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Bioorganic & Medicinal Chemistry 16 (2008) 1796–1815
Bioorganic & Medicinal Chemistry

Synthesis and structure–activity relationships of 16-ene-22-thia-1α,25-dihydroxy-26,27-dimethyl-19-norvitamin D_3 analogs having side chains of different sizes

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Received 8 October 2007; revised 31 October 2007; accepted 2 November 2007
Available online 6 November 2007

Abstract—We have synthesized eight novel 16-ene-22-thia-26,27-dimethyl-19-norvitamin D_3 analogs **1**–**5** bearing side chains of different sizes, in combination with 20*S*- and 20*R*-isomers. The target compounds were prepared by Wittig-Horner reaction of A-ring phosphine oxide with 16-ene-22-thia-25-hydroxy Grundmann's ketones having different sized side chains, which were derived from the S-phenyloxycarbonyl derivative **13** as key intermediates. The binding affinity to the vitamin D receptor (VDR), VDR-mediated transcriptional activity, and osteoclast-inducing activity of synthetic 22-thia-19-norvitamin D analogs **1**–**5** were investigated. The (20*S*)-22-thia-19,24-dinorvitamin D analog **1a** is as active as the natural hormone 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) in terms of biological activities tested in vitro. The analogs **2a** and **3a** exhibited almost the same potency as 1α,25-$(OH)_2D_3$ in binding to the VDR, were about 20 times more potent than 1α,25-$(OH)_2D_3$ in terms of transcriptional activity, and **3a** was approximately 100 times as potent as 1α,25-$(OH)_2D_3$ in eliciting osteoclast formation. The biological activities of (20*S*)-22-thia compounds were more potent (by more than 10-fold) than those of the corresponding 20*R*-counterparts, but the activity of (20*R*)-compounds **1b**, **2b**, and **3b** in stimulating the formation of osteoclasts was similar to that of 1α,25-$(OH)_2D_3$, and the 24-dihomo- and trihomo-analogs **4a** and **5a** showed low transcriptional activity. These results suggest that elongation of the side chain in 22-thia analogs by up to one carbon can be stably accommodated in the VDR ligand binding pocket.

1. Introduction

Vitamin D_3, originally discovered as an antirachitic factor, is converted in the liver to 25-hydroxyvitamin D_3 and then further hydroxylated in the kidney to an active metabolite, 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$]. Currently it is well recognized that 1α,25-$(OH)_2D_3$ is an important hormone in the regulation of calcium and phosphorus homeostasis and bone mineralization.[1] In addition, 1α,25-$(OH)_2D_3$ plays a role in controlling differentiation and growth inhibition of a variety of cell types including malignant cells and keratinocytes, and has immunomodulatory activity on B and T cells.[1,2]

1α,25-$(OH)_2D_3$ has been used for the treatment of osteoporosis, rickets, secondary hyperparathyroidism, psoriasis, and renal osteodystrophy. However, application of 1α,25-$(OH)_2D_3$ as an anticancer drug induces significant hypercalcemia. Analogs having weak calcemic activity while retaining strong cell differentiation and antiprolifer-

Keywords: 16-Ene-22-thia-19-norvitamin D; VDR binding affinity; Transcriptional activity; Osteoclast formation; Structure–activity relationships.
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doi:10.1016/j.bmc.2007.11.013

ation activities have been developed, and a number of 1α,25-$(OH)_2D_3$ analogs have been synthesized.[3–5] Analogs exhibiting lower calcemic activity while retaining or showing improved cell differentiation activity have certain structural characteristics: (1) deletion of the 19-exomethylene group,[6] (2) introduction of heteroatoms such as oxygen or sulfur at the C-22 position,[3,7,8] and (3) incorporation of a double bond at the C-16 or C-22 position.[5,9] Among them, 1α,25-dihydroxy-22-oxavitamin D_3 (maxacalcitol)[3] and 1α,25-dihydroxy-19-norvitamin D_2 (paricalcitol)[10] have been shown to have desirably low calcemic activity, and have been developed for clinical treatment of psoriasis and secondary hyperparathyroidism. On the other hand, structural modifications enhancing the transcriptional activity and cell differentiation activity include inversion of stereochemistry at the C-20 position and homologation at C-24 or at C-26 and C-27.[11,12] KH1060 shows extremely strong transcriptional activity and dramatically enhanced cell differentiation potency in comparison with 1α,25-$(OH)_2D_3$.[13] It is also reported that 22-oxavitamin D analogs with a 16,17-double bond is susceptible to oxidation due to a newly generated allylic center at C-20, and are assumed to exhibit reduced calcemic activity due to faster oxidative metabolism in vivo.[14,15]

We have reported that structural modifications at the C-2 position of the A-ring, or on both the A-ring and the side chain, produced more than 100 synthetic 19-norvitamin D_3 analogs, some of which have interesting biological profiles.[16–22] Among them, we found that (20*S*)-2α-methyl-2β-hydroxy-1α,25-dihydroxy-19- norvitamin D_3 showed equivalent binding affinity to the VDR and ligand-dependent transcriptional activity (30 fold) compared with the natural ligand 1α,25-$(OH)_2D_3$.[18,20,22] As a continuation of our studies of structural modifications of the A-ring and/or the side chain and the structure–activity relationships (SAR) of 19-norvitamin D analogs, we have designed highly modified analogs of 1α,25-dihydroxy-19-norvitamin D_3 containing an anticalcemic 16,17-double bond and a 22-thia group, and also a powerful antiproliferative 26,27-dimethyl group. We also became interested in the effect of the anticalcemic and antiproliferative groups, the length of the side chain, and the stereochemistry of C-20 center on the biological activities of the 19-norvitamin D analogs.

We report herein the synthesis of 16-ene-22-thia-1α,25-dihydroxy-26,27-dimethyl-19-norvitamin D analogs **1**–**5** using a strategy based on a convergent approach using the Wittig-Horner reaction of the A-ring phosphine oxide with the 25-hydroxy-Grundmann's ketones (Fig. 1). We also examine the biological activity of the synthetic 22-thia-19-nor derivatives on the affinity of binding to the VDR, in activating gene transcription, and effects on osteoclast formation.

2. Results and discussion

2.1. Chemistry

The synthesis of the 16-ene-22-thia-19-norvitamin D analogs **1**–**5** bearing different-sized side chains was carried out employing Wittig-Horner coupling of 22-thia-C/D-ring Grundmann's ketones with the A-ring phosphine oxide in a convergent manner. The C/D-ring synthons (**26**, **39**–**42**) with a normal- or epi-configuration (*S*- or *R*-) at the C-20 position (steroid numbering) were prepared via key reaction of *O*-phenyl chlorothionoformate with the 16-alcohol **10** or **12**, which was derived from the known 20-keto derivative **6**,[23] as illustrated in Figures 2 and 3.

The 20-oxo compound **6**, which was obtained from vitamin D_2 in five steps according to published procedures,[23] was reduced with sodium borohydride ($NaBH_4$) to obtain epimeric alcohols **7a** and **7b** at C-20 in a ratio of ca. 8:1.[24,25] The reduction of **6** with metallic potassium by refluxing with 2-propanol afforded a slight excess of (20*S*)-alcohol **7b** relative to (20*R*)-**7a** (**7a**:**7b** = ca. 4:5).[26,27] These alcohols were separated by column chromatography. Dehydration of **7** is dependent on the configuration of the C-20 center. Treatment of (20*R*)-**7a** with $POCl_3$ in pyridine proceeded stereospecifically to provide the known 17(20)*Z*-ethylidene derivative **8**,[25,28] whereas similar treatment of (20*S*)-**7b** gave the *E*-ethylidene **9**. Comparison with an authentic **8** on the basis of spectral data confirmed the designated 17(20)*Z*-ethylidene structure. Stereoselective hydroxylation of the *Z*-ethylidene **8** with selenium dioxide in the presence of *tert*-butyl hydroperoxide (TBHP) under Sharpless conditions afforded an allylic 16α-hydroxyl (OH) compound **10**.[29,30] Oxidation of **10** under Swern conditions gave the corresponding 16-keto compound **11**, which upon reduction with $NaBH_4$ afforded a mixture of allylic alcohols in which the 16β-OH isomer **12** predominated (**10**:**12** = ca. 1:8).[29] The stereochemistry of **10** was unambiguously assigned on the basis of NMR spectroscopy and mechanistic considerations of compounds with similar structural features. The 18-methyl group *cis* to the 16β-OH moiety appeared at 0.17 ppm lower field than the 18-methyl *trans* to the 16α-OH group. It is well known that an axial proton resonates at a higher field than the equatorial counterpart. Upfield shift of an axial 16α-proton in **12** by ca. 0.09 ppm provides supporting evidence for the stereochemistry. Introduction of both the 16,17-double bond and side chains bearing a sulfur atom at the C-22 position was accomplished by employing an efficient method described by the Chugai group.[7] Reaction of the16α-alcohol **10** with *O*-phenyl chlorothionoformate resulted in formation of *O*-phenyl thiocarbonate, which underwent [3,3]-sigmatropic rearrangement to afford exclusively a homoallylic (20*S*)-S-phenyloxycarbonyl derivative **13a** as a sole product,[7,31] whereas under the same conditions the epimeric 16β-alcohol **12** was converted stereoselectively to the 20-*epi* compound **13b**. This reaction is highly dependent on the configuration of the OH group at the C-16 center. In the reaction of the 16α-alcohol **10** with PhOC(S)Cl, initially formation of a 16α-thiocarbonate, followed by a [3,3]-shift, would take place through the less hindered α-face to produce (20*S*)-S-phenyloxycarbonyl derivative **13a**. 16-Ene-22-thia-C/D-ring ketones **26**, **39**–**42** having side chains of different sizes were synthesized by elongation reaction of the thiol carbonates **13** with bromoester

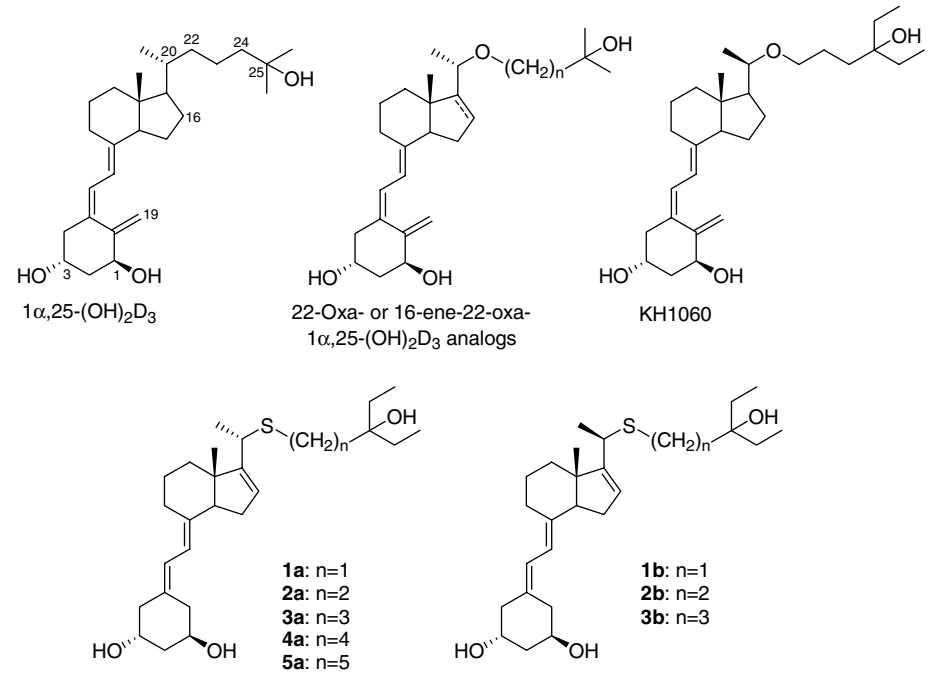

Figure 1. The structures of $1\alpha,25\text{-}(OH)_2D_3$ and its analogs.

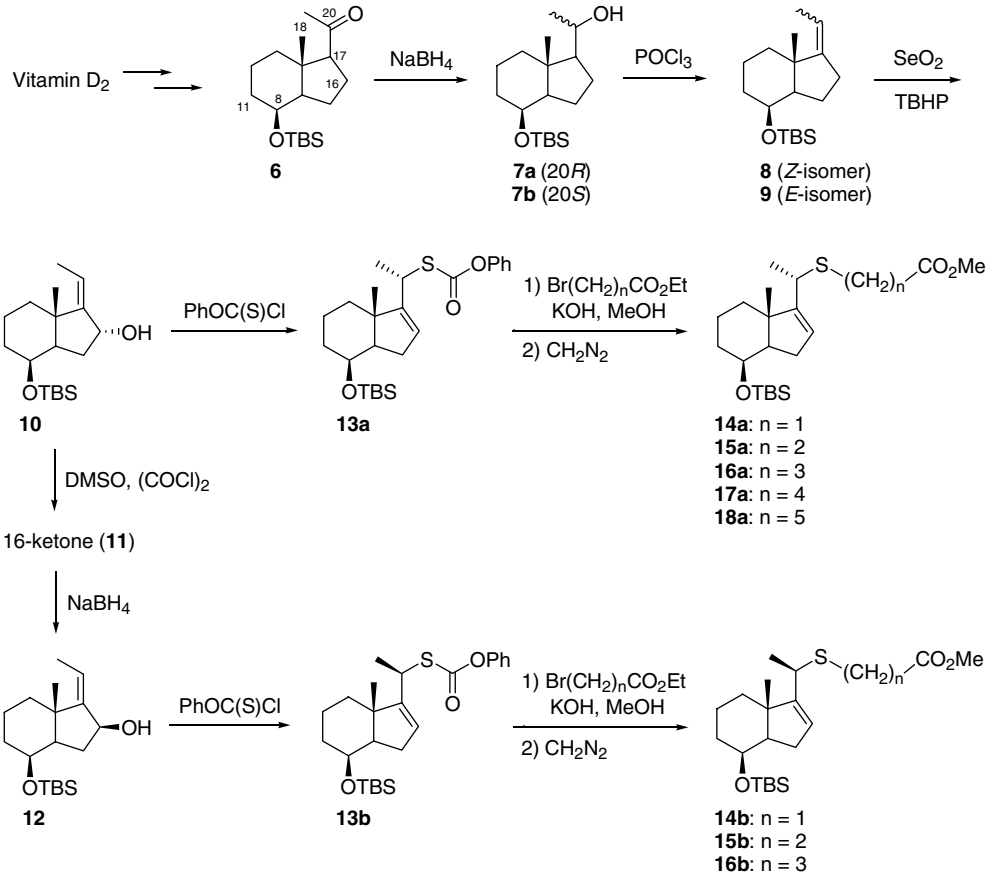

Figure 2. Synthesis of 16-ene-sulfanyl-carboxylic acid methyl esters.

derivatives bearing alkyl chains of different lengths. The reaction of **13a** with five bromoesters $Br(CH_2)_nCOOEt$ (n = 1–5) under alkaline solvolysis conditions, and subsequent methylation by diazomethane, gave sulfanyl carboxylic acid methyl esters **14a**–**18a** in good yields. The 20*R*-epimers **14b**–**16b** were prepared from **13b** in a

