

Synthesis of C-2 substituted vitamin D derivatives having ringed side chains and their biological evaluation, especially biological effect on bone by modification at the C-2 position†

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In order to obtain vitamin D derivatives, which have strong activity for enhancing bone growth, we designed vitamin D derivatives with various substitutions at the C-2 position. Novel 2 α -substituted vitamin D derivatives were synthesized starting from D-glucose as a chiral template of the A-ring with a CD-ring bromoolefin unit using the Trost coupling method. We evaluated these compounds by two *in vitro* assays, affinity to VDR and transactivation assays, using human osteosarcoma (Hos) cells, and demonstrated the SAR of the C-2 position of VD₃. Furthermore, by using the OVX model, we found that compound **5c**, which has a hydroxypropoxy side chain at C-2 and 2,2-dimethyl cyclopentanone in the CD-ring side chain, has a strong activity for enhancing bone growth, same as the reported compound, 2 α -(3-hydroxypropoxy)-1 α ,25-dihydroxyvitamin D₃ **1d**, and this derivative shows a possibility that calcemic activity is less than **1d** *in vivo*.

1. Introduction

1 α ,25-Dihydroxyvitamin D₃ (Fig. 1, **1a**) shows biological activities through the interaction with a vitamin D receptor (VDR), which is a member of the nuclear hormone receptor superfamily, and acts as a ligand-dependent gene transcription factor with co-activators.^{1,2} Although the biological activities of **1a** vary,

such as the proliferation and differentiation of various types in tumor cells, and the regulation of immune reactions,^{3,4} the most important physiological role of **1a** is the regulation of calcium and phosphorus metabolism as well as bone remodeling *via* its action in the bone, intestine and kidney; therefore, **1a** is used as a therapeutic drug for osteoporosis, a systemic skeletal disease characterized by low bone mineral density (BMD) leading to reduced bone strength and fracture.

However, the use of vitamin D as a therapeutic agent is limited because of its calcemic and phosphatemic activities; therefore, in order to find new vitamin D analogs, which are more efficacious, safer and more selective than natural **1a**, numerous analogs of **1a** have been developed.^{4,5} Most of the analogs have a modified side chain. As an example, 1 α ,25-dihydroxyvitamin D₃-26,23-lactones **2**, which was reported by Ishizuka *et al.*, has a unique structure

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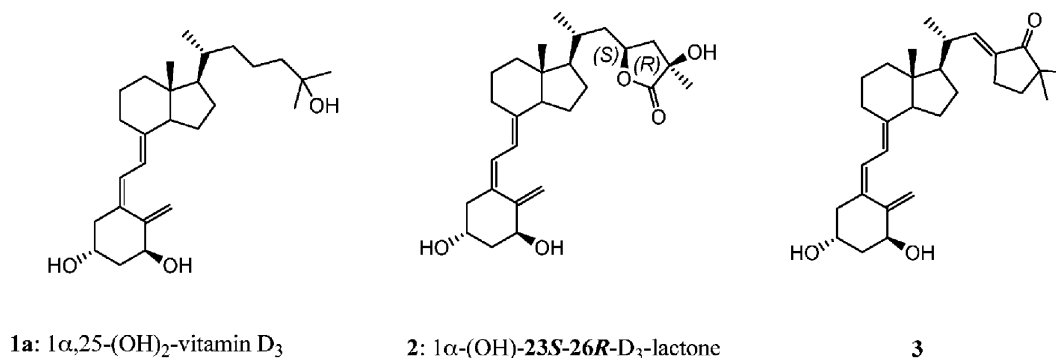


Fig. 1 Structures of 1 α ,25-dihydroxyvitamin D₃ (**1**) and its derivatives.

with a lactone ring on the side chain.⁶ We therefore synthesized and evaluated vitamin D derivatives with a ringed side chain in their structures. In a series of derivatives, we report that compound **3**, which has a cyclopentanone in the side chain, showed strong affinity with VDR.⁷

On the other hand, Kittaka *et al.* developed the first systematic synthesis of novel analogs of vitamin D₃ based on the structural modification of the A-ring in order to investigate A-ring structure–activity relationships.^{8–13} During the course of their studies, they found that the introduction of methyl (**1b**),⁸ 3-hydroxypropyl (**1c**),⁹ and 3-hydroxypropoxy (**1d**)¹⁰ groups at the C-2 α position showed 2- to 4-fold higher binding affinity relative to the natural hormone **1a** (Fig. 2). In addition, Saito *et al.* reported that 2 α modification of TEI-9647 (**4a**), which is the first vitamin D₃ antagonist, increases antagonistic activity significantly.¹⁴

Against this background, we modified **3** with 2 α substitution in order to obtain VD₃ derivatives, which have strong activity for enhancing bone growth. As mentioned above, the 2 α -methyl group (**1b**), 2 α -3-hydroxypropyl group (**1c**), and 2 α -3-hydroxypropoxy group (**1d**) were appropriate substituted groups for the higher affinity to VDR. Recent research about co-crystals of these ligands and the ligand-binding domain (LBD) of VDR clarified the reason for the higher activity of these compounds.¹⁵ Namely, the C-2 α methyl group of **1b** interacts with amino acid residues Ser237, Leu233, and Phe150 in the LBD, and each C-2 α terminal hydroxy group of **1c** and **1d** forms a new hydrogen bond between the hydroxy group and Arg274; therefore, we were interested in the effect of these substitutions for enhancing bone growth and we designed **5a–5c** (Fig. 3). In addition, we designed **5d–5h** as new 2 α substituted groups. As stated above, the co-crystals of **1c** and **1d**

only showed an interaction between the terminal hydroxy group and Arg274 of VDR, but there remained a cavity around the alkyl side chain of **1c** and **1d**. We therefore designed **5d** and **5e** because we expected that more occupation of the cavity around the C-2 position of the A-ring would increase the affinity to VDR and the biological activities. Furthermore, we designed **5f–5h** in order to investigate the effects for the affinity to VDR and the biological activities by the difference of the terminal group in 2 α -substituted groups.

2. Results

Our synthesis plan of 2 α -substituted vitamin D₃ derivatives is shown in Scheme 1. The triene skeleton of the desired vitamin D₃ derivatives (**5a–5h**) was constructed by Trost's Pd-catalyzed alkenylative cyclization¹⁶ of A-ring precursor enynes (**7a–7h**) with the CD-ring bromoolefin counterpart (**6**).⁷ The A-ring precursor enynes (**7a–7h**) could be synthesized from D-glucose derivatives on the basis of the reported scheme.¹⁰

2.1. Synthesis

2.1.1. Preparation of A-ring precursors 7a–7h. We synthesized the A-ring precursors **7d** and **7g**, in which the cyclopropyl group is introduced into the center of the 2 α side chain from the D-glucose derivative (Scheme 2).

Treatment of the known epoxide **8**¹⁷ with 2,2-cyclopropane-1,3-diol and KO^tBu under heat conditions gave 3-(3-hydroxy-2,2-ethanopropyl)oxyaltropyranoside **9**. Protection of the primary alcohol using pivaloyl chloride, followed by O-silylation, gave

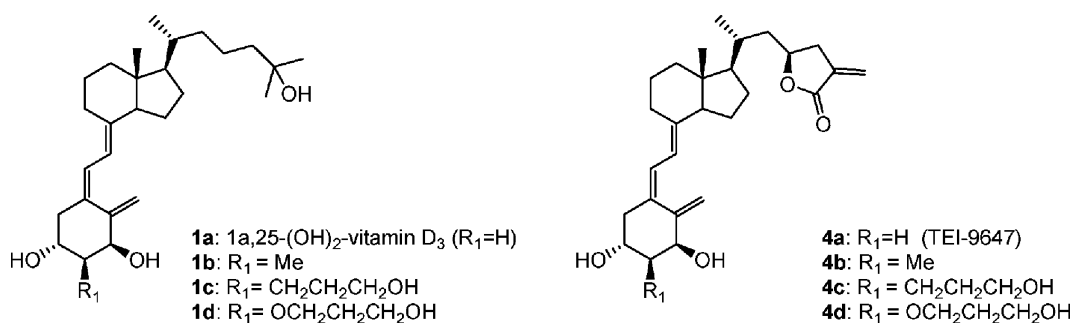


Fig. 2 Structures of 1 α ,25-dihydroxyvitamin D₃ (**1a**), 1 α -hydroxyvitamin D₃-26,23-lactones (TEI-9647:**4a**) and their representative C-2 α -modified analogs (**1b–d**, **4b–d**).

