The Enantiospecific Synthesis of an Isoxazoline. A RGD Mimic Platelet GPIIb/IIIa Antagonist

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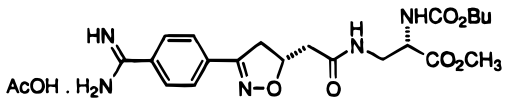
A convergent, large-scale, chiral synthesis of isoxazoline **1** has been achieved in 37% overall yield and >99.6% optical purity, starting from L-asparagine and 4-cyanobenzaldehyde. Hofmann reaction of N_α-*n*-Boc-L-asparagine with iodosobenzene diacetate provides optically pure N_α-*n*-Boc-L-α,*β*-diaminopropionic acid (**8**) in 75% yield. A process of lipase resolution−base catalyzed epimerization gives the single enantiomer **5**. Reaction of acid **5** with amine **9** in the presence of thionyl chloride forms the framework of **1**. A Pinner reaction of intermediate **4** in methyl acetate or anisole, followed by an amidination with ammonium acetate, gives optically pure product **1**.

The application of platelet glycoprotein IIb/IIIa (GPIIb/IIIa) antagonists as antithrombotic agents has been an active research area in recent years.[1] The binding of adhesive proteins such as fibrinogen to GPIIb/IIIa causes platelets to aggregate. This binding is mediated in part by an Arg-Gly-Asp (RGD) recognition sequence. A number of peptides containing the Arg-Gly-Asp sequence (RGD) have been tested as platelet GPIIb/IIIa antagonists;[2] however, development of peptides into therapeutic agents has been hindered by their oral bioavailability and rapid degradation by peptidases.[3] The search for an RGD peptide mimic led to a number of chiral isoxazoline compounds that are very potent GPIIb/IIIa antagonists.[4]

In order to perform clinical studies with these potent antithrombotic agents, an effective large-scale chiral synthesis was required. In addition, we needed to find a stable, pharmaceutically suitable solid drug substance as a development candidate.[5] This paper describes the process used to provide isoxazoline **1**, a crystalline nonpeptide GPIIb/IIIa binding antagonist.



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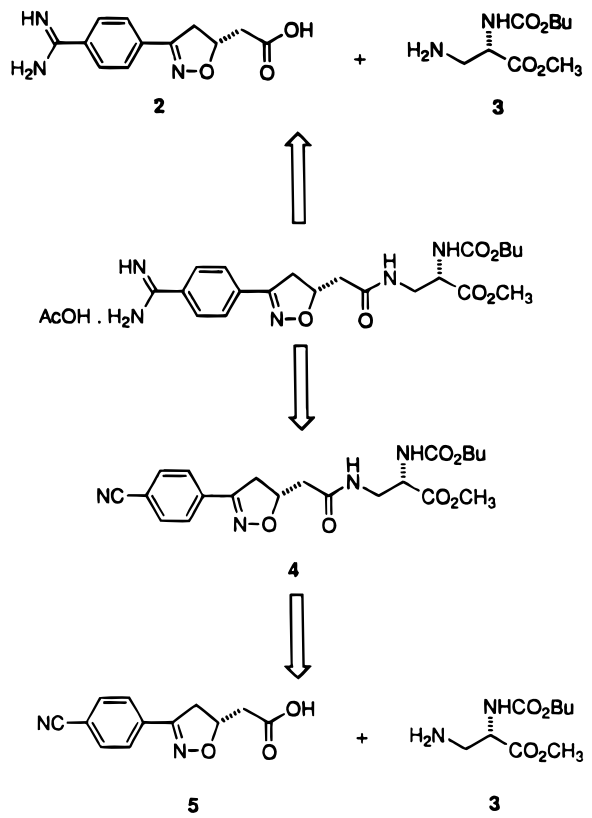
A simple method to produce **1** would be to form the central amide bond as the final step. Accordingly, the product could be synthesized from amidine acid **2** and *N*-Boc-diaminopropionic ester (3) (Scheme 1). However, preparation of optically pure **2** was a tedious process and gave poor overall yield. In addition, isolation of product **1** from the reaction mixture containing **2** was not possible without resorting to preparative reversed-phase HPLC. Because of this, the central amide bond was formed earlier in the sequence from cyano acid **5** and *N*-*n*-Boc-diaminopropionic ester (**3**), making the terminal amidine formation the last step.

