Process Development for the Practical Production of Eldecalcitol by Linear, Convergent and Biomimetic Syntheses

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Abstract. *Eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3], an analog of calcitriol (1α,25-dihydroxyvitamin D_3), possesses a hydroxypropoxy substituent at the 2β-position of calcitriol. Eldecalcitol has potent biological effects on bone disease such as osteoporosis. The marketing of eldecalcitol has very recently started in Japan. In consideration of this, we have been investigating practical synthesis of eldecalcitol for industrial-scale production. Eldecalcitol was initially synthesized in a linear manner. The 27-step linear sequence was, however, suboptimal due to its lengthiness and low overall yield (ca. 0.03%). Next, we developed a convergent approach based on the Trost coupling reaction, in which the A-ring fragment (ene-yne part obtained in 10.4% overall yield) and the C/D-ring fragment (bromomethylene part obtained in 27.1% overall yield) are coupled to produce the triene system of eldecalcitol (15.6%). Although the overall yield of the convergent synthesis was better than that of the linear synthesis, significant improvements were still necessary. Therefore, additional biomimetic studies were investigated. Process development for the practical production of eldecalcitol is described herein.*

It is well-established that cholecalciferol (vitamin D_3, Figure 1, **1**), ingested in foods or synthesized in the skin, is metabolized to calcifediol (25-hydroxyvitamin D_3, **2**) in the liver, which is further hydroxylated at the 1α-position in the kidney to produce active vitamin D_3, calcitriol (1α,25-dihydroxyvitamin D_3, **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 (2). In Japan, calcitriol and its synthetic prodrug, alfacalcidol (1α-hydroxyvitamin D_3, **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 quarter of a century (3). Calcitriol 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. Eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (**5**), developing code: ED-71], which possesses a hydroxypropoxy substituent at the 2β-position of calcitriol, is such an analog that shows potent effects on bone therapy (4-9). The recent completion of a phase III clinical trial of eldecalcitol in comparison with alfacalcidol for bone fracture prevention produced excellent results (10, 11). The marketing of eldecalcitol with the sales name of Edirol as an excellent medicine for the treatment of osteoporosis has very recently started in Japan.

Considering the marketing of eldecalcitol, we have been investigating its practical synthesis for industrial-scale production. Eldecalcitol was initially synthesized in a linear manner in which 1,2α-epoxide **28**, prepared from lithocholic acid (**6**) *via* 25-hydroxycholesterol (**17**), served as a key intermediate for the introduction of the hydroxypropoxy substituent at the 2β-position (4, 12). The 27-step linear sequence was, however, suboptimal due to its lengthiness and low overall yield (*ca.* 0.03%). Next, we developed a convergent approach based on the Trost coupling reaction, in which A-ring fragment **37** (ene-yne part prepared from C_2 symmetrical epoxide **30** in 10.4% overall yield) and C/D-ring fragment **40** (bromomethylene part obtained from calcifediol (**2**) in 27.1% overall yield) are coupled to produce the triene system of eldecalcitol (15.6%) (13, 14). Although the overall yield of the convergent synthesis was better than that of the linear synthesis, significant improvements were still necessary. Therefore, additional biomimetic studies on the microbial 25-hydroxylation of the steroidal side chain using cholesterol (**42**) as a starting material were investigated (15). In this paper, process development for the practical

