Stereoselective Syntheses of (22*R*)- and (22*S*)-22-Methyl-1α,25-dihydroxyvitamin D_3: Active Vitamin D_3 Analogs with Restricted Side Chain Conformation

Keiko Yamamoto, Jun Takahashi, Kazuhiko Hamano, and Sachiko Yamada*

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan

Kentaro Yamaguchi

School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

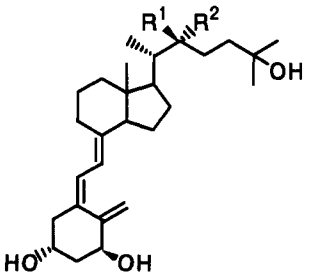
Hector F. DeLuca

Department of Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706

Received November 23, 1992

(22*R*)- and (22*S*)-22-methyl-1α,25-dihydroxyvitamin D_3 (**1b** and **1c**) were synthesized stereoselectively from 1α-hydroxylated C(22)-steroid **2**. The two new vitamin D analogs, which have a side chain with restricted flexibility, were designed to allow the study of the stereochemical structure required to bind to the receptor of the active vitamin D_3 (VDR). According to force-field calculations, the side chain of (22*R*)- and (22*S*)-methylated active vitamin D_3 analogs (**1b** and **1c**) adopts with more than 90% of the population gauche(+) and anti conformations, respectively, at the C(17–20–22–23) dihedral angle. Either the (22*R*)- or (22*S*)-methylated steroidal side chain was constructed with high stereoselectivity via a kinetically controlled conjugate addition of methylcopper reagent to (22*E*)- or (22*Z*)-22-en-24-ones (**6** or **7**), respectively, as a key step. The ability of the two analogs to bind to VDR was examined and only the (22*S*)-isomer (**1c**) showed significant activity. From the results, the side chain conformation best fitted to VDR was suggested to be the anti with respect to the C(17–20–22–23) dihedral angle.

Structure and function studies of the active vitamin D analogs are important not only to develop a clinically useful medicine but also to clarify the mechanism of action of the active vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, **1a**). The active vitamin D_3 is a multifunctional



1a: $R^1=R^2=H$
1b: $R^1=H$, $R^2=Me$
1c: $R^1=Me$, $R^2=H$
1d: $R^1=H$, $R^2=OMe$
1e: $R^1=OMe$, $R^2=H$

hormone[1] which is believed to exert its activities by a mechanism mediated by the nuclear vitamin D receptor (VDR).[2] Extensive studies on the structure and function of vitamin D analogs have shown that the separation of the multiple activities of the active vitamin is possible.[3] However the mechanism of the separation of the actions is unclear. In the case of 22-oxa-1α,25-dihydroxyvitamin D_3, the separation of the differentiating action from calcemic activities can be explained by a pharmacokinetics.[4] But in the case of 24-di- and trihomo-1,25-dihydroxyvitamin D_3 analogs, the separation of the two actions in the intestine, calcium transport, and synthesis of calcium binding protein (CaBP), cannot be explained by the same mechanism.[5] In this case, no calcium transporting activity was elicited even when these vitamin D analogs reached the target tissue, and the synthesis of CaBP was proved at the mRNA level. In any case it should be noted that these analogs played an important role in understanding the mechanism of the expression of biological activities.

In contrast to general steroid compounds, vitamin D has a flexible structure. The A ring with two exocyclic double bonds, the opened B ring, and the side chain can adopt a wide range of conformations. So it is interesting to synthesize vitamin D analogs with a fixed conformation and to test their activities to know the essential structure required for expressing each of the activities. The C(17–20–22–23) torsion angle of the side chain is the key to

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Table I. Minimum Energy Conformations of the Side Chain of 1α,25-Dihydroxyvitamin D_3 and Its (22R)- and (22S)-Methyl Analogs[a]

	conformation at C(17–20–22–23)					
	anti		gauche(+)		gauche(−)	
compound	energy[b] (population,[d] %)	angle[c]	energy[b] (population,[d] %)	angle[c]	energy[b] (population,[d] %)	angle[c]
1'a	33.005 (38)	−168°	32.723 (62)	64°		
1'b (22R)			35.732 (95)	68°	37.454 (5)	−73°
1'c (22S)	36.076 (90)	−167°	37.403 (10)	55°		

[a] The energies were calculated for model system (1', Figure 1) by MM2PP force-field method.[8] [b] Steric energy in kcal/mol. [c] C(17–20–22–23) torsion angle. [d] Boltzmann distribution at 25 °C.

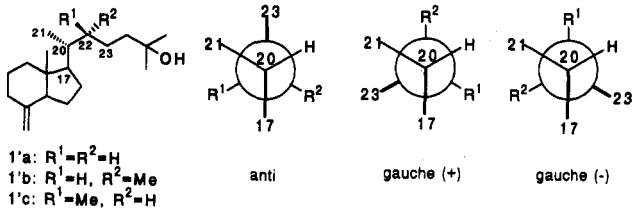

Figure 1.

determine the conformation of the side chain. It is known from the X-ray analysis that vitamin D_3 adopts two major side chain conformations, anti and gauche(+) conformations with respect to C(17–20–22–23) torsion angle (Figure 1).[6] But in the X-ray structure of 25-hydroxyvitamin D_3, only the anti conformer was observed.[7] This is in contrast to the results of force-field calculations. By MM2PP calculations,[8] the 25-hydroxyvitamin D_3 side chain has two minimum energy conformations with respect to the torsion angle at C(17–20–22–23): the gauche(+) and the anti conformations, the former being 62:38 more favorable than the latter at 25 °C (Table I). In any case these two conformations are similar in energy. Therefore it is interesting to know which is the appropriate conformation to bind to VDR. According to force-field calculations, introduction of a methyl group to the 22-position makes one of the staggered conformations exceedingly favorable (Table I). Thus (22R)- and (22S)-22-methyl-1,25-dihydroxyvitamin D_3 (**1b** and **1c**) adopt gauche(+) and anti forms, respectively, with more than 90% of the population. Introduction of a methyl group was expected to cause minimum effect on the electronic and geometric structure of the parent vitamin D molecule. On the same idea Ikekawa's group have already synthesized two epimeric 22-methoxy derivatives of 1,25-$(OH)_2D_3$ (**1d** and **1e**) and their biological activity was tested.[9] From the results, they concluded that the anti conformation is responsible for the activities. However because of the ability of oxygen to form a hydrogen bond with VDR, introduction of methoxyl group was considered not to be appropriate for the purpose of the study. Here we report stereoselective syntheses of (22R)- and (22S)-22-methyl-1α,25-dihydroxyvitamin D_3 (**1b** and **1c**) and the competitive binding study of these analogs to intestinal VDR.

Results and Discussion

Strategies for the syntheses of two epimeric 22-methyl active vitamin D_3 analogs (**1b** and **1c**) are as follows (Scheme I). The 22-methyl group was planned to be introduced by conjugate addition of methylcopper reagent to Δ^{22}-24-ketone (**6** or **7**). Because of the bulky steroid skeleton at the γ-position, the conjugate addition reaction was expected to be highly stereospecific. In fact, as described below, when the reaction was conducted under kinetically controlled conditions, 22R- and 22S-methylated products (**9** and **11**) were obtained with high selectivity from (22E)- and (22Z)-enones (**6** and **7**), respectively. The enone **6** can be synthesized easily as reported[10] and the (Z)-enone **7** is obtainable by photochemical isomerization of the (E)-enone (**6**).

