Tetrahedron 68 (2012) 7691–7694

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Improved chemoenzymatic asymmetric synthesis of (*S*)-Rivastigmine

Michael Fuchs, Dominik Koszelewski, Katharina Tauber, Johann Sattler, Wilfried Banko, Anja K. Holzer, Mathias Pickl, Wolfgang Kroutil, Kurt Faber *

Department of Chemistry, Organic & Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria

ARTICLE INFO

Article history:
Received 25 February 2012
Received in revised form 21 May 2012
Accepted 8 June 2012
Available online 21 June 2012

Keywords:
Rivastigmine
Transaminase
Biocatalysis
Asymmetric synthesis

ABSTRACT

(*S*)-Rivastigmine [(*S*)-**1**] was obtained via a four-step synthesis using an asymmetric enzymatic transamination protocol as the key step. An early introduction of the carbamate pharmacophore side chain avoided the use of protective group strategies and hence led to a considerable shortcut. This strategy required a novel ω-transaminase from *Paracoccus denitrificans*, which could transform the highly polar key substrate 3-acetylphenyl ethyl(methyl)carbamate (**4**) to the corresponding amine (*S*)-**5** in 99% ee and >80% conversion.

1. Introduction

(*S*)-Rivastigmine {(*S*)-3-[1-(dimethylamino)-ethyl]phenyl ethyl(methyl)carbamate (**1**)} acts as potent cholinesterase inhibitor and represents one of the most potent agents for the treatment of Alzheimer's and Parkison's disease at early stages.[1,2] However, only the (*S*)-enantiomer exhibits the desired biological activity,[2] which intrinsically requires the drug to be administered in enantiomerically pure form (Fig. 1).

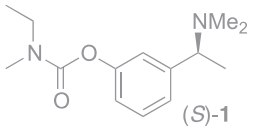


Fig. 1. (*S*)-Rivastigmine (**1**).

To date, several asymmetric methods have been developed for the preparation of enantiopure Rivastigmine (e.g., racemate resolution using chiral acids,[3] asymmetric addition of organozinc species onto imines using transition metal catalysis,[4a] diastereoselective reductive amination[4b] or lipase-catalyzed (dynamic) kinetic resolution of a hydroxy-precursor[5]). We have recently reported an asymmetric total synthesis of Rivastigmine by forming the chiral amine moiety via enzymatic amination of the corresponding ketone employing ω-transaminases (ω-TAs).[6] The latter enzymes transfer the amine functionality from an amino acid (e.g., alanine) onto a ketone. The main obstacle to overcome is the equilibrium of this reaction, which is far on the alanine/ketone side.[7] In this context, various approaches have been undertaken, mainly via removal of the formed co-product pyruvate, e.g., through reduction (using lactate dehydrogenase) or decarboxylation (catalysed by pyruvate decarboxylase).[8–10] The most attractive alternative represents a formal reductive amination, where alanine is recycled from pyruvate using an amino acid dehydrogenase together with an appropriate NADH-recycling system and ammonia as amine donor.[10,11]

2. Results and discussion

During our previous study, we were unable to identify an ω-TA, which would transform the 'ideal' ketone precursor **4** bearing the polar carbamate pharmacophor at the *m*-phenolic group at satisfying conversions of >30% (Table 1). The problem was circumvented by a cumbersome protection–deprotection strategy using a MOM-analog. However, the rapid growth of readily available ω-TAs[12,13] prompted us to search for a suitable alternative enzyme. Thus, ω-TAs from *Paracoccus dentrificans* (Pd-ωTA),[12] *Pseudomonas putida* KT2440 [Pp-ωTA_1 (Gen PP5182) and Pp-ωTA_2 (Gen PP2180)],[14] *Chromobacterium violaceum* DSM 30191 (CV-ω-TA),[15] *Bacillus megaterium* SC6394 (BM-ω-TA),[16] *Arthrobacter* sp. CNB05-01 (ArS-ω-TA)[17] and *Vibrio fluvialis* (Vf-ω-TA) as well as two commercially available ω-TAs (ATA-113, ATA-114, Codexis) were

