Synthetic Studies of Vitamin D Analogues. IX.[1] Synthesis and Differentiation-Inducing Activity of 1α,25-Dihydroxy-23-oxa-, thia-, and azavitamin D_3[2]

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Three vitamin D_3 analogues, 1α,25-dihydroxy-23-oxavitamin D_3 (3), 1α,25-dihydroxy-23-thiavitamin D_3 (4) and 1α,25-dihydroxy-23-azavitamin D_3 (5) were synthesized. In the differentiation-inducing activity of human myeloid leukemia cells into macrophages *in vitro*, the 23-aza analogue (5) showed the least activity, while no remarkable differences were observed between the 23-oxa analogue (3) and the 23-thia analogue (4), which were less active than 1α,25-dihydroxyvitamin D_3 (1).

Keywords vitamin D_3 analogue; 1α,25-dihydroxy-23-oxavitamin D_3; 1α,25-dihydroxy-23-thiavitamin D_3; 1α,25-dihydroxy-23-azavitamin D_3; differentiation-inducing activity; 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-22-oxavitamin D_3; vitamin D_3 synthesis

In the course of our study of modification of the side chain of 1α,25-dihydroxyvitamin D_3 (**1**), with the aim of separating the differentiation-inducing activity and the potential hypercalcemic action of **1**, we recently found that an almost complete separation of these types of physiological actions was successfully realized with 1α,25-dihydroxy-22-oxavitamin D_3 (**2**). We found that **2** was about 10 times more active than **1** in suppressing proliferation and inducing differentiation of human myeloid leukemia cells (HL-60), and about 1/20 more active in inducing the release of ^{45}Ca from prelabeled fetal mouse calvaria.[1,3] These findings stimulated our interest in a compound oxygenated at a different position, namely 1α,25-dihydroxy-23-oxavitamin D_3 (**3**). From the point of view of bioisosterism,[4] we also focused our attention on compounds bearing other heteroatoms at the 23 position, 1α,25-dihydroxy-23-thiavitamin D_3 (**4**) and 1α,25-dihydroxy-23-azavitamin D_3 (**5**). Although these vitamin D_3 analogues (**3**, **4** and **5**) have already been synthesized by Hesse starting from a degradation product of vitamin D_2,[5] no biological information is yet available. This paper deals with an alternative synthesis of **3**, **4** and **5**, and the preliminary evaluation of their differentiation-inducing activities.

In our synthesis of **3**, **4** and **5**, the diene alcohol (**15**) was chosen as the common intermediate. For the synthesis of **15**, the readily available 17(*Z*)-ethylidene diacetate (**6**)[6] seemed to be a reasonable starting material. Thus, the ene reaction of **6** with paraformaldehyde in the presence of boron trifluoride etherate[7] stereoselectively gave the 20(*S*)-alcohol (**7**) in 89% yield. Subsequent catalytic hydrogenation of the C-16 double bond in **7** was quantitatively achieved from the less congested α-face to afford the alcohol (**8**). After protection of the hydroxy group in **8** as the tetrahydropyranyl ether (**9**), the 1,3-diacetoxy groups in **9** were converted to the 1,3-disilyl ethers in **11** *via* the diol (**10**) in 58% overall yield from **7**. The tetrahydropyranyl ether moiety in **11** was then selectively cleaved by dimethylaluminum chloride[8] to give the alcohol (**12**), in 70% yield, which was transformed into the diene alcohol (**15**) by the following procedure in 63% overall yield; i) acetylation giving **13**, ii) bromination with *N*-bromosuccinimide then dehydrobromination with γ-collidine giving **14**, iii) hydrolysis with potassium carbonate in aqueous ethanol giving **15**.