Scheme 1



Derivatives of L-2,3-diaminopropionic acid are components of many natural products and synthetic drugs.[6a] Syntheses of N_α-CBZ and N_α-*t*-Boc-diaminopropionic acid *via* Mitsunobu reaction from serine or ring opening of serine *β*-lactone are known.[6b] Additionally, derivatives of L-2,3-diaminopropionic acid have been synthesized from aspartic acid by Schmidt (Curtius) reaction[6c] or from asparagine by Hofmann rearrangement with bis(trifluoroacetoxy)iodobenzene. In an early preparation of product **1**, this right-hand segment was prepared from the commercially available amino acid, N_α-(*n*-butoxycarbonyl)-1,2-diaminopropionic acid (**8**).[6d] Because compound **8** is quite expensive and not readily available in bulk quantities, we decided to develop our own route to this necessary intermediate.

Reaction of L-asparagine (**6**) with *n*-butoxycarbonyl chloride gave *N*-*n*-Boc-L-asparagine (**7**) in 80% yield.

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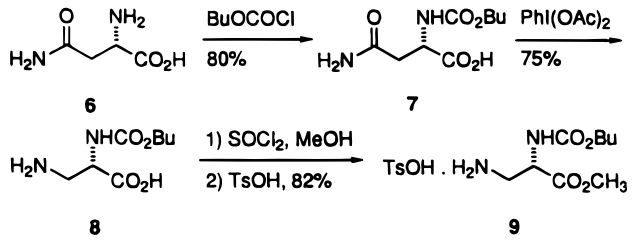
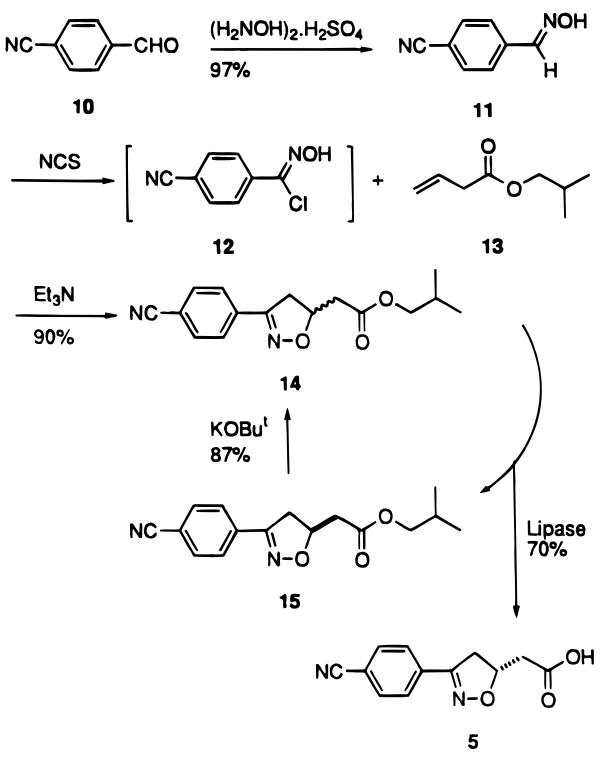
Scheme 2



Standard Hofmann reaction of **7** with the usual reagents such as hypochlorites[7] or NBS/KOH[8] gave little product. Only partial conversion to **8** was observed when **7** reacted with dichloroisocyanuric acid[9] under alkaline conditions. Reaction of **7** with iodobenzene bis(trifluoroacetate)/pyridine[10] produced **8** in about 40% isolated yield. Finally, we found that the Hofmann degradation of asparagine **7** with iodosobenzene diacetate[11] provided the desired product **8** in 75% yield. This chemistry allowed us to prepare optically pure **8** in hundred kilogram quantities. Esterification of **8** with thionyl chloride/methanol, followed by a replacement of HCl with toluenesulfonic acid, gave crystalline **9** in 82% yield (Scheme 2).

