Advances in the Enzymatic Reduction of Ketones

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ABSTRACT

Historically, biocatalytic ketone reductions involved the use of Baker's yeast. Within the last five years, a significant and growing number of isolated ketoreductases have become available that have rendered yeast-based reductions obsolete. The broad substrate range and exquisite selectivities of these enzymes repeatedly outperform other ketone reduction chemistries, making biocatalysis the general method of choice for ketone reductions. Presented here is a summary of our understanding of the capabilities and limitations of these enzymes.

Introduction

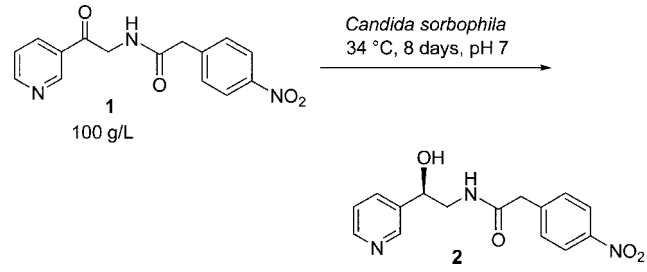
Biocatalysis is well-matched to chemical synthesis in the pharmaceutical industry. A significant number of pharmaceuticals products are chiral in at least one center, and many compounds have multiple chiral centers. The efficient syntheses of these chiral centers require enantio- and regioselective catalysts, and enzymes are consistently the most selective catalysts available. Enzymes that catalyze ketone reductions (known as ketoreductases) are a reliable source of high enantiomeric excess chiral alcohols matching and often exceeding the ability of chemical catalysts to perform the same reactions.

Background: Whole Cell Bioreductions

Much of the historical impact of ketone bioreductions on synthetic chemistry derives from the use of Baker's yeast or similar naturally existing whole cell catalysts. Using the diverse collection of yeast strains available at Merck, a wide variety of ketones have been stereoselectively reduced to give desired alcohol products.[1] Because the cells are self-replicating from simple nutrients, significant amounts of whole cell catalysts have been made inexpensively. However, the factors regulating expression of the ketoreductase enzyme responsible for catalysis in these natural isolate organisms were poorly understood, and extensive fermentation optimization was required to ensure enzyme activity was produced consistently. Once the ketone was added to the active cells, nutrient feeding continued the fermentation throughout the reaction to replace enzyme lost to inactivation and to provide reducing equivalents, typically through the aerobic oxidation of glucose. Often the compounds we wanted to reduce showed some toxicity to these organisms and the need for metabolic activity throughout the reaction limited ketone concentrations to below toxic effect levels in the reaction and resulted in dilute processes.

When the ketone and alcohol toxicity were minimal and time was available to develop a reliable fermentation, whole cell wild-type yeast were productive and cost efficient catalysts. One such process is shown below on a ketoamide substrate **1** (Scheme 1).[2,3] A screen identified

Scheme 1. Ketoamide Whole Cell Reduction



the yeast *Candida sorbophila* as producing the (*R*)-hydroxy amide **2** in high enantiomeric excess (>98% ee). Extensive optimization provided a culture medium consisting of inexpensive ingredients (glucose, monosodium glutamate, and a few salts including trace amounts of $CuCl_2$) that consistently produced active yeast. Both **1** and **2** had low solubility (less than 0.5 g/L) at neutral pH but had good solubility in acid. Optimized bioconversion conditions incorporated a substrate feed as an acid solution to maintain the ketone concentration just below its solubility limit, avoiding reduced reaction rates resulting from the slow dissolution rate of precipitated ketoamide. End of reaction alcohol concentrations reached 100 g/L as a precipitate. To isolate the alcohol, addition of acid dissolved the precipitated alcohol, filtration removed the yeast, and addition of base induced crystallization of the alcohol. Multi-kilogram quantities of alcohol **2** were produced and isolated with good enantiomeric excess, yield, and economics, validating the time the optimization required. In addition, none of the chemical hydrogenation

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catalysts available were able to achieve the enantioselectivity required, further highlighting the value of the bioreduction.

Improvements in methodology allowed whole cell processes to be developed more quickly. Screening was scaled down to 96 well plates, rapid HPLC and data analysis automation were added, and instrumentation measuring the effects of toxicity was implemented. However, these improvements did not reduce the time required to optimize scalable fermentations or improve the toxicity of many ketones and alcohols to whole cells.[4] The lack of improvement in these key areas continued to make wild-type whole cell processes time-consuming in an industry where synthetic process development is continually pressed for time improvements.

