Synthesis of All Possible A-ring Diastereomers at the 1- and 3-Positions of 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) Using C_2-Symmetrical Epoxide as a Common Starting Material

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Abstract. *The active vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), is now well recognized as a potent regulator of cell proliferation and differentiation in addition to possessing a regulatory effect on calcium and phosphorus metabolism. From research on the synthesis of 1,25$(OH)_2D_3$ analogs with the goal of separating these biological activities, we have already reported two characteristic analogs of active vitamin D_3, namely 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) and 1α,25-dihydroxy-2β-(3-hydroxypropoxy) vitamin D_3 (ED-71). OCT, a 22-oxa analog obtained from modification of the side chain of 1,25$(OH)_2D_3$, has been used clinically as an injection for the treatment of secondary hyperparathyroidism underlying renal insufficiency and as an ointment for the skin disease, psoriasis. OCT has also been reported to exhibit antiangiogenic activity, exerting antitumor effects without producing serious side effects such as hypercalcemia. On the other hand, ED-71, which possesses a hydroxypropoxy substituent at the 2β-position of the A-ring of 1,25$(OH)_2D_3$, has more potent biological effects on bone compared to 1 and phase III clinical studies for bone-fracture prevention have been completed. To explore structure activity relationship between ED-71 and related analogs, significant attention was now focused on the diastereomer of 3 at both the 1- and 3-positions of the A-ring, namely 3-epi-ED-71, 1-epi-ED-71 and 1,3-diepi-ED-71. All possible A-ring diastereomers at the 1- and 3-positions of ED-71 were synthesized using C_2-symmetrical epoxide as a common starting material by convergent Trost methodology.*

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Key Words: 1α,25-Dihydroxyvitamin D_3, 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3, ED-71, 1-*epi*-ED-71, 3-*epi*-ED-71, 1,3-di*epi*-ED-71.

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 in addition to possessing regulatory effects on calcium and phosphorus metabolism (1). Various analogs of 1,25$(OH)_2D_3$ (**1**) have been synthesized to separate differentiation-induction and antiproliferation activities from calcemic activity with the aim of obtaining useful analogs for the medical treatment of psoriasis, cancer, *etc*., without risk of hypercalcemia (2). 1α,25-Dihydroxy-22-oxavitamin D_3 (OCT, **2**), which contains an oxygen atom at the 22-position in the side chain of **1**, was synthesized for this purpose. OCT has been shown to be highly potent in stimulating monocytic differentiation of human promyelocytic leukemic HL-60 cells but is less calcemic than **1**. OCT has been used clinically as an injection for the treatment of secondary hyperparathyroidism underlying renal insufficiency and as an ointment for the skin disease, psoriasis (3). OCT has also been reported to exhibit antiangiogenic activity, exerting antitumor effects without producing serious side effects such as hypercalcemia. Furthermore, a number of *in vitro* and *in vivo* studies carried out in different animal species have demonstrated the antitumor properties of OCT for breast cancer, pancreatic cancer, prostate cancer, salivary cancer, lung cancer, *etc*. (4).

There is also intense interest in obtaining analogs more potent than **1** in regulating calcium and phosphorus metabolism with the objective of treating bone disease such as osteoporosis. 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71, **3**), which possesses a hydroxypropoxy substituent at the 2β-position of the A-ring of **1**, is such an analog that shows more potent effects in bone therapy compared to **1**. A phase III clinical trial with ED-71 has been completed with very good results as an oral medication for treating osteoporosis. The hope is that it will, in the near future, help osteoporosis patients as a characteristic new active vitamin D_3 analog (Figure 1) (3, 5).

Recently, it has been reported that the epimerization of **1** at the 3-position of the A-ring plays a major role in

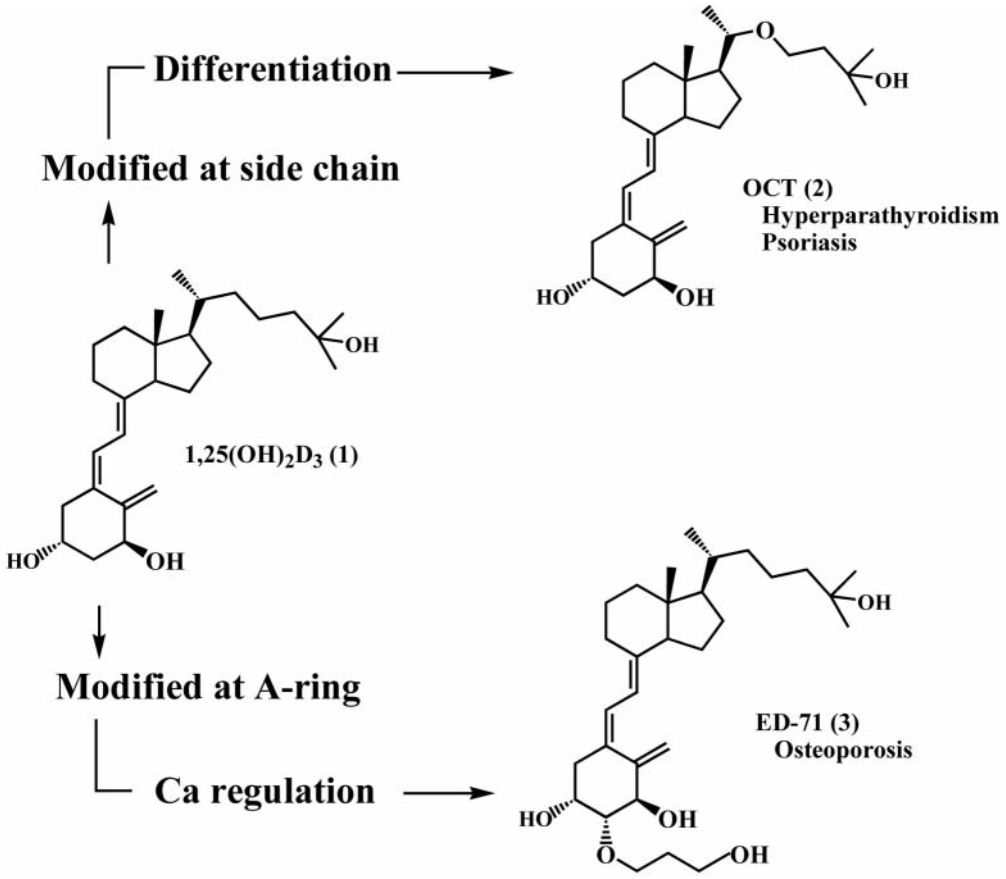

Figure 1. *Modification of 1,25(OH)$_2$D$_3$ (**1**), chemical structure and therapeutic indication of OCT (**2**) and ED-71 (**3**).*

