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Revisiting the 7,8-*cis*-vitamin D_3 derivatives: synthesis, evaluating the biological activity, and study of the binding configuration

Daisuke Sawada [a,†], Shinji Kakuda [b], Midori Kamimura-Takimoto [b], Akiko Takeuchi [b], Yotaro Matsumoto [a,‡], Atsushi Kittaka [a,*]

[a] Faculty of Pharmaceutical Sciences, Teikyo University, Kaga, Itabashi, Tokyo 173-8605, Japan
[b] Teijin Institute for Bio-medical Research, Teijin Pharma Ltd., Hino, Tokyo 191-8512, Japan

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ABSTRACT

Four 7,8-*cis*-1α,25-dihydroxyvitamin D_3 derivatives, 7,8-*cis*- and 7,8-*cis*-14-*epi*-1α,25-dihydroxy-19-norvitamin D_3 as well as 7,8-*cis*- and 7,8-*cis*-14-*epi*-1α,25-dihydroxyvitamin D_3 were synthesized, and their chemical stability was characterized. In our previous work, we disclosed that 14-*epi*-19-nortachysterol showed the unprecedented binding configuration in human vitamin D receptor (hVDR), that is, 5,6- and 7,8-*s*-*trans* configuration. However, this configuration is variable because of the rotation at the single bond between C7 and C8. For the precise discussion of the 7,8-*s*-*trans* configuration, we designed and synthesized the 7,8-*cis*-locked skeleton of vitamin D_3 derivatives. Among four analogs, the 19-nor derivatives were stable at ambient temperature, and their hVDR binding affinity and co-crystallographic analysis of their hVDR complexes were studied. The other derivatives with the triene system were isomerized to corresponding previtamin D_3 and vitamin D_3.

1. Introduction

In recent years, various papers concerning the biological study of vitamin D have been published, and continue to increase in number. These papers have disclosed new functions of vitamin D in our body; inevitably, it is thought to be concerned with many diseases, and to be a potential therapeutic agent.[1] For medical treatments, new vitamin D analogs which selectively work against specific diseases are now a topic of intense interest.

Recently, we reported a new type of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$ (**1**)] analog, 14-*epi*-1α,25-dihydroxy-19-nortachysterol (**2**), which showed an unprecedented binding configuration in human vitamin D receptor (hVDR), that is, 5,6- and 7,8-*s*-*trans* configuration with 15% of binding affinity for hVDR (Fig. 1).[2] This opposite binding configuration to natural active vitamin D_3 drove us to search further for this 7,8-*s*-*trans* skeleton, which would afford unique biological activities. However, the configuration is variable because of the rotation at the single bond between C7 and C8, and a precise discussion of the 7,8-*s*-*trans* configuration might be difficult. To treat this configuration as a more stable and rigid form, we planned to fix this *s*-*trans* triene moiety, i.e., we focused on the 7,8-*cis*-locked skeleton of vitamin D_3. We also would like to disclose the effects of the stereochemistry at C14, because the tachysterol skeleton allows evaluation of biological activity only with the stable C14-*epi*-form, and we know that it is difficult to compare both stereochemistries at C14.[2] Here, we designed compounds **3**–**6** as target compounds, in which compound **5** was known,[3] and by comparison of these compounds (**3**–**6**), along with **1** and **2**, it should be possible to discuss the biological effects of this new binding mode, 7,8-*s*-*trans* form of **2**, and 7,8-*cis*-skeleton of **3**–**6**.

The vitamin D_3 analogs with 7,8-*cis*-skeleton have already been reported: Okamura et al. proposed the 7,8-*cis*-derivatives starting from vinylallene thermolysis in 1978,[4a] and the other 7,8-*cis*-derivatives with the different triene system via thermal rearrangements of vitamin D_3 isomers including vinylallenes were reported.[4] The first example with 7,8-*cis*-skeleton of vitamin D_3 was disclosed in 1986 by Barrack and Okamura,[5] and the 9α-OH substituted derivative (**7**) was synthesized by Moriño et al. in 1991[6] and by Dauben and Greenfield in 1992.[7] In 1994, Okamura et al. reported the synthesis and thermodynamic profile of 7,8-*cis*-vitamin D_3 (**5**, **8**),[3] and Vandewalle et al. synthesized the 14-*epi*-19-nor derivative **9** as a side product in their 14-*epi*-19-nor-

* Corresponding author. Tel.: +81 3 3964 8109; fax: +81 3 3964 8117; e-mail address: akittaka@pharm.teikyo-u.ac.jp (A. Kittaka).
† Present address: Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan.
‡ Present address: Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan.

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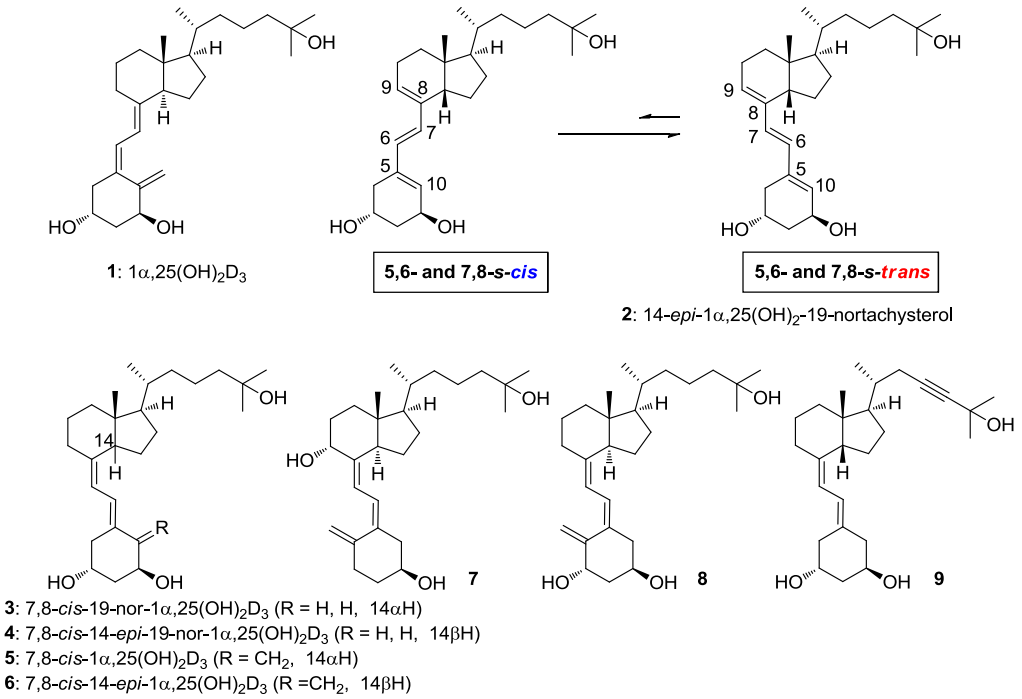
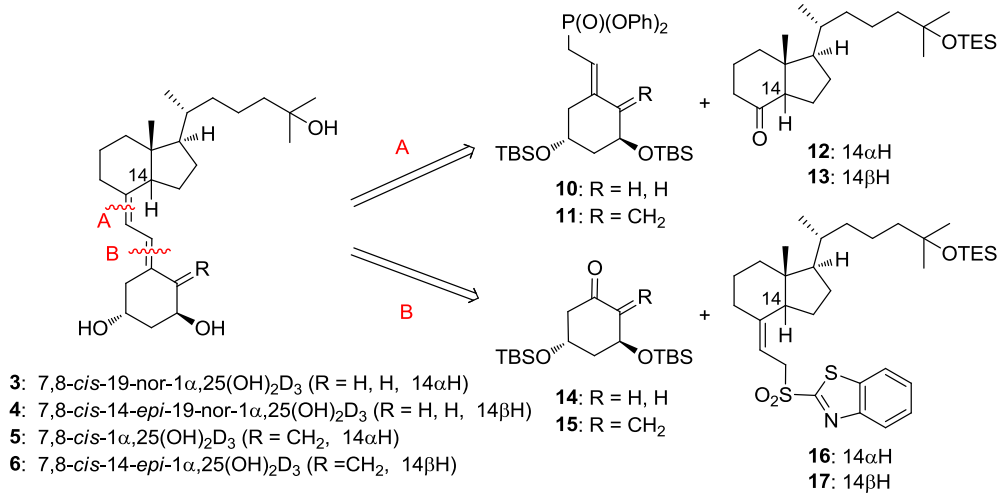


Fig. 1. Single bond rotation in the triene moiety of 14-*epi*-19-nortachysterol and the structures of active vitamin D_3 **1** and 7,8-*cis*-vitamin D_3 derivatives **3**–**9**.

$1\alpha,25(OH)_2D_3$ synthetic work in 2001.[8] In the above papers, there were no precise discussions about the binding configuration in VDR, and little information about biological activity. This time, we report on comprehensive studies of synthesis, hVDR binding affinity, and binding configuration in hVDR for 7,8-*cis*-vitamin D_3.



Scheme 1. Retrosynthetic analysis of 7,8-*cis*-vitamin D_3 derivatives.

