Synthesis and Pharmacokinetics of 1α-Hydroxyvitamin D_3 Tritiated at 22 and 23 Positions Showing High Specific Radioactivity

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A novel synthesis of a radioactive compound of 1α-hydroxyvitamin D_3 (1αOHD_3) (1) and its pharmacokinetics are described. Radioactive 1αOHD_3 tritiated at 22 and 23 positions ([22,23-$^3H_4$]1αOHD_3) (5) was prepared *via* key reactions of the reduction of acetylenic side chain in the ketone (12) with tritium gas in the presence of palladium-charcoal and the subsequent Wittig reaction with the A-ring synthon (16). [22,23-$^3H_4$]1αOHD_3 (5) showed high specific radioactivity (111.5 Ci/mmol) and was used successfully in pharmacokinetics studies with rats. In the pharmacokinetics studies, the plasma concentration level of the active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$], after oral or intravenous administration of [22,23-$^3H_4$]1αOHD_3 (5), showed longer half-life, lower maximum concentration, and lower area under the curve than those after treatment of 1α,25(OH)$_2D_3$ tritiated at 26 and 27 positions (4). These results might suggest a beneficial therapeutic utility of 1αOHD_3 (1) over the treatment of 1α,25(OH)$_2D_3$ (2).

Key words 1α,25-dihydroxyvitamin D_3; 1α-hydroxyvitamin D_3; pharmacokinetics; tritiated compound; tritium gas; specific radioactivity

Background 1α-Hydroxyvitamin D_3 (1αOHD_3) (**1**) is now well known as a synthetic prodrug of 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$] (**2**), a hormonally active form of vitamin D_3,[1] and has been used clinically for the treatment of rickets, hypovitaminosis, hypocalcemia, chronic renal failure, and osteoporosis.[2] Regarding pharmacokinetics and distribution studies of 1αOHD_3 (**1**),[3] administered radioactivity was simply traced using 1αOHD_3 tritiated at 2 position ([2-^{3}H]1αOHD_3) (**3**) in which specific radioactivity was shown to be very low (4.2 Ci/mmol).[4] Detailed plasma concentration level of bioconverted 1α,25(OH)$_2D_3$ (**2**) by the hydroxylation at 25 position of dosed 1αOHD_3 (**1**) has not been compared with 1α,25(OH)$_2D_3$ level after administration of 1α,25(OH)$_2D_3$ (**2**) itself. The non-availability of such important information about the bioconversion from a prodrug, 1αOHD_3 (**1**), to an active form, 1α,25(OH)$_2D_3$ (**2**), has been due to lack of tritiated 1αOHD_3 possessing high specific radioactivity. We have also been interested in the relevance of tissue distribution studies and cytopharmacology with cellular autoradiography of 1αOHD_3 (**1**) to determine its mode-of-action in bone.[5] In microautoradiography experiments, [2-^{3}H]1αOHD_3 (**3**) is, however, far from satisfactory due to quite low specific radioactivity.

Although the synthesis of 1α,25(OH)$_2D_3$ tritiated at 26 and 27 positions ([26,27-$^3H_6$]1α,25(OH)$_2D_3$) (**4**) possessing high specific radioactivity is known, the preparative method for tritiated 1αOHD_3 with high specific radioactivity has never, to our knowledge, been reported. In this paper we describe: 1) a novel procedure for the preparation of 1αOHD_3 tritiated at 22 and 23 positions ([22,23-$^3H_4$]1αOHD_3) (**5**) showing high specific radioactivity and 2) pharmacokinetics results of 1α,25(OH)$_2D_3$ levels after administration of [22,23-$^3H_4$]1αOHD_3 (**5**) to rats in comparison with after administration of [26,27-$^3H_6$]1α,25(OH)$_2D_3$ (**4**) (Chart 1).

Synthetic Results After many unsuccessful trials to convert commercially available [26,27-$^3H_6$]1α,25(OH)$_2D_3$ (**4**) to 1αOHD_3 tritiated at 26 and 27 positions by removal of the hydroxy group at 25 position, we focused our synthetic strategy on the tritiation of the acetylenic side chain by the catalytic reduction using tritium gas. The key intermediate for the tritiation reaction should be the acetylenic derivative **12**, which would be transformed to [22,23-$^3H_4$]1αOHD_3 (**5**) *via* the Wittig reaction with the A-ring synthon (**16**) of active vitamin D_3 after tritiation (Chart 2).

