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SURVEYING THE EFFECTS OF ELDECALCITOL AND RELATED ANALOGS FROM A BIOLOGICAL PERSPECTIVE*

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Abstract – In the previous review paper, explorative and developmental researches of eldecalcitol (1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3), an analog of active vitamin D_3, calcitriol (1α,25-dihydroxyvitamin D_3), were introduced. Eldecalcitol possesses potent effects on bone disease such as osteoporosis. The completion of a phase III clinical trial of eldecalcitol for bone fracture prevention in comparison with alfacalcidol (1α-hydroxyvitamin D_3), prodrug of calcitriol, produced excellent results. Although clinically, eldecalcitol showed greater potency than calcitriol/alfacalcidol, the detailed physiological properties and mechanism of action of the enhanced activity of eldecalcitol toward bone remains to be clarified. To explore structure-activity relationships, related analogs of eldecalcitol have been synthesized with inherent biological background of each targeted analogs. These include epimeric analogs at 1, 2, 3, and 20 positions, nor analog at 19 position and deoxy analogs at 1 and 25 positions. This review discusses eldecalcitol and related analogs in a biological perspective. The synthetic features of analogs are also outlined.

CONTENTS

**This paper is dedicated to Professor Dr. Ei-ichi Negishi on the occasion of his 77th birthday.*

1. INTRODUCTION

It is well-established that vitamin D_3 (cholecalciferol **1**) ingested into foods or synthesized in the skin is metabolized to 25-hydroxyvitamin D_3 (calcifediol **2**) in the liver, which is further hydroxylated at the 1α position in the kidney to produce the active form, 1α,25-dihydroxyvitamin D_3 (calcitriol **3**).[1] Calcitriol (**3**) is well recognized as a potent regulator of calcium and phosphorous metabolism while also possessing regulatory effects on cell proliferation and differentiation processes.[2] In Japan, calcitriol (**3**) and its synthetic prodrug, 1α-hydroxyvitamin D_3 (alfacalcidol **4**), which is also activated to **3** in the body (liver and bone), have been widely used for the treatment of osteoporosis for more than a

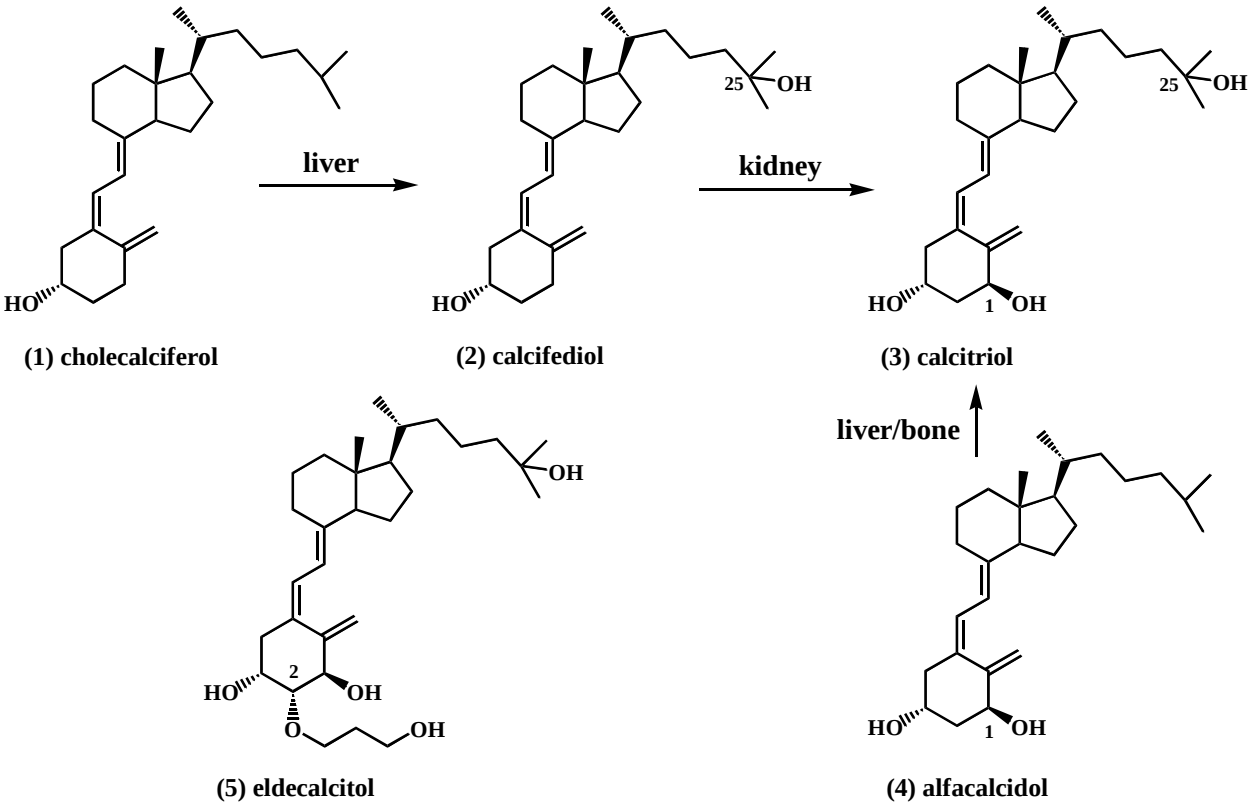


Figure 1. Activation of cholecalciferol and alfacalcidol to calcitriol and structure of eldecalcitol

quarter-century.[3] Calcitriol (**3**) and alfacalcidol (**4**) have been recognized as very safe medicines that show mild or moderate increase in bone mineral density (BMD) in osteoporotic patients. There exists intense interest in obtaining active vitamin D_3 analogs more potent than **3** and **4** towards increasing BMD and preventing bone fracture with less calcemic activity for treating osteoporosis. 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (eldecalcitol **5**, developing code; ED-71), which possesses a hydroxypropoxy substituent at the 2β position of the A-ring of **3**, is such an analog that shows potent effects on bone therapy.[4-6] Recent completion of a phase III trial of **5** for bone fracture prevention and BMD increase in comparison with alfacalcidol (**4**) produced excellent results.[7,8] The marketing of eldecalcitol (**5**) with the sales name of Edirol as an excellent medicine for the treatment of osteoporosis has started very recently in Japan by Chugai Pharmaceutical Co., Ltd (Figure 1).

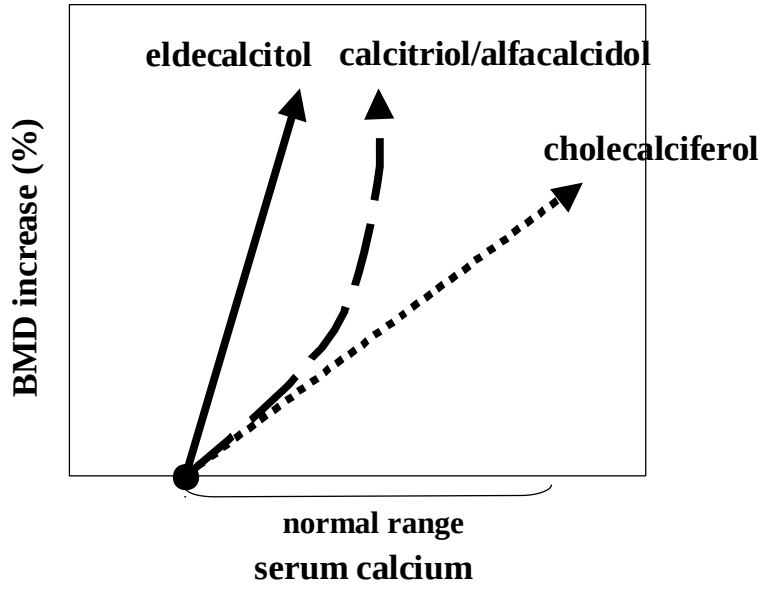


Figure 2. Basic relationship between calcemic activity (serum calcium) and effect on bone (BMD increase) with cholecalciferol, calcitriol/alfacalcidol, and eldecalcitol

Figure 2 illustrates the basic relationship between calcemic activity (serum calcium) and the targeted effect on bone (BMD increase) with cholecalciferol (**1**), calcitriol (**3**)/alfacalcidol (**4**), and eldecalcitol (**5**).[9] The potent effect on bone is highest with **5** followed by **3** and **4** and then **1**, at doses that induce approximately the same level of serum calcium (Figure 2). The question is what is the different mode-of-action between calcitriol (**3**)/alfacalcidol (**4**) and eldecalcitol (**5**). To explore structure-activity relationships, related analogs of eldecalcitol (**5**) have been synthesized by Chugai group or other groups with inherent biological background of each targeted analogs. These include

epimeric analogs at 1, 2, 3, and 20 positions, nor analog at 19 position and deoxy analogs at 1 and 25 positions. This review discusses eldecalcitol (**5**) and related analogs, 20-epieldecalcitol (**6**), 3-epieldecalcitol (**7**), 1-epieldecalcitol (**8**), 1,3-diepieldecalcitol (**9**), 2-epieldecalcitol (**10**), 2,20-diepieldecalcitol (**11**), 19-noreldecalcitol (**12**), 25-deoxyeldecalcitol (**13**), and 1-deoxyeldecalcitol (**14**), from a biological perspective (Figure 3). The synthetic features of the analogs are also outlined.

