Synthesis and HDAC Inhibitory Activity of Largazole Analogs: Alteration of the Zinc-Binding Domain and Macrocyclic Scaffold

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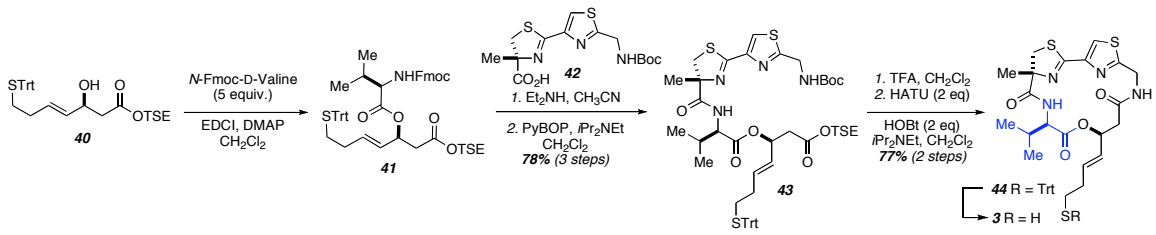
Supporting Information



(3*S*,4*E*)-2-(Trimethylsilyl)ethyl-3-[(*R*)-2-((*R*)-2-{2-[(*tert*-butoxycarbonyl)methyl]thiazol-4-yl}-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyloxy]-7-(tritylthio)hept-4-enoate (43). 0.104g (0.2 mmol) of β-hydroxy ester **40**[1] and 0.340g (1.0 mmol, 5 equiv.) *N*-Fmoc-D-valine were dissolved in 5 mL dry CH_2Cl_2. The reaction was cooled to 0º C and 0.192g (1.0 mmol, 5 equiv.) EDCI and 0.003g (0.02 mmol, cat.) DMAP were added in ~5 mL CH_2Cl_2, followed by 0.2 mL iPr$_2$NEt. The reaction was allowed to warm to room temperature and stirred over night, when TLC showed complete disappearance of **40**. The reaction was concentrated and passed through a short plug of silica, washing with 100% EtOAc. The product diester eluted with a by-product from the excess amino acid used, which was not separated at this time. Instead, the crude diester was taken up in 15 mL CH_3CN (to ~0.01M) and treated with 1 mL diethylamine (to ~0.2M). The resulting solution was stirred for two hours and then concentrated, taken up in EtOAc, and concentrated again.

0.061g (0.22 mmol, 1.1 equiv.) acid **42** was dissolved in 5 mL dry CH_2Cl_2 and treated with 0.151g (0.44 mmol, 2.0 equiv) PyBOP and 0.076 mL (0.66 mmol, 3.0 equiv.) iPr$_2$NEt. After stirring for ~5 min., the crude amine in 5 mL CH_2Cl_2 was added to the mixture dropwise. After 2hr., the reaction was concentrated and submitted immediately to column chromatography, 0.056g (0.058 mmol, 30% from **40**) of peptide **43** eluting

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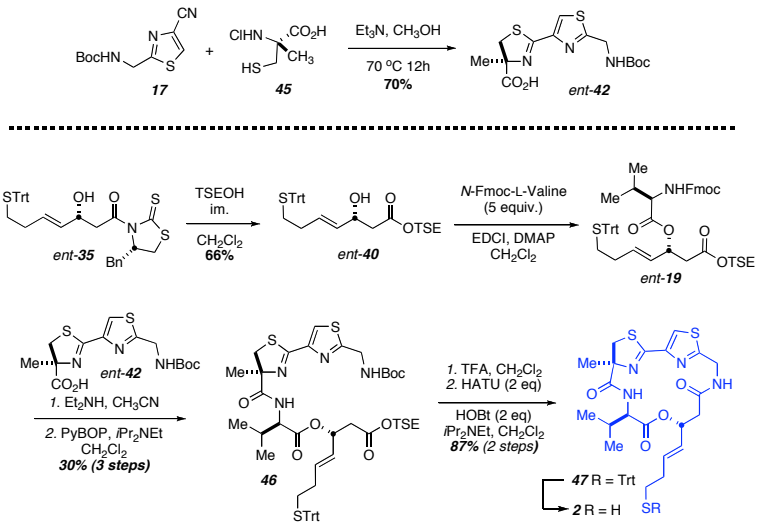
cleanly in (2:1 hexanes:EtOAc). Clear oil. $[\alpha]^{24}_D$: -6.0 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 9H), 0.88 (d *J* = 6.9 Hz, 3H), 0.95 (d *J* = 6.9 Hz, 3H), 1.32-1.38 (m, 2H), 1.47 (s, 9H), 1.59 (s, 3H), 1.96-2.05 (m, 2H), 2.12-2.21 (m, 3H), 2.45 (dd *J* = 5.1, 15.9 Hz, 1H), 2.59 (dd *J* = 8.4, 15.9 Hz, 1H), 3.29 (d *J* = 11.4 Hz, 1H), 3.73 (d *J* = 11.4 Hz, 1H), 4.05-4.17 (m, 2H), 4.52 (dd *J* = 4.5, 9.0 Hz, 1H), 4.63 (d *J* = 6.3 Hz, 1H), 5.26-5.35 (m, 2H), 5.54-5.61 (m, 2H), 7.18-7.39 (m, 16H), 7.93 s, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ -1.3, 11.3, 12.0, 17.5, 17.6, 18.8, 19.2, 19.3, 25.4, 28.5, 29.9, 31.2, 31.5, 31.7, 39.8, 41.2, 42.1, 42.5, 53.7, 57.0, 63.3, 66.8, 71.8, 80.6, 85.3, 121.9, 126.8, 127.8, 128.0, 129.7, 133.4, 145.0, 148.8, 155.8, 163.5, 169.9, 170.5, 174.7. HRMS (ESI): *m/z* calcd. for $C_{50}H_{64}N_4NaO_7S_3Si$ $(M + Na)^+$ 979.35986, found 979.35980.

General procedure for macrocyclization. 0.056g (0.058 mmol) Acyclic precursor **43** was dissolved in 5 mL CH_2Cl_2 (to ~0.03M), cooled to 0 ºC and treated with 1 mL TFA (to ~0.6M). The reaction was allowed to warm to room temperature and stirred overnight. Solvents were evaporated and the crude amino acid redissolved in toluene and concentrated a second time to remove residual TFA. The crude amino acid was then taken up in ~5 mL CH_2Cl_2 and added dropwise to a stirred solution of 0.061 mL (6.0 equiv.) *i*Pr_2Net in 60 mL dry CH_3CN (to ~0.001M). The resulting moderately opaque solution was allowed to stir ~10 min., before 0.044g (0.12 mmol, 2 equiv.) HATU and 0.016g (0.12 mmol, 2 equiv.) HOBt were added dropwise in ~5 mL CH_3CN. The reaction was allowed to stir for 16 hr., then concentrated and submitted immediately to column chromatography. Macrocycle **44** (0.020g, 57% yield) eluted quickly in EtOAc, after a general wash with 10:1 hexanes: EtOAc. Clear oil. $[\alpha]^{24}_D$: +16.1 (c = 1, CH_3OH). ^{1}H NMR (300 MHz, 5:1 $CDCl_3$:CD_3OD) δ 0.70 (d *J* = 6.6 Hz, 3H), 0.80 (d *J* = 6.6 Hz, 3H), 1.71 (s, 3H), 1.90-2.03 (m, 3H), 2.05-2.12 (m, 2H), 2.41 (d *J* = 16.8 Hz, 1H), 2.80 (dd *J* = 10.5, 16.8 Hz, 1H), 3.14 (d *J* = 11.4 Hz, 1H), 4.08-4.19 (m, 3H), 4.90 (d *J* = 17.1 Hz, 1H), 5.21 (dd *J* = 8.4, 15.3 Hz, 1H), 5.57-5.71 (m, 2H), 7.09-7.22 (m, 10H), 7.28-7.32 (m, 6H), 7.67 (s, 1H). ^{13}C NMR (100.6 MHz, 5:1 $CDCl_3$:CD_3OD): δ 18.0, 18.8, 26.4, 31.0, 31.4, 32.2, 38.7, 39.9, 40.7, 41.5, 59.8, 59.9, 66.8, 73.0, 77.5, 84.6, 125.0, 126.8, 127.9, 128.0, 129.7, 135.3, 144.9, 147.1, 163.8, 167.9, 168.5, 170.4, 174.0. HRMS (ESI): *m/z* calcd. for $C_{40}H_{42}N_4NaO_4S_3$ $(M + Na)^+$ 761.22604, found 761.22478.

