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Design and Synthesis of Potent Vitamin D Receptor Antagonists with A-Ring Modifications: Remarkable Effects of 2α-Methyl Introduction on Antagonistic Activity

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Abstract—Novel A-ring analogues of the vitamin D receptor (VDR) antagonist (**3a**), ZK-159222, and its 24-epimer (**3b**) were convergently synthesized. Preparation of the CD-ring portions with the side chains of **3a**,**b**, followed by palladium-catalyzed cross-coupling with the A-ring enyne precursors (**15a**,**b**), (3*S*,4*S*,5*R*)- and (3*S*,4*S*,5*S*)-bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne, afforded the 2α-methyl-introduced analogues (**4a**,**b**) and their 3-epimers (**5a**,**b**). The biological profiles of the hybrid analogues were assessed in terms of affinity for VDR, and antagonistic activity to inhibit HL-60 cell differentiation induced by the natural hormone, 1α,25-dihydroxyvitamin D_3. The analogue **4a** showed an approximately fivefold higher antagonistic activity compared with **3a**. The 2α-methyl introduction into **3a** increased the receptor affinity, thereby enhancing VDR antagonism. This approach to design potent antagonists based on hybridization of structural motifs in the A-ring and in the side chain may prove to be valuable.

Introduction

Cholecalciferol, known as vitamin D_3, is metabolized via 25-hydroxyvitamin D_3 to afford the hormonally active form, 1α,25-dihydroxyvitamin D_3 (**1**), formation of which is strictly regulated. In addition to its classical role in calcium and phosphorus homeostasis, 1α,25-dihydroxyvitamin D_3 dominates the cell cycle in many malignant cells, regulating proliferation, differentiation and apoptosis.[1] The broad spectrum of biological activities of **1** is considered to be mediated by a ligand-inducible transcriptional factor, vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily.[2] The specific interaction of ligands with the ligand-binding domain (LBD) of VDR has been a major focus of attention, since the X-ray crystal structure of deletion mutant VDR complexed with the natural ligand **1** was solved in 2000.[3] The binding of ligands to VDR triggers conformational changes of the transactivation domain of the receptor C-terminal region (AF-2) in helix-12, which provides an interface for binding to other proteins, such as retinoid X receptors (RXRs), coactivators and corepressors.[4] Thus, ligand-specific structural changes of the receptor, particularly in helix-12, are of great importance, because they can determine whether ligands would function as agonists or antagonists.[5]

Most of the analogues of **1** synthesized so far were modified in the side chain, providing many useful compounds with high potency or selective therapeutic agents, such as 22-oxa-1α,25-dihydroxyvitamin D_3 (OCT),[6] 26,27-cyclo-22-ene-1α,24*S*-dihydroxyvitamin D_3 (MC-903),[7] and super-agonist 20-*epi*-1α,25-dihydroxyvitamin D_3 (MC-1288).[8] Modification of **1** in the A-ring, which bears two critical hydroxyl groups at C1 and C3, has recently become of interest, because the other three A-ring stereoisomers of **1** have proven to exhibit unique activity profiles, distinct from that of the natural hormone **1**.[9] Our study of all eight possible A-ring stereoisomers of 2-methyl-1,25-dihydroxyvitamin D_3 revealed that 2α-methyl-1α,25-dihydroxyvitamin D_3 (**2**) was a four fold better binder to VDR with a two fold higher cell differentiation-inducing activity in HL-60 cells than the natural hormone **1**.[10] In addition, we synthesized the 2-methyl analogues with side-chain

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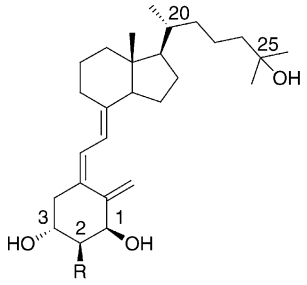
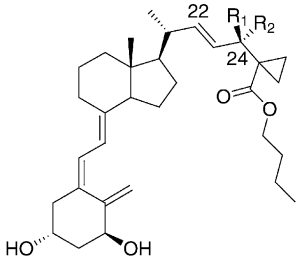
modification, including 20-epimerization, to find that combined modifications both in the A-ring and in the side chain yield super-agonists with exceptionally high potency.[10,11]



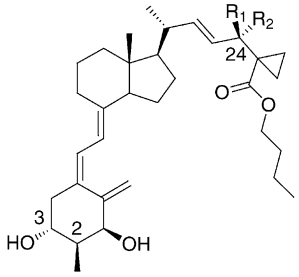
1: R = H
2: R = CH_3



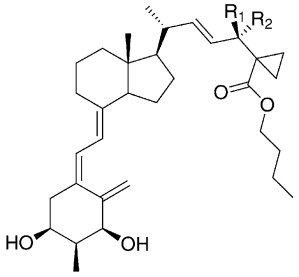
3a: R_1 = OH, R_2 = H (ZK-159222)
3b: R_1 = H, R_2 = OH

Up to date, among a large number of side chain modified analogues of **1**, only two types of VDR antagonists have been identified.[12,13] Initially, analogue **3a** (ZK-159222), which possesses a characteristic long butyl ester at the C25 position, attracted much attention, because it exhibited functional antagonism in many cell lines in the presence of **1**.[12] Interestingly, its chemical structure is identical to that of the potent psoriasis agent, MC-903, apart from the 25-carboxylic acid butyl ester. The second type consists of analogues that antagonize the actions of **1**, as exemplified by TEI-9647 and its 23-epimer TEI-9648, having the 26,23-lactone structure in the side chain.[13] Potent VDR antagonists may find application in the treatment of certain disease states characterized by hypersensitivity to **1**, as observed in patients with Paget's disease of bone.[14]

In this study, we have designed the novel 2α-methyl analogues (**4a**,**5a**) as hybrid analogues modified both in the A-ring and in the side chain. To identify the effects of the stereochemistry at the C24 position on the biological activity, the corresponding 24-epimers (**3b**,**4b**,**5b**) were also synthesized. The major catabolic pathway of **1** is via 24-hydroxylation, followed by side-chain cleavage, to afford virtually inactive metabolites.[1] Recent studies have indicated that the natural hormone **1** and its synthetic analogues are also metabolized to afford their 3-epimers in many cell lines, and their unique activity has recently become a focus of attention.[15] In view of these important results, we also synthesized the 3-*epi* analogues (**5a**,**b**), not only as metabolic probes of **4a**,**b**, but also to investigate how these modifications in the A-ring at the C3 position affect the activity profiles.



4a: R_1 = OH, R_2 = H
4b: R_1 = H, R_2 = OH



5a: R_1 = OH, R_2 = H
5b: R_1 = H, R_2 = OH

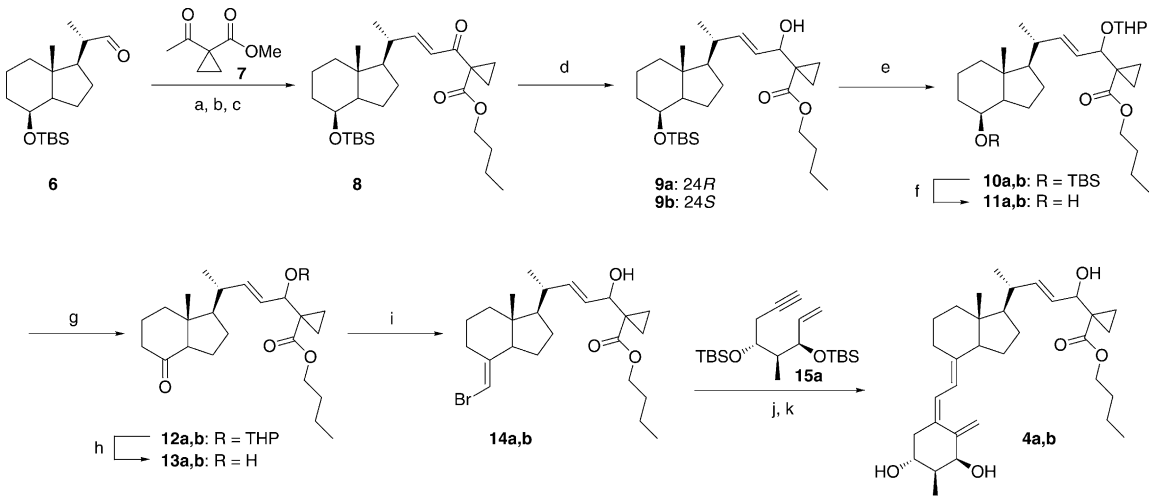
Results and Discussion

Convergent synthesis can be more effective and flexible for preparation of a variety of hybrid analogues than the classical steroidal approach.[16] In particular, a convergent procedure using palladium-catalyzed coupling of the A-ring enyne precursors with the CD-ring portions, pioneered by Trost et al.[17] and successfully applied by us[10,11] and by others,[18] seems most advantageous.

