SUPPORTING INFORMATION

Siderocalin outwits the coordination chemistry of vibriobactin, a siderophore of *Vibrio cholerae*

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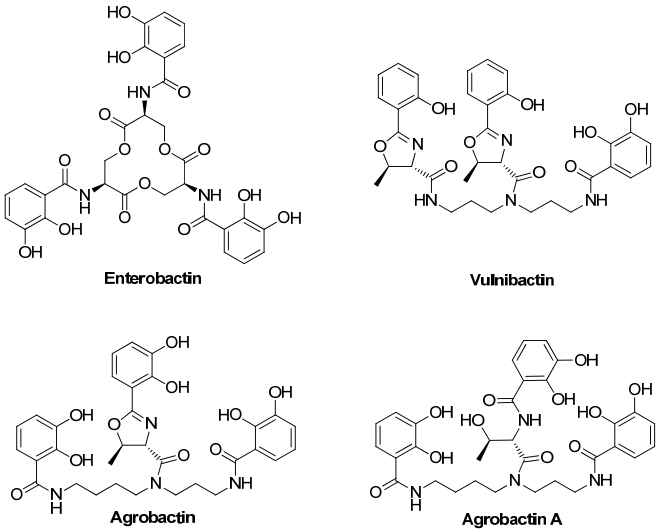
SUPPLEMENTAL FIGURES AND SCHEMES

Figure S1. Chemical structure of four catechol siderophores



Scheme S1. Synthesis of siderophore library precursors

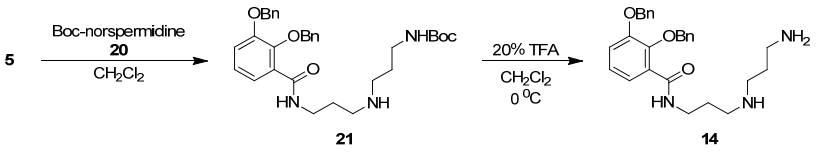


Figure S2. Fluorescence quenching curves of Scn at pH 7.4. The titration data (points) are the average of three independent titrations. The lines represent the one-to-one binding model fit by DYNAFIT to the compiled (not averaged) titration data.(*1*) The K_d for each Fe- or apo-siderophore is listed in Table 1.

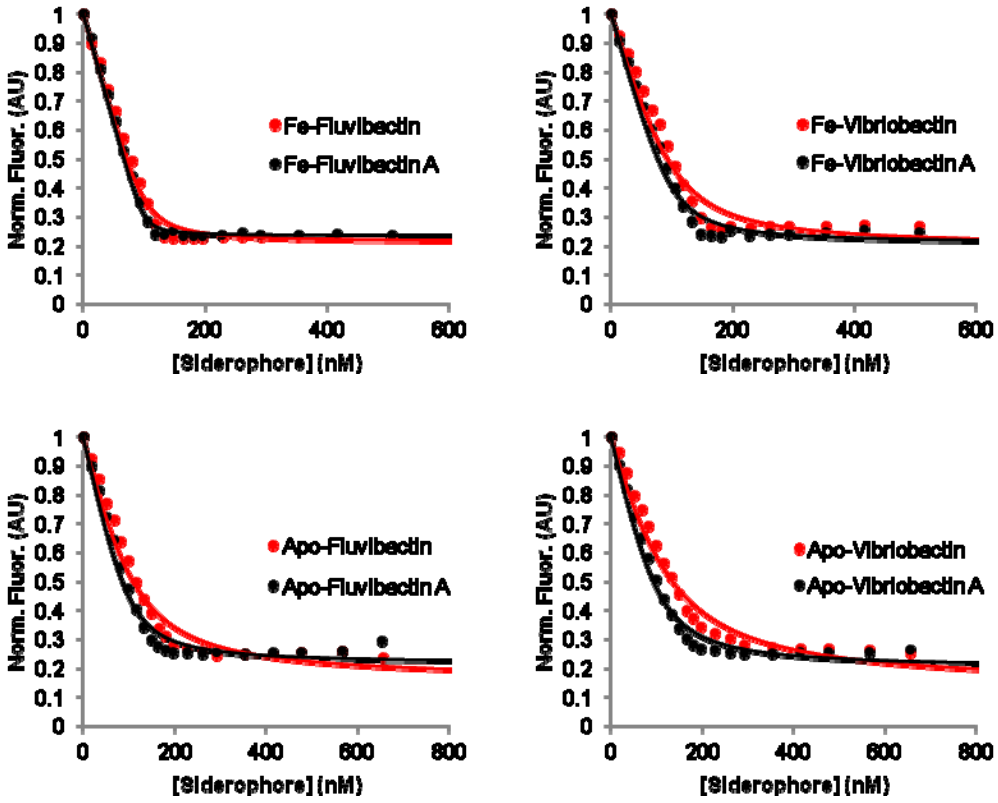


Figure S3. pH dependence of Fe-vibriobactin speciation. The calculation was made using measured protonation constant in the program HySS.(*2*) It shows that at physiological pH (7.4) 87% of the complex is in the phenolate-oxazoline coordination mode.

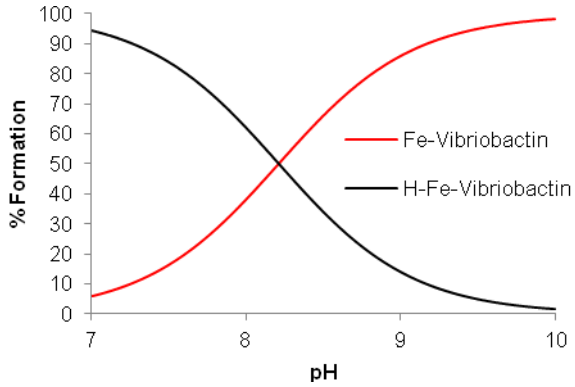
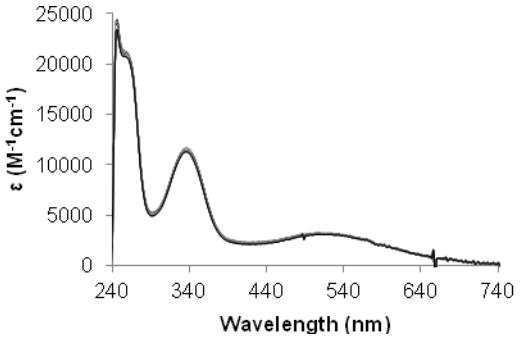


Figure S4. Spectrophotometric titration of Fe-fluvibactin. No change in the absorbance from pH 10 to pH 6 indicates that the first protonation of Fe-fluvibactin and the resulting phenolate-oxazoline coordination mode is not present at physiological pH.



METHODS

General Synthesis Procedures. Starting materials and reagents were used as provided from commercial sources. Flash chromatography was performed using silica gel (40-7 mesh). Thin layer chromatography (TLC) was performed with aluminum backed plates of silica gel 60 F_{254}. The Mass Spectrometry Facility at University of California, Berkeley, recorded the FABLR-MS and ESI-MS, while the Microanalytical Services Laboratory of the same institution performed the microanalyses. The ^{1}H and ^{13}C spectra were measured using the noted Bruker spectrometers at room temperature unless otherwise indicated. The solvent for each spectrum is noted, and the spectra were calibrated the appropriate solvent peak.

Benzyl 2,3-bis(benzyloxy)benzoate (2). A white slurry of 2,3-dihydroxybenzoic acid (**1**) (5.22 g, 33.9 mmol), benzyl chloride (12.5 mL, 109 mmol), KI (18.0 g, 109 mmol), K_2CO_3 (32.8 g, 237 mmol) in acetone (250 mL) was stirred at reflux (60 °C) for 3 days. After cooling, the acetone was evaporated and the solid was dissolved in water and extracted three times with CH_2Cl_2. The organic layer was dried with Na_2SO_4 before purification by flash chromatography. The silica column was packed with hexanes, and a purple side product eluted was with the same solvent. The benzyl ester (14.0 g, 97% yield by weight) was eluted with a 15-100% (v/v) gradient of CH_2Cl_2 in hexanes: R_f = 0.96 (CH_2Cl_2); ^{1}H NMR (AV-300, $CDCl_3$) δ 7.43-7.05 (m, 18H), 5.292 (s, 2H), 5.116 (s, 2H), 5.041 (s, 2H); ^{13}C NMR (AVQ-400, $CDCl_3$) δ 166.37, 152.98, 148.49, 137.53, 136.70, 136.08, 128.75, 128.55, 128.39, 128.29, 128.05, 127.76, 127.03, 124.17, 123.15, 118.12, 75.78, 71.42, 67.11; ESI-MS *m/z* calcd for (M+H) $C_{28}H_{25}O_4$ 425.1753, found 425.1750.

