

**Synthesis and Biological Evaluation of Epidithio-, Epitetrahydro- and bis-(Methylthio)diketopiperazines. Synthetic Methodology, Enantioselective Total Synthesis of Epicoccin G, 8,8'-*epi-ent*-Rostratin B, Gliotoxin, Gliotoxin G, Emethallicin E and Haematocin, and Discovery of New Antiviral and Antimalarial Agents**

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**Supporting Information Available**

- I. Experimental Section**
- II. <sup>1</sup>H and <sup>13</sup>C NMR Spectra of Compounds**
- III. Protocols of Biological Tests**
- IV. References**
- V. Complete List of Authors of Abbreviated References**

## I. Experimental Section

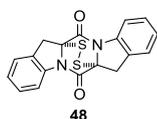
### General Methods

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), toluene, benzene, diethyl ether (Et<sub>2</sub>O), *N,N'*-dimethylformamide (DMF), and methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (<sup>1</sup>H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate, and heat as developing agents. E. Merck silica gel (60, particle size 0.040 – 0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on Bruker DRX-500 or DRX-600 instruments and calibrated using residual undeuterated solvent (CDCl<sub>3</sub>: δ<sub>H</sub> = 7.26 ppm, δ<sub>C</sub> = 77.0 ppm) as an internal reference. Boc-protected compounds show <sup>1</sup>H spectra for two rotamers. The chemical shift of the peak of the major rotamer is listed and coupling constants are given in Hz only for the major rotamer. In <sup>13</sup>C spectra, peaks listed together in parentheses are assigned to rotamers. The following abbreviations were used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Perkin–Elmer 100 FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded on an Agilent ESI-TOF (time of flight) mass spectrometer using MALDI (matrix-assisted laser desorption ionisation) or ESI (electrospray ionization). Melting points are uncorrected and were recorded on a Thomas-Hoover Unimelt capillary melting point apparatus. Optical rotations were recorded on a Perkin-Elmer Model 343 polarimeter at 589 nm, and are reported in units of 10<sup>-1</sup> (deg cm<sup>2</sup> g<sup>-1</sup>).

For experimental procedures, physical data and spectra of previously described compounds<sup>1,3</sup> the reader is referred to the original communications.<sup>1,3</sup>

### General Procedure A. Preparation of epidithiodiketopiperazines.<sup>1</sup>

**Dithiodiketopiperazine 48.** To a suspension of elemental sulfur S<sub>8</sub> (88 mg, 2.75 mmol, 8.0

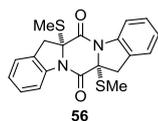


equiv) in THF (0.2 M, 1.8 mL) at 25 °C under argon was added NaHMDS (0.6 M in PhMe, 1.72 mL, 1.03 mmol, 3.0 equiv) dropwise over a period of 2 min. During the addition, the insoluble yellow S<sub>8</sub> quickly changed color, initially into a dark blue solution, then dark orange and finally light orange solution. This solution was stirred for an additional 1 min, and diketopiperazine **47**<sup>2</sup> (100 mg, 0.344 mmol, 1.0 equiv) dissolved in THF (0.2 M, 1.8 mL) was added dropwise at 25 °C over a 2 min period, at which time the reaction mixture turned to light brown. The mixture was stirred for an additional 1 min, then additional NaHMDS (0.6 M in PhMe, 1.14 mL, 0.689 mmol, 2.0 equiv) was added and the resulting mixture was stirred for 0.5 h at 25 °C. The reaction mixture was quenched with sat. aq. NH<sub>4</sub>Cl solution (6 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 6 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and concentrated to afford a brownish residue which was taken to the next step without purification. The residue was dissolved in a mixture of degassed THF:EtOH (0.05 M, 3.4 mL, 1:1) at 0 °C and to the stirred solution under argon was added NaBH<sub>4</sub> (327 mg, 8.61 mmol, 25 equiv) in small portions over a period of 1 min. The resulting mixture was stirred for 45 min while it was allowed to reach ambient temperature. After this time, the solution was cooled to 0 °C and quenched by careful addition of sat. aq. NH<sub>4</sub>Cl solution (3 mL). The resulting mixture was extracted with EtOAc (3 × 3 mL) and to the combined organic extracts was added an aq. solution of KI<sub>3</sub> (1.4 M, 3 mL). This mixture was stirred for 10 min and then quenched with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution (4 mL), and the resulting mixture was extracted with EtOAc (3 × 3 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated in vacuo. The so-obtained residue was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:10) to afford pure epidithiodiketopiperazine **48** (82 mg, 0.234 mmol, 68% yield). **48**: R<sub>f</sub> = 0.45 (silica, EtOAc:hexanes, 1:5); m.p. = 168 – 170 °C (CH<sub>2</sub>Cl<sub>2</sub>); IR ν<sub>max</sub> (film): 2923w, 1695s, 1481s, 1433s, 1388s, 753s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 8.03 (d, J = 7.8

Hz, 2 H), 7.36 (m, 4 H), 7.22 (t,  $J = 7.5$  Hz, 2 H), 4.36 (d,  $J = 18.4$  Hz, 2 H), 3.36 (d,  $J = 18.4$  Hz, 2 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 160.8, 138.5, 129.0, 128.1, 125.9, 125.4, 116.1, 76.9, 36.1$  ppm; HRMS calcd for  $\text{C}_{18}\text{H}_{12}\text{N}_2\text{O}_2\text{S}_2\text{H}^+$  [ $M+\text{H}^+$ ] 353.0413 found 353.0408.

### General procedure B. Preparation of epidithiodiketopiperazines.<sup>1</sup>

**bis-Methylthiodiketopiperazine 56.** To a suspension of elemental sulfur  $\text{S}_8$  (88 mg, 2.75



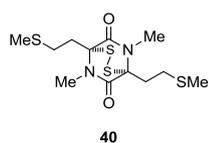
mmol, 8.0 equiv) in THF (0.2 M, 1.8 mL) at 25 °C under argon was added NaHMDS

(0.6 M in PhMe, 1.72 mL, 1.03 mmol, 3.0 equiv) dropwise over a period of 2 min.

During the addition, the insoluble yellow  $\text{S}_8$  quickly changed color initially into a dark blue solution, then dark orange and finally light orange solution. This solution was stirred for an additional 1 min, and diketopiperazine **47**<sup>2</sup> (100 mg, 0.344 mmol, 1.0 equiv) dissolved in THF (0.2 M, 1.8 mL) was added dropwise at 25 °C over a 2 min period, at which time the reaction mixture turned light brown. The mixture was stirred for an additional 1 min, then additional NaHMDS (0.6 M in PhMe, 1.14 mL, 0.689 mmol, 2.0 equiv) was added and the resulting mixture was stirred for 0.5 h at 25 °C. The reaction mixture was quenched with sat. aq.  $\text{NH}_4\text{Cl}$  solution (6 mL) and extracted with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  6 mL). The combined organic layers were dried over  $\text{MgSO}_4$ , filtered, and concentrated to afford a brownish residue which was taken to the next step without purification. The residue was dissolved in a mixture of degassed THF:EtOH (0.05 M, 3.4 mL, 1:1) at 0 °C and to the stirred solution under argon was added  $\text{NaBH}_4$  (327 mg, 8.61 mmol, 25 equiv) in small portions over a period of 1 min. The resulting mixture was stirred for 45 min while it was allowed to reach ambient temperature. After this time, the solution was cooled to 0 °C and then MeI (1.11 mL, 17.9 mmol, 50 equiv) was added and the solution stirred at 25 °C for 15 h. After this time, the solution was quenched by careful addition of sat. aq.  $\text{NH}_4\text{Cl}$  solution (5 mL) and extracted with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  4 mL). The combined organic layers were dried ( $\text{MgSO}_4$ ), filtered, and concentrated in vacuo. The residue so-obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:4) to afford pure bis-methylthiodiketopiperazine **56** (80 mg, 0.210 mmol, 61% yield). **56**:  $R_f = 0.21$  (silica, EtOAc:hexanes, 1:5); IR  $\nu_{\text{max}}$  (film): 2921w, 1677s, 1463s, 1390s, 753s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ ,

600 MHz)  $\delta = 8.08$  (d,  $J = 8.0$  Hz, 2 H), 7.36 – 7.31 (m, 4 H), 7.20 (t,  $J = 7.4$  Hz, 2 H), 3.73 (d,  $J = 17.1$  Hz, 2 H), 3.61 (d,  $J = 17.1$  Hz, 2 H), 2.34 (s, 6 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 162.8$ , 140.4, 128.9, 128.2, 126.1, 125.3, 118.0, 73.1, 39.8, 14.4 ppm; HRMS calcd for  $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_2\text{S}_2\text{Na}^+$  [ $M+\text{Na}^+$ ] 405.0702 found 405.0719.

**Epidithiodiketopiperazine 40.** Following general procedure A using substrate **39**<sup>3</sup>, the crude



residue obtained was purified by flash column chromatography (silica gel,

EtOAc:hexanes, 1:2) to afford pure epidithiodiketopiperazine **40** as colorless

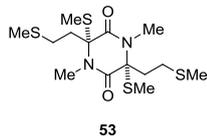
crystals (43% yield). **40**:  $R_f = 0.35$  (silica, EtOAc:hexanes, 1:2); IR  $\nu_{\text{max}}$  (film):

2917w, 1705s, 1442w, 1358s, 1170s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 2.86$  (s, 6 H), 2.73 – 2.65 (m, 2 H), 2.61 – 2.54 (m, 2 H), 2.44 – 2.37 (m, 2 H), 2.34 – 2.28 (m, 2 H), 2.10 (s, 6 H) ppm;

$^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 162.4$ , 75.5, 34.2, 29.1, 28.8, 15.7 ppm; HRMS calcd for

$\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_2\text{S}_4\text{H}^+$  [ $M+\text{H}^+$ ] 353.0480 found 353.0488.

**bis-Methylthiodiketopiperazine 53.** Following general procedure B using substrate **39**<sup>3</sup>, the



crude residue obtained was purified by flash column chromatography (silica gel,

EtOAc:hexanes, 1:3) to afford pure *bis*-methylthiodiketopiperazine **53** as a

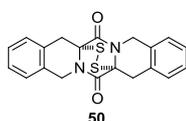
colorless amorphous solid (51% yield). **53**:  $R_f = 0.42$  (silica, EtOAc:hexanes,

1:2); IR  $\nu_{\text{max}}$  (film): 2914w, 1677s, 1433w, 1357s, 1169s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 3.03$  (s, 6 H), 2.77 – 2.70 (m, 2 H), 2.42 – 2.33 (m, 2 H), 2.35 – 2.23 (m, 2 H), 2.22 – 2.10 (m, 2 H),

2.19 (s, 6 H), 2.11 (s, 6 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 159.8$ , 72.1, 31.3, 28.9, 28.6,

18.5, 10.1 ppm; HRMS calcd for  $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}_4\text{Na}^+$  [ $M+\text{Na}^+$ ] 405.0769 found 405.0758.

**Epidithiodiketopiperazine 50.** Following general procedure A using substrate **49**<sup>4</sup>, the crude



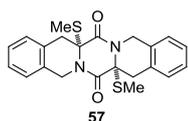
residue obtained was purified by flash column chromatography (silica gel,

EtOAc:hexanes, 1:10) to afford pure epidithiodiketopiperazine **50** as a colorless

amorphous solid (70% yield). **50**:  $R_f = 0.48$  (silica, EtOAc:hexanes, 1:5); IR  $\nu_{\text{max}}$  (film): 2920w,

1705s, 1463s, 1447s, 1388s, 751s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 7.29 - 7.17$  (m, 8 H), 5.41 (d,  $J = 17.1$  Hz, 2 H), 4.36 (d,  $J = 17.1$  Hz, 2 H), 3.67 (d,  $J = 16.2$  Hz, 2 H), 3.31 (d,  $J = 16.2$  Hz, 2 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 164.4, 132.3, 131.4, 128.8, 127.1, 127.1, 126.4, 84.8, 43.5, 41.2$  ppm; HRMS calcd for  $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2\text{H}^+$  [ $M+\text{H}^+$ ] 381.0726 found 381.0734.

**bis-Methylthiodiketopiperazine 57.** Following general procedure B using substrate **49**<sup>4</sup>, the



crude residue obtained was purified by flash column chromatography (silica gel,

EtOAc:hexanes, 1:4) to afford pure *bis*-methylthiodiketopiperazine **57** as a colorless

amorphous solid (67% yield). **57**:  $R_f = 0.27$  (silica, EtOAc:hexanes, 1:5); IR  $\nu_{\text{max}}$  (film): 2925w, 1682s, 1470s, 1390s, 751s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 7.29 - 7.16$  (m, 8 H), 5.40 (d,  $J = 16.8$  Hz, 2 H), 4.34 (d,  $J = 16.8$  Hz, 2 H), 3.77 (d,  $J = 16.0$  Hz, 2 H), 3.33 (d,  $J = 16.0$  Hz, 2 H), 2.16 (s, 6 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 163.8, 132.2, 131.5, 128.6, 127.1, 127.0, 126.5, 86.8, 41.6, 41.1, 12.8$  ppm; HRMS calcd for  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_2\text{S}_2\text{Na}^+$  [ $M+\text{Na}^+$ ] 433.1015 found 433.1018.

**Influence of the Base in the sulfenylation of selected Epidithiodiketopiperazines.** The

effect of the alkali metal in the base on the efficiency of the sulfenylation reaction was examined. Thus, NaHMDS, KHMDS and LiHMDS were used in the sulfenylation protocol in procedure A using diketopiperazines **24**, **41** and *2-epi-43* to generate epidithiodiketopiperazines **27**, **42** and **44**, respectively. For NaHMDS (0.6 M in PhMe) and KHMDS (0.5 M in PhMe), procedure A was followed on 50 mg scale. For LiHMDS (1.0 M in THF) the following procedure was followed:

To a suspension of elemental sulfur  $\text{S}_8$  (25 mg, 0.781 mmol, 8.0 equiv) in THF:PhMe (0.7 mL, 2:5) at 25 °C under argon was added LiHMDS (1.0 M in THF, 0.46 mL, 0.46 mmol, 3.0 equiv) dropwise over a period of 2 min. During the addition, the insoluble yellow  $\text{S}_8$  quickly changed color, initially into a dark blue solution, then dark orange and finally light orange solution. This solution was stirred for an additional 1 min, and diketopiperazine **24** (50 mg, 0.155 mmol, 1.0 equiv) dissolved in THF:PhMe (0.63 mL, 3:4) was added dropwise at 25 °C over a 2 min period, at which time the reaction mixture turned to light brown. The mixture was stirred for an additional 1 min, then

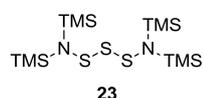
additional LiHMDS (1.0 M in THF, 0.31 mL, 0.31 mmol, 2.0 equiv) was added and the resulting mixture was stirred for 0.5 h at 25 °C. The reaction mixture was quenched with sat. aq. NH<sub>4</sub>Cl solution (2 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 2 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and concentrated to afford a brownish residue which was taken to the next step without purification. The residue was dissolved in a mixture of degassed THF:EtOH (3.2 mL, 1:1) at 0 °C and to the stirred solution under argon was added NaBH<sub>4</sub> (147 mg, 3.87 mmol, 25 equiv) in small portions over a period of 1 min. The resulting mixture was stirred for 45 min while it was allowed to reach ambient temperature. After this time, the solution was cooled to 0 °C and quenched by careful addition of sat. aq. NH<sub>4</sub>Cl solution (2 mL). The resulting mixture was extracted with EtOAc (3 × 2 mL) and to the combined organic extracts was added an aq. solution of KI<sub>3</sub> (1.4 M, 3 mL). This mixture was stirred for 10 min and then quenched with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution (3 mL), and the resulting mixture was extracted with EtOAc (3 × 3 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated in vacuo. The so-obtained residue was purified by PTLC (silica gel, EtOAc:hexanes, 1:4) to afford pure epidithiodiketopiperazine **27** (26 mg, 0.068 mmol, 45% yield).<sup>1</sup>

**Table 1.** Influence of the Base in the sulfenylation of selected Epidithiodiketopiperazines

Entry	Substrate	Product <sup>a</sup>	LiHMDS	Yield [%]	
				NaHMDS	KHMDS
1	<b>24</b>	<b>27</b>	45	69	50
2	<b>41</b>	<b>42</b>	50	65	53
3	<i>2-epi-43</i>	<b>44</b>	53	69	43

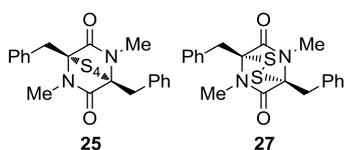
<sup>a</sup>Physical properties match those previously reported.<sup>1</sup>

*N,N'*-trithio-bis-trimethylsilyl compound **23**.<sup>5</sup> To a stirred solution of NaHMDS (1.0 M in THF, 50.0 mmol, 50.0 mL, 2.0 equiv) was added a solution of S<sub>2</sub>Cl<sub>2</sub> (2 mL, 25.0



mmol, 1.0 equiv) in THF (50 mL) at -50 °C. The resulting mixture was stirred for

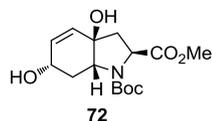
additional 8 h at  $-50\text{ }^{\circ}\text{C}$ , and then it was allowed to gradually warm to room temperature and stir for another 15 h. The reaction mixture was concentrated in vacuo and the desired compound was isolated by distillation under reduced pressure ( $135\text{ }^{\circ}\text{C}$  at 10 Pa) to afford pure *N,N'*-trithio-bis-trimethylsilyl compound **23** as a yellow syrup (2.0 g, 4.8 mmol, 18% yield). **23**:  $R_f = 0.68$  (silica, hexanes); IR  $\nu_{\text{max}}$  (film): 2954w, 1256s, 902s, 846s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 0.22$  (s, 36 H), ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 2.6$  (6C); HRMS calcd for  $\text{C}_{12}\text{H}_{36}\text{N}_2\text{S}_3\text{Si}_4\text{H}^+$  [ $M+\text{H}^+$ ] 417.1190 found 417.1187.



**Reaction of diketopiperazine 24 with  $(\text{TMS})_2\text{S}_3(\text{TMS})_2$ .** To a

stirred solution of diketopiperazine **24** (48 mg, 0.149 mmol, 1.0 equiv) in THF (2 mL) were added  $(\text{TMS})_2\text{S}_3(\text{TMS})_2$  (248 mg, 0.596 mmol, 4.0 equiv) in one portion and NaHMDS (0.6 M in PhMe, 0.99 mL, 0.596 mmol, 4.0 equiv) dropwise at ambient temperature and the reaction mixture turned to red. The reaction mixture was stirred for additional 2 h and then quenched with sat. aq.  $\text{NaHCO}_3$  solution (3 mL) and extracted with EtOAc ( $3 \times 4$  mL). The combined organic phases dried over  $\text{MgSO}_4$ , and concentrated in vacuo. The residue obtained was purified by PTLC (silica gel, benzene) to give epidithiodiketopiperazine **27** as a white solid (35 mg, 0.091 mmol, 43% yield) and epitetrathiodiketopiperazine **25** as a white solid (15 mg, 0.033, 22%). Physical properties match those reported.<sup>1</sup>

**Diol 72.** To a stirred solution of enone **63**<sup>6</sup> (32.8 g, 105.5 mmol, 1.0 equiv) in MeOH (800



mL) was added  $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$  (50.0 g, 135.0 mmol, 1.3 equiv) at ambient

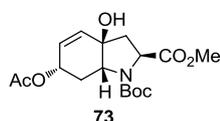
temperature. The resulting mixture was cooled to  $-20\text{ }^{\circ}\text{C}$ ,  $\text{NaBH}_4$  (8.0 g, 211.0

mmol, 2.0 equiv) was added portionwise over 1 h, and the resulting mixture was

stirred for additional 3 h, while it was allowed to gradually warm to  $0\text{ }^{\circ}\text{C}$ . The reaction mixture was concentrated in vacuo, EtOAc (400 mL) was added, and the resulting mixture was carefully quenched with sat. aq.  $\text{NH}_4\text{Cl}$  (300 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc ( $4 \times 200$  mL). The combined organic layers were dried over anhydrous

MgSO<sub>4</sub>, concentrated in vacuo, and the residue so-obtained was purified by flash column chromatography (silica gel, EtOAc) to give alcohol **72** as a white foam (33.0 g, 105.4 mmol, 99% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 1.1:1) that did not coalesce on heating at 60 °C. **72**: R<sub>f</sub> = 0.42 (silica, EtOAc); [α]<sub>D</sub><sup>25</sup> = -49.2 (*c* = 0.95, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3385s, 2975m, 1755m, 1731m, 1676s, 1395s, 1367s, 1171s, 1131s, 1060m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz, signals for major rotamer) δ = 5.81 (t, *J* = 9.3 Hz, 1 H), 5.71 (t, *J* = 9.2 Hz, 1 H), 4.46 (br s, 1 H), 4.37 (d, *J* = 10.0 Hz, 1 H), 4.04 – 3.96 (m, 1 H), 3.78 (s, 3 H), 2.59 – 2.51 (m, 1 H), 2.50 – 2.38 (m, 1 H), 2.10 (dd, *J* = 20.7, 14.2 Hz, 1 H), 1.91 (s, 1 H), 1.41 (s, 9 H), 1.33 (s, 1 H), 1.30 (d, *J* = 10.7 Hz, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz, signals for both rotamers) δ = (175.8, 175.5), (154.0, 153.1), (133.7, 133.4), (129.0, 128.7), (80.9, 80.8), (77.5, 76.5), (66.5, 66.2), (64.9, 64.6), (58.6, 58.4), (53.1, 52.7), (41.0, 40.1), (38.4, 37.9), 28.7, (28.3, 28.2) (3C) ppm; HRMS calcd for C<sub>15</sub>H<sub>23</sub>NO<sub>6</sub>H<sup>+</sup> [*M*+H<sup>+</sup>] 314.1598 found 314.1604.

**Hydroxy acetate 73.** To a stirred solution of diol **72** (33.0 g, 105.4 mmol, 1.0 equiv) in



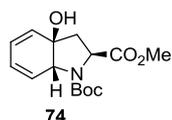
CH<sub>2</sub>Cl<sub>2</sub> (800 mL) at 0 °C were added Et<sub>3</sub>N (44.4 mL, 316.2 mmol, 3.0 equiv), 4-

DMAP (2.6 g, 21.1 mmol, 0.2 equiv), and Ac<sub>2</sub>O (21.5 mL, 210.8 mmol, 2.0

equiv) dropwise. The mixture was stirred at 0 °C for 1 h. The reaction mixture

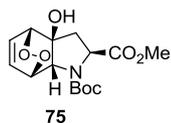
was then quenched with sat. aq. NaHCO<sub>3</sub> solution (200 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 150 mL). The combined organic phases were washed with brine (2 × 150 mL), dried over MgSO<sub>4</sub>, and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:1) to give acetate **73** as a white foam (34.0 g, 95.8 mmol, 91% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 1.2:1) that did not coalesce on heating at 60 °C. **73**: R<sub>f</sub> = 0.51 (silica gel, EtOAc:hexanes, 1:1); [α]<sub>D</sub><sup>25</sup> = -61.4 (*c* = 1.1, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3445m, 2976m, 1735s, 1698s, 1391s, 1367s, 1236s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz, signals for major rotamer) δ = 5.83 – 5.76 (m, 1 H), 5.75 – 5.67 (m, 1 H), 4.50 – 4.32 (m, 2 H), 4.19 – 4.00 (m, 1 H), 3.81 (s, 3 H), 2.59 – 2.53 (m, 1 H), 2.49 – 2.38 (m, 1 H), 2.12 (dd, *J* = 20.4, 14.3 Hz, 1 H), 2.07 (s, 3 H), 1.69 (s, 1 H), 1.47 (s, 9 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>,

150 MHz, signals for both rotamers)  $\delta = (175.6, 175.3), (170.4, 170.2), (153.9, 152.9), (130.6, 130.4), (129.7, 129.3), (81.1, 80.8), (77.2, 76.2), (68.8, 68.6), (64.5, 64.3), (58.6, 58.4), (53.1, 52.7), (40.9, 39.9), (34.3, 33.8), (28.3, 28.2)$  (3C), (21.2, 21.1) ppm; HRMS calcd for  $C_{17}H_{25}NO_7H^+$  [ $M+H^+$ ] 356.1704 found 356.1700.



**Hydroxy diene 74.** To a stirred solution of hydroxyl acetate **73** (35.0 g, 98.5 mmol, 1.0 equiv) in degassed toluene (500 mL) were added  $Ph_3P$  (2.6 g, 9.85 mmol, 0.1 equiv) and  $Pd(OAc)_2$  (1.33 g, 1.97 mmol, 0.02 equiv). Then  $Et_3N$  (16.6 mL, 118.2 mmol, 1.2 equiv) was added at ambient temperature and left stirring for 20 min. After 20 min, the resulting mixture was heated at reflux for 3 h, and then allowed to cool down at ambient temperature. EtOAc (200 mL) and water (200 mL) were added and the organic layer was separated. The aqueous phase was extracted with EtOAc ( $3 \times 100$  mL). The combined organic layers were dried over anhydrous  $MgSO_4$  and concentrated in vacuo. The so-obtained residue was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:1) to give diene **74** as an orange oil (25.0 g, 84.7 mmol, 86% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 1.2:1) that did not coalesce on heating at 60 °C. **74**:  $[\alpha]_D^{25} = -98.5$  ( $c = 1.1, CHCl_3$ );  $R_f = 0.55$  (silica, EtOAc:hexanes, 1:1); IR  $\nu_{max}$  (film): 3443m, 2977w, 1758w, 1727m, 1697s, 1677m, 1384s, 1366s, 1172s  $cm^{-1}$ ;  $^1H$  NMR: ( $CDCl_3$ , 600 MHz, signals for major rotamer)  $\delta = 6.06 - 5.70$  (m, 4 H), 4.68 (s, 1 H), 4.18 (ddd,  $J = 26.8, 10.1, 1.0$  Hz, 1 H), 3.81 (s, 1 H), 2.31 (td,  $J = 13.6, 10.1$  Hz, 1 H), 2.12 (ddd,  $J = 13.9, 12.1, 7.5$  Hz, 1 H), 1.48 (s, 9 H) ppm;  $^{13}C$  NMR: ( $CDCl_3$ , 150 MHz, signals for both rotamers)  $\delta = (176.6, 176.3) (154.4, 153.6), (130.8, 130.4), (127.7, 127.3), (122.3, 122.2), (122.0, 121.6), (80.9, 80.7), (78.9, 77.8), (66.9, 66.7), (55.6, 55.4), (53.0, 52.7), (42.7, 41.5), (28.4, 28.2)$  (3C) ppm; HRMS calcd for  $C_{15}H_{21}NO_5H^+$  [ $M+H^+$ ] 296.1492 found 296.1492.

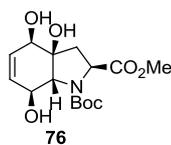
**Endoperoxide 75.** Hydroxy diene **74** (19.0 g, 68.1 mmol, 1.0 equiv) and tetraphenylporphine



(150 mg, 0.244 mmol, 0.0036 equiv) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1000 mL). Oxygen was bubbled through the solution and the flask was irradiated with a 400 W (Philips-MH400/U) sunlamp at ambient temperature for 24 h. The solvent was evaporated

and the residue was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:1) to give endoperoxide **75** as a brown foam (21.2 g, 68.2 mmol, 73% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 2:1) that did not coalesce on heating at 60 °C. **75**:  $[\alpha]_D^{25} = -118.3$  ( $c = 1.0$ , CHCl<sub>3</sub>);  $R_f = 0.33$  (silica, EtOAc:hexanes, 1:1); IR  $\nu_{\max}$  (film): 3455m, 2977w, 1748m, 1698s, 1391s, 1368m, 1174m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz, signals for major rotamer)  $\delta = 6.92 - 6.59$  (m, 1 H), 6.60 – 6.10 (m, 1 H), 6.92 – 6.59 (m, 1 H), 4.60 – 4.41 (m, 1 H), 4.38 – 4.15 (m, 1 H), 4.08 – 4.01 (m, 1 H), 3.77 (s, 3 H), 3.13 (s, 1 H), 2.35 – 2.04 (m, 2 H), 1.47 (s, 9 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz, signals for both rotamers)  $\delta = 196.1, 172.8, 172.6, 172.1, 156.3, 154.0, 153.9, 150.5, 131.9, 131.3, 130.8, 125.5, 82.3, 81.7, 81.1, 78.8, 78.3, 78.2, 77.5, 73.7, 73.5, 73.0, 72.6, 64.8, 64.3, 59.2, 59.0, 58.0, 52.5, 52.4, 52.3, 41.0, 39.4, 38.6, 28.3, 28.2, 28.1$  ppm; HRMS calcd for C<sub>15</sub>H<sub>21</sub>NO<sub>7</sub>H<sup>+</sup> [ $M+H^+$ ] 328.1391 found 328.1395.

**Triol 76.** Endoperoxide **75** (25.6 g, 78.3 mmol, 1.0 equiv) was dissolved in MeOH (500 mL).

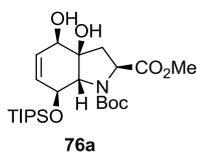


Thiourea (11.9 g, 156.6 mmol, 2.0 equiv) was added and the solution was stirred at ambient temperature for 2 h. The solvent was evaporated and the residue was purified by flash column chromatography (silica gel, EtOAc:Et<sub>2</sub>O, 7:3) to give triol

**76** as a white foam (21.6 g, 65.7 mmol, 84% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 4:1) that did not coalesce on heating at 60 °C. **76**:  $[\alpha]_D^{25} = -94.8$  ( $c = 1.0$ , CHCl<sub>3</sub>);  $R_f = 0.52$  (silica, EtOAc); IR  $\nu_{\max}$  (film): 3402m, 2978w, 1751m, 1675s, 1395s, 1368s, 1173m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz, signals for major rotamer)  $\delta = 6.02 - 5.84$  (m, 2 H), 4.75 (d,  $J = 1.7$  Hz, 1 H), 4.33 (dd,  $J = 9.7, 1.6$  Hz, 1 H), 4.25 (s, 1 H), 4.12 – 4.07 (m, 1 H), 4.02 (t,  $J = 4.7$  Hz, 1 H), 3.98 – 3.92 (m, 1 H), 3.79 (s, 3 H), 3.14 (d,  $J = 5.4$  Hz, 1 H), 2.19 – 2.04 (m, 2 H), 1.44 (s, 9 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz, signals for both rotamers)  $\delta =$

174.1, 156.3, 130.7, 128.1, 81.9, 78.2, 72.8, 69.7, 67.4, 58.0, 52.6, 39.5, 28.2(3C) ppm; HRMS calcd for C<sub>15</sub>H<sub>23</sub>NO<sub>7</sub>Na<sup>+</sup> [*M*+Na<sup>+</sup>] 352.1367 found 352.1371.

**Dihydroxy TIPS ether 76a.** To a stirred solution of triol **76** (18.5 g, 56.2 mmol, 1.0 equiv)



in CH<sub>2</sub>Cl<sub>2</sub> (400 mL) at 0 °C was added Et<sub>3</sub>N (15.8 mL, 112.4 mmol, 2.0 equiv),

followed by TIPSOTf (13.9 mL, 61.8 mmol, 1.1 equiv) dropwise over 1 h. The

mixture was stirred at 0 °C for an additional 30 min. The reaction was then

quenched with a sat. aq. NaHCO<sub>3</sub> solution (150 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL). The

combined organic layers were washed with brine (2 × 100 mL), dried over MgSO<sub>4</sub> and concentrated

in vacuo. The residue obtained was purified by flash column chromatography (silica gel,

EtOAc:hexanes, 3:7) to give dihydroxy TIPS ether **76a** as a colorless oil (26.2 g, 54.0 mmol, 96%

yield). **76a**: R<sub>f</sub> = 0.62 (silica gel, EtOAc:hexanes, 1:1); [α]<sub>D</sub><sup>25</sup> = -33.1 (*c* = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub>

(film): 3513w, 3414w, 2945m, 2868m 1757m, 1708s, 1366s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ =

6.38 (dd, *J* = 9.1, 6.4 Hz, 1 H), 6.24 (dd, *J* = 9.3, 5.7 Hz, 1 H), 4.80 (d, *J* = 5.6 Hz, 1 H), 4.22 (dd, *J*

= 9.6, 1.7 Hz, 1 H), 4.13 (s, 1 H), 3.91 (d, *J* = 11.2 Hz, 1 H), 3.85 (dd, *J* = 11.1, 6.2 Hz, 1 H), 3.75 (s,

3 H), 3.71 (s, 1 H), 2.24 – 2.09 (m, 1 H), 2.01 (dd, *J* = 13.7, 1.6 Hz, 1 H), 1.39 (s, 9 H), 1.20 – 1.12

(m, 3 H), 1.09 – 1.06 (m, 18 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 173.9, 154.1, 133.8, 133.5,

80.6, 80.2, 70.9, 68.5, 66.1, 59.2, 52.1, 39.9, 28.1 (3C), 17.9 (6C), 12.0 (3C) ppm; HRMS calcd for

C<sub>24</sub>H<sub>43</sub>NO<sub>7</sub>SiH<sup>+</sup> [*M*+H<sup>+</sup>] 486.2881 found 486.2886.

**Thionocarbonate 77.** Dihydroxy TIPS ether **76a** (20.5 g, 42.2 mmol, 1.0 equiv) and 1,1'-

thiocarbonyldiimidazole (9.0 g, 50.6 mmol, 1.2 equiv) were dissolved in toluene (450 mL) and

heated at reflux for 3 h. The solution was cooled to ambient temperature, diluted

with EtOAc (200 mL), washed with water (2 × 150 mL), dried over MgSO<sub>4</sub> and

concentrated in vacuo. The residue obtained was purified by flash column

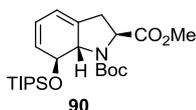
chromatography (silica gel, EtOAc:hexanes, 3:7) to give thionocarbonate **77** as a yellow foam (20.1

g, 38.1 mmol, 90% yield). **77**: R<sub>f</sub> = 0.75 (silica gel, EtOAc:hexanes, 3:7); [α]<sub>D</sub><sup>25</sup> = -33.4 (*c* = 1.0,

CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 2945m, 2867m 1750s, 1719s, 1303s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ =

6.31 (d,  $J = 6.6$  Hz, 1 H), 6.01 – 5.88 (m, 1 H), 5.02 (d,  $J = 3.3$  Hz, 1 H), 4.87 (t,  $J = 4.7$  Hz, 1 H), 4.28 (d,  $J = 4.8$  Hz, 1 H), 4.20 (d,  $J = 6.6$  Hz, 1 H), 3.79 (s, 3 H), 2.59 (dd,  $J = 13.8, 6.4$  Hz, 1 H), 2.44 (dd,  $J = 13.6, 8.6$  Hz, 1 H), 1.38 (s, 9 H), 1.15 – 1.00 (m, 21 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 189.0, 171.6, 152.7, 137.8, 121.3, 89.6, 81.1, 80.0, 66.1, 63.4, 57.5, 52.5, 39.7, 28.0$  (3C), 17.9 (3C), 12.3 (6C) ppm; HRMS calcd for  $\text{C}_{25}\text{H}_{41}\text{NO}_7\text{SSiH}^+$  [ $M+\text{H}^+$ ] 528.2446 found 528.2440.

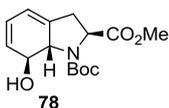
**TIPS ether diene 90.** Thionocarbonate **77** (20.0 g, 37.9 mmol, 1.0 equiv) was dissolved in



trimethylphosphite (70 mL) and heated at reflux for 16 h. The solution was cooled and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:9) to give TIPS ether diene **90** as a

colorless oil (14.0 g, 31.0 mmol, 82% yield). **90**:  $R_f = 0.52$  (silica gel, EtOAc:hexanes, 2:8);  $[\alpha]_{\text{D}}^{25} = -31.7$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (film): 2943m, 2866m 1752s, 1721s, 1173m, 1116s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 5.88 - 5.83$  (m, 1 H), 5.80 (dd,  $J = 9.5, 0.7$  Hz, 1 H), 5.72 (d,  $J = 1.9$  Hz, 1 H), 4.75 (d,  $J = 13.8$  Hz, 1 H), 4.57 (d,  $J = 14.0$  Hz, 1 H), 4.46 (dd,  $J = 8.2, 7.1$  Hz, 1 H), 3.72 (s, 3 H), 2.92 (dd,  $J = 16.4, 7.9$  Hz, 1 H), 2.75 – 2.68 (m, 1 H), 1.39 (s, 9 H), 1.13 – 1.05 (m, 21 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 173.3, 154.2, 140.2, 135.8, 124.2, 117.7, 80.1, 75.8, 64.8, 62.3, 52.0, 34.2, 28.2$  (3C), 18.2 (3C), 12.6 (6C) ppm; HRMS calcd for  $\text{C}_{24}\text{H}_{41}\text{NO}_5\text{SiH}^+$  [ $M+\text{H}^+$ ] 452.2827 found 452.2835.

**Hydroxy diene 78.** To a stirred solution of TIPS ether diene **90** (10.0 g, 22.2 mmol, 1.0



equiv) in  $\text{CH}_2\text{Cl}_2$  (50 mL) at 0 °C was added HCl (1 M in  $\text{Et}_2\text{O}$ , 50 mL) dropwise over 5 min. The resulting mixture was stirred at 0 °C for 10 min, and then concentrated in vacuo. The residue obtained was purified by flash column

chromatography (silica gel, EtOAc:hexanes, 2:8) to give hydroxyl diene **78** as a colorless oil (6.2 g, 21.0 mmol, 98% yield). **78**:  $R_f = 0.31$  (silica gel, EtOAc:hexanes, 2:8);  $[\alpha]_{\text{D}}^{25} = -128.8$  ( $c = 0.5$ ,  $\text{CH}_2\text{Cl}_2$ ); IR  $\nu_{\text{max}}$  (film): 3362w, 2978w, 1749s, 1683s, 1396s, 1368s, 1169s, 1136s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 6.60$  (s, 1 H), 5.90 – 5.77 (m, 1 H), 5.76 – 5.65 (m, 2 H), 4.66 (d,  $J = 12.7$

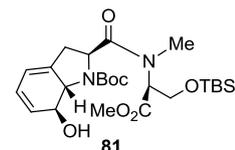
Hz, 1 H), 4.61 – 4.54 (m, 1 H), 4.51 (dd,  $J = 9.4, 6.6$  Hz, 1 H), 3.74 (s, 3 H), 2.96 (dd,  $J = 17.4, 9.4$  Hz, 1 H), 2.64 – 2.57 (m, 1 H), 1.43 (s, 9 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 173.2, 156.0, 134.9, 130.2, 122.7, 117.5, 82.1, 73.6, 66.7, 60.7, 52.1, 32.6, 28.1(3\text{C})$  ppm; HRMS calcd for  $\text{C}_{15}\text{H}_{21}\text{NO}_5\text{Na}^+$  [ $M+\text{Na}^+$ ] 318.1312 found 318.1311.