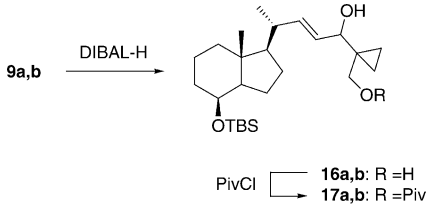
Scheme 1 outlines the synthesis of the CD-ring portion **14a** and its 24-epimer **14b**, and the subsequent coupling with an A-ring enyne **15a**,[19] as exemplified by the (1α,2α,3β)-isomer (steroidal numbering), to produce the 2α-methyl analogues **4a**,**b**. The aldol condensation of the aldehyde **6**[20] with the β-keto ester **7**,[21] followed by trans-esterification via the mesylate, gave the requisite butyl ester **8** in 61% yield in three steps. Regioselective reduction of **8** with $NaBH_4$ in the presence of $CeCl_3{\cdot}7H_2O$ furnished a 24-epimeric mixture of **9a**,**b** (49/51) in 93% yield. Separation of the mixture by using silica gel column chromatography gave each of the diastereomers as a pure specimen, which was then converted to the corresponding derivative to determine the absolute configuration. First, the modified Mosher's method was examined.[22] Reduction of **9a**,**b** with DIBAL-H, followed by selective esterification of the primary alcohol, gave **17a**,**b**, respectively (Scheme 2). The absolute configuration at the C24 position of each isomer was determined by the ^{1}H NMR analysis of its MTPA esters (Fig. 1). Thus, the more polar isomer **9a** has the (24*R*)-configuration, whereas the less polar isomer **9b** has the (24*S*)-configuration. The application of the circular dichroic (CD) exciton chirality method to determine absolute configurations using benzoates of allylic alcohols supported the above conclusion.[23] The CD spectra of the benzoates **18a**,**b** (Scheme 3) showed complementary signs of the Cotton effect; **18a** showed a positive Cotton effect at 230 nm, whereas **18b** showed a negative Cotton effect at 230 nm (Fig. 2).[24] Molecular mechanics calculation using the Merck molecular force field (MMFF) indicated that the dihedral angles of C22-C23-C24-O of these benzoates in the most stable conformations, which determine the signs of the Cotton effects, were −120° in **18a** and +116° in **18b** in accordance with the above observation.[25] In this way, the stereochemistry of **9a**,**b** at the C24 position was confirmed by two separate methods.

Protection of the allylic alcohols in **9a**,**b** with THP gave **10a**,**b** in 89-91% yield. The TBS protecting groups in **10a**,**b** were removed by TBAF treatment to afford **11a**,**b** in 91–99% yield. Oxidation of the secondary alcohols in **11a,b** with tetrapropylammonium perruthenate (TPAP) gave the ketones **12a**,**b** in 91–99% yield. Removal of the THP groups in **12a**,**b** proceeded smoothly in the presence of Me_2AlCl to afford **13a**,**b** in 76% yield.[26] Finally, Wittig reaction of **13a**,**b** furnished the requisite CD-ring bromoolefin **14a**,**b**, respectively, in 36–42% yield.

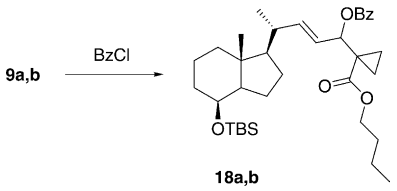
Each of the A-ring enyne precursors **15a**,**b**,[10a–c] prepared separately as we previously reported, was coupled



Scheme 1. Reagents and conditions: (a) LDA/THF, −78–0 °C; (b) MsCl, Et_3N/CH_2Cl_2, 0 °C; (c) *n*-BuOH, DMAP/CH_2Cl_2, −10 °C, 61% (three steps); (d) $NaBH_4$, $CeCl_3{\cdot}7H_2O$/THF-MeOH, 0 °C, 93%; (e) DHP, PPTS/CH_2Cl_2, rt, 89% for **10a**, 91% for **10b**; (f) TBAF/THF, reflux, 99% for **11a**, 86% for **11b**; (g) TPAP, NMO, 4A MS/CH_2Cl_2, rt, 91% for **12a**, 99% for **12b**; (h) Me_2AlCl/CH_2Cl_2, −40 °C, 76% for **12a**, 76% for **12b**; (i) $Ph_3P^+CH_2Br{\cdot}Br^-$, NaHMDS/THF, −60 °C, 36% for **14a**, 42% for **14b**; (j) $(Ph_3P)_4Pd$, Et_3N/toluene, reflux; (k) TBAF/THF, reflux, 22% for **4a**, 15% for **4b** (two steps).



Scheme 2.



Scheme 3.

with each of the CD-ring portions **14a**,**b** by using a palladium catalyst, followed by deprotection with TBAF to give the 2α-methyl analogues **4a**,**b** and their 3-epimers **5a**,**b**. For comparison, ZK-159222 (**3a**) and its 24-epimer **3b** were synthesized by this convergent method using the A-ring enyne, (3*S*,5*R*)-bis[(*tert*-butyldimethylsilyl)oxy]-oct-1-en-7-yne,[10d] instead of **15a**,**b**. In this way, six vitamin D analogues (**3a**,**b**,**4a**,**b**,**5a**,**b**) were obtained, and each of them was purified for biological evaluation by using recycling HPLC.

The VDR binding affinity of the synthesized analogues was examined by using bovine thymus VDR,[27] whose amino acid residues in the LBD are identical with those of human VDR.[28] Table 1 summarizes the relative VDR binding affinity of the synthesized compounds (**3**,**4**,**5**) in comparison with the natural hormone **1** (normalized to 100), together with the 2α-methyl analogue (**2**). The analogue **4a** showed 80% of the affinity of **1**, which suggests that introduction of the 2α-methyl group into **3a** results in an approximately 10-fold enhancement of the affinity for VDR. The 2α-methyl introduction into the other stereoisomer at the C24 position also positively affected the affinity for VDR, as in the case of **4b** and **3b**. The 3-*epi* isomers **5a**,**b** showed weaker affinity than the corresponding C3-natural isomers **4a**,**b**, in accordance with our previous results.[10] Each (24*R*)-isomer (**3a**,**4a**,**5a**) was shown to be a better binder to VDR than its (24*S*)-isomer (**3b**,**4b**,**5b**), suggesting that the 'up' hydroxyl substitution at position 24 would be advantageous.

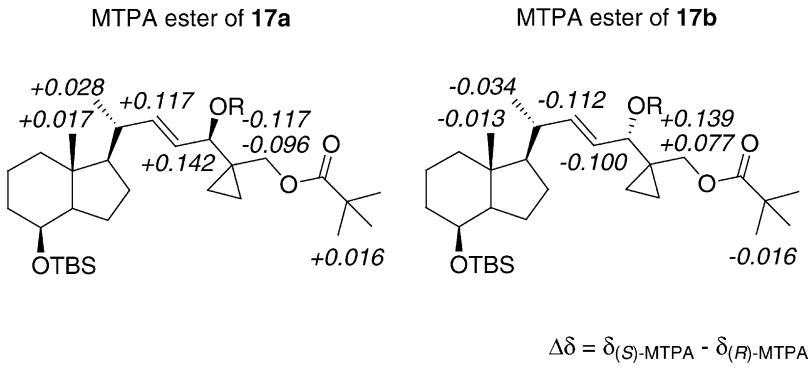


Figure 1. Determination of absolute configuration at the C24 position by ^1H NMR analysis of the MTPA esters.

