DOI: 10.1002/adsc.200800496

Asymmetric Synthesis of Optically Pure Pharmacologically Relevant Amines Employing ω-Transaminases

Dominik Koszelewski,[a,b] Iván Lavandera,[a,b] Dorina Clay,[a,b] David Rozzell,[c] and Wolfgang Kroutil[a,b,*]

[a] Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria
Fax: (+43)-316-380-9840; e-mail: wolfgang.kroutil@uni-graz.at
[b] Research Centre Applied Biocatalysis, Graz, Austria
[c] Codexis, Inc., Redwood City, California, USA

Received: August 8, 2008; Published online: November 17, 2008

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.200800496.

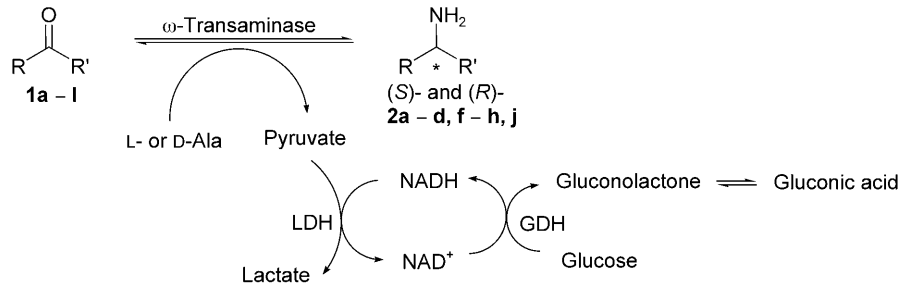
Abstract: Various ω-transaminases were tested for the synthesis of enantiomerically pure amines from the corresponding ketones employing D- or L-alanine as amino donor and lactate dehydrogenase to remove the side-product pyruvate to shift the unfavourable reaction equilibrium to the product side. Both enantiomers, (*R*)- and (*S*)-amines, could be prepared with up to 99% *ee* and >99% conversions within 24 h at 50 mM substrate concentration. The activity and stereoselectivity of the amination reaction depended on the ω-transaminase and substrate employed; furthermore the co-solvent significantly influenced both the stereoselectivity and activity of the transaminases. Best results were obtained by employing ATA-117 to obtain the (*R*)-enantiomer and ATA-113 or ATA-103 to access the (*S*)-enantiomer with 15% v v^{-1} DMSO.

Keywords: amination; amines; asymmetric catalysis; biotransformations; transaminase

Introduction

Optically active amines are required for the preparation of a broad range of biologically active compounds showing various pharmacological properties.[1,2,3] For instance, chiral amines have been used as building blocks in the synthesis of neurological, cardiovascular, immunological, anti-hypertensive, anti-infective, and anti-emetic drugs. In most cases, the pharmacological activities of these amines are related to the configuration of the stereogenic center. For example, (*S*)-amphetamine has higher pharmacologically activity as stimulant[4] and hyperthermic agent[5] than its (*R*)-enantiomer; (*R*)-4-phenylbutan-2-amine (**2a**) is a precursor of the anti-hypertensive dilevalol,[6] and (*R*)-*p*-methoxyamphetamine (**2d**) is a constituent of (*R*,*R*)-formoterol, a potent bronchodilator.[7] Furthermore 1-phenyl-1-propylamine (**2c**) is a precursor of the corticotropin-releasing factor type-1 receptor antagonist, a potent anti-depressive agent.[8] Consequently, there is a need for efficient methods to obtain the desired (*R*)- or (*S*)-enantiomer in optically pure form starting from easily accessible ketone. Resolution of some of these amines has been carried out by fractional crystallization or distillation of the diastereomeric salts,[9,10] chromatographic separation of diastereomeric amides,[11] microbial hydrolysis of an *N*-acyl derivative[12–14] or by enantioselective acylation of racemic amines catalyzed by lipase B from *Candida antarctica*.[15–17] Among several enzymatic methods that have been employed for the synthesis of optically active amino acids[18] and amines, ω-transaminases have recently received increased attention.[12,19–20]