**Amide 81.** To a stirred solution of hydroxy diene **78** (470 mg, 1.59 mmol, 1.0 equiv) in THF

(2 mL) at 0 °C was added LiOH (1 M aq., 12 mL) in one portion. The resulting

mixture was stirred at ambient temperature for 5 h. The reaction was then

quenched with  $\text{KHSO}_4$  solution (1 M aq.) until pH = 3, and then extracted with



EtOAc (3 × 15 mL). The combined organic layers were dried over  $\text{MgSO}_4$  and concentrated to give carboxylic acid **79** (443 mg, 1.57 mmol, 99%).

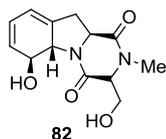
To a stirred solution of the above carboxylic acid and amine **80**<sup>7</sup> (785 mg, 3.18 mmol, 2.0 equiv) in  $\text{CH}_2\text{Cl}_2$  (16 mL) at 0 °C were added diisopropylethylamine (0.85 mL, 4.77 mmol, 3.0 equiv) and HOAt (238 mg, 1.75 mmol, 1.1 equiv) in one portion, followed by HATU (665 mg, 1.75 mmol, 1.1 equiv) portionwise over 1 min. The resulting mixture was stirred at 25 °C for 15 h. The solvent was removed under vacuo and the crude product was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:1) to give amide **81** as white foam (706 mg, 1.38 mmol, 88% yield). **81**:  $R_f = 0.68$  (silica, EtOAc:hexanes, 1:1);  $[\alpha]_D^{25} = -15.2$  ( $c = 1.0, \text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (film): 3354w, 2954m, 2930m, 1748m, 1684s, 1669s, 1394s, 1131m  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 6.73$  (s, 1 H), 5.85 – 5.73 (m, 1 H), 5.67 (d,  $J = 9.7$  Hz, 2 H), 5.02 (dd,  $J = 5.7, 2.8$  Hz, 1 H), 4.92 (dd,  $J = 10.0, 5.0$  Hz, 1 H), 4.63 (s, 2 H), 4.22 (dd,  $J = 10.9, 5.8$  Hz, 1 H), 3.90 (dd,  $J = 10.9, 2.8$  Hz, 1 H), 3.69 (s, 3 H), 3.17 (s, 3 H), 3.00 (dd,  $J = 17.9, 10.0$  Hz, 1 H), 2.58 (d,  $J = 17.6$  Hz, 1 H), 1.40 (s, 9 H), 0.85 (s, 9 H), 0.03 (s, 6 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 173.6, 169.6, 156.8, 135.8, 130.3, 122.7, 117.3, 81.3, 73.5, 66.9, 62.3, 59.3, 58.4, 52.0, 33.7, 31.6, 28.1(3\text{C}), 25.6(3\text{C}), 17.9, -5.7, -5.8$  ppm; HRMS calcd for  $\text{C}_{25}\text{H}_{42}\text{N}_2\text{O}_7\text{SiH}^+$  [ $M+\text{H}^+$ ] 511.2834 found 511.2834.

**Diketopiperazine 82.** To a stirred solution of amide **81** (560 mg, 1.1 mmol, 1.0 equiv) in

CH<sub>2</sub>Cl<sub>2</sub> (8 mL) at 0 °C was added TFA (8 mL) in one portion. The resulting mixture

was stirred at ambient temperature for 3 h. The solvent was removed in vacuo and the

residue was coevaporated with toluene (2 × 10 mL) to remove all traces of TFA.



The crude product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 mL), and then Et<sub>3</sub>N (0.77 mL, 5.5 mmol, 5.0 equiv) was added dropwise at 0 °C. The resulting mixture was stirred at ambient temperature for 15 h. The solvent was concentrated in vacuo and the residue was purified by flash column chromatography (silica gel, MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 1:9) to give diketopiperazine **82** as white crystals (183 mg, 0.69 mmol, 63% yield). **82**: R<sub>f</sub> = 0.35 (silica, MeOH:EtOAc, 1:9); m.p. = 137.0 °C (EtOH); [α]<sub>D</sub><sup>25</sup> = -136.0 (*c* = 1.0, MeOH); IR ν<sub>max</sub> (film): 3353m, 2952w, 1631s, 1197m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 5.84 (dd, *J* = 9.1, 6.6 Hz, 2 H), 5.70 (d, *J* = 8.9 Hz, 1 H), 4.72 (d, *J* = 13.5 Hz, 1 H), 4.62 (d, *J* = 13.7 Hz, 1 H), 4.42 (dd, *J* = 11.2, 6.5 Hz, 1 H), 4.31 (dd, *J* = 12.2, 1.9 Hz, 1 H), 4.06 (s, 1 H), 4.00 (dd, *J* = 12.2, 3.4 Hz, 1 H), 3.08 (s, 3 H), 2.97 (dd, *J* = 15.1, 6.4 Hz, 1 H), 2.73 (dd, *J* = 14.2, 12.8 Hz, 1 H), 1.30 (t, *J* = 7.3 Hz, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 167.3, 167.0, 133.3, 129.6, 123.2, 118.5, 73.1, 68.1, 62.2, 61.0, 59.1, 33.7, 30.5 ppm; HRMS calcd for C<sub>13</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>H<sup>+</sup> [*M*+H<sup>+</sup>] 265.1183 found 265.1185.

**Gliotoxin (3) and gliotoxin G (4):** To a suspension of sulfur S<sub>8</sub> (32 mg, 1.0 mmol, 8.0 equiv)

in THF (1 mL) at ambient temperature under argon was added LiHMDS (1.0 M in

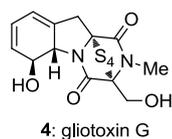
THF, 0.5 mL, 0.5 mmol, 4.0 equiv) dropwise over 2 min. During the addition, the

insoluble yellow S<sub>8</sub> turned to a homogeneous light red solution. This solution was

stirred for an additional 5 min, and diketopiperazine **82** (33 mg, 0.125 mmol, 1.0

equiv) dissolved in THF (2 mL) was added dropwise at ambient temperature over 2

min, at which time the reaction mixture turned into cloudy yellow. The mixture was stirred for an additional 5 min, then more LiHMDS (1.0 M in THF, 0.5 mL, 4.0 equiv) was added, at which time the reaction turned into dark brown. Stirring was continued for 1.5 h at ambient temperature. The reaction mixture was quenched with sat. aq. NaHCO<sub>3</sub> solution (5 mL) and extracted with EtOAc (3



× 10 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated. The resulting brownish colored residue was purified by PTLC (silica gel, MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 1:20) to give gliotoxin (**3**) (9.4 mg, 0.028 mmol, 23% yield), gliotoxin G (**4**) (16 mg, 0.041 mmol, 33% yield), and recovered starting material **82** (2 mg, 0.007 mmol, 6%).

Gliotoxin (**3**): white powder;  $R_f = 0.36$  (silica, MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 1:20);  $[\alpha]_D^{25} = -286$  ( $c = 0.31$ , CHCl<sub>3</sub>), lit.<sup>8b</sup>  $[\alpha]_D^{25} = -255$  ( $c = 0.103$ , CHCl<sub>3</sub>); IR  $\nu_{\max}$  (film): 3366w, 1662s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz)  $\delta = 6.00$  (d,  $J = 2.6$  Hz, 1 H), 5.94 (dd,  $J = 9.4, 4.8$  Hz, 1 H), 5.79 (d,  $J = 9.9$  Hz, 1 H), 5.58 (s, 1 H), 4.82 (s, 2 H), 4.41 (dd,  $J = 12.8, 5.8$  Hz, 1 H), 4.27 (dd,  $J = 12.8, 9.8$  Hz, 1 H), 3.80 – 3.67 (m, 1 H), 3.45 – 3.37 (m, 1 H), 3.19 (s, 3 H), 2.96 (d,  $J = 18.0$  Hz, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz)  $\delta = 166.1, 165.5, 130.7, 130.2, 123.4, 120.4, 77.1, 75.6, 73.3, 69.7, 60.9, 36.7, 27.6$  ppm; HRMS calcd for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>H<sup>+</sup> [ $M+H^+$ ] 327.0468 found 327.0478.

Gliotoxin G (**4**): white powder;  $R_f = 0.24$  (silica, MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 1:20);  $[\alpha]_D^{25} = -432$  ( $c = 0.27$ , CHCl<sub>3</sub>), lit.<sup>9c</sup>  $[\alpha]_D^{25} = -499$  ( $c = 1.25$ , CHCl<sub>3</sub>); IR  $\nu_{\max}$  (film): 3349w, 1654s, 1378m, 1252m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz)  $\delta = 5.94$  (s, 1 H), 5.89 (d,  $J = 9.1$  Hz, 1 H), 5.78 (d,  $J = 9.5$  Hz, 1 H), 5.42 (s, 1 H), 5.07 (d,  $J = 12.9$  Hz, 1 H), 4.77 (d,  $J = 13.1$  Hz, 1 H), 4.36 (d,  $J = 12.1$  Hz, 1 H), 4.09 (d,  $J = 11.9$  Hz, 1 H), 4.00 (s, 1 H), 3.25 (d,  $J = 17.2$  Hz, 1 H), 3.13 (s, 3 H), 3.06 (d,  $J = 16.7$  Hz, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz)  $\delta = 169.1, 168.1, 130.1, 129.2, 122.9, 120.3, 78.2, 73.8, 72.6, 70.5, 62.5, 40.1, 28.7$  ppm; HRMS calcd for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S<sub>4</sub>H<sup>+</sup> [ $M+H^+$ ] 390.9909 found 390.9914.

**Table 2.** <sup>1</sup>H NMR (CDCl<sub>3</sub>) Spectroscopic Data Comparison of Natural<sup>8a</sup> and Synthetic Gliotoxin (**3**)

Natural <sup>a</sup> $\delta$ <sup>1</sup> H [ppm, mult, $J$ (Hz)] 500 MHz	Synthetic $\delta$ <sup>1</sup> H [ppm, mult, $J$ (Hz)] 600 MHz
2.95 (d, 17.8)	2.96 (d, 18.0)
3.19 (s)	3.19 (s, 3 H)
– <sup>b</sup>	3.37 – 3.45 (m)
3.72 (d, 17.8)	3.72 (m)
4.24 (d, 12.8)	4.27 (dd, 12.8, 9.8)
4.43 (d, 12.8)	4.41 (dd, 12.8, 5.8)
4.82 (s)	4.82 (s)
– <sup>b</sup>	5.58 (s)
5.77 (d, 9.8)	5.79 (d, 9.9)
5.93 (dd, 9.8, 4.4)	5.94 (dd, 9.4, 4.8)
5.99 (d, 4.4)	6.00 (d, 2.6)

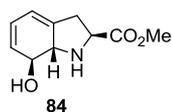
<sup>a</sup>After wash. <sup>b</sup>Value not tabulated.

**Table 3.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) Spectroscopic Data Comparison of Natural<sup>9a</sup> and Synthetic Gliotoxin G (4)

Natural $\delta$ $^1\text{H}$ [ppm, mult, $J$ (Hz)] 300 MHz	Synthetic $\delta$ $^1\text{H}$ [ppm, mult, $J$ (Hz)] 600 MHz
3.05 (d, 16.6)	3.06 (d, 16.7)
3.12 (s)	3.13 (s)
3.26 (d, 16.6)	3.25 (d, 17.2)
4.00 (s)	– <sup>a</sup>
4.08 (d, 12.5)	4.09 (d, 11.9)
4.36 (d, 12.5)	4.36 (d, 12.1)
4.78 (d, 13.0)	4.77 (d, 13.1)
5.06 (d, 13.0)	5.07 (d, 12.9)
5.50 (s)	5.42 (s)
5.75 – 5.95(m)	5.78 (d, 9.5)
5.75 – 5.95(m)	5.89 (d, 9.1)
5.75 – 5.95(m)	5.94 (s)

<sup>a</sup>OH group is exchanged.

**Hydroxy diene amine 84.** To a stirred solution of hydroxy diene Boc derivative **78** (6.5 g,



22.0 mmol, 1.0 equiv) in  $\text{CH}_2\text{Cl}_2$  (50 mL) at 0 °C was added TFA (20 mL) dropwise

over 5 min. The resulting mixture was stirred at ambient temperature for 4 h. The

solvent was removed in vacuo and the resulting crude product was purified by flash

column chromatography (silica gel, MeOH:EtOAc, 1:9) to give hydroxyl diene amine **84** as an

amorphous solid (4.1 g, 21.0 mmol, 95% yield). **84**:  $R_f = 0.47$  (silica, MeOH:EtOAc, 1:9);  $[\alpha]_D^{25} = -$

36.5 ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (film): 3348w, 2960w, 1749m, 1671s, 1199s, 1135s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR:

( $\text{CDCl}_3$ , 600 MHz)  $\delta = 5.95 - 5.79$  (m, 1 H), 5.74 (d,  $J = 2.2$  Hz, 1 H), 5.68 (d,  $J = 9.6$  Hz, 1 H), 4.46

(d,  $J = 14.5$  Hz, 1 H), 4.15 – 4.08 (m, 1 H), 3.98 (d,  $J = 14.2$  Hz, 1 H), 3.75 (s, 3 H), 2.92 (dd,  $J =$

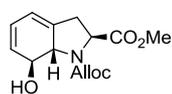
17.2, 8.5 Hz, 1 H), 2.76 (d,  $J = 18.9$  Hz, 1 H), 2.05 (br s, 2 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta$

$= 174.5, 141.3, 130.3, 124.9, 116.4, 75.5, 65.3, 59.4, 52.4, 33.8$  ppm; HRMS calcd for  $\text{C}_{10}\text{H}_{13}\text{NO}_3\text{H}^+$

$[M+\text{H}^+]$  196.0968 found 196.0977.

**Alloc derivative 78a.** To a stirred solution of amine **84** (3.3 g, 11.3 mmol, 1.0 equiv) in

dioxane: $\text{H}_2\text{O}$  (50 mL, 1:1) at 0 °C was added  $\text{NaHCO}_3$  (1.0 g, 113 mmol, 10.0 equiv) portionwise,

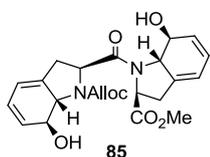


followed by dropwise addition of allylchloroformate (1.8 mL, 19.5 mmol, 1.7 equiv)

**78a**

over 15 min. The resulting mixture was stirred at ambient temperature for 3 h. The mixture was quenched with H<sub>2</sub>O (25 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (2 × 25 mL), dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:3) to give alloc derivative **78a** as a yellow oil (2.7 g, 9.7 mmol, 88% yield). **78a**: R<sub>f</sub> = 0.82 (silica gel, EtOAc:hexanes, 1:2); [α]<sub>D</sub><sup>25</sup> = -181.5 (*c* = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3380w, 2953w, 1746s, 1688s, 1403s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 6.36 (s, 1 H), 5.90 – 5.80 (m, 2 H), 5.75 (d, *J* = 1.2 Hz, 1 H), 5.70 (d, *J* = 9.6 Hz, 1 H), 5.28 (d, *J* = 17.2 Hz, 1 H), 5.22 (d, *J* = 10.5 Hz, 1 H), 4.68 (d, *J* = 12.4 Hz, 1 H), 4.62 (dt, *J* = 15.5, 7.6 Hz, 3 H), 4.58 – 4.52 (m, 1 H), 3.71 (s, 3 H), 3.01 (dd, *J* = 17.3, 9.5 Hz, 1 H), 2.66 (d, *J* = 17.3 Hz, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 172.7, 156.6, 134.5, 131.8, 130.0, 122.7, 118.1, 117.6, 73.3, 67.0, 66.8, 60.2, 52.3, 32.5 ppm; HRMS calcd for C<sub>14</sub>H<sub>17</sub>NO<sub>5</sub>H<sup>+</sup> [*M*+H<sup>+</sup>] 280.1179 found 280.1180.

**Carboxylic acid 83 and amide 85.** To a stirred solution of alloc derivative **78a** (530 mg, 1.9

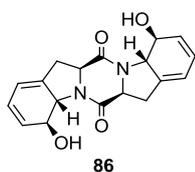


mmol, 1.0 equiv) in THF (5 mL) at 0 °C was added LiOH (1 M aq., 5 mL) in 3 portions, and the resulting mixture was stirred at ambient temperature for 5 h. The reaction mixture was then quenched with KHSO<sub>4</sub> (1 M aq.) solution until pH = 3,

and then extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated in vacuo. The obtained crude carboxylic acid **83** was used for the next step without further purification. An analytically pure sample of **83** was obtained by PTLC (silica gel, MeOH:EtOAc, 1:9) as a light yellow foam. **83**: R<sub>f</sub> = 0.19 (silica, MeOH:EtOAc, 1:9); [α]<sub>D</sub><sup>25</sup> = -83.0 (*c* = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3385w, 2922w, 1738m, 1683s, 1404s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 6.59 (br s, 1 H), 5.84 (d, *J* = 5.1 Hz, 2 H), 5.79 – 5.66 (m, 2 H), 5.34 – 5.22 (m, 1 H), 5.19 (d, *J* = 9.4 Hz, 1 H), 4.69 (s, 1 H), 4.61 (dd, *J* = 14.9, 7.4 Hz, 4 H), 3.13 – 3.01 (m, 1 H), 2.74 – 2.72 (m, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 176.9, 156.7, 134.4, 131.7, 129.7, 122.9, 118.2, 117.8, 73.3, 67.0, 66.8, 59.8, 32.5 ppm; HRMS calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>5</sub>H<sup>+</sup> [*M*+H<sup>+</sup>] 266.1023 found 266.1029.

To a stirred solution of the so-obtained carboxylic acid **83** and amine **84** (370 mg, 1.9 mmol, 1.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) at 0 °C were added sequentially diisopropylethylamine (1.0 mL, 5.7 mmol, 3.0 equiv) and BOP-Cl (530 mg, 2.0 mmol, 1.1 equiv) portionwise over 2 h. The resulting mixture was stirred at 25 °C for 15 h. The resulting mixture was quenched with sat. aq. NaHCO<sub>3</sub> solution (10 mL) and extracted with EtOAc (4 × 10 mL). The combined organic phases were dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:1) to give amide **85** as colorless oil (697 mg, 1.57 mmol, 83% yield). The NMR spectra of this compound showed signals corresponding to two rotamers (ca. 6:1) that did not coalesce on heating at 60 °C. **85**: R<sub>f</sub> = 0.28 (silica, EtOAc:hexanes, 1:1); [α]<sub>D</sub><sup>25</sup> = -38.6 (*c* = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3348w, 2923m, 2851m, 1743m, 1687s, 1648m, 1405s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz, signals for major rotamer) δ = 5.95 – 5.81 (m, 4 H), 5.80 (s, 1 H), 5.77 – 5.69 (m, 3 H), 5.25 (dd, *J* = 29.3, 13.8 Hz, 2 H), 4.78 – 4.50 (m, 9 H), 3.77 (s, 3 H), 3.21 – 3.08 (m, 2 H), 2.82 (d, *J* = 18.4 Hz, 1 H), 2.59 (d, *J* = 16.5 Hz, 1 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz, signals for both rotamers) δ = 174.0, 171.3, 157.3, 134.1, 133.5, 132.3, 129.9, 129.8, 123.1, 122.8, 118.4, 118.3, 117.9, 73.4, 71.7, 69.0, 67.2, 67.1, 60.0, 59.1, 53.0, 33.0, 32.5 ppm; HRMS calcd for C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>7</sub>H<sup>+</sup> [*M*+H<sup>+</sup>] 443.1813 found 443.1810.

**Diketopiperazine 86.** Argon was bubbled through a stirred solution of amide **85** (580 mg,



1.3 mmol, 1.0 equiv) in THF:Et<sub>2</sub>NH (12 mL, 2:1) at ambient temperature for 10 min, then dppb was added (28 mg, 0.065 mmol, 0.05 equiv), followed by Pd<sub>2</sub>(dba)<sub>3</sub> (24 mg, 0.026 mmol, 0.02 equiv). The reaction mixture was stirred at

ambient temperature for 2 h, and then quenched with a sat. aq. NH<sub>4</sub>Cl solution (12 mL) and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (2 × 5 mL), dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:1 → 1% MeOH in EtOAc:CH<sub>2</sub>Cl<sub>2</sub>, 1:1) to give diketopiperazine **86** as a white crystalline solid (360 mg, 0.841 mmol, 84% yield). **86**: R<sub>f</sub> = 0.68 (silica gel, EtOAc); m.p. = 156.0 °C (EtOH), [α]<sub>D</sub><sup>25</sup> = -74.6 (*c* = 0.385, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film):

3337w, 2945m, 2868m 1732w, 1646s, 1436s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  = 6.00 (s, 2 H), 5.92 – 5.86 (m, 4 H), 5.76 (d,  $J$  = 9.4 Hz, 2 H), 4.75 (d,  $J$  = 13.1 Hz, 2 H), 4.72 – 4.60 (m, 4 H), 2.98 (dd,  $J$  = 15.8, 7.1 Hz, 2 H), 2.93 – 2.81 (m, 2 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta$  = 168.2, 132.6, 130.0, 122.9, 119.0, 73.6, 68.1, 62.4, 32.2 ppm; HRMS calcd for  $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_4\text{H}^+$  [ $M+\text{H}^+$ ] 327.1339 found 327.1339.

**Optimization study of the sulfenylation of diketopiperazine 86.** Following general procedure A on 5 mg scale and alternating the solvent system (THF,  $\text{CH}_2\text{Cl}_2$ , PhMe,  $\text{Et}_2\text{O}$ ), the crude residue obtained before the  $\text{NaBH}_4$  reduction was analyzed by  $^1\text{H}$  NMR for yield determination.

**Table 4.** Optimization Study of the Sulfenylation of Diketopiperazine **86**

Entry	Solvent	LiHMDS	NaHMDS	KHMDS	Yield of <b>87</b> (%) [rsm]
1	THF	20[70]	10[40]	<5[35]	
2	$\text{CH}_2\text{Cl}_2$	<5[<5]	<5[<5]	<5[<5]	
3	PhMe	15[70]	<5[45]	<5[30]	
4	$\text{Et}_2\text{O}$	25[60]	15[35]	<5[35]	
5	THF <sup>a</sup>	46[43]	28[30]	<5[40]	

<sup>a</sup>Experimental information is reported below.

**Epitetrathiodiketopiperazine 87:** To a suspension of sulfur (180 mg, 5.6 mmol, 37 equiv)

in  $\text{Et}_2\text{O}$  (3 mL) at ambient temperature under argon was added LiHMDS (1.0 M in

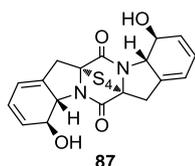
THF, 3.0 mL, 3.0 mmol, 20 equiv) dropwise over 2 min. During the addition, the

yellow  $\text{S}_8$  suspension turned to a homogeneous orange solution. This solution was

stirred for an additional 5 min, and then it was cannulated at ambient temperature over 2 min to a

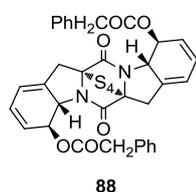
THF: $\text{Et}_2\text{O}$  (9 mL, 2:1) solution of diketopiperazine **86** (50 mg, 0.15 mmol, 1.0 equiv). The reaction

mixture turned to dark green and then to brown. The mixture was stirred for an additional 5 min,



then more LiHMDS (1.0 M in THF, 3.0 mL, 3.0 mmol, 20 equiv) was added and the resulting solution (dark red) was stirred for 5 h at ambient temperature. The reaction mixture was quenched with sat. aq. NaHCO<sub>3</sub> solution (10 mL), extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated. The resulting residue was purified by PTLC (silica gel, EtOAc:hexanes, 4:3) to give epitetrathiodiketopiperazine **87** as a yellow solid (31 mg, 0.069 mmol, 46% yield) and recovered starting material **86** (21 mg, 0.064 mmol, 43%). **87**: R<sub>f</sub> = 0.31 (silica gel, EtOAc:hexanes, 1:1); [α]<sub>D</sub><sup>25</sup> = -28.9 (c = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3386w, 2924m, 1654s 1397m, 1061m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 5.95 (s, 2 H), 5.89 (d, J = 12.1 Hz, 2 H), 5.80 (d, J = 9.7 Hz, 2 H), 5.24 (s, 2 H), 5.05 (d, J = 12.7 Hz, 2 H), 4.78 (d, J = 13.7 Hz, 2 H), 3.20 (d, J = 16.2 Hz, 2 H), 3.03 (d, J = 16.5 Hz, 2 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 170.2, 131.0, 129.6, 122.8, 121.1, 78.1, 72.8, 69.9, 40.7 ppm; HRMS calcd for C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>S<sub>4</sub>H<sup>+</sup> [M+H<sup>+</sup>] 453.0066 found 453.0070.

**bis-Ester epitetrathiodiketopiperazine 88.** To a stirred solution of



epitetrathiodiketopiperazine **87** (15 mg, 0.033 mmol, 1.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL)

at 0 °C were added BnCO<sub>2</sub>H (136 mg, 1.0 mmol, 30 equiv) and 4-DMAP (12.2 mg,

0.1 mmol, 3.0 equiv), followed by DCC (206 mg, 1.0 mmol, 30 equiv) in one

portion. The mixture was stirred at ambient temperature for 5 h, and then quenched with sat. aq.

NaHCO<sub>3</sub> solution (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic phases

were washed with brine (2 × 5 mL), dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue

obtained was purified by PTLC (silica gel, EtOAc:hexanes, 4:3) to give *bis*-ester **88** as a white solid

(16.2 mg, 0.023 mmol, 71% yield) and recovered starting material **87** (3.9 mg, 0.008 mmol, 26%

yield). **88**: R<sub>f</sub> = 0.56 (silica gel, EtOAc:hexanes, 1:1); [α]<sub>D</sub><sup>25</sup> = -13.3 (c = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film):

2921m, 2851w, 1735m, 1686s 1369m, 1215m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 7.43 – 7.31

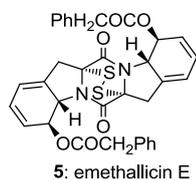
(m, 10 H), 6.02 – 5.87 (m, 4 H), 5.84 (d, J = 15.0 Hz, 2 H), 5.62 (d, J = 9.4 Hz, 2 H), 5.43 (d, J =

14.6 Hz, 2 H), 3.79 (q, J = 15.6 Hz, 4 H), 3.21 (d, J = 16.8 Hz, 2 H), 3.04 (d, J = 16.7 Hz, 2 H) ppm;

<sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 171.1, 166.7, 134.1, 132.9, 129.7(2C), 128.9, 128.6(2C), 127.1,

125.0, 121.0, 79.3, 74.9, 64.2, 41.48, 41.46 ppm; HRMS calcd for  $C_{34}H_{28}N_2O_6S_4Na^+$  [ $M+Na^+$ ] 711.0722 found 711.0717.

**Emethallicin E (5).**<sup>10a</sup> To a stirred solution of *bis*-ester epitetrathiodiketopiperazine **88** (15



mg, 0.022 mmol, 1.0 equiv) in  $CH_2Cl_2$  (0.5 mL) and MeCN (12 mL) at ambient temperature was added a solution of  $Et_3N$  (0.001 mL, 0.007 mmol, 0.32 equiv) in MeCN (0.25 mL), followed by propanedithiol (0.22 mL, 2.0 mmol, 90 equiv) in

one portion. The resulting mixture was stirred at ambient temperature for 30 min and then washed with hexanes ( $5 \times 4$  mL). The combined hexane layers were washed with MeCN (10 mL) and the combined MeCN fractions were concentrated in vacuo. The residue obtained was passed through a short plug of silica gel (EtOAc:hexanes, 1:4  $\rightarrow$  EtOAc) and the combined EtOAc fractions (25 mL), were diluted with MeOH (25 mL). Oxygen was bubbled through the solution in the dark for 2 h to effect oxidation of the dithiol to the epidithiodiketopiperazine. The resulting solution was then concentrated under vacuo and the residue was purified by PTLC (silica gel, EtOAc:hexanes, 1:1) to give emethallicin E (**5**) (7.3 mg, 0.011 mmol, 54% yield).

Emethallicin E (**5**): yellow powder;  $R_f = 0.27$  (silica, EtOAc:hexanes, 3:7);  $[\alpha]_D^{25} = -99$  ( $c = 0.36$ ,  $CHCl_3$ ), lit.<sup>10b</sup>  $[\alpha]_D^{20} = -104$  ( $c = 0.3$ ,  $CHCl_3$ ); IR  $\nu_{max}$  (film): 2924w, 1738m, 1703s  $cm^{-1}$ ;  $^1H$  NMR: ( $CDCl_3$ , 600 MHz)  $\delta = 7.25$  (ddd,  $J = 8.0, 3.7, 2.1$  Hz, 10 H), 6.05 (d,  $J = 13.0$  Hz, 2 H), 5.89 (ddt,  $J = 7.6, 5.0, 3.8$  Hz, 4 H), 5.48 (d,  $J = 9.5$  Hz, 2 H), 5.00 (d,  $J = 12.9$  Hz, 2 H), 3.74 – 3.68 (m, 6 H), 2.85 (d,  $J = 17.8$  Hz, 2 H) ppm;  $^{13}C$  NMR: ( $CDCl_3$ , 150 MHz)  $\delta = 170.9, 162.7, 134.1, 132.3, 129.5$  (2C), 128.4 (2C), 127.5, 126.9, 124.5, 119.8, 78.2, 74.4, 64.2, 41.2, 36.3 ppm; HRMS calcd for  $C_{34}H_{28}N_2O_6S_2H^+$  [ $M+H^+$ ] 625.1461 found 625.1445.

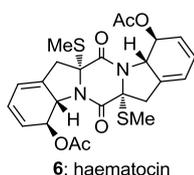
**Table 5.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) Spectroscopic Data Comparison of Natural<sup>10b</sup> and Synthetic Emethallicin E (5)

<b>Natural</b> $\delta$ $^1\text{H}$ [ppm, mult, $J$ (Hz)] 400 MHz	<b>Synthetic</b> $\delta$ $^1\text{H}$ [ppm, mult, $J$ (Hz)] 600 MHz
2.87 (d, 17.7)	2.85 (d, 17.8)
3.72 (dd, 17.7, 1.8)	3.68 – 3.74 (m)
3.74 (br s)	–
5.02 (ddd, 13.4, 1.8, 1.8)	5.00 (d, 12.9)
5.50 (br d, 9.8)	5.48 (d, 9.5)
5.90 – 6.05 (m)	5.89 (ddt, 7.6, 5.0, 3.8)
6.05 (br d, 13.4)	6.05 (d, 13.0)
7.25 – 7.35 (m)	7.25 (ddd, 8.0, 3.7, 2.1)

**Table 6.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) Spectroscopic Data Comparison of Natural<sup>10b</sup> and Synthetic Emethallicin E (5)

<b>Natural</b> $\delta$ $^{13}\text{C}$ [ppm, mult, $J$ (Hz)] 100 MHz	<b>Synthetic</b> $\delta$ $^{13}\text{C}$ [ppm, mult, $J$ (Hz)] 150 MHz
170.9	170.9
162.7	162.7
134.1	134.1
132.5	132.3
129.5	129.5
128.4	128.4
127.6	127.5
126.9	126.9
124.5	124.5
119.8	119.8
78.2	78.2
74.5	74.4
64.2	64.2
41.3	41.2
36.4	36.3

**Haematocin (6).** To a stirred solution of epitetrathiodiketopiperazine **87** (15 mg, 0.033



mmol, 1.0 equiv) in  $\text{CH}_2\text{Cl}_2$  (10 mL) at 0 °C was added glacial AcOH (0.057 mL,

1.0 mmol, 30.3 equiv), followed by 4-DMAP (12.2 mg, 0.1 mmol, 3.0 equiv) and

DCC (106 mg, 1.0 mmol, 30.3 equiv) in one portion. The mixture was stirred at

ambient temperature for 15 h and then it was quenched with a sat. aq.  $\text{NaHCO}_3$  solution (5 mL) and

extracted with EtOAc ( $3 \times 10$  mL). The residue obtained was purified by PTLC (silica gel,

EtOAc:hexanes, 4:3) to give *bis*-ester **89** as a white solid (12.6 mg, 0.023 mmol, 71% yield) and

recovered starting material **87** (3.6 mg, 0.008 mmol, 24% yield).

The *bis*-ester **89** was dissolved in pyridine (1 mL) and MeOH (1 mL) and cooled to 0 °C. NaBH<sub>4</sub> (100 mg, 2.6 mmol, 80 equiv) was added in one portion, followed by careful addition of MeI (1 mL, 16.0 mmol, 485 equiv). The mixture was stirred at ambient temperature for 4 h. The reaction mixture was then quenched with a sat. aq. NH<sub>4</sub>Cl solution (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 5 mL), dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue obtained was purified by by PTLC (silica gel, EtOAc:benzene, 3:7) to give haematocin (**6**) (11 mg, 0.022 mmol, 97% yield).

Haematocin (**6**): yellow solid;  $R_f = 0.43$  (silica, EtOAc:benzene, 3:7);  $[\alpha]_D^{25} = -208$  ( $c = 0.18$ , CHCl<sub>3</sub>), lit.<sup>11</sup>  $[\alpha]_D^{25} = -216$  ( $c = 0.076$ , CHCl<sub>3</sub>); IR  $\nu_{\max}$  (film): 2924w, 2854w, 1738m, 1668s, 1382s, 1232s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz)  $\delta = 6.14$  (d,  $J = 14.2$  Hz, 2 H), 5.96 (dd,  $J = 11.4$ , 2.4 Hz, 4 H), 5.59 (d,  $J = 9.2$  Hz, 2 H), 5.17 (d,  $J = 13.0$  Hz, 2 H), 3.01 (d,  $J = 16.1$  Hz, 2 H), 2.83 (d,  $J = 16.1$  Hz, 2 H), 2.26 (s, 6 H), 2.11 (s, 6 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz)  $\delta = 170.4$ , 164.9, 133.9, 128.0, 125.1, 120.0, 75.3, 74.1, 64.2, 40.1, 21.3, 14.3 ppm; HRMS calcd for C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>Na<sup>+</sup> [ $M+Na^+$ ] 525.1124 found 525.1120.

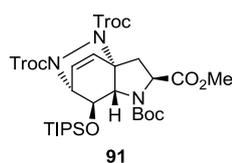
**Table 7.** <sup>1</sup>H NMR (CDCl<sub>3</sub>) Spectroscopic Data Comparison of Natural<sup>11</sup> and Synthetic Haematocin (**6**)

<b>Natural</b> $\delta$ <sup>1</sup> H [ppm, mult, $J$ (Hz)] 400 MHz	<b>Synthetic</b> $\delta$ <sup>1</sup> H [ppm, mult, $J$ (Hz)] 600 MHz
2.11 (s)	2.11 (s)
2.25 (s)	2.26 (s)
2.83 (br d, 16.1)	2.83 (d, 16.1)
3.01 (dd, 16.1, 1.2)	3.01 (d, 16.1)
5.16 (br d, 14.8)	5.17 (d, 13.0)
5.59 (br d, 9.2)	5.59 (d, 9.2)
5.94 (m)	5.96 (dd, 11.4, 2.4)
5.96 (m)	–
6.13 (br d, 14.8)	6.14 (d, 14.2)

**Table 8.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) Spectroscopic Data Comparison of Natural<sup>11</sup> and Synthetic Haematocin (6)

Natural $\delta^{13}\text{C}$ [ppm, mult, $J$ (Hz)] 100 MHz	Synthetic $\delta^{13}\text{C}$ [ppm, mult, $J$ (Hz)] 150 MHz
170.4	170.4
164.9	164.9
133.9	133.9
128.0	128.0
125.1	125.1
119.9	120.0
75.3	75.3
74.1	74.1
64.2	64.2
40.1	40.1
21.3	21.3
14.3	14.3

**Olefin TIPS ether 91.** Diene TIPS ether **90** (11.4 g, 25.4 mmol, 1.0 equiv) was dissolved in

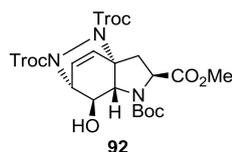


$\text{CH}_2\text{Cl}_2$  (150 mL) and to the resulting solution was added TrocN=NTroc (11.6 g, 30.5 mmol, 1.2 equiv). The reaction mixture was heated at reflux for 4 h. The resulting solution was cooled to room temperature and concentrated under

vacuo. The residue obtained was purified by flash column chromatography (silica gel,  $\text{Et}_2\text{O}$ :hexanes, 2:8) to give olefin TIPS ether **91** as a white foam (19.6 g, 23.6 mmol, 93% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 4:1) that did not coalesce on heating at 60 °C. **91**:  $R_f = 0.71$  (silica gel,  $\text{EtOAc}$ :hexanes, 2:8);  $[\alpha]_D^{25} = -27.2$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (film): 2944m, 2866m 1751s, 1720s, 1173m, 1125s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz, signals for major rotamer)  $\delta = 6.81$  (d,  $J = 8.2$  Hz, 1 H), 6.36 (dd,  $J = 8.1, 6.2$  Hz, 1 H), 5.01 (dd,  $J = 5.5, 4.5$  Hz, 1 H), 4.94 (d,  $J = 3.6$  Hz, 1 H), 4.89 (d,  $J = 11.8$  Hz, 1 H), 4.77 (d,  $J = 11.9$  Hz, 1 H), 4.69 (d,  $J = 11.9$  Hz, 1 H), 4.55 (d,  $J = 11.8$  Hz, 1 H), 4.49 (dd,  $J = 9.6, 7.3$  Hz, 1 H), 3.82 (dd,  $J = 13.5, 7.3$  Hz, 1 H), 3.75 (s, 3 H), 3.51 (s, 1 H), 2.15 (dd,  $J = 13.5, 9.6$  Hz, 1 H), 1.36 (s, 9 H), 1.27 – 1.19 (m, 3 H), 1.06 (s, 18 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz, signals for both rotamers)  $\delta = 173.7, 158.3, 153.9, 152.8, 136.1, 128.8, 94.9, 94.5, 80.5, 80.2, 75.4, 75.2, 72.9, 68.0, 64.4, 59.7, 54.3, 52.1, 52.0, 35.3, 34.7, 28.1, 28.0, 18.12, 18.10, 18.09, 17.6, 12.2, 12.18, 12.11$  ppm; HRMS calcd for  $\text{C}_{30}\text{H}_{45}\text{Cl}_6\text{N}_3\text{O}_9\text{SiH}^+$  [ $M+\text{H}^+$ ] 830.1129 found 830.1125.