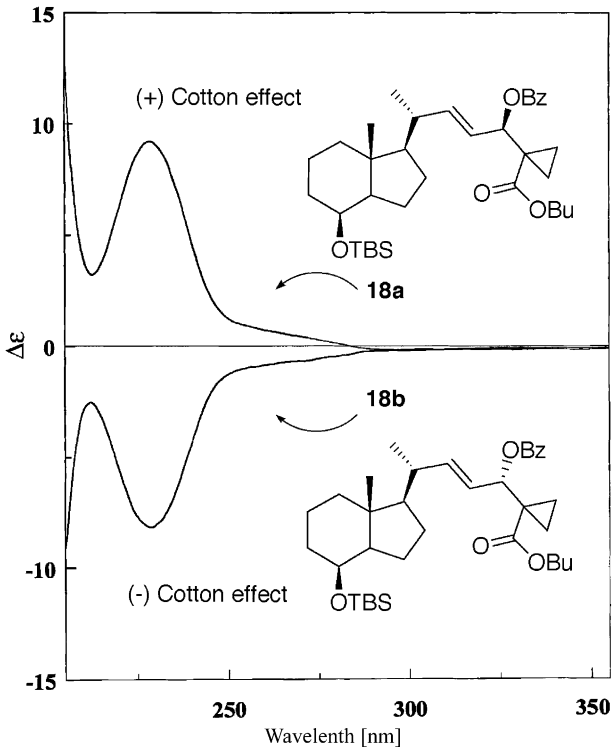


Figure 2. The CD spectra of the allylic benzoates **18a**,**b** in ethanol at 22 °C. The concentration of **18a**,**b** was adjusted to 95 μM using the value of ε 16,400 at 230 nm obtained by UV spectroscopy.[23]

Table 1. Biological activity of the synthesized analogues

Compd	VDR[a] affinity	Antagonistic activity[b] (IC_{50})
1	100[c]	—
2	400[d]	—
3a	7	29 nM
3b	0.03	NA[e]
4a	80	5.6 nM
4b	0.16	NA
5a	0.07	>1 μM
5b	<0.001	NA

[a]Bovine thymus vitamin D receptor.
[b]Antagonistic activity was assessed in terms of 50%-inhibitory concentration (IC_{50}) for differentiation of HL-60 cells induced with 10 nM **1**.
[c]The potency of 1α,25-dihydroxyvitamin D_3 (**1**) is taken as 100.
[d]Refs 10a–c.
[e]Not active; These compounds showed no antagonistic activity at 10^{-6} M in the presence of 10^{-8} M **1**.

The treatment of human promyelocytic leukemia (HL-60) cells with **1** causes monocyte/macrophage differentiation,[29] which is now widely accepted as one of the important actions of **1** through the VDR.[30] The antagonistic activity of the synthesized analogues was examined in terms of the concentration that inhibits 50% of cell differentiation induced by 10 nM **1** in HL-60 cells.[13] The antagonist ZK-159222 (**3a**) gave the IC_{50} value of 29 nM. Interestingly, the 2α-methyl analogue **4a** exhibited the IC_{50} value of 5.6 nM, showing an approximately five fold increase in antagonistic activity compared with **3a**. The 2α-methyl substitution in the A-ring worked as a positive motif to elevate receptor affinity, affording a highly potent antagonist. It is also important to note that the 3-*epi* analogue **5a** showed a weak but distinctive antagonistic activity. On the other hand, the analogues having the (24*S*)-stereochemistry (**3b**,**4b**,**5b**) showed no antagonistic activity. These results suggest that the C24 stereochemistry, not only determines the magnitude of the VDR affinity, but also plays a key role in the shift from agonistic to antagonistic activity.

In summary, we have efficiently synthesized novel vitamin D analogues by employing a convergent method using a palladium catalyst. The procedure should be versatile for the synthesis of a variety of analogues, particularly those with A-ring and side-chain modifications. Biological evaluation has revealed that 2α-methyl introduction into antagonists increases the receptor affinity to yield compounds with an enhanced VDR antagonistic activity. This approach to design unique analogues based on hybridization of the structural motifs in the A-ring and in the side chain may prove to be valuable. The results of broad-spectrum biological screening of the analogues will be reported in due course.

Experimental

General

NMR spectra were recorded on a JEOL ECP-600 or an AL-400 spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JMS-SX 102A. Infrared spectra were recorded on a Jasco FT/IR-8000 spectrometer and are expressed in cm^{-1}. Ultraviolet spectra were recorded with a Shimadzu UV-1600 spectrophotometer. Optical rotations were determined by using a Jasco DIP-370 digital polarimeter. Circular dichroism (CD) spectra were measured on a Jasco J-720 spectropolarimeter. Recycling preparative HPLC was performed on a Shimadzu LC equipped with an LC-6AD HPLC pump and an SPD-10A VP absorbance detector.

Methyl 1-acetyl-cyclopropane-1-carboxylate (7).[21] Bp 85–90 °C/30 mmHg; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.47 (4H, s), 2.46 (3H, s), 3.74 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 19.2, 29.8, 34.9, 52.22, 52.25, 171.5, 202.9; MS *m*/*z* 142 $(M)^+$, 127 $(M-Me)^+$; HRMS calcd for $C_7H_{10}O_3$ 142.0630, found 142.0623.

Butyl (22*E*)-(8*S*)-de-A,B-8-(*tert*-butyldimethylsilyl)oxy-26,27-cyclo-24-oxo-22-cholestene-25-carboxylate (8). To a solution of diisopropylamine (2.6 mL, 18.3 mmol) in dry THF (5 mL) was added with stirring *n*-BuLi (1.6 M in *n*-hexane, 10.4 mL, 16.6 mmol) at 0 °C under an argon atmosphere. The mixture was stirred at 0 °C for 15 min, then a solution of **7**[21] (2.36 g, 16.6 mmol) in THF (5 mL) was introduced into the resulting solution at −78 °C. The whole reaction mixture was stirred at −78 °C for 45 min, then transferred to a solution of aldehyde **6**[20] (895 mg, 2.76 mmol) in THF (5 mL), and the combined solution was stirred at −78 °C for 2 h. After the addition of saturated aqueous NH_4Cl (8 mL),

the reaction mixture was allowed to reach 0 °C. The mixture was diluted with brine (30 mL), and the whole was stirred at room temperature for 30 min. Extraction with ethyl acetate after the addition of 5% oxalic acid, followed by evaporation of the solvent, afforded a crude mixture, which was subjected to the subsequent esterification without further purification. The crude mixture thus obtained was dissolved in CH_2Cl_2 (25 mL) and treated with triethylamine (2.0 mL, 14 mmol) and mesyl chloride (0.58 mL, 7.5 mmol) at 0 °C. The solution was stirred for 45 min, then *n*-butanol (2.2 mL, 24 mmol) and DMAP (118 mg, 0.97 mmol) were added, and the whole was stirred at −10 °C for 1 h. The reaction mixture was poured into cooled saturated aqueous sodium bicarbonate, and the whole was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated. The crude mixture was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/20) to afford **8** (489 mg) as a colorless oil in 61% yield.

8. $[\alpha]_D^{18}$ + 57.6 (*c* 0.95, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.88 (9H, s), 0.92 (3H, t, *J* = 7.4 Hz), 0.95 (3H, s), 1.06 (3H, d, *J* = 6.3 Hz), 4.00 (1H, m), 4.13 (2H, m), 6.41 (1H, d, *J* = 15.4 Hz), 6.75 (1H, dd, *J* = 15.4, 5.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ −5.2, −4.8, 13.7, 14.0, 17.3, 17.4, 16.3, 18.0, 19.1, 19.2, 23.0, 25.8, 27.3, 30.6, 34.1, 39.5, 40.6, 42.5, 52.9, 55.7, 65.2, 69.3, 126.1, 153.1, 171.6, 194.2; IR (neat) 2957, 2934, 2859, 1730, 1682, 1622, 1460 cm^{-1}; MS *m*/*z* 490 $(M)^+$, 475 $(M-Me)^+$, 433 $(M-Bu)^+$; HRMS calcd for $C_{29}H_{50}O_4Si$ 490.3478, found 490.3473.

Butyl (22*E*)-(8*S*,24*R*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-24-hydroxy-22-cholestene-25-carboxylate (9a) and butyl (22*E*)-(8*S*,24*S*)-de-A,B-8-(*tert*-butyldimethylsilyl)oxy-26,27-cyclo-24-hydroxy-22-cholestene-25-carboxylate (9b). A solution of **8** (585 mg, 1.19 mmol) and $CeCl_3 \cdot 7H_2O$ (711 mg, 1.90 mmol) dissolved in THF (2.5 mL) and methanol (7.5 mL) was treated with $NaBH_4$ (131 mg, 3.45 mmol) at 0 °C under an argon atmosphere. It was stirred for 40 min, then water was added, and the whole was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **9a** (more polar, 267 mg) and **9b** (less polar, 278 mg) were separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/9), both as colorless oils, in 93% yield.

9a. $[\alpha]_D^{19}$ +52.5 (*c* 0.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.00 (3H, s), 0.88 (9H, s), 0.92 (3H, s), 0.93 (3H, t, *J* = 7.4 Hz), 1.00 (3H, d, *J* = 6.6 Hz), 3.14 (1H, br.d, *J* = 6.3 Hz), 3.98 (2H, m), 4.09 (2H, t, *J* = 6.2 Hz), 5.38 (1H, dd, *J* = 15.5, 6.7 Hz), 5.48 (1H, dd, *J* = 15.4, 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.6, 11.9, 13.8, 14.1, 14.9, 17.8, 18.2, 19.3, 20.3, 23.2, 25.9, 27.9, 28.6, 30.7, 34.5, 39.6, 40.7, 42.2, 53.1, 56.2, 64.6, 69.4, 74.7, 125.9, 140.0, 174.5; IR (neat) 3536, 2932, 1721, 1460 cm^{-1}; MS *m*/*z* 492 $(M)^+$; HRMS calcd for $C_{29}H_{52}O_4Si$ 492.3635, found 492.3625.

