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Bioorganic & Medicinal Chemistry 14 (2006) 4277–4294
Bioorganic & Medicinal Chemistry

Synthesis and biological activities of new 1α,25-dihydroxy-19-norvitamin D_3 analogs with modifications in both the A-ring and the side chain

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Received 22 December 2005; revised 21 January 2006; accepted 23 January 2006
Available online 24 February 2006

Abstract—In a series of studies on structure–activity relationships of 2-substituted 19-norvitamin D analogs, we found that 1α,25-dihydroxy-19-norvitamin D_3 analogs with 2β-hydroxyethoxy or 2*E*-hydroxyethylidene moieties show strong binding affinity for the vitamin D receptor (VDR) as well as marked transcriptional activity. To further examine the effects of side chain structure on the activity of 2-substituted 19-norvitamin D analogs, we have synthesized new 19-norvitamin D_3 analogs with modifications in both the A-ring at the C(2) position and the side chain. The side chains of these analogs contained a double bond between C(22) and C(23) or an oxygen atom at C(22). The biological activity of the analogs was evaluated in vitro. All the side chain-modified analogs were less active than 1α,25-dihydroxyvitamin D_3 **1e** and the parent compounds **3**–**6e** possessing a natural 20*R*-configuration in binding to the VDR, but, except for the (20*R*)-22-oxa analogs **3**–**6d**, were significantly more potent in transcriptional activity. Of the side-chain-modified analogs **4** and **5**, the 2β-hydroxyethoxy- and 2*E*-hydroxyethylidene-22,24-diene-24a,26a,27a-trihomo analogs showed markedly higher transcriptional activity (25- and 17.5-fold, respectively) compared with **1e**. Elongation of the side chain at the C-24, C-26, and C-27 positions and introduction of a 22,24-diene moiety strongly increased transcriptional activity, as seen in the 20-*epi* analogs **3**–**6f**.

1. Introduction

1α,25-Dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] (**1e**) exerts pleiotropic effects, including regulation of bone mineralization and calcium homeostasis as well as regulation of cell proliferation and differentiation, and has immunomodulatory activity.[1] 1α,25-$(OH)_2D_3$ exerts most of its effects via the vitamin D receptor (VDR), which belongs to the large superfamily of nuclear receptors. The VDR has been shown to heterodimerize with the retinoid X receptor (RXR) and binds to vitamin D response elements (VDREs), which are located in promoter regions of certain genes, such as those encoding osteopontin, osteocalcin, and 1α,25-D_3 24-hydroxylase (CYP24).[1]

1α,25-Dihydroxy-19-norvitamin D_3 [1,25-$(OH)_2$-19-nor-VD] **2e** is a highly stable compound because of its lack of the labile conjugated triene moiety characteristic of vitamin D; it possesses the desirable properties of low calcemic activity but preserved cell differentiation activity.[2,3] Introduction of a substituent into the C(2) position dramatically changes the biological activity of vitamin D derivatives.[4–12] During the last decade, a number of 2-substituted vitamin D analogs have been synthesized and studied intensively for biological activity.[13] (20*S*)-2-Methylene-1α,25-dihydroxy-19-norvitamin D_3 (2MD, Fig. 1), developed by DeLuca and co-workers, shows preferential activity on bone compared with intestine and also stimulates bone formation in ovariectomized rats without causing toxic hypercalcemia.[14,15] ED-71

Keywords: 19-Norvitamian D; Side-chain-modified vitamin D analog; Vitamin D receptor; Transcriptional activity.
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doi:10.1016/j.bmc.2006.01.061

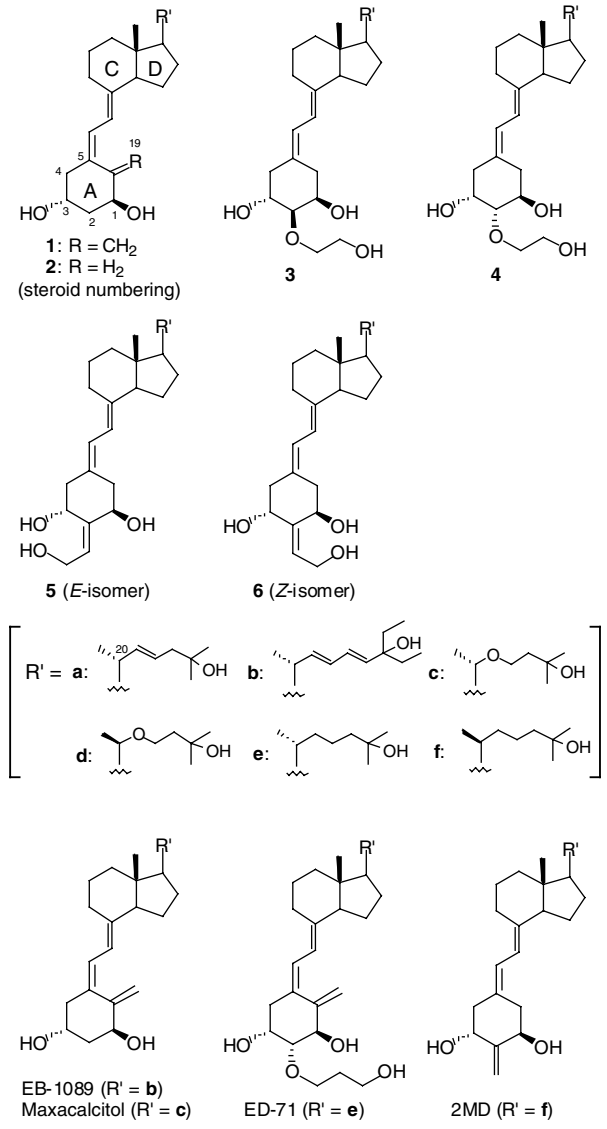

Figure 1. Structures of 1α,25-dihydroxyvitamin D_3 and its analogs.

(Fig. 1), which contains a 2β-hydroxypropoxyl group, also increases bone mass.[16] Both 2MD and ED-71 are promising therapeutic candidates for the treatment of osteoporosis and are currently in phase II and phase III clinical trials, respectively. In our examination of the structure–activity relationships (SAR) of the A-ring-modified 19-norvitamin D analogs, we synthesized 19-norvitamin D analogs containing hydroxyethoxy or hydroxyethylidene moieties at the C(2) position and demonstrated that these analogs show strong binding affinity for the VDR and enhanced transcriptional activity.[7,8] We also synthesized novel 2,2-disubstituted 19-norvitamin D analogs; among these, (20*S*)-2-methyl-2-hydroxy-1α,25-dihydroxy-19-norvitamin D is as potent as 2MD in inducing osteoclast formation.[9,17]

Up to now, more than 3000 vitamin D analogs have been synthesized. Most of the structural modifications of **1e** occur in the side chain, followed by A-ring alterations.[13] Some of these analogs have low calcemic activity, and such modifications are of key importance in modulating the biological activities of vitamin D. To elucidate further the SAR of 19-norvitamin D analogs, we focused our attention on examining the effect of side chain structure on the biological activities of 2-substituted analogs. Limited biological data are available for vitamin D analogs with structural modifications on both the A-ring and the side chain,[18–21] although such modifications could provide additional information regarding the SAR of vitamin D. Biological activity is markedly affected by the size, chemical properties (hydrophilicity or hydrophobicity), and position of the substituent, as well as by the stereochemistry of substitution. From the active space group concept proposed by Yamamoto et al.,[22,23] analogs bearing a double bond at C(22), an oxygen atom at the C(22) position or a 22,24-diene moiety are predicted to have high potency in cell differentiation and transcriptional activities but weak calcemic activity. Methylation at C(26) and C(27) is also expected to increase biological activity. 22-Oxa-1,25-$(OH)_2D_3$ (Maxacalcitol) is currently marketed for the treatment of psoriasis and 22,24-diene-24,26,27-trihomo-1,25-$(OH)_2D_3$ (EB-1089) is under development as an anticancer drug (Fig. 1).

Here, we report the synthesis and biological activities of a new class of highly modified (20*R*)- and (20*S*)-19-norvitamin D analogs **3**–**6** with hydroxyethoxy or hydroxyethylidene groups at C(2) and a double bond or oxygen atom at C(22).

2. Results and discussion

2.1. Synthesis

For the synthesis of both A-ring- and side-chain-modified 1α,25-$(OH)_2$-19-nor VD analogs **3**–**6**, Wittig–Horner coupling of the A-ring phosphine oxides **7** or **8** with the protected 25-hydroxy Grundmann's ketones **22a**–**d** was employed.

(3*R*,5*R*)-A-ring phosphine oxide **7** (based on sugar numbering) was prepared from D-glucose as reported,[7] and we synthesized the A-ring synthon **8** with a hydroxyethylidene group at the C(4) position as shown in Figure 2. The cyclohexanone derivative **9**, which was synthesized from D-glucose in 26% yield,[7] was reduced with sodium borohydride ($NaBH_4$) to give **10** (ca. 2:1 diastereomeric mixture, quantitative yield). Benzylation of **10** with benzyl bromide afforded the benzyl ether **11** (85%), which was selectively hydrolyzed by treatment with a mixture of aqueous acetic acid in THF to yield the 4-hydroxy compound **12** (80%). Swern oxidation of **12** gave the 4-keto derivative **13** (quantitative yield) as a single compound. Cyanomethylation of **13** with diethyl(cyanomethyl)phosphonate [$(EtO)_2P(O)CH_2CN$] afforded **14** (96%) as an approximate 2:1 diastereomeric mixture, which on reduction with diisobutylaluminum hydride (DIBAL-H) followed by $NaBH_4$ gave the hydroxyethylidene derivative **16** in excellent yield. Protection of the free hydroxyl group of **16** with *tert*-butyldimethylsilyl chloride (TBSCl) followed by hydrogenolysis of the

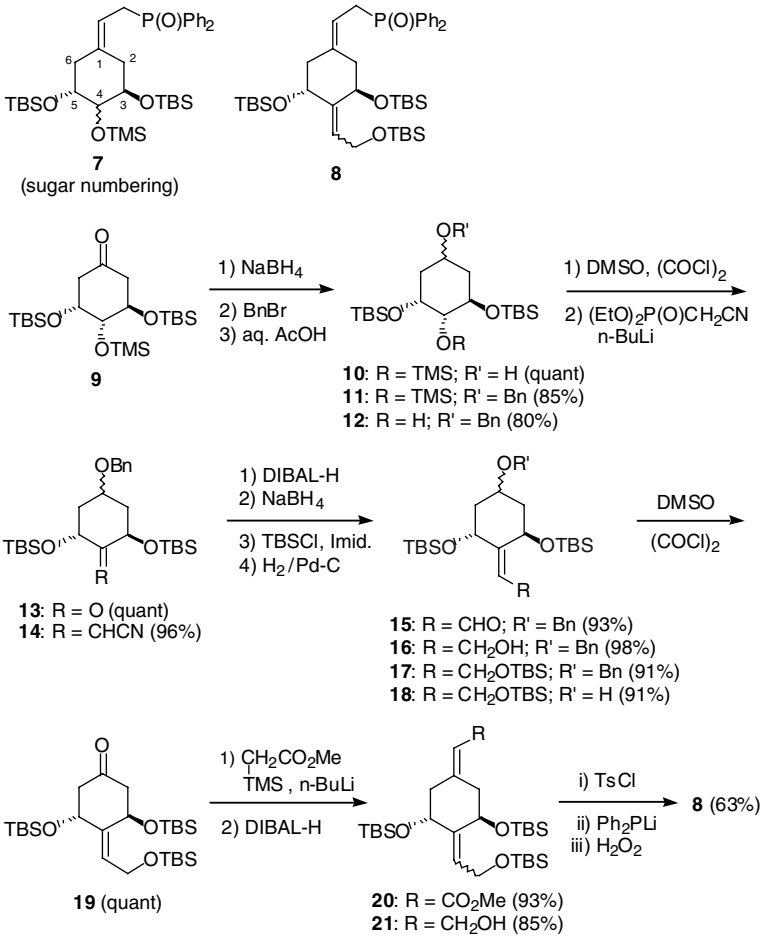

Figure 2. Synthetic scheme of the A-ring phosphine oxides with the hydroxyethylidene moiety.

benzyl ether **17** catalyzed by 10 wt % palladium on carbon gave **18**, which upon Swern oxidation yielded the 1-keto compound **19** (quantitative yield) as the sole product. Transformation of **19** to the desired phosphine oxide **8** was accomplished according to the procedures reported by DeLuca and co-workers.[4]

Protected 25-hydroxy Grundmann's ketones **22a** and **22b** (based on steroid numbering) possessing 22-ene or 22,24-diene moieties were prepared as reported.[24–26] (20*S*)- or (20*R*)-22-Oxa C/D-ring ketones **22c** or **22d** were synthesized from the 20-hydroxy compound **23** derived from vitamin D_2 as illustrated in Figure 3. Briefly, the tosylate **24** with a 5-carbon fragment was prepared from a standard Grignard reaction of 4-hydroxy-2-butanone, tosylation of the resulting diol, and then protection by *tert*-butyldimethyl trifluoromethanesulfonate (TBSOTf). Williamson's etherification of (20*R*)-**23** with **24** in the presence of a large excess of sodium hydride afforded (20*R*)-22-oxa ketone **25** (86%). Attempts to alkylate **23** with the corresponding O-silyl-protected 25-hydroxylated bromide or iodide led mainly to the recovery of **23** with a low yield of the desired C/D-ring ketones (approx. 20%). Deprotection of TBS protecting groups with *p*-toluenesufonic acid, followed by tetrapropylammonium perruthenate (Pr_4NRuO_4)-catalyzed oxidation of the diol **26**, then protection of the 25-hydroxy group with chlorotriethylsilane (TESCl) yielded **22d** (94% from **25**). The same sequence of reaction with (20*S*)-20-ol **23** afforded the (20*S*)-22-oxa derivatives **22c** and **22′c**.

Eight 2-hydroxyethoxy-19-norvitamin D analogs **3a–d** and **4a–d** bearing four different side chains were synthesized according to the sequence developed in Figure 4. The protected 25-hydroxy Grundmann's ketones **22a–d** were treated with the A-ring phosphine oxide **7** (ca. 2:1 isomeric mixture) in the presence of *n*-butyl lithium to afford derivatives with the 19-norvitamin D structures **28a–d** (52–88%) as a mixture of diastereomers in the ratios depicted in Figure 4. Deprotection of the TMS-protecting group in **28a** and **28c** with aqueous acetic acid in THF gave 2-hydroxy compounds **29a** (98%) and **29c** (72%), whereas under the same conditions **28d** yielded the mono-ol **29d** (27%) accompanied by the 2,25-dihydroxy compound **30d** (65%) as the major product. The derivative **28b** possessing a 22,24-diene-24,26,27-trihomo group is not stable in the above acidic conditions and was found to undergo dehydration to afford a side product containing a conjugated triene group in the side chain. Silyl-protected **28b** was treated with tetrabutylammonium fluoride (TBAF) to yield the tetra-ol **29b** (97%), which was reprotected regioselectively by TBSCl in the presence of triethylamine to give **30b** (66%).

