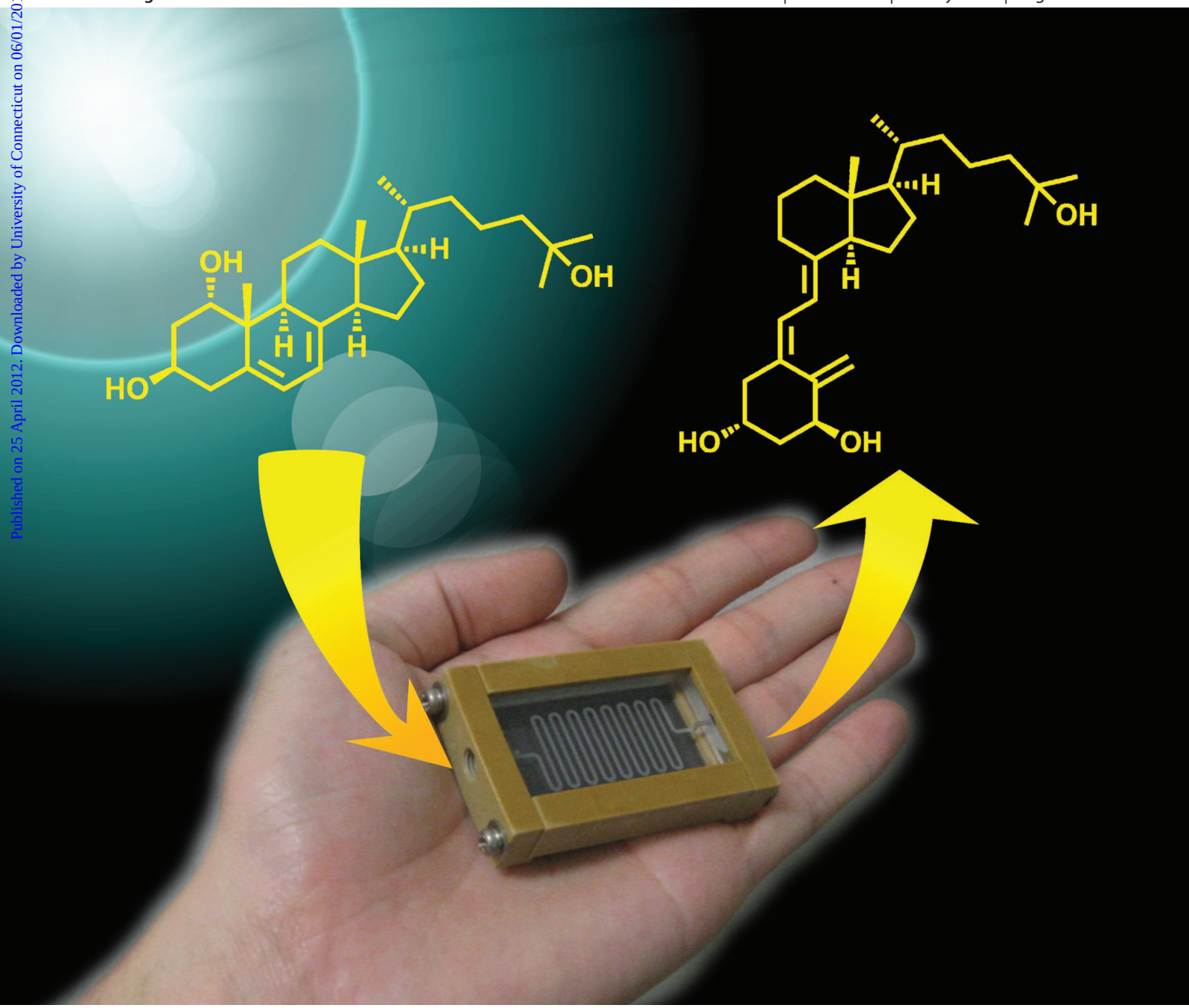
Organic & Biomolecular Chemistry

www.rsc.org/obc

Volume 10 | Number 27 | 21 July 2012 | Pages 5153–5316



ISSN 1477-0520

RSC Publishing

PAPER

Takashi Takahashi *et al.*

Continuous-flow synthesis of activated vitamin D_3 and its analogues

1477-0520(2012)10:27;1-A

Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 5205

www.rsc.org/obc

PAPER

Continuous-flow synthesis of activated vitamin D_3 and its analogues†

Shinichiro Fuse, Yuto Mifune, Nobutake Tanabe and Takashi Takahashi*

Received 9th March 2012, Accepted 24th April 2012
DOI: 10.1039/c2ob25511a

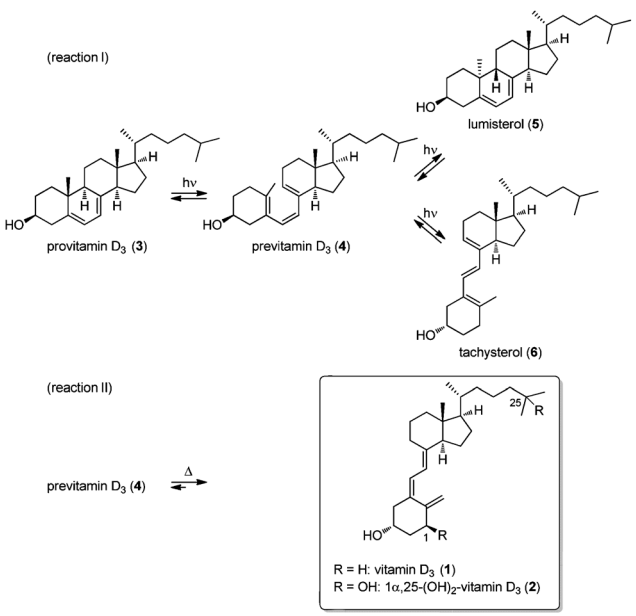
An efficient, two-stage, continuous-flow synthesis of 1α,25-$(OH)_2$-vitamin D_3 (activated vitamin D_3) and its analogues was achieved. The developed method afforded the desired products in satisfactory yields using a high-intensity and economical light source, *i.e.*, a high-pressure mercury lamp. In addition, our method required neither intermediate purification nor high-dilution conditions.