The synthesis of the 23-oxa analogue (**3**) from **15** was first carried out. The alkylation of the hydroxy part in **15** was effected with isobutylene oxide to give the ether (**17**) in 47% yield. Subsequent irradiation of **17** with a 400 W mercury lamp through a Vycor filter, followed by thermal isomerization in refluxing ethanol and elimination of the silyl group with tetrabutylammonium fluoride, gave rise to 1α,25-dihydroxy-23-oxavitamin D_3 (**3**) in 6% yield. Subsequently, two other analogues (**4** and **5**) were prepared from **15**. Treatment of the mesylate (**16**), prepared from **15** in the usual manner, with 1-mercapto-2-methyl-2-hydroxypropane or 1-amino-2-methyl-2-hydroxypropane, afforded the sulfide (**18**) or the amine (**19**) in 47 and 65% yields, respectively. Both **18** and **19** were irradiated, thermally isomerized and desilylated yielding 1α,25-dihydroxy-23-thia- and 23-azavitamin D_3 (**4** and **5**), in 10 and 13% yields, respectively.

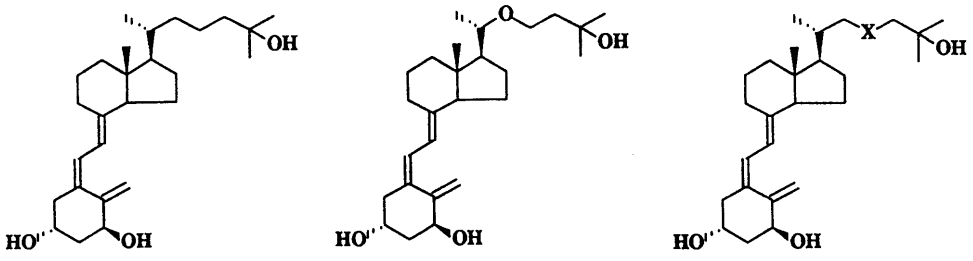

Chart 1

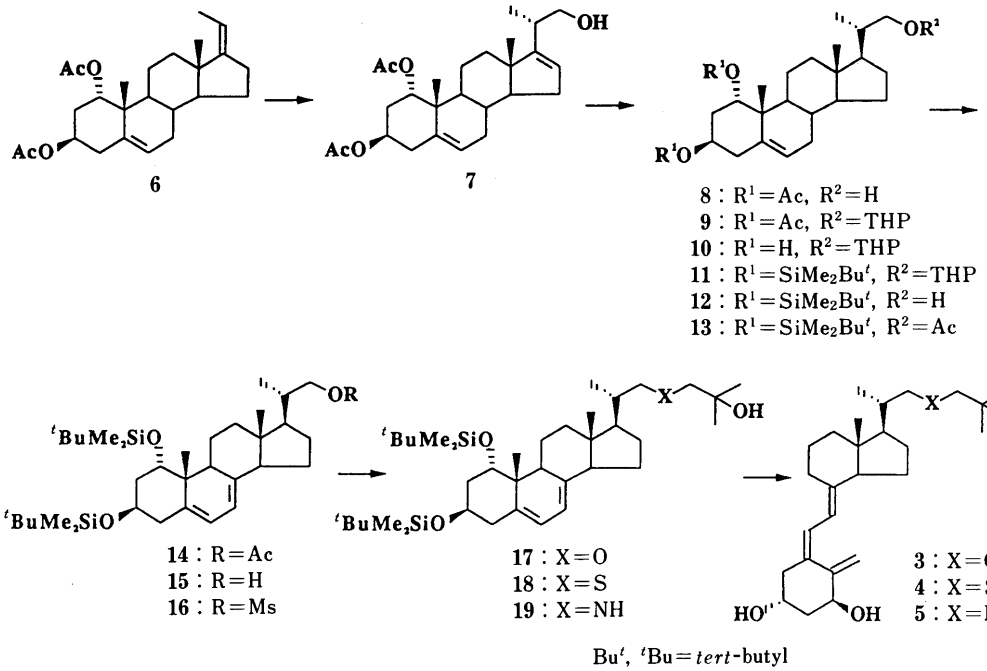

Chart 2

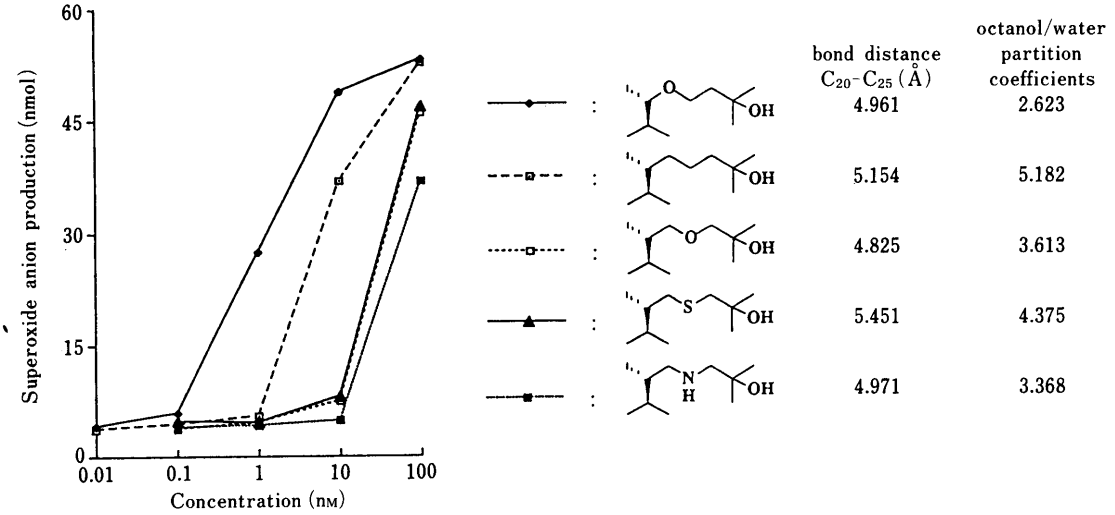

Fig. 1. Comparative Effects of Vitamin D_3 Analogues on the Induction of Superoxide Anion Production of HL-60

Figure 1 shows the preliminary results of the differentiation-inducing activity of HL-60 into macrophages *in vitro* estimated by superoxide anion production.[9] Among the three analogues synthesized, the 23-aza analogue (**5**) showed the least activity (about 1/8 of **1**), while no remarkable differences were observed between the 23-oxa analogue (**3**) and the 23-thia analogue (**4**), which had about 1/5 as much activity as 