The syntheses were started with readily available 1α-hydroxylated C(22) steroid **2**.[11] Parikh oxidation of C(22)-alcohol **2** provided aldehyde **3**. The (E)-enone **6** was obtained (73%) from the aldehyde **3** by a modification of the reported procedure.[10] Thus aldol condensation of **3** with 3-hydroxy-3-methylbutanone THP ether (LDA, THF) produced adduct **4** as an isomeric mixture at C(22) which was dehydrated (TsOH) to (E)-enone **5** with concomitant deprotection of the THP group. The 25-hydroxyl group was protected (MOMCl, *N,N*-diisopropylethylamine) to give enone **6**. The reaction of the (E)-enone **6** with Me_2CuLi under the condition of a normal Gilman reaction (0 °C, THF) gave the 22-methylated compound **9** with high stereoselectivity (98:2) together with a trace of its 22-epimer **11** in high yield (91%). The conjugate addition reaction conducted in the presence of TMSCl[12] (Me_2CuLi, TMSCl, HMPA, THF, −78 °C),[13] which is supposed to proceed under kinetic control,[12a] gave a single 22-methylated enol silyl ether **8** (100% selectivity, 95% yield). Desilylation of **8** gave the same 22-methyl ketone **9** (94%) as that obtained by the above Gilman reaction. The change of the conditions did not switch the selectivity but just improved it. The stereochemistry at C(22) of **9** was determined to be R by the X-ray crystal-

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(8) Conformational analyses of the vitamin D compounds were carried out on model compounds 1'a–c using a program MM2PP (Toray Systems Center Inc., Tokyo), which is a hybrid type of MMPI (see ref 8a) and MM2' (see ref 8b). Starting geometry was obtained with 3D-MOL (Toray Systems Center Inc., Tokyo) based on the X-ray crystallographic structure of 25-hydroxyvitamin D_3.[7] (a) Allinger, N. L.; Sprague, J. T. *J. Am. Chem. Soc.* **1973**, *95*, 3893–3907. (b) Jaime, C.; Osawa, E. *Tetrahedron* **1983**, *39*, 2769–2778.

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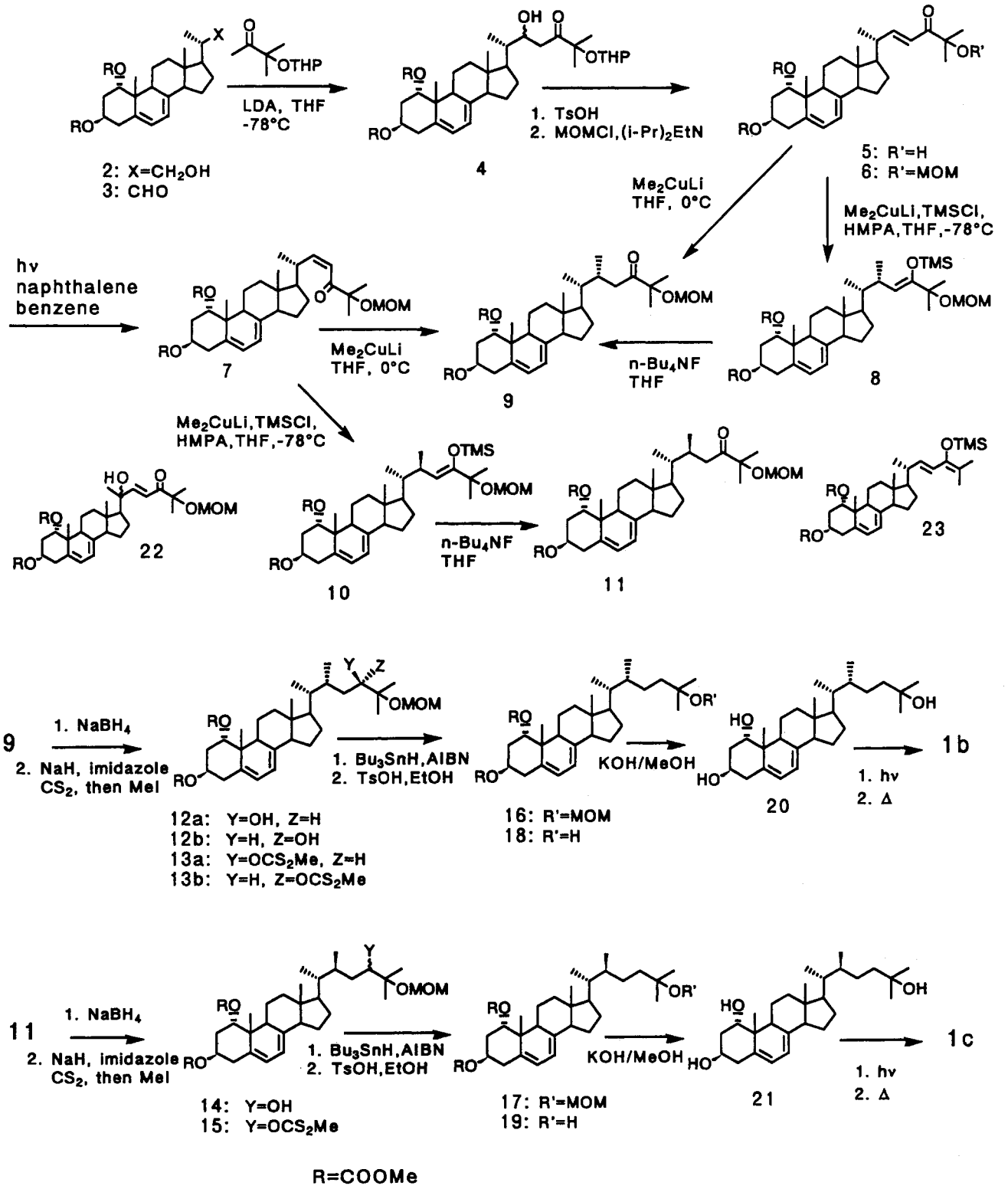
Scheme I

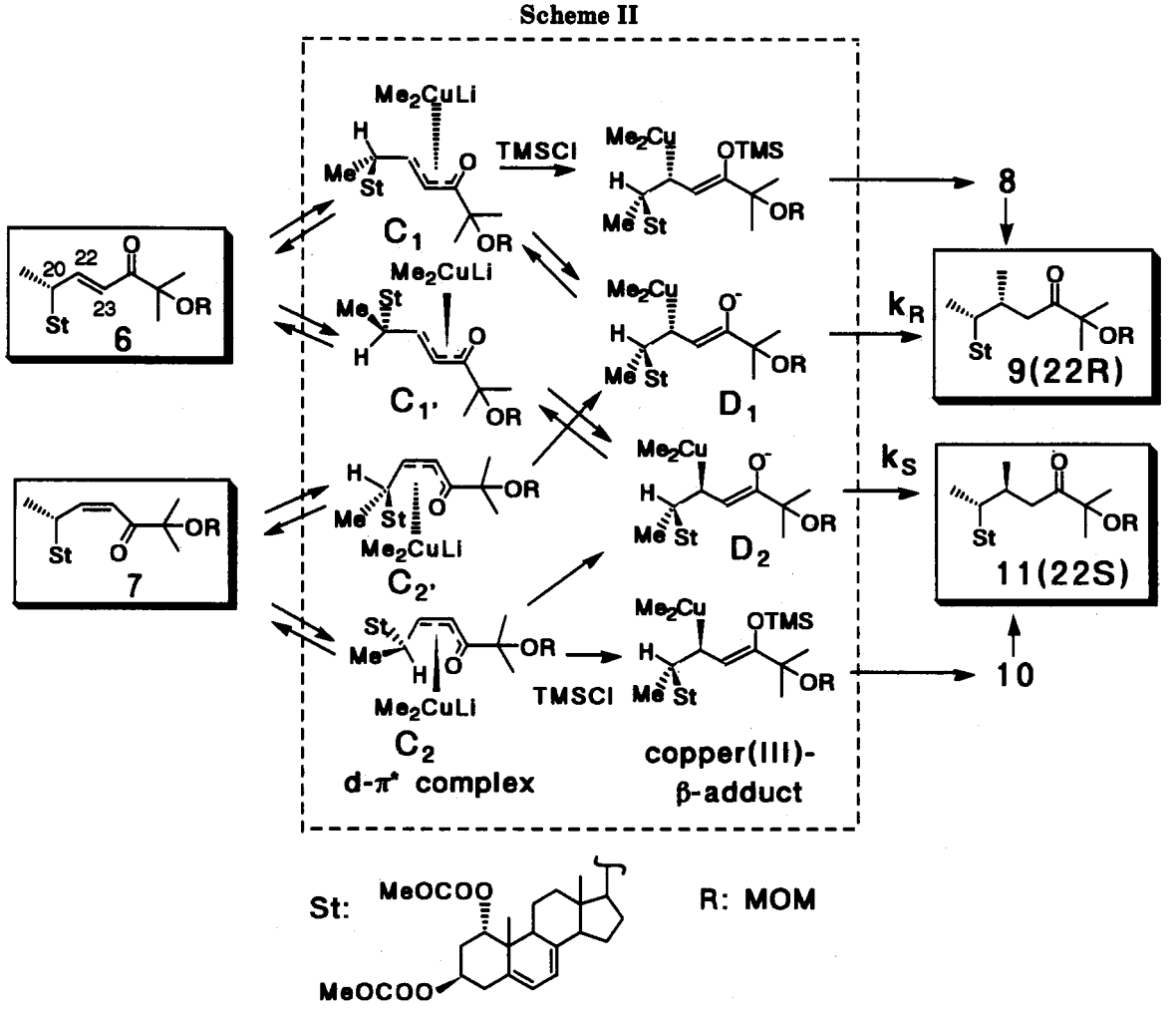


lographic analysis of its derivative **12a**, which is one of the epimers of the $NaBH_4$ reduction products of **9**. The PLUTO drawing of the X-ray structure of **12a** is shown in Figure 2.[14] As shown in the Figure 2 the side chain carrying (22*R*)-methyl group has gauche(+) conformation at C(17–20–22–23) in accord with the force-field calculation (Table I).

