Development of Efficient Chemical Syntheses of Vitamin D Degradation Products

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Abstract. *In recent years it has been recognized that vitamin D deficiency is associated with a wide range of diseases, including various types of cancers. Due to the enormous medical importance of vitamin D and its metabolites, their status in blood serum has to be accurately measured. Thus, the metabolites actually used as reference standards and also others of relevant biological activity have to be provided for validation and continuous improvement of appropriate diagnostic devices. Efficient chemical syntheses of vitamin D derivatives described in the literature are herein proven in a comparative study and applied to the synthesis of some of the most relevant natural metabolites as representative examples.*

Vitamin D and its metabolites are known for many distinguished roles in human physiology and pharmacology (1-4). Vitamin D is responsible for intestinal absorption of calcium and phosphate, and is thus particularly critical to the process of bone mineralization. Vitamin D deficiency is considered to be associated with a wide range of diseases (5), such as rickets, osteoporosis, psoriasis, leukemia, breast and prostate cancer, Alzheimer disease, AIDS, cardiovascular diseases, as well as autoimmune disorders, such as multiple sclerosis. On the other hand, vitamin D toxicity, caused by high-dose supplementation, may lead to calcemia and presumably also other disorders.

Due to this enormous medical importance for disease prevention, and therapy (6), and because it has become obvious in recent years that approximately 60% of all citizens in industrialized countries suffer from vitamin D deficiency, the status of vitamin D and its natural metabolites in human serum has to be carefully and regularly analyzed and monitored. However, the biological role of more than 40 natural vitamin D metabolites (1) known to date is still not fully understood although the metabolic pathways involved are quite well-established (1, 4). Vitamin D (**1**), generated either from 7-dehydrocholesterol in the skin by sunlight irradiation or supplied by food ingredients, is hydroxylated in the liver to 25-hydroxyvitamin D_3 (**2**), and then in the kidney (and also other tissues) to the most relevant metabolite 1,25-dihydroxyvitamin D_3 (calcitriol) (**3**) (Figure 1). Compounds **2** and **3** are degraded separately following two pathways, usually by successive oxidation and degradation of their side-chains, respectively. In both pathways, the same enzyme 24-hydroxylase (CYP24) is mainly involved, as also in the metabolism of vitamin D analogs.

In most common assays for determining a vitamin D status (7-10) in blood serum or plasma, the inactive metabolite **2** is measured instead of **1** or **3**, because the concentration of **2** is considered to correlate well to that of **1** and **3** (1000-fold to 3) and is maintained in the serum for many days at comparably high concentrations (18-36 μg/l, half life 4-6 h). However, other metabolites, such as **4-6** and their corresponding 1α-hydroxylated as well as D_2 derivatives should not be neglected, mainly due to their interference with metabolites assayed (7, 8), and also due to their potential, but widely uncertain, biological activity.

We have proven and optimized various alternative approaches described in the literature towards the synthesis of metabolites **2-6**, as well as their corresponding 1α-hydroxylated and partly D_2 derivatives, and review herewith the most efficient chemical syntheses of the naturally occurring vitamin D degradation products **4-6** as representative examples, particularly because the metabolites **2-6** are all actually measured in currently available clinical assays. The aim of our work was mainly to explore the most efficient approach for the synthesis of these metabolites, to make them available in gram quantities to be employed either as reference standards, as starting materials or building blocks for the synthesis of further metabolites or analogs, or to be used for biological studies.

