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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 1221–1225

Enzyme-catalyzed preparation of methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate: a key intermediate for (*R*)-metalaxyl

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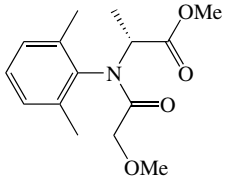
Received 3 January 2005; accepted 25 January 2005

Abstract—A biocatalytic approach for the production of (*R*)-metalaxyl, mefenoxam, has been developed. A practical synthesis of methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate, a key intermediate for (*R*)-metalaxyl, has been developed by the use of lipase-catalyzed hydrolytic kinetic resolution and chemical racemization of the remaining ester. At high concentrations in aqueous media (300 g/L) lipases were stable and gave moderate to good conversions and excellent enantioselectivities (>98% ee). A simple extraction procedure was used to separate the acid product from the remaining ester and the acid was esterified with methanol to give methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate without any reduction in enantiomeric excess (>98% ee). Subsequent chemical coupling with methoxyacetyl chloride provided enantiomerically pure (*R*)-metalaxyl (>98% ee) without racemization.

1. Introduction

A large number of chiral chemicals in the pharmaceutical sector, and increasingly also in the agrochemical and nonlife science sectors, contain at least one stereogenic center.[1] Chiral chemicals are gaining increasing attention in the context of biological activity.[2,5] This has led to the discovery of a wide range of chiral technologies, such as large-scale chiral chromatography and catalytic asymmetric reactions and resolutions, both chemical and enzymatic. Catalytic asymmetric synthesis has distinct advantages over stoichiometric reactions for economic and environmental reasons. Resolution approaches have played a central role in the production of optically active compounds despite the inherent disadvantage of a maximum yield of 50% based on a racemic starting material. Classical resolution uses stoichiometric amounts of a chiral resolving agent. Kinetic resolutions employing chiral catalysts or reagents to produce enantiomerically enriched forms must always be evaluated against any asymmetric synthesis.[1] Enzymes have been used to catalyze reactions with high chemo-, regio-, and stereoselectivity. Furthermore, enzyme-catalyzed reactions are less hazardous, polluting, and energy-intensive than conventional chemistry-based transformations. Enantioselective hydrolysis of esters and enantioselective esterification of carboxylic acids using hydrolases are among the most commonly used methods for the preparation of enantiomerically pure alcohols, acids, and esters. Among various commercial enzymes, lipases and esterases are attractive in terms of ready availability, no need of cofactors, high stability, and activity in organic media. Lipase-catalyzed hydrolytic kinetic resolutions are still popular for manufacturing chiral chemicals.[6]

Metalaxyl is a fungicide, which is effective in the control of phytopathogenic fungi and one of the few pesticides marketed in a single enantiomer formulation (as well as a racemic formulation). The biological activity resides with (*R*)-metalaxyl, mefenoxam, (***R*)-1** (Fig. 1).[2] Currently racemic metalaxyl is being replaced in many



(***R*)-1** [(*R*)-Metalaxyl]

Figure 1. Structure of (*R*)-metalaxyl.

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doi:10.1016/j.tetasy.2005.01.033

countries by metalaxyl-M, the product enriched with the (*R*)-enantiomer, although the use of generic racemic metalaxyl may continue in some countries. Metalaxyl-M typically consists of 97.5% of the (*R*)-isomer and 2.5% of (*S*)-isomer. Asymmetric catalytic hydrogenation of prochiral imine and enamide was reported to give (***R***)-**1** of 95.6% ee, which is not satisfactory in terms of enantiomeric excess.[3–5] Herein we report a practical lipase-catalyzed synthesis of optically active methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***R***)-**3**, a key intermediate for (*R*)-metalaxyl, mefenoxam, (***R***)-**1** (>98% ee).