2,3-Bis(benzyloxy)benzoic acid (3). Benzyl ester **2** was stirred at room temperature overnight in a solution of 6 g NaOH, 50 mL methanol, 20 mL CH_2Cl_2. After removing the solvent, the white solid was dissolved in water and acidified with concentrated HCl to precipitate bis-protected carboxylic acid **3**. Filtration and drying gave 11.9 g of material with quantitative conversion of the ester to the carboxylic acid. Characterization of the carboxylic acid matches a previous characterization of the same compound prepared by another method:(*3*) IR (neat) 3031, 2567, 1686, 1577, 1472, 1455, 1415, 1376, 1302, 1258, 1213 cm^{-1}; ^1H NMR (AV-300, $CDCl_3$) δ 11.30 (br, 1H), 7.72 (d, *J* = 7.5 Hz, 1H), 7.50-7.17 (m, 12H), 5.25 (s, 2H), 5.18, (s, 2H); ^{13}C NMR (AVQ-400, $CDCl_3$) δ 165.48, 151.51, 147.27, 136.05, 134.85, 129.51 129.05, 129.02, 128.75, 127.99, 125.24, 124.64, 123.31, 119.13, 71.72; ESI-MS *m/z* calcd for (M+Na) $C_{21}H_{18}O_4Na$ 357.1103, found 357.1099.

2,3-Bis(benzyloxy)benzoyl chloride (4) was synthesized according to the procedure of Schuda et al.(*4*): mp = 51-54°C; ^1H NMR δ (AV-300, $CDCl_3$) 7.57-7.09 (m, 13H), 5.14 (s, 2H), 5.10 (s, 2H).

3-[2,3-Bis(benzyloxy)benzoyl]-1,3-thiazolidine-2-thione (5) was synthesized by using a modified procedure from Samuel et al.(*5*) Acid chloride **4** (20.7 mmol) was dissolved in THF and cooled to 0 °C. A solution of 2-mercaptothiazoline (3.21 g, 26.9 mmol) and triethylamine (5.74 mL, 41.4 mmol) in 70 mL of THF was added over five minutes. The solution became a yellow cloudy mixture while it warmed to room temperature with stirring overnight. The reaction mixture was chromatographed on a silica gel column with CH_2Cl_2. The product was recrystallized with EtOAc/hexanes to give bright yellow crystals: R_f = 0.43 (CH_2Cl_2); ^1H NMR (AV-300, $CDCl_3$) δ 7.42-7.29 (m, 10H), 7.10-7.05 (m, 2H), 6.98-6.95 (m, 1H), 5.12 (s, 4H), 4.33 (t, *J* = 7.4 Hz, 2H), 2.84 (t, *J* = 7.4, 2H); ^{13}C NMR (AVQ-400, $CDCl_3$) δ 201.33, 168.27, 151.45,

145.85, 138.02, 136.67, 130.39, 128.81, 128.59, 128.42, 128.31, 128.26, 127.78, 124.53, 121.60, 117.52, 71.50, 55.90, 28.78.

***N*^{1},*N*^{7}-bis[2,3-bis(benzyloxy)benzoyl]norspermidine (6)** was made using a modified procedure of Miyasaka et al.(*3*) Norspermidine replaced spermidine, and diamide **6** was purified by flash chromatography using 1% NH_4OH/10% methanol in CH_2Cl_2 (v/v) to give a clear yellow oil. The characterization matches the previous report of the same compound that Bergeron et al. prepared using a different method:(*6*) R_f = 0.23 (5% (v/v) methanol in CH_2Cl_2) ; ^{1}H NMR (AVQ-400, $CDCl_3$) δ 8.09 (t, *J* = 5.4 Hz, 2H), 7.69-7.64 (m, 2H), 7.47-7.32 (m, 20H), 7.13-7.01 (m, 4H), 5.15 (s, 4H), 5.07 (s, 4H), 3.32 (q, *J* = 6.4 Hz, 4H), 2.63 (br), 2.46 (t, *J* = 6.8 Hz, 4H), 1.57 (quin, *J* = 6.7 Hz, 4H); ^{13}C NMR (AVQ-400 $CDCl_3$) δ 165.76, 151.85, 146.84, 136.54, 128.94, 128.84, 128.42, 127.80, 127.54, 124.59, 123.26, 117.06, 71.38, 46.94, 37.52, 29.06; FABLR *m/*z calcd for $C_{48}H_{49}N_3O_6$ 763.36, found 764.5.

***N*^{4}-(*N*-carbobenzyloxy-L-threonyl)-*N*^{1},*N*^{7}-bis[2,3-bis(benzyloxy)benzoyl]norspermidine (7).** Diamide **6** (1.09 g, 1.43 mmol) was stirred with *N*-carbobenzyloxy-L-threonine (0.40 g, 1.58 mmol) in DMF (30 mL dried over 4 Å molecular sieves) under N_2(g) and cooled to 0 °C. 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) was added (0.65 g, 1.72 mmol) to the solution, followed by an excess of *N,N*-diisopropylethylamine (DIEA) (0.60 mL, 3.44 mmol). The solution turned bright yellow. The reaction mixture was allowed to warm to room temperature as the ice bath melted while stirring overnight. Water (60 mL) was added to the solution and extracted three times with CH_2Cl_2 (70 mL). The organic layer was washed with 0.5 N HCl (250 mL), then dried, filtered and condensed with rotary evaporation to give a yellow oil. This was purified by flash chromatography using 10% (v/v) methanol in CH_2Cl_2 to elute a yellow oil. The ^{1}H NMR spectrum showed residual DMF, so the oil was

dissolved in CH_2Cl_2 (30 mL) and washed three times with 0.5 N HCl (30 mL). The organic layer was dried, filtered, and concentrated under vacuum to yield a pale yellow solid (685 mg, 48% yield by weight). The ^{1}H NMR spectrum matches the previously reported spectrum of the same compound prepared by a different method:(*6*) R_f = 0.56 (10% (v/v) methanol in CH_2Cl_2); ^{1}H NMR δ (AVQ-400, $CDCl_3$) 8.05 (t, 1H), 7.94 (t, 1H), 7.64 (m, 2H) 7.48-7.25 (m, 25H) 7.15-7.07 (m, 4H), 5.64 (d, *J* = 9.2 Hz, 1H), 5.18-4.96 (m, 10H), 4.33 (d, *J* = 8.8 Hz, 1H), 4.10 (br, 1H), 3.94 (q, *J* = 6.4 Hz, 1H), 3.35-3.00 (m, 8H), 1.60-1.49 (m, 4H), 1.10 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (AVQ-400, $CDCl_3$) δ 172.38, 165.79, 165.58, 156.76, 151.88, 146.95, 136.77, 136.70, 136.62, 129.02, 128.90, 128.86, 128.79, 128.67, 128.48, 129.42, 128.29, 128.14, 127.84, 124.57, 123.37, 123.18, 117.14, 76.65, 76.46, 71.45, 67.76, 67.24, 45.23, 42.93, 37.07, 36.65, 29.09, 27.47, 19.07; FABLR *m/z* calcd for (M+H) $C_{60}H_{63}N_4O_{10}$ 1000, found 1000; Anal. Calcd (Found) for $C_{60}H_{62}N_4O_{10}$ • 2/3H_2O •1/6 $C_3H_{12}N_2O$ (urea): C, 70.90 (70.88); H, 6.39 (6.51); N, 5.89 (5.91).

***N*^{4}-(L-threonyl)-*N*^{1},*N*^{7}-bis(2,3-dihydroxybenzoyl)norspermidine (8).** Ethanol was degassed by exchanging vacuum and N_2(g) on a Schlenk line. Triamide **7** (0.21 g, 0.21 mmol) was added to the solvent, followed by 5% Pd-C (0.22 g wet, 0.05 mmol). The black slurry was stirred under 1 atm H_2(g) for 30 hours. The slurry was filtered with an acid-washed fine frit, and the solvent was removed to give the deprotected product **3** (0.10 g, 94 % yield) The ^{1}H NMR spectrum matches the previously reported spectrum of the same compound prepared by a different method:(*6*) R_f = baseline (10% (v/v) methanol in CH_2Cl_2, spot is red on silica); ^{1}H NMR (AV-300, CD_3OD) δ 7.24 (t, *J* = 9 Hz, 2H), 6.93 (d, *J* = 7.8 Hz, 2H), 6.75-6.68 (m, 2H), 4.25 (d, J_1 = 5.4 Hz, 1H), 4.07 (quin, *J* = 6.0 Hz, 1H), 3.77-3.60 (m, 2H), 3.5-3.39 (m, 6H), 2.05-1.78 (m, 4H), 1.30-1.20 (m, 3H); ^{13}C NMR (AV-300, CD_3OD) δ 172.15, 171.79, 171.59, 150.69, 147.62, 147.56, 133.66,

132.52, 129.97, 119.64, 119.56, 119.03, 118.81, 117.04, 116.88, 69.36, 69.21, 57.22, 50.00, 48.30, 46.91, 44.88, 40.22, 39.00, 37.90, 31.69, 30.56, 30.22, 30.07, 28.46, 25.03, 24.13, 20.12, 14.55, 11.54.