General procedure for trityl deprotection. 0.010g (0.04 mmol) *S*-Trityl macrocycle **44** was dissolved in 5 mL dry CH_2Cl_2 and cooled to 0 ºC. The mixture was successively treated with 0.017 mL (0.08 mmol, 2 equiv.) *i*Pr_3SiH and 0.200 mL TFA (to ~0.2M in **44**). The reaction mixture was allowed to warm to room temperature and stirred for 2hr., before being concentrated and chromatographed (EtOAc) to provide 0.019g (0.038 mmol, 95%) thiol **9**. Clear oil. $[\alpha]^{24}_D$: +11.0 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (d *J* = 6.9 Hz, 3H), 0.97 (d *J* =6.9 Hz, 3H), 1.42 (t *J* = 7.8 Hz, 1H), 2.10-2.18 (m, 1H), 2.29-2.40 (m, 2H), 2.53-2.63 (m, 3H), 2.82-2.94 (m, 1H), 3.22 (d *J* = 11.4 Hz, 1H), 4.23-4.35 (m, 3H), 5.10 (dd *J* = 7.8, 16.5 Hz, 1H), 5.45 (dd *J* = 8.7, 15.9 Hz, 1H), 5.82 (t *J* = 9.9 Hz, 1H), 5.90 (dt *J* = 7.8, 15.3 Hz, 1H), 6.43 (s, 1H), 7.25 (s, 1H), 7.72 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.3, 19.1, 23.9, 26.7, 32.5, 36.6, 38.8, 40.6, 41.0, 41.9, 59.9, 72.9, 85.0, 124.8, 124.9,

129.1, 134.8, 167.9, 168.1, 169.8, 173.4. HRMS (ESI): *m/z* calcd. for $C_{21}H_{28}N_4NaO_4S_3$ $(M + Na)^+$ 519.11649, found 519.11777.

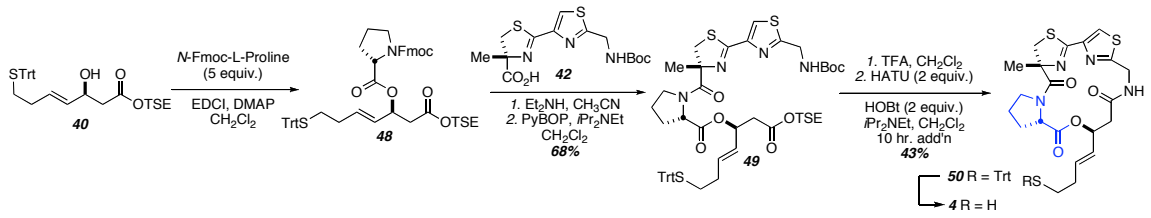


(*S*)-2-(2-((*tert*-Butoxycarbonylamino)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylic acid (*ent*-42). To a solution of sodium bicarbonate (0.65 g, 7.68 mmol) in CH_3OH (22 ml) and pH 7 phosphate buffer (14.4 ml) was added **17** and **45**. The mixture was stirred overnight at 70 °C and then cooled to room temperature. The solvent was evaporated, and the residue dissolved in ether and water. Following extraction into ether, the organic layers were discarded and the aqueous layer was acidified to pH 2 with 3 N HCl. This was then extracted into EtOAC (3 x 20 ml), washed with brine, and dried over sodium sulfate to give ***ent*-42** as a light brown foam (0.96 g, 70%yield). $[\alpha]^{24}_D$: +22.0 (c = 1, CH_3OH). Both ^1H and ^{13}C NMR spectra of ***ent*-42** matched our previously published spectra of **42** itself.

(*R*,*E*)-2-(Trimethylsilyl)ethyl 3-hydroxy-7-(tritylthio)hept-4-enoate (*ent*-40). To a stirred solution of ***ent*-35** (0.88 g, 1.44 mmol) in CH_2Cl_2 (14 ml) was added 2-(trimethylsilyl)ethanol (2 ml, 14.4 mmol) and imidazole (0.15 g, 2.16 mmol). The mixture was stirred overnight, after which the solvent was evaporated and the residue purified by column chromatography (10:1 to 4:1 hexanes/ethyl acetate) to give ***ent*-40** as a clear oil (0.49 g, 66%). $[\alpha]^{24}_D$: +5.0 (c = 2, $CHCl_3$). Both ^1H and ^{13}C NMR spectra of ***ent*-40** matched our previously published spectra of **40** itself.

(3*R*,4*E*)-2-(Trimethylsilyl)ethyl-3-[(*S*)-2-((*S*)-2-{2-[(*tert*-butoxycarbonyl)methyl]thiazol-4-yl}-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyloxy]-7-(tritylthio)hept-4-enoate (46). **46** was prepared from ***ent*-40** (0.14g, 0.28 mmol) in the same fashion as **43**, to give **46** in 50% yield (0.12 g, 0.14 mmol). $[\alpha]^{24}_D$: +20.0, (c = 0.2, $CHCl_3$. Both ^1H and ^{13}C NMR spectra of **43** matched our previously published spectra of ***ent*-43**.

(-)-Largazole thiol (2). The general procedure for both cyclization and deprotection described above was followed to give trityl protected macrocycle **47** in 87% yield (0.07 g, 0.14 mmol), $[\alpha]_D$ = -6, c=0.0.1 in methanol. E*nt*-Largazole (**2**) was completed in 90% yield (0.03 g, 0.05 mmol), $[\alpha]_D$ = -21.0 (c = 0.1, $CHCl_3$). ^1H and ^{13}C NMR of both **47** and **2** match those of (+)-Largazole.

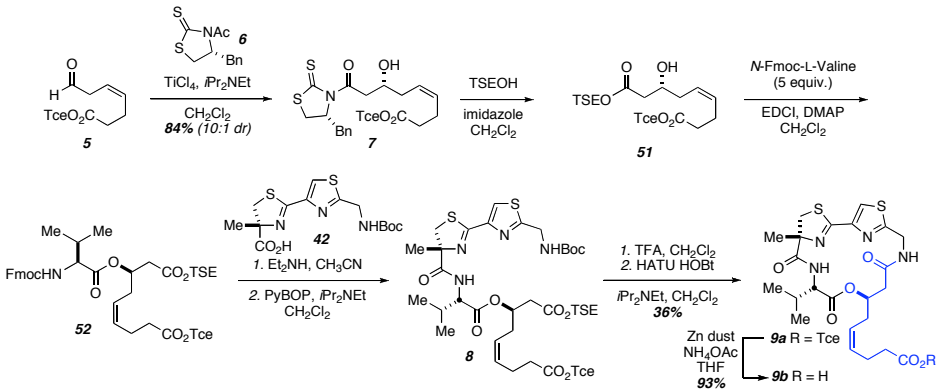


Acyclic precursor 49. 0.185g (0.36 mmol, 1.0 equiv.) of β-hydroxy ester **40** and 0.601g (1.8 mmol, 5 equiv.) *N*-Fmoc-L-proline were dissolved in 10 mL dry CH_2Cl_2. The reaction was cooled to 0º C and 0.341g (1.8 mmol, 5 equiv.) EDCI and 0.004g (0.036 mmol, 0.1 equiv.) DMAP were added in ~5 mL CH_2Cl_2, followed by 0.370 mL (2.1 mmol, 6 equiv.) *i*Pr_2NEt. The reaction was allowed to warm to room temperature and stirred over night, when TLC showed complete disappearance of β-hydroxy ester **40**. The reaction was concentrated and submitted immediately to column chromatography. 0.234g (0.28 mmol, 78% yield) Fmoc-protected diester **48** eluted in 4:1 hexanes:EtOAc.