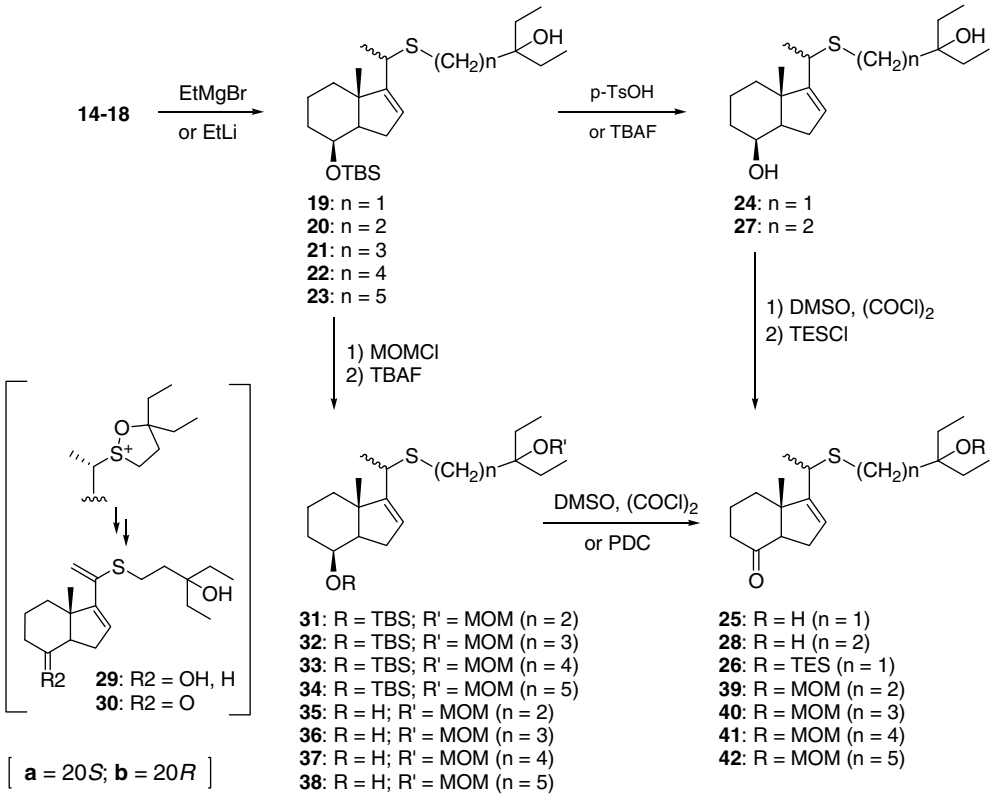

Figure 3. Synthesis of 16-ene-22-thia-C/D ring ketones.

synthetic sequence similar to that of the (20*S*)-counterparts.

The (20*S*)-methyl ester **14a** ($n = 1$) was treated with ethyl magnesiumbromide, affording the tertiary alcohol **19a**, which was deprotected with *para*-toluenesulfonic acid (*p*-TsOH) or tetrabutylammonium fluoride (TBAF) to provide the diol **24a**. The oxidation of **24a** under Swern conditions, followed by protection of the 25-hydroxyl group with chlorotriethylsilane (TESCl), gave the C/D-ring synthon **26a**. Since more than 20% of a mono-ethylated ketone was recovered after ethylation of **14** by Grignard reagent, we employed ethyllithium (EtLi) instead of EtMgBr in the following reaction. Ethylation of **15a** ($n = 2$) with freshly prepared EtLi afforded **20a** in an excellent yield, and removal of the *tert*-butyldimethyl silyl (TBS) protecting group of **20a** by TBAF gave **27a**. The Swern oxidation of **27a** was unsuccessful, leading to unexpected diene compounds **29** and **30**, and the desired ketone **28a** was not produced. Although the exact mechanism responsible for generating the unwanted dienes is unclear, they may be produced via the five-membered ring oxosulfonium ion as an intermediate, resulting in the intramolecular cyclization reaction of the sulfur atom to the activated tertiary alcohol group by treatment of **27a** with DMSO/$(COCl)_2$ (Fig. 3). It was considered that the side reaction would be avoided by protecting the free 25-OH group in **27**. The 25-OH group in **20a**–**23a** was protected by chloromethyl methyl ether (MOMCl), affording methoxymethyl (MOM) ethers **31a**–**34a**, which upon deprotection of the silyl ether group by TBAF yielded the C(8)-alcohols **35a**–**38a**. Oxidation of **35a**–**38a** under Swern conditions or by pyridinium dichromate (PDC) gave the C/D-ring kentones **39a**–**42a**. The (20*R*)-methyl esters **14b**–**16b** were converted to the corresponding C/D-ring ketones **26b**, **39b**, and **40b** utilizing the same sequence of reactions as described for the 20*S*-series compounds.

The synthesis of 16-ene-22-thia-19-norvitamin D analogs **1**–**5** bearing side chains of different sizes was carried out by Wittig-Horner reaction as shown in Figure 4: the coupling of C/D-ring ketones **26**, **39**–**42** with the conjugate base of A-ring allylic phosphine oxide **43** obtained by the reported method,[32] followed by cleavage of both silyl and MOM ether groups with camphor sulfonic acid (CSA), gave the desired compounds **1**–**5** with a 20*S*- or 20*R*-configuration.

2.2. Biological activity

We have synthesized eight new 22-thia-19-norvitamin D analogs with high structural modifications in both the D-ring and the side chain. The side chains of these analogs have the sulfur atom at C-22, methylation at C-26 and C-27, and different-sized side chains in combination with epimerization at C-20. The biological activity of these analogs **1**–**5** was evaluated in vitro.

We first tested the binding affinity for the recombinant rat VDR ligand-binding domain (LBD)[33] and VDR-mediated transcriptional activity in COS-7 cells. The results are summarized in Table 1 and the activities were shown as percentages of that of the natural hormone,

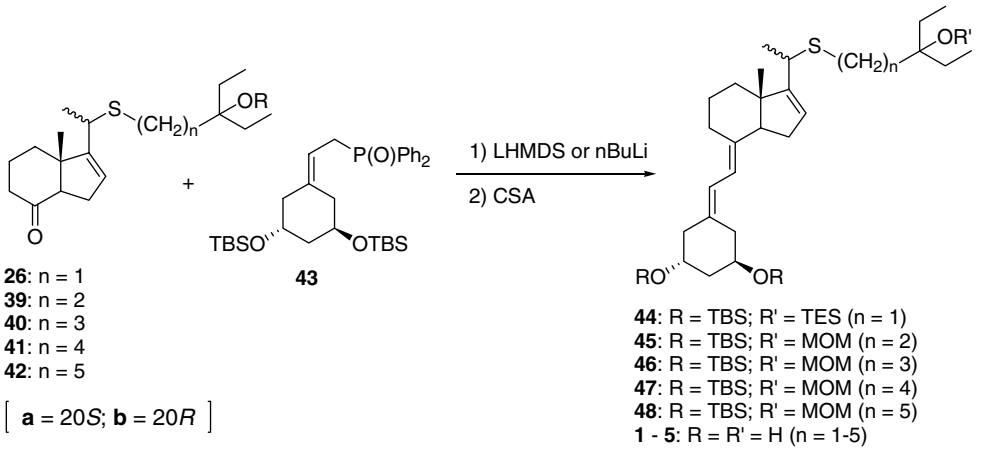

Figure 4. Synthesis of 16-ene-22-thia-19-norvitamin D_3 analogs bearing side chains of different sizes.

1α,25-$(OH)_2D_3$. The amino acid sequence of rat VDR LBD shows approximately 93% similarity with that of human VDR LBD, and the rat VDR is four amino acids shorter than the human counterpart. Rat VDR LBD binds the natural hormone with similar affinity to that of the full-length VDR. In the evaluation of VDR-binding affinity, the length of the side chain in pairs of three analogs **1**–**3** did not influence the affinity. The stereochemistry at C-20 appeared critical: the analogs **1a**–**3a** with a 20*S*-configuration showed binding potency similar to that of 1α,25-$(OH)_2D_3$, and the 20*R*-epimers **1b**–**3b** were 10- to 20-fold less potent than the corresponding 20*S*-epimers. The VDR-mediated transcriptional activity of the eight 22-thia-19-norvitamin D analogs was tested by luciferase assay with the mouse osteopontin vitamin D response element (VDRE) in COS-7 cells. The relative activities of new analogs **1**–**5** were assessed in terms of the ED_{50} values calculated from their dose-response curves. Among the compounds bearing 20*S*-configurations, the 24-nor analog **1a** was comparable in potency to the natural ligand. The analog **2a**, with a normal-sized side chain (containing three atoms between the C-20 and C-25 positions) and carrying a 26,27-dimethyl group, and the 24-homo analog **3a** were approximately 20 times more active than 1α,25-$(OH)_2D_3$ in stimulating transcriptional activity. The 24-dihomo- and 24-trihomo analogs **4a** and **5a** displayed markedly (50- to 100-fold) decreased transcriptional activity. Epimerization at the C-20 position decreased transcriptional potency relative to the 20*S*-epimers 10- to 20-fold in accordance with the effect on VDR-binding affinity.[19] It should be noted that (20*R*)-22-thia-1α,25-dihydroxyvitamin D_3 analogs without the 16,17-double bond have high biological activities compared with their 20*S*-counterparts.[8] The 24-nor analog **1a** showed almost equivalent activity with respect to the natural ligand in terms of VDR binding affinity and transcriptional activity. It is reported that deletion of the methylene group of 1α,25-$(OH)_2D_3$ in the side chain results in about a 10-fold reduction of HL-60 differentiating potency.[34] This might be due to the lack of hydrogen bonds between the terminal 25-OH group and the amino acids lining the ligand binding pocket (LBP).[35,36] The preserved biological activities of 22-thia-24-norvitamin D **1a** may reflect the hydrogen bond of the terminal 25-OH moiety with histidine 305 (His305) and His397 due to the sulfur atom with a large van der Waals radius. The X-ray crystallographic data for human or rat VDR LBD complexed with 1α,25-$(OH)_2D_3$ and its analogs including the 20-epimers revealed that the 25-OH groups contact the same amino acid residues His305 and His397 within the LBP.[35–37] This interaction is crucial for stimulating VDR responses. Elimination of the 25-OH group lowers the VDR binding affinity to approximately 1/1000,[38] and it is well known that hereditary vitamin D-resistant rickets (HVDRR) is caused by a missense mutation of VDR which results in the replacement of histidine with glutamine (Gln) at amino acid 305 (His305Gln VDR).[39] Quite recently, we succeeded in determining the crystal structure of rat VDR LBD in complex with an analog of **1a** having a substituent at the A-ring (data not shown).[31] Analysis of the crystal structure of the rat VDR LBD/ligand complex revealed contacts of the 25-OH group of the ligand with the LBP-lining amino acid residues His305 and His397: the distances between the oxygen atoms and His305 and His397 were 2.65 and 2.82 Å, respectively. The 22-thia-analogs **2a** and **3a** are about 20 times more effective than 1α,25-$(OH)_2D_3$. Elongation of the side chain by up to two carbons compared with 1α,25-$(OH)_2D_3$ can be accommodated in the LBP, forming hydrogen bonds with the same two histidines, and increases the hydrophobic interactions with the amino acid residue lining the LBP.[40] Methylation at C-26 and C-27 has similar effects to this elongation.[12] The extremely lower transcriptional activity of the 24-dihomo- and 24-trihomo-analogs **4a** and **5a** might be explained by interference with formation of the active conformation of the VDR/ligand complex due to the longer side chains containing the large sulfur atom at C-22. Upon ligand binding in the VDR LBP, the VDR changes its conformation to the transcriptionally active form, in which helix 12 (H12) located in the C-terminal region seals the LBP and forms a transactivation function 2 (AF-2) surface.[41,42] Coactivator proteins bind this hydrophobic AF-2 surface through the leucine (Leu) x x Leu Leu (LxxLL, x = any amino acids) motif in their nuclear receptor interacting domain.[36,44] It is known for the

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

Compound	VDR affinity	Transcription
1α,25-$(OH)_2D_3$	100	100[b]
1a	60	104[b]
1b	6	9[b]
2a	100	2000[b]
2b	4	27[b]
3a	100	2000[b]
3b	8	20[b]
4a	N.D.	1[b]
5a	N.D	2[b]

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

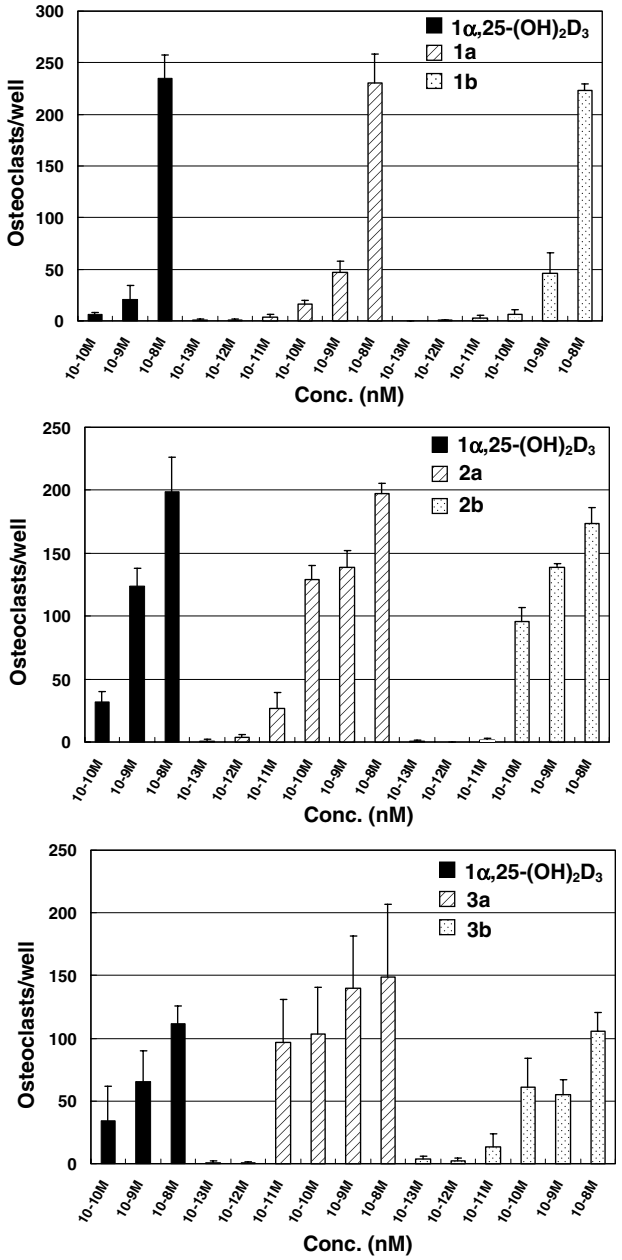

Figure 5. Effects of 1α,25-$(OH)_2D_3$ and its derivatives on osteoclast formation in co-culture.

estrogen receptor that when ligands bearing a bulky side chain are accommodated in the LBP, H-12 cannot adopt the active conformation. These ligands act as antagonists.[43–45]

Next, we examined the effects of 22-thia-19-norvitamin D analogs **1**–**3** on osteoclast formation in a mouse co-culture system, and the results are shown in Figure 5. Treatment of the culture with the 22-thia analogs resulted in a dose-dependent increase in the number of osteoclasts formed. 1α,25-$(OH)_2D_3$ and 22-thia analogs **1**–**3** induced multinucleated, tartrate-resistant acid phosphatase (TRAP, a marker enzyme of osteoclasts)-positive cells typical of osteoclasts. 1α,25-$(OH)_2D_3$ and its analogs caused a dose-dependent increase in the number of osteoclasts. The 22-thia-24-nor analogs **1a** and **1b** showed similar activity to 1α,25-$(OH)_2D_3$ regardless of the configuration of the C-20 center. Analogs **2a** and **2b** strongly stimulated osteoclast formation at 10^{-10} M and were more active than the natural ligand. The 24-homo analog **3a** was more than 10 times as potent as 1α,25-$(OH)_2D_3$ in stimulating osteoclast formation, whereas its 20-epimer **3b** exhibited almost the same activity as the natural hormone. Effects of (20*S*)-22-thia analogs on osteoclast-inducing activity correlate with the transcriptional activities. The transcriptional activity of analogs **1b**, **2b**, and **3b** having the 20*R*-configuration was about 10% as potent as that of their corresponding counterparts, and quite different from their effects on osteoclastogenesis.

