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Synthesis and evaluation of a 3-position diastereomer of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71)☆

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Abstract—A 3-position diastereomer of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71, **2**), 3-*epi*-ED-71 (**4**), was synthesized by the convergent method coupling the A-ring fragment (**5**) with the C/D-ring fragment (**6**). As the results of preliminary in vitro biological evaluation of 3-*epi*-ED-71 (**4**), the inhibition of parathyroid hormone secretion in bovine parathyroid cells and binding affinity to human recombinant vitamin D receptor and to human vitamin D binding protein in comparison with ED-71 (**2**), 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$, **1**), and 3-*epi*-1,25$(OH)_2D_3$ (**3**) are described.

1. Introduction

Active vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$, **1**), is well recognized as a potent regulator of cell proliferation and differentiation processes in addition to possessing regulatory effects on calcium and phosphorus metabolism.[2] Various analogs of 1,25$(OH)_2D_3$ (**1**) have been investigated in attempts to separate differentiation-induction and antiproliferation activities from calcemic activity with the aim of obtaining useful analogs for the medical treatment of psoriasis, secondary hyperparathyroidism, cancer, etc.[3] There is also intense interest in obtaining analogs more potent than 1,25$(OH)_2D_3$ (**1**) in terms of regulatory effects on calcium and phosphorus metabolism with the objective of treating bone diseases such as osteoporosis. 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71, **2**), an analog of 1,25$(OH)_2D_3$ (**1**) from which a hydroxypropoxy substituent at the 2β-position is appended, is such an analog that shows potent effects on bone therapy.[4–8] ED-71 (**2**) is currently under phase III clinical studies in Japan as a promising candidate for the treatment of osteoporosis and bone fracture prevention.[9]

It is well known that the synthesis and secretion of parathyroid hormone (PTH) is regulated by 1,25$(OH)_2D_3$ (**1**).[10,11] Interestingly during our clinical development of ED-71 (**2**), serum intact PTH in osteoporotic patients did not change significantly upon treatment with **2**, although the reason remains unclear, however.[12] Brown et al. reported that epimerization of 1,25$(OH)_2D_3$ (**1**) at the 3-position of the A-ring plays a major role in hormone activation and inactivation, especially in the case of parathyroid cells.[13] It has been also reported that 3-*epi*-1,25$(OH)_2D_3$ (**3**), an epimer of 1,25$(OH)_2D_3$ (**1**) at the 3-position, shows equipotent and prolonged activity compared to **1** at suppressing PTH secretion.[14] Since ED-71 (**2**) has a bulky hydroxypropoxy substituent at the 2-position in the A-ring, epimerization of **2** at the adjacent and sterically hindered 3-position might be prevented. This could be the reason why ED-71 (**2**) showed weak potency in PTH suppression during clinical studies. We have significant interest in ED-71 (**2**) epimerization at the 3-position and the biological potency of 3-*epi*-ED-71 (**4**) in suppressing PTH production.

In this paper, therefore, we describe the synthesis of 3-*epi*-ED-71 (**4**) and its in vitro suppression of PTH

Keywords: 1α,25-Dihydroxyvitamin D_3; 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3; ED-71; 3-*epi*-ED-71.
☆ See Ref. 1.
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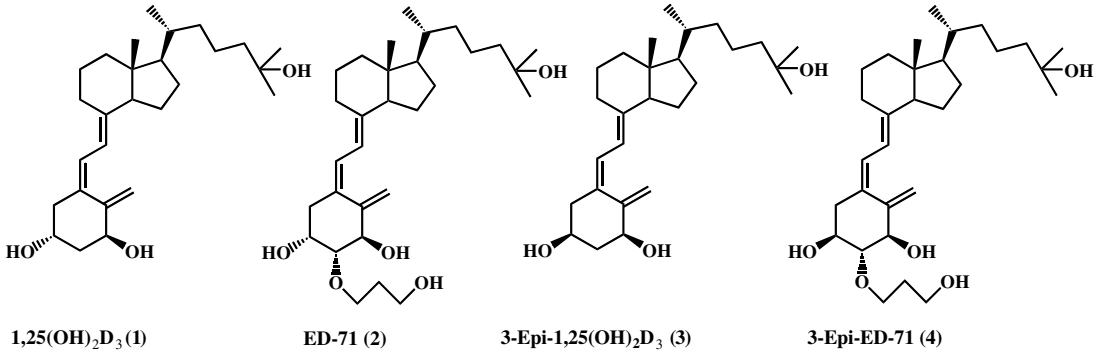


Figure 1. Structures of active vitamin D_3 analogs.

compared to ED-71 (**2**), 3-*epi*-1,25$(OH)_2D_3$ (**3**), and 1,25$(OH)_2D_3$ (**1**) (Fig. 1).

