Concise, Improved Procedure for the Synthesis of Brassinolide and Some Novel Side-Chain Analogues

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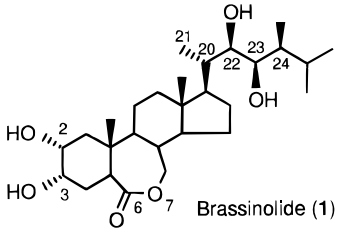
Brassinolide (**1**) is a plant growth promoter that was first isolated in 1979 by workers at the U.S. Department of Agriculture.[1] It exhibits activity at the nanogram/individual plant level and is thus active at concentrations several orders of magnitude lower than other known classes of plant growth promoters. Brassinolide and related brassinosteroids have attracted considerable attention because of reports that they significantly increase the yields of several types of commercially important crops, even at exceptionally low dosage levels.[2] Moreover, recent progress in the elucidation of the role of **1** and related brassinosteroids at the molecular level in signal transduction and the regulation of gene expression in plants has stimulated additional interest.[3] Although brassinolide is relatively ubiquitous in the plant kingdom, its extremely low abundance makes natural sources an impractical source of supply. A number of syntheses of **1** have been reported,[4,5] but they are generally lengthy and expensive. The side chain, with its four contiguous chiral centers and vicinal diol functionality, provides the main challenge in such endeavors. The relative unavailability of **1** has necessitated the use of less potent, but more easily synthesized brassinosteroids, such as 24-epibrassinolide, in the majority of field trials and related biological experiments to date.[6] However, brassinolide remains the most active of the naturally occurring brassinosteroids, thereby providing a strong incentive for the development of improved methods for its synthesis. The preparation of novel analogues is also of importance, both for the purpose of structure−activity studies and for developing compounds with improved or altered biological properties.[7]



Brassinolide (**1**)

We recently reported a new synthesis of brassinolide, in which the side chain was elaborated from the C-22 aldehyde **2** in just three steps.[8] Unfortunately, our original procedure suffered from poor stereoselectivity in the generation of the chiral center at C-22, necessitating a difficult chromatographic separation of the resulting epimers and inconsistent results in the other steps when attempts were made to scale them up. We now report an improved procedure that provides excellent stereoselectivity at C-22 and optimized conditions for the remaining steps that permit the preparation of brassinolide on a multigram scale. The method is also versatile in permitting variations in the structure of the C-25 to C-27 portion of the brassinolide side chain, which is introduced in the penultimate step. This is useful for the preparation of new analogues and for structure−activity studies.[9] To illustrate, we report the preparation of two novel side-chain analogues by this approach.

Results and Discussion

The new synthesis of brassinolide is shown in Scheme 1. The key intermediate aldehyde **2** is readily available from stigmasterol, the principal sterol of soy bean oil, by an eight-step sequence[10] in an overall yield of 42%. Aldehyde **2** was treated with the selenium-stabilized anion **3**[11] to produce a mixture of selenide stereoisomers at C-23, but with no detectable amount (by NMR) of the corresponding undesired C-22 epimer. The high Cram selectivity[12] of this process obviates the need for the difficult separation of the C-22 alcohols. Although the selenide epimers at C-23 could be separated, it proved more convenient to subject the unseparated crude mixture directly to an oxidative workup with hydrogen peroxide, thereby effecting an *in situ* selenoxide *syn* elimination.[13] Thus, both C-23 epimers afforded exclusively the *trans*-allylic alcohol **4**, which was easily purified by chromatography. The anion **3** was conveniently generated by cleavage of the selenoacetal **8** with *n*-

(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 (London)* **1979**, *281*, 216.

(2) *Brassinosteroids: Chemistry, Bioactivity and Applications*; Cutler, H. G., Yokota, T., Adam, G., Eds.; ACS Symposium Series 474; American Chemical Society: Washington, DC, 1991.

(3) For recent examples, see: (a) Li, J.; Nagpal, P.; Vitart, V.; McMorris, T. C.; Chory, J. *Science* **1996**, *272*, 398. (b) Clouse, S. D.; Langford, M.; McMorris, T. C. *Plant Physiol.* **1996**, *111*, 671.

