

Synthesis of Valuable Chiral Intermediates by Isolated Ketoreductases: Application in the Synthesis of α -Alkyl- β -hydroxy Ketones and 1,3-Diols

Dimitris Kalaitzakis,^a J. David Rozzell,^b Ioulia Smonou,^{a,*} and Spiros Kambourakis^{b,*}

^a Department of Chemistry, University of Crete, Voutes-Iraklio 71003, Crete, Greece
Fax: (+30)-2810-545001; e-mail: smonou@chemistry.uoc.gr

^b BioCatalytics Inc, 129 North Hill Ave, Suite 103, Pasadena, CA 91106, USA
Fax: (+1)-626-356-3999; e-mail: skambourakis@biocatalytics.com

Received: April 18, 2006; Accepted: July 24, 2006



Supporting information for this article is available on the WWW under <http://asc.wiley-vch.de/home/>.

Abstract: Regio- and stereoselective reductions of α -substituted 1,3-diketones to the corresponding β -keto alcohols or 1,3-diols by using commercially available ketoreductases (KREDs) are described. A number of α -monoalkyl- or dialkyl-substituted symmetrical as well as non-symmetrical diketones were reduced in high optical purities and chemical yields, in one or two enzymatic reduction steps. In most cases, two or even three out of the four possible diastereomers of α -alkyl- β -keto alcohols were synthesized by using different enzymes, and in two exam-

ples both ketones were reduced to the 1,3-diol. By replacing the α -alkyl substituent with the OAc group, 1-keto-2,3-diols, as well as 1,2,3-triols were synthesized in high optical purities. These enzymatic reactions provide a simple, highly stereoselective and quantitative method for the synthesis of different diastereomers of valuable chiral synthons from non-chiral, easily accessible 1,3-diketones.

Keywords: 1,2-diols; 1,3-diols; enzymatic reduction; ketoreductase; β -hydroxy ketones

Introduction

Asymmetric reduction of double bonds ($C=X$ where $X=C, O, N$) represents a very useful and widely applicable method for introducing chirality into a prochiral compound in high yield (up to the theoretical 100%). Asymmetric reduction of ketones is particularly important, since chiral alcohols are present in a wide spectrum of pharmaceutical and other high-value compounds, or can be used as chiral building blocks in the synthesis of larger, more complicated compounds. Optically active α -alkyl- β -hydroxy ketones and their analogues represent important classes of chiral synthons that have been used in the synthesis of many natural products and pharmaceuticals^[1] and have been identified in a spectrum of biologically active compounds^[2] including polyketides. We now report a two-step chemo-enzymatic route to a number of these compounds by starting from prochiral α -alkyl-1,3-diketones (Figure 1).

Several methods for the stereoselective synthesis of β -hydroxy ketones have been developed. They include asymmetric reductions using chiral ruthenium

$Ru(II)$ ^[3] or rhodium^[4] catalysts or hydroboration using chiral $Co(II)$ catalysts.^[4] Methods requiring chiral starting precursors such as amides^[5] and ketones,^[6] or aldol condensations starting from achiral ketones,^[7] through boron enolates in the presence of tertiary amines,^[8] or through zirconium enolates^[9] and bases like LDA, LTMP and LHMDs^[10] have also been used. Compounds with high optical purities have been obtained; however, these methods are frequently not generally applicable for the synthesis of other structurally related compounds or different diastereomers of the same compounds.

Biocatalytic asymmetric reductions using whole cells of baker's yeast^[11a-d] or isolated enzymes^[11e-j] have also been reported. For the whole cell reactions, yields and/or enantiomeric purities of products are low, because of the large number of ketoreductases present in this yeast. An isolated reductase from *Lactobacillus brevis* (LBADH) was found to be selective in the reduction of C-5 of a 3,5-dioxocarboxylate,^[11e,f] while one ketoreductase from *Saccharomyces cerevisiae* was selective in the reduction of the C-3 ketone of the same compound.^[11i] The latter enzyme was active

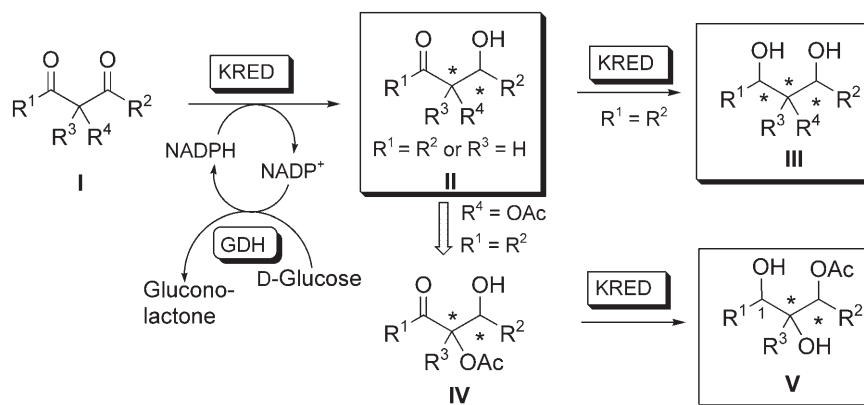


Figure 1. Enzymatic reduction of α -alkyl-1,3-diketones with NADPH-dependent ketoreductases. KRED = ketoreductase, GDH = glucose dehydrogenase.

against a number of substrates including two more 1,3-diketones.^[11i] Recently, whole cells of lyophilized *Rhodococcus ruber* were found to be active against a number of ketones including some 1,2- and 1,3-diketones.^[11g] One broad substrate and solvent tolerant dehydrogenase was isolated from this organism.^[11h] Even though a few 1,3-diketones have been reduced by the previous biocatalysts, none of them contained substituents at the α -position as in the examples presented in this report. In every case only one active enzyme was found giving one (of the possible two) enantiomers.

It has been clearly shown in a number of recent reports^[12–15] that commercially available ketoreductases are chemo-, stereo-, and regioselective catalysts that often reduce a wide range of ketones and represent an important addition that complements and expands the existing technology of ketone reductions. Cloned and overexpressed enzymes (as in the case of all ketoreductases described herein) are easily and cost-effectively synthesized on a commercial scale by fermentations. The required cofactor is no longer a significant addition to the cost of the process since NAD(P)H can be added in catalytic amounts and be efficiently recycled by various enzymatic methods, such as glucose dehydrogenase (GDH),^[13a,b] formate dehydrogenase (FDH),^[13b] or phosphite dehydrogenase.^[13c]

In this report we present a regio- and stereoselective synthesis of different diastereomers of a large number of α -alkyl- β -hydroxy ketones (II) in good to excellent optical purities, from readily available non-chiral α -alkyl-1,3-diketones (I) by the use of thirty-four commercially available ketoreductase enzymes. All prochiral α -alkyl-1,3-diketones (I) were chemically synthesized from cheap and readily available starting materials in one or two steps in high yields. We also show that various diastereomers of the corresponding 1,3-diols (III, V) can be synthesized by stepwise reduction by using different enzymes. As a result

two, and potentially three chiral centers are created from a non-chiral diketone substrate in high yields (> 90%) in one or two enzymatic steps (Figure 1). The chiral center of the α -carbon bearing the alkyl group(s) is created by the selective reduction of one of the two enantiotopic ketones when $R^1 = R^2$ (Figure 1). When R^1 and R^2 are different and $R^3 = H$ both chiral centers are set in one enzymatic reduction step and in high yield (up to the theoretical 100%) due to the rapid equilibrium of two enantiomeric diketones.^[12a,b,14,15] In this latter case the enzyme shows both regio- and stereoselectivity.

