Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: *Asymmetry* 18 (2007) 2133–2138
Tetrahedron: *Asymmetry*

Reductions of cyclic β-keto esters by individual *Saccharomyces cerevisiae* dehydrogenases and a chemo-enzymatic route to (1*R*,2*S*)-2-methyl-1-cyclohexanol

Santosh Kumar Padhi,[a,†] Iwona A. Kaluzna,[a,†,‡] Didier Buisson,[b,*] Robert Azerad[b] and Jon D. Stewart[a,*]

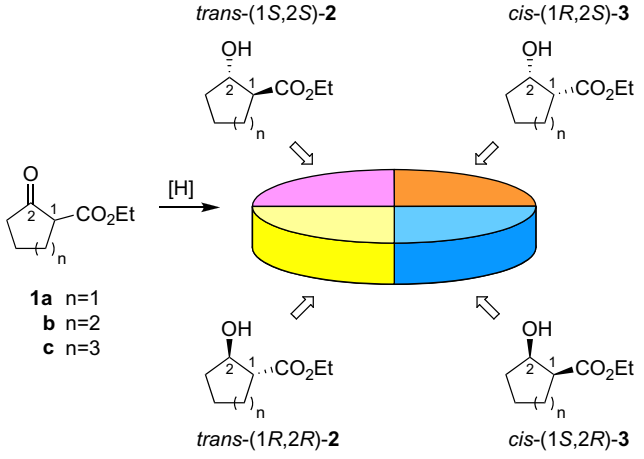
[a]*Department of Chemistry, University of Florida, Gainesville, FL 32611, USA*
[b]*Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601 CNRS, Université Paris Descartes, 45 Rue des Saints-Pères, 75006 Paris, France*

Received 1 June 2007; accepted 3 August 2007
Available online 25 September 2007

Abstract—Twenty purified dehydrogenases cloned from bakers' yeast (*Saccharomyces cerevisiae*) and expressed as fusion proteins with glutathione (*S*)-transferase were tested for their ability to reduce three homologous cyclic β-keto esters. The majority of dehydrogenases reduced ethyl 2-oxo-cyclopentanecarboxylate, yielding a pair of diastereomeric alcohols with consistent (1*R*)-stereochemistry. Ethyl 2-oxo-cyclohexanecarboxylate reductions afforded only *cis*-alcohol enantiomers. Ethyl 2-oxo-cycloheptanecarboxylate was accepted by two enzymes in the collection, and both yielded mainly the *cis*-(1*R*,2*S*)-alcohol. *Escherichia coli* cells overexpressing the YDL124w gene were used in a dynamic kinetic resolution of ethyl 2-oxo-cyclohexanecarboxylate to produce the key intermediate in a chemo-enzymatic synthesis of (1*R*,2*S*)-2-methyl-1-cyclohexanol, an important chiral building block.

1. Introduction

Asymmetric reductions of substituted β-keto esters allow simple access to densely-functionalized chiral building blocks. Such reductions can be carried out by hydrogenation in the presence of chiral transition metal catalysts[1,2] or by using biological methods.[3,4] In cases where the β-keto ester contains an α-substituent, the biocatalytic strategy can sometimes deliver higher stereoselectivities. This is particularly true for cyclic β-keto esters, and extensive microbial screening programs have uncovered several whole-cell biocatalysts that afford several stereoisomeric alcohols from representative substrates (Scheme 1).[5–9] Surprisingly, whole cells of bakers' yeast (*Saccharomyces cerevisiae*) reduced substrates **1a**–**c** with only modest stereoselectivities.[6,8] Our prior experience suggested that this may result from multiple dehydrogenases with overlapping substrate specificities but divergent stereopreferences.[3,10,11] We have therefore evaluated twenty individual, purified *S. cerevisiae* dehydrogenases as catalysts for reducing **1a**–**c**. These enzymes were selected after displaying relatively broad substrate specificities in earlier studies.



Scheme 1.