1α,25-dihydroxyvitamin D_3 (**1**). The calculated bond distance from C-20 to C-25 of each side chain,[10] and octanol/water partition coefficients,[11] are also shown in Fig. 1. It is suggestive that there are no strict relations between the differentiation-inducing activity and side chain length or hydrophilicity. The reason for the greatest potential for differentiation-inducing activity being in the 22-oxa analogue (**2**) (in this experiment, **2** was 6 times more active than **1**), is still ambiguous. Further pharmacological and physicochemical properties of these analogues are now under investigation, and will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and uncorrected. Infrared (IR) spectra were recorded with a Hitachi 270-30 spectrometer, proton nuclear magnetic resonance (^{1}H-NMR) spectra with a JEOL FX-200 spectrometer in $CDCl_3$ with tetramethylsilane as an internal reference, mass spectra (MS) with a Shimadzu GCMS-QP 1000 spectrometer and ultraviolet (UV) spectra with a Shimadzu UV-240 spectrometer. All reactions with the exception of hydrogenation were carried out under an atmosphere of dry argon. Flash column chromatography was carried out with Merck Silica gel 60, 230—400 mesh ASTM. Thin layer chromatography (TLC) was carried out with Merck Silica gel 60 F254 (0.25 mm thickness) pre-coated TLC plates, and preparative TLC was performed on 20 × 20 cm plates coated with 0.5 mm thickness of Merck Silica gel 60 F254. The phrase "residue upon work-up" refers to the residue when the organic layer was separated, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. All new compounds described in this experimental section were homogeneous on TLC.