Enantioenriched amines can be produced *via* two reaction strategies employing ω-transaminases: that is, (i) the asymmetric synthesis starting from ketones (Scheme 1), and (ii) the kinetic resolution starting from racemic amines.[21–23] Although ω-transaminases exhibit good enantioselectivity in general, they have not been used frequently in asymmetric synthesis, although in this case a 100% yield is theoretically possible.[24–27] One challenge in asymmetric synthesis employing ω-transaminases is to shift the equilibrium to the product side, especially when using an amino acid like alanine as amino donor, since in this case the equilibrium is on the side of the substrates (ketone,



Scheme 1. Stereoselective amination of ketones employing ω-transaminases. Removal of pyruvate was achieved *via* its reduction employing lactate dehydrogenase (LDH) to shift the equilibrium to the product side.

alanine) and not on the side of the products (amine, pyruvate);[27] another problem is that the stereoselectivity of the enzyme has to be perfect, which is not always the case for ω-transaminases. For this reason, ω-transaminases are mainly used for the kinetic resolution of racemic amines,[28–33] where enantioselectivity does not necessarily need to be perfect, since at a certain conversion >50% the *ee* of the substrate will always approximate >99%.

Results and Discussion

In this paper, we report the asymmetric amination of various prochiral ketones employing commercial ω-transaminases ATA-103, ATA-113, ATA-117 in combination with alanine as amino donor. To shift the equilibrium[34,35] the pyruvate formed was removed by reduction using lactate dehydrogenase (LDH) in a coupled reaction system as illustrated in Scheme 1.

For initial testing 4-phenyl-2-butanone (**1a**) was used as model substrate due to the synthetic significance of amine **2a** (Figure 1) as a building block.[6] To find a suitable enzyme, 19 commercially available ω-transaminases were tested employing LDH, NADH-recycling system, and L- or D-alanine, respectively. We found that only ω-transaminases ATA-103, ATA-113, ATA-114 and ATA-117 led to reasonable conversion for the transamination of this ketone **1a** (Table 1). Transaminases ATA-103, ATA-113, ATA-114 showed (*S*)-preference while ATA-117 displayed (*R*)-preference; furthermore, ATA-114 and ATA-117 showed perfect stereoselectivity (>99% *ee*). The reactions were performed in phosphate buffer pH 7.0 at 30 and 40 °C. By using a 5-fold excess of the amino donor (250 mM, D- or L-alanine), the conversion reached a plateau after 24 h. The excess of amino donor was required to achieve a reasonably fast reaction; using less amino donor resulted in longer reaction times. Increasing the reaction temperature from 30 °C to 40 °C caused a loss of stereoselectivity only in case of ATA-113 but enhanced its activity; in all other cases a decrease of activity was observed.

The effect of organic solvents on ω-transaminases has been scarcely studied,[36–38] and since 4-phenyl-2-butanone (**1a**) shows low solubility in water, 15% $v\,v^{-1}$ water-miscible organic solvents (THF, DME, 1,4-dioxane, methanol, DMF, and DMSO) were

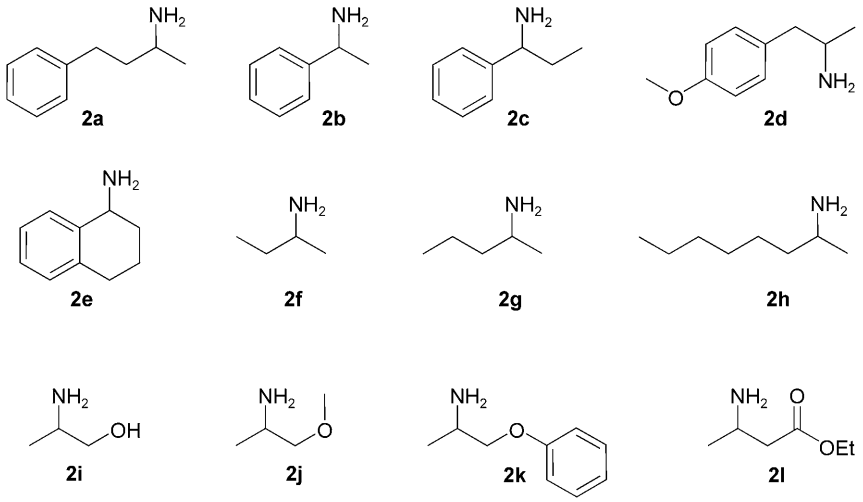


Figure 1. Target amines **2a–l** for the biocatalytic asymmetric synthesis starting from the corresponding ketone employing ω-transaminase and LDH.

