Synthesis of a Conformationally Restricted Substrate Analog of Siderophore Biosynthetases

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Supporting Information

Experimental

General Methods. ^1H NMR and ^{13}C NMR spectra were obtained on a Varian 300 spectrometer and were referenced to residual DMSO, $CHCl_3$, or H_2O. Analytical TLC was carried out using Merck aluminum-backed 0.2 mm silica gel 60 F-254 plates. Column chromatography was conducted using Merck silica gel 60 (230-400) mesh.

All reactions were periodically monitored by TLC and worked up after the complete consumption of starting materials unless specified otherwise. Anhydrous tetrahydrofuran was freshly distilled from sodium and stored under argon. Anhydrous acetonitrile was freshly distilled from CaH_2 and stored under argon. All purchased reagents were of reagent grade quality and were used without further purification.

1,4-*syn*-Hydroxamic acid (2). A suspension of *N*-acetyl cycloadduct **1** (206 mg, 1.48 mmol) and $CuSO_4$ (25 mg, 0.153 mmol) in THF (1 mL) was treated with a solution of methylnitroacetate (344 mg, 2.89 mmol) in THF (4 mL) and a solution of $Pd(OAc)_2$ (33 mg, 0.147 mmol) and PPh_3 (158 mg, 0.601 mmol) in THF (10 mL) and refluxed under argon for 50 min. The mixture was diluted with EtOAc and washed with a saturated solution of Na_2EDTA. The aqueous layer was extracted with EtOAc and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo* to an oil. Chromatography (silica gel; eluted with 3% MeOH-CH_2Cl_2) provided 268 mg (70%) of 1,4-*syn*-hydroxamic acid **2** as a 1:1 mixture of diastereomers as an oil: ^1H NMR of diastereomeric mixture (300 MHz, DMSO-d_6) δ 1.66 (overlapping ddd, J = 5.7, 5.7, 13.5 Hz, 1H), 1.97 (s, 3H), 2.35 (overlapping ddd, J = 8.4, 8.4, 13.5 Hz, 1H), 3.48 (m, 1H), 3.77 (d, 3H), 5.66 (m, 4H), 9.51 (d, 1H); ^{13}C NMR of diastereomeric mixture (75 MHz, DMSO-d_6) δ 20.76, 29.08, 44.46, 53.53, 59.77, 90.11, 131.78, 133.72, 164.45, 170.62; HRMS (FAB) calcd for $C_{10}H_{14}N_2O_6$ $(M+H)^+$ 259.0930, found 259.0919.

1,4-*syn*-*O*-Bn Hydroxamate (3). A suspension of 1,4-*syn*-hydroxamic acid **2** (44 mg, 0.169 mmol), Na_2CO_3 (72 mg, 0.683 mmol), and a catalytic amount of NaI in CH_3CN (3 mL) was treated with BnBr (34 µL, 0.286 mmol) and heated at 60 ˚C under argon for 48 h. The mixture was diluted with EtOAc and washed with 1 M HCl. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo* to an oil. Flash chromatography (silica gel; eluted with 2:1 hexanes-EtOAc) provided 11 mg of starting material and 23 mg (53%) of a 1:1 mixture of diastereomers of 1,4-*syn*-*O*-Bn hydroxamate **3** as an oil: ^1H NMR of diastereomeric mixture (300 MHz, $CDCl_3$) δ 1.74 (m, 1H), 2.18 (d, 3H), 2.56 (m, 1H), 3.58 (m, 1H), 3.78 (d, 3H), 4.81 (m, 2H), 5.03 (m, 1H), 5.61 (bm, 1H), 5.92 (dm, 1H), 6.00 (m, 1H), 7.38 (m, 5H); ^{13}C NMR of diastereomeric mixture (75 MHz, $CDCl_3$) δ 21.28, 30.01, (30.83), 44.76, (45.11), 53.35, (53.52), 62.41, 79.34, (79.53), 90.22, (90.50), 128.72, (128.80), 129.06, (129.15), 129.18, (129.39), 132.83, (133.24), 133.47, (133.55), 133.90, (134.00), 164.00, (164.07), 170.62; HRMS (FAB) calcd for $C_{17}H_{20}N_2O_6$ $(M+H)^+$ 349.1400, found 349.1420.

