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Assessing substrate acceptance and enantioselectivity of yeast reductases in reactions with substituted α-keto β-lactams

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Abstract

A number of yeast (*Saccharomyces cerevisiae*) strains and yeast reductases overexpressed in *Escherichia coli* were investigated as bioreductants for several α-keto-β-lactams substituted with aryl and alkyl groups in the 4-position. Two of the yeast reductases were found to accept a majority of the substrates tested, while others were more limited. Although none of the reductases investigated showed *both* diastereo- and enantio-selectivity under the screening conditions, these bioreagents are still useful in reductions of α-keto β-lactams.

Keywords: Bioreductions; Yeast-catalyzed reductions; Yeast reductases; α-Keto β-lactams; α-Hydroxy β-lactams

1. Introduction

The high-biological activity of many 3-hydroxy-4-substituted β-lactams [1,2], combined with their importance as building blocks for a variety of targets [3], has encouraged a search for selective methods for the synthesis of these compounds [3,4]. Access to optically pure β-lactams has been achieved via asymmetric synthesis by using combinations of an enantiopure imine and an achiral ketene or an achiral imine and an enantiopure ketene [5,6], or via directed cyclization of a ketene–imine pair in the presence of a chiral catalyst [7]. Lipase-catalyzed resolutions of acylated 3-hydroxy β-lactams was introduced by Sih and co-workers [8] and has been employed by a number of investigators [9]. We have investigated the possibility of introducing the desired chirality via baker's yeast-mediated reductions of α-keto β-lactams. This strategy was quite successful in the preparation of the enantiopure *cis*-(3*R*,4*S*) and *trans*-(3*R*,4*R*) diastereomers of 4-*tert*-butyl-3-hydroxy β-lactam [10]. On the other hand, baker's yeast-catalyzed reduction of 3-oxo-4-phenyl β-lactam gave only moderate enantioselectivity for the *cis*-(3*R*,4*S*) alcohol [11]. Analyzing the product composition as a function of fractional conversion suggested that more than one enzyme was involved in reduction of this substrate [11].

The asymmetric reduction of ketones to chiral alcohols is one of the most fundamental reactions that can be accomplished through biocatalysis and baker's yeast (*Saccharomyces cerevisiae*) has been the microorganism of choice for decades. Yeast-catalyzed reductions have been extensively studied and reviewed and simple empirical rules have been proposed to predict their stereochemical outcome [12]. Because the organism harbours a large number of reductase enzymes, these conversions are remarkable for their tolerance of a large and diverse collection of carbonyl compounds. The downside to this plethora of reducing enzymes is that several catalysts with overlapping substrate acceptabilities may have opposite enantioselectivities, which leads

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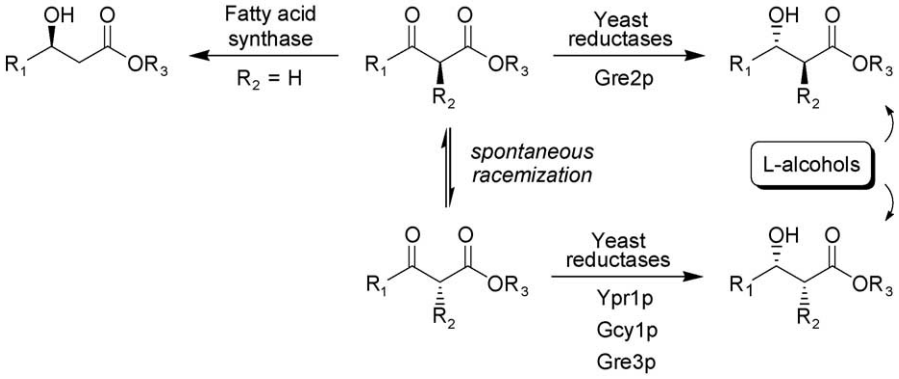


Scheme 1.

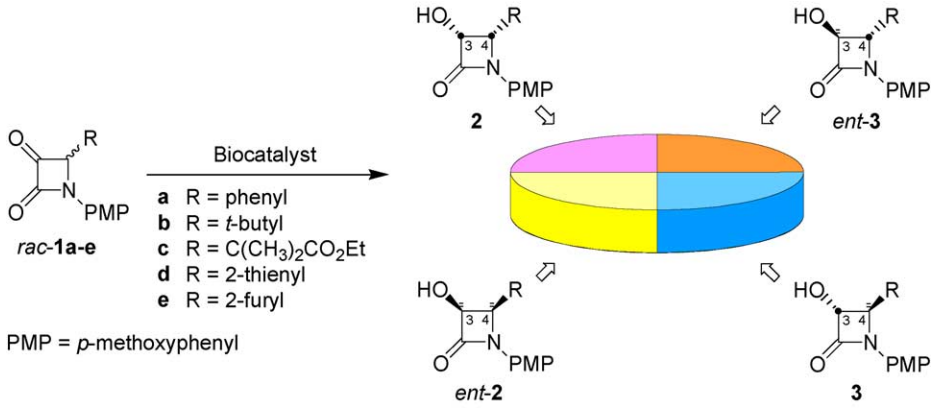
to lower enantiopurities of the alcohol products; that, we suspected, was the problem in the above mentioned reduction of 3-oxo-4-phenyl β-lactam. The ideal solution to finding a "perfect-for-the-reaction" enzyme is to identify and characterize all yeast reductases and overexpress them in appropriate host organisms. While several yeast reductases have been isolated and studied [13–15], and systematic investigations of their properties have commenced [16], numerous putative reductases are still awaiting full characterization [17].

