An Asymmetric Synthesis of L-694,458, a Human Leukocyte Elastase Inhibitor, via Novel Enzyme Resolution of β-Lactam Esters

Raymond J. Cvetovich,*,† Michel Chartrain,‡ Frederick W. Hartner, Jr.,† Christopher Roberge,‡ Joseph S. Amato,† and Edward J. J. Grabowski†

Departments of Process Research and Bioprocess Research and Development, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received April 2, 1996[⊗]

A convergent synthesis of [*S*-(*R**,*S**)]-2-[4-[(4-methylpiperazin-1-yl)carbonyl]phenoxy]-3,3-diethyl-*N*-[1-[3,4-(methylenedioxy)phenyl]butyl]-4-oxo-1-azetidinecarboxamide (L-694,458, **1**), a potent human leukocyte elastase inhibitor, was achieved via chiral synthesis of key intermediates: (*S*)-3,3-diethyl-4-[4′-[(*N*-methylpiperazin-1-yl)carbonylphenoxy]-2-azetidinone (**2**) and (*R*)-α-propylpiperonyl isocyanate (**3**). Synthesis of β-lactam **2** was achieved by a novel enantioselective lipase hydrolysis of ester **5** to produce (*S*)-3,3-diethyl-4-(4′-carboxyphenoxy)-2-azetidinone (**6**) (60% yield, three cycles, 93% ee) with isolation, epimerization, and recycling of the undesired (*R*)-ester **5**. Isocyanate **3** was prepared by chiral addition of Zn(*n*-Pr)$_2$ to piperonal (98% yield, 99.2% ee), azide displacement and reduction to (*R*)-α-propylpiperonylamine (**11**) (58% yield, 85% ee), crystallization as the D-pyroglutamic acid salt (92% yield, 98.2% ee), and isocyanate formation (98% yield) with phosgene.

Introduction

Human leukocyte elastase (HLE, EC 3.4.21.37) is a serine protease which is stored in the azurophilic granules of polymorphonuclear leukocytes (PMNs). Upon its release into extracellular space, HLE is capable of hydrolyzing the connective tissue component elastin as well as collagen. Excessive release of HLE or the inhibition of its natural inhibitors has been implicated in several disease processes.[1] The controlled inhibition of HLE, normally in balance with its natural inhibitors α_1-proteinase inhibitor (α_1PI) and α_2-macroglobulin (α_2M), by an exogenous low molecular weight inhibitor might, therefore, have a positive therapeutic effect in the treatment of emphysema,[1a,2a] rheumatoid arthritis,[2b] and cystic fibrosis.[2c] Among the β-lactam-based inhibitors reported,[3−5] L-694,458[6] (**1**) has been shown to be a remarkably potent (K_{obs}/[I] = 3.8×10^6 M^{-1} s^{-1}), selective, and orally active HLE inhibitor and has been selected for further development.[7] Herein we report a convergent, enantioselective synthesis suitable for large scale application (Figure 1).

Results and Discussion

A synthesis of L-694,458 (**1**), whose absolute configuration was determined by X-ray analysis,[6] logically proceeds via the coupling of β-lactam **2** and isocyanate **3**. Our initial preparation of chiral β-lactam **2** was achieved via resolution of benzoic acid **6** (Scheme 1). Racemic acid **6** was prepared by (1) displacement of the propionate group of 3,3-diethyl-4-(propionyloxy)-2-azetidinone[8] (**4**) by benzyl 4-hydroxybenzoate (benzyl paraben) and Cs_2CO_4 in 10% aqueous CH_3CN to give crystalline benzyl ester **5** (93% yield) followed by (2) hydrogen transfer reduction using Pd/C and cyclohexene in EtOH and crystallization from *tert*-butyl methyl ether (MTBE) to give racemic acid **6** (82% yield). Racemic acid **6** was

† Department of Process Research.
‡ Department of Bioprocess Research and Development.
[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.

(1) (a) Snider, G. L. Protease-Antiprotease Imbalance in the Pathogenesis of Emphysema and Chronic Bronchial Injury: A Potential Target for Drug Development. *Drug. Dev. Res.* **1987**, *10*, *235*. (b) Weinbaum, G.; Giles, R. E.; Krell, R. D., Eds. Pulmonary Emphysema, the Rationale for Intervention. *Ann. N.Y. Acad. Sci.* **1991**, *624*, 1. (c) Bender, J. G.; van Epps, D. E.; Searles, R.; Williams, R. C., Jr. Altered Function of Synovial Fluid Granulocytes in Patients with Acute Inflammatory Arthritis; Evidence for Action of Neutrophils and its Mediation by a Factor Present in Synovial Fluid. *Inflammation* **1986**, *10*, 443.

(2) (a) Janoff, A. Elastases and Emphysema. Current Assessment of the Protease-Antiprotease Hypothesis. *Am. Rev. Respir. Dis.* **1985**, *132*, 417. (b) Jackson, A. H.; Hill, S. L., Afford, S. C.; Stockley, R. A. Sputum Soluble Phase Proteins and Elastase Activity in Patients with Cystic Fibrosis. *J. Respir. Dis.* **1984**, *65*, 114. (c) Janoff, A. In *Neutral Proteases of Human Polymorphonuclear Leukocytes*; Havermann, K., Janoff, A. Eds.; Urban and Schwartzenberg: Baltimore, MD, 1978; pp 390−417

(3) Doherty, J. B.; Shah, S. K.; Finke, P. E.; Dorn, C. P., Jr.; Hagman, W. K.; Hale, J. J.; Kissinger, A. L.; Thompson, K. R.; Brause, K.; Chandler, G. O.; Knight, W. B.; Maycock, A. L.; Ashe, B. M.; Weston, H.; Gale, P.; Mumford, R. A.; Anderson, O. F.; Williams, H. R.; Nolan, T. E.; Frankenfield, D. L.; Underwood, D.; Vyas, K. P.; Kari, P. H.; Dahlgren, M. E.; Mao, J.; Fletcher, D. S.; Dellea, P. S.; Hand, K. M.; Osinga, D. G.; Peterson, L. A.; Williams, D. T.; Metzger, J. M., Bonney, R. J.; Humes, J. L.; Pacholok, S. P.; Hanlon, W. A.; Opas, E.; Stolk, J.; Davies, P. *Proc. Nat. Acad. Sci. U.S.A.* **1993**, 8727.

(4) Finke, P. E.; Shah, S. K.; Ashe, B. M.; Ball, R. G.; Blacklock, T. J.; Bonney, R. J.; Brause, K. A.; Chandler, G. O.; Cotton, M.; Davies, P.; Dellea, P. S.; Dorn, C. P., Jr.; Fletcher, D. S.; O'Grady, L. A.; Hagman, W. K.; Hand, K. M.; Knight, W. B.; Maycock, A. L.; Mumford, R. A.; Osinga, D. G.; Sohar, P.; Thompson, K. R.; Weston, H. *J. Med. Chem.* **1992**, *35*, 3731.