**Hydroxyl olefin 92.** To a stirred solution of TIPS ether olefin **91** (19.6 g, 23.6 mmol, 1.0

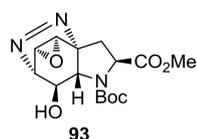
equiv) in THF (120 mL) at 0 °C was added TBAF (1 M in THF, 47.2 mL, 47.2 mmol, 2.0 equiv) dropwise. The mixture was stirred at 0 °C until completion indicated by TLC analysis. The reaction mixture was then quenched with a sat.



aq. NH<sub>4</sub>Cl solution (50 mL) and extracted with EtOAc (3 × 50 mL) and the combined organic layers were washed with brine (2 × 20 mL), dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 4:6) to give hydroxyl olefin **92** as a white foam (14.6 g, 21.7 mmol, 92% yield). The NMR spectra of this product showed signals corresponding to two carbamate rotamers (ca. 1:1) that did not coalesce on heating at 60 °C. **92**: R<sub>f</sub> = 0.42 (silica gel, EtOAc:hexanes, 1:1); [α]<sub>D</sub><sup>25</sup> = -40.9 (c = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3493w, 2979m, 2956m 1748s, 1719s, 1149m cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz, signals for major rotamer) δ = 6.88 (d, J = 8.2 Hz, 1 H), 6.41 (dd, J = 8.1, 6.2 Hz, 1 H), 5.22 (d, J = 11.9 Hz, 1 H), 5.13 – 5.04 (m, 2 H), 4.88 (d, J = 11.9 Hz, 1 H), 4.61 (d, J = 11.9 Hz, 1 H), 4.51 (dd, J = 10.1, 6.6 Hz, 1 H), 4.37 (d, J = 11.9 Hz, 1 H), 3.88 (dd, J = 13.6, 6.6 Hz, 1 H), 3.76 (s, 3 H), 3.52 (d, J = 2.6 Hz, 1 H), 2.27 (dd, J = 13.6, 10.1 Hz, 2 H), 1.37 (s, 9 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz, signals for both rotamers) δ = 173.0, 158.3, 154.7, 154.3, 137.3, 127.6, 95.0, 94.4, 81.6, 75.5, 74.9, 73.0, 67.5, 66.1, 60.2, 53.1, 52.3, 52.2, 36.4, 28.2, 28.0 ppm; HRMS calcd for C<sub>21</sub>H<sub>25</sub>Cl<sub>6</sub>N<sub>3</sub>O<sub>9</sub>H<sup>+</sup> [M+H<sup>+</sup>] 673.9795 found 673.9781.

**Diazo epoxide 93.**<sup>12a</sup> To a stirred solution of hydroxyl olefin **92** (600 mg, 0.892 mmol, 1.0

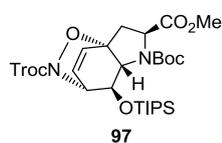
equiv) in MeCN (24 mL) at 0 °C was added Na<sub>2</sub>EDTA (4 × 10<sup>-4</sup> M aq., 3.4 mL), followed by cold (4 °C) trifluoroacetone (5 mL, 55.7 mmol, 62 equiv), NaHCO<sub>3</sub> (1.1 g, 13.1 mmol, 15 equiv) and Oxone<sup>®</sup> (5.2 g, 34.2 mmol, 38 equiv) portionwise



over a 2 min period. The resulting mixture was stirred at ambient temperature for 15 h in the absence of light. The mixture reaction was then quenched with H<sub>2</sub>O (25 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated in vacuo.

The obtained residue was dissolved in degassed MeOH (9 mL), and to the resulting solution at room temperature was added sequentially activated Zn (1.2 g, 18.7 mmol, 21 equiv) and degassed NH<sub>4</sub>Cl (1 M aq., 2.1 mL). The mixture was stirred at the same temperature in the absence of light for 2 h. The reaction mixture was filtered through a frit, and in the resulting solution at 0 °C was added NH<sub>4</sub>OH (40% aq., 1 mL), followed by CuCl<sub>2</sub> (1 M aq.) dropwise until change of color (green→blue). The reaction mixture was then quenched with sat. aq. NaHCO<sub>3</sub> solution (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated in vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:2) to give diazo epoxide **93** as a white solid (230 mg, 0.678 mmol, 76% yield). **93**: R<sub>f</sub> = 0.42 (silica gel, EtOAc:hexanes, 1:1); m.p. = 196.0 °C (CHCl<sub>3</sub>); [α]<sub>D</sub><sup>25</sup> = -20.3 (c = 1.0, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film): 3495w, 2978m, 1748s, 1690s, 1149s cm<sup>-1</sup>; <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 600 MHz) δ = 5.81 – 5.69 (m, 1 H), 4.52 (dd, J = 9.0, 7.6 Hz, 1 H), 3.81 (s, 3 H), 3.78 – 3.76 (m, 1 H), 3.74 (dd, J = 13.2, 7.6 Hz, 1 H), 3.62 – 3.59 (m, 2 H), 3.53 (br s, 1 H), 3.50 (d, J = 4.0 Hz, 1 H), 2.40 (dd, J = 13.2, 9.0 Hz, 1 H), 1.37 (s, 9 H) ppm; <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 150 MHz) δ = 172.8, 154.4, 81.8, 75.5, 70.0, 69.0, 66.4, 60.5, 52.4, 47.2, 43.3, 35.6, 28.0 (3C) ppm; HRMS calcd for C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>H<sup>+</sup> [M+H<sup>+</sup>] 340.1503 found 340.1508.

**Olefin TIPS ether 97.**<sup>12b</sup> To a stirred solution of diene TIPS ether **90** (45 mg, 0.1 mmol, 1.0

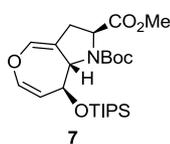


equiv) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) at 0 °C were added tetrabutyl ammonium iodide (36.9 mg, 0.1 mmol, 1.0 equiv), a solution of NaIO<sub>4</sub> (17.1 mg, 0.1 mmol, 1.0 equiv) in H<sub>2</sub>O (0.5 mL) in one portion, followed by TrocNHOH (52 mg, 0.25 mmol, 2.5

equiv) portionwise over 5 min. The resulting mixture was stirred for another 10 min and then quenched with sat. aq. NaS<sub>2</sub>O<sub>3</sub> (4 mL) solution, washed with a sat. aq. NaHCO<sub>3</sub> solution (4 mL) and brine (4 mL). The aqueous phases were back-extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the combined organic layers were dried over MgSO<sub>4</sub> and concentrated under vacuo. The residue obtained was purified by flash column chromatography (silica gel, EtOAc:hexanes, 1:6→1:4) to give olefin TIPS ether **97** as a white foam (57.9 mg, 0.088 mmol, 88% yield). **97**: R<sub>f</sub> = 0.44 (silica gel,

EtOAc:hexanes, 2:8);  $[\alpha]_D^{25} = -14.6$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (film): 2945m, 2867m, 1753s, 1713s, 1130s  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz)  $\delta = 6.52$  (d,  $J = 5.4$  Hz, 1 H), 6.52 (d,  $J = 5.4$  Hz, 1 H), 4.87 (s, 1 H), 4.80 (d,  $J = 11.9$  Hz, 1 H), 4.71 (d,  $J = 11.8$  Hz, 1 H), 4.66 (s, 1 H), 4.56 (t,  $J = 8.3$  Hz, 1 H), 3.73 (s, 3 H), 3.44 (s, 1 H), 2.67 (br s, 1 H), 2.10 (dd,  $J = 13.9, 8.8$  Hz, 1 H), 1.37 (s, 9 H), 1.29 – 1.17 (m, 3 H), 1.06 (s, 9 H), 1.05 (s, 9 H) ppm;  $^{13}\text{C}$  NMR: ( $\text{CDCl}_3$ , 150 MHz)  $\delta = 173.7, 155.2, 152.5, 131.2, 129.9, 95.0, 84.0, 80.0, 75.1, 71.6, 66.5, 59.7, 55.9, 52.0, 34.0, 28.0$  (3C), 18.2 (6C), 12.2 (3C) ppm; HRMS calcd for  $\text{C}_{27}\text{H}_{43}\text{Cl}_3\text{N}_2\text{O}_8\text{SiH}^+$  [ $M+\text{H}^+$ ] 657.1927 found 657.1933.

**Oxepine 7.** To a stirred solution of olefin TIPS ether **97** (10 mg, 0.015 mmol, 1.0 equiv) in



MeCN (1 mL) at ambient temperature was added  $\text{Na}_2\text{EDTA}$  ( $4 \times 10^{-4}$  M aq., 1 mL).

The resulting solution was cooled to 0 °C and cold (4 °C) trifluoroacetone (0.1 mL,

1.05 mmol, 7.0 equiv) was added, followed by  $\text{NaHCO}_3$  (73 mg, 0.9 mmol, 60

equiv) in one portion and Oxone<sup>®</sup> (200 mg, 0.3 mmol, 20 equiv) portionwise over 5 h. The mixture was stirred at 0 °C for 15 h, then poured into  $\text{H}_2\text{O}$  (4 mL), and extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 4$  mL).

The combined organic layers were dried over  $\text{MgSO}_4$  and concentrated in vacuo. The residue

obtained was purified by PTLC (silica gel pretreated with  $\text{Et}_3\text{N}$ , EtOAc:hexanes, 1:8) to give

oxepine **7** as a yellow oil (2.8 mg, 0.006 mmol, 40% yield). The NMR spectra of this product

showed signals corresponding to carbamate rotamers (ca. 1:1) that did not coalesce on heating at 60

°C. **7**:  $R_f = 0.47$  (silica gel, EtOAc:hexanes, 2:8);  $[\alpha]_D^{25} = -4.0$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (film):

2953w, 2923m, 1773w, 1726s, 1686m, 1409m  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 600 MHz, signals for major

rotamer)  $\delta = 6.47$  (d,  $J = 2.2$  Hz, 1 H), 5.97 (d,  $J = 5.9$  Hz, 1 H), 4.58 (s, 1 H), 4.33 (t,  $J = 7.8$  Hz, 1

H), 4.22 – 4.10 (m, 2 H), 3.80 (s, 3 H), 2.96 (dd,  $J = 15.2, 8.6$  Hz, 1 H), 2.67 (dd,  $J = 14.9, 7.3$  Hz, 1

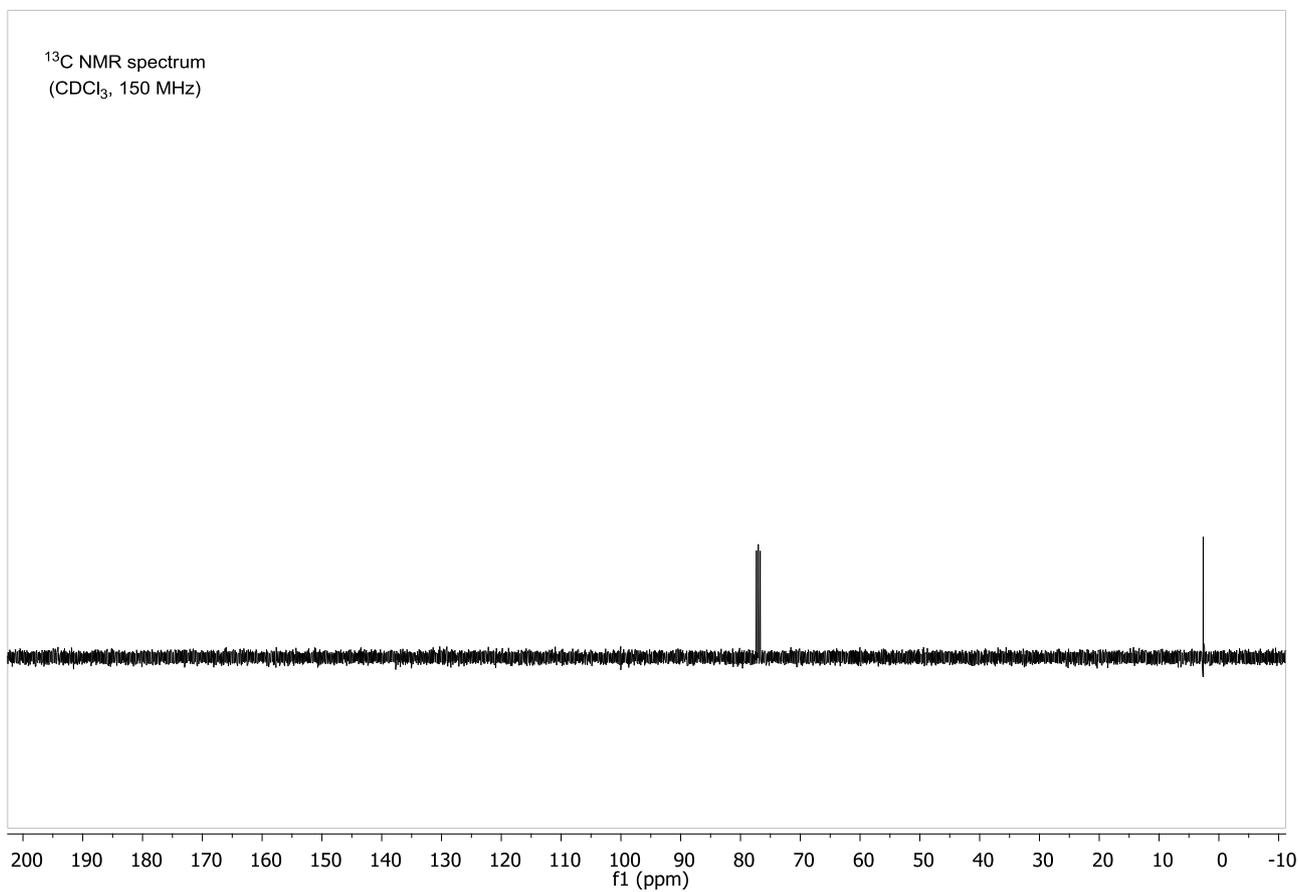
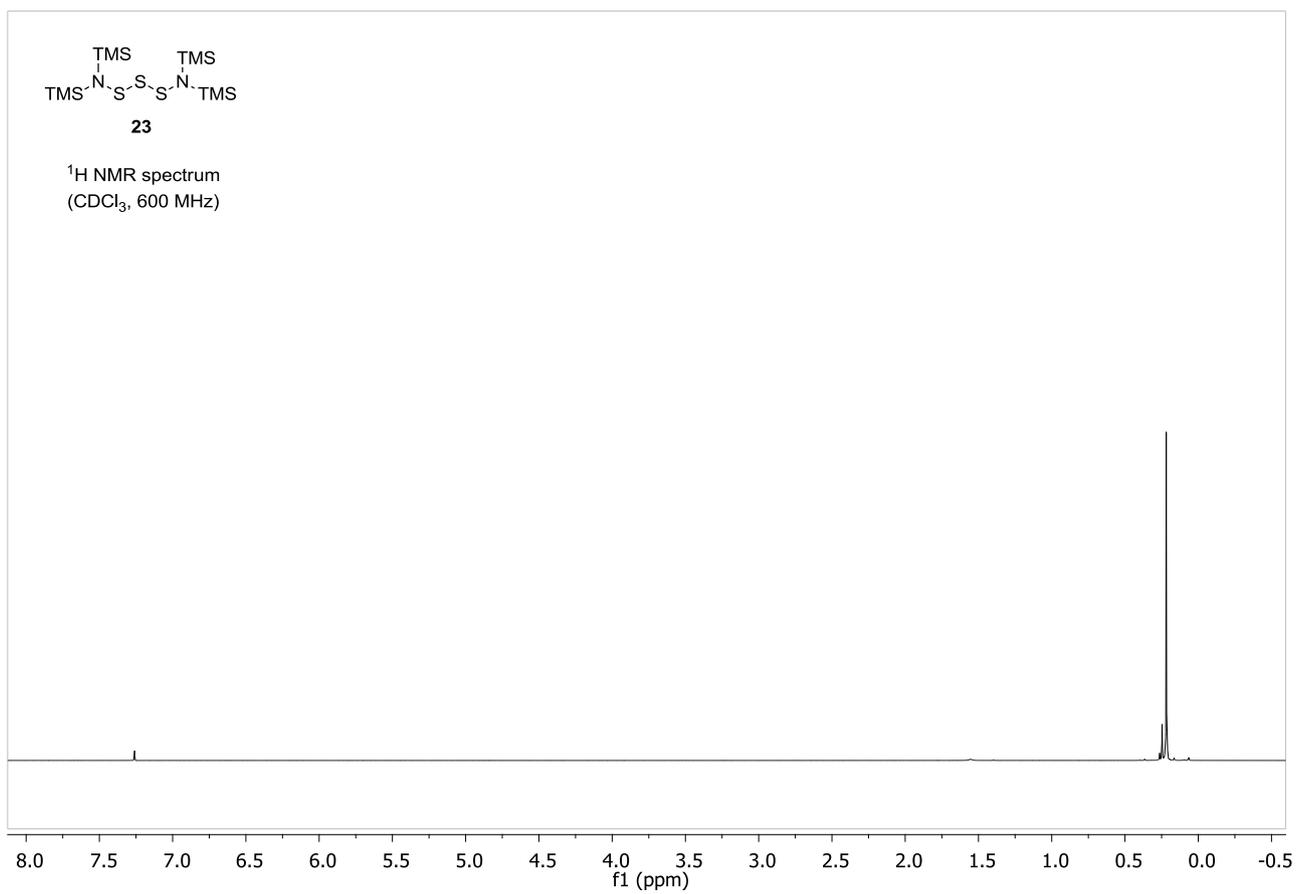
H), 1.44 (s, 9 H), 1.30 – 1.27 (m, 3 H), 1.10 (d,  $J = 7.4$  Hz, 9 H), 1.06 (d,  $J = 7.4$  Hz, 9 H) ppm;  $^{13}\text{C}$

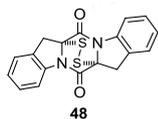
NMR: ( $\text{CDCl}_3$ , 150 MHz, signals for major rotamer)  $\delta = 173.2, 154.8, 149.1, 144.7, 126.1, 114.1,$

80.7, 75.2, 63.3, 61.7, 52.2, 33.5, 20.1 (3C), 18.4 (3C), 12.9 (6C) ppm; HRMS calcd for

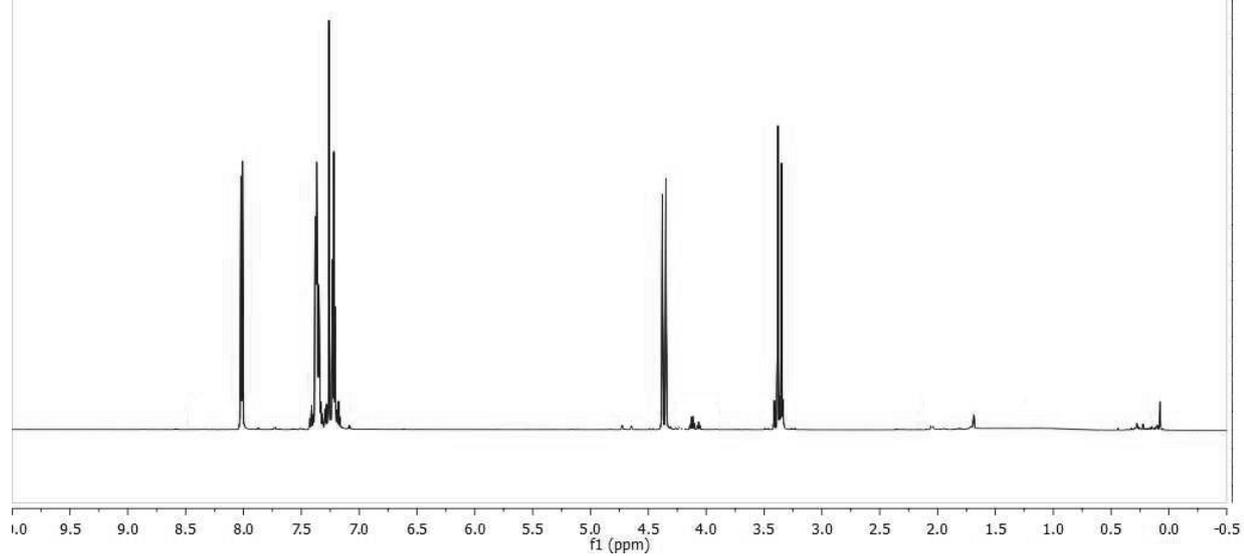
$\text{C}_{24}\text{H}_{41}\text{NO}_6\text{SiH}^+$  [ $M+\text{H}^+$ ] 468.2776 found 468.2794.