(20*S*)-20-Methyl-5,16-pregnadien-1α,3β,21-triol 1,3-Diacetate (7) BF_3·

OEt_2 (80 μl) was added dropwise to a stirred solution of the 17(*Z*)-ethylidene diacetate (**6**) (2.59 g, 6.5 mmol) and paraformaldehyde (0.32 g, 6.5 mmol) in CH_2Cl_2 (20 ml). The mixture was stirred at room temperature for 1.5 h and poured into saturated $NaHCO_3$ (20 ml). The separated organic layer was washed with saturated NaCl, and the residue upon work-up was chromatographed using *n*-hexane–AcOEt (3 : 1, v/v) as the eluent to afford the (20*S*)-alcohol (**7**) (2.48 g, 89%) as a colorless foam. IR (neat): 3460, 1730, 1240, 1030 cm^{-1}. ^{1}H-NMR δ: 0.83 (3H, s), 1.05 (3H, d, J=7.0 Hz), 1.15 (3H, s), 2.03 (3H, s), 2.07 (3H, s), 3.58 (2H, br d, J=6.5 Hz), 5.35—5.65 (2H, br). MS *m/z*: 310 ($M^+ - CH_3COOH \times 2$, 100%).

(20*S*)-20-Methyl-5-pregnen-1α,3β,21-triol 1,3-Diacetate (8) The (20*S*)-alcohol (**7**) (1.72 g, 4.0 mmol) in AcOEt (90 ml) was hydrogenated in the presence of 5% platinum on carbon (250 mg) at room temperature. After the absorption of equimolar hydrogen, the insoluble material was filtered off. The filtrate was concentrated under reduced pressure to give the practically pure alcohol (**8**) (1.72 g) as a colorless foam, which was used without further purification. Preparative TLC developed with CH_2Cl_2–AcOEt (9 : 1, v/v) gave the analytically pure (**8**). IR (neat): 3510, 1735, 1245, 1050 cm^{-1}. ^{1}H-NMR δ: 0.69 (3H, s), 1.04 (3H, d, J=6.6 Hz), 1.08 (3H, s), 2.02 (3H, s), 2.05 (3H, s), 3.27—3.41 (1H, br), 3.63 (1H, br d, J=9.0 Hz), 4.80—5.00 (1H, m), 5.03—5.09 (1H, br), 5.54 (1H, br d, J=6.0 Hz). MS *m/z*: 312 ($M^+ - CH_3COOH \times 2$), 118 (100%). *Anal.* Calcd for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.13; H, 9.22.

(20*S*)-20-Methyl-21-(tetrahydropyran-2-yl)oxy-5-pregnen-1α,3β-diol 1,3-Diacetate (9) A mixture of the crude alcohol (**8**) (1.45 g), 3,4-dihydropyrane (0.42 g, 5.0 mmol) and Amberlyst-15 (200 mg) in CH_2Cl_2 (30 ml) was stirred at room temperature for 15 h. The insoluble material was filtered off. The filtrate was concentrated under reduced pressure to give the practically pure tetrahydropyranyl ether (**9**) (1.70 g), as a colorless foam, which was used without further purification. Preparative TLC developed with CH_2Cl_2 gave the analytically pure (**9**). IR (neat): 1740, 1240 cm^{-1}. ^{1}H-NMR δ: 0.69 (3H, s), 1.04 (3H, s), 2.01 (3H, s), 2.04 (3H, s), 4.44—4.60 (1H, br), 4.80—4.96 (1H, br), 5.04 (1H, br s), 5.44—5.58 (1H, br). MS *m/z*: 457 ($M^+ - CH_3COO$), 85 (100%).

(20*S*)-20-Methyl-21-(tetrahydropyran-2-yl)oxy-5-pregnen-1α,3β-diol (10) A solution of the crude tetrahydropyranyl ether (**9**) (1.07 g) in tetrahydrofuran (THF) (50 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (0.64 g, 16.8 mmol) in THF (50 ml). The mixture was refluxed for 1 h, quenched with 10% NaOH and the organic layer was separated. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with saturated NaCl and the residue upon work-up was chromatographed using CH_2Cl_2–AcOEt (1 : 2, v/v) as the eluent to afford the diol (**10**) (1.13 g, 78% from **8**) as a colorless foam. IR (neat): 3400 cm^{-1}. ^{1}H-NMR δ: 0.69 (3H, s), 1.03 (3H, s), 1.10 (3H, d, J=7.0 Hz), 3.04 (1H, br t), 3.30—3.56 (2H, br), 3.72—4.08 (3H, br), 4.48—4.60 (1H, br d), 5.55—5.64 (1H, br d). MS *m/z*: 432 (M^+), 85 (100%).