0.100g (0.12 mmol, 1.0 equiv.) Fmoc-protected diester **48** was taken up in 12 mL CH_3CN (to ~0.01M) and treated with 0.600 mL diethylamine (to ~0.2M). The resulting solution was stirred for 2 hr. and then concentrated, taken up in EtOAc, reconcentrated, and dried on a mechanical pump to remove residual diethylamine. Meanwhile, 0.046g (0.13 mmol, 1.1 equiv.) acid **42** was dissolved in 5 mL dry CH_2Cl_2 and treated with 0.124g (0.24 mmol, 2.0 equiv) PyBOP and 0.0.62 mL (0.36 mmol, 3.0 equiv.) *i*Pr_2NEt. After stirring for ~5 min., the crude amine in 10 mL CH_3CN was added to the mixture dropwise. After 2hr., the reaction was assumed complete, concentrated, and submitted immediately to column chromatography. 0.078g (0.081 mmol, 68% yield) of acyclic precursor **49** eluted cleanly in 2:1 hexanes:EtOAc. **(49):** Clear oil. Compound NMRs display highly complex mixtures of conformational isomers; the room temperature ^1H NMR spectrum (300 MHz, $CDCl_3$) and ^{13}C NMR spectrum (100.6 MHz, $CDCl_3$), as well as elevated temperature ^1H NMR spectra (300 MHz, DMSO-d_6) are illustrated below. HRMS (ESI): *m/z* calcd. for $C_{50}H_{62}N_4NaO_7S_3Si$ $(M + Na)^+$ 977.34476, found 977.34522.

***S*-Trityl macrocycle 50 and thiol 4.** 0.091g (0.095 mmol, 1.0 equiv.) acyclic precursor **49** was dissolved in 5 mL (to ~0.03M in substrate) dry CH_2Cl_2 at 0 °C and treated with 1 mL (to ~0.3M in substrate) TFA. The mixture was then warmed to room temperature and stirred overnight. The solvents were removed *in vaccuo*. The crude salt was dissolved in toluene, concentrated, and dried on mechanical pump to remove residual TFA. It was then dissolved in 5 mL dry CH_2Cl_2 and added dropwise to a solution of 0.100 mL (0.57 mmol, 6.0 equiv.) *i*Pr_2NEt in 10 mL CH_3CN at 0 °C. The solution was stirred ~0.5 hr., then taken up in syringe and added via syringe pump over 10 hr. to a solution of 0.072g (0.19 mmol, 2.0 equiv.) HATU, 0.026g (0.19 mmol, 2.0 equiv.), and 0.100 mL

(0.57 mmol, 6.0 equiv.) *i*Pr_2NEt in 100 mL (to ~0.001M) CH_3CN. Upon completion of the addition, the solution was stirred a further 6 hr., then concentrated and redissolved in ~2 mL CH_2Cl_2. Solids were removed by filtration through a cotton plug and the product macrocycle was purified via chromatotron. 0.030g (0.041 mmol, 43% yield) macrocycle **50** eluted in 30:1 CH_2Cl_2:CH_3OH. **(50):** $[\alpha]^{24}_D$: +29.1 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 3H), 1.56-1.60 (m, 2H), 1.70-1.79 m, 2H), 1.89-1.95 (m, 1H), 2.03-2.19 (m, 4H), 2.26 (d *J* = 17.6 Hz, 1H), 2.56 (dd *J* = 2.8, 14.0 Hz, 1H), 2.81 (dd *J* = 5.2, 14.0 Hz, 1H), 3.51 (dd *J* = 11.6, 16.4 HZ, 1H), 3.52 (d *J* = 11.6 Hz, 1H), 3.76-3.80 (m, 1H), 3.86 (d *J* = 11.6 Hz, 1H), 3.88 (dd *J* = 4.8, 17.7 Hz, 1H), 4.72 (dd *J* = 6.4, 17.6 Hz, 1H), 5.12 (dd *J* = 2.8, 8.4 Hz, 1H), 5.21-5.25 (m, 1H), 5.70 (dt *J* = 6.4, 16.0 Hz, 1H), 5.91 (dd *J* = 5.6, 16.0 Hz, 1H), 7.15-7.23 (m, 10H), 7.59 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.7, 31.5, 31.8, 32.5, 41.1, 42.8, 44.9, 49.6, 60.8, 67.0, 73.0, 77.4, 86.9, 124.0, 127.0, 128.1, 128.5, 129.8, 130.6, 130.8, 144.7, 147.9, 158.4, 167.2, 170.1, 172.3, 173.8. HRMS (ESI): *m/z* calcd. for $C_{40}H_{40}N_4NaO_4S_3$ $(M + Na)^+$ 759.21039, found 759.21059. 0.009g **50** was deprotected according to the general procedure to provide **4**, which was purified by preparative thin layer chromatography.



(*R*,*Z*)-2,2,2-trichloroethyl 9-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-7-hydroxy-9-oxonon-4-enoate 7. A solution of the chiral auxiliary (887mg, 3.53 mmol) in CH_2Cl_2 (28.5 mL) was cooled to 0°C, followed by addition of $TiCl_4$ (0.47 mL, 4.39 mmol). The reaction was allowed to stir for 5 minutes then cooled to -78°C, before *i*Pr_2NEt (0.76 mL, 4.37 mmol) were slowly added and stirred for 2 hours. The aldehyde was dissolved in CH_2Cl_2 (2.2 mL) and added drop wise to the auxiliary solution, then stirred for 1.5 hours. The reaction was quenched with saturated *aq* NH_4Cl, and diluted with CH_2Cl_2, and warmed to room temperature. The reaction was extracted with CH_2Cl_2, then washed with brine and dried over Na_2SO_4, filtered and condensed. Purification was accomplished with silica gel chromatography (30% EtOAc/Hex) to afford yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) d 3.83m, 1H), 1.23 (s, 1H), 2.35 (m, 2 H), 2.45 (m, 2H), 2.53 (m, 2H), 2.88 (dd, 3.2, 11.6 Hz, 1 H), 3.02 (dd, 10.4, 13.2 Hz, 1 H), 3.17 (m, 2 H), 3.42 (m, 2 H), 3.62 (dd, 2.8, 17.6 Hz, 1 H) 4.15 (m, 2 H), 4.73 (s, 2H), 5.37 (m, 1 H), 5.52 (m, 2 H), 7.30 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$) d 22.8, 29.8, 32.2, 33.8, 34.3, 34.5, 36.9, 45.1, 45.5, 67.7, 68.2, 68.4, 68.5, 95.1, 126.8, 127.4, 129.1, 129.6, 130.5, 136.5, 169.1, 171.6, 173.1, 173.7. HRMS (ESI): *m/z* calcd. for $C_{21}H_{25}Cl_3NO_4S_2$ $(M + H)^+$ 524.02742, found 524.02851 $[a]_D$ = -74.2 (c 2, $CHCl_3$)

(*R*,*Z*)-1-(2,2,2-trichloroethyl) 9-(2-(trimethylsilyl)ethyl) 7-hydroxynon-4-enedioate 51. The alcohol (764 mg, 1.45 mmol) dissolved in CH_2Cl_2 (2.9 mL) was treated with imidazole (148mg, 2.175 mmol) followed by the addition of 2-(trimethylsilyl)ethanol (2.08 mL, 14.5 mmol). The reaction was stirred overnight, then condensed and purified by silica gel chromatography (30% EtOAc/ Hex) to give the protected β-hydroxy acid as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) ḍ0.04 (s, 9 H), 0.99 (m, 2H), 2.30 (m, 2 H), 2.45 (m, 4 H), 2.54 (m, 2 H), 4.06 (m, 1H), 4.20 (m, 2 H), 4.73 (s, 2H), 5.52 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) d -.127, 17.5, 22.8, 33.8, 34.5, 41.0,63.3, 67.9, 74.1, 95.1, 126.8, 130.3 171.7, 173.2. $[a]_D$ = -1.7 (c 2, $CHCl_3$)

