Enantioselective enzymatic aminolysis of a racemic 2-isoxazolylacetate alkyl ester

Amy E. Sigmund, Kenneth C. McNulty, Dzuy Nguyen, Charlotte E. Silverman, Philip Ma, Jaan A. Pesti, and Robert DiCosimo

Abstract: *Pseudomonas cepacia* lipase or *Candida antartica* lipase B catalyzes the enantioselective aminolysis of a racemic 2-isoxazolylacetate alkyl ester, isobutyl 2-[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetate, by 3-amino-*N*-(butoxycarbonyl)-L-alanine methyl ester, mono(4-methylbenzenesulfonate) to produce the corresponding amide, (*R*)-methyl-3-[[[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine, which is an intermediate in the preparation of an isoxazoline-based platelet glycoprotein IIb/IIIa antagonist.

Key words: enantioselective aminolysis, *Pseudomonas cepacia* lipase, *Candida antartica* lipase B.

Résumé : La lipase de *Pseudomonas cepacia* ou la lipase B de *Candida antartica* catalyse l'aminolyse énantiosélective d'un ester 2-isoxazolylylacétate d'alkyle, le 2-[3-(4-cyanophényl)-4,5-dihydro-5-isoxazolyl]acétate d'isobutyle, par le mono(4-méthylbenzènesulfonate) de l'ester méthylique du 3-amino-*N*-(butoxycarbonyl)-L-alanine pour conduire à la formation de l'amide correspondant, la (*R*)-méthyl-3-[[[3-(4-cyanophényl)-4,5-dihydro-5-isoxazolyl]acétyl]amino]-*N*-(butoxycarbonyl)-L-alanine, un intermédiaire dans la préparation d'un antagoniste IIb/IIIa glycoprotéinique des plaquettes à base d'isoxazoline.

Mots clés : aminolyse énantiosélective, lipase de *Pseudomonas cepacia*, lipase B de *Candida antartica*.

[Traduit par la Rédaction]

Introduction

A variety of enzymes catalyze the enantioselective ammoniolysis or aminolysis of racemic esters. Several lipases have been used for the enantioselective ammoniolysis of free amino acid esters (1), and *Candida antartica* lipase B (CAL B) catalyzed the kinetic resolution of a racemic ester via ammoniolysis (2). D-Aminopeptidase (3), thiolsubtilisin (4), and papain (5) each catalyzed the aminolysis of α-amino acid esters to produce the corresponding chiral α-amino acid amides.

The lipase-catalyzed enantioselective aminolysis of racemic esters to produce chiral amides has been reviewed (6). Aminolysis of racemic 2-methyloctanoate by (*R*)-1-phenylethylamine in the presence of CAL B produced (*R*)-2-methyloctanoic-(*R*)-1-phenylethylamide in 30–40% diastereomeric excess (de) at ca. 100% conversion (7). Resolution of chiral amines by reaction with an isoalkyl ester in the presence of CAL B produced the corresponding chiral amides in high enantiomeric excess (8). A systematic study of the enzymatic aminolysis of racemic 2-methyl butyrate by benzylamine found that CAL B and the lipases from *Rhizopus niveus* and porcine pancreas were highly enantioselective (84–87% ee), while *Candida rugosa* and *Pseudomonas cepacia* lipases were neither highly active nor enantioselective (15% ee and 12% ee, respectively) (9). CAL B (Novo SP435) catalyzed the enantioselective aminolysis of racemic 3-hydroxyesters (10), or racemic 2-alkylesters or 2-haloesters (11), by aliphatic amines, where the degree of enantioselectivity was dependent on the substrate and nucleophile. For the enzymatic aminolysis of dimethyl 3-hydroxyglutarate by aliphatic and aromatic amines (12), CAL B yielded enantiopure monoamidation products in very high yield, while no amidation products were observed in the presence of lipases from *Candida cylindracea* and *P. cepacia* under identical reaction conditions.