* Corresponding author. Tel.: +43 316 380 5332; fax: +43 316 380 9840; e-mail address: Kurt.Faber@Uni-Graz.at (K. Faber).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.06.031

Table 1
Transamination of ketone **4** to (*S*)-**5**

Entry	ω-TA	Conv. [%][a]	ee [%][b]
1	BM-ω-TA[c]	4	n.d.
2	CV-ω-TA[c]	8	n.d.
3	ATA 113[d]	7	n.d.
4	ATA 114[d]	9	n.d.
5	Pp-ω-TA_1[c]	7	n.d.
6	ArS-ω-TA[c]	27	77
7	Vf-ω-TA[d]	29	99
8	Pp-ω-TA_2[c]	35	98
9	Pd-ω-TA[c]	83 (76)	99 (99)

Reaction conditions: Substrate **4** (50 mM, 11 mg, 0.05 mmol), phosphate buffer (1.0 mL, 100 mM, pH 7.0, 1 mM pyridoxal 5′-phosphate), L-alanine (250 mM, 22 mg, 0.25 mmol), ω-TA (10 mg of crude enzyme preparation, or 20 mg of lyophilized whole *E. coli* host cells containing overexpressed ω-TA), LDH mix (30 mg/mL, contains LDH, GDH, glucose, NAD^+), shaking at 30 °C and 120 rpm for 24 h.

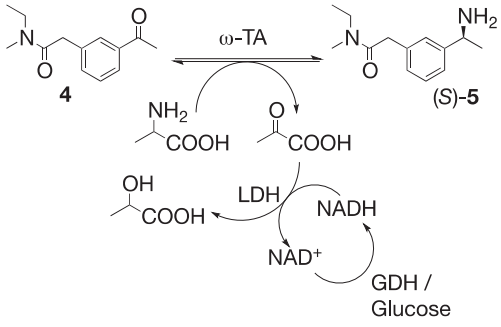
[a] Conversions were determined via GC–MS peak area integration; isolated yield and ee of prep-scale experiment are given in brackets.

[b] Ee was determined via HPLC-UV after derivatisation (for details see experimental section).

[c] Lyophilized whole cells of *E. coli* BL21(DE3) containing overexpressed ω-TA.

[d] Lyophilized crude enzyme preparation.

investigated. For shifting the equilibrium towards the desired amine **5**, the co-product pyruvate was removed via reduction to the lactate using lactate dehydrogenase in presence of glucose dehydrogenase (GDH)/glucose NADH-recycling (Scheme 1).[6,9]



ω-TA = ω-Transaminase, GDH = glucose dehydrogenase, LDH = lactate dehydrogenase

Scheme 1. Asymmetric enzymatic transamination of Rivastigmine key precursor **4**.

Most of the ω-TAs showed trace activities with substrate **4** (entries 1–5), also (*S*)-selective ω-TAs from *Arthrobacter* sp. and *V. fluvialis* gave modest, but synthetically insufficient conversions of ~30% (entries 6 and 7). The latter is presumably caused by the high polarity of the carbamate group, which is heavily hydrated in the aqueous reaction medium. On the contrary, the results from the novel ω-TAs were encouraging: Although Pp-ωTA_2 was only slightly better than Vf-ω-TA (entry 8), ω-TA from *Paracoccus denitrificans* (Pd-ωTA), which shows a remarkably high sequence identity of 94% to Vf-ω-TA, exhibited dramatically increased activities leading to >80% conversion (entry 9).

For reason of comparison, the pyruvate–lactate reduction system was exchanged with alanine dehydrogenase in combination with the formate dehydrogenase/formate NADH-recycling system, which allows the regeneration of L-alanine at the expense of cheap ammonium formate.[11] However, the conversion dropped to 29% in comparison to the LDH system.

For the total synthesis of (*S*)-Rivastigmine (**1**) the biotransformation was performed on a preparative scale, which gave amine (*S*)-**5** in 76% isolated yield and 99% e.e. Reductive amination of the latter with formaldehyde in the presence of sodium triacetoxyborohydride gave (*S*)-Rivastigmine (**1**) quantitatively, leading to an isolated overall yield of 66% over four steps (Scheme 2).

