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Highly Stereoselective Reductions of α-Alkyl-1,3-diketones and α-Alkyl-β-keto Esters Catalyzed by Isolated NADPH-Dependent Ketoreductases

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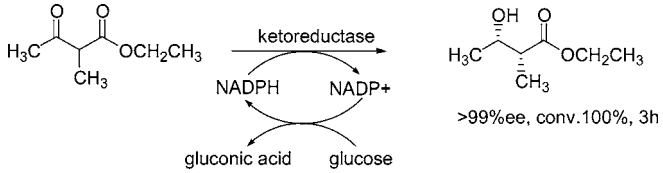
ABSTRACT



The biocatalytic reduction of α-alkyl-1,3-diketones and α-alkyl-β-keto esters employing 1 of 20 different isolated NADPH-dependent ketoreductases proved to be a highly efficient method for the preparation of optically pure keto alcohols or hydroxy esters.

Optically active α-alkyl-β-hydroxy ketones and α-alkyl-β-hydroxy esters are important compounds in asymmetric organic synthesis, where they are used as building blocks for synthesis of polyketides, statins, protease inhibitors, and other important pharmaceuticals.[1] They are of relatively small molecular weight, bear chirality at two stereogenic centers, and contain at least two reactive functionalities (an alcohol, a ketone, and other reactive groups potentially present in the side chain of the substituent).

Enzyme-based approaches are being increasingly explored for the synthesis of these classes of compounds. Whole cells of microorganisms[2] with ketoreductase enzyme activities (particularly Baker's yeast) have been frequently utilized; however, many problems are associated with their use.[2] Significant efforts are required to grow and screen large libraries of microorganisms in order to identify those with useful reductases for every particular application.[2] Whole cells typically contain multiple ketoreductase enzymes, leading to mixed stereoselectivity and side reactions by other competing ketoreductases. Whole-cell reactions also suffer from low reaction rates, limited concentrations of product per liter of culture, and inhibition due to the toxicity of

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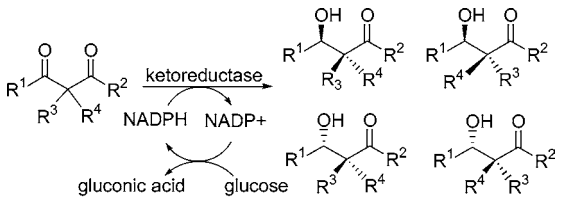
reactants and/or products to the cells. Various empirical approaches have been tried to minimize these problems in specific cases, but no generally applicable solutions exist.[3,4]

Use of isolated enzymes can minimize, and even eliminate, all these problems.[5] Screening sets containing a variety of different ketoreductase enzymes covering broad ranges of different ketones are now available,[6] and screening of these enzymes in parallel to identify the best enzyme for a given target ketone can be carried out rapidly. In this paper, we present the results of highly diastereo- and enantioselective reductions of α-alkyl-1,3-diketones and α-alkyl-β-keto esters utilizing twenty different commercially available ketoreductase enzymes[6] (Figure 1).



	R^1	R^2	R^3	R^4
1	Me	Me	Me	H
2	Me	Me	Et	H
3	Me	Me	*iso*-Bu	H
4	Me	Me	allyl	H
5	Me	Me	2-Buten-3-Me	H
6	Et	Me	Me	H
7	Me	OEt	Me	H
8	Me	OEt	Et	H

Figure 1. Enzymatic reduction of α-alkyl-1,3-diketones **1**−**6** and α-alkyl-β-keto esters **7** and **8** with NADPH-dependent ketoreductases

Using α-alkyl-1,3-diketones and α-alkyl-β-keto esters as starting materials, ketoreductases offer an opportunity to carry out reductions that are stereoselective and regioselective. Consequently, as we show here, it is possible to prepare single diastereomers of the product keto alcohols and hydroxy esters by tailoring the choice of enzyme. In addition, the product is frequently formed in quantitative yield. The yields and stereochemical purities achieved in the reduction of six α-alkyl-1,3- diketones and two α-alkyl-β-keto esters are summarized in Table 1.

In an achiral environment, the reduction of the diketones chosen as substrates for this study will yield mixture of all four possible diastereomers (Figure 1). Reductive enzymes, on the other hand, are both stereo- and regioselective and can distinguish between the faces of prochiral ketones reducing them in a highly stereoselective fashion.