The Inhoffen-Lythgoe diol (**6**),[6] prepared from vitamin D_2 by ozonolysis, was converted to the known aldehyde (**7**)[6] by acetylation of the primary hydroxy moiety in **6**, silylation of the secondary hydroxy group, deacetylation by the reduction with lithium aluminum hydride ($LiAlH_4$), and oxidation of the resulting primary hydroxy group with pyridinium chlorochromate (PCC). The formyl group in **7** was transformed to the ketene dibromide (**8**) in 96% yield by Corey's method[7] using carbon tetrabromide (CBr_4) and triphenyl phosphine (PPh_3) in dichloromethane (CH_2Cl_2). The ketene dibromide (**8**) was treated with *n*-butyllithium (*n*-BuLi) at −78 °C, followed by a reaction with isobutylaldehyde to yield the acetylenic alcohol (**9**), quantitatively.[7] Removal of the secondary hydroxy group in **9** by the Barton method,[8] *i.e.* initial formation of thioester by treatment with pheny chlorothionoformate (PhOCSCl) and the subsequent reduction of the resulting thioester with tri-*n*-butyltin hydride (*n*-Bu_3SnH), was accomplished clearly to give a 98% yield of the acetylene (**10**). Desilylation of the protecting group in **10** by aqueous hydrochloric acid (HCl) in tetrahydrofuran (THF) gave the alcohol **11** in 68% yield, which was oxidized by PCC to afford the acetylenic ketone (**12**) in 56% yield.

Having obtained the key intermediate **12**, catalytic hydrogenation was first carried out as a model experiment for tritiation reaction using tritium gas. Thus, hydrogenolysis of **12** in the presence of palladium-charcoal (Pd-C) in ethyl acetate (AcOEt) afforded the ketone (**13**), quantitatively. The ketone (**13**) was allowed to react with the A-ring synthon (**16**), prepared by Hatakeyama's method,[9] followed by desilylation with tetra-*n*-butylammonium fluoride (TBAF) to give

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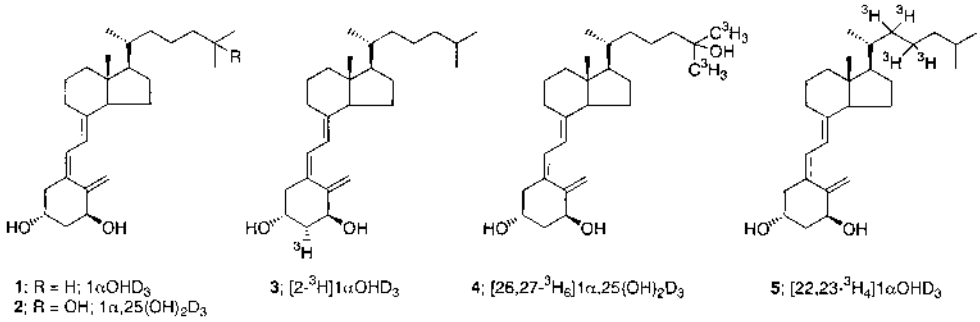


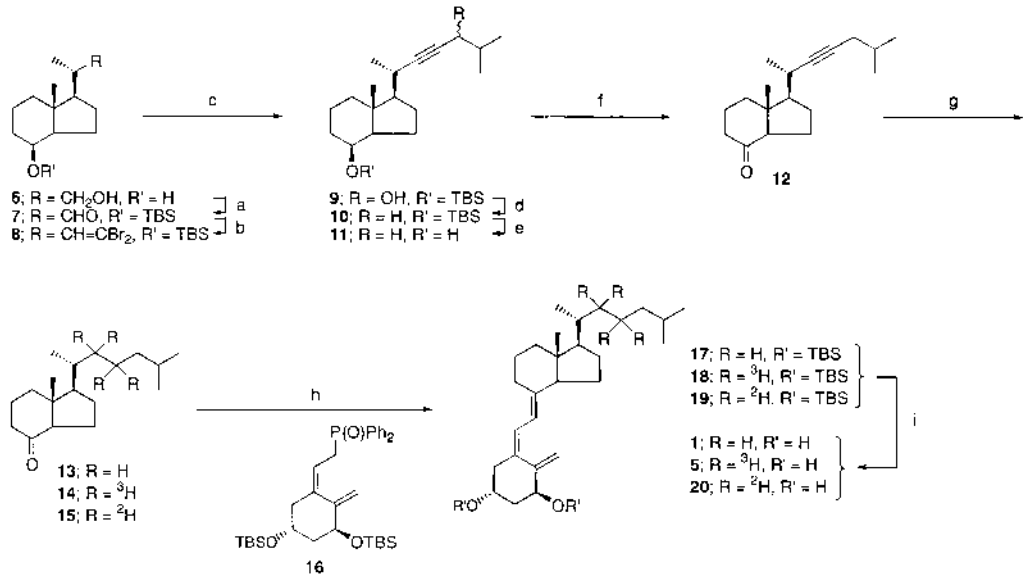
1; R = H; 1αOHD$_3$
2; R = OH; 1α,25(OH)$_2$D$_3$

