would fill the space formed upon substitution of the Trp to Arg (Figure S4C, Supporting Information). Thus, we synthesized vitamin D compounds substituted with a butyl (**4a**), allyl (**4b**), or 3-hydroxypropyl (**4d**) group at the 9α-position (Scheme 3).

Grundman's ketone was converted to enol trimethylsilyl ether (**23**), which was treated in situ with MeLi followed by butyl iodide or allyl iodide to give the 9α-substituted ketones **24a** or **24b** (71% or 52%, respectively). The ketones (**24a** and **24b**) were treated with vinyl magnesium chloride (**25a** 88% and **25b** 79%, respectively), oxidized with PCC to give aldehydes **26a** (80%) and **26c** (89%), respectively, which were then reduced to give alcohols **26b** (86%) and **26d** (61%), respectively. Compound **26b** was converted to Wittig reagent **27** (77%), combined with A-ring fragment **28** (21%, 74% based on the recovery of the starting material **27**), and deprotected with CSA to give **4a** (96%). The low yield of the Wittig–Horner reaction was probably due to the steric inhibition of the 9α-butyl group. For the synthesis of **4b–e**, we employed the Julia-Kociensky reaction to couple the A-ring and CD-ring fragments. Allyl alcohol **26d** was treated with 2-mercaptobenzothiazole in the presence of triphenyl phosphine and DIAD and then oxidized with H_2O_2 to give **30** (97%). Compound **30** was treated with A-ring fragment **31** to give **32** (57%, 83%, on the basis of the recovered **30**) as a 5:3 mixture of *E*- and *Z*-isomers at C(2), which were deprotected and separated by HPLC to give **4b** (2*E*-isomer) and **4c** (2*Z*-isomer) (5:3, 90% total). To obtain 9-hydroxypropyl compounds, **32** was allowed to undergo hydroboration followed by oxidation and deprotection to give **4d** (17% in three steps, 2*E*-isomer) and **4e** (10% in three steps, 2*Z*-isomer).

Biological Evaluation of Synthetic Ligands. *Ligands for R274L.* Ligands **2a** and **2b** were less active than the natural hormone in the luciferase assay with hR274L, and ligands **2c–e** have almost no potency (Figure 5A). Molecular modeling predicted a space that would be generated in the LBP by the mutation of Arg270 to Leu (Figure S4A, Supporting Information). However, the actual space in the LBP of R270L crystal structure differed slightly from the predicted space (Figure S4B, Supporting Information). The R270L had a pocket (Figure S4B, Supporting Information, yellow) that was narrower than we expected on the basis of the model (Figure S4B, Supporting Information, green), and accordingly, ligand **2b** did not fit correctly in the LBP of R270L. This difference was likely due to a slight movement of helix H5, which is difficult to predict by modeling. Therefore, the 1α-substituents of ligands **2a–e** were unable to enter the LBP due to steric congestion.

The ligands (**3a–3d**), which were initially designed as super agonists,[16] showed excellent activity for R274L: **3a**, **3b**, **3c**, and **3d** were 15, 4, 130, and 46 times more potent than **1** for R274L in transactivation (Figure 5B). Interestingly, while 2β-hydroxyethoxy-D analogue (**3b**) was the most potent for the WT VDR,[16] the 2*E*-hydroxyethylidene analogue (**3c**) appeared to be the most potent for the R274L mutant. Ligand **3c** had the highest activity for R274L because its terminal hydroxyl group directly interacted with the main chain carbonyl group of Asp144 (Figure 2B). Thus, **3c** may be a good candidate for the treatment of HVDRR caused by the R274L mutation.

Ligand **3d** was less active than **3c** for R274L because it interacted with Asp144 via a water molecule (Figure 2D). These results indicate that direct interactions are much stronger than indirect interactions via water.

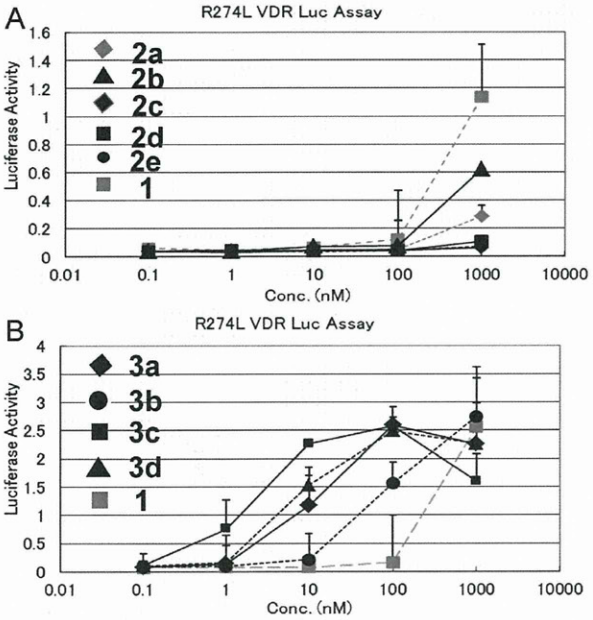

Figure 5. Transcriptional activities of **1** and synthetic ligands on R274L hVDR. The activities of **2a–e** and **1** (A) and **3a–d** and **1** (B) were evaluated by dual luciferase assay using a R274L full-length hVDR expression plasmid (pCMX-hVDR) and a luciferase reporter gene with a mouse osteopontin VDRE at the promoter (SPPx3-TK-Luc) in COS7 cells.

Ligands for W286R. Despite our design (Figures S3B and S4C, Supporting Information) and efforts, the 9-substitued compounds we synthesized had little to no potency on W286R hVDR (**4a** and **4c** to **4e**, data not shown; **4b**, see Figure S6, Supporting Information). The hydrophobic 9-substituent groups did not induce better folding of the disordered β-strands. The temperature factors shown on the ligand (Figure S3C and D, Supporting Information) suggest that shorter substituents would be better. Our findings also suggest that a negatively charged group introduced at the 9α-position may hold the Arg282 side chain inside the LBP, thereby maintaining the normal folding of the β-sheet part.

■ DISCUSSION

Structures of the R270L and W282R Mutant Proteins Would Be Trapped under Our Crystallization Conditions and Represent a Minor Population in Biological Solutions. Regardless of the mutants' low potency, the crystal structures of the ternary complexes of the mutant rVDRs adopted canonical active conformations. Because of the importance of the DRIP205 peptide in crystallization, it is clear that the peptide trapped the ligand-bound active conformation. Packing modes in the unit cell also supported crystallization of the active conformation (Figure S1, Supporting Information). In the most N-terminal part of the R270L complexed with the natural hormone (**1**), electron density of the residues of the expression tag sequence (Asn-Ser-Pro) was observed (Figure S1A and C, Supporting Information, red helix). These residues interact with Asp137 and His140 in the neighboring molecule in the crystal (Figure S1C, Supporting Information). In the crystal of W282R, the interactions of His130 with Glu304 and Glu307, and of Arg248 with Glu392 in the neighboring molecule were also

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observed (Figure S1E, Supporting Information). These interactions between neighboring molecules were not observed in WT rVDR-LBD.

In contrast, thermal transition experiments in the solution state using CD spectra indicated that R270L was present in a nearly ligand-free and W282R completely ligand-free states, whereas WT was completely in the ligand-bound state. From all of these results, we concluded that these mutants R270L and W282R were crystallized as the complexes with ligands in the active conformations probably because the coactivator peptide binds to the AF-2 surface thereby stabilizing the complex. It is also clear that these mutants are free of the ligands in biological solution, as supported by the CD spectral analysis and by their biological activities.[9,10]

Substitution Mutation R270L Causes a Significant Change in the Main Chain Structure between Helices H1 and H2. The substitution mutation led to a local conformational change in the protein structure. A small difference (rmsd 0.13−0.28) in the coordinates of R270L/1,25$(OH)_2D_3$ compared with those of WT/1,25$(OH)_2D_3$ would be important. Arg274 in hVDR is essential for its interaction with the 1α-OH group of the ligand.[22] However, Arg274 also has an important interaction with the main chain carbonyl group of Thr142. The loop 1−2 of agonist-bound VDR-LBDs derived from humans, rats, and zebra fish, shares common secondary structural characteristics:[11a,14,15d] Glu126 to Thr142 displays a typical α-helix (H1), Thr142 to Pro145 forms a β-strand-like structure, Pro145 and Thr146 form a hydrogen-bonded turn, and Ala148 to Asp152 displays a 3_{10}-helix (H2). Thr142 and Tyr143 are particularly notable because they are tethered to the natural hormone. The Tyr143 side chain interacts directly with the 3β-OH of the ligand, and the Thr142 main chain carbonyl group interacts with Arg270, which interacts with the 1α-OH of the ligand. In the R270L mutant, Thr142 and 1α-OH are connected by interactions via two water molecules. Thus, one of the two interactions is weakened by the mutation. We previously performed a mutational analysis of all of the LBP residues of the VDR and reported that the single mutation of Y143A causes a marked reduction in ligand-dependent transactivation.[22,23] These results suggested that the local area structures between H1 and H2 influence ligand-dependent VDR functions. We confirmed in the present studies that water-mediated interactions are weaker than direct interactions between ligands and residues.

In the present study, the 2-substituted-19-norvitamin D_3 analogues **3a**, **3b**, **3c**, and **3d** showed potent agonistic activity against the hVDR R274L mutant (Figure 5B). Of these four derivatives, **3c** was the most effective agonist against the R274L mutant, whereas **3b** was the most effective against the WT VDR. We, therefore, suggest that **3c** is the most promising candidate among these analogues for the treatment of HVDRR caused by the R274L mutation.

