Synthesis of side-chain homologated analogs of 1,25-dihydroxycholecalciferol and 1,25-dihydroxyergocalciferol

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*A novel synthesis of side-chain homologated analogs of vitamin D isomers has been described. The synthesis allows for the insertion of the double bond into the C-24 position of the side chain. The key synthetic step involves the coupling of a new C_{24}-vitamin D synthon with the respective side-chain fragment. The method is illustrated by the preparation of (24E)-24,24a-dehydro-24,24-dihomo-1,25-dihydroxycholecalciferol (**1**) and (24b R)- and (24b S)-24,24-dihomo-1,25-dihydroxyergocalciferols (**2** and **3**).* Trans *geometry of the newly formed double bond in the side chain was confirmed by high field nuclear magnetic resonance spectra.* (*Steroids* **56**:311–315, 1991)

Keywords: steroids; cholecalciferol; ergocalciferol; side-chain analogs; vitamin D isomers; skin disorders; Julia olefination of vitamin D aldehyde; vitamin D analogs

Introduction

Vitamin D analogs modified in the aliphatic side chain have recently become interesting targets for numerous synthetic approaches.[1–3] This might be due to the increased activity of these analogs in causing differentiation of human promyelocytes[4] and in treatment of skin disorders,[5] while having substantially diminished activity in mobilization of calcium. Special interest has been focused on analogs with the side chain extended by one to three carbon units in a straight or branched manner.[6] Insertion of a *trans* double bond at C-22 of 24-homologated analogs did not significantly affect the activities[7] compared with the respective analogs with saturated side chain. However, the fast inactivating metabolism of a side chain-modified analog may involve not only the typical C-24 oxidation, but also the reduction of C-22 double bond.[8]

We assumed that the transposition of the double bond in the side chain from the natural C-22 to the metabolically important C-24 position might cause further decrease in inborn vitamin D-type activity, retaining the properties associated with the extended side chain.

To investigate the biologic consequences of the new location of the unsaturation in the side chain, we devised a modified synthetic approach that provides access to the novel group of vitamin D analogs with the double bond at C-24. In this report, we have exemplified our approach by the synthesis of vitamin D_3 analog **1** (Figure 1) as well as by the first preparation of vitamin D_2 analogs with the homologated side chain (compounds **2** and **3**).

Experimental

Starting vitamin D intermediate **10** (Scheme 1) was prepared in the Hormone Department of this institute from methyl hyodeoxycholate by the known methods. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl. *n*-Butyllithium (*n*-BuLi) in hexane was titrated with *n*-propanol against 1,10-phenanthroline. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrophotometer model 1725X as films of oily substances or in KBr pellets. Ultraviolet (UV) spectra were taken on a Shimadzu Model 160A UV-VIS spectrophotometer in solvents indicated. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 100 MHz on a Bruker WP-100SY or at 500 MHz on a Bruker AM 500 spectrometer in solvents indicated, downfield from the internal TMS. Mass spectra (MS) were recorded on a Finnigan MAT spectrometer model 8200. Column chromatography was performed on silica gel Si 60 (230 to 400 mesh, Merck). Thin-layer chromatography (TLC) precoated plates were used in solvent systems: A, hexane/ethyl acetate 7 : 3; B, hex-

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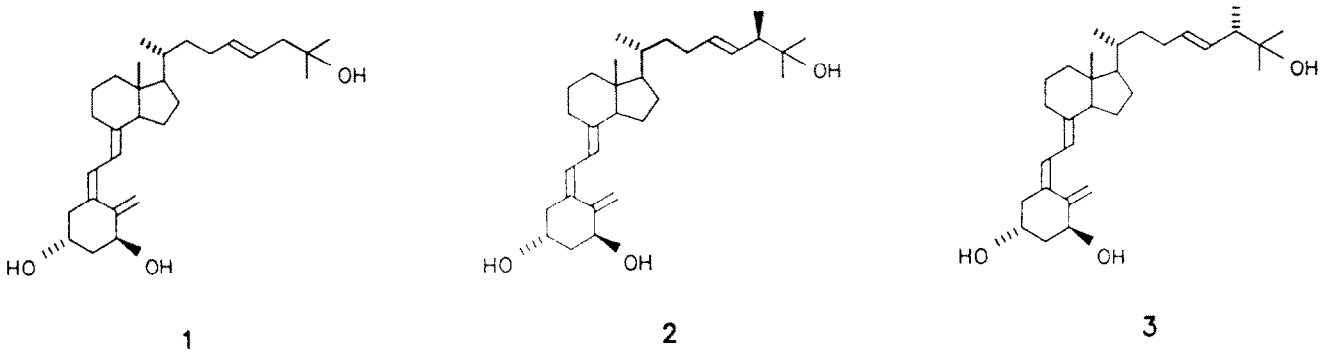