Preliminary results of the enzymatic reductions of some mono- α -alkyl-substituted diketones that are discussed in this report have already been published.^[14,15] In particular the reduction of 4-methyl-3,5-heptadione (entry 8, Table 1) by ketoreductase EXP-A1B forms the natural pheromone (+)-sitophilure, so the synthesis of this compound was reported separately.^[15] In the present work the substrate complexity of the diketones was expanded to include dialkyl-substituted as well as non-symmetrical 1,3-diketones (Table 1). The possibility of increasing even more the number and the structural diversity of the compounds that can be synthesized from the same α -alkyl 1,3-diketone scaffold was investigated by introducing an *O*-acetyl group as one of the α -substituents, which upon reduction would form α,β -dihydroxy ketones (IV) or 1,2,3-triols (V).

Results and Discussion

Asymmetric synthesis is moving into an area where not only high enantiomeric purities of products are required, but also in the development of methods that give easy access to both enantiomers (or a number of diastereomers if possible) of a targeted compound. This is particularly important in the synthesis of biologically active compounds (such as pharmaceuticals

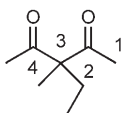
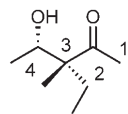
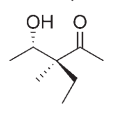
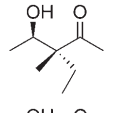
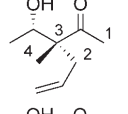
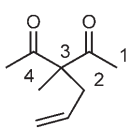
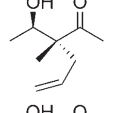
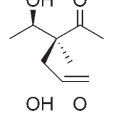
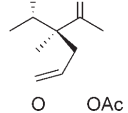
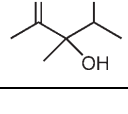
Table 1. Enzyme-catalyzed stereoselective reduction of diketones to keto alcohols.

Entry	Substrate	KRED	Diastereomeric ratio %				Yield ^[d] (Time)	Product	
			A	B	C	D			
1		102 and 106 ^[a]	> 99 3 <i>R</i> ,4 <i>S</i>	-	-	-	100 % (24 h)		1a
		A1B	74 3 <i>R</i> ,4 <i>S</i>	-	26 3 <i>S</i> ,4 <i>R</i>	-	100 % (10 min)		
		127	3	94 3 <i>S</i> ,4 <i>S</i>	-	3	90 % (24 h)		1b
2		102 and 106 ^[a]	> 99 3 <i>R</i> ,4 <i>S</i>	-	-	-	100 % and 90 % (12 h)		2a
		112 ^[a]	96 3 <i>R</i> ,4 <i>S</i>	4	-	-	100 % (3 h)		
		A1B	-	-	95 3 <i>S</i> ,4 <i>R</i>	5	100 % (1 h)		2c
		107 ^[a]	-	-	90 3 <i>S</i> ,4 <i>R</i>	10	100 % (6 h)		
		118 and 119 ^[a]	-	> 98 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		2b
		102 and 106	> 99 3 <i>R</i> ,4 <i>S</i>	-	-	-	100 % and 93 % (12 h)		3a
3		112	97 3 <i>R</i> ,4 <i>S</i>	-	3	-	100 % (6 h)		3a
		A1B	-	-	96 3 <i>S</i> ,4 <i>R</i>	4	100 % (1 h)		3c
		107	8	-	79 3 <i>S</i> ,4 <i>R</i>	10	82 % (24 h)		
		118 and 119	5	95 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		3b
		101 ^[a]	96 3 <i>R</i> ,4 <i>S</i>	-	-	4	100 % (6 h)		4a
		102 and 106 ^[a]	> 99 3 <i>R</i> ,4 <i>S</i>	-	-	-	94 % and 76 % (24 h)		4a
4		123	96 3 <i>R</i> ,4 <i>S</i>	-	1	3	100 % (6 h)		4a
		A1B	-	-	90 3 <i>S</i> ,4 <i>R</i>	10	100 % (24 h)		4c
		107 ^[a]	-	-	72 3 <i>S</i> ,4 <i>R</i>	28	40 % (24 h)		
		102 and 106 ^[a]	> 99 3 <i>R</i> ,4 <i>S</i>	-	-	-	100 (3 h and 6 h)		5a
		112 ^[a]	95 3 <i>R</i> ,4 <i>S</i>	2	-	3	100 % (1 h)		
		A1B	-	-	99 3 <i>S</i> ,4 <i>R</i>	1	100 (20 min)		5c
5		107 ^[a]	2	2	90 3 <i>S</i> ,4 <i>R</i>	6	100 % (12 h)		5c
		108 ^[a]	1	99 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		5b
		118 and 119 ^[a]	1 and 1	99 and 97 3 <i>S</i> ,4 <i>S</i>	-	0 and 2	100 % (24 h and 12 h)		

Table 1. (Continued)

Entry	Substrate	KRED	Diastereomeric ratio %				Yield ^[d] (Time)	Product	
			A	B	C	D			
6		102 and 106	>99 3 <i>R</i> ,4 <i>S</i>	-	-	-	100 % (12 h and 24 h)		6a
		A1B	-	-	92 3 <i>S</i> ,4 <i>R</i>	8	100 % (2 h)		6c
		107	-	-	84 3 <i>S</i> ,4 <i>R</i>	26	100 % (6 h)		6c
		118 and 119	7 and 7	93 and 90 3 <i>S</i> ,4 <i>S</i>	-	0 and 3	100 % (24 h)		6b
		124	4	96 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		6b
		102 and 106 ^[a]	>99 3 <i>R</i> ,4 <i>S</i>	-	-	-	100 % (12 h)		7a
7		131	93 3 <i>R</i> ,4 <i>S</i>	-	7	-	100 % (24 h)		7a
		107 ^[a]	-	-	97 3 <i>S</i> ,4 <i>R</i>	3	100 % (12 h)		7c
		A1B	-	-	>98 3 <i>S</i> ,4 <i>R</i>	-	100 % (0.5 h)		7c
		118 and 119 ^[a]	10 and 8	90 and 92 3 <i>S</i> ,4 <i>S</i>	-	-	93 % (24 h)		7b
		124	5	95 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		7b
		A1B ^[a]	-	-	97 4 <i>S</i> ,5 <i>R</i>	3	100 % (40 min)		8c
8		118 and 119 ^[a]	4 & < 1	96 and > 99 4 <i>S</i> ,5 <i>S</i>	-	-	93 % and 100 % (24 h and 12 h)		8b
		102 and 106 ^[a]	>99 2 <i>S</i> ,3 <i>R</i>	-	-	-	100 % and 85 % (24 h)		9a
		103 ^[a]	>99 2 <i>S</i> ,3 <i>R</i>	-	-	-	85 % (24 h)		9a
9		127	3	96 2 <i>S</i> ,3 <i>S</i>	-	1	92 % (24 h)		9b
		102	91 2 <i>S</i> ,3 <i>R</i>	8	1	-	75 % (24 h)		10a
10		106	95 2 <i>S</i> ,3 <i>R</i>	3	2	-	46 % (24 h)		10a
		126	94 2 <i>S</i> ,3 <i>R</i>	4	-	-	100 % (12 h)		10a
		107	1	96 2 <i>S</i> ,3 <i>S</i>	3	-	75 % (24 h)		10b
		127	6	94 2 <i>S</i> ,3 <i>S</i>	-	-	100 % (24 h)		10b