## II. $^1\text{H}$ and $^{13}\text{C}$ NMR Spectra of Compounds

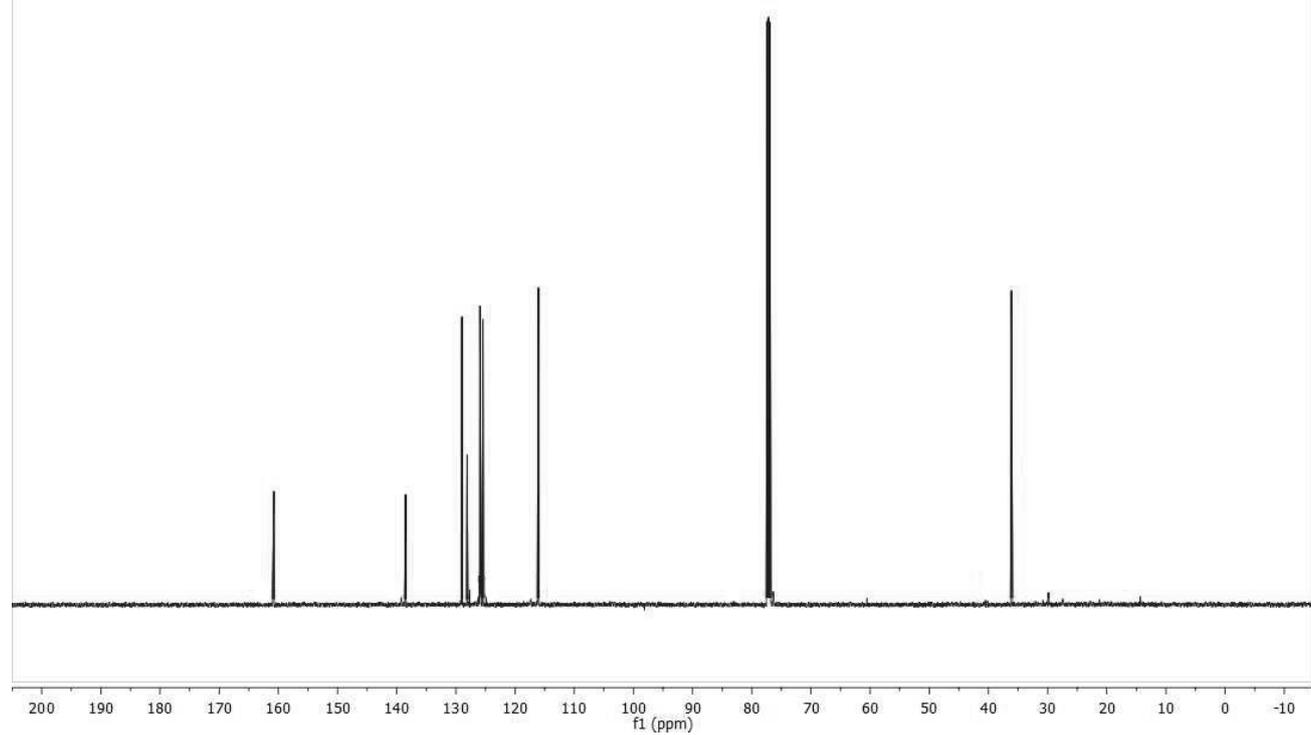


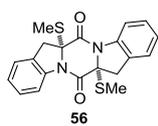


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

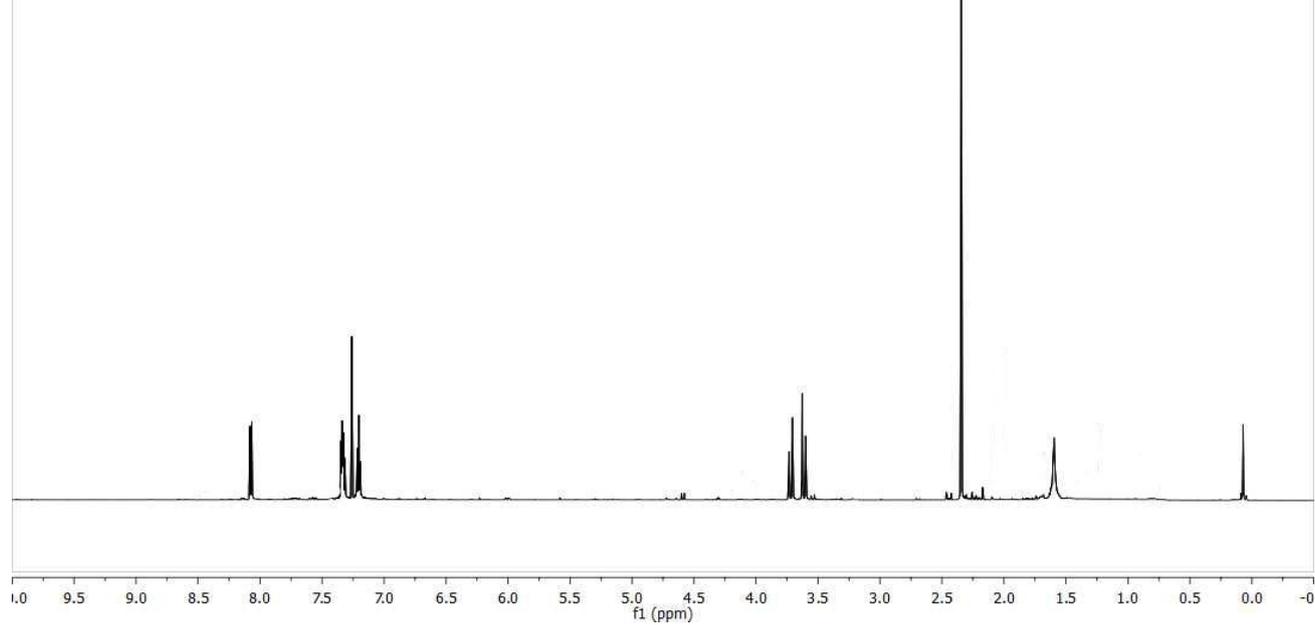


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

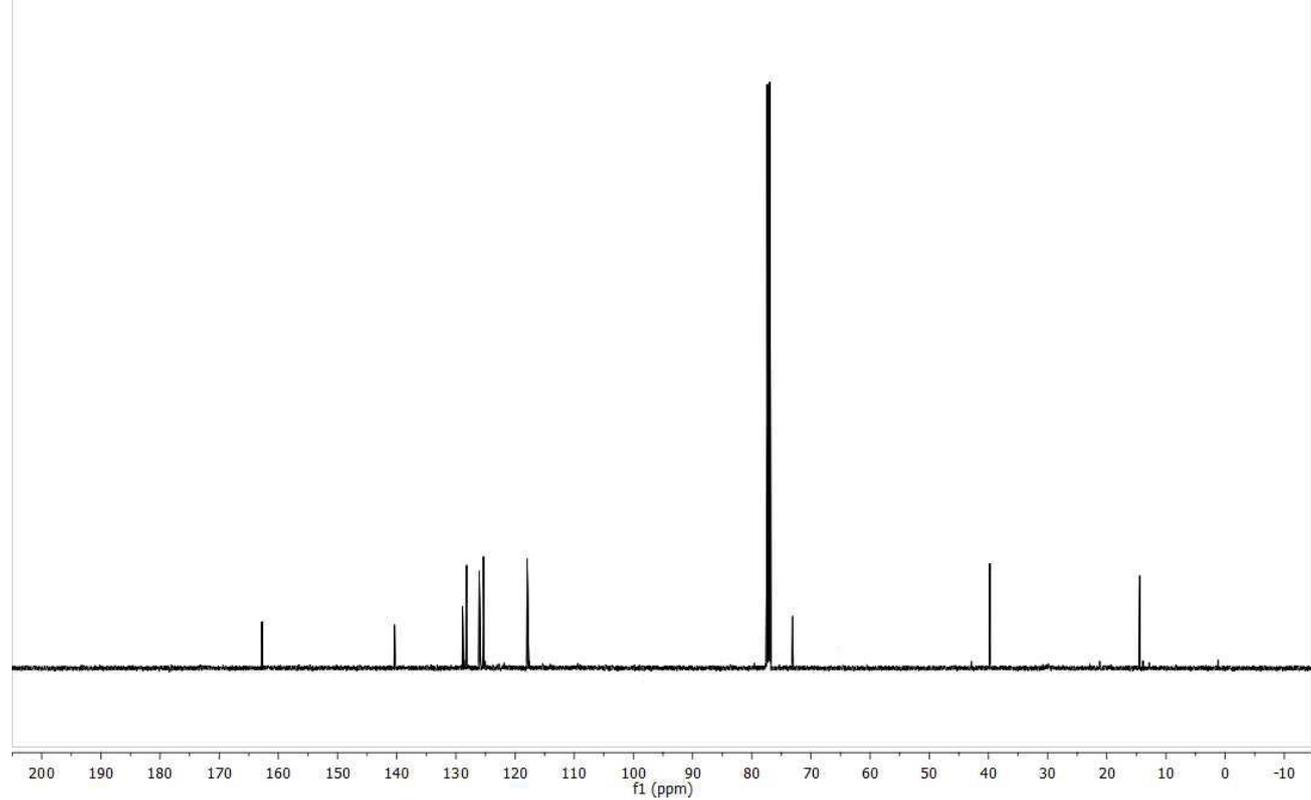




<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

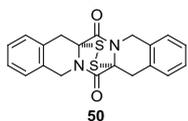


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

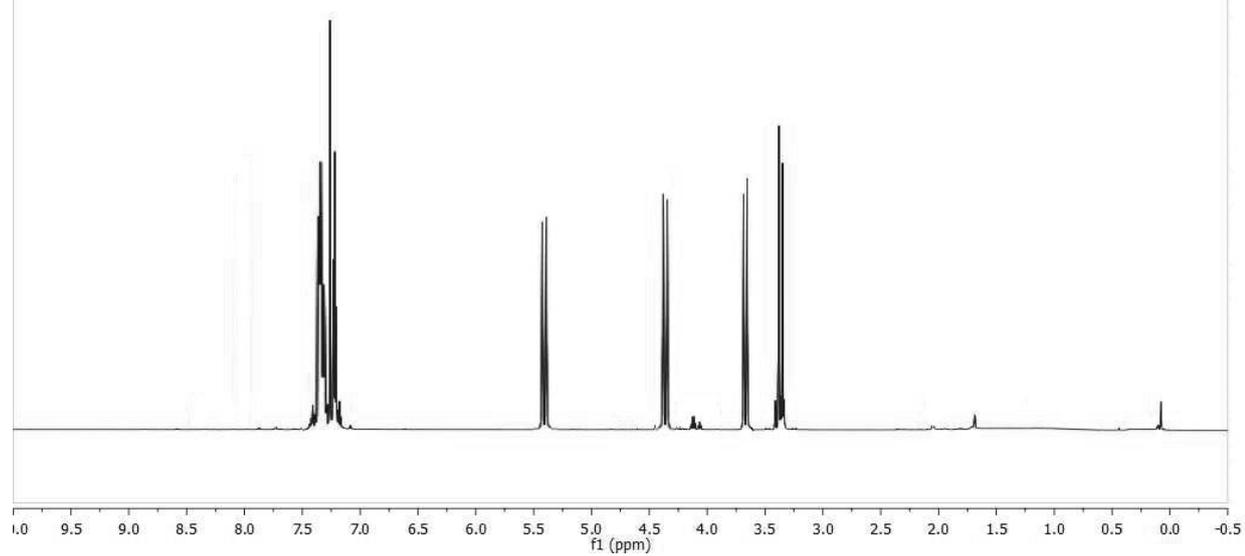




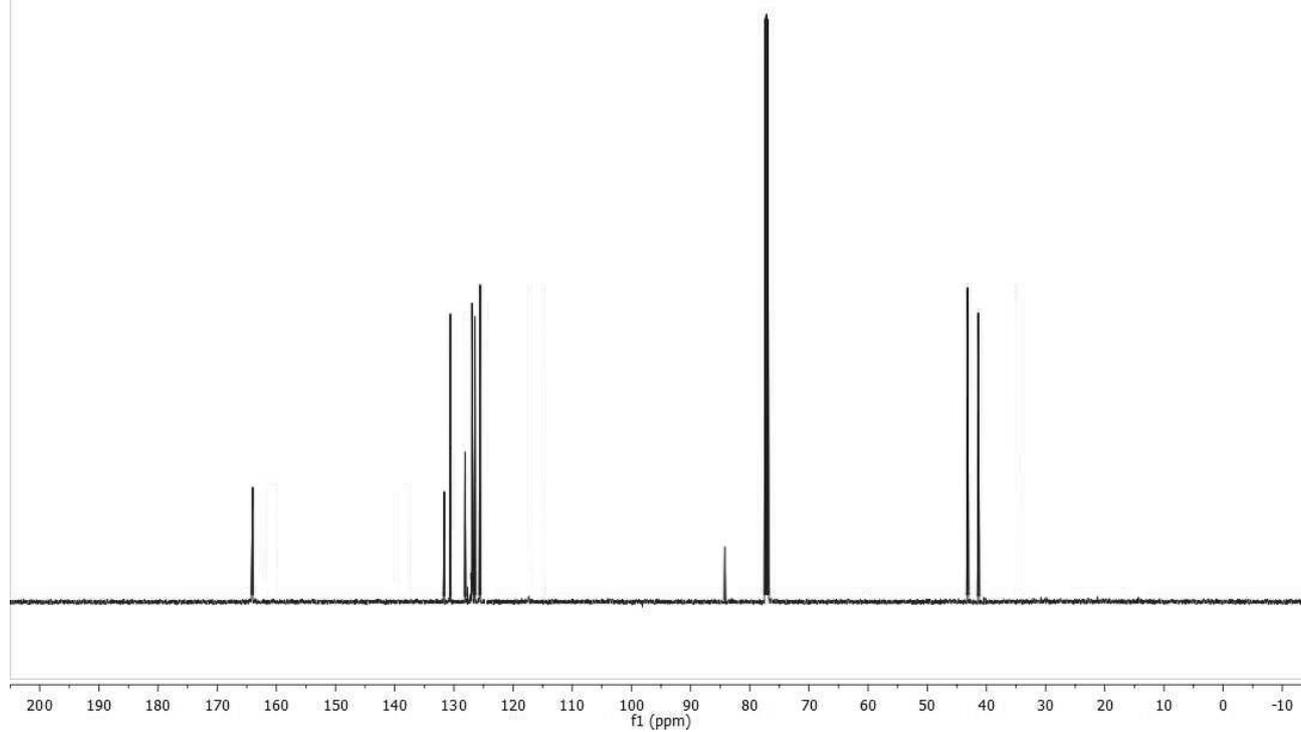


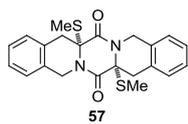


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

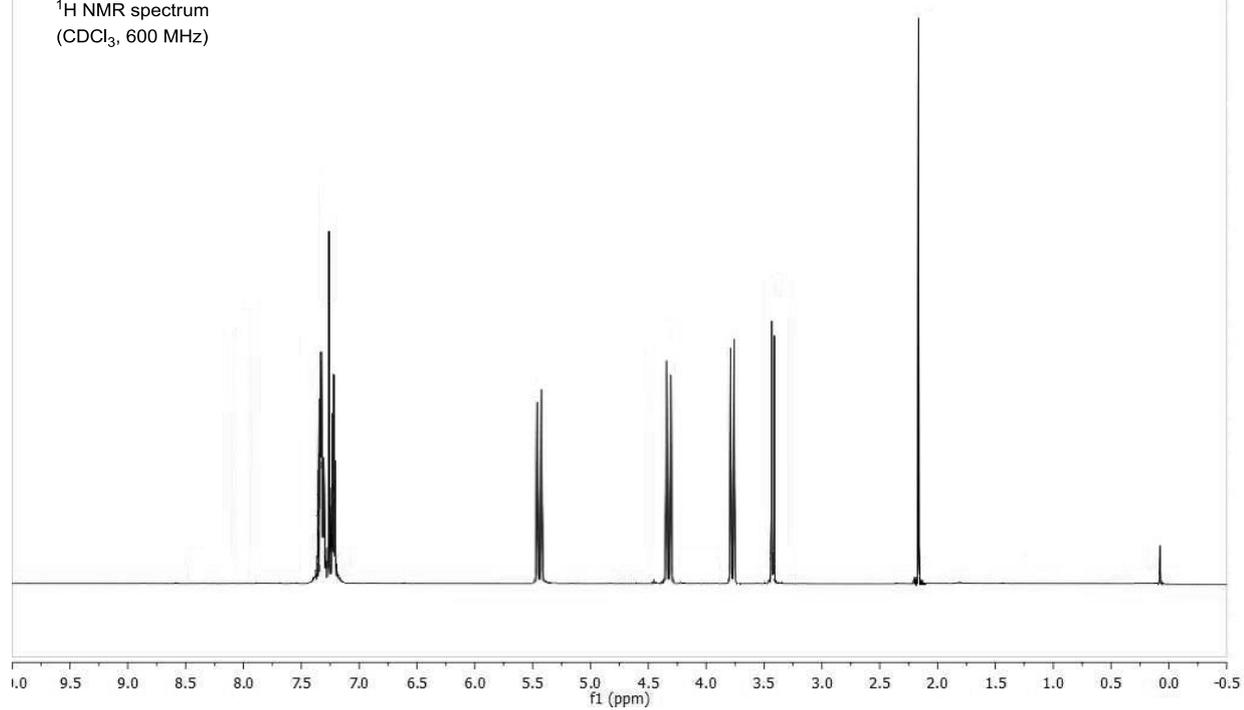


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

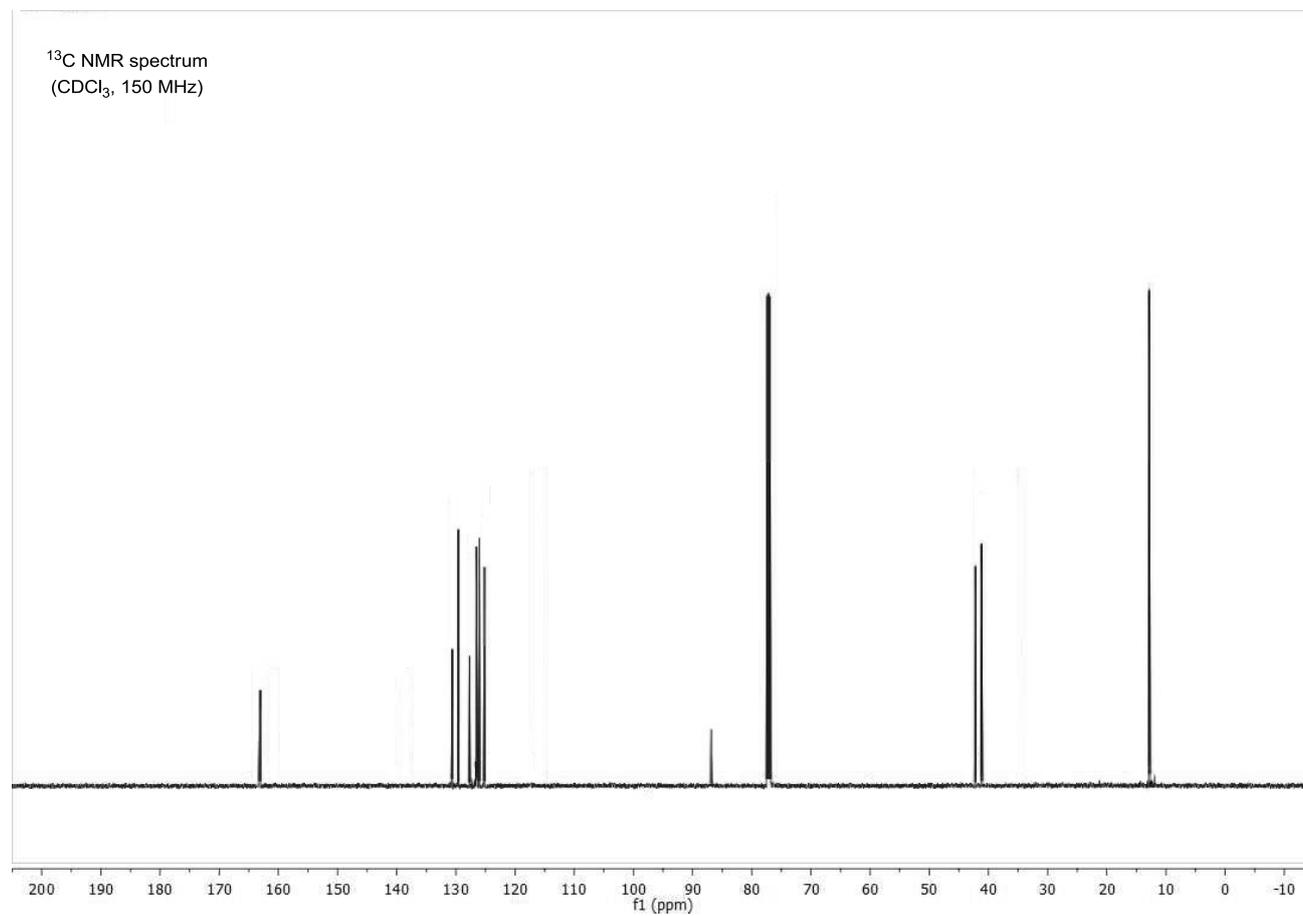


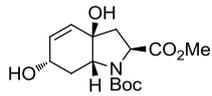


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

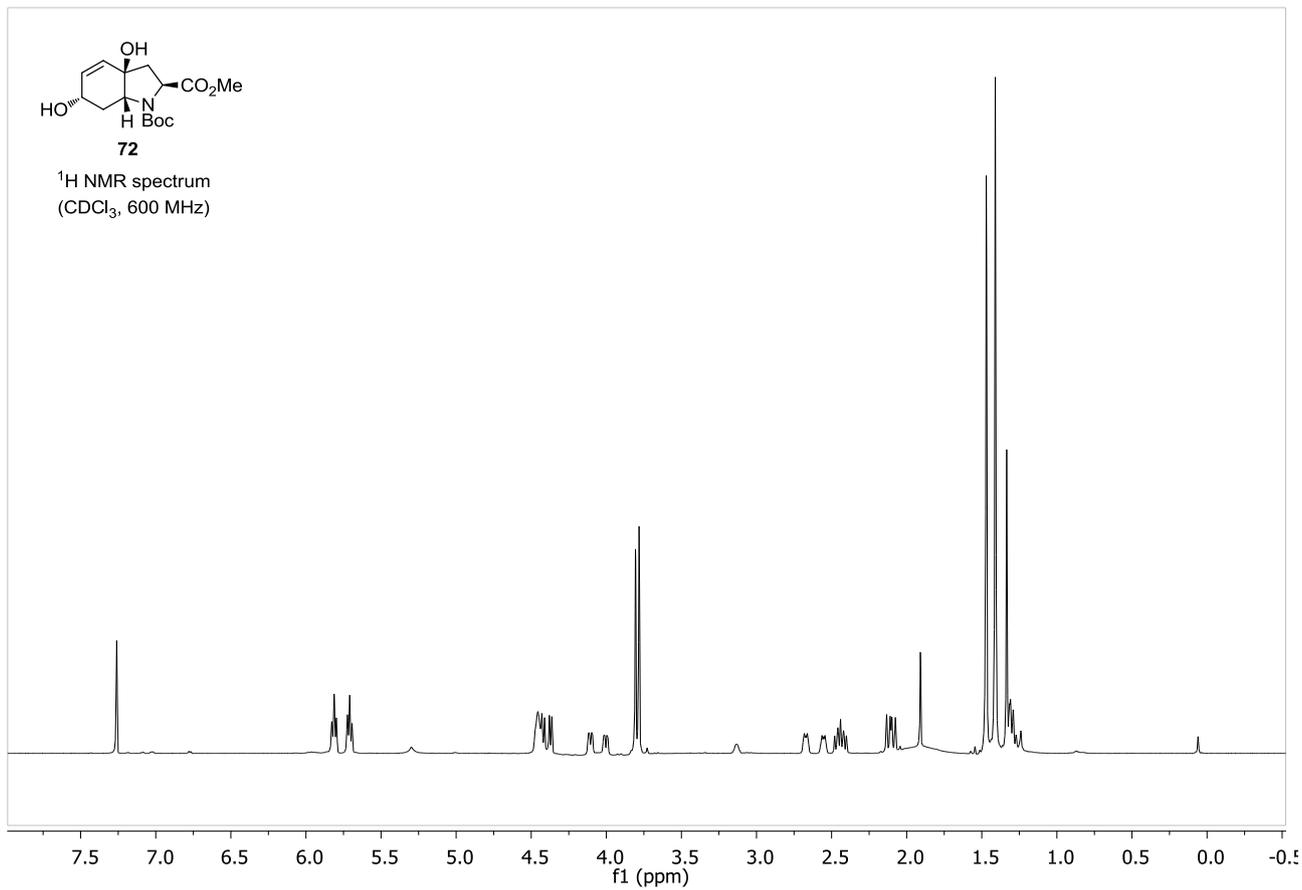


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

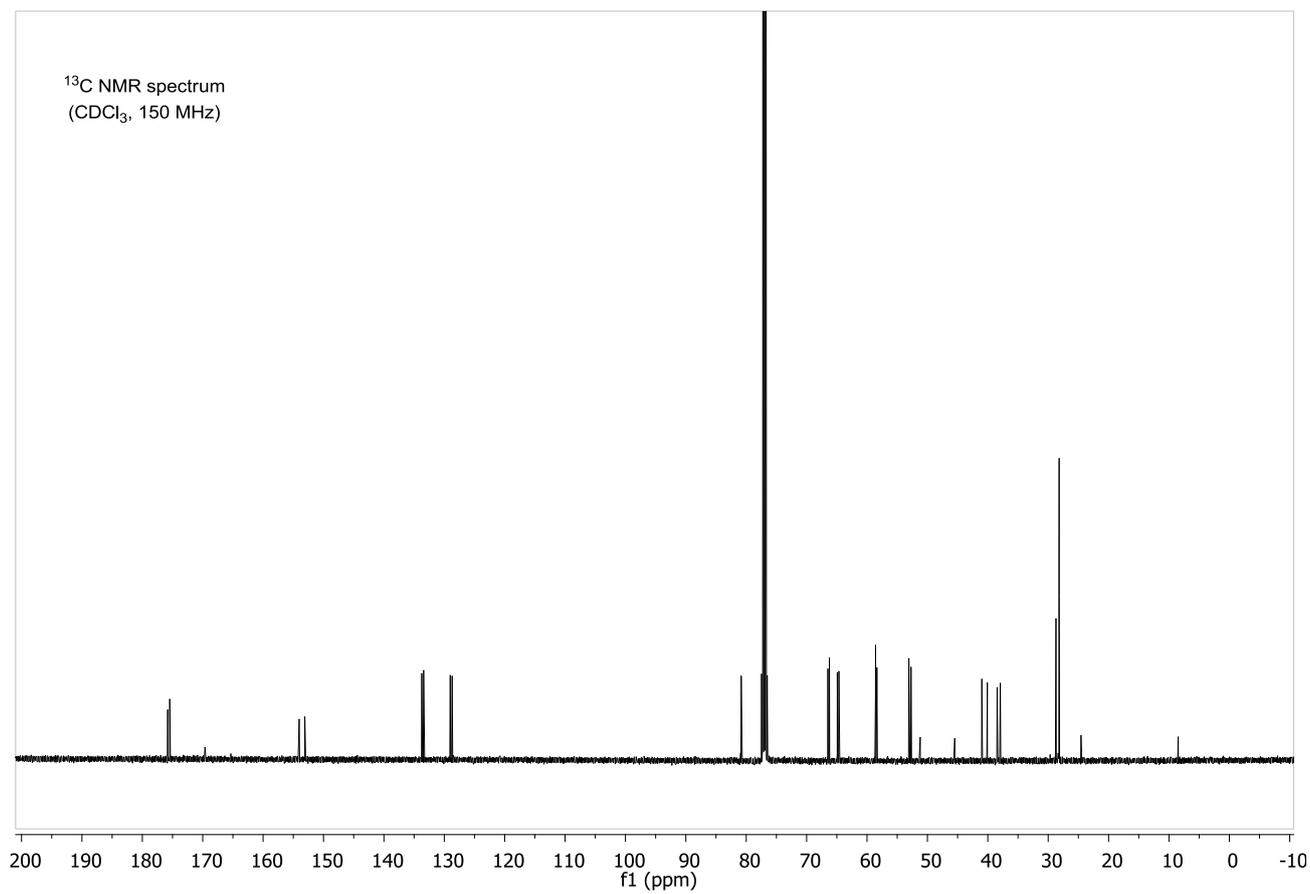


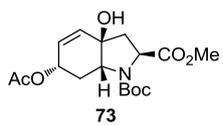


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)



<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)



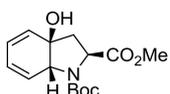


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5  
f1 (ppm)

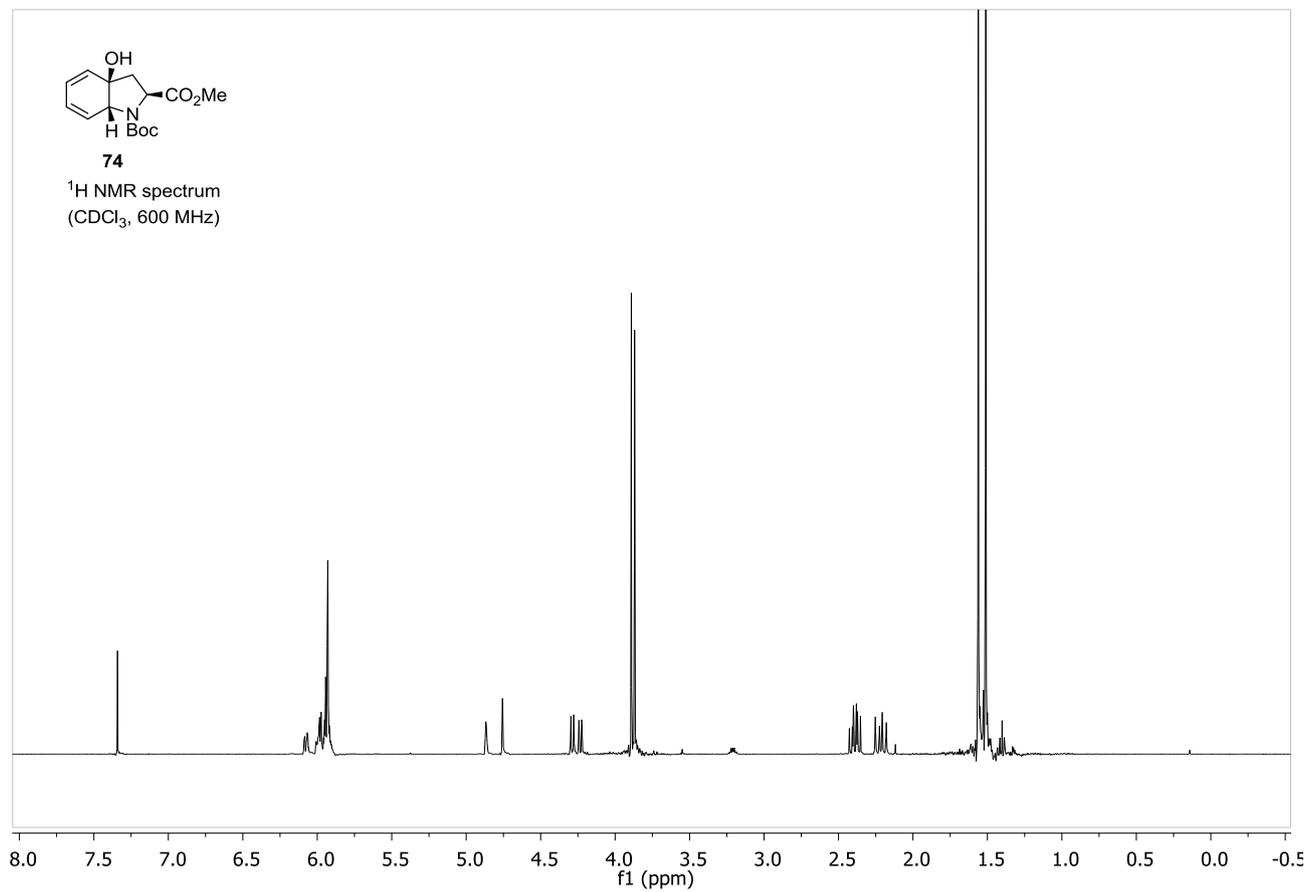
<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10  
f1 (ppm)

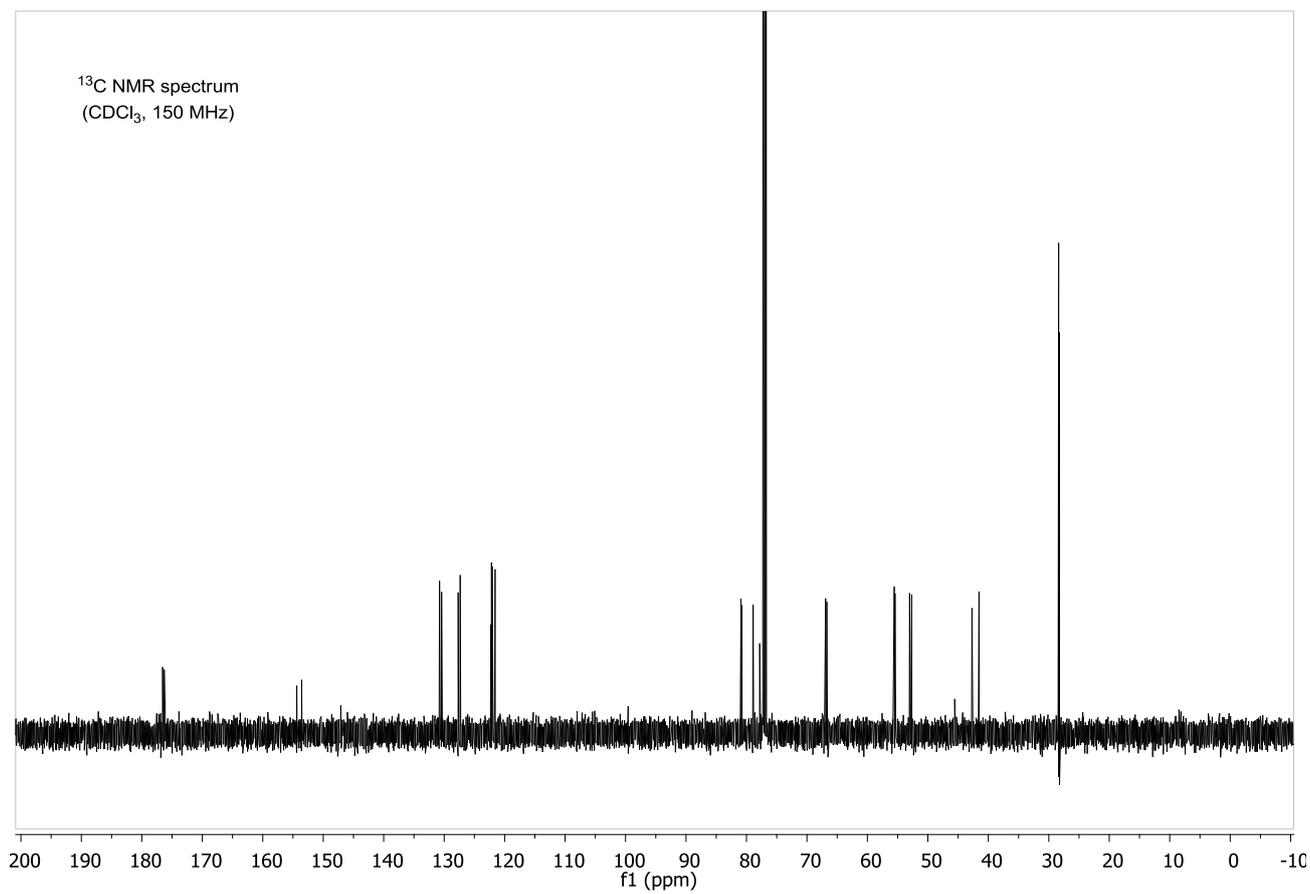


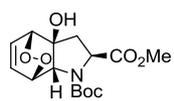
74

<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)



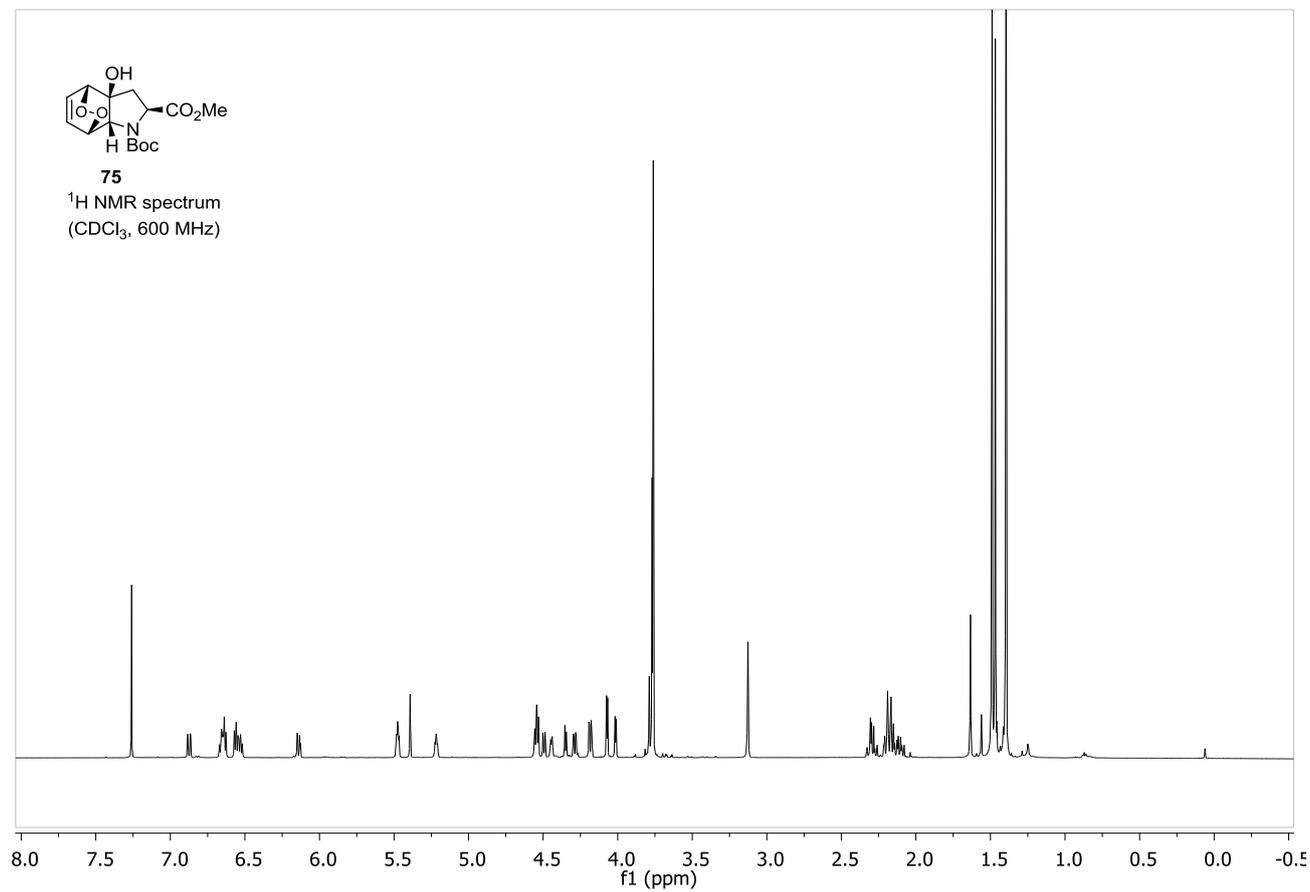
<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)



