Synthesis of Enantiomerically and Diastereomerically Pure 2(*S*)-Amino-6(*R*)-hydroxy-1,7-heptanedioic Acid Dimethyl Ester Hydrochloride from Cycloheptadiene

Brock T. Shireman and Marvin J. Miller*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

Marvin.J.Miller.2@nd.edu

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The complete carbon framework of enantiomerically and diastereomerically pure 2(*S*)-amino-6(*R*)-hydroxy-1,7-heptanedioic acid dimethyl ester hydrochloride was derived from cycloheptadiene in six steps utilizing an amino acid-derived acylnitroso Diels−Alder reaction as the key step. This versatile amino diester has been previously used to synthesize amino-differentiated diaminopimelic acid (DAP) and biologically active analogues. In addition, after formation of a novel aminoxy diketopiperazine, the newly formed carboxyl groups were differentiated by a novel transpeptidation of the amino acid that directed the stereochemistry of the initial cycloaddition.

Introduction

meso-Diaminopimelic acid (DAP) (Figure 1) is a strengthening constituent of Gram-negative bacterial cell walls.[1] Gram-positive bacteria utilize lysine in a similar role. L,L-DAP serves as a biosynthetic precursor to *meso*-DAP, which in turn is a biosynthetic precursor to lysine in Gram-positive bacteria. Since mammals obtain lysine from their diet and, thus, do not rely on the pimelic acid pathway for the synthesis of lysine, inhibition of *meso*-DAP biosynthesis can effectively repress proliferation of both Gram-negative or Gram-positive bacteria without detriment to the mammalian host. Therefore, syntheses of functionally differentiated DAP analogues[1] and DAP-containing peptides[2] are of interest for the potential development of antibiotics.

Amino alcohol **3** (Figure 2)[1k,3] has been shown to be readily functionalized to L,L-DAP, *meso*-DAP,[3d] or inhibitors of enzymes of the DAP pathway.[1k,3a] Previously, a 1:1 diastereomeric mixture of **3** was synthesized in 46−65% yields utilizing an ene reaction between allylglycine (**1**) and methylglyoxylate (**2**) as the key step.[3a] Hydrogenation of the initial ene product provided **3** as a diastereomeric mixture. Chiral glyoxylates derived from **2** also have been utilized to give **3** with up to 76% diastereoselectivity.[3d]

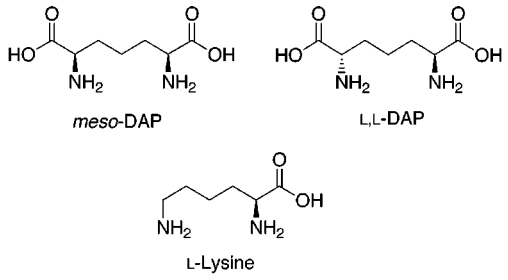

Figure 1. Structures of *meso*-DAP, L,L-DAP, and L-Lysine.

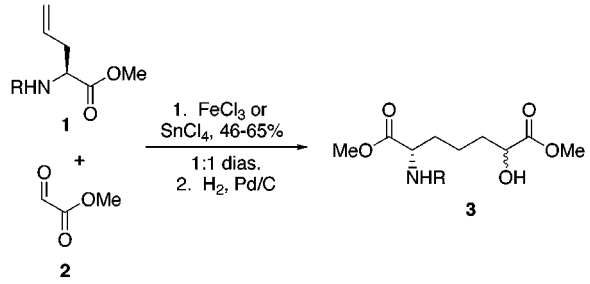

Figure 2. Ene Reaction for the Synthesis of **3**.

We reasoned that enantiomerically pure amino acid-derived acylnitroso Diels−Alder cycloadduct **6** (derived from cycloheptadiene (**4**))[4] and the acylnitroso species **5** (derived from Boc-L-Ala-NHOH) would serve as an efficient precursor to 2(*S*)-amino-6(*R*)-hydroxy-1,7-heptanedioic acid dimethyl ester hydrochloride (**8**, Figure 3).