Introduction

Vitamin D_3 (**1**) is metabolized sequentially in the liver and kidney into 1α,25-$(OH)_2$-vitamin D_3 (**2**) (activated vitamin D_3), which has a broad spectrum of biological activities such as cell differentiation, regulation of calcium metabolism, and immune function.[1–3] Activated vitamin D_3 and its analogues are clinically used as drugs for various kinds of diseases including renal failure, osteoporosis, psoriasis, and secondary hyperparathyroidism.[4] Therefore, development of a facile method for the synthesis of vitamin D_3 and its analogues[5–7] is highly important. The most conventional synthesis of vitamin D_3 (**1**) with fewest steps includes photo-reaction of provitamin D_3 (**3**) into previtamin D_3 (**4**) using high-pressure or medium-pressure mercury lamp and the subsequent thermal-reaction of previtamin D_3 (**4**) into vitamin D_3 (**1**) (Scheme 1).[4,8] This method is also used for the industrial preparation of vitamin D_3 (**1**). The most serious problem with this method is the low over-all yield (< 20%).[9–12] The photo-reaction is not selective because previtamin D_3 (**4**) has an absorption wavelength that is similar to that of provitamin D_3 (**3**). The undesired products lumisterol (**5**) and tachysterol (**6**) result from the equilibrium between the products.[8,13] Therefore, with the present industrial method, it is necessary to interrupt the irradiation after a relatively low conversion (10 to 20%) of provitamin D_3 (**3**) to previtamin D_3 (**4**). The unconverted provitamin D_3 (**3**) is recycled while the previtamin D_3 (**4**) must be purified using a tedious work-up procedure. Synthesis of various vitamin D_3 analogues for drug discovery has been hampered by the low yield of the conventional method.

Various other sources of UV irradiation have been considered to improve the yield of previtamin D_3. In fact, the use of excimer or exciplex lasers with a narrow-band spectra have reportedly been effective in the photo-reaction of provitamin D_3 into previtamin D_3.[14–16] However, the use of a laser requires a specialized equipment set-up, and the light source is expensive. The use of a solution filter with an economical light source to generate a narrow-band spectrum has been reported.[17] However, the need to dispose of a large amount of waste is problematic. The use of a sensitizer[18] or a filter compound[19] has been reported. However, a tedious work-up procedure to remove these compounds from the reaction mixture is necessary.

Micro-flow reactors[20–33] have advantages for both photo- and thermal-reactions. In photo-reactions, micro-flow reactors have a thin reaction space. Therefore, light penetration efficiency is much higher than conventional batch reactors.[23,34–44] In thermal-reactions, the surface-to-volume ratio of micro-flow reactors is much higher than that of conventional batch reactors and



Scheme 1 Two-step conversion of provitamin D_3 (**3**) to vitamin D_3 (**1**).

Department of Applied Chemistry, Tokyo Institute of Technology, 2-12-1, Ookayama, Meguro-ku, Tokyo 152-8552, Japan.
E-mail: ttak@apc.titech.ac.jp; Fax: +81-3-5734-2884;
Tel: +81-3-5734-2120

†Electronic supplementary information (ESI) available: including ^1H and ^{13}C spectra. See DOI: 10.1039/c2ob25511a

therefore, the heat transfer is quick and the reaction temperature can be precisely controlled using micro-flow reactors.

Recently, we reported a highly efficient, two-stage,[8,14,15,19] continuous-flow synthesis of vitamin D_3 (**1**) from provitamin D_3 (**3**) as shown in Fig. 1.[45,46] The mixture of previtamin D_3 (**4**) and tachysterol (**6**) prepared from provitamin D_3 (**3**) using the photo-microflow reactor (313–578 nm) was converted into the desired vitamin D_3 (**1**) by using a photo- and thermal-microflow reactor (360 nm, 100 °C). Consequently, the equilibrium for the photo-isomerization of tachysterol (**6**) to previtamin D_3 (**4**) was shifted to produce more previtamin D_3 (**4**). Desired vitamin D_3 (**1**) was obtained in a 32% isolated yield. Herein, we report the first continuous-flow synthesis of activated vitamin D_3 (**2**) and its analogues **11–13** (Fig. 2) using a high-intensity and economical light source, *i.e.*, a high-pressure mercury lamp, with no intermediate purifications.

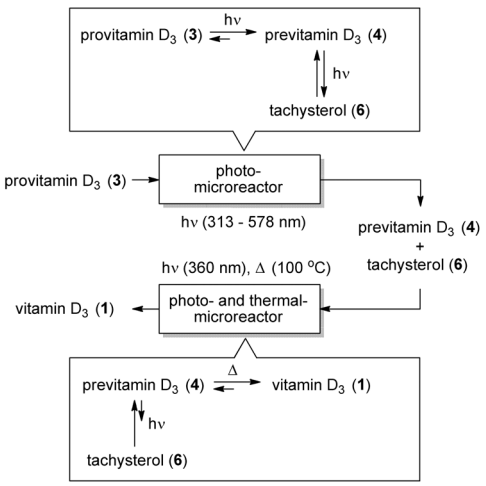
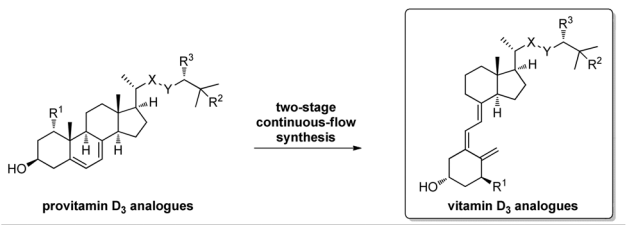


Fig. 1 Two-stage, continuous-flow synthesis of vitamin D_3 (**1**) from provitamin D_3 (**3**).



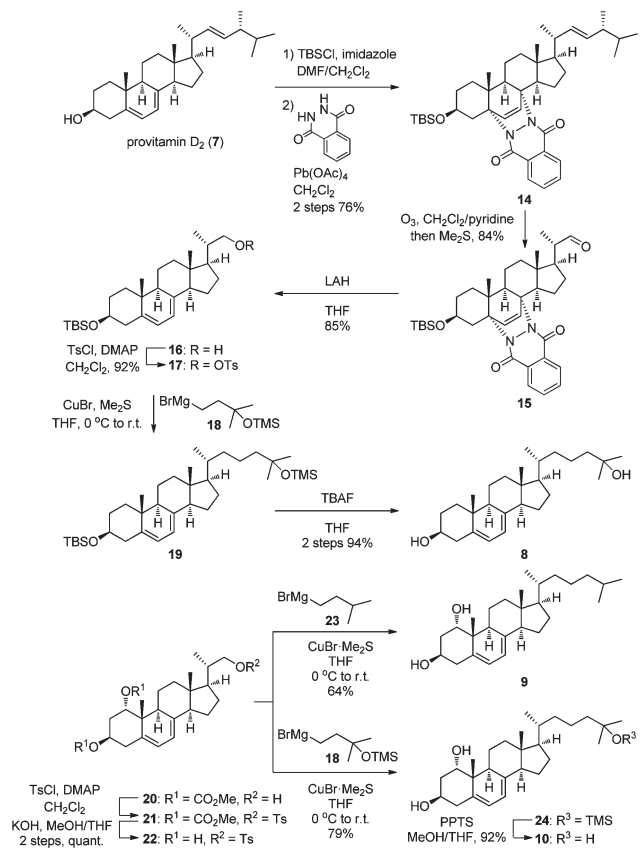
provitamin D_3 analogues	vitamin D_3 analogues	R^1	R^2	R^3	X-Y
7	**11** vitamin D_2	H	H	Me	CH=CH
8	**12** 25-OH vitamin D_3	H	OH	H	CH_2-CH_2
9	**13** 1α-OH vitamin D_3	OH	H	H	CH_2-CH_2
10	**2** activated vitamin D_3	OH	OH	H	CH_2-CH_2