Enzyme Development

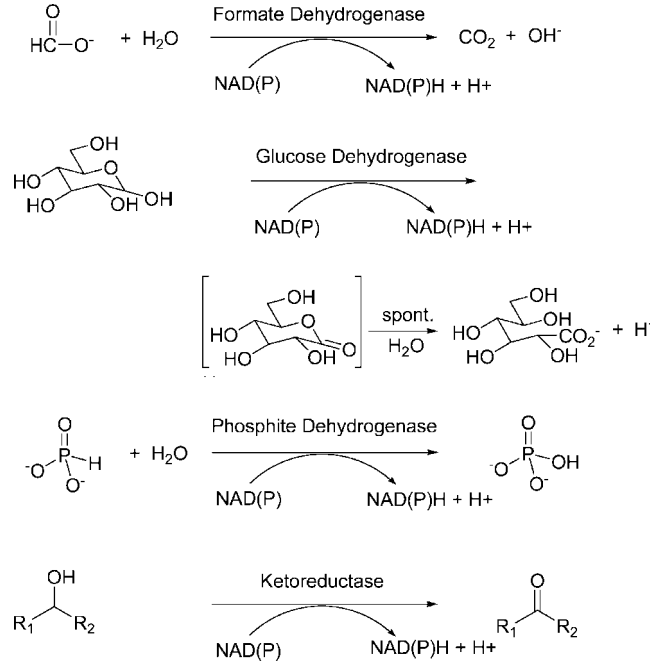
Improving issues of toxicity and development time required a change in approach. Isolating the ketoreductase enzymes from cells and using them directly in reactions eliminated problems of compound toxicity by eliminating the need for viable cells, and it accelerated alcohol synthesis by requiring robust fermentations for enzyme production prior to screening the biocatalyst for ketone reductions. The accumulation of genetic information through genome sequencing projects combined with enzyme expression systems made the isolated enzyme approach feasible. With this information every enzyme made in a genome could be inspected computationally and, if predicted to be a ketoreductase, produced in amounts 100−1000-fold more concentrated than in its original whole cell context. In fact, the analysis of Baker's yeast indicated the potential for 50 ketoreductases of which 19 were overexpressed, isolated, and studied.[5] This new ketoreductase collection reduced a wide array of ketones and produced both enantiomers of most products. In addition, conversions of moderate enantioselectivity from Baker's yeast were often the result of two or more enzymes, each with very good to excellent selectivity, operating in opposition to make enantiomeric mixtures. These ketoreductases expressed individually provided products in high enantiomeric excess. Extending this technology to the entire genetic database allowed for the commercialization of larger collections of ketoreductase enzymes and enabled the rapid production of large quantities of enzyme on demand.[6] Our primary suppliers of ketoreductases and the source of all enzymes described in this paper are Biocatalytics, Inc. (Pasadena, CA; listed in catalog as KRED-###, an otherwise unidentified number designation), and Julich Fine Chemicals (Julich, Germany; listed in catalog as ADH-XX where XX refers to the organism from which the enzyme was originally discovered; for example, ADH-RE is the alcohol dehydrogenase (a ketoreductase) from *Rhodococcus erythropolis*). Both companies have recently been purchased by Codexis, Inc. (Redwood City, CA).

With the appearance of ketoreductase enzymes, the process of screening, development, and reaction scale-up improved dramatically. Fermentation development and substrate toxicity issues disappeared; substrate concentrations rose to consistently greater than 50 g/L. Reactions could now be developed quickly and run in any standard chemical facility. Mass balance issues in isolation disappeared, and processes immediately became dependably very productive.

Cofactor Recycling Systems

The hydride source all ketoreductases use is either of the two forms of nicotinamide adenine dinucleotide cofactor, NADH or NADPH [abbreviated NAD(P)H]. NADH and NADPH are not readily available in the amounts or at the costs necessary to use stoichiometrically. In order to supply a feasible source of hydride and to drive the reaction to completion, a second reaction is added to recycle the oxidized cofactor $NAD(P)^+$ back to NAD(P)H. Choices for this second reaction that have been used at Merck are illustrated in Scheme 2. Glucose dehydrogenase

Scheme 2. NAD(P)H Cofactor Recycling Systems



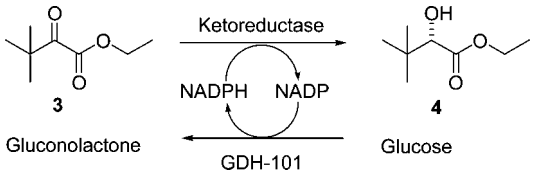
(GDH), formate dehydrogenase (FDH), and phosphite dehydrogenase (PDH) are second enzymes added to the process in a coupled enzyme approach.[7] GDH has been the preferred recycling system because it is highly stable and active and recycles both NAD and NADP. Neutralizing the gluconic acid formed requires base addition to maintain pH, and monitoring the amount of base added allows for rapid evaluation of reaction progress. FDH has historically recycled only NAD and has been less active and less stable than GDH, but it is a reasonable alternative when the ketone or alcohol product is sensitive to base addition or when GDH competes with the ketoreductase to nonselectively perform the primary ketone reduction. FDH requires pH control through acid addition, and new mutants of FDH demonstrate improved stability, activity, and NADP acceptance. Phosphite dehydrogenase repre-