(5) (a) Shah, S. K.; Dorn, C. P., Jr.; Finke, P. E.; Hale, J. J.; Hagman, W. K.; Brause, K.; Chandler, G. O.; Kissinger, A. L.; Ashe, B. M.; Weston, H.; Knight, W. B.; Maycock, A. L.; Dellea, P. S.; Fletcher, D. S.; Hand, K. M.; Mumford, R. A; Underwood, D. J.; Doherty, J. B. *J. Med. Chem.* **1992**, *35*, 3745. (b) Finke, P. E.; Shah, S. K.; Fletcher, D. S.; Ashe, B. M.; Brause, K. A.; Chandler, G. O.; Dellea, P. S.; Hand, K. M.; Maycock, A. L.; Osinga, D. G.; Underwood, D. J.; Weston, H.; Davies, P.; Doherty, J. B. *J. Med. Chem.* **1995**, *38*, 2449.

(6) Finke, P. E.; Dorn, C. P., Jr.; Kissinger, A. L.; Shah, S. K.; Ball, R. G.; Chabin, R.; Davies, P.; Dellea, P. S.; Doherty, J. B.; Durette, P. L.; Fletcher, D. S.; Griffen, P. R.; Green, B. G.; Hale, J. J.; Hanlon, W. A.; Hand, K. M.; Humes, J. L.; Kieczykowski, G. R.; Klatt, T. D.; Lanza, T. J.; Knight, W. B.; MacCoss, M.; Miller, D. S.; Mumford, R. A.; Pacholok, S. G.; Peterson, L. P.; Poe, M.; Underwood, D. J.; Williams, D. T.; Williams, H. R. Orally Active, Intracellular Inhibitors of Human Leukocyte Elastase (HLE). Presented at the 22nd National Meeting of the American Chemical Society, Chicago, 1995, MEDI 083.

(7) L-694,458 has been licensed to DuPont Merck Pharmaceutical Company (DMP-777).

(8) Claus, M.; Grimm, D.; Prossel, G. *Liebigs Ann. Chem.* **1974**, 539.

S0022-3263(96)00618-4 CCC: $12.00

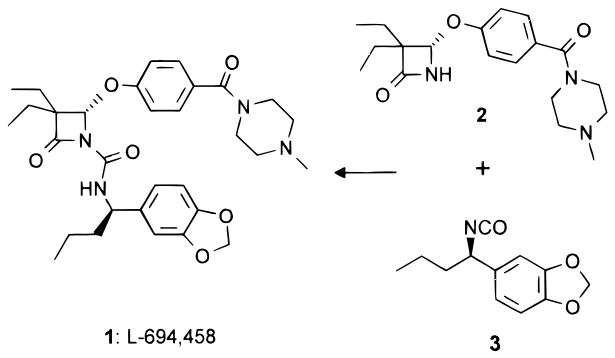
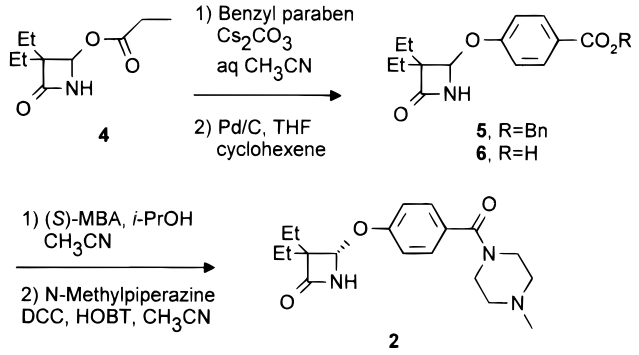


Figure 1. L-694,458 (**1**) and key intermediates.

Scheme 1. Synthesis of (*S*)-*β*-Lactam 2



resolved by crystallization of the (*S*)-enantiomer as the salt of (*S*)-α-methylbenzylamine (α-MBA) followed by recrystallization from 2-propanol:acetonitrile. Maximum recovery of acid (*S*)-**6** was achieved by first crystallizing and filtering the (*R*)-**6**:(*R*)-α-MBA salt. Treatment of the mother liquour with (*S*)-α-MBA gave on crystallization the (*S*)-**6**:(*S*)-α-MBA salt directly. A recrystallization from 2-propanol:acetonitrile gave acid (*S*)-**6**:(*S*)-α-MBA salt in 27% yield (based on racemic **6**) in 96.4% ee.

Greater efficiency in the production of the chiral acid (*S*)-**6** was a necessary and major goal of the synthesis. Attempts to racemize either the (*R*)- or (*S*)-enantiomer of acid **6** via displacement with either 4-hydroxybenzoic acid or with the benzyl paraben gave mixtures of acid, ester, and phenols (via displacement by the carboxylate of 4-hydroxybenzoic acid). A kinetic enzymatic resolution of the ester was proposed as an attractive alternative to the classical salt resolution. The synthesis of optically active *β*-lactams via enzyme resolution has been reported, via the lipase hydrolysis of *N*-acyloxymethyl *β*-lactams.[9] Our goal was to discover an enzyme to evoke a resolution upon the hydrolysis of esters of *β*-lactam **5**, an ambitious goal involving an aromatic ring as spacer between the prochiral center and the site of hydrolysis.

Racemic benzyl ester **5** was exposed to a series of enzyme sources, and two promising candidates, *Pseudomonas aeruginosa* lipase (MB5001) and lipase PS-800 (Amano), were identified. Some lipases (wheat germ, *Chromobacterium viscosum*, *Rhizopus arrhizus*, *Pseudomonas sp.*, and pig pancreas) failed to effect hydrolysis over a 20 h incubation at 28 °C, while pig liver esterase gave a rapid nonselective hydrolysis. After preliminary development of the hydrolysis (pH, concentration, surfactant, surfactant concentration, temperature), a variety of esters were exposed to the selected enzymes (*P. aeruginosa* lipase (MB-5001) and lipase PS-800), and rates of acid **6** production and enantiomeric excess were measured (see Table 1). While the *P. aeruginosa* lipase showed sensitivity in the rate of acid production to the type of ester, little differentiation was observed in the overall enantioselectivity, with similar 85−88% ee values observed. Hydrolysis using lipase PS-800 exhibited lower production rates than *P. aeruginosa* lipase, but a sensitivity to the ester structure was observed, with maximum enantioselectivity occurring when the benzyl ester was employed.

Commercially available lipase PS-800 (Amano) was selected for further development. Bioconversion of ester **5** to the acid **6** with lipase PS-800 was found to be insensitive to pH in the range of 5−8, but highly dependent on the surfactant concentration, the charge of ester (the ester demonstrated limited solubility in the broth, but the presence of excess solid was necessary to keep the solution saturated with racemic ester), and the charge of enzyme. The hydrolysis exhibited a decline in acid production after 4 days (see Figure 2). The addition of benzyl alcohol to an enzyme hydrolysis resulted in an inhibition of hydrolysis. When racemic acid **6** was exposed to the enzyme hydrolysis conditions in the presence of benzyl alcohol, no production of benzyl ester **5** was detected. Inhibition of the PS-800 lipase due to alcohol production was an effect observed with all of the ester derivatives. The loss in rate of hydrolysis due to production of alcohol was paralleled with gradually lower ee (96−93%) as the hydrolysis was allowed to proceed beyond 4 days, such that maximal productivity and enantioselectivity were achieved at a 25% conversion of racemic ester.