* Corresponding authors. Tel.: +33 1 42 86 21 70; fax: +33 1 42 86 83 87 (D.B.); tel.: +1 352 846 0743; fax: +1 352 846 2095 (J.D.S.); e-mail addresses: didier.buisson@univ-paris5.fr; jds2@chem.ufl.edu
† Both authors contributed equally to this work.
‡ Present address: BioCatalytics, Inc., 129 North Hill Avenue, Suite 103, Pasadena, CA 91106, USA.

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.08.010

Our current studies had two major goals. The first was to compare dehydrogenase substrate- and stereopreferences for structurally-related acyclic and cyclic β-keto esters. These comparisons provide valuable information in predicting the likely outcomes of proposed reductions and also shed light on how substrates interact with the active sites. Second, we hoped to provide simple routes to the majority of the stereoisomeric alcohol building blocks in enantiomerically pure form. Our prior experience has shown that if a suitable dehydrogenase can be identified, *Escherichia coli* cells that overproduce this protein can be used to supply practical quantities of chiral alcohols for chemical synthesis. In particular, we hoped to develop a chemo-enzymatic route to homochiral *cis*-(1*R*,2*S*)-2-methylcyclohexanol.

Despite its simple structure, *cis*-(1*R*,2*S*)-2-methylcyclohexanol is difficult to prepare in enantiomerically pure form. Several routes requiring kinetic resolutions involving ester hydrolysis[12–14] or synthesis,[15] and enantioselective oxidation[16] or reduction[17–19] have been reported. Unfortunately, the enantioselectivities of these processes are modest, which lessens their practical utility. In addition, separating the desired alcohol from the structurally similar by-product is undesirable on larger scales. The facile enolization of β-keto esters allows carbonyl reductions to proceed via dynamic kinetic resolutions that convert 100% of the input starting material to the desired product, thereby side-stepping the separation issue.

2. Results and discussion

Three representative cyclic β-keto esters **1a**–**c** were tested as substrates for our collection of twenty *S. cerevisiae* dehydrogenases and the results are collected in Table 1. All proteins were expressed and purified as fusions with glutathione *S*-transferase.[3] NADPH served as cofactor for all the dehydrogenases, which was supplied by enzymatic glucose-6-phosphate oxidation. Reactions were carried out at 30 °C in phosphate buffer (pH 7.0) and reaction progress was determined by periodic GC analysis. All four alcohol stereoisomers derived from **1a** and **1b** were resolved by chiral-phase GC; products from **1c** required prior derivatization by trifluoroacetic anhydride for resolution by chiral-phase GC. The identities of all peaks were assigned using authentic standards available from our previous studies.[6,8] Since two numbering systems have appeared in the literature, carbons are numbered unambiguously in Scheme 1.