At the beginning of this project, three yeast enzymes were believed to play a major role in reductions of β-keto esters: Ypr1p, Gre2p, and the fatty acid synthase complex (FAS) [13,14]. Eventually, it was demonstrated that aldose reductases Ypr1p, Gre3p and Gcy1p often yield *syn*-(2*R*,3*S*) alcohols, while α-acetoxy ketone reductase, Gre2p, catalyzes formation of *anti*-(2*S*,3*S*) alcohols. Fatty acid synthase was found to produce D-alcohols (R configuration at the hydroxyl) but accepted only unsubstituted (R_2 = H) β-keto esters as substrates [8,13,14,18,19]. These results are summarized in Scheme 1.

We considered the possibility that the same enzymes might be involved in reductions of α-keto β-lactams as seen in Scheme 2. To test this hypothesis, we screened several racemic 3-oxo-4-substituted β-lactams against whole cell biocatalysts with altered levels of the reductases discussed above. These included several recombinant yeast strains and *Escherichia coli* overexpression systems for Ypr1p and three other related aldose reductases.

2. Experimental

The X-ray diffraction measurements of the three *p*-bromobenzoyl derivatives of the compounds (3*S*,4*S*)-**2d**, (3*R*,4*S*)-**3d** and (3*S*,4*S*)-**2e** were performed at room temperature on a Siemens P4 diffractometer using graphite-monochromatized Mo Kα (λ = 0.71073 Å) radiation. The data collections were made by the $2\theta/\omega$ scan technique using the XSCANS program (XSCANS, PC Version 5, Bruker AXS Inc., Madison, WI, 1995). The coordinates of the bromine atom were determined by direct methods and all the other non-hydrogen atoms were found by the usual Fourier methods. The refinement of the structures was done on F^2 by full matrix least-squares analysis. The hydrogen atom positions were fixed in their calculated position with $U_{eq} = 1.2U_{eq}$ (or 1.5 for methyl groups) of the carbon to which they are bonded. Corrections were made for absorption (empirical ψ scan), Lorentz and polarization effects. The calculations were done using the Siemens SHELXTL system (SHELXTL, Release 5.10, Bruker AXS Inc., Madison, WI, 1997) [20]. Chiral-phase HPLC analyses were performed on a Chiracel



Scheme 2.

OD-H column (4.6 mm × 150 mm) using hexane:*iso*-propanol (90:10) as the mobile phase and detection at 254 nm. Capillary gas chromatography was performed on a DB-1301 (15 m × 0.53 mm × 1.0 μm) column from J&W Scientific. Lipases were generous gifts from Amano Enzyme U.S.A. Co. Ltd. Commercial baker's yeast was obtained from a local grocery chain. The protocol for the preparation of 3-oxo-4-substituted β-lactams has been described [21,24].

2.1. General procedure for biotransformations with commercial baker's yeast

Dry baker's yeast (0.5 g) was added to a solution of sucrose (2 g) in sterile water (25 mL) contained in a 250 mL Erlenmeryer flask. The mixture was stirred at 30 °C for 30 min to activate the yeast. β-Lactam (25 mg, finely ground with 25 mg β-cyclodextrin when the solubility was low) was added to initiate the reaction. The conversion was monitored by GC and chiral HPLC as described above.

2.2. General procedure for biotransformations with laboratory yeast strains

Preparation of yeast cells and the general procedure for reductions with yeast strains have been described in detail [22]. Analytical samples were prepared by mixing 300 μL of the reaction mixture with 300 μL of ethyl acetate. After vortex mixing for 1 min, the sample was centrifuged in a microcentrifuge for 1 min, then the organic layer was removed and dried under nitrogen. The residue was dissolved in 200 μL of *iso*-propanol and 1 μL was used for GC analysis (where applicable) and 20 μL was used for HPLC analysis.

2.3. General procedure for biotransformations with recombinant E. coli strains

The general procedure for reductions with *E. coli* strains has been described [23]. Analytical samples were treated and analyzed as described above.