2. Results and discussion

The synthetic strategy is described in Scheme 1. We prepared two synthetic plans, and the target compounds were divided into two fragments, the A-ring and CD-ring moieties. The critical point was how to construct the *cis* geometry at the C7,8 double bond, and for this purpose we adopted the Horner–Wadsworth–Emmons (HWE) reaction[9] with ketone using a modification of Ando's method.[10] Using this reaction, the olefin geometry might not be completely controlled, however, the undesired 7,8-*trans* compounds are of interest.[3]

As the first trial, we used the synthetic route A, and the CD-ring fragments were the known compounds, which were prepared from vitamin D_3 (Scheme 1).[11] To prepare the 19-nor-A-ring fragment **10**, we started from the known allyl alcohol **18**, which was once transformed into allyl chloride (Scheme 2).[12] This was dissolved immediately in DMF with Cs_2CO_3 and Bu_4NI (TBAI), and then $HP(O)(OPh)_2$ was slowly added to the mixture over 1 h.[13] After stirring for a further hour, the desired phosphonate **10**, the 19-nor-A-ring fragment was afforded in good yield. For the A-ring fragment **11**, we planned the stereoselective ring closing reaction from en-ynol **20**.[14] According to the literature,[15] once the acetyl group was introduced

at the hydroxy group, the resultant acetate reacted with $Pd(OAc)_2$, PPh_3, and Et_3N to afford the desired *E*-allylacetate, whose acetyl group was successively removed by methanolysis (**21**).[16] Using the above strategy, **21** was transformed into the phosphonate **11**, the A-ring fragment. Now each of the two A-ring and CD-ring fragments was in hand, we tried the HWE reaction. The normal reaction conditions using nBuLi with **10** and **12** afforded the coupling products, and after deprotection of silyl groups, the *cis* isomer **3** was appeared due to the effects of the (OPh) groups at the phosphonate moiety in the A-ring fragment [*cis*(**3**)/*trans*(**22**[12])=1/2 ratio].[9] After several experiments, we found that the addition of HMPA to the THF solvent brought a better stereoselectivity [*cis*(**3**)/*trans*(**22**)=4/5, y. 46%, conv. y. 53%]. In contrast, in the case of **11** as the A-ring fragment, HMPA had negative effects on the stereoselectivity, and the normal conditions afforded the coupled compounds in *cis*(**5**)[3]/*trans*(**24**)[3,17]=1/2 ratio (y. 37%, conv. y. 40%). Using ketones as substrates, the desired *cis*-selective reactions were not realized, and these results should depend on the steric factors of the transition states in the reaction.

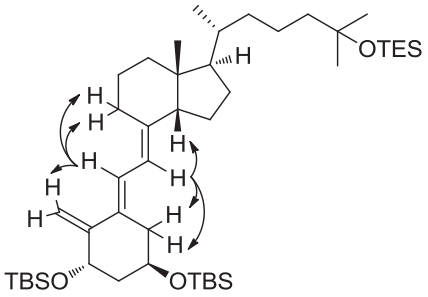
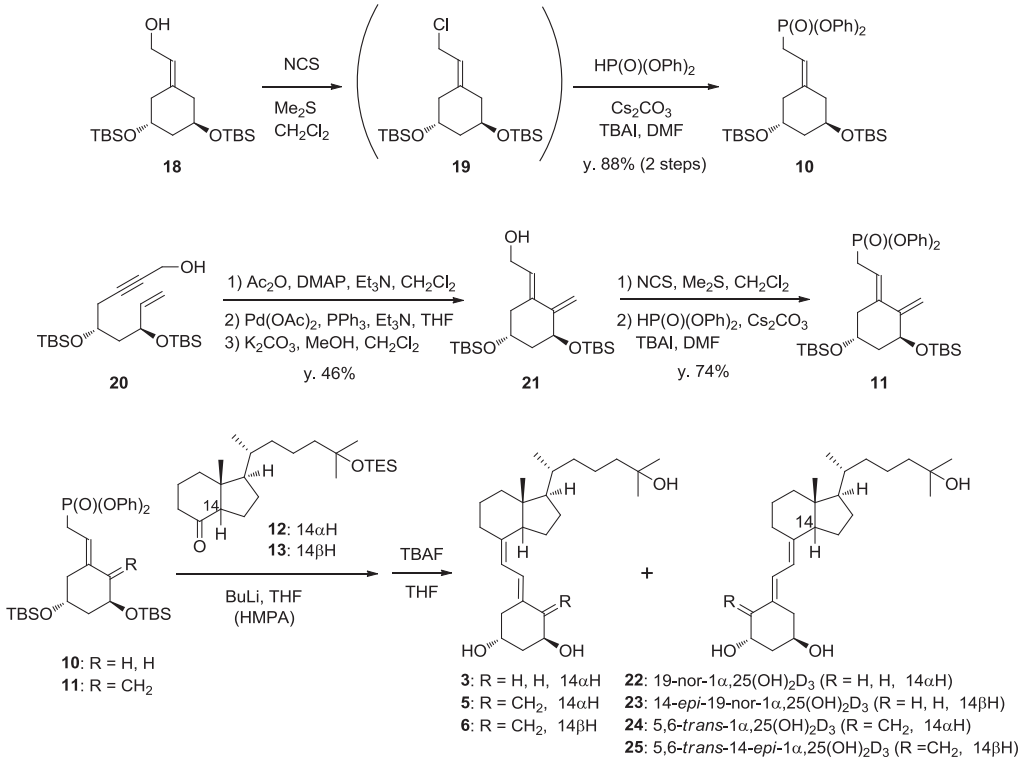


Fig. 2. Representative NOE observed.

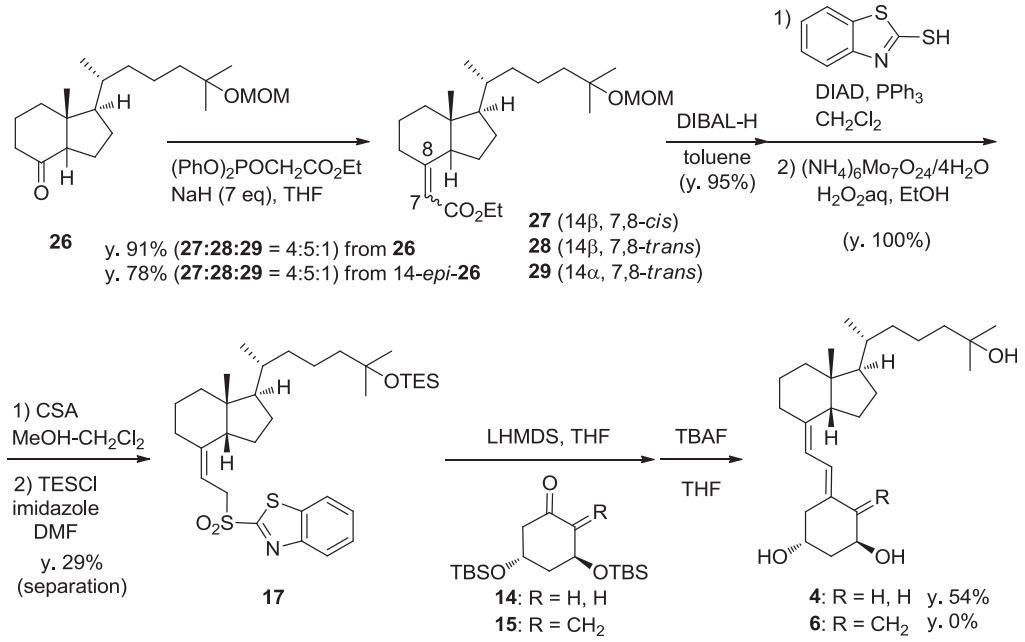
compounds **28**, **29**, and 14α-7,8-*cis*-alkene were known in Refs. 20 and 21, we could determine the structure **27** should have the 14β-7,8-*cis*-geometry as shown in Scheme 3. Interestingly, this reaction caused C14-epimerization dominantly over the HWE reaction, and the ratio of the products was not related to the



Scheme 2. Synthetic plan A.

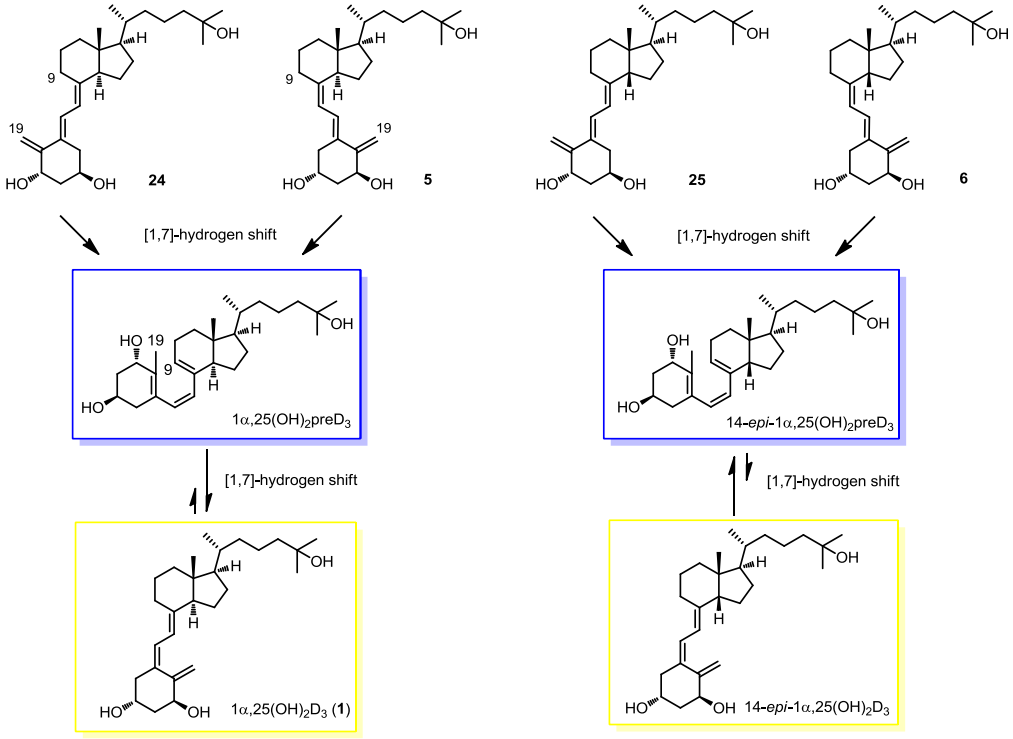
Similarly, with **13** as the 14-*epi*-CD-ring fragment, unfortunately, the reaction with **10** gave only *trans* product (**23**,[8,18] y. 75%, conv. y. 80%) in spite of much effort, and the reaction with **11** afforded slightly better results [*cis*(**6**)/*trans*(**25**)[19]=1/15, y. 36%]. The stereochemistry at the C7,8 double bond of **25** was determined by NOE experiments using the silyl protected compound, which did not indicate any isomerization (Fig. 2).

