath was measured.

4.3 Synthesis

*(22E)-3β-bromo-5α-stigmasta-22-en-6-one (**6**)*

To a solution of compound 5 (0.50 g, 0.12 mmol) in AcOH (3 mL) was added 48% HBr (15 mL). The reaction mixture was stirred for 1 h, diluted with water (15 mL) and extracted with $CHCl_3$ (3x15 mL). The organic layers were combined and washed with sat. $NaHCO_3$ (20 mL)

and brine (20 mL). Evaporation of the solvent and purification of the crude product by column chromatography (silica gel, 2:1 hexane/EtOAc) yielded 80% of the product (**6**). Mp. 144-146 ^{o}C; lit. 140-141 ^{o}C [11]. ^{1}H NMR ($CDC1_3$): δ 5.10 (2H, m, 22-H and 23-H), 3.94 (1H, m, 3α-H), 2.40-1.10 (25H, m), 1.30-1.00 (6H, m), 0.98-0.78 (9H, m), 0.70 (3H, s). ^{13}C NMR ($CDC1_3$): δ 209.8 (CO), 137.9 (C-22), 129.6 (C-23), 59.0, 56.8, 55.9, 53.9, 51.3, 50.6, 46.6, 42.9, 40.7, 40.4, 39.3, 39.2, 37.8, 33.4, 32.4, 31.9, 28.7, 25.4, 24.0, 21.3, 21.2, 21.1, 19.0, 13.1, 12.3, 12.2. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{29}H_{48}BrO$: 491.2883, found: 491.2894.

*(22E,24S)-5α-stigmasta-2,22-diene-6-one (**7**)*

A solution of (*22E*)-3β-bromosigmasta-22-en-6-one (**6**) (0.30 g, 0.60 mmol) in DMF (5 mL) was slowly added Li_2CO_3 (44 mg, 0.60 mmol). The reaction mixture was heated under reflux for 4 h. Evaporation of the solvent under reduced pressure gave the residue, water (15 mL) was added and extracted with CH_2Cl_2 (3x15 mL) . The organic extracts were combined, dried with anh. Na_2SO_4 and concentrated to give the crude product which was purified by column chromatography (silica gel, 2:1 hexane/EtOAc). The compound **7** was obtained in 38% yield. Mp. 119-120 ^{o}C; lit. 119-120 ^{o}C [5]. ^{1}H NMR ($CDCl_3$): 5.60 (2H, m, 2-H and 3-H), 5.10 (2H, m, 22-H and 23-H), 2.40-1.40 (28H, m), 1.00-0.78 (8H, m), 0.77 (3H, s), 0.75 (3H, s). ^{13}C NMR ($CDC1_3$): 211.8 (CO), 138.0 (C-22), 129.5 (C-23), 124.5 and 125.0 (C-2 and C-3), 56.9, 55.9, 53.8, 53.5, 51.3, 47.0, 42.7, 40.4, 40.0, 39.4, 39.3, 37.7, 31.9, 28.7, 25.4, 24.0, 21.7, 21.4, 21.2, 21.1, 19.0, 13.5, 12.3, 12.1. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{29}H_{47}O$: 411.3621, found: 411.3627.

Osium-catalysed asymmetric dihydroxylation

A mixture of (*22E,24S*)-5α-stigma-2,22-diene-6-one (**7**) (0.200 g, 0.490 mmol), $K_3Fe(CN)_6$ (0.968 g, 2.94 mmol), anh. K_2CO_3 (0.406 g, 2.94 mmol), methanesulfonamide (0.093 g, 0.98 mmol), dihydroquinidine 4-chlorobenzoate (0.046 g, 0.98 mmol) and OsO_4 (0.25 ml 2.5% in BuOH, 0.020 mmol) in *t*-BuOH : H_2O (1:1, 30 mL) was stirred at room temperature for 10 days. Sodium sulfite (0.50 g) was added, and the mixture was stirred at room temperature for 18 h. *t*-BuOH was removed under reduced pressure, and the residue was extracted with EtOAc (3x20 mL). The combined organic extracts were washed with water (20 mL), 0.25 M H_2SO_4 (20 mL), brine (20 mL), dried, concentrated *in vacuo* to give a pale colorless oil which was purified by flash column chromatography (silica gel, 2:1 hexane/EtOAc) to give the first