(4) For recent reviews, see: (a) Back, T. G. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1995; Vol. 16, pp 321−364. (b) McMorris, T. C.; Donaubauer, J. R.; Silveira, M. H.; Molinski, T. F. In ref 2; Chapter 4. (c) Khripach, V. A.; Zhabinskii, V. N.; Litvinovskaya, R. P. In ref 2; Chapter 5.

(5) For a more recent synthesis, see: McMorris, T. C.; Chavez, R. G.; Patil, P. A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 295.

(6) Ikekawa, N.; Zhao, Y.-J. In ref 2; Chapter 24.

(7) For lead references to structure−activity studies of brassinosteroids, see: (a) Adam, G.; Marquardt, V.; Vorbrodt, H. M.; Hörhold, C.; Andreas, W.; Gartz, J. In ref 2; Chapter 7. (b) Brosa, C.; Capdevila, J. M.; Zamora, I. *Tetrahedron* **1996**, *52*, 2435.

(8) Back, T. G.; Blazecka, P. G.; Krishna, M. V. *Can. J. Chem.* **1993**, *71*, 156.

(9) One side-chain analogue (25-homobrassinolide) was recently reported to have greater activity than brassinolide itself: Mori, K.; Takeuchi, T. *Liebigs Ann. Chem.* **1988**, 815.

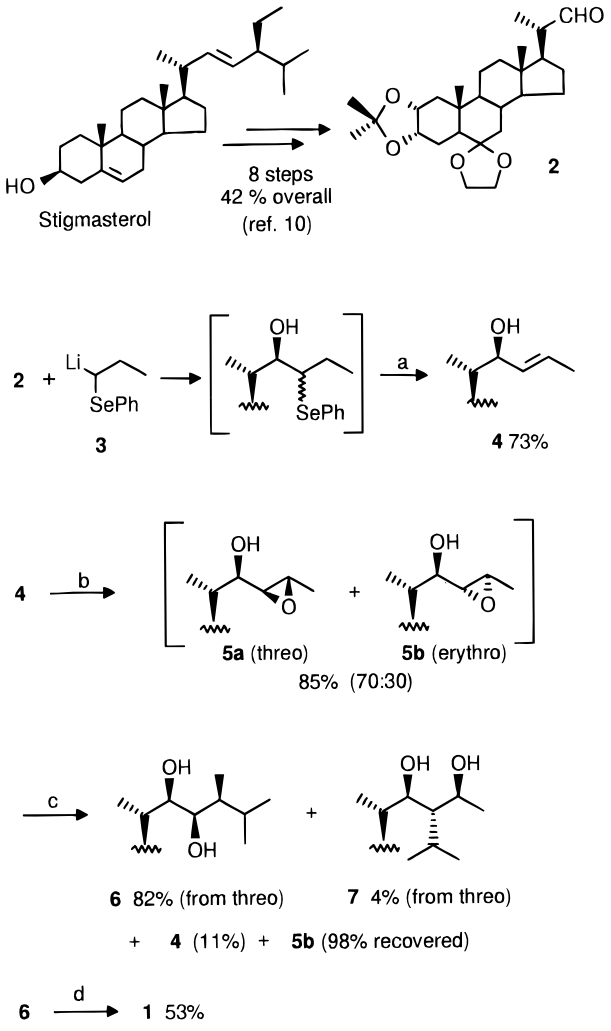
(10) The preparation of aldehyde **2** was achieved by minor variations of procedures reported previously: (a) Aburatani, M.; Takeuchi, T.; Mori, K. *Synthesis* **1987**, 181. (b) Sakakibara, M.; Okada, K.; Ichikawa, Y.; Mori, K. *Heterocycles* **1982**, *17*, 301. The yields obtained in our hands for individual steps are as follows: (i−iii) (ref 10a) tosylation and solvolysis of stigmasterol to the corresponding 6-hydroxycyclosterol, followed by Jones oxidation to the 6-ketone, 83%; (iv) (ref 10a) isomerization of the cyclosterol to the 2-ene, 86%; (v) (ref 10b) *cis*-dihydroxylation to the 2,3-diol, 81%; (vi) (ref 10b) protection of the 2,3-diol as the acetonide, 93%; (vii) (ref 10b) protection of the 6-ketone as the ethylene ketal, 92%; (viii) (ref 10b) ozonolysis with Me_2S workup, 85%.

