Bioorganic & Medicinal Chemistry 16 (2008) 6949–6964

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

2-Substituted-16-ene-22-thia-1α,25-dihydroxy-26,27-dimethyl-19-norvitamin D_3 analogs: Synthesis, biological evaluation, and crystal structure

Masato Shimizu [a], Yukiko Miyamoto [a], Hajime Takaku [a], Mayumi Matsuo [a], Makoto Nakabayashi [a], Hiroyuki Masuno [b], Nobuyuki Udagawa [c], Hector F. DeLuca [d], Teikichi Ikura [e,*], Nobutoshi Ito [e,*]

[a] Laboratory of Medicinal Chemistry, School of Biomedical Science, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Institute of Biomaterial and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[c] Department of Biochemistry, Matsumoto Dental University, Nagano 399-0781, Japan
[d] Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706-1544, USA
[e] Laboratory of Structural Biology, School of Biomedical Science, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

ARTICLE INFO

Article history:
Received 17 April 2008
Accepted 21 May 2008
Available online 27 May 2008

Keywords:
16-Ene-22-thia-19-norvitamin D
VDR-binding affinity
Transcriptional activity
Structure–activity relationships
Crystal structure

ABSTRACT

Recently, we have found that 16-ene-22-thia-26,27-dimethyl-19-norvitamin D_3 analogs **1a** ($n = 2, 3$) are 20 times more active than the natural hormone 1α,25-dihydroxyvitamin D_3 in terms of transcriptional activity. To further investigate the effects of the A-ring modification of **1a**, **b** on the biological activity profile, novel 22-thia-19-norvitamin D analogs **2–11** bearing a hydroxyethoxy-, hydroxyethylidene- or methyl group at C-2 in combination with 20*S*- and 20*R*-isomers were prepared and tested for their in vitro biological activities. All of the synthesized analogs showed 0.5–140% of the activity of the natural hormone in binding to the vitamin D receptor (VDR). When compared with the transcriptional activity of C-2 or C-20 isomeric pairs of the 22-thia analogs, the 20*S*-isomers **2–11a** were more potent than the 20*R*-isomers **2**, **3**, **8–11b**, and the 2β-hydroxyethoxy, 2*E*-hydroxyethylidene, and 2α-methyl-2β-hydroxy-22-thia isomers showed higher potency than their corresponding counterparts. In particular, **3a** exhibited an extremely higher level of potency (210-fold) than the natural hormone. To elucidate the action mode of superagonist **3a** at the molecular level, we determined the crystal structures of the rat VDR-ligand-binding domain complexed with **3a** or **3b** in the presence of peptide containing a nuclear box motif (LxxLL) at 1.9–2.0 Å resolution. The crystal structures demonstrated that the 1α-OH, 3β-OH, and 25-OH groups of the natural hormone and **3a** were anchored by the same amino acid residues in the ligand-binding pocket, and the terminal OH moiety of the substituent at C-2 formed hydrogen bonds with Arg270 and a water molecule to create a tight water molecule network. Moreover, the methyl groups at C-26a and C-27a make additional contact with hydrophobic residues such as Leu223, Ala227, Val230, and Ala299. These hydrophilic and hydrophobic interactions in **3a** may underlie the induction of superagonistic activity.

1. Introduction

The natural hormone 1α,25-dihydroxyvitamin D_3[1α,25-$(OH)_2D_3$] is now recognized as a calcium- and phosphorous-regulating hormone, and plays a pivotal role in bone homeostasis.[1] In addition, it elicits differentiation and proliferation activities in various cell types including malignant cells and keratinocytes.[1,2] Most of the known biological effects of 1α,25-$(OH)_2D_3$ are exerted through the direct transcriptional regulation of specific binding to the vitamin D receptor (VDR).[3] 1α,25-$(OH)_2D_3$ binding to the VDR causes a conformational changes in the receptor. The activated VDR/1α,25-$(OH)_2D_3$ complex binds as a heterodimer with the retinoid X receptor (RXR) to vitamin D response elements (VDREs), which are located in the promoter region of the target gene. Recruitment of coactivator proteins to this heterodimer is critical for the transactivation. The conjugated triene structure characteristic of vitamin D_3 is highly labile toward acid, heat, light, and oxygen. 1α,25-Dihydroxy-19-norvitamin D_3[1α,25-$(OH)_2$-19ND], developed by DeLuca's group, is an analog of 1α,25-$(OH)_2D_3$ that lacks an exomethylene group at the C-19 position, and is extremely stable due to lack of a labile conjugated triene moiety.[4,5] The chemical synthesis of 19ND analogs is easier than that of vitamin D derivatives, and 19NDs are therefore attractive compounds from an industrial viewpoint.

The 19-exomethylene group of the natural hormone is pivotal for the binding affinity for VDR. 1α,25-$(OH)_2$-19ND shows slightly

* Corresponding authors. Tel./fax: +81 03 5803 4594 (T. Ikura); tel.: +81 03 5803 4594 (N. Ito).
E-mail addresses: ikura.str@tmd.ac.jp (T. Ikura), ito.str@tmd.ac.jp (N. Ito).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.05.043

weak VDR binding and low calcemic activities, and has a cell-differentiation activity similar to that of 1α,25-$(OH)_2D_3$.[4,5] Thus, 19NDs have received much attention as compound characterized by dissociation of cell-differentiation and calcemic activities. Paricalcitol is a vitamin D_2 derivative of 1α,25-$(OH)_2$-19ND, and is currently used for treatment of psoriasis and hyperparathyroidism. In the course of systematic studies of the structure–activity relationships (SAR) of a number of 19ND analogs, DeLuca and co-workers have found 2MD in which the 19-exomethylene group transposes to the C-2 position and the stereochemistry at C-20 possesses an unnatural *S*-configuration.[6,7] 2MD induces strong bone formation in vitro and in vivo and is now being evaluated in a phase II clinical trial as a therapeutic drug for osteoporosis. Recently, the crystal structure of the rat VDR-ligand-binding domain (rVDR-LBD) in complex with 2MD and coactivator peptide has been clarified.[8]

In our previous paper,[9] we reported studies on the SAR of 16-ene-22-thia-1α,25-dihydroxy-26,27-dimethyl-19-norvitamin D analogs bearing different-sized side chains **1a** and **1b** in combination with 20*S*- and 20*R*-isomers. (20*S*)-22-Thia-19-24-dinorvitamin D analog (**1a**, $n = 1$) is as active as the natural hormone in terms of binding affinity to the VDR and transcriptional activity. (20*S*)-24-Normal- or 24-homo-22-thia-19ND analogs **1a** ($n = 2$ or 3, respectively) exhibited potency 1–2 orders of magnitude greater than that of 1α,25-$(OH)_2D_3$ in stimulating transcriptional activity and the formation of osteoclasts. In SAR studies of a series of 19-norvitamin D analogs, we have synthesized more than a hundred 19ND analogs with structural modification at the A-ring, or both the A-ring and the side chain.[10–16] We have found that introduction of substituents at the C-2 position causes dramatic changes in the activity profile compared with the parent 19-norvitamin D, and that 2β-hydroxyethoxy-, (2*E*)-hydroxyethylidene-, or 2α-methyl-2β-hydroxy-19ND analogs have strong-binding affinity for the VDR and ligand-dependent transcriptional activity. Therefore, we anticipated that the introduction of 2-hydroxyethoxyl, 2-hydroxyethylidene, or 2-methyl-2-hydroxyl groups into the 16-ene-22-thia-19ND skeleton as a scaffold would enhance the biological activities. Furthermore, methylation at C-26a and C-27a is also expected to increase biological activity.[17] In this paper, we report our SAR studies aimed at the development of novel biological profiles utilizing 16-ene-22-thia-26,27-dimethyl-19-norvitamin D analogs **2**–**11** having a 2-hydroxyethoxyl, 2-hydroxyethylidene, or 2-methyl-2-hydroxyl group at the C-2 position. To elucidate the molecular interactions between the synthetic ligands and the VDR, we studied the crystal structure of a ternary complex of rVDR-LBD-coactivator peptide with two 2β-hydroxyethoxy-22-thia-19-norvimtain D analogs **3a** and **3b** (Fig. 1).

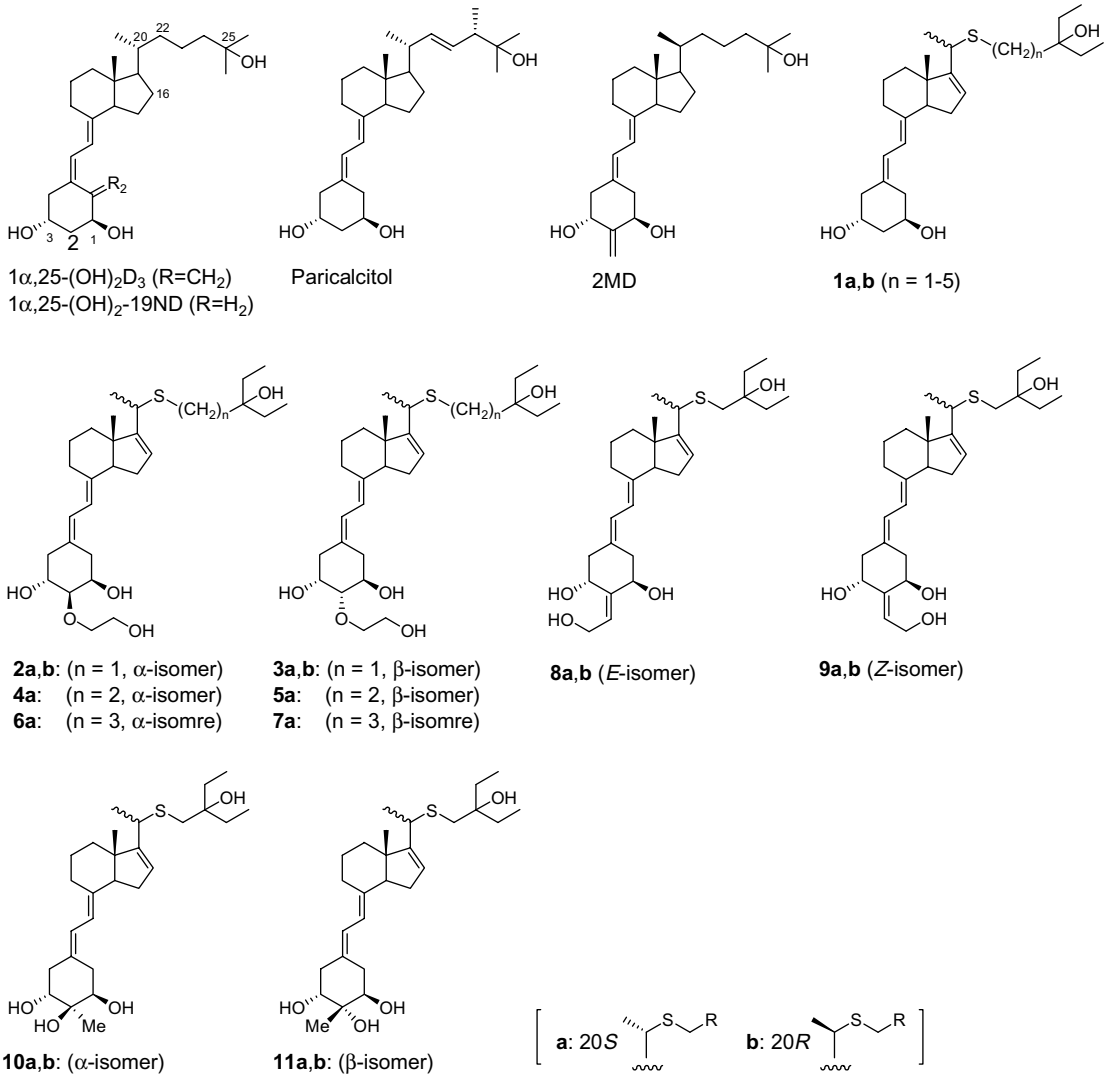

Figure 1. The structures of 1α,25-$(OH)_2D_3$ and its19-norvitamin D analogs.

2. Results and discussion

2.1. Chemistry

For the synthesis of 2-substituted-16-ene-22-thia-19-norvitamin D analogs **2**–**11**, we employed a Wittig–Horner coupling of the appropriately substituted A-ring phosphine oxide **12** or **37** with the 25-hydroxy Grundmann's ketone **13**–**15** as shown in Figures 2 and 3. The A-ring phosphine oxide **12** (ca. 2:1 diastereomeric mixture) was prepared from D-glucose as reported,[10] and we synthesized a new 2,2-disubstituted A-ring synthon **37** (ca. 1:1 diastereomeric mixture) with methyl and hydroxyl moieties at C-2 (based on steroid numbering) as shown in Figure 3. The synthesis of the 16-ene-22-thia-C/D-ring ketones bearing side chains of different sizes was reported in our recently published paper.[9]

The phosphine oxide **12** was treated with the C/D-ring synthon **13a** in the presence of LiHMDS to give a 19-norvitamin D derivative **16a** (ca. 3:2 diastereomeric mixture, 38%), which was selectively hydrolyzed by treatment with a mixture of aqueous acetic acid in THF, yielding the 2-hydroxy compound **19a**. Compound **19a** was allowed to react with excess (2-bromoethoxy)-*tert*-butyldimethylsilane in the presence of excess sodium hydride, and then all the protecting groups in **22a** were removed by treatment with camphor sulfonic acid (CSA) to give the isomeric 2-hydroxyethoxy-19-norvitamin D analogs **2a** and **3a** in good yield (71%). The other target analogs **2–3b**, and **4**–**7a** bearing elongated side chains or a 20-epi configuration were synthesized using the same sequence of reactions with the C/D-ring synthons **13b**, **14a**, and **15a** and the A-ring synthon **12**.

(*E*)- and (*Z*)-2-Hydroxyethylidene-19-norvitamin D derivatives **28** were prepared by employing the 2-hydroxy compounds **19**. Oxidation of **19** under Swern conditions afforded the 2-keto derivative **25** as a single product. Cyanomethylation of **25** with diethyl(cyanomethyl)phosphonate gave **26** as an approximately 1:1 diastereomeric mixture, which on reduction with diisobutylaluminum hydride (DIBAL-H), followed by sodium borohydride yielded the 2-hydroxyethylidene derivative **28**. All the protecting groups in **28** were cleaved by CSA to afford the desired 19-norvitamin D analogs **8** and **9** with an overall yield of 54% and 65%, respectively.

2,2-Disubstituted 19-norvitamin D analogs **38a** and **39b** were successfully synthesized according to the reaction sequences shown in Figure 3. We prepared the A-ring phosphine oxide **37** from the spiro-oxirane **30** (ca. 9:1 mixture), which was obtained using the published procedure via the key intermediate **29**.[12] Reductive ring cleavage of **30** with lithiumaluminum hydride to give **31** (74%), following protection by trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave the 2-TMS ether **32** (98%), which upon Pd–C-catalyzed hydrogenolysis afforded the alcohol **33** (quantitative yield). Oxidation of **33** under Swern conditions and subsequent Peterson olefination of the obtained **34** (91%) with methyl(trimethylsilyl)acetate yielded the allylic ester **35** (96%) as an approximately 1:1 diastereomeric mixture due to the newly generated double bond isomerism, which on reduction with DIBAL-H gave the corresponding allylic alcohol **36** (95%). Transformation of **36** into the A-ring phosphine oxide **37** was achieved in a one-pot process according to the reported procedures.[6] The 16-ene-22-thia-Grundmann ketone **13a** was treated with the phosphine oxide **37** (ca. 1:1 isomeric mixture), affording the 2,2-disubstituted 19-norvitamin D analog **38a** at low yield as an approximately 3:2 mixture of diastereomers, which after deprotection with CSA, provided **10a** and **11a** (93%). The 20-epi analogs **10b** and **11b** were obtained as a ca. 5:1 diastereomeric mixture by coupling of **13b** with **37** through the same sequence of reactions with **13a** and **37**.