fraction of (*22E,24S*)-2α,3α-dihydroxy-5α-stigmast-22-en-6-one (**8**), the second fraction of (*22S,23S*)-28-homocastasterone (**9**) and the last fraction of 28-homocastasterone (**3**).

*(22E,24S)-2α,3α-dihydroxy-5α-stigmast-22-en-6-one (**8**)* as a white solid (0.035 g, 16.1%), Mp. 235-237 °C; lit. 237-239 [17]. $[\alpha]^{28}$ = -6.7 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.15 (dd, *J* = 15.1, 8.4 Hz, 1H, H-22), 5.02 (dd, *J* = 15.2, 8.3 Hz, 1H, H-23), 4.05 (br s, 1H, H-3), 3.77 (d, *J* = 10.8 Hz, 1H, H-2), 2.68 (dd, *J* = 12.6, 3.4 Hz, 1H, H-5), 2.30 (dd, *J* = 13.1, 4.6 Hz, 1H, H-7a), 2.20-1.10 (m, 23H), 1.02 (d, *J* = 6.6 Hz, 3H, H-21), 0.84 (d, *J* = 6.6 Hz, 3H, H-26), 0.81 (t, *J* = 7.6 Hz, 3H, H-29), 0.79 (d, *J* = 6.8 Hz, 3H, H-27), 0.76 (s, 3H, H-19), 0.68 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.0 (CO), 138.0 (C-22), 129.6 (C-23), 68.4, 68.3, 56.8, 55.9, 53.8, 51.2, 50.7, 46.8, 42.8, 42.6, 40.4, 40.3, 39.3, 37.7, 31.9, 28.7, 26.3, 25.4, 24.0, 21.2, 21.1, 21.05, 19.0, 13.6, 12.2 (2). HRES-MS *m/z* calcd for $[M+H]^+$ $C_{29}H_{48}O_3$: 445.3667, found: 445.3676.

*(22S,23S)-28-Homocastasterone (**9**)* (0.040 g, 17.1%), as a white solid. Mp. 207-208 °C; lit. 206-208 °C [17]. $[\alpha]^{28}$ = -2.1 (*c* = 1.52, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (br s, 1H,), 3.83 – 3.71 (m, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.68 (dd, *J* = 12.4, 3.1 Hz, 1H, H-5), 2.30 (dd, *J* = 13.0, 4.4 Hz, 1H), 1.04 (d, *J* = 6.8 Hz, 3H, H-21), 0.96 (t, *J* = 7.5 Hz, 3H, H-29), 0.95 (d, *J* = 7.2 Hz, 3H, H-26), 0.88 (d, *J* = 6.9 Hz, 3H, H-27), 0.76 (s, 3H, H-19), 0.70 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.5 (CO), 72.1 and 70.5 (C-22 and C-23), 68.3 and 68.2 (C-2 and C-3), 56.2, 53.6, 52.5, 50.8, 49.6, 46.7, 43.5, 42.5, 42.2, 40.1, 39.4, 37.6, 27.8, 26.8, 26.3, 24.2, 21.8, 21.2, 18.5, 17.7, 14.5, 14.2, 13.5, 11.9. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{29}H_{51}O_3$: 479.3737, found: 479.3733.

