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Synthesis of 26,27-bisnorcastasterone analogs and analysis of conformation–activity relationship for brassinolide-like activity

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Abstract—Three castasterone (CS) derivatives with varied side-chain moieties, 26,27-bisnorcastasterone (20*S*-bisnorCS), 20-*epi*-26,27-bisnorcastasterone (20*R*-bisnorCS), and 21,26,27-trisnorcastasterone (trisnorCS), were synthesized stereoselectively from either stigmasterol or dehydroisoandrosterone. The 50% effective doses (ED_{50}, nmol/plant) in the concentration–response curve for brassinolide-like activity in the rice lamina inclination assay were determined to be 0.020 nmol (pED_{50} = 10.7) for 20*S*-bisnorCS, 3.2 nmol (pED_{50} = 8.5) for 20*R*-bisnorCS, and 2.0 nmol (pED_{50} = 8.7) for trisnorCS. An analog containing an ester linkage between the steroid and the side-chain moiety of 20*S*-bisnorCS was also synthesized and its activity was evaluated to be 3.2 nmol (pED_{50} = 8.5), being equipotent to 20*R*-bisnorCS and trisnorCS. The activity of 20*S*-bisnorCS was 1/40 that of CS. The conformation analysis was conducted using a systematic search, showing that the activity decreases with an increase in the degree of freedom of the side chain of the steroidal skeleton.

1. Introduction

Brassinolide (BL, **1**; Fig. 1) is a steroid hormone, which is essential for the growth and development of plants, and its structure was determined by Grove and co-workers a quarter century ago.[1] BL has a number of interesting substructures, including a seven-membered ring lactone structure as part of the B-ring moiety and four asymmetric carbons in the side-chain moiety. Various physiological effects on plant growth and improvement of stress tolerance are associated with BL structure. Later, castasterone (CS, **2**; Fig. 1) was isolated in chestnut insect gall by Yokota and co-workers,[2] which is the biosynthetic immediate precursor of BL.[3] Since the discovery of BL, more than 50 BL analogs have been identified in the plant kingdom, and these BL-like compounds are collectively called brassinosteroids (BRs).[4,5]

It is well known that steroid hormones bind to nuclear receptors and the ligand–receptor complexes act on genome in animals, but no nuclear receptor has been identified in plants to date. In plants, a transmembrane receptor kinase, brassinosteroid-insensitive1 (BRI1), functions as a receptor of brassinolide.[6] The binding domain of BRI1 was recently determined in *Arabidopsis* using a biotin-tagged photo-affinity CS.[7] Numerous efforts have also been devoted to the chemical synthesis and structure–activity relationship (SAR) studies of BL analogs to obtain potent compounds and good probes for the function analysis of biosynthetic and metabolic enzymes and receptors.[8–16]

Our group synthesized new BRs containing the ester linkage between the steroid skeleton and the side chain such as compound **3** (BL-ester, Fig. 1) and discussed the structure–activity relationship for the BL-activity of compounds with various side-chain moieties.[15] The activity of ester analogs was drastically enhanced by the introduction of an OH group at the α-position of the ester moiety. The compound with *R*-configuration with respect to the α-carbon was 10 times more potent than the corresponding *S*-form. However, even for the most potent compound, its activity was 1/1000 that of BL. We assumed that the reduction in activity is possibly

Abbreviations: BL, brassinolide; CS, castasterone; ED_{50}, 50% effective dose; BR, brassinosteroid; TsOH, toluenesulfonic acid; BuOH, butanol; HRMS, high-resolution mass spectrum; THF, tetrahydrofuran; DMAP, 4-dimethylaminopyridine; PPTS, pyridinium *p*-toluenesulfonate; PDC, pyridinium dichromate.

Keywords: Brassinosteroids; Castasterone; Rice lamina inclination; Conformation analysis.

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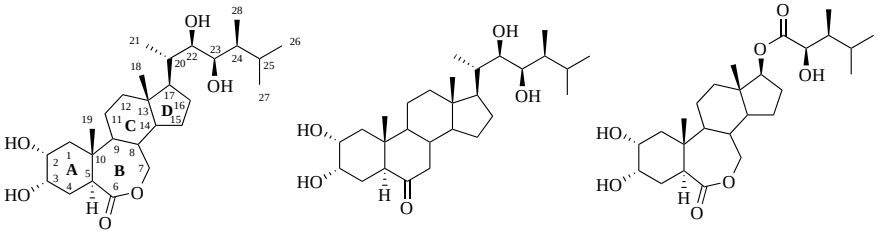


Figure 1. Chemical structures of brassinosteroids.

due to the lack of the substituent at the position corresponding to C21 position of BRs. We also demonstrated that the replacement of the side chain of CS to that of ponasterone A (PonA), which is an insect molting hormone agonist, eliminated the activity, but its molting hormonal activity remained.[17,18] It is also reported that the configurations of C22 and C23 with hydroxyl groups are both *R* for the high BL-like activity.[19]

It is known that steroid hormones and their agonists change the conformation of their receptor proteins, triggering a series of molecular events leading to various biological responses.[20,21] It is generally accepted that ligands with a stable conformation are able to bind tightly to the receptor and induce strong biological activity. To date, the conformational flexibility of the side chain of epimeric vitamins has been examined and the contribution of the conformers was discriminated using the energy window concept.[22,23] Based on this concept, Yamamoto and co-workers studied the relationship between the conformation and function of vitamin D to demonstrate to clarify the conformation of vitamin D responsible for the receptor binding.[24]

In this report, we synthesized both 20*S* (**4**) and 20*R* (**5**) enantiomers of 26,27-bisnorcastasterone analog and 21,26,27-trisnorcastasterone (**6**, Fig. 2) to examine the effect of C21 methyl group on the BL-like activity. Even though it is appropriate to use the BL- or CS-type compounds to examine the effect of C21 methyl group, the construction of the original side chain is not easy as compared with that of 26,27-bisnor type compounds. In compounds **4**–**6**, the carbon at 24-position is not asymmetric anymore, and the decrease in the number of asymmetric carbons dramatically lower the labor of synthetic chemists. The corresponding ester analog **7** with CS-type steroidal skeleton was also synthesized, and its BL-like activity was compared with those of compounds **4**–**6**. In the further study, we performed the conformation analysis for these compounds to discuss the relationship between the conformation and the activity.

2. Materials and methods

2.1. General for chemical synthesis

Reactions requiring anhydrous conditions were conducted under argon atmosphere using oven-dried glass wares. Anhydrous solvents used in this study were either commercially available or prepared conventionally in this laboratory. Chemicals were purchased from Aldrich Chemical Corp. (Milwaukee, WI, USA), Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Nacalai Tesque Inc. (Kyoto, Japan), unless noted. Starting materials in Schemes 1–3 were synthesized previously in our laboratory. Column chromatography was conducted using Kieselgel 60 (Merck, Darmstadt, Germany) as the absorbent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 or ARX-500 NMR spectrometer in deuteriochloroform ($CDCl_3$) with tetramethylsilane as the internal standard. Optical rotations were measured on a JASCO P-1000 polarimeter. High-resolution mass spectrum (HRMS) was recorded on a JEOL JMS 700 spectrometer (Tokyo, Japan). Elemental analyses were performed at the Microanalytical Center of Kyoto University. Melting points were determined with a Yanako melting point apparatus (Yanagimoto Seisakusho Co. Ltd., Kyoto, Japan) and are uncorrected.