3. Conclusion

The synthesis of newly designed hybrid analogs having the sulfur atom at C-22, the 16,17-double bond, 26,27-dimethyl groups, and different-sized side chains, has been achieved successfully. The isomeric pairs, (20*S*)- and (20*R*)-22-thia-19-norvitamin D analogs **1**–**3**, showed distinct biological profiles, and the 20*S*-isomers were more active than the 20*R*-isomers in terms of VDR binding and transcriptional activity. 20*S*-Isomers were comparable in potency to 1α,25-$(OH)_2D_3$ in terms of binding affinity for the VDR, and 1-20 times more active than 1α,25-$(OH)_2D_3$ in stimulating transcriptional activity. The most promising analog is **3a**, in which incorporation of a sulfur atom at C-22 is combined with homologation at C-24, C-26 and C-27. We have demonstrated some synthetic 19-norvitamin D analogs with various substituents at the C-2 position, and among them, 2β-hydroxyethoxy-, or 2α-methyl-2β-hydroxy-19-norvitamin D derivatives have powerful biological activities. The synthesis of the current 22-thia analogs in combination with C-2 substituents, which is expected to yield some interesting biological properties, is now in progress.

4. Experimental

^{1}H 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) down-

field from tetramethylsilane as an internal standard (δ 0 ppm) for ^{1}H NMR. Abbreviations used are: singlet (s), doublet (d), triplet (t), multiplet (m), broad signal (br). Low- and high-resolution mass spectra (LR-MS and HR-MS) 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 was separated by HPLC equipped with a Model PU-980 pump, a Rheodyne Model 7125 injector, and a Model MD-910 multiwavelength UV detector from JASCO. Column chromatography was carried out on silica gel (Wako Pure Chem. Ind. Ltd. Wakogel C-200). All reactions were conducted under an atmosphere of argon gas. Yields were not optimized.

4.1. (20*R*)- and (20*S*)-Alcohols (7a) and (7b)

4.1.1. Method I. To a stirred, ice-cooled solution of **6** (1.90 g, 6.118 mmol) in methanol (MeOH, 20 mL) was added sodium borohydride ($NaBH_4$, 231.9 mg, 6.130 mmol). After being stirred for 1 h at the same temperature, the mixture was poured into ice-water and extracted with AcOEt. The organic phase was washed with brine, dried over anhydrous magnesium sulfate ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on silica gel (70 g) with 2% AcOEt in hexane to give (20*R*)-**7a** (1.67 g) and (20*S*)-**7b** (208 mg) in a total yield of 98% (ca. **7a**:**7b** = 8:1).

4.1.2. Method II. A solution of **6** (750.5 mg, 2.42 mmol) in anhydrous 2-propanol (2-PrOH, 65 mL) was stirred under reflux and metallic potassium (6.6 g, 169 mmol) was added in small pieces in a period of 0.5 h, and the mixture was refluxed for another 1 h. The mixture was cooled to room temperature, poured into ice-water, and extracted with AcOEt. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel with 2% AcOEt in hexane to afford (20*R*)-**7a** (293.3 mg, 39%) and (20*S*)-**7b** (357.6 mg, 47%). (ca. **7a**:**7b** = 4:5).

(20*R*)-**7a** ^{1}H NMR ($CDCl_3$) δ: −0.003, 0.01 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.01 (3H, s, H-18), 1.12 (3H, d, J = 6.1 Hz, H-21), 3.73 (1H, m, H-20), 4.01 (1H, m, H-8). MS *m*/*z* (%): 312 (M^+, 1), 255 (19), 237 (100), 161 (38), 75 (72). HRMS *m*/*z*: 310.2487 (Calcd for $C_{18}H_{36}O_2Si$: 312.2485). (20*S*)-**7b** ^{1}H NMR ($CDCl_3$) δ: −0.003, 0.01 (each 3H, s, 2× Si–Me), 0.88 (9H, s, Si-*t*-Bu), 0.91 (3H, s, H-18), 1.20 (3 H, d, J = 6.2 Hz, H-21), 3.68 (1H, m, H-20), 4.02 (1H, m, H-8). MS *m*/*z* (%): 312 (M^+, 1), 255 (5), 237 (14), 161 (18), 75 (100). HRMS *m*/*z*: 310.2470 (Calcd for $C_{18}H_{36}O_2Si$: 312.2485).

4.2. 17(20) *Z*-Ethylidene (8)

To a stirred, ice-cooled solution of **7a** (1.33 g, 4.281 mmol) in anhydrous pyridine (15 mL) was added phosphoryl chloride ($POCl_3$, 0.8 mL, 8.583 mmol). The mixture was stirred for 1 h at 0 °C and for 21 h at ambient temperature, poured into ice-water. The whole mixture was acidified with 2 N HCl and extracted with AcOEt. The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (30 g) with hexane to give **8** (975.6 mg, 78%).

^{1}H NMR ($CDCl_3$) δ: 0.01, 0.02 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.11 (3H, s, H-18), 1.64 (3H, dt, J = 7.1, 2.0 Hz, H-21), 4.07 (1H, m, H-18), 5.04 (1H, qt, J = 7.1, 2.0 Hz, H-20). ^{13}C NMR ($CDCl_3$) δ: −4.9, −4.6, 13.2, 18.1, 18.2, 19.8, 24.0, 26.0 (3×), 30.6, 34.6, 38.3, 44.4, 52.9, 69.9, 112.5, 151.1. MS *m*/*z* (%): 294 (M^+, 6), 279 (4), 237 (100), 163 (20), 162 (60), 161 (85), 75 (75). HRMS *m*/*z* 294.2385 (Calcd for $C_{18}H_{34}OSi$: 294.2379).

4.3. 17(20)*E*-Ethylidene (9)

Following the same procedure as described above, treatment of **7b** (453.1 mg, 1.45 mmol) with $POCl_3$ (337.8 μL, 3.62 mmol) gave **9** (402.3 mg, 94%).

^{1}H NMR ($CDCl_3$) δ: 0.02 (6H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 0.98 (3H, s, H-18), 1.52 (3H, d, J = 6.7 Hz, H-21), 4.07 (1H, m, H-8), 4.97 (1H, qt, J = 6.7, 2.5 Hz, H-20). MS *m*/*z* (%): 294 (M^+, (3), 279 (3), 237 (100), 163 (18), 162 (41), 161 (76), 75 (76).

4.4. 17(20)*E*-Ethylidene-16α-ol (10)

To a stirred suspension of selenium dioxide (177.5 mg, 1.600 mmol) in methylene chloride (CH_2Cl_2, 5 mL) at 0 °C was added dropwise 70 wt % *tert*-butyl hydroperoxide in H_2O (552 μL, 5.696 mmol). Stirring was continued for 1 h, and a solution of **8** (942.3 mg, 3.199 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was stirred for 4 h at ambient temperature and poured into ice-water with 10% sodium hydroxide (NaOH). After extraction with AcOEt, the organic phase was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography on silica gel (30 g) with 5% AcOEt in hexane to afford **10** (847.0 mg, 85%).

^{1}H NMR ($CDCl_3$) δ: 0.01, 0.02 (each 3H, s, 2× Si–Me), 0.88 (9H, s, Si-*t*-Bu), 1.10 (3H, s, H-18), 1.72 (3H, dd, J = 7.2, 1.0 Hz, H-21), 4.10 (1H, m, H-8), 4.44 (1H, m, H-16), 5.51 (1H, qd, J = 7.2, 1.2 Hz, H-20). MS *m*/*z* (%): 310 (M^+, 5), 292 (5), 253 (37), 235 (100), 178 (24), 161 (89), 160 (75). HRMS *m*/*z*: 310.2337 (Calcd for $C_{18}H_{34}O_2Si$: 310.2328).

4.5. 17(20)*E*-Ethylidene-16-one (11)

To a stirred solution of oxalyl chloride [$(COCl)_2$, 314 μL, 3.599 mmol] in dry CH_2Cl_2 (3 mL) at −78 °C was added dimethylsulfoxide (DMSO, 509 μL, 7.173 mmol) in dry CH_2Cl_2 (1 mL). After being stirred for 10 min, a solution of **10** (845.6 mg, 2.723 mmol) in dry CH_2Cl_2 (7 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 15 min, and triethylamine (Et_3N, 2.09 mL, 14.995 mmol) was added. The

entire mixture was stirred at −78 °C for 10 min, and at 0 °C for 30 min, quenched with ice water, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, and dried over anhydrous $MgSO_4$, solvent was evaporated in vacuo, and. the residue was chromatographed on silica gel (25 g) with 5% AcOEt in hexane to give **11** (792.8 mg, 94%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.05 (each 3H, s, 2× Si–Me), 0.90 (9H, s, Si-*t*-Bu), 1.25 (3H, s, H-18), 1.83 (3H, d, J = 7.5 Hz, H-21), 4.13 (1H, m, H-8), 6.43 (1H, q, J = 7.5 Hz, H-20). ^{13}C NMR ($CDCl_3$) δ: −4.9, −4.6, 13.3, 18.0, 20.6 (2×) 34.4, 35.0, 38.4, 49.3, 69.7, 73.7, 118.5. MS *m*/*z* (%): 308 (M^+, 12), 293 (3), 251 (100). HRMS *m*/*z*: 294.2165 (Calcd for $C_{18}H_{32}O_2Si$: 308.2172).

4.6. 17(20)*E*-Ethylidene-16β-ol (12)

To a solution of **11** (144.7 mg, 0.469 mmol) in MeOH (1 mL) at 0 °C were successively added cerium (III) chloride heptahydrate (17.5 mg, 0.047 mmol) and $NaBH_4$ (17.7 mg, 0.469 mmol). After being stirred at 0 °C for 30 min, the mixture was poured into ice-water and extracted with AcOEt. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (Wacogel C-300, 5 g) with 2% AcOEt in hexane to give **10** (16.7 mg) and **12** (126.4 mg) in a total yield of 98%.

^{1}H NMR ($CDCl_3$) δ: 0.02 (6H, s, 2× Si–Me), 0.90 (9H, s, Si-*t*-Bu), 1.27 (3H, s, H-18), 1.71 (3H, dd, J = 7.2, 1.6 Hz, H-21), 4.04 (1H, m, H-8), 4.35 (1H, m, H-16), 5.44 (1 H, qd, J = 7.2, 1.6 Hz, H-20). ^{13}C NMR ($CDCl_3$) δ: −4.9, −4.6, 13.3, 18.1, 18.2, 21.0, 26.0 (3×), 34.4, 34.6, 37.9, 44.2, 48.6, 69.2, 75.0, 118.2, 151.5. MS *m*/*z* (%): 310 (M^+, 5), 292 (9), 253 (17), 235 (51), 178 (11), 161 (100), 160 (28). HRMS *m*/*z*: 310.2299 (Calcd for $C_{18}H_{34}O_2Si$: 310.2328).

4.7. (20*S*)-16-Ene-thionocarbonic acid *O*-phenyl ester (13a)

To a stirred solution of **10** (630.2 mg, 2.029 mmol) in dry pyridine (6 mL) at 0 °C was added *O*-phenyl chlorothionoformate [PhOC(S)Cl, 337 μL, 2.436 mmol] and the mixture was stirred for 3.5 h. An additional PhOC(S)Cl (220 μL, 1.590 mmol) was added and stirring was continued for another 2.5 h at 0 °C. The reaction mixture was poured into ice-water containing 2 N HCl and then extracted with AcOEt. The AcOEt layer was washed with brine and dried over anhydrous $MgSO_4$. Solvent was evaporated to dryness, and the residue was chromatographed on silica gel (30 g) with 3% AcOEt in hexane to yield **13a** (772.5 mg, 85%).

^{1}H NMR ($CDCl_3$) δ: 0.026, 0.030 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.19 (3H, s, H-18), 1.54 (3H, d, J = 6.9 Hz, H-21), 2.31 (1H, m, H-15), 4.07 (1H, q, J = 6.9 Hz, H-20), 4.10 (1H, m, H-8), 5.71 (1H, m, H-16), 7.14–7.39 (5 H, m, arom H). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 18.1, 18.2, 19.7, 23.4, 26.0 (3×), 31.3, 34.7, 35.4, 38.9, 47.3, 55.3, 69.1, 121.6 (2×), 126.2, 126.9, 129.7 (2×), 151.4, 154.8, 170.3. MS *m*/*z* (%): 446 (M^+, 2), 431 (1), 389 (3), 292 (10), 235 (100), 161 (80), 160 (66). HRMS *m*/*z*: 446.2299 (Calcd for $C_{25}H_{38}O_3SSi$: 446.2311).

4.8. (20*R*)-16-Ene-thionocarbonic acid *O*-phenyl ester (13b)

Following the same procedure as mentioned above, treatment of **12** (289.2 mg, 0.931 mmol) with PhOC(S)Cl (258 μL, 1.865 mmol) gave **13b** (313.0 mg, 75%).

^{1}H NMR($CDCl_3$) δ: 0.026, 0.033 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.07 (3H, s, H-18), 1.61 (3H, d, J = 6.9 Hz, H-21), 4.04 (1H, q, J = 6.9 Hz, H-20), 4.10 (1H, m, H-8), 5.72 (1H, m, H-16), 7.14–7.40 (5H, m, arom-H). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 18.16, 18.22, 19.3, 22.9, 26.0 (3×), 31.2, 34.7, 35.5, 38.6, 47.0, 54.8, 69.1, 121.6 (2×), 126.2, 126.8, 129.7 (2×), 151.4, 153.7, 170.4. MS *m*/*z* (%): 446 (M^+, 3), 431 (1), 389 (4), 293 (17), 235 (13), 161 (100). HRMS *m*/*z*: 446.2318 (Calcd for $C_{25}H_{38}O_3SSi$: 446.2311).

4.9. (20*S*)-16-Ene-sulfanyl-acetic acid methyl ester (14a)

To a stirred solution of **13a** (740.5 mg, 1.658 mmol) in 10% KOH in MeOH (10 mL) at ambient temperature was added ethyl bromoacetate (0.55 mL, 4.978 mmol). After being stirred for 4 h, the reaction mixture was poured into ice-water containing 2 N HCl, and extracted with AcOEt. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The resulting residue was dissolved in CH_2Cl_2 (10 mL), cooled to 0 °C, and diazomethane in ether (about 0.5 M in ether, 10 mL) was added. Upon stirring for 30 min at the same temperature, solvent was evaporated in vacuo. The residue was chromatographed on silica gel (20 g) with 3% AcOEt in hexane to afford **14a** (661.0 mg, quantitative yield).