*(22R,23R)-28-Homocastasterone (**3**)* (0.030 g, 12.8%), as a white solid. Mp. 254-256 °C; lit. 253-256 °C [21]. $[\alpha]^{28}$ = -9.3 (*c* = 0.13, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.04 (d, *J* = 2.5 Hz, 1H), 3.80-3.73 (m, 1H), 3.72 (d, *J* = 8.5 Hz, 1H), 3.59 (d, *J* = 8.7 Hz, 1H), 2.69 (dd, *J* = 12.5, 2.9 Hz, 1H), 2.31 (dd, *J* = 13.0, 4.4 Hz, 1H), 0.97 (d, *J* = 6.7 Hz, 3H, H-26), 0.96-0.92 (m, 6H, H-27, H-29), 0.92 (d, *J* = 6.2 Hz, 3H, H-21), 0.76 (s, 3H, H-19), 0.68 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.0 (CO), 74.5 and 72.8 (C-22 and C-23), 68.4 and 68.3 (C-2 and C-3), 56.6, 53.7, 52.5, 50.7, 46.7, 46.4, 42.8, 42.6, 40.6, 39.5, 37.7, 37.0, 28.9, 27.6, 26.3, 23.8, 21.2, 19.4, 18.8, 13.6, 13.5, 11.9x2. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{29}H_{51}O_3$: 479.3737, found: 479.3733.

General procedure for the protection of diols

To a solution of compounds **3** or **9** (1 equiv.) in acetone was added *p*-TsOH (2.5 mol%) and stirred at room temperature for 40 h. The solvent was removed by evaporatation and the residue was dissolved in dichloromethane. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give a brown oil. Purification by column chromatography gave the acetonide **(3a, 9a).**

*(22R,23R,24S)-2α,3α,22,23-Tetrahydroxy-5α, stigmastan-6-one 2,3-acetonide (**3a**)*

Prepared from compound **3** (60.0 mg, 0.12 mmol) in acetone (5.0 mL), after the protection reaction compound **3a** was obtained as a white solid (27.0 mg, 43.3%) with recovery of some compound **3** (25.0 mg, 41.7%) R*f* 0.19 (hexane/EtOAc, 1:1). Mp. 248-250 ^{o}C. IR (cm^{-1}): 3054, 2986, 1706, 1421, 1262. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (s, 1H, H-3), 3.85-3.66 (m, 3H, H-2, H-22, H-23), 2.69 (d, *J* = 10.5 Hz, 1H, H-5), 2.30 (dd, *J* = 13.0, 4.3 Hz, 1H, H-7a), 1.35 (s, 6H, CH_3x2), 0.98 (d, *J* = 6.4 Hz, 3H, H-26), 0.96 (d, *J* = 6.8 Hz, 3H, H-27), 0.91 (d, *J* = 6.8 Hz, 3H, H-21), 0.90 (t, *J* = 6.8 Hz, 3H, H-29), 0.76 (s, 3H, H-19), 0.67 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.1 (CO), 107.5 (C-acetal), 80.3, 77.3, 68.4, 68.3, 56.5, 53.7, 53.4, 50.7, 46.8, 46.7, 42.9, 42.6, 40.3, 39.2, 37.8, 35.7, 28.9, 27.8, 27.4, 27.2, 26.4, 23.8, 21.2, 20.2, 19.6, 19.2, 14.2, 13.6, 12.6, 11.8. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{32}H_{55}O_5$: 519.4044, found: 519.4039.

*(22S,23S,24S)-2α,3α,22,23-Tetrahydoxy-5α-stigmastan-6-one 2,3:22,23-diacetonide (**9a**)*

