

Robust Enzymatic Resolution of 3-Fluoromandelic Acid with Lipase PS Supported on Celite

Javier Mendiola,* Susana García-Cerrada,* Óscar de Frutos, and María Luz de la Puente

Centro de Investigación, Lilly S.A., Avda. de la Industria, 30, Alcobendas-Madrid 28108, Spain

S Supporting Information

ABSTRACT: The resolution of different mandelic acids using the lipase PS “Amano” SD enzyme is described. By supporting the lyophilized enzyme over Celite, both the activity and the stability of lipase PS in organic media were significantly improved, enabling the robust resolution scale-up of 3-fluoromandelic acid. The methodology was extended to produce a range of optically pure (*R*)-mandelic acids, avoiding tedious extractions or chromatography.

1. INTRODUCTION

Mandelic acid and its derivatives are important building blocks in bioactive compounds.¹ They have been widely utilized as chiral pool starting material and reagents for synthetic purposes. Although an ample range of derivatives are commercially available, optically pure materials are often expensive, thus limiting their use in large- and medium-scale processes.

For that reason, in the last 20 years, a range of different approaches (Scheme 1) have been developed for the preparation of mandelic acids in enantiomerically pure form: (a) chemoselective synthesis (through the reduction of α -keto-acid derivatives),² (b) classical resolution (salt screening),³ (c) chromatography (of racemic or diastomeric derivatives),⁴ and (d) biotransformation (*via* enzymatic resolution),⁵ deracemization,⁶ and reduction with yeast.^{2b}

3-(*R*)-Fluoromandelic acids were identified in our chemistry group to cover a structure–activity relationship (SAR) study in a project under development. After an extensive bibliographic search, our group compiled a significant number of references dealing with the enzymatic resolution of mandelic derivatives.^{5–7} However, to the best of our knowledge, only two papers^{6d,7} reported the direct resolution of such α -hydroxy acids (in both cases achieved *via* lipase PS acetylation).⁷

On the basis of our findings, we envisaged the enzymatic resolution of 3-fluoromandelic acid (**2**) *via* acetylation, using lipase PS. Initially promising results were obtained at small scale (50 mg, 50% conversion, >95% ee). However, attempts to scale up the resolution to 1 g failed due to low conversion, under a range of different temperatures or enzyme/substrate ratios.

In the present contribution the development of a robust resolution of 3-fluoromandelic acid and the application of this methodology to a further nine mandelic acids using lipase PS supported over a ceramic support is described.

2. RESULTS AND DISCUSSION

The conditions reported in the literature for the resolution of 3-fluoromandelic acid failed during the scale-up studies. On the basis of our previous experience on enzymatic transformations, we designed two resolution screens (saponification and acetylation) for the resolution of 3-fluoromandelic acid with

an internal kit of 24 hydrolytic enzymes.⁸ Both processes (see Schemes 2 and 3) were carried out following our standard protocols.⁹

Saponification (from **1** to **2**) was run in an aqueous phosphate buffer at 25 °C for 24 h. HPLC/MS monitoring showed conversion close to 50% in 6 out of the 24 tests. Those hit reactions were worked up and analyzed by chiral HPLC/MS (Table 1). Only CAL-A (either lyophilized or immobilized) showed optical purity exceeding of 98% for the ester derivative; however, over-reaction (>50% of conversion) was mandatory to ensure the desired optical purity (>98% ee).

Acetylation screen (from **2** to **1**) was performed in MTBE at 25 °C for 24 h using vinyl acetate as the source of acetate (Table 2). All the reactions were monitored by HPLC/MS. Among them, five showed a conversion close to 50%, and were worked up for further chiral HPLC/MS analysis. Lipase PS “Amano” SD and lipase AK “Amano” 20 showed very promising results with optical purity ranging between 97% and 98% ee for both the ester and the alcohol derivatives. While CAL-A lyophilized enzyme showed adequate optical purity (97% ee) for the ester due to a low conversion, the immobilized CAL-A over-reacted and provided ee >98% for the hydroxyl acid. Immobilized *Thermomyces lanuginosus* was nonselective. As reported in the literature, conversion and optical purity results confirmed lipase PS “Amano” SD as the enzyme of choice for the enzymatic resolution of 3-fluoromandelic acid.