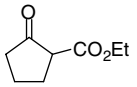
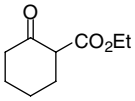
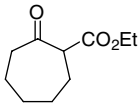
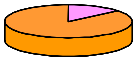
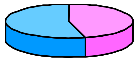
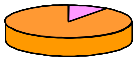
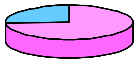
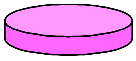
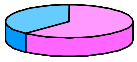
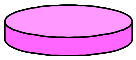
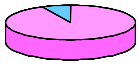
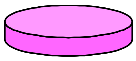
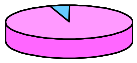
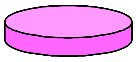
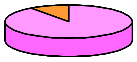
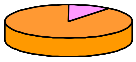
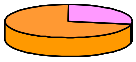
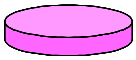
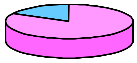
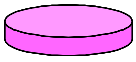
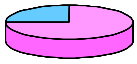
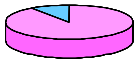
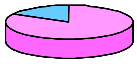
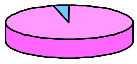
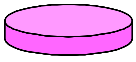
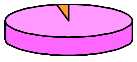
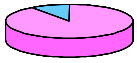
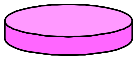
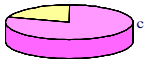
The majority of the yeast dehydrogenases examined reduced ethyl 2-oxo-cyclopentanecarboxylate **1a**. Despite the diversity in dehydrogenase sequences, the same two alcohol products were always observed (*trans*-(1*R*,2*R*)-**2a** and *cis*-(1*R*,2*S*)-**3a**). In no case did a yeast reductase afford a single diastereomer, although YPR1 afforded the best result (91% de; >98% ee, >98% ee). Our previous experience with acyclic β-keto ester reductions suggested that the configuration of the newly-created hydroxyl stereocenter would be consistent in the alcohol products from a given dehydrogenase.[3,10,11] This was not the case for **1a**. Every yeast dehydrogenase afforded mixtures of (*R*)- and (*S*)-alcohols whereas the carboxyethyl substituent maintained a consistent configuration. Whether this was due to two, flipped substrate binding orientations being accessible in all the enzyme active sites, or whether a single substrate binding mode permitted hydride addition from both the *Si*- and *Re*-faces could not be determined. Whole cells of commercial bakers' yeast also provided two diastereoisomeric alcohols from **1a**, although only one was also produced by our collection of isolated dehydrogenases *cis*-(1*R*,2*S*)-**3a**. By contrast, the *trans*-alcohol product was the enantiomer of that produced by the isolated enzymes. This implies that at least one additional dehydrogenase accepting **1a** is missing from our current collection.

Reductions of cyclohexanone **1b** were more complex. All of the medium chain dehydrogenases and two of the four short chain dehydrogenases failed to reduce **1b** to a significant extent. In all cases where **1b** was accepted as a substrate, only *cis*-alcohols were formed; however, the enantiomeric compositions of the product mixtures differed. Four enzymes (encoded by the YJR096w, YDL124w, YGL185c, and YNL274c genes) produced mainly *cis*-(1*S*,2*R*)-**3b**, although with a maximum of 90% ee (YDL124w). By contrast, eight other enzymes produced the opposite enantiomer in >98% de and >98% ee. This behavior is reminiscent of the results obtained when the same collection of dehydrogenases was used to reduce ethyl 2-chloropropionylacetate.[11] It is not clear why this acyclic substrate's behavior should resemble that of **1b**, which lacks a polar group at the α-position. This further underscores the advantages of screening individual dehydrogenases to determine reaction outcomes directly, rather than by relying on analogous substrates.

Only two reductases in our collection accepted cycloheptanone **1c** and the same alcohol stereoisomer predominated in both cases [*cis*-(1*R*,2*S*)]. In addition to this major product, aldose reductase Ypr1 also afforded a small quantity of its enantiomer, while the short chain dehydrogenase encoded by the YGL039w gene produced a small quantity of the *trans*-(1*S*,2*S*)-alcohol. The observation that relatively few yeast dehydrogenases accept larger substrates parallels our results from structurally diverse acyclic β-keto esters, suggesting that simple sterics is the major contributor to this phenomenon.[20]

Our initially planned synthetic route to *cis*-(1*R*,2*S*)-2-methylcyclohexanol featured a kinetic resolution of racemic 2-methylcyclohexanone by a yeast dehydrogenase. It was abandoned after a screen of our enzyme collection failed to uncover one with the necessary stereoselectivity for this substrate. We therefore adopted the chemo-enzymatic strategy outlined in Scheme 2, which was adapted from our earlier work.[6,8,21] While screening reactions were carried out with purified fusion proteins and a cofactor regeneration system, preparative reductions of **1b** used whole *E. coli* cells that overexpressed the YDL124w protein under non-growing conditions.[22] NADPH was supplied by glucose metabolism by intrinsic *E. coli* enzymes. Under non-optimized conditions, ca. 3 g/L of **1b** was reduced in 24 h. After product isolation by continuous extraction[23]