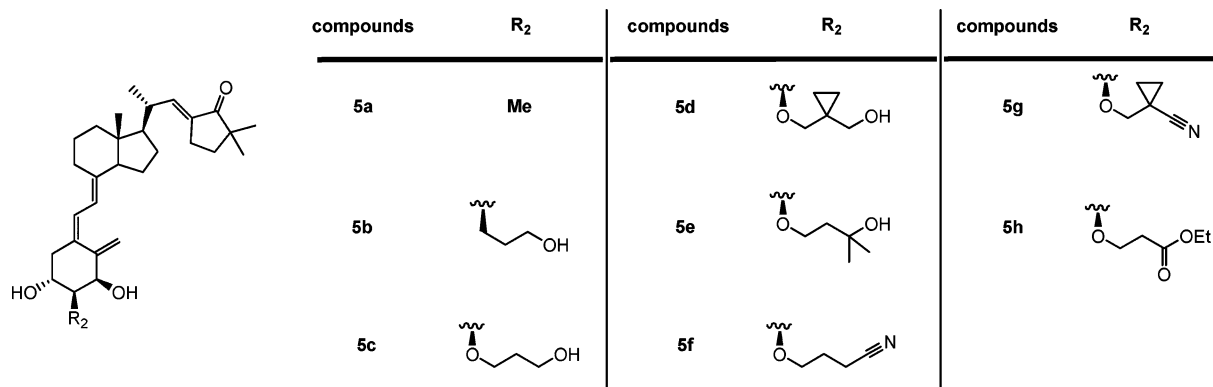
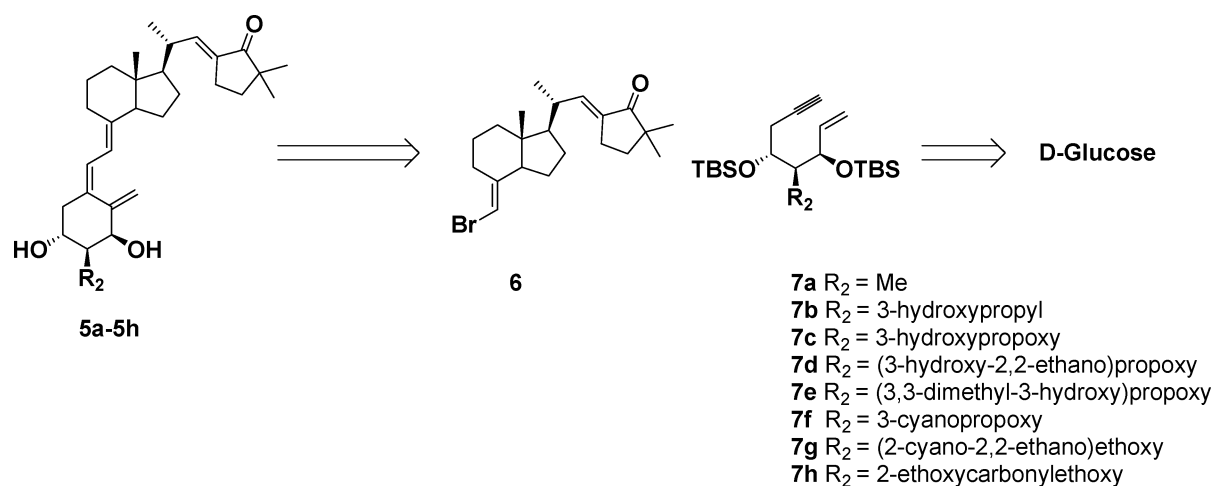
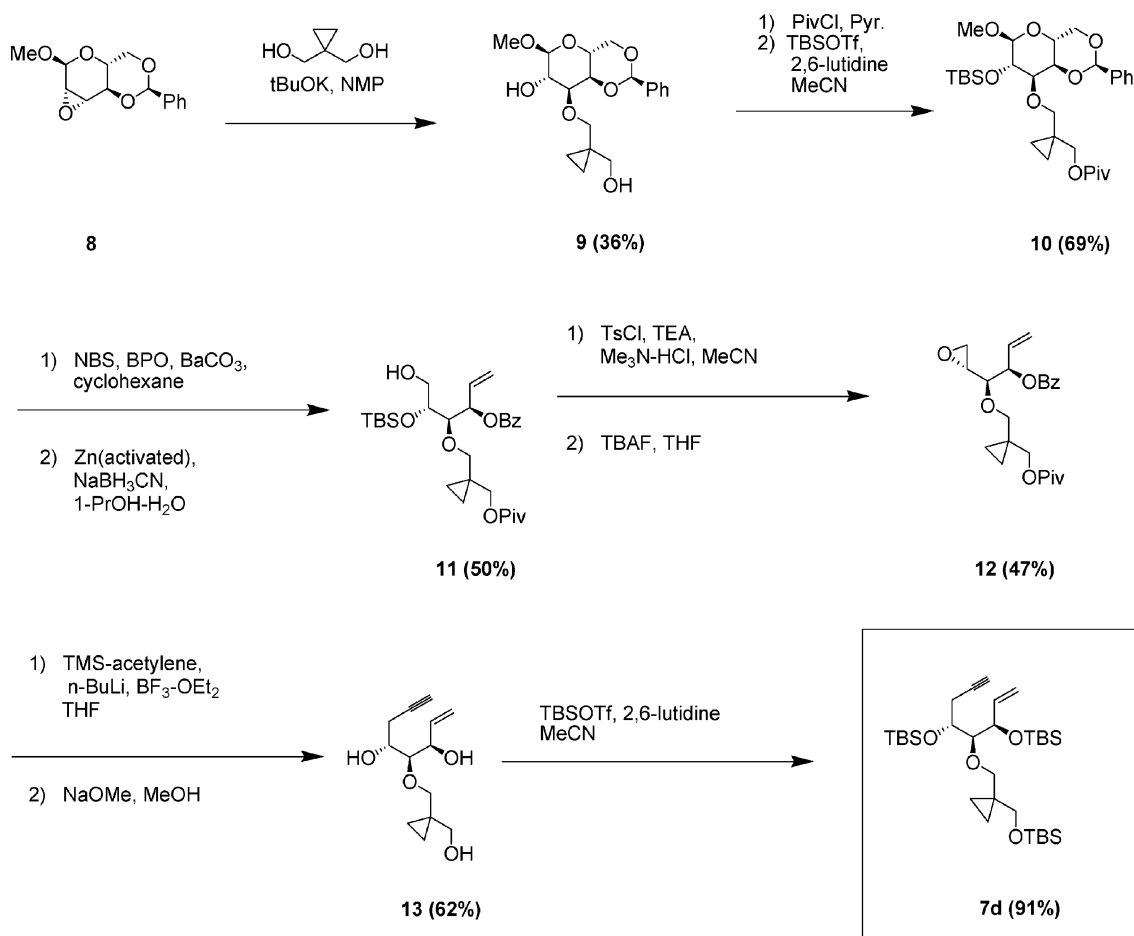


Fig. 3 Design of derivatives of **3** (R₂ = H).



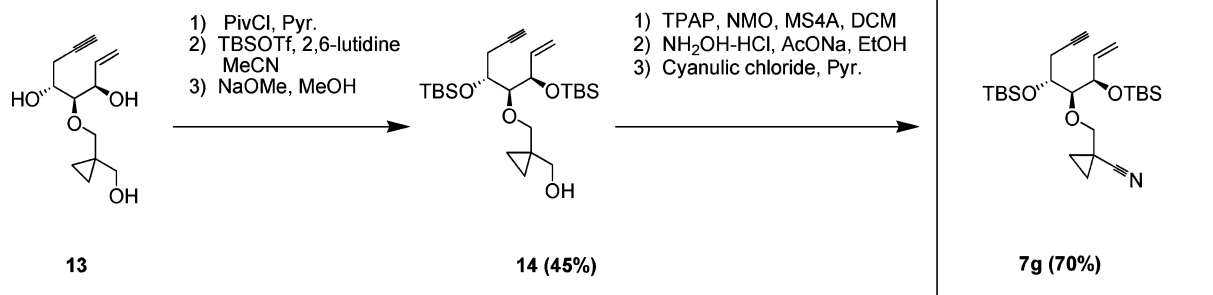
Scheme 1 Retrosynthesis.



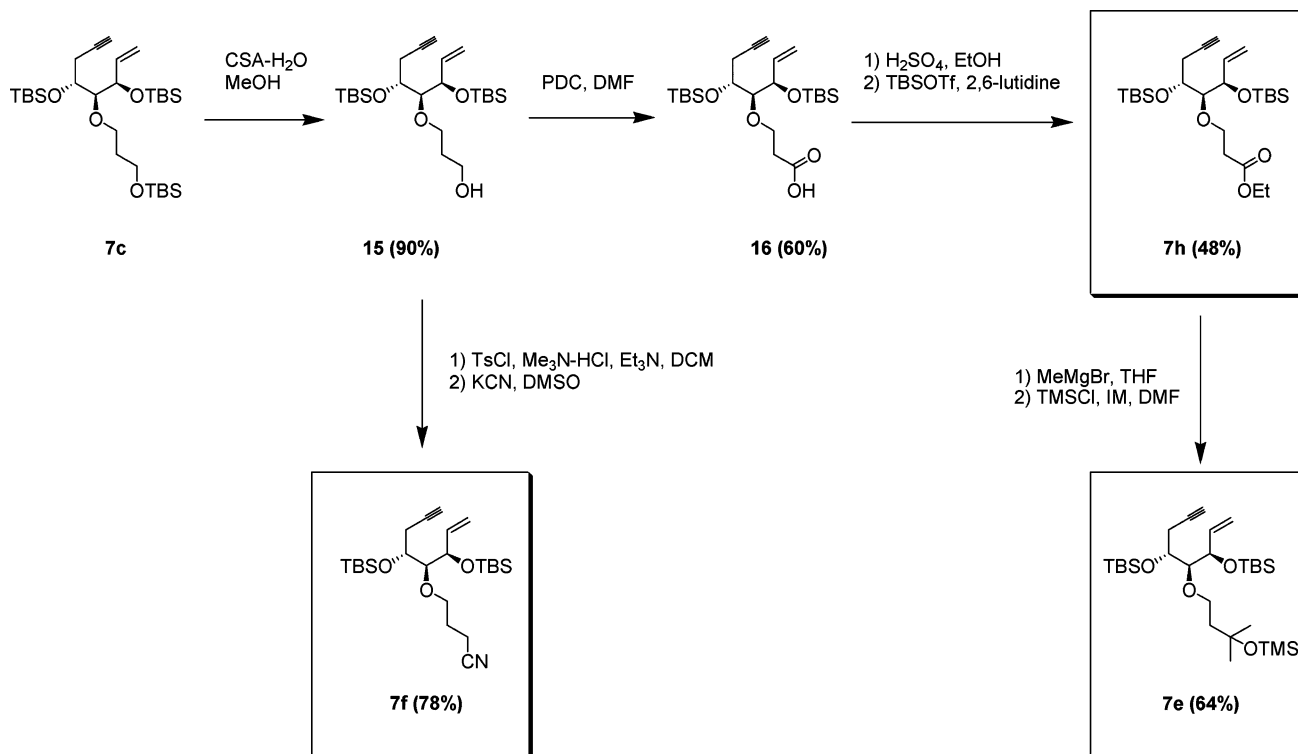
Scheme 2 Synthetic route of **7d**.

protected atropyranoside **10**. Treatment with NBS, followed by reduction using activated zinc powder and NaBH₃CN gave alcohol **11**. Tosylation and subsequent deprotection with TBAF provided epoxide **12**. Ethynylation using lithium TMS acetylide in the presence of BF₃-Et₂O in THF and methanolysis gave triol **13**. Finally, protection of triol by TBS groups gave the desired enyne A-ring precursor **7d**.