parathyroid hormone (PTH) synthesis and secretion. Epimerized 3-*epi*-1,25(OH)$_2$D$_3$ shows equipotent and prolonged activities in comparison with **1** at suppressing PTH secretion (6, 7). During our clinical development of ED-71, the serum PTH in osteoporotic patients, however, was found not to change significantly upon treatment with ED-71 (5). A bulky hydroxylpropoxy substituent at the 2-position of the A-ring was assumed to interfere with epimerization of ED-71 at the adjacent and sterically hindered 3-position leading to the lack of epimerized 3-*epi*-ED-71 (**4**) in the parathyroid glands. This could explain why ED-71 showed weak potency in PTH suppression during clinical studies. Therefore, the synthesis and the biological evaluation of **4** were of interest. On the other hand, it has also been reported that the epimerization of **1** at the 1-position of the A-ring renders it devoid of activity as an agonist for transcaltachia concerning non-genomic intestinal calcium absorption (8). Therefore, 1-*epi*-1,25(OH)$_2$D$_3$ might be considered a potent stereospecific antagonist of **1** stimulating a transcaltachia response (8). Considering the structure-activity relationship between **1** and 1-*epi*-1,25(OH)$_2$D$_3$, the synthesis and biological activity of 1-*epi*-ED-71 (**5**) were also of interest. Moreover to further explore the structure-activity relationship between ED-71 and related analogs, significant attention was focused on the diastereomer of **3** at both the 1- and 3-positions of the A-ring, namely 1,3-di*epi*-ED-71 (**6**). In this paper, we describe the synthesis of all possible A-ring diastereomers at the 1- and 3-positions of ED-71, **4**, **5** and **6**, using C_2-symmetrical epoxide (**8**) as a common starting material (Figure 2).

Materials and Methods

General methods. All reactions were performed under argon atmosphere. All extracts were dried over magnesium sulfate and evaporated under reduced pressure with a rotary evaporator. Anhydrous tetrahydrofuran (THF) was purchased from Kanto Chemical Co. Inc., dichloromethane (CH_2Cl_2), triethylamine (Et_3N), 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 (IR) spectra were measured on a JASCO FTIR-230

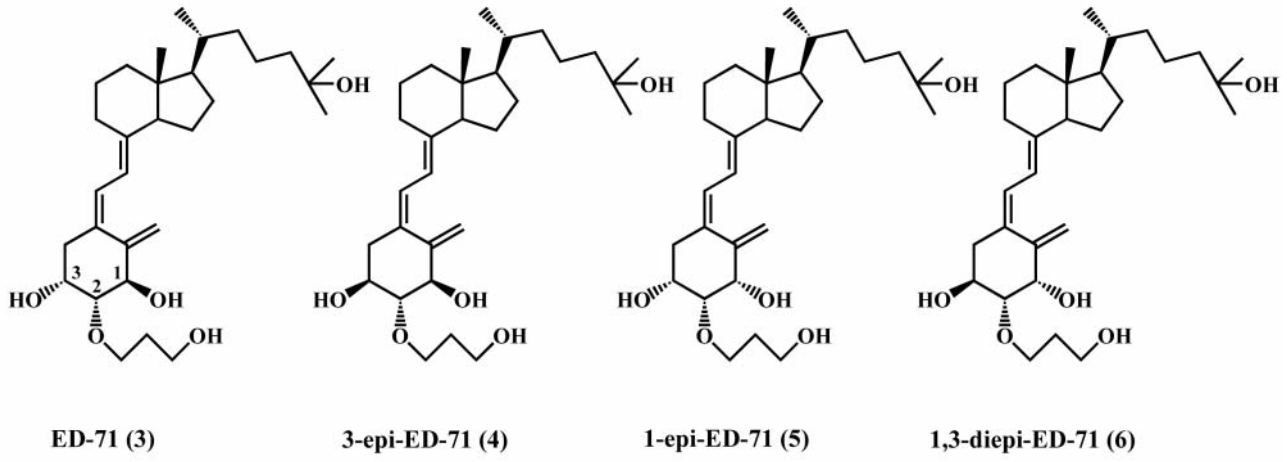

Figure 2. *Structure of ED-71 (**3**), 3-epi-ED-71 (**4**), 1-epi-ED-71 (**5**) and 1,3-diepi-ED-71 (**6**).*

