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24,24-Dimethylvitamin D_3-26,23-lactones and their 2α-functionalized analogues as highly potent VDR antagonists☆

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Abstract—Novel vitamin D receptor (VDR) antagonists, 24,24-dimethyl-1α-hydroxyvitamin D_3-26,23-lactones (**8** and **9**) and their C2α functionalized analogues (**8a**–**c** and **9a**–**c**) were efficiently synthesized and their biological activities were evaluated. The construction of vitamin D_3 triene skeleton was achieved by palladium-catalyzed alkenylative cyclization of A-ring precursor enyne (**22** and **22a**–**c**) with CD-ring bromoolefin having a 24,24-dimethyl-α-methylene-γ-lactone unit on the side chain (**13** and **14**). The CD-ring precursors **13** and **14** were prepared by using chromium-mediated allylation of the aldehyde **10** derived from vitamin D_2. On the other hand, the A-ring enyne having 2α-(3-hydroxypropyl) group (**22b**) was newly synthesized from epoxide **15** using regio- and stereoselective alkylation methodology. The potency of the antagonistic activity of the newly designed analogues (**8** and **9**) increased up to 12 times that of TEI-9647 (**2**). Furthermore, introduction of the three motifs, that is, a methyl (**8a** and **9a**), an ω-hydroxypropyl (**8b** and **9b**) or an ω-hydroxypropoxyl group (**8c** and **9c**) into the C2α position of **8** and **9**, respectively, resulted in remarkable enhancement, up to 89 times, of the antagonistic activity on VDR.

1. Introduction

1α,25-Dihydroxyvitamin D_3 (**1**), which is a hormonally active form of vitamin D, exerts various biological profiles including calcium and phosphorous homeostasis, cell proliferation and differentiation of various types of tumor cells, and immune reaction.[1,2] Most of the biological responses of **1** are mediated by its specific receptor, vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily and acts as a ligand-dependent gene transcription factor with coactivators.[3,4] Recently, we have synthesized several 1α,25-dihydroxyvitamin D_3 analogues, which systematically introduced an alkyl, ω-hydroxyalkyl, and ω-hydroxyalkoxyl group into the C2α position of **1**.[5] Some of these 2α-modified vitamin D_3 analogues exhibited unique biological activities with potent agonistic activity.[6,7] In particular, introduction of the 2α-methyl (**1a**),[5a,b] 2α-(3-hydroxypropyl) (**1b**),[5c,d] and 2α-(3-hydroxypropoxy) (**1c**)[5e] groups showed 2- to 4-fold higher binding affinity to the bovine thymus VDR relative to **1** (Fig. 1).

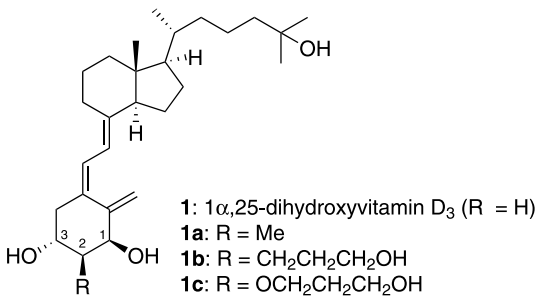

Figure 1. Structures of 1α,25-dihydroxyvitamin D_3 (**1**) and its C2α-modified analogues **1a**–**c**.

In 1999, the first VDR antagonists, 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones, TEI-9647 (**2**) and TEI-9648 (**3**) were discovered during the course of studies on the side-chain modification of the 1α,25-dihydroxyvitamin D_3-26,23-lactone metabolite[8] derived from **1** (Fig. 2).[9,10] Both vitamin D_3 analogues **2** and **3** specifically antagonize the VDR-mediated genomic action of **1**.[11] That is, **2** and **3** inhibit differentiation of human leukemia cells (HL-60 cells)[9a] as well as 25-hydroxyvitamin D_3-24-hydroxylase

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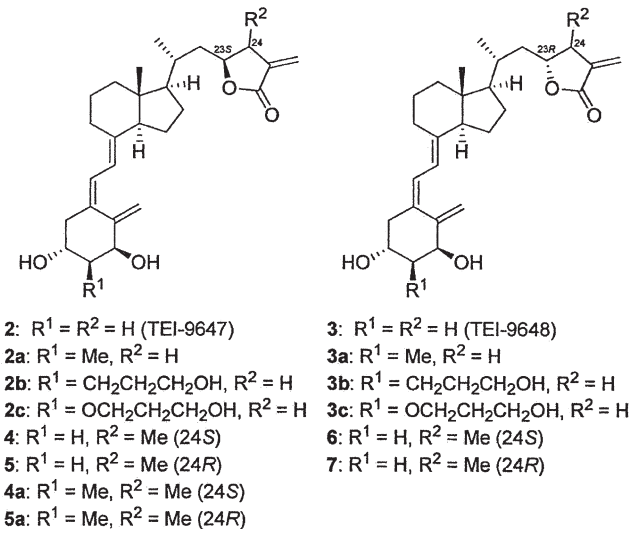


Figure 2. Structures of 1α-hydroxyvitamin D_3-26,23-lactones (TEI-9647: **2** and TEI-9648: **3**), their 2α-modified (**2a**–**c** and **3a**–**c**), 24-modified (**4**–**7**), and 2,24-double modified analogues (**4a** and **5a**).

gene expression in human osteosarcoma cells[9b] and in HL-60 cells[9d] induced by **1**. Furthermore, TEI-9647 (**2**) antagonizes the genomic-mediated calcium metabolism regulated by **1** in vivo in rat.[9e] The interesting biological profiles of the vitamin D_3 analogues **2** and **3** prompted us to investigate structure–activity relationship of the vitamin D_3 lactones from the standpoint of developing more potent anti-D molecules.

Quite recently, we found that some pertinent modifications of **2** and **3** enhanced their biological activities.[12] Namely, introduction of the above three motifs, that is, the methyl, the 3-hydroxypropyl or the 3-hydroxypropoxy group, into the C2α position of **2** and **3** (**2a**–**c** and **3a**–**c**) raised the potential of the antagonistic activity up to 30-fold.[12a] On the other hand, it was also found that the VDR binding affinity and antagonistic activity of **2** and **3** were affected by the structure including stereochemistry of the lactone part (**4**–**7**).[12b] Especially, introducing the methyl group into the C24 position on the lactone ring improved the antagonistic activity to be up to 2.5-fold more potent than that of TEI-9647 (**2**). Furthermore, we disclosed that simultaneous double functionalization of the C2α and C24 positions of **2** (**4a** and **5a**) remarkably increased the antagonistic activity of **2** to 62-fold stronger than that of the original **2**.

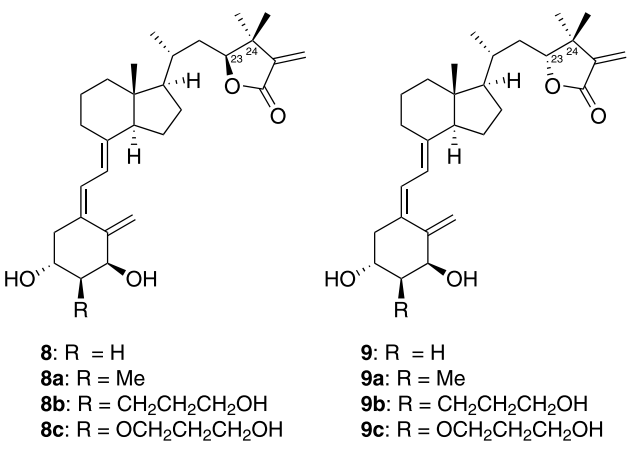


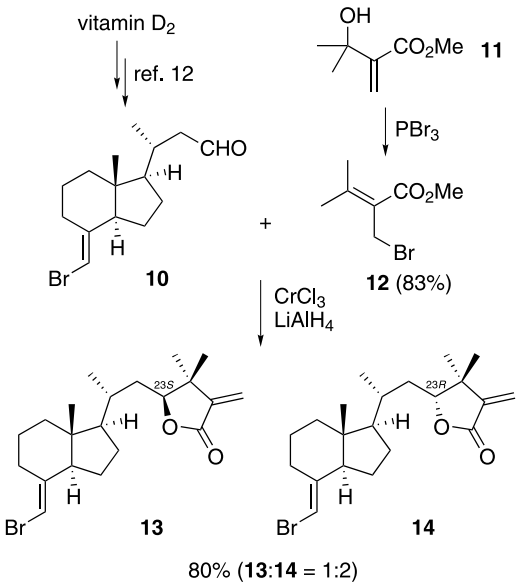
Figure 3. 24,24-Dimethylvitamin D_3 lactones (**8** and **9**) and their 2α-modified analogues (**8a**–**c** and **9a**–**c**).

On the basis of our previous results, we newly designed novel vitamin D_3 lactone analogues **8** and **9**, which have the dimethyl groups at the C24 position, to investigate further structure–activity relationships on the lactone core structure (Fig. 3). From the point of manufacturing new drug candidates, reduction of the number of chiral centers is favorable. Moreover, we expected that biological activity of the vitamin D_3 lactone analogues would be enhanced by introduction of the above three motifs, that is, the methyl (**8a** and **9a**), the 3-hydroxypropyl (**8b** and **9b**) and the 3-hydroxypropoxyl group (**8c** and **9c**) as in our previous studies.[6,7,12] Here, we report the synthesis and biological evaluation of the novel 24,24-dimethylvitamin D_3-26,23-lactones and their 2α-modified analogues.

2. Results

2.1. Synthesis and biological evaluation of 24,24-dimethylvitamin D_3-26,23-lactones

For the synthesis of the 24,24-dimethylvitamin D_3 lactone analogues, we utilized the Pd-catalyzed A-ring/CD-ring connective strategy.[13] First of all, we prepared the CD-ring precursors having the 24,24-dimethyl-α-methylene-γ-lactone side chain via the low-valent Cr-mediated allylation–lactonization process[14] (Scheme 1). The aldehyde **10** was synthesized from vitamin D_2,[12] and the alcohol **11**[15] was treated with PBr_3 to give allylic bromide **12** in 83% yield. When aldehyde **10** reacted with **12** in the presence of the Cr(II) complex generated from $CrCl_3$ and $LiAlH_4$, two hydrindan derivatives **13** and **14**, which were diastereomers with respect to the C23 position on the lactone ring (based on steroidal numbering), were obtained in 80% yield in the ratio of 1 to 2. The absolute stereochemistries at C23 position



Scheme 1. Preparation of CD-ring precursors.