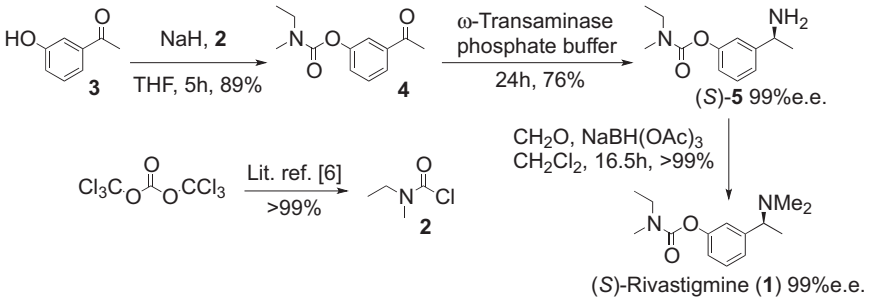
3. Conclusion

In conclusion, we have shown that ω-transaminase from *Paracoccus denitrificans* (Pd-ωTA) shows a unique ability to accept the precursor substrate **4** bearing the highly polar carbamate pharmacophore side chain of Rivastigmine. This protocol eliminates the use of protective group strategies and gives the target compound (*S*)-**1** in 66% overall yield via four steps, which (to the best of our knowledge) depicts the shortest route to enantiopure Rivastigmine reported to date.

4. Experimental

4.1. General

All chemicals were purchased from Sigma Aldrich or Acros Organics and were used as received. All solvents were purchased from Roth. Dry THF was freshly distilled from sodium/benzophenone. All moisture sensitive reactions were operated using standard Schlenk techniques with dry argon. Biocatalytic reactions and rehydration of enzymes were accomplished in a HT Infors Unitron AJ 260 shaker at 120 rpm and 30 °C (horizontal position). Centrifugation was done at 13,000 rpm in a Heraeus Biofuge pico or at 4000 rpm in a Heraeus Biofuge primo. Derivatisation of amines was performed in an Eppendorf thermomixer comfort. NMR spectra were recorded on a Bruker NMR unit at 300 (^{1}H) and 75 (^{13}C) MHz, shifts are given in parts per million and coupling constants (*J*) are given in Hertz. All GC–MS measurements were carried out with an Agilent 7890A GC system, equipped with an Agilent 5975C mass-selective detector (electron impact, 70 eV) and a HP-5-MS column (30 m×0.25 mm×0.25 μm film) using He as carrier gas at a flow of 0.55 mL/min. The following temperature program was used in all GC–MS measurements: initial temperature 100 °C, hold for 0.5 min, 10 °C/min, to 300 °C. High resolution mass spectra were recorded on a Waters Synapt HDMS Q-TOF mass spectrometer (ESI



Scheme 2. Chemoenzymatic asymmetric synthesis of (*S*)-Rivastigmine (**1**).

ion source, positive mode, capillary voltage 2.6 kV) using a syringe pump to directly infuse the sample dissolved in MeCN. Chiral HPLC analysis of (*S*)-Rivastigmine (**1**) was performed on a Shimadzu HPLC system using a modified method of Srinivasu et al.[18] Optical rotation values were measured on a Perkin Elmer Polarimeter 341. Ethyl(methyl)carbamic chloride (**2**) was prepared as described previously.[6] The following enzyme preparations were obtained from Codexis: Vf-ω-TA (020207KVP, 49 mg mL^{-1}, 7.3 U mg^{-1}), ATA-113 (102907WW, 0.46 U mg^{-1}), ATA-114 (1091108 MW, 2.7 U mg^{-1}), ATA-117 (102907WW, 1.9 U mg^{-1}), LDH mix (PRM-102, 101807KVP) and FDH (24.11, H62411.01, 220 U/mL). Lyophilized whole cells of *E. coli* containing overexpressed BM-ω-TA, CV-ω-TA and AS-ω-TA were obtained as previously reported.[19] In case of Pd-ω-TA,[12] Pp-ω-TA_1 (Gen PP5182) and Pp-ω-TA_2 (Gen PP2180) the plasmids were transformated into *E. coli* BL21(DE3) cells, which were grown according to the manufactor's manual in LB/Amp medium. Induction was done using anhydrotetracycline according to the manual of the used vector (pASK-IBA35+) as recently described.[13]