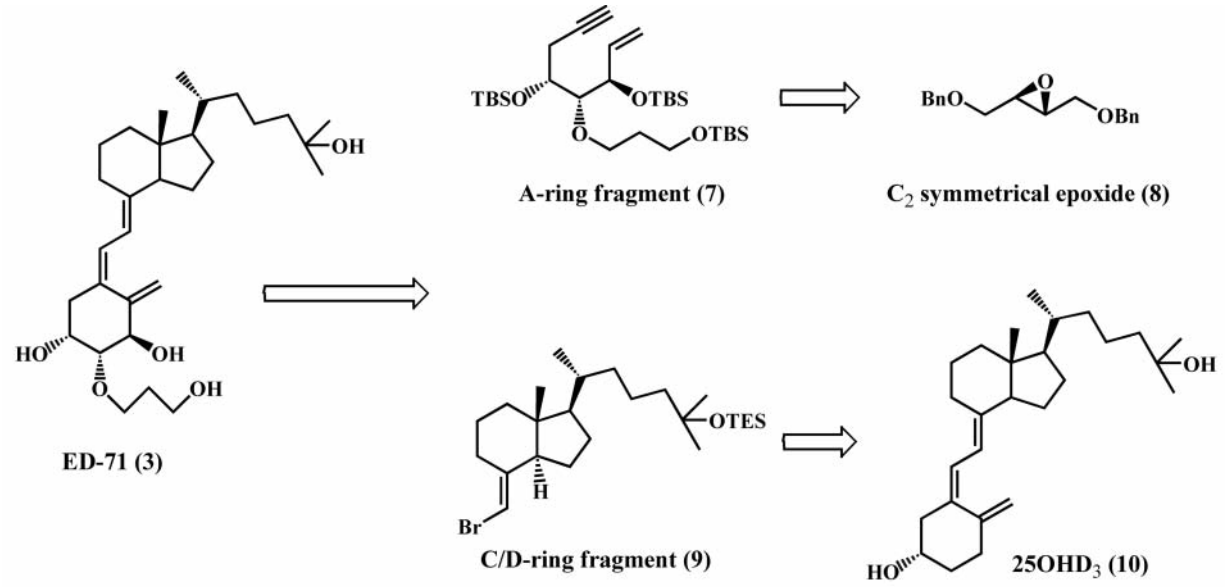

Figure 3. *Retrosynthesis of ED-71 (**3**).*

spectrometer. Optical rotations were recorded on a JASCO DIP-370 polarimeter at ambient temperature. ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian Gemini 300, JEOL JNM-AL 400, or Varian Unity plus 500 spectrometers. For ^{1}H 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 $CHCl_3$ or MeOH. Mass spectra (MS) were measured with JEOL JMS-HX-100, Shimadzu GCMS QP-1000, and Hitachi M1200H instruments. High-resolution mass spectra (HRMS) were recorded on JEOR JMS-AX-500 and VG Auto Spec Q instruments. Ultraviolet (UV) spectra obtained with Shimadzu UV-240 spectrometer using ethanol (EtOH) as a solvent.

*(5Z,7E)-(1R,2R,3R)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (**3**).* ED-71 (**3**) was prepared from A-ring fragment (**7**) and C/D-ring fragment (**9**) as a colorless foam; IR (nujol): ν 3360, 1100, 1060, 910 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.36 (1H, d, *J*=10.5 Hz), 6.04 (1H, d, *J*=10.5 Hz), 5.49 (1H, s), 5.08 (1H, s), 4.36-4.12 (2H, m), 4.02-3.60 (5H, br), 1.21 (6H, s), 0.91 (3H, d, *J*=6.1 Hz), 0.55 (3H, s). MS (EI) *m/z* 490 (M^+), 472, 454, 396, 59 (100%). HRMS (EI) calcd for $C_{30}H_{50}O_5$ (M^+) 490.3658, found 490.3678. UV λmax; 263 nm.

*(5Z,7E)-(1S,2R,3R)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (**4**).* 1-Epi-ED-71 (**4**) was prepared from A-ring fragment (**17**) and C/D-ring fragment (**9**) as a white

