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Articles

Process Development in the Synthesis of the ACE Intermediate MDL 28,726

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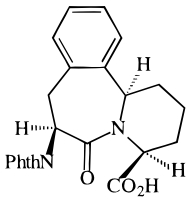
Abstract:

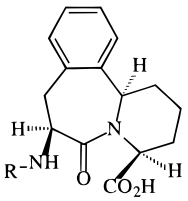
MDL 28,726 is a key intermediate in the synthesis of the ACE inhibitors MDL 27,210A and MDL 100,240. An efficient nine-step synthesis of this tricyclic acid, which has three chiral centers, was developed beginning with 3,4-dihydro-2*H*-pyran. A key step in the synthesis features an enzyme-catalyzed resolution of the lithium salt of the *N*-trifluoroacetamide of (*R*,*S*)-6-hydroxynorleucine. All of the steps were optimized and completed in reactor equipment using environmentally acceptable processes. Process development of this route is described.

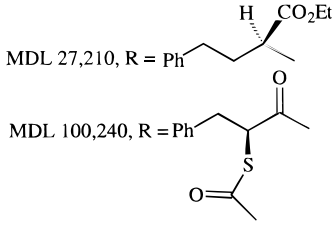
Introduction

The tricyclic acid MDL 28,726 is a key intermediate in the synthesis of the ACE inhibitors MDL 27,210A[1] and MDL 100,240.[2] The initial synthesis of MDL 28,726 utilized a

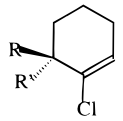


MDL 28,726

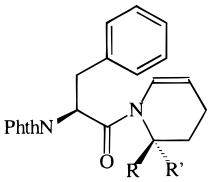




racemic mixture of amines **1a** and **1b** and required the separation of the diastereomic acylenamines **2a** and **2b** by preparative chromatography.[1] This route was improved by



1a R = NH_2, R' = H
1b R = H, R' = NH_2



2a R = CO_2CH_3, R' = H
2b R = H, R' = CO_2CH_3

resolving the racemic mixture of amines with (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate to provide the needed (*S*)-amine **1a**. However, an optimized large-scale (1 kg) resolution provided amine **1a** with an optical purity of only 93% ee.

The subject of this paper is an improved synthesis of MDL 28,726, starting from 3,4-dihydro-2*H*-pyran[3] (Scheme 1), which ultimately was optimized and scaled up to reactor equipment.

Results and Discussion

The first step of the process was the synthesis of 5-(4-hydroxybutyl)hydantoin, **3**. The preparation of **3** was optimized in 5-L glassware after determining the effect of varying the unit ratios of hydrochloric acid, potassium cyanide, acetic acid, and ammonium carbonate on the yield of the hydantoin. Laboratory studies revealed that the most critical variable in the process was the unit ratio of potassium cyanide. When the process was run with a 25% excess of potassium cyanide, a lower yield of off-colored hydantoin, which was difficult to filter, was obtained. Changes in the unit ratios of the other raw materials had little effect on the

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[⊥] Present address: Alza Corp., 1015 Joaquin Rd., Mountainview, CA 94043.
[∥] Present address: Roche Colorado Corp., 2075 N. 55th St., Boulder, CO 80301.

(1) Flynn, G.; Giroux, E.; Dage, R. *J. Am. Chem. Soc.* **1987**, *109*, 7914.
(2) Flynn, G.; Warshawsky, A. M.; Mehdi, S.; Bey, P.; Beight, D. W.; Giroux, E.; Burkholder, T. P. (Merrell Dow Pharmaceuticals Inc.). U.S. Patent 5,430,145, July 4, 1995.
(3) Gaudry, R. *Can. J. Res., Sect. B* **1948**, *26*, 387.

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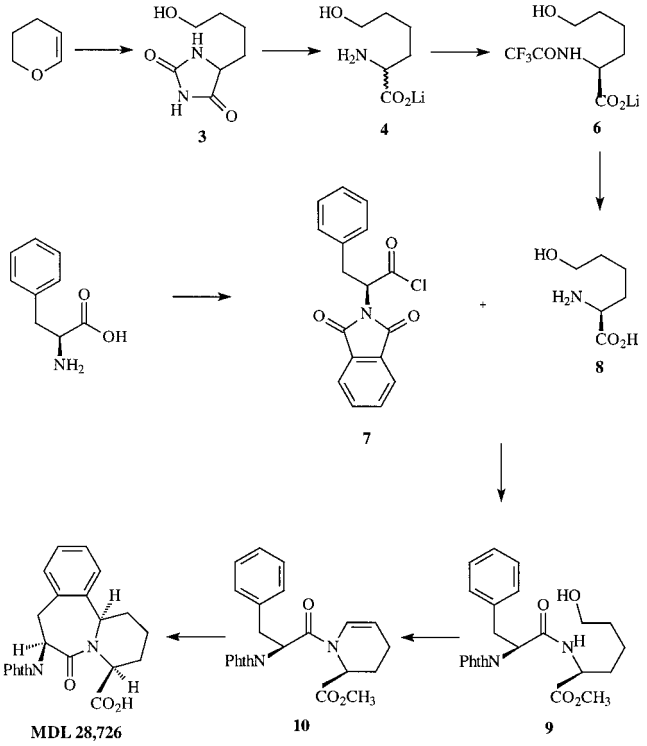
Scheme 1



yield of **3**. Ultimately, this process was used in 4000-gal equipment to produce as much as 854 kg of hydantoin in a single run in a 54% yield, mimicking the results obtained in 5-L glassware.[4]

During the initial small-scale studies, **3** was hydrolyzed with barium hydroxide in an autoclave at 160 °C to give racemic 6-hydroxynorleucine in 94% yield. Barium salts were isolated as barium carbonate after treatment of the reaction mixture with ammonium carbonate.