The synthesis of the isoxazoline portion of product **1** started from 4-cyanobenzaldehyde (**10**). Reaction of **10** with hydroxylamine sulfate in methanol gave 4-cyanobenzaldoxime (**11**) in 97% yield. Reaction of oxime **11** with *N*-chlorosuccinimide formed the chloride **12**, which converted to the nitrile oxide in the presence of triethylamine. Cycloaddition of **12** with isobutyl vinyl-

Scheme 3



acetate (**13**) *in situ* furnished the racemic **14**.[12] This one-pot reaction provided isoxazoline **14** in 90% yield from oxime **11**. We screened a number of enzymes[13] in the resolution of isobutyl ester **14** and found that enzymatic hydrolysis with lipase PS30 in phosphate buffer (pH 7.4−8.0) provided optically pure acid **5** with the *R* configuration[4,5] and the corresponding chiral ester **15** possessing the *S* configuration. A similar reaction with the methyl ester of **14** failed to produce optically pure material due to concomitant chemical hydrolysis. The recovered optically active ester **15** racemized to **14**, in 1 h, with a catalytic amount of potassium *tert*-butoxide in toluene at 40 °C. This enzymatic resolution−base epimerization sequence provided the optically pure isoxazoline acid (**5**) in 70% yield from raceme **14** on a multikilogram scale (Scheme 3).

Originally, coupling of diamine **9** with isoxazoline **5** to provide optically pure **4** was completed by using typical peptide coupling conditions with the PyBop reagent.[5,14] Because PyBop is an expensive reagent, we looked for an alternative method. Typically, thionyl chloride is not considered a good reagent for amide bond formation because epimerization occurs. We found that thionyl chloride, under a specific set of conditions, can be used for this reaction and still maintain stereochemical integrity. Thus, reaction of acid **5** with amine **9** in the presence of thionyl chloride gave optically pure **4** in 82% yield (Scheme 4).

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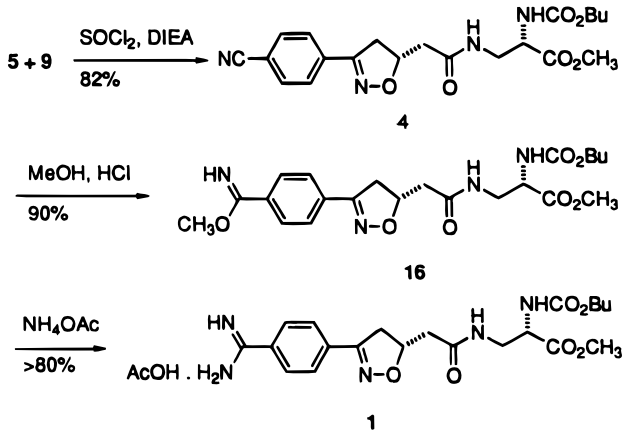
Scheme 4

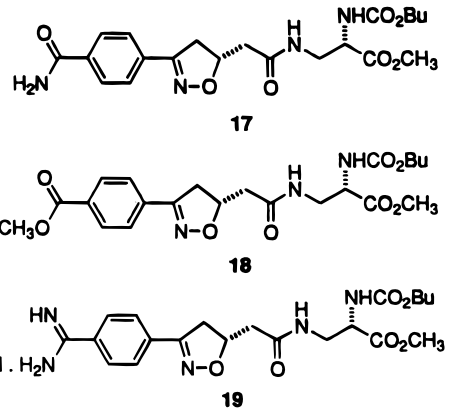


There are a number of reports that detail the synthesis of the amidine functional group.[15] The Pinner reaction, followed by amination, is probably the best known method to prepare amidines from cyano compounds.[16] Reaction of (cyanophenyl)isoxazoline **4** with saturated methanolic hydrogen chloride provided imidate **16** in good yield. However, the imidate was not stable under strongly acidic conditions and partially decomposed during workup. Reaction of **4** in other solvents such as ethanol and 2-propanol effected transesterification as well as decomposition. However, reaction of (cyanophenyl)isoxazoline **4** with HCl in methyl acetate containing a small amount of methanol gave imidate **16** in better than 90% yield and greatly reduced the need for a huge molar excess of HCl. Similar results were also obtained when the reaction was carried out with anisole replacing the methyl acetate.