(11) For reviews of selenium-stabilized carbanions, see: (a) Krief, A. In *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S., Ed.; Wiley: Chichester, 1987; Volume 2, Chapter 17. (b) Reich, H. J. In *Organoselenium Chemistry*; Liotta, D., Ed.; Wiley: New York, 1987; Chapter 5. (c) Paulmier, C. *Selenium Reagents and Intermediates in Organic Synthesis*; Pergamon Press: Oxford, 1986; Chapter 9.

(12) (a) For a discussion of the stereochemistry of additions of nucleophiles to aldehydes, see: Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994; pp 876−888. (b) For an example of a different stereoselective propenylation of a less elaborated steroidal C-22 aldehyde, see: Tsukamoto, M.; Iio, H.; Tokoroyama, T. *Tetrahedron Lett.* **1987**, *28*, 4561.

S0022-3263(96)01854-3 CCC: $14.00

Scheme 1[a]

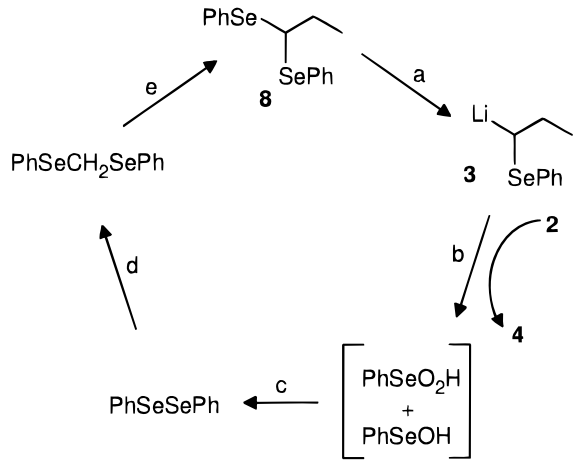


[a] (a) Excess H_2O_2; (b) $Ti(O\text{-}iPr)_4$, L-(+)-diethyl tartrate, cumene hydroperoxide; (c) iPrMgCl, CuCN (cat.), Et_2O; (d) CF_3CO_2H, CF_3CO_3H.

butyllithium.[11] The selenoacetal was in turn easily prepared from the reduction of diphenyl diselenide with sodium borohydride, followed by the reaction of the resulting selenolate with diiodomethane and alkylation of the anion of the product with ethyl iodide. The byproduct of the selenoxide elimination is a disproportionating mixture of diphenyl diselenide, benzeneselenenic acid (PhSeOH), and benzeneseleninic acid ($PhSeO_2H$).[14] Reduction of the reaction mixture with sodium sulfite during workup regenerated diphenyl diselenide, which can be easily recovered and recycled (Scheme 2).

An alternative approach was also investigated, where phenyl *n*-propyl selenide was first oxidized at low temperature to its corresponding selenoxide, followed by deprotonation with LDA and addition to an appropriate C-22 aldehyde.[15] Unfortunately, this procedure afforded lower yields and poorer Cram vs anti-Cram stereoselectivity than the method outlined in Schemes 1 and 2.

Scheme 2[a]



[a] (a) nBuLi; (b) H_2O_2; (c) Na_2SO_3; (d) (1) $NaBH_4$, (2) CH_2I_2; (e) (1) LDA, (2) EtI.