2. Results and discussion

2.1. Synthesis of 3-*epi*-ED-71 (*4*)

The synthesis of 3-*epi*-ED-71 (**4**) was envisioned using the convergent method via palladium-catalyzed coupling of the A-ring fragment (**5**) prepared from the C_2 symmetrical epoxide (**7**) with known C/D-ring fragment (**6**) obtained from the Inhoffen-Lythgoe diol (**8**) (Fig. 2). [15,16]

The synthesis of the A-ring fragment (**5**) began with inversion of the C_3 configuration of alcohol (**9**) which was prepared from the C_2 symmetrical epoxide (**7**) according to our previously established procedure.[17] Thus, reaction of **9** with *p*-nitrobenzoic acid in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine gave the *p*-nitrobenzoate (**10**) in 84% yield.[18] Treatment of **10** with $NaHCO_3$ in methanol allowed selective methanolysis of the *p*-nitrobenzoate group to give the inverted alcohol (**11**) in 86% yield. After hydrogenolysis of the benzyl ether functionalities in **11**, the resulting diol was protected as its acetonide to afford acetonide (**12**) in 88% yield. Swern oxidation of **12** followed by Grignard reaction of the resulting aldehyde with vinylmagnesium bromide produced alcohol (**13**) as an epimeric mixture (*S*:*R* = 3:2, determined by ^{1}H NMR) in 66% yield.[19] To separate this epimeric mixture, **13** was subjected to lipase-catalyzed acetylation using vinyl acetate and Novozyme in *tert*-butyl methyl ether.[20] As a result, the *R*-epimer preferentially underwent acetylation to give the acetate (**14**) and *S*-**13** (*R*:*S* = 1:20) in 40% and 57% yields, respectively. Acidic hydrolysis of **14** gave diol (**15**) in 90% yield, which, upon Mitsunobu reaction using DEAD and triphenylphosphine in boiling toluene, afforded epoxide (**16**) in 75% yield.[21] Reaction of **16** with lithium trimethylsilylacetylide in the presence of $BF_3{\cdot}OEt_2$ at −78 °C followed by saponification provided eneyne (**17**) in 65% yield.[22] Protection of **17** as its triethylsilyl ether produced A-ring fragment (**5**) quantitatively.

Having secured A-ring fragment (**5**), we then performed its coupling with C/D-ring fragment (**6**) using Trost's methodology.[15,16] Thus, the A-ring fragment (**5**) was allowed to react with C/D-ring fragment (**6**) in the presence of $(Ph_3P)_4Pd$ and triethylamine in boiling toluene to give the coupling product which was desilylated with ammonium fluoride in boiling methanol to produce 3-*epi*-ED-71 (**4**) in 46% yield (Scheme 1).

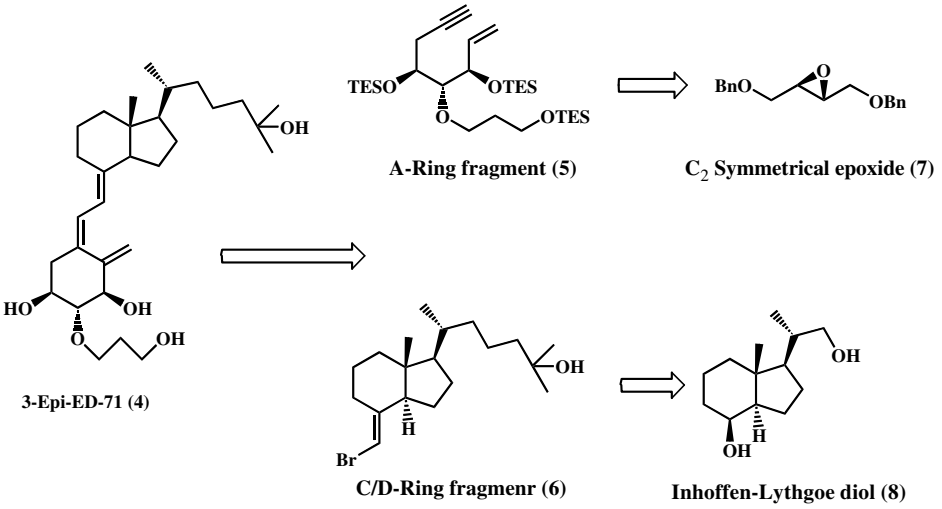
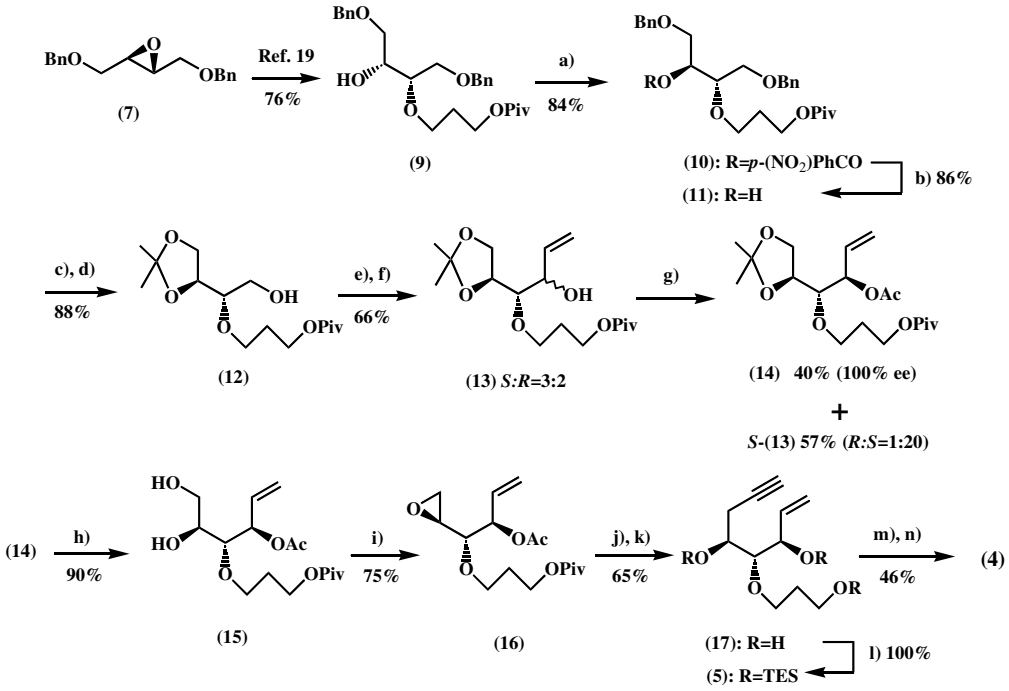