sents the latest technology in cofactor recycling; it simply converts the reaction buffer from phosphite to phosphate with no significant pH change.[8,9] In our limited experience with this enzyme, it has activity similar to the original FDH, but it is much more stable, recycles both NAD and NADP, and works well through at least the 50 L scale. The final recycling system is a substrate-coupled approach and takes advantage of the ketoreductase's ability to not only reduce the ketone of interest but also oxidize a secondary alcohol (typically isopropanol).[10–12] This method requires a large molar excess of isopropanol relative to ketone (and when necessary acetone removed) to drive the reaction. This method of cofactor recycling is thus limited to enzymes that tolerate high solvent alcohol concentrations. We have used this system to good effect when the reactions require harsh conditions (i.e., high temperature, or where the substrate or product is a catalyst poison), where the probability of finding both a ketoreductase and a recycling enzyme able to withstand these conditions is low. These cofactor recycling enzymes were purchased from Biocatalytics, Inc. [Pasadena, CA; listed in catalog by abbreviation and three digit number (e.g., GDH-101); the 101 designates the original enzyme, and subsequent numbers indicate variants with improved properties (e.g., GDH-103 is a thermostable variant)].

Initial Reaction

The first compound screened against the initial ketoreductase library (consisting of 10 enzymes) was ethyl trimethylpyruvate **3** to identify a catalyst that made the corresponding *R* enantiomer alcohol **4** (Scheme 3). We

Scheme 3. Ethyl Trimethylpyruvate Reduction to (*R*)-2-Hydroxy-3,3-dimethylbutyric Acid



screened **3** against both the whole cell and isolated enzyme catalyst libraries and found that ten yeasts and one enzyme produced **4**. The enzyme KRED-101 demonstrated a selectivity of 500:1 in favor of the *R*-alcohol and did not suffer from the low concentration limits and limited extent of conversion that the whole cells did. Process development utilizing this enzyme quickly converged on 50 g/L substrate charge as a second phase oil with phosphate buffer (pH 7.0) and 0.1 g/L KRED-101, corresponding to 0.2% catalyst loading by weight and a molar ratio of substrate to catalyst of ~150 000 to 1. GDH-101 (0.5 g/L) was used as the recycling system enzyme and required 1.3 mol equiv of glucose as the hydride donor and 0.12 g/L NADP cofactor (2000 to 1 molar ratio of substrate to cofactor). With substrate feeding over the course of the reaction, 100 g/L alcohol concentration could be reached without altering enzyme or cofactor amounts. As shown in Figure 1, the reaction could be stressed to the point of failure through incomplete mixing

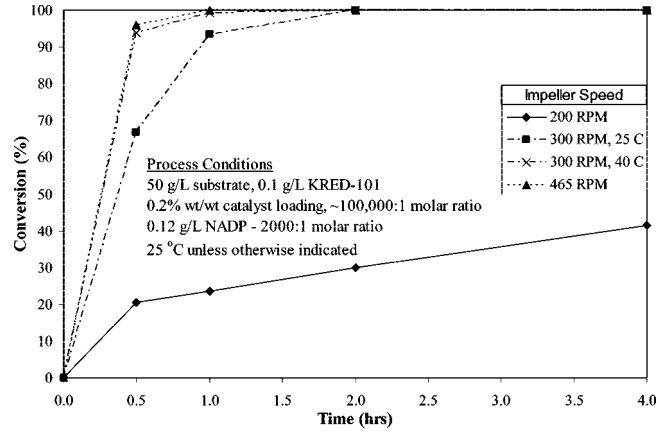


FIGURE 1. Process characteristics of ethyl trimethylpyruvate reduction.

(200 rpm leaves a visible second phase) or by agitation to the point where air is entrained by the impeller (well beyond the data shown in Figure 1) as this reaction also did not complete. When pushed to the point of failure, however, the highly selective nature of the biocatalyst did not change; the reaction simply stopped. Addition of fresh enzyme restarted the reaction and produced the same highly enantioselective results as before. This is typical of enzymatic reductions; selectivity is maintained throughout the reaction, and events that alter the enzyme's ability to function (e.g., temperature, pH excursions, overaggressive agitation) typically result in absence of activity rather than alteration of selectivity.