For the A-ring precursor **7g**, the end of 2 α side chain of **7d** was substituted with a cyano group and synthesized as follows (Scheme 3). Protection of the primary alcohol of **13** with a pivaloyl group, followed by silylation and deprotection of the pivaloyl group gave alcohol **14**. Oxidation of alcohol followed by conversion to oxime and dehydration provided nitrile **7g**.



Scheme 3 Synthetic route of **7g**.



Scheme 4 Synthetic route of **7e**, **7f** and **7h**.

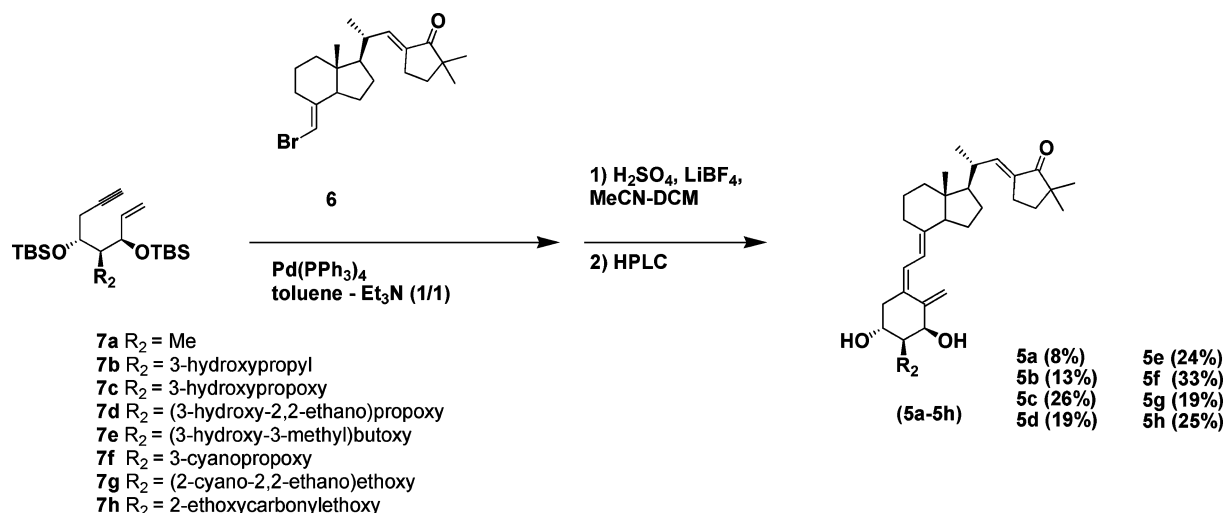
For the A-ring precursor **7e**, the primary alcohol of the 2 α side chain of **7c** was changed to the tertiary alcohol, was synthesized as follows (Scheme 4). The known enyne A-ring precursor **7c**¹⁰ was treated with camphorsulfonic acid to give alcohol **15**. Oxidation of **15** with excess PDC (5 mol. equiv.) provided carboxylic acid **16**. Esterification followed by re-protection gave the A-ring precursor **7h**. Moreover, the desired A-ring precursor **7e** was provided by treatment of **7h** with methyl magnesium bromide, followed by protection. Furthermore, the A-ring precursor **7f** was obtained by tosylation of **15** and substitution to the cyano group.

2.1.2. Synthesis of 2-substituted vitamin D₃ derivatives. The palladium-catalyzed coupling reaction of the above enynes **7a–7h** and bromoolefin **6**, followed by deprotection of silyl groups and purification by reversed-phase HPLC provided the desired compounds **5a–5h**, respectively (Scheme 5).

2.2. Biological evaluation

2.2.1. In vitro evaluation (affinity to VDR and transactivation assays on Hos cells). The newly synthesized derivatives were evaluated *in vitro*. We evaluated new derivatives by two *in vitro* assays, affinity to VDR and transactivation assays, using human osteosarcoma (Hos) cells. The evaluation results are shown in Table 1.

As described above, it was reported that the methyl group, hydroxypropyl group, and 3-hydroxypropoxy group at C-2 contributed to increased affinity to VDR, but when the side chain in the CD-ring was the ring, there was a difference in activity between the above appropriate substitution groups. Namely, modification by the 3-hydroxypropoxy group at C-2, compound **5c**, increased the affinity to VDR, but 2 α -Me modification, **5a**, and 3-hydroxypropyl modification, **5b**, did not contribute to improve the affinity to VDR markedly. Additionally, new derivatives **5d** and **5e**, which have branched 3-hydroxypropoxy side chains at



Scheme 5 Coupling and deprotection of A-ring precursor and CD-bromoolefin.

Table 1 VDR binding affinity and osteocalcin promoter transactivation in Hos cells

compd	VDR affinity (%) ^a	Transactivation assay (EC ₅₀ , pM)	compd	VDR affinity (%)	Transactivation assay (EC ₅₀ , pM)
1	100	30	5d	5.5	36.9
3	22.7	16.2	5e	2	630
5a	11.6	20	5f	10	59.0
5b	32.3	11.2	5g	18	105
5c	58.8	6.7	5h	1	7460

^a The potency of **1** is normalized to 100.

C-2, also decreased the affinity to VDR. In particular, **5e** led to a significantly decreased affinity. In the case of compounds **5f–5h**, in which terminal groups in the side chain are substituted from the hydroxy group to other groups, the affinity to VDR of compounds **5f** and **5g** was moderate, but that of **5h** was decreased.

With respect to *trans* activity assay on Hos cells, it was found that there was a moderate correlation between activity of *trans* activity assay and the affinity to VDR (Fig. 4). Especially, compound **5c** showed a stronger activity (6.9 pM) than that of natural hormone **1** and compound **5e**.

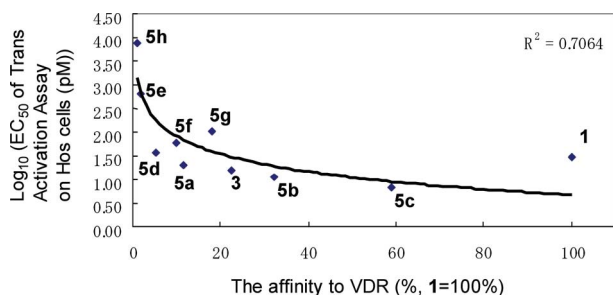


Fig. 4 Relationship between the affinity to VDR and the transactivation assay on Hos cells of **1**, **3** and **5a–5h**.

2.2.2. In vivo evaluation: Evaluation of effects of derivatives on bone mineral loss in ovariectomized rats. As described above, we found that compound **5c** had strong activity on Hos cells; therefore, we evaluated the *in vivo* therapeutic effect of compound **5c** using an ovariectomized rat therapeutic model (OVX). Twelve-

week-old Sprague-Dawley female rats (Charles River, Japan) were ovariectomized and fed a normal diet containing 1.0% Ca *ad libitum* for 4 weeks. The rats were then administered vitamin D₃ derivatives at doses from 0.006 μg kg⁻¹ to 1 μg kg⁻¹, 5 times a week for 4 weeks. The sham and OVX groups were administered MCT alone. Twenty-four hours after the final administration, blood was collected under ether anesthesia, the rats were then euthanized, and BMD of the spine (L4–L5) bone mass was measured by dual X-ray absorptiometry. The results of BMD and serum Ca density of 3 vitamin D₃ derivatives are shown in Fig. 5, 6 and 7, respectively.

As shown in Fig. 5a and 5b, the starting compound **3** did not show such a strong increase of BMD, although it showed strong activity *in vitro*, and the high dose of **3** especially showed hypercalcemia. In the case of 2α-hydroxypropoxy-1,25-(OH)₂-vitamin D₃ **1d** (Fig. 6a and 6b), it showed an increase of BMD, which was not linear. Furthermore, the calcemic activity was observed from the lowest dose. It was found that by stepping up the dose, the calcemic activity was stronger than the increase of BMD.

On the other hand, newly synthesized derivative **5c** (Fig. 7a and 7b) showed a significant increase of BMD at 0.025 μg kg⁻¹, and the significant difference of the calcemic activity was not observed at this dose.

3. Discussion

In this research, we designed novel vitamin D derivatives with the reported and new 2α side chains. We designed and synthesized compounds **5d** and **5e** because we expected that the terminal

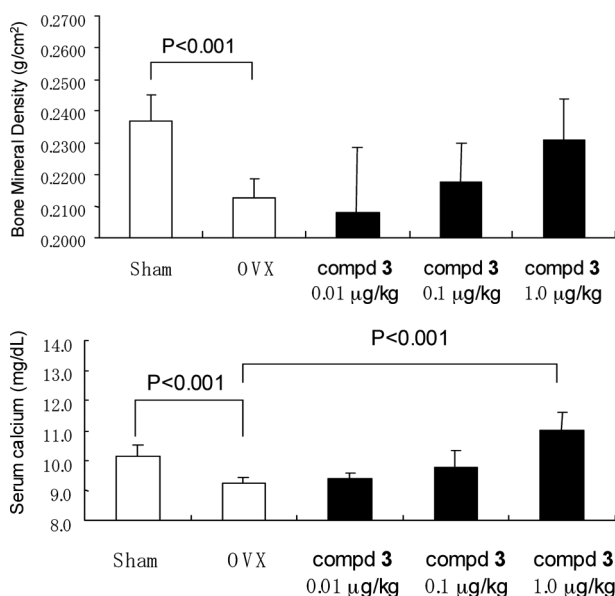


Fig. 5 (a) Spiral bone mineral density (BMD) in the OVX rat therapeutic model administered compound **3** orally (b) Serum calcium level in the OVX rat therapeutic model administered compound **3** orally.

