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Synthesis and preliminary biological evaluation of 20-epi-eldecalcitol [20-epi-1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3: 20-epi-ED-71]☆

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This paper is dedicated to the memory of the late Dr. Etsuro Ogata, Professor Emeritus, Tokyo University/Director Emeritus, Cancer Institute Hospital, Japan, who passed away on November 1, 2009.

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ABSTRACT

Eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3, developing code: ED-71] is an analog of active vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] that possesses a hydroxypropoxy substituent at the 2β-position of 1,25$(OH)_2D_3$. Eldecalcitol has potent biological effects on bone and is now in preparation for approval as a promising medicine for the treatment of osteoporosis in Japan. To explore chemical structure–biological activity relationships between eldecalcitol and related analogs, we have already synthesized 1-epi-eldecalcitol, 3-epi-eldecalcitol, and 1,3-diepi-eldecalcitol with inherent biological interests of each targeted analog and evaluated their biological responses. It has been reported that 20-epi-1,25$(OH)_2D_3$, a diastereomer of 1,25$(OH)_2D_3$ that possesses an inverted methyl substituent at the 20-position of the side chain, shows remarkably enhanced biological activities compared to parental compound, 1,25$(OH)_2D_3$. As a continuation of our modification studies on eldecalcitol, we took great interest in 20-epi-eldecalcitol and its biological responses. In this paper, the synthesis of 20-epi-eldecalcitol by the Trost coupling reaction between the A-ring fragment and the C/D-ring fragment as well as *in vitro* preliminary biological evaluation of 20-epi-eldecalcitol are described. In the induction of human myeloid leukemia cell (HL-60) differentiation, inhibition of the human histiocytic lymphoma cell (U937) proliferation, and increase in osteocalcin concentration in the human osteosarcoma cell (MG-63), 20-epi-eldecalcitol showed significantly enhanced activity compared to eldecalcitol.

1. Introduction

There is intense interest in obtaining analogs more potent than active vitamin D_3, 1α,25-dihydroxyvitamin D_3 [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 [1]. Eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3, developing code: 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 [2]. Eldecalcitol (**2**) is now in preparation for approval as a promising medicine for the treatment of osteoporosis in Japan. Recent completion of phase III clinical trials of **2** for bone fracture prevention produced excellent results [3,4].

To explore chemical structure–biological activity relationships between eldecalcitol (**2**) and related analogs, we have already synthesized 1-epi-eldecalcitol [5], 3-epi-eldecalcitol [6], and 1,3-diepi-eldecalcitol [7] with inherent biological interests of each targeted analogs and evaluated their biological responses [8]. It has been reported that 20-epi-1,25$(OH)_2D_3$ (**3**), a diastereomer of 1,25$(OH)_2D_3$ (**1**) which possesses an inverted C-21 methyl substituent at the 20-position of the side chain, shows remarkably enhanced biological activities compared to parental compound, **1**. For example, 20-epi-1,25$(OH)_2D_3$ (**3**) exhibits 18 times the potency of induction of human myeloid leukemia cell (HL-60) differentiation [9], 50 times the inhibition of the human histiocytic lymphoma cell (U937) proliferation [10], and 4.5 times the increase in osteocalcin concentrations in the human osteosarcoma cell (MG-63) [11] compared to **1**. These findings prompted our interest in analog of eldecalcitol (**2**) epimerized at the 20-position and its biological responses [12]. In this paper, we describe the synthesis of 20-epi-eldecalcitol (**4**) as a continuation of our modification studies on **2** and its preliminary biological evalua-

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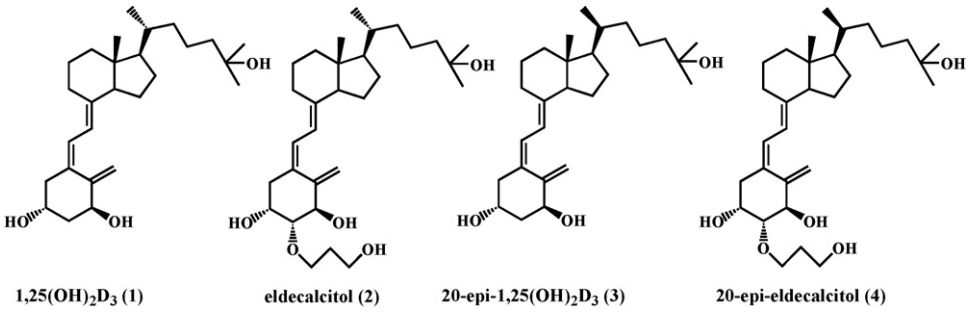

Fig. 1. Chemical structures of active vitamin D_3 analogs.

