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Biocatalytic synthesis towards both antipodes of 3-hydroxy-3-phenylpropanitrile a precursor to fluoxetine, atomoxetine and nisoxetine

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Abstract—The bakers' yeast reduction of 3-oxo-3-phenylpropanenitrile (**1**) has been difficult to achieve due to a dominant alkylating mechanism. A library of 20 bakers' yeast reductases, that are overexpressed in *Escherichia coli*, were screened against (**1**). Four enzymes were found to reduce this substrate and by varying the enzyme both enantiomers of 3-hydroxy-3-phenylpropanitrile (**2**) could be prepared with a high enantiomeric excess. In addition, the *Escherichia coli* whole-cell system can be optimized to nearly eliminate the competing alkylating mechanism. By using this system, a formal biocatalytic synthesis of both antipodes of fluoxetine, atomoxetine and nisoxetine has been demonstrated.

1. Introduction

The treatment of a major depressive disorder using serotonin/norepinephrine reuptake inhibitors has become a billion dollar industry since their inception. These inhibitors have also been used to treat other disorders such as anxiety, alcoholism, chronic pain, migraine headaches, urinary incontinence, sleep disorders, memory disorders, obesity and bulimia. Specific interest has been placed on Prozac™ (fluoxetine) (**3**), Straterra™ (atomoxetine) (**4**) and nisoxetine (**5**) because these inhibitors show little effect on dopaminergic and noradrenergic receptors.[1,2] This initiated our interest in the reduction of 3-oxo-3-phenylpropanenitrile (**1**), because 3-hydroxy-3-phenylpropanitrile (**2**) is a simple precursor to these popular serotonin/norepinephrine reuptake inhibitors (Scheme 1).[3]

Bakers' yeast (*Saccharomyces cerevisiae*) is a popular biocatalytic tool for organic chemists. It has been shown to catalyze the forming and breaking of C–C bonds, oxidations, hydrolysis and a variety of reductions.[4] Its ability to reduce an assortment of substrates can be explained by the many reductases it contains.[5] Unfortunately, this large number of reductases often leads to a mixture of products formed by competing enzymes. To circumvent this problem, GST-reductase chimeras were engineered and placed into *Escherichia coli* creating a bakers' yeast reductase library.[5–7] We have used this system to screen the stereospecificity of a single reductase for a given substrate by the use of the pure fusion protein or used directly in whole cells.[7–9]

2. Results and discussion

The asymmetric synthesis of (**2**) has been accomplished by the oxidation of styrene,[10] ruthenium catalyzed reductions of (**1**),[11–13] enzyme reduction of (**1**),[14] aldol-type addition of alkylnitriles to benzaldehyde,[15–17] and kinetic resolutions using lipase.[18–21] The biocatalytic reduction of (**1**) has also been achieved by both bakers' yeast and the fungus *Curvularia lunata* (Scheme 2).[22–30] Surprisingly, these whole-cell systems did not afford the expected alcohol (**2**) as the dominant product, but instead these organisms gave a mixture of α-alkylated product 2-cyano-1-phenylbutanone (**6**) and alcohol *S*-(**2**). This led to the screening of (**1**) against the library of bakers' yeast reductases, using our *E. Coli* whole-cell overexpression system. Out of the 20 reductases

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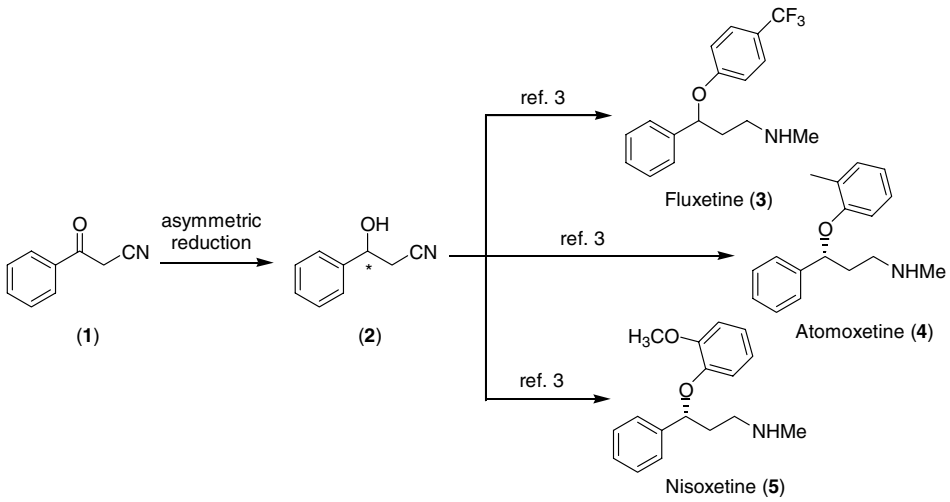
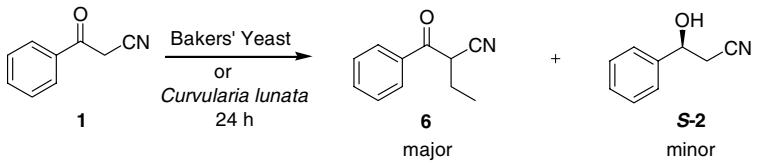


Scheme 1. Overall strategy towards Prozac™ (fluoxetine) (**3**), Straterra™ (atomoxetine) (**4**) and nisoxetine (**5**).