2.2. Synthesis of (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-26,27-bisnor-5α-ergostan-6-one (4; Scheme 1)

2.2.1. Ethyl (22*R*,23*S*)-2α,3α-isopropylidenedioxy-22,23-dihydroxy-6-oxo-5α-cholan-24-oate (10). Triethyl phosphonoacetate (11 ml, 55 mmol) was added dropwise to a stirred suspension of NaH (60% oil suspension;

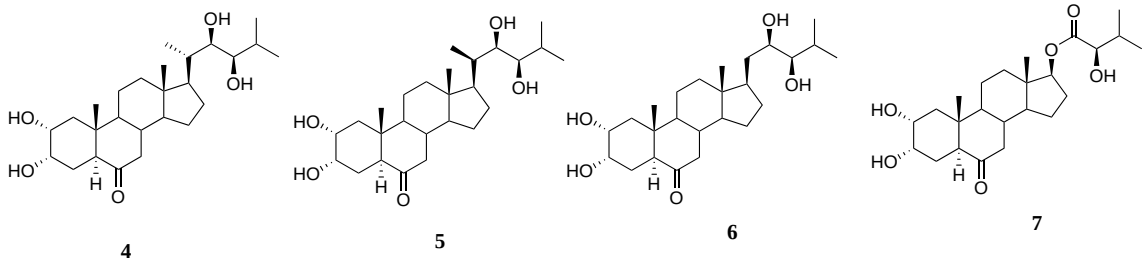
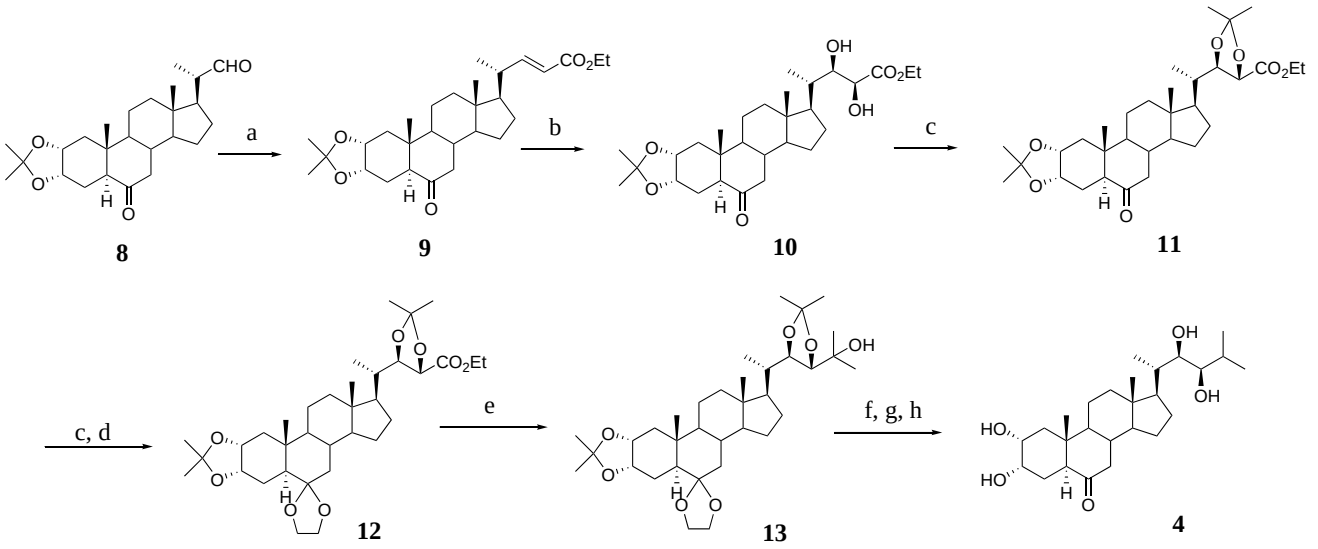
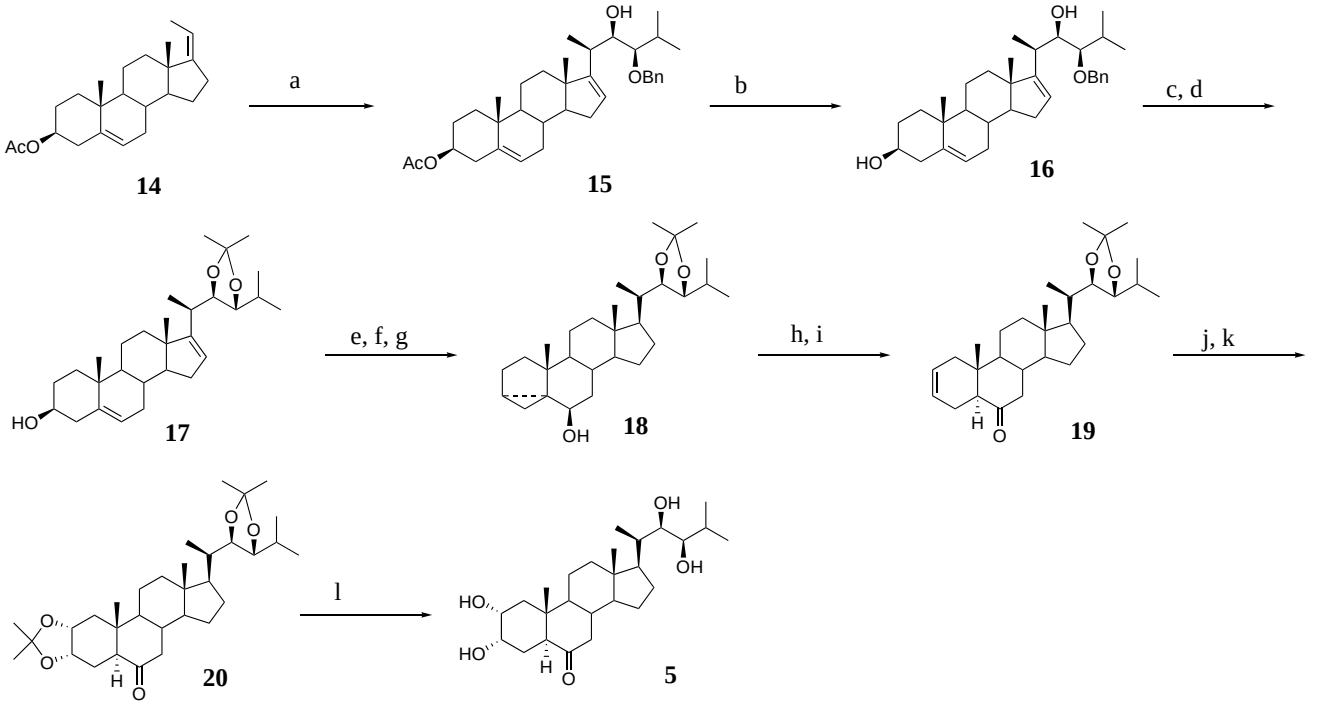


Figure 2. Newly synthesized brassinosteroids.

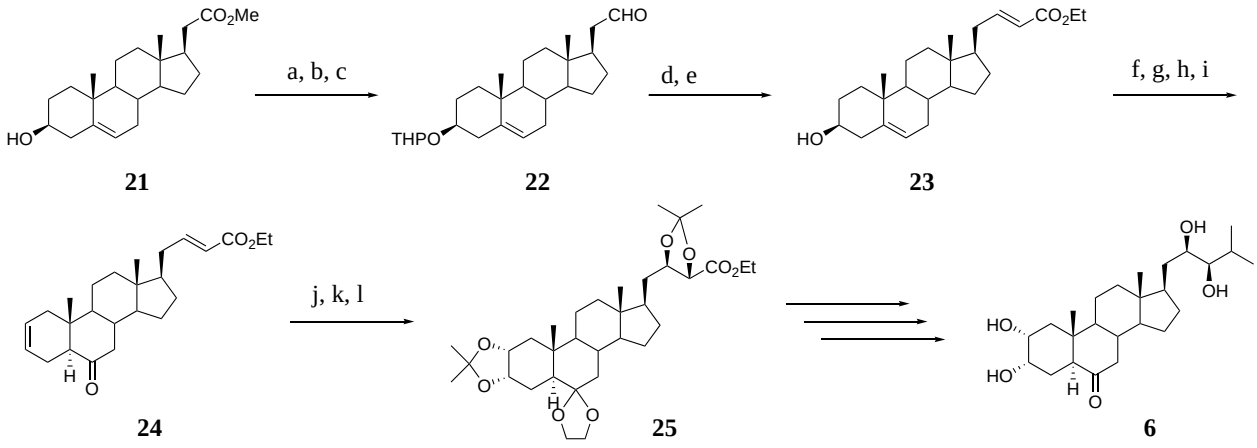


Scheme 1. Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (b) OsO_4, $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, $MeSO_2NH_2$, *t*-BuOH–H_2O; (c) 2,2-dimethoxypropane, *p*-TsOH, $CHCl_3$; (d) 2,2-dimethyl-1,3-dioxolane, *p*-TsOH, reflux; (e) MeLi, THF, −78 °C; (f) MsCl, Et_3N, DMAP, CH_2Cl_2; (g) H_2, Pd/C, EtOH; (h) 70% aq AcOH, reflux.

