Synthesis of 24,24-Dihomo-1α,25-dihydroxyvitamin D_3

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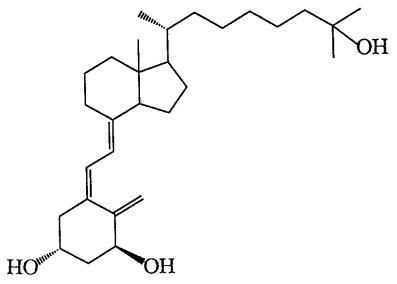
An alternative synthesis of 24,24-dihomo-1α,25-dihydroxyvitamin D_3 was achieved starting from stigmasterol or cholenic acid.

Keywords vitamin D_3 analogue; 24,24-dihomo-1α,25-dihydroxyvitamin D_3; enol esterification; irradiation; thermal isomerization; cell differentiation

The active form of vitamin D_3 namely 1α,25-dihydroxyvitamin D_3 has cell-differentiation-inducing activity.[1] Since this discovery, many vitamin D_3 compounds have been synthesized in order to investigate the structure–activity relationship.[2] DeLuca *et al.* synthesized 24,24-dihomo-1α,25-dihydroxyvitamin D_3 (**1**) and found that it has strong cell-differentiation-inducing ability.[3] Thus, vitamin D derivatives are candidates for the therapy of some cancers.[4] DeLuca *et al.* synthesized **1** by the selective 1α-hydroxylation of a C-22 vitamin D derivative followed by construction of the side chain,[5] but many steps were required. In addition, this process gave a poor yield of **1**, probably because of the instability of the C-22 vitamin D itself and the resulting coupling product under the reaction conditions employed.



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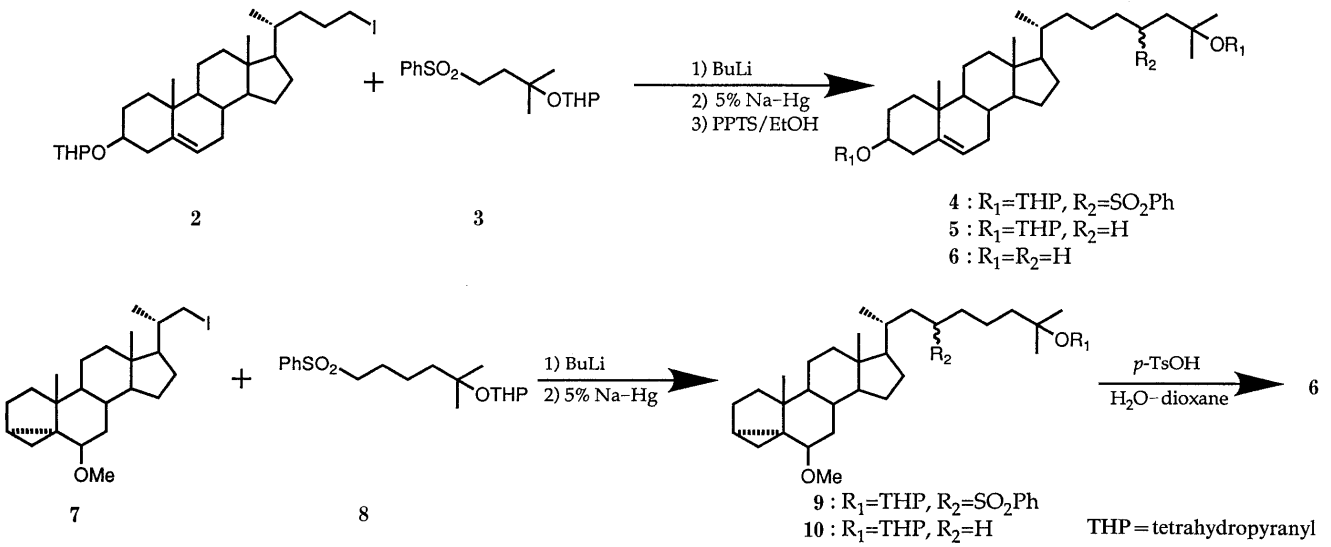
In the present paper, an alternative synthesis of **1** by using commercially available stigmasterol or cholenic acid as the starting material is described. Thus, 24,24-dihomo-25-hydroxycholesterol (**6**) was synthesized by utilizing a Julia coupling strategy[6] to connect the steroid part with the side chain part. The phenylsulfone derivative (**3**)[7] chosen as the side chain segment to constitute the C-5 synthon was condensed with the C-24 iodide derivative (**2**) obtainable from cholenic acid.[8] Reductive desulfonylation of the coupling product (**4**) with sodium amalgam in buffered methanol followed by the cleavage of tetrahydropyranyl ethers (**5**) under acidic conditions afforded **6** in 39% yield. Similar treatment of the C-22 iodide (**7**)[9] derived from stigmasterol with the C-7 sulfone derivative (**8**)[5] also resulted in the formation of **6** (23%).