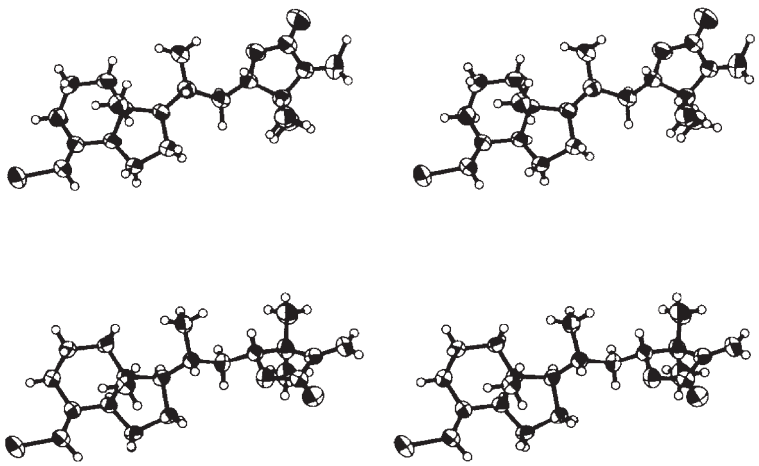
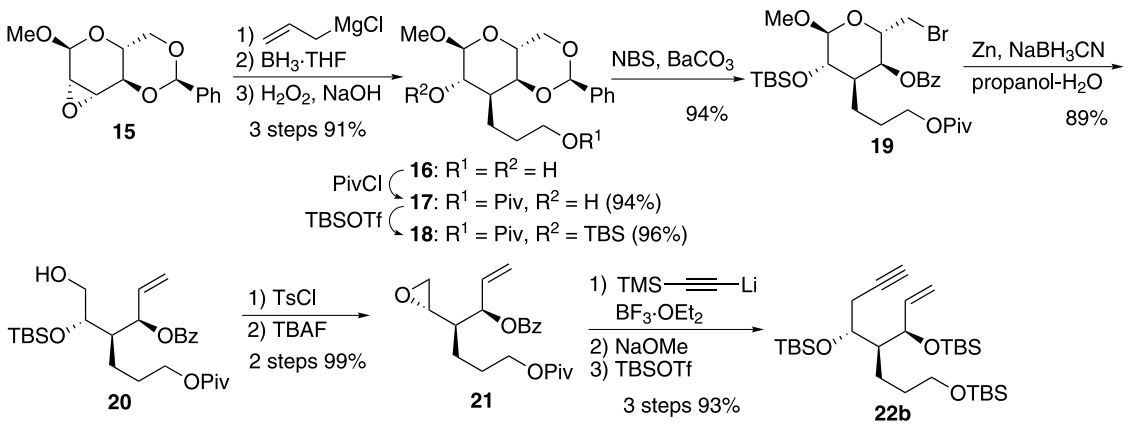


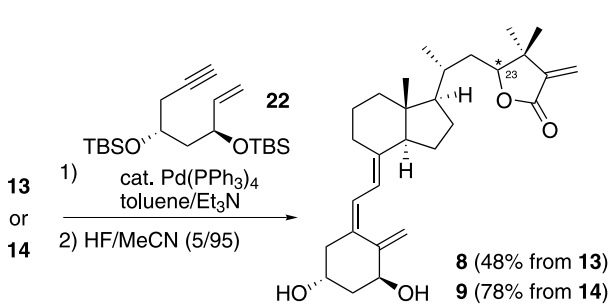
Figure 4. Stereoscopic views of the crystal structure of compounds **13** (upper) and **14** (lower). The thermal displacement parameters are drawn at both 50% probability (**13** and **14**).



Scheme 2. Improved synthesis of A-ring precursor having 3-hydroxypropyl side chain **22b**.

of **13** and **14** were confirmed by X-ray structual determination, respectively (Fig. 4).[16]

Next, the A-ring precursor having the ω-siloxypropyl side-chain **22b** was synthesized in an improved manner from known epoxide **15**[17] by using our regio- and stereoselective alkylation methodology reported previously[5e,18] (Scheme 2). The epoxide **15** was treated with allyl magnesium chloride[19] followed by hydroboration–oxidation to give diol **16** in 3 steps in 91% yield. Protection of two hydroxyl groups with Piv and TBS provided **18**, which was then converted into bromide **19** via radical benzylidene acetal cleavage by NBS. Pyranose ring-opening reaction of **19** with activated-zinc in the presence of $NaBH_3CN$ gave **20** in 89% yield. The alcohol **20** reacted with TsCl, then the resulting sulfonate derivative was treated with TBAF to afford epoxide **21**. Introduction of the TMS-ethynyl group into **21** followed by deprotection under basic conditions gave a triol. The resulting triol was protected by TBS groups to provide the desired A-ring precursor **22b** in totally 12 steps from epoxide **15**.[20]



Scheme 3. Synthesis of 24,24-dimethylvitamin D_3-26,23-lactones **8** and **9**.

Construction of the vitamin D_3 triene unit was achieved by Pd-catalyzed alkenylative cyclization of **22**[5a] with **13** or **14**;

Table 1. Biological activities of 24,24-dimethylvitamin D_3 lactones **8** and **9**

Compounds	VDR binding affinity[a]	Antagonistic activity[b] (IC_{50}, nM)
1	100	—
TEI-9647 (**2**)	12	8.3
4[c]	29	3.7
5[c]	22	3.2
8	37	0.71
TEI-9648 (**3**)	7	111.6
6[c]	12	160.0
7[c]	5	51.0
9	18	51.5

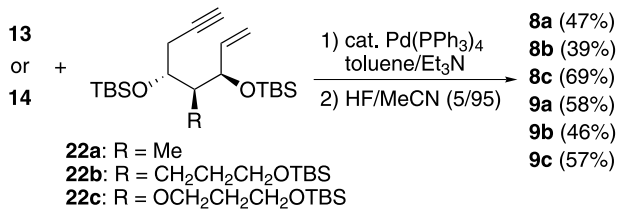
[a] The potency of **1** is normalized to 100.
[b] Antagonistic activity was assessed in terms of IC_{50} for differentiation of HL-60 cells induced by 10 nM of **1**.
[c] See: Ref. 12b.

and then deprotection of the silyl groups by HF gave the desired 24,24-dimethylvitamin D_3 lactones **8** and **9** (Scheme 3).

First, the receptor binding affinity and antagonistic activity of 24,24-dimethylvitamin D_3 lactones **8** and **9** were evaluated to see the biological effects of the 24,24-dimethyl groups (Table 1). We also show the data of 24-methylvitamin D_3 lactones (**4**–**7**) for comparison. Binding affinities of **8** and **9** to the chick intestinal VDR were examined as described previously.[21] The affinity of (23*S*)-24,24-dimethylactone derivative **8** increased to be 3.1-fold more potent than that of **2**. In the case of TEI-9648 type analogue **9**, introducing the dimethyl unit into the C24 position raised the binding affinity to 2.6 times higher compared with that of **3**. The antagonistic activities of **8** and **9** were assessed by the NBT-reduction method[22] in terms of inhibition of HL-60 cell differentiation induced by **1** (10 nM). Surprisingly, introduction of the dimethyl groups into the original antagonist **2** resulted in marked enhancement of the antagonistic activity to be ca. 12 times stronger than that of **2**. Although TEI-9648 (**3**) type analogue **9** showed weaker antagonistic activity than that of **2**, the dimethyl analogue **9** exhibited 2.2-fold higher activity compared to the original compound **3**. These results indicated that the vitamin D_3 lactone derivatives having the two-methyl groups on the C24 position (**8** and **9**) altered binding affinity for the VDR and worked on antagonism on the VDR more effectively than the mono methyl analogues (**4**–**7**).

2.2. Effect of C2α modifications of 24,24-dimethylvitamin D_3 lactones

Next, we turned our attention to C2α-functionalization of 24,24-dimethylvitamin D_3-lactone analogues **8** and **9**. According to our previous results, C2α-functionalization of 24-methylvitamin D_3 lactones effectively enhanced both binding affinity to the VDR and antagonistic activity.[12b] Therefore, we expected a high increase in VDR binding affinity and marked improvement of the VDR antagonistic activity through such functionalizations of the new 24,24-dimethylvitamin D_3 lactones. The 2α-modified vitamin D_3 analogues (**8a**–**c** and **9a**–**c**) were similarly synthesized from the corresponding CD-ring unit **13** and **14** with the A-ring precursor **22a**,[17a] **22b** and **22c**,[5e] respectively (Scheme 4).



Scheme 4. Synthesis of 2α-modified 24,24-dimethylvitamin D_3 lactones **8a**–**c** and **9a**–**c**.

The evaluation of biological activities of **8a**–**c** and **9a**–**c** disclosed that C2α modifications were also effective to enhance the biological potency of 24,24-dimethylvitamin D_3 lactones (Table 2). That is, VDR binding affinity of TEI-9647 (**2**) type analogues increased to 3.0–5.9 times stronger than that of **2** by introduction of the three motifs into the C2α position of **8** (**8a**–**c**). Such C2α modification exhibited remarkable effect on antagonistic activity. Especially, VDR antagonistic activity of 2α-methyl analogue **8a** increased to be ca. 89-fold more potent than that of the original **2** (7.6 times stronger than **8**). The other lactone derivatives **8b** and **8c** also showed 12- and 28-fold stronger antagonistic activity than that of **2**, respectively. In the case of TEI-9648 type derivatives (**9a**–**c**), receptor binding affinity was raised to be 1.7–7.6 fold more potent than TEI-9648 (**3**). Although **9a**–**c** generally showed weaker antagonistic activities than **8a**–**c**, C2α functionalization of **9** significantly increased the activities in comparison with the original compound **3** (4–19 more potent than **3**) and 24,24-dimtehylvitamin D_3 lactone derivative **9** (1.8–8.9 times stronger than **9**).

