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Review

Recent Developments Towards the Synthesis of Vitamin D Metabolites

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Abstract. *Due to latest advancements in the development of LC-MS/MS based assays and their applications in clinical chemistry, low abundant vitamin D metabolites can be detected at low concentration with high specificity. Consequently, there is a growing need for their synthesis as stable isotopically labeled compounds to be used as calibration and reference standards. Most commonly, ex chiral pool synthesis, starting with vitamin D_2, and palladium(0) catalyzed coupling reactions are applied in vitamin D synthesis. By contrast, little is known so far regarding the application of cobalt complexes for this purpose. In fact, the Pausen-Khand reaction can be applied for the synthesis of a wide range of isotopically labeled vitamin D metabolites that are hardly accessible by other known methods. Applications, scope and limitations of the most recent vitamin D synthesis methods, with a particular focus on this new promising methodology using Co-complexes, are discussed.*

The function of vitamin D metabolites in human physiology is considered a major health issue (1, 2). Consequently, enormous efforts have been undertaken in the past decades in vitamin D synthesis (3-4), mainly due to the rationale that analogs of vitamin D (5) could be developed as new drugs to treat vitamin D-dependent diseases, particularly various types of cancer (6-8). These endeavors apparently have largely been disappointing, because most analogs of vitamin D are not soluble under physiological conditions, and have been shown to exhibit toxic (calcemic) effects or tend to be metabolized too rapidly. Although, some recently developed analogs [**1-3**], shown in Figure 1, appear promising to overcome these problems (9-12).

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Consequently, vitamin D synthesis is still of significant importance. Particularly, the development of new LC-MS/MS based assays to measure the concentration of vitamin D metabolites, not only for assessment of vitamin D deficiency but also for the diagnosis of vitamin D related diseases, is of growing interest for academia as well as for the pharmaceutical industry (13-17).

Metabolism of Vitamin D

The main pathways of vitamin D metabolism are shown in Figure 2 (2, 18). By UV radiation and thermal exposure, 7-dehydrocholesterol **4** is converted in the skin to Vitamin D_3 **5**, that is hydroxylated in the liver to 25OHVitD_3 **6**, which in turn is hydroxylated in the kidneys to the presumably most active metabolite **7** (1,25$(OH)_2$VitD_3, calcitriol). Metabolism occurs mainly *via* two pathways, starting with hydroxylation at C24, either by formation of 1,24,25$(OH)_3$VitD_3 **8** or 24,25$(OH)_2$VitD_3 **9**. Additionally, 23,26-lactones **10** and **11** can be formed along these two pathways, although these metabolites appear not to be relevant to humans due to a lack of 23-hydroxylase. Additionally, 3-*epi*-isomers **12-15** have been identified (19-22). As an endpoint of the metabolism process, calcitroic acid **16** is considered as the major metabolite, bearing a carboxyl group for renal clearance (23). Finally, vitamin D_2 [**17**] and its corresponding metabolites have to be considered as well, because they also appear in human blood and tissues and exhibit significant activity as compared to their vitamin D_3 counterparts (24).

Synthesis of Vitamin D Metabolites

It appears that it is not feasible to start the synthesis in a chemical lab with 7-dehydrocholesterol **4**, due to low yields in the B-ring opening step of the steroid skeleton. Most practical methods (Figure 3) start with readily available vitamin D_2 [**17**].

In a linear synthesis (upper branch in Figure 3) (25), the vitamin D skeleton is left intact, the C3-OH and the C5/6-