Figure 2. Retrosynthesis of 3-*epi*-ED-71 (**4**).



Scheme 1. Synthesis of 3-*epi*-ED-71 (**4**). Reagents and conditions: (a) *p*-$(NO_2)PhCO_2H$, DEAD, Ph_3P, toluene; (b) $NaHCO_3$, MeOH; (c) $Pd(OH)_2$, H_2, MeOH; (d) 2,2-dimethoxypropane, TsOH, acetone; (e) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N; (f) CH_2=CHMgBr, THF, −20 °C; (g) Novozyme, CH_2=CHOAc, *t*-BuOMe; (h) 60% AcOH; (i) DEAD, Ph_3P, dioxane, reflux; (j) $TMSC_2H$, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C; (k) 10 M NaOH, MeOH; (l) TESOTf, NEt_3, CH_2Cl_2, −40 °C; (m) **6**, $(Ph_3P)_4Pd$, Et_3N, toluene, reflux; (n) NH_4F, MeOH, reflux.

2.2. Biological evaluation of 3-*epi*-ED-71 (4)

The results of preliminary in vitro biological evaluation of 3-*epi*-ED-71 (**4**) in comparison with ED-71 (**2**), $1,25(OH)_2D_3$ (**1**), and 3-*epi*-$1,25(OH)_2D_3$ (**3**) are summarized in Table 1, which contains affinity to human vitamin D receptors (VDR) and human vitamin D binding protein (DBP), and inhibition of PTH in cultured bovine parathyroid cells. The dose-responsive effects of analogs on PTH suppression are also shown in Figure 3. 3-*Epi*-ED-71 (**4**) showed only slight inhibition of PTH secretion in bovine parathyroid cells compared to ED-71 (**2**). In our assay systems, 3-*epi*-$1,25(OH)_2D_3$ (**3**) did not show greater activity than $1,25(OH)_2D_3$ (**1**) in suppressing PTH secretion. The inhibitory potency of vitamin D_3 analogs was $1,25(OH)_2D_3$ (**1**) >ED-71 (**2**) ⩾3-*epi*-$1,25(OH)_2D_3$ (**3**) ⩾3-*epi*-ED-71 (**4**), and they were well responsive for affinity to human recombinant VDR as shown in Table 1. Regarding affinity to human DBP as reported previously in the rat DBP case, ED-71 (**2**) showed more potent affinity than $1,25(OH)_2D_3$ (**1**). This increase in DBP affinity is due to the existence of a hydroxypropoxy substituent at the 2β-position and was also observed in the 3-*epi* series: 3-*epi*-$1,25(OH)_2D_3$ (**3**): 8.3 and 3-*epi*-ED-71 (**4**): 113.1, as shown in Table 1.

Table 1. Biological evaluation of 3-*epi*-ED-71 (**4**)

	VDR	DBP	PTH
$1,25(OH)_2D_3$ (**1**)	100	100	100
3-*epi*-$1,25(OH)_2D_3$ (**3**)	9.62	8.3	1.25
ED-71 (**2**)	44.6	421.9	3.54
3-*epi*-ED-71 (**4**)	0.02	113.1	0.11

VDR, relative affinity normalized by the potency of $1,25(OH)_2D_3$ (=100) using human vitamin D receptors.

DBP, relative affinity normalized by the potency of $1,25(OH)_2D_3$ (=100) using human vitamin D binding protein.

PTH, relative inhibitory activity of parathyroid hormone secretion normalized by the potency of $1,25(OH)_2D_3$ (=100) in cultured bovine parathyroid cells.

ED-71 (**2**) and its 3-position epimer, 3-*epi*-ED-71 (**4**), appear to be inherently weak agents toward PTH suppression. This should be examined further with in vivo evaluation systems using renal insufficiency animal models such as 5/6 nephrectomized rats showing high level of serum PTH.[23] Nevertheless, the less potent activity of ED-71 (**2**) toward native PTH suppression compared to $1,25(OH)_2D_3$ (**1**) might be a beneficial characteristic of **2** for treating osteoporotic patients.