3. Results and discussion

Five representative 3-oxo-4-substituted β-lactams, **1a**–**e**, with aromatic and aliphatic groups in position 4 (Scheme 2), were synthesized according to protocols already described [11,21,24]. Samples of the enantiopure alcohols were prepared via lipase-mediated resolutions of the corresponding acetates or yeast reductions of the corresponding α-keto-β-lactams. The structures of **2b**, **3b**, *ent*-**2c**, and *p*-bromobenzoyl derivative of **3a**, established by X-ray crystallographic analyses, in combination with chiral phase HPLC, allowed unambiguous identification of the absolute configuration of all isomers [10,24]. The crystal structures of the *p*-bromobenzoyl derivatives of *cis*-(3*S*,4*S*)-**2d**, *trans*-(3*R*,4*S*)-**3d** and *cis*-(3*S*,4*S*)-**2e** which have not been previously reported, are shown in Fig. 1.

The biocatalysts used in this study included commercial baker's yeast and two fatty acid synthase mutants: ATCC 26403, which is defective in Claisen condensation activity but has wild-type β-keto thioester reduction activity [25] and mutant yeast strain 2B, in which the gene encoding one of the fatty acid synthase subunits (*FAS2*) was completely deleted [22]. We also investigated the following engineered yeast and *E. coli* overexpression strains: (a) strain 2B expressing α-acetoxy ketone reductase (Gre2p); (b) yeast strain 15C overexpressing Ypr1p; (c) yeast strain InvSc1 overexpressing Ypr1p; (d) *E. coli* BL21(DE3) overproducing Ypr1p; (e) *E. coli* BL21(DE3) overexpressing Ara1p; (f) *E. coli* JM105 overexpressing reductase Gre3p; and (g) *E. coli* JM105 overexpressing reductase Gcy1p. The construction and evaluation of the recombinant biocatalysts has been reported previously [22,26,27].

An earlier study [11] showed that, in the reduction catalyzed by commercial baker's yeast, racemic α-keto-β-lactam **1a** was converted completely to a mixture of *cis*-**2a** enantiomers and optically pure (>99% e.e.) *trans*-(3*R*,4*R*)-**3a**. Since reduction carried out with the fatty acid synthase-deficient *S. cerevisiae* strain ATCC 26403 produced a significantly lower proportion of the *trans* isomer **3a**, we tentatively concluded that FAS was a major contributor to the formation of the *trans* isomer. Moreover, a quantitative analysis of the conversion suggested that either, (1) a single enzyme with low enantioselectivity, or (2) multiple reductases with similar stereoselectivities might be responsible for the formation of **2a** [11]. To discover the contributing enzyme(s), we screened our collection of well-characterized yeast enzymes involved in reductions of α- and β-keto esters [11,22,23,26,27]. Initial screenings with the isolated enzymes revealed that "the single enzyme" hypothesis was incorrect since both Ypr1p and the related enzyme Ara1p reduced β-lactam **1a**. Not every enzyme, however, was a catalyst: neither short-chain dehydrogenase Gre2p nor two aldose reductases, Gcy1p and Gre3p, which share high-amino acid sequence identity with Ypr1p, accepted **1a** [27]. In view of these results, all of the strains listed above were screened for the ability to reduce each of the α-keto β-lactams depicted in Scheme 2.

Data for the reductions are collected in Table 1 [28]. For compounds **1a**–**c**, both the fractional conversion and ratios of *cis*:*trans* alcohol products were obtained from baseline-resolved GC traces. Conditions for chiral-phase HPLC analysis and data from X-ray crystallography allowed complete resolution and assignment of peaks for all four alcohol-stereoisomers [10,24]. The reduction products of compounds **1d** and **1e** could not be analyzed by GC and these reactions were therefore monitored by TLC and chiral-phase HPLC with UV detection. Both, the ketones and alcohols were resolved by HPLC and standard curves were established to assess the degree of conversion [29]. The HPLC elution pattern of furyl-substituted β-lactam **3e** was assigned by analogy with the other compounds in the series.

The results presented in Table 1 emphasize differences between various baker's yeast strains. While commercial