Table 1. Effect of Ester Variation on Rate of Acid Production and Stereospecificity with MB-5001 and PS-800 Lipases

	enzyme			
	MB-5001		PS-800	
ester[a]	mg/L (h)	% ee	mg/L (h)	% ee
methyl	26 (24)		2 (24)	
	36 (80)	85.5	85 (80)	37
propyl	270 (24)		21 (24)	
	251 (80)	88.6	48 (80)	88
hexyl	545 (24)		32 (24)	
	644 (80)	87.8	58 (80)	91
benzyl	45 (24)		43 (24)	
	60 (80)	86.8	72 (80)	95

[a] The isopropyl ester failed to hydrolyze with either lipase.

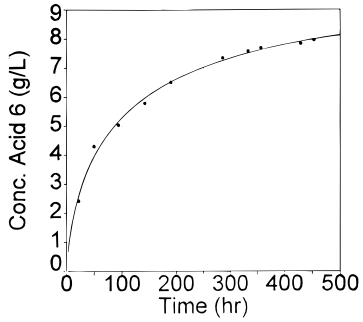
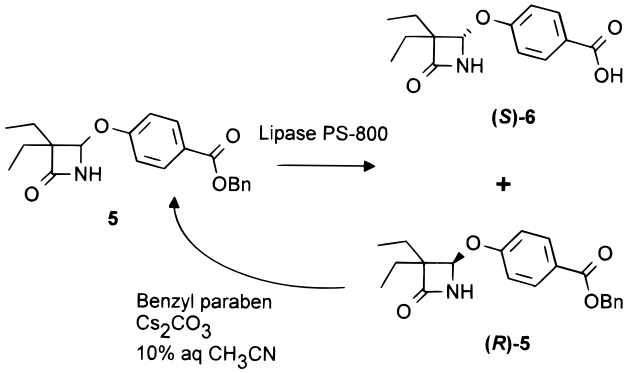


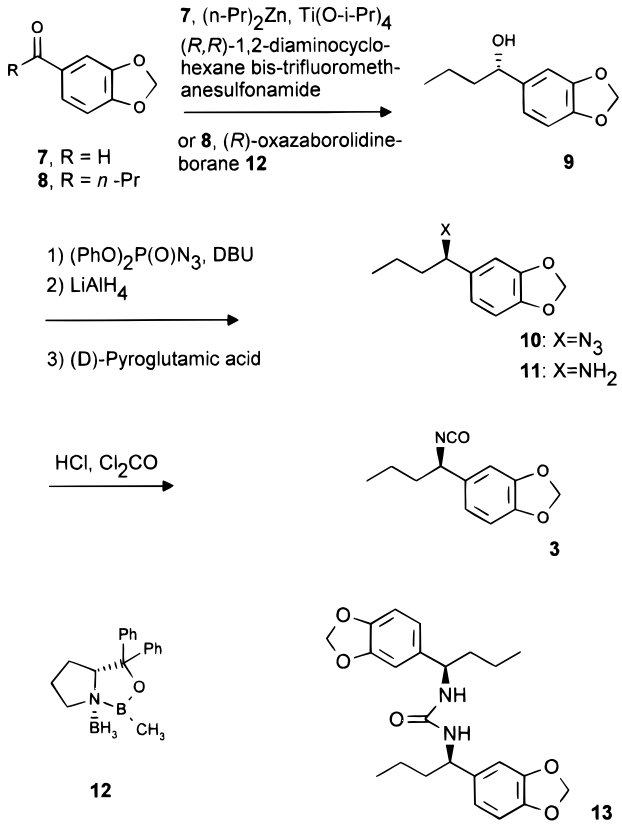
Figure 2. Rate of acid **6** production.

(9) (a) Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull. Jpn.* **1992**, *40*, 2227. (b) Jouglet, B.; Rousseau, G. *Tetrahedron Lett.* **1993**, *34*, 2307. (c) Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull. Jpn.* **1992**, *41*, 1933. (d) Oumoch, S.; Rousseau, G. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2841. (e) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351 (and references therein).

Scheme 2. Enzyme Hydrolysis of Ester 5



Scheme 3. Synthesis of (*R*)-Isocyanate 3



After considerable experimentation, the following process was developed for optimal production of acid **6**, isolation and racemization of recovered ester **5**, and recovery and reuse of enzyme (Scheme 2). Racemic ester **5** was treated with lipase enzyme PS-800[10] in aqueous pH 7 phosphate medium in the presence of Triton X-100 and terminated at ~25% conversion to acid (*S*)-**6** (93% ee). Insoluble, unreacted ester **5** was recovered by filtration as a 70:30 mixture of *R:S* enantiomers, giving a milky white filtrate. The isolated benzyl ester was racemized in 90% yield via displacement with benzyl 4-hydroxybenzoate and Cs_2CO_4 in 10% aqueous acetonitrile. The PS-800 lipase enzyme was isolated by passage of the filtrate through a 10 kD cartridge and washing with water. Reuse of the enzyme indicated that the lipase regained >90% of its original activity and all of its enantioselectivity. Acid (*S*)-**6** was isolated from the second filtrate by salting (NaCl), pH adjustment (5.0, with phosphoric acid), extraction with *i*-PrOAc, and crystallization from MTBE. Racemized ester **5** was hydrolyzed with recovered enzyme, and the isolation procedure was repeated. This recycling procedure continued to provide chiral acid from racemized ester after three cycles in a 60% overall yield based on ester **5** with retention of the activity and selectivity of the lipase (93% ee).

(*S*)-Acid **6** was converted to amide **2** in 90% yield using DCC with *N*-methylpiperazine. Crystallization provided intermediate amide **2** in 66% yield with an increase in optical purity to 99.4% ee, using (*S*)-acid **6** with optical purity in the 93−96% ee range.

The preparation of the second fragment of the convergent synthesis of L-694,458 is described. (*R*)-Isocyanate **3** was obtained from (*S*)-alcohol **9**, which was prepared via chiral addition of Zn(*n*-Pr)$_2$ (mediated with titanium isopropoxide and the bis-triflamide of (*R,R*)-1,2-diaminocyclohexane)[11] to piperonal **7** in 98% yield and 99.2% ee (Scheme 3). Alternatively, ketone **8** was reduced with stoichiometric amounts of (*S*)-oxazaborolidine−borane ((*S*)-OAB−BH_3) complex[12] (*S*)-**12** to give the alcohol (*R*)-**9** in 97.5% ee. The desired (*S*)-enantiomer should then be accessible by the reduction with (*R*)-OAB−BH_3. (*S*)-Alcohol **9** was reacted with diphenylphosphoryl azide[13] in the presence of DBU to produce (*R*)-azide **10**, which was reduced to (*R*)-amine **11** with lithium aluminum hydride in 57% yield and 85% ee. The loss of optical purity of this benzylic alcohol was similar to the loss in a reported[13] example of a benzylic alcohol containing a *p*-OCH_3 vs an unsubstituted aromatic or *p*-CH_3 substitution. The use of alternate bases, as well as the use of $Zn(N_3)_2$,[14] failed to improve upon this result. The optical purity of (*R*)-amine **11** was upgraded to 98% ee by crystallization as the D-pyroglutamic acid salt in 92% yield. (*R*)-Amine **11** was then converted to the (*R*)-isocyanate **3** in 98% yield and in 98.2% ee.