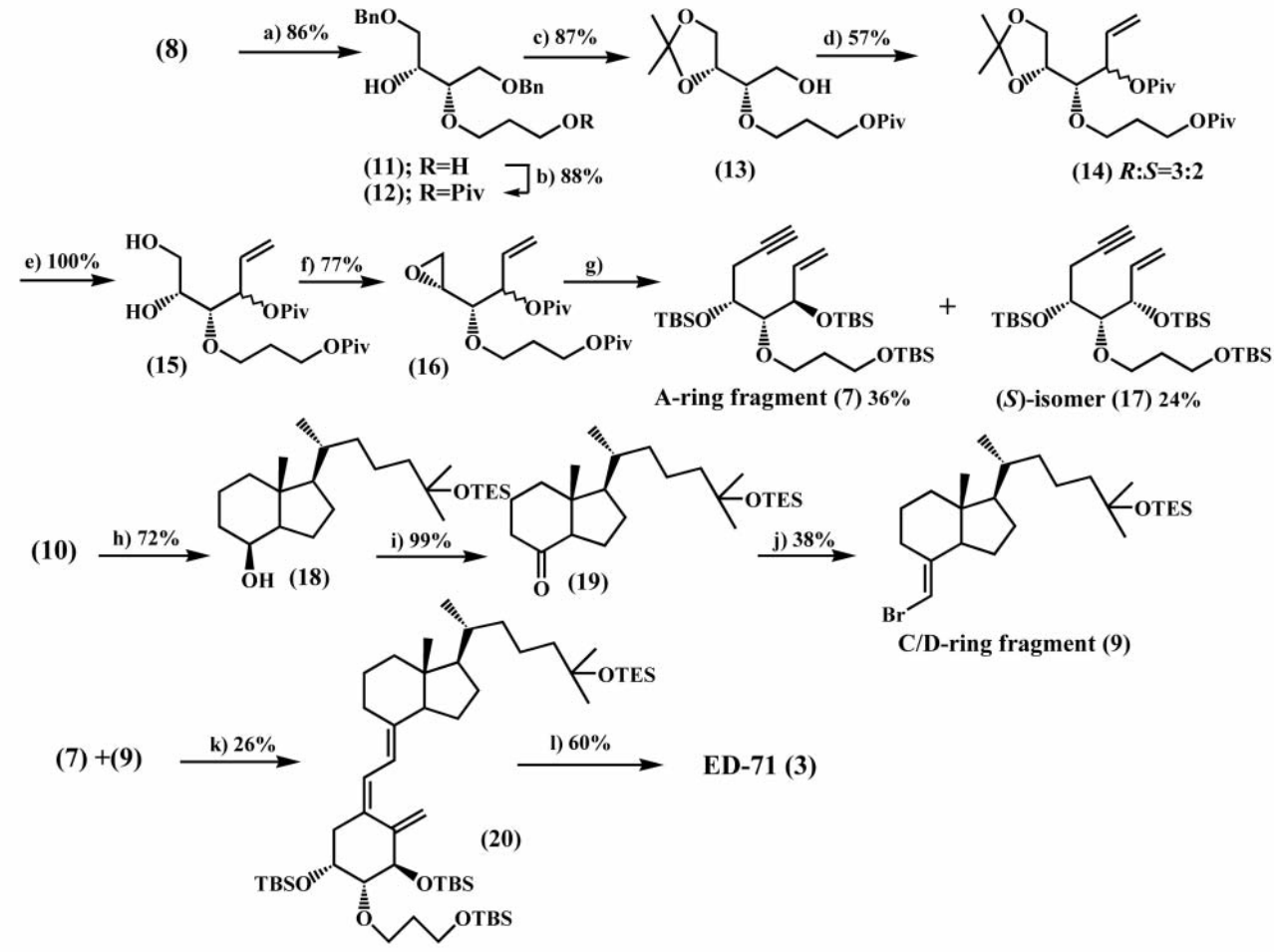

Figure 4. *Synthesis of ED-71 (**3**). Reagents and conditions: a) $HO(CH_2)_3OH$/t-BuOK, 120˚C. b) t-BuCOCl/pyridine/CH_2Cl_2, rt. c) i) H_2/$Pd(OH)_2$/MeOH, rt. ii) $Me_2C(OMe)_2$/TsOH/acetone, rt. d) i) DMSO/$(COCl)_2$/CH_2Cl_2, –60˚C. ii) CH_2=CHMgBr/THF, –60˚C. iii) t-BuCOCl/Et_3N/DMAP/CH_2Cl_2, rt. e) 1 M HCl/MeOH, rt. f) Ph_3P/DEAD/benzene, reflux. g) i) LiC CTMS/BF_3-OEt_2, –78˚C. ii) 10 N NaOH/MeOH, rt. iii) TBSOTf/Et_3N/CH_2Cl_2, 0˚C. h) i) TESOTf/Et_3N/CH_2Cl_2, 0˚C. ii) O_3/CH_2Cl_2/MeOH, –78˚C then $NaBH_4$/MeOH, –78˚C. i) NMO/TPAP/4Ams/CH_2Cl_2, rt. j) $Ph_3P^+CH_2BrBr^-$/NaHMDS/THF, –60˚C – rt. k) $(dba)_3Pd_2$-$CHCl_3$/PPh_3/Et_3N/toluene, reflux. l)TBAF/THF/toluene, reflux.*

powder; IR (neat): ν 3400, 2930, 2850 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.43 (1H, d, *J*=12.0 Hz), 6.03 (1H, d, *J*=12.0 Hz), 5.38 (1H, s), 5.08 (1H, s), 4.33-4.28 (1H, br s), 4.10-4.01 (1H, m), 3.91 (2H, t, *J*=5.4 Hz), 3.84 (2H, t, *J*=5.4 Hz), 3.64-3.58 (1H, m), 1.22 (6H, s), 0.94 (3H, d, *J*=6.5 Hz), 0.54 (3H, s). HRMS (EI) calcd for $C_{30}H_{50}O_5$ (M^+) 490.3658, found 490.3706. UV λ_{max}: 264 nm, λ_{min}: 227 nm.

*(5Z,7E)-(1R,2R,3S)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10 (19)-triene-1,3,25-triol (**5**).* 3-*Epi*-ED-71 (**5**) was prepared from A-ring fragment (**30**) and C/D-ring fragment (**9**) as colorless crystals; mp 126-128˚. $[\alpha]_D^{26}$ –61.6˚ (*c*, 0.39, CH3OH). IR (KBr): ν 3332, 2939, 1641, 1442, 1375, 1082 cm^{-1}. ^1H NMR (CD3OD) δ 6.32 (1H, d, *J*=9.8 Hz), 5.99 (1H, d, *J*=11.2 Hz), 5.13 (2H, dt, *J*=2.4, 24.3 Hz), 3.96-3.87 (2H, m), 3.79 (1H, dt, *J*=2.2, 8.9 Hz), 3.71 (2H, t, *J*=6.1 Hz), 3.51-3.46 (1H, m), 2.97 (1H, t, *J*=8.9 Hz), 2.84 (1H, dd, *J*=5.0, 11.2 Hz), 2.50 (1H, dd, *J*=5.2, 12.8 Hz), 2.17 (1H, t, *J*=11.1), 2.04-1.99 (2H, m), 1.99-1.87 (1H, m), 1.82 (2H, quint, *J*=6.1 Hz), 1.70-1.66 (2H, m), 1.58-1.40 (7H, m), 1.37-1.28 (4H, m), 1.26-1.22 (1H, m), 1.16 (6H, s), 1.10-1.03 (1H, m), 0.96 (3H, d, *J*=6.4 Hz), 0.58 (3H, s). ^{13}C NMR (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^+) 490.3658, found 490.3658.

*(5Z,7E)-(1S,2R,3S)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (**6**).* 1,3-Di*epi*-ED-71 (**6**) was prepared from A-ring fragment (**36**) and C/D-ring fragment (**9**) as a colorless oil. $[\alpha]_D^{22}$ -18.3˚ (*c* 0.12, MeOH). IR (neat): ν 3359, 2941, 1375, 1076 cm^{-1}. ^1H NMR (CD3OD): δ 6.25 (1H, d, *J*=11.2 Hz), 5.99 (1H, d, *J*=11.2 Hz), 5.24 (1H, s), 4.88 (1H, s), 4.32 (1H, d, *J*=3.0 Hz), 3.88-3.87 (1H, m), 3.70-3.67 (1H, m), 3.60-3.56 (3H, m), 2.76 (1H, dd, *J*=3.4, 11.2 Hz), 2.51 (1H, dd, *J*=4.9, 13.6 Hz),