3; [2-^{3}H]1αOHD$_3$

4; [26,27-^{3}H$_6$]1α,25(OH)$_2$D$_3$

5; [22,23-^{3}H$_4$]1αOHD$_3$

Chart 1



6; R = CH_2OH, R' = H
7; R = CHO, R' = TBS
8; R = CH=CBr_2, R' = TBS

9; R = OH, R' = TBS
10; R = H, R' = TBS
11; R = H, R' = H

12

13; R = H
14; R = ^{3}H
15; R = ^{2}H

16

17; R = H, R' = TBS
18; R = ^{3}H, R' = TBS
19; R = ^{2}H, R' = TBS

1; R = H, R' = H
5; R = ^{3}H, R' = H
20; R = ^{2}H, R' = H

TBS=*tert*-butyldimethylsilyl. a) 1) Ac_2O/pyridine, 2) TBSCl, 3) $LiAlH_4$, 4) PCC; b) CBr_4/PPh_3; c) *n*-BuLi/isobutylaldehyde; d) 1) PhOCSCl/pyridine, 2) *n*-Bu_3SnH; e) aqueous HCl; f) PCC/Celite; g) H_2, $^2H_2$ or $^3H_2$/Pd–C/AcOEt; h) *n*-BuLi; i) TBAF

Chart 2

1αOHD_3 (**1**), which was completely identical with authentic material.[10] The described thirteen-step synthesis from the Inhoffen-Lythgoe diol (**6**) to 1αOHD_3 (**1**) provides a novel convergent method for the preparation of clinically important **1**.

The same reaction conditions as used in the hydrogenolysis of **12** were applied to prepare the tritiated ketone (**14**). The tritiation of the acetylenic part in **12** with tritium gas in the presence of Pd-C afforded the tritiated ketone (**14**) in 95% radiochemical purity with 116 Ci/mmol specific radioactivity. [22,23-$^3H_4$]1αOHD_3 (**5**) was obtained with 111.5 Ci/mmol (4125.5 GBq/mmol) specific radioactivity and 98% radiochemical purity by the Wittig reaction (17% yield) with the A-ring synthon (**16**) and the subsequent desilylation (48% yield) with TBAF. When deuterium gas was used instead of tritium gas, 1αOHD_3 deutrated at 22 and 23 positions ([22,23-$^2H_4$]1αOHD_3) (**20**) was also obtained in a comparable yield (Chart 2). Since [22,23-$^3H_4$]1αOHD_3 (**5**) has high specific radioactivity, microautoradiography experiments of 1αOHD_3 were carried out successfully using **5** and the results have been reported recently.[11]

Pharmacokinetics Results [22,23-$^3H_4$]1αOHD_3 (**5**) or [26,27-$^3H_6$]1α,25(OH)$_2$D$_3$ (**4**) were given to Sprague-Dawley male rats (6-week-old) orally or intravenously at a dose of 5 nmol (*ca.* 2 μg)/kg/50 μCi, respectively. The plasma levels of total radioactivity, 1α,25(OH)$_2$D$_3$ fraction and 1αOHD_3 fraction (in case of [22,23-$^3H_4$]1αOHD_3 (**5**) administration) were determined at 5 min, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h after fractionation by HPLC. The results are shown in Fig. 1 for the administration of [22,23-$^3H_4$]1αOHD_3 (**5**) and Fig. 2 for [26,27-$^3H_6$]1α,25(OH)$_2$D$_3$ (**4**). Calculated pharmacokinetics parameters, plasma half-life ($T_{1/2}$), maximum concentration (C_{max}), time at C_{max} (T_{max}) and area under the curve (*AUC*) are also summarized in Table 1.