2.2 g, 55 mmol) in tetrahydrofuran (THF; 80 ml) at 0 °C. After stirring the mixture for 30 min at room temperature, a THF solution of **8** (7.47 g, 18.6 mmol), which is derived from stigmasterol according to the conventional method,[25] was added and stirred for 2 h. Saturated aqueous NH_4Cl (200 ml) was added to the solution and extracted with ethyl acetate (4×100 ml). The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to give a crude yellow solid, which was purified by flash column chromatography (hexane/ethyl acetate = 7:2, v/v) to provide **9** as a colorless solid (7.35 g). To a solution of **9** in a mixture of *t*-BuOH (85 ml) and H_2O (85 ml), dihydroquinidine–phthalazine ligands $(DHQD)_2PHAL$ (1.17 g, 1.5 mmol), $K_3Fe(CN)_6$ (8.69 g, 26 mmol), K_2CO_3 (3.65 g, 26 mmol), 10% (w/v) OsO_4/*t*-BuOH (9 ml, OsO_4: 0.35 mmol), and $CH_3SO_2NH_2$ (1.67 g, 17.6 mmol) were added successively. After stirring overnight at room temperature, Na_2SO_3 (5.0 g, 40 mmol) was added to the reaction mixture, which was then



Scheme 2. Reagents and conditions: (a) (*R*)-2-benzyloxy-3-methylbutanal, $EtAlCl_2$, CH_2Cl_2–hexane; (b) K_2CO_3, MeOH–H_2O; (c) H_2, Pd/C, EtOAc; (d) 2,2-dimethoxypropane, *p*-TsOH, $CHCl_3$; (e) MsCl, Et_3N, DMAP, CH_2Cl_2; (f) $KHCO_3$, acetone–H_2O, reflux; (g) H_2, Pd/C, EtOH; (h) CrO_3, H_2SO_4, acetone; (i) NaBr, *p*-TsOH, DMF, reflux; (j) OsO_4, NMO, THF–H_2O; (k) 2,2-dimethoxypropane, *p*-TsOH, $CHCl_3$; (l) 80% aq AcOH, 60 °C.



Scheme 3. Reagents and conditions: (a) 3,4-dihydro-2*H*-pyran, PPTS, CH_2Cl_2; (b) $LiAlH_4$, THF; (c) PDC, Celite, CH_2Cl_2; (d) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (e) PPTS, EtOH, reflux; (f) MsCl, Et_3N, DMAP, CH_2Cl_2; (g) $KHCO_3$, acetone–H_2O, reflux; (h) CrO_3, H_2SO_4, acetone; (i) NaBr, *p*-TsOH, DMF, reflux; (j) OsO_4, $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, $MeSO_2NH_2$, *t*-BuOH–H_2O; (k) 2,2-dimethoxypropane, *p*-TsOH, $CHCl_3$; (l) 2,2-dimethyl-1,3-dioxolane, *p*-TsOH, reflux.

extracted with $CHCl_3$ (1×150 ml, 3×50 ml). The combined organic layer was washed with 2 M KOH (150 ml) and brine (150 ml), dried over anhydrous $MgSO_4$, and the solvent was evaporated. The residue was purified by flash column chromatography ($CHCl_3/CH_3OH$ = 50:1, v/v) to afford **10** (3.62 g, 68% for two steps) as a colorless solid. Mp: 220 °C. NMR δ_H ($CDCl_3$): 0.66 (3H, s), 0.67 (3H, s), 1.05 (3H, d, J = 6.1), 1.31 (3H, t, J = 7.2), 1.34 (3H, s), 1.51 (3H, s), 2.13 (1H, m), 2.32 (1H, dd, J = 4.3, 13.0), 2.38 (1H, d, J = 6.7), 2.55 (1H, dd, J = 4.0, 13.0), 3.02 (1H, d, J = 5.8), 3.80 (1H, m), 4.07–4.13 (1H, m), 4.20–4.37 (3H, m). NMR δ_C ($CDCl_3$): 11.7, 12.61, 12.64, 14.2, 21.1, 22.5, 23.9, 26.5, 27.7, 28.6, 37.6, 39.3, 39.8, 41.1, 42.5, 42.7, 46.8, 51.5, 52.4, 53.2, 56.4, 62.1, 72.1, 72.3, 73.1, 73.8, 107.9, 173.6, 211.4. $[\alpha]_D^{23}$ +34.0 (*c* 1.13, $CHCl_3$).

2.2.2. Ethyl (22*R*,23*S*)-6,6-ethylenedioxy-2α,3α,22,23-bis(isopropylidenedioxy)-5α-cholan-24-oate (12). To a solution of **10** (3.61 g, 7.1 mmol) in $CHCl_3$ (50 ml) were added 2,2-dimethoxypropane (5 ml) and catalytic amount of *p*-TsOH, and then the mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with $CHCl_3$ (200 ml) and washed with saturated aqueous $NaHCO_3$ (100 ml). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated to afford **11**. Compound **11** was dissolved in 2,2-dimethyl-1,3-dioxolane (10 ml) with catalytic amount of *p*-TsOH. After stirring for 2 h at 90 °C, the mixture was diluted with $CHCl_3$ (200 ml), and washed with saturated aqueous $NaHCO_3$ (100 ml). The organic layer was dried over anhydrous $MgSO_4$, and the solvent was evaporated. The residue was purified by flash column chromatography (hexane/ethyl acetate = 7:2) to provide **12** (3.02 g, 72% for two steps) as colorless solid. Mp 199 °C. NMR δ_H ($CDCl_3$): 0.68 (3 H, s), 0.84 (3H, s), 0.98 (3H, d, J = 6.6), 1.29 (3H, t, J = 7.1), 1.33 (3H, s), 1.40 (3H, s), 1.46 (3H, s), 1.48 (3H, s), 3.72–3.79 (1H, m), 3.87–3.99 (3H, m), 4.06–4.14 (1H, m), 4.18–4.33 (5H, m). NMR δ_C ($CDCl_3$): 11.8, 12.2, 13.4, 14.3, 20.7, 22.0, 24.1, 25.4, 26.6, 26.6, 27.7, 28.6, 32.9, 36.9, 38.0, 39.4, 41.0, 42.5, 42.7, 45.5, 52.9, 53.1, 55.7, 61.2, 64.2, 65.5, 72.8, 72.9, 76.4, 80.9, 107.5, 109.7, 110.9, 171.8. $[\alpha]_D^{23}$ +45.9 (*c* 0.98, $CHCl_3$).

2.2.3. (22*R*,23*S*)-6,6-Ethylenedioxy-2α,3α,22,23-bis(isopropylidenedioxy)-26,27-bisnor-5α-ergostan-24-ol (13). To a solution of **12** (2.00 g, 3.4 mmol) in THF (30 ml) was added dropwise 1.14 M CH_3Li/Et_2O (12 ml, 13.7 mmol) at −78 °C under argon. After 30 min, saturated aqueous NH_4Cl (5 ml) was added dropwise, and the reaction mixture was warmed to room temperature. Saturated aqueous NH_4Cl (100 ml) was added and extracted with ethyl acetate (1×100 ml, 3×50 ml). The combined organic layer was washed with saturated aqueous NaCl (100 ml) and dried over anhydrous $MgSO_4$. After evaporating the solvent, the residue was purified with flash column chromatography (hexane/ethyl acetate = 3:1) to provide **13** (1.37 g, 70%) as a colorless amorphous solid. NMR δ_H ($CDCl_3$): 0.66 (3H, s), 0.83 (3H, s), 1.00 (3H, d, J = 6.1), 1.16 (3H, s), 1.25 (3H, s), 1.33 (3H, s), 1.39 (6H, s), 1.48 (3H, s), 2.08 (1H, s), 3.61 (1H, d, J = 8.6), 3.72–3.78 (1H, m), 3.87–3.99 (3H, m), 4.06–4.13 (2H, m), 4.27 (1H, m). NMR δ_C ($CDCl_3$): 11.8, 12.6, 13.4, 20.8, 22.0, 24.0, 24.7, 26.6, 27.3, 27.5, 27.6, 28.3, 28.6, 33.0, 36.9, 38.0, 39.5, 41.0, 42.4, 42.7, 45.5, 52.9, 53.6, 55.6, 64.2, 65.5, 69.8, 72.8, 73.0, 78.2, 83.1, 107.5, 107.9, 109.7. HR-MS *m*/*z* (M^+) Calcd for $C_{34}H_{56}O_7$: 576.4026. Found: 576.4034. $[\alpha]_D^{22.6}$ +41.0 (*c* 0.13, MeOH).