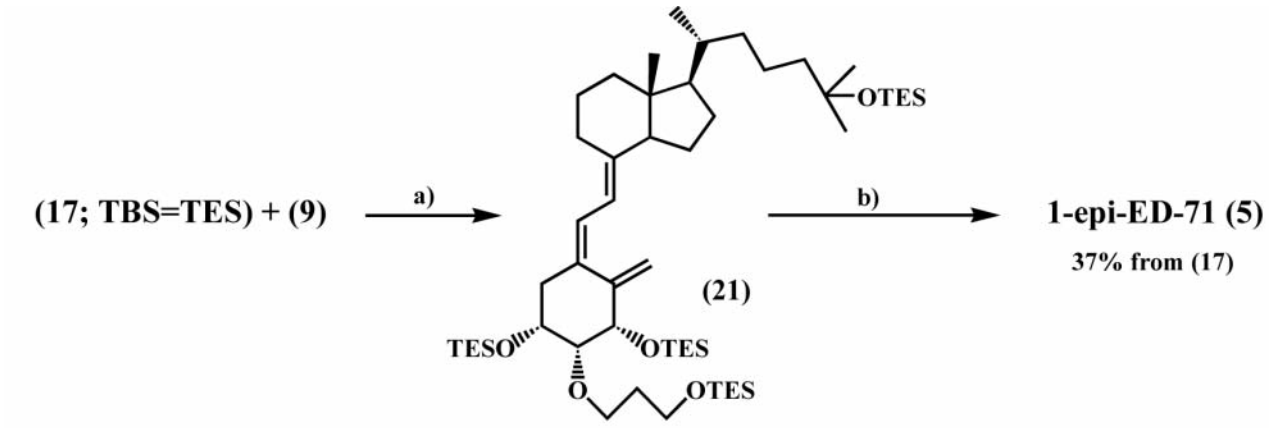

Figure 5. *Synthesis of 1-epi-ED-71 (**5**). Reagents and conditions: a) $Pd(PPh_3)_4/Et_3N$/toluene, reflux. b) 47% HF/MeCN, rt.*

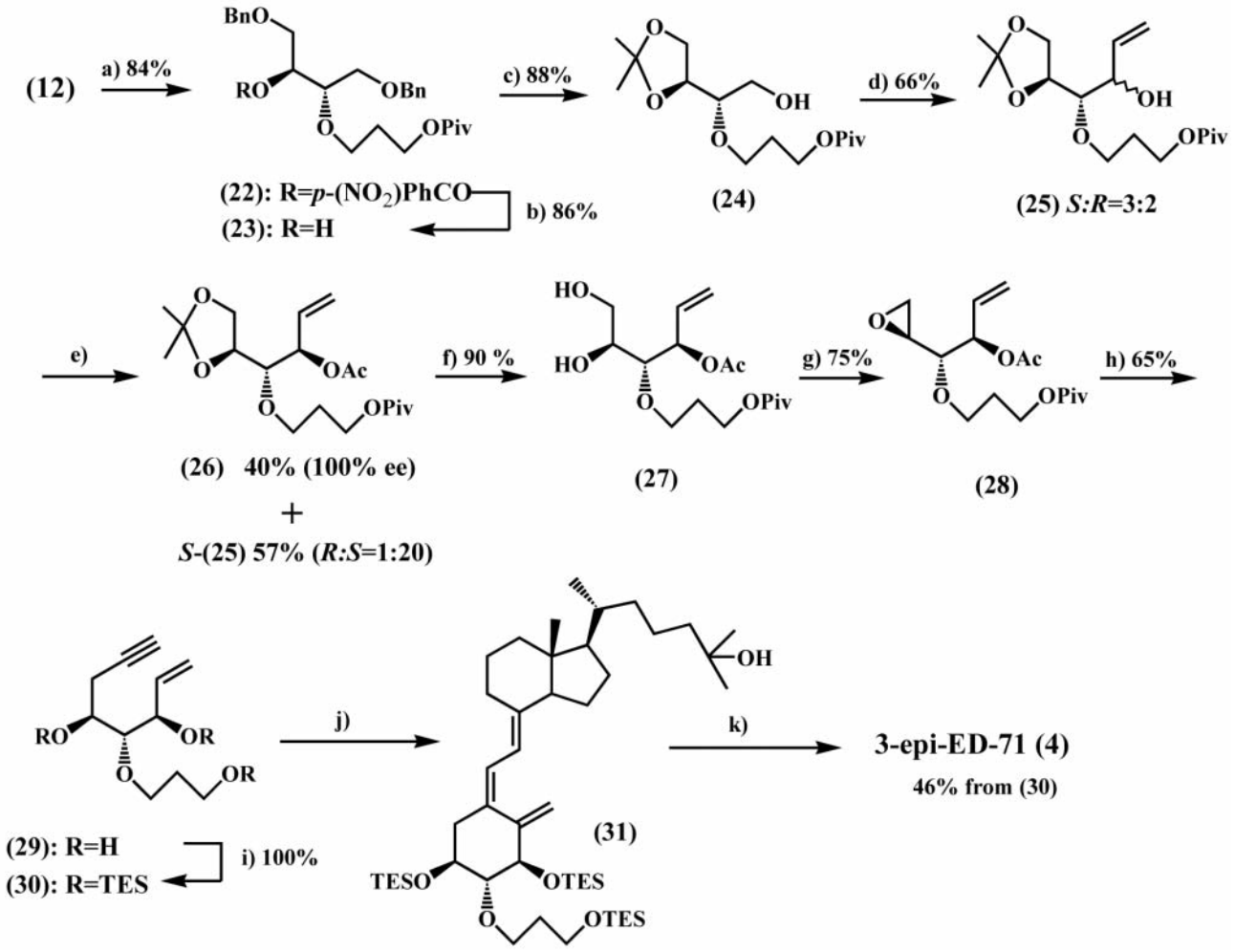

Figure 6. *Synthesis of 3-epi-ED-71 (**4**). Reagents and conditions: a) p-$(NO_2)PhCO_2H$/DEAD/PPh_3/toluene, rt. b) $NaHCO_3$/MeOH, rt. c) i) $Pd(OH)_2/H_2$/MeOH, rt. ii) 2,2-dimethoxypropane/TsOH/acetone, rt. d) i) $(COCl)_2$/DMSO/CH_2Cl_2, –78°C then Et_3N. ii) CH_2=CHMgBr/THF, –40°C. e) Novozyme/CH_2=CHOAc/t-BuOMe, 30°C. f) 60% AcOH/H_2O, rt. g) DEAD/PPh_3/dioxane, reflux. h) i) LiC=CTMS/BF_3-OEt_2, –78°C. ii) 10 M NaOH/MeOH, rt. i) TESOTf/Et_3N/CH_2Cl_2, –40°C. j) (**9**; TES=H)/$Pd(PPh_3)_4/Et_3N$/toluene, reflux. k) NH_4F/MeOH, reflux.*