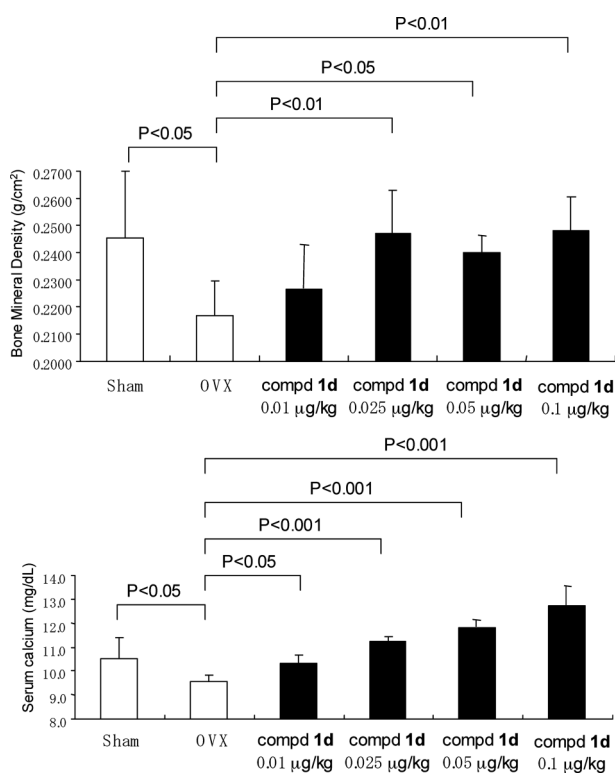


Fig. 6 (a) Spiral bone mineral density (BMD) in the OVX rat therapeutic model administered compound **1d** orally (b) Serum calcium level in the OVX rat therapeutic model administered compound **1d** orally.

hydroxy group of these compounds would form hydrogen bonds with Arg274 as reported with compound **1d**; however, the affinity to VDR of compounds **5d** and **5e** was decreased more than that of compound **5c**. From these results, we consider that the terminal hydroxy group of 2α side chains in compounds **5d** and **5e** might not be able to form a hydrogen bond with Arg274 because the

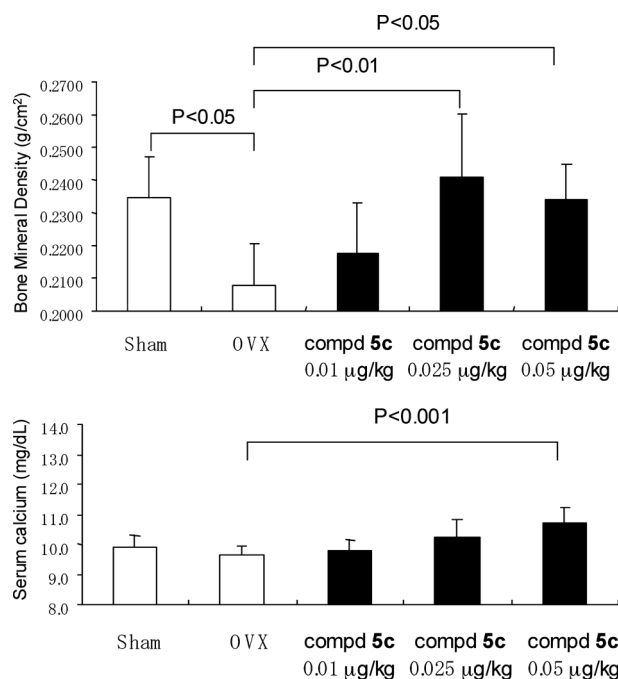


Fig. 7 (a) Spiral bone mineral density (BMD) in the OVX rat therapeutic model administered compound **5c** orally (b) Serum calcium level in the OVX rat therapeutic model administered compound **5c** orally.

terminal hydroxy groups of these compounds might move by insertion of the substituted groups into the alkyl side chains. In addition, we designed compounds **5f–5h**, in which the terminal groups were substituted. In these compounds, the affinity to VDR of compounds **5f** and **5g** did not markedly decrease. In the previous paper,^{9b,10c} it was reported that VD₃ derivatives, in which alkyl side chains were introduced at C-2, markedly decreased the affinity to VDR except for the 2α -methyl substituted VD₃ derivative.^{9a} From the difference between the activity of the cyano side chains and alkyl side chains, it was supposed that compounds **5f** and **5g** might interact with amino acids in VDR.

About the activity for enhancing bone growth, we evaluated newly synthesized derivatives by transactivation assays on Hos cells. The activity showed a moderate correlation with the affinity to VDR. So, we consider the activity for enhancing bone growth was based on the affinity to VDR.

Furthermore, we evaluated 2α -substituted vitamin D₃ derivatives *in vivo*. It was confirmed that compound **1d** has strong activity for enhancing bone growth (Fig. 6a), but the calcemic activity was also observed from the lowest dose (Fig. 6b), so we consider that compound **1d** has a strong calcemic activity. On the other hand, compound **5c** showed a strong activity for enhancing bone growth, same as **1d** (Fig. 7a), but the significant difference of the calcemic activity was not observed at $0.025 \mu\text{g kg}^{-1}$ (Fig. 7b). From these results, we consider that compound **5c** possibly has a calcemic activity less than that of **1d**. It was supposed that the structure of the side chain in the CD-ring would contribute to the separate activity for enhancing bone growth from calcemic activity.

4. Conclusion

We designed and synthesized novel vitamin D₃ derivatives with various substituted side chains at the C-2 position in order to

obtain vitamin D₃ derivatives with strong activity for enhancing bone growth. As the result of *in vitro* experiments, we demonstrated the SAR of the C-2 position of VD₃. Furthermore, by using the OVX model, we found that compound **5c**, which has a hydroxypropoxy side chain at C-2 and 2,2-dimethyl cyclopentanone in the CD-ring side chain, has a strong activity for enhancing bone growth, same as the reported compound, 2 α -(3-hydroxypropoxy)-1 α ,25-dihydroxyvitamin D₃ **1d**, and this derivative shows a possibility that the calcemic activity is less than that of **1d** *in vivo*.

5. Experimental section

5.1. Synthesis

5.1.1. General. NMR spectra were measured using a JEOL AL-400 magnetic resonance spectrometer. Infrared spectra data were recorded on a JASCO FTIR-5300 spectrometer. Mass spectra were measured on a Shimadzu LC-MS-IT-TOF. Specific optical rotations were measured using a JASCO P-1030 polarimeter. Purification by flash column chromatography on silica gel was carried out using a Biotage FLASH system. Preparative thin layer chromatography was performed using Merck Kieselgel F²⁵⁴ plates. Reversed-phase HPLC was carried out on a Shimadzu LC-2010 system.

5.1.2. Methyl 4,6-O-benzylidene-3-[(3-hydroxy-2,2-ethanopropyl)oxy- α -D-altoxypranoside (9**).** To a stirred solution of epoxide **8** (6.0 g, 22.7 mmol) in *N*-methyl-pyrrolidone (60 mL) was added 2,2-cyclopropane-1,3-propanediol (15 g, 146.8 mmol) and KO^tBu (10.19 g, 90.8 mmol) at room temperature. The solution was stirred at 130 °C for 8 h. The reaction mixture was cooled to room temperature, the solution was diluted with H₂O (240 mL), and Dia-ion HP-20SS (Mitsubishi Chemical Industries, 30 g) was added to the solution and stirred at room temperature overnight. Filtration was followed by washing with saturated aq. NH₄Cl, H₂O, and elution by acetone. The elution was evaporated, the residue was diluted with EtOAc, and the solution was washed with brine. The organic layer was dried over MgSO₄, followed by filtration and concentration. The residue was purified by flash column chromatography on silica gel (*n*-hexane–EtOAc = 20/80) to provide 2.97 g of **9** as a colorless syrup (8.1 mmol, 36%). [α]_D²⁵ +71.1 (*c* 1.0, CHCl₃); IR (film, CHCl₃) 3020, 1217, 1099, 1045 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 7.51–7.36 (5H, m), 5.54 (1H, s), 4.61 (1H, s), 4.40–4.29 (2H, m), 4.08 (1H, t, *J* = 4.3 Hz), 4.01 (1H, dd, *J* = 9.3, 2.7 Hz), 3.93 (1H, br s), 3.83–3.75 (3H, m), 3.60–3.50 (3H, m), 3.41 (3H, s), 0.59–0.41 (3H, m); ¹³C NMR (100 MHz, CDCl₃) δ : 137.6, 129.8, 129.2, 128.4, 126.3, 126.2, 102.6, 101.2, 80.1, 76.0, 70.4, 69.8, 69.3, 59.1, 55.5, 22.8, 9.2, 9.1; EI-LRMS *m/z* 389.1 (M+Na); EI-HRMS calcd for C₁₉H₂₆O₇Na 389.1571 (M+Na), found 389.1576.

5.1.3. Methyl 4,6-O-Benzylidene-2-(*tert*-butyldimethylsilyloxy-3-[3-(2,2-dimethylpropionyl)oxy-2,2-ethanopropyl]oxy- α -D-altoxypranoside (10**).** To a solution of **9** (2.97 g, 8.10 mmol) in pyridine (30 mL), pivaloyl chloride (1.15 mL, 9.32 mmol) was added at 0 °C and stirred at the same temperature for 1 h. Dry MeOH (3 mL) was added to the reaction mixture and stirred at room temperature for 5 min. The reaction mixture was evaporated and diluted with toluene. The solution was washed with brine, and the organic layer was dried over MgSO₄, followed

by filtration and concentration. The residue was dissolved in dry CH₂Cl₂ (20 mL) and cooled to 0 °C. To the solution were added 2,6-lutidine (1.3 mL, 11.6 mmol) and *t*-butyldimethylsilyl trifluoromethanesulfonate (2.14 mL, 9.32 mmol), and the reaction mixture was stirred at room temperature for 1 h. Dry MeOH (5 mL) was added to the reaction mixture and stirred at room temperature for 5 min. The reaction mixture was evaporated and diluted with toluene. The solution was washed with brine, and the organic layer was dried over MgSO₄, followed by filtration and concentration. Purification by flash silica gel chromatography (*n*-hexane–EtOAc = 95/5, then 90/10) gave **10** (3.19 g, 5.61 mmol) in 69% as a colorless syrup. [α]_D²⁵ +36.71 (*c* 1.0, CHCl₃); IR (film, CHCl₃) 3020, 1716, 1217, 1107, 1047 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 7.49–7.34 (5H, m), 5.56 (1H, s), 4.45 (1H, s), 4.29–4.25 (2H, m), 4.18 (1H, d, *J* = 11.2 Hz), 3.98–3.92 (3H, m), 3.75 (1H, t, *J* = 12.0 Hz), 3.65 (1H, t, *J* = 2.7 Hz), 3.60 (1H, d, *J* = 9.5 Hz), 3.52 (1H, d, *J* = 9.5 Hz), 3.35 (3H, s), 1.19 (9H, s), 0.91 (9H, s), 0.61–0.51 (4H, m), 0.10 (3H, s), 0.10 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ : 178.5, 137.8, 129.0, 128.2, 126.2, 102.3, 102.2, 73.5, 70.0, 69.4, 67.6, 58.4, 55.3, 38.8, 27.2, 25.7, 25.5, 20.4, 18.0, 8.2, 8.1, –5.0; EI-LRMS *m/z* 587.2 (M+Na), 582.2 (M+H₂O); EI-HRMS calcd for C₃₀H₄₈O₈SiNa 587.3011 (M+Na), found 587.3012.

