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Synthesis of a 1α-*C*-methyl analogue of 25-hydroxyvitamin D_3: interaction with a mutant vitamin D receptor Arg274Leu

Shinobu Honzawa [a,†], Naoyuki Takahashi [a], Atsushi Yamashita [b], Takayuki Sugiura [b], Masaaki Kurihara [c], Midori A. Arai [a,‡], Shigeaki Kato [d], Atsushi Kittaka [a,*]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 229-0195, Japan*
[b] *Department of Hygienic Chemistry and Nutrition, Faculty of Pharmaceutical Sciences, Teikyo University, Kanagawa 229-0195, Japan*
[c] *National Institute of Health Sciences, Tokyo 158-8501, Japan*
[d] *Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113-0032, Japan*

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ABSTRACT

Vitamin D_3 analogues have been developed for a mutant vitamin D receptor (VDR), Arg274Leu. The mutant VDR has a mutation at Arg274, which forms an important hydrogen bond with 1α-OH of 1α,25-dihydroxyvitamin D_3 to anchor the ligand tightly in the VDR ligand binding pocket. Stereoselective synthesis of the A-ring part of the novel vitamin D analogue, 2α-(3-hydroxypropyl)-1α-methyl-25-hydroxyvitamin D_3 (**4**), from D-galactose was accomplished with the key steps of the introduction of the methyl and allyl groups to the chiral building blocks. The new analogue **4** is ca. 7.3-fold more active than the natural hormone 1α,25-dihydroxyvitamin D_3 (**1**).

1. Introduction

Hydrogen bonding is one of the most important interactions between biomacromolecules such as proteins and nucleic acids, and biologically active small molecules such as hormones and drug molecules.[1] Also very important is the hydrophobic interactions between such molecules.[2] The latter interactions are weaker than the former, but cannot be neglected when they account for much of the biological system.

Vitamin D_3, a lipophilic hormonal molecule of low molecular weight, has long been known to play a significant role in the sustainment of life through calcium and phosphate homeostasis.[3] Recently, it has been clarified that this molecule is also involved in controlling cell differentiation and proliferation through binding with a specific nuclear hormone receptor, vitamin D receptor (VDR).[4] The identity of the vitamin D_3 action is the dihydroxylated metabolite, 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$, **1**). X-ray crystallographic experiments[5] have shown that the three hydroxy groups of **1**, that is, 1α-OH, 3β-OH and 25-OH, interact with hydrophilic amino acid residues in the ligand binding pocket (LBP) of VDR through hydrogen bonds. However, hydrophobic interactions between the hydrophobic moieties of the ligand and hydrophobic amino acid residues in the LBP of VDR seem to be important. In the case of the human VDR, the triene moiety connecting the C-ring and A-ring of **1** is sandwiched between Ser-275 and Trp-286 on one side, and Leu-233 on the other side, and the D-ring side chain is surrounded by hydrophobic residues, for example, Leu-227, Val-234, Val-300, Leu-309, Leu-404, and Val-418. Recently, adamantyl vitamin D analogues have been reported that showed VDR partial agonism and antagonism, in which hydrophobic interactions of the rigid and bulky adamantyl side chain to helixes and loops of the VDR ligand binding domain affected stability of the VDR active conformation.[6] Alanine scanning mutational analysis of VDR afforded important information of ligand recognition by the VDR and transactivation potency of the ligand. These were also related to hydrophobic interactions (Fig. 1).[7]

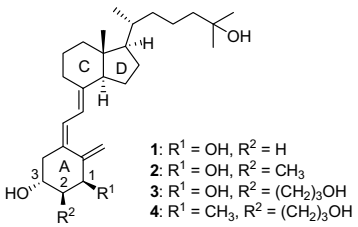

Figure 1. Vitamin D analogues.

* Corresponding author. Tel./fax: +81 42 685 3713.
E-mail address: akittaka@pharm.teikyo-u.ac.jp (A. Kittaka).
† Present address: Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences (NUPALS), Niigata 956-8603, Japan.
‡ Present address: Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan.

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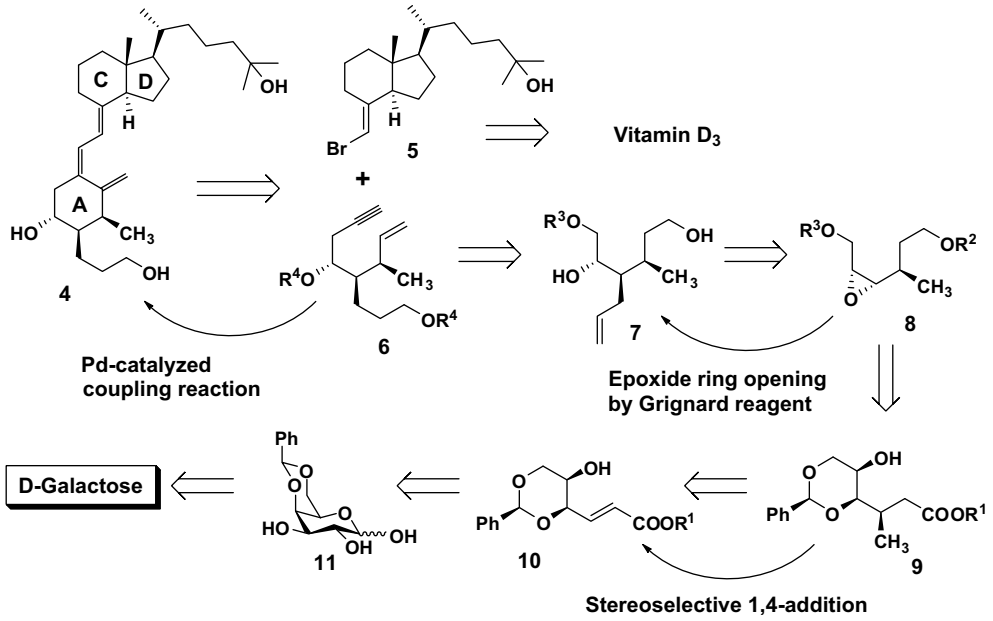


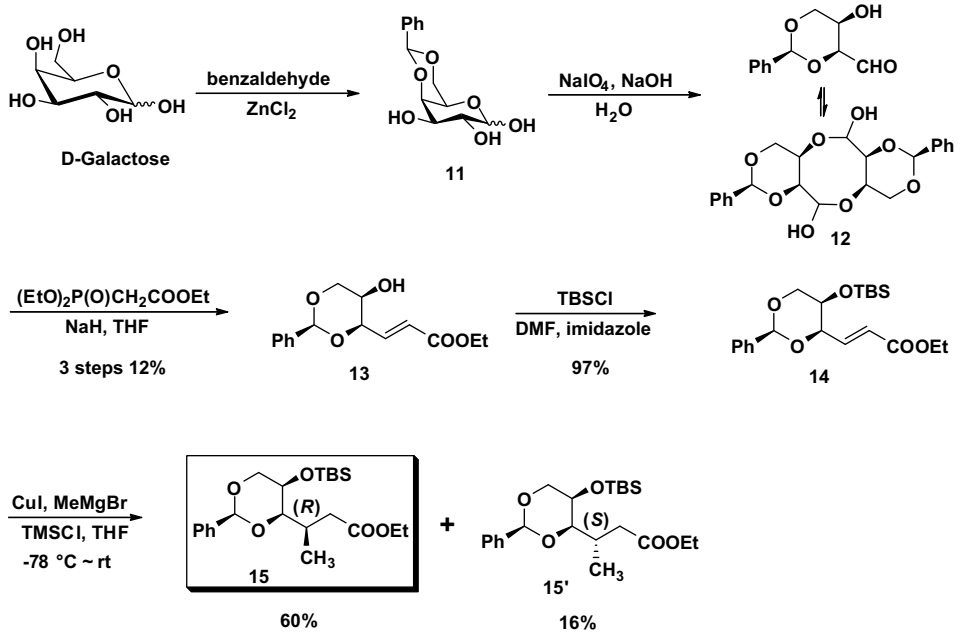
Scheme 1. Retrosynthetic analysis of the analogue.

Mutations of hydrophilic amino acid residues would diminish affinity, and have been found to cause hereditary vitamin D resistant rickets (HVDRR).[8] We focused on the mutation of Arg-274, which forms a hydrogen bond with the 1α-OH group of **1**. The replacement of Arg-274 with Leu diminished affinity to ca. 1/1000.[9]

We anticipated that the loss of affinity would be recovered through hydrophobic interactions between the hydrophobic pocket formed by the mutation and the hydrophobic substituent of the modified ligand. Such analogues, which possess a hydrophobic 1α-benzyloxy group, have been reported by Koh's group.[10] Other analogues have been reported by us,[11] which have the 1α-hydroxymethyl group reported by Posner's group to be hypocalcemic.[12] So we decided to design and synthesize the 1α-methylated analogue **4** as a candidate for an alternative ligand for the mutant VDR (Arg274Leu) (Fig. 1).

2. Results and discussion

The analogue **4** was synthesized by a convergent method (Scheme 1). A 2α-(3-hydroxypropyl) group was introduced to enhance affinity for the VDR.[13–16] 2α-(3-Hydroxypropyl) group is one of the active motifs that we have been developing, and we have already shown that the moiety itself recovers affinity for the mutant VDR (Arg274Ala).[17] The CD-ring moiety **5** was prepared from vitamin D_3 as reported,[18] and the A-ring enyne **6** was synthesized from D-galactose in order to introduce efficiently all of the chiral carbon centers in **6**. The key steps are (1) regioselective introduction of the allyl group that corresponds to the 2α-(3-hydroxypropyl) group for step **8** to **7**, (2) stereoselective 1,4-addition using an organocopper reagent in order to introduce the 1α-methyl group for step **10** to **9**.[19] Coupling of the CD-ring **5** and the A-ring enyne **6** was carried out with a palladium-catalyzed Trost coupling reaction.[18]

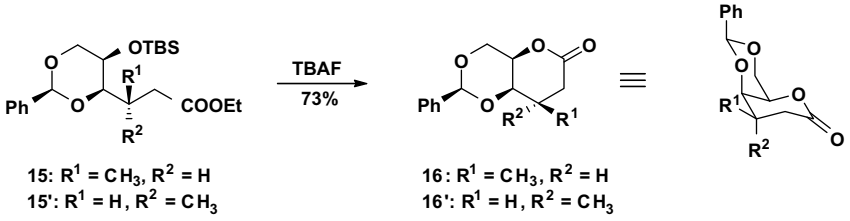


Scheme 2. Synthesis of A-ring enyne [part 1].

