Baker's Yeast-Mediated Reductions of α-Keto Esters and an α-Keto-*β*-Lactam. Two Routes to the Paclitaxel Side Chain

Margaret M. Kayser,*[,†,‡] Marko D. Mihovilovic,[†,§,⊥] Jeff Kearns,[†] Anton Feicht,[†] and Jon D. Stewart*[,§,∥]

Department of Chemistry, University of New Brunswick, P.O. Box 5050, Saint John, New Brunswick E2L 4L5, Canada, and Department of Chemistry, University of Florida, Gainesville, Florida 32611

Received January 14, 1999

Baker's yeast (*Saccharomyces cerevisiae*) has been used to reduce a series of alkyl esters derived from pyruvate and benzoylformate. Both the yield and enantioselectivities of these reductions were maximized when methyl esters were used, and the (*R*)-alcohols were isolated in all instances. Yeast-mediated ester hydrolysis was a significant side reaction for products derived from long-chain alcohols. In the case of ethyl benzoylformate, the addition of methyl vinyl ketone increased the enantioselectivity of the reduction. These reductions were applied to two syntheses of the paclitaxel C_{13} side chain [(2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine]. In the first, a racemic α-keto-*β*-azido ester was reduced by whole cells of Baker's yeast to afford a diastereomeric mixture in which the desired product predominated and could be isolated chromatographically. In the second, an easily synthesized α-keto-*β*-lactam was reduced by yeast cells to afford the desired cis isomer as well as the undesired trans diastereomer. Substituting a yeast strain deficient in fatty acid synthase in this reduction suppressed formation of the trans diastereomer. These results suggest that a single enzyme is responsible for both the D- and L-*cis*-alcohols resulting from reduction of the α-keto-*β*-lactam. All of the yeast strains used in this project are available commercially, and these biocatalytic reductions require only common laboratory equipment.

Introduction

Baker's yeast (*Saccharomyces cerevisiae*) has been the most popular whole-cell biocatalyst, particularly for asymmetric reductions of carbonyl compounds.[1−5] Reductions catalyzed by this organism tolerate a large diversity of carbonyl substrates, and side reactions are rarely observed. This broad substrate acceptance is due to the presence of a number of reductase enzymes, several of which have been isolated and characterized.[6−17] Unfortunately, some of these reductases possess overlapping substrate specificities but with opposite enantioselectivities, which often diminishes the optical purities of the alcohol products when a compound is accepted by multiple enzymes. Traditional approaches to overcoming these problems have included variations in substrate structure[18] and concentration,[19] the use of organic solvents in place of water,[20−24] heat treatment,[25] and the addition of exogenous modifiers.[12,26−30] With the recent

* To whom correspondence should be addressed.
† University of New Brunswick.
‡ Phone: (506)648-5576. Fax: (506)648-5650. E-mail: kayser@unbsj.ca.
§ University of Florida.
⊥ Present address: Institute for Organic Chemistry, Vienna University of Technology, Getreidemarkt 9, A-1060 Vienna, Austria.
∥ Phone: (352) 846-0743. Fax: (352) 846-2095. E-mail: jds2@chem.ufl.edu.

(1) Neuberg, C. *Adv. Carbohydr. Chem.* **1949**, *4*, 75−117.
(2) Servi, S. *Synthesis* **1990**, 1−25.
(3) Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49.
(4) D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. *Adv. Appl. Microbiol.* **1997**, *44*, 81−123.
(5) Stewart, J. D. *Curr. Opin. Drug Discov. Dev.* **1998**, *1*, 278−289.
(6) Francke, A. *Biochem. J.* **1965**, *95*, 633−640.
(7) King, H. L., Jr.; Dyar, R. E.; Wilken, D. R. *J. Biol. Chem.* **1974**, *249*, 4689−4695.
(8) Furuichi, A.; Akita, H.; Matsukura, H.; Oishi, T.; Horikoshi, K. *Agric. Biol. Chem.* **1985**, *49*, 2563−2570.
(9) Murata, K.; Fukuda, Y.; Simosaka, M.; Watanabe, K.; Saikusa, T.; Kimura, A. *Eur. J. Biochem.* **1985**, *151*, 631−636.
(10) Shieh, W.-R.; Gopalin, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993−2994.
(11) Heidlas, J.; Engel, K.-H.; Tressl, R. *Eur. J. Biochem.* **1988**, *172*, 633−639.
(12) Nakamura, K.; Kawai, Y.; Nakajima, N.; Ohno, A. *J. Org. Chem.* **1991**, *56*, 4778−4783.
(13) Ishihara, K.; Nakajima, N.; Tsuboi, S.; Utaka, M. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3314−3319.
(14) Nakajima, N.; Ishihara, K.; Kondo, S.; Tsuboi, S.; Utaka, M.; Nakamura, K. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 2080−2081.
(15) Nakamura, K.; Kondo, S.; Kawai, Y.; Nakajima, N.; Ohno, A. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 2236−2240.
(16) Kuhn, A.; van Zyl, C.; van Donder, A.; Prior, B. A. *Appl. Environ. Microbiol.* **1995**, *61*, 1580−1585.
(17) Wada, M.; Kataoka, M.; Kawabata, H.; Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Shimizu, S. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 280−285.
(18) Zhou, B.-N.; Gopalin, A. S.; VanMiddlesworth, F.; Shieh, W.-R.; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 5925−5926.
(19) D'Arrigo, P.; Pedrocchi Fantoni, G.; Servi, S.; Strini, A. *Tetrahedron: Asymmetry* **1997**, *8*, 2375−2379.
(20) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *J. Org. Chem.* **1988**, *53*, 2589−2593.
(21) Naoshima, Y.; Maeda, J.; Munakata, Y.; Nishiyama, T.; Kamezawa, M.; Tachibana, H. *J. Chem. Soc., Chem. Commun.* **1990**, 964−965.
(22) Nakamura, K.; Kondo, S.-i.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.* **1991**, 7075−7078.
(23) Nakamura, K.; Kondo, S.-i.; Kawai, Y.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2738−2743.
(24) Medson, C.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron: Asymmetry* **1997**, *8*, 1049−1054.
(25) Kawai, Y.; Kondo, S.-i.; Tsujimoto, M.; Nakamura, K.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2244−2247.
(26) Nakamura, K.; Inoue, K.; Oshio, K.; Oka, S.; Ohno, A. *Chem. Lett.* **1987**, 679−682.
(27) Hayakawa, R.; Shimizu, M.; Fujisawa, T. *Tetrahedron: Asymmetry* **1997**, *8*, 3201−3204.
(28) Hayakawa, R.; Nozawa, K.; Shimizu, M.; Fujisawa, T. *Tetrahedron Lett.* **1998**, 67−70.
(29) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836−6841.
(30) Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 875−879.