Oxidation of the diol (**6**) with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (80%) and the subsequent acetylation of the hydroxyl group at the C-25 position of the formed trienone (**11**) afforded the 25-acetate (**12**) (87%). Enol esterification of the **4**, 6-dienone system of **12** with isopropenyl acetate in the presence of *p*-toluenesulfonic acid and the reduction of the enol acetate (**13**) with calcium borohydride gave the trienol. Protection of the 5,7-diene with 4-phenyl-1,2,4-triazoline (PTAD) as a Diels–Alder adduct and the subsequent protection of the hydroxyl group as a *tert*-butyldimethylsilyl ether to perform selective α-epoxidation afforded **15** (29% from



2 3 7 8

Chart 1

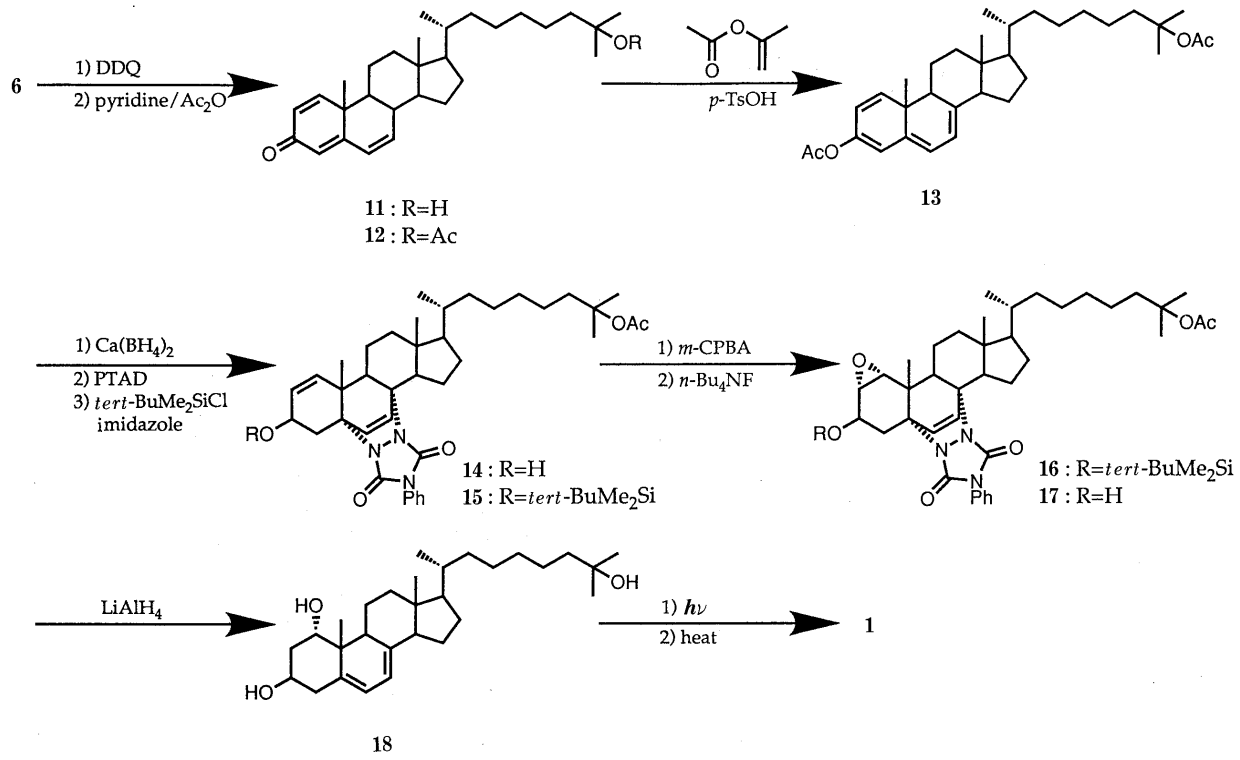


Chart 2

12).

Epoxidation of **15** with *m*-chloroperbenzoic acid (*m*-CPBA), removal of the silyl group, and reduction with $LiAlH_4$ produced the 5,7-diene (**18**) (56% from **15**). Irradiation of **18** with a high-pressure mercury lamp and thermal isomerization of the resultant previtamin D derivative afforded the target compound (**1**) in 24% yield.

Experimental

All the melting points are uncorrected. ^{1}H-NMR spectra were recorded with TMS as an internal standard and $CDCl_3$ as a solvent at 200 MHz on a JEOL JNM-FX200 spectrometer unless otherwise stated. Mass spectra were measured on a Hitachi M80 spectrometer at 70 eV. IR spectra were recorded on a Jasco IR-810 spectrometer. UV spectra were taken on a Hitachi 320 spectrometer. Merck Kieselgel 60 (Art. 7734, 70—230 mesh) was used for SiO_2 column chromatography. Solvents were removed under reduced pressure.