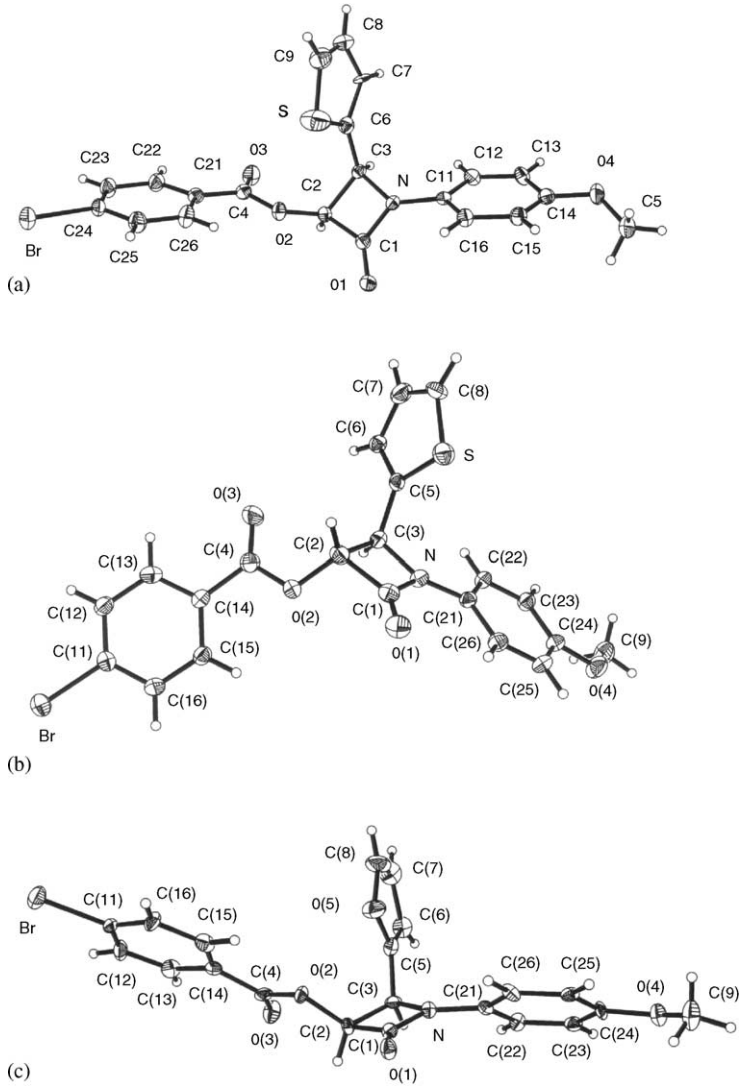
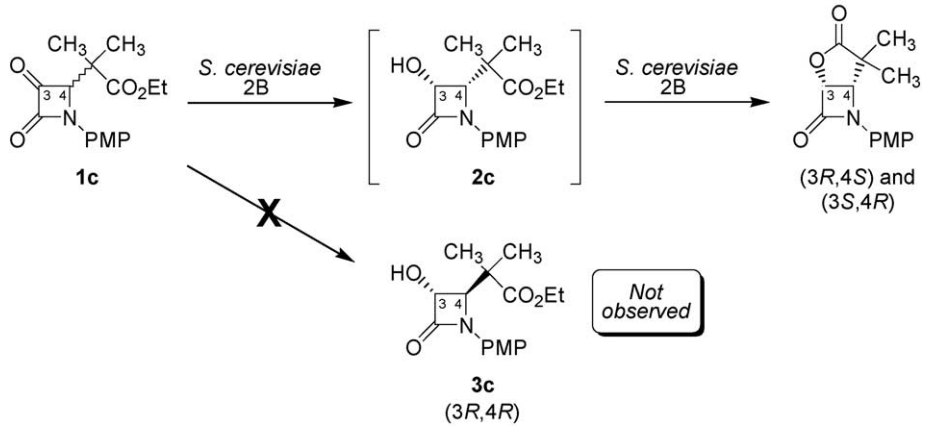
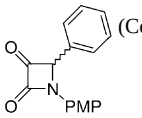
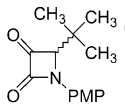
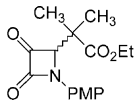
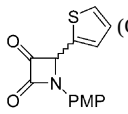
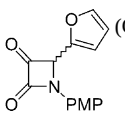
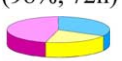
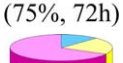
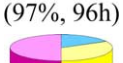
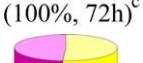
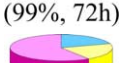
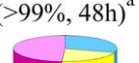


Fig. 1. X-ray crystal structures of *p*-bromobenzoyl derivatives of compounds *ent*-**2d**, **3d**, and *ent*-**2e**: (a) *cis*-(3*S*,4*S*)-**2dX**, (b) *trans*-(3*R*,4*S*)-**3dX**, and (c) *cis*-(3*S*,4*S*)-**2eX** [20].



Scheme 3.