10.1021/jo9900681 CCC: $18.00
Published on Web 08/10/1999

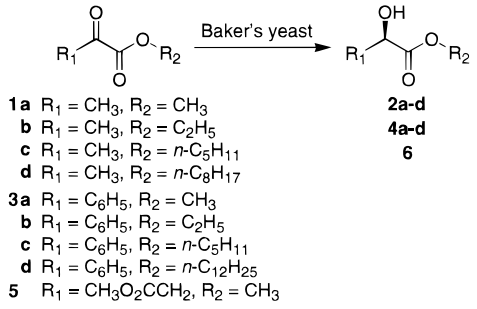
determination of the complete genome sequence of *S. cerevisiae*,[31] altering the enantioselectivities of whole-cell-mediated reductions by genetic manipulation has become a feasible goal. For example, we have already shown that the catalytic repertoire of Baker's yeast can be expanded to include asymmetric Baeyer−Villiger oxidations by creating a recombinant strain that expresses bacterial cyclohexanone monooxygenase.[32] The use of similar techniques to improve the enantioselectivity of Baker's yeast reductions requires that the yeast enzymes catalyzing the reactions be identified.

In this paper, we focus on Baker's yeast-mediated reductions of α-keto esters. While this class of compounds has received relatively less attention than *β*-keto esters and acids,[33] the product α-hydroxy acids and esters are equally valuable chiral building blocks, and several routes to these compounds have been devised.[34] Reductions of α-keto esters catalyzed by Baker's yeast are experimentally simple and avoid the yield limitations associated with kinetic resolutions. The major drawback is less-than-optimal stereoselectivity, the result of multiple reductase enzymes produced simultaneously by the yeast. Three such enzymes have been isolated by Nakamura and co-workers,[15] although other enzymes may be important for specific substrates. Our goals in this study were to investigate further the yeast-mediated reductions of α-keto carbonyl compounds and apply this knowledge to synthesize the paclitaxel side chain by reduction of α-keto carbonyl compounds.[35]

Results and Discussion

Four pyruvate esters and four benzoylformate esters bearing various alkoxy chains were synthesized and reduced by Baker's yeast (Scheme 1), and the results are shown in Table 1. The (*R*)-alcohols were isolated in all cases, although the enantiomeric purities depended significantly on the length of the alkoxy chain. These results stand in contrast to earlier reports that the (*S*)-alcohols predominated.[20−23] This discrepancy likely results from the very different reaction conditions used in the present study and the existence of an enantioselective yeast-mediated hydrolysis of (*R*)-lactate alkyl esters.[23,36] For both **1** and **3**, the best results were obtained from the reductions of methyl esters, suggesting that these are poor substrates for the yeast esterase(s). Consistent with this notion, a high yield and optical purity were obtained for ester **4b**, which has been reported to be inert with respect to Baker's yeast-mediated hydrolysis.[36]

Scheme 1



1a $R_1 = CH_3$, $R_2 = CH_3$
b $R_1 = CH_3$, $R_2 = C_2H_5$
c $R_1 = CH_3$, $R_2 = n\text{-}C_5H_{11}$
d $R_1 = CH_3$, $R_2 = n\text{-}C_8H_{17}$
3a $R_1 = C_6H_5$, $R_2 = CH_3$
b $R_1 = C_6H_5$, $R_2 = C_2H_5$
c $R_1 = C_6H_5$, $R_2 = n\text{-}C_5H_{11}$
d $R_1 = C_6H_5$, $R_2 = n\text{-}C_{12}H_{25}$
5 $R_1 = CH_3O_2CCH_2$, $R_2 = CH_3$

2a-d
4a-d
6

Table 1. Yeast Reductions of α-Keto Esters Using Commercial Baker's Yeast (Yeast:substrate = 20:1 (by weight); 30 °C, 24 h)

starting material	product	recovered starting material	product yield (%)	ee[a] (%)	byproduct(s)
1a	**2a**	<2	36	92	none
1b	**2b**	<2	38	90	none
1c	**2c**	41	24	48	$n\text{-}C_5H_{11}OH$, 7%
1d	**2d**	44	17	18	$n\text{-}C_8H_{17}OH$, 11%
3a	**4a**	<2	91	98	none
3b	**4b**	<2	68	82	none
3b[b]	**4b**	<2	72	92	none
3b[c]	**4b**	90	<2		none
3c	**4c**	32	47	52	$n\text{-}C_5H_{11}OH$, 12%; $PhCH_2OH$, 8%
3e	**4e**	30	46	20	$n\text{-}C_{12}H_{25}OH$, 6%; $PhCH_2OH$, 5%
5	**6**	<2	64	>98	none

[a] All products possessed the (*R*)-configuration. [b] Methyl vinyl ketone (126 mM) was included in the reaction mixture. [c] Methyl vinyl ketone (126 mM) and ethyl chloroacetate (66 mM) were included in the reaction mixture.