2.2.4. (22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-26,27-bisnor-5α-ergostan-6-one (4). To a solution of **13** (478 mg, 0.83 mmol), triethylamine (0.58 ml, 4.16 mmol) in CH_2Cl_2 (10 ml) with catalytic amount of 4-dimethylaminopyridine (DMAP) was added methanesulfonyl chloride (0.13 ml, 1.7 mmol) at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was diluted with $CHCl_3$ (30 ml) and washed with saturated aqueous $NaHCO_3$ (30 ml). The aqueous layer was extracted with $CHCl_3$ (3×10 ml), and the combined organic layer was washed with brine (30 ml). After being dried over anhydrous Na_2SO_4, the solvent was evaporated. The residue was purified by flash column chromatography

(hexane/ethyl acetate = 5:1) to afford the olefin compounds (296 mg), a mixture of 23- and 24-ene compounds. This olefin mixture (285 mg) was dissolved in EtOH (2 ml) and hydrogenated with Pd/C to provide a colorless amorphous solid, which was dissolved in 70% aqueous acetic acid (5 ml). After stirring for 5 h at 80 °C, the reaction mixture was concentrated. The residue was azeotropically concentrated with toluene and purified by flash column chromatography ($CHCl_3$/MeOH = 15:1–9:1) to provide **4** (70 mg, 20% for three steps) as a colorless solid. Mp: 291–292 °C (EtOH). (lit.,[26] 290–295) NMR δ_H ($CDCl_3/C_5D_5N$ = 1/1): 0.69 (3H, s), 0.79 (3H, s), 0.98 (3H, d, J = 6.7), 1.06 (3H, d, J = 6.6), 1.10 (3H, d, J = 6.8), 2.29 (1H, dd, J = 4.4, 12.9), 2.89 (1H, dd, J = 2.5, 12.3), 3.56 (1H, dd, J = 2.4, 7.6), 3.72 (1H, d, J = 7.5), 3.86 (1H, m), 4.20 (1H, m). NMR δ_C ($CDCl_3/C_5D_5N$ = 1/1): 12.0, 12.5, 13.6, 15.2, 20.8, 21.3, 23.9, 27.2, 27.8, 29.8, 37.7, 38.2, 39.6, 40.7, 42.5, 42.8, 46.8, 51.1, 52.6, 53.8, 56.6, 68.0, 68.4, 73.4, 76.3, 212.1. HRMS m/z (M^+) Calcd for $C_{26}H_{44}O_5$: 436.3189. Found: 436.3192.

2.3. Synthesis of (20*R*,22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-26,27-bisnor-5α-ergostan-6-one (5; Scheme 2)

2.3.1. (20*R*,22*R*,23*R*)-23-Benzyloxy-26,27-bisnorergost-5,16-diene-3β,22-diol (16). To a solution of compound **14** (1.41 g, 4.1 mmol) prepared according to the conventional method,[27] and (*R*)-2-benzyloxy-3-methyl-butanal (1.05 g, 5.5 mmol) in CH_2Cl_2 (30 ml) was added dropwise 1 M $EtAlCl_2$/hexane (15 ml, 15 mmol) at −78 °C, and the reaction mixture was stirred for 30 min. One molar HCl (10 ml) was added to the mixture and then warmed to room temperature. One molar HCl (150 ml) was added and extracted with CH_2Cl_2 (1 × 50 ml, 4 × 20 ml). The combined extract was washed with brine (80 ml) and dried over anhydrous $MgSO_4$. The solvent was evaporated to afford a yellow oil, which was purified by flash column chromatography (hexane/ethyl acetate = 15:1–10:1) to provide colorless oil (1.27 g) containing **15**. This material was hydrolyzed with K_2CO_3 in CH_3OH/H_2O at 80 °C and then purified by flash column chromatography (hexane/ethyl acetate = 5/2) to afford compound **16** (1.08 g, 53% for two steps) as a colorless amorphous solid. NMR δ_H ($CDCl_3$) δ: 0.80 (3 H, s), 0.97 (3H, d, J = 6.8), 0.98 (3H, d, J = 6.8), 1.05 (3H, s), 1.048 (3H, d, J = 6.9), 2.13 (1H, ddd, J = 3.0, 6.6, 14.9), 2.53 (1H, d, J = 6.9), 3.29 (1H, dd, J = 2.1, 5.8), 4.56 (1H, d, J = 11.1), 4.70 (1H, d, J = 11.1), 5.37 (1H, m), 5.49 (1H, m), 7.36 (5H, m). NMR δ_C ($CDCl_3$): 16.5, 18.3, 18.7, 18.8, 19.3, 20.8, 30.1, 30.5, 31.51, 31.56, 31.62, 34.8, 35.9, 36.7, 37.2, 42.3, 47.5, 50.7, 56.5, 71.7, 73.2, 73.7, 82.0, 121.5, 123.7, 127.7, 127.8, 128.4, 138.5, 141.0, 158.2. $[\alpha]_D^{25}$ +39.7 (c 0.56, $CHCl_3$).

2.3.2. (20*R*,22*R*,23*R*)-2α,3α,22,23-Bis(isopropylidenedioxy)-26,27-bisnor-5α-ergostan-6-one (20). To the solution of **16** (0.98. 1.98 mmol) in ethyl acetate (10 ml) was added 10% Pd/C (Aldrich Co., Degussa type, 200 mg), then the mixture was stirred at room temperature for 30 min under H_2 atmosphere. After filtration of Pd/C, the solvent was evaporated to give the corresponding triol. This triol was dissolved in $CHCl_3$ (10 ml) and then 2,2-dimethoxypropane (2 ml) and catalytic amount of *p*-TsOH were added to this solution. After stirring for 5 min, MeOH (5 ml) was added to the reaction mixture and stirred for further 5 min. The mixture was diluted with $CHCl_3$ (100 ml) and washed with saturated aqueous $NaHCO_3$ (50 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford crude **17** (0.89 g), which was used for the next step without purification.

To a mixture of crude **17** (0.89 g, synthesized from 1.98 mmol of **16**), Et_3N (0.56 ml, 4.0 mmol), and catalytic DMAP in CH_2Cl_2 (10 ml) was added methanesulfonyl chloride (0.23 ml, 3.0 mmol) at 0 °C. After stirring for 30 min at room temperature, the reaction mixture was diluted with $CHCl_3$ (100 ml) and then washed successively with 1 M HCl (50 ml) and saturated aqueous $NaHCO_3$ (50 ml). The organic layer was concentrated to give crude mesylate. The mixture of this mesylate and $KHCO_3$ (0.92 g, 9.2 mmol) in acetone/H_2O (48 ml–12 ml) was stirred for 1.5 h at 75 °C and then concentrated. The precipitate was dissolved in ethyl acetate (60 ml), washed with water (30 ml), and the aqueous layer was extracted with ethyl acetate (3 × 15 ml). The combined organic layer was washed with 0.5 M HCl (50 ml), saturated aqueous $NaHCO_3$ (50 ml), and brine (50 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated to give crude 3α,5-cycloalcohol. This cycloalcohol was dissolved in EtOH (10 ml). To this solution was added 10% Pd/C (0.25 g) and the resulting mixture was stirred overnight at room temperature under H_2 atmosphere. Pd/C was filtered off and the filtrate was concentrated to give crude **18** (0.84 g) as a colorless amorphous solid, which was used for the next step without purification.