Fig. 2 Target compounds **11–13** and **2**.

Results and discussion

Four known bioactive compounds, vitamin D_2 (**11**) (calciferol), 25-OH vitamin D_3 (**12**) (calderol), 1α-OH vitamin D_3 (**13**) (alfa-calcidol), and 1α,25-$(OH)_2$-vitamin D_3 (**2**) (rocaltrol), were selected as target molecules. It is well known that the 1-OH and/or 25-OH vitamin D_3 analogues **2**, **12** and **13** (Fig. 2), and their precursors of the thermal-reaction, *i.e.*, 1-OH and/or 25-OH previtamin D_3 analogues, are highly unstable to heat, light and oxygen.[47] Therefore, the micro-flow synthesis of these oxygenated vitamin D_3 analogues is a challenging task. Except for provitamin D_2 (**7**), provitamin D_3 analogues **8–10** were not commercially available. These substrates were synthesized as shown in Scheme 2. Some modification of the reported 7-step procedure[48] led to the synthesis of 25-OH provitamin D_3 (**8**) from **7** in a good yield. 1α-OH provitamin D_3 (**9**)[49] and 1α,25-$(OH)_2$ provitamin D_3 (**10**)[50] were prepared from the alkylation of the common starting material **22** with Grignard reagents **23** or **18**.[7] Tosylate **22** was readily prepared from alcohol **20**[51,52] in high yield.

We prepared a micro-flow system (Fig. 3). Two micro-flow reactors and a syringe pump were connected with PEEK tubing. The first photo-microflow reactor (length: 250 mm, depth: 200 μm, width: 1 mm, volume: 50 μL) was irradiated using a 313–578 nm light source (400 W high-pressure mercury lamp with a Vycor filter). The second photo- and thermal-microflow reactor (length: 500 mm, depth: 200 μm, width: 1 mm, volume:



Scheme 2 Preparation of 25-OH provitamin D_3 (**8**), 1α-OH provitamin D_3 (**9**), and 1α,25-$(OH)_2$ provitamin D_3 (**10**).

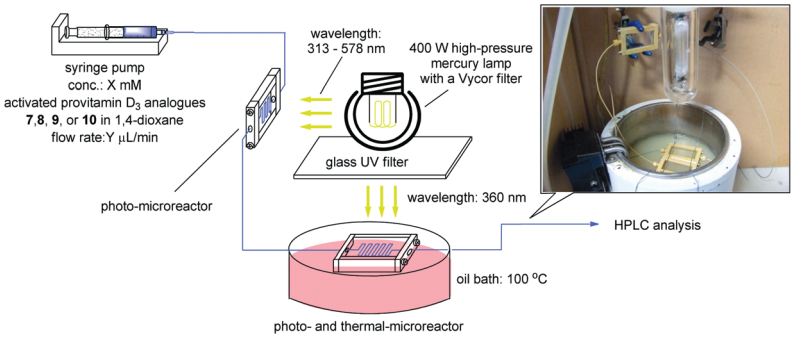


Fig. 3 Two-stage, continuous-flow synthesis of activated vitamin D_3 (**2**) and its analogues **11–13** using a micro-flow system.

Table 1 Isolated yields of products obtained from a two-stage continuous-flow synthesis

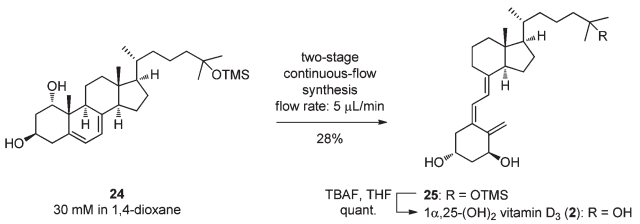
Entry	Substrate/conc. X	Flow rate Y	Product/yield[b]
1	**7**/30 mM	5 μL min^{-1}	**11**/23%
2	**8**/20 mM[a]	10 μL min^{-1}	**12**/25%
3	**9**/30 mM	5 μL min^{-1}	**13**/23%
4	**10**/5 mM[a]	10 μL min^{-1}	**2**/8%

[a] 30 mM solutions of substrates **8** and **10** in 1,4-dioxane could not be prepared because of their poor solubility. A 20 mM solution of **8** and a 5 mM solution of **10** were employed in the two-stage, continuous-flow synthesis. [b] Isolated yield.

100 μL) was irradiated with 360 nm light (400 W high-pressure mercury lamp with a Vycor filter and a glass UV filter) and it was put on hot oil (100 °C). Then, solutions of activated provitamin D_3 analogues **7–10** were introduced with a syringe pump at the indicated flow rates in Table 1. According to our previous optimization in micro-flow vitamin D_3 (**1**) synthesis, 20 and 30 mM solutions of substrate in 1,4-dioxane solvent was injected at a flow rate of 5 or 10 μL min^{-1}.

All the expected products were obtained by the two-stage, continuous-flow synthesis. The observed spectral data of **11–13** and **2** were in good agreement with those reported previously.[53–56] The desired vitamin D_2 (**11**), 25-OH vitamin D_3 (**12**) and 1α-OH vitamin D_3 (**13**) were obtained in satisfactory yields (Table 1, entries 1–3). In the case of 1α,25-$(OH)_2$ provitamin D_3 (**10**), a 30 mM solution could not be prepared due to its poor solubility in 1,4-dioxane. Therefore, a 5 mM solution of **10** in 1,4-dioxane was used for the two-stage continuous-flow synthesis of **2** and a severe yield reduction was observed (entry 4).