The Sharpless oxidation[16] of **4** gave more consistent results when *tert*-butyl hydroperoxide[8] was replaced by cumene hydroperoxide and the reaction was performed at −20 °C. The unseparated mixture of the resulting *threo*- and *erythro*-epoxides **5a** and **5b** was then treated with isopropylmagnesium chloride in the presence of a catalytic amount of cuprous cyanide in ether to afford the desired diol **6** in 82% yield from the *threo*-epoxide, along with traces of the 1,3-diol regioisomer **7** and the deoxygenated allylic alcohol **4**.[17] Under these conditions, the much less reactive *erythro*-epoxide **5b** can be recovered intact and is easily separated from the more polar diol products. The substantial difference in the reactivity of the two epoxide isomers can therefore be exploited to avoid their difficult chromatographic separation. The completion of the synthesis was achieved in one step by the treatment of **6** with triflouroacetic and trifluoroperoxyacetic acids, resulting in the removal of the protecting groups, followed by Baeyer−Villiger oxidation[18] (Scheme 1).

Since the side-chain portion comprising C-25 to C-27 is introduced in the penultimate step of the synthesis, variation of this moiety is easily accomplished by choice of the appropriate Grignard reagent. Thus, when the mixture of epoxides **5a** and **5b** was subjected to the same protocol with cyclohexyl or *n*-dodecyl Grignard reagents, instead of isopropylmagnesium chloride, the novel side-chain analogues **11** and **12** were similarly obtained via diols **9** and **10**, respectively (Scheme 3).

This route permits the preparation of brassinolide in 8% overall yield from commercially available stigmasterol

(13) Selenoxide *syn* eliminations proceed with high regioselectivity away from oxygen substituents and generally favor the *trans* isomer when the product is a 1,2-disubstituted olefin. See (a) ref 11c, Chapter 5. (b) Back, T. G. In *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S., Ed.; Wiley: Chichester, 1987; Volume 2, Chapter 3.

(14) (a) Hori, T.; Sharpless, K. B. *J. Org. Chem.* **1978**, *43*, 1689. (b) Reich, H. J.; Wollowitz, S.; Trend, J. E.; Chow, F.; Wendelborn, D. F. *J. Org. Chem.* **1978**, *43*, 1697.

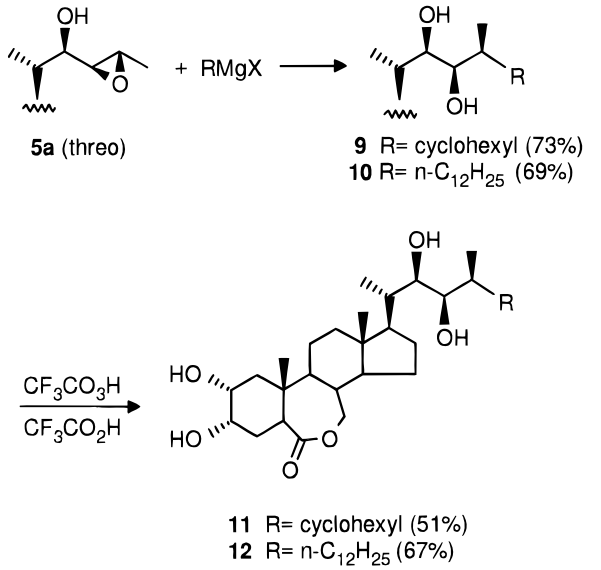
(15) Attempts to deprotonate phenyl *n*-propyl selenide directly were unsuccessful, necessitating the prior formation of the more acidic selenoxide. See: Reich, H. J.; Shah, S. K.; Chow, F. *J. Am. Chem. Soc.* **1979**, *101*, 6648.

(16) (a) Sharpless, K. B.; Verhoeven, T. R. *Aldrichimica Acta* **1979**, *12*, 63. (b) Johnson, R. A.; Sharpless, K. B. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, 1991; Volume 7, Chapter 3.2. (c) Rossiter, B. E. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: Orlando, FL, 1985; Vol. 5, Chapter 7. (d) Finn, M. G.; Sharpless, K. B. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: Orlando, FL, 1985; Vol. 5, Chapter 8.

(17) When the reaction was run under more concentrated conditions, substantial amounts of the allylic alcohol **4** were also produced, resulting in poorer yields of **6**.

(18) Trifluoroperoxyacetic acid is the reagent of choice for the Baeyer−Villiger oxidation of many 6-ketosteroids; for examples see ref 4a, pp 329−330. A similar procedure was employed by McMorris et al. (ref 5) for the Baeyer−Villiger oxidation of a 2,3,22,23-tetrahydroxycampestan-6-one diacetonide.