We explored two approaches to increasing the enantioselectivities of α-keto ester reductions even further. In the case of **3b**, known inhibitors of yeast reductases were included in the reaction mixture (Table 1).[37] Methyl vinyl ketone increased slightly both the yield and optical purity of alcohol **4b**, although the simultaneous presence of methyl vinyl ketone and ethyl chloroacetate was toxic to the yeast cells, and only starting material was recovered. Two other yeast strains were also tested for the reduction of **3a** (Table 2). Strain ATCC 26403 is unable to produce fatty acid synthase.[38] Substitution of this strain for commercial Baker's yeast had little effect on the enantioselectivity of the reduction, suggesting that this enzyme is not responsible for the production of **4a**. Another laboratory yeast strain (INVSc1) was also used for the reduction of **3a**. While this strain possessed markedly different enantioselectivities for reductions of

(31) Mewes, H. W.; Albermann, K.; Bahr, M.; Frishman, D.; Gleissner, A.; Hani, J.; Heumann, K.; Kleine, K.; Maierl, A.; Oliver, S. G.; Pfeiffer, F.; Zollner, A. *Nature* **1997**, *387*, 7−8.

(32) Stewart, J. D.; Reed, K. W.; Martinez, C. A.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3541−3548.

(33) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071−1140.

(34) Chemical syntheses: (a) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346−4348. (b) Corey, E. J.; Link, J. O.; Shao, Y. *Tetrahedron Lett.* **1992**, 3435−3438. Biocatalytic routes: (c) Reference 36. (d) Wong, C.-H.; Matos, J. R. *J. Org. Chem.* **1985**, *50*, 1992−1994. (e) Effenberger, F. *Ang. Chem., Int. Ed. Engl.* **1994**, *33*, 1555−1564. (f) Adam, W.; Fell, R. T.; Hoch, U.; Saha-Möller, C. R.; Schreier, P. *Tetrahedron: Asymmetry* **1995**, *6*, 1047−1050. (g) Adam, W.; Lazarus, M.; Boxx, B.; Saha-Möller, C. R.; Humpf, H.-U.; Schreier, P. *J. Org. Chem.* **1997**, *62*, 7841−7843. (h) Adam, W.; Boland, W.; Hartmann-Schreier, J.; Humpf, H.-U.; Lazarus, M.; Saffert, A.; Saha-Möller, C. R.; Schreier, P. *J. Am. Chem. Soc.* **1998**, *120*, 11044-11048. (i) Sugai, T.; Ohta, H. *Tetrahedron Lett.* **1991**, 7063−7064.

(35) Other synthetic routes: (a) Reference 40. (b) Reference 41. (c) Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R. *J. Org. Chem.* **1991**, *56*, 1681−1683. (d) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985−7012. (e) Denis, J.-N.; Greene, A. E.; Serra, A. A.; Luche, M.-J. *J. Org. Chem.* **1986**, *51*, 46. (f) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1990**, *55*, 1957. (g) Denis, J. N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1991**, *56*, 6939−6942. (h) Commerçon, A.; Bézard, D.; Bernard, F.; Bourzat, J. D. *Tetrahedron Lett.* **1992**, 5185−5188. (i) Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. *J. Med. Chem.* **1992**, *35*, 4230−4237. (j) Gou, D. M.; Liu, Y. C.; Chen, C. S. *J. Org. Chem.* **1993**, *58*, 1287−1289. (k) Hattori, K.; Miyata, M.; Yamamoto, H. *J. Am. Chem. Soc.* **1993**, *115*, 1151−1152.

(36) Ushio, K.; Yamauchi, S.; Masuda, K. *Biotechnol. Lett.* **1991**, *13*, 495−500.

(37) Nakamura, K. In *Stereochemical Control in Microbial Reduction*; Nakamura, K., Ed.; Kluwer Academic Publishers: Boston, 1992; Vol. 381, pp 389−398.

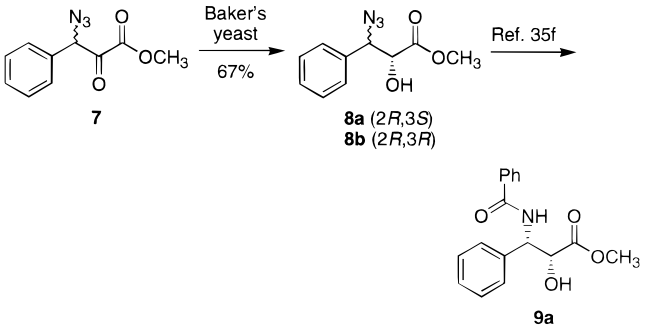
(38) Schweizer, E.; Bolling, H. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 660−666.

Table 2. Yeast Reductions of Methyl Benzoyl Formate 3a Catalyzed by Different *S. cerevisiae* Strains

yeast strain	reaction time (h)	product yield (%)	ee[a] (%)
commercial Baker's yeast	24	91	>98
26403	36	46[b]	>98
INVSc1	24	91	96

[a] The (*R*)-alcohol was isolated in all cases. [b] Significant quantities of yeast metabolites were also isolated from this reaction mixture.

Scheme 2



β-keto esters,[39] these cells displayed nearly identical behavior toward the reduction of **3a**.