Prepared from compound **11** (60.0 mg, 0.12 mmol) in acetone (5.0 mL), after the protection reaction compound **11a** was obtained as a white solid (60.0 mg, 85.0%). Mp. 186-187 ^{o}C. R*f* 0.79 (hexane/EtOAc, 1:1). IR (cm^{-1}): 2952, 2872, 1711, 1462, 1378, 1367, 1241, 1055. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.20 (br s, 1H, H-3), 4.07-3.98 (m, 1H, H-2), 3.92 (dd, *J* = 8.5, 1.8 Hz, 1H, H-22), 3.83 (dd, *J* = 8.5, 3.2 Hz, 1H, H-23), 2.46 (dd, *J* = 12.4, 4.0 Hz, 1H, H-5), 2.25 (dd, *J* = 12.9, 4.3 Hz, 1H, H-7a), 1.42 (s, 3H, CH_3), 1.30 (s, 6H, CH_3x2), 1.27 (s, 3H, CH_3), 0.96 (d, *J* = 6.8 Hz, 3H, H-21), 0.90 (t, *J* = 6.5 Hz, 3H, H-29), 0.89 (d, *J* = 5.6 Hz, 3H, H-26), 0.88 (d, *J* = 6.9 Hz, 3H, H-27), 0.61 (s, 3H, H-19), 0.60 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.3 (CO), 106.9 and 105.7 (C-acetals), 78.7, 76.3, 71.3, 71.1, 55.3, 52.3, 51.8, 50.5, 45.8, 45.5, 42.5, 41.4, 40.1, 38.3, 37.3, 36.5, 27.6, 27.0, 26.5, 26.2, 25.8, 25.5, 23.2, 22.4, 21.5, 20.1, 18.6, 17.6, 13.0, 12.3, 11.6, 10.5. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{35}H_{59}O_5$: 559.4357, found: 559.4346.

General procedure for the Grignard reaction

To a solution of allylmagnesium bromide (0.2 M in Et_2O, 5 equiv.) at 0 °C was added a solution of ketone-brassinosteroids **(8, 3a, 19a)** (1 equiv.) in Et_2O or CH_2Cl_2. The mixture was stirred at room temperature for 40 min and quenched with saturated aqueous NH_4Cl. The solvent was evaporated and the residue was dissolved in dichloromethane. The combined organic extracts were washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give a pale colorless oil which was prified by column chromatography giving the allyl products **(8a, 3b, 9b).**

*(22E,24S)-6α-Allyl-2α,3α,6β-trihydroxy-5α-sitostane (**8a**)*

Prepared from compound **8** (80.0 mg, 0.18 mmol) in CH_2Cl_2 (10.0 mL), after the Grignard reaction compound **8a** was obtained as a white wax like-solid (88.0 mg, quantitative yield). $[\alpha]^{28}$ = -20.4 (*c* = 1.77, $CHCl_3$). R*f* 0.35 (hexane/EtOAc, 1:1). IR (cm^{-1}): 3054, 2987, 1421, 1265, 896. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.89-5.72 (m, 1H, H-2′), 5.26-4.91 (m, 4H, H-22, H-23, H-3′), 4.08 (br s, 1H, H-3), 3.81 (br s, 1H, H-2), 2.40-1.05 (m), 1.04 (s, 3H, H-19), 1.03 (d, *J* = 6.6 Hz, 3H, H-21), 0.86 (d, *J* = 6.4 Hz, 3H, H-26), 0.82 (t, *J* = 6.6 Hz, 3H, H-29), 0.81 (d, *J* = 6.6 Hz, 3H, H-27), 0.72 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.2 (C-22), 133.9 (C-2′), 129.4 (C-23), 118.6 (C-3′), 73.2 (C-6), 69.1, 68.7, 56.1, 56.0, 53.8, 51.2, 46.9, 42.8, 42.6, 42.5, 40.5, 39.7, 37.6, 31.9, 30.8, 29.7, 28.9, 26.5, 25.4, 24.3, 21.2, 21.1, 20.7, 19.0, 15.8, 12.3, 12.2. HRES-MS *m/z* calcd for $[M+Cl]^+$ $C_{32}H_{54}ClO_3$: 521.3761, found: 521.3758.