An enantioselective enzymatic aminolysis of a racemic 2-isoxazolylacetate alkyl ester has now been employed to produce an intermediate in the preparation of roxifiban ((*R*)-methyl-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine monoacetate (Scheme 1)), a non-peptide platelet glycoprotein IIb/IIIa antagonist that has antithrombotic activity (13, 14). The aminolysis reaction produces the desired product with

Received 16 October 2001. Published on the NRC Research Press Web site at http://canjchem.nrc.ca on 10 April 2002.

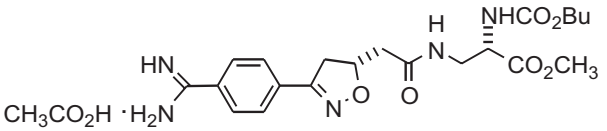
Dedicated to Professor J. Bryan Jones on the occasion of his 65th birthday.

A.E. Sigmund and R. DiCosimo.[1] DuPont Central Research and Development, Experimental Station, P.O. Box 80328, Wilmington, DE 19880-0328, U.S.A.
K.C. McNulty, D. Nguyen, C.E. Silverman, P. Ma, and J.A. Pesti. Chemical Process Research and Development, Bristol-Meyers Squibb Co., Chambers Works, Deepwater, NJ 08023, U.S.A.

[1]Corresponding author (e-mail: Robert.DiCosimo@usa.dupont.com).

 DOI: 10.1139/V02-004

Scheme 1.



Roxifiban

high selectivity and de, despite the presence of a second alkyl ester functionality on both the reacting amine and the product amide.

Results and discussion

The 2-isoxazolylacetate alkyl ester intermediate (*R*)-methyl-3-[[[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine ((*R*,*S*)-**1**) is currently synthesized in two steps (Scheme 2) (13*b*). Racemic isobutyl 2-[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetate ((*R*/*S*)-**2**) is first converted into the chiral acid (*R*)-2-[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetic acid ((*R*)-**3**) in an aqueous reaction mixture using *P. cepacia* (formerly *Pseudomonas sp.)* lipase (15), leaving the chiral isobutyl ester (*S*)-**2** unreacted. After isolation, (*R*)-**3** is reacted with thionyl chloride and diamine (*S*)-**4** (3-amino- *N*-(butoxycarbonyl)-L-alanine methyl ester, mono(4-methylbenzenesulfonate)), followed by the addition of diisopropylethylamine (DIEA) to produce (*R*,*S*)-**1**. In a separate step, recovered (*S*)-**2** is racemized for recycle by a catalytic amount of potassium *tert*-butoxide in toluene. This sequence of reactions provides (*R*)-**3** in 70% yield (after one recycle) from racemate (*R*/*S*)-**2**, and (*R*)-**3** is subsequently converted to (*R*,*S*)-**1** in 82% yield.

As an alternative to the two-step procedure described above, the lipase-catalyzed aminolysis of racemic (*R*/*S*)-**2** by diamine (*S*)-**4** to directly produce (*R*,*S*)-**1** was examined (Scheme 3). Several potential by-product-forming reactions which could have resulted in yield loss or a decrease in de of (*R*,*S*)-**1** included: (*i*) nonenzymatic aminolysis of (*R*/*S*)-**2** by (*S*)-**4**; (*ii*) enzymatic hydrolysis of (*R*/*S*)-**2** to (*R*)-**3** (if water is present in the organic solvent); (*iii*) enzymatic hydrolysis of the product (*R*,*S*)-**1** (if water is present in the organic solvent); (*iv*) enzyme-catalyzed aminolysis of (*S*)-**4** by an additional equivalent of (*S*)-**4**; or (*v*) enzymatic or nonenzymatic aminolysis of the product (*R*,*S*)-**1** to produce an aminated by-product. Regioselective aminolysis of non-chiral diesters catalyzed by PS30 lipase has been reported, where regioselectivity was attributed to a preference for aminolysis of a β-alkoxy ester relative to the remaining ester functionality (16).