Table 1. Asymmetric amination of 4-phenyl-2-butanone (**1a**).

Entry	ATA[a]	T [°C]	Conv. [%][a]	ee_{amine} [%][b]
1	113	30	61	15 (*S*)
2	113	40	82	5 (*S*)
3	114	30	15	>99 (*S*)
4	114	40	11	>99 (*S*)
5	117[c]	30	37	>99 (*R*)
6	117[c]	40	17	>99 (*R*)
7	103	30	44	87 (*S*)
8	103	40	34	89 (*S*)

[a] *Reaction conditions:* 4-phenyl-2-butanone (**1a**) (50 mM), L-alanine (250 mM), and crude ω-transaminase (ATA-103: 20 μL, ATA-113: 2 mg, ATA-114: 2 mg, ATA-117: 2 mg,), LDH mix (40 mg), shaking at 30 or 40 °C for 24 h.
[b] Enantiomeric excess was determined by GC analysis on a chiral phase.
[c] D-Alanine employed.

tested to improve the availability of the substrate in the buffer. In the case of ω-transaminase ATA-103, the co-solvent influenced both the conversion and the stereoselectivity (Table 2). Since, in the presence of DMSO, the *ee* value was highest, actually as high as without co-solvent (entry 7, Table 1), DMSO was employed for our following studies (see also below). Changes of stereoselectivity have frequently been observed with enzymes, for example, for the hydrolysis of nitriles.[39]

In contrast to ATA-103, ATA-117 showed perfect stereoselectivity (>99% *ee*) with and without DMSO. Furthermore, the conversions achieved depended strongly on the co-solvent concentration used (Figure 2). The activity of the asymmetric amination of **1a** catalyzed by ATA-117 was highest in the presence of DMSO in a concentration range between 5 to 15% v v^{-1}. Since, ATA-117 gave the highly desired

Table 2. Effect of the co-solvent on the asymmetric amination of 4-phenyl-2-butanone (**1a**) catalyzed by ω-transaminase ATA-103.

Entry	Solvent[a,b]	Conv. [%]	ee_{amine} [%]
1	–	44	87 (*S*)
2	THF	<1	nd
3	DME	19	74 (*S*)
4	1,4-Dioxane	<1	nd
5	MeOH	56	73 (*S*)
6	DMF	5	79 (*S*)
7	DMSO	12	87 (*S*)

[a] Abbreviation: DME = 1,2-dimethoxyethane.
[b] *Reaction conditions:* 4-phenyl-2-butanone (**1a**) (50 mM), L-alanine (250 mM), 15% v v^{-1} of each co-solvent, phosphate buffer (100 mM, pH 7.0), crude ω-transaminase ATA-103 (20 μL), LDH mix (40 mg), with shaking at 30 °C for 24 h.

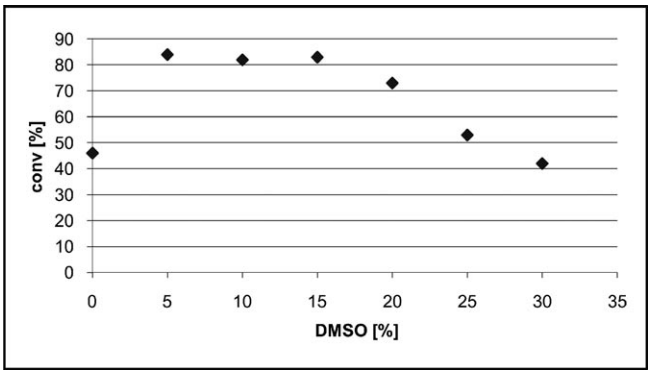


Figure 2. Amination of 4-phenyl-2-butanone (**1a**) catalyzed by ω-transaminase ATA-117 at varied DMSO concentrations.

Table 3. Effect of the pH and PLP concentration on the asymmetric amination of 4-phenyl-2-butanone (**1a**) catalyzed by ω-transaminase ATA-117.