Scheme 3



in 12 steps and is therefore the most concise and efficient synthesis of **1** reported to date. Moreover, this approach is particularly well-suited for the preparation of side-chain analogues of brassinolide.

Experimental Section

Stigmasterol was purchased from the Aldrich Co. Phenyl *n*-propyl selenide[19] and bis(phenylseleno)methane[19] were prepared by literature methods. All other reagents were obtained from commercial sources and used without further purification. NMR spectra were recorded in deuteriochloroform unless otherwise indicated. Assignment of ^{13}C-NMR signals was confirmed by DEPT experiments. Elemental analyses and mass spectra were obtained by Ms. D. Fox and Ms. Q. Wu at the University of Calgary.

1,1-Bis(phenylseleno)propane (8). A solution of LDA (0.087 mol) in 40 mL of THF was added via cannula over 30 min to a solution containing 26.0 g (0.0796 mol) of bis(phenylseleno)methane in 140 mL of THF at −78 °C. After 10 min, iodoethane (6.96 mL, 0.087 mol) was added, and the solution was permitted to warm to room temperature and allowed to stir overnight. The mixture was poured into 300 mL of 1 M HCl solution, which was then extracted twice with 50% ether−hexanes. The organic extracts were washed with 10% $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated under vacuum. The oily residue was subjected to Kugelrohr distillation, bp 170 °C at 0.1 Torr, to afford 25.0 g (89%) of the selenoacetal **8**. It was identical to an authentic sample[20a] prepared from propanal and benzeneselenol by the general method of Krief et al.[20b]

(2*R*,3*S*,5α,22*S*,23*E*)-6,6-(Ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholest-23-en-22-ol (4). *n*-Butyllithium (12.4 mL, 2.5 M, 31.0 mmol) was added slowly to a solution of bis(phenylseleno)propane (10.7 g, 30.3 mmol) in 60 mL of dry THF at −78 °C. After 20 min, aldehyde **2** (10.7 g, 23.9 mmol) in 60 mL of dry THF was added dropwise via cannula over 15 min. The mixture stirred for an additional 3 h at −78 °C and then acetic acid (1.8 mL, 31 mmol) was added; the mixture was warmed to 0 °C, H_2O_2 (35 mL of a 30% solution, ca. 0.3 mol) was slowly added, and stirring was continued at room temperature for 15 h. The solution was then diluted with ether, washed with 10% Na_2SO_3 solution, saturated $NaHCO_3$ solution, and saturated NaCl solution, and dried (Na_2SO_4), and the solvent was evaporated. Flash chromatography over silica gel (elution with 0−15% acetonitrile−dichloromethane) afforded a yellow component consisting chiefly of diphenyl diselenide,[21] followed by 8.60 g (73%) of allylic alcohol **4**, identical to an authentic sample prepared by a different route.[8] Neither the 22*R* epimer, nor the (*Z*)-olefin could be detected by NMR analysis of the crude reaction mixture.