The free amine of (*S*)-**4** was unstable, and readily dimerized and trimerized in both aqueous solution and in organic solvents in the absence of lipase. As a *p*-toluenesulfonate salt, (*S*)-**4** was stable in organic solvents, and it was first demonstrated that in the presence or absence of *P. cepacia* lipase (Amano PS30) or CAL B there was no significant change in concentration of (*S*)-**4** in diisopropyl ether (DIPE), methyl-*tert*-butyl ether (MTBE), heptane, dioxane, or toluene after 24 h at 25°C. In aqueous solution, a significant (>75%) decomposition of the *p*-toluenesulfonate salt of (*S*)-**4** occurred at 25°C in 0.40 M sodium phosphate buffer (pH 8.0) after 24 h, whereas the salt was stable for at least 18 h in aqueous 5 mM methanesulfonic acid (pH 2.5).

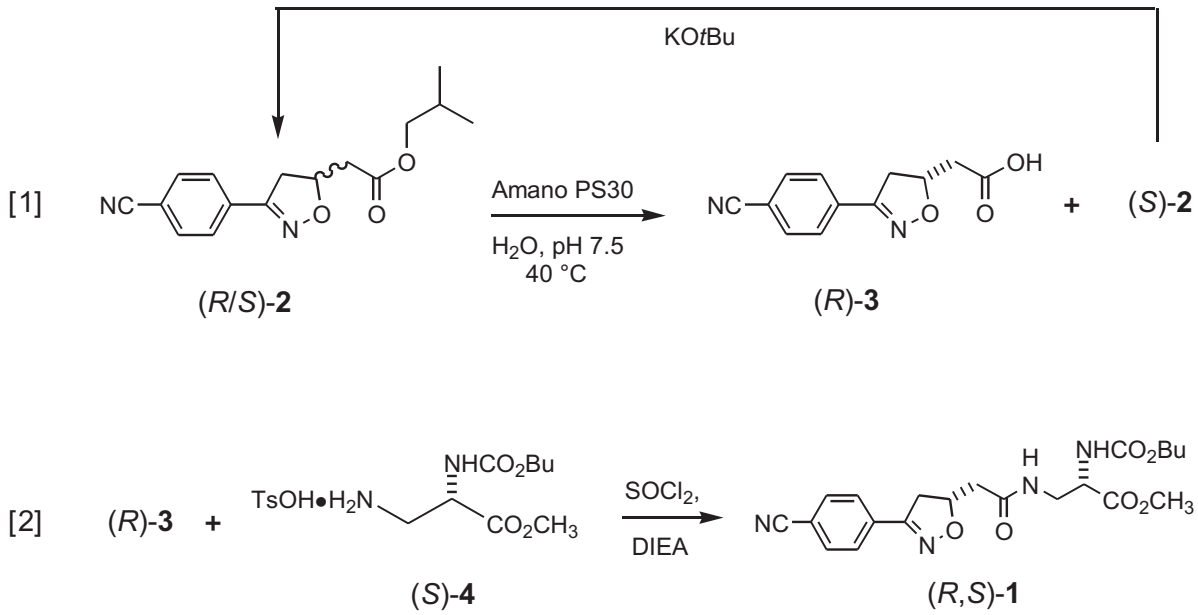
Prior to use in aminolysis reactions, a solution of 30–60 mg mL^{-1} of lipase in 0.10 M sodium phosphate buffer was adjusted to pH 8.0, and then lyophilized to obtain a "dry" enzyme catalyst. This catalyst was subsequently used in aminolysis reactions to produce (*R*,*S*)-**1**, where 52 mM (*R*/*S*)-**2** and from 37.5 to 100 mM (*S*)-**4** were reacted with the dried lipase in DIPE, MTBE, heptane, toluene, or acetonitrile, at temperatures of from 24– 40°C. Initially, the resulting product mixtures contained significant amounts of the ester hydrolysis product (*R*)-**3** as the sole reaction by-product, indicating that water was available in the reaction mixtures, possibly originating from the solvent, enzyme preparation, and (or) starting materials. None of the other potential reaction by-products described above occurred in significant amounts (<1%). Drying the solvents over activated molecular sieves prior to use improved selectivity to (*R*,*S*)-**1** over (*R*)-**3**, and the inclusion of 10–35 mg mL^{-1} of powdered, activated molecular sieves in the reaction mixture (17) further improved selectivity to (*R*,*S*)-**1**.