Ethyl 2,3-dihydroxybenzimidate (9) was made according to the procedure of Bergeron.(*7*)

Fluvibactin (10) was synthesized using a procedure given by Bergeron et al.(*6*) and purified on a LH-20 column eluting with 20% (v/v) ethanol in benzene. The product was precipitated in diethyl ether to give a fine, light brown solid. Characterization of **5** matched the report of the natural product by Yamamoto et al.(*8*): IR (neat) 3331, 2936, 1633, 1590, 1539, 1456, 1321, 1259, 1236, 1168, 1146 cm^{-1}; ^{1}H NMR (AVQ-400, CD_3OD) δ 7.25-7.10 (m, 3H), 6.97-6.83 (m, 3H), 6.76-6.60 (m, 3H) 5.26 (quin, J = 6.4 Hz, 1H), 4.81 (d, J = 6.4 Hz, 1H), 3.95-3.80 (m, 2H), 3.75-3.60 (m, 2H), 3.60-3.35 (m, 4H), 2.15-2.00 (m, 2H), 1.89 (quin, J = 6.8 Hz, 2H), 1.39 (d, J = 6.4 Hz, 3H); ^{13}C NMR (AVQ-400, CD_3OD) δ 171.95, 171.64, 171.49, 167.96, 150.45, 147.48, 146.82, 120.35, 120.02, 119.75, 118.75, 118.70, 116.78, 111.98, 79.90, 73.01, 46.87, 45.14, 38.03, 37.96, 30.40, 28.54, 20.35; ESI-MS *m/z* calcd for (M+H) $C_{31}H_{35}N_4O_{10}$ 623, found 623; Anal. Calcd (Found) for $C_{31}H_{34}N_4O_{10}$ • 4/5 H_2O: C, 58.45 (58.46); H, 5.63 (5.67); N, 8.79 (8.72).

***N*-[2,3-bis(benzyloxy)benzoyl]-L-threonine (11)** was synthesized following the procedure of Peterson et al.(*9*) As a modification to the cited procedure, acid chloride **4** was prepared following the procedure of Schuda et al.(*4*) The ^{1}H NMR spectra of **11** resembles the literature characterization with slight differences from using a different solvent and instrument: ^{1}H NMR δ (AV-300 $CDCl_3$) 8.94 (d, J = 9.0 Hz, 1H), 7.67 (dd, J_1 = 7.65 Hz, J_2 = 2.1 Hz, 1H), 7.45-7.09 (m, 12H), 5.20-5.11 (m, 4H), 4.60 (dd, J_1 = 7.35 Hz, J_2 = 2.7 Hz, 1H), 4.37 (qd, J_1 = 6.45 Hz, J_2

= 3 Hz, 1H), 1.11 (d, J = 6.6 Hz, 3H); ESI-MS *m/z* calcd for $C_{25}H_{25}NO_6$ (M+H) 436.1760, found 436.1753.

Benzyl-Protected Fluvibactin A (12). Carboxylic acid **11** (0.72 g, 1.6 mmol) was dissolved in THF at room temperature with *N*,*N*-diisopropylethylamine (DIEA) (0.62 mL, 3.6 mmol). The coupling agent 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) (0.68 g, 1.8 mmol) was added to the reaction solution with stirring, and a fine white precipitate formed. Diamide **6** (0.72 g, 1.6 mmol) was added and the reaction was stirred for 24 hours. Over time the precipitate dissolved and the solution turned light yellow. The THF was removed, and the reaction mixture was redissolved in CH_2Cl_2 (~100 mL) and washed sequentially with 0.5 N HCl, brine, and water. The water wash rested overnight to break an emulsion. The organic layer was dried with Na_2SO_4, filtered, and evaporated to give a yellow oil. The oil was purified with flash chromatography using 100% EtOAc as the eluant to give **12** (1.87 g, quantitative yield by weight): R_f = 0.375 (100% EtOAc) or R_f = 0.625 (10% (v/v) methanol in CH_2Cl_2); ^{1}H NMR (AV-600, $CDCl_3$) δ 8.625 (d, J = 9.0 Hz, 1H), 8.021 (dt, J_1 = 16.2 Hz, J_2 = 6.0 Hz, 2H), 7.7-7.6 (m, 3H), 7.5-7.0 (m, 36H), 5.2-5.0 (m, 12H), 4.876 (d, J = 8.4 Hz, 1H), 4.016 (q, J = 6.2 Hz, 1H), 3.5-3.4 (m, 1H), 3.36-3.15 (m, 7H), 1.602 (quin, J = 7.5 Hz, 2H), 1.531 (quin, J = 6.8 Hz, 2H), 1.122 (d, J = 6.6 Hz, 3H); ^{13}C NMR (AV-600, $CDCl_3$) δ 172.38, 165.76, 165.54, 165.51, 151.99, 151.81, 147.18, 146.86, 146.82, 136.64, 146.58, 136.34, 136.21, 129.17, 128.91, 128.80, 128.78, 128.75, 128.73, 128.65, 128.34, 128.30, 128.01, 127.91, 127.84, 127.74, 127.71, 124.48, 124.40, 124.33, 123.25, 123.18, 123.08, 117.51, 117.09, 116.96, 76.52, 76.24, 76.11, 72.45, 71.38, 71.32, 67.91, 45.44, 43.38, 37.09, 36.83, 29.04, 27.50, 19.21, 14.31; ESI-MS *m/z* calcd for $C_{73}H_{72}N_4O_{11}Na$ 1203.5095 (M+Na), found 1203.5126.

General deprotection of benzyl-protected siderophores or siderophore precursor (13, 16, 19). The benzyl protected siderophore or siderophore precursor (**12**, **15**, or **18**) was dissolved in methanol with 1 drop of concentrated HCl. The catalyst, 10 wt% Pd-C, was then added (5 mol% per benzyl protecting group). The reaction was stirred under 1 atm H_2(g) for at least 24 hours. The H_2(g) was removed and the solution was filtered with a fine frit to give a clear to light orange solution. The methanol was removed and the oil was precipitated in cold diethyl ether. The beige solid was isolated by centrifugation and dried under high vacuum to give the reported compounds.

Fluvibactin A (13). See general deprotection procedure above: ^{1}H NMR (AV-600, CD_3OD) δ 7.349 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H) 7.240 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H), 7.204 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H), 6.948 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H), 6.94-6.89 (m, 2H), 6.745 (t, J = 8.1 Hz, 1H), 6.697 (t, J = 8.1 Hz, 1H), 5.058 (d, J = 4.2 Hz, 1H), 4.161 (dq, J_1 = 6.3 Hz, J_2 = 4.2 Hz, 1H), 3.77-3.32 (m, 8H), 2.12-1.99 (m, 2H), 1.93-1.83 (m, 2H), 1.195 (d, J = 6 Hz, 3H); ^{13}C NMR (AV-600, CD_3OD) δ 172.92, 172.02, 171.67, 170.54, 150.44, 149.45, 147.45, 147.35, 120.09, 120.04, 119.98, 119.84, 119.79, 119.77, 118.88, 118.76, 117.53, 116.90, 116.87, 69.06, 55.97, 47.05, 44.78, 38.11, 37.90, 29.98, 28.50, 20.33; ESI-MS *m/z* calcd for $C_{31}H_{36}N_4O_{11}Na$ (M+Na) 663.2278, found 663.2265; Anal. Calcd (Found) for $C_{31}H_{36}N_4O_{11} \cdot H_2O \cdot CH_3OH \cdot$ NaCl: C, 51.30 (51.54); H, 5.65 (5.54); N, 7.48 (7.32).

