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Synthesis of 2,2-dimethyl-1,25-dihydroxyvitamin D_3: A-ring structural motif that modulates interactions of vitamin D receptor with transcriptional coactivators

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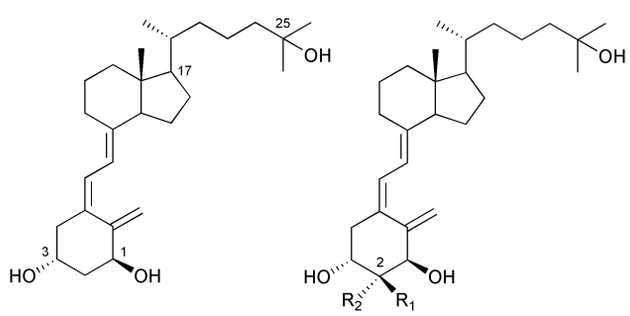
A concise synthesis of all four possible A-ring stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 and characterization of their distinct transcriptional features, which appear to have been inherited from the corresponding 2α-methyl derivatives, is reported.

Introduction

Cholecalciferol, known as vitamin D_3, is metabolized to afford the hormonally active form, 1α,25-dihydroxyvitamin D_3 (**1**), through 25-hydroxyvitamin D_3. 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 in recent years, since the X-ray crystal structure of deletion mutant VDR complexed with the natural ligand **1** was solved in 2000.[3] Insight into the structure–function relationships of a variety of ligands is essential to understand how the subtype-free, singular VDR can deliver the diverse biological activities of **1**, as well as to allow the development of potential therapeutic agents with selective activity profiles for the treatment of cancer or osteoporosis.



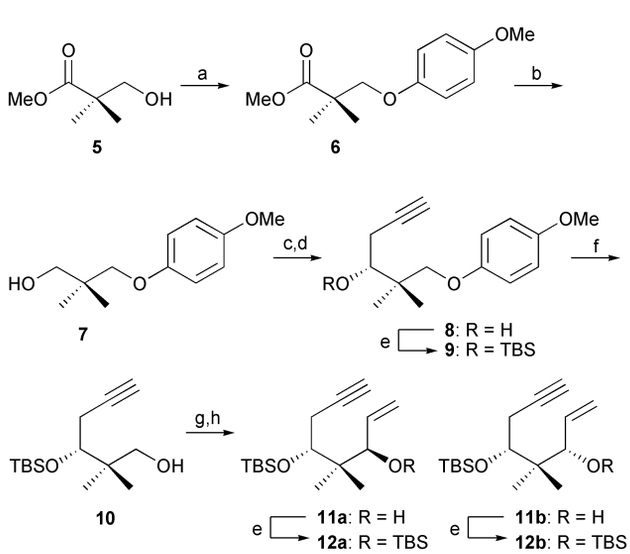
1: 1α,25-dihydroxyvitamin D_3

2: $R_1 = CH_3$, $R_2 = H$
3: $R_1 = H$, $R_2 = CH_3$
4a: $R_1 = R_2 = CH_3$

Modification of **1** in the A-ring, which possesses two critical hydroxy groups at C1 and C3, has become of interest in recent years, because the other three A-ring stereoisomers of **1** have proven to exhibit unique activity profiles, being different from the natural hormone **1**.[4] In addition, some of our synthesised 2-substituted analogues exhibited remarkably high affinity for VDR.[5,6] The X-ray crystal structure of VDR complexed with **1** indicated, in addition to the cavity in which the flexible C17 side-chain moiety of **1** is accommodated, the presence of an extra space in the vicinity of the A-ring.[3a] It was suggested that substituents of synthetic A-ring analogues could occupy this additional space.[5h,6c] 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 fourfold better binder to VDR, whereas its 2-epimer, 2β-methyl-1α,25-dihydroxyvitamin D_3 (**3**) showed one-eighth of the affinity of **1**.[5a–c] In view of these important results, we have now synthesised all four possible A-ring stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4a**–**d**) as A-ring analogues having methyl substituents projecting in both directions in the cavity, to investigate how the second methyl group affects the activity profiles of the parent compounds.

Results and discussion

Synthesis was carried out by using a convergent method[7] pioneered by Trost *et al.*[8] The synthetic route to the 2,2-disubstituted A-ring precursors (**12a**,**b**) is shown in Scheme 1. Protection of the primary alcohol in methyl 3-hydroxy-2,2-dimethylpropionate (**5**) with a *p*-anisyl group gave **6** in excellent yield.[9] Reduction of the methyl ester **6** using $LiAlH_4$, followed by PDC oxidation of the resultant alcohol **7** furnished the



Scheme 1 *Reagents and conditions*: (a) 4-methoxyphenol, DEAD, Ph_3P–THF, 98%; (b) $LiAlH_4$–THF; 97%; (c) PDC, 4 Å MS–CH_2Cl_2, 89%; (d) allenylmagnesium bromide–ether, 68%; (e) TBSOTf, 2,6-lutidine–CH_2Cl_2, 81% for **9**, quant. for **12a**,**b**; (f) CAN–CH_3CN, H_2O, 77%; (g) TPAP, NMO, 4 Å MS–CH_2Cl_2, 69%; (h) vinylmagnesium bromide–toluene, 60%.

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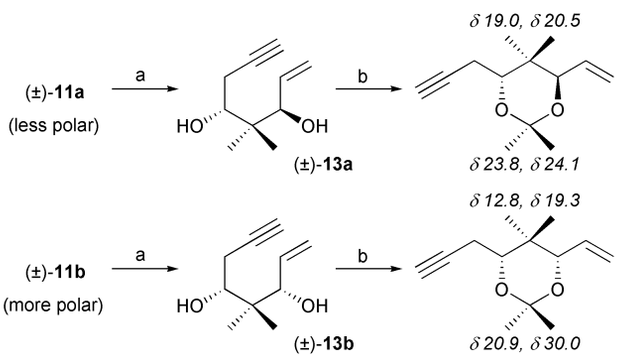
corresponding aldehyde, which was reacted with allenylmagnesium bromide[10] to give an enantiomeric mixture of the propargyl alcohol (±)-**8**. Protection of the secondary alcohol of (±)-**8** with a *tert*-butyldimethylsilyl (TBS) group afforded (±)-**9** in 81% yield, and deprotection of the *p*-anisyl group with cerium(IV) diammonium nitrate (CAN) furnished the primary alcohol (±)-**10** in a satisfactory yield. Introduction of a vinyl group into the aldehyde, obtained from (±)-**10** with tetrapropylammonium perruthenate (TPAP) in 69% yield, proceeded smoothly to give the allylic alcohols (±)-**11a**,**b** in 60% yield as an approximately 1 : 2 mixture of the diastereomers. These isomers were readily separable by silica gel column chromatography and the relative stereochemistry of the 1,3-diol in each enantiomeric mixture was determined by ^{13}C NMR analysis of the acetonides (Scheme 2).[11] The data for the dimethyl groups, in addition to the acetal methyl groups, supported the conformational preference.[12]



Scheme 2 Determination of relative stereochemistry of the 1,3-diols by ^{13}C NMR analysis. *Reagents and conditions*: (a) TBAF–THF; (b) 2,2-dimethoxypropane, CSA.