Crude compound **19** derived from **18** was converted to **20** as a colorless amorphous solid (overall yield: 9% for nine steps from **16**) according to conventional methods.[28] NMR δ_H ($CDCl_3$): 0.62 (3H, s), 0.68 (3H, s), 0.94 (6H, d, J = 6.9, 2× CH_3), 0.98 (3H, d, J = 6.6), 1.34 (3H, s), 1.36 (3H, s), 1.38 (3H, s), 1.50 (3H, s), 2.33 (1H, dd, J = 4.3, 12.9), 2.55 (1H, dd, J = 4.0, 12.4), 3.54 (1H, dd, J = 6.0, 6.5), 3.64 (1H, dd, J = 4.8, 6.6), 4.06 – 4.13 (1H, m), 4.28 (1H, m). NMR δ_C ($CDCl_3$): 12.7, 13.2, 15.8, 17.6, 20.0, 20.8, 22.4, 22.5, 24.0, 26.5, 27.4, 27.7, 28.6, 31.8, 33.8, 37.2, 37.4, 41.1, 42.5, 43.3, 46.9, 48.9, 51.5, 53.8, 55.6, 72.1, 72.3, 84.3, 84.4, 107.9, 108.0, 211.4. $[\alpha]_D^{25}$ +26.0 (c 0.37, $CHCl_3$). HRMS m/z (M^+) Calcd for $C_{32}H_{52}O_5$: 516.3815. Found: 516.3802.

2.3.3. (20*R*,22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-26,27-bisnor-5α-ergostan-6-one (5). Compound **20** (44 mg, 84 μmol) was dissolved in 80% aqueous acetic acid (5 ml) and stirred for 6 h at 60 °C. The reaction mixture was concentrated and azeotropically concentrated with toluene. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 20:1) to provide **5** (21.7 mg, 59%) as a colorless amorphous solid. NMR δ_H ($CDCl_3$): 0.65 (3H, s), 0.76 (3H, s), 0.93 (3H, d, J = 7.5), 0.96 (3H, d, J = 7.1), 0.97 (3H, d, J = 6.7), 2.31 (1H, dd, J = 4.5, 13.6), 2.69 (1H, dd, J = 3.1, 12.5), 3.36 (1H, m), 3.43 (1H, m), 3.78 (1H, m), 4.05

(1H, m). NMR δ_C ($CDCl_3$): 13.2, 13.6, 14.6, 17.4, 19.7, 21.0, 23.2, 24.0, 26.4, 31.4, 35.1, 37.7 (2× C), 40.1, 42.7, 43.5, 46.8, 49.9, 50.9, 54.2, 55.9, 68.1, 68.2, 74.9, 75.3, 213.1. HRMS *m/z* (M^+) Calcd for $C_{26}H_{44}O_5$: 436.3189. Found: 436.3178.

2.4. Synthesis of (22*R*,23*R*)-2α,3α,22,23-tetrahydro-21,26,27-trisnor-5α-ergostan-6-one (6; Scheme 3)

2.4.1. 3β-Tetrahydropyranyloxypregn-5-en-21-al (22). To a solution of **21**[29] (10.61 g, 30.6 mmol) in CH_2Cl_2 were added 3,4-dihydro-2*H*-pyran (3.1 ml, 34 mmol) and catalytic amount of pyridinium *p*-toluenesulfonate (PPTS), and stirred overnight at room temperature. The reaction mixture was diluted with $CHCl_3$ (200 ml) and washed with saturated aqueous $NaHCO_3$ (100 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the crude THP ether. The residue was dissolved in THF (100 ml), and $LiAlH_4$ (2.90 g, 76.4 mmol) was added to this solution at 0 °C. After stirring for 1 h at room temperature, water (2.9 ml), 10% aqueous NaOH (2.9 ml), and water (8.7 ml) were added dropwise at 0 °C, successively. The precipitate was filtered off, and the filtrate was concentrated to afford the crude 21-hydroxy compound. This alcohol was dissolved in CH_2Cl_2, and Celite® (10 g) and pyridinium dichromate (PDC; 17.0 g, 45 mmol) were added. After stirring for 2 h, *i*-PrOH (10 ml) was added, and the precipitate was filtered off. The filtrate was washed with 10% aqueous NaOH (100 ml) and brine (100 ml). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (hexane/ethyl acetate = 10:1) to provide **22** (4.86 g, 40% for three steps) as a colorless solid. NMR δ_H ($CDCl_3$): 0.62 (3H, s), 1.02 (3H, s), 2.24 (1H, ddd, J = 2.3, 9.6, 15.7), 2.50 (1H, ddd, J = 2.3, 4.5, 15.7), 3.45–3.58 (2H, m), 3.88–3.94 (1H, m), 4.72 (1H, m), 5.35 (1H, m), 9.77 (1H, t, J = 2.3).

2.4.2. Ethyl (22*E*)-3β-hydroxy-21-norchol-5,22-diene-24-oate (23). To a stirred suspension of NaH (60% oil suspension; 0.97 g, 24 mmol) in THF was added dropwise triethyl phosphonoacetate (4.8 ml, 24 mmol). After 10 min, a solution of **22** (4.86 g, 12.1 mmol) in THF was added and stirred for 3 h. Saturated aqueous NH_4Cl (100 ml) was added to the solution and extracted with ethyl acetate (5 × 50 ml). The combined extract was washed with brine (100 ml), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexane/ethyl acetate = 10:1) to afford the 5,22-dien. This diene was dissolved in EtOH (50 ml), and PPTS (0.30 g) was added to this solution. After stirring for 1 h at 80 °C, the reaction mixture was concentrated to the oil, which was dissolved with $CHCl_3$ (200 ml) and washed with saturated aqueous $NaHCO_3$ (100 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexane/Ethyl acetate = 2:1) to provide **23** (3.93 g, 85% for two steps) as a colorless solid. NMR δ_H ($CDCl_3$): 0.63 (3H, s), 1.02 (3H, s), 1.28 (3H, t, J = 7.1), 3.52 (1H, m), 4.18 (2H, q, J = 7.1), 5.35 (1H, m), 5.81 (1H, d, J = 15.6), 6.95 (1H, dt, J = 15.5, 7.4). NMR δ_C ($CDCl_3$): 12.4, 14.3, 19.4, 20.8, 24.6, 28.2, 31.6, 31.9, 33.4, 36.6, 37.3, 37.7, 42.1, 42.3, 49.6, 50.4, 55.9, 60.1, 71.7, 121.3, 121.6, 140.8, 149.4, 166.8. $[\alpha]_D^{24}$ −71.7 (*c* 0.26, $CHCl_3$). HRMS *m/z* (M^+) Calcd for $C_{25}H_{38}O_3$: 386.2821. Found: 386.2827.

2.4.3. Ethyl (22*E*)-21-nor-5α-chol-2,22-dien-24-oate (24). Compound **24** was synthesized according to the similar method as shown in Scheme 2. NMR δ_H ($CDCl_3$): 0.63 (3H, s), 0.72 (3H, s), 1.29 (3H, t, J = 7.1), 4.18 (2H, q, J = 7.1), 5.57 (1H, m), 5.69 (1H, m), 5.82 (1H, dt, J = 15.6, 1.4), 6.94 (1H, dt, J = 7.4, 15.5). NMR δ_C ($CDCl_3$): 12.4, 13.5, 14.3, 20.8, 21.7, 24.2, 28.1, 33.4, 37.4, 37.7, 39.4, 40.0, 42.6, 47.0, 49.6, 53.6, 53.8, 55.9, 60.1, 121.5, 124.5, 124.9, 149.0, 166.7, 211.8. $[\alpha]_D^{24}$ +13.2 (*c* 0.78, $CHCl_3$).

