Electronic Supplementary Material (ESI) for Metallomics

SUPPORTING INFORMATION

Characterization of iron(III) Sequestration by an analog of the cytotoxic siderophore brasilibactin A: Implications for the iron transport mechanism in mycobacteria

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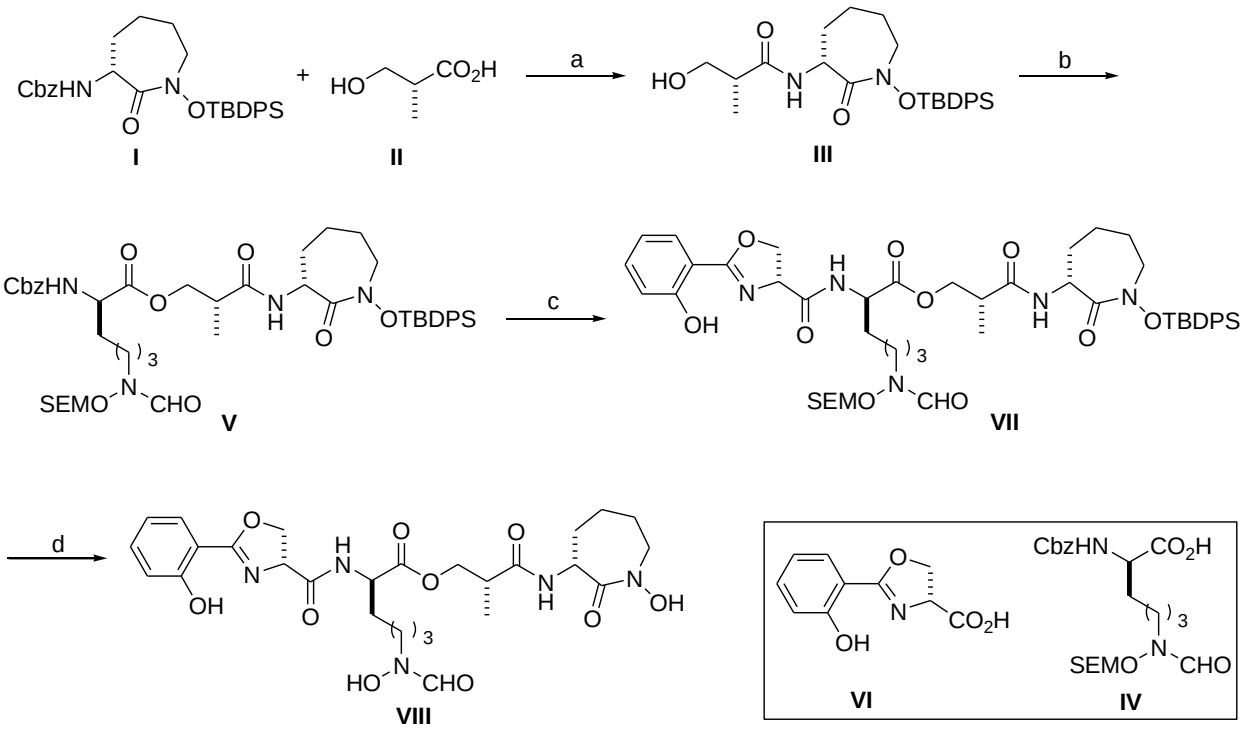
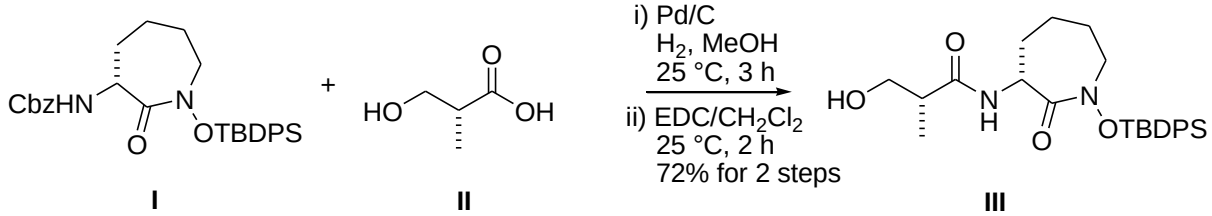
Overall reaction scheme for the synthesis of Bbtan



Scheme S1. Synthesis of a water-soluble brasilibactin A analogue **VIII** or Bbtan. Reagents and conditions: (a) 10% Pd/C, H_2, MeOH, 25 ℃, 3 h; then **II**, EDC, CH_2Cl_2,25 ℃, 2 h, 72%; (b) **IV**, DCC, DMAP, toluene, 25 ℃, 22 h, 66%; (c) 10% Pd/C, H_2, MeOH, 25 ℃, 5 h; then **VI**, EDC, CH_2Cl_2, 25 ℃, 16 h, 87%; (d) TFA, CH_2Cl_2, 25 ℃, 2 h, 78%.

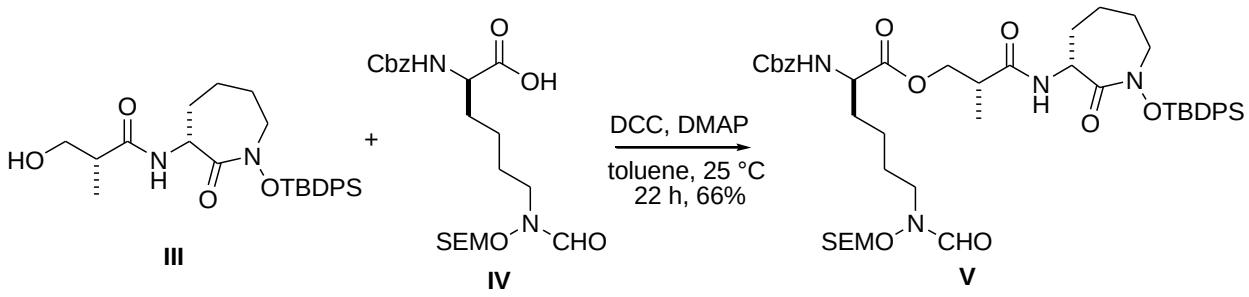
Preparation of cyclic hydroxamate alcohol (III)



Scheme S2

To a solution of Cbz protected hydroxamate **I** (255 mg, 0.49 mmol) in MeOH (3.0 mL) was added Pd/C (10%, 50 mg) at 25 ℃. The reaction mixture was flushed with H_2, then stirred under H_2 atmosphere for 3 h. After filtration through celite, the reaction mixture was concentrated under reduced pressure to afford the corresponding free amine. To a solution of free amine in CH_2Cl_2 (20.0 mL) was added *β*-hydroxy carboxylic acid **II** (79 mg, 0.76 mmol) and EDC·HCl (284 mg, 1.48 mmol) at 25 ℃. After being stirred for 2 h at 25 ℃, the reaction mixture was concentrated and purified by column chromatography (silica gel, EtOAc/hexanes, 1/2) to afford cyclic hydroxamate alcohol **III** (168 mg, 72% for 2 steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (d, *J* = 7.2 Hz, 3H), 1.13 (s, 9H), 1.18–1.29 (m, 1H), 1.36–1.46 (m, 1H), 1.52–1.61 (m, 2H), 1.78 (d, *J* = 10.0 Hz, 2H), 2.39–2.47 (m, 1H), 3.30 (br, s, 1H), 3.46–3.47 (m, 2H), 3.54–3.61 (m, 2H), 4.26 (dd, *J* = 6.8, 10.0 Hz, 1H), 6.73 (d, *J* = 6.8 Hz, 1H), 7.34–7.47 (m, 6H), 7.71–7.75 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.15, 170.03, 136.25, 136.15, 132.15, 131.68, 130.37, 130.33, 127.69, 127.63, 65.26, 54.31, 51.71, 42.39, 30.64, 27.40, 26.98, 25.44, 19.66, 13.71.

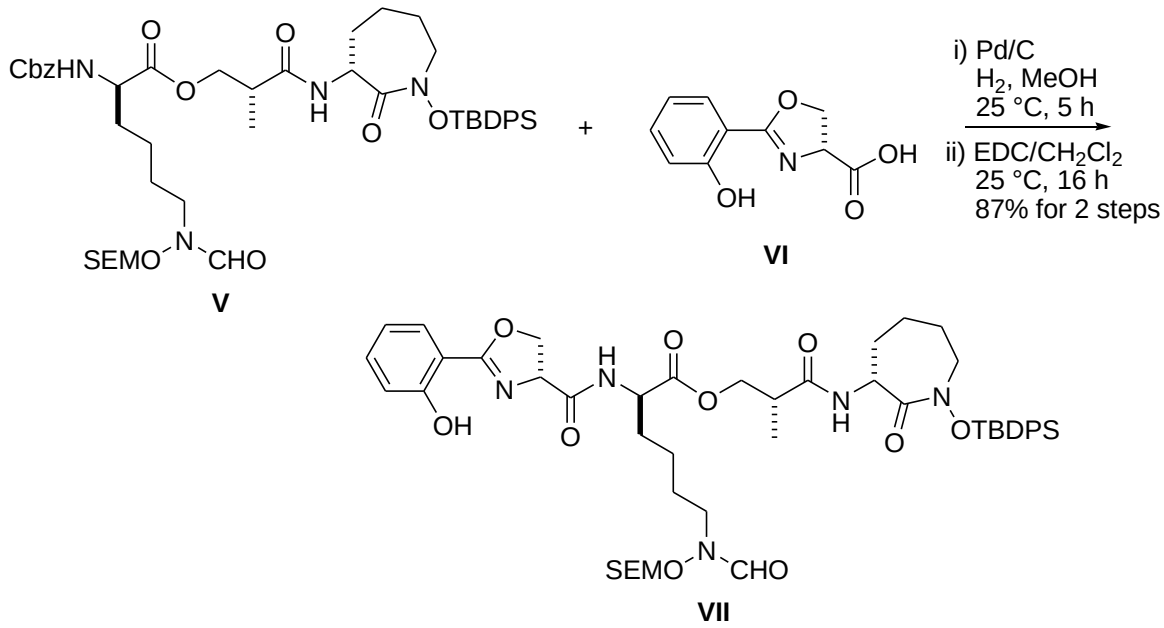
Preparation of cyclic hydroxamate ester (V)



Scheme S3

To a solution of **III** (78 mg, 0.17 mmol) in toluene (4.0 mL) was added a solution of **IV** (96 mg, 0.21 mmol) in toluene (4.0 mL) and followed by the addition of DCC (138 mg, 0.69 mmol) and DMAP (82 mg, 0.69 mmol) at 25 ℃. After being stirred for 22 h at 25 ℃, the reaction mixture was concentrated and purified by column chromatography (silica gel, EtOAc/hexanes, 1/1) to afford cyclic hydroxamate ester **V** (100 mg, 66%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 9H), 0.95 (t, *J* = 8.4 Hz, 3H), 0.97–1.91 (m, 27H), 2.53 (m, 1H), 3.42–3.57 (m, 4H), 3.72 (t, *J* = 8.0 Hz, 2H), 4.09–4.20 (m, 4H), 4.30–4.32 (m, 1H), 4.84 (s, 1H), 5.10 (s, 2H), 5.58 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 4.8 Hz, 1H), 7.29–7.38 (m, 11H), 7.71–7.73 (m, 4H), 8.30 (s, 0.8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.31, 172.05, 169.60, 163.71, 156.21, 136.57, 136.33, 136.21, 132.16, 131.73, 130.50, 130.37, 128.66, 128.25, 127.70, 114.21, 98.25, 54.38, 54.03, 51.78, 44.82, 40.21, 39.00, 33.35, 32.08, 31.84, 31.16, 30.44, 31.00, 29.84, 29.50, 27.44, 27.06, 26.88, 26.45, 26.06, 25.43, 22.83, 22.38, 19.73, 18.21, 14.30, -1.31.