9b. $[\alpha]_D^{17}$ +45.9 (*c* = 0.23, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.00 (3H, s), 0.88 (9H, s), 0.92 (3H, s), 0.93 (3H, t, *J* = 7.3 Hz), 0.99 (3H, d, *J* = 6.6 Hz), 3.09 (1H, br.d, *J* = 7.3 Hz), 3.99 (1H, d, *J* = 2.4 Hz), 4.03 (1H, t, *J* = 6.2 Hz), 4.08 (2H, t, *J* = 6.6 Hz), 5.37 (1H, d, *J* = 15.6, 6.1 Hz), 5.54 (1H, ddd, *J* = 15.4, 8.4, 1.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.6, 11.7, 13.8, 14.1, 14.8, 17.8, 18.2, 19.3, 20.4, 23.2, 26.0, 27.8, 28.5, 30.7, 34.5, 39.5, 40.7, 42.2, 53.1, 56.4, 64.6, 69.4, 74.1, 125.8, 139.5, 174.5; IR (neat) 3480, 2932, 2859, 1719, 1458 cm^{-1}; MS *m*/*z* 492 $(M)^+$; HRMS calcd for $C_{29}H_{52}O_4Si$ 492.3635, found 492.3646.

Butyl (22*E*)-(8*S*,24*R*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-24-(2-tetrahydropyranyl)oxy-22-cholestene-25-carboxylate (10a). A stirred solution of **9a** (884 mg, 1.79 mmol) in CH_2Cl_2 (18 mL) was treated with DHP (0.21 mL, 2.3 mmol) and PPTS (60 mg, 0.23 mmol) at room temperature for 1 h. The reaction mixture was diluted with ether, washed with brine and dried over sodium sulfate. Evaporation of the filtrate gave a residue, from which **10a** (921 mg) was separated by silica gel column chromatography (ethyl acetate: *n*-hexane = 1/9) as a colorless oil in 89% yield.

10a. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02 (3H, s), −0.01 (3H, s), 0.88 (9H, s), 0.91 (3H, s), 0.92 (3H, t, *J* = 7.3 Hz), 0.98 (3H×1/2, d, *J* = 6.3 Hz), 1.00 (3H×1/2, d, *J* = 6.6 Hz), 3.44 (1H, m), 3.86 (1H, m), 3.98 (1H, br. s), 4.05 (2H, t, *J* = 6.6 Hz), 4.43 (1H×1/2, d, *J* = 8.3 Hz), 4.48 (1H×1/2, d, *J* = 7.6 Hz), 4.65 (1H×1/2, m), 4.79 (1H×1/2, t, *J* = 3.2 Hz), 5.17 (1H×1/2, dd, *J* = 15.4, 8.3 Hz), 5.30 (1H×1/2, dd, *J* = 15.4, 7.6 Hz), 5.47 (1H×1/2, dd, *J* = 15.8, 8.8 Hz), 5.51 (1H×1/2, dd, *J* = 15.6, 8.8 Hz)]; IR (neat) 2936, 2859, 1725 cm^{-1}; MS *m*/*z* 519 $(M-t\text{-}Bu)^+$; HRMS calcd for $C_{30}H_{51}O_5Si$ 519.3506, found 519.3505.

Butyl (22*E*)-(8*S*,24*S*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-24-(2-tetrahydropyranyl)oxy-22-cholestene-25-carboxylate (10b). This compound was obtained by the same procedure as described for **10a** by using **9b** instead of **9a**, in 91% yield.

10b. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.00 (3H, s), 0.88 (9H, s), 0.92 (3H, s), 0.93 (3H, t, *J* = 7.3 Hz), 0.97 (3H, d, *J* = 6.6 Hz), 3.46 (1H, m), 3.87 (1H, m), 3.99 (1H, br. s), 4.05 (2H, t, *J* = 6.6 Hz), 4.45 (1H×1/2, d, *J* = 8.3 Hz), 4.53 (1H×1/2, d, *J* = 7.1 Hz), 4.67 (1H×1/2, m), 4.78 (1H×1/2, t, *J* = 3.4 Hz), 5.17 (1H×1/2, dd, *J* = 15.4, 8.3 Hz), 5.32 (1H×1/2, dd, *J* = 15.5, 7.2 Hz), 5.54 (1H, dd, *J* = 15.4, 8.8 Hz); IR (neat) 2934, 2861, 1727 cm^{-1}; MS *m*/*z* 519 $(M-t\text{-}Bu)^+$; HRMS calcd for $C_{30}H_{51}O_5Si$ 519.3506, found 519.3502.

Butyl (22*E*)-(8*S*,24*R*)-de-A,B-26,27-cyclo-8-hydroxy-24-(2-tetrahydropyranyl)oxy-22-cholestene-25-carboxylate (11a). A solution of **10a** (515 mg, 0.89 mmol) in THF (5 mL) was treated with TBAF (1.0 M in THF, 8.9 mL, 8.9 mmol) at 50 °C for 5 days. The reaction mixture was cooled and water was added. After extraction with ethyl acetate, the organic layer was washed with brine and dried over sodium sulfate. Evaporation of the filtrate

gave a residue, from which **11a** (407 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/9) as a colorless oil in 99% yield.

11a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, t, J = 7.3 Hz), 0.92 (3H×1/2, s), 0.94 (3H×1/2, s), 1.00 (3H×1/2, d, J = 6.3 Hz), 1.01 (3H×1/2, d, J = 6.6 Hz), 3.45 (1H, m), 3.86 (1H, m), 4.06 (2H, t, J = 6.6 Hz), 4.07 (1H, m), 4.42 (1H×1/2, d, J = 8.1 Hz), 4.48 (1H×1/2, d, J = 7.3 Hz), 4.66 (1H×1/2, t, J = 3.4 Hz), 4.79 (1H×1/2, t, J = 3.2 Hz), 5.21 (1H×1/2, dd, J = 15.4, 8.3 Hz), 5.34 (1H×1/2, dd, J = 15.4, 7.3 Hz), 5.48 (1H×1/2, dd, J = 15.4, 8.8 Hz), 5.52 (1H×1/2, dd, J = 15.4, 8.8 Hz); IR (neat) 3494, 2934, 2870, 1722 cm^{-1}; MS m/z 377 $(M-THP)^+$; HRMS calcd for $C_{23}H_{37}O_4$ 377.2692, found 377.2687.

Butyl (22*E*)-(8*S*,24*S*)-de-A,B-26,27-cyclo-8-hydroxy-24-(2-tetrahydropyranyl)oxy-22-cholestene-25-carboxylate (11b). This compound was obtained by the same procedure as described for **11a** by using **10b** instead of **10a**, in 86% yield.

11b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, t, J = 7.3 Hz), 0.94 (3H, s), 0.98 (3H, d, J = 6.6 Hz), 3.45 (1H, m), 3.86 (1H, m), 4.05 (2H×1/2, t, J = 6.6 Hz), 4.06 (2H×1/2, t, J = 6.6 Hz), 4.07 (1H, m), 4.44 (1H×1/2, d, J = 8.3 Hz), 4.53 (1H×1/2, d, J = 7.1 Hz), 4.67 (1H×1/2, t, J = 3.4 Hz), 4.77 (1H×1/2, t, J = 3.0 Hz), 5.20 (1H×1/2, dd, J = 15.4, 8.3 Hz), 5.34 (1H×1/2, dd, J = 15.4, 7.1 Hz), 5.50 (1H×1/2, dd, J = 15.6, 8.3 Hz), 5.51 (1H×1/2, dd, J = 15.4, 8.8 Hz); IR (neat) 3532, 2934, 2869, 1723 cm^{-1}; MS m/z 462 $(M)^+$; HRMS calcd for $C_{28}H_{46}O_5$ 462.3345, found 462.3359.

Butyl (22*E*)-(24*R*)-de-A,B-26,27-cyclo-8-oxo-24-(2-tetrahydropyranyl)oxy-22-cholestene-25-carboxylate (12a). Solid tetrapropylammonium perruthenate (TPAP) (188 mg, 0.53 mmol) was added to a stirred mixture of **11a** (411 g, 0.89 mmol), 4-methylmorpholine *N*-oxide (NMO) (270 mg, 2.31 mmol) and powdered 4A MS (93 mg) in CH_2Cl_2 (9 mL) at room temperature under argon. The resulting mixture was stirred for 5 min, and separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/4) to give **12a** (373 mg) as a colorless oil in 91% yield.