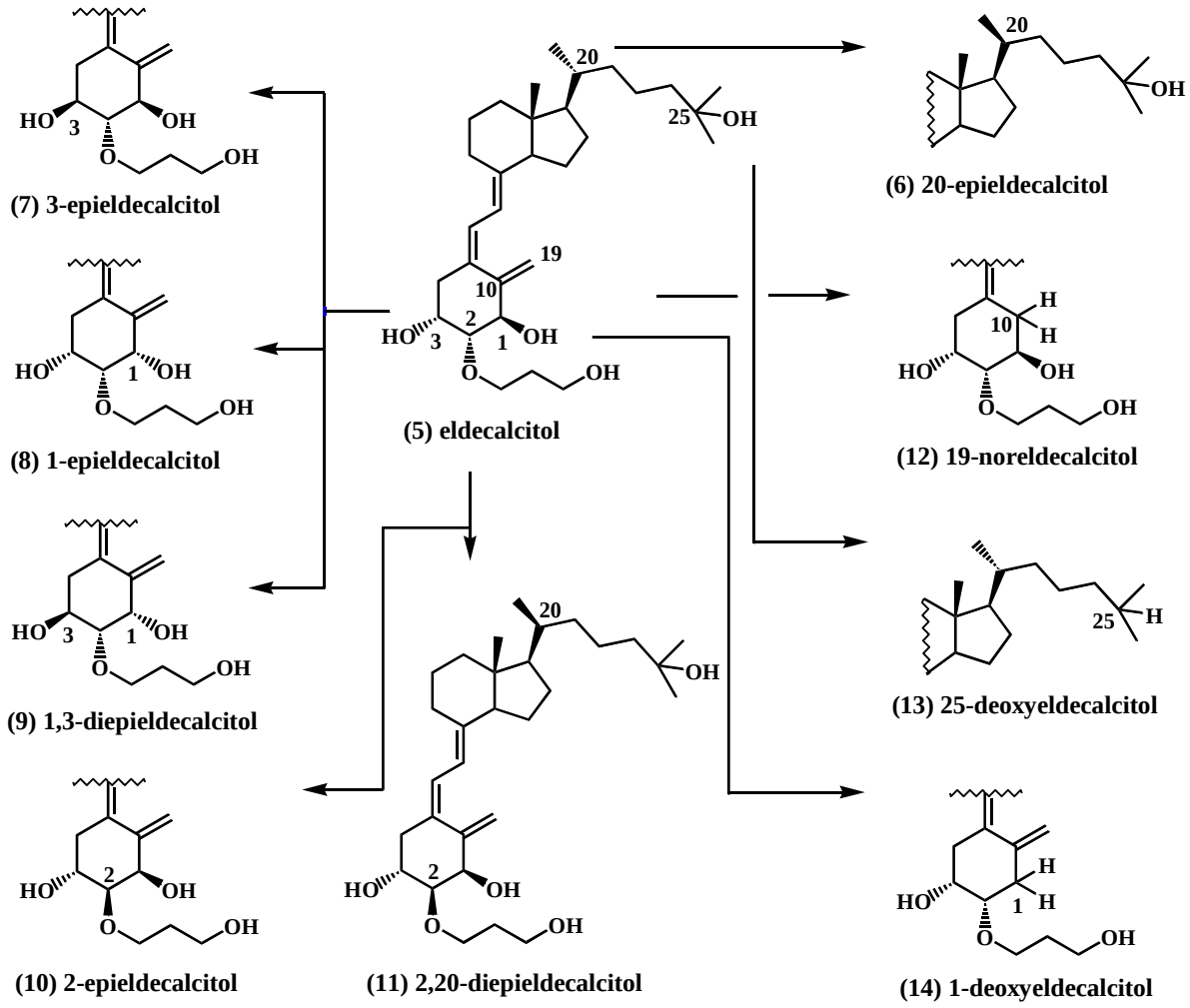


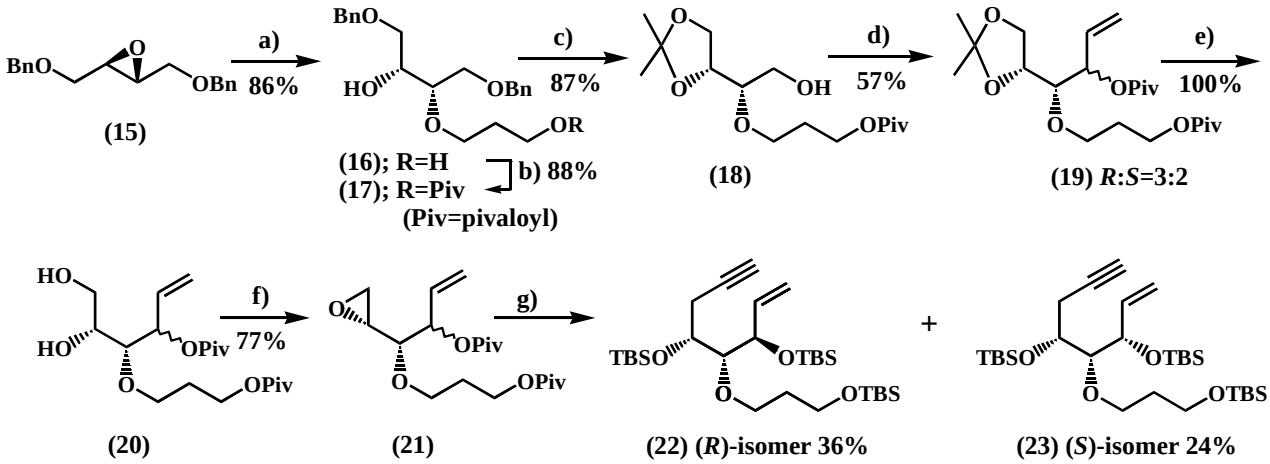
Figure 3. Structures of eldecalcitol and related analogs

2. EPIMERIC ANALOGS AT 1, 2, 3, AND 20 POSITIONS

2-1. 20-EPIELDECALCITOL (6)[10,11]

It has been reported that 20-epicalcitriol, a diastereomer of calcitriol (**3**), which possesses an inverted C_{21} methyl substituent at the 20 position of the side chain of **3**, shows remarkably enhanced biological activities compared to parent compound **3**.[12] For example, 20-epicalcitriol exhibits 18 times the potency of induction of differentiation in human myeloid leukemia cells (HL-60).[13] Furthermore

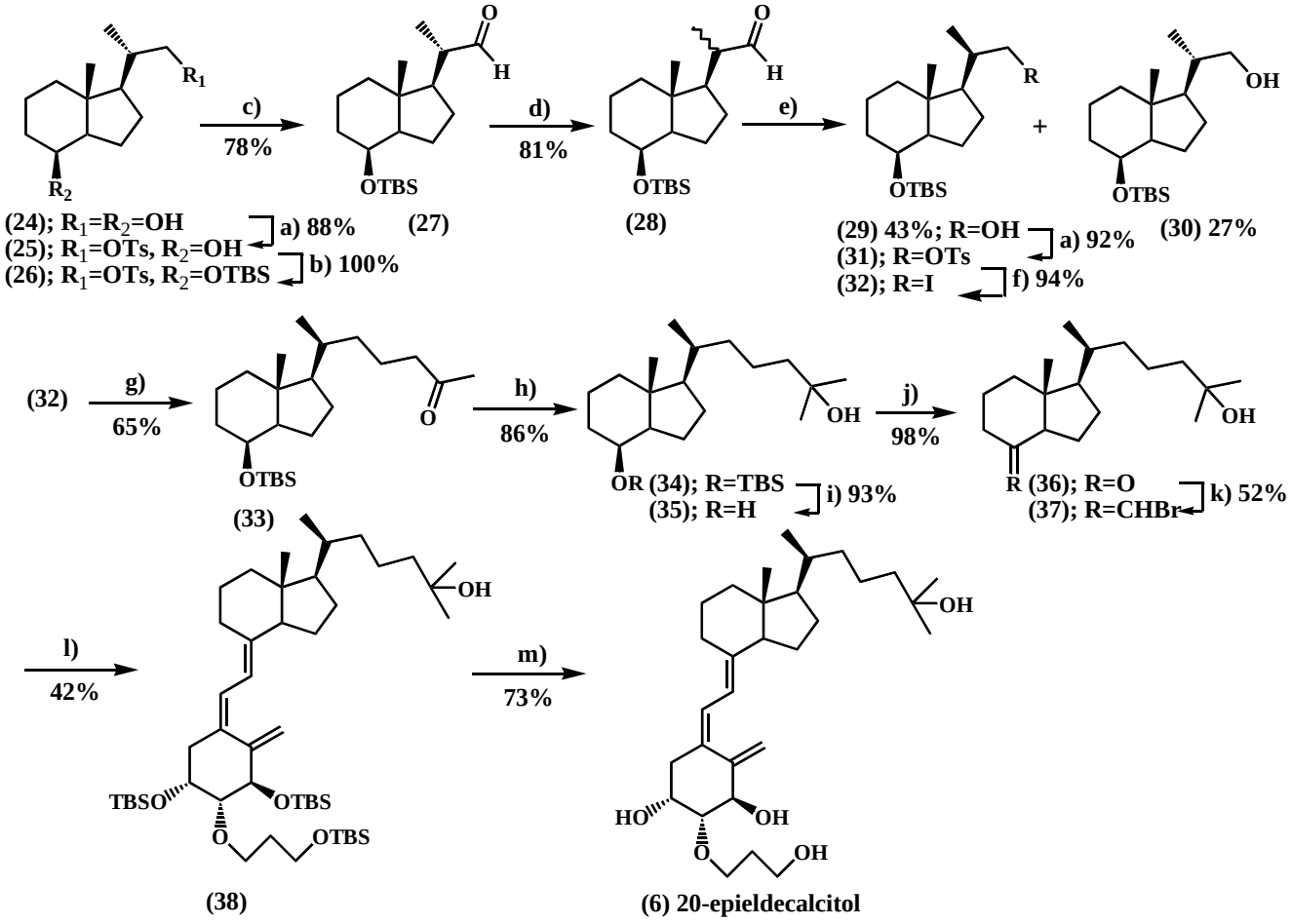
20-epicalcitriol shows 50 times the inhibition of proliferation in human histiocytic lymphoma cells (U937)[14] and a 4.5 fold increase in osteocalcin concentration in human osteosarcoma cells (MG-63)[15] compared to calcitriol (**3**). These findings prompted our interest in an analog of eldecalcitol (**5**) epimerized at the 20 position and its biological responses. We, therefore, synthesized 20-epieldecalcitol (**6**) and investigated preliminary biological activities using HL-60, U937, and MG-63 compared to **5**.



