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SYNTHESIS OF 1α,25-DIHYDROXY-2β-(3-HYDROXYPROPOXY)-VITAMIN D_3 (ELDECALCITOL) AND RELATED COMPOUNDS BY THE TROST CONVERGENT METHODOLOGY*

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Abstract – Using Trost convergent methodology, the synthesis of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (eldecalcitol) for the improved and industrial scale production and related putative metabolites of eldecalcitol is summarized. In addition, A-ring diastereomers at the 1- and 3-positions of eldecalcitol are described. The synthesis centers on a key palladium-catalyzed alkylative cyclization and coupling of ene-ynes that constitute the A-ring fragment to bromomethylenes comprising the C/D-ring fragment which affords the requisite triene framework of vitamin D_3 analogs.

INTRODUCTION

Active vitamin D_3, 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**), and its synthetic prodrug, 1α-hydroxyvitamin D_3 (alfacalcidol, **2**), are well recognized as potent regulators of cell proliferation and differentiation in addition to possessing regulatory effects on calcium and phosphorus metabolism.[1] Various analogs of calcitriol (**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, secondary hyperparathyroidism, cancer, etc., without risk of hypercalcemia.[2] There is also intense interest in obtaining analogs more potent than calcitriol (**1**) and alfacalcidol (**2**) in regulating calcium and phosphorus metabolism with the objective of treating bone disease such as

**Dedicated to the memory of the late Dr. John W. Daly, Scientist Emeritus, National Institute of Health.*

osteoporosis. 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (eldecalcitol, **3**, developing code: ED-71), which possesses a hydroxypropoxy substituent at the 2β-position of the A-ring of calcitriol (**1**), is such an analog that shows more potent effects on bone therapy.[3,4,5,6,7,8] Phase III clinical studies of eldecalcitol (**3**) as a promising candidate for the treatment of osteoporosis and bone fracture prevention are now being successfully conducted in Japan and will be completed by the end of 2008 after 3 years of medication of osteoporotic patients (Figure 1).[9]

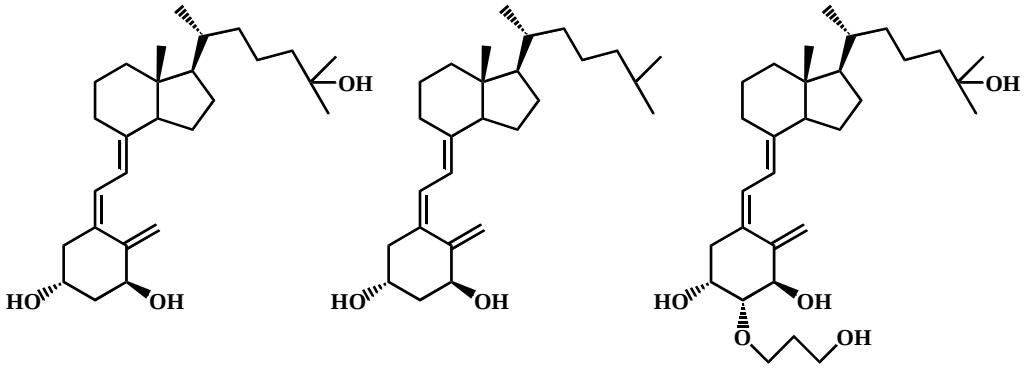
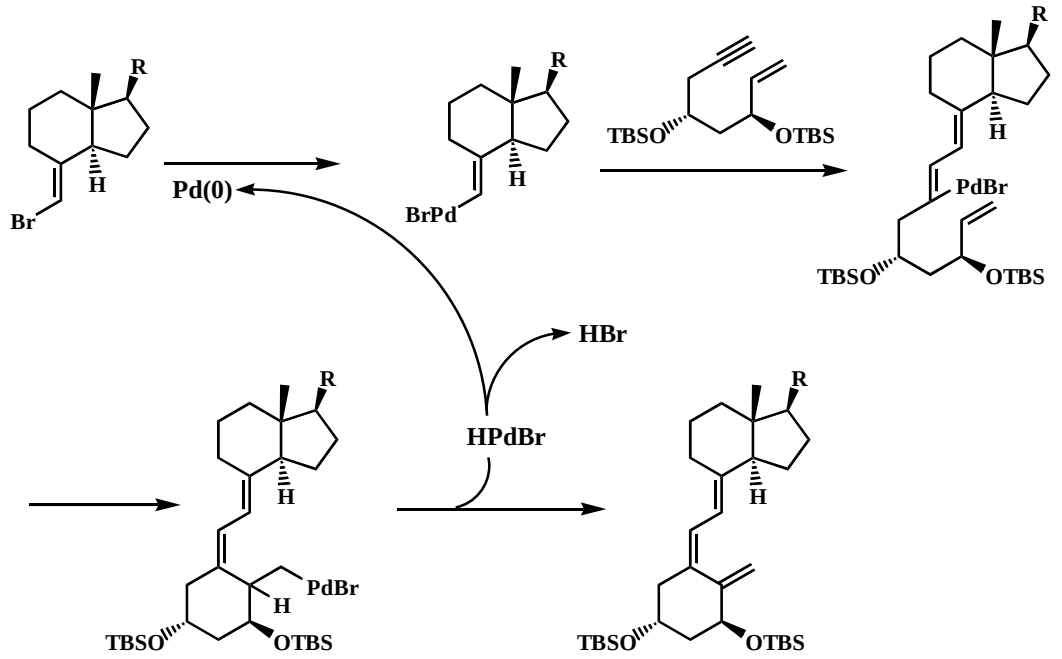


Figure 1. Structure of calcitriol (1), alfacalcidol (2), and eldecalcitol (3)

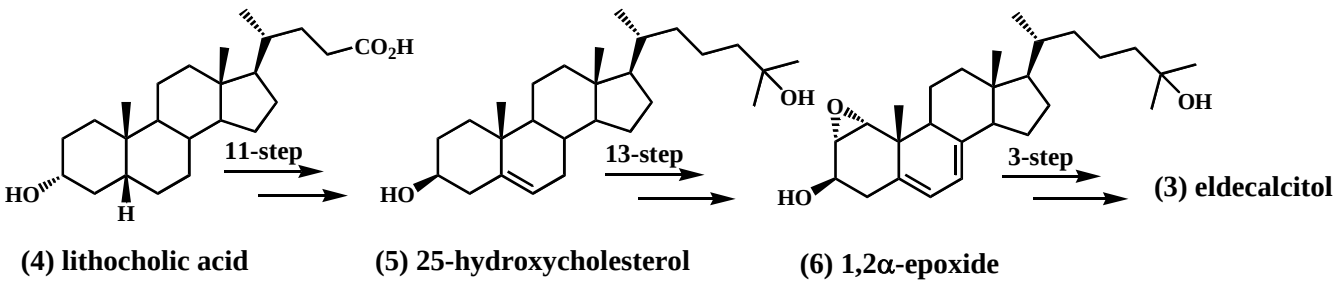
Synthetic studies on related compounds such as putative metabolites for structural elucidation of actual metabolites obtained in pharmacokinetic and metabolic studies, biologically interesting diastereomers for investigation of detailed mode of action, etc., in addition to improvement of practical synthesis for industrial scale production are generally indispensable to the development of new medicines.[10] Since during the development of eldecalcitol (**3**) as an anti-osteoporotic agent,[11] various related compounds were also necessary to be synthesized, we adopted the Trost convergent methodology to construct triene framework of **3** and related analogs as a common key reaction. The Trost reaction, which involves tandem palladium-catalyzed alkylative cyclization of ene-ynes as the A-ring fragment with bromomethylenes as C/D-ring fragment seemed to be versatile and applicable to our synthetic strategy (Scheme 1).[12,13] In this review, we describe an improved practical synthesis of eldecalcitol (**3**) and the synthesis of related analogs of **3**, A-ring diastereomers at the 1- and 3-positions and putative metabolites, which focuses on the Trost convergent coupling reaction. The detailed biological background of each targeted analog of **3** is also described.