Why Does the W286R Mutant Never Respond to Vitamin D Ligands? We also obtained the crystal structures of the W282R mutant as the ligand-bound active conformation. These structures would also represent a minor population in a biological solution because previous studies have shown that W286R hVDR never binds to the natural hormone and never responds to vitamin D-dependent gene transactivation.[10] However, the structures of these complexes have a notable feature. Ten to seven residues around the mutated part of the protein complexed with either 1,25$(OH)_2D_3$ or ligand **3a** or **3b** were not visible. Every VDR-LBD, including those of rats, humans, and zebra fish, has a set of antiparallel β-sheets. From the center of the β-sheet, Trp282 (or Trp286 in hVDR) protrudes to the seco-B and C ring parts of the ligand, where they strongly interact (Figure S3A, Supporting Information). In general, proteins favor hydrophobic residues on the inside and hydrophilic residues on the outside. Thus, unlike Trp, the hydrophilic Arg282 would favor the outside rather than the inside. This disruption of the β-strand conformation could lead to a partial unfolding of this part of the complex. We conclude that correct folding at the β-strand part is important for the VDR action. It has been reported[24] in the crystal structural study of intact peroxisome proliferator activating receptor γ (PPARγ)-RXRα complex on DNA that the β-sheet parts of the PPARγ form hydrophobic interactions with RXRα DBD and that these interactions can contribute to DNA recognition and affinity. The β-sheet part of the VDR may have similar function. A CD spectral study showed the stabilities of the WT, and the mutant widened drastically when the ligand was present. The mutation spoils the stability gain of the VDR upon ligand binding.

We designed the agonists (**4a**, **4b**, and **4d**) for the W286R mutant by paying attention to the position (C-9) of modification in the ligand. In future studies, we plan to examine the electronic compatibility of the ligand with the Arg side chain. For example, it would be interesting to introduce a negatively charged group at the 9α-position of the ligand.

■ CONCLUSIONS

We solved the crystal structures of two rVDR-LBD mutants implicated in HVDRR, R270L, and W282R, complexed with natural and synthetic vitamin D ligands and the coactivator DRIP205 peptide. All of the crystal structures adopted canonical active conformations. However, these active conformations are assumed to be minor conformations in biological solutions because the responsiveness of these mutants to the natural hormone are severely reduced or even eliminated. The binding of the coactivator peptide to the AF-2 surface may have facilitated the crystallization of the complex.

The mutations caused only local conformational changes. In R270L, we observed small Cα rmsd changes in the residues near the R270L mutation and the loss of direct interactions of Arg270 with the ligand and with Thr142. The hydroxyethylidene side chain at C(2) of analogue **3c** formed a hydrogen bond with Asp144 and restored the potency that was reduced by the mutation. We suggest that **3c** has potential as an agent for the treatment of HVDRR caused by the R274L mutation.

The W282R mutation disrupted the structure around the β-strands so that 7 to 10 residues around that position became invisible. This occurred probably because the Trp to Arg mutation changed the property of this portion from hydrophobic to hydrophilic. However, it should be noted that in the W282R/ligand complexes, all three hydroxyl groups of the ligands (**1**, **4a**, and **4b**) were doubly anchored by a pair of hydrogen bonds to the protein residues, suggesting that the six hydrogen bonds are not enough for the VDR in biological solution to anchor the ligands. Maintenance of the conformation around the β-strands is important for VDR function. We, therefore, suggest that vitamin D derivatives with a 9α-substituent that has a negative charge may be suitable agents to keep the arginine side chain inside the LBP to activate W286R.

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EXPERIMENTAL PROCEDURES

Protein Expression and Purification. To obtain the mutant VDR-LBD genes, we used QuikChange Site-Directed Mutagenesis Kit (Stratagene) and performed PCR with pET14b/rVDR-LBD plasmid[11b] as a template.

The rat VDR-LBDs (residues116–423 and Δ165–211) with or without the mutations were cloned as an N-terminal His6-tagged fusion protein into the pET14b expression vector and overexpressed in *Escherichia coli* C41, which is a modified strain from BL21. The cells were grown at 37 °C in LB medium (including ampicilin-Na 100 mg/L) and subsequently induced for 6 h with 15 μM isopropyl-β-D-thiogalactopyranoside (IPTG) at 23 °C. The purification procedure included affinity chromatography on a Ni-NTA column, followed by dialysis and ion-exchange chromatography (SP-sepharose). After tag removal by thrombin digestion, protease was removed by filtration through a HiTrap benzamidine column, and the protein was further purified by gel filtration on a Superdex200 column. The purity and homogeneity of the rVDR-LBD were assessed by SDS–PAGE.

Crystallization. Crystallization conditions for WT, R270L, and W282R have some difference. Purified WT solution was concentrated to about 0.75 mg/mL by ultrafiltration. To an aliquot (800 μL) of the protein solution was added each ligand (ca. 10 equivalents), the solution was further concentrated to attain about 100 μL, and then a solution (25 mM Tris-HCl, pH 8.0; 50 mM NaCl; 10 mM DTT; and 0.02% NaN_3) of coactivator peptide (H_2N-KNHPMLMNLLKDN-$CONH_2$, ca. 5 equivalents) derived from DRIP205 was added. These solutions of WT/ligands/peptide were allowed to crystallize by the vapor diffusion method using a series of precipitant solutions containing 0.1 M MOPS-NaOH (pH 7.0), 0.1–0.4 M sodium formate, 12–22% (w/v) PEG4000, and 5% (v/v) ethylene glycol.

Purified R270L solution was also concentrated to about 0.75 mg/mL by ultrafiltration. To an aliquot (800 μL) of the protein solution was added each ligand (ca. 10 or 20 equivalents), the solution was further concentrated to attain about 100 μL, and then the peptide solution was added. These solutions of R270L/ligands/peptide were allowed to crystallize using a series of precipitant solutions containing 0.1 M MOPS-NaOH (pH 7.0) (or 0.1 M Tris-HCl (pH 8.0), or 0.1 M glycine-NaOH (pH 9.0)), 0.4 M sodium formate, and 12–22% (w/v) PEG4000.

Purified W282R solution was concentrated to about 1.7 mg/mL by ultrafiltration. To an aliquot (350 μL) of the protein solution was added each ligand (ca. 10 or 20 equivalents), the solution was further concentrated to attain about 100 μL, and then the peptide solution was added. Solutions of W282R/natural hormone (**1**) or ligand **4a**/peptide were allowed to crystallize using a series of precipitant solutions containing 0.1 M MOPS-NaOH (pH 7.0), 0.05–0.2 M diammonium citrate, 14–26% (w/v) PEG4000, and 4% (v/v) 2-propanol. The other solution of W282R/ligand **4b**/peptide was allowed to crystallize using a series of precipitant solutions containing 0.1 M MOPS-NaOH (pH 7.0), 0.1 M sodium formate, 18–22% (w/v) PEG4000, and 5%(v/v) ethylene glycol.

Droplets for the crystallizations were prepared by mixing 2 μL of complex solution and 1 μL of precipitant solution, and droplets were equilibrated against 500 μL of precipitant solution at 20 °C. It took 1 to 3 days to obtain crystals with X-ray diffraction quality. The peptide derived from DRIP205 was essential for all of our crystallizations.

Diffraction Experiment and Structure Analysis. Prior to diffraction data collection, crystals were soaked in cryoprotectant solutions containing 17–20% ethylene glycol and the other reagents. Diffraction data sets were collected at 100 K in a stream of nitrogen gas at beamline BL-6A of KEK-PF and NW12A of PF-AR (Tsukuba, Japan). Reflections were recorded with an oscillation range per image of 1.0°. Diffraction data were indexed, integrated, and scaled using the program HKL2000.[25] The structures of complexes were solved by molecular replacement with the program CNS,[26] and finalized sets of atomic coordinates were obtained after iterative rounds of model modification with the program XtalView[27] and refinement with CNS by rigid body refinement, simulated annealing, positional minimization, water molecule identification, and individual isotropic B-value refinement.

Thermal Unfolding Measurement. Circular dichloism (CD) spectra were recorded on a Jasco J-820 spectropolarimeter at 20 °C in 10 mM Na-phosphate buffer (pH 7.0) containing 1 mM Tris 2-carboxyethyl phosphine (TCEP) and 1% ethanol. The samples analyzed in a 10 mm optical path length cell were 1 μM rVDR-LBD proteins in the absence or presence of 5 μM $1,25(OH)_2D_3$. CD spectra in the region of 200 to 250 nm were obtained using a scanning speed of 20 nm/min, a time response of 1 s, a bandwidth of 1 nm, a data interval of 0.2 nm, and an average of four scans. Thermal transition curves were determined by monitoring the CD values at 222 nm as the temperature was increased by 1.0 °C/min from 20 to 85 °C.

Luciferase Assays. COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum. Cells were seeded on 24-well plates at a density of 2×10^4 per well. After 24 h, the cells were transfected with a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc), a mutant hVDR expression plasmid [pCMX-hVDR], and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) by the lipofection method as described previously.[28] After 4 h of incubation, the medium was replaced with fresh DMEM containing 5% charcoal-treated FCS (HyClone, UT, USA). The next day, the cells were treated with either the ligand or ethanol vehicle and cultured for 24 h. Cells in each well were harvested with a cell lysis buffer, and the luciferase activity was measured with a luciferase assay kit (Toyo Ink, Inc., Japan). Transactivation measured by luciferase activity was normalized with the internal control. All experiments were done in triplicate.

Synthesis of Ligands. *General.* All nonaqueous reactions were carried out under argon or nitrogen in freshly distilled anhydrous solvents. We conducted high-pressure liquid chromatography (HPLC) by using Jasco 880-PU pumps equipped with an 801-SC solvent programmer and a Uvidec-100 V variable-length UV–vis detector. All samples for biological assays were purified by HPLC and shown to have a purity of >95% [YMC-Pack ODS-AM SH-342–5, 15–20% H_2O/MeOH, 8 mL/min]. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded in $CDCl_3$ solution on a Bruker ARX 400 MHz spectrometer. Low (MS)- and high-resolution mass spectra (HRMS) were obtained by electronic ionization (70 eV) on a JEOL JMS-AX505HA spectrometer. Ultraviolet spectra were recorded on a Hitachi U-3200 spectrophotometer.