12a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (3H, s), 0.92 (3H, t, J = 7.3 Hz), 1.06 (3H×1/2, d, J = 6.6 Hz), 1.07 (3H×1/2, t, J = 6.6 Hz), 2.45 (1H, m), 3.46 (1H, m), 3.85 (1H, m), 4.05 (2H, t, J = 6.7 Hz), 4.42 (1H×1/2, d, J = 8.1 Hz), 4.48 (1H×1/2, d, J = 7.1 Hz), 4.65 (1H×1/2, t, J = 3.2 Hz), 4.77 (1H×1/2, t, J = 3.0 Hz), 5.26 (1H×1/2, dd, J = 15.4, 8.1 Hz), 5.38 (1H×1/2, dd, J = 15.4, 7.3 Hz), 5.51 (1H×1/2, dd, J = 15.4, 8.5 Hz), 5.54 (1H×1/2, dd, J = 15.4, 8.8 Hz); IR (neat) 2957, 2872, 1717 cm^{-1}; MS m/z 460 $(M)^+$; HRMS calcd for $C_{28}H_{44}O_5$ 460.3189, found 460.3139.

Butyl (22*E*)-(24*S*)-de-A,B-26,27-cyclo-8-oxo-24-(2-tetrahydropyranyl)oxy-22-cholestene-25-carboxylate (12b). This compound was obtained by the same procedure as described for **12a** by using **11b** instead of **11a**, in 99% yield.

12b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (3H, s), 0.92 (3H, t, J = 7.3 Hz), 1.04 (3H, d, J = 6.6 Hz), 2.45 (1H, m), 3.46 (1H, m), 3.85 (1H, m), 4.05 (2H, t, J = 6.6 Hz), 4.44 (1H×1/2, d, J = 8.1 Hz), 4.52 (1H×1/2, d, J = 7.0 Hz), 4.65 (1H×1/2, t, J = 3.4 Hz), 4.75 (1H×1/2, t, J = 3.9 Hz), 5.25 (1H×1/2, dd, J = 15.4, 8.1 Hz), 5.39 (1H×1/2, dd, J = 15.4, 6.8 Hz), 5.53 (1H, dd, J = 15.4, 8.8 Hz); IR (neat) 2955, 2872, 1717 cm^{-1}; MS m/z 460 $(M)^+$; HRMS calcd for $C_{28}H_{44}O_5$ 460.3189, found 460.3191.

Butyl (22*E*)-(24*R*)-de-A,B-26,27-cyclo-24-hydroxy-8-oxo-22-cholestene-25-carboxylate (13a). To a solution of **12a** (130 mg, 0.28 mmol) in dry CH_2Cl_2 (5 mL) was added with stirring Me_2AlCl (1.0 M in *n*-hexane, 0.56 mL, 0.56 mmol) at −40 °C under an argon atmosphere. The mixture was stirred for 1.5 h, then saturated aqueous sodium bicarbonate (9 mL) was added, and the whole was extracted with CH_2Cl_2. The organic layer was dried over magnesium sulfate and filtered. Evaporation of the solvent gave a residue, from which **13a** (81 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/4) as a colorless oil in 76% yield.

13a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (3H, s), 0.92 (3H, t, J = 7.3 Hz), 1.06 (3H, d, J = 6.6 Hz), 3.92 (1H, br. d, J = 4.9 Hz), 4.08 (2H, t, J = 6.6 Hz), 5.44 (1H, dd, J = 15.6, 5.9 Hz), 5.50 (1H, dd, J = 15.6, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 12.8, 13.8, 14.9, 19.2, 19.3, 20.5, 24.1, 27.8, 28.5, 30.7, 38.9, 39.6, 41.0, 49.8, 56.1, 62.0, 64.6, 74.7, 127.0, 138.9, 174.4, 211.4; IR (neat) 3499, 2961, 2874, 1715 cm^{-1}; MS m/z 376 $(M)^+$; HRMS calcd for $C_{23}H_{36}O_4$ 376.2614, found 376.2604.

Butyl (22*E*)-(24*S*)-de-A,B-26,27-cyclo-24-hydroxy-8-oxo-22-cholestene-25-carboxylate (13b). This compound was obtained by the same procedure as described for **13a** by using **12b** instead of **12a**, in 76% yield.

13b. $[\alpha]_D^{18}$ −16.4 (c = 1.37, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (3H, s), 0.92 (3H, t, J = 7.4 Hz), 1.05 (3H, d, J = 6.8 Hz), 3.97 (1H, br. d, J = 5.9 Hz), 4.07 (2H, t, J = 6.6 Hz), 5.43 (1H, dd, J = 15.4, 5.9 Hz), 5.50 (1H, ddd, J = 15.6, 8.5, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.9, 13.8, 14.8, 19.2, 19.3, 20.6, 24.1, 27.8, 28.4, 30.7, 38.9, 39.5, 41.0, 49.8, 56.2, 61.9, 64.6, 74.1, 126.8, 138.2, 174.4, 211.4; IR (neat) 3505, 2959, 2874, 1715 cm^{-1}; MS m/z 376 $(M)^+$; HRMS calcd for $C_{23}H_{36}O_4$ 376.2614, found 376.2614.

Butyl (8*E*,22*E*)-(24*R*)-de-A,B-8-bromomethylene-26,27-cyclo-24-hydroxy-22-cholestene-25-carboxylate (14a). To a solution of (bromomethyl)triphenylphosphonium bromide (636 mg, 1.48 mmol) in THF (3 mL) was added NaHMDS (1.0 M in THF, 1.4 mL, 1.4 mmol) under an argon atmosphere at −60 °C. The mixture was stirred for 1 h at −40 °C, then a solution of **13a** (37 mg, 0.098 mmol) in THF (3 mL) was introduced at −60 °C, and stirring was continued at −40 °C for 1.5 h, and then at −10 °C for 1 h. After another 30 min at room temperature, the reaction mixture was diluted with ether and the

whole was filtered over Celite™. Evaporation of the solvent gave a residue, from which **14a** (16 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/5) as a pale yellow oil in 36% yield.

14a. $[\alpha]_D^{18}$ +94.5 (c = 0.48, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (3H, s), 0.93 (3H, t, J = 7.3 Hz), 1.04 (3H, d, J = 6.3 Hz), 1.97 (2H, br. d, J = 12.2 Hz), 2.07 (1H, m), 2.87 (1H, m), 3.18 (1H, d, J = 7.1 Hz), 3.94 (1H, br. t, J = 6.7 Hz), 4.09 (2H, t, J = 6.6 Hz), 5.43 (1H, dd, J = 15.4, 6.1 Hz), 5.50 (1H, dd, J = 15.5, 7.9 Hz), 5.64 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 12.2, 13.9, 14.9, 19.3, 20.6, 22.1, 22.6, 27.8, 28.5, 30.7, 31.1, 39.8, 40.1, 45.5, 55.3, 55.9, 64.6, 74.7, 97.4, 126.6, 139.2, 144.7, 174.5; IR (neat) 3412, 2953, 2869, 1703 cm^{-1}; MS m/z 452 $(M)^+$; HRMS calcd for $C_{24}H_{37}O_3{}^{79}Br$ 452.1926, found 452.1921.

Butyl (8*E*,22*E*)-(24*S*)-de-A,B-8-bromomethylene-26,27-cyclo-24-hydroxy-22-cholestene-25-carboxylate (14b). This compound was obtained by the same procedure as described for **14a** by using **13b** instead of **13a**, in 42% yield.

14b. $[\alpha]_D^{17}$ +62.4 (c = 0.46, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (3H, s), 0.93 (3H, t, J = 7.4 Hz), 1.03 (3H, d, J = 6.6 Hz), 1.97 (2H, m), 2.08 (1H, m), 2.87 (1H, m), 3.15 (1H, d, J = 7.3 Hz), 4.00 (1H, br. t, J = 6.6 Hz), 4.08 (2H, t, J = 6.6 Hz), 5.42 (1H, dd, J = 15.4, 6.1 Hz), 5.54 (1H, dd, J = 15.5, 8.4 Hz), 5.64 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.2, 13.8, 14.8, 19.3, 20.6, 22.2, 22.6, 27.8, 28.5, 30.7, 31.1, 39.8, 40.0, 45.5, 55.3, 55.9, 64.6, 74.2, 97.4, 126.4, 138.9, 144.7, 174.5; IR (neat) 3400, 2957, 2930, 2853, 1717 cm^{-1}; MS m/z 452 $(M)^+$; HRMS calcd for $C_{24}H_{37}O_3{}^{79}Br$ 452.1926, found 452.1921.