To overcome this problem, we employed TMS ether **24** as a substrate instead of **10** (Scheme 3). The TMS ether **24** is the synthetic precursor of **10** (Scheme 2), thus, the total number of reaction steps was not changed. A 30 mM solution of **24** in 1,4-dioxane was injected into the micro-flow system shown in Fig. 3 at a flow rate of 5 μL min^{-1}. To our delight, the TMS-protected product **25** was obtained in a 28% yield. The desilylation of **25** was performed in accordance with the previously described condition (**19** to **8**) to afford the desired 1α,25-$(OH)_2$-vitamin D_3 (**2**) in a quantitative yield. As described before, the conventional photo- and thermal-reactions to synthesize activated vitamin D_3 and its analogues in low yields (<20%) required high-dilution



Scheme 3 Two-stage, continuous-flow synthesis of TMS-protected activated vitamin D_3 **25** from **24**, and the subsequent desilylation to afford 1α,25-$(OH)_2$ vitamin D_3 (**2**).

conditions (*ca.* 0.1 mM)[14–16] in order to suppress the undesired filter effect in the photo-reaction. Thus, the observed yields (23–28%) were satisfactory. It should be noted that higher concentrations (20–30 mM) can be used for our developed method. In addition, our developed method requires no purification of unstable intermediates, thereby reducing waste.

Conclusions

In summary, we achieved a two-stage continuous-flow synthesis of activated vitamin D_3 (**2**) and its analogues **11–13** in satisfactory yields using a high-intensity and economical light source, *i.e.*, a high-pressure mercury lamp. This is the first application of a micro-flow system to the synthesis of activated vitamin D_3 and its analogues. One of the advantages of using micro-flow reactors is the ease of scaling-up. It should be possible to scale-up our developed process by either continuous running or by the numbering-up of the micro-flow reactors. It should be noted that the continuous micro-flow synthesis of activated vitamin D_3 required no purification of intermediates or high-dilution conditions, thereby reducing waste.

Experimental section

General

NMR spectra were recorded on a JEOL Model ECP-400 (400 MHz for ^1H, 100 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in units of parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (7.26 ppm for ^1H, 77.0 ppm for ^{13}C) or DMSO (2.50 ppm for ^1H, 39.5 ppm for ^{13}C).

Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz (Hz). IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. Optical rotations were measured with JASCO model P-1020 polarimeter. HRMS (ESI-TOF) were measured with a Waters LCT Premier^{TM} XE. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light, visualized by 5% ethanolic *p*-anisaldehyde solution. Flash column chromatography was performed on Silica Gel 60 N, purchased from Kanto Chemical Co. Preparative HPLC was carried out on a Waters 515 HPLC pump using a Senshu Pak Silica-3301-N column (8ϕ × 300 mm) with a SHISEIDO SI-2/3002 and a Shodex RI-71. CH_2Cl_2 was dried by a Glass Contour. THF and 1,4-dioxane were dried by distillation from sodium benzophenone ketyl.

3β-*tert*-Butyldimethylsilyloxy-5α,8α-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazine-2,3-diyl)ergosta-6,22-diene (14)

To a solution of provitamin D_2 (**7**) (2.00 g, 5.04 mmol, 1.0 eq.) and imidazole (1.03 g, 15.1 mmol, 3.0 eq.) in DMF (20 mL) and CH_2Cl_2 (20 mL) was added *tert*-butyldimethylsilyl chloride (1.52 g, 10.1 mmol, 2.0 eq.) at 0 °C under argon. After being stirred at room temperature for 24 h, the reaction mixture was poured into water at 0 °C and the aqueous layer was extracted twice with hexane. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the crude diene and phthalic hydrazide (3.27 g, 20.2 mmol, 4.0 eq.) in CH_2Cl_2 (60 mL) was added dropwise a solution of $Pb(OAc)_4$ (3.35 g, 7.56 mmol, 1.5 eq.) in CH_2Cl_2 (40 mL) at −5 to 0 °C under argon. After being stirred at 0 °C for 1 h, Al_2O_3 (9.0 g) was added to the reaction mixture at the same temperature and stirred for a further 30 min. After filtering through a pad of Celite, the organic layer was washed with water, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The obtained mixture was filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (10% Et_2O in hexane) to give 3β-*tert*-butyldimethylsilyloxy-5α,8α-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazine-2,3-diyl)-ergosta-6,22-diene (**14**) (2.58 g, 3.84 mmol, 2 steps 76%) as a yellow amorphous solid. The observed spectral data were in good agreement with those reported previously.[48]

3β-*tert*-Butyldimethylsilyloxy-5α,8α-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazine-2,3-diyl)-23,24-bisnorchol-6-en-22-al (15)

A mixture of O_3 and O_2 was bubbled through a solution of 3β-*tert*-butyldimethylsilyloxy-5α,8α-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazine-2,3-diyl)ergosta-6,22-diene (**14**) (1.04 g, 1.55 mmol, 1.0 eq.) in CH_2Cl_2 (90 mL) and pyridine (2.64 mL) at −78 °C for 3 h. Then, dimethyl sulfide (0.56 mL) was added to the reaction mixture at −78 °C. After being stirred at the same temperature for 30 min, the mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (15% EtOAc in hexane) to give 3β-*tert*-butyldimethylsilyloxy-5α,8α-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazine-2,3-diyl)-23,24-bisnorchol-6-en-22-al (**15**) (783 mg, 1.30 mmol, 84%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.56 (d, J = 3.4 Hz, 1H), 8.13 (m, 2H), 7.70 (m, 2H), 6.67 (d, J = 8.3 Hz, 1H), 6.23 (d, J = 8.3 Hz, 1H), 4.02 (dd, J = 11.7, 7.4 Hz, 1H), 3.89 (dd, J = 14.1, 4.9 Hz, 1H), 3.59 (m, 1H), 1.14 (d, J = 6.8 Hz, 3H), 1.03 (s, 3H), 0.88 (s, 3H), 0.86 (s, 9H), 0.09 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.5, 161.8, 159.6, 138.9, 132.7, 132.6, 130.4, 130.2, 128.2, 127.0, 126.5, 77.2, 68.1, 67.4, 67.1, 51.7, 50.6, 48.9, 48.4, 44.7, 40.4, 39.1, 35.6, 34.7, 30.5, 26.5, 25.9, 24.4, 22.2, 18.5, 18.0, 13.5, −4.4, −4.9; IR (neat): 2954, 2856, 2706, 1725, 1653, 1311, 1092, 1079, 837 cm^{-1}; $[\alpha]_D^{26}$ = −103.4 (*c* 0.96, $CHCl_3$); mp 110–115 °C; HRMS (ESI-TOF): calcd for $[C_{36}H_{50}N_2O_4Si + H]^+$ 603.3618, found 603.3615.