Materials and Methods

Although a wide variety of chemical syntheses of vitamin D metabolites and their analogs are described in the literature (1, 11, 12), not all of them are suitable for producing gram quantities of the desired target molecule. In Figure 2, three general synthetic

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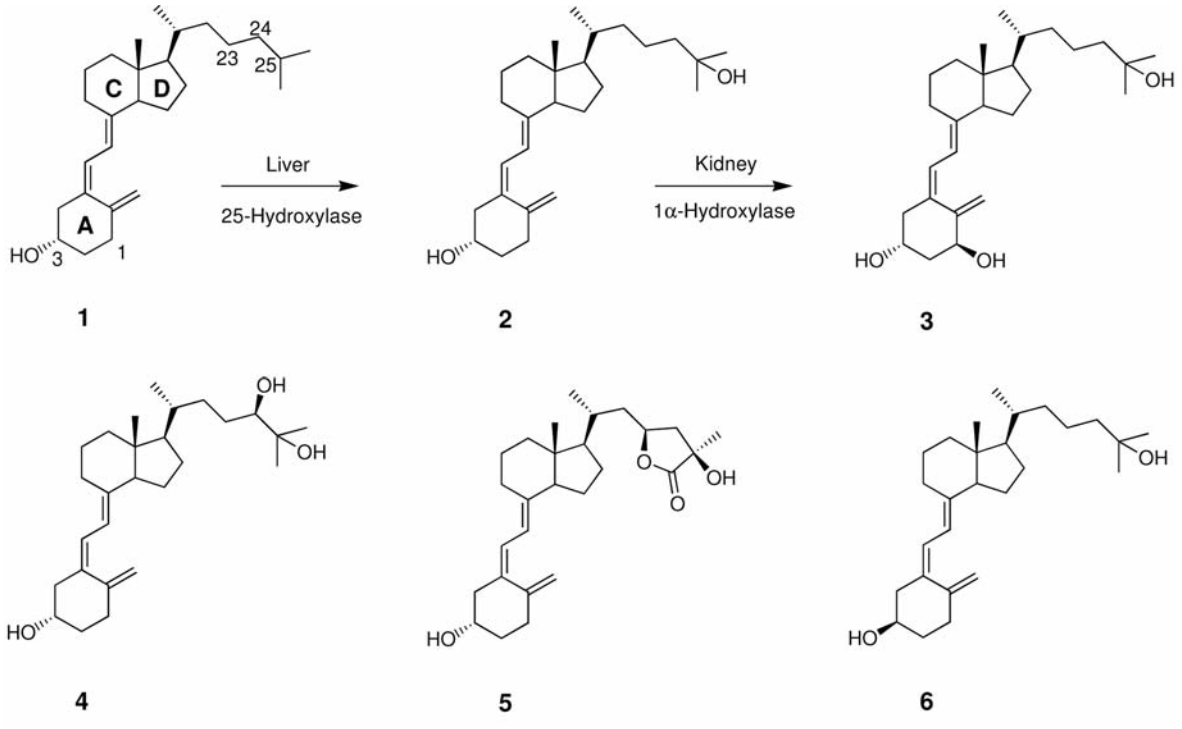

Figure 1. *Metabolites of vitamin D_3.*

strategies are outlined: The first one (route A) is the biosynthetic pathway (also technically applied for the synthesis of vitamin D_3 and D_2). In this approach, a 7-dehydrocholesterol derivative is irradiated by UV light, and the intermediate product (previtamin D) is isomerized thermally to a corresponding vitamin D derivative. By application of the second strategy (routes B-E), an A-ring and a CD-ring precursor are synthesized independently, which are finally connected after appropriate chemical modifications in the course of a convergent synthesis. In the third approach (routes F-G) a vitamin D_3 or D_2 molecule is appropriately modified in the course of a linear synthesis, leaving the vitamin D skeleton intact. We applied all three approaches in order to explore the most efficient one for the synthesis of each metabolite of interest.