Temperature constraints in plant equipment limited the hydantoin hydrolysis temperature to <140 °C. In addition, leaching tests on barium carbonate indicated that barium levels in the leachate were 55 times greater than that permitted for landfill disposal. Therefore, the use of lithium hydroxide at 135 °C was examined for the hydrolysis of the hydantoin. Hydrolysis of **3** with excess lithium hydroxide occurred in 20 h in glassware to provide the desired **4** in high yield (>90%). The carbon dioxide used to convert the excess lithium hydroxide to lithium carbonate in the laboratory runs was supplied by the addition of a large excess of dry ice to the vented reaction flask. When the hydrolysis was carried out in pilot plant equipment, with subsequent addition of CO_2(g), the desired lithium salt, **4**, contained about 15% of the free carboxylic acid, **5.** Apparently, the solution concentration of carbon dioxide was higher in plant equipment because a pressurized vessel was used. As shown in Scheme 2, as the concentration of CO_2 increases, the lithium salt **4** can be converted to **5** and lithium carbonate, and the amount converted depends on the relative solubilities of the lithium carbonate and **4**, as well as the concentration of carbon dioxide.

Subsequently, the need for the CO_2 addition was eliminated by hydrolyzing the hydantoin with a stoichiometric amount (3 equiv) of lithium hydroxide at 135 °C using a reaction time of 20 h. The lithium carbonate was precipitated with *n*-propanol and removed by filtration, and the resulting solution of **4** was concentrated to remove water. Solvent exchange into *n*-butanol precipitated **4,** which was used as a wet cake in the subsequent trifluoroacetamide formation step.

During the initial small-scale work, the *N*-trifluoroacetamide was obtained by treating **4** with methyl trifluoroacetate in the presence of 1,1,3,3-tetramethylguanidine. Using an improved procedure, a slurry of **4** in methanol at 8 °C was treated, in the presence of a small amount of sodium methoxide (0.2 equiv), with 1.2 equiv of methyl trifluoroacetate. This resulted in the complete conversion of **4** to the lithium salt **6**. It was crucial that conversion be complete at this point, since any unconverted material negatively impacted the optical purity obtained in the subsequent enzymatic resolution step. After the pH was adjusted to 5.0 with acetic acid, water was distilled off in vacuo to aid in the removal of residual methanol and *n*-butanol, which can act as inhibitors for the enzymatic resolution. It was particularly important in plant equipment to minimize the amount of time required for this operation, since excessive heating led to small amounts (1−2%) of **4**, which subsequently lowered the optical purity of **8**.

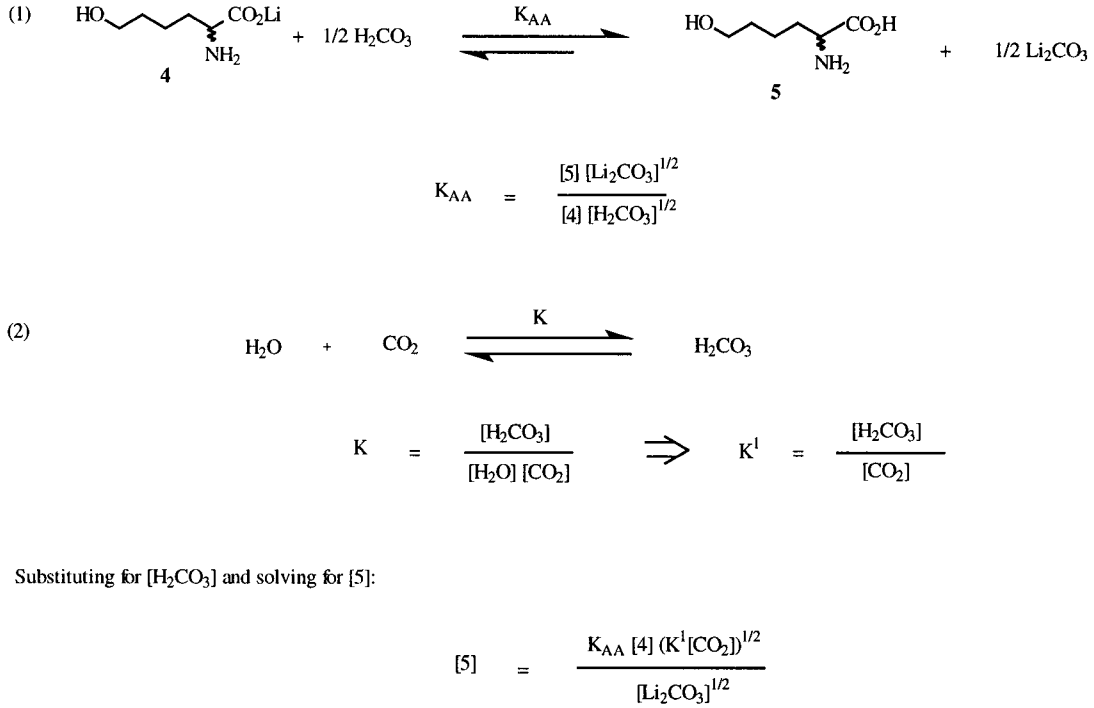
On a small scale, the *N*-trifluoroacetamide was purified by crystallization from methylene chloride. Scale-up to pilot plant equipment presented enviromental problems, particularly with vapor containment on centrifugation of the product. On a large scale, lithium salt **6** was used in situ for the enzymatic resolution.