Coupling reaction of the protected A-ring enynes (±)-**12a**,**b** with the CD-ring portion **14**[8] in the presence of the tetrakis(triphenylphosphine)palladium, followed by deprotection with TBAF,[13] proceeded smoothly to give the vitamin analogues **4a**,**b** and **4c**,**d**, respectively (Scheme 3). The absolute stereochemistry of the diols was determined by ^{1}H NMR analyses of their bis-MTPA esters (Fig. 1).[14] In this way, four stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4a**–**d**) were obtained, and each of them was purified by using recycling reversed-phase HPLC for biological evaluation.

Table 1 Vitamin D receptor binding affinity of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4a**–**d**)[a]

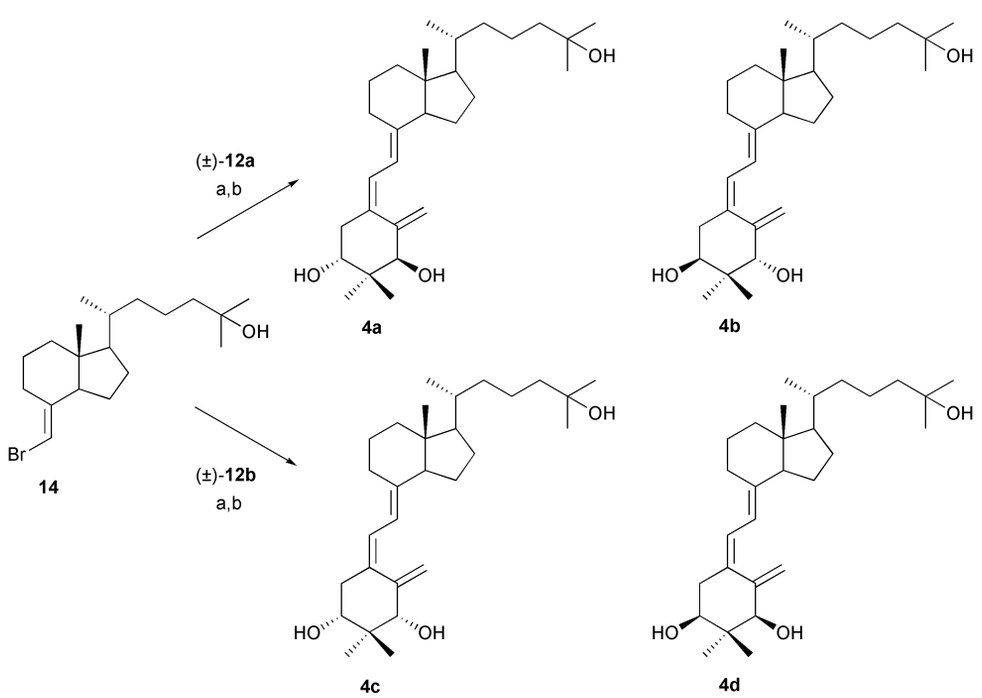
Compounds	VDR[b] affinity
1	100
2	400[c]
3	13[c]
4a	3
4b	0.005
4c	<0.001
4d	0.06

[a] The potency of 1α,25-dihydroxyvitamin D_3 (**1**) is taken as 100. [b] Bovine thymus vitamin D receptor. [c] Ref 5*a*,*c*.

Table 1 summarizes the relative VDR binding affinity of the synthesised compounds (**4a**–**d**) in comparison with the natural hormone **1**, together with the 2-methyl analogues (**2**, **3**).[5] The analogue **4a** showed 3% of the affinity of **1**, which suggests that introduction of the second methyl group into **2** results in an approximately 100-fold reduction of the affinity to VDR. Other diastereomers (**4b**–**d**) showed weaker affinity compared with their mono-methyl parents.[5]

Transcriptional machinery of the nuclear receptors is highly conserved among the nuclear receptor superfamily.[2] The modulation of the transcriptional function by ligands depends on conformational changes of the transactivation domain of the receptor C-terminal region (AF-2), which provides an interface for binding of cofactors.[15,16] Modifications that can influence the interface of VDR should include those in the side chain moiety of **1**, which would be embedded in the vicinity of the AF-2 region.[15a,16a] In particular, 22-oxa-1α,25-dihydroxyvitamin D_3 (OCT) used to treat psoriasis, was reported to induce selective interaction of VDR with coactivator TIF2, which could be responsible for its unique activity profile.[15]

Using the A-ring analogues, we investigated ligand-specific potentiation of VDR transcriptional activity with two coactivators, TIF2 and SRC-1, both of which belong to the 160 kDa protein family of coactivators (Fig. 2).[15] Treatment with **1** at 10^{-8} M in the presence of TIF2 induced approximately twice the transcriptional potency compared with the result with no



Scheme 3 *Reagents and conditions*: (a) $(Ph_3P)_4Pd$, Et_3N–toluene, 63–66%; (b) TBAF–THF, 29–63%.

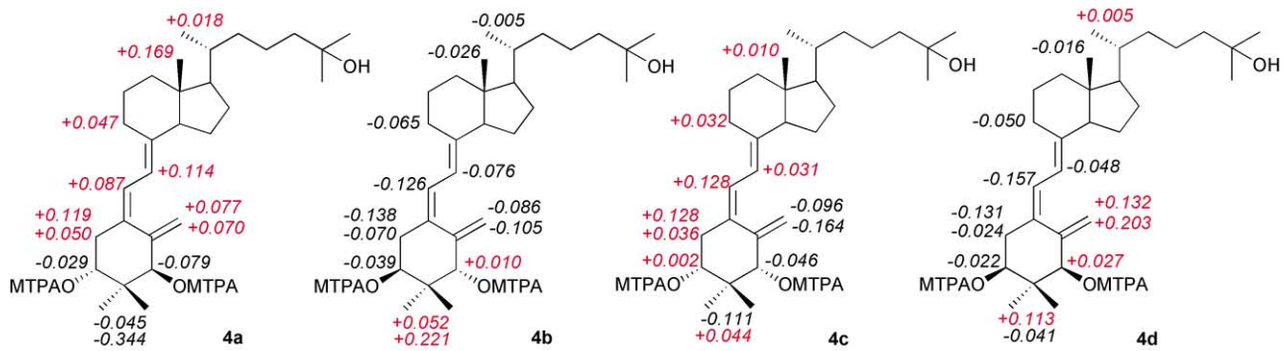


Fig. 1 Determination of absolute configuration at the C1 and C3 positions of **4a**–**d** by ^{1}H NMR analysis of their bis-MTPA esters.