Entry	pH	PLP [mM]	Conv. [%][b]	ee_{amine} [%] (*R*)
1	7.0	0.5	55	>99
2	7.0	1.0	76	>99
3	8.0	0.5	76	>99
4	8.0	1.0	91	>99
5	9.0[a]	0.5	51	>99
6	9.0[a]	1.0	82	>99

[a] Carbonate buffer (100 mM).
[b] *Reaction conditions:* 4-phenyl-2-butanone (**1a**) (50 mM), D-alanine (250 mM), 15% v v^{-1} DMSO, phosphate buffer (100 mM), crude ω-transaminase ATA-117 (2 mg), LDH mix (40 mg), with shaking at 30 °C for 24 h.

(*R*)-enantiomer, (*R*)-**2a**, the further optimization was performed employing this ω-transaminase.

Optimization of the reaction conditions with respect to pH and PLP concentration for ATA-117 led to 91% conversion at 50 mM substrate concentration at pH 8.0 and 1 mM PLP (Table 3). In all cases the *ee* remained constantly perfect (>99%).

Finally, the influence of the substrate concentration **1a** on the transamination with ATA-117 was investigated. Even at a substrate concentration of 100 mM a conversion of 84% was reached within 24 h (Figure 3). Interestingly, the reaction got faster in the case when more substrate was added; however, at substrate concentration below 10 mM the conversion of ketone **1a** was very low (<1%). First, we suspected that ω-transaminase ATA-117 shows "interfacial activation" like lipases,[40,41] however additional studies in biphasic systems (organic solvent-buffer) did not give a clear picture. Probably this observation can only be explained when a crystal structure becomes available.

Having optimized the amination, a preparative transformation of 100 mg 4-phenyl-2-butanone (**1a**) at 33 mM substrate concentration yielded (*R*)-**2a** at 92%

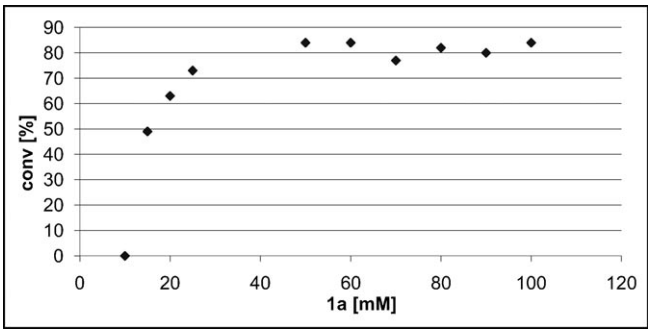


Figure 3. Conversion at varied substrate concentrations for the asymmetric amination of 4-phenyl-2-butanone (**1a**) catalyzed by ω-transaminase ATA-117. The system became biphasic between 30–40 mM. The reaction mixture contained 4-phenyl-2-butanone (**1a**) (1 equiv.), D-alanine (5 equiv.), 15% v v^{-1} of DMSO, phosphate buffer (100 mM, PLP 1 mM, pH 8.0), and crude ω-transaminase ATA-117 (2 mg), LDH mix (40 mg), with shaking at 30 °C for 24 h.

conversion after 24 h with 99% *ee* and 63% isolated yield.

A variety of aromatic and aliphatic ketones was tested to study the substrate spectrum of ω-transaminases ATA-103, 113 and 117 (Table 4). In most cases good to excellent conversions to amines with excellent *ee* values were achieved. While aliphatic ketones such as 2-butanone (**1f**), 2-pentanone (**1g**), or 2-octanone (**1h**) reacted smoothly, aromatic amines containing a substituted (**2d**) or non-substituted (**2b**, **c** and **2e**) phenyl ring were obtained with slightly lower yields. In the case where the substrate contained an unprotected hydroxy group (**1i**), no product was formed. In the cases of alkoxy- (**1j**) or aryloxy ketones (**1k**) the corresponding amines were formed with very high conversions (>99%). In most cases, high to excellent stereoselectivities were obtained using ATA-117 to afford the (*R*)-amines and ATA-113 to give the enantiocomplementary product.

Since chiral β-amino acid derivatives are important building blocks for the synthesis of numerous biologically active compounds such as β-peptides, β-lactams antibiotics and many chiral drugs,[42,43] ethyl acetoacetate (**1l**) was tested. Using ATA-117 the chiral β-amino ester (*R*)-**2l** was obtained with excellent isolated yield (98%) and high optical purity (98% *ee*).