2.4.4. Ethyl (22*R*,23*R*)-6,6-ethylenedioxy-2α,3α,22,23-bis(isopropylidenedioxy)-21-nor-5α-cholan-24-oate (25). To a mixture of **24** (2.30 g, 6.0 mmol), $(DHQD)_2PHAL$ (0.94 g, 1.2 mmol), $K_3Fe(CN)_6$ (9.88 g, 30 mmol), and K_2CO_3 (4.15 g, 30 mmol) were added 10% (w/v) OsO_4/*t*-BuOH (7.6 ml, OsO_4: 0.30 mmol), *t*-BuOH (60 ml), H_2O (60 ml), and $CH_3SO_2NH_2$ (1.71 g, 18 mmol), successively. After stirring for 2 days at room temperature, Na_2SO_3 (8.0 g, 64 mmol) was added to the reaction mixture, which was then extracted with $CHCl_3$ (1 × 200 ml, 3 × 100 ml). The combined extract was washed with 2 M KOH (150 ml) and brine (150 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the crude 2,3,22,23-tetraol (3.29 g). This tetraol compound (3.19 g) was dissolved in $CHCl_3$ (30 ml), and 2,2-dimethoxypropane (10 ml) and catalytic amount of *p*-TsOH were added to the solution. After 3 h, the reaction mixture was diluted with $CHCl_3$ (100 ml) and washed with saturated aqueous $NaHCO_3$ (50 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexane/ethyl acetate = 3:1) to afford the bisacetonide, which was protected by 2,2-dimethyl-1,3-dioxolane (7 ml) with catalytic amount of *p*-TsOH, in a similar manner to that used for the preparation of **12** in Scheme 1, to give the pure colorless solid **25** (0.83 g, 37% for three steps). Mp: 220 °C. NMR δ_H ($CDCl_3$): 0.59 (3H, s), 0.85 (3H, s), 1.30 (3H, t, J = 7.1), 1.33 (3H, s), 1.43 (3H, s), 1.46 (3H, s), 1.48 (3H, s), 3.73–3.77 (1H, m), 3.88–3.97 (3H, m), 4.07–4.14 (3H, m), 4.21–4.28 (3H, m). NMR δ_C ($CDCl_3$): 12.5, 13.4, 14.2, 20.5, 22.0, 24.6, 25.8, 26.6, 27.3, 27.8, 28.6, 32.9, 34.3, 37.1, 38.1, 41.2, 42.1, 42.7, 45.6, 46.6, 53.4, 55.0, 61.2, 64.2, 65.5, 72.8, 73.0, 78.2, 79.5, 107.6, 109.6, 110.8, 171.0. $[\alpha]_D^{24}$ +57.3 (*c* 1.04, $CHCl_3$). Anal. Calcd for $C_{33}H_{52}O_8$: C, 68.72; H, 9.09. Found: C, 68.68; H, 8.80.

2.4.5. (22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-21,26,27-trisnor-5α-ergostan-6-one (6). The conversion of compound **25** to compound **6** was done in a similar manner to that used for the preparation of **4** from **13** in Scheme 1. Colorless amorphous solid (31% for four steps). NMR δ_H ($CDCl_3$): 0.59 (3H, s, 18-H), 0.77 (3H, s, 19-H), 0.93 (3H, d, J = 6.8, 25-H or 28-H), 0.97 (3H, d, J = 6.8, 28-H or 25-H), 1.35 (1H, dt, J = 3.5, 12.7, 11-H), 1.43 (1H, dt, J = 3.7, 12.3, 9-H), 1.53–1.59 (2H, m, 1-H and 20-H), 1.92 (1H, td, J = 3.4, 15.2, 16-H), 1.95–1.98 (1H, m,

4-H), 2.03 (1H, t, J = 12.8, 7-H), 2.26 (3H, br s, 2-OH, 22-OH, and 23-OH), 2.32 (1H, dd, J = 4.5, 13.0), 7-H), 2.49 (1H, br s, 3-OH), 2. 70 (1H, dd, J = 3.0, 12.6, 5-OH), 3.12 (1H, m, 23-OH), 3.58 (1H, m, 22-H), 3.76 (1H, m, 2-H), 4.04 (1H, m, 3-H). NMR δ_C ($CDCl_3$): 12.7, 13.6, 16.7, 19.8, 21.0, 24.4, 26.3, 27.8, 30.0, 34.3, 37.0, 37.7, 40.3, 42.5, 42.6, 46.2, 46.8, 50.8, 54.2, 55.9, 68.3, 68.4, 70.5, 80.0, 212.1. HRMS m/z (M^+) Calcd for $C_{25}H_{42}O_5$: 422.3032. Found: 422.3045.

2.4.6. 2α,3α-Dihydroxy-17β-[(2*R*,3*S*)-2-hydroxy-3-methylpentanoyl]oxy-5α-androstan-6-one (7). Compound **7** was synthesized according to the previously reported method.[15] Mp: 235–237 °C. NMR δ_H ($CDCl_3$): 0.77 (3H, s), 0.81 (3H, s), 0.87 (3H, d, J = 6.9), 1.02 (3H, d, J = 6.9), 2.32 (1H, dd, J = 13.0, 4.4), 2.71 (1H, dd, J = 12.7, 3.0), 2.74 (1H, d, J = 6.1), 3.77 (1H, m), 4.03–4.06 (2H, m), 4.71 (1H, dd, J = 9.0, 7.7). NMR δ_C ($CDCl_3$): 12.11, 13.58, 16.60, 18.69, 20.67, 23.28, 26.25, 27.36, 32.18, 36.30, 37.33, 40.15, 42.49, 43.08, 46.13, 50.71, 50.91, 53.59, 68.20, 68.29, 74.82, 83.64, 174.92, 211.36. $[\alpha]_D^{23}$ −2.0 (c 0.93, $CHCl_3$). Anal. Calcd for $C_{24}H_{38}O_6$: C, 68.22; H, 9.06. Found: C, 67.95; H, 8.87.

2.5. Computations

All computations such as constructions of structures, structural optimization, and conformational analysis were executed using SYBYL ver. 6.91 (Tripos Co., St. Louis, MO, USA). Analog structures were constructed by modifying the structure of (22*S*, 23*S*)-homobrassinolide whose X-ray crystallographic data are available in Cambridge Database.[30] The side-chain moiety of optimized (22*S*,23*S*)-28-homobrassinolide was replaced with each of compounds **4**–**7**, and each structure was fully optimized by the semi-empirical molecular orbital method, PM3 in MOPAC 5.0. The optimized structures of the corresponding BL derivatives of **4**–**7** are shown in Figure 3. Conformation analyses were executed by a SYBYL module, Systematic Search. To execute the systematic search, each molecule was minimized by molecular mechanics using the 'Powell method' with SYBYL default parameter. The mobility of the side chain of synthetic analogs was examined using the SYBYL systematic search, in which C17–C20 bond was rotated 360° with 10° intervals, and each C20–C22, C22–C23, and C23–C24 bond was done 360° with 30° intervals to yield $360/10 \times (360/30)^3 = 62{,}208$ for each compound. From these conformers, infeasible conformations suffering from van der Waals repulsion were eliminated. Systematic searches were also executed for BL, 20-*epi*BL, and 20-norBL, in which the C17–C20 bond was rotated 360° with 10° intervals, and each C20–C22, C22–C23, C23–C24, and C24–C25 bond was done 360° with 30° intervals to yield $360/10 \times (360/30)^4 = 746{,}496$.

3. Result and discussion

3.1. Chemistry

Compound **4** was synthesized according to Zhou et al's method.[31] First, Horner–Wadsworth–Emmons (HWE) reaction between steroidal 22-aldehyde **8** and triethyl phosphonoacetate provided the geometrically pure (*E*)-α,β-unsaturated ester **9**. This ester compound **9** was oxidized to 22*R*,23*S*-diol **10** (68% yield for two steps) by Sharpless asymmetric dihydroxylation reaction using $(DHQD)_2PHAL$ as a chiral catalyst.[32] The 22,23-dihydroxy and 6-carbonyl groups were protected as acetonide and ketal, respectively, giving compound **12** in 72% yield for two steps. Methylation of compound **12** with CH_3Li gave compound **13** in 70% yield, which was then transformed to **4** in three steps.

Next we synthesized **5**, the 20-epimer of **4**, using carbonylene reaction reported by Watanabe et al.[33] in order to examine the effect of configuration of symmetric 20-carbon. Even though 20-*epi*BL and 20-*epi*CS were synthesized by Kametani et al.,[34] their activity values have not been reported and no discussion was made for the effect of C21 methyl group. Steroidal (*Z*)-17(20)-olefin **14** and (*R*)-2-benzyloxy-3-methylbutanal were condensed in the presence of $EtAlCl_2$ to provide **15**. The acetyl group of **15** was removed by alkaline hydrolysis to give **16** in 53% for two steps. Regioselective hydrogenation of $\Delta^{5,16}$-steroidal compound with Pd/C was reported by Hershberg et al.,[35] but it failed for **16**. Instead, deprotection of 23-benzyloxy group without hydrogenation of double bond successfully occurred to give 3,22,23-triol compound, which was then protected with acetonide to provide **17**. Compound **17** was converted to 3α,5-cycloalcohol via mesylation and alkaline hydrolysis, and the following hydrogenation with Pd/C effected the reduction of 16(17)-double bond to afford **18**. Compound **18** was converted to **19** according to the conventional methods in 19% yield from **16**. Dihydroxylation of **19** provided the mixture of 2α,3α- and 2β,3β-diol, which were separated after derivatization to acetonided by column chromatography to provide **20** in 47% yield for two steps. Deprotection of **20** under acidic condition afforded **5** in 59% yield. Hydroxy group of the known steroid **21** was protected as THP ether, then reduction of methoxycarbonyl moiety followed by PDC-oxidation afforded 22-aldehyde **22** in 40% for three steps. The HWE reaction of **22** provided the (*E*)-α,β-unsaturated ester, which was treated with acid to provide **23** in 85% yield for two steps. Steroidal part of **23** was modified by conventional methods to give 2,22-dien **24** in 62% yield for four steps. Asymmetric dihydroxylation of **24** followed by protection of 22,23-diol and 6-ketone provided compound **25**, which was then converted into **6** in the similar manner as that for **4** from **13**.