***N*^{1}-[2,3-bis(benzyloxy)benzoyl]norspermidine (14).** Boc-protected amine **21** (0.629 g, 1.15 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. TFA (5 mL) was added and the reaction was monitored by TLC using alumina plates with 5% (v/v) methanol in CH_2Cl_2. The reaction was quenched after 40 min by adding 1 M NaOH until the pH was basic. The mixture was extracted with CH_2Cl_2 three times, dried with Na_2SO_4, and filtered and evaporated. The

residue was dissolved in a small amount of CH_2Cl_2 and stirred with K_2CO_3(s) to absorb and residual water and hydroxide. The slurry was filtered to give a clear solution. Evaporation gave monoamide **9** (0.45 g, 87.5% yield by weight): ^{1}H NMR (AV-300, $CDCl_3$) δ 8.068 (m, 1H), 7.75-7.55 (m, 1H), 7.50-7.18 (m, 10H), 7.18-7.00 (m, 2H), 5.095 (s, 2H), 5.016 (s, 2H), 3.312 (q, *J* = 6.4 Hz, 2H), 2.660 (t, *J* = 6.8, 2H), 2.491 (q, *J* = 6.6 Hz), 1.60-1.45 (m, 4H); ^{13}C NMR (AV-600, $CDCl_3$) δ 165.218, 151.778, 146.688, 136.537, 136.463, 128.811, 128.699, 128.652, 128.285, 127.709, 124.454, 123.204, 116.791, 76.339, 71.260, 47.772, 47.576, 40.533, 37.901, 33.770, 29.590; ESI-MS *m/z* calcd for $C_{27}H_{34}N_3O_3$ (M+H) 448.2600, found 448.2593.

***N*^{1},*N*^{4}-bis(*N*-carbobenzyloxy-L-threonyl)]-*N*^{7}-[2,3-bis(benzyloxy)benzoyl]norspermidine (15).** The bis-TFA salt of monoamide **14** (1.30 g, 1.92 mmol), Cbz-Thr (1.23 g, 4.87 mmol), DIEA (2.76 mL, 15.8 mmol) were stirred in CH_2Cl_2 at 0 °C. HATU (2.01g, 5.28 mmol) was added and the reaction was allowed to warm to room temperature. Stirring continued for 36 hours. The solution was diluted with CH_2Cl_2 (to 50 mL) and washed twice with 5% (w/v) citric acid in brine (50 mL) and once with brine (50 mL). The slightly cloudy organic layer was dried, filtered and evaporated. The resulting mixture was applied to a column (2 in. O.D.) layered with alumina (1.5 in.) on top of silica (5 in.) packed with CH_2Cl_2 and eluted with a gradient of 0-3% (v/v) methanol in CH_2Cl_2. The product-containing fractions were then applied to a silica column and eluted with 2-5% (v/v) methanol in ethyl acetate to give pure **15** (0.44 g, 23.5% yield): R_f = 0.42 (5% methanol in CH_2Cl_2); ^{1}H NMR (AV-300, $(CD_3)_2SO$) δ 8.35-8.15 (m, 1H), 8.05-7.80 (m, 1H), 7.60-7.10 (m, 24H), 6.925 (d, *J* = 8.1 Hz, 1H), 5.30-4.90 (m, 8H), 4.766 (m, 2H), 4.50-4.30 (m, 1H), 4.05-3.75 (m, 3H), 3.35-2.90 (m, 6H), 1.95-1.45 (m, 4H), 1.15-0.95 (m, 6H); ^{13}C NMR (AV-300, $(CD_3)_2SO$) δ 170.263, 170.081, 165.905, 165.522, 164.616, 156.123, 151.624, 145.151, 137.121, 136.979, 136.819, 131.346, 130.997, 128.490, 128.341, 128.223, 128.016,

127.791, 127.689, 124.181, 120.731, 115.787, 75.088, 70.180, 67.090, 66.661, 65.568, 60.823, 56.241, 44.865, 42.926, 38.260, 36.736, 36.593, 36.217, 28.578, 27.263, 20.122, 19.814; ESI MS *m/z* calcd for (M+H) $C_{51}H_{60}N_5O_{11}$ 918.4289, found 918.4284.

***N*^{1},*N*^{4}-bis(L-threonyl)-*N*^{7}-(2,3-dihydroxybenzoyl)norspermidine (16).** See general deprotection procedure above: ^{1}H NMR (AV-600, CD_3OD) δ 7.230 (dt, J_1 = 8.0 Hz, J_2 = 1.4 Hz, 1H), 6.918 (d, J = 7.8 Hz, 1H), 6.72-6.67 (m, 1H), 4.04-3.93 (m, 1H), 3.851 (quin, J = 6.3 Hz, 1H), 3.751 (t, J = 6.3 Hz, 1H), 3.69-3.56 (m, 2H), 3.52- 3.18 (m, 7H), 2.10-1.75 (m, 4H), 1.24-1.13 (m, 6H); ^{13}C NMR (AV-600, CD_3OD) δ 175.061, 174.346, 173.866, 171.844, 171.668, 150.931, 150.866, 147.794, 147.687, 119.583, 119.470, 119.360, 119.098, 118.862, 117.195, 117.008, 114.271, 70.573, 70.248, 69.497, 69.425, 67.040, 61.809, 61.716, 57.379, 49.999, 49.719, 47.000, 44.960, 44.830, 44.464, 37.955, 37.877, 37.792, 30.233, 30.148, 28.576; ESI MS *m/z* calcd for (M+H) $C_{21}H_{36}N_5O_7$ 470.2615, found 470.2609.

Vibriobactin (17) was synthesized following the procedure of Bergeron et al.(*10*) The siderophore was purified using an LH-20 column with ethyl acetate as the eluant. The product was further purified by precipitation in water and ethanol to give a fine tan powder. Characterization matched the previous reports:(*10*, *11*) ^{1}H NMR (AV-600, CD_3OD) δ 7.21-7.08 (m, 3H), 6.96-6.86 (m, 3H), 6.73-6.62 (m, 3H), 5.30-5.19 (m, 1H), 4.90-4.75 (m, 1H), 4.425 (dd, J_1 = 14.7 Hz, J_2 = 7.4 Hz, 1H), 3.95-3.10 (m, 8H), 2.12-1.94 (m, 2H), 1.92-1.76 (m, 2H), 1.56-1.12 (m, 6H); ^{13}C NMR δ (AV-300, CD_3OD) 173.444, 173.149, 171.924, 171.625, 171.328, 171.234, 168.522, 167.956, 150.428, 149.492, 147.484, 146.871, 146.786, 120.393, 120.022, 119.731, 118.747, 116.775, 111.971, 111.893, 111.825, 80.782, 79.848, 75.913, 73.054, 67.050, 46.858, 46.681, 44.993, 44.678, 38.030, 37.795, 30.920, 30.333, 28.391, 21.537, 20.479, 20.318,

15.589; ESI-MS *m/z* calcd for $C_{35}H_{38}N_5O_{11}$ (M-H) 704.2573, found 704.2556; Anal. Calcd (Found) for $C_{35}H_{39}N_5O_{11}$: C, 59.57 (59.23); H, 5.57 (5.88); N, 9.92 (9.64).

Bn-Vibriobactin A (18). Mono-amide **14** (0.789 g, 1.23 mmol), carboxylic acid **11** (1.29 g, 2.95 mmol), DIEA(diisopropylethyl amine) (1.67 mL, 9.59 mmol) were stirred in 40 mL of CH_2Cl_2 under N_2(g) and cooled to 0 °C. HATU (1.21 g, 3.19 mmol) was added, and the solution was allowed to warm to room temperature. After stirring for 2 days, 40 mL of CH_2Cl_2 was added, and the solution was washed twice with 5% (w/v) citric acid in brine (80 mL) and once with H_2O (80 mL). The organic phase was dried, filtered, and evaporated. The resulting mixture was applied to a column (2 in. diameter) layered with alumina (1.5 in.) on top of silica (5 in.) packed with CH_2Cl_2. The protected siderophore was eluted with a gradient of 0-3% (v/v) methanol in CH_2Cl_2 (0.423 g, 26.8 % yield): ^{1}H NMR (AV-500, $(CD_3)_2SO$, 373K) δ 8.40-8.35 (m, 1H), 8.28-8.25 (m, 1H), 8.05-7.80 (br, 1H), 7.71-7.50 (br, 1H) 7.50-7.06 (m, 38H), 5.19-5.03 (m, 12H), 4.93 (m, 1H), 4.63-4.57 (m, 2H), 4.44 (m, 1H), 4.10 (m, 1H), 3.95 (m, 1H), 3.60-3.05 (m, 8H), 1.95-1.50 (m, 4H), 1.09 (d, J = 6.0 Hz, 6H); ^{13}C NMR (AV-500, $(CD_3)_2SO$, 373K) δ 169.827, 164.535, 164.123, 151.284, 151.202, 145.737, 145.374, 136.605, 136.420, 136.243, 136.082, 128.489, 128.114, 128.039, 127.784, 127.595, 127.555, 127.314, 127.269, 127.241, 127.219, 127.059, 123.368, 123.322, 121.614, 121.455, 120.930, 116.905, 116.725, 116.251, 74.770, 70.454, 70.428, 66.863, 66.150, 36.418, 36.044, 19.349, 19.159; ESI-MS *m/z* calcd for $C_{77}H_{78}N_5O_{13}$ (M-H) 1280.5596, found 1280.5629.