Table 1
Compositions of product mixtures from reductions catalyzed by commercial and laboratory baker's yeast strains (including FAS deficient mutants and cells that overproduce either Ypr1p or Gre2p) and *E. coli* cells overexpressing Ypr1p, Ara1p, Gcy1p or Gre3p

Biocatalyst	 (Conversion)	 (Conversion)	 (Conversion)	 (Conversion)	 (Conversion)
Commercial baker's yeast	 (>98%, 72h)	 (>98%, 32h)	 (>98%, 24h)	 (>98%, 24h)	 (>98%, 24h)[a]
S. cerevisiae InvSc1	 (>98%, 72h)	 (>98%, 72h)	 (>98%, 24h)	 (>99%, 48h)	 (>99%, 24h)[a]
S. cerevisiae 15C	 (75%, 72h)	 (>99%, 96h)	 (73%, 24h)	 (99%, 72h)	 (99%, 48h)[a]
S. cerevisiae 26403 (FAS-)	 (>98%, 52h)	 (96%, 96h)	 (>98%, 24h)[c]	 (>99%, 48h)	 (>99%, 48h)[a]
S. cerevisiae 2B (ΔFAS)	 (98%, 72h)	 (99%, 72h)	 (98%, 72h)[b]	 (98%, 72h)	 (98%, 72h)[a]
S. cerevisiae InvSc1 (Ypr1p$^+$)	 (75%, 72h)	 (97%, 96h)	 (100%, 72h)[c]	 (99%, 72h)	 (>99%, 48h)[a]
S. cerevisiae 15C (Ypr1p$^+$)	 (88%, 48h)	 (96%, 72h)	 (98%, 39h)[c]	 (98%, 48h)	 (>99%, 48h)[a]
S. cerevisiae 2B (Gre2p$^+$)	(93%, 96h)	(>99%, 96h)	(97%, 24h)[c]	(90%, 96h)	(>99%, 48h)[a]
E. coli BL21(DE3) (Ypr1p$^+$)	(55%, 72h)	N.D.[d] (0%, 72 h)	(9%, 72h)	(94%, 72h)	(90%, 72h)[a]
E. coli BL21(DE3) (Ara1p$^+$)	(81%, 72h)	(20%, 48h)	N.D.[d] (12%, 48 h)	(93%, 72h)	(98%, 72h)[a]
E. coli JM105 (Gre3p$^+$)	N.D. (0%, 72 h)	N.D. (0%, 72 h)	N.D. (0%, 72 h)	(92%, 72h)	(44%, 96h)[a]
E. coli JM105 (Gcy1p$^+$)	N.D. (0%, 72 h)	N.D. (0%, 72 h)	N.D. (0%, 72 h)	(20%, 72h)	(57%, 72h)

[a]The peaks for the *trans* isomers were not well resolved on chiral phase HPLC and the enantiomeric composition of the two enantiomers is tentatively assigned.
[b]The *cis* isomers produced as lactones (Scheme 3).
[c]The *cis* isomers include lactones: InvSc1(Ypr1p), 38%; 15C(Ypr1p), 9%; 26403, 9%; 2B(Gre2p), 6%; BL21(DE3)Ypr1p, 5%.
[d]Not determined.

baker's yeast and laboratory strains InvSc1 and 15C gave comparable results for **1c–e**, 15C-catalyzed reductions resemble those carried out with the FAS deficient mutant ATCC 26403 for **1a** and **1b**. Interestingly, strain 2B (FAS deletion strain), afforded product distributions quite different from those obtained with the ATCC 26403 FAS point mutant (Table 1). Nonetheless, both FAS-deficient strains showed diminished production of the *trans*-(3*R*,4*R*)-alcohols and consequently we concluded that fatty acid synthase accepts preferentially (4*R*)-ketones ((4*S*)-configuration in compounds **1d** and **1e**)) and converts them to (3*R*)-alcohols. This agrees with our earlier conclusions based on yeast-catalyzed reductions of **1a** [11]. Reduction of **1c** mediated by the 2B strain produced a mixture of alcohols and the corresponding lactones as shown in Scheme 3. This was the only strain that efficiently catalyzed cyclization of the product alcohols to lactones.

Comparing the results from yeast strains 2B (FAS knockout) and the same strain with the Gre2p short-chain dehydrogenase overexpressed showed considerably different product distribution profiles. This suggests that Gre2p *does* participate in reduction of the β-lactams studied, contributing to the formation of enantiopure or highly enriched *trans* products in four out of five compounds studied.

The large number of reductase genes present [17], and the possibility that patterns of gene expression might change as a result of gene knockout and/or overexpression, makes it difficult to interpret studies with whole yeast cells unambiguously. We therefore examined the reductions of **1a–e** by four *E. coli* strains that each overproduced a single yeast dehydrogenase (Ypr1p, Gre3p Gcy1p, or Ara1p; Table 1). Control reactions carried out with the two *E. coli* host strains showed no conversion of any of the α-keto β-lactams over the experimental time-frame (72 h).