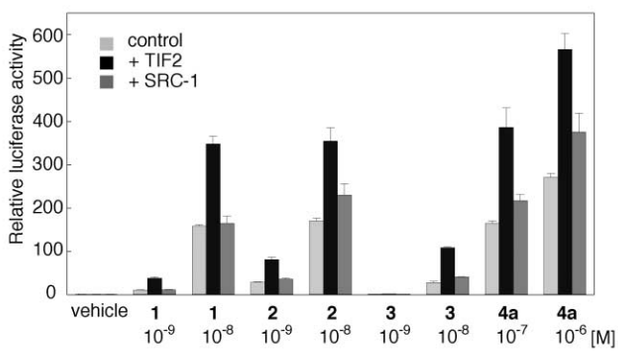


Fig. 2 Ligand-specific potentiation of VDR transactivation function by coactivators. COS-1 cells were transfected with a reporter plasmid [heptadecamer(×2)-β-globin-luciferase] and pM(GAL4-DBD)-VDR(DEF) with or without TIF2 or SRC-1 expression vectors in the presence or absence of 1α,25-dihydroxyvitamin D_3 (**1**), or its analogues (**2**,**3**,**4a**) and harvested for luciferase assay at 24 h post-transfection.

coactivator (control), whereas the treatment had little effect in the presence of SRC-1. On the other hand, in the presence of SRC-1, the 2α-methyl analogue **2** at 10^{-8} M potentiated the VDR transcription function by 35% compared with its control. The 2β-methyl analogue **3** showed a weaker potency, but exhibited a significant preference for TIF2 over SRC-1. Treatment with **4a** at 10^{-7} M in the presence of SRC-1 caused a 32% increase of the transcriptional potency, which is similar to the result with **2** at 10^{-8} M. The character of **4a** may be inherited from the 2α-methyl compound **2**.

The present results suggest that ligands with a modification in the A-ring, which appears to be located far from AF-2, can alter the VDR-coactivator interaction, resulting in selective potentiation of the transcription function. This strategy to modulate the functions of the receptor based on an altered protein–protein interaction by A-ring modification may prove to be valuable.

In summary, we have efficiently synthesised all four possible A-ring stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4a**–**d**) by employing a convergent method using a palladium catalyst. The transcriptional function of the dimethyl analogue **4a** in the presence of coactivators of the 160 kDa protein family suggested that **4a** retained the unique activity afforded by 2α-methyl substitution, despite the reduced VDR binding affinity. Differential recruitment of coactivators could be an interesting approach for the development of selective therapeutic agents.

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. Recycling preparative HPLC was performed on a Waters LC equipped with a 510 HPLC pump and a 484 tunable absorbance detector.

Methyl 3-(4-methoxyphenoxy)-2,2-dimethylpropionate (6). A solution of methyl hydroxypivalate **5** (3.00 g, 22.7 mmol), 4-methoxyphenol (8.45 g, 68.1 mmol) and triphenylphosphine (7.74 g, 29.5 mmol) in THF (50 mL) was treated with diethyl azodicarboxylate (DEAD) (40% in toluene solution, 13 mL) at 0 °C under an argon atmosphere. The resulting homogeneous solution was heated at reflux for 2 h, cooled to room temperature and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 9) to afford **6** (5.30 g) as a colourless oil in 98% yield.

6: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (6 H, s), 3.69 (3 H, s), 3.76 (3 H, s), 3.91 (2 H, s), 6.82 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4 (q), 43.4 (s), 52.0 (q), 55.7 (q), 75.3 (t), 114.6 (d), 115.7 (d), 153.3 (s), 176.5 (s); IR (neat) 2951, 2835, 1734, 1510, 1471, 1396, 1367, 1232, 1151, 1107, 1049, 1003, 825, 742 cm^{-1}; MS *m*/*z* 238 $(M)^+$, 207 $(M - OMe)^+$; HRMS calcd for $C_{13}H_{18}O_4$ 238.1205, found 238.1206.

3-(4-Methoxyphenoxy)-2,2-dimethylpropanol (7). A solution of **6** (2.07 g, 8.39 mmol) in THF (15 mL) was added dropwise to a suspension of lithium aluminium hydride (478 mg, 12.6 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 1.5 h at this temperature, then ethyl acetate, followed by water, was added, and the whole was filtered over Celite™. The filtrate was extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and concentrated. The residue was subjected to silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 3) to afford **7** (1.71 g) as a colourless solid in 97% yield.

7: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (6 H, s), 2.01 (1 H, br s), 3.54 (2 H, m), 3.73 (2 H, s), 3.77 (3 H, s), 6.84 (4 H, m); IR (neat) 3265, 2959, 2937, 2909, 2874, 2835, 1516, 1460, 1359, 1292, 1242, 1180, 1111, 1035, 821 cm^{-1}; MS *m*/*z* 210 $(M)^+$; HRMS calcd for $C_{12}H_{18}O_3$ 210.1256, found 210.1265.

1-(4-Methoxyphenoxy)-2,2-dimethylhex-5-yn-3-ol (8). A stirred mixture of **7** (1.67 g, 7.94 mmol) and powdered 4 Å MS (500 mg) in CH_2Cl_2 (20 mL) was treated with PDC (7.45 g, 19.9 mmol) at 0 °C under an argon atmosphere. The resulting mixture was stirred overnight at room temperature, and separated by silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 3) to give the corresponding aldehyde (1.47 g) as a colourless oil in 89% yield. The aldehyde was used immediately in the following step.

3-(4-Methoxyphenoxy)-2,2-dimethylpropanal. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.20 (6 H, s), 3.77 (3 H, s), 3.91 (2 H, s), 6.82 (4 H, s), 9.64 (1 H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 19.0 (q), 21.8 (q), 46.8 (s), 55.7 (q), 73.3 (t), 114.6 (d), 115.5 (d), 152.9 (s),

154.0 (s), 204.6 (d); IR (neat) 2964, 2910, 2874, 2835, 1734, 1510, 1471, 1442, 1402, 1377, 1290, 1230, 1182, 1107, 1049, 1005, 929, 898, 825 cm^{-1}; MS *m/z* 208 $(M)^+$; HRMS calcd for $C_{12}H_{16}O_3$ 208.1099, found 208.1079.

To a solution of the aldehyde (4.73 g, 22.7 mmol) in ether (40 mL) was added with stirring allenylmagnesium bromide (*ca.* 2 M in ether, 66 mL) at −78 °C under argon. The reaction mixture was stirred for 1.5 h, and the reaction was quenched by addition of saturated aqueous NH_4Cl. After extraction with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the solvent afforded a residue, from which the 3-epimeric alcohols **8** (3.82 g) were obtained in 68% total yield.

8: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.03 (3 H, s), 1.04 (3 H, s), 2.04 (1 H, t, J = 2.8 Hz), 2.38 (1H, ddd, J = 16.5, 9.3, 2.8 Hz), 2.50 (1 H, dt, J = 16.5, 2.8 Hz), 2.63 (1 H, br d, J = 2.8 Hz), 3.68 (1 H, d, J = 8.8 Hz), 3.77 (3 H, s), 3.83 (1 H, dt, J = 9.3, 2.8 Hz), 3.84 (1 H, d, J = 8.8 Hz), 6.83 (4 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 19.6 (q), 21.9 (q), 22.5 (t), 38.5 (s), 55.7 (q), 70.3 (s), 74.8 (d), 75.8 (t), 82.2 (d), 114.6 (d), 115.4 (d), 153.0 (s), 153.9 (s); MS *m/z* 248 $(M)^+$; HRMS calcd for $C_{15}H_{20}O_3$ 248.1413, found 248.1408.

4-(*tert*-Butyldimethylsilyl)oxy-6-(4-methoxyphenoxy)-5,5-dimethylhex-1-yne (9). To a stirred mixture of **8** (3.77 g, 15 mmol) and 2,6-lutidine (4.2 mL, 45 mmol) in CH_2Cl_2 (30 mL) was added TBSOTf (6.5 mL, 23 mmol) at 0 °C under argon. The resulting mixture was stirred at 0 °C for 5 min, diluted with ethyl acetate and washed with water. The organic layer was dried over magnesium sulfate and filtered. Removal of the solvent afforded a residue, from which **9** (4.45 g) was separated by silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 12) as a colourless oil in 81% yield.