Vibriobactin A (19). See general deprotection procedure above: ^{1}H NMR (AV-300 CH_3OD) δ 7.45-7.18 (m, 3H), 7.05-6.85 (m, 3H), 6.80-6.65 (m, 3H), 5.09 (d, J = 5.1 Hz, 0.7H), 5.00 (d, J = 4.2 Hz, 0.3H), 4.48 (dd, J_1 = 8.4 Hz, J_2 = 3.3 Hz, 1H), 4.42-4.28 (m, 1H), 3.88-3.15 (m, 12H), 2.18-1.65 (m, 4H), 1.34-1.08 (m, 6H); ^{13}C NMR (AV-300 CH_3OD) δ 173.47, 172.92, 172.76,

171.98, 171. 63, 170.81, 170.41, 150.47, 149.22, 147.46, 147.30, 120.40, 120.24, 120.13, 119.76, 118.87, 118.74, 117.96, 117.53, 116.81, 69.24, 69.02, 68.43, 61.01, 60.88, 56.08, 55.90, 46.67, 44.62, 38.10, 37.90, 29.90, 29.43, 28.45, 28.19, 20.82, 20.69, 20.35, 20.27; ESI-MS *m/z* calcd for $C_{35}H_{42}N_5O_{13}$ (M-H) 740.2779, found 740.2765; Anal. Calcd (Found) for $C_{35}H_{43}N_5O_{13} \bullet 2H_2O \bullet ¼(CH_3CH_2)_2O$: C, 54.30 (54.24); H, 6.27 (6.10); N, 8.79 (8.79).s

***N*^{1}-(*tert*-butyl carbamate)norspermidine** (**20**) was synthesized according to the procedure of Krapcho et al.(*12*)

***N*^{1}-[2,3-bis(benzyloxy)benzoyl]-*N*^{7}-(*tert*-butyl carbamate)norspermidine (21).** Amine **20** (2.45 g, 10.6 mmol) and thiazolide **5** (5.08 g, 11.7 mmol) were dissolved in CH_2Cl_2 and stirred at room temperature overnight. The solvent was removed and mixture was purified by flash chromatography by eluting with 0-8% (v/v) methanol in CH_2Cl_2 to give **21** (3.174 g, 54% yield by mass). The product gives two spots by TLC using 10% (v/v) methanol in CH_2Cl_2 (R_f = 0.13 and R_f = 0.38) likely due to two protonation states of the product amine. Only one spot is observed on basic alumina plates: ^{1}H NMR (AV-600, $CDCl_3$) δ 8.077 (m, 1H), 7.670 (t, *J* = 10.8 Hz, 1H), 7.48-7.26 (m, 10H), 7.122 (d, *J* = 8.4 Hz, 2H), 5.251 (s, 2H), 5.125 (s, 2H), 3.315 (q, *J* = 6.4 Hz, 2H), 2.497 (t, *J* = 6.6 Hz, 2H), 2.459 (t, *J* = 6.6 Hz, 2H), 1.954 (br), 1.561 (quin, *J* = 6.3 Hz, 2H), 1.499 (quin, *J* = 6.6 Hz), 1.395 (s, 9H); ^{13}C NMR (AV-600, $CDCl_3$) δ 165.507, 156.310, 151.867, 146.864, 136.599, 136.551, 128.902, 128.816, 128.400, 127.793, 124.588, 123.356, 117.062, 76.526, 71.422, 50.650, 47.432, 47.262, 38.932, 37.679, 29.809, 29.620, 18.578; ESI-MS *m/z* calcd for $C_{32}H_{42}N_3O_5$ (M+H) 548.3124, found 548.3125.

Fluorescence quenching titrations. The fluorescence measurements were performed as previously reported with small modifications.(*13*) The excitation slits on the Cary Eclipse fluorescence spectrophotometer were 20 nm and the emission slits were 5 nm. The ligand solutions (10 μM siderophore, TBS with 5% (v/v) DMSO, pH 7.4) were prepared from 4 mM stock solutions in DMSO. Absorbance measurements of the ligand solutions were performed after every titration to confirm the ligand concentration. The protein solution was prepared as previously reported. Small amounts of ligand solution were added to the protein solution, mixed, and allowed to equilibrate for at least 4 min before measuring the fluorescence. The data was fit to a one-to-one binding model with two parameters, K_d and fluorescence response, using the program DYNAFIT.(*1*) The K_d values are reported with the calculated standard error in parentheses.

Spectrophotometric Titration Procedures. All solutions were made with Milli-Q water that is degassed by boiling for one hour while bubbling Ar(g) in the water. Volumes of fluid were dispensed with glass volumetric pipets (100 mL, 50 mL, 25 mL) or adjustable volume pipets with disposable tips (< 1 mL) (Eppendorf). All titrations were controlled by the LabVIEW computer programs handsome.vi (for manual titrations) or punish.vi (for automatic titrations). Absorbance values from 190-820 nm were measured with an HP 8452A Diode Array Spectrophotometer. A Brinkmann 665 Dosimat controls the addition of titrant. The temperature were maintained at 25 °C with a water bath pumping water through the jacketed spectrophotometric cell. The cell pathlength is 6.6 cm. The titration solutions were stirred with a magnetic stirrer. Solutions of HCl and KOH in 5% (v/v) DMSO were made separately using the appropriate vial of analytical concentrate (J. T. Baker) and DMSO (50 mL) mixed in a

volumetric flask (1L) with water. The HCl titrant were standardized with potassium hydrogen phthalate using phenolphthalein as the endpoint indicator. The KOH titrant was standardized with tris(hydroxymethyl)aminomethane using bromocresol green as the endpoint indicator. The semi-micro glass electrode (OI Analytical) was calibrated in the spectrophotometric cell with electrolyte (100 mL, 0.1 M KCl, 5% (v/v) DMSO). An aliquot of standardized HCl (2 mL) was added to the electrolyte followed by a titration with standardized KOH. The data were analyzed in a Gran plot by the computer program GLEE to give the E° and slope values needed to calibrate the electrode. A blank spectrum was recorded before adding vibriobactin to the solution. Vibriobactin was stored at -20 °C as a 50 mM stock solution in DMSO that was thawed before each titration. To prepare the titration cell electrolyte (100 mL, 0.1 M KCl, 5% (v/v) DMSO) was buffered with HEPES, MES, and CHES (10-15 mg of each). A small amount of base was added to make the starting pH above 10. Vibriobactin or fluvibactin (6-10 μL of 50 mM stock solution) was added to the stirring, temperature controlled solution. Then one equivalent of iron was added from a stock solution (27.1 mM $FeCl_3$ in 1 M HCl), and the solution became faintly red. Standardized HCl was added to the solution in 5 μL steps as the absorbance was measured at every pH change of 0.05. The titration was stopped once the pH was below 7. The absorbance vs. pH data was baseline corrected at 800 nm and analyzed in pHab.

Calculating the Dissociation Constant for Triscatecholate Fe-Vibriobactin. The data from fluorescence quenching titration of Scn with Fe-vibriobactin were refit using the SOLVER tool found in Microsoft Excel. The fitting model included the equilibrium between Fe-vibriobactin and the protonated complex and a one-to-one binding of Scn to Fe-vibriobactin. The spreadsheet was set up following the example shown by Brown.(*14*)

Absorbance Measurements. Solutions of 20 μM Scn, 20 μM Fe-vibriobactin, and 20 μM Scn and Fe-vibriobactin were made (TBS buffered at pH 7.4 with 5% (v/v) DMSO).A **s**olution of 20 μM Fe-vibriobactin (TBS with 5% (v/v) DMSO at pH 7) was made and the absorbance was measured at room temperature. Based on the measured K_d, more than 97% of the Fe-vibriobactin is bound by Scn in this solution.