The A-ring enyne **6** was synthesized as follows (Scheme 2); the aldehyde **12**[20]was subjected to Horner–Wadsworth–Emmons olefination to give an α,β-unsaturated ester, **13**, whose secondary hydroxy group was protected by a TBS group. A methyl group was introduced into the α,β-unsaturated ester **14** with good stereoselectivity to give the (*R*)-configured ester **15** as the major isomer. The configuration of the methyl group of **15** was confirmed by NMR experiments (COSY, differential NOE) with the lactones **16** and **16′** (Scheme 3 and Fig. 2). The stereoselectivity could be explained by a 'modified' Felkin–Ahn model (Fig. 3).[21]

The benzylidene acetal of **15** was deprotected by hydrogenolysis, followed by lactonization to give the 5-membered lactone **17**. The primary hydroxy group was protected as a trityl ether, **18**, and a 2-step reduction of lactone gave the diol **19**, whose primary hydroxy group was protected as a pivaloyl ester, **20** (Scheme 4).

The set up was now ready for the introduction of the allyl group. The secondary hydroxy group of **20** was mesylated, and treatment with TBAF in the presence of zeolite gave the epoxide **22** in good yield, which was treated with excess allyl Grignard reagent in THF



Scheme 3. Formation of lactones **16** and **16′**.

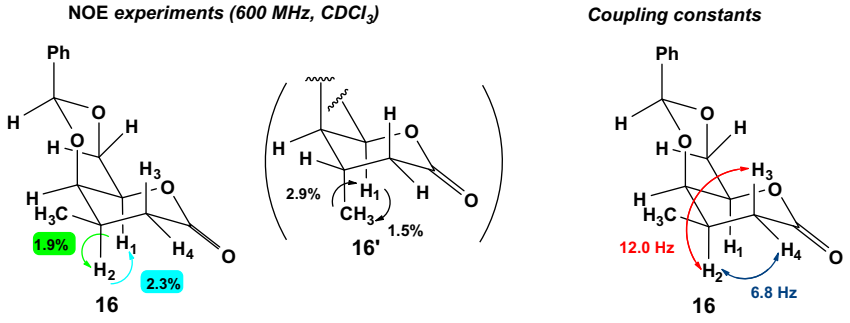


Figure 2. Determination of the configuration of the methyl group.

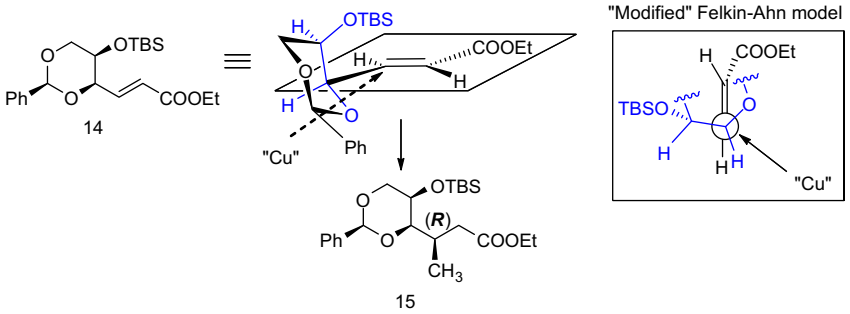
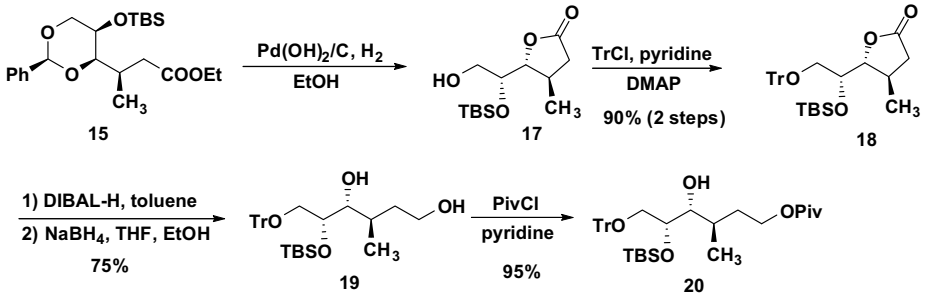
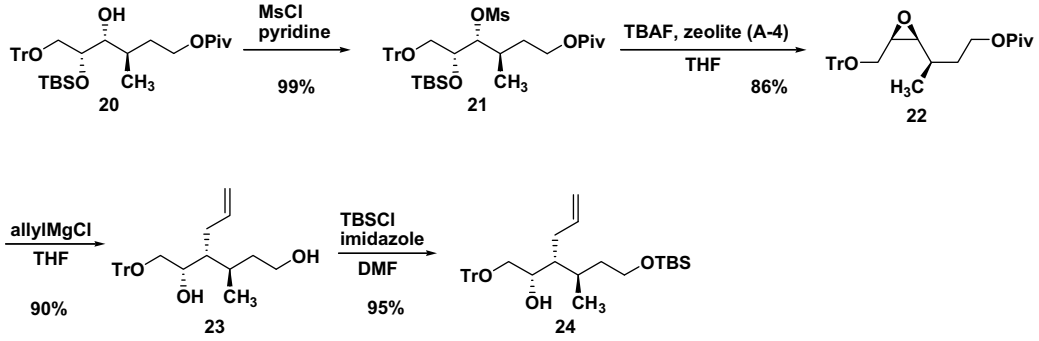


Figure 3. Modified Felkin–Ahn model to explain the diastereoselective methylation of **14**.



Scheme 4. Synthesis of A-ring enyne [part 2].

to give **23**. The allyl group was introduced regioselectively into a site far from the trityloxy group by this method.[22] The position of the allyl group could be assigned by 2D-NMR (COSY and HMBC). At the same time, the pivaloyl group was also deprotected during the allylation, so reprotection as a TBS ether needed to be carried out to afford **24** (Scheme 5).



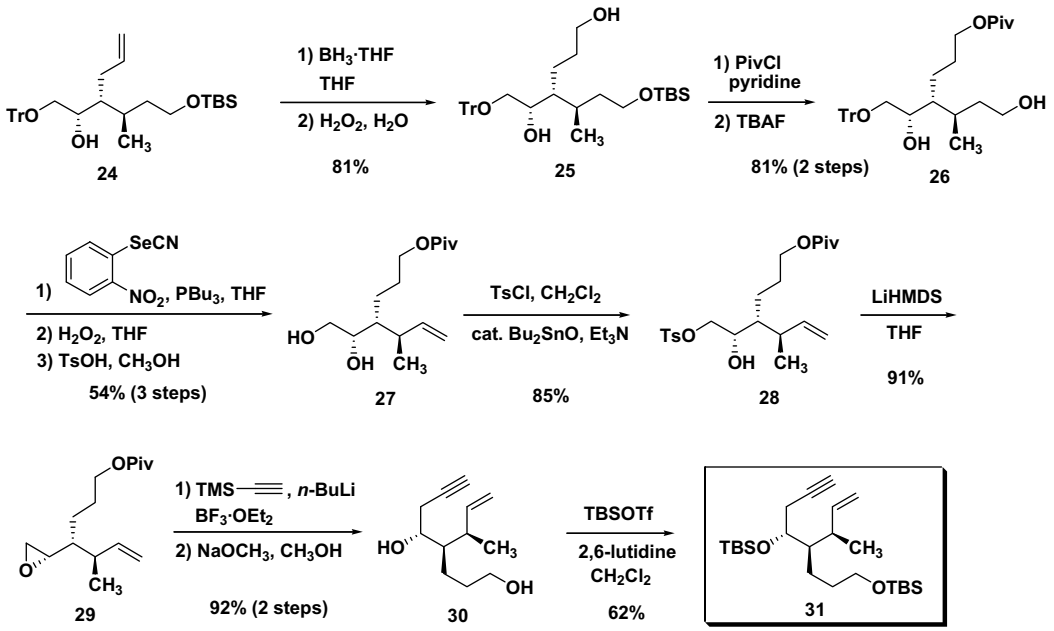
Scheme 5. Synthesis of A-ring enyne [part 3].

Transformation to the A-ring enyne could be carried out in a relatively conventional manner (Scheme 6). Hydroboration at the terminal olefin of **24** led to the diol **25**, whose primary hydroxy group was protected as a pivaloyl ester. The TBS group was deprotected, and then dehydration according to Greeco's procedure[23] afforded the terminal alkene, whose trityl group was removed by TsOH to give **27**. The primary hydroxy group could be selectively esterified as the tosylate **28**,[24] and treatment with base gave the epoxide **29**. Alkynylation[25] followed by persilylation gave the target enyne **31**.

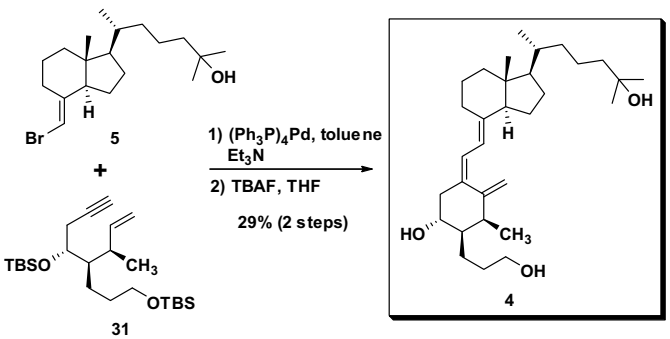
Trost coupling between **5** and **31** followed by deprotection afforded the target molecule **4** in 29% yield. This compound could be purified by reverse phase HPLC for biological assays (Scheme 7).