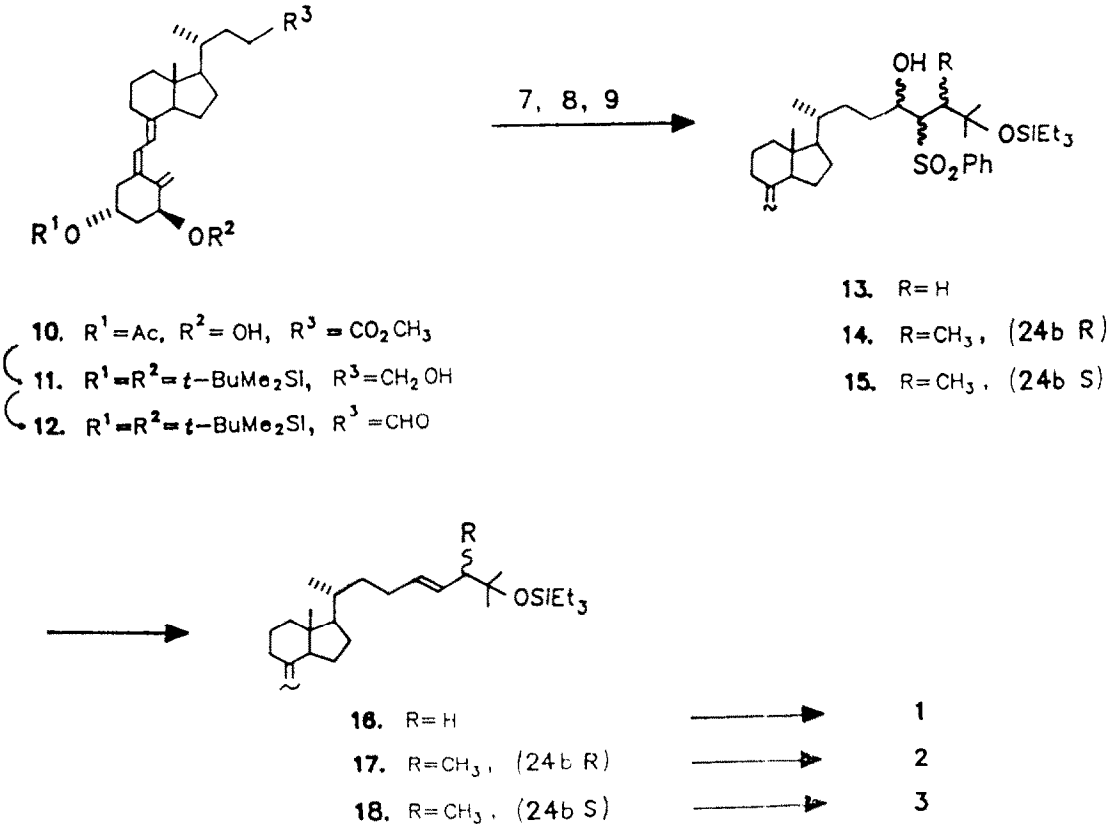
1 2 3

Figure 1

ane/ethyl acetate 6 : 1; C, hexane/ethyl acetate 1 : 1. High-performance liquid chromatography (HPLC) was done using a Knauer instrument model 64 and Lichrosorb Si 60 columns, 5 μm, 10 × 25 cm, and 4 × 25 cm, and Nucleosil 5 C-18 column, 4 × 25 cm, in solvent systems: A, methanol/water 4 : 1; B, methanol/water 95 : 5; C, hexane/ethyl acetate 98 : 2; D, hexane/2-propanol 85 : 15; E, hexane/ethyl acetate 96 : 4.