Scheme 1. Preparation of A-ring fragment for the synthesis of 20-epieldecalcitol. Reagents and conditions: 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) 1M 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.

The synthesis of 20-epieldecalcitol (**6**) was envisioned using the Trost coupling reaction of A-ring fragment **22** with C/D-ring fragment **37**.[16,17] First, the required A-ring fragment **22** was synthesized from C_2-symmetrical epoxide **15** based upon the methodology that has been previously established by us (Scheme 1).[18] Thus, cleavage of **15** with 1,3-propanediol in the presence of *t*-BuOK gave diol **16** in 86% yield. After protection of the primary hydroxy group to give pivalate **17** in 88% yield, cleavage of the benzyl ether moiety in **17** and subsequent protection of the resulting 1,2-diol as the acetonide gave alcohol **18** in 87% overall yield. Swern oxidation of **18** and subsequent Grignard reaction of the resulting aldehyde with vinylmagnesium bromide followed by pivaloylation of the alcohol afforded dipivalate **19** as an epimeric mixture (*R*/*S*=3/2). Without separation of the epimeric mixture, the acetonide moiety in **19** was cleaved quantitatively to give diol **20**. Exposure of **20** to Mitsunobu conditions afforded epoxide **21** in 77% yield. The acetylene unit was successfully installed by the

regioselective epoxide opening of **21** with lithium TMS acetylide in the presence of BF_3-OEt_2 to provide enyne **22** as the A-ring fragment in 36% yield after protecting group exchange from pivalate to TBS ether. The accompanied (*S*)-isomer **23**, which consists of the requisite stereochemistry to obtain 1-epieldecalcitol (**8**), was separated in 24% yield by simple column chromatography (Scheme 1). Next, we performed the synthesis of C/D-ring fragment **37** from the Inhoffen-Lythgoe diol (**24**), which is obtained by ozonolysis of vitamin D_2.[19] Based on the reported route to **37** from **24**,[20,21] we developed a convenient approach for the facile introduction of the C_{23} – C_{27} side chain moiety as shown in Scheme 2. Upon treatment of A-ring fragment **22** and C/D-ring fragment **37** with $Pd(PPh_3)_4$ and Et_3N, the coupled product **38** was obtained in 42% yield. Deprotection of the TBS group with 47% HF afforded 20-epieldecalcitol (**6**) in 73% yield (Scheme 2).[10,11]



Scheme 2. Preparation of C/D-ring fragment and the Trost coupling reaction with A-ring fragment to 20-epieldecalcitol. Reagents and conditions: 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) NaI, DMF, 85°C. g) MVK, Zn, CuI, EtOH, H_2O, 20-30°C. h) MeMgBr, THF, 0°C. i) 47% HF, MeCN, THF, 0°C. j) TPAP, NMO, CH_2Cl_2, rt. k) $Ph_3P^+CH_2Br/Br^-$, NaHMDS, rt. l) **22**, $Pd(PPh_3)_4$, Et_3N, toluene, reflux. m) 47% HF, MeCN, rt.

The results of preliminary *in vitro* biological evaluation of 20-epieldecalcitol (**6**) in comparison with eldecalcitol (**5**) and calcitriol (**3**) are summarized in Table 1. As anticipated, 20-epieldecalcitol (**6**) showed greatly enhanced activity toward the induction of HL-60 differentiation (6085.9/49.6=122.7 times), inhibition of U937 proliferation (738.74/4.15=178.0 times), and increase in osteocalcin concentration in MG-63 (2980/15=198.7 times), compared to eldecalcitol (**5**).[10,11] Based on these encouraging *in vitro* results, we are very interested in *in vivo* biological activity of 20-epieldecalcitol (**6**) on bone.

Table 1. Biological evaluation of 20-epieldecalcitol (**6**)

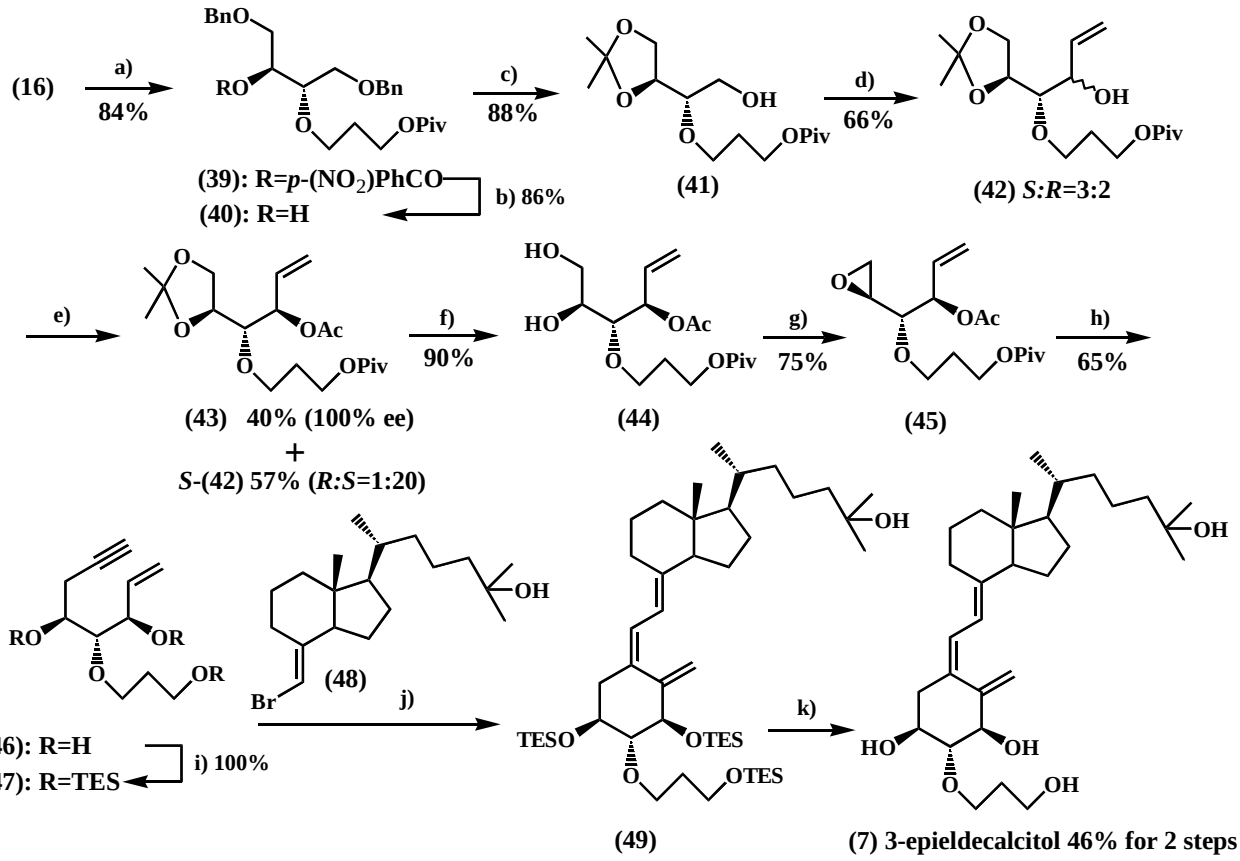
	HL-60	U937	MG-63
calcitriol (**3**)	100	100	100
eldecalcitol (**5**)	49.6	4.15	15
20-epieldecalcitol (**6**)	6085.9	738.74	2980

HL-60: Relative potency of induction of human myeloid leukemia cell differentiation.[13]
U937: Relative potency of inhibition of human histiocytic lymphoma cell proliferation.[14]
MG-63: Relative potency of transcriptional activity of osteocalcin of human osteosarcoma cells.[15]

2-2. 3-EPIELDECALCITOL (7)[22,23]

It is well-known that the synthesis and secretion of parathyroid hormone (PTH) is regulated by calcitriol (**3**).[24] Interestingly during the clinical development of eldecalcitol (**5**), serum intact PTH in osteoporotic patients did not change significantly upon treatment with **5**, although the reason remains unclear.[25] Brown group reported that epimerization of calcitriol (**3**) at the 3 position plays a major role in hormone activation and inactivation, especially in parathyroid cells.[26] It has been also reported that 3-epicalcitriol, an epimer of calcitriol (**3**) at the 3 position, shows equipotent and prolonged activity compared to **3** at suppressing PTH secretion.[27] Since eldecalcitol (**5**) has a bulky hydroxypropoxy substituent at the 2 position, epimerization of **5** at the adjacent and sterically hindered 3 position might be prevented. This could be the reason why eldecalcitol (**5**) showed weak potency in PTH suppression during clinical studies. Therefore, we have significant interest in eldecalcitol (**5**) epimerization at the 3 position and the biological potency of 3-epieldecalcitol (**7**) in suppressing PTH production.

