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Efficient Synthesis and Biological Evaluation of All A-Ring Diastereomers of 1α,25-Dihydroxyvitamin D_3 and its 20-Epimer

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Abstract—An improved synthesis of the diastereomers of 1α,25-dihydroxyvitamin D_3 (**1**) was accomplished utilizing our practical route to the A-ring synthon. We applied this procedure to synthesize for the first time all possible A-ring diastereomers of 20-*epi*-1α,25-dihydroxyvitamin D_3 (**2**). Ten-step conversion of 1-(4-methoxyphenoxy)but-3-ene (**6**), including enantiomeric introduction of the C-3 hydroxyl group to the olefin by the Sharpless asymmetric dihydroxylation, provided all four possible stereoisomers of A-ring enynes (**3**), i.e., (3*R*,5*R*)-, (3*R*,5*S*)-, (3*S*,5*R*)- and (3*S*,5*S*)-bis[(*tert*-butyldimethylsilyl)oxy]oct-1-en-7-yne, in good overall yield. Palladium-catalyzed cross-coupling of the A-ring synthon with the 20-*epi* CD-ring portion (**5**), (*E*)-(20*S*)-de-A,B-8-(bromomethylene)cholestan-25-ol, followed by deprotection, afforded the requisite diastereomers of 20-*epi*-1α,25-dihydroxyvitamin D_3 (**2**). The biological profiles of the synthesized stereoisomers were assessed in terms of affinities for vitamin D receptor (VDR) and vitamin D binding protein (DBP), HL-60 cell differentiation-inducing activity and in vivo calcium-regulating potency in comparison with the natural hormone.

Introduction

The biologically active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (**1**), plays a major role in calcium–phosphorus homeostasis, and its functions, such as cell differentiation-inducing and antiproliferative activities, attracted us to explore analogues which might have therapeutic potential for cancer, psoriasis and osteoporosis.[1,2] In order to investigate the structure–function relationships of this hormone, a number of analogues have been synthesized and biologically evaluated. For reasons of synthetic convenience and for metabolic studies of its inactivation process, most of the analogues synthesized so far have been altered in the side chain. Among them, 20-*epi*-1α,25-dihydroxyvitamin D_3 (**2**) is noteworthy due to its high activity in cell differentiation with relatively low calcemic effects, having a clearly different biological profile from its parent hormone.[3,4]

The A-ring moiety of **1**, which bears two hydroxyl groups on the six-membered ring, is of interest in connection with metabolic studies as well as analogue synthesis. The orientations of the two hydroxyl groups on the A-ring in the natural hormone are 1α and 3β (the vitamin D numbering system was used). The other three diastereomers on the A-ring moiety are depicted in Chart 1. The 1-epimer of 1α,25-dihydroxyvitamin D_3, i.e., the (20*R*)-(1β,3β) isomer, was shown to be an antagonist of nongenomic, but not genomic, actions by Norman and co-workers.[5] Most recently, the 3-epimer of **1**, i.e., the (20*R*)-(1α,3α) isomer, was shown to be produced in the vitamin D metabolic pathway in certain cell lines, though its functions remain unknown.[6–8] Therefore, studies on A-ring diastereomers with all possible hydroxyl configurations, as well as on 1α,25-dihydroxyvitamin D_3 itself have become of interest in vitamin D research.

Convergent synthesis can be more effective and flexible for the synthesis of a variety of analogues than the classical steroidal approach.[9,10] In particular, Trost's convergent method[11] using palladium-catalyzed coupling of the A-ring enyne synthon (**A**) with the CD-ring portion (**4**) seems most useful (Scheme 1). We applied this procedure to an improved synthesis of the diastereomers of 1α,25-dihydroxyvitamin D_3.[12] We also disclose herein the first synthesis of all four possible A-ring diastereomers of 20-*epi*-1α,25-dihydroxyvitamin D_3

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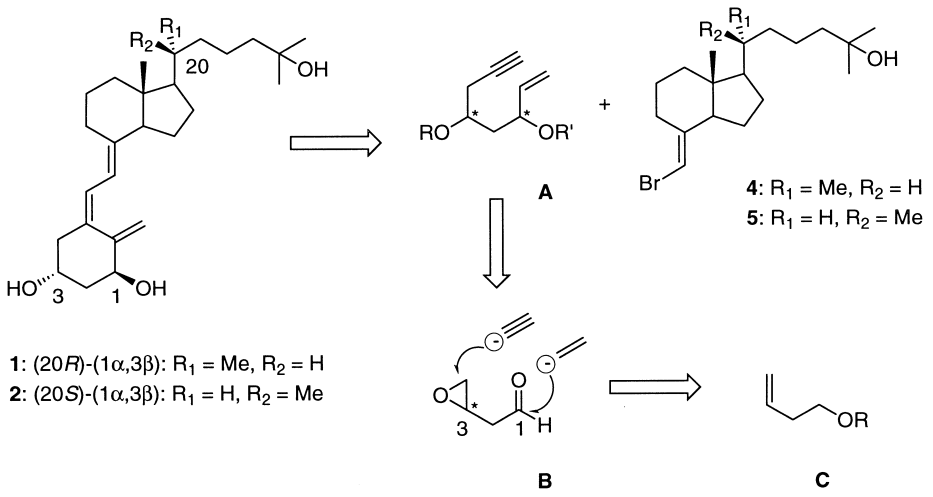


Scheme 1.

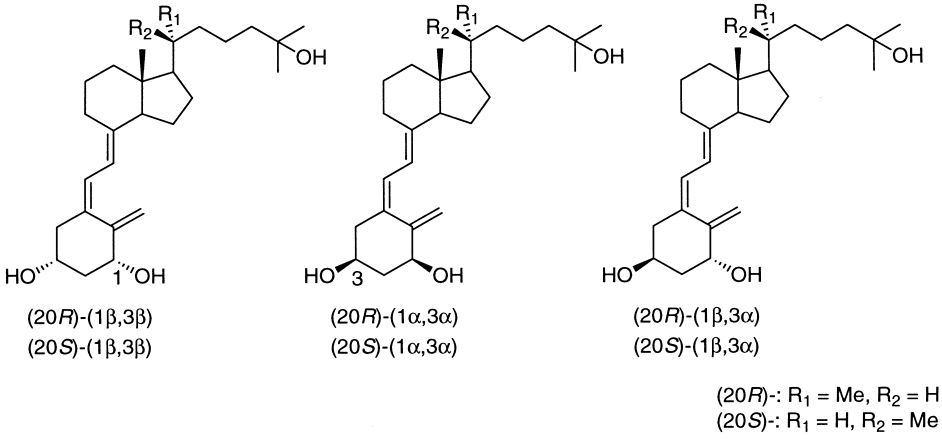


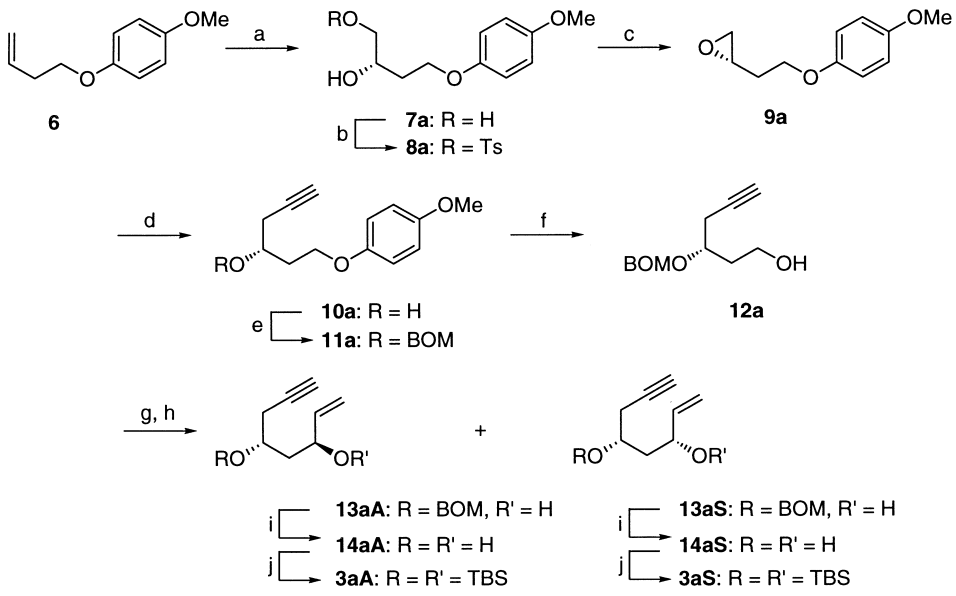
Chart 1.

(**2**) using the 20-*epi* CD-ring (**5**) instead of **4**, and their biological activities in comparison with those of the 20-natural counterparts.

Synthesis

The retrosynthetic analysis is shown in Scheme 1. Introduction of an acetylene unit and a vinyl group into the chiral epoxy-aldehyde precursor (**B**) would lead to the desired A-ring enyne (**A**). Therefore, the 3-buten-1-ol derivative (**C**) was chosen as a starting material. The 20-natural CD-ring portion (**4**) can be prepared from vitamin D_3 by known methods,[11] while the 20-*epi* CD-ring portion (**5**) can be obtained according to our procedure, a part of which was reported in ref 13.