To compare the activity between the acyl side-chain analogs and alkyl side-chain analogs, we also synthesized the corresponding ester analog **7**. Although we previously prepared some analogs with acyl side chain,[15] their steroid moiety and the length of side chains are different from those synthesized in this study.

3.2. Bioassays

The synthesized analogs were subjected to the rice lamina inclination assay under the synergistic condition

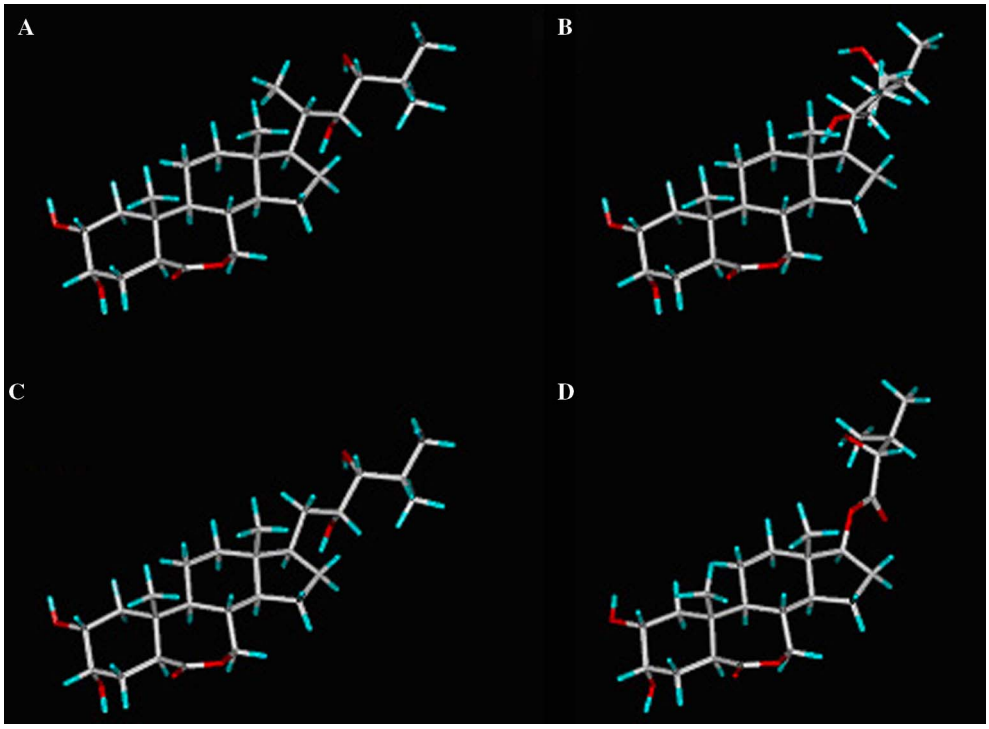


Figure 3. Initial conformations of 26,27-bisnorBL (A), 21-*epi*-25,26-bisnorBL (B), 21,25,26-trisnorBL (C), and compound **3** (D).

with indole-3-acetic acid (IAA). Biological activity was evaluated quantitatively as the reciprocal logarithm of the ED_{50} (ED_{50}: 50% effective dose per plant in moles), pED_{50}, calculated in the same manner as reported by Uesusuki et al.[15] The pED_{50} values of the synthesized compounds are listed in Table 1.

Compound **4** showed the highest activity among compounds tested in this study, but it was about 1/50 of CS (**2**). By removing the C21-methyl group, the activity decreased by 100-fold (**4** vs **6**). This finding is consistent with the consideration by Uesusuki et al.[15] that the existence of the 21-methyl group is important to enhance the activity based on the SAR for a series of alkoxy BL analogs. Strnad and Kohout also synthesized 17-alkoxy analogs of BL lacking C21 methyl group such as 17β-3-methylbutyloxy and 17β-2-methylbutyloxy, and reported that their activity is 100 times less potent than 24-*epi*BL in the bean second internode bioassay.[36] Besides, a remarkable reduction of the activity has been reported for these ether BL analogs lacking C21 methyl group in the bean second internode bioassay. These analogs, however, also lack 22,23-diol that are critically important for the hormonal activity, so the contribution of C21 methyl to the activity was unclear. The low activity of ester analogs is probably due to the lack of C21 substituent like in the case of **6**.

Table 1. Brassinolide-like activity of newly synthesized compounds in the rice lamina inclination assay

Compound	pED_{50} (nmol)
1 (Brassinolide)	13.6[a]
2 (Castasterone)	12.3[a]
3 (BL-ester)	10.1[a]
4	10.7 ± 0.4[b]
5	8.5 ± 0.2[b]
6	8.7 ± 0.2[b]
7	8.5

[a] From Ref. 15.
[b] Means ± standard deviation (n = 2) for **4**–**6**.

The activity of **5** was 100 times lower than that of **4**. Interestingly **5**, **6**, and the corresponding ester analog **7** were equipotent. These facts indicate that C21 methyl group with a proper stereochemistry is important to enhance the activity. It is assumed that C21 methyl group influences on the conformational flexibility of the side chain at C17, as reported for the conformation–function relationship of vitamin D.[37]

3.3. Systematic search

We analyzed the relationship between the hormonal activity of the analogs and the spatial area occupied by C25. The possible conformations of each analog were searched using 'Systematic Search' module with molecular mechanics method. Thirty four possible conformations were obtained for **4**, 40 for **5**, and 385 for **6**. In this search experiment, infeasible conformations suffering from van der Waals repulsion were omitted. The positions of 25-carbon were plotted for all possible conformers, in which steroid moiety was overlapped. One cross sign corresponds to one conformation of the side chain. Figure 4 indicates that the side chains of **4** mainly occupy a certain limited area, but that of **5** occupy two areas. However, in the case of **6**, the corresponding terminal carbon of the side chain covers a wider area than those for **4** and **5** as depicted with green crosses. The wide area is also occupied for ester analog **7** (data not