Table 1. Yeast dehydrogenase reductions of cyclic β-keto esters

Entry	Yeast ORF	Gene name			
1	YJR096w	—[a]	—		—
2	YDL124w	—			—
3	YBR149w	ARA1			—
4	YCR107w	*AAD3*			—
5	YNL331c	AAD14	—	—	—
6	YOR120w	*GCY1*			—
7	YHR104w	*GRE3*	—	N.D.[b]	—
8	YDR368w	*YPR1*			
9	YGL185c	—	—		—
10	YNL274c	*GOR1*	—		—
11	YPL275w	*FDH2*	—		—
12	YPL113c	—			—
13	YLR070c	*XYL2*	—	—	—
14	YAL060w	*BDH1*		—	—
15	YAL061w	—	—	—	—
16	YGL157w	—		—	—
17	YDR541c	—		—	—
18	YGL039w	—			
19	YOL151w	*GRE2*			—
20	YMR226c	—	—	—	—
21	Yeast cells	—	 [c]	 [c]	 [d]

Product compositions of reactions proceeding to >20% completion are indicated graphically using the patterns shown in Scheme 1. Yeast enzymes are grouped by superfamily (aldose reductase, D-hydroxy acid dehydrogenase, medium chain dehydrogenase, short-chain dehydrogenase); results from using commercial bakers' yeast cells are included for comparison.

[a] Conversion <20% after 24 h.

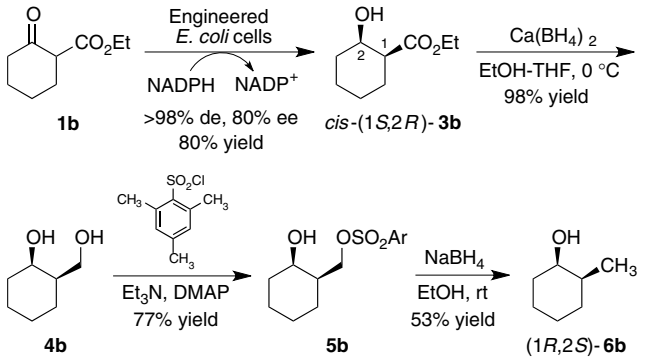
[b] Not determined.

[c] Ref. 6.

[d] Ref. 8.

and purification by silica gel chromatography, *cis*-(1*S*,2*R*)-**3b** was obtained in 80% yield. The stereochemical purity was identical to that observed in the screening reaction, demonstrating that the catalytic activities of any *E. coli*

dehydrogenases were negligible under these reaction conditions.



Scheme 2.

The remainder of the synthesis paralleled our earlier route.[6] Complete carboethoxy reduction was accomplished by a three-step procedure. Calcium borohydride reduction afforded diol **4b** in nearly quantitative yield. Reaction with tosyl chloride occurred mainly at the primary alcohol, although some bis-tosylate was formed. By contrast, mesityl sulfonyl chloride reacted with only the primary alcohol to produce **5b**. Many methods for reducing **5b** to the target were evaluated. Using optimized conditions (excess $NaBH_4$ in EtOH at room temperature), the reaction proceeded cleanly; the modest yield was caused by difficulties in isolating the volatile product. As expected, the enantiomeric purity of **6b** (80% ee) matched that of the bio-reduction product, indicating that no racemization had taken place during the subsequent synthetic operations.

3. Conclusion

A collection of dehydrogenases from bakers' yeast previously shown to reduce acyclic β-keto esters with high stereoselectivities also shows activity against representative cyclic β-keto esters. Interestingly, the product mixtures from yeast cell-mediated reductions of **1a**–**c** do not match the aggregate observed from twenty individual *S. cerevisiae* dehydrogenases. This mismatch undoubtedly reflects the absence of one or more participating yeast enzymes, but the possibility that the natural expression levels of some enzymes in our collection may be very low should not be overlooked. Individually expressing and isolating yeast reductases eliminates low native expression levels, and is a key advantage of the library approach.