The coupling of (*S*)-*β*-lactam **2** and (*R*)-isocyanate **3** did not proceed without added base. The use of powdered K_2CO_3 generated 1−2% of the symmetrical urea **13**, an impurity that was difficult to remove during crystallization (but could be removed by dissolving the product in aqueous acetic acid and filtering the insoluble urea). The addition of (*S*)-*β*-lactam **2** to (*R*)-isocyanate **3** was best achieved in CH_3CN in the presence of a catalytic amount of DBU. The use of other solvents (MTBE, *i*-PrOAc) gave the symmetrical urea **13** in 2−15% yield. L-694,458, **1**, was ultimately isolated and crystallized from MTBE in 80% yield and >99.5% de (SFC-HPLC).

An asymmetric and large scale synthesis of the potent elastase inhibitor L-694,458 was achieved. A recyclable enzymatic chiral synthesis of the *β*-lactam acid intermediate **6** was demonstrated, as well as a resolution of the acid. The chiral piperonyl isocyanate **3** was prepared via chiral addition of Zn(*n*-Pr)$_2$ to piperonal, conversion of alcohol **9** to azide **10**, and reduction to amine **11**. Coupling of the intermediates was highly successful using DBU in acetonitrile, and the final product was crystallized in high diastereomeric purity.

(10) A full discussion of the enzyme hydrolysis will be the subject of a future publication. Roberge, C.; Cvetovich, R. J.; Amato, J. S.; Hartner, F.; Greasham, R.; Chartrain, M., to be submitted.

(11) Takahashi, H.; Kawakita, T.; Yoshioka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1989**, *30*, 7095.

(12) Mathre, D. J., Thompson, A. S., Douglas, A. W., Hoogsteen, K., Carroll, J. D., Corley, E. G., Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 2880.

(13) Thompson, A.; Humphrey, G.; DeMarco, A.; Mathre, D.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 5886.

(14) Viaud, M. C.; Rollin, P. *Synthesis* **1990**, 130.

Experimental Section

General. HPLC analyses were performed using a Spectra-Physics SP8700 ternary solvent delivery system. All reactions were carried out in Pyrex glass vessels under an atmosphere of N_2, and solvents and reagents were dried where appropriate over 3 Å molecular sieves prior to use. Other solvents and reagents were used as received. Karl Fisher water analyses were carried out on a Metrohm 684 KF coulometer. Optical rotations were measured in a 1 dm cell. PS-800 lipase was purchased from Amano (Troy, VA). High-resolution mass spectroscopy studies were performed in the FAB mode. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. Dipropylzinc was obtained from Akzo Chemicals, Inc.

3,3-Diethyl-4-((4′-benzylcarboxy)phenoxy)-2-azetidinone (5). Into CH_3CN:H_2O (1:1, 40 L) were charged benzyl 4-hydroxybenzoate (6.03 kg, 26.4 mol) and Cs_2CO_4 (13 kg, 39.9 mol). The resulting two-phase mixture was heated to 30 °C, and 3,3-diethyl-4-(propionyloxy)-2-azetidinone[8] (**4**) (7 kg, 35.2 mol) was added dropwise over 60 min. The mixture was aged for 90 min at 30−35 °C, cooled to rt, and partitioned with H_2O (19 L) and MTBE (19 L). The organic phase was washed with H_2O (3 × 19 L) and then concentrated *in vacuo* (40 °C, 28 in. of Hg) to a volume of 10 L. The concentrate was diluted with EtOH (10 L) and reconcentrated *in vacuo*. The batch was diluted to a volume of 26 L with EtOH (20 L) and assayed by HPLC. Ester **5** (16.8 kg, 295 g/L) was obtained in 93% yield (based on benzyl paraben). HPLC: Altex Ultrasphere Octyl, 250 × 4.6 mm, 5 μm; CH_3CN:H_2O (with 0.1% H_3PO_4 in each), gradient elution 50:50 to 90:10 over 30 min, 254 nm, 25 °C, 2.0 mL/min; t_R {min} ester **5**, 12.0; benzyl paraben, 5.4. Ester **5** can be crystallized from EtOH:H_2O (1:1). Mp 78.5−80.9 °C. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 8.04 (m, 2H), 7.45−7.30 (om, 5H), 6.91−6.84 (om, 3H), 5.41 (s, 1H), 5.33 (s, 2H), 2.00−1.67 (om, 4H), 1.04 (t, J = 7.5, 3H), 1.02 (t, J = 7.4, 3H). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 172.9, 165.7, 160.1, 135.9, 131.8 (2C), 128.4, 128.0, 127.9 (2C), 123.7, 115.0, 82.8, 66.4, 64.69, 23.5, 21.5, 8.7, 8.5. IR (film): 3270, 2915, 2840, 1770, 1716, 1606, 1508, 1273, 1243, 1053 cm^{-1}.

Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.22; H, 6.45; N, 3.92.

3,3-Diethyl-4-(4′-carboxyphenoxy)-2-azetidinone (6). To benzyl ester **5** (7.0 kg, 20.5 mol) in EtOH (15 L) were added cyclohexene (10 L) and 5% Pd/C (500 g). The reaction was stirred at reflux for 2 h. The mixture was filtered through Solka-Floc (1 kg), and the cake was washed with EtOH (2 × 1 L). The EtOH solution was evaporated *in vacuo* (30 °C, 29 in. of Hg) to a volume of 10 L. The concentrate was diluted with MTBE (5 L) and reconcentrated *in vacuo* to a slurry. MTBE (5 L) was added, and the product was filtered, washed with MTBE (10 L), and dried with a nitrogen flow giving 4.38 kg of acid **6** for an 82% yield. Mp: 168.5−170.7 °C. HPLC assay: Altex Ultrasphere Octyl, 250 × 4.6 mm, 5 μm; CH_3CN:H_2O (with 0.1% H_3PO_4 in each), gradient elution 50:50 to 90:10 over 30 min, 254 nm, 25 °C, 2 mL/min; t_R {min} acid **6**, 2.3; ester **5**, 12.0. ^{1}H NMR (250.13 MHz, CD_3OD): δ 7.99 (d, J = 8.9, 2H), 6.98 (d, J = 8.9, 2H), 5.50 (s, 1H), 4.99 (br s, exchangeable H), 1.96−1.68 (om, 4H), 1.04 (t, J = 7.6, 3H), 1.00 (t, J = 7.6, 3H). ^{13}C NMR (62.89 MHz, CD_3OD): δ 175.6, 169.4, 161.7, 133.0 (2C), 125.5, 116.3 (2C), 83.8, 65.3, 24.8, 22.9, 9.4, 8.9. IR (film): λ_{max} 3220, 2915, 2840, 1717, 1692, 1605, 1238 cm^{-1}.

Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found C, 63.62; H, 6.54; N. 5.23.

Resolution of Acid 6. Racemic acid **6** (3.9 kg, 14.8 mol) was dissolved in *i*-PrOH:CH_3CN (1:1, 70 L) at 70 °C. (*R*)-α-Methylbenzylamine (883 mL) was added. The solution was cooled to room temperature over 4 h, and the slurry was aged 16 h at room temperature. The *R*,*R* salt was filtered, washed with *i*-PrOH:CH_3CN (1:1, 7 L), and dried with a nitrogen flow to give 1.88 kg with an enantiomeric ratio *R*:*S* = 80:20. The desired (*S*)-enantiomer was enriched in the mother liquors as a 76:24 mixture. Chiral SFC-HPLC: Chiracel OD(H), 250 × 4.6 mm, 28 vol % EtOH (containing 20 mM $HClO_4$) modifier, 1.0 mL/min, 300 bar, 35 °C, 248 nm; t_R {min} (*S*)-**6**, 7.6; (*R*)-**6**, 9.5. To the mother liquors from the *R*,*R* salt crystallization was added (*S*)-α-methylbenzylamine (850 mL). The slurry was aged 16 h at room temperature. The *S*,*S* salt was filtered, washed with *i*-PrOH:CH_3CN (1:1, 5 L), and dried with a nitrogen flow to give product as a wet cake (enantiomeric ratio, *S*:*R* = 77:23). Into *i*-PrOH:CH_3CN (1:1, 80 L) was charged the *S*,*S* diasteriomeric salt. The slurry was heated to reflux to obtain a clear solution, which was cooled to room temperature over 6 h and then aged 16 h. The *S*,*S* salt was filtered, washed with *i*-PrOH:CH_3CN (1:1, 6 L), and dried with a nitrogen flow giving (*S*)-**6**:(*S*)-MBA salt (1.2 kg, 21.5% yield from racemic acid, 96.4% ee). By recombining all the filtrates and isolating the acid free of amine by extraction, a racemic mixture of the acid is recovered which was resolved as above, to give an additional 310 g of (*S*)-**6**:(*S*)-MBA salt, for a total isolated yield of 27%: $[\alpha]^{25}_{589}$ −78.6° (*c* = 1.0, MeOH); mp = 117.5−118.8 °C. NMR (250.13 MHz, CD_3OD): δ 7.93 (br d, J = 8.7, 1H), 7.6−7.32 (om, 5H), 6.90 (br d, J = 8.7, 2H), 5.45 (s, 1H), 5.0 (br s, 1H), 4.42 (q, J = 6.8, 1H), 1.97−1.58 (om, 4H), 1.61 (d, J = 6.8, 3H), 1.05 (t, J = 7.5, 3H), 1.01 (t, J = 7.5, 3H). ^{13}C NMR (62.89 MHz, CD_3OD): δ 175.7, 174.6, 159.9, 140.4, 132.5, 132.2 (2C), 130.2 (2C), 129.9, 127.6 (2C), 115.8 (2C), 84.1, 65.2, 52.2, 24.8, 22.9, 21.1, 9.4, 8.9. IR (film): λ_{max} 3175, 3131, 1775, 1608, 1514, 1396, 1247 cm^{-1}.

Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 68.6; H, 7.26; N, 7.28.

A sample of salt was partitioned between dilute phosphoric acid and *i*-PrOAc and then crystallized from *i*-PrOAc after partial concentration: $[\alpha]^{25}_{589}$ −115.8° (*c* 1.00, MeOH); mp 156.5−158 °C.

Following the same procedure, the (*R*)-**6**:(*R*)-MBA salt was upgraded by recrystallization (94.6% ee): $[\alpha]^{25}_{589}$ +75.2° (*c* = 1.0, MeOH).

Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 69.0; H, 7.29; N, 7.27.

Enzyme Hydrolysis of Benzyl Ester 5. Racemic ester **5** (43 g) was added to a phosphate-buffered aqueous solution (1.5 L) of Triton X-100 (2.7 g) and stirred (200 rpm) at 37 °C. Lipase PS-800 (12 g) was added, and the mixture was agitated for 96 h. The enzyme hydrolysis mixture, containing benzyl ester and enzyme in suspension and carboxylic acid (8.09 g, by HPLC assay) in solution, was filtered through a coarse sintered glass funnel that was overlaid with filter paper. The filtered ester **5** was washed with H_2O (3 × 200 mL), dried, and set aside for racemization. The combined aqueous suspension of the enzyme hydrolysis medium was pumped (Cole-Parmer Masterflex centrifugal pump) at ∼300 mL/min through a Millipore PLTK Prep/Scale-TFF 1 ft^2 10 kD regenerated cellulose filter cartridge. The retentate return line was restricted to allow a permeate flow of ∼700 mL/h. The final reduction in volume was followed by a dilution to 250 mL with H_2O and recirculation of the enzyme slurry through the filter. The enzyme slurry was removed and drained from the filter, and fresh H_2O (250 mL) was recirculated through the filter (twice) to recover the enzyme. The combined aqueous enzyme washes were set aside for assay and reuse. Assay of the recovered aqueous enzyme suspension (olive oil hydrolysis)[15] indicated that 90% activity of enzyme was recovered. The permeate (3 L) was adjusted to pH 5.0 with H_3PO_4, NaCl (1.2 kg) was added, and the mixture was extracted with *i*-PrOAc (3 × 200 mL). The combined extracts were washed with H_2O (200 mL), azeotropically dried by distillation, and filtered to remove inorganic solids. This material was evaporated, and the concentrate was crystallized from MTBE (100 mL). The slurry was filtered, washed with MTBE, and dried to give 7.0 g of acid (*S*)-**6**. The mother liquors were concentrated to 15 mL, aged for 5 h, filtered, washed with MTBE, and dried to give an additional 1.02 g of acid (*S*)-**6**. The first and second crops were assayed (HPLC) and found to be 91 wt % and 96 wt % pure, respectively, with a 93% ee by chiral SFC-HPLC assay. Isolated acid **6** (6.4 g) was dissolved in *i*-PrOH:CH_3CN (1:1, 120 mL) and warmed to 55 °C. (*S*)-α-Methylbenzylamine was added, and crystallization began immediately. The

(15) Tiez, N.; Rex-Astles, J.; Shuey, D. *Clin. Chem.* **1989**, *35*, 1688.

slurry was aged 18 h at 20 °C, cooled to 2 °C, filtered, washed with *i*-PrOH:CH_3CN (1:1, 20 mL), and dried (*in vacuo*, 45 °C, 25 in. of Hg) to give (*S*)-**6**:(*S*)-MBA salt (8.4 g, 90% yield, 96.1% ee). Chiracel OD(H), 250 × 4.6 mm; 28 vol % EtOH (containing 20 mM $HClO_4$) modifier, 1.0 mL/min, 300 bar, 35 °C, 248 nm; t_R {min} (*S*)-**6**, 7.6; (*S*)-**5**, 8.3; (*R*)-**6**, 9.5; (*R*)-**5**, 11.6.