The analogues were assayed for the wild-type human VDR (hVDR) and the mutant hVDR(Arg274Leu). Assays were carried out with luciferase as reported previously.[11] The analogue **32**[11] without substitution at the 1-position was also assayed as a reference. For the wild-type hVDR, the analogue synthesized this time showed only 6% affinity as compared with 1α,25(OH)$_2$D$_3$ (**1**) (Table 1). This result is reasonable because the 1α-methyl group of **4** is not able to form a hydrogen bond with the wild-type receptor.

Next, an assay was carried out using the mutant hVDR (Arg274Leu) (Table 2). As shown previously, the 2α-hydroxypropyl group itself could recover the loss of affinity for the mutant (the

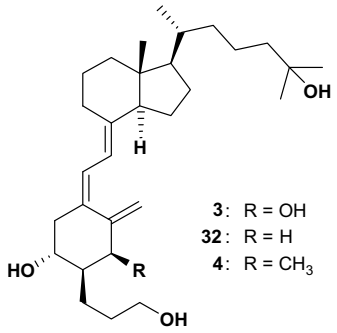


Scheme 6. Synthesis of A-ring enyne [part 4].



Scheme 7. Construction of the analogue **4**.

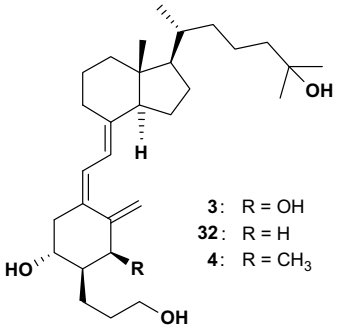
Table 1
Transcriptional activity for wild-type hVDR



Compounds	Relative luciferase activity
1α,25(OH)$_2$D$_3$ (**1**)	100
3	210
32	3
4	*6*

The potency of 1α,25(OH)$_2$D$_3$ is normalized to 100.

Table 2
Transcriptional activity for mutant hVDR (Arg274Leu)



Compounds	Relative luciferase activity
1α,25(OH)$_2$D$_3$ (**1**)	0.8[a] (100)[b]
3	3.9[a] (570)[b]
32	3.8[a] (560)[b]
4	*6.2*[a] *(730)*[b]

[a] The potency of 1α,25(OH)$_2$D$_3$ for wild type VDR is normalized to 100.
[b] The potency of 1α,25(OH)$_2$D$_3$ for mutant VDR (Arg274Leu) is normalized to 100.

analogue **32** is 5.6-fold more potent than the natural hormone **1**).[17] **4** was found to be more potent than **32**, and the most potent of the analogues tested this time (7.3-fold more potent than the natural hormone **1**). This is consistent with our expectation that the 1α-methyl group of **4** would fit the hydrophobic pocket in the LBP of the mutant VDR, thus enhancing affinity for the mutant VDR.

A hydrophobic interaction is one of the most important ones between proteins such as receptors and ligands and between enzymes and their substrates. We have examined 1α,25(OH)$_2$D$_3$ analogues which possess 2α-alkyl, 2α-alkoxy, 2α-(ω-hydroxyalkyl) or 2α-(ω-hydroxyalkoxy) groups in order to develop much more effective vitamin D agonists and to consider the effects of the A-ring conformation on biological activity.[13–16] In the ligand binding domain (LBD) of VDR, there is a hydrophobic cavity around the A-ring of 1α,25(OH)$_2$D$_3$ in a complex with the receptor and a water channel near the C-2 position of the ligand.[5,26] Among the analogues synthesized, the 2α-methyl analogue has been shown to have increased affinity for the VDR, and more potent HL-60 cell differentiation activity and transcriptional activity in reporter assays. Recently, an X-ray crystallographic analysis[26] showed the structure of the complex of the hVDR LBD with the 2α-methyl 1α,25(OH)$_2$D$_3$ analogue **2**.[27] From the results, the 2α-methyl group seemed to interact with Phe-150, Leu-233, and Ser-237 to make additional van der Waals contacts. These contacts should increase the affinity for the VDR up to 4-fold. The role of the 2α-(3-hydroxypropyl) group[14b,c] for compound **4** was also clarified in the same study. Interestingly, this moiety comes into the water channel, and the terminal OH group replaces one of the water molecules constituting the water channel to maintain hydrogen bond networks, thus stabilizing the complex up to 3-fold.

Similar effects appear to work for the complex of the mutant hVDR (Arg274Leu) with **4**. Molecular modeling demonstrated that around the 1α-methyl group there appears a cavity, more hydrophobic than that of the wild-type hVDR because of the mutation. The distance from the 1α-methyl group to the side chain of Leu-274 was calculated to be 3.2 Å, which is reasonable for a van der Waals contact (Fig. 4). The terminal hydroxyl group of the 2α-substitution of **4** would work as one of the water molecules which binds to the Asp-144 peptide backbone in the LBP.

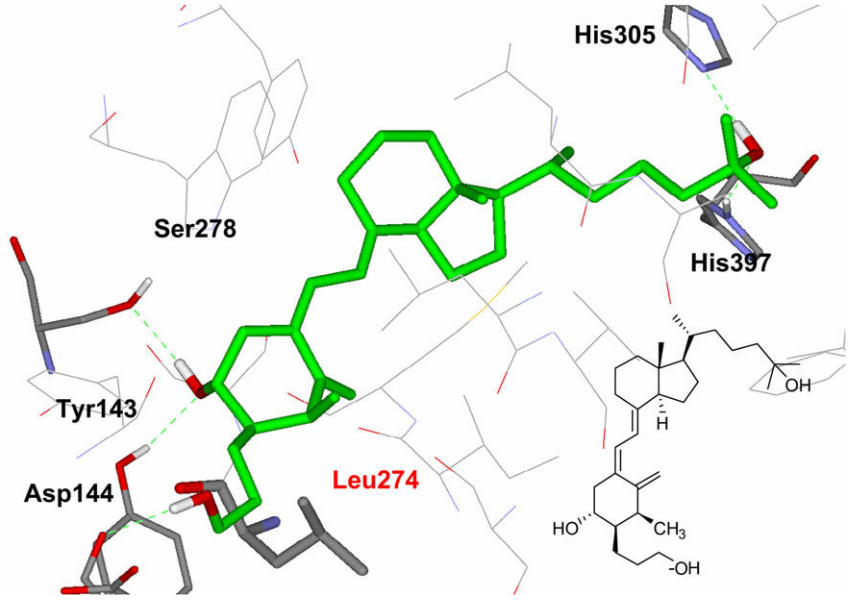


Figure 4. Molecular modeling of the complex of the analogue **4** and the mutant hVDR (Arg274Leu).

3. Conclusion

We have developed the agonist **4** for a mutant hVDR (Arg274Leu), in which the A-ring part was synthesized from D-galactose in a stereoselective manner. The analogue has a 1α-methyl group that is anticipated to interact with the hydrophobic pocket formed by the mutation. **4** is ca. 7.3-fold and 1.6-fold more active than the natural hormone **1** and 1α-hydroxy analogue **3**, respectively, and the latter enhancement might be caused by hydrophobic interactions.

4. Experimental section

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on JEOL AL-400 NMR (400 MHz) and ECP-600 NMR (600 MHz) spectrometers. ^{1}H NMR spectra were referenced with $(CH_3)_4Si$ (δ 0.00 ppm) as an internal stantard. ^{13}C NMR spectra were referenced with deuterated solvent (δ 77.0 ppm for $CDCl_3$). IR spectra were recorded on JASCO FT-IR-800 Fourier Transform Infrared Spectrophotometer. Low- and high resolution mass spectra were recorded on a JEOL JMX-SX 102A spectrometer. FAB mass spectra were measured using *m*-nitrobenzyl alcohol matrix. Elemental analyses were conducted with a Perkin Elmer PE 2400II CHNS/O analyzer. Melting points were determined with a Yanagimoto micromelting point apparatus without correction. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Column chromatography was performed on silica gel 60 N (Kanto Chemical Co., Inc., 100–210 μm) or silica gel 60 (Merck, 0.040–0.063 mm). Preparative thin layer chromatography was performed on silica gel 60 F_{254} (Merck, 0.5 mm).

4.2. Ethyl (*E*)-3-[(2*S*,4*R*,5*R*)-5-hydroxy-2-phenyl-1,3-dioxan-4-yl]acrylate (13)

Under an Ar atmosphere, a mixture of D-galactose (10.0 g, 55.5 mmol), $ZnCl_2$ (3.8 g, 27.8 mmol) and benzaldehyde (8.4 mL, 83.3 mmol) was stirred at room temperature overnight. Benzaldehyde (8.4 mL, 83.3 mmol) was added further, and stirring was continued at the same temperature overnight. The reaction was quenched by adding ice-cold water (200 mL). Layers were separated, and the organic layer was extracted with water (500 mL). Combined aqueous layers were basified by 10% Na_2CO_3, and precipitate was filtered off through Celite. The filtrate was washed with hexane (700 mL), and concentrated under reduced pressure to a volume of 1/10. The resulting benzylidene acetal solution was basified (pH>10) by 1 M aqueous NaOH, and the solution was cooled with an ice bath. $NaIO_4$ (47.5 g, 0.22 mol) was added, and the mixture was stirred at room temperature for 6 h. Water (300 mL) and NaCl were added, and precipitates were removed by filtration through Celite. The filtrate was extracted with AcOEt (2.5 L), and the organic layer was washed with brine (200 mL) and dried (Na_2SO_4). The solvent was removed in vacuo to give pale yellow oil of **12**.