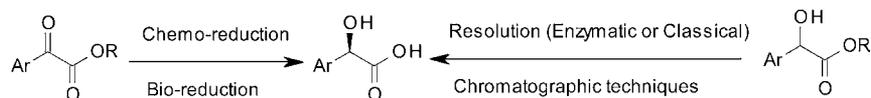
In order to improve the results of the resolution, more dilute conditions (20 vol of MTBE) and addition of a higher excess of vinyl acetate (5 equiv) were initially explored. Unexpectedly, very low conversion (20%) was observed at both 1:1 and 2:1 enzyme/substrate ratio. Furthermore, conversion could not be increased by modifying the temperature, the time of reaction, or the solvent (dichloromethane, acetonitrile, toluene).

It is well-known that enzyme activity is compromised in organic solvents or extreme conditions. However, it has been reported that the immobilized enzymes are more stable and active than the corresponding lyophilized forms. On the basis of literature references about the activity of lipases over ceramic

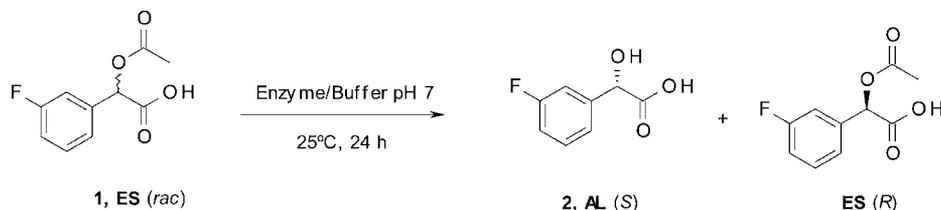
Received: May 24, 2012

Published: June 26, 2012

Scheme 1



Scheme 2



Scheme 3

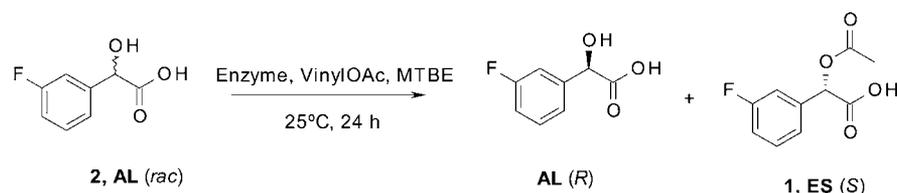


Table 1. Saponification screening for affinity enzyme/substrate for 2 (24 h)

enzyme	ES (1) (ee, %) ^a	AL (2) (ee, %)	E ^b
acylase "Amano"	34 (ES1)	51 (AL2)	4
lipase AS "Amano" SD	7 (ES2)	6 (AL1)	1
D-aminoacylase "Amano"	81 (ES2)	77 (AL1)	19
CAL-A	>98 (ES1)	81 (AL2)	49
CAL-A-IMB	>98 (ES1)	75 (AL2)	31
protease from <i>Aspergillus oyzae</i>	15 (ES1)	49 (AL2)	3

^aCalculated by chiral HPLC, see Supporting Information. ^bE value calculated according to Rackels et al. (*Enzyme Microb. Technol* 1993, 15, 1051).

Table 2. Esterification screening for affinity enzyme/substrate for 1 (24 h)

enzyme	ES (1) (ee, %) ^a	AL (2) (ee, %)	E ^b
lipase PS "Amano" SD	>98 (ES2)	>98 (AL1)	458
lipase AK "Amano" 20	97 (ES2)	>98 (AL1)	303
CAL-A	97 (ES2)	49 (AL1)	107
CAL-A-IMB	47 (ES2)	>98 (AL1)	11
<i>Thermomyces lanuginosus</i> IMB	85 (ES2)	57 (AL1)	22

^aCalculated by chiral HPLC, see Supporting Information. ^bE value calculated according to Rackels et al. (*Enzyme Microb. Technol* 1993, 15, 1051).