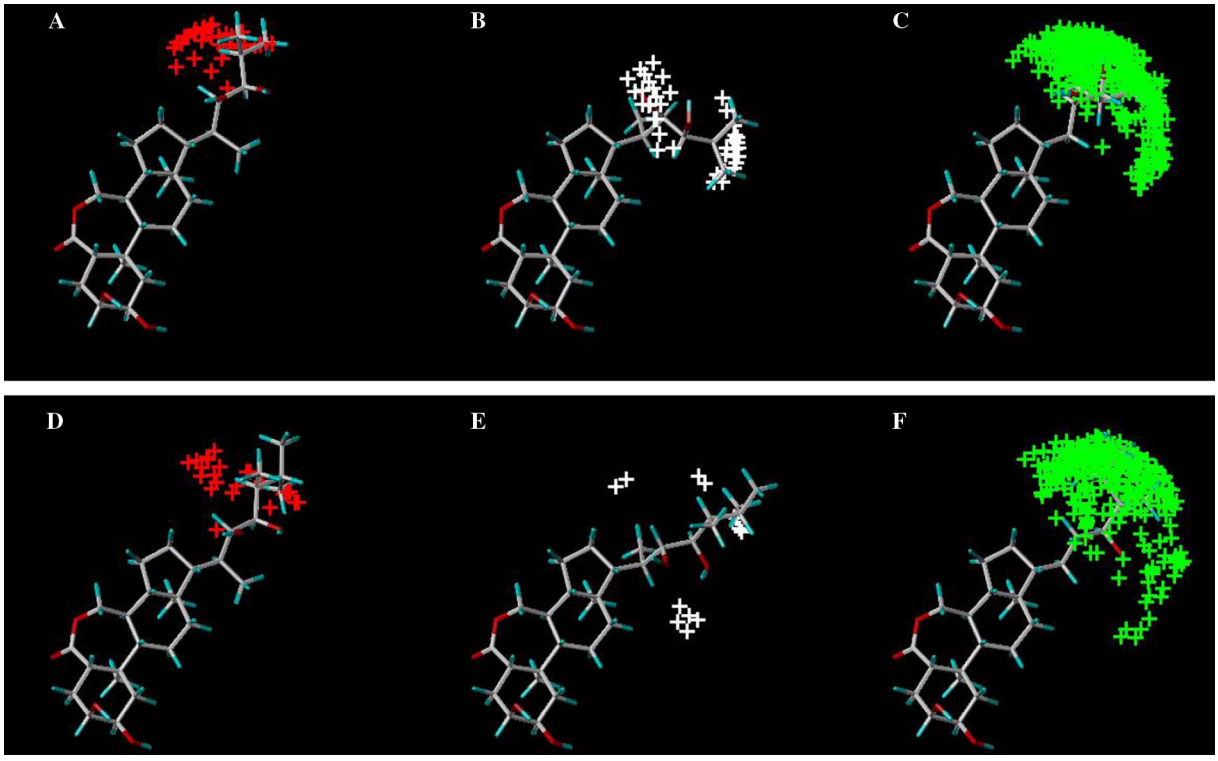


Figure 4. The mobile areas of the side chains are shown by (A) red cross for **4**, (B) white cross for **5**, and (C) green cross for **6**. The mobile areas of the side chain of corresponding BL analogs are also plotted by (D) red cross for BL, (E) white cross for 20-*epi*BL, and (F) green cross for 20-norBL.

shown). The results for the systematic searches for BL and its analogs (20-*epi*BL and 21norBL) gave the similar results as shown in Figure 4D–F, although the number of conformers was smaller than those for **4**–**6** (24 for BL, 13 for 20-*epi*BL, and 274 for 21-norBL). The introduction of a CH_3 group at the C20 position prevents any free rotation with respect to the C17–C20 bond, resulting in the entire molecule having a rigid conformation. Thus, we can assume that the activity increases with the poor conformational flexibility of the side chain. The replacement of side-chain moiety with a benzene structure may decrease the flexibility of side-chain moiety. For the insect steroid hormone, 20-hydroxyecdysone, it is demonstrated that the side-chain moiety of 20-hydroxyecdysone corresponds to the benzoyl moiety of non-steroidal dibenzoylhydrazine-type compounds.[38]

In conclusion, we synthesized three CS analogs **4**, **5**, and **6** as well as the corresponding ester analog **7**. The hormonal activity of both **5** and **6** was 100 times lower than that of **4** which has the same configuration at C20 as natural brassinosteroids. The corresponding ester analog **7** has similar hormonal activity as **5** and **6**. Results of a systematic search for the conformation-activity relationship for these compounds as well as BL analogs (BL, 20-norBL, and 20-*epi*BL) suggested that the hormonal activity decreases with the degree of freedom of the side chain for the steroidal skeleton.

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References and notes

1. Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen, J. D., Jr.; Steffens, G. L.; Flippen-Anderson, J. L.; Cook, J. C., Jr. *Nature* **1979**, *281*, 216.
2. Yokota, T.; Arima, M.; Takahashi, N. *Tetrahedron Lett.* **1982**, *23*, 1275.
3. Bishop, G. J.; Yokota, T. *Plant Cell Physiol.* **2001**, *42*, 114.
4. Fujioka, S. *Brassinosteroids*; Springer: Tokyo, 1999, pp 21.
5. Fujioka, S.; Yokota, T. *Annu. Rev. Plant Biol.* **2003**, *54*, 137.
6. Wang, Z. Y.; Seto, H.; Fujioka, S.; Yoshida, S.; Chory, J. *Nature* **2001**, *410*, 380.
7. Kinoshita, T.; Cano-Delgado, A.; Seto, H.; Hiranuma, S.; Fujioka, S.; Yoshida, S.; Chory, J. *Nature* **2005**, *433*, 167.
8. Luo, W.; Janzen, L.; Pharis, R. P.; Back, T. G. *Phytochemistry* **1998**, *49*, 637.
9. Fujioka, S.; Noguchi, T.; Takatsuto, S.; Yoshida, S. *Phytochemistry* **1998**, *49*, 1841.
10. Brosa, C. *Brassinosteroids*; Springer: Tokyo, 1999, pp 191.
11. Sung, G. C.; Janzen, L.; Pharis, R. P.; Back, T. G. *Phytochemistry* **2000**, *55*, 121.
12. Back, T. G.; Janzen, L.; Nakajima, S. K.; Pharis, R. P. *J. Org. Chem.* **2000**, *65*, 3047.

13. Back, T. G.; Janzen, L.; Pharis, R. P.; Yan, Z. *Phytochemistry* **2002**, *59*, 627.
14. Back, T. G.; Pharis, R. P. *J. Plant Growth Regul.* **2003**, *22*, 350.
15. Uesusuki, S.; Watanabe, B.; Yamamoto, S.; Otsuki, J.; Nakagawa, Y.; Miyagawa, H. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1097.
16. Sisa, M.; Hnilickova, J.; Swaczynova, J.; Kohout, L. *Steroids* **2005**.
17. Watanabe, B.; Nakagawa, Y.; Miyagawa, H. *J. Pestic. Sci.* **2003**, *28*, 188.
18. Watanabe, B.; Nakagawa, Y.; Ogura, T.; Miyagawa, H. *Steroids* **2004**, *69*, 483.
19. Brosa, C.; Capdevila, J. M.; Zamora, I. *Tetrahedron* **1996**, *52*, 2435.
20. Allan, G. F.; Leng, X.; Tsai, S. Y.; Weigel, N. L.; Edwards, D. P.; Tsai, M. J.; O'Malley, B. W. *J. Biol. Chem.* **1992**, *267*, 19513.
21. Beekman, J. M.; Allan, G. F.; Tsai, S. Y.; Tsai, M. J.; O'Malley, B. W. *Mol. Endocrinol.* **1993**, *7*, 1266.
22. Okamura, W. H.; Palenzuela, J. A.; Plumet, J.; Midland, M. M. *J. Cell. Biochem.* **1992**, *49*, 10.
23. Midland, M. M.; Plumet, J.; Okamura, W. H. *Bioorg. Med. Chem.* **1993**, *3*, 1799.
24. Yamamoto, K.; Sun, W. Y.; Ohta, M.; Hamada, K.; DeLuca, H. F.; Yamada, S. *J. Med. Chem.* **1996**, *39*, 2727.
25. McMorris, T. C.; Chavez, R. G.; Patil, P. A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 295.
26. Takatsuto, S.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1984**, 439.
27. Drefahl, G.; Ponsold, K.; Schick, H. *Chem. Ber.* **1965**, *98*, 604.
28. Aburatani, M.; Takeuchi, T.; Mori, K. *Synthesis* **1987**, 181.
29. Zarecki, A.; Wicha, J. *Synthesis* **1996**, 455.
30. Kutschabsky, L.; Adam, G.; Vorbrodt, H.-M. *Z. Chem.* **1990**, 30.
31. Zhou, W.-s.; Jiang, B.; Pan, X.-f. *Tetrahedron* **1990**, *46*, 3173.
32. Sharpless, K. B.; Michaelson, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6136.
33. Watanabe, B.; Yamamoto, S.; Sasaki, K.; Nakagawa, Y.; Miyagawa, H. *Tetrahedron Lett.* **2004**, *45*, 2767.
34. Kametani, T.; Katoh, T.; Fujio, J.; Nogiwa, I.; Tsubuki, M.; Honda, T. *J. Org. Chem.* **1988**, *53*, 1982.
35. Hershberg, E. B.; Oliveto, E. P.; Gerold, C.; Johnson, L. *J. Am. Chem. Soc.* **1951**, *73*, 5073.
36. Strnad, M.; Kohout, L. *J. Plant Growth Regul.* **2003**, *40*, 39.
37. Yamada, S.; Yamamoto, K.; Masuno, H.; Ohta, M. *J. Med. Chem.* **1998**, *41*, 1467.
38. Billas, I. M. L.; Iwema, T.; Garnier, J. M.; Mitschler, A.; Rochel, N.; Moras, D. *Nature* **2003**, *426*, 91.