Preparation of SEM and TBDPS protected depsipeptide (VII)

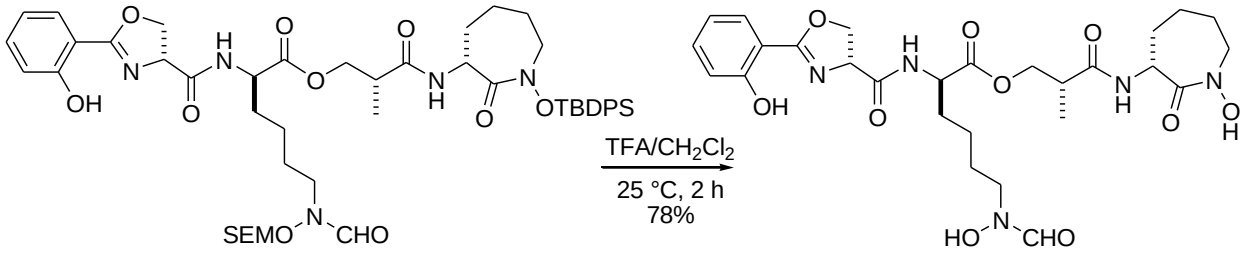


Scheme S4

To a solution of Cbz protected hydroxamate ester **V** (101 mg, 0.11 mmol) in MeOH (2.0 mL) was added Pd/C (10%, 15 mg) at 25 ℃. The reaction mixture was flushed with H_2, then stirred under H_2 atmosphere for 5 h. After filtration through celite, the reaction mixture was concentrated under reduced pressure to afford the corresponding free amine. To a solution of free amine in CH_2Cl_2 (10.0 mL) was added oxazoline carboxylic acid **VI** (60 mg, 0.20 mmol) and EDC·HCl (65 mg, 0.34 mmol) at 25 ℃. After being stirred for 16 h at 25 ℃, the reaction mixture was concentrated and purified by column chromatography (silica gel, acetone/hexanes, 2/7) to afford SEM and TBDPS protected depsipeptide **VII** (94 mg, 87%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.02 (s, 9H), 0.94 (t, *J* = 11.2 Hz, 3H), 1.04 – 1.91 (m, 27H), 2.61 (dd, *J* = 11.2, 17.6 Hz, 1H), 3.49–3.57 (m, 4H), 3.70 (t, *J* = 11.2 Hz, 2H), 4.17–4.26 (m, 2H), 4.47–4.53 (m, 1H), 4.61–4.68 (m, 2H), 4.80 (s, 1H), 4.95 (dd, *J* = 10.4, 14.0 Hz, 1H), 6.91 (t, *J* = 7.6 Hz, 1H), 6.97–7.03 (m, 3H), 7.33–7.46 (m, 7H), 7.68–7.76 (m, 5H), 7.96 (s, 0.2H), 8.22 (s, 0.8H),

11.29 (br, s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.24, 171.42, 170.48, 169.76, 163.73, 159.95, 136.25, 136.16, 134.27, 132.08, 131.76, 130.37, 128.68, 127.66, 119.11, 117.07, 98.20, 69.56, 68.09, 67.22, 54.39, 52.42, 51.80, 40.11, 31.00, 27.38, 26.99, 26.24, 25.42, 22.30, 19.70, 18.14, 14.41, -1.35.

Preparation of brasilibactin A analogue (Bbtan; VIII)



VII **VIII**

Scheme S5

Depsipeptide **VII** (50 mg, 0.052 mmol) in TFA/CH_2Cl_2 (1.5 mL, 1:4) was stirred under N_2 atmosphere at 25 ℃ for 2 h. The reaction mixture was concentrated and washed with Et_2O (1.0 mL × 3). The crude mixture was then purified by reverse phase semi-HPLC (Luna C18, Phenomenex, 250 mm × 10 mm × 5 μm; eluent, CH_3CN/H_2O, 40:60; flow rate, 3 mL/min; UV detection, 230 nm and 254 nm) to afford iron-free brasilibactin A analogue **VIII** (24 mg, 78%) as a white sold: ^{1}H NMR (400 MHz, CD_3OD) δ 1.14 (d, 3H, *J* = 7.2 Hz), 1.29–1.98 (m, 12H), 2.80–2.90 (m, 1H), 3.47 (t, 1H, *J* = 6.8 Hz), 3.54 (t, 1H, *J* = 6.8 Hz), 3.67 (dd, 1H, *J* = 4.8, 16.0 Hz), 3.93 (dd, 1H, *J* = 11.2, 16.0 Hz), 4.14–4.25 (m, 2H), 4.48 (ddd, *J* = 5.2, 8.6, 8.6 Hz 1H), 4.57–4.63 (m, 3H), 5.07 (t, *J* = 8.8 Hz, 1H), 6.90 (t, *J* = 7.6 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.68 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.90 (br s, 0.6H), 8.22 (br s, 0.4H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.58, 172.91, 170.83, 168.50, 163.93, 160.94, 159.43, 135.02,

129.48, 120.04, 117.67, 111.51, 70.43, 69.15, 67.75, 54.08, 53.84, 52.69, 51.00, 47.08, 40.91, 31.86, 28.69, 27.49, 26.91, 26.80, 23.64, 23.46, 14.41; LCMS (FAB) *m/z* calcd for $C_{27}H_{37}N_5O_{10}$ $(M + H)^+$ 592.25, found 592.21.

Stability of Brasilibactin A analog, Bbtan, in aqueous solution

A direct determination of the protonation constants of Bbtan was attempted through spectrophotometric titration of the molecule with standardized NaOH (Figure S1). A 1.4 x 10^{-4} mol dm^{-3} solution was titrated with measured volumes of standardized 0.10 M NaOH and the UV-Visible spectrum was recorded as the pH changed over the range of 2.2 to 10.2. All pH measurements were made with an Orion 230 A+ pH/ion meter equipped with an Orion Ross pH electrode filled with 3 mol dm^{-3} NaCl solution. The electrode was calibrated by the "classical method," titration of standardized 0.10 mol dm^{-3} $HClO_4$ with standardized 0.10 mol dm^{-3} NaOH. Calibration data was analyzed using the computer program, GLEE. (*1*, *2*) UV-visible spectra were recorded with the Cary-50 spectrophotometer equipped with an external dip probe (Hellma, USA).

The spectra measured during the titration of Bbtan show two separate spectral transitions over distinct pH ranges, one from pH 2.2 to pH 6.1 characterized by a gradual shift in the λ_{max} and a slight decrease in the intensity of the spectrum, and a second spectral transition from pH 6.1 to pH 10.2, characterized by a major shift in the λ_{max} and in spectral intensity (Figure S1). However, it was found that the system exhibited a gradual shift in the solution pH to lower values when allowed 15-60 minutes equilibration time, accompanied by a corresponding shift in the spectrum. Also, upon returning the solution pH to the initial value (pH 2.3), the spectral

shift was found to be irreversible (Figure S2). This observation indicates the occurrence of some irreversible chemical reaction of Bbtan, possibly including hydrolysis of the molecule.

To test the stability of Bbtan under basic conditions, a ^{1}H-NMR experiment was performed to mimic the spectrophotometric titration (Figures S3-S15, below). After addition of 20% (v/v) of 0.1 N NaOD to Bbtan in D_2O, ^{1}H NMR spectra were acquired every 25 min to monitor the hydrolysis of Bbtan. For comparison, ^{1}H NMR spectra of fragment **IX** and **X** were also obtained after addition of 20% (v/v) of 0.1 N NaOD to **IX** and **X** solution in D_2O, respectively.

Peaks in the H-NMR spectrum of Bbtan were observed at pH 11 at 7.78, 7.05, 5.09, and 2.91 ppm, but not at low solution pH. These peaks correspond to analogous peaks independently obtained for the H-NMR spectra of fragments **IX** and **X** at pH 11. Additionally, the peaks corresponding to one C14 proton and two C17 protons were upfield-shifted from 4.20–4.23 ppm at acidic pH to 3.93–3.98 ppm at pH 11 and from 4.57–4.62 ppm in acidic pH to 3.58–3.72 ppm at pH 11, respectively. The peak shifts observed at pH 11 were also observed in the spectra of the fragment **IX** and **X** at pH 11 (see Figures S9-S14 for details). The shift in NMR spectra at basic pH and comparison to the fragments **IX** and **X** (Figure S15) demonstrates that irreversible hydrolysis of Bbtan likely occurs during the spectrophotometric titration at basic conditions, cleaving the ester bond of the molecule (see main paper, Scheme 1). This type of ester hydrolysis reaction under basic conditions was reported for another siderophore, vibrioferrin, although at higher concentrations of NaOH, higher temperatures, and longer equilibration times than those seen here.(*3*)

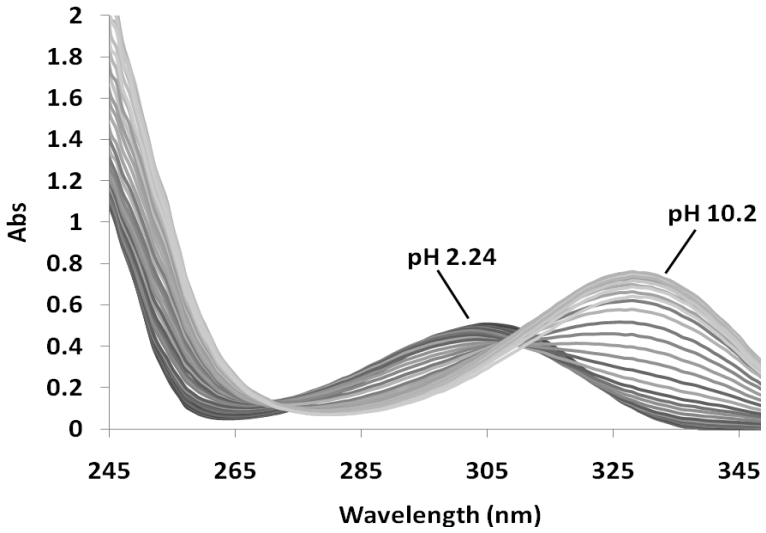


Figure S1. Spectrophotometric titration of the synthetic analog of Brasilibactin A, Bbtan, over the pH range of 2.24 to 10.2. Conditions: [Bbtan] = 1.4 x 10^{-4} M, μ = 0.10 ($NaClO_4$), T = 25 °C.