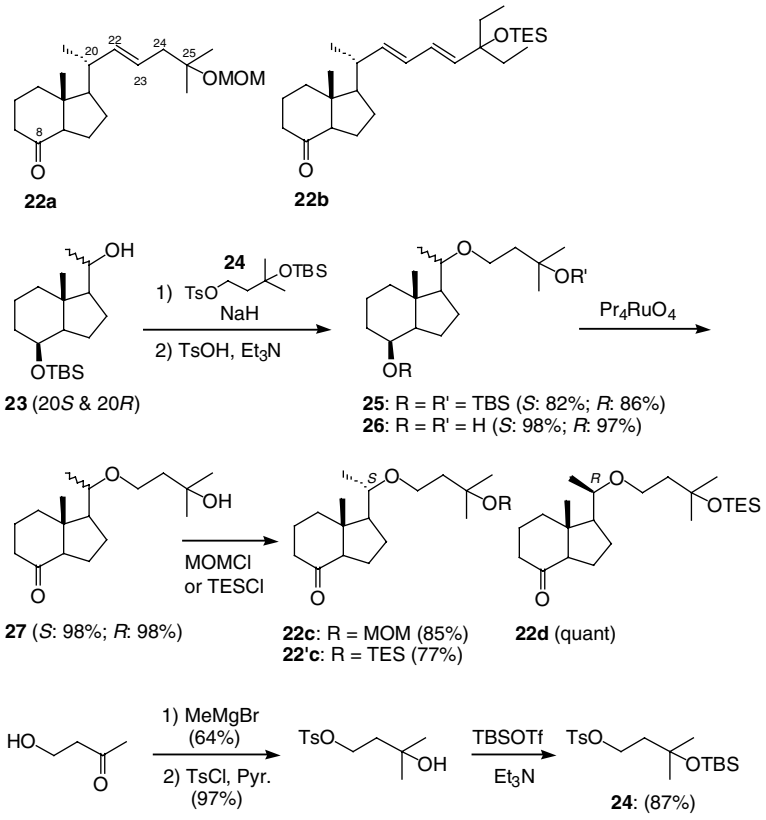

Figure 3. Synthetic scheme of the 25-hydroxy Grundmann's ketones.

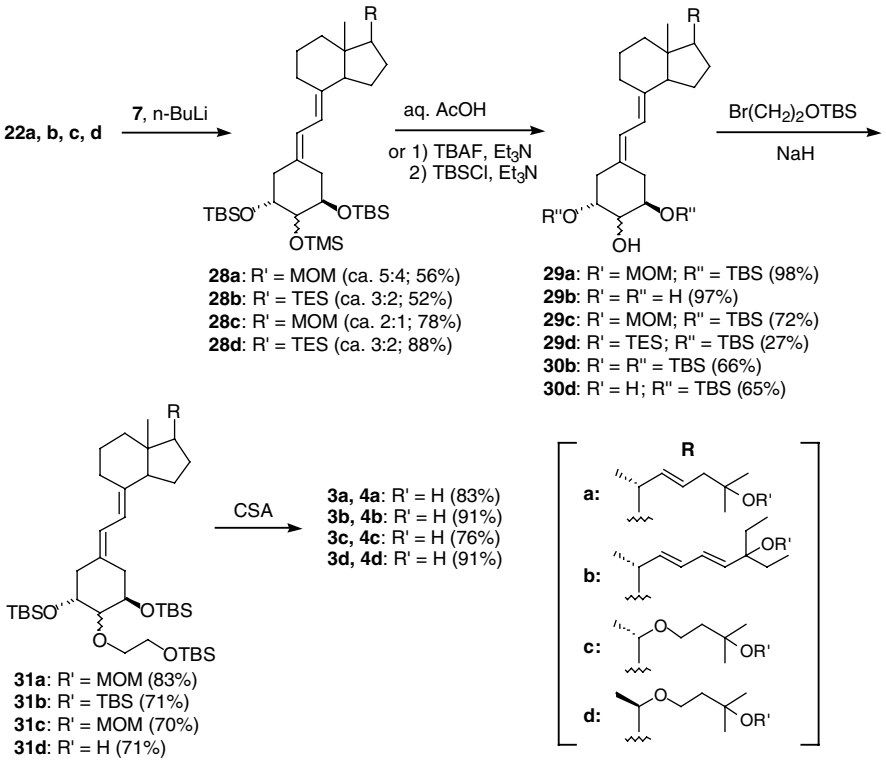

Figure 4. Synthetic scheme of the A-ring- and side-chain-modified 2-hydroxyethoxy-19-norvitamin D analogs.

2-Hydroxy-19-norvitamin D derivatives **29a**,**c**,**d** and **30b** were allowed to react with (2-bromoethoxy)-*tert*-butyldimethylsilane in the presence of excess sodium hydride, and then all the protecting groups were removed by treatment with camphor sulfonic acid (CSA) to afford the 2-hydroxyethoxy-19-norvitamin D analogs **3a**–**d**

and **4a**–**d** in good yields. All C(2)-epimeric pairs were separated by HPLC. The C(2)-configuration of 2-hydroxyethoxy analogs was determined as reported.[7,8]

(*E*)- and (*Z*)-1α,25-Dihydroxy-2-(2-hydroxyethylidene)-19-norvitamin D_3 analogs **5a**–**d** and **6a**–**d** were synthesized as illustrated in Figure 5. Wittig–Horner reaction of **22'c** with the A-ring synthon **7** gave 19-norvitamin D derivative **32c** (ca. 2:1; 59%) as a mixture of diastereomers, and deprotection of the TMS-protecting group of **32c** afforded **33c** (79%). The 2-hydroxy compounds **29a**, **33c**, and **30d** obtained above were oxidized to the 2-keto derivatives **34a**, **34c**, and **34d**, respectively, in excellent yield. Cyanomethylation of 2-ketones **34a**, **c**, **d** with $(EtO)_2P(O)CH_2CN$ gave **35a** (85%), **35c** (96%), and **35d** (97%) as approximately 1:1 mixtures of *E*- and *Z*-isomers. 2-(2-Hydroxyethylidene) derivatives **37a**, **c**, **d** were obtained in reasonable yields by two-step reductions of cyanomethyl derivatives **35a**, **c**, **d** with DIBAL-H followed by $NaBH_4$. The MOM or silyl-protecting groups were cleaved by CSA to give the desired 2-hydroxyethylidene-19-norvitamin D analogs **5a**, **c**, **d** and **6a**, **c**, **d** (74–96%) as geometrical isomers. All 1:1 mixtures of *E*- and *Z*-isomers in **5** and **6** were separated by HPLC. 19-Norvitamin D analogs **5b** and **6b** with elongated side chains were prepared by the reaction of the phosphine oxide **8** possessing the desired 2-hydroxyethylidene group at C(2) with the C/D-ring ketone **22b**, followed by deprotection of **38b** with TBAF. The coupling yield of **38b** (*E*:*Z* = ca. 6:1; 27%) was significantly low, probably as a result of the bulkiness of the side chain of the C/D-ring part, and the *E*-isomer was the major product.[6]

2.2. Biological activity

Analogs **3a**–**d** to **6a**–**d**, with the structural modifications described above, are expected to show dramatic changes in activity pattern compared with the parent compounds **3**–**6e**. We evaluated the affinity of the 19-norvitamin D analogs (**3**–**12**) for the recombinant rat VDR ligand-binding domain (LBD)[27] and VDR-mediated

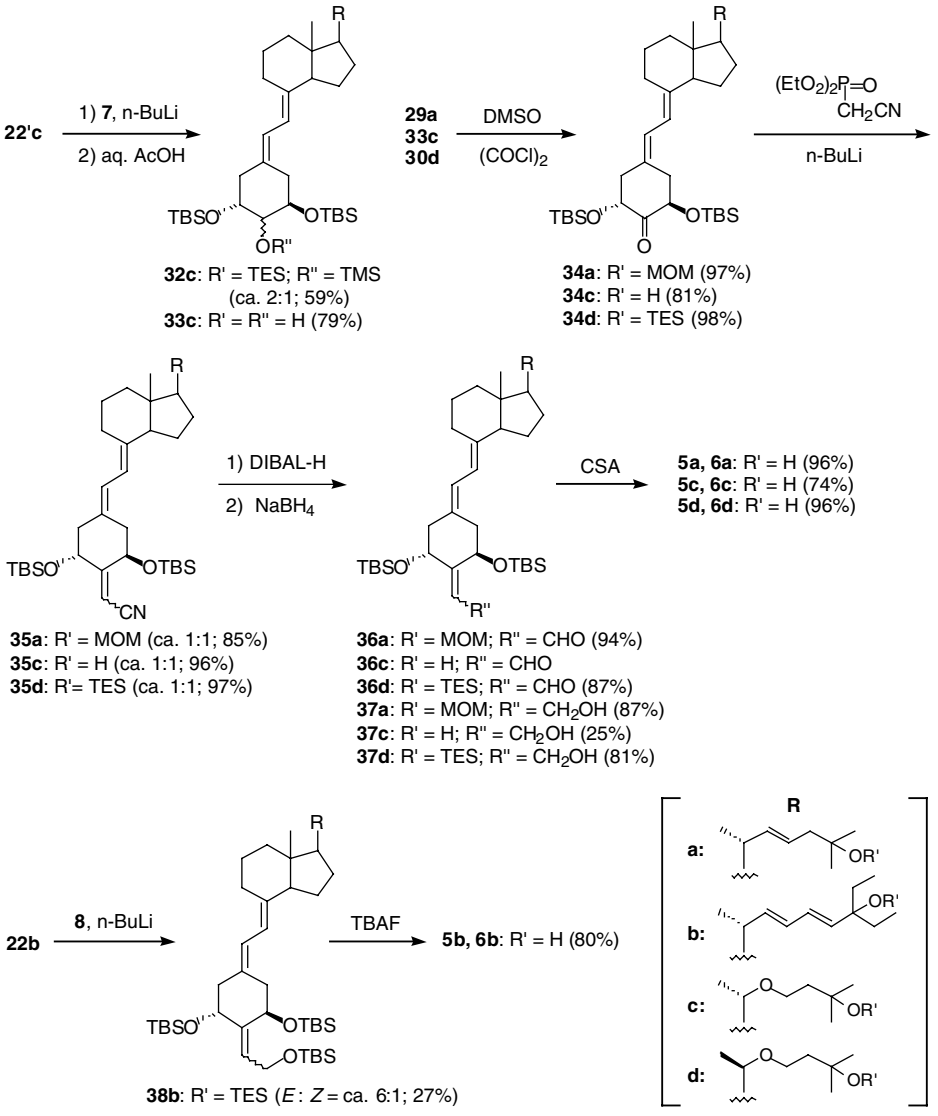

Figure 5. Synthetic scheme of the A-ring- and side-chain-modified 2-hydroxyethylidene-19-norvitamin D analogs.

transcriptional activity was tested in COS-7 cells. The results obtained are shown in Table 1.

Introduction of a double bond at C(22) had variable and weak effects on VDR binding, although most of the 22,24-diene and 22-oxa analogs showed decreased binding to the VDR as compared with the parent compounds **3**–**6e**. Replacement of C(22)–H_2 by oxygen reduced both VDR affinity and transcriptional activity, which were further decreased by epimerization at C(20). The two isomeric pairs **3/4** and **5/6** exhibited distinct biological profiles. In spite of structural modification of the side chain, analogs containing 2β-hydroxyethoxy or 2*E*-hydroxyethylidene substituents at C(2) displayed enhanced binding affinity for the VDR as well as enhanced gene-activating properties compared with their counterparts. Analogs **4a**, **5a**, and **5b** had VDR-binding affinity similar to that of **1e**. Comparison of the two isomers **3** and **4** showed that the *E*-isomer **3** is more potent than the *Z*-isomer **4**. 1α,25-$(OH)_2D_3$ in solution exists as a mixture of two equilibrating A-ring chair conformations, the α-conformation with an axial 1α-OH and an equatorial 3β-OH and the β-conformation with an equatorial 1α-OH and an axial 3β-OH.[28] In the crystal structure of VDR LBD complexed with 1α,25-$(OH)_2D_3$ and its derivatives, the native ligand docked in the ligand-binding pocket (LBP) adopts the β-conformation at the A-ring.[29–33] In the solution state, the *E*-isomer **3** predominantly takes the β-conformation, whereas the *Z*-isomer **4** prefers the α-conformation. The low-energy A-ring α-conformation of the *Z*-isomer must change to the high-energy β-conformation before it can be accommodated in the VDR LBP. This might explain the decreased VDR-binding affinity for the *Z*-isomer.

Table 1. Relative VDR affinity and transcriptional activity of A-ring- and side-chain-modified 19-norvitamin D analogs[a]

Compound	VDR affinity[b]	Transcription[c]
1e	1	1
3a	0.27	1.7
3b	0.04	5.6
3c	0.04	2.5
3d	0.02	0.13
3e	0.1[d]	3.5[d]
3f	1.0[d]	10.0[d]
4a	0.94	3.1
4b	0.14	25.0
4c	0.17	2.8
4d	0.03	1.25
4e	1.0[d]	7.0[d]
4f	5.0[d]	30.0[d]
5a	0.96	11.7
5b	0.99	17.5
5c	0.48	25.0
5d	0.12	0.26
5e	2.0[d]	2.0[d]
5f	1.6[d]	12.5[d]
6a	0.05	1.4
6b	0.04	2.8
6c	0.006	0.6
6d	0.0007	0.22
6e	0.007[d]	0.3[d]
6f	0.02[d]	4.2[d]

[a] Activities are shown as % of that of **1e**.
[b] Rat vitamin D receptor ligand-binding domain.
[c] Activity was assessed in terms of ED_{50}.
[d] Ref. 7 and 8.

It should be noted that for all analogs tested transcriptional activity was amplified relative to VDR affinity. The transcriptional activity of analogs containing 22,24-diene groups was higher than that of the parent compounds **3**–**6e** and similar to that of the 20-*epi* analogs **3**–**6f**. In contrast, the transcriptional activity of the (20*R*)-22-oxa analogs **3**–**6d** was weaker than that of the parent compounds **3**–**6e**, in accordance with the effect on receptor binding. In the crystal structure of the VDR LBD/1α,25-$(OH)_2D_3$ complex, the aliphatic side chain of 1α,25-$(OH)_2D_3$ exhibits the *gauche* (−) conformation with the C16–C17–C20–C22 torsion angle close to −30°.[29,32] Analogs with 22-ene or (20*S*)-22-oxa moieties take a similar *gauche* (−) conformation in the free state and can be accommodated in the LBP in their major conformations. In the transcriptional assay, the diene-trihomo analogs **3**–**6b** showed the highest potency among the test compounds. Elongation of the side chain increases hydrophobic interaction with the amino acid residues lining the LBP. When accommodated in the VDR LBD, the side chain of the diene-trihomo analogs **3**–**6b** takes a compact conformation in the LBP, forming hydrogen bonds with His305 and His397, as observed in the crystal structures of VDR/KH1060 or VDR/seocalcitol complexes.[30,31] Docking studies of the diene-trihomo analogs **4b** and **5b** using the docking software FlexX (Tripos, St. Louis) indicate that the terminal hydroxyl groups of the substituents at C(2) can form a hydrogen bond with the backbone carbonyl group of Asp144. Additional hydrophobic interactions of methyl groups at the terminal carbons 26 and 27 with Phe422, Leu414, and Leu227 are observed. Such additional interactions with the LBD around the A-ring and the side chain could stabilize the transcriptionally active receptor conformation.