Platform Technology

Isolated ketoreductases have made tremendous strides from this first project. The number of enzymes commercially available have increased from the 10 in the original screen to 35 for much of the work reviewed here to most recently 130, and these catalysts reduce a wide range of ketones and generally produce enantiocomplimentary alcohol products. The screening technology developed for use in the whole cell efforts has been implemented on the enzyme platform, allowing for rapid (1 day) determination of reaction feasibility. Automation has been added through Merck's collaboration with Symyx (Santa Clara, CA), who provided an automated powder dispensing robot to prearray catalysts, liquid handling robots for automated generation and sampling of reactions, and control of analytical equipment for automated data handling to create a standard screening process that could be run as a template. The cofactor recycling systems have been evolved to be more stable and more active (GDH-103, for example), thereby requiring less recycle catalyst to perform the reduction and allowing for broader ranges of temperature, pH, and solvent conditions.[13] Taken together, we had a library of ketoreductases that could be screened quickly, produced hits of good enantiomeric excess (ee) on the majority of compounds, and could be scaled readily from a few grams of alcohol product for route scouting through many kilograms for clinical testing. This provided the basis for bringing

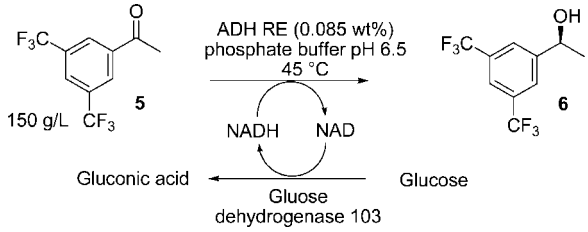
enzymatic ketone reduction from a chemistry that contributed rarely and only late in the development pipeline to one that is part of the mainstream, everyday Process Chemistry culture at Merck. At scale, the costs of enzymatic ketone reduction catalysts today are often better than their chemocatalytic counterparts, and we expect pricing in the enzyme supply market to continue to improve. In addition, the enzyme catalysts are produced from renewable resources, biodegradable, and less toxic than alternatives.

Acetophenone Reductions

Over the past few years, this library of ketoreductases has been successfully used to reduce ketones across several structural classes. Substituted acetophenones comprise one of the more important structural classes, and reduction has been clearly demonstrated by chemocatalysis.[14] Biocatalysts have been equally successful and often lead to significantly higher selectivity. Much of the current ketoreductase library is highly active on acetophenone.[15]

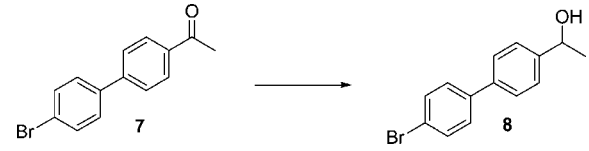
An example of this library's impact on the substituted acetophenone class is the reduction of 3,5-bistrifluoromethylacetophenone **5** to (*S*)-3,5-bistrifluoromethylphenyl ethanol **6** (Scheme 4).[16] Alcohol **6** is difficult to upgrade

Scheme 4. Reduction of 3,5-Bistrifluoromethyl Acetophenone



by crystallization; the biocatalytic route was chosen over chemocatalytic methods as a result of the superior selectivity displayed by ketoreductases. The ketoreductase ADH-RE gave the *S*-enantiomer product in >99.9% enantiomeric excess. Both the FDH and GDH recycling systems were considered; GDH was chosen based on a pH optimum that matched ADH-RE and the availability of a highly thermostable variant (GDH-103). As in many of the acetophenone derivatives, substrate **5** is an oil with low solubility (<2 mM) in water. ADH-RE appears to work on these compounds effectively at low substrate concentrations, but the reactions are substrate limited, and increased solubility increases the reaction rate. Often we improve solubility by cosolvent addition, but the good thermostability of GDH-103 and ADH-RE allowed us to improve the solubility sufficiently with elevated temperature (45 °C) to eliminate the need for cosolvent. The process characteristics rival that of the ethyl trimethylpyruvate reduction described earlier: this process employs a substrate charge of 150 g/L, catalyst loading of 0.085% by weight, molar ratios of substrate to enzyme approximately 250 000:1 and 0.125 g/L GDH-103, 4-fold less than the optimized ethyl trimethylpyruvate reduction employing the original GDH.

Scheme 5. Reduction of 4-Br-biarylacetophenone



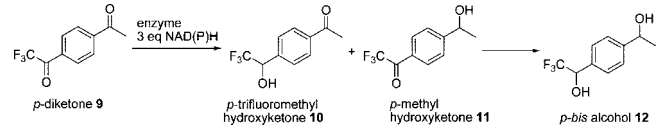
The *R*-enantiomer of 3,5-bistrifluoromethylphenyl ethanol **6** was also an important intermediate and was produced enantioselectively using the same process but with a different ketoreductase, KRED-101.[16] Like ADH-RE, KRED-101 is highly stable and highly active.

