

## Optimization of Chemical Syntheses of Vitamin D C3-Epipimers

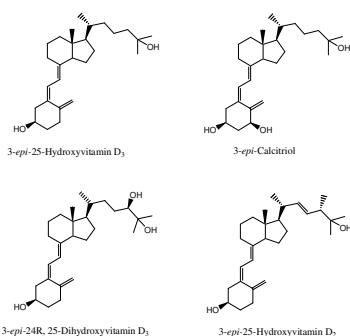
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### Introduction

Due to the widespread impact of vitamin D metabolites on human health, the development of various assays to measure their status is continuously being improved, either with the aim to detect and thus prevent vitamin D deficiency, or to identify new medicinally relevant metabolites in order to develop novel therapeutic rationales. Over 50 natural metabolites of vitamin D are known to date, although only very few of them are routinely measured in commercially available assays, thus neglecting the impact of most other metabolites of relevance. Particularly, naturally occurring C3-epimers of vitamin D<sub>3</sub> (and D<sub>2</sub>) deserve attention, because some of them not only may interfere in analytical measurements with other metabolites of interest, but also have controversial and not yet fully understood physiological functions [1] (Figure 1).

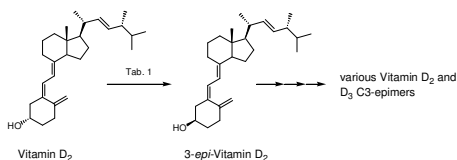


**Figure 1: Naturally occurring Vitamin D C3-Epipimers**

### Methods

In this study we have explored several synthetic methods to invert the configuration of the stereogenic center at C3 of the intact vitamin D skeleton with the aim to apply the most efficient method to the synthesis of various vitamin D<sub>3</sub> and D<sub>2</sub> C3-epimers.

It has already been explored previously, that readily available vitamin D<sub>2</sub> is a suitable starting material for the chemical synthesis of many vitamin D metabolites [2]. The inversion of the configuration of the C3-OH group (from β to α) at the A-ring of vitamin D<sub>2</sub>, leading to the corresponding C3-epimer, can be accomplished most appropriately under so-called Mitsunobu conditions [3]. Thus, vitamin D<sub>2</sub> or a related derivative thereof is treated with an aromatic acid, a diazo dicarboxylate and triphenylphosphine, leading to the formation of a corresponding ester, concomitant to the inversion of the configuration of the stereogenic center at C3. Reduction or saponification of the resulting ester finally leads to the corresponding C3-epimer, which may serve as starting material for a wide variety of other vitamin D<sub>3</sub> and D<sub>2</sub> C3-epimers (Figure 2).



**Figure 2: Synthesis of Vitamin D C3-Epipimers**

### Results and Discussion

The exploration of various starting materials, reagents and reaction conditions towards the synthesis of C3-*epi*-vitamin D derivatives is shown in Table 1.

Vitamin D<sub>2</sub>, vitamin D<sub>3</sub> and 7,8-bishydroxylated vitamin D<sub>2</sub> served as starting material. Different acids (benzoic acid, 3-chloro benzoic acid, 4-nitro benzoic acid, 2-picolinic acid, various azoesters, such as diethyl- and diisopropyl diazo dicarboxylate (DEAD, DIAD), as well as different solvents were employed. Additionally, reaction time and temperature were optimized.

With regard to ester formation, bishydroxylated vitamin D<sub>2</sub> appeared as the most suitable starting material, leading to >60% yield, although cleavage of the corresponding ester gave only poor yields. Favourably, saponification was carried out using potassium hydroxide, starting with vitamin D<sub>2</sub> and formation of a corresponding 3-chlorobenzoate.

Starting material	Reaction conditions and reagents, yield	Product 1	Reaction conditions and reagents, yield	Product 2
	benzoic acid, DIAD, PPh <sub>3</sub> , toluene, RT, 19%		lithiumaluminumhydride diethylether, 48%	
	4-nitro benzoic acid, DIAD, PPh <sub>3</sub> , THF, RT, 18%-33%		lithiumaluminumhydride diethylether, 74%	
	3-chlorobenzoic acid, DEAD, PPh <sub>3</sub> , toluene, RT, 30% 3-chlorobenzoic acid, DEAD, PPh <sub>3</sub> , toluene, RT, 30% 3-chlorobenzoic acid, DIAD, PPh <sub>3</sub> , toluene, RT, 30%		KOH, methanol-diethylether (1:1), 88% / 57% / 75%	
	2-picolinic acid, DIAD, PPh <sub>3</sub> , toluene, RT, 47% (contains elimination product)		copper(II)-acetate, chloroform-methanol (95:5), 22% over 2 steps	
	2-picolinic acid, DIAD, PPh <sub>3</sub> , toluene, RT, 64%		copper(II)-acetate, chloroform-methanol (95:5), 28% over 2 steps	

**Table 1: Starting Materials, Reagents, Conditions and Products**

### Conclusion

Inversion of the configuration at the C3 stereogenic center on the intact skeleton of vitamin D<sub>2</sub> or an appropriate vitamin D<sub>2</sub> derivative under "Mitsunobu conditions" was optimized and can finally be carried out in gram scale, leading to products suitable to synthesize a wide variety of natural 3-*epi* vitamin D metabolites and analogs.

### Acknowledgements

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### References

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