Butyl (5*Z*,7*E*,22*E*)-(1*S*,3*R*,24*R*)-26,27-cyclo-1,3,24-trihydroxy-9,10-*seco*-5,7,10(19),22-cholestatetraene-25-carboxylate (3a). **3a**: $[\alpha]_D^{18}$ +25.3 (c 0.14, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.84 (1H, m), 0.93 (3H, t, J = 7.4 Hz), 1.04 (3H, d, J = 6.3 Hz), 2.31 (1H, dd, J = 13.4, 6.6 Hz), 2.60 (1H, dd, J = 13.7, 3.2 Hz), 2.83 (1H, dd, J = 12.2, 3.7 Hz), 3.17 (1H, d, J = 7.1 Hz), 3.97 (1H, t, J = 6.8 Hz), 4.08 (2H, t, J = 6.8 Hz), 4.23 (1H, m), 4.43 (1H, m), 4.99 (1H, s), 5.32 (1H, s), 5.41 (1H, dd, J = 15.4, 6.3 Hz), 5.51 (1H, dd, J = 15.4, 8.5 Hz), 6.01 (1H, d, J = 11.2 Hz), 6.37 (1H, d, J = 11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 12.4, 13.9, 14.9, 19.3, 20.6, 22.4, 23.7, 27.9, 28.5, 29.1, 30.7, 40.2, 40.4, 43.0, 45.3, 46.0, 56.0, 56.4, 64.6, 66.9, 70.8, 74.7, 111.6, 117.0, 124.8, 126.3, 132.8, 139.5, 142.7, 147.5, 174.5; IR (neat) 3422, 2947, 2930, 2870, 1701 cm^{-1}; MS m/z 512 $(M)^+$, 494 $(M-H_2O)^+$, 476 $(M-2H_2O)^+$; HRMS calcd for $C_{32}H_{48}O_5$ 512.3502, found 512.3494.

Butyl (5*Z*,7*E*,22*E*)-(1*S*,3*R*,24*S*)-26,27-cyclo-1,3,24-trihydroxy-9,10-*seco*-5,7,10(19),22-cholestatetraene-25-carboxylate (3b). **3b**: $[\alpha]_D^{18}$ +15.9 (c = 0.24, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.84 (1H, m), 0.93 (3H, t, J = 7.3 Hz), 1.02 (3H, d, J = 6.6 Hz), 2.31 (1H, dd, J = 13.4, 6.6 Hz), 2.60 (1H, dd, J = 13.7, 3.4 Hz), 2.83 (1H, dd, J = 12.9, 4.4 Hz), 3.15 (1H, d, J = 6.8 Hz), 4.01 (1H, t, J = 6.3 Hz), 4.08 (2H, t, J = 6.9 Hz), 4.23 (1H, m), 4.43, (1H, m), 5.00 (1H, s), 5.32 (1H, s), 5.40 (1H, dd, J = 15.4, 6.4 Hz), 5.50 (1H, dd, J = 15.9, 8.3 Hz), 6.01 (1H, d, J = 11.0 Hz), 6.38 (1H, d, J = 11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.4, 13.9, 14.8, 19.3, 20.7, 22.4, 23.7, 27.8, 28.5, 29.2, 30.7, 40.1, 40.4, 42.9, 45.3, 46.0, 56.1, 56.4, 64.6, 66.9, 70.8, 74.2, 111.7, 117.0, 124.8, 126.2, 132.8, 139.2, 142.8, 147.4, 174.5; IR (neat) 3337, 2932, 2870, 1703 cm^{-1}; MS m/z 512 $(M)^+$, 494 $(M-H_2O)^+$, 476 $(M-2H_2O)^+$; HRMS calcd for $C_{32}H_{48}O_5$ 512.3502, found 512.3507.

Butyl (5*Z*,7*E*,22*E*)-(1*S*,2*S*,3*R*,24*R*)-26,27-cyclo-1,3,24-trihydroxy-2-methyl-9,10-*seco*-5,7,10(19),22-cholestatetraene-25-carboxylate (4a). A mixture of the CD-ring portion **14a** (14 mg, 32 μmol), tetrakis(triphenylphosphine)palladium (12 mg, 11 μmol) and triethylamine (2 mL) in toluene (1 mL) was stirred for 20 min at room temperature, then a solution of the A-ring enyne precursor **15a**[10a–c] (23 mg, 61 μmol) in toluene (1 mL) was added. After having been heated at reflux for 7 h and diluted with ether, the reaction mixture was filtered through a pad of silica gel with ether. After evaporation of the solvent, the residue was subjected to silica gel preparative TLC (ethyl acetate/*n*-hexane = 1/4) to give a crude mixture of the protected vitamins (14 mg). The crude mixture, dissolved in THF (1 mL), was treated with TBAF (1.0 M in THF, 0.1 mL, 0.1 mmol) at reflux for 8 h. Brine was added to the cooled reaction mixture and the whole was extracted with ethyl acetate. The combined organic layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel preparative TLC (ethyl acetate/*n*-hexane = 2/3) to give **4a** (3.7 mg, 22% in two steps). Further purification of **4a** was conducted by using a recycling HPLC (Shim-Pack PREP-SIL column, 20×250 mm, 10.0 mL/min, ethyl acetate/*n*-hexane = 2/1).

4a. UV (EtOH) λ_{max} 267 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.83 (1H, m), 0.93 (3H, t, J = 7.6 Hz), 1.04 (3H, d, J = 6.6 Hz), 1.08 (3H, d, J = 6.8 Hz), 1.92 (1H, dd, J = 7.8, 3.4, 6.8 Hz), 2.23 (3H, dd, J = 13.9, 7.6 Hz), 2.67 (1H, dd, J = 13.4, 3.9 Hz), 2.82 (1H, dd, J = 12.9, 4.9 Hz), 3.17 (1H, br. s), 3.84 (1H, dt, J = 4.4, 7.8 Hz), 3.94 (1H, d, J = 6.6 Hz), 4.09 (2H, t, J = 6.6 Hz), 4.30 (1H, d, J = 3.4 Hz), 5.00 (1H, d, J = 2.2 Hz), 5.27 (1H, s), 5.41 (1H, dd, J = 15.4, 6.6 Hz), 5.51 (1H, dd, J = 15.6, 8.3 Hz), 6.00 (1H, d, J = 11.0 Hz), 6.39 (1H, d, J = 11.5 Hz); MS m/z 526 $(M)^+$; HRMS calcd for $C_{33}H_{50}O_5$ 526.3658, found 526.3659.

Butyl (5*Z*,7*E*,22*E*)-(1*S*,2*S*,3*R*,24*S*)-26,27-cyclo-1,3,24-trihydroxy-2-methyl-9,10-*seco*-5,7,10(19),22-cholestatetraene-25-carboxylate (4b). This compound was obtained by the same procedure as described for **4a** by using **15b** instead of **15a**, in 15% yield.

4b. UV (EtOH) λ_{max} 267 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.83 (1H, m), 0.93 (3H, t, J = 7.4 Hz), 1.02 (3H, d, J = 6.6 Hz), 1.08 (3H, d, J = 6.8 Hz), 1.92 (1H, dd, J = 7.8, 3.4, 6.8 Hz), 2.31 (1H,

dd, $J=13.4$, 6.6 Hz), 2.60 (1H, dd, $J=13.7$, 3.4 Hz), 2.83 (1H, dd, $J=12.9$, 4.4 Hz), 3.15 (1H, d, $J=7.1$ Hz), 4.01 (1H, t, $J=6.4$ Hz), 4.08 (2H, t, $J=6.8$ Hz), 4.23 (1H, m), 4.43 (1H, m), 5.00 (1H, s), 5.33 (1H, s), 5.40 (1H, dd, $J=15.4$, 6.4 Hz), 5.50 (1H, dd, $J=15.9$, 8.6 Hz), 6.01 (1H, d, $J=11.0$ Hz), 6.38 (1H, d, $J=11.2$ Hz); MS m/z 526 $(M)^+$; HRMS calcd for $C_{33}H_{50}O_5$ 526.3658, found 526.3645.

Butyl (5*Z*,7*E*,22*E*)-(1*S*,2*S*,3*S*,24*R*)-26,27-cyclo-1,3,24-trihydroxy-2-methyl-9,10-*seco*-5,7,10(19),22-cholestatetraene-25-carboxylate (5a). This compound was obtained by the same procedure as described for **4a** by using **14b** instead of **14a**, in 48% yield.