Table 1 lists the conversion of (*R*/*S*)-**2**, selectivity to (*R*,*S*)-**1** (vs. hydrolysis to produce (*R*)-**3**), and the corresponding de of (*R*,*S*)-**1** (where there was significant conversion) obtained using lyophilized PS30 lipase in dry DIPE, MTBE, heptane, toluene, or acetonitrile. There was no significant conversion of (*R*/*S*)-**2** in the absence of lipase under the reported reaction conditions. With lipase present, there was very little conversion of starting material in toluene or acetonitrile relative to the other solvents, and the reaction rate was considerably slower in heptane relative to MTBE or DIPE. The solubilities of (*R*/*S*)-**2**, (*R*,*S*)-**1**, and reaction by-products were low in all of these solvents, and reactions were run as heterogeneous mixtures (18). Following the progress of a reaction by sampling the heterogeneous mixture over time gave inconsistent results, so measurements of selectivity and de vs. conversion of (*R*/*S*)-**2** were performed by setting up a series of identical reactions, then working up individual reactions for analysis at predetermined times.

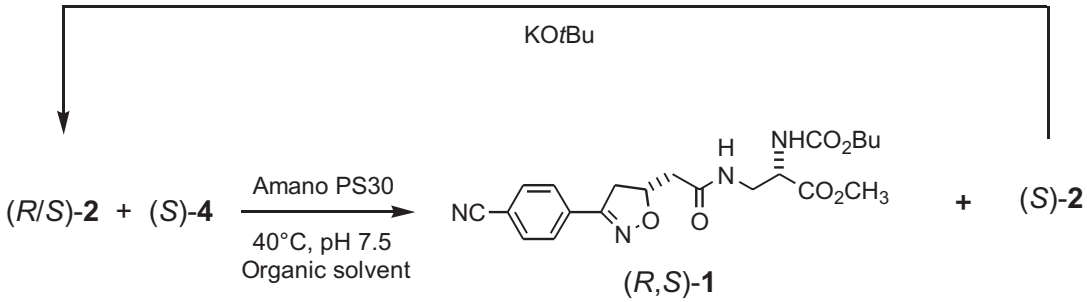
The de and selectivity for production of (*R*,*S*)-**1** at conversions of (*R*/*S*)-**2** between 20 and 55% in either DIPE or MTBE are depicted in Fig. 1. The selectivity to (*R*,*S*)-**1** in DIPE averaged ca. 95% at conversions of up to 45%, whereas in MTBE the selectivity was only ca. 90% at conversions of up to 52%. The de of (*R*,*S*)-**1** at a given conversion was similar in either solvent; for MTBE, the de decreased from 86 to 78% as conversion increased from 27 to 46%, and in DIPE, the de decreased from 86 to 74% as the conversion increased from 23 to 44%.

CAL B (19) also produced the desired diastereomer (*R*,*S*)-**1** in high de (Table 2), while *C. antartica* lipase A (CAL A) had the opposite enantioselectivity for (*R*/*S*)-**2** (producing (*S*,*S*)-**1** instead of (*R*,*S*)-**1**), demonstrating that not all enzymes capable of catalyzing the aminolysis of (*R*,*S*)-**1** by (*S*)-**4** produce the desired diastereomer. Although the de obtained using CAL B was moderately higher than that obtained with PS30 lipase in the same solvent (ca. 8% higher de at 35% conversion), the selectivity to the aminolysis product relative to hydrolysis product was significantly lower (81 vs. 89% at 35% conversion); all attempts to improve selectivity by removal of additional water from the

Scheme 2.



Scheme 3.



individual components of the reaction mixture, or from the reaction mixture itself, were unsuccessful. *Pseudomonas fluorescens* lipase (Fluka or Amano AK) gave no significant conversion of (*R*/*S*)-**2** to (*R*,*S*)-**1** under these same reaction conditions.