Table 2. Biological activities of 2α-modified 24,24-dimethylvitamin D_3 lactones **8a**–**c** and **9a**–**c**

Compounds	VDR binding affinity[a]	Antagonistic activity[b] (IC_{50}, nM)
TEI-9647 (**2**)	12	8.3
8a	67	0.093
8b	71	0.7
8c	36	0.3
TEI-9648 (**3**)	7	111.6
9a	48	5.8
9b	53	7.7
9c	12	28.0

[a] The potency of **1** is normalized to 100.
[b] Antagonistic activity was assessed in terms of IC_{50} for differentiation of HL-60 cells induced by 10 nM of **1**.

3. Discussion

In the generally accepted mechanism of transactivation mediated by VDR, a ligand first binds to the ligand binding-domain (LBD) of an apo form of VDR. Next, the VDR–ligand complex changes the conformation into a transcriptionally active holo form, which binds to the coactivators to activate transcription of the target gene.[23] In this conformational change process, the appropriate positioning of helix 12 of VDR, which is the most C-terminal α-helix and presents an interaction site with the other proteins such as coactivators in the active holo form, is essential, and regulates whether the function of the ligand on the VDR exhibits agonism or antagonism.[24]

The antagonist **2** binds to the LBD of a VDR, and the binding of **2** changes the conformation of the VDR into an unusual transcriptionally inactive form.[25] We at present speculate that some amino acid residues in the LBD participate in the unusual conformational change of the VDR through the interaction with the *exo*-methylene lactone moiety of **2**. Namely, there are two cysteines, that is, Cys403 on helix 11 and Cys410 on the hinge region between helix 11 and helix 12, in the LBD of the hVDR.[26] The nucleophilic thiol groups of the cysteines could attack on the α-methylene-γ-lactone of **2** via 1,4-addition to give the corresponding cysteine adduct.[27] Such interaction between the LBD and the ligand might prevent the usual positioning of helix 12. As the result, the VDR-**2** complex

could not form transcriptionally active conformation. Therefore, it is thought that the antagonist, whose *exo*-methylene unit is located on more favorable position to interact with Cys403 and/or Cys410, would show more potent antagonistic activity.

Although it is yet unclear why the newly synthesized 24,24-dimethylvitamin D_3 lactones (**8** and **9**) and their C2α modified analogues (**8a**–**c** and **9a**–**c**) exhibited more potent antagonistic activity than the original **2** and **3**, they might be situated in the above mentioned preferable position to interact with the cysteine residues after the binding to the LBD of the VDR.

4. Conclusion

We have succeeded in developing highly potent vitamin D receptor antagonists (**8**, **8a**–**c**, **9** and **9a**–**c**), 24,24-dimethyl-1α-hydroxyvitamin D_3-26,23-lactones and their C2α-functionalized analogues. Recently, the VDR antagonists are expected to be potent therapeutic agents for some diseases caused by hypersensitivity to 1α,25-dihydroxyvitamin D_3, such as Paget's bone disease.[28] We expect that these analogues with potent anti-vitamin D activity would contribute to understanding the mechanisms involved in the expression of antagonistic activity on VDR as well as to finding the seeds of new medicines for treating Paget's bone disease.

5. Experimental

5.1. General

All manipulations were performed under an argon atmosphere unless otherwise mentioned. All solvents and reagents were purified when necessary using standard procedures. Column chromatography was performed on silica gel 60 N (Kanto Chemical Co., Inc., 100–210 μm), and flash column chromatography was performed on silica gel 60 (Merck, 40–63 μm). NMR spectra were measured on a JEOL AL-400 magnetic resonance spectrometer. Infrared spectra were recorded on JASCO FTIR-8000 spectrometer. Mass spectra were measured on JEOL JMX-SX 102 mass spectrometer. Specific optical rotations were measured on JASCO DIP-370 digital polarimeter.

5.1.1. Methyl 2-(bromomethyl)-3-methylbut-2-enoate (12). To a solution of **11** (200 mg, 1.4 mmol) in Et_2O was added PBr_3 (80 μL, 0.83 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. To the mixture was added H_2O at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=20/1) to give **12** (240 mg, 83%) as a colorless oil. IR (neat) 1723, 1628, 1373 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.99, (s, 3H), 2.16 (s, 3H), 3.79 (s, 3H), 4.31 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.0, 24.0, 29.4, 51.7, 124.6, 153.8, 166.9; EI-LRMS *m/z* 205 (M^+), 191, 175; EI-HRMS Calcd for $C_7H_{11}O_2^{79}Br$ 205.9942, found 205.9951.

5.1.2. (*S*)-5-{(*R*)-2-[(1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-7a-methylperhydroinden-1-yl]propyl}-4,4-dimethyl-3-methylenedihydrofuran-2-one (13) and (*R*)-5-{(*R*)-2-[(1*R*,4*E*,3a*R*,7a*R*)-4-bromomethylene-7a-methylperhydroinden-1-yl]propyl}-4,4-dimethyl-3-methylenedihydrofuran-2-one (14). To a suspension of $CrCl_3$ (739 mg, 4.7 mmol) in THF (23 mL) was added $LiAlH_4$ (94 mg, 2.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. To the mixture were added a solution of **10** (486 mg, 2.3 mmol) in THF (8 mL) and a solution of **12** (350 mg, 1.2 mmol) at room temperature, and the resulting mixture was stirred at the same temperature for 1 h. To the mixture was added H_2O at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=10/1) to give a mixture of **13** and **14** (390 mg, 80% in the ratio of 2 to 1). Further separation was performed by recycle-HPLC (column: SHIMADZU Shim-pack PREP-SIL(H)-KIT, eluent: hexane/AcOEt=8/1, flow rate: 10 mL/min, detector: UV (235 nm)). **13**: mp. 130 °C (recrystallized from Et_2O–hexane); $[\alpha]_D^{25}$=+31.4 (*c* 0.85, $CHCl_3$); IR (film, $CHCl_3$) 1767, 1653, 1634, 1371, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3H), 1.05 (s, 3H), 1.08 (d, *J*=6.6 Hz, 3H), 1.21 (s, 3H), 1.25–1.70 (m, 11H), 1.95–2.04 (m, 3H), 2.88 (dd, *J*=15.9, 3.9 Hz, 1H), 4.10 (dd, *J*=9.0, 2.9 Hz, 1H), 5.46 (s, 1H), 5.65 (s, 1H), 6.14 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 19.7, 22.1, 22.6, 23.0, 24.3, 27.9, 31.0, 35.3, 35.5, 39.8, 42.6, 45.6, 55.7, 55.9, 86.1, 97.5, 118.9, 114.8, 146.1, 170.5; EI-LRMS *m/z* 394 (M^+), 315, 256, 227; EI-HRMS Calcd for $C_{21}H_{31}O_2^{79}Br$ 394.1507, found 394.1508. Anal. Calcd for $C_{21}H_{31}O_2Br$: C, 63.79; H, 7.90. Found: C, 64.13; H, 8.27. **14**: mp. 117 °C (recrystallized from Et_2O–hexane); $[\alpha]_D^{25}$=+141.2 (*c* 0.38, $CHCl_3$); IR (film, $CHCl_3$) 1767, 1651, 1638, 1458, 1190 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3H), 1.00 (d, *J*=6.3 Hz, 3H), 1.05 (s, 3H), 1.11 (dd, *J*=13.4, 11.2 Hz, 1H), 1.21 (s, 3H), 1.25–1.36 (m, 3H), 1.44–1.66 (m, 7H), 1.89 (m, 1H), 1.96–2.04 (m, 2H), 2.89 (dd, *J*=15.6, 6.8 Hz, 1H), 4.14 (d, *J*=10.5 Hz, 1H), 5.47 (s, 1H), 5.65 (s, 1H), 6.15 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 18.6, 22.1, 22.5, 22.8, 25.1, 27.6, 31.0, 32.9, 35.9, 39.9, 41.9, 45.6, 55.9, 56.2, 84.2, 97.6, 119.1, 144.7, 146.1, 170.3; EI-LRMS *m/z* 394 (M^+), 315, 256, 227; EI-HRMS Calcd for $C_{21}H_{31}O_2^{79}Br$ 394.1507, found 394.1508. Anal. Calcd for $C_{21}H_{31}O_2Br$: C, 63.79; H, 7.90. Found: C, 63.57; H, 8.20.

5.1.3. Methyl 4,6-*O*-benzylidene-3-allyl-3-deoxy-α-D-altropyranoside. To a solution of **15** (264 mg, 1.0 mmol) in THF (1.5 mL) was added a solution of allylmagnesium chloride in THF (2.0 M, 1.5 mL, 3.0 mmol) at room temperature, and the mixture was stirred at 80 °C for 1 h. To the mixture was added water at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=2/1) to give the desired allylated compound (314 mg, quant.) as an amorphous solid. IR (neat) 3422, 2930, 1642, 1456, 1381 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.90 (m, 1H), 2.26 (m, 1H), 2.53 (dd, *J*=7.1, 7.1 Hz, 2H), 3.39 (s, 3H),

3.78 (dd, J=10.2, 10.2 Hz, 1H), 3.97 (m, 1H), 4.00 (ddd, J=10.2, 10.2, 5.1 Hz, 1H), 4.12 (dd, J=10.2, 5.1 Hz, 1H), 4.29 (dd, J=10.2, 5.1 Hz, 1H), 4.60 (s, 1H), 5.06 (d, J=10.2 Hz, 1H), 5.11 (d, J=17.2 Hz, 1H), 5.60 (s, 1H), 5.83 (ddt, J=17.2, 10.2, 7.1 Hz, 1H), 7.36–7.37 (m, 3H), 7.48–7.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 42.8, 55.6, 59.9, 69.7, 69.9, 76.4, 102.3, 102.7, 116.9, 126.4 (2C), 128.5 (2C), 129.2, 137.3, 137.9; EI-LRMS m/z 306 (M^+); EI-HRMS Calcd for $C_{17}H_{22}O_5$ 306.1467, found 306.1469.