5a. UV (EtOH) λ_{max} 267 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.83 (1H, m), 0.93 (3H, t, $J=7.3$ Hz), 1.04 (3H, d, $J=6.6$ Hz), 1.22 (3H, d, $J=7.1$ Hz), 2.49 (1H, d, $J=13.4$ Hz), 2.57 (1H, dd, $J=13.9$, 3.7 Hz), 2.79 (1H, d, $J=7.8$ Hz), 2.85 (1H, m), 3.17 (1H, d, $J=7.3$ Hz), 3.91 (1H, m), 3.97 (1H, t, $J=6.8$ Hz), 4.09 (2H, t, $J=6.6$ Hz), 4.17 (1H, m), 4.96 (1H, d, $J=2.0$ Hz), 5.22 (1H, d, $J=2.0$ Hz), 5.41 (1H, dd, $J=15.6$, 6.4 Hz), 5.51 (1H, dd, $J=15.1$, 8.1 Hz), 6.02 (1H, d, $J=11.5$ Hz), 6.47 (1H, d, $J=10.5$ Hz); MS m/z 526 $(M)^+$; HRMS calcd for $C_{33}H_{50}O_5$ 526.3658, found 526.3664.

Butyl (5*Z*,7*E*,22*E*)-(1*S*,2*S*,3*S*,24*S*)-26,27-cyclo-1,3,24-trihydroxy-2-methyl-9,10-*seco*-5,7,10(19),22-cholestatetraene-25-carboxylate (5b). This compound was obtained by the same procedure as described for **4a** by using **14b** and **15b**, instead of **14a** and **15a**, respectively, in 35% yield.

5b. UV (EtOH) λ_{max} 266 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.83 (1H, m), 0.93 (3H, t, $J=7.2$ Hz), 1.02 (3H, d, $J=6.6$ Hz), 1.22 (3H, d, $J=7.3$ Hz), 2.49 (1H, br. d, $J=13.4$ Hz), 2.58 (1H, dd, $J=13.9$, 3.4 Hz), 2.81 (1H, d, $J=8.1$ Hz), 2.84 (1H, m), 3.14 (1H, d, $J=6.8$ Hz), 3.91 (1H, m), 4.02 (1H, t, $J=6.4$ Hz), 4.08 (3H, t, $J=6.4$ Hz), 4.17 (1H, m), 4.94 (1H, d, $J=2.2$ Hz), 5.23 (1H, d, $J=2.0$ Hz), 5.40 (1H, dd, $J=15.4$, 6.1 Hz), 5.50 (1H, dd, $J=15.6$, 8.8 Hz), 6.02 (1H, d, $J=11.5$ Hz), 6.47 (1H, d, $J=11.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.5, 13.9, 14.2, 14.8, 19.3, 20.7, 20.9, 23.5, 27.9, 28.5, 29.1, 30.7, 40.1, 40.3, 40.5, 45.9, 53.5, 56.1, 56.3, 64.6, 72.5, 74.2, 78.6, 114.0, 117.0, 126.0, 126.1, 130.8, 139.3, 142.5, 146.9, 174.5; MS m/z 526 $(M)^+$; HRMS calcd for $C_{33}H_{50}O_5$ 526.3658, found 526.3652.

Butyl (22*E*)-(8*S*,24*R*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-25-hydroxymethyl-22-cholesten-24-ol (16a). To a solution of **9a** (92 mg, 0.19 mmol) in toluene (0.8 mL) was added with stirring DIBAL-H (1.0 M in toluene, 0.87 mL, 0.87 mmol) at −78 °C under an argon atmosphere, and the resulting mixture was stirred for 100 min. To the reaction mixture was added aqueous 1N HCl (3 mL) and the whole was extracted with ether. The organic layer was washed with brine and dried over sodium sulfate. Evaporation of the filtrate gave a residue, from which **16a** (41 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/5) as a colorless oil in 51% yield.

16a. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.41 (1H, m), 0.55 (3H, m), 0.88 (9H, m), 0.93 (3H, s), 1.02 (3H, d, $J=6.6$ Hz), 3.33 (1H, d, $J=11.2$ Hz), 3.83 (1H, d, $J=5.4$ Hz), 3.85 (1H, d, $J=10.3$ Hz), 3.99 (1H, d, $J=2.0$ Hz), 5.44 (1H, dd, $J=15.4$, 6.3 Hz), 5.52 (1H, d, $J=15.4$, 8.3 Hz); MS m/z 404 $(M-H_2O)^+$, 386 $(M-2H_2O)^+$, 347 $(M-H_2O-t\text{-Bu})^+$; HRMS calcd for $C_{25}H_{44}O_2Si$ 404.3111, found 404.3117.

Butyl (22*E*)-(8*S*,24*S*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-25-hydroxymethyl-22-cholesten-24-ol (16b). This compound was obtained by the same procedure as described for **16a** by using **9b** instead of **9a**, in 38% yield.

16b. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.41 (1H, m), 0.50 (1H, m), 0.60 (1H, m), 0.89 (9H, s), 0.94 (3H, s), 1.01 (3H, d, $J=6.6$ Hz), 3.28 (1H, d, $J=11.5$ Hz), 3.83 (1H, d, $J=5.6$ Hz), 3.89 (1H, d, $J=11.2$ Hz), 4.00 (1H, d, $J=2.2$ Hz), 5.44 (1H, dd, $J=15.4$, 5.6 Hz), 5.58 (1H, ddd, $J=15.4$, 8.5, 1.2 Hz); MS m/z 404 $(M-H_2O)^+$, 386 $(M-2H_2O)^+$, 347 $(M-H_2O-t\text{-Bu})^+$; HRMS calcd for $C_{25}H_{44}O_2Si$ 404.3111, found 404.3113.

Butyl (22*E*)-(8*S*,24*R*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-25-(pivalyloxy)methyl-22-cholesten-24-ol (17a). To a solution of **16a** (41 mg, 97 μmol) in pyridine (0.4 mL) was added with stirring pivalyl chloride (59 μL, 0.49 mmol) at 0 °C under an argon atmosphere, and the resulting mixture was stirred for 1 h. The reaction mixture was diluted with ether. The organic layer was washed with aqueous 1N HCl, followed by with brine, and dried over sodium sulfate. Evaporation of the filtrate gave a residue, from which **17a** (25 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1/9) as a colorless oil in 51% yield.

17a. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02 (3H, s), 0.00 (3H, s), 0.48 (1H, m), 0.58 (3H, m), 0.88 (9H, s), 0.92 (3H, s), 1.00 (3H, d, $J=6.6$ Hz), 1.21 (9H, s), 3.81 (1H, d, $J=7.1$ Hz), 3.90 (1H, d, $J=11.5$ Hz), 3.99 (1H, br. d, $J=2.4$ Hz), 4.20 (1H, d, $J=11.7$ Hz), 5.36 (1H, dd, $J=15.3$, 7.0 Hz), 5.50 (1H, dd, $J=15.9$, 8.6 Hz); MS m/z 506 $(M)^+$; HRMS calcd for $C_{30}H_{54}O_4Si$ 506.3791, found 506.3796.

Butyl (22*E*)-(8*S*,24*S*)-de-A,B-8-(*tert*-butyldimethylsilyl)-oxy-26,27-cyclo-25-(pivalyloxy)methyl-22-cholesten-24-ol (17b). This compound was obtained by the same procedure as described for **17a** by using **16b** instead of **16a**, in 38% yield.

17b. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.00 (3H, s), 0.48 (1H, m), 0.56 (1H, m), 0.60 (1H, m), 0.65 (1H, m), 0.88 (9H, s), 0.93 (3H, s), 1.00 (3H, d, $J=6.3$ Hz), 1.22 (9H, s), 3.83 (1H, br. d, $J=5.2$ Hz), 3.88 (1H, d, $J=11.5$ Hz), 3.99 (1H, br. d, $J=2.5$ Hz), 4.21 (1H, d, $J=11.8$ Hz), 5.37 (1H, ddd, $J=15.4$, 6.0, 0.6 Hz), 5.55 (1H, ddd, $J=15.4$, 8.7, 1.2 Hz); MS m/z

449 (M−*t*-Bu)$^+$; HRMS calcd for $C_{26}H_{45}O_4Si$ 449.3087, found 449.3081.

General procedure for synthesis of MTPA esters. A solution of each of the above compounds **17a**,**b** dissolved in dry CH_2Cl_2 was treated with DMAP (5 equiv) and (*R*)- or (*S*)-MTPACl (4 equiv) at room temperature under an argon atmosphere. The reaction mixture was purified by silica gel preparative TLC (ethyl acetate/*n*-hexane = 1/9) without pretreatment to afford the corresponding MTPA ester.

(*S*)-MTPA ester of 17a. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.012 (3H, s), 0.006 (3H, s), 0.597 (4H, m), 0.884 (9H, s), 0.920 (3H, s), 0.991 (3H, d, *J* = 6.4 Hz), 1.206 (9H, s), 3.531 (3H, s), 3.828 (1H, d, *J* = 12.0 Hz), 3.916 (1H, d, *J* = 11.7 Hz), 3.992 (1H, br, s), 5.356 (1H, dd, *J* = 15.0, 8.7 Hz), 5.458 (1H, d, *J* = 8.5 Hz), 5.699 (1H, dd, *J* = 15.1, 8.8 Hz), 7.382 (3H, m), 7.496 (2H, m); MS *m*/*z* 722 (M)$^+$; HRMS calcd for $C_{40}H_{61}O_6F_3Si$ 722.4190, found 722.4196.