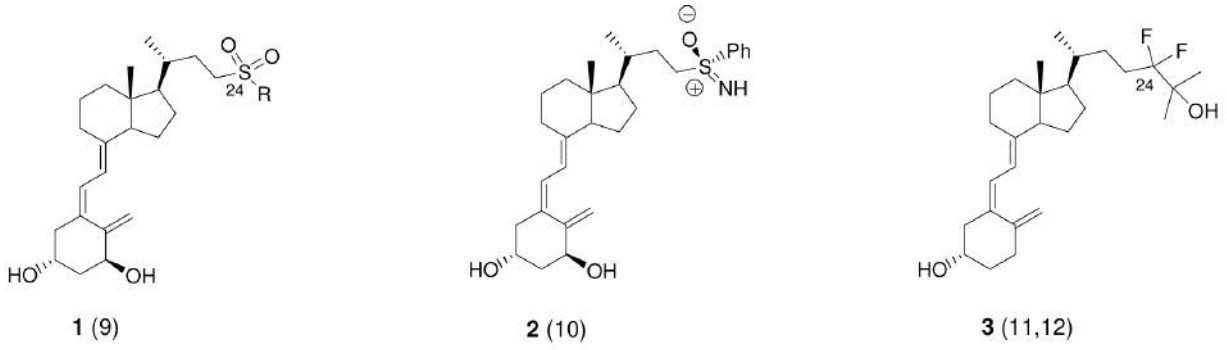

Figure 1. *Vitamin D analogs*.