Knowing that ADH-RE and KRED-101 typically provide enantiocomplimentary results on larger acetophenones enabled us to move quickly on other intermediates. When we examined the 4-Br-biarylacetophenone reduction **7** (Scheme 5) that needed a few grams of material within 1 week, we initiated a screen and at the same time started gram-scale reactions of ADH-RE and KRED-101. Within 24 h, we isolated high ee alcohol of both enantiomers.

Chemoselective Reductions

The ketoreductases are also chemoselective. When we screened the *para* aryl diketone **9** (Scheme 6), the reduc-

Scheme 6. Regioselective Reduction of a *para*-Diketone



tion of the electron-deficient trifluoromethyl ketone was generally the favored product (**10**), but in addition a few enzymes were selective for the methyl ketone reduction (**11**).[17] Table 1 contains the product distribution and

Table 1. Enzymatic Chemoselective Reduction of *para*-Diketone

	product distribution (%)			
enzyme	**10**	**11**	**12**	ee of major product (%)
KRED-129	100	0	0	>99 (*R*)
KRED-131	100	0	0	>99 (*R*)
KRED-A1n	100	0	0	>99 (*R*)
KRED-A1x	100	0	0	>99 (*R*)
KRED-112	100	0	0	>99 (*S*)
KRED-A1i	100	0	0	>99 (*S*)
KRED-113	86	0	14	>99 (*S*)
ADH-RE	47	10	43	>99 (*R*)
ADH-CP	0	100	0	98 (*S*)

selectivity of the best enzymes identified. Six enzymes gave only **10**, leaving the methyl ketone untouched and providing access to the enantiopure *R* and *S* alcohols. ADH-CP demonstrated a strong preference for the more electron-rich ketone and provided (*S*)-**11**. The ability to produce three of the four enantiomerically pure products without the use of protection/deprotection steps represents a significant advance in convenience and in decreased environmental impact over chemocatalytic reductions, which on this compound generate racemates of **10** or **11**[18] or on related β-diketone compounds poor to moderate ee for the trifluoromethyl alcohol only.[19] The data also

suggest that through judicious choice of enzymes, the four possible diastereomers of **12** could also be produced. By combining two enzymes, ADH-CP to set the methyl alcohol center to *S* and KRED-129 or KRED-112 to produce the *R* or *S* trifluoromethyl alcohol, respectively, two of the four diastereomers (*S, R* and *S, S*) could be made selectively. In addition, the enzymes that selectively produce primarily the *S* trifluoromethyl alcohol ((*S*)-**10**) but also reduce the methyl ketone to produce small amounts of diol (such as KRED-113) are likely to retain the same facial selectivity as they reduce the methyl ketone and would therefore selectively produce the *R* methyl alcohol, providing access to a third diasteromer (*R, S*). The fourth diasteromer might be made by first setting the *R* trifluoromethyl alcohol with KRED-129 and then finishing the reaction with KRED-113 to produce the *R* methyl alcohol (*R, R* diastereomer). This speculation is still under investigation. The data clearly show the ability to make three of the four possible enantiomers and suggests access to all four diastereomers without chemical protection of either ketone.

Aryl−Alkyl Ketone Reductions

When the methyl group was elaborated on the acetophenone structure by adding a halide (**13**) or a short alkyl halide (**14**) (Figure 2), the enantioselectivities were consistent with the previous description and with the literature, with KRED-112 and KRED-130 providing either enantiopure alcohol.[20] Similar to chemocatalysis, bio-

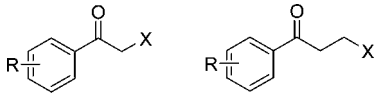


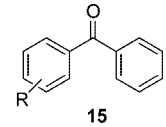
FIGURE 2. Generic substituted haloalkyl acetophenones.

reduction catalysts are subject to poisoning, generally by alkylating agents. Chemocatalyst poisons are typically organic soluble compounds; biocatalyst poisons are typically water soluble. Generally, washing low water solubility substrates with water prior to the reduction successfully removes the poisoning compounds. Under some substitution patterns, the haloalkyl acetophenones can act as alkylating agents, reducing the ability of many enzymes and most recycling systems to perform. In these instances, ADH-T proved highly valuable because of its naturally high stability and its ability to recycle cofactor using the isopropanol recycling system.