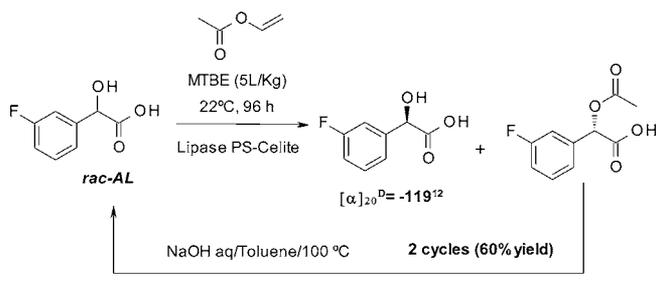
supports,¹⁰ inexpensive Celite was selected as the immobilizing support in our laboratories. A 1-to-1 w/w Celite/lipase PS "Amano" SD mixture was suspended in 1 volume of water, homogenized with mechanical stirring, and dried in an oven at 40 °C for 24 h, to provide the "Celite supported enzyme lipase PS Amano SD" (KF¹¹ analysis indicated <2% of water). Acetylation was repeated at 300-mg scale using the freshly supported immobilized enzyme. Under previous conditions (20 vol MTBE, 5 equiv vinyl acetate), 50% conversion in 72 h at 25 °C was obtained when a 2/1 lipase PS–Celite/substrate (1/1 enzyme:substrate) ratio was used. The procedure was repeated

at 20- and 200-g scale (reaction time was 96 h) to give consistent results.

Additionally, an extensive solubility screen of the products identified that the desired alcohol could be precipitated directly from the reaction mixture by the addition of dichloromethane. This finding gave an efficient and cost-effective process for the preparation of desired pure 3-(R)-fluoromandelic acid. The configuration of the product isolated was established as (R) by comparison of the specific optical rotation value with the one described in the literature.¹²

Furthermore, it was identified that 3-fluoromandelic acid could be epimerized in strong basic media. This finding was used to increase the yield of this simple and robust process. Thus, the isolated undesired (S)-acetyl derivative was hydrolyzed with 15% aq NaOH/toluene at reflux to yield a racemic mixture that could be resubjected to enzymatic resolution to provide further 3-(R)-fluoromandelic acid (Scheme 4).

Scheme 4



To evaluate the potential recycling of the supported enzyme as a further improvement to the protocol, immobilized lipase PS "Amano" SD–Celite from the previous resolution was used for a second cycle under identical reaction conditions. Analysis of the results from this test showed that the activity of the enzyme had not been impacted, from 20 g of racemic 3-fluoromandelic acid, 11.9 g (60%) of 3-(R)-fluoromandelic acid¹² was isolated after two cycles (Scheme 4).

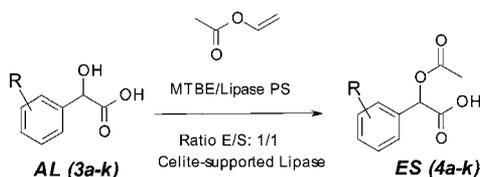
Table 3. Resolution of different mandelic acids (3) using lipase PS “Amano” supported on Celite

entry	Ar–X	ee (AL) (25 °C) (%)	ee (ES) (25 °C) (%)	E^d	ee (AL) (40 °C) (%)	ee (ES) (40 °C) (%)	E^d
1	4-Br (3a)	88	98	290	99	98	525
2	3-Cl (3b)	79	97	159	97	96	207
3	4-CF ₃ (3c)	86	98	276	99	98	525
4	3-MeO (3d)	84	93	73	97	83	45
5	4-Me (3e)	88	98	290	96	97	259
6	4-MeO (3f)	91	98	316	99	97	347
7	2-F (3g)	45	99	310	60	99	367
8	H (3h)	97	96	207	<i>a</i>	<i>a</i>	–
9	4-F (3i)	98	98	458	<i>a</i>	<i>a</i>	–
10	4-OH (3j)	<i>b</i>	<i>b</i>	–	<i>a</i>	<i>a</i>	–
11	OCH ₂ O (3k)	<i>c</i>	<i>c</i>	–	<i>a</i>	<i>a</i>	–

^aResolution was not performed. ^bThere were solubility issues. ^cDecomposition products were detected. ^d E value calculated according to Rackels et al. (*Enzyme Microb. Technol* 1993, 15, 1051).