A key feature of the synthesis of MDL 28,726 was the enzyme-catalyzed resolution of the *N*-trifluoroacetamide with

(4) **Use of KCN requires extreme caution!** (a) Laboratory Preparation: This reaction was run in a well-ventilated hood. The reactor was vented to a bleach/caustic scrubber during the cyanohydrin-forming reaction. One should be completely familiar with the symptoms of cyanide poisoning and the first aid treatment procedures for cyanide poisoning before attempting to repeat this procedure. Complete familiarity with the Material Safety Data Sheet for potassium cyanide is recommended. (b) Plant Reaction: A potassium hydroxide-stabilized (pH 12−12.5) solution of potassium cyanide in water, sufficient for the entire plant campaign, was prepared from potassium cyanide briquettes and stored in a stainless steel tank. The line from the tank to the cyanohydrin reactor was welded stainless steel (no flanges to eliminate the possibility of leaks). The operating area was equipped with a central hydrogen cyanide-monitoring system, with sensors located at points where high potential for leaks existed (flanges, seals, etc.). All operating personnel were equipped with Compur Monitox HCN personal monitors with 5/10 ppm alarm settings. A dedicated caustic/bleach scrubber was used to treat the vent stream from the cyanohydrin reactor. The pH of the scrubber was maintained above 12 to prevent the rapid formation of ammonia and carbon dioxide gas due to the decomposition of cyanate which can occur below pH 9. A dedicated venturi water scrubber was used to treat the vent stream from the hydantoin reactor. Under no circumstances was any stream containing ammonia allowed to come in contact with the caustic/bleach scrubber. The thermochemistry of all reactions was completely evaluated by differential scanning calorimetry (DSC) and thermodynamic calculations. In addition, the thermochemistry of the entire process was evaluated using an RC-1 calorimeter. (c) The safety and pre-startup issues involved in the pilot plant preparation of the hydantoin have been presented: DesJardin, M. A.; Foot, K. J.; Goralski, C. T.; Ressler, R. J.; Striebel, G. R.; Legge. J. B. Challenges of the 500X Scale-up: Process Design and Scale-up Issues for the Synthesis of a Hydantoin. Presented at the 1994 Annual Meeting of the American Institute of Chemical Engineers, San Francisco, CA, Nov 13−18, 1994; Abstract 26b.

(5) Hydrolysis of 5-(4-hydroxybutyl)hydantoin with barium carbonate has been previously described: see ref 3.

Scheme 2



acylase I.[6,7] After the pH of an aqueous solution of **6** was adjusted to 7.5 with LiOH, acylase I was added, and the pH was continuously readjusted to 7.1−7.5[8] by the addition of 0.09 N LiOH. When the pH stabilized, it was adjusted to 5.0 with acetic acid, and then the mixture was heated to 70 °C to denature the enzyme. The desired (*S*)-enantiomer was subsequently isolated in high optical purity (97% ee) and very good yield (42.7%) by diluting a concentrated aqueous phase with ethanol.

A process was developed for recovery of (*R*)-6-hydroxynorleucine from the mother liquor, with subsequent racemization to (*R*,*S*)-6-hydroxynorleucine lithium salt, **4**, suitable for recycle to the desired (*S*)-enantiomer. The process consisted of treating the mother liquor with lithium hydroxide while exchanging the ethanol crystallization solvent with water to effect hydrolysis of the trifluoroacetamide. The excess lithium hydroxide was precipitated as lithium carbonate and filtered off. After water was exchanged for methanol by distillation, (*R*)-6-hydroxynorleucine was precipitated by addition of acetic acid. The (*R*)-amino acid was heated with aqueous lithium hydroxide at 130 °C for 4 days to provide the racemic amino acid **4** in good yield (70−80%).

Amino acid **8** was converted to its corresponding methyl ester without difficulty on a small scale by using trimethyl orthoformate with HCl in methanol. After concentration, this ester was coupled with *N*-phthaloyl-L-phenylalanine acid chloride to give recrystallized **9** in very good yield (85−87%). The initial scale-up of this process in pilot plant equipment gave a very low (27%) yield of **9**. It was determined that excess HCl present in the methanol during the esterification produced water during the evaporation step by forming methyl chloride or dimethyl ether. This water then hydrolyzed the methyl ester back to acid **8**.

This problem was solved by quenching the reaction mixture with triethylamine after methyl ester formation. Methanol was replaced by toluene, and the triethylamine hydrochloride that precipitated was filtered off, providing a solution of the amino methyl ester in toluene. Subsequent coupling of the free base with an equivalent amount of acid chloride, **7**, provided **9** in very good yield (85.5%).

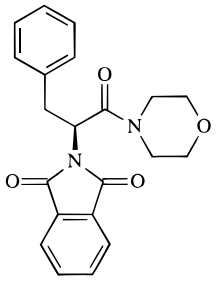
Acid chloride **7** was prepared from L-phenylalanine by reaction with phthalic anhydride in toluene in very high yield (97%) in pilot plant equipment, employing a Dean−Stark trap for the azeotropic removal of water. Conversion to the acid chloride was accomplished with oxalyl chloride. HPLC analysis was used to ensure complete conversion to the acid chloride. The solvent was then replaced with ethyl acetate prior to coupling the resulting **7** with the amino methyl ester.

Coupling of the amino methyl ester with an equivalent amount of the acid chloride **7** proceeded in good yield (86%) using excess *N*-methylmorpholine in acetonitrile/ethyl acetate/toluene. Acetonitrile was necessary as a cosolvent to minimize coprecipitation of the amino methyl ester with *N*-methylmorpholine hydrochloride. The solution of acid chloride **7** was titrated immediately before use with excess morpholine, and the quantity of amide **11** formed was used to estimate the concentration of the acid chloride. Use of excess acid chloride led to formation of bis-adduct **12**.