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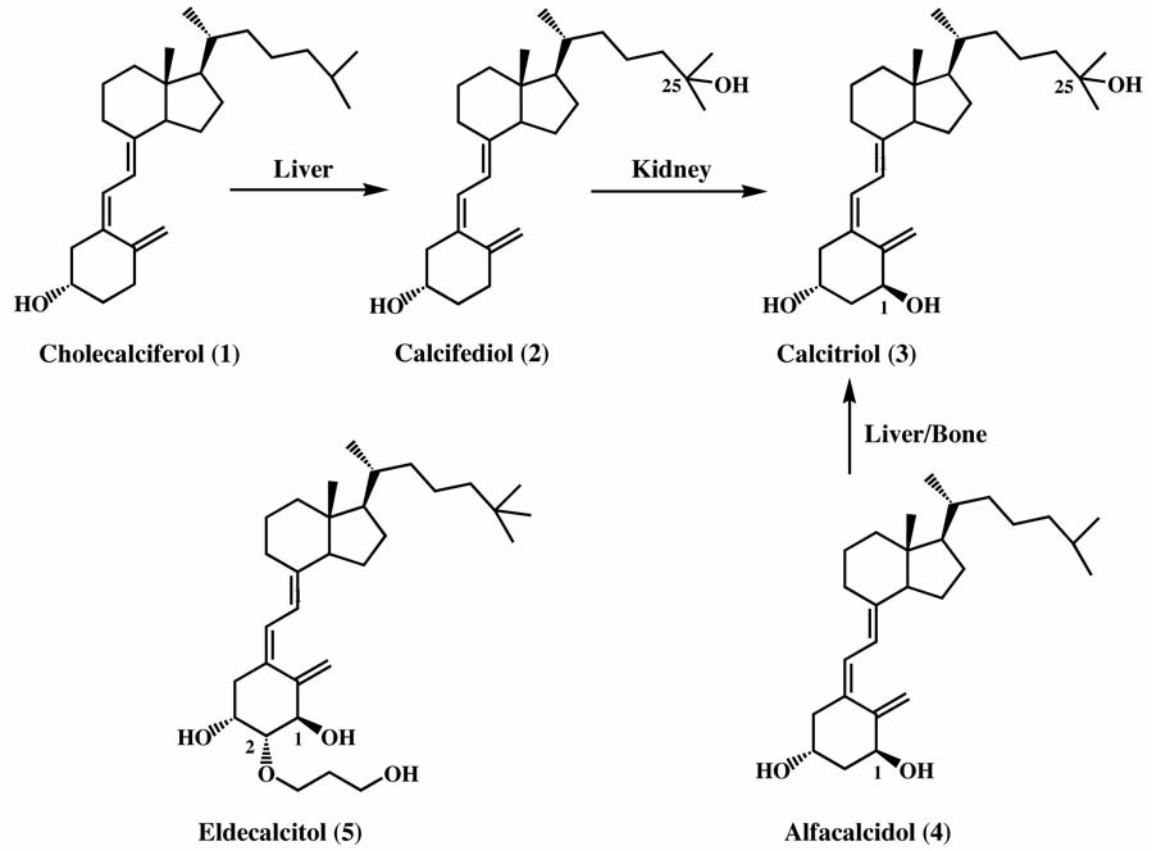

Figure 1. *Activation of cholecalciferol (**1**), calcifediol (**2**), and alfacalcidol (**4**) to calcitriol (**3**) and the structure of eldecalcitol (**5**).*

production of eldecalcitol, the linear synthesis, convergent synthesis, and biomimetic synthesis are described.

Materials and Methods

*Eldecalcitol (**5**) by linear synthesis*. Detailed experimental procedures for the linear synthesis have been described elsewhere (4, 12).

*Eldecalcitol (**5**) by convergent synthesis*. Detailed experimental procedures for the convergent synthesis have also been described elsewhere (13).

*Eldecalcitol (**5**) by biomimetic synthesis*. Conversion of cholesterol (**42**) to 1,2α-epoxide **53** has been previously described (16) and detailed experimental procedures for microbial 25-hydroxylation of **55** have been described in a patent (17).