9: ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.01 (3 H, s), 0.15 (3 H, s), 0.88 (9 H, s), 1.00 (3 H, s), 1.04 (3 H, s), 1.98 (1 H, t, J = 2.8 Hz), 2.28 (1H, ddd, J = 17.0, 4.9, 2.8 Hz), 2.57 (1 H, ddd, J = 17.0, 4.9, 2.8 Hz), 3.57 (1 H, d, J = 8.8 Hz), 3.74 (1 H, d, J = 8.8 Hz), 3.76 (1 H, s), 3.93 (1 H, t, J = 4.9 Hz), 6.81 (4 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.8 (q), −3.8 (q), 18.2 (s), 19.8 (q), 21.9 (q), 23.2 (t), 26.0 (q), 40.1 (s), 55.7 (q), 70.1 (s), 74.5 (t), 74.8 (d), 83.3 (d), 114.5 (d), 115.1 (d), 153.4 (s), 153.5 (s); MS *m/z* 362 $(M)^+$, 347 $(M - Me)^+$, 305 $(M - {}^tBu)^+$; HRMS calcd for $C_{21}H_{34}O_3Si$ 362.2278, found 362.2285.

3-(*tert*-Butyldimethylsilyl)oxy-2,2-dimethylhex-5-yn-1-ol (10). A solution of **9** (2.38 g, 6.6 mmol) in CH_3CN (68 mL) and water (17 mL) was treated with diammonium cerium nitrate (CAN) (8.66 g, 15.8 mmol) at 0 °C. After 15 min, ethyl acetate (50 mL) and brine (30 mL) were added to the solution. The aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous $NaHCO_3$, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **10** (1.30 g) was separated by silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 9) as a pale yellow oil in 77% yield.

10: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.12 (3 H, s), 0.17 (3 H, s), 0.87 (3 H, s), 0.92 (9 H, s), 1.03 (3 H, s), 2.04 (1 H, t, J = 2.7 Hz), 2.34 (1H, ddd, J = 17.6, 4.4, 2.7 Hz), 2.58 (1 H, ddd, J = 17.6, 6.0, 2.7 Hz), 3.35 (1 H, dd, J = 11.0, 6.0 Hz), 3.70 (1 H, d, J = 11.0 Hz), 3.72 (1 H, dd, J = 6.0, 4.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.9 (q), −4.1 (q), 18.1 (s), 21.6 (q), 22.8 (q), 23.3 (t), 25.9 (q), 39.9 (s), 69.7 (t), 70.6 (s), 78.1 (d), 82.5 (d); MS *m/z* 199 $(M - {}^tBu)^+$; HRMS calcd for $C_{10}H_{19}O_2Si$ 199.1154, found 199.1156.

(3*RS*,5*RS*)-5-(*tert*-Butyldimethylsilyl)oxy-4,4-dimethyloct-1-en-7-yn-3-ol (11a: 1,3-*anti*-diol) and (3*RS*,5*SR*)-5-(*tert*-butyldimethylsilyl)oxy-4,4-dimethyloct-1-en-7-yn-3-ol (11b: 1,3-*syn*-diol). Solid tetrapropylammonium perruthenate (TPAP) (483 mg, 0.23 eq.) was added to a stirred mixture of **10** (1.50 g, 5.86 mmol), 4-methylmorpholine *N*-oxide (NMO) (1.79 g, 2.6 eq.) and powdered 4 Å MS (150 mg) in CH_2Cl_2 (15 mL) at room temperature under argon. The resulting mixture was stirred for 1.5 h, and separated by silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 9) to give the corresponding aldehyde (1.03 g) as a colourless oil in 69% yield. The aldehyde was used immediately in the following step.

3-(tert-Butyldimethylsilyl)oxy-2,2-dimethylhex-5-ynal. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.09 (3 H, s), 0.15 (3 H, s), 0.87 (9 H, s), 1.08 (3 H, s), 1.09 (3 H, s), 2.02 (1 H, t, J = 2.8 Hz), 2.33 (1H, ddd, J = 17.6, 4.9, 2.8 Hz), 2.45 (1 H, ddd, J = 17.6, 6.0, 2.8 Hz), 3.97 (1 H, t, J = 5.5 Hz), 9.67 (1 H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.8 (q), −4.0 (q), 18.05 (q), 18.09 (s), 18.9 (q), 23.8 (t), 25.8 (q), 51.4 (s), 71.7 (d), 75.0 (d), 81.4 (s), 205.4 (d); IR (neat) 3314, 2957, 2932, 2887, 2858, 2710, 2121, 1730, 1469, 1388, 1361, 1255, 1101, 1006, 918, 837, 777 cm^{-1}; MS *m/z* 239 $(M - Me)^+$; HRMS calcd for $C_{13}H_{23}O_2Si$ 239.1468, found 239.1472.

To a solution of the aldehyde (230 mg, 0.91 mmol) in toluene (2 mL) was added with stirring vinylmagnesium bromide (1.6 M in THF, 0.57 mL, 0.91 mmol) at −78 °C under argon. The reaction mixture was stirred for 1 h, and the reaction was quenched by addition of saturated aqueous NH_4Cl. After extraction with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the solvent afforded a residue, from which **11a** (53 mg, 20%, less polar) and **11b** (102 mg, 40%, more polar) were obtained by silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 9), both as colourless oils.

11a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.15 (3 H, s), 0.20 (3 H, s), 0.82 (3 H, s), 0.93 (9 H, s), 0.98 (3 H, s), 2.04 (1 H, t, J = 2.7 Hz), 2.41 (1H, ddd, J = 17.6, 4.9, 2.7 Hz), 2.66 (1 H, ddd, J = 17.6, 4.9, 2.7 Hz), 3.76 (1 H, t, J = 4.9 Hz), 3.86 (1 H, br s), 4.31 (1 H, dt, J = 6.3, 1.1 Hz), 5.18 (1H, ddd, J = 10.4, 1.9, 1.1 Hz), 5.28 (1H, ddd, J = 17.0, 1.9, 1.1 Hz), 5.84 (1H, ddd, J = 17.0, 10.4, 6.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.8 (q), −4.1 (q), 18.1 (s), 20.1 (q), 22.5 (q), 23.0 (t), 25.9 (q), 41.3 (s), 70.8 (s), 77.2 (d), 80.6 (d), 82.3 (d), 116.8 (t), 137.1 (d); IR (neat) 3464, 3314, 2957, 2932, 2885, 2858, 2120, 1639, 1471, 1425, 1390, 1361, 1255, 1082, 1005, 922, 837, 810, 777 cm^{-1}; MS *m/z* 282 $(M)^+$; HRMS calcd for $C_{16}H_{30}O_2Si$ 282.2015, found 282.2012.