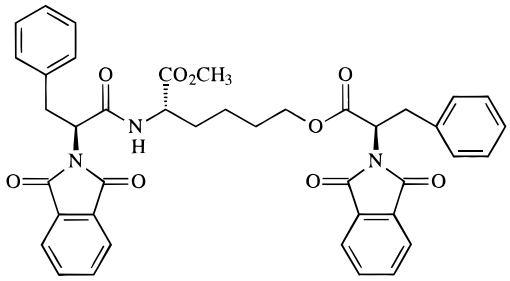
(6) The high selectivity of the cleavage of *N*-acyl-L-amino acids is well documented, e.g.: (a) Sabioni, G.; Shea, M. L.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 236. (b) Schneider, M.; Engel, N.; Honicke, P.; Heineman, G.; Gorisch, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 67. (c) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354.

(7) Synthesis of (*S*)-6-hydroxynorleucine via an enzymatic resolution of the *N*-acetyl derivative of (*R*,*S*)-6-hydroxynorleucine using a similar procedure has been described: Bodanszky, M.; Martinez, J.; Priestly, G. P.; Gardner, J. D.; Mutt, V. *J. Med. Chem.* **1978**, *21*, 1030.

(8) The pH dependence of maximum velocity has a bell-shaped profile with the maximum at pH 7.5: Galaev, I. Y.; Svedas, V. K. *Biochim. Biophys. Acta* **1982**, *701*, 389.



11



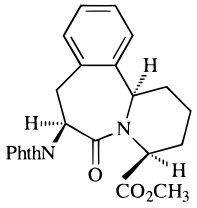
12

Hydroxy ester **9** was isolated by crystallization from toluene. The isolated yield of **9** decreased as the concentration of **12** increased. Crystallization of **9** proved to be problematic in one of the 20-kg-scale reactions in pilot plant equipment. As a consequence, it was decided to isolate **9** as a solution in methylene chloride, which was advantageous since the next two steps were performed in this solvent.

Hydroxy ester **9** was converted to an aldehyde using a Swern oxidation.[9] The initial small-scale oxidations were performed at −50 °C by adding **9** to a mixture of dimethyl sulfoxide (DMSO)/oxalyl chloride, followed by the addition of triethylamine. Scale-up (1.5 kg) of the oxidation was accomplished at higher temperature (−20 to −15 °C) by treating a mixture of **9** and DMSO in methylene chloride with oxalyl chloride, followed by the addition of triethylamine. The dimethyl sulfide generated during this oxidation was conveniently oxidized by treating the reaction mixture with OXONE. The intermediate aldehyde was converted in situ to the acylenamine **10** by addition of trifluoroacetic acid. When the Swern oxidation of **9** was attempted in pilot plant equipment at −15 °C, a lower than expected yield (31% vs 55%) of **10** was obtained. Subsequent laboratory experiments revealed that the addition times for the oxalyl chloride and triethylamine in pilot plant equipment, each over 2 h in order to maintain a −15 °C reaction temperature, were sufficient to allow the activated DMSO to decompose before all of the triethylamine was added. Further reactions in pilot plant equipment were done below −40 °C, which permitted faster addition of oxalyl chloride and triethylamine. The expected yield (52%) of **10** was obtained.

Attempted conversion of **10** to MDL 28,726 using CH_3SO_3H gave unidentified byproducts and none of the desired product. While H_2SO_4 would effect the desired cyclization, the yield was very low (<25%) and nonreproducible, as well.

Treatment of **10** with triflic acid[10,11] gave the desired tricyclic acid MDL 28,726. Initial experiments revealed the presence of significant quantities (5−10%) of ester **13**.



13

Addition of trifluoroacetic acid anhydride (TFAA) to the reaction medium served as a methanol "sponge" and completely eliminated the formation of this impurity. The use of either trifluoromethanesulfonic acid anhydride or acetic acid anhydride gave a cyclization reaction that was slower than in the case where neither anhydride was present. In contrast, TFAA did not impede the reaction rate.

The overall yield for the conversion of amino acid **8** to MDL 28,726 was 38% when the intermediates were isolated. On scale-up to pilot plant equipment, this process was simplified by not isolating intermediates **9** and **10**, but instead carrying them forward as their crude reaction mixtures. This approach eliminated a solvent exchange, the use of two solvents, and the solids handling operations associated with two crystallization steps.

When **10** was isolated, the cyclization reaction was complete in about 22 h with a triflic acid loading of 7 mol of triflic acid/mol of **10**. By not isolating **10**, this ratio of reactants resulted in a reaction time of 88 h to reach completion. Upon increasing the triflic acid loading to 11 mol/mol of **10**, the reaction reached completion in about 24 h. While not isolating intermediates was less efficient in the use of triflic acid, very importantly, the overall yield for the last three steps improved to 55%, and the quality of the resultant MDL 28,726 was not compromised.

Conclusions

An efficient synthesis of the tricyclic acid MDL 28,726 was developed beginning from dihydropyran, requiring nine steps and proceeding in an overall yield of 11%. The synthesis established the proper stereochemistry[12] for the three chiral centers in this molecule and permitted the preparation of bulk quantities of this key intermediate using an environmentally acceptable process.

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded using a Varian XL 300 or Gemini 300 spectrometer operating at

(9) Mancuso, A. J.; Brownfain, D. S.; Swern, D. *J. Org. Chem.* **1979**, *44*, 4148.

(10) The facile acylation of arenes using triflic acid has been known for some time: Effebberger, F.; Epple, G. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 300.

(11) Acylation of benzene using higher concentrations of triflic acid has been described: Corriu, R.; Dobosi, G.; Germain, A. *Bull. Soc. Chim. Fr.* **1972**, *4*, 617.

(12) The relative stereochemistry was confirmed by conversion of MDL 28,726 to a benzhydryl ester, whose structure was determined by X-ray crystallography: see ref 1.