(*R*)-MTPA ester of 17a. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.011 (3H, s), 0.006 (3H, s), 0.883 (9H, s), 0.903 (3H, s), 0.963 (3H, d, *J* = 6.6 Hz), 1.190 (9H, s), 3.551 (3H, s), 3.945 (1H, d, *J* = 11.7 Hz), 3.997 (1H, s), 4.012 (1H, d, *J* = 12.0 Hz), 5.214 (1H, dd, *J* = 15.1, 8.1 Hz), 5.336 (1H, d, *J* = 8.1 Hz), 5.582 (1H, dd, *J* = 15.3, 8.9 Hz), 7.376 (3H, m), 7.487 (2H, m); MS *m*/*z* 722 (M)$^+$; HRMS calcd for $C_{40}H_{61}O_6F_3Si$ 722.4190, found 722.4193.

(*S*)-MTPA ester of 17b. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.007 (3H, s), 0.007 (3H, s), 0.886 (9H, s), 0.902 (3H, s), 0.958 (3H, d, *J* = 6.6 Hz), 1.190 (9H, s), 3.549 (3H, s), 3.961 (1H, d, *J* = 12.0 Hz), 3.987 (1H, s), 4.002 (1H, d, *J* = 11.7 Hz), 5.238 (1H, dd, *J* = 14.9, 8.1 Hz), 5.303 (1H, d, *J* = 7.8 Hz), 5.555 (1H, dd, *J* = 14.9, 9.0 Hz), 7.625 (3H, m), 7.507 (2H, m); MS *m*/*z* 665 (M−*t*-Bu)$^+$; HRMS calcd for $C_{36}H_{52}O_6F_3Si$ 665.3485, found 665.3481.

(*R*)-MTPA ester of 17b. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.006 (3H, s), 0.007 (3H, s), 0.552 (4H, br. s), 0.619 (2H, m), 0.889 (9H, s), 0.992 (3H, d, *J* = 6.6 Hz), 1.206 (9H, s), 3.538 (3H, s), 3.822 (1H, d, *J* = 11.7 Hz), 3.925 (1H, d, *J* = 11.7 Hz), 3.983 (1H, br. d, *J* = 2.0 Hz), 5.338 (1H, dd, *J* = 15.0, 8.4 Hz), 5.420 (1H, d, *J* = 8.5 Hz), 5.667 (1H, dd, *J* = 15.0, 9.2 Hz), 7.376 (3H, m), 7.501 (2H, m); MS *m*/*z* 722 (M)$^+$; HRMS calcd for $C_{40}H_{61}O_6F_3Si$ 722.4190, found 722.4191.

Butyl (22*E*)-(8*S*,24*R*)-de-A,B-24-benzoyloxy-8-(*tert*-butyldimethylsilyl)oxy-26,27-cyclo-22-cholestene-25-carboxylate (18a). To a solution of **9a** (5.3 mg, 11 μmol) and DMAP (4.0 mg, 33 μmol) in CH_2Cl_2 (1 mL) was added with stirring benzoyl chloride (2 μL, 17 μmol) at room temperature under an argon atmosphere, and the resulting mixture was stirred for 1.5 h. The reaction mixture was purified by silica gel preparative TLC (ethyl acetate/*n*-hexane = 1/3) to give **18a** (1.7 mg) as a colorless oil in 26% yield.

18a. UV (EtOH) λ_{max} 274 nm, 230 nm, λ_{min} 260 nm, 211 nm; CD (EtOH) λ_{ext} 229 nm (Δε +9.2); ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.02 (3H, s), 0.00 (3H, s), 0.88 (9H, s), 0.98 (3H, d, *J* = 6.6 Hz), 3.98 (1H, s), 4.08 (2H, m), 5.40 (1H, dd, *J* = 15.3, 7.6 Hz), 5.64 (1H, dd, *J* = 15.3, 8.9 Hz), 5.82 (1H, d, *J* = 7.4 Hz), 7.43 (2H, t, *J* = 7.7 Hz), 7.55 (1H, t, *J* = 7.3 Hz), 8.03 (2H, d, *J* = 7.7 Hz); MS *m*/*z* 596 (M)$^+$; HRMS calcd for $C_{36}H_{56}O_5Si$ 596.3897, found 596.3903.

Butyl (22*E*)-(8*S*,24*S*)-de-A,B-24-benzoyloxy-8-(*tert*-butyldimethylsilyl)oxy-26,27-cyclo-22-cholestene-25-carboxylate (18b). This compound was obtained by the same procedure as described for **18a** by using **9b** instead of **9a**, in 41% yield.

18b. UV (EtOH) λ_{max} 274 nm, 229 nm, λ_{min} 253 nm, 211 nm; CD (EtOH) λ_{ext} 229 nm (Δε −8.2); ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.04 (3H, s), −0.01 (3H, s), 0.86 (9H, s), 0.97 (3H, d, *J* = 6.6 Hz), 3.97 (1H, s), 4.07 (2H, m), 5.37 (1H, dd, *J* = 15.4, 7.1 Hz), 5.66 (1H, dd, *J* = 15.4, 8.8 Hz), 5.87 (1H, d, *J* = 7.4 Hz), 7.43 (2H, t, *J* = 7.7 Hz), 7.55 (1H, t, *J* = 7.3 Hz), 8.02 (2H, d, *J* = 7.1 Hz); MS *m*/*z* 596 (M)$^+$; HRMS calcd for $C_{36}H_{56}O_5Si$ 596.3897, found 596.3892.

Vitamin D receptor (VDR) binding assay[27]

Bovine thymus VDR receptor was obtained from Yamasa Biochemical (Chiba, Japan) and dissolved in 0.05 M phosphate buffer (pH 7.4) containing 0.3 M KCl and 5 mM dithiothreitol just before use. The receptor solution (500 μL) was pre-incubated with 50 μL of ethanol solution of 1α,25-dihydroxyvitamin D_3 or an analogue at various concentrations for 60 min at 25 °C. Then, the receptor mixture was left to stand overnight with 0.1 nM [^{3}H]-1α,25-dihydroxyvitamin D_3 at 4 °C. The bound and free [^{3}H]-1α,25-dihydroxyvitamin D_3 were separated by treatment with dextran-coated charcoal for 30 min at 4 °C and centrifuged at 3000 rpm for 10 min. The supernatant (500 μL) was mixed with ACS-II (9.5 mL) (Amersham, UK) and the radioactivity was counted. The relative potency of the analogues was calculated from their concentration needed to displace 50% of [^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3 (assigned a 100% value).

Assay for HL-60 cell differentiation

Nitro blue tetrazolium (NBT)-reducing activity was used as a cell differentiation marker. HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FCS. Exponentially proliferating cells were collected, suspended in fresh medium and seeded in culture plates (Falcon, Becton Dickinson and Company, Franklin Lakes, NJ). Cell concentration at seeding was adjusted to 2×10^4 cells/mL and the seeding volume was 1 mL/well. An ethanol solution of 1α,25-dihydroxyvitamin D_3 (final concentration: 10^{-8} M) and an analogue (final concentration: 10^{-11}–10^{-6} M) was added to the culture medium at 0.1% volume and culture was continued for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without medium change. The same amount of vehicle was added to the control

culture. NBT-reducing assay was performed according to the method of Collins et al.[30] Briefly, cells were collected, washed with PBS, and suspended in serum-free medium. NBT/TPA solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng/mL, respectively. Then, the cell suspensions were incubated at 37 °C for 25 min. After incubation, cells were collected by centrifugation and resuspended in PBS. Cytospin smears were prepared, and the counter-staining of nuclei was done with Kemechrot solution. At least 500 cells per preparation were observed.

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(3*S*,4*S*,5*R*)- and (3*S*,4*S*,5*S*)-bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne: See refs 10a–c.
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24. The exciton theory predicts that, if the two long axes of benzoate and double-bond chromophores constitute a positive exciton chirality, i.e., right-handed screwness, the Cotton effect at 230 nm is positive. On the other hand, if the allylic benzoate constitutes a negative exciton chirality, the Cotton effect should be negative: see ref 23.
25. The side-chain conformations of **18a**,**b** were calculated using model compounds having a 9-(trimethylsilyl)oxy group, instead of the 9-(*tert*-butyldimethylsilyl)oxy group, and methyl ester at the C25 position, instead of butyl ester. The Monte Carlo conformational searches based on the MMFF 94 force field were performed using Spartan '02 for Linux, Wavefunction, Inc.
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