In view of these results, Celite-supported lipase PS “Amano” SD was utilized to determine the potential scope and generality of this protocol for the resolution of other substituted mandelic acids (Table 3, Scheme 5). As observed for 3-fluoromandelic acid, the immobilized enzyme showed very high selectivity towards one enantiomer.

Scheme 5



The temperature and the reaction time were adjusted for each substrate. First, a screen at 25 °C was performed (Table 3). Two samples failed (entries 10, 11) due to solubility issues and cross reactivity and two samples (entries 8, 9) showed high selectivity and accurate conversion for a success resolution (>97% ee for hydroxyl acid, and >96% for the acetyl derivative). A second screen at 40 °C showed excellent selectivity and an accurate conversion (40–50%) for all examples except for the *o*-fluoro derivative (entry 7, 60% ee for hydroxyl acid, which performed with good ester selectivity profile (99% ee) but poor conversion). This finding confirmed the general trend that, in general, minor steric hindrance was the major influence in the reactivity with supported enzyme, and this was reflected in the need for further optimization for the resolution of the *o*-fluoro-substituted mandelic acid. Functionalization at ortho/meta/para required a reaction temperature ~40 °C, but reactivity was found as para > ortho on the basis of conversion data at 25 and 40 °C. Small substituents such as F in the para/meta position or no substitution provided good conversion at 25 °C.

3. CONCLUSIONS

A robust protocol for the enzymatic resolution of α -hydroxyl mandelic acids has been designed, developed and validated. A more stable form of the immobilized lipase PS “Amano” SD enzyme was prepared for use in organic media by simply supporting the enzyme on Celite. The stability and activity of the new supported form was successfully utilized for the resolution of a range of mandelic acids and the enzyme could be recycled. The yield of the resolution of 3-fluoromandelic

acid could be increased via chemical racemization in basic media, separation and subsequent acetylation resolution, recycling the previous supported enzyme (two separate steps). There is a reactivity trend for the enzymatic resolution of substituted mandelic acids, reactivity was found as *para* > *meta* > *ortho*.

4. EXPERIMENTAL SECTION

Enzymes and reagents. This work has been conducted with hydrolytic enzymes procured from Biocatalytics Co. (USA) (Hydrolytic enzyme screening kit, ICR-2400), from Amano Co. (Japan) and from Sigma (USA).

Analytical conditions. ¹H NMR spectra were acquired on a Bruker Avance DPX 300 MHz spectrometer.

HPLC(DAD)/MS was used for the determination of reaction conversion and enantiomeric excess of both remaining substrate and resulting product.

All analytical studies were performed on a Series 1100 Liquid Chromatography/Mass Selective Detector LC/MSD (Agilent, Waldbronn, Germany) driven by ChemStation software (Rev. A10.02, Agilent Technologies).

Sample solution of reference materials and those products yielded by enzymatic screen was prepared by dissolving 2 mg in 1 mL of a 9/1 hexane/ethanol mixture.

High Performance Liquid Chromatography coupled with Diode Array and Mass Detection (HPLC/UV(DAD)/MS) was used for the optical purity assessment of enzymatic hits (both remaining substrate and resulting product) and isolated products.

HPLC grade *n*-hexane and ethanol (EtOH) were purchased from Merck (Darmstadt, Germany) while 2-propanol (isopropanol; IPA) was supplied by LabScan (Dublin, Ireland). Spectrophotometric grade trifluoroacetic acid (TFA) was provided by Sigma-Aldrich (Steinheim, Germany). Chromatography was performed on Chiralpak AD [amylose tris (3,5-dimethyl-phenyl carbamate)] and Chiralcel OJ [cellulose tris (4-methyl benzoate)] columns from Daicel (Chiral Technologies Europe). The dimension of the analytical columns was 150 × 4.6 mm. with the enantioselective phase coated onto a 5 μ m silica-gel substrate. (Further information is compiled in the Supporting Information).