Under an Ar atmosphere, to a suspension of NaH (60% in oil, 823 mg, 34.3 mmol) in THF (50 mL) was added ethyl diethylphosphonoacetate (6.8 mL, 34.3 mmol), and stirred at 0 °C for 15 min. A solution of crude aldehyde **12** above prepared in THF (30 mL) was added via cannula, and further stirred at the same temperature for 10 min. The reaction was quenched by adding saturated aqueous NH_4Cl solution (50 mL), and the mixture was extracted with AcOEt (400 mL). The organic layer was washed with brine (30 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (2:1)) gave the ester **13** (2.3 g, 16% for 3 steps) as a white solid. Analytically pure **13** was obtained by recrystallization from EtOH.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (3H, t, *J*=7.2 Hz), 2.64 (1H, d, *J*=11.2 Hz), 3.70 (1H, dddd, *J*=1.2, 1.6, 1.6, 11.2 Hz), 4.15 (1H, dd, *J*=1.2, 12.0 Hz), 4.21 (2H, q, *J*=7.2 Hz), 4.28 (1H, dd, *J*=1.6, 12.0 Hz), 4.67 (1H, ddd, *J*=1.6, 1.6, 4.0 Hz), 5.67 (1H, s), 6.20 (1H, dd, *J*=1.6, 15.6 Hz), 6.96 (1H, dd, *J*=4.0, 15.6 Hz), 7.39–7.41 (3H, m), 7.52–7.54 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 60.9, 65.8, 72.7, 78.9, 101.6, 123.2, 126.2, 128.6, 129.5, 137.5, 143.2, 166.1. IR (KBr): 698, 750, 808, 1028, 1150, 1186, 1244, 1302, 1402, 1659, 1699, 2975, 3507 cm^{-1}. MS *m*/*z*: 278 (M^+), 172. HRMS calcd for [$C_{15}H_{18}O_5$] 278.1155, found 278.1151. $[\alpha]_D^{19}$ −46.7 (*c* 1.12, $CHCl_3$). Mp 107.0–108.0 °C (EtOH). Anal. Calcd for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52. Found: C, 64.69; H, 6.66.

4.3. Ethyl (*E*)-3-[(2*S*,4*R*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-phenyl-1,3-dioxan-4-yl]acrylate (14)

Under an Ar atmosphere, to an ice cold solution of **13** (5.7 g, 20.5 mmol) in DMF (50 mL) was added imidazole (2.8 g, 41.0 mmol) and TBSCl (4.6 g, 30.8 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched by adding saturated aqueous $NaHCO_3$ (50 mL), and the mixture was extracted with Et_2O (500 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (15:1 to 9:1)) gave the TBS ether **14** (7.8 g, 97%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (3H, s), 0.08 (3H, s), 0.91 (9H, s), 1.27 (3H, t, *J*=7.2 Hz), 3.70 (1H, ddd, *J*=1.6, 1.6, 1.6 Hz), 4.07 (1H, dd, *J*=1.6, 12.0 Hz), 4.15 (1H, dd, *J*=1.6, 12.0 Hz), 4.20 (2H, q, *J*=7.2 Hz), 4.56 (1H, ddd, *J*=1.6, 1.6, 4.0 Hz), 5.60 (1H, s), 6.13 (1H, dd, *J*=1.6, 15.6 Hz), 6.90 (1H, dd, *J*=4.0, 15.6 Hz), 7.35–7.40 (3H, m), 7.52–7.54 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.4, 14.3, 18.2, 25.8, 60.3, 66.1, 72.3, 78.8, 101.1, 122.4, 126.3, 128.2, 129.0, 138.0, 144.3, 166.0. IR (neat): 777, 1026, 1097, 1163, 1267, 1364, 1400, 1460, 1666, 1720, 2859, 2932 cm^{-1}. MS *m*/*z*: 392 (M^+), 335, 305, 259. HRMS calcd for [$C_{21}H_{32}O_5Si$] 392.2025, found 392.2022. $[\alpha]_D^{19}$ −35.8 (*c* 1.17, $CHCl_3$).

4.4. Ethyl (*R*)-3-[(2*S*,4*R*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-phenyl-1,3-dioxan-4-yl]butylate (15) and ethyl (*S*)-3-[(2*S*,4*R*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-phenyl-1,3-dioxan-4-yl]butylate (15′)

Under an Ar atmosphere, a suspension of CuI (97 mg, 0.50 mmol) in THF (2 mL) was stirred at −78 °C, and CH_3MgBr (0.93 M in THF, 3.2 mL, 2.98 mmol) was added. After stirred at −40 °C for 30 min, the mixture was cooled to −78 °C, and $(CH_3)_3SiCl$ (65 μL, 0.50 mmol) was added, followed by a solution of **14** (100 mg, 0.25 mmol) in THF (4 mL). Stirring was continued at the same temperature for 1.5 h, and the temperature was gradually raised up to room temperature. The reaction was quenched by adding saturated aqueous NH_4Cl solution buffered to pH 9 (5 mL), and the mixture was extracted with Et_2O (40 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$), and concentrated. Purification by preparative TLC (hexane/AcOEt (20:1)) gave **15** (61 mg, 60%) and **15′** (17 mg, 16%) as colorless syrupy oils.

*4.4.1. Compound **15***

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11 (3H, s), 0.14 (3H, s), 0.95 (9H, s), 0.97 (3H, d, *J*=6.8 Hz), 1.16 (3H, t, *J*=7.2 Hz), 2.19 (1H, dd, *J*=8.0, 15.2 Hz), 2.46–2.56 (1H, m), 2.65 (1H, dd, *J*=4.8, 15.2 Hz), 3.50 (1H, dd, *J*=0.8, 9.6 Hz), 3.71 (1H, ddd, *J*=0.8, 1.2, 1.6 Hz), 3.95 (1H, dd, *J*=1.2, 12.0 Hz), 4.00 (2H, q, *J*=7.2 Hz), 4.19 (1H, dd, *J*=1.6, 12.0 Hz), 5.47 (1H, s), 7.30–7.37 (3H, m), 7.47–7.49 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.5, −3.8, 14.2, 15.2, 18.3, 25.9, 30.8, 37.7, 59.9,

64.4, 72.3, 83.4, 101.1, 126.3, 128.0, 128.7, 138.6, 173.1. IR (neat): 775, 1026, 1074, 1252, 1304, 1364, 1460, 1734, 2857, 2932, 2957 cm^{-1}. MS *m*/*z*: 408 (M^+). HRMS calcd for [$C_{22}H_{36}O_5Si$] 408.2332, found 408.2323. $[\alpha]_D^{19}$ +4.1 (*c* 1.02, $CHCl_3$).

*4.4.2. Compound **15′***

^1H NMR (400 MHz, $CDCl_3$) δ 0.12 (3H, s), 0.15 (3H, s), 0.95 (9H, s), 1.07 (3H, d, *J*=6.8 Hz), 1.26 (3H, t, *J*=6.8 Hz), 2.17 (1H, dd, *J*=8.6, 14.2 Hz), 2.42–2.46 (1H, m), 2.49 (1H, dd, *J*=4.2, 14.2 Hz), 3.57 (1H, dd, *J*=1.2, 8.6 Hz), 4.13 (1H, ddd, *J*=1.2, 1.2, 1.5 Hz), 3.96 (1H, dd, *J*=1.2, 12.5 Hz), 4.20 (2H, q, *J*=6.8 Hz), 4.20 (1H, dd, *J*=1.5, 12.5 Hz), 5.51 (1H, s), 7.33–7.38 (3H, m), 7.49–7.51 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.4, −3.7, 14.3, 15.7, 18.3, 25.9, 31.3, 36.9, 60.3, 64.8, 72.2, 83.0, 101.5, 126.2, 128.1, 128.7, 138.7, 172.5. IR (neat): 775, 835, 1028, 1101, 1179, 1254, 1364, 1462, 1734, 2930 cm^{-1}. MS *m*/*z*: 408 (M^+). HRMS calcd for [$C_{22}H_{36}O_5Si$] 408.2332, found 408.2342. $[\alpha]_D^{21}$ −2.9 (*c* 0.62, $CHCl_3$).

4.5. (2*R*,4*R*,8*R*,9*R*)-8-Methyl-2-phenyltetrahydropyrano[3,2-*d*]-1,3-dioxin-6-one (16)

Under an Ar atmosphere, to a cold (0 °C) solution of **15** (290 mg, 0.71 mmol) in THF (5 mL) was added TBAF (1 M in THF, 1.1 mL, 1.1 mmol), and the mixture was stirred at room temperature for 21 h. The mixture was diluted with Et_2O (20 mL), and washed with brine (5 mL). The organic layer was dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (3:2)) gave the lactone **16** (129 mg, 73%) as a colorless syrupy oil.

^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (3H, d, *J*=6.8 Hz), 2.22 (1H, m), 2.52 (1H, dd, *J*=6.8, 18.0 Hz), 2.58 (1H, dd, *J*=12.0, 18.0 Hz), 4.06 (1H, dd, *J*=1.2, 1.2 Hz), 4.11 (1H, dd, *J*=1.2, 12.8 Hz), 4.22 (1H, ddd, *J*=1.2, 1.2, 1.2 Hz), 4.42 (1H, dd, *J*=1.2, 12.8 Hz), 5.61 (1H, s), 7.37–7.39 (3H, m), 7.46–7.48 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.3, 31.2, 32.7, 69.5, 72.8, 73.7, 100.6, 126.0, 128.2, 129.1, 137.5, 170.1. IR (neat): 702, 882, 1013, 1094, 1237, 1456, 1399, 1732, 2926 cm^{-1}. MS *m*/*z*: 248 (M^+), 190, 171, 142. HRMS calcd for [$C_{14}H_{16}O_4$] 248.1048, found 248.1052. $[\alpha]_D^{19}$ +4.4 (*c* 0.59, $CHCl_3$).