Results

*Synthesis of 24R,25-dihydroxyvitamin D_3 (**4**) (Figure 3).* The most feasible synthesis of this metabolite, which is described in detail in (13) and references therein, follows a convergent approach (route D in Figure 2). It starts with readily available vitamin D_2 **7**. $KMnO_4$ oxidation and silylation of the 3-OH group with *tert*-butyldimethylsilyl chloride yielded **8**. Oxidative cleavage of **8** with Pb(IV) acetate, and subsequent reductive workup with sodium borohydride ($NaBH_4$) gave separately appropriate precursors of A-ring (**9**) and CD-ring (**10**). **9** was treated with N-chlorosuccinimide to yield **11**, which was converted to **12** by adding lithium diphenylphosphide and hydrogen peroxide. **10** was submitted to an ozonolysis, to yield after reductive workup with $NaBH_4$ diol **13**. In order to produce sufficient quantities of diol **13**, vitamin D_2 was preferentially directly submitted to an ozonolysis, followd by reductive workup with $NaBH_4$. Subsequently, **13** was converted into iodide **14**, which was added in a Zn/Cu-mediated coupling, supported by ultrasound, to enone **15**. **15** was obtained by addition of pivaldehyde to lactic acid, in turn available in both enantiomeric configurations, either one or the other to be employed depending on the desired configuration at the later 24 position in the product, and subsequent bromination and elimination. The resulting coupling product **16** was treated with methylmagnesium bromide in the course of a Grignard reaction, and the resulting 1,2 diol moiety of the resulting triol **17** was protected as an isopropylidene ketal. The remaining free alcohol group was oxidized with pyridinium dichromate to yield ketone **18**, which was coupled in the course of a Wittig reaction with A-ring phosphine oxide **12** to give **19**. Removal of the silyl protective group with tetra-*n*-butylammonium fluoride and acidic hydrolysis of the ketal finally led to production of **4**.

*Synthesis of (23S,25R)-25-dihydroxyvitamin D_3 26,23-lactone (**5**) (Figure 4) (14-23).* Analogously to the successful previously described synthesis of **4**, a convergent approach appeared to be most suitable, using A-ring precursor **12** and CD-ring building block **10**, both obtained by oxidative