The PS30 or CAL B lipase-catalyzed aminolysis of racemic (*R*/*S*)-**2** by (*S*)-**4** to directly produce (*R*,*S*)-**1** has several advantages when compared to the two-step, resolution and aminolysis method. The reaction proceeds with significant enantioselectivity, reduces the number of process steps, eliminates several costly reagents, generates less waste, and permits facile product recovery. The heterogeneous product mixture can be diluted with additional solvent, which will dissolve (*R*,*S*)-**1** and unreacted (*R*)-**2** and (*S*)-**2**, and leave unreacted (*S*)-**4** (as the *p*-toluenesulfonate salt), molecular sieves, and the enzyme as filterable solids. The product (*R*,*S*)-**1** can then be isolated by column chromatography, selective precipitation, or fractional crystallization. Unreacted (*R*)-**2** and (*S*)-**2** can be recovered and racemized (13*b*) and this racemate recycled in a second enzymatic aminolysis reaction, thereby increasing the overall yield of (*R*,*S*)-**1** produced, and decreasing the amount of waste. (*R*,*S*)-**1** was produced in 78– 86% de, which corresponds to a yield of the desired diastereomer of 89–93%, respectively, (based on total (*R*,*S*)-**1** and (*S*,*S*)-**1** produced); the subsequent separation of these two diastereomers is readily performed by recrystallization, and produces the desired diastereomer in very high purity. Further improvement in de might be obtained by examining the dependence of enantioselectivity on the choice of alkyl ester substituent of (*R*/*S*)-**2**.

Experimental section

Starting materials ((*R*/*S*)-**2** and (*S*)-**4**) and products ((*R*,*S*)-**1**, (*S*,*S*)-**1**, (*S*)-**2**, (*R*)-**3**, and (*S*)-**3**) for the enzymatic aminolysis reactions were prepared and characterized according to published procedures (13*b*), or obtained from commercial sources. Enzymes were obtained from Amano, Sigma, Fluka, or Roche Molecular Biochemicals, and were used as received without further purification. Relative specific activities of commercial enzyme preparations were determined by measuring the rate of hydrolysis of *p*-nitrophenyl acetate (PNPA) (20).

HPLC analysis of reaction components

Analysis of mixtures of (*R*/*S*)-**2**, (*R*,*S*)-**1**, and (*S*,*S*)-**1**, and (*R*)-**3** and (*S*)-**3** (without separation of enantiomers or diastereomers) were performed by HPLC using UV detection at 260 nm on an Eclipse XDB-C8 column (4.6 ×

Table 1. Enantioselective amination of (*R*/*S*)-**2** by (*S*)-**4** using *P. cepacia* lipase in different organic solvents.

Solvent	Time (h)	(*R*/*S*)-**2** Conversion (%)	(*R*,*S*)-**1**, (*S*,*S*)-**1** Selectivity (%)[a]	(*R*,*S*)-**1**:(*S*,*S*)-**1**	de (*R*,*S*)-**1** (%)
DIPE[b]	24	23	95	93:7	86
MTBE[c]	24	24	95	91:9	82
Heptane[b]	72	24	90	86:14	72
Toluene[b]	72	4	—	—	—
Acetonitrile[b]	72	3	—	—	—