5.1.4. Methyl 4,6-*O*-benzylidene-3-deoxy-3-(3-hydroxypropyl)-α-D-altropyranoside (16). To a solution of the above allylated compound (1.8 g, 5.9 mmol) in THF (12 mL) was added a solution of BH_3·THF in THF (1.0 M, 14.7 mL, 14.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 23 h. To the mixture was added 3N NaOH aq. solution (7.7 mL) and 30% H_2O_2 aq. solution (7.7 mL) at 0 °C, and the mixture was stirred at room temperature for 3 h. To the mixture was added saturated NH_4Cl aq. solution at 0 °C, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=1/2) to give alcohol (1.72 g, 91%) as a colorless oil. $[\alpha]_D^{19}$=+84.2 (c 2.31, $CHCl_3$); IR (neat) 3403, 2936, 1103, 1051 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52–1.86 (m, 4H), 2.17–2.18 (m, 3H), 3.36 (s, 3H), 3.61 (t, J=6.3 Hz, 2H), 3.76 (dd, J=10.1, 10.1 Hz, 1H), 3.92 (m, 1H), 3.97 (ddd, J=10.1, 10.1, 5.2 Hz, 1H), 4.11 (dd, J=10.1, 5.2 Hz, 1H), 4.27 (dd, J=10.1, 5.2 Hz, 1H), 4.57 (s, 1H), 5.57 (s, 1H), 7.34–7.39 (m, 3H), 7.45–7.48 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 31.4, 42.5, 55.2, 59.5, 62.8, 69.5, 70.2, 76.4, 102.0, 102.1, 126.1 (2C), 128.2 (2C), 129.0, 137.5; EI-LRMS m/z 324 (M^+), 292, 274, 215, 162, 143, 105, 77; EI-HRMS Calcd for $C_{17}H_{24}O_6$ 324.1573, found 324.1575.

5.1.5. Methyl 4,6-*O*-benzylidene-3-deoxy-3-[(3-pivaloyloxy)propyl]-α-D-altropyranoside (17). To a solution of **16** (227 mg, 0.7 mmol) in pyridine (3.5 mL) was added pivaloyl chloride (95 μL, 0.77 mmol) at 0 °C, and the mixture was stirred at room temperature for 12 h. To the mixture was added water at 0 °C, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=4/1) to give **17** (270 mg, 94%) as a colorless oil. $[\alpha]_D^{19}$=+66.1 (c 2.38, $CHCl_3$); IR (neat) 3474, 2963, 1725, 1287, 1103, 1049 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (s, 9H), 1.63–1.90 (m, 4H), 2.18 (m, 1H), 2.32 (br s, 1H), 3.36 (s, 3H), 3.77 (dd, J=10.2, 10.2 Hz, 1H), 3.91 (m, 1H), 3.97 (ddd, J=10.2, 10.2, 5.0 Hz, 1H), 4.01–4.14 (m, 3H), 4.27 (dd, J=10.2, 5.0 Hz, 1H), 4.58 (s, 1H), 5.58 (s, 1H), 7.33–7.38 (m, 3H), 7.44–7.47 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.9, 27.2 (3C), 27.5, 38.8, 42.5, 55.2, 59.5, 64.3, 69.5, 70.1, 76.2, 101.9, 102.1, 126.1 (2C), 128.1 (2C), 128.9, 137.6, 178.6; EI-LRMS m/z 408 (M^+), 390, 358, 241, 162, 105; EI-HRMS Calcd for $C_{22}H_{32}O_7$ 408.2140, found 408.2140.

5.1.6. Methyl 4,6-*O*-benzylidene-2-(*tert*-butyldimethylsilyloxy)-3-deoxy-3-[(3-pivaloyloxy)propyl]-α-D-altropyranoside (18). To a solution of **17** (270 mg, 0.66 mmol) in CH_2Cl_2 (6.6 mL) were added 2,6-lutidine (0.39 mL, 3.3 mmol) and TBSOTf (0.23 mL, 0.99 mmol) at 0 °C, and the mixture was stirred at room temperature for 13 h. To the mixture was added water at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=9/1) to give **18** (332 mg, 96%) as a colorless oil. $[\alpha]_D^{19}$=+35.9 (c 0.62, $CHCl_3$); IR (neat) 2955, 1728, 1258, 1107, 1051 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.086 (s, 3H), 0.092 (s, 3H), 0.92 (s, 9H), 1.19 (s, 9H), 1.60–1.89 (m, 4H), 2.05 (m, 1H), 3.34 (s, 3H), 3.77 (dd, J=10.1, 10.1 Hz, 1H), 3.87 (br s, 1H), 3.92 (ddd, J=10.1, 10.1, 5.0 Hz, 1H), 4.03–4.14 (m, 3H), 4.26 (dd, J=10.1, 5.0 Hz, 1H), 4.45 (s, 1H), 5.59 (s, 1H), 7.34–7.37 (m, 3H), 7.47–7.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.8, 18.1, 21.1, 25.8 (3C), 27.2 (3C), 27.8, 38.8, 43.4, 55.0, 59.4, 64.4, 69.7, 70.7, 76.4, 101.9, 102.6, 126.2 (2C), 128.6 (2C), 128.9, 137.8, 178.4; EI-LRMS m/z 522 (M^+), 491, 358, 244, 159; EI-HRMS Calcd for $C_{28}H_{46}O_7$ 522.3013, found 522.3015.

5.1.7. Methyl 4-*O*-benzoyl-6-bromo-2-[(*tert*-butyldimethylsilyl)oxy]-3-deoxy-3-[(3-pivaloyloxy)propyl]-6-deoxy-α-D-altropyranoside (19). To a solution of **18** (2.28 g, 4.4 mmol) in CCl_4 (22 mL) were added $BaCO_3$ (861 mg, 4.4 mmol) and NBS (932 mg, 5.2 mmol) at room temperature, and the mixture was stirred at 80 °C for 30 min. To the mixture was added saturated $Na_2S_2O_3$ aq. solution and saturated $NaHCO_3$ aq. solution, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=9/1) to give **19** (2.46 g, 94%) as a colorless oil. $[\alpha]_D^{18}$=+18.4 (c 2.92, $CHCl_3$); IR (neat) 2957, 1727, 1267, 1116, 1042 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.12 (s, 3H), 0.93 (s, 9H), 1.08 (s, 9H), 1.43–1.73 (m, 3H), 1.87 (m, 1H), 2.16 (ddt, J=6.0, 5.9, 6.3 Hz, 1H), 3.43 (s, 3H), 3.58 (dd, J=10.7, 7.6 Hz, 1H), 3.66 (dd, J=10.7, 2.8 Hz, 1H), 3.82 (dd, J=6.0, 2.4 Hz, 1H), 4.00 (t, J=6.1 Hz, 2H), 4.10 (ddd, J=7.8, 7.6, 2.8 Hz, 1H), 4.55 (d, J=2.4 Hz, 1H), 5.38 (dd, J=7.8, 5.9 Hz, 1H), 7.43–7.47 (m, 2H), 7.59 (m, 1H), 7.99–8.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.6, 18.1, 21.7, 25.8 (3C), 26.9, 27.1 (3C), 33.4, 38.7, 42.1, 55.4, 64.2, 69.0, 71.0, 71.1, 103.7, 128.5 (2C), 129.5, 129.6 (2C), 133.3, 165.3, 178.3; EI-LRMS m/z 569 (M^+-OMe), 423, 339, 322, 213; EI-HRMS Calcd for $C_{27}H_{42}O_6{}^{79}Br$ 569.1934, found 569.1931.

5.1.8. (2*S*,3*R*,4*R*)-4-[(Benzoyl)oxy]-2-[(*tert*-butyldimethylsilyl)oxy]-3-[3-(pivaloyloxy)propyl]hexa-5-ene-1-ol (20). To a solution of **19** (2.40 g, 4.0 mmol) in 1-propanol/H_2O (5/1, 24 mL) were added activated Zn dust (45.5 g, 0.7 mol) and $NaBH_3CN$ (9.8 g, 0.14 mol) at 95 °C, and the mixture was stirred at the same temperature for 1.5 h. To the mixture was added saturated NH_4Cl aq. solution, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=6/1) to give **20** (1.76 g, 89%) as a colorless

oil. $[\alpha]_D^{19}$=+25.4 (*c* 2.62, $CHCl_3$); IR (neat) 3495, 2957, 1725, 1598, 1271, 1111, 1069 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 1.18 (s, 9H), 1.42–1.63 (m, 2H), 1.67–1.90 (m, 3H), 2.06 (ddt, *J*=6.3, 5.6, 6.1 Hz, 1H), 3.62 (dd, *J*=11.0, 4.9 Hz, 1H), 3.72 (dd, *J*=11.0, 5.3 Hz, 1H), 3.95 (ddd, *J*=5.6, 5.3, 4.9 Hz, 1H), 4.04 (t, *J*=6.1 Hz, 2H), 5.28 (ddd, *J*=10.5, 1.2, 1.2 Hz, 1H), 5.37 (ddd, *J*=17.1, 1.2, 1.2 Hz, 1H), 5.56 (dd, *J*=6.7, 6.3 Hz, 1H), 5.92 (ddd, *J*=17.1, 10.5, 6.7 Hz, 1H), 7.42–7.46 (m, 2H), 7.56 (m, 1H), 8.02–8.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.6, −4.2, 18.2, 22.8, 25.9 (3C), 27.2 (3C), 27.9, 38.8, 44.8, 64.4, 65.0, 73.0, 75.8, 118.4, 128.3 (2C), 129.5 (2C), 130.3, 132.9, 134.6, 165.4, 178.4; EI-LRMS *m/z* 391 (M^+-OPiv), 239, 105; EI-HRMS Calcd for $C_{22}H_{35}O_4Si$ 391.2305, found 391.2307.