24,24-Dihomo-5-cholestene-3β,25-diol (6) from 2 2-Methyl-4-phenylsulfonyl-2-(tetrahydropyranyloxy)butane (**3**) (8.1 g, 26.0 mmol) was dissolved in tetrahydrofuran (THF) (20 ml) and cooled at −20 °C. A hexane solution of *n*-BuLi (1.6 M) (18 ml, 28.8 mmol) was added to the above solution. The whole was further stirred at −20 °C for 45 min. After the addition of 1,3-dimethyl-2-imidazolidinone (DMI) (3 ml, 29.0 mmol), a THF solution (40 ml) of 24-iodo-3-tetrahydropyranyloxy-5-cholestene (**2**) (12.0 g, 21.7 mmol) was added dropwise at −20 °C. The solution was stirred at −20 °C for 1 h and at room temperature for 2 h. After the addition of saturated NH_4Cl solution, the mixture was extracted with ethyl acetate. The solution was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (chloroform–hexane, 2 : 1, v/v) to give the 23-phenylsulfonyl derivative (**4**) (12.0 g, 75%). MS *m/z*: 637 ($M^+ - THP - H_2O$). ^{1}H-NMR δ: 7.60, 7.91 (5H, m, phenyl), 5.31 (1H, m, 6-H), 4.71 (2H, br s, CH(THP)), 3.86 (2H, m, CH_2(THP)), 3.49 (4H, m, 3-H, CH_2(THP), 23-H).

A Na_2HPO_4–saturated methanol solution (200 ml) of **4** (12.0 g, 21.7 mmol) and 5% Na–Hg (40 g) was stirred at room temperature overnight. After removal of the Hg by filtration, the methanol was evaporated off. The residue was extracted with ethyl acetate. This solution was washed with brine and evaporated. After removal of ethyl acetate, the residue was chromatographed on silica gel (chloroform–hexane, 1 : 1, v/v) to afford **5** (7.5 g, 77%). MS *m/z*:394 ($M^+ - 2THP - 2H_2O$). ^{1}H-NMR δ: 5.30 (1H, m, 6-H), 4.70 (2H, m, CH(THP)), 3.49, 3.91 (5H, m, CH_2(THP), 3-H).