*1α-(tert-Butyldimethylsilyloxy)-25-(methoxymethoxy)-19-norvitamin D_3 3-Pivaloyl Ester (**7**) and 1α-(Pivaloyloxy)-25-(methoxymethoxy)-19-norvitamin D_3 3-(tert-butyl dimethylsilyl) Ether (**8**).* A 1.0 M THF solution of LiHMDS (912 μL, 0.912 mmol) was added to a solution of arylsulphone **5** (486.8 mg, 0.912 mmol) in THF (3 mL) at −78 °C, and the mixture was stirred for 30 min. A solution of ketone **6** (228.8 mg, 0.696 mmol) in THF (3 mL) was added to the mixture at −78 °C, the mixture was stirred for 1 h, and then the temperature was raised to −40 °C for 2 h. Saturated NH_4Cl solution was added to the reaction, the mixture was extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel and eluted with 2–3% AcOEt/hexane to give **7** and **8** (334.8 mg, 74%) as a 2:1 mixture and with 10% AcOEt/hexane to give **5** (187.1 mg, 38%). A mixture (2:1) of **7** and **8**: ^1H NMR ($CDCl_3$) δ 0.07 (6 H, s, Si-Me × 2), 0.53, 0.50 (2: 1) (3 H, s, H-18), 0.88–0.89 (9 H, s, Si-*t*Bu), 0.92 (3 H, d, J = 6.4 Hz, H-21), 1.15, 1.14 (2: 1) (9 H, s, CO*t*Bu), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 3.96 (1 H, m), 4.71 (2 H, s, OCH_2O), 5.08, 5.15 (2: 1) (1 H, m), 5.82, 5.71 (2: 1) (1 H, d, J = 11.2 and 11.5 Hz, H-7), 6.15, 6.23 (2: 1) (1 H, d, J = 11.2 and 11.5 Hz, H-6). Mass m/z (%) 646 (M^+, 0.1), 584 (0.4), 544 (11), 482 (100).

*1α-(tert-Butyldimethylsilyloxy)-25-(methoxymethoxy)-19-norvitamin D_3 (**9**) and 1α-Hydroxy-25-(methoxymethoxy)-19-norvitamin D_3 3-(tert-Butyldimethylsilyl) Ether (**10**).* A solution of DIBALH (441 μL, 0.441 mmol, 1.01 M toluene solution) was added to a solution of **7** and **8** (2:1 mixture, 95.2 mg, 0.147 mmol) in toluene (1 mL) at −78 °C and stirred for 1.5 h. The reaction was quenched by adding saturated sodium potassium tartrate, and the mixture was

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extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 10% AcOEt/hexane to give a 2:1 mixture of **9** and **10** (71.7 mg, 87%). The mixture was further chromatographed on fine silica gel (C-300, 5 g) and eluted with 5% AcOEt/hexane to give **9** (25.2 mg), a mixture of **9** and **10** (21.8 mg), and then **10** (10.7 mg). **9**: ^{1}H NMR ($CDCl_3$) δ 0.06, 0.07 (each 3 H, s, Si-Me × 2), 0.54 (3 H, s, H-18), 0.88 (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.14–2.24 (2 H, m, H-4, 10), 2.47 (1 H, dd, J = 13.1, 3.2 Hz, H-4), 2.56 (1 H, dd, J = 13.0, 3.5 Hz, H-10), 2.80 (1 H, dd, J = 12.5, 4.2 Hz, H-9), 3.37 (3 H, s, OCH_3), 4.00 (1 H, m, H-1), 4.12 (1 H, m, H-3), 4.71 (2 H, s, OCH_2O), 5.84 (1 H, d, J = 11.2 Hz, H-7), 6.25 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 562 (M^+, 9), 544 (8), 500 (62), 482 (83), 443 (32), 425 (42), 350 (42), 75 (100). **10**: ^{1}H NMR ($CDCl_3$) δ 0.06, 0.07 (each 3 H, s, Si-Me × 2), 0.54 (3 H, s, H-18), 0.87 (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.14 (1 H, dd, J = 12.7, 7.7 Hz, H-4), 2.39 (1 H, dd, J = 13.1, 4.2 Hz, H-4), 2.41 (2 H, m, H-10, OH), 2.80 (1 H, dd, J = 12.1, 3.6 Hz, H-9), 3.37 (3 H, s, OCH_3), 4.04 (1 H, m, H-3), 4.11 (1 H, m, H-1), 4.71 (2 H, s, OCH_2O), 5.82 (1 H, d, J = 11.1 Hz, H-7), 6.27 (1 H, d, J = 11.1 Hz, H-6). MS m/z (%) 562 (M^+, 13), 500 (77), 482 (80), 443 (27), 425 (35), 350 (35), 75 (100).

*1α-(tert-Butyldimethylsilyloxy)-25-(methoxymethoxy)-19-norvitamin D_3 3-Methoxymethyl Ether (**11a**).* To a solution of **9** (53.7 mg, 0.095 mmol) in CH_2Cl_2 (400 μL) at 0 °C were added ethyldiisopropylamine (50.1 μL, 0.286 mmol) and MOMCl (14.5 μL, 0.191 mmol), and the mixture was stirred at room temperature for 3 h. HCl solution (1 N) was added to the reaction mixture, the mixture was extracted with CH_2Cl_2, and the extract was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (4 g) and eluted with 10% AcOEt/hexane to give **11a** (50.6 mg, 87%), and then **9** was recovered (6.0 mg, 11%). **11a**: ^{1}H NMR ($CDCl_3$) δ 0.06 (6 H, s, Si-Me × 2), 0.54 (3 H, s, H-18), 0.87 (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.22 (1 H, dd, J = 14.2, 7.3 Hz, H-4), 2.32 (1 H, dd, J = 13.4, 6.8 Hz, H-10), 2.43 (2 H, m, H-4, 10), 2.80 (1 H, dd, J = 12.4, 3.9 Hz, H-9), 3.36, 3.37 (each 3 H, s, OCH_3), 3.96, 4.06 (each 1 H, m, H-1, 3), 4.66, 4.68 (each 1 H, s, OCH_2O), 4.71 (2 H, s, OCH_2O), 5.83 (1 H, d, J = 11.3 Hz, H-7), 6.23 (1 H, d, J = 11.3 Hz, H-6). MS m/z (%) 606 (M^+, 31), 576 (39), 544 (96), 482 (92), 73 (100).

*1α-Hydroxy-25-(methoxymethoxy)-19-norvitamin D_3 3-Methoxymethyl Ether (**11b**).* To a solution of **11a** (77.0 mg, 0.127 mmol) was added TBAF (380.6 μL, 0.381 mmol), and the mixture was stirred for 3 h at room temperature. Then additional TBAF (190.3 μL, 0.190 mmol) was added, and the mixture was stirred for 5.5 h. The mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3.5 g) and eluted with 30% AcOEt to give **11b** (59.4 mg, 95%). **11b**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.27 (1 H, dd, J = 13.4, 7.1 Hz, H-4), 2.36 (1 H, dd, J = 13.5, 7.0 Hz, H-10), 2.47 (1 H, dd, J = 13.4, 3.7 Hz, H-4), 2.61 (1 H, dd, J = 13.5, 3.6 Hz, H-10), 2.80 (1 H, dd, J = 12.3, 4.0 Hz, H-9), 3.36, 3.37 (each 3 H, s, OCH_3), 3.96 (1 H, m, H-3), 4.10 (1 H, m, H-1), 4.666, 4.669 (each 1 H, s, OCH_2O), 4.71 (2 H, s, OCH_2O), 5.84 (1 H, d, J = 11.2 Hz, H-7), 6.31 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 492 (M^+, 21), 462 (29), 430 (98), 400 (59), 368 (100), 350 (18).

*1α-{(2-tert-Butyldimethylsilyloxy)-ethyloxy}-25-(methoxymethoxy)-19-norvitamin D_3 3-Methoxymethyl Ether (**13**).* To a solution of **11b** (24.0 mg, 0.0487 mmol) in DMF (500 μL) at 0 °C were added NaH (60% in oil, 58.4 mg, 0.293 mmol) and then bromide **12** (70.0 mg, 0.293 mmol), and the mixture was stirred 15 h at room temperature. The reaction was quenched by adding ice water and extracted with 50% AcOEt/hexane. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (4 g) and eluted with 6% AcOEt/hexane to give **13** (6.0 mg, 19%) and then eluted with 8% AcOEt/hexane to give **11b** (4.7 mg, 16%). **13**: ^{1}H NMR ($CDCl_3$) δ 0.07 (6 H, s, Si-Me × 2), 0.54 (3 H, s, H-18), 0.89 (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.28 (1 H, dd, J = 13.6, 6.6 Hz, H-4), 2.35 (1 H, dd, J = 13.6, 7.5 Hz, H-10), 2.44 (1 H, dd, J = 13.6, 3.5 Hz, H-4), 2.60 (1 H, dd, J = 13.6, 3.4 Hz, H-10), 2.80 (1 H, dd, J = 12.2, 3.7 Hz, H-9), 3.36, 3.37 (each 3 H, s, OCH_3), 3.53 (2 H, m, OCH_2), 3.72 (3 H, m, H-1, OCH_2), 3.96 (1 H, m, H-3), 4.66, 4.71 (each 2 H, s, OCH_2O), 5.85 (1 H, d, J = 11.2 Hz, H-7), 6.23 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 650 (M^+, 5), 588 (7), 526 (3), 73 (100).

*1α-(2-Hydroxyethoxy)-25-hydroxy-19-norvitamin D_3 (**2a**).* CSA (12.8 mg, 0.055 mmol) was added to a solution of **13** (6.0 mg, 9.2 μmol) in MeOH (700 μL), and the mixture was stirred for 2.5 h at room temperature. Additional CSA (12.8 mg, 0.055 mmol) was added and stirred for 20 h. 5% $NaHCO_3$ was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3.5 g) and eluted with 1% MeOH/AcOEt to give **2a** (2.9 mg, 71%). **2a**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.23 (2 H, m, H-4, 10), 2.49 (1 H, dd, J = 13.5, 3.0 Hz, H-4), 2.73 (1 H, dd, J = 13.2, 3.4 Hz, H-10), 2.80 (1 H, dd, J = 12.5, 4.1 Hz, H-9), 3.59, 3.70 (2 H, 3 H, m, H-1, OCH_2 × 2), 4.13 (1 H, m, H-3), 5.85 (1 H, d, J = 11.2 Hz, H-7), 6.26 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 448 (M^+, 100), 430 (74), 412 (10). UV λ_{max} (EtOH) 244, 252, and 262 nm.