5.1.9. (3*R*,4*R*)-3-Benzoyloxy-4-[(1*S*)-1-{(*tert*-butyldimethylsilyl)oxy}-2-(*p*-toluenesulfonyl)ethyl]-7-(pivaloyloxy)hept-1-ene. To a solution of **20** (1.72 g, 3.4 mmol) in pyridine (7 mL) was added TsCl (3.0 g, 16 mmol) at 0 °C, and the mixture was stirred at room temperature for 17.5 h. To the mixture was added water at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=9/1) to give the desired sulfonate (2.24 g, 99%) as a colorless oil. $[\alpha]_D^{19}$=+18.6 (*c* 0.46, $CHCl_3$); IR (neat) 2957, 1725, 1599, 1269, 1107 cm^{-1};^1H NMR (400 MHz, $CDCl_3$) δ −0.06 (s, 3H), −0.04 (s, 3H), 0.83 (s, 9H), 1.17 (s, 9H), 1.33–1.79 (m, 4H), 1.95 (m, 1H), 2.43 (s, 3H), 3.92–4.05 (m, 4H), 4.14 (ddd, *J*=6.2, 5.5, 2.8 Hz, 1H), 5.26 (ddd, *J*=10.4, 1.2, 1.2 Hz, 1H), 5.35 (ddd, *J*=17.3, 1.2, 1.2 Hz, 1H), 5.43 (dd, *J*=7.3, 7.1 Hz, 1H), 5.84 (ddd, *J*=17.3, 10.4, 7.1 Hz, 1H), 7.30–7.34 (m, 2H), 7.42–7.48 (m, 2H), 7.59 (m, 1H), 7.78–7.80 (m, 2H), 8.01–8.03 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.3, 18.0, 21.7, 22.3, 25.8 (3C), 27.2 (3C), 27.6, 38.8, 45.0, 64.1, 70.0, 71.3, 75.5, 118.9, 127.9 (2C), 128.4 (2C), 129.5 (2C), 129.8 (2C), 130.1, 132.7, 133.0, 134.6, 144.8, 165.2, 178.3; EI-LRMS *m/z* 475 (M^+-OTs), 329, 229; EI-HRMS Calcd for $C_{27}H_{43}O_5Si$ 475.2880, found 475.2887.

5.1.10. (3*R*,4*R*)-3-[(Benzoyl)oxy]-4-(*S*)-oxiranyl-7-(pivaloyloxy)hept-1-ene (21). To a solution of the above sulfonate (110 mg, 0.17 mmol) in THF (1.7 mL) was added a solution of TBAF in THF (1.0 M, 0.26 mL, 0.26 mmol) at 0 °C, and the mixture was stirred at room temperature for 6 h. To the mixture was added water at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=9/1) to give **21** (64 mg, quant.) as a colorless oil. $[\alpha]_D^{20}$=+28.7 (*c* 2.31, $CHCl_3$); IR (neat) 2973, 1725, 1601, 1269, 1157 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (s, 9H), 1.50 (m, 1H), 1.59–1.78 (m, 2H), 1.79–1.92 (m, 2H), 2.59 (dd, *J*=4.9, 2.6 Hz, 1H), 2.77 (dd, *J*=4.9, 3.9 Hz, 1H), 2.93 (ddd, *J*=8.3, 3.9, 2.6 Hz, 1H), 4.06 (t, *J*=6.3 Hz, 2H), 5.28 (ddd, *J*=10.5, 1.2, 1.0 Hz, 1H), 5.37 (ddd, *J*=17.1, 1.2, 1.0 Hz, 1H), 5.62 (dddd, *J*=6.6, 6.6, 1.0, 1.0 Hz, 1H), 5.90 (ddd, *J*=17.1, 10.5, 6.6 Hz, 1H), 7.44–7.47 (m, 2H), 7.58 (m, 1H), 8.03–8.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 26.2, 27.2 (3C), 38.7, 45.9, 46.8, 53.1, 64.1, 75.5, 118, 2, 128.4 (2C), 129.5 (2C), 129.9, 133.1, 134.2, 165.3, 178.4; EI-LRMS *m/z* 360 (M^+), 259, 136, 80; EI-HRMS Calcd for $C_{21}H_{28}O_5$ 360.1937, found 360.1935.

5.1.11. (3*R*,4*R*,5*S*)-4-(3-Hydroxypropyl)oct-1-en-7-yne-3,5-diol. To a solution of ethynyltrimethylsilane (0.32 mL, 2.2 mmol) in THF (3 mL) was added a solution of *n*-BuLi in hexane (1.56 M, 1.1 mL, 1.7 mmol) at −78 °C, and the mixture was stirred at the same temperature for 30 min. To the mixture were added a solution of **21** (400 mg, 1.1 mmol) in THF (8 mL) and $BF_3{\cdot}OEt_2$ (0.15 mL, 1.2 mmol) at −78 °C, and the resulting mixture was stirred at the same temperature for 3 h. To the mixture was added saturated NH_4Cl aq. solution, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was dissolved in MeOH (11 mL). To the mixture was added NaOMe (300 mg, 5.6 mmol) at 0 °C, and the mixture was stirred at 40 °C for 15.5 h. To the mixture was added saturated NH_4Cl aq. solution at 0 °C, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt=1/4) to give the desired triol (217 mg, 99%) as a colorless oil. $[\alpha]_D^{20}$=+9.88 (*c* 0.77, $CHCl_3$); IR (neat) 3304, 2940, 2118, 1630, 1051 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45–1.77 (m, 5H), 2.02 (dd, *J*=2.7, 2.7 Hz, 1H), 2.33 (ddd, *J*=16.8, 7.1, 2.7 Hz, 1H), 2.48 (ddd, *J*=16.8, 7.1, 2.7 Hz, 1H), 3.03 (br s, 3H), 3.62–3.74 (m, 2H), 4.20 (dt, *J*=1.5, 7.1 Hz, 1H), 4.41 (m, 1H), 5.25 (ddd, *J*=10.5, 1.6, 1.6 Hz, 1H), 5.37 (ddd, *J*=17.1, 1.7, 1.7 Hz, 1H), 5.91 (ddd, *J*=17.1, 10.5, 4.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 24.3, 30.4, 44.8, 62.8, 70.3, 70.4, 73.6, 81.0, 115.5, 139.5; EI-LRMS *m/z* 180 (M^+-H_2O), 161, 105, 79; EI-HRMS Calcd for $C_{11}H_{16}O_2$ 180.1150, found 180.1149.

5.1.12. (3*R*,4*R*,5*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-[3-{(*tert*-butyldimethylsilyl)oxy}propyl]oct-1-en-7-yne (22b). To the solution of the above triol (14 mg, 71 μmol) in CH_2Cl_2 (0.7 mL) were added 2,6-lutidine (49 μL, 0.42 mmol) and TBSOTf (65 μL, 0.28 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. To the mixture was added water at 0 °C, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane) to give **22b** (36 mg, 94%) as a colorless oil. $[\alpha]_D^{22}$=+8.32 (*c* 0.77, $CHCl_3$); IR (neat) 3314, 2932, 1256, 1102 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.04 (s, 6H), 0.05 (s, 3H), 0.07 (s, 3H), 0.09 (s, 3H), 0.89 (s, 27H), 1.32 (m, 2H), 1.56 (m, 2H), 1.75 (ddt, *J*=6.4, 4.0, 6.8 Hz, 1H), 1.95 (t, *J*=2.8 Hz, 1H), 2.38 (ddd, *J*=16.8, 6.2, 2.8 Hz, 1H), 2.42 (ddd, *J*=16.8, 6.2, 2.8 Hz, 1H), 3.56 (t, *J*=6.8 Hz, 2H), 4.03 (dt, *J*=4.0, 6.2 Hz, 1H), 4.12 (dd, *J*=7.6, 6.4 Hz, 1H), 5.08 (d, *J*=10.0 Hz, 1H), 5.14 (d, *J*=17.2 Hz, 1H), 5.84 (ddd, *J*=17.2, 10.0, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1 (2C), −4.4, −4.2, −3.9, −3.4, 18.25, 18.32, 18.5, 22.2, 26.0 (3C), 26.08 (3C), 26.12 (3C), 26.2, 32.6, 49.2, 63.6, 69.9, 71.5, 75.9, 82.1, 115.5, 140.4; EI-LRMS *m/z* 483 (M^+-*t*Bu);

EI-HRMS Calcd for $C_{25}H_{51}O_3Si_3$ 483.3146, found 483.3141.

5.2. General procedure for the synthesis of vitamin D_3 lactones

To a solution of an A-ring precursor (1.5 equiv. to a CD-ring precursor), and the CD-ring precursor in toluene were added Et_3N and $Pd(PPh_3)_4$ (30 mol% to the CD-ring precursor) and the mixture was stirred at 110 °C. After the mixture was filtered through a silica gel pad, the filtrate was concentrated. The crude product was dissolved in MeCN (1 mL). To the solution was added 10% solution of conc. HF in MeCN (1 mL) at 0 °C, the mixture was stirred at room temperature. To the mixture was added saturated $NaHCO_3$ aq. solution, and the aqueous layer was extract with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel or thin-layer chromatography on silica gel to give the vitamin D_3 derivative. Further purification for biological assays was conducted by reversed-phase recycle HPLC (YMC-Pack ODS column, 20×150 mm, 9.9 mL/min, eluent: CH_3CN/H_2O=90/10).