Since methyl α-keto esters were reduced by whole Baker's yeast cells to (*R*)-alcohols in high optical purities, we applied this methodology to a short synthesis of the paclitaxel C_{13} side chain [(2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine] (Scheme 2). This work builds on our earlier route to this compound that featured a yeast-mediated reduction of a chiral α-keto ester to generate the correct stereochemistry at C_2.[40] In the present synthesis, we attempted to use a Baker's yeast-mediated reduction to kinetically resolve racemic β-azido-α-keto ester **7**, which was converted to a separable diastereomeric mixture of known (*R*)-alcohols **8a** and **8b**. Spectral data obtained for materials prepared by this route were completely consistent with literature data.[41] The desired product **8a** was converted to the paclitaxel C_{13} side chain by the method of Greene and co-workers.[35f] While this approach yielded the desired target compound, the limited diastereoselectivity observed for the enzymatic reduction of **7** was a significant drawback.

We also explored a second route to the paclitaxel side chain (Scheme 3). The activated β-lactam derivative of (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine allows it to be directly coupled to readily available 10-deacetylbaccatin III,[42] and we anticipated that the rigid skeleton might also improve the enantioselectivity of carbonyl reduction. The key step in our approach is the Baker's yeast-mediated reduction of racemic α-keto-β-lactam **13**, which was assembled in two steps from readily available starting materials in 77% overall yield by a modification of the procedure of Cainelli et al.[43] The ketone was reduced by Baker's yeast, and the compositions of the reaction mixtures were determined as a function of time (Table 3).[44] When commercial Baker's yeast was used for the reduction of **13**, the desired diastereomer **14** was the major product, consistent with the results described above for acyclic α-keto esters; however, significant quantities of its enantiomer (*ent*-**14**) and *trans*-alcohol **15** were also formed (Figure 1). All of these are known compounds.[45] The level of *ent*-**15**, if formed, was below our detection limits. The structure of **15**, including its absolute configuration, was further confirmed by X-ray crystallography after its chromatographic separation from the product mixture and derivitization as a *p*-bromobenzoate ester.[46]

The reduction of racemic α-keto-β-lactam **13** allows for a kinetic resolution, and in the case of commercial Baker's yeast, both substrate enantiomers were reduced to alcohol products. While the enantiomers of **13** were not resolved by chiral-phase HPLC, it was possible to calculate its optical purity from the observed product composition (Table 3). When these values were plotted as a function of fractional conversion, the data clearly did not fit the expected pattern for a kinetic resolution catalyzed by a single enzyme,[47] suggesting that more than one enzyme was responsible for the three observed products (**14**, *ent*-**14**, and **15**). Substitution of the laboratory strain INVSc1 in place of commercial Baker's yeast improved the optical purity of **14**; however, significant quantities of the undesired *trans*-alcohol were also formed by this strain (Table 3). Again, quantitative analysis of the enantiomeric purity of the starting α-keto-β-lactam during the course of the reaction was consistent with more than one enzyme catalyzing the reduction of **13**. By contrast, the use of fatty acid synthase-deficient *S. cerevisiae* ATCC 26403 almost completely suppressed formation of the undesired trans isomer **15** (Table 3). Moreover, a quantitative analysis of the optical purity of the starting material as a function of fractional conversion was consistent with reduction by only a single enzyme (enantioselectivity value 4; Figure 2). This suggests that yeast fatty acid synthase is primarily responsible for the production of trans isomer **15**.

If a single enzyme is responsible for the formation of both **14** and *ent*-**14**, it must be capable of producing both a D- and an L-alcohol. This initially surprising lack of enantioselectivity may be a consequence of the nearly symmetrical structure of β-lactam **13**. The calculated structures of **14** and *ent*-**14** can be overlaid on one another so that the aromatic groups are nearly coincident (Figure 3). The hydrides added during the enzymatic reduction of **13** are within 0.5 Å and aligned nearly parallel to one another, suggesting that a single enzyme active site could accommodate production of both **14** and *ent*-**14**, as suggested by our data. The identity of the enzyme(s) responsible for the production of **14** and *ent*-**14** remains to be established, however.

(39) Kayser, M. M.; Mihovilovic, M. D.; Rodriguez, S.; Stewart, J. D. Unpublished results.

(40) Kearns, J.; Kayser, M. M. *Tetrahedron Lett.* **1994**, 2845−2848.

(41) Thijs, L.; Porskamp, J. J. M.; van Loon, A. A. W. M.; Derks, M. P. W.; Feenstra, R. W.; Legters, J.; Zwanenburg, B. *Tetrahedron* **1990**, *46*, 2611−2622.

(42) Holton, R. A.; Liu, J. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2475−2478.

(43) Cainelli, G.; Panunzio, M.; Giacomini, D.; DiSimone, B.; Camerini, R. *Synthesis* **1994**, 805−808.

(44) Initial experiments indicated that the low solubility of **13** in the aqueous reaction medium posed difficulties in accurate sampling for analysis by chiral-phase HPLC. On the other hand, complete extractions of reaction mixtures provided >90% mass recovery. Parallel reactions were therefore started simultaneously, and then each reaction mixture was extracted completely after a given period of time. All samples were analyzed by chiral-phase HPLC, which cleanly resolved **13** and the four possible reduced products.

(45) Srirajan, V.; Deshmukh, A. R. A. S.; Bhawal, B. M. *Tetrahedron* **1996**, *52*, 5585−5590.

(46) Details of this crystal structure will be published elsewhere.