1,4-*syn*-Hydroxamic acid (4). To a tartaric acid buffer solution (8.8 mL, made by dissolving 49 g of tartaric acid and 33 g of NaOH in 200 mL H_2O) was added aqueous $TiCl_3$ (8.6%, 2.4 mL, 1.59 mmol). The resulting green slurry was adjusted to pH 7.0 using 1 N NaOH to form a clear green solution. To this solution was added $NaBH_4$ (76 mg, 2.01 mmol) quickly followed by a solution of **3** (112 mg, 0.322 mmol, obtained from several iterations of the previous reaction) in methanol (2 mL). The reaction was stirred under argon for 30 min and then exposed to air and stirred overnight. The reaction mixture was saturated with K_2HPO_4 and extracted several times with ethyl acetate. The combined organics were dried with Na_2SO_4, filtered, and concentrated *in vacuo* to provide a yellow residue. The residue was diluted with THF (7.5 mL) and treated with $NaHCO_3$ (84 mg, 0.995 mmol) and benzyl chloroformate (50 mL, 0.350 mmol). The reaction was stirred under argon for 3 h and then diluted with water. The pH was adjusted to ~5 with 1 M HCl and the aqueous phase was extracted with ethyl acetate. The combined organics were dried with Na_2SO_4, filtered, and concentrated *in vacuo* to an oil. Chromatography (silica gel; eluted with 1:1 hexanes-EtOAc) provided 37 mg of starting material **3** and 45 mg (49%) of a 1:1 mixture of diastereomers of the methyl ester of **4** as an oil: ^{1}H NMR of diastereomeric mixture (300 MHz, $CDCl_3$) δ 1.83 (m, 1H), 2.07 (d, 3H), 2.36 (dm, 1H), 3.24 (m, 1H), 3.72 (s, 3H), 4.49 (dm, 1H), 4.81 (m, 2H), 5.05 (m, 2H), 5.41 (d, J = 7.5, 1H), 5.51 (m, 1H), 5.81 (m, 1H), 5.92 (m, 1H), 7.34 (m, 5H); ^{13}C NMR of diastereomeric mixture (75 MHz, $CDCl_3$) δ 21.22, (21.37), 27.85, (29.69), 46.12, (46.32), 52.35, 56.11, (56.44), 63.37, (63.88), 66.92, 79.14, 127.96, (128.05), 128.42, 128.66, 128.91, (128.96), 129.15, (129.35), 132.00, 132.65, 133.45, 134.52, 136.26, 171.98; HRMS (FAB) calcd for $C_{25}H_{28}N_2O_6$ $(M+H)^+$ 453.2026, found 453.2025.

A solution of the methyl ester (52 mg, 0.114 mmol, obtained from several iterations of the previous reaction sequence) in methanol (6 mL) and water (2 mL) was treated with lithium hydroxide and stirred under argon for 4 h. The mixture was concentrated *in vacuo* and the residue was taken up in ethyl acetate and water. The pH was adjusted to ~4 with 1 M HCl and the solution was extracted with ethyl acetate. The combined organics were dried with Na_2SO_4, filtered, and concentrated *in vacuo* to an oil. Column chromatography (silica gel; eluted with 6% MeOH-1% AcOH-CH_2Cl_2) provided 9 mg of starting material and 38 mg (91%) of 1,4-*syn*-hydroxamic acid **4** as a 1:1 mixture of diastereomers as an oil: ^{1}H NMR of diastereomeric mixture (300 MHz, $CDCl_3$) δ 1.85 (m, 1H), 2.07 (d, 3H), 2.42 (dm, 1H), 3.27 (m, 1H), 4.48 (dm, 1H), 4.81 (m, 2H), 5.05 (m, 2H), 5.39 (dm, 1H), 5.82 (dm, 1H), 5.81 (m, 1H), 5.85 (m, 1H), 5.91 (m, 1H), 6.39 (bs, 1H), 7.30 (m, 5H); ^{13}C NMR of diastereomeric mixture (75 MHz, $CDCl_3$) δ 21.09, (21.24), 28.18, (29.99), 45.89, (46.19), 55.95, (56.33), 63.80, (64.30), 67.06, 79.12, 127.93, (128.06), 128.42, 128.69, 128.98, (129.03), 129.21, (129.38), 131.68, 132.78, 133.23, 134.05, 134.73, 136.16, 156.67, 174.58, 174.80.

1,4-*syn*-Hydroxamic acid (5). To an argon purged solution of 1,4-*syn*-hydroxamic acid **4** (24 mg, 0.54 mmol) in methanol (3 mL) was added palladium on carbon (10 mg). The suspension was purged with H_2 and then stirred under H_2 for 3 h. The mixture was filtered through a plug of Celite 521 and concentrated to an oil. The oil was taken up in methanol and flushed through a plug of C_{18} silica gel to give 12 mg (quantitative) of 1,4-*syn*-hydroxamic acid **5** as a 1:1 mixture of diastereomers as an oil: ^{1}H NMR of diastereomeric mixture (300 MHz, DMSO-d_6) δ 1.68 (m, 6H), 2.00 (s, 3H), 2.20 (m, 1H), 3.53 (overlapping dd, J = 6, 7.2, 1H), 4.77 (m, 1H); ^{13}C NMR of diastereomeric mixture (75 MHz, $CDCl_3$) δ 21.09, (21.24), 28.18, (29.99), 45.89, (46.19), 55.95, (56.33), 63.80, (64.30), 67.06, 79.12, 127.93, (128.06), 128.42, 128.69, 128.98, (129.03), 129.21, (129.38), 131.68, 132.78, 133.23, 134.05, 134.73, 136.16, 156.67, 174.58, 174.80; HRMS (FAB) calcd for $C_9H_{16}N_2O_4$ $(M+H)^+$ 217.1189, found 217.1163.