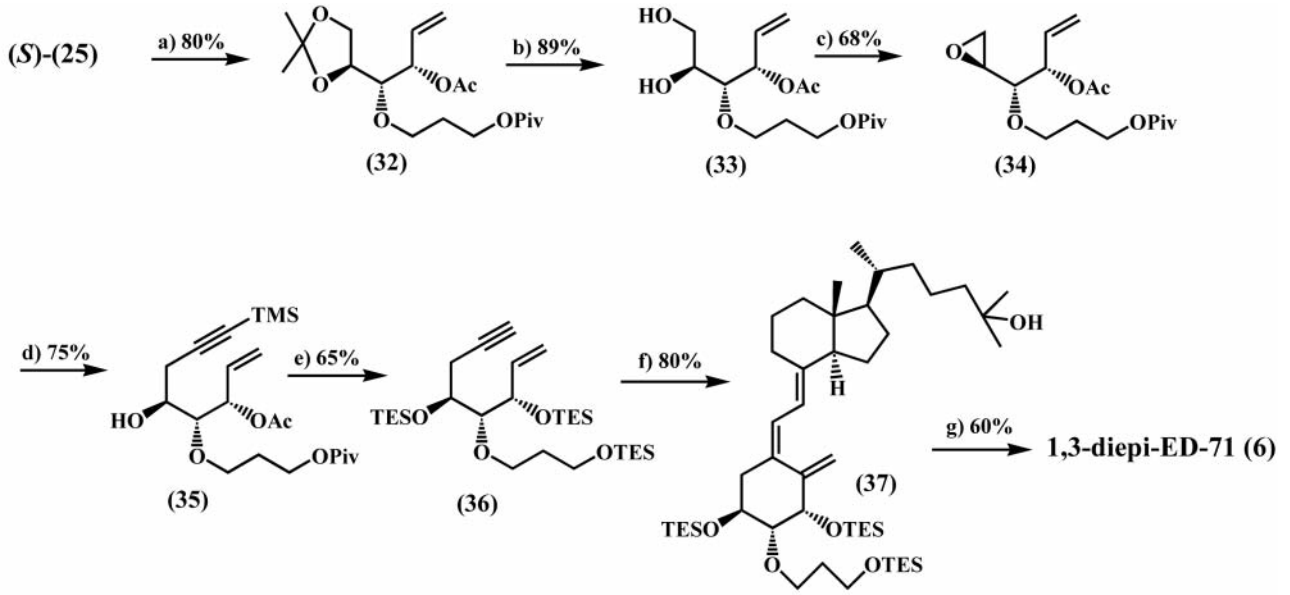

Figure 7. *Synthesis of 1,3-diepi-ED-71 (**6**). Reagents and conditions: a) $Ac_2O/Et_3N/DMAP/CH_2Cl_2$, rt. b) 60% $AcOH/H_2O$, rt. c) PPh3/DEAD/dioxane, reflux. d) LiC=CTMS/BF_3-OEt_2/THF, –78°C. e) i) 10 M NaOH/MeOH, rt. ii) TESOTf/Et_3N/CH_2Cl_2, –40°C. f) (9; TES=H)/$Pd(PPh_3)_4/Et_3N$/toluene, reflux. g) 46% HF/MeCN, rt.*

2.07 (1H, dd, *J*=6.8, 13.2 Hz), 1.95-1.87 (2H, m), 1.82-1.79 (1H, m), 1.72 (2H, quint, *J*=5.8 Hz), 1.58 (2H, d, *J*=11.2 Hz), 1.41-1.31 (7H, m), 1.26-1.19 (4H, m), 1.07 (6H, s), 0.87 (3H, d, *J*=6.8 Hz), 0.80-0.77 (1H, m), 0.47 (3H, s). ^{13}C NMR (CD_3OD): δ 147.1, 134.6, 125.2, 119.0, 115.0, 85.8, 73.0, 71.5, 69.4, 68.6, 60.4, 58.0, 57.6, 45.3, 42.4, 41.9, 37.8, 33.7, 30.8, 30.0, 29.3, 29.1, 28.7, 24.7, 23.4, 21.9, 19.4, 12.3. HRMS (FAB) calcd for $C_{30}H_{50}O_5$ (M^+) 490.3658 found 490.3648.

Results and Discussion

The synthesis of all the possible A-ring diastereomers at the 1- and 3-positions of ED-71 (**3**) was envisioned using convergent methodology described by Trost *et al*. (9, 10). The key step involves palladium-catalyzed coupling of A-ring fragment (**7**) prepared from C_2-symmetrical epoxide (**8**) with C/D-ring fragment (**9**) obtained from 25-hydroxyvitamin D_3 ($25OHD_3$, **10**). This methodology was evaluated as an improved method for industrial scale production of ED-71, considering the potential clinical application of **3** as a useful drug in the near future (Figure 3) (11).

*Synthesis of ED-71(**3**) and 1-epi-ED-71 (**5**)*. The required A-ring fragment (**7**) for the synthesis of ED-71 (**3**) was synthesized based on the methodology which we have previously established (11). Thus, cleavage of the known C_2-symmetrical epoxide (**8**) (12) with 1,3-propanediol in the presence of potassium *tert*-butoxide (*t*-BuOK) gave the diol (**11**) in 86% yield. After protection of the primary hydroxyl group to give the pivalate (**12**) in 88% yield, cleavage of the benzyl ether moiety in **12** and subsequent protection of the resulting 1,2-diol as the acetonide gave the alcohol (**13**) in 87% overall yield. Swern oxidation of **13** and subsequent Grignard reaction of the resulting aldehyde with vinylmagnesium bromide (CH_2=CHMgBr) followed by pivaloylation of the resulting alcohol afforded the dipivalate (**14**) as an epimeric mixture (*R*/*S*=3/2). Without separation of the epimeric mixture, the acetonide moiety in **14** was cleaved quantitatively to give the diol (**15**). Exposure of **15** to Mitsunobu conditions (13) afforded the epimeric epoxide (**16**) in 77% yield. The acetylene unit was successfully installed by the regioselective epoxide-opening of **16** with lithium trimethylsilylacetylide (LiC=CTMS) to provide the ene-yne (**7**) as the A-ring fragment for ED-71 (**3**) in 36% yield after protecting group exchange from the pivalate to the *tert*-butyldimethylsilyl (TBS) ether. The accompanying (*S*)-epimer (**17**), which consists of the requisite stereochemistry to obtain 1-epi-ED71 (**6**), was separated in 24% yield by simple column chromatography. Next, the synthesis of the C/D-ring fragment (**9**) from readily and commercially available $25OHD_3$ (**10**) was performed (14). $25OHD_3$ (**10**) was protected as the *bis*-triethylsilyl (TES) ether using triethylsily trifluoromethanesulfonate (TESOTf), and was then converted to the alcohol (**18**) by ozonolysis and treatment with sodium borohydride ($NaBH_4$) (72% yield from **10**). The hydroxyl moiety in **18** was oxidized to the ketone (**19**) with tetrapropylammonium perruthenate (TPAP) and *N*-methylmolpholine *N*-oxide (NMO) in 99% yield. Wittig reaction of **19**