*1α-{4-tert-Butyldimethylsilyloxy)butoxy}-25-(methoxymethoxy)-19-norvitamin D_3 3-(tert-Butyldimethylsilyl) Ether (**15**).* NaH (60% in oil, 41.8 mg, 1.04 mmol) and then tosylate **14** (60.2 mg, 0.168 mmol) in dry DMF (300 μL) were added to a solution of **10b** (19.6 mg, 0.035 mmol) in DMF (500 μL) at 0 °C, and the mixture was stirred for 15 h. The reaction was quenched by ice water and extracted with 50% AcOEt/hexane, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (4 g) and eluted with 5% AcOEt/hexane to give **15** (20.6 mg, 79%). **15**: ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05, 0.06 (3 H, 3 H, 6 H, s, Si-Me × 4), 0.54 (3 H, s, H-18), 0.87, 0.89 (each 9 H, s, Si-*t*Bu × 2), 0.93 (3 H, d, J = 6.5 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.80 (1 H, m, H-9), 3.37 (3 H, s, OCH_3), 3.42, 3.62 (2 H, 3 H, m, H-1, OCH_2 × 2), 4.05 (1 H, m, H-3), 4.71 (2 H, s, OCH_2O), 5.83 (1 H, d, J = 11.2 Hz, H-7), 6.16 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%): 748 (M^+, 5), 686 (50), 554 (13), 482 (73), 73 (100).

*1α-(4-Hydroxybutoxy)-25-hydroxy-19nor-vitamin D_3 (**2b**).* CSA (38.3 mg, 0.165 mmol) was added to a solution of **15** (20.6 mg, 0.027 mmol) in MeOH (600 mL), and the mixture was stirred at room temperature for 1 h. 5% $NaHCO_3$ solution was added, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3 g) and eluted with 1% MeOH/AcOEt to give **2b** (12.9 mg, 99%). **2b**: ^{1}H NMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.20 (1 H, dd, J = 13.3, 6.1 Hz, H-4), 2.26 (1 H, dd, J = 13.3, 8.1 Hz, H-10), 2.48 (1 H, dd, J = 13.3, 3.1 Hz, H-4), 2.69 (1 H, dd, J = 13.3, 3.5 Hz, H-10), 2.80 (1 H, dd, J = 12.3, 3.9 Hz, H-9), 3.48, 3.54 (each 1 H, OCH_2), 3.63 (3 H, m, H-1, OCH_2), 4.11 (1 H, m, H-3), 5.85 (1 H, d, J = 11.2 Hz, H-7), 6.26 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%): 476 (M^+, 19), 458 (23), 386 (100), 368 (74). UV λ_{max} (EtOH): 244 nm, 252 nm, 262 nm.

*1α-(tert-Butyldimethylsilyloxy)-25-methoxymethoxy-2-spiro-[oxirane]-19-norvitamin D_3 (**17**).* To a solution of sulphone **5** (159.7 mg, 0.299 mmol) in THF (1 mL) at −78 °C was added a 1.0 M THF solution of LiHMDS (299 μL, 0.299 mmol). The mixture was stirred for 30 min at −78 °C, and then a solution of A ring ketone **16** (67.3 mg, 0.174 mmol) in THF (1.5 mL) was added. The mixture was stirred at −78 °C for 1 h, and then the temperature was raised to −10 °C during 2.5 h. A saturated NH_4Cl solution was added to the reaction, the mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (6 g) and eluted with 2–3% AcOEt/hexane to give **17** (88.7 mg, 72%) as a mixture of epimers at C(2) and recovered **7** (30.3 mg, 19%). **17**: ^{1}H NMR ($CDCl_3$) δ 0.02–0.08 (12 H, s, Si-Me × 4), 0.55 (3 H, s, H-18), 0.86, 0.88 (each 9 H, s, Si-*t*Bu × 2), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.75 and 2.82, 2.57, and 2.92 (3:2) (each 1 H, d, J = 5.5 Hz, $-CH_2OC-$), 3.37 (3 H, s, OCH_3), 3.68 (minor) (1 H, dd, J = 4.9, 2.8 Hz, H-1 or −3), 3.81, 3.88 (major) (each 1 H, dd, J = 7.0, 3.8 Hz, H-1

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and −3), 4.04 (minor) (1 H, dd, J = 9.3, 4.6 Hz, H-1 or 3), 5.82 (1 H, d, J = 11.2 Hz, H-7), 6.21, 6.27 (3:2) (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 704 (M^+, 24), 642 (12), 585 (74), 73 (100).

*1α-Hydroxy-25-methoxymethoxy-2-spiro[oxirane]-19-norvitamin D_3 (**18**).* A 1.0 M THF solution of TBAF (377 μL, 0.377 mmol) was added to a solution of **17** (88.7 mg, 0.126 mmol) in THF (1 mL), and the mixture was stirred at room temperature for 4 h. The mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (6 g) and eluted with 50% AcOEt/hexane to give 1,3-diol **18** (58.9 mg, 98%) as a mixture of epimers at C2. **18**: ^{1}HNMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.62, 2.72 (3:2) (1 H, dd, J = 13.6, 3.9 Hz), 2.85 and 3.08, 2.94, and 2.99 (3:2) (each 1 H, d, J = 4.7 Hz, $-CH_2OC-$), 3.37 (3 H, s, OCH_3), 3.81 (1 H, m, H-1 or −3), 3.91, 3.98 (3:2)(1 H, m, H-1 or −3), 4.71 (2 H, s, OCH_2O), 5.87 (1 H, m, H-7), 6.39 (1 H, m, H-6). MS m/z (%) 476 (M^+, 25), 414 (100).

*1α,2-Dihydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 (**19**).* $LiAlH_4$ (3.3 mg, 0.088 mmol) was added to a solution of diol **18** (20.9 mg, 0.044 mmol) in THF (500 μL), and the mixture was stirred at room temperature for 16 h. A solution of saturated potassium sodium tartrate was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 70% AcOEt/hexane to give triol **19** (17.2 mg, 82%) as a 3:2 mixture of epimers at C2. **19**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.27, 1.30 (3:2) (3 H, s, H-2Me), 3.37 (3 H, s, OCH_3), 3.74 (2 H, m, H-1, 3), 4.71 (2 H, s, OCH_2O), 5.83 (1 H, m, H-7), 6.32 (1 H, m, H-6). MS m/z (%) 478 (M^+, 45), 416 (100), 398 (13).

*1α,2-Dihydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**20a**) and 1,2-Dimethyl Acetal (**20b**).* To a solution of triol **19** (34.6 mg, 0.072 mmol) in 2,2-dimethoxypropane (1 mL) was added TsOH·H_2O (1.4 mg, 7.23 μmol), and the mixture was stirred at room temperature for 1 h. The mixture was extracted with AcOEt, and the extract was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 20% AcOEt/hexane to give **20** (28.4 mg, 64%) as a mixture of 2,3- and 1,2-diol acetonides (3:2). **20**: ^{1}H NMR ($CDCl_3$) δ 0.54, 0.55 (3:2) (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.30, 1.34 (3:2) (3 H, s, H-2Me), 1.38, 1.45 (major) (each 3 H, s, $C(\underline{CH_3})_2$), 1.39, 1.46 (minor) (each 3 H, s, $C(\underline{CH_3})_2$), 3.37 (3 H, s, OCH_3), 3.88, 3.82 (3:2) (1 H, m, H-1 or −3), 4.04 (minor) (1 H, t, J = 4.1 Hz, H-1 or −3), 4.10 (major) (1 H, t, J = 4.4 Hz, H-1 or −3), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.26 (1 H, m, H-6). MS m/z (%) 518 (M^+, 97), 456 (100), 413 (11), 398 (33), 380 (23).

*1α-(2-tert-Butyldimethylsilyloxyethoxy)-2-hydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**22a**) and 1α,2-Dihydroxy-25-(methoxylmethoxy)-2-methyl-19-norvitamin D_3 3-(2-tert-Butyldimethylsilyloxyethyl) Ether 1,2-Dimethyl Acetal (**22a′**).* To a solution of alcohol **20** (11.8 mg, 0.028 mmol) in DMF (400 μL) were added NaH (60% in oil, 33.1 mg, 0.827 mmol) and a solution of tosylate **21a** (62.5 mg, 0.189 mmol) in DMF (500 μL). The mixture was stirred for 4 h at room temperature and then quenched with ice water. The mixture was extracted with 50% AcOEt/hexane, washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 5% AcOEt/hexane to give **22a** and **22a′** (11.8 mg, 63%) as a mixture (3:2) of isomers. **22a** and **22a′**: ^{1}H NMR ($CDCl_3$) δ 0.07, 0.05 (3:2)(6 H, s, Si-Me × 2), 0.54, 0.55 (3:2)(3 H, s, H-18), 0.90, 0.89 (3:2) (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.325, 1.334 (3:2)(3 H, s, H-2Me), 1.37, 1.45 (major) (each 3 H, s, $C(\underline{CH_3})_2$) 1.38, 1.47 (minor) (each 3 H, s, $C(\underline{CH_3})_2$), 3.37 (3 H, s, OCH_3), 3.75−4.06 (6 H, m, H-1 and 3, $O(\underline{CH_2})_2$OTBS), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.23 (1 H, m, H-6). MS m/z (%) 676 (M^+, 2), 614 (4), 599 (3), 556 (2), 438 (100).