(*threo*-2*R*,3*S*,5α,22*R*,23*R*,24*S*)-23,24-Epoxy-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestan-22-ol (5a) and Its *erythro* Isomer 5b. (+)-L-Diethyl tartrate (L-DET) (774 mg, 3.76 mmol) in 15 mL of dichloromethane was added to a suspension of powdered 4 Å molecular sieves (8.0 g) in dichloromethane (80 mL) under argon at −30 °C, followed by $Ti(O\text{-}iPr)_4$ (890 mg, 3.14 mmol) in 15 mL of dichloromethane and cumene hydroperoxide (80%, 6.0 mL, 31 mmol). The mixture was stirred for 1 h at −30 °C, after which a solution of allylic alcohol **4** (15.3 g, 31.3 mmol) in dichloromethane (100 mL) was added dropwise over a period of 1 h. The reaction mixture was allowed to stand at −25 °C for 3 days. An additional 6.0 mL of cumene hydroperoxide was added in portions over this period. The reaction mixture was then poured into 5% aqueous tartaric acid solution (500 mL) and extracted four times with dichloromethane. The organic layers were combined, washed with 5% aqueous $NaHCO_3$ solution and with aqueous NaCl solution, dried (Na_2SO_4), and concentrated under vacuum. The crude product was flash chromatographed over silica gel (elution with a 50:45:5 mixture of hexanes−dichloromethane−acetonitrile and then with 5−20% acetonitrile−dichloromethane) to afford 3.88 g (25%) of unreacted allylic alcohol **4**, followed by 11.00 g (70%; 93% based on recovered starting material) of a mixture of *threo*- and *erythro*-epoxy alcohols **5a** and **5b** in the ratio of 70:30 as determined by NMR integration. Alternatively, when the reaction was allowed to proceed similarly for 6 days at −25 °C, the mixture of **5a** and **5b** was obtained in 85% yield in the same ratio, with only traces of recovered strarting material. This corresponds to a yield of 58% of **5a**. The unseparated mixture of epoxides was employed directly in the next step.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-6,6-(Ethylenedioxy)-2,3-(isopropylidenedioxy)ergostane-22,23-diol (6). Isopropylmagnesium chloride (23.4 mL of a 2.0 M solution in ether, 47 mmol) was added to a suspension of CuCN (421 mg, 4.68 mmol) in 30 mL of ether at −78 °C. The mixture was stirred for 1 h, followed by the dropwise addition of a solution of epoxy alcohols **5a** and **5b** (4.72 g, 9.35 mmol; *threo*/*erythro* ratio of 70:30) in 40 mL of ether over 40 min. The reaction mixture was stirred for 2 h at −78 °C and at 0 °C for 3 h, followed by the addition of 20% aqueous NH_4Cl solution and extraction three times with ether. The organic extracts were washed with 5% aqueous $NaHCO_3$ solution and saturated NaCl solution, dried (Na_2SO_4), and concentrated under vacuum. The crude product was chromatographed over silica gel (elution with 30−60% ether−hexanes) to afford 343 mg (8%) of allylic alcohol **4**, followed by 1.39 g (98%) of unreacted *erythro*-epoxy alcohol **5b**, 141 mg (4%, based on the *threo*-epoxide **5a**) of the 22,24-diol **7**, and 2.94 g (82%, based on the *threo*-epoxide **5a**) of the desired 22,23-diol **6**. The product was identical to an authentic sample.[8]

Brassinolide (1). Aqueous hydrogen peroxide (9.8 mL of a 30% solution, ca. 85 mmol) was slowly added to 62.6 mL (0.443 mol) of trifluoroacetic anhydride over 10 min at 0 °C. After 30 min, the mixture was diluted with 100 mL of chloroform. In a separate vessel, trifluoroacetic acid (30 mL) was added to a solution of diol **6** (5.85 g, 10.7 mmol) in 240 mL of chloroform. The latter solution was stirred at room temperature for 40 min and then was added dropwise to the pregenerated trifluoroperoxyacetic acid solution at 0 °C over a period of 45 min, followed by warming to room temperature and stirring for an additional 3 h. The mixture was diluted with water and extracted five times with chloroform. The organic extracts were washed with 10% aqueous Na_2SO_3 solution, dried (Na_2SO_4), and evaporated to dryness. The resulting white solid was stirred with 0.500 g of Na_2CO_3 in 300 mL of methanol for 14 h at room temperature. The mixture was concentrated under reduced pressure, taken up in chloroform, washed with water and aqueous NaCl solution, and dried (Na_2SO_4). The solvent was evaporated in vacuo, and the residue was recrystallized from methanol to afford 2.73 g (53%) of brassinolide (**1**), mp 275−277 °C (lit.[1] mp 274−275 °C), identical in all respects to an authentic sample.[8] Flash chro-

(19) Duddeck, H.; Wagner, P.; Rys, B. *Magn. Reson. Chem.* **1993**, *31*, 736.