4.2. 3-Acetylphenyl ethyl(methyl)carbamate (4)

Sodium hydride (180 mg, 7.5 mmol, prewashed with *n*-pentane to remove mineral oil) was suspended in dry THF (80 mL). Under a stream of Ar 3′-hydroxyacetophenone (**3**, 234 mg, 1.7 mmol) was added and the mixture was stirred for 20 min at rt. Ethyl(methyl) carbamic chloride (**2**, 310 μL, 322 mg, 2.7 mmol) was dissolved in dry THF (20 mL) and added to the reaction mixture, which was stirred for 5 h. The reaction was quenched by addition of NaOH (1.1 M, 40 mL) and extracted with EtOAc (3x 20 mL). The combined organic phase was washed with NaOH (1.1 M, 20 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give 3-acetylphenyl ethyl(methyl)carbamate (**4**) as yellow oil (337 mg, 1.52 mmol, 89%): υ_{max} 2973, 2935, 1716, 1683, 1587, 1476, 1426, 1396, 1357, 1258, 1191, 1151 cm^{-1}; ^1H NMR (DMSO) 7.84–7.80 (m, 1H), 7.66 (bs, 1H), 7.54 (t, 1H, *J*=8.1), 7.42–7.39 (m, 1H), 3.42 (q, 1H, *J*=6.9, 1 rotamer), 3.32 (q, 1H, *J*=7.2, 1 rotamer), 3.04 (s, 1.5H, 1 rotamer), 2.91 (s, 1.5H, 1 rotamer), 2.59 (s, 3H), 1.20 (t, 1.5H, *J*=7.2), 1.11 (t, 1.5H, *J*=6.9); ^{13}C NMR (DMSO) 197.7, 153.9 (1 rotamer), 153.8 (1 rotamer), 152.0, 138.5, 130.1, 127.3 (1 rotamer), 127.3 (1 rotamer), 125.5, 121.8, 44.0, 34.4 (1 rotamer), 34.1 (1 rotamer), 27.3, 13.5 (1 rotamer), 12.7 (1 rotamer); GC–MS (EI): t_{ret} 13.02, *m*/*z* (relative intensity [%]): 221 (8), 121 (5), 95 (4), 86 (100), 58 (54); HRMS (ESI): MH^+ found 222.1120, $C_{12}H_{16}NO_3^+$ requires 222.1130.

4.3. General procedure for enzymatic transamination using the LDH/GDH system

Crude enzyme preparations (10 mg) were suspended for 5 min in sodium phosphate buffer (1 mL, 100 mM, pH 7.0) containing pyridoxal 5′-phosphate (1 mM). Whole lyophilised cells (20 mg) were rehydrated for 20 min in the same buffer. LDH-mix (30 mg, containing LDH, GDH, glucose, NAD^+), L-alanine (22 mg, 0.25 mmol) and substrate **4** (11 mg, 0.05 mmol) were added and the suspension was shaken for 24 h. Saturated K_2CO_3 solution was added (200 μL) and the aqueous layer was extracted with EtOAc (2×500 μL), denaturated enzymes were removed by centrifugation. The combined organic phase was dried over Na_2SO_4 and subjected to GC–MS-analysis.

4.4. General procedure for enzymatic transamination using the AlaDH/FDH system

Pd-ωTA (lyophilized whole cells, 20 mg) was rehydrated for 20 min in sodium phosphate buffer (1 mL, 100 mM, pH 7.0) containing pyridoxal 5′-phosphate (1 mM) and NAD^+ (1 mM). Alanine dehydrogenase (20 μL, 7.5 mg protein/mL stock solution), ammonium formate (9.5 mg, 0.15 mmol), formate dehydrogenase (20 μL, 220 U/mL stock solution), L-alanine (22 mg, 0.25 mmol) and substrate **4** (11 mg, 0.05 mmol) were added and the suspension was shaken for 24 h. Saturated K_2CO_3 solution was added (200 μL) and the aqueous layer was extracted with EtOAc (2×500 μL, denaturated enzyme was removed by centrifugation). The combined organic phase was dried over Na_2SO_4 and subjected to GC–MS-analysis.