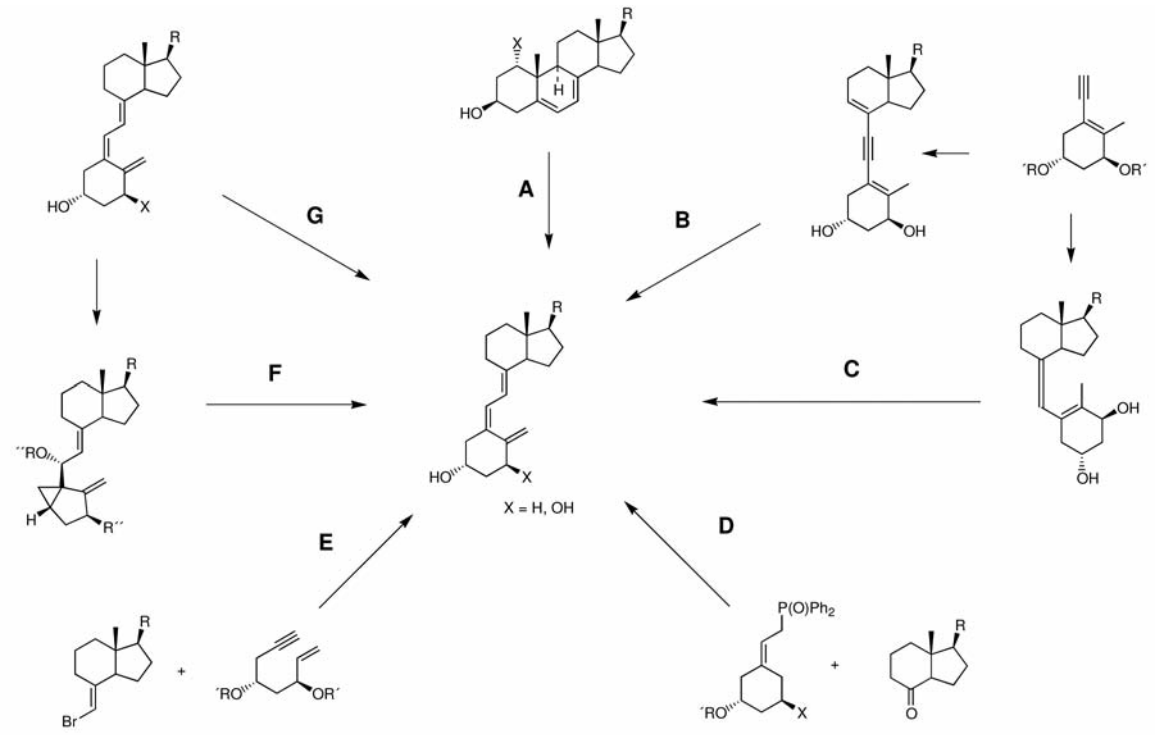

Figure 2. *Approaches towards the synthesis of vitamin D metabolites.*

degradation from vitamin D_2 (**7**). In order to introduce the appropriate side chain, isopentenol **22** was submitted to a stereoselective Sharpless dihydroxylation (20). The resulting 1,2 diol moiety was protected as an isopropylidene ketal, yielding **23**, which was transformed into sulfone **24** by successive treatment of **23** with *p*-toluene sulfonyl chloride, thiophenol/potassium t-butoxide and *m*-chloroperoxybenzoic acid. In the course of a Julia olefination (23), **24** was added to the corresponding CD-ring aldehyde **21**, in turn available from **10** by silylation and oxidative degradation of **20** by ozonolysis. Surprisingly, a Wittig reaction of a phosphine oxide corresponding to **24**, with **21** was not successful. The isopropylidene ketal at **25** was removed under acidic conditions using pyridinium *p*-toluenesulfonate, and the primary alcohol moiety was oxidized to yield **26**, which was submitted to an iodolactonization, yielding after removal of the iodo substituent at **27** by tributyltin hydride to a mixture of diastereomers **28**, which were employed in the next steps without further purification. Desilylation of **28** and oxidation of the resulting alcohol **29** with pyridinium dichromate gave **30**, which was coupled as previously described in the course of a Wittig reaction with A-ring phoshine oxide **12** to yield **31**. Final desilylation and submission of the resulting product mixture to preparative High Pressure Liquid Chromatography (HPLC) led to diastereomerically pure (23S,25R) diastereomer **5**.

*Synthesis of 3-epi-25-hydroxyvitamin D_3 (**6**) (Figure 5).* For the synthesis of this metabolite, a linear, non-convergent approach, corresponding to a well-established technical calcitriol (**3**) synthesis (24-29) was chosen. By contrast, in the first step the 3-OH group was inverted under Mitsunobu conditions by treatment with diethyl azodicarboxylate and -nitrobenzoic acid, followed by lithium aluminium hydride, leading to **32**, although obtained in poor yield (21%). Protection of the 3-OH group as a *tert*-butyldimethylsilyl ether, conversion in a SO_2 adduct and subsequent degradation of the side-chain by ozonolysis and reductive workup with $NaBH_4$ gave **33**. Tosylation and removal of the SO_2 protective group led to **34**, which was coupled by a Grignard reaction with **35**, leading to **36** after prior desilylation of the coupling product. Alternatively, **33** was converted into the iodide **37**, which was added in a Ni/Zn-mediated Michael-type reaction to ethyl acrylate. The resulting ethyl ester was treated with methyl magnesium bromide to give **36** in comparably good yields. **36** was finally isomerized photochemically (25, 26) to give **6**.

Discussion

Although the synthesis according to the biosynthetic pathway, starting from a 7-dehydrocholesterol derivative, is technically applied, at least for the synthesis of vitamin D_3 and