AN IMPROVED PRACTICAL SYNTHESIS OF ELDECALCITOL (3)



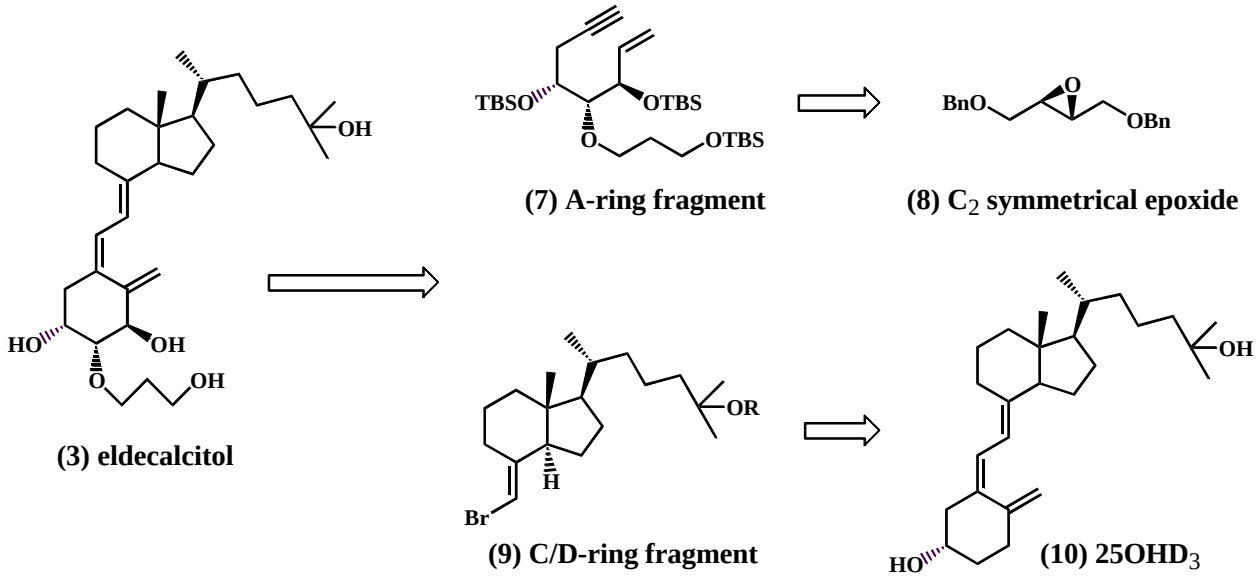
Scheme 1. Tandem palladium-catalyzed alkylative cyclization (the Trost convergent reaction)

Considering the potential clinical applications of eldecalcitol (**3**) as a useful drug in the near future, we investigated a practical synthesis of **3** for industrial scale production. Eldecalcitol (**3**) was initially synthesized in a linear manner in which the 1,2α-epoxide **6**, prepared from lithocholic acid (**4**) *via* 25-hydroxycholesterol (**5**),[14] served as a key intermediate for the introduction of the characteristic hydroxypropoxy substituent at the 2β-position (Scheme 2).[3] The 27-step linear sequence, however, was suboptimal due to its lengthiness and low overall yield. We, therefore, sought out to develop a more efficient and practical route that involves a convergent approach for the preparation of eldecalcitol (**3**) based on the Trost coupling reaction.



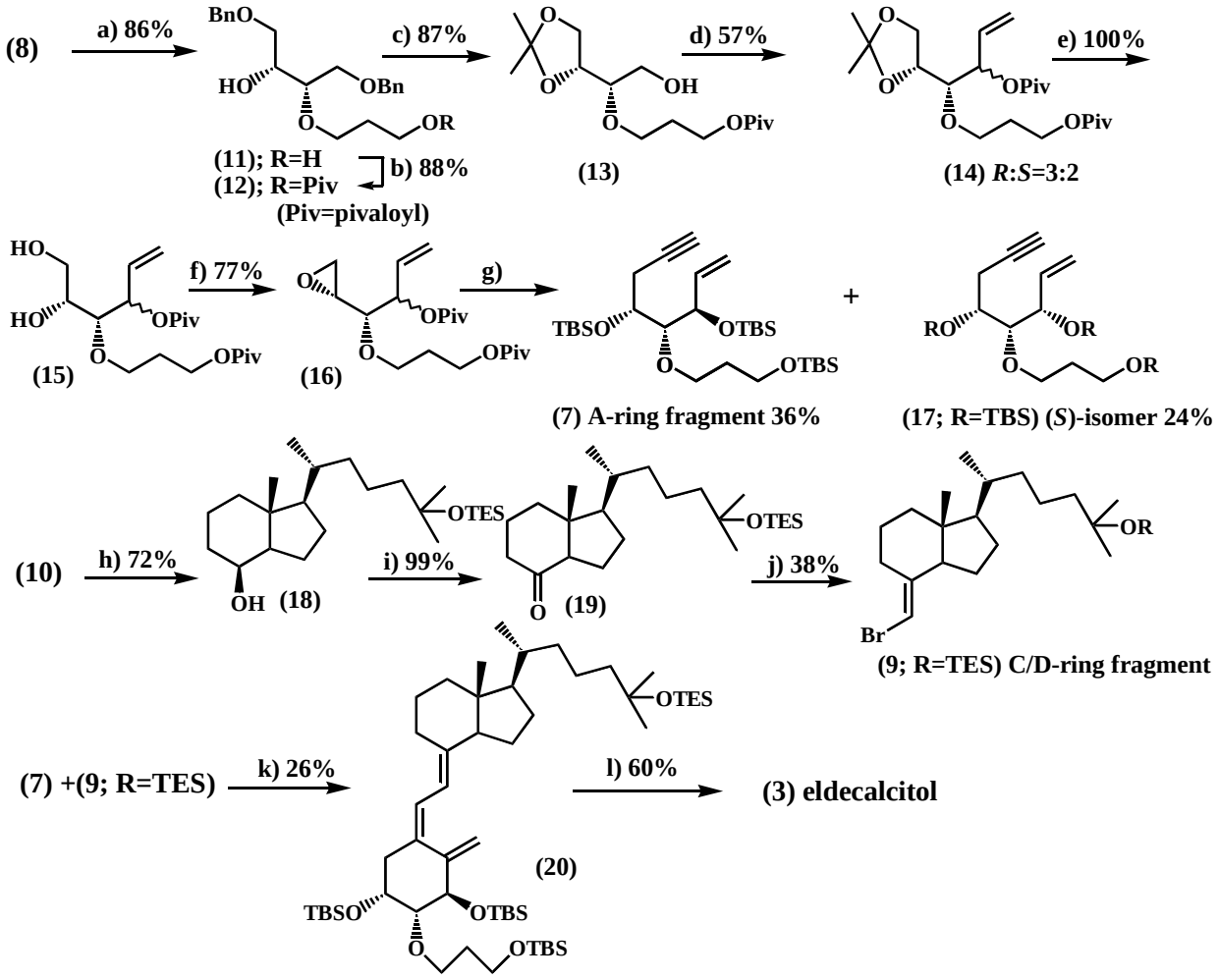
Scheme 2. Linear synthesis of eldecalcitol (3) from lithocholic acid (4)

Our synthesis of eldecalcitol (**3**) was envisioned using the coupling reaction of A-ring fragment **7** derived from C_2-symmetrical epoxide **8** with C/D-ring fragment **9** which can be obtained from 25-hydroxyvitamin D_3 (25OHD_3, **10**) (Scheme 3).