*1α-(3-tert-Butyldimethylsilyloxypropoxy)-2-hydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**22b**) and 1α,2-Dihydroxy-25-(methoxylmethoxy)-2-methyl-19-norvitamin D_3 3-(3-tert-Butyldimethylsilyloxypropyl) Ether 1,2-Dimethyl Acetal (**22b′**).* Monohydroxy compound **20** (16.9 mg, 0.033 mmol) dissolved in DMF (900 μL) was treated with NaH (60% in oil, 52.0 mg, 1.30 mmol) and then tosylate **21b** (90.6 mg, 0.263 mmol) as in the synthesis of **22a** and **22a′**. The ethers **22b** and **22b′** (11.1 mg, 49%) were obtained after similar workup as a 3:2 mixture of isomers. **22b** and **22b′**: ^{1}H NMR ($CDCl_3$) δ 0.05, 0.04 (3:2) (6 H, s, Si-Me × 2), 0.54, 0.55 (3:2) (3 H, s, H-18), 0.89, 0.88 (3:2)(9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.32, 1.33 (3:2) (3 H, s, H-2Me), 1.37, 1.44 (major) (each 3 H, s, $C(\underline{CH_3})_2$) 1.39, 1.47 (minor) (each 3 H, s, $C(\underline{CH_3})_2$), 3.37 (3 H, s, OCH_3), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.21 (1 H, m, H-6).

*1α-(4-tert-Butyldimethylsilyloxybutoxy)-2-hydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**22c**) and 1α,2-Dihydroxy-25-(methoxylmethoxy)-2-methyl-19-norvitamin D_3 3-(4-tert-Butyldimethylsilyloxybutyl) Ether 1,2-Dimethyl Acetal (**22c′**).* Monohydroxy compound **20** (11.9 mg, 0.023 mmol) in DMF (700 μL) was similarly treated with NaH (60% in oil, 27.5 mg, 0.688 mmol) and tosylate **21c** (49.4 mg, 0.138 mmol). The ethers **22c** and **22c′** (10.6 mg, 65%) were obtained after similar workup as a mixture (3:2) of isomers. **22c** and **22c′**: ^{1}H NMR ($CDCl_3$) δ 0.05, 0.04 (3:2) (6 H, s, Si-Me × 2), 0.54, 0.55 (3:2) (3 H, s, H-18), 0.891, 0.887 (3:2) (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.32, 1.33 (3:2)(3 H, s, H-2Me), 1.37, 1.45 (major) (each 3 H, s, $C(\underline{CH_3})_2$), 1.39, 1.47 (minor) (each 3 H, s, $C(\underline{CH_3})_2$), 3.37 (3 H, s, OCH_3), 3.39−3.70 (5 H, m, H-1 or 3, $O\underline{CH_2}(CH_2)_2\underline{CH_2}$OTBS), 4.00, 4.05 (3:2)(1 H, m, H-1 or 3), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.23 (1 H, m, H-6).

*1α-(2-Hydroxyethoxy)-2β,25-dihydroxy-2α-methyl-19-norvitamin D_3 (**2c**).* CSA (24.3 mg, 0.105 mmol) was added to a solution of **22a** and **22a′** (11.8 mg, 0.017 mmol) in MeOH (300 μL), and the mixture was stirred for 1.5 h at room temperature. Then, 5% $NaHCO_3$ was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3 g) and eluted with 70% AcOEt/hexane to give a 3:2 mixture of regioisomers **2c** and **2c′** (7.2 mg, 87%). The mixture was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min] to give **2c** (2.59 mg, RT 21.08) and **2c′** (1.67 mg, RT 23.75). **2c**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.29 (3 H, s, H-2Me), 2.37, 2.49 (each 1 H, m, H-4), 2.79 (1 H, dd, J = 12.6, 4.1 Hz, H-9), 2.91 (1 H, dd, J = 13.7, 4.4 Hz, H-10), 3.48−3.81 (6 H, m), 5.83 (1 H, d, J = 11.2 Hz, H-7), 6.26 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 478 (M^+, 100), 460 (58), 442 (18). UV λ_{max} (EtOH): 244, 252, and 261 nm. **2c′** ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.94 (3 H, d, J = 6.5 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.30 (3 H, s, H-2Me), 2.60 (1 H, dd, J = 13.7, 4.2 Hz), 2.73 (1 H, dd, J = 14.7, 5.5 Hz),2.79 (1 H, m), 3.55−3.74 (6 H, m), 5.81 (1 H, d, J = 11.1 Hz, H-7), 6.32 (1 H, d, J = 11.1 Hz, H-6). MS m/z (%) 478 (M^+, 100), 460 (55), 442 (24). UV λ_{max} (EtOH): 244 nm, 252 nm, 261 nm.

*1α-(3-Hydroxypropoxy)-2β,25-dihydroxy-2α-methyl-19-norvitamin D_3 (**2d**).* Deprotection of **22b** and **22b′** (11.1 mg, 0.016 mmol) by the procedure described above gave a 3:2 mixture of **2d** and **2d′** (4.3 mg, 54%). The mixture was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min to give **2d** (2.37 mg, RT: 20.18) and **2d′** (1.37 mg, RT: 21.60). **2d** ^{1}H NMR ($CDCl_3$) δ: 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.27 (3 H, s, H-2Me), 2.09 (1 H, dd, J = 13.1, 9.8 Hz, H-10), 2.36 (1 H, dd, J = 14.0, 5.2 Hz, H-4), 2.47 (1 H, m, H-4), 2.80 (1 H, dd, J = 12.3, 4.1 Hz, H-9), 2.89 (1H, dd, J = 13.7, 4.2 Hz, H-10), 3.44−3.86 (6 H, m), 5.83 (1 H, d, J = 11.2 Hz, H-7), 6.27 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 492 (M^+, 100), 474 (65), 456 (26). UV λ_{max} (EtOH): 244, 252, and 261 nm. **2d′** ^{1}H NMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.5 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.25 (3 H, s, H-2Me), 2.60−2.81 (3H, m), 3.50−3.88 (6 H, m), 5.81 (1 H, d, J = 11.2 Hz, H-7), 6.32 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%): 492 (M^+, 38), 474 (45), 456 (33), 135 (100). UV λ_{max} (EtOH): 244 nm, 252 nm, 262 nm.

*1α-(4-Hydroxybutoxy)-2β,25-dihydroxy-2α-methyl-19-norvitamin D_3 (**2e**).* Deprotection of **22c** and **22c′** (10.6 mg, 0.015 mmol) was similarly carried out by treatment with CSA (20.9 mg, 0.090

mmol) in THF/MeOH (1:1 400 μL) to give a 3:2 mixture of **2e** and **2e′** (4.6 mg, 60%). The isomers were separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min] to give **2e** (3.68 mg, RT: 28.66) and **2e′** (0.90 mg, RT: 32.68). **2e** ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.93 (3 H, d, *J* = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.28 (3 H, s, H-2Me), 2.09 (1H, dd, *J* = 13.3, 9.7 Hz, H-10), 3.43–3.64 (2 H, OCH_2), 3.65–3.74 (4 H, m), 5.82 (1 H, d, *J* = 11.2 Hz, H-7), 6.26 (1 H, d, *J* = 11.2 Hz, H-6). Mass *m/z* (%) 507 (M^+, 6), 489 (4), 471 (1), 75 (100). UV λ_{max} (EtOH): 244 nm, 252 nm, 261 nm. **2e′** ^{1}H NMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, *J* = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.27 (3 H, s, H-2Me), 2.60–2.81 (3 H, m), 3.49 (2 H, OCH_2), 3.67 (3 H, m), 3.76 (1 H, m), 5.82 (1 H, d, *J* = 11.2 Hz, H-7), 6.31 (1 H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%) 507 (M^+, 2), 489 (1), 75 (100). UV λ_{max} (EtOH): 244, 252, and 262 nm.

*5α-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-one (**24a**).* To a solution of iPr_2NH (706 μL, 5.04 mmol) in THF (10 mL) at −20 °C was added *n*-BuLi (2.8 mL, 4.37 mmol, 1.58 M hexane), and the mixture was stirred for 15 min. Grundman's ketone (1.09 g, 3.36 mmol) in THF (5 mL) was added to the LDA solution at −78 °C, the mixture was stirred for 1 h at that temperature, and then TMSCl (436 μL, 5.04 mmol) and Et_3N (702 μL, 5.04 mmol) were added. The temperature of the reaction was raised to −20 °C for 1.5 h. The solvent was evaporated, the residue was dissolved in hexane, and the mixture was passed through Celite to give **23** (1.34 g). To a solution of **23** (500.5 mg, 1.262 mmol) in THF (3 mL) at 0 °C was added MeLi (1.2 M Et_2O solution, 1.16 mL, 1.388 mmol) and stirred for 1 h. This solution of enolate was added to a solution of iodobutane (718 μL, 6.31 mmol) and HMPA (439 μL, 2.524 mmol) in THF (2 mL) at 0 °C, and the mixture was stirred at 0 °C for 3 h. A saturated NH_4Cl solution was added to the reaction, the solution was extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (20 g) and eluted with 5% AcOEt/hexane to give **24a** (249.2 mg, 52%). **24a**: ^{1}H NMR ($CDCl_3$) δ 0.64 (3 H, s, H-18), 0.87 (3 H, t, J = 7.1 Hz, $(CH_2)_3\underline{CH_3}$), 0.95 (3 H, d, J = 6.1 Hz, H-21), 1.21 (6 H, s, H-26,27), 2.02 (1 H, m, H-9), 2.60 (1 H, dd, *J* = 11.6, 7.4 Hz, H-14), 3.37 (3 H, OMe), 4.70 (2 H, s, $O\underline{CH_2}O$). MS *m/z* (%) 380(M^+, 2), 365 (7), 318 (45), 227 (24), 262 (100), 219 (209, 103 (41).

*5-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-4-vinyl-octahydro-inden-4-ol (**25a**).* A solution of **24a** (303 mg, 0.80 mmol) in THF (3 mL) at 0 °C was treated with vinyl magnesium bromide (1.66 mL, 1.662 mmol THF solution), and the mixture was stirred for 3 h. The reaction was quenched by adding a 1 N HCl solution and extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 10% AcOEt/hexane to give **25a** (297.5 mg, 88%). **25a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3 H, t, *J* = 7.1 Hz, $(CH_2)_3\underline{CH_3}$), 0.91 (3 H, d, *J* = 6.5 Hz, H-21), 0.96(3 H, s, H-189, 1.21 (6 H, s, H-26,27), 2.03 (1 H, m, H-14), 3.37 (3 H, s, OMe), 4.70 (2 H, s, OCH_2O), 5.02 (1 H, dd, *J* = 10.8, 1.6 Hz, H-6), 5.23 (1 H, dd, *J* = 17.2, 1.6 Hz, H-6), 5.94 (1 H, dd, *J* = 17.2, 10.8 Hz, H-7).