(47) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

Scheme 3

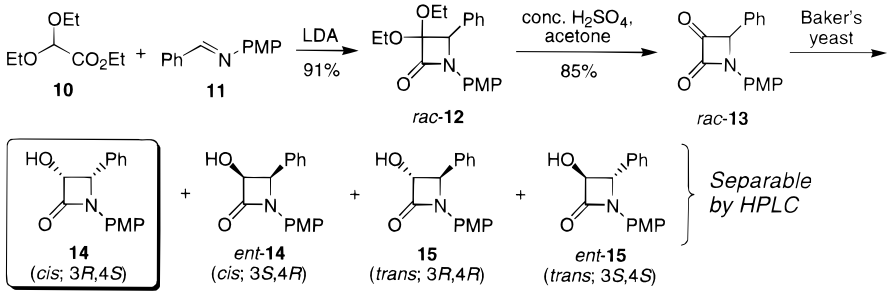


Table 3. Baker's Yeast-Mediated Reductions of Racemic α-Keto-β-lactam 13

	reaction time (h)	starting material **13**[a] (%)	conversion[b] (%)	ee (**13**)[c] (%)	**14** (3*R*,4*S*) (%)	*ent*-**14** (3*S*,4*R*) (%)	ee (**14**)[d] (%)	**15** (3*R*,4*R*) (%)	*ent*-**15** (3*S*,4*S*) (%)	ee (**15**)[c] (%)
commercial yeast	3	61	39	18	25	5	67	9	<1	>89
	9	43	57	26	34	9	58	14	<1	>93
	20	13	87	38	46	16	48	25	<1	>96
	72	<1	100		48	14	55	38	<1	>97
INVSc1 (laboratory strain)	3	77	23	14	17	2	79	4	<1	>75
	6	71	29	21	22	2	83	5	<1	>80
	9	47	53	62	41	4	82	8	<1	>88
	20	21	79	1	47	15	52	17	<1	>94
	72	5	95	60	49	14	56	32	<1	>97
26403 (FAS deficient)	21	81	17	10	13	4	53	<1	<1	
	46	61	39	31	29	8	57	2	<1	>50
	68	53	48	46	36	9	60	3	<1	>67
	119	47	53	53	39	10	59	4	<1	>75
	96[f]	<1	100		55	35	22	10	<1	>90

[a] Reaction compositions were obtained by integrations of HPLC peaks. [b] Calculated as (**14** + *ent*-**14** + **15** + *ent*-**15**)/(**13** + **14** + *ent*-**14** + **15** + *ent*-**15**). [c] Calculated as ([**14** + *ent*-**15**] − [**15** + *ent*-**14**])/(100 − [**14** + *ent*-**14** + **15** + *ent*-**15**]). [d] Calculated as (**14** − *ent*-**14**)/(**14** + *ent*-**14**) × 100. [e] Calculated as (**15** − *ent*-**15**)/(**15** + *ent*-**15**) × 100. [f] These data were obtained from a separate reaction that was allowed to proceed to completion. This reaction required less time than the previous set.

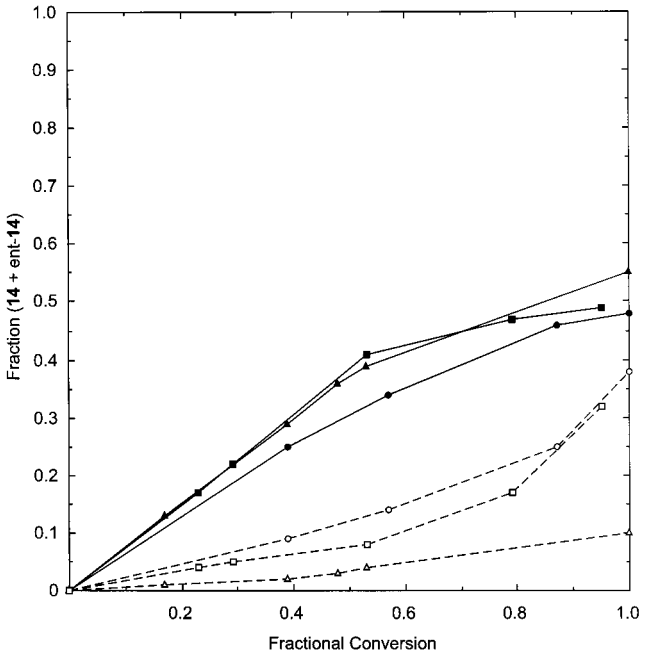


Figure 1. Reduction of α-keto-β-lactam **13** by Baker's yeast strains. Compositions of the reaction mixtures and values of fractional conversion were determined by HPLC analysis. Commercial Baker's yeast: ●, **14**; ○, **15**. *S. cerevisiae* INVSc1: ■, **14**; □, **15**. *S. cerevisiae* ATCC 26403: ▲, **14**; △, **15**.

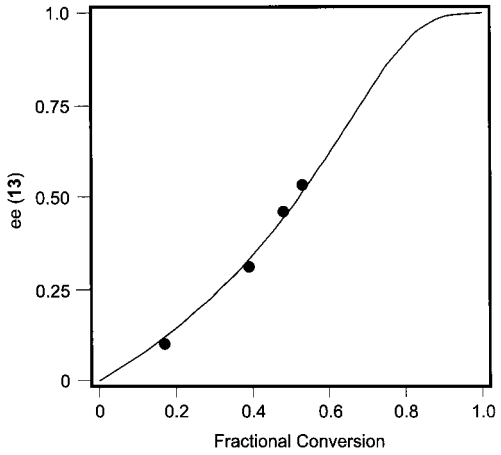


Figure 2. Enantioselectivity of the reduction of **13** by fatty acid synthase-deficient Baker's yeast. Values for the enantiomeric excess and fractional conversion were calculated from the data in Table 3. The data were fit by nonlinear least-squares methods to an equation derived from that of Sih and co-workers in which the enantioselectivity was the only adjustable parameter.[44] The close correspondence between the calculated and observed data is consistent with the production of both **14** and *ent*-**14** by a single enzyme.