Optical rotation was measured in a Perkin-Elmer Polarimeter 343 system. Specific rotation was calculated based on the equation $[\alpha]_D^T = (100 \cdot \alpha)/(l \cdot c)$ where l is the cell path length in decimeters ($l = 1$ in our unit); c is the concentration in g/100 mL ($c = 1$ for 10 mg mL⁻¹); T is the temperature (given in

degrees Celsius) at which experiments have been run and λ is the wavelength of light used for the observation in nanometers (589 nm, the D line of a sodium lamp, in our protocol). Concentration and solvent data is included.

General procedure for the enzymatic hydrolytic screening. To 10 mg of each enzyme in a test tube, is added 0.1 mmol of substrate dissolved/suspended in 1 mL of phosphate buffer pH 7.5 0.1 M (a stock solution/suspension can be used). The mixtures are incubated with agitation at 30 °C, and the progress of the reaction is monitored by any preferred method over a 24 h period (HPLC and/or TLC), to check the conversion of the ester to the alcohol (recommended: 8 h, 16 h, 24 h). After 24 h, HCl 1N (1 mL) and MTBE (2 mL) are added to each mixture. The organic phases are collected and filtered through a 0.45 nylon filter. The solvent is removed, and the conversion is monitored by LCMS.

General procedure for supporting the enzyme over Celite. Lipase PS "Amano" SD (40 g) and Celite (40 g) in water (40 mL) were well stirred inside a plastic container until homogenization. This mixture was dried in the vacuum oven at 40 °C and 4 mbar until the quantity of water was below 2%.¹¹

General procedure for the resolution of mandelic acids. The enzyme supported over Celite (40 g) was suspended in MTBE (400 mL) and then, racemic 3-fluoromandelic acid (20 g, 0.118 mol) and vinyl acetate (54.54 mL, 0.558 mol) were added. Reaction mixture was stirred overnight at 23 °C for 96 h. The supported enzyme was filtered and washed with TBME (93 mL) and the filtrate was evaporated to dryness. The residue was stirred with DCM (10 mL) for 10 min. The white solid obtained was filtered and washed with DCM (1.5 mL). Pure 3-(R)-fluoromandelic acid was obtained (first batch). The fraction soluble in DCM was concentrated and triturated again with DCM to obtain a second batch of pure 3-(R)-fluoromandelic acid (Purity >99%, 100% ee). Total amount: 7.02 g (70.2% corrected yield, 41.4 mmol). Fraction soluble in DCM was concentrated to give a colourless oil (76.6 mmol of mixture). It was dissolved in toluene (62 mL) and 15% aq. NaOH (66 mL) was added. The mixture was heated at 100 °C for 8 h. Then, reaction was cooled to 22 °C, toluene was removed under vacuum, and 2 N aq. HCl was added until pH: 3–4. The mixture was extracted with MTBE (2 × 100 mL), washed with brine (50 mL) and dried over Na₂SO₄. It was filtered and solvent was removed under vacuum to give a white solid (13.03 g, 76.6 mmol). For the second resolution, the previous recovered supported enzyme mixture was suspended in MTBE (400 mL) and then, racemic 3-fluoromandelic acid (76.6 mmol) and vinyl acetate (37.44 mL, 0.383 mol) were added. Reaction mixture was stirred overnight at 23 °C for 96 h. The supported enzyme was filtered and washed with MTBE (50 mL) and the filtrate was evaporated to dryness. The residue was stirred with DCM (6 mL) for 10 min. The white solid obtained was filtered and washed with DCM (1 mL). Pure 3-(R)-fluoromandelic acid was obtained (first batch). The soluble fraction in DCM was concentrated and triturated again with DCM to obtain a second batch of pure 3-(R)-fluoromandelic acid (Purity >99%, 100% ee). Total amount: 4.89 g (75.0% corrected yield, 28.7 mmol). Combining the entire processes, after a first enzymatic resolution, subsequent epimerization, and final second enzymatic resolution, 11.91 g (60% overall yield, 79% corrected yield, 70.0 mmol) of 3-(R)-fluoromandelic acid¹² was isolated as a white solid. ¹H NMR (DMSO-*d*₆, 300 MHz): 12.55 (br, 1H), 7.43–7.36 (m, 1H),