Table 1. (Continued)

Entry	Substrate	KRED	Diastereomeric ratio %				Yield ^[d] (Time)	Product	
			A	B	C	D			
11		112	91 3 <i>R</i> ,4 <i>S</i>	-	9	-	100 % (1 h)		11a
		108	1	99 3 <i>S</i> ,4 <i>S</i>	-	-	70 % (24 h)		11b
		118 and 119	-	> 99 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		
		A1B	-	-	11	89 3 <i>R</i> ,4 <i>R</i>	100 % (1.5 h)		11d
		107	-	-	49	51	34 % (24 h)		
		101 and 112	98 3 <i>R</i> ,4 <i>S</i>	-	2	-	100 % (1 h)		12a
		113 and 115	94 3 <i>R</i> ,4 <i>S</i>	-	6	-	100 % (6 h)		
12		A1B	-	-	10	90 3 <i>R</i> ,4 <i>R</i>	100 % (24 h)		12d
		107	-	-	65 3 <i>S</i> ,4 <i>R</i>	35	100 % (1 h)		12c
		108 and 119	-	> 99 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (24 h)		12b
		118	-	> 99 3 <i>S</i> ,4 <i>S</i>	-	-	100 % (6 h)		
		112 ^[c] 113 ^[c]	98 97	- -	2 3	- -	100 % (0.2 h) 100 % (0.2 h)		13a

[a] Ref.^[14][b] Ref.^[15]

[c] The absolute stereochemistry of this reaction product has not been determined, the assignment of the major diastereomer as A was based on its retention time in chiral GC.

[d] The yields were calculated from the integration on GC of crude reaction extracts. Scaling of a few reactions and isolation of keto-alcohols gave very similar yields as those reported in this Table.

and pesticides), when libraries of compounds are synthesized in order to be tested for biological activity, or when improvement of the properties of an already active compound is made by the synthesis of closely related analogues. As a result, the development and discovery of catalysts that are chemo-, regio- and stereoselective and can give access to a number of isomers of closely related compounds are highly desirable.

The results presented in this report show that ketoreductases fulfill all the previous requirements. In addition, enzymatic reductions using the isolated enzymes described herein have been scaled to laboratory, preparative, and industrial scales for the synthesis of chiral intermediates.^[13a,b] In order to demonstrate the wide range of keto alcohols that can be accessed by using different ketoreductases, eight mono-alkyl-

substituted, **1–8**, three disubstituted, **11**, **12** and **13**, and two unsymmetrical diketones, **9** and **10**, were selected as substrates for stereoselective reduction (Table 1). All these compounds were synthesized from the corresponding 1,3-diketone after alkylation with the appropriate alkyl halide. High yields of isolated diketone were routinely obtained in reaction processes that require cheap and readily available starting materials and simple and scalable reaction conditions (details in Experimental Section).

With only a couple of exceptions, all enzymes gave the keto alcohols shown in Table 1 as the only product, after all diketone had completely reacted. Addition of excess enzyme and long incubation times resulted in the formation of the diol in some cases, but in the majority of cases enzymes were completely unreactive towards the keto alcohol they produced. In

all but one substrate (**13**, Table 1) presented in this report, more than one diastereomer was synthesized in good to excellent optical purities with the action of different enzymes. For example, in five out of the eight symmetrical monoalkyl-substituted diketones (entries **2**, **3**, **5**, **6**, **7**) and in two out of the three dialkyl-substituted diketones (entries **11** and **12**), three out of the four possible diastereomers were synthesized in good to excellent optical purities with the action of different enzymes. In the rest of the cases two out of the four diastereomers were produced (substrates **1**, **4**, **8**, **9**, **10**). The data reported in Table 1 represent the best reactions obtained after screening thirty-one ketoreductases of the commercially available KRED30,000 kit (Biocatalytics) and three of the thirty-three newly discovered enzymes (EXP33,000 kit, BioCatalytics Inc). The only exception is KRED107 where all its reaction products are reported (even though this enzyme did not give high selectivity with all reported substrates) as a representative example and in order to illustrate general enzyme selectivities that will be discussed later.

As predicted in our previous report^[14] and now confirmed experimentally, in every previously reported diketone more diastereomers were synthesized, or an improvement of the optical purities was achieved after the screening of fourteen newly discovered enzymes. In particular, the keto alcohol (3*S*,4*R*) that was accessed in moderate optical purities by KRED107 in substrates **2**, **5** and **7** and in low optical purities in **3**, **4** and **6**, can now be synthesized very efficiently in improved optical purities by EXP-A1B (Table 1). Additional diastereomers can now be synthesized from substrates **1**, **4** and **9**. The utility of this methodology was expanded by the reduction of non-symmetrical α,α -dialkyl-substituted **11**, **12** and one α -alkyl- α -*O*-acetyl-substituted diketone **13** (Table 1). Reduction of the disubstituted ketones resulted in the production of an otherwise difficult to synthesize chiral tertiary carbon, while replacing the α -alkyl group with other functionalities increased the number of important intermediates that can be accessed. When the two keto groups were not chemically equivalent, as in the case of the non-symmetrical substrates **9** and **10**, all active enzymes were selective for the reduction of the less substituted ketone.

Besides being regioselective, many enzymes showed good to excellent stereoselectivities of reduction regardless of the α -alkyl group. In a previous report the general *R* or *S* preference of reduction for the enzymes of KRED30,000 kit was identified by using two standard ketones.^[13a] Most enzymes retained this stereoselectivity for the reduction of the diketone discussed herein, and most gave the same absolute configuration in the formation of both chiral carbons of every keto alcohol reduced. For example, both KRED102 and KRED106 gave high optical purities

of the keto alcohol containing the *S*-hydroxy, *R*-alkyl configuration in all monoalkyl-substituted diketones (**1–7**, **9** and **10**, Table 1), while neither enzyme reacted with the dialkyl-substituted substrates **11–13**. The same trend in the formation of the same major diastereomer, though without always perfect selectivity, was also observed in most active enzymes that are not shown in Table 1. With the exception of the α -methyl diketone **1**, where enzymes KRED118 and KRED119 gave an almost equimolar mixture of (3*S*,4*S*) and (3*R*,4*S*) keto alcohols (data not shown), both enzymes gave good to excellent optical purities of keto alcohols containing the *S*-configuration for both the alcohol and alkyl groups with all the monosubstituted and the disubstituted diketones (Table 1). Both enzymes showed almost no activity for substrates **4**, **9** and **10**. Note also that even in the case of substrate **1**, both KRED118 and KRED119 gave the *S*-alcohol upon reduction of the ketone; however, both were not selective for the configuration of the α -methyl group. In contrast, KRED102 and KRED106 formed keto alcohols in high optical purities with the same stereopreference for all the substrates they reduced, including **1** (Table 1). In general, substrate **1** was reactive with almost all enzymes tested; however, only three enzymes (Table 1) showed good to excellent diastereoselectivity of product formation.