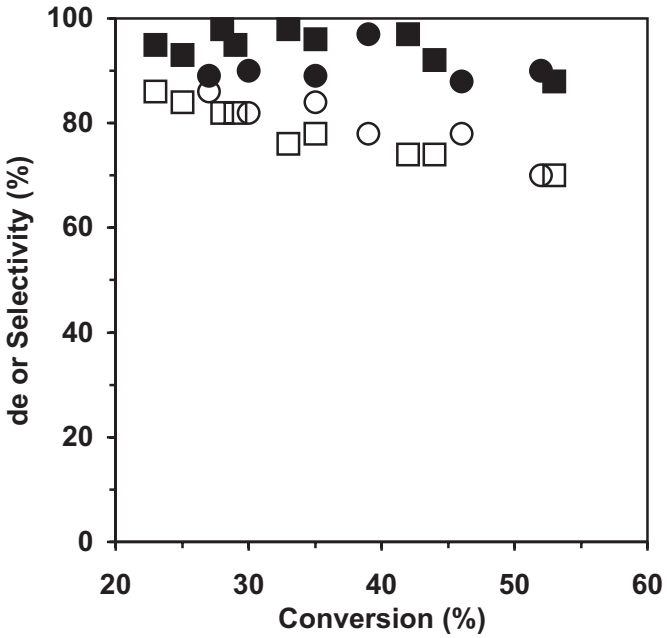
[a]Selectivity = 100([(*R*,*S*)-**1** + (*S*,*S*)-**1**]/[(*R*,*S*)-**1** + (*S*,*S*)-**1** + (*R*)-**3** + (*S*)-**3**]).
[b]Reaction conditions: 40°C, 3 Å sieves (20 mg mL^{-1}), 30 mg lyophilized PS30 mL^{-1}.
[c]Reaction conditions: 24°C, 3 Å sieves (25 mg mL^{-1}), 30 mg lyophilized PS30 mL^{-1}.

Table 2. Enantioselective amination of (*R*/*S*)-**2** by (*S*)-**4** in MTBE using *C. antartica* lipase A or B (CAL A or CAL B).

Enzyme	(*R*/*S*)-**2** Conversion (%)	(*R*,*S*)-**1**, (*S*,*S*)-**1** Selectivity (%)	(*R*,*S*)-**1**:(*S*,*S*)-**1**	de (*R*,*S*)-**1** (%)	de (*S*,*S*)-**1** (%)
CAL A	35	86	12:88	—	76
CAL B	31	81	96:4	92	—

Note: Reaction conditions: 24 h, 24°C, 3 Å sieves (20 mg mL^{-1}), 30 mg lyophilized CAL A or CAL B mL^{-1}.

Fig. 1. Selectivity and de for the conversion of (*R*/*S*)-**2** to (*R*,*S*)-**1** using *P. cepacia* lipase (PS30) in DIPE or MTBE: selectivity (DIPE (■), MTBE (●)); de (DIPE (□), MTBE (○)). Reaction conditions: (*a*) DIPE, 40°C, 3 Å sieves (20 mg mL^{-1}), 30 mg lyophilized PS30 mL^{-1}; (*b*) MTBE, 40°C, 3 Å sieves (20–25 mg mL^{-1}), 60 mg lyophilized PS30 mL^{-1}.



250 mm) at 40°C and a flow rate of 1.0 mL min^{-1}, using a gradient of two eluents: mobile phase A (0.1% trifluoroacetic acid – 0.1% triethylamine in distilled deionized water), mobile phase B (0.1% trifluoroacetic acid in THF (unstabilized)). The mobile phase gradient employed was: t = 0 min, 85% A, 15% B; t = 10 min, 85% A, 15% B; t = 32 min, 50% A, 50% B; t = 40 min, 50% A, 50% B; t = 42 min, 85% A, 15% B; t = 52 min, 85% A, 15% B.

Chiral HPLC analysis of (*R*)-**2** and (*S*)-**2**, and (*R*)-**3** and (*S*)-**3**, was performed using UV detection at 280 nm on a Daicel Chiralcel OJ column (4.6 × 250 mm) at 38°C and a flow rate of 0.90 mL min^{-1} of 0.25% trifluoroacetic acid in 81% hexane – 19% ethanol.

Chiral HPLC analysis of (*R*,*S*)-**1** and (*S*,*S*)-**1** was performed using UV detection at 280 nm on two Daicel Chiralcel OJ columns (4.6 × 250 mm) in series at 40°C and a flow rate of 0.75 mL min^{-1} of 80% heptane – 20% ethanol. The production of (*R*,*S*)-**1** and (*S*,*S*)-**1** as aminolysis reaction products was confirmed by preparing authentic samples of (*R*,*S*)-**1**, (*S*,*S*)-**1**, (*S*,*R*)-**1**, and (*R*,*R*)-**1**, separating all four diastereomers by chiral HPLC, and comparing product retention times with the authentic standards.