Preparation of 2-methyl-4-(phenylsulfonyl)-2-[(triethylsilyl)oxy]butane (7)

A solution of 13.48 g (88.1 mmol) of 3-bromopropionic acid (**4**) in 30 g (980 mmol) of methanol, containing 0.5 ml of concentrated H_2SO_4, was refluxed for 5 hours. Standard work-up procedure gave 10.3 g (70%) of respective methyl ester. Methylmagnesium bromide (50 ml, 3 M solution in ethyl ether) was added under argon to the solution of 7.3 g of this ester in 100 ml of THF. The mixture was stirred for 48 hours at room temperature (RT) and then acidified to pH 1 with 2 N HCl saturated with NaCl. Extraction with benzene and standard work-up gave 6.5 g (64%) of alcohol **5**. Potassium *t*-butylate (6 g) was added to the solution of alcohol **5** in DMF, followed by 5.5 g of thiophenol. The mixture was stirred for 24 hours under argon. Then, 20 ml of water was added and the product was extracted with benzene. Column chromatography yielded 5.2 g



Scheme 1

(68%) of sulfide **6**. *m*-Chloroperoxybenzoic acid (3.6 g) was added to the solution of this sulfide in 30 ml of CH_2Cl_2. Usual work-up gave 5.0 g (84%) of the respective sulfone. Then, 1.4 g of triethylsilyl chloride and 1.0 g of imidazole was added to the solution of 1 g of this sulfone in 5 ml of DMF. The mixture was stirred at RT for 2 hours and diluted with dichloromethane. Usual work-up and column chromatography gave 1.8 g of protected sulfone **7**: HPLC (system E) retention volume (R_V) 39 ml; UV (hexane/2-propanol 9 : 1) λ_{max} 270.4, 263.4, 257.8, nm, λ_{min} 235.8, 260.2, 267.8 nm; IR (KBr) 2,964, 2,914, 2,884, 1,458, 1,309, 1,160, 1,125, 1,050 cm^{-1}: MS m/z 342 (M^+, 6), 313 (100), 227 (8); ^1H NMR ($CDCl_3$) δ 0.58 (6H, m, $SiCH_2CH_3$), 0.86 (9H, m, $SiCH_2CH_3$), 1.18 (6H, s, 1-CH_3, 2-CH_3), 7.6, and 7.9 (5H, m, Ar).

*Preparation of (5Z,7E)-(1S,3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-9,10-secochola-5,7,10(19)-trien-24-ol (**11**)*

A solution of KOH in methanol (10 ml, 0.1 N) was added with stirring to the solution of ester **10** (100 mg, 0.23 mmol) in 10 ml of anhydrous THF. Stirring was continued for 80 minutes at RT (TLC, system A). Extraction with ethyl acetate gave 84.5 mg (93%) of respective 1,3-dihydroxyester as a pale yellow oil. To this oil dissolved in 5 ml of DMF, 250 mg (3.6 mmol) of imidazole was added, followed by solution of 250 mg (1.6 mmol) of *t*-butyldimethylsilyl chloride in 2 ml of DMF. The mixture was stirred at 55 C for 15 minutes under argon (TLC, system A). The product was extracted with hexane, worked up in the usual way, and filtered through Sep-Pak. Removal of solvents gave 135 mg (97%) of respective silyl ester as a pale yellow oil. Solution of 135 mg (0.21 mmol) of this product in 5 ml of THF was cooled to 0 C under argon, and 25 mg (0.65 mmol) of $LiAlH_4$ was added. This was vigorously stirred for 15 minutes at 0 C. Then 1 ml of 10% water in THF was added, and stirring was continued for 15 minutes. The product was extracted with ethyl acetate. Sep-Pak filtration and removal of solvents afforded 85 mg (67%) of (5Z,7E)-(1S,3R)-1,3-bis[(*t*-butyldimethyl-silyl)oxy]-9,10-secochola-5,7,10(19)-trien-24-ol (**11**) as a colorless oil: IR (KBr) 3,331, 1,483, 1,388, 1,269 cm^{-1}; UV (hexane/2-propanol 9 : 1) λ_{max} 264 nm, λ_{min} 227 nm; NMR (CD_3COCD_3) δ 0.00 (12H, s, Si-CH_3), 0.11 (18H, s, $SiC(CH_3)_3$), 0.55 (3H, s, 18-CH_3), 0.95 (3H, d, 21-CH_3), 4.28 (1H, m, 3-H), 4.45 (1H, m, 1-H), 4.84 (1H, m, 19Z-H), 5.25 (1H, m, 19 E-H), 6.08 (1H, d, 7-H), 6.31 (1H, d, 6-H); MS m/z 602 (M^+, 11), 587 (2), 470 (55), 248 (100); HPLC (system B) R_V 16 ml.