In our previous modification studies of $1,25(OH)_2D_3$ (**1**) and ED-71 (**2**) at the 1-position of the A-ring, 1-*epi*-$1,25(OH)_2D_3$ and 1-*epi*-ED-71 showed enhanced affinity to rat DBP compared to parent compounds, **1** and **2**.[24] The relative binding affinities of $1,25(OH)_2D_3$, 1-*epi*-$1,25(OH)_2D_3$, ED-71, and 1-*epi*-ED-71 to DBP were 100, 290, 410, and 670, respectively.[24] Interestingly, epimerization of $1,25(OH)_2D_3$ (**1**) and ED-71 (**2**) at the 3-position in the present study did not result in enhanced affinity to human DBP. At the present time, however, the reasons for this remain unclear. Future studies along these lines should provide clarification and will be reported elsewhere.

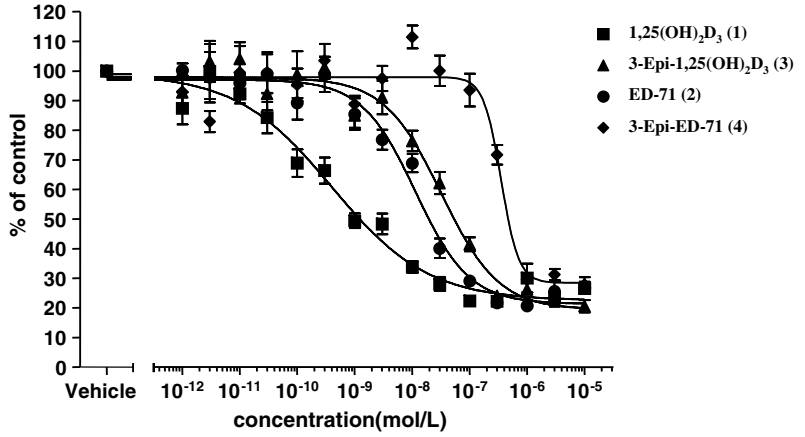


Figure 3. Dose-responsive effects of active vitamin D_3 analogs on PTH secretion in bovine parathyroid cells in vitro.

3. Experimental

3.1. General methods

Where appropriate, reactions were performed in flame-dried glassware under argon atmosphere. All extracts were dried over $MgSO_4$ and concentrated by rotary evaporation below 30 °C at 25 Toor. Anhydrous tetrahydrofuran (THF) was purchased from Kanto Chemical Co., Inc., dichloromethane (CH_2Cl_2), triethylamine, dimethylsulfoxide (DMSO), toluene, and acetonitrile (MeCN) were distilled from CaH_2. Methanol (MeOH) was distilled from sodium. Thin-layer chromatography was performed with Merck F-254 TLC plates. Column chromatography was performed using Kanto Chemical Co., Inc., silica gel 60 N (spherical, neutral). Infrared spectra were measured on a JASCO FTIR-230 spectrometer. Optical rotations were recorded on a JASCO DIP-370 polarimeter at ambient temperature. ^1H and ^{13}C NMR spectra were measured on a Varian Gemini 300, JEOL JNM-AL 400, or Varian Unity plus 500 spectrometer. For ^1H NMR spectra, chemical shifts are reported as δ values in ppm downfield from tetramethylsilane. For ^{13}C NMR spectra, chemical shifts are reported as δ values in ppm relative to chloroform or methanol. EI Mass spectra were measured on a JEOL JMS-700N. The melting point was measured on a YANACO Melting Point Apparatus MP-83 and are uncorrected.

3.2. (2*S*,3*R*)-3-[(4-Nitrobenzoyl)oxy-1,4-bisbenzyloxybutan-2-yloxy]propyl pivalate (10)

To a mixture of triphenylphosphine (23.6 g, 90.0 mmol), *p*-nitrobenzoic acid (15.0 g, 90.0 mmol), and **9** (20.0 g, 45.0 mmol) in toluene (600 mL) was added DEAD (2.2 M in toluene, 40.9 mL, 90.0 mmol). After stirring at room temperature for 20 h, most of the toluene was evaporated. The residue was diluted with Et_2O, washed with H_2O and brine, dried, concentrated, and chromatographed (SiO_2 500 g, hexane/AcOEt = 6:1) to give **10** (22.4 g, 84%) as a colorless oil; $[\alpha]_D^{24}$ +5.9° (*c* 1.00, $CHCl_3$); IR (neat) 3808, 3739, 3455, 2863, 1718, 1465, 1282, 1159, 1091 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, 2H, J = 8.7 Hz), 8.25 (d, 2H, J = 9.0 Hz), 7.45–7.15 (m, 10H), 5.51 (br q, J = 2.1 Hz), 4.53–4.45 (m, 4H), 4.09 (dt, 2H, J = 6.3, 1.6 Hz), 3.83 (br quint, 1H, J = 5.2 Hz), 3.78–3.45 (m, 7H), 2.64 (br d, 1H, J = 4.8 Hz), 1.88 (quint, 2H, J = 6.3 Hz), 1.18 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 163.9, 150.4, 137.6, 135.4, 129.5, 128.2, 127.5, 127.4, 127.3, 123.4, 74.1, 73.5, 73.2, 68.9, 67.9, 67.7, 61.2, 38.7, 29.4, 27.2; HRMS (EI) *m*/*z* calcd for $C_{33}H_{39}NO_9$ (M^+) 593.2625, found 593.2607.