3β-*tert*-Butyldimethylsilyloxy-22-hydroxy-23,24-bisnorchola-5,7-diene (16)

To a solution of $LiAlH_4$ (493 mg, 13.0 mmol, 10 eq.) in THF (9.5 mL), a solution of 3β-*tert*-butyldimethylsilyloxy-5α,8α-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazine-2,3-diyl)-23,24-bisnorchol-6-en-22-al (**15**) (783 mg, 1.30 mmol, 1.0 eq.) in THF (10 mL) was added dropwise at 0 °C under argon. After being stirred at 45 °C for 2.5 h, the reaction mixture was quenched with saturated aqueous potassium sodium tartrate at 0 °C and stirred for further 1 h. The aqueous layer was extracted twice with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (20% EtOAc in hexane) to give 3β-*tert*-butyldimethylsilyloxy-22-hydroxy-23,24-bisnorchola-5,7-diene (**16**) (491 mg, 1.10 mmol, 85%) as a white solid. The observed spectral data were in good agreement with those reported previously.[48]

3β-*tert*-Butyldimethylsilyloxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (17)

To a solution of 3β-*tert*-butyldimethylsilyloxy-22-hydroxy-23,24-bisnorchola-5,7-diene (**16**) (242 mg, 0.544 mmol, 1.0 eq.) in CH_2Cl_2 (4.0 mL), tosyl chloride (207 mg, 1.09 mmol, 2.0 eq.) and DMAP (199 mg, 1.63 mmol, 3.0 eq.) were added at 0 °C under argon. After being stirred at the same temperature for 3.5 h, the reaction mixture was poured into water at 0 °C and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (15% EtOAc in hexane) to give 3β-*tert*-butyldimethylsilyloxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (**17**) (301 mg, 0.503 mmol, 92%) as a white solid. The observed spectral data were in good agreement with those reported previously.[48]

3β,25-Dihydroxycholesta-5,7-diene {25-hydroxyprovitamin D_3 (8)}

To stirred magnesium turnings (200 mg, 8.21 mmol, 10 eq.), two drops of 4-bromo-2-methyl-2-[(trimethylsilyl)oxy]butane were added and a solution of 4-bromo-2-methyl-2-[(trimethylsilyl)-oxy]butane (1.96 g, 8.21 mmol, 10 eq.) in THF (11 mL) was

added dropwise at 50 °C under N_2. After being stirred at 50 °C for 10 min, the reaction mixture was cooled at 0 °C and a suspension of CuBr·Me_2S (169 mg, 0.821 mmol, 1.0 eq.) in THF (3 mL) was added and a solution of 3β-*tert*-butyldimethylsilyloxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (**17**) (492 mg, 0.821 mmol, 1.0 eq.) in THF (11 mL) was added dropwise at 0 °C under N_2. After being stirred at room temperature for 1 h, the reaction mixture was poured into saturated aqueous NH_4Cl at 0 °C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by short path column chromatography on silica gel (5% Et_2O in hexane) and used for the next reaction without further purification.

To a solution of crude silyl ether **19** in THF (16.4 mL), a solution of TBAF (1.0 M in THF, 12.3 mL, 12.3 mmol, 15 eq.) was added at 0 °C under argon. After being stirred at room temperature for 10 h, the reaction mixture was poured into water at 0 °C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (2% MeOH in $CHCl_3$) to give 3β,25-dihydroxycholesta-5,7-diene (**8**)[48] (309 mg, 0.771 mmol, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.57 (m, 1H), 5.39 (m, 1H), 3.64 (m, 1H), 1.22 (s, 6H), 0.95 (d, J = 8.8 Hz, 3H), 0.94 (s, 3H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.3, 139.8, 119.6, 116.3, 71.1, 70.4, 55.8, 54.5, 46.2, 44.4, 42.9, 40.8, 39.2, 38.4, 37.0, 36.4, 36.1, 32.0, 29.4, 29.2, 28.1, 23.0, 21.1, 20.1, 18.8, 16.3, 11.8; IR (neat): 3306, 2962, 2875, 1456, 1363, 1068, 911, 829 cm^{-1}; $[\alpha]_D^{27}$ = −102.6 (*c* 0.47, $CHCl_3$); mp 168–171 °C; HRMS (ESI-TOF): calcd for $[C_{27}H_{44}O_2 + H]^+$ 401.3420, found 401.3418.

1α,3β-Bis(methoxycarbonyloxy)-22-hydroxy-23,24-bisnorchola-5,7-diene (20)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.67 (m, 1H), 5.37 (m, 1H), 4.89 (m, 1H), 4.83 (br, 1H), 3.78 (s 3H), 3.77 (s, 3H), 3.64 (dd, J = 10.6, 3.2 Hz, 1H), 3.38 (dd, J = 10.7, 6.8 Hz, 1H), 1.06 (d, J = 6.8 Hz, 3H), 1.00 (s, 3H), 0.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.0, 155.0, 140.9, 133.8, 122.1, 115.5, 78.4, 72.2, 67.8, 54.8, 54.6, 54.2, 52.1, 42.9, 41.2, 39.0, 38.7, 37.7, 35.5, 31.8, 27.5, 23.0, 20.3, 16.8, 16.0, 12.0; IR (neat): 3545, 2959, 2875, 1747, 1444, 1281, 1255, 984, 755 cm^{-1}; $[\alpha]_D^{28}$ = −40.6 (*c* 1.22, $CHCl_3$); mp 83–86 °C; HRMS (ESI-TOF): calcd for $[C_{26}H_{38}O_7 + NH_4]^+$ 480.2961, found 480.2966.