An ethanol solution (150 ml) of **5** (7.5 g, 12.6 mmol) and PPTS (0.3 g, 1.2 mmol) was stirred at 60 °C for 1 h. Extraction with ethyl acetate, washing of the extract with brine, evaporation and chromatography on silica gel provided the diol **6** (4.5 g, 83%). mp 166—167 °C (acetone). MS *m/z*: 430 (M^+), 412 ($M^+ - H_2O$). ^{1}H-NMR δ: 5.36 (1H, m, 6-H), 3.51 (1H, m, 3-H), 1.17, 1.20 (each 3H, s, 26-H_3, 27-H_3), 1.00 (3H, s, 19-H_3), 0.90 (3H, d, J=6.0 Hz, 21-H_3), 0.71 (3H, s, 18-H_3). IR (Nujol): 3350, 1645, 1155, 1060 cm^{-1}. *Anal.* Calcd for $C_{29}H_{50}O_2$: C, 80.85; H, 11.72. Found: C, 80.69; H, 10.45.

24,24-Dihomo-5-cholestene-3β,25-diol (6) from 7 A *n*-BuLi hexane solution (1.6 M) (10 ml, 16 mmol) was added to a THF solution (50 ml) of 2-methyl-6-phenylsulfonyl-2-(tetrahydropyranyloxy) hexane (**8**) (4.8 g, 14.3 mmol) at −20 °C. The solution was stirred for 45 min at −20 °C. DMI (3 ml, 29.0 mmol) and a THF solution (50 ml) of (6*R*)-22-iodo-6-methoxy-23,24-dinor-3,5-cyclocholestene (**7**) (5.0 g, 11.0 mmol) were added dropwise at −20 °C. The mixture was stirred at −20 °C for 3 h. After the addition of saturated NH_4Cl solution, the mixture was extracted with ethyl acetate. The solution was washed with brine and evaporated. The residue was chromatographed on silica gel (ethyl acetate–hexane, 1 : 9, v/v) to give the 23-phenylsulfonyl derivative **9** (3.7 g, 51%).

A Na_2HPO_4–saturated methanol solution (200 ml) of **9** (3.7 g, 5.5 mmol) and 5% Na–Hg (40 g) was stirred at room temperature overnight. After separation of Hg by filtration, the methanol was evaporated off. The residue was extracted with ethyl acetate and the solution was washed with brine. After evaporation of the ethyl acetate, the residue was purified on silica gel (ethyl acetate–hexane, 1 : 9, v/v) to afford **10** (1.7 g, 58%). MS *m/z*: 526 (M^+). ^{1}H-NMR δ: 4.70 (1H, m, CH(THP)), 3.44, 3.94 (each 1H, m, CH_2(THP)), 3.31 (3H, s, OCH_3), 2.76 (1H, m, 6-H), 1.17, 1.21 (each 3H, 26-H_3, 27-H_3), 1.01 (3H, s, 19-H_3), 0.90 (3H, d, J=6.0 Hz, 21-H_3), 0.70 (3H, s, 18-H_3).

A dioxane–H_2O solution (80 ml, 5 : 3, v/v) containing **10** (1.7 g, 3.2 mmol) and *p*-toluenesulfonic acid (0.2 g, 1.1 mmol) was heated to reflux for 1 h, then extracted with chloroform. The chloroform solution was washed with brine and evaporated. The residue was chromatographed on silica gel (5% ethyl acetate in chloroform) to afford **6** (1.1 g,

79%).

25-Acetoxy-24,24-dihomo-1,4,6-cholestatrien-3-one (12) A dioxane solution (200 ml) of the diol (**6**) (5.1 g, 11.6 mmol) and DDQ (15.0 g, 66.1 mmol) was heated under reflux for 16 h. After evaporation of the dioxane, the residue was purified by silica gel chromatography (5% ethyl acetate in chloroform) to give **11** (4.0 g, 80%). mp 132—133 °C (ether–hexane). MS *m/z*: 424 (M^+), 406 ($M^+ - H_2O$). *Anal.* Calcd for $C_{29}H_{44}O_2$: C, 82.00; H, 10.46. Found: C, 81.82; H, 10.68.