At laboratory scale, the workup of the Pinner reaction involved a vacuum distillation to remove the excess HCl after conversion to the imidate. At preparative scale, HCl could not be completely removed by vacuum distillation; therefore, imidate **16** converted to the diester **18** due to long-term exposure to acidic conditions. In order to prevent this problem, a neutralization of residual HCl with either ammonia or ammonium acetate was added, followed by filtration to remove the precipitated salts. However, we found that long filtration time or poor temperature control led to partial conversion of **16** to amidine **19**, which then coprecipitated with the undesired ammonium chloride salts. This amidine **19** can be recovered by washing the filter cake with methanol and converted to product **1** by reaction of **19** with ammonium acetate.

Alternatively, other weak bases including sodium or potassium acetate can be used as the neutralizing agent. This allows an easy filtration of salts, keeping the imidate in solution for subsequent controlled conversion to the amidine. However, the acetic acid produced affects the yield of product **1** from the subsequent step, so further optimization is necessary to improve the recovery of the product.

Typically, imidates are converted to amidines by reaction with ammonia or ammonium carbonate. Reaction of imidate **16** with ammonia in methanol did convert the imidate to amidine, but the product was a mixture of



diastereomers. As might be expected, these isoxazoline compounds are very sensitive to base and epimerize easily under alkaline conditions (pH > 10.5). Presumably, the epimerization of the isoxazoline is through a reversible Michael reaction. Although the epimerization was not as severe when ammonia was replaced with ammonium carbonate, the side products amide **17** and diester **18** were generated in the reaction. The amidine product **19**, an HCl salt, existed as an amorphous solid that could not be crystallized. In earlier SAR studies isoxazoline **19** was purified by chromatographic means[4] and the overall yield from **16** to **19** was low.

We have previously described our conversion of this imidate to the acetate salt of the amidine, compound **1**.[5] This reaction worked very well at scale and allowed us to obtain the desired amidine in 74% yield from the cyano compound **4**. The optical purity of product **1** was verified by chiral SFC analysis.

In summary, we describe a convergent nine-step synthesis of isoxazoline compound **1**, at multikilogram scale. Compound **1** was produced in 37% overall yield (based on 4-cyanobenzaldehyde) and greater than 99.6% optical purity. The substance is currently undergoing clinical trial.

Experimental Section

General Methods. All melting points are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, and are reported in DMSO-d_6 (except where noted). Mass spectra were recorded by the analytical laboratory, Chemical Process R&D, DuPont Merck Research Laboratories. The elemental analyses were determined by Quantitative Technologies Inc., Whitehouse, NJ. Solvents, starting materials, and reagents were used as purchased without further purification. Lipase PS30 was purchased from Amano Enzyme USA Co., Ltd.

***N*$_\alpha$-*n*-Boc-L-asparagine (7).** A mixture of L-asparagine monohydrate (67.5 kg, 449.58 mol), water (337.5 L), sodium carbonate (59.4 kg, 560.43 mol), and THF (57.4 L) was heated to 50−55 °C under agitation. A solution of *n*-butyl chloroformate (73.65 kg, 539.24 mol) in THF (57.4 L) was added into the reactor over a period of 3 h. The reaction was held at 50−55 °C for 1 h after addition and then cooled to 15 °C. The pH of the reaction mixture was adjusted to 2.5−3.5 with aqueous HCl (11%). The slurry was agitated at 15−20 °C for 1 h. The product was isolated by filtration, washed with water (3 × 222.8 L), and dried under vacuum (70−75 °C) to constant weight: 83.0 kg, 80% yield; mp 148−9 °C; ^{1}H NMR δ 0.89 (3H), 1.39−1.25 (2H), 1.45−1.57 (2H), 2.37−2.57 (2H), 3.93 (1H), 4.24−4.34 (1H), 6.91 (1H), 7.21 (1H), 7.32 (1H), 12.60 (1H). Anal. Calcd for $C_9H_{16}N_2O_5$: C, 46.55; H, 6.94; N, 12.06. Found: C, 46.68; H, 7.03; N, 12.01.