Structure Determination. Prior to crystallization, purified Scn (C87S mutant) was loaded with a molar excess of Fe-fluvibactin, washed multiple times with buffer and concentrated to 10 mg/mL in a 10 kDa concentrator. Crystals were grown by vapor diffusion over reservoirs of 1.0-1.4 M ammonium sulfate, 200 mM lithium sulfate, 50-100 mM sodium chloride and 100 mM sodium acetate (pH = 4.5) and cryopreserved in mother liquor (reservoir solution plus 15% (v/v) glycerol). Diffraction data were collected at the Advanced Light Source, beamline 5.0.1, and processed with HKL2000 software. (*15*) Initial phase information was determined by molecular replacement using a previous Scn structure as the search model (PDB ID:1L6M) in PHASER and refined using REFMAC, both part of the CCP4i program suite. (*16, 17, 18*) All model building was performed with COOT. (*19*)

Accession Code. Coordinates and structure factors have been deposited in the Protein Data Bank under accession code 4K19.

Table 2-2: Crystallographic Statistics for Scn/Fe-Fluvibactin Complex

Data collection	
Space group	$P4_12_12$
Lattice constants (Å)	a = b = 114.20; c = 119.30
Resolution (Å)	50.00-2.75 (2.85-2.75)
Unique reflections	21299
Average redundancy	14.4 (14.7)
Completeness (%)	100 (100)
R_{merge} (%)	8.3 (34.5)
I/σ(I)	33.9 (8.3)
Refinement statistics	
R_{work} (%)	23.7
R_{free} (%)	29.5
Number of atoms	
Protein	3906
Ligand (Fe + Fluvibactin)	138
Solvent	67
R.M.S deviations	
Bond lengths (Å)	0.0052
Bond angles (°)	1.074
Ramachandran	
Favored (%)	88.4
Allowed (%)	10.0
Generously allowed (%)	0.9
Disallowed (%)	0.7

Values in parentheses are for the highest resolution shell.

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Figure S5. $^{1}H/^{13}C$ NMR spectrum of benzyl-protected fluvibactin A (**12**)

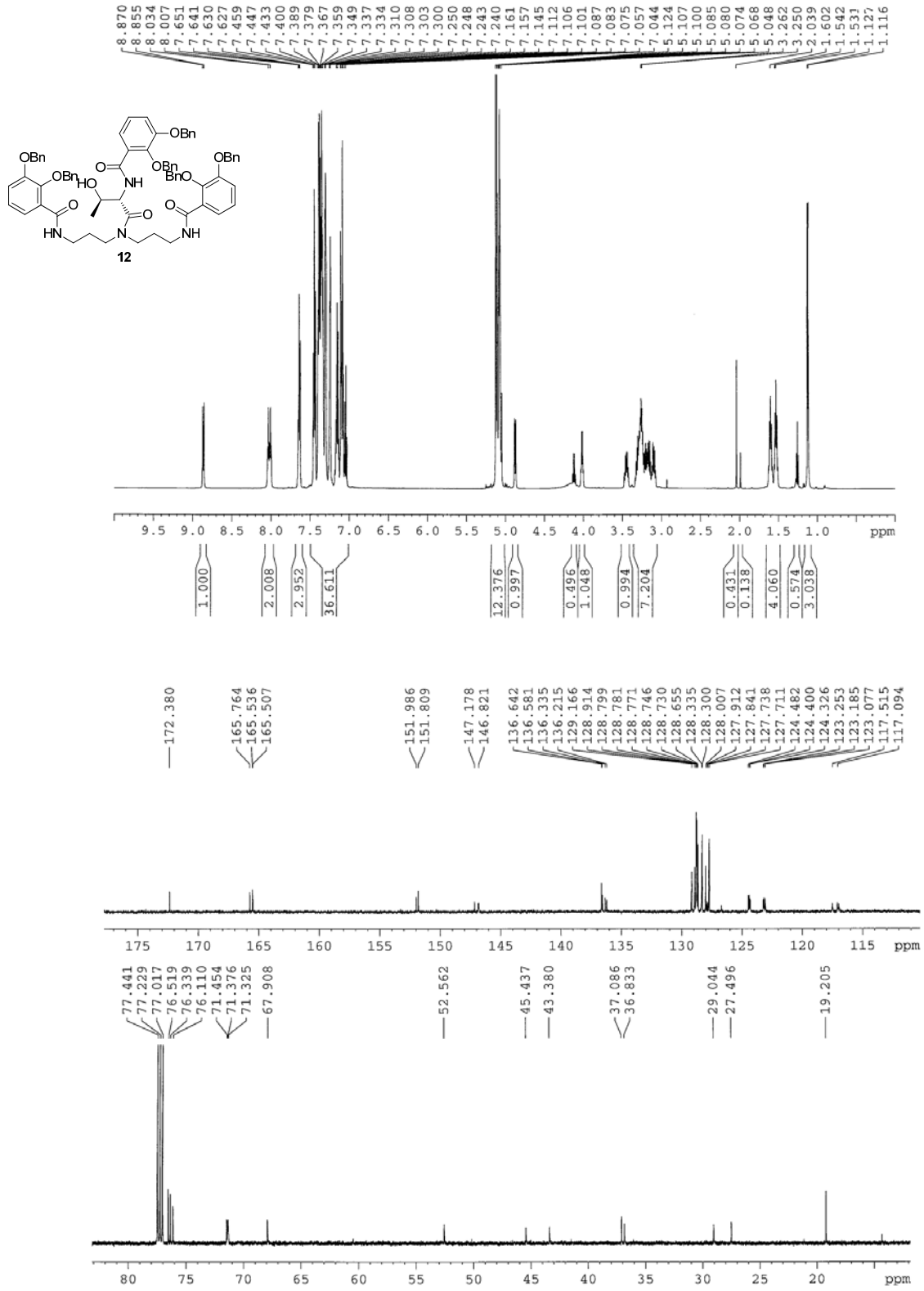

Figure S6. $^{1}H/^{13}C$ NMR spectrum of fluvibactin A (**13**)

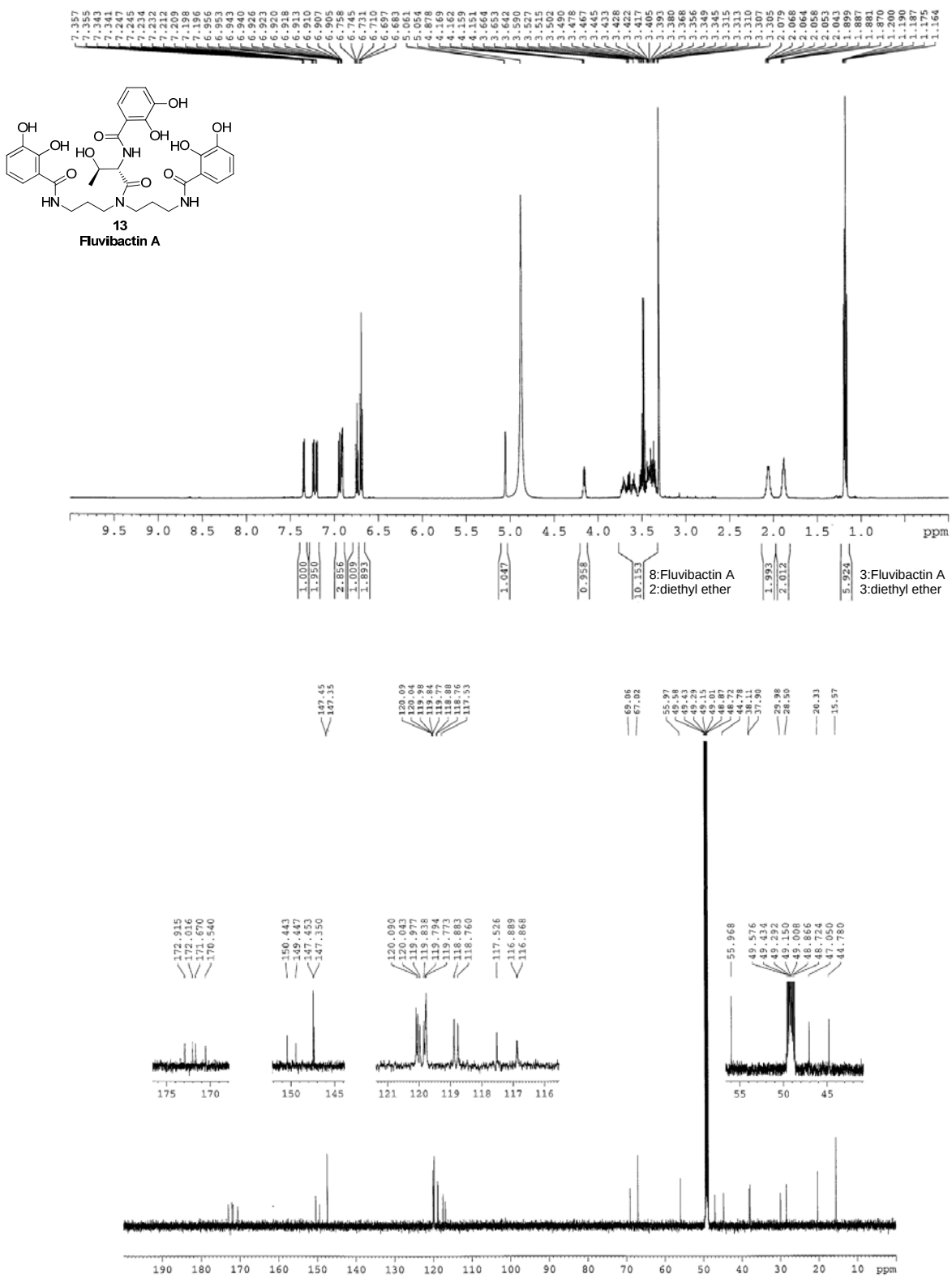

Figure S7. $^{1}H/^{13}C$ NMR Spectrum of N^{1}-[2,3-bis(benzyloxy)benzoyl]norspermidine (**14**).