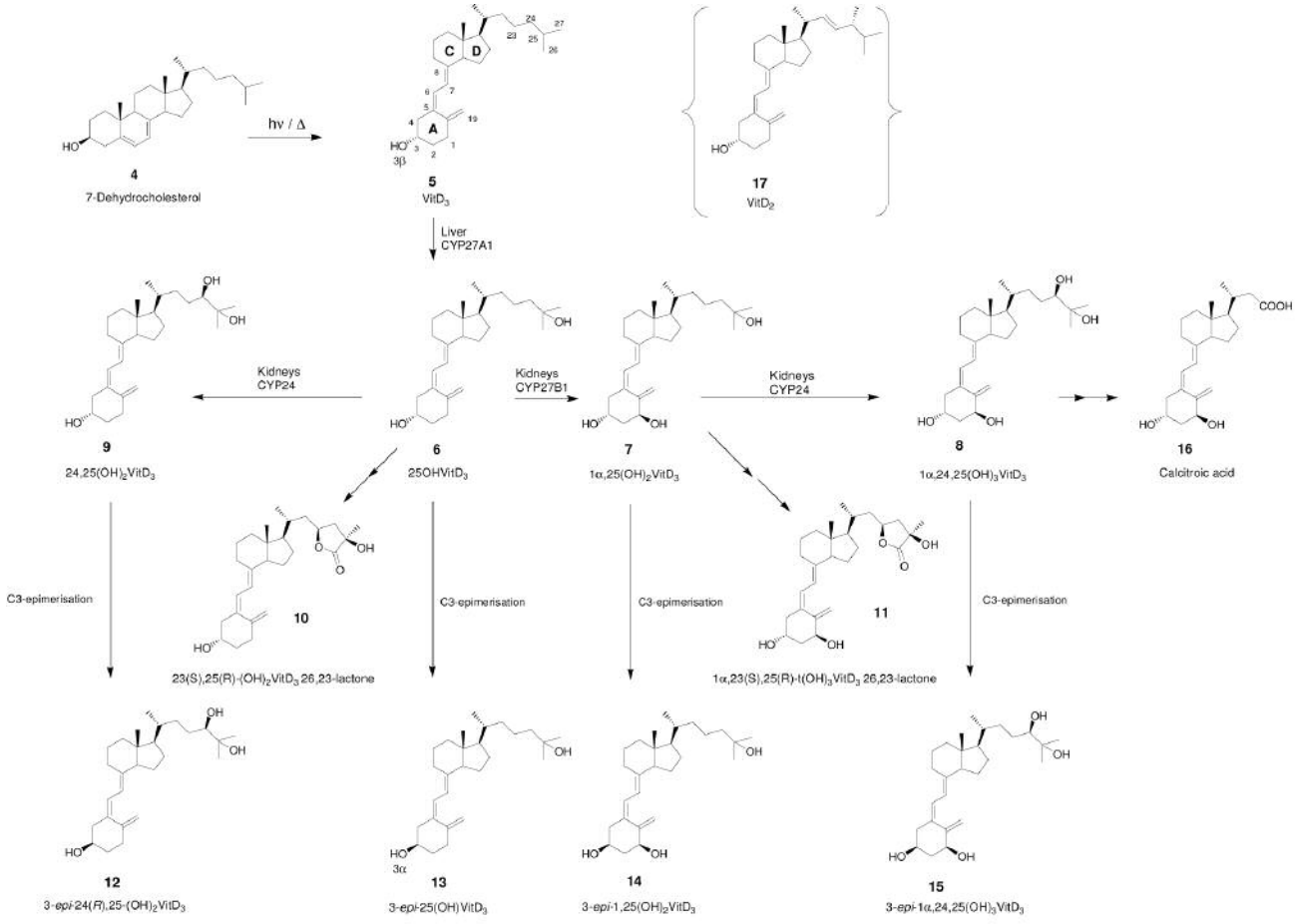

Figure 2. *Metabolism of vitamin D*.

C10/19 cis-diene moiety are protected and the side chain is oxidatively removed, leading to **18**. The C1-position can be hydroxylated in the course of the synthesis, and an accordingly modified side chain is attached, giving after photoisomerization **19a/b**. Alternatively, as a more flexible approach (lower branch in Figure 3) (26-28), in the course of a connective (*i.e.* convergent) synthesis, vitamin D_2 **17** is cleaved in an A-ring and a CD-ring, both parts are modified separately and are finally connected. By application of this strategy, vitamin D_2 is converted in its *tert*-butyldimethylsilyl (TBDMS) ether and cleaved into an A-ring allylic alcohol **20** and a CD ring diol **21**. The A-ring and