(20*S*)-20-Methyl-1α,3β-bis(*tert*-butyldimethylsilyloxy)-21-(tetrahydropyran-2-yl)oxy-5-pregnene (11) A mixture of the diol (**10**) (1.13 g, 2.6 mmol), imidazole (3.56 g, 52.0 mmol), hydroxybenzotriazole (136 mg, 0.8 mmol) and *tert*-BuMe_2SiCl (3.36 g, 15.6 mmol) in dimethylformamide (DMF) (35 ml) was stirred at 65—70 °C for 48 h. Then the mixture was poured into H_2O and extracted with ether. The extract was washed with saturated NaCl and the residue upon work-up was chromatographed using *n*-hexane–CH_2Cl_2 (3 : 1, v/v) as the eluent to afford the 1,3-disilyl ether (**11**) (1.27 g, 74%) as a colorless foam. ^{1}H-NMR δ: 0.04 (9H, s), 0.05 (3H, s), 0.69 (3H, s), 0.89 (18H, s), 0.95 (3H, s), 1.15 (3H, d, J=6.0 Hz), 4.45—4.65 (1H, br), 5.36—5.55 (1H, br). MS *m/z*: 660 (M^+), 85 (100%).

(20*S*)-20-Methyl-1α,3β-bis(*tert*-butyldimethylsilyloxy)-5-pregnen-21-ol (12) To a stirred solution of the 1,3-disilyl ether (**11**) (1.27 g, 1.9 mmol) in CH_2Cl_2 (20 ml), was added Me_2AlCl (1 mol/l in *n*-hexane, 3.84 ml, 3.8 mmol) dropwise at −5—−10 °C. The mixture was then stirred at room temperature for 15 h and quenched by saturated $NaHCO_3$. The separated organic layer was washed with saturated NaCl and the residue upon work-up was chromatographed using CH_2Cl_2 as the eluent to afford the alcohol (**12**) (0.79 g, 71%) as colorless glasses: mp 165—167 °C. IR (Nujol): 3250 cm^{-1}. ^{1}H-NMR δ: 0.05 (9H, s), 0.06 (3H, s), 0.70 (3H, s), 0.88 (18H, s), 0.96 (3H, s), 1.10 (3H, d, J=6.0 Hz), 5.30—5.55 (1H, br). MS *m/z*: 576 (M^+), 387 (100%). *Anal.* Calcd for $C_{34}H_{64}O_3Si_2$: C, 70.77; H, 11.18. Found: C, 70.24; H, 11.17.

(20*S*)-20-Methyl-1α,3β-bis(*tert*-butyldimethylsilyloxy)-5-pregnen-21-ol Acetate (13) A mixture of the alcohol (**12**) (785 mg, 1.36 mmol), 4,4-dimethylaminopyridine (100 mg), pyridine (20 ml) and acetic anhydride (20 ml) was stirred at room temperature for 20 h. Then the mixture was poured into H_2O and extracted with AcOEt. The extract was washed with 5% HCl, saturated $NaHCO_3$ and saturated NaCl, and the residue upon work-up was chromatographed using *n*-hexane–CH_2Cl_2 (2 : 1, v/v) as the eluent to afford the acetate (**13**) (702 mg, 84%) as colorless glasses: mp 104—105 °C. IR (Nujol): 1740, 1250 cm^{-1}. ^{1}H-NMR δ: 0.05 (9H, s), 0.06 (3H, s), 0.70 (3H, s), 0.88 (18H, s), 0.96 (3H, s), 1.05 (3H, d, J=6.0 Hz), 2.03 (3H, s), 5.22—5.50 (1H, br). MS *m/z*: 618 (M^+), 429 (100%). *Anal.* Calcd for $C_{36}H_{66}O_4Si_2$: C, 69.84; H, 10.75. Found: C, 69.86; H, 10.51.