The synthetic route to the A-ring enynes (**3aA**, **3aS**) is outlined in Scheme 2.[14] Introduction of the C-3 hydroxyl group to **6** was accomplished by the Sharpless asymmetric dihydroxylation (AD). Corey and co-workers reported that, in the AD reaction of 3-buten-1-ol derivatives, the combination of the chiral ligand $(DHQD)_2PYDZ$ and the *p*-anisyl protecting group at the primary alcohol gave high enantioselectivity (91% ee).[15,16] We found, however, that the use of $(DHQ)_2$PHAL as a ligand instead resulted in comparable yield with slightly better enantioselectivity.[17] Thus, treatment of *p*-anisyl ether **6** with AD-mix-α in *t*-BuOH:H_2O (1:1) at 0 °C for 3 h afforded the diol **7a** of the desired 3(*S*)-configuration in 95% yield with 93% ee. This was recrystallized from CH_2Cl_2-*n*-hexane to yield an enantiomerically pure specimen. The enantiopurity and the absolute configuration were determined by ^1H NMR analyses of the MTPA esters of the mono-tosylate **8a** (Chart 2).[18] The primary alcohol of **7a** was converted in 94% yield to the tosylate **8a**, which upon treatment with lithium hexamethyldisilazide afforded the epoxide **9a** in 84% yield. The acetylene unit was introduced by the reaction of **9a** with lithium acetylide–ethylenediamine complex in DMSO to give the alcohol **10a** in 86% yield. Protection of the resulting secondary alcohol of **10a** with a benzyloxymethyl (BOM) group afforded **11a** in 90% yield, and deprotection of the *p*-anisyl group with cerium (IV) diammonium nitrate (CAN) furnished the primary alcohol **12a** in quantitative yield. Introduction of a vinyl group into the aldehyde, obtained from **12a** with PDC in 96% yield, proceeded smoothly to give the allyl alcohols **13aA** and **13aS** in 62% yield as an approximately 1:1 mixture of the diastereomers. These isomers were readily separable by silica gel column



Scheme 2. (a) AD-mix-α/ *t*-BuOH-H_2O, 0 °C, 95%; (b) TsCl/pyridine, 0 °C, 94%; (c) LiHMDS/THF, 0 °C, 84%; (d) Lithium acetylide-EDA/DMSO, r.t., 86%; (e) BOMCl, DIPEA/CH_2Cl_2, r.t., 90%; (f) CAN/MeCN-H_2O, 0 °C, 99%; (g) PDC/CH_2Cl_2, r.t., 96%; (h) VinylMgBr/toluene, −78 °C, 62%; (i) PhSH-BF_3.Et_2O/CH_2Cl_2, 0 °C, 86%; (j) TBSOTf, 2,6-Lutidine/CH_2Cl_2, 0 °C. 99%.

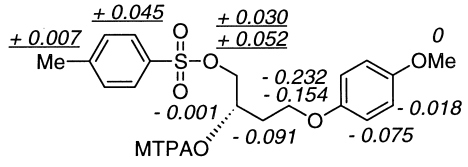


Chart 2.

chromatography and the C-1 configuration of both isomers was determined by ^1H NMR analyses of their MTPA esters (Chart 3).[18] Removal of the BOM protecting group by PhSH-BF_3·OEt_2[19] afforded the desired enynes **14aA** and **14aS**, from **13aA** and **13aS**, respectively. Finally, the diols (**14aA**, **14aS**) were protected using TBSOTf in 99% yield to give the requisite A-ring enyne synthons (**3aA**, **3aS**). Oxidation of **6** using AD-mix-β instead of AD-mix-α gave the diol **7b** in 94% yield with 93% ee, which led to the enantiomers **3bA** and **3bS**, corresponding to **3aA** and **3aS**, respectively, in the same way as above (Scheme 3). Thus, we have synthesized all four possible stereoisomers of A-ring enyne synthon in good overall yield, 29% for 10 steps, starting from **6**.

The 20-*epi* CD-ring portion (**5**) was synthesized as illustrated in Scheme 4.[13] The aldehyde **15**[20] was equilibrated under basic conditions to give an approximately 2:3 mixture of the aldehydes in favor of the 20-epimer. Reduction of this aldehyde mixture with $NaBH_4$ afforded the corresponding C-20 epimeric alcohols, which were then separated by chromatography to obtain the desired 20-*epi* alcohol **16** in 52% yield. Tosylation of the primary alcohol **16** afforded **17** in 98% yield, and treatment with NaI gave the iodide **18** in 92% yield. Condensation of **18** with the side chain moiety **19**[21] using *n*-BuLi as a base in the presence of HMPA furnished a mixture of C-23 epimeric sulfones **20** in 98% yield. Desulfonylation with sodium amalgam in a buffered mixture of methanol and THF[22] produced the desired CD-ring portion **21**. Removal of both protecting groups in **21** with TsOH afforded the corresponding diol **22** in high yield. The resulting secondary alcohol was oxidized with TPAP-NMO[23] to give the ketone **23** in 96% yield. Finally, bromomethylenation of **23** furnished the requisite 20-*epi* CD-ring synthon **5**.

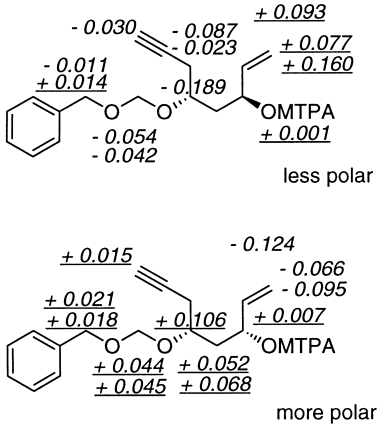
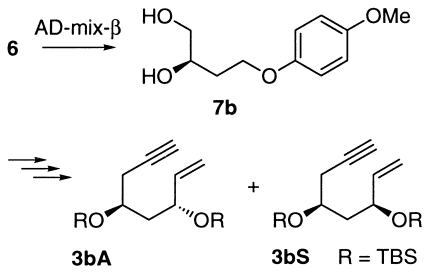
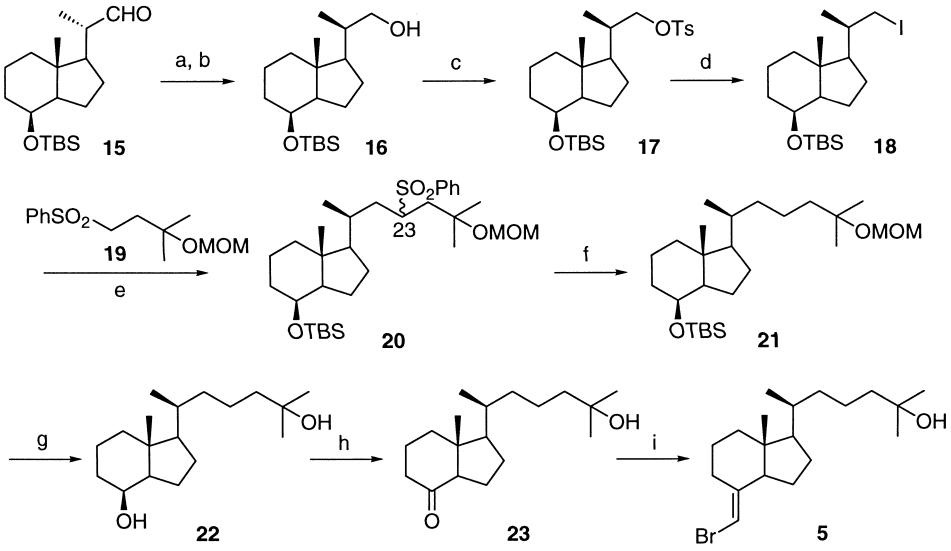


Chart 3.



Scheme 3.



Scheme 4. (a) DBU/THF, reflux; (b) $NaBH_4$/MeOH, 0 °C, 52% (two steps); (c) TsCl/pyridine, r.t., 98%; (d) NaI/DMF, 50 °C, 92%; (e) *n*-BuLi, HMPA/THF, −78 °C, 98%; (f) Na-Hg/Na_2HPO_4-MeOH-THF, r.t., 98%; (g) TsOH/MeOH, r.t., 85%; (h) TPAP-NMO-4Å M.S./CH_2Cl_2, 96%; (i) $Ph_3P^+CH_2Br{\cdot}Br^-$, NaHMDS/THF, 57%.

The coupling reaction of the disilyl ether (**3aA**) with the 20-*epi* CD-ring portion (**5**) catalyzed by $Pd_2(dba)_3$-PPh_3-Et_3N in toluene at 120 °C for 2 h, followed by deprotection with camphorsulfonic acid (CSA) in MeOH gave the (20*S*)-(1α,3β) isomer (**2**) in 52% yield.[11] The same treatment of **3aS**, **3bS** and **3bA** afforded the other diastereomers, (20*S*)-(1β,3β), (20*S*)-(1α,3α) and (20*S*)-(1β,3α), respectively. Use of the CD-ring part (**4**)[11] instead of **5** with the A-ring enyne (**3**) in the same way gave 1α,25-dihydroxyvitamin D_3 (**1**) and the other three diastereomers, (20*R*)-(1β,3β), (20*R*)-(1α,3α) and (20*R*)-(1β,3α).[24] In this way, the two sets of all four possible A-ring diastereomers of 1α,25-dihydroxyvitamin D_3 (**1**) and its 20-epimer (**2**) were synthesized.

Biological evaluation

The biological activities of the synthesized analogues are summarized in Table 1. In the vitamin D receptor (VDR) binding assay using bovine thymus VDR,[25] (20*S*)-(1α,3β) (**2**) exhibited 4-fold higher affinity than 1α,25-dihydroxyvitamin D_3. The VDR binding potency of **2** relative to that of 1α,25-dihydroxyvitamin D_3 (**1**) (normalized to 100) was reported to be 120 for chick intestinal VDR[3] and 500 for bovine thymus VDR.[4] Our results were consistent with this estimation. The (20*S*)-(1α,3α) isomer, the 3-epimer of **2**, showed rather high affinity to VDR with half the potency of the natural hormone **1**. The (20*S*)-(1β,3β) and (20*S*)-(1β,3α) isomers exhibited much lower affinity for VDR, implying that 1β-hydroxyl orientation greatly decreases the potency irrespective of the configuration of the C-3 hydroxyl group and the C-20 stereochemistry.