3.3. (2*S*,3*S*)-3-(1,4-Bisbenzyloxy-3-hydroxybutan-2-yloxy)propyl pivalate (11)

To a solution of **10** (22.3 g, 38.0 mmol) in MeOH (380 mL) was added $NaHCO_3$ (19.0 g, 22.6 mmol) at 0 °C, and the mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O and brine, dried, concentrated, and chromatographed (SiO_2 400 g, hexane/AcOEt = 6:1) to afford **11** (14.6 g, 86%) as a colorless oil: $[\alpha]_D^{24}$ +8.3° (*c* 1.00, $CHCl_3$); IR (neat) 3781, 3712, 2867, 1724, 1600, 1529, 1481, 1351, 1272, 1105 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43–7.19 (m, 10H), 4.52 (s, 4H), 4.13 (dt, 2H, J = 6.0, 1.6 Hz), 3.91 (br quint, 1H, J = 5.3 Hz), 3.80–3.46 (m, 6H), 2.62 (br d, 1H, J = 4.8 Hz), 1.87 (quint, 2H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 137.9, 128.3, 127.6, 127.5, 78.4, 73.4, 70.8, 70.7, 69.9, 67.5, 61.3, 38.7, 29.4, 27.2; HRMS (EI) *m*/*z* calcd for $C_{26}H_{36}O_6$ (M^+) 444.2512, found 444.2506.

3.4. [(*S*)-2-Hydroxy-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy]propyl pivalate (12)

A mixture of **11** (12.3 g, 29.2 mmol) and $Pd(OH)_2$ (1.30 g, 1.76 mmol) in MeOH (210 mL) was stirred under H_2 atmosphere at room temperature for 24 h. The reaction mixture was filtered through Celite pad, and the filtrate was concentrated to give the corresponding triol (7.41 g) as a colorless oil. To a solution of the crude triol (7.41 g) in acetone (31 mL) were added 2,2-dimethoxypropane (4.37 g, 42.0 mmol) and *p*-toluenesulfonic acid monohydrate (13.3 mg, 0.07 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with AcOEt, washed with

saturated $NaHCO_3$ and brine, dried, concentrated, and chromatographed (SiO_2, 200 g, hexane/AcOEt = 4:1) to afford **12** (7.78 g, 88%) as a colorless oil; $[\alpha]_D^{24}$ +8.30° (*c* 1.00, $CHCl_3$); IR (neat) 3500, 2973, 1720, 1473, 1375, 1288, 1216, 1164, 1052 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.25 (dt, 1H, *J* = 11.2, 6.1 Hz), 4.14 (quint, 1H, *J* = 5.6 Hz), 4.01 (dd, 1H, *J* = 7.6, 6.8 Hz), 3.78–3.62 (m, 4H), 3.56 (quint, 1H, *J* = 6.0 Hz), 3.41 (dd, 1H, *J* = 8.1, 6.0 Hz), 2.32 (br t, 1H, *J* = 6.0 Hz), 1.93 (quint, 2H, *J* = 6.3 Hz), 1.43 (s, 3H), 1.36 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 109.5, 80.7, 76.7, 67.5, 65.8, 61.9, 61.4, 36.0, 29.8, 27.5, 26.7, 25.6; HRMS (EI) *m*/*z* calcd for $C_{15}H_{28}O_6$ (M^+) 304.1886, found 304.1885.

3.5. A 3:2 mixture of 3-[(1*S*,2*R*)-2-hydroxy-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)but-3-enyloxy]propyl pivalate and 3-[(1*S*,2*S*)-2-hydroxy-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)but-3-enyloxy]propyl pivalate (13)

To a solution of oxalyl chloride (624 mg, 4.92 mmol) in CH_2Cl_2 (10 mL) was added DMSO (768 mg, 9.84 mmol) at −78 °C. After stirring for 15 min at −78 °C, a solution of **12** (500 mg, 1.64 mmol) in CH_2Cl_2 (7 mL) was added and stirring was continued at −78 °C for 30 min. Triethylamine (1510 mg, 14.8 mmol) was added, and the mixture was allowed to warm to 0 °C and stirred at 0 °C for 1 h. The reaction mixture was diluted with AcOEt, washed with saturated NH_4Cl (10 mL), H_2O, and brine, dried, concentrated, and chromatographed (SiO_2 10 g, hexane/AcOEt = 1:1) affording the corresponding aldehyde (580 mg) which was used for the next reaction without purification. To a solution of the crude aldehyde (580 mg) in THF (17 mL) was added vinylmagnesium bromide (1 M in THF, 5.05 mL, 5.05 mmol) at −40 °C, and the mixture was stirred at −40 °C for 3 h. The reaction was quenched with saturated NH_4Cl and the reaction mixture was extracted with AcOEt. The extract was washed with H_2O and brine, dried, concentrated, and chromatographed (SiO_2 20 g, hexane/AcOEt = 6:1) to give **13** (330 mg, 66%), a colorless oil, as a diastereomer mixture (*R*:*S* = 2:3); IR (neat) 3488, 2981, 1729, 1481, 1371, 1286, 1168 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.91 (ddd, 1H, *J* = 16.2, 10.5, 5.7 Hz), 5.42 (dd, 1H, *J* = 15.6, 3.3 Hz), 5.23 (dd, 1H, *J* = 12.0, 3.6 Hz), 4.29–4.15 (m, 4H), 4.05 (t, 1H, *J* = 8.5 Hz), 3.65 (m, 2H), 3.30 (dd, 1H, *J* = 5.2, 4.3 Hz), 3.25 (dd, 1H, *J* = 6.4, 3.6 Hz), 2.83 (d, 0.6H, *J* = 3.6 Hz), 2.55 (d, 0.4H, *J* = 3.6 Hz), 1.92 (quint, 2H, *J* = 6.3 Hz), 1.40 (s, 3H), 1.34 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.6, 178.5, 137.9, 137.1, 128.4, 116.6, 115.0, 109.4, 109.3, 82.5, 82.2, 77.6, 77.1, 76.8, 72.9, 72.9, 69.5, 68.2, 66.3, 66.1, 61.4, 61.3, 39.0, 39.0, 29.7, 29.7, 27.6, 26.8, 26.6, 25.8; HRMS (EI) *m*/*z* calcd for $C_{17}H_{30}O_6$ (M^+) 330.2642, found 330.2036.