Therefore, we attempted synthetic plan B in Scheme 1. In plan B, the CD-ring fragment should equip the 7,8-*cis* geometry. Practically, the known ketone **26**[19] was reacted with Ando's reagent in the presence of NaH in THF,[10] and three products were obtained: desired **27** (14β-7,8-*cis*), undesired **28** (14β-7,8-*trans*)[20] as well as **29** (14α-7,8-*trans*)[21] in a 4:5:1 ratio, but another desired compound (14α-7,8-*cis*)[21] did not appear (Scheme 3). Since the above stereochemistry at C14 of the substrate. The mixture of the unsaturated esters **27**–**29** was further transformed into alcohol by DIBAL-H and then benzothiazole-sulfone. In this mixture, the protective group was changed to TES from MOM, and we were able to separate the stereoisomers by silica-gel column chromatography to give compound **17**. The A-ring fragments **14**[12] and **15**[22] were prepared according to the literature, and we tried the Julia olefination with the CD-ring fragment **17**.[21] Using **17** and **14**, the coupled compound was smoothly obtained, and after deprotection the desired compound **4** was afforded in 54% yield (2 steps), which was not available in plan A. However, using **17** and **15**, the A-ring fragment with 19-methylene, the coupled compound was not produced, and some unknown compounds appeared (Scheme 3).



Scheme 3. Synthetic plan B.

Here, we synthesized four desired compounds **3**–**6**, although their stereoselectivities were not sufficient. Among them, the compounds with 19-methylene group, i.e., **5** and **6**, and the byproducts in HWE reactions in Scheme 2, 5,6-*trans*-vitamin D_3 (**24**), 14-*epi*-5,6-*trans*-vitamin D_3 (**25**) indicated noteworthy profiles: after isolation by HPLC, each compound gradually decreased during NMR operations, and by contrast, some other compounds increased. By careful NMR observation in $CDCl_3$ or CD_3OD at ambient temperature, we found that all compounds **5**, **6**, **24**, and **25** isomerized to the mixture of the corresponding previtamin D and, finally, vitamin D skeleton that were all known compounds,[23,24] probably due to double [1,7]-hydrogen shift between C9 and C19. This process includes the vitamin D-previtamin D equilibrium.[11,23] In this isomerization, 7,8-*cis* compounds (**5**, **6**) showed faster transformation than those of 7,8-*trans* compounds (**24**, **25**), and of course, 14-*epi*-analogs (**6**, **25**) preferred staying in the previtamin D form (Scheme 4).[3,11,24] These phenomena were different observation from the literature published previously.[3] We were therefore not able to evaluate their biological activity, and using 19-nor-compounds **3** and **4**, we estimated the hVDR binding affinity and



Scheme 4. Isomerism of 7,8-*cis*-vitamin D_3 (**5**,**6**) and 5,6-*trans*-vitamin D_3 (**24**,**25**).

their binding configuration in the ligand binding domain of hVDR by X-ray co-crystallographic analysis. Table 1 describes the relative binding affinities of compounds **2**, **3**, and **4** to 1α,25(OH)$_2$D$_3$ (**1**),[2,25] and 19-nor-7,8-*cis*-vitamin D derivatives indicated lower affinity than 14-*epi*-19-nor-1α,25(OH)$_2$-tachysterol (**2**), regardless of the stereochemistry at C14.

The X-ray co-crystallographic analyses of **3** and **4** in the ligand binding domain (LBD) of the hVDR were accomplished, and the stereochemistry of **3** and **4** was also clear. In Fig. 3, the superimposed structure of **3** and the natural hormone **1**[26] is shown. The diene part of **3** occupies actually different positions from that of **1** in the LBD of the hVDR. Next, the structures of **3** and **4** compared to 2α-methyl substituted 14-*epi*-19-nor-1α,25(OH)$_2$-tachysterol (**30**),[2] respectively, and the superimposed structures of them are in Figs. 4 and 5. In both figures, the A-ring and C5-8 alkene moieties showed relatively good overlaps, however, the CD-ring moiety indicated apparent conflicts. It is noteworthy that although the stereochemistry at C14 of compounds **4** and **30** was the same, their configurations of the CD-ring moiety were remarkably different, for example, the position of the 18-methyl group, and these differences should explain the decreased binding affinity.[27] In our previous work, an appropriate distribution of the hydroxy groups at C1 and C3 was thought to be essential for the binding affinity, and in this case similar distributions were observed.[2] In this 7,8-*cis*-vitamin D$_3$ skeleton, the C7,8 double bond fixed by the A-ring moiety should determine the CD-ring configuration, while the single bond at C7,8 in the tachysterol skeleton would bring flexibility and induced fitting for the binding pocket of hVDR, and that is why the configuration of the CD-ring moieties showed such differences. The results in Table 1 clearly indicate the lower binding affinity of the 7,8-*cis* fixed molecule. Nevertheless, 14-*epi*-19-nor-1α,25(OH)$_2$-tachysterol (**2**) had moderate binding affinity and bound to hVDR in 7,8-*s*-*trans* form; therefore, the tachysterol skeleton was thought to have unusual flexibility and attractive potential for binding to the receptor.

3. Conclusions

The 7,8-*cis*-vitamin D$_3$ derivatives were synthesized, and among them we disclosed the unique isomerization of compounds **5**, **6**, **24**, and **25** to the corresponding previtamin D and vitamin D skeleton. Also, we evaluated the binding affinity and configuration of the 19-nor-compounds, **3** and **4**, for hVDR. By comparing them and 14-*epi*-19-nor-1α,25(OH)$_2$-tachysterol (**2**), the 7,8-*cis*-skeleton indicated low binding affinity regardless of the stereochemistry at C14, and the tachysterol skeleton should have outstanding flexibility and potential. Further studies of the new tachysterol analogs to make the most of their merits are ongoing.

4. Experimental

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$_3$)$_4$Si (δ 0.00 ppm) as an internal standard. ^{13}C NMR spectra were referenced with deuterated solvent (δ 77.0 ppm for CDCl$_3$, and 128.0 ppm for C$_6$D$_6$). IR spectra were recorded on JASCO FTIR-800 Fourier Transform Infrared Spectrophotometer. High resolution mass spectra were obtained on SHIMADZU LCMS-IT-TOF mass spectrometer in positive electrospray ionization (ESI) method. 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). High performance liquid chromatography (HPLC) was carried out on a SHIMADZU HPLC system consisting of the following equipments: pump, LC-6AD; detector, SPD-10A; column, YMC-Pack ODS-A. All experiments were performed under anhydrous conditions in an atmosphere of argon, unless otherwise mentioned.

Table 1
Relative binding affinity for hVDR

Compound	hVDR binding affinity[a]
1α,25(OH)$_2$D$_3$ (**1**)	100
14-*epi*-1α,25(OH)$_2$-19-Nortachysterol (**2**)	15[b]
7,8-*cis*-19-Nor-1α,25(OH)$_2$D$_3$ (**3**)	<0.5
7,8-*cis*-14-*epi*-19-Nor-1α,25(OH)$_2$D$_3$ (**4**)	<0.5

[a] The potency of 1α,25(OH)$_2$D$_3$ is normalized to 100.
[b] Ref. 2.

4.2. Synthesis of 7,8-*cis*-1α,25-dihydroxy-19-norvitamin D$_3$ (3)

*4.2.1. Diphenyl (2-[(3R,5R)-3,5-bis{(tert-butyldimethylsilyl)oxy}cyclohexylidene]ethyl)phosphonate (**10**).* To a solution of NCS (539 mg, 4.04 mmol) in dry CH$_2$Cl$_2$ (6 mL) was added dimethylsulfide (0.297 mL, 4.04 mmol) at 0 °C, and stirred at the same temperature for 20 min. To the mixture was added **18** (223 mg, 0.577 mmol) in dry CH$_2$Cl$_2$ (2 mL) at −20 °C and stirred for 30 min at the same temperature. Then, the mixture was warmed up to 0 °C, further stirred for 30 min, and added water at the same temperature, and extracted with hexane. The organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated. The residue was purified by short column chromatography on neutral silica gel (hexane/AcOEt=30/1) to give a crude product, which was used without further separation. To a solution of a part of the above product (135 mg, 0.333 mmol) in dry DMF (4 mL) were added tetrabutylammonium iodide (615 mg, 1.67 mmol), Cs$_2$CO$_3$ (542 mg, 1.67 mmol) at room temperature. Then, to the mixture was slowly added (PhO)$_2$P(O)H (0.128 mL 0.666 mmol) over 75 min. After 1 h, the reaction was quenched by saturated aqueous NH$_4$Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=15/1–8/1) to give **10** (201 mg, 88% in 2 steps) as a colorless oil:

$[\alpha]_D^{25}$ +21.5 (*c* 1.00, CHCl$_3$); IR (neat) 3066, 2951, 2931, 2889, 2858, 1593, 1489, 1385, 1281, 1254, 1192, 1080, 1053, 1007, 933, 837, 775, 687 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.001 (s, 3H), 0.003 (s, 3H), 0.02 (s, 3H), 0.03 (s, 3H), 0.84 (s, 9H), 0.86 (s, 9H), 1.69–1.72 (m, 2H), 2.09–2.14 (m, 2H), 2.22–2.26 (m, 1H), 2.32–2.35 (m, 1H), 2.85 (ddd, *J*=6.9, 15.9, 47.6 Hz, 1H), 2.94 (ddd, *J*=8.7, 15.9, 48.1 Hz, 1H), 4.03–4.05 (m, 2H), 5.38 (dd, *J*=7.3, 15.1 Hz, 1H), 7.12–7.18 (m, 6H), 7.27–7.31 (m, 4H); ^{13}C NMR (100 MHz, CDCl$_3$) δ −4.9, −4.84, −4.86, −4.7, 18.0, 18.1, 25.3, 25.7, 25.8, 26.7, 37.1, 43.4, 45.1, 67.48, 67.50, 67.78, 67.80, 77.2, 112.5, 112.6, 120.55, 120.57, 120.59, 120.62, 125.0, 129.7, 139.5, 139.6, 150.34, 150.36, 150.42, 150.5; HRFABMS calcd for C$_{32}$H$_{51}$NaO$_5$Si$_2$P 625.2905, found 625.2927.