(20) (a) Krief, A.; Dumont, W.; Clarembeau, M.; Bernard, G.; Badaoui, E. *Tetrahedron* **1989**, *45*, 2005. (b) Clarembeau, M.; Cravador, A.; Dumont, W.; Hevesi, L.; Krief, A.; Lucchetti, J.; Van Ende, D. *Tetrahedron* **1985**, *41*, 4793.

(21) Recrystallization of the nonpolar fractions from ethanol permitted recovery of 70−90% of diphenyl diselenide.

matography of the concentrated mother liquor (elution with 5−12% methanol−chloroform) yielded an additional 1.20 g of a mixture of **1** and its 6-oxa regioisomer in the ratio of 5:2, as determined by NMR integration.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-24-Cyclohexyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (9). The procedure for the preparation of diol **6** was followed, employing cyclohexylmagnesium chloride instead of the isopropyl derivative. Flash chromatography (elution with 25−60% ether−hexanes) afforded 81% of recovered *erythro*-epoxy alcohol **5b** and 73% (based on the *threo*-epoxide **5a**) of the 22,23-diol **9** as a colorless oil: IR (KBr) 3510, 3458, 1240, 1217, 1083, 1051, 972, 753 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.27 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.73 (m, 2 H), 3.55 (m, 1 H), 1.47 (s, 3 H), 1.32 (s, 3 H), 0.88 (d, J = 5.8 Hz, 3 H), 0.83 (d, J = 6.0 Hz, 3 H), 0.83 (s, 3 H), 0.67 (s, 3 H); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.5 (C), 74.7 (CH), 72.9 (CH), 72.8 (CH), 72.4 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.8 (CH), 52.9 (CH), 52.3 (CH), 45.4 (CH), 42.6 (CH_2), 42.3 (C), 40.9 (CH_2), 40.4 (CH), 39.7 (CH_2), 38.8 (CH), 38.0 (C), 36.8 (CH), 32.9 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.6 (CH_3), 27.7 (CH_2), 26.6 (CH_2), 26.6 (CH_3), 24.0 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 13.4 (CH_3), 11.9 (2 CH_3), 10.0 (CH_3); mass spectrum, *m*/*z* (relative intensity %) 588 (2), 573 (8), 446 (16), 431 (60), 235 (100). Exact mass calcd for $C_{36}H_{60}O_6$: 588.4390. Found: 588.4371.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-24-*n*-Dodecyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (10). The procedure for the preparation of diol **6** was followed, except that *n*-dodecylmagnesium chloride was employed instead of the isopropyl derivative, and the reaction was performed at −40 °C. Flash chromatography (elution with 25−60% ether−hexanes) afforded 88% of recovered *erythro*-epoxy alcohol **5b** and 69% (based on the *threo*-epoxide **5a**) of the 22,23-diol **10** as a colorless oil: IR (KBr) 3409, 1238, 1218, 1082, 1051 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 1 H), 3.55 (br s, 2 H), 1.49 (s, 3 H), 1.32 (s, 3 H), 0.91 (d, J = 5.6 Hz, 3 H), 0.89 (d, J = 6.1 Hz, 3 H), 0.85 (t, J = 7.9 Hz, 3 H), 0.84 (s, 3 H), 0.69 (s, 3 H); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.6 (C), 75.0 (CH), 74.4 (CH), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.8 (CH), 53.0 (CH), 52.3 (CH), 45.4 (CH), 42.6 (CH_2), 42.3 (C), 40.9 (CH_2), 39.7 (CH_2), 38.0 (C), 37.0 (CH), 34.4 (CH_2), 33.8 (CH), 32.9 (CH), 31.9 (CH_2), 29.8 (CH_2), 29.6 (five CH_2), 29.3 (CH_2), 28.6 (CH_3), 27.7 (CH_2), 27.3 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 22.6 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 14.1 (CH_3), 13.4 (CH_3), 12.7 (CH_3), 12.0 (CH_3), 11.9 (CH_3); mass spectrum, *m*/*z* (relative intensity %) 674 (2), 659 (6), 431 (29), 235 (85), 58 (100). Exact mass calcd for $C_{42}H_{74}O_6$: 674.5485. Found: 674.5521.