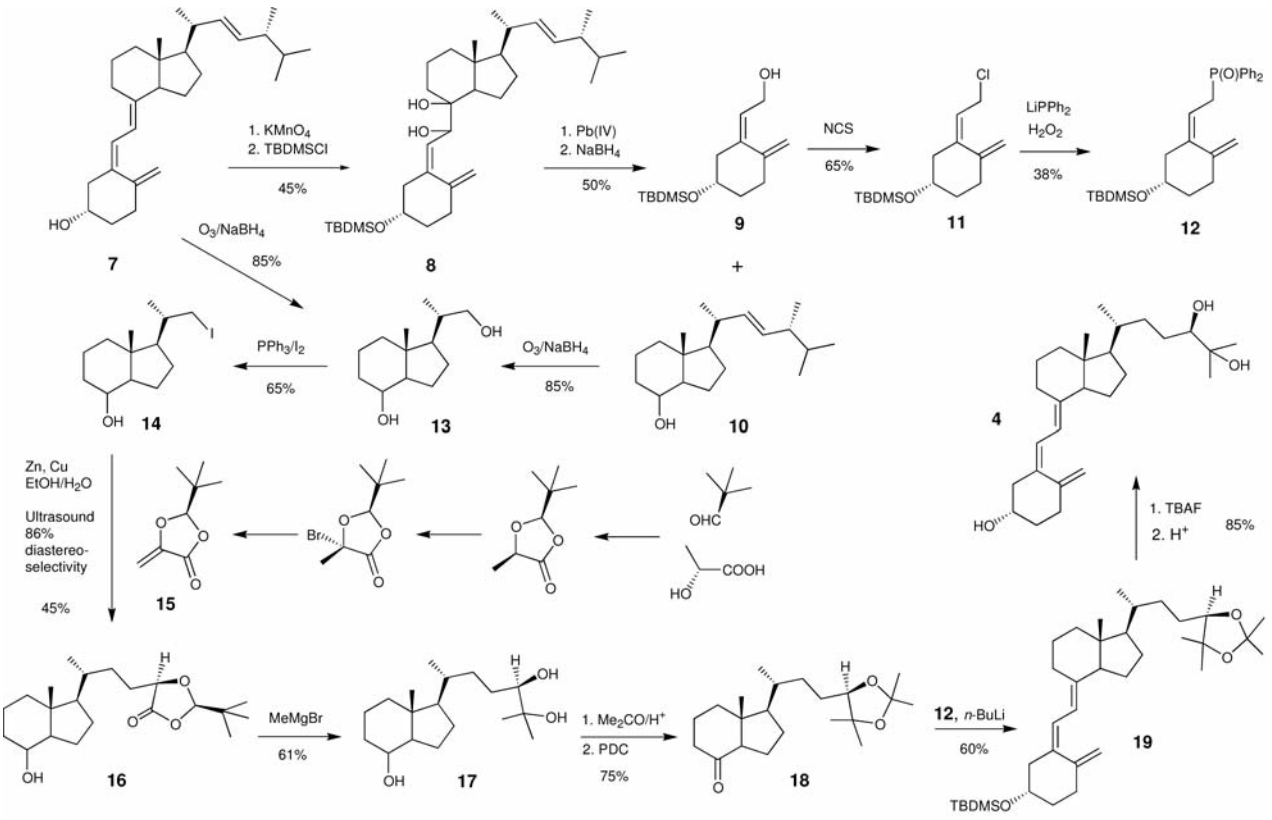

Figure 3. *Synthesis of 24R,25-dihydroxyvitamin D_3. TBDMSCl: tert-butyldimethylsilyl chloride, NCS: N-chlorosuccinimide, $LiPPh_2$: lithium diphenylphosphide, PDC: pyridinium dichromate, n-BuLi: n-butyllithium, TBAF: tetra-n-butylammonium fluoride.*

analogously for vitamin D_2, this approach turned-out to be less favorable for the synthesis of their metabolites, because the two-step transformation (photochemical ring opening and thermal isomerization), a synthesis sequence to be performed rather at the end than at the beginning of a suitable synthesis, led to very low yields (*i.e.* 20%). All most feasible syntheses successfully applied on laboratory scale started from readily available vitamin D_2. Depending on substituents to be introduced and transformations necessary, the most straightforward approach appeared to be the option leaving the vitamin D skeleton intact, and to employ an SO_2 adduct as an intermediate, as established for the technical calcitriol synthesis and analogously for the described synthesis of 3-*epi*-25-hydroxyvitamin D_3 (**6**). However, because the SO_2 adduct does not tolerate strong bases, acids, elevated temperatures or otherwise harsh reaction conditions, which have to be applied to synthesize most other metabolites, this approach has its limitations. Consequently, the most flexible, although more lengthy route involves the cleavage of vitamin D_2 into an A-ring and a CD-ring, appropriate modification of the CD-ring precursor and subsequent coupling with accordingly modified A-ring phoshine oxide **12**. Finally it should be noted that the corresponding 1α-hydroxylated derivatives of **2** and **4-6** can easily be synthesized by allylic selenium dioxide oxidation at an appropriate synthesis step (29).