(13) Acylase I has moderate thermal stability: Gentzen, I.; Loffler, H.-G.; Schneider, F. *Z. Naturforsch.* **1980**, *35c*, 544.

300 or 75 MHz, respectively. Mass spectra (MS) were obtained on a Finnigan MAT 4600 spectrometer. Melting points were recorded using a Thomas-Hoover melting point apparatus and are uncorrected.

5-(4-Hydroxybutyl)hydantoin, 3. A total of 1.0 L of 0.2 N HCl was charged to a 3-L three-necked flask fitted with a stirrer, thermometer, and continuous nitrogen purge. The stirred solution was heated to 40 °C, and 250 g (2.97 mol) of 3,4-dihydro-2*H*-pyran was added. The temperature of the reaction mixture rose to 60 °C. After the mixture was cooled to 5 °C and stirred for 2 h, 190 g (3.16 mol) of acetic acid was added, followed by a solution of 195 g (2.99 mol) of KCN (**Use of KCN requires extreme caution! See footnote 4.**) in 325 mL of H_2O, stabilized with 1.09 g of 90% KOH. The temperature of the reaction mixture rose to 30 °C. The mixture was stirred for 1.5 h, during which time it cooled to 20 °C.

A separate 5-L three-necked flask, fitted with a stirrer, condenser, thermometer, continuous nitrogen purge, and pressure-equalizing addition funnel, was charged with a mixture of 503 g (4.62 mol) of ammonium carbonate and 1 L of H_2O. The stirred mixture was heated to 60 °C, and the cyanide mixture was added over 20 min. After the reaction mixture was heated and stirred at 60 °C for 2 h, it was concentrated by distillation until the pot temperature reached 102−103 °C. A total of 850 mL of distillate was collected. The contents of the pot were stirred and cooled to 5 °C and held there for 2 h. Crude product was filtered off, washed with 1 L of ice water, and then dried at 65 °C/50 Torr to give 286.1 g, 55.9% yield, of **3**.

Crude product (140.21 g) was dissolved in 700 mL of H_2O at reflux. The stirred solution was allowed to cool to room temperature, and then the mixture was cooled and stirred at 5 °C for 2 h. Solid which crystallized was filtered off, washed with 100 mL of ice water, and then dried at 75 °C/50 Torr to give 124.4 g (88.7% recovery) of **3**, mp 154−156 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.53 (s, 1H), 7.90 (s, 1H), 4.38 (s,1H), 4.02−3.95 (m, 1H), 3.37 (s, 2H), 1.74−1.20 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.8, 157.2, 60.2, 57.3, 31.8, 30.8, 20.5. MS (CI, CH_4): *m*/*z* (relative intensity) 148 (68), 130 (15), 102 (100), 85 (19), 84 (23).

(*R*,*S*)-6-Hydroxynorleucine Lithium Salt, 4. A 600-mL stainless steel autoclave was charged with 86.1 g (0.5 mol) of (*R*,*S*)-5-(4-hydroxybutyl)hydantoin and 64.2 g (1.53 mol) of lithium hydroxide monohydrate in 250 mL of H_2O. The stirred mixture was heated at 135 °C under an atmosphere of N_2 for 20 h. After cooling to ambient temperature, the reaction mixture was transferred to a 1-L, round-bottom flask. The autoclave was rinsed with 3 × 75 mL of H_2O, and the rinse was added to the reaction mixture. The suspension was concentrated by atmospheric distillation of 380 mL of H_2O. The residue was cooled to ambient temperature and diluted with 100 mL of H_2O and 300 mL of *n*-propanol. The stirred suspension was heated to 90 °C and then cooled to ambient temperature. Li_2CO_3 was filtered off and rinsed with 3 × 100 mL of *n*-propanol. The filtrate was charged to a 2-L, round-bottom flask and distilled while simultaneously adding *n*-butanol so as to maintain a constant volume of 1 L. Crystallization began when the boiling point reached 110 °C. The distillation was continued with the simultaneous addition of *n*-butanol until the boiling point reached 115 °C. After the stirred mixture was cooled to room temperature, the product was filtered off, washed with 3 × 75 mL of *n*-butanol, and then dried at 100 °C/50 Torr to give 73.1 g of **4**, mp 178−180 °C, 95.4% yield.

^{1}H NMR (300 MHz, D_2O): δ 3.57 (t, J = 6.3 Hz, 2H), 3.26 (t, J = 6.5 Hz, 1H), 1.73−1.48 (m, 4H), 1.4−1.26 (m, 2H). ^{13}C NMR (75 MHz, D_2O): δ 182.7, 61.5, 55.8, 33.9, 31.2, 21.3. MS (CI, CH_4): *m*/*z* (relative intensity) 148 (75), 130 (16), 102 (100), 85 (20), 84 (24).