A pyridine solution (40 ml) of **12** (4.0 g, 9.4 mmol) and acetic anhydride (9.6 g, 94 mmol) was heated at 100 °C for 15 h. The mixture was extracted with ethyl acetate, washed with 10% hydrochloric acid, saturated $NaHCO_3$ solution and brine, and evaporated. The residue was purified by silica gel chromatography (hexane–chloroform, 1 : 1, v/v) to afford **12** (3.8 g, 87%). MS *m/z*: 466 (M^+), 406 ($M^+ - CH_3COOH$). ^{1}H-NMR δ: 5.96—7.06 (5H, m, 1-H, 2-H, 4-H, 6-H, 7-H), 1.94 (3H, s, $COCH_3$), 1.43 (6H, s, 26-H_3, 27-H_3), 1.10 (3H, s, 19-H_3), 0.95 (3H, d, J=6.1 Hz, 21-H_3), 0.84 (3H, s, 18-H_3). IR (Nujol): 1760, 1720, 1605, 1280, 1205 cm^{-1}.

25-Acetoxy-3β-*tert*-butyldimethylsilyloxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidino)-24,24-dihomo-1,6-cholestadiene (15) A butyl acetate solution (100 ml) of **12** (3.8 g, 8.2 mmol), *p*-toluenesulfonic acid (3.8 g, 22.1 mmol) and isopropenyl acetate (40 ml) was refluxed for 6 h. The solution was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to give crude **13** (4.0 g). An ethanol solution (100 ml) of $NaBH_4$ (6.0 g, 162.1 mmol) was added to a methanol solution (100 ml) of $CaCl_2$ (12.0 g, 125 mmol) at 0—5 °C for 1 h. An ether solution (100 ml) of **13** (4.0 g) was added to the above mixture at −10 to −15 °C and the whole was stirred for 2 h at the same temperature and then at room temperature overnight. After the addition of 50% acetic acid, the mixture was extracted with ethyl acetate. This solution was washed with saturated $NaHCO_3$ solution and brine, then 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) was added until the red color persisted. Evaporation of ethyl acetate and silica gel chromatography of the residue (10% ethyl acetate in chloroform) afforded **14** (2.4 g, 48%).

A dimethylformamide solution (10 ml) of **14** (2.4 g, 3.7 mmol), *tert*-butyldimethylsilyl chloride (1.2 g, 7.9 mmol) and imidazole (1.2 g, 15.0 mmol) was warmed at 45 °C for 1 h. The mixture was extracted with ether and washed with brine. Evaporation of the ether and the addition of methanol to the residue afforded **15** as crystals (1.8 g, 29% from **12**). mp 144—145 °C. MS *m/z*: 582 (M^+ −PTAD), 522 (582 − AcOH). ^{1}H-NMR δ: 7.37 (5H, m, phenyl) 6.24, 6.43 (2H, ABq, J=8.0 Hz, 6-H, 7-H), 5.63 (2H, m, 1-H, 2-H), 4.94 (1H, m, 3-H), 1.97 (3H, s, $COCH_3$), 1.43 (6H, s, 26-H_3, 27-H_3) 1.09 (3H, s, 19-H_3), 0.94 (3H, d, J=6.0 Hz, 21-H_3), 0.90 (9H, s, *tert*-Bu), 0.81 (3H, s, 18-H_3), 0.09, 0.14 (each 3H, s, Si–H_3). IR (Nujol): 1755, 1740, 1695, 1300, 1225, 1040 cm^{-1}. *Anal.* Calcd for $C_{45}H_{67}N_3O_5Si$: C, 71.28; H, 8.92; N, 5.54. Found: C, 71.23; H, 8.97; N, 5.52.