*4.2.2. 7,8-cis-1α,25-Dihydroxy-19-norvitamin D$_3$ (**3**) and 1α,25-dihydroxy-19-norvitamin D$_3$ (**22**).* To a solution of **10** (38 mg, 0.063 mmol) in THF (0.7 mL) and HMPA (0.05 mL) was added nBuLi (1.65 M hexane solution, 0.042 mL, 0.069 mmol) at −78 °C and stirred at the same temperature for 25 min. To the mixture was added **12** (16 mg, 0.042 mmol) in THF (0.3 mL) at −78 °C and the whole mixture was gradually warmed up to 0 °C over 3 h. After stirring for further 40 min at the same temperature, the reaction was quenched by aqueous NH$_4$Cl at 0 °C, and the mixture was extracted with AcOEt, washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by flush column

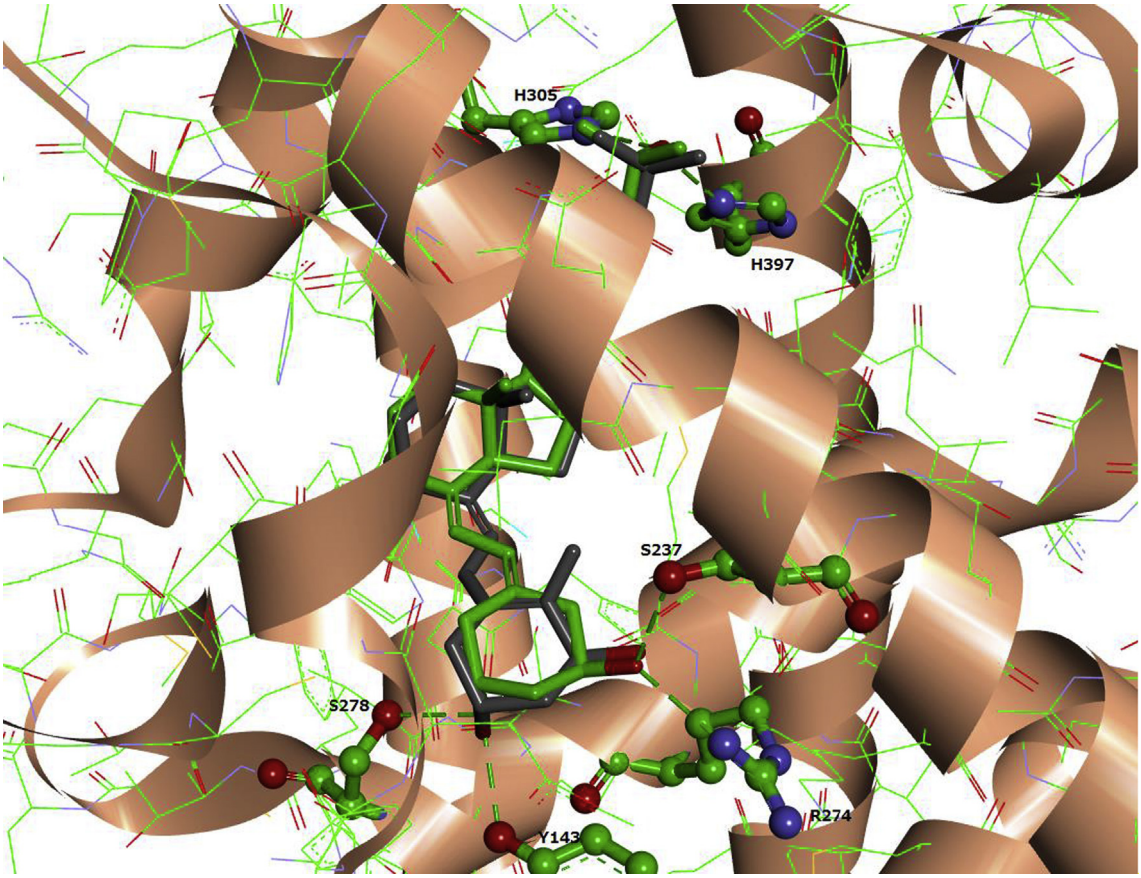


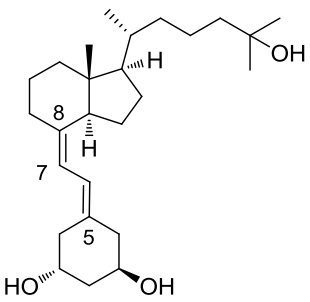
Fig. 3. Superimposed three dimensional structures of **3** (green) and **1** (gray) based on X-ray co-crystallographic analysis in the hVDR ligand binding domain. Protein Data Bank accession numbers are 3X36 for **3** and 1DB1[26] for **1**.

chromatography on silica gel (hexane/AcOEt=50/1–7/1) to give crude protected compounds as an inseparable mixture of isomers with the substrate **12** (1.7 mg). Then, to a solution of the crude compounds obtained above in THF (1.0 mL) was added TBAF (0.17 mL, 1.0 M THF solution, 0.17 mmol) at 0 °C and stirred at room temperature for 17 h. After the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=1:1–0/1) followed by purification on preparative silica gel TLC plate (AcOEt) to give **3** and **22** as an inseparable mixture. The isomers were separated by reverse-phase HPLC (YMC-Pack ODS column) using MeOH/H_2O (9/1) as a solvent system to give **3** (3.0 mg, 20% in two steps) as a colorless oil and **22**[12] (3.7 mg, 26% in two steps). **Compound 3**: $[\alpha]_D^{25}$ +52.5 (c 0.20, $CHCl_3$); UV (EtOH) λ_{max} 261.6, 252.0, 243.4 nm, λ_{min} 258.2, 247.2, 202.4 nm; IR (neat) 3365, 2950, 2855, 1596 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.63 (s, 3H), 0.95 (d, J=6.9 Hz, 3H), 1.04–1.08 (m, 1H), 1.23 (s, 3H), 1.23 (s, 6H), 1.18–1.34 (m, 3H), 1.33–1.48 (m, 6H), 1.52–1.62 (m, 2H), 1.80–1.99 (m, 6H), 2.01–2.09 (m, 2H), 2.11–2.19 (m, 2H), 2.37 (dd, J=6.9, 13.7 Hz, 1H), 2.42 (dd, J=3.4, 13.1 Hz, 1H), 2.53 (dd, J=3.4, 13.7 Hz, 1H), 4.02–4.11 (m, 2H), 6.10 (d, J=11.7 Hz, 1H), 6.45 (d, J=11.7 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.7, 19.2, 20.9, 24.2, 26.5, 28.4, 29.3, 29.5, 36.0, 36.2, 36.6, 39.3, 40.8, 42.3, 44.5, 45.5, 46.7, 55.0, 56.1, 67.4, 67.6, 71.2, 119.6, 125.0, 131.6, 142.2; HRFABMS calcd for $C_{26}H_{44}NaO_3$ 427.3183, found 427.3186.

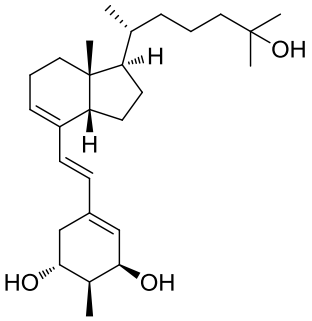
*4.2.3. 14-epi-1α,25-Dihydroxy-19-norvitamin D_3 (**23**).* To a solution of **10** (32 mg, 0.053 mmol) in THF (0.7 mL) was added nBuLi (1.65 M hexane solution, 0.035 mL, 0.058 mmol) at −78 °C and stirred at the same temperature for 15 min. To the mixture was added **13** (14 mg, 0.035 mmol) in THF (0.3 mL) at −78 °C and the whole mixture was gradually warmed up to 0 °C over 2.5 h. After stirring for further 40 min at the same temperature, the reaction was quenched by aqueous NH_4Cl at 0 °C, and the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flush column chromatography on silica gel (hexane/AcOEt=50/1–7/1) to give crude protected compounds as an inseparable mixture of isomers with the substrate **13** (1.0 mg). Then, to a solution of the crude compounds obtained above in THF (1.0 mL) was added TBAF (0.28 mL, 1.0 M THF solution, 0.28 mmol) at 0 °C and stirred at room temperature for 16 h. After the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=1:1–0/1) followed by purification on preparative silica gel TLC plate (AcOEt) to give **23**, which was further purified by reverse-phase HPLC (YMC-Pack ODS column) using MeOH/H_2O (9/1) as a solvent system to give **23**[8] (10.2 mg, 75% in two steps).

