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Biocatalytic strategy toward asymmetric β-hydroxy nitriles and γ-amino alcohols

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ABSTRACT

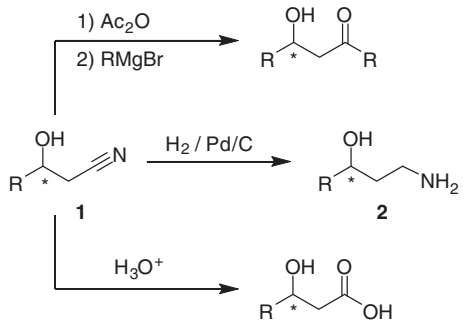
A library of 20 bakers' yeast reductases, that are overexpressed in *Escherichia coli*, were screened against a variety of β-keto nitriles. Enzymes from the aldose reductase and the short chain dehydrogenase family displayed activity toward these substrates. All of the seven substrates were reduced with high enantioselectivities and in some cases both antipodes could be synthesized in high ees. These whole-cell reactions afforded gram quantities of asymmetric compounds that could ultimately lead to scaleable and simple synthesis to new drug analogs of serotonin reuptake inhibitors and β-adrenergic blocking agents.

1. Introduction

Asymmetric β-hydroxy nitriles are excellent chiral precursors due to their reactive diversity (Scheme 1). These asymmetric products have been used to make a variety of chiral natural products and pharmaceuticals.[1–3] The most popular example is the utilization of β-hydroxy nitriles **1** or γ-amino alcohols **2** in the synthesis of the serotonin reuptake inhibitors[4–6] and β-adrenergic blocking agents.[7–9]

Chiral organic[10–12] and inorganic[13–20] catalysts have both been investigated in the synthesis of these molecules. The main disadvantage to these strategies is that the catalysts often involve a difficult synthesis and have toxicity issues especially when considering the use of heavy metals for the synthesis of pharmaceuticals. In addition, high enantioselectivity is often difficult to achieve.

Biocatalytic strategies utilizing isolated enzymes have also been a strategy toward asymmetric β-hydroxy nitriles **1**.[21–23] The most popular examples use a lipase or nitrilase to catalyze the kinetic resolution of the racemic β-hydroxy nitrile.[4,24–27] However, these enzymes are inherently limited to a 50% yield when resolving the racemate. The reduction of β-keto nitriles using isolated keto reductases has recently been investigated; however, the cofactor must be supplied or a cofactor regeneration system used when working with pure enzymes.[28,29]



Scheme 1. Examples of β-hydroxy nitriles synthetic utility.

Whole-cell biocatalytic strategies have also been investigated for the reduction of β-keto nitriles. This is advantageous because the cell will supply the cofactors needed for the reduction resulting in affordable and simple reaction conditions. However, studies using *Curvularia lunata* and bakers' yeast (*Saccharomyces cerevisiae*) to reduce these substrates have been plagued by a dominant alkylating mechanism (Scheme 2).[30–35]

Bakers' yeast (*S. cerevisiae*) has been a popular biocatalytic tool that has been investigated to achieve a variety of reductions.[36] Its ability to reduce an assortment of ketone substrates is ultimately due to the many keto reductases this organism contains.[37] Unfortunately, this large number of reductases often leads to a mixture of products formed by competing enzymes. To circumvent this

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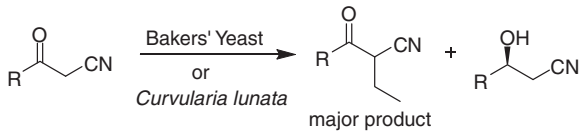


Scheme 2. Ethylated product is formed during wild-type whole-cell reactions using *Curvularia lunata* and bakers' yeast.

problem, GST-reductase chimeras were engineered and placed into *Escherichia coli* creating a bakers' yeast reductase library.[37–39] We have used this system to screen the stereospecificity of a single reductase for a given substrate by use of the pure fusion protein or in whole-cells.[39–41]

2. Results and discussion

This manuscript reports the screening of this heterologous library for its ability to asymmetrically reduce a variety of β-keto nitriles. The simplicity of this system lies in the fact that it uses whole-cells to supply the cofactors, minimizes competing reactions by overexpressing a single yeast reductase, and is scaleable thus it is well suited for the synthetic chemist who desires gram quantities.

The asymmetric reduction of a variety of alkyl and aryl substituted β-keto nitriles were investigated (Scheme 3). Many of these were commercially available or they were synthesized using acid chlorides and cyanoacetic acid following the procedure by Kirsten and Schrader.[42] Each substrate was screened using whole-cell reaction conditions with the engineered *E. coli* and each enzyme's stereoselectivity was recorded (Table 1).

This enzyme library can be broken down into four families (D-hydroxy dehydrogenase, medium chain dehydrogenase, short chain dehydrogenase, and aldose reductase). The shaded area on Table 1 represents the aldose reductase family and the non-shaded area represents the short chain dehydrogenase family. Interestingly, the other two families did not show activity toward this class of substrates. Comparison shows that the short chain dehydrogenase family tends to accept more of these substrates when compared to the aldose reductase family. In previous investigations YNL331c has been considered part of the aldose reductase family;[43] however, based on our observed broad range of substrate selectivity YNL331c acts more like the short-chain dehydrogenase family members because it reduces all of the ketone substrates. This is in contrast to the aldose family members which exhibited a narrow range of substrate acceptance. Interestingly, comparison of YNL331c amino acid sequence to the members of the short-chain dehydrogenase family or the aldose reductase family does not exhibit a high degree of similarity to either reductase family. However, when the amino acid relationship is viewed in terms of a phylogenetic tree it suggests that YNL331c is more closely related to the short-chain dehydrogenase family than the aldose reductase family (see Supplementary data).