***N*$_\alpha$-*n*-Boc-L-α,β-diaminopropionic Acid (8).** A solution of *n*-propanol (81.2 kg), methyl acetate (50.4 kg), and water

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(10.8 kg) was cooled to 5 °C. To this solution were added N_α-Boc-L-asparagine (23.5 kg, 101.21 mol) and iodosobenzene diacetate (37.5 kg, 116.39 mol) under agitation. This mixture was warmed to 25 °C over a period of 1 h and stirred at the same temperature for additional 2 h. The reaction mixture was slowly heated to 50 °C over 90 min and then cooled to 3−5 °C. The reaction mixture was held at 3−5 °C for 30 min. The product was isolated by filtration, washed with methyl acetate (2 × 22 kg), and dried under vacuum (50−55 °C) to constant weight: 15.5 kg, 75% yield; mp 215 °C dec; ^{1}H NMR δ 0.90 (3H), 1.27−1.42 (1H), 1.47−1.61 (2H), 3.03 (1H), 3.23 (1H), 3.98 (2H), 4.22−4.32 (1H), 7.56 (1H), 8.05 (3H). Anal. Calcd for $C_8H_{16}N_2O_4$: C, 47.05; H, 7.90; N, 13.72. Found: C, 47.17; H, 8.01; N, 13.72.

Methyl N_α-Boc-L-α,β-diaminopropionate·TsOH (9). A mixture of methanol (130 kg) and N_α-Boc-L-α,β-diaminopropionic acid (22.0 kg, 107.74 mol) was cooled to 10 °C. To this mixture was added thionyl chloride (15.4 kg, 129.44 mol) over a period of 35 min. The reaction was stirred at 20−25 °C for 3−4 h, the solvent was removed under reduced pressure, and toluene (47.6 kg) was added. The residual methanol was removed by azeotropic distillation, and a solution of TsOH (anhyd, 117.23 mol) in methyl acetate (41.5 kg) was added. The solution was distilled to an oil, and methyl acetate (113 kg) and heptane (101 kg) were added. The solution was cooled to 0−5 °C and held at 0−5 °C for 4 h. The product was isolated by filtration, washed with methyl acetate/heptane (40 kg, 1/1 ratio), and dried under vacuum (50−55 °C) to constant weight: 34.9 kg, 82% yield; mp 95−96 °C; ^{1}H NMR δ 0.88 (3H), 1.25−1.40 (2H), 1.47−1.59 (2H), 2.28 (3H), 3.03 (1H), 3.21 (1H), 3.66 (3H), 3.97 (2H), 4.30−4.41 (1H), 7.11 (2H), 7.47 (2H), 7.66 (1H), 7.92 (3H). Anal. Calcd for $C_{16}H_{27}N_2O_7S$: C, 49.09; H, 6.95; N, 7.16. Found: C, 49.32; H, 6.76; N, 7.13.

4-Cyanobenzaldoxime (11). A solution of methanol (272.1 L), 4-cyanobenzaldehyde (50 kg, 381.3 mol), and hydroxylamine sulfate (36.1 kg, 219.7 mol) was stirred at 55−60 °C for 3 h, and then water (272 L) was added. The mixture was cooled to 0−5 °C and held for 30 min. The crude product was collected by filtration. The filter cake was washed with a mixed solvent of cold methanol and water (2/3 ratio, 735.0 L) and water (750.0 L) and dried under vacuum (60−70 °C) to constant weight: 54.1 kg, 97% yield; mp 174−6 °C; ^{1}H NMR δ 7.82 (2H), 7.88 (2H), 8.26 (1H), 12.00 (1H). Anal. Calcd for $C_8H_6N_2O$: C, 65.75; H, 4.14; N, 19.17. Found: C, 65.73; H, 4.26; N, 19.14.