Scheme 3. Retrosynthesis of eldecalcitol (3)

The required A-ring fragment **7** for the synthesis of eldecalcitol (**3**) was synthesized based on the methodology that has been previously established.[15] Thus, cleavage of the known C_2-symmetrical epoxide **8**[16] with 1,3-propanediol ($HO(CH_2)_3OH$) in the presence of potassium *tert*-butoxide (*t*-BuOK) gave diol **11** in 86% yield. After protection of the primary hydroxyl group to give 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 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 diol **15**. Exposure of **15** to Mitsunobu conditions[17] afforded epoxide **16** in 77% yield. The acetylene unit was successfully installed by the regioselective epoxide-opening of **16** with lithium trimethylsilylacetylide (LiC≡CTMS) in the presence of boron trifluoride diethyl etherate (BF_3-OEt_2) at –78 °C to provide ene-yne **7** as the A-ring fragment of eldecalcitol (**3**) in 36% yield after protecting group exchange from pivalate to *tert*-butyldimethylsilyl (TBS) ether. The accompanied (*S*)-isomer **17**, which consists of the requisite stereochemistry to obtain 1-epi-eldecalcitol (**21**), was separated in 24% yield by simple column



Scheme 4. Improved convergent synthesis of eldecalcitol (3) by the Trost coupling reaction 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/acetone, 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. h) 1) TESOTf/Et_3N/CH_2Cl_2, 0 °C. 2) 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.

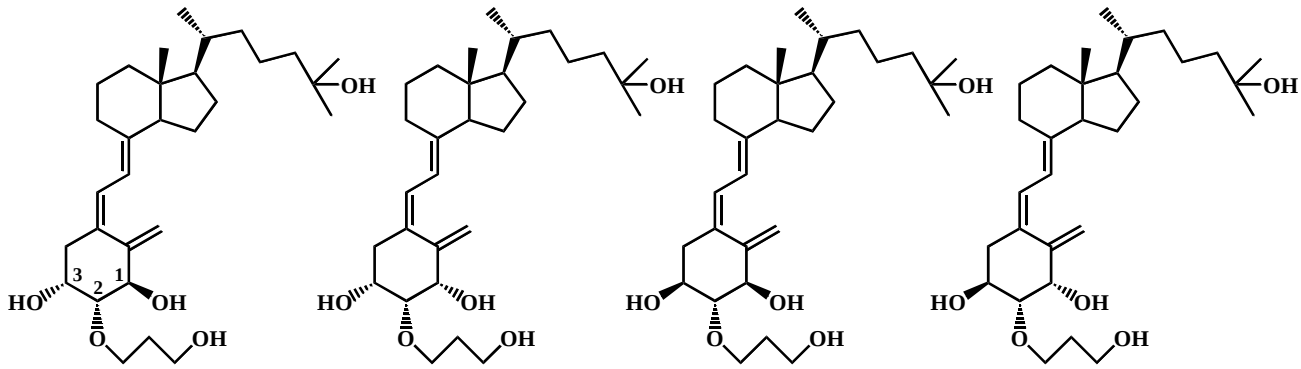
chromatography. Next, we performed the synthesis of C/D-ring fragment **9** from readily and commercially available $25OHD_3$ (**10**). $25OHD_3$ (**10**) was protected as the bis-triethylsilyl (TES) ether using triethylsily trifluoromethanesulfonate (TESOTf), and was then converted to alcohol **18** by ozonolysis and treatment with sodium borohydride ($NaBH_4$) (72% yield from **10**). The hydroxyl moiety in **18** was oxidized to ketone **19** with tetrapropylammonium perruthenate (TPAP) and *N*-methylmolpholine *N*-oxide (NMO) in 99% yield. Wittig reaction of **19** with (bromomethylene)triphenylphosphonium bromide ($Ph_3P^+CH_2Br/Br^-$) and sodium hexamethyldisilazide (NaHMDS) gave rise to C/D-ring fragment bromomethylene **9** (R=TES) in 38% yield. With A-ring fragment **7** and C/D-ring fragment **9** in hand, we

next investigated the Trost coupling reaction. Upon treatment of **7** and **9** with triethylamine (Et_3N), triphenylphosphine (PPh_3) and tris(di-benzylideneacetone)dipalladium-chloroform [$(dba)_3Pd_2$-$CHCl_3$] in boiling toluene, the desired 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 eldecalcitol (**3**) in 60% yield (Scheme 4).

Although the overall yield of eldecalcitol (**3**) by the Trost convergent synthesis was better than the previous linear approach, significant improvements were still demanded for a practical production of **3** and further investigation are ongoing.[18]

SYNTHESIS OF DIASTEREOMERS AT THE 1- AND 3-POSITIONS OF ELDECALCITOL (3)

To explore the biological mode-of-action of eldecalcitol (**3**) toward bone and related tissues such as parathyroid glands, intestines, and kidneys, diastereomers at the 1- and 3-positions of the A-ring seemed to be very helpful with inherent reasons described below. We, therefore, synthesized 1-epi-eldelacitol (**21**), 3-epi-eldecalcitol (**22**), and 1,3-diepi-eldecalcitol (**23**) (Figure 2).[19]



(3) eldecalcitol **(21) 1-epi-eldecalcitol** **(22) 3-epi-eldecalcitol** **(23) 1,3-diepi-eldecalcitol**

Figure 2. Structure of eldecalcitol (3), 1-epi-eldecalcitol (21), 3-epi-eldecalcitol (22), and 1,3-diepi-eldecalcitol (23)

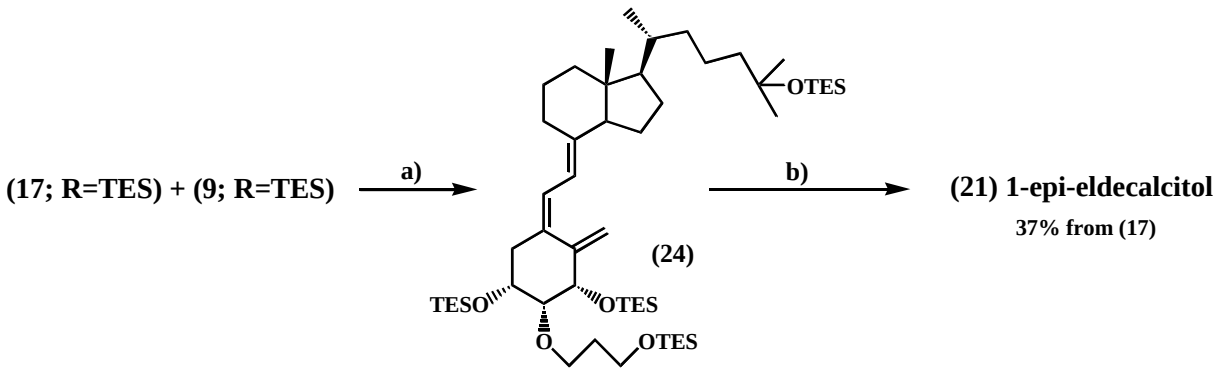
SYNTHESIS OF 1-EPI-ELDECALCITOL (21)