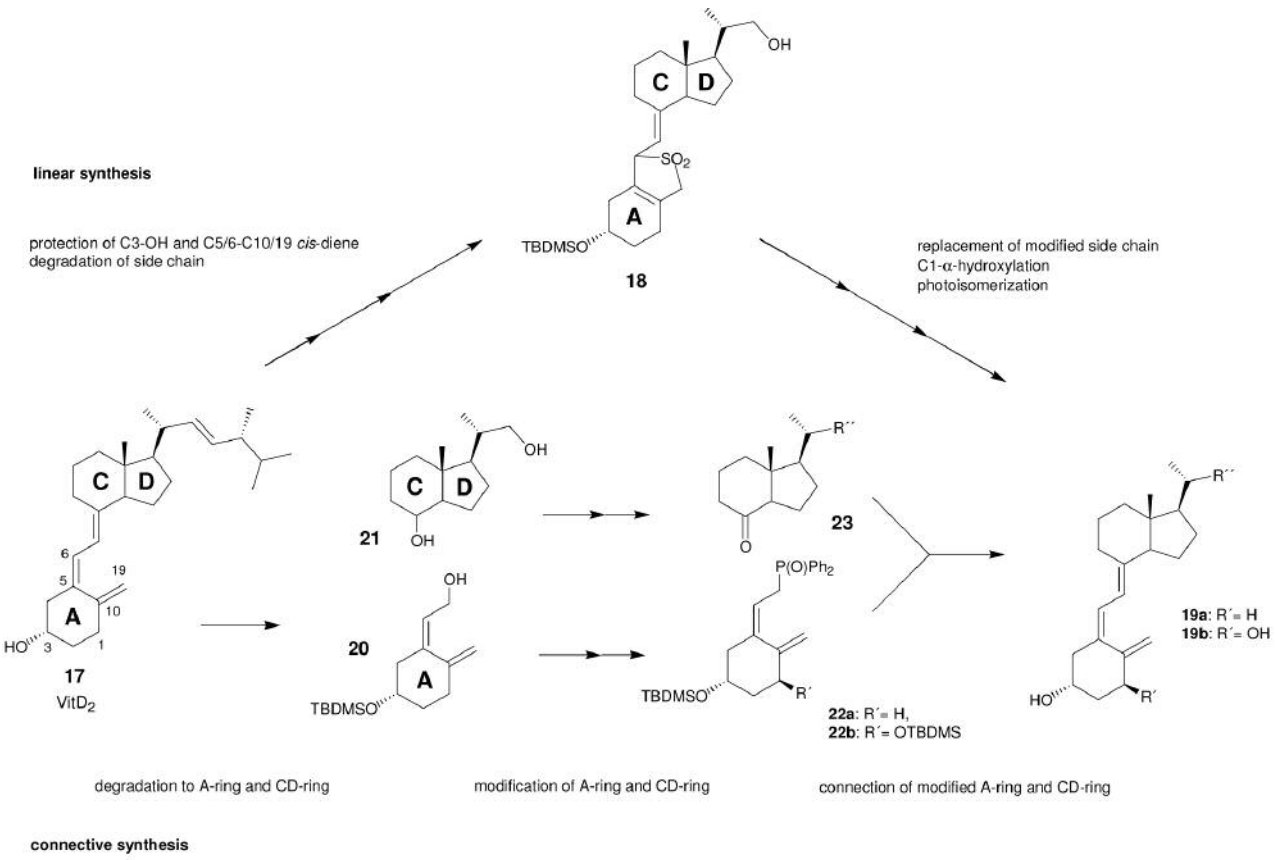

Figure 3. *Practical synthesis of vitamin D metabolites starting from vitamin D_2. TBDMS: tert-butyldimethylsilyl.*

the CD-ring are modified accordingly. The allylic alcohol **20** is converted in a phosphine oxide **22a/b**. After the side chain of the CD-ring is prepared separately, it is attached to the CD ring building block, leading to ketone **23**. Finally, both parts are connected by a Wittig-Horner reaction to obtain **19a/b**. If isotopically labeled compounds are needed, as for drug monitoring studies or as reference standards for measurement of any vitamin D metabolite in LC-MS/MS assays, accordingly labeled starting material can be used, leading to metabolites labeled by deuterium (D) or ^{13}C in the side chain. As a representative example, the synthesis of 25OHVitD$_3$-d6 [**6**-*d*6] is shown in Figure 4.

Diol **21** is converted in its corresponding iodide **24**, that is connected with ethyl acrylate in a copper/zinc mediated reaction to **25**. In this step, ^{13}C labeled acrylate can be employed to obtain the corresponding ^{13}C labeled metabolite. By Grignard addition of deuterated methyl iodide, the precursor **26**, bearing a 6-fold labeled C25-OH side chain, is obtained. Following oxidation with pyridinium chlorochromate (PCC) and protection of the C25-OH group of **27** as its *tert*-butyldimethylsilyl ether, the resulting ketone **28** is coupled by Wittig-Horner reaction with diphenylphoshine oxide **22a**, giving, after removal of the silyl protective groups, 25OHVitD$_3$-*d6* (**6**-*d6*).

As an alternative approach, pioneered by Trost in the early 90´s, palladium (Pd(0)) catalyzed tandem-reactions can be applied (Figure 5) (29). In this method, an acyclic enyne **29** is coupled with a CD-ring vinyl bromide **31**, in turn obtained from the corresponding ketone **30**. Closure of the A-ring and its connection with CD-ring can be carried out in one pot, yielding **32**. Alternatively, the corresponding trifluoro sulfonate **33** can be employed in a Zn-mediated reaction, which was invented by Negishi (30). Additionally, the CD-ring alkene boranates **34** can be used *via* an analogous coupling method originally invented by Suzuki (31). Finally, a cyclic A-ring alkene **35** can be coupled with CD ring triflate **36**, according to a coupling method invented by Sonogashira (32).

In all these cases, the CD-ring vinyl bromide, boranate or triflate are usually carrying the modified side chain already, before coupling with the acyclic or cyclic A-ring enyne. A major drawback of these methods is the fact that synthesis of acyclic