4.6. (2*R*,4*R*,8*S*,9*R*)-8-Methyl-2-phenyltetrahydropyrano[3,2-*d*]-1,3-dioxin-6-one (16′)

Under an Ar atmosphere, to a cold (0 °C) solution of **15′** (18 mg, 44 μmol) in THF (0.5 mL) was added TBAF (1 M in THF, 0.1 mL, 0.1 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with Et_2O (20 mL), and washed with water (5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), and concentrated. Purification by preparative TLC (hexane-AcOEt (1: 1)) gave the lactone **16′** (7 mg, 54%) as a colorless syrupy oil.

^1H NMR (600 MHz, $CDCl_3$) δ 1.15 (3H, d, *J*=7.2 Hz), 2.29 (1H, dd, *J*=3.6, 17.4 Hz), 2.35–2.41 (1H, m), 2.94 (1H, dd, *J*=6.0, 17.4 Hz), 4.01 (1H, dd, *J*=1.8, 3.6 Hz), 4.11 (1H, dd, *J*=1.8, 12.6 Hz), 4.28 (1H, ddd, *J*=1.8, 1.8, 1.8 Hz), 4.45 (1H, dd, *J*=1.8, 12.6 Hz), 5.60 (1H, s), 7.36–7.40 (3H, m), 7.47–7.49 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 30.8, 32.9, 69.4, 70.2, 74.4, 100.9, 126.2, 128.3, 129.3, 137.3, 170.2. IR (neat): 808, 932, 1030, 1229, 1366, 1450, 1715, 2928 cm^{-1}. MS *m*/*z*: 248 (M^+). HRMS calcd for [$C_{14}H_{16}O_4$] 248.1048, found 248.1050. $[\alpha]_D^{19}$ −9.3 (*c* 0.46, $CHCl_3$).

4.7. (4*R*,5*R*)-5-[(*R*)-1-(*tert*-Butyldimethylsilyloxy)-2-trityloxyethyl]-4-methyldihydrofuran-2-one (18)

A mixture of **15** (449 mg, 1.10 mmol), $Pd(OH)_2$ (20 wt % on charcoal, 154 mg, 0.22 mmol) in EtOH (5 mL) was stirred at room temperature under a H_2 atmosphere. After stirred for 1 h, the catalyst was removed by filtration, and the filtrate was concentrated to give the crude lactone **17** (301 mg) as colorless syrup. **17** was dissolved in pyridine (10 mL), and TrCl (460 mg, 1.65 mmol) and DMAP (134 mg, 1.10 mmol) were added. After stirred at 75 °C overnight, the mixture was cooled to room temperature, and diluted with water (30 mL). The mixture was extracted with Et_2O (170 mL), and the organic layer was washed with brine (20 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (15:1)) gave **18** (514 mg, 90% for 2 steps) as a white wax.

^1H NMR (400 MHz, $CDCl_3$) δ −0.18 (3H, s), −0.06 (3H, s), 0.78 (9H, s), 1.14 (3H, d, *J*=6.8 Hz), 2.10 (1H, dd, *J*=6.0, 17.6 Hz), 2.38–2.46 (1H, m), 2.70 (1H, dd, *J*=8.8, 17.6 Hz), 3.09 (1H, dd, *J*=4.8, 9.2 Hz), 3.40 (1H, dd, *J*=8.4, 9.2 Hz), 3.61 (1H, ddd, *J*=2.4, 4.8, 8.4 Hz), 4.41 (1H, dd, *J*=2.4, 5.2 Hz), 7.22–7.32 (10H, m), 7.42–7.45 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.4, 18.0, 19.6, 25.7, 31.0, 37.0, 64.0, 71.9, 86.5, 87.1, 127.1, 127.8, 128.5, 143.8, 176.8. IR (neat): 1076, 1175, 1213, 1255, 1451, 1491, 1781, 2930, 2955, 3061 cm^{-1}. MS *m*/*z*: 516 (M^+), 459 (M^+−*t*-Bu), 439 (M^+−C_6H_5). HRMS calcd for [$C_{32}H_{40}O_4Si$] 516.2683, found 516.2711. $[\alpha]_D^{23}$ −19.7 (*c* 1.11, $CHCl_3$).

4.8. (3*R*,4*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-3-methyl-6-trityloxyhexane-1,4-diol (19)

Under an Ar atmosphere, to a cold (−78 °C) solution of **18** (514 mg, 0.99 mmol) in toluene (10 mL) was added DIBAL-H (1 M in toluene, 3.0 mL, 3.0 mmol), and stirred at the same temperature for 30 min. The reaction was quenched by adding 10% aqueous Rochelle salt solution (20 mL) at −78 °C, and the mixture was extracted with AcOEt (180 mL). The organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. The residue was dissolved in THF (5 mL) and EtOH (5 mL), and cooled to 0 °C. $NaBH_4$ (112 mg, 2.97 mmol) was added, and the mixture was stirred at the same temperature for 30 min, and room temperature for 40 min. The mixture was cooled in an ice bath, and water was added. The mixture was extracted with AcOEt (170 mL), and the organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (5:1)) gave the diol **19** (363 mg, 75%) as a colorless syrupy oil.

^1H NMR (400 MHz, $CDCl_3$) δ −0.13 (3H, s), −0.02 (3H, s), 0.81 (9H, s), 0.91 (3H, d, *J*=6.4 Hz), 1.55–1.63 (1H, m), 1.65–1.73 (1H, m), 1.78–1.88 (1H, m), 2.11 (1H, br s), 2.37 (1H, br s), 3.04 (1H, dd, *J*=4.8, 9.6 Hz), 3.33 (1H, dd, *J*=7.2, 9.6 Hz), 3.51 (1H, dd, *J*=2.4, 8.4 Hz), 3.61 (1H, ddd, *J*=5.1, 7.8, 10.0 Hz), 3.74 (1H, ddd, *J*=5.6, 5.6, 10.0 Hz), 3.79 (1H, ddd, *J*=2.4, 4.8, 7.2 Hz), 7.21–7.32 (10H, m), 7.42–7.45 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.1, 17.4, 18.1, 25.8, 33.7, 36.9, 61.2, 64.7, 71.2, 75.7, 87.0, 127.0, 127.8, 128.6, 143.9. IR (neat): 706, 837, 1073, 1256, 1449, 2361, 2886, 2955, 3384 cm^{-1}. FABMS *m*/*z*: 543 $(M+Na)^+$. HRFABMS calcd for [$C_{32}H_{44}O_4SiNa$] 543.2906, found 543.2913. $[\alpha]_D^{22}$ −4.1 (*c* 0.96, $CHCl_3$).

4.9. (3*R*,4*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-4-hydroxy-3-methyl-6-trityloxyhexyl pivalate (20)

To a cold (0 °C) solution of **19** (344 mg, 0.66 mmol) in pyridine (7 mL) was added PivCl (97 μL, 0.79 mmol), and the mixture was stirred at room temperature for 3 h. The reaction was quenched by adding saturated aqueous NH_4Cl solution (10 mL) and extracted with AcOEt (100 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (20:1)) gave the ester **20** (378 mg, 95%) as a colorless syrupy oil.

^1H NMR (400 MHz, $CDCl_3$) δ −0.17 (3H, s), −0.05 (3H, s), 0.79 (9H, s), 0.90 (3H, d, *J*=6.8 Hz), 1.18 (9H, s), 1.42–1.52 (1H, m), 1.60–1.64 (1H, m), 2.14 (1H, d, *J*=9.2 Hz), 2.11–2.19 (1H, m), 3.03 (1H, dd, *J*=4.8, 9.2 Hz), 3.34 (1H, dd, *J*=8.8, 9.2 Hz), 3.46 (1H, apparent t,

J=8.8 Hz), 3.76 (1H, ddd, J=4.8, 8.8, 9.2 Hz), 4.07–4.20 (2H, m), 7.21–7.30 (10H, m), 7.32–7.44 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.1, 16.3, 18.0, 25.8, 27.3, 31.7, 33.0, 38.7, 63.2, 64.5, 70.8, 75.3, 87.0, 127.0, 127.7, 128.6, 143.9, 178.5. IR (neat): 775, 837, 1076, 1161, 1254, 1285, 1366, 1395, 1449, 1475, 1723, 2932, 2959, 3555 cm^{-1}. FABMS m/z: 627 (M+Na)$^+$. HRFABMS calcd for [$C_{37}H_{52}O_5SiNa$] 627.3482, found 627.3471. $[\alpha]_D^{22}$ +0.3 (c 1.02, $CHCl_3$).

4.10. (3*R*,4*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-4-methanesulfonyloxy-3-methyl-6-trityloxyhexyl pivalate (21)

Under an Ar atmosphere, to a cold (0 °C) solution of **20** (330 mg, 0.55 mmol) in pyridine (5 mL) was added MsCl (105 μL, 1.38 mmol), and the mixture was stirred at the same temperature for 4 h, and at room temperature for 50 min. The reaction was quenched by adding water (10 mL) and the mixture was extracted with AcOEt (100 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (9:1 to 5:1)) gave the mesylate **21** (372 mg, 99%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04 (3H, s), 0.02 (3H, s), 0.89 (9H, s), 0.97 (3H, d, J=6.8 Hz), 1.14 (9H, s), 1.37–1.46 (1H, m), 1.89–2.01 (2H, m), 2.87 (3H, s), 3.23 (1H, dd, J=6.0, 10.0 Hz), 3.29 (1H, dd, J=6.4, 10.0 Hz), 3.96 (1H, ddd, J=3.2, 6.0, 6.4 Hz), 3.99–4.10 (2H, m), 4.66 (1H, dd, J=3.2, 6.8 Hz), 7.22–7.30 (10H, m), 7.32–7.45 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.7, −4.2, 16.6, 18.1, 25.8, 27.2, 30.8, 31.2, 38.5, 38.6, 62.2, 64.4, 71.3, 86.1, 87.4, 127.1, 127.8, 128.7, 143.6, 178.3. IR (neat): 777, 837, 918, 1174, 1342, 1360, 1450, 1479, 1728, 2905, 2959 cm^{-1}. FABMS m/z:706 (M+Na)$^+$. HRFABMS calcd for [$C_{38}H_{54}O_7SiSNa$] 705.3258, found 705.3239. $[\alpha]_D^{22}$ +11.0 (c 0.70, $CHCl_3$).