Racemization of (*R*)-3,3-Diethyl-4-[4-(benzylcarboxy)-phenoxy]-2-azetidinone (5). Benzyl ester **5** (49.5 g), recovered from enzyme hydrolyses as a 70:30 mixture of *R:S* enantiomers, was added to 10% aqueous CH_3CN (165 mL) and warmed to 50 °C to give a homogeneous solution. Benzyl paraben (0.38 g) and Cs_2CO_3 (0.5 g) were added, and the mixture was aged at 50−55 °C for 6 h. After the solution was cooled to room temperature, H_2O (500 mL) and MTBE (100 mL) were added. The aqueous phase was extracted with MTBE (100 mL), and the combined organic phases were washed with saturated aqueous NaCl (50 mL), concentrated *in vacuo* (40 °C, 28 in. of Hg) to a volume of ∼80 mL, diluted with EtOH (75 mL), reevaporated to ∼80 mL, and then diluted with EtOH to a 150 mL volume, whereupon the benzyl ester began to slowly crystallize after seeding. H_2O (100 mL) was added dropwise over 1 h, and the mixture was aged for 1 h, filtered, washed with 33% (v/v) aqueous EtOH (100 mL), and then dried *in vacuo* (45 °C, 20 h) to give 45.2 g of crystalline racemic ester **5** (98.7 wt % by HPLC assay, see above, 90% yield).

Racemized ester was resubmitted to enzyme hydrolysis, using recycled enzyme, and identical results were achieved.

(*S*)-3,3-Diethyl-4-(4′-((*N*-methylpiperazin-1-yl)carbonyl)phenoxy)-2-azetidinone (2). (*S*)-**6**:(*S*)-MBA salt (3.30 kg, 8.58 mol, 96.4% ee) was suspended in *i*-PrOAc (30 L). H_2O (1 L) was added. To this mixture, maintained at 25 °C was added a 1 N aqueous H_3PO_4 solution (11.8 L) dropwise until all the solids were dissolved, and a constant pH of 2.0 was achieved. NaCl (1.0 kg) was added, and the phases were separated. The *i*-PrOAc solution was concentrated *in vacuo* to ∼16 L (KF ∼8 mg/mL) whereupon acid (*S*)-**6** began to crystallize. *i*-PrOAc (10 L) was added, and the solution was dried by azeotropic distillation *in vacuo* to KF = 0.4 mg/mL. The mixture was warmed to 55 °C, *N*-methylpiperazine (1.05 kg, 10.46 mol) and HOBT (146 g, 1.08 mol) were added, and then a solution of DCC (2.97 kg, 14.4 mol) in *i*-PrOAc (3 L) was added over 5 min. The reaction temperature was adjusted to 48−50 °C and aged for 1.5 h. The reaction mixture was cooled to 18 °C and filtered. The cake was washed with *i*-PrOAc (3 L), and the filtrate was concentrated *in vacuo* to a volume of ∼8 L. Crystallization began during distillation. The mixture was aged for 18 h at 18 °C and 2 h at 10 °C and then filtered and washed with cold *i*-PrOAc (3 L). The cake was dried with a stream of nitrogen for 40 h to give *β*-lactam **2** (1.98 kg, 99.5 area %, 66% isolated yield) as a white crystalline solid. Chiral SFC assay showed that the crystallization enriched the (*S*)-enantiomer (solids, 99.4% ee; mother liquour, 87.2% ee). HPLC assay: Inertsil C8, 250 × 4.6 mm, 5 μm; CH_3CN:H_2O (with 0.1% H_3PO_4); gradient elution 3:97 to 80:20 over 20 min, 248 nm, 25 °C, 2.0 mL/min; t_R{min} *β*-lactam **2**, 7.9; **6**, 14.2. Chiral SFC HPLC assay: Chiralcel OD(H), 250 × 4.6 mm, 20% MeOH (containing 0.1% TEA) modifier; 1.0 mL/min, 300 bar, 248 nm; t_R {min} (*S*)-**2**, 7.3, (*R*)-**2**, 10.1. $[\alpha]^{25}_{589}$ −81.2° (*c* = 5.0, MeOH). ^{1}H NMR (250.13 MHz): δ 8.09 (s, 1H), 7.23 (d, *J* = 8.7, 2H), 6.72 (d, *J* = 8.7, 2H), 5.14 (s, 1H), 3.75−3.10 (br m, 4H), 2.43−2.00 (br m, 4H), 2.13 (s, 3H), 1.88−1.51 (om, 4H), 0.91−0.81 (om, 6H). ^{13}C NMR (62.89 MHz): δ 172.6, 169.5, 157.3, 128.8, 128.5, 115.1, 82.7, 64.0, 54.4, 45.5, 23.4, 21.3, 8.6, 8.3. Mp: 117.5−120.7 °C. IR (film): 3550, 3200, 2915, 2840, 1767, 1607, 1462, 1437, 1237, 1053 cm^{-1}.

Anal. Calcd for $C_{19}H_{27}N_3O_3$: C, 66.06; H, 7.88; N, 12.16. Found: C, 66.00; H, 8.12; N, 12.10.

(*S*)-α-Propylpiperonyl Alcohol (9). Titanium(IV) isopropoxide (226 mL) was charged to a slurry of (*R*,*R*)-1,2-diaminocyclohexane ditrifluoromethanesulfonamide[11] (29.6 g) in toluene (1.25 L) at room temperature. The mixture was heated to 40 °C for 20 min and then cooled to 20 °C. In a separate flask, Zn(*n*-Pr)$_2$ (850 g, 5.60 mol) was added to cold (−5 °C) hexanes (5.6 L). The titanium catalyst mixture was added to the Zn(*n*-Pr)$_2$ solution; then a solution of piperonal (**7**, 619 g, 4.12 mol) in toluene (1.9 L) was added while a temperature of ∼0 °C was maintained. The mixture was stirred at 0 °C for 2−4 h; then the reaction was quenched by the slow addition of cold 2 N HCl (8.8 L) while a temperature of <5 °C was maintained. The aqueous layer was extracted with a 1:1 mixture of hexanes:toluene (2 L). The combined organic layers were washed with aqueous $NaHCO_3$ (1.5 L) and 10% aqueous NaCl (1.5 L). The organic layer was dried with Na_2SO_4 (700 g) and was filtered to provide a solution of alcohol **9** (784 g, 4.03 mol) in 98% yield and 99.2% ee. Chiral HPLC assay: Chiralcel-OD, 250 × 4.6 mm, 280 nm, *i*-PrOH:hexane, isocratic 7.5:92.5, 1.5 mL/min; t_R {min} (*R*)-**9**, 5.3; (*S*)-**9**, 7.5. HPLC assay: Zorbax Phenyl, 250 × 4.6 mm, 5 μm, 210 nm; CH_3CN: 0.1% H_3PO_4, gradient, 50:50 at *t* = 0 min, 90:10 at *t* = 18 min, 1.0 mL/min; t_R {min} **9**, 5.8; **7**, 3.8 min; toluene, 7.7. A sample was concentrated for analysis. ^{1}H NMR (250.13 MHz): δ 6.82 (s, 1H), 6.74 (s, 2H), 5.91 (s, 2H), 4.53 (t, *J* = 6.64, 1H), 2.39 (br s, 1H), 1.80−1.51 (om, 2H), 1.45 (om, 2H), 0.90 (t, *J* = 7.3, 3H). ^{13}C NMR (62.89 MHz): δ 147.5, 146.6, 139.0, 119.2, 107.8, 106.3, 100.8, 74.0, 41.0, 18.9, 13.8. IR (film): λ_{max} 3381, 2857, 1504, 1487, 1442, 1244, 1040, 939, 811 cm^{-1}. HRMS: [M$^+$] = 1 94.0952 (calcd = 194.0943).