Since the conjugate addition to (*E*)-enone **6** was found to give (22*R*)-methyl epimer **9** nearly exclusively regardless of the conditions, we next examined the addition reaction to (*Z*)-enone **7**. (*Z*)-Enone **7** was obtained from the (*E*)-enone **6** by dye-sensitized photoisomerization. The proportion of (*Z*)-enone **7** to (*E*)-enone **6** at the photostationary state was maximum with a sensitizer of a triplet energy

(14) Crystal data: $C_{34}H_{54}O_9$, FW = 606.80, space group $P2_1$ (monoclinic), $Z = 2$, $a = 20.346(2)$, $b = 6.368(1)$, $c = 13.1256(8)$ Å, $\beta = 92.685(6)°$, $V = 1698.6(3)$ Å^3, $D_c = 1.186$ g cm^{-3}. The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Scheme II



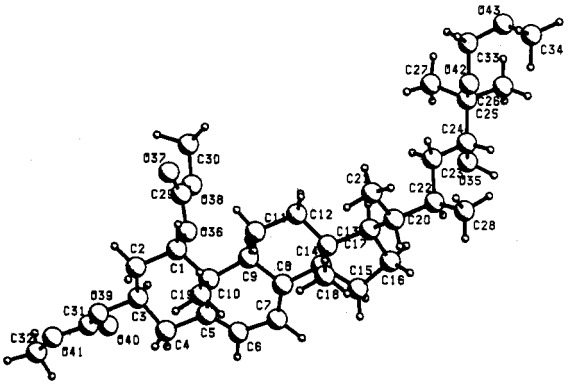


Figure 2. X-ray crystal structure of compound **12a**.

(E_T) of 50–60 kcal/mol. However the ratio (Z/E) did not exceed 1. Also the photochemical reaction produced a byproduct **22** oxygenated at C(20) as a mixture of C(20)-epimers. The C(20)-alcohol **22** might be produced by the reaction of trace oxygen with $\Delta^{20(22),23}$-dienol which was formed by photochemical enolization of (Z)-enone **7**.[15] In the preparative reaction, isomerization with naphthalene (E_T 60.9 kcal/mol) as a sensitizer (high-pressure mercury lamp, Pyrex filter, benzene) gave a best result (68% yield at 50% conversion). Gilman reaction of the (Z)-enone **7** (Me_2CuLi, THF, 0 °C) gave the 22-methylated ketones **9** and **11** with the same stereoselectivity (98:2) as in the reaction with the (E)-enone (**6**). The results indicate that the addition of Me_2CuLi under these conditions proceeded by a thermodynamically controlled mechanism. We then conducted the addition reactions under kinetic conditions (Me_2CuLi, TMSCl, HMPA, THF, −78 °C). The reaction gave a new 22-methylated enol silyl ether **10** as a major product (97% selectivity) together with **8** (3% selectivity) in 75% yield.[16] Deprotection of **10** gave isomeric 22-methyl ketone **11** (92%) to which we assigned the 22*S* configuration.

Thus highly stereoselective methods for introducing the methyl group to the steroidal 22-position were established. The cuprate additions with reversed stereoselectivity provided not only stereoselective methods for synthesizing (22*R*)- and (22*S*)-alkyl steroids but also intriguing evidence for the mechanism of the cuprate addition reactions.[12a,17] Here we discuss briefly a mechanism suggested for the cuprate additions (Scheme II).[18] Cuprate addition under normal conditions appears to be thermodynamically controlled, since both (E)- and (Z)-enones (**6** and **7**) gave the same products (**9** and **11**) in the same ratio (98:2). In the case of the (E)-enone, the whole steps up to the Cu(III)-β-adducts (D) are supposed to be in rapid equilibrium

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(16) Under these conditions, a byproduct **23** where the 25-OMOM group was eliminated was also obtained in low yield.[13]

(17) (a) House, H. O.; Wilkins, J. M. *J. Org. Chem.* **1978**, *43*, 2443–2454. (b) Krauss, S. R.; Smith, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 141–148. (c) Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1984**, *25*, 3063–3066. (d) Ullenius, C.; Christenson, B. *Pure Appl. Chem.* **1988**, *60*, 57–64. (e) Corey, E. J.; Hannon, F. J.; Boaz, N. W. *Tetrahedron* **1989**, *45*, 545–555.

(18) (a) Studies of the cuprate additions and a proposed mechanism for the reactions have been reported.[13] (b) Full account of the detailed studies will be reported elsewhere.

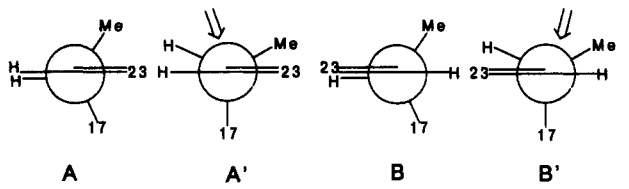

Figure 3.

and the last reductive elimination steps are the rate-determining steps with relative rate r_R/r_S being 49. In the case of the (*Z*)-enone **7** it probably reacts after being isomerized to the (*E*)-enone through the routes shown in Scheme II.[19] Addition of TMSCl accelerates the steps following the d-π* complex (C) formation and drives the reaction all the way to the silyl enol ether (**8** or **10**). Thus the reaction is kinetically controlled and the stereoselectivity is assumed to be determined by the stereochemistry of the starting enones. The (*E*)-enone **6** adopts two equally stable eclipsed conformations (A and B, Figure 3) by a molecular mechanics calculation.[13] Applying Felkin–Anh concept,[20] we assumed that the cuprate approaches the conformation A′ as shown in Figure 3 to yield d-π* complex C_1. In the case of the (*Z*)-enone **7**, B is the only global minimum energy conformation and the reaction is assumed to proceed via a transition state B′ to give complex C_2.

The carbonyl oxygen at C(24) of **9** and **11** was removed via methyl thiocarbonate[21] of the 24-alcohols (**12** and **14**) to give desired side chain structure. Other methods, such as reduction of tosylhydrazone,[22] reduction of 24-alcohol tosylate (mesylate), and radical deoxygenation via 24-phenyl thiocarbonate,[21b] gave unsatisfactory results. The 24-ketones were reduced ($NaBH_4$), the resulting epimeric 24-alcohols **12** and **14** were converted to methyl thiocarbonates **13** and **15** (imidazole, NaH, CS_2, and then MeI), and then they were reductively deoxygenated (Bu_3SnH, AIBN) to give **16** and **17** in 74 and 50% yields from **9** and **11**, respectively. The hydroxyl protecting groups at C(1), C(3), and C(25) were removed in two steps to give provitamin D (**20** and **21**) in 80–90% overall yield from **16** and **17**. The provitamin D (**20** and **21**) were converted to (22*R*)- and (22*S*)-22-methyl-1α,25-dihydroxyvitamin D_3 (**1b** and **1c**) by photochemical reaction followed by thermal isomerization.

The ability of these two 22-methylvitamin D analogs (**1b** and **1c**) to bind to pig intestinal receptor was examined in compared with 1,25-$(OH)_2D_3$ (**1a**). The 22*S*-epimer **1c** is about a third as active as the active vitamin D (**1a**) but the 22*R*-epimer (**1b**) was considerably less active (1/50) than **1a**. The results suggest that the side chain conformation required to bind to the receptor is the anti at C(17–20–22–23). Details of the biological activities and the structure–activity studies will be reported elsewhere.