(+)-Isobutyl 2-[3-(4-Cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetate (14). To a solution of DMF (262.0 L), 4-cyanobenzaldoxime **(11)** (46 kg, 342.1 mol), and *N*-chlorosuccinimide (52 kg, 389.4 mol) was added isobutyl vinylacetate (95 kg, 665.7 mol). The solution was cooled to 2−6 °C, and triethylamine (40 kg, 388.6 mol) was slowly added over a period of 4 h. The reaction was stirred at the same temperature for an additional 1 h. Water (330.0 L) and hydrochloric acid (1 N, 49 L) were added. The crude product was collected by filtration, washed with water (555.0 L), and redissolved in toluene (500.0 L, 40 °C). The organic layer was washed with water (291.0 L) and dried by azeotropic distillation (removing about 250 L of toluene). Heptane (300.0 L) was added, and the reaction was cooled at 0−5 °C for 3 h. The product was collected by filtration and washed with toluene/heptane (150.0 L, 1/2 ratio). The product was dried under vacuum at 55−60 °C to constant weight: 81.8 kg, 90% yield; mp 98−100 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (6H), 1.96 (1H), 2.70 (1H), 2.92 (1H), 3.15 (1H), 3.56 (1H), 3.90 (2H), 5.20 (1H), 7.70 (2H), 7.80 (2H). Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 67.06; H, 6.20; N, 9.76.

(*R*)-2-[3-(4-Cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetic Acid (5). A suspension of H_2O (597.0 L), $NaH_2PO_4·H_2O$ (60.0 kg), aqueous NaOH (33%, 36.0 L), Triton X-100 (3.2 kg), compound **14** (40.0 kg, 139.7 mol), and lipase PS30 (4.0 kg, enzyme content 8%) was slowly heated to 40 °C and held in the temperature range of 40−43 °C until the resolution was completed (∼16 h). The pH of the reaction mixture was maintained between 7.4 and 8.0 and adjusted by the addition of 33% aqueous NaOH. The batch was cooled to 20−25 °C when the reaction was completed, and the pH of the reaction mixture was adjusted to between 8.0 and 8.2 by the addition of aqueous NaOH (33%, 11.0 L). The crude **15** was collected by a filtration through a layer of Celite (20 kg) and washed with water (70 L). This crude **15** was recycled through a racemization step: ^{1}H NMR ($CDCl_3$) δ 0.95 (6H), 1.80 (1H), 2.69 (1H), 2.91 (1H), 3.14 (1H), 3.54 (1H), 3.91 (2H), 5.13−5.23 (1H), 7.68−7.78 (4H).

The pH of the solution of filtrate (∼800 L) and isopropyl acetate (20 L) was adjusted to 2.8−3.2 with concentrated hydrochloric acid (∼57 kg). The crude product **5** was precipitated, collected by filtration, and washed with water (70 L). This crude product was crystallized from hot ethanol (525.0 L) to give optically pure **5**. Isoxazoline **5** was collected by filtration, washed with ethanol (76.0 L), and dried to constant weight: 12.3 kg, 77% yield based on the amount of **5** in **14**; mp 198−200 °C; ^{1}H NMR δ 2.70 (2H), 3.20 (1H), 3.59 (1H), 5.00−5.10 (1H), 7.78−7.91 (4H), 12.44 (1H). Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.61; H, 4.38; N, 12.17. Found: C, 62.39; H, 4.49; N, 11.98.

Racemization of 15 to 14. A solution of toluene (414.0 L) and crude **15** (∼120 kg wet cake) was heated to 50 °C and filtered to remove Celite (∼40 kg). The filter cake was washed by toluene (72.0 L). The organic layers were combined and washed by brine (108.0 L). After removal of the aqueous layer, the organic layer was dried by azeotropic distillation to constant boiling point (111 °C). The batch was cooled to 40 °C, and potassium *tert*-butoxide in *tert*-butyl alcohol (1 N, 1.6 L) was added. The reaction was agitated (200 rpm) at 40 °C until racemization was completed. The batch was cooled to 20−25 °C, and water (108.0 L) was added. The reaction mixture was neutralized by the addition of aqueous hydrochloric acid (1 N, 1.6 L). The aqueous bottom layer was removed, and the organic layer was concentrated by distillation. The reaction was cooled to 60 °C when ∼380.0 L of toluene was removed. To this solution was added heptane (115 L), and the reaction was held at 50 °C for 1 h. The mixture was cooled to 0−5 °C and held for 2 h. The product **14** was collected by filtration and washed with a mixed solvent of toluene and heptane (1/2 ratio, 70 L). The product **14** was dried under vacuum (50−55 °C) to constant weight: 17.3 kg, 87% yield based on the *S*-isomer in **14**.