The synthesis of 3-epieldecalcitol (**7**) was also accomplished using the Trost coupling methodology. As shown in Scheme 3, the preparation of the A-ring fragment **47** began with inversion of the C_3 configuration of alcohol **16** which was obtained from C_2-symmetrical epoxide **15** during the synthesis of 20-epieldecalcitol (**6**). Thus, reaction of **16** with *p*-$(NO_2)PhCO_2H$ in the presence of diethylazodicarboxylate (DEAD) and Ph_3P gave the *p*-nitrobenzoate **39** in 84% yield. After hydrolysis



Scheme 3. Synthesis of 3-epieldecalcitol. Reagents and conditions: a) *p*-$(NO_2)PhCO_2H$, DEAD, Ph_3P, toluene. b) $NaHCO_3$, MeOH. c) 1) H_2, $Pd(OH)_2$, MeOH, rt. 2) $Me_2C(OMe)_2$, TsOH, acetone. d) 1) DMSO, $(COCl)_2$, CH_2Cl_2 -78 °C. 2) CH_2=CHMgBr, THF -20 °C. e) Novozyme, CH_2=CHOAc, *t*-BuOMe. f) 60% AcOH. g) Ph_3P, DEAD, dioxane, reflux. h) 1) LiC≡CTMS, BF_3-OEt_2, THF, -78 °C. 2) 10N NaOH, MeOH. i) TESOTf, Et_3N, CH_2Cl_2 -40 °C. j) $Pd(PPh_3)_4$, Et_3N, toluene, reflux. k) NH_4F, MeOH, reflux.

of **39** (86%) and subsequent acetonide **41** formation (88%), Swern oxidation of **41** followed by Grignard reaction of the resulting aldehyde produced alcohol **42** as an epimeric mixture (*S*/*R*=3/2) in 66% yield. To separate this epimeric mixture, **42** was subjected to lipase-catalyzed acetylation using vinyl acetate and Novozyme. As a result, the *R*-epimer preferentially underwent acetylation to give acetate **43** and ***S*-42** (*R*/*S*=1/20) in 40% and 57% yields, respectively. Acetate **43** was converted to A-ring fragment **47** by a similar reaction sequence as in the preparation of the A-ring fragment **22** for 20-epieldecalcitol (**6**). The A-ring fragment **47** was allowed to react with C/D-ring fragment **48**, obtained from the Inhoffen-Lythgoe diol by known method, in the condition of the Trost coupling reaction to give the desired coupling product **49**. Deprotection of the TES groups afforded 3-epieldecalcitol (**7**) (Scheme 3).[22,23]

The results of preliminary *in vitro* biological evaluation of 3-epieldecalcitol (**7**) in comparison with eldecalcitol (**5**), calcitriol (**3**), and 3-epicalcitriol are summarized in Table 2. 3-Epieldecalcitol (**7**)

showed only slight inhibition of PTH secretion in cultured bovine parathyroid cells compared to eldecalcitol (**5**). In our assays, 3-epicalcitriol did not show greater activity than calcitriol (**3**) in suppressing PTH secretion. The inhibitory potency of analogs were calcitriol (**3**) > eldecalcitol (**5**) ≥ 3-epicalcitriol >> 3-epieldecalcitol (**7**), and well-responsible for affinity to human recombinant vitamin D receptor (VDR) as also shown in Table 2. Regarding the affinity to human vitamin D binding protein (DBP) which was previously known in the rat DBP case, eldecalcitol (**5**) showed more potent affinity than calcitriol (**3**). 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-episeries – 3-epicalcitriol: 8.3 and 3-epieldecalcitriol (**7**): 113.1 – as shown in Table 2.[22,23] Eldecalcitol (**5**) and 3-epieldecalcitol (**7**) appear to be inherently weak agents toward PTH suppression. This should be examined further with *in vivo* studies using renal insufficient animal models such as 5/6 nephrectomized rats showing high level of serum PTH.[28] Nevertheless, the less potent activity of eldecalcitol (**5**) toward PTH suppression compared to calcitriol (**3**) might be a beneficial characteristic of **5** for treating osteoporosis.

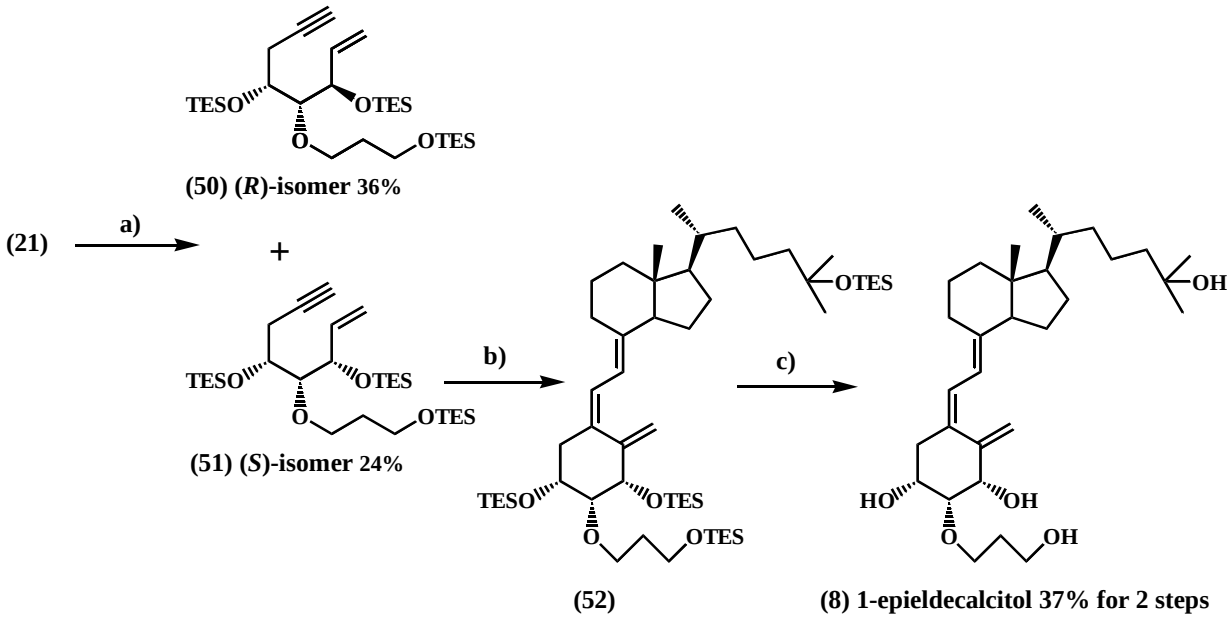
Table 2. Biological evaluation of 3-epieldecalcitol (**7**)

	VDR	DBP	PTH
calcitriol (**3**)	100	100	100
3-epicalcitriol	9.62	8.3	1.25
eldecalcitol (**5**)	44.6	421.9	3.54
3-epieldecalcitol (**7**)	0.02	113.1	0.11

VDR: Relative affinity for human vitamin D receptors.
DBP: Relative affinity for human vitamin D binding protein.
PTH: Relative inhibition of parathyroid hormone secretion in cultured bovine parathyroid cells.

2-3. 1-EPIELDECALCITOL (8)[29,30]

Although the detailed mode of action of enhanced activity of eldecalcitol (**5**) beyond calcitriol (**3**) and alfacalcidol (**4**) toward bone remains to be clarified, the long duration of **5** in the blood stream arises from its strong affinity for DBP (2-fold~4-fold in comparison with **3**) and might explain, in part, the enhanced biological effects of **5**. We, therefore, were highly interested in an analog with strong affinity for DBP. It was reported that the epimerization of calcitriol (**3**) at the 1 position remarkably enhances the affinity for DBP. Norman and co-workers reported that 1-epicalcitriol shows a 65.7-fold increase in affinity for DBP as comparered to **3**.[31] These findings stimulated our interest in the biological profile of epimeraized eldecalcitol at the 1 position namely, 1-epieldecalcitol (**8**), including DBP affinity and its effects on bone.



Scheme 4. Synthesis of 1-epieldecalcitol. Reagents and conditions: a) 1) LiC≡CTMS, BF_3-OEt_2, THF, -78 °C. 2) 10M NaOH, MeOH, rt. 3) TESOTf, Et_3N, -40 °C. b) **48** (OH=OTES), $Pd(Ph_3P)_4$, Et_3N, toluene, reflux. c) 47% HF, MeCN, rt.

As previously mentioned, in our preparation of A-ring fragment **22** for convergent route to 20-epieldecalcitol (**6**), epimeric epoxide **21** produced the (*R*)-isomer **22** as separable diastereomeric mixture along with (*S*)-isomer **23** in a 3:2 ration (Scheme 1). The A-ring fragment **51**, obtained from **21**, possesses the requisite stereochemistry for 1-epieldecalcitol (**8**) (Scheme 4). Thus, the Trost coupling reaction of **51** with excess bromomethylene **48** gave triene **52** which was desilylated to 1-epieldecalcitol (**8**) in 37% yield from **51** (Scheme 4).[30]

As anticipated, 1-epieldecalcitol (**8**) showed enhanced affinity for DBP (1.6-fold in comparison with eldecalcitol) (Table 3). Further *in vivo* biological evaluations of **8** using ovariectomized rats model for osteoporosis in comparison with eldecalcitol (**5**) would be highly interesting.