Results and Discussion

Synthesis of eldecalcitol by the linear method. Eldecalcitol (**5**) was originally synthesized by the linear method using lithocholic acid (**6**) as a starting material during the exploratory research for **5** (4). Thus, oxidative bromination of **6** with *N*-bromosuccinimide (NBS) in aqueous dioxane afforded ketobromide **7**, quantitatively, which was then dehydrobrominated to enone **8**, quantitatively, by treatment with lithium carbonate. The carboxylic acid moiety in **8** was converted to the methyl ester **9**, which was treated with acetic anhydride (Ac_2O) in the presence of *p*-toluenesulfonic acid (TsOH) to give dienolacetate **10** in 87% yield from **6**. Stereoselective reduction of the enolacetate group in **10** was achieved with excess sodium borohydride ($NaBH_4$) in methanol (MeOH) and tetrahydrofuran (THF), affording **11** in 54% yield. Bromide **14**, prepared from **11** in 74% overall yield by a known procedure (18, 19), was treated with lithio-2-methyl-1,3-dithiane in THF to give dithiane **15** in 88% yield. The conversion of **15** into 25-hydroxycholesterol (**17**) was effected in 62% yield by a two-step sequence: hydrolysis of the dithiane and THP groups in **15** with methyl iodide in aqueous acetone, followed by the reaction with methylmagnesium bromide (MeMgBr) (12). An important intermediate, 1,2α-epoxide **28**, was obtained from **17** by a 13-step sequence, which is basically the same procedure as the conversion of cholesterol (**42**) to epoxide **53** described in Figure 4. Treatment of **28** with 1,3-propanediol in the presence of potassium *tert*-butoxide (*t*-BuOK) resulted in