All C(2)-epimeric pairs and geometrical isomers were carefully separated by HPLC. The C(2)-stereochemistry of all synthetic compounds **2**–**11** was determined as reported previously.[10–15]

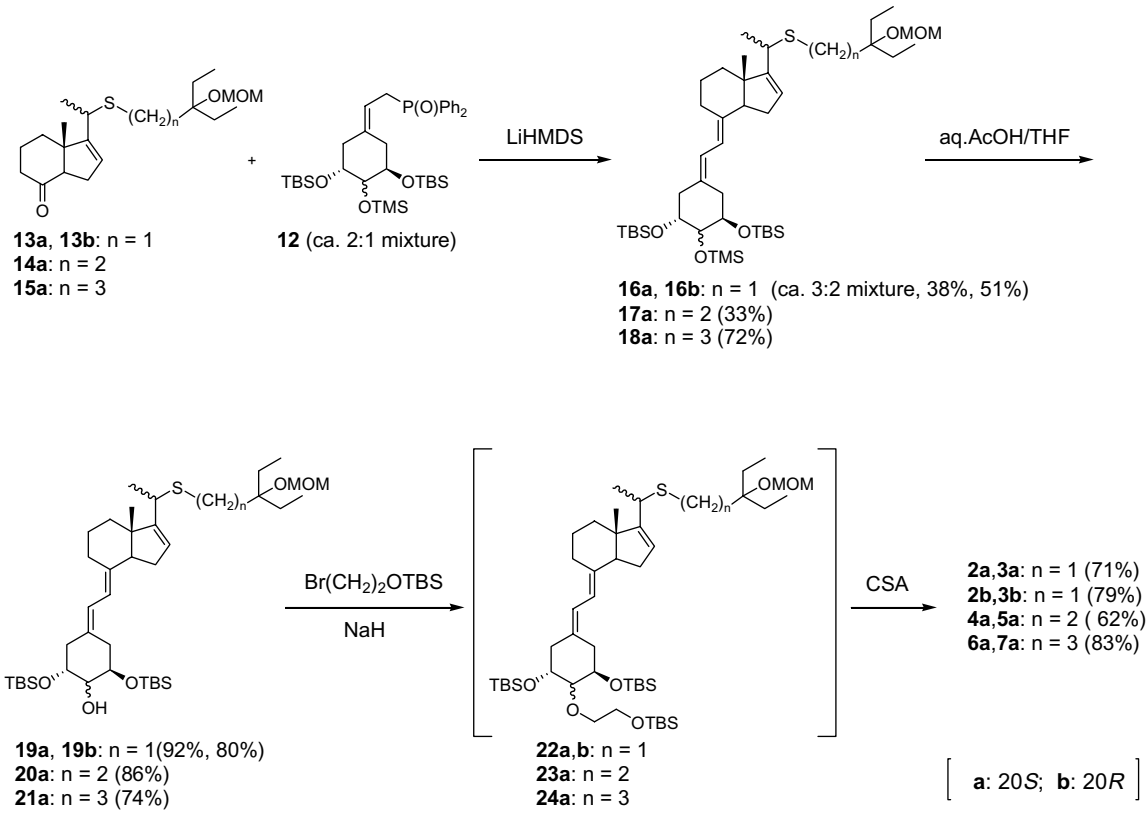

Figure 2. Synthesis of 2-hydroxyethoxy-22-thia-19-norvitamin D analogs.

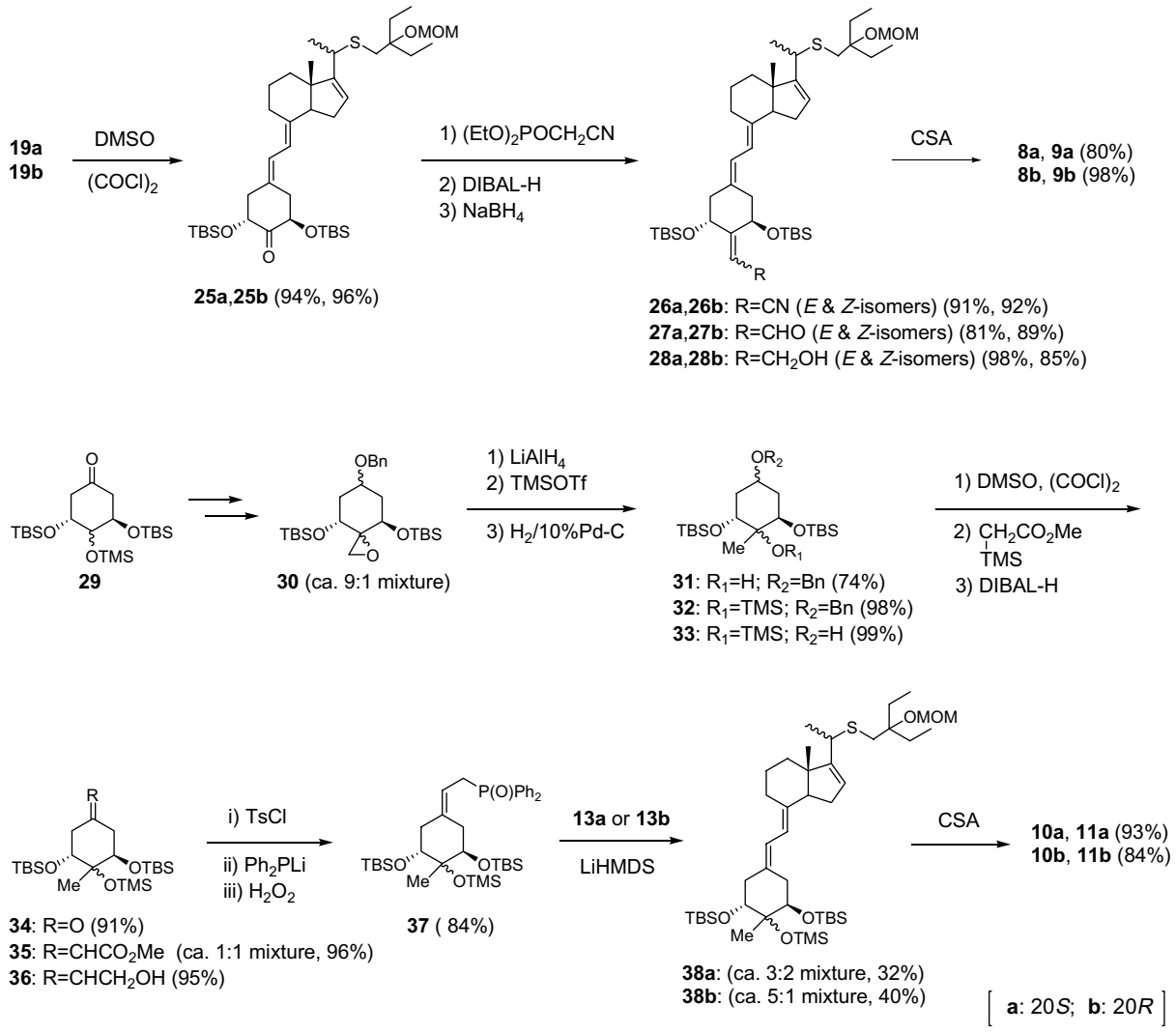

Figure 3. Synthesis of 2-hydroxyethylidene- and 2-methyl-2-hydroxy-22-thia-19-norvitamin D analogs.

2.2. Biological activity

The synthesized 2-substituted-22-thia-19-norvitamin D analogs **2**–**11**, with the structural modifications at the A-ring, D-ring, and side chains, are expected to exhibit dramatic changes in activity pattern compared with the parent compounds without the substituent at the C-2 positions **1a** and **1b**. The novel 22-thia-19-norvitamin D analogs were examined for the following in vitro biological activities: (1) binding affinity for the VDR, (2) VDR-mediated transcriptional activity, and (3) osteoclast formation. The results are summarized in Tables 1 and 2 and Figures 4 and 5, the activities being shown as percentages of that of the natural ligand, 1α,25-$(OH)_2D_3$.

First, the binding affinity for the VDR was determined by a competitive-binding assay using rat recombinant VDR-LBD (Table 1).[18] The 22-thia analogs **2**–**11a** with a 20*S*-configuration displayed higher-binding potency than that of their 20*R*-counterparts **2**–**3b**, **8**–**11b**. When compared with the binding affinity of the C-2 isomeric pairs of the 2-substituted-22-thia-19-norvitamin D analogs **2**–**11**, the isomers having 2β-hydroxyethoxy, 2*E*-hydroxyethylidene, or 2α-methyl-2β-hydroxy moieties **3a**–**b**, **5a**, **7a**, **8a**–**b**, **11a**–**b** proved to be more active than their corresponding counterparts. These results are in accordance with those of our previous SAR studies employing 19-norvitamin D analogs having various types of side chains.[13] Introduction of a substituent at C-2 and elongation of the side chain had variable effects on VDR binding. The 2-hydroxyethoxy-22-thia analogs **2**–**7** showed 10–140% activity with respect to the natural hormone in terms of VDR-binding affinity. Among the compounds with the same stereochemistry at C-20 (**2a** vs **4a** vs **6a**; **3a** vs **5a** vs **7a**), elongation of the side chain decreased the binding affinity. Introduction of a 2-hydroxyethoxy group into the 24-nor compounds **1a**, **b** ($n = 1$) increased the binding potency regardless of the C-2 configuration. In contrast, the binding affinity of the 24-normal or 24-homo analogs **4**–**7** was weaker than that of the parent compounds **1a** ($n = 2$ or 3). Of the geometrical isomers **8** and **9**, the *E*-isomers **9** showed higher-binding potency, while the *Z*-isomers **10** showed lower affinity than the natural hormone. Except for **9a**, introduction of a 2-hydroxyethylidene moiety increased the binding potency, but replacement of $C(2)H_2$ by methyl and hydroxyl groups markedly reduced the affinity for the VDR, except in the case of **11b**.

Next, the VDR-mediated transcriptional activity of the 2-substituted-22-thia analogs **2**–**11** was tested by luciferase assay with mouse osteopontin vitamin D response element (VDRE) in COS-7 cells (Table 1 and Fig. 4). The relative activities of test compounds were assessed by the ED50 values calculated from their dose–response curves. Most of the 22-thia analogs except for **10** were 2.1- to 210-fold more potent than 1α,25-$(OH)_2D_3$ in stimulating

Table 1
Relative VDR affinity and transcriptional activity of 2-substituted-16-ene-22-thia-19-norvitamin D analogs[a]

Compound	VDR affinity	Transcription	EC_{50} (nM)
1α,25-$(OH)_2D_3$	100	100[b]	
1a ($n = 1$)	60	104[b]	
1b ($n = 1$)	6	9[b]	
1a ($n = 2$)	100	2000[b]	
1b ($n = 2$)	4	27[b]	
1a ($n = 3$)	100	2000[b]	
1b ($n = 3$)	8	20[b]	
2a	92	1630[c]	0.027
2b	24	380[c]	0.115
3a	140	21,000[c]	0.0021
3b	84	1830[c]	0.024
4a	30	1450[d]	0.058
5a	90	2800[d]	0.03
6a	10	930[d]	0.09
7a	47	930[d]	0.09
8a	142	3650[e]	0.026
8b	137	3280[e]	0.029
9a	40	1120[e]	0.085
9b	14	480[e]	0.2
10a	1	13[f]	1.9
10b	0.5	6[f]	4.5
11a	18	1470[f]	0.017
11b	12	210[f]	0.12

[a] Activities are shown as percentages of that of 1α,25-$(OH)_2D_3$.
[b] Ref. [9].
[c,d,e,f] Activity was assessed in terms of EC_{50} of 1α,25-$(OH)_2D_3$. (c) 0.44 nM, (d) 0.84 nM, (e) 0.95 nM, (f) 0.25 nM.

Table 2
Relative potency of 1α,25-$(OH)_2D_3$ and its analogs in osteoclast formation[a]

Compound	EC_{50} (nM)	Osteoclast formation
1α,25-$(OH)_2D_3$	2.0	100
2MD	0.048	4200[b]
2a	0.04	5000[b]
2b	0.43	470[b]
3a	0.035	5700[b]
3b	0.22	900[b]

[a] Activities are shown as percentages of that of 1α,25-$(OH)_2D_3$.
[b] Activity was assessed in terms of EC_{50}.

transcriptional activity. Comparison of the 22-thia analogs with a different configuration at C-20 showed that the (20*S*)-isomers had more potent transcriptional activity than the (20*R*)-isomers in accordance with the effect on VDR binding. In addition, the activity enhancement of analogs with a 2β-hydroxyethoxy, 2*E*-hydroxyethylidene, or 2α-methyl-2β-hydroxy group with respect to their corresponding isomeric counterparts was consistent with the findings for VDR binding.

In our previous SAR studies of 22-thia analogs **1a**, **b** with different-sized side chains,[9] the 24-normal and 24-homo analogs **1a** ($n = 2$ and 3) displayed markedly increased (20-fold) transcriptional potency compared with the natural ligand and the 24-nor analog **1a** ($n = 1$) as shown in Table 1. In comparing transcriptional activity of the analogs carrying a 2-hydroxyethoxy substituent, the 2β-hydroxyethoxy-24-nor analog **3a** exhibited a 210-fold increase in potency and the highest potency among the test compounds. It should be noted that, to our knowledge this is the strongest transcriptional activity among the known 19-norvitamin D analogs. Introduction of the hydroxyethoxy group into the C-2 position of the 24-nor analogs **1a** and **1b** ($n = 1$) caused markedly increased transcriptional activity, but contrary to expectation the same structural modification of the 22-thia analogs with elongated side chains **4**–**7** did not have strong effects on transcriptional activity. The two isomeric pairs **4a** and **5a** or **6a** and **7a** exhibited no distinct transcriptional activity. It is reported that the HL-60 differentiation activity of 24-nor-1α,25-$(OH)_2D_3$, which lacks the 24-methylene group of 1α,25-$(OH)_2D_3$, is about one order of magnitude lower than that of the natural hormone. To investigate the cause of the marked transcriptional activity enhancement of the 24-nor-22-thia analog **3a** compared with the analog **1a** ($n = 1$) with no substituent at C-2 and the natural ligand, we performed crystallization of rat VDR-LBD complexed with **3a** or **3b** in the presence of coactivator peptide and succeeded in determining their crystal structures. Analysis of these crystal structures of the ternary complex of rVDR-LBD/ligand/peptide will be described later in detail. The 2-hydroxyethylidene-22-thia analogs **8** and **9** were 4.8–36.5 times more active than the natural hormone in stimulating transcriptional activity. Introduction of a 2-hydroxyethylidene group also increased transcriptional potency markedly relative to the parent compounds **1** ($n = 1$). Docking studies of **8a** and **9a** using the docking software FlexX (Tripos, St. Louis) indicated that the backbone carbonyl group of Asp144 formed a hydrogen bond with the OH group of the 2*E*-substituent in **8a**, whereas Arg274 exhibits a hydrophilic interaction with the 2*Z*-hydroxyethylidene group in **9a**. Such additional interactions could stabilize the transcriptionally active VDR conformation. These may explain the much more pronounced effect of the 2-hydroxyethylidene analogs on transcriptional activity. The two isomeric pairs **10a** and **11a** or **10b** and **11b** exhibited large difference in transcriptional activity. 2α-Methyl-2β-hydroxy-22-thia analogs **11** had higher activities, while the corresponding isomers **10** were about 10-fold less potent than 1α,25-$(OH)_2D_3$. The stereochemistry of the substituent at C-2 is crucial for VDR-mediated gene transcription. The crystal data for the VDR-LBD/1α,25-$(OH)_2D_3$ complex indicated that the hydrophobic amino acid residues surround the area above the A-ring of the ligand, whereas the hydrophilic residues surround the area below the A-ring. The order of the two substituents in **11** is complementary to the amino acid residues surrounding the A-ring causing markedly increased transcriptional activity. In contrast, the 2β-methyl-2α-hydroxy group in **10** forms unfavorable interactions within the LBP, explaining the decreased transcriptional potency.