7.27–7.19 (m, 2H), 7.15–7.09 (m, 1H), 6.14 (br, 1H), 5.07 (s, 1H).

■ ASSOCIATED CONTENT

📄 Supporting Information

Table 1 - retention time (in minutes) of the isomers of each racemic alcohol (1, 3a–g) and its corresponding racemic acetyl derivative (2, 4a–g); Table 1 - results from the optical purity assessment in (1, 2, 3a–g, 4a–g). This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*mendiola_javier@lilly.com (J.M.); susana.garcia@lilly.com (S.G.-C.)

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Lorraine Murtagh for her assessment and helpful discussion, Dr. Carlos Jaramillo, Dr. Marta Cifuentes, and Dr. Cristina García Paredes for their continuous support, and Alcalá Pilot Plant for confirming the robustness of the enzymatic resolution at 200-g scale.

■ REFERENCES

- (1) (a) Patel, R. N. *Stereoselective Biocatalysis for Synthesis of Some Chiral Pharmaceutical Intermediates*. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, Basel; 2000; pp 87–130 (b) Patel, R. N. *Curr. Org. Chem.* **2006**, *10*, 1289–1321.
- (2) (a) Aizpurua, J. M.; Palomo, C.; Fratila, R. M.; Ferrón, P.; Miranda, J. I. *Tetrahedron* **2010**, *66*, 3187–3194. (b) Zeror, S.; Collin, J.; Fiaud, J.-C.; Zouioueche, L. A. *Tetrahedron: Asymmetry* **2010**, *21*, 1211–1215.
- (3) (a) Guo, H.-S.; Kim, J.-M.; Pham, X.-H.; Chang, S.-M.; Kim, W.-S. *Cryst. Growth Des.* **2011**, *11*, 53–58. (b) Sakai, K.; Sakurai, R.; Nohira, H. *Novel Opt. Resolution Technol.* **2007**, *269*, 199–231. (c) Martin, A.; Cocero, M. J. *J. Supercrit. Fluids* **2007**, *40*, 67–73. (d) Marchand, P.; Lefebvre, L.; Querniard, F.; Cardinael, P.; Perez, G.; Counieux, J. J.; Coquerel, G. *Tetrahedron: Asymmetry* **2004**, *15*, 2455–2465.
- (4) Ma, C.; Xu, X.-L.; Ai, P.; Xie, S.-M.; LV, Y.-C.; Shan, H.-Q.; Yuan, L.-M. *Chirality* **2011**, *23*, 319–382.
- (5) (a) Ebert, C.; Ferluga, G.; Gardossi, L.; Gianferrara, T.; Linda, P. *Tetrahedron: Asymmetry* **1992**, *3*, 903–312. (b) Basavaiah, D.; Krishna, P. R. *Tetrahedron* **1998**, *51*, 2403–2416. (c) Itoh, T.; Nishimura, Y.; Ouchi, N.; Hayase, S. *J. Mol. Catal. B: Enzym.* **2003**, *26*, 41–45. (d) Itoh, T.; Matshusita, Y.; Abe, Y.; Han, S.-h.; Wada, S.; Hayase, S.; Kawatsura, M.; Takai, S.; Marimoto, M.; Hirose, Y. *Chem.—Eur. J.* **2006**, *12*, 9228–9237. (e) Uhm, K.-N.; Lee, S.-J.; Kim, H.-k.; Kang, H.-Y.; Lee, Y. *J. Mol. Catal. B: Enzym.* **2007**, *45*, 34–38. (f) Cabrera, Z.; Fernandez-Lorente, G.; Fernandez-Lafuente, R.; Palomo, J. M.; Guisan, J. M. *J. Mol. Catal. B: Enzym.* **2009**, *57*, 171–176. (g) Mishra, M. K.; Kumaraguru, T.; Sheelu, G.; Fadnavis, N. W. *Tetrahedron: Asymmetry* **2009**, *20*, 2854–2860. (h) Ju, X.; Yu, H.-L.; Pan, J.; Wei, D.-Z.; Xu, J.-H. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 83–91.
- (6) (a) Vanttinen, E.; Kanerva, T. *Tetrahedron: Asymmetry* **1995**, *6*, 1779–1786. (b) Strauss, U. T.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 4079–4081. (c) Fernando, F. F.; Laxmi, Y. R. S.; Bäckvall, J.-E. *Org. Lett.* **2000**, *2*, 1037–1040. (d) Kaftzik, N.; Kroutil, W.; Faber, K.; Kragl, U. *J. Mol. Catal. A: Chem.* **2004**, *214*, 107–112.
- (7) Campbell, R. F.; Fitzpatrick, K.; Inghardt, T.; Karlsson, O.; Nilsson, K.; Reilly, J. E.; Yet, L. *Tetrahedron* **2003**, *44*, 5477–5481.
- (8) Acylase "Amano", lipase AS "Amano", protease N "Amano", lipase AYS "Amano", protease S "Amano", lipase PS "Amano" SD, lipase AK "Amano" 20, D-aminoacylase "Amano", ICR-106 (*Candida*