We designed and synthesized active vitamin D analogs (**1b** and **1c**) having a side chain with restricted conformational flexibility. The syntheses used highly diastereoselective conjugate addition of Me_2CuLi to steroidal (*E*)- and (*Z*)-22-en-24-ones (**6** and **7**) as the key step. We suggested a mechanism for the stereoselective cuprate addition. Of the two analogs (**1b** and **1c**) synthesized only the 22*S*-isomer **1c** showed high affinity for VDR indicating that the anti conformer with respect to the C(17–20–22–23) bond is responsible for binding to VDR. This conclusion is in accord with that obtained with 22-methoxy analogs.[9]

Experimental Section

General. ^1H NMR spectra were measured at 270 MHz on a commercially available instrument. Low and high resolution mass spectra were measured at 70 eV. Relative intensities are given in parentheses. All air-sensitive reactions were run under argon atmosphere, and reagents were added through septa using oven-dried syringes. The phrase "dried and evaporated" indicates drying with Na_2SO_4, followed by evaporation of the solvents under house vacuum.

1α,3β-Bis[(methoxycarbonyl)oxy]-23,24-dinor-5,7-choladien-22-al (3). Dimethyl sulfoxide (3.67 mL, 51.8 mmol) in CH_2Cl_2 (10 mL) was added to a solution of oxalyl chloride (2.07 mL, 23.7 mmol) in CH_2Cl_2 (50 mL) at −78 °C. The mixture was stirred at −78 °C for 15 min, and then a solution of alcohol **2** (10 g, 21.6 mmol) in CH_2Cl_2 (40 mL) was added. After 15 min, triethylamine (15 mL, 108 mmol) was added at that temperature, and then the reaction mixture was allowed to warm to room temperature. The mixture was poured into ice–water and extracted with CH_2Cl_2. The extract was washed with water, dried, and evaporated to give a crystalline residue. The residue was recrystallized from ether–CH_2Cl_2 to give aldehyde **3** (8.18 g, 82%) as colorless needles. The filtrates were evaporated and chromatographed on silica gel (30 g) with $CHCl_3$ to give **3** (1.40 g, 14%): mp 171–173 °C; ^1H NMR δ 0.66 (3 H, s, H-18), 1.02 (3 H, s, H-19), 1.14 (3 H, d, J = 6.9 Hz, H-21), 3.78 and 3.79 (each 3 H, s, CH_3O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.40 and 5.69 (each 1 H, m, H-6 and -7), 9.59 (1 H, d, J = 3.0 Hz, H-22); IR (KBr) 2962, 2878, 1746, 1723, 1441, 1280, 1257, 1147, 990, 963, 940 cm^{-1}; MS m/z 384 (M^+ − CH_3OCO_2H, 15), 308 (99), 293 (14), 251 (17), 235 (45), 209 (22), 197 (24), 155 (35), 141 (81), 59 (100). Anal. Calcd for $C_{26}H_{36}O_7 \cdot 1/4H_2O$: C, 67.15; H, 7.91. Found: C, 67.25; H, 7.85.

1α,3β-Bis[(methoxycarbonyl)oxy]-22-hydroxy-25-(tetrahydropyranyloxy)-5,7-cholestadien-24-one (4). To a solution of diisopropylamine (1.84 mL, 13.0 mmol) in dry THF (6 mL) was added dropwise *n*-BuLi (1.6 M in hexane, 6.54 mL, 10.5 mmol) at −78 °C and the solution was stirred at that temperature. After 20 min, to the LDA solution was added dropwise a solution of 3-hydroxy-3-methyl-2-butanone tetrahydropyranyl ether (2.42 g, 13.0 mmol) in dry THF (3 mL) at −78 °C and the mixture was stirred for 15 min. The solution of the enolate was then added to a solution of aldehyde **3** (3.0 g, 6.52 mmol) in dry THF (6 mL) via a double-headed needle at −78 °C, and the mixture was stirred for 90 min at that temperature. The mixture was quenched with aqueous NH_4Cl and extracted with AcOEt. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (70 g) with 5–15% AcOEt–benzene to give the coupling product **4** (4.01 g, 95.2%) as a mixture of 22-epimers. **4**: ^1H NMR δ 3.78 and 3.79 (each 3 H, s, CH_3O), 4.60 (1 H, m, acetal H), 4.85 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38

(19) If this mechanism is correct, isomerization of the starting enone **7** to (*E*)-enone **6** must be observed in the thermodynamically controlled cuprate addition reactions. In the conjugate additions of organocuprates to enones, isomerization of (*Z*)-enone to (*E*)-enone has frequently been observed.[12a,17e] In the reaction of (*Z*)-enone **7** with Me_2CuLi under thermodynamically controlled conditions, however, no *Z*–*E* isomerization was detected: When the reaction of the (*Z*)-enone **7** with Me_2CuLi under thermodynamic conditions was terminated at the middle of the reaction, the recovered enone contained no *E*-isomer **6**. The results can be explained as follows: isomerization of the (*Z*)-enone **7** to the *E*-isomer **6** did occur under the reaction conditions but since the *E*-isomer **6** is far more reactive than the *Z*-isomer **7** no *E*-enone remained in the reaction mixture. This assumption was substantiated by a competition experiment. When a mixture of the (*E*)- and (*Z*)-enones (**6** and **7**, 1:1) was treated with 1 equiv of Me_2CuLi (THF, −10 °C), all the *E*-isomer was consumed within 5 min but only 20% of the *Z*-isomer reacted even after 40 min. These results suggest that in the case of the reaction of the *Z*-isomer **7**, a considerably high energy barrier exists between the d-π* complexes (C) and the Cu(III)-β-adducts (D). This means that the rate determining step of the reaction of the (*Z*)-enone is the isomerization to the (*E*)-enone thus supporting the proposed mechanism.[18b]

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and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 3496, 2962, 1748, 1444, 1286, 1151, 1129, 1077, 1023, 984 cm^{-1}; MS *m/z* 410 ($M^+ - CH_3OCO_2H \times 2$ - dihydropyran, 0.6), 392 (0.8), 384 (3), 343 (1), 308 (25), 141 (22), 85 (100); HRMS *m/z* calcd for $C_{36}H_{54}O_{10}$ 646.3713 (M^+), found 646.3701.

(22*E*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-hydroxy-5,7,-22-cholestatrien-24-one (5). To a solution of β-hydroxy ketone **3** (4.0 g, 6.2 mmol) in toluene–$CHCl_3$ (3:1, 80 mL) were added *p*-toluenesulfonic acid monohydrate (115 mg, 0.60 mmol) and magnesium sulfate (2.6 g, 21.6 mmol) and the solution was stirred at 50 °C for 1 h. The reaction mixture was cooled to room temperature, diluted with AcOEt, and washed with 5% $NaHCO_3$ and water. The organic layer was dried and evaporated. The residue was chromatographed on silica gel (100 g, 5–10% AcOEt–benzene) to afford (*E*)-enone **5** (2.86 g, 85%): mp 177–178.5 °C (benzene–hexane); ^1H NMR δ 0.66 (3 H, s, H-18), 1.02 (3 H, s, H-19), 1.13 (3 H, d, *J* = 6.4 Hz, H-21), 1.38 (6 H, s, H-26 and -27), 3.78 and 3.79 (each 3 H, s, CH_3O),3.98 (1 H, s, OH), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7), 6.35 (1 H, d, *J* = 15.3 Hz, H-23), 7.00 (1 H, dd, *J* = 15.3 and 8.9 Hz, H-22); IR (KBr) 3440, 2962, 1744, 1698, 1626, 1444, 1282, 1147, 1067, 994, 791 cm^{-1}; MS *m/z* 468 ($M^+ - CH_3OCO_2H$, 6), 392 (17), 349 (17), 334 (9), 59 (100); HRMS *m/z* calcd for $C_{31}H_{44}O_8$ 544.3033 (M^+), found 544.3058. Anal. Calcd for $C_{31}H_{44}O_8 \cdot 1/2H_2O$: C, 67.25; H, 8.19. Found: C, 67.66; H, 8.17.