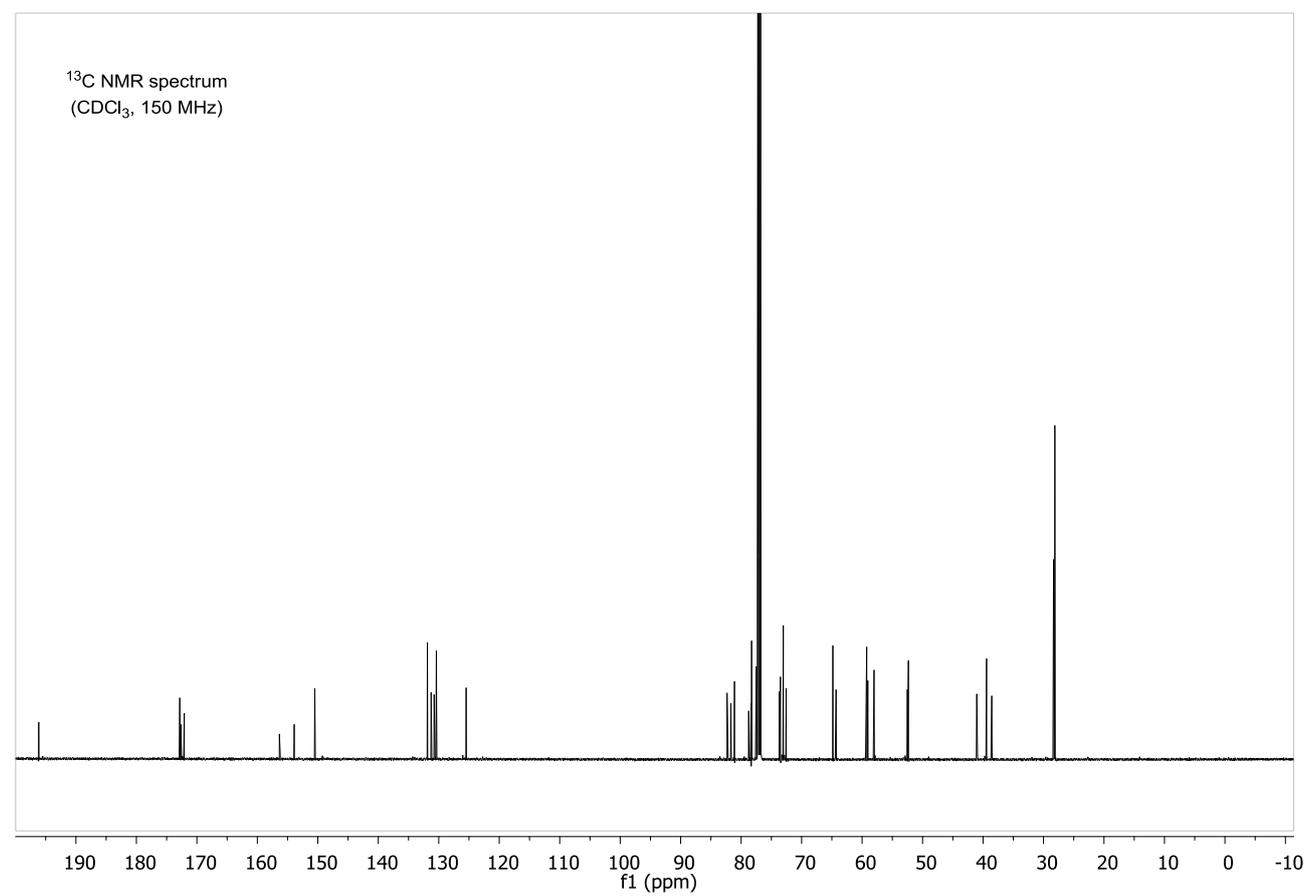


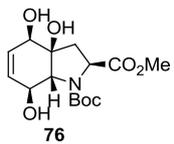
75

<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

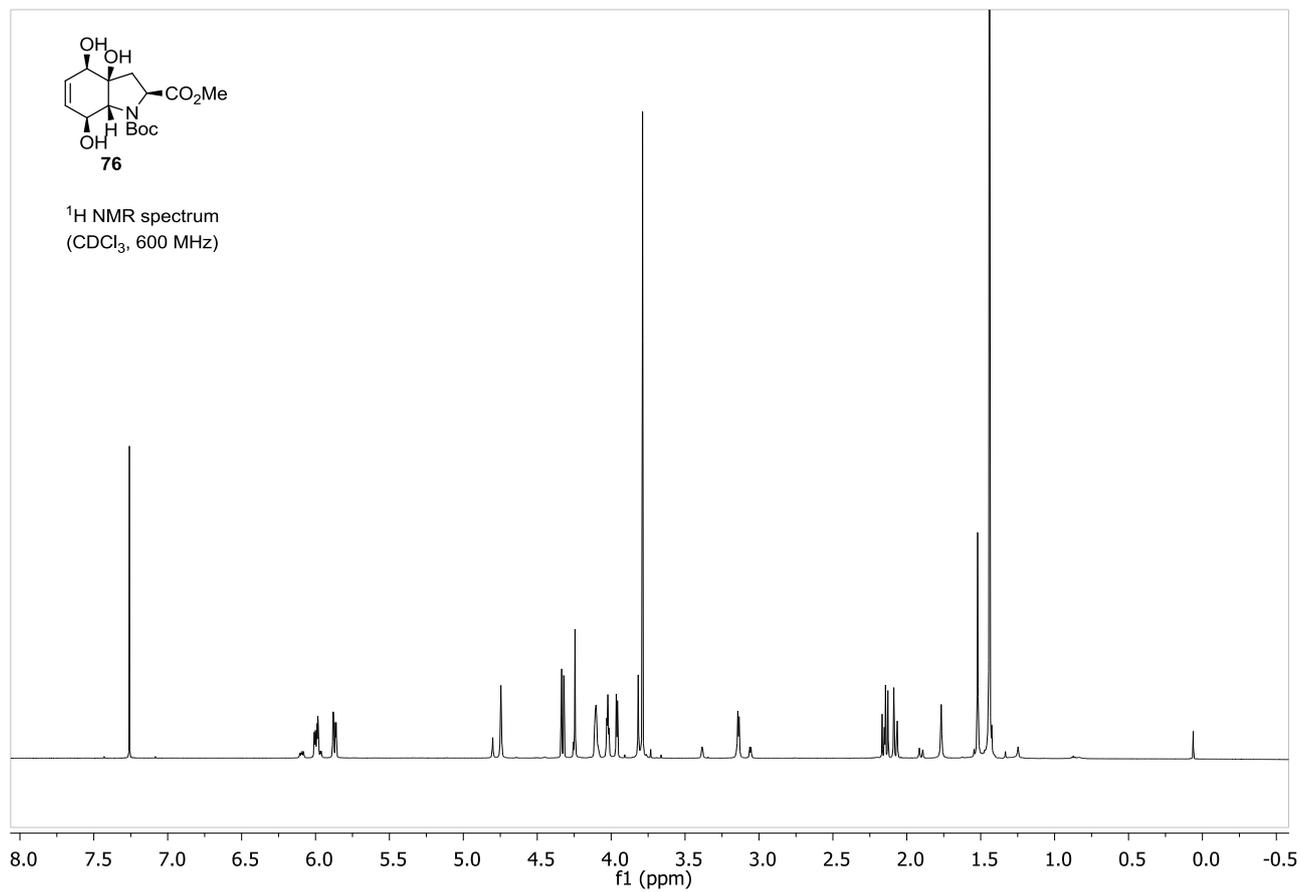


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

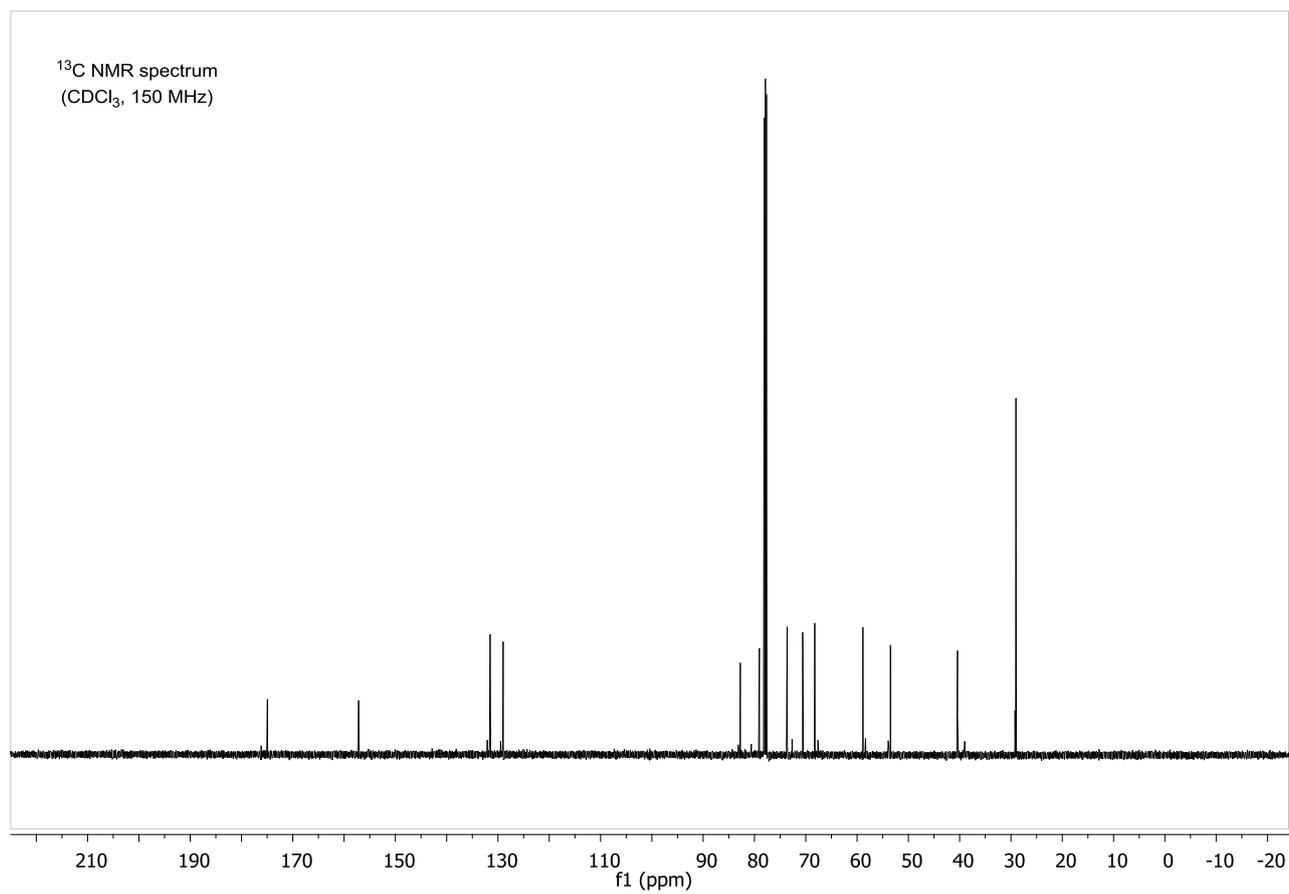


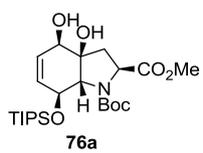


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

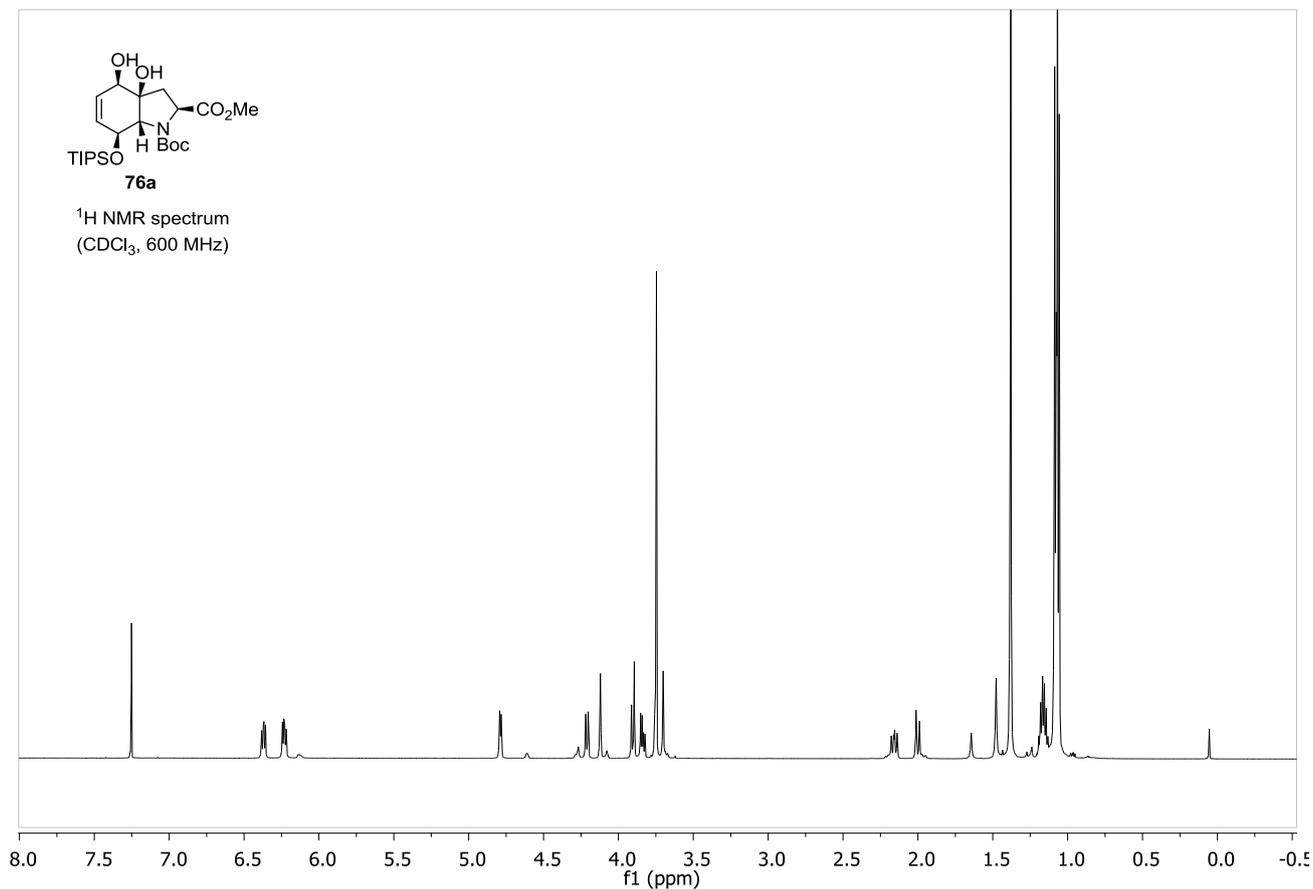


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

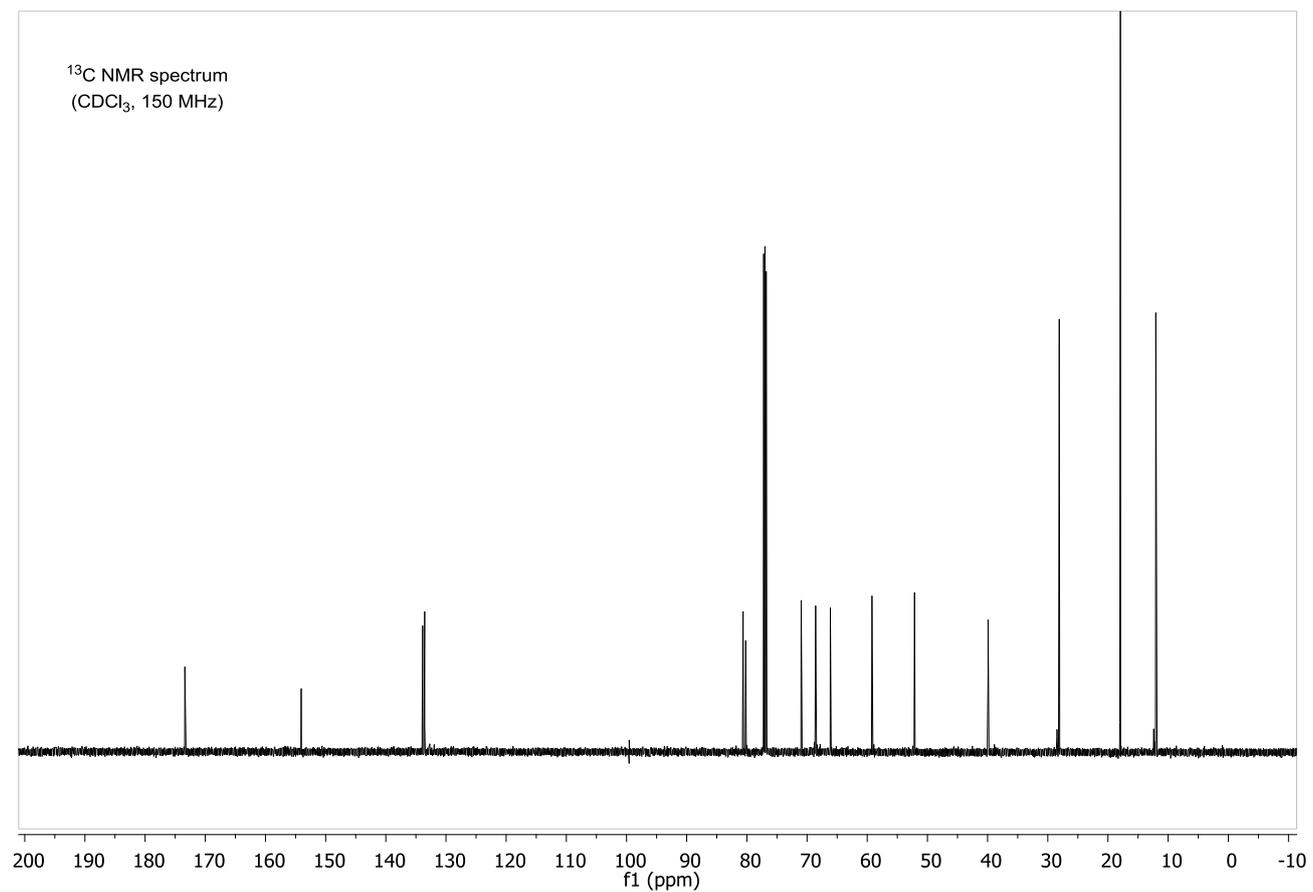


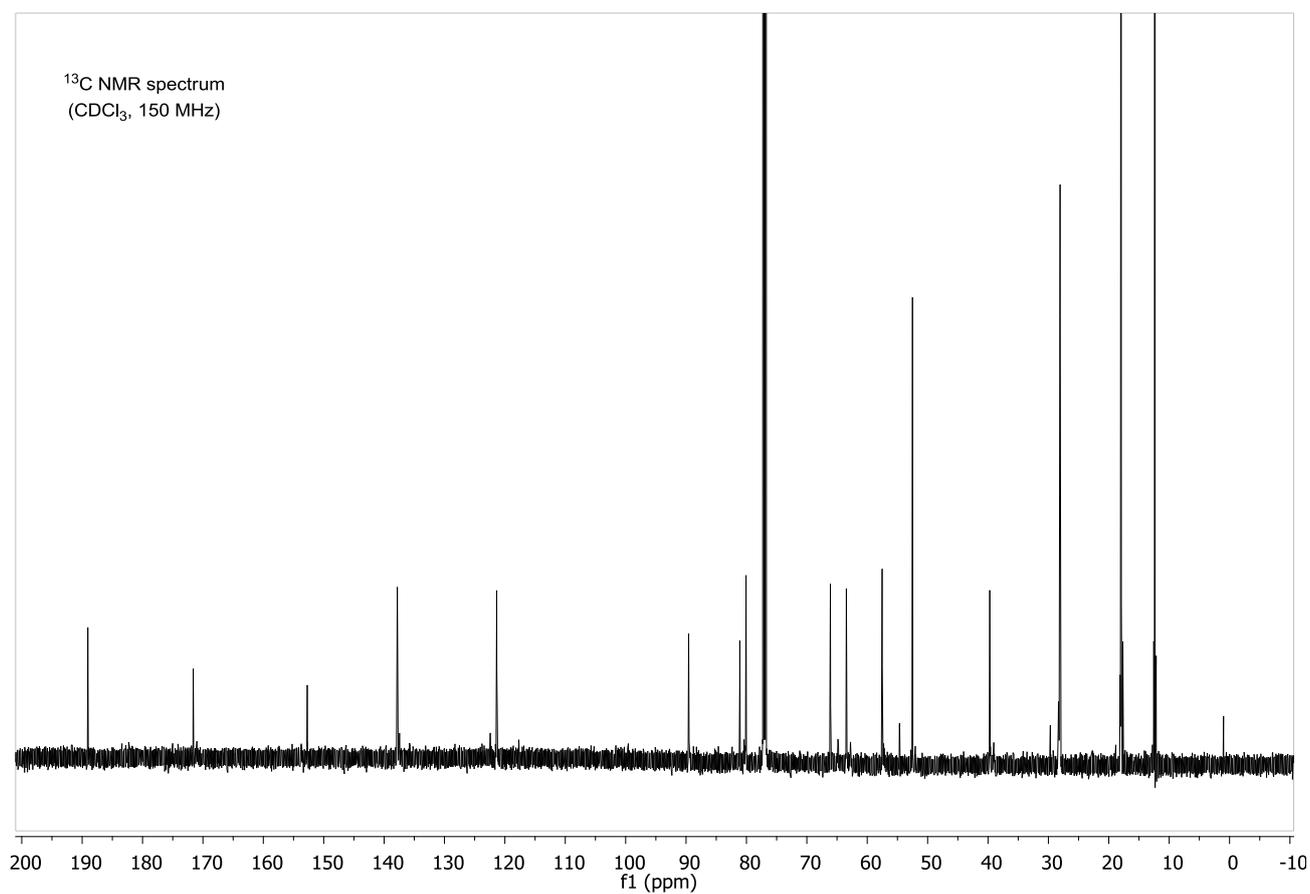
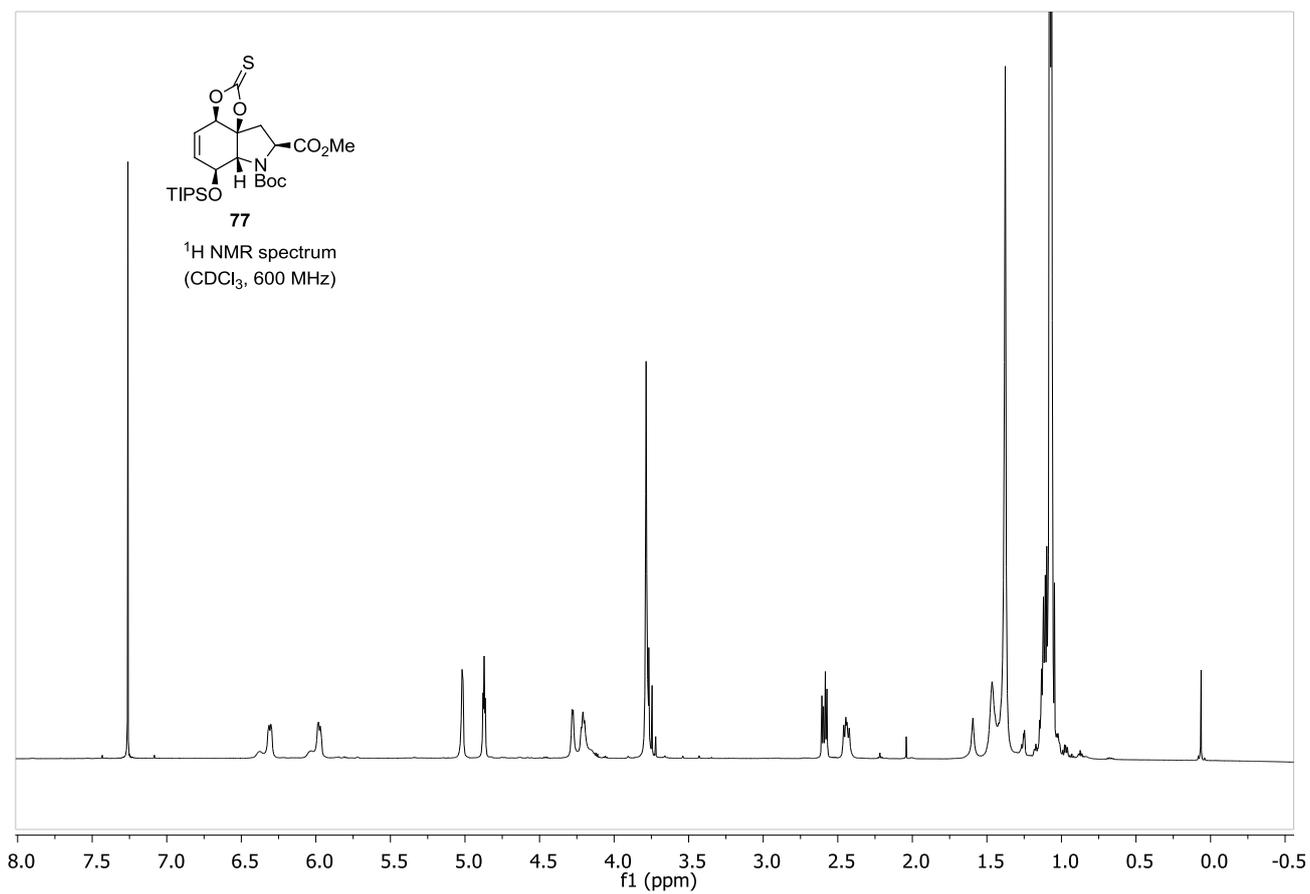


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

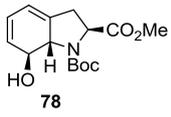


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

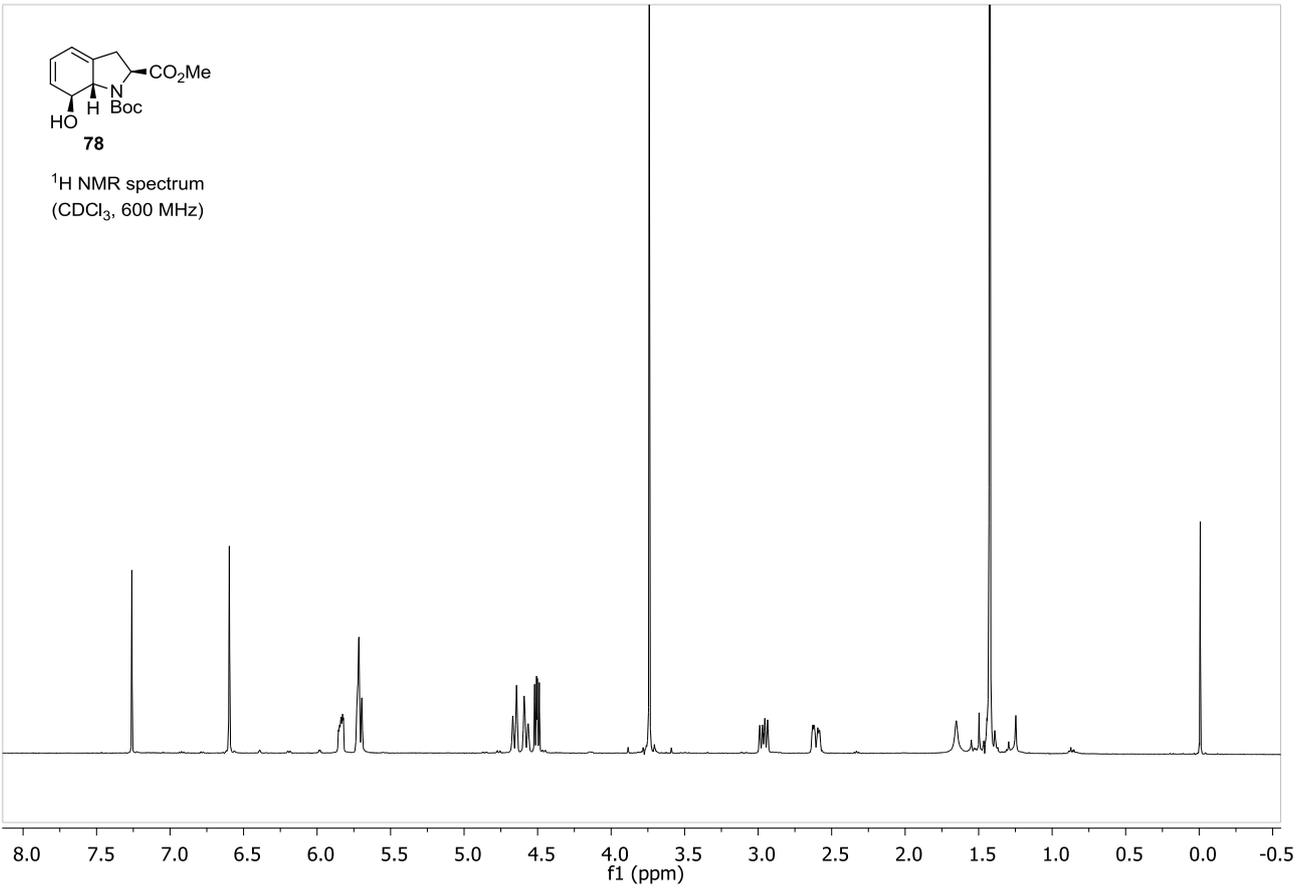




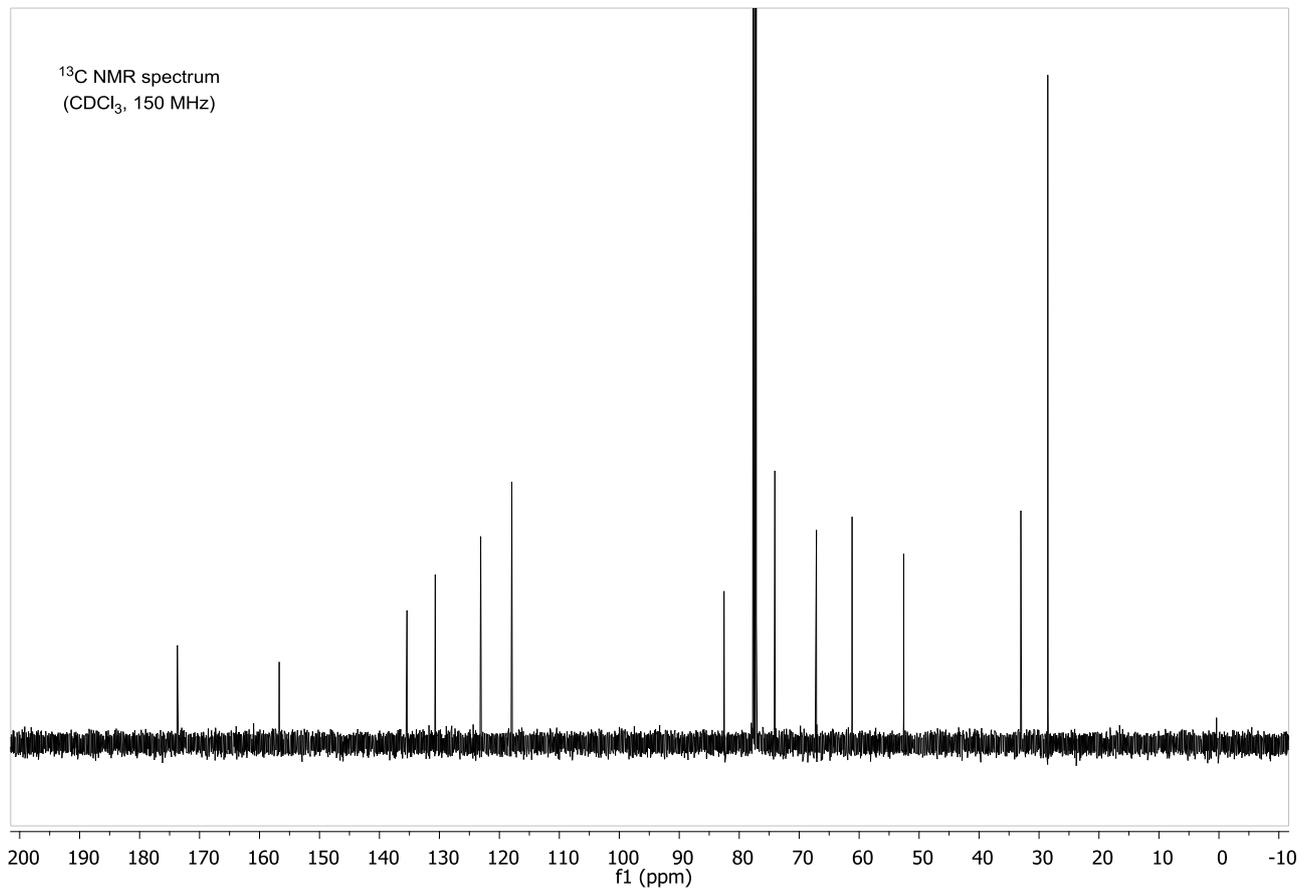




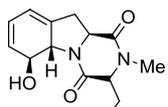
<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)



<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

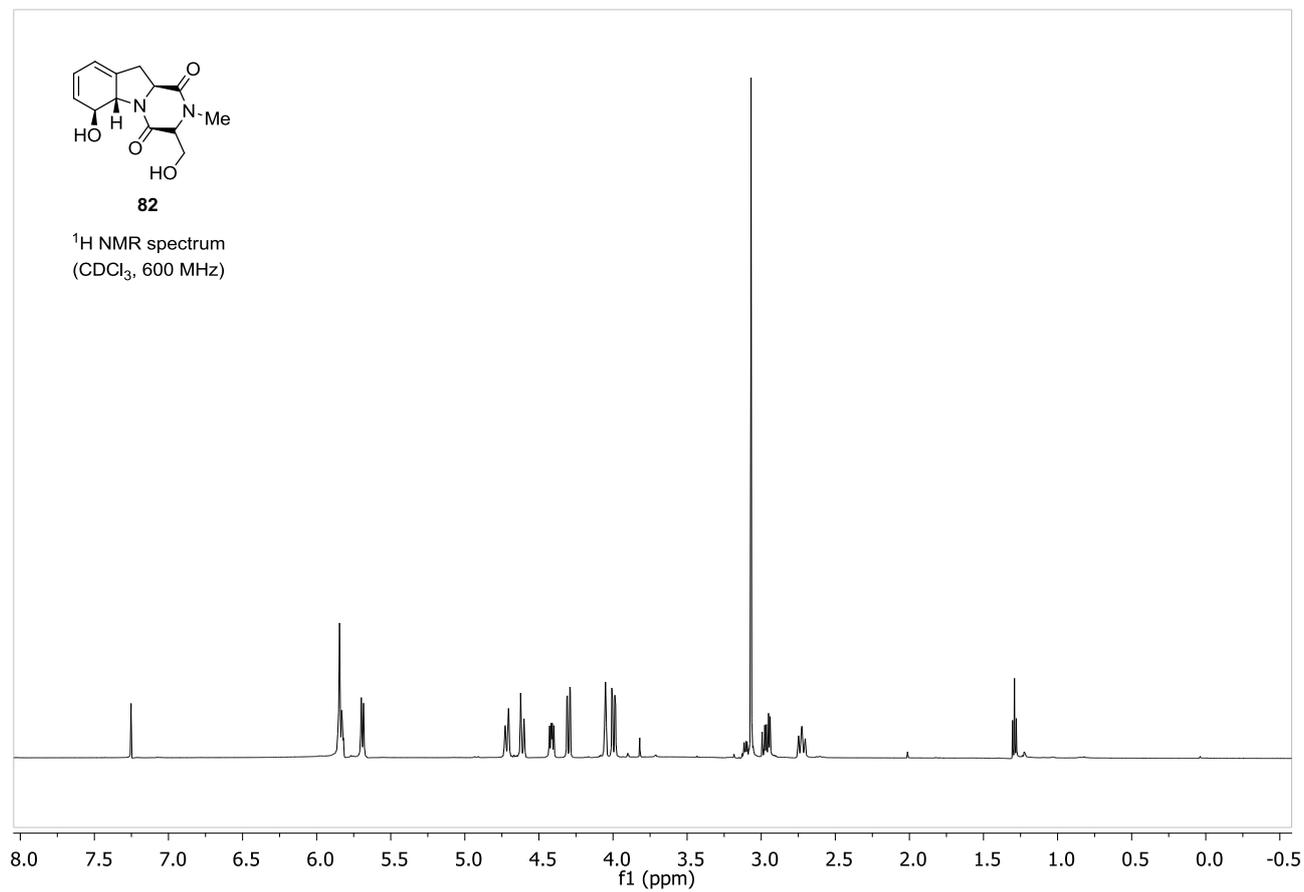




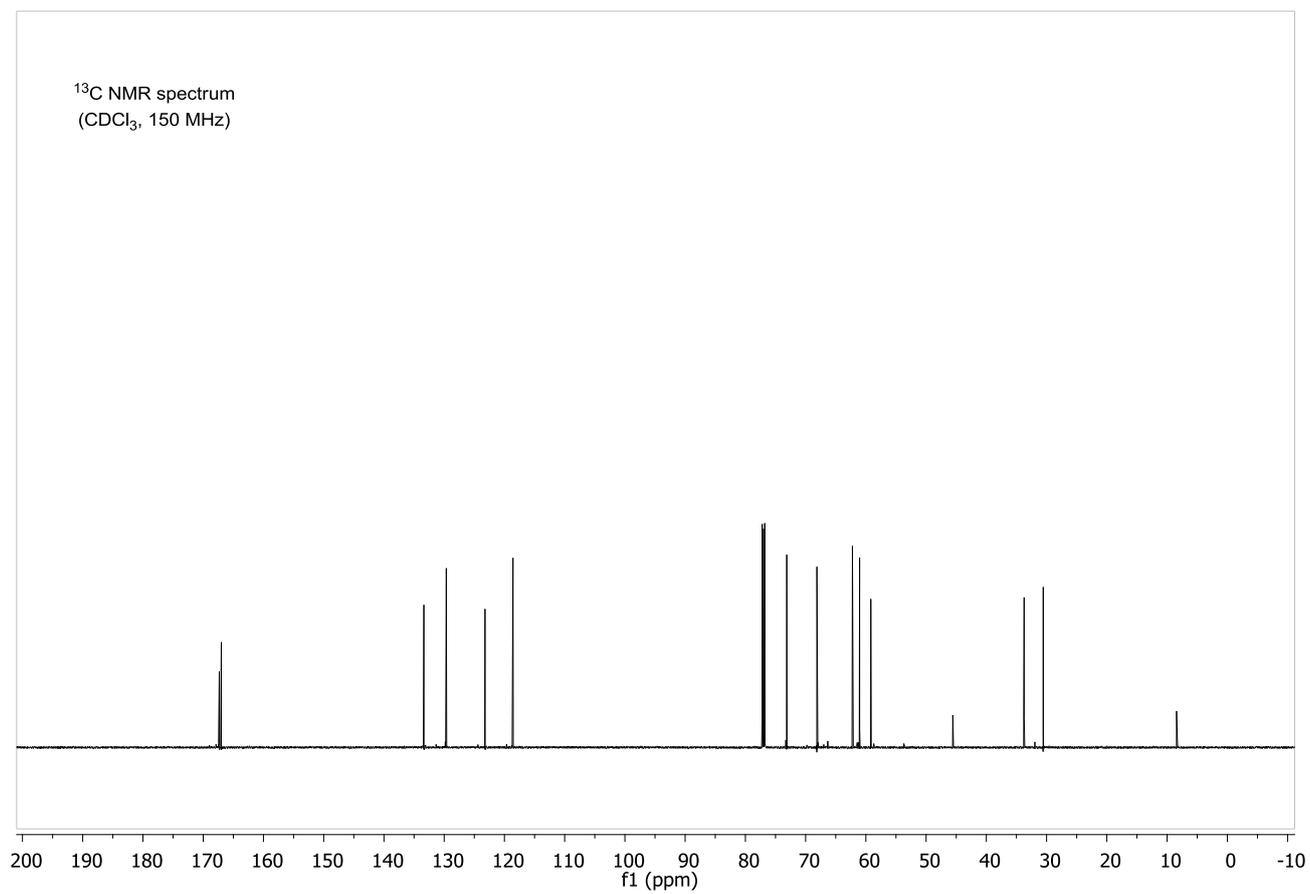


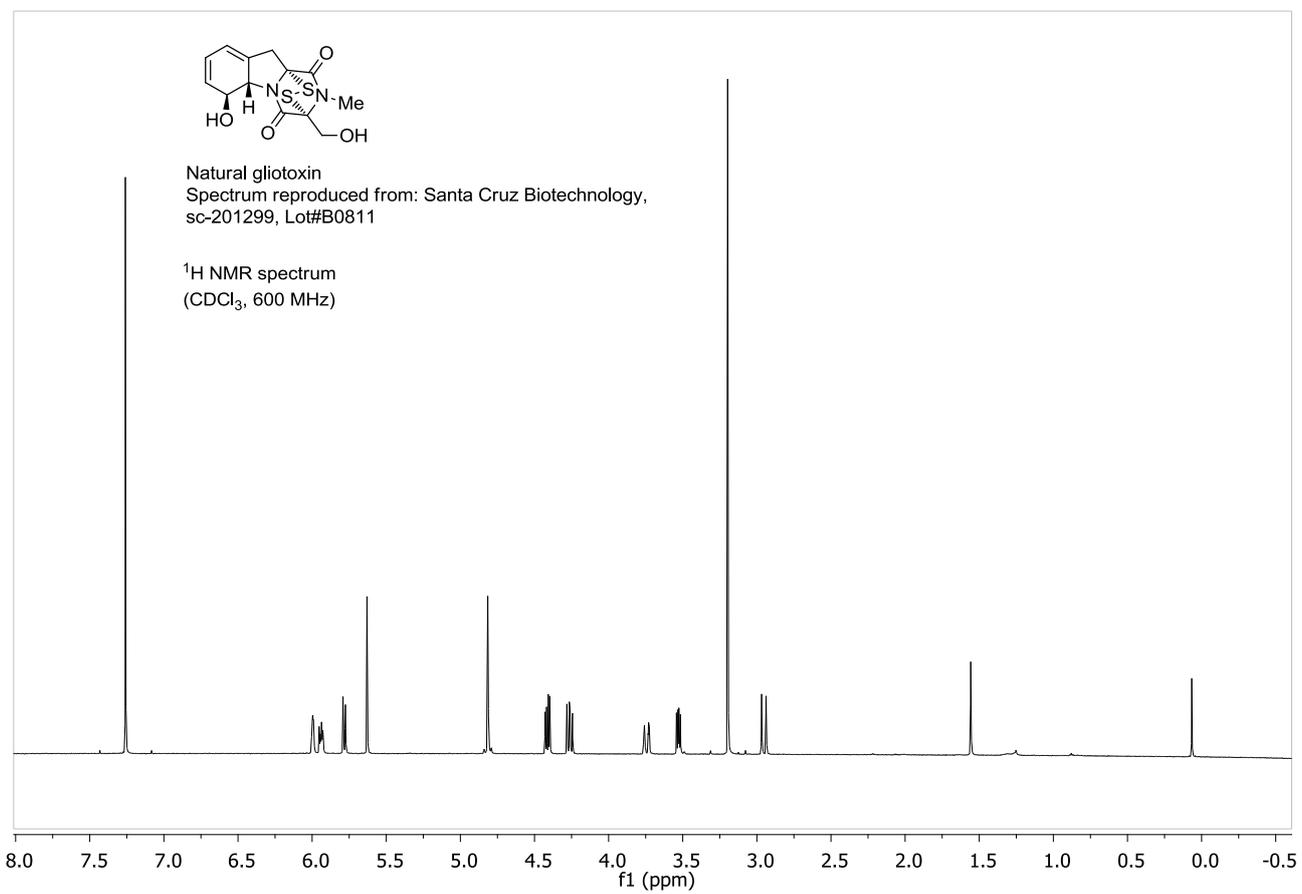
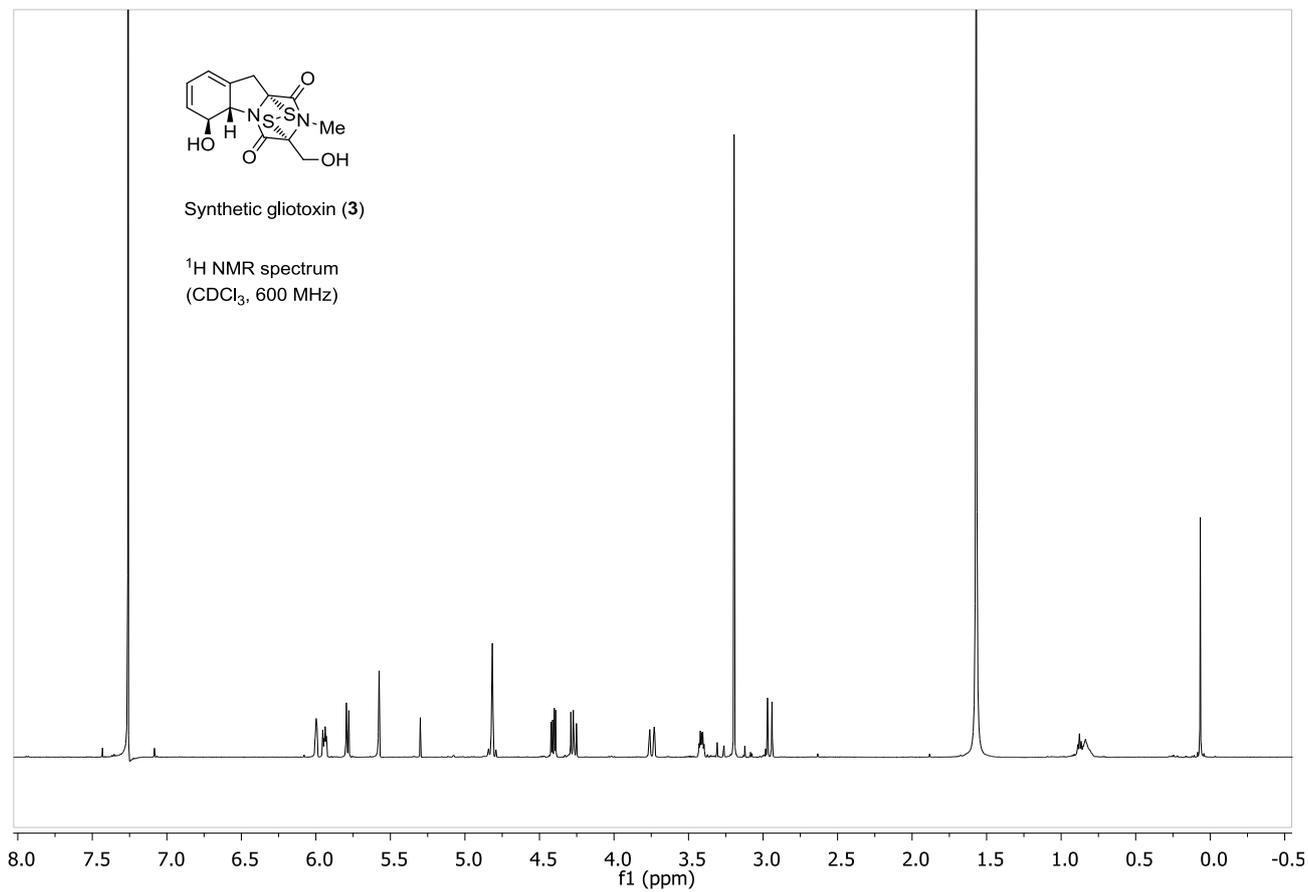
82

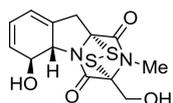
<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)



<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

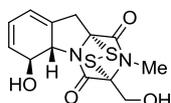
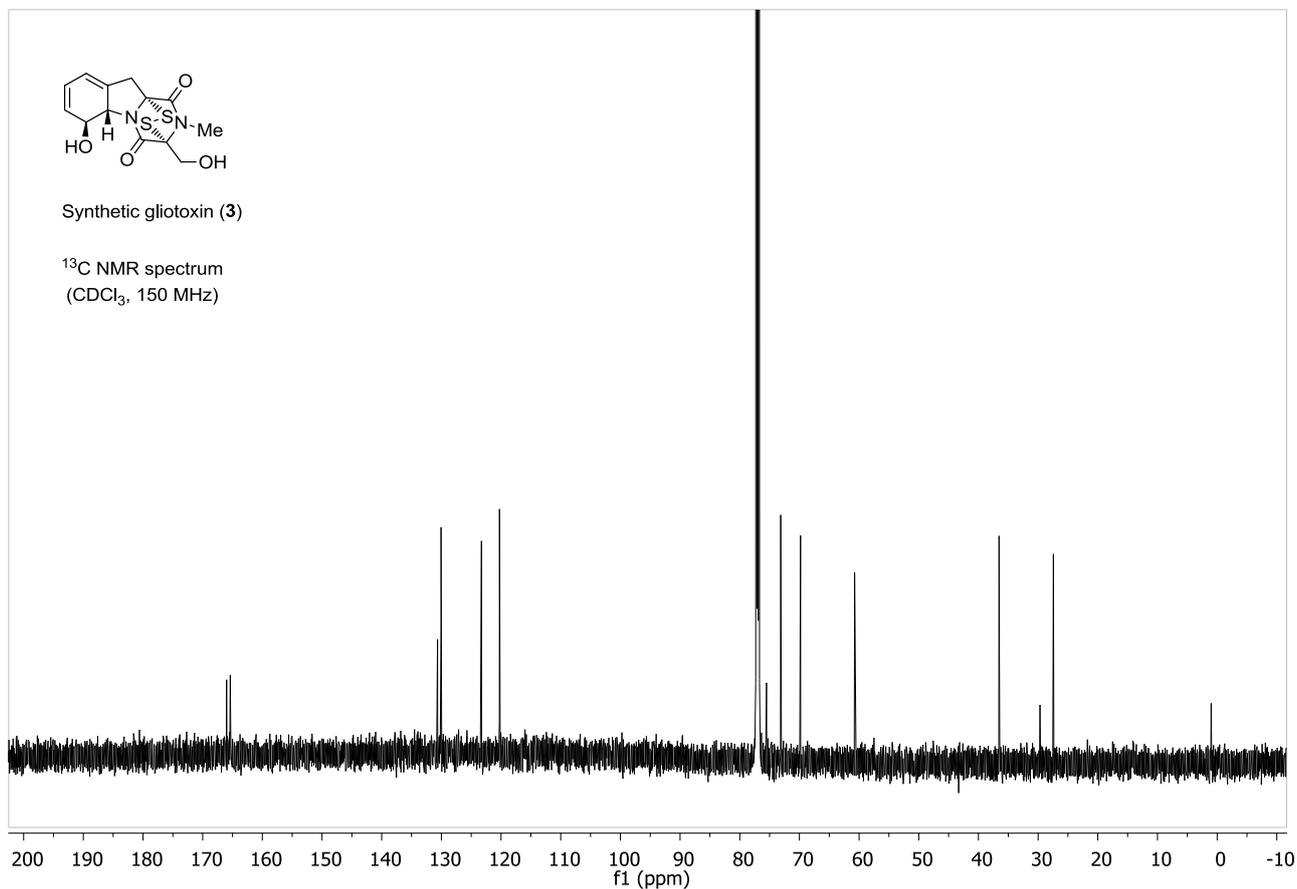






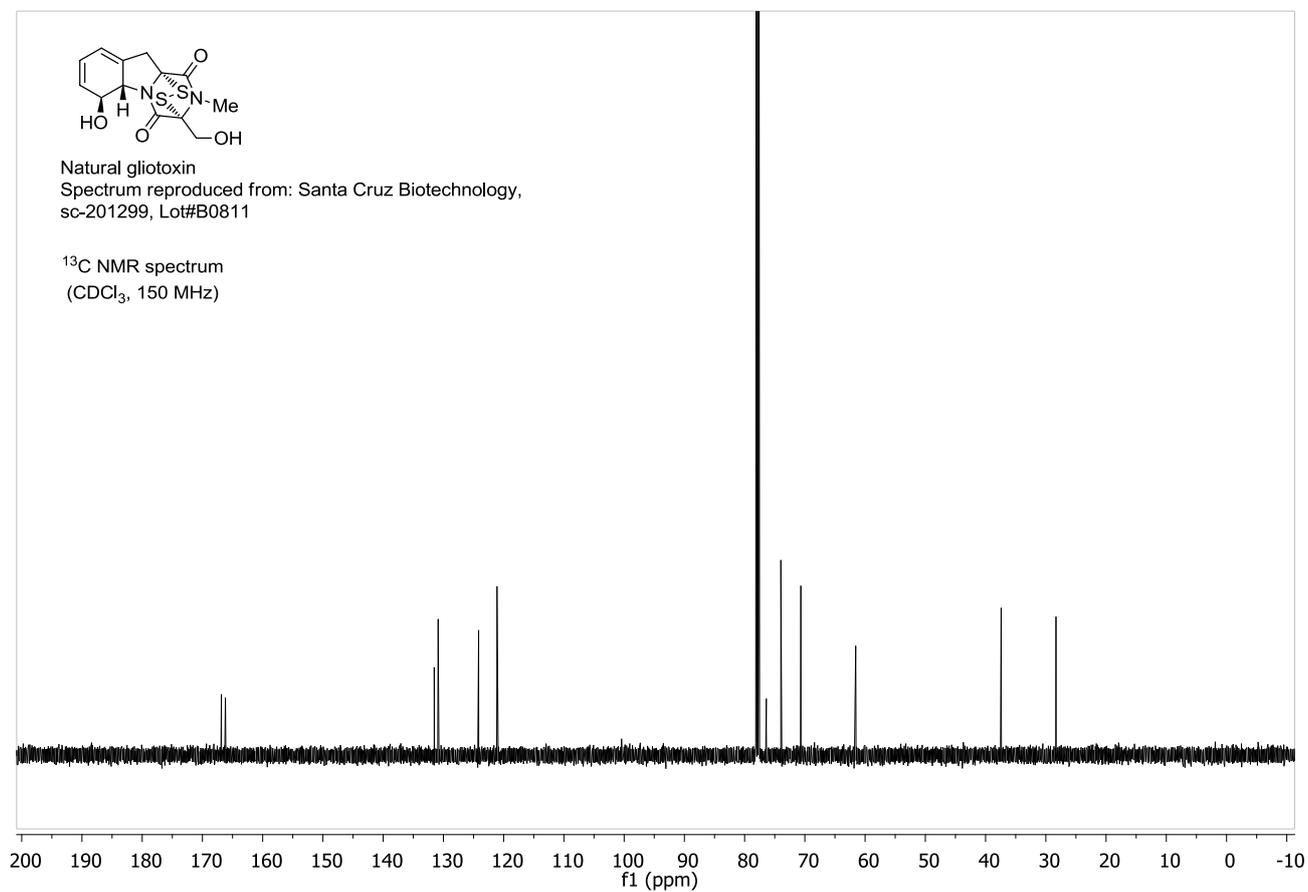
Synthetic gliotoxin (3)