On a preparative scale, 100 mg (0.6 mmol) of 4-methoxyphenylacetone (**1d**) and 100 mg (0.65 mmol) phenoxy-2-propanone (**1k**) were transformed into optically pure amines (*R*)-**1d** and (*R*)-**1k** with complete conversion and >99% *ee* and 99% isolated yield.

Conclusions

In summary, enantioselective amination of prochiral ketones was demonstrated for the synthesis of pharmacologically interesting amines employing ω-transaminases. Due to the excellent stereoselectivity of selected enzymes for the amination of certain ketones the corresponding optical pure amines could be prepared with excellent yields.[44]

Table 4. Amination of various ketones with ω-transaminases.[a]

Entry	Substrate	ATA	Conv. [%]	ee_{amine} [%][b]
1	**1b**	117	43	96 (*R*)
2		103	21	>99 (*S*)
3		113	5	>99 (*S*)
4	**1c**	117	3	42 (*R*)
5		103	3	>99 (*S*)
6		113	28	>99 (*S*)
7	**1d**	117	98	>99(*R*)
8		103	52	40 (*S*)
9		113	21	40 (*S*)
10	**1e**	117	<1	nd[c]
11		103	3	nd[c]
12		113	<1	nd[c]
13	**1f**	117	98	>99 (*R*)
14		113	97	98 (*S*)
15	**1g**	117	89	>99 (*R*)
16		103	19	13 (*S*)
17		113	82	>99 (*S*)
18	**1h**	117	70	90 (*R*)
19		103	24	90 (*S*)
20		113	52	98 (*S*)
21	**1i**	117	<1	nd[c]
22		103	<1	nd[c]
23		113	<1	nd[c]
24	**1j**	117	>99	>99 (*R*)
25		103	86	5 (*R*)
26		113	>99	77 (*S*)
27	**1k**	117	>99	>99 (*R*)
28		103	29	81 (*S*)
29		113	>99	96 (*S*)
30	**1l**	117	>99	98 (*R*)
31		103	83	50 (*S*)
32		113	98	61 (*S*)

[a] *Reaction conditions:* ketone (**1b–l**) (50 mM), D- or L-alanine (250 mM), 15% v v^{-1} of DMSO, phosphate buffer (100 mM, pH 7.0), crude ω-transaminase (ATA-113: 2 mg, ATA-117: 2 mg, ATA-103: 20 μL), LDH mix (40 mg), with shaking at 30 °C for 24 h.

[b] Determined by GC on a chiral stationary phase.

[c] nd: not determined due to low conversion.

Experimental Section

Initial Tests

All biocatalytic reactions were performed at 30 °C for 24 h in sodium phosphate buffer (100 mM, pH 7) containing pyridoxal 5′-phosphate (1 mM) in a 2-mL Eppendorf tube. The reaction buffer (850 μL) was mixed with ω-transaminase

(2 mg in the cases of ATA-113, ATA-114 and ATA-117, and 20 μL for ATA-103), L- or D-alanine (250 mM), pyruvate reductase mix (=LDH mix) (40 mg, mixture of lactate dehydrogenase, glucose dehydrogenase, glucose, NAD^+), and DMSO (150 μL). The reaction mixture contained 50 mM of the corresponding ketone **1**. The reaction was stopped by addition of aqueous NaOH solution (200 μL, 10 N), followed by extraction with ethyl acetate (600 μL, twice), the organic phase was dried (Na_2SO_4) and the conversion was measured by GC.

Analysis of Optical Purity of Products

The enantiomeric excess of amines **2a–l** was analyzed by gas chromatography on a chiral phase after derivatization to the acetoamides, which was performed by addition of DMAP and a 20-fold excess of acetic anhydride. After washing with water and drying (Na_2SO_4), the *ee* of the derivatized compound was measured (see Supporting Information).