(*R*,*Z*)-1-(2,2,2-trichloroethyl) 9-(2-(trimethylsilyl)ethyl) 7-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)non-4-enedioate 52. The protected acid (97mg, 0.224 mmol) and N-Fmoc-L-Val (380mg, 1.123 mmol) were dissolved in CH_2Cl_2 (4.08 mL) and cooled to 0°C. EDCI (258 mg, 1.347 mmol) and DMAP (2.7mg, 0.0225 mmol) were dissolved in CH_2Cl_2 (1.02 mL) and added to the cooled reaction followed by the slow addition of *i*Pr_2NEt (0.23 mL, 1.347 mmol). The reaction was allowed to warm to room temperature and stirred over night, then condensed and purified with column chromatography (30% EtOAc/ Hex) ^{1}H NMR (300 MHz $CDCl_3$) d̃ḍ3s, 9 H), 0.88 (d, 3 H), 0.97 (m, 5 H) 2.15 (m, 1 H), 2.43 (m, 3 H), 2.50 (d, 2 H), 2.59 (m, 3 H), 4.25 (m, 3 H), 4.38 (m, 2 H), 4.73 (s, 2H), 5.32 (m, 2 H) 5.43, (m, 1 H), 5.53 (m, 1 H), 7.35 (m, 4 H), 7.60 (d, 2 H), 7.76 (d, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) d -1.27, 17.5, 19.2, 22.8, 31.4, 31.7, 33.7, 38.7, 47.4, 59.1, 63.3, 67.2, 71.5, 71.6, 74.1, 95.1, 120.2, 125.1, 125.3, 127.2, 127.9, 131.1, 131.2, 141.5, 143.9, 144.1, 156.3, 156.4. ($M+Na^=$) calcd for $C_{36}H_{46}Cl_3NO_8Si$ 753.20627, found 776.19549. $[a]_D$ = 0.0 (c 2, $CHCl_3$)

(*R*,*Z*)-1-(2,2,2-trichloroethyl) 9-(2-(trimethylsilyl)ethyl) 7-((*S*)-2-((*R*)-2-(2-((*tert*-butoxycarbonylamino)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyloxy)non-4-enedioate 8. The protected amine (118mg 0.157 mmol) was dissolved in CH_2Cl_2 (7.85 mL) and treated with Et_2NH, then allowed to stir for 2 hrs. The reaction was concentrated, then taken up in EtOAc and re-concentrated again to remove any left over Et_2NH. The thiazolinethiazole (61.7 mg, 0.172 mmol) and PyBOP (164mg, .471 mmol) were dissolved in CH_2Cl_2 (2.87 mL) and treated with *i*Pr_2NEt and allowed to stir for 5 minutes. Then the crude amine in CH_3CN (1.42 mL) was slowly added to the PyBOP solution and allowed to stir overnight. The reaction was then condensed and purified with column chromatography (30-50% EtOAc/ Hex). ^{1}H NMR (300 MHz $CDCl_3$) d̃ḍ3s, 9 H), 0.82 (d, *J*= 3 H), 0.88 (m, 3 H), 0.97 (m, 2 H), 1.47 (s, 9 H), 1.62 (s, 3 H), 2.15 (m, 1 H), 2.45 (m, 3 H), 2.57 (m, 5 H), 3.34 (d, *J*= 11.4 1 H), 3.81 (d, *J*= 11.4, 1 H), 4.15 (m, 2 H), 4.48 (m, 1H), 4.63 (d, *J*= 6.3 Hz, 2 H), 4.74 (s, 2 H), 5.31 (m, 2 H), 5.43 (m, 1 H), 5.54 (m, 1 H), 8.06 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) d -1.3, 17.5, 17.7, 19.3, 22.8, 24.9, 28.5, 31.2, 33.7, 38.7, 38.8, 41.6, 42.6, 51.4, 57.3, 63.3, 71.5, 74.1, 80.7, 85.0, 95.1, 125.1, 125.3, 131.0, 131.1, 148.5, 155.9, 170.3, 170.4, 170.8, 170.9, 171.4, 171.5, 174.4. ($M+H^=$) calcd for $C_{35}H_{53}Cl_3N_4O_8S_2Si$ 870.21108, found 871.21836. $[a]_D$ = -19.86 (c 2, $CHCl_3$)

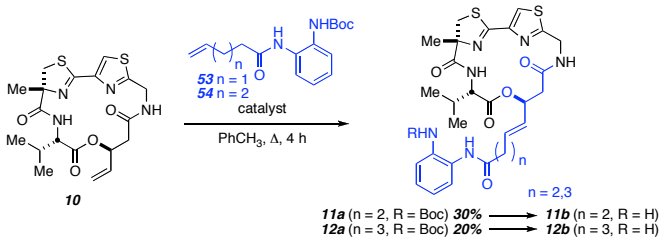
Formation of the Trichloroethyl ester protected Largazole Azumamide hybrid 9a. The acyclic precursor (77mg, 0.088 mmol) was dissolved in CH_2Cl_2 (2.95 mL) and cooled to 0°C. TFA (0.15 mL) was slowly added to the cooled solution. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was then condensed, and re-dissolved in toluene and condensed again to remove any excess TFA. The crude amino acid was dissolved in CH_2Cl_2 (4.4 mL) then cooled to 0°C and treated with *i*Pr_2NEt (0.093mL, 0.53 mmol) and stirred for 30 min. In a separate flask HOBt (23 mg, 0.177 mmol), HATU (67 mg, 0.177 mmol) were dissolved in CH_3CN (88.7 mL) and treated with *i*Pr_2NEt (0.093 mL, 0.53 mmol). The crude amino acid solution was then added via syringe pump addition to the HATU solution in a 10-hour addition. The reaction was allowed to stir for an additional 6 hours before solvents were removed and purified with silica gel chromatography (30%-50% EtOAc/ Hex). ^{1}H NMR (300 MHz $CDCl_3$) ḍ0. 47 d, *J*= 6.9 Hz, 3 H), 0.69 (d, *J*= 8.7 Hz, 3 H), 1.24 (s, 1H), 1.87 (s, 3 H), 2.13 (m, 1 H), 2.43 (m, 3 H), 2.52 (d, *J*= 6.6 Hz, 2 H), 2.72 (m, 4 H), 3.29 (d, *J*= 12.3 Hz, 1 H), 4.05 (d, *J*= 11.4 Hz, 1 H), 4.27 (dd, *J*= 3, 17.7 Hz, 1 H), 4.64 (dd, *J*= 3, 9.3 Hz, 1 H), 4.75 (s, 2 H), 5.25 (m, 2 H), 5.40 (m, 1 H), 5.53 (m, 1 H), 6.33 (m, 1 H), 7.10 (d, *J*= 9.3 Hz, 1 H), 7.78 (s, 1 H) ^{13}C NMR (75 MHz, $CDCl_3$) d 14.4, 16.5, 18.5, 18.7, 19.3, 22.8, 24.1, 24.9, 29.4, 31.2, 32.7, 33.7, 33.9, 34.3, 35.5, 39.2, 41.2, 43.4, 43.7, 57.8, 59.7, 60.6, 72.5, 74.1, 83.8, 95.1, 110.5, 118.4, 124.9, 125.1, 127.3, 131.2, 146.8, 167.0, 168.7, 169.5, 170.6, 171.5, 173.2. ($M+H^=$) calcd for $C_{25}H_{31}Cl_3N_4O_6S_2$ 675.0637, found 675.06428 $[a]_D$ = -0.94 (c 2, $CHCl_3$)

Largazole Auzumamide E Hybrid 9b. The macrocycle (40) (9.8 mg, 0.015 mmol) was dissolved in dry THF (0.5 mL, 0.03M) and vigorously stirred. Then Zn dust (35mg, 36 mmol) was added to the solution followed by 1M NH_4OAc (0.083 mL, .18M) and allowed to stir for 24 hours under argon. The reaction was then filtered and taken up in EtOAc and washed with 5% aq $KHSO_4$ (2x 2 mL) and Brine (2x 2 mL) then dried over Na_2SO_4, filtered and solvents removed. Purification by PTLC (MeOH / CH_2Cl_2 10%). ^{1}H NMR (300 MHz $CDCl_3$) ḍ0.47 (d, *J*= 6.9 Hz, 3 H), 0.69 (d, *J*= 6.9 Hz, 3 H), 0.85 (m, 2 H), 1.88 (s, 3 H), 2.14 (m, 1 H), 2.41 (m, 5 H), 2.71 (m, 4 H), 3.29 (d, *J*= 11.4 Hz, 1 H), 4.06 (d, *J*=11.4 Hz, 1 H), 4.27 (dd, *J*= 3.3, 17.4 Hz, 1 H), 4.45 (d, *J*= 6.0 Hz, 2 H), 4.64 (dd, *J*= 3.0, 9.3 Hz, 1 H), 5.29 (m, 2 H), 5.42 (m, 1H), 5.51 (m, 1 H), 5.85 (t, *J*= 6.0 Hz, 1 H), 6.40 (m, 1 H), 7.11 (d, *J*= 9.3 Hz, 1 H), 7.79 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) d 14.4, 16.6, 19.2, 21.8, 24.2, 29.9, 31.1, 33.7, 39.3, 41.3, 43.5, 57.8, 60.6, 68.4, 68.6, 72.4, 124.8, 131.4, 168.2, 169.6, 170.2, 172.1, 173.6. ($M-H^=$) calcd for $C_{23}H_{30}N_4O_6S_2$ 521.1534, found 521.15292. $[a]_D$ = +10.107 (c 2, $CHCl_3$)