Table 3. Biological evaluation of 1-epieldecalcitol (**8**)

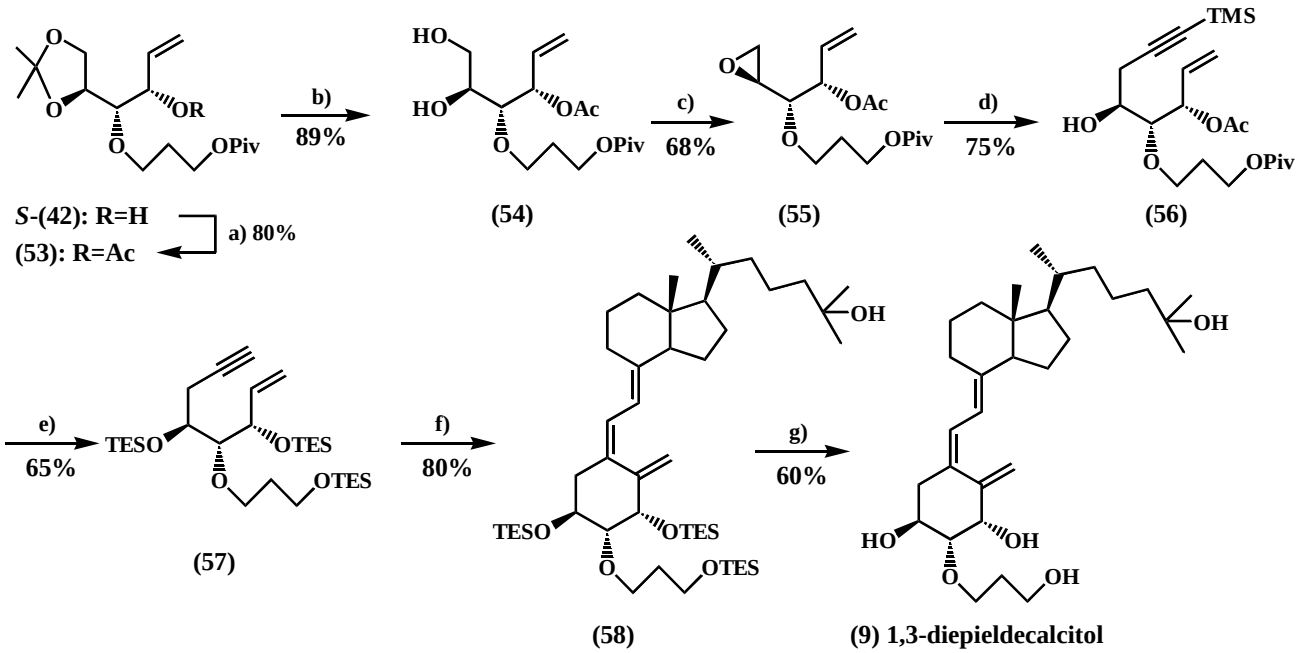
	VDR	DBP
calcitriol (**3**)	100	100
eldecalcitol (**5**)	70	410
1-epieldecalcitol (**8**)	0.3	670

VDR: Relative affinity for bovine thymus vitamin D receptors.
DBP: Relative affinity for rat vitamin D binding protein.

2-4. 1,3-DIEPIELDECALCITOL (9)[32]

With completion of the synthesis of 3-epieldecalcitol (**7**) and 1-epieldecalcitol (**8**) and to further explore structure-activity relationships between eldecalcitol (**5**) and related analogs, we focused significant attention on the epimer of **5**, at both 1 and 3 positions of the A-ring, namely 1,3-diepieldecalcitol (**9**). The synthesis of the A-ring fragment **57** of 1,3-diepieldecalcitol (**9**) started from the alcohol ***S*-42** which was obtained from the previous lipase-catalyzed acetylation of **41** as the unreacted (*S*)-isomer. The alcohol ***S*-42** possesses the requisite stereochemistry at positions 1, 2 and 3 of the A-ring that comprises **9**. Acetylation of ***S*-42** gave acetate **53** in 80% yield which was converted to A-ring fragment **57** by a similar reaction sequence to the preparation of the A-ring fragment **47** for 3-epieldecalcitol (**7**). The A-ring fragment **57** was allowed to react with C/D-ring fragment **48** under the Trost coupling conditions to give the coupled product **58**, which was desilylated to 1,3-diepieldecalcitol (**9**) (Scheme 5).

Although 1,3-diepieldecalcitol (**9**), in combination with others, is anticipated to enhance our understanding of the mode-of-action of medicinally important eldecalcitol (**5**), the detailed biological characterization of **9** in comparison with eldecalcitol (**5**), 3-epieldecalcitol (**7**) and 1-epieldecalcitol (**8**) remains to be clarified.[32]

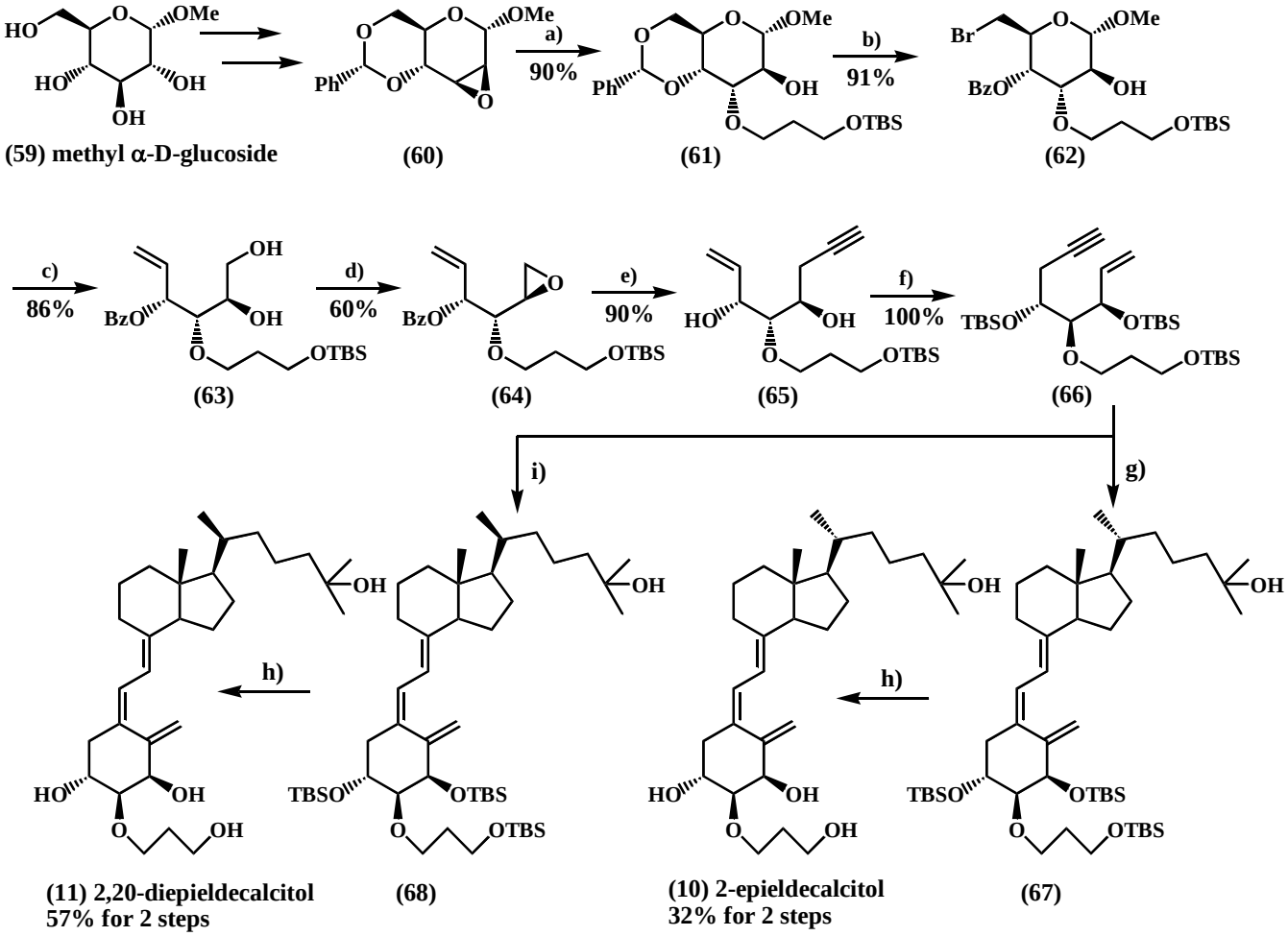


Scheme 5. Synthesis of 1,3-diepieldecalcitol. Reagents and conditions: a) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt. b) 60% AcOH, rt. c) Ph_3P, DEAD, dioxane, reflux. d) LiC≡CTMS, BF_3-OEt_2, THF, -78 °C. e) 1) 10M NaOH, MeOH, rt. 2) TESOTf, Et_3N, CH_2Cl_2 -40 °C. f) **48** (OH=OTES), $Pd(PPh_3)_4$, Et_3N, toluene, reflux. g) 46% HF, MeCN, rt.

2-5. 2-EPIELDECALCITOL (10) AND 2,20-DIEPIELDECALCITOL (11)[13]

2-Epieldecalcitol (**10**) and 2,20-diepieldecalcitol (**11**) were synthesized by Kittaka group during their

own modification studies on A-ring part of calcitriol (**3**) to obtain analogs with high VDR affinity.[13] Methyl α-D-glucoside (**59**) was converted to the known epoxide **60**.[33] Treatment of **60** with 1,3-propanediol in the presence of *t*-BuOK at 110 °C followed by *O*-silylation afforded protected methyl 3-*O*-(3-hydroxypropoxy)altropyranoside (**61**) in 90% yield, in which the chiralities of C_2, C_3, and C_4 satisfy the 3β, 2α, and 1α stereochemistry of the targeted molecules, **10** and **11**. NBS treatment of benzylidene acetate **61** produced bromide **62** in 91% yield. Reaction of **62** with activated zinc powder and $NaBH_3CN$ provided diol **63** in 86% yield. Diol **63** was converted to epoxide **64** through sulfonylation of the primary hydroxy moiety followed by LiHDMS treatment in 60% yield.



Scheme 6. Synthesis of 2-epieldecalcitol and 2,20-diepieldecalcitol. Reagents and conditions: a) 1) $HO(CH_2)_3OH$, *t*-BuOK, 110 °C. 2) TBSCl, Et_3N, DMAP, CH_2Cl_2. b) NBS, $BaCO_3$, CCl_4, reflux. c) Zn powder, $NaBH_3CN$, 1-propanol/H_2O (9/1), 95 °C. d) 1) 2,4,6-trimethylbenzenesulfonyl chloride, pyridine. 2) LiHMDS, THF, -78 °C to 0 °C. e) 1) LiC≡CTMS, BF_3-OEt_2, THF, -78 °C to rt. 2) K_2CO_3, MeOH. f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C. g) **48**, $Pd(Ph_3P)_4$, Et_3N/toluene (1/1), reflux. h) TBAF, THF. i) **37**, $Pd(Ph_3P)_4$, Et_3N/toluene (1/1), reflux.