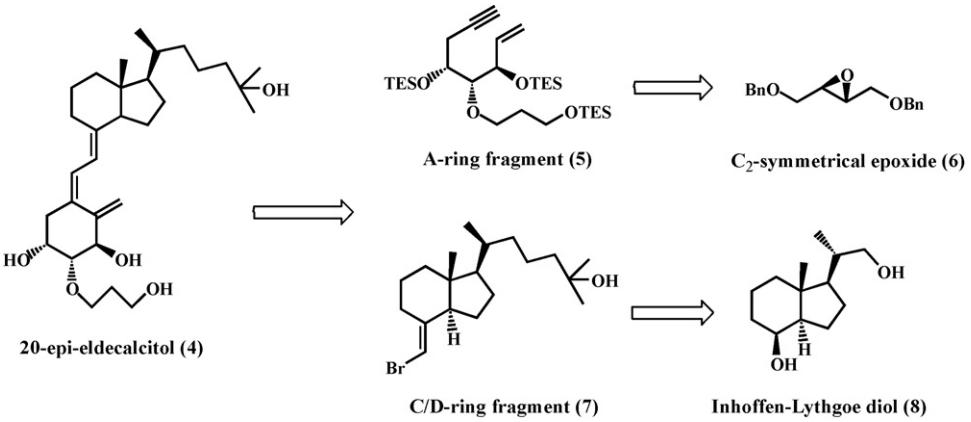

Fig. 2. Retrosynthesis of 20-epi-eldecalcitol (**4**).

tion using HL-60, U937, and MG-63 compared to eldecalcitol (**2**) (Fig. 1).

2. Materials and methods

*2.1. (1R,2R,3R,Z)-5-((E)-2-((1R,3aS,7aR)-1-((S)-6-Hydroxy-6-methylheptan-2-yl)-7a-methyldihydro-1H-inden-4-(2H,5H,6H,7H,7aH)-ylidene)ethylidene)-2-(3-hydroxypropoxy)-4-methylenecyclohexane-1,3-diol (**4**)*

20-Epi-eldecalcitol (**4**) was prepared from the coupling reaction of A-ring fragment **5** with C/D-ring fragment **7** and the subsequent desilylation reaction as a colorless oil; $[\alpha]_D^{20}$ −74.6° (*c*, 0.54, CH_3OH); FTIR (neat) 3407, 1463, 1375, 1260, 1215, 1105 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.30 (d, 1H, *J* = 11.0 Hz), 6.06 (d, 1H, *J* = 11.0 Hz), 5.42 (t, 1H, *J* = 2.0 Hz), 4.98 (t, 1H, *J* = 2.0 Hz), 4.23 (d, 1H, *J* = 8.0 Hz), 4.19 (m, 1H), 3.75–3.71 (m, 2H), 3.71–3.60 (m, 6H), 3.10 (dd, 1H, *J* = 14.8, 4.5 Hz), 2.86 (dd, 1H, *J* = 10.0, 4.0 Hz), 2.47 (dd, 1H, *J* = 14.0, 6.0 Hz), 2.35 (d, 1H, *J* = 13.5 Hz), 2.04–1.98 (m, 2H), 1.88–1.79 (m, 4H), 1.72–1.65 (m, 2H), 1.56–1.24 (m, 12H), 1.17 (m, 6H), 0.87 (d, 3H, *J* = 7.0 Hz), 0.57 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.2, 142.9, 132.1, 124.4, 117.2, 111.8, 85.4, 71.5, 71.1, 68.2, 66.5, 61.1, 60.4, 56.2, 45.9, 44.3, 40.3, 36.6, 35.4, 31.8, 29.7, 29.2, 27.3, 23.7, 22.2, 20.9, 18.5, 14.2, 12.2, 1.0; EIMS *m*/*z* 490 (M^+), 472 (100%); HRMS (EI) *m*/*z* calcd for $C_{30}H_{50}O_5$ (M^+) 490.3676, found 490.3641.

2.2. Induction of HL-60 differentiation

The induction of HL-60 differentiation activity by eldecalcitol (**2**), 20-epi-eldecalcitol (**4**), and 1,25$(OH)_2D_3$ (**1**) was measured according to Kittaka et al. [9].