As a general rule, most enzymes showed good to excellent stereoselectivity for the reduction of the keto group. However, the selectivity for the α -alkyl stereocenter was not always high. As a result, most enzymatic reductions would either give a single diastereomer or a mixture of products where the two major (or sometimes the only two) diastereomers would contain the same absolute configuration for the alcohol and opposite configurations for the alkyl substituted compound. This selectivity has already been discussed for KRED118 and 119 with substrate **1**. Another example includes KRED107, an enzyme that would always give the *R*-configuration in the synthesis of the alcohol (except substrate **10**) but mixed *S*- and *R*-configurations for the α -alkyl group, the *R*-configuration product always being the major one (Table 1). Although this selectivity was true for most enzymes and for all the substrates that were active (symmetrical and non-symmetrical mono-substituted compounds **1–10**), there were a couple of exceptions when comparing the reductions of mono- versus disubstituted substrates. This trend is illustrated by the reductions of EXP-A1B which gave keto alcohol (3*S*,4*R*) as the major diastereomer in the monosubstituted symmetrical diketones **2–8**, (3*R*,4*S*) with the methyl-containing diketone **1**, and (3*R*,4*R*) in the disubstituted (**11**, **12**) analogues (Table 1). These results clearly show that prediction of a ketoreductase selectivity cannot always be done with absolute certainty, even when the results of similar substrates are known.

Replacing the α -alkyl group, or one of the alkyl groups in the dialkyl substrates with side chains containing heteroatoms such as oxygen can give access to an even larger class of important intermediates. For example, we synthesized substrate **13** where one alkyl group was replaced with an *O*-acetyl group. Substrate **13** represents a very interesting and potentially very useful class of substrates since its reduction product, α,β -dihydroxy ketone, contains a secondary and a tertiary 1,2-diol. Stereoselective synthesis of such compounds is very difficult and is usually achieved by asymmetric epoxidation of an alkene followed by hydrolysis. Here the stereochemistry of both hydroxy groups is produced in one step starting from a non-chiral, easy to synthesize starting material (**13**, Table 1).

Upon enzymatic reduction of diketone **13**, the α -oxo-acetyl keto alcohol **13i** was forming first. Under the reaction conditions however, this intermediate rearranged very fast to the final product β -oxo-acetyl keto alcohol **13a**, probably by an intramolecular trans-

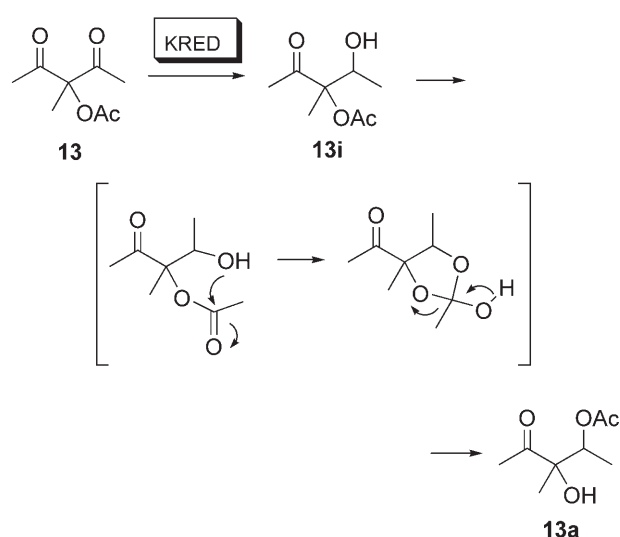


Figure 2. Product formation and rearrangement of substrate **13**.

esterification (Figure 2). This reaction progress was monitored by ^1H NMR at three different pH values (6.5, 6.9 and 7.5). In every case, the β -*O*-acetylated alcohol **13a** was the only observed final product even though in early time points of the reaction, a mixture of the α - and the β -oxo-acetylated alcohols (**13i** and **13a**) was observed. Many enzymes gave product with substrate **13**. However, only two gave high stereoselectivity of product formation (Table 1). The absolute configuration of the product has not yet been determined. The assignment of the major product as **A** in Table 1 was based on the relative time of elution of this product from the chiral GC column (first to come out).

High regioselectivity and, most of the times, medium to excellent stereoselectivity were obtained in all reactions. Most enzymes gave the keto alcohol as the only product, and only a couple of them (A1B for example) were able to reduce further the keto alcohol they produced, upon addition of excess enzyme and after longer reaction times. Even in these cases, the keto alcohol could be isolated as a pure product in quantitative yields, if the reaction progress was monitored. This selectivity was quite surprising considering the wide substrate range that most of these enzymes reduce.^[12–15] In order to determine whether such selectivity was dependent on the keto alcohol stereochemistry or the keto alcohol chemical composition, we investigated the reduction of various diastereomers of the same keto alcohol (substrate **2**). This keto alcohol was selected because three out of the four possible diastereomers can be synthesized in high optical purities. As a result, the dependence of reactivity on absolute stereochemistry could be more thoroughly checked. Each keto alcohol **2a–c** (Table 2) was synthesized and was then tested for reduction by using selected KREDs. Most KREDs tested were either inactive or had lower activities for the reduction of keto-alcohols **2a–c** than for the reduction of diketone **2**.

In Table 2 are shown some representative examples of enzyme reactivity. In one example, KRED101 showed small stereoselectivity for the formation of

Table 2. Enzymatic reduction of diketones **2** and **13** and subsequent formation of their corresponding 1,3-diols.

Substrate	First Reduction: keto alcohol (% <i>de</i>)					Product	Second Reduction: 1,3-diol (% <i>de</i>)					Product
	101	102	112	118	A1B		101	102	112	118	A1B	
2	58 %	99 %	96 %			3 <i>R</i> ,4 <i>S</i> 2a	99 %	NR	99 %	NR		2 <i>S</i>
								NR		NR	99 %	2 <i>R</i>
	27 %			99 %		3 <i>S</i> ,4 <i>S</i> 2b	95 %	NR	99 %	NR		2 <i>S</i>
								NR		NR	99 %	2 <i>R</i>
13					95 %	3 <i>S</i> ,4 <i>R</i> 2c	95 %	NR	99 %	NR	99 %	2 <i>R</i>
	95 %	95 %	98 %	93 %	30 %	13a	97 %	99 %	98 %	NR		A
										NR	> 99 %	B

NR = no reaction was detected.

keto alcohol by giving a mixture of **2a** and **2b**. However, it reduced all three diastereomers **2a–c** to the corresponding 1,3-diols in high optical purities. EXP-A1B on the other hand, gave products in high optical purities after the reduction of both diketone **2** and all diastereomers **2a–c**. KRED102 and KRED118 that led to optically pure **2a** and **2b** respectively, were inactive with all keto alcohols **2a–c** (Table 2). Just as the enzyme reactivity was not easily predictable, the stereoselectivity of ketone reduction also varied depending on substrate and enzyme. For example, KRED112 and KRED101 catalyzed the *S* reduction of the keto group of **2a** and **2b**, and *R*- for the keto alcohol **2c**. Both enzymes showed *S* enantipreference for the first reduction of diketone **2** (Table 2). In contrast, enzyme A1B showed *R* stereoselectivity in the reduction of both diketones and all keto alcohols (Table 2).