(22*E*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-5,7,22-cholestatrien-24-one (6). To a solution of (*E*)-enone **5** (2.24 g, 4.12 mmol) in dry CH_2Cl_2 (10 mL) were added *N,N*-diisopropylethylamine (21.5 mL, 123 mmol) and chloromethyl methyl ether (6.25 mL, 82.3 mmol), and the mixture was stirred at ambient temperature. After 3 h, water was added and the mixture was extracted with AcOEt. The extracts were washed with 5% $NaHCO_3$ and water, dried, and evaporated. The residue was chromatographed on silica gel (50 g, 5% AcOEt–benzene) to afford **6** (2.18 g, 90%): mp 152–155 °C (AcOEt–benzene); ^1H NMR δ 0.65 (3 H, s, H-18), 1.02 (3 H, s, H-19), 1.12 (3 H, d, *J* = 6.9 Hz, H-21), 1.38 (6 H, s, H-26 and -27), 3.38 (3 H, s, CH_3OCH_2), 3.78 and 3.79 (each 3 H, s, CH_3OCO), 4.66 (2 H, s, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7), 6.61 (1 H, d, *J* = 15.3 Hz, H-23), 6.88 (1 H, dd, *J* = 15.3, 8.9 Hz, H-22); IR (KBr) 3422, 2956, 1742, 1700, 1632, 1446, 1286, 1257, 1147, 1040 cm^{-1}; UV (95% EtOH) λ_{max} 235, 261, 271, 281.5, 293.5 nm, λ_{min} 255.5, 264.5, 276, 289 nm; MS *m/z* 512 ($M^+ - CH_3OCO_2H$, 4), 437 (4), 378 (18), 333 (5), 155 (18), 103 (100). Anal. Calcd for $C_{33}H_{48}O_9$: C, 67.32; H, 8.22. Found: C, 67.44; H, 8.28.

(22*Z*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-5,7,22-cholestatrien-24-one (7). A solution of (*E*)-enone **6** (180 mg, 0.306 mmol) and naphthalene (100 mg, 0.781 mmol) in benzene (33 mL) in Pyrex vessel was flushed with Ar for 15 min and then irradiated externally with a 100-W high-pressure mercury lamp (Shigemi Standard, Tokyo) at room temperature under Ar, until approximately half the (*E*)-enone was consumed (analyzed by HPLC). The solvent was evaporated and the residue was chromatographed on silica gel (30 g, 3% AcOEt–benzene) to give (*Z*)-enone **7** (61 mg, 34%), starting (*E*)-enone **6** (85 mg, 47%), and 20-alcohol **22** (22 mg, 12%) as a 1:1 mixture of 20-epimers, which could not be separated on TLC or HPLC. **7**: mp 139–141 °C (MeOH–AcOEt); ^1H NMR δ 0.68 (3 H, s, H-18), 1.02 (3 H, s, H-19), 1.03 (3 H, d, *J* = 7.4 Hz, H-21), 1.37 (6 H, s, H-26 and -27), 3.38 (3 H, s, CH_3OCH_2), 3.78 and 3.79 (each 3 H, s, CH_3OCO), 4.67 (2 H, s, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.36 and 5.68 (each 1 H, m, H-6 and -7), 6.00 (1 H, dd, *J* = 11.4, 10.9 Hz, H-22), 6.49 (1 H, d, *J* = 11.4 Hz, H-23); IR (KBr) 3436, 2958, 1748, 1694, 1620, 1444, 1288, 1257, 1147, 1038 cm^{-1}; UV (95% EtOH) λ_{max} 235, 261, 271, 281.5, 293.5 nm, λ_{min} 255.5, 264, 276, 289 nm; MS *m/z* 512 ($M^+ - CH_3OCO_2H$, 6), 436 (8), 378 (6), 155 (20), 103 (100), 81 (23). Anal. Calcd for $C_{33}H_{48}O_9$: C, 67.32; H, 8.22. Found: C, 67.36; H, 8.31. **22** (1:1 mixture): ^1H NMR δ 0.65 and 0.69 (3 H (1:1), s, H-18), 0.99 (3 H, s, H-19), 1.386 and 1.391 (6 H (1:1), s, H-26 and -27), 1.50 and 1.52 (3 H (1:1), s, H-21), 3.39 (3 H, s, CH_3OCH_2), 3.77 (3 H, s, CH_3OCO), 3.78 and 3.79 (3 H (1:1), s, CH_3OCO), 4.66 and 4.67 (2 H (1:1), s, OCH_2O), 4.82 (1 H, m, H-1), 4.89 (1 H, m, H-3), 5.36 and 5.68 (each 1 H, m, H-6 and -7), 6.81 and 6.86, (1 H (1:1), d, *J* = 16 Hz, H-22 or -23), 7.02 and 7.09 (1 H (1:1), d, *J* = 16 Hz, H-23 or -22); IR (KBr) 3420, 2962, 2878, 1748, 1698, 1628, 1446, 1367, 1284, 1149, 1083, 1035, 994, 957, 938 cm^{-1}; UV (10% 2-propanol/hexane) λ_{max} 237, 262, 272, 282, 294 nm; MS *m/z* 528 ($M^+ - CH_3OCO_2H$, 4), 452 (4), 394 (9), 349 (6), 251 (24), 155 (34), 141 (41), 103 (100); HRMS *m/z* calcd for $C_{33}H_{48}O_{10}$ 604.3247 (M^+), found 604.3240.

(22*S*,23*Z*)-22-Methyl-5,7,23-cholestatriene-1α,3β,24,-25-tetrol 1,3-Bis(methyl carbonate) 25-(Methoxymethyl) 24-(Trimethylsilyl) Diether (8). Methyllithium (1.4 M in ether, 970 μL, 1.36 mmol) was added to a suspension of cuprous iodide (130 mg, 0.68 mmol) in THF (400 μL) at 0 °C and the solution was stirred for 15 min at that temperature. After being cooled to −78 °C, TMSCl (108 μL, 0.85 mmol), HMPA (148 μL, 0.85 mmol), and a solution of (*E*)-enone **6** (100 mg, 0.17 mmol) in THF (400 μL) were added in this order and the mixture was stirred at that temperature. After 30 min, triethylamine (2 mL) was added and then the mixture was diluted with AcOEt. The mixture was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g) with 3% AcOEt–benzene to afford TMS ether **8** (109 mg, 95%): ^1H NMR δ 0.20 (9 H, s, TMS), 0.60 (3 H, s, H-18), 0.88 (3 H, d, *J* = 6.9 Hz, H-21 or 22-Me), 0.94 (3 H, d, *J* = 6.9 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.33 and 1.34 (each 3 H, s, H-26 and -27), 3.36 (3 H, s, CH_3OCH_2), 3.77 and 3.79 (each 3 H, s, CH_3OCO), 4.62 and 4.66 (each 1 H, d, *J* = 6.9 Hz, OCH_2O), 4.67 (1 H, d, *J* = 9.4 Hz, H-23), 4.83 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 2964, 1750, 1657, 1444, 1259, 1149, 1098, 1040, 963, 845 cm^{-1}; MS *m/z* 538 ($M^+ - CH_3OCO_2H - CH_3OCH_2OH$, 15), 462 (6), 372 (3), 354 (5), 185 (41), 183 (44), 141 (35), 131 (31), 111 (100), 83 (25), 73 (98). Anal. Calcd for $C_{37}H_{60}O_9Si \cdot 1/4H_2O$: C, 65.21; H, 8.95. Found: C, 65.17; H, 9.10.

(22*R*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-22-methyl-5,7-cholestadien-24-one (9). Deprotection of Enol Silyl Ether 8. To a solution of TMS ether **8** (100 mg, 0.15 mmol) in dry THF (0.8 mL) was added *n*-Bu_4NF (1.0 M in THF, 150 μL, 0.15 mmol) and the mixture was stirred at room temperature. After 1.5 h, the reaction was quenched with saturated NH_4Cl and extracted with AcOEt. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g) with 4% AcOEt–benzene to afford **9** (84 mg, 94%): mp 162–165 °C (hexane–AcOEt); ^1H NMR δ 0.63 (3 H, s, H-18), 0.853 (3 H, d, *J* = 5.9 Hz, H-21 or 22-Me), 0.855 (3 H, d, *J* = 6.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.33 and 1.36 (each 3 H, s, H-26 and -27), 3.39 (3 H, s, CH_3OCH_2), 3.78 and 3.79 (each 3 H, s, CH_3OCO_2), 4.72 (2 H, s, OCH_2O), 4.85 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (KBr) 2962, 1746, 1717, 1444, 1377, 1286, 1149, 1035, 992, 963, 936, 868, 791 cm^{-1}; MS *m/z* 572 (M^+ – MeOH, 1), 528 (1), 496 (2), 483 (3), 452 (5), 420 (4), 407 (4), 394 (7), 141 (24), 103 (100). Anal. Calcd for $C_{34}H_{52}O_9$: C, 67.52; H, 8.67. Found: C, 67.47; H, 8.70.