11b: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.12 (3 H, s), 0.17 (3 H, s), 0.85 (3 H, s), 0.92 (9 H, s), 0.93 (3 H, s), 2.04 (1 H, t, J = 2.8 Hz), 2.30 (1H, ddd, J = 17.6, 4.4, 2.8 Hz), 2.34 (1 H, br d, J = 3.8 Hz), 2.63 (1 H, ddd, J = 17.6, 6.0, 2.8 Hz), 3.82 (1 H, dd, J = 6.0, 4.4 Hz), 4.14 (1 H, m), 5.19 (1H, ddd, J = 10.4, 1.7, 1.1 Hz), 5.27 (1H, dt, J = 17.0, 1.7 Hz), 5.94 (1H, ddd, J = 17.0, 10.4, 6.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.8 (q), −3.8 (q), 18.2 (s), 18.4 (q), 20.3 (q), 23.6 (t), 26.0 (q), 42.7 (s), 70.4 (s), 77.5 (d), 77.6 (d), 83.3 (d), 116.3 (t), 137.6 (d); IR (neat) 3454, 3312, 2957, 2932, 2885, 2858, 2120, 1732, 1641, 1471, 1425, 1388, 1363, 1255, 1087, 1005, 924, 837, 810, 775 cm^{-1}; MS *m/z* 282 $(M)^+$; HRMS calcd for $C_{16}H_{30}O_2Si$ 282.2015, found 282.1994.

(3*RS*,5*RS*)-Bis[(*tert*-butyldimethylsilyl)oxy]-4,4-dimethyloct-1-en-7-yne (12a). To a stirred mixture of **11a** (91 mg, 0.32 mmol) and 2,6-lutidine (90 μL, 0.96 mmol) in CH_2Cl_2 (1 mL) was added TBSOTf (140 μL, 0.48 mmol) at 0 °C under argon. The resulting mixture was stirred at 0 °C for 1 h, diluted with ethyl acetate and washed with water. The organic layer was dried over magnesium sulfate and filtered. Removal of the solvent afforded a residue, from which **12a** (126 mg) was separated by silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 12) as a colourless oil in quantitative yield.

12a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.00 (3 H, s), 0.04 (3 H, s), 0.08 (3 H, s), 0.15 (3 H, s), 0.82 (3 H, s), 0.86 (3 H, s), 0.89 (9 H, s), 0.91 (9 H, s), 1.97 (1 H, t, J = 3.1 Hz), 2.22 (1H, ddd, J = 17.3, 5.8, 3.1 Hz), 2.56 (1 H, ddd, J = 17.3, 4.1, 3.1 Hz), 3.75

(1 H, dd, J = 5.8, 4.1 Hz), 4.01 (1 H, d, J = 8.0 Hz), 5.12 (1H, d, J = 18.7 Hz), 5.13 (1H, d, J = 10.4 Hz), 5.83 (1H, ddd, J = 18.7, 10.4, 8.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.7 (q), −4.5 (q), −3.6 (q), −3.4 (q), 18.2 (s), 18.3 (s), 19.6 (q), 19.9 (q), 23.6 (t), 25.9 (q), 26.1 (q), 44.1 (s), 70.1 (s), 75.7 (d), 78.9 (d), 83.8 (d), 116.6 (t), 138.7 (d); IR (neat) 3314, 3078, 2957, 2932, 2887, 2858, 2121, 1471, 1388, 1361, 1253, 1074, 1005, 925 cm^{-1}; MS m/z 396 $(M)^+$, 381 $(M - Me)^+$, 339 $(M - {}^tBu)^+$; HRMS calcd for $C_{22}H_{44}O_2Si_2$ 396.2880, found 396.2910.

(3*RS*,5*SR*)-Bis[(*tert*-butyldimethylsilyl)oxy]-4,4-dimethyloct-1-en-7-yne (12b). This compound was obtained by the same procedure as described for **12a**, starting from **11b** instead of **11a**.

12b: ^{1}H NMR (600 MHz, $CDCl_3$) δ − 0.01 (3 H, s), 0.04 (3 H, s), 0.09 (3 H, s), 0.17 (3 H, s), 0.76 (3 H, s), 0.86 (3 H, s), 0.90 (9 H, s), 0.92 (9 H, s), 1.96 (1 H, t, J = 2.7 Hz), 2.20 (1H, ddd, J = 17.3, 6.3, 2.7 Hz), 2.60 (1 H, dt, J = 17.3, 2.7 Hz), 3.80 (1 H, dd, J = 6.3, 2.7 Hz), 4.03 (1 H, d, J = 7.1 Hz), 5.13 (1H, d, J = 10.4 Hz), 5.14 (1H, d, J = 17.3 Hz), 5.81 (1H, ddd, J = 17.3, 10.4, 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.9 (q), −4.6 (q), −3.7 (q), −3.3 (q), 18.2 (s), 18.4 (s), 18.5 (q), 20.0 (q), 23.3 (t), 25.9 (q), 26.2 (q), 43.8 (s), 70.0 (s), 75.7 (d), 78.2 (d), 84.1 (d), 116.3 (t), 138.4 (d); IR (neat) 3316, 3078, 2957, 2932, 2887, 2858, 2121, 1471, 1386, 1361, 1253, 1084, 1005, 924 cm^{-1}; MS m/z 396 $(M)^+$, 339 $(M - {}^tBu)^+$; HRMS calcd for $C_{22}H_{44}O_2Si_2$ 396.2880, found 396.2889.

(3*RS*,5*RS*)-4,4-Dimethyloct-1-en-7-yne-3,5-diol (13a). To a stirred solution of **11a** (50 mg, 0.18 mmol) in THF (1 mL) was added TBAF (1.0 M in THF, 0.27 mL, 0.27 mmol), and the resulting mixture was stirred at 0 °C for 30 min. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **13a** (29 mg) was separated by silica gel column chromatography (ethyl acetate–*n*-hexane = 2 : 3) as a colourless oil in 94% yield.

13a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.89 (3 H, s), 0.95 (3 H, s), 2.06 (1 H, t, J = 2.7 Hz), 2.37 (1H, ddd, J = 16.5, 9.3, 2.7 Hz), 2.42 (1 H, ddd, J = 16.5, 3.6, 2.7 Hz), 3.78 (1 H, dd, J = 9.3, 3.6 Hz), 3.99 (1 H, br d, J = 6.3 Hz), 5.23 (1H, dt, J = 10.4, 1.7 Hz), 5.30 (1H, dt, J = 17.3, 1.7 Hz), 5.96 (1H, ddd, J = 17.3, 10.4, 6.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 19.9 (q), 21.2 (q), 22.5 (t), 40.2 (s), 70.6 (s), 75.9 (d), 80.1 (d), 81.8 (d), 116.9 (t), 137.0 (d); IR (neat) 3354, 3308, 3080, 2966, 2928, 2880, 2120, 1734, 1641, 1469, 1425, 1392, 1369, 1280, 1203, 1115, 1043, 955, 927 cm^{-1}; MS m/z 150 $(M - H_2O)^+$; HRMS calcd for $C_{10}H_{14}O$ 150.1044, found 150.1048.

(3*RS*,5*SR*)-4,4-Dimethyloct-1-en-7-yne-3,5-diol (13b). This compound was obtained by the same procedure as described for **13a**, starting from **11b** instead of **11a**.