3.6. 3-[(1*S*,2*R*)-2-Acetoxy-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)but-3-enyloxy]propyl pivalate (14) and 3-[(1*S*,2*S*)-2-hydroxy-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)but-3-enyloxy]propyl pivalate (*S*-13)

A solution of vinyl acetate (2.3 g, 26.7 mmol) and **13** (4.40 g, 13.3 mmol) in *tert*-butyl methyl ether (133 mL) was added Novozyme (2.2 g), and the mixture was stirred at 30 °C for 7 d. The reaction mixture was filtered through Celite pad, concentrated, and chromatographed (SiO_2 150 g, hexane/AcOEt = 10:1) to afford **14** (2.03 g, 40%) and *S*-**13** (*R*:*S* = 1:20, 2.50 g, 57%) each as a colorless oil. Compound **14**: $[\alpha]_D^{26}$ −9.1° (*c* 1.10, $CHCl_3$); IR (neat) 2985, 1752, 1644, 1481, 1378, 1251 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.90 (ddd, 1H, *J* = 17.2, 10.4, 5.6 Hz), 5.35 (d, 1H, *J* = 17.2 Hz), 5.25 (d, 1H, *J* = 10.8 Hz), 4.17 (quint, 3H, *J* = 6.0 Hz), 3.97 (t, 1H, *J* = 8.5 Hz), 3.81–3.65 (m, 3H), 3.35 (dd, 1H, *J* = 5.2, 4.3 Hz), 2.10 (s, 3H), 1.92 (quint, 2H, *J* = 6.0 Hz), 1.43 (s, 3H), 1.36 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.1, 170.4, 133.6, 109.9, 82.2, 77.0, 74.5, 69.8, 66.5, 62.0, 39.6, 30.2, 28.0, 27.4, 23.4, 21.9; HRMS (EI) *m*/*z* calcd for $C_{19}H_{33}O_7$ (M^+) 372.2148, found 372.2137. Compound *S*-**13**: $[\alpha]_D^{25}$ − 21.7° (*c* 0.42, $CHCl_3$); IR (neat) 3487, 2976, 1722, 1471, 1375, 1286, 1216, 1163, 1065 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.93 (ddd, 1H, *J* = 17.4, 10.7, 6.2 Hz), 5.34(dt, 1H, *J* = 17.4, 1.5 Hz), 5.23 (dt, 1H, *J* = 10.2, 1.5 Hz), 4.29–4.09 (m, 4H), 4.00 (dd, 1H, *J* = 8.4, 6.3 Hz), 3.79–3.65 (m, 3H), 3.30 (dd, 2H, *J* = 6.0, 5.0 Hz), 2.89 (d, 1H, *J* = 6.3 Hz), 1.92 (quint, 2H, *J* = 6.3 Hz), 1.42 (s, 3H), 1.36 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 137.1, 116.4, 108.9, 82.4, 72.5, 67.9, 65.8, 61.0, 38.5, 29.2, 27.0, 26.2, 25.4, 20.8, 14.0; HRMS (EI) *m*/*z* calcd for $C_{17}H_{30}O_6$ (M^+) 330.2048, found 330.2039.

3.7. 3-[(2*S*,3*S*,4*R*)-4-Acetoxy-1,2-dihydroxyhex-5-en-3-yloxy]propyl pivalate (15)

A solution of **14** (300 mg, 0.805 mmol) in 60% aqueous AcOH (2 mL) was stirred at room temperature for 22 h. The reaction mixture was carefully basified by the addition of $NaHCO_3$, diluted with CH_2Cl_2, washed with H_2O and brine, dried, and concentrated. Purification of the residue by chromatography (SiO_2 20 g, hexane/AcOEt = 2:1) gave **15** (240 mg, 90%) as a colorless oil; $[\alpha]_D^{26}$ +18.5° (*c* 1.00, $CHCl_3$); IR (neat) 3478, 2969, 1729, 1481, 1371, 1286, 1238, 1162, 1079 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.90 (ddd, 1H, *J* = 17.2, 10.4, 5.6 Hz), 5.39 (t, 1H, *J* = 6.0 Hz), 5.34 (d, 1H, *J* = 17.2 Hz), 5.27 (d, 1H, *J* = 10.4 Hz), 4.28 (dt, 1H, *J* = 10.8, 6.4 Hz), 4.12 (dt, 1H, *J* = 11.2, 6.0 Hz), 3.81 (dt, 1H, *J* = 8.0, 4.8 Hz), 3.80–3.55 (m, 3H), 3.42 (t, 1H, *J* = 5.2 Hz), 2.68 (d, 1H, *J* = 4.0 Hz), 2.10 (s, 4H), 1.90 (quint, 2H, *J* = 6.0 Hz), 1.43 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5, 169.7, 132.4, 118.4, 80.0, 73.7, 70.9, 68.8, 63.5, 60.9, 38.7, 29.5, 27.2, 21.1; HRMS (EI) *m*/*z* calcd for $C_{16}H_{29}O_7$ [$(M+H)^+$] 333.1913, found 333.1910.