5.2.1. (23*S*)-25-Dehydro-1α-hydroxy-24,24-dimethylvitamin D_3-26,23-lactone (8). According to the general procedure, a crude product, which was obtained from **13** (31 mg, 78 μmol), **22** (43 mg, 117 μmol), Et_3N (0.8 mL) and $Pd(PPh_3)_4$ (33 mg, 28 μmol) in toluene (0.8 mL) at 110 °C for 1 h, was treated with conc. HF in MeCN for 1 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/1) to give **8** (17 mg, 48% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=264.0 nm; $[\alpha]_D^{24}$=−21.8 (*c* 0.85, $CHCl_3$); IR (film, $CHCl_3$) 3382, 1765, 1663, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 1.05 (s, 3H), 1.07 (d, *J*=6.6 Hz, 3H), 1.21 (s, 3H), 1.25–1.72 (m, 13H), 1.88–2.06 (m, 5H), 2.32 (dd, *J*=13.4, 6.3 Hz, 1H), 2.60 (dd, *J*=13.4, 3.5 Hz, 1H), 2.83 (dd, *J*=10.4, 3.8 Hz, 1H), 4.10 (dd, *J*=10.4, 3.4 Hz, 1H), 4.23 (br s, 1H), 4.43 (br s, 1H), 5.00 (dd, *J*=1.6, 1.5 Hz, 1H), 5.33 (dd, *J*=1.7, 1.6 Hz, 1H), 5.45 (s, 1H), 6.02 (d, *J*=11.2 Hz, 1H), 6.13 (s, 1H), 6.38 (d, *J*=11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 19.7, 22.3, 23.0, 23.6, 24.4, 27.9, 29.1, 35.4, 35.6, 40.4, 42.6, 42.9, 45.3, 46.0, 56.2, 56.7, 66.8, 70.8, 86.3, 111.6, 117.1, 118.8, 124.8, 133.0, 142.8, 146.2, 147.6, 170.5; EI-LRMS *m/z* 454 (M^+), 418, 403; EI-HRMS Calcd for $C_{29}H_{42}O_4$ 454.3083, found 454.3083.

5.2.2. (23*R*)-25-Dehydro-1α-hydroxy-24,24-dimethylvitamin D_3-26,23-lactone (9). According to the general procedure, a crude product, which was obtained from **14** (30 mg, 76 μmol), **22** (48 mg, 114 μmol), Et_3N (0.8 mL) and $Pd(PPh_3)_4$ (26 mg, 23 μmol) in toluene (0.8 mL) at 110 °C for 30 min, was treated with conc. HF in MeCN for 30 min. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/1) to give **9** (27 mg, 78% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=265.0 nm; $[\alpha]_D^{24}$=+56.2 (*c* 1.15, $CHCl_3$); IR (film, $CHCl_3$) 3426, 1759, 1672, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 0.99 (d, *J*=6.6 Hz, 3H), 1.05 (s, 3H), 1.11 (dd, *J*=10.5, 1.47 Hz, 1H), 1.21 (s, 3H), 1.26 (s, 3H), 1.45–1.76 (m, 9H), 1.83–2.04 (m, 5H), 2.31 (dd, *J*=13.4, 6.5 Hz, 1H), 2.60 (dd, *J*=13.4, 3.4 Hz, 1H), 2.83 (dd, *J*=12.0, 3.9 Hz, 1H), 4.14 (dd, *J*=11.6, 1.6 Hz, 1H), 4.24 (br s, 1H), 4.43 (br s, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.47 (s, 1H), 6.12 (d, *J*=11.4 Hz, 1H), 6.15 (s, 1H), 6.37 (d, *J*=11.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 18.6, 22.3, 22.8, 23.6, 25.1, 27.6, 29.1, 32.9, 35.9, 40.5, 42.0, 42.9, 45.3, 46.0, 56.4, 57.0, 66.8, 70.8, 84.3, 111.7, 117.2, 119.1, 124.7, 133.1, 142.6, 146.2, 147.5, 170.4; EI-LRMS *m/z* 454 (M^+), 418, 403; EI-HRMS Calcd for $C_{29}H_{42}O_4$ 454.3083, found 454.3083.

5.2.3. (23*S*)-25-Dehydro-1α-hydroxy-2α,24,24-trimethylvitamin D_3-26,23-lactone (8a). According to the general procedure, a crude product, which was obtained from **13** (25 mg, 63 μmol), **22a** (41 mg, 107 μmol), Et_3N (0.6 mL) and $Pd(PPh_3)_4$ (37 mg, 32 μmol) in toluene (0.6 mL) at 110 °C for 1.5 h, was treated with conc. HF in MeCN for 1 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/1) to give **8a** (14 mg, 47% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=266.0 nm; $[\alpha]_D^{26}$=+11.6 (*c* 1.08, $CHCl_3$); IR (film, $CHCl_3$) 3441, 1752, 1671, 1636, 1051 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3H), 1.04 (s, 3H), 1.06 (d, *J*=6.7 Hz, 3H), 1.07 (d, *J*=6.7 Hz, 3H), 1.21 (s, 3H), 1.25–1.70 (m, 13H), 1.88–2.04 (m, 4H), 2.23 (dd, *J*=13.8, 8.1 Hz, 1H), 2.66 (dd, *J*=13.8, 4.0 Hz, 1H), 2.82 (m, 1H), 3.84 (ddd, *J*=7.6, 7.6, 4.2 Hz, 1H), 4.10 (dd, *J*=8.8, 3.4 Hz, 1H), 4.31 (d, *J*=3.2 Hz, 1H), 5.00 (d, *J*=1.7 Hz, 1H), 5.27 (dd, *J*=2.0, 1.0 Hz, 1H), 5.45 (s, 1H), 6.01 (d, *J*=11.2 Hz, 1H), 6.13 (s, 1H), 6.38 (d, *J*=11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 12.6, 19.7, 22.3, 23.0, 23.5, 24.3, 27.9, 29.1, 35.4, 35.6, 40.4, 42.6, 43.5, 44.2, 46.0, 56.2, 56.7, 71.7, 75.4, 86.2, 113.1, 117.0, 118.8, 124.7, 133.1, 142.8, 146.2, 146.5, 170.5; EI-LRMS *m/z* 468 (M^+), 451, 434, 419; EI-HRMS Calcd for $C_{30}H_{44}O_4$ 468.3240, found 468.3248.

5.2.4. (23*R*)-25-Dehydro-1α-hydroxy-2α,24,24-trimethylvitamin D_3-26,23-lactone (9a). According to the general procedure, a crude product, which was obtained from **14** (26 mg, 66 μmol), **22a** (40 mg, 105 μmol), Et_3N (1 mL) and $Pd(PPh_3)_4$ (24 mg, 21 μmol) in toluene (1 mL) at 110 °C for 2.5 h, was treated with conc. HF in MeCN for 1 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/1) to give **9a** (18 mg, 58% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=266.5 nm; $[\alpha]_D^{26}$=+78.5 (*c* 1.38, $CHCl_3$); IR (film, $CHCl_3$) 3476, 1750, 1649, 1051 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 1.00 (d, *J*=6.7 Hz, 3H), 1.06 (s, 3H), 1.08 (d, *J*=6.7 Hz, 3H), 1.12 (m, 1H), 1.21 (s, 3H), 1.26–1.34 (m, 3H), 1.46–1.72 (m, 10H), 1.90–2.04 (m, 3H), 2.23 (dd, *J*=13.4, 7.9 Hz, 1H), 2.67 (dd, *J*=13.4, 4.0 Hz, 1H), 2.83 (m, 1H), 3.85 (ddd, *J*=7.5, 7.5, 4.2 Hz, 1H), 4.15 (dd, *J*=11.6, 1.3 Hz, 1H), 4.31 (br s, 1H), 5.01 (d, *J*=2.0 Hz, 1H), 5.28 (s, 1H), 5.47 (s, 1H), 6.01 (d, *J*=11.2 Hz, 1H), 6.15 (s, 1H), 6.38 (s, *J*=11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 12.6, 18.6, 22.3, 22.9, 23.5, 25.1, 27.6, 29.1, 32.9, 35.9, 40.6, 42.0, 43.4, 44.2, 46.0, 56.4, 57.0, 71.7, 75.3, 84.3, 113.1, 117.1, 119.1, 124.6, 133.2, 142.6, 146.2, 146.5, 170.4; EI-LRMS *m/z* 468 (M^+), 451, 434, 419, 404; EI-HRMS Calcd for $C_{30}H_{44}O_4$ 468.3240, found 468.3264.

5.2.5. (23*S*)-25-Dehydro-1α-hydroxy-2α-(3-hydroxypropyl)-24,24-dimethylvitamin D_3-26,23-lactone (8b). According to the general procedure, a crude product, which was obtained from **13** (18 mg, 46 μmol), **22b** (37 mg, 68 μmol), Et_3N (0.4 mL) and $Pd(PPh_3)_4$ (30 mg, 26 μmol) in toluene (0.4 mL) at 110 °C for 4 h, was treated with conc. HF in MeCN for 4 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/4) to give **8b** (9 mg, 39% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=267.5 nm; $[\alpha]_D^{26}$=+13.7 (*c* 0.85, $CHCl_3$); IR (film, $CHCl_3$) 3380, 1763, 1653, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3H), 1.05 (s, 3H), 1.07 (d, *J*=6.6 Hz, 3H), 1.21 (s, 3H), 1.24–1.54 (m, 10H), 1.58–1.77 (m, 7H), 1.92–2.02 (m, 5H), 2.25 (dd, *J*=13.5, 8.9 Hz, 1H), 2.66 (dd, *J*=13.6, 4.3 Hz, 1H), 2.83 (m, 1H), 3.69–3.71 (m, 2H), 3.89 (ddd, *J*=8.3, 8.3, 4.4 Hz, 1H), 4.11 (dd, *J*=9.0, 3.2 Hz, 1H), 4.38 (d, *J*=2.9 Hz, 1H), 5.00 (d, *J*=1.6 Hz, 1H), 5.28 (d, *J*=1.6 Hz, 1H), 5.46 (s, 1H), 6.00 (d, *J*=11.4 Hz, 1H), 6.14 (s, 1H), 6.40 (d, *J*=11.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 12.6, 19.7, 22.3, 23.0, 23.5, 24.3, 27.9, 29.1, 35.4, 35.6, 40.4, 42.6, 43.5, 44.2, 46.0, 56.2 (2C), 56.7 (2C), 71.7, 75.4, 86.2, 113.1, 117.0, 118.8, 124.7, 133.1, 142.8, 146.2, 146.5, 170.5; EI-LRMS *m/z* 512 (M^+), 495, 478, 461; EI-HRMS Calcd for $C_{32}H_{48}O_5$ 512.3502, found 512.3490.