Plasma concentration of 1α,25(OH)$_2$D$_3$ after oral or intravenous administration of [22,23-$^3H_4$]1αOHD_3 (**5**) showed longer $T_{1/2}$, lower C_{max}, and lower *AUC* than those after treatment of [26,27-$^3H_6$]1α,25(OH)$_2$D$_3$ (**4**). In our radioassay experiments which were carried out separately from the present pharmacokinetics studies, 1α,25(OH)$_2$D$_3$ fraction in rat bone after 1αOHD_3 treatment was sustained for longer than that after 1α,25(OH)$_2$D$_3$ treatment.[11] We also confirmed in the microautoradiography studies that localization of radioactivity in bone after treatment with 1αOHD_3 or 1α,25(OH)$_2$D$_3$ is observed in osteoblast nuclei.[11] Radioactivity in the nuclei after 1αOHD_3 treatment was also sustained longer than that after 1α,25(OH)$_2$D$_3$ treatment. Taking the results obtained in

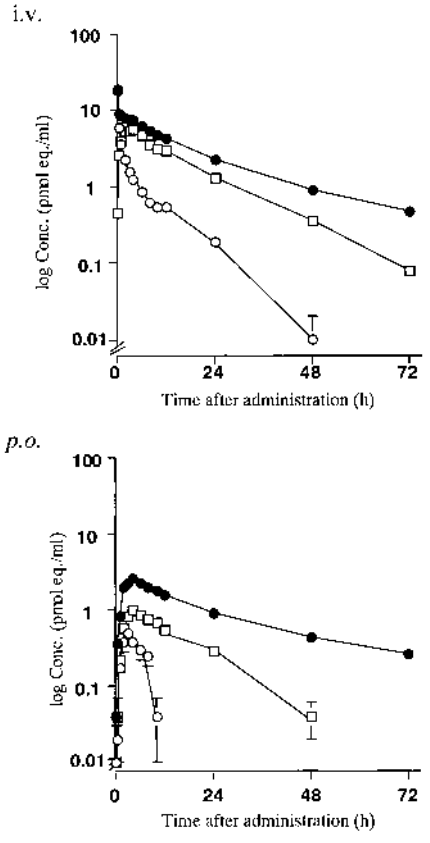


Fig. 1. Plasma Concentration of Radioactivity after Oral (*p.o.*) or Intravenous (i.v.) Administration of [22,23-$^3H_4$]1αOHD$_3$ (**5**)
–●–: total radioactivity, –○–: 1αOHD$_3$ fraction, –□–: 1α,25(OH)$_2$D$_3$ fraction.

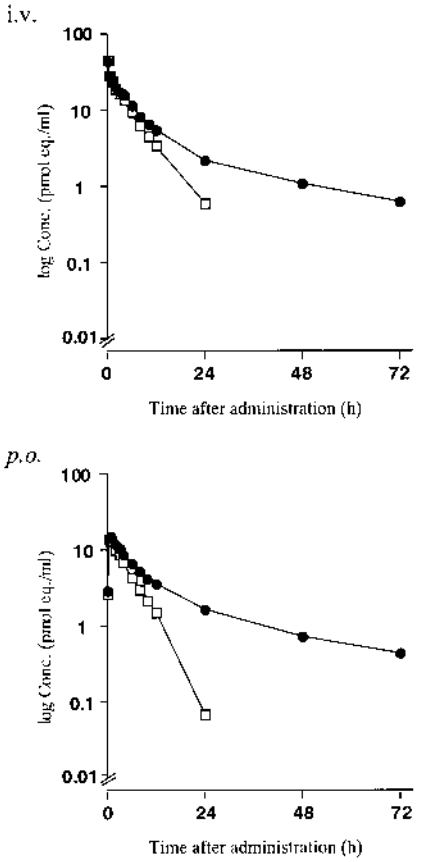


Fig. 2. Plasma Concentration of Radioactivity after Oral (*p.o.*) or Intravenous (i.v.) Administration of [26,27-$^3H_6$]1α,25(OH)$_2$D$_3$ (**4**)
–●–: total radioactivity, –□–: 1α,25(OH)$_2$D$_3$ fraction.

the present pharmacokinetics studies and the above-mentioned radioassay in bone and microautoradiography in osteoblast nuclei into consideration, treatment of a prodrug [1αOHD$_3$ (**1**)] supplies bone with an active form of drug [1α,25(OH)$_2$D$_3$ (**2**)] more steadily and stably than those of 1α,25(OH)$_2$D$_3$ (**2**) treatment. Although these results might suggest a beneficial therapeutic utility of a prodrug [1αOHD$_3$ (**1**)] over treatment of an active form of vitamin D$_3$ [1α,25(OH)$_2$D$_3$ (**2**)], further basic and clinical trials are necessary to clarify the disparate characters of 1αOHD$_3$ (**1**) on bone from 1α,25(OH)$_2$D$_3$ (**2**).