1α,3β-Dihydroxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (22)

To a solution of 1α,3β-bis(methoxycarbonyloxy)-22-hydroxy-23,24-bisnorchola-5,7-diene (**20**) (300 mg, 0.649 mmol, 1.0 eq.) in CH_2Cl_2 (3.25 mL), tosyl chloride (247 mg, 1.30 mmol, 2.0 eq.) and DMAP (238 mg, 1.95 mmol, 3.0 eq.) were added at 0 °C under argon. After being stirred at 0 °C for 4 h, the reaction mixture was poured into water at 0 °C and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of crude tosylate **21** in MeOH (6.49 mL) and THF (3.25 mL), potassium hydroxide (164 mg, 2.92 mmol, 4.5 eq.) was added at 0 °C under argon. After being stirred at room temperature for 12 h, the reaction mixture was poured into 1 M HCl at 0 °C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (3% MeOH in $CHCl_3$) to give 1α,3β-dihydroxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (**22**) (352 mg, 0.703 mmol, quant.) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 5.72 (m, 1H), 5.36 (m, 1H), 4.07 (m, 1H), 3.99 (dd, J = 9.3, 3.4 Hz, 1H), 3.82 (dd, J = 9.6, 6.6 Hz, 1H), 3.76 (br, 1H), 2.45 (s, 3H), 1.02 (d, J = 6.8 Hz, 3H), 0.93 (s, 3H), 0.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.6, 140.6, 136.3, 133.0, 129.8, 127.9, 121.8, 115.5, 75.5, 72.7, 65.3, 60.4, 54.2, 51.5, 43.1, 42.2, 39.9, 38.8, 38.5, 37.7, 36.5, 27.3, 22.9, 21.6, 20.7, 16.9, 16.2, 14.1, 11.8; IR (neat): 3359, 2941, 1457, 1360, 1175, 1052, 942, 845, 668, 555 cm^{-1}; $[\alpha]_D^{24}$ = −50.6 (*c* 1.03, $CHCl_3$); mp 80–82 °C; HRMS (ESI-TOF): calcd for $[C_{29}H_{40}O_5S + H]^+$ 501.2675, found 501.2657.

1α,3β-Dihydroxycholesta-5,7-diene {1α-hydroxyprovitamin D_3 (9)}

To stirred magnesium turnings (90.7 mg, 3.73 mmol, 10 eq.), two drops of 1-bromo-3-methylbutane were added and a solution of 1-bromo-3-methylbutane (563 mg, 3.73 mmol, 10 eq.) in THF (5.2 mL) was added dropwise at 50 °C under N_2. After being stirred at the same temperature for 10 min, the reaction mixture was cooled at 0 °C and a suspension of CuBr·Me_2S (76.7 mg, 0.373 mmol, 1.0 eq.) in THF (2 mL) was added and a solution of 1α,3β-dihydroxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (**22**) (187 mg, 0.373 mmol, 1.0 eq.) in THF (3.2 mL) was added dropwise at 0 °C under N_2. After being stirred at room temperature for 30 min, the reaction mixture was poured into saturated aqueous NH_4Cl at 0 °C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The obtained mixture was filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (40% EtOAc in hexane) to give 1α,3β-dihydroxycholesta-5,7-diene (**9**)[49] (95.0 mg, 0.237 mmol, 64%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73 (m, 1H), 5.38 (m, 1H), 4.06 (m, 1H), 3.77 (br, 1H), 0.94 (s, 3H), 0.94 (d, J = 4.9 Hz, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.8, 135.8, 122.1, 115.2, 72.9, 65.5, 55.9, 54.7, 43.1, 42.3, 40.0, 39.5, 39.2, 38.5, 38.0. 36.1, 36.1, 28.1, 28.0, 23.9, 23.0, 22.8, 22.5, 20.9, 18.8, 16.3, 11.9; IR (neat): 3381, 2954, 2872, 1464, 1377, 1366, 1052, 826, 690 cm^{-1}; $[\alpha]_D^{27}$ = −50.2 (*c* 1.14, $CHCl_3$); mp 114–117 °C;

HRMS (ESI-TOF): calcd for $[C_{27}H_{44}O_2 + H]^+$ 401.3420, found 401.3420.

1α,3β-Dihydroxy-25-trimethylsilyloxycholesta-5,7-diene (24)

To stirred magnesium turnings (81.2 mg, 3.34 mmol, 10 eq.), two drops of 4-bromo-2-methyl-2-[(trimethylsilyl)oxy]butane were added and a solution of 4-bromo-2-methyl-2-[(trimethylsilyl)oxy]butane (799 mg, 3.34 mmol, 10 eq.) in THF (5 mL) was added dropwise at 50 °C under N_2. After being stirred at 50 °C for 10 min, the reaction mixture was cooled at 0 °C and a suspension of CuBr·Me_2S (68.7 mg, 0.334 mmol, 1.0 eq.) in THF (2 mL) was added and a solution of 1α,3β-dihydroxy-22-tosyloxy-23,24-bisnorchola-5,7-diene (**22**) (167 mg, 0.334 mmol, 1.0 eq.) in THF (2.6 mL) was added dropwise at 0 °C under N_2. After being stirred at room temperature for 1 h, the reaction mixture was poured into saturated aqueous NH_4Cl at 0 °C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The obtained mixture was filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (50% EtOAc in hexane) to give 1α,3β-dihydroxy-25-trimethylsilyloxycholesta-5,7-diene (**24**) (129 mg, 0.264 mmol, 79%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.68 (m, 1H), 5.36 (m, 1H), 4.04 (m, 1H), 3.73 (br, 1H), 1.19 (s, 6H), 0.94 (d, J = 6.3 Hz, 3H), 0.91 (s, 3H), 0.62 (s, 3H), 0.09 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.5, 136.1, 121.9, 115.2, 74.1, 72.8, 65.3, 56.0, 54.6, 45.2, 43.0, 42.2, 39.9, 39.2, 38.4, 37.9, 36.4, 36.2, 29.9, 29.8, 28.1, 23.0, 21.0, 20.8, 18.8, 16.2, 11.9, 2.6; IR (neat): 3372, 2946, 2873, 1461, 1364, 1249, 1150, 1045, 838 cm^{-1}; $[\alpha]_D^{23}$ = −45.4 (c 0.69, $CHCl_3$); mp 141–144 °C; HRMS (ESI-TOF): calcd for $[C_{30}H_{52}O_3Si + H]^+$ 489.3764, found 489.3742.