Comparison of the 22-oxa analogs having a different configuration at C(20) showed that the (20*S*)-22-oxa analogs **3**–**6c** have more potent transcriptional activity than the (20*R*)-22-oxa analogs **3**–**6d**, in spite of the structural modification at C(2). The active space group concept[22,23] suggests that the side chain of the (20*S*)-22-oxa analogs is located in a region showing strong transcriptional activity (or cell differentiating activity). The side chain of the (20*S*)-22-oxa analogs occupies a narrow spatial region and these analogs can be docked in the VDR LBP with a small energy loss.

3. Conclusion

We have described the synthesis and biological evaluation of 16 novel A-ring- and side-chain-modified 19-norvitamin D analogs. Our present data demonstrate that structural modifications on the side chain reduce the affinity for the VDR of nearly all analogs examined and that, the gene-activating activity of the analogs, except for the (20*R*)-22-oxa compounds **3**–**6d**, was similar to or stronger than that of the parent compounds

3–**6e**. In addition, introduction of a 22,24-diene-24,26,27-trihomo group into the side-chain enhanced transcriptional activity to a similar extent as that caused by C(20) epimerization. A more extensive biological evaluation is in progress in our laboratory.

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. A mixture of diastereomers were separated by HPLC equipped with a Model PU-980 pump, a Rhedyne Model 7125 injector and a Model MD-910 multiwavelength UV detector from Jasco (Tokyo, Japan). 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.

In the following experiments, the numbering of the compounds **7**–**21** and **24** corresponding to the A-ring part of the 19-norvitamin D was expressed based on the IUPAC nomenclature of organic chemistry. The nomenclature of the compounds **3** to **6a**–**d** and **22**–**38** was expressed on the basis of the steroidal numbering.

4.1. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)oxy]-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D$_3$ *tert*-butyldimethylsilyl ether (28a)

To a stirred solution of **7** (268.2 mg, 0.407 mmol, a mixture of ca. 2:1) in dry THF (2 mL) at −78 °C was added slowly *n*-BuLi (261 μL, 0.407 mmol, 1.56 M solution in hexane), and the resulting dark orange solution was stirred for 15 min. To this colored solution was added a solution of **22a** (87.5 mg, 0.271 mmol) in dry THF (1 mL), the reaction mixture was stirred for 2 h at −78 °C, quenched with saturated NH_4Cl and extracted with AcOEt. The AcOEt layer was washed with brine dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (10 g) using 2% AcOEt in hexane to afford **28a** (116.1 mg, 56% based on **22a**) as a mixture of two isomers in a ca. 5:4 ratio, and 10% AcOEt in hexane to give the unreacted starting material **22a** (11.6 mg, 13%).

MS *m*/*z* (%): 762 (M^+, 18), 700 (28), 630 (39), 568 (57), 511 (18), 465 (25), 309 (36), 147 (35), 109 (56), 75 (100). HRMS *m*/*z*: 762.5474 (Calcd for $C_{43}H_{82}O_5Si_3$: 762.5470). 2α-Isomer (major): ^{1}H NMR ($CDCl_3$) δ: 0.04–0.06 (12H, s), 0.12 (9H, s), 0.55 (3H, s), 0.87, 0.88 (each 9H, s), 1.02 (3H, d, J = 6.6 Hz), 1.20 (6H, s), 2.80 (1H, m), 3.37 (3H, s), 3.54 (1H, m), 3.80 (1H, m), 3.87 (1H, m), 4.73 (2H, s), 5.33 (2H, m), 5.81 (1H, d, J = 11.1 Hz), 6.10 (1H, d, J = 11.1 Hz). 2β-Isomer (minor): ^{1}H NMR ($CDCl_3$) δ: 0.04–0.06 (12H, s), 0.12 (9H, s), 0.54 (3H, s), 0.86, 0.89 (each 9H, s), 1.02 (3H, d, J = 6.6 Hz), 1.02 (6H, s), 2.80 (1H, m), 3.37 (3H, s), 3.60 (1H, m), 3.80 (1H, m), 3.93 (1H, m), 4.73 (2H, s), 5.33 (2H, m), 5.78 (1H, d, J = 11.2 Hz), 6.13 (1H, d, J = 11.2 Hz).

4.2. 24a,26a,27a-Trihomo-1α-[(*tert*-butyldimethylsilyl)oxy]-2-[(trimethylsilyl)oxy]-22,24-diene-25-[(triethylsilyl)oxy]-19-norvitamin D$_3$ *tert*-butyldimethylsilyl ether (28b)

The same procedure as described above, but using **7** (206.0 mg, 0.394 mmol, a mixture of ca. 2:1) and **22b** (101.8 mg, 0.235 mmol) gave **28b** (106.4 mg, 52% based on **22b**) as a mixture of two isomers in a ca. 3:2 ratio.

MS *m*/*z* (%): 872 (no M^+), 740 (33), 683 (7), 608 (65), 551 (17), 505 (43), 459 (18), 324 (31), 149 (100), 75 (99). HRMS *m*/*z*: 740.5422 (M^+−TBSOH) (Calcd for $C_{44}H_{80}O_3Si_3$: 740.5415). 2α-Isomer (major): ^{1}H NMR ($CDCl_3$) δ: 0.039, 0.051, 0.059, 0.064 (each 3H, s, 4× Si–Me), 0.119 (9H, s, 3× Si–Me), 0.57 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.06 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 3.53 (1H, m, H-2), 3.80 (1H, m, H-3), 3.88 (1H, m, H-1), 5.52 (1H, d, J = 15.2 Hz, H-24a, overlapped with H-22), 5.81 (1H, d, J = 11.1 Hz, H-7), 5.94 (1H, dd, J = 14.9, 10.4 Hz, H-23), 6.05 (1H, dd, J = 15.2, 10.4 Hz, H-24), 6.10 (1H, d, J = 11.1 Hz, H-6). 2β-Isomer (minor): ^{1}H NMR ($CDCl_3$) δ: 0.039, 0.051, 0.059, 0.064 (each 3H, s, 4× Si–Me), 0.124 (9H, s, 3× Si–Me), 0.56 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.06 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 3.59 (1H, m, H-2), 3.80 (1H, m, H-3), 3.94 (1H, m, H-1), 5.52 (1H, d, J = 15.2 Hz, H-24a, overlapped with H-22), 5.78 (1H, d, J = 11.1 Hz, H-7), 5.94 (1H, dd, J = 14.9, 10.4 Hz, H-23), 6.05 (1H, dd, J = 15.2, 10.4 Hz, H-24), 6.13 (1H, d, J = 11.1 Hz, H-6).

4.3. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)oxy]-22-oxa-25-[(methoxymethyl)oxy]-19-norvitamin D$_3$ *tert*-butyldimethylsilyl ether (28c)

The same procedure as described above, but using **7** (244.4 mg, 0.38 mmol, a mixture of ca. 2:1) and **22c** (79.8 mg, 0.19 mmol) gave **28c** (111.8 mg, 78% based on **22c**) as a mixture of two isomers in a ca. 3:2 ratio.

MS *m*/*z* (%): 766 (M^+, 12), 704 (15), 634 (33), 572 (47), 515 (12), 75 (100). HRMS *m*/*z*: 766.5442 (Calcd for $C_{42}H_{82}O_6Si_3$: 766.5419). 2α-Isomer (major) ^{1}H NMR ($CDCl_3$) δ: 0.03–0.08 (12H, s, 4× Si–Me), 0.13 (9H, Si–Me_3), 0.52 (3H, s, H-18), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 1.16 (3H, d, J = 6.0 Hz, H-21), 1.25 (6H, s, H-26, 27), 2.78 (1H, m, H-9), 3.21 (1H, m, H-20), 3.33

(1H, m, H-23), 3.36 (3H, s, OMe), 3.53 (1H, m, H-2), 3.66 (1H, m, H-23), 3.80 (1H, m, H-3), 3.87 (1H, m, H-1), 4.70 (2H, s, OCH_2O), 5.82 (1H, m, d, $J = 11.2$ Hz, H-7), 6.09 (1H, d, $J = 11.2$ Hz, H-6). 2β-Isomer (minor) ^{1}H NMR ($CDCl_3$) δ: 0.03–0.08 (12H, s, 4× Si–Me), 0.12 (9H, Si–Me_3), 0.51 (3H, s, H-18), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 1.16 (3H, d, $J = 6.0$ Hz, H-21), 1.25 (6H, s, H-26, 27), 2.78 (1H, m, H-9), 3.21 (1H, m, H-20), 3.33 (1H, m, H-23), 3.36 (3H, s, OMe), 3.60 (1H, m, H-2), 3.66 (1H, m, H-23), 3.80 (1H, m, H-3), 3.92 (1H, m, H-1), 4.70 (2H, s, OCH_2O), 5.80 (1H, m, d, $J = 11.2$ Hz, H-7), 6.10 (1H, d, $J = 11.2$ Hz, H-6).

4.4. 20-*epi*-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)oxy]-22-oxa-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (28d)

The same procedure as described above, but using **7** (212.0 mg, 0.321 mmol, a mixture of ca. 2:1) and **22d** (85.0 mg, 0.214 mmol) gave **28d** (158.8 mg, 88% based on **22d**) as a mixture of two isomers in a ca. 3:2 ratio.

MS *m*/*z* (%): 836 (no M^+), 704 (10), 647 (3), 618 (7), 572 (19), 486 (20), 469 (13), 383 (17), 309 (19), 75 (100). HRMS *m*/*z*: 704.5032 (M^+−TBSOH) (Calcd for $C_{40}H_{76}O_4Si_3$: 704.5051). 2α-Isomer (major): ^{1}H NMR ($CDCl_3$) δ: 0.04–0.06 (12H, 4× Si–Me), 0.13 (9H, s, 3× Si–Me), 0.56 (3H, s, H-18), 0.57 (6H, q, $J = 7.9$ Hz, 3× $SiCH_2$), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, $J = 7.9$ Hz, 3× $SiCH_2CH_3$), 1.08 (3H, d, $J = 5.9$ Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.80 (1H, m, H-9), 3.26 (1H, m, H-20), 3.32, 3.69 (each 1H, m, H-23), 3.53 (1H, m, H-2), 3.80 (1H, m, H-3), 3.89 (1H, m, H-1), 5.79 (1H, d, $J = 11.1$ Hz, H-7), 6.11 (1H, d, $J = 11.1$ Hz, H-6). 2β-Isomer (minor): ^{1}H NMR ($CDCl_3$) δ: 0.04–0.06 (12H, 4× Si–Me), 0.12 (9H, s, 3× Si–Me), 0.54 (3H, s, H-18), 0.57 (6H, q, $J = 7.9$ Hz, 3× $SiCH_2$), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, $J = 7.9$ Hz, 3× $SiCH_2CH_3$), 1.08 (3H, d, $J = 5.9$ Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.80 (1H, m, H-9), 3.26 (1H, m, H-20), 3.32, 3.69 (each 1H, m, H-23), 3.59 (1H, m, H-2), 3.80 (1H, m, H-3), 3.93 (1H, m, H-1), 5.77 (1H, d, $J = 11.2$ Hz, H-7), 6.14 (1H, d, $J = 11.2$ Hz, H-6).

4.5. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-hydroxy-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (29a)

A solution of **28a** (60.0 mg, 0.0786 mmol, ca. 5:4 isomeric mixture) in THF/AcOH/H_2O (v/v/v, 8:8:1, 4.25 mL) was stirred at ambient temperature for 18 h, and diluted with AcOEt. The organic phase was successively washed with 5% $NaHCO_3$ and brine and dried over Na_2SO_4. After evaporation of the solvent, the resulting residue was purified by chromatography on silica gel (6 g) using 5% AcOEt in hexane to afford **29a** (53.4 mg, 98%) as a mixture of two isomers in a ca. 5:4 ratio.

MS *m*/*z* (%): 690 (M^+, 6), 628 (9), 571 (7), 501 (5), 439 (29), 309 (11), 237 (11), 109 (63) 75 (100). HRMS *m*/*z*: 690.5084 (Calcd for $C_{40}H_{74}O_5Si_3$: 690.5075). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.01 (12H), 0.56 (3H, s), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 1.02 (3H, d, $J = 6.6$ Hz, H-21), 1.20 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 3.37 (3H, s, OMe), 3.51 (1H, m, H-2), 3.90 (1H, m, H-3), 3.99 (1H, m, H-1), 4.73 (2H, s, OCH_2O), 5.33 (2H, m, H-22, 23), 5.79 (1H, d, $J = 11.1$ Hz, H-7), 6.15 (1H, d, $J = 11.1$ Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si–Me), 0.54 (3H, s, H-18), 0.86, 0.90 (each 9H, s, 2× Si–*t*-Bu), 1.02 (3H, d, $J = 6.6$ Hz, H-21), 1.20 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 3.37 (3H, s, OMe), 3.59 (1H, m, H-2), 3.99 (2H, m, H-1, 3), 4.73 (2H, s, OCH_2O), 5.33 (2H, m, H-22, 23), 5.79 (1H, d, $J = 11.2$ Hz, H-7), 6.18 (1H, d, $J = 11.2$ Hz, H-6).

4.6. 1α- [(*tert*-Butyldimethylsilyl)oxy]-2-hydroxy-22-oxa-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (29c)

In a same way, deprotection of **28c** (63.2 mg, 0.13 mmol, ca. 3:2 isomeric mixture) in aq AcOH in THF afforded **29c** (41.4 mg, 72%) as a mixture of two isomers in a ca. 2:1 ratio.

MS *m*/*z* (%): 694 (M^+, 3), 632 (5), 575 (4), 505 (2), 443 (12), 75 (100). HRMS *m*/*z*: 694.5002 (Calcd for $C_{39}H_{74}O_6Si_2$: 694.5024). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.09 (12H, s, 4× Si–Me), 0.52 (3H, s, H-18), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 1.16 (3H, d, $J = 6.0$ Hz, H-21), 1.24 (6H, s, H-26, 27), 2.78 (1H, m, H-9), 3.22 (1H, m, H-20), 3.32 (1H, m, H-23), 3.35 (3H, s, OMe), 3.50 (1H, m, H-2), 3.66 (1H, m, H-23), 3.92 (1H, m, H-3), 3.99 (1H, m, H-1), 4.70 (2H, s, OCH_2O), 5.80 (1H d, $J = 11.1$ Hz, H-7), 6.14 (1H, d, $J = 11.1$ Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.09 (12H, s, 4× Si–Me), 0.51 (3H, s, H-18), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 1.16 (3H, d, $J = 6.0$ Hz, H-21), 1.24 (6H, s, H-26, 27), 2.78 (1H, m, H-9), 3.22 (1H, m, H-20), 3.32 (1H, m, H-23), 3.35 (3H, s, OMe), 3.58 (1H, m, H-2), 3.66 (1H, m, H-23), 3.99 (2H, m, H-1, 3), 4.70 (2H, s, OCH_2O), 5.80 (1H, m, d, $J = 11.1$ Hz, H-7), 6.17 (1H, d, $J = 11.1$ Hz, H-6).