*Preparation of (5Z,7E)-(1S,3R)-1,3-bis[t-butyldimethylsilyloxy]-9,10-secochola-5,7,10(19)-trien-24-al (**12**)*

A solution of oxalyl chloride (60 μl, 0.68 mmol) in 1 ml of CH_2Cl_2 was added to the solution of 100 μl (1.4 mmol) of DMSO in 3 ml of CH_2Cl_2 at −60 C. The mixture was stirred for 10 minutes at −60 C, then a solution of 60 mg (0.1 mmol) of alcohol **11** in 1 ml of CH_2Cl_2 was added dropwise. Stirring at −60 C was continued until no starting material was detected (TLC, system B). Triethylamine (0.2 ml) was then added and the product was extracted with ethyl acetate. Standard work-up and Sep-Pak filtration gave 38 mg (63%) of aldehyde **12** as a pale yellow oil: HPLC (system C) R_V 18.2 ml; IR (KBr) 1,736, 1,478, 1,388, 1,269, 1,090 cm^{-1}; UV (hexane/2-propanol 9 : 1) λ_{max} 264 nm, λ_{min} 228 nm; NMR (CD_3COCD_3) δ 0.00 (12H, s, $SiCH_3$), 0.12 (18H, s, $SiC(CH_3)_3$), 0.69 (3H, s, 18-CH_3), 0.99 (3H, d, 21-CH_3), 4.26 (1H, m, 3-H), 4.46 (1H, m, 1-H), 4.85 (1H, m, 19Z-H), 5.25 (1H, m, 19E-H), 6.07 (1H, d, 7-H), 6.31 (1H, d, 6-H), 9.74 (1H, m, 24-H); MS m/z 600 (M^+, 12), 468 (54), 411 (7), 248 (100).

*Preparation of (24E)-24,24a-dehydro-24,24-dihomo-1,25-dihydroxycholecalciferol (**1**)*

Diisopropylamine (40 μl, 0.28 mmol) was added to 85 μl (0.123 mmol) of 1.5 M *n*-BuLi in hexane at −75 C under argon in the presence of 1,10-phenanthroline. After 20 minutes of stirring at −75 C, a solution of 50 mg (0.16 mmol) of sulfone **7** in 200 μl of THF was added. Stirring was continued for 30 minutes and a solution of 20 mg (0.033 mmol) of aldehyde **12** in 200 μl of THF was added. The mixture was stirred at −70 C for 1.5 hours. Then 1 ml of saturated NH_4Cl aqueous was added at RT. Products were extracted with ethyl acetate. Preparative HPLC (system C, column 10 × 25 cm) gave the recovered aldehyde **12** (3 mg, R_V 12 ml), sulfone **7** (22 mg, R_V 64 ml), and product **13** (6.2 mg, 20%, R_V 56 ml) as a colorless oil. Anhydrous Na_2HPO_4 (80 mg) was added to 0.5 ml of saturated solution of Na_2HPO_4 in methanol, and the mixture was cooled to 0 C under argon. To this mixture, a solution of 3 mg of vitamin D sulfone **13** in 0.5 ml of THF was added. The mixture was stirred for 5 minutes and 200 mg of 5% sodium amalgam was added. The mixture was stirred for 3 hours at 5 C under argon. Hexane (2 ml) was then added, supernatant decanted, and the precipitate washed with hexane. Standard work-up gave protected triol **16**. Tetrabutylammonium fluoride (20 μl, 1 M solution in THF) was added to the solution of compound **16** in 0.5 ml of THF, and the mixture was stirred at 55 C for 2 hours under argon. The same amount of fluoride was then added, and the mixture was stirred until no substrate or intermediates were detected (TLC, system C). Ethyl acetate extraction and preparative HPLC (system D, column 10 × 25 cm, R_V 33 ml) gave 645 μg of triol **1** (46% from sulfone **13**) as a colorless oil: UV (hexane/2-propanol 9 : 1) λ_{max} 263.6 nm, λ_{min} 237.6 nm; MS m/z 442 (M^+, 5), 424 (12), 285 (12), 267 (6), 251 (4), 152 (24), 134 (62), 59 (100); NMR (CD_3COCD_3) δ 0.57 (3H, s, 18-CH_3), 0.96 (3H, d, 21-CH_3), 4.17 (1H, m, 3-H), 4.39 (1H, m, 1-H), 4.86 (1H, s, 19Z-H), 5.31 (1H, s, 19E-H), 5.48 (2H, m, 24-H, 24a-H), 6.09 (1H, d, 7-H), 6.28 (1H, d, 6-H).