(20*S*)-20-Methyl-1α,3β-bis(*tert*-butyldimethylsilyloxy)-5,7-pregnadien-21-ol Acetate (14) A mixture of the acetate (**13**) (702 mg, 1.13 mmol) and *N*-bromosuccinimide (262 mg, 1.47 mmol) in *n*-hexane (10 ml) was refluxed for 1.25 h. After cooling to room temperature, the precipitate was filtered out. The filtrate was concentrated under reduced pressure. The residue was dissolved in xylene (10 ml) and γ-collidine (1.5 ml). The resulting mixture was refluxed for 1.5 h, then cooled to room temperature, and diluted with toluene and H_2O. The organic layer was washed with saturated NaCl and the residue upon work-up was chromatographed using *n*-hexane–CH_2Cl_2 (3 : 2, v/v) as the eluent to afford the 5,7-diene (**14**) (590 mg, 87%) as a colorless semi-solid softening at 43—46 °C. IR (Nujol): 1745, 1250 cm^{-1}. ^{1}H-NMR δ: 0.05 (9H, s), 0.06 (3H, s), 0.64 (3H, s), 0.89 (18H, s), 0.96 (3H, s), 1.05 (3H, d, J=6.0 Hz), 2.02 (3H, s), 5.15—5.70 (2H, br). MS *m/z*: 616 (M^+), 427 (100%). UV λ_{max} nm: 293, 281, 270. *Anal.* Calcd for $C_{36}H_{64}O_4Si_2$: C, 70.07; H, 10.45. Found: C, 69.85; H, 10.69.

(20*S*)-20-Methyl-1α,3β-bis(*tert*-butyldimethylsilyloxy)-5,7-pregnadien-21-ol (15) A mixture of the 5,7-diene (**14**) (580 mg, 0.94 mmol) and K_2CO_3 (390 mg, 2.82 mmol) in EtOH (30 ml) was stirred at 30 °C for 21 h. The solvent was then evaporated under reduced pressure. The residue was taken up with CH_2Cl_2 and H_2O. The organic layer was washed with saturated NaCl and the residue upon work-up was chromatographed using CH_2Cl_2 as the eluent to afford the diene alcohol (**15**) as colorless glasses: mp 154—156 °C. IR (Nujol): 3270 cm^{-1}. ^{1}H-NMR δ: 0.05 (3H, s), 0.06 (3H, s), 0.11 (3H, s), 0.64 (3H, s), 0.89 (18H, s), 0.91 (3H, s), 1.09 (3H, d, J=6.0 Hz), 3.33—3.49 (1H, m), 3.59—3.76 (2H, br d), 3.94—4.14 (1H, br), 5.29—5.39 (1H, m), 5.60 (1H, d, J=6.0 Hz). MS *m/z*: 574 (M^+), 385 (100%). UV λ_{max} nm: 293, 281, 270. *Anal.* Calcd for $C_{34}H_{62}O_3Si_2$: C, 71.02; H, 10.87. Found: C, 71.20; H, 10.64.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-23-oxa-5,7-cholestadien-25-ol (17) To a stirred mixture of the diene alcohol (**15**) (65 mg, 0.11 mmol), dibenzo-18-crown-6 (25 mg) and isobutylene oxide (250 μl) in benzene (3 ml), was added *tert*-BuOK (139 mg, 1.24 mmol) at room temperature. The resulting mixture was then refluxed for 1 h and diluted with toluene. The mixture was washed with H_2O and saturated NaCl, and the residue upon work-up was chromatographed using *n*-hexane–AcOEt (9 : 1, v/v) as the eluent to afford the ether (**17**) (34 mg, 47%) as a colorless powder. ^{1}H-NMR δ: 0.07 (9H, s), 0.11 (3H, s), 0.65 (3H, s), 0.89 (21H, s), 1.06 (3H, d, J=6.0 Hz), 1.20 (6H, s), 5.15—5.39 (1H, m), 5.55 (1H, br d, J=6.0 Hz). MS *m/z*: 646 (M^+), 457 (100%). UV λ_{max} nm: 293, 282, 271.