4.7. 20-*epi*-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,25-dihydroxy-22-oxa-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (29d) and 20-*epi*-1α-[(*tert*-butyldimethylsilyl)oxy]-2-hydroxy-22-oxa-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (30d)

In a same way, deprotection of **28d** (153.0 mg, 0.183 mmol, ca. 3:2 isomeric mixture) in aq AcOH in THF afforded **29d** (77.1 mg, 65%) and **30d** (37 0 mg, 27%) as a mixture of two isomers in a ca. 3:2 ratio, respectively.

Compound **29d**: MS *m*/*z* (%): 650 (M^+, 2), 632 (8), 546 (6), 489 (8), 443 (10), 357 (8), 265 (22), 113 (30), 75 (100). HRMS *m*/*z*: 650.4776 (Calcd for $C_{37}H_{70}O_5Si_2$: 650.4762). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si–Me), 0.56 (3H, s, H-18), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 1.14 (3H, d, $J = 5.9$ Hz, H-21), 1.22, 1.24 (each 3H, s, H-26, 27), 2.80 (1H, m, H-9), 3.28 (1H, m, H-20), 3.46 (1H, m, H-23), 3.51 (1H, m,

H-2), 3.59 (1H, s, OH), 3.85 (1H, m, H-23), 3.92 (1H, m, H-3), 4.00 (1H, m, H-1), 5.78 (1H, d, J = 11.1 Hz, H-7), 6.16 (1H, d, J = 11.1 Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si–Me), 0.54 (3H, s, H-18), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 1.14 (3H, d, J = 5.9 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.80 (1 H, m, H-9), 3.28 (1H, m, H-20), 3.46 (1H, m, H-23), 3.59 (1H, m, H-2), 3.85 (1H, m, H-23), 4.00 (2H, m, H-1, 3), 5.78 (1H, d, J = 11.2 Hz, H-7), 6.19 (1H, d, J = 11.2 Hz, H-6).

Compound **30d**: MS *m*/*z* (%): 764 (M^+, 1), 707 (1), 632 (4), 575 (2), 546 (3), 489 (4), 443 (5), 357 (20), 265 (11), 103 (31), 75 (100). HRMS *m*/*z*: 764.5643 (Calcd for $C_{43}H_{84}O_5Si_3$: 764.5627). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si–Me), 0.56 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.81 (1H, m, H-9), 3.26 (1H, m, H-20), 3.32 (1H, m, H-23), 3.51 (1H, m, H-2), 3.70 (1H, m, H23), 3.91 (1H, m, H-3), 4.01 (1H, m, H-1), 5.78 (1H, d, J = 11.1 Hz, H-7), 6.18 (1H, d, J = 11.1 Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (12H, 4× Si–Me), 0.55 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.81 (1H, m, H-9), 3.26 (1H, m, H-20), 3.32 (1H, m, H-23), 3.59 (1H, m, H-2), 3.70 (1H, m, H-23), 4.01 (2H, m, H-1, 3), 5.78 (1H, d, J = 11.2 Hz, H-7), 6.20 (1H, d, J = 11.2 Hz, H-6).

4.8. 24a,26a,27a-Trihomo-1α,2α,25-trihydroxy- and 24a,26a,27a-trihomo-1α,2β,25-trihydroxy-22,24-diene-19-norvitamin D_3 (29b)

A mixture of **28b** (55 mg, 0.063 mmol, ca. 3:2 isomeric mixture), Et_3N (20 μL), and tetrabutylammonium fluoride (504 μL, 0.504 mmol, 1.0 M solution in THF) in dry THF (1 mL) was stirred at ambient temperature for 4 h. The mixture was poured into ice water 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 purified by chromatography on silica gel (5 g) with 2% MeOH in AcOEt to yield **29b** (28.0 mg, 97%) as a mixture of two isomers in a ca. 3:2 ratio.

MS *m*/*z* (%): 458 (M^+, 33), 440 (95), 422 (14), 404 (16), 386 (52), 318 (40), 289 (90), 237 (44), 149 (100). HRMS *m*/*z*: 458.3383 (Calcd for $C_{29}H_{46}O_4$: 458.3396). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, H-18), 0.87 (6H, t, J = 7.4 Hz, H-26a, 27a), 1.05 (3H, d, J = 6.6 Hz, H-21), 1.55, 1.56 (each 2H, q, J = 7.4 Hz, H-26, 27), 2.62 (1H, dd, J = 12.8, 4.1 Hz, H-4), 2.80 (1H, m, H-9), 2.89 (1H, dd, J = 14.7, 4.3 Hz, H-10), 3.53 (1H, dd, J = 8.2, 2.9 Hz, H-2), 3.79 (1H, m, H-3), 4.09 (1H, m, H-1), 5.53 (1H, d, J = 15.2 Hz, H-24a, overlapped with H-22), 5.80 (1H, d, J = 11.1 Hz, H-7), 5.98 (1H, dd, J = 15.0, 10.3 Hz, H-23), 6.15 (1H, dd, J = 15.2, 10.3 Hz, H-24), 6.37 (1H, d, J = 11.1 Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, H-18), 0.87 (6H, t, J = 7.4 Hz, H-26a, 27a), 1.05 (3H, d, J = 6.6 Hz, H-21), 1.55, 1.56 (each 2H, q, J = 7.4 Hz, H-26, 27), 2.43 (2H, m, H-4), 2.80 (1H, m, H-9), 3.07 (1H, dd, J = 13.2, 4.9 Hz, H-10), 3.48 (1H, dd, J = 8.8, 3.0 Hz, H-2), 3.67 (1H, m, H-3), 4.09 (1H, m, H-1), 5.53 (1H, d, J = 15.2 Hz, H-24a, overlapped with H-22), 5.83 (1H, d, J = 11.1 Hz, H-7), 5.98 (1H, dd, J = 15.0, 10.3 Hz, H-23), 6.15 (1H, dd, J = 15.2, 10.3 Hz, H-24), 6.29 (1H, d, J = 11.1 Hz, H-6).

4.9. 24a,26a,27a-Trihomo-1α-[(*tert*-butyldimethylsilyl)oxy]-2-hydroxy-22,24-diene-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (30b)

To a stirred solution of **29b** (48.0 mg, 0.105 mmol, ca. 3:2 isomeric mixture) in dry DMF (1 mL) were added Et_3N (117 μL, 0.840 mmol), *tert*-butyldimethylsilyl chloride (63.9 mg, 0.424 mmol), and 4-(dimethylamino)pyridine (6.4 mg, 0.052 mmol) at ambient temperature. After 2 h stirring, the reaction mixture was poured into ice water, and extracted with AcOEt. Organic extracts were washed with brine dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by chromatography on silica gel (5 g) using 5% AcOEt in hexane to yield **30b** (55.3 mg, 66%) as a mixture of two isomers in a ca. 3:2 ratio.

MS *m*/*z* (%): 800 (no M^+), 668 (6), 611 (2), 536 (3), 479 (12), 386 (6), 149 (100), 75 (79). HRMS *m*/*z*: 668.5021 (M^+−TBSOH) (Calcd for $C_{41}H_{72}O_3Si_2$: 668.5020). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (18H, 6× Si–Me), 0.57 (3H, s, H-18), 0.87 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.87–0.92 (27H, 3× Si–*t*-Bu), 1.06 (3H, d, J = 6.6 Hz, H-21), 1.54, 1.55 (each 2H, q, J = 7.5 Hz, H-26, 27), 2.80 (1H, m, H-9), 3.51 (1H, m, H-2), 3.92 (1H, m, H-3), 4.00 (1H, m, H-1), 5.53 (1H, d, J = 15.3 Hz, H-24a), 5.55 (1H, dd, J = 15.2, 8.5 Hz, H-22), 5.79 (1H, d, J = 11.1 Hz, H-7), 5.98 (1H, dd, J = 15.2, 10.4 Hz, H-23), 6.15 (1H, dd, J = 15.3, 10.4 Hz, H-24), 6.18 (1H, d, J = 11.1 Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.10 (18H, 6× Si–Me), 0.56 (3H, s, H-18), 0.87 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.87–0.92 (27H, 3× Si–*t*-Bu), 1.06 (3H, d, J = 6.6 Hz, H-21), 1.54, 1.55 (each 2H, q, J = 7.5 Hz, H-26, 27), 2.80 (1H, m, H-9), 3.59 (1H, m, H-2), 4.00 (2H, m, H-1, 3), 5.53 (1H, d, J = 15.3 Hz, H-24a), 5.55 (1H, dd, J = 15.2, 8.5 Hz, H-22), 5.79 (1H, d, J = 11.1 Hz, H-7), 5.98 (1H, dd, J = 15.2, 10.4 Hz, H-23), 6.14 (1H, d, J = 11.1 Hz, H-6), 6.15 (1H, dd, J = 15.3, 10.3 Hz, H-24).

4.10. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(*tert*-butyldimethylsilyl)oxy-ethoxy]-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (31a)

A suspension of **29a** (44.3 mg, 0.064 mmol, ca. 5:4 isomeric mixture), NaH (77.0 mg, 1.925 mmol, 60% dispersion in mineral oil), and (2-bromoethoxy)-*tert*-butyldimethylsilane (69 μL, 0.320 mmol) in dry DMF (1 mL) was stirred vigorously for 20 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 $MgSO_4$.

Following evaporation of the solvent in vacuo, the residue was purified by chromatography on silica gel (10 g) using 2% AcOEt in hexane to afford **31a** (45.0 mg, 83%) as a mixture of two isomers in a ca. 1:1 ratio.

^{1}H NMR ($CDCl_3$) δ: 0.05–0.09 (18H, 6× Si–Me), 0.54, 0.56 (ca. 1:1) (3H, s, H-18), 0.86–0.91 (27H, 3× Si–*t*-Bu), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.24 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 3.19, 3.28 (ca. 1:1) (1H, m, H-2), 3.37 (3H, s, OMe), 3.5–4.1 (7H, m, OCH_2CH_2O, H-1, 3), 4.73 (2H, s, OCH_2O), 5.32 (2H, m, H-22, 23), 5.77, 5.80 (ca. 1:1) (1H, d, J = 11.0 Hz, H-7), 6.12, 6.14 (ca. 1:1) (1H, d, J = 11.0 Hz, H-6). MS *m*/*z* (%): 848 (no M^+), 786 (1), 716 (4), 654 (8), 610 (8), 553 (5), 522 (10), 465 (12), 75 (100). HRMS *m*/*z*: 786.5856 ($M^+-MeOCH_2OH$) (Calcd for $C_{46}H_{86}O_4Si_3$: 786.5834).

4.11. 24a,26a,27a-Trihomo-1α-[2-(*tert*-butyldimethylsilyl)-oxy]-2-[2-(*tert*-butyldimethylsilyl)oxy]-ethoxy-22,24-diene-25-[(*tert*-butyldimethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (31b)

Following the same procedure as described above, treatment of **30b** (52.4 mg, 0.065 mmol, ca. 3:2 isomeric mixture) with the bromide (68 μL, 0.317 mmol) yielded **31b** (44.7 mg, 71%) as a mixture of two isomers in a ca. 3:2 ratio.

^{1}H NMR ($CDCl_3$) δ: 0.05–0.10 (24H, 8× Si–Me), 0.55, 0.57 (ca. 2:3) (3 H, s, H-18), 0.85–0.92 (42H, 4× Si–*t*-Bu, H-26a, 27a), 1.05 (3H, d, J = 6.6 Hz, H-21), 1.54, 1.55 (each 2H, d, J = 7.5 Hz, H-26, 27), 2.80 (1H, m, H-9), 3.18–4.45 (7 H, m, OCH_2CH_2O, H-1, 3), 5.52 (1H, d, J = 15.1 Hz, H-24a), 5.54 (1H, dd, J = 15.0, 8.6 Hz, H-22), 5.78, 5.81 (ca. 3:2) (1H, d, J = 11.2 Hz, H-7), 5.97 (1H, dd, J = 15.0, 10.4 Hz, H-23), 6.11, 6.14 (ca. 2:3) (1H, d, J = 11.2 Hz, H-6), 6.15 (1H, dd, J = 15.1, 10.4 Hz, H-24). MS *m*/*z* (%): 958 (no M^+), 826 (1), 769 (2), 649 (14), 651 (11), 562 (12), 519 (24), 233 (100). HRMS *m*/*z*: 826.6146 ($M^+-TBSOH$) (Calcd for $C_{49}H_{90}O_4Si_3$: 826.6147).

4.12. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxy]-ethoxy]-22-oxa-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (31c)

Following the same procedure as described above, treatment of **29c** (41.4 mg, 0.57 mmol, ca. 2:1 isomeric mixture) with the bromide (781.4 μL, 3.64 mmol) yielded **31c** (35.0 mg, 70%) as a mixture of two isomers in a ca. 3:1 ratio.

^{1}H NMR ($CDCl_3$) δ: 0.04–0.07 (18H, s, 6× Si–Me), 0.51, 0.52 (ca. 1:3) (3H, s, H-18), 0.85–0.95 (27H, s, 3× Si–*t*-Bu), 1.16 (3H, d, J = 6.0 Hz, H-21), 1.25 (6H, s, H-26, 27), 2.79 (1H, m, H-9), 3.21 (1H, m, H-23), 3.33 (1H, m, H-20), 3.36 (3H, s, OMe), 3.60–3.90 (5H, m, OCH_2CH_2), 3.93 (1H, m), 4.03 (1H, m), 4.71 (2H, s, OCH_2O), 5.79, 5.81 (ca. 1:3) (1H, m, d, J = 11.1 Hz, H-7), 6.11, 6.13 (ca. 3:1) (1H, d, J = 11.1 Hz, H-6). MS *m*/*z* (%): 852 (no M^+), 790 (1), 720 (3), 658 (4), 614 (4), 557 (2), 526 (4), 469 (5), 75 (100). HRMS *m*/*z*: 790.5798 ($M^+-CH_3OCH_2OH$) (Calcd for $C_{45}H_{86}O_5Si_3$: 790.5783).

4.13. 20-*epi*-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxy]-ethoxy]-22-oxa-25-hydroxy-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (31d)

Following the same procedure as described above, treatment of **29d** (73.5 mg, 0.113 mmol, ca. 3:2 isomeric mixture) with the bromide (118μL, 0.550 mmol) yielded **31d** (65.0 mg, 71%) as a mixture of two isomers in a ca. 3:2 ratio.

^{1}H NMR ($CDCl_3$) δ: 0.05–0.07 (18 H, 6× Si–Me), 0.54, 0.55 (ca. 2:3) (3H, s, H-18), 0.86–0.89 (27H, 3× Si–*t*-Bu), 1.13 (3H, d, J = 5.5 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.80 (1H, m, H-9), 3.2–4.1 (10H, m, OCH_2-CH_2O, H-1, 2, 3, 20, 23), 5.77 (1H, H-7), 6.14 (1H, H-6). MS *m*/*z* (%): 808 (no M^+), 790 (1), 676 (4), 658 (5), 572 (6), 526 (5), 397 (18), 233 (74), 75 (100). HRMS *m*/*z*: 790.5766 (M^+-H_2O) (Calcd for $C_{45}H_{86}O_5Si_3$: 790.5783).