Diaryl Ketones and Beyond

Elaborating the methyl group of acetophenone still further produces the diaryl ketones (**15**) (Table 2), an important class of compounds because the corresponding chiral alcohols are intermediates in a variety of pharmaceutical applications.[21] Currently, chemocatalysts are limited in scope to diaryl ketones with substitutions sufficient to create enantioselectivity through steric effects or to cases where the aryl rings are electronically different.[22–27] In contrast, the ketoreductase library generated greater than 80% ee of at least one

Table 2. Ketoreductase Catalyzed Reduction of Diaryl Ketones



15

R=CH_3, NO_2, OH, NH_2, Cl

	(*R*)-alcohol		(*S*)-alcohol		yield %
ketone R	% ee	KRED	% ee	KRED	1 g scale
o-CH_3	**98**	121	**95**	119	95
m-CH_3	72	121			
p-CH_3	**85**	101	9	119	
m-NO_2	34	111	>**99**	108	90
p-NO_2	**96**	128	**97**	119	
o-OH	**84**	111			
m-OH	61	112	13	119	
p-OH	69	113	55	117	
o-NH_2	**91**	101	64	114	92
p-NH_2	60	111	51	119	
o-Cl	64	121	>**99**	118	
m-Cl	39	111	>**99**	108	95
p-Cl	64	101			

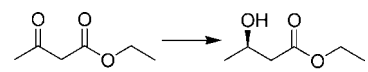
enantiomer on 14 of the 20 compounds studied (8 of the 13 shown here), including the more challenging *meta* and *para* substituted compounds (Table 2).[21] The best screening result for each compound is shown in the table; across all compounds, the set of enzymes KRED-101, -108, -111–119, -121, and -123 consistently demonstrate activity. The ketoreductase collection does not appear to be negatively influenced by substitution position or the electron-withdrawing or -donating nature of the substitution. Additionally, examples exist in the literature where enantioselectivity and other enzyme properties have been improved toward a given substrate through amino acid changes within the enzyme.[28] This approach might be used to improve the selectivity on any particular compound if higher ee values are required.

Further elaboration of the methyl group beyond diaryl ketones leads to compounds like the β-ketoamide **1** and others. The current library of ketoreductases contains a few enzymes active on molecules of this size, and these enzymes consist of KRED-101, -108, and -111−120. On the β-ketoamide structure all of these enzymes give good selectivity to produce (*R*)-**2**. Appending large groups to the methyl group and the aryl ring simultaneously often results in no activity.

Alkyl Ketone Reduction

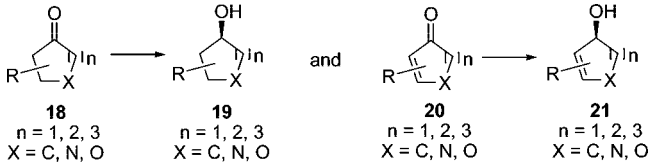
The ketoreductases also effectively reduce the alkyl ketones, such as the ethyl trimethylpyruvate **3** example. A number of publications address the reduction of these short chain α and β keto esters.[29–31] On the few projects

Scheme 7. Ethyl Acetoacetate Reduction



that we have examined (**3** and ethyl acetoacetate **16**, Scheme 7, as primary examples), KRED-101 and -107 gave a high ee value and good rates on the α-keto ester and on the β-keto ester respectively, both making the *R*

Scheme 8. Reduction of Cyclic Alkyl Ketones

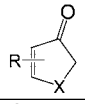
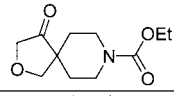
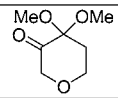
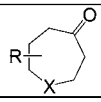


enantiomer similar to literature results.[29] In the case of **16**, the rate of the KRED-107 catalyzed reaction decreased significantly when changing from the ethyl to the methyl ester. We have observed sensitivity of rates and selectivity to changes between methyl and ethyl esters on a variety of alkyl ketones.

Cyclic Alkyl Ketone Reductions

Our efforts in the alkyl ketones class have focused more closely on cyclic alkyl ketones. Typical cyclic substrates are five- to seven-membered rings with an R group α or β to the carbonyl, often with a heteroatom in the ring (**18**) and occasionally with C=C double bond in conjugation with the ketone (**20**, Scheme 8). In many cases, the R group is duplicated so that the carbon atom containing the R group is not a stereocenter. Enzymatic reductions on these compounds typically occur with high selectivity and produce enantiocomplimentary results across the different ring sizes and heteroatoms as shown in Table 3.