We extended our studies to the reduction of keto alcohol **13a** which, upon further reduction, would give chiral 1,2,3-triols (Figure 1). Again many enzymes reacted with keto alcohol **13a** but only some are shown in Table 2. Looking at this Table, a few interesting observations can be made. First, KRED102, which was inactive towards all three diastereomers of keto alcohol **2**, now gave a product of high optical purity. Although only one diastereomer could be accessed in high optical purities in the first reduction of diketone **13**, both possible enantiomers were formed in the second step for the reduction of the keto alcohol (**A** and **B** in Table 2). The absolute configuration of these products was not determined. However on the basis of the previous stereoselectivity in the second reduction step, enantiomer **A** should have the *2S* alcohol configuration and enantiomer **B** should have the *2R* configuration.

All reactions shown in Table 1 were performed on a small scale (1 mL, 25 mM substrate) by using 10–25% (g/g) of enzyme relative to the diketone and 10% (mol/mol) of cofactor. Selected reactions (#1 with KRED-102 and #8 with KRED-118 and KRED-A1B) were performed in larger scale (100 mL 50 mM substrate) by using the same relative concentration of KRED to substrate (10–20% g/g) and 1% (mol/mol) of NADPH relative to substrate. Quantitative yields were obtained within 24 h of reaction time, and isolated yields of >85% were obtained (details in Experimental Section). In general, high yields and purities of products are usually obtained from the enzymatic reductions since byproducts resulting from harsh reaction conditions (i.e., excessive heating, use of strong acid/bases) do not form under the mild enzymatic reaction conditions (near neutral pH and 25–35 °C).

It is important to note at this point that these reactions have not been optimized in terms of substrate, enzyme and cofactor. Improvement of the relative amounts of KRED to substrate to give high reaction

yields in a timely fashion (<24 h) can be achieved in most cases. The parameters that need to be optimized in order to scale enzymatic reductions to preparative (10–50 g) and pilot (24 kg) scale have been discussed previously.^[13a,b] In all the reactions presented here, the NADPH cofactor was very efficiently recycled by using glucose and glucose dehydrogenase (GDH), an enzyme that has been cloned and overexpressed and can regenerate NADPH from NADP⁺ by the concomitant oxidation of glucose to gluconolactone. Product isolation and purification in all these reactions was very simple and consisted of an aqueous organic extraction, where all the salts, cofactors and glucose products remain in the aqueous layer and all the organic products (and starting materials if present) are extracted in the organic layer.

The diastereomeric ratio of products presented in Tables 1 and 2 was derived by chiral GC analysis. The relative configuration of keto alcohols **1**, **8**, **9** and **10** was found by comparing their ¹H NMR spectra with literature data. The relative configuration of keto alcohols **2–7** was found by measuring the $J_{vic(H,H)}$ after decoupling experiments. The relative configuration of keto alcohols **11** and **12** was found by comparing the ¹³C NMR spectrum of the diastereomers and by applying the Heathcock rule.^[16] The absolute configuration of keto alcohols **1–12** was found by transforming them to their corresponding MPA (*R* or *S* methoxyphenylacetic acid) esters.^[17]

Conclusions

The reduction of a large number of α -monoalkyl and dialkyl symmetrical as well as non-symmetrical 1,3-diketones by thirty four NADPH-dependent commercially available ketoreductase enzymes (KREDs) is presented. All diketones described in this report were synthesized in high yields from readily available starting materials. Chiral keto alcohols and diols, such as the ones described herein, represent very useful synthons in organic synthesis. Such compounds have been used as precursors in the synthesis (or are structural elements) of various biologically active compounds and pharmaceuticals.

At least two, and in many cases three out of the four possible diastereomers of all the α -alkyl-substituted β -keto alcohols (symmetrical, non-symmetrical mono- or dialkyl-substituted) were selectively produced by the use of different enzymes. With only two exceptions, all enzymes gave the same absolute configuration for the reduction of the first keto group regardless of the α -alkyl substitution. The stereoselectivity of the second chiral center (that of the α -alkyl position), however, was not always high and was dependent on the enzyme and the diketone substrate.

Most enzymes were either inactive or possessed much lower activity for the reduction of the keto-alcohol that they produced. As a result, the isolation of chemically pure keto alcohols in high reaction yields and optical purities was possible in every successful enzymatic reduction. This selectivity is impressive if one considers that most diketones (with the exception of non-symmetrical **9** and **10**) possess a plane of symmetry making the two keto groups enantiotopic. To our knowledge, no chemical catalysts exist that can distinguish between so closely related keto groups and give only one of the four potential diastereomeric keto alcohols and without further reduction to the diol. In the case of the two non-symmetrical diketones **9** and **10**, the less hindered ketone was reduced by all active enzymes.

Formation of the 1,3-diols was achieved in a second enzymatic step. Almost every enzyme tested showed lower activity in the reduction of the keto alcohol compared to the corresponding diketone. In all cases investigated, high stereoselectivity in the 1,3-diol formation was achieved even by enzymes that were not very stereoselective in the first reduction step of the same diketone (Table 2).

By changing the functionality at the α -alkyl position other useful classes of compounds can be synthesized. For example, we replaced one alkyl group with an acetyl group (compound **13**), and upon enzymatic reduction we obtained either the 1-keto-2,3-diol or the 1,2,3-triols (**IV**, **V**, Figure 1). One can speculate that by replacing of the acetyl with a free or protected amine, various different 1,2-amino alcohols can be synthesized by using the same methodology. In addition, other functionalities can be present in the α -alkyl substituent as in substrates **5–7** and **12**. Clearly, a large number of useful and reactive chiral synthons can be synthesized by modifications of the 1,3-diketone scaffold. Experiments are underway to further extend the applicability of this method.

The results that are presented in this report do not only show an easy, high yielding and stereoselective synthesis of various important classes of compounds (Figure 1), but also represent an excellent example of the usefulness of enzymes as catalysts in chiral organic synthesis. The general applicability of enzymatic reductions in the synthesis of a wide range of chiral alcohols as well as their scalability has recently been shown.^[12–14] An additional advantage of using isolated enzymes for screening that is not usually applicable to chemical or whole cell microbial catalysts, is their rapid identification of activity towards any substrate. After an active enzyme has been identified, scaling the process to laboratory-scale quantities is usually fast and straightforward.^[13a,b] Further improvement of the enzymatic activity, stability or selectivity is also possible using modern techniques of enzyme evolution. This is a unique advantage that only enzymes

have, making them an even more appealing biocatalyst for large-scale synthesis. The use of isolated enzymes as catalysts for organic reactions is becoming a more standardized and practical tool in the hands of synthetic chemists, mainly because of the increasing need for environmentally benign methods, the commercial availability and low price of purified enzymes.