13b: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77 (3 H, s), 0.91 (3 H, s), 2.06 (1 H, t, J = 2.7 Hz), 2.34 (1H, ddd, J = 16.5, 9.6, 2.7 Hz), 2.47 (1 H, dt, J = 16.5, 2.7 Hz), 3.76 (1 H, dd, J = 9.6, 2.7 Hz), 4.04 (1 H, dt, J = 7.1, 1.1 Hz), 5.20 (1H, ddd, J = 10.4, 1.9, 1.1 Hz), 5.26 (1H, ddd, J = 17.0, 1.9, 1.1 Hz), 5.90 (1H, ddd, J = 17.0, 10.4, 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.5 (q), 21.4 (q), 22.7 (t), 40.6 (s), 70.6 (s), 77.5 (d), 80.9 (d), 81.9 (d), 117.4 (t), 137.1 (d); IR (neat) 3368, 3304, 3080, 2972, 2937, 2880, 2118, 1734, 1639, 1463, 1423, 1394, 1373, 1273, 1116, 1060, 995, 929 cm^{-1}; MS m/z 168 $(M)^+$; HRMS calcd for $C_{10}H_{16}O_2$ 168.1150, found 168.1144.

General procedure for synthesis of acetonides. To each of the above enyne compounds (25 mg, 0.15 mmol) dissolved in dimethoxypropane (0.5 mL) was added CSA (7 mg, 0.2 eq.) at room temperature under argon. The resulting mixture was stirred overnight at room temperature. Evaporation of the solvent, followed by separation using silica gel column chromatography (ethyl acetate–*n*-hexane = 1 : 9) gave the corresponding acetonide as a colourless oil in 52–54% yield.

Acetonide of 13a. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.82 (3 H, s), 0.84 (3 H, s), 1.38 (3 H, s), 1.41 (3 H, s), 1.98 (1 H, t, J = 2.8 Hz), 2.29 (1H, ddd, J = 17.1, 8.0, 2.8 Hz), 2.32 (1 H, ddd, J = 17.1, 5.8, 2.8 Hz), 3.63 (1 H, dd, J = 8.0, 5.8 Hz), 3.84 (1 H, d, J = 6.9 Hz), 5.19 (1H, d, J = 10.7 Hz), 5.24 (1H, d, J = 17.0 Hz), 5.78 (1H, ddd, J = 17.0, 10.7, 6.9 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 19.0 (q), 20.0 (t), 20.5 (q), 23.8 (q), 24.1 (q), 39.8 (s), 69.1 (s), 74.9 (d), 77.7 (d), 82.0 (d), 101.3 (s), 117.0 (t), 134.1 (d); IR (neat) 3314, 3080, 2988, 2961, 2928, 2856, 2365, 2123, 1736, 1649, 1541, 1466, 1379, 1228, 1176, 1128, 1082, 1059, 1014, 924 cm^{-1}; MS m/z 208 $(M)^+$, 193 $(M - Me)^+$; HRMS calcd for $C_{13}H_{20}O_2$ 208.1464, found 208.1492.

Acetonide of 13b. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.81 (3 H, s), 0.84 (3 H, s), 1.45 (3 H, s), 1.49 (3 H, s), 1.96 (1 H, t, J = 2.8 Hz), 2.23 (1H, ddd, J = 17.0, 8.8, 2.8 Hz), 2.40 (1 H, dt, J = 17.0, 2.8 Hz), 3.77 (1 H, dd, J = 8.8, 2.8 Hz), 3.99 (1 H, d, J = 6.6 Hz), 5.23 (1H, d, J = 9.3 Hz), 5.26 (1H, d, J = 16.0 Hz), 5.77 (1H, ddd, J = 16.0, 9.3, 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.8 (q), 19.3 (q), 20.2 (t), 20.9 (q), 30.0 (q), 35.9 (s), 68.9 (s), 77.2 (d), 79.5 (d), 82.4 (d), 99.0 (s), 118.3 (t), 134.0 (d); IR (neat) 3310, 3080, 2991, 2968, 2939, 2874, 2123, 1861, 1740, 1645, 1468, 1425, 1381, 1263, 1201, 1174, 1126, 1089, 1060, 1037, 993 cm^{-1}; MS m/z 208 $(M)^+$, 193 $(M - Me)^+$; HRMS calcd for $C_{13}H_{20}O_2$ 208.1464, found 208.1484.

(5*Z*,7*E*)-(1*R*,3*R*)-2,2-Dimethyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (4a) and (5*Z*,7*E*)-(1*S*,3*S*)-2,2-dimethyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (4b). A mixture of the CD-ring portion **14** (57 mg, 0.16 mmol), tetrakis-(triphenylphosphine)palladium (55 mg, 48 μmol) and triethylamine (2.5 mL) in toluene (1 mL) was stirred for 20 min at room temperature, then a solution of the A-ring enyne precursor **12a** (63 mg, 0.16 mmol) in toluene (2 mL) was added. After having been heated at reflux for 1 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 : 3) to give a mixture of the protected vitamins (71 mg, 66%). The crude vitamins dissolved in THF (3 mL) were treated with tetrabutylammonium fluoride (TBAF) (1.0 M in THF, 0.5 mmol) at room temperature for 15 h. Brine was added to the 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 = 1 : 2) to give **4a**,**b** (14 mg, 29%) with recovery of mono-silylated alcohols (21 mg, 34%). Separation and further purification of **4a**,**b** were conducted by using a reversed-phase recycling HPLC (YMC-Pack ODS column, 20 × 150 mm, 9.0 mL min^{-1}, acetonitrile–water = 9 : 1).

4a: UV (EtOH) λ_{max} 268 nm, λ_{min} 229 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.54 (3 H, s), 0.93 (3 H, d, J = 6.6 Hz), 0.98 (3 H, s), 1.04 (3 H, s), 1.21 (6 H, s), 1.48 (1 H, d, J = 6.0 Hz), 1.49 (1 H, d, J = 5.8 Hz), 2.28 (1 H, dd, J = 14.0, 6.6 Hz), 2.64 (1 H, dd, J = 14.0, 3.6 Hz), 2.81 (1 H, dd, J = 12.4, 4.4 Hz), 3.76 (1 H, dt, J = 3.8, 6.3 Hz), 3.99 (1 H, d, J = 5.5 Hz), 5.05 (1 H, t, J = 1.7 Hz), 5.31 (1 H, t, J = 1.7 Hz), 6.03 (1 H, d, J = 11.3 Hz), 6.36 (1 H, d, J = 11.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 18.8, 20.5, 20.8, 21.0, 22.3, 23.6, 27.6, 29.1, 29.2, 29.4, 36.1, 36.4, 40.5, 40.8, 41.4, 44.4, 45.9, 56.3, 56.5, 71.1, 74.8, 78.8, 113.1, 116.9, 124.8, 133.1, 143.1, 145.0; MS m/z 444 $(M)^+$, 426 $(M - H_2O)^+$, 408 $(M - 2H_2O)^+$, 393 $(M - 2H_2O - Me)^+$, 390 $(M - 3H_2O)^+$, 375 $(M - 3H_2O - Me)^+$; HRMS calcd for $C_{29}H_{48}O_3$ 444.3604, found 444.3610.