The effects of the four diasteoisomers of 2-hydroxyethoxy-22-thia-24-nor analogs **2**–**3** on osteoclast formation were examined in a mouse co-culture system, and the results are summarized in Table 2 and Figure 5. Treatment of cultures with the 22-thia analogs resulted in a dose-dependent increase in the number of osteoclasts. 1α,25-$(OH)_2D_3$ and 2MD induced multinucleated cells that were positive for tartrate-resistant acid phosphatase (TRAP), a feature typical of osteoclasts. We have reported that the 22-thia analogs **1a**, **b** ($n = 1$) exhibited similar activity to 1α,25-$(OH)_2D_3$ with respect to ability to induce osteoclast formation.[9] 2MD stimulates bone formation both in vitro and in vivo, and is expected to be a promising candidate for the treatment of osteoporosis (now being tested in phase II clinical trials).[7] Osteoclast formation by 2MD and 2β-hydroxyethoxy-22-thia analogs **2a** and **3a** was dose-dependent, and their activities were approximately 50 times greater than that of the natural hormone, whereas the relative activities of the corresponding isomers **2b** and **3b** compared with 1α,25-$(OH)_2D_3$ were 4.7- and 9-fold, respectively. As seen in Figure 5, the effect of 2MD and 2β-hydroxyethoxy-22-thia analogs on osteoclastogenesis at 10^{-10} M was almost as equally potent as 1α,25-$(OH)_2D_3$ at 10^{-8} M, and **3a** was more active than 2MD.

2.3. Crystal structure of rat VDR-LBD/ligand (3a or 3b)/DRIP peptide complex

As mentioned above, the 2β-hydroxyethoxy-22-thia-19,24-dinorvitamin D analog **3a** was 210 times more potent than 1α,25-$(OH)_2D_3$ in terms of transcriptional activity, and epimeriza-

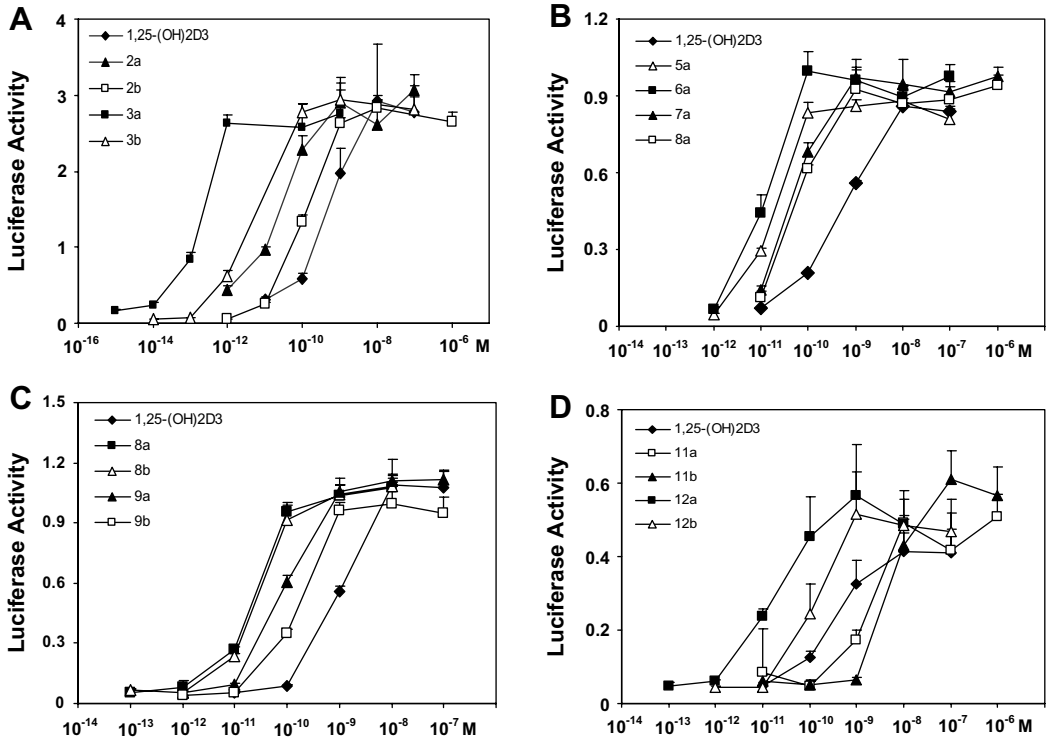

Figure 4. Dose–response effects of 1α,25-$(OH)_2D_3$ and 2-substituted-22-thia-19-norvitamin D analogs on transcriptional activity.

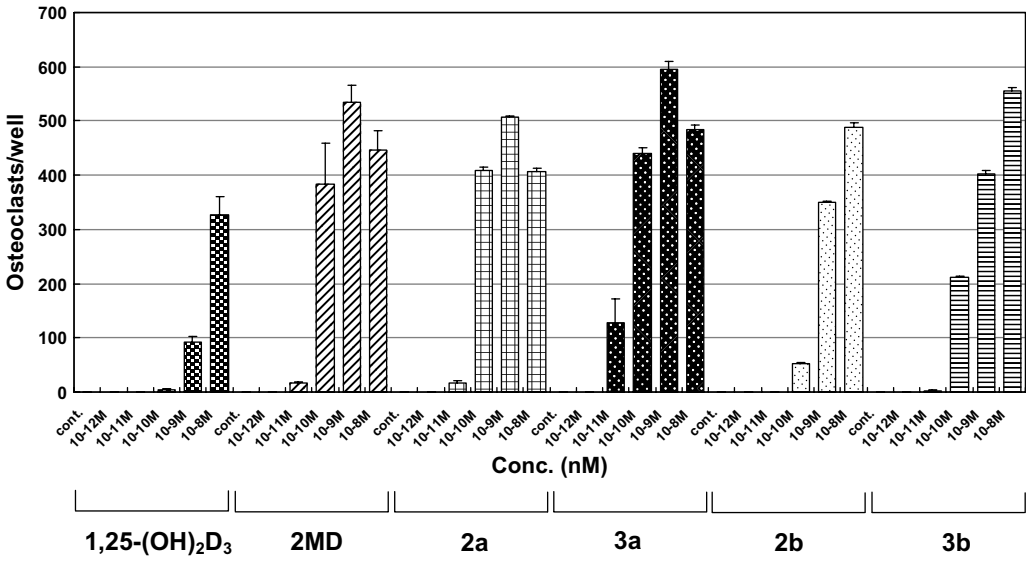

Figure 5. Effects of 1α,25-$(OH)_2D_3$, 2MD, and 2-hydroxyethoxy-22-thia-19-norvitamin D analogs (**2a**, **2b**, **3a**, and **3b**) on osteoclast formation.

tion at C-20 caused an extreme decrease of the activity. To understand, on a molecular level, the mode of action of the superagonist, 2β-hydroxyethoxy-22-thia analog **3a** mediated by VDR, and the activity difference between 1α,25-$(OH)_2D_3$ and **3** or (20*S*)-isomer **3a** and (20*R*)-isomer **3b** at the transcriptional level, the crystal structures of VDR-LBD in complex with the agonist **3a**, **3b**, or 1α,25-$(OH)_2D_3$ will provide valuable information. The crystal structures for the complexes of VDR-LBD with eighteen VDR agonists have been published so far.[19–21,8,22–26] In 2004, DeLuca's group reported the crystal structure of the rVDR-LBD mutant lacking about 50 amino acid residues in the flexible loop connecting helices 1 and 3, in ternary complexes with 19-norvitamin D agonists and a synthetic coactivator peptide including the Leu-x-x-Leu-Leu (x: any amino acids) consensus sequence.[8] The amino acid sequence of human and rat VDR-LBD is highly conserved (approximately 93% similarity), and the rat VDR is four amino acids shorter than its human counterpart. Rat VDR-LBD binds the natural hormone with similar affinity to that of the full-length VDR. We have created a rVDR-LBD mutant containing a hexahistidine tag at the amino-terminus, which is slightly different from the construct created by DeLuca's group.[8] We have solved the crystal structures of rVDR-LBD bound to the 22-thia analog **3a**, **3b**, or 1α,25-$(OH)_2D_3$

in the presence of coactivator peptide at 1.9, 2.0, or 2.0 Å resolution, respectively. A ribbon presentation of holo-rVDR-LBD complexes is shown in Figure 6. The results of the crystallographic refinement are summarized in Table 3. The electron density for rVDR-LBD is well defined, except for several residues at the N-terminus (Ala115-Pro122) and the C-terminus (Asn420-Ser423). The overall structure of the rVDR-LBD framework is identical in the two ligands **3a** and **3b**, and similar to that observed for rVDR-LBD bound to 1α,25-$(OH)_2D_3$. The coactivator peptide is tightly bound to the activation function 2 (AF-2) surface formed by the helices H3, 4, and 12 of the LBD and occupies the typical agonist position. (The refined model contains 190, 176, and 82 water molecules in **3a**, 3b, and 1α,25-$(OH)_2D_3$ complexes.) Six hydrogen bonds that anchor the 1α-OH, 3β-OH, and 25-OH groups of the natural ligand are preserved in the two analog **3a** and **3b** complexes as shown in Figure 7: the 1α-OH group forms a tight hydrogen bonds with Ser233 and Arg270, the 3β-OH group with Tyr143 and Ser274, and the 25-OH group with His301 and His393. In the rVDR-LBD/**3** complex, in spite of the shortened side chain, ligand molecules and two His301 and His393 residues are each shifted to maintain the hydrogen bonds with the 25-OH group of **3a** or **3b**. In addition, the 2β-hydroxyethoxy group in the **3a** or **3b** complex shows further stabilizing interactions via hydrogen bonds between the terminal OH moiety of the 2-substituent and both Arg270 and a water molecule. These interactions may provide significant stabilization of the active receptor conformation.

Superimposition of 1α,25-$(OH)_2D_3$ and **3a** or **3a** and **3b** is shown in Figure 8. The observed positioning of 1α,25-$(OH)_2D_3$ and **3a** in the LBP differs markedly. In the rVDR-LBD/**3a** complex, introduction of the hydroxyethoxy group at the C-2β position causes the whole 19-norvitamin D scaffold to be moved significantly. The 22-thia analog **3a** is notably shifted toward the right-hand side and upwards along the long axis of the 1α,25-$(OH)_2D_3$ ligand to allow access to two His301 and His393 residues to the binding pocket. The oxygen atoms at C-1, C-3, and C-25 in the two ligands **3a** and 1α,25-$(OH)_2D_3$ are separated by 0.46 Å, 0.35 Å and 1.27 Å, respectively, in the structures. The distance between the 1-OH and the 25-OH groups varies from 12.30 Å for **3a** to 13.08 Å for 1α,25-$(OH)_2D_3$ complexes. The hydrophobic interactions of ligand with the amino acid residue surrounding the LBP allow increased stabilization of the liganded receptor complex and are important for biological activity. KH1060 bearing the 20-epi-22-oxo-24a,26a,27a-trihomo side chain and EB1089 with the 22,23-diene-24a,26a,27a-trihomo side chain shows very potent inhibition of cell proliferation.[27,28] The 22-thia analog **3a** contains methyl groups at C-26a and C-27a, and additional contacts of the two methyl groups with neighboring amino acid residues (Leu226, Ala227, Ala299, and Phe418), which do not interact with 1α,25-$(OH)_2D_3$ ligand, are observed in the VDR-LBD/**3a** structure. In the superimposed structures of **3a** and **3b**, four OH groups from the ligand **3a** occupy almost the same positions as those of **3b**, as seen in the case of superimposition of 1α,25-$(OH)_2D_3$ and 20-epi-1α,25-$(OH)_2D_3$ (MC1288) structures.[20] The major differences observed are the positioning of the substituent at the side chain part. The positioning of the two terminal ethyl groups from the ligands **3a** and **3b** is each the same, resulting in common contacts with neighboring hydrophobic amino acid residues: both ethyl groups interact with Leu223, Leu226, Ala227, Val230, Ala299, Tyr397, and Phe418. The number of ligand–receptor contacts including hydrogen bonding and hydrophobic interactions is 17 for 1α,25-$(OH)_2D_3$, 23 for **3a**, and 21 for 20-epi-22-thia analog **3b** at a distance cutoff of 4.0 Å. Sufficient ligand–receptor contacts are necessary to form an energetically favorable conformation of the ligand

Table 3
Data collection and refinement statistics

Ligand	YI-III-690A	YI-III-725B	1,25-$(OH)_2D_3$
Data collection			
Unit cell dimension			
a	153.9	154.0	153.86
b	42.3	42.0	42.00
c	42.1	42.2	41.95
β	95.7	95.8	96.06
Resolution	50–1.9	50–2.0	50–2.0
Completeness (%)	98.3 (98.9)	98.1 (93.6)	98.8 (99.9)
Redundancy	2.6 (2.5)	3.6 (2.9)	3.7 (3.6)
$I/\sigma(I)$	30.9 (2.9)	30.6 (3.9)	21.0 (8.7)
R-factor (%)	3.7 (36.4)	4.3 (30.3)	3.7 (16.2)
Refinement			
R_{cryst} (%)	21.6	21.3	22.5 (37.3)
R_{free} (%)	27.6	26.3	26.1 (38.9)
RMS bond length	0.0072	0.0060	0.0062
RMS bond angles	2.24	1.14	0.86
Atoms			
Protein	1911	1911	1926
Peptide	83	92	92
Ligand	34	34	30
Water	190	176	82
Average B factor	43.4	39.8	40.0

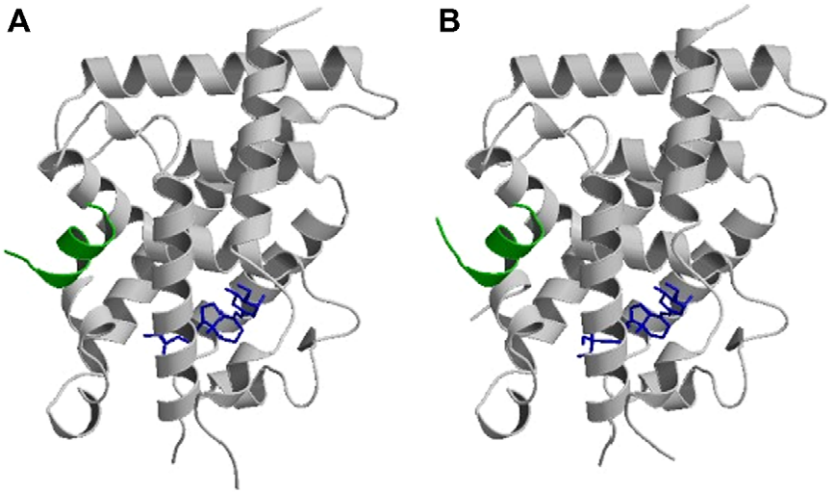

Figure 6. Overall crystal structures of rat VDR-LBD (gray) in complex with 2β-hydroxyethoxy-22-thia-19-norvitamin D analogs **3a** (A, blue) and **3b** (B, blue) in the presence of coactivator peptide (green).

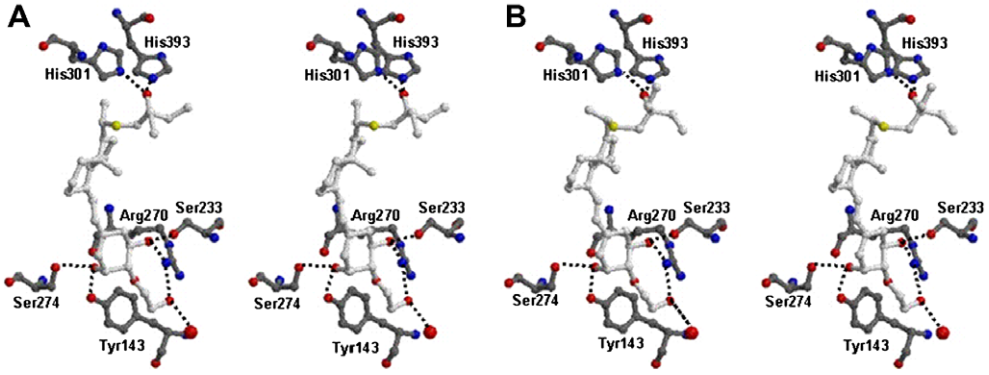

Figure 7. Stereo view of interactions between rat VDR-LBD and 2β-hydroxyethoxy-22-thia-19-norvitamin D analogs **3a** (A) or **3b** (B) in complex. Dotted lines represent hydrogen bonding pairs. Labeled residues belong to rat VDR-LBD. A red sphere represent a water molecule forming hydrogen bond to the ligands **3a** or **3b**.