*(22R,23R,24S)- 6α-Allyl-2α,3α,6β,22,23-pentahydroxy-5α-sitostane 2,3-acetonide (**3b**)*

Prepared from compound **3a** (52.0 mg, 0.10 mmol) in CH_2Cl_2 (5.0 mL), after the Grignard reaction compound **3b** was obtained as a white solid (23.0 mg, 43%) and with recovery of compound **3a** (20.0 mg, 36%). Mp. 109-110 °C. R*f* 0.24 (hexane/EtOAc, 1:1). IR (cm^{-1}): 3419, 2942, 1377, 1235. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90-5.71 (m, 1H, H-2′), 5.13 (d, *J* = 8.0 Hz, 1H, H-3′a), 5.09 (d, *J* = 15.3 Hz, 1H, H-3′b), 4.06 (br s,1H, H-2), 3.83-3.76 (m, 3H, H-2, H-22, H-23) 1.35 (s, 6H, CH_3x2), 1.03 (s, 3H, H-19), 0.98-0.95 (m, 6H), 0.92-0.88 (m, 6H), 0.69 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.9 (C-2), 118.7 (C-3′), 107.4, 80.4, 77.3, 73.2, 69.1, 68.6, 55.8, 53.8, 53.5, 46.9, 46.6, 43.3, 42.8, 42.5, 39.6, 37.6, 35.7, 30.9, 28.9, 28.0, 27.4, 27.2, 26.5, 24.1, 20.8, 20.2, 19.5, 19.2, 15.8, 14.1, 12.6, 11.8. HRES-MS m/z calcd for $[M+H]^+$ $C_{35}H_{61}O_5$: 561.4514, found: 561.4516.

*(22S,23S,24S)-6α-Allyl-2α,3α,6β,22,23-pentahydoxy-5α-stigmastan-6-one 2,3:22,23-diacetonide (**9b**)*

Prepared from compound **9a** (40.0 mg, 0.072 mmol) in Et_2O (5.0 mL), after the Grignard reaction compound **9b** was obtained as a white solid (43.0 mg, quantitative yield). Mp. 97-99 ^oC. R*f* 0.83 (hexane/EtOAc, 1:1). IR (cm^{-1}): 2986, 2873, 2305, 1421, 1379, 1264, 1047, 895. ^1H NMR (300 MHz, $CDCl_3$) δ 5.91-5.74 (m, 1H, H-2′), 5.14 (d, *J* = 8.1 Hz, 1H, H-3′a), 5.10 (d, *J* = 15.8 Hz, 1H, H-3′b), 4.31 (t, *J* = 3.9 Hz, 1H, H-3), 4.16-4.05 (m, 1H, H-2), 4.00 (dd, *J* = 8.5, 1.6 Hz, 1H, H-22), 3.93 (dd, *J* = 8.6, 3.0 Hz, 1H, H-23), 1.48 (s, 3H, CH_3), 1.38 (s, 6H, CH_3x2), 1.35 (s, 3H, CH_3), 1.04 (d, *J* = 6.9 Hz, 3H, H-21), 1.00-0.94 (m, 9H, H-26, H-27, H-29), 0.93 (s, 3H, H-19), 0.71 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.7 (C-2′), 118.9 (C-3′), 107.4, 106.6, 79.6, 77.2, 73.0, 72.8x2, 55.7, 53.3, 52.8, 46.4, 44.2, 43.4, 43.2x2, 39.8, 38.4, 37.2, 30.4, 29.7, 28.6, 28.3, 27.5, 27.3, 26.8, 26.6, 24.4, 23.2, 23.1, 20.8, 19.6, 18.6, 14.6, 13.9, 13.3, 11.5. HRES-MS *m/z* calcd for $[M+H]^+$ $C_{38}H_{65}O_5$: 601.4827, found: 601.4819.

General procedure for the deprotection of acetals

A solution of allyl **(3b, 9b)** (0.10 mmol) in 70% AcOH (5.0 mL) was heated at 90 °C for 16 h. After cooling, ice-water was added, the solid filtered, then washed with cold water and dried, giving the tetraol **(3c, 9c)**.