2. Results and discussion

Readily accessible racemic methyl ester **3** was used in the preliminary enzyme screening with enzymes chosen from the collection of more than 20 hydrolytic enzymes (lipases, esterases, and proteases), which are known to hydrolyze ester bonds. The hydrolytic enzyme reactions were performed by incubating a racemic substrate (50 mg) with enzymes (25 mg) in a 0.1 M phosphate buffer (pH 7.0) at 30 °C. Reactions were stopped at intervals to check the conversion and enantiomeric excess of the remaining ester and the product acid **2**. Although under the enzyme screening the substrate was suspended in the aqueous mixture (the substrate esters are liquid), the reactions proceeded because of lipophilicity of the lipases. There was no reaction with organic solvents added. Determination of the enantiomeric excess of the remaining ester and the acid product was carried out with HPLC on a Chiralcel OD column. The reduced reaction rate after 50% conversion indicated the selectivity of the enzyme used (Table 1). Separate tests of enzyme screening with methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***R***)-**3** and methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***S***)-**3** showed that Lipase PS had preference for (***R***)-**3**.

Lipase PS was chosen from the enzyme screening for further study. Lipase PS from *Burkholderia cepacia*, one of the most frequently employed lipases in kinetic resolutions, was selected based on enantiopreference and its readily availability. Lipase PS preparation contains insoluble inorganic Celite and active proteins. Separate preparation of (***R***)-**3** and (***S***)-**3**, and reaction of them with Lipase PS showed that (***R***)-**3** was hydrolyzed much faster than (***S***)-**3** (data not shown). After enzymatic hydrolysis, both the acid product (***R***)-**2** and the remaining ester **3** [enriched as (***S***)-**3**] were extracted with ethyl acetate. These two compounds can be easily separated by partitioning in ethyl acetate or toluene/H_2O. This simple extraction/partition procedure is especially convenient and suitable for a large-scale operation. The remaining ester can be recycled by chemical racemization. Racemization was achieved through treatment with acid–butyraldehyde mixture in toluene (Scheme 1).

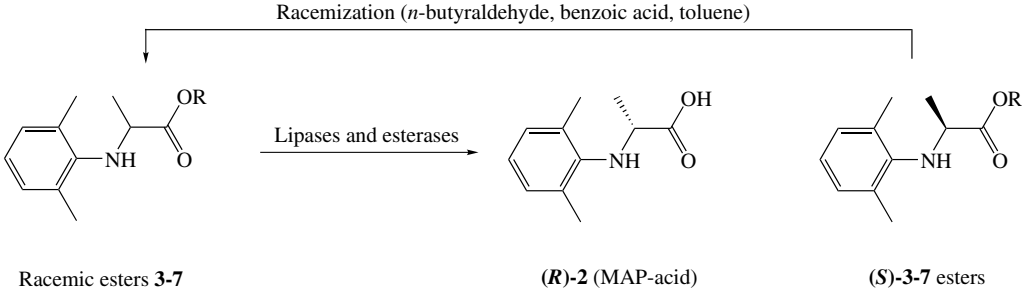
Table 1. Enzyme screening for enantioselective hydrolysis of racemic ester **3**

Enzyme	Source	Conversion (%)	
		1 day	2 days
Alcalase	*Bacillus licheniformis*	60	83
Lipase PS	*Burkholderia cepacia*	50	56
Novozym 435	*Candida antarctica*	100	—
Lipase OF	*Candida rugosa*	47	57
Lipase QLM	*Alcaligenes* sp.	60	76
Acylase Amano	*Aspergillus melleus*	51	65
Protease PS	*Bacillus* sp.	100	—
PLE-AL Amano	Porcine liver	100	—

The conversion of the ester was analyzed by HPLC on a C18 reverse phase column.

For a large-scale preparation, the activity of Lipase PS with methyl ester **3** was still too low. In some cases, the reaction rate and substrate specificity depend on the substituents by partially modifying the structure of the substrate.[7,8] We prepared various racemic esters **4**–**7**. Their syntheses were achieved by esterification of racemic acid chloride with alcohols. Under the substrate screening with Lipase PS, the reaction rates highly depend on the kind of racemic esters employed (Table 2). These interesting results indicate that an electronegative moiety can increase the reaction rates. However, ester substituents do not influence the enantiomeric excess significantly in this case. After 50% conversion, the enantiomeric excess of the acid product decreases as observed in other enzyme-catalyzed kinetic resolutions. 2-Chloroethyl ester **4**, allyl ester **5**, methoxyethyl ester **6**, and ethoxyethyl ester **7** all gave higher reaction rate than methyl ester **3**.