5.1.4. (2*R*,3*R*,4*R*)-4-(Benzoyloxy)-2-(*tert*-butyldimethylsilyloxy)-3-[3-(2,2-dimethylpropionyl)oxy-2,2-ethanopropyl]oxyhex-5-en-1-ol (11**).** To a solution of **10** (3.17 g, 5.61 mmol) in cyclohexane (63 mL) were added BaCO₃ (775 mg, 3.92 mmol), benzoyl peroxide (136 mg, 0.56 mmol), and *N*-bromo succinimide (1.21 g, 6.73 mmol) at room temperature, and the reaction mixture was refluxed for 1 h. The reaction mixture was cooled to room temperature followed by filtration with celite. The residue was washed with cyclohexane. The combined organic layer was washed with brine and then dried over MgSO₄, followed by filtration and concentration. The residue (4.0 g) was dissolved in a mixture of 1-PrOH (36 mL) and H₂O (4 mL). To the solution were added activated zinc powder (7.38 g, 112.2 mmol) and NaBH₃CN (1.42 g, 22.4 mmol), and the mixture was stirred vigorously at 110 °C for 1 h. The reaction mixture was cooled to room temperature, followed by filtration. Almost all 1-PrOH was removed by evaporation and the residue was diluted with EtOAc, washed with brine, and dried over MgSO₄. After filtration and concentration, purification by flash chromatography on silica gel (*n*-hexane–EtOAc = 90/10 then 80/20) provided alcohol **11** (1.50 g, 2.80 mmol) in 50% yield (2 steps). [α]_D²⁵ +31.21 (*c* 1.0, CHCl₃); IR (film, CHCl₃) 3020, 1716, 1215 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 8.05–8.02 (2H, m), 7.59–7.43 (3H, m), 6.11 (1H, ddd, *J* = 11.0, 17.3, 6.0 Hz), 5.78–5.75 (1H, m), 5.41 (1H, dt, *J* = 17.3, 1.3 Hz), 5.30 (1H, dt, *J* = 10.4, 1.2 Hz), 4.17 (1H, d, *J* = 11.4 Hz), 3.96–3.93 (2H, m), 3.81 (1H, dd, *J* = 11.4, 5.1 Hz), 3.73–3.68 (2H, m), 3.64 (1H, d, *J* = 9.7 Hz), 3.50 (1H, d, *J* = 9.7 Hz), 1.18 (9H, s), 0.90 (9H, s), 0.55 (4H, t, *J* = 1.9 Hz), 0.09 (3H, s), 0.07 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ : 178.5, 165.3, 133.1, 133.0, 130.2, 129.5, 128.4, 118.9, 82.4, 75.1, 74.9, 72.6, 67.4, 63.9, 38.8, 27.1, 25.8, 20.7, 18.0, 8.6, –4.6; EI-LRMS *m/z* 557.2 (M+Na); EI-HRMS calcd for C₂₉H₄₆O₇SiNa 557.2905, found 557.2914.

5.1.5. (3*R*,4*R*,5*R*)-3-(Benzoyloxy)-4-[3-(2,2-dimethylpropionyl)oxy-2,2-ethanopropyl]oxy-5,6-epoxyhex-1-ene (12**).** To a solution of **11** (2.41 g, 4.5 mmol) in dry MeCN (25 mL) were added

triethylamine (1.26 mL, 9 mmol), trimethylamine hydrochloride salt (86 mg, 0.9 mmol), and *p*-toluenesulfonyl chloride (1.30 g, 6.8 mmol) at room temperature and stirred at the same temperature for 1 h. The reaction mixture was quenched with saturated aq. NaHCO₃ followed by evaporation. The residue was diluted with EtOAc and washed with brine. The organic layer was dried over MgSO₄, followed by filtration and concentration. The residue (3.31 g) was diluted with THF (18 mL) and tetrabutyl ammonium fluoride (1 M in THF, 13.5 mL, 13.5 mmol) was added to the solution and refluxed for 1.5 h. The reaction mixture was cooled to room temperature and diluted with EtOAc, washed with brine. The organic layer was dried over MgSO₄, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 90/10) to give **12** (851 mg, 2.1 mmol) in 47% yield. [α]_D²⁵ +31.21 (*c* 1.0, CHCl₃); IR (film, CHCl₃) 3020, 1718, 1271, 1217, 1165, 1111 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 8.05–8.02 (2H, m), 7.59–7.43 (3H, m), 6.11 (1H, ddd, *J* = 11.0, 17.3, 6.0 Hz), 5.78–5.75 (1H, m), 5.41 (1H, dt, *J* = 17.3, 1.3 Hz), 5.30 (1H, dt, *J* = 10.4, 1.2 Hz), 4.17 (1H, d, *J* = 11.4 Hz), 3.96–3.93 (2H, m), 3.81 (1H, dd, *J* = 11.4, 5.1 Hz), 3.73–3.68 (2H, m), 3.64 (1H, d, *J* = 9.7 Hz), 3.50 (1H, d, *J* = 9.7 Hz), 1.18 (9H, s), 0.90 (9H, s), 0.55 (4H, t, *J* = 1.9 Hz), 0.09 (3H, s), 0.07 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ : 178.5, 165.3, 133.1, 133.0, 130.2, 129.5, 128.4, 118.9, 82.4, 75.1, 74.9, 72.6, 67.4, 63.9, 38.8, 27.1, 25.8, 20.7, 18.0, 8.6, –4.6; EI-LRMS *m/z* 425.1 (M+Na), 420.2 (M+H₂O), 403.1 (M+H); EI-HRMS calcd for C₂₃H₃₀O₆Na 425.1935, found 425.1929

5.1.6. (3R,4S,5R)-4-[3-Hydroxy-2,2-ethanopropyl]oxyoct-1-en-7-yne-3,5-diol (13). A solution of trimethylsilyl acetylene (1.62 mL, 11.5 mmol) in THF (3 mL) under N₂ gas atmosphere was cooled in dry ice–acetone. To the solution was added *n*-butyl lithium in hexane (2.64 M, 3.97 mL, 10.5 mmol) and the solution was stirred at the same temperature for 45 min. To the solution were added a solution of **12** (846 mg, 2.1 mmol) in THF (6 mL) and BF₃–diethylether complex (0.343 mL, 2.73 mmol) successively and the reaction mixture was stirred at the same temperature for 2 h, and then at 0 °C for 1 h. Saturated aq. NH₄Cl was added to the solution and the reaction mixture was warmed to room temperature and extracted with EtOAc. The organic layer was washed with saturated aq. NaHCO₃, brine, dried over MgSO₄, and evaporated. The residue was dissolved in dry MeOH (10 mL) and sodium methoxide (870 mg, 6.3 mmol) was added to the solution. The solution was stirred at 50 °C for 1 h. The reaction mixture was cooled to room temperature and evaporated. The residue was diluted with EtOAc and the solution was washed with brine, dried over MgSO₄, and evaporated. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 60/40, then 50/50 then 35/65) to give **13** (311.5 mg, 1.30 mmol) in 62% yield.

[α]_D²⁵ –15.06 (*c* 0.5, CHCl₃); IR (film, CHCl₃) 3020, 1217, 1041 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ : 5.57 (1H, ddd, *J* = 17.0, 11.0, 6.0 Hz), 4.88 (1H, dt, *J* = 17.0, 1.7 Hz), 4.73 (1H, dt, *J* = 11.0, 1.7 Hz), 3.85–3.81 (1H, m), 3.51 (1H, ddd, *J* = 8.4, 5.7, 2.1 Hz), 3.16 (1H, d, *J* = 9.5 Hz), 3.05 (1H, d, *J* = 9.5 Hz), 2.85 (2H, dd, *J* = 4.6, 2.2 Hz), 2.12–1.92 (2H, m), 1.85 (1H, t, *J* = 2.7 Hz); ¹³C NMR (100 MHz, CD₃OD) δ : 139.95, 116.22, 83.51, 81.92, 77.77, 71.46, 71.07, 67.23, 24.31, 23.77, 9.14, 9.02; EI-LRMS *m/z*

263.1 (M+Na); EI-HRMS calcd for C₁₃H₂₀O₄Na 263.1254, found 263.1244.

5.1.7. (3R,4S,5R)-3,5-Bis-[(*tert*-butyldimethylsilyloxy)-4-[3-(*tert*-butyldimethylsilyloxy)-2,2-ethanopropyl]oxyoct-1-en-7-yne (7d). To a solution of **13** (257.0 mg, 1.07 mmol) in CH₂Cl₂ (5 mL) were added 2,6-lutidine (0.748 mL, 0.42 mmol) and *t*-butyldimethylsilyl trifluoromethanesulfonate (1.11 mL, 4.82 mmol) at 0 °C and they were stirred at room temperature for 1 h. Dry MeOH (2 mL) was added to the reaction mixture at room temperature and stirred at the same temperature for 10 min. The reaction mixture was diluted with hexane, washed with H₂O and dried over MgSO₄, followed by filtration and evaporation. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 96/4) to give **7d** (571 mg, 0.979 mmol) in 91% yield. [α]_D²⁵ +4.62 (*c* 0.5, CHCl₃); IR (film, CHCl₃) 3020, 1253, 1217, 1086 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 6.01–5.92 (1H, m), 5.20 (1H, d, *J* = 17.3 Hz), 5.11 (1H, d, *J* = 12.2 Hz), 4.32 (1H, dd, *J* = 7.2, 3.8 Hz), 3.96 (1H, dd, *J* = 11.4, 5.4 Hz), 3.62 (2H, dd, *J* = 14.4, 10.0 Hz), 3.49 (2H, dd, *J* = 9.9, 7.4 Hz), 3.36 (1H, t, *J* = 4.3 Hz), 2.53 (1H, ddd, *J* = 16.8, 6.6, 2.7 Hz), 2.36 (1H, ddd, *J* = 16.8, 6.6, 2.7 Hz), 1.94 (1H, t, *J* = 2.7 Hz), 0.90 (9H, s), 0.88 (9H, s), 0.88 (9H, s), 0.40 (4H, d, *J* = 7.3 Hz), 0.12 (3H, s), 0.09 (3H, s), 0.06 (3H, s), 0.03 (3H, s), 0.02 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 139.1, 116.0, 84.9, 82.5, 75.7, 75.5, 74.5, 71.7, 69.6, 65.5, 26.0, 25.8, 25.7, 23.8, 22.8, 18.4, 18.2, 18.1, 7.8, 7.4, –4.0, –4.1, –4.2, –4.7, –5.3, –5.3; EI-LRMS *m/z* 605.7 (M+Na); EI-HRMS calcd for C₃₁H₆₂O₄Si₃Na 605.3848, found 605.3862.