Preparation of (24b R)-24,24-dihomo-1,25-dihydroxyergocalciferol (2)

Analog **2** was obtained by the method described above from 30 mg (0.096 mmol) of sulfone **8**[12] ($[\alpha]_D^{20}$ = +23.4 [c 0.71 2-propanol in hexane]) and 8.5 mg (0.014 mmol) of aldehyde **12**. Preparative HPLC (system C, column 10 × 25 cm) gave recovered aldehyde **12** (1 mg, R_V 12 ml), sulfone **8** (10 mg, R_V 64 ml), and product **14** (3.0 mg, 19%, R_V 55 ml) as a colorless oil. Vitamin D sulfone **14** (3 mg, 3.1 mmol) was transformed into protected triol **17**, and then deprotected to give triol **2**. Preparative HPLC (system D, column 10 × 25 cm, R_V 32 ml) gave 420 μg of triol **2** (30% from sulfone **14**) as a colorless oil: UV (hexane/2-propanol 9 : 1) λ_{max} 263.2 nm, λ_{min} 229.0 nm; MS m/z 438 ($M^+ - H_2O$), 420 (31), 405 (8), 195 (11), 152 (6), 93 (24), 59 (88); NMR (CD_3COCD_3) δ 0.57 (3H, s, 18-CH_3), 0.96 (3H, d, 21-CH_3), 0.99 (3H, d, 28-CH_3), 4.17 (1H, m, 3-H), 4.39 (1H, m, 1-H), 4.87 (1H, s, 19Z-H), 5.31 (1H, s, 19E-H), 5.47 (2H, m, 24-H, 24a-H), 6.09 (1H, d, 7-H), 6.28 (1H, d, 6-H).

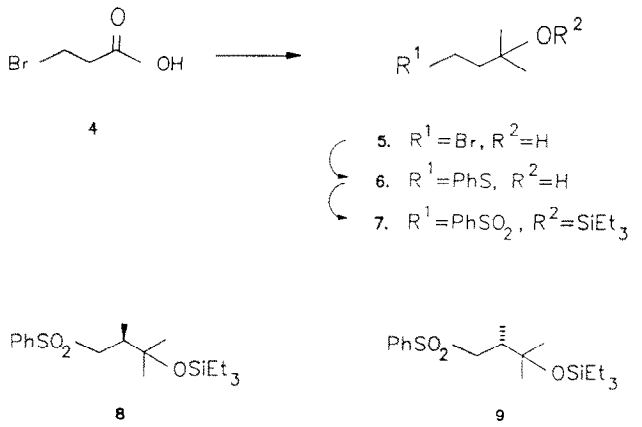
Preparation of (24b S)-24,24-dihomo-1,25-dihydroxyergocalciferol (3)