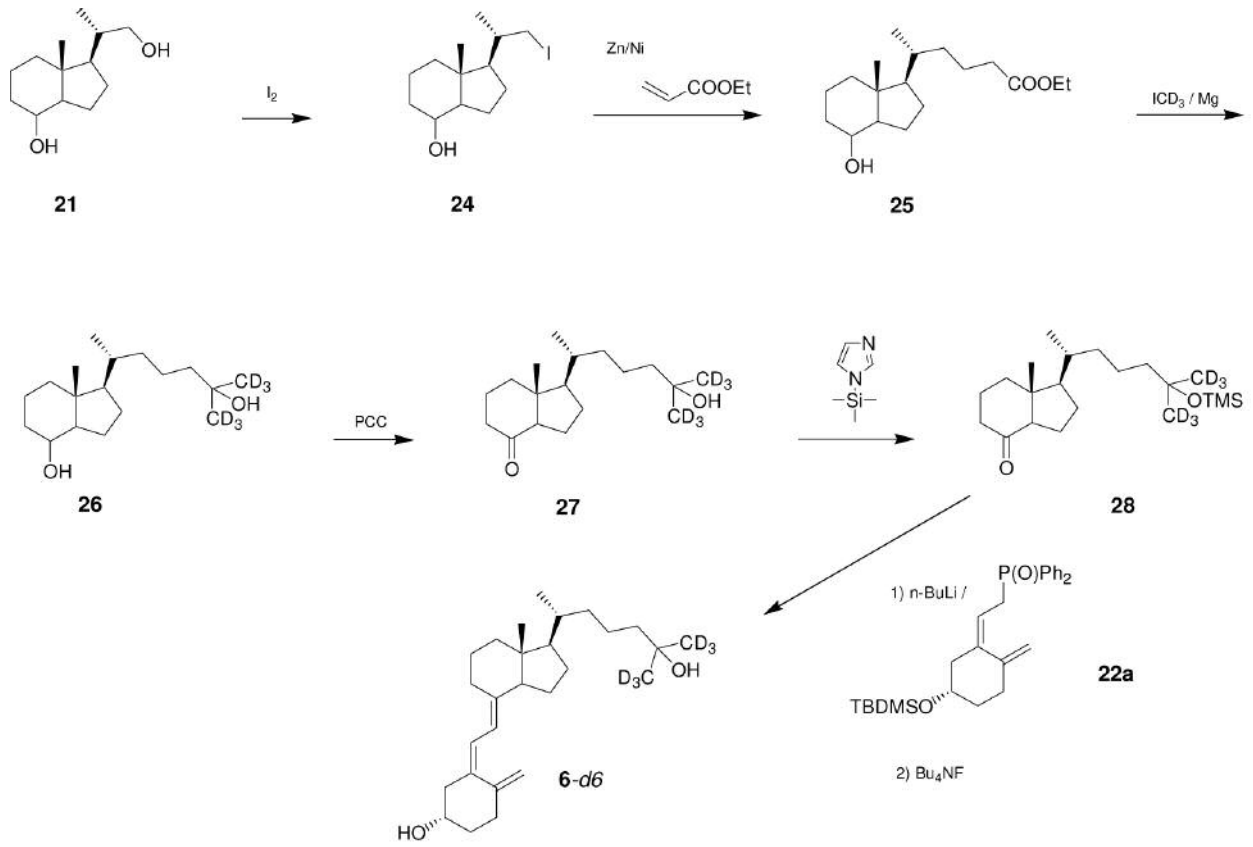

Figure 4. *Synthesis of 25OHVitD$_3$-d6. TBDMS: tert-butyldimethylsilyl; TMS: trimethylsilyl, PCC: pyridinium chlorochromate; n-BuLi: n-butyllithium; Ph: phenyl; Bu$_4$NF: tetra-n-butylammonium fluoride.*

or cyclic enynes or other A-ring building-blocks not derived from vitamin D_2 involves many synthesis steps, and in some cases separation of diastereomeric mixtures is also necessary to obtain enantiomerically and diastereomerically pure product. However, suitable starting materials can usually be obtained from the natural chiral pool (*i.e.* terpenes, malic acid, quinic acid or carbon hydrates such as D-glucose or D-xylose), which are usually readily available. Consequently, these advanced strategies involving Pd(0) catalyzed reactions have widely been applied in recent years and are by now well established.