with (bromomethylene) triphenyl-phosphonium bromide ($Ph_3P^+CH_2Br/Br^-$) and sodium hexamethyldisilazide (NaHMDS) gave rise to the C/D-ring fragment bromomethylene (**9**) in 38% yield. With A-ring fragment (**7**) and C/D-ring fragment (**9**) in hand, next the Trost coupling reaction was investigated. Thus, upon treatment of **7** and **9** with Et_3N, triphenyphosphine (PPh_3) and tris (dibenzylideneacetone)dipalladium-chloroform [$(dba)_3Pd_2$-$CHCl_3$] in boiling toluene, the coupled product (**20**) was obtained in 26% yield together with recovered **7** (45%) and **9** (56%). Deprotection of the silyl moiety in **20** with tetrabutylammonium fluoride (TBAF) afforded ED-71 (**3**) in 60% yield (Figure 4) (14).

On the other hand, as described above, when the ene-yne (**7**) was prepared from the epimeric epoxide (**16**) as the (*R*)-isomer, the separable (*S*)-isomer (**17**) was accompanied as a by-product, which was used to obtain 1-epi-ED-71 (**5**). Upon treatment of excess bromomethylene (**9**) and ene-yne (**17**, TBS=TES) in the presence of tetrakis (triphenylphosphine)-palladium (0) [$Pd(PPh_3)_4$] and Et_3N in boiling toluene, the coupled product (**21**) was obtained as an inseparable mixture with recovered **9**. The mixture was desilylated using 47% hydrofluoric acid (HF) in MeCN and purified to afford 1-epi-ED-71 (**5**) in 37% yield from **17** (Figure 5) (15, 16).

*Synthesis of 3-epi-ED-71 (**4**) and 1,3-diepi-ED-71 (**6**).* The synthesis of the A-ring fragment (**30**) for 3-epi-ED-71 (**4**) began with inversion of the C_3 configuration of the alcohol (**12**). Reaction of **12** with *p*-nitrobenzoic acid in the presence of diethyl azodicarboxylate (DEAD) and PPh3 gave *p*-nitrobenzoate (**22**) in 84% yield (17). Treatment of **22** with sodium bicarbonate ($NaHCO_3$) in MeOH allowed selective methanolysis of the *p*-nitrobenzoate group to give the inverted alcohol (**23**) in 86% yield. After hydrogenolysis of the benzyl ether functionalities in **23** the resulting diol was protected as its acetonide to afford the acetonide (**24**) in 88% yield. Swern oxidation of **24** followed by Grignard reaction of the resulting aldehyde with CH_2=CHMgBr produced the alcohol (**25**) as an epimeric mixture (*S*:*R*=3:2) in 66% yield. To separate this epimeric mixture, **25** was subjected to lipase-catalyzed acetylation using vinyl acetate (CH_2=CHOAc) and Novozyme in *t*-butyl methyl ether (*t*-BuOMe) (18). As a result, the *R*-epimer preferentially underwent acetylation to give the acetate (**26**) and ***S*-25** (*R*:*S*=1:20) in 40% and 57% yields, respectively. Acidic hydrolysis of **26** gave the diol (**27**) in 90% yield, which upon Mitsunobu reaction using DEAD and PPh_3 in boiling toluene afforded the epoxide (**28**) in 75% yield (12). Reaction of **28** with LiC=CTMS in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) at –78˚C followed by saponification provided the ene-yne (**29**) in 65% yield (19). Protection of **29** as its TES ether produced the A-ring fragment (**30**) quantitatively. Having secured the A-ring fragment (**30**), its coupling with the C/D-ring fragment (**9**) was performed using the Trost methodology. Thus, the A-ring fragment (**30**) was allowed to react with the C/D-ring fragment (**9**, TES=H) in the presence of $Pd(Ph_3P)_4$ and Et_3N in boiling toluene to give the coupling product (**31**) which was desilylated with ammonium fluoride (NH_4F) in boiling MeOH to produce 3-*epi*-ED-71 (**4**) in 46% yield from **30** (Figure 6) (20).

The synthesis of the A-ring fragment (**36**) for 1,3-di*epi*-ED-71 (**6**) started from the alcohol (**25**) which was obtained in our previous lipase-catalyzed acetylation of **25** as the unreacted (*S*)-isomer. The alcohol (**25**) possesses the requisite stereochemistry at positions 1, 2 and 3 of the A-ring that comprises **6**. Acetylation of **25** gave the acetate (**32**) in 80% yield from which the diol (**33**) was obtained in 89% yield after deprotection of the acetonide moiety using 60% acetic acid (AcOH). Mitsunobu reaction of **33** with DEAD and PPh_3 in boiling toluene afforded the epoxide (**34**) in 68% yield. Reaction of **34** with LiC=CTMS in the presence of $BF_3{\cdot}OEt_2$ at –78˚C gave the ene-yne (**35**) in 75% yield, which was then converted to the A-ring fragment (**36**) by saponification with 10 M sodium hydroxide (NaOH) and subsequent protection of the hydroxyl groups as their TES ether in 65% overall yield. Next the A-ring fragment (**36**) was coupled to the C/D-ring fragment (**9**) using Trost's methodology. Thus, **36** was coupled with **9** (TES=H) in the presence of $Pd(PPh_3)_4$ and Et_3N in boiling toluene to produce the desired coupling product (**37**) in 80% yield. Desilylation of **37** with 46% HF in MeCN at room temperature gave rise to 1,3-diepi-ED-71 (**6**) in 60% yield (Figure 7) (21).