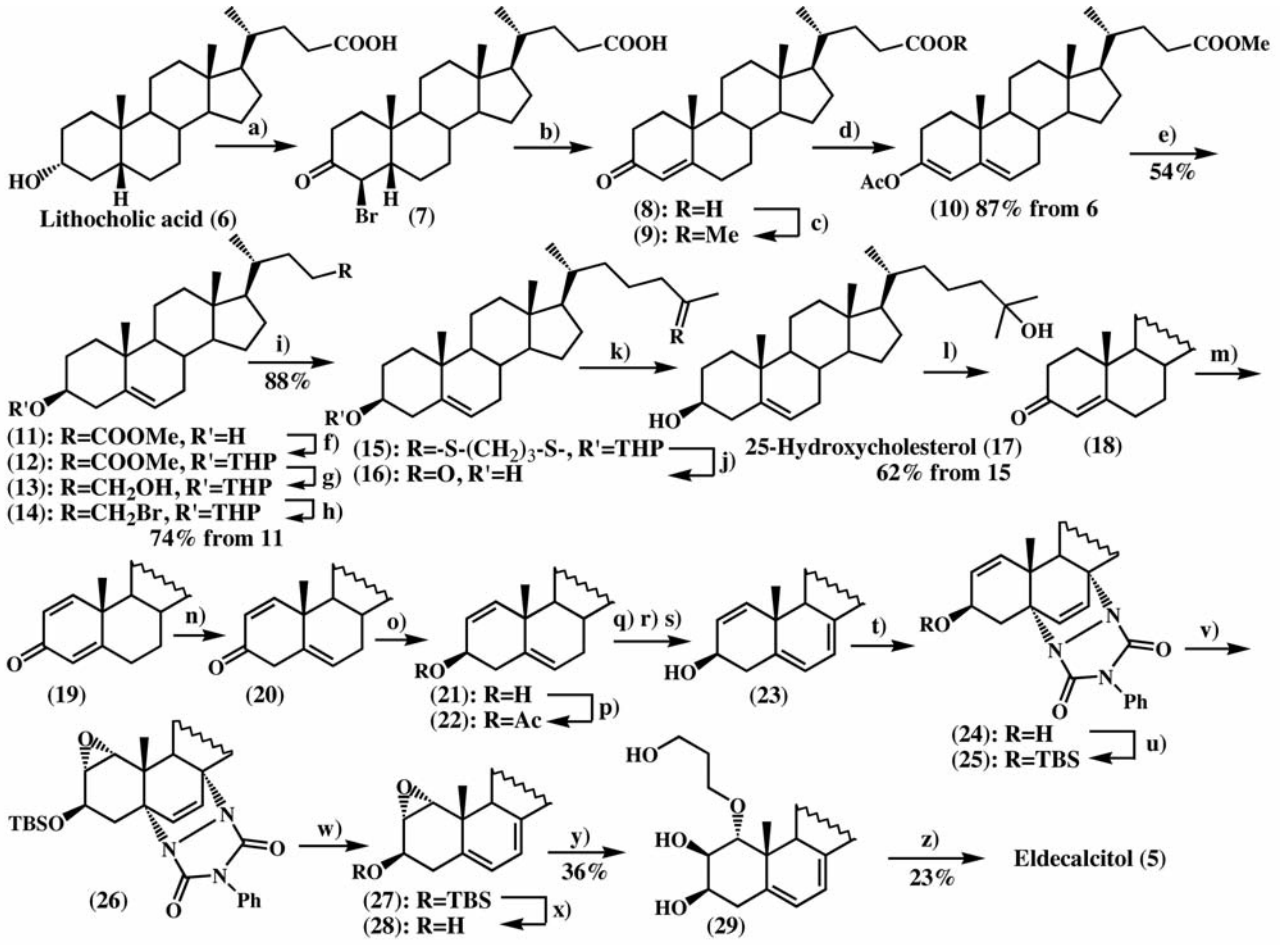

Figure 2. *Linear synthesis of eldecalcitol (**5**) from lithocholic acid (**6**) via 25-hydroxycholesterol (**17**). Reagents and conditions: a: NBS/dioxane, 40°C to 55°C. b: $LiCO_3$/DMF, 90°C. c: HCl/MeOH, rt. d: TsOH/Ac_2O, 85°C. e: $NaBH_4$/MeOH/THF, 0°C. f: DHP/Amberlyst 15/CH_2Cl_2, rt. g: Red-Al/benzene, reflux. h: NBS/Ph_3P/$NaHCO_3$/DMF, 0°C. i: 2-methyl-1,3-dithiane/n-BuLi/THF/n-hexane, –78°C to 4°C. j: MeI/acetone/H_2O, reflux. k: MeMgBr/THF, –5°C to 0°C. l: $(Oi\text{-}Pr)_3Al$/cyclohexanone. m: DDQ/AcOEt. n: NaOEt/EtOH. o: $NaBH_4$/MeOH/THF. p: Ac_2O/DMPA/pyridine, rt. q: NBS/AIBN/n-hexane, reflux. r: γ-collidine/toluene, reflux. s: KOH/MeOH, rt. t: PTAD/CH_2Cl_2, rt. u: TBSCl/imidazole. v: MCPBA/CH_2Cl_2. w: DMI, 140°C. x: TBAF/THF. y: $HO(CH_2)_3OH$/t-BuOK, 110°C. z: 400 W high pressure mercury lamp/THF, 0°C then reflux without mercury lamp.*

stereo- and regioselective introduction of the characteristic 3-hydroxypropoxy group into the 2β-position to give proeldecalcitol (**29**) in 36% yield. Finally **29** was converted to eldecalcitol (**5**) in 23% yield by irradiation at 0°C using a high pressure mercury lamp (400 W, Vycor filter), followed by thermal isomerization in boiling THF (Figure 2) (4). In the linear synthesis from lithocholic acid (**6**) to eldecalcitol (**5**), the 27-step sequence was, however, suboptimal due to its lengthiness and low overall yield (*ca*. 0.03%).

Synthesis of eldecalcitol by the convergent method. Next, we developed a convergent approach based on the Trost coupling reaction (20, 21), in which A-ring fragment **37** and C/D-ring fragment **40** are coupled to produce the triene system of eldecalcitol (**5**) (13, 14). Thus, cleavage of the C_2 symmetrical epoxide **30** (22) with 1,3-propanediol in the presence of potassium *t*-BuOK gave diol **31** in 86% yield. After protection of the primary hydroxyl group to give pivalate **32** in 88% yield, cleavage of the benzyl ether moiety in **32** and subsequent protection of the resulting 1,2-diol as the acetonide gave alcohol **33** in 87% overall yield. Swern oxidation of **33** and subsequent Grignard reaction of the resulting aldehyde with vinylmagnesium bromide (CH_2=CHMgBr) followed by pivaloylation of the resulting alcohol afforded pivalate **34** as an epimeric mixture (*R*/*S*=3/2). Without separation of the epimeric mixture, the acetonide moiety in **34** was cleaved quantitatively to give the diol **35**. Exposure of **35** to Mitsunobu conditions (23) afforded the epimeric epoxide **36** in 77% yield. The acetylene unit was successfully installed by regioselective epoxide-opening of **36** with lithium trimethylsilylacetylide