Experimental Section

General Analytical Methods

Flash chromatography was carried out with 60 Å silica gel according to the procedure of Still.^[19] The progress of the enzymatic reactions and the selectivities were determined by gas chromatography of crude EtOAc extracts (for the small scale reactions) and of isolated products (in the larger scale reactions) using HP5890II gas chromatograph equipped with an FID detector; (column: 30 m $\times$ 0.25 mm $\times$ 0.25 μ m chiral capillary column, 20% permethylated β -cyclodextrin HP Part No 19091G-8233). Some keto alcohols were acetylated prior to GC analysis. The exact methodology used to separate every racemic keto alcohol is presented in the supplementary material. In addition representative chiral GC chromatograms for racemic and enzyme-produced keto alcohols **1**, **2**, **4**, **5**, **7**, **9** are presented in the supplementary material of ref.^[14]

¹H NMR and ¹³C NMR spectra were recorded on a 300 or 500 MHz Bruker spectrometer in CDCl₃ solutions, by using Me₄Si as internal standard. Chemical shifts are reported in ppm downfield from Me₄Si. Yields refer to isolated and spectroscopically pure materials. All ¹H NMR data and absolute stereochemistry assignments data are included in the Supporting Information.

Synthesis of 1,3-Diketones

α -Alkyl-1,3-diketones **1–7** and **11**, **12** were prepared from commercially available 2,4-pentanedione (acetylacetone) by alkylation with the proper alkyl halide. α -Alkyl 1,3-diketone **10** was prepared from commercially available 1-phenyl-1,3-butadione by alkylation with methyl iodide. Diketones **8** and **9** were prepared by oxidation of their corresponding racemic keto alcohols. Diketone **13** was prepared from commercially available 3-chloro-2,4-pentanedione by treatment with sodium acetate following by alkylation with methyl iodide.

All diketones presented in this report are literature compounds. The ¹H NMR spectra of the synthesized diketones **1–13** were compared to those reported in the literature.

Synthesis of α -Alkyl 1,3-Diketones, **1–7** and **10**

Under a nitrogen atmosphere acetyl acetone (50 mmol) or 1-phenyl-1,3-butadione (50 mmol) were dissolved in anhydrous acetone (40 mL) and pre-dried potassium carbonate (46.5 mmol) was added. After stirring the solution at room temperature for 5 min, the proper alkyl iodide [**1**, **2**, **10**] or alkyl bromide (61.5 mmol) [**3**, **4**, **5**, **6**, **7**] was added drop wise over a period of 2 min and the reaction mixture was refluxed overnight. Complete reaction was observed by thin

layer chromatography analysis of reaction aliquots. After condensation of the reaction mixture, diethyl ether (50 mL) was added, the mixture was filtered and the organic layer was evaporated to dryness. Pure products were obtained using silica gel chromatography (hexane/EtOAc, v/v, 8/1), 70% to 90% isolated yield. Exact reaction yields and product characterization for each diketone are presented in the supplementary material.

Synthesis of Diketones, 11 and 12

Under a nitrogen atmosphere 3-ethyl-2,4-pentanedione **2** (39 mmol, 5 g) or 3-allyl-2,4-pentanedione **5** (39 mmol, 5.54 g) were dissolved in anhydrous acetone (40 mL) followed by the addition of pre-dried potassium carbonate (40 mmol, 5.5 g). After stirring the solution at room temperature for 5 min, methyl iodide (45 mmol, 2.72 mL) was added dropwise in each reaction over a period of 2 min and the reaction mixture was refluxed overnight, where complete reaction was observed by thin layer chromatography. After condensation of the reaction mixture, diethyl ether (50 mL) was added, the mixture was filtered and the organic layer was evaporated to dryness. Pure diketone (4.5 g) were obtained using silica gel chromatography (hexane/EtOAc, v/v, 8/1), 90% isolated yield for both reactions.

Synthesis of Diketones 8, and 9, by Oxidation of the Corresponding Hydroxy Ketone with PCC

A typical protocol is described. Under a nitrogen atmosphere pyridinium chlorochromate (PCC, 4.9 g, 22.5 mmol) was added in dry methylene chloride (10 mL) and the mixture was cooled to 0°C. Then a solution of hydroxy ketone (15 mmol, 2.16 g of keto alcohol **8** or 1.95 g of keto alcohol **9**, whose synthesis is described in section 2.2), in dry CH₂Cl₂ (5 mL) was slowly added and the reaction mixture was stirred at 0°C for 2 h where completion of the reaction was observed by TLC analysis. Diethyl ether (100 mL) was then added followed by double filtration over solid Floricil. Washing of the solids with Et₂O, and evaporation of the combined organic layers gave the product, which was used for enzymatic reduction without any further purification. Pure products were also obtained using silica gel chromatography (hexane/EtOAc, v/v, 5/1), 70% isolated yield in both cases.

Synthesis of Diketone 13

a) 1-Acetyl-2-oxopropyl acetate was prepared from commercially available 3-chloro-2,4-pentanedione according to the following procedure: under a nitrogen atmosphere dry sodium acetate (902 mg, 11 mmol) and 3-chloro-2,4-pentanedione (1.25 mL, 11 mmol) were dissolved in 20 mL dry DMF. The reaction mixture was left under stirring at 50°C overnight. After completion of the reaction, 50 mL H₂O were added and the mixture was extracted three times with CHCl₃ (60 mL). The organic layer was washed three times with H₂O (60 mL), dried over MgSO₄ and evaporated to dryness. The pure product, 1-acetyl-2-oxopropyl acetate, was obtained using silica gel chromatography (hexane/EtOAc, v/v, 8/1) 60% isolated yield.

b) 1-Acetyl-1-methyl-2-oxopropyl acetate, diketone **13**, was prepared by following the same procedure as in the

preparation of diketones **10–12**, by alkylation of the prepared 1-acetyl-2-oxopropyl acetate with methyl iodide in 90% isolated yield. Combined isolated yield for both steps: 54%. Without any further purification diketone **13** was subjected to enzymatic reduction.

Synthesis of Racemic β -Keto Alcohols as Standards for Chiral GC Analysis

Racemic keto alcohols **1–7** and **11–13** were prepared as standards by reduction with sodium borohydride. The corresponding keto alcohols **8** and **9** were prepared from 3-pentanone by aldol condensation with the corresponding aldehyde. Keto alcohol **10** was prepared from 1-phenyl-1-propanone by aldol condensation with acetaldehyde.

Synthesis of Racemic β -Keto Alcohols 1–7 and 11, 12 and 13

Under a nitrogen atmosphere sodium borohydride (0.098 mmol) was added in dry ethanol (10 mL) and the mixture was cooled to 0°C for 5 min. A solution of dry ethanol (5 mL) containing diketone (0.3 mmol) was then added drop wise. After stirring at 0°C for 2–4 h, TLC and GC analysis confirmed reaction completion. The reaction mixture was then cooled to 0°C, saturated ammonium chloride (1 mL) was added and the mixture was evaporated to dryness. After addition of 15 mL water, the mixture was extracted twice with ethyl acetate (2 $\times$ 20 mL). The combined organic layers were dried over MgSO₄, and evaporated to dryness. Pure products were obtained using silica gel chromatography (hexane/EtOAc, v/v, 9/1), 70% to 80% isolated yield.