The affinity of vitamin D binding protein (DBP) was tested using rat serum DBP.[26] Each 20-*epi* analogue showed lower affinity than its 20-natural counterpart. The range of potencies among the isomers was narrower

Table 1. Biological activities of A-ring diastereomers of 1α,25-dihydroxyvitamin D_3 (**1**) and 20-*epi*-1α,25-dihydroxyvitamin D_3 (**2**)[a]

	VDR[b] binding	DBP[c] binding	HL-60 cell[d]	Ca mobilization[e]
(20*S*)-(1α,3β)(**2**)	400	< 0.1	3571	360
(20*S*)-(1α,3α)	50	5	208	110
(20*S*)-(1β,3β)	0.3	15	< 10	ND[f]
(20*S*)-(1β,3α)	0.3	26	< 10	ND
(20*R*)-(1α,3β)(**1**)	100	100	100	100
(20*R*)-(1α,3α)	(24)[g]	45 (800)[h]	< 10	NT[i]
(20*R*)-(1β,3β)	(0.2)[g]	243 (450)[h]	< 10	NT
(20*R*)-(1β,3α)	(0.8)[g]	410 (6570)[h]	< 10	NT

[a]The potencies of 1α,25-dihydroxyvitamin D_3 (**1**) are taken as 100.
[b]Bovine thymus.
[c]Rat serum.
[d]Cell differentiation was assessed in terms of expression of antigen CD11b.
[e]Rat serum calcium level.
[f]Not detected.
[g]Ref 5, chick intestinal.
[h]Ref 27, human DBP.
[i]Not tested.

than the results using human DBP reported by Norman et al.[27] However, as they reported,[27] the A-ring configuration of (1β,3α) was preferred, independent of the C-20 stereochemistry.

Cell differentiation-inducing activity of (20*S*)-(1α,3β) (**2**) towards HL-60 cells[28] was 36 times higher than that of the natural hormone (**1**). The potency of **2** towards U-937 cells was reported to be 27-fold higher than that of **1**,[3] which is in reasonable agreement with our result considering the difference in cell lines. The 3-epimer of **2**, i.e., (20*S*)-(1α,3α), was approximately 2.1 times more active than **1**. Thus, the 3-epimerization of **2** resulted in approximately 17-fold reduction in cell differentiation-inducing activity, which is high compared with the 8-fold reduction in the VDR affinity. Those results might be explained by a difference in DBP binding, which is believed to correlate to availability of the compounds to target cells. The 1β-isomers, as well as (20*R*)-(1α,3α) showed virtually no cell-differentiating activity even at the concentration of 10^{-8} M.

In vivo calcium-regulating activities of the diastereomers were tested using normal SD male rats.[29] The (20*S*)-(1α,3β) (**2**) and (20*S*)-(1α,3α) compounds (single dose) showed similar time courses with peak calcemic activity at 48 h after administration, while the natural hormone (**1**) showed the peak activity at 24 h under the same experimental conditions (data not shown). The potencies, based on the ability to elevate serum calcium level by 1 mg/dL, were calculated at the time when the effects were maximal. The effective concentrations of (20*S*)-(1α,3β) (**2**), (20*S*)-(1α,3α) and (20*R*)-(1α,3β) (**1**) were 0.8, 2.7 and 3.0 μg/kg, respectively, and Table 1 shows the data as relative potencies with respect to **1**, defined as 100. The 20-*epi* modification of **1** increased the calcemic activity concomitantly with the VDR binding potency. However, in contrast to the 36-fold difference of relative potency of **2** to **1** in cell differentiation, the 20-epimerization had much less effect on the calcemic activity (3.6-fold). The (20*S*)-(1α,3α) compound, the 3-epimer of **2**, exhibited similar calcemic activity to 1α,25-dihydroxyvitamin D_3. Thus, alteration in both C-3 and C-20 stereochemistry retained calcemic and cell-differentiating activities similar to those of the natural hormone. These results imply that the 3-epimerization of **2** would produce the (20*S*)-(1α,3α) compound with relatively high calcium-regulating potency but much less cell differentiation activity than the parent compound **2**. It is of interest to know whether 3-epimerization is involved in the metabolic pathway of **2** or not.[6–8] On the other hand, the 1β-isomers showed no serum calcium-elevating activity under these conditions.

In summary, we have synthesized two sets of all four possible A-ring diastereomers of 1α,25-dihydroxyvitamin D_3 (**1**) and its 20-epimer (**2**) by employing the convergent method using palladium catalyst. The above procedure should be versatile for the synthesis of a variety of A-ring analogues, as well as those with CD-ring and/or side chain modifications. Biological evaluation revealed that the 20-epimerization of the analogues with a 1α-hydroxyl group increased the potencies of VDR binding, HL-60 cell differentiation and in vivo calcemic activity. Each 20-*epi* analogue with a 1β-hydroxyl group exhibited weak activity similar to that of its 20-natural counterpart, implying that 1β-hydroxyl orientation greatly decreases the potency irrespective of the configuration of the C-3 hydroxyl group and the C-20 stereochemistry. In contrast, the DBP binding potency of each analogue appeared to be reduced by the 20-epimerization independently of the A-ring stereochemistry. These results shed some light on the structure–activity relationships of the A-ring and the side chain. Some of these compounds would make useful tools in research on the biology of vitamin D.

Experimental

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. NMR spectra were recorded on a JEOL GSX-400 spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra and high-resolution mass spectra (HRMS) were recorded on a JMS-SX 102A. Fast atom bombardment mass spectra (FAB–MS) were recorded with 3-nitrobenzyl alcohol (NBA) as a matrix. Infrared spectra were recorded on a Jasco FT/IR-8000 spectrometer and are expressed in cm^{-1}. Ultraviolet spectra were recorded with a Hitachi 200-10 spectrophotometer. Optical rotations were determined by using a Jasco DIP-370 digital polarimeter. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within 0.3% of the theoretical values.

1-(4-Methoxyphenoxy)but-3-ene (6).[15,16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52 (2H, qt, J = 6.7, 1.2 Hz), 3.77 (3H, s), 3.97 (2H, t, J = 6.7 Hz), 5.10 (1H, dq, J = 10.0, 1.2 Hz), 5.16 (1H, dq, J = 17.0, 1.2 Hz), 5.99 (1H, ddt, J = 17.0, 10.0, 6.7 Hz), 6.83 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.7 (t), 55.7 (q), 68.0 (t), 114.7 (d), 115.7 (d), 116.9 (t), 134.7 (d), 153.2 (s), 153.9 (s); MS 178 $[M]^+$, 124 $[CH_3OC_6H_4OH]^+$; HRMS calcd. for $[C_{21}H_{24}O_4]$ 178.0994, found 178.0995.

(*S*)-4-(4-Methoxyphenoxy)butane-1,2-diol (7a). A mixture of AD-mix-α (Aldrich, 1.4 g) in *t*-BuOH (5 mL) and H_2O (5 mL) was cooled to 0 °C. The resulting suspension was treated with the olefin **6** (178 mg, 1.0 mmol), then stirred for 3 h, and quenched by addition of sodium sulfite (1.5 g, 6.0 mmol). The mixture was stirred for 30 min at room temperature, and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 4:1) to afford the desired diol (202 mg) as a solid in 95% yield with 93% ee. It was recrystallized from CH_2Cl_2:*n*-hexane to yield optically pure **7a** as colorless fine needles.

Mp 64 °C (recry. from CH_2Cl_2-*n*-hexane); $[\alpha]_D^{26}$ −5.2 (c = 1.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (2H, m), 2.61 (1H, d, J = 4.7 Hz), 3.56 (1H, m), 3.72

(1H, ddd, $J=11.0$, 6.6, 3.7 Hz), 3.77 (3H, s), 4.01 (1H, m), 4.12 (2H, m), 6.84 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.7 (t), 55.7 (q), 66.1 (t), 66.7 (t), 70.4 (d), 114.7 (d), 115.5 (d), 152.6 (s), 154.1 (s); FTIR (Nujol) 3252, 1215, 1290, 1242, 1116, 1072, 1032, 993, 966 cm^{-1}; MS 212 $[M]^+$, 124 $[CH_3OC_6H_4OH]^+$; HRMS calcd. for $[C_{11}H_{16}O_4]$ 212.1049, found 212.1049. Anal. calcd for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 61.97; H, 7.48.

(*R*)-4-(4-Methoxyphenoxy)butane-1,2-diol (7b). This compound was obtained by the same procedure as described for **7a** using AD-mix-β instead of AD-mix-α. Mp 64 °C (recry. from ethyl acetate:*n*-hexane); $[\alpha]_D^{26}$ +5.4 ($c=1.15$, $CHCl_3$).

(*S*)-2-Hydroxy-4-(4-methoxyphenoxy)butan-1-ol *p*-toluenesulfonate (8a). To a solution of **7a** (200 mg, 0.94 mmol) in pyridine (3 mL) was added TsCl (216 mg, 1.1 mmol) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 22 h, then poured into water and extracted with ether. The organic layer was washed with 2 N HCl aq and brine, dried over magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:2) to give **8a** (324 mg) in 94% as a solid, which was recrystallized from ethyl acetate:*n*-hexane to give analytically pure **8a** as colorless needles.