Conclusions

Taken together, our results show that yeast-mediated reduction of α-keto carbonyl compounds provides a simple route to optically active alcohols. In the case of the open-chain α-keto esters, enzymatic reductions can provide high enantioselectivity, although the levels of diastereoselectivity can be relatively modest, e.g., in the reduction of **7** to **8**. For an α-keto-β-lactam such as **13**, the commercially available laboratory strain INVSc1 provided the desired alcohol diastereomer **14** with the highest optical purity (up to ca. 80% ee at 50% conversion), although significant quantities of the trans dias-

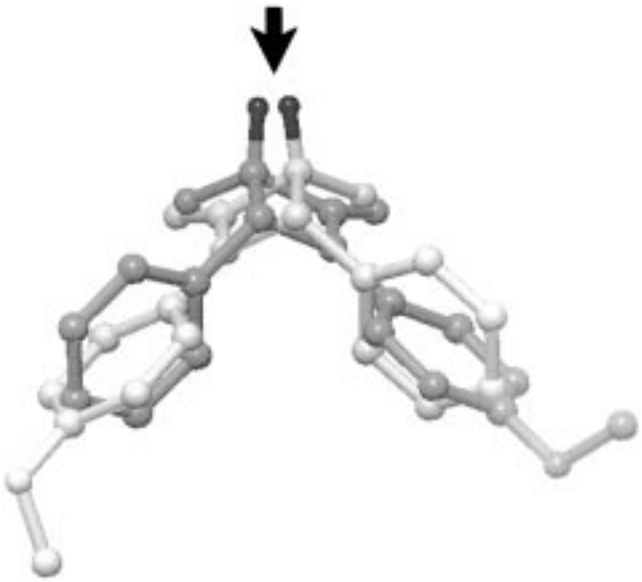


Figure 3. Overlay of calculated structures for **14** and *ent*-**14**. Both structures were sketched and energy-minimized using the Tripos force field (Sybyl 6.4). Dummy atoms were created in the centers of both aromatic rings, and these, along with the C_3 proton, were used for rigid-body superpositioning. Atoms of **14** are shown in light gray, while those of *ent*-**14** are depicted in dark gray. The hydrides delivered by the reductase(s) are shown in black and indicated by an arrow. Note the pseudosymmetry inherent in these structures.

tereomer **15** were also formed. Interestingly, while inactivation of the fatty acid synthase by genetic mutation largely suppressed the formation of **15**, it also diminished the optical purity of the desired alcohol **14**. From a quantitative analysis of the data, it appears that both **14** and *ent*-**14** are products of a single reductase enzyme with relatively low enantioselectivity that favors (*S*)-**13**. In the presence of fatty acid synthase, however, the apparent enantioselectivity of this enzyme is increased because fatty acid synthase competes effectively for the undesired (*R*)-**13** and converts it to a different diastereomer that can be removed by simple chromatographic separation. This suggests that further genetic manipulations of yeast reductases might lead to whole-cell catalysts with greater enantio- and diastereoselectivities for reductions of such compounds.

Experimental Section

Chemicals used were purchased from Aldrich Chemical Co. All solvents and ethyl pyruvate were distilled immediately prior to use. Melting points are uncorrected. Proton and carbon NMR spectra were recorded from $CDCl_3$ solutions unless otherwise indicated. Optical rotations were measured from $CHCl_3$ solutions. Chiral-phase GC analyses were performed using a chiral Cyclosilb column (0.25 mm × 30 m, film thickness 0.25 μm). Chiral-phase HPLC analyses were performed using a nonchiral Econosphere silica 5U column (4.6 × 250 mm) connected in series with a Chiracel OD-H column (4.6 × 150 mm) using a gradient of petroleum ether: *i*-propanol from 95:5 to 80:20 at 1% per min as the mobile phase. Detection was accomplished by UV monitoring at 254 nm.

Commercial Baker's yeast was obtained from a local bakery in a vacuum-packed form, and these large blocks were divided into 20 g portions and stored under argon at 4 °C. *S. cerevisiae* strains INVSc1 and ATCC 26403 were obtained from Invitrogen, Corp. and the American Type Culture Collection, respectively. For routine culturing, these strains were grown on YPD medium (1% Bacto-Yeast Extract, 2% Bacto-Peptone, 2% glucose). For culturing ATCC 26403, the YPD medium was supplemented with K_2HPO_4 (5 g/L), KH_2PO_4 (5 g/L), TWEEN 40 (10 g/L), myristic acid (70 mg/L), palmitic acid (70 mg/L), and stearic acid (70 mg/L).

General Procedure for the Preparation of Benzoylformate and Pyruvate Esters. To a stirred solution of α-keto acid (30 mmol) in dry alcohol (150 mmol) at −10 °C (for methyl and ethyl alcohols; 0 °C for pentyl alcohol; 10 °C for octyl and dodecyl alcohols) was added freshly distilled thionyl chloride (33 mmol) dropwise over a period of 15 min. The solution was then brought to room temperature, and stirring was continued for an additional 30 min. Excess thionyl chloride was removed by rotary evaporation, and the esters were purified by distillation.