General procedure for the cross-metathesis reactions. Adapting the procedure described by Luesch and co-workers,[2] macrocycle **10** was dissolved in the indicated solvent (to ~0.026M) and heated to reflux under argon. Solutions of sacrificial olefin (2.0 equiv., ~0.26M) and catalyst (0.2 equiv., ~0.052M) were then added to the reaction. The resulting mixture was stirred at reflux for a further 3 hr., with equivalent portions of olefin (2.0

[2] Taori, K.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 13506-13506.

equiv., ~0.26M in toluene) and catalyst (0.2 equiv., ~0.052M in toluene) being added each hour. After the last addition of olefin and catalyst, the reaction was refluxed for 1 hr. and then cooled to room temperature. Several drops of DMSO were added and the mixture was stirred overnight. Concentration *in vaccuo*, followed by column chromatography provided the substituted olefins as products.



Boc-protected benzamide 11a and amine 11b. According to the general procedure, 0.025g (0.057 mmol) macrocycle **10** was combined with olefin **53**[3] to yield 0.012g (0.017 mmol, 30% yield) compound **11a**, which eluted slowly in 100% EtOAc. Clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.62 (d *J* = 6.9 Hz, 3H), 0.73 (d *J* = 6.9 Hz, 3H), 1.53 (s, 9H), 1.87 (s, 3H), 1.98-2.00 (m, 1H), 2.42-2.65 (m, 5H), 2.77 (dd *J* = 6.0, 15.6 Hz, 1H), 3.31 (d *J* = 11.4 Hz, 1H), 3.91 (dd *J* = 4.2, 16.8 Hz, 1H), 4.00 (d *J* = 11.4 Hz, 1H), 4.54 (dd *J* = 4.8, 9.3 Hz, 1H), 4.86 (dd *J* = 8.1, 16.8 Hz, 1H), 5.69-5.79 (m, 2H), 5.86-5.97 (m, 1H), 6.61-6.67 (m, 1H), 6.90 (t *J* = 7.8 Hz, 1H), 6.98 (d *J* = 7.2 Hz, 1H), 7.08 (td *J* = 1.2, 8.1 Hz, 1H), 7.19 (d *J* = 9.0 Hz, 1H), 7.48 (d *J* = 7.8 HZ, 1H), 7.67 (s, 1H), 7.98 (bs, 1H), 8.40 (bs, 1H). HRMS (ESI): *m/z* calcd. for $C_{33}H_{42}N_6NaO_7S_2$ $(M + Na)^+$ 721.24541, found 721.24526. 0.010g Benzamide **11a** was deprotected in 1mL CH_2Cl_2 and 0.2mL TFA. After 2 hr., the solvents were removed and the product amine **11b** purified by preparative thin layer chromatography.

Boc-protected benzamide 12a and amine 12b. According to the general procedure, 0.039g (0.089 mmol) macrocycle **10** was combined with olefin **54** to yield 0.012g (0.017 mmol, 20% yield) compound **12a**, which eluted slowly in 100% EtOAc. Clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.59 (d *J* = 6.9 Hz, 3H), 0.73 (d *J* = 6.9 Hz, 3H), 1.50 (s, 9H), 1.86 (s, 3H), 1.99-2.00 (m, 1H), 2.15-2.32 (m, 3H), 2.32-2.41 (m, 3H), 2.66-2.73 (m, 2H), 3.30 (d *J* = 11.4 Hz, 1H), 4.02 (d *J* = 11.4 Hz, 1H), 4.28 (dd *J* = 3.6, 17.7 Hz, 1H), 4.57 (dd *J* = 4.5, 9.6 Hz, 1H), 4.99 (dd *J* = 6.9, 17.7 Hz, 1H), 5.61-5.72 (m, 2H), 5.86 (dt *J* = 7.2, 14.4 Hz, 1H), 6.59-6.66 (m, 1H), 7.01 (t *J* = 8.4 Hz, 1H), 7.11-7.16 (m, 3H), 7.26 (d *J* = 9.0 Hz, 1H), 7.49 (d *J* = 8.1 Hz, 1H), 7.67 (s, 1H), 8.48 (s, 1H). HRMS (ESI): *m/z* calcd. for $C_{34}H_{44}N_6NaO_7S_2$ $(M + Na)^+$ 735.26106, found 735.2609. 0.004g Benzamide **12a** was deprotected in 1mL CH_2Cl_2 and 0.2mL TFA. After 2 hr., the solvents were removed and the product amine **12b** purified by preparative thin layer chromatography.

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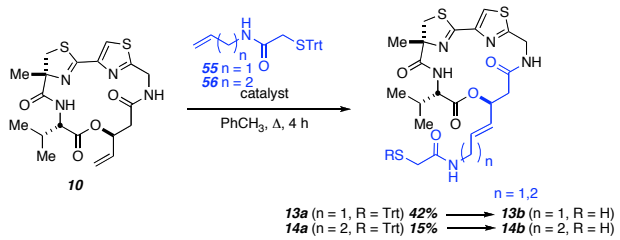

***S*-Trityl-α-thioamide 13a.** According to the general procedure, 0.060g (0.14 mmol) macrocycle **10** was combined with olefin **55**[4] in presence of the Hoveyda-Grubbs second generation catalyst in toluene to yield 0.046g (0.058 mmol, 42% yield) compound **13a**, which eluted in 10:1 CH_2Cl_2:CH_3OH. Clear oil. **(13a):** $[\alpha]^{24}_D$: +12.4 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (d *J* = 6.8 Hz, 3H), 0.69 (d *J* = 6.8 Hz, 3H), 1.81 (s, 3H), 1.96-2.10 (m, 3H), 2.64 (dd *J* = 3.6, 15.6 Hz, 1H), 2.76 (dd *J* = 8.8, 15.6 Hz, 1H), 3.03 (s, 2H), 3.25 (d *J* = 11.2 Hz, 1H), 3.52 (t *J* = 5.6 Hz, 1H), 4.00 (d *J* = 11..2 Hz, 1H), 4.25 (dd *J* = 3.2, 17.2 Hz, 1H), 4.55 (dd *J* = 4.0, 9.6 Hz, 1H), 5.20 (dd *J* = 8.8, 17.2 Hz, 1H), 5.49 (dd *J* = 6.4, 15.6 Hz, 1H), 5.63-5.96 (m, 1H), 5.71 (dt *J* = 4.2, 15.6 Hz, 1H), 6.00 (t *J* = 4.2 Hz, 1H), 6.50-6.52 (m, 1H), 7.15-7.29 (m, 9H), 7.37-7.39 (m, 6H), 7.70 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 14.3, 17.1, 19.1, 24.4, 29.9, 34.1, 35.9, 40.8, 41.2, 41.4, 43.5, 58.2, 68.0, 71.5, 127.3, 128.1, 128.4, 129.6, 130.0, 144.1, 168.4, 168.9, 169.3. HRMS (ESI): *m/z* calcd. for $C_{41}H_{43}N_5NaO_5S_3$ $(M + Na)^+$ 804.23185, found 804.23259.