Addition of Me_2CuLi to (*E*)-Enone 6. Methyllithium (1.4 M in ether, 2.91 mL, 4.07 mmol) was added to a suspension of CuI (389 mg, 2.04 mmol) in dry THF (0.6 mL) at 0 °C and the solution was stirred for 15 min. (*E*)-Enone **6** (300 mg, 0.51 mmol) in THF (1.8 mL) was added to the resulting solution of Me_2CuLi and the mixture was stirred at 0 °C for 25 min. The reaction was quenched with saturated NH_4Cl and extracted with AcOEt. The organic extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 5% AcOEt–benzene) to afford **9** together with a small amount of its 22-epimer **11** (98:2 by HPLC analysis, 281 mg, 91%).

Addition of Me_2CuLi to (*Z*)-Enone 7. (*Z*)-Enone **7** (100 mg, 0.17 mmol) was treated with Me_2CuLi under similar conditions described above for (*E*)-enone **6** to yield **9** together with a trace of its 22-epimer **11** (98:2, 80 mg, 78%).

(22*R*,23*Z*)-22-Methyl-5,7,23-cholestatriene-1α,3β,24,-25-tetrol 1,3-Bis(methyl carbonate) 25-(Methoxymethyl) 24-(Trimethylsilyl) Diether (10). Methyllithium (1.4 M in ether, 2.14 mL, 3.00 mmol) was added to a suspension of cuprous iodide (285 mg, 1.50 mmol) in THF (400 μL) at 0 °C and the solution was stirred for 15 min at that temperature. The mixture was cooled to −78 °C, TMSCl (237 μL, 1.87 mmol), HMPA (325 μL, 1.87 mmol), and a solution of (*Z*)-enone **7** (110 mg, 0.187 mmol) in THF (400 μL) were added in this order, and the mixture was stirred for 40 min at that temperature. Triethylamine (2 mL)

was added at −78 °C and then the mixture was diluted with AcOEt. The mixture was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (12 g, 2% AcOEt–benzene) to afford byproduct **23** (7 mg, 6%) and TMS ether **10** containing a trace of its 22-epimer **8** (97:3 by HPLC, 95 mg, 75%) in this order. **10**: ^{1}H NMR δ 0.20 (9 H, s, TMS), 0.61 (3 H, s, H-18), 0.79 (3 H, d, J = 6.9 Hz, H-21 or 22-Me), 0.86 (3 H, d, J = 5.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.35 (6 H, s, H-26 and -27), 3.36 (3 H, s, CH_3OCH$_2$), 3.78 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.63 and 4.66 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.84 (1 H, m, H-1), 4.85 (1 H, d, J = 9.4 Hz, H-23), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 2964, 1748, 1657, 1446, 1282, 1149, 1042, 963, 843 cm^{-1}; MS m/z 538 (M$^+$ − CH$_3$OCO$_2$H − CH$_3$OCH$_2$OH, 1), 462 (1), 390 (1), 372 (1), 183 (100), 141 (9), 111 (11), 73 (74); HRMS m/z calcd for C$_{37}$H$_{60}$O$_9$Si 676.4002 (M$^+$), found 676.3962. **23**: ^{1}H NMR δ 0.20 (9 H, s, TMS), 0.65 (3 H, s, H-18), 1.02 (3 H, s, H-19), 1.07 (3 H, d, J = 6.4 Hz, H-21), 1.68 and 1.72 (each 3 H, s, H-26 and -27), 3.77 and 3.79 (each 3 H, s, CH_3OCO), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7), 5.59 (1 H, dd, J = 15.3 and 8.9 Hz, H-22), 6.20 (1 H, d, J = 15.3 Hz, H-23); IR (KBr) 2960, 2876, 1748, 1626, 1446, 1282, 1199, 1149, 984, 963, 940 cm^{-1}; UV (95% EtOH) λ_{max} 251, 260, 270.5, 281.5, 293.5 nm, λ_{min} 221.5, 267, 277, 289 nm; MS m/z 524 (M$^+$ − CH$_3$OCO$_2$H, 5), 448 (12), 433 (2), 376 (2), 251 (8), 249 (13), 197 (36), 170 (30), 73 (100); HRMS m/z calcd for C$_{34}$H$_{52}$O$_7$Si 600.3483 (M$^+$), found 600.3477.

(22*S*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-22-methyl-5,7-cholestadien-24-one (11). Deprotection of Enol Silyl Ether 10. TMS ether **10** (160 mg, 0.24 mmol) was deprotected similarly to give **11** (131 mg, 92%): ^{1}H NMR δ 0.60 (3 H, s, H-18), 0.73 (3 H, d, J = 6.9 Hz, H-21 or 22-Me), 0.82 (3 H, d, J = 5.4 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.34 (6 H, s, H-26 and -27), 3.39 (3 H, s, CH_3OCH$_2$), 3.78 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.71 (2 H, s, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (KBr) 2966, 1746, 1717, 1444, 1284, 1149, 1033, 938, 793 cm^{-1}; MS m/z 572 (M$^+$ − CH$_3$OH, 1), 528 (3), 497 (1), 452 (7), 394 (8), 141 (19), 103 (100); HRMS m/z calcd for C$_{34}$H$_{52}$O$_9$ 604.3608 (M$^+$), found 604.3611.

(22*R*,24*R*)- and (22*R*,24*S*)-22-Methyl-5,7-cholestadiene-1α,3β,24,25-tetrol 1,3-Bis(methyl carbonate) 25-(Methoxymethyl) Ether (12a and 12b). To a solution of **9** (213 mg, 0.35 mmol) in CH$_2$Cl$_2$ (2 mL) at room temperature were added methanol (1 mL) and NaBH$_4$ (27 mg, 0.71 mmol) and the mixture was stirred for 70 min. The solution was diluted with CH$_2$Cl$_2$ and water and extracted with CH$_2$Cl$_2$. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 5% AcOEt–benzene) to afford **12a** (158 mg, 74%) and its 24*S*-epimer **12b** (52 mg, 24%) in this order. **12a**: mp 156–157.5 °C (MeOH); ^{1}H NMR δ 0.63 (3 H, s, H-18), 0.81 (3 H, d, J = 5.4 Hz, H-21 or 22-Me), 0.91 (3 H, d, J = 6.4 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.17 and 1.20 (each 3 H, s, H-26 and -27), 3.40 (3 H, s, CH_3OCH$_2$), 3.42 (1 H, m, H-24), 3.77 and 3.79 (each 3 H, s, CH$_3$OCO$_2$), 4.71 and 4.78 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (KBr) 3466, 2960, 2878, 1748, 1444, 1373, 1270, 1147, 1038 cm^{-1}; MS m/z 530 (M$^+$ − CH$_3$OCO$_2$H, 8), 498 (5), 454 (16), 422 (48), 392 (41), 351 (38), 251 (34), 224 (24), 223 (24), 209 (72), 197 (39), 155 (59), 141 (60), 103 (100), 72 (91), 59 (60), 55 (73). Anal. Calcd for C$_{34}$H$_{54}$O$_9$: C, 67.30; H, 8.97. Found: C, 67.48; H, 9.15. **12b**: ^{1}H NMR δ 0.62 (3 H, s, H-18), 0.84 (3 H, d, J = 5.4 Hz, H-21 or 22-Me), 1.01 (3 H, s, H-19), 1.04 (3 H, d, J = 6.9 Hz, 22-Me or H-21), 1.17 and 1.21 (each 3 H, s, H-26 and -27), 3.39 (3 H, s, CH_3OCH$_2$), 3.42 (1 H, m, H-24), 3.77 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.70 and 4.77 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 3448, 2962, 1746, 1444, 1274, 1147, 1036 cm^{-1}; MS m/z 530 (M$^+$ − CH$_3$OCO$_2$H, 3), 498 (6), 454 (8), 422 (29), 392 (48), 351 (33), 251 (32), 224 (20), 223 (21), 209 (55), 197 (34), 155 (53), 141 (50), 103 (100), 72 (82), 59 (53), 55 (65); HRMS m/z calcd for C$_{34}$H$_{54}$O$_9$ 606.3767 (M$^+$), found 606.3763.