General Procedure for the Reduction of α-Keto Esters Using Commercial Baker's Yeast. The ester (1.0 g) was added with vigorous stirring to a suspension of dry yeast (20 g) in distilled water (800 mL) in a round-bottom flask whose volume was at least twice the total volume of the reaction mixture. Sucrose (20 g) was then added over a period of 1 h (to minimize foaming). The reaction was allowed to proceed at room temperature for 24 h. After this time, the reaction mixture was centrifuged at 4000*g* for 15 min. Alternatively, Celite was added to the reaction mixture suspension, and then this was vacuum-filtered through no.1 filter paper. The isolated supernatant (or filtrate) was saturated with NaCl, acidified with 10% HCl, and extracted continuously with dichloromethane for 16 h. The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo to give a yellow liquid. The composition of the crude product was established by ^1H NMR, followed by the addition of diazomethane to determine whether any acids were present. Chromatography on a short silica gel column using 10% ethyl acetate−hexanes afforded pure reduction products. The enantiomeric excess values were determined by optical rotation (sodium D-line, 25 °C, 2 dm path length, c = 2 g/100 mL, $CHCl_3$). For those compounds where a literature value for the specific rotation was unavailable, the ester was hydrolyzed by being stirred for 10 min in an aqueous solution containing 10% potassium hydroxide and 5% zinc chloride. The solution was made slightly acidic by the addition of 10% HCl and extracted with dichloromethane. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residual acid was converted to the ester with diazomethane, and the specific rotation was determined for the methyl ester.

Yeast-Mediated Reductions in the Presence of Inhibitors. The general procedure for yeast reductions described was followed. Ethyl chloroacetate was added to the reaction mixture at a final concentration of 66 mM, and methyl vinyl ketone was used to a final concentration of 126 mM. The reactions were worked up as usual, and the inhibitors were removed during the final chromatographic separation.

General Procedure for Reductions Using Laboratory Strains of *S. cerevisiae*. YPD medium (40 mL) was inoculated with the appropriate *S. cerevisiae* strain, and the culture was incubated at 30 °C in an orbital shaker for 24 h. A 100 mg portion of the ketone was added, and the reduction was carried out at 30 °C. After complete consumption of the substrate (as determined by HPLC), the cells were separated by centrifugation at 3000*g* for 5 min, and then they were washed once with water and once with EtOAc. The aqueous layers were extracted with EtOAc, and the combined organic layers were dried and evaporated. Products were isolated by flash chromatography.

Methyl 2-Oxo-3-azido-3-phenylpropionate (7). Sodium azide (3.801 g, 58.45 mmol) was added in a single portion to a solution of methyl phenylglycidate (2.006 g, 11.26 mmol) in a mixture of methanol (40 mL), water (5 mL), and dimethyl formamide (8 mL). The mixture was warmed to 60 °C and stirred vigorously for 46 h. The crude product was extracted with ether, dried over $MgSO_4$, and concentrated by rotary evaporation, and then the mixture was chromatographed on silica gel using EtOAc−hexanes (1:9) to afford 2.048 g of methyl 2-hydroxy-3-azido-3-phenylpropionate as an oil (82%

yield) whose spectral data were identical to those reported previously.[35f] A portion (1.23 g, 10.5 mmol) of this material was treated with Jones' reagent (3 mL) in a 60 °C water bath and stirred for 12 h. The reaction mixture was filtered, neutralized with 10% sodium carbonate solution, and extracted with diethyl ether. The organic layer was washed with brine and dried over $MgSO_4$, and the solvent was evaporated. The crude material was purified by silica gel chromatography using EtOAc−hexanes (1:9) to yield 0.402 g of the title compound as a yellow oil (27% yield). IR ($CHCl_3$): 2130, 1750, 1730, 1720 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.75 (s, 3H), 6.16 (s, 1H), 7.11−7.46 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$): δ 51.5, 124.3−133.8, 159.5, 169.6 ppm. MS (EI, 70 eV): m/z 219 (M^+, 2%), 192 (7%), 135 (8%).

Methyl (2*R*,3*S*)-2-Hydroxy-3-azido-3-phenylpropionate (8a). Sucrose (20 g) was added slowly to a stirred suspension of dry yeast (20 g) in water (400 mL). Once active fermentation had begun, **7** (0.505 g, 2.29 mmol) was added. After 48 h, Celite (5 g) was added, and then the mixture was filtered through no. 1 filter paper. The crude product was isolated using the general procedure described above to give 0.334 g (67% yield) of a 7:3 ratio of (2*R*,3*S*)-**8a** and (2*R*,3*R*)-**8b** (as determined by ^1H NMR analysis). The two diastereomers were separated by spinning-plate chromatography on a 2 mm silica plate using EtOAc−pentane (15:85) as the solvent. Spectral data were consistent with those reported previously.[41]

3,3-Diethoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (12). A solution of diisopropylamine (4.21 g, 41.6 mmol) in 42 mL of dry THF under nitrogen was treated with *n*-BuLi (41.6 mmol, 1.6 M hexanes solution) at −20 °C. After the solution was warmed to 0 °C, stirring was continued for 15 min to complete formation of LDA. A solution of ketal **10** (6.65 g, 37.8 mmol) in 35 mL of dry THF was added at −70 ± 5 °C, and the resulting reaction mixture was stirred for 2.5 h at this temperature. After addition of a solution of **11** (4.00 g, 18.9 mmol) in 20 mL of dry THF, the solution was stirred for an additional 1.5 h at −70 ± 5 °C, and then it was allowed to warm slowly to room temperature. After stirring for an additional 18 h at room temperature, the reaction mixture was quenched with brine and extracted with Et_2O. The combined organic layers were washed with 2 M HCl, saturated $NaHCO_3$, and brine, then dried over $MgSO_4$, and concentrated in vacuo. Removal of all volatiles by Kugelrohr distillation enabled recovery of some excess **10**. The crude product was purified by recrystallization from diisopropyl ether to afford 5.77 g of the title compound as colorless crystals (89%, mp 98−99 °C). ^1H NMR: δ 0.85 (t, J = 7.0 Hz, 3H,), 1.30 (t, J = 7.0 Hz, 3H), 3.10 (dq, J_1 = 7.0 Hz, J_2 = 9.6 Hz, 1H), 3.55 (dq, J_1 = 7.0 Hz, J_2 = 9.6 Hz, 1H), 3.65 (s, 3H), 3.80 (dq, J_1 = 7.0 Hz, J_2 = 9.6 Hz), 4.00 (dq, J_1 = 7.0 Hz, J_2 = 9.6 Hz, 1H), 5.05 (s, 1H), 6.70 (d, J = 9.2 Hz, 2H), 7.20 (d, J = 9.2 Hz, 2H), 7.32−7.39 (m, 5H) ppm. ^{13}C NMR: δ 14.7 (q), 15.2 (q), 55.4 (q), 59.8 (t), 60.7 (t), 69.4 (d), 107.9 (s), 114.2 (d), 119.0 (d), 128.0 and 128.5 (2d), 128.6 (d), 130.4 (s), 133.7 (s), 156.3 (s), 163.1 (s) ppm.