4.14. 1α,25-Dihydroxy-2α-(2-hydroxyethoxy)- and 1α,25-dihydroxy-2β-(2-hydroxyethoxy)-22-ene-19-norvitamin D_3 (3a and 4a)

A mixture of **31a** (45.0 mg, 0.053 mmol, ca. 1:1 isomeric mixture) and (−)-10-camphor sulfonic acid (73.8 mg, 0.318 mmol) in dry MeOH (1 mL) was stirred at ambient temperature for 2 h. The reaction mixture was poured into 5% $NaHCO_3$ and extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed on silica gel (4 g) using 2% MeOH in AcOEt to give the isomeric mixture of **3a** and **4a** (20.3 mg, 83%). The mixture was separated by HPLC (YMC-Pack ODS-AM SH-342-5, 150 × 20 mm, 25% H_2O in MeOH) to afford pure **3a** (8.3 mg) and **4a** (7.9 mg), respectively.

Compound **3a**: ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, H-18), 1.04 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.62 (1H, dd, J = 13.5, 4.5 Hz, H-4), 2.67 (each 1H, br s, 2× OH), 2.79 (1H, m, H-9), 2.86 (1H, dd, J = 14.4, 4.9 Hz, H-10), 3.03 (1H, br s, OH), 3.33 (1H, dd, J = 8.0, 2.8 Hz, H-2), 3.68–3.83 (4H, m, OCH_2CH_2O), 3.94 (1H, m, H-3), 4.15 (1H, m, H-1), 5.38 (2H, m, H-22, 23), 5.82 (1H, d, J = 11.2 Hz, H-7), 6.33 (1H, d, J = 11.2 Hz, H-6). UV λ_{max} (EtOH): 244 (ε 27,400), 252 (ε 32,000), 261 (ε 21,700) nm. MS *m*/*z* (%): 462 (M^+, 55), 444 (58), 426 (43), 408 (22), 346 (32), 317 (68), 299 (39), 255 (69), 237 (76), 133 (100). HRMS *m*/*z*: 462.3348 (Calcd for $C_{28}H_{46}O_5$: 462.3345).

Compound **4a**: ^{1}H NMR ($CDCl_3$) δ: 0.56 (3H, s, H-18), 1.04 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.34 (1H, d, J = 14.2 Hz, H-4), 2.48 (1H, dd, J = 14.2, 2.0 Hz, H-4), 2.62 (1H, br s, OH), 2.79 (1H, m, H-9), 3.07 (1H, dd, J = 13.2, 3.8 Hz, H-10), 3.28 (1H, dd, J = 8.7, 2.7 Hz, H-2), 3.42 (1H, br s, OH), 3.67 (1H, m, H-1), 3.75–3.87 (4 H, m, OCH_2CH_2O), 4.17 (1H, m, H-3), 5.39 (2H, m, H-22, 23), 5.84 (1H, d,

J = 11.2 Hz, H-7), 6.27 (1H, d, J = 11.2 Hz, H-6). UV λ_{max} (EtOH): 243 (ε 27,700), 251 (ε 32,300), 261 (ε 21,600) nm. MS *m/z* (%): 462 (M^+, 41), 444 (44), 426 (37), 408 (17), 346 (39), 317 (55), 299 (29), 255 (59), 237 (75), 133 (100). HRMS *m/z*: 462.3362 (Calcd for $C_{28}H_{46}O_5$: 462.3345).

4.15. 1α,25-Dihydroxy-2α-(2-hydroxyethoxy)- and 1α,25-dihydroxy-2β-(2-hydroxyethoxy)-22-oxa-19-norvitamin D_3 (3c and 4c)

Following the same procedure as described above, treatment of **31c** (25.1 mg, 0.039 mmol, ca. 3:1 isomeric mixture) with (−)-10-camphor sulfonic acid (73.3 mg, 0.32 mmol) gave a mixture of **3c** and **4c** (13.8 mg, 76%), which was separated by the reversed-phase HPLC to give pure **3c** (6.23 mg) and **4c** (1.83 mg), respectively.

Compound **3c**: ^{1}H NMR ($CDCl_3$) δ: 0.54 (3H, s, H-18), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.19 (2H, m), 2.62 (1H, dd, J = 13.3, 4.6 Hz, H-4), 2.80 (1H, m, H-9), 2.86 (1H, dd, J = 14.5.0, 5.0 Hz, H-10), 3.25 (1H, m, H-20), 3.35 (1H, dd, J = 7.9, 2.9 Hz, H-2), 3.49 (1H, m, H-23), 3.75–3.90 (5H, m, OCH_2CH_2OH, OH), 3.95 (1H, m, H- 3), 4.15 (1H, m, H-1), 5.82 (1H, d, J = 11.2 Hz, H-7), 6.32 (1H, d, J = 11.2 Hz, H-6). MS *m/z* (%): 466 (M^+, 13), 448 (18), 430 (10), 412 (2), 317 (10), 237 (9), 133 (18), 113 (36), 69 (100). HRMS *m/z*: 466.3282 (Calcd for $C_{27}H_{46}O_6$: 466.3294). UV λ_{max} (EtOH): 243, 251, 261 nm.

Compound **4c**: ^{1}H NMR ($CDCl_3$) δ: 0.53 (3H, s, H-18), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.35 (1 H, d, J = 15.0 Hz, H-4), 2.48 (1H, dm, J = 15.0 Hz, H-4), 2.80 (1H, m, H-9), 3.07 (1H, d, J = 13.2, 3.4 Hz, H-10), 3.25 (1H, m, H-20), 3.32 (1H, dd, J = 8.0, 2.8 Hz, H-2), 3.50 (1H, m, H-23), 3.65–3.90 (6H, m, OCH_2CH_2OH, OH, H-3, 23), 4.17 (1H, m, H-1), 5.85 (1H, d, J = 11.2 Hz, H-7), 6.27 (1H, d, J = 11.2 Hz, H-6). MS *m/z* (%): 466 (M^+, 10), 448 (10), 430 (8), 412 (3), 317 (10), 237 (13), 133 (21), 113 (31), 69 (100). HRMS *m/z*: 466.3311 (Calcd for $C_{27}H_{46}O_6$: 466.3294). UV λ_{max} (EtOH): 243, 251, 260 nm.

4.16. 20-*epi*-1α,25-Dihydroxy-2α-(2-hydroxyethoxy)- and 1α,25-dihydroxy-2β-(2-hydroxyethoxy)-22-oxa-19-norvitamin D_3 (3d and 4d)

Following the same procedure as described above, treatment of **31d** (63.0 mg, 0.078 mmol, ca. 3:2 isomeric mixture) with (−)-10-camphor sulfonic acid (108.5 mg, 0.467 mmol) gave a mixture of **3d** and **4d** (33.0 mg, 91%), which was separated by the reversed-phase HPLC to give pure **3d** (13.9 mg) and **4d** (10.3 mg), respectively.

Compound **3d**: ^{1}H NMR ($CDCl_3$) δ: 0.55 (3H, s, H-18), 1.12 (3H, d, J = 5.9 Hz, H-21), 1.22, 1.23 (each 3H, s, H-26, 27), 2.60 (1H, dd, J = 13.4, 4.5 Hz, H-4), 2.79 (1H, m, H-9), 2.86 (1H, dd, J = 14.5, 4.8 Hz, H-10), 3.26 (1H, m, H-20), 3.31 (1H, dd, J = 8.1, 2.7 Hz, H-2), 3.45 (1H, m, H-23), 3.57 (1H, s, OH), 3.66–3.86 (5H, m, OCH_2CH_2O, H-23), 3.92 (1H, m, H-3), 4.14 (1H, m, H-1), 5.79 (1H, d, J = 11.2 Hz, H-7), 6.33 (1H, d, J = 11.2 Hz, H-6). UV λ_{max} (EtOH): 243 (ε 29,600), 251 (ε 34,500), 261 (ε 23,200) nm. MS *m/z* (%): 466 (M^+, 39), 448 (30), 430 (13), 362 (14), 345 (12), 317 (13), 237 (9), 133 (20), 113 (50), 69 (100). HRMS *m/z*: 466.3267 (Calcd for $C_{27}H_{46}O_6$: 466.3294).

Compound **4d**: ^{1}H NMR ($CDCl_3$) δ: 0.55 (3H, s, H-18), 1.13 (3H, d, J = 5.9 Hz, H-21), 1.22, 1.24 (each 3H, s, H-26, 27), 2.34 (1H, d, J = 14.1 Hz, H-4), 2.48 (1H, dd, J = 14.1, 2 Hz, H-4), 2.67 (1H, br s, OH), 2.79 (1H, m, H-9), 3.07 (1H, dd, J = 13.4, 3.8 Hz, H-10), 3.27 (2H, m, H-2, 20), 3.45 (1H, m, H-23), 3.56 (1H, s, OH), 3.64–3.87 (6H, m, OCH_2CH_2O, H-1, 23), 4.17 (1H, m, H-3), 5.82 (1H, d, J = 11.2 Hz, H-7), 6.27 (1H, d, J = 11.2 Hz, H-6). UV λ_{max} (EtOH): 243 (ε 32,500), 251 (ε 37,900), 261 (ε 25,100) nm. MS *m/z* (%): 466 (M^+, 28), 448 (22), 430 (11), 362 (9), 345 (9), 317 (9), 237 (11), 133 (19), 113 (43), 69 (100). HRMS *m/z*: 466.3300 (Calcd for $C_{27}H_{46}O_6$: 466.3294).

4.17. 24a,26a,27a-Trihomo-1α,25-dihydroxy-2α-(2- hydroxyethoxy)- and 24a,26a,27a-trihomo-1α,25-dihydroxy-2β-(2-hydroxyethoxy)-22, 24-diene-19-norvitamin D_3 (3b and 4b)

A mixture of **31b** (42.0 mg, 0.044 mmol, ca. 3:2 isomeric mixture), Et_3N (30 μL), and tetrabutylammonium fluoride (0.350 mL, 0.350 mmol, 1.0 M solution in THF) in dry THF (1 mL) was stirred at ambient temperature for 5 h. The mixture was poured into ice water and extracted with AcOEt. The organic phase was washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel (5 g) with 2% MeOH in AcOEt to yield the isomeric mixture of **3b** and **4b** (20.0 mg, 91%). The mixture was separated by HPLC (YMC-Pack ODS-AM SH-342-5, 150 × 20 mm, 20% H_2O in MeOH) to give pure **3b** (9.4 mg) and **4b** (8.3 mg), respectively.

Compound **3b**: ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, H-18), 0.86 (6H, t, J = 7.4 Hz, H-26a, 27a), 1.04 (3H, d, J = 6.6 Hz, H-21), 1.54, 1.56 (each 2H, q, J = 7.4 Hz, H-26, 27), 2.61 (1H, dd, J = 13.4, 4.4 Hz, H-4), 2.72 (1H, br s, OH), 2.80 (1H, m, H-9), 2.86 (1H, dd, J = 14.4, 4.8 Hz, H-10), 3.12 (1H, br s, OH), 3.32 (1H, dd, J = 8.0, 2.5 Hz, H-2), 3.48 (1H, br s, OH), 3.67–3.81 (4H, m, OCH_2CH_2O), 3.93 (1H, m, H-3), 4.15 (1H, m, H-1), 5.53 (1H, d, J = 15.3 Hz, H-24a, overlapped with H-22), 5.81 (1H, d, J = 11.1 Hz, H-7), 5.97 (1H, dd, J = 15.0, 10.3 Hz, H-23), 6.14 (1H, dd, J = 15.3, 10.3 Hz, H-24), 6.33 (1H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH): 235 (ε 44,000), 243 (ε 44,200), 251 (ε 38,000), 261 (ε 23,800) nm. MS *m/z* (%): 502 (M^+, 11), 484 (62), 466 (33), 448 (7), 386 (17), 333 (40), 237 (29), 149 (100), 133 (43), 93 (49). HRMSs *m/z*: 502.3658 (Calcd for $C_{31}H_{50}O_5$: 502.3658).

Compound **4b**: ^{1}H NMR ($CDCl_3$) δ: 0.56 (3H, s, H-18), 0.86 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.05 (3H, d, J = 6.6 Hz, H-21), 1.55, 1.56 (each 2H, q, J = 7.5 Hz, H-26, 27), 2.34 (1H, d, J = 14 Hz, H-4), 2.47 (1H, d, J = 14, 2 Hz, H-4), 2.64 (1H, br s, OH), 2.80 (1H, m, H-9), 3.07 (1H, dd, J = 13.2, 4.0 Hz, H-10), 3.27 (1H,

dd, $J = 8.7$, 2.6 Hz, H-2), 3.64–3.86 (5H, m, OCH_2-CH_2O, H-1), 4.16 (1H, m, H-3), 5.53 (1H, d, $J = 15.3$ Hz, H-24a, overlapped with H-22), 5.83 (1H, d, $J = 11.1$ Hz, H-7), 5.97 (1H, dd, $J = 15.0$, 10.3 Hz, H-23), 6.15 (1H, dd, $J = 15.3$, 10.3 Hz, H-24), 6.26 (1H, d, $J = 11.1$ Hz, H-6). UV λ_{max} (EtOH): 235 (ε 44,000), 243 (ε 44,500), 251 (ε 38,900), 261 (ε 24,000) nm. MS *m/z* (%): 502 (M^+, 13), 484 (78), 466 (39), 448 (7), 386 (13), 333 (48), 237 (27), 149 (100), 133 (46), 93 (49). HRMS *m/z*: 502.3664 (Calcd for $C_{31}H_{50}O_5$: 502.3658).

4.18. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[(trimethylsilyl)oxy]-22-oxa-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (32c)

To a stirred solution of **7** (222.0 mg, 0.34 mmol, a mixture of ca. 2:1) in dry THF (1 mL) at −78 °C was added *n*-BuLi (215.2 μL, 0.34 mmol, 1.56 M solution in hexane) and the resulting dark orange solution was stirred for 15 min. To this cooled solution was added a solution of **22′c** (61.2 mg, 0.17 mmol) in dry THF (0.5 + 0.2 mL), and the whole mixture was stirred at −78 to −20 °C for 2.5 h. The reaction mixture was quenched with saturated NH_4Cl and extracted with AcOEt. The organic extract was washed with brine and 5% $NaHCO_3$, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (10 g) using 2% AcOEt in hexane to afford **32c** (39.8 mg, 59% based on **22′c**) as a mixture of two isomers in a ca. 2:1 ratio, 15% AcOEt in hexane to give the unreacted starting material **22′c** (36.1 mg, 33%), and 40% AcOEt in hexane to afford the unreacted starting material **7** (123.8 mg).