5.2.6. (23*R*)-25-Dehydro-1α-hydroxy-2α-(3-hydroxypropyl)-24,24-dimethylvitamin D_3-26,23-lactone (9b). According to the general procedure, a crude product, which was obtained from **14** (39 mg, 76 μmol), **22b** (68 mg, 126 μmol), Et_3N (0.8 mL) and $Pd(PPh_3)_4$ (25 mg, 22 μmol) in toluene (0.8 mL) at 110 °C for 4 h, was treated with conc. HF in MeCN for 1.5 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/4) to give **9b** (18 mg, 46% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=268.0 nm; $[\alpha]_D^{22}$=+69.4 (*c* 1.38, $CHCl_3$); IR (film, $CHCl_3$) 3393, 1757, 1649, 1638 1076 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.99 (d, *J*=6.3 Hz, 3H), 1.06 (s, 3H), 1.11 (m, 1H), 1.21 (s, 3H), 1.26–1.35 (m, 5H), 1.48–1.86 (m, 11H), 1.97–2.05 (m, 3H), 2.25 (dd, *J*=13.3, 8.7 Hz, 2H), 2.28 (br s, 1H), 2.66 (dd, *J*=13.3, 4.2 Hz, 1H), 2.83 (m, 1H), 3.69–3.70 (m, 2H), 3.90 (ddd, *J*=8.2, 8.2, 4.3 Hz, 1H), 4.15 (dd, *J*=11.4, 1.1 Hz, 1H), 4.38 (d, *J*=2.9 Hz, 1H), 4.99 (d, *J*=1.7 Hz, 1H), 5.28 (d, *J*=1.7 Hz, 1H), 5.47 (s, 1H), 6.00 (d, *J*=11.2 Hz, 1H), 6.15 (s, 1H), 6.39 (d, *J*=11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 14.2, 18.6, 22.3, 22.9, 23.5, 25.1, 27.6, 29.0, 30.1, 33.0, 35.9, 40.6, 42.0, 44.3, 46.0, 49.0, 56.4, 56.9, 62.7, 70.3, 73.5, 84.3, 113.6, 117.1, 119.1, 124.5, 133.0, 142.6, 146.2, 146.4, 170.4; EI-LRMS *m/z* 512 (M^+), 495, 478, 461; EI-HRMS Calcd for $C_{32}H_{48}O_5$ 512.3502, found 512.3502.

5.2.7. (23*S*)-25-Dehydro-1α-hydroxy-2α-(3-hydroxypropoxy)-24,24-dimethylvitamin D_3-26,23-lactone (8c). According to the general procedure, a crude product, which was obtained from **13** (14 mg, 35 μmol), **22c** (35 mg, 63 μmol), Et_3N (0.4 mL) and $Pd(PPh_3)_4$ (13 mg, 11 μmol) in toluene (0.4 mL) at 110 °C for 2 h, was treated with conc. HF in MeCN for 1.5 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/4) to give **8c** (13 mg, 69% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=267.0 nm; $[\alpha]_D^{23}$=+13.3 (*c* 0.69, $CHCl_3$); IR (neat) 3330, 1763, 1649, 1624, 1096 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.70 (s, 3H), 1.05 (s, 3H), 1.06 (d, *J*=6.6 Hz, 3H), 1.21 (s, 3H), 1.23–1.72 (m, 11H), 1.84–2.04 (m, 5H), 2.24 (dd, *J*=13.6, 9.2 Hz, 1H), 2.53 (br s, 3H), 2.68 (dd, *J*=13.6, 4.6 Hz, 1H), 2.82 (m, 1H), 3.38 (dd, *J*=7.4, 3.3 Hz, 1H), 3.83 (m, 4H), 4.05 (m, 1H), 4.10 (dd, *J*=8.9, 3.3 Hz, 1H), 4.45 (d, *J*=2.9 Hz, 1H), 5.10 (d, *J*=1.5 Hz, 1H), 5.39 (d, *J*=1.5 Hz, 1H), 5.46 (s, 1H), 6.02 (d, *J*=11.2 Hz, 1H), 6.13 (s, 1H), 6.42 (d, *J*=11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 19.7, 22.3, 23.0, 23.5, 24.3, 27.9, 29.1, 32.0, 35.6, 40.4, 41.0, 42.6, 46.0, 52.5, 56.2, 56.7, 61.2, 68.4, 68.5, 71.9, 84.5, 86.2, 116.1, 117.2, 118.8, 125.4, 131.6, 143.1, 144.2, 146.2, 170.5; EI-LRMS *m/z* 528 (M^+), 511, 494, 477, 435; EI-HRMS Calcd for $C_{32}H_{48}O_6$ 528.3451, found 528.3451.

5.2.8. (23*R*)-25-Dehydro-1α-hydroxy-2α-(3-hydroxypropoxy)-24,24-dimethylvitamin D_3-26,23-lactone (9c). According to the general procedure, a crude product, which was obtained from **14** (30 mg, 76 μmol), **22c** (71 mg, 128 μmol), Et_3N (0.8 mL) and $Pd(PPh_3)_4$ (27 mg, 23 μmol) in toluene (0.8 mL) at 110 °C for 3 h, was treated with conc. HF in MeCN for 1 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt=1/4) to give **9c** (23 mg, 57% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max}=267.0 nm; $[\alpha]_D^{23}$=+64.9 (*c* 1.77, $CHCl_3$); IR (film, $CHCl_3$) 3397, 1763, 1638, 1076 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.99 (d, *J*=6.6 Hz, 3H), 1.05 (s, 3H), 1.11 (m, 1H), 1.21 (s, 3H), 1.23–1.35 (m, 4H), 1.47–1.56 (m, 3H), 1.66–1.88 (m, 6H), 1.96–2.05 (m, 2H), 2.24 (dd, *J*=13.6, 8.8 Hz, 1H), 2.68 (dd, *J*=13.6, 4.4 Hz, 1H), 2.73 (br s, 3H), 2.83 (m, 1H), 3.37 (dd, *J*=7.6, 3.2 Hz, 1H), 3.74–3.91(m, 4H), 4.06 (ddd, *J*=8.2, 8.2, 4.4 Hz, 1H), 4.15 (dd, *J*=11.5, 1.2 Hz, 1H), 4.45 (d, *J*=2.9 Hz, 1H), 5.09 (d, *J*=1.7 Hz, 1H), 5.39 (s, 1H), 5.47 (s, 1H), 6.12 (d, *J*=11.2 Hz, 1H), 6.15 (s, 1H), 6.41 (d, *J*=11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 18.6, 22.3, 22.9, 23.5, 25.1, 27.6, 29.1, 31.9, 32.9, 35.9, 40.6, 41.0, 42.0, 46.0, 56.4, 56.9, 61.1, 68.3, 68.4, 71.8, 84.3, 84.4, 116.0, 117.3, 119.1, 125.2, 131.8, 142.9, 144.2, 146.2, 170.4; EI-LRMS *m/z* 528 (M^+), 511, 494, 477, 435; EI-HRMS Calcd for $C_{32}H_{48}O_6$ 528.3451, found 528.3449.

5.3. Vitamin D receptor (VDR) binding assay

[26,27-*Methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 (specific activity 6.623 TBq/mmol, 15,000 dpm, 15.7 pg) and various amounts of 1α,25-dihydroxyvitamin D_3 and an analogue to be tested were dissolved in 50 μL of absolute ethanol in 12×75-mm polypropylene tubes. The chick intestinal VDR (0.2 mg) and 1 mg of gelatin in 1 mL of phosphate buffer solution (25 nM KH_2PO_4, 0.1 M KCl, 1 mM dithiothreitol, pH 7.4) were added to each tube in an ice bath. The assay tubes were incubated in shaking water bath for 1 h at 25 °C and then chilled in an ice bath. 1 mL of 40% polypropylene glycol 6000 in distilled water was added to each tube, which was the mixed vigorously and centrifuged at 2,260×*g* for 60 min at 4 °C. After the supernatant was decanted, the bottom of the tube containing the pellet was cut off into a scintillation vial containing 10 mL of

dioxane-based scintillation fluid and the radioactivity was counted with a Beckman liquid scintillation counter (Model LS6500). The relative potency of the analogues were calculated from their concentration needed to displace 50% of [26,27-*methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3 (assigned a 100% value).

5.4. Assay for HL-60 cell differentiation

Nitro blue tetrazolium (NBT)-reducing activity was used as a cell differentiation marker. HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FCS. Exponentially proliferating cells were collected, suspended in fresh medium and seeded in culture plates (Falcon, Becton Dickinson and Company, Franklin Lakes, NJ). Cell concentration at seeding was adjusted to 2×10^4 cells/mL and the seeding volume was 1 mL/well. An ethanol solution of 1α,25-dihydroxyvitamin D_3 (final concentration: 10^{-8} M) and an analogue (final concentration: 10^{-11} to 10^{-6} M) was added to the culture medium at 0.1% volume and culture was continued for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without medium change. The same amount of vehicle was added to the control culture. NBT-reducing assay was performed according to the method of Collins.[22] Briefly, cells were collected, washed with PBS, and suspended in serum-free medium. NBT/TPA solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng/mL, respectively. Then, the cell suspensions were incubated at 37 °C for 25 min. After incubation, cells were collected by centrifugation and resuspended in FCS. Cytospin smears were prepared, and the counter-staining of nuclei was done with Kemechrot solution. At least 500 cells per preparation were observed.

6. Supporting Information Available

Charts of vitamin D receptor binding assay of compounds **8**, **8a–c**, **9**, and **9a–c**, and assay for HL-60 cell differentiation to test antagonistic activity of compounds **8**, **8a–c**, **9**, and **9a–c**. This material is available online with the paper in Science Direct.