^{1}H NMR ($CDCl_3$) δ: 0.02 (6H, s, 2× Si–Me), 0.88 (9H, s, Si-*t*-Bu), 1.12 (3H, s, H-18), 1.36 (3H, d, J = 7.0 Hz, H-21), 2.28 (1H, m), 3.16 (2H, s, H-23), 3.58 (1H, q, J = 7.0 Hz, H-20), 3.71 (3H, s, OMe), 4.07 (1H, m, H-8), 5.64 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 18.1, 18.2, 19.3, 21.9, 26.0 (3×), 31.0, 33.1, 34.7, 35.7, 38.6, 46.8, 52.5, 55.5, 69.2, 125.9, 155.1, 171.6. MS *m*/*z* (%): 398 (M^+, 35), 383 (15), 341 (28), 309 (9), 292 (9), 235 (92), 161 (100), 160 (48). HRMS *m*/*z*: 398.2307 (Calcd for $C_{21}H_{38}O_3SSi$: 398.2311).

4.10. (20*R*)-16-Ene-sulfanyl-acetic acid methyl ester (14b)

Following the same procedure as described above, treatment of **13b** (345.3 mg, 0.773 mmol) with ethyl bromoacetate (258 μL, 2.327 mmol), followed by methylation with ethereal diazomethane, afforded **14b** (296.3 mg, 96%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.05 (3H, s, H-18), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.26 (1H, m), 3.16, 3.24 (each 1H, d, J = 14.7 Hz, H-23), 3.47 (1H, q, J = 7.0 Hz, H-20),

3.72 (3H, s, OMe), 4.07 (1H, m, H-8), 5.59 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 18.2 (2×), 19.6, 22.0, 26.0 (3×), 31.1, 32.5, 34.7, 35.6, 37.1, 47.0, 52.5, 54.8, 69.1, 125.4, 154.7, 171.1. MS *m*/*z* (%): 398 (M^+, 37), 383 (16), 341 (24), 292 (7), 235 (62), 161 (100), 160 (33). HRMS *m*/*z*: 398.2298 (Calcd for $C_{21}H_{38}O_3SSi$: 398.2311).

4.11. (20*S*)-16-Ene-sulfanyl-propionic acid methyl ester (15a)

Following the same procedure as described above, treatment of **13a** (331.9 mg, 0.763 mmol) with ethyl 3-bromopropionate (292 μL, 2.278 mmol), followed by methylation with ethereal diazomethane, afforded **15a** (262.4 mg, 86%).

^{1}H NMR ($CDCl_3$) δ: 0.01 (6H, s, 2× Si–Me), 0.88 (9H, s, Si-*t*-Bu), 1.12 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 2.27 (1H, m, H-15), 2.57 (2H, m, H-24), 2.68 (2H, m, H-23), 3.41 (1H, q, J = 7.0 Hz, H-20), 3.68 (3H, s, –OMe), 4.07 (1H, m, H-8), 5.61 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.7, 18.1, 18.2, 19.5, 22.3, 26.0 (3×), 26.2, 31.0, 34.69, 34.73, 35.7, 37.9, 46.8, 51.9, 55.5, 69.2, 125.3, 155.8, 172.8. MS *m*/*z* (%): 412 (M^+, 17), 355 (10), 292 (6), 235 (100), 161 (76), 160 (53). HRMS *m*/*z*: 412.2449 (Calcd for $C_{22}H_{40}O_3SSi$: 412.2467).

4.12. (20*R*)-16-Ene-sulfanyl-propionic acid methyl ester (15b)

Following the same procedure as described above, treatment of **13b** (207.2 mg, 0.464 mmol) with ethyl 3-bromopropionate (178 μL, 1.388 mmol), followed by methylation with ethereal diazomethane, afforded **15b** (142.2 mg, 74%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.04 (3H, s, H-18), 1.47 (3H, d, J = 7.0 Hz, H-21), 2.24 (1H, m, H-15), 2.58 (2H, m, H-24), 2.72 (2 H, m, H-23), 3.32 (1H, q, J = 7.0 Hz, H-20), 3.69 (3H, s, OMe), 4.08 (1H, m, H-8), 5.54 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 18.2 (2×), 19.6, 22.5, 25.5, 26.0 (3×), 31.0, 34.7, 35.0, 35.8, 36.5, 47.0, 51.9, 54.8, 69.2, 124.4, 155.4, 172.8. MS *m*/*z* (%): 412 (M^+, 8), 355 (5), 292 (3), 235 (100), 161 (82), 160 (63). HRMS *m*/*z*: 412.2457 (Calcd for $C_{22}H_{40}O_3SSi$: 412.2467).

4.13. (20*S*)-16-Ene-sulfanyl-butyric acid methyl ester (16a)

Following the same procedure as described above, treatment of **13a** (124.5 mg, 0.279 mmol) with ethyl 4-bromobutyrate (127 μL, 0.887 mmol), followed by methylation with ethereal diazomethane, afforded **16a** (118.5 mg, 99%).

^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 1.89 (2H, t, J = 7.2 Hz, H-24), 2.28 (1H, m, H-15), 2.43, 2.47 (each 2H, t, J = 7.2 Hz, H-23, 24a), 3.38 (1H, q, J = 7.0 Hz, H-20), 3.67 (3H, s, OMe), 4.08 (1H, m, H-8), 5.61 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.7, 18.11, 18.16, 19.4, 22.3, 24.8, 25.9 (3×), 30.4, 30.9, 33.2, 34.7, 35.7, 37.5, 46.7, 51.7, 55.5, 69.2, 125.0, 156.0, 173.8. MS *m*/*z* (%): 426 (M^+, 20), 411 (9), 369 (10), 292 (10), 235 (100), 161 (86), 160 (70). HRMS *m*/*z*: 426.2629 (Calcd for $C_{23}H_{42}O_3SSi$: 426.2624).

4.14. (20*R*)-16-Ene-sulfanyl-butyric acid methyl ester (16b)

Following the same procedure as described above, treatment of **13b** (331.2 mg, 0.741 mmol) with ethyl 4-bromobutyrate (338 μL, 2.362 mmol), followed by methylation with ethereal diazomethane, afforded **16b** (292.2 mg, 92%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.04 (3H, s, H-18), 1.44 (3H, d, J = 7.0 Hz, H-21), 2.24 (1H, m, H-15), 2.40–2.60 (4H, m, H-23, 24a), 3.30 (1H, q, J = 7.0 Hz, H-20), 3.67 (3H, s, OMe), 4.09 (1H, m, H-8), 5.53 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 18.2 (2×), 19.6, 22.5, 25.0, 26.0 (3×), 29.9, 31.1, 33.2, 34.8, 35.8, 36.3, 47.0, 51.8, 54.9, 69.2, 124.2, 155.7, 173.9. MS *m*/*z* (%): 426 (M^+, 24), 411 (11), 369 (9), 292 (5), 235 (70), 161 (100), 160 (44). HRMS *m*/*z*: 426.2617 (Calcd for $C_{23}H_{42}O_3SSi$: 426.2624).

4.15. (20*S*)-16-Ene-sulfanyl-pentanoic acid methyl ester (17a)

Following the same procedure as described above, treatment of **13a** (115.4 mg, 0.258 mmol) with ethyl 5-bromovalerate (122.7 μL, 0.775 mmol), followed by methylation with ethereal diazomethane, afforded **17a** (110.0 mg, 97%).

^{1}H NMR ($CDCl_3$) δ: 0.030 (6H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 2.32 (2H, t, J = 7.5 Hz, 24aa), 2.43 (2H, t, J = 7.2 Hz, H-23), 3.39 (1H, m, H-20), 3.67 (3H, s, –CO_2Me), 4.08 (1H, m, H-8), 5.61 (1H, m, H-16). MS *m*/*z* (%): 440 (M^+, 16), 383 (9), 292 (9), 235 (100), 161, (69), 160 (83). HRMS *m*/*z*: 440.2766 (Calcd for $C_{24}H_{44}O_3SSi$: 440.2780).

4.16. (20*S*)-16-Ene-sulfanyl-hexanoic acid methyl ester (18a)

Following the same procedure as described above, treatment of **13a** (82.7 mg, 0.185 mmol) with ethyl 6-bromohexanoate (99.8 μL, 0.555 mmol), followed by methylation with ethereal diazomethane, afforded **18a** (74.1 mg, 88%).

^{1}H NMR($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 2.29 (1H, m), 2.32 (2H, t,J = 7.5 Hz), 2.42 (2H, t, J = 7.3 Hz, H-23), 3.37 (1H, q, J = 7.0 Hz, H-20), 3.67 (3H, s, CO_2Me), 4.08 (1H, m, H-8), 5.61 (1H, m, H-16). MS *m*/*z* (%): 454 (M^+, 3), 439 (1), 397 (6), 365 (1), 292 (3), 235(61), 217 (9), 161 (50), 160 (47), 75 (100). HRMS *m*/*z*: 454.2928 (Calcd for $C_{25}H_{46}O_3SSi$: 454.2937).

4.17. (20*S*)-16-Ene-22-thia-24-nor-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (19a)

To a stirred solution of **14a** (661.0 mg, 1.658 mmol) in dry tetrahydrofuran (THF, 5 mL) at 0 °C was added ethylmagnesium bromide (EtMgBr, 4.97 mL, 4.974 mmol, 1.0 M solution in THF). After 3 h stirring, an additional EtMgBr (1.66 mL, 1.66 mmol) was added and the mixture was stirred for 2 h. The entire reaction mixture was poured into ice-water containing 2 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (20 g) with 2% AcOEt in hexane to afford **19a** (494.0 mg, 70%) and the ethyl-ketone (154.2 mg, 23%).

19a: ^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.87 (6H, t, J = 7.4 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.15 (3H, s, H-18), 1.38 (3H, d, J = 7.0 Hz, H-21), 2.56 (2H, s, H-23), 3.37 (1H, q, J = 7.0 Hz, H-20), 4.08 (1H, m, H-8), 5.63 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 8.2 (2×), 18.1, 18.2, 19.6, 22.7, 26.0 (3×), 31.0 (3×), 34.7, 35.8, 39.5, 41.6, 46.9, 55.5, 69.2, 74.0, 125.3, 156.3. MS *m*/*z* (%): 426 (M^+, 8), 408 (20), 393 (2), 351 (2), 325 (11), 309 (5), 292 (9), 235 (91), 217 (17), 193 (8), 161 (100), 160 (57). HRMS *m*/*z*: 426.2990 (Calcd for $C_{24}H_{46}O_2SSi$: 426.2988). **Ethyl-ketone**: ^{1}H NMR ($CDCl_3$) δ: 0.022, 0.024 (each 3H, s, 2× Si–Me), 0.89 (9H, s, Si-*t*-Bu), 1.08 (3H, t, J = 7.3 Hz, H-26), 1.12 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 2.61 (2H, m, H-25), 3.18 (2H, m, H-23), 3.39 (1H, q, J = 7.0 Hz, H-20), 4.08 (1H, m, H-8), 5.64 (1H, m, H-16). MS *m*/*z* (%): 396 (M^+, 35), 381 (6), 339 (8), 324 (32), 292 (9), 267 (37), 235 (73), 161 (100), 160 (52).

4.18. (20*R*)-16-Ene-22-thia-24-nor-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (19b)

Following the same procedure as described above, treatment of **14b** (355.7 mg, 0.892 mmol) with EtMgBr (2.68 mL, 2.68 mmol) afforded **19b** (264.9 mg, 70%) and the ethyl-ketone (97 mg, 27%).

19b: ^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.86, 0.88 (each 3H, t, J = 7.2 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.04 (3H, s, H-18), 1.45 (3H, d, J = 7.0 Hz, H-21), 2.57, 2.63 (each 1H, d,J = 12.7 Hz, H-23), 3.31 (1H, q, J = 7.0 Hz, H-20), 4.09 (1H, m, H-8), 5.55 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, 4.6, 8.22, 8.26, 18.2 (2×), 19.6, 22.7, 26.0 (3×), 31.0, 31.07, 31.11, 34.7, 35.9, 37.7, 40.6, 47.0, 54.8, 69.1, 73.8, 124.5, 155.6. MS *m*/*z* (%): 426 (M^+, 7), 408 (6), 351 (2), 325 (5), 309 (15), 292 (6), 235 (79), 217 (7), 193 (4), 161 (46), 160 (58), 75 (100). HRMS *m*/*z*: 426.2973 (Calcd for $C_{24}H_{46}O_2SSi$: 426.2988). Ethyl-ketone: ^{1}H NMR ($CDCl_3$) δ: 0.018, 0.024 (each 3H, s, 2× Si–Me), 0.88 (9H, s, Si-*t*-Bu), 1.08 (3H, t, J = 7.4 Hz, H-26), 1.03 (3H, s, H-18), 1.42 (3H, d, J = 6.9 Hz, H-21), 2.53–2.74 (2H, m, H-25), 3.18, 3.23 (each 1H, d, J = 14.0 Hz, H-23), 3.32 (1H, q, J = 6.9 Hz, H-20), 4.08 (1H, m, H-8), 5.58 (1H, m, H-16). MS *m*/*z* (%): 396 (M^+, 5), 339 (2), 324 (4), 292 (4), 267 (6), 235 (60), 161 (69), 160 (39), 75 (100).

4.19. (20*S*)-16-Ene-22-thia-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (20a)

To a stirred solution of **15a** (262.4 mg, 0.639 mmol) in dry THF (2 mL) at −78 °C was added ethyllithium (EtLi, 1.28 mL, 1.920 mmol, about 1.5 M solution in ether). After being stirred for 1 h, the reaction mixture was quenched with saturated ammonium chloride (NH_4Cl), poured into ice-water, and extracted with AcOEt. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (15 g) with 8% AcOEt in hexane to give **20a** (274.9 mg, 98%).

^{1}H NMR ($CDCl_3$) δ: 0.023, 0.025 (each 3H, s, 2× Si–Me), 0.856, 0.861 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.14 (3H, s, H-18), 1.37 (3H, d, J = 7.0 Hz, H-21), 1.69 (4H, m, H-26, 27), 2.29 (1H, m, H-15), 2.49 (2H, m, H-23), 3.43 (1H, q, J = 7.0 Hz, H-20), 4.08 (1H, m, H-8), 5.62 (1 H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.7, 7.96, 8.02, 18.1 (2×), 19.6, 22.3, 25.5, 25.9 (3×), 30.9, 31.1, 34.7, 35.7, 37.7, 37.8, 46.8, 55.5, 69.2, 74.9, 77.5, 125.3, 156.2. MS *m*/*z* (%): 440 (M^+, 9), 422 (10), 365 (3), 292 (5), 235 (100), 161 (82), 160 (61). HRMS *m*/*z*: 440.3146 (Calcd for $C_{25}H_{48}O_2SSi$: 440.3144).

4.20. (20*R*)-16-Ene-22-thia-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (20b)

Following the same procedure as described above, treatment of **15b** (142.2 mg, 0.346 mmol) with EtLi (0.69 mL, 1.035 mmol, 1.5 M solution in ether) afforded **20b** (141.1 mg, 93%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.86 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.05 (3H, s, H-18), 1.46 (3H, d, J = 6.9 Hz, H-21, overlapped with H-26, 27), 2.25 (1H, m, H-15), 2.51 (2H, m, H-23), 3.34 (1H, q, J = 6.9 Hz, H-20), 4.09 (1H, m, H-8), 5.54 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 8.0 (2×), 18.2, 19.6, 22.5, 24.8, 26.0 (3×), 31.04, 31.11, 31.12, 34.8, 35.9, 36.5, 38.0, 47.0, 54.9, 69.2, 75.0, 77.4, 124.2, 155.6. MS *m*/*z* (%): 440 (M^+, 15), 422 (11), 407 (2), 365 (3), 292 (3), 235 (42), 161 (100), 160 (30). HRMS *m*/*z*: 440.3157 (Calcd for $C_{25}H_{48}O_2SSi$: 440.3144).