3.8. 3-[(1*S*,2*R*)-2-Acetoxy-1-((*S*)-oxiran-2-yl)but-3-enyloxy]propyl pivalate (16)

To a solution of **15** (1.80 g, 5.12 mmol) in dioxane (50 mL) were added triphenylphosphine (2.01 g, 7.57 mmol) and DEAD (2.2 M in toluene, 3.49 mL, 7.67 mmol), and the mixture was refluxed for 17 h. The reaction mixture was concentrated and chromatographed (SiO_2 150 g, hexane/AcOEt = 10:1) to give **16**

(1.20 g, 75%) as a colorless oil; $[\alpha]_D^{24}$ +11.3° (*c* 1.00, $CHCl_3$); IR (neat) 2969, 1735, 1473, 1371, 1284, 1236, 1162, 1108, 1029 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.90 (ddd, 1H, *J* = 17.2, 10.6, 6.4 Hz), 5.39 (t, 1H, *J* = 6.0 Hz), 5.32 (d, 1H,, *J* = 17.6 Hz), 5.27 (d, 1H, *J* = 10.8 Hz), 4.17 (dt, 3H, *J* = 6.4, 1.2 Hz), 3.84 (dt, 1H, *J* = 9.6, 6.0 Hz), 3.60 (dt, 1H, *J* = 10.0, 6.0 Hz), 3.05–3.00 (m, 2H), 2.77 (t, 1H, *J* = 2.0 Hz), 2.55 (quant, 1H, *J* = 4.4 Hz), 2.11 (s, 3H), 1.90 (quint, 2H, *J* = 3.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.0, 170.3, 133.1, 119.1, 82.7, 75.0, 68.0, 61.9, 52.8, 44.3, 29.9, 28.0, 21.8; HRMS (EI) *m/z* calcd for $C_{14}H_{23}O_5$ [$(M-Ac)^+$] 271.1546, found 271.1541.

3.9. (3*R*,4*R*,5*S*)-4-(3-Hydroxypropoxy)oct-1-en-7-yne-3,5-diol (17)

To a solution of trimethylsilylacetylene (1.72 g, 17.5 mmol) in THF (20 mL) was added *n*-BuLi (1.45 M in THF, 11.3 mL, 17.5 mmol) at −78 °C, and the mixture was stirred at −78 °C for 30 min. $BF_3{\cdot}Et_2O$ (2.49 g, 17.5 mmol) and a solution of **16** (1.11 g, 3.53 mmol) in THF (50 mL) were added and stirring was continued at −78 °C for 1 h. After the mixture was allowed to warm to room temperature over 1 h, the reaction was quenched with saturated $NaHCO_3$. The reaction mixture was extracted with CH_2Cl_2, washed with H_2O and brine, dried, and concentrated. The residue was dissolved in MeOH (8 mL) and 10 M aqueous NaOH (8 mL) was added. After being stirred at room temperature for 6 h, the reaction mixture was concentrated and extracted with AcOEt. The aqueous layer was concentrated and extracted with THF. Combined organic extracts were concentrated and chromatographed (SiO_2, 35 g, hexane/AcOEt = 1:1) to give **17** (503 mg, 65%) as a colorless oil; $[\alpha]_D^{24}$ +13.3° (*c* 0.50, $CHCl_3$); IR (neat) 3754, 3417, 2884, 2235, 2117, 1643, 1423, 1072 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.90 (ddd, 1H, *J* = 17.2, 10.6, 6.4 Hz), 5.41 (d, 1H, *J* = 17.6 Hz), 5.25 (d, 1H, *J* = 10.5 Hz), 4.31 (dd, 1H, *J* = 9.0, 5.2 Hz), 3.93–3.80 (m, 5H), 3.42 (dd, 1H, 6.3, 3.0 Hz), 2.52 (m, 2H), 2.06 (s, 1H), 1.85 (quant, *J* = 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2, 116.9, 82.7, 80.3, 73.7, 72.0, 70.7, 70.4, 60.5, 31.9, 24.0; HRMS (EI) *m/z* calcd for $C_{14}H_{23}O_5$ [$(M+H)^+$] 215.1283, found 215.1274.