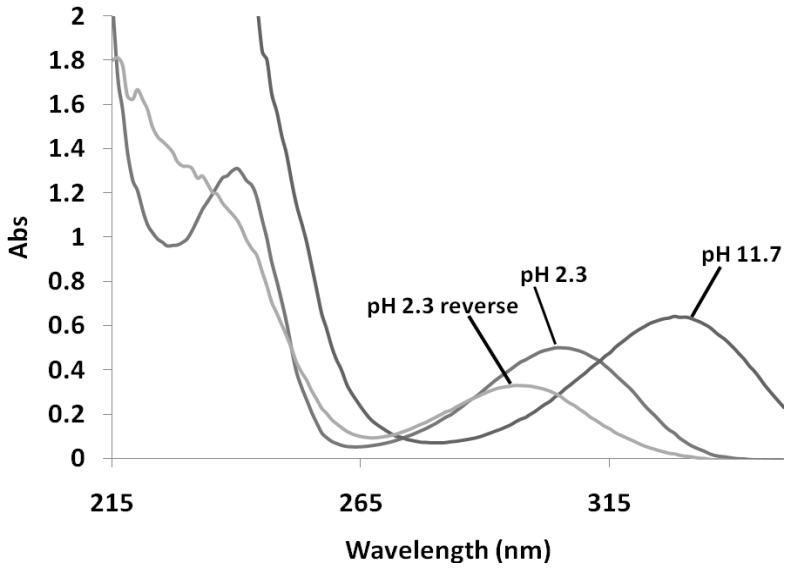
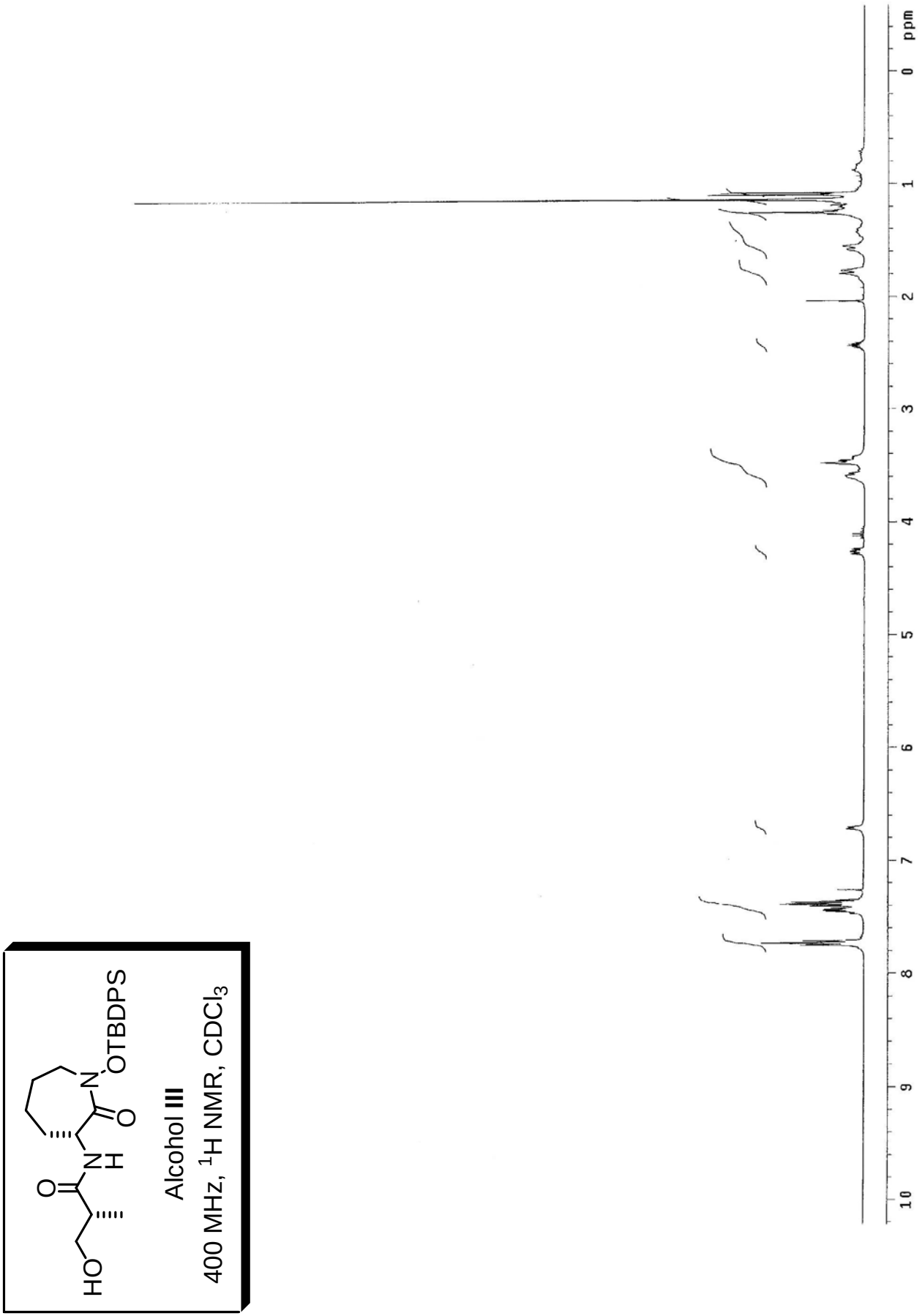


Figure S2. UV-Visible spectra obtained from spectrophotometric titration of Bbtan showing the lack of reversibility of the titration. Initial spectrophotometric tiration proceeded from pH 2.3 to 11.7. pH 2.3 reverse label represents the spectrum subsequent to returning the solution pH to 2.3.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N
H
O
N
OTBDPS
Alcohol III
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0
1
2
3
4
5
6
7
8
9
10