Ethnylation of **64** using lithium TMS acetylide in the presence of BF_3-OEt_2 in THF and subsequent solvolysis in K_2CO_3/MeOH supplied enyne **65** in 90% yield. Persilylation with TBSOTf afforded the desired product enyne **66**, quantitatively. The seco-steroidal structure was constructed using the Trost coupling cyclization strategy with C/D-ring fragment **48** or 20-epiC/D-ring fragment **37**. Subsequent deprotection of the resulting products, **67** and **68**, furnished 2-epieldecalcitol (**10**) and 2,20-diepieldecalcitol (**11**) in 32% and 57% yields, respectively (Scheme 6).

The VDR binding affinities and potencies of induction of HL-60 differentiation of the synthesized analogs, **10** and **11**, are summarized in Table 4 in comparison with those of calcitriol (**3**). In inducing HL-60 differentiation, 2-epieldecalcitol (**10**) exhibited a rather lower effect while 2,20-diepieldecalcitol (**11**) showed quite a high potency, compared to calcitriol (**3**).[13]

Table 4. Biological evaluation of 2-epieldecalcitol (**10**) and 2,20-diepieldecalcitol (**11**)

	VDR	HL-60
calcitriol (**3**)	100	100
2-epieldecalcitol (**10**)	180	70
2,20-diepieldecalcitol (**11**)	165	2120

VDR: Relative affinity for bovine thymus vitamin D receptors.
HL-60: Relative potency of induction of human myeloid leukemia cell differentiation.

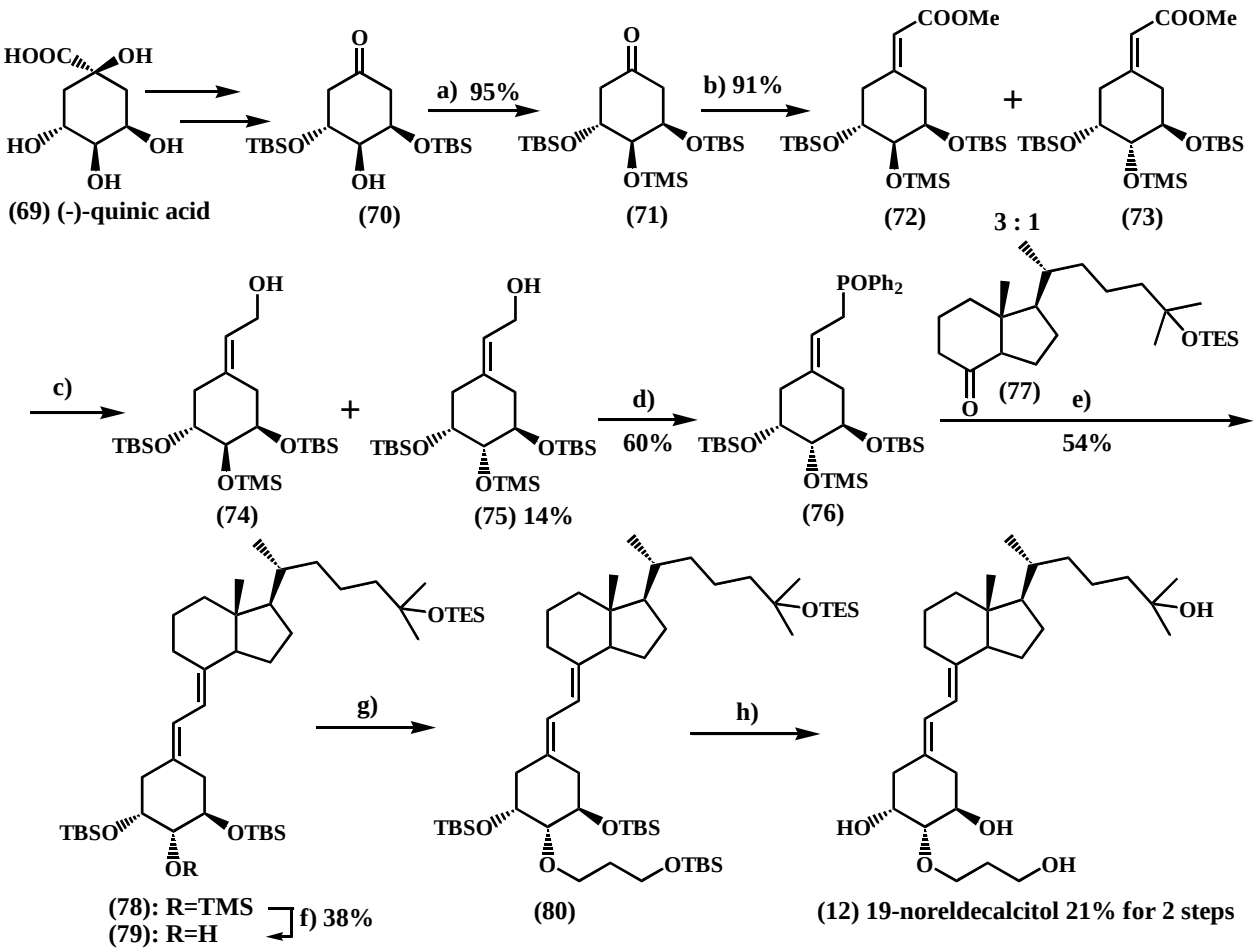
3. NOR ANALOG AT 19 POSITION AND DEOXY ANALOGS AT 1 AND 25 POSITIONS

3-1. 19-NORELDECALCITOL (12)[34]

19-Noreldecalcitol (**12**) was synthesized by DeLuca group as an analog of 19-norseries of calcitriol (**3**) in the hope of obtaining a selective activity profile that exhibits high potency in inducing differentiation of malignant cells with very low or no bone calcification activity. DeLuca group considered that 19-noreldecalcitol (**12**) might retain potent bone formation activity without hypercalcemia resulting from bone calcium mobilization.[34]

The cyclohexanone derivative **70** was prepared from commercially available (-)-quinic acid (**69**).[35,36] The 4-hydroxy group of **70** was protected as TMS ether in 95% yield. Peterson reaction of **71** with $TMSCH_2CO_2Me$ in the presence of LDA gave a 3:1 mixture of the two isomeric cyclohexylidene esters **72** and **73** in 91% yield. The formation of the isomeric mixture was the result of the newly created axial chirality of the methyl 2-(4-hydroxycyclohexylidene)ethanoate system. Isomeric esters **72** and **73** were reduced to the allylic alcohols **74** and **75** which were easily separated by preparative HPLC. The separated alcohol **75** was transformed to the A-ring phosphine oxide **76** by *in situ* tosylation and

conversion into corresponding phosphine followed by oxidation in ca. 60% overall yield. Wittig-Horner coupling of **76** with the protected Windaus-Grundmann ketone (**77**) gave 19-nor type compound **78** in 54% yield. The TMS protecting group in **78** was selectively hydrolyzed under carefully controlled condition to alcohol **79** in 38% yield, which was converted to ether **80** by alkylation with $Br(CH_2)_3OTBS$. Finally deprotection of **80** with TBAF gave 19-noreldecalcitol (**12**) in 21% yield from **79** (Scheme 7).



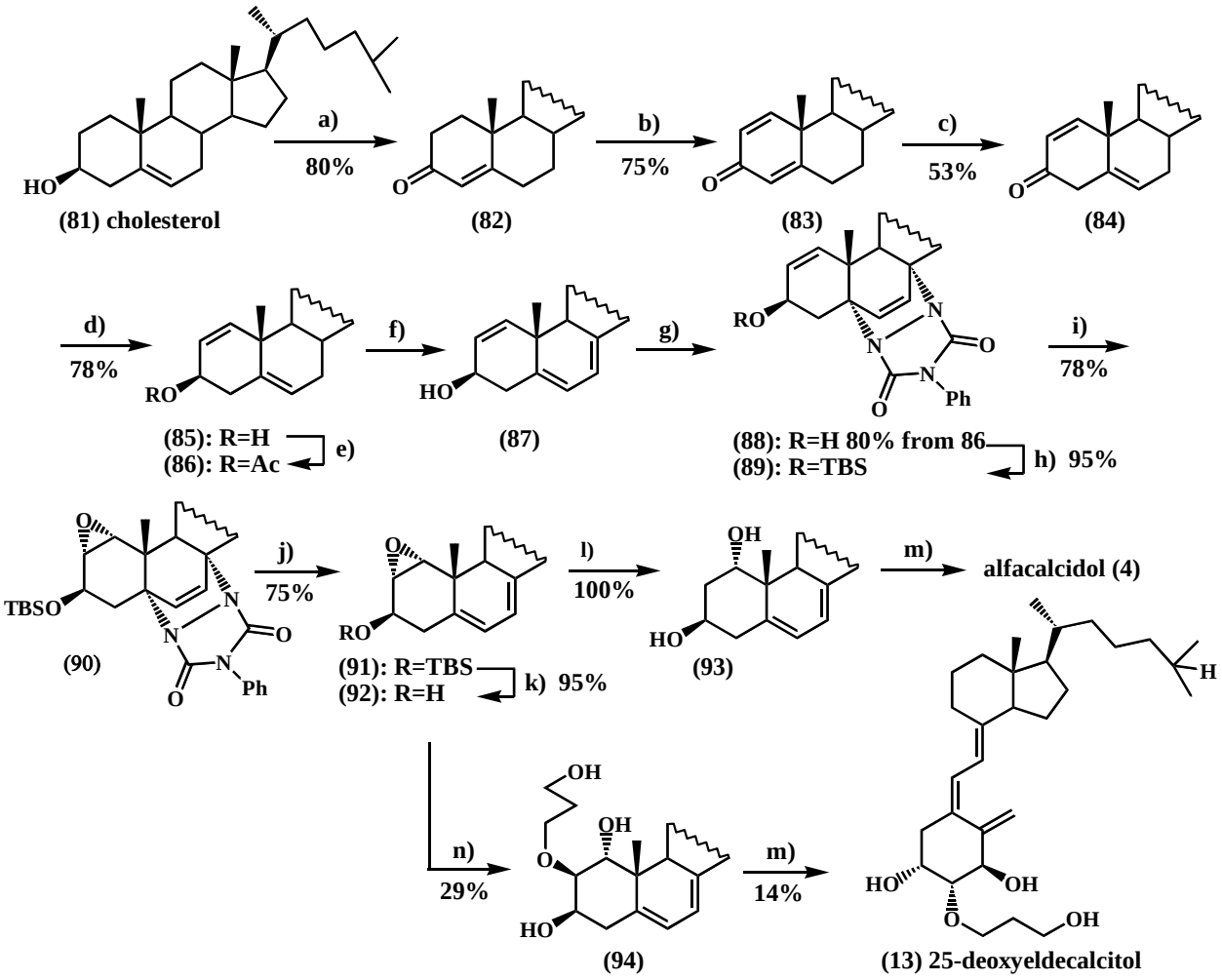
Scheme 7. Synthesis of 19-noreldecalcitol. Reagents and conditions: a) TMSCl, imidazole. b) $TMSCH_2CO_2Me$, LDA, -78 °C. c) DIBAH, toluene, -78 °C. d) 1) TsCl, *n*-BuLi, THF, 0 °C. 2) Ph_2PH, *n*-BuLi, THF, 0 °C. 3) 10% H_2O_2, CH_2Cl_2, 0 °C. e) *n*-BuLi, THF, 0 °C. f) AcOH, THF, H_2O, rt. g) $Br(CH_2)_3OTBS$, NaH, DMF, 18-Crown-6, rt. h) TBAF, THF.