(*R*)-Methyl-3-[[[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetyl] amino]-*N*-(butoxycarbonyl)-L-alanine (4). A solution of acetonitrile (204.0 L), acid **5** (12.0 kg, 52.10 mol), amine **9** (22.4 kg, 57.30 mol), and thionyl chloride (6.8 kg, 57.30 mol) was stirred at 0−5 °C for 1 h. To this solution was added diisopropylethylamine (22.2 kg, 172.00 mol) at 20 °C over a period of 90 min. Water (612.0 L) was added after the reaction. The crude product **4** precipitated out. This crude **4** was collected by filtration and washed with water (96.0 L). The wet cake was dissolved in hot methanol (50−60 °C, 311.0 L), and any insoluble particles were removed by filtration. The solution was cooled at 0−5 °C for 3 h, and the product was collected by filtration and washed with methanol (75.0 L). The product was dried under vacuum (55−60 °C) to constant weight: 18.3 kg, 82% yield; mp 154−6 °C; ^{1}H NMR δ 0.92 (3H), 1.37 (2H), 1.59 (2H), 1.67 (1H), 2.58 (1H), 2.71 (1H), 3.22 (1H), 3.51 (1H), 3.67 (2H), 3.77 (3H), 4.06 (2H), 4.44 (1H), 5.14 (1H), 5.70 (1H), 6.38 (1H), 7.70 (2H), 7.77 (2H). Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.61; H, 4.38; N, 12.17. Found: C, 62.39; H, 4.49; N, 11.98.

(*R*)-Methyl-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Monoacetate (1). A solution of methyl acetate (55.8 L), methanol (4.8 L), HCl (9.6 kg), and compound **4** (12.0 kg, 27.88 mol) was cooled to −20 °C and stirred under 3−5 psi (HCl) at 10 °C for 27 h. After the reaction, the HCl was removed under vacuum, and the methyl acetate (21.5 L) and methanol (63.2 L) were added. The residual HCl was neutralized with ammonia (2.5 kg) under 10 °C. The resulting ammonium chloride was removed by filtration. The filter cake was washed with methyl acetate and methanol (20.0 L). To the filtrate was added ammonium acetate (6 kg), and the reaction was stirred at room temperature overnight. The crude product was collected by filtration and recrystallized from hot methanol to give **1**: 10.4 kg, 74% yield; mp 213−4

°C; $[\alpha]^{25}_D$ −86.55° (methanol, $c = 0.6$); ^{1}H NMR δ 0.86 (3H), 1.30 (1H), 1.51 (1H), 1.71 (3H), 2.42 (1H), 2.55 (1H), 3.18 (2H), 3.27 (1H), 3.54 (2H), 3.62 (3H), 3.93 (2H), 4.16 (1H), 5.01 (1H), 7.49 (1H), 7.82 (4H), 8.20 (1H), 10.24 (4H); ^{13}C NMR δ 13.52, 18.48, 24.56, 30.61, 38.88, 39.66, 40.58, 51.97, 53.61, 63.83, 78.41, 126.67, 127.93, 130.93, 133.42, 156.07, 156.13, 165.12, 169.29, 171.06, 176.35. Anal. Calcd for $C_{23}H_{33}N_5O_8$: C, 54.53; H, 6.55; N, 13.80. Found: C, 54.45; H, 6.61; N, 13.67.

Alternate Synthesis of 1 from 4. A solution of anisole (2.5 L), methanol (141 mL), HCl (375 g), and compound **4** (0.5 kg, 1.16 mol) was stirred at 0−5 °C for 14 h. After the reaction, HCl was removed by decantation of anisole (50% of the volume) and vacuum distillation. The residual HCl was further neutralized by a saturated solution of ammonium acetate in methanol under 5 °C. The resulting ammonium chloride (314 g) was removed by filtration. To this filtrate was added ammonium acetate (425 g, 4.75 mol). The reaction was stirred at 25−30 °C for 15 h. Product **1** (0.45 kg, 76% yield) was isolated by filtration, washed with methanol, and dried under vacuum (65 °C). The spectral data of **1** were identical to those from above.

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