Figure S3

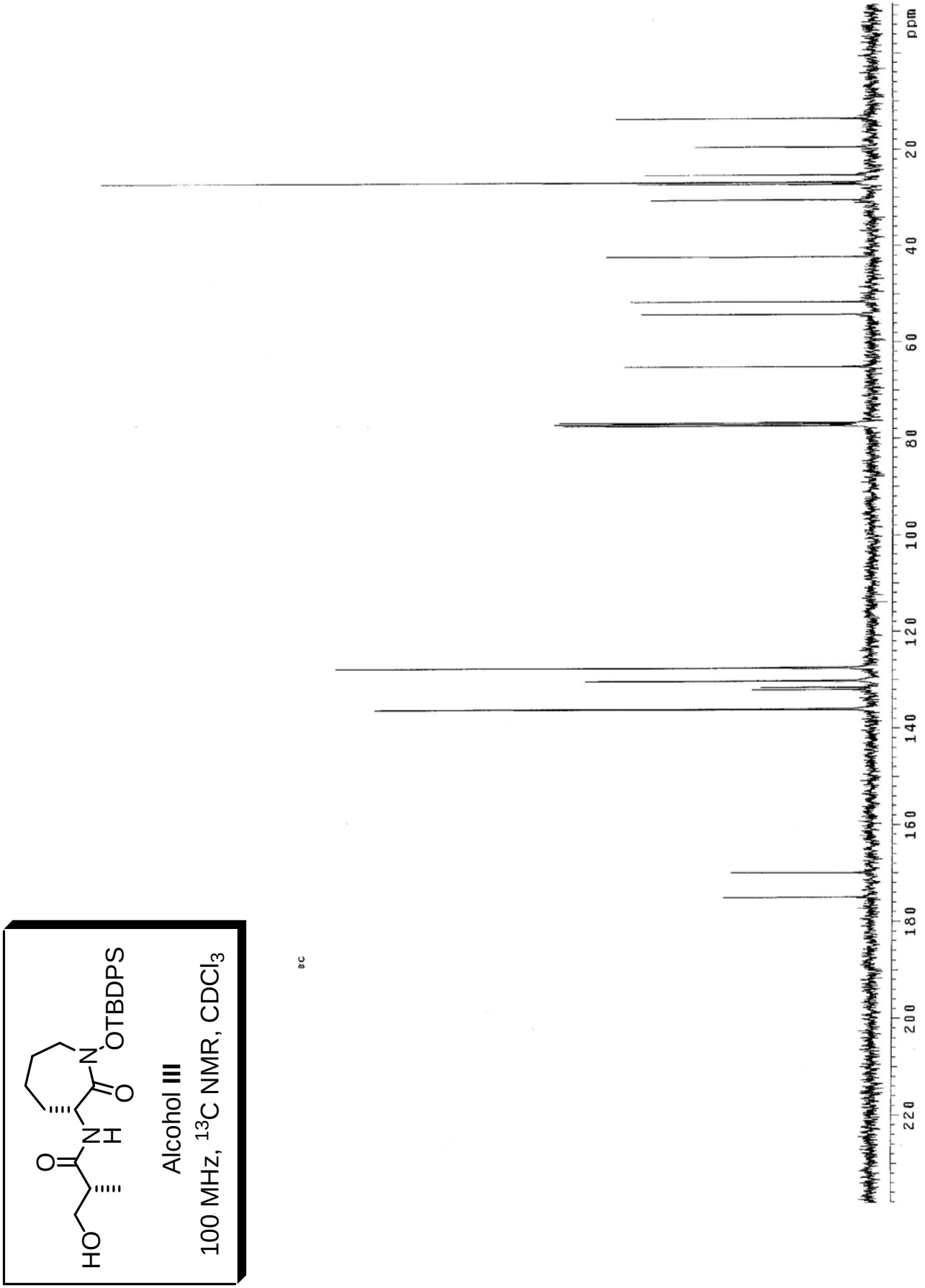


Figure S4

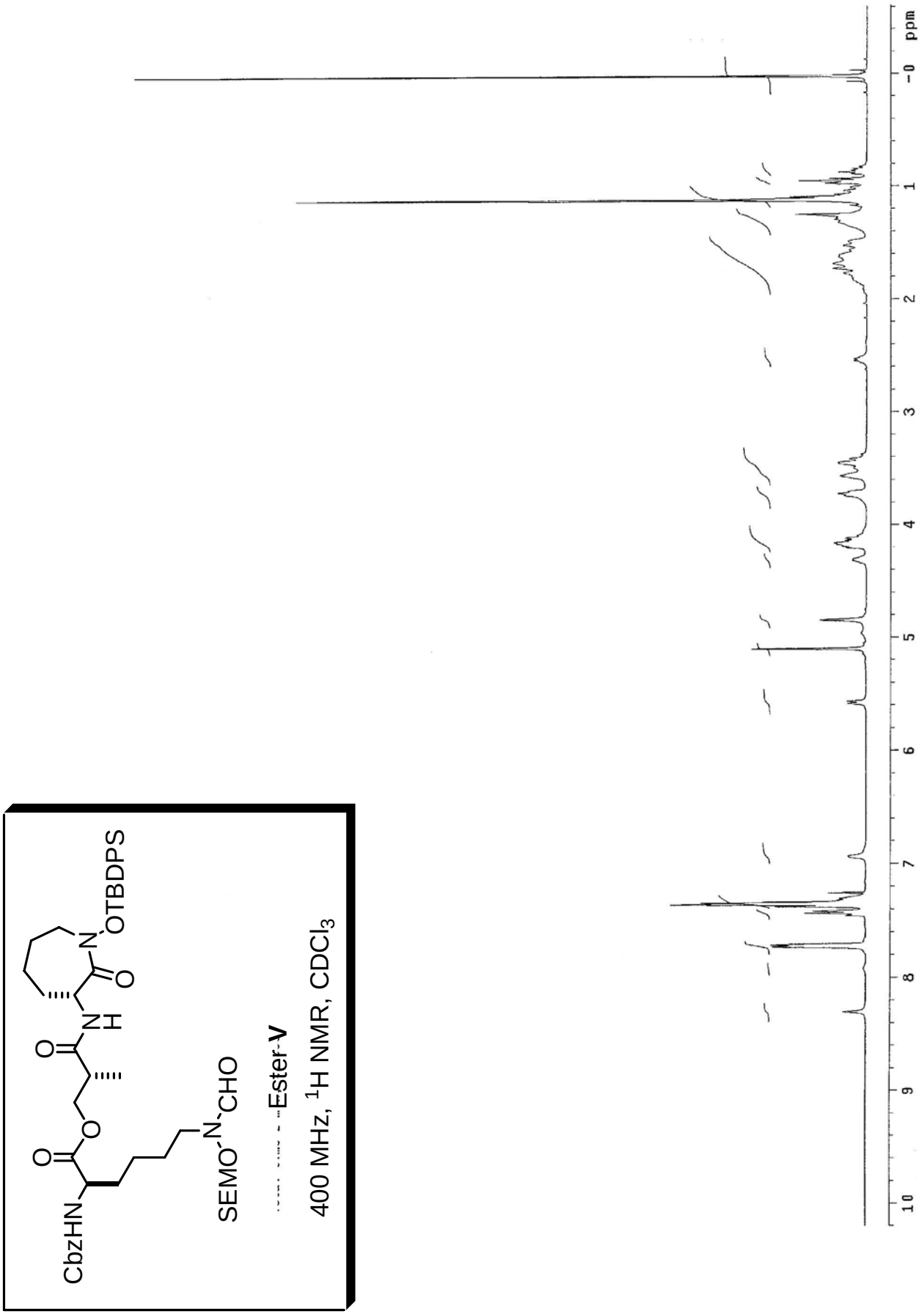


Figure S5

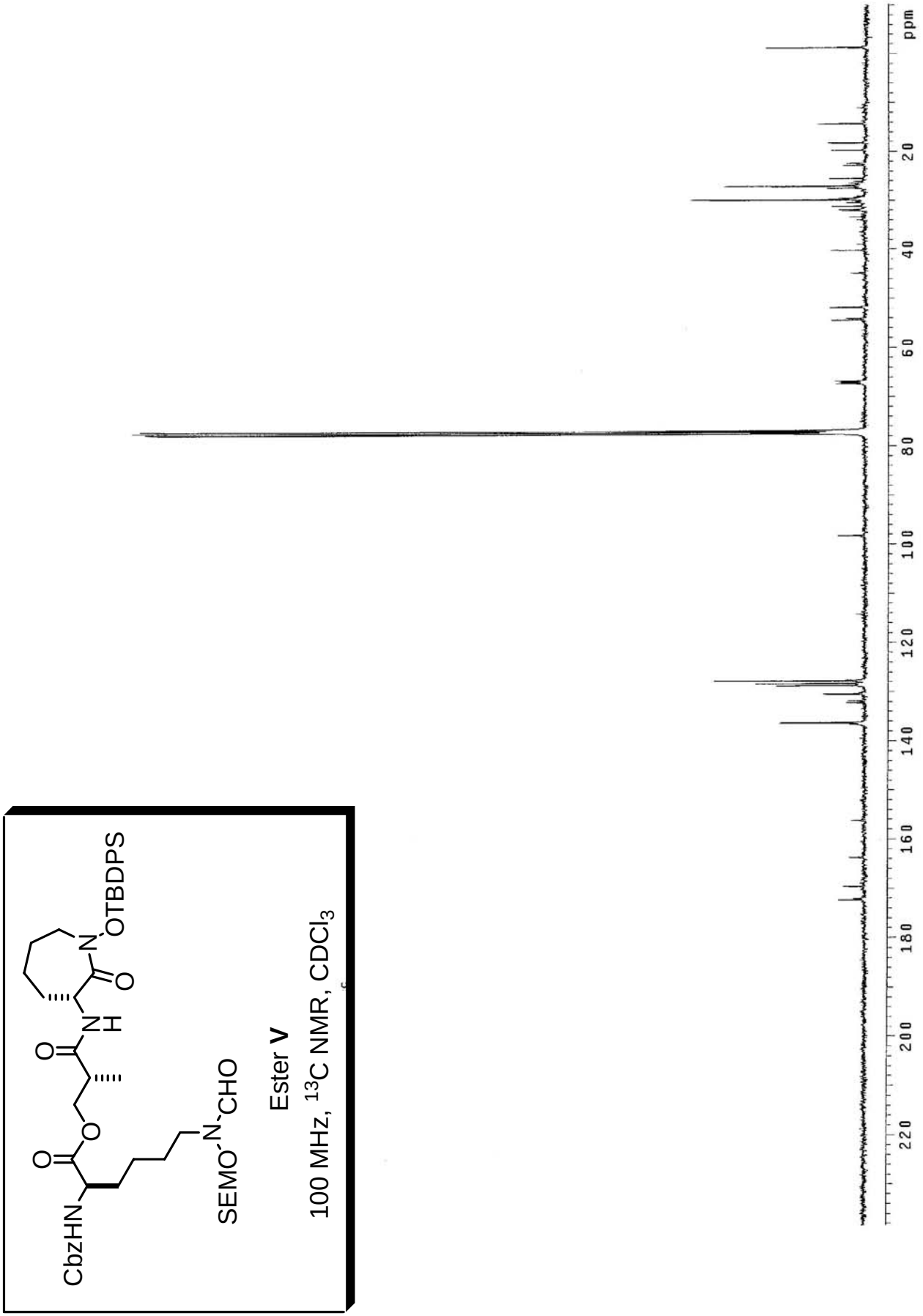


Figure S6