*(22R,23R,24S)- 6α-Allyl-2α,3α,6β,22,23-pentahydroxy-5α-sitostane **(3c)***

Prepared from compound **3b** (55.0 mg, 0.10 mmol), after the deprotection reaction compound **3c** was obtained as a white solid (30.0 mg, 57%). Mp. 106-107 ^oC. $[\alpha]^{28}$ = +3.8 (*c* = 0.52, $CHCl_3$). R*f* 0.61 (hexane/EtOAc, 1:20). IR (cm^{-1}): 3419, 2935, 2871, 1462, 1381, 1043. ^1H NMR (300 MHz, $CDCl_3$) δ 5.88-5.75 (m, 1H, H-2′), 5.13 (d, *J* = 7.6 Hz, 1H, H-3′a), 5.09 (d, *J* = 15.4 Hz, 1H, H-3′b), 4.07 (br s, 1H, H-3), 3.80 (d, *J* = 11.5 Hz, 1H, H-2), 3.72 (d, *J* = 8.4 Hz, 1H, H-23), 3.61 (d, *J* = 8.5 Hz, 1H, H-22), 1.03 (s, 3H, H-19), 1.00-0.86 (m, 12H), 0.70 (s, 3H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.8 (C-2′), 118.6 (C-3′), 74.8, 73.2, 72.8, 69.1, 55.9, 53.7, 52.6, 46.9, 46.3, 43.3, 42.8, 42.7, 42.4, 39.9, 37.6, 37.0, 30.9, 29.7, 28.9, 27.8, 26.5, 24.1, 21.2, 20.8, 19.5, 18.9, 15.8, 13.5, 11.9. HRES-MS *m/z* calcd for $[M+NH_4]^+$ $C_{32}H_{60}NO_5$: 538.4466, found: 538.4456.

*(22S,23S,24S)-6α-Allyl-2α,3α,6β,22,23-pentahydoxy-5α-stigmastan-6-one (**11c**)*

Prepared from compound **11b** (60.0 mg, 0.10 mmol), after the deprotection reaction compound **11c** was obtained as a white solid (35.0 mg, 67%). Mp. 127-129 °C. $[\alpha]^{28}$ = -4.7 (*c* = 1.52, $CHCl_3$). R*f* 0.66 (hexane/EtOAc, 1:20). IR (cm^{-1}): 3410, 2986, 2305, 1421, 1264, 1156, 896. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.88-5.72 (m, 1H, H-2′), 5.12 (d, *J* = 6.2 Hz, 1H, H-3′a), 5.08 (d, *J* = 15.0 Hz, 1H, H-3′b), 4.06 (br s, 1H, H-3), 3.85 – 3.75 (m, 1H, H-2), 3.60-3.59 (m, 2H, H-22, H-23), 1.03 (s, 3H, H-18), 1.02 (d, *J* = 6.2 Hz, 3H, H-21), 0.96 (t, *J* = 6.7 Hz, 3H, H-29), 0.94 (d, *J* = 7.3 Hz, 3H, H-26), 0.88 (d, *J* = 6.8 Hz, 3H, H-27), 0.72 (s, 3H, H-19). ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.9 (C-2′), 118.6 (C-3′), 73.2, 72.2, 70.6 69.1, 68.6, 55.7, 53.8, 52.7, 49.7, 46.9, 43.4, 43.1, 42.8, 42.5, 42.4, 39.8, 37.6, 30.8, 28.0, 26.9, 26.5, 24.4, 21.8, 20.7, 18.6, 17.7, 15.7, 14.4, 14.1, 12.0. HRES-MS *m/z* calcd for $[M+NH_4]^+$ $C_{32}H_{60}NO_5$: 538.4466, found: 538.4456.

General procedure for dimerization

To a solution of allyl-brassinosteroids **(8a, 3c, 9c)** (1 equiv.) in anh. CH_2Cl_2 was added 2nd generation Grubbs catalyst 2.5 %mol at the room temperature under argon. The mixture was heated under pressured tube at 90 °C for 20 h and purified by column chromatography giving the dimer products **(8b, 3d, 9d)**.