5.1.8. (3R,4S,5R)-3,5-Bis-[(*tert*-butyldimethylsilyloxy)-4-[2-(cyano)-2,2-ethanopropyl]oxyoct-1-en-7-yne (7g). To a solution of **14** (155 mg, 0.33 mmol) in CH₂Cl₂ (3 mL) were added Dess–Martin reagent (212 mg, 0.5 mmol) at 0 °C and it was stirred at the same temperature for 1.5 h. The reaction mixture was diluted with EtOAc and washed with aq. Na₂S₂O₃, aq. NaHCO₃ successively. The organic layer was dried over MgSO₄, filtered and evaporated. The residue (251.3 mg) was dissolved in EtOH (4 mL), and hydroxylamine hydrochloride (46 mg, 0.66 mmol) and sodium acetate (82 mg, 1.0 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried over MgSO₄, followed by filtration and evaporation. The residue (290 mg) was dissolved in pyridine (6 mL), and cyanuric chloride (243 mg, 1.32 mmol) was added to the solution. The reaction mixture was stirred at 60 °C overnight. The reaction mixture was cooled to room temperature and diluted with toluene. The solution was washed with H₂O, brine successively. The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 95/5) to give **7g** (107.2 mg, 0.23 mmol) in 70% yield. [α]_D²⁵ –3.3 (*c* 1.0, CHCl₃); IR (film, CHCl₃) 3314, 2930, 1637, 1253, 1095 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 5.98 (1H, ddd, *J* = 17.0, 10.0, 6.0 Hz), 5.24 (1H, ddd, *J* = 17.2, 1.5, 1.0 Hz), 5.18 (1H, ddd, *J* = 10.0, 1.5, 1.0 Hz), 4.35–4.32 (1H, m), 3.86 (1H, q, *J* = 5.5 Hz), 3.74 (1H, d, *J* = 10.0 Hz), 3.65 (1H, d, *J* = 10.0 Hz), 3.48 (1H, dd, *J* = 5.7, 3.3 Hz), 2.51 (1H, dq, *J* = 16.9, 2.8 Hz), 2.37 (1H, dq, *J* = 17.1, 2.6 Hz), 1.98 (1H, t, *J* = 2.7 Hz), 1.23–1.20 (2H, m), 1.02–0.98 (2H, m), 0.91 (9H, s), 0.89 (9H, s), 0.12 (3H, s), 0.12 (3H, s), 0.08 (3H, s), 0.04 (3H, s); ¹³C-NMR (100 MHz, CDCl₃) δ : 137.9,

122.6, 116.7, 85.1, 81.2, 74.8, 74.2, 71.1, 70.3, 31.5, 25.9, 25.8, 24.1, 22.7, 18.1, 18.0, 14.1, 12.3, 11.8, 10.6, -4.2, -4.4, -4.8; EI-LRMS m/z 557.2 (M+Na); EI-HRMS calcd for C₂₅H₄₅NO₃Si₂ 464.3011, found 464.3021.

5.1.9. (3R,4S,5R)-3,5-Bis-[(*tert*-butyldimethylsilyloxy]-5-(3-cyanopropyl)oxyoct-1-en-7-yne (7f). To a solution of **15** (1.64 g, 3.70 mmol) in CH₂Cl₂ (15 mL) were added triethylamine (1.03 mL, 7.40 mmol) and trimethylamine hydrochloride (35.4 mg, 0.370 mmol) and it was stirred under an argon atmosphere at 0 °C. *p*-Toluenesulfonyl chloride (1.06 g, 5.56 mmol) was added to the reaction mixture, which was warmed to room temperature overnight. H₂O was added to the reaction mixture at room temperature, it was stirred for 30 min, and CH₂Cl₂ was removed by evaporation. The residue was diluted with EtOAc, and washed with H₂O, saturated aq. NaHCO₃ and brine. The organic layer was dried over Na₂SO₄, followed by filtration and evaporation. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 95/5) to give a tosylate (1.85 g, 3.10 mmol). To the tosylate (1.85 g, 3.10 mmol) in *N,N*-dimethylformamide (15 mL) were added potassium cyanide (404 mg, 6.20 mmol) and 18-crown-6-ether (78.7 mg, 0.31 mmol), and it was stirred at 50 °C overnight. The reaction mixture was cooled to room temperature and diluted with H₂O. The mixture was extracted with EtOAc, and the organic layer was washed with brine and dried over Na₂SO₄, followed by filtration and evaporation. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 95/5 then 90/10) to give **7f** (1.31 g, 2.88 mmol) in 78%. [α]_D²⁵ -5.37 (*c* 1.0, CHCl₃); IR (film) 3314, 2930, 1095 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 5.97–5.89 (1H, m), 5.23–5.18 (2H, m), 4.30 (1H, dd, *J* = 7.0, 3.0 Hz), 3.84–3.68 (3H, m), 3.44 (1H, dd, *J* = 5.6, 3.2 Hz), 2.53–2.39 (3H, m), 2.35 (1H, dq, *J* = 16.9, 2.6 Hz), 1.99 (1H, t, *J* = 2.7 Hz), 1.92–1.85 (2H, m), 0.91 (9H, s), 0.90 (9H, s), 0.10 (3H, s), 0.08 (6H, s), 0.05 (3H, s); ¹³C-NMR (100 MHz, CDCl₃) δ : 138.0, 119.9, 116.6, 85.4, 81.2, 74.6, 71.3, 70.3, 70.1, 26.5, 25.9, 25.8, 24.3, 18.2, 18.1, 14.2, -4.1, -4.3, -4.4, -4.7; EI-LRMS m/z 474.2 (M+Na), EI-HRMS calcd for C₂₄H₄₅O₃Si₂Na 474.2830, found 474.2832.

5.1.10. 3-[(3R,4S,5R)-3,5-Bis-(*t*-butyldimethylsilyloxy)oct-1-en-7-yn-4-yl]oxypropanoic acid (16). To a solution of **15** (540.5 mg, 1.22 mmol) in DMF (5 mL) was added PDC (2.25 g, 6.10 mmol) at room temperature and it was stirred at the same temperature overnight. Water was added to the reaction mixture and extracted twice with EtOAc. The combined organic layer was washed with brine and dried over MgSO₄, followed by filtration and evaporation. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 91/9 then 85/15 then 67/33) to give **16** (335.3 mg, 0.73 mmol) in 60% yield. [α]_D²⁵ +0.58 (*c* 0.5, CHCl₃); IR (film, CHCl₃) 3020, 1215 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 5.91 (1H, ddd, *J* = 11.0, 17.0, 6.0 Hz), 5.30–5.18 (2H, m), 4.32 (1H, dd, *J* = 6.8, 3.4 Hz), 3.98–3.93 (2H, m), 3.82 (1H, q, *J* = 5.5 Hz), 3.52 (1H, dd, *J* = 6.1, 3.7 Hz), 2.64–2.61 (2H, m), 2.46 (1H, ddd, *J* = 17.1, 2.8, 6.0 Hz), 2.36 (1H, ddd, *J* = 17.2, 2.5, 6.0 Hz), 2.01 (1H, t, *J* = 2.7 Hz), 0.91 (18H, s), 0.10 (3H, s), 0.09 (3H, s), 0.08 (3H, s), 0.07 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 174.4, 137.1, 117.4, 85.7, 80.7, 74.3, 70.9, 70.7, 68.0, 35.3, 29.7, 25.9, 25.8, 24.3, 18.2, 18.1, 13.0, -4.2, -4.4, -4.5, -4.8; EI-LRMS m/z 479.1 (M+Na). EI-HRMS calcd for C₂₅H₄₅NO₃Si₂Na 479.2644, found 479.2640.

5.1.11. (3R,4S,5R)-3,5-Bis-[(*tert*-butyldimethylsilyloxy]-4-[2-(ethoxycarbonyl)ethyl]oxyoct-1-en-7-yne (7h). To a solution of **16** (180 mg, 0.394 mmol) in EtOH (2 mL) was added sulfonic acid (44 μ L, 0.79 mmol) at 0 °C and it was refluxed for overnight. The reaction mixture was cooled to room temperature and quenched with saturated aq. NaHCO₃. After evaporation of the reaction mixture, the residue was diluted with EtOAc and washed with brine. The organic layer was dried over MgSO₄, filtered and evaporated. The residue (88 mg) was dissolved in dry CH₂Cl₂ (2 mL) and cooled to 0 °C. 2,6-Lutidine (177 μ L, 1.576 mmol) and *t*-butyldimethylsilyl trifluoromethanesulfonate (272 μ L, 1.182 mmol) were added to the solution and stirred at room temperature for 1 h. The reaction mixture was quenched with dry MeOH (2 mL), diluted with EtOAc and washed with H₂O. The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 95/5) to give **7h** (92.7 mg, 0.191 mmol) in 48% yield. [α]_D²⁵ +0.58 (*c* 0.5, CHCl₃); IR (film, CHCl₃) 3020, 1215 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 5.99–5.91 (1H, m), 5.21 (1H, ddd, *J* = 17.3, 1.1, 2.0 Hz), 5.14 (1H, ddd, *J* = 10.4, 1.0, 2.0 Hz), 4.32–4.29 (1H, m), 4.13 (2H, q, *J* = 7.2 Hz), 4.02–3.95 (1H, m), 3.89–3.81 (2H, m), 3.40 (1H, dd, *J* = 5.5, 3.5 Hz), 2.56 (2H, t, *J* = 6.7 Hz), 2.48 (1H, ddd, *J* = 17.0, 3.0, 6.0 Hz), 2.35 (1H, ddd, *J* = 17.0, 3.0, 6.0 Hz), 1.96 (1H, t, *J* = 2.7 Hz), 1.26 (3H, t, *J* = 7.1 Hz), 0.90 (9H, s), 0.89 (9H, s), 0.09 (3H, s), 0.08 (3H, s), 0.07 (3H, s), 0.03 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 171.7, 138.6, 116.1, 85.4, 81.9, 74.7, 71.4, 69.9, 67.9, 60.3, 35.6, 25.9, 25.8, 24.0, 18.2, 18.1, 14.2, -4.1, -4.2, -4.4, -4.8; EI-LRMS m/z 507.2 (M+Na). EI-HRMS calcd for C₂₅H₄₈O₅Si₂Na 507.2933, found 507.2919.