(*S*)-6-Hydroxynorleucine, 8. A suspension of 76.6 g (0.5 mol) of (*R*,*S*)-6-hydroxynorleucine lithium salt and 1.5 g of lithium carbonate in 40 mL of *n*-butanol and 300 mL of methanol was heated at reflux for 10 min and then cooled in an ice bath to 17 °C. To the suspension was added 73.6 g (0.575 mol) of methyl trifluoroacetate, and within 5 min the solution warmed to 30 °C. The solution was held at reflux for 45 min, and an additional 10.0 g (0.08 mol) of methyl trifluoroacetate was added. After an additional 1 h at reflux, 0.5 g of Li_2CO_3 and 10.0 g (0.08 mol) of methyl trifluoroacetate were added. After an additional 1 h at reflux, the reaction mixture was concentrated by the atmospheric distillation of about 175 mL of solvent, followed by the addition of 6.6 g (0.11 mol) of acetic acid at 75 °C. The distillation was continued until a total of 325 mL of solvent was removed and the bp reached 82 °C. The residue was diluted with H_2O to a total volume of 500 mL. The solution was cooled to 25 °C. The pH was adjusted to 7.6 with 0.09 N aqueous LiOH, and 0.125 g of acylase I was added. The suspension was warmed to 35 °C, and after 15 min the pH had dropped to 7.0. The pH was again adjusted to 7.5 with 0.09 N LiOH, and an additional 0.1 g of acylase I was added. After 0.5 h the pH was 7.2, where it remained for an additional 1.0 h. The pH was adjusted to 5.0 with acetic acid. Insolubles were filtered off on dicalite, and the filter cake was washed with 2 × 100 mL of H_2O. The filtrate was concentrated on a rotary evaporator at 70 °C to a residual weight of 200 g and then diluted with 1.0 L of EtOH. After heating at reflux for a few minutes, the stirred mixture was allowed to cool to ambient temperature, and then it was stirred and cooled in an ice bath for 0.5 h. Solid was filtered off, washed with 3 × 200 mL of EtOH, and then dried at 50 °C/50 Torr to give 31.4 g of **8**, mp 263−267 °C, optical purity 97.0% ee (HPLC), 42.7% yield.

^{1}H NMR (300 MHz, D_2O): δ 3.79 (t, J = 6.3 Hz, 1H), 3.67 (t, J = 6.3 Hz, 2H), 2.04−1.84 (m, 2H), 1.72−1.60 (m, 2H), 1.58−1.38 (m, 2H). ^{13}C NMR (75 MHz, D_2O): δ 174.7, 61.1, 54.6, 30.8, 30.1, 20.7. MS (CI, CH_4): *m*/*z* (relative intensity) 148 (79), 130 (18), 102 (100), 85 (17), 84 (23).

HPLC analysis was done using the following conditions: column, 15 cm × 4 mm Daicel Chrom Pak (+); mobile phase, 0.11 M $HClO_4$; flow rate, 1.0 mL/min; detection, 200 nm; retention times, 1.85 min, (*R*), 2.87 min, (*S*).

(*R*,*S*)-6-Hydroxynorleucine from (*S*)-6-Hydroxynorleucine Mother Liquor. A 475-g sample of (*S*)-6-hydroxynorleucine mother liquor, containing the potential equivalence of 0.27 mol of (*R*,*S*)-6-hydroxynorleucine, was concentrated on a rotary evaporator to give 87.8 g of a viscous oil. The residue was chased with 500 mL of H_2O on the rotary evaporator at 90 °C and then diluted with 250 mL of H_2O. The solution was placed in a 600-mL stainless steel autoclave with 16.8 g (0.4 mol) of $LiOH \cdot H_2O$ and heated to 135 °C. After 4 days at 135 °C, the reaction mixture was cooled to ambient temperature, giving a residual pressure of 75 psig. After the pressure was released, the suspension was diluted with 400 mL of H_2O and 200 mL of MeOH. About 100 g of dry ice was added to the suspension over a 20-min period at about 10 °C. The suspension was filtered through Celite, and the filtrate was concentrated to about one-half of its volume at 90 °C on a rotary evaporator, whereupon a precipitate began to form. The suspension was filtered through Celite and washed with 3 × 75 mL of H_2O. The filtrate was diluted with 300 mL of MeOH, and 25 g (0.34 mol) of propionic acid was added. The solution was distilled and displaced with *n*-butanol until the boiling point reached 114 °C and the residue temperature reached 117 °C. During the distillation, the pot volume was maintained at about 1 L. To the hot solution was added 50 g (0.68 mol) of propionic acid. The stirred solution was cooled to ambient temperature, with crystallization beginning at about 50 °C. After the suspension was stirred at ambient temperature for 12 h and cooled in an ice bath for 1 h, the precipitate was filtered off, washed with 100 mL of *n*-butanol and 2 × 100 mL of MeOH, and dried at 50 °C/50 Torr to give 31.1 g of product containing 49.1% (*S*)-enantiomer (HPLC), mp 250−252 °C, 78% yield.

***N*-Phthaloyl-(*S*)-phenylalanine.** A solution of 165.2 g (1.0 mol) of (*S*)-phenylalanine and 148.1 g (1.0 mol) of phthalic anhydride in 1.6 L of toluene was heated to and held at reflux while removing H_2O using a Dean−Stark trap. After 2.5 h, a total of 18.0 mL had been collected. The stirred mixture was cooled to and maintained at ambient temperature for 15 h. Product was filtered off, washed with 3 × 100 mL of toluene, and then dried at 60 °C/50 Torr to give 277.0 g, 93.9% yield, mp 180−183 °C, optical purity 100% (*S*)-isomer (HPLC). HPLC analysis was done using the following conditions: column, 250 × 4.6 mm ASTEC Cyclobond I, cyclodextrin; mobile phase, blend of 340 mL of 1% TEAA (pH 4.1), 84 mL of H_2O, 336 mL of MeOH, and 20 mL of EtOH; flow rate, 0.5 mL/min; detection, 230 nm; retention times, 20.54 min (*R*), 22.30 min (*S*).

***N*-Phthaloyl-(*S*)-phenylalanine Acid Chloride, 7.** A total of 121.8 g (0.96 mol) of oxalyl chloride was added dropwise over 15 min at 15 °C to a suspension of 236.2 g (0.8 mol) of *N*-phthaloyl-(*S*)-phenylalanine and 2.0 mL of DMF in 1.2 L of toluene. After being stirred at ambient temperature for 16 h, the resulting solution was concentrated by removal of 500 mL of solvent at 60 °C/50 Torr. The residue was diluted with 250 mL of EtOAc, and a small quantity of insolubles was removed by filtration. The solid was washed with 100 mL of EtOAc. The filtrate was held for use in the subsequent reaction.