In Japan, calcitriol (**1**) and alfacalcidol (**2**) have been widely used for the treatment of osteoporosis for more than 25 years.[20] Calcitriol (**1**) and alfacalcidol (**2**) have been recognized as very safe medicines showing mild or moderate increase in bone mineral density (BMD) in osteoporotic patients. In the US and Europe, bisphosphonates such as sodium alendronate, sodium risedronate and sodium ibandronate, are mainly prescribed for treating osteoporosis because of strong increment of BMD.[21] Therefore, there has

been strong interest in obtaining active vitamin D_3 analogs more potent than calcitriol (**1**)/alfacalcidol (**2**) or comparable to bisphosphonates in increasing BMD and preventing bone fracture. Eldecalcitol (**3**) has been shown to be more effective than calcitrol (**1**) and alfacalcidol (**2**) in increasing BMD and mechanical bone strength in ovariectomized model rats.[7,22] The detailed mode of action of the enhanced activity of eldecalcitol (**3**) beyond calcitriol (**1**) and alfacalcidol (**2**) toward bone remains to be clarified. The long duration of **3** in the blood stream arises from its strong affinity for vitamin D binding protein (DBP) (2-fold ~ 4-fold v. **1**) and might explain, in part, the enhanced biological effects of **3**.[6] We, therefore, were highly interested in developing an active vitamin D_3 analog with strong affinity for DBP. It was reported that the epimerization of calcitriol (**1**) at the 1-position of the A-ring remarkably enhances the affinity for DBP. Norman and co-workers reported that 1-epi-calcitriol shows a 65.7-fold increase in affinity for DBP as compared to **1**.[23] These findings stimulated our interest in the biological profile of epimerized eldecalcitol (**3**), particularly at the 1-position. Thus our attention turned to 1-epi-eldecalcitol (**21**) and studying its affects on DBP affinity and on bone.

As described above, when ene-yne **7** was prepared from 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-eldecalcitol (**21**). Upon treatment of excess bromomethylene **9** (R=TES) and ene-yne **17** (R=TES) in the presence of tetrakis(triphenylphosphine)palladium (0) [$Pd(PPh_3)_4$] and Et_3N in boiling toluene, the coupled product **24** was obtained as an inseparable mixture with recovered **9.** The mixture was desilylated using 47% hydrofluoric acid (HF) in acetonitrile (MeCN) and purified to afford 1-epi-eldecalcitol (**21**) in 37% yield from **17** (Scheme 5).

As anticipated, 1-epi-eldecalcitol (**21**) showed enhanced affinity for DBP (1.6-fold v. eldecalcitol) and further *in vivo* biological studies with **21** including effects on bone are currently under investigation.[24,25]



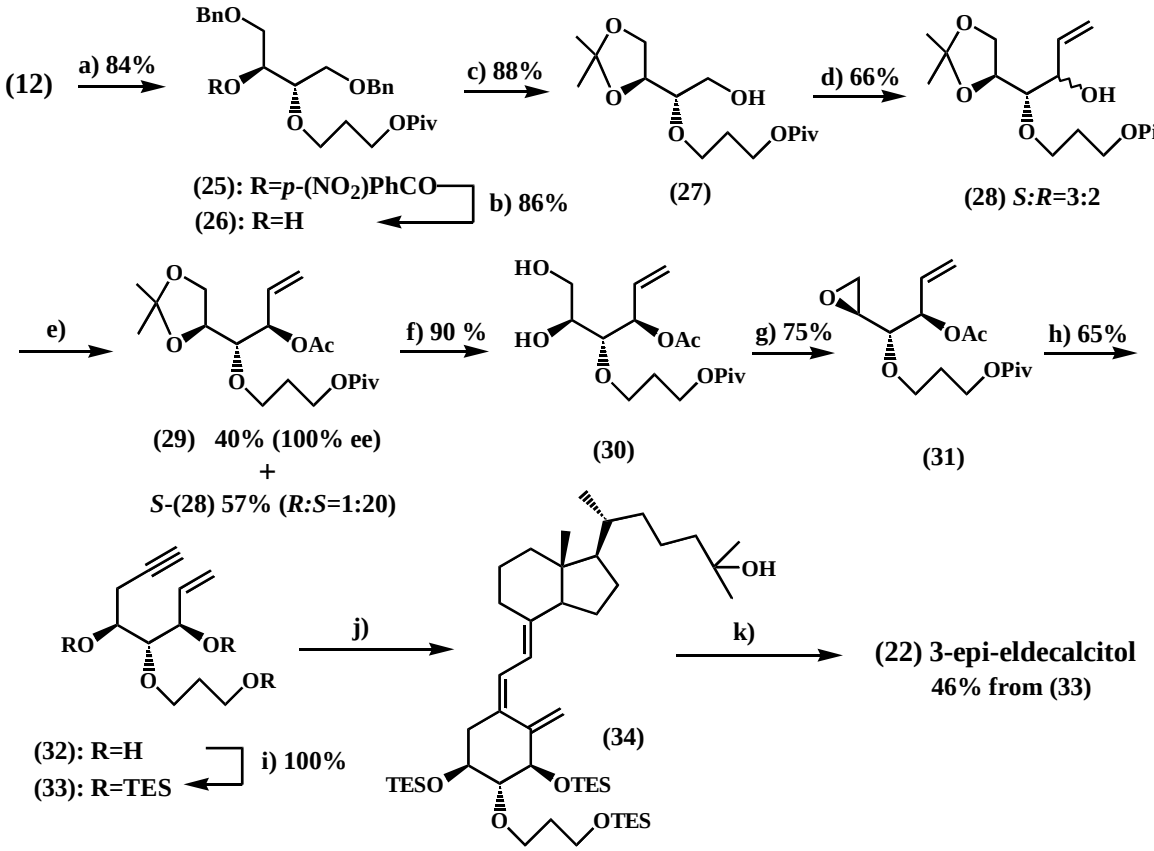
Scheme 5. Synthesis of 1-epi-eldecalcitol (21) Reagents and conditions: a) $Pd(PPh_3)_4/Et_3N$/toluene, reflux. b) 47% HF/MeCN, rt.

SYNTHESIS OF 3-EPI-ELDECALCITOL (22)

Recently, it was reported that the epimerization of calcitriol (**1**) at the 3-position of the A-ring plays a major role in parathyroid hormone (PTH) synthesis and secretion. Epimerized 3-epi-calcitriol shows equipotent and prolonged activities in comparison with **1** at suppressing PTH secretion.[26,27] During our clinical development of eldecalcitol (**3**), serum PTH in osteoporotic patients, however, did not change significantly upon treatment with **3**.[9] We assumed that a bulky hydroxylpropoxy substituent at the 2-position of the A-ring would interfere with epimerization of eldecalcitol (**3**) at the adjacent and sterically hindered 3-position leading to the absence of epimerized 3-epi-eldecalcitol (**22**) in parathyroid glands. This could explain why eldecalcitol (**3**) showed weak potency in PTH suppression during clinical studies. We, therefore, were interested in the synthesis and biological evaluation of 3-epi-eldecalcitol (**22**).