4.5. Derivatisation of compound 5 for chiral analysis

EtOAc from the GC–MS sample was removed under a stream of air. The residue was dissolved in CH_2Cl_2 (800 μL) and Na_2SO_4 (20 mg), $NaBH(OAc)_3$ (50 mg) and formaldehyde (37% in water, 30 μL) were added and the mixture was shaken at rt for 24 h. The reaction was quenched with HCl (1 M, 100 μL), the pH was adjusted to >10 (NaOH, 10 M) and the phases were separated. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under a stream of air. The residue was dissolved in HPLC eluent (*n*-heptane/2-propanol/TFA 8/2/0.2, 1 mL) and subjected to chiral HPLC analysis.

4.6. Prep-scale synthesis of (*S*)-3-(1-aminoethyl)phenyl ethyl(methyl)carbamate [(*S*)-5]

Pd-ωTA (200 mg whole cells) was rehydrated in sodium phosphate buffer (10 mL, 100 mM, pH 7.0) containing pyridoxal 5′-phosphate (1 mM). LDH-mix (300 mg, containing LDH, GDH, glucose, NAD^+), L-alanine (220 mg, 2.5 mmol) and substrate **4** (99 mg, 0.45 mmol) were added and the suspension was shaken for 24 h. The mixture was extracted with EtOAc (2×10 mL) to remove traces of remaining starting material. The pH was adjusted to >10 with saturated K_2CO_3 solution. Subsequent extraction with EtOAc (3×10 mL), drying of the combined organic phase and concentration under reduced pressure gave (*S*)-5 (75 mg, 0.34 mmol, 76%): $[\alpha]_D^{20}$ −11.2 (c 1.0, MeOH); υ_{max} 2968, 2930, 1710, 1609, 1589, 1476, 1433, 1397, 1236, 1217, 1158 cm^{-1}; ^1H NMR (MeOD) 7.34 (t, 1H, *J*=7.8), 7.24 (d, 1H, *J*=7.5), 7.14 (t, 1H, *J*=0.9), 7.01–6.98 (m, 1H), 4.07 (q, 1H, *J*=6.9), 3.52 (q, 1H, *J*=7.2, 1 rotamer), 3.41 (q, 1H, *J*=7.2, 1 rotamer), 3.11 (s, 1.5H, 1 rotamer), 2.99 (s, 1.5H, 1 rotamer), 1.41 (s, 1.5H, 1 rotamer), 1.39 (s, 1.5H, 1 rotamer), 1.28 (t, 1.5H, *J*=6.9, 1 rotamer), 1.20 (t, 1.5H, *J*=7.2, 1 rotamer); ^{13}C NMR (MeOD) 155.0, 151.6, 148.2, 129.1, 122.7, 120.1 (1 rotamer), 120.0 (1 rotamer), 119.1 (1 rotamer), 119.0 (1 rotamer), 50.6, 43.8, 33.2 (1 rotamer), 33.0 (1 rotamer), 23.6, 12.1 (1 rotamer), 11.3 (1 rotamer); GC–MS (EI): t_{ret} 12.65, *m*/*z* (relative intensity [%]): 222 (1), 180 (5), 120 (7), 86 (100), 77 (5), 58 (60); HRMS (ESI): M-NH_2 found 206.1188, $C_{12}H_{16}NO_2^+$ requires 206.1181.

4.7. (*S*)-3-[1-(Dimethylamino)ethyl]phenyl ethyl(methyl) carbamate (Rivastigmine) [(*S*)-1]