Mp 70 °C (recry. from ethyl acetate:*n*-hexane); $[\alpha]_D^{26}$ +2.4 ($c=1.00$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (2H, m), 2.52 (1H, d, $J=4.0$ Hz), 3.77 (3H, s), 3.99–4.13 (4H, m), 4.15 (1H, m), 6.80 (4H, m), 7.35 (2H, d, $J=8.5$ Hz), 7.81 (2H, dt, $J=8.5$, 1.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7 (q), 32.4 (t), 55.8 (q), 65.3 (t), 67.6 (d), 73.6 (t), 114.7 (d), 115.5 (d), 128.0 (d), 130.0 (d), 132.7 (s), 145.1 (s), 152.6 (s), 154.1 (s); FTIR (Nujol) 3547, 2725, 1714, 1508, 1350, 1290, 1226, 1172, 1124, 1039, 962 cm^{-1}; MS 366 $[M]^+$; HRMS calcd. for $[C_{18}H_{22}O_6S]$ 366.1137, found 366.1137. Anal. calcd for $C_{18}H_{22}O_6S$: C, 59.00; H, 6.05. Found: C, 58.71; H, 6.12.

(*S*)-MTPA ester of 8a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.041 (2H, m), 2.440 (3H, s), 3.505 (3H, s), 3.631 (1H, ddd, $J=9.5$, 8.2, 4.6 Hz), 3.773 (3H, s), 3.783 (1H, m), 4.176 (1H, dd, $J=11.3$, 6.1 Hz), 4.348 (1H, dd, $J=11.3$, 2.6 Hz), 5.525 (1H, m), 6.668 (2H, m), 6.800 (2H, m), 7.253–7.376 (5H, m), 7.461 (2H, d, $J=7.9$ Hz), 7.753 (2H, d, $J=8.5$ Hz).

(*R*)-MTPA ester of 8a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.132 (2H, m), 2.434 (3H, s), 3.411 (3H, s), 3.773 (3H, s), 3.863 (1H, ddd, $J=9.5$, 7.6, 5.2 Hz), 3.937 (1H, m), 4.146 (1H, dd, $J=11.0$, 5.8 Hz), 4.296 (1H, dd, $J=11.0$, 3.4 Hz), 5.526 (1H, m), 6.743 (2H, m), 6.818 (2H, m), 7.258–7.394 (5H, m), 7.432 (2H, d, $J=8.1$ Hz), 7.708 (2H, d, $J=8.5$ Hz).

(*R*)-2-Hydroxy-4-(4-methoxyphenoxy)butan-1-ol *p*-toluenesulfonate (8b). Mp 71 °C (recry. from ethyl acetate:*n*-hexane); $[\alpha]_D^{27}$ +2.1 ($c=1.15$, $CHCl_3$).

(*S*)-4-(4-Methoxyphenoxy)-1,2-epoxybutane (9a). A solution of **8a** (1.00 g, 2.7 mmol) in dry THF (10 ml) was treated with LiHMDS (1.0 M in THF, 3.3 mL, 3.3 mmol) at 0 °C under argon. The mixture was stirred for 1 h at 0 °C, then poured into satd NH_4Cl aq and extracted with ether. The organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude mixture was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:4) to give **9a** (443 mg) as a colorless oil in 84% yield.

$[\alpha]_D^{25}$ −13.6 ($c=1.65$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (1H, dq, $J=14.0$, 5.8 Hz), 2.08 (1H, m), 2.57 (1H, dd, $J=4.9$, 2.8 Hz), 2.82 (1H, dd, $J=4.9$, 4.3 Hz), 3.15 (1H, m), 3.77 (3H, s), 4.07 (2H, m), 6.84 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.4 (t), 47.1 (t), 49.8 (d), 55.7 (q), 65.3 (t), 114.7 (d), 115.5 (d), 152.9 (s), 154.0 (s); FTIR (neat) 1232 cm^{-1}; MS 194 $[M]^+$, 124 $[CH_3OC_6H_4OH]^+$; HRMS calcd for $[C_{11}H_{14}O_3]$ 194.0943, found 194.0941.

(*R*)-4-(4-Methoxyphenoxy)-1,2-epoxybutane (9b). $[\alpha]_D^{25}$ +15.6 ($c=1.35$, $CHCl_3$).

(*R*)-1-(4-Methoxyphenoxy)hex-5-yn-3-ol (10a). To a solution of **9a** (250 mg, 1.3 mmol) in DMSO (3 mL) was added with stirring lithium acetylide ethylenediamine complex (175 mg, 1.9 mmol) at room temperature. The mixture was stirred for 30 min, then diluted with water and extracted with ether. The organic layer was washed with brine, and dried over magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:3) to give **10a** (245 mg) in 86% as a solid, which was recrystallized from CH_2Cl_2:*n*-hexane to give analytically pure **10a** as colorless fine needles.

Mp 45 °C (recry. from CH_2Cl_2:*n*-hexane); $[\alpha]_D^{27}$ −9.8 ($c=1.18$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95–2.10 (2H, m), 2.07 (1H, t, $J=2.8$ Hz), 2.44 (1H, ddd, $J=16.0$, 6.1, 2.8 Hz), 2.50 (1H, ddd, $J=16.0$, 6.1, 2.8 Hz), 2.54 (1H, d, $J=4.3$ Hz), 3.77 (3H, s), 4.08 (2H, m), 4.15 (1H, ddd, $J=9.5$, 7.3, 4.9 Hz), 6.84 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3 (t), 35.4 (t), 55.8 (q), 66.2 (t), 68.2 (d), 70.9 (d), 80.7 (s), 114.7 (d), 115.5 (d), 152.8 (s), 154.1 (s); FTIR (neat) 3919, 3422, 2932, 2118, 2056, 1508, 1290, 1230 cm^{-1}; MS 220 $[M]^+$, 124 $[CH_3OC_6H_4OH]^+$; HRMS calcd. for $[C_{13}H_{16}O_3]$ 220.1100, found 220.1099. Anal. calcd for $C_{13}H_{18}O_3$: C, 70.89; H, 7.32. Found: C, 70.77; H, 7.32.

(*S*)-1-(4-Methoxyphenoxy)hex-5-yn-3-ol (10b). Mp 44 °C (recry. from CH_2Cl_2:*n*-hexane; $[\alpha]_D^{26}$ +10.6 ($c=0.97$, $CHCl_3$).

(*R*)-3-[(Benzyloxymethyl)oxy]-1-(4-methoxyphenoxy)hex-5-yne (11a). To a mixture of **10a** (100 mg, 0.45 mmol) and diisopropylethylamine (0.32 mL, 1.8 mmol) in CH_2Cl_2 (2 mL) was added with stirring BOMCl (213 mg, 1.4 mmol) under argon at room temperature. The reaction mixture was stirred for 16 h, and diluted with CH_2Cl_2. The organic layer was washed with 1 N HCl aq, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **11a** (138 mg) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:3) in 90% as a colorless oil.

$[\alpha]_D^{28}$ −19.4 (c = 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (1H, t, J = 2.7 Hz), 2.12 (2H, m), 2.51 (1H, ddd, J = 16.8, 4.6, 2.7 Hz), 2.57 (1H, ddd, J = 16.8, 6.1, 2.7 Hz), 3.76 (3H, s), 4.01–4.12 (3H, m), 4.58 (1H, d, J = 11.6 Hz), 4.66 (1H, d, J = 11.6 Hz), 4.81 (1H, d, J = 7.0 Hz), 4.89 (1H, d, J = 7.0 Hz), 6.81 (4H, m), 7.30 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7 (t), 34.0 (t), 55.7 (q), 64.7 (t), 69.8 (t), 70.5 (d), 72.6 (d), 80.6 (s), 94.0 (t), 114.7 (d), 115.4 (d), 127.8 (d), 128.4 (d), 137.7 (s), 153.0 (s), 153.8 (s); FTIR (neat) 3289, 2951, 2118, 1591, 1508, 1470, 1385, 1290, 1232 cm^{-1}; MS 340 $[M]^+$; HRMS calcd. for $[C_{21}H_{24}O_4]$ 340.1675, found 340.1676.

(*S*)-3-[(Benzyloxymethyl)oxy]-1-(4-methoxyphenoxy)hex-5-yne (11b). $[\alpha]_D^{28}$ +23.0 (c = 1.20, $CHCl_3$).

(*R*)-3-[(Benzyloxymethyl)oxy]hex-5-yn-1-ol (12a). A solution of **11a** (138 mg, 0.41 mmol) in CH_3CN (4.8 mL) and H_2O (1.2 mL) was treated with diammonium cerium nitrate (CAN) (553 mg, 0.98 mmol) with stirring at 0 °C. After 15 min, ethyl acetate (8 mL) and brine (8 mL) were added to the solution and separated. The aqueous layer was extracted with ethyl acetate. The combined organic phases were washed with satd $NaHCO_3$ aq, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **12a** (95 mg) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:1) in 95% yield as a pale yellow oil.

$[\alpha]_D^{27}$ −50.3 (c = 1.74, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (2H, m), 2.02 (1H, t, J = 2.4 Hz), 2.05 (1H, t, J = 5.8 Hz), 2.50 (1H, ddd, J = 17.1, 5.2, 2.4 Hz), 2.55 (1H, ddd, J = 17.1, 6.1, 2.4 Hz), 3.74–3.88 (2H, m), 4.02 (1H, dq, J = 8.2, 5.2 Hz), 4.67 (2H, s), 4.84 (1H, d, J = 7.0 Hz), 4.90 (1H, d, J = 7.0 Hz), 7.28–7.37 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.6 (t), 36.5 (t), 59.5 (t), 70.0 (t), 70.4 (d), 74.3 (d), 80.6 (s), 94.1 (t), 127.8 (d), 128.5 (d), 137.4 (s); FTIR (neat) 3383, 2964, 2872, 1456, 1377 cm^{-1}; FAB–MS (NBA-NaI) 257 $[M + Na]^+$.