25-Acetoxy-1α,2α-epoxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolizino)-24,24-dihomo-6-cholesten-3β-ol (17) A chloroform solution of **15** (1.8 g, 2.4 mmol) and *m*-CPBA (2.0 g, 11.9 mmol) was stirred at room temperature overnight. The solution was washed with 10% K_2CO_3 solution and brine. Evaporation of the solvent afforded **16** (1.8 g). A THF solution (20 ml) of **16** (1.8 g, 2.3 mmol) and 1 M *n*-Bu_4NF THF solution (7 m) was stirred at room temperature for 2 h. The solution was extracted with ethyl acetate, washed with brine and dried over $MgSO_4$. Evaporation of ethyl acetate and purification by silica gel chromatography (10% ethyl acetate in chloroform) afforded **17** (1.2 g, 76%). MS *m/z*: 484 (M^+ −PTAD). ^{1}H-NMR δ: 7.34 (5H, m, phenyl) 6.16, 6.43 (2H, ABq, J=8.0 Hz, 6-H, 7-H), 4.97 (1H, m, 3-H), 3.20 (2H, m, 1-H, 2-H), 1.96 (3H, s, $COCH_3$), 1.43 (6H, s, 26-H_3, 27-H_3) 1.06 (3H, s, 19-H_3), 0.93 (3H, d, J=6.0 Hz, 21-H_3), 0.83 (3H, s, 18-H_3). IR (Nujol): 1750, 1700, 1025 cm^{-1}.

24,24-Dihomo-5,7-cholestadiene-1α,3β,25-triol (18) A THF solution (40 ml) of **17** (1.2 g, 1.8 mmol) was added to a THF solution (40 ml) of $LiAlH_4$ (0.6 g, 18.0 mmol) and the mixture was refluxed for 2 h. Excess $LiAlH_4$ was decomposed by the addition of H_2O and the THF solution was filtered on Celite. The filtrate was extracted with chloroform and washed with brine. After evaporation of the solvent, the residue was crystallized from methanol to provide **18** (600 mg, 74%). mp 180—182 °C. MS *m/z*: 444 (M^+), 426 ($M^+ - H_2O$), 408 (426 − H_2O). UV: λ_{max} 282 nm (ε=11300, methanol). ^{1}H-NMR δ: 5.37, 5.71 (2H, m, 6-H, 7-H), 4.07 (1H, m, 3-H), 3.76 (1H, s, 1-H), 1.21 (6H, s, 26-H_3, 27-H_3), 0.93 (6H, m, 19-H_3, 21-H_3), 0.61 (3H, s, 18-H_3). IR (Nujol): 3300, 1065, 1025 cm^{-1}. *Anal.* Calcd for $C_{29}H_{48}O_3$: C, 78.31; H, 10.90. Found: C, 77.88; H, 10.72.

24,24-Dihomo-1α,25-dihydroxyvitamin D_3 (1) An ether–THF solution (500 ml) (1 : 4, v/v) of **18** (50 mg, 0.1 mmol) was irradiated with a 450 W high-pressure mercury lamp (Ushio Co. Ltd., UM-452) for 2 min using 1.2% KNO_3 solution as filter. The solution was evaporated. An ethanol solution (5 ml) of the residue was refluxed for 1 h. After evaporation of the ethanol, the residue was purified by HPLC (10% ethyl acetate in chloroform) to yield 12 mg of the target compound (**1**). Yield 24% (oil). MS *m/z* (relative intensity, %): 444 (M^+, 25), 426 ($M^+ - H_2O$, 100), 418 (426 − H_2O, 50). UV: λ_{max} 264 nm (ε=17200, methanol). ^{1}H-NMR δ: 6.03, 6.40 (2H, ABq, J=12.0 Hz, 6-H, 7-H), 5.34 (1H, s, 19E-H), 5.00 (1H, s, 19Z-H), 4.43 (1H, m, 1-H), 4.21 (1H, m, 3-H), 1.21 (6H, s, 26-H_3, 27-H_3), 0.89 (3H, d, J=6.0 Hz, 21-H_3), 0.55 (3H, s, 18-H_3). IR (Nujol): 3360, 1625, 1125, 1050 cm^{-1}.

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