The synthesis of A-ring fragment **33** for 3-epi-eldecalcitol (**22**) began with inversion of the C_3 configuration of alcohol **12**. Reaction of **12** with *p*-nitrobenzoic acid (*p*-$(NO_2)PhCO_2H$) in the presence of diethyl azodicarboxylate (DEAD) and PPh_3 gave *p*-nitrobenzoate **25** in 84% yield.[28] Treatment of **25** with sodium hydrogen carbonate ($NaHCO_3$) in methanol (MeOH) allowed selective methanolysis of the *p*-nitrobenzoate group to give inverted alcohol **26** in 86% yield. After hydrogenolysis of the benzyl ether functionality in **26,** the resulting diol was protected as its acetonide to afford **27** in 88% yield. Swern oxidation of **27** followed by Grignard reaction of the resulting aldehyde with CH_2=CHMgBr produced alcohol **28** as an epimeric mixture (*S:R* = 3:2) in 66% yield. To separate this epimeric mixture, **28** was subjected to lipase-catalyzed acetylation using vinyl acetate (CH_2=CHOAc) and Novozyme in *t*-butyl methyl ether (*t*-BuOMe).[29] The (*R*)-isomer preferentially underwent acetylation to give the acetate **29** and ***S*-28** (*R*:*S* = 1:20) in 40% and 57% yields, respectively. Acidic hydrolysis of **29** gave diol **30** in 90% yield, which upon Mitsunobu reaction using DEAD and PPh_3 in boiling toluene afforded epoxide **31** in 75% yield. Reaction of **31** with LiC≡CTMS in the presence of BF_3-OEt_2 at –78 °C followed by saponification provided ene-yne **32** in 65% yield.[30] Protection of **32** as its TES ether produced A-ring fragment **33** quantitatively. Having secured A-ring fragment **33**, we performed its coupling with C/D-ring fragment **9** using Trost methodology. Thus, A-ring fragment **33** was allowed to react with C/D-ring fragment **9** (R=H) in the presence of $Pd(Ph_3P)_4$ and Et_3N in boiling toluene to give coupling product **34** which was desilylated with ammonium fluoride (NH_4F) in boiling MeOH to produce 3-epi-eldecalcitol (**22**) in 46% yield from **33** (Scheme 6).

3-Epi-eldecalcitol (**22**) showed only slight inhibition of PTH secretion in bovine parathyroid cells compared to eldecalcitol (**3**). Eldecalcitol (**3**) and 3-epi-eldecalcitol (**22**) might be inherently weak agents toward PTH suppression, which might be a beneficial characteristic of **3** for treating osteoporotic patients, although further studies with **3** will be necessary.[31,32]



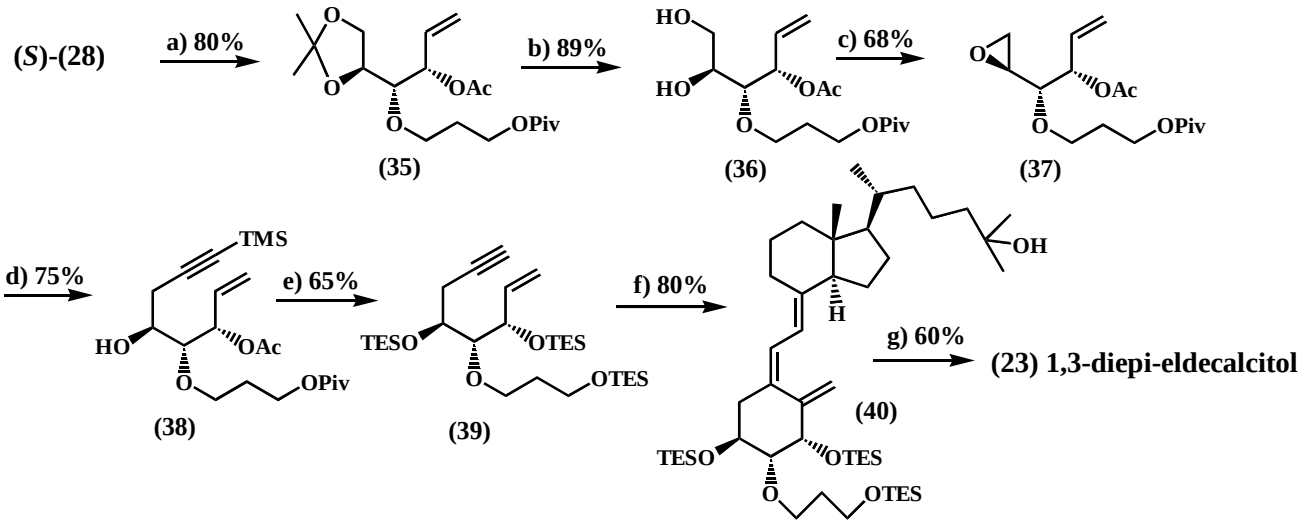
Scheme 6. Synthesis of 3-epi-eldecalcitol (23) Reagents and conditions: a) *p*-(NO_2)$PhCO_2H$/PPh_3/toluene, rt. b) $NaHCO_3$/MeOH, rt. c) 1) $Pd(OH)_2/H_2$/MeOH, rt. 2) $Me_2C(OMe)_2$/TsOH/acetone, rt. d) 1) $(COCl)_2$/DMSO/CH_2Cl_2, -78 °C then Et_3N. 2) CH_2=CHMgBr/THF, -40 °C. e) Novozyme/CH_2=CHOAc/*t*-BuOMe, 30 °C. f) 60% AcOH/H_2O, rt. g) DEAD/PPh_3/dioxane, reflux. h) 1) LiC≡CTMS/BF_3-OEt_2/THF, -78 °C. 2) 10M NaOH/MeOH, rt. i) TESOTf/Et_3N/CH_2Cl_2, -40 °C. j) (**9**; R=H)/$Pd(PPh_3)_4$/Et_3N/toluene, reflux. k) NH_4F/MeOH, reflux.

SYNTHESIS OF 1,3-DIEPI-ELDECALCITOL (23)

To further explore structure-activity-relationships between eldecalcitol (**3**) and related analogs, we focused significant attention to the diastereomer of **3** at both the 1- and 3-positions of the A-ring. We proceeded with the synthesis of structurally and biologically relevant 1,3-diepi-eldecalcitol (**23**), which represents the final structural member that completes the set all possible A-ring diastereomers at the 1- and 3-positions of eldecalcitol (**3**). This analog, in combination with others, is anticipated to enhance our understanding of the mode-of-action of medicinally important **3**. The synthesis of A-ring fragment **39** commenced from (*S*)-alcohol **28** which was obtained in our previous lipase-catalyzed acetylation of **28** as the unreacted (*S*)-isomer.[31] (*S*)-Alcohol **28** possesses the requisite stereochemistry at positions 1, 2, and 3 of the A-ring that comprises **23**. Acetylation of **28** gave acetate **35** in 80% yield, from which diol **36** was obtained in 89% yield after deprotection of the acetonide moiety using 60% acetic acid (AcOH). Mitsunobu reaction of **36** with DEAD and PPh_3 in boiling toluene afforded epoxide **37** in 68% yield. Reaction of **37** with

LiC≡CTMS in the presence of BF_3-OEt_2 at -78 °C gave ene-yne **38** in 75% yield. Conversion of **38** to A-ring fragment **39** was achieved by saponification with 10 M sodium hydroxide (NaOH) followed by subsequent protection of the hydroxyl groups as their TES ether in 65% overall yield. With A-ring fragment **39** in hand, the key coupling step to C/D-ring fragment **9** using the Trost methodology was investigated. Thus, **39** was coupled with **9** (R=H) in the presence of $Pd(PPh_3)_4$ and Et_3N in boiling toluene to produce desired coupling product **40** in 80% yield. Desilylation of **40** with 46% HF in MeCN at rt afforded 1,3-diepi-eldecalcitol (**23**) in 60% yield (Scheme 7).