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References and notes

1. (a) In *Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic: New York, 1997. (b) Ettinger, R. A.; DeLuca, H. F. *Adv. Drug Res.* **1996**, *28*, 269.
2. Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200.
3. (a) Evans, R. M. *Science* **1988**, *240*, 889. (b) Umezono, K.; Murakami, K. K.; Thompson, C. C.; Evans, R. M. *Cell* **1991**, *65*, 1255.
4. Takeyama, K.; Masuhiro, Y.; Fuse, H.; Endoh, H.; Murayama, A.; Kitanaka, S.; Suzawa, M.; Yanagisawa, J.; Kato, S. *Mol. Cell Biol.* **1999**, *19*, 1049.
5. (a) Konno, K.; Maki, S.; Fujishima, T.; Liu, Z.; Miura, D.; Chokki, M.; Takayama, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 151. (b) Konno, K.; Fujishima, T.; Maki, S.; Liu, Z.; Miura, D.; Chokki, M.; Ishizuka, S.; Yamaguchi, K.; Kan, Y.; Kurihara, M.; Miyata, N.; Smith, C.; DeLuca, H. F.; Takayama, H. *J. Med. Chem.* **2000**, *43*, 4247. (c) Suhara, Y.; Nihei, K.-i.; Tanigawa, H.; Fujishima, T.; Konno, K.; Nakagawa, K.; Okano, T.; Takayama, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1129. (d) Suhara, Y.; Nihei, K.-i.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. *J. Org. Chem.* **2001**, *66*, 8760. (e) Kittaka, A.; Suhara, Y.; Takayanagi, H.; Fujishima, T.; Kurihara, M.; Takayama, H. *Org. Lett.* **2000**, *2*, 2619.
6. For our account, see: Takayama, H.; Kittaka, A.; Fujishima, T.; Suhara, Y. In *Vitamin D Analogs in Cancer Prevention and Therapy*. Recent Results in Cancer Research 164; Reichrath, J., Friedrich, M., Tilgen, W., Eds.; Springer: Berlin, 2003; pp 289–317.
7. This concept was also good for 19-nor **1**, see: (a) Ono, K.; Yoshida, A.; Saito, N.; Fujishima, T.; Honzawa, S.; Suhara, Y.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *J. Org. Chem.* **2003**, *68*, 7407. (b) Yoshida, A.; Ono, K.; Suhara, Y.; Saito, N.; Takayama, H.; Kittaka, A. *Synlett* **2003**, 1175. (c) For the alternative synthesis of ED-71, see: Hatakeyama, S.; Ikeda, T.; Maeyama, J.; Esumi, T.; Iwabuchi, Y.; Irie, H.; Kawase, A.; Kubodera, N. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2871.
8. (a) Ishizuka, S.; Ishimoto, S.; Norman, A. W. *Biochemistry* **1984**, *23*, 1473. (b) Ishizuka, S.; Ohba, T.; Norman, A. W. In *Vitamin D: Molecular, Cellular and Chemical Endocrinology*; Norman, A. W., Schaefer, K., Grigoleit, H. G., von Herrath, D., Eds.; Walter de Gruyter: Berlin, 1988; pp 143–144.
9. (a) Miura, D.; Manabe, K.; Ozono, K.; Saito, M.; Gao, Q.; Norman, A. W.; Ishizuka, S. *J. Biol. Chem.* **1999**, *274*, 16392. (b) Ozono, K.; Saito, M.; Miura, D.; Michigami, T.; Nakajima, S.; Ishizuka, S. *J. Biol. Chem.* **1999**, *274*, 32376. (c) Miura, D.; Manabe, K.; Gao, Q.; Norman, A. W.; Ishizuka, S. *FEBS Lett.* **1999**, *460*, 297. (d) Ishizuka, S.; Miura, D.; Eguchi, H.; Ozono, K.; Chokki, M.; Kamimura, T.; Norman, A. W. *Arch. Biochem. Biophys.* **2000**, *380*, 92. (e) Ishizuka, S.; Miura, D.; Ozono, K.; Chokki, M.; Mimura, H.; Norman, A. W. *Endocrinology* **2001**, *142*, 59. (f) Ishizuka, S.; Miura, D.; Ozono, K.; Saito, M.; Eguchi, H.; Chokki, M.; Norman, A. W. *Steroids* **2001**, *66*, 227.
10. The other type of VDR antagonist of 25-carboxylic esters ZK159222 and ZK168281, see: (a) Herdick, M.; Steinmeyer, A.; Carlberg, C. *J. Biol. Chem.* **2000**, *275*, 16506. (b) Bury, Y.; Steinmeyer, A.; Carlberg, C. *Mol. Pharmacol.* **2000**, *58*, 1067. (c) Herdick, M.; Steinmeyer, A.; Carlberg, C. *Chem. Biol.* **2000**, *7*, 885. (d) Toell, A.; Gonzalez, M. M.; Ruf, D.; Steinmeyer, A.; Ishizuka, S.; Carlberg, C. *Mol. Pharmacol.* **2001**, *59*, 1478. (e) Väisänen, S.; Peräkylä, M.; Kärkkäinen, J. I.; Steinmeyer, A.; Carlberg, C. *J. Mol. Biol.* **2002**, *315*, 229.
11. Carlberg, C. *J. Cell. Biochem.* **2003**, *88*, 274.

12. (a) Saito, N.; Matsunaga, T.; Fujishima, T.; Anzai, M.; Saito, H.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takayama, H.; Kittaka, A. *Org. Biomol. Chem.* **2003**, *1*, 4396. (b) Saito, N.; Saito, H.; Anzai, M.; Yoshida, A.; Fujishima, T.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takayama, H.; Kittaka, A. *Org. Lett.* **2003**, *5*, 4859.
13. Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.* **1992**, *114*, 9836.
14. Okuda, Y.; Nakatsukasa, S.; Oshima, K.; Nozaki, H. *Chem. Lett.* **1985**, 481.
15. Xu, L.-H.; Kündig, E. P. *Helv. Chim. Acta* **1994**, *77*, 1480.
16. Crystal data of **13** are as follows: space group $P2_1$, Z=2, a=9.394(2), b=7.571(1), c=14.713(2) Å, β=106.10(1)°, V=1005.3(3) Å^3, D_c=1.445 g/cm^3. Crystal data of **14** are as follows: space group $P2_1$, Z=2, a=11.137(2), b=7.609(2), c=12.433(2) Å, β=100.02(1)°, V=1037.5(2) Å^3, D_c=1.400 g/cm^3. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 239774 for **13** and CCDC 239775 for **14**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: dposit@ccdc.cam.ac.uk).
17. Wiggins, L. S. *Methods Carbohydr. Chem.* **1963**, *2*, 181.
18. (a) Suhara, Y.; Kittaka, A.; Kishimoto, S.; Calverly, M. J.; Fujishima, T.; Saito, N.; Sugiura, T.; Waku, K.; Takayama, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3255. (b) Honzawa, S.; Suhara, Y.; Nihei, K.-i.; Saito, N.; Kishimoto, S.; Fujishima, T.; Kurihara, M.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3503.
19. (a) Wood, A. J.; Jenkins, P. R.; Fawcett, J.; Russell, D. R. *J. Chem. Soc., Chem. Commun.* **1995**, 1567. (b) Wood, A. J.; Holt, D. J.; Dominguez, M.-C.; Jenkins, P. R. *J. Org. Chem.* **1998**, *63*, 8522.
20. Previously, enyne **22b** was prepared from 1,2-*O*-isopropylidene-D-xylofuranose in 29 steps. See, Ref. 5c,d.
21. (a) Eisman, J. A.; Hamstra, A. J.; Kream, B. E.; DeLuca, H. F. *Arch. Biochem. Biophys.* **1976**, *176*, 235. (b) Inaba, M.; DeLuca, H. F. *Biochim. Biophys. Acta* **1989**, *1010*, 20.
22. Collins, S. J.; Ruscetti, F. W.; Gallagher, R. E.; Gallo, R. C. *J. Exp. Med.* **1979**, *149*, 969.
23. For recent description of VDR regulated transactivation mechanism, see: Masuno, H.; Yamamoto, K.; Wang, X.; Choi, M.; Ooizumi, H.; Shinki, T.; Yamada, S. *J. Med. Chem.* **2002**, *45*, 1825.
24. Recent review on the function of VDR in response to **1** and the other synthetic ligands, see: Carlberg, C. In *Vitamin D Analogs in Cancer Prevention and Therapy*. *Recent Results in Cancer Research 164*; Reichrath, J., Friedrich, M., Tilgen, W., Eds.; Springer: Berlin, 2003; pp 29–42; See also, Ref. 11.
25. Bula, C. M.; Bishop, J. E.; Ishizuka, S.; Norman, A. W. *Mol. Endocrinol.* **2000**, *14*, 1788.
26. Ochiai, E.; Miura, D.; Eguchi, H.; Takenouchi, K.; Harada, Y.; Azuma, Y.; Kamimura, T.; Ishizuka, S. *J. Bone Miner. Res.* **2003**, *18*(Suppl. 2), S103.
27. Takenouchi, K.; Sogawa, R.; Manabe, K.; Saito, H.; Gao, Q.; Miura, D.; Ishizuka, S. *J. Steroid Biochem. Mol. Biol.* **2004**, in press.
28. Recent reports on Paget's bone disease, see: (a) Menaa, C.; Barsony, J.; Reddy, S. V.; Cornish, J.; Cundy, T.; Roodman, G. D. *J. Bone Miner. Res.* **2000**, *15*, 228. (b) Kurihara, N.; Reddy, S. V.; Menaa, C.; Anderson, D.; Roodman, G. D. *J. Clin. Invest.* **2000**, *105*, 607. (c) Menaa, C.; Reddy, S. V.; Kurihara, N.; Maeda, H.; Anderson, D.; Cundy, T.; Cornish, J.; Singer, F. R.; Bruder, J. M.; Roodman, G. D. *J. Clin. Invest.* **2000**, *105*, 1833. (d) Leach, R. J.; Singer, F. R.; Roodman, G. D. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 24. (e) Reddy, S. V.; Kurihara, N.; Menaa, C.; Landucci, G.; Forthal, D.; Koop, B. A.; Windle, J. J.; Roodmann, G. D. *Endocrinology* **2001**, *142*, 2898. (f) Friedrichs, W. E.; Reddy, S. V.; Bruder, J. M.; Cundy, T.; Cornish, J.; Singer, F. R.; Roodman, G. D. *J. Bone Miner. Res.* **2002**, *17*, 145. (g) Reddy, S. V.; Kurihara, N.; Menaa, C.; Roodman, G. D. *Rev. Endocr. Metab. Disord.* **2001**, *2*, 195.