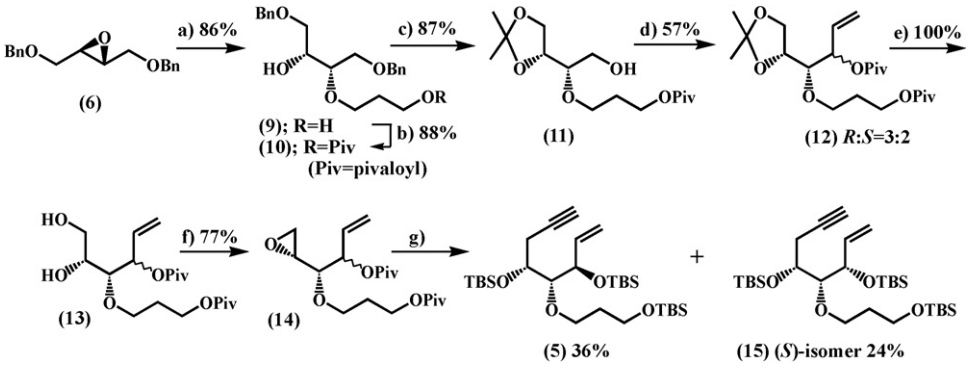

Fig. 3. Synthesis of A-ring fragment **5**. (a) $HO(CH_2)_3OH$, *t*-BuOK, 120 °C; (b) *t*-BuCOCl, pyridine, CH_2Cl_2, rt; (c) (1) H_2, $Pd(OH)_2$, MeOH, rt; (2) $Me_2C(OMe)_2$, TsOH, rt; (d) (1) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C; (2) CH_2=CHMgBr, THF, −60 °C; (3) *t*-BuCOCl. Et_3N, DMAP, CH_2Cl_2, rt; (e) 1 M HCl, MeOH, rt; (f) Ph_3P, DEAD, benzene, reflux; (g) (1) LiC≡CTMS, BF_3-OEt_2, THF, −78 °C; (2) 10N NaOH, MeOH, rt; (3) TBSOTf, Et_3N, CH_2Cl_2, 0 °C.

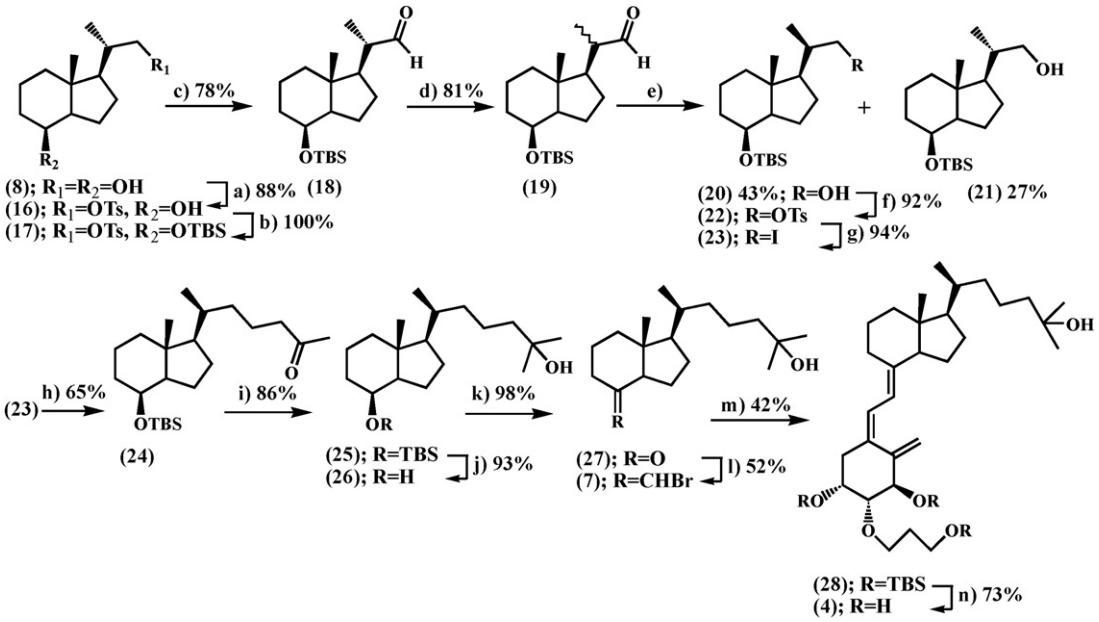

Fig. 4. Synthesis of C/D-ring fragment **7** and coupling with A-ring fragment **5** to obtain 20-epi-eldecalcitol (**4**). (a) TsCl, DMAP, pyridine, rt; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2, −40 °C; (c) DMSO, *s*-collidine, 150 °C; (d) *n*-Bu_4NOH, CH_2Cl_2, rt; (e) $NaBH_4$, EtOH, THF, 0 °C; (f) TsCl, DMPA, pyridine, rt; (g) NaI, DMF, 85 °C; (h) MVK, Zn, CuI, EtOH, H_2O, 20–30 °C; (i) MeMgI, THF, 0 °C; (j) 47% HF, MeCN, THF, 0 °C; (k) TPAP, NMO, CH_2Cl_2, rt; (l) $Ph_3P^+CH_2Br/Br^-$, NaHMDS, rt; (m) A-ring fragment **5**, $Pd(PPh_3)_4$, Et_3N, toluene, reflux; (n) 47% HF, MeCN, rt.