DeLuca group mentioned that 19-noreldecalcitol (**12**) possessed intestinal calcium transport activity but much less than that of calcitriol (**3**) and **12** showed also bone calcium mobilizing activity.[34]

3-2. 25-DEOXYELDECALCITOL (13)[4]

25-Deoxyeldecalcitol (**13**) was synthesized during our exploratory research for eldecalcitol (**5**).[4] As

previously described, alfacalcidol (**4**) was launched as a prodrug of calcitriol (**3**) in Japan by Chugai and Teijin in 1981. Scheme 8 depicts the synthetic route to alfacalcidol (**4**) from inexpensive cholesterol (**81**) as a starting material, in which α-epoxide **92** served as a key intermediate for the introduction of biologically important 1α hydroxy moiety of **4** by hydride reduction of **92**.[37] The start of an industrial scale production of alfacalcidol (**4**) provided us an abundant amount of α-epoxide **92**. Treatment of **92** with 1,3-propanediol in the presence of *t*-BuOK resulted in stereo and regioselective introduction of a hydroxypropoxy group into 2β position to give **94**, which was then converted to 25-deoxyeldecalcitol (**13**) by irradiation using a high pressure mercury lamp followed by thermal isomerization in 14% yield (Scheme 8).[4]



Scheme 8. Industrial synthesis of alfacalcidol and synthesis of 25-deoxyeldecalcitol. Reagents and conditions: a) Al(*i*-PrO)$_3$, cyclohexanone. b) DDQ, AcOEt. c) NaOEt, EtOH. d) $NaBH_4$, MeOH, THF. e) Ac_2O, DMPA, pyridine, rt. f) 1) NBS, AIBN, *n*-hexane, reflux. 2) γ-collidine, toluene, reflux. 3) KOH, MeOH, rt. g) 4-phenyl-1,2,4-triazoline-3,5-dione, CH_2Cl_2. h) TBSCl, imidazole. i) MCPBA, CH_2Cl_2. j) 1,3-dimethyl-2-imidazolidinone, 140 °C. k) TBAF, THF. l) $NaBH_4$, EtOH. m) 1) 400W high pressure mercury lamp, THF, 0 °C. 2) THF, reflux. n) $HO(CH_2)_3OH$, *t*-BuOK, 110 °C.

Table 5 compares the plasma calcium levels in rats (Sprague-Dawley rats) on a low calcium (0.003%) and vitamin D deficient diet after oral administration of calcitriol (**3**), alfacalcidol (**4**), eldecalcitol (**5**), and 25-deoxyeldecalcitol (**13**) (6.25μg/kg/day for 5 days, respectively). 25-Deoxyeldecalcitol (**13**) significantly increased plasma calcium levels, which reached an almost normal range.[4] Although the structural relationship between 25-deoxyeldecalcitol (**13**) and eldecalcitol (**5**) corresponds to that between alfacalcidol (**4**) and calcitriol (**3**), the possible hydroxylation of **13** at 25 position in liver or bone to produce **5**, such as metabolic conversion from **4** to **3**, has not been investigated until now.

Table 5. Biological evaluation of 25-deoxyeldecalcitol (**13**)

	Plasma calcium levels (mg/dl)
control	4.79 ± 0.23
calcitriol (**3**)	5.25 ± 0.21[b)]
alfacalcidol (**4**)	5.31 ± 0.37[a)]
eldecalcitol (**5**)	9.60 ± 0.54[c)]
25-deoxyeldecalcitol (**13**)	8.22 ± 0.87[c)]

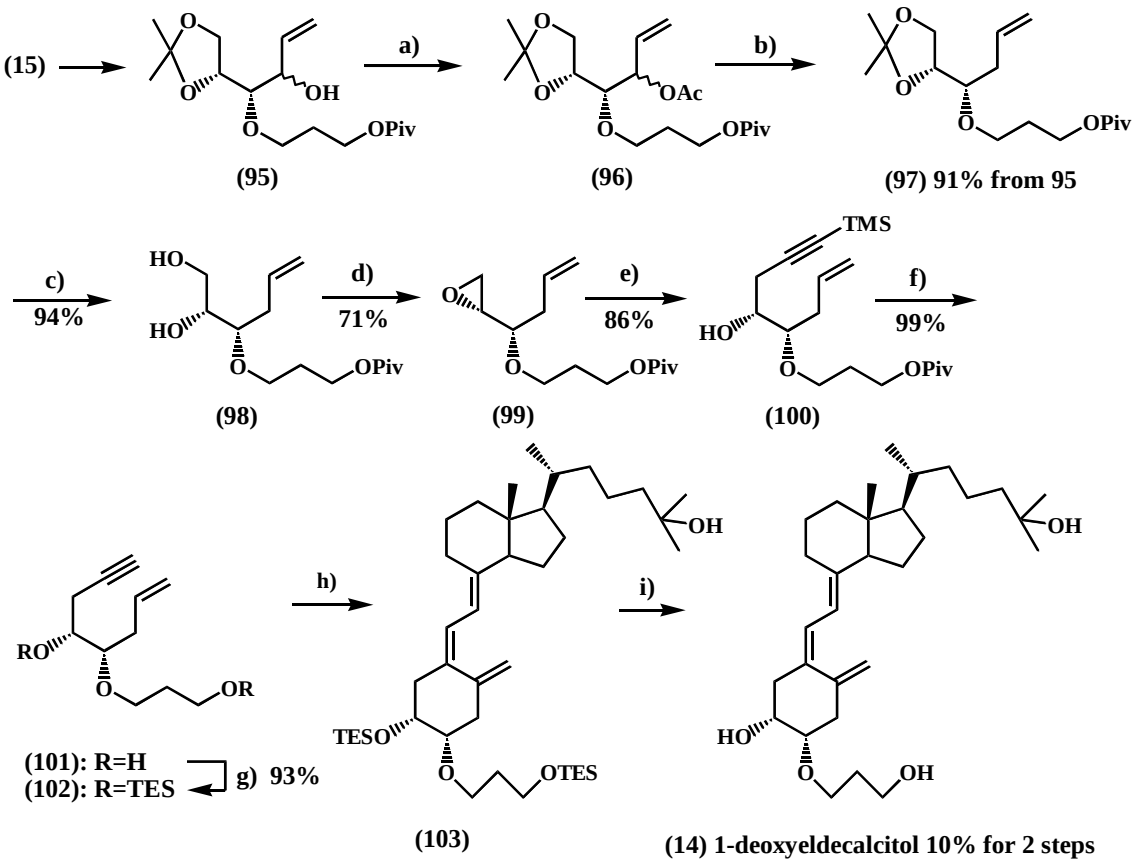
Comparison of plasma calcium levels in rats. a) $p<0.05$, b) $p<0.01$, c) $p<0.001$ compared to control.

3-3. 1-DEOXYELDECALCITOL (14)[38]

Considering the metabolic pathway of cholecalciferol (**1**) and biological effects of **1**, an idea was recently presented that calcitriol (**3**) is responsible for calcemic activity whereas the strong binding of calcifediol (**2**) to DBP and therefore long existence of **2** in blood are responsible for an anabolic effect on bone resulting in BMD increase.[39] Since the structural relationship between **3** and **2** corresponds to that between eldecalcitol (**5**) and 1-deoxyeldecalcitol (**14**), we have been very interested in the biological action of **14**, e.g. possible hydroxylation of **14** to **5** in the kidney, affinity for DBP, duration in blood stream, and anabolic effect on bone.

The requisite A-ring fragment **102** for the synthesis of 1-deoxyeldecalcitol (**14**) corresponds to the deoxygenated enyne of (*R*)-isomer **22** and (*S*)-isomer **23** in Scheme 1. The epimeric alcohol **95**, prepared from C_2-symmetrical epoxide **15** as shown in Scheme 1, was acetylated to acetate **96**, which was converted to diol **98** after several steps. Mitsunobu reaction of **98** afforded epoxide **99** in 71% yield. Reaction of **99** with lithium TMS acetylide gave the enyne **100** in 86% yield, which was converted to the A-ring fragment **102** by saponification and subsequent protection of the hydroxy groups in **101** as their TES ether. Based on the Trost coupling methodology involving A-ring fragment **102**

and C/D-ring fragment **48**, 1-deoxyeldecalcitol (**14**) was obtained in 10% yield after desilylation of the resulting coupled product **103** (Scheme 9). The detailed biological action of recently synthesized **14** in comparison with eldecalcitol (**5**) will be investigated and reported in due course.