4.3. Synthesis of 7,8-*cis*-1α,25-dihydroxyvitamin D_3 (5)

*4.3.1. (E)-2-[(3S,5R)-3,5-Bis{(tert-butyldimethylsilyl)oxy}-2-methylenecyclohexylidene]ethan-1-ol (**21**).* To a solution of **20** (470 mg, 1.18 mmol) in CH_2Cl_2 (5 mL) were added triethylamine (329 μL, 2.36 mmol), acetic anhydride (181 μL, 1.77 mmol), and then dimethylaminopyridine (7.2 mg, 0.059 mmol) at 0 °C and stirred at room temperature for 45 min. After the reaction was quenched by NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with



3: 7,8-*cis*-19-nor-1α,25(OH)$_2$D$_3$



30: 2α-methyl-14-*epi*-1α,25(OH)$_2$-19-nortachysterol

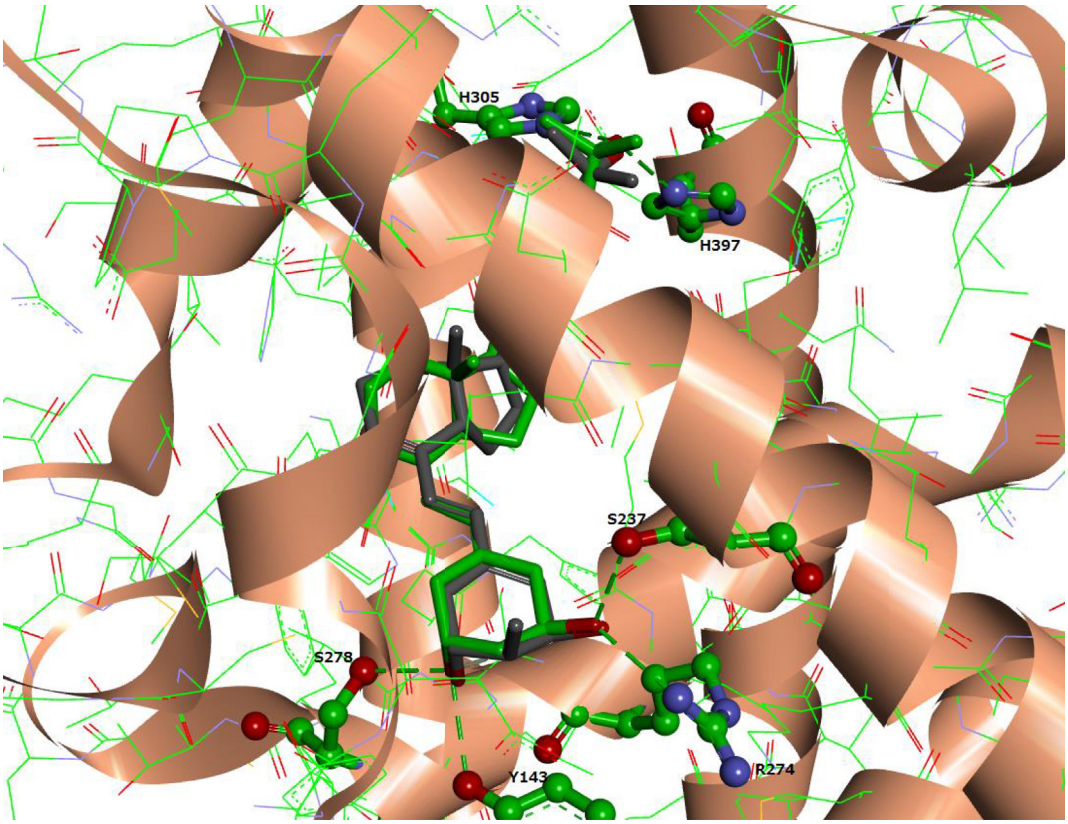
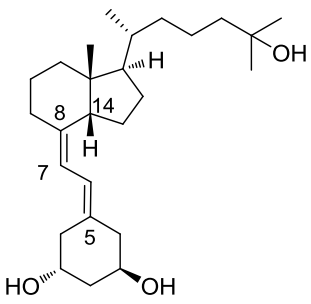


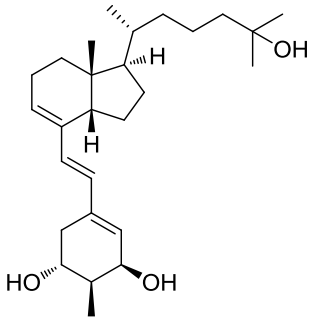
Fig. 4. Superimposed three dimensional structures of **3** (green) and **30** (gray) based on X-ray co-crystallographic analysis in the hVDR ligand binding domain. Protein Data Bank accession number is 3X36 for **3**.

brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by short column chromatography on neutral silica gel (hexane/AcOEt=50/1–10/1) to give a crude product, which was used without further separation. To a solution of a part of the above product (21 mg, 0.0476 mmol) in dry THF (2 mL) were added triethylamine (100 μL, 0.714 mmol), triphenylphosphine (12.5 mg, 0.0476 mmol), and then $Pd(OAc)_2$ (5.3 mg, 0.0238 mmol) at room temperature, and stirred at 50 °C. After 24 h, the reaction mixture was concentrated, and the residue was purified by short column chromatography on neutral silica gel (hexane/AcOEt=50/1–40/1) to give a crude product, which was used without further separation. To a solution of a part of the product (10.5 mg, 0.0238 mmol) in CH_2Cl_2 (1 mL) and MeOH (1 mL) were added K_2CO_3 (7 mg, 0.036 mmol) at 0 °C and stirred at room temperature for 30 min. After the reaction was quenched by NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=10/1–8/1) to give **21**[16] (201 mg, 88% in 2 steps) as a colorless oil.

*4.3.2. Diphenyl ((E)-2-[(3S,5R)-3,5-bis{(tert-butyldimethylsilyl)oxy}-2-methylenecyclohexylidene]ethyl)phosphonate (**11**).* To a solution of NCS (263 mg, 1.97 mmol) in dry CH_2Cl_2 (4 mL) was added dimethylsulfide (0.144 mL, 1.97 mmol) at 0 °C, and stirred at the same temperature for 20 min. To the mixture was added **21** (112 mg, 0.281 mmol) in dry CH_2Cl_2 (1 mL) at −20 °C and stirred for 30 min at the same temperature. Then, the mixture was warmed up to 0 °C, further stirred for 1.5 h, and added water at the same



4: 7,8-*cis*-14-*epi*-19-nor-1α,25(OH)$_2$D$_3$



30: 2α-methyl-14-*epi*-1α,25(OH)$_2$-19-nortachysterol

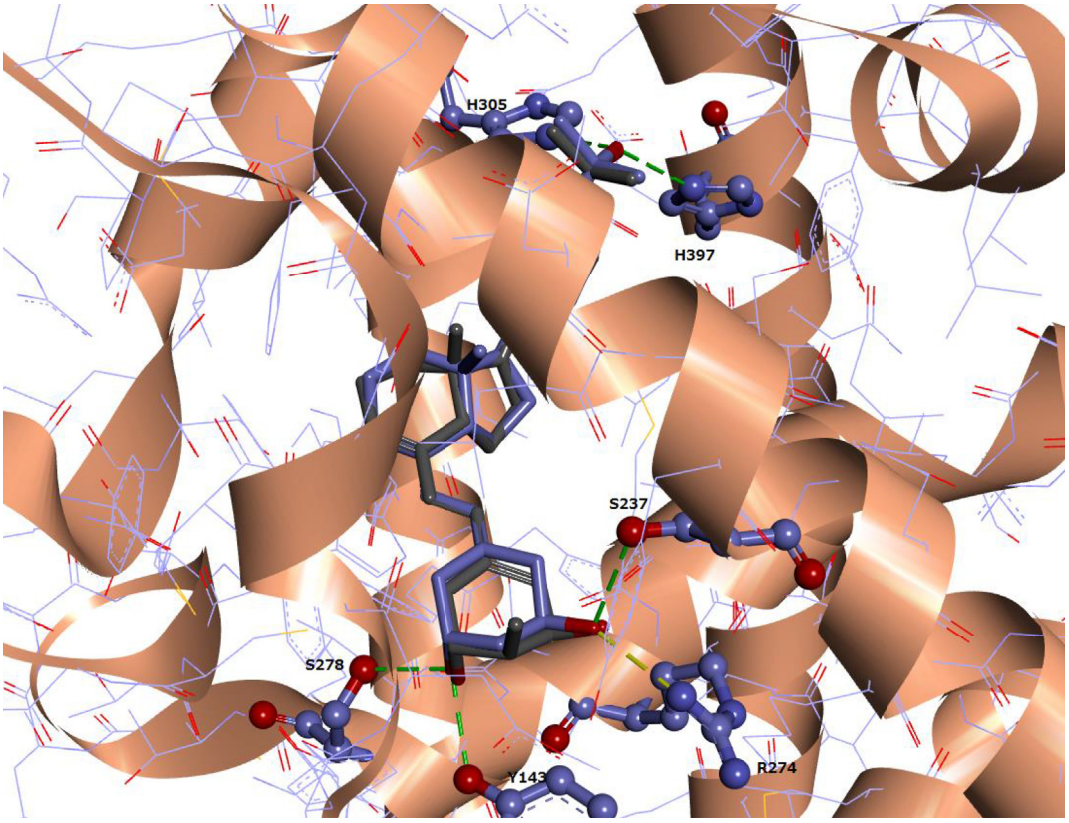


Fig. 5. Superimposed three dimensional structures of **4** (purple) and **30** (gray) based on X-ray co-crystallographic analysis in the hVDR ligand binding domain. Protein Data Bank accession number is 3X31 for **4**.