MS *m/z* (%): 836 (no M^+), 704 (7), 647 (2), 618 (5), 572 (12), 486 (13), 469 (7), 383 (9), 309 (10), 75 (100). HRMS *m/z*: 704.5066 (M^+−TESOH) (Calcd for $C_{40}H_{76}O_4Si_4$: 704.5051). 2α-Isomer (major) ^{1}H NMR ($CDCl_3$) δ: 0.056, 0.063 (each 6H, s, 2× Si–Me), 0.124 (9H, Si–Me_3), 0.52 (3H, s, H-18), 0.57 (6H, q, $J = 8.0$ Hz, 3× Si–CH_2CH$_3$), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, $J = 8.0$ Hz, 3× Si–CH$_2$CH_3), 1.16 (3H, d, $J = 6.0$ Hz, H-21), 1.22 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 3.20 (1H, m, H-20), 3.35 (1H, m, H-23), 3.54 (1H, m, H-2), 3.67 (1H, m, H-23), 3.80 (1H, m, H-3), 3.88 (1H, m, H-1), 5.83 (1H, m, d, $J = 11.0$ Hz, H-7), 6.09 (1H, d, $J = 11.0$ Hz, H-6). 2β-Isomer (minor) ^{1}H NMR ($CDCl_3$) δ: 0.036, 0.044 (each 6H, s, 2× Si–Me), 0.120 (9H, Si–Me_3), 0.53 (3H, s, H-18), 0.57 (6H, q, $J = 8.0$ Hz, 3× Si–CH_2CH$_3$), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, $J = 8.0$ Hz, 3× Si–CH$_2$CH_3), 1.16 (3H, d, $J = 6.0$ Hz, H-21), 1.22 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 3.20 (1H, m, H-20), 3.35 (1H, m, H-23), 3.59 (1H, m, H-2), 3.67 (1H, m, H-23), 3.80 (1H, m, H-3), 3.94 (1H, m, H-1), 5.80 (1H, m, d, $J = 11.0$ Hz, H-7), 6.12 (1H, d, $J = 11.0$ Hz, H-6).

4.19. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2,25-dihydroxy-22-oxa-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (33c)

A solution of **32c** (111.8 mg, 0.13 mmol, ca. 2:1 isomeric mixture) in THF/AcOH/H_2O (4.25 mL, 8:8:1, v/v/v) was stirred at ambient temperature for 22 h and diluted with AcOEt. The organic phase was successively washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (5 g) using 6% AcOEt in hexane to give **33c** (69.3 mg, 79%) as a mixture of two isomers in a ca. 3:2 ratio.

MS *m/z* (%): 650 (M^+, 2), 632 (10), 546 (7), 489 (7), 357 (55), 75 (100). HRMS *m/z*: 650.4754 (Calcd for $C_{37}H_{70}O_5Si_2$: 650.4762). 2α-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.09 (12H, s, 4× Si–Me), 0.53 (3H, s, H-18), 0.87, 0.88 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, $J = 5.9$ Hz, H-21), 1.24, 1.25 (each 3H, s, H-26, 27), 2.79 (1H, m, H-9), 3.25 (1H, m, H-20), 3.45–3.60 (2H, m, H-2, 23), 3.84 (1H, m, H-23), 3.92 (1H, m, H-3), 3.99 (1H, m, H-1), 5.80 (1H, m, d, $J = 11.1$ Hz, H-7), 6.14 (1H, d, $J = 11.1$ Hz, H-6). 2β-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06–0.09 (12H, s, 4× Si–Me), 0.52 (3H, s, H-18), 0.86, 0.89 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, $J = 5.9$ Hz, H-21), 1.24, 1.25 (each 3H, s, H-26, 27), 2.79 (1H, m, H-9), 3.25 (1H, m, H-20), 3.45–3.60 (2H, m, H-2, 23), 3.84 (1 H, m, H-23), 3.99 (2H, m, H-1, 3), 5.80 (1H, m, d, $J = 11.1$ Hz, H-7), 6.17 (1H, d, $J = 11.1$ Hz, H-6).

4.20. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-oxo-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (34a)

To a stirred solution of oxalyl chloride (7.8 μL, 0.088 mmol) in dry CH_2Cl_2 (1 mL) at −78 °C was added a solution of DMSO (12.4 μL, 0.175 mmol) in dry CH_2Cl_2 (0.2 mL). After being stirred for 5 min, a solution of **29a** (50.4 mg, 0.073 mmol, ca. 5:4 isomeric mixture) in dry CH_2Cl_2 (1.2 mL) was added dropwise. The reaction mixture was stirred for 15 min at −78 °C, and Et_3N (51 μL, 0.365 mmol) was added. The whole mixture was stirred at −78 °C for 40 min and at 0 °C for 20 min, quenched with ice water, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel (5 g) using 5% AcOEt in hexane to afford **34a** (49.0 mg, 97%) as a single compound.

^{1}H NMR ($CDCl_3$) δ: 0.055, 0.065, 0.069, 0.094 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.88, 0.89 (each 9H, s, 2× Si–*t*-Bu), 1.03 (3H, d, $J = 6.6$ Hz, H-21), 1.20 (6H, s, H-26, 27), 2.44 (1H, dd, $J = 13.3$, 8.9 Hz), 2.52 (1H, dd, $J = 14.2$, 3.8 Hz), 2.69 (2H, m), 2.81 (1H, m, H-9), 3.37 (3H, s, OMe), 4.35 (1H, dd, $J = 6.4$, 4.2 Hz), 4.55 (1H, dd, $J = 8.7$, 5.5 Hz), 4.73 (2H, s, OCH_2O), 5.34 (2H, m, H-22, 23), 5.80 (1H, d, $J = 11.2$ Hz, H-7), 6.34 (1H, d, $J = 11.2$ Hz, H-6). MS *m/z* (%): 688 (no M^+), 631 (5), 599 (16), 569 (100), 437 (22), 325 (17), 109 (81), 75 (52). HRMS *m/z*: 569.3848 (M^+−Bu–MeOCH$_2$OH) (Calcd for $C_{34}H_{57}O_3Si_2$: 569.3846).

4.21. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-oxo-22-oxa-25-hydroxy-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (34c)

The Swern oxidation of **33c** (69.3 mg, 0.1 mmol, ca. 2:1 isomeric mixture) was carried out according to the same

procedure described for the preparation of **34a** to give **34c** (50.8 mg, 81%) as a single compound.

^{1}H NMR ($CDCl_3$) δ: 0.05, 0.09 (each 3H, s, Si–Me), 0.07 (6H, s, 2× Si–Me), 0.53 (3H, s, H-18), 0.87, 0.89 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.44 (1 H, dd, J = 13.0, 8.7 Hz), 2.54 (1H, dd, J = 14.1, 3.9 Hz), 2.65 (1H, dd, J = 13.0, 5.4 Hz), 2.70 (1H, dd, J = 14.1, 6.6 Hz), 2.82 (1H, m, H-9), 3.49 (1H, m, H-20), 3.25, 3.84 (each 1H, m, H-23), 4.36 (1H, m, H-3), 4.53 (1H, m, H-1), 5.82 (1H, m, d, J = 11.2 Hz, H-7), 6.33 (1H, d, J = 11.2 Hz, H-6). MS *m*/*z* (%): 648 (M^+, 2), 591 (5), 573 (100), 441 (32). HRMS *m*/*z*: 648.4621 (Calcd for $C_{37}H_{68}O_5Si_2$: 648.4605).

4.22. 20-*epi*-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-oxo-22-oxa-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (34d)

The Swern oxidation of **30d** (60.7 mg, 0.079 mmol, ca. 3:2 isomeric mixture) was carried out according to the same procedure described for the preparation of **34a** to afford **34d** (59.5 mg, 98%) as a single compound.

^{1}H NMR ($CDCl_3$) δ: 0.057, 0.066, 0.070, 0.097 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.57 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.87, 0.89 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.45 (1H, dd, J = 13.2, 8.7 Hz), 2.52 (1H, dd, J = 14.0, 4.0 Hz), 2.67 (1H, dd, J = 13.2, 5.3 Hz), 2.72 (1 H, dd, J = 14.0, 6.6 Hz), 2.83 (1H, m, H-9), 3.26 (1H, m, H-20), 3.32, 3.70 (each 1H, m, H-23), 4.36 (1H, dd, J = 6.3, 4.2 Hz), 4.55 (1H, dd, J = 8.7, 5.5 Hz), 5.79 (1H, d, J = 11.0 Hz, H-7), 6.37 (1H, d, J = 11.0 Hz, H-6). MS *m*/*z* (%): 762 (no M^+), 705 (8), 573 (12), 487 (22), 355 (12), 103 (51), 75 (100). HRMS *m*/*z*: 705.4740 (M^+−Bu) (Calcd for $C_{39}H_{73}O_5Si_3$: 705.4766).

4.23. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-cyanomethylene-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (35a)

To a stirred solution of diethyl (cyanomethyl)phosphonate (32 μL, 0.197 mmol) in dry THF (1 mL) at −40 °C was added *n*-BuLi (126 μL, 0.197 mmol, 1.56 M solution in hexane). The mixture was stirred for 15 min, and a solution of **34a** (68.0 mg, 0.099 mmol) in dry THF (1.2 mL) was added dropwise. Stirring was continued for 2 h at −40 °C after which time the reaction mixture was quenched with saturated NH_4Cl and extracted with AcOEt. The AcOEt layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave the residue, which was purified by chromatography on silica gel (5 g) using 3% AcOEt in hexane to afford **35a** (59.6 mg, 85%) as a mixture of *E*- and *Z*-isomers in a ca. 1:1 ratio.

MS *m*/*z* (%): 711 (M^+, 5), 649 (18), 592 (61), 565 (76), 517 (20), 408 (26), 109 (92), 75 (99), 73 (100). HRMS *m*/*z*: 711.5054 (Calcd for $C_{42}H_{73}O_4Si_2$: 711.5078). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.054, 0.065, 0.094, 0.120 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.84, 0.92 (each 9H, s, 2× Si–*t*-Bu), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 3.12 (1H, m, H-10), 3.37 (3H, s, OMe), 4.46 (1H, m, H-1), 4.73 (2H, s, OCH_2O), 4.99 (1H, m, H-3), 5.33 (2H, m, H-22, 23), 5.47 (1H, d, J = 1.8 Hz, C=CH), 5.82 (1H, d, J = 11.1 Hz, H-7), 6.18 (1H, d, J = 11.1 Hz, H-6). *Z*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.065, 0.075, 0.111, 0.133 (each 3H, s, 4× Si–Me), 0.55 (3H, s, H-18), 0.84, 0.92 (each 9H, s, 2× Si–*t*-Bu), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.80 (1H, m, H-9), 2.99 (1H, m, H-10), 3.37 (3H, s, OMe), 4.57 (1H, m, H-3), 4.73 (2H, s, OCH_2O), 5.04 (1H, m, H-1), 5.33 (2H, m, H-22, 23), 5.47 (1H, d, J = 1.8 Hz, C=CH), 5.78 (1H, d, J = 11.1 Hz, H-7), 6.31 (1H, d, J = 11.1 Hz, H-6).

4.24. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-cyanomethylene-22-oxa-25-hydroxy-19- norvitamin D_3 *tert*-butyldimethylsilyl ether (35c)

Following the same procedure as described above, treatment of **34c** (50.8 mg, 0.07 mmol)with diethyl (cyanomethyl)phosphonate (24.0 μL, 0.14 mmol) afforded **35c** (45.9 mg, *E*:*Z* = ca. 1: 1, 96%).

MS *m*/*z* (%): 671 (M^+, 8), 653 (12), 614 (10), 596 (80), 569 (100), 521 (21), 464 (15), 410 (5). HRMS *m*/*z*: 671.4775 (Calcd for $C_{39}H_{69}NO_4Si_2$: 671.4765). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.05, 0.06, 0.09, 0.11 (each 3H, s, 4× Si–Me), 0.54 (3H, s, H-18), 0.83, 0.92 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.24, 1.25 (6H, s, H-26, 27), 2.30, 2.36 (each 1H, d, J = 14.0 Hz), 2,80 (1H, m, H-9), 3.10 (1H, m, H-10), 3.25 (1H, m, H-20), 3.50, 3.85 (each 1H, m, H-23), 4.44 (1H, m, H-1), 4.98 (1H, t, J = 2.8 Hz, H-3), 5.47 (1H, m, C=CH), 5.83 (1H, m, d, J = 11.2 Hz, H-7), 6.17 (1H, d, J = 11.2 Hz, H-6). *Z*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06, 0.07, 0.10, 0.13 (each 3H, s, 4× Si–Me), 0.52 (3H, s, H-18), 0.83, 0.92 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.25, 1.27 (6H, s, H-26, 27), 2.60 (1H, m), 2.82 (1H, m, H-9), 2.98 (1H, m, H-10), 3.25 (1H, m, H-20), 3.50, 3.85 (each 1H, m, H-23), 4.55 (1H, m, H-1), 5.03 (1H, t, J = 2.8 Hz, H-3), 5.47 (1H, m, C=CH), 5.79 (1H, d, J = 11.2 Hz, H-7), 6.30 (1H, d, J = 11.2 Hz, H-6).

4.25. 20-*epi*-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-cyanomethylene-22-oxa-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (35d)

Following the same procedure as described above, treatment of **34d** (120.3 mg, 0.157 mmol) with diethyl (cyanomethyl)phosphonate (51 μL, 0.315 mmol) afforded **35d** (120.6 mg, *E*:*Z* = ca. 1:1, 97%).

MS *m*/*z* (%): 785 (M^+, 2), 728 (8), 701 (12), 653 (6), 596 (9), 569 (16), 510 (17), 483 (11), 103 (66), 75 (100). HRMS *m*/*z*: 785.5637 (Calcd for $C_{45}H_{83}O_4NSi_3$: 785.5630). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.05, 0.07, 0.10, 0.12 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.84, 0.92 (each 9H, s, 2× Si–*t*-Bu), 0.93 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23

(each 3H, s, H-26, 27), 2.31, 2.37 (each 1H, br d, J = 14 Hz, H-4), 2.80 (1H, m, H-9), 3.12 (1H, m, H-10), 3.26 (1H, m, H-20), 3.32, 3.69 (each 1H, m, H-23), 4.46 (1H, m, H-1), 4.99 (1H, m, H-3), 5.47 (1H, d, J = 1.8 Hz, C=CH), 5.80 (1H, d, J = 11.1 Hz, H-7), 6.20 (1 H, d, J = 11.1 Hz, H-6). Z-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.06, 0.08, 0.11, 0.13 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.84, 0.92 (each 9H, s, 2× Si–t-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.61 (1H, m, H-4), 2.82 (1H, m, H-9), 2.99 (1H, m, H-10), 3.26 (1H, m, H-20), 3.32, 3.70 (each 1H, m, H-23), 4.58 (1H, ddd, J = 11.0, 5.9, 1.9 Hz, H-3), 5.04 (1H, m, H-1), 5.47 (1H, d, J = 1.9 Hz, C=CH), 5.77 (1H, d, J = 11.2 Hz, H-7), 6.33 (1 H, d, J = 11.2 Hz, H-6).