4.21. (20*S*)-16-Ene-22-thia-24-homo-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (21a)

Following the same procedure as described above, treatment of **16a** (470.3 mg, 1.102 mmol) with EtLi (2.204 mL, 3.306 mmol, 1.5 M solution in ether) afforded **21a** (498.3 mg, 99%).

^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.86 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 1.46 (4H, q,

J = 7.5 Hz, H-26, 27), 2.28 (1H, m, H-15), 2.44 (2H, t, J = 7.0 Hz, H-23), 3.39 (1H, q, J = 7.0 Hz, H-20), 4.08 (1H, m, H-8), 5.62 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 7.97, 8.01, 18.2, 19.5, 22.5, 23.7, 26.0 (3×), 30.96, 31.17, 31.24, 31.9, 34.8, 35.8, 37.76, 37.84, 46.8, 55.5, 69.2, 74.7, 77.5, 124.9, 156.2. MS m/z (%): 454 (M^+, 8), 436 (5), 379 (2), 292 (5), 235 (100), 161 (100), 160 (65). HRMS m/z: 436.3214 (M^+-H_2O) (Calcd for $C_{26}H_{48}OSSi$: 436.3195).

4.22. (20*R*)-16-Ene-22-thia-24-homo-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (21b)

Following the same procedure as described above, treatment of **16b** (292.3 mg, 0.685 mmol) with EtLi (1.37 mL, 2.055 mmol, 1.5 M solution in ether) afforded **21b** (306.1 mg, 98%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.86 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.05 (3H, s, H-18), 1.44 (3H, d, J = 7.0 Hz, H-21), 1.45 (4H, q, J = 7.5 Hz, H-26, 27), 2.24 (1H, m, H-15), 2.47 (2H, m, H-23), 3.30 (1H, q, J = 7.0 Hz, H-20), 4.09 (1H, m, H-8), 5.53 (1 H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 8.0 (2×), 18.2, 19.6, 22.7, 23.9, 26.0 (3×), 31.05, 31.18, 31.24 (2×), 34.8, 35.9, 36.4, 37.9, 47.0, 54.9, 69.2, 74.7, 77.4, 124.0, 155.9. MS m/z (%): 454 (M^+, 7), 436 (3), 379 (2), 292 (5), 235 (100), 161 (73), 160 (65). HRMS m/z: 454.3325 (Calcd for $C_{26}H_{50}O_2SSi$: 454.3301).

4.23. (20*S*)-16-Ene-22-thia-24,24-dihomo-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (22a)

Following the same procedure as described above, treatment of **17a** (89.9 mg, 0.204 mmol) with EtLi (0.544 mL, 0.816 mmol, 1.5 M solution in ether) afforded **22a** (86.3 mg, 90%).

^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.85 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.36 (3H, d, J = 6.9 Hz, H-21), 1.46 (4H, q, J = 7.5 Hz, H-26, 27), 2.28 (1H, m), 2.44 (2H, t, J = 7.4 Hz, H-23), 3.38 (1H, q, J = 6.9 Hz, H-20), 4.08 (1H, m, H-8), 5.61 (1 H, m, H-16). MS m/z (%): 468 (M^+, 7), 450 (3), 393 (2), 292 (8), 235 (100), 161 (82), 160 (71). HRMS m/z: 468.3470 (Calcd for $C_{27}H_{52}OSSi$: 468.3457).

4.24. (20*S*)-16-Ene-22-thia-24,24,24-trihomo-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (23a)

Following the same procedure as described above, treatment of **18a** (144.8 mg, 0.318 mmol) with EtLi (0.849 mL, 1.274 mmol, 1.5 M solution in ether) afforded **23a** (99.9 mg, 82%).

^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.85 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.36 (3H, d, J = 7.0 Hz, H-21), 2.29 (1H, m), 2.43 (2H, t, J = 7.3 Hz, H-23), 3.37 (1H, q, J = 7.0 Hz, H-20), 4.08 (1H, m, H-8), 5.61 (1H, m, H-16). MS m/z (%): 482 (M^+, 11), 464 (3), 292 (5), 235 (98), 217 (8), 161 (100), 160 (64). HRMS m/z: 482.3630 (Calcd for $C_{28}H_{54}O_2SSi$: 482.3614).

4.25. (20*S*)-16-Ene-22-thia-24-nor-26,27-dimethyl-8-ol (24a)

A mixture of **19a** (541.0 mg, 1.268 mmol), p-toluenesulfonic acid monohydrate (p-TsOH, 723.4 mg, 3.803 mmol) in MeOH (5 mL) was stirred for 5 h at ambient temperature, poured into ice-water, and extracted with AcOEt. The organic phase was washed with 5% sodium bicarbonate ($NaHCO_3$), and brine, and then dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the residue, which was purified by column chromatography on silica gel (20 g) with 20% AcOEt in hexane to yield **24a** (328.8 mg, 83%).

^{1}H NMR ($CDCl_3$) δ: 0.87 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.19 (3H, s, H-18), 1.39 (3H, d, J = 7.0 Hz, H-21), 2.33 (1H, m), 2.57 (2H, s, H-23), 3.39 (1H, q, J = 7.0 Hz, H-20), 4.18 (1H, m, H-8), 5.67 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.19, 8.22, 17.9, 19.0, 22.6, 30.5, 31.0, 31.1, 34.0, 35.5, 39.4, 41.5, 46.5, 54.9, 69.3, 74.0, 125.4, 156.0. MS m/z (%): 312 (M^+, 4), 294 (6), 276 (16), 178 (13), 160 (73), 145 (100). HRMS m/z: 312.2112 (Calcd for $C_{18}H_{32}O_2S$: 312.2123).

4.26. (20*R*)-16-Ene-22-thia-24-nor-26,27-dimethyl-8-ol (24b)

Following the same procedure as described above, treatment of **19b** (286.4 mg, 0.671 mmol) with p-TsOH (255.3 mg, 1.342 mmol) afforded **24b** (181.5 mg, 84%).

^{1}H NMR ($CDCl_3$) δ: 0.86, 0.88 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.07 (3H, s, H-18), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.26 (1H, m), 2.57, 2.63 (each 1H, d, J = 12.7 Hz, H-23), 3.31 (1H, q, J = 7.0 Hz, H-20), 4.18 (1H, m, H-8), 5.59 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.20, 8.25, 17.9, 19.0, 22.6, 30.6, 31.0, 31.1, 34.0, 35.7, 37.6, 40.5, 46.7, 54.2, 69.2, 73.8, 124.5, 155.3. MS m/z (%): 312 (M^+, 8), 294 (2), 276 (15), 178 (7), 160 (71), 145 (100).

4.27. (20*S*)-16-Ene-22-thia-24-nor-26,27-dimethyl-8-one (25a)

To a stirred solution of $(COCl)_2$ (187 μL, 2.144 mmol) in dry CH_2Cl_2 (2 mL) at −78 °C was added DMSO (303 μL, 4.270 mmol) in dry CH_2Cl_2 (0.5 mL). After being stirred for 10 min, a solution of **24a** (304.7 mg, 0.975 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 10 min, and Et_3N (1.36 mL, 9.757 mmol) was added. The entire mixture was stirred at −78 °C for 15 min, and at 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 chromatographed on silica gel (15 g) with 15% AcOEt in hexane to give **25a** (251.2 mg, 83%).

^{1}H NMR ($CDCl_3$) δ: 0.87, 0.88 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 0.97 (3H, s, H-18), 1.45 (3H, d, J = 7.0 Hz, H-21), 2.54, 2.57 (each 1H, d, J = 12.9 Hz, H-23), 2.87

(1H, dd, J = 10.5, 6.3 Hz, H-14), 3.46 (1H, q, J = 7.0 Hz, H-20), 5.59 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.4 (2×), 18.4, 22.0, 24.3, 27.6, 31.3 (2×), 34.6, 40.0, 40.8, 41.3, 53.6, 64.0, 74.2, 125.8, 153.8, 210.9. MS *m/z* (%): 310 (M^+, 13), 292 (49), 209 (29), 177 (100), 176 (50), 161 (27). HRMS *m/z*: 310.1944 (Calcd for $C_{18}H_{30}O_2S$: 310.1966).

4.28. (20*R*)-16-Ene-22-thia-24-nor-26,27-dimethyl-8-one (25b)

The Swern oxidation of **24b** (162.3 mg, 0.519 mmol) was carried out according to the same procedure described for the preparation of **25a** to give **25b** (125.6 mg, 78%).

^{1}H NMR ($CDCl_3$) δ: 0.85 (3H, s, H-18), 0.88, 0.89 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.48 (3H, d, J = 7.0 Hz, H-21), 2.60, 2.62 (each 1H, d, J = 13.2 Hz, H-23), 2.91 (1H, dd, J = 10.6, 6.6 Hz), 3.35 (1H, q, J = 7.0 Hz, H-20), 5.56 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.20, 8.23, 17.7, 22.5, 24.2, 27.4, 30.98, 31.14, 34.8, 38.5, 40.5, 40.6, 53.9, 62.8, 74.0, 124.6, 153.3, 210.9. MS *m/z* (%): 310 (M^+, 16), 292 (24), 209 (29), 177 (78), 176 (100), 161 (63).

4.29. (20*S*)-16-Ene-22-thia-24-nor-25-(triethylsilyl)oxy-26,27-dimethyl-8-one (26a)

To a stirred solution of **25a** (38.7 mg, 0.125 mmol) in dry *N*,*N*-dimethylformamide (DMF, 0.8 mL) were added imidazole (50.9 mg, 0.748 mmol) and chlorotriethylsilane (TESCl, 62.8 μL, 0.374 mmol) at 0 °C and the mixture was stirred for 5 h at ambient temperature. Additional imidazole (50.9 mg) and TESCl (62.8 μL) were added, the whole mixture was stirred for 16 h at ambient temperature, poured into ice water, and then extracted with AcOEt: hexane (1:1, v/v). The organic phase was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (6 g) with 5% AcOEt in hexane to give **26a** (35.4 mg, 67%).

^{1}H NMR($CDCl_3$) δ: 0.58 (6H, q, J = 7.9 Hz, 3× Si–CH_2CH_3), 0.85, 0.86 (each 3H, t, J = 7.3 Hz, H-26a, 27a), 0.95 (9H, t, J = 8.0 Hz, 3× Si–CH_2CH_3), 0.97 (3H, s, H-18), 1.42 (3H, d, J = 7.0 Hz, H-21), 2.50, 2.53 (each 1H, d, J = 11.7 Hz, H-23), 2.87 (1H, dd, J = 10.6, 6.4 Hz), 3.43 (1H, q, J = 7.0 Hz, H-20), 5.57 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.2 (3×), 7.4 (3×), 8.3, 8.5, 18.0, 21.5, 24.1, 27.3, 31.9, 32.3, 34.4, 39.3, 40.1, 40.6, 53.4, 63.9, 78.5, 125.1, 153.8, 210.9. MS *m/z* (%): 424 (M^+, 1), 395 (3), 338 (8), 292 (10), 263 (1), 201 (100), 177 (14). HRMS *m/z*: 424.2842 (Calcd for $C_{24}H_{44}O_2SSi$: 424.2831).

4.30. (20*R*)-16-Ene-22-thia-24-nor-25-(triethylsilyl)oxy-26,27-dimethyl-8-one (26b)

Following the same procedure as described above, treatment of **25b** (21.3 mg, 0.069 mmol) with TESCl (69.1 μL, 0.412 mmol) in the presence of imidazole (56.0 mg, 0.823 mmol) afforded **26b** (17.7 mg, 61%).

^{1}H NMR ($CDCl_3$) δ: 0.60 (6H, q, J = 7.9 Hz, 3× Si–CH_2CH_3), 0.86 (3H, s, H-18), 0.867, 0.873 (each 3H, t, J = 7.3 Hz, H-26a, 27a), 0.96 (9H, t, J = 7.9 Hz, 3× Si–CH_2CH_3), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.54, 2.58 (each 1H, d, J = 11.6 Hz, H-23), 2.90 (1H, dd, J = 10.6, 6.4 Hz), 3.32 (1H, q, J = 7.0 Hz, H-20), 5.50 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.2 (x 3), 7.5 (3×), 8.3, 8.4, 17.8, 22.1, 24.3, 27.4, 32.1 (2×), 34.7, 37.9, 39.3, 40.7, 54.0, 62.9, 78.4, 123.9, 153.5, 211.0.

4.31. (20*S*)-16-Ene-22-thia-26,27-dimethyl-8,25-diol (27a)

A mixture of **20a** (140.7 mg, 0.319 mmol), Et_3N (50 μL, 0.359 mmol), and tetrabutylammonium fluoride (0.957 mL 0.957 mmol, 1.0 M solution in THF) in dry THF (1.5 mL) was stirred at 50 °C for 70 h. The mixture was poured into ice water and extracted with AcOEt. The organic phase was washed with brine and dried over anhydrous $MgSO_4$. Removal of the solvent in vacuo afforded the residue, which was purified by chromatography on silica gel (8 g) with 8% MeOH in AcOEt to yield **27a** (94.0 mg, 90%).

^{1}H NMR ($CDCl_3$) δ: 0.858, 0.861 (each 3H, t, J = 7.5 Hz, H-26a, H-27a), 1.19 (3H, s, H-18), 1.38 (3H, d, J = 7.0 Hz, H-21), 1.47 (4H, q, J = 7.4 Hz, H-26, H-27), 2.34 (1H, m, H-15), 2.49 (2H, m, H-23), 3.43 (1H, q, J = 7.0 Hz, H-20), 4.13 (1H, m, H-8), 5.66 (1H, m, H-16).

4.32. 16,20-Diene-22-thia-26,27-dimethyl-8,25-diol (29) and 16,20-diene-22-thia-25-hydroxy-26,27-dimethyl-8-one (30)

To a stirred solution of $(COCl)_2$ (54.7 μL, 0.627 mmol) in dry CH_2Cl_2 (1 mL) at −78 °C was added a solution of DMSO (88.7 μL, 1.250 mmol) in dry CH_2Cl_2 (0.5 mL). After being stirred for 10 min, a solution of **27a** (93 mg, 0.285 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred for 15 min at −78 °C, and Et_3N (397.2 μL, 2.850 mmol) was added. The whole mixture was stirred at −78 °C for 15 min and at 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 $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel (8 g) using 10% AcOEt in hexane to afford **30** (32.2 mg, 35%), and 15% AcOEt in hexane to give **29** (55.3 mg, 60%).

29: ^{1}H NMR ($CDCl_3$) δ: 0.86, 0.88 (each 3 H, t, J = 7.5 Hz, H-26a, 27a), 1.24 (3H, s, H-18), 1.50 (4H, q, J = 7.5 Hz, H-26, H-27), 2.36 (1H, m, H-15), 4.19 (1H, m, H-8), 5.00, 5.32 (each 1H, s, H-21), 6.05 (1H, m, H-16). MS *m/z* (%): 324 (M^+, 84), 306 (41), 288 (28), 277 (31), 210 (56). **30** ^{1}H NMR ($CDCl_3$) δ: 0.87, 0.89 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 1.00 (3H, s, H-18), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.53 (1H, m, H-15), 2.96 (1H, dd, J = 10.9, 6.5 Hz), 5.03, 5.29 (each 1H, s, H-21), 5.98 (1H, m, H-16). MS *m/z* (%): 322 (M^+, 67), 304 (69), 275 (54), 233 (23), 208 (51).