4.11. (*R*)-3-[(1*S*,2*R*)-2-(Trityloxymethyl)oxiranyl]butyl pivalate (22)

Under an Ar atmosphere, to a mixture of zeolite A-4 (pre-dried in vacuo at 130 °C for 2 h, 5 mg), TBAF (1 M in THF, 0.1 mL, 0.1 mmol), in THF (0.5 mL) was added at 0 °C a solution of **21** (37 mg, 54 μmol) in THF (0.6 mL) via cannula, and the mixture was stirred at the same temperature for 30 min, and at room temperature for 2.5 h. The mixture was diluted with Et_2O (10 mL) and filtered through Celite. The filtrate was diluted with water (5 mL), and the mixture was extracted with Et_2O (50 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1 to 10:1)) gave the epoxide **22** (22 mg, 86%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, d, J=6.4 Hz), 1.10 (9H, s), 1.46–1.54 (1H, m), 1.60–1.70 (2H, m), 2.70 (1H, dd, J=4.0, 9.6 Hz), 3.12 (1H, dd, J=4.8, 9.6 Hz), 3.20 (1H, ddd, J=4.0, 4.8, 10.0 Hz), 3.38 (1H, dd, J=6.0, 10.0 Hz), 3.39 (2H, t, J=6.8 Hz), 7.21–7.32 (10H, m), 7.44–7.47 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.5, 27.2, 29.8, 32.3, 38.6, 56.1, 61.4, 62.0, 62.1, 87.0, 127.0, 127.8, 128.5, 143.6, 178.1. IR (neat): 756, 767, 1071, 1156, 1283, 1451, 1728, 2964 cm^{-1}. MS m/z: 472 (M$^+$), 395 (M$^+$−C_6H_5). HRMS calcd for [$C_{31}H_{36}O_4$] 472.2610, found 472.2626. $[\alpha]_D^{24}$ −8.0 (c 0.92, $CHCl_3$).

4.12. (3*R*,4*R*,5*S*)-4-Allyl-3-methyl-6-trityloxyhexane-1,5-diol (23)

Under an Ar atmosphere, to a cold (0 °C) solution of the epoxide **22** (184 mg, 0.39 mmol) in THF (4 mL) was added dropwise allyl magnesium chloride (2 M in THF, 1.0 mL, 2.0 mmol), and the mixture was stirred at the same temperature for 15 min, and at room temperature for 1 h. The reaction was quenched by adding saturated aqueous NH_4Cl solution (5 mL), and the mixture was extracted with Et_2O (40 mL). The organic layer was washed with brine (5 mL), dried (Na_2SO_4) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (5:1)) gave **23** (150 mg, 90%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, d, J=6.8 Hz), 1.24–1.37 (1H, m), 1.40–1.44 (1H, m), 1.45–1.56 (1H, m), 1.64–1.69 (1H, m), 2.03–2.18 (2H, m), 3.14 (1H, dd, J=4.0, 9.2 Hz), 3.17 (1H, dd, J=8.0, 9.2 Hz), 3.49 (1H, ddd, J=5.6, 7.6, 10.4 Hz), 3.60 (1H, ddd, J=5.6, 7.6, 10.4 Hz), 3.91 (1H, ddd, J=4.0, 4.0, 8.0 Hz), 4.89 (1H, dd, J=1.6, 10.0 Hz), 4.93 (1H, dd, J=1.6, 17.2 Hz), 5.72 (1H, ddt, J=7.2, 10.0, 17.2 Hz), 7.23–7.33 (10H, m), 7.42–7.44 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 18.1, 30.6, 37.2, 44.8, 61.3, 66.7, 71.3, 86.8, 115.4, 127.0, 127.8, 128.6, 138.8, 143.8. IR (neat): 908, 1069, 1248, 1373, 1449, 1493, 2878, 2932, 3427 cm^{-1}. MS m/z: 430 (M$^+$). HRMS calcd for [$C_{29}H_{34}O_3$] 430.2508, found 430.2488. $[\alpha]_D^{24}$ +5.4 (c 0.95, $CHCl_3$).

4.13. (2*S*,3*R*)-3-[(*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-methylpropyl]-1-trityloxyhex-5-en-2-ol (24)

Under an Ar atmosphere, to a cold (0 °C) solution of diol **23** (688 mg, 1.60 mmol) in DMF were added imidazole (327 mg, 4.80 mmol) and TBSCl (289 mg, 1.92 mmol), and stirred at room temperature for 1 h. The reaction mixture was cooled in an ice bath, and saturated aqueous $NaHCO_3$ solution (10 mL) was added. The mixture was extracted with Et_2O (150 mL), and the organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave the TBS ether **24** (832 mg, 95%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ −0.05 (6H, s), 0.81 (9H, s), 0.84 (3H, d, J=6.8 Hz), 1.24–1.28 (1H, m), 1.32–1.37 (1H, m), 1.39–1.47 (1H, m), 1.57–1.64 (1H, m), 1.99 (1H, ddd, J=7.2, 7.2, 14.4 Hz), 2.08 (1H, ddd, J=7.2, 7.2, 14.4 Hz), 2.16 (1H, br s), 3.05 (1H, dd, J=4.0, 9.2 Hz), 3.12 (1H, dd, J=8.4, 9.2 Hz), 3.42 (1H, dt, J=7.2, 10.0 Hz), 3.53 (1H, dt, J=7.2, 10.0 Hz), 3.85 (1H, ddd, J=4.0, 4.0, 8.4 Hz), 4.82 (1H, d, J=10.0 Hz), 4.86 (1H, d, J=17.2 Hz), 5.64 (1H, ddt, J=7.2, 10.0, 17.2 Hz), 7.16–7.27 (10H, m), 7.37–7.39 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −5.2, 16.8, 18.4, 26.0, 30.6, 31.2, 37.5, 44.8, 61.7, 66.8, 71.0, 86.8, 115.3, 127.0, 127.8, 128.6, 138.9, 143.8. IR (neat): 706, 775, 835, 1091, 1254, 1448, 1491, 1597, 1640, 2930, 3061, 3505 cm^{-1}. MS m/z: 487 (M$^+$−t-Bu). HRMS calcd for [$C_{31}H_{39}O_3Si$] [(M−t-Bu)$^+$] 487.2668, found 487.2693. $[\alpha]_D^{25}$ +7.2 (c 1.40, $CHCl_3$).

4.14. (4*R*,5*S*)-4-[(*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-methylpropyl]-6-trityloxyhexane-1,5-diol (25)

Under an Ar atmosphere, to a cold (0 °C) solution of the terminal alkene **24** (458 mg, 0.84 mmol) in THF (5 mL) was added BH_3·THF complex (1 M in THF, 1.7 mL, 1.7 mmol), and the mixture was stirred at room temperature for 1.25 h. The reaction mixture was cooled in an ice bath, and to this solution were added successively water (1 mL), 15% aqueous NaOH (1 mL) and 30% aqueous H_2O_2 (1 mL). The mixture was stirred at room temperature for 1 h. After cooled in an ice bath, 10% aqueous $Na_2S_2O_3$ solution (20 mL) was added, and the mixture was extracted with AcOEt (60 mL). The organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (5:1)) gave the diol **25** (382 mg, 81%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (6H, s), 0.86 (9H, s), 0.87 (3H, d, J=6.8 Hz), 1.21–1.29 (1H, m), 1.31–1.47 (3H, m), 1.53–1.64 (4H, m), 3.13 (1H, dd, J=8.4, 9.2 Hz), 3.18 (1H, dd, J=4.4, 9.2 Hz), 3.45 (1H, dt, J=6.8, 10.0 Hz), 3.55 (2H, t, J=6.0 Hz), 3.57 (1H, dt, J=6.2, 10.0 Hz), 3.81 (1H, ddd, J=4.4, 4.4, 8.4 Hz), 7.22–7.32 (10H, m), 7.41–7.44 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, −5.2, 17.0, 18.3, 22.5, 26.0, 30.6, 32.3, 36.7, 44.7, 61.6, 62.8, 66.8, 71.5, 86.9, 127.1, 127.8, 128.5, 143.8. IR (neat): 706, 733, 775, 835, 907, 1094, 1254, 1377, 1449, 2858, 2930, 3389 cm^{-1}. FABMS m/z: 585 (M+Na)$^+$. HRFABMS calcd for [$C_{35}H_{50}O_4SiNa$] 585.3376, found 585.3375. $[\alpha]_D^{23}$ +2.2 (c 0.99, $CHCl_3$).