The detailed biological properties of 1,3-diepi-eldecalcitol (**23**) in comparison with eldecalcitol (**3**), 1-epi-eldecalcitol (**21**) and 3-epi-eldecalcitol (**22**) are currently under investigation.[33]

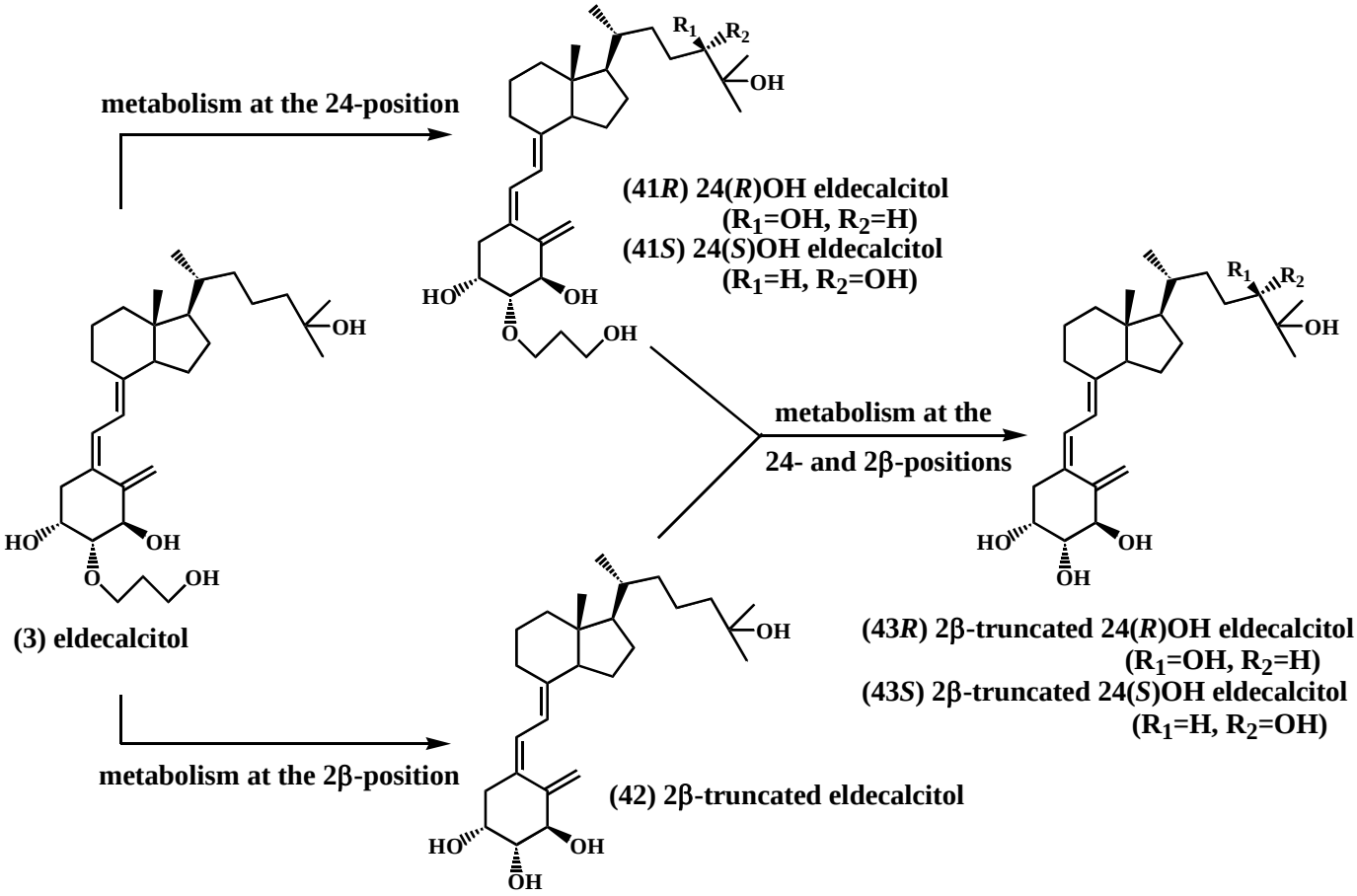


Scheme 7. Synthesis of 1,3-diepi-eldecalcitol (23) Reagents and conditions: a) Ac_2O/Et_3N/DMAP/CH_2Cl_2, rt. b) 60% AcOH/H_2O, rt. c) PPh_3/DEAD/dioxane, reflux. d) LiC≡CTMS/BF_3-OEt_2/THF, -78°C. e) 1) 10 M NaOH/MeOH, rt. 2) TESOTf/Et_3N/CH_2Cl_2, -40 °C. f) (**9**; R=H)/$Pd(PPh_3)_4$/Et_3N/toluene, reflux. g) 46% HF/MeCN, rt.

SYNTHESIS OF PUTATIVE METABOLITES OF ELDECALCITOL (3)

It is known that calcitriol (**1**) is hydroxylated at the 24-position of the side chain as a first step in its metabolism to produce 24-hydroxylated calcitriol.[34,35,36,37,38,39] On the assumption that hydroxylation pathway of eldecalcitol (**3**) and **1** would be similar, we undertook the synthesis of 24-hydroxylated eldecalcitol in 24(*R*) and 24(*S*) forms (**41*R*** and **41*S***).[15] In the case of calcitriol (**1**), 24-hydroxylated calcitriol is further hydroxylated at the 23- and 26-positions of the side chain and oxidized to keto-alcohol, lactone (calcitriol lactone), or carboxylic acid (calcitroic acid).[34,35,36,37,38,39] Since eldecalcitol (**3**) has a substituent at the 2β-position of the A-ring, it is recognized that the metabolic pathway of **3** might be more

complicated than calcitriol (**1**) because of metabolism at the 2β-position substituent in addition to the inherent metabolism of the side chain. In fact, preliminary metabolic studies on eldecalcitol (**3**) revealed the presence of 24-hydroxylated eldecalcitol (**41*R*** and **41*S***) as metabolites of **3** which were also accompanied by several other metabolic associates that may be derived from metabolism of both the hydroxypropoxy substituent of the 2β-position and a combination of metabolism between the side chain and 2β-position. In order to assist in the confirmation and structure elucidation of putative metabolic analogs of eldecalcitol (**3**), the synthesis of 2β-truncated eldecalcitol (**42)** and 24-hydroxylated and 2β-truncated eldecalcitol (**43*R*** and **43*S***) was pursued (Scheme 8).



Scheme 8. Putative metabolic pathway of eldecalcitol (3)

SYNTHESIS OF 24-HYDROXYLATED ELDECALCITOL (41*R* and 41*S*)

The synthesis of C/D-ring fragments in both 24(*R*)- and 24(*S*)-hydroxylated series, respectively, was performed. Inhoffen-Lythgoe diol (**44**), prepared from vitamin D_2 by ozonolysis,[40] was converted to sulfone **46** *via* **45** by known methodology.[41,42] Sulfone **46** was alkylated independently with (*R*)- and (*S*)-2,3-dihydroxy-3-methylbutyl *p*-toluenesulfonate **47*R*** and **47*S***[43,44] to afford alcohols **48*R*** in 71% yield

and **48*S*** in 28% yield, respectively. The (*R*)- and (*S*)-alcohols **48*R*** and **48*S*** were converted to C/D-ring fragments **55*R*** and **55*S*** as follows:

1. Desulfurization of **48*R*** and **48*S*** with sodium amalgam (Na-Hg) produced **49*R*** and **49*S*** in 97% and 68% yields, respectively.
2. Acetonide formation of **49*R*** and **49*S*** with 2,2-dimethoxypropane ($Me_2C(OMe)_2$) and subsequent acetylation giving **50*R*** and **50*S*** in 83% and 64% yields, respectively.
3. Deprotection of the acetonide moiety in **50*R*** and **50*S*** with iodine (I_2) in MeOH produced **51*R*** and **51*S*** in 61% and 83% yields, respectively.
4. Silylation of **51*R*** and **51*S*** with TBSOTf gave **52*R*** and **52*S*** in 92% and 91% yields, respectively.
5. Reductive cleavage of the acetyl groups in **52*R*** and **52*S*** with lithium aluminum hydride ($LiAlH_4$) afforded **53*R*** and **53*S*** in 96% and 100% yields, respectively.
6. Oxidation of the hydroxyl groups in **53*R*** and **53*S*** with NMO and TPAP produced **54*R*** and **54*S*** in 96% and 100% yields, respectively.
7. Wittig reaction of **54*R*** and **54*S*** with $Ph_3P^+CH_2Br/Br^-$ in the presence of NaHMDS generated **55*R*** and **55*S*** in 49% and 57% yields, respectively.