Table 1. Pharmacokinetics Parameters of Plasma 1α,25(OH)$_2$D$_3$ Fraction after Oral (*p.o.*) or Intravenous (i.v.) Administration of [22,23-$^3H_4$]1αOHD$_3$ (**5**) or [26,27-$^3H_6$]1α,25(OH)$_2$D$_3$ (**4**)

	Route	$T_{1/2}$ (h)	C_{max} (pmol/ml)	T_{max} (h)	*AUC* (pmol·h/ml)
[22,23-$^3H_4$]1αOHD$_3$ (**5**)	*p.o.*	10.2	0.88	6.00	18.0
	i.v.	11.4	5.99	3.25	102
[26,27-$^3H_6$]1α,25(OH)$_2$D$_3$ (**4**)	*p.o.*	3.17	14.9	0.75	75.8
	i.v.	4.31	46.4	—	175

Experimental

General Methods for Synthetic Studies Infrared (IR) spectra were recorded with a Hitachi 270-30 spectrometer, proton nuclear magnetic resonance (NMR) spectra with a JEOL FX-200 or JEOL FX-270, mass (MS) spectra with a Shimadzu GCMS-QP 1000, and ultraviolet (UV) spectra with a Shimadzu UV-240. Gas chromatography was performed with a Shimadzu GC-17A (Capillary GC, FID) using column DB-17 (J & W Scientific 0.53 mm 15 m). Flash column chromatography was carried out with Merck Kieselgel 60 (Art 9385), and preparative TLC was performed on 20×20 cm plates coated with 0.25 mm thickness of Merck Kieselgel 60 coating F254. Reverse phase high-performance liquid chromatography (HPLC) was carried out on YMC ODS A-312 at a flow rate of 1 ml/min with EtOH/H_2O (85 : 15). Radioactivity was measured with an Aloka LSC-900.

(8β)-De-A,B-23,23-dibromo-8-(*tert*-butyldimethylsilyloxy)-24-norchol-22-ene (8) To a stirred solution of the aldehyde (7)[6] (4.69 g, 14.4 mmol) in CH_2Cl_2 (25 ml) was rapidly added CBr_4 (9.55 g, 28.8 mmol) and PPh_3 (15.1 g, 57.6 mmol) in CH_2Cl_2 (25 ml). The mixture was then stirred at room temperature for 3 min and diluted with *n*-hexane. The insoluble material was filtered out. The filtrate was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$, and evaporated. The residue was taken up with *n*-hexane. The insoluble material was removed by filtration, and the filtrate was evaporated. The crude product was purified by flash column chromatography with *n*-hexane as the eluant to give the ketene dibromide (**8**) (6.63 g, 96%) as a colorless oil. NMR ($CDCl_3$) δ: 6.17 (1H, d, J=9.8 Hz), 4.00 (1H, br s), 2.48 (1H, m), 1.00 (3H, d, J=6.8 Hz), 0.96 (3H, s), 0.89 (9H, s), 0.01 (6H, s).

(8β)-De-A,B-8-(*tert*-butyldimethylsilyloxy)-22-cholestyne-24-ol (9) To a stirred solution of the ketene dibromide (**8**) (1.00 g, 2.08 mmol) in THF (15 ml) was added *n*-BuLi (1.61 M solution in hexane, 2.71 ml, 4.37 mmol) dropwise at −78 °C under argon. The mixture was stirred at −78 °C for 1 h and at room temperature for 30 min. Isobutylaldehyde (0.62 ml, 6.86 mmol) was added dropwise to the mixture at −78 °C. The resulting mixture was stirred at −78 °C for 20 min, poured into H_2O at room temperature and extracted with AcOEt. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (15 : 1) as the eluant to give the acetylene (**9**) (838 mg, quantitatively) as a colorless oil. NMR ($CDCl_3$) δ: 4.14 (1H, br s), 4.00 (1H, br s), 2.46 (1H, m), 1.18 (3H, d, J=6.8 Hz), 0.99 (6H, d, J=6.8 Hz), 0.96 (6H, d, J=6.8 Hz), 0.93 (3H, s), 0.89 (9H, s), 0.01 (6H, s). IR (neat): 3430, 2950, 2860, 1470, 1375, 1250, 1160, 1080, 1025 cm^{-1}.