(*S*)-3-[(Benzyloxymethyl)oxy]hex-5-yn-1-ol (12b). $[\alpha]_D^{27}$ +55.5 (c = 1.54, $CHCl_3$).

(3*S*,5*R*)-5-[(Benzyloxymethyl)oxy]oct-1-en-7-yn-3-ol (13aA) and (3*R*,5*R*)-5-[(Benzyloxymethyl)oxy]oct-1-en-7-yn-3-ol (13aS). A stirred mixture of **12a** (73 mg, 0.31 mmol) and powdered 4 Å MS (50 mg) in CH_2Cl_2 (2 mL) was treated with PDC (292 mg, 0.78 mmol) at room temperature. The mixture was stirred at room temperature for 1 day under argon, and separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:1) to give the corresponding aldehyde (70 mg, 96%) as a colorless oil, which was used immediately in the following step.

To a solution of the above aldehyde (30 mg, 0.13 mmol) in dry toluene (3 mL) was added with stirring vinyl magnesium bromide (1.0 M in THF, 390 μL, 0.39 mmol) at −78 °C under argon. The reaction mixture was stirred for 40 min, and quenched by addition of satd NH_4Cl aq After extraction with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the solvent afforded a crude mixture of **13aA** and **13aS**. This was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:2) to give **13aA** (9 mg) and **13aS** (12 mg) both as colorless oils in 62% total yield.

13aA. $[\alpha]_D^{26}$ −33.2 (c = 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78 (1H, ddd, J = 14.3, 9.5, 3.1 Hz), 1.93 (1H, ddd, J = 14.3, 9.5, 3.1 Hz), 2.03 (1H, t, J = 2.7 Hz), 2.37 (1H, d, J = 4.6 Hz), 2.50 (1H, ddd, J = 16.8, 4.6, 2.7 Hz), 2.57 (1H, ddd, J = 16.8, 6.4, 2.7 Hz), 4.08 (1H, ddt, J = 6.4, 4.6, 3.1 Hz), 4.41 (1H, m), 4.66 (1H, d, J = 11.6 Hz), 4.69 (1H, d, J = 11.6 Hz), 4.85 (1H, d, J = 7.0 Hz), 4.91 (1H, d, J = 7.0 Hz), 5.12 (1H, dt, J = 10.4, 1.5 Hz), 5.29 (1H, dt, J = 17.4, 1.5 Hz), 5.91 (1H, ddd, J = 17.4, 10.4, 5.5 Hz), 7.28–7.37 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8 (t), 41.0 (t), 69.1 (d), 70.1 (t), 70.5 (d), 73.5 (d), 80.5 (s), 94.5 (t), 114.3 (t), 127.9 (d), 128.0 (d), 128.6 (d), 137.5 (s), 140.8 (d); FTIR (neat) 3448, 3289, 2918, 2120, 1647, 1496, 1456, 1419 cm^{-1}; FAB–MS (NBA-NaI) 283 $[M + Na]^+$.

(*S*)-MTPA ester of 13aA. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.000 (1H, t, J = 2.4 Hz), 1.96–2.05 (2H, m), 2.392 (1H, ddd, J = 16.8, 3.7, 2.4 Hz), 2.540 (1H, ddd, J = 16.8, 6.4, 2.4 Hz), 3.524 (3H, d, J = 1.2 Hz), 3.596 (1H, m), 4.574 (1H, d, J = 11.9 Hz), 4.675 (1H, d, J = 7.3 Hz), 4.730 (1H, d, J = 11.9 Hz), 4.818 (1H, d, J = 7.3 Hz), 5.292 (1H, dt, J = 10.4, 0.9 Hz), 5.414 (1H, dt, J = 17.4, 0.9 Hz), 5.690 (1H, m), 5.869 (1H, ddd, J = 17.4, 10.4, 7.3 Hz), 7.278–7.403 (8H, m), 7.516–7.548 (2H, m).

(*R*)-MTPA ester of 13aA. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.998 (1H, ddd, J = 14.7, 9.5, 3.4 Hz), 2.030 (1H, t, J = 2.4 Hz), 2.080 (1H, ddd, J = 14.5, 9.5, 3.4 Hz), 2.479 (1H, ddd, J = 16.8, 4.0, 2.4 Hz), 2.563 (1H, ddd, J = 16.8, 6.4, 2.4 Hz), 3.538 (1H, d, J = 1.2 Hz), 3.758 (1H, ddd, J = 6.4, 4.0, 3.4 Hz), 4.585 (1H, d, J = 11.9 Hz), 4.716 (1H, d, J = 11.9 Hz), 4.729 (1H, d, J = 7.3 Hz), 4.860 (1H, d, J = 7.3 Hz), 5.215 (1H, dt, J = 10.4, 0.9 Hz), 5.308 (1H, dt, J = 17.1, 0.9 Hz), 5.689 (1H, m), 5.776 (1H, ddd, J = 17.1, 10.4, 7.0 Hz), 7.276–7.410 (8H, m), 7.511-7.534 (2H, m).

13aS. $[\alpha]_D^{27}$ −31.5 (c = 1.09, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (2H, m), 2.03 (1H, t, J = 2.8 Hz), 2.53 (1H, ddd, J = 16.8, 4.6, 2.8 Hz), 2.59 (1H, ddd, J = 16.8, 6.1, 2.8 Hz), 4.00 (1H, m), 4.35 (1H, m), 4.64 (1H, d, J = 11.6 Hz), 4.70 (1H, d, J = 11.6 Hz), 4.83 (1H, d, J = 7.0 Hz), 4.91 (1H, d, J = 7.0 Hz), 5.13 (1H, dt, J = 10.4, 1.5 Hz), 5.28 (1H, dt, J = 17.1, 1.5 Hz), 5.89 (1H, ddd, J = 17.1, 10.4, 6.1 Hz), 7.28–7.37 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.6 (t), 41.1 (t), 70.1 (t), 70.6 (d), 71.3 (d), 75.0 (d), 80.4 (s), 93.8 (t), 114.9 (t), 127.9 (d), 128.0 (d), 128.6 (d), 137.5 (s), 140.5 (d); FTIR (neat) 3445, 3296, 2947, 2120, 1649, 1498, 1456, 1421 cm^{-1}; FAB–MS (NBA-NaI) 283 $[M + Na]^+$.

(*S*)-MTPA ester of 13aS. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.030 (1H, t, J = 2.4 Hz), 2.052 (1H, ddd, J = 14.0, 7.6, 4.6 Hz), 2.150 (1H, ddd, J = 14.0, 8.2, 5.8 Hz), 2.501 (1H, m), 2.555 (1H, m), 3.557 (3H, d, J = 1.2 Hz), 3.777 (1H, m), 4.609 (1H, d, J = 11.9 Hz), 4.693 (1H, d, J = 11.9 Hz), 4.747 (1H, d, J = 7.3 Hz), 4.838 (1H, d,

$J = 7.3$ Hz), 5.250 (1H, dt, $J = 10.7$, 0.9 Hz), 5.310 (1H, dt, $J = 17.8$, 0.9 Hz), 5.647 (1H, m), 5.740 (1H, ddd, $J = 17.8$, 10.7, 7.0 Hz), 7.282–7.412 (8H, m), 7.503–7.526 (2H, m).

(*R*)-MTPA ester of 13aS. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.000 (1H, ddd, $J = 14.0$, 7.3, 5.2 Hz), 2.015 (1H, t, $J = 2.8$ Hz), 2.082 (1H, ddd, $J = 14.0$, 7.6, 6.4 Hz), 2.434 (1H, ddd, $J = 17.1$, 4.6, 2.8 Hz), 2.504 (1H, ddd, $J = 17.1$, 6.1, 2.8 Hz), 3.543 (3H, d, $J = 1.2$ Hz), 3.671 (1H, m), 4.588 (1H, d, $J = 11.9$ Hz), 4.675 (1H, d, $J = 11.9$ Hz), 4.703 (1H, d, $J = 7.0$ Hz), 4.793 (1H, d, $J = 7.0$ Hz), 5.316 (1H, d, $J = 9.8$ Hz), 5.405 (1H, dt, $J = 17.1$, 0.9 Hz), 5.640 (1H, m), 5.864 (1H, ddd, $J = 17.1$, 9.8, 7.6 Hz), 7.271–7.409 (8H, m), 7.510–7.533 (2H, m).

(3*R*,5*S*)-5-[(Benzyloxymethyl)oxy]oct-1-en-7-yn-3-ol (13bA). $[\alpha]_D^{25}$ +33.7 ($c = 1.00$, $CHCl_3$).

(3*S*,5*S*)-5-[(Benzyloxymethyl)oxy]oct-1-en-7-yn-3-ol (13bS). $[\alpha]_D^{26}$ +32.5 ($c = 1.08$, $CHCl_3$)

(3*S*,5*R*)-Oct-1-en-7-yn-3,5-diol (14aA). A solution of $BF_3{\cdot}OEt_2$ (420 μL, 3.4 mmol) in dry CH_2Cl_2 (2 mL) was added slowly to a solution of **13aA** (296 mg, 1.1 mmol) and thiophenol (176 μL, 1.7 mmol) in dry CH_2Cl_2 (10 mL) with stirring at 0 °C under argon. Stirring was continued at 0 °C for 15 min, then the reaction mixture was diluted with phosphate buffer and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:1) to give **14aA** (132 mg) as a colorless oil in 86% yield.