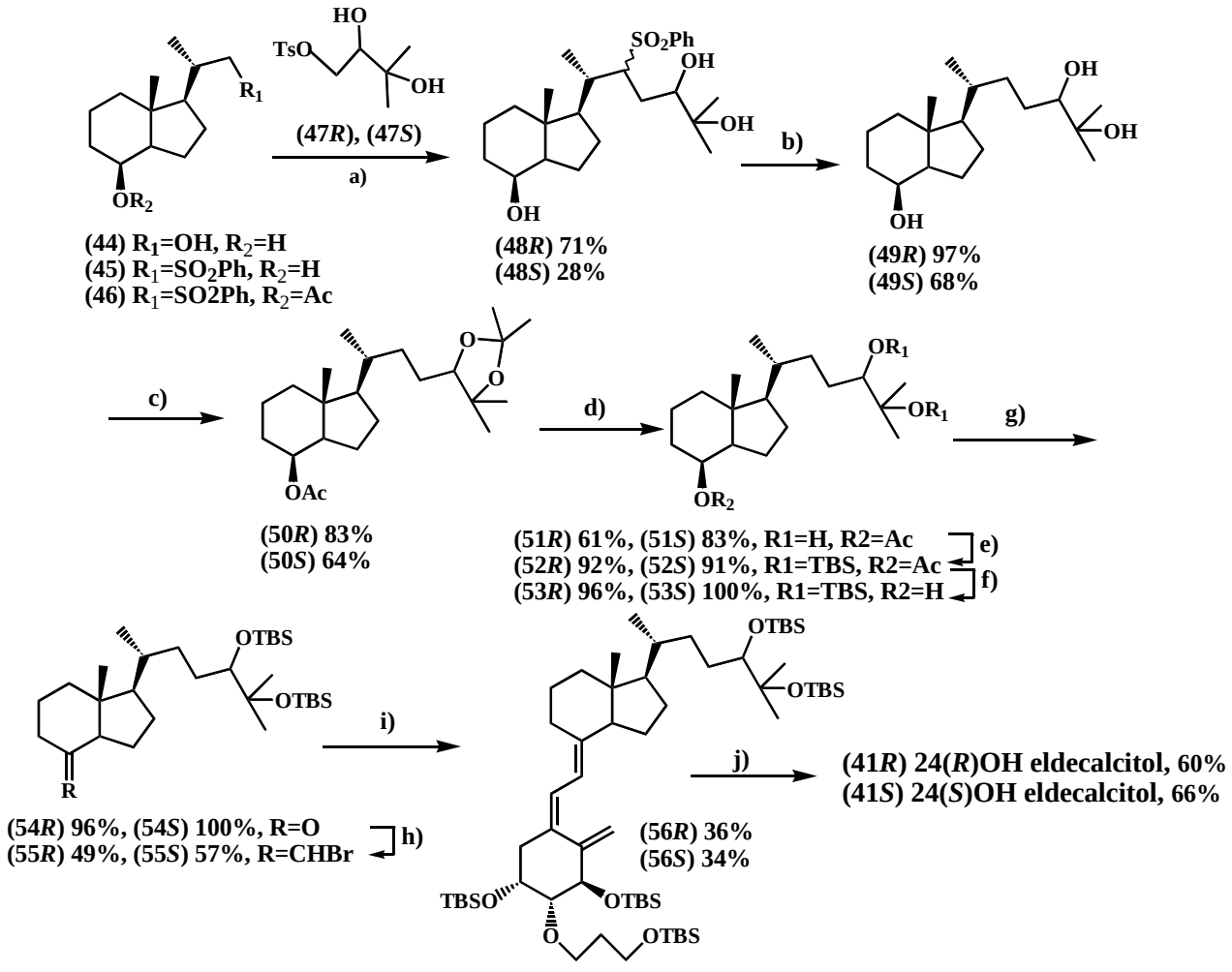
Using Trost methodology, 24-hydroxylated C/D-ring fragments **55*R*** and **55*S*** were coupled with A-ring fragment **7** which produced coupled products **56*R*** and **56*S*** in 36% and 34% yields, respectively. Deprotection with TBAF gave 24(*R*)-hydroxylated eldecalcitol (**41*R***) and 24(*S*)-hydroxylated eldecalcitol (**41*S***) in 60% and 66% yields, respectively (Scheme 9).

When eldecalcitol (**3**) was orally administered to rats, 24-hydroxylated eldecalcitol (**41*R*** and **41*S***) were found in rat plasma as actual metabolites of **3**, for which the synthesized **41*R*** and **41*S*** were conveniently used as authentic samples for structure elucidation.[15]

SYNTHESIS OF 2β-TRUNCATED ELDECALCITOL (42)

Next, the synthesis of the A-ring fragment **66** for the preparation of 2β-truncated eldecalcitol (**42**) was undertaken. Diol **57**, obtained from D-mannitol,[45] was protected as pivalate **58** in 72% yield. Pivalate **58** was subjected to hydrolysis with 1N hydrochloric acid (HCl) to give diol **59** (50% yield) accompanied by tetraol **60** (34% yield) as well as recovered starting material **58** (16% yield), which were easily separated by silica gel column chromatography. Diol **59** was converted to orthoester **61** (88% yield) with triethyl orthoformate ($HC(OEt)_3$) and *p*-toluenesulfonic acid monohydrate (TsOH). Upon treatment of orthoester **61** with boiling acetic anhydride (Ac_2O), terminal olefin formation took place cleanly to produce olefin **62** in 93% yield.[46,47,48,49] Deketalization of **62** afforded diol **63** in 93% yield.