*[5-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-acetaldehyde (**26a**).* To a solution of **25a** (268.7 mg, 0.657 mmol) in CH_2Cl_2 (15 mL) was added PCC (723 mg, 3.288 mmol) and Celite (0.8 g), and the mixture was stirred at room temperature for 23 h. The reaction mixture was directly chromatographed on silica gel (10 g) and eluted with 5% AcOEt/hexane to give aldehyde **26a** (172.8 mg, 86%). **26a**: ^{1}H NMR ($CDCl_3$) δ 0.61 (3 H, s, H-18), 0.88 (3H, t, *J* = 7.2 Hz, $(CH_2)_3\underline{CH_3}$), 0.94 (3 H, d, *J* = 5.7 Hz,H-21), 1.21 (6 H, s, H-26,27), 2.36 (1 H, m, H-14), 3.37 (3 H, s, OMe), 3.39 (1 H, m, H-9), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, d, *J* = 8.3 Hz, H-7), 10.06 (1 H, d, *J* = 8.3 Hz, CHO).

*2-[5-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-ethanol (**26b**).* $NaBH_4$ (13.9 mg, 0.367 mmol) in EtOH (2 mL) was added to a solution of aldehyde **26a** (200.5 mg, 0.493 mg) in EtOH (1.5 mL) at 0 °C, and the mixture was stirred for 1 h. Water was added to the reaction, and the mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g) and eluted with 20–25% AcOEt/hexane to give **26b** (172.8 mg, 86%). **26b**: ^{1}HNMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.88 (3 H, t, *J* = 7.3 Hz, $(CH_2)_3\underline{CH_3}$), 0.94 (3 H, d, *J* = 6.4 Hz, H-21), 1.21 (6 H, s, H-26,27), 2.12 (1 H, m, H-14), 2.62 (1 H, m, H-9), 3.37 (3 H, s, OMe), 4.15, 4.25 (each 1 H, m, H-6), 4.71 (2 H, s, OCH_2O), 5.23 (1 H, m, H-7). MS e/z (%) 408 (M^+, 0), 346 (11), 328 (58), 313 (23), 271 (19), 243 (33), 217 (100).

*5-Butyl-4-[2-(diphenyl-phosphinoyl)-ethylidene]-1-(5-methoxy-methoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-indene (**27**).* To a solution **26b** (156 mg, 0.382 mmol) in THF (2 mL) at 0 °C were added a solution of *n*-BuLi (1.58 M hexane, 266 μL, 0.420 mmol) and then a solution of TsCl (87.4 mg, 0.458 mmol) in THF (0.2 mL), and the mixture was stirred for 5 min. In another flask, to a solution of diphenylphosphine (133 μL, 0.764 mmol) in THF (1 mL) at 0 °C was added a solution of *n*-BuLi (1.58 M hexane, 484 μL, 0.764 mmol) to yield a red solution. This red solution was slowly added via a double headed needle to the above solution of tosylate until the red color did not disappear in the solution, and the mixture was stirred for 30 min. Water (20 μL) was added to the reaction, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C, 10% H_2O_2 (3 mL) was added to this solution, and the mixture was stirred at 0 °C for 1 h. Sodium thiosulfate (2 N) was added to the reaction, the mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (8 g) and eluted with 40% AcOEt/hexane to give Wittig–Horner reagent **27** (173.7 mg, 77%). **27**: ^{1}H NMR ($CDCl_3$) δ 0.23 (3 H, s, H-18), 0.86 (3 H, t, *J* = 7.4 Hz, $(CH_2)_3\underline{CH_3}$ overlapped with H-21), 1.20 (6 H, s, H-26,27), 2.02 (1 H, m, H-14), 2.49 (1 H, m, H-9), 2.98, 3.34 (each 1 H, m, H-6), 3.36 (3 H, s, OMe), 4.70 (2 H, s, OCH_2O), 5.04 (1 H, m, H-7), 7.43–7.78 (10 H, m, aromatic H). MS *m/z* (%) 592 (M^+, 2), 530 (100), 473 (33), 419 (4), 216 (74), 202 (91).

*9α-Butyl-1α-(tert-butyldimethylsilyloxy)-25-methoxymethoxy-19-norvitamin D_3 3-(tert-Butyldimethylsilyl) Ether (**29**).* To a solution of phosphine oxide **27** (124.1 mg, 0.209 mmol) in THF (2 mL) at −78 °C were added HMPA (36 μL, 0.209 mmol) and *n*-BuLi (1.58 M hexane solution, 132 μL, 0.209 mmol), and the mixture was stirred for 15 min. To this solution was slowly added ketone **28** (37.5 mg, 0.105 mmol) in THF (1 mL), the mixture was stirred for 1 h at −78 °C, and then the temperature of the reaction was slowly raised to room temperature, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by adding saturated NH_4Cl and extracted with AcOEt, and the organic layer was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (8 g) and eluted with 2% AcOEt/hexane to yield **29** (15.7 mg, 21%, 81% on the basis of recovered **27**) and then eluted with 40% AcOEt/hexane to give starting phosphine oxide **27** (91.3 mg, 74%). **29**: ^{1}H NMR ($CDCl_3$) δ 0.048, 0.053 (each 6H, s, SiMe), 0.54 (3 H, s, H-18), 0.865, 0.87 (each 9H, s, *t*-BuSi overlapped with $(CH)_2\underline{CH_3}$), 0.92 (3 H, d, *J* = 6.3 Hz, H-21), 1.21 (6 H, s, H-26,27), 2.83 (1 H, m),3.37 (3 H, s, OMe), 4.71 (2 H, s, OCH_2O), 4.07 (2 H, m, H-1,3), 5.83 (1 H, d, *J* = 11.2 Hz, H-7), 6.14 (1 H, d, *J* = 11.2 Hz, H-6).

*9α-Butyl-1α,25-dihydroxy-19-norvitamin D_3 (**4a**).* To a solution of **29** (28.5 mg, 0.039 mmol) in MeOH (1 mL) was added CSA (54.2 mg, 0.233 mmol), and the mixture was stirred for 1.5 h at room temperature. A 5% $NaHCO_3$ solution was added to the reaction, the solution was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (4 g) and eluted with 70% AcOEt/hexane to give **4a** (17.2 mg, 96%). Since **4a** contains a minor product, it was purified by HPLC (YMC-Pack ODS-AM SH-342-5, 15% H_2O/MeOH, 8 mL/min) to yield **4a** (11.8 mg) and its geometrical isomer **4a′** (1.0 mg) at the 7-position. **4a**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.87 (3 H, t, 7.2 Hz, $(CH_2)_3\underline{CH_3}$), 0.93 (3 H, d, *J* = 6.4 Hz, H-21),1.22 (6 H, s, H-26,27), 2.82 (1 H, m, H-9), 4.07 (2 H, m, H-1,3), 5.87 (1 H, d, *J* = 11.2 Hz, H-7), 6.31 (1 H, d, *J* = 11.2 Hz, H-6). UV (EtOH) λ_{max} (ε) 244 (27,000), 252 (31,000) and 261 nm (21,000). MS z/e (%) 460 (M^+, 21), 442 (100), 424 (36), 406 (15), 331 (31), 313 (32), 295 (21). **4a′** [(7Z)-9α-Butyl-1α,25-dihydroxy-19-norvita-

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min D_3]: ^{1}HNMR ($CDCl_3$) δ 0.64 (3 H, s, H-18), 0.87 (3 H, t, J = 7.2 Hz, $(CH_2)_3\underline{CH_3}$), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26,27), 4.07 (2 H, m, H-1,3), 6.12 (1 H, d, J = 11.5 Hz, H-7), 6.46 (1 H, d, J = 11.5 Hz, H-6). UV (EtOH) λ_{max} 244, 252, and 261 nm. MS z/e (%) 460 (M^+, 18), 442 (100), 424 (33), 406 (17), 331 (36), 313 (33), 295 (22).

*5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-one (**24b**).* To a solution of iPr$_2$NH (706 μL, 5.04 mmol) in THF (10 mL) at −20 °C was added n-BuLi (2.8 mL, 4.37 mmol, 1.58 M hexane), and the mixture was stirred for 15 min. Ketone **5** (1.09 g, 3.36 mmol) in THF (5 mL) was added to the LDA solution at −78 °C, the mixture was stirred for 1 h at that temperature, and then TMSCl (436 μL, 5.04 mmol) and Et_3N (702 μL, 5.04 mmol) were added. The temperature of the reaction was raised to −20 °C for 1.5 h. The solvent was evaporated, the residue was dissolved in hexane, and the mixture was passed through Celite to give **23** (1.34 g). To a solution of silyl enol ether **23** (1.3 g, 3.36 mmol) in THF (5 mL) at 0 °C was added a solution of MeLi (1.2 M Et_2O, 3.4 mL, 4.06 mmol), and the mixture was stirred for 1 h. This mixture was added to a solution of allyl iodide (631 μL, 6.77 mmol) in THF (2 mL) at −78 °C. The mixture was stirred for 1 h while raising the temperature to −45 °C. NH_4Cl solution was added to the reaction, and the mixture was extracted with AcOEt, the extract was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was chromatographed on silica gel (30 g) with 8−10% AcOEt/hexane to give **24b** (887.7 mg, 73%, 2 steps). **24b** ^{1}H NMR ($CDCl_3$) δ 0.66 (3 H, s, H-18), 0.96 (3 H, d, J = 6.2 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.57 (1 H, dd, J = 11.5, 7.4 Hz, H-14), 3.37 (3 H, s, OCH_3), 4.70 (2 H, s, OCH_2O), 5.09 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.68 (1 H, m, $CH_2\underline{CH}{=}CH_2$). MS m/z (%) 364 (M^+, 6), 323 (8), 302 (76), 261 (17), 219 (68), 191 (25), 55 (100).