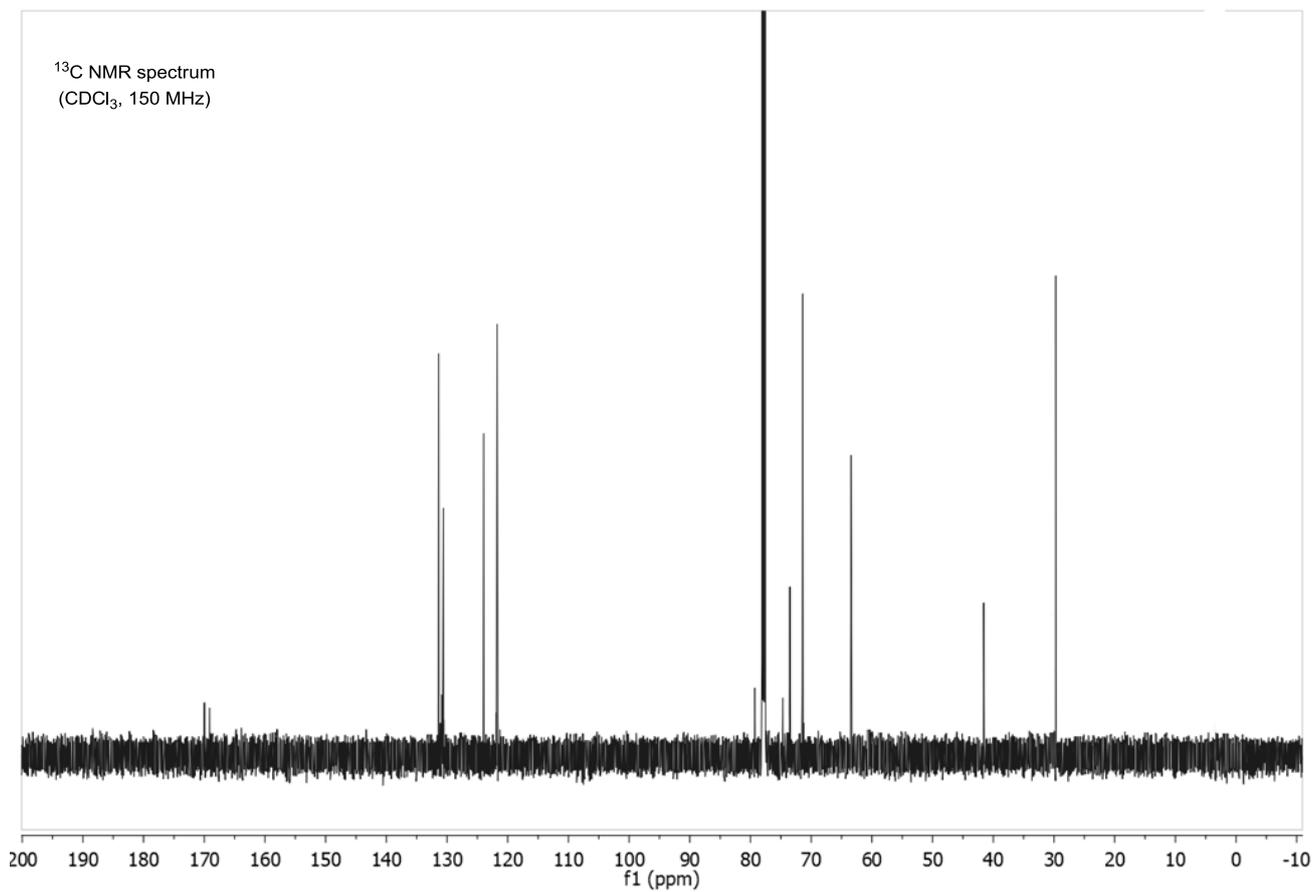
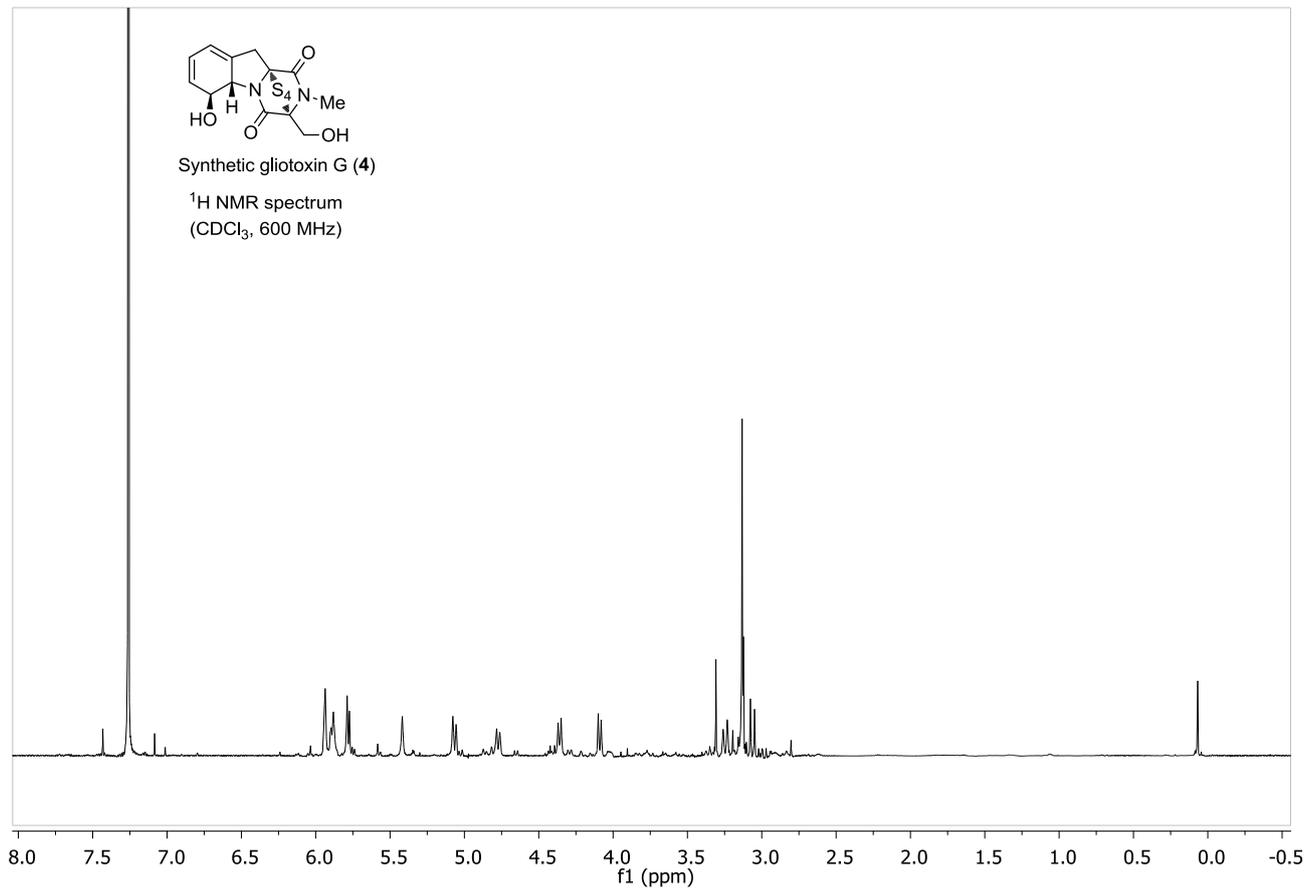
$^{13}\text{C}$  NMR spectrum  
( $\text{CDCl}_3$ , 150 MHz)

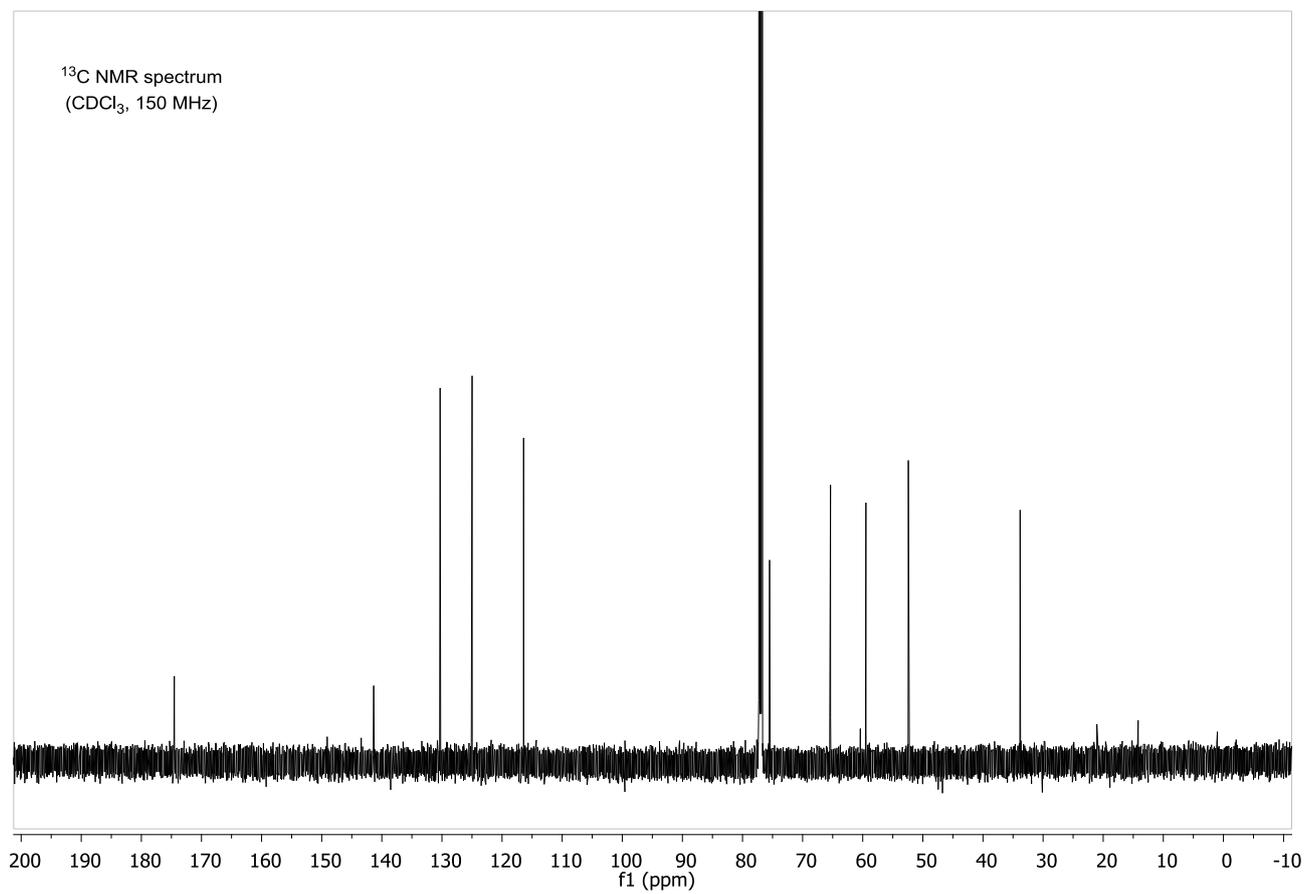
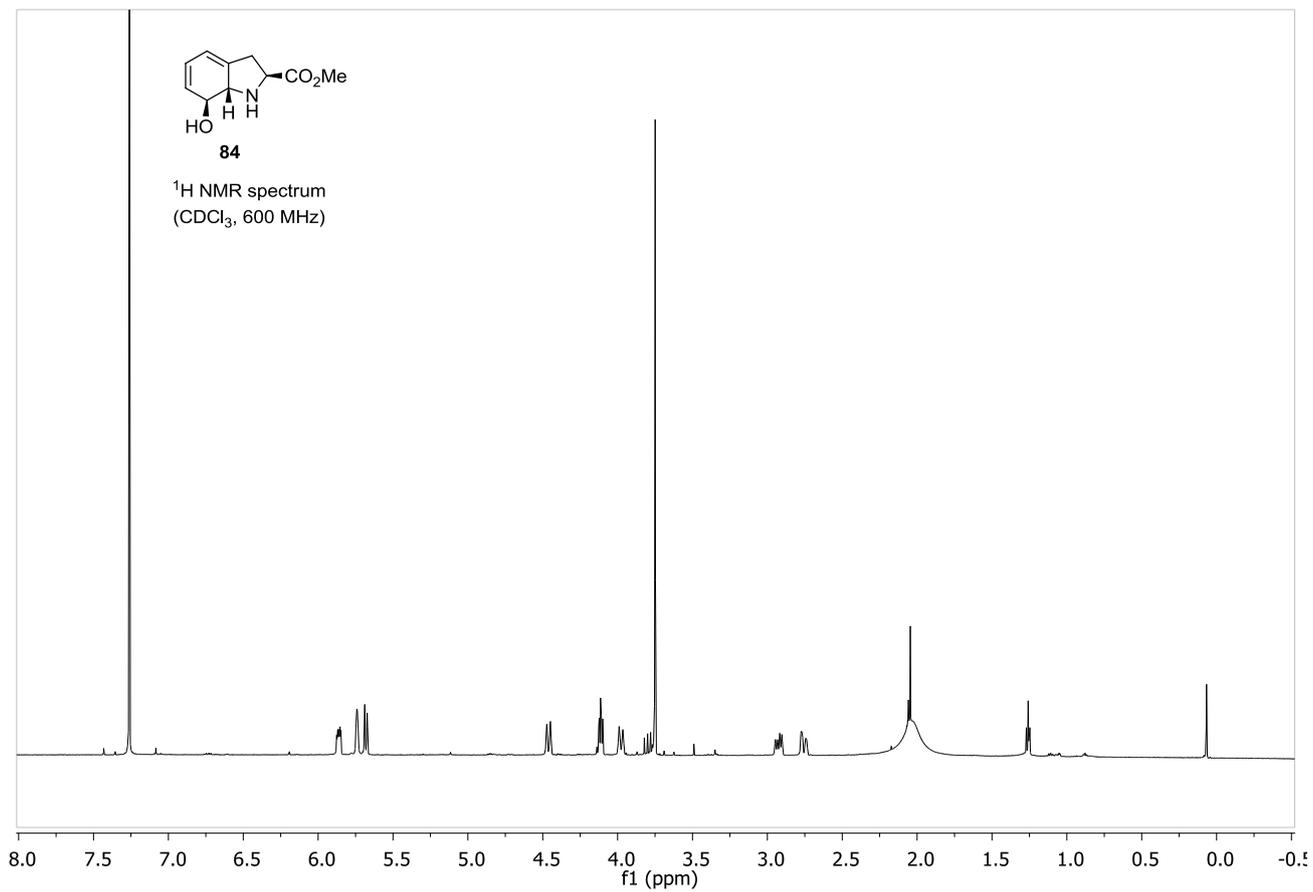


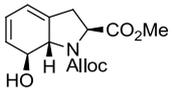
Natural gliotoxin  
Spectrum reproduced from: Santa Cruz Biotechnology,  
sc-201299, Lot#B0811

$^{13}\text{C}$  NMR spectrum  
( $\text{CDCl}_3$ , 150 MHz)

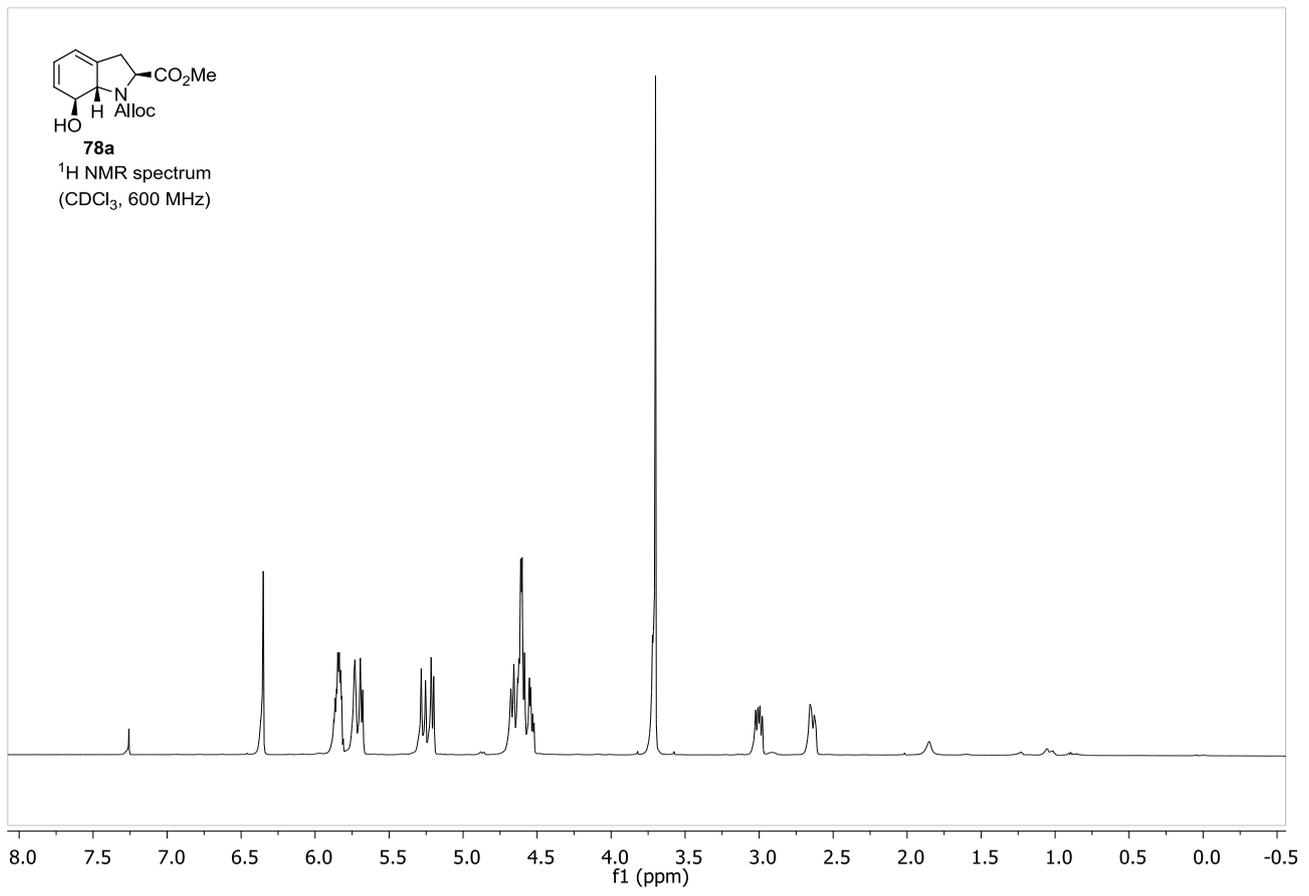




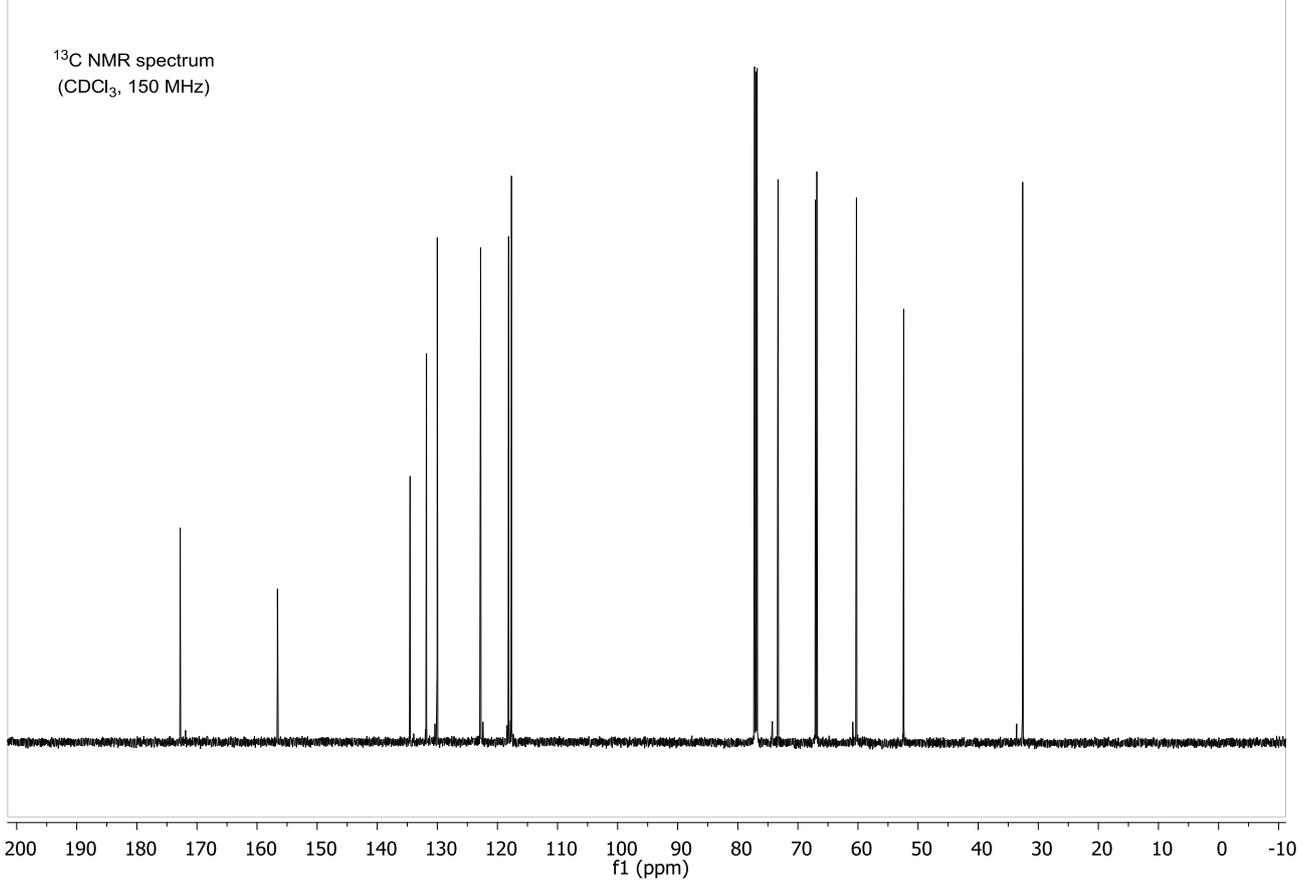


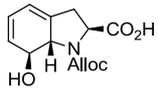


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)



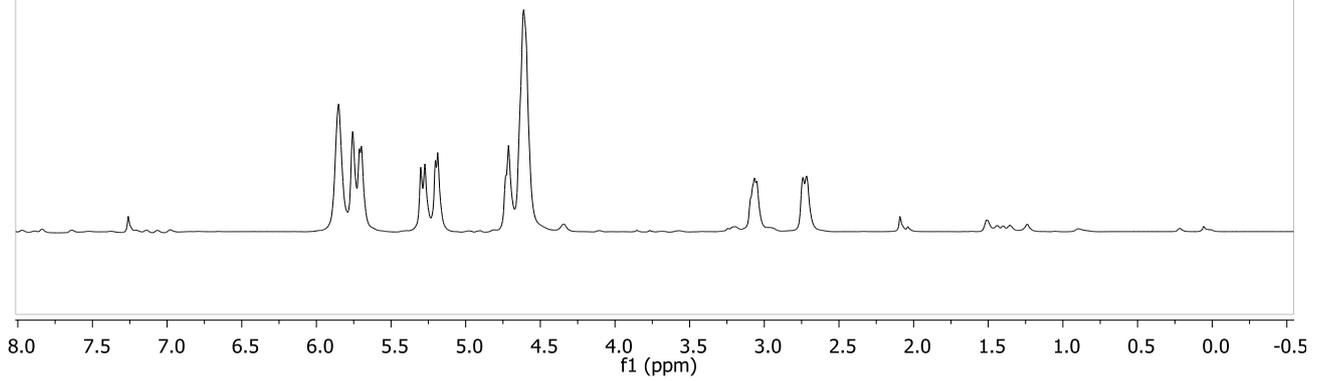
<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)