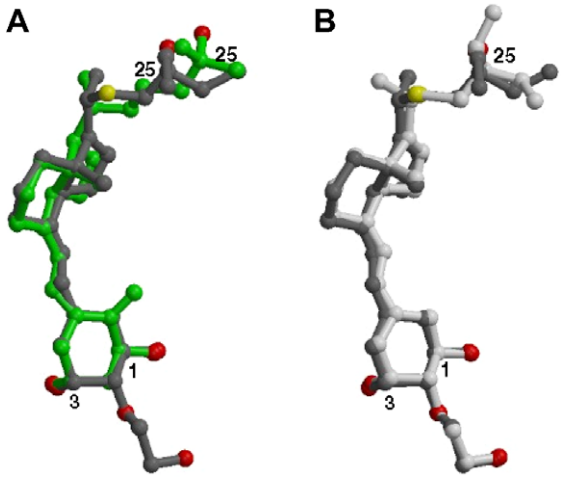

Figure 8. Conformation of the bound ligands (A) Comparison of the ligand conformations of 1α,25-$(OH)_2D_3$ (green) and 2β-hydroxyethoxy-22-thia analog **3a** (gray) in their VDR-LBD. (B) Comparison of the ligand conformation of **3a** (gray) and its 20-epimer**3b** (white).

in the LBP. In this case, this conformation of superagonist ligand **3a** might lead to a transcriptionally active and stable receptor conformation.

3. Conclusion

We have described the synthesis and biological evaluation of newly designed hybrid 19-norvitamin D analogs bearing substituents at C-2, the 16,17-double bond, the sulfur atom at C-22, 26,27-dimethyl groups, and the different-sized side chains. 2-Substituted 22-thia analogs have significant biological activities, in particular the 2β-hydroxyethoxy-22-thia-24-nor analog **3a** being characterized by an extremely high ability (210-fold with respect to the natural hormone) to activate gene transcription, and to our knowledge this is among the most potent of 19-norvitamin D analogs. Our present data demonstrate that in most cases, introduction of substituents into the C-2 position of 22-thia-19-norvitamin D analogs enhanced both transcriptional activity and osteoclast differentiation to an extent similar to, or greater than that caused by C(20)-epimerization of 1α,25-$(OH)_2D_3$. Analysis of the crystal data of rVDR-LBD/**3a** or **3b**/coactivator peptide ternary complex revealed that three OH groups in **3a** interact with the same amino acid residues in the VDR-LBP, and that the terminal OH moiety of the substituent at C-2 forms a hydrogen bond with Arg270 and the water molecule near the C-2 position to create a tight water molecule network. We expect that 24-nor-22-thia analog **3a** will have a much broader spectrum of biological activities.

4. Experimental

NMR spectra were obtained on a Bruker ARX-400 spectrometer, operating at 400 MHz for ^{1}H. Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane as an internal standard (δ 0 ppm) for ^{1}H NMR. Abbreviations used are singlet (s), doublet (d), triplet (t), multiplet (m), aromatic (arom), broad signal (br). Low- and high-resolution mass spectra (MS and HRMS) were obtained with electronic ionization (EI) on a JEOL JMS-AX505HA spectrometer run at 70 eV for EI; *m/z* values are given with relative intensities in parentheses. UV spectra were obtained on a Beckmann DU-7500 spectrophotometer. HPLC system equipped with JASCO MD-910 multi-wavelength UV detector was used to separate the isomeric mixture, which was difficult to separate each other employing usual open column methods. Column chromatography was carried out on silica gel (Wakogel C-200), unless otherwise indicated. All reactions, unless specifically mentioned, were conducted under an atmosphere of argon gas. Yields are not optimized.

4.1. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)-oxy]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (16a)

To a stirred solution of **12** (380.4 mg, 0.577 mmol, a mixture of ca. 2:1) in dry tetrahydrofuran (THF, 3 mL) at −78 °C was added lithium bis(trimethylsilyl)amide (LHMDS, 577 μL, 0.577 mmol, 1.0 M solution in THF), and the resulting dark orange solution was stirred for 30 min. To this colored solution was added a solution of **13a** (136.4 mg, 0.385 mmol) in dry THF (1.5 mL), the reaction mixture was stirred for 1 h at −78 °C and at 0 °C for 1 h. The reaction mixture was quenched with saturated ammonium chloride (NH_4Cl), and extracted with ethyl acetate (AcOEt). The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (15 g) using 3% AcOEt in hexane to afford **16a** (116.5 mg, 38% based on **13a**, a mixture of two isomers in a ca. 3:2 ratio), and 10% AcOEt in hexane to give the unreacted starting material **13a** (82.3 mg, 60%).

^{1}H NMR ($CDCl_3$) δ: 0.04–0.07 (12H, 4× Si-Me), 0.12, 0.13 (2:3) (9H, s, Si-Me_3), 0.82–0.90 (27H, m, H-18, 26a, 27a, 2× Si-*t*-Bu), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 2.62 (2H, m, H-23), 2.79 (1H, m, H-9), 3.40 (3H, s, OMe, overlapped with H-20), 3.54, 3.61 (3:2) (1H, m, H-2), 3.80 (1H, m), 3.88, 3.93 (3:2) (1H, m), 4.69 (2H, s, OCH_2O), 5.61 (1H, m, H-16), 5.87, 5.90 (2:3) (1H, d, *J* = 11.5 Hz, H-7), 6.10, 6.13 (3:2) (1H, d, *J* = 11.5 Hz, H-6). MS *m/z* (%): 794 (M^+, 1), 732

(5), 675 (1), 616 (53), 559 (12), 484 (94), 427 (47), 381 (63), 309 (36), 263 (34), 73 (100).

4.2. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)-oxy]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (16b)

The same procedure as described above, but using **12** (299.3 mg, 0.454 mmol, a mixture of ca. 2:1) and **13b** (80.5 mg, 0.227 mmol) gave **16b** (92.7 mg, 51% based on **13b**, a mixture of two isomers in a ca. 3:2 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.04–0.07 (12H, s, 4× Si-Me), 0.124, 0.127 (2:3) (9H, s, Si-Me_3), 0.71, 0.72 (2:3) (3H, s, H-18), 0.84–0.90 (24H, m, H-26a, 27a, 2× Si-*t*-Bu), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 3.32 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 3.54, 3.60 (3:2) (1H, m, H-2), 3.80 (1H, m), 3.88, 3.93 (3:2) (1H, m), 4.69 (2H, s, OCH_2O), 5.57 (1H, m, H-16), 5.88, 5.91 (2:3) (1H, d, *J* = 11.1 Hz, H-7), 6.10, 6.12 (3:2) (1H, d, *J* = 11.1 Hz, H-6). MS *m/z* (%): 794 (no M^+), 616 (18), 559 (4), 484 (36), 427 (18), 381 (25), 309 (53), 75 (100).

4.3. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)-oxy]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (17a)

To a stirred, cold (−20 °C) solution of **12** (119.3 mg, 0.181 mmol, a mixture of ca. 2:1) and **14a** (60.8 mg, 0.165 mmol) was added LHMDS (180 μL, 0.180 mmol, 1.0 M solution in THF) in a 15 min period. Stirring was continued at −20 °C for 1 h and at 0 °C for 1 h. The reaction mixture was quenched with saturated NH_4Cl, and extracted with AcOEt. The AcOEt extracts were rinsed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (5 g) with 3% AcOEt in hexane to give **17a** (43.5 mg, 33% based on **14a**, as a mixture of two isomers in a ca. 3:2 ratio) and with 10% AcOEt in hexane to afford the unreacted **14a** (39.5 mg).

^{1}H NMR ($CDCl_3$) δ: 0.04–0.07 (12H, 4× Si-Me), 0.12, 0.13 (2:3) (9H, s, Si-Me_3), 0.81–0.90 (27H, m, 2× Si-*t*-Bu, H-18, 26a, 27a), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 2.78 (1H, m, H-9), 3.38 (3H, s, OMe), 3.46 (1H, q, *J* = 7.0 Hz, H-20), 3.53, 3.60 (3:2) (1H, m, H-2), 3.78–3.97 (2H, m, H-1, 3), 4.64, 4.66 (each 1H, d, *J* = 7.4 Hz, OCH_2O), 5.60 (1H, m, H-16), 5.87, 5.90 (2:3) (1H, d, *J* = 11.0 Hz, H-7), 6.10, 6.13 (3:2) (1H, d, *J* = 11.0 Hz, H-6). MS *m/z* (%): 808 (no M^+), 746 (2), 676 (1), 616 (27), 559 (7), 484 (61), 427 (31), 395 (18), 381 (49), 75 (100).

4.4. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)-oxy]-16-ene-22-thia-24-homo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (18a)

The same procedure as described above, but using **12** (144.8 mg, 0.220 mmol, a mixture of ca. 2:1) and **15a** (76.4 mg, 0.200 mmol) gave **18a** (117.8 mg, 72%, a mixture of two isomers in a ca. 3:2 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.04–0.07 (12H, 4× Si-Me), 0.13 (9H, s, Si-Me_3), 0.81–0.90 (27H, m, 2× Si-*t*-Bu, H-18, 26a, 27a), 1.41 (3H, d, *J* = 7.0 Hz, H-21), 2.79 (1H, m, H-9), 3.39 (3H, s, OMe), 3.45 (1H, q, *J* = 7.0 Hz, H-20), 3.54, 3.61 (3:2) (1H, m, H-2), 3.80–4.00 (2H, m), 4.65 (2H, s, OCH_2O), 5.60 (1H, m, H-16), 5.88, 5.91 (2:3) (1H, d, *J* = 11.2 Hz, H-7), 6.10, 6.13 (1H, d, *J* = 11.2 Hz, H-6).

4.5. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-hydroxy-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (19a)

A solution of **16a** (122.5 mg, 0.154 mmol, ca. 3:2 isomeric mixture) in THF/acetic acid (AcOH)/H_2O (v/v/v, 8:8:1, 8.5 mL) was stirred at ambient temperature for 17 h, and diluted with AcOEt. The organic phase was successively washed with 5% sodium bicarbonate ($NaHCO_3$) and brine, and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the resulting residue was purified by chromatography on silica gel (7 g) using 3% AcOEt in hexane to afford **19a** (102.3 mg, 92%, a mixture of two isomers in a ca. 3:2 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si-Me), 0.82–0.90 (27H, m, H-18, 26a, 27a, 2× Si-*t*-Bu), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 2.64 (2H, m, H-23), 2.80 (1H, m, H-9), 3.40 (3H, s, OMe, overlapped with H-20), 3.51, 3.60 (3:2) (1H, m, H-2), 3.89–4.02 (2H, m, H-1, 3), 4.69 (2H, s, OCH_2O), 5.62 (1H, m, H-16), 5.89 (1H, d, *J* = 11.2 Hz, H-7), 6.15, 6.19 (3:2) (1H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%): 722 (no M^+), 660 (1), 603 (1), 544 (6), 487 (5), 412 (6), 355 (35), 309 (32), 75 (100).

4.6. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-hydroxy-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (19b)

In a same way, deprotection of **16b** (128.9 mg, 0.162 mmol, ca. 3:2 isomeric mixture) in aq AcOH in THF afforded **19b** (93.8 mg, 80%, a mixture of two isomers in a ca. 2:1 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, s, 4× Si-Me), 0.71, 0.72 (1:2) (3H, s, H-18), 0.85–0.90 (24H, m, H-26a, 27a, 2× Si-*t*-Bu), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.78 (1H, m, H-9), 3.32 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 3.52, 3.60 (2:1) (1H, m, H-2), 3.90–4.02 (2H, m, H-1, 3), 4.69 (2H, s, OCH_2O), 5.58 (1H, m, H-16), 5.89 (1H, d, *J* = 11.1 Hz, H-7), 6.15, 6.18 (2:1) (1H, d, *J* = 11.1 Hz, H-6). MS *m/z* (%): 722 (no M^+), 660 (1), 544 (54), 487 (20), 412 (16), 355 (100).

4.7. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-hydroxy-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (20a)

In a same way, deprotection of**17a** (55.0 mg, 0.068 mmol, ca. 3:2 isomeric mixture) in aq AcOH in THF afforded **20a** (43.0 mg, 86%, a mixture of two isomers in a ca. 3:2 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.05–0.09 (12H, 4× Si-Me), 0.82–0.89 (27H, m, H-18, 26a, 27a, 2× Si-*t*-Bu), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 2.78 (1H, m, H-9), 3.37 (3H, s, OMe), 3.46 (1H, q, *J* = 7.0 Hz, H-20), 3.51, 3.60 (3:2) (1H, m, H-2), 3.88–4.02 (2H, m, H-1, 3), 4.64 (2H, s, OCH_2O), 5.60 (1H, m, H-16), 5.88 (1H, d, *J* = 11.2 Hz, H-7), 6.15, 6.18 (3:2) (1H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%): 736 (no M^+), 544 (5), 487 (4), 430 (4), 412 (3), 355 (19), 75 (100). HRMS *m/z*: 544.3768 [M^+−$HSCH_2CH_2CH(Et)_2OMOM$] (Calcd for $C_{32}H_{56}O_3Si_2$: 544.3768).

4.8. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-hydroxy-16-ene-22-thia-24-homo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (21a)

In a same way, deprotection of **18a** (117.6 mg, 0.143 mmol, ca. 3:2 isomeric mixture) in aq AcOH in THF afforded **21a** (79.2 mg, 74%, a mixture of two isomers in a ca. 3:2 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si-Me), 0.81–0.90 (27H, m, H-18, 26a, 27a, 2× Si-*t*-Bu), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 1.51 (4H, q, *J* = 7.5 Hz, H-26, 27), 2.79 (1H, m, H-9), 3.38 (3H, s, OMe), 3.45 (1H, q, *J* = 7.0 Hz, H-20), 3.51, 3.60 (3:2) (1H, m, H-2), 3.88–4.03 (2H, m, H-1, 3), 4.65 (2H, s, OCH_2O), 5.60 (1H, m, H-16), 5.89 (1H, d, *J* = 11.2 Hz, H-7), 6.15, 6.18 (3:2) (1H, d, *J* = 11.2 Hz, H-6).

4.9. (20*S*)-1α,25-Dihydroxy-2α-(2-hydroxyethoxy)- and (20*S*)-1α,25-dihydroxy-2β-(2-hydroxyethoxy)-16-ene-22-thia-26,27-dimethyl-19,24-dinorvitamin D_3 (2a and 3a)

A suspension of **19a** (72.4 mg, 0.100 mmol, ca. 3:2 isomeric mixture), sodium hydride (NaH, 120.1 mg, 3.003 mmol, 60% dis-

persion in mineral oil), and 2-bromoethoxy-*tert*-butyldimethylsilane ($BrCH_2CH_2OTBS$, 107 μL, 0.500 mmol) in dry *N*,*N*-dimethylformamide (DMF, 1 mL) was stirred vigorously for 22 h at 0 °C, and the reaction mixture was poured into ice water, and then extracted with AcOEt/hexane (v/v, 1:1). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. Following evaporation of the solvent in vacuo, the residue was purified by chromatography on silica gel (7 g) using 2% AcOEt in hexane to afford a crude **22a** (89.0 mg, quantitative).

The above crude product was dissolved in methanol (MeOH, 1.5 mL) and camphor sulfonic acid (CSA, 139.4 mg, 0.600 mmol) was added. The reaction mixture was stirred for 2 h at ambient temperature, poured into 5% $NaHCO_3$, and extracted with AcOEt. The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. Removal of the solvent afforded the crude product, which was purified by column chromatography on silica gel (5 g) with 2% MeOH in AcOEt to give **2a** and **3a** (35.2 mg, 71% based on **19a**, ca. 2:1 isomeric mixture). The compounds **2a** and **3a** was further purified by HPLC [YMC-Pack ODS-AM SH-342-5, 25% H_2O/MeOH, 8 mL/min] to yield **2a** (14.6 mg) and **3a** (6.7 mg).