***N*-Phthaloyl-(*S*)-phenylalanine Morpholine Amide, 11.** A solution of 54.9 g (0.63 mol) of morpholine and 100 mL of DMF in 200 mL of CH_2Cl_2 was cooled to −40 °C and treated dropwise over 15 min with 94.1 g (0.3 mol) of *N*-phthaloyl-(*S*)-phenylalanine acid chloride in 500 mL of CH_2Cl_2. The stirred mixture was allowed to warm to ambient temperature over 1.5 h, and then it was diluted with 600 mL of H_2O. The organic phase was separated, washed with 1 L of 1 N HCl and 2 × 1 L of H_2O, and then dried ($MgSO_4$). Drying agent was filtered off and washed with 3 × 100 mL of CH_2Cl_2, and then the filtrate was evaporated at 30 °C/50 Torr to give a solid residue. The residue was slurried in 500 mL of heptane and then filtered off and dried at 60 °C/50 Torr to give 98.4 g of crude product. Crude product was dissolved in 1.5 L of refluxing IPA. After the stirred solution cooled to ambient temperature, product was filtered off, washed with 3 × 150 mL of IPA, and then dried at 80 °C/50 Torr to give 87.5 g, 80.5% yield, mp 144−146 °C.

Assay of *N*-Phthaloyl-(*S*)-phenylalanine Acid Chloride, 7. To 275.2 g (about 0.2 mol) of a CH_2Cl_2 solution of *N*-phthaloyl-(*S*)-phenylalanine acid chloride in 300 mL of EtOAc stirred in an ice bath was added 52.3 g (0.5 mol) of morpholine at 10−20 °C over 20 min. The resulting precipitate was heated to 40 °C, cooled to ambient temperature, and then diluted with 200 mL of H_2O. The organic phase was separated, washed with 1 L of saturated NaCl and 2 × 500 mL of saturated NaCl, and then dried ($MgSO_4$). Drying agent was filtered off and washed with 3 × 100 mL of EtOAc. The filtrate was concentrated to dryness at 100 °C/50 Torr, followed by drying at 100 °C/0.2 Torr to give 71.7 g of product, mp 144−146 °C. The purity relative to a reference standard was determined to be 99.0% (HPLC). HPLC analysis was done using the following system: column, ZORBAX C8 (4.5 mm × 15 cm); mobile phase, 65% acetonitrile in H_2O; flow rate, 1.0 mL/min; detection, 254 nm; retention time of morpholine amide, 212 s.

(*S*)-*N*-[2-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-1-oxo-3-phenylpropyl]-6-hydroxy-(*S*)-norleucine Methyl Ester, 9. A total of 10.0 g (0.274 mol) of HCl (g) was sparged into MeOH initially at 25 °C (warmed to approximately 40 °C). (*S*)-6-Hydroxynorleucine (20.0 g, 0.136 mol), **8**, was added with stirrring, and the resulting mixture was heated at reflux for 2 h. A total of 20.6 mL (20.0 g, 0.222 mol) of trimethyl orthoformate was added, and reflux was maintained for an additional 6 h. The reaction mixture was concentrated to a volume of 200 mL at 25 °C/130 Torr. Triethylamine (42 mL, 30.4 g, 0.30 mol) and 150 mL of toluene were added, and the mixture was concentrated further at 100 Torr while allowing the overhead temperature to reach 35 °C. When the internal volume was again reduced to about 200 mL, additional triethylamine (5 mL, 3.65 g, 0.035 mol) and toluene (150 mL) were added, and the mixture was again concentrated (40 °C/ 60 Torr) to approximately 200 mL. While at 40 °C, the mixture was treated with 15 mL of triethylamine (10.95 g, 0.105 mol), 20 mL of acetonitrile, and 150 mL of toluene for 2 h. The mixture was cooled to

and maintained at 10 °C for 30 min. Insolubles were filtered off and washed with 200 mL of toluene. The filtrate was evaporated at 45 °C/50 Torr to give 22.3 g of crude methyl ester free base. This material was diluted with 80 mL of EtOAc, 40 mL of acetonitrile, and 48 mL of *N*-methylmorpholine. The mixture was cooled to −15 °C and treated with a total of 188 mL of 0.722 M acid chloride **7** in portions over 1 h while maintaining a reaction temperature of between −5 and −10 °C. After the addition of 1.00 equiv of acid chloride, 1.36% of bis-adduct **11** was observed by HPLC. The stirred mixture was allowed to warm to 0 °C, and then it was treated with 200 mL of 2 N HCl. The aqueous phase was separated and extracted with 50 mL of EtOAc. Organic extracts were combined, washed with 300 mL of saturated $NaHCO_3$, and 200 mL of saturated NaCl, and then dried with $MgSO_4$. Drying agent was filtered off and washed with EtOAc. The filtrate was transferred to a 500-mL, three-necked flask fitted with a stirrer, internal thermometer, and distillation apparatus. Solvent was distilled off until the internal temperature reached 100 °C. Toluene (300 mL) was added, and the mixture was concentrated by distillation to a total volume of about 270 mL. The stirred mixture was cooled to 25 °C over 2 h and then cooled to and held at 5 °C for 1 h. Solid which crystallized was filtered off, washed with 200 mL of toluene, and then air-dried to give 51 g of **9**, 85.5% yield, 97.8% pure (HPLC).