Analog **3** was prepared by the method described previously from 35 mg (0.112 mmol) of sulfone **9**[12] ($[\alpha]_D^{20}$ = −23.6 [c 0.70 5% 2-propanol in hexane]) and 15 mg (0.112 mmol) of aldehyde **12**. Preparative HPLC (system C, column 10 × 25 cm) gave recovered aldehyde **12** (2 mg, R_V 12 ml), sulfone **9** (12 mg, R_V 65 mL), and product **15** (6.1 mg, 21%, R_V 54 ml) as a colorless oil. This was converted to protected triol **18**, then to triol **3**. Preparative HPLC (system D, column 10 × 25 cm, R_V 34 ml) gave 810 μg of triol **3** (31% from sulfone **15**) as a colorless oil: UV (hexane/2-propanol 9 : 1) λ_{max} 265.2 nm, λ_{min} 228.0 nm; NMR (CD_3COCD_3) δ 0.57 (3H, s, 18-CH_3), 0.96 (3H, d, 21-CH_3), 1.00 (3H, d, 28-CH_3), 4.16 (1H, m, 3-H), 4.38 (1H, m, 1-H), 4.86 (1H, s, 19Z-H), 5.31 (1H, s, 19E-H), 5.47 (2H, m, 24-H and 24a-H), 6.09 (1H, d, 7-H), 6.28 (1H, d, 6-H).

Results and discussion

Our present method for the preparation of side-chain homologated analogs is a modification of the general synthetic strategy[9] that has already found several synthetic applications.[7,10,11] The method described here is specifically designed for analogs with the extended side chain. To obtain 24-dehydro compounds, we have now prepared a new C-24 vitamin D synthon (**12**, Scheme 1) as a key intermediate for the facile preparation of analogs **1**, **2**, and **3** (Figure 1).

Side chain fragments **7**, **8**, and **9** (Scheme 2) have been prepared by the known methods.[9,12] Thus, the preparation of the protected sulfone **7** started from readily available 3-bromopropionic acid (**4**). This was esterified in acidic methanol and reacted with methylmagnesium bromide to give tertiary alcohol **5**. Standard construction of the sulfone moiety (i.e., sulfide **6** formation with potassium thiophenoxide and oxidation



Scheme 2

with 3-chloroperbenzoic acid) followed by the protection of hydroxyl provided silylated sulfone **7** in 26% overall yield from **4**. Our preparation of protected sulfones **8** and **9** followed the previously described synthesis[9] from (R)-(−)- and (S)-(+)-3-hydroxy-2-methylproprionate, respectively.

Starting vitamin D compound **10** was obtained by the classic route from methyl hyodeoxycholate in 1.5% yield. Both hydroxyls of ester **10** were protected as silyl ethers. Hydride reduction of the ester followed by Swern oxidation of the resulting alcohol **11** provided aldehyde **12** in 42% yield from ester **10**. The construction of the side chain part of analogs **1**, **2**, and **3** was accomplished by the condensation of aldehyde **12** with lithiated sulfones **7**, **8**, or **9** (Julia olefination), leading to vitamin D hydroxy sulfones **13**, **14**, and **15**, respectively. Dehydroxy desulfonylation with sodium amalgam in buffered methanol followed by fluoride promoted deprotection of silyl ethers provided final compounds **1**, **2**, and **3** (9.2%, 5.7%, and 6.5% yield from aldehyde **12**, respectively). As was anticipated, Julia olefination of aldehyde **12** afforded exclusively 24E isomer of each analog. Additionally, single peak of condensation products was observed on HPLC in chromatographic conditions, which allows for the separation of both isomers.[13] Furthermore, multiplets at 5.47 ppm in NMR spectra, originated from side-chain olefinic protons,[14] provided additional proof for the geometry assigned. However, the final assignment of the structure of analogs **1**, **2**, and **3** with the use of x-ray crystallography remains to be performed. Biologic activity of analogs **1**, **2**, and **3** will be reported elsewhere.

Application of the present method leading to other side-chain homologated analogs of vitamins D is under way in this laboratory.

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Appendix

The following trivial names of steroids were used in this report:

methyl hyodeoxycholate	3α,6α-dihydroxy-5β-cholan-24-oic acid methyl ester
1,25-dihydroxycholecalciferol	(5Z,7E)-(1S,3R)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol
1,25-dihydroxyergocalciferol	(5Z,7E)-(1S,3R)-9,10-secoergosta-5,7,10(19)-triene-1,3,25-triol
vitamin D$_3$	(5Z,7E)-(3R)-9,10-secocholesta-5,7,10(19)-trien-3-ol
vitamin D$_2$	(5Z,7E)-(3R)-9,10-secoergosta-5,7,10(19)-trien-3-ol

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