As a drawback of all methods starting the synthesis with vitamin D_2, isotopic labeling is installed in the side chain, leading to loss of labeling during enzymatic degradation. To overcome this problem, labeling of the CD-ring system is highly desirable, but challenging. An alternative approach is a *de novo* synthesis of both ring systems, where a labeling by deuterium (D) or ^{13}C can be installed at the CD-ring system. As a practical alternative, the CD-ring can be synthesized *via* the Pausen-Khand reaction (Figure 6). In this reaction, a $Co_2(CO)_8$ complex in the presence of CO mediates the coupling of an alkene and an alkyne, by concomitant insertion of CO, leading to the formation of a cyclopentenone. Thus, by employing an appropriate enyne **37**, that is subsequently silylated to **38**, a bicyclic ring system **39** is built in the course of a Pausen-Khand reaction, that can be transformed *via* **40** in the methylated CD-ring precursor **41** by addition of methyl cuprate ($(CH_3)_2CuLi$). If isotopically labeled cuprate, such as $(CD_3)_2CuLi$ or $(^{13}CH_3)_2CuLi$ is employed, the corresponding C18-labeled CD-ring can be obtained. However, methylation leading to the desired *trans*-hydrindane is challenging to achieve. Due to constraints of the transition state of the methylation reaction, usually by application of common methods exclusively cis-hydrindane is formed, or the reaction fails at all. However, a Si assisted reaction using isocyanates, invented by Mouriño (33, 34), actually leads to the formation of the desired product, that can be converted in diol **21** by a few routine steps.

Conclusion

Pauson-Khand reaction of enynes, following by Si-assisted allylic substitution, employing isocyanates, enables the synthesis of a wide range of vitamin D metabolites,