2.3. Inhibition of U937 proliferation

The inhibitory activity of U937 proliferation by eldecalcitol (**2**), 20-epi-eldecalcitol (**4**), and 1,25$(OH)_2D_3$ (**1**) was measured according to Binderup et al. [10].

2.4. Osteocalcin concentration in MG-63

The osteocalcin concentrations in MG-63 with eldecalcitol (**2**), 20-epi-eldecalcitol (**4**), and 1,25$(OH)_2D_3$ (**1**) were measured according to Ryhanen et al. [11].

3. Results

3.1. Synthesis of 20-epi-eldecalcitol (*4*)

Our synthesis of 20-epi-eldecalcitol (**4**) was envisioned using the coupling reaction of A-ring fragment **5**, derived from C_2-symmetrical epoxide **6**, with C/D-ring fragment **7**, which can be obtained from Inhoffen-Lythgoe diol (**8**) [13,14] (Fig. 2). The required A-ring fragment **5** for the synthesis of 20-epi-eldecalcitol (**4**) was synthesized based on the methodology that has been previously established by us [15–17] (Fig. 3). Next, we performed the synthesis of C/D-ring fragment **7** from the Inhoffen-Lythgoe diol (**8**) obtained by ozonolysis of vitamin D_2 as shown in Fig. 4 [18–20]. With A-ring fragment **5** and C/D-ring fragment **7** in hand, we finally investigated the Trost coupling reaction. Upon treatment of an excess **5** with **7** in the presence of tetrakis(triphenylphosphine)palladium (0) [$Pd(PPh_3)_4$] and triethylamine (Et_3N) in toluene, the coupled product **28** was obtained in 42% yield, which was desilylated using 47% HF in acetonitrile (MeCN) to afford 20-epi-eldecalcitol (**4**) in 73% yield (Fig. 4). The ^1H NMR chemical shift of the C-21 methyl resonance in 20-epi-eldecalcitol (**4**) (0.87 ppm) shifted upfield by 0.04 ppm relative to natural configuration, eldecalcitol (**2**) (0.91 ppm) [2] and is consistent with this general pattern [21].

Table 1
Preliminary biological evaluation of 20-epi-eldecalcitol (**4**) in comparison with 1,25$(OH)_2D_3$ (**1**) and eldecalcitol (**2**).

	HL-60	U937	MG-63
1,25$(OH)_2D_3$ (**1**)	100	100	100
Eldecalcitol (**2**)	49.6	4.15	15
20-Epi-eldecalcitol (**4**)	6085.99	738.74	2980

HL-60: relative potency of induction of human myeloid leukemia cell (HL-60) differentiation [9].
U937: relative potency of inhibition of the human histiocytic lymphoma (U937) proliferation [10].
MG-63: relative potency of transcriptional activity of osteocalcin of human osteosarcoma cell (MG-63) [11].

3.2. Preliminary biological evaluation of 20-epi-eldecalcitol (*4*)

The results of preliminary *in vitro* biological evaluation of synthetic 20-epi-eldecalcitol (**4**) in comparison with eldecalcitol (**2**) and 1,25$(OH)_2D_3$ (**1**) are summarized in Table 1. As anticipated, 20-epi-eldecalcitol (**4**) showed greatly enhanced activity toward the induction of HL-60 differentiation (6085.99/49.6 = 122.7 times), inhibition of U937 proliferation (738.74/4.15 = 178.0 times), and increase in osteocalcine concentrations in MG-63 (2980/15 = 198.7 times), compared to eldecalcitol (**2**).

4. Conclusion

Based on the Trost coupling methodology involving A-ring fragment **5** and C/D-ring fragment **7**, the synthesis of 20-epi-eldecalcitol (**4**) has been successfully achieved. In preliminary biological evaluations, 20-epi-eldecalcitol (**4**) showed greatly enhanced potencies toward HL-60, U937, and MG-63 cell lines. We are very much interested in *in vivo* biological activity of 20-epi-eldecalcitol (**4**) on bone. Detailed experimental conditions and further biological studies will be reported elsewhere.

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