3.10. (3*R*,4*R*,5*S*)-4-(3-(Triethylsilyloxy)propoxy)-3,5-di(triethylsilyloxy)oct-1-en-7-yne (5)

To a solution of triethylamine (1.52 g, 14.9 mmol) and **17** (400 mg, 1.87 mmol) in CH_2Cl_2 (20 mL) was added triethylsilyl triflate (2.47 g, 9.33 mmol) at −40 °C, and stirring was continued at −40 °C for 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O and brine, dried, concentrated, and chromatographed (SiO_2 40 g, hexane) to afford **5** (1.10 g, 100%) as a colorless oil; $[\alpha]_D^{27}$ +16.8° (*c* 0.98, $CHCl_3$); IR (neat) 3313, 2954, 2877, 1459, 1415, 1240, 1095, 1006 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.94 (ddd, 1H, *J* = 17.0, 10.8, 6.4 Hz), 5.24 (d, 1H, *J* = 17.2 Hz), 5.10 (d, 1H, *J* = 10.4 Hz), 4.34 (dd, 1H, *J* = 12.0, 6.8 Hz), 3.85 (dd, 1H, *J* = 10.8 8.0 Hz), 3.74–3.64 (m, 4H), 3.20 (dd, 1H, *J* = 6.0, 5.2 Hz), 2.60 (ddd, 1H, *J* = 15.6, 6.0, 2.4 Hz), 2.32 (ddd, 1H, *J* = 15.6, 6.0, 2.4 Hz), 2.16 (s, 1H), 1.92 (quint, 2H, 8.0 Hz), 0.94–0.91 (m, 30H), 0.62–0.55 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7, 115.6, 84.6, 82.6, 74.0, 71.7, 70.1, 70.1, 34.0, 24.3, 6.7, 6.6, 5.7, 5.4, 4.8; HRMS (EI) *m/z* calcd for $C_{29}H_{60}O_4Si_3$ (M^+) 556.3799, found 556.3798.

3.11. (5*Z*,7*E*)-(1*R*,2*R*,3*S*)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (4)

To a solution of **5** (144.3 mg, 0.26 mmol) and **6** (134.6 mg, 0.38 mmol) in degassed toluene (6.2 mL) were added triethylamine (3.7 mL, 26.5 mmol) and $(Ph_3P)_4Pd$ (87.9 mg, 0.076 mmol). After being refluxed for 2 h, the reaction mixture was diluted with Et_2O, filtered through Celite pad, concentrated, and chromatographed (SiO_2 18 g, hexane/AcOEt = 10:1) to give a mixture of the coupling product and **6** (183.2 mg) as an yellow oil. The mixture (183.2 mg) thus obtained was dissolved in MeOH (16 ml) and NH_4F (55.9 mg, 1.51 mmol) was added. After being refluxed for 4 h, the reaction mixture was concentrated, extracted with AcOEt, washed with H_2O and saturated $NaHCO_3$, dried, and concentrated. The residue was purified by preparative TLC (AcOEt) followed by HPLC (ODS-M80) (MeCN/H_2O = 1:1) to afford **4** (58.4 mg, 46%) as a colorless oil. This material was crystallized from a small amount of MeCN to form colorless crystals; mp 126–128°; $[\alpha]_D^{26}$ −61.6° (*c* 0.39, MeOH); IR (KBr) 3332, 2939, 1641, 1442, 1375, 1082 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.32 (d, 1H, *J* = 9.8 Hz), 5.99 (d, 1H, *J* = 11.2 Hz), 5.13 (dt, 2H, *J* = 24.3, 2.4 Hz), 3.96–3.87 (m, 2H), 3.79 (dt, 1H, *J* = 8.9, 2.2 Hz), 3.71 (t, 2H, *J* = 6.1 Hz), 3.51–3.46 (m, 1H), 2.97 (t, 1H, *J* = 8.9 Hz), 2.84 (dd, 1H, *J* = 5.0, 11.2 Hz), 2.50 (dd, 1H, *J* = 12.8, 5.2 Hz), 2.17 (t, 1H, *J* = 11.1), 2.04–1.99 (m, 2H), 1.99–1.87 (m, 1H), 1.82 (quint, 2H, *J* = 6.1 Hz), 1.70–1.66 (m, 2H), 1.58–1.40 (m, 7H), 1.37–1.28 (m, 4H), 1.26–1.22 (m, 1H), 1.16 (s, 6H), 1.10–1.03 (m, 1H), 0.96 (d, 3H, *J* = 6.4 Hz), 0.58 (s, 3H); ^{13}C NMR (100 MHz, CD_3OH) δ 147.3, 143.6, 134.0, 124.5, 118.7, 111.8, 90.2, 75.3, 73.0, 71.5, 71.4, 60.5, 58.0, 57.6, 47.1, 45.3, 43.6, 41.8, 37.7, 33.7, 30.0, 29.3, 29.1, 28.7, 24.8, 23.4, 21.9, 19.4, 12.3; HRMS (EI) *m/z* calcd for $C_{30}H_{50}O_5$ (M^+) 490.3659, found 490.3658.

3.12. Inhibition of PTH secretion in cultured bovine parathyroid cells

The inhibitory activity of analogs (**1**–**4**) in cultured bovine parathyroid cells was analyzed according to Brown et al.[25]

3.13. VDR binding assay

The binding affinity of analogs (**1**–**4**) with human recombinant VDR was analyzed according to Wecksler et al.[26]

3.14. DBP binding assay

The binding affinity of analogs (**1**–**4**) with human DBP was analyzed according to Preece et al.[27]

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