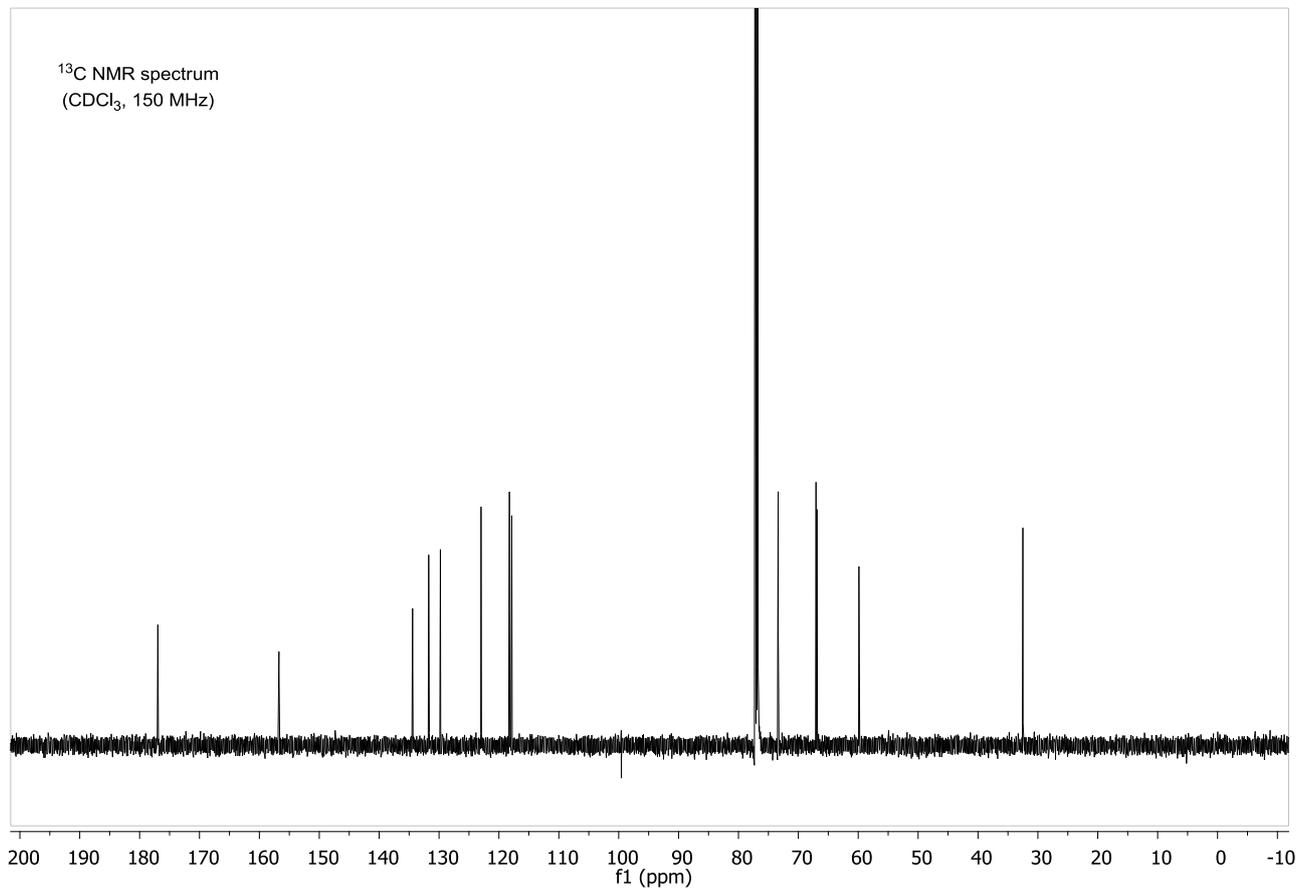


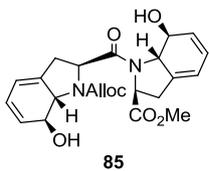
83

<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

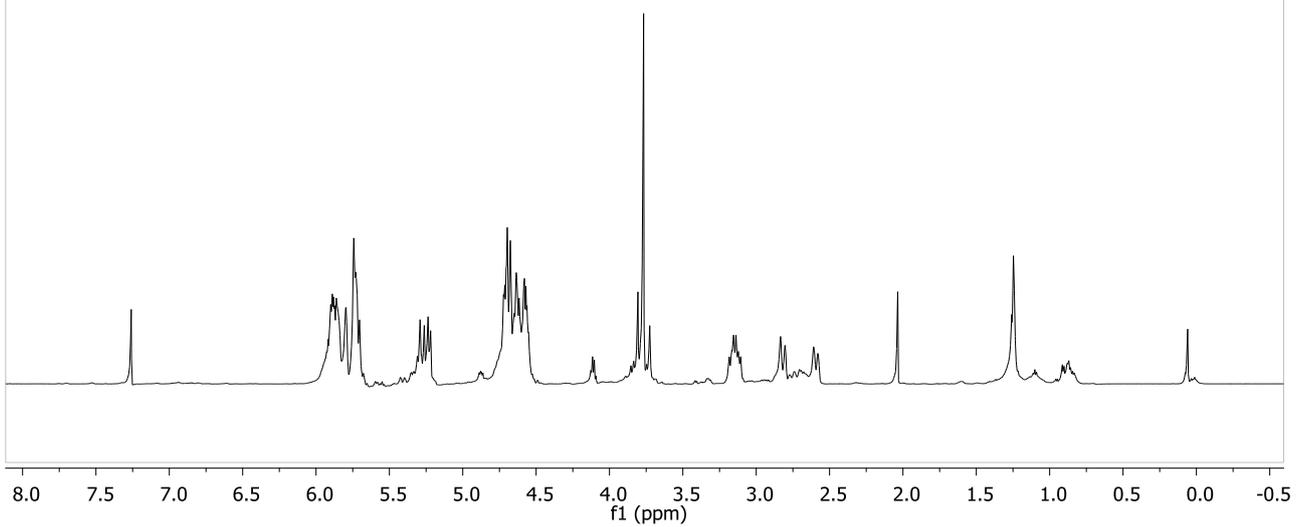


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

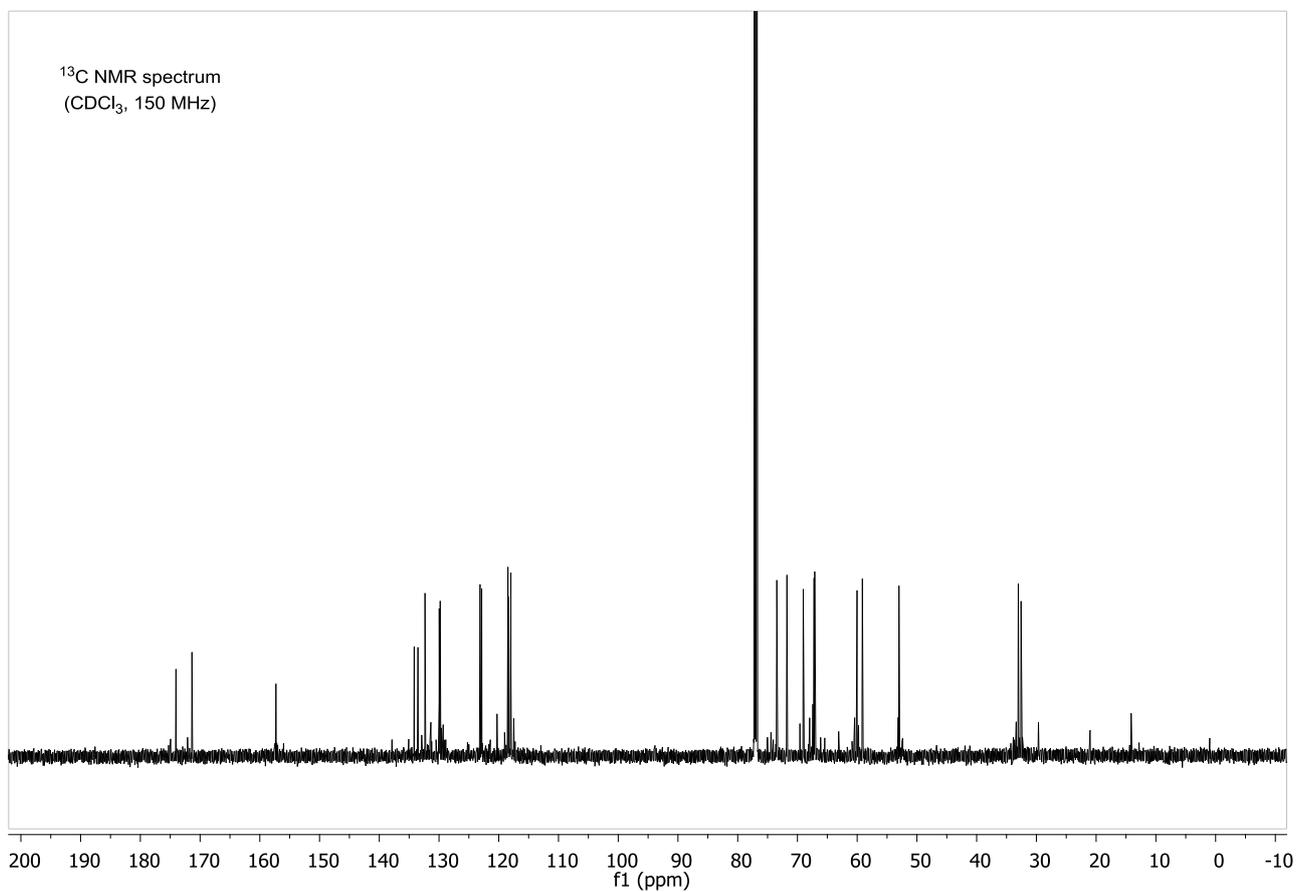


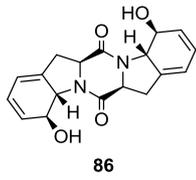


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

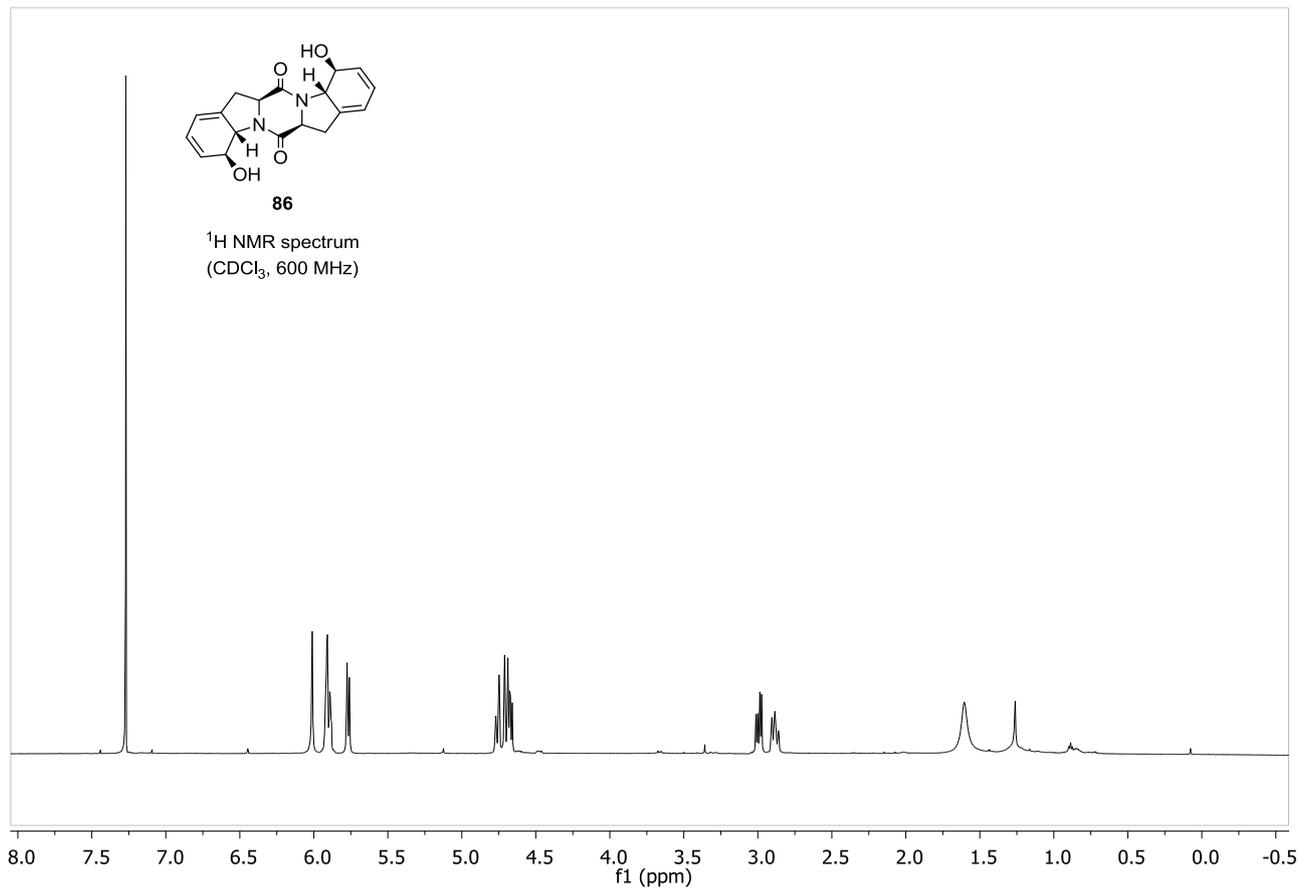


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

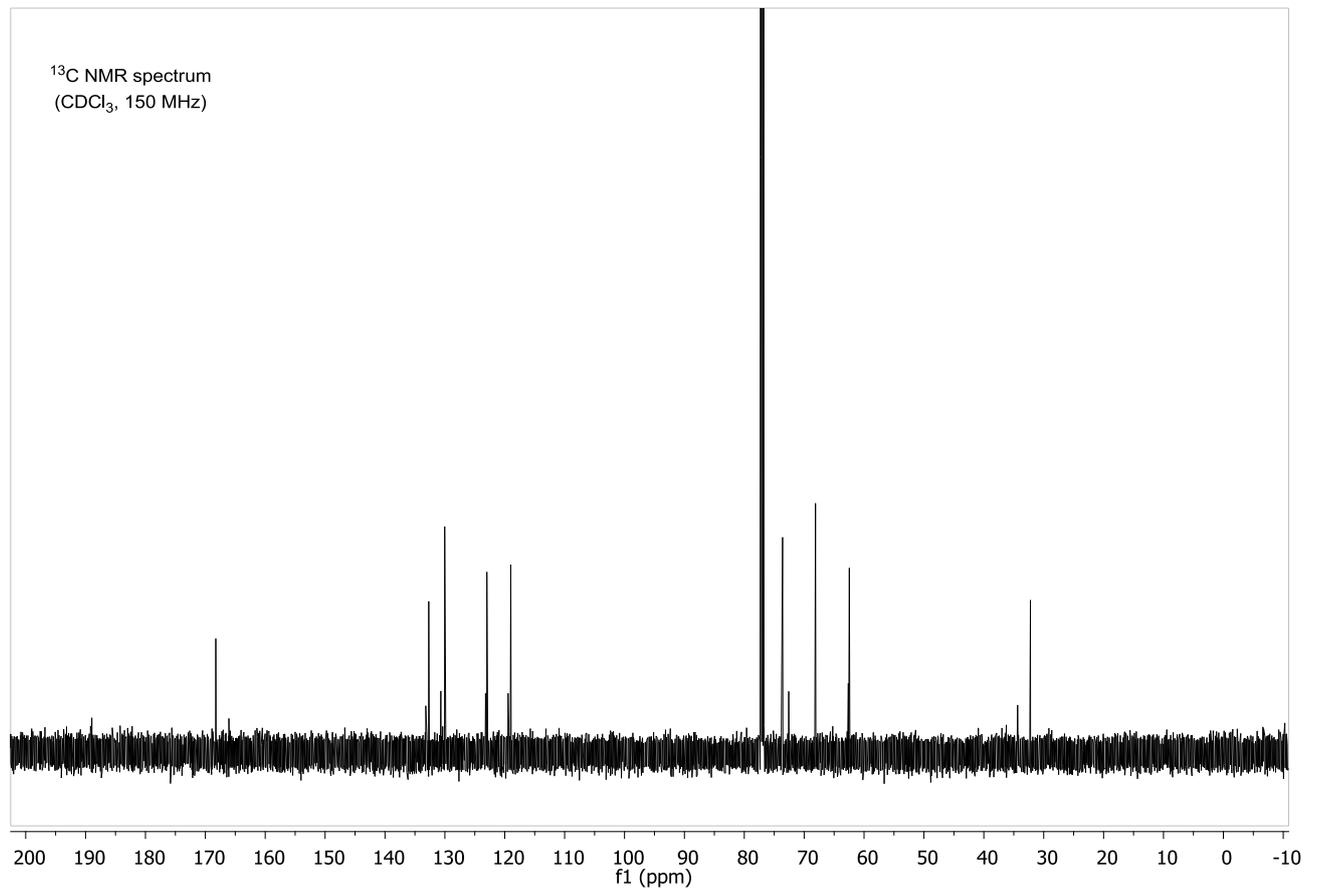


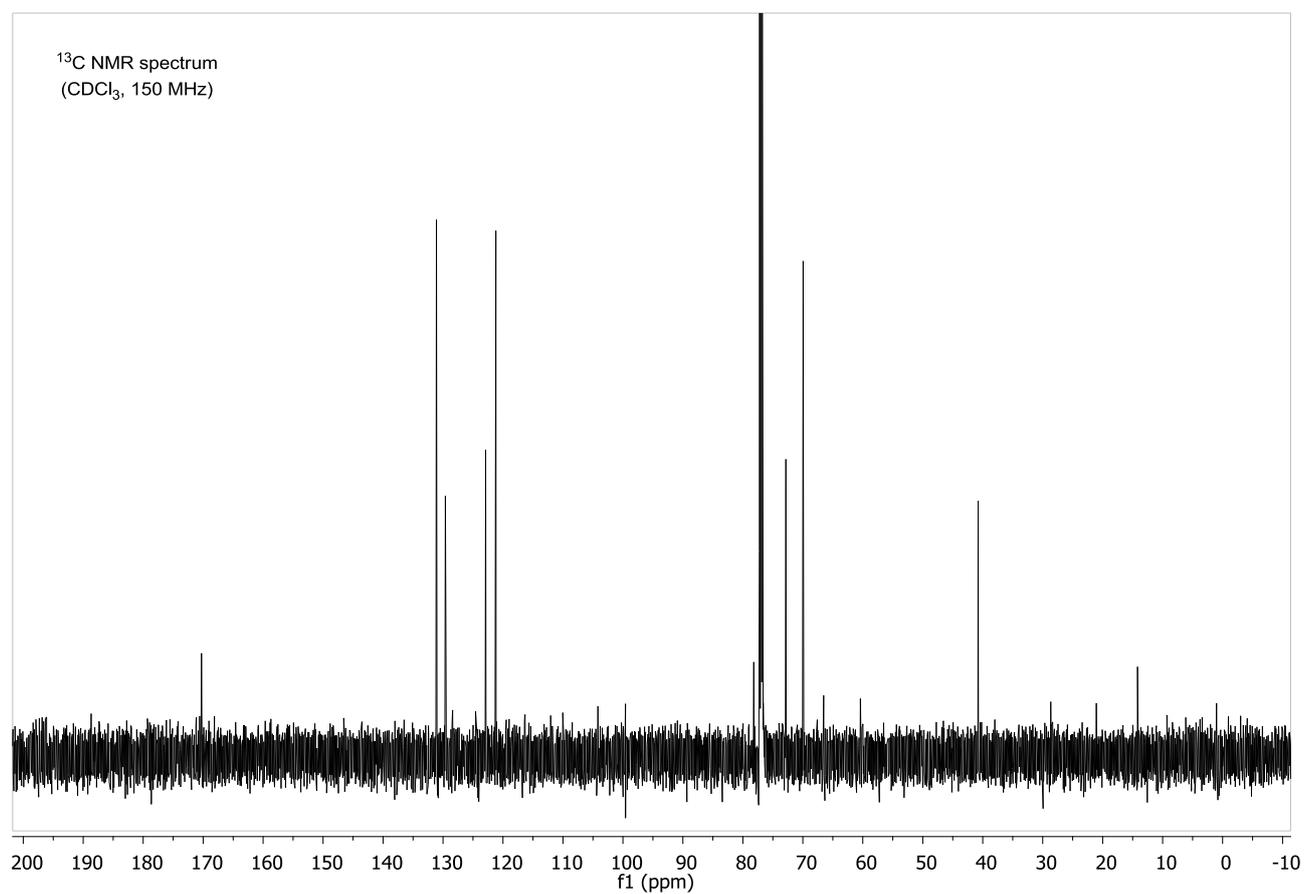
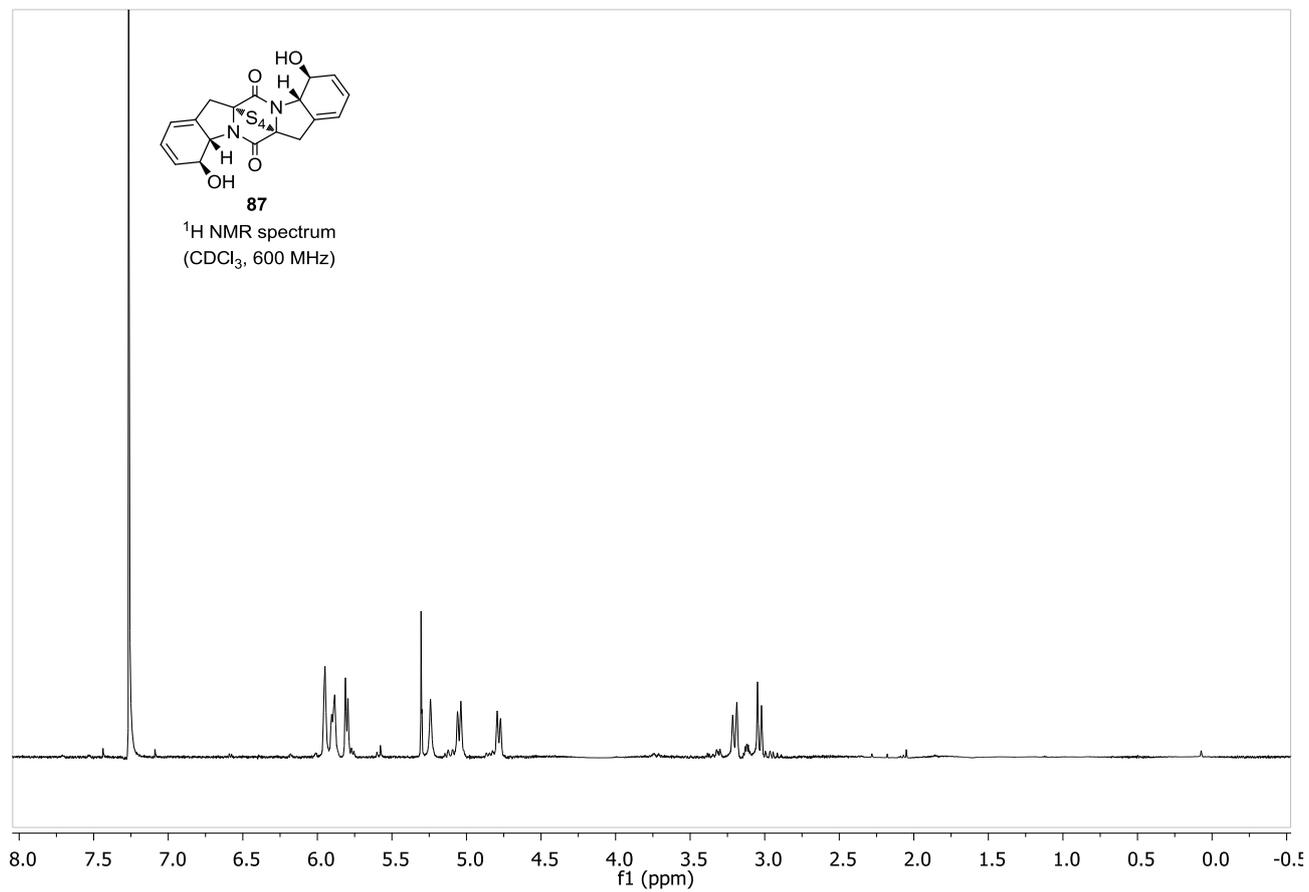


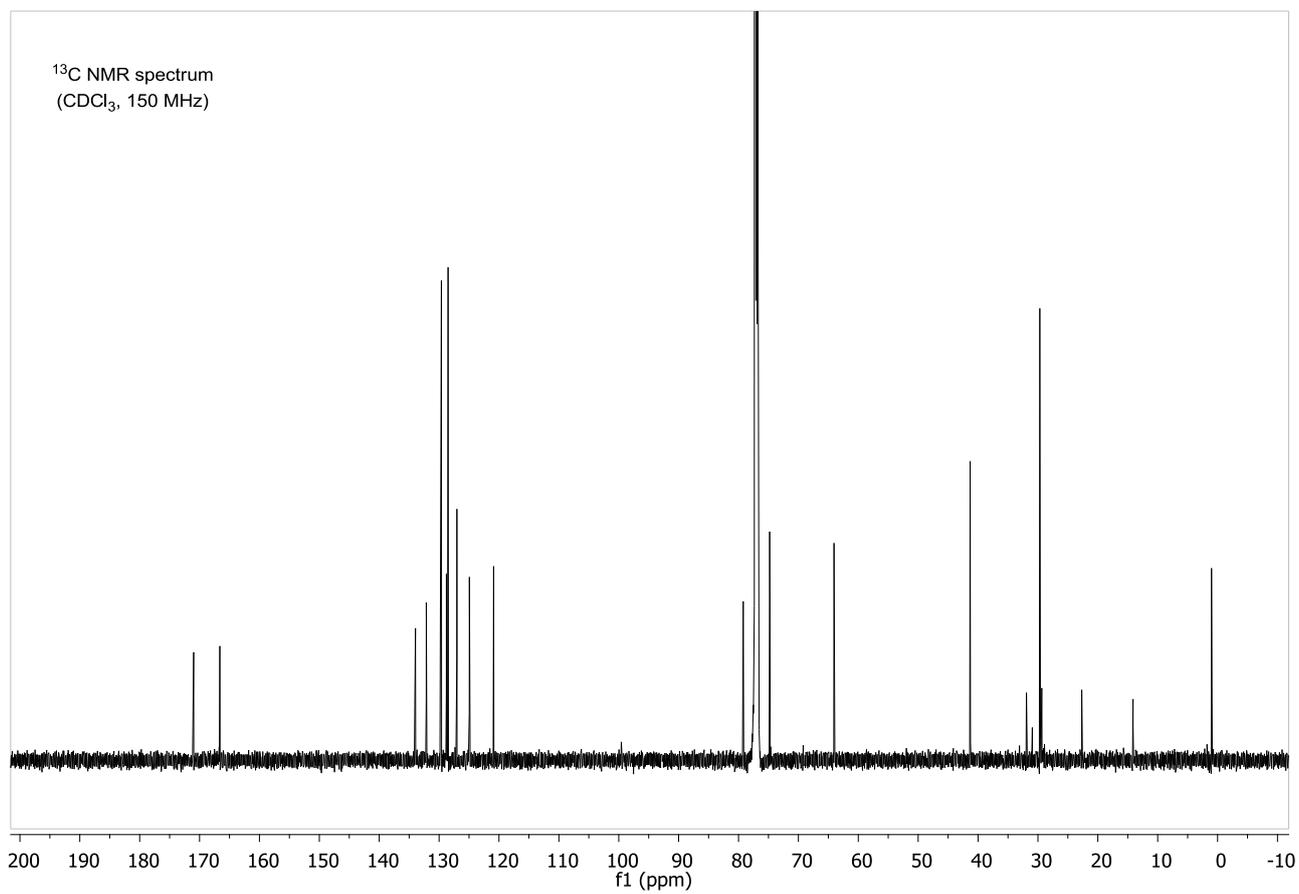
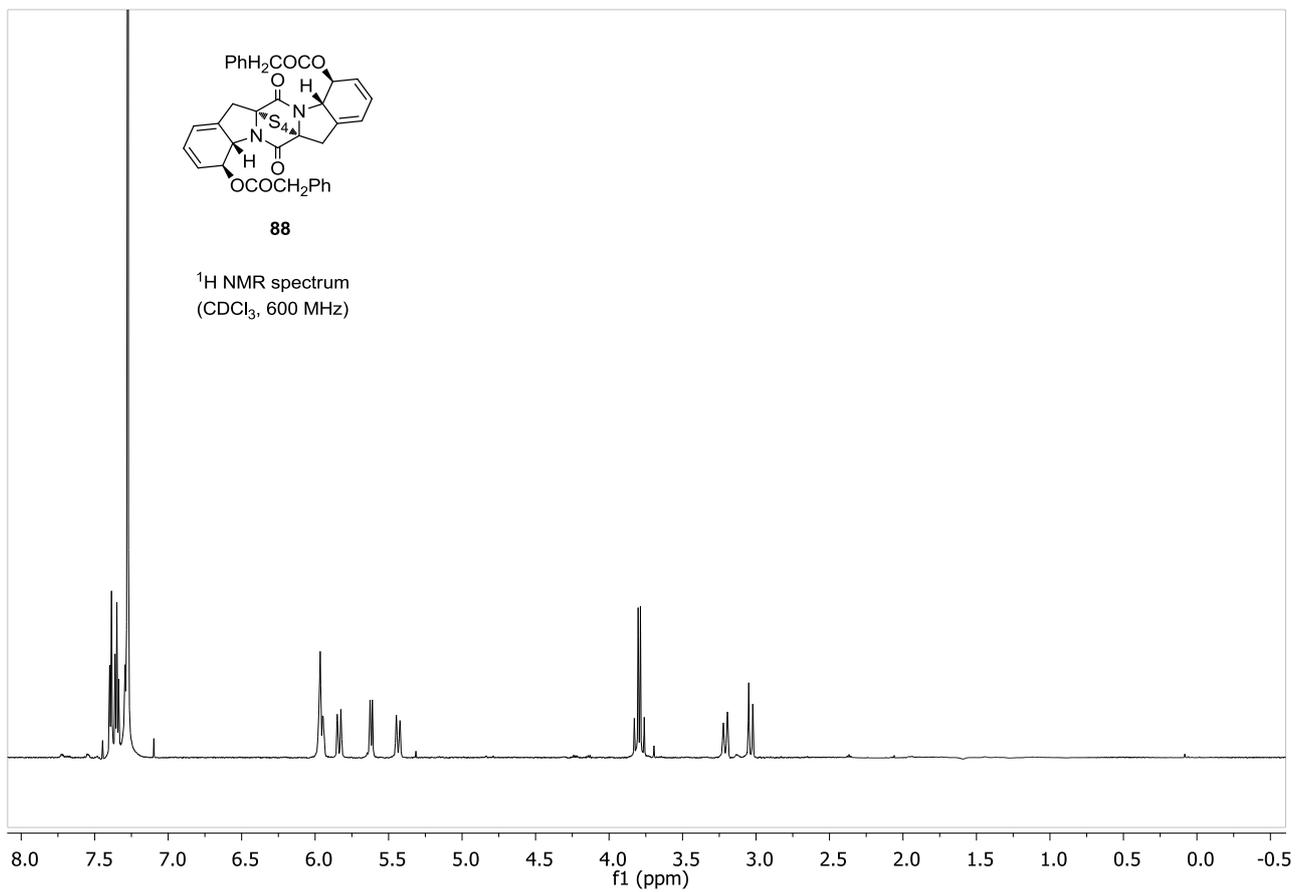
<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

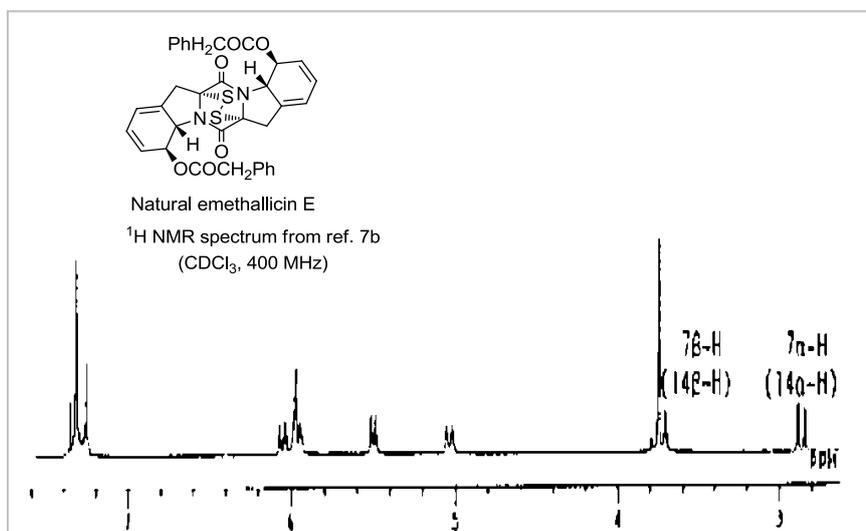
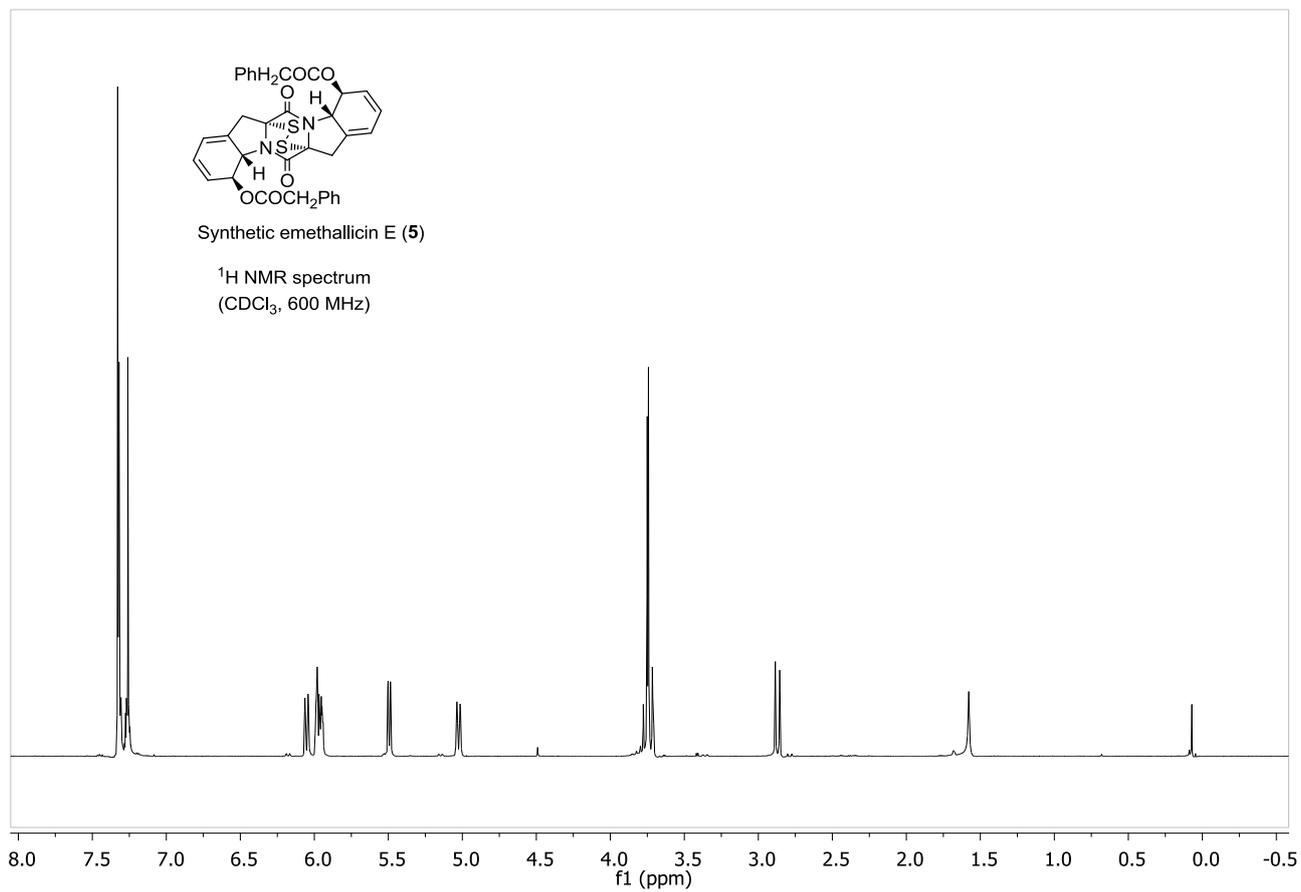


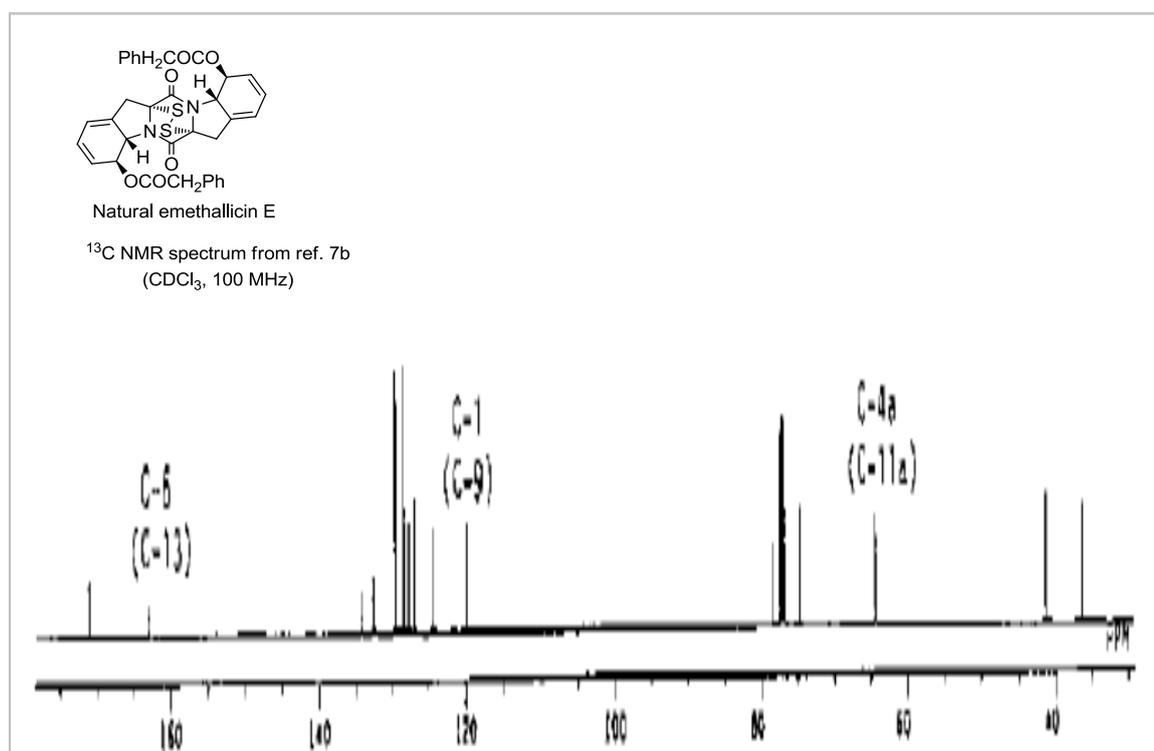
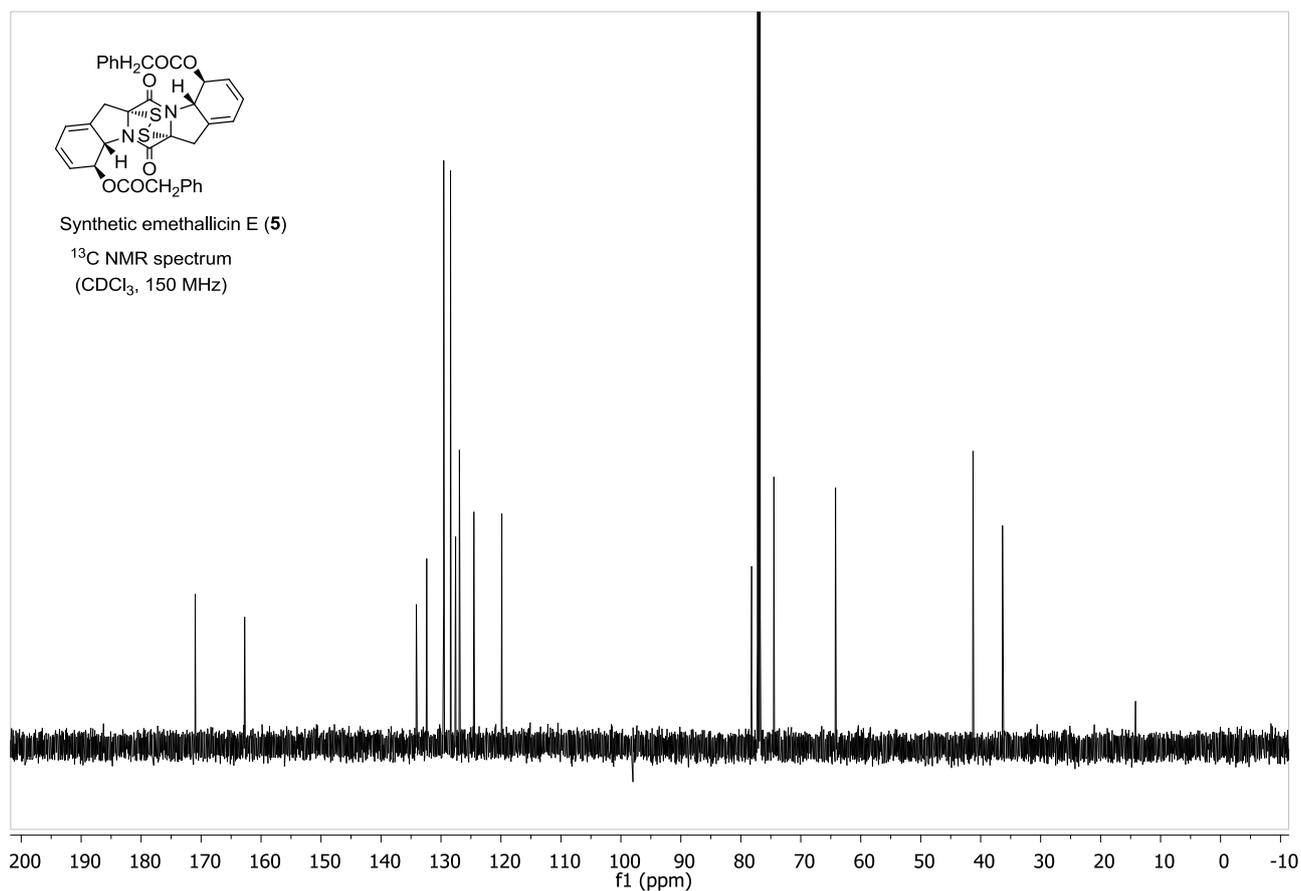
<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

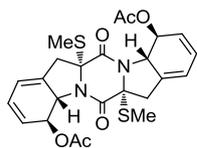






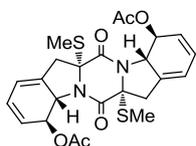
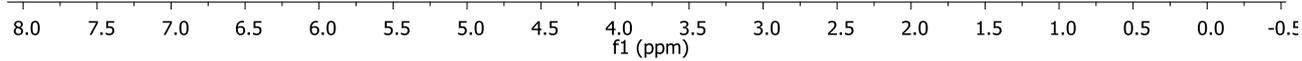






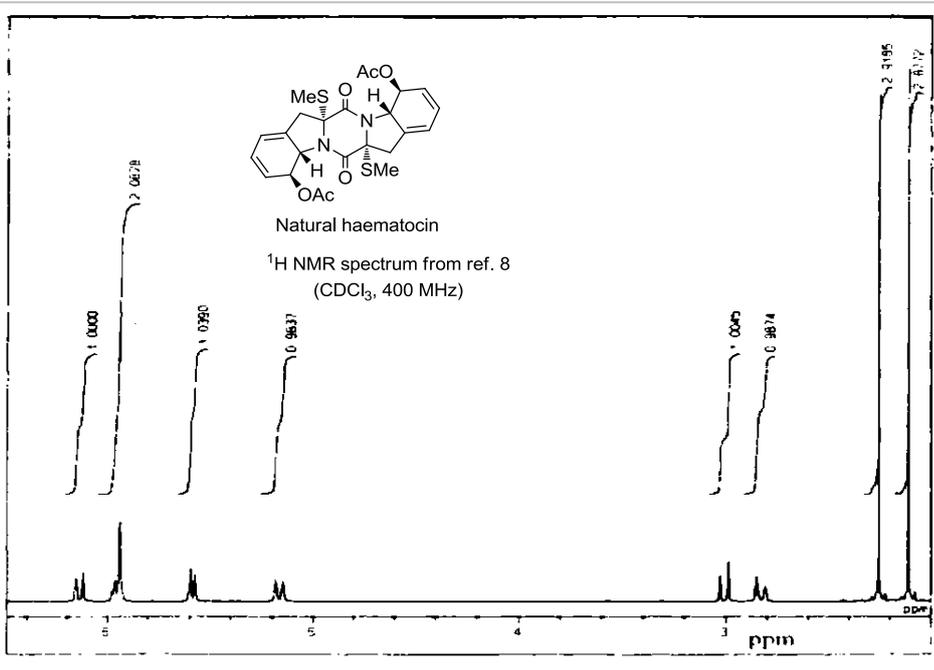
Synthetic haematocin (6)

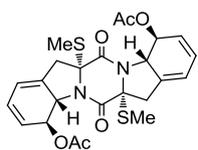
$^1\text{H}$  NMR spectrum  
( $\text{CDCl}_3$ , 600 MHz)



Natural haematocin

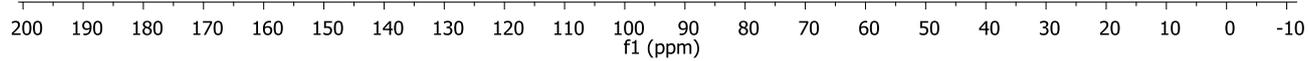
$^1\text{H}$  NMR spectrum from ref. 8  
( $\text{CDCl}_3$ , 400 MHz)



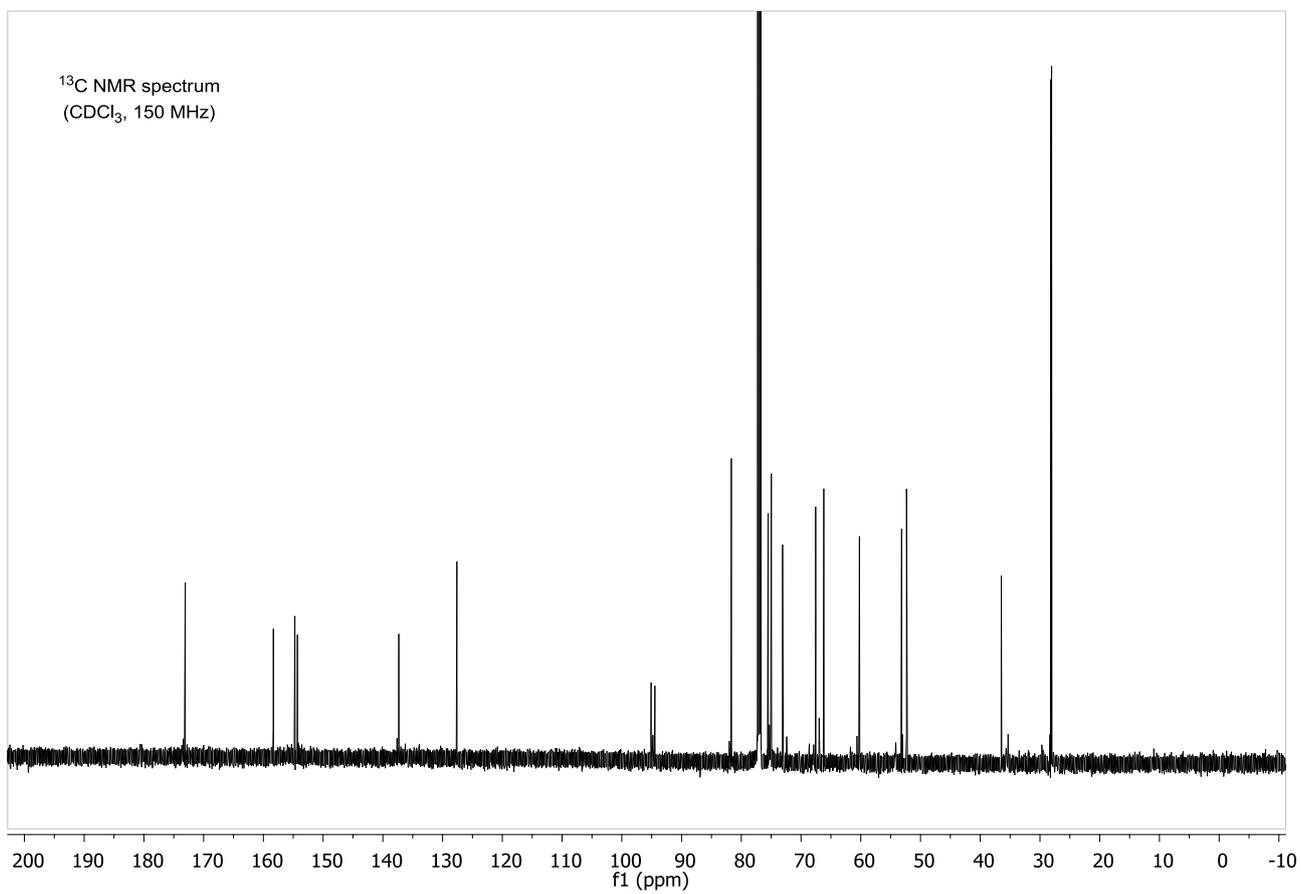
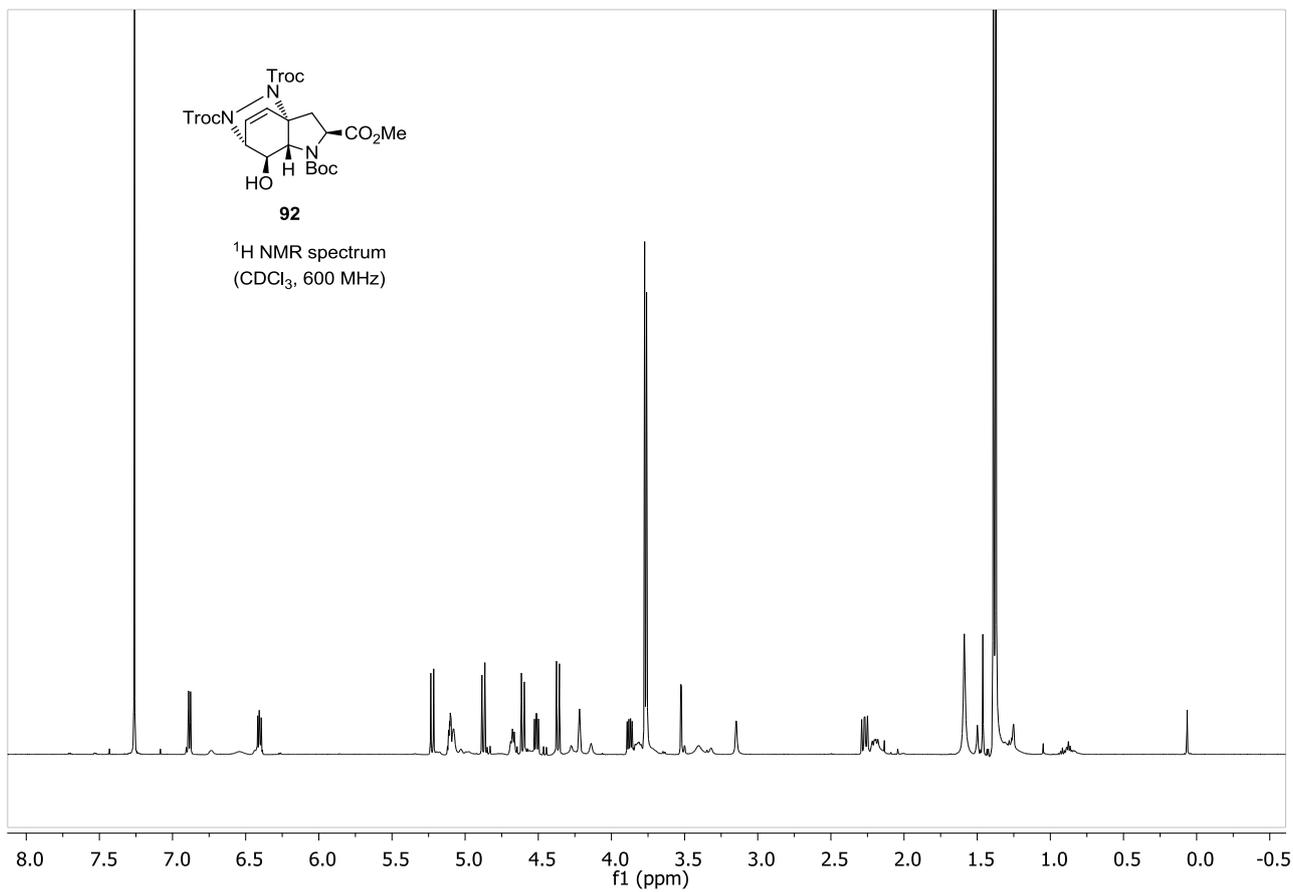