1α,25-Dihydroxy-23-oxavitamin D_3 (3) A solution of the ether (**17**) (33.4 mg, 0.05 mmol) in EtOH (400 ml) was irradiated with a 400 W high pressure mercury lamp through a Vycor filter at 0 °C under argon bubbling for 3.5 min, then refluxed for 1.5 h. Removal of the solvent under reduced pressure gave an oil, which was dissolved in THF (5 ml) and *n*-Bu_4NF (1 mol/l in THF) (750 μl, 0.75 mmol). The resulting mixture was stirred at room temperature for 15 h, then diluted with AcOEt and washed with H_2O and saturated NaCl. The residue upon work-up was submitted to a two-stage purification: 1) flash column chromatography using CH_2Cl_2–EtOH (5 : 0.3, v/v) as the eluent, 2) preparative TLC developed twice with CH_2Cl_2–EtOH (10 : 1, v/v), to afford the 23-oxavitamin D_3 (**3**) (1.19 mg, 6%). ^{1}H-NMR δ: 0.57 (3H, s), 1.05 (3H, d, J=6.2 Hz), 1.21 (6H, s), 3.11—3.47 (4H, m), 4.03—4.27 (1H, br), 4.35—4.47 (1H, br), 4.99 (1H, s), 5.35 (1H, s), 6.00 (1H, d, J=11.4 Hz), 6.36 (1H, d, J=11.4 Hz). MS *m/z*: 418 (M^+), 59 (100%). UV λ_{max}: 263 nm, λ_{min}: 227 nm.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-23-thia-5,7-cholestadien-25-ol (18) To a stirred solution of the diene alcohol (**15**) (57.5 mg, 0.10 mmol) in pyridine (2 ml), was added MsCl (24 μl, 0.30 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 45 min, then poured into H_2O and extracted with AcOEt. The extract was washed with saturated $CuSO_4$, H_2O, saturated $NaHCO_3$, H_2O and saturated NaCl, and the residue upon work-up was the practically pure mesylate (**16**) (67 mg) which was used without further purification. To a stirred mixture of the

above-mentioned mesylate (**16**) (67 mg; crude), 1-mercapto-2-methyl-2-hydroxypropane (300 μl) and hexamethylphosphoramide (HMPA, 300 μl) in THF (20 ml), was added NaH (60%) (180 mg, 4.50 mmol). The resulting mixture was stirred at room temperature for 4 h, then poured into H_2O and extracted with AcOEt. The extract was washed with saturated NaCl and the residue upon work-up was chromatographed using *n*-hexane–AcOEt (9 : 1, v/v) as the eluent to afford the sulfide (**18**) (31 mg, 47%) as a colorless oil. ^{1}H-NMR δ: 0.06 (9H, s), 0.11 (3H, s), 0.63 (3H, s), 0.87 (21H, s), 1.08 (3H, d, J=6.0 Hz), 1.26 (6H, s), 5.20—5.44 (1H, m), 5.48—5.75 (1H, br). MS *m/z*: 662 (M^+), 473 (100%). UV λ_{max} nm: 293, 282, 271.