Table 3. Enantioselective Reduction of Monocyclic Ketones[a]

Entry	n		%ee	Enzyme
1	1		98% (*R*) > 99% (*S*)	KRED-119 ADH RE
2 (**22**)	1		87% (+) -89% (-)	KRED-101 KRED-108
3 (**23**)	2		> 99% (*R*) > 99% (*S*)	KRED-101 ADH T
4	3		94% (*R*) 92% (S)	KRED-112 KRED-124

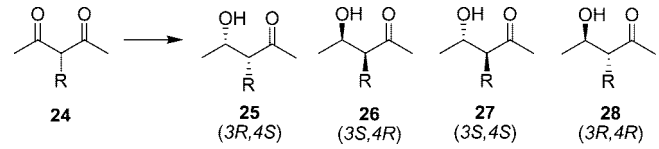
[a] X= C, N, or O.

Additionally, glucose dehydrogenase is active on many of the compounds in this class, presumably because these compounds have similar size and shape and can adopt similar conformations as glucose. As a result, scalable processes for the reduction of these ketones often require phosphite and formate dehydrogenases to recycle NAD(P).

Diastereomeric Ketone Reductions

When the R groups on these cyclic alkyl ketones are not symmetric, the carbon atom containing the R group is a stereocenter, and the reduction generates diasteromers. A number of dynamic diasteromeric reductions of α-substituted β-diketones (Scheme 9) have been published using Baker's yeast and ketoreductase enzymes as catalysts.[30–33]

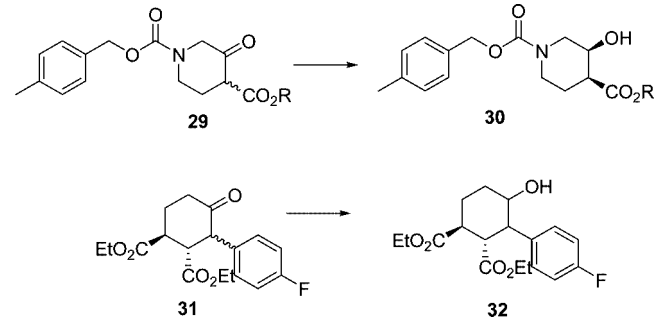
Scheme 9. α-Substituted β-Ketoester Reduction and the Four Possible Products



As a quick summary of this work, KRED-102, -106, and -112 make predominantly the *syn* isomer **25**, while KRED-A1B makes the other *syn* isomer **26**. KRED-118 and -119 make the *anti* isomer **27**. The *anti* isomer **28** is generally not selectively produced by these enzymes.

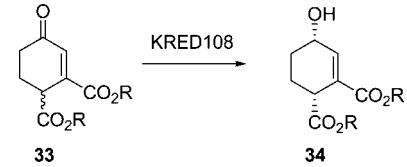
Examples of racemic substitutions α to a carbonyl that were candidates for dynamic kinetic reductions are shown in Scheme 10. Compound **29** is a cyclic example of **24** and was screened only against the first 10 KREDs. KRED-101, -102, -106, and -107 gave low conversion; KRED-108 gave high conversion to the single *syn* stereoisomer **30** (98% *syn*, 99% ee).

Scheme 10. Diastereomeric Reduction with *in Situ* Racemization



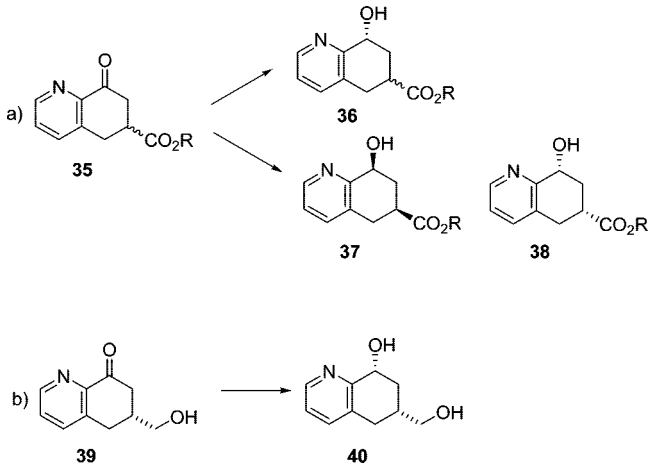
Ketone **31** was reduced to a single *syn* diasteromer by KRED-101 and others, while KRED-108 made racemic *syn* enantiomers. KRED-118 and -119 showed modest (40% ee) selectivity for *syn* isomers only. This is in clear contrast to the selectivity described in the α-substituted β-ketoester literature, where KRED-118 and 119 were noted as selective for a single *anti* diastereomer.[31]