$[\alpha]_D^{25}$ −1.2 ($c = 1.25$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76 (1H, ddd, $J = 14.7$, 7.6, 3.1 Hz), 1.86 (1H, ddd, $J = 14.7$, 8.9, 3.1 Hz), 2.06 (1H, t, $J = 2.8$ Hz), 2.43 (3H, m), 2.80 (1H, br.s), 4.10 (1H, m), 4.49 (1H, m), 5.17 (1H, d, $J = 10.4$ Hz), 5.31 (1H, d, $J = 17.4$ Hz), 5.94 (1H, ddd, $J = 17.4$, 10.4, 5.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3 (t), 41.2 (t), 67.2 (d), 70.3 (d), 70.9 (d), 80.6 (s), 114.7 (t), 140.4 (d); FTIR (neat) 3298, 2918, 2118, 1647, 1419, 1336, 1292, 1126 cm^{-1}; FAB–MS (NBA-NaI) 163 $[M + Na]^+$.

(3*R*,5*S*)-Oct-1-en-7-yn-3,5-diol (14bA). $[\alpha]_D^{29}$ +1.3 ($c = 1.58$, $CHCl_3$).

(3*R*,5*R*)-Oct-1-en-7-yn-3,5-diol (14aS). $[\alpha]_D^{28}$ −10.3 ($c = 0.70$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70 (1H, dt, $J = 14.7$, 9.8 Hz), 1.84 (1H, dt, $J = 14.7$, 3.1 Hz), 2.06 (1H, t, $J = 2.8$ Hz), 2.41 (2H, m), 2.73 (1H, d, $J = 3.1$ Hz), 3.28 (1H, d, $J = 3.1$ Hz), 4.05 (1H, m), 4.41 (1H, m), 5.13 (1H, dt, $J = 10.4$, 1.2 Hz), 5.28 (1H, dt, $J = 17.4$, 1.2 Hz), 5.90 (1H, ddd, $J = 17.4$, 10.4, 6.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.6 (t), 41.9 (t), 70.3 (d), 70.9 (d), 73.3 (d), 80.5 (s), 114.9 (t), 140.4 (d); FTIR (neat) 3298, 2916, 2120, 1649, 1417, 1331, 1126 cm^{-1}; FAB–MS (NBA-NaI) 163 $[M + Na]^+$.

(3*S*,5*S*)-Oct-1-en-7-yn-3,5-diol (14bS). $[\alpha]_D^{25}$ +9.2 ($c = 0.52$, $CHCl_3$).

(3*S*,5*R*)-Bis[(*tert*-butyldimethylsilyl)oxy]oct-1-en-7-yne (3aA). To a stirred mixture of **14aA** (30 mg, 0.21 mmol) and 2,6-lutidine (73 μL, 0.63 mmol) in dry CH_2Cl_2 (2 mL) was added TBSOTf (123 μL, 0.53 mmol) at 0 °C under argon. The resulting mixture was stirred at 0 °C for 30 min, diluted with CH_2Cl_2 and washed with water. The organic layer was dried over magnesium sulfate and filtered. Removal of the solvent afforded a residue, from which **3aA** (76 mg) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:8) as a colorless oil in 99% yield.

$[\alpha]_D^{27}$ −7.5 ($c = 1.01$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (3H, s), 0.07 (3H, s), 0.086 (3H, s), 0.093 (3H, s), 0.890 (9H, s), 0.894 (9H, s), 1.66 (1H, ddd, $J = 13.7$, 6.4, 4.9 Hz), 1.88 (1H, ddd, $J = 13.7$, 7.6, 5.2 Hz), 1.97 (1H, t, $J = 2.8$ Hz), 2.33 (1H, ddd, $J = 16.8$, 5.2, 2.8 Hz), 2.38 (1H, ddd, $J = 16.8$, 6.1, 2.8 Hz), 3.93 (1H, quintet, $J = 5.5$ Hz), 4.23 (1H, m), 5.04 (1H, ddd, $J = 10.1$, 1.5, 0.5 Hz), 5.14 (1H, ddd, $J = 17.1$, 1.5, 0.9 Hz), 5.82 (1H, ddd, $J = 17.1$, 10.1, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.6 (q), −4.6 (q), −4.4 (q), −3.8 (q), 18.1 (s), 18.2 (s), 25.86 (q), 25.93 (q), 28.0 (t), 45.9 (t), 68.1 (d), 70.1 (d), 71.7 (d), 81.5 (s), 114.3 (t), 141.9 (d); FTIR (neat) 3314, 2955, 2932, 2887, 2858, 1471, 1464, 1361, 1255, 1091 cm^{-1}; MS 311 $[M\text{-}^tBu]^+$; HRMS calcd. for $[C_{16}H_{31}O_2Si_2]$ 311.1862, found 311.1857.

(3*R*,5*S*)-Bis[(*tert*-butyldimethylsilyl)oxy]oct-1-en-7-yne (3bA). $[\alpha]_D^{25}$ +10.2 ($c = 0.55$, $CHCl_3$).

(3*R*,5*R*)-Bis[(*tert*-butyldimethylsilyl)oxy]oct-1-en-7-yne (3aS). $[\alpha]_D^{25}$ −10.9 ($c = 1.08$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.09 (3H, s), 0.90 (18H, s), 1.80 (2H, t, $J = 6.1$ Hz), 1.97 (1H, t, $J = 2.8$ Hz), 2.34 (1H, ddd, $J = 16.8$, 5.5, 2.8 Hz), 2.40 (1H, ddd, $J = 16.8$, 6.1, 2.8 Hz), 3.88 (1H, quintet, $J = 6.1$ Hz), 4.25 (1H, m), 5.06 (1H, ddd, $J = 10.4$, 1.6, 1.2 Hz), 5.18 (1H, ddd, $J = 16.8$, 1.8, 1.2 Hz), 5.81 (1H, ddd, $J = 16.8$, 10.4, 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8 (q), −4.6 (q), −4.3 (q), −4.2 (q), 18.1 (s), 18.2 (s), 25.86 (q), 25.90 (q), 27.6 (t), 45.2 (t), 68.0 (d), 70.0 (d), 71.1 (d), 81.5 (s), 114.3 (t), 141.2 (d); FTIR (neat) 3316, 2955, 2932, 2889, 2858, 1471, 1464, 1361, 1255, 1087 cm^{-1}; MS 311 $[M\text{-}^tBu]^+$; HRMS calcd for $[C_{16}H_{31}O_2Si_2]$ 311.1862, found 311.1864.

(3*S*,5*S*)-Bis[(*tert*-butyldimethylsilyl)oxy]oct-1-en-7-yne (3bS). $[\alpha]_D^{25}$ +15.9 ($c = 0.41$, $CHCl_3$).

(20*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-20-(hydroxymethyl)pregnane (16). A solution of **15** (6.15 g, 19 mmol) in THF (50 mL) was refluxed under an argon atmosphere in the presence of DBU (3.4 mL, 23 mmol) for 12 h. The reaction mixture was cooled and diluted with ether (400 mL). The combined mixture was washed with water, 1 N HCl aq, then brine, dried over magnesium sulfate and concentrated under reduced pressure to afford a mixture of 20-epimeric aldehydes.

The above residue was dissolved in MeOH (50 mL) and treated with $NaBH_4$ (3.57 g, 95 mmol) with stirring at 0 °C. The reaction mixture was stirred for 1 h at 0 °C,

then water was added to destroy the excess reagent. To the resulting mixture was added brine (50 mL), followed by extraction with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and concentrated. Purification by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:8) afforded the desired alcohol **16** (3.14 g, 52%) and the more polar epimer (2.49 g, 39%), both as colorless oils.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.01 (3H, s), 0.89 (9H, s), 0.93 (3H, s), 0.94 (3H, d, J = 6.7 Hz), 3.45 (1H, dd, J = 10.6, 7.0 Hz), 3.71 (1H, dd, J = 10.6, 3.7 Hz), 4.00 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1 (q), −4.8 (q), 14.1 (q), 16.6 (q), 17.7 (t), 18.0 (s), 22.9 (t), 25.9 (q), 26.7 (t), 34.4 (t), 37.5 (d), 40.2 (t), 42.0 (s), 53.0 (d), 53.1 (d), 66.9 (t), 69.4 (d); FTIR (neat) 3333, 2930, 2858, 1471, 1375, 1253, 1167 cm^{-1}; MS 326 $[M]^+$; HRMS calcd for $[C_{19}H_{38}O_2Si]$ 326.2641, found 326.2642.

(20*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-20-[(*p*-tolylsulfonyl)oxymethyl]pregnane (17). To a solution of **16** (3.14 g, 9.6 mmol) in pyridine (20 mL) was added *p*-tosyl chloride (2.20 g, 12 mmol) with stirring at 0 °C under an argon atmosphere. The resulting mixture was stirred for 13 h at room temperature. The reaction mixture was cooled in an ice bath, then water was added to the mixture. After extraction with ether, the organic layer was washed with 1 N HCl aq and brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a crude mixture, from which **17** (4.53 g) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:4) in 98% yield as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02 (3H, s), 0.00 (3H, s), 0.81 (3H, s), 0.87 (9H, s), 0.88 (3H, d, J = 6.7 Hz), 2.45 (3H, s), 3.78 (1H, dd, J = 9.2, 7.2 Hz), 3.97 (1H, m), 4.11 (1H, dd, J = 9.2, 3.4 Hz), 7.34 (2H, d, J = 8.2 Hz), 7.78 (2H, d, J = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2 (q), −4.8 (q), 14.1 (q), 16.7 (q), 17.6 (t), 18.0 (s), 21.6 (q), 22.7 (t), 25.8 (q), 26.6 (t), 34.2 (t), 34.7 (d), 39.9 (t), 41.8 (s), 52.7 (d), 52.8 (d), 69.2 (d), 74.3 (t), 128.0 (d), 129.7 (d), 133.3 (s), 144.6 (s); FTIR (neat) 2930, 2856, 2361, 1473, 1458, 1361, 1251, 1176 cm^{-1}; MS 465 [M-Me]$^+$, 423 [M-^{t}Bu]$^+$; HRMS calcd for $[C_{25}H_{41}O_4SSi]$ 465.2495, found 465.2493.