1α,25-Dihydroxy-23-thiavitamin D_3 (4) A mixture of the sulfide (**18**) (40.6 mg, 0.06 mmol) in EtOH (400 ml) was irradiated with a 400 W high pressure mercury lamp through a Vycor filter at 0 °C under argon bubbling for 4.5 min, then refluxed for 1.5 h. Removal of the solvent under reduced pressure gave an oil, which was dissolved in THF (7 ml) and *n*-Bu_4NF (1 mol/l in THF) (1 ml, 1 mmol). The resulting mixture was stirred at room temperature for 16 h, then diluted with AcOEt and washed with H_2O and saturated NaCl. The residue upon work-up was submitted to a two-stage purification: 1) flash column chromatography using CH_2Cl_2–EtOH (5 : 0.3, v/v) as the eluent, 2) preparative TLC developed twice with CH_2Cl_2–EtOH (10 : 1, v/v), to afford the 23-thiavitamin D_3 (**4**) (2.67 mg, 10%). ^{1}H-NMR δ: 0.56 (3H, s), 1.10 (3H, d, J=6.2 Hz), 1.28 (6H, s), 2.53—2.89 (2H, m), 2.63 (2H, s), 4.09—4.29 (1H, br), 4.36—4.49 (1H, br), 4.99 (1H, s), 5.32 (1H, s), 6.01 (1H, d, J=11.4 Hz), 6.37 (1H, d, J=11.4 Hz). MS *m/z*: 434 (M^+), 134 (100%). UV λ_{max}: 263 nm, λ_{min}: 227 nm.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-23-aza-5,7-cholestadiene-25-ol (19) A mixture of the crude mesylate (**16**) [70 mg; prepared from the alcohol (**15**) (57.5 mg, 1 mmol) in the same manner described in the preparation of the sulfide (**18**)] and 1-amino-2-methyl-2-hydroxypropane (1 ml) was stirred at 60 °C for 3 h. The mixture was then taken up with H_2O and CH_2Cl_2. The organic layer was washed with saturated NaCl and the residue upon work-up was chromatographed using CH_2Cl_2–EtOH (10 : 1, v/v) as the eluent to afford the amine (**19**) (42 mg, 65%) as a pale yellow gum. ^{1}H-NMR δ: 0.05 (3H, s), 0.07 (3H, s), 0.66 (3H, s), 0.90 (18H, s), 0.91 (3H, s), 1.09 (3H, d, J=6.0 Hz), 1.23 (6H, s), 3.68—3.76 (1H, br), 3.96—4.16 (1H, br), 5.28—5.36 (1H, br), 5.59 (1H, br d, J=6.0 Hz). MS *m/z*: 645 (M^+), 43 (100%). UV λ_{max} nm: 293, 281, 270.

1α,25-Dihydroxy-23-azavitamin D_3 (5) A mixture of the amine (**19**) (38.2 mg, 0.059 mmol) in EtOH (400 ml) was irradiated with a 400 W high pressure mercury lamp through a Vycor filter at 0 °C under argon bubbling for 4 min, then refluxed for 1.5 h. Removal of the solvent under reduced pressure gave an oil, which was dissolved in THF (7 ml) and *n*-Bu_4NF (1 mol/l in THF) (1 ml, 1 mmol). The resulting mixture was stirred at room temperature for 15 h, then diluted with AcOEt and washed with H_2O and saturated NaCl. The residue upon work-up was purified by preparative TLC developed once with CH_2Cl_2–EtOH (3 : 1, v/v) to afford the 23-azavitamin D_3 (**5**) (3.2 mg, 13%). ^{1}H-NMR δ: 0.57 (3H, s), 1.02 (3H, d, J=6.2 Hz), 1.23 (6H, s), 2.51 (2H, s), 4.11—4.28 (1H, br), 4.31—4.50 (1H, br), 4.99 (1H, s), 5.32 (1H, s), 6.01 (1H, d, J=11.4 Hz), 6.37 (1H, d, J=11.4 Hz). MS *m/z*: 417 (M^+), 43 (100%). UV λ_{max}: 263 nm, λ_{min}: 227 nm.

Differentiation-Inducing Activity HL-60, kindly provided by Dr. T. Suda (Showa University, Tokyo, Japan), was cultured in an RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and 20 μg/ml gentamicin at 37 °C in a humidified atmosphere of 5% CO_2 in air. Induction of differentiation was estimated by the ability of the cell to generate superoxide anion. Vitamin D-induced cells were obtained by seeding HL-60 cells at 1×10^5/ml in a growth media and culturing it for 4 d in the presence of various concentrations of vitamin D_3 analogues. The cells were washed free of the compounds and suspended in a 1.5 ml reaction mixture containing 80 μM ferricytochrome c (Sigma Chemical Co., St. Louis, MO) and 500 ng/ml phorbol myristate acetate (Sigma) in a 0.1% gelatin Hanks' balanced salt solution without phenol red. The mixture was incubated at 37 °C for 60 min and centrifuged for 10 min at $400 \times g$ at 4 °C. The optical density of the supernates was determined with a Hitachi U-3200 dual-wavelength (550 *versus* 540 nm) spectrophotometer. The amount of superoxide anion generated was calculated with a molar extinction coefficient of 19.1×10^3/cm.

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References and Notes

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