Scheme 11. Stereoselective Reduction of a Vinyloguous Keto-ester



A similar structure **33** with no α-substitution but with a racemizable γ-stereocenter (Scheme 11) showed similar preferences for the *syn* diastereomers.[34] KRED-101, -111−115, -121, and -123 "read" the stereochemistry of the ester and match that with the stereochemistry of the alcohol, making predominantly equal mixtures of both *syn* enantiomers (substrate-controlled reduction), while ADH-RE makes the *S* alcohol without regard to the stereochemistry of the ester (catalyst-controlled reduction). KRED-108, -116, and -120 do both, providing the *S*-alcohol in the *syn* configuration with 9:1 selectivity (and therefore *R* ester) in high ee. When the ethyl esters were switched to

Scheme 12. Diastereomeric Reduction (a) without *in Situ* Racemization and (b) with Set Conformation of One Center



methyl esters, the 9:1 selectivity increased to 20:1, again highlighting the dramatic effects simple ester changes can have. ADH-LB is also highly selective for a *syn* isomer, but instead makes the enantiomer of the KRED-108 product in 52% ee. Additionally, one of the *anti* diastereomers is made with modest selectivity (2:1 *anti* to *syn*) in 99% ee by KRED-106. Taken together, these enzymes provide access to three of the four diastereomers with moderate to excellent selectivity. Chemocatalysts screened on the same compound could not generate any of the single isomers in excess.

The strong preponderance of *syn* alcohol reduction products on α- and γ-substituted cyclic alkyl ketones continues with the β-substituted cyclic alkyl ketones shown in Scheme 12. Ketones **35** and **39** show no evidence of racemization under mild pH reaction conditions. The ketoester **35** was reduced in catalyst-controlled fashion by KRED-101 to give the *R* alcohol regardless of the position of the ester, leading to equal mixtures of the *syn* and *anti* diasteromers (**36**). KRED-118 and -119 reductions proceed in substrate-controlled fashion; these enzymes "read" the position of the ester and match that configuration with the alcohol to make a racemic mixture of the two *syn* diasteromers (**37** and **38**). No enzyme exhibited substrate and catalyst control to produce a single diasteromer. On **39**, the β-substitution chirality is already set, and we would therefore expect catalyst-controlled KRED-101 and related enzymes to produce a single diasteromer, and they do, making the expected *syn* (*R*, *R*) diasteromer. Surprisingly, KRED-118 is inactive, and more surprisingly, KRED-119 preferentially makes the *anti* product in excess of 5:1 over the *syn*.

Biocatalyst Summary

On examination of the data from all of the substrate classes, some consistent themes emerge. First, ADH-RE performs reactions under catalyst control, meaning that the enzyme consistently delivers the hydride to one face of the ketone to make the *S* enantiomer alcohol (unless a substitution changes the naming of the enantiomer as in the trifluoromethyl alcohols; the enzyme's facial selectivity remains the same) on a wide array of compounds in various classes regardless of other stereocenter configurations in the molecule. This makes ADH-RE a catalyst of choice for enantioselective reductions but problematic for diastereoselective reductions where two centers would be set simultaneously. Additionally, ADH-RE is inactive on diaryl or larger ketones. KRED-108 and -116–120 appear to be related based on similar activity profiles against a wide array of compounds, and these plus KRED-130 and ADH-T will often give the *S* enantiomer, but the specificities of this group are affected by other structural features of the ketone substrate. As a result, this group contains some of the better diastereomerically selective catalysts on cyclic ketones and will occasionally generate the difficult to make *anti* diastereomers (e.g., KRED-118 and -119). KRED-101, -111–115, -121, and -123 also appear to be related and generate the *R* enantiomers consistently, as do ADH-LK and ADH-LB. This group of enzymes is often diastereomerically selective on cyclic ketones, making one or both *syn* diasteromers.

Conclusion

Isolated enzymes have clearly supplanted whole cell bioreductions and in most instances chemocatalytic ketones reductions at Merck. The substrate range and enantioselectivity for ketone reductions are excellent, providing high ee of either alcohol on the majority of ketone substrates. The enzymes also demonstrate valuable chemoselectivity and diastereoselectivity as described on the *para*-diketone **10** and several other substrates. The enzymes can be screened and scaled-up as rapidly as their chemocatalytic counterparts, and their cost to use at large scale and the environmental impact of their use is less. They have been used at Merck to economically deliver kilogram quantities of chiral intermediates with excellent yields and ee values. As a result, ketoreductases are the preferred catalyst for ketone reductions at Merck.

Future

The development of ketoreductases from sporadic use as whole cell catalysts to mainstream chemistry as isolated enzymes at Merck took place over the last five years. Looking forward, two additional reduction chemistries, the conversion of ketones to amines (via transaminases and reductive aminases) and the alkene reductions of α/β-unsaturated carbonyls (via enoate reductases), are preparing to make similar transitions to mainstream chemistry. These new reductions combined with the growing impact of the ketone reductions shown here highlight the expanding role enzymes will play in the field of asymmetric reduction chemistry.

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