The *E. coli* Ypr1p overexpression strain catalyzed reduction of substrates **1a** and **1c–e**. The reactions were slow under the screening conditions; however, conversions of 50–94% could be achieved after 72h. No reduction of *tert*-butyl substituted **1b** was observed even after 96 h. Ypr1p-catalyzed reduction of **1a** gave a 1:1 mixture of *cis*-alcohols and the enantiopure *trans*-(3*S*,4*S*)-product. The conversion of (4*S*)-**1a** to the *trans*-(3*S*,4*S*)-alcohol proceeded at a slower pace, as was deduced from kinetic runs. Interestingly, reductions of **1a** by yeast strains overexpressing Ypr1p consistently provided increased quantities of the (3*S*,4*R*)-alcohol compared to the host strains; however, with these biocatalysts, no (3*S*,4*S*)-product could be detected, probably because the (4*S*)-ketone is more rapidly converted to the (3*R*,4*S*)-alcohol by one or more enzymes present in the yeast cells (Table 1).

The reduction of **1d** by the *E. coli* strain overexpressing Ypr1p was almost complete (94% conversion), relatively rapid, and the product alcohols were uncontaminated by any cellular metabolites. The product ratio (79% of the combined (3*R*,4*R*)- and (3*S*,4*R*)-products compared with 21% (3*R*,4*S*)-alcohol at 94% conversion) indicates that **1d** must have epimerized under the biotransformation conditions and that the (4*R*)-ketone was accepted preferentially by the enzyme. Similar conclusions can be reached in the case of several other transformations depicted in Table 1.

Ara1p, which reduced α-keto β-lactam **1a**, also converted **1d** and **1e** to mixtures of *cis*- and *trans*-alcohols. These are potentially useful transformations as they provide access to the enantiopure, separable diastereomers, which are otherwise very difficult to obtain. Compounds **1b** and **1c** are poor substrates and the conversions were very low, even after 72 h. Moreover, as is frequently the case with poor substrates, large quantities of metabolites contaminated the isolated products.

Gre3p and Gcy1p overexpressed in *E. coli* strain JM105 converted thienyl and furyl substituted compounds **1d** and **1e** to a mixture of alcohols, although the other substrates showed no conversion after 72 h. Interestingly, both enzymes showed enhanced proportions of the enantiopure *trans*-products. The identity of (3*R*,4*S*)-**3d** was confirmed by X-ray crystallography and the absolute configuration of alcohol **3e** was tentatively assigned by analogy to its close relative **3d**.

4. Conclusions

This study has shown that yeast-catalyzed reductions of 3-keto β-lactams provide a practical route to some enantiopure or highly enriched 3-hydroxy derivatives (for example, (3*R*,4*S*)-**2b** and (3*R*,4*R*)-**3b**). More frequently, however, these reductions lead to mixtures of products. Our initial suspicion that this was due to a large complement of yeast reductases with overlapping substrates but different enatioselectivities was only partially correct. Of the five yeast reductases studied here, three aldose reductase family members (Ypr1p, Gre3p and Gcy1p) were relatively non-selective in reducing 3-keto β-lactams, although both Gre3p and Gcy1p-catalyzed reactions yielded high proportions of *trans*-products from **1e**. The Ara1p reductase showed promise in producing optically enriched diastereomers not accessible via transformations with other reductases.

Contrary to our initial expectations, short-chain dehydrogenase Gre2p appears to accept α-keto β-lactams and significantly contributes (along with fatty acid synthase) to the formation of *trans*-(3*R*,4*R*)-products. A recombinant *E. coli* strain overexpressing this enzyme might be useful in cleanly reducing these substrates.

It appears that all reductases studied so far contribute only partially to the reductions of β-lactams by whole cells of baker's yeast. This can be deduced from the fact that while both Ara1p and Ypr1p afforded significant quantities of (3*S*,4*S*)-alcohols (when overexpressed invidually in *E. coli*), the reactions mediated by wild-type yeast strains did not result in observable quantities of this isomer. Thus, one or more additional enzymes that reduce these substrates have yet to be identified.

In summary, while easy-to-perform baker's yeast-catalyzed reductions may be useful with some of these substrates, manipulating expression levels of the various

reductases involved by gene knockout and overexpression in order to enhance selectivity does not appear to be practical. With such a large number of reductases present, and an apparent lack of discrimination shown by the few that we have studied, "background" contributions are always likely to compromise stereoselectivity. *E. coli* overexpression of single yeast reductases is far more promising. Fermentation experiments designed to manipulate product distribution and increase the rate of conversion by these recombinant strains are in progress.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2004.10.005.

References

[1] M.I. Page, Acc. Chem. Res. 17 (1984) 144;
S. Gérard, G. Dive, B. Clamot, R. Touillaux, J. Marchand-Brynaert, Tetrahedron 58 (2002) 2423.