Despite their apparent structural similarities, the outcomes of cyclic β-keto ester reductions often diverged from expectations based on acyclic analogs. In particular, reductions of cyclopentanone **1a** proceed with a surprising lack of stereoselectivity with respect to the hydroxyl configuration. Reductions of cyclohexanone **1b** and cycloheptanone **1c** were more in line with predictions from previous studies. In both cases, *cis*-alcohols were the predominant (usually sole) products. These observations could be put to use in a concise synthesis of *cis*-(1*R*,2*S*)-**6b**, an important chiral building block.

4. Experimental

4.1. General procedures

All reagents were obtained from commercial suppliers and used as received. ^1H and ^{13}C NMR spectra were recorded at 300 MHz using a Varian VXR instrument and referenced to residual protonated solvent. FT-IR spectra were recorded from thin films on a Bruker Vector 22 instrument. Mass spectra were determined with an HP 5971 instrument interfaced to an HP 5890 GC. Optical rotations were measured at room temperature using a Perkin–Elmer 241 polarimeter. GC analysis was carried out using 0.25 mm × 30 m DB-17 and 0.25 × 25 m Chirasil-Dex CB columns for normal and chiral-phase separations, respectively. Racemic standards were prepared by treating **1a**–**c** with excess $NaBH_4$ in EtOH. While conditions allowing adequate resolution of all four diastereomeric alcohols from **1a**,**b** using chiral-phase GC were identified, products from **1c** required prior conversion to trifluoroacetate derivatives. Standard media and techniques for growth and maintenance of *E. coli* were used and LB medium contained 1% Bacto-Tryptone, 0.5% Bacto-Yeast Extract, and 1% NaCl. GST-fusion proteins were isolated as described previously and stored at −20 °C in the presence of 50% glycerol.[3] *E. coli* strain BL21(DE3)(pIK8) produced the GST-YDL124w fusion protein under control of a T7 promoter, as described previously.[3] Glucose-6-phosphate dehydrogenase (Sigma type XV from bakers' yeast) was used for NADPH regeneration. When required, glucose concentrations were determined using Trinder reagent in a commercially-available kit.

4.2. Screening yeast dehydrogenase fusion proteins

Screening reaction mixtures contained $NADP^+$ (0.15 mg, 0.20 μmol), glucose-6-phosphate (4.3 mg, 14 μmol), glucose-6-phosphate dehydrogenase (5 μg) and a yeast dehydrogenase fusion protein (12–60 μg) in a total volume of 1.0 mL of 100 mM KP_i (pH 7.0). Reactions were incubated at 30 °C for 24 h, then extracted with Et_2O for GC analysis.

4.3. *cis*-(1*S*,2*R*) Ethyl 2-hydroxycyclohexanecarboxylate 3b

A single colony of BL21(DE3)(pIK8) was cultured overnight in 50 mL of LB supplemented with 25 μg/mL kanamycin at 37 °C. This preculture was diluted into 4 L of the same medium in a New Brunswick M19 fermenter. The culture was grown at 37 °C with vigorous stirring (700 rpm) and an air flow of 4 L/min until the $O.D._{600}$ reached 0.59, then it was cooled to 30 °C and supplemented with isopropylthio-β-D-galactoside (final concentration of 100 μM) and kept under these conditions for an additional 6 h. Cells were harvested by centrifugation at 6000*g* for 10 min at 4 °C, then they were resuspended in 100 mL of buffer (per liter: Na_2HPO_4, 12.8 g; KH_2PO_4, 3 g; NaCl, 0.50 g) and added to 900 mL of the same buffer in a B. Braun Biostat B fermenter employing a 1 L vessel. Glucose

was added to a final concentration of 4 g/L. The reduction was carried out at 30 °C at pH 7.0 (maintained with 3 M KOH) with an air flow of 0.5 L/min and stirring set at 800 rpm. Ketone **1b** (final quantity 20 mmol, 3.4 g) was added in two 10 mmol portions at 0 and 2 h. The glucose concentration was maintained at ca. 4 g/L by periodic manual additions of a sterile 20% stock solution. After 24 h, the product was isolated by continuous extraction with CH_2Cl_2, drying with $MgSO_4$, and concentration under reduced pressure followed by silica gel chromatography (1:4 EtOAc/hexanes) to yield 2.3 g of **3b** as a pale yellow oil (80% yield). Chiral-phase GC analysis showed 80% ee. Spectral data matched those reported previously.[24] $[\alpha]_D = -27.5$ (*c* 0.6, $CHCl_3$); lit.[5] for enantiomer $[\alpha]_D = +20.0$ (*c* 0.6, $CHCl_3$).