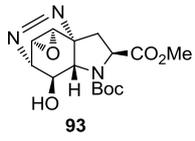
Synthetic haematocin (6)

$^{13}\text{C}$  NMR spectrum  
( $\text{CDCl}_3$ , 150 MHz)

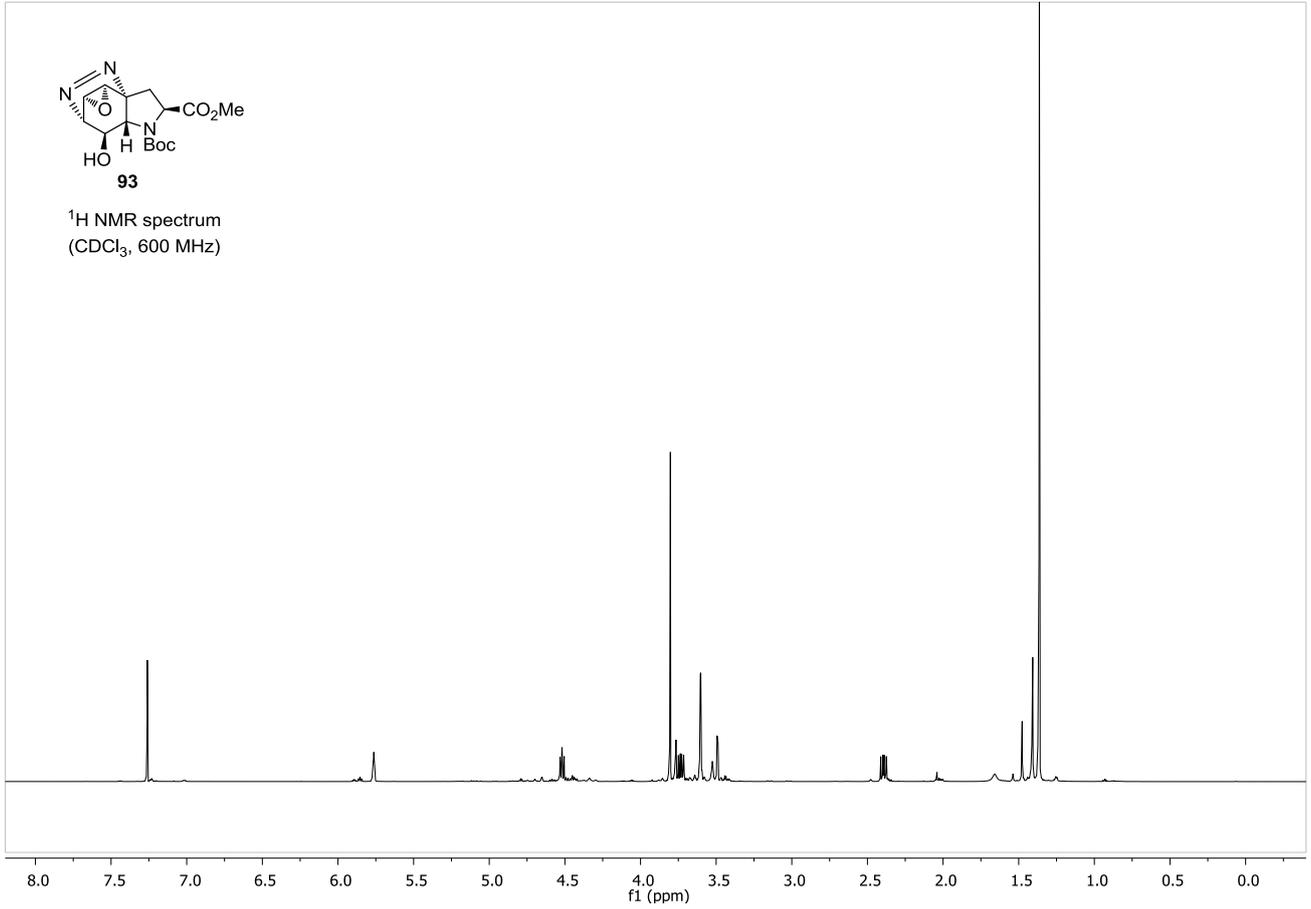




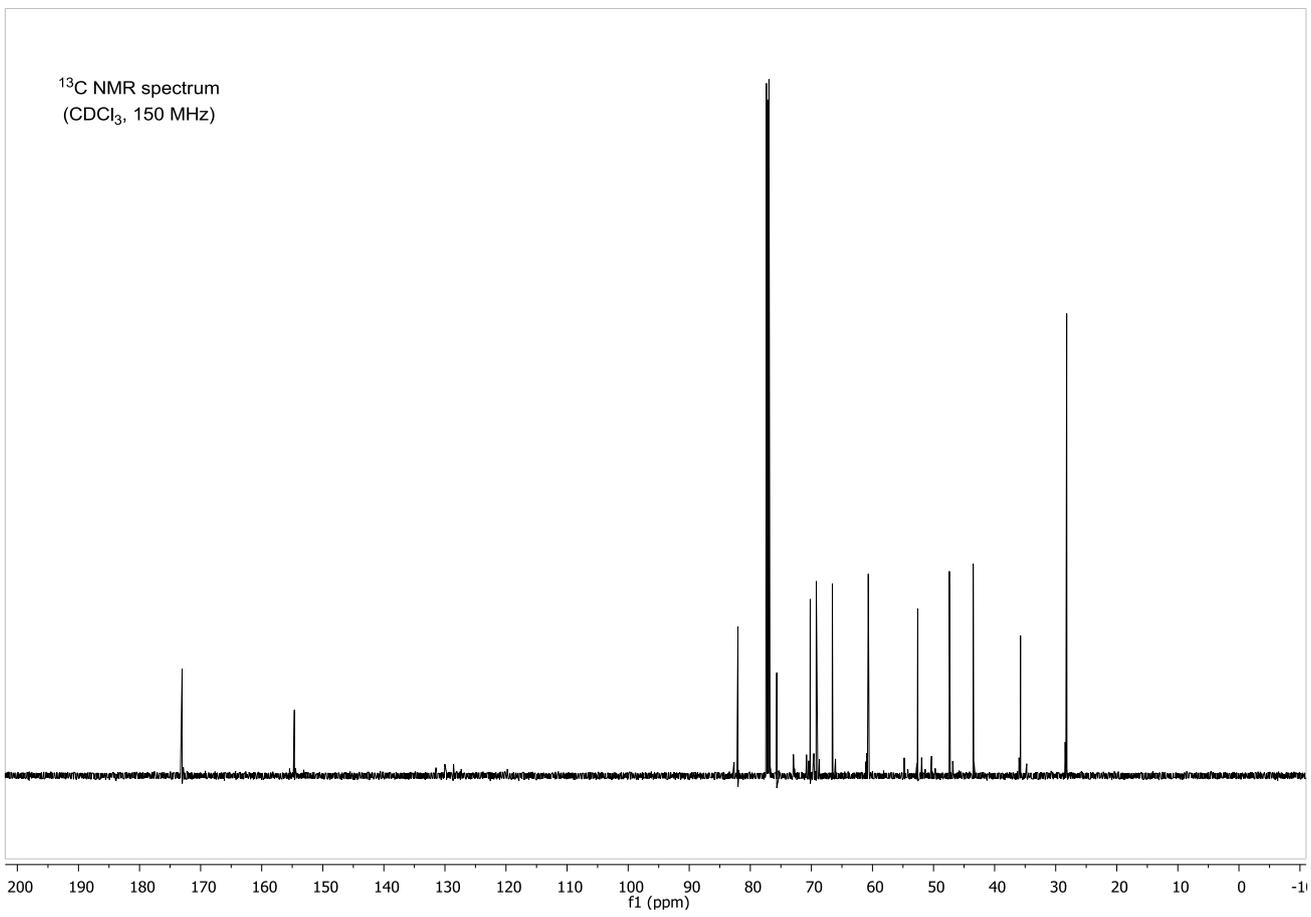


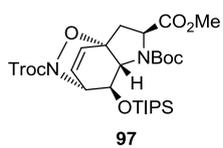


<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)

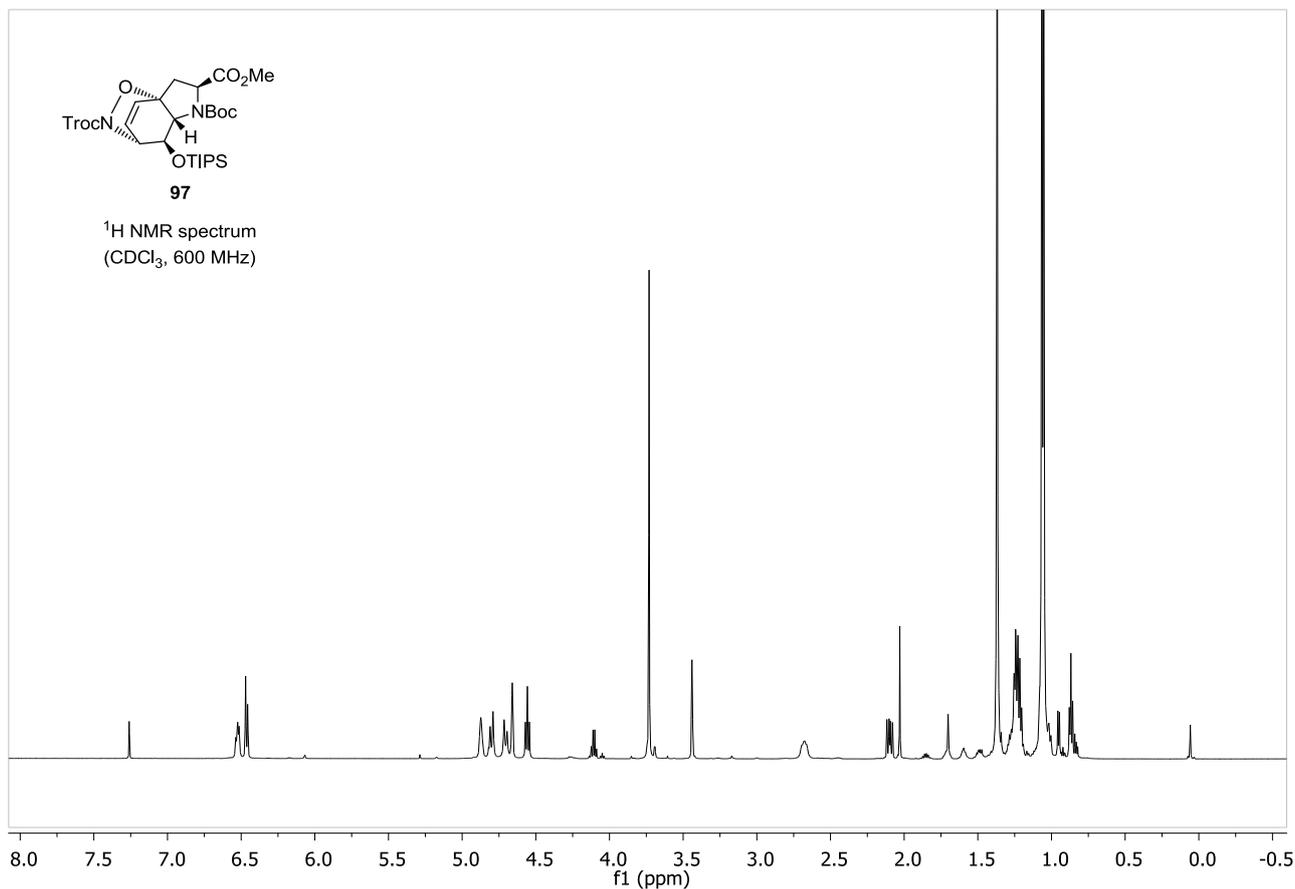


<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)

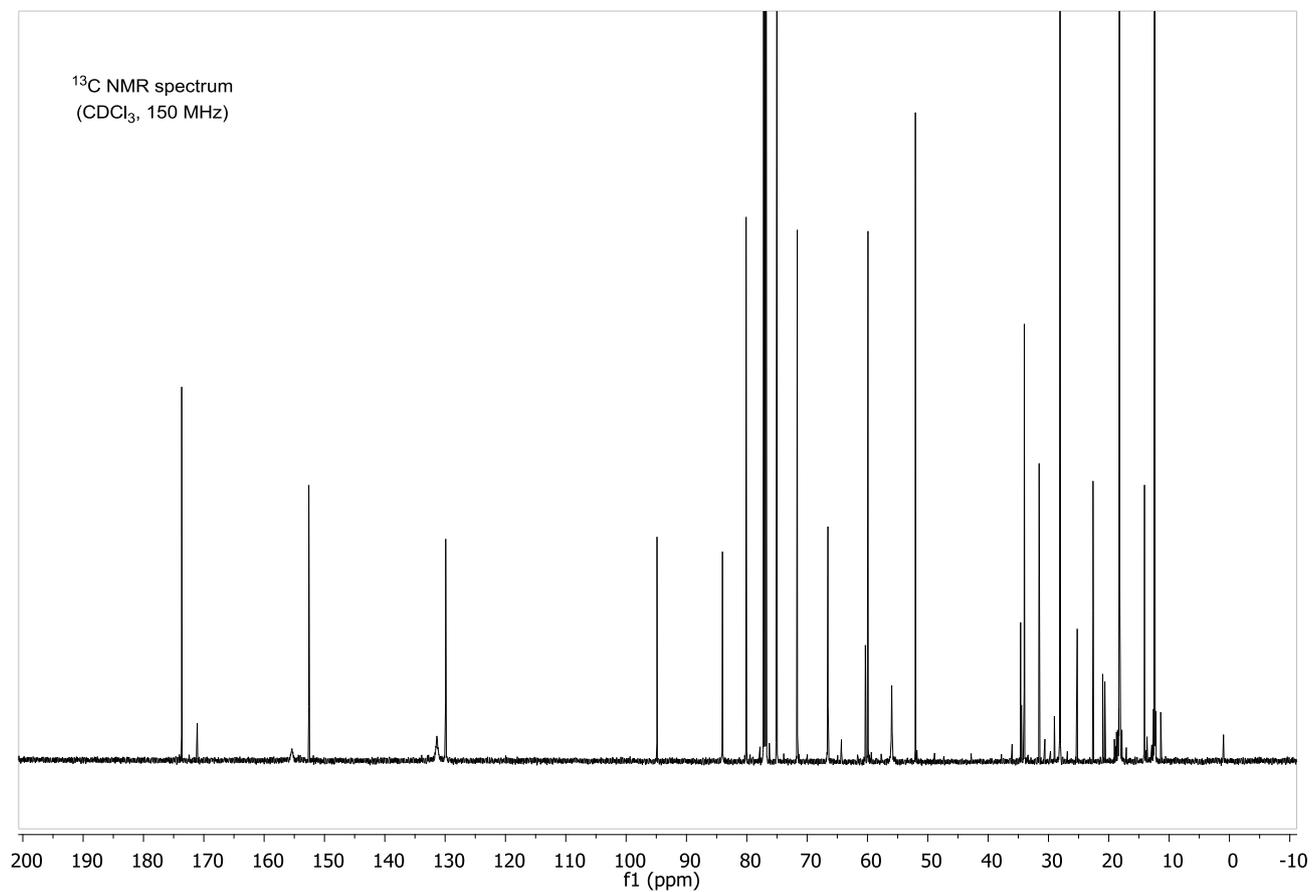


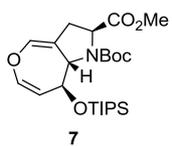


$^1\text{H}$  NMR spectrum  
( $\text{CDCl}_3$ , 600 MHz)

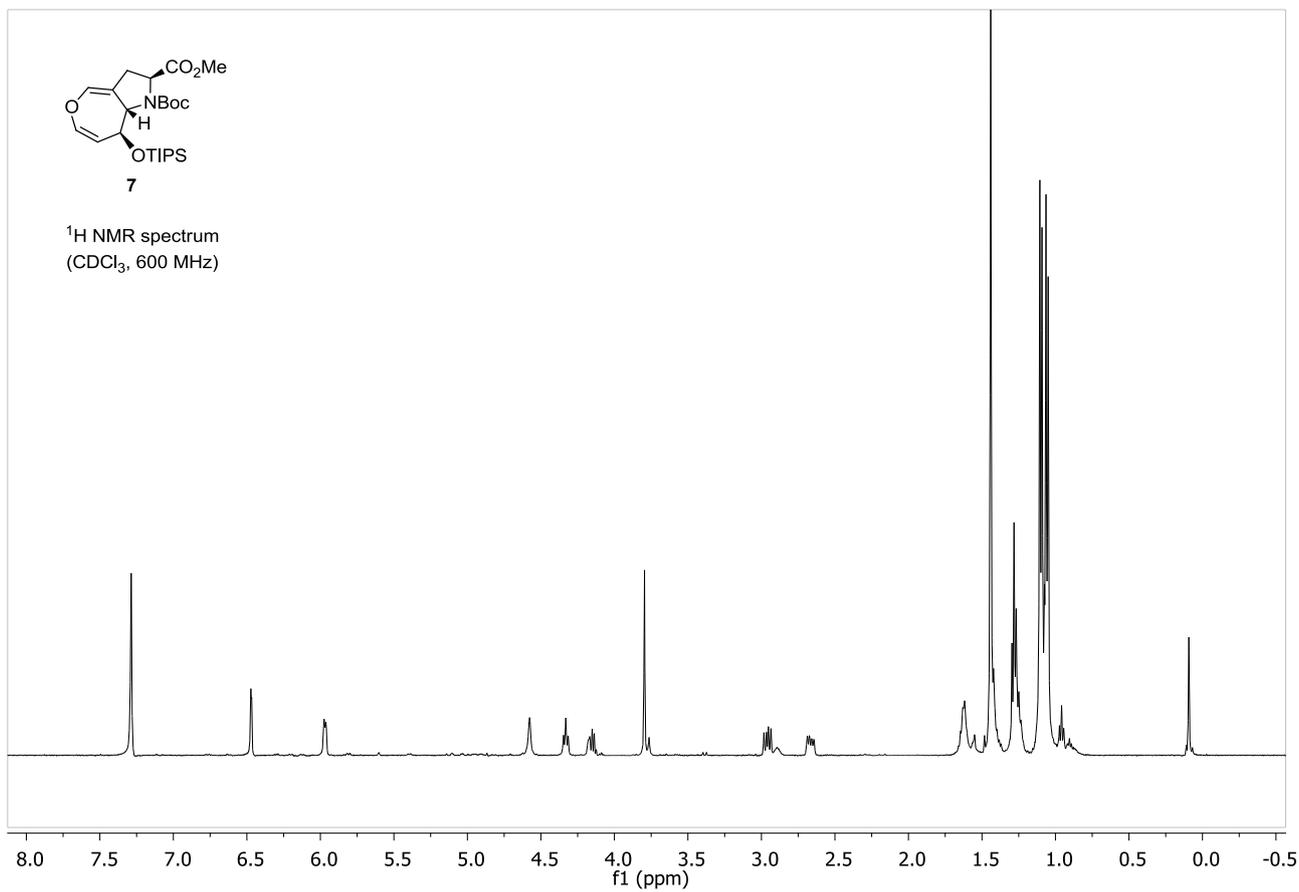


$^{13}\text{C}$  NMR spectrum  
( $\text{CDCl}_3$ , 150 MHz)

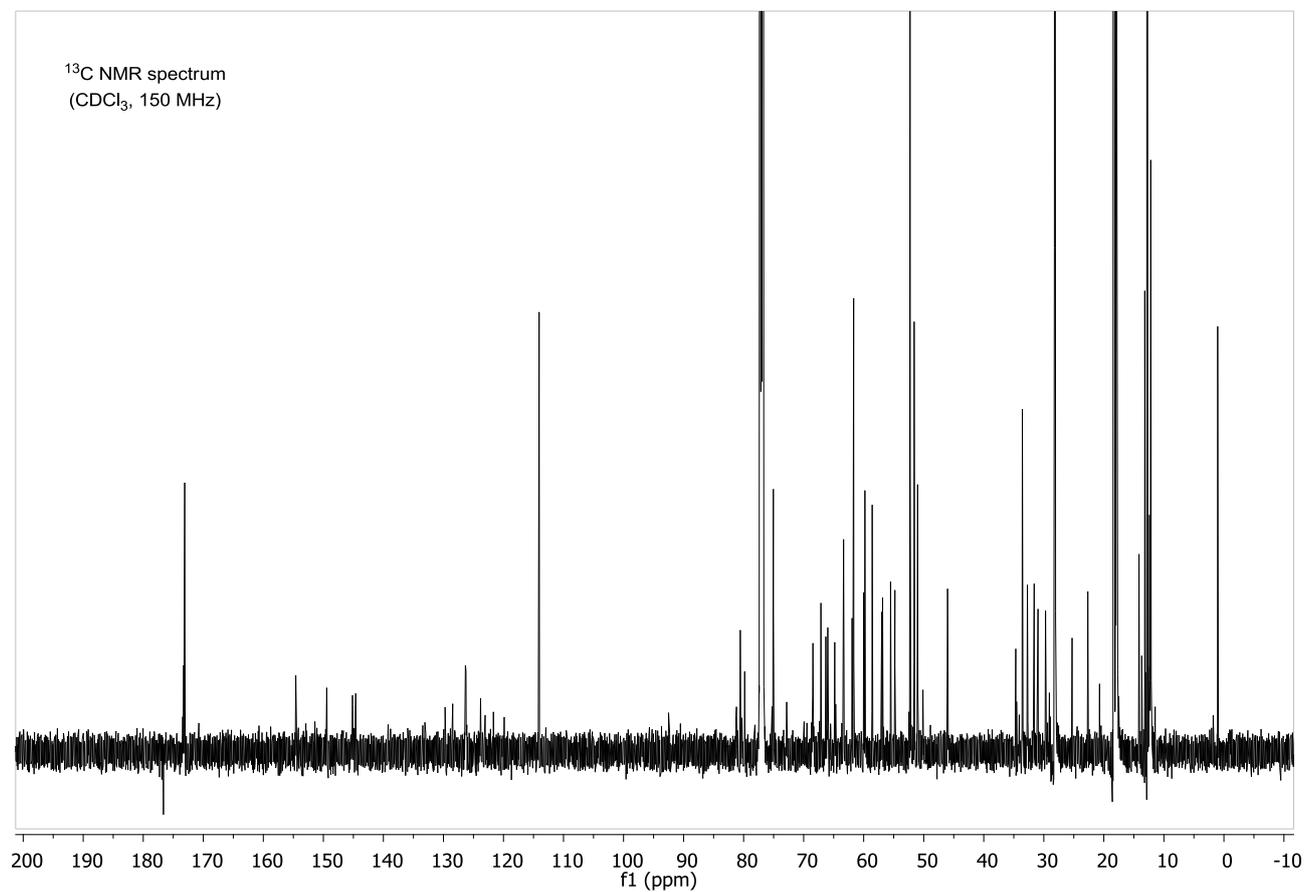




<sup>1</sup>H NMR spectrum  
(CDCl<sub>3</sub>, 600 MHz)



<sup>13</sup>C NMR spectrum  
(CDCl<sub>3</sub>, 150 MHz)



### III. Protocols for Biological Tests

#### A) Anti-Poliovirus Test:

Standard Assay: Inhibition of Viral Cytopathic Effect (CPE): This test, run in 96 well flat-bottomed microplates, was used for the initial antiviral evaluation of all new test compounds. In this CPE inhibition test, four  $\log_{10}$  dilutions of each test compound were added to 3 cups containing African green monkey kidney (Vero) cell monolayers; within 5 min, the poliovirus was then added (at 50-100 cell culture infectious doses per well) and the plate covered, incubated at 37 °C and CPE read microscopically when infected controls developed 100% CPE (approximately 72 hrs). The degree of CPE in each well was estimated by a trained investigator, using a scale of 0 to 4 in 0.5 unit increments. The known positive control drug Pirodavir<sup>(R)</sup> was evaluated in parallel with test drugs in each test. Follow-up testing with compounds found active in initial screening tests were run in the same manner except 8 one-half  $\log_{10}$  dilutions of each compound were used in 4 cups containing the cell monolayer per dilution. The data are expressed as 50% effective concentrations ( $EC_{50}$ ).

Standard Assay: Increase in Neutral Red (NR) Dye Uptake: This test was run to validate the CPE inhibition seen in the initial test, and utilized the same 96-well micro plates after the CPE was read. Neutral red was added to the medium at a final concentration of 0.011% for 2 hrs at 37 °C; cells not damaged by virus take up a greater amount of dye, which is read on a computerized micro plate autoreader. The method as described by McManus<sup>13</sup> was used. An  $EC_{50}$  was determined from this dye uptake method.

Decrease in Virus Yield Assay: Compounds considered active by CPE inhibition and by NR dye uptake were re-tested using both CPE inhibition and, using the same plate, effect on reduction of virus yield by assaying frozen and thawed eluates from each cup for virus titer by serial dilution onto monolayers of susceptible cells, using four microwells per dilution and calculating virus titer by an endpoint dilution method.<sup>14</sup> Development of CPE in these cells is the indication of presence of infectious virus. As in the initial tests, Pirodavir<sup>(R)</sup> is run in parallel as a positive control. The 90% effective concentration ( $EC_{90}$ ), which is that test drug concentration that inhibits virus yield by 1  $\log_{10}$ , is determined from these data.

Methods for Assay of Cytotoxicity:

A) Visual Observation: In the CPE inhibition test, two wells of uninfected cells treated with each concentration of test compound were run in parallel with infected, treated wells. At the time CPE was determined microscopically, the toxicity control cells were also examined microscopically for any changes in cell appearance compared to normal control cells run in the same plate. These changes may be enlargement, granularity, cells with ragged edges, a filmy appearance, rounding, detachment from the surface of the well, or other changes. The degree of cytotoxicity was determined at the same time that viral CPE was evaluated, using a grading scale of 0 to 5. A 50% cell inhibitory (cytotoxic) concentration (IC<sub>50</sub>) was determined by regression analysis of these data.

B) Neutral Red Uptake: In the neutral red dye uptake phase of the antiviral test described above, the two toxicity control wells also receive neutral red and the degree of color intensity was determined spectrophotometrically. A neutral red IC<sub>50</sub> (NR IC<sub>50</sub>) was subsequently determined.

	Compound	Visual			Neutral Red			Virus Yield Reduction		
		EC <sub>50</sub> (µg/mL)	CC <sub>50</sub> (µg/mL)	SI	EC <sub>50</sub> (µg/mL)	CC <sub>50</sub> (µg/mL)	SI	EC <sub>90</sub> (µg/mL)	CC <sub>50</sub> (µg/mL)	SI
Trial 1	<b>46</b>	0.057	1.8	18	0.058	1.6	17	0.071	1.6	22.5
Trial 2	<b>46</b>	0.036	2.9	81	0.047	2.1	45	0.062	2.1	33.9
Trial 3	<b>46</b>	0.016	1.8	113	0.017	1.8	106	0.027	1.8	66.7
	Mean	0.036	2.17	70	0.041	1.83	56	0.053	1.83	41
	SD	0.021	0.64	48	0.021	0.25	45	0.023	0.25	23
Trial 1	<i>2,2'-epi-46</i>	0.057	1	18	0.073	1.5	14	0.078	1.5	19.2
Trial 2	<i>2,2'-epi-46</i>	0.048	1.5	31	0.05	1.5	30	0.065	1.5	23.1
Trial 3	<i>2,2'-epi-46</i>	0.0087	1.1	126	0.01	1.8	180	0.046	1.8	39.1
	Mean	0.038	1.20	59	0.044	1.60	75	0.063	1.60	27
	SD	0.026	0.26	59	0.032	0.17	92	0.016	0.17	11
Trial 1	<b>61</b>	0.015	0.32	21	0.0086	0.47	55	0.018	0.47	26.1
Trial 2	<b>61</b>	0.0062	0.49	79	0.01	0.23	23	0.016	0.23	14.4
Trial 3	<b>61</b>	0.0068	0.52	76	0.008	0.37	46	0.013	0.37	28.5
	Mean	0.009	0.44	59	0.009	0.36	41	0.016	0.36	23
	SD	0.005	0.11	33	0.001	0.12	17	0.003	0.12	8

SI = CC<sub>50</sub>/EC<sub>50</sub> or EC<sub>90</sub>. The Virus Yield Reduction CC<sub>50</sub> came from the Neutral Red test.

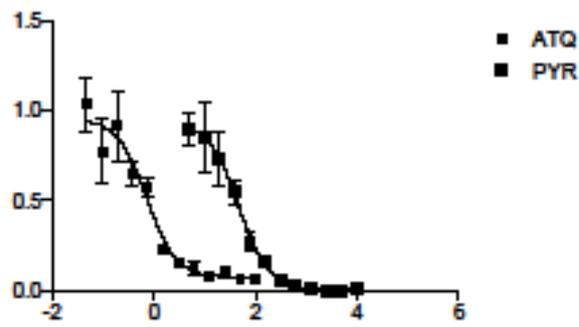
## **B) Malaria parasite growth assays:**

The compounds to be tested were diluted 1:500 with sterile screening media [RPMI (without phenol red, with L-glutamine), 4.16 mg/mL albumax, 0.013 mg/mL hypoxanthine, 1.73 mg/mL glucose, 0.18% NaHCO<sub>3</sub>, 0.031 M HEPES, 2.60 mM NaOH, 0.043 mg/mL gentamicin] from the stock solution. 150 µL were added to the first column of the screening plate (white with clear bottom). Into the subsequent columns (2 to 12) 75 µL sterile screening medium were loaded. A 1:2 dilution of the compounds throughout the plate was achieved by mixing 75 µL of the first column with 75 µL of the subsequent column, and so on. The parasitemia was adjusted to 0.3% and the hematocrit to 5% in screening media. 75 µL of this suspension was added to each well, resulting in a final hematocrit of 2.5% and a compound dilution of 1:1000. The assay plates were transferred to off-line incubators that contained airtight incubation units. The units were gassed daily with 93% nitrogen, 4% carbon dioxide, and 3% oxygen during the 72-hour incubation at 37 °C. 30 µL of detection reagent, consisting of 10x SYBR Green I (Invitrogen; supplied in 10,000x concentration) in lysis buffer (20 mM Tris·HCl, 5 mM EDTA, 0.16% Saponin weight/vol, 1.6% Triton X vol/vol) was dispensed into the assay plates. The assay plates were left at room temperature for 24 hours and read off-line by using several Acquest GT multimode readers (Molecular Devices).

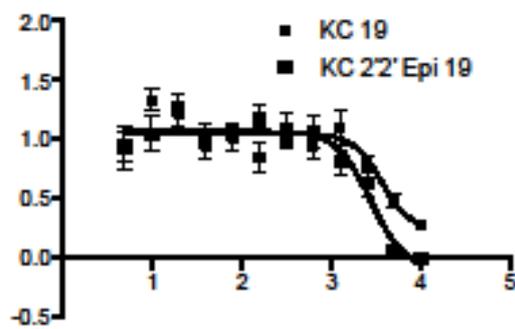
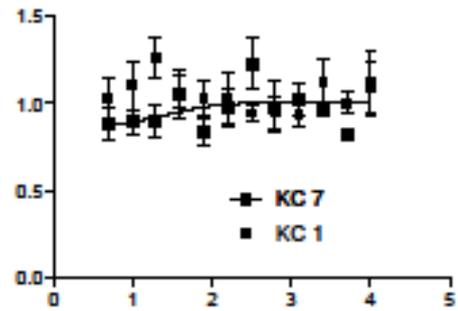
The starting concentration was 10 µM for all compounds and dilution was by 1:2 steps. Each compound was run in duplicates on a plate and all plates were duplicated, so an average and mean from 4 measurements for each data point was obtained. As controls atovaquone (ATQ, starting concentration 100 nM) and pyrimethamine (PYR, 10 µM) were used.

The EC<sub>50</sub> assays were repeated with a higher starting concentration of 30 µM for all compounds.

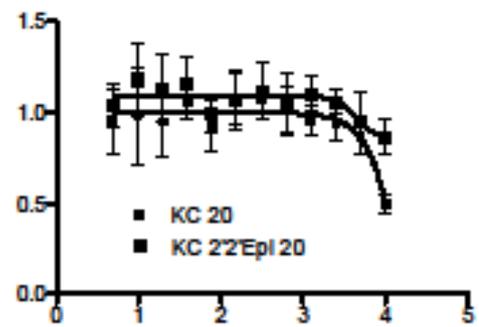
Transform of controls



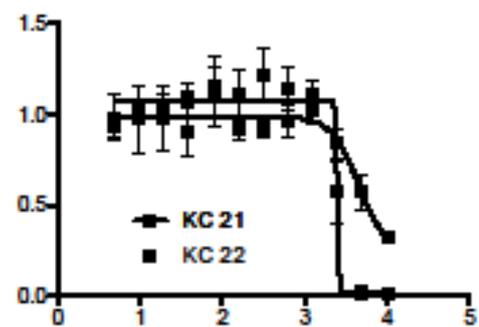
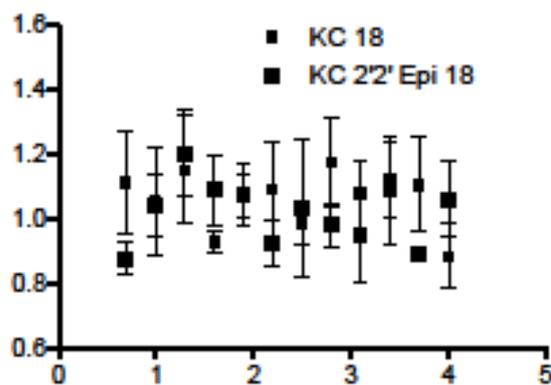
ATQ	PYR
0.7300	47.35



KC 19	KC 2'2'Epi 19
3602	2739

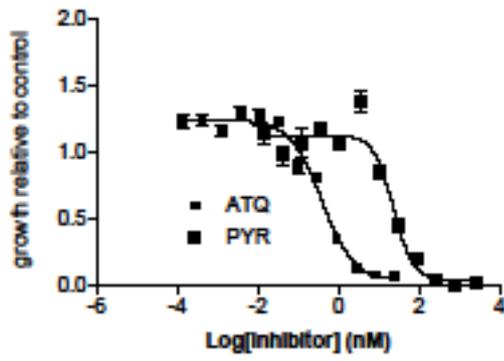


KC 20	KC 2'2'Epi 20
1.229e+006	4389

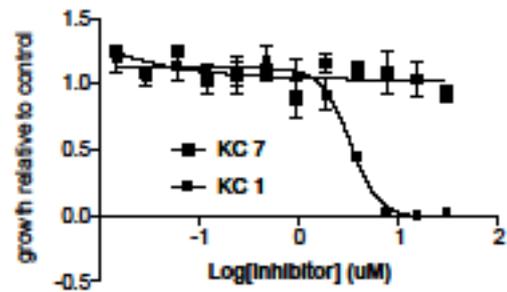


KC 21	KC 22
~ 2506	4536

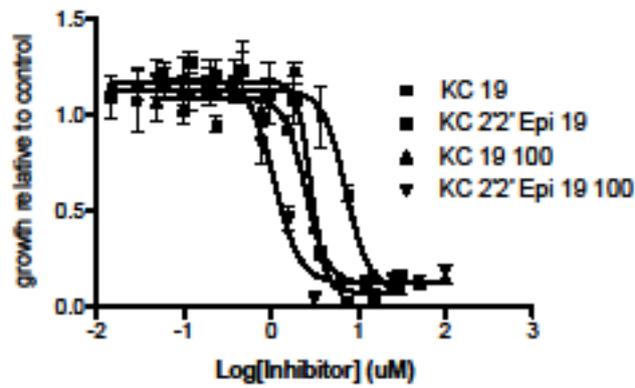
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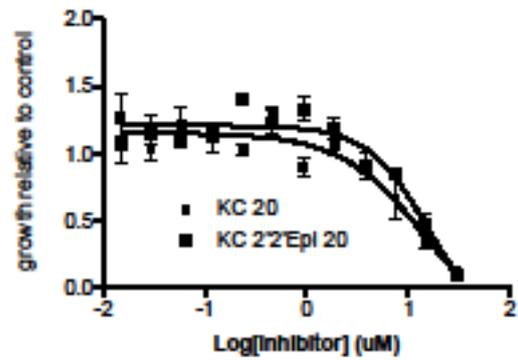
ATQ	PYR
0.3693	23.60



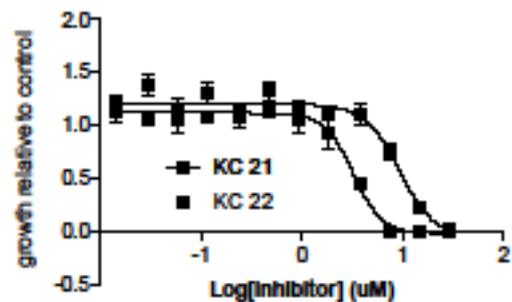
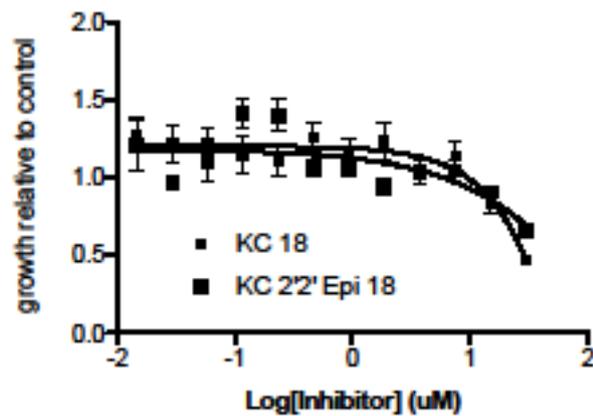
	KC 1
IC50	3.241



KC 19	KC 2'2'Epi 19
7.230	2.870
KC 19 100	KC 2'2'Epi 19 100
2.495	1.092



KC 20	KC 2'2'Epi 20
15.27	15.79



KC 21	KC 22
3.241	9.382

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