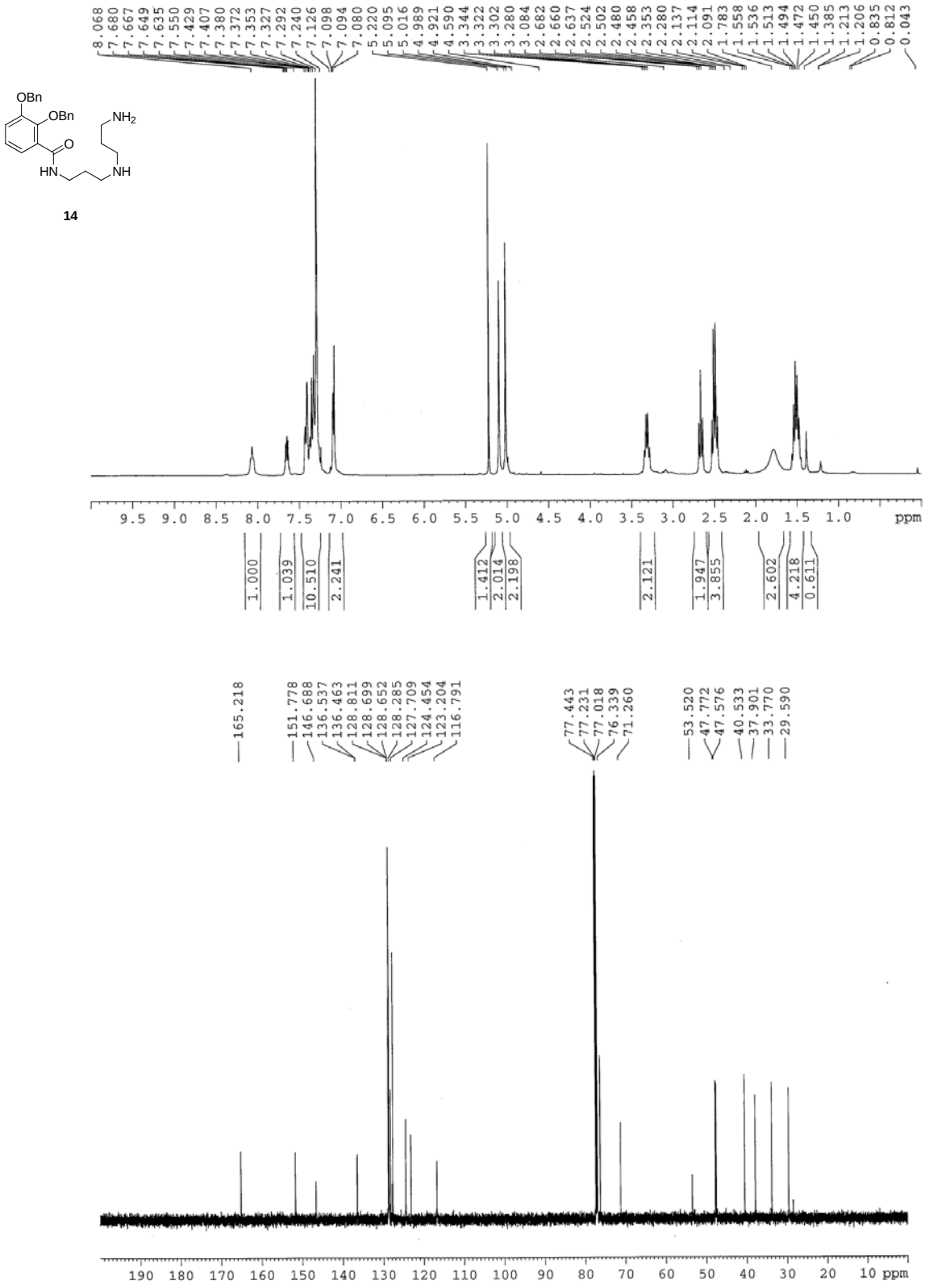

Figure S8. $^{1}H/^{13}C$ NMR spectrum of $N1,N^{4}$-bis(*N*-carbobenzyloxy-L-threonyl)]-N^{7}-[2,3-bis(benzyloxy)benzoyl]norspermidine (**15**).

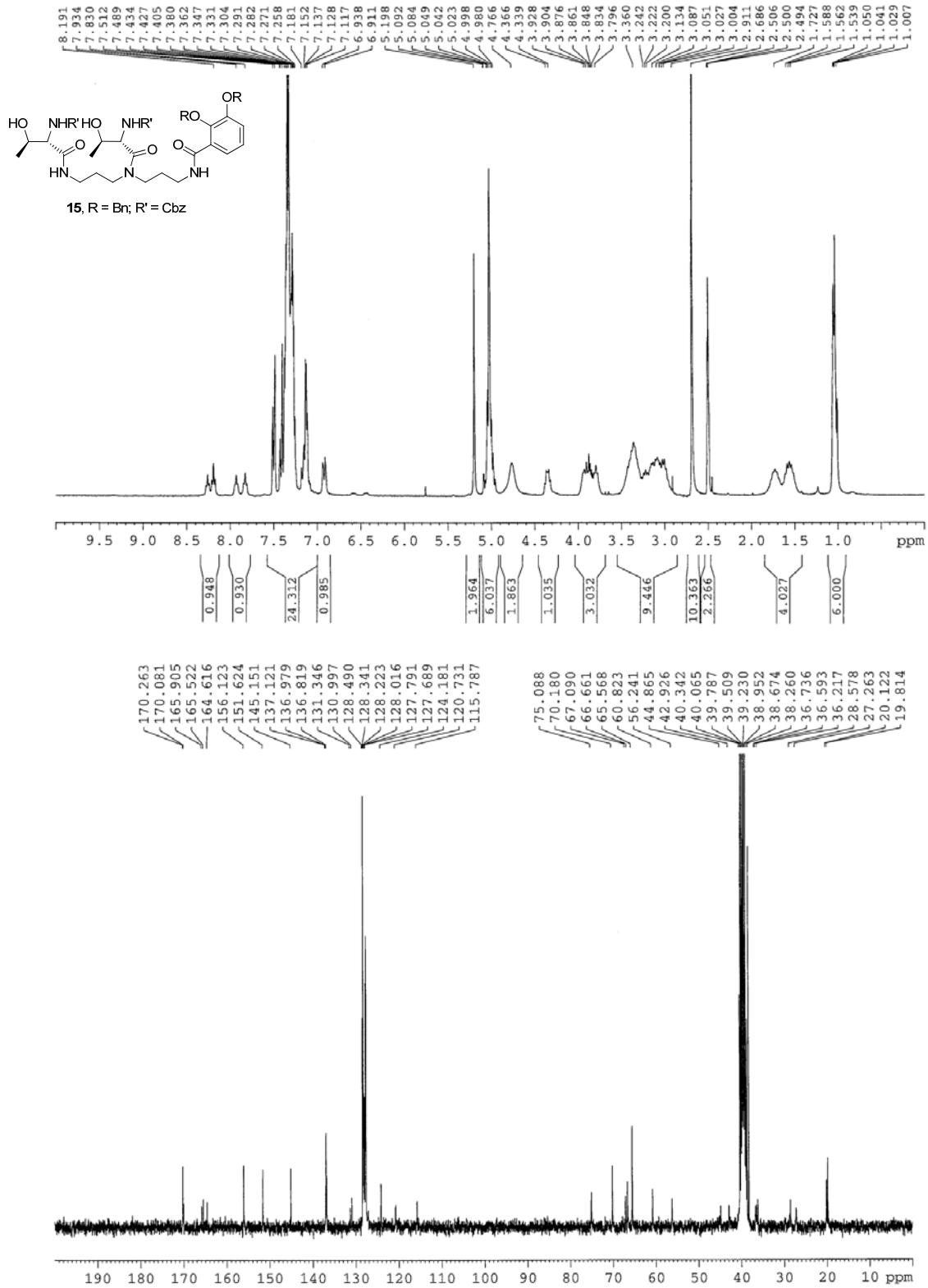

Figure S9. $^{1}H/^{13}C$ NMR spectrum of benzyl-protected vibriobactin A (**18**).