(22*S*)-22-Methyl-5,7-cholestadiene-1α,3β,24,25-tetrol 1,3-Bis(methyl carbonate) 25-(Methoxymethyl) Ether (14). 22-Methyl ketone **11** (91 mg, 0.15 mmol) was similarly reduced with NaBH$_4$ to give 24-alcohol **14** (84 mg, 92%) as a mixture of 24-epimers (1:1 by NMR). **14**: ^{1}H NMR δ 0.62 and 0.64 (3 H (1:1), s, H-18), 1.01 and 1.02 (3 H (1:1), s, H-19), 3.39 (3 H, s, CH$_3$OCH$_2$), 3.77 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.72 and 4.76 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 3464, 2964, 1744, 1446, 1255, 1147, 1033, 955, 795 cm^{-1}; MS m/z 530 (M$^+$ − CH$_3$OCO$_2$H, 6), 498 (5), 454 (12), 422 (51), 392 (29), 351 (32), 251 (44), 224 (35), 223 (27), 209 (87), 197 (64), 155 (72), 141 (76), 103 (100), 72 (99), 59 (52), 55 (66); HRMS m/z calcd for C$_{34}$H$_{54}$O$_9$ 606.3767 (M$^+$), found 606.3751.

(22*R*,24*R*)-22-Methyl-5,7-cholestadiene-1α,3β,24,-25-tetrol 1,3-Bis(methyl carbonate) 24-(*S*-Methyl dithiocarbonate) 25-(Methoxymethyl) Ether (13a). To a solution of 24-alcohol **12a** (100 mg, 0.16 mmol) and imidazole (0.2 mg) in THF (1.5 mL) at room temperature was slowly added NaH (50% oil dispersion, 12 mg, 0.25 mmol). After the mixture was stirred for 20 min, carbon disulfide (30 μL, 0.50 mmol) was added all at once. Stirring was continued for 30 min, and then iodomethane (20 μL, 0.32 mmol) was added in a single portion. The mixture was stirred another 30 min and quenched with water. The mixture was extracted with AcOEt, and the extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 2% AcOEt–benzene) to afford **13a** (102 mg, 89%): ^{1}H NMR δ 0.60 (3 H, s, H-18), 0.83 (3 H, d, J = 5.4 Hz, H-21 or 22-Me), 0.98 (3 H, d, J = 6.4 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.27 (6 H, s, H-26 and -27), 2.58 (3 H, s, SCH$_3$), 3.37 (3 H, s, CH_3OCH$_2$), 3.77 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.70 and 4.79 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.85 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7), 5.90 (1 H, d, J = 10.4 Hz, H-24); MS m/z 467 (M$^+$ − CH$_3$OCO$_2$H × 2 − CH$_3$OCH$_2$OH − CH$_3$, 38), 392 (36), 325 (31), 251 (42), 235 (21), 223 (29), 209 (76), 197 (50), 183 (28), 155 (83), 141 (68), 123 (31), 113 (34), 91 (89), 69 (100), 55 (81).

(22*R*,24*S*)-22-Methyl-5,7-cholestadiene-1α,3β,24,-25-tetrol 1,3-Bis(methyl carbonate) 24-(*S*-Methyl dithiocarbonate) 25-(Methoxymethyl) Ether (13b). The epimeric alcohol **12b** was converted to *S*-methyl dithiocarbonate **13b** under similar conditions. **13b**: ^{1}H NMR δ 0.61 (3 H, s, H-18), 0.83 (3 H, d, J = 5.9 Hz, H-21 or 22-Me), 0.93 (3 H, d, J = 6.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.27 (6 H, s, H-26 and -27), 2.58 (3 H, s, SCH$_3$), 3.37 (3 H, s, CH_3OCH$_2$), 3.78 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.71 and 4.77 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7), 5.88 (1 H, dd, J = 8.9, 3.0 Hz, H-24).

(22*S*)-22-Methyl-5,7-cholestadiene-1α,3β,24,25-tetrol 1,3-Bis(methyl carbonate) 24-(*S*-Methyl dithiocarbonate) 25-(Methoxymethyl) Ether (15). (22*S*)-24-Alcohol **14** (26 mg, 0.043 mmol) was converted to dithiocarbonate **15** (18 mg, 60%) under similar conditions. **15**: ^{1}H NMR δ 0.60 and 0.62 (3 H (1:1), s, H-18), 1.01 (3 H, s, H-19), 1.27 (6 H, s, H-26 and -27), 2.565 and 2.569 (3 H (1:1), s, SCH$_3$), 3.367 and 3.370 (3 H (1:1), s, CH$_3$OCH$_2$), 3.775 and 3.782 (each 3 H, s, CH_3OCO$_2$), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.36 and 5.68 (each 1 H, m, H-6 and -7), 5.91 (1 H, m, H-24); IR (KBr) 2964, 1748, 1267, 1058 cm^{-1}; MS m/z 512 (M$^+$ − CH$_3$OCO$_2$H × 2 − CH$_3$OH, 4), 467 (3), 450 (5), 392 (20), 374 (18), 359 (12), 325 (7), 277 (22), 251 (32), 249 (35), 223 (31), 209 (75), 197 (55), 183 (28), 155 (64), 141 (64), 123 (29), 95 (50), 69 (63), 60 (100), 55 (75); HRMS m/z calcd for C$_{36}$H$_{56}$O$_9$S$_2$ 696.3366 (M$^+$), found 696.3358.

(22*R*)-22-Methyl-5,7-cholestadiene-1α,3β,25-triol 1,3-Bis(methyl carbonate) 25-(Methoxymethyl) Ether (16). To a solution of dithiocarbonate **13a** (140 mg, 0.20 mmol) in dry toluene (2.5 mL) were added azobis(isobutyronitrile) (8 mg, 0.05 mmol) and *n*-Bu$_3$SnH (161 μL, 0.60 mmol), and the mixture was refluxed for 15 min. The reaction mixture was directly chromatographed on silica gel (7 g, 15% AcOEt–hexane) to afford **16** (101 mg, 85%): ^{1}H NMR δ 0.61 (3 H, s, H-18), 0.82 (3 H, d, J = 6.4 Hz, H-21 or 22-Me), 0.88 (3 H, d, J = 6.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.21 (6 H, s, H-26 and -27), 3.37 (3 H, s, CH_3OCH$_2$), 3.77 and 3.79 (each 3 H, s, CH_3OCO$_2$), 4.70 (2 H, s, OCH$_2$O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 2958, 1744, 1444, 1255, 1147, 1044, 955, 868, 793 cm^{-1}; MS m/z 452 (M$^+$ − CH$_3$OCO$_2$H − CH$_3$OCH$_2$OH, 14), 438 (7), 376 (100), 361 (12), 325 (14), 277 (25), 249 (25), 209 (55),

197 (27), 155 (39), 141 (41), 103 (43), 69 (50), 55 (57); HRMS *m/z* calcd for $C_{34}H_{54}O_8$ 590.3819 (M^+), found 509.3809.

(22S)-22-Methyl-5,7-cholestadiene-1α,3β,25-triol 1,3-Bis(methyl carbonate) 25-(Methoxymethyl) Ether (17). Under similar conditions, dithiocarbonate **15** (34 mg, 0.049 mmol) was reduced to give **17** (26 mg, 90%): ^1H δ 0.62 (3 H, s, H-18), 0.72 (3 H, d, *J* = 6.9 Hz, H-21 or 22-Me), 0.79 (3 H, d, *J* = 5.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.21 (6 H, s, H-26 and -27), 3.37 (3 H, s, CH_3OCH_2), 3.78 and 3.79 (each 3 H, s, CH_3OCO_2), 4.71 (2 H, s, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7); IR (KBr) 2962, 1744, 1444, 1284, 1147, 1042, 955, 866, 793 cm^{-1}; MS *m/z* 452 ($M^+ - CH_3OCO_2H - CH_3OCH_2OH$, 11), 438 (8), 376 (100), 361 (16), 277 (28), 251 (32), 249 (29), 224 (31), 209 (78), 197 (49), 155 (51), 141 (59), 103 (42), 69 (81), 55 (79); HRMS *m/z* calcd for $C_{34}H_{54}O_8$ 590.3818 (M^+), found 590.3826.