*Dimeric compound of **8a***

Prepared from compound **8a** (50.0 mg, 0.10 mmol) in CH_2Cl_2 (10.0 mL) following the general procedure, compound **8b** was obtained as a white solid (12.0 mg, 13 %) with recovery of compound **8a** (27.0 mg, 55.5 %). Mp. 155-157 °C. $[\alpha]^{28}$ = -65.6 (*c* = 0.52, $CHCl_3$). R*f* 0.40 (hexane/EtOAc, 1:20). IR (cm^{-1}): 3410, 2987, 1421, 1265, 896. ^{1}H NMR (300 MHz, $CDCl_3$-MeOD) δ 5.43 (br s, 2H, H-2′), 5.17 (dd, *J* = 15.1, 8.4 Hz, 2H, H-22), 5.02 (dd, *J* = 15.1, 8.4 Hz, 2H, H-23), 4.01 (br s, 2H, H-2), 3.73 (d, *J* = 10.6 Hz, 2H, H-3), 2.27 (d, *J* = 10.2 Hz, 2H, H-5), 1.03 (d, *J* = 6.4 Hz, 6H, H-21), 1.02 (s, 6H, H-19), 0.85 (d, *J* = 6.4 Hz, 6H, H-26), 0.81 (t, *J* = 7.4 Hz, 6H, H-29), 0.80 (d, *J* = 6.7 Hz, 6H, H-27), 0.71 (s, 6H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$-MeOD) δ 138.2 (C-22), 129.3 (C-2′), 129.2 (C-23), 73.7 (C-6), 69.0 (C-2), 68.3 (C-3), 56.2, 56.1, 54.0, 51.2, 45.1, 43.1, 42.5, 42.4, 41.5, 40.4, 39.8, 37.4, 31.8, 30.8, 28.8, 26.4, 25.3, 24.4, 21.1, 21.0, 20.7, 18.9, 15.7, 12.2, 12.1. HRES-MS *m/z* calcd for $[M+Cl]^+$ $C_{62}H_{104}ClO_6$: 979.7521, found: 979.7516.

*Dimeric compound of **3c***

Prepared from compound **3c** (100.0 mg, 0.20 mmol) in CH_2Cl_2 (10.0 mL) following the general procedure, compound **3d** was obtained as a white solid (35 mg, 17 %) with recovery of compound **3c** (52.0 mg, 52 %). Mp. 212-214 ^oC. $[\alpha]^{28}$ = +22.0 (*c* = 0.55, $CHCl_3$). R*f* 0.21 (hexane/EtOAc, 1:20). IR (cm^{-1}): 3419, 3056, 2986, 2842, 1650, 1404, 1016. ^1H NMR (300 MHz, $CDCl_3$-MeOD) δ 5.48 (br s, 2H, H-2'), 4.02 (br s, 2H, H-3), 3.78 (br s, 2H, H-2), 3.69 (d, *J* = 8.7 Hz, 2H, H-23), 3.54 (d, *J* = 8.5 Hz, 2H, H-22), 1.01 (s, 6H, H-19), 0.99-0.84 (m, 24H), 0.70 (s, 6H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$-MeOD) δ 129.9 (C-2′), 77.3, 74.5, 74.4, 72.2, 68.7, 68.3, 56.7, 54.0, 52.6, 46.2, 45.1, 42.8, 42.0, 41.1, 39.9, 37.1, 30.7, 29.6, 29.0, 27.7, 26.2, 23.9, 21.1, 20.7, 19.4, 18.8, 15.6, 13.5, 11.9, 11.8. HRES-MS *m/z* calcd for $[M+Na]^+$ $C_{62}H_{108}O_{10}Na$: 1035.7835, found: 1035.7825.