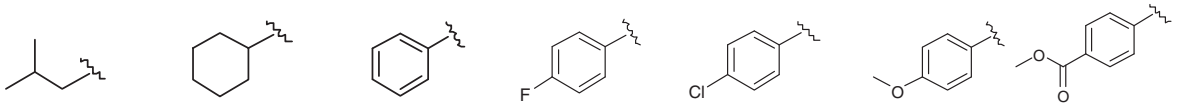
Scheme 3. The bakers' yeast reductase library was screened against a variety of β-keto nitrile substrates.

Most importantly, this screening shows that using these enzymes to reduce β-keto nitriles is synthetically useful. Every substrate can be reduced affording a product that is at least 99% ee. Moreover, every substrate apart from **4a**, we find that the enzyme library can select for both the L and the D isomers, which is in contrast to similar substrates that have been reduced by these enzymes in which they predominantly select for the L isomer only.[43] In fact, the aldose reductase family only yields the D enantiomer when reducing this class of substrates. Even more interesting, is that for substrates **5a**, **7a**, **8a**, and **9a** an enzyme can be chosen to afford either enantiomer in high optical purity. These results suggest that β-keto nitriles may be more synthetically useful with these reductases than other ketone substrates investigated because of the diverse stereoselectivity that is observed.

After each screening, the enzyme that affords the best enantioselectivity was then chosen for scale up using whole-cell non-growing conditions (Table 2), which has been previously shown to result in gram quantities and clean reactions.[40,41]

These whole-cell reactions were conducted in a non-growing media (100 mM PO_4^-, pH = 7, 5 g/L glucose, OD_{600} = 18). The final concentration of these reactions varied between 0.5 and 1.0 g/L. Since a 1 L reaction yielded enough product for characterization we did not work on condition optimization by manipulating the protein expression, non-growing reaction conditions, or found it necessary to utilize cell lysates with a cofactor regeneration system. As mentioned previously, whole-cell reactions of β-keto

Table 1
Enzyme library screening results reported in ee[a]



Substrate	3b	4b	5b	6b	7b	8b	9b
Enzyme							
YOL151w	99%D	99%D	99%D	99%D	78%D	99%D	74%D
YGL039w	––	––	97%L	75%L	99%L	47%L	99%L
YGL157w	93%D	44%D	92%L	70%L	79%L	79%L	99%L
YNL331c	11%L	90%D	33%L	22%D	68%L	26%L	99%D
YJR096w	––	––	––	96%D	99%D	––	99%D
YDR368w	99%D	––	––	––	99%D	––	––
YOR120w	99%D	––	––	––	99%D	––	99%D
YBR149w	99%D	––	––	––	99%D	––	––
BY	97%L	99%D	99%D	99%D	99%D	90%L	93%D

White row––Short chain dehydrogenase family. Light gray row––undetermined. Dark gray row––Aldose reductase family. Absolute configuration was determined by NMR and each product was fully characterized (see Supplementary data) and is represented as L and D in the table to better identify enzyme–substrate binding patterns.

[a] Percent calculated by chiral GC analysis.

Table 2
Results from the whole-cell reaction scale up

Substrate	Enzyme	Yield (%)	Final concentration	Ethylated product[a] (%)	Bakers yeast (%) ethylated[a]
3a	YOL151w	98	0.66 g/L	<1	38
4a	YOL151w	86	0.69	<1	28
5a	YOL151w	96	1.01	4	90
6a	YOL151w	89	0.41	2	78
7a	YOL151w	91	0.55	2	63
8a	YOL151w	89	0.57	<1	71
9a	YGL039w	91	0.49	<1	41

The percent of ethylated product formed is compared to the percent ethylated by bakers' yeast.
[a] Percent calculated by GC analysis.

nitriles afford an alkylated major product when using *C. lunata* and bakers' yeast. We found that this heterologous whole-cell system can limit the alkylated product to less than 5% and in some cases no ethylated product is found. We observed the bakers yeast conversion to be very slow and after 24 h there was usually less than 15% conversion (3 mM); whereas, using the overexpression system usually achieved 100% conversion in 12 h. This is most likely due to the difference in expression levels of the reductases in the bakers' yeast versus the engineered cells.

3. Conclusions

Asymmetric β-hydroxy nitriles are chiral precursors which have been applied to the synthesis of serotonin reuptake inhibitors[4–6] and β-adrenergic blocking agents.[7–9] We have shown a simple and scaleable approach to make a variety of asymmetric β-hydroxy nitriles while avoiding the alkylated product often seen with whole-cell biocatalysis. In most cases these products are reduced with very high stereospecificity and there are also examples in which both antipodes can be synthesized by using the appropriate enzyme. Thus, we feel that these enzymes can be used in a simple and scalable approach toward the synthesis of chiral drug analogs of serotonin reuptake inhibitors and β-adrenergic blocking agents by varying the substituent of these ketone substrates. Lastly, by studying the enzymes in the library and correlating the data with this class of substrates lend more insight into these reductases and their families.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.03.009.

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