Amine (*S*)-**4** (64 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (6 mL) and Na_2SO_4 (42 mg, 0.30 mmol), $NaBH(OAc)_3$ (504 mg, 2.4 mmol) and formaldehyde (37% in water, 88 μL, 1.0 mmol) were added and the reaction mixture was stirred at room temperature for 16.5 h. The reaction was quenched with satd K_2CO_3 solution. The phases were separated, the aqueous phase was washed with EtOAc (2×10 mL) and the combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give (*S*)-**1** (72 mg, 0.29 mmol, >99%): $[\alpha]_D^{20}$ −32.8 (c 1.0, CH_2Cl_2), (lit. −28.5, *c* 1.0, CH_2Cl_2);[5c] υ_{max} 2974, 2934, 2817, 2768, 1716, 1607, 1589, 1454, 1435, 1396, 1227, 1156 cm^{-1}; ^1H NMR (DMSO) 7.30 (t, 1H, *J*=7.8), 7.12 (d, 1H, *J*=7.8), 7.02–6.96 (m, 2H), 3.45–3.23 (m, 3H), 3.01 (s, 1.5H, 1 rotamer), 2.89 (s, 1.5H, 1 rotamer), 2.09 (s, 6H), 1.26 (s, 1.5H, 1 rotamer), 1.24 (s. 1.5H, 1 rotamer), 1.15 (t, 1.5H, *J*=6.9, 1 rotamer), 1.10

(t, 1.5H, J=7.2, 1 rotamer); ^{13}C NMR (DMSO) 154.1 (1 rotamer), 154.0 (1 rotamer), 151.7, 146.1, 129.2, 124.4, 121.0, 120.7, 64.8, 43.9, 43.0, 34.3 (1 rotamer), 34.0 (1 rotamer), 20.1, 13.6 (1 rotamer), 12.8 (1 rotamer); GC-EIMS: t_{ret} 12.65, m/z (relative intensity [%]): 2502 (5), 235 (100), 206 (2), 164 (2), 150 (5), 86 (18), 72 (35), 58 (18); HRMS (ESI): MH^+ found 251.1752, $C_{14}H_{23}N_2O_2^+$ requires 251.1760; chiral HPLC analysis {Daicel Chiracel OD-H, *n*-heptane/2-propanol/TFA 8/2/0.2, 0.8 mL/min, 25 °C, UV 215 nm, t_{ret}[(*R*)-**1**]=10.5 min, t_{ret}[(*S*)-**1**=16.0 min]}: t_{ret}=16.1 min, >99% ee.

Acknowledgements

Financial support by Evonik Degussa GmbH and the BMBF (Germany) is gratefully acknowledged. We would like to thank Klaus Zangger and his group for NMR measurements and Gerald N. Rechberger for high resultion mass spectroscopy. Jan Pfeffer (Evonik, Marl), Rolf Breinbauer (Graz) and Arne Skerra (TU Munich) are cordially thanked for ω-transaminase plasmids and for fruitful discussions.

Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2012.06.031.