There are other reports in which haloesters and vinyl esters were employed as activated esters for enzymatic acylation of alcohols.[8] However, the use of activated esters for hydrolytic kinetic resolutions has been less applied. Recently various esters of acetyl-*N*-*tert*-leucine



Scheme 1. Lipase-catalyzed hydrolysis of racemic esters and chemical racemization.

Table 2. Results of Lipase PS-catalyzed hydrolysis of racemic esters **3**–**7**

Esters (R)	Conversion[a] (%)		Enantiomeric excess (ee_p)[b] (%)	
	3 h	24 h	3 h	24 h
3 CH_3	17.1	50.0	96.6	97.2
4 ClC_2H_4	48.3	52.6	97.0	90.2
5 $H_2C{=}CHCH_2$	32.5	50.5	99.6	98.0
6 $CH_3OC_2H_4$	39.6	52.1	99.0	92.0
7 $C_2H_5OC_2H_4$	48.1	51.2	98.8	95.4

[a] $c = ee_s/(ee_s + ee_p)$, where ee_s and ee_p represent the enantiomeric excess of starting ester and acid product, respectively.

[b] The enantiomeric excess of the remaining ester and acid was determined by HPLC on a Chiralcel OD column.

were compared with alcalase-catalyzed hydrolytic resolutions.[7] Faster reaction rates of acylation of racemic amines were also reported by changing the acylating agents as well.[7] We suppose the enabling effect of the methoxy-group is caused by the enhanced activity of the carbonyl-group induced by the electronegative substituent. With methoxyethyl *N*-(2,6-dimethylphenyl)-alaninate **6**, the enzymatic reaction was optimized. Even at high concentrations of **6** (300 g/L), the Lipase PS-catalyzed reaction gave enantiomerically pure (***R***)-**2** (>98% ee), and the recovery of acid product with extraction and esterification with methanol gave (***R***)-**3** (>98% ee) without racemization.

Chemical coupling of (***R***)-**3** with methoxyacetyl chloride was carried out in toluene and gave (***R***)-**1** with quantitative yield without racemization. Racemic (*RS*)-*N*-(2,6-dimethylphenyl)alaninate and their ester compounds are useful as precursors for the synthesis of benalaxyl, furalaxyl, etc. having antifungal activity with different amide moiety other than methoxyacetyl as well as metalaxyl. Hence methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***R***)-**3** can be a common intermediate of these enantiomerically enriched fungicides (Scheme 2).

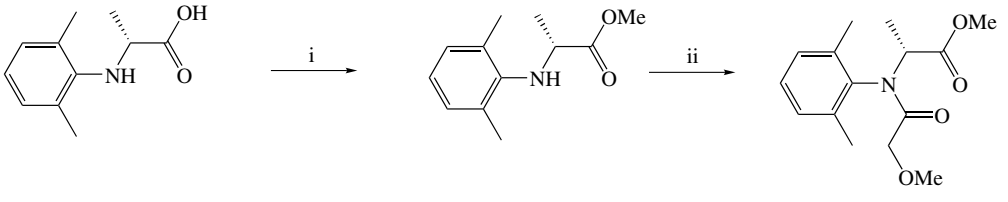
3. Conclusion

In conclusion, we have successfully developed a convenient and scalable chemoenzymatic procedure of the synthesis of enantiomeric version of metalaxyl (***R***)-**1**, taking advantage of the excellent enantioselectivity of Lipase PS and chemical racemization of the remaining ester. The kind of esters strongly influenced the reaction rate and the methoxyethyl ester was found to be the best substrate in terms of reaction rate and enantiomeric excess. This example along with recent examples of hydrolytic kinetic resolutions of drug intermediates supports the usefulness of lipases and esterases in organic synthesis.[6,9] The method is easy to perform with standard equipment and can be widely applied. We will report the immobilization of enzymes, recycling of the enzymes, and scale-up results of the process with racemic **6** in due course.