temperature, and extracted with hexane. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by short column chromatography on neutral silica gel (hexane/AcOEt=30/1) to give a crude product, which was used without further separation. To a solution of the above product in dry DMF (4 mL) were added tetrabutylammonium iodide (519 mg, 1.41 mmol), Cs_2CO_3 (458 mg, 1.41 mmol) at room temperature. Then, to the mixture was slowly added $(PhO)_2P(O)H$ (0.108 mL 0.562 mmol) over 2 h. After 1 h, the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=15/1–8/1) to give **11** (128 mg, 74% in 2 steps) as a colorless oil: $[\alpha]_D^{25}$ +2.1 (*c* 1.69, $CHCl_3$); IR 3070, 2951, 2931, 2889, 2858, 1593, 1489, 1385, 1281, 1254, 1192, 1076, 1026, 933, 837, 771, 687 (neat) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.87 (s, 9H), 0.90 (s, 9H), 1.80–1.85 (m, 2H), 2.28–2.32 (m, 2H), 2.93 (ddd, *J*=7.3, 15.8, 45.8 Hz, 1H), 3.19 (ddd, *J*=8.7, 15.8, 44.2 Hz, 1H), 4.18–4.22 (m, 1H), 4.45–4.49 (m, 1H), 4.93 (brs, 1H), 4.95 (brs, 1H), 5.64–5.71 (m, 1H), 7.12–7.18 (m, 6H), 7.27–7.33 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.05, −4.93, −4.89, −4.79, 18.0, 18.2, 25.7, 25.8, 37.1, 43.8, 66.7, 70.5, 108.6, 113.5, 120.6, 125.1, 129.7, 141.7, 141.9, 150.2, 150.28, 150.34, 150.4, 151.98, 152.01; HRFABMS calcd for $C_{33}H_{51}NaO_5Si_2P$ 637.2905, found 637.2897.

*4.3.3. 7,8-cis-1α,25-Dihydroxyvitamin D$_3$ (**5**) and 5,6-trans-1α,25-dihydroxyvitamin D$_3$ (**24**).* To a solution of **11** (33 mg,

0.054 mmol) in THF (0.7 mL) was added nBuLi (1.64 M hexane solution, 0.036 mL, 0.059 mmol) at −78 °C and stirred at the same temperature for 20 min. To the mixture was added **12** (14 mg, 0.036 mmol) in THF (0.3 mL) at −78 °C and the whole mixture was gradually warmed up to 0 °C over 3 h. After stirring for further 40 min at the same temperature, the reaction was quenched by aqueous NH_4Cl at 0 °C, and the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flush column chromatography on silica gel (hexane/AcOEt=50/1–7/1) to give crude protected compounds as an inseparable mixture of isomers with the substrate **12** (1.0 mg). Then, to a solution of the crude compounds obtained above in THF (1.0 mL) was added TBAF (0.13 mL, 1.0 M THF solution, 0.13 mmol) at 0 °C and stirred at room temperature for 18 h. After the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=1:1–0/1) followed by purification on preparative silica gel TLC plate (AcOEt) to give **5** and **24** as an inseparable mixture. The isomers were separated by reverse-phase HPLC (YMC-Pack ODS column) using MeOH/H_2O (9/1) as a solvent system to give **5**[3] (1.8 mg, 12% in two steps) as a colorless oil and **24**[3] (3.7 mg, 25% in two steps).

NOTE: By the NMR observation in $CDCl_3$, 5,6-*trans*-7,8-*cis*-1α,25(OH)$_2$D$_3$ (**5**) was immediately transformed into 1α,25(OH)$_2$D$_3$ ($t_{1/2}$ ca.15 min), and it was difficult to isolate pure 5,6-*trans*-7,8-*cis*-1α,25(OH)$_2$D$_3$. According to **24**, transformation to the previtamin D form in $CDCl_3$ was slow with producing unknown decomposed compounds; therefore, it was difficult to determine its $t_{1/2}$.

4.4. Synthesis of 7,8-*cis*-14-*epi*-1α,25-dihydroxyvitamin D$_3$ (6)

*4.4.1. 7,8-cis-14-epi-1α,25-Dihydroxyvitamin D$_3$ (**6**) and 5,6-trans-14-epi-1α,25-dihydroxyvitamin D$_3$ (**25**).* To a solution of **11** (12 mg, 0.020 mmol) in THF (0.3 mL) was added nBuLi (1.64 M hexane solution, 0.013 mL, 0.021 mmol) at −78 °C and stirred at the same temperature for 30 min. To the mixture was added **13** (5 mg, 0.013 mmol) in THF (0.1 mL) at −78 °C and the whole mixture was gradually warmed up to 0 °C over 3 h. After stirring for further 40 min at the same temperature, the reaction was quenched by aqueous NH_4Cl at 0 °C, and the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flush column chromatography on silica gel (hexane/AcOEt=50/1–7/1) to give crude protected compounds as an inseparable mixture of isomers. Then, to a solution of the crude compounds obtained above in THF (1.0 mL) was added TBAF (0.047 mL, 1.0 M THF solution, 0.047 mmol) at 0 °C and stirred at room temperature for 23 h. After the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt=1:1–0/1) followed by purification on preparative silica gel TLC plate (AcOEt) to give **6** and **25** as an inseparable mixture. The isomers were separated by reverse-phase HPLC (YMC-Pack ODS column) using MeOH/H_2O (9/1) as a solvent system to give **6** (0.12 mg, 2% in two steps) and **25** (1.9 mg, 34% in two steps), as a colorless oil, respectively.

NOTE: By the NMR observation in $CDCl_3$, 7,8-*cis*-14-*epi*-1α,25(OH)$_2$D$_3$ (**6**) was immediately transformed into 14-*epi*-1α,25(OH)$_2$D$_3$ ($t_{1/2}$ ca. 15 min), and it was difficult to isolate pure 7,8-*cis*-14-*epi*-1α,25(OH)$_2$D$_3$. According to **25**, transformation to the previtamin D form in $CDCl_3$ was very slow with producing unknown decomposed compounds; therefore, it was also difficult to determine $t_{1/2}$ (ca. ~a day).

Compound 25: $[\alpha]_D^{20}$ +84.6 (c 0.46, $CHCl_3$); UV (EtOH) λ_{max} 274.5, 210.0 nm, λ_{min} 230.0 nm; IR (neat) 3370, 2932, 1710, 1644, 1455, 1370, 1259, 1050, 903 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, *J*=6.4 Hz, 3H), 0.93 (s, 3H), 0.99–1.19 (m, 1H), 1.21 (s, 6H), 1.22–1.75 (m, 13H), 1.79–1.92 (m, 3H), 2.02–2.18 (m, 1H), 2.13–2.20 (m, 2H), 2.27–2.35 (m, 1H), 2.50 (ddd, *J*=4.1, 5.0, 15.1 Hz, 1H), 2.84 (dd, *J*=4.1, 13.7 Hz, 1H), 4.19–4.27 (m, 1H), 4.49 (dd, *J*=4.1, 6.0 Hz, 1H), 4.96 (brs, 6H), 5.10 (brs, 6H), 6.06 (d, *J*=11.5 Hz, 1H), 6.50 (d, *J*=11.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 19.8, 21.8, 22.3, 25.1, 27.0, 29.2, 29.4, 29.9, 34.0, 34.6, 36.8, 37.9, 42.0, 44.4, 45.5, 54.5, 58.1, 66.0, 71.0, 71.1, 77.2, 109.5, 119.2, 123.6, 132.8, 145.7, 151.7; HRFABMS calcd for $C_{27}H_{44}NaO_3$ 439.3204, found 439.3200.

4.5. Synthesis of 7,8-*cis*-14-*epi*-1α,25-dihydroxy-19-norvitamin D$_3$ (4)

*4.5.1. Ethyl 2-[(1R,3aR,7aR,Z)-1-{(R)-6-(methoxymethoxy)-6-methylheptan-2-yl}-7a-methyloctahydro-4H-inden-4-ylidene]acetate (**27**), ethyl 2-[(1R,3aR,7aR,E)-1-{(R)-6-(methoxymethoxy)-6-methylheptan-2-yl}-7a-methyloctahydro-4H-inden-4-ylidene]acetate (**28**), and ethyl 2-[(1R,3aS,7aR,E)-1-{(R)-6-(methoxymethoxy)-6-methylheptan-2-yl}-7a-methyloctahydro-4H-inden-4-ylidene]acetate (**29**).* To a suspension of NaH (40 mg (60% in oil), 1.00 mmol) in THF (2 mL) was added $(PhO)_2POCH_2CO_2Et$ (283 μL, 1.08 mmol) at 0 °C and stirred for 15 min. Then, a solution of **26** (50 mg, 0.154 mmol) in THF (1 mL) was added to the mixture and stirred at room temperature for 36 h. After the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane:AcOEt=9:1) to give **27**, **28**, and **29** (55 mg (4:5:1), 91%) as an inseparable mixture, which was used without further separation.

*4.5.2(1R,2E,6R,7R)-2-{2-(Benzothiazole-2-sulfonyl)ethylidene}-7-{(R)-6-(triethylsylyloxy)-6-methylheptan-2-yl}-6-methylbicyclo[4,3,0]nonane (**17**).* To a solution of the above mixture of compounds **27**, **28**, and **29** (55 mg, 0.14 mmol) in toluene (3 mL) was added DIBAL-H (415 μL, 1.01 M toluene solution, 0.42 mmol) at −78 °C and stirred at the same temperature for 1 h. After the reaction was quenched by saturated aqueous Na_2SO_4, the mixture was stirred over night, and then dried over Mg_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane:AcOEt=5:1–4:1) to give a mixture of compounds (47 mg, 95%), which was used without further separation.