Thiol 13b. According to the general procedure, 0.035g **13a** was deprotected to give 0.022g **13b** after preparative thin layer chromatography. **(13b):** $[\alpha]^{24}_D$: +6.1 (c = 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (d *J* =6.9 Hz, 3H), 0.71 (d *J* =6.9 Hz, 3H), 1.88 (s, 3H), 1.95 (t *J* = 9.0 Hz, 1H), 2.04-2.16 (m, 1H), 2.26-2.33 (m, 2H), 2.70 (dd *J* = 3.0, 16.2 Hz, 1H), 2.88 (dd *J* = 9.9, 16.2 Hz, 1H), 3.24-3.32 (m, 2H), 3.19 (d *J* = 8.7 Hz, 1H), 3.30 (d *J* = 11.4 Hz, 1H), 3.42-3.50 (m, 1H), 4.06 (d *J* = 11.4 Hz, 1H), 4.35 (dd *J* = 3.6, 17.7 Hz, 1H), 4.61 (dd *J* = 3.3, 9.3 Hz, 1H), 5.25 (dd *J* = 9.3, 17.7 Hz, 1H), 5.56 (dd *J* = 7.2, 15.3 Hz, 1H), 5.64-5.70 (m, 1H), 5.83 (dt *J* = 7.2, 15.3 Hz, 1H), 6.44 (d *J* = 6.0 Hz, 1H), 6.74 (s, 1H), 7.19 (d *J* = 9.6 Hz, 1H), 7.80 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.0, 19.1, 24.5, 28.5, 32.3, 34.4, 38.8, 40.8, 41.3, 43.6, 58.1, 72.6, 77.4, 84.6, 124.6, 129.8, 131.8, 147.7, 168.2, 169.4, 169.5, 169.6, 173.7. HRMS (ESI): *m/z* calcd. for $C_{23}H_{31}N_5NaO_5S_3$ $(M + Na)^+$ 576.13795, found 576.13795.

***S*-Trityl-α-thioamide 14a.** According to the general procedure, 0.052g (0.12 mmol) macrocycle **10** was combined with olefin **56** in presence of the Grubbs second generation catalyst in toluene to yield 0.014g (0.018 mmol, 15% yield) compound **14a**, which eluted in 10:1 CH_2Cl_2:CH_3OH. Clear oil. **(14a):** $[\alpha]^{24}_D$: +8.1 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.50 (d *J* = 6.9 Hz, 3H), 0.67 (d *J* = 6.9 Hz, 3H), 1.89 (s, 3H), 2.00-2.11 (m, 3H), 2.61 (dd *J* = 2.7, 16.5 Hz, 1H), 2.78 (dd *J* = 10.2, 16.5 Hz, 1H), 2.94-3.05 (m, 2H), 3.10 (s, 2H), 3.27 (d *J* = 11.4 Hz, 1H), 4.04 (d *J* = 11.4 Hz, 1H), 4.25 (dd *J* = 3.0, 17.7 Hz, 1H), 4.58 (dd *J* =3.6, 9.6 Hz, 1H), 5.25 (dd

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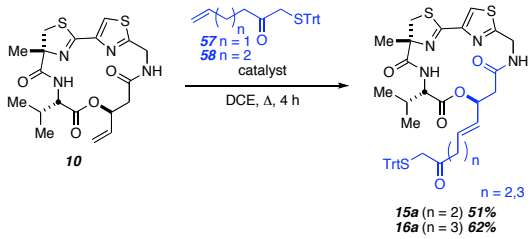
J = 9.3, 17.4 Hz, 1H), 5.43 (dd *J* = 6.9, 15.6 Hz, 1H), 5.57-5.63 (m, 1H), 5.69 (dt *J* = 6.9, 11.1 Hz, 1H), 6.06 (t *J* = 5.4 Hz, 1H), 6.33 (dd *J* = 3.0, 9.3 Hz, 1H), 7.14 (d *J* = 9.6 Hz, 1H), 7.20-7.37 (m, 9H), 7.38-7.42 (m, 6H), 7.75 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.9, 19.1, 24.4, 32.2, 34.4, 36.1, 38.9, 40.7, 41.3, 43.6, 58.0, 68.0, 72.4, 77.5, 84.5, 124.7, 127.3, 128.4, 129.2, 129.7, 131.8, 144.2, 147.6, 168.1, 169.1, 169.5, 173.7. HRMS (ESI): *m/z* calcd. for $C_{42}H_{45}N_5NaO_5S_3$ $(M + Na)^+$ 818.2475, found 818.2469.

Thiol 14b. According to the general procedure, 0.035g **14a** was deprotected to give 0.022g **14b** after preparative thin layer chromatography. **(14b):** $[\alpha]^{24}_D$: +15.6 (c = 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (d *J* =6.9 Hz, 3H), 0.71 (d *J* =6.9 Hz, 3H), 1.88 (s, 3H), 1.95 (t *J* = 9.0 Hz, 1H), 2.04-2.16 (m, 1H), 2.26-2.33 (m, 2H), 2.70 (dd *J* = 3.0, 16.2 Hz, 1H), 2.88 (dd *J* = 9.9, 16.2 Hz, 1H), 3.24-3.32 (m, 2H), 3.19 (d *J* = 8.7 Hz, 1H), 3.30 (d *J* = 11.4 Hz, 1H), 3.42-3.50 (m, 1H), 4.06 (d *J* = 11.4 Hz, 1H), 4.35 (dd *J* = 3.6, 17.7 Hz, 1H), 4.61 (dd *J* = 3.3, 9.3 Hz, 1H), 5.25 (dd *J* = 9.3, 17.7 Hz, 1H), 5.56 (dd *J* = 7.2, 15.3 Hz, 1H), 5.64-5.70 (m, 1H), 5.83 (dt *J* = 7.2, 15.3 Hz, 1H), 6.44 (d *J* = 6.0 Hz, 1H), 6.74 (s, 1H), 7.19 (d *J* = 9.6 Hz, 1H), 7.80 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.0, 19.1, 24.5, 28.5, 32.3, 34.4, 38.8, 40.8, 41.3, 43.6, 58.1, 72.6, 77.4, 84.6, 124.6, 129.8, 131.8, 147.7, 168.2, 169.4, 169.5, 169.6, 173.7. HRMS (ESI): *m/z* calcd. for $C_{23}H_{31}N_5NaO_5S_3$ $(M + Na)^+$ 576.13795, found 576.13795.



***S*-Trityl-α-thioketone 15a.** According to the general procedure, 0.044g (0.10 mmol) macrocycle **10** was combined with olefin **57**[5] in presence of the Hoveyda-Grubbs second generation catalyst in 1,2-dichloroethane to yield 0.040g (0.051 mmol, 51% yield) compound **15a**, which eluted in EtOAc. Clear oil. **(15a):** $[\alpha]^{24}_D$: +11.0 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.55 (d *J* = 6.9 Hz, 3H), 0.71 (d *J* = 6.9 Hz, 3H), 1.85 (s, 3H), 2.04-2.41 (m, 7H), 2.66 (dd *J* = 3.6, 15.9 Hz, 1H), 2.79 (dd *J* = 8.4, 15.9 Hz, 1H), 2.93 (d *J* = 14.7 Hz, 1H), 3.00 (d *J* = 14.7 Hz, 1H), 3.28 (d *J* = 11.4 Hz, 1H), 4.03 (d *J* = 11.4 Hz, 1H), 4.29 (dd *J* = 6.3, 17.7 Hz, 1H), 4.57 (dd *J* = 3.9, 9.6 Hz, 1H), 5.25 (dd *J* = 9.3, 17.7 Hz, 1H), 5.45 (dd *J* = 6.3, 15.3 Hz, 1H), 5.62-5.68 (m, 1H), 5.79 (dt *J* = 6.3, 15.3 Hz, 1H), 6.54 (dd *J* = 2.4, 9.0 Hz, 1H), 7.18-7.33 (m, 10H), 7.36-7.41 (m, 6H), 7.72 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.1, 19.1, 24.5, 26.3, 34.2, 40.4, 40.8, 41.4, 42.8, 43.5, 58.2, 67.3, 71.8, 84.7, 124.4, 127.2, 127.4, 128.3, 129.8, 133.7, 144.3, 147.7, 164.8, 168.3, 168.9, 169.5, 173.7, 205.5. HRMS (ESI): *m/z* calcd. for $C_{42}H_{44}N_4NaO_5S_3$ $(M + Na)^+$ 803.2366, found 803.23654.