[2] W.D. Vaccaro, R. Sher, H.R. Davis Jr., Bioorg. Med. Chem. Lett. 8 (1998) 35;
W.D. Vaccaro, H.R. Davis Jr., Bioorg. Med. Chem. Lett. 8 (1998) 313.

[3] C. Palomo, J.M. Aizpurua, I. Ganboa, M. Oiarbide, Synlett (2001) 1813;
C. Palomo, J.M. Aizpurua, I. Ganboa, M. Oiarbide, Pure Appl. Chem. 72 (2000) 1763;
R. Herranz, J. Castro-Pichel, S. Vinuesa, M.T. Garcia-Lopez, J. Org. Chem. 55 (1990) 2232, and references cited therein.

[4] (a) L. Bianchi, C. Dell'Erba, M. Maccagno, A. Mugnoli, M. Novi, G. Petrillo, F. Sancassan, C. Tavani, Tetrahedron 59 (2003) 10195 (Staudinger reaction) ;
(b) M. Arun, S.N. Joshi, V.G. Puranik, B.M. Bhawal, A.R.A.S. Deshmukh, Tetrahedron 59 (2003) 2309 (2+2 cycloaddition) ;
(c) I. Banik, F.F. Becker, B.K. Banik, J. Med. Chem. 46 (2003) 12 (cyclization) ;
(d) T.B. Durham, M.J. Miller, J. Org. Chem. 68 (2003) 35 (*N*-tosyloxy β-lactams) ;
(e) T.B. Durham, M.J. Miller, J. Org. Chem. 68 (2003) 27 (β-lactams from β-keto esters) ;
(f) C. Palomo, M. Oiarbide, A. Landa, A. Esnal, A. Linden, J. Org. Chem. 66 (2001) 4180;
(g) A.K. Bose, B.K. Banik, C. Mathur, D.R. Wagle, M.S. Manhas, Tetrahedron 56 (2000) 5603.

[5] G.I. Georg, V.T. Ravikumar, Stereocontrolled ketene-imine cycloaddition reactions, in: G.I. Georg (Ed.), The Organic Chemistry of β-Lactams, VCH, New York, NY, 1993, pp. 295–368.

[6] M. Arun, S.N. Joshi, V.G. Puranik, B.M. Bhawal, A.R.A.S. Deshmukh, Tetrahedron 59 (2003) 2309;
R. Fernández, A. Ferrete, J.M. Lassaletta, J.M. Llera, E.M. Zamora, Angew. Chem. Int. Ed. 41 (2002) 831;
S.N. Joshi, A.R.A.S. Deshmukh, B.M. Bhawal, Tetrahedron: Asymmetry 11 (2000) 1477;
S. Brown, A.M. Jordan, N.J. Lawrence, R.G. Pritchard, A.T. McGown, Tetrahedron Lett. 39 (1998) 3559;
I. Ojima, F. Delaloge, Chem. Soc. Rev. 26 (1997) 377;
S. Matsui, Y. Hashimoto, K. Saigo, Synthesis (1998) 1161;
Y. Hashimoto, A. Kai, K. Saigo, Tetrahedron Lett. 36 (1995) 8821;
T. Fujisawa, A. Shibuya, D. Sato, M. Shimizu, Synlett (1995) 1067.

[7] P.A. Magriotis, Angew. Chem. Int. Ed. 40 (2001) 4377;
B.L. Hodous, G.C. Fu, J. Am. Chem. Soc. 124 (2002) 1578;
A.E. Taggi, A.M. Hafez, H. Wack, B. Young, W.J. Drury III, T. Lectka, J. Am. Chem. Soc. 122 (2000) 7831;
H. Wack, W.J. Drury III, A.E. Taggi, D. Ferraris, T. Lectka, Org. Lett. 1 (1999) 1985.

[8] R. Brieva, J.Z. Crich, C.J. Sih, J. Org. Chem. 58 (1993) 1068.

[9] E. Forró, F. Fülöp, Tetrahedron: Asymmetry 12 (2001) 2351;
W. Adam, P. Groer, H.-U. Humpf, C.R. Saha-Möller, J. Org. Chem. 65 (2000) 4919;
A. Basak, T. Mahato, G. Bhattacharya, B. Mukherjee, Tetrahedron Lett. 38 (1997) 643;
J.A. Carr, T.F. Al-Azemi, T.E. Long, J.-Y. Shim, C.M. Coates, E. Turos, K.S. Bisht, Tetrahedron 59 (2003) 9147.

[10] M.M. Kayser, Y. Yang, M.D. Mihovilovic, A. Feicht, F.D. Rochon, Can. J. Chem. 80 (2002) 796.

[11] M.M. Kayser, M.D. Mihovilovic, J. Kearns, A. Feicht, J.D. Stewart, J. Org. Chem. 64 (1999) 6603.