rugosa), ICR-110 (CAL-B), ICR-112 (CAL-A), ICR-115 (*Thermomyces lanuginosus*), ICR-116 (*Mucor miehei*), NZL-101-IMB (CAL-A IMB), IMB-102 (CAL-B IMB), NZL-104-IMB (*T. lanuginosus* IMB), NZL-103-IMB (*Rhizomucor miehei* IMB), protease from *Bacillus licheniformis*, protease from *Aspergillus oryzae*, protease from *Bacillus* sp., protease from *Bacillus amyloliquefaciens*, esterase from porcine liver, lipase type II from porcine pancreas, CAL-B-IMB (Sprin Tech).

(9) (a) Mendiola, J.; García-Cerrada, S.; de Frutos, O.; de la Puente, M. L.; Gu, R. L.; Khau, V. V. *Org. Process Res. Dev.* **2009**, *13*, 292–296.

(10) (a) Lee, K.-T.; Akoh, C. C. *Biotech. Techniques* **1998**, *12*, 381–384. (b) Yamano, T.; Kikumoto, F.; Yamamoto, S.; Miwa, K.; Kawada, M.; Ito, T.; Ikemoto, T.; Tomimatsu, K.; Mizuno, Y. *Chem. Lett.* **2000**, *29*, 448–449. (c) Queiroz, N.; Nacimento, M. G. *Tetrahedron Lett.* **2002**, *43*, 5225–5227. (d) Kamori, M.; Yamashita, Y.; Naoshima, Y. *Chirality* **2002**, *14*, 558–561. (e) Palomo, J. M.; Muñoz, G.; Fernandez-Lorente, G.; Mateo, C.; Fuentes, M.; Guisan, J. M.; Fernandez-Lafuente, R. *J. Mol. Catal. B: Enzym.* **2003**, *21*, 201–210. (f) Segura, R. L.; Palomo, J. M.; Mateo, C.; Cortes, A.; Terreni, M.; Fernandez-Lafuente, R.; Guisan, J. M. *Biotechnol. Prog.* **2004**, *20*, 825–829. (g) Palomo, J. M.; Fernandez-Orente, G.; Guisan, J. M.; Fernandez-Lafuente, R. *Adv. Synth. Catal.* **2007**, *349*, 1119–1127.

(11) KF: Karl Fischer titration for water determination.

(12) Colon, D. F.; Pickard, S. T.; Smith, H. E. *J. Org. Chem.* **1991**, *56*, 2322–2326.