*5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-4-vinyl-octahydro-inden-4-ol (**25b**).* To a solution of **24b** (887.7 mg, 2.4 mmol) in THF (7 mL) at 0 °C was added a solution of vinyl magnesium bromide (1 M THF solution, 4.87 mL, 4.87 mmol), and the mixture was stirred for 2 h. The reaction mixture was treated as described above for the synthesis of **25a**. The residue was chromatographed on silica gel (10 g) and eluted with 10% AcOEt/hexane to give **25b** (759.1 mg, 79%). **25b**: ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, J = 6.5 Hz, H-21), 0.97 (3 H, s, H-18), 1.21(6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 4.71 (2 H, s, OCH_2O), 4.99 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.05 (1 H, dd, J = 10.8, 1.5 Hz, H-6), 5.25 (1 H, dd, J = 17.2, 1.5 Hz, H-6), 5.69 (1 H, m, $CH_2\underline{CH}{=}CH_2$), 5.91 (1 H, dd, J = 17.2, 10.8 Hz, H-7). MS m/z (%) 392 (no M^+), 330 (53), 312 (50), 297 (14), 271 (20), 247 (87), 219 (20), 201 (49), 55 (100).

*[5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-acetaldehyde (**26c**).* To a solution of **25b** (759.1 mg, 1.93 mmol) in CH_2Cl_2 (30 mL) were added PCC (1.67 g, 7.73 mmol) and Celite (1.7 g), and the mixture was stirred at room temperature for 23 h. The reaction mixture was chromatographed on silica gel (20 g) and eluted with 2% AcOEt/hexane to give aldehyde **26c** (669.3 mg, 89%). **26c**: ^{1}H NMR ($CDCl_3$) δ 0.62 (3 H, s, H-18), 0.95 (3 H, d, J = 5.7 Hz, H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 3.47 (1 H, m, H-9), 4.71 (2 H, s, OCH_2O), 5.03 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.68 (1 H, m, $CH_2\underline{CH}{=}CH_2$), 5.76 (1 H, dd, J = 8.3, 1.5 Hz, H-7), 10.01 (1 H, d, J = 8.2 Hz, CHO). MS m/z (%) 390 (M^+, 6), 328 (71), 287 (52), 217 (28), 215 (100).

*2-[5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-ethanol (**26d**).* $NaBH_4$ (63.9 mg, 1.69 mmol) was added to a solution of aldehyde **26c** (660.1 mg, 1.69 mmol) in EtOH (10 mL) at 0 °C, and the mixture was stirred for 1 h. The mixture was treated as described above for the synthesis of **26b**. Chromatography of the product on silica gel (20 g) and elution with 20−25% AcOEt/hexane gave alcohol **26d** (400.6 mg, 61%). **26d**: ^{1}H NMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.73 (1 H, dd, J = 13.9, 7.1 Hz, H-9), 3.37 (3 H, s, OCH_3), 4.14 (2 H, d, J = 6.8 Hz, H-6), 4.71 (2 H, s, OCH_2O), 4.97 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.28 (1 H, dd, J = 7.1, 1.6 Hz, H-7), 5.70 (1 H, m, $CH_2\underline{CH}{=}CH_2$). MS m/z (%) 392 (no M^+), 330 (100), 312 (53), 299 (64), 289 (19), 271 (41), 245 (47), 219 (32), 217 (49), 201 (72).

*2-{2-[5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-ethanesulfonyl}-benzothiazole (**30**).* To a solution of alcohol **26d** (108.8 mg, 0.277 mmol) in CH_2Cl_2 (2 mL) were added Ph_3P (109 mg, 0.416 mmol), 2-mercaptobenzothiazole (69.5 mg, 0.416 mmol), and DIAD (57.4 μL, 0.277 mmol), and the mixture was stirred at 1 h. The solvent was evaporated, and the residue was dissolved in EtOH (2.5 mL) and cooled to 0 °C. To this solution were added 30% H_2O_2 (300 μL) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (68.5 mg, 0.055 mmol), and the mixture was stirred for 1 h at room temperature. The reaction was quenched with 2 N Na_2SO_3 solution, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 7% AcOEt/hexane to give **30** (158 mg, 97%). **30**: ^{1}H NMR ($CDCl_3$) δ: 0.17 (3 H, s, H-18), 0.84 (3 H, d, J = 6.1 Hz, H-21), 1.20 (6 H, s, H-26, 27), 2.70 (1 H, m, H-9), 3.36 (3 H, s, OCH_3), 4.06 (1 H, ddd, J = 14.4, 5.9, 2.0 Hz, H-6), 4.54 (1 H, dd, J = 14.4, 9.8 Hz, H-6), 4.69 (2 H, s, OCH_2O), 4.94 (2 H, m, $CH{=}\underline{CH_2}$), 5.06 (1 H, m, H-7), 5.64 (1 H, m, $\underline{CH}{=}CH_2$), 7.61, 8.00, 8.21 (2 H, 1 H, 1 H, m, arom-H).

*9α-Allyl-1α-(tert-butyldimethylsilyloxy)-2-[2-(tert-butyldimethylsilyloxy)-ethylidene]-25-methoxymethoxy-19-norvitamin D_3 3-(tert-Butyldimethylsily) Ether (**32**).* A solution of LHMDS (1.0 M THF, 230 μL, 0.23 mmol) was added to a solution of sulphone **30** (158.0 mg, 0.269 mmol) in THF (1.5 mL) at −78 °C, the solution was stirred for 30 min, and to this solution, a solution of A-ring ketone **31** (69.3 mg, 0.155 mmol) in THF (1 mL) was added. The mixture was stirred for 1 h at −78 °C, the temperature was raised to 0 °C, and the mixture was stirred at that temperature for 1 h. Saturated NH_4Cl solution was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g) and eluted with 3% AcOEt/hexane to give **32** (112.4 mg, 83% on the basis of recovered **30**) as a mixture of E- and Z-isomers (5:3) and with 10% AcOEt/hexane sulphone **30** (64.8 mg, 41%). **32a** (E isomer): ^{1}H NMR ($CDCl_3$) δ 0.05−0.09 (18 H, Si-Me × 6), 0.56 (3 H, s, H-18), 0.82−0.94 (30 H, Si-tBu × 3, H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 4.25−4.45 (3 H, m, H-1, $\underline{CH_2}$OTBS), 4.71 (2 H, s, OCH_2O), 4.78 (1 H, m, H-3), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.61 (1 H, m, $C{=}CH$), 5.72 (1 H, m, $\underline{CH}{=}CH_2$), 5.89 (1 H, d, J = 11.1 Hz, H-7), 6.12 (1 H, d, J = 11.1 Hz, H-6). **32b** (Z isomer): ^{1}H NMR ($CDCl_3$) δ 0.05−0.09 (18 H, Si-Me × 6), 0.54 (3 H, s, H-18), 0.82−0.94 (30 H, Si-tBu × 3, H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 4.25−4.45 (3 H, m, H-3, $\underline{CH_2}$OTBS), 4.71 (2 H, s, OCH_2O), 4.78 (1 H, m, H-1), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.61 (1 H, m, $C{=}CH$), 5.72 (1 H, m, $\underline{CH}{=}CH_2$), 5.83 (1 H, d, J = 10.4 Hz, H-7), 6.22 (1 H, d, J = 10.4 Hz, H-6). MS m/z (%) 872 (M^+, 3), 740 (68), 678 (100), 621 (8).

*9α-Allyl-1α,25-dihydroxy-2-(2-hydroxyethylidene)-19-norvitamin D_3 (**4b** and **4c**).* CSA (77 mg, 0.332 mmol) was added to a solution of **32** (48.0 mg, 55 μmol) in MeOH (1 mL), and the mixture was stirred for 1.5 h at room temperature. A 5% $NaHCO_3$ solution was added to the reaction, the mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 2% MeOH/AcOEt to give a mixture of **4b** and **4c** (E- and Z-isomers at the position 2) (24.2 mg, 90%). The mixture was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min] to give **4b** (E-isomer) (14.15 mg, RT 34.80) and **4c** (Z-isomer) (8.49 mg, RT 37.10). **4b**: ^{1}H NMR ($CDCl_3$) δ: 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.2 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.42 (2 H, m, H-4), 2.92 (1 H, m, H-9), 3.13 (1 H, dd, J = 13.0, 4.8 Hz, H-10), 4.15 (1 H, dd, J = 12.5, 5.8 Hz, $\underline{CH_2}$OH), 4.37 (1 H, dd, J = 12.5, 8.1 Hz, $C\underline{H_2}$OH), 4.40 (1 H, m, H-1), 4.82 (1 H, m, H-3), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.73 (1 H, m, $\underline{CH}{=}CH_2$), 5.80 (1 H, m, $C{=}CH$), 5.90 (1 H, d, J = 11.1 Hz, H-7), 6.26 (1 H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH) 247, 255, and 264 nm. **4c**: ^{1}H NMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.94 (3 H, d, J = 6.2 Hz, H-21), 1.22 (6 H, s, H-26, 27), 4.19 (1 H, dd, J = 12.5, 6.0 Hz, $\underline{CH_2}$OH), 4.37 (1 H, dd, J = 12.5, 7.9 Hz, $C\underline{H_2}$OH), 4.45 (1 H, m, H-3), 4.87 (1 H, m, H-1), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.72 (1 H, m,

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CH=CH_2), 5.81 (1 H, m, C=CH), 5.87 (1 H, d, J = 11.2 Hz, H-7), 6.37 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%): 486 (M^+, 17), 468 (17), 450 (30), 432 (61), 391 (100). UV λ_{max} (EtOH) 247, 255, and 264 nm.