4.4. (1*R*,2*R*)-2-(Hydroxymethyl)-cyclohexanol 4b

Sodium borohydride (0.48 g, 13 mmol) was added to a stirred solution of **3b** (0.56 g, 3.2 mmol) and anhydrous $CaCl_2$ (0.72 g, 6.5 mmol) in 9.5 mL of EtOH and 5.6 mL of THF at 0 °C. The reaction mixture was slowly warmed to rt over 16 h, then it was quenched with 1 M HCl and extracted with Et_2O (3×20 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated under reduced pressure to afford 414 mg of **4b** as a thick, colorless oil that required no further purification (98% yield). ^1H NMR data matched those reported previously.[25] $[\alpha]_D = -32.1$ (*c* 0.24, H_2O); lit.[26] $[\alpha]_D = -36.0$ (*c* 0.42, H_2O).

4.5. ((1*R*,2*R*)-2-Hydroxycyclohexyl)methyl 2,4,6-trimethylbenzenesulfonate 5b

To a stirred solution of **4b** (50 mg, 0.38 mmol) in CH_2Cl_2 (1.0 mL) was added Et_3N (160 μL, 1.15 mmol) followed by 2,4,6-trimethylbenzenesulfonyl chloride (92 mg, 0.42 mmol) and DMAP (5 mg, 0.04 mmol). After stirring for 12 h at rt, additional 2,4,6-trimethylbenzenesulfonyl chloride (92 mg, 0.42 mmol) and CH_2Cl_2 (0.50 mL) were added, and stirring was continued for an additional 14 h. The mixture was washed with 1 M HCl and saturated $NaHCO_3$, then dried with $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by silica chromatography (1:1 EtOAc/hexanes) to yield 93 mg of **5b** as a colorless oil (77% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.16–1.86 (m, 9H), 2.31 (s, 3H), 2.63 (s, 6H), 3.77 (dd, 1H, $J = 9.6$, 6.3 Hz), 3.98–4.05 (m, 2H), 6.98 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 19.8, 21.0, 22.5, 22.6, 24.7, 32.6, 41.0, 65.4, 70.9, 130.5, 131.7, 139.8, 143.2 ppm. IR *v* 3549, 2934, 2858, 1714, 1604, 1567, 1449, 1406 cm^{-1}. MS *m*/*z* (relative intensity) 312 (3.5%), 213 (13%), 200 (69%), 134 (46%), 119 (68%), 118 (76%), 95 (100%). $[\alpha]_D = -9.5$ (*c* 1.0, $CHCl_3$).

4.6. (1*R*,2*S*)-2-Methyl-cyclohexanol 6b

To a solution of **5b** (0.31 g, 1.0 mmol) in 10 mL of absolute EtOH was added $NaBH_4$ (0.37 g, 10 mmol) portionwise over 10 min at rt. The reaction was stirred for an additional 6 h until conversion was judged complete by GC/MS. The reaction was quenched by 1 M HCl, then extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and brine, then dried with $MgSO_4$, and concentrated under reduced pressure. Pure **5b** was obtained after micro-distillation as a colorless oil (60 mg, 53% yield). Spectral data matched those reported previously.[27] $[\alpha]_D = -16.2$ (*c* 2.12, $CHCl_3$); lit.[12] $[\alpha]_D = -8.3$ (*c* 2.1, $CHCl_3$).