(8β)-De-A,B-8-(*tert*-butyldimethylsilyloxy)-22-cholestyne (10) To a stirred solution of the acetylene (**9**) (810 mg, 2.06 mmol) in CH_2Cl_2 (15 ml) were added pyridine (0.58 ml, 7.21 mmol) and PhOCSCl (0.43 ml, 3.09 mmol) under argon. The resulting mixture was stirred at room temperature for 2 h and extracted with AcOEt. The extract was washed with cold 0.5 N HCl, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$ and evaporated to give a yellow oil which was dissolved in toluene (30 ml). To the resulting solution was added *n*-Bu_3SnH (0.83 ml, 3.09 mmol) and 2,2-azobisisobutyronitrile (67.7 mg, 0.41 mmol). The mixture was refluxed for 3 h and evaporated. The residue was purified by flash column chromatography with *n*-hexane as the eluant to give the acetylene (**10**) (760 mg, 98%) as a colorless oil. NMR ($CDCl_3$) δ: 4.00 (1H, br s), 2.66 (1H, m), 1.15 (3H, d, J=6.8 Hz), 0.95 (6H, dd, J=6.8, 1.5 Hz), 0.93 (3H, s), 0.89 (9H, s), 0.01 (6H, s).

(8β)-De-A,B-22-cholestyne-8-ol (11) A solution of the acetylene (**10**) (740 mg, 1.96 mmol) and 6 N HCl (10 ml) in THF (20 ml) was refluxed for 7 h. The mixture was then diluted with AcOEt, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (10 : 1) as the eluant to give the alcohol (**11**) (351 mg, 68%) as a colorless oil. NMR ($CDCl_3$) δ: 4.09 (1H, br s), 2.68 (1H, m), 2.42 (1H, m), 1.16 (3H, d, J=6.8 Hz), 0.95 (6H, d, J=6.3 Hz), 0.95 (3H, s). IR (neat): 3450, 2950, 2860, 1460, 1375, 1280, 1165, 1065 cm^{-1}.

De-A,B-22-cholestyne-8-one (12) A mixture of the alcohol (**11**) (340 mg, 1.30 mmol), Celite (1.0 g) and PCC (356 mg, 1.95 mmol) in CH_2Cl_2 (5 ml) was stirred at room temperature for 1.5 h. The mixture was diluted with Et_2O, treated with Florisil column chromatography and evaporated. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (15 : 1) as the eluant to give the ketone (**12**) (189 mg, 56%) as a colorless oil. NMR ($CDCl_3$) δ: 2.47 (1H, m), 2.26 (1H, m), 1.21 (3H, d, J=6.9 Hz), 0.95 (6H, d, J=6.6 Hz), 0.68 (3H, s). IR (neat): 2950, 2875, 1715, 1460, 1380, 1305, 1220 cm^{-1}. MS *m/z*: 260 (M^+), 133 (100%).

De-A,B-8-oxocholestane (13) A mixture of the ketone (**12**) (12.5 mg, 48.0 μmol) and 5% Pd-C (10.5 mg) in AcOEt (1 ml) was stirred at room temperature for 30 min under hydrogen. The mixture was treated with Celite/silica gel column chromatography using AcOEt as a solvent to give the ketone (**13**) (12.5 mg, quantitatively) as a colorless oil. NMR ($CDCl_3$) δ: 0.95 (3H, d, J=5.8 Hz), 0.86 (6H, d, J=6.3 Hz), 0.64 (3H, s). IR (neat): 2950, 2875, 1715, 1470, 1380, 1310, 1240, 1055 cm^{-1}. MS *m/z*: 264 (M^+), 125 (100%).

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-9,10-secocholesta-5,7,10(19)-triene (17) To a stirred solution of the A-ring synthon (**16**) (58 mg, 99.7 μmol) in THF (0.75 ml) was added *n*-BuLi (1.63 M solution in THF, 130 μl, 212 μmol) dropwise at −75 °C under argon. The mixture was stirred at −75 °C for 5 min. The ketone (**13**) (4.6 mg, 17.4 μmol) in THF (0.3 ml) was added dropwise to the mixture at −75 °C. The resulting mixture was stirred at −75 °C for 105 min and at room temperature for 15 min, poured into NaCl and extracted with AcOEt. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt (24 : 1) to give **17** (5.0 mg, 46%) as a colorless oil, whose TLC, NMR, IR, UV and MS were completely identical with those of authentic material.[12]

1α-Hydroxyvitamin D_3 (1) A solution of **17** (29.3 mg, 46.6 μmol) and TBAF (1 M solution in THF, 500 μl, 500 μmol) in THF (1 ml) was refluxed mildly for 2 h. The mixture was diluted with AcOEt, washed with 0.5 M HCl, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$ and evapolated. The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt/EtOH (20 : 10 : 1) to give 1αOHD_3 (**1**) (18.4 mg, 98%) as a colorless foam, whose HPLC, TLC, NMR and UV were completely identical with those of authentic material.[10]

