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Enzymatic Reductions for the Regio- and Stereoselective Synthesis of Hydroxy-keto Esters and Dihydroxy Esters

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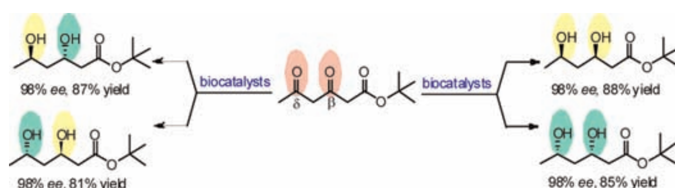
ABSTRACT



Ketoreductases were utilized for the stereoselective synthesis of δ-hydroxy-β-keto esters, β-hydroxy-δ-keto esters, and β,δ-dihydroxy esters. Seven out of eight possible stereoisomers were obtained from the enzymatic reduction of the corresponding β,δ-diketo ester in high enantio- and diastereomeric excess.

Optically active δ-hydroxy-β-keto esters, β-hydroxy-δ-keto esters and β,δ-dihydroxy esters are very useful intermediates in asymmetric organic synthesis and have been used for the synthesis of many natural products, pharmaceuticals, and other high value chemical compounds.[1] Although several methods have been developed for their synthesis such as chemical,[2] chemoenzymatic,[3] and enzymatic,[4] there is not a simple and straightforward method for the stereoselective synthesis of all possible stereoisomers.

In recent years, the challenge to simplify a multistep synthetic procedure to one step and if possible to combine consecutive catalytic transformations in the same reaction vessel has attracted interest in academic research and in the chemical industry.[5,6] In general, enzymes can facilitate an otherwise complicated conventional chemical route, since they offer great opportunities to control the enantioselectivity of the products and to accomplish domino reactions in the same vessel.[7]

Our continuing interest in enzymatic reductions for the stereoselective synthesis of various chiral synthons[8] led us to present herein a straightforward enzymatic reduction of *tert*-butyl 3,5-dioxohexanoate **1**. This simple one-step

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reaction proceeded in a totally regio- and stereoselective manner, affording in excellent yields optically and chemically pure δ-hydroxy-β-keto esters **2**, β-hydroxy-δ-keto esters **3**, or β,δ-dihydroxy esters **4**, Figure 1.

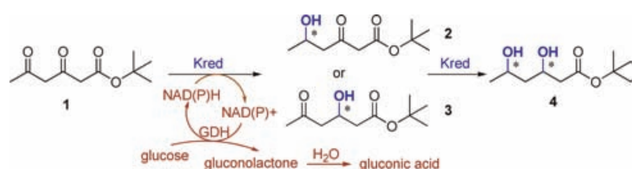

Figure 1. Enzymatic reduction of *tert*-butyl 3,5-dioxohexanoate **1**.

The eight possible products of this reduction are the two enantiomers of δ-hydroxy-β-keto ester **2a,b**, two enantiomers of β-hydroxy-δ-keto ester **3a,b**, and four steroisomers of β,δ-dihydroxy ester **4a**–**d**, as shown in Figure 2. Our challenging goal was the one-step preparation of every single stereoisomer out of these eight products, in optically pure form.

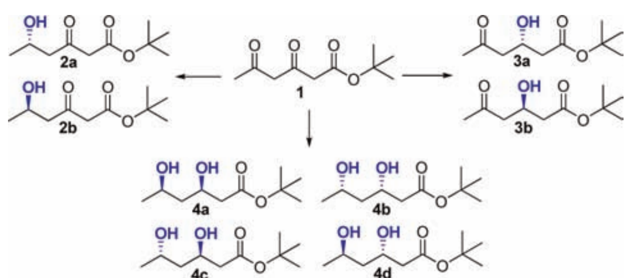

Figure 2. All possible products from the keto reduction of *tert*-butyl 3,5-dioxohexanoate (**1**).

To this purpose, we explored the enzymatic reduction of only one carbonyl group in order to produce either the δ-hydroxy-β-keto ester **2a** and **2b** or the β-hydroxy-δ-keto ester **3a** and **3b**. A series of 23 enzymes were screened for this transformation, and the most representative results are shown in Figure 3.

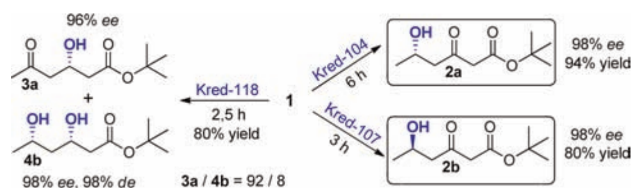

Figure 3. Enzymatic reduction of diketo ester **1** catalyzed by the enzymes Kred-104, Kred-107, and Kred-118.