2a (2α-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.84 (3H, s, H-18), 0.86, 0.87 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.42 (3H, d, J = 7.0 Hz, H-21), 2.54, 2.57 (each 1H, d, J = 12.9 Hz, H-23), 2.62 (1H, m, H-4), 2.79 (1H, m, H-9), 2.88 (1H, dd, J = 14.4, 5.0 Hz, H-10), 3.33 (1H, dd, J = 8.0, 2.8 Hz, H-2), 3.44 (1H, q, J = 7.0 Hz, H-20), 3.62–3.84 (4H, m, OCH_2CH_2O), 3.95 (1H, m, H-3), 4.16 (1H, m, H-1), 5.62 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.32 (1H, d, J = 11.2 Hz, H-6). MS *m/z* (%): 494 (M^+, 1), 476 (12), 458 (6), 360 (100), 342 (61). HRMS *m/z*: 494.3091 (Calcd for $C_{28}H_{46}O_5S$: 494.3066). UV λ_{max} (EtOH): 243 (ε 28,400), 251 (ε 33,200), 261 (ε 22,300) nm. **3a** (2β-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.867, 0.875 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.42 (3H, d, J = 7.0 Hz, H-21), 2.49 (1H, m, H-4), 2.54, 2.58 (each 1H, d, J = 12.9 Hz, H-23), 2.78 (1H, m, H-9), 3.09 (1H, dd, J = 13.5, 3.4 Hz, H-10), 3.30 (1H, dd, J = 8.7, 2.8 Hz, H-2), 3.43 (1H, q, J = 7.0 Hz, H-20), 3.65–3.89 (5H, m, H-1, OCH_2CH_2O), 4.18 (1H, m, H-3), 5.63 (1H, m, H-16), 5.94 (1H, d, J = 11.3 Hz, H-7), 6.27 (1H, d, J = 11.3 Hz, H-6). MS *m/z* (%): 494 (M^+, 1), 476 (13), 458 (19), 440 (8), 360 (64), 342 (100), 324 (41), 280 (28). HRMS *m/z*: 494.3072 (Calcd for $C_{28}H_{46}O_5S$: 494.3066). UV λ_{max} (EtOH): 243, 251, 261 nm.

4.10. (20*R*)-1α,25-Dihydroxy-2α-(2-hydroxyethoxy)- and (20*R*)-1α,25-dihydroxy-2β-(2-hydroxyethoxy)-16-ene-22-thia-26,27-dimethyl-19,24-dinorvitamin D_3 (2b and 3b)

The same procedure as described above, but using $Br(CH_2)_2OTBS$ (50 μL, 0.233 mmol) and **19b** (25.0 mg, 0.0346 mmol, ca. 2:1 isomeric mixture) gave **22b** (30.6 mg, 72%).

Following the deprotection of **22b** (30.6 mg, 0.0347 mmol, ca. 2:1 isomeric mixture) with CSA (48.3 mg,, 0.208 mmol) gave **2b** and **3b** (13.5 mg, 79%, ca. 2:1 isomeric mixture), which were separated by HPLC [YMC-Pack ODS-AM SH-342-5, 25% H_2O/MeOH, 8 mL/min] to afford **2b** (6.8 mg) and **3b** (3.5 mg).

2b (2α-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.73 (3H, s, H-18), 0.87, 0.88 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.59, 2.63 (each 1H, d, J = 12.8 Hz, H-23, overlapped with H-4), 2.78 (1H, m, H-9), 2.87 (1H, dd, J = 14.4, 5.0 Hz, H-10), 3.35 (2H, m, H-2, 20), 3.69–3.84 (4H, m, OCH_2CH_2O), 3.95 (1H, m, H-3), 4.16 (1H, m, H-1), 5.60 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.33 (1H, d, J = 11.2 Hz, H-6). MS *m/z* (%): 494 (M^+, 1), 476 (11), 458 (17), 440 (7), 360 (47), 342 (100), 324 (38). HRMS *m/z*: 494.3084 (Calcd for $C_{28}H_{46}O_5S$: 494.3066).

UV λ_{max} (EtOH): 243, 251, 261 nm. **3b** (2β-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.72 (3H, s, H-18), 0.87, 0.88 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.58, 2.63 (each 1H, d, J = 12.8 Hz, H-23), 2.78 (1H, m, H-9), 3.09 (1H, dd, J = 13.3, 3.4 Hz, H-10), 3.31 (1H, dd, J = 8.8, 2.9 Hz, H-2), 3.35 (1H, q, J = 7.0 Hz, H-20), 3.66–3.90 (5H, m, H-1, OCH_2CH_2O), 4.18 (1H, m, H-3), 5.61 (1H, m, H-16), 5.93 (1H, d, J = 11.3 Hz, H-7), 6.28 (1H, d, J = 11.3 Hz, H-6). MS *m/z* (%): 494 (M^+, 1), 476 (11), 458 (5), 360 (100), 342 (58). HRMS *m/z*: 494.3069 (Calcd for $C_{28}H_{46}O_5S$: 494.3066). UV λ_{max} (EtOH): 243, 251, 261 nm.

4.11. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxy]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (23a), (20*S*)-1α,25-dihydroxy-2α-(2-hydroxyethoxy)- and (20*S*)-1α,25-dihydroxy-2β-(2-hydroxyethoxy)-16-ene-22-thia-26,27-dimethyl-19-norvitamin D_3 (4a and 5a)

The same procedure as described above, but using $Br(CH_2)_2OTBS$ (58 μL, 0.2703 mmol) and **20a** (40.8 mg, 0.0553 mmol, ca. 3:2 isomeric mixture) gave **23a** (22.3 mg, 45%).

Following the deprotection of **23a** (20.7 mg, 0.023 mmol, ca. 1:1 isomeric mixture) with CSA (43.0 mg, 0.185 mmol) gave **4a** and **5a** (7.3 mg, 62%, ca. 1:1 isomeric mixture), which was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 25% H_2O/MeOH, 8 mL/min] to afford **4a** (2.8 mg) and **5a** (2.6 mg).

23a ^{1}H NMR ($CDCl_3$) δ: 0.03–0.09 (18H, m, 6× Si-Me), 0.82–0.94 (36H, m, H-18, 26a, 27a, 3× Si-*t*-Bu), 1.42 (3H, d, J = 7.0 Hz, H-21), 3.19. 3.29 (1:1) (1H. m. H-10), 3.38 (3H, s, OMe), 3.46 (2H, q, J = 7.0 Hz, H-23), 3.6–4.1 (7H, m, H-1, 3, OCH_2CH_2O), 4.65 (2H, s, OCH_2O), 5.60 (1H, m, H-16), 5.88 (1H, m, H-7), 6.13 (1H, m, H-6). MS *m/z* (%): 894 (no M^+), 702 (2), 570 (5), 526 (5), 438 (6), 394 (7), 263 (7), 233 (38), 75 (100). **4a** (2α-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.84 (3H, s, H-18), 0.86 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.42 (3H, d, J = 7.0 Hz, H-21), 1.47 (4H, q, J = 7.5 Hz, H-26, 27), 2.63 (1H, m, H-4), 2.78 (1H, m, H-9), 2.85 (1H, dd, J = 14.2, 5.1 Hz, H-10), 3.37 (1H, dd, J = 7.7, 2.6 Hz, H-2), 3.48 (1H, q, J = 7.0 Hz, H-20), 3.72–3.83 (4H, m, OCH_2CH_2O), 3.96 (1H, m, H-3), 4.15 (1H, m, H-1), 5.61 (1H, m, H-16), 5.92 (1H, d, J = 11.1 Hz, H-7), 6.33 (1H, d, J = 11.1 Hz, H-6). MS *m/z* (%): 508 (no M^+), 490 (10), 472 (8), 360 (71), 342 (47), 324 (14), 262 (56), 55 (100). HRMS *m/z*: 490.3103 (M^+-H_2O) (Calcd for $C_{29}H_{46}O_4S$: 490.3117). UV λ_{max} (EtOH): 243, 251, 261 nm. **5a** (2β-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.858, 0.860 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.42 (3H, d, J = 7.0 Hz, H-21), 2.78 (1H, m, H-9), 3.10 (1H, m, H-10), 3.32 (1H, dd, J = 8.7, 2.8 Hz, H-2), 3.48 (1H, q, J = 7.0 Hz, H-20), 3.66–3.90 (5H, m, H-1, OCH_2CH_2O), 4.18 (1H, m, H-3), 5.62 (1H, m, H-16), 5.94 (1H, d, J = 11.3 Hz, H-7), 6.28 (1H, d, J = 11.3 Hz, H-6). MS *m/z* (%): 508 (no M^+), 490 (8), 472 (7), 360 (69), 342 (58), 324 (19), 262 (50), 55 (100). HRMS *m/z*: 490.3147 (M^+-H_2O) (Calcd for $C_{29}H_{46}O_4S$: 490.3117). UV λ_{max} (EtOH): 243, 251, 261 nm.

4.12. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxy]-16-ene-22-thia-24-homo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (24a), (20*S*)-1α,25-dihydroxy-2α-(2-hydroxyethoxy)- and (20*S*)-1α,25-dihydroxy-2β-(2-hydroxyethoxy)-16-ene-22-thia-24-homo-26,27-dimethyl-19-norvitamin D_3 (6a and 7a)

The same procedure as described above, but using $Br(CH_2)_2OTBS$ (110 μL, 0.513 mmol) and **21a** (79.2 mg, 0.105 mmol, ca. 3:2 isomeric mixture) gave **24a** (68.2 mg, 71%).

Following the deprotection of**24a** (68.2 mg, 0.750 mmol, ca. 1:1 isomeric mixture) with CSA (139.3 mg,, 0.600 mmol) gave **6a** and **7a** (32.5 mg, 83%, ca. 1:1 isomeric mixture), which were separated by HPLC [YMC-Pack ODS-AM SH-342-5, 25% H_2O/MeOH, 8 mL/min] to afford **6a** (10.9 mg) and **7a** (8.8 mg).

24a ^{1}H NMR ($CDCl_3$) δ: 0.05–0.07 (12H, 4× Si-Me), 0.82–0.90 (27H, m, H-18, 26a, 27a, 3× Si-*t*-Bu), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.4 Hz, H-26, 27), 2.79 (1H, m, H-9), 3.19,

3.29 (1:1) (1H, m), 3.38 (3H, s, OMe), 3.44 (1H, q, J = 7.0 Hz, H-20), 3.52–4.05 (6H, m, H-1, 3, OCH_2CH_2O), 4.64 (2H, s, OCH_2O), 5.59 (1H, m, H-16), 5.87, 5.89 (1:1) (1H, d, J = 11.1 Hz, H-7), 6.11, 6.14 (1:1) (1H, d, J = 11.1 Hz, H-6). **6a** (2α-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.85 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.45 (4H, q, J = 7.5 Hz, H-26, 27), 2.63 (1H, dd, J = 12.4, 4.5 Hz, H-4), 2.78 (1H, m, H-9), 2.87 (1H, dd, J = 14.3, 5.0 Hz, H-10), 3.34 (1H, dd, J = 8.0, 2.7 Hz, H-2), 3.45 (1H, q, J = 7.0 Hz, H-20), 3.64–3.83 (4H, m, OCH_2CH_2O), 3.95 (1H, m, H-3), 4.16 (1H, m, H-1), 5.60 (1H, m, H-16), 5.92 (1H, d, J = 11.2 Hz, H-7), 6.33 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 522 (M^+, 1), 504 (2), 486 (2), 360 (36), 342 (34), 324 (7), 262 (14), 143 (100). HRMS m/z: 504.3292 (M^+-H_2O) (Calcd for $C_{30}H_{48}O_4S$: 504.3273). UV λ_{max} (EtOH): 243, 251, 261 nm. **7a** (2β-isomer) ^{1}H NMR ($CDCl_3$) δ: 0.82 (3H, s, H-18), 0.85 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.45 (4H, q, J = 7.5 Hz, H-26, 27), 2.79 (1H, m, H-9), 3.10 (1H, m, H-10), 3.29 (1H, dd, J = 8.7, 2.3 Hz, H-2), 3.45 (1H, q, J = 7.0 Hz, H-20), 3.65–3.88 (5H, m, H-1, OCH_2CH_2O), 4.18 (1H, m, H-3), 5.61 (1H, m, H-16), 5.94 (1H, d, J = 11.2 Hz, H-7), 6.27 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 522 (no M^+), 504 (2), 486 (3), 360 (31), 342 (38), 324 (12), 262 (27), 143 (87), 115 (100). HRMS m/z: 504.3282 (M^+-H_2O) (Calcd for $C_{30}H_{48}O_4S$: 504.3273). UV λ_{max} (EtOH): 243, 251, 261 nm.

4.13. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-oxo-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (25a)

To a stirred solution of oxalyl chloride [$(COCl)_2$, 14 μL, 0.160 mmol] in dry methylene chloride (CH_2Cl_2, 1 mL) at −78 °C was added dimethyl sulfoxide (DMSO, 22 μL, 0.310 mmol) in dry CH_2Cl_2 (0.5 mL). After being stirred for 10 min, a solution of **19a** (94.0 mg, 0.130 mmol, ca. 2:1 isomeric mixture) in dry CH_2Cl_2 (1 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 15 min, and triethylamine (Et_3N, 91 μL, 0.653 mmol) was added. The entire mixture was stirred at −78 °C for 15 min, and 0 °C for 30 min, quenched with ice water, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (7 g) with 3% AcOEt in hexane to give **25a** (88.1 mg, 94%).

^{1}H NMR ($CDCl_3$) δ: 0.059, 0.065, 0.070, 0.101 (each 3H, s, 4× Si-Me), 0.87, 0.90 (each 9H, s, 2× Si-*t*-Bu, overlapped with H-18, 26a, 27a), 1.43 (3H, d, J = 7.0 Hz, H-21), 2.60, 2.66 (each 1H, d, J = 12.3 Hz, H-23), 2.81 (1H, m, H-9), 3.40 (3H, s, OMe, overlapped with H-20), 4.35 (1H, dd, J = 6.2, 4.1 Hz), 4.56 (1H, dd, J = 8.9, 5.6 Hz), 4.69 (2H, s, OCH_2O), 5.62 (1H, m, H-16), 5.90 (1H, d, J = 11.2 Hz, H-7), 6.35 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 720 (no M^+), 663 (5), 601 (28), 542 (14), 485 (100), 353 (78).

4.14. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-oxo-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (25b)

The Swern oxidation of **19b** (60.3 mg, 0.083 mmol, ca. 2:1 isomeric mixture) was carried out according to the same procedure described for the preparation of **19a** to give **25b** (57.5 mg, 96%).

^{1}H NMR ($CDCl_3$) δ: 0.059, 0.068, 0.069, 0.100 (each 3H, s, 4× Si-Me), 0.73 (3H, s, H-18), 0.87, 0.90 (each 9H, s, 2× Si-*t*-Bu, overlapped with H-26a, 27a), 1.47 (3H, d, J = 7.0 Hz, H-21), 1.64 (4H, q, J = 7.4 Hz, H-26, 27), 2.65 (2H, s, H-23), 2.82 (1H, m, H-9), 3.32 (1H, q, J = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.36 (1H, dd, J = 6.3, 4.1 Hz), 4.56 (1H, dd, J = 8.7, 5.5 Hz), 4.69, 4.70 (each 1H, d, J = 7.4 Hz, OCH_2O), 5.58 (1H, m, H-16), 5.90 (1H, d, J = 11.2 Hz, H-7), 6.35 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 720 (M^+, 1), 601 (13), 542 (8), 485 (100), 353 (38).

4.15. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-cyanomethylene-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (26a)

To a stirred solution of diethyl (cyanomethyl)phosphonate (39 μL, 0.241 mmol) in dry THF (1 mL) at −40 °C was added *n*-butyllithium (*n*-BuLi, 150 μL, 0.238 mmol, 1.59 M solution in hexane). The mixture was stirred for 15 min after which time a solution of **25a** (86.1 mg, 0.119 mmol) in dry THF (1 mL) was added dropwise. Stirring was continued for 2 h at −40 °C, the mixture was quenched with saturated NH_4Cl, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (5 g) using 3% AcOEt in hexane to afford **26a** (80.9 mg, 91%) as a mixture of two isomers in a ratio of ca. 1:1.