Lipase-catalyzed aminolysis of racemic isobutyl 2-[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetate ((*R*/*S*)-2) by 3-amino-*N*-(butoxycarbonyl)-L-alanine methyl ester, mono(4-methylbenzenesulfonate) ((*S*)-4)

In a typical procedure, a series of 4 mL glass vials were charged with 30–60 mg of lipase and 1.0 mL of 100 mM NaH_2PO_4 (pH 8.0, adjusted with 50% aq NaOH), and the resulting solution was frozen and lyophilized. To each vial was then added 15 mg of (*S*)-**4** (38.3 mM), 15.0 mg of (*R*,*S*)-**2** (52.3 mM), 10–35 mg of powdered, activated 3 Å molecular sieves, 1.0 mL of diisopropyl ether (dried over activated 3 Å molecular sieves), and a magnetic stir bar. The vials were capped and the reaction mixtures stirred at 24–40°C for 18–48 h.

After the desired reaction time, the individual reactions were prepared for analysis by cooling the product mixtures to 24°C and then evaporating the solvent by flowing a

stream of dry nitrogen over the product mixture. The resulting solid was mixed with: (*i*) 18.0 mL of a 1:1 (v/v) mixture of tetrahydrofuran and 0.1% (v/v) acetic acid in distilled, deionized water (adjusted to pH 2.0 with 6.0 N HCl); (*ii*) 2.0 mL of 30 mM *p*-toluamide (HPLC external standard) in a 1:1 (v/v) mixture of tetrahydrofuran and 0.1% (v/v) acetic acid in distilled, deionized water (adjusted to pH 2.0 with 6.0 N HCl); and (*iii*) 0.108 mL of 6.0 N HCl. A 1 mL sample of the resulting mixture (mixture A) was centrifuged to remove undissolved solids, and the supernatant analyzed for (*R*/*S*)-**2**, (*R*,*S*)-**1**, and (*R*/*S*)-**3** by HPLC.

A second 2.0 mL sample of mixture A was evaporated to dryness under vacuum, then 1 mL of ethanol was added to the resulting solid and the suspension heated at 50°C for 1.0 min. The resulting suspension was filtered (0.45 μm) and analyzed by chiral HPLC for (*R*)-**2** and (*S*)-**2**, (*R*,*S*)-**1** and (*S*,*S*)-**1**, and (*R*)-**3** and (*S*)-**3**.

Acknowledgment

The authors thank Michael B. D'Amore for helpful discussions regarding lipase-catalyzed reactions.

