AN EFFICIENT SYNTHESIS OF 1α,25-DIHYDROXYVITAMIN D_3 LC-BIOTIN

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Introduction

In recent years, the dramatic impact of vitamin D deficiency on human health has gained increased awareness. Consequently, the development of appropriate assays to measure the status of medicinally relevant vitamin D metabolites in human blood or relevant tissue is continuously being improved. Particularly, assaying of 1α,25-dihydroxyvitamin D_3 (calcitriol), in turn considered as the most active metabolite, is indicated mainly in disorders resulting from impaired 1α-hydroxylation, which may lead to vitamin D dependent diseases such as chronic kidney disease (CDK), cases of rickets or hypercalcemia. In advanced competitive protein binding assays (RIA or ELISA), biotin-linked 1α,25-dihydroxyvitamin D_3 (1α,25-dihydroxyvitamin D_3 LC-biotin) **1** (Figure 1) is employed, in turn competing with the corresponding unlabeled counterpart for binding to an antibody or DBP in order to measure actual calcitriol concentration.

In this study we have explored several synthetic strategies to access 1α,25-dihydroxyvitamin D_3 LC-biotin **1** in order to identify a most efficient approach.

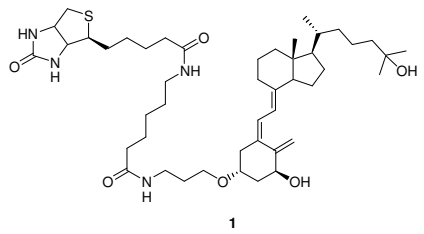

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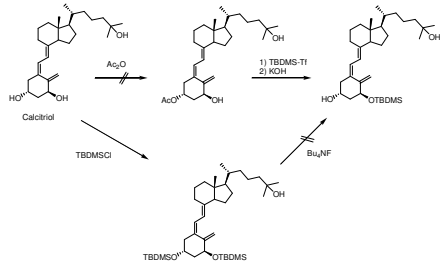
Figure 1: 1α,25-Dihydroxyvitamin D_3 LC-Biotin

Methods

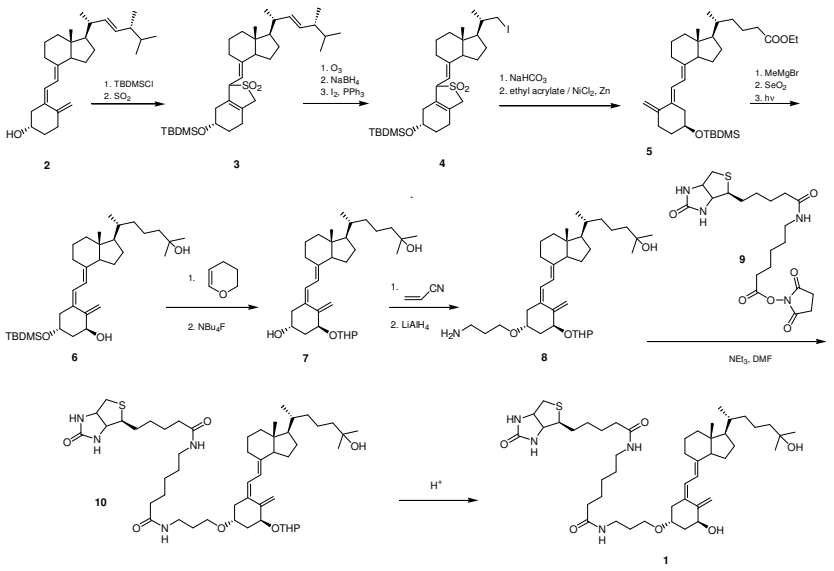
A suitable calcitriol derivative is aminopropylated at the C-3-OH-position, following by connection with a biotin labeled spacer [1]. The most obvious strategy, starting with calcitriol (Scheme 1) was not successful, due to its preexisting hydroxyl groups at C-1 and C-3, which are difficult to differentiate because of their similar reactivity. We have chosen a linear synthesis instead, starting from readily available vitamin D_2, utilizing a classical approach to access 1α,25-dihydroxyvitamin D_3 [2], and by application of appropriate OH-protective group transformations, involving a tetrahydropyranyl ether at C-1 [3].



Scheme 1. Initial Strategy to approach 1 from Calcitriol

Results and Discussion

The linear 15 steps synthesis of **1** (Scheme 2) utilizes a classical approach to access calcitriol, developed by a group from Hoffmann-La Roche and others. Thus, the C-3-OH group of vitamin D_2 (**2**) is protected as a *t*-butyldimetylsilyl ether, following by formation of an SO_2-adduct (**3**) to protect the *cis*-diene moiety. Ozonolysis leads after reductive workup with sodium borohydride and treatment with iodine to iodide **4**, which is submitted to a nickel-mediated conjugate addition to ethyl acrylate, leading to **5**. Grignard reaction with methyl magnesium bromide, allylic oxidation with selenium dioxide, and photochemical isomerization leads to **6**, which is converted in its corresponding THP-ether **7**. The linker is installed by reaction of **7** with acrylonitrile, reduction to the corresponding amine by lithium aluminium hydride, and final coupling with commercially available biotin-X-NHS (**9**). Final removal of the THP protective group leads to **1**.



Scheme 2. Linear Synthesis of 1α,25-Dihydroxyvitamin D_3 LC-Biotin

Conclusion

As shown for the synthesis of 1α,25-dihydroxyvitamin D_3 LC-biotin as a representative example, the developed methodology is applicable to the synthesis of a wide variety of 1,25-dihydroxyvitamin D_3 and D_2 LC-biotin- and also otherwise C-3-OH linked/labeled derivatives.

References

[1] Roy A, Ray R: Aminopropylation of Vitamin D hormone (1α,25-dihydroxyvitamin D_3), its biological precursors, and other steroidal alcohols: an anchoring moiety for affinity studies of steroids. Steroids 60(88): 530-3, 1995; EP1097132B1, 1999.

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