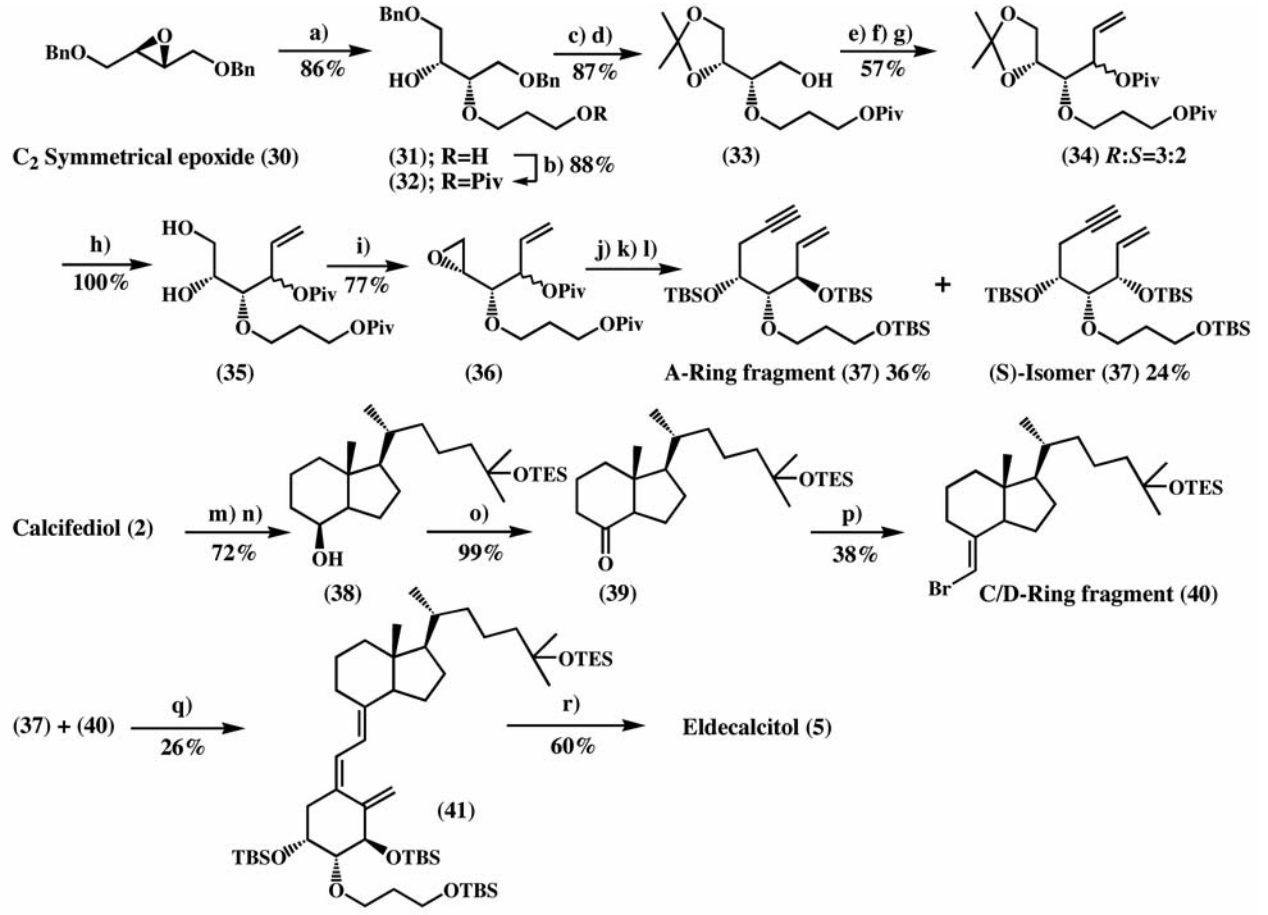

Figure 3. *Convergent synthesis of eldecalcitol (**5**) by coupling A-ring fragment **37** with C/D-ring fragment **40**. Reagents and conditions: a: $HO(CH_2)_3OH$/t-BuOK, 120˚C. b: t-BuCOCl/pyridine/CH_2Cl_2, rt. c: H_2/$Pd(OH)_2$/MeOH, rt. d: $Me_2C(OMe)_2$/TsOH/acetone, rt. e: DMSO/$(COCl)_2$/CH_2Cl_2, –60˚C. f: CH_2=CHMgBr/THF, –60˚C. g: t-BuCOCl/Et_3N/DMAP/CH_2Cl_2, rt. h: 1 M HCl/MeOH, rt. i: Ph_3P/DEAD/benzene, reflux. j: LiC ≡ CTMS/BF_3-OEt_2, –78˚C. k: 10 N NaOH/MeOH, rt. l: TBSOTf/Et_3N/CH_2Cl_2, 0˚C. m: TESOTf/Et_3N/CH_2Cl_2, 0˚C. n: O_3/CH_2Cl_2/MeOH, –78˚C then $NaBH_4$/MeOH, –78˚C. o: NMO/TPAP/4Ams/CH_2Cl_2, rt. p: $Ph_3P^+CH_2BrBr^-$/NaHMDS/ THF, –60˚C to rt. q: $(dba)_3Pd_2$-$CHCl_3$/PPh_3/Et_3N/toluene, reflux. r: TBAF/THF/toluene, reflux.*

(LiC ≡ CTMS) to provide ene-yne **37** as the A-ring fragment for eldecalcitol in 36% yield after protecting group exchange from the pivalate to the *tert*-butyldimethylsilyl (TBS) ether. The accompanying (*S*)-epimer **37** was separated in 24% yield by simple column chromatography (24). The synthesis of the C/D-ring fragment **40** from readily and commercially available calcifediol (**2**) was performed (13). Calcifediol (**2**) was protected as the *bis*-triethylsilyl (TES) ether using triethylsilyl trifluoromethanesulfonate (TESOTf), and was then converted to the alcohol **38** by ozonolysis and treatment with $NaBH_4$ in 72% yield. The hydroxyl moiety in **38** was oxidized to ketone **39** with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) in 99% yield. Wittig reaction of **39** with (bromomethylene)triphenylphosphonium bromide ($Ph_3P^+CH_2Br/Br^-$) and sodium hexamethyldisilazide (NaHMDS) gave rise to bromomethylene **40** as the C/D-ring fragment in 38% yield. Thus, upon treatment of **37** and **40** with triethylamine (Et_3N), triphenyphosphine (PPh_3) and *tris*(dibenzylideneacetone)dipalladium-chloroform [$(dba)_3Pd_2$-$CHCl_3$] in boiling toluene, the coupled product **41** was obtained in 26% yield together with recovered **37** (45%) and **40** (56%). Deprotection of the silyl moiety in **41** with tetrabutylammonium fluoride (TBAF) afforded eldecalcitol (**5**) in 60% yield (Figure 3). Although the overall yield of the convergent synthesis (A-ring fragment **37** from **30**: 10.4%, C/D-ring fragment **40** from **2**: 27.1% and coupling **37** with **40**: 15.6%) was better than the linear synthesis (*ca.* 0.03%), significant improvements were still required for large-scale production. The convergent methodology of production of eldecalcitol, however, proved quite useful for the synthesis of related compounds, such as putative metabolites (25).