1α,3β,25-Trihydroxycholesta-5,7-diene {1α,25-dihydroxyprovitamin D_3 (10)}

To a solution of 1α,3β-dihydroxy-25-trimethylsilyloxycholesta-5,7-diene (**24**) (272 mg, 0.556 mmol, 1.0 eq.) in THF (1.99 mL) and MeOH (0.98 mL), PPTS (14 mg, 0.056 mmol, 0.1 eq.) was added at 0 °C under argon. After being stirred at the same temperature for 1 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ at 0 °C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (3% MeOH in $CHCl_3$) to give 1α,3β,25-trihydroxycholesta-5,7-diene (**10**)[50] (213 mg, 0.551 mmol, 92%) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 5.50 (m, 1H), 5.27 (m, 1H), 4.63 (d, J = 4.4 Hz, 1H), 4.45 (d, J = 4.9 Hz, 1H), 4.04 (s, 1H), 3.80 (m, 1H), 3.54 (br, 1H), 1.05 (s, 6H), 0.93 (d, J = 6.3 Hz, 3H), 0.79 (s, 3H), 0.57 (s, 3H); ^{13}C NMR (100 MHz, DMSO): δ 140.6, 138.7, 119.6, 114.8, 70.8, 68.8, 63.3, 55.3, 54.2, 44.1, 42.6, 41.5, 36.8, 36.1, 35.6, 29.4, 29.2, 27.7, 22.7, 20.3, 19.8, 18.7, 15.8, 11.7; IR (neat): 3785, 3367, 3019, 2941, 2872, 1377, 1220, 1054, 772, 667 cm^{-1}; $[\alpha]_D^{18}$ = −7.5 (c 0.23, CH_3OH); mp 187–190 °C; HRMS (ESI-TOF): calcd for $[C_{27}H_{44}O_3 + H]^+$ 417.3369, found 417.3371.

Synthesis of activated vitamin D_3 (2), and its analogues 11–13

Solutions of provitamin D_3 analogue **7**, **8**, **9**, **10**, or **24** in 1,4-dioxane were introduced into the syringe pump. The 400 W high-pressure mercury lamp with a Vycor filter was turned on 10 min before starting the reaction. Then, the solution of substrate in 1,4-dioxane was injected at a flow rate of 5 or 10 μL min^{-1}. The mixture that was eluted during the first 400 μL was discarded, and the portion that followed was collected for 750 μL. After removal of solvent, the obtained residue was purified by preparative HPLC to give activated vitamin D_3 analogues **2**, **11**, **12**, **13**, or **25**. The desilylation of **25** was performed in accordance with the previously described condition (**19** to **8**) to afford the desired 1α,25-$(OH)_2$-vitamin D_3 (**2**) in a quantitative yield. The conditions for HPLC purification of obtained compounds, and the observed ^{1}H NMR spectra are shown in the supporting information. The spectral data of synthetic **2**, **11**, **12** and **13** were in good agreement with those reported previously.[53–56]

1α,25-Dihydroxyvitamin D_3 (2)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.38 (d, J = 11.1 Hz, 1H), 6.02 (d, J = 11.1 Hz, 1H), 5.33 (br, 1H), 5.00 (br, 1H), 4.43 (m, 1H), 4.24 (m, 1H), 1.22 (s, 6H), 0.93 (d, J = 6.3 Hz, 3H), 0.54 (s, 3H); IR (neat): 3361, 2924, 2852, 1660, 1634, 1468, 1378, 1144, 1057, 911, 773, 647 cm^{-1}; HRMS (ESI-TOF): calcd for $[C_{27}H_{44}O_3 + H]^+$ 417.3369, found 417.3332.

Vitamin D_2 (11)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.23 (d, J = 11.7 Hz, 1H), 6.03 (d, J = 11.7 Hz, 1H), 5.19 (m, 2H), 5.05 (br, 1H), 4.81 (br, 1H), 3.95 (m, 1H), 1.01 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 7.3 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H), 0.55 (s, 3H); IR (neat): 3329, 2955, 2871, 1647, 1457, 1371, 1050, 970, 894 cm^{-1}; HRMS (ESI-TOF): calcd for $[C_{28}H_{44}O + H]^+$ 397.3470, found 397.3476.

25-Hydroxyvitamin D_3 (12)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.23 (d, J = 11.7 Hz, 1H), 6.03 (d, J = 11.2 Hz, 1H), 5.05 (br, 1H), 4.82 (br, 1H), 3.95 (m, 1H), 1.21 (s, 6H), 0.93 (d, J = 6.4 Hz, 3H), 0.54 (s, 3H); IR (neat): 3361, 2942, 2872, 1644, 1440, 1378, 1215, 1051, 909, 757 cm^{-1}; HRMS (ESI-TOF): calcd for $[C_{27}H_{44}O_2 + H]^+$ 401.3420, found 401.3418.

1α-Hydroxyvitamin D_3 (13)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.38 (d, J = 11.2 Hz, 1H), 6.01 (d, J = 11.2 Hz, 1H), 5.33 (br, 1H), 5.00 (br, 1H), 4.43 (br, 1H), 4.22 (br, 1H), 0.92 (d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.4 Hz, 3H) , 0.86 (d, J = 6.8 Hz, 3H), 0.54 (s, 3H); IR (neat): 3351, 2951, 2870, 1647, 1467, 1378, 1220, 1055, 914, 773 cm^{-1}; HRMS (ESI-TOF): calcd for $[C_{27}H_{44}O_2 + H]^+$ 401.3420, found 401.3419.

Acknowledgements

The authors thank Prof. Takayuki Doi (Tohoku University) for his support in the micro-flow synthesis of activated vitamin D_3. The authors also thank Kuraray Co., Ltd, for kindly providing compound **20**, and Grant-in-Aid for Young Scientists (B) for financial support.