4.26. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(formyl)-ethylidene]-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (36a) and 1α-[(*tert*-butyldimethylsilyl)oxy]-2-[2-(hydroxy)-ethylidene]-22-ene-25-[(methoxymethyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (37a)

To a stirred solution of **35a** (59.6 mg, 0.084 mmol, ca. 1:1 isomeric mixture) in dry toluene (1 mL) at −78 °C was added dropwise diisobutylaluminum hydride (126 μL, 0.126 mmol, 1.0 M solution in hexane), and the mixture was stirred for 1.5 h at the same temperature. The reaction mixture was diluted with hexane and directly loaded onto silica gel column (5 g). The column was eluted with 5% AcOEt in hexane to afford **36a** (56.0 mg, 94%) as a mixture of E- and Z-isomer in a ca. 1:1 ratio.

$NaBH_4$ (3.6 mg, 0.094 mmol) was added to a solution of **36a** (56.0 mg, 0.078 mmol, ca. 1:1 isomeric mixture) in MeOH/THF (v/v, 2:1, 1.5 mL) at 0 °C. After being stirred for 1 h at 0 °C, the mixture was poured into ice water, and 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 (6 g) using 8% AcOEt in hexane to give **37a** (E-isomer, 26.3 mg, 47%, and Z-isomer, 22.7 mg, 40%). The total yield was 87%.

Compound **36a**: MS m/z (%): 714 (M^+, 9), 652 (13), 595 (20), 582 (13), 520 (34), 491 (23), 463 (14), 411 (17), 109 (33), 75 (100). HRMS m/z: 714.5074 (Calcd for $C_{42}H_{74}O_5Si_2$: 714.5075). E-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.10 (12H, 4× Si–Me), 0.57 (3H, s, H-18), 0.84, 0.92 (each 9H, s, 2× Si–t-Bu), 1.03 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.42 (2H, m, H-4), 2.82 (1H, m, H-9), 3.05 (1H, m, H-10), 3.37 (3H, s, OMe), 4.56 (1H, m, H-1), 4.73 (2H, s, OCH_2O), 5.35 (2H, m, H-22, 23), 5.46 (1H, m, H-3), 5.84 (1H, d, J = 11.4 Hz, H-7), 6.16 (1H, m, C=CH), 6.19 (1H, d, J = 11.4 Hz, H-6), 10.18 (1H, d, J = 7.9 Hz, CHO). Z-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.10 (12H, 4× Si–Me), 0.56 (3H, s, H-18), 0.84, 0.93 (each 9H, s, 2× Si–t-Bu), 1.03 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.65 (1H, m, H-4), 2.82 (1H, m, H-9), 3.00 (1H, m, H-10), 3.37 (3H, s, OMe), 4.70 (1H, m, H-3), 4.73 (2H, s, OCH_2O), 5.35 (2H, m, H-22, 23), 5.53 (1H, m, H-1), 5.79 (1H, d, J = 11.3 Hz, H-7), 6.17 (1H, m, C=CH), 6.31 (1H, d, J = 11.3 Hz, H-6), 10.16 (1H, d, J = 7.9 Hz, CHO).

Compound **37a**: MS m/z (%): 716 (M^+, 1), 584 (39), 522 (14), 491 (9), 147 (8), 109 (19), 75 (100). HRMS m/z: 584.4277 (M^+−TBSOH) (Calcd for $C_{36}H_{60}O_4Si$: 584.4261). E-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.02, 0.06 (each 3H, s, 2× Si–Me), 0.08 (6H, s, 2× Si–Me), 0.56 (3H, s, H-18), 0.85, 0.92 (each 9H, s, 2× Si–t-Bu), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.30 (2H, m, H-4), 2.80 (1H, m, H-9), 2.88 (1H, dd, J = 12.7, 4.6 Hz, H-10), 3.37 (3H, s, OMe), 4.20, 4.30 (each 1H, m, CH_2OH), 4.37 (1H, dd, J = 9.5, 4.0 Hz, H-1), 4.73 (2H, s, OCH_2O), 4.81 (1H, m, H-3), 5.33 (2H, m, H-22, 23), 5.72 (1H, m, C=CH), 5.85 (1H, d, J = 11.1 Hz, H-7), 6.14 (1H, d, J = 11.1 Hz, H-6). Z-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01, 0.08, 0.08, 0.09 (each 3H, s, 4× Si–Me), 0.55 (3H, s, H-18), 0.84, 0.93 (each 9 H, s, 2× Si–t-Bu), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.20 (6H, s, H-26, 27), 2.55 (1H, dd, J = 12.5, 4.9 Hz, H-4), 2.83 (2H, m, H-9, 10), 3.37 (3H, s, OMe), 4.22 (1H, dd, J = 12.4, 7.0 Hz, CH_2OH), 4.30 (1H, dd, J = 12.4, 7.0 Hz, CH_2OH), 4.48 (1H, m, H-3), 4.73 (2 H, s, OCH_2O), 4.86 (1H, m, H-1), 5.33 (2H, m, H-22, 23), 5.72 (1H, td, J = 7.0, 1.3 Hz, C=CH), 5.80 (1H, d, J = 11.1 Hz, H-7), 6.25 (1H, d, J = 11.1 Hz, H-6).

4.27. 1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(formyl)-ethylidene]-22-oxa-25- hydroxy-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (36c) and 1α-[(*tert*-butyldimethylsilyl)oxy]-2-[2-(hydroxy)-ethylidene]-22-oxa-25- hydroxy-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (37c)

Following the same procedure as described above, treatment of **35c** (34.8 mg, 0.05 mmol, ca. 1:1 isomeric mixture) with diisobutylaluminum hydride (100 μL, 0.10 mmol) gave **36c**, then the treatment of **36c** and $NaBH_4$ (1.9 mg, 0.05 mmol) yielded **37c** (6.25 mg, 25%) as a mixture of two isomers in a ca. 1: 1 ratio.

Compound **36c**: E-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01, 0.07 (each 3H, s, 2× Si–Me), 0.09 (6H, s, 2× Si–Me), 0.54 (3H, s, H-18), 0.84, 0.92 (each 9H, s, 2× Si–t-Bu), 1.20 (3H, d, J = 6.0 Hz, H-21), 1.24, 1.25 (6H, s, H-26, 27), 2.40 (2H, m, H-4), 2.80 (1H, m, H-9), 3.06 (1H, dd, J = 13.2, 4.9 Hz, H-10), 3.26 (1H, m, H-20), 3.50, 3.85 (each 1H, m, H-23), 4.57 (1H, m, H-1 or 3), 5.46 (1H, m, H-1 or 3), 5.86 (1H, m, d, J = 11.0 Hz, H-7), 6.15 (1H, dd, J = 8.0, 1.3 Hz, C=CH), 6.17 (1H, d, J = 11.0 Hz, H-6), 10.18 (1H, d, J = 7.9 Hz, CHO). Z-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.02, 0.07, 0.099, 0.102 (each 3H, s, 4× Si–Me), 0.53 (3H, s, H-18), 0.84, 0.93 (each 9H, s, 2× Si–t-Bu), 1.20 (3H, d, J = 6.0 Hz, H-21), 1.24, 1.25 (6H, s, H-26, 27), 2.24 (1H, m, H-4), 2.83 (1H, m, H-9), 2.99 (1H, m, H-10), 3.26 (1H, m, H-20), 3.50, 3.85 (each 1H, m, H-23), 4.68 (1H, m, H-1 or 3), 5.53 (1H, m, H-1 or 3), 5.80 (1H, m, d, J = 11.0 Hz, H-7), 6.16 (1H, dd, J = 8.0, 1.5 Hz, C=CH), 6.30 (1H, d, J = 11.0 Hz, H-6), 10.17 (1H, d, J = 7.9 Hz, CHO).

Compound **37c**: MS *m*/*z* (%): 676 (M^+, 2), 619 (5), 544 (37), 513 (11), 412 (7), 394 (9), 75 (100). HRMS *m*/*z*: 676.4904 (Calcd for $C_{39}H_{72}O_5Si_2$: 676.4918). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.12 (12H, s, 4× Si–Me), 0.52 (3H, s, H-18), 0.82, 0.93 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.23, 1.24 (6H, s, H-26, 27), 2.79 (1H, m, H-9), 3.04 (1H, m, H-10), 3.27 (1H, m, H-20), 3.49, 3.83 (each 1H, m, H-23), 4.15–4.32 (2H, m, CH_2OH), 4.35 (1H, m, H-1), 4.81 (1H, m, H-3), 5.59 (1H, m, C=CH), 5.88 (1H, m, d, J = 11.1 Hz, H-7), 6.13 (1H, d, J = 11.1 Hz, H-6). *Z*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01–0.12 (12H, s, 4× Si–Me), 0.54 (3H, s, H-18), 0.82, 0.93 (each 9H, s, 2× Si–*t*-Bu), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.23, 1.24 (6H, s, H-26, 27), 2.54 (1H, dd, J = 12.0, 4.5 Hz), 2.83 (2H, m, H-9, 10), 3.27 (1H, m, H-20), 3.49, 3.83 (each 1H, m, H-23), 4.15–4.32 (2H, m, CH_2OH), 4.47 (1H, m, H-1), 4.86 (1H, m, H-3), 5.59 (1H, m, C=CH), 5.82 (1H, m, d, J = 11.1 Hz, H-7), 6.23 (1H, d, J = 11.1 Hz, H-6).

4.28. 20-*epi*-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[2-(formyl)-ethylidene]-22-oxa-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (36d) and 20-*epi*-1α-[(*tert*-butyl-dimethylsilyl)oxy]-2-[2-(hydroxy)-ethylidene]-22-oxa-25-[(tri-ethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (37d)

Following the same procedure as described above, treatment of **35d** (77.0 mg, 0.098 mmol, a mixture of ca. 1:1) with diisobutylaluminum hydride (147 μL, 0.147 mmol) afforded **36d**, then the treatment of **36d** and $NaBH_4$ (3.9 mg, 0.102 mmol) yielded **37d** (*E*-isomer, 30.2 mg; *Z*-isomer, 24.2 mg; 81%).

Compound **36d**: MS *m*/*z* (%): 788 (M^+, 5), 731 (5), 656 (8), 627 (7), 599 (4), 524 (5), 495 (3), 409 (5), 103 (42), 75 (100). HRMS *m*/*z*: 788.5649 (Calcd for $C_{45}H_{84}O_5Si_3$: 788.5627). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01, 0.07, 0.09, 0.10 (each 3H, s, 4× Si–Me), 0.57 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.84, 0.92 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.42 (2H, m, H-4), 2.80 (1H, m, H-9), 3.05 (1H, dd, J = 12.8, 5.3 Hz, H-10), 3.26 (1H, m, H-20), 3.32, 3.69 (each 1H, m, H-23), 4.57 (1H, m, H-1), 5.46 (1H, m, H-3), 5.83 (1H, d, J = 11.1 Hz, H-7), 6.15 (1H, dd, J = 7.9, 1.1 Hz, C=CH), 6.19 (1H, d, J = 11.1 Hz, H-6), 10.18 (1H, d, J = 7.9 Hz, CHO). *Z*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.02, 0.08, 0.10, 0.11 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.57 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.84, 0.93 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.65 (1H, dd, J = 12.0, 4.8 Hz, H-4), 2.80 (1H, m, H-9), 3.00 (1H, m, H-10), 3.26 (1H, m, H-20), 3.32, 3.71 (each 1H, m, H-23), 4.69 (1H, m, H-3), 5.54 (1H, m, H-1), 5.78 (1H, d, J = 11.2 Hz, H-7), 6.16 (1H, dd, J = 7.9, 1.1 Hz, C=CH), 6.32 (1H, d, J = 11.1 Hz, H-6), 10.16 (1H, d, J = 7.9 Hz, CHO).

Compound **37d**: MS *m*/*z* (%): 790 (M^+, 1), 772 (1), 733 (1), 658 (45), 627 (11), 526 (7), 508 (7), 376 (5), 103 (33), 75 (100). HRMS *m*/*z*: 658.4830 (M^+−TBSOH) (Calcd for $C_{39}H_{70}O_4Si_2$: 658.4813). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01, 0.07 (each 3H, s, 2× Si–Me), 0.08 (6H, s, 2× Si–Me), 0.56 (3H, s, H-18), 0.56 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.85, 0.92 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.31 (2H, m, H-4), 2.80 (1H, m, H-9), 2.88 (1H, dd, J = 12.6, 4.6 Hz, H-10), 3.26 (1H, m, H-20), 3.32, 3.70 (each 1H, m, H-23), 4.18, 4.31 (each 1H, m, CH_2OH), 4.37 (1H, m, H-1), 4.82 (1H, m, H-3), 5.72 (1H, m, C=CH), 5.83 (1H, d, J = 11.0 Hz, H-7), 6.15 (1H, d, J = 11.0 Hz, H-6). *Z*-Isomer: ^{1}H NMR ($CDCl_3$) δ: 0.01, 0.07, 0.08, 0.10 (each 3H, s, 4× Si–Me), 0.56 (3H, s, H-18), 0.57 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.84, 0.93 (each 9H, s, 2× Si–*t*-Bu), 0.94 (9H, *t*, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.09 (3H, d, J = 5.9 Hz, H-21), 1.21, 1.23 (each 3H, s, H-26, 27), 2.55 (1H, dd, J = 12.5, 5.0 Hz, H-4), 2.83 (2H, m, H-9, 10), 3.26 (1H, m, H-20), 3.32, 3.70 (each 1H, m, H-23), 4.22, 4.27 (each 1 H, m, CH_2OH), 4.48 (1H, m, H-3), 4.86 (1H, m, H-1), 5.72 (1H, dt, J = 7.0, 1.4 Hz, C=CH), 5.79 (1H, d, J = 11.1 Hz, H-7), 6.26 (1H, d, J = 11.1 Hz, H-6).

4.29. 1α,25-Dihydroxy-2-[2-(hydroxy)-ethylidene]-22-ene-19-norvitamin D_3 (5a, *E*-isomer; 6a, *Z*-isomer)

A mixture of the *E*-isomer **37a** (26.3 mg, 0.037 mmol) and (−)-10-camphor sulfonic acid (51.2 mg, 0.220 mmol) in dry MeOH (1 mL) was stirred at ambient temperature for 2 h. Cold 5% $NaHCO_3$ was added, and the mixture was extracted with AcOEt. The organic phase was washed with brine and dried over $MgSO_4$. Solvents were evaporated in vacuo, and the residue was purified by chromatography on silica gel (5 g) using 2% MeOH in AcOEt to afford **5a** (15.6 mg, 96%). The desired product was further purified by HPLC (YMC-Pack ODS-AM SH-342-5, 150 × 20 mm, 20% H_2O in MeOH) to give pure **5a** (12.5 mg).

Deprotection using CSA was carried out as described for (2*E*)-**37a** and **6a** was obtained in 96% yield.