***E*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.13 (12H, 4× Si-Me), 0.84 (9H, s, Si-*t*-Bu, overlapped with H-18, 26a, 27a), 0.930 (9H, s, Si-*t*-Bu), 1.43 (3H, d, J = 7.0 Hz, H-21), 2.79 (1H, m, H-9), 3.12 (1H, m, H-10), 3.40 (3H, s, OMe, overlapped with H-20), 4.69 (2H, s, OCH_2O), 4.47 (1H, m, H-1), 5.00 (1H, m, H-3), 5.48 (1H, d, J = 1.7 Hz, C=CHCN), 5.62 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.18 (1H, d, J = 11.2 Hz, H-6). ***Z*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.13 (12H, 4× Si-Me), 0.82 (9H, s, Si-*t*-Bu, overlapped with H-18, 26a, 27a), 0.926 (9H, s, Si-*t*-Bu), 1.43 (3H, d, J = 7.0 Hz, H-21), 2.79 (1H, m, H-9), 3.00 (1H, m, H-10), 3.40 (3H, s, OMe, overlapped with H-20), 4.69 (2H, s, OCH_2O), 4.58 (1H, m, H-3), 5.05 (1H, m, H-1), 5.48 (1H, d, J = 1.7 Hz, C=CHCN), 5.62 (1H, m, H-16), 5.88 (1H, d, J = 11.2 Hz, H-7), 6.32 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 743 (M^+, 1), 681 (3), 565 (60), 508 (79), 481 (60), 433 (33), 407 (35), 376 (15), 73 (100).

4.16. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-cyanomethylene-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (26b)

The same procedure as described above, but using diethyl (cyanomethyl)phosphonate (26 μL, 0.161 mmol) and **25b** (57.5 mg, 0.0797 mmol) gave **26b** (54.6 mg, 92%, a mixture of two isomers in a ca. 1:1 ratio).

***E*–isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.13 (12H, 4× Si-Me), 0.72 (3H, s, H-18), 0.84, 0.92 (each 9H, s, 2× Si-*t*-Bu), 0.87 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 3.12 (1H, m, H-10), 3.32 (1H, q, J = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.47 (1H, m, H-1), 4.69 (2H, s, OCH_2O), 5.00 (1H, m, H-3), 5.47 (1H, d, J = 1.9 Hz, C=CH), 5.58 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.18 (1H, d, J = 11.2 Hz, H-6). ***Z*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.13 (12H, 4× Si-Me), 0.71 (3H, s, H-18), 0.82, 0.92 (each 9H, s, 2× Si-*t*-Bu), 0.87 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.47 (3H, d, J = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 3.00 (1H, m, H-10), 3.32 (1H, q, J = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.59 (1H, m, H-3), 4.69 (2H, s, OCH_2O), 5.04 (1H, m, H-1), 5.47 (1H, d, J = 1.9 Hz, C=CH), 5.58 (1H, m, H-16), 5.88 (1H, d, J = 11.2 Hz, H-7), 6.31 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 743 (no M^+), 681 (1), 565 (56), 508 (100), 481 (72), 433 (37), 407 (37), 376 (19).

4.17. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(formyl)-ethylidene]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (27a)

To a stirred solution of **26a** (75.8 mg, 0.102 mmol, E/Z = ca. 1:1) in dry toluene (1 mL) at −78 °C was added dropwise diisobutylaluminum hydride (DIBAL-H, 152 μL, 0.152 mmol, 1.0 M solution in toluene). The mixture was stirred for 1.5 h at −78 °C and diluted with hexane. The solution was loaded directly on top of column

on silica gel (5 g), and the column was eluted with 5% AcOEt in hexane to give **27a** (61.6 mg, 81%, a mixture of two isomers in a ca. 1:1 ratio).

***E*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.02–0.11 (12H, 4× Si-Me), 0.83, 0.93 (each 9H, s, 2× Si-*t*-Bu, overlapped with H-18, 26a, 27a), 1.43 (3H, d, *J* = 7.0 Hz, H-21), 2.82 (1H, m, H-9), 3.05 (1H, m, H-10), 3.40 (3H, s, OMe, overlapped with H-20), 4.58 (1H, m, H-1), 4.691 (2H, s, OCH_2O), 5.46 (1H, m, H-3), 5.62 (1H, m, H-16), 5.93 (1H, d, *J* = 11.4 Hz, H-7), 6.16 (1H, m, C=CH), 6.19 (1H, d, *J* = 11.4 Hz, H-6), 10.19 (1H, d, *J* = 7.8 Hz, CHO). ***Z*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.02–0.11 (12H, 4× Si-Me), 0.84, 0.93 (each 9H, s, 2× Si-*t*-Bu, overlapped with H-18, 26a, 27a), 1.43 (3H, d, *J* = 7.0 Hz, H-21), 2.82 (1H, m, H-9), 3.02 (1H, m, H-10), 3.40 (3H, s, OMe, overlapped with H-20), 4.694 (2H, s, OCH_2O, overlapped with H-3), 5.55 (1H, m, H-1), 5.62 (1H, m, H-16), 5.89 (1H, d, *J* = 11.4 Hz, H-7), 6.16 (1H, m, C=CH), 6.31 (1H, d, *J* = 11.4 Hz, H-6), 10.16 (1H, d, *J* = 7.7 Hz, CHO).

4.18. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(formyl)-ethylidene]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (27b)

The same procedure as described above, but using DIBAL-H (103 μL, 0.103 mmol, 1.0 M solution in toluene) and **26b** (51.1 mg, 0.0686 mmol) gave **27b** (45.6 mg, 89%, a mixture of two isomers in a ca. 1:1 ratio).

***E*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.02–0.10 (12H, 4× Si-Me), 0.73 (3H, s, H-18), 0.85, 0.93 (each 9H, s, 2× Si-*t*-Bu), 0.89 (6H, t, *J* = 7.5 Hz, H-26a, 27a), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 3.05 (1H, m, H-10), 3.32 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.58 (1H, m, H-1), 4.69 (2H, s, OCH_2O), 5.46 (1H, m, H-3), 5.58 (1H, m, H-16), 5.94 (1H, d, *J* = 11.2 Hz, H-7), 6.16 (1H, m, C=CH), 6.19 (1H, d, *J* = 11.2 Hz, H-6), 10.19 (1H, d, *J* = 7.9 Hz, CHO). ***Z*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.02–0.11 (12H, 4× Si-Me), 0.72 (3H, s, H-18), 0.83, 0.93 (each 9H, s, 2× Si-*t*-Bu), 0.89 (6H, t, *J* = 7.5 Hz, H-26a, 27a), 1.47 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 3.00 (1H, m, H-10), 3.32 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.69 (2H, s, OCH_2O, overlapped with H-3), 5.54 (1H, m, H-1), 5.58 (1H, m, H-16), 5.89 (1H, d, *J* = 11.2 Hz, H-7), 6.16 (1H, m, C=CH), 6.31 (1H, d, *J* = 11.2 Hz, H-6), 10.16 (1H, d, *J* = 7.9 Hz, CHO).

4.19. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(hydroxy)-ethylidene]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (28a)

To the stirred solution of **27a** (60.1 mg, 0.080 mmol, ca. 1:1 isomeric mixture) in ethanol (EtOH, 1 mL) was added sodium borohydride ($NaBH_4$, 3.0 mg, 0.080 mmol) portionwise at 0 °C. After being stirred for 1 h, the mixture was poured into ice water and extracted with AcOEt. The organic layer was washed with brine, and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by chromatography on silica gel (5 g) using 15% AcOEt in hexane to give **28a** (59.3 mg, 98%, a mixture of two isomers in a ca. 1:1 ratio).

***E*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.10 (12H, 4× Si-Me), 0.85, 0.91 (each 9H, s, 2× Si-*t*-Bu, overlapped with H-18, 26a, 27a), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 3.40 (3H, s, OMe, overlapped with H-20), 4.38 (1H, m, H-1), 4.69 (2H, s, OCH_2O), 4.17–4.34 (2H, m, CH_2OH), 4.82 (1H, m, H-3), 5.61 (1H, m, H-16), 5.72 (1H, m, C=CH), 5.94 (1H, d, *J* = 11.0 Hz, H-7), 6.14 (1H, d, *J* = 11.0 Hz, H-6). ***Z*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.10 (12H, 4× Si-Me), 0.82, 0.93 (each 9H, s, 2× Si-*t*-Bu, overlapped with H-18, 26a, 27a), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 3.40 (3H, s, OMe, overlapped with H-20), 4.17–4.34 (2H, m, CH_2OH), 4.48 (1H, m, H-3), 4.69 (2H, s, OCH_2O), 4.88 (1H, m, H-1), 5.61 (1H, m, H-16), 5.72 (1H, m, C=CH), 5.90 (1H, d, *J* = 11.0 Hz, H-7), 6.25 (1H, d, *J* = 11.0 Hz, H-6).

4.20. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(hydroxy)-ethylidene]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (28b)

The same procedure as described above, but using $NaBH_4$ (2.3 mg, 0.0608 mmol) and **27b** (45.6 mg, 0.0610 mmol) gave **28b** (38.8 mg, 85%, a mixture of two isomers in a ca. 1:1 ratio).

***E*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.10 (12H, 4× Si-Me), 0.73 (3H, s, H-18), 0.85, 0.92 (each 9H, s, 2× Si-*t*-Bu), 0.87 (6H, t, *J* = 7.3 Hz, H-26a, 27a), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 2.88 (1H, dd, *J* = 12.8, 4.4 Hz, H-10), 3.32 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.17–4.33 (2H, m, CH_2OH), 4.38 (1H, m, H-1), 4.69 (2H, s, OCH_2O), 4.82 (1H, m, H-3), 5.58 (1H, m, H-16), 5.72 (1H, m, C=CH), 5.94 (1H, d, *J* = 11.1 Hz, H-7), 6.14 (1H, d, *J* = 11.0 Hz, H-6). ***Z*-isomer**: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.10 (12H, 4× Si-Me), 0.72 (3H, s, H-18), 0.83, 0.93 (each 9H, s, 2× Si-*t*-Bu), 0.87 (6H, t, *J* = 7.3 Hz, H-26a, 27a), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 2.79 (1H, m, H-9), 2.82 (1H, m, H-10), 3.32 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 4.69 (2H, s, OCH_2O), 4.17–4.33 (2H, m, CH_2OH), 4.48 (1H, m, H-3), 4.87 (1H, m, H-1), 5.58 (1H, m, H-16), 5.72 (1H, m, C=CH), 5.90 (1H, d, *J* = 11.1 Hz, H-7), 6.25 (1H, d, *J* = 11.1 Hz, H-6).

4.21. (20*S*)-1α,25-Dihydroxy-2-[2-(hydroxy)-ethylidene]-16-ene-22-thia-26,27-dimethyl-19,24-dinorvitamin D_3 (8a: *E*-isomer; 9a: *Z*-isomer)

A mixture of **28a** (55.3 mg, 0.074 mmol), CSA (102.9 mg, 0.443 mmol) in MeOH (1 mL) was stirred for 1.5 h at ambient temperature, poured into 5% $NaHCO_3$, and extracted with AcOEt. The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. Removal of the solvent afforded the crude product, which was purified by column chromatography on silica gel (3 g) with 2% MeOH in AcOEt to yield **8a** and **9a** (28.3 mg, 80%, as ca. 1:1 isomeric mixture). The compounds **8a** and **9a** were further purified by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O-MeOH, 8 mL/min] to give **8a** (11.8 mg) and **9a** (11.5 mg).

8a (*E*-isomer): ^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.866, 0.874 (each 3H, t, *J* = 7.4 Hz, H-26a, 27a), 1.43 (3H, d, *J* = 7.0 Hz, H-21), 2.54, 2.58 (each 1H, d, *J* = 12.8 Hz, H-23), 2.79 (1H, m, H-9), 3.16 (1H, dd, *J* = 12.6, 4.7 Hz, H-10), 3.43 (1H, q, *J* = 7.0 Hz, H-20), 4.11 (1H, dd, *J* = 12.4, 5.3 Hz, CH_2OH), 4.38 (2H, m, H-1, CH_2OH), 4.82 (1H, m, H-3), 5.64 (1H, m, H-16), 5.77 (1H, m, C=CH), 5.98 (1H, d, *J* = 11.2 Hz, H-7), 6.27 (1H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%): 476 (M^+, 1), 458 (3), 440 (8), 422 (28), 404 (9), 342 (32), 324 (57), 306 (100). HRMS *m/z*: 476.2959 (Calcd for $C_{28}H_{44}O_4S$: 476.2960). UV λ_{max} (EtOH): 245 (ε 31,600), 254 (ε 35,800), 263 (ε 24,100) nm. **9a** (*Z*-isomer): ^{1}H NMR ($CDCl_3$) δ: 0.85 (3H, s, H-18), 0.865, 0.873 (each 3H, t, *J* = 7.4 Hz, H-26a, 27a), 1.43 (3H, d, *J* = 7.0 Hz, H-21), 2.54, 2.58 (each 1H, d, *J* = 12.9 Hz, H-23), 2.69 (1H, dd, *J* = 12.7, 4.7 Hz, H-4), 2.80 (1H, m, H-9), 2.88 (1H, dd, *J* = 14.2, 4.0 Hz, H-10), 3.44 (1H, q, *J* = 7.0 Hz, H-20), 4.16 (1H, dd, *J* = 12.5, 5.7 Hz, CH_2OH), 4.36 (1H, dd, *J* = 12.5, 8.1 Hz, CH_2OH), 4.45 (1H, m, H-3), 4.85 (1H, m, H-1), 5.64 (1H, m, H-16), 5.77 (1H, m, C=CH), 5.94 (1H, d, *J* = 11.2 Hz, H-7), 6.37 (1H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%): 476 (M^+, 1), 458 (5), 440 (11), 422 (29), 404 (9), 342 (4), 324 (63), 306 (100). HRMS *m/z*: 476.2953 (Calcd for $C_{28}H_{44}O_4S$: 476.2960). UV λ_{max} (EtOH): 245, 254, 263 nm.

4.22. (20*R*)-1α,25-Dihydroxy-2-[2-(hydroxy)-ethylidene]-16-ene-22-thia-26,27-dimethyl-19,24-dinorvitamin D_3 (**8b**: *E*-isomer; **9b**: *Z*-isomer)

The same procedure as described above, but using CSA (72.0 mg, 0.310 mmol) and **28b** (38.8 mg, 0.0518 mmol) gave **8b** and **9b** (24.3 mg, 98%, as a mixture of two isomers in a ca. 1:1 ratio), which was further purified by HPLC [YMC-Pack ODS-AM SH-342-5, 25% H_2O/MeOH, 8 mL/min] to give **8b** (11.8 mg) and **9b** (9.4 mg).

8b (*E*-isomer): ^{1}H NMR ($CDCl_3$) δ: 0.72 (3H, s, H-18), 0.87, 0.88 (each 3H, t, J = 7.4 Hz, H-26a, 27a), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.59, 2.63 (each 1H, d, J = 12.8 Hz, H-23), 2.79 (1H, m, H-9), 3.17 (1H, dd, J = 12.6, 4.6 Hz, H-10), 3.35 (1H, q, J = 7.0 Hz, H-20), 4.10 (1H, dd, J = 12.5, 5.3 Hz, CH_2OH), 4.37 (2H, m, H-1, CH_2OH), 4.82 (1H, m, H-3), 5.61 (1H, m, H-16), 5.76 (1H, m, C=CH), 5.98 (1H, d, J = 11.2 Hz, H-7), 6.27 (1H, d, J = 11.2 Hz, H-6). MS *m/z* (%): 476 (M^+, 1), 458 (5), 440 (10), 422 (28), 404 (8), 342 (42), 324 (66), 306 (100). HRMS *m/z*: 476.2949 (Calcd for $C_{28}H_{44}O_4S$: 476.2960). UV λ_{max} (EtOH): 245, 254, 263 nm. **9b** (*Z*-isomer): ^{1}H NMR ($CDCl_3$) δ: 0.74 (3H, s, H-18), 0.87, 0.88 (each 3H, t, J = 7.4 Hz, H-26a, 27a), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.59, 2.63 (each 1H, d, J = 12.8 Hz, H-23), 2.68 (1H, dd, J = 12.8, 4.5 Hz, H-4), 2.79 (1H, m, H-9), 2.88 (1H, m, H-10), 3.35 (1H, q, J = 7.0 Hz, H-20), 4.15 (1H, dd, J = 12.4, 5.3 Hz, CH_2OH), 4.36 (1H, dd, J = 12.4, 8.3 Hz, CH_2OH), 4.44 (1H, m, H-3), 4.85 (1H, m, H-1), 5.60 (1H, m, H-16), 5.76 (1H, m, C=CH), 5.93 (1H, d, J = 11.1 Hz, H-7), 6.37 (1H, d, J = 11.1 Hz, H-6). MS *m/z* (%): 476 (M^+, 1), 458 (4), 440 (10), 422 (28), 404 (10), 342 (30), 324 (58), 306 (100). HRMS *m/z*: 476.2969 (Calcd for $C_{28}H_{44}O_4S$: 476.2960). UV λ_{max} (EtOH): 245, 254, 263 nm.