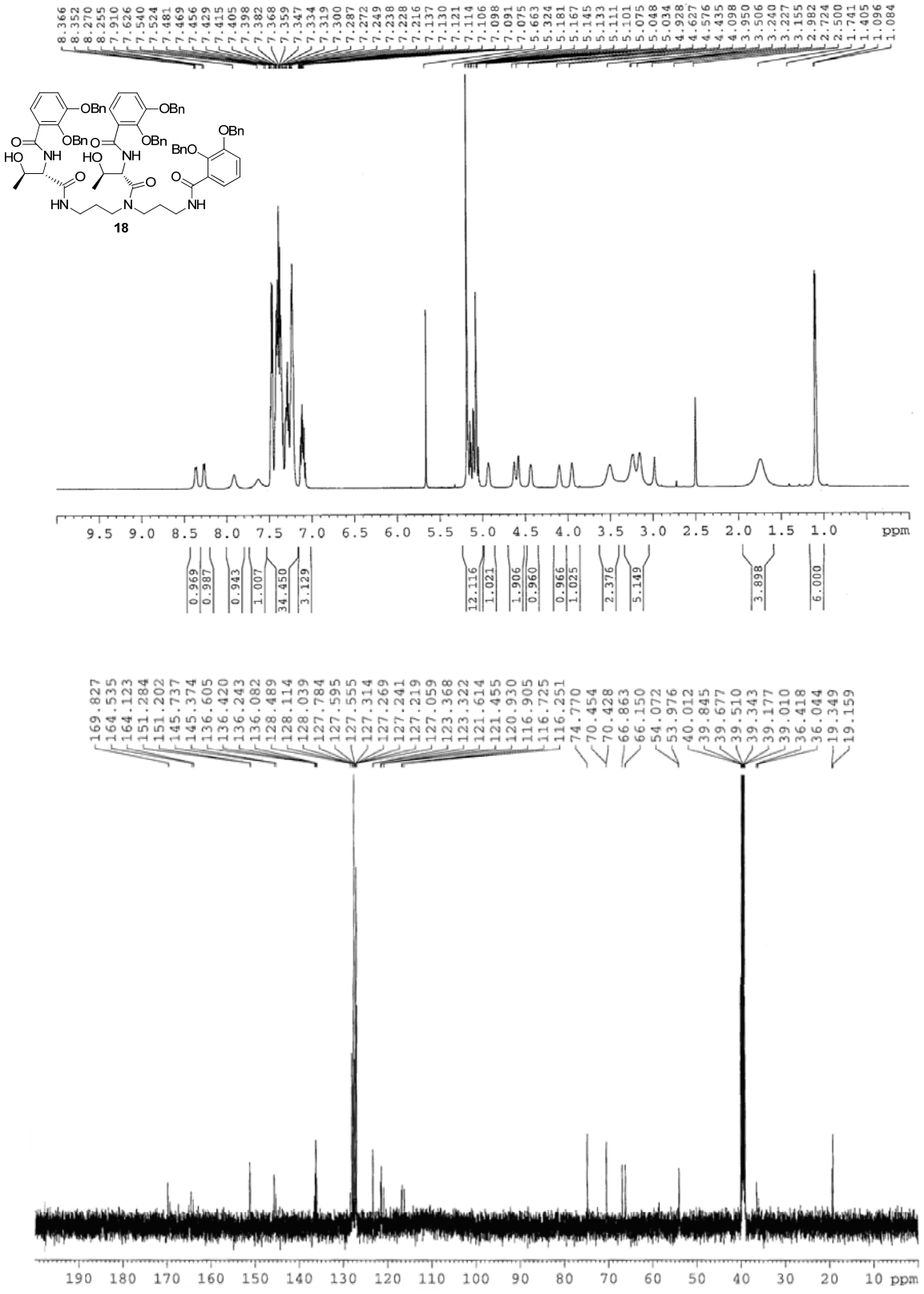

Figure S10. $^{1}H/^{13}C$ NMR spectrum of vibriobactin A (**19**).

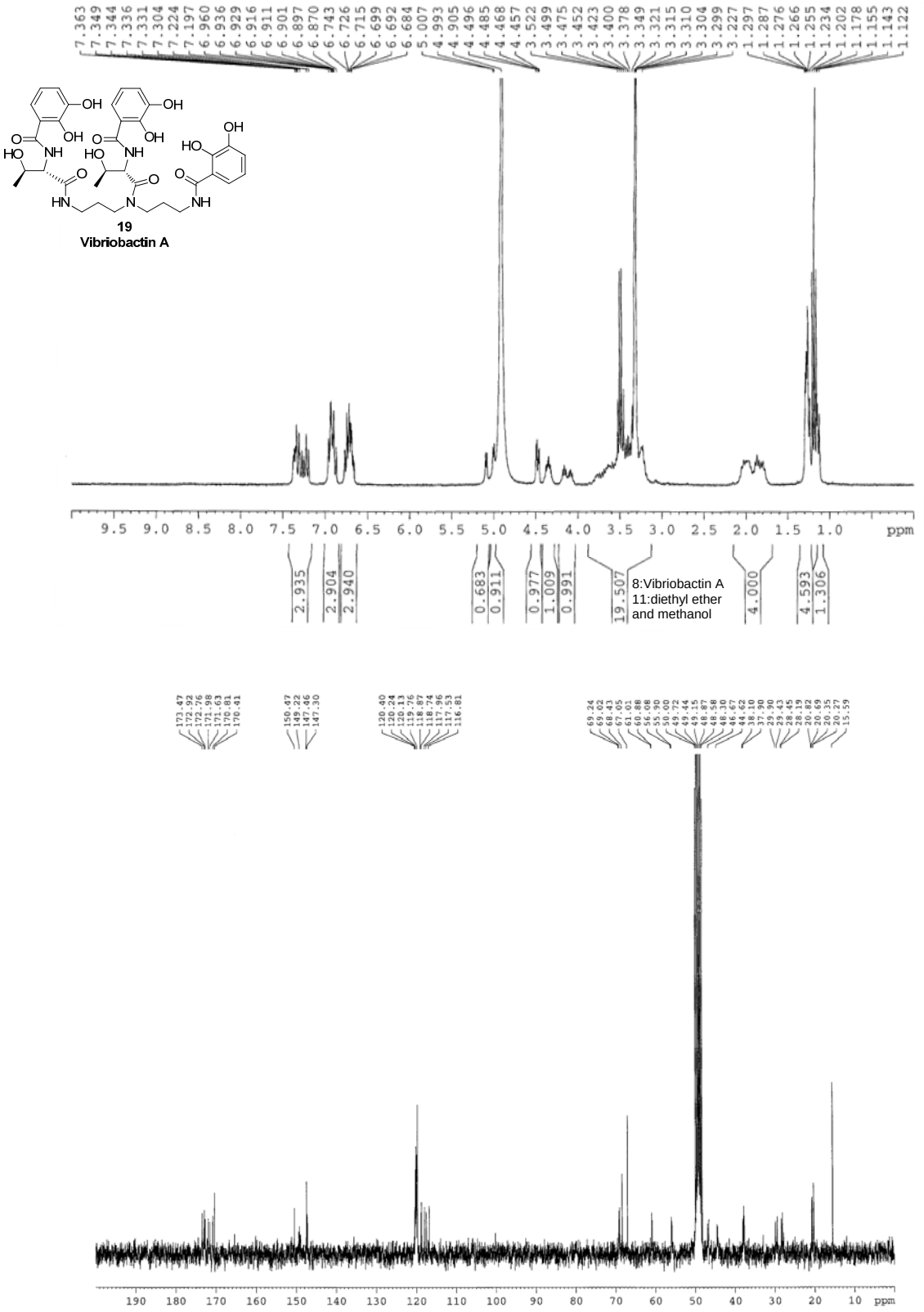

Figure S11. $^{1}H/^{13}C$ NMR spectrum N^{1}-[2,3-bis(benzyloxy)benzoyl]-N^{7}-(*tert*-butyl carbamate)norspermidine (**21**)