Acknowledgments

Financial support from Pfizer, Inc. (S.K.P.), the Ruegamer Foundation (I.A.K.), and the National Science Foundation (CHE-0130315) is gratefully acknowledged.

References

1. Blaser, H.-U.; Malan, C.; Pugin, B.; Spindler, F.; Steiner, H.; Studer, M. *Adv. Synth. Catal.* **2003**, *345*, 103–151.
2. Genet, J.-P. *Acc. Chem. Res.* **2003**, *36*, 908–918.
3. Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827–12832.
4. Zhu, D.; Mukherjee, C.; Rozzell, J. D.; Kambourakis, S.; Hua, L. *Tetrahedron* **2006**, *62*, 901–905.
5. Deol, B. S.; Ridley, D. D.; Simpson, G. W. *Aust. J. Chem.* **1976**, *29*, 2459–2467.
6. Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1986**, *27*, 2631–2634.
7. Spiliotis, V.; Papahatjis, D.; Ragoussis, N. *Tetrahedron Lett.* **1990**, *31*, 1615–1616.
8. Danchet, S.; Bigot, C.; Buisson, D.; Azerad, R. *Tetrahedron: Asymmetry* **1997**, *8*, 1735–1739.
9. Abalain, C.; Buisson, D.; Azerad, R. *Tetrahedron: Asymmetry* **1996**, *7*, 2983–2996.
10. Kaluzna, I.; Andrew, A. A.; Bonilla, M.; Martzen, M. R.; Stewart, J. D. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 101–105.
11. Kaluzna, I. A.; Feske, B. D.; Wittayanan, W.; Ghiviriga, I.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 342–345.
12. Kasai, M.; Kawai, K.-i.; Imuta, M.; Ziffer, H. *J. Org. Chem.* **1984**, *49*, 675–679.
13. Fantin, G.; Fogagnolo, M.; Guerrini, A.; Medici, A.; Pedrini, P.; Fontana, S. *Tetrahedron: Asymmetry* **2001**, *12*, 2709–2713.
14. Utsumi, R.; Izumi, S.; Hirata, T. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 439–443.
15. Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385–2394.
16. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Fontana, S. *Tetrahedron: Asymmetry* **2000**, *11*, 2367–2373.
17. Miyazawa, M.; Okamura, S.; Kameoka, H. *J. Chem. Tech. Biotechnol.* **1996**, *65*, 171–175.
18. Zarevúcka, M.; Rejzek, M.; Saman, D.; Streinz, L.; Zdenek, W. *Biocatal. Biotrans.* **1996**, *13*, 233–243.
19. De Conti, R. M.; Porto, A. L. M.; Augusto, J.; Rodrigues, R.; Moran, P. J. S.; Manfio, G. P.; Marsaioli, A. J. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 233–236.
20. Stewart, J. D. *Adv. Appl. Microbiol.* **2006**, *59*, 31–52.
21. Buisson, D.; Azerad, R. *Tetrahedron: Asymmetry* **1996**, *7*, 9–12.
22. Walton, A. Z.; Stewart, J. D. *Biotechnol. Prog.* **2004**, *20*, 403–411.
23. Feske, B. D.; Kaluzna, I. A.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 9654–9657.
24. Panunzio, M.; Camerini, R.; Pachera, R.; Donati, D.; Marchioro, C.; Perboni, A. *Tetrahedron: Asymmetry* **1996**, *7*, 2929–2938.

25. Lubineau, A.; Queneau, Y. *J. Org. Chem.* **1987**, *52*, 1001–1007.
26. Lemieux, R. U.; Brewer, J. T. *Adv. Chem. Ser.* **1973**, *117*, 121–146.
27. Marino, S. T.; Stachurska-Buczek, D.; Huggins, D. A.; Krywult, B. M.; Sheehan, C. S.; Nguyen, T.; Choi, N.; Parsons, J. G.; Griffiths, P. G.; James, I. W.; Bray, A. M.; White, J. M.; Boyce, R. S. *Molecules* **2004**, *9*, 405–426.