^{1}H NMR (300 MHz, DMSO-d_6): δ 8.60 (d, J = 8.0 Hz, 1H), 7.83 (s, 4H), 7.23−7.06 (m,5H), 5.04 (dd, J = 4.5 Hz, J' = 11.7 Hz, 1H), 4.43−4.30 (m, 2H), 3.67 (s, 3H), 3.57 (dd, J = 4.6 Hz, J' = 13.7 Hz, 1H), 3.47−3.23 (m, 3H), 1.80−1.17 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.4, 167.7, 167.1, 137.2, 134.2, 131.1, 128.5, 128.0, 126.2, 122.7, 60.2, 53.8, 52.0, 51.6, 33.7, 31.5, 30.1, 21.6. MS (CI, CH_4): *m*/*z* (relative intensity) 439 (M + H, 100), 407 (27), 295 (12), 261 (22), 251 (20), 199 (12), 165 (96), 150 (56), 148 (32), 101 (10).

HPLC analysis was done using the following conditions: column, 250- × 4.6-mm Adsorbosphere Phenyl 5 μm; mobile phase, (A) acetonitrile, (B) 0.02 M aqueous ammonium phosphate monobasic solution, adjusted to pH 2.5 with 85 wt % phosphoric acid, gradient, isocratic 50% A for 10 min, increment to 70% A over 2 min, isocratic at 70% A for 8 min, back to 50% A over 2 min; flow rate, 1.0 mL/min; detection, 238 nm; retention times, **9**, 6.2 min, *N*-phthaloyl-L-phenylalanine, 6.7 min.

[4*S*-(4α,7α,12b*β*)]-7-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-1,2,3,4,6,7,8,12b-octahydro-6-oxopyrido[2,1-*a*][2]-benzazepine-4-carboxylic Acid, MDL 28,726. A mixture of 14.9 g of **9** and 78 mL of CH_2Cl_2 was charged to a 500-mL, three-necked flask fitted with a stirrer, condenser, thermometer, and continuous N_2 purge. DMSO (28.8 g, 0.637 mol) was added, and the solution was cooled to −60 °C using a dry ice/acetone bath. The bath was removed, and oxalyl chloride (7.5 g, 0.059 mol) was added in less than 1 min, resulting in a temperature rise of about 30 °C. The solution was stirred for 10 min at −30 to −35 °C and then cooled to −40 °C. Triethylamine (20.0 g, 0.20 mol) was added over 1 min, resulting in a temperature rise of about 40 °C. The slurry was stirred for 10 min at about 0 °C. H_2O (110 mL) was added to dissolve the triethylamine hydrochloride, and the phases were separated. The organic phase was cooled to about −10 °C, and a solution of OXONE (40.0 g in 250 mL of H_2O) was added, resulting in a temperature rise of about 30 °C. The mixture was stirred for about 15 min at 20−25 °C, and then the organic phase was separated, washed with 120 mL of 3% HCl, and transferred to a 250-mL three-necked flask fitted with a stirrer, condenser, thermometer, and continuous N_2 purge. Anhydrous $MgSO_4$ (9.0 g) and trifluoroacetic acid (1.5 g) were added, and the mixture was stirred at 20−25 °C for 3 h. The $MgSO_4$ was filtered off, and the filter cake was washed with 10 mL of CH_2Cl_2. To a 250-mL three-necked flask fitted with a magnetic stirrer, condenser, thermometer, and continuous N_2 purge was charged triflic acid (45.1 g, 0.3 mol), trifluoroacetic anhydride (8.8 g, 0.042 mol), and 15 mL of CH_2Cl_2. The stirred mixture was cooled to about 15 °C, and the solution of **10** was added, resulting in a temperature rise of about 8 °C. After the mixture was stirred at 20−25 °C for 25 h, HPLC analysis showed the presence of 70.4% MDL 28,726 and 0.8% **10**. The mixture was washed with 200 mL of H_2O, and then 100 mL of H_2O, and then evaporated to a residue at 25 °C/50 Torr. Acetonitrile (25 g) was added, and the solution was concentrated at 35 °C/50 Torr to give a solid foam. Acetonitrile (30 g) was added, and the mixture was heated to 80 °C to give a solution. The solution was allowed to cool to ambient temperature, with crystallization beginning at 55 °C. The stirred mixture was cooled at 5 °C for 1 h, and then solid was filtered off, washed with 20 mL of cold (<5 °C) 50/50 (v/v) acetonitrile/H_2O, and dried at 50 °C/50 Torr to give 9.9 g of MDL 28,726 acetonitrile solvate (9.2% acetonitrile by weight), 65.4% yield, >99% chemical purity.

^{1}H NMR (300 MHz, DMSO-d_6): δ 7.98−7.86 (m, 4H), 7.36−7.10 (m, 5H), 5.83 (dd, J = 6.2 Hz, J' = 11.8 Hz, 1H), 5.48 (m, 1H), 4.77 (m, 1H), 4.19 (dd, J = 12.0 Hz, J' = 17.0 Hz, 1H), 3.45 (dd, J = 6.2 Hz, J' = 16.8 Hz, 1H), 2.40−2.25 (m, 1H), 2.19−2.05 (m, 1H), 2.04−1.89 (m, 2H), 1.88−1.63 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.4, 168.6, 167.5, 137.4, 136.6, 134.5, 129.8, 127.0, 125.6, 125.5, 122.9, 53.4, 52.9, 51.1, 32.4, 25.9, 24.7, 18.2. MS (CI, CH_4): *m*/*z* (relative intensity) 405 (M + H, 19), 387 (30), 361 (15), 317 (14), 289 (82), 149 (100), 142 (7). MDL 28,726 was analyzed by HPLC using the following system: column, ZORBAX C8 (4.5 mm × 15 cm); mobile phase, 65% acetonitrile in H_2O; flow rate, 1.0 mL/min; detection, 254 nm; retention times, MDL 28,726, 207 s, **10**, 410 s.

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