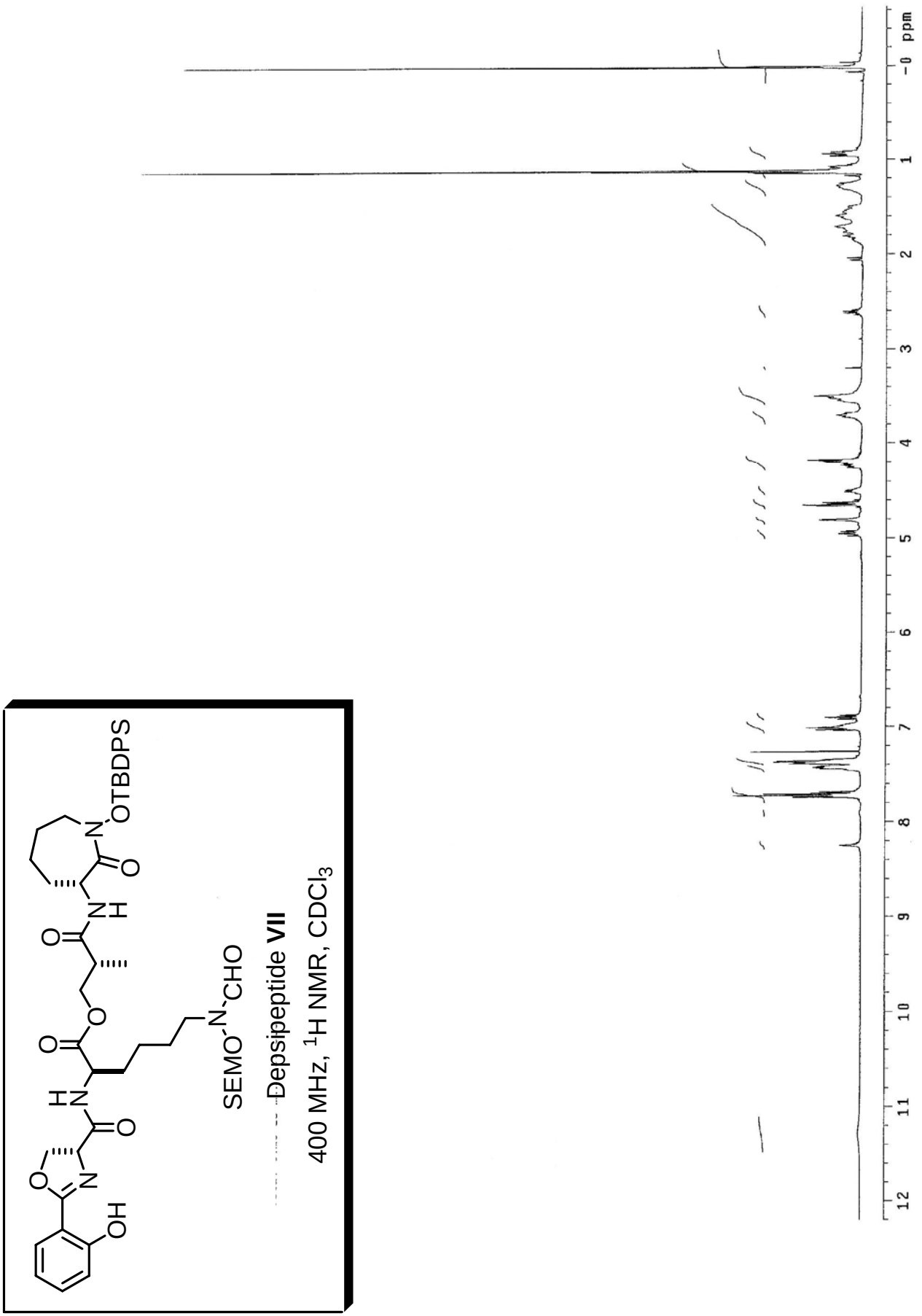
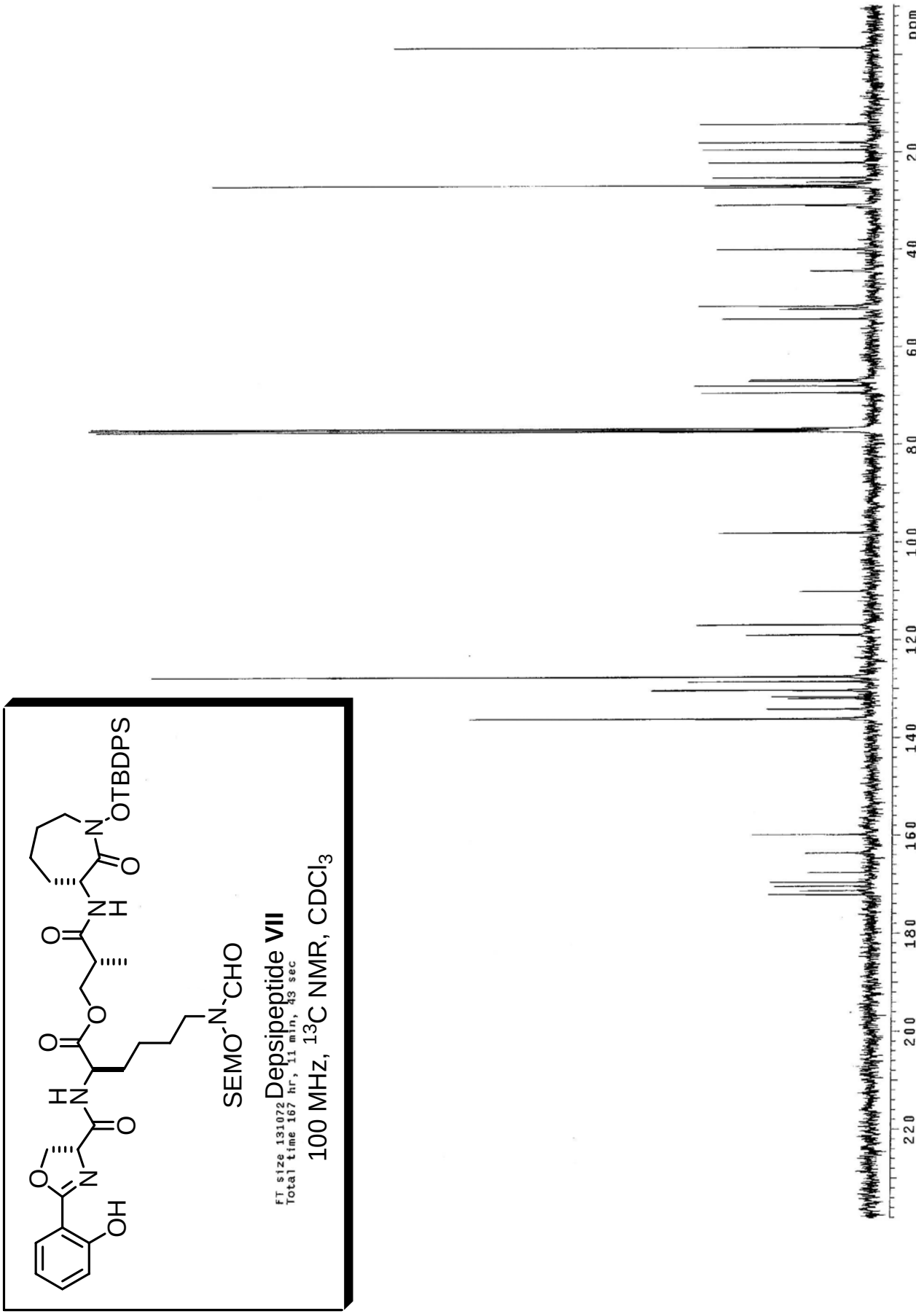


Figure S7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ppm
220
200
180
160
140
120
100
80
60
40
20