1-(4-Methoxyphenyl)-4-phenyl-azetidine-2,3-dione (13). A suspension of **12** (0.492 g, 1.44 mmol) in 5 mL of water was cooled to 5 °C and slowly treated with 20 mL of concentrated H_2SO_4. After complete addition of the acid, 2 vol % of acetone was added, and the suspension was stirred at the above temperature. Another 20 vol % of acetone was added after 2 h, and stirring was continued until TLC analysis indicated complete hydrolysis (usually 1−1.5 h). The suspension was poured into ice/water and extracted with EtOAc. The combined organic extracts were neutralized with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to give 0.33 g of the title compound as yellow crystals (85%, mp 124−126 °C). ^1H NMR: δ 3.78 (s, 3H), 5.55 (s, 1H), 6.87 (d, J = 8.8 Hz, 2H), 7.29−7.40 (m, 5H), 7.42 (d, J = 8.8 Hz, 2H) ppm. ^{13}C NMR: δ 55.5 (q), 74.9 (d), 114.8 (d), 119.8 (d), 126.4 and 129.4 (2d), 129.5, (d), 129.9 (s), 131.7 (s), 158.0 (s), 160.0 (s), 190.7 (s) ppm.

Reduction of 13 by *S. cerevisiae* ATCC 26403. A frozen culture of ATCC 26403 containing 0.1 g of yeast cells was thawed and added to 100 mL of YPD medium containing fatty acids in a baffled 250 mL flask. The mixture was shaken at 30 °C for 30 min at 250 rpm on an orbital shaker, and then solid **13** (270 mg, 1.0 mmol) was added. The mixture was shaken at 30 °C at 250 rpm and sampled periodically for HPLC analysis.[48] When all of the starting material had been consumed, the mixture was transferred to a 500 mL centrifuge bottle. Solid NaCl was added to saturation followed by 60 mL of EtOAc. After shaking, the mixture was centrifuged at 3000*g* at 0 °C for 10 min. The supernatant was decanted carefully from the cell pellet into a separatory funnel, and the aqueous layer was removed and subsequently extracted with EtOAc (2 × 60 mL). The combined organic layers were washed with brine (75 mL), dried with anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel chromatography using a gradient elution from hexanes to EtOAc−hexanes (1:4) to afford 120 mg of **14** as beige crystals (45% yield, mp 204−208 °C; lit.[45] mp 198−201 °C). Spectral data were consistent with those reported previously.[45]

***trans*-3-Hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetindinone (15).** The general procedure for Baker's yeast-mediated reductions of **13** was followed, and the crude products from three separate reactions were combined to afford 304 mg of a mixture containing **14**, *ent*-**14**, and **15**. Chromatography on silica gel using petroleum ether−EtOAc (3:1) afforded 90 mg of **15** as colorless crystals (11% yield, mp 152−155 °C; lit.[49] mp 155−156 °C). ^1H NMR (DMSO-d_6): δ 3.71 (s, 3H), 4.70 (d, J = 1.5 Hz, 1H), 4.87 (d, J = 1.5 Hz, 1H), 6.69 (d, J = 9.1 Hz, 2H), 7.13 (d, J = 9.1 Hz, 2H), 7.29−7.36 (m, 5H) ppm. ^{13}C NMR (DMSO-d_6): δ 55.3 (q), 65.7 (d), 76.7 (d), 114.2 (d), 119.0 (d), 126.1 (d), 129.8 (d), 128.7 (d), 130.1 (s), 136.0 (s), 156.4 (s), 166.8 (s) ppm.

Acknowledgment. Financial support by the Natural Sciences and Engineering Research Council and the University of New Brunswick (M.M.K.), the National Science Foundation (J.D.S.; Grant CHE-9816318), and the FWF for a Schrödinger Fellowship (M.D.M.; Grant J1471-CHE) is gratefully acknowledged. We also thank Cerestar, Inc. for supplying the cyclodextrins used in this work. J.D.S. is a New Faculty Awardee of the Camille and Henry Dreyfus Foundation (1994−1999). We thank Gang Chen for his assistance throughout this project and Dr. Fernande Rochon for performing the X-ray crystallographic analysis associated with this work.

Supporting Information Available: NMR spectra for the compounds used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9900681

(48) Analytical samples were prepared by vortexing 0.5 mL of the reaction mixture with 0.5 mL of EtOAc. After the mixture was centrifuged at 7500 rpm for 1 min, the organic layer was removed, filtered, and analyzed by HPLC.

(49) Cossío, F. P.; Palomo, C. *Tetrahedron Lett.* **1985**, 4239−4242.