4.15. (4*R*,5*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-[(*S*)-1-hydroxy-2-(trityloxy)ethyl]-5-methylheptyl pivalate

Under an Ar atmosphere, to a cold (0 °C) solution of diol **25** (653 mg, 1.16 mmol) in pyridine (10 mL) was added PivCl (0.16 mL, 1.39 mmol), and stirred at the same temperature for 1.25 h, and then at room temperature for 1.5 h. The reaction was quenched by adding water (20 mL), and the mixture was extracted with AcOEt (150 mL). The organic layer was washed with brine (10 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave the ester (734 mg, 98%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (6H, s), 0.86 (9H, s), 0.88 (3H, d, J=7.2 Hz), 1.15 (9H, s), 1.29–1.63 (8H, m), 2.25 (1H, br s), 3.09 (1H, dd, J=4.0, 9.2 Hz), 3.15 (1H, dd, J=8.0, 9.2 Hz), 3.47 (1H, dt, J=6.8, 10.0 Hz), 3.57 (1H, dt, J=6.4, 10.0 Hz), 3.84 (1H, ddd, J=4.0, 4.0, 8.0 Hz), 3.92 (1H, dt, J=6.4, 10.8 Hz), 3.96 (1H, dt, J=6.4, 10.8 Hz), 7.22–7.31 (10H, m), 7.41–7.43 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −5.2, 16.8, 18.3, 22.7, 26.0, 27.3, 28.2, 30.5, 37.2, 38.7, 44.6, 61.6, 64.6, 66.9, 71.1, 86.8, 127.1, 127.8, 128.5, 143.8, 178.4. IR (neat): 704, 774, 835, 1093, 1161, 1254, 1287, 1390, 1450, 1460, 1728, 2932, 2955, 3501 cm^{-1}. FABMS m/z: 669 $(M+Na)^+$. HRFABMS calcd for $[C_{40}H_{58}O_5SiNa]$ 669.3951, found 669.3940. $[\alpha]_D^{23}$ −0.4 (c 0.89, $CHCl_3$).

4.16. (4*R*,5*R*)-7-Hydroxy-4-[(*S*)-1-hydroxy-2-trityloxyethyl]-5-methylheptyl pivalate (26)

Under an Ar atmosphere, to a cold (0 °C) solution of the above pivalate (734 mg, 1.13 mmol) in THF (10 mL) was added TBAF (1 M in THF, 2.3 mL, 2.3 mmol), and stirred at room temperature for 1.5 h. The reaction mixture was partitioned between water (20 mL) and AcOEt (150 mL), and the organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (4:1)) gave the diol **26** (583 mg, 97%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, d, J=6.8 Hz), 1.16 (9H, s), 1.16–1.65 (8H, m), 3.14 (2H, d, J=6.0 Hz), 3.49 (1H, dt, J=7.2, 10.4 Hz), 3.62 (1H, dt, J=6.0, 10.4 Hz), 3.85 (1H, dt, J=4.0, 6.0 Hz), 3.93 (1H, dt, J=6.8, 10.8 Hz), 3.96 (1H, dt, J=6.8, 10.8 Hz), 7.22–7.32 (10H, m), 7.42–7.44 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0, 22.7, 27.3, 27.3, 28.2, 30.5, 36.9, 44.5, 61.3, 64.5, 66.8, 71.4, 86.9, 127.1, 127.8, 128.5, 143.7, 178.5. IR (neat): 704, 1076, 1154, 1254, 1289, 1480, 1724, 2878, 2955, 3456 cm^{-1}. FABMS m/z: 555 $(M+Na)^+$. HRFABMS calcd for $[C_{34}H_{44}O_5Na]$ 555.3086, found 555.3083. $[\alpha]_D^{24}$ −0.9 (c 0.99, $CHCl_3$).

4.17. (4*R*,5*R*)-4-[(*S*)-1,2-Dihydroxyethyl]-5-methylhept-6-enyl pivalate (27)

Under an Ar atmosphere, to a cold (0 °C) solution of **27** (242 mg, 0.45 mmol) and 2-nitrophenyl selenocyanate (204 mg, 0.90 mmol) in THF (4 mL) was added n-Bu_3P (0.22 mL, 0.90 mmol), and the mixture was stirred at the same temperature for 35 min, and then at room temperature for 35 min. 2-Nitrophenyl selenocyanate (51 mg, 0.23 mmol) and n-Bu_3P (0.05 mL, 0.23 mmol) were added further at 0 °C. Reaction temperature was raised gradually up to room temperature, and the mixture was stirred overnight. The reaction was quenched by adding water (10 mL), and the mixture was extracted with Et_2O (80 mL). The organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. The residue was dissolved in THF (4 mL), and the mixture was cooled in an ice bath. To the mixture was added 30% aqueous H_2O_2 (2.5 mL), and the mixture was stirred at the same temperature for 10 min, and then at room temperature for 2.3 h. The mixture was cooled in an ice bath, and 10% aqueous $Na_2S_2O_3$ solution (20 mL) was added. The mixture was extracted with Et_2O (80 mL), and the organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. The residue was dissolved in MeOH (4 mL), and cooled in an ice bath. $TsOH\cdot H_2O$ (270 mg, 1.35 mmol) was added, and the mixture was stirred at room temperature for 3.5 h. The reaction mixture was cooled in an ice bath, diluted with AcOEt (20 mL) and saturated aqueous $NaHCO_3$ solution (10 mL) was added. The mixture was extracted with AcOEt (80 mL), and the organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (toluene/AcOEt (1:0 to 4:1)) gave the terminal alkene **27** (67 mg, 54% for 3 steps) as a pale yellow syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, d, J=6.8 Hz), 1.93 (9H, s), 1.34–1.40 (1H, m), 1.42–1.53 (2H, m), 1.63–1.74 (2H, m), 1.90 (2H, br s), 2.34–2.42 (1H, m), 3.56 (1H, dd, J=8.0, 10.8 Hz), 3.60 (1H, dd, J=3.6, 10.8 Hz), 3.79 (1H, ddd, J=3.6, 3.6, 8.0 Hz), 4.04 (2H, t, J=6.4 Hz), 5.02 (1H, ddd, J=0.8, 1.6, 10.4 Hz), 5.04 (1H, ddd, J=1.6, 2.4, 18.0 Hz), 5.77 (1H, ddd, J=7.6, 10.4, 18.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.4, 22.8, 27.3, 28.5, 38.8, 39.2, 45.0, 64.5, 65.6, 73.6, 114.6, 142.3, 178.5. IR (neat): 912, 1034, 1055, 1287, 1368, 1399, 1462, 1640, 1728, 2934, 3428 cm^{-1}. MS m/z: 272 (M^+). HRMS calcd for $[C_{15}H_{28}O_4]$ 272.1948, found 272.2003. $[\alpha]_D^{24}$ +13.1 (c 1.28, $CHCl_3$).

4.18. (4*R*,5*R*)-4-[(*S*)-1-Hydroxy-2-(*p*-toluenesulfonyloxy)-ethyl]-5-methylhept-6-enyl pivalate (28)

Under an Ar atmosphere, to a cold (0 °C) solution of **27** (67 mg, 0.24 mmol) in CH_2Cl_2 (1 mL) were added n-Bu_2SnO (1 mg, 4.8 μmol), Et_3N (33 μL, 0.24 mmol) and TsCl (46 mg, 0.24 mmol), and stirred at room temperature for 2 h. TsCl (23 mg, 0.12 mmol) was added further, and the mixture was stirred at the same temperature for 2.3 h. The mixture was filtered through Celite, and the filtrate was concentrated. Purification by silica gel column chromatography (hexane/AcOEt (6:1)) gave the tosylate **28** (87 mg, 85%) as a pale yellow syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (3H, d, J=6.8 Hz), 1.18 (9H, s), 1.34–1.45 (3H, m), 1.57–1.68 (2H, m), 1.91 (1H, br s), 2.28–2.35 (1H, m), 2.46 (3H, s), 3.92 (1H, ddd, J=3.8, 3.8, 9.6 Hz), 3.96 (1H, dd, J=6.4, 10.4 Hz), 3.97–4.00 (2H, m), 4.03 (1H, dd, J=3.8, 10.4 Hz), 4.98 (1H, ddd, J=1.2, 1.2, 17.6 Hz), 4.99 (1H, ddd, J=1.2, 1.2, 10.0 Hz), 5.64–5.73 (1H, m), 7.36 (2H, d, J=8.0 Hz), 7.80 (2H, d, J=8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.3, 21.7, 22.7, 27.2, 28.2, 38.7, 38.8, 44.6, 64.3, 70.6, 73.1, 114.8, 127.9, 129.9, 132.6, 141.8, 145.0, 178.4. IR (neat): 814, 970, 1178, 1289, 1362, 1480, 1725, 2973, 3507 cm^{-1}. FABMS m/z: 449 $(M+Na)^+$. HRFABMS calcd for $[C_{22}H_{34}O_6SNa]$ 449.1962, found 449.1983. $[\alpha]_D^{26}$ +15.9 (c 0.96, $CHCl_3$).

4.19. (4*R*,5*R*)-5-Methyl-4-[(*S*)-oxiranyl]-hept-6-enyl pivalate (29)

Under an Ar atmosphere, to a cooled (−78 °C) solution of the tosylate **28** (85 mg, 0.20 mmol) in THF (2 mL) was added LiHMDS (1 M in THF, 0.24 mL, 0.24 mmol), and stirred at the same temperature for 40 min, and then at room temperature for 20 min. The reaction was cooled in an ice bath, and saturated aqueous NH_4Cl solution (5 mL) was added. The mixture was extracted with Et_2O (40 mL), and the organic layer was washed with brine (5 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (5:1)) gave the epoxide **29** (46 mg, 91%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, d, J=7.2 Hz), 1.20 (9H, s), 1.45–1.54 (1H, m), 1.58–1.64 (1H, m), 1.63–1.71 (1H, m), 1.68–1.76 (1H, m), 1.78–1.89 (1H, m), 2.30 (1H, m), 2.49 (1H, dd, J=2.8, 4.8 Hz), 2.72–2.78 (2H, m), 4.06 (2H, t, J=6.4 Hz), 5.00 (1H, ddd, J=0.8, 1.2, 10.4 Hz), 5.01 (1H, ddd, J=1.2, 2.0, 17.2 Hz), 5.73 (1H, ddd, J=8.0, 10.4, 17.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.2, 26.3, 26.8, 27.2, 38.6, 40.0, 46.1, 46.9, 54.8, 64.4, 114.2, 142.0, 178.4. IR (neat): 916, 1159, 1285, 1460, 1482, 1730, 2971 cm^{-1}. MS m/z: 254 (M^+). HRMS calcd for $[C_{15}H_{26}O_3]$ 254.1883, found 254.1881. $[\alpha]_D^{25}$ +10.4 (c 1.25, $CHCl_3$).