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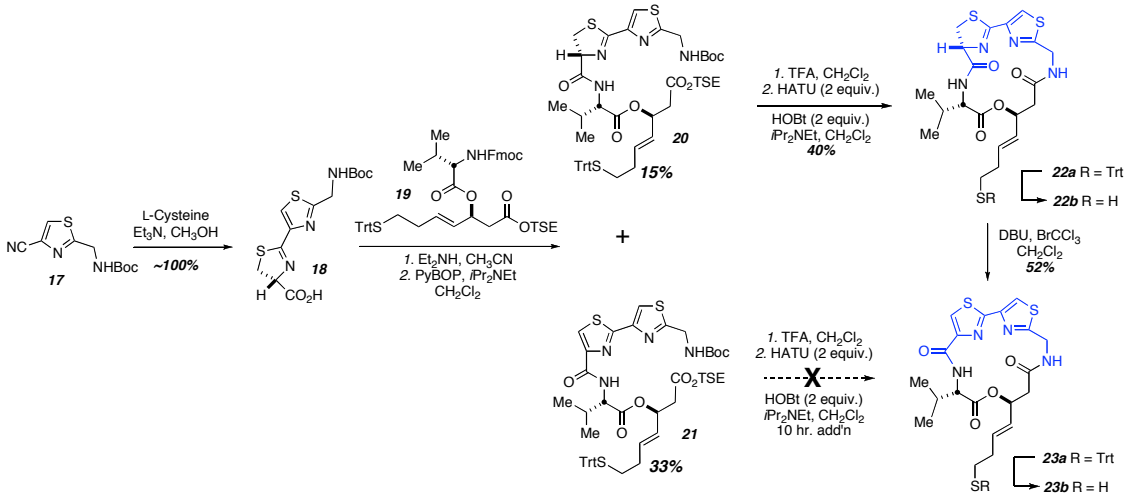
***S*-Trityl-α-thioketone 16a.** According to the general procedure, 0.048g (0.11 mmol) macrocycle **10** was combined with olefin **58** in presence of the Hoveyda-Grubbs second generation catalyst in 1,2-dichloroethane to yield 0.054g (0.067 mmol, 62% yield) compound **16a**, which eluted in EtOAc. Clear oil. **(16a):** $[\alpha]^{24}_{D}$: +5.1 (c = 2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.52 (d *J* = 6.9 Hz, 3H), 0.70 (d *J* = 6.9 Hz, 3H), 1.43-1.53 (m, 2H), 1.87 (s, 3H), 1.89-1.97 (m, 2H), 2.06-2.23 (m, 3H), 2.65 (dd *J* = 2.7, 16.5 Hz, 1H), 2.82 (dd *J* = 10.5, 16.5 Hz, 1H), 3.06 (s, 2H), 3.28 (d *J* = 11.4 Hz, 1H), 4.05 (d *J* = 11.4 Hz, 1H), 4.26 (dd *J* = 2.4, 17.7 Hz, 1H), 4.60 (dd *J* = 3.3, 9.3 Hz, 1H), 5.27 (dd *J* = 9.0, 17.7 Hz, 1H), 5.38 (dd *J* = 6.9, 17.7 Hz, 1H), 5.60-5.66 (m, 1H), 5.74 (dt *J* = 6.6, 15.3 Hz, 1H), 6.43 (d *J* = 7.8 Hz, 1H), 7.15-7.33 (m, 10H), 7.37-7.44 (m, 6H), 7.77 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.9, 19.2, 22.9, 24.4, 31.5, 34.5, 40.8, 41.0, 41.3, 42.9, 43.6, 57.9, 67.3, 72.6, 84.6, 124.5, 127.2, 127.3, 128.3, 129.8, 134.8, 144.4, 147.7, 164.8, 168.2, 169.2, 169.7, 173.8, 205.8. HRMS (ESI): *m/z* calcd. for $C_{43}H_{46}N_4NaO_5S_3$ $(M + Na)^+$ 817.25225, found 817.25292.



2-{2-[(tert-Butoxycarbonyl)methyl]thiazol-4-yl}-4,5-dihydrothiazole-4-carboxylic acid (18). 0.800g (3.3 mmol) Thiazole nitrile **17** and 0.446g (3.6 mmol, 1.1 equiv.) cysteine were dissolved 33 mL dry CH_3OH and 0.5 mL dry Et_3N was added dropwise. The resulting solution was heated at reflux overnight. The reaction was subsequently cooled to room temperature and the solvents removed in vaccuo. The crude reaction mixture was then dissolved in sat. aqu. $NaHCO_3$ and washed with diethyl ether. The aqueous layer was then acidified to pH ~3-4 by dropwise addition of 3N HCl and extracted with EtOAc (3x 30mL). The combined organics were dried over Na_2SO_4, filtered, and concentrated to provide 1.15g (3.3 mmol, ~100% yield from **17**) of acid **18** in spectroscopically pure form. Pale orange foam. $[\alpha]^{24}_{D}$: +30.9 (c = 1, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (bs, 1H), 7.98 (s, 1H), 5.59 (s, 1H), 4.59 (d *J* = 6.3 Hz, 2H), 3.88 (d *J* = 11.4 Hz, 1H), 3.30 (d *J* = 11.4 Hz, 1H), 1.66 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 175.74, 170.38, 170.16, 165.03, 155.91, 147.77, 123.3, 84.23, 80.70, 42.39, 41.31, 28.51, 27.17, 26.67, 24.30. HRMS (ESI): *m/z* calcd. for $C_{14}H_{19}N_3NaO_4S_2$ $(M + Na)^+$ 380.07147, found 380.07165.

Thiazoline-thiazole acyclic precursor 20 and thiazole-thiazole acyclic precursor 21. 0.300g (0.36 mmol, 1.0 equiv.) diester **19** and 0.147g (0.42 mmol, 1.2 equiv.) acid **18** were coupled according to the same procedure described above for synthesis of **43**. The two resulting products could be separated via column chromatography, washing first with 4:1, then with 2:1 hexanes:EtOAc. 0.110g (33% yield) **21** eluted first, followed quickly by 0.052g (15% yield) **20**. **(20):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 9H), 0.75 (d *J* = 6.9 Hz, 3H), 0.82 (d *J* = 6.9 Hz, 3H), 0.93-0.99 (m, 2H), 1.49 (s, 9H), 1.99-2.21 (m, 5H), 2.55 (dd *J* = 5.4, 15.6 Hz, 1H), 2.69 (dd *J* = 7.8, 15.6 Hz, 1H), 3.61-3.74 (m, 2H), 4.12-4.18 (m, 2H), 4.53 (dd *J* = 4.8, 9.0 Hz, 1H), 4.62 (d *J* = 6.0 Hz, 1H), 5.19 (t *J* = 9.0 Hz, 1H), 5.25-5.32 (m, 1H), 5.37 (dd *J* = 7.5, 15.3 Hz, 1H), 5.59-5.74 (m, 2H), 7.16-7.29 (m, 9H), 7.37-7.40 (m, 6H), 7.92 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ -1.2, 17.5, 17.7, 19.2, 28.6, 31.3, 31.5, 31.7, 35.8, 39.9, 42.6, 57.0, 63.4, 66.8, 72.1, 79.4, 80.7, 121.7, 126.8, 128.0, 128.1, 129.8, 134.3, 145.0, 148.7, 155.9, 165.7, 169.9, 170.6, 171.2 HRMS (ESI): *m/z* calcd. for $C_{49}H_{63}N_4O_7S_3Si$ $(M + H)^+$ 943.35463, found 943.3619. **(21):** $[\alpha]^{24}_D$: -1.1 (c = 2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.01 (s, 9H), 0.90-0.98 (m, 8H), 1.49 (s, 9H), 2.01-2.08 (m, 2H), 2.14-2.21 (m, 2H), 2.23-2.30 (m, 1H), 2.56 (dd *J* = 5.4, 15.6 Hz, 1H), 2.70 (dd *J* = 8.1, 15.6 Hz, 1H), 4.11-4.16 (m, 2H), 4.66 (d *J* = 5.7, 2H), 4.72 (dd *J* = 4.8, 9.3 Hz, 1H), 5.32-5.43 (m, 1H), 5.39 (dd *J* = 8.7, 15.6 Hz, 1H), 5.63-5.76 (m, 2H), 7.19-7.30 (m, 8H), 7.37-7.44 (m, 7H), 7.88 (d *J* = 9.0 Hz, 2H), 7.92 (s, 1H), 8.11 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ -1.3, 17.5, 18.0, 19.4, 28.6, 31.3, 31.6, 32.0, 40.0, 42.6, 57.2, 63.5, 66.9, 72.3, 77.5, 80.8, 117.6, 124.4, 126.9, 127.9, 128.1, 129.8, 134.5, 145.1, 148.4, 150.4, 156.0, 161.2, 162.7, 170.5, 171.0. HRMS (ESI): *m/z* calcd. for $C_{49}H_{60}N_4NaO_7S_3Si$ $(M + Na)^+$ 963.32911, found 963.32983.