Conclusion

Based on the Trost coupling methodology involving A-ring fragments (**7**), (**17**), (**30**) and (**36**) and C/D-ring fragment (**9**), the synthesis of ED-71 (**3**), 3-*epi*-ED-71 (**4**), 1-*epi*-ED-71 (**5**) and 1,3-di*epi*-ED-71 (**6**) was successfully accomplished, completing the preparation of the full complement of A-ring 1- and 3-positional diasteromers. The detailed biological properties of these analogs are currently under investigation and will be reported elsewhere.

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References

1 Bouillon R, Okamura WH and Norman AW: Structure-function relationships in the vitamin D endocrine system. Endocr Rev *16*: 200-257, 1995.

2 Posner GH and Kahraman M: Overview: rational design of 1α,25-dihydroxyvitamin D_3 analogs (deltanoids). *In*: Vitamin D Second Edition. Feldman D, Pike JW and Glorieux FH (eds.). New York, Elsevier Academic Press, pp. 1405-1422, 2005.

3 Kubodera N: Development of OCT and ED-71. *In*: Vitamin D Second Edition. Feldman D, Pike JW and Glorieux FH (eds.). New York, Elsevier Academic Press, pp. 1525-1541, 2005.

4 Kubodera N, Sato K and Nishii Y: Characteristics of 22-oxacalcitriol (OCT) and 2β-(3-hydroxypropoxy)calcitriol (ED-71). *In*: Vitamin D. Feldman D, Glorieux FH and Pike JW (eds.). New York, Academic Press, pp. 1071-1086, 1997.

5 Matsumoto T, Miki T, Hagino H, Sugimoto T, Okamoto S, Hirota T, Tanigawara Y, Hayashi Y, Fukunaga M, Shiraki M and Nakamura T: A new active vitamin D, ED-71, increases bone mass in osteoporotic patients under vitamin D supplementation: a randomized, double-blind, placebo-controlled clinical trial. J Clin Endocrinol Metab *90*: 5031-5036, 2005.

6 Brown AJ, Ritter C, Slatopolsky E, Muralidharan KR, Okamura WH and Reddy GS: 1α,25-Dihydroxy-3-epi-vitamin D_3, a natural metabolite of 1α,25-dihydroxyvitamin D_3, is a potent suppressor of parathyroid hormone secretion. J Cell Biochem *73*: 106-113, 1999.

7 Brown AJ, Ritter C, Weiskopf AS, Vouros P, Sasso GJ, Uskokovic MR, Wang G and Reddy GS: Isolation and identification of 1α-hydroxy-3-*epi*-vitamin D_3 a potent suppressor of parathyroid hormone secretion. J Cell Biochem *96*: 569-578, 2005.

8 Norman AW, Nemere I, Muralidharan KR and Okamura WH: 1β,25$(OH)_2$-vitamin D_3 is an antagonist of 1α,25$(OH)_2$-vitamin D_3 stimulated transcaltachia (the rapid hormonal stimulation of intestinal calcium transport). Biochem Biophys Res Commun *189*: 1450-1456, 1992.

9 Trost BM and Dumas J: New strategy for the total synthesis of 1α-hydroxyvitamin D derivatives. J Am Chem Soc *114*: 1924-1925, 1992.

10 Trost BM, Dumas J and Villa M: New strategies for the synthesis of vitamin D metabolites. J Am Chem Soc *114*: 9836-9845, 1992.

11 Hatakeyama S, Kawase A, Uchiyama Y, Maeyama J, Iwabuchi Y and Kubodera N: Synthesis and biological characterization of 1α,24,25-trihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (24-hydroxylated ED-71). Steroids *66*: 267-276, 2001

12 Nicolau KC, Papahatjis DP, Claremon DA, Magolda RL and Dolle RE: Total synthesis of ionophore antibiotic X-14547A. J Org Chem *50*: 1440-1456, 1985.

13 Mitsunobu O: The use of diethyl azocarboxylate and triphenylphosphine in synthesis and transformation of natural products. Synthesis pp. 1-28, 1981.

14 Maeyama J, Hiyamizu H, Takahashi K, Ishihara J, Hatakeyama S and Kubodera N: Two convergent approaches to the synthesis of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) by the Lythgoe and the Trost coupling reactions. Heterocycles *70*: 295-307, 2006.

15 Eto K, Fujiyama A, Kaneko M, Takahashi K, Ishihara J, Hatakeyama S, Ono Y and Kubodera N: An improved synthesis of 1-epi-ED-71, a biologically interesting diastereomer of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71). Heterocycles *77*: 323-331, 2009.

16 Ono Y, Watanabe H, Kawase A Kubodera N, Okano T, Tsugawa N and Kobayashi T: Synthesis and biological character of 1β-hydroxylated vitamin D_3 analogues. Bioorg Med Chem Lett *4*: 1523-1526, 1994.

17 Martin SF and Dodge JA: Efficacious modification of the Mitsunobu reaction for inversions of sterically hindered secondary alcohols. Tetrahedron Lett *32*: 3017-3020, 1991.

18 Nakamura K and Hirose Y: Fitness of lipases and substrates in lipase-catalyzed resolution. J Synth Org Chem Jpn *53*: 668-677, 1995.

19 Yamaguchi M and Hirao I: An efficient method for the alkynylation of oxiranes using alkynyl boranes. Tetrahedron Lett *24*: 391-394, 1983.

20 Hatakeyama S, Nagashima S, Imai N, Takahashi K, Ishihara J, Sugita A, Nihei T, Saito H, Takahashi F and Kubodera N: Synthesis and evaluation of a 3-position diastereomer of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71). Bioorg Med Chem *14*: 8050-8056, 2006.

21 Fujiyama A, Kaneko M, Takahashi K, Ishihara J, Hatakeyama S and Kubodera N: Synthesis of 1,3-diepi-ED-71, a biologically important diastereomer of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71). Heterocycles *71*: 2263-2271, 2007.

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