Represenative Example for Amination: Preparation of (*R*)-2a

In a 50-mL screw-topped tube **1a** (100 mg, 0.67 mmol) was suspended in phosphate buffer (17 mL, 100 mM, pH 8.0, 1 mM PLP, 15% v v^{-1} DMSO). D-Alanine (3.35 mmol) and a crude preparation of lactate dehydrogenase mixture (500 mg) were added. The reaction was started by the addition of ω-transaminase ATA-117 (40 mg) and the tube was shaken at 30 °C (300 rpm). Conversion and enantiomeric excess were analyzed as described above. After 24 h, the pH of the mixture was adjusted to pH 1 with aqueous HCl (5 M), and the ketone **1a** was extracted five times with dichloromethane (5×10 mL). After the extraction, **1a** was not detectable in the residual aqueous phase. The pH was now adjusted to pH 12, and the amine **2a** was extracted four times with dichloromethane (4×10 mL). The solvent of the combined organic phases was evaporated under reduced pressure and **2a** was obtained; yield: 63%; $[\alpha]_D$ −6.73 (*c* 0.5, chloroform), [lit. 6.40 (c 0.47, chloroform) for the (*S*)-enantiomer, 98% *ee*];[16] ^{1}H NMR (200 MHz): δ=1.10 (d, 3H, J=6.70 Hz), 1.32 (s, 2H), 1.52–1.75 (m, 2H), 2.52–2.74 (m, 2H), 2.87–2.91 (m, 1H), 7.10–7.52 (m, 5H).

References

[1] H. Y. Aboul-Enein, I. W. Wainer, *The Impact of Stereochemistry on Drug Development and Use*, Wiley & Sons Inc., New York, **1997**.

[2] E. J. Ariëns, W. Soudijn, P. B. M. W. M. Timmermans, *Stereochemistry and Biological Activity of Drugs*, Blackwell Scientific Publishers, Oxford, **1983**.

[3] L. Sutin, S. Andersson, L. Bergquist, V. M. Castro, E. Danielsson, S. James, M. Henriksson, L. Johansson, C. Kaiser, K. Flyrén, M. Williams, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4837–4840.

[4] S. H. Snyder, K. M. Tayler, *Science* **1970**, *168*, 1487–1489.

[5] G. T. Hajos, S. Garattin, *J. Pharm. Pharmacol.* **1973**, *25*, 418–419.

[6] J. E. Clifton, I. Collins, P. Hallett, D. Hartley, L. H. C. Lunts, P. D. Wicks, *J. Med. Chem.* **1982**, *25*, 670–679.

[7] K. Murase, T. Mase, H. Ida, K. Takahashi, M. Murakami, *Chem. Pharm. Bull.* **1978**, *26*, 1123–1129.

[8] J. W. Corbett, M. R. Rauckhorst, F. Qian, R. L. Hoffman, C. S. Knauer, L. W. Fitzgerald, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6250–6256.

[9] C. Franchini, C. Cellucci, F. Corbo, G. Lentini, A. Scilimati, V. Tortorella, F. Stasi, *Chirality* **1994**, *6*, 590–595.

[10] M. Acs, T. Szili, E. Fogassy, *Tetrahedron Lett.* **1991**, *32*, 7325–7328.

[11] R. Aav, O. Parve, T. Pehk, A. Claesson, I. Martin, *Tetrahedron: Asymmetry* **1999**, *10*, 3033–3038.

[12] S. Buchholz, H. Gröger, in: *Biocatalysis in the Pharmaceutical and Biotechnology Industries*, (Ed.: R. N. Patel), CRC Press, Boca Raton, **2007**, pp 829–847.

[13] M.-J. Kim, Y. Ahn, J. Park, in: *Biocatalysis in the Pharmaceutical and Biotechnology Industries*, (Ed.: R. N. Patel), CRC Press, Boca Raton, **2007**, pp 249–272.

[14] J. Ogawa, S. Shimizu, H. Yamada, *Bioorg. Med. Chem.* **1994**, *2*, 429–432.

[15] B. Martin-Matute, J.-E. Bäckvall, *Curr. Opin. Chem. Biol.* **2007**, *11*, 226–232.

[16] J. González-Sabín, V. Gotor, F. Rebolledo, *Tetrahedron: Asymmetry* **2002**, *13*, 1315–1320.

[17] V. Gotor-Fernández, E. Busto, V. Gotor, *Adv. Synth. Catal.* **2006**, *348*, 797–812.

[18] J. Ager, T. Li, D. P. Pantaleone, R. F. Senkpeil, P. P. Taylor, I. G. Fotheringham, *J. Mol. Catal. B: Enzym.* **2001**, *11*, 199–205.

[19] J. D. Stewart, *Curr. Opin. Chem. Biol.* **2001**, *5*, 120–129.