Notes and references

1 R. Bouillon, W. H. Okamura and A. W. Norman, *Endocr. Rev.*, 1995, **16**, 200–257.
2 R. H. Ettinger and H. F. DeLuca, *Adv. Drug Res.*, 1996, **28**, 269–312.
3 J. M. Lappe, D. Travers-Gustafson, K. M. Davies, R. R. Recker and R. P. Heaney, *Am. J. Clin. Nutr.*, 2007, **85**, 1586–1591.
4 G. H. Posner and M. Kahraman, *Eur. J. Org. Chem.*, 2003, 3889–3895.
5 T. Doi, I. Hijikuro and T. Takahashi, *J. Am. Chem. Soc.*, 1999, **121**, 6749–6750.
6 I. Hijikuro, T. Doi and T. Takahashi, *J. Am. Chem. Soc.*, 2001, **123**, 3716–3722.
7 T. Doi, M. Yoshida, I. Hijikuro and T. Takahashi, *Tetrahedron Lett.*, 2004, **45**, 5727–5729.
8 G. D. Zhu and W. H. Okamura, *Chem. Rev.*, 1995, **95**, 1877–1952.
9 N. Kubodera and H. Watanabe, JP188061, 1991.
10 M. Katoh, T. Mikami and H. Watanabe, JP72994, 1994.
11 N. Kubodera, H. Watanabe and K. Miyamoto, JP80626, 1994.
12 D. R. Rafael and P. Andreas, WO128783, 2008.
13 M. P. Rappoldt and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, 1960, **79**, 369–381.
14 V. Malatesta, C. Willis and P. A. Hackett, *J. Am. Chem. Soc.*, 1981, **103**, 6781–6783.
15 W. G. Dauben and R. B. Phillips, *J. Am. Chem. Soc.*, 1982, **104**, 355–356.
16 W. G. Dauben and R. B. Phillips, *J. Am. Chem. Soc.*, 1982, **104**, 5780–5781.
17 T. Sato, H. Yamauchi, Y. Ogata, T. Kunii, K. Kagei, G. Katsui, S. Toyoshima, M. Yasumura and T. Kobayashi, *J. Nutr. Sci. Vitaminol.*, 1980, **26**, 545–556.
18 S. C. Eyley and D. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1975, 858–858.
19 M. Okabe, R. C. Sun, M. Scalone, C. H. Jibilian and S. D. Hutchings, *J. Org. Chem.*, 1995, **60**, 767–771.
20 J. Yoshida, A. Nagaki and T. Yamada, *Chem.–Eur. J.*, 2008, **14**, 7450–7459.
21 C. Wiles and P. Watts, *Eur. J. Org. Chem.*, 2008, 1655–1671.
22 *Microreactors in organic synthesis and catalysis*, ed. T. Wirth, Wiley-VCH, Weinheim, 2008.
23 T. Fukuyama, T. Rahman, M. Sato and I. Ryu, *Synlett*, 2008, 151–163.
24 K. Geyer, T. Gustafsson and P. H. Seeberger, *Synlett*, 2009, 2382–2391.
25 *Handbook of microreactors*, ed. V. Hessel, J. C. Schouten, A. Renken, Y. Wang and J. Yoshida, Wiley-VCH, Weinheim, 2009.
26 *Chemical reactions and processes under flow conditions*, ed. S. V. Luis and E. Carcia-Verdugo, Royal Society of Chemistry, Cambridge, 2010.
27 S. Suga, D. Yamada and J. Yoshida, *Chem. Lett.*, 2010, **39**, 404–406.
28 J. P. McMullen and K. F. Jensen, *Annu. Rev. Anal. Chem.*, 2010, **3**, 19–42.
29 J. Yoshida, *Chem. Rec.*, 2010, **10**, 332–341.
30 J. Yoshida, H. Kim and A. Nagaki, *ChemSusChem*, 2011, **4**, 331–340.
31 M. Baumann, I. R. Baxendale and S. V. Ley, *Mol. Diversity*, 2011, **15**, 613–630.
32 S. Fuse, N. Tanabe and T. Takahashi, *Chem. Commun.*, 2011, **47**, 12661–12663.
33 K. Kaizuka, K.-Y. Lee, H. Miyamura and S. Kobayashi, *J. Flow Chem.*, 2012, **2**, 1–4.
34 B. D. A. Hook, W. Dohle, P. R. Hirst, M. Pickworth, M. B. Berry and K. I. Booker-Milburn, *J. Org. Chem.*, 2005, **70**, 7558–7564.
35 A. Sugimoto, Y. Sumino, M. Takagi, T. Fukuyama and I. Ryu, *Tetrahedron Lett.*, 2006, **47**, 6197–6200.
36 Y. Matsushita, T. Ichimura, N. Ohba, S. Kumada, K. Sakeda, T. Suzuki, H. Tanibata and T. Murata, *Pure Appl. Chem.*, 2007, **79**, 1959–1968.
37 H. Mukae, H. Maeda, S. Nashihara and K. Mizuno, *Bull. Chem. Soc. Jpn.*, 2007, **80**, 1157–1161.
38 F. F. Lv, X. W. Li, U. Z. Wu and C. H. Tung, *Tetrahedron*, 2008, **64**, 1918–1923.
39 K. Tsutsumi, K. Terao, H. Yamaguchi, S. Yoshimura, T. Morimoto, K. Kakiuchi, T. Fukuyama and I. Ryu, *Chem. Lett.*, 2010, **39**, 828–829.
40 T. Horie, M. Sumino, T. Tanaka, Y. Matsushita, T. Ichimura and J. Yoshida, *Org. Process Res. Dev.*, 2010, **14**, 405–410.
41 M. Oelgemoller and O. Shvydkiv, *Molecules*, 2011, **16**, 7522–7550.
42 J. Wegner, S. Ceylan and A. Kirschning, *Adv. Synth. Catal.*, 2012, **354**, 17–57.
43 M. Oelgemöller, *Chem. Eng. Technol.*, 2012, DOI: 10.1002/ceat.201200009, *in press*.
44 M. Nettekoven, B. Pullmann, R. E. Martin and D. Wechsler, *Tetrahedron Lett.*, 2012, **53**, 1363–1366.
45 S. Fuse, N. Tanabe, M. Yoshida, H. Yoshida, T. Doi and T. Takahashi, *Chem. Commun.*, 2010, **46**, 8722–8724.
46 S. Fuse, K. Machida and T. Takahashi, in *New strategies in chemical synthesis and catalysis*, ed. B. Pignataro, Wiley-VCH, 2012, pp. 33–56.
47 Y. Makino and Y. Suzuki, US5158944, 1992.
48 I. Scherlitz-Hofmann, M. Dubs, R. Prousa, B. Schonecker, P. Droescher, H. Schick and E. Schrotter, *Synthesis*, 1999, 1331–1334.
49 D. W. Guest and D. H. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1695–1697.
50 M. Reichenbacher, S. Gliesing, C. Lange, M. Gonschior and B. Schonecker, *J. Prakt. Chem./Chem.-Ztg.*, 1996, **338**, 634–641.
51 T. Takahashi, N. Nakagawa, T. Minoshima, H. Yamada and J. Tsuji, *Tetrahedron Lett.*, 1990, **31**, 4333–4336.
52 N. Nakagawa, *Ph.D. Thesis*, The University of Tokyo, 1990.
53 J. A. Campbell, D. M. Squires and J. C. Babcock, *Steroids*, 1969, **13**, 567–577.
54 K. R. Muralidharan, A. R. Delera, S. D. Isaeff, A. W. Norman and W. H. Okamura, *J. Org. Chem.*, 1993, **58**, 1895–1899.
55 M. D. Mizhiritskii, L. E. Konstantinovskii and R. Vishkautsan, *Tetrahedron*, 1996, **52**, 1239–1252.
56 C. Gomez-Reino, C. Vitale, M. Maestro and A. Mourino, *Org. Lett.*, 2005, **7**, 5885–5887.