*Dimeric compound of **8c***

Prepared from compound **8c** (100.0 mg, 0.20 mmol) in CH_2Cl_2 (10.0 mL) following the general procedure, compound **8d** was obtained as a white solid (40 mg, 20 %) with recovery of compound **8c** (55.0 mg, 55.5 %). Mp. 182-184 ^oC. $[\alpha]^{28}$ = -76.5 (*c* = 0.48, $CHCl_3$). R*f* 0.24 (hexane/EtOAc, 1:20). IR (cm^{-1}): 3410, 2949, 2839, 1650, 1404, 1016. ^1H NMR (300 MHz, $CDCl_3$-MeOD) δ 5.43 (br s, 2H, H-2'), 4.00 (br s, 2H, H-3), 3.74-3.70 (m, 2H, H-2), 3.52-3.49 (m, 4H, H-22, H-23), 1.02 (s, 6H, H-19), 1.00-0.93 (m, 18H, H-21, H-26, H-29), 0.94 (d, *J* = 7.1 Hz, 6H), 0.86 (d, *J* = 6.8 Hz, 6H, H-27), 0.73 (s, 6H, H-18). ^{13}C NMR (75 MHz, $CDCl_3$-MeOD) δ 133.2 (C-2′), 77.8, 76.4, 74.3, 72.9, 72.1, 59.8, 57.9, 56.5, 53.0, 49.1, 47.0, 46.7, 46.4, 46.2, 45.2, 43.8, 41.2, 34.7, 32.1, 30.7, 30.4, 28.4, 25.4, 24.6, 22.6, 21.2, 19.6, 18.4, 18.2, 15.7. HRES-MS *m/z* calcd for $[M+Na]^+$ $C_{62}H_{108}O_{10}Na$: 1035.7835, found: 1035.7818.

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Graphic Abstract

Synthesis and Preliminary Evaluation of Dimeric-28-homobrassinosteroids for Plant Growth Regulators

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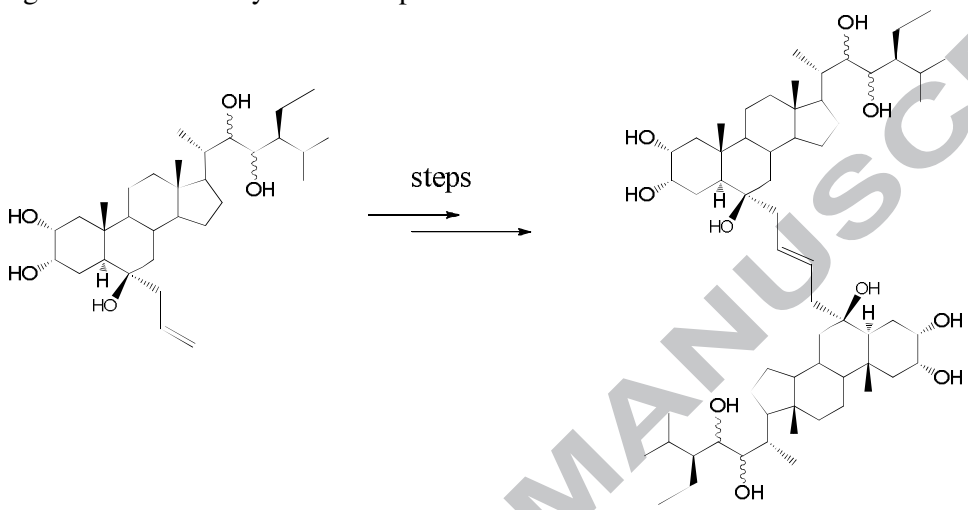
The synthesis and bioactivity of the synthetic analogues of 28-homobrassinosteroids including the dimeric compounds of 28-homocastasterone were studied. Evaluation of the rice lamina inclination activity revealed that the dimeric analogues possessed a reduced but significant bioactivity when compared to the nature 28-homocastasterone.



Research Highlights

-Preparation of (22R, 23R)- and (22S, 23S)-homocastasterone are reported.
-Nucleophilic additions of C6 carbonyl group by allylic Grignard reagent were designed.
-Olefin metathesis of the allyl analogues using Grubbs catalyst resulted in the dimeric analogues.
-Rice lamina inclination assay of the dimeric analogues showed the significant activity compared to the natural 28-homocastasterone.