To a solution of the above mixture of compounds (47 mg, 0.134 mmol) in CH_2Cl_2 (2 mL) were added PPh_3 (53 mg, 0.20 mmol), 2-mercaptobenzothiazole (34 mg, 0.20 mmol), and DIAD (97 μL, 40% toluene solution, 0.20 mmol) at 0 °C and stirred at room temperature for 1 h. Then, the mixture was concentrated, and the residue was purified by short column chromatography on silica gel (hexane:AcOEt=30:1–20:1) to give a crude product. The above obtained crude product was dissolved in EtOH (2 mL), and to the solution were added 30% H_2O_2 (0.6 mL) and $(NH_4)_6Mo_7O_{24}/H_2O$ (33 mg, 0.027 mmol) at 0 °C and stirred at room temperature for 1.5 h. After the reaction was carefully quenched by saturated aqueous $Na_2S_2O_3$ at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane:AcOEt=5:1) to give a mixture of compounds (70 mg, 100%), which was used without further separation.

To a solution of the above mixture of compounds in CH_2Cl_2 (1.5 mL) and MeOH (3 mL) was added CSA (365 mg, 1.57 mmol) at 0 °C and stirred at room temperature for 7.5 h. After the reaction was quenched by saturated aqueous $NaHCO_3$ at 0 °C, the mixture

was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (AcOEt/hexane=1:3) to give alcohols (430 mg, 84%) as an inseparable mixture. The above obtained alcohols were dissolved in DMF (4 mL), and to the solution were added imidazole (180 mg, 2.65 mmol) and TESCl (222 μL, 1.32 mmol) at 0 °C and the whole mixture was stirred at room temperature for 5 h. After the reaction was quenched by saturated aqueous $NaHCO_3$ at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane:AcOEt=30:1) to give compound **17** (24 mg, 33% in five steps) and the isomer (7,8-*trans*-14-*epi*, 40 mg, 56% in five steps).

Compound **17**: $[\alpha]_D^{27}$ +33.7 (c 1.0, $CHCl_3$); IR (neat) 2952, 2873, 1652, 1472, 1380, 1333, 1236, 1153, 1041, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (q, *J*=7.8 Hz, 6H), 0.68 (s, 3H), 0.82 (d, *J*=6.3 Hz, 3H), 0.93 (t, *J*=7.8 Hz, 9H), 0.96–1.03 (m, 1H), 1.17–1.51 (m, 14H), 1.19 (s, 6H), 1.73–1.81 (m, 1H), 2.01–2.05 (m, 1H), 2.11–2.20 (m, 1H), 2.36 (m, 1H), 4.21 (dd, *J*=7.8, 17.1 Hz, 1H), 4.25 (dd, *J*=8.1, 17.1 Hz, 1H), 5.30 (dd, *J*=7.8, 8.1 Hz, 1H), 7.53–7.62 (m, 2H), 7.96–7.99 (m, 1H), 8.18–8.21 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 6.8. 7.1, 19.8, 20.9, 21.8, 23.0, 27.4, 29.6, 29.8, 30.1, 32.4, 33.7, 34.3, 38.1, 45.1, 45.4, 49.4, 53.8, 55.7, 73.3, 107.7, 122.1, 125.2, 127.4, 127.7, 136.8, 152.1, 152.5, 165.7; HRFABMS calcd for $C_{26}H_{44}NaNO_3SiS_2$, 626.3128, found 626.3131.

The isomer (7,8-*trans*-14-*epi*): $[\alpha]_D^{24}$ +31.5 (c 1.0, $CHCl_3$); IR (neat) 2956, 2873, 1653, 1472, 1380, 1332, 1236, 1151, 1041, 910, 729 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.54 (q, *J*=7.8 Hz, 6H), 0.70 (s, 3H), 0.74 (d, *J*=6.6 Hz, 3H), 0.92 (t, *J*=7.8 Hz, 9H), 0.91–0.95 (m, 1H), 1.15 (s, 6H), 1.16–1.46 (m, 14H), 1.68–1.72 (m, 1H), 1.76–1.85 (m, 1H), 2.04 (dd, *J*=8.5, 8.8 Hz, 1H), 2.08–2.15 (m, 1H), 4.20 (dd, *J*=7.8, 14.4 Hz, 1H), 4.27 (dd, *J*=8.1, 14.4 Hz, 1H), 5.20 (dd, *J*=7.8, 8.1 Hz, 1H), 7.52–7.61 (m, 2H), 7.92–7.97 (m, 1H), 8.15–8.19 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 6.8. 7.1, 19.5, 21.3, 21.8, 21.9, 24.9, 26.7, 29.0, 29.7, 29.9, 33.9, 34.5, 37.1, 45.2, 45.3, 53.9, 54.1, 57.1, 73.2, 107.0, 122.0, 125.1, 127.4, 127.7, 136.8, 152.2, 152.6, 165.6; HRFABMS calcd for $C_{33}H_{53}NaNO_3SiS_2$, 626.3128, found 626.3127.

*4.5.3. 7,8-cis-14-epi-1α,25-Dihydroxy-19-norvitamin D₃ (**4**).* To a solution of **17** (25 mg, 0.041 mmol) in THF (0.4 mL) was added LiHMDS (46 μL, 1.0 M THF solution, 0.46 mmol) at −78 °C. After the mixture was stirred at the same temperature for 20 min, **14** (15 mg, 0.0414 mmol) in THF (0.15 mL) was added, and the whole mixture was gradually warmed up to −40 °C for 4 h. After the reaction was quenched by saturated aqueous NH_4Cl, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on neutral silica gel (hexane:AcOEt=50:1). To a solution of the coupling product obtained above in THF (1.2 mL) was added TBAF (0.42 mL, 1.0 M THF solution, 0.42 mmol) at 0 °C and stirred at room temperature for 16 h. After the reaction was quenched by saturated aqueous NH_4Cl at 0 °C, the mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (AcOEt-AcOEt/MeOH=20:1) followed by purification on preparative silica gel TLC plate (AcOEt) to give compound **4** as an inseparable mixture, which was further purified by reverse-phase HPLC (YMC-Pack ODS column) using MeOH/H_2O (9/1) as a solvent system to give compound **4** (9.0 mg, 54% in two steps) as a colorless oil: $[\alpha]_D^{25}$ +71.9 (c 0.23, $CHCl_3$); UV (EtOH) λ_{max} 261.5, 252.5, 244.0 nm, λ_{min} 258.5, 247.5 nm; IR (neat) 3362, 2952, 2858, 1602 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, *J*=6.3 Hz, 3H), 0.93 (s, 3H), 1.02–1.09 (m, 1H), 1.23 (s, 3H), 1.23 (s, 3H), 1.22–1.30 (m, 2H), 1.33–1.64 (m, 12H), 1.82–1.91 (m, 3H), 1.99–2.05 (m, 1H), 2.18 (dd, *J*=7.6, 12.9 Hz, 1H), 2.22–2.29 (m, 1H), 2.32 (dd, *J*=7.6, 12.9 Hz, 1H), 2.48 (dd, *J*=3.7, 13.7 Hz, 1H), 2.62 (dd, *J*=3.7, 13.7 Hz, 1H), 2.68 (dd, *J*=7.3, 11.5 Hz, 1H), 4.05–4.11 (m, 2H), 6.09 (d, *J*=11.2 Hz, 1H), 6.21 (d, *J*=11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.0, 21.3, 22.0, 23.5, 27.4, 29.3, 29.4, 29.6, 32.8, 33.9, 34.5, 36.9, 38.6, 42.2, 44.3, 44.9, 45.0, 49.3, 56.0, 67.3, 67.5, 71.1, 119.6, 123.6, 131.4, 143.4; HRFABMS calcd for $C_{26}H_{44}NaO_3$ 427.3183, found 427.3182.

4.6. Human VDR binding assay

Binding affinity to hVDR was evaluated using a 1α,25(OH)$_2$D$_3$ assay kit (Polarscreen Vitamin D Receptor Competitor Assay, RED, Cat. No. PV4569) purchased from Invitrogen. The solution of test compound (1 mM in EtOH) was diluted to 10 times with DMSO. The solution was diluted to 50 times with the assay buffer included in the kit further. The solution was defined as the compound solution. On the other hand, VDR/Fluoromone and VDR RED, both of which are included in the kit, were diluted with the assay buffer included in the kit so that the concentration of VDR/Fluoromone was 2.8 nM, and that of VDR RED was 2 nM in the mixture. The solution was defined as the VDR/Fluoromone and VDR RED complex. To a 384 well Black plate (Coring, #3677) was added the compound solution (10 μL), and the VDR/Fluoromone and VDR RED complex (10 μL) was added to each wells. The mixture was incubated under 20–25 °C for 2 h. The polarized fluoresce in each wells was measured (384 nm, emission: 595 nm, excitation: 535 nm, time: 250 ms/well). All compounds were evaluated with *N*=2 within the range from 10^{-6} M to 10^{-10} M. IC_{50} values were calculated by using the average of the measured value. The activities of each compound were shown as relative value, in which the activity of the natural hormone **1** was normalized to 100%.