Conclusion

Efficient chemical syntheses of vitamin D degradation products in gram quantities are favorably carried-out successfully starting from vitamin D_2, either in a convergent or linear approach, depending on substitution pattern and structural elements to be envisaged.

Acknowledgements

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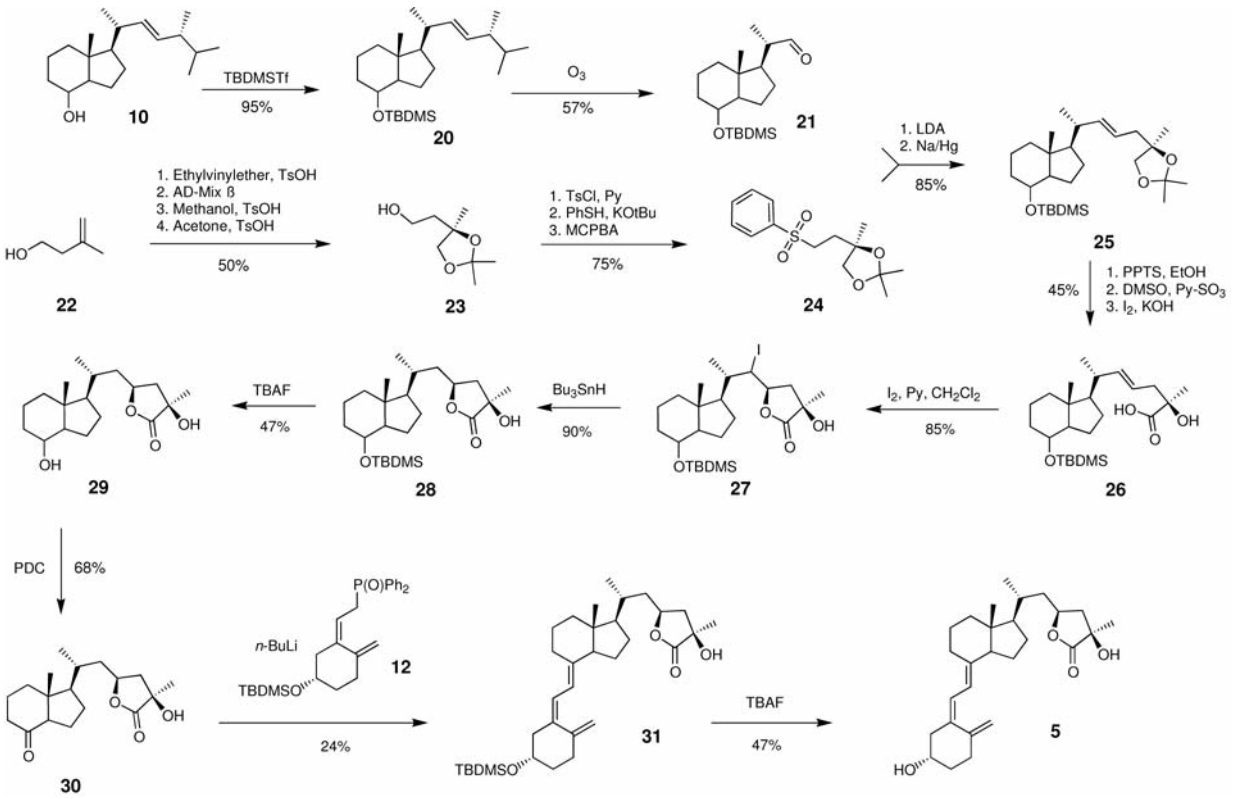