Subsequent

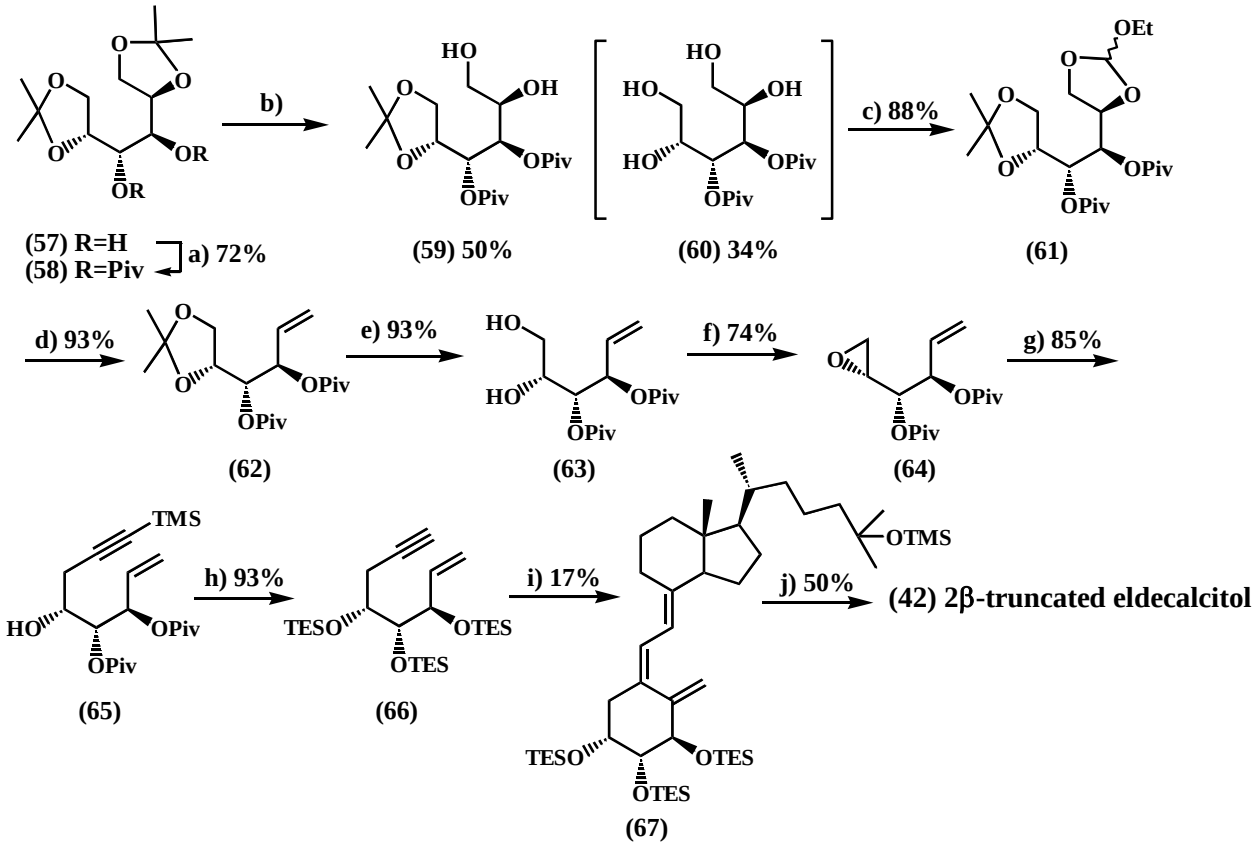


Scheme 9. Synthesis of 24-hydroxylated eldecalcitol (41*R* and 41*S*) Reagents and conditions: a) *n*-BuLi/THF, -20 °C. b) Na-Hg/THF/MeOH, rt. c) 1) $Me_2C(OMe)_2$/TsOH/acetone, rt. 2) Ac_2O/pyridine/DMAP, rt. d) I_2/MeOH, reflux. e) TBSOTf/2,6-lutidine/CH_2Cl_2, rt. f) $LiAlH_4$/THF, 0 °C. g) TPAP/NMO/4A molecular sieves, rt. h) $Ph_3P^+CH_2BrBr^-$/NaHMDS/THF, -60 °C. i) (**7**)/$(dba)_3Pd_2$-$CHCl_3$/PPh_3/Et_3N/toluene, reflux. j) TBAF/THF/toluene, reflux.

Mitsunobu reaction of **63** with diisopropyl azodicarboxylate (DIAD) and Ph_3P in dioxane gave epoxide **64** in 74% yield. Treatment of epoxide **64** with LiC≡CTMS in the presence of BF_3-OEt_2 provided 1,7-ene-yne **65** in 85% yield. Then, 2β-truncated A-ring fragment **66** was obtained from **65** by hydrolysis and subsequent TES ether formation in 93% yield. With A-ring fragment **66** in hand, the coupling reaction with C/D-ring fragment **9** (R=TMS) following the Trost methodology gave rise to **67** in 17% yield, which was deprotected using TBAF to afford 2β-truncated eldecalcitol (**42**) in 50% yield (Scheme 10).

The synthesized 2β-truncated eldecalcitol (**42**) is currently in use as an authentic reference sample for

metabolic studies of eldecalcitol (**3**).[50]



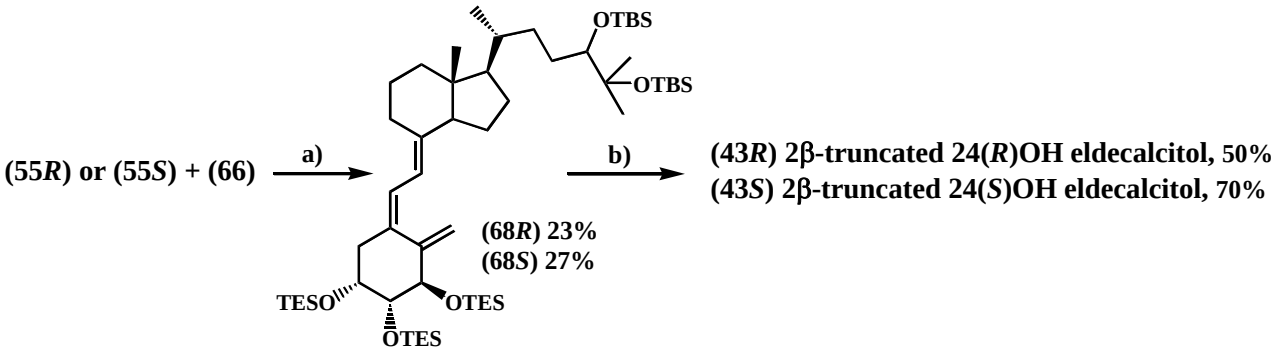
Scheme 10. Synthesis of 2β-truncated eldecalcitol (42) Reagents and conditions: a) *t*-BuCOCl/pyridine/DMPA, reflux. b) 1N HCl/THF, 0 °C~rt. c) $HC(OEt_3)$/TsOH/CH_2Cl_2, 0 °C~rt. d) Ac_2O, reflux. e) 1N HCl/THF, rt. f) Ph_3P/DIAD, reflux. g) LiC≡CTMS/BF_3-OEt_2/THF, -78 °C. h) 1) 1N NaOH/MeOH, rt. 2) TESOTf/Et_3N, -40 °C. i) (**9**; R=TMS), $Pd(Ph_3)_4$/Et_3N, reflux. j) TBAF/THF, rt.

SYNTHESIS OF 24-HYDROXYLATED AND 2β-TRUNCATED ELDECALCITOL (43*R* and 43*S*)

2β-Truncated A-ring fragment **66** and 24-hydroxylated C/D-ring fragments **55*R*** and **55*S*** consist of the requisite components for the synthesis of 24-hydroxylated and 2β-truncated eldecalcitol (**43*R*** and **43*S***). The coupling reaction between **55*R*** or **55*S*** and **66** under Trost conditions produced coupled products **68*R*** and **68*S*** in 23% and 27% yields, respectively. Deprotection using TBAF afforded 24(*R*)-hydroxylated and 2β-truncated eldecalcitol (**43*R***) and 24(*S*)-hydroxylated and 2β-truncated eldecalcitol (**43*S***) in 50% and 70% yields, respectively (Scheme 11).

The synthesized putative metabolites, **43*R*** and **43*S***, are currently in use as authentic reference samples for

metabolic studies of eldecalcitol (**3**).[50]



Scheme 11. Synthesis of 24-hydroxylated and 2β-truncated eldecalcitol (43*R* and 43*S*) Reagents and conditions: a) $Pd(PPh_3)_4/Et_3N$, reflux. b) TBAF/THF, rt.

CONCLUSION

Based on the Trost coupling methodology involving A-ring fragments **7**, **17**, **33**, **39**, and **66** and C/D-ring fragments **9**, **55*R***, and **55*S***, the successful total synthesis of eldecalcitol (**3**) and related compounds, 1-epi-eldecalcitol (**21**), 3-epi-eldecalcitol (**22**), 1,3-diepi-eldecalcitol (**23**), 24-hydroxylated eldecalcitol (**41*R*** and **41*S***), 2β-truncated eldecalcitol (**42**), and 24-hydroxylated and 2β-truncated eldecalcitol (**43*R*** and **43*S***) has been achieved. These analogs play important roles as indispensable structural entities during the development of eldecalcitol (**3**) as an anti-osteoporotic agent.

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