4.33. (20*S*)-16-Ene-22-thia-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (31a)

To a stirred solution of **20a** (366.9 mg, 0.832 mmol) in dry CH_2Cl_2 (3 mL) were added *N*,*N*-diisopropylethylamine (^{i}Pro$_2$NEt, 725 μL, 4.160 mmol) and chloromethyl methyl ether (MOMCl, 158 μL, 2.080 mmol) at 0 °C, the mixture was stirred at amb. temperature for 6 h, poured into a mixture of 2 N HCl and ice water, and then extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel (22 g) with 3% AcOEt in hexane to afford **31a** (309.1 mg, 77%).

^{1}H NMR ($CDCl_3$) δ: 0.021, 0.025 (each 3H, s, 2× Si–Me), 0.83 (6H, t, J = 7.5 Hz, H-26a, H-27a), 0.88 (9H, s, Si-*t*-Bu), 1.15 (3H, s, H-18), 1.37 (3H, d, J = 7.0 Hz, H-21), 2.28 (1H, m, H-15), 2.42 (2H, m, H-23), 3.38 (3H, s, –OMe), 3.40 (1H, q, J = 7.0 Hz, H-20), 4.08 (1H, m, H-8), 4.65 (2H, s, OCH_2O), 5.62 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 8.0 (2×), 18.2, 19.6, 22.4, 25.2, 26.0 (4×), 27.9, 28.0, 31.0, 34.7, 35.6, 35.8, 37.9, 46.9, 55.6, 55.9, 69.2, 81.0, 90.6, 125.0, 156.1. MS *m*/*z* (%): 484 (M^+, 3), 452 (2), 422 (5), 407 (1), 365 (1), 292 (4), 235 (100), 161 (63), 160 (67). HRMS *m*/*z*: 484.3429 (Calcd for $C_{27}H_{52}O_3SSi$: 484.3406).

4.34. (20*R*)-16-Ene-22-thia-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (31b)

Following the same procedure as described above, treatment of **20b** (113.7 mg, 0.258 mmol) with MOMCl (39 μL, 0.513 mmol) in the presence of ^{i}Pro$_2$NEt (180 μL, 1.033 mmol) yielded **31b** (124.0 mg, 99%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.84 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.05 (3H, s, H-18), 1.46 (3H, d, J = 7.0 Hz, H-21), 2.25 (1H, m, H-15), 2.47 (2H, m, H-23), 3.32 (1H, q, J = 7.0 Hz, H-20), 3.39 (3H, s, OMe), 4.09 (1H, m, H-8), 4.65 (2H, s, OCH_2O), 5.53 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 8.0 (2×), 18.2, 19.6, 22.6, 24.5, 26.0 (4×), 27.9 (2×), 31.0, 34.8, 35.8, 35.9, 36.5, 47.0, 54.9, 55.9, 69.2, 81.0, 90.6, 124.0, 155.7. MS *m*/*z* (%): 484 (M^+, 7), 452 (3), 422 (11), 407 (1), 365 (2), 292 (15), 235 (100), 161 (56), 160 (86). HRMS *m*/*z*: 484.3417 (Calcd for $C_{27}H_{52}O_3SSi$: 484.3406).

4.35. (20*S*)-16-Ene-22-thia-24-homo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol *tert*-butylodimethylsilyl ether (32a)

Following the same procedure as described above, treatment of **21a** (469.4 mg, 1.032 mmol) with MOMCl (235 μL, 3.094 mmol) in the presence of ^{i}Pro$_2$NEt (1.08 mL, 6.200 mmol) gave **32a** (492.1 mg, 96%).

^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.28 (1H, m, H-15), 2.41 (2H, m, H-23), 3.39 (3H, s, OMe, overlapped with H-20), 4.08 (1H, m, H-8), 4.65 (2H, s, OCH_2O), 5.61 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 7.93, 7.96, 18.17, 18.21, 19.5, 22.5, 23.6, 26.0 (3×), 28.0 (2×), 31.0, 31.8, 34.8, 34.9, 35.8, 37.8, 46.8, 55.5, 55.9, 69.2, 80.9, 90.6, 124.9, 156.2. MS *m*/*z* (%): 498 (M^+, 4), 466 (2), 436 (7), 292 (5), 235 (100), 161 (61), 160 (61). HRMS *m*/*z*: 498.3575 (Calcd for $C_{28}H_{54}O_3SSi$: 498.3563).

4.36. (20*R*)-16-Ene-22-thia-24-homo-25-(methoxymethyl) oxy-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (32b)

Following the same procedure as described above, treatment of **21b** (290.1 mg, 0.638 mmol) with MOMCl (121 μL, 1.593 mmol) in the presence of ^{i}Pro$_2$NEt (556 μL, 3.192 mmol) afforded **32b** (294.8 mg, 93%).

^{1}H NMR ($CDCl_3$) δ: 0.02, 0.03 (each 3H, s, 2× Si–Me), 0.83 (6H, t, J = 7.4 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.05 (3H, s, H-18), 1.44 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.4 Hz, H-26, 27), 2.24 (1H, m, H-15), 2.45 (2H, m, H-23), 3.30 (1H, q, J = 7.0 Hz, H-20), 3.39 (3H, s, OMe), 4.08 (1 H, m, H-8), 4.65 (2H, s, OCH_2O), 5.53 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: −5.0, −4.6, 8.0 (2×), 18.2 (2×), 19.6, 22.7, 23.8, 26.0 (3×), 28.0 (2×), 31.1, 31.2, 34.8, 35.0, 35.9, 36.5, 47.0, 54.9, 55.9, 69.2, 81.0, 90.6, 124.0, 155.9. MS *m*/*z* (%): 498 (M^+, 4), 466 (2), 436 (9), 292 (4), 235 (74), 161 (73), 160 (48), 75 (100). HRMS *m*/*z*: 498.3559 (Calcd for $C_{28}H_{54}O_3SSi$: 498.3563).

4.37. (20*S*)-16-Ene-22-thia-24,24-dihomo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (33a)

Following the same procedure as described above, treatment of **22a** (57.4 mg, 0.122 mmol) with MOMCl (37.1 μL, 0.488 mmol) in the presence of ^{i}Pro$_2$NEt (170.3 μL, 0.978 mmol) yielded **33a** (60.8 mg, 97%).

^{1}H NMR ($CDCl_3$) δ: 0.02 (6H, s, 2× Si–Me), 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.35 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.44 (2H, t, J = 7.3 Hz, H-23), 3.39 (3 H, s, –CH_2OCH_3, overlapped with H-20), 4.08 (1 H, m, H-8), 4.65 (2H, s, –CH_2OCH_3), 5.61 (1 H, m, H-16). MS *m*/*z* (%): 512 (M^+, 6), 480 (3), 450 (9), 292 (3), 235 (100), 161 (83), 160 (58). HRMS *m*/*z*: 512.3749 (Calcd for $C_{29}H_{56}O_3SSi$: 512.3719).

4.38. (20*S*)-16-Ene-22-thia-24,24-24-trihomo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol *tert*-butyldimethylsilyl ether (34a)

Following the same procedure as described above, treatment of **23a** (66.0 mg, 0.137 mmol) with MOMCl (31.1 μL, 0.409 mmol) in the presence of ^{i}Pro$_2$NEt (142.9 μL, 0.820 mmol) gave **34a** (70.5 mg, 98%).

^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s, 2× Si–Me), 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.89 (9H, s, Si-*t*-Bu), 1.13 (3H, s, H-18), 1.36 (3H, d, J = 7.0 Hz, H-21), 2.29 (1H, m), 2.43 (2H, t, J = 7.3 Hz, H-23), 3.39 (3H, s, –CH_2OCH_3,

overlapped with H-20), 4.08 (1H, m, H-8), 4.65 (2H, s, –CH_2OCH_3), 5.61 (1H, m, H-16). MS *m/z* (%): 526 (M^+, 8), 494 (4), 464 (9), 292 (4), 235 (100), 217 (6), 161 (100), 160 (56). HRMS *m/z*: 526.3884 (Calcd for $C_{30}H_{58}O_3SSi$: 526.3876).

4.39. (20*S*)-16-Ene-22-thia-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol (35a)

A mixture of **31a** (309.1 mg, 0.638 mmol), Et_3N (100 μL, 0.717 mmol), and TBAF (1.914 mL, 1.914 mmol, 1.0 M solution in THF) in dry THF (3 mL) was stirred at 50 °C for 91 h. The mixture was poured into ice water and extracted with AcOEt. The organic phase was washed with brine and dried over anhydrous $MgSO_4$. Removal of the solvent in vacuo afforded the residue, which was purified by chromatography on silica gel (15 g) with 30% AcOEt in hexane to yield **35a** (207.9 mg, 88%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (6H, t, J = 7.5 Hz, H-26a, H-27a), 1.19 (3H, s, H-18), 1.38 (3H, d, J = 7.0 Hz, H-21), 2.33 (1H, m, H-15), 2.42 (2H, m, H-23), 3.39 (3H, s, –OMe, overlapped with H-20), 4.18 (1H, m, H-8), 4.65 (2H, s, OCH_2O), 5.66 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.0 (x 2), 17.9, 18.9, 22.2, 25.1, 27.9, 28.0, 30.4, 34.0, 35.4, 35.5, 37.8, 46.5, 55.0, 55.9, 69.3, 80.9, 90.6, 125.0, 155.9. MS *m/z* (%): 370 (M^+, 4), 338 (3), 308 (6), 178 (16), 160 (62), 145 (100). HRMS *m/z*: 370.2570 (Calcd for $C_{21}H_{38}O_3S$: 370.2542).

4.40. (20*R*)-16-Ene-22-thia-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol (35b)

Following the same procedure as described above, treatment of **31b** (120.0 mg, 0.247 mmol) with TBAF (742 μL, 0.742 mmol) gave **35b** (87.5 mg, 95%).

^{1}H NMR ($CDCl_3$) δ: 0.84 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.09 (3H, s, H-18), 1.47 (3H, d, J = 7.0 Hz, H-21), 2.29 (1H, m, H-15), 2.44 (2H, m, H-23), 3.32 (1H, q, J = 7.0 Hz, H-20), 3.39 (3H, s, OMe), 4.17 (1H, m, H-8), 4.66 (2H, s, OCH_2O), 5.56 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.9 (2×), 17.9, 18.9, 22.5, 24.5, 27.9 (2×), 30.5, 34.0, 35.6, 35.7, 36.4, 46.6, 54.3, 55.9, 69.2, 80.9, 90.6, 123.9, 155.5. MS *m/z* (%): 370 (M^+, 2), 338 (5), 308 (4), 178 (21), 160 (78), 145 (100). HRMS *m/z*: 370.2558 (Calcd for $C_{21}H_{38}O_3S$: 370.2542).

4.41. (20*S*)-16-Ene-22-thia-24-homo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol (36a)

Following the same procedure as described above, treatment of **32a** (476.3 mg, 0.955 mmol) with TBAF (2.86 mL, 2.864 mmol) gave **36a** (360.0 mg, 98%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.18 (3H, s, H-18), 1.37 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.33 (1H, m, H-15), 2.41 (2H, m, H-23), 3.39 (3H, s, OMe, overlapped with H-20), 4.17 (1 H, m, H-8), 4.65 (2H, s, OCH_2O), 5.61 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.91, 7.94, 17.9, 18.8, 22.3, 23.5, 28.0 (2×), 30.4, 31.7, 34.0, 34.9, 35.5, 37.7, 46.4, 55.0, 55.9, 69.3, 80.9, 90.6, 125.0, 155.9.MS *m/z* (%): 384 (M^+, 2), 322 (3), 304 (2), 160 (61), 145 (100). HRMS *m/z*: 384.2695 (Calcd for $C_{22}H_{40}O_3S$: 384.2698).

4.42. (20*R*)-16-Ene-22-thia-24-homo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol (36b)

Following the same procedure as described above, treatment of **32b** (294.8 mg, 0.591 mmol) with TBAF (1.77 mL, 1.773 mmol) yielded **36b** (222.5 mg, 98%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.08 (3H, s, H-18), 1.46 (3H, d, J = 7.0 Hz, H-21), 1.52 (4H, q, J = 7.5 Hz, H-26, 27), 2.29 (1H, m, H-15), 2.45 (2H, m, H-23), 3.31 (1H, q, J = 7.0 Hz, H-20), 3.39 (3H, s, OMe), 4.18 (1H, m, H-8), 4.65 (2H, s, OCH_2O), 5.56 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.9 (2×), 17.9, 18.9, 22.5, 23.7, 28.00, 28.02, 30.5, 31.1, 34.1, 35.0, 35.6, 36.4, 46.6, 54.3, 55.9, 69.3, 80.9, 90.6, 124.0, 155.6. MS *m/z* (%): 384 (M^+, 1), 352 (1), 322 (2), 304 (1), 178 (6), 160 (58), 145 (100). HRMS *m/z*: 384.2686 (Calcd for $C_{22}H_{40}O_3S$: 384.2698).

4.43. (20*S*)-16-Ene-22-thia-24,24-dihomo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol (37a)

Following the same procedure as described above, treatment of **33a** (62.8 mg, 0.122 mmol) with TBAF (489 μL, 0.489 mmol) gave **37a** (46.3 mg, 95%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.18 (3H, s, H-18), 1.37 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.33 (1H, m), 2.44 (2H, t, J = 7.2 Hz, H-23), 3.39 (3H, s, –CH_2OCH_3, overlapped with H-20), 4.17 (1H, m, H-8), 4.65 (2H, s, –CH_2OCH_3), 5.65 (1H, m, H-16). MS *m/z* (%): 398 (M^+, 6), 336 (8), 178 (20), 160 (60), 145 (100). HRMS *m/z*: 398.2828 (Calcd for $C_{23}H_{42}O_3S$: 398.2855).

4.44. (20*S*)-16-Ene-22-thia-24,24,24-trihomo-25-(methoxymethyl)oxy-26,27-dimethyl-8-ol (38a)

Following the same procedure as described above, treatment of **34a** (73.4 mg, 0.139 mmol) with TBAF (557 μL, 0.557 mmol) gave **38a** (52.7 mg, 92%).

^{1}H NMR ($CDCl_3$) δ: 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.18 (3H, s, H-18), 1.37 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.33 (1H, m), 2.43 (2H, t, J = 7.3 Hz, H-23), 3.39 (3H, s, –CH_2OCH_3, overlapped with H-20), 4.18 (1H, m, H-8), 4.65 (2H, s, –CH_2OCH_3), 5.64 (1H, m, H-16). MS *m/z* (%): 412 (M^+, 9), 380 (4), 350 (11), 178 (32), 161 (73), 160 (32), 145 (100). HRMS *m/z*: 412.3008 (Calcd for $C_{24}H_{44}O_3S$: 412.3011).

4.45. (20*S*)-16-Ene-22-thia-25-(methoxymethyl)oxy-26,27-dimethyl-8-one (39a)

A mixture of **35a** (98.7 mg, 0.266 mmol), Celite (400.8 mg), and pyridinium dichromate (PDC, 200.4 mg, 0.533 mmol) in dry CH_2Cl_2 (2.5 mL) was stir-

red for 3 h at ambient temperature and a reaction mixture was applied to the top of the silica gel column (15 g). The column was eluted with 50% AcOEt in hexane to give **39a** (75.3 mg, 77%).