Compound **5a**: ^{1}H NMR (CD_3OD) δ: 0.59 (3H, s, H-18), 1.05 (3H, d, J = 6.6 Hz, H-21), 1.16 (6H, s, H-26, 27), 2.35 (1H, d, J = 14.0 Hz, H-4), 2.43 (1H, dd, J = 14.0, 2.9 Hz, H-4), 2.86 (1H, m, H-9), 3.11 (1H, d, J = 12.8, 5.0 Hz, H-10), 4.24 (2H, m, CH_2OH), 4.30 (1H, m, H-1), 4.83 (1H, m, H-3), 5.31, 5.42 (each 1H, m, H-22, 23), 5.79 (1H, td, J = 6.9, 1.8 Hz, C=CH), 5.91 (1H, d, J = 11.1 Hz, H-7), 6.23 (1H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH): 246 (ε 37,000), 254 (ε 42,000), 263 (ε 27,800) nm. MS *m*/*z* (%): 444 (M^+, 7), 426 (5), 408 (22), 390 (9), 372 (14), 281 (4), 263 (11), 252 (100), 147 (9), 109 (12). HRMS *m*/*z*: 444.3246 (Calcd for $C_{28}H_{44}O_4$: 444.3240).

Compound **6a**: ^{1}H NMR (CD_3OD) δ: 0.61 (3H, s, H-18), 1.04 (3H, d, J = 6.6 Hz, H-21), 1.16 (6H, s, H-26, 27), 2.65 (1H, dd, J = 12.4, 5.0 Hz, H-4), 2.85 (1H, m, H-9), 2.93 (1H, d, J = 14.4, 3.0 Hz, H-10), 4.25 (2H, m, CH_2OH), 4.39 (1H, m, H-3), 4.87 (1H, m, H-1), 5.31, 5.42 (each 1H, m, H-22, 23), 5.77 (1H, td,

J = 6.9, 1.7 Hz, C=CH), 5.89 (1H, d, J = 11.1 Hz, H-7), 6.32 (1H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH): 246 (ε 32,500), 254 (ε 37,200), 263 (ε 24,500) nm. MS m/z (%): 444 (M^+, 10), 426 (5), 408 (23), 390 (27), 372 (91), 281 (54), 263 (79), 252 (57), 147 (86), 109 (100). HRMS m/z: 444.3227 (Calcd for $C_{28}H_{44}O_4$: 444.3240).

4.30. 1α,25-Dihydroxy-[2-(hydroxy)-ethylidene]-22-oxa-19-norvitamin D_3 (5c and 6c)

Following the same procedure as described above, treatment of **37c** (6.25 mg, 0.009 mmol) with CSA (22.2 mg, 0.036 mmol) yielded **5c** and **6c** (4.80 mg, 74%) in ca. 1:1 ratio, which was separated by the reversed-phase HPLC to give pure **5c** (1.77 mg) and **6c** (1.85 mg), respectively.

Compound **5c**: ^{1}H NMR ($CDCl_3$) δ: 0.54 (3H, s, H-18), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.24, 1.25 (each 3H, s, H-26, 27), 2.44 (2H, m, H-4), 2.81 (1H, m, H-9), 3.12 (1H, m, J = 13.1, 5.0 Hz, H-10), 3.26 (1H, m, H-20), 3.78 (1H, br s, OH), 3.49, 3.85 (each 1H, m, H-23), 4.23 (1H, dd, J = 12.4, 6.4 Hz, CH_2OH), 4.38 (1H, dd, J = 12.4, 7.4 Hz), 4.44 (1H, m, H-1), 4.87 (1H, m, H-3), 5.87 (1H, m, C=CH), 5.89 (1H, d, J = 11.2 Hz, H-7), 6.30 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 448 (M^+, 3), 430 (5), 412 (10), 394 (28), 376 (18), 308 (19), 263 (18), 131 (26), 113 (33), 69 (100). HRMS m/z: 448.3186 (Calcd for $C_{27}H_{44}O_5$: 448.3189). UV λ_{max} (EtOH): 246, 253, 263 nm.

Compound **6c**: ^{1}H NMR ($CDCl_3$) δ: 0.54 (3H, s, H-18), 1.19 (3H, d, J = 6.0 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.70 (1 H, dd, J = 12.8, 4.8 Hz, H-4), 2.82 (2H, m, H-9, 10), 3.26 (1H, m, H-20), 3.49 (1H, m, H-23), 3.79 (1H, br s, OH), 3.85 (1H, m, H-23), 4.26 (1H, dd, J = 12.6, 6.5 Hz, CH_2OH), 4.39 (1H, dd, J = 12.6, 7.2 Hz), 4.46 (1H, m, H-3), 4.88 (1H, m, H-1), 5.84 (1H, m, C=CH), 5.85 (1H, d, J = 11.2 Hz, H-7), 6.39 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 448 (M^+, 3), 430 (6), 412 (17), 394 (38), 376 (19), 308 (27), 263 (21), 131 (29), 113 (36), 69 (100). HRMS m/z: 448.3162 (Calcd for $C_{27}H_{44}O_5$: 448.3189). UV λ_{max} (EtOH): 245, 254, 263 nm.

4.31. 20-*epi*-1α,25-Dihydroxy-[2-(hydroxy)-ethylidene]-22-oxa-19-norvitamin D_3 (5d and 6d)

Following the same procedure as described above, treatment of **37d** (*E*-isomer, 43.6 mg, 0.055 mmol) with CSA (76.8 mg, 0.331 mmol) yielded **5d** (23.7 mg, 96%), which was separated by the reversed-phase HPLC to give pure **5d** (20.1 mg), and treatment of **37d** (*Z*-isomer, 35.5 mg, 0.045 mmol) with CSA (62.5 mg, 0.269 mmol) gave **6d** (19.3 mg, 96%), which was separated by the reversed-phase HPLC to afford pure **6d** (17.6 mg).

Compound **5d**: ^{1}H NMR ($CDCl_3$) δ: 0.54 (3H, s, H-18), 1.13 (3H, d, J = 5.9 Hz, H-21), 1.22, 1.23 (each 3H, s, H-26, 27), 2.33, 2.43 (each 1H, br d, J = 13 Hz, H-4), 2.79 (1H, m, H-9), 3.12 (1H, d, J = 12.5, 4.4 Hz, H-10), 3.26 (1H, m, H-20), 3.44 (1H, m, H-23), 3.51, 3.58, 3.90 (each 1H, br s, 3× OH), 3.84 (1H, dt, J = 9.4, 4.3 Hz, H-23), 4.08 (1 H, dd, J = 12.4, 5.2 Hz, CH_2OH), 4.33 (2H, m, H-1, CH_2OH), 4.79 (1H, m, H-3), 5.74 (1 H, m, C=CH), 5.84 (1H, d, J = 11.1 Hz, H-7), 6.26 (1H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH): 246 (ε 34,600), 254 (ε 39,700), 263 (ε 26,500) nm. MS m/z (%): 448 (M^+, 9), 430 (8), 412 (14), 394 (26), 376 (12), 308 (13), 263 (12), 131 (20), 113 (39), 69 (100). HRMS m/z: 448.3188 (Calcd for $C_{27}H_{44}O_5$: 448.3189).

Compound **6d** (Z-isomer): ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, H-18), 1.13 (3H, d, J = 5.9 Hz, H-21), 1.23, 1.24 (each 3H, s, H-26, 27), 2.68 (1H, dd, J = 12.6, 4.5 Hz, H-4), 2.81 (1H, m, H-9), 2.88 (1H, d, J = 14.2, 3.5 Hz, H-10), 3.28 (1H, m, H-20), 3.45, 3.84 (each 1H, m, H-23), 3.61 (1H, s, OH), 4.14 (1H, dd, J = 12.5, 5.6 Hz, CH_2OH), 4.34 (1H, dd, J = 12.5, 8.4 Hz, CH_2OH), 4.44 (1H, m, H-3), 4.84 (1H, m, H-1), 5.75 (1H, m, C=CH), 5.82 (1H, d, J = 11.1 Hz, H-7), 6.38 (1H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH): 246 (ε 32,300), 254 (ε 37,100), 263 (ε 24,600) nm. MS m/z (%): 448 (M^+, 7), 430 (7), 412 (14), 394 (25), 376 (12), 308 (13), 263 (12), 131 (21), 113 (39), 69 (100). HRMS m/z: 448.3214 (Calcd for $C_{27}H_{44}O_5$: 448.3189).

4.32. 24a,26a,27a-Trihomo-1α-[(*tert*-butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxy]-ethylidene]-22,24-diene-25- [(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (38b)

To a stirred solution of **8** (119.5 mg, 0.164 mmol, a mixture of two isomers) in dry THF (2 mL) at −78 °C was added dropwise *n*-BuLi (105 μL, 0.164 mmol, 1.56 M solution in hexane), and the resulting dark orange solution was stirred for 15 min. To this colored solution was added dropwise a solution of **22b** (47.4 mg, 0.109 mmol) in dry THF (1 mL), and the mixture was stirred for 2 h at −78 °C at which point it was quenched with saturated NH_4Cl, and extracted with AcOEt. The AcOEt layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent in vacuo yielded the residue, which was chromatographed on silica gel (10 g) with 1% AcOEt in hexane to afford **38b** (27.7 mg, 27% based on **22b**) as a mixture of two isomers in a ca. 6:1 ratio, and 5% AcOEt in hexane to give the unreacted starting material **22b** (27 mg, 57%).

^{1}H NMR ($CDCl_3$) δ: 0.01–0.08 (18H, 6× Si–Me), 0.569 (6H, q, J = 7.9 Hz, 3× $SiCH_2$), 0.571 (3H, s, H-18), 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.84, 0.90, 0.91 (each 9H, s, 3× Si–*t*-Bu), 0.94 (9H, t, J = 7.9 Hz, 3× $SiCH_2CH_3$), 1.06 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 2.96, 3.04 (ca. 6:1) (1H, dd, J = 12.5, 4.7 Hz, H-10), 4.24–4.47 (3H, m, H-1 or 3, CH_2OH), 4.77, 4.83 (ca. 6:1) (1H, m, H-1 or 3), 5.51 (1H, dd, J = 15.0, 8.2 Hz, H-22), 5.52 (1H, d, J = 15.3 Hz, H-24a), 5.60 (1H, m, C=CH), 5.86 (1H, d, J = 11.1 Hz, H-7), 5.94 (1H, dd, J = 15.0, 10.4 Hz, H-23), 6.05 (1H, dd, J = 15.3, 10.4 Hz, H-24), 6.12 (1H, d, J = 11.1 Hz, H-6). MS m/z (%): 940 (no M^+), 826 (1), 883 (1), 808 (6), 751 (10), 676 (21), 544 (11), 75 (100). HRMS m/z: 808.6029 (M^+−TBSOH) (Calcd for $C_{49}H_{88}O_3Si_3$: 808.6041).

4.33. 24a,26a,27a-Trihomo-1α,25-dihydroxy-2-[2-(hydroxy)-ethylidene]-22,24-diene-19-norvitamin D_3 (5b and 6b)

A mixture of **38b** (56 mg, 0.0595 mmol, ca. 6:1 isomeric mixture), Et_3N (40 μL), and tetrabutylammonium fluoride (476 μL, 0.476 mmol, 1.0 M solution in THF) in dry THF (1 mL) was stirred at ambient temperature for 20 h. The mixture was poured into ice water and extracted with AcOEt. The organic phase was washed with brine and dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded the residue, which was chromatographed on silica gel (5 g) with 2% MeOH in AcOEt to yield a mixture of **5b** and **6b** (23.0 mg, 80%) in a 10:1 ratio. The mixture was separated by HPLC (LiChrosorb Si 60, Hibar RT 250-10, 250 × 10 mm, hexane/CH_2Cl_2/MeOH 50:50:4) to give pure **5b** (17.1 mg, *E*-isomer) and **6b** (1.9 mg, *Z*-isomer), respectively.

Compound **5b**: ^{1}H NMR ($CDCl_3$) δ: 0.56 (3H, s, H-18), 0.87 (6H, t, *J* = 7.5, H-26a, 27a), 1.05 (3H, d, *J* = 6.5 Hz, H-21), 1.55, 1.56 (each 2H, q, *J* = 7.5 Hz, H-26, 27), 2.34 (1H, d, *J* = 13.3 Hz, H-4), 2.44 (1H, dd, *J* = 13.3, 2.0 Hz, H-4), 2.81 (1H, m, H-9), 3.14 (1H, d, *J* = 12.5, 4.3 Hz, H-10), 3.38, 3.74 (each 1H, br s, 2× OH), 4.09 (1H, dd, *J* = 12.3, 5.2 Hz, CH_2OH), 4.34 (2 H, m, H-1, CH_2OH), 4.80 (1H, m, H-3), 5.53 (1H, d, *J* = 15.4 Hz, H-24a, overlapped with H-22), 5.75 (1H, m, C=CH), 5.88 (1H, d, *J* = 11.1 Hz, H-7), 5.97 (1H, dd, *J* = 15.0, 10.4 Hz, H-23), 6.15 (1H, dd, *J* = 15.4, 10.4 Hz, H-24), 6.27 (1 H, d, *J* = 11.1 Hz, H-6). UV λ_{max} (EtOH): 236 (ε 47,300), 245 (ε 48,000), 254 (ε 43,200), 264 (ε 27,200) nm. MS *m*/*z* (%): 484 (M^+, 7), 466 (15), 448 (17), 430 (44), 412 (34), 279 (33), 263 (25), 149 (100), 133 (35), 93 (38). HRMS *m*/*z*: 484.3526 (Calcd for $C_{31}H_{48}O_4$: 484.3553).

Compound **6b**: ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, H-18), 0.87 (6H, t, *J* = 7.5 Hz, H-26a, 27a), 1.05 (3H, d, *J* = 6.6 Hz, H-21), 1.55, 1.56 (each 2H, q, *J* = 7.5 Hz, H-26, 27), 2.22 (1H, dd, *J* = 13.0, 9.7 Hz, H-4), 2.33 (1H, m, H-10), 2.70 (1H, dd, *J* = 13.0, 4.7 Hz, H-4), 2.82 (2H, m, H-9, 10), 4.25 (1H, dd, *J* = 12.6, 6.4 Hz, CH_2OH), 4.38 (1 H, dd, *J* = 12.6, 7.3 Hz, CH_2OH), 4.46 (1H, m, H-3), 4.87 (1H, m, H-1), 5.54 (1H, d, *J* = 15.3 Hz, H-24a, overlapped with H-22), 5.84 (2H, m, H-7, C=CH), 5.98 (1H, dd, *J* = 15.0, 10.3 Hz, H-23), 6.15 (1H, dd, *J* = 15.3, 10.3 Hz, H-24), 6.40 (1H, d, *J* = 11.1 Hz, H-6). MS *m*/*z* (%): 484 (M^+, 4), 466 (12), 448 (16), 430 (44), 412 (38), 279 (35), 263 (32), 149 (100), 133 (39), 93 (42). HRMS *m*/*z*: 484.3561 (Calcd for $C_{31}H_{48}O_4$: 484.3553).

4.34. Vitamin D receptor-binding assay

The rat 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 supernatants were diluted—1000 times in 50 mM Tris buffer (100 mM KCl, 5 mM DTT, and 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 2–3 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 assays

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 or pSG5-hVDR (Δ165-215)], and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) by the lipofection method as described previously.[34] 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 1,25-$(OH)_2D_3$, 19-nor-vitamin 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.

Supplementary data

Experimental details for preparation of compounds **10**–**27** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.01.061.

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