(22R)-22-Methyl-5,7-cholestadiene-1α,3β,25-triol 1,3-Bis(methyl carbonate) (18). A solution of MOM ether **16** (101 mg, 0.17 mmol) and *p*-toluenesulfonic acid monohydrate (90 mg, 0.47 mmol) in 95% ethanol (4 mL) was refluxed for 10 min. The solvent was evaporated, the residue was dissolved in AcOEt, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 3% AcOEt–benzene) to afford 25-alcohol **18** (86 mg, 92%): ^1H NMR δ 0.61 (3 H, s, H-18), 0.82 (3 H, d, *J* = 6.4 Hz, H-21 or 22-Me), 0.88 (3 H, d, *J* = 6.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.210 and 1.216 (each 3 H, s, H-26 and -27), 3.78 and 3.79 (each 3 H, s, CH_3O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (KBr) 3552, 2966, 1744, 1731, 1446, 1344, 1292, 1265, 1098, 1025, 797 cm^{-1}; MS *m/z* 470 ($M^+ - CH_3OCO_2H$, 8), 394 (81), 379 (17), 376 (19), 361 (6), 251 (18), 224 (26), 209 (67), 197 (39), 155 (49), 141 (58), 69 (69), 59 (100), 55 (75).

(22S)-22-Methyl-5,7-cholestadiene-1α,3β,25-triol 1,3-Bis(methyl carbonate) (19). Under similar conditions, MOM ether **17** (11 mg, 0.019 mmol) was converted to 25-alcohol **19** (8 mg, 80%): ^1H NMR δ 0.62 (3 H, s, H-18), 0.73 (3 H, d, *J* = 6.9 Hz, H-21 or 22-Me), 0.79 (3 H, d, *J* = 5.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.21 (6 H, s, H-26 and -27), 3.78 and 3.79 (each 3 H, s, CH_3O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and -7).

(22R)-22-Methyl-5,7-cholestadiene-1α,3β-25-triol (20). To a solution of biscarbonate **18** (80 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) was added 5% KOH/MeOH (3.5 mL) and the mixture was stirred at 40 °C for 45 min. The mixture was concentrated in vacuo and diluted with $CHCl_3$, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 60% AcOEt–CH_2Cl_2) to afford provitamin D **20** (60 mg, 95%): ^1H NMR δ 0.63 (3 H, s, H-18), 0.84 (3 H, d, *J* = 6.4 Hz, H-21 or 22-Me), 0.88 (3 H, d, *J* = 6.9 Hz, 22-Me or H-21), 0.95 (3 H, s, H-19), 1.216 and 1.223 (each 3 H, s, H-26 and -27), 3.78 (1 H, m, H-1), 4.07 (1 H, m, H-3), 5.38 and 5.73 (each 1 H, m, H-6 and -7); IR (KBr) 3426, 2934, 1460, 1383, 1263, 1031, 909, 801 cm^{-1}; UV (95% EtOH) λ_{max} 272, 282.5, 294.5 nm, λ_{min} 235, 277, 290 nm; MS *m/z* 430 (M^+, 18), 412 (18), 394 (19), 251 (20), 227 (32), 197 (26), 171 (35), 157 (39), 69 (88), 59 (94), 55 (100); HRMS *m/z* calcd for $C_{28}H_{46}O_3$ 430.3447 (M^+), found 430.3434.

(22S)-22-Methyl-5,7-cholestadiene-1α,3β,25-triol (21). Under similar conditions, biscarbonate **19** (19 mg, 0.036 mmol) was hydrolyzed to give provitamin D **21** (14 mg, 93%): ^1H NMR δ 0.64 (3 H, s, H-18), 0.74 (3 H, d, *J* = 6.9 Hz, H-21 or 22-Me), 0.81 (3 H, d, *J* = 5.9 Hz, 22-Me or H-21), 0.95 (3 H, s, H-19), 1.22 (6 H, s, H-26 and -27), 3.77 (1 H, m, H-1), 4.07 (1 H, m, H-3), 5.38 and 5.74 (each 1 H, m, H-6 and -7); IR (KBr) 3408, 2966, 1460, 1381, 1154, 1054, 909, 760 cm^{-1}; UV (95% EtOH) λ_{max} 272, 282.5, 294.5 nm, λ_{min} 233, 277, 290 nm; MS *m/z* 430 (M^+, 18), 412 (12), 394 (17), 251 (19), 227 (31), 197 (26), 171 (35), 157 (42), 69 (82), 59 (100), 55 (89); HRMS *m/z* calcd for $C_{28}H_{46}O_3$ 430.3447 (M^+), found 430.3442.

(22R)-22-Methyl-1α,25-dihydroxyvitamin D_3 (1b). A solution of the provitamin D **20** (60 mg, 0.14 mmol) in benzene–EtOH (150:20, 170 mL) was flushed with Ar for 15 min and then irradiated at 0 °C under Ar with a 100-W high-pressure mercury lamp (Shigemi Standard, Tokyo) through a Vycor filter until most of the provitamin D was consumed (the reaction was monitored by HPLC). The solvent was evaporated and the residue was chromatographed on Sephadex LH-20 (20 g) with $CHCl_3$/hexane/MeOH (70:30:1) to give the previtamin D (30.6 mg, yield 51%). The previtamin D was dissolved in 95% ethanol (5 mL) and stored in the dark at room temperature under Ar for 2 weeks. The solvent was evaporated and the residue was chromatographed on Sephadex LH-20 (12 g) with the same solvent system as above to give **1b** (24.2 mg, 79%) as a colorless glass: ^1H NMR δ 0.54 (3 H, s, H-18), 0.82 and 0.88 (each 3 H, d, *J* = 6.9 Hz, H-21 and 22-Me), 1.218 and 1.225 (each 3 H, s, H-26 and -27), 4.23 (1 H, m, H-3), 4.43 (1 H, m, H-1), 5.01 and 5.33 (each 1 H, m, H-19), 6.02 and 6.38 (each 1 H, d, *J* = 11.4 Hz, H-7 and -6, respectively); IR (KBr) 3398, 2952, 2878, 1657, 1543, 1460, 1381, 1261, 1060, 959, 911, 801 cm^{-1}; UV (95% EtOH) λ_{max} 264 nm, λ_{min} 228 nm; MS *m/z* 430 (M^+, 3), 412 (7), 394 (6), 379 (3), 152 (26), 134 (100), 69 (69), 59 (71), 55 (86); HRMS *m/z* calcd for $C_{28}H_{46}O_3$ 430.3447 (M^+), found 430.3436.

(22S)-22-Methyl-1α,25-dihydroxyvitamin D_3 (1c). Under similar conditions described above, provitamin D **21** (32.7 mg, 0.076 mmol) was converted to the previtamin D (12.4 mg, yield 38%) and then isomerized to vitamin D (**1c**) (8.8 mg, 71%): ^1H NMR δ 0.55 (3 H, s, H-18), 0.73 (3 H, d, *J* = 6.9 Hz, H-21 or 22-Me), 0.78 (3 H, d, *J* = 5.9 Hz, 22-Me or H-21), 1.21 (6 H, s, H-26 and -27), 4.23 (1 H, m, H-3), 4.43 (1 H, m, H-1), 5.01 and 5.33 (each 1 H, m, H-19), 6.02 and 6.38 (each 1 H, d, *J* = 10.9 Hz, H-7 and -6, respectively); IR (KBr) 3400, 2940, 1638, 1381, 1156, 1058, 909 cm^{-1}; UV (95% EtOH) λ_{max} 265 nm, λ_{min} 228 nm; MS *m/z* 430 (M^+, 3), 412 (6), 394 (5), 379 (3), 152 (24), 134 (100), 69 (60), 59 (67), 55 (71); HRMS *m/z* calcd for $C_{28}H_{46}O_3$ 430.3447 (M^+), found 430.3451.

Acknowledgment. We are grateful to Kuraray Co., Ltd. for kindly providing C(22)-alcohol **2**.

Supplementary Material Available: ^1H NMR spectra of compounds **1b**, **1c**, **4**, **10**, **11**, **12b**, **13a**, **13b**, and **14–23** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.