[20] S.-S. Yi, C.-w. Lee, J. Kim, D. Kyung, B.-G. Kim, Y.-S. Lee, *Process Biochem.* **2007**, *42*, 895–898.

[21] B.-Y. Hwang, B.-K. Cho, H. Yun, K. Koteshwar, B.-G. Kim, *J. Mol. Catal B: Enzym.* **2005**, *37*, 47–55.

[22] U. Kaulmann, K. Smithies, M. E. B. Smith, H. C. Hailes, J. M. Ward, *Enzyme Microbiol. Technol.* **2007**, *41*, 628–637.

[23] H. Yun, S. Lim, B.-K. Cho, B.-G. Kim, *Appl. Environ. Microbiol.* **2004**, *70*, 2529–2534.

[24] A. Iwasaki, Y. Yamada, Y. Ikenaka, J. Hasegawa, *Biotechnol. Lett.* **2003**, *25*, 1843–1846.

[25] K. Nakamichi, T. Shibatani, Y. Yamamoto, T. Sato, *Appl. Microbiol. Biotechnol.* **1990**, *33*, 637–640.

[26] A. Iwasaki, Y. Yamada, N. Kizaki, Y. Ikenaka, J. Hasegawa, *Appl. Microbiol. Biotechnol.* **2006**, *69*, 499–505.

[27] M. Höhne, S. Kühl, K. Robins, U. T. Bornscheuer, *ChemBioChem.* **2008**, *9*, 363–365.

[28] C. U. Ingram, M. Bommer, M. E. B. Smith, P. A. Dalby, J. M. Ward, H. C. Hailes, G. J. Lye, *Biotechnol. Bioeng.* **2006**, *96*, 559–569.

[29] R. L. Hanson, B. L. Davis, Y. Chen, S. L. Goldberg, W. L. Parker, T. P. Tully, M. A. Montana, R. N. Patel, *Adv. Synth. Catal.* **2008**, *350*, 1367–1375.

[30] B.-K. Cho, H.-Y. Park, J.-H. Seo, J. Kim, T.-J. Kang, B.-S. Lee, B.-G. Kim, *Biotechnol. Bioeng.* **2008**, *99*, 275–284.

[31] J.-S. Shin, B.-G. Kim, D.-H. Shin, *Enzyme Microbiol. Technol.* **2001**, *29*, 232–239.

[32] K. Juhan, D. Kyung, H. Yun, B.-K. Cho, B.-G. Kim, *J. Microbiol. Biotechnol.* **2006**, *16*, 1832–1836.

[33] M. Höhne, K. Robins, U. T. Bornscheuer, *Adv. Synth. Catal.* **2008**, *350*, 807–812.
[34] J.-S. Shin, B.-G. Kim, *Biotechnol. Bioeng.* **2002**, *77*, 832–837.
[35] H. Yun, B.-Y. Hwang, J.-H. Lee, B.-G. Kim, *Appl. Environ. Microbiol.* **2005**, *71*, 4220–4224.
[36] J. S. Shin, B.-G. Kim, *Biotechnol. Bioeng.* **1997**, *55*, 348–358.
[37] B.-K. Cho, H. J. Cho, H. Yun, B.-G. Kim, *J. Mol. Catal. B: Enzym.* **2003**, *26*, 273–285.
[38] H. Yun, B.-Y. Hwang, J.-H. Lee, B.-G. Kim, *Biotechnol. Bioeng.* **2006**, *93*, 391–395.
[39] M.-X. Wang, C.-S. Liu, J.-S. Li, O. Meth-Cohn, *Tetrahedron Lett.* **2000**, *41*, 8549–8552.
[40] B. Rubin, *Struct. Biol.* **1994**, *1*, 568–572.
[41] I. Mingarro, C. Abad, L. Braco, *Proc. Natl. Acad. Sci.* **1995**, *92*, 3308–3312.
[42] E. Juaristi, *Enantioselective Synthesis of β-Amino Acids*, Wiley-VCH, New York, **1997**.
[43] K. Gademann, T. Hintermann, J. V. Schreiber, *Curr. Med. Chem.* **1999**, *6*, 905–925.
[44] Similar approaches are currently being investigated by N. Turner (personal communication, 2008).