4b: UV (EtOH) λ_{max} 268 nm, λ_{min} 229 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.54 (3 H, s), 0.93 (3 H, d, J = 6.6 Hz), 1.01 (3 H, s), 1.02 (3 H, s), 1.21 (6 H, s), 1.45 (1 H, d, J = 4.9 Hz),

1.49 (1 H, d, J = 6.0 Hz), 2.30 (1 H, dd, J = 14.0, 7.4 Hz), 2.60 (1 H, dd, J = 14.0, 3.8 Hz), 2.82 (1 H, dd, J = 12.4, 4.4 Hz), 3.78 (1 H, ddd, J = 7.7, 6.0, 4.4 Hz), 3.96 (1 H, d, J = 5.2 Hz), 5.05 (1 H, dd, J = 1.9, 1.1 Hz), 5.29 (1 H, dd, J = 1.9, 1.1 Hz), 6.02 (1 H, d, J = 11.3 Hz), 6.37 (1 H, d, J = 11.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 18.8, 20.3, 20.8, 21.0, 22.3, 23.6, 27.6, 29.0, 29.2, 29.4, 36.1, 36.4, 40.5, 40.9, 41.3, 44.4, 45.9, 56.3, 56.5, 71.1, 74.3, 79.6, 113.8, 116.9, 124.9, 133.0, 143.1, 145.7; MS m/z 426 $(M - H_2O)^+$, 408 $(M - 2H_2O)^+$, 393 $(M - 2H_2O - Me)^+$, 390 $(M - 3H_2O)^+$, 375 $(M - 3H_2O - Me)^+$; HRMS calcd for $C_{29}H_{46}O_2$ 426.3498, found 426.3498.

(5*Z*,7*E*)-(1*S*,3*R*)-2,2-Dimethyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (4c) and (5*Z*,7*E*)-(1*R*,3*S*)-2,2-dimethyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (4d). A mixture of the CD-ring portion **14** (57 mg, 0.16 mmol), tetrakis(triphenylphosphine)palladium (55 mg, 48 μmol) and triethylamine (2.5 mL) in toluene (1 mL) was stirred for 20 min at room temperature, then a solution of the A-ring enyne precursor **12b** (63 mg, 0.16 mmol) in toluene (2 mL) was added. After having been heated at reflux for 1 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 : 3) to give a mixture of the protected vitamins (68 mg, 63%). The crude vitamins dissolved in THF (3 mL) were treated with tetrabutylammonium fluoride (TBAF) (1.0 M in THF, 0.5 mmol) at room temperature for 19 h. Brine was added to the 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 = 3 : 2) to give **4c**,**d** (28 mg, 63%). Separation and further purification of **4c**,**d** were conducted by using a reversed-phase recycling HPLC (YMC-Pack ODS column, 20 × 150 mm, 9.0 mL min^{-1}, acetonitrile–water = 9 : 1).

4c: UV (EtOH) λ_{max} 266 nm, λ_{min} 228 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.55 (3 H, s), 0.94 (3 H, d, J = 6.6 Hz), 0.96 (3 H, s), 1.17 (3 H, s), 1.21 (6 H, s), 2.24 (1 H, d, J = 5.0 Hz), 2.39 (1 H, dd, J = 14.6, 4.4 Hz), 2.71 (1 H, br d, J = 14.0 Hz), 2.81 (1 H, d, J = 8.8 Hz), 2.84 (1 H, dd, J = 11.5, 3.3 Hz), 3.57 (1 H, dd, J = 8.8, 4.4 Hz), 3.82 (1 H, d, J = 4.1 Hz), 5.07 (1 H, d, J = 2.2 Hz), 5.26 (1 H, d, J = 1.9 Hz), 6.07 (1 H, d, J = 11.3 Hz), 6.46 (1 H, d, J = 11.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 18.8, 20.7, 20.8, 22.4, 23.7, 24.5, 27.6, 29.1, 29.2, 29.3, 36.1, 36.4, 39.9, 40.5, 41.2, 44.4, 45.9, 56.4, 56.5, 71.1, 76.5, 82.4, 115.0, 116.9, 125.8, 131.1, 143.0, 145.6; MS m/z 444 $(M)^+$, 426 $(M - H_2O)^+$, 408 $(M - 2H_2O)^+$, 393 $(M - 2H_2O - Me)^+$, 390 $(M - 3H_2O)^+$, 375 $(M - 3H_2O - Me)^+$; HRMS calcd for $C_{29}H_{48}O_3$ 444.3604, found 444.3610.

4d: UV (EtOH) λ_{max} 267 nm, λ_{min} 229 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.53 (3 H, s), 0.93 (3 H, d, J = 6.6 Hz), 0.98 (3 H, s), 1.13 (3 H, s), 1.21 (6 H, s), 2.12 (1 H, d, J = 5.2 Hz), 2.40 (1 H, dd, J = 14.3, 5.2 Hz), 2.66 (1 H, dd, J = 14.3, 2.2 Hz), 2.71 (1 H, d, J = 7.1 Hz), 2.84 (1 H, dd, J = 11.3, 2.8 Hz), 3.56 (1 H, ddd, J = 7.1, 5.2, 2.2 Hz), 3.80 (1 H, d, J = 4.9 Hz), 5.04 (1 H, d, J = 2.2 Hz), 5.26 (1 H, d, J = 2.2 Hz), 6.03 (1 H, d, J = 11.3 Hz), 6.43 (1 H, d, J = 11.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 18.8, 19.6, 20.8, 22.2, 23.5, 24.5, 27.7, 29.1, 29.2, 29.4, 36.1, 36.4, 40.2, 40.5, 41.3, 44.4, 45.9, 56.3, 56.5, 71.1, 76.4, 81.8, 114.5, 117.0, 125.6, 131.1, 143.0, 145.6; MS m/z 444 $(M)^+$, 426 $(M - H_2O)^+$, 408 $(M - 2H_2O)^+$, 393 $(M - 2H_2O - Me)^+$, 390 $(M - 3H_2O)^+$, 375 $(M - 3H_2O - Me)^+$; HRMS calcd for $C_{29}H_{48}O_3$ 444.3604, found 444.3611.

General procedure for synthesis of MTPA esters[14]. A solution of each of the above vitamins dissolved in dry CH_2Cl_2 was treated with DMAP (4 eq.) and (*R*)- or (*S*)-MTPACl (2.5 eq.) at room temperature under an argon atmosphere. The reaction mixture was purified by preparative TLC (ethyl acetate–*n*-hexane = 1 : 2) without pretreatment to afford the corresponding MTPA ester.

(*S*)-MTPA ester of 4a. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.439 (3 H, s), 0.609 (3 H, s), 0.908 (3 H, s), 0.929 (3 H, d, J = 6.6 Hz), 1.219 (6 H, s), 2.507 (1 H, t, J = 11.8 Hz), 2.729 (1 H, dd, J = 13.7, 4.9 Hz), 2.806 (1 H, dd, J = 12.9, 4.1 Hz), 3.485 (3 H, s), 3.516 (3 H, s), 5.047 (1 H, dd, J = 10.4, 4.9 Hz), 5.178 (1 H, s), 5.264 (1 H, s), 5.498 (1 H, s), 5.918 (1 H, d, J = 11.5 Hz), 6.449 (1 H, d, J = 11.5 Hz), 7.35–7.53 (10 H, m); FABMS m/z 900 $(M + Na)^+$, 877 $(M + H)^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4299.