As shown in Figure 3, Kred-104 and Kred-107 catalyzed the reduction of substrate **1**, affording the *S* or *R* enantiomer of hydroxy ketoesters **2a,b** respectively as the only product. The enantiomers of choice **2a** and **2b** were formed in a relatively short time, 6 and 3 h respectively, free of any byproducts. The absolute configuration of these two enantiomers was determined utilizing chiral derivatizing agents.[9] It should be emphasized here that the reduction catalyzed by Kred-118 took place mostly at the β-carbonyl group of **1** affording **3a** as the major product (92%). This important pathway led to the optically pure β-hydroxy-δ-keto ester **3a**, a useful chiral synthon and building block, difficult to be synthesized otherwise.[10] Along with major product **3a**, a small amount of dihydroxy ester **4b** (8%) was also identified in high enantiomeric and diastereomeric excess (Figure 3).

At this point, to investigate the selectivity of Kred-118, we tested the enzymatic reduction of optically pure δ-hydroxy-β-keto ester **2a**, synthesized earlier (Figure 3). To our delight, this reduction afforded dihydroxy ester **4b** in optically pure form (Figure 4). Furthermore, in a control experiment we also found that attempts to reduce **3a** (separated by flash column chromatography from the **3a** and **4b** mixture) utilizing the same enzyme Kred-118 failed (Figure 4). This result indicates that the reduction of diketo ester **1** by Kred-118 initially leads to the formation of **3a** and **2a**. A further reduction of **2a** leads to the observed minor product **4b**.

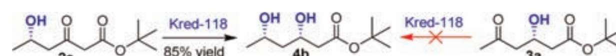

Figure 4. Pathway for the formation of *syn*-dihydroxy ester **4b**, catalyzed by Kred-118.

After this unexpected and significant regio- and enantioselectivity of Kred-118, attempts to improve the yield of the β-hydroxy-δ-keto ester **3a** by minimizing the formation of product **4b** were successful. Specifically, we combined the enzymatic reduction of diketo ester **1** by Kred-118 with an enzymatic oxidation utilizing Kred-104 in the same reaction vessel. As shown before (Figure 3), Kred-104 catalyzes the reduction only at the δ-carbonyl group of **1**. Consequently, the reverse oxidation procedure is expected to convert dihydroxy ester **4b** to **3a**. Indeed, after completion of the reduction of **1** by Kred-118, an oxidation step was followed in the same vessel utilizing Kred-104 and $NADP^+$ (oxidant) converting dihydroxy ester **4b** into the desired product **3a** (Figure 5). An excess of 2-pentanone

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was used for $NADP^+$ recycling. Specifically, after the first reduction step, the reaction was left for 24 h. This period of time is needed for NADPH and Kred-118 to be inactive. Next, we used 2-pentanone for regeneration of $NADP^+$ since we have already demonstrated that this compound is a good substrate for Kred-104.[11] Thus, this novel cascade enzymatic transformation led to the preparation of β-hydroxy-δ-keto ester **3a** as the only product, with high chemical and optical purity (Figure 5).

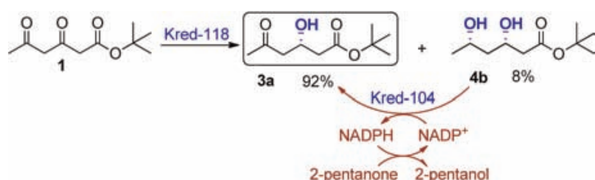

Figure 5. Preparation of β-hydroxy-δ-keto ester **3a** in >98% chemical purity and 82% isolated yield.

Furthermore, the preparation of optically pure dihydroxy esters was accomplished by the enzymatic reduction of the optically pure hydroxy keto esters **2a**, **2b**, and **3a**, which were previously synthesized enzymatically (Figure 3). These reactions are shown in Figure 6.

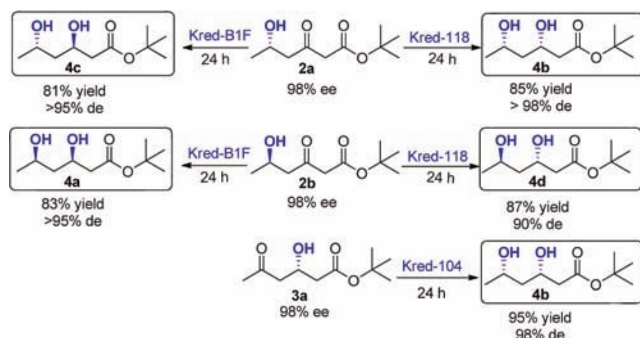

Figure 6. Enzymatic synthesis of optically pure dihydroxy esters **4a**–**4d**.