4.23. 6-Benzyloxy-2,6-bis-(*tert*-butyl-dimethyl-silanyloxy)-1-methyl-cyclohexanol (31)

To a stirred suspension of $LiAlH_4$ (352.7 mg, 9.294 mmol) in dry THF (15 mL) was added a solution of **30** (4.45 g, 9.294 mmol, a mixture of ca. 9:1) in dry THF (15 mL) and the mixture was stirred for 6 h at ambient temperature. An additional $LiAlH_4$ (176.4 mg, 4.648 mmol) was added, and the whole mixture was further stirred for 7 h. Excess $LiAlH_4$ was destroyed by addition of saturated potassium sodium tartrate, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (150 g) using 50% AcOEt in hexane to afford the major isomer **31** (3.31 g, 74%), and the minor isomer was not isolated. The stereochemistry of the major product **31** was not elucidated.

^{1}H NMR ($CDCl_3$) δ: 0.042 (6H, s, 2× Si-Me), 0.06, 0.08 (each 3H, s, 2× Si-Me), 0.84, 0.90 (each 9H, s, 2× Si-*t*-Bu), 1.17 (3H, s, Me), 1.66 (1H, m), 1.88 (2H, m), 2.00 (1H, m), 2.19 (1H, s, OH), 3.70 (2H, m), 3.81 (1H, m), 4.51, 4.54 (each 1H, d, J = 12.0 Hz, CH_2Ph), 7.26–7.35 (5H, m, arom. H). MS *m/z* (%): 480 (M^+, 1), 423 (5), 366 (5), 309 (46), 291 (33), 91 (100). HRMS *m/z*: 480.3090 (Calcd for $C_{26}H_{48}O_4Si_2$: 480.3091).

4.24. [3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4-methyl-4-trimethylsilanyloxy-cyclohexyloxymethyl]-benzene (32)

To a stirred solution of **31** (3.27 g, 6.80 mmol, a single isomer) in dry toluene (30 mL) at −40 °C were added Et_3N (2.84 mL, 20.4 mmol) and trimethylsilyl trifluoromethanesulfonate (1.85 mL, 10.2 mmol), and the reaction mixture was stirred, and allowed to warm to −20 °C in a period of ca. 2.5 h. The mixture was poured into 5% $NaHCO_3$, and extracted with AcOEt. The AcOEt layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed on silica gel (100 g) with 5% AcOEt in hexane to yield **32** (3.69 g, 98%).

^{1}H NMR ($CDCl_3$) δ: 0.017, 0.023, 0.04, 0.05 (each 3H, s, 4× Si-Me), 0.11 (9H, s, Si-Me_3), 0.83, 0.91 (each 9H, s, 2× Si-*t*-Bu), 1.20 (3H, s, Me), 1.77 (2H, m), 1.89 (2H, m), 3.57 (1H, m), 3.61 (1H, dd, J = 11.6, 4.2 Hz), 3.63 (1H, m), 4.52 (2H, s, CH_2Ph), 7.26–7.35 (5H, m, arom. H).

MS *m/z* (%): 552 (M^+, 1), 537 (2), 495 (10), 461 (2), 387 (64), 363 (10), 91 (100). HRMS *m/z*: 552.3466 (Calcd for $C_{29}H_{56}O_4Si_3$: 552.3486).

4.25. 3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4-methyl-4-trimethylsilanyloxy-cyclohexanol (33)

A mixture of **32** (3.69 g, 6.673 mmol, single isomer) and palladium, 10 wt. % on carbon (369 mg) in AcOEt (15 mL) and EtOH (15 mL) was hydrogenated under an atmospheric pressure of H_2 at ambient temperature. After vigorous stirring for 1 h, the reaction mixture was filtered through a pad of Celite. The pad was washed with AcOEt, and the combined filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (80 g) using 10% AcOEt in hexane to afford **33** (3.06 g, 99%).

^{1}H NMR ($CDCl_3$) δ: 0.044, 0.055, 0.063, 0.070 (each 3H, s, 4× Si-Me), 0.12 (9H, s, Si-Me_3), 0.88, 0.91 (each 9H, s, 2× Si-*t*-Bu), 1.22 (3H, s, Me), 1.73 (2H, m), 1.84 (2H, m), 3.58 (1H, m), 3.68 (1H, dd, J = 11.4, 4.1 Hz), 3.92 (1H, m). MS *m/z* (%): 462 (M^+, 2), 405 (2), 387 (100), 315 (9), 273 (26). HRMS *m/z*: 462.3007 (Calcd for $C_{22}H_{50}O_4Si_3$: 462.3017).

4.26. 3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4-methyl-4-trimethylsilanyloxy-cyclohexanone (34)

To a stirred solution of $(COCl)_2$ (692 μL, 7.932 mmol) in dry CH_2Cl_2 (5 mL) at −78 °C was added a solution of DMSO (1.12 mL, 15.8 mmol) in dry CH_2Cl_2 (2 mL). After being stirred for 5 min, a solution of **33** (3.06 g, 6.611 mmol, single isomer) in dry CH_2Cl_2 (20 mL) was added dropwise. The reaction mixture was stirred for 15 min at −78 °C, and Et_3N (4.61 mL, 33.1 mmol) was added. The whole mixture was warmed from −78 °C to ambient temperature over 1.5 h after which time it was quenched with ice water, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (200 g) using 2% AcOEt in hexane to afford **34** (2.78, 91%) as a single compound.

^{1}H NMR ($CDCl_3$) δ: 0.050, 0.057 (each 3H, s, 2× Si-Me), 0.064 (6H, s, 2× Si-Me), 0.16 (9H, s, Si-Me_3), 0.84, 0.91 (each 9H, s, 2× Si-*t*-Bu), 1.35 (3H, s, Me), 2.17 (1H, dt, J = 14.6, 2.5 Hz), 2.37 (1H, ddd, J = 14.0, 5.0, 2.1 Hz) 2.69 (1H, dd, J = 14.0, 11.3 Hz), 2.94 (1H, dd, J = 14.6, 3.2 Hz), 3.81 (1H, t, J = 3.1 Hz), 3.98 (1H, dd, J = 11.3, 5.0 Hz). MS *m/z* (%): 460 (M^+, 1), 445 (5), 403 (100), 313 (15), 271 (56), 143 (82). HRMS *m/z*: 460.2886 (Calcd for $C_{22}H_{48}O_4Si_3$: 460.2860).

4.27. [3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4-methyl-4-trimethylsilanyloxy-cyclohexylidene]-acetic acid methyl ester (35)

To a stirred solution of lithium diisopropylamide in dry THF [freshly prepared by diisopropylamine (1.69 mL, 12.1 mmol) in dry THF (5 mL) and *n*-BuLi (7.64 mL, 12.1 mmol, 1.58 M solution in hexane) at −78 °C] was added methyl trimethylsilylacetate (1.98 mL, 12.1 mmol). After stirring for 10 min, to this solution was added dropwise a solution of **34** (2.78 g, 6.032 mmol, single compound) dissolved in dry THF (20 mL), and stirring was continued for 2 h at −78 °C. The mixture was quenched with saturated NH_4Cl, and extracted with AcOEt. The organic phase was washed with brine, and dried over $MgSO_4$. Removal of the solvent in vacuo afforded the residue, which was chromatographed on silica gel (80 g) using 2% AcOEt in hexane to give **35** (2.98 g, 96%) as a mixture of two isomers in ca. 1:1 ratio due to newly generated double bond isomerism. The stereochemistry of **35** remains unassigned.

^{1}H NMR ($CDCl_3$) δ: 0.02–0.073 (12H, 4× Si-Me), 0.126, 0.130 (ca. 1:1) (9H, s, Si-Me_3), 0.81, 0.84 (ca. 1:1) (9H, s, Si-*t*-Bu), 0.91, 0.93 (ca. 1:1) (9H, s, Si-*t*-Bu), 1.25 (3H, s, Me), 1.90–2.85 (3H, m), 3.60–3.84 (3H, m), 3.65, 3.68 (ca. 1:1) (3H, s, CO_2Me), 5.56, 5.71 (ca. 1:1) (1H, s, C=CH). MS *m/z* (%): 516 (M^+, 1), 501 (3), 459 (100), 427 (13), 399 (4), 369 (8), 327 (46), 295 (60). HRMS *m/z*: 516.3148 (Calcd for $C_{25}H_{52}O_5Si_3$: 516.3123).

4.28. 2-[3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4-methyl-4-trimethylsilanyloxy-cyclohexylidene]-ethanol (36)

To a stirred solution of **35** (2.98 g, 5.765 mmol, a mixture of ca. 1:1) in dry toluene (25 mL) at −78 °C was added DIBAL-H (14.4 mL, 14.4 mmol, 1.0 M solution in toluene), and the mixture was stirred for 1.5 h. Excess reducing reagents were decomposed by adding saturated potassium sodium tartrate. The mixture was poured into ice water, and extracted with AcOEt. The organic layer was successively washed with water and brine, and dried over $MgSO_4$. Solvents were evaporated in vacuo, and the residue was purified by chromatography on silica gel (80 g) using 10% AcOEt in hexane to afford **36** (2.69 g, 95%, a mixture of two isomers in ca. 1:1 ratio).

^{1}H NMR ($CDCl_3$) δ: 0.03–0.07 (12H, 4× Si-Me), 0.119, 0.121 (ca. 1:1) (9H, s, Si-Me_3), 0.85 (9H, s, Si-*t*-Bu), 0.91, 0.92 (ca. 1:1) (9H, s, Si-*t*-Bu), 1.23 (3H, s, Me), 1.85–2.75 (4H, m), 3.56–3.67 (2H, m), 4.08–4.16 (2H, m), 5.36, 5.49 (ca. 1:1) (1H, m, C mL,CH). MS *m/z* (%): 488 (M^+, 1), 470 (5), 455 (4), 431 (10), 413 (54), 380 (9), 341 (17), 307 (27), 299 (22), 260 (59), 73 (100). HRMS *m/z*: 470.3048 (M^+-H_2O) (Calcd for $C_{24}H_{50}O_3Si_3$: 470.3068).

4.29. 2-[3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4-methyl-4-trimethylsilanyloxy-cyclohexylidene]-ethyl-diphenylphosphine oxide (37)

To a stirred solution of **36** (2.10 g, 4.295 mmol, a mixture of ca. 1:1 isomeric mixture) in dry THF (20 mL) at 0 °C was added dropwise *n*-BuLi (3.03 mL, 4.725 mmol, 1.56 M solution in hexane), a solution of freshly recrystallized *p*-toluenesulfonyl chloride (982.6 mg, 5.154 mmol) in dry THF (3 mL) was added dropwise, and the mixture was allowed to stand at 0 °C for 5 min. To this solution of the tosylate was added dropwise a red solution freshly prepared from diphenylphosphine (1.12 mL, 6.443 mmol) in THF (5 mL) and *n*-BuLi (4.13 mL, 6.443 mmol, 1.56 M solution in hexane) at 0 °C until the orange color persisted. The entire mixture was stirred for 30 min at 0 °C, and quenched by adding water (0.5 mL). The solvent was evaporated in vacuo, and the crude product was dissolved in CH_2Cl_2 (20 mL). To this mixture was added 10% hydrogen peroxide (30 mL). The mixture was stirred for 1 h at 0 °C, and CH_2Cl_2 phase was separated. The organic layer was successively washed with cold 2 N Na_2SO_3, water and brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by chromatography on silica gel (80 g) using 40% AcOEt in hexane to afford **37** (2.44 g, 84%, a mixture of two isomers in a ratio of ca. 1:1).

^{1}H NMR ($CDCl_3$) δ: −0.04 to 0.02 (12H, 4× Si-Me), 0.07, 0.08 (ca. 1:1) (9H, s, Si-Me_3), 0.80, 0.83 (ca. 1:1) (9H, s, Si-*t*-Bu), 0.88, 0.89 (ca. 1:1) (9H, s, Si-*t*-Bu), 1.17, 1.18 (ca. 1:1) (3H, s, Me), 1.6–2.6 (4H, m), 2.9–3.2 (2H, m), 3.45–3.64 (2H, m), 5.17, 5.27 (ca. 1:1) (1H, m, CH=C), 7.4–7.8 (10H, m, arom. H). MS *m/z* (%): 672 (M^+, 1), 657 (5), 615 (59), 540 (100), 483 (44), 451 (20), 408 (9), 338 (26). HRMS *m/z*: 672.3613 (Calcd for $C_{36}H_{61}O_4PSi_3$: 672.3615).

4.30. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)-oxy]-2-methyl-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (38a)

To a stirred solution of **37** (312.5 mg, 0.464 mmol, a mixture of ca. 1:1) in dry THF (1.5 mL) at −78 °C was added slowly LHMDS (464 μL, 0.464 mmol, 1.0 M solution in THF), and the resulting dark orange solution was stirred for 30 min. To this colored solution was added a solution of **13a** (82.3 mg, 0.232 mmol) in dry THF (1 mL), the reaction mixture was stirred for 1 h at −78 °C and at 0 °C for 1 h. The reaction mixture was quenched with saturated NH_4Cl, and extracted with AcOEt. The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (15 g) using 3% AcOEt in hexane to afford **38a** (59.2 mg, 32% based on **13a**, a mixture of two isomers in a ca. 3:2 ratio), and 15% AcOEt in hexane to give the unreacted starting material **13a** (43.6 mg).

^{1}H NMR ($CDCl_3$) δ: 0.02–0.09 (12H, 4× Si-Me), 0.11, 0.12 (3:2) (9H, s, Si-Me_3), 0.82, 0.84 (3:2) (9H, m, 2× Si-*t*-Bu), 0.86, 0.87 (each 3H, t, *J* = 7.5 Hz, H-26, 27), 0.92, 0.93 (3:2) (9H, s, Si-*t*-Bu), 1.23, 1.24 (2:3) (3H, s, 2-Me), 1.42 (3H, d, *J* = 7.0 Hz, H-21), 3.399, 3.403 (3:2) (3H, s, OMe), 3.57–3.73 (2H, m, H-1, 3), 4.69 (2H, s, OCH_2O), 5.61 (1H, m, H-16), 5.87, 5.92 (3:2) (1H, d, *J* = 11.2 Hz, H-7), 6.02, 6.14 (2:3) (1H, d, *J* = 11.2 Hz, H-6).

4.31. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)-oxy]-2-methyl-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (38b)

Following the same procedure as described above, treatment of **37** (78.2 mg, 0.116 mmol, ca. 1:1 isomeric mixture) with **13b** (20.6 mg, 0.058 mmol) in the presence of LHMDS (116 μL, 0.116 mmol) gave **38b** (19.0 mg, 40%, ca. 5:1 isomeric mixture) and the unreacted starting ketone **13b** (12 mg).