5.1.12. (3R,4S,5R)-3,5-Bis-[(*tert*-butyldimethylsilyloxy]-5-[[3-methyl-3-(trimethylsilyloxy)butyloxy]oct-1-en-7-yne (7e). To a solution of **7h** (68 mg, 0.14 mmol) in THF (2.1 mL) was added MeMgBr (1 M in THF, 0.58 mL, 0.58 mmol) at room temperature and it was stirred at the same temperature for 2h. The reaction mixture was quenched with saturated aq. NH₄Cl, diluted with EtOAc and washed with brine. The organic layer was dried over MgSO₄, followed by filtration and evaporation. To a solution of crude product in DMF (1 mL) were added imidazole (29 mg, 0.42 mmol) and trimethylsilyl chloride (26.6 μ L, 0.21 mmol) at 0 °C and it was stirred at room temperature for 1 h. The reaction was quenched with dry MeOH (1 mL). The reaction mixture was diluted with EtOAc, washed with brine and dried over MgSO₄. After filtration and evaporation, the residue was purified by flash column chromatography on silica gel (hexane–EtOAc = 98/2) to give **7e** (49 mg, 0.09 mmol) in 64% yield. [α]_D²⁵ +4.62 (*c* 0.5, CHCl₃); IR (film, CHCl₃) 3020, 1215 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 6.00–5.92 (1H, m), 5.23–5.11 (2H, m), 4.33–4.29 (1H, m), 3.87–3.60 (3H, m), 3.37 (1H, dd, *J* = 5.6, 3.4 Hz), 2.52–2.32 (2H, m), 1.96 (1H, t, *J* = 2.3 Hz), 1.75 (2H, t, *J* = 7.6 Hz), 1.23 (3H, s), 1.22 (3H, s), 0.91 (9H, s), 0.89 (9H, s), 0.13 (3H, s), 0.10 (3H, s), 0.10 (9H, d, *J* = 1.7 Hz), 0.07 (3H, s), 0.03 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 138.8, 115.9, 85.3, 82.1, 74.7, 73.0, 71.6, 69.8, 69.4, 30.4, 30.2, 25.9, 25.8, 14.1, 18.2, 18.1, 2.6, -4.1, -4.2, -4.4, -4.7; EI-LRMS m/z 507.2 (M-TMS+2H₂O), 493.2 (M-TMS+Na); EI-HRMS calcd for C₂₅H₅₀O₄Si₂Na (M-TMS+Na) 493.3140, found 493.3136.

5.1.13. (5Z,7E)-(1S,2S,3R)-2-Methyl-20-[(2,2-dimethylcyclopentanone-(5E)-ylidene)] methyl-9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5a). Under N₂ atmosphere, a solution of **6** (40 mg, 0.105 mmol), **7a** (52 mg, 0.148 mmol) and Pd(PPh₃)₄ (20 mg, 0.019 mmol) in toluene (1 mL) and Et₃N (1 mL) was stirred at 110 °C for 2 h. The reaction mixture was evaporated and purified with PTLC (Merck Kieselgel plate Art. 113794 1 mm, the eluent was hexane–EtOAc = 95/5) to give a crude product (32.4 mg), which was dissolved in dry MeCN (1 mL) and CH₂Cl₂ (1 mL). To the solution was added LiBF₄ (20 mg, 0.21 mmol) and 1 M H₂SO₄ in MeCN (20 µL, 0.02 mmol) at 0 °C and it was stirred at the same temperature for 1.5 h. After the work up, the crude product (6.0 mg) was obtained with preparative TLC (Merck Kieselgel plate Art. 113794 1 mm, the eluent was hexane–EtOAc = 50/50). Further purification with reversed-phase HPLC (YMC-Pack ODS column, 30–250 mm, 10 mL min⁻¹, eluent A: MeCN–H₂O = 5/95, eluent B: MeCN–MeOH–H₂O = 59.5/40/0.5, eluent A/B = 15/85) gave **5a** (4.0 mg, 0.008 mmol, 8% yield). [α]_D²⁵ +128.8 (*c* 0.1, CHCl₃); IR (film, CHCl₃) 3348, 2974, 2885, 1454, 1381, 1089, 1051 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ : 6.43–6.37 (2H, m), 6.00 (1H, d, *J* = 11.2 Hz), 5.27 (1H, s), 4.99 (1H, d, *J* = 1.9 Hz), 4.30 (1H, br s), 3.84 (1H, br s), 2.84 (1H, dd, *J* = 12.2, 3.9 Hz), 2.67 (1H, dd, *J* = 13.4, 4.2 Hz), 2.53–2.33 (3H, m), 2.23 (1H, dd, *J* = 13.4, 8.1 Hz), 2.03–1.90 (3H, m), 1.76–1.65 (5H, m), 1.54–1.34 (7H, m), 1.17–1.11 (1H, m), 1.08 (3H, d, *J* = 6.8 Hz), 1.07 (3H, s), 1.05 (3H, s), 1.04 (3H, d, *J* = 6.0 Hz), 0.57 (3.0H, s); ¹³C-NMR (100 MHz, CDCl₃) δ : 211.2, 146.5, 142.6, 152.5, 133.7, 133.2, 124.7, 117.1, 113.2, 75.4, 71.7, 56.5, 55.9, 46.0, 45.6, 44.2, 43.5, 40.3, 37.2, 35.4, 29.0, 26.8, 23.8, 23.5, 23.3, 22.2, 19.2, 12.5, 12.4; EI-LRMS *m/z* 475.2 (M+Na) 435.2 (M–H₂O+H), 417.2 (M–2H₂O+H); EI-HRMS calcd for C₃₀H₄₄O₃Na (M+Na) 475.3183, found 475.3171.

5.2. Biology

5.2.1. Evaluation of affinity to vitamin D receptor (VDR).

The binding affinity to VDR was evaluated using a 1 α ,25-(OH)₂VD₃ assay kit (POLARSCREEN VITAMIN D RECEPTOR COMPETITOR ASSAY, RED, Cat.No.PV4569; Invitrogen). The solution of test compound (1 mM in EtOH) was diluted 10 times with DMSO. The solution was diluted 50 times with the assay buffer included in the kit. The solution was defined as the compound solution. On the other hand, VDR/Fluoromone and VDR RED, both of which are included in the kit, were diluted with the assay buffer included in the kit so that the concentration of VDR/Fluoromone was 2.8 nM, and that of VDR RED was 2 nM in the mixture. The solution was defined as the VDR/Fluoromone and VDR RED complex. To a 384-well Black plate (Corning, #3677) was added the compound solution (10 µL), and the VDR/Fluoromone and VDR RED complex (10 µL) was added to each well. The mixture was incubated at 20–25 °C for 2 h. The polarized fluorescence in each well was measured (384 nm, emission: 595 nm, excitation: 535 nm, time: 250 ms/well). All compounds were evaluated with *N* = 2 within the range from 10⁻⁶ M to 10⁻¹⁰ M. IC₅₀ values were calculated using the average measured value. The activities of each compound were shown as a relative value in which the activity of natural hormone **1** was normalized to 100%.

5.2.2. General procedure for transactivation assay of human osteocalcin promoter. The human osteocalcin gene promoter fragment –838/+10 was cloned into the reporter plasmid pGL3 (Promega) as reported. Human VDR and RXR genes were cloned into expression vector pcDNA3 (Invitrogen). Hos cells were maintained in phenol red-free DMEM (Invitrogen) containing 10% FCS (Invitrogen). Prior to transfection, the cells were plated in a 96-well plate at a density of 400,000 cells per well in Opti-MEM (Invitrogen). The cells were transfected with human osteocalcin reporter vector (pGL3-hOc: 100 ng/well), human VDR and RXR expression vector (pcDNA-hVDR, pcDNA-hRXR: 10 ng/well) and phRL-TK (Promega: 25 ng/well) using 0.45 µL Lipofectamine 2000 reagent (Invitrogen). After incubation at 37 °C for 3 h, the culture media were replaced with phenol red-free DMEM containing 10% FCS. The cells were treated with ethanol vehicle or various concentrations of compounds (from 0.1 pM to 100 nM). After incubation at 37 °C for 24 h, the luciferase activity of the cells was quantitated by a luminometer (Berthold) using the Dual-Glo luciferase assay system (Promega).

5.2.3. Evaluation of derivatives on bone mineral loss in ovariectomized rats. Twelve-week-old Sprague-Dawley female rats (Charles River, Japan) were ovariectomized and fed *ad libitum* with a normal diet containing 1.0% Ca for 4 weeks. The rats were orally administrated vitamin D derivatives at various doses in MCT as the vehicle 5 times a week for 4 weeks. The sham and OVX groups were administrated MCT alone. Twenty-four hours after the final administration, blood was collected under ether anesthesia, and the rats were euthanized. The BMD of spine (L4–L5) bone mass was measured by a dual X-ray absorptiometer (QDR-2000; HOLOGIC, USA). The results are expressed as the mean \pm standard error of the mean. The statistical significance of differences between the OVX and experimental groups was analyzed by Student's *t*-test.