The same method was used for the reduction of diketone **13**. After purification with flash column chromatography (hexane/EtOAc, v/v, 9/1), 30% of the racemic keto-alcohol was isolated.

Synthesis of Racemic Keto Alcohols 8, 9 and 10 by Aldol Condensation

These substrates were prepared by aldol condensation starting from pentan-3-one or 1-phenyl-1-propanone and addition of propionaldehyde for compound **8** and acetaldehyde for **9** and **10**. A typical protocol for their synthesis is described as follows. Under a nitrogen atmosphere, diisopropylamine (4.2 mL, 30 mmol) was dissolved in dry THF (15 mL). The mixture was cooled to 0°C and BuLi 1.45 M in hexane (13.8 mL, 20 mmol) was added dropwise. After stirring for 15 min at room temperature, the mixture was cooled to –78°C and a solution of pentan-3-one (2.1 mL, 20 mmol) in dry THF (10 mL) or 1-phenyl-1-propanone (2.66 mL, 20 mmol) was added and the mixtures were stirred for 20 min. A solution of aldehyde (propionaldehyde 1.44 mL or acetaldehyde 1.12 mL, 20 mmol) in dry THF (10 mL) was then added over a period of 20 min and the solution was stirred for another 3 h (temperature was slowly increased to 0°C). Completion of the reaction was observed by TLC analysis of reaction aliquots. The reaction mixture was quenched with saturated NH₄Cl (50 mL) and was then extracted with diethyl ether (60 mL). The organic layer was washed with saturated NaCl (100 mL) followed by distilled water (100 mL). After drying over MgSO₄ evaporation to

dryness, and silica gel chromatography (hexane/EtOAc, v/v, 5/1), 70 % of pure keto alcohols were isolated.

Enzymatic Reductions

Thirty-four different ketoreductases (KRED-101–131 kit; and three from the KRED-EXP-A1B kit; BioCatalytics, Inc. Pasadena, CA USA) were screened for reduction. In addition to the ketoreductases, both NADPH and glucose dehydrogenase (GDH) that was used for cofactor recycling, are products available from BioCatalytics.

Small-Scale Screening of Enzymatic Reactions (Table 1)

The screening was performed in small-scale reactions where in a phosphate buffered solution (1 mL, 200 mM, pH 6.9) each substrate (25 mM) was mixed with NADPH (2.5 mM, 2 mg) and 2 mg mL⁻¹ of each ketoreductase. The mixture also contained glucose (100 mM, 18 mg) and glucose dehydrogenase (GDH, 2 mg mL⁻¹) for cofactor recycling. The reactions were incubated at 37 °C and reaction aliquots were taken at various time intervals. After extraction with ethyl acetate the crude extracts were analyzed by GC chromatography. All the small-scale enzymatic reductions were performed according to the previous protocol, except for diketones **1** and **9**, where the reaction was buffered at pH 6.5 and diketone **13** at pH 7.5. Reaction times and yields are shown in Table 1. Isolated products were analyzed by ¹H NMR and are shown in the supplementary material.

Larger-Scale Enzymatic Reductions

Larger-scale enzymatic reductions were prepared according to the following protocol. A phosphate-buffered solution (100 mL, pH 6.5 or 6.9, 200 mM) containing 50 mM substrate, NaCl (200 mM), glucose (120 mM), NADPH (0.5 mM), glucose dehydrogenase (50 mg) and the appropriate ketoreductase (50–150 mg) was stirred at 37 °C for 24 h, until GC analysis of crude extracts showed complete reaction. Periodically the pH was readjusted to 6.5 or 6.9 with NaOH (2 M). Product was isolated by extracting the crude reaction mixture with EtOAc (70 mL × 2). Sometimes centrifugation (6000 rpm, 10 min) was required for the aqueous and the organic layers to separate as clear solutions. The combined organic layers were then extracted with saturated NaCl solution, dried over MgSO₄ and evaporated to dryness. Pure, optically active alcohols were obtained in 85 % to 90 % yield. The products were analyzed by NMR spectroscopy. Their optical purity was determined by chiral GC chromatography using 20 % permethylated cyclodextrin column.

According to this protocol diketone **1** (570 mg, 50 mM) was reduced with KRED-102 (150 mg) at pH 6.5. Isolated yield 513 mg, 90 %. Diketone **8** (710 mg, 50 mM) was reduced with KRED-119 (100 mg) at pH 6.9. Isolated yield 618 mg, 87 %. Diketone **8** was also reduced with KRED-A1B (50 mg) at pH 6.9. Isolated yield: 604 mg, 85 %.

Enzymatic Reduction of Keto Alcohols **2a–c** to the Corresponding 1,3-Diols (Table 2)

After the enzymatic production of α -ethyl- β -hydroxy ketone to each **2a–c** diastereomers using KRED102 (**2b**), KRED118 (**2b**) and EXP-A1B (**2c**) reduction to the 1,3-diol was performed. In the same buffer and after the synthesis of **2a–c** (1 mL, 200 mM, pH 6.9), 4 mg of a different ketoreductase, 2 mg of glucose dehydrogenase, 2 mg NADPH and 18 mg of glucose were added. The reactions were incubated at 37 °C and reaction aliquots were taken at various time points. After extraction with ethyl acetate they were analyzed by GC chromatography. ¹H NMR and absolute configuration analysis data are included in the Supporting Information.

Enzymatic Reduction of Substrate **13** with KRED 112

In 8 mL phosphate buffer (200 mM, pH 7.5) were added 2 mg of KRED112, 2 mg GDH, 2 mg NADPH, 200 mg glucose (140 mM) and then 200 mg substrate (145 mM) were added periodically (10 mg every 5 min). The reaction was incubated at room temperature for two hours until completion of the reaction. The product was isolated by extracting the crude reaction mixture with EtOAc (10 mL × 2). The combined organic layers were then extracted with saturated NaCl solution, dried over MgSO₄ and evaporated to dryness to give 180 mg (90 %) of pure keto alcohol **13a**.

Enzymatic Reduction of Keto Alcohol **13a**

The enzymatic reduction of keto alcohol **13a** was accomplished by following the same procedure as in enzymatic reductions of keto alcohols in small scale.

Acknowledgements

IS thanks Prof Frances H. Arnold for her generous hospitality during her sabbatical stay at Caltech (2006). Financial support for this work came from the Greek Secretariat of Research and Technology (Pythagoras I, 2004 and PENED 2003). D. K. thanks the Greek National Scholarships Foundation (IKY) for providing a three year fellowship. We also thank Prof. G. J. Karabatsos for his valuable comments.