^{1}H NMR ($CDCl_3$) δ: 0.829, 0.832 (each 3H, t, J = 7.5 Hz, H-26a, 27a), 0.97 (3H, s, H-18), 1.44 (3H, d, J = 7.0 Hz, H-21), 2.29 (1H, m), 2.42 (2H, m), 2.87 (1H, dd, J = 10.6, 6.4 Hz, H-15), 3.38 (3H, s, OMe), 3.47 (1H, q, J = 7.0 Hz, H-20), 4.63, 4.65 (each 1H, d, J = 7.4 Hz, OCH_2O), 5.57 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.97, 7.99, 18.1, 21.5, 24.1, 24.6, 27.3, 27.92, 27.96, 34.3, 35.5, 38.4, 40.6, 53.4, 55.9, 63.9, 80.9, 90.6, 125.2, 153.6, 210.9. MS *m*/*z* (%): 368 (M^+, 5), 336 (6), 306 (17), 176 (100), 161 (90). HRMS *m*/*z*: 368.2408 (Calcd for $C_{21}H_{36}O_3S$: 368.2385).

4.46. (20*R*)-16-Ene-22-thia-25-(methoxymethyl)oxy-26,27-dimethyl-8-one (39b)

Following the same procedure as described above, treatment of **35b** (48.7 mg, 0.131 mmol) with PDC (98.9 mg, 0.263 mmol) and Celite (200 mg) afforded **39b** (39.0 mg, 81%).

^{1}H NMR ($CDCl_3$) δ: 0.84 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86 (3H, s, H-18), 1.48 (3H, d, J = 7.0 Hz, H-21), 2.90 (1H, m, H-14), 3.39 (3H, s, OMe, overlapped with H-20), 4.66 (2H, s, OCH_2O), 5.53 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.2 (2×), 17.9, 22.5, 24.5, 24.8, 27.6, 28.1 (2×), 35.0, 35.9, 37.6, 40.9, 54.2, 56.1, 63.2, 81.1, 90.8, 124.3, 153.7, 211.1. MS *m*/*z* (%): 368 (M^+, 5), 336 (3), 306 (20), 176 (100), 161 (90). HRMS *m*/*z*: 368.2397 (Calcd for $C_{21}H_{36}O_3S$: 368.2385).

4.47. (20*S*)-16-Ene-22-thia-24-homo-25-(methoxymethyl) oxy-26,27-dimethyl-8-one (40a)

To a stirred solution of $(COCl)_2$ (82 μL, 0.940 mmol) in dry CH_2Cl_2 (2 mL) at −78 °C was added a solution of DMSO (134 μL, 1.888 mmol) in dry CH_2Cl_2 (1 mL). After being stirred for 5 min, a solution of **36a** (303.0 mg, 0.788 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred for 15 min at −78 °C, and Et_3N (549 μL, 3.885 mmol) was added. The whole mixture was stirred at −78 °C for 15 min and at 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 concentrated in vacuo. The residue was purified by chromatography on silica gel (12 g) using 10% AcOEt in hexane to afford **40a** (287.4 mg, 95%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.96 (3H, s, H-18), 1.43 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.87 (1H, dd, J = 10.6, 6.4 Hz, H-14), 3.38 (3H, s, OMe), 3.45 (1H, q, J = 7.0 Hz, H-20), 4.65 (2H, s, OCH_2O), 5.56 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 7.9 (2×), 18.0, 21.5, 23.4, 24.0, 27.3, 27.9 (2×), 31.2, 34.2, 34.9, 38.3, 40.6, 53.3, 55.9, 63.8, 80.8, 90.5, 125.1, 153.5, 210.8. MS *m*/*z* (%): 382 (M^+, 31), 350 (44), 320 (50), 177 (100), 176 (84). HRMS *m*/*z*: 382.2531 (Calcd for $C_{22}H_{38}O_3S$: 382.2542).

4.48. (20*R*)-16-Ene-22-thia-24-homo-25-(methoxymethyl) oxy-26,27-dimethyl-8-one (40b)

The Swern oxidation of **36b** (210.4 mg, 0.547 mmol) was carried out according to the same procedure described for the preparation of **36a** to give **40b** (178.5 mg, 85%).

^{1}H NMR ($CDCl_3$) δ: 0.84 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86 (3H, s, H-18), 1.47 (3H, d, J = 7.0 Hz, H-21), 1.52 (4H, q, J = 7.5 Hz, H-26, 27), 2.91 (1H, dd, J = 10.6, 6.5 Hz, H-14), 3.35 (1H, q, J = 7.0 Hz, H-20), 3.39 (3H, s, OMe), 4.66 (2H, s, OCH_2O), 5.53 (1H, m, H-16). ^{13}C NMR ($CDCl_3$) δ: 8.2 (2×), 17.9, 22.5, 23.9, 24.5, 27.6, 28.2 (2×), 31.4, 35.0, 35.2, 37.6, 40.9, 54.2, 56.1, 63.1, 81.1, 90.8, 124.3, 153.8, 211.2. MS *m*/*z* (%): 382 (M^+, 7), 350 (9), 320 (26), 177 (100), 176 (33). HRMS *m*/*z*: 382.2523 (Calcd for $C_{22}H_{38}O_3S$: 382.2542).

4.49. (20*S*)-16-Ene-22-thia-24,24-dihomo-25-(methoxymethyl)oxy-26,27-dimethyl-8-one (41a)

The Swern oxidation of **37a** (46.3 mg, 0.116 mmol) was carried out according to the same procedure described for the preparation of **36a** to give **41a** (42.9 mg, 93%).

^{1}H NMR ($CDCl_3$) δ: 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.96 (3H, s, H-18), 1.43 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.86 (1H, dd, J = 10.6, 6.4 Hz), 3.39 (3H, s, –CH_2OCH_3), 3.44 (1H, q, J = 7.0 Hz, H-20), 4.65 (2H, s, –CH_2OCH_3), 5.56 (1H, m, H-16). MS *m*/*z* (%): 396 (M^+, 7), 364 (6), 334 (14), 176 (100). HRMS *m*/*z*: 396.2670 (Calcd for $C_{23}H_{40}O_3S$: 396.2698).

4.50. (20*S*)-16-Ene-22-thia-24,24,24-trihomo-25-(methoxymethyl)oxy-26,27-dimethyl-8-one (42a)

The Swern oxidation of **38a** (94.6 mg, 0.234 mmol) was carried out according to the same procedure described for the preparation of **36a** to give **42a** (75.8 mg, 79%).

^{1}H NMR ($CDCl_3$) δ: 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.96 (3H, s, H-18), 1.43 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.86 (1H, dd, J = 10.6, 6.4 Hz), 3.39 (3H, s, –CH_2OCH_3), 3.44 (1H, q, J = 7.0 Hz, H-20), 4.65 (2H, s, –CH_2OCH_3), 5.56 (1H, m, H-16). MS *m*/*z* (%): 410 (M^+, 4), 378 (4), 348 (12), 177 (100), 176 (75). HRMS *m*/*z*: 410.2874 (Calcd for $C_{24}H_{42}O_3S$: 410.2855).

4.51. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-25-[(triethylsilyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (44a)

To a stirred solution of **43** (85.2 mg, 0.149 mmol) in dry THF (0.7 mL) at −78 °C was added slowly n-BuLi (95.7 μL, 0.149 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 **26a** (31.7 mg, 0.074 mmol) in dry THF (0.8 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 anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (5 g) using 2% AcOEt in hexane to afford **44a** (22.8 mg, 48% based on **26a**) and 10% AcOEt in hexane to give the unreacted starting material **26a** (8.5 mg, 27%).

^{1}H NMR ($CDCl_3$) δ: 0.046, 0.049, 0.055, 0.063 (each 3H, s, 4× Si–Me), 0.59 (6H, q, J = 7.9 Hz, 3× Si–CH_2CH_3), 0.83 (3H, s, H-18), 0.85, 0.88 (each 9H, s, 2× Si-*t*-Bu), 0.95 (9H, t, J = 7.9 Hz, 3× Si–CH_2CH_3), 1.40 (3H, d, J = 7.0 Hz, H-21), 2.53 (2H, m, H-23), 2.80 (1H, m), 3.42 (1H, q, J = 7.0 Hz, H-20), 4.06 (1H, m), 4.11 (1H, m), 5.60 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.17 (1H, d, J = 11.2 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: −4.70, −4.62, −4.52, −4.43, 7.2, 7.5, 8.3, 8.4, 17.7, 18.3, 18.4, 21.7, 23.5, 26.0, 28.5, 29.6, 31.9, 32.3, 35.4, 36.9, 39.5, 40.1, 43.9, 46.3, 49.6, 59.1, 68.2, 68.3, 78.5, 116.6, 121.8, 125.1, 134.3, 140.0, 155.8. MS *m*/*z* (%): 776 (M^+, 3), 747 (1), 719 (1), 644 (4), 615 (1), 528 (18), 471 (6), 396 (32), 339 (18), 201 (100). HRMS *m*/*z*: 776.5471 (Calcd for $C_{44}H_{84}O_3SSi$: 776.5449).

4.52. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-25-[(triethylsilyl)oxy]-26,27-dimethyl-19,24-dinorvitamin D_3 *tert*-butyldimethylsilyl ether (44b)

Following the same procedure as described above, treatment of **43** (69.4 mg, 0.122 mmol) with **26b** (10.4 mg, 0.024 mmol) in the presence of n-BuLi (77.9 μL, 0.122 mmol) gave **44b** (2.8 mg, 18%) and the unreacted starting ketone **26b** (5.5 mg, 53%).

^{1}H NMR ($CDCl_3$) δ: 0.05–0.07 (12H, 4× Si–Me), 0.59 (6H, q, J = 7.9 Hz, 3× Si–CH_2CH_3), 0.72 (3H, s, H-18), 0.86, 0.88 (each 9H, 2× Si-*t*-Bu), 0.96 (9H, t, J = 7.9 Hz, 3× Si–CH_2CH_3), 1.45 (3H, d, J = 7.0 Hz, H-21), 2.56 (2H, s, H-23), 2.79 (1H, m), 3.33 (1H, q, J = 7.0 Hz, H-20), 4.07, 4.10 (each 1H, m, H-1, 3), 5.56 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.17 (1H, d, J = 11.2 Hz, H-6). MS *m*/*z* (%): 776 (M^+, 5), 644 (11), 528 (38), 471 (11), 396 (66), 339 (31), 201 (100). HRMS *m*/*z*: 776.5449 (Calcd for $C_{44}H_{84}O_3SSi$: 776.5449).

4.53. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (45a)

Following the same procedure as described above, treatment of **43** (125.8 mg, 0.220 mmol) with **39a** (40.6 mg, 0.11 mmol) in the presence of lithium bis(trimethylsilyl)amide (LHMDS, 220 μL, 0.220 mmol, 1.0 M solution in THF) gave **45a** (33.5 mg, 42%).

^{1}H NMR ($CDCl_3$) δ: 0.046 (6H, s, 2× Si–Me), 0.05, 0.06 (each 3 , s, 2× Si–Me), 0.83 (3H, s, H-18) 0.85, 0.88 (each 9H, s, 2× Si-*t*-Bu), 1.42 (3H, d, J = 7.0 Hz, H-21), 2.80 (1 H, m, H-9), 3.38 (3H, s, OMe), 3.46 (1H, q, J = 7.0 Hz, H-20), 4.06, 4,11 (each 1H, m, H-1, 3), 4.64, 4.66 (each 1H, d, J = 7.2 Hz, OCH_2O), 5.60 (1H, m, H-16), 5.91 (1H, d, J = 11.2 Hz, H-7), 6.17 (1H, d,J = 11.2 Hz, H-6). MS *m*/*z* (%): 720 (no M^+), 528 (12), 471 (3), 396 (22), 339 (11), 264 (8), 75 (100).

4.54. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (45b)

Following the same procedure as described above, treatment of **43** (79.9 mg, 0.140 mmol) with **39b** (25.8 mg, 0.070 mmol) in the presence of LHMDS (140 μL, 0.140 mmol, 1.0 M solution in THF) gave **45b** (25.6 mg, 51%).

^{1}H NMR ($CDCl_3$) δ: 0.048, 0.062 (each 3H, s, 2× Si–Me), 0.052 (6H, s, 2× Si–Me), 0.73 (3H, s, H-18), 0.84 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86, 0.88 (each 9H, s, 2× Si-*t*-Bu), 1.47 (3H, d, J = 7.0 Hz, H-21), 2.80 (1H, m, H-9), 3.39 (3 H, s, OMe, overlapped with H-20), 4.07, 4.10 (each 1H, m, H-1, 3), 4.65 (2H, s, OCH_2O), 5.58 (1H, m, H-16), 5.91 (1H, d,J = 11.2 Hz, H-7), 6.17 (1H, d,J = 11.2 Hz, H-6). MS *m*/*z* (%): 720 (no M^+), 528 (19), 471 (5), 396 (30), 339 (17), 264 (13), 75 (100).

4.55. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-24-homo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (46a)

Following the same procedure as described above, treatment of **43** (89.5 mg, 0.157 mmol) with **40a** (40.0 mg, 0.105 mmol) in the presence of LHMDS (157 μL, 0.157 mmol, 1.0 M solution in THF) gave **46a** (33.6 mg, 44%).

^{1}H NMR ($CDCl_3$) δ: 0.048, 0.061 (each 3H, s, 2× Si–Me), 0.052 (6H, s, 2× Si–Me), 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a, overlapped with H-18), 0.86, 0.88 (each 9H, s, 2× Si-*t*-Bu), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.80 (1H, m, H-9), 3.38 (3H, s, OMe), 3.45 (1H, q, J = 7.0 Hz, H-20), 4.08 (2H, m, H-1, 3), 4.65 (2H, s, OCH_2O), 5.59 (1H, m, H-16), 5.90 (1H, d,J = 11.2 Hz, H-7), 6.17 (1H, d, J = 11.2 Hz, H-6). MS *m*/*z* (%): 734 (no M^+), 528 (28), 471 (8), 396 (50), 339 (26), 264 (16), 75 (100).

4.56. (20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-24-homo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (46b)

Following the same procedure as described above, treatment of **43** (92.4 mg, 0.162 mmol) with **40b** (41.3 mg, 0.108 mmol) in the presence of LHMDS (162. μL, 0.162 mmol, 1.0 M solution in THF) gave **46b** (22.5 mg, 28%).

^{1}H NMR ($CDCl_3$) δ: 0.05 (9H, s, 3× Si–Me), 0.06 (3H, s, Si–Me), 0.72 (3H, s, H-18), 0.83 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86, 0.88 (each 9H, s, 2× Si-*t*-Bu), 1.45 (3H, d, J = 7.0 Hz, H-21), 1.52 (4H, q, J = 7.5 Hz, H-26, 27), 2.80 (1 H, m, H-9), 3.36 (1H, q, J = 7.0 Hz, H-

20), 3.39 (3H, s, OMe), 4.09 (2H, m, H-1, 3), 4.65 (2H, s, OCH_2O), 5.58 (1H, m, H-16), 5.91 (1H, d,J = 11.2 Hz, H-7), 6.17 (1H, d, J = 11.2 Hz, H-6). MS m/z (%): 734 (no M^+), 528 (57), 471 (16), 396 (86), 339 (38), 264 (20), 75 (100).