Table 1. Enzyme-Catalyzed Stereoselective Reduction of Diketones/Keto Esters to Keto Alcohols/Hydroxy Esters

	KRED	Diastereomeric ratio (%)[a]				Yield(time)	Product[d]
		A	B	C	D		
1	102	>99[b]	-	-	-	100 (24h)	
2	102	>99	-	-	-	100 (12h)	
	107	-	-	90	10	100 (6h)	
	118	-	>99	-	-	100 (6h)	
3	102	>99	-	-	-	94 (24h)	
	114	96	-	-	4	100 (24h)	
4	102	>99	-	-	-	100 (24h)	
	107	-	93	7	-	100 (6h)	
	120	-	-	-	>99	100 (12h)	
5	102	>99	-	-	-	100 (12h)	
	107	-	97	-	3	100 (24h)	
	119	8	-	92	-	93 (24h)	
6	102	>99	-	-	-	100 (24h)	
7	102	>99[c]	-	-	-	100 (3h)	
	107	-	15	-	85	100 (6h)	
8	102	>99	-	-	-	100 (3h)	
	116	10	-	90	-	100 (24h)	

[a] Diastereomeric ratio was determined by chiral GC. In each case, the racemic product was prepared for calibration. [b,c] Syn stereochemistry was assigned by comparing ^{1}H NMR results with literature data.[9] [d] Determination of absolute configuration of the stereogenic center bearing the OH group, was made by ^{1}H NMR analysis of the corresponding MPA esters.[10] The relative stereochemistry (*syn/anti*) was assigned by ^{1}H NMR analysis on the basis of literature method.[11]

This is clearly demonstrated in the reduction of compounds **2** and **4** (Table 1), where two out of the four stereoisomers are formed in optically pure form (>99% ee) using different enzymes while the third diastereomer in both compounds was also accessed in lower enantiomeric purities (Table 1). This result is very impressive in showing that potentially all diastereomers can be accessed using different enzymes. Two out of the four diastereomers were also obtained in substrates **7** and **8** (Table 1), and in three of the substrates studied one diastereomer was produced (entries 1, 3, and 6, Table 1). The diastereomeric ratio, presented in Table 1, was derived from chiral GC analysis.

In all but one of the substrates shown in Table 1, both carbonyl carbons are enantiotopic, chemically equivalent, as

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(6) KRED-101-120, BioCatalytics, Inc., Pasadena, CA.

a result of the symmetrical structure of the molecule (plane of symmetry). In an asymmetric environment, a regio- and stereoselective reduction of the keto group leads to the formation of a single diastereomer. In 3-methyl-2,4 hexadione (entry 6, Table 1), however, the two keto groups are chemically different because the molecule is not symmetrical. Upon reduction with KRED102, hydroxy ketone **6** was obtained in quantitative chemical yield and with excellent optical purity (ee >99%). This is only possible if an equilibrium between the two enantiomeric diketones was taking place during the enzymatic reaction conditions,[7] while the enzyme was selective for the reduction of only one enantiomer. Furthermore, the enzyme was regiospecific with respect to the two carbonyl groups, reducing the less substituted one. Such an equilibrium has been previously observed in the enzymatic reduction of 2-alkyl-3-ketoglutarate diester precursors in the synthesis of statines.[8]

Quantitative yields of product were obtained in 3−24 h of reaction time, using 1−10% (g/g) of enzyme relative to substrate. For example, diketone **1** and keto ester **7** (50−100 mM) were reduced on a larger scale (100 mL) with KRED102 affording optically pure keto alcohol **1** and hydroxy ester **7**, respectively, in high isolated yield and optical purities (90% yield, >99% ee) (details in the Supporting Information). In every reaction discussed in this paper, the NADPH cofactor was used in catalytic amounts (1% relative to the substrate) and was recycled in situ using glucose dehydrogenase (GDH).[12] Determination of the absolute configuration of the produced keto alcohols and hydroxy esters was accomplished based on literature methods.[10,11] In all of its reactions KRED102 shows *syn* selectivity (entries 1−8b), whereas KREDs116, 118, 119, and 120 show *anti* selectivity (Table 1). All of the ketoreductases used in this study follow Prelog's rule, except KRED107, which showed anti-Prelog selectivity in the reduction of the diketones.

We have shown herein that the enzymatic transformation of the above diketones and keto esters shows excellent chemical and optical yields and can be tailored to afford most of the four possible diastereomers from the same starting substrate at will depending on the chosen enzyme.

These results clearly demonstrate the power of bioreductions compared to traditional chemical transformations. Besides being regio- and stereoselective, these enzymes exhibited high chemoselectivity by giving a keto alcohol and not the diol. To the best of our knowledge, no such group of related chemical catalysts exists that is both regio- and stereoselective enough to reduce a diketone to an optically pure single keto alcohol. Finally, the ability to synthesize various single diastereomers of the same compound, such as the keto alcohols and hydroxy esters presented in this report, is desirable when libraries of pharmaceutical compounds are synthesized and tested for biological activity.

In conclusion, the biocatalytic reduction of α-alkyl-1,3-diketones and α-alkyl-β-keto esters employing 1 of 20 different isolated NADPH-dependent ketoreductases proved to be a highly efficient method for the preparation of optically pure keto alcohols or hydroxy esters. Using isolated enzymes as catalysts for organic reactions is becoming a more standardized and practical tool in the hands of synthetic chemists. Further investigations are underway in our laboratories.

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

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