*1α-(tert-Butyldimethylsilyloxy)-2-[2-(tert-butyldimethylsilyloxy)-ethylidene]-9α-(3-hydroxypropyl)-25-methoxymethoxy-19-norvitamin D_3 3-(tert-Butyldimethylsily) Ether (**33**).* To a solution of **32** (57.7 mg, 0.0661 mmol, *E:Z*, 5:3) in THF (300 μL) was added 9-borabicyclo[3.3.1]nonane (9-BBN) (1.32 mL, 0.661 mmol, 10.0 equiv) at room temperature, and the mixture was stirred for 1.5 h. MeOH (220 μL) was added to the reaction, and the mixture was stirred for 15 min. The mixture was cooled to 0 °C, and 6 M NaOH (220.2 μL, 1.32 mmol, 20.0 equiv) and 30% H_2O_2 (220 μL) were added. The mixture was stirred at room temperature for 1 h. To the reaction, 2 N HCl was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g) and eluted with 8% EtOAc/hexane to give **33a** (2*E*-isomer, 13.1 mg, 22%) and with 10% AcOEt/hexane to give **33b** (2*Z*-isomer, 11.1 mg, 19%). **33a**: ^{1}H NMR ($CDCl_3$) δ 0.05–0.08 (18 H, Si-Me × 6), 0.55 (3 H, s, H-18), 0.83, 0.89, 0.93 (each 9 H, s, Si-*t*Bu × 3, overlapped with H-21), 1.22 (6 H, s, H-26, 27), 2.86 (1 H, m, H-9), 3.00 (1 H, dd, J = 12.7, 4.7 Hz, H-10), 3.37 (3 H, s, OCH_3), 3.61 (2 H, m, CH_2OH), 4.25–4.38 (3 H, m, H-1, CH_2OTBS), 4.71 (2 H, s, OCH_2O), 4.78 (1 H, m, H-3), 5.61 (1 H, m, C=CH), 5.93 (1 H, d, J = 11.1 Hz, H-7), 6.11 (1 H, d, J = 11.1 Hz, H-6). MS m/z (%) 890 (M^+, 2), 828 (5), 758 (52), 696 (100), 626 (10), 75 (93). **33b**: ^{1}H NMR ($CDCl_3$) δ 0.06–0.09 (18 H, Si-Me × 6), 0.54 (3 H, s, H-18), 0.82, 0.90, 0.93 (each 9 H, s, Si-*t*Bu × 3, overlapped with H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 3.61 (2 H, m, CH_2OH), 4.30 (2 H, m, CH_2OTBS), 4.44 (1 H, m, H-3), 4.71 (2 H, s, OCH_2O), 4.83 (1 H, m, H-1), 5.61 (1 H, m, C=CH), 5.87 (1 H, d, J = 10.4 Hz, H-7), 6.22 (1 H, d, J = 10.4 Hz, H-6). MS m/z (%) 890 (M^+, 1), 828 (1), 758 (20), 696 (22), 626 (4), 75 (100).

*(2E)-1α,25-Dihydroxy-2-(2-hydroxyethylidene)-9α-(3-hydroxypropyl)-19-norvitamin D_3 (**4d**).* To a solution of **33a** (13.1 mg, 0.0147 mmol) in MeOH (300 μL) was added CSA (34.1 mg, 0.147 mmol, 10.0 equiv), and the mixture was stirred for 2 h. Then, 5% $NaHCO_3$ was added to the reaction, the mixture was extracted with AcOEt, and the organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 5% MeOH/AcOH to give **4d** (5.7 mg, 77%). **4d**: ^{1}H NMR ($CDCl_3$) δ: 0.56 (3 H, s, H-18), 0.94 (3 H, d, J = 6.0 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.17 (1 H, t, J = 8.7 Hz), 2.29, 2.41 (each 1 H, m, H-4), 2.89 (1 H, m, H-9), 3.16 (1 H, m, H-10), 3.48, 3.60 (each 1 H, m, CH_2CH_2OH), 4.09 (1 H, m, CH_2OH), 4.31 (3 H, m, H-1, CH_2OH, OH), 4.40 (1 H, br.s, OH), 4.80 (1 H, m, H-3), 5.74 (1 H, m, C=CH), 5.97 (1 H, d, J = 10.6 Hz, H-7), 6.11 (1 H, d, J = 10.6 Hz, H-6). MS m/z (%): 504 (M^+, 1), 486 (10), 468 (34), 450 (57), 432 (24), 386 (100), 339 (14). UV λ_{max} (EtOH): 246, 254, and 264 nm.

*(2Z)-1α,25-Dihydroxy-2-(2-hydroxyethylidene)-9α-(3-hydroxypropyl)-19-norvitamin D_3 (**4e**).* **33b** (2*Z*-isomer, 11.1 mg, 0.0124 mmol) was deprotected similarly to give **4e** (3.4 mg, 54%). **4e**: ^{1}H NMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.93 (3 H, d, J = 6.3 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.38 (1 H, m, H-10), 2.66 (1 H, dd, J = 13.0, 4.6 Hz, H-4), 2.76 (1 H, dd, J = 14.0, 5.7 Hz, H-10), 2.89 (1 H, m, H-9), 3.61 (2 H, m, CH_2CH_2OH), 4.26 (1 H, dd, J = 12.6, 6.4 Hz, CH_2OH), 4.36 (1 H, dd, J = 12.6, 7.1 Hz, CH_2OH), 4.43 (1 H, m, H-3), 4.84 (1 H, m, H-1), 5.84 (1 H, m, C=CH), 5.91 (1 H, d, J = 11.0 Hz, H-7), 6.37 (1 H, d, J = 11.0 Hz, H-6). MS m/z (%) 504 (M^+, 1), 486 (17), 468 (58), 450 (100), 432 (41), 339 (23). UV λ_{max} (EtOH): 246, 254, and 264 nm.

Graphical Manipulations and Ligand Docking. Graphical manipulations were performed using SYBYL (Tripos, St. Louis).

■ ASSOCIATED CONTENT

Supporting Information

Figures S1–S6 and Tables S1–S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

VDR, vitamin D receptor; HVDRR, hereditary vitamin D-resistant rickets; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; RXR, 9-*cis*-retinoic acid receptor; VDRE, vitamin D-responsive elements; AF-2, activation function 2; DBD, DNA binding domain; LBD, ligand binding domain; LBP, ligand-binding pocket; PPARγ, peroxisome proliferator-activating receptor γ; CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5

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Crystal structures of complexes of vitamin D receptor ligand-binding domain with lithocholic acid derivatives

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Abstract The secondary bile acid lithocholic acid (LCA) and its derivatives act as selective modulators of the vitamin D receptor (VDR), although their structures fundamentally differ from that of the natural hormone 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. Here, we have determined the crystal structures of the ligand-binding domain of rat VDR (VDR-LBD) in ternary complexes with a synthetic partial peptide of the coactivator MED1 (mediator of RNA polymerase II transcription subunit 1) and four ligands, LCA, 3-keto LCA, LCA acetate, and LCA propionate, with the goal of elucidating their agonistic mechanism. LCA and its derivatives bind to the same ligand-binding pocket (LBP) of VDR-LBD that 1,25$(OH)_2D_3$ binds to, but in the opposite orientation; their A-ring is positioned at the top of the LBP, whereas their acyclic tail is located at the bottom of the LBP. However, most of the hydrophobic and hydrophilic interactions observed in the complex with 1,25$(OH)_2D_3$ are reproduced in the complexes with LCA and its derivatives. Additional interactions between VDR-LBD and the C-3 substituents of the A-ring are also observed in the complexes with LCA and its derivatives. These may result in the observed difference in the potency among the LCA-type ligands.—Masuno, H., T. Ikura, D. Morizono, I. Orita, S. Yamada, M. Shimizu, and N. Ito. **Crystal structures of complexes of vitamin D receptor ligand-binding domain with lithocholic acid derivatives.** *J. Lipid Res.* **2013.** 54: **2206–2213.**

Supplementary key words nuclear receptor • structure-function relationship • bile acid • hypercalcemia

The active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], regulates calcium homeostasis (1). It also promotes cellular differentiation, inhibits cellular proliferation, and suppresses the immune system (2–7). It has been used clinically to treat renal osteodystrophy, vitamin D-dependent rickets type I, and X-linked hypophosphatemic rickets, among other conditions (8–14). Most of its effects are mediated by its specific binding to the vitamin D receptor (VDR), which is a member of nuclear receptor (NR) super family (15). When 1,25$(OH)_2D_3$ is bound to VDR, it activates it by inducing conformational changes. The activated complex, VDR/1,25$(OH)_2D_3$, binds as a heterodimer with the retinoid X receptor (RXR) to vitamin D response elements located in the promoter region of the target genes. Recruitment of coactivator proteins to this heterodimer is also essential to the transactivation. However, clinical use of 1,25$(OH)_2D_3$ is limited because therapeutic doses can give rise to significant hypercalciuria and hypercalcemia (16). A number of synthetic ligands to VDR have been developed for medical use; however, most of them can also cause similar problems because they are derived from 1,25$(OH)_2D_3$.

Several synthetic compounds without the vitamin D_3 scaffold have been reported to bind to VDR and have VDR-modulating activities, including growth inhibition of cancer cells and keratinocytes and induction of leukemic cell differentiation, with less calcium mobilization side effects than 1,25$(OH)_2D_3$ (17). Therefore, these synthetic compounds are expected to be therapeutics for cancer, leukemia, and psoriasis. Subsequently, Makishima et al. discovered that secondary bile acids, including lithocholic acid (LCA) and its derivatives, also behaved as VDR agonists (18–20). LCA acts as a detergent to stabilize fats for absorption, and it has been implicated in human and experimental animal carcinogenesis. However, the agonistic behavior of LCA as a ligand recognized by VDR was not common knowledge because the structure of LCA is completely different from that of vitamin D_3. Additional studies showed that VDR had dual functions as a metabolic sensor of bile acids and as an endocrine receptor for 1,25$(OH)_2D_3$.

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Abbreviations: AcOEt, ethyl acetate; AF2, activation function 2; LBD, ligand-binding domain; LBP, ligand-binding pocket; LCA, lithocholic acid; MED1, mediator of RNA polymerase II transcription subunit 1; NR, nuclear receptor; 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; RMSD, root-mean-square deviation; RXR, retinoid X receptor; VDR, vitamin D receptor.

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