4. Experimental

4.1. General remarks

^{1}H NMR spectra were recorded on a NMR spectrometer (400 MHz) using tetramethylsilane as the internal standard. Column chromatography was conducted using silica gel 60 (230–400) mesh. All reactions were periodically monitored by TLC and worked up after the complete consumption of starting materials unless specified otherwise. GC analyses were performed on a chromatograph equipped with an FID detector using the capillary column AT-5 (Altech). Mass spectra were recorded using an electrospray (4000 V, positive mode) as ionization source. Optical rotation was measured with JASCO Digital Polarimeter at 589 nm and 24 °C. Mass spectra were recorded using an electrospray (4000 V, positive mode) as ionization source. Enzymatic reactions were performed in a 0.1 M phosphate buffer (1.0 mL, pH 7.0) with 50 mg substrate at 30 °C with an adequate amount of enzymes. HPLC was carried out on a C18 Capcell Pak column for the substrate conversion (250 × 4.6 mm, acetonitrile/water/trifluoroacetic acid = 70/30/0.1) and a Chiralcel OD column for enantiomeric excess [250 × 4.6 mm, *n*-hexane/*i*-PrOH/trifluoroacetic acid = 95/5/0.1 for acid, *n*-hexane/*i*-PrOH = 100/1 for esters, and *n*-hexane/*i*-PrOH = 30/70 for (***R***)-**1** and (***S***)-**1**] at UV 254 nm. The conversion can be also calculated from the equation $c = ee_s/(ee_s + ee_p)$, where ee_s and ee_p represent the enantiomeric excess of the starting ester and the acid product, respectively.[10] The retention times of the two pairs of enantiomers **1**, **2**, **3**, and **6** are shown in parentheses (min): (***R***)-**1** (10.6), (***S***)-**1** (4.7), (***R***)-**2** (7.7), (***S***)-**2** (9.5), (***R***)-**3** (7.9), (***S***)-**3** (8.9), (***R***)-**6** (11.2), and (***S***)-**6** (12.4).



(***R***)-**2** (MAP-acid) (***R***)-**3** (***R***)-**1** (Mefenoxam)

Scheme 2. Synthesis of mefenoxam from enzymatically-prepared acid. Reagents and conditions: (i) methanol, thionyl chloride, reflux, 3 h; (ii) $NaHCO_3$, toluene, methoxyacetyl chloride, rt, 1 h.

4.2. Procedure for the preparation of the compounds 4–7

Racemic methyl *N*-(2,6-dimethylphenyl)alaninate **3** was hydrolyzed with aqueous HCl and the acid, *N*-(2,6-dimethylphenyl)alaninate was extracted with ethyl acetate. After evaporation, thionyl chloride was added dropwise in the presence of each alcohol. *N*-(2,6-Dimethylphenyl)alaninate was esterified with each alcohol in the presence of thionyl chloride. Various esters were obtained in quantitative yield. The purity of compounds was verified with GC and MS. After work-up, the silica gel column chromatography gave each racemic ester (confirmed with GC).

4.2.1. Chloroethyl *N*-(2,6-dimethylphenyl)alaninate 4. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (d, 3H), 2.30 (s, 6H), 3.61 (m, 2H), 3.72 (br, 1H), 4.08 (q, 1H), 4.36 (m, 2H), 6.82 (m, 1H), 6.97 (m, 2H), ESIMS *m*/*z*: 256.2 $[M+H]^+$.

4.2.2. Allyl *N*-(2,6-dimethylphenyl)alaninate 5. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, 3H), 2.30 (s, 6H), 3.77 (br, 1H), 4.02 (q, 1H), 4.58 (m, 2H), 5.26 (m, 2H), 5.87 (m, 1H), 6.82 (m, 1H), 6.97 (m, 2H), ESIMS *m*/*z*: 234.2 $[M+H]^+$.