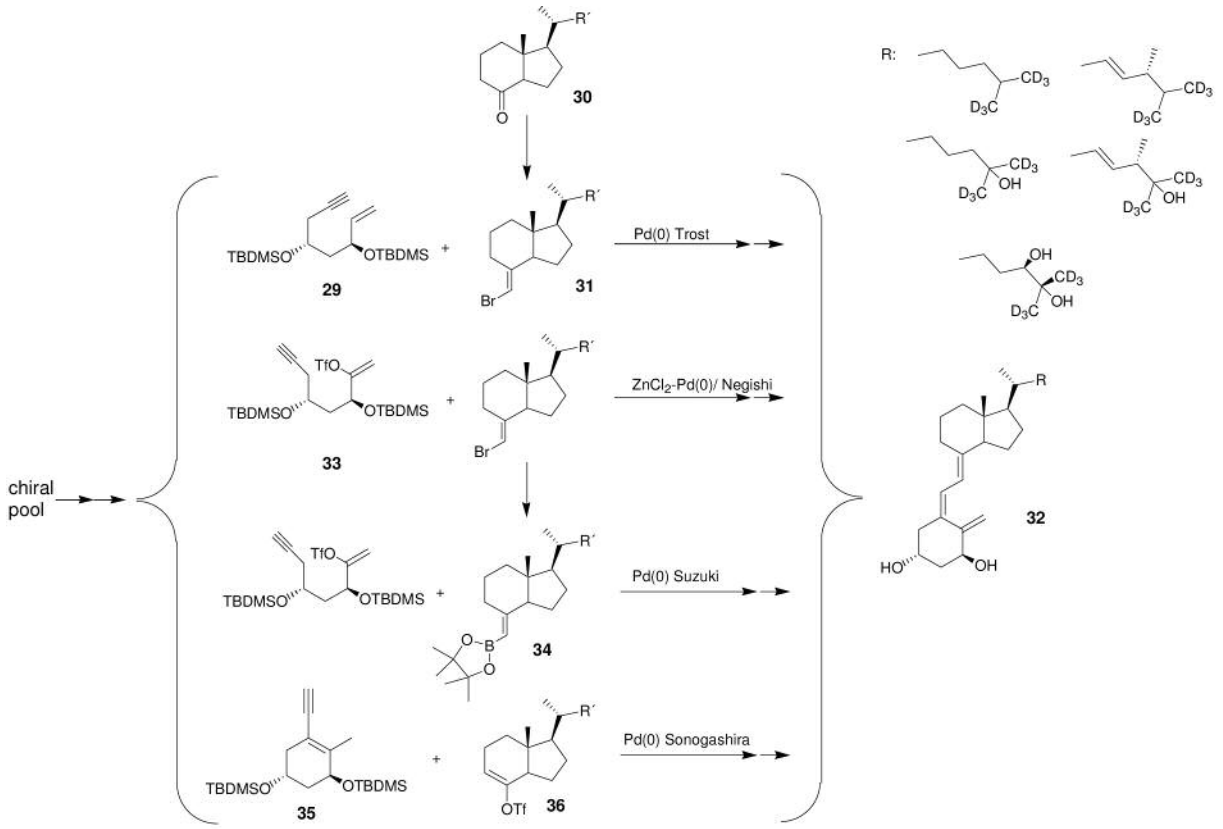

Figure 5. *Pd(0) catalyzed vitamin D synthesis. TBDMS: tert-butyldimethylsilyl; Tf: trifluoromethanesulfonyl.*

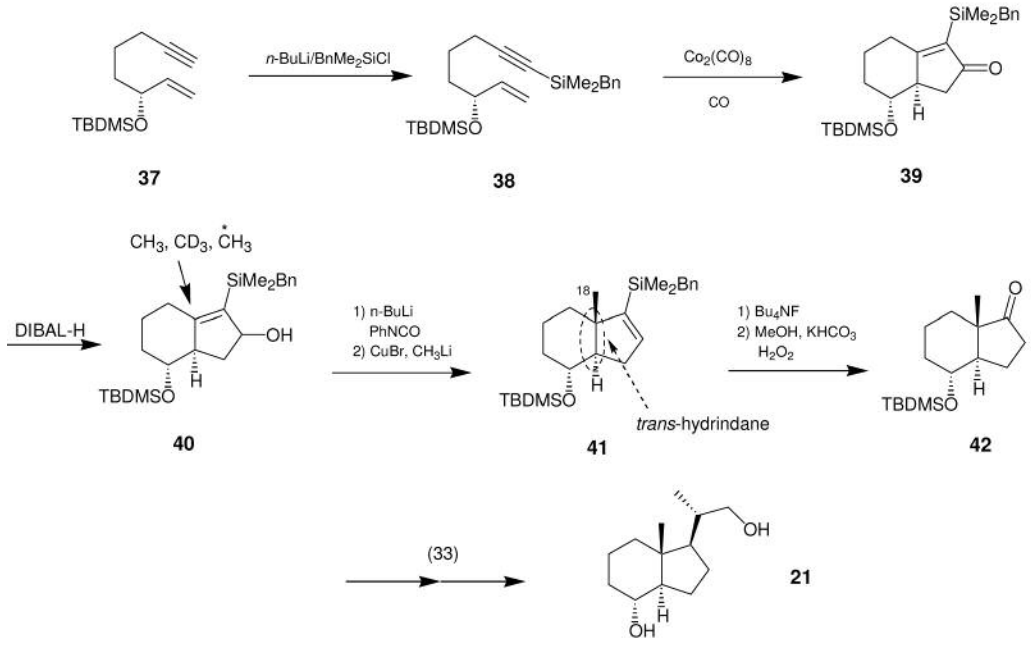

Figure 6. *Application of the Pauson-Khand reaction to the synthesis of vitamin D metabolites. n-BuLi: n-Butyllithium; $BnMe_2SiCl$: benzyldimethylsilyl chloride; $Co_2(CO)_8$: cobalt octacarbonyl; DIBAL-H: diisobutylaluminiumhydride; PhNCO: phenylisocyanate; CuBr: copper bromide; Bu_4NF: tetra-n-butylammonium fluoride.*

particularly those isotopically labeled at C18, opening up the access to vitamin D metabolites labeled by deuterium or ^{13}C that are hardly accessible by conventional methods. The products could be used as valuable reference standards in LC-MS/MS based assays or for metabolism studies if detection of a metabolite even after its enzymatic degradation is of interest.

Conflicts of Interest

The Author declares no conflict of interest regarding this study.

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