4.20. (4*R*,5*R*)-4-[(*S*)-1-Methylprop-2-enyl]-oct-7-yne-1,5-diol (30)

Under an Ar atmosphere, to a cooled (−78 °C) solution of ethynyltrimethylsilane (0.13 mL, 0.90 mmol) in THF (2 mL) was added *n*-BuLi (1.45 M in hexane, 1.86 mL, 2.70 mmol), and the mixture was stirred at the same temperature for 10 min. To this was added the epoxide **29** (7 mg, 0.30 mmol) in THF (3 mL) via cannula, and $BF_3{\cdot}OEt_2$ (0.11 mL, 0.90 mmol) was added. The mixture was stirred at the same temperature for 10 min, and then at room temperature for 3.3 h. The reaction was quenched by adding water (5 mL), and the mixture was extracted with Et_2O (50 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and concentrated. The residue was dissolved in MeOH (5 mL), and to this was added NaOMe (49 mg, 0.90 mmol). The mixture was stirred at 40 °C overnight, and cooled in an ice bath. Saturated aqueous NH_4Cl solution (5 mL) was added, and the mixture was extracted with AcOEt (50 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (3:1)) gave the diol **30** (54 mg, 92%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (3H, d, *J*=6.8 Hz), 1.40–1.73 (5H, m), 2.05 (1H, t, *J*=2.4 Hz), 2.35–2.38 (1H, m), 2.42 (2H, dd, *J*=2.4, 6.0 Hz), 3.64 (1H, ddd, *J*=6.8, 10.4, 16.8 Hz), 3.65 (1H, ddd, *J*=6.8, 10.4, 16.8 Hz), 3.80 (1H, dt, *J*=6.0, 11.2 Hz), 5.00 (1H, ddd, *J*=1.6, 1.6, 10.4 Hz), 5.03 (1H, ddd, *J*=1.6, 1.6, 17.6 Hz), 5.77 (1H, ddd, *J*=8.0, 10.4, 17.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 22.1, 25.8, 32.4, 39.3, 46.6, 62.7, 70.8, 71.6, 81.2, 114.6, 141.9. IR (neat): 916, 1036, 1059, 1418, 1451, 1640, 2932, 3308 cm^{-1}. MS *m*/*z*: 169 (M^+). HRMS calcd for [$C_{12}H_{20}O_2$] 196.1447, found 196.1467. $[\alpha]_D^{25}$ +15.0 (*c* 1.27, $CHCl_3$).

4.21. (3*R*,4*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-4-[3-(*tert*-butyldimethylsilyloxy)propyl]-3-methyloct-1-en-7-yne (31)

Under an Ar atmosphere, to a cold (−78 °C) solution of diol **30** (6 mg, 33 μmol) in CH_2Cl_2 (0.5 mL) were added 2,6-lutidine (31 μL, 264 μmol) and TBSOTf (30 μL, 132 μmol), and stirred at the same temperature for 50 min, and then at 0 °C for 45 min. The reaction was quenched by adding saturated aqueous $NaHCO_3$ solution (5 mL), and the mixture was extracted with CH_2Cl_2 (30 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel flash column chromatography (hexane/AcOEt (150:1)) gave the bisTBS ether **31** (9 mg, 62%) as a colorless syrupy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.07 (3H, s), 0.08 (3H, s), 0.89 (9H, s), 0.89 (9H, s), 1.02 (3H, d, *J*=6.8 Hz), 1.34–1.60 (5H, m), 1.96 (1H, t, *J*=2.4 Hz), 2.30–2.35 (1H, m), 2.37 (2H, dd, *J*=2.4, 6.4 Hz), 3.56 (2H, t, *J*=6.8 Hz), 3.95 (1H, dt, *J*=3.2, 6.4 Hz), 4.95 (1H, ddd, *J*=1.2, 1.2, 10.0 Hz), 4.99 (1H, ddd, *J*=1.2, 1.2, 17.2 Hz), 5.74 (1H, ddd, *J*=8.0, 10.0, 17.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −4.5, −3.9, 18.1, 18.4, 18.8, 23.1, 25.4, 25.9, 26.0, 32.4, 38.8, 47.0, 63.6, 69.9, 72.0, 82.1, 113.6, 143.3. IR (neat): 775, 835, 916, 1005, 1096, 1256, 1362, 1389, 1462, 2240, 2363, 2957 cm^{-1}. MS *m*/*z*: 424 (M^+). HRMS calcd for [$C_{24}H_{48}O_2Si_2$] 424.3181, found 424.3201. $[\alpha]_D^{24}$ −2.9 (*c* 0.91, $CHCl_3$).

4.22. 2α-(3-Hydroxypropyl)-25-hydroxy-1α-methylvitamin D_3 (4)

Under an Ar atmosphere, a mixture of A-ring enyne **31** (26 mg, 61 μmol), CD-ring bromoolefin **5**[18] (33 mg, 91 μmol), $Pd(PPh_3)_4$ (35 mg, 31 μmol) in Et_3N (2 mL) and toluene (2 mL) was stirred at room temperature for 10 min, and then at 80 °C for 1 h. After cooled to room temperature, the mixture was diluted with Et_2O (10 mL), and filtered through Celite. The filtrate was concentrated, and partially purified by silica gel column chromatography (hexane/AcOEt (10:1)). The residue was dissolved in THF (2 mL), and cooled in an ice bath. TBAF (1 M in THF, 0.3 mL, 0.3 mmol) was added, and the mixture was stirred at room temperature for 5 h. The mixture was diluted with Et_2O (10 mL) and water (5 mL), and extracted with Et_2O (30 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and concentrated. Purification by preparative TLC (hexane/AcOEt (1:1)) gave the target **4** (6 mg, 29% for 2 steps) as a white solid. This compound was further purified by preparative reverse phase HPLC. HPLC (YMC-Pack ODS column, 20×150 mm, 10 mL min^{-1}, acetonitrile/water=95:5). UV (EtOH) λ_{max} 264 nm, λ_{min} 228 nm. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (3H, s), 0.94 (3H, d, *J*=6.0 Hz), 0.94 (3H, d, *J*=7.2 Hz), 1.05–1.06 (1H, m), 1.22 (6H, s), 1.25–1.70 (19H, m), 1.84–1.91 (1H, m), 1.69–1.97 (1H, m), 1.99–2.01 (1H, m), 2.22 (1H, dd, *J*=7.8, 12.6 Hz), 2.61–2.64 (1H, m), 2.64 (1H, dd, *J*=3.6, 7.8 Hz), 2.81 (1H, dd, *J*=4.8, 12.6 Hz), 3.67 (2H, t, *J*=6.0 Hz), 3.77 (1H, dt, *J*=4.2, 7.8 Hz), 4.80 (1H, d, *J*=2.4 Hz), 5.00 (1H, d, *J*=1.8 Hz), 5.98 (1H, d, *J*=10.8 Hz), 6.27 (1H, d, *J*=10.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 14.3, 18.9, 20.9, 22.3, 23.6, 23.8, 27.7, 29.1, 29.3, 29.4, 30.6, 36.1, 36.4, 38.9, 40.6, 44.4, 44.6, 45.9, 47.9, 56.3, 56.5, 63.1, 70.7, 71.1, 110.9, 117.4, 122.8, 135.0 141.9, 149.4. IR (film): 605, 731, 909, 1038, 1377, 1455, 1472, 1632, 2934, 3401 cm^{-1}. MS *m*/*z*: 472 (M^+). HRMS calcd for [$C_{31}H_{52}O_3$] 472.3907, found 472.3925. $[\alpha]_D^{23}$ +44.1 (*c* 0.22, $CHCl_3$).

4.23. Reporter assays using luciferase as a reporter

Human breast cancer cell line MCF7 cells were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) in an atmosphere of 95% air and 5% CO_2. Cells were collected, suspended in the DMEM supplemented with 5% FBS (stripped with dextran-coated charcoal) and 1% P/S without phenol red, and plated in 24-well plate (2.5×10^4 cells/well). Cells were incubated in CO_2 incubator at 37 °C overnight. Ligand stock solutions were prepared at various concentrations in DMSO (10^{-7}–10^{-3} M). DMSO itself was used as vesicle. Plasmids used in our assays were as follows; receptor plasmids pM(GAL4-hVDR(DEF)) for wild type hVDR, and pM(GAL4-hVDR(R274L)(DEF)) for mutant hVDR, the latter prepared by site-directed mutagenesis using QuikChange II XL Site-Directed Mutagenesis Kits (Stratagene), reporter plasmid (17M2-G-Luc) and internal standard plasmid (pRL-CMV). Plasmids were diluted in OPTI-MEM medium at concentrations of 50 ng/well for receptor plasmid, 0.2 μg/well for reporter plasmid, and 2.5 ng/well for internal plasmid. Transfections were carried out by using TransFast reagent (Promega) according to the manufacture's instruction. After 3–6 h of transfection, ligand stock solutions were added at the final concentrations of 10^{-10}–10^{-6} M, and cells were further incubated overnight. Luciferase assays were performed by using Dual-Luciferase Reporter Assay System Kit (Promega). All experiments were carried out at least 3 times.

4.24. Molecular modeling

The mutant binding-site model was generated by changing the side chain of Arg274 to that of Leu from wild type VDR(1DB1), and the docking model of ligands bound to mutant VDR(Arg274Leu) was constructed by conformational search with *MacroModel* (ver. 8.1). Conformational search was performed by fixing receptor protein without ligands and Leu274 residue. AMBER* was used as force field.

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