4.2.3. Methoxyethyl *N*-(2,6-dimethylphenyl)alaninate 6. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, 3H), 2.30 (s, 6H), 3.33 (s, 3H), 3.53 (m, 2H), 3.79 (br, 1H), 4.04 (q, 1H), 4.26 (m, 2H), 6.81 (m, 1H), 6.97 (m, 2H), ESIMS *m*/*z*: 252.2 $[M+H]^+$.

4.2.4. Ethoxyethyl *N*-(2,6-dimethylphenyl)alaninate 7. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, 3H), 1.40 (d, 3H), 2.30 (s, 6H), 3.50 (m, 2H), 3.57 (m, 2H), 3.78 (br, 1H), 4.06 (q, 1H), 4.24 (m, 2H), 6.81 (m, 1H), 6.97 (m, 2H), ESIMS *m*/*z*: 266.2 $[M+H]^+$.

4.2.5. (*R*)-*N*-(2,6-Dimethylphenyl)alaninate (*R*)-2. Racemic methyl *N*-(2,6-dimethylphenyl)alaninate **3** (20 g, 96.5 mmol) and Lipase PS (750 mg) were added to distilled water (30 mL). Triton X-100 (0.1 mL) was added for effective mixing. The reaction was performed at 40 °C with magnetic stirring. The pH of the reaction mixture was adjusted to pH 7.0 by adding 1 M NaOH solution with Mettler Toledo DL 70 pH stat. The progress of the reaction was monitored by HPLC. After 40% conversion, the reaction mixture was filtered and both the acid product **2** and the remaining ester **3** were extracted with ethyl acetate. The pH of the aqueous layer was adjusted to pH 9.0 by the careful addition of 1 M NaOH. Ethyl acetate was removed under reduced pressure to give the crude **3**. The pH of aqueous layer was adjusted to pH 2–3 with concentrated HCl and extracted with ethyl acetate. The layers were separated and the organic layer was dried with anhydrous $MgSO_4$. Ethyl acetate was removed in vacuo to give a pale brown solid (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***R*)-2**. (6.1 g, 32.7% yield, *R*/*S* = 98.2/1.4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (d, 3H), 2.30 (s, 6H), 4.01 (q, 1H), 6.82 (m, 1H), 6.97 (m, 2H), $[\alpha]_D^{24} = +11.7$ (*c* 1.325, C_2H_5OH), ESIMS *m*/*z*: 194.1 $[M+H]^+$.

4.2.6. Methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (*R*)-3. *N*-(2,6-Dimethylphenyl)alaninate (***R*)-2** (19.32 g, 0.1 mol, *R*/*S* = 99/1) prepared by enzymatic hydrolysis, was dissolved in methanol (58 g). Thionyl chloride (13.5 g, 0.1 mol) was added dropwise at 0 °C for 10 min and the reaction mixture refluxed for 3 h. After the reaction the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (60 mL) and washed with 5% Na_2CO_3 (30 mL × 2 times) and H_2O (20 mL). The layers were separated and the organic layer dried over anhydrous $MgSO_4$. The organic layer was filtered and concentrated in vacuo to give a pale yellow oil, methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***R*)-3** (19.3 g, 93% yield, *R*/*S* = 99/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (d, 3H), 2.30 (s, 6H), 3.67 (s, 3H), 4.01 (q, 1H), 6.82 (m, 1H), 6.97 (m, 2H), $[\alpha]_D^{24} = +32.1$ (*c* 1.08, CH_3OH), ESIMS *m*/*z*: 208.2 $[M+H]^+$.

4.2.7. Racemization of methyl *N*-(2,6-dimethylphenyl)alaninate (*S*)-3. A solution of methyl *N*-(2,6-dimethylphenyl)alaninate (***S*)-3** (15 g, 72.4 mmol, *R*/*S* = 2/98) in toluene (20 mL) was added to a mixture of *n*-butyraldehyde (5.22 g, 72.4 mmol) and benzoic acid (3.54 g, 28.9 mmol, 0.4 equiv). The mixture was refluxed for 2 h under N_2 gas. After the reaction, the mixture was cooled to 20 °C and washed with 5% Na_2CO_3 (20 mL × 3 times) and H_2O (10 mL). The layers were separated and the organic layer dried over anhydrous $MgSO_4$. The organic layer was filtered and concentrated under reduced pressure. The residue (a reddish brown oil) was distilled (1 Torr) at 110 °C to give a pale yellow oil (13.8 g, 92% yield, *R*/*S* = 50/50).