References and notes

1. Emre, M. *CNS Drugs* **2006**, *20*, 748–750; Farlow, M. R.; Cummings, J. L. *Am. J. Med.* **2007**, *120*, 388–397; Pan, Y.; Xianghua, X.; Wang, X. *Br. J. Pharmacol.* **2003**, *140*, 907–912; Polinsky, R. *J. Clin. Ther.* **1998**, *20*, 634–647.
2. Enz, A., *Ger. Offen.* **1988**, DE 3805744. *Chem. Abstr.* **1989**, *111*, 187620.
3. Gaitonde, A.; Mangle, M.; Pawar, S., PCT 2005, WO 2005/061446 *Chem. Abstr.* **2005**, *143*, 77963; Garrido, M. J. V.; Montserrat, A. M.; Juárez, M. J., PCT 2007, WO2007/014973 *Chem. Abstr.* **2007**, *146*, 206113; Ma, D. W.; Pan, Q. B.; Pan, S., PCT 2007, WO 2007/025481 *Chem. Abstr.* **2007**, *146*, 295621; Stepankova, H.; Hajicek, J.; Simek, S., PCT. 2004, WO 2004/037771 A1 *Chem. Abstr.* **2004**, *142*, 6315.
4. (a) Boezio, A. A.; Pytkowicz, J.; Côté, A.; Charette, A. B. *J. Am. Chem. Soc.* **2003**, *125*, 14260–14261; (b) Hu, M.; Zhang, F.-L.; Xie, M.-H. *Synth. Commun.* **2009**, *39*, 1527–1533.
5. (a) Arunkumar, K.; Reddy, M. A.; Kumar, T. S.; Kumar, B. V.; Chandrasekhar, K. B.; Kumar, P. R.; Pal, M. *Beilstein J. Org. Chem.* **2010**, *6*, 1174–1179; (b) Han, K.; Kim, C.; Park, J.; Kim, M. J. *J. Org. Chem.* **2010**, *75*, 3105–3108; (c) Mangas-Sánchez, J.; Rodríguez-Mata, M.; Busto, E.; Gotor-Fernández, V.; Gotor, V. *J. Org. Chem.* **2009**, *74*, 5304–5310.
6. Fuchs, M.; Koszelewski, D.; Tauber, K.; Kroutil, W.; Faber, K. *Chem. Commun.* **2010**, 5500–5502.
7. Ager, D. J.; Li, T.; Pantaleone, D. P.; Senkpeil, R. F.; Taylor, P. P.; Fortheringham, I. G. *J. Mol. Catal. B* **2001**, *11*, 199–205; Hoehne, M.; Bornscheuer, U. T. *ChemCatChem* **2009**, *1*, 42–51; Koszelewski, D.; Tauber, K.; Faber, K.; Kroutil, W. *Trends Biotechnol.* **2010**, *28*, 324–332; Stewart, J. D. *Curr. Opin. Chem. Biol.* **2001**, *5*, 120–129.
8. Hoehne, M.; Robins, K.; Bornscheuer, U. T. *Adv. Synth. Catal.* **2008**, *350*, 807–812; Shin, J.-S.; Kim, B.-G. *Biotechnol. Bioeng.* **1999**, *65*, 206–211.
9. Koszelewski, D.; Lavandera, I.; Clay, D.; Rozzell, D.; Kroutil, W. *Adv. Synth. Catal.* **2008**, *350*, 2761–2766.
10. Truppo, M. D.; Rozzell, J. D.; Moore, J. C.; Turner, N. J. *Org. Biomol. Chem.* **2009**, *7*, 395–398.
11. Koszelewski, D.; Lavandera, I.; Clay, D.; Guebitz, G. M.; Rozzell, D.; Kroutil, W. *Angew. Chem., Int. Ed.* **2008**, *47*, 9337–9340.
12. Park, E.; Kim, M.; Shin, J.-S. *Adv. Synth. Catal.* **2010**, *352*, 3391–3398.
13. Mutti, F. G.; Fuchs, C.; Pressnitz, D.; Turrini, N. G.; Sattler, J. H.; Lerchner, A.; Skerra, A.; Kroutil, W. *Eur. J. Org. Chem.* **2012**, 1003–1007.
14. Sieber, V.; Grammann, K.; Rühmann, B.; Haas, T.; Pfeffer, J.; Doderer, K.; Rollmann, C.; Skerra, A.; Rausch, C.; Lerchner, A., PCT 2010, WO 2010/089171 A2 *Chem. Abstr.* **2010**, *153*, 284693.
15. Kaulmann, U.; Smithies, K.; Smith, M. E. B.; Hailes, H. C.; Ward, J. M. *Enzyme Microb. Technol.* **2007**, *41*, 628–637.
16. Hanson, R. L.; Davis, B. L.; Chen, Y.; Goldberg, S. L.; Parker, W. L.; Tully, T. P.; Montana, M. A.; Patel, R. N. *Adv. Synth. Catal.* **2008**, *350*, 1367–1375.
17. Pannuri, S.; Kamat, S. V.; Garcia, A. R. M., PCT 2006, WO 2006/063336 *Chem. Abstr.* **2006**, *145*, 42156.
18. Srinivasu, M. K.; Rao, B. M.; Reddy, B. S. S.; Kumarb, P. R.; Chandrasekhar, K. B.; Mohakhud, P. K. *J. Pharm. Biomed. Anal.* **2005**, *38*, 320–325.
19. Koszelewski, D.; Göritzer, M.; Clay, D.; Seisser, B.; Kroutil, W. *ChemCatChem* **2010**, *2*, 73–77; Koszelewski, D.; Pressnitz, D.; Clay, D.; Kroutil, W. *Org. Lett.* **2009**, *11*, 4810–4812.