4.57. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-24,24-dihomo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (47a)

Following the same procedure as described above, treatment of **43** (73.3 mg, 0.128 mmol) with **41a** (29.5 mg, 0.074 mmol) in the presence of LHMDS (128 μL, 0.128 mmol, 1.0 M solution in THF) gave **47a** (26.8 mg, 48%).

^{1}H NMR ($CDCl_3$) δ: 0.05–0.06 (12H, 4× Si–Me), 0.820 (3H, s, H-18), 0.823 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86, 0.88 (each 9H, s, Si-*t*-Bu), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz, H-26, 27), 2.80 (1H, m), 3.39 (3H, s, $-CH_2OCH_3$), 3.44 (1H, q, J = 7.0 Hz, H-20), 4.08 (2H, m, H-1, 3), 4.65 (2H, s, $-CH_2OCH_3$), 5.60 (1H, m, H-16), 5.90 (1H, d, J = 11.2 Hz, H-6), 6.16 (1H, d, J = 11.2 Hz, H-7).

4.58. (20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-16-ene-22-thia-24,24,24-trihomo-25-[(methoxymethyl)oxy]-26,27-dimethyl-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (48a)

Following the same procedure as described above, treatment of **43** (82.0 mg, 0.1437 mmol) with **42a** (29.5 mg, 0.072 mmol) in the presence of LHMDS (144 μL, 0.144 mmol, 1.0 M solution in THF) gave **48a** (15.0 mg, 27%).

^{1}H NMR ($CDCl_3$) δ: 0.048, 0.061 (each 3H, s, 2× Si–Me), 0.052 (6H, s, 2× Si–Me), 0.82 (3H, s, H-18), 0.82 (6H, t, J = 7.5 Hz, H-26a, 27a), 0.86, 0.88 (each 9H, s, Si-*t*-Bu), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.51 (4H, q, J = 7.5 Hz), 2.80 (1H, m), 3.38 (3H, s, $-CH_2OCH_3$), 3.44 (1H, q, J = 7.0 Hz, H-20), 4.08 (2H, m, H-1, 3), 4.65 (2H, s, $-CH_2OCH_3$), 5.60 (1H, m, H-16), 5.90 (1H, d, J = 11.2 Hz, H-6), 6.16 (1H, d, J = 11.2 Hz, H-7).

4.59. (20*S*)-16-Ene-22-thia-26,27-dimethyl-1α,25-dihydroxy-19,24-dinorvitamin D_3 (1a)

A mixture of **44a** (22.2 mg, 0.029 mmol) and (−)-10-camphor sulfonic acid (39.8 mg, 0.171 mmol) in MeOH (1 mL) was stirred at ambient temperature for 1.5 h. The reaction mixture was poured into 5% $NaHCO_3$ and extracted with AcOEt. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was chromatographed on silica gel (5 g) using 80% AcOEt in hexane to give **1a** (12.2 mg, 98%).

^{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), 1.43 (3H, d, J = 7.0 Hz, H-21), 2.56, (2H, m, H-23), 2.78 (2H, m), 3.44 (1H, q, J = 7.0 Hz, H-20), 4.06, 4.13 (each 1H, m, H-1, 3), 5.63 (1H, m, H-16), 5.95 (1H, d, J = 11.3 Hz, H-7), 6.30 (1H, d, J = 11.3 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.2 (2×), 17.7, 21.9, 23.6, 28.7, 29.7, 31.1 (2×), 35.3, 37.4, 39.9, 41.1, 42.4, 44.8, 49.7, 59.0, 67.4, 67.6, 74.0, 115.8, 123.9, 125.6, 131.8, 142.0, 155.5. MS m/z (%): 434 (M^+, 2), 416 (11), 398 (11), 380 (9), 300 (100), 282 (32). HRMS m/z: 416.2766 (M^+-H_2O) (Calcd for $C_{26}H_{40}O_2S$: 416.2749).

4.60. (20*R*)-16-Ene-22-thia-26,27-dimethyl-1α,25-dihydroxy-19,24-dinorvitamin D_3 (1b)

Following the same procedure as described above, treatment of **44b** (4.4 mg, 0.0057 mmol) with (−)-10-camphor sulfonic acid (7.9 mg, 0.034 mmol) gave **1b** (2.4 mg, 98%).

^{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), 2.77 (2H, m), 3.36 (1H, q, J = 7.0 Hz, H-20), 4.06, 4.13 (1H, m, H-1, 3), 5.61 (1H, m, H-16), 5.95 (1H, d, J = 11.3 Hz, H-7), 6.31 (1H, d, J = 11.3 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.3, 17.4, 22.6, 23.7, 23.9, 28.8, 31.1, 35.5, 37.4, 38.9, 40.8, 42.4, 44.8, 49.6, 50.2, 58.1, 67.5, 67.6, 73.9, 115.6, 123.9, 124.8, 131.6, 142.2, 155.4. MS m/z (%): 434 (M^+, 2), 416 (8), 398 (12), 380 (8), 300 (100), 282 (50), 264 (48). HRMS m/z: 434.2859 (Calcd for $C_{26}H_{42}O_3S$: 434.2855).

4.61. (20*S*)-16-Ene-22-thia-26,27-dimethyl-1α,25-dihydroxy-19-norvitamin D_3 (2a)

Following the same procedure as described above, treatment of **45a** (26.5 mg, 0.0367 mmol) with (−)-10-camphor sulfonic acid (51.2 mg, 0.220 mmol) gave **2a** (14.6 mg, 89%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.859, 0.861 (each 3H, t, J = 7.6 Hz, H-26a, H-27a), 1.43 (3H, d, J = 7.0 Hz, H-21), 1.47 (4H, q, J = 7.6 Hz, H-26, 27), 2.78 (2H, m), 3.49 (1H, q, J = 7.0 Hz, H-20), 4.07. 4.13 (each 1H, m, H-1, 3), 5.62 (1H, m, H-16), 5.95 (1H, d, J = 11.3 Hz, H-7), 6.30 (1H, d,J = 11.3 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.0 (2×), 17.7, 21.6, 23.9, 25.0, 28.7, 29.6, 31.0, 31.1, 35.2, 37.4, 37.8, 38.5, 42.4, 44.8, 49.7, 59.1, 67.3, 67.6, 75.0, 115.8, 123.8, 125.4, 131.8, 142.0, 155.4. MS m/z (%): 448 (M^+, 1), 430 (11), 412 (7), 394 (2), 300 (100), 282 (23), 264 (10). HRMS m/z: 430.2893 (M^+-H_2O) (Calcd for $C_{27}H_{42}O_2S$: 430.2905).

4.62. (20*R*)-16-Ene-22-thia-26,27-dimethyl-1α,25-dihydroxy-19-norvitamin D_3 (2b)

Following the same procedure as described above, treatment of **45b** (25.6 mg, 0.035 mmol) with (−)-10-camphor sulfonic acid (49.5 mg, 0.213 mmol) gave **2b** (15.2 mg, 96%).

^{1}H NMR ($CDCl_3$) δ: 0.74 (3H, s, H-18), 0.86 (6 H, t, J = 7.5 Hz, H-26a, 27a), 1.47 (3H, d, J = 7.0 Hz, H-21), 1.48 (4H, q, J = 7.5 Hz, H-26, 27), 2.78 (2H, m,

H-9, 10), 3.39 (1 H, q, J = 7.0 Hz, H-20), 4.05 (1H, m, H-1), 4.10 (1H, m, H-3), 5.60 (1H, m, H-16), 5.95 (1H, d, J = 11.2 Hz, H-7), 6.30 (1H, d, J = 11.2 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.0 (2×), 17.4, 22.4, 23.7, 25.0, 28.8, 29.7, 31.06, 31.13, 35.6, 37.4, 37.7, 37.9, 42.4, 44.8, 50.2, 58.2, 67.4, 67.6, 75.0, 115.5, 123.9, 124.5, 131.7, 142.2, 155.3. MS m/z (%): 448 (M^+, 1), 430 (9), 412 (7), 394 (4), 300 (100), 282 (33), 249 (23). HRMS m/z: 430.2879 (M^+-H_2O) (Calcd for $C_{27}H_{42}O_2S$: 430.2905). UV λ_{max} (EtOH): 244, 252 (ε 28650), 262 nm.

4.63. (20*S*)-16-Ene-22-thia-24-homo-26,27-dimethyl-1α, 25-dihydroxy-19-norvitamin D_3 (3a)

Following the same procedure as described above, treatment of **46a** (30.3 mg, 0.0412 mmol) with (−)-10-camphor sulfonic acid (57.4 mg, 0.247 mmol) afforded **3a** (18.6 mg, 97%).

^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 0.85 (6 H, t, J = 7.5 Hz, H-26a, 27a), 1.41 (3H, d, J = 7.0 Hz, H-21), 1.47 (4H, q, J = 7.5 Hz, H-26, 27), 2.77 (2H, m, H-9, 10), 3.45 (1 H, q, J = 7.0 Hz, H-20), 4.05 (1H, m, H-1), 4.12 (1H, m, H-3), 5.60 (1H, m, H-16), 5.95 (1H, d, J = 11.2 Hz, H-7), 6.30 (1H, d,J = 11.2 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.0 (2×), 17.6, 21.7, 23.6, 23.7, 28.7, 29.6, 31.1, 31.2, 31.4, 35.2, 37.4, 37.9, 38.4, 42.2, 44.8, 49.7, 59.0, 67.3, 67.6, 74.7, 115.8, 123.8, 125.1, 131.8, 142.1, 155.5. MS m/z (%): 462 (no M^+), 444 (3), 426 (4), 408 (3), 300 (46), 282 (28), 264 (33), 143 (100). HRMS m/z: 444.3066 (M^+-H_2O) (Calcd for $C_{28}H_{44}O_2S$: 444.3062). UV λ_{max} (EtOH): 244, 252, 262 nm.

4.64. (20*R*)-16-Ene-22-thia-24-homo-26,27-dimethyl-1α, 25-dihydroxy-19-norvitamin D_3 (3b)

Following the same procedure as described above, treatment of **46b** (22.5 mg, 0.031 mmol) with (−)-10-camphor sulfonic acid (42.6 mg, 0.183 mmol) gave **3b** (13.7 mg, 97%).

^{1}H NMR ($CDCl_3$) δ: 0.73 (3H, s, H-18), 0.86 (6H, t, J = 7.5 Hz, H-26a, 27a), 1.45 (3H, d, J = 7.0 Hz, H-21), 1.46 (4H, q, J = 7.5 Hz, H-26, 27), 2.77 (2H, m, H-9, 10), 3.36 (1H, q, J = 7.0 Hz, H-20), 4.06 (1H, m, H-1), 4.13 (1H, m, H-3), 5.58 (1H, m, H-16), 5.95 (1H, d, J = 11.3 Hz, H-7), 6.30 (1H, d,J = 11.3 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.0 (2×), 17.4, 22.5, 23.7, 23.8, 28.8, 29.7, 31.16, 31.22, 31.4, 35.5, 37.4, 37.5, 37.9, 42.4, 44.8, 50.2, 58.2, 67.4, 67.6, 74.7, 115.5, 123.9, 124.3, 131.6, 142.2, 155.6. MS m/z (%): 462 (M^+, 1), 444 (2), 426 (3), 408 (2), 300 (36), 282 (22), 264 (22), 143 (100). HRMS m/z: 444.3060 (M^+-H_2O) (Calcd for $C_{28}H_{44}O_2S$: 444.3062). UV λ_{max} (EtOH): 244, 252, 262 nm.

4.65. (20*S*)-16-Ene-22-thia-24,24-dihomo-26,27-dimethyl-1α,25-dihydroxy-19-norvitamin D_3 (4a)

Following the same procedure as described above, treatment of **47a** (26.8 mg, 0.0358 mmol) with (−)-10-camphor sulfonic acid (49.8 mg, 0.2146 mmol) yielded **4a** (17.2 mg, 99%), which was further purified by HPLC (YMC-Pack ODS-AM SH-342-5AM, 32% H_2O/MeOH, 8 mL/min) to give pure **4a** (5.2 mg).

^{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), 2.77 (2H, m, H-9, 10), 3.43 (1H, q, J = 7.0 Hz, H-20), 4.06, 4.13 (each 1H, m, H-1, 3), 5.60 (1 H, m, H-16), 5.95 (1H, d, J = 11.2 Hz, H-6), 6.30 (1H, d, J = 11.2 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 8.0 (x 2), 17.6, 21.7, 23.1, 23.6, 28.7, 29.6, 30.2, 30.7, 31.2 (2×), 35.2, 37.4, 38.0, 38.3, 42.4, 44.8, 49.7, 59.0, 67.4, 67.6, 74.8, 115.8, 123.9, 125.1, 131.7, 142.1, 155.6. MS m/z (%): 476 (no M^+), 440 (4), 300 (26), 282 (21), 264 (22), 95 (100). HRMS m/z: 440.3110 (M^+-2H_2O) (Calcd for $C_{29}H_{44}OS$: 440.3113).

4.66. (20*R*)-16-Ene-22-thia-24,24,24-trihomo-26,27-dimethyl-1α,25-dihydroxy-19-norvitamin D_3 (5a)

Following the same procedure as described above, treatment of **48a** (15.0 mg, 0.0196 mmol) with (−)-10-camphor sulfonic acid (27.4 mg, 0.1179 mmol) yielded **5a** (6.2 mg, 65%), which was further purified by HPLC [Hibar RT 250-10 LiChrosorb Si 60 (7 μm), hexane/$CHCl_3$/MeOH = 55/42/3 (v/v/v), 5 mL/min] to give pure **5a** (1.2 mg).

^{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), 1.43 (4H, q, J = 7.5 Hz), 2.77 (2H, m, H-9, 10), 3.43 (1H, q, J = 7.0 Hz, H-20), 4.06, 4.13 (each 1H, m, H-1, 3), 5.60 (1H, m, H-16), 5.95 (1H, d, J = 11.2 Hz, H-6), 6.30 (1H, d, J = 11.2 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 8.0 (2×), 17.6, 21.7, 23.3, 23.6, 28.7, 29.7 (2×), 29.9, 30.7, 31.2 (2×), 35.2, 37.4, 38.3 (2×), 42.4, 44.8, 49.7, 59.1, 67.4, 67.6, 74.8, 115.8, 123.9, 125.1, 131.7, 142.2, 155.6. MS m/z (%): 490 (no M^+), 472 (7), 454 (4), 300 (71), 282 (34), 264 (42), 55 (100). HRMS m/z: 472.3380 (M^+-H_2O) (Calcd for $C_{30}H_{48}O_2S$: 472.3375).

4.67. 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 E. coli BL21 (DE3) pLys S (Novagen).[33] 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 15łμl 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, U.K.) and submitted for radioactivity counting. Each assay was performed at least twice in duplicate.

4.68. 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 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.[46] 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 performed in triplicate.

4.69. Osteoclast differentiation assay

Bone marrow cells were obtained from tibiae of 5- to 8-wk-old male mice of the ddY strain. Primary osteoblastic cells were prepared from the calvariae of newborn ddY mice as previously described.[47,48] Briefly, mouse bone marrow cells (1.5×10^5 cell/well) and primary osteoclasts (3×10^3 cells/well) were cocultured for 7 days in the presence of 1,25-$(OH)_2D_3$, 2MD or 19-norvitamin D analogs (10^{-8} M 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.[48,49] TRAP-positive multinucleated cells containing three or more nuclei were counted as osteoclasts, under microscopic examination. The results were expressed as means ± SEM of three cultures.

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