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-24-Cyclohexyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (11). Compound **9** was treated with triflouroperoxyacetic acid as in the procedure for the preparation of **1**. Flash chromatography (elution with 10−15% isopropyl alcohol−chloroform) provided 69% of a mixture of **11** and its 6-oxa regioisomer in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 51% of pure **11**: mp 277−280 °C; IR (KBr) 3452, 1697, 1185, 1066, 1027, 979 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.09 (m, 2 H), 4.03 (m, 1 H), 3.74 (m, 2 H), 3.55 (br d, J = 8.0 Hz, 1 H), 3.15 (m, 1 H), 0.93 (s, 3 H), 0.90 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 3 H), 0.72 (s, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$−CD_3OD) δ 177.3 (C), 74.2 (CH), 72.6 (CH), 70.5 (CH_2), 67.8 (two CH), 58.0 (CH), 52.1 (CH), 51.2 (CH), 42.3 (C), 41.1 (CH_2), 40.9 (CH), 40.2 (CH), 39.5 (CH_2), 39.0 (CH), 38.9 (CH), 38.1 (C), 36.8 (CH), 31.1 (CH_2), 30.8 (two CH_2), 27.4 (CH_2), 26.5 (two CH_2), 26.4 (CH_2), 24.6 (CH_2), 22.1 (CH_2), 15.3 (CH_3), 11.7 (CH_3), 11.6 (CH_3), 9.9 (CH_3); mass spectrum of the 2,3,22,23-tetraacetate,[22] *m*/*z* (relative intensity, %) 688 (0.3), 568 (8), 506 (91), 463 (96), 404 (60), 361 (75), 123 (100). Anal. Calcd for $C_{31}H_{52}O_6$: C, 71.50; H, 10.07. Found: C, 71.16; H, 10.14.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-24-*n*-Dodecyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (12). Compound **11** was treated with triflouroperoxyacetic acid as in the procedure for the preparation of **1**. Flash chromatography (elution with 10−15% isopropyl alcohol−chloroform) provided 86% of a mixture of **12** and its 6-oxa regioisomer in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 67% of pure **12**: mp 208−211 °C; IR (KBr) 3440, 1695, 1187, 1062, 1044, 981 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.09 (m, 2 H), 4.03 (m, 1 H), 3.74 (m, 1 H), 3.57 (br s, 2 H), 3.13 (dd, J = 12.1, 4.1 Hz, 1 H), 0.93 (s, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.90 (t, J = 7.0 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 3 H), 0.72 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD) δ 177.3 (C), 74.8 (CH), 73.9 (CH), 70.5 (CH_2), 67.8 (two CH), 58.0 (CH), 52.1 (CH), 51.2 (CH), 42.3 (C), 41.1 (CH_2), 40.9 (CH), 39.5 (CH_2), 39.0 (CH), 38.1 (C), 37.0 (CH), 34.3 (CH_2), 33.8 (CH), 31.8 (CH_2), 31.1 (CH_2), 29.8 (CH_2), 29.6 (five CH_2), 29.2 (CH_2), 27.4 (CH_2), 27.2 (CH_2), 24.6 (CH_2), 22.6 (CH_2), 22.1 (CH_2), 15.3 (CH_3), 14.0 (CH_3), 12.5 (CH_3), 11.8 (CH_3), 11.6 (CH_3); mass spectrum of the 2,3,22,23-tetraacetate,[22] *m*/*z* (relative intensity, %) 774 (0.6), 654 (13), 506 (69), 463 (91), 404 (58), 361 (62), 43 (100). Anal. Calcd for $C_{37}H_{66}O_6$: C, 73.22; H, 10.96. Found: C, 73.00; H, 10.70.

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Supporting Information Available: ^{1}H-NMR and ^{13}C-NMR spectra of brassinolide (**1**) and of the side-chain analogues **9**−**12** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microform version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(22) The electron impact mass spectra of the tetrols **11** and **12** did not show their molecular ions. The mass spectra are therefore reported for the corresponding tetraacetates, which were prepared by treating **11** and **12** with acetic anhydride and 4-(dimethylamino)pyridine in pyridine for 4 h at room temperature.