4.2.8. Methyl (*R*)-*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alaninate (*R*)-1. $NaHCO_3$ (2.43 g, 28.9 mmol, 1.2 equiv) was added to a solution of methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (***R*)-3** (5 g, 24.1 mmol, *R*/*S* = 99/1, in toluene 10 g) and the temperature reduced to 0 °C. Methoxyacetyl chloride (2.88 g, 26.5 mmol, 1.1 equiv) was slowly added at 0 °C and the mixture stirred for 1 h at room temperature. The mixture was washed with 5% Na_2CO_3 (20 mL) and H_2O (20 mL). The layers were separated and the organic layer dried over anhydrous $MgSO_4$. The organic layer was filtered and concentrated in vacuo to give a pale brown oil, methyl (*R*)-*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alaninate (***R*)-1** (6.7 g, 99% yield, *R*/*S* = 99/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (d, 3H), 2.16 (s, 3H), 2.47 (s, 3H), 3.34 (s, 3H), 3.63 (dd, 2H), 3.80 (s, 3H), 4.54 (q, 1H), 7.10–7.24 (m, 3H), $[\alpha]_D^{24} = -55.4$ (*c* 1.88, CH_3COCH_3), ESIMS *m*/*z*: 280.4 (10%) $[M+H]^+$, 302.3 (15%) $[M+Na]^+$, 581.1 (100%) $[2M+H]^+$.

Acknowledgments

We wish to thank Amano Enzymes Inc. and Meito Sangyo for kindly supplying enzyme samples for the research. We especially thank Dr. J. H. Chung, W. K. Chung, B. W. Seo, and J. H. Bang, for scientific assistance and for fruitful discussions.

References

1. (a) Federsel, H. J. *Chirality* **2003**, *15*, S128–S142; (b) Keith, J. M.; Larrow, J. F.; Jacobsen, E. N. *Adv. Synth. Catal.* **2001**, *343*, 5–26; (c) Zang, M.; Kazlauskas, R. *J. Org. Chem.* **1999**, *64*, 7498–7503; (d) Shundo, R.; Saito, S.; Okabe, E.; Saito, H. (Chisso Co., Japan) U.S. Patent 6,045,720, 2000.
2. Garrison, A. W. *Chim. Oggi* **2002**, *20*, 28–32.
3. (a) Blaser, H. U.; Malan, C.; Pugin, B.; Spindler, F.; Steiner, H.; Studer, M. *Adv. Synth. Catal.* **2003**, *345*, 103–151; (b) Spindler, F.; Blaser, H. U. *Enantiomer* **1999**, *4*, 557–568.
4. Spindler, F.; Pugin, B.; Buser, H.; Jalett, H. P.; Pittelkow, U.; Blaser, H. U. *Pestic. Sci.* **1998**, *48*, 302–304.
5. Crosby, J. *Pestic. Sci.* **1996**, *46*, 11–31.
6. Chikusa, Y.; Hirayama, Y.; Ikunaka, M.; Inoue, T.; Kamiyama, S.; Moriwaki, M.; Nishimoto, Y.; Nomoto, F.; Ogawa, K.; Ohno, T.; Otsuka, K.; Sakota, A. K.; Shirasaka, N.; Uzura, A.; Uzura, K. *Org. Process Res. Dev.* **2003**, *7*, 289–296.
7. (a) Laumen, K.; Ghisalba, O.; Auer, K. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1977–1980; (b) Balkenhohl, F.; Ditrich, K.; Hauer, B.; Ladner, W. *J. Prakt. Chem.* **1997**, *339*, 381–384.
8. (a) Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205; (b) Park, O. J.; Kim, D. Y.; Dordick, J. S. *Biotechnol. Bioeng.* **2000**, *70*, 208–216.
9. Gais, H. J.; Theil, F. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; pp 335–578.
10. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.