(*R*)-α-Propylpiperonylamine (11). In a Pyrex glass vessel, alcohol **9** (1.20 kg, 6.2 mol) in toluene (12 L) was cooled to 5 °C. Diphenylphosphoryl azide (1.60 L, 7.42 mol) was added; then DBU (1.11 L) was added at such a rate as to maintain a temperature of ≤5 °C. The reaction was allowed to warm to rt over 2−3 h, forming two liquid layers upon 16 h of aging. [*Caution:* Tests have shown that azide **10** and toluene solutions of the azide are shock sensitive and undergo exothermic decomposition beginning at ∼50 °C.] The two liquid layers were diluted with H_2O (7 L) and separated. The aqueous layer was extracted with toluene (1 L); then the combined organic extracts were washed sequentially with H_2O (7 L), cold 1 N aqueous HCl (4 L), H_2O (4 L), and 10% aqueous NaCl (4 L). The organic layer was dried with anhydrous Na_2SO_4 and filtered. On a large scale, no attempt was made to concentrate the azide solution. HPLC conditions: Inertsil Phenyl, 250 × 4.6 mm; 5 μm; 210 nm; CH_3CN:0.1% H_3PO_4, gradient 50:50 at *t* = 0 min, 90:10 at *t* = 18 min, 1.0 mL/min; t_R {min} **9**, 5.9; toluene 7.8; $(PhO)_2P(O)N_3$, 9.3; **10**, 11.4. To dry THF (6.3 L) cooled to 10 °C was added a 1 M LAH/THF solution (6.0 L). An azide **10** solution was added to the LAH over ∼2 h at such a rate as to maintain the temperature at 23 ± 2 °C. The reaction was aged until gas evolution (N_2) ceased (6 h). Then the reaction mixture was cooled to 0 °C, and the excess LAH was quenched by the slow addition of H_2O (400 mL). A 17.5 wt % aqueous potassium sodium tartrate solution (8 L) was added to the reaction, and the mixture was stirred at room temperature for 16 h. The aqueous layer was extracted with toluene (2 L), and the combined organic phases were washed with H_2O (7 L). The organic phase was extracted with cold 1 N HCl (7 L). The aqueous phase was adjusted to pH 13 with 25 wt % aqueous NaOH and then extracted with toluene (4 L). After the toluene layer was washed with 10% aqueous NaCl (4 L) and dried with anhydrous Na_2SO_4 (500 g), it was filtered and concentrated to give amine **11** as an oil (740 g, 57% yield of (*R*)-**11** over two steps, 85% ee). HPLC assay: Inertsil ODS-2, 250 × 4.6 mm, 5 μm; 210 nm; CH_3CN:10 mM pH 6.5 potassium phosphate buffer:MeOH; gradient 36:60:6 at *t* = 0 min, 64:30:6 at *t* = 12 min, 67:27:6 at *t* = 18 min, 74:20:6 at *t* = 19 min, 74:20:6 at *t* = 25 min; 1.0 mL/min; 30 °C; t_R {min} **11**, 5.0; toluene, 14.7. The ratio of enantiomers was determined by either of two Chiral HPLC methods: (1) Chiralcel OD-R, 250 × 4.6 mm, 238 nm, CH_3CN:0.1% $HClO_4$; isocratic 15:85; 1.0 mL/min; 23 °C; t_R {min} (*R*)-**11**, 7.3; (*S*)-**11**, 15.0; alternatively, (2) SFC HPLC: Chiracel OD(H); 250 × 4.6 mm; 238 nm; 22% MeOH modifier (containing 0.1 vol % of 70% $HClO_4$); 1 mL/min; 35 °C; 300 Bar; t_R{min}: (*R*)-**11**, 6.1; (*S*)-**11**, 8.8. Azide **10**. ^{1}H NMR (300.133 MHz, $CDCl_3$): δ 6.81 (d, *J* = 1.6, 1H), 6.80 (d, *J* = 7.9, 1H), 6.75 (dd, *J* = 7.9, 1.6, 1H), 5.99 (s, 2H), 4.34 (t, *J* = 7.3, 1H), 1.86−1.61 (m, 2H), 1.45−1.24 (m, 2H), 0.92 (t, *J* = 7.3, 3H). ^{13}C NMR (75.469 MHz, $CDCl_3$): δ 148.0, 147.4, 133.7, 120.6, 108.2, 107.0, 101.2, 66.0, 38.2, 19.5, 13.7. Amine **11**: ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 6.76 (s, 1H), 6.66 (s, 2H), 5.83 (s, 2H), 3.73 (t, *J* =

6.9, 1H), 1.62−1.46 (om, 2H), 1.41 (br s, 2H), 1.36−1.07 (m, 2H), 0.83 (t, J = 7.3, 3H). ^{13}C NMR (62.896 MHz, $CDCl_3$): δ 147.4, 146.0, 140.7, 119.2, 107.7, 106.3, 100.5, 55.5, 41.6, 19.4, 13.7. IR (film): λ_{max} 3330, 2956, 1486, 1440, 1245, 1040, 937, 810 cm^{-1}. HRMS: [M$^+$] = 193.1073 (calcd = 193.1103).

Optical Purification of (*R*)-α-Propylpiperonylamine (11). A solution α-propylpiperonylamine (**11**) (1.523 kg, 7.88 mol, 85% ee) in EtOAc (30.5 L) and EtOH (1.5 L) was heated to 50−55 °C. D-Pyroglutamic acid (150 g) was added, and the solution was seeded with amine/D-pyroglutamic acid salt (5 g). Additional D-pyroglutamic acid (769 g) was added in portions over 30 min as the salt crystallized. The mixture was allowed to cool to 20−22 °C over 2−3 h and was aged for 16 h. The slurry was filtered, and the cake was washed with a mixture of EtOAc (5 L) and EtOH (0.25 L). The cake was dissolved in a mixture of toluene (6 L) and cold 2.5% aqueous NaOH (15 L). The toluene layer was washed with 10% aqueous NaCl (3 L), dried with anhydrous Na_2SO_4 (500 g), filtered, and concentrated to give amine **11** as an oil (1.315 kg, 92% yield, 98.2% ee). (*R*)-α-Propylpiperonylamine D-pyroglutamic acid salt. Mp: 106.5−107.5 °C, $[\alpha]^{25}_{405}$ −17.7° (c = 1.0, MeOH). ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 8.2 (br s, 3H), 7.82 (s, 1H), 6.92 (s, 1H), 6.8−6.7 (om, 2H), 5.91 (s, 2H), 3.95 (dd, J = 9.3, 5.5, 1H), 3.76 (m, 1H), 2.28−2.05 (om, 3H), 1.96−1.70 (om, 3H), 1.27−1.02 (om, 2H), 0.83 (t, J = 7.1, 3H). ^{13}C NMR (62.896 MHz, $CDCl_3$): δ 179.2, 178.1, 148.0, 147.6, 132.0, 121.3, 108.2, 107.5, 101.2, 58.4, 55.0, 36.9, 30.6, 25.4, 19.0, 13.6.

Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.62; H, 6.88; N, 8.69. Found: C, 59.48; H, 6.87; N, 8.63.

(*R*)-α-Propylpiperonyl Isocyanate (3). To a solution of (*R*)-**11** (1.16 kg, 6.14 mol) in toluene (24 L) was added 12 N HCl (582 mL) over 5−10 min to form a thick slurry. The slurry was azeotropically dried using a Dean−Stark trap. When the distillate was clear, additional toluene (2.4 L) was distilled. The mixture was then allowed to cool to 100 °C, and a solution of phosgene in toluene (1.93 M, 9.54 L) was added over 1 h while a temperature of 100 °C was maintained. The resulting homogeneous solution was heated at 100 °C for an additional 20 min, cooled to 0 °C, washed with 5% $NaHCO_3$ (1 × 18 L, 2 × 9 L) and H_2O (2 × 9 L), and then dried with Na_2SO_4 (2.4 kg). The mixture was filtered, and the filtrate was concentrated to give isocyanate **3** as an oil (1.346 kg, 98% yield). HPLC assay: Inertsil ODS-2, 250 × 4.6 mm; 5 μm; 210 nm; CH_3CN:10 mM pH 6.5 potassium phosphate buffer:MeOH; isocratic, 64:30:6, 1.0 mL/min; t_R {min} **11**, 4.9; **3**, 10.4. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 6.81−6.71 (om, 3H), 5.96 (s, 2H), 4.49 (dd, J = 8.0, 6.0, 1H), 1.88−1.64 (om, 2H), 1.52−1.26 (om, 2H), 0.94 (t, J = 7.4, 3H). ^{13}C NMR (75.469 MHz, $CDCl_3$): δ 147.9, 147.1, 135.5, 119.2, 108.1, 106.2, 101.2, 58.9, 41.7, 19.4, 13.5. IR (film): λ_{max} 2980, 2950, 2890, 2270, 1510, 1495, 1500, 1250, 1040, 935, 810 cm^{-1}. HRMS: [M$^+$] = 219.0880 (calcd = 219.0895).

[*S*-(*R,*S**)]-2-[4-[(4-Methylpiperazin-1-yl)carbonyl]phenoxy]-3,3-diethyl-*N*-[1-(3,4-(methylenedioxy)phenyl)butyl]-4-oxo-1-azetidinecarboxamide (1).** A slurry of (*S*)-β-lactam **2** (1.77 kg, 5.12 mol) and (*R*)-isocyanate **3** (1.12 kg, 5.11 mol) in CH_3CN (23.5 L) was cooled to 4 °C. DBU (76 g, 0.5 mol) dissolved in CH_3CN (0.5 L) was added to the mixture over 1 min with cooling. The mixture was aged for a total of 60 min and then poured into a stirring mixture of H_2O (100 L, containing 1 wt % sodium chloride) and *i*-PrOAc (50 L). The organic phase was washed with H_2O (2 × 20 L, containing 1 wt % sodium chloride) and then saturated aqueous sodium chloride (10 L). The organic phase was concentrated *in vacuo* to 40 L, diluted with *i*-PrOAc (20 L), and reconcentrated to ∼6 L. The concentrate was diluted with MTBE (4 L), reconcentrated, and diluted with MTBE (4 L). The product was crystallized by being heated to reflux and cooled to 0 °C. The mixture was aged for 1 h and filtered. The cake was washed with cold (−10 °C) MTBE (6 L) and dried at rt with a nitrogen flow for 18 h, to give L-694,458 (**1**, 2.25 kg, >99.5% de) as a crystalline white solid. HPLC assay: Inertsil C8, 250 × 4.6 mm; 5 μm; CH_3CN:H_2O (0.1% $HClO_4$); gradient: 25:75 to 100:0 over 20 min; 2.0 mL/min, 230 nm, 25 °C; t_R {min} **2**, 3.6; L-694,458,**1**, 9.9; **3**, 13.8. Chiral SFC HPLC assay: Chiralcel OD(H), 250 × 4.6 mm, MeOH (containing 0.1% TEA) modifier; gradient (8−32%, rate 1%/min); 30 min, 300 bar, 1.0 mL/min, 35 °C, 230 nm; t_R {min} (*R*,*R*)-diastereomer, 11.65 min; (*R*,*S*)-diastereomer, 12.19 min; L-694,458, **1** (*S*,*R*), 14.76 min; (*S*,*S*)-diastereomer, 20.37 min. $[\alpha]^{25}_{589}$ +57.7° (c = 5.0, MeOH). Mp: 117.5−118.8 °C. ^{1}H NMR (399.872 MHz, CD_3CN): δ 7.39 (m, 2H), 7.27 (m, 2H), 6.88 (d, J ∼7, 1H), 6.87 (s, 1H), 6.82 (m, 2H), 5.97 (s, 2H), 5.79 (s, 1H), 4.74 (q, J = 7.0, 1H), 3.54 (v br s, 4H), 2.36 (br s, 4H), 2.26 (s, 3H), 1.97 (m, 1H), 1.89−1.69 (om, 5H), 1.44−1.21 (om, 2H), 1.06 (t, J = 7.5, 3H), 0.95 (t, J = 7.2, 3H), 0.93 (t, J = 7.2, 3H). ^{13}C NMR (100.558 MHz, CD_3CN): δ 172.9, 170.1, 159.0, 150.2, 148.9, 147.7, 137.9, 131.9, 129.8 (2C), 118.0$_5$ (2C), 108.9, 107.6, 102.3, 86.8, 65.2, 55.7 (2C), 54.8, 48 (v br, 2C), 46.2, 43 (v br), 39.4, 24.0, 21.8, 20.2, 14.0, 9.2, 8.8. IR (film): λ_{max} 3350, 1770, 1710, 1630, 1295, 1230 cm^{-1}.

Anal. Calcd for $C_{31}H_{40}N_4O_6$: C, 65.94; H, 7.14; N, 9.92. Found: C, 65.85; H, 7.20; N, 9.84.

Acknowledgment. We are grateful to Ms. Debbie Zink for providing us with the high-resolution mass spectra.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra for compounds **1**−**3**, **4**−**6**, and **9**−**11** (19 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO960618K