References

- [1] a) D. Sawada, M. Kanai, M. Shibasaki, *J. Am. Chem. Soc.* **2000**, 122, 10521; b) I. Paterson, D. Y.-K. Chen, J. L. Acena, A. S. Franklin, *Org. Lett.* **2000**, 2, 1513; c) F. Eustache, P. I. Dalko, J. Cossy, *Tetrahedron Lett.* **2003**, 44, 8823.
- [2] a) M. Kobayashi, K. Higuchi, M. Murakami, Y. Tajima, S. Aoki, *Tetrahedron Lett.* **1997**, 38, 2859; b) J. D. White, R. Hanselmann, R. W. Jackson, W. J. Porter, Y. Ohba, T. Tiller, S. Wang, *J. Org. Chem.* **2001**, 66, 5217; c) Y. Che, J. B. Gloer, D. T. Wicklow, *Org. Lett.* **2004**, 6, 1249; d) M. Magnin-Lachaux, Z. Tan, B. Liang, E.-i. Negishi, *Org. Lett.* **2004**, 6, 1425; e) G. F. Liou, C. Khosla, *Curr. Opin. Chem. Biol.* **2003**, 7, 279; f) R. W.

- Hoffmann, *Angew. Chem. Int. Ed.* **1987**, 26, 489; g) I. Paterson, V. A. Doughty, G. Florence, K. Gerlach, M. D. McLeod, J. P. Scott, T. Trieselmann, *ACS Symposium Series* **2001**, 783 (Organoboranes for Syntheses), 195–206; h) M. Chartrain, P. M. Salmon, D. K. Robinson, B. C. Buckland, *Curr. Opin. Biotechnol.* **2000**, 11, 209; i) J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **2001**, 18, 380; j) R. W. Hoffmann, W. Helbig, W. Ladner, *Tetrahedron Lett.* **1982**, 23, 3479; k) J. L. Vicario, A. Job, M. Wolberg, M. Müller, D. Enders, *Org. Lett.* **2002**, 4, 1023; l) N. Arai, N. Chikaraishi, S. Omura, I. Kuwajima, *Org. Lett.* **2004**, 6, 2845; m) F. J. Schmitz, S. P. Gunasekera, G. Yalamanchili, M. B. Hos-sain, D. Van der Helm, *J. Am. Chem. Soc.* **1984**, 106, 7251.
- [3] F. Eustache, P. J. Dalko, J. Cossy, *Org. Lett.* **2002**, 4, 1263.
- [4] Y. Ohtsuka, K. Koyasu, T. Ikeno, T. Yamada, *Org. Lett.* **2001**, 3, 2543.
- [5] J. L. Vicario, D. Badia, E. Dominguez, M. Rodriguez, L. Carrillo, *J. Org. Chem.* **2000**, 65, 3754.
- [6] N. A. Van Draanen, S. Arseniyadis, M. T. Crimmins, H. C. Heathcock, *J. Org. Chem.* **1991**, 56, 2499.
- [7] a) H-X. Wei, R. L. Jasoni, H. Shao, J. Hu, P. W. Pare, *Tetrahedron* **2004**, 60, 11829; b) J. Jankowska, J. Mlynarski, *J. Org. Chem.* **2006**, 71, 1317; c) R. Mahrwald, B. Schetter, *Org. Lett.* **2006**, 8, 281; d) N. Mase, Y. Nakai, N. Ohara, H. Yoda, K. Takabe, F. Tanaka, C. F. Barbas III, *J. Am. Chem. Soc.* **2006**, 128, 734.
- [8] D. A. Evans, J. V. Nelson, E. Vogel, T. R. Taber, *J. Am. Chem. Soc.* **1981**, 103, 3099.
- [9] Y. Yamamoto, K. Maruyama, *Tetrahedron Lett.* **1980**, 21, 4607.
- [10] C. H. Heathcock, C. T. Buse, W. A. Kleshick, M. C. Pir-rung, J. E. Sohn, J. Lampe, *J. Org. Chem.* **1980**, 45, 1066.
- [11] a) D. Buisson, S. Henrot, M. Larcheveque, R. Azerad, *Tetrahedron Lett.* **1987**, 28, 5033; b) A. Fauve, H. Veschambre, *J. Org. Chem.* **1988**, 53, 5215; c) For a recent review see: K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, *Tetrahedron:Asymmetry* **2003**, 14, 2659; d) M. J. Homann, R. B. Vail, E. Previte, M. Tamarez, B. Morgan, D. R. Dodds, A. Zaks, *Tetrahedron* **2004**, 60, 789; e) M. Muller *Angew. Chem. Int. Ed.* **2005**, 44, 362; f) M. Wolberg, W. Hummel, C. Wandrey, M. Muller *Angew. Chem. Int. Ed.* **2000**, 39, 4306; g) K. Edegger, W. Stampfer, B. Seisser, K. Faber, S. F. Mayer, R. Oehr-lein, A. Hafner, W. Kroutil *Eur. J. Org. Chem.* **2006**, 1904; h) B. Kosjek, W. Stampfer, M. Pogorevc, W. Goessler, K. Faber, W. Kroutil *Biotechnol. Bioeng.* **2004**, 86, 55; i) T. Ema, H. Moriya, T. Kofukuda, T. Ishida, K. Maehara, M. Utaka, T. Sakai *J. Org. Chem.* **2001**, 66, 8682.
- [12] a) S. Kambourakis, J. D. Rozzell, *Adv. Synth. Catal.* **2003**, 345, 699; b) S. Kambourakis, J. D. Rozzell, *Tetra-hedron* **2004**, 60, 663; c) D. Zhu, C. Mukherjee, J. D. Rozzell, S. Kambourakis, L. Hua, *Tetrahedron* **2006**, 62, 901; d) D. Zhu, B. E. Rios, J. D. Rozzell, L. Hua, *Tetra-hedron: Asymmetry* **2005**, 16, 1541; e) D. Zhu, C. Mar-kherjee, L. Hua, *Tetrahedron:Asymmetry* **2005**, 16, 3275.
- [13] a) I. A. Kaluzna, J. D. Rozzell, S. Kambourakis, *Tetra-hedron:Asymmetry* **2005**, 16, 3682; b) D. Pollard, M. Truppo, J. Pollard, C. Chen, J. Moore *Tetrahedron:A-symmetry* **2006**, 17, 554; c) T. W. Johannes, R. D. Wood-yer, H. Zhao *Appl. Environ. Microbiol.* **2005**, 71, 5728.
- [14] D. Kalaitzakis, J. D. Rozzell, S. Kambourakis, I. Smonou, *Org. Lett.* **2005**, 7, 4799.
- [15] D. Kalaitzakis, J. D. Rozzell, S. Kambourakis, I. Smonou, *Eur. J. Org. Chem.* **2006**, 2309.
- [16] C. H. Heathcock, M. C. Pirrung, J. E. Sohn, *J. Org. Chem.* **1979**, 44, 4294–4299.
- [17] J. M. Seco, E. Quinoa, R. Riguera, *Chem. Rev.* **2004**, 104, 17.
- [18] a) I. Paterson, J. M. Goodman, M. A. Lister, R. C. Schumann, C. K. McClure, R. D. Norcross, *Tetrahedron* **1990**, 46, 4663; b) A. Fauve, H. Veschambre, *Tetrahe-dron Lett.* **1987**, 28, 5037.
- [19] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, 14, 2923–2925.