As shown in Figure 6, ketoreductases were active toward the reduction of **2a**, **2b**, and **3a** producing all possible stereoisomers of the product **4a**–**4d**. In particular, Kred-118 catalyzed the reduction of both substrates **2a** and **2b**, producing in high optical purity the (3*S*,5*S*)-**4b** and (3*S*,5*R*)-**4d** enantiomer respectively. Furthermore, we were able to synthesize stereoselectively the enantiomers (3*R*,5*S*)-**4c** and (3*R*,5*R*)-**4a** utilizing Kred-B1F, which catalyzed the reduction of both substrates **2a** and **2b**. Finally, β-hydroxy-δ-keto ester **3a** was reduced stereoselectively to compound (3*S*,5*S*)-**4b** by Kred-104. All the above enzymatic reactions were completed at 24 h, and the products were isolated without any chromatographic purification. The relative and absolute configuration of **4a**–**4d** were assigned by comparison with literature data.[1e]

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To this end, having accomplished the preparation of **4a**–**4d**, starting from the optically pure hydroxy keto esters **2a** and **2b**, we investigated the proper combination of specific biocatalysts for the one-pot synthesis of all stereoisomers of dihydroxy esters **4a**–**4d** starting from diketo ester **1**. Indeed, we managed to accomplish the one-pot synthesis of all these compounds, utilizing two sequential reduction steps, in the same vessel, without the isolation of the intermediates. The results are summarized in Figure 7.

This figure shows that the reduction of *tert*-butyl 3,5-dioxohexanoate **1** by Kred-107 followed by Kred-B1F led to stereoisomer (3*R*,5*R*)-**4a**. The enantiomer (3*R*,5*S*)-**4c** was produced by the reduction of substrate **1** by Kred-104, followed by Kred-B1F. The sequential reduction of substrate **1**, by Kred-107 and Kred-118, afforded (3*S*,5*R*)-**4d**. Finally, the stereoisomer (3*S*,5*S*)-**4b** was prepared utilizing Kred-104 and Kred-118 simultaneously in the same vessel. It should be emphasized here that the simultaneous reduction by both enzymes led to **4b** because both intermediate products **2a** and **3a** were reduced by these enzymes leading to the same product **4b**. In all these cases the dihydroxy esters **4a**–**4d** were synthesized in high optical and chemical purity without any chromatographic purification process.

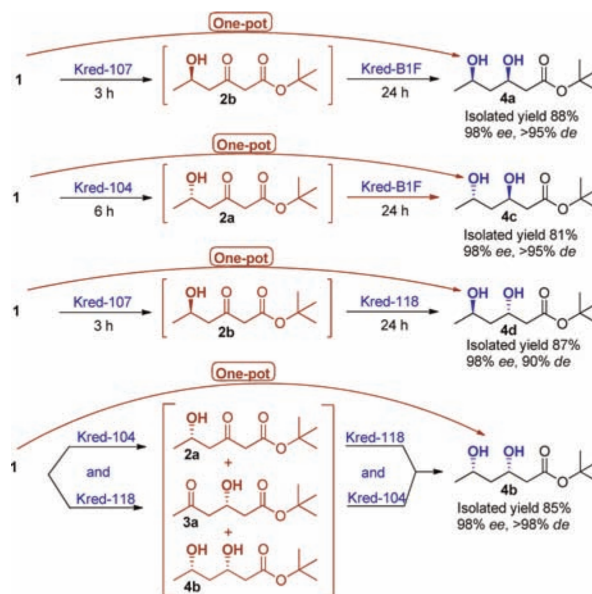

Figure 7. Enzymatic one-pot synthesis of all the stereoisomers of *tert*-butyl 3,5-dihydroxyhexanoate (dihydroxy esters **4a**–**4d**).

Furthermore, an interesting result was obtained from the reduction of diketo ester **1** catalyzed by Kred-B1F (Figure 8). In this case, the stereoisomer (3*R*,5*S*)-**4c** was formed directly, indicating that only one enzyme, Kred-B1F stereoselectively catalyzed the reduction of both β and

δ carbonyl groups. This is a very impressive result because of the direct formation of the desired stereoisomer in high optical purity and chemical yield, utilizing only one enzyme.