^{1}H NMR ($CDCl_3$) δ: 0.03–0.09 (12H, 4× Si-Me), 0.11, 0.12 (5:1) (9H, s, Si-Me_3), 0.71, 0.72 (5:1) (3H, s, H-18), 0.82, 0.84 (5:1) (9H, s, Si-*t*-Bu), 0.87 (6H, t, *J* = 7.5 Hz, H-26a, 27a), 0.92, 0.93 (5:1) (9H, s, Si-*t*-Bu), 1.24 (3H, s, 2-Me), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.65 (2H, s, H-23), 3.34 (1H, q, *J* = 7.0 Hz, H-20), 3.41 (3H, s, OMe), 3.56–3.73 (2H, m, H-1, 3), 4.69 (2H, s, OCH_2O), 5.58 (1H, m, H-16), 5.87, 5.93 (5:1) (1H, d, *J* = 11.1 Hz, H-7), 6.02, 6.13 (1:5) (1H, d, *J* = 11.1 Hz, H-6).

4.32. (20*S*)-1α,2α,25-Trihydroxy-2β-methyl- and (20*S*)-1α,2β,25-trihydroxy-2α-methyl-16-ene-22-thia-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (10a and 11a)

A mixture of **38a** (59.2 mg, 0.073 mmol, ca. 3:2 isomeric mixture) and CSA (101.9 mg, 0.439 mmol) in MeOH (1 mL) was stirred for 4 h at ambient temperature, poured into 5% $NaHCO_3$, and extracted with AcOEt. The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. Removal of the solvent afforded the crude product, which was purified by column chromatography on silica gel (5 g) with 80% AcOEt in hexane to afford **10a** and **11a** (31.6 mg, 93%, ca. 3:2 isomeric mixture). The compounds **10a** and **11a** were further purified by HPLC [LiChrosorb Si 60 (7 μm), hexane: $CHCl_3$/MeOH/THF = 80:20:3:12, 5 mL/min] to give **10a** (7.6 mg) and **11a** (7.2 mg).

10a ^{1}H NMR ($CDCl_3$) δ: 0.84 (3H, s, H-18), 0.87, 0.88 (each 3H, t, *J* = 7.5 Hz, H-26a, 27a), 1.31 (3H, s, 2-Me), 1.43 (3H, d, *J* = 7.0 Hz, H-21), 2.55, 2.57 (each 1H, d, *J* = 13.0 Hz), 2.79 (1H, m, H-9), 3.44 (1H, q, *J* = 7.0 Hz, H-20), 3.74, 3.79 (each 1H, m, H-3, 1), 5.63 (1H, m, H-16), 5.91 (1H, d, *J* = 11.3 Hz, H-7), 6.33 (1H, d, *J* = 11.3 Hz, H-6). MS *m/z* (%): 464 (M^+, 2), 446 (20), 428 (8), 410 (7), 392 (6), 330 (100), 312 (48), 294 (31). HRMS *m/z*: 464.2969 (Calcd for $C_{27}H_{44}O_4S$: 464.2960). **11a** ^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.87, 0.88 (each 3H, t, *J* = 7.4 Hz, H-26a, 27a), 1.28 (3H, s, 2-Me), 1.43 (3H, d, *J* = 7.0 Hz, H-21), 2.55, 2.58 (each 1H, d, *J* = 12.9 Hz, H-23), 2.78 (1H, m, H-9), 2.96 (1H, dd, *J* = 13.6, 4.7 Hz, H-10), 3.44 (1H, q, *J* = 7.0 Hz, H-20), 3.76 (2H, m, H-1, 3), 5.64 (1H, m, H-16), 5.94 (1H, d, *J* = 11.2 Hz, H-7), 6.29 (1H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%): 464 (M^+, 2), 446 (23),

428 (10), 410 (8), 392 (7), 330 (100), 312 (54), 294 (31). HRMS *m/z*: 464.2953 (Calcd for $C_{27}H_{44}O_4S$: 464.2960).

4.33. (20*R*)-1α,2α,25-Trihydroxy-2β-methyl- and (20*R*)-1α,2β,25-trihydroxy-2α-methyl-16-ene-22-thia-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (10b and 11b)

Following the same procedure as described above, treatment of **38b** (19.0 mg, 0.0235 mmol, ca. 5:1 isomeric mixture) with CSA (43.6 mg,, 0.188 mmol) gave **10b** and **11b** (9.1 mg, 84%, ca. 1:5 isomeric mixture). The compounds **10b** and **11b** were further purified by HPLC [LiChrosorb Si 60 (5 μm), hexane/chloroform/MeOH = 20:80:2.5, 1 mL/min] to give **10b** (1.1 mg) and **11b** (5.0 mg).

10b ^{1}H NMR ($CDCl_3$) δ: 0.73 (3H, s, H-18), 0.87, 0.88 (each 3H, t, *J* = 7.4 Hz, H-26a, 27a), 1.31 (3H, s, 2-Me), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.79 (1H, m, H-9), 3.35 (1H, q, *J* = 7.0 Hz, H-20), 3.72 (2H, m, H-3, 1), 5.61 (1H, m, H-16), 5.91 (1H, d, *J* = 11.2 Hz, H-7), 6.33 (1H, d, *J* = 11.2 Hz, H-6). MS *m/z* (%): 464 (M^+, 2), 446 (17), 428 (9), 410 (8), 392 (6), 330 (100), 312 (57), 294 (34). HRMS *m/z*: 464.2963 (Calcd for $C_{27}H_{44}O_4S$: 464.2960). **11b** ^{1}H NMR ($CDCl_3$) δ: 0.72 (3H, s, H-18), 0.87, 0.88 (each 3H, t, *J* = 7.4 Hz, H-26a, 27a), 1.27 (3H, s, 2-Me), 1.46 (3H, d, *J* = 7.0 Hz, H-21), 2.59, 2.63 (each 1H, d, *J* = 12.8 Hz), 2.78 (1H, m, H-9), 2.95 (1H, dd, *J* = 13.5, 4.4 Hz, H-10), 3.35 (1H, q, *J* = 7.0 Hz, H-20), 3.76 (2H, m, H-1, 3), 5.61 (1H, m, H-16), 5.94 (1H, d, *J* = 11.3 Hz, H-7), 6.29 (1H, d, *J = 11.3 Hz, H-6). MS m/z* (%): 464 (M^+, 2), 446 (21), 428 (11), 410 (8), 392 (6), 330 (100), 312 (55), 294 (30). HRMS *m/z*: 464.2938 (Calcd for $C_{27}H_{44}O_4S$: 464.2960).

4.34. Vitamin D receptor-binding assay

The rat recombinant VDR ligand-binding domain (LBD) (amino acids 115–423) was expressed as an amino-terminal His-tagged protein in *Escherichia* coli BL21 (DE3) pLys S (Novagen). The cells were lysed by sonication. The supernatants were diluted approximately 1000 times in 50 mM Tris buffer (100 mM KCl, 5 mM DTT, 0.5% CHAPS, pH 7.5) containing bovine serum albumin (100 μg/mL) and were pipetted into glass culture tubes. A solution containing an increasing amount of 1α,25-$(OH)_2D_3$ or the synthetic analogs in 15 μL of EtOH was added to the receptor solution in each tube and the mixture was vortexed 1–2 times. The mixture was incubated for 1 h at room temperature. [^{3}H]1α,25-$(OH)_2D_3$ (specific activity, 6.62 TBq/mmol, ca. 5000 dpm) in 15 μL of EtOH was added, vortexed 2–3 times, and the whole mixture was then allowed to stand at 4 °C for 18 h. At the end of the second incubation, 200 μL of dextran-coated charcoal suspension (purchased from Yamasa Shoyu) was added to bind any free ligands (or to remove free ligands) and the sample was vortexed. After 30 min at 4 °C, bound and free [^{3}H]1α,25-$(OH)_2D_3$ were separated by centrifugation at 3000 rpm for 15 min at 4 °C. Aliquots (500 μL) of the supernatant were mixed with 9.5 mL of ACS-II scintillation fluid (Amersham, Buckinghamshire, UK) and submitted for radioactivity counting. Each assay was performed at least twice in duplicate.

4.35. Transactivation assay

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS). Cells were seeded on 24-well plates at a density of $\sim 2 \times 10^4$ per well. After 24 h, cells were transfected with a reporter plasmid containing three copies of the mouse osteopontin VDRE (5′-GGTTCAcgaGGTTCA, SPPx3-TK-LUC), a wild-type or mutant hVDR expression plasmids (pCMX-hVDR), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) by the lipofection method as described previously.[29] After 4 h incubation, the medium was replaced with fresh DMEM containing 1% FCS (HyClone, UT). The next day, the cells were treated with either indicated concentration of 1α,25-$(OH)_2D_3$, 19-norvitamin D analogs, 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 (Tokyo Ink, Inc., Japan) according to the manufacturer's instructions. Transactivation measured by the luciferase activity was normalized with the internal control. All experiments were done in triplicate.

4.36. Osteoclasts differentiation assay

Bone marrow cells were obtained from tibiae of 5- to 8-week-old male mice of the ddY strain. Primary osteoblastic cells were prepared from the calvariae of newborn ddY mice as previously described.[30,31] Briefly, mouse bone marrow cells (1.5×10^5 cell/well) and primary osteoclasts (3×10^3 cells/well) were co-cultured for 7 days in the presence of 1α,25-$(OH)_2D_3$, 2MD or 19-norvitamin D analogs (10^{-8} to 10^{-12} M) in 0.3 mL of α-MEM (Sigma, St. Louis, USA) supplemented with 10% FBS in 48-well plates. Cells were replenished on day 3 with fresh medium. Cells were then fixed with 10% formaldehyde in PBS and stained for tartrate-resistant acid phosphatase (TRAP) as described.[31,32] TRAP-positive multinucleated cells containing three or more nuclei were counted as osteoclasts, under microscopic examination. The results were expressed as the mean ± SEM of three cultures.

4.37. X-ray crystallographic analysis of the complex structure

The ternary complex of rat VDR-LBD/1α,25-$(OH)_2D_3$, ligand **3a** or **3b**/DRIP peptide was prepared for the X-ray diffraction studies as described by Vanhooke et al.[8] The crystals of the complexes were grown at 20 °C by vapor diffusion in the 'hanging drop' configuration. The precipitant solution contained 0.1–0.4 M formic acid, 15–20% polyethylene glycol (PEG) 4000, and 0–20% ethylene glycol. The crystals were flash-frozen using mother liquor supplemented with 20% ethylene glycol. Diffraction data for the complex were collected at beamline BL-6A at the Photon Factory and were integrated and scaled with HKL2000 (HKL Research Inc., VA). The structure was solved by molecular replacement by using a crystal structure of the ternary complexes (PDB code: 1RJK) as the search model in CNS (Brünger et al. 1998). Refinement was performed with CNS and XtalView (McRee 1999). The coordinates of the three complexes were deposited in the Protein Data Bank with accession numbers 2ZLC for 1α,25-$(OH)_2D_3$, 2ZL9 for the ligand **3a**, and 2ZLA for the ligand **3b**.

References and notes

1. *Vitamin D*; Feldman, D., lorrieux, F. H., Pike, J. W., Eds.; Academic: New York, 1997.
2. Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4990.
3. Yamada, S.; Shimizu, M.; Yamamoto, K. *Med. Res. Rev.* **2003**, *23*, 89.
4. Perlman, K. L.; Sicinski, R. R.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1990**, *31*, 1823.
5. Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1991**, *32*, 7663.
6. Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.* **1998**, *41*, 4662.
7. Shevde, N. K.; Plum, L. A.; Clagett-Dame, M.; Yamamoto, H.; Pike, J. W.; DeLuca, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13487.
8. Vanhooke, J. L.; Benning, M. M.; Bauer, C. B.; Pike, J. W.; DeLuca, H. F. *Biochemistry* **2004**, *43*, 4101.
9. Takaku, H.; Miyamoto, Y.; Asami, S.; Shimazaki, M.; Yamada, S.; Yamamoto, K.; Udagawa, N.; DeLuca, H. F.; Shimizu, M. *Bioorg. Med. Chem.* **2008**, *16*, 1796.
10. Shimizu, M.; Iwasaki, Y.; Shibamoto, Y.; Sato, M.; DeLuca, H. F.; Yamada, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 809.
11. Shimizu, M.; Yamamoto, K.; Mihori, M.; Iwasaki, Y.; Morizono, D.; Yamada, S. *J. Steroid Biochem. Mol. Biol.* **2004**, *89–90*, 75.

12. Shimizu, M.; Iwasaki, Y.; Shimazaki, M.; Amano, Y.; Yamamoto, K.; Reischl, W.; Yamada, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1451.
13. Shimizu, M.; Miyamoto, Y.; Kobayashi, E.; Shimazaki, M.; Yamamoto, K.; Wolfgang, R.; Yamada, S. *Bioorg. Med. Chem.* **2006**, *14*, 4277.
14. Shimazaki, M.; Miyamoto, Y.; Yamamoto, K.; Yamada, S.; Takami, M.; Shinki, T.; Udagawa, N.; Shimizu, M. *Bioorg. Med. Chem.* **2006**, *14*, 4645.
15. Kobayashi, E.; Shimazaki, M.; Miyamoto, Y.; Masuno, H.; Yamamoto, K.; DeLuca, H. F.; Yamada, S.; Shimizu, M. *Bioorg. Med. Chem.* **2007**, *15*, 1475.
16. Sato, M.; Nakamichi, Y.; Nakamura, M.; Sato, N.; Ninomiya, T.; Muto, A.; Nakamura, H.; Iwasaki, Y.; Kobayashi, E.; Shimizu, M.; DeLuca, H. F.; Takahashi, N.; Udagawa, N. *Bone* **2007**, *40*, 293.
17. Honda, A.; Mori, Y.; Otomo, S.; Ishizuka, S.; Ikekawa, N. *Steroid* **1991**, *56*, 142.
18. Strugnell, S. A.; Hill, J. J.; McCaslin, D. R.; Wiefling, B. A.; Royer, C. A.; DeLuca, H. F. *Arch. Biochem. Biophys.* **1999**, *364*, 42.
19. Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. *Mol. Cell* **2000**, *5*, 173.
20. Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5491.
21. Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Moras, D. *J. Med. Chem.* **2004**, *47*, 1956.
22. Eelen, G.; Verlinden, L.; Rochel, N.; Claessens, F.; De Clercq, P.; Vandewalle, M.; Tocchini-Valentini, G.; Moras, D.; Bouillon, R.; Verstuyf, A. *Mol. Pharmacol.* **2005**, *67*, 1566.
23. Hourai, S.; Fujishima, T.; Kittaka, A.; Suhara, Y.; Takayama, H.; Rochel, N.; Moras, D. *J. Med. Chem.* **2006**, *49*, 5199.
24. Ciesielski, F.; Rochel, N.; Moras, D. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 235.
25. Vanhooke, J. L.; Tade, B. P.; Benning, M. M.; Plum, L. A.; DeLuca, H. F. *Arch. Biochem. Biophys.* **2007**, *460*, 161.
26. ochel, N.; Hourai, S.; Pérez-Garcia, X.; Rumbo, A.; Mourino, A.; Moras, D. *Arch. Biochem. Biophys.* **2007**, *460*, 172.
27. Peleg, S.; Sastry, M.; Collins, E. D.; Bishop, J. E.; Norman, A. W. *J. Biol. Chem.* **1995**, *270*, 10551.
28. Hansen, C. M.; Hamberg, K. J.; Binderup, E.; Binderup, L. *Curr. Pharm. Des.* **2000**, *6*, 803.
29. Choi, M.; Yamamoto, K.; Masuno, H.; Nakashima, K.; Taga, T.; Yamada, S. *Bioorg. Med. Chem.* **2001**, *9*, 1721.
30. Koga, T.; Nishihara, T.; Fujiwara, T.; Nishizawa, T.; Okahashi, N.; Noguchi, T.; Hamada, S. *Infect. Immun.* **1985**, *47*, 638.
31. Suda, T.; Jimi, E.; Nakamura, I.; Takahashi, N. *Methods Enzymol.* **1997**, *282*, 223.
32. Li, X.; Udagawa, N.; Itoh, K.; Suda, K.; Murase, Y.; Nishihara, T.; Suda, T.; Takahashi, N. *Endocrinology* **2002**, *143*, 3105.