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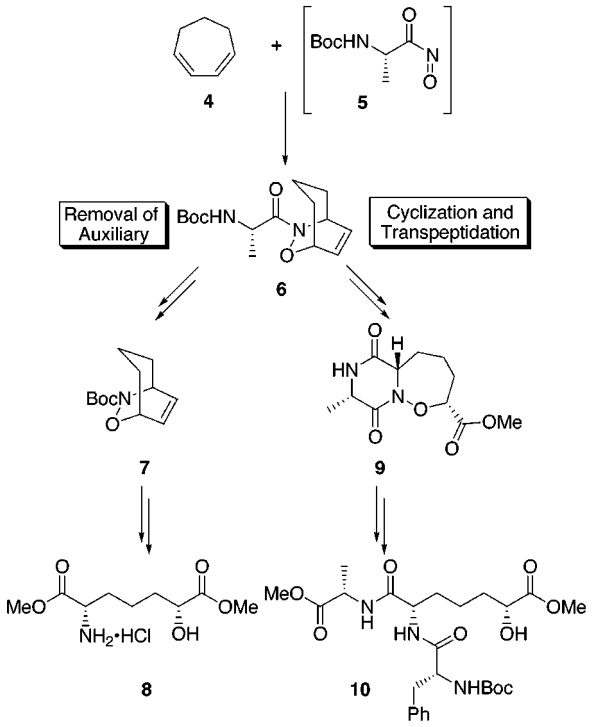


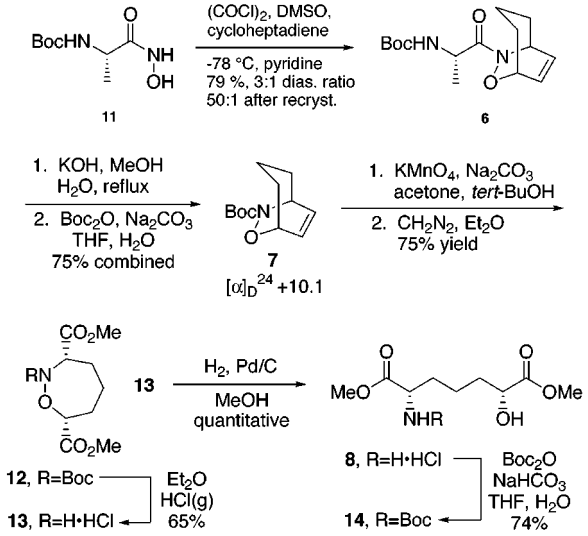
Figure 3. Planned Syntheses of **8** and **10** from Cycloheptadiene (**4**).

Key to the synthesis would be the efficient removal of the amino acid chiral auxiliary and reprotection as the Boc carbamate **7**.[5,6] Furthermore, after conversion to aminoxydiketopiperazine **9**, the amino acid initially utilized to control both newly generated stereogenic centers in **6** should be transferred through a novel transpeptidation to provide tripeptide **10** after subsequent manipulations. Herein, we demonstrate the synthesis of enantiomerically and diastereomerically pure **8**, in addition to differentiation of the carbomethoxy groups to give tripeptide **10**.

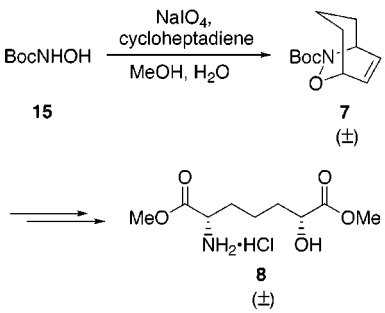
Results and Discussion

Oxidation of hydroxamic acid **11** under Swern conditions[7] in the presence of cycloheptadiene provided cycloadduct **6** in 79% combined yield as a 3:1 diastereomeric mixture, as determined by HPLC analysis (Scheme 1, major diastereomer shown). After a single recrystallization, the diastereomeric ratio was enhanced to 50:1 and

Scheme 1



Scheme 2



utilized as such in subsequent steps. When **6** was reacted with KOH in wet refluxing MeOH, the intermediate substituted hydroxylamine was isolated and treated with Boc_2O, in the presence of Na_2CO_3 in THF/H_2O, to give (+)-**7** in 75% combined yield for the two steps.[8] With **7** in hand, oxidation with $KMnO_4$ in acetone/*t*-BuOH in the presence of Na_2CO_3[9] gave diester **12** in 75% yield after treatment with ethereal CH_2N_2. The Boc group of **12** was removed by treatment with HCl(g)/Et_2O to give HCl salt **13** in 65% yield as a white solid. Then, N−O bond reduction of **13** with H_2/Pd−C in MeOH proceeded smoothly to give **8** quantitatively. For ease of characterization and compatibility with peptide chemistry, amino alcohol **8** was converted to Boc derivative **14** by treatment with Boc_2O and $NaHCO_3$ in THF/H_2O.

The enantiomeric purity of (+)-**8** was confirmed by comparison with (±)-**8** derived from racemic cycloadduct (±)-**7** (Scheme 2). Oxidation of BocNHOH (**15**) with $NaIO_4$ in MeOH/H_2O in the presence of cycloheptadiene gave (±)-**7**. Conversion of (±)-**7** into racemic (±)-**8** followed that of the enantiomerically pure cycloadduct illustrated previously. ^{19}F NMR analyses of the Mosher's amides of (+)-**8** and the corresponding racemate confirmed that (+)-**8** from the amino acid-derived acylnitroso Diels−Alder reaction was of >98% ee.

To increase the versatility of **8** or Boc derivative **14**, differentiation of the two similar ester constituents was deemed necessary. We envisioned that the amino acid that controlled the initial cycloaddition stereochemistry

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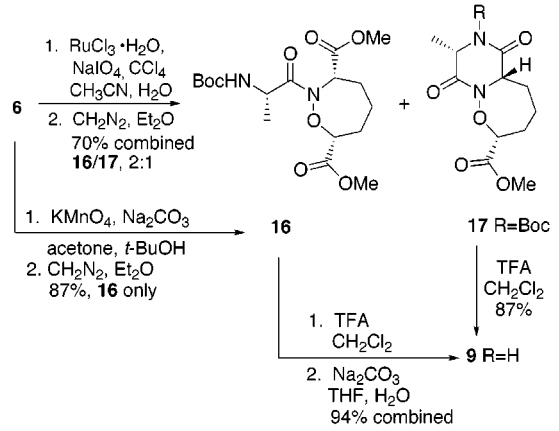
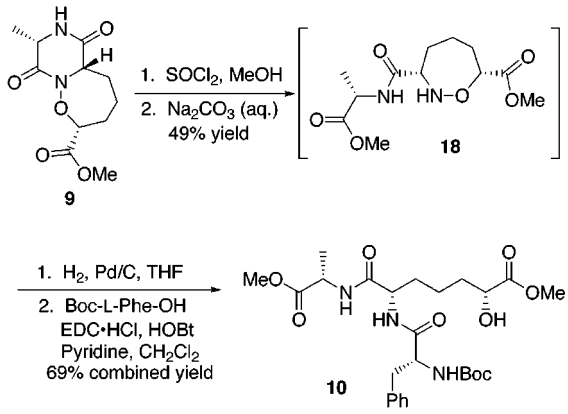
Scheme 3



Scheme 4



could be selectively transferred to the α-carboxyl of the newly generated α-amino ester by diketopiperazine formation, followed by ring opening of the aminoxycarbonyl compound as shown in Schemes 3 and 4.

Subjection of **6** as a 50:1 diastereomeric mixture to RuO_4, generated in situ from $NaIO_4/RuCl_3 \cdot H_2O$[5c,10] and followed by treatment with CH_2N_2, provided dimethyl ester **16** and diketopiperazine **17**. The two compounds were isolated in a 2:1 ratio in 70% combined yield as confirmed by ^1H NMR analysis of the crude product mixture. Separation of **16** and **17**, followed by removal of the Boc-protecting group from **17** with TFA in CH_2Cl_2, provided diketopiperazine **9** in 87% yield. Analysis of the X-ray crystal structure of **9** secured the stereochemistry and structural assignment. Alternatively, **6** was oxidized with $KMnO_4$ in the presence of Na_2CO_3 to give dimethyl ester **16** selectively in 85% yield after treatment with ethereal CH_2N_2. Dimethyl ester **16** was converted to diketopiperazine **9** in 94% yield after treatment with TFA in CH_2Cl_2 followed by neutralization of the corresponding TFA salt with Na_2CO_3 in THF/H_2O.

Net transpeptidation from diester **16** to diester **18** occurred when **9** was refluxed in methanolic HCl generated from $SOCl_2$/MeOH (Scheme 4). Immediately after isolation of **18**, the N−O bond was reduced with H_2−Pd/C in THF to give the corresponding primary amine. As a representative example for more extensive investigations, EDC·HCl/HOBt-mediated coupling of Boc-L-Phe-OH with **18** was performed to give tripeptide **10** in 69% yield from the intermediate-substituted hydroxylamine **18**.[3b]

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Conclusions

The amino acid-derived acylnitroso Diels−Alder reaction has been shown to provide rapid access to **8**. Derivatives of the versatile amino ester **8** have been previously utilized to synthesize amino-differentiated DAP and DAP analogues. In addition, through the formation of novel aminoxydiketopiperazine **17**, the carbomethoxy groups of the derived DAP framework were differentiated by selective migration of the amino acid initially used to induce the ultimate stereochemistry in **8**. Because of the versatility of the acylnitroso Diels−Alder reaction, this methodology should provide access to differentiated and selectively elaborated ω-hydroxy, ω-carboxy, and α-amino acids.

Experimental Section

General Methods. Et_3N, CH_3CN, and CH_2Cl_2 were distilled directly before use from CaH_2 under Ar. Pyridine was stored over KOH and utilized without further purification. Melting points were performed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 300 or 500 MHz, as indicated, on Varian Unity spectrometers. ^{13}C NMR were recorded at 75 or 125 MHz on Varian Unity spectrometers. ^1H NMR are reported in ppm relative to tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm), or residual CD_3SOCD_2H (2.49 ppm). ^{13}C NMR are reported in ppm relative to residual $CDCl_3$ (77.0 ppm) or residual C_2D_6SO (39.5 ppm). Coupling constants are reported in Hertz. Optical rotations were obtained utilizing a Rudolph Research Autopol III polarimeter with a 1.0-dm cell length at ambient temperature. TLC was performed utilizing silica gel 60 F_{254} aluminum-backed plates. Visualization was typically performed utilizing UV light, $KMnO_4$, ninhydrin, or PMA stain. Flash chromatography was performed utilizing silica gel 60 (30−70 μm irregular particles). HPLC was performed monitoring at 254 nm on an Alltech Econosil column (5 μm, 4.6 × 250 mm).

(1*R*,4*S*)-*N*-[*N*-(*tert*-Butyloxycarbonyl)-L-alanine]-2,3-oxazabicyclo[3.2.2]non-5-ene (6). Hydroxamic acid **11** (9.0 g, 44.1 mmol) in CH_2Cl_2/DMSO (146:45 mL) was cooled to −78 °C, and cycloheptadiene (4.32 g, 4.98 mL, 45.9 mmol) was added. In a separate flask, $(COCl)_2$ (22.3 g, 15.3 mL, 176.0 mmol) in CH_2Cl_2 (161.0 mL) was cooled to −78 °C, and DMSO (20.7 g, 18.8 mL, 264.0 mmol) was added. After 3 min, the $(COCl)_2$/DMSO solution was transferred by cannula into the hydroxamic acid solution. After the transfer was complete, the solution was allowed to stir at −78 °C for 15 min. Pyridine (34.9 g, 35.7 mL, 441 mmol) was added dropwise over 10 min. The cooling bath was removed, the reaction was allowed to warm slowly to rt, and Et_2O was then added followed by 1 N HCl. The layers were separated, and the aqueous layer was extracted with Et_2O (2×). The combined organic layers were then washed with saturated $NaHCO_3$ and brine and then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a residue. Purification on silica gel using 50% EtOAc/hexanes gave 10.5 g (79%) of a 3:1 diastereomeric mixture of **6** as a white solid. The major diastereomer was further purified by recrystallization from hexanes and a minimal amount of EtOAc to give 5.35 g (37% based on hydroxamic acid **11**) of **6** as a 50:1 diastereomeric ratio. An analytical sample was obtained by an additional recrystallization to provide a single diastereomer for characterization. HPLC (10% *i*PrOH/hexanes, 1.5 mL/min) t_R: 5.1 (major) and 6.3 (minor) min. Major diastereomer **6**: mp 111−113 °C; R_f 0.44 (60% EtOAc/hexanes). IR (KBr): 3267, 2976, 1705, 1646, 1629, 1533, 1366, 1250, 1172, 1014, 863 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.25 (d, J = 6.89 Hz, 3H), 1.44 (s, 9H), 1.57 (m, 2H), 1.86 (m, 4H), 4.68 (m, 1H), 4.75 (m, 1H), 5.16 (m, 3.29 Hz, 1H), 5.41 (d, J = 7.79 Hz, 1H), 6.20 (t, J = 8.39 Hz, 1H), 6.36 (dd, J = 6.45, 8.85 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 18.2, 18.5, 28.4, 28.6, 29.3, 47.1, 51.0, 76.8, 79.2, 126.6, 130.0, 155.1, 168.5. HRMS−FAB (m/z): $[M + H]^+$ calcd for $C_{15}H_{25}N_2O_4$, 297.1814; found, 297.1828.

(1*R*,4*S*)-*N*-[*tert*-Butyloxycarbonyl]-2,3-oxazabicyclo-[3.2.2]non-5-ene (7). To cycloadduct **6** (1.0 g, 3.38 mmol) in MeOH/H_2O (9:1, 5 mL) was added KOH (0.750 g, 8.0 mmol), and the reaction was refluxed for 1.75 h. The reaction was allowed to cool to rt and then partitioned between EtOAc (15 mL) and H_2O (10 mL). The layers were separated, and the aqueous layer was saturated with NaCl and then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine and concentrated to give a pale yellow solid that was dissolved in THF/H_2O (1:1, 16 mL). Then, Na_2CO_3 (0.753 g, 7.10 mmol) was added followed by Boc_2O (0.811 g, 3.72 mmol). After 5 h, H_2O (10 mL) was added, and the reaction was extracted with Et_2O (2 × 10 mL). The combined organic layers were washed with brine and then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an oil that could be further purified with silica gel chromatography utilizing 30% EtOAc/hexanes to give 0.628 g (83%) of **7** as a white solid. The sample was further purified by recrystallization from hexanes. Characterization data of **7** was comparable to that previously reported.[8] $[\alpha]^{24}_D$ +10.1 (*c* 1.22, $CHCl_3$); mp 47−48 °C; R_f 0.38 (30% EtOAc/hexanes). IR (KBr): 2978, 2931, 2863, 1737, 1698, 1369, 1280 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.26−1.60 (m, 2H), 1.48 (s, 9H), 1.69−1.93 (m, 4H), 4.77 (m, 2H), 6.17 (ddd, J = 0.75, 6.0, 8.5 Hz, 1H), 6.36 (dd, J = 7.0, 9.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 18.4, 27.5, 28.2, 30.6, 54.3, 74.9, 81.1, 127.5, 129.4, 156.2. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{12}H_{20}NO_3$, 226.1443; found, 226.1453.

2-(*tert*-Butyloxycarbonyl)-3(*S*),7(*R*)-[dicarbomethoxy]-oxazepine (12). To cycloadduct **7** (0.459 g, 2.04 mmol) in acetone/*t*-BuOH (40:1, 20.5 mL) was added Na_2CO_3 (0.193 g, 1.82 mmol). The flask was cooled to −10 °C, and $KMnO_4$ (0.904 g, 5.72 mmol) was added portionwise such that the internal temperature of the reaction did not exceed 0 °C. The reaction proceeded at −10 °C for 15 min and then was allowed to warm to rt and stir an additional 4 h. The reaction was partitioned between EtOAc and H_2O. Then, 10% aqueous $Na_2S_2O_5$ was added until the aqueous layer gave a negative KI/starch paper test. Then, the aqueous layer was acidified to pH 3 (pH paper) with 1 N HCl. The layers were separated, and the aqueous layer was saturated with NaCl and then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine and then dried ($MgSO_4$), filtered, and concentrated to give a yellow residue that was dissolved in Et_2O (15.0 mL) and treated with excess CH_2N_2 in Et_2O at 0 °C. After 1 h, excess CH_2N_2 was quenched with 10% AcOH/Et_2O, and the reaction was washed with saturated $NaHCO_3$. The aqueous layer was extracted with Et_2O (1×). The combined organic layers were washed with brine and then dried ($MgSO_4$), filtered, and concentrated to give an oil that was chromatographed on silica gel utilizing 50% EtOAc/hexanes to give 0.437 g (75%) of **12** as a clear oil. $[\alpha]^{24}_D$ −39.2 (*c* 1.01, $CHCl_3$); R_f 0.44 (60% EtOAc/hexanes). IR (thin film): 2954, 1743, 1705, 1369, 1664 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz, 55 °C): δ 1.40 (m, 9H), 1.61−1.79 (m, 3H), 1.86−2.04 (m, 2H), 2.16−2.25 (m, 1H), 3.65 (s, 3H), 3.67 (s, 3H), 4.30 (dd, J = 3.6, 10.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 22.8, 27.7, 28.5, 32.5, 51.8, 52.0, 60.7, 62.3, 80.9, 85.0, 154.4 (broad), 169.3, 171.0. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{14}H_{24}NO_7$, 318.1553; found, 318.1561.

3(*S*),7(*R*)-[Dicarbomethoxy]oxazepine Hydrochloride (13). To Boc-amine **12** (0.135 g, 0.425 mmol) in Et_2O at 0 °C was bubbled HCl(g) until a white precipitate formed. The solid was filtered to give 0.067 g (67%) of **13**. $[\alpha]^{24}_D$ −23.3 (*c* 0.86, $CHCl_3$); mp 123−125 °C dec. IR (KBr): 3483, 2956, 2840, 2626, 2540, 2418, 1755, 1444, 1303, 1225 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.53−1.67 (m, 2H), 1.68−1.87 (m, 2H), 1.94−2.09 (m, 2H), 3.61 (s, 3H), 3.62 (s, 3H), 3.68 (dd, J = 5.5, 9.1 Hz, 1H), 4.31 (dd, J = 6.4, 9.4 Hz, 1H), 8.01 (bs, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 21.9, 31.1, 31.6, 51.5, 51.7, 62.9, 79.4, 171.6, 171.7. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_9H_{16}NO_5$, 218.1028; found, 218.1018.

2(*S*)-Amino-6(*R*)-hydroxy-1,7-heptanedioic Acid Dimethyl Ester Hydrochloride (8). HCl salt **13** (0.075 g, 0.296 mmol) in MeOH (3.0 mL) was hydrogenated utilizing Pd/C under an atmosphere of H_2. After 2.5 h, the reaction was purged with Ar and filtered through a pad of Celite. The MeOH filtrate was concentrated to give 0.073 g (97%) of **8** as a clear oil. $[\alpha]^{24}_D$ +16.7 (*c* 0.87, MeOH). IR (thin film): 3476, 3369, 3306, 2954, 1736, 1208 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.30−1.63 (m, 6H), 3.25−3.29 (m, 1H), 3.60 (s, 3H), 3.61 (s, 3H), 3.84 (dd, J = 4.8, 7.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 20.2, 29.9, 33.3, 51.4, 51.9, 52.6, 69.3, 170.2, 174.4. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_9H_{18}NO_5$, 221.1263; found, 221.1247.

(±)-*N*-[*tert*-Butyloxycarbonyl]-2,3-oxazabicyclo[3.2.2]-non-5-ene (7). To **15** (1.0 g, 7.5 mmol) in MeOH (70 mL) cooled to 0 °C was added cycloheptadiene (0.708 g, 7.5 mmol, 0.815 mL). Then, $NaIO_4$ (1.61 g, 7.5 mmol) in H_2O (10 mL) was added over 10 min. After 30 min, the reaction mixture was allowed to warm to rt and was partitioned between Et_2O and H_2O. The layers were separated, and the aqueous layer was extracted with Et_2O (2×). The combined organic layers were washed with saturated $NaHCO_3$ and brine and then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a brown oil that was chromatographed on silica gel utilizing 30% EtOAc/hexanes to give 0.851 g (50% yield) of (±)-**7** as a white solid whose spectral data was identical to that of (+)-**7**.

(±)-*syn*-2-Amino-6-hydroxy-1,7-heptanedioic Acid Dimethyl Ester Hydrochloride (8). Compound **8** was prepared from (±)-**7** following the procedure for (+)-**8**. Spectral data of all intermediates matched those of previous samples.

Mosher's Amide Analysis of (±)-8. To **(±)-8** in CH_2Cl_2 at 0 °C was added DMAP (200 mol %) followed by Mosher's Cl (150 mol %). After 1 h, the reaction was partitioned between Et_2O and 1 N HCl. The layers were separated, and the aqueous layer was extracted with Et_2O (1×). The combined organic layers were washed with saturated $NaHCO_3$ and brine and then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an oil that was analyzed without further purification by ^{19}F NMR. Because excess Mosher's Cl was utilized, four absorptions were identified corresponding to the diastereomeric Mosher's amides and Mosher's esters. Utilizing an identical protocol, the % ee of the Mosher's amide derived from (+)-**8** was shown to be >98% ee by ^{19}F NMR and spiking with the Mosher's amide derived from (±)-**8**.

2(*S*)-Amino[(*tert*-butyloxycarbonyl)]-6(*R*)-hydroxy-1,7-heptanedioic Acid Dimethyl Ester Hydrochloride (14). HCl salt **8** (0.028 g, 0.110 mL) in THF/H_2O (1:1, 1.0 mL) was treated with $NaHCO_3$ (0.019 g, 0.231 mmol) followed by Boc_2O (0.026 g, 0.121 mmol). After 2 h, the reaction was extracted with EtOAc (1×). The organic layer was washed with brine and then dried ($MgSO_4$), filtered, and concentrated to give an oil that was chromatographed on silica gel utilizing 60% EtOAc/hexanes to give 0.026 g (74%) of **14** as a clear oil; R_f 0.31 (55% EtOAc/hexanes). IR (thin film): 3379, 2956, 1740, 1716, 1367, 1166 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.45 (s, 9H), 1.46−1.68 (m, 4 H), 1.80−1.86 (m, 2 H), 2.76 (d, J = 5.5 Hz, 1H), 3.74 (s, 3H), 3.79, (s, 3H), 4.18 (ddd, J = 5.5, 7.5, 9.5 Hz, 1H), 4.31 (m, 1H), 5.03 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.7, 28.3, 32.4, 33.7, 52.3, 52.6, 53.2, 70.1, 79.9, 155.4, 173.2, 175.4. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{14}H_{25}NO_7$, 320.1709; found, 320.1690.

2-[*N*-(Carbo-*tert*-butoxy)-L-alanyl]-3(*S*),7(*R*)-[dicarbomethoxy]oxazepine (16). Cycloadduct **6** (0.100 g, 0.338 mmol) was dissolved in *t*-BuOH/acetone (0.18 mL/4.4 mL), and solid Na_2CO_3 (0.036 g, 0.338 mmol) was added. The flask was cooled to −10 °C, and $KMnO_4$ (0.171 g, 1.08 mmol) was added portionwise such that the internal temperature of the reaction did not exceed 0 °C. The reaction proceeded at −10 °C for 6 h. It was then allowed to warm to rt and stirred an additional 16 h. EtOAc (10 mL) was added followed by 10% aqueous $Na_2S_2O_5$ (10 mL), and the aqueous layer was acidified to pH 3 (pH paper) with 1 N HCl. The layers were separated, and the aqueous layer was saturated with NaCl and then extracted with EtOAc (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give a yellow residue that was dissolved in THF (8.0 mL) and treated with excess CH_2N_2 in Et_2O at 0 °C. After 1 h, excess CH_2N_2 was removed with a stream of Ar that passed through an AcOH

trap, and the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel utilizing 50% EtOAc/hexanes to give 0.111 g (87%) of **16** as a white solid. $[\alpha]^{24}_D$ −31.8 (*c* 0.63, $CHCl_3$); mp 112−115 °C; R_f 0.33 (50% EtOAc/hexanes). IR (KBr): 3381, 2980, 2951, 2869, 1755, 1694, 1677, 1518, 1458, 1366, 1296, 1224, 1169, 1066, 1018, 861, 788 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.43 (d, J = 7.19 Hz, 3H), overlapping with 1.43 (s, 9H), 1.80 (m, 3H), 2.04 (m, 1H), 2.31 (m, 2H), 3.70 (s, 3H), 3.80 (s, 3H), 4.60 (dd, J = 3.30, 11.4 Hz, 1H), 4.72 (m, 1H), 4.86 (dd, J = 6.29, 11.1 Hz, 1H), 5.08 (d, J = 7.79 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 17.0, 23.2, 28.27, 28.30, 32.5, 47.6, 52.3, 52.5, 61.1, 79.5, 86.6, 155.3, 168.8, 170.4, 176.3. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{17}H_{29}N_2O_8$, 389.1924; found, 389.1919.

Boc-Protected Diketopiperazine 17. To cycloadduct **6** (0.800 g, 2.70 mmol) in $CCl_4/CH_3CN/H_2O$ (1:1:2, 22.0 mL) was added $NaIO_4$ (2.37 g, 11.1 mmol) followed by $RuCl_3 \cdot H_2O$ (0.028 g, 0.135 mmol). The biphasic mixture was allowed to stir at rt, open to the air, for 7 h. The reaction was partitioned between EtOAc (50 mL) and H_2O (50 mL). The aqueous layer was saturated with NaCl and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated to give a brown oil.

The residue was dissolved in THF (20.0 mL) and treated with excess CH_2N_2 in Et_2O at 0 °C. After 1 h, excess CH_2N_2 was removed with a stream of Ar that passed through an AcOH trap, and the reaction mixture was concentrated under reduced pressure. The residue was then chromatographed on silica gel utilizing 50% EtOAc/hexanes to give 1.88 g (70%) of a 2:1 mixture of **16**:**17**. Recrystallization of the mixture from Et_2O/cyclohexane provided diketopiperazine **17**. $[\alpha]^{24}_D$ +39.2 (*c* 1.02, $CHCl_3$); mp 140−141.5 °C; R_f 0.24 (50% EtOAc in hexanes). IR (KBr): 2953, 2925, 1742, 1725, 1682, 1297, 1159 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.53 (s, 9H), 1.61 (d, J = 7.19 Hz, 3H), 1.76 (m, 2H), 2.15 (m, 3H), 2.51 (m, 1H), 3.84 (s, 3H), 4.30 (dd, J = 3.89, 10.48 1H), 4.44 (m, 1H), 4.67 (q, J = 7.19 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 21.7, 22.4, 27.6, 27.8, 34.5, 52.6, 55.1, 66.6, 82.1, 85.0, 149.7, 164.0, 165.5, 169.4. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{16}H_{25}N_2O_7$, 357.1662; found, 357.1676.

Diketopiperazine 9. Dimethyl ester **16** (0.238 g, 0.613 mmol) was dissolved in CH_2Cl_2 (5.0 mL) under Ar. The flask was cooled to 0 °C, and TFA (2.52 g, 1.70 mL, 0.022 mmol) was added. After being stirred at 0 °C for 1 h, the reaction was diluted with toluene (5.0 mL) and concentrated under reduced pressure. The resulting yellow oil was dissolved in THF/H_2O (1:2, 6 mL), and Na_2CO_3 (0.129 g, 1.22 mmol) was added. The reaction was allowed to proceed for 1 h and then extracted with CH_2Cl_2 (5 × 7 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give 0.148 g (94%) of **9** as a white foam. $[\alpha]^{24}_D$ −5.37 (*c* 1.08, $CHCl_3$); mp 151−153 °C; R_f 0.34 (5% MeOH in EtOAc). IR (KBr): 3264, 3171, 2987, 2942, 1749, 1685, 1447, 1433, 1307, 1214, 1174, 1074, 1015, 932, 914, 784, 746 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.54 (d, J = 6.89 Hz, 3H), 1.78 (m, 2H), 2.12 (m, 3H), 2.50 (m, 1H), 3.83 (s, 3H), 4.04 (dq, J = 2.70, 6.89 Hz, 1H), 4.2 (dd, J = 3.60, 11.09 Hz, 1H), 4.45 (dd, J = 5.99, 10.79 Hz, 1H), 6.43 (bs, 1H). ^{13}C NMR ($CDCl_3$): δ 22.3, 22.4, 27.9, 34.5, 51.2, 52.6, 65.2, 81.9, 165.1, 167.3, 169.4. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{11}H_{17}N_2O_5$, 257.1137; found, 257.1143.

Tripeptide 10. To MeOH (4.0 mL) at 0 °C was added $SOCl_2$ (0.213 g, 0.131 mL, 1.79 mmol) dropwise. Then diketopiperazine **9** (0.90 g, 0.351 mmol) was added, and the heterogeneous mixture was allowed to stir at 0 °C for 15 min and then allowed to warm to rt over 1 h. The reaction was heated at reflux for 30 min and then cooled to rt. The reaction was diluted with EtOAc and treated with 5% Na_2CO_3 until the aqueous layer remained basic. The layers were separated, and the aqueous layer was saturated with NaCl and extracted with EtOAc (4×). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give a mixture of starting material **9** and substituted hydroxylamine **18**. Expedient chomatography utilizing silica gel with 100% EtOAc afforded 0.049 g (49%) of **18** as a film that was utilized without further purification.

Substituted hydroxylamine **18** (0.049 g, 0.170 mmol) in MeOH (2.0 mL) was hydrogenated with Pd/C under an atmosphere of H_2. After 30 min, the reaction was purged with Ar and filtered through a pad of Celite. The MeOH filtrate was concentrated to give 0.045 g (91% crude mass recovery) of the reduced amine as a clear oil.

To the reduced amine (0.045) in CH_2Cl_2 (1.5 mL) at 0 °C was added Boc-L-Phe-OH (0.045, 0.171 mmol) followed by pyridine (0.014 g, 0.014 mL, 0.171 mmol), HOBt (0.023 g, 0.171 mmol), and EDC·HCl (0.033 g, 0.171 mmol). The reaction was allowed to warm to rt and after 12 h was partitioned between EtOAc and 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$ and brine and then dried ($MgSO_4$), filtered, and concentrated to give an oil that was chromatographed on silica gel utilizing 100% EtOAc/hexanes to give 0.066 g (69% from **18**) of **10** as a clear glass; R_f 0.38 (100% EtOAc). IR (thin film): 3309 (broad), 2954, 1735, 1702, 1648 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.39 (s, 9H), 1.39−1.51 (m, 4H), 1.58−1.69 (m, 2H), 1.77−1.90 (m, 3H), 3.00−3.14 (m, 2H), 3.18 (d, J = 4.20 Hz, 1H), 3.74 (s, 3H), 3.77 (s, 3H), 4.15 (m, 1H), 4.38−4.56 (m, 3H), 5.09 (d, J = 7.5, 1H), 6.72 (d, J = 7.8 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 7.18−7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.9, 20.8, 28.2, 31.8, 33.5, 38.1, 48.1, 52.4, 52.5, 52.7, 55.8, 70.2, 80.4, 127.0, 128.7, 129.3, 136.4, 155.5, 170.6, 171.3, 173.0, 175.4. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{26}H_{40}N_3O_9$, 538.2765; found, 538.2757.

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Supporting Information Available: ^1H NMR and ^{13}C NMR for **6**−**14**, **16**, and **17** and plot of the X-ray structure **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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