4.7. X-ray co-crystallographic studies

The human VDR LBD protein was the same construct and was purified and complexed by the same protocol as described in the literature.[26] Crystallization experiments were performed using the hanging-drop vapor diffusion method. The ligand **3** or **4** was added to aliquots of the purified protein in a 5-fold molar excess. Crystallization conditions were similar conditions for the VDR LBD-1α,25(OH)$_2$D$_3$ complex crystals by mixing 1 μL of protein solution (10 mg/mL) with an equal volume of the reservoir solution, which contained 1.4 M ammonium sulfate with 0.1 M MES, pH 6.5 and equilibrating against 1 mL reservoir solution. Single crystals grew to suitable dimensions in 2–4 days. hVDR complex crystals with **3** or **4** were cryo-protected in 30% glycerol and cooled at 79 K, and X-ray data were collected using beamline MX2 at the Australian Synchrotron and beamline NW12 at the Photon Factory (PF), respectively. The data for the complex with compound **3** was processed using the XDS software package[28] and the complex with compound **4** was processed the HKL2000 software package.[29] We carried out molecular replacement using MOLREP[30] from CCP4 (Collaborative Computational Project, Number 4, 1994) with the coordinates of the VDR LBD-1α,25(OH)$_2$D$_3$ complex (PDB code 1DB1; the solvent molecules and 1α,25(OH)$_2$D$_3$ were removed) as the initial model. Refinement was carried out using the programs REFMAC.[31] A sample containing a random 5% of the total reflections in the data set was excluded for R_{free} calculations. After rigid-body refinement, electron density for ligand **3** or **4** was clearly constructed using COOT.[32] Statistics of the data collection and final

Table 2
Data collection and refinement statistics values in parentheses are for the highest resolution shell

Crystal data	hVDR LBD-3	hVDR LBD-4
PDB ID	3X36	3X31
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell (Å) *a*	44.82	44.37
b	51.27	51.51
c	132.17	131.52
Data collection		
Beam line	ASR MX2	PF NW12
Wavelength (Å)	0.9537	1.0000
Resolution (Å)	47.80–1.93 (2.03–1.93)	50.0–2.10 (2.18–2.10)
Total number of reflections	102,670	119,575
Unique reflections	18,445	18,033
R_{merge}[a] (%)	5.9 (24.8)	8.6 (28.5)
Completeness (%)	78.2 (24.8)	99.5 (100.0)
Multiplicity	5.5 (2.0)	6.6 (6.9)
Average I/σ (I)	22.8 (3.1)	33.2 (8.7)
Refined statistics		
R_{factor}[b] (%)	17.7	23.7
R_{free}[b] (%)	22.1	29.9
Ramachandran statistics		
Bond lengths (Å)	0.018	0.017
Bond angles (°)	2.116	2.0

[a] $R_{merge}=\Sigma\Sigma i|Ii-<I>|/\Sigma<I>$, where $<I>$ is the mean intensity of N reflections with intensities I*i* and common indices *h*, *k* and *l*.

[b] $R_{factor}=\Sigma_{hkl}||F_{obs}|-k|F_{calc}||/\Sigma_{hkl}|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, R_{free} is calculated for a randomly chosen 5% of reflections and R_{factor} is calculated for the remaining 95% of reflections. ASR means Australian synchrotron radiation and PF means Photon factory in Japan.

structure are summarized in Table 2. Figures were produced using DS Visualizer (Accelrys, http://accelrys.co.jp/).

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Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra for new compounds (**3**, **4**, **10**, **11**, **17**, and **25**)) related to this article can be found at http://dx.doi.org/10.1016/j.tet.2016.03.081.

References and notes

1. *Vitamin D*, 3rd ed.; Feldman, D., Pike, J. W., Adams, J. S., Eds.; Elsevier Academic: New York, 2011; For mechanisms of action, chapters 7–12 and 15; for mineral and bone homeostasis, chapters 16–19, 22, and 23; for cancer, chapters 82–90; for immunity and inflammation, chapters 91–93 and 95–97.
2. Sawada, D.; Tsukuda, Y.; Saito, H.; Kakuda, S.; Takimoto-Kamimura, M.; Ochiai, E.; Takenouchi, K.; Kittaka, A. *J. Am. Chem. Soc.* **2011**, *133*, 7215.
3. VanAlstyne, E. M.; Norman, A. W.; Okamura, W. H. *J. Am. Chem. Soc.* **1994**, *116*, 6207.
4. (a) Hammond, M. L.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1978**, *100*, 4907; (b) Verloop, A.; Corts, G. J. B.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1960**, *79*, 164; (c) Onisko, B. L.; Schnoes, H. K.; DeLuca, H. F. *J. Org. Chem.* **1978**, *43*, 3441; (d) Cordran, P.; Hammond, M. L., Jr.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1980**, *102*, 6259; (e) Jeganathan, S.; Johnston, A. D.; Kuenzel, E. A.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1984**, *49*, 2152; (f) Hoeger, C. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1985**, *107*, 268; (g) Hoeger, C. A.; Johnston, A. D.; Okamura, W. H. *J. Am. Chem. Soc.* **1987**, *109*, 4690; (h) Wu, K.-M.; Okamura, W. H. *J. Org. Chem.* **1990**, *55*, 4025.
5. Barrack, S. A.; Okamura, W. H. *J. Org. Chem.* **1986**, *51*, 3201.
6. Maestro, M. A.; Sardina, F. J.; Castedo, L.; Mouriño, A. *J. Org. Chem.* **1991**, *56*, 3582.
7. Dauben, W. G.; Greenfield, L. J. *J. Org. Chem.* **1992**, *57*, 1597.
8. Wu, Y.; Zhao, Y.; Tian, H.; De Clercq, P.; Vandewalle, M.; Berthier, M.; Pellegrino, G.; Maillos, P.; Pascal, J.-C. *Eur. J. Org. Chem.* **2001**, 3779.
9. Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Uskoković, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 2945.
10. (a) Ando, K. *J. Org. Chem.* **1998**, *63*, 8411; (b) Ando, K. *J. Org. Chem.* **1999**, *64*, 6815; (c) Ando, K. *J. Org. Chem.* **1999**, *64*, 8406; (d) Ando, K.; Oishi, T.; Hirama, M.; Ohno, H.; Ibuka, T. *J. Org. Chem.* **2000**, *65*, 4745.
11. (a) Sawada, D.; Katayama, T.; Tsukuda, Y.; Saito, N.; Takano, M.; Saito, H.; Takagi, K.; Ochiai, E.; Ishizuka, S.; Takenouchi, K.; Kittaka, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5397; (b) Sawada, D.; Tsukuda, Y.; Saito, H.; Takagi, K.; Ochiai, E.; Ishizuka, S.; Takenouchi, K.; Kittaka, A. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 20; (c) Sawada, D.; Katayama, T.; Tsukuda, Y.; Saito, N.; Saito, H.; Takagi, K.; Ochiai, E.; Ishizuka, S.; Takenouchi, K.; Kittaka, A. *Tetrahedron* **2010**, *66*, 5407.
12. Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1991**, *32*, 7663.
13. Cohen, R. J.; Fox, D. L.; Eubank, J. F.; Salvatore, R. N. *Tetrahedron Lett.* **2003**, *44*, 8617.
14. Hatakeyama, S.; Irie, H.; Shintani, T.; Noguchi, Y.; Yamada, H.; Nishizawa, M. *Tetrahedron* **1994**, *50*, 13369.
15. Castro, J.; Balme, G.; Gore, J. *J. Chem. Res. (S)* **1995**, 504.
16. Daniewski, A. R.; Garofalo, L. M.; Hutchings, S. D.; Kabat, M. M.; Liu, W.; Okabe, M.; Radinov, R.; Yiannikouros, G. P. *J. Org. Chem.* **2002**, *67*, 1580.
17. Fujishima, T.; Konno, K.; Nakagawa, K.; Tanaka, M.; Okano, T.; Kurihara, M.; Miyata, N.; Takayama, H. *Chem. Biol.* **2001**, *8*, 1011.
18. Bouillon, R.; Vandewalle, M.; De Clercq, P. J. *PCT Int. Appl.* **1995** WO9501960 A1 19950119.
19. Torneiro, M.; Fall, Y.; Castedo, L.; Mouriño, A. *Tetrahedron Lett.* **1992**, *33*, 105.
20. Kittaka, A.; Hara, H.; Takano, M.; Sawada, D.; Arai, M. A.; Takagi, K.; Chida, T.; Harada, Y.; Saito, H.; Takenouchi, K.; Ishizuka, S.; Hayashi, K.; Ikushiro, S.; Sakaki, T.; Sugiura, T.; Chen, T. C. *Anticancer Res.* **2009**, *29*, 3563.
21. Ono, K.; Yoshida, A.; Saito, N.; Fujishima, T.; Honzawa, S.; Suhara, Y.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *J. Org. Chem.* **2003**, *68*, 7407.
22. Parker, K.; Dermatakis, A. *J. Org. Chem.* **1997**, *62*, 6692.
23. Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958–6966.
24. Maynard, D. F.; Trankle, W. G.; Norman, A. W.; Okamura, W. H. *J. Med. Chem.* **1994**, *37*, 2387–2393.
25. Sawada, D.; Kittaka, A. *Curr. Top. Med. Chem.* **2014**, *14*, 2454.
26. Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. *Mol. Cell* **2000**, *5*, 173.
27. The 14-*epi* molecule **4** can have a compact contact with Leu313 of the hVDR by the molecular distortion compared with 14-normal molecule **3** and affect slight movement of Helix-7 to the compound **4**.
28. Kabsch, W. *J. Appl. Cryst.* **1988**, *21*, 67.
29. Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307–326.
30. Vagin, A.; Teplyakov, A. *J. Appl. Cryst.* **1997**, *30*, 1022.
31. Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. *Acta Cryst.* **1997**, *D53*, 240.
32. Emsley, P.; Cowtan, K. *Acta Cryst.* **2004**, *D60*, 2126.