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References

- (a) R. M. Evans, The Steroid and Thyroid Hormone Receptor Superfamily, *Science*, 1988, **240**, 889–895; (b) K. Umezono, K. K. Murakami, C. C. Thompson and R. M. Evans, Direct, Repeats as Selective Response Elements for the Thyroid Hormone, Retinoic acid, and Vitamin D₃ Receptors, *Cell*, 1991, **65**, 1255–1266; (c) P. Chambon, The Nuclear Receptor Superfamily: A Personal Retrospect on the First Two Decades, *Mol. Endocrinol.*, 2005, **19**, 1418–1428.
- K. Takeyama, Y. Masuhiro, H. Fuse, H. Endoh, A. Murayama, S. Kitanaka, M. Suzawa, J. Yanagisawa and S. Kato, Selective, Interaction

- of Vitamin D Receptor with Transcriptional Coactivators by a Vitamin D Analog, *Mol. Cell Biol.*, 1999, **19**, 1049–1055.
- 3 (a) *Vitamin D 2nd Edition*, D. Feldman, F. H. Glorieux, J. W. Pike Eds, Academic Press, New York, 2005; (b) R. A. Ettinger and H. F. DeLuca, The Vitamin D Endocrine System and Its Therapeutic Potential, *Adv. Drug Res.*, 1996, **28**, 269–312.
 - 4 R. Bouillon, W. H. Okamura and A. W. Norman, Structure-Function, Relationships in the Vitamin D Endocrine System, *Endocr. Rev.*, 1995, **16**, 200–257.
 - 5 For selected papers on the synthesis of vitamin D analogs, see: (a) G. Jones and M. J. Calverley, A Dialogue on Analogues Newer Vitamin-D Drugs for Use in Bone Disease, Psoriasis, and Cancer, *Trends Endocrinol. Metab.*, 1993, **4**, 297–303; (b) G. D. Zhu and W. H. Okamura, Synthesis, of Vitamin D (Calciferol), *Chem. Rev.*, 1995, **95**, 1877–1952.
 - 6 (a) S. Ishizuka, S. Ishimoto and A. W. Norman, Isolation, and Identification of $1\alpha,25$ -Dihydroxy-24-oxovitamin D_3 , $1\alpha,25$ -Dihydroxyvitamin D_3 , 26,23-lactone, and $1\alpha,24(S),25$ -Trihydroxyvitamin D_3 : in vivo Metabolites of $1\alpha,25$ -Dihydroxyvitamin D_3 , *Biochemistry*, 1984, **23**, 1473–1478; (b) S. Ishizuka, T. Ohba, A. W. Norman $1\alpha,25(OH)_2D_3$ -26,23-Lactone is a Major Metabolite of $1\alpha,25(OH)_2D_3$ under Physiological Conditions. *Vitamin D: Molecular, Cellular and Clinical Endocrinology*, A. W. Norman, K. Schaefer, H. G. Grigoleit, D. von Herrath, ed., Walter de Gruyter, Berlin, 1988, pp 143–144.
 - 7 *US Pat.*, 6 028 208, 2000.
 - 8 (a) K. Konno, S. Maki, T. Fujishima, Z. Liu, D. Miura, M. Chokki and H. Takayama, A Novel and Practical Route to A-Ring Enyne Synthons for $1\alpha,25$ -Dihydroxyvitamin D_3 Analogs: Synthesis of A-Ring Diastereomers of $1\alpha,25$ -Dihydroxyvitamin D_3 and 2-Methyl-1,25-dihydroxyvitamin D_3 , *Bioorg. Med. Chem. Lett.*, 1998, **8**, 151–156; (b) K. Konno, T. Fujishima, S. Maki, Z. Liu, D. Miura, M. Chokki, S. Ishizuka, K. Yamaguchi, Y. Kan, M. Kurihara, N. Miyata and C. Smith, DeLuca, H. F., Takayama, H. Synthesis, Biological Evaluation, and Conformational Analysis of A-Ring Diastereomers of 2-Methyl-1,25-dihydroxyvitamin D_3 and Their 20-Epimers: Unique Activity Profiles Depending on the Stereochemistry of the A-Ring and at C-20, *J. Med. Chem.*, 2000, **43**, 4247–4265.
 - 9 (a) Y. Suhara, K.-i. Nihei, H. Tanigawa, T. Fujishima, K. Konno, K. Nakagawa, T. Okano and H. Takayama, Syntheses, and Biological Evaluation of Novel 2α -Substituted $1\alpha,25$ -Dihydroxyvitamin D_3 Analogues, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1129–1132; (b) Y. Suhara, K.-i. Nihei, M. Kurihara, A. Kittaka, K. Yamaguchi, T. Fujishima, K. Konno, N. Miyata and H. Takayama, Efficient, and Versatile Synthesis of Novel 2α -Substituted $1\alpha,25$ -Dihydroxyvitamin D_3 Analogues and Their Docking to Vitamin D Receptor, *J. Org. Chem.*, 2001, **66**, 8760–8771.
 - 10 (a) A. Kittaka, Y. Suhara, H. Takayanagi, T. Fujishima, M. Kurihara and H. A. Takayama, Concise and Efficient Route to 2α -(ω -Hydroxyalkoxy)- $1\alpha,25$ -dihydroxyvitamin D_3 : Remarkably High Affinity to Vitamin D Receptor, *Org. Lett.*, 2000, **2**, 2619–2622; (b) N. Saito, Y. Suhara, M. Kurihara, T. Fujishima, S. Honzawa, H. Takayanagi, T. Kozono, M. Matsumoto, M. Ohmori, N. Miyata and H. Takayama, Kittaka, A. Design, and Efficient Synthesis of 2α -(ω -Hydroxyalkoxy)- $1\alpha,25$ -dihydroxyvitamin D_3 Analogues Including 2-*epi*-ED-71 and Their 20-Epimers with HL-60 Cell Differentiation Activity, *J. Org. Chem.*, 2004, **69**, 7463–7471; (c) N. Saito, Y. Suhara, D. Abe, T. Kusudo, M. Ohta, K. Yasuda, T. Sakaki, S. Honzawa, T. Fujishima and A. Kittaka, Synthesis, of 2α -propoxy- $1\alpha,25$ -dihydroxyvitamin D_3 and comparison of its metabolism by human CYP24A1 and rat CYP24A1, *Bioorg. Med. Chem.*, 2009, **17**, 4296–4301.
 - 11 For our account, see: H. Takayama, A. Kittaka, T. Fujishima and Y. Suhara, Design, Synthesis, and Biological Studies of the A-ring-Modified $1,25$ -Dihydroxyvitamin D_3 Analogues, *Vitamin D Analogs in Cancer Prevention and Therapy, Recent Results in Cancer Research*, **164**; J. Reichrath, M. Friedrich, W. Tilgen, ed., Springer-Verlag, Berlin-Heidelberg, 2003, pp 289–317.
 - 12 This concept was also applicable to $1\alpha,25$ -dihydroxy-19-norvitamin D_3 , see: (a) A. Yoshida, K. Ono, Y. Suhara, N. Saito, H. Takayama and A. Kittaka, Efficient, and Convergent Coupling Route for the Short-step Synthesis of Enantiopure 2α - and 2α -Alkylated $1\alpha,25$ -Dihydroxy-19-norvitamin D_3 Analogues, *Synlett*, 2003, 1175–1179; (b) K. Ono, A. Yoshida, N. Saito, T. Fujishima, S. Honzawa, Y. Suhara, S. Kishimoto, T. Sugiura, K. Waku, H. Takayama and A. Kittaka, Efficient, Synthesis of 2-Modified $1\alpha,25$ -Dihydroxy-19-norvitamin D_3 with Julia Olefination: High Potency in Induction of Differentiation on HL-60 Cells, *J. Org. Chem.*, 2003, **68**, 7407–7415.
 - 13 For the pioneering works on A-ring modification with C- 2α -functionalization, see: (a) G. H. Posner and N. Johnson, Stereocontrolled, Total Synthesis of Calcitriol Derivatives: $1,25$ -Dihydroxy-2-(4'-hydroxybutyl)vitamin D_3 Analogs of an Osteoporosis Drug, *J. Org. Chem.*, 1994, **59**, 7855–7861; (b) G. H. Posner, C.-G. Cho, T. E. N. Anjeh, N. Johnson, R. L. Horst, T. Kobayashi, T. Okano and N. Tsugawa, 2-Fluoroalkyl A-Ring Analogs of $1,25$ -Dihydroxyvitamin D_3 , Stereocontrolled Total Synthesis via Intramolecular and Intermolecular Diels–Alder Cycloadditions. Preliminary Biological Testing, *J. Org. Chem.*, 1995, **60**, 4617–4628.
 - 14 (a) N. Saito, T. Matsunaga, T. Fujishima, M. Anzai, H. Saito, K. Takenouchi, D. Miura, S. Ishizuka, H. Takayama and A. Kittaka, Remarkable, Effect of 2α -Modification on the VDR Antagonistic Activity of 1α -Hydroxyvitamin D_3 -26,23-lactones, *Org. Biomol. Chem.*, 2003, **1**, 4396–4402; (b) N. Saito, M. Masuda, T. Matsunaga, H. Saito, M. Anzai, K. Takenouchi, D. Miura and S. Ishizuka, Takimoto-Kamimura, M., Kittaka, A. 24,24-Dimethylvitamin D_3 -26,23-lactones and Their 2α -Functionalized Analogues as Highly Potent VDR Antagonists, *Tetrahedron*, 2004, **60**, 7951–7961.
 - 15 S. Hourai, T. Fujishima, A. Kittaka, Y. Suhara, H. Takayama, N. Rochel and D. Moras, Probing, a Water Channel near the A-ring of Receptor-Bound $1\alpha,25$ -Dihydroxyvitamin D_3 with Selected 2α -Substituted Analogs, *J. Med. Chem.*, 2006, **49**, 5199–5205.
 - 16 B. M. Trost, J. Dumas and M. Villa, New, Strategies for the Synthesis of Vitamin D Metabolites via Pd-Catalyzed Reactions, *J. Am. Chem. Soc.*, 1992, **114**, 9836–9845.
 - 17 L. S. Wiggins, Ethylene, oxide-type anhydrosugars, *Methods Carbohydr. Chem*, 1963, **2**, 188–191.