(20*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-20-(iodomethyl)pregnane (18). To a solution of **17** (127 mg, 0.26 mmol) in DMF (3 mL) was added NaI (126 mg, 0.84 mmol) and the mixture was stirred at 85 °C for 3 h. It was then cooled, and brine was added. After extraction with ether, the organic layer was dried over magnesium sulfate, filtered and concentrated. Purification by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:4) afforded the iodide **18** (104 mg) as a colorless oil in 92% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.88 (9H, s), 0.92 (3H, s), 0.95 (3H, d, J = 6.4 Hz), 3.18 (1H, dd, J = 9.4, 6.4 Hz), 3.46 (1H, dd, J = 9.4, 3.1 Hz), 4.00 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2 (q), −4.8 (q), 14.1 (q), 17.6 (t), 18.0 (s), 19.5 (t), 21.4 (q), 22.8 (t), 25.8 (q), 26.9 (t), 34.2 (t), 36.2 (d), 40.5 (t), 42.0 (s), 52.7 (d), 55.2 (d), 69.3 (d); FTIR (neat) 2930, 2856, 1730 cm^{-1}; MS 436 $[M]^+$; HRMS calcd for $[C_{19}H_{37}IOSi]$ 436.1658, found 436.1658.

The 23-epimeric sulfones (20). To a solution of **19** (1.50 g, 5.5 mmol) in dry THF (2 mL) and HMPA (2.2 mL, 13 mmol), *n*-BuLi solution (1.6 M in *n*-hexane, 3.4 mL, 5.5 mmol) was added dropwise with stirring at −78 °C. The resulting mixture was stirred for 10 min, then a solution of **18** (800 mg, 1.8 mmol) in dry THF (5 mL) was added. The mixture was stirred at −78 °C for 40 min, then the reaction was quenched by adding saturated aqueous NH_4Cl solution. The whole was extracted with ethyl acetate. The organic solution was dried over magnesium sulfate, filtered and concentrated. The crude product was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:4) to give a mixture of 23-epimeric sulfones **20** (1.04 g) in 98% yield.

23-Epimeric mixture: ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02 (3H, s), 0.00 (3H, s), 0.66 (3H, d, J = 6.4 Hz), 0.85 and 0.88 (9H, s), 1.23 and 1.27 (6H, s), 2.32 (1H, dd, J = 15.3, 4.3 Hz), 3.26 (1H, m), 3.30 (3H, s), 3.96 (1H, m), 4.57 (1H, d, J = 7.3 Hz), 4.67 (1H, d, J = 7.3 Hz), 7.55 (2H, t, J = 6.3 Hz), 7.63 (1H, t, J = 6.3 Hz), 7.88 (2H, t, J = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3 (q), −4.9 (q), 14.2 (q), 17.5 (t), 17.8 (q), 17.9 (s), 22.7 (t), 24.9 (q), 25.7 (q), 26.7 (t), 27.7 (q), 30.8 (d), 34.3 (t), 38.0 (t), 40.8 (t), 42.1 (t), 52.9 (d), 55.1 (q), 56.9 (d), 58.9 (d), 69.3 (d), 75.1 (s), 90.9 (t), 129.0 (d), 129.1 (d), 133.4 (d), 138.4 (s); FTIR (neat) 2932, 2856, 2361, 1469, 1448, 1375, 1304, 1145 cm^{-1}; MS 580 $[M]^+$; HRMS calcd for $[C_{32}H_{56}O_5SSi]$ 580.3618, found 580.3618.

(20*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-25-[(methoxymethyl)oxy]cholestane (21). A saturated solution of Na_2HPO_4 (20 g) in MeOH (30 mL) was added to a stirred solution of **20** (1.50 g, 2.6 mmol) in THF (30 mL). To this mixture was added 5% sodium amalgam (35 g) at 0 °C. The resulting suspension was stirred under argon for 1 h at room temperature. The mixture was diluted with ether and the whole was filtered over Celite® and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:8) to give **21** (1.10 g) as a colorless oil in 98% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.81 (3H, d, J = 6.7 Hz), 0.89 (9H, s), 0.91 (3H, s), 1.21 (6H, s), 3.36 (3H, s), 3.99 (1H, m), 4.70 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1 (q), −4.8 (q), 14.0 (q), 17.8 (t), 18.1 (s), 18.6 (t), 20.6 (t), 23.0 (t), 25.8 (q), 26.4 (q), 27.2 (t), 34.5 (t), 34.8 (d), 35.8 (t), 40.7 (t), 42.1 (t), 42.2 (s), 53.2 (d), 55.1 (q), 56.5 (d), 69.5 (d), 76.4 (s), 91.0 (t); FTIR (neat) 2932, 2858, 1469, 1377, 1251, 1165, 1145, 1086, 1041, 922 cm^{-1}; MS 440 $[M]^+$, 425 [M-Me]$^+$; HRMS calcd for $[C_{26}H_{52}O_3Si]$ 440.3686, found 440.3687.

(20*S*)-De-A,B-cholestane-8β,25-diol (22). A solution of **21** (80 mg, 0.18 mmol) in MeOH (3 mL) was treated with *p*-TsOH·H_2O (174 mg, 0.91 mmol) at room temperature. The reaction mixture was stirred at room

temperature for 1 day. After the reaction was completed, the mixture was concentrated and purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:2) to give **22** (43 mg) as a colorless oil in 85% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (3H, d, J = 6.7 Hz), 0.93 (3H, s), 1.21 (6H, s), 4.07 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8 (q), 17.5 (t), 18.5 (q), 20.9 (t), 22.4 (t), 27.1 (t), 29.2 (q), 29.3 (q), 33.6 (t), 34.7 (d), 35.7 (t), 40.4 (t), 41.9 (s), 44.3 (t), 52.7 (d), 56.3 (d), 69.4 (d), 71.1 (s); FTIR (neat) 3385, 2932, 2870, 1468, 1375, 1265, 1165, 1082, 1068, 989, 947 cm^{-1}; MS 264 [M-H_2O]$^+$, 246 [M-2H_2O]$^+$; HRMS calcd for [$C_{18}H_{32}O$] 264.2453, found 264.2453.

(20*S*)-De-A,B-25-hydroxycholestan-8-one (23). Solid tetrapropylammonium perruthenate (TPAP, 135 mg, 0.38 mmol) was added to a stirred mixture of **22** (186 mg, 0.66 mmol), 4-methylmorpholine *N*-oxide (NMO, 198 mg, 1.7 mmol), and 4 Å MS (50 mg) in dry CH_2Cl_2 (10 mL) at room temperature under argon. Upon completion of the reaction, the mixture was directly purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:3) to give the ketone **23** (178 mg) as a colorless oil in 96% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (3H, s), 0.87 (3H, d, J = 6.1 Hz), 1.22 (6H, s), 2.45 (1H, dd, J = 11.6, 7.3 Hz), 4.07 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8 (q), 18.5 (q), 19.0 (t), 20.9 (t), 24.1 (t), 29.3 (q), 29.4 (d), 34.9 (t), 36.0 (t), 41.0 (t), 44.2 (t), 50.0 (s), 56.3 (d), 62.0 (d), 71.0 (s), 212.0 (s); FTIR (neat) 3445, 2964, 2876, 1711, 1468, 1427, 1305, 1269, 1224, 1157 cm^{-1}; MS 280 [M]$^+$, 262 [M-H_2O]$^+$; HRMS calcd. for [$C_{18}H_{32}O_2$] 280.2402, found 280.2401.

(*E*)-(20*S*)-De-A,B-8-(bromomethylene)cholestan-25-ol (5). Sodium hexamethyldisilazide (NaHMDS) (1.0 M in THF, 0.86 mL, 0.86 mmol) was added to (bromomethylene)triphenylphosphonium bromide (389 mg, 0.90 mmol) in THF (1.5 mL) at −60 °C under argon. After 1 h, a solution of **23** (50 mg, 0.18 mmol) in THF (1.5 mL) was added. After an additional 1 h at room temperature, the reaction mixture was diluted with *n*-hexane and the whole was filtered over Celite®. The filtrate was concentrated, then purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:8, then 1:3) to give **5** (36 mg) as a colorless oil in 56% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.56 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 1.22 (6H, s), 2.88 (1H, m), 5.64 (1H, d, J = 1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1 (q), 18.5 (q), 19.0 (t), 20.9 (t), 21.9 (t), 22.6 (t), 27.3 (t), 29.2 (q), 29.3 (q), 31.1 (t), 35.3 (d), 36.0 (t), 39.8 (t), 44.3 (t), 45.6 (s), 55.4 (d), 55.9 (d), 71.1 (s), 97.4 (d), 145.1 (s); FTIR (neat) 3383, 2964, 2872, 1631, 1466, 1377 cm^{-1}; MS 356 and 358 [M]$^+$, 338 and 340 [M-H_2O]$^+$; HRMS calcd. for [$C_{19}H_{33}{}^{79}BrO$] 356.1716, found 356.1715.