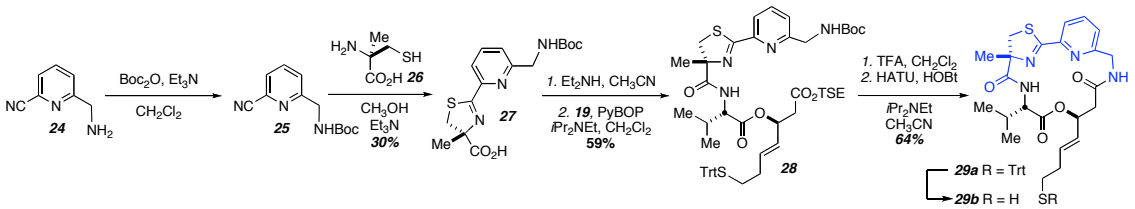
***S*-Trityl macrocycle 22a.** According to the general procedure, 0.054g acyclic precursor **20** was deprotected and cyclized to provide 0.030g (72% yield) macrocycle **22a** after purification by column chromatography. Eluent: EtOAc. **(22a)** Light yellow foam. $[\alpha]^{24}_D$: +30.0 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, 1:1 $CDCl_3$:CD_3OD) δ 0.39 (d *J* = 6.9 Hz, 3H), 0.63 (d *J* = 6.9 Hz, 3H), 1.97-2.18 (m, 5H), 2.54 (dd *J* = 2.4, 16.5 Hz, 1H), 3.66 (dd *J* = 9.0, 14.7 Hz, 1H), 3.95 (dd *J* = 1.2, 11.4 Hz, 1H), 4.12 (d *J* = 17.4 Hz, 1H), 4.54 (dd *J* = 3.6, 9.6 Hz, 1H), 5.15 (d *J* = 17.4 Hz, 1H), 5.29-5.34 (m, 1H), 5.35 (dd *J* = 6.9, 15.6 Hz, 1H), 5.50-5.57 (m, 1H), 5.65 (dt *J* = 6.9, 15.3 Hz, 1H), 6.98 (d *J* = 9.6 Hz, 1H), 7.14-7.26 (m, 10H), 7.32-7.36 (m, 6H), 7.77 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.6, 19.4, 31.6, 31.8, 34.4, 37.8, 38.9, 40.1, 41.2, 58.0, 73.2, 78.2, 125.7, 127.1, 127.3, 128.3, 130.0, 133.8, 145.2, 147.0, 166.4, 169.0, 169.5, 171.0, 171.5. HRMS (ESI): *m/z* calcd. for $C_{39}H_{40}N_4NaO_4S_3$ $(M + Na)^+$ 747.21039, found 747.21042.

Thiol 22b. According to the general procedure, 0.035g **22a** was deprotected to give 0.022g **22b** after preparative thin layer chromatography. Clear oil. **(22b)** $[\alpha]^{24}_D$: -1.1 (c = 0.2, $CHCl_3$). ^{1}H NMR (300 MHz, 1:1 $CDCl_3$:CD_3OD) δ 0.52 (d *J* = 6.9 Hz, 3H), 0.70 (d *J* = 6.9 Hz, 3H), 1.42 (t *J* = 4.8 Hz, 1H), 2.08-2.14 (m, 1H), 2.32-2.40 (m, 2H), 2.53-2.61 (m, 2H), 2.89 (dd *J* = 10.2, 16.8 Hz, 1H), 3.68 (dd *J* = 8.7, 14.1 Hz, 1H), 4.02 (d *J* = 11.4 Hz, 1H), 4.30 (dd *J* = 3.3, 17.7 Hz, 1H), 4.63 (dd *J* = 3.6, 9.6 Hz, 1H), 5.28 (dd *J* = 9.3, 17.7 Hz, 1H), 5.40 (d *J* = 6.0 Hz, 1H), 5.55 (ddt *J* = 1.5, 6.6, 15.3 Hz, 1H), 6.45-6.50 (m, 1H), 7.13 (d *J* = 9.0 Hz, 1H), 7.79 (s, 1H). ^{13}C

NMR (100.6 MHz, $CDCl_3$): δ 16.8, 19.2, 24.1, 34.3, 36.6, 37.8, 38.9, 40.8, 41.4, 57.9, 72.3, 124.7, 129.0, 132.9, 147.5, 168.0, 169.1, 169.6, 170.8. HRMS (ESI): *m/z* calcd. for $C_{20}H_{26}N_4NaO_4S_3$ $(M + Na)^+$ 505.10139, found 505.10156.

***S*-Trityl-thiazole-thiazole macrocycle 23a.** 0.020g (0.028 mmol, 1.0 equiv.) **22a** was dissolved in 1 mL dry CH_2Cl_2 and cooled to 0 °C. 0.021mL (0.14 mmol, 5.0 equiv.) DBU was added dropwise, followed by 0.014mL (0.14 mmol, 5 equiv.) $BrCCl_3$ in 1 mL CH_2Cl_2. The reaction was allowed to warm to room temperature and stirred overnight. The resulting solution was then poured over cold (0 °C) saturated aqueous $NaHCO_3$, extracted, concentrated, and dried. The product was then purified by column chromatography, eluting in 1:2 hexanes: EtOAc (0.010g, 52% yield). **(23a)** $[\alpha]^{24}_D$: -7.0 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.70 (d *J* = 6.9 Hz, 3H), 0.79 (d *J* = 6.9 Hz, 3H), 1.91-1.99 (m, 2H), 2.07-2.12 (m, 2H), 2.16-2.24 (m, 1H), 2.33 (d *J* = 14.1 Hz, 1H), 2.61 (dd *J* = 10.8, 15.0 Hz, 1H), 4.39 (dd *J* = 5.1, 17.4 Hz, 1H), 4.77 (dd *J* = 7.2, 17.4 Hz, 1H), 5.17 (dd *J* = 3.9, 9.9 Hz, 1H), 5.35 (dd *J* = 7.5, 15.0 Hz, 1H), 5.50-5.65 (m, 2H), 7.19-7.28 (m, 3H), 7.28-7.35 (m, 12H), 7.62 (d *J* = 9.6 Hz, 1H), 8.30 (s, 1H), 8.32 (s, 1H), 8.72 (t *J* = 6.3 Hz, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 16.6, 19.4, 31.6, 31.8, 34.4, 37.8, 38.9, 40.1, 41.2, 58.0, 73.2, 78.2, 125.7, 127.1, 127.3, 128.3, 130.0, 133.8, 145.2, 147.0, 166.4, 169.0, 169.5, 171.0, 171.5. HRMS (ESI): *m/z* calcd. for $C_{39}H_{38}N_4NaO_4S_3$ $(M + Na)^+$ 745.19474, found 745.19430.

Thiol 23b. According to the general procedure, 0.010g **23a** was deprotected to give 0.005g **23b** after preparative thin layer chromatography. **(23b):** Clear oil. $[\alpha]^{24}_D$: -1.1 (c = 0.2, $CHCl_3$). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82-0.85 (m, 6H), 2.21-2.29 (m, 4H), 2.39 (d *J* = 14.1 Hz, 1H), 2.65 (dd *J* = 10.5, 14.7 Hz, 1H), 4.40 (dd *J* = 4.8, 17.1 Hz, 1H), 4.77 (dd *J* = 7.2, 18.3 Hz, 1H), 5.21 (dd *J* = 3.9, 9.9 Hz, 1H), 5.47-5.61 (m, 2H), 5.75 (dt *J* = 6.9, 14.7 Hz, 1H), 7.68 (d *J* = 9.9 Hz, 1H), 8.31 (s, 1H), 8.33 (s, 1H), 8.74 (t *J* = 5.4 Hz, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 16.8, 19.2, 24.1, 34.3, 36.6, 37.8, 38.9, 40.8, 41.4, 57.9, 72.3, 124.7, 129.0, 132.9, 147.5, 168.0, 169.1, 169.6, 170.8. HRMS (ESI): *m/z* calcd. for $C_{20}H_{24}N_4NaO_4S_3$ $(M + Na)^+$ 503.08519, