Total Synthesis of (-)- and (+)-Dysiherbaine

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Supporting Material

General Procedures. NMR spectra were recorded at 400 MHz, chemical shifts are reported in δ, coupling constants in Hz, and IR spectra were obtained as thin film and are reported in cm^{-1}.

Ethyl *(R)*-Cyano[[(oxiranyl)carbonyl]amino]acetate (6R). A solution of *(R)*-glycidic acid (**8R**) (2.90 g, 32.9 mmol) and ethyl amino(cyano)acetate (**7**) (4.43 g, 34.6 mmol) in EtOAc (250 mL) was treated with dicyclohexylcarbodiimide (DCC) (7.13 g, 34.6 mmol) at 0 °C for 1 h and at room temperature for 12. The white precipitated DCU was filtered off and washed with EtOAc (3 × 50 mL). The filtrate and the washings were combined and washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexane/EtOAc) gave 5.54 g (85%) of **6R** as a mixture of 1:1 diastereomers: ^{1}H NMR ($CDCl_3$) 6.98 (d, 0.5 × 1, *J* = 7.6, NH), 6.96 (d, 0.5 × 1, *J* = 7.6, NH), 5.50 (d, 0.5 × 1, *J* = 7.6), 5.48 (d, 0.5 × 1, *J* = 7.6), 4.40-4.33 (m, 2), 3.58-3.54 (m, 1), 3.10-3.07 (m, 1), 2.92-2.89 (m, 1), 1.39-1.35 (m, 3); ^{13}C NMR ($CDCl_3$) (168.62, 168.52), (162.82, 162.68), (113.44, 113.32), (64.44, 64.37), (49.15, 49.05), (47.44, 47.35), (42.21, 41.99), 13.87; IR 3325, 1757, 1689, 1626; HRMS (CI/CH_4) calculated for $C_8H_{11}N_2O_4$ (MH^+) 199.0719, found, 199.0724.

Ethyl *(2R,4R)*-2-Cyano-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate (5R) and Ethyl *(2S,4R)*-2-Cyano-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate (11R). A solution of NaOEt in EtOH (1.58 M, 0.33 mL, 0.517 mmol) was added under N_2 to a solution of **6R** (1.024 g, 5.17 mmol) in THF (250 mL) at room temperature. The resulting solution was heated at 60 °C for 12 h, cooled to room temperature and treated with additional NaOEt solution (1.58 M, 0.16 mL, 0.253 mmol). The solution was heated at 60 °C for 12 h. The reaction was quenched by pouring it into saturated NH_4Cl (100 mL), and the aqueous layer was extracted with EtOAc

(3 × 75 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Flash chromatography of the residue on silica gel (3:1 to 1:1 hexane/EtOAc) gave 0.082 g (8%) of recovered **6R** followed by 0.594 g (58%) of **5R** and **11R** as a 45:55 difficulty separable mixture of diastereomers. Careful flash chromatography of the mixture on silica gel (3:1 hexane/EtOAc) gave a small amount of pure **11R** followed by mixed fractions.

Data for **5R** were determined from the mixture: ^{1}H NMR ($CDCl_3$) 7.78 (s, 1, NH), 4.48 (dd, 1, *J* = 7.9, 7.9), 4.35 (q, 2, *J* = 7.3), 2.97 (dd, 1, *J* = 13.4, 7.9), 2.70 (dd, 1, *J* = 13.4, 7.9), 1.37 (t, 3, *J* = 7.3); ^{13}C NMR ($CDCl_3$) 177.1, 166.1, 116.4, 67.1, 64.5, 55.4, 39.7, 13.8; IR 3331, 2216, 1729.

Data for **11R**: ^{1}H NMR ($CDCl_3$) 7.57 (s, 1, NH), 4.63 (dd, 1, *J* = 9.2, 7.9), 4.39 (q, 2, *J* = 7.3), 3.15 (dd, 1, *J* = 12.8, 7.9), 2.47 (dd, 1, *J* = 12.8, 9.2), 1.38 (t, 3, *J* = 7.3); ^{13}C NMR ($CDCl_3$) 176.4, 165.3, 116.5, 67.6, 64.7, 54.6, 40.3, 13.8; IR 3331, 2216, 1729.

Ethyl *(2R,4R)*-2-Cyano-4-*tert*-butyldimethylsilyloxy-5-oxo-2-pyrrolidinecarboxylate (12R) and Ethyl *(2S,4R)*-2-Cyano-4-*tert*-butyldimethylsilyloxy-5-oxo-2-pyrrolidinecarboxylate (13R). TBSOTf (226 mg, 0.853 mmol) was added to a solution of **5R** and **11R** (65 mg, 0.328 mmol) and 2,6-lutidine (93 mg, 0.869 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture warmed to room temperature, stirred for 6 h, and quenched by pouring into a mixture of Et_2O (15 mL) and H_2O (5 mL). The organic layer was separated, washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue on silica gel (9:1 hexane/EtOAc) gave 53 mg (52%) of **13R** followed by 44 mg (43%) of **12R**.

Data for **12R**: ^{1}H NMR ($CDCl_3$) 6.60 (br s, 1, NH), 4.39-4.32 (m, 3), 2.79 (dd, 1, *J* = 13.6, 6.8), 2.63 (dd, 1, *J* = 13.6, 5.2), 1.37 (t, 3, *J* = 7.4), 0.92 (s, 9), 0.17 (s, 3), 0.15 (s, 3); ^{13}C NMR ($CDCl_3$) 174.5, 166.0, 116.5, 68.3, 64.4, 55.3, 41.3, 25.6 (3 C), 18.1, 13.9, -4.6, -5.3; IR 3190, 1746, 1728; $[\alpha]_D$ = +17.4 (c = 2.2, $CHCl_3$); HRMS (CI/CH_4) calculated for $C_{14}H_{25}N_2O_4Si$ (MH^+) 313.1584, found, 313.1595.

Data for **13R**: ^{1}H NMR ($CDCl_3$) 6.44 (br s, 1, NH), 4.51 (dd, 1, *J* = 8.8, 7.6), 4.37 (q, 2, *J* = 7.4), 3.01 (dd, 1, *J* = 12.8, 7.6), 2.47 (dd, 1, *J* = 12.8, 8.8), 1.37 (t, 3, *J* = 7.4), 0.90 (s, 9),

0.18 (s, 3), 0.16 (s, 3); ^{13}C NMR ($CDCl_3$) 174.1, 165.0, 116.8, 68.4, 64.4, 54.3, 41.4, 25.6 (3 C), 18.1, 13.9, -4.6, -5.3; $[\alpha]_D$ = +47.5 (c = 1.4, $CHCl_3$).

Ethyl *(2R,4R)*-2-Aminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (14R). A solution of **12R** (33.0 mg, 0.106 mmol) and conc HCl (0.030 mL) in EtOH (1 mL) was hydrogenated over PtO_2 (5 mg) under 50 psi H_2 at room temperature for 14 h. The reaction mixture was filtered through Celite, which was washed with EtOH. The filtrate was concentrated to give 25.2 mg (100%) of pure **14R**: ^{1}H NMR (D_2O, HOD at δ 4.65) 4.44 (dd, 1, *J* = 8.4, 8.4), 4.13 (q, 2, *J* = 7.2), 3.38 (d, 1, *J* = 14.0), 3.34 (d, 1, *J* = 14.0), 2.77 (dd, 1, *J* = 14.0, 8.4), 1.95 (dd, 1, *J* = 14.0, 8.4), 1.13 (t, 3, *J* = 7.2); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.8, 172.7, 68.9, 64.9, 61.3, 44.4, 37.9, 14.0; IR 3220, 1710; $[\alpha]_D$ = +49.5 (c = 1.3, CH_3OH); HRMS (DCI/NH_3) calculated for $C_8H_{15}N_2O_4$ (M^+) 203.1032, found, 203.1031.

Ethyl *(2R,4R)*-2-(*N,N*-Dimethylaminomethyl)-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (15R). A solution of **14R** (23.1 mg, 0.096 mmol) and CH_2O (37% in H_2O, 16.4 mg, 0.20 mmol) in H_2O (1.2 mL) was hydrogenated over 10% Pd-C under 50 psi H_2 for 12 h. The reaction mixture was filtered through Celite and the filtrate was concentrated to give 25.7 mg (100%) of pure **15R**: ^{1}H NMR (D_2O, HOD at δ 4.65) 4.42 (dd, 1, *J* = 8.0, 7.9), 4.15 (q, 2, *J* = 7.3), 3.65 (d, 1, *J* = 14.0), 3.58 (d, 1, *J* = 14.0), 2.85 (s, 6), 2.81 (dd, 1, *J* = 14.0, 7.9), 1.98 (dd, 1, *J* = 14.0, 8.0), 1.14 (t, 3, *J* = 7.3); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.9, 172.6, 68.7, 65.2, 63.0, 61.3, 46.4 (br, 2 C), 39.4, 14.0; IR 3286, 1723; HRMS (DCI/NH_3) calculated for $C_{10}H_{19}N_2O_4$ (M^+) 231.1345, found, 231.1350.

***(2R,4R)*-2-Ethoxycarbonyl-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium iodide (16R)**. A solution of **15R** (25.7 mg, 0.096 mmol) in EtOH (1 mL) was neutralized by adding 2-3 drops of saturated $NaHCO_3$ until the pH was 8. The solvent was evaporated to dryness and $CHCl_3$ (5 mL) was added. The mixture was stirred vigorously for 30 min to dissolve the amine and the NaCl was filtered off. The filtrate was concentrated to give 22.0 mg of amine. A solution of amine and excess CH_3I (410 mg,

2.88 mmol) in THF (1.5 mL) was stirred at room temperature for 36 h. The solvent and excess CH_3I were evaporated to gave 33.9 mg (95%) of **16R**: ^{1}H NMR (D_2O, HOD at δ 4.65) 4.29 (dd, 1, *J* = 7.9, 5.5), 4.23-4.15 (m, 2), 4.08 (d, 1, *J* = 14.0), 3.79 (d, 1, *J* = 14.0), 3.09 (s, 9), 2.77 (dd, 1, *J* = 14.0, 7.9), 2.05 (dd, 1, *J* = 14.0, 5.5), 1.17 (t, 3, *J* = 7.3); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.8, 172.6, 71.5, 68.5, 65.6, 62.5, 55.9 (3 C), 41.4, 14.0; IR 3418, 1714; $[\alpha]_D$ = +20.7 (c = 1.0, CH_3OH); HRMS (DCI/NH_3) calculated for $C_{11}H_{21}N_2O_4$ (M^+) 245.1501, found, 245.1509.

***ent*-Dysibetaine [*(2R,4R)*-2-carboxy-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium (1R)]**. A solution of **16R** (20.2 mg, 0.054 mmol) and Dowex 550A resin in the hydroxide form (150 mg) in CH_3OH (2 mL) was heated at 55 °C for 10 h. The resin was filtered off and filtrate was concentrated to give 11.2 mg (97%) of **1R**: ^{1}H NMR (D_2O, HOD at δ 4.65, 20.0 °C) 4.21 (dd, 1, *J* = 8.0, 5.5), 3.90 (d, 1, *J* = 14.0), 3.60 (d, 1, *J* = 14.0), 3.07 (s, 9), 2.53 (dd, 1, *J* = 13.9, 8.0), 1.86 (dd, 1, *J* = 13.9, 5.5); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.5, 176.8, 73.1, 69.1, 64.1, 55.6 (3 C), 42.4; IR 3361, 1713, 1626; $[\alpha]_D$ = +5.3 (c = 0.26, H_2O); HRMS (FAB) calculated for $C_9H_{16}N_2O_4$ (MH^+) 217.1188, found, 217.1190.

The ^{1}H NMR spectra is identical to that reported for the natural product in D_2O (HOD at δ 4.65, 25.6 °C) except that all peaks absorb 0.02 ppm upfield in the synthetic material. The ^{13}C NMR correspond closely except for the quaternary carbon, which absorbs at 66.0, not 64.1, in the natural product.[1]

Ethyl *(2S,4R)*-2-Aminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (17R). Reaction of **13R** (16.0 mg, 0.0512 mmol) and conc HCl (15 μL) and PtO_2 (2 mg) in EtOH (0.5 mL) as described above for the preparation of **14R** gave 12.2 mg (100%) of **17R**: ^{1}H NMR (D_2O, HOD at δ 4.65) 4.38 (dd, 1, *J* = 8.5, 7.3), 4.16-4.10 (m, 2), 3.35 (d, 1, *J* = 14.0), 3.21 (d, 1, *J* = 14.0), 2.56 (dd, 1, *J* = 14.0, 8.5), 2.17 (dd, 1, *J* = 14.0, 7.3), 1.12 (t, 3, *J* = 7.3); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.2, 172.6, 68.5, 64.9, 61.5, 45.0, 38.3, 14.0; $[\alpha]_D$ = +16.3 (c = 2.1, CH_3OH).

Ethyl *(2S,4R)*-2-*N,N*-Dimethylaminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (18R). Reaction of **17R** (9 mg, 0.038 mmol), CH_2O (37% in H_2O, 6.4 mg, 0.079 mmol), and 10% Pd-C (2 mg) in H_2O (0.4 mL) as described above for the preparation of **15R** gave 10 mg (100%) of **18R**: ^{1}H NMR (D_2O, HOD at δ 4.65) 4.43 (dd, 1, *J* = 8.4, 7.9), 4.17 (q, 2, *J* = 7.3), 3.74 (d, 1, *J* = 14.0), 3.42 (d, 1, *J* = 14.0), 2.77 (s, 6), 2.56 (dd, 1, *J* = 14.0, 7.9), 2.13 (dd, 1, *J* = 14.0, 8.4), 1.14 (t, 3, *J* = 7.3); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.7, 172.7, 68.1, 65.4, 62.8, 60.8, 46.0 (br, 2 C), 40.5, 14.0.

***(2S,4R)*-2-Ethoxycarbonyl-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium iodide (19R)**. Reaction of **18R** (10.0 mg, 0.0375 mmol) and CH_3I (160 mg, 1.13 mmol) in THF (1 mL) as described above for the preparation of **16R** gave 13.2 mg (95%) of **19R**: ^{1}H NMR (D_2O, HOD at δ 4.65) 4.47 (dd, 1, *J* = 9.2, 7.9), 4.24-4.15 (m, 2), 4.08 (d, 1, *J* = 14.0), 3.61 (d, 1, *J* = 14.0), 3.04 (s, 9), 2.57 (dd, 1, *J* = 13.4, 7.9), 2.13 (dd, 1, *J* = 13.4, 9.2), 1.16 (t, 3, *J* = 7.3); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.4, 172.5, 70.5, 67.8, 65.6, 60.8, 55.5 (3 C), 42.3, 13.9; $[\alpha]_D$ = +5.9 (c = 0.64, CH_3OH).

***(2S,4R)*-2-carboxy-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium (20R)**. Reaction of **19R** (9.6 mg, 0.0258 mmol) and Dowex 550A resin in the hydroxide form (75 mg) in CH_3OH (1 mL) as described above for the preparation of **1R** gave 5.7 mg (97%) of **20R**: ^{1}H NMR (D_2O, HOD at δ 4.65ppm, 19.2 °C) 4.42 (dd, 1, *J* = 9.8, 7.9), 3.90 (d, 1, *J* = 14.0), 3.45 (d, 1, *J* = 14.0), 3.02 (s, 9), 2.46 (dd, 1, *J* = 13.4, 7.9), 1.90 (dd, 1, *J* = 13.4, 9.8); ^{13}C NMR (D_2O, CD_3OD at δ 49.0 as internal standard) 179.2, 176.8, 71.6, 68.6, 62.0, 55.4 (3 C), 43.1; $[\alpha]_D$ = +9.5 (c = 0.30, CH_3OH).

Ethyl *(S)*-cyano[[(oxiranyl)carbonyl]amino]acetate (6S) (3.5 g, 85%) was prepared from *S*-glycidic acid.

Ethyl *(2S,4S)*-2-cyano-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate (5S) and ethyl *(2R,4S)*-2-cyano-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate (11S) (55%, 0.40 g) were prepared from **6S** as a 45:55 mixture of diastereomers

Ethyl *(2S,4S)*-2-cyano-4-(*tert*-butyldimethylsilyl)oxy-5-oxo-2-pyrrolidinecarboxylate (12S) [83 mg, 40%, $[\alpha]_D$ = -19.9 (c = 3.0, $CHCl_3$)] and **ethyl *(2R,4S)*-2-cyano-4-(*tert*-butyldimethylsilyl)oxy-5-oxo-2-pyrrolidinecarboxylate (13S)** [105 mg, 51%. $[\alpha]_D$ = -47.9 (c = 3.2, $CHCl_3$)] were prepared from the mixture of **5S** and **11S**.

Ethyl *(2S,4S)*-2-aminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (14S) [43 mg, 97%, $[\alpha]_D$ = -48.9 (c = 4.0, CH_3OH)] was prepared from **12S**.

Ethyl *(2S,4S)*-2-*N,N*-dimethylaminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (15S) (46 mg, 100%) was prepared from **14S**.

***(2S,4S)*-2-Ethoxycarbonyl-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium iodide (16S)** [38 mg, 92%, $[\alpha]_D$ = -21.0 (c = 1.9, CH_3OH)] was prepared from **15S**.

Dysibetaine [*(2S,4S)*-2-carboxy-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium (1S)] [20 mg, 97%, $[\alpha]_D$ = -7.1 (c = 0.26, H_2O] was prepared from **15S** The ^1H and ^{13}C NMR data of **1S** are identical to those of **1R**.

Ethyl *(2R,4S)*-2-aminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (17S) [77 mg, 97%, $[\alpha]_D$ = -19.5 (c = 5.5, CH_3OH)] was prepared from **13S**.

Ethyl *(2R,4S)*-2-*N,N*-Dimethylaminomethyl-4-hydroxy-5-oxo-2-pyrrolidinecarboxylate hydrochloride (18S) (80 mg, 100%) was prepared from **17S**.

***(2R,4S)*-2-Ethoxycarbonyl-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium iodide (19S)** [50 mg, 93%, $[\alpha]_D$ = -7.2 (c = 2.0, CH_3OH)] was prepared from **18S**.

***(2R,4S)*-2-carboxy-4-hydroxy-*N,N,N*-trimethyl-5-oxo-2-pyrrolidinemethanaminium (20S)** [28 mg, 97%, $[\alpha]_D$ = -11.2 (c = 0.34, CH_3OH)] was prepared from **19S**.