Scheme 2. Whole-cell reduction of (**1**) with bakers' yeast or *C. lunata* over 24 h.

screened, four were found to reduce (**1**) (Table 1). Unlike the results from bakers' yeast and *C. lunata,* the heterologous *E. coli* system afforded both *R*-(**2**) and *S*-(**2**) with a high enantiomeric excess. The asymmetric synthesis of *R*-(**2**) is of particular importance because it is a stereochemical mimic and building block for the synthesis of (*R*)-atomoxetine (**4**) and (*R*)-nisoxetine (**5**). Additionally, synthesis of optically pure Prozac™ (**3**) may be advantageous since it is currently delivered as the racemate, even though the stereochemistry of the alcohol has shown to effect its inhibitory properties.[1,29,30]

The organisms overexpressing YOL151w and YGL039w were chosen for the *E. coli* whole-cell scale-up because of their products high enantiomeric excess (Table 1). These reactions were investigated in two different growth conditions: A nutrient rich growth media (Luria–Bertani) and a nitrogen deficient non-growing media (phosphate buffer pH = 6, 4 mM glucose).

The whole-cell reduction of (**1**) by YOL151w and YGL039w in a nutrient rich growth media afforded ethylated product (**6**) at about 39% of the overall yield as shown by GC analysis (Table 2). The two whole-cell reactions were then performed in a nitrogen deficient media (Scheme 3), which is a system that has proven successful for previous substrates.[8,9] Both non-growing systems yielded ~1 g/L final concentration of (**2**) and the formation of (**6**) was decreased to a 5% overall yield. Compared to the whole-cell bakers' yeast reduction, these results show a major improvement in the yield and it also allows for the synthesis of optically pure *R*-(**2**). In addition, these data further suggest that the heterologous *E. coli* system is able to focus more of the host bacteria's metabolism towards reducing substrates while

Table 1. Enantiomeric excess and configuration of the reduction products from the four bakers' yeast reductases compared to bakers' yeast

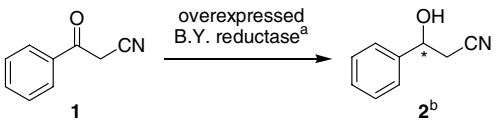
Overexpressed gene	ee	Configuration[a]
YOL151w	99%	*S*[c]
YGL039w	97	*R*[d]
YGL157w	92	*R*
YNL331c	33	*R*
Bakers' yeast[b]	98	*S*

[a] Configuration confirmed by NMR.[31]
[b] Isolated in a 10% yield.
[c] $[\alpha]_D$ −60.0 (*c* 1.6, $CHCl_3$); lit.[3] $[\alpha]_D$ −60.5 (*c* 1.0, $CHCl_3$).
[d] $[\alpha]_D$ +57.5 (*c* 1.2, $CHCl_3$).

Table 2. Triplicate average of the whole-cell reaction in Scheme 3 for YOL151w and YGL039w

Overexpressed gene	Final concn of (**2**)[a] (g/L)	% (**2**)[b]	% (**6**)[b]
YOL151w[c]	0.60	60	40
YGL039w[c]	0.51	62	38
YOL151w[d]	1.01	96	4
YGL039w[d]	0.96	94	6
Bakers' yeast[e]	0.11	10	90

[a] Final concentration is the amount of isolated product per reaction volume (g/L).
[b] Percent calculated by GC analysis.
[c] LB (1 L, pH 6) shaken for 24 h at 20 °C.
[d] Phosphate buffer (1L, pH 6) shaken for 24 h at 20 °C.
[e] Tap water (100 mL) shaken for 24 h at 20 °C.



Scheme 3. Whole-cell reduction of (**1**) using engineered *E. coli*.
[a]LB medium with 30 ug/mL kanamycin was inoculated with a single colony of *E. coli* (containing either YOL151w or YGL039w overexpressed gene) and shaken 15 h at 37 °C. This preculture was diluted 1:100 into 2 L of the same medium and shaken 2.5 h at 37 °C with a stir rate of 400 rpm. Upon reaching an $O.D._{600} = 0.6$, the cell culture was cooled to 20 °C, and isopropylthio-β-D-galactoside was added to a final concentration of 100 μM and shaken for 14 h. The cells were collected by centrifugation (5000 g for 10 min at 4 °C) and then resuspended in 1 L of 100 mM phosphate buffer (pH = 7) containing 4 g/L glucose. The bioconversion was carried out at 20 °C with a stir rate of 400 rpm. The portions of neat (**1**) were added in small increments (50 mg) and monitored by GCMS for conversion.
[b]IR (Neat) 3421, 3032, 2915, 2256, 1454, 1054 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.38 (s, 5H), 5.01 (t, 1H, $J = 6.0$ Hz), 3.23 (br s, 1H), 2.73 (d, 2H, $J = 6.0$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 141.1, 129.0, 128.9, 125.6, 117.4, 70.1, 28.0; mass (EI) 147, 129, 107, 79, 77.

under non-growing conditions versus conditions that allow bacterial reproduction and growth.

3. Conclusions

The reduction of β-keto nitriles using whole-cell catalysis has been a difficult task due to the dominating mechanism affording alkylated products. By using the heterologous overexpression system the alkylated product (**6**) is nearly eliminated. In addition, this method allows for the synthesis of both antipodes of 3-hydroxy-3-phenylpropanitrile (**2**), which demonstrates the advantage of using a single overexpressed bakers' yeast reductase versus bakers' yeast alone. By using this system, a formal biocatalytic synthesis of both antipodes of fluoxetine (**3**), atomoxetine (**4**) and nisoxetine (**5**) has been demonstrated.

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