Figure S8

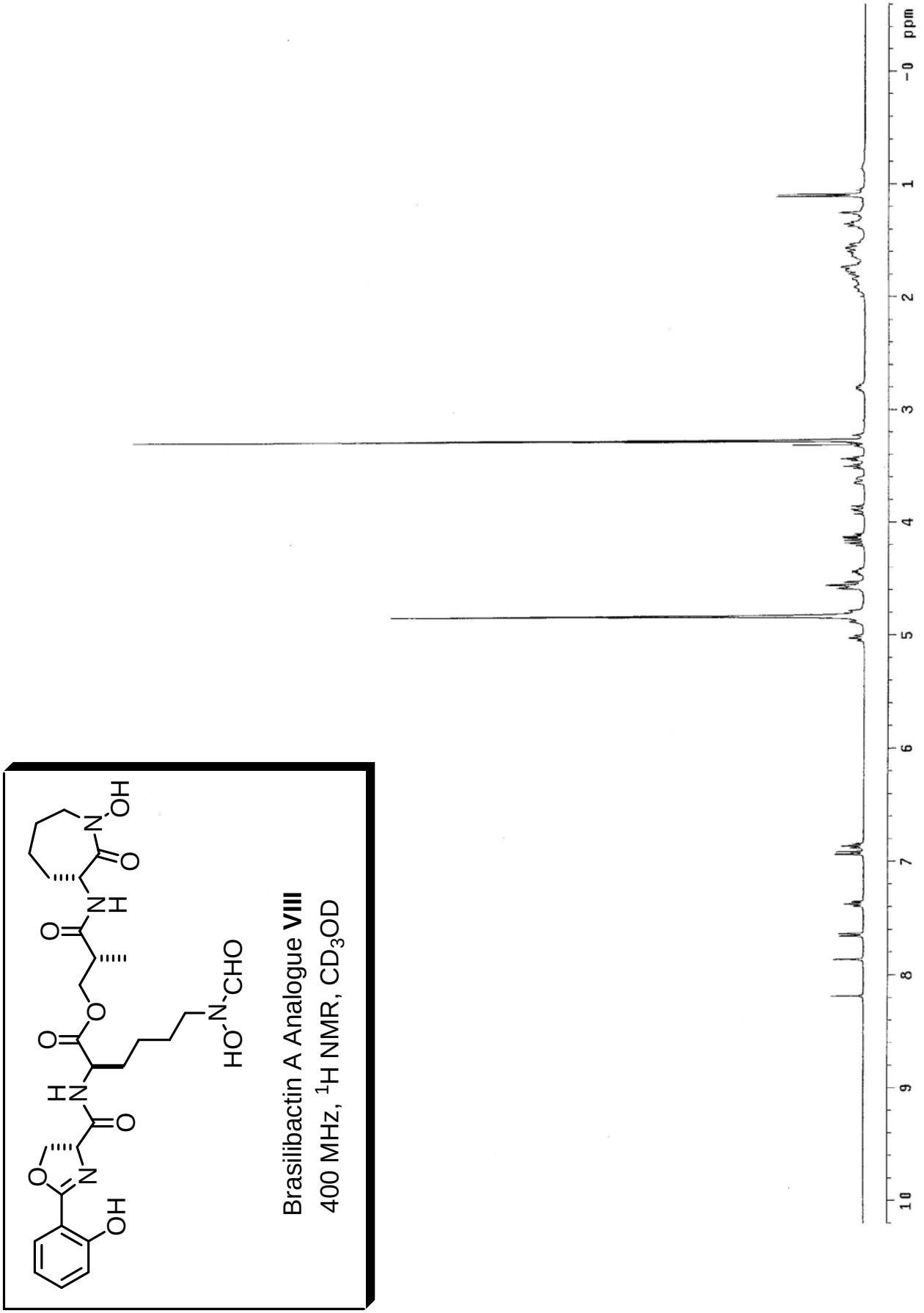

Figure S9

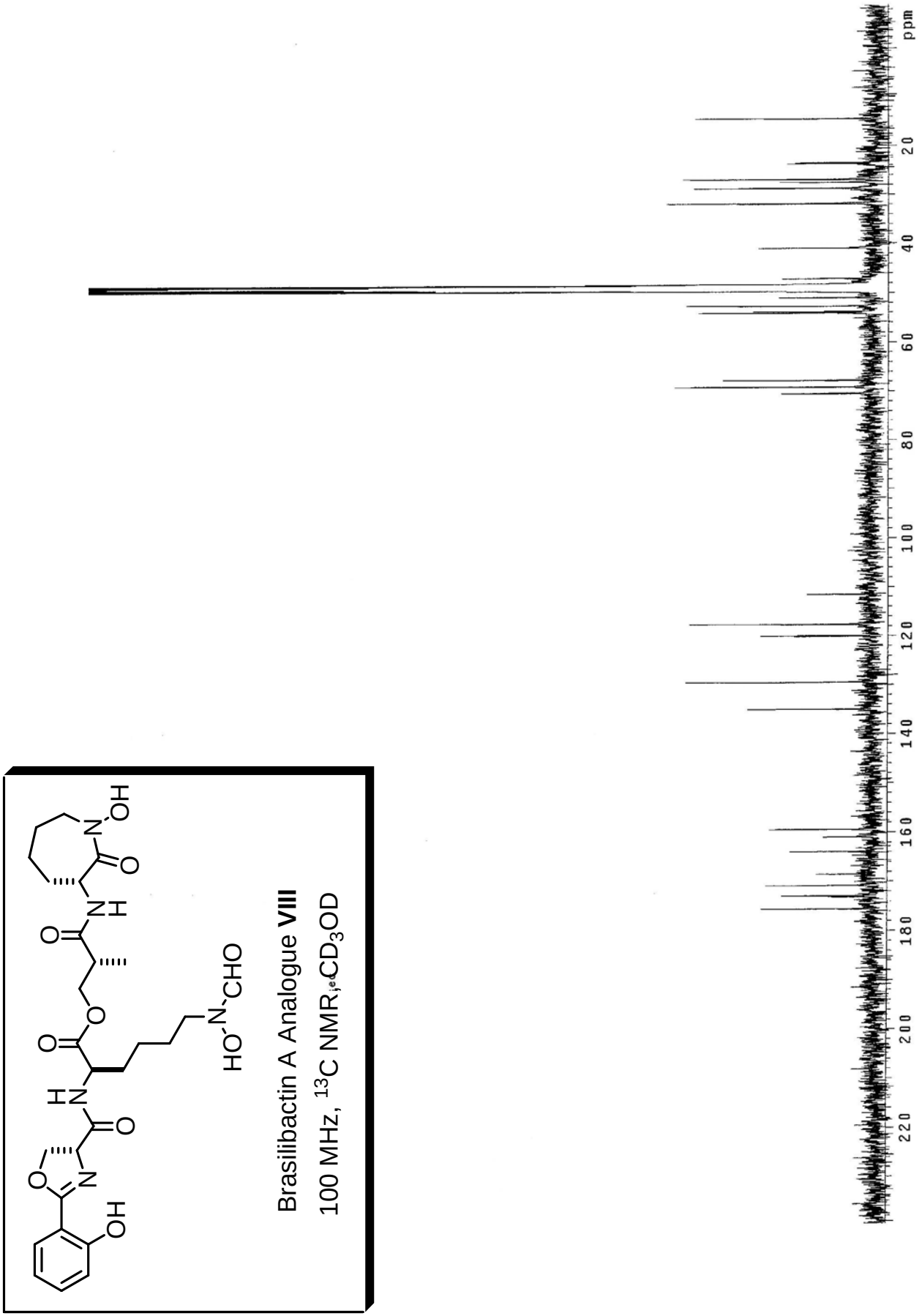

Figure S10

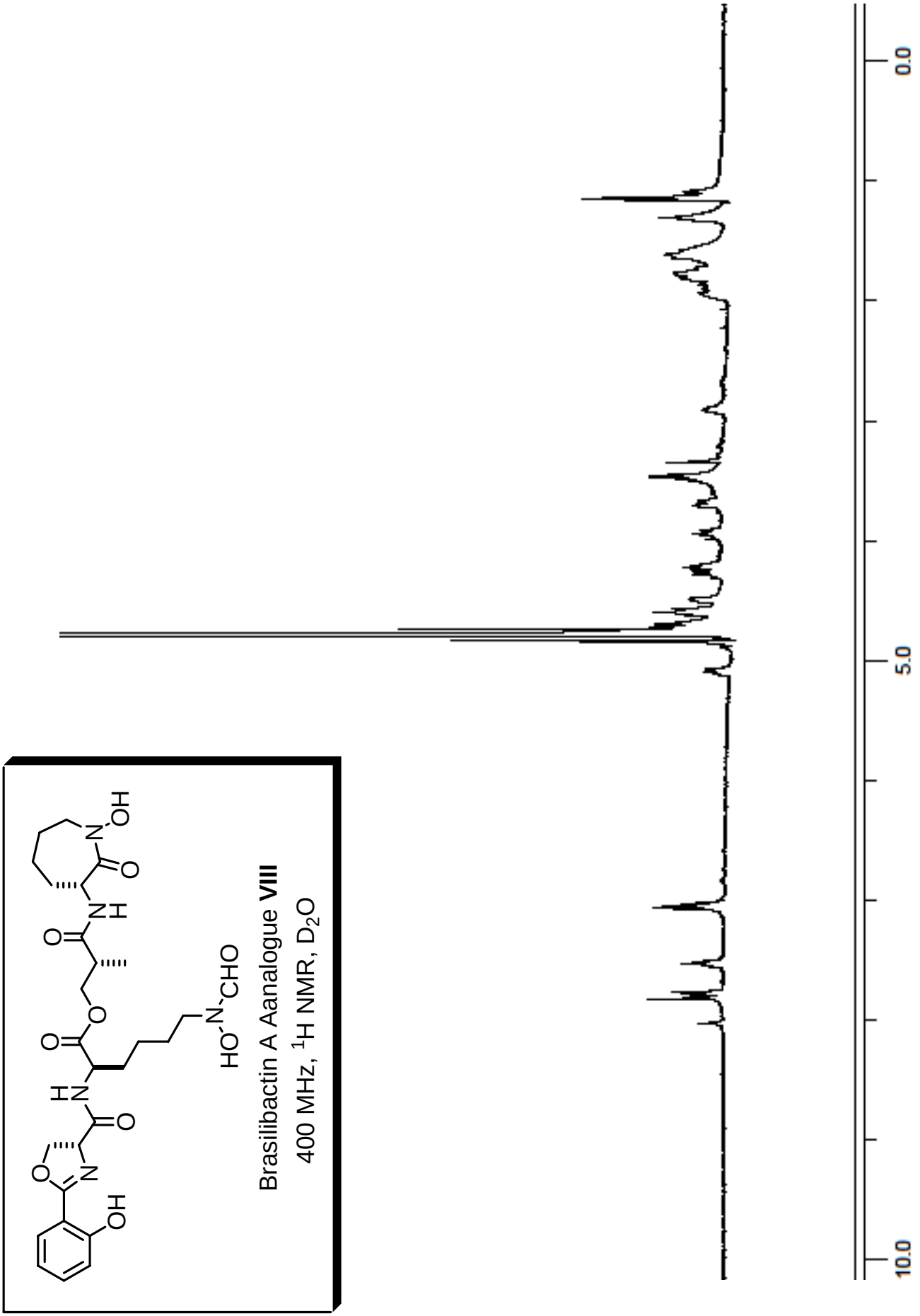

Figure S11

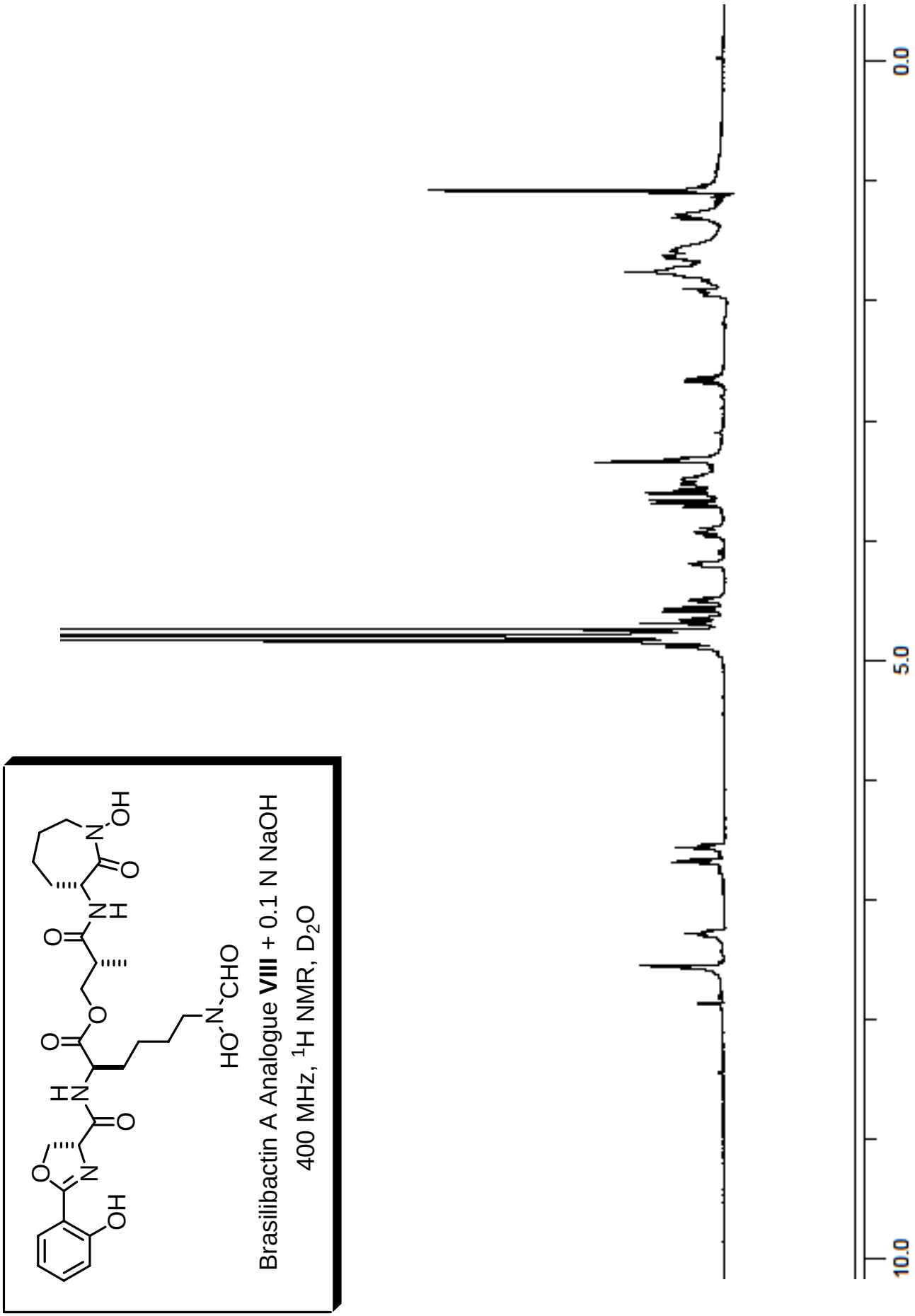

Figure S12

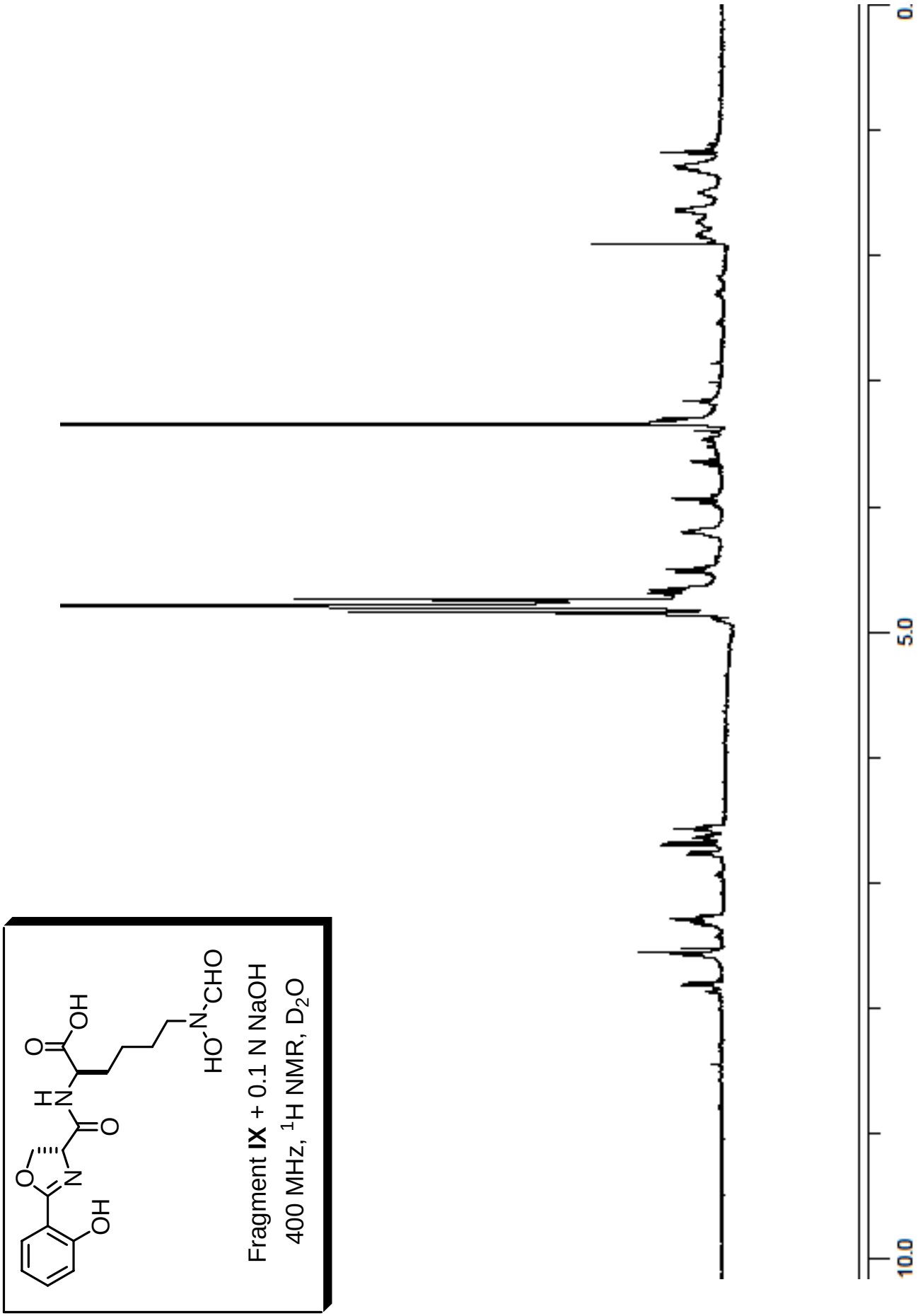

Figure S13

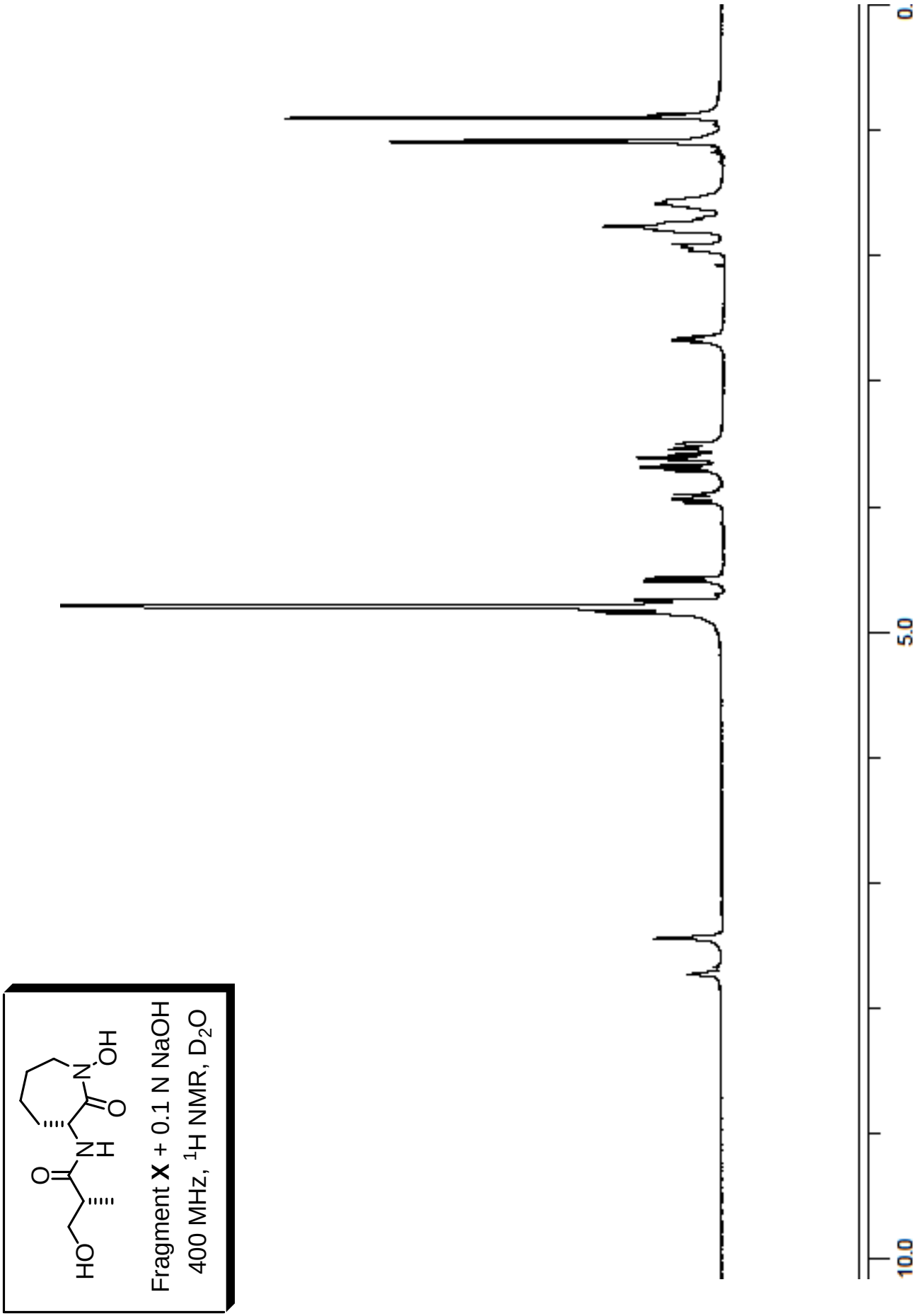

Figure S14

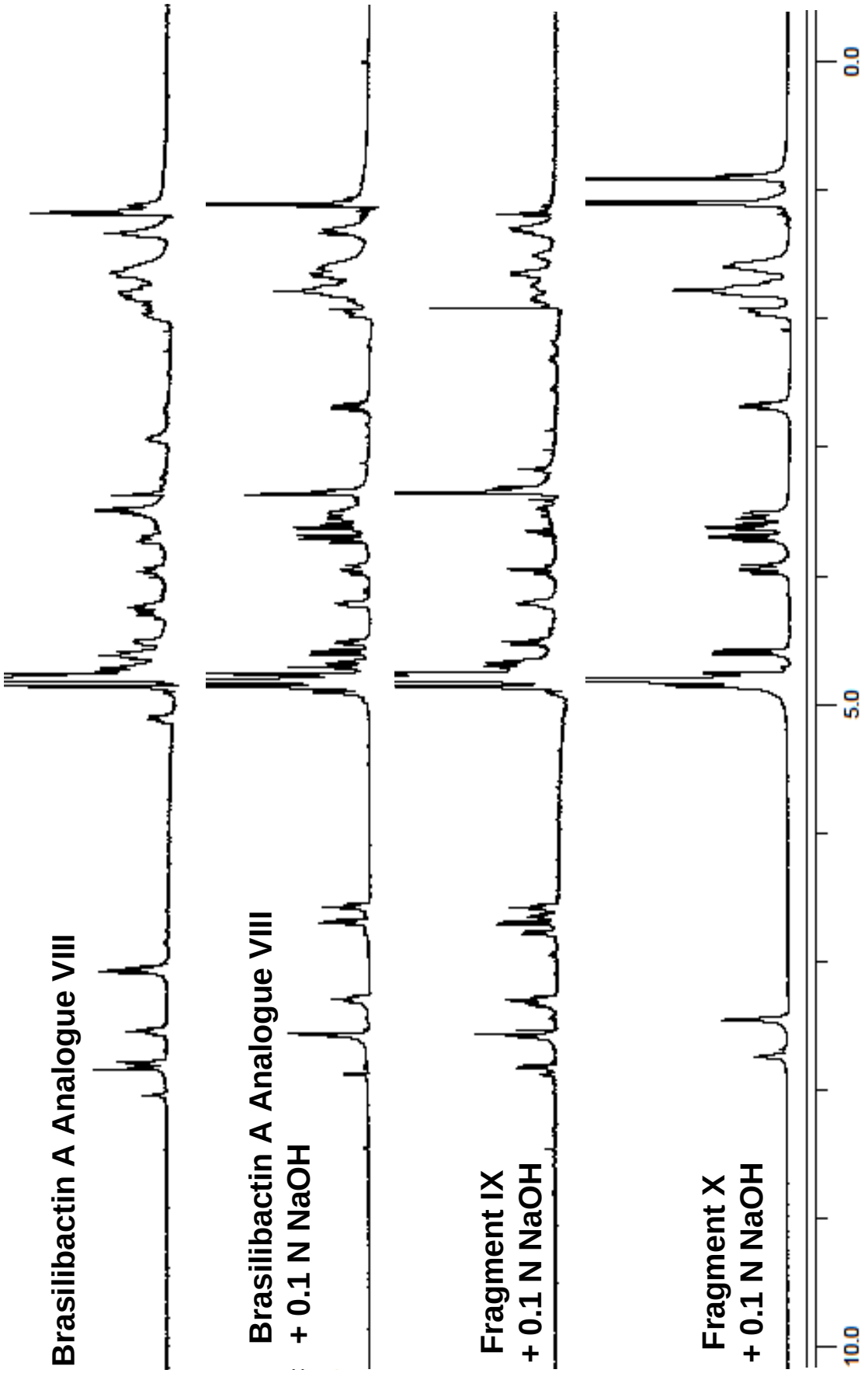

Figure S15

Fe-Bbtan complex pH-dependent spectrophotometric titration

The stability of the iron(III)-Bbtan complex was characterized using spectrophotometric titrations. Two spectrophotometric titrations of the iron(III)-Bbtan complex were performed, one going from low pH to high pH and another going from moderate pH to low pH. The low-to-high pH titration was performed by increasing the solution pH of a 1:1 Fe:Bbtan molar ratio solution through measured additions of standardized 0.1 M NaOH over the pH range of 2.1 to 11.3. The moderate-to-low pH titration was performed by titrating a 1:1 Fe:Bbtan molar ratio solution with 1.0 M $HClO_4$ over the pH range of 6.7 to ~ 0.5. To measure low pH values, the electrode was calibrated assuming Nernstian behavior with a junction potential. Calibration data was analyzed using the program, VLpH. (*4*)