De-A,B-[22,22,23,23-$^3H_4$]-8-oxocholestane (14) A mixture of the ketone (**12**) (12.5 mg, 48.0 μmol) and 10% Pd-C (10.5 mg) in AcOEt (1 ml) was stirred at room temperature under tritium gas (10 Ci) in a tritiation vessel for 3 h. The insoluble material was filtered out and the filtrate was evaporated with EtOH (10 ml×4) to give the crude ketone (**14**) (3 Ci). The crude **14** (3 Ci) was purified by preparative TLC developed with *n*-hexane/AcOEt (9 : 1) to give the analytically pure **14** (2.1 Ci). This was dissolved in EtOH (50 ml) and analyzed. Specific radioactivity: 116 Ci/mmol. Radiochemical purity: 95%. The behavior of **14** on TLC and HPLC was identical with cold authentic **13**.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-[22,22,23,23-$^3H_4$]-9,10-secocholesta-5,7,10(19)-triene (18) To a stirred solution of the A-ring synthon (**16**) (45 mg, 77.3 μmol) in THF (0.4 ml) was added *n*-BuLi (1.69 M solution in THF, 92 μl, 155 μmol) dropwise at −75 °C under argon. The mixture was stirred at −75 °C for 5 min. The ketone (**14**) (900 mCi) in THF (0.25 ml) was added dropwise to the mixture at −75 °C, and the resulting mixture was stirred at −75 °C for 1 h and at room temperature for 10 min, poured into aqueous NaCl and extracted with AcOEt. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt (24 : 1) to give **18** (149 mCi, 17%), which was identical with cold authentic **17** on TLC and HPLC.

1α-Hydroxy-[22,22,23,23-$^3H_4$]vitamin D_3 (5) A solution of **18** (201 mCi) and TBAF (1 M solution in THF, 100 μl, 100 μmol) in THF (0.5 ml) was refluxed mildly for 2.5 h. The mixture was diluted with AcOEt, washed with 0.5 M HCl, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by preparative TLC developed with CH_2Cl_2/EtOH (50 : 3) to give [22,22,23,23-$^3H_4$]1αOHD_3 (**5**) (95.7 mCi, 48%), which was identical with cold authentic **1** on TLC and HPLC. Specific radioactivity: 111.5 Ci/mmol (4125.5 GBq/mmol). Radiochemical purity: 98%.

De-A,B-[22,22,23,23-$^2H_4$]-8-oxocholestane (15) A mixture of the ketone (**12**) (10.4 mg, 39.9 μmol) and 5% Pd–C (10.5 mg) in AcOEt (1 ml) was stirred at room temperature for 30 min under deuterium gas. The mixture was treated with Celite/silica gel column chromatography to give the ketone (**15**) (10.7 mg, quantitatively) as a colorless oil. NMR ($CDCl_3$) δ: 0.94 (3H, d, J=6.3 Hz), 0.87 (6H, d, J=6.8 Hz), 0.64 (3H, s). IR (neat): 2950, 2875, 1710, 1460, 1380, 1310, 1220, 1050 cm^{-1}. MS *m/z*: 268 (M^+), 125 (100%).

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-[22,22,23,23-$^2H_4$]-9,10-secocholesta-5,7,10(19)-triene (19) To a stirred solution of the A-ring synthon (**16**) (56 mg, 95.6 μmol) in THF (0.75 ml) was added *n*-BuLi (1.63 M solution in THF, 88 μl, 143 μmol) dropwise at −78 °C under argon. The mixture was stirred at −78 °C for 5 min. The ketone (**15**) (5.6 mg, 21.2 μmol) in THF (0.3 ml) was added dropwise to the mixture at −78 °C. The resulting mixture was stirred at −78 °C for 1.75 h and at room temperature for 15 min, poured into aqueous NaCl and extracted with AcOEt. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt (24 : 1) to give **19** (11.2 mg, 84%) as a colorless oil. NMR ($CDCl_3$) δ: 6.24 (1H, d, J=11.5 Hz), 6.02 (1H, d, J=11.5 Hz), 5.18 (1H, d, J=2.2 Hz), 4.87 (1H, d, J=2.2 Hz), 4.44—4.32 (1H, m), 4.27—4.12 (1H, m), 0.96—0.82 (24H, m), 0.53 (3H, s), 0.06 (12H, s). IR (neat): 2940, 2850, 1465, 1375, 1360, 1245, 1080 cm^{-1}. MS *m/z*: 632 (M^+), 249 (100%). UVλ_{max} nm 265.