Scheme 9. Synthesis of 1-deoxyeldecalcitol. Reagents and conditions: a) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt. b) $Pd(PPh_3)_4$, HCO_2NH_4, 1,4-dioxane, THF, 80 °C. c) 60% aq. AcOH, rt. d) Ph_3P, DEAD, 1,4-dioxane, 130 °C. e) LiC≡CTMS, BF_3-OEt_2, THF, -78 °C to -40 °C. f) 3M NaOH, MeOH, rt. g) TESOTf, 2,6-lutidine, CH_2Cl_2 -40 °C. h) **48**, $Pd(PPh_3)_4$, Et_3N, toluene, reflux. i) 47% HF, MeCN, rt.

4. CONCLUSION

There are still many challenges ahead in attempting to gain a full understanding of the mode-of-action of eldecalcitol (**5**) with the objective of developing an even more effective and sophisticated pharmaceutical product. This demands the need for new improvements to achieve a more effective and safer vitamin D_3 analog for osteoporosis based on the assessment of its limitations. Nevertheless, it is expected that eldecalcitol (**5**), a promising new medicine, will contribute to the treatment of patients with osteoporosis.[40]

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6. REFERENCES

1. R. L. Horst, T. A. Reinhardt, and G. S. Reddy, 'Vitamin D Metabolism' Vitamin D Second Edition, ed. by D. Feldman, J. W. Pike, and F. H. Glorieux, Elsevier Academic Press, Burlington, 2005, pp. 15-36.
2. R. Bouillon, W. H. Okamura, and A. Norman, *Endocr. Rev.*, 1995, **16**, 200.
3. R. Eastell and B. L. Riggs, 'Vitamin D and Osteoporosis' Vitamin D Second Edition, ed. by D. Feldman, J. W. Pike, and F. H. Glorieux, Elsevier Academic Press, Burlington, 2005, pp. 1101-1120.
4. K. Miyamoto, E. Murayama, K. Ochi, H. Watanabe, and N. Kubodera, *Chem. Pharm. Bull.*, 1993, **41**, 1111.
5. Y. Ono, H. Watanabe, A. Shiraishi, S. Takeda, Y. Higuchi, K. Sato, N. Tsugawa, T. Okano, T. Kobayashi, and N. Kubodera, *Chem. Pharm. Bull.*, 1997, **45**, 1626.
6. Y. Ono, A. Kawase, H. Watanabe, A. Shiraishi, S. Takeda, Y. Higuchi, K. Sato, T. Yamauchi, T. Mikami, M. Kato, N. Tsugawa, T. Okano, and N. Kubodera, *Bioorg. Med. Chem.*, 1998, **6**, 2517.
7. T. Matsumoto, M. Ito, Y. Hayashi, T. Hirota, Y. Tanigawara, T. Sone, M. Fukunaga, M. Shiraki, and T. Nakamura, *Bone*, 2011, **49**, 605.
8. T. Matsumoto, T. Takano, S. Yamakido, F. Takahashi, and N. Tsuji, *J. Steroids Biochem. Mol. Biol.*, 2010, **121**, 261.
9. N. Kubodera, *Mini-Reviews Med. Chem.*, 2009, **9**, 1416.
10. S. Hatakeyama, M. Yoshino, K. Eto, K. Takahashi, J. Ishihara, Y. Ono, H. Saito, and N. Kubodera, *J. Steroids Biochem. Mol. Biol.*, 2010, **121**, 25.
11. M. Yoshida, K. Eto, K. Takahashi, J. Ishihara, S. Hatakeyama, Y. Ono, H. Saito, and N. Kubodera, *Heterocycles*, 2010, **81**, 381.
12. L. Binderup, E. Binderup, W. O. Godtfredsen, and A.-M. Kissmeyer, 'Development of New Vitamin D Analogs' Vitamin D Second Edition, ed. by D. Feldman, J. W. Pike, and F. H. Glorieux, Elsevier Academic Press, Burlington, 2005, pp. 1489-1510.
13. N. Saito, Y. Suhara, M. Kurihara, T. Fujishima, S. Honzawa, H. Takayanagi, T. Kozono, M.

Matsumoto, M. Ohmori, N. Miyata, H. Takayama, and A. Kittaka, *J. Org. Chem.*, 2004, **69**, 7463.

14. L. Binderup, S. Latini, E. Binderup, C. Bretting, M. Calverley, and K. Hansen, *Biochem. Pharmacol.*, 1991, **42**, 1569.
15. S. Ryhanen, A. Mahonen, T. Jaaskelainen, and P. H. Maenpaa, *Eur. J. Biochem.*, 1996, **238**, 97.
16. B. M. Trost and J. Dumas, *J. Am. Chem. Soc.*, 1992, **114**, 1924.
17. B. M. Trost, J. Dumas, and M. Villa, *J. Am. Chem. Soc.*, 1992, **114**, 9836.
18. J. Maeyama, H. Hiyamizu, K. Takahashi, J. Ishihara, S. Hatakeyama, and N. Kubodera, *Heterocycles*, 2006, **70**, 295.
19. H. H. Inhoffen, G. Quinkert, S. Schutz, G. Friedrich, and E. Tober, *Chem. Ber.*, 1958, **91**, 781.
20. T. Fujishima, K. Konno, K. Nakagawa, M. Kurobe, T. Okano, and H. Takayama, *Bioorg. Med. Chem.*, 2000, **8**, 123.
21. G. H. Posner, Z. Li, M. C. White, V. Vinader, K. Takeuchi, S. E. Guggino, P. Dolan, and T. W. Kensler, *J. Med. Chem.*, 1995, **38**, 4529.
22. S. Hatakeyama, S. Nagashima, N. Imai, K. Takahashi, J. Ishihara, A. Sugita, T. Nihei, H. Saito, F. Takahashi, and N. Kubodera, *J. Steroid Biochem. Mol. Biol.*, 2007, **103**, 222.
23. S. Hatakeyama, S. Nagashima, N. Imai, K. Takahashi, J. Ishihara, A. Sugita, T. Nihei, H. Saito, F. Takahashi, and N. Kubodera, *Bioorg. Med. Chem.*, 2006, **14**, 8050.
24. J. Silver and T. Naveh-Many, 'Vitamin D and the Parathyroids' Vitamin D Second Edition, ed. by D. Feldman, J. W. Pike, and F. H. Glorieux, Elsevier Academic Press, Burlington, 2005, pp. 537-549.
25. T. Matsumoto, T. Miki, H. Hagino, T. Sugimoto, S. Okamoto, T. Hirota, Y. Tanigawara, Y. Hayashi, M. Fukunaga, M. Shiraki, and T. Nakamura, *J. Clin.Endocrinol. Metab.*, 2005, **90**, 5031.
26. A. J. Brown, C. Ritter, A. S. Weiskopf, P. Vouros, G. J. Sasso, M. R. Uskokovic, G. Wang, and G. S. Reddy, *J. Cell. Biochem.*, 2005, **96**, 569.
27. A. J. Brown, C. Ritter, E. Slatopolsky, K. R. Muralidharan, W. H. Okamura, and G. S. Reddy, *J. Cell. Biochem.*, 1999, **73**, 106.
28. M. Hirata, K. Endo, K. Katsumura, F. Ichikawa, N. Kubodera, and M. Fukagawa, *Nephrol. Dial. Transplant.*, 2002, **17 (Suppl. 10)**, 41.
29. Y. Ono, H. Watanabe, A. Kawase, N. Kubodera, T. Okano, N. Tsugawa, and T. Kobayashi, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1523.
30. K. Eto, A. Fujiyama, M. Kaneko, K. Takahashi, J. Ishihara, S. Hatakeyama, Y. Ono, and N. Kubodera, *Heterocycles*, 2009, **77**, 323.
31. A. W. Norman, R. Bouillon, M. C. Farach-Carson, J. E. Bishop, L.-X. Zhou, I. Nemere, J. Zhao, K.

R. Muralidharan, and W. H. Okamura, *J. Biol. Chem*., 1993, **268**, 20022.

32. A. Fujiyama, M. Kaneko, K. Takahashi, J. Ishihara, S. Hatakeyama, and N. Kubodera, *Heterocycles*, 2007, **71**, 2263.
33. L. F. Wiggins, *Methods Carbohydr. Chem*., 1963, **2**, 188.
34. R. R. Sicinski, K. L. Perlman, and H. F. DeLuca, *J. Med. Chem*., 1994, **37**, 3730.
35. K. L. Perlman, R. E. Swenson, H. E. Paaren, H. K. Schnoes, and H. F. DeLuca, *Tetrahedron Lett*., 1991, **32**, 7663.
36. D. Desmaele and S. Tanier, *Tetrahedron Lett*., 1985, **26**, 4941.
37. N. Kubodera, *Molecules*, 2009, **14**, 3869.
38. H. Sasaki, K. Eto, K. Takahashi, J. Ishihara, S. Hatakeyama, and N. Kubodera, *Heterocycles*, 2011, **83**, 1385.
39. A. G. Need and B. E. C. Nordin, *Bone*, 2008, **42**, 1021.
40. N. Kubodera and F. Takahashi, 'Analogs for the Treatment of Osteoporosis' Vitamin D Third Edition, ed. by D. Feldman, J. W. Pike, and J. S. Adams, Elsevier Academic Press, Burlington, 2011, pp. 1489-1496.

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