The spectra obtained from the high pH titration are shown in Figure S16, where no change in the wavelength of maximum absorbance (λ_{max}) is observed through pH 8.1, suggesting no change in the inner coordination sphere of the complex from pH 2.1 to 8.1. Above pH 8.1, the complex spectrum change suggests dissociation of the complex, as the absorbance in the visible range decreases to the baseline. This spectral change was irreversible, suggesting hydrolysis of the ligand upon complex dissociation. The spectra obtained from the low pH spectrophotometric titration are shown in Figure S17. During this titration, there appears to be little spectral change aside from dilution until very low pH, at which point the degree of dilution due to addition of titrant, and slow kinetics of complex dissociation made it difficult to determine the spectral characteristics of the complex. The Fe-Bbtan complex dissociation reaction was reversible.

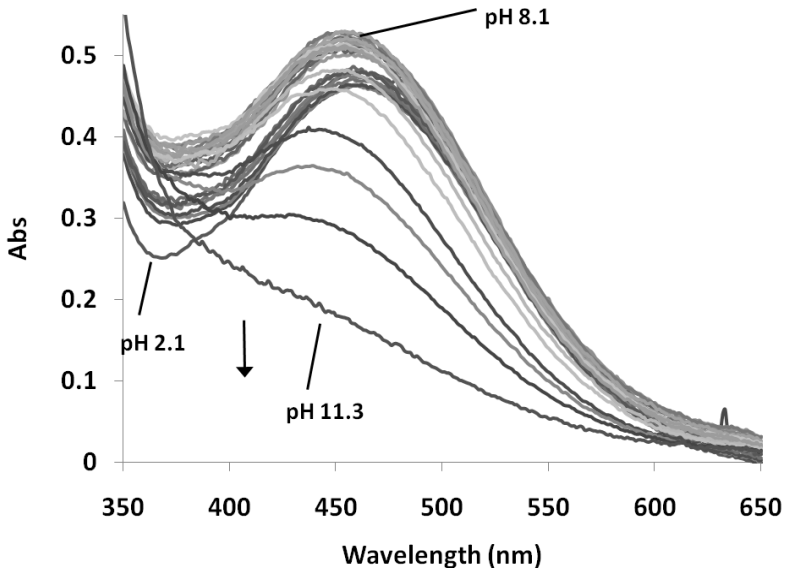

Figure S16. Spectrophotometric titration of the Fe(III)-Bbtan system at a 1:1 M:L molar ratio. [Bbtan] = 2.4 x 10^{-4} M, [Fe^{3+}] = 2.3 x 10^{-4} M, T = 25 °C, and μ = 0.10 ($NaClO_4$). Each trace refers to UV-Visible spectrum measured at different solution pH.

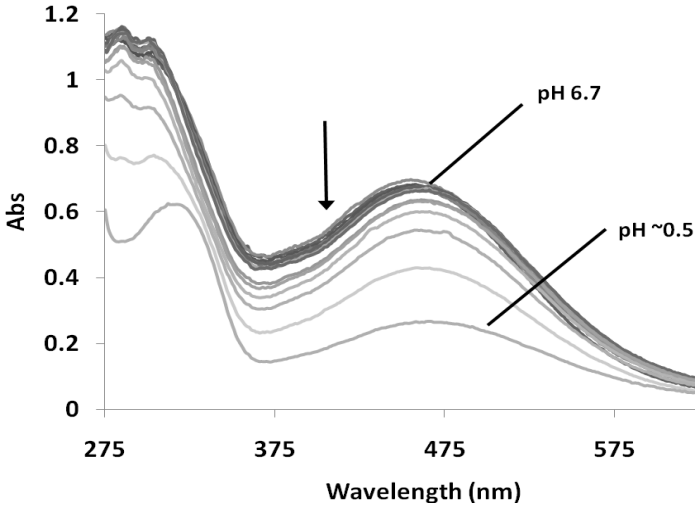

Figure S17. Spectrophotometric titration of the Fe(III)- Bbtan system at a 1:1 molar ratio. [Bbtan] = 2.1 x 10^{-4} M, [Fe^{3+}] = 2.1 x 10^{-4} M, T = 25 °C, and μ = 0.10 ($NaClO_4$).

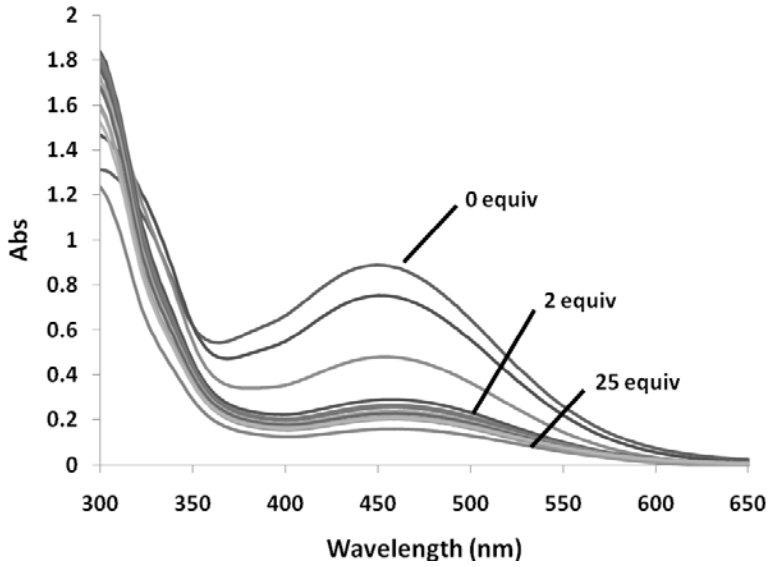

Figure S18. Competition titration reaction between the Fe(III)- Bbtan complex and EDTA. $[Fe^{3+}]$ = 2.5 x 10^{-4} M, [Bbtan] = 2.6 x 10^{-4} M, T = 25 °C, μ = 0.10 ($NaClO_4$), and pH = 7.03 (HEPES buffer). Spectra measured at 0 equivalents of EDTA, 2 equivalents of EDTA, and 25 equivalents of EDTA are marked for clarity.

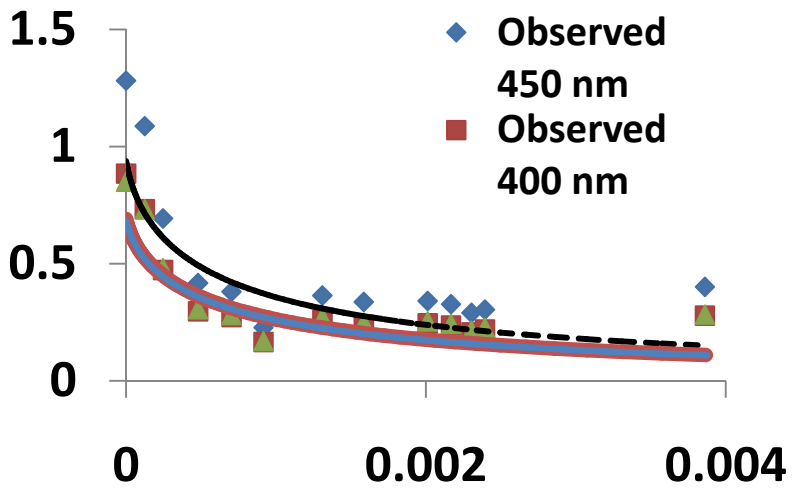

Figure S19. Plot comparing predicted absorbance at three wavelengths to observed absorbances corrected for dilution over a range of total EDTA concentrations added for the EDTA/Bbtan competition reaction shown in Eq 4 .

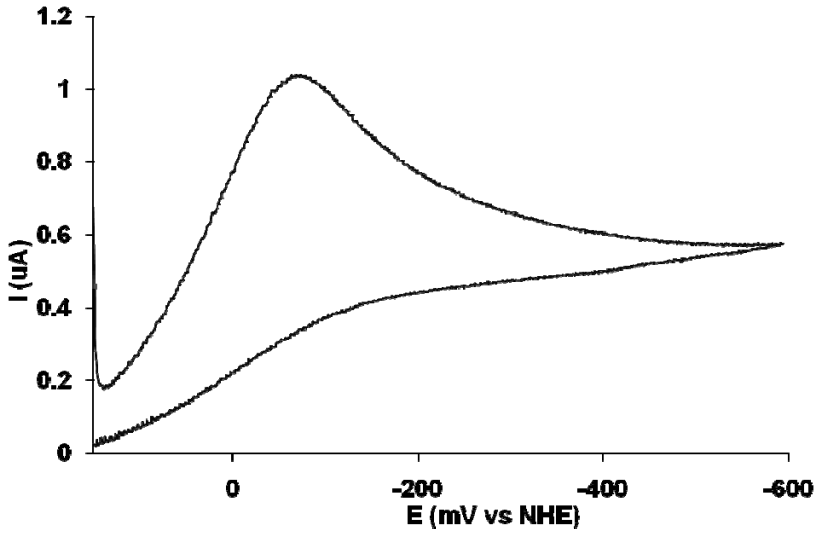

Figure S20. Cyclic voltammogram measured for Bbtan in the absence of iron(III) at pH 7.24. [Bbtan] = 4.2 x 10^{-4} M, T = 25 °C, μ = 0.10 M ($NaClO_4$). Working electrode: HDME, auxiliary electrode: Pt wire, reference electrode: Ag/AgCl, 3.5 M KCl, scan rate = 50 mV/sec.

References

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