(*R*)-MTPA ester of 4a. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.270 (3 H, s), 0.911 (3 H, d, J = 6.3 Hz), 0.953 (6 H, s), 1.231 (6 H, s), 2.388 (1 H, t, J = 11.8 Hz), 2.679 (1 H, dd, J = 13.7, 4.9 Hz), 2.759 (1 H, dd, J = 13.5, 4.4 Hz), 3.394 (3 H, s), 3.513 (3 H, s), 5.076 (1 H, dd, J = 10.2, 4.9 Hz), 5.187 (1 H, s), 5.257 (1 H, s), 5.428 (1 H, s), 5.804 (1 H, d, J = 11.3 Hz), 6.362 (1 H, d, J = 11.5 Hz), 7.39–7.56 (10 H, m); FABMS m/z 900 $(M + Na)^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4298.

(*S*)-MTPA ester of 4b. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.513 (3 H, s), 0.934 (3 H, d, J = 6.3 Hz), 0.939 (3 H, s), 0.978 (3 H, s), 1.220 (6 H, s), 2.380 (1 H, dd, J = 12.4, 11.3 Hz), 2.662 (1 H, dd, J = 13.7, 4.7 Hz), 2.742 (1 H, dd, J = 13.5, 4.9 Hz), 3.428 (3 H, s), 3.503 (3 H, s), 5.068 (1 H, dd, J = 9.9, 4.7 Hz), 5.204 (1 H, s), 5.220 (1 H, s), 5.425 (1 H, s), 5.890 (1 H, d, J = 11.3 Hz), 6.320 (1 H, d, J = 11.3 Hz), 7.35–7.43 (6 H, m), 7.48–7.50 (4 H, m); FABMS m/z 900 $(M + Na)^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4303.

(*R*)-MTPA ester of 4b. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.539 (3 H, s), 0.718 (3 H, s), 0.926 (3 H, s), 0.939 (3 H, d, J = 6.3 Hz), 1.217 (6 H, s), 2.518 (1 H, t, J = 11.8 Hz), 2.732 (1 H, dd, J = 13.7, 4.9 Hz), 2.807 (1 H, dd, J = 12.4, 4.1 Hz), 3.472 (3 H, s), 3.528 (3 H, s), 5.107 (1 H, dd, J = 10.7, 4.9 Hz), 5.194 (1 H, s), 5.306 (1 H, s), 5.530 (1 H, s), 5.966 (1 H, d, J = 11.3 Hz), 6.446 (1 H, d, J = 11.3 Hz), 7.29–7.50 (10 H, m); FABMS m/z 900 $(M + Na)^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4308.

(*S*)-MTPA ester of 4c. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.553 (3 H, s), 0.850 (3 H, s), 0.857 (3 H, s), 0.940 (3 H, d, J = 6.3 Hz), 1.220 (6 H, s), 2.484 (1 H, t, J = 11.8 Hz), 2.655 (1 H, dd, J = 13.2, 4.9 Hz), 2.790 (1 H, dd, J = 12.6, 3.9 Hz), 3.520 (3 H, s), 3.535 (3 H, s), 4.896 (1 H, s), 4.912 (1 H, s), 4.943 (1 H, dd, J = 11.3, 5.0 Hz), 5.235 (1 H, s), 5.888 (1 H, d, J = 11.3 Hz), 6.402 (1 H, d, J = 11.3 Hz), 7.38–7.43 (6 H, m), 7.51–7.55 (4 H, m); FABMS m/z 900 $(M + Na)^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4304.

(*R*)-MTPA ester of 4c. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.543 (3 H, s), 0.806 (3 H, s), 0.940 (3 H, d, J = 6.3 Hz), 0.968 (3 H, s), 1.220 (6 H, s), 2.356 (1 H, dd, J = 12.4, 10.7 Hz), 2.619 (1 H, dd, J = 12.7, 4.7 Hz), 2.758 (1 H, dd, J = 13.7, 4.9 Hz), 3.425 (6 H, s), 4.941 (1 H, dd, J = 9.6, 4.7 Hz), 4.992 (1 H, s), 5.076 (1 H, s), 5.281 (1 H, s), 5.857 (1 H, d, J = 11.3 Hz), 6.280 (1 H, d, J = 11.3 Hz), 7.39–7.43 (6 H, m), 7.49–7.55 (4 H, m); FABMS m/z 900 $(M + Na)^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4312.

(*S*)-MTPA ester of 4d. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.513 (3 H, s), 0.934 (3 H, d, J = 6.3 Hz), 0.939 (3 H, s), 0.978 (3 H, s), 1.220 (6 H, s), 2.380 (1 H, dd, J = 12.4, 11.3 Hz), 2.662 (1 H, dd, J = 13.7, 4.7 Hz), 2.742 (1 H, dd, J = 13.5, 4.9 Hz), 3.428 (3 H, s), 3.503 (3 H, s), 5.068 (1 H, dd, J = 9.9, 4.7 Hz), 5.204 (1 H, s), 5.220 (1 H, s), 5.425 (1 H, s), 5.890 (1 H, d, J = 11.3 Hz), 6.320 (1 H, d, J = 11.3 Hz), 7.35–7.43 (6 H, m), 7.48–7.50 (4 H, m);

FABMS *m*/*z* 900 (M + Na)$^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4303.

(*R*)-MTPA ester of 4d. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.539 (3 H, s), 0.718 (3 H, s), 0.926 (3 H, s), 0.939 (3 H, d, J = 6.3 Hz), 1.217 (6 H, s), 2.518 (1 H, t, J = 11.8 Hz), 2.732 (1 H, dd, J = 13.7, 4.9 Hz), 2.807 (1 H, dd, J = 12.4, 4.1 Hz), 3.472 (3 H, s), 3.528 (3 H, s), 5.107 (1 H, dd, J = 10.7, 4.9 Hz), 5.194 (1 H, s), 5.306 (1 H, s), 5.530 (1 H, s), 5.966 (1 H, d, J = 11.3 Hz), 6.446 (1 H, d, J = 11.3 Hz), 7.29–7.50 (10 H, m); FABMS *m*/*z* 900 (M + Na)$^+$; HRFABMS calcd for $C_{49}H_{62}O_7F_6Na$ 899.4297, found 899.4308.

Vitamin D receptor (VDR) binding assay. 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 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).

Transactivation assay.[15] COS-1 cells were maintained in Dulbecco's modified Eagle's medium without phenol red, supplemented with 5% fetal calf stripped with dextran-coated charcoal. Cells were transfected by means of lipofection. The following plasmids were used for transfection: reporter plasmid pGL-GAL4-UAS (0.5 μg) containing the *GAL4* upstream activation sequence (UAS) (heptadecamer ×2, β-globin promoter, and luciferase) cotransfected with 0.1 μg of pM(GAL4-DBD)-VDR(DEF) with or without TIF2 or SRC-1 expression vector (0.1 μg). As a reference plasmid for normalization, 0.1 ng of plasmid pRL-CMV plasmid (Promega) was used. Either **1** or an analogue was added to the medium at 6 h after transfection. After 24 h, firefly luciferase activity (from GAL4-UAS) was used to measure transfection efficiency by *Renilla* luciferase activity (from pRL-CMV).

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