Figure 4. *Synthesis of (23S,25R)-25-dihydroxyvitamin D_3 26,23-Lactone. TBDMSTf: Trifluoromethanesulfonic acid tert-butyldimethylsilyl ester, TsOH: p-toluenesulfonic acid, TsCl: p-toluenesulfonyl chloride, Py: pyridine, PhSH: thiophenol, KOtBu: potassium tert-butoxide, MCPBA: meta-chloroperoxybenzoic acid, LDA: lithium diisopropylamide, PPTS: pyridinium p-toluenesulfonate, DMSO: dimethyl sulfoxide, Bu3SnH: tributyltin hydride, TBAF: tetra-n-butylammonium fluoride, PDC: pyridinium dichromate.*

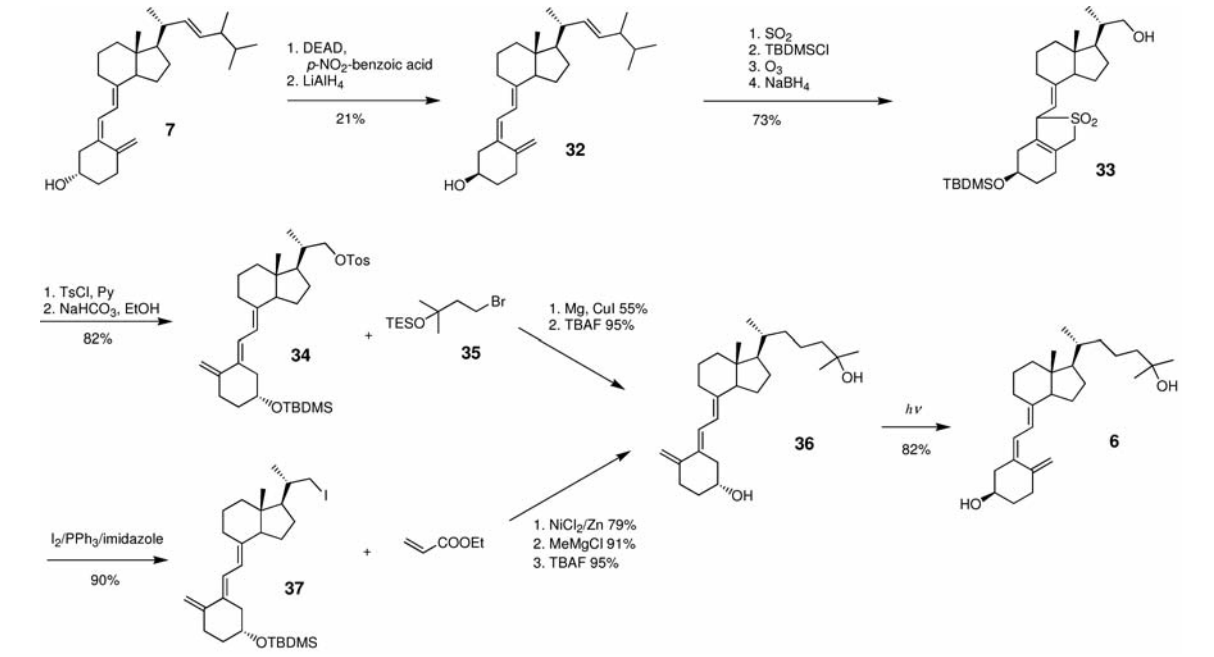

Figure 5. *Synthesis of 3-epi-25-hydroxyvitamin D_3. DEAD: Diethyl azodicarboxylate, TBDMSCl: tert-butyldimethylsilyl chloride, TsCl: p-toluenesulfonyl chloride, Py: pyridine, TES: triethylsilyl, TBAF: tetra-n-butylammonium fluoride.*

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