(5*Z*,7*E*)-(1*S*,3*R*,20*S*)-9,10-Seco-5,7,10(19)-cholestatriene-1,3,25-triol (2: (20*S*)-(1α,3β)). A mixture of $(dba)_3Pd_2\cdot CHCl_3$ (2.0 mg, 0.0013 mmol), Ph_3P (5.0 mg, 0.019 mmol) and triethylamine (0.5 mL) in toluene (0.5 mL) was stirred for 10 min at room temperature, then a solution of the A-ring moiety **3aA** (21 mg, 0.057 mmol) and the 20-*epi* CD-ring **5** (34 mg, 0.095 mmol) in toluene (0.5 mL) was added. After having been heated at reflux for 2 h and diluted with pentane, the reaction mixture was filtered through a pad of silica gel with ether. After evaporation of the solvent, the crude mixture was dissolved in methanol (2 mL) and treated with CSA (15 mg, 0.065 mmol) at room temperature for 12 h. After removal of the solvent, the residue was subjected to silica gel column chromatography (ethyl acetate:*n*-hexane = 1:1) to give the vitamin **2** (12 mg) as a white solid in 52% yield. Further purification for biological evaluation was conducted by using reversed-phase recycle HPLC (YMC-Pack ODS column, 20×150 mm, 9.0 mL/min, acetonitrile:water = 9:1).

UV (EtOH) λ_{max} 263 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 1.21 (6H, s), 2.31 (1H, dd, J = 13.4, 6.4 Hz), 2.60 (1H, dd, J = 13.4, 3.1 Hz), 2.83 (1H, dd, J = 12.5, 4.3 Hz), 4.23 (1H, m), 4.43 (1H, m), 5.01 (1H, s), 5.33 (1H, s), 6.02 (1H, d, J = 11.3 Hz), 6.38 (1H, d, J = 11.3 Hz); MS 416 [M]$^+$, 398 [M-H_2O]$^+$, 380 [M-2H_2O]$^+$; HRMS calcd for [$C_{27}H_{44}O_3$] 416.3291, found 416.3286.

(5*Z*,7*E*)-(1*R*,3*R*,20*S*)-9,10-Seco-5,7,10(19)-cholestatriene-1,3,25-triol ((20*S*)-(1β,3β)). UV (EtOH) λ_{max} 261 nm, λ_{min} 225 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.85 (3H, d, J = 6.7 Hz), 1.21 (6H, s), 2.47 (1H, dd, J = 13.4, 4.3 Hz), 2.56 (1H, d, J = 14.0 Hz), 2.85 (1H, dd, J = 12.5, 4.3 Hz), 4.11 (1H, m), 4.36 (1H, m), 5.01 (1H, d, J = 1.8 Hz), 5.30 (1H, s), 6.06 (1H, d, J = 11.3 Hz), 6.45 (1H, d, J = 11.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1 (q), 18.5 (q), 20.8 (t), 22.1 (t), 23.6 (t), 27.2 (t), 29.0 (t), 29.2 (q), 35.4 (d), 36.0 (t), 40.0 (t), 40.3 (t), 44.2 (t), 45.4 (t), 45.9 (s), 56.1 (d), 56.4 (d), 68.2 (d), 71.1 (s), 73.6 (d), 113.4 (t), 117.0 (d), 125.8 (d), 131.7 (s), 143.2 (s), 147.3 (s); FTIR (neat) 3356, 2943, 2870, 1437, 1377, 1064, 1016, 908, 725 cm^{-1}; MS 416 [M]$^+$, 398 [M-H_2O]$^+$, 380 [M-2H_2O]$^+$; HRMS calcd for [$C_{27}H_{44}O_3$] 416.3291, found 416.3292.

(5*Z*,7*E*)-(1*S*,3*S*,20*S*)-9,10-Seco-5,7,10(19)-cholestatriene-1,3,25-triol ((20*S*)-(1α,3α)). UV (EtOH) λ_{max} 261 nm, λ_{min} 225 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 1.22 (6H, s), 2.44 (1H, dd, J = 13.4, 5.5 Hz), 2.56 (1H, d, J = 13.4 Hz), 2.84 (1H, dd, J = 11.6, 4.3 Hz), 4.06 (1H, m), 4.31 (1H, m), 5.00 (1H, d, J = 1.5 Hz), 5.30 (1H, s), 6.03 (1H, d, J = 11.3 Hz), 6.44 (1H, d, J = 11.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2 (q), 18.5 (q), 20.8 (t), 22.1 (t), 23.4 (t), 27.2 (t), 29.0 (t), 29.2 (q), 35.4 (d), 36.0 (t), 40.4 (t), 40.7 (t), 44.3 (t), 45.5 (t), 45.9 (s), 56.1 (d), 56.4 (d), 68.2 (d), 71.1 (s), 73.1 (d), 112.9 (t), 117.1 (d), 125.6 (d), 131.7 (s), 143.2 (s), 147.3 (s); FTIR (neat) 3356, 2941, 2872, 1456, 1377, 1064, 1016, 908, 733 cm^{-1}; MS 416 [M]$^+$, 398 [M-H_2O]$^+$, 380 [M-2H_2O]$^+$; HRMS calcd for [$C_{27}H_{44}O_3$] 416.3291, found 416.3283.

(5*Z*,7*E*)-(1*R*,3*S*,20*S*)-9,10-Seco-5,7,10(19)-cholestatriene-1,3,25-triol ((20*S*)-(1β,3α)). UV (EtOH) λ_{max} 263 nm,

λ_{min} 225 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.85 (3H, d, J = 6.7 Hz), 1.22 (6H, s), 2.30 (1H, dd, J = 13.1, 7.6 Hz), 2.62 (1H, dd, J = 13.1, 3.7 Hz), 2.83 (1H, dd, J = 12.5, 4.3 Hz), 4.22 (1H, m), 4.44 (1H, m), 5.01 (1H, s), 5.32 (1H, s), 6.01 (1H, d, J = 11.3 Hz), 6.39 (1H, d, J = 11.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2 (q), 18.5 (q), 20.8 (t), 22.1 (t), 23.5 (t), 27.2 (t), 29.0 (t), 29.2 (q), 35.4 (d), 36.0 (t), 40.3 (t), 42.8 (t), 44.2 (t), 45.4 (t), 45.9 (s), 56.1 (d), 56.3 (d), 66.8 (d), 71.1 (s), 71.4 (d), 112.6 (t), 117.1 (d), 125.1 (d), 132.8 (s), 143.3 (s), 147.4 (s); FTIR (neat) 3358, 2945, 2872, 1456, 1375, 1057, 910, 733 cm^{-1}; MS 416 $[M]^+$, 398 $[M\text{-}H_2O]^+$, 380 $[M\text{-}2H_2O]^+$; HRMS calcd for $[C_{27}H_{44}O_3]$ 416.3291, found 416.3291.

Binding to vitamin D receptor (VDR). Bovine thymus 1α,25-dihydroxyvitamin D_3 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, 0.23 mg protein) 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 radioactivity of the supernatant (500 μL) with ACS-II (9.5 mL) (Amersham, UK) was then counted.

Binding to vitamin D binding protein (DBP). Serum from vitamin D-deficient Wistar male rats was diluted (×70,000) with 3.5 mM barbiturate buffer (pH 8.6) containing 0.13 M NaCl and 0.1% (w/v) bovine serum albumin (BSA) and used as a source of DBP. The diluted serum (1 mL) was incubated with 0.1 nM [^{3}H]-25-hydroxyvitamin D_3 and 100 μL of ethanol solution of 1α,25-dihydroxyvitamin D_3 or an analogue at various concentrations for 60 min at 4 °C. Unbound [^{3}H]-25-hydroxyvitamin D_3 was removed by treatment with dextran-coated charcoal for 10 min at 4 °C and centrifuged at 3000 rpm for 10 min. The radioactivity of the supernatant (1 mL) was measured.

Cell surface antigen expression analysis. HL-60 cells were seeded at 10^5 cells/well in 24-well plates, and incubated for 72 h with between 10^{-12} and 10^{-7} (or 10^{-8}) M of 1α,25-dihydroxyvitamin D_3 or an analogue at 37 °C in a humidified atmosphere of 5% carbon dioxide in air. The cells were then washed with PBS and adjusted to 2×10^6 cells/100 μL of Diluent solution [phosphate-buffered saline (minus Mg^{2+}, minus Ca^{2+}) containing 1% BSA and 1% NaN_3]. Aliquots of cell suspension (100 μL) were incubated with 10 μL of the human monoclonal fluorescein isothiocyanate-conjugated CD11b antibody for 30 min at room temperature without light. The cells were washed once with Diluent solution and then fixed in 500 μL of PBS containing 2% paraformaldehyde. Fluorescence was read on a Becton Dickinson FACSan™ at an excitation wavelength of 490 nm and emission wavelength of 520 nm. Results were recorded as the mean fluorescence index, which is the product of the % fluorescence and the mean fluorescence intensity, with 10^4 cells being counted per treatment.

In vivo bone mineral mobilization assay. Six-week-old normal SD male rats were divided into several groups of five rats each receiving either an iv injection of 1.0, 10.0 or 100.0 μg/kg of 1α,25-dihydroxyvitamin D_3 or an analogue in 2 mL/kg of 0.1% Triton X-100 solution. The resulting increases in serum calcium were measured at 8, 24, 48 and 72 h after administration by the *o*-cresolphthalein complexone method. Relative activity of each analogue with respect to 1α,25-dihydroxyvitamin D_3 was evaluated as follows: dose response effect of each analogue was calculated at the time when the effect was maximum and expressed as the dosage required to elevate the serum calcium level by 1 mg/dL.

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