1α-Hydroxy-[22,22,23,23-$^2H_4$]vitamin D_3 (20) A solution of **19** (6.1 mg, 9.6 μmol) and TBAF (1 M solution in THF, 200 μl, 200 μmol) in THF (1.5 ml) was refluxed mildly for 2 h. The mixture was diluted with AcOEt, washed with 0.5 M HCl, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$ and evaporated. The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt/EtOH (20 : 10 : 1) to give [22,22,23,23-$^2H_4$]1αOHD_3 (**20**) (3.3 mg, 84%) as a colorless oil. NMR ($CDCl_3$) δ: 6.39 (1H, d, J=11.2 Hz), 6.02 (1H, d, J=11.2 Hz), 5.33 (1H, br s), 4.51—4.37 (1H, m), 4.31—4.13 (1H, m), 2.90—2.76 (1H, m), 2.68—2.52 (1H, m), 2.40—2.24 (1H, m), 0.91 (3H, d, J=5.9 Hz), 0.87 (6H, d, J=6.3 Hz), 0.54 (3H, s). IR (neat): 3350, 2950, 2850, 1460, 1375, 1210, 1050 cm^{-1}. MS *m/z*: 404 (M^+), 134 (100%). UVλ_{max}nm 264.

Pharmacokinetics Studies Six-week-old male Sprague-Dawley rats were purchased from S.L.C. Japan Co., Ltd., (Shizuoka, Japan). After an acclimation period of one week with standard rodent chow containing 1.25% calcium and 1.06% phosphate (CE-2, Clea Japan Inc.), rats were starved overnight prior to administration. [22,22,23,23-$^3H_4$]1αOHD_3 (**5**) or [26,26,26,27,27,27-$^3H_6$]1α,25$(OH)_2D_3$ (**4**) (purchased from Amersham International plc.), was administered orally or intravenously in saline or intravenously in saline containing 1% EtOH and 1% Tween 20 as a solvent at a dose of 5 nmol (*ca.* 2 μg)/kg/50 μCi. Blood was taken periodically at 5 min, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h from the tail vein. Total radioactivity was determined by liquid scintillation counter (Tri–Carb 2500TR, Packard). Radioactive fractions were separated by HPLC (SCL-10A, Shimadzu) and detected by liquid scientillation counter. The radioactivity of 1α,25$(OH)_2D_3$ fraction was fitted to the least squares method to calculate elimination rate constant (*ke*). Plasma $T_{1/2}$ was calculated as ln2/*ke* and *AUC* was determined by the trapezoidal rule with extrapolation using the *ke*.

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References

1) Bouillon R., Okamura W. H., Norman A. W., *Endocrine Rev*., **16**, 200—257 (1995).
2) Higuchi Y., Sato K., Nanjo M., Isogai T., Takeda S., Kumaki K., Nishii Y., *Vitamins*, **68**, 87—93 (1994).
3) Tohira Y., Ochi K., Matsunaga I., Fukushima M., Takanashi S., Hata K., Kaneko C., Suda T., *Anal. Biochem*., **77**, 495—502 (1977).
4) Tohira Y., Nakano Y., Ogawa M., Kamiyama H., Nakano H., Takanashi S., Suda T., *Vitamins*, **52**, 341—352 (1978).
5) Stumpf W. E., *Drug Metab. Dispos*., **23**, 885—886 (1995).
6) Wovkulich P. M., Barcelos F., Batcho A. D., Sereno J. F., Baggiolini E. G., Hennessy B. M., Uskokovic M. R., *Tetrahedron*, **40**, 2283—2296 (1984).
7) Corey E. J., Fuchs P. L., *Tetrahedron Lett*., **36**, 3769—3772 (1972).
8) Robins M. J., Wilson J. S., Hausske F., *J. Am. Chem. Soc*., **105**, 4059—4065 (1983).
9) Hatakeyama S., Numata H., Osanai K., Takano S., *J. Org. Chem*., **54**, 3515—3517 (1989).
10) Kaneko C., Yamada S., Sugimoto A., Eguchi Y., Ishikawa M., Suda T., Suzuki M., Kakuta S., Sasaki S., *Steroids*, **23**, 75—92 (1974).
11) Koike N., Ichikawa F., Nishii Y., Stumpf W. E., *Calcif. Tissue Int*., **63**, 391—395 (1998).
12) Matsuura F., Kato M., Shimizu H., Michishita T., Japan. Patent 62-29875, Dec. 25 (1987) [*Chem. Abstr*., **109**, 93445 (1988)].