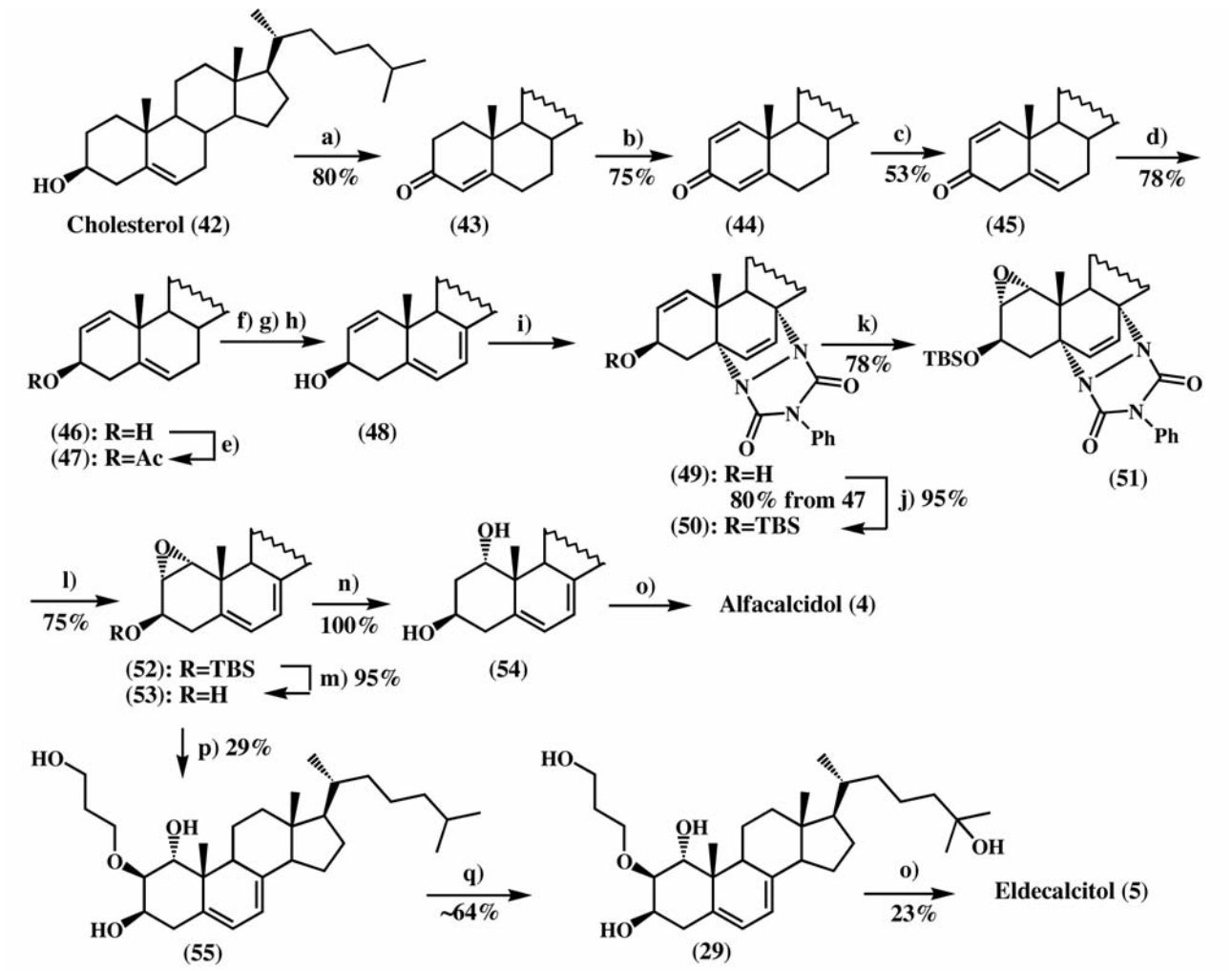

Figure 4. *Industrial synthesis of alfacalcidol (**4**) and biomimetic synthesis of eldecalcitol (**5**) from cholesterol (**42**). Reagents and conditions: a: [Al(Oi-Pr)$_3$]/cyclohexanone. b: DDQ/AcOEt. c: NaOEt/EtOH. d: NaBH$_4$/MeOH/THF. e: Ac$_2$O/DMPA/pyridine, rt. f: NBS/AIBN/n-hexane, reflux. g: γ-collidine/toluene, reflux. h: KOH/MeOH, rt. i: PTAD/CH$_2$Cl$_2$, rt. j: TBSCl/imidazole. k: MCPBA/CH$_2$Cl$_2$. l: DMI, 140°C. m: TBAF/THF. n: NaBH$_4$/EtOH. o: 400 W high pressure mercury lamp/THF, 0°C then reflux without mercury lamp. p: HO(CH$_2$)$_3$OH/t-BuOK, 110°C. q: Microbial 25-hydroxylation.*