[12] S. Servi, Synthesis (1990) 1;
R. Csuk, B.I. Glänzer, Chem. Rev. 91 (1991) 49;
P. D'Arrigo, G. Pedrocchi-Fantoni, S. Servi, Adv. Appl. Microbiol. 44 (1997) 81;
J.D. Stewart, Curr. Opin. Drug Discovery Dev. 1 (1998) 278;
K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, Tetrahedron: Asymmetry 14 (2003) 2659.

[13] W.-R. Shieh, A.S. Gopalan, C.J. Sih, J. Am. Chem. Soc. 107 (1985) 2993.

[14] K. Nakamura, Y. Kawai, N. Nakajima, A. Ohno, J. Org. Chem. 56 (1991) 4778.

[15] N. Nakajima, H. Ishihara, S. Tsuboi, M. Utaka, K. Nakamura, Biosci. Biotech. Biochem. 58 (1994) 2080;
K. Nakamura, S. Kondo, Y. Kawai, N. Nakajima, A. Ohno, Biosci. Biotech. Biochem. 58 (1994) 2236;
A. Kuhn, C. van Zyl, A. van Donder, B.A. Prior, Appl. Environ. Microbiol. 61 (1995) 1580;
M. Wada, M. Kataoka, H. Kawabata, Y. Yasohara, N. Kizaki, J. Hasegawa, S. Shimizu, Biosci. Biotech. Biochem. 62 (1998) 280;
S.T. Kim, W.H. Huh, B.H. Lee, S.O. Kang, Biochim. Biophys. Acta 1429 (1998) 29;
D.-Y. Yum, B.-Y. Lee, J.-G. Pan, Appl. Environ. Microbiol. 65 (1999) 3341;
G. Ford, E.M. Ellis, Chem. Biol. Interact. 130–132 (2001) 685;
G. Ford, E.M. Ellis, Yeast 19 (2002) 1087;
I. Kaluzna, A.A. Andrew, M. Bonilla, M.M. Martzen, J.D. Stewart, J. Mol. Catal. B: Enzym. 17 (2002) 101;
M. Katz, T. Frejd, B. Hahn-Hägerdal, M.F. Gorwa-Grauslund, Biotechnol. Bioeng. 84 (2003) 573;
M. Katz, B. Hahn-Hägerdal, M.F. Gorwa-Grauslund, Enzyme Microb. Technol. 33 (2003) 163.

[16] I.A. Kaluzna, T. Matsuda, A.K. Sewell, J.D. Stewart, J. Am. Chem. Soc. 126 (2004) 12827.

[17] J.D. Stewart, S. Rodríguez, M.M. Kayser, Cloning, structure and activity of ketone reductases, in: H.A. Kirst, W.-K. Yeh, M.J. Zmijewski (Eds.), Enzyme Technology for Pharmaceutical and

Biotechnological Applications, Marcel Dekker Inc., New York, NY, 2001, pp. 175–207.
[18] W.-R. Shieh, C.J. Sih, Tetrahedron: Asymmetry 4 (1993) 1259.
[19] Y. Kawai, M. Tsujimoto, S. Kondo, K. Takanobe, K. Nakamura, A. Ohno, Bull. Chem. Soc. Jpn. 67 (1994) 524.
[20] Bruker XSCANS, SHELXS-97, SHELXL-97 and SHELXTL, Bruker Analytical X-Ray Systems, Madison, Wisconsin, USA. The CIF tables have been deposited with the Cambridge Data File Centre. The deposit numbers are CCDC 247144–247146. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK [Fax: +44 (1223) 336 033; E-mail: deposit@ccdc.cam.ac.uk].
[21] M.D. Mihovilovic, A. Feicht, M.M. Kayser, J. Prakt. Chem. 342 (2000) 585.
[22] S. Rodríguez, M.M. Kayser, J.D. Stewart, J. Am. Chem. Soc. 123 (2001) 1547.
[23] S. Rodríguez, K.T. Schroeder, M.M. Kayser, J.D. Stewart, J. Org. Chem. 65 (2000) 2586.
[24] Y. Yang, F.-Y. Wang, F.D. Rochon, M.M. Kayser, Can. J. Chem. (2005) in press.
[25] E. Schweizer, H. Bolling, Proc. Natl. Acad. Sci. U.S.A. 67 (1970) 660.
[26] S. Rodríguez, M.M. Kayser, J.D. Stewart, Org. Lett. 1 (1999) 1153.
[27] S. Rodríguez Giordano, Ph.D. Dissertation, University of Florida, Gainesville, FL, USA, 2000.
[28] The corresponding numerical values are available in the Supplementary data.
[29] The ketones have lower UV absorptions than the alcohols, and the margin of error in the determining the extent of conversion and the product distribution for compounds **1d** and **1e** is therefore slightly larger than for **1a–c**. The trends observed, however, are quite consistent.