References

1. (*a*) P. Lopez-Serrano, M.A. Wegman, F. van Rantwijk, and R.A. Sheldon. Tetrahedron: Asymmetry, **12**, 235 (2001); (*b*) M.A. Wegman, M.A.P.J. Hacking, J. Rops, P. Pereira, F. Van Rantwijk, and R.A. Sheldon. Tetrahedron: Asymmetry, **10**, 1739 (1999); (*c*) M.A.P.J. Hacking, M.A. Wegman, J. Rops, F. van Rantwijk, and R.A. Sheldon. J. Mol. Catal. B: Enzym. **5**, 155 (1998).
2. E. Garcia-Urdiales, F. Rebolledo, and V. Gotor. Tetrahedron: Asymmetry, **10**, 721 (1999).
3. (*a*) Y. Kato, Y. Asano, A. Nakazawa, and K. Kondo. Tetrahedron, **45**, 5743 (1989); (*b*) Y. Asano, A. Nakazawa, Y. Kato, and K. Kondo. J. Biol. Chem. **264**, 14 233 (1989).
4. T. Nakatsuka, T. Sasaki, and E.T. Kaiser. J. Am. Chem. Soc. **109**, 3808 (1987).
5. Y.V. Mitin, N.P. Zapevalova, and E.Y. Gorbunova. Int. J. Pept. Protein Res. **23**, 528 (1984).
6. V. Gotor. Bioorg. Med. Chem. **7**, 2189 (1999).
7. C. Vörde, H.E. Högsberg, and E. Hedenström. Tetrahedron: Asymmetry, **7**, 1507 (1996).
8. C.D. Reeve. PCT Int. Appl. WO 9931264 A1 (1999).
9. M.S. de Castro and J.V.S. Gago. Tetrahedron, **54**, 2877 (1998).
10. M.J. Garcia, F. Rebolledo, and V. Gotor. Tetrahedron: Asymmetry, **4**, 2199 (1993).
11. M. Quiros, V.M. Sanchez, R. Brieva, F. Rebolledo, and V. Gotor. Tetrahedron: Asymmetry, **4**, 1105 (1993).
12. S. Puertas, F. Rebolledo, and V. Gotor. J. Org Chem. **61**, 6024 (1996).
13. (*a*) L.-H. Zhang, L. Anzalone, P. Ma, G.S. Kauffman, L. Storace, and R. Ward. Tetrahedron Lett. **37**, 4455 (1996); (*b*) L.-H. Zhang, J.C. Chung, T.D. Costello, I. Valvis, P. Ma, G.S. Kauffman, and R. Ward. J. Org. Chem. **62**, 2466 (1997).
14. (*a*) J. Wityak, T.M. Sielecki, D.J. Pinto, G. Emmett, J.Y. Sze, J. Liu, E. Tobin, S. Wang, B. Jiang, P. Ma, S.A. Mousa, R.A. Wexler, and R.E. Olson. J. Med. Chem. **40**, 50 (1997); (*b*) C.B. Xue and S.A. Mousa. Drugs Future, **23**, 707 (1998); (*c*) C.B. Xue, J. Wityak, T.M. Sielecki, D.J. Pinto, D.G. Batt, G.A. Cain, M. Sworin, A.L. Rockwell, J.J. Rodercik, S. Wang, M.J. Orwat, W.E. Frietze, L.L. Bostrom, J. Liu, C.A. Higley, F.W. Rankin, A.E. Tobin, G. Emmett, G.K. Lalka, J.Y. Sze, S.V. Di Meo, S.A. Mousa, M.J. Thoolen, A.L. Racanelli, E.A. Hausner, T.M. Reilly, W.F. DeGrado, R.R. Wexler, and R.E. Olson. J. Med. Chem. **40**, 2064 (1997).
15. S.A. Patkar, F. Bjoerking, M. Zundel, M. Schulein, A. Svendsen, H.P. Heldt-Hansen, and E. Gormsen. Indian J. Chem. Sect. B: Org. Chem. Incl. Med. Chem. **32B**, 76 (1993).
16. M. Adamczyk and J. Grote. Bioorg. Med. Chem. Lett. **9**, 245 (1999).
17. (*a*) W. Lu and C.J. Sih. Tetrahedron Lett. **40**, 4965 (1999); (*b*) S. Conde, P. Lopez-Serrano, M. Fierros, M.I. Biezma, A. Martinez, and M.I. Rodriguez-Franco. Tetrahedron, **53**, 11 745 (1997).
18. A. Wolff, V. von Asperen, A.J.J. Straathof, and J.J. Heijnen. Biotechnol. Prog. **15**, 216 (1999).
19. U. Bornscheuer, O.W. Reif, R. Lausch, R. Freitag, T. Scheper, K.N. Fragiskos, and U. Menge. Biochim. Biophys. Acta, **1201**, 55 (1994).
20. (*a*) J. Anderson, T. Byrne, K.J. Woelfel, J.E. Meany, G.T. Spyridis, and Y. Pocker. J. Chem. Ed. **71**, 715 (1994); (*b*) T. Furutani, R. Su, H. Ooshima, and J. Kato. Enzyme Microb. Technol. **17**, 1067 (1995); (*c*) J. Zhou, R.J. Ain, C.M. Riley, and R.L. Schowen. Anal. Biochem. **231**, 265 (1995).