Synthesis of eldecalcitol by the biomimetic method. Figure 4 shows the practical synthesis of alfacalcidol (**4**), which has been completely established during the manufacturing production (16). Thus, cholesterol (**42**) was oxidized with aluminum isopropoxide [Al(O*i*-Pr)$_3$] in 80% yield to 4-en-3-one **43**, which was further oxidized with 2,3-dichloro-5,6-dicyano-1,4- benzoquinone (DDQ) to 1,4-dien-3-one **44** in 75% yield. Treatment of **44** with sodium ethoxide (NaOEt) gave 1,5-diene-3-one **45** in 53% yield, which was reduced with NaBH$_4$ yielding 3β-hydroxy-1,5-diene 46 in 78% yield. After protection of hydroxyl moiety in **46** as acetate **47**, the 5,7-diene system in **48** was fashioned through bromination with NBS/2,2-azobisisobutyronitrile (AIBN) in hexane and dehydrobromination with γ-collidine in toluene follwed by deacetylation. The 5,7-diene moiety in **48** was protected *via* adduct formation with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to give the PTAD adduct **49**, in 80% yield from **47**. The hydroxyl group in **49** was protected as its TBS ether **50** in 95% yield, which was then regio- and stereoselectively epoxidized with m-chloroperbenzoic acid (MCPBA) to give 1,2α-epoxide **51** in 78% yield. Retro-cycloaddition of PTAD adduct **51** to regenerate the 5,7-diene system in 52 was carried out by simply heating (140°C) **51** in 1,3-dimethyl-2-imidazolidinone (DMI) in 75% yield (26). The 3β-hydroxyl moiety in **53**, obtained by deprotection of TBS group in 52 with TBAF, contributed to the regio- and stereoselective cleavage of epoxide ring with NaBH$_4$ to give proalfacalcidol (**54**), quantitatively. Finally, **54** was subjected to photolysis

and thermal isomerization to afford alfacalcidol (**4**) (Figure 4) (16). Since microbial 25-hydroxylation of steroidal and secosteroidal side chain was known (27, 28), we applied this methodology to the preparation of proeldecalcitol (**29**). Thus, 1,2α-epoxide **53** was cleaved by 1,3-propanediol in the presence of *t*-BuOK to introduce the 3-hydroxypropoxy group at the 2β-position giving **55** in 29% yield (4). By culturing *Amycolata autotrophica* ATCC 33796, hydroxylation of **55** at the 25-position was successfully carried out to afford proeldecalcitol (**29**) in moderate yield (17), which was converted to eldecalcitol (**5**) by irradiation and thermal isomerization as described above (Figure 4). The yields for converting steroidal framework in **29** to secosteroidal structure in **5** by photolysis and thermal isomerization are usually moderate to low, since several structural variants such as lumisterol, tachisterol, *etc*. are also formed.

Conclusion

The summary of process development for eldecalcitol (Figure 1, **5**) between the linear synthesis, convergent synthesis, and biomimetic synthesis is shown in Table I. The biomimetic synthesis was adopted for the practical production of **5** and has been used in industrial-scale preparations. The detailed methodology of the biomimetic synthesis including microbial hydroxylation of **55** will be reported in due course.

Table I. *Summary of process development for eldecalcitol (**5**).*

Synthesis	Steps	Total yield (%)
Linear	27	~0.03
Convergent	4 (A), 12 (C/D), 2 (CP)	10.4 (A), 27.1 (C/D), 15.6 (CP)
Biomimetic	16	~0.5

A: Preparation of A-ring fragment. C/D: Preparation of C/D-ring fragment. CP: Coupling reaction.

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