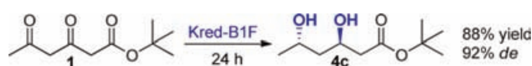

Figure 8. Direct formation of *anti* dihydroxy ester **4c** by enzymatic reduction of **1** with Kred-B1F.

It is also interesting to note that this reduction afforded the *anti* diastereomer indicating that the enzyme shows an opposite selectivity to the β-carbonyl group compared to that of the δ-carbonyl group. In order to determine the course of this reaction, we managed to identify the intermediate products by terminating the reduction at an early stage (1 and 2 h respectively). As shown in Figure 9, there were two intermediate mixtures consisting of a δ-hydroxy-β-keto ester **2**, a β-hydroxy-δ-keto ester **3**, and the corresponding dihydroxy ester **4**. These results indicate that, in the initial step, the enzyme did not exhibit any regioselectivity toward the reduction of β- or δ-carbonyl groups of substrate **1**. However, this enzyme showed high stereoselectivity toward the formation of only one final product **4c** after 24 h of reaction.

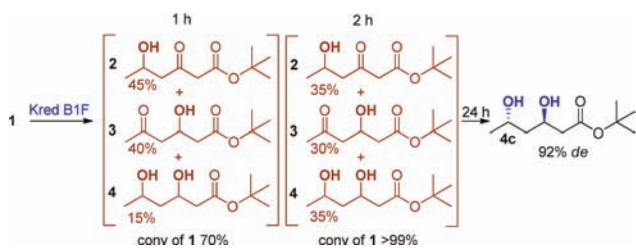

Figure 9. Investigation of the reduction of diketo ester **1** catalyzed by Kred-B1F.

To determine the absolute configuration of intermediates **2**−**4** and the final product **4c**, we accomplished enzymatic reductions of the optically pure compounds (5*S*)-**2a**, (5*R*)-**2b**, and (3*S*)-**3a** with Kred-B1F (Figure 10).

These experiments showed that Kred-B1F was active only toward the reduction of substrates **2a** and **2b**. Moreover, only **2a** afforded the *anti*-dihydroxy ester (3*R*,5*S*)-**4c**. This crucial observation led us to conclude that the intermediate δ-hydroxy-β-keto ester (**2**, Figure 9) was the (5*S*)-**2a** stereoisomer. Furthermore, Kred-B1F was inactive toward substrate (3*S*)-**3a** indicating that the intermediate β-hydroxy-δ-keto ester observed during the reduction of **1** (**3**, Figure 9) was the (3*R*)-**3b** enantiomer shown in Figure 11. Thus, by determining the stereochemistry of intermediates **2** and **3** as 5*S* and 3*R* respectively, we were able to assign with confidence the absolute configuration of dihydroxy ester **4c** (Figure 11) as the *anti* dihydroxy ester (3*R*,5*S*).

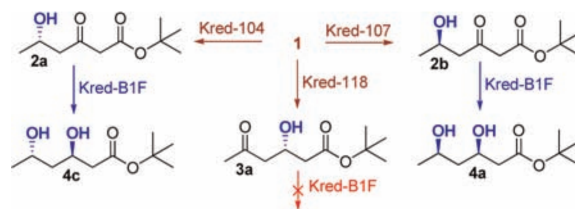

Figure 10. Kred-B1F mediated reduction of compounds **2a**, **2b**, and **3a**.

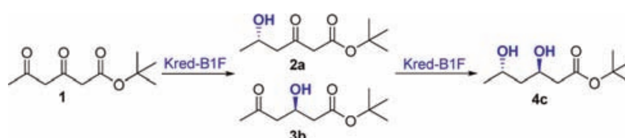

Figure 11. Route for the enzymatic formation of **4c** by Kred-B1F.

In summary, NADPH dependent ketoreductases were used as catalysts for the stereoselective synthesis of δ-hydroxy-β-keto esters, β-hydroxy-δ-keto ester, and β,δ-dihydroxy esters. This method proved very efficient for the preparation of seven out of the eight stereoisomers of these highly valuable chiral molecules depending on the choice of the enzyme. Cascade enzymatic reactions were performed for the one-pot synthesis of optically pure β,δ-dihydroxy esters by avoiding time-, effort-, and solvent-intensive steps starting from the same diketo ester **1**. This green procedure afforded these stereoisomers in high stereoselectivity, with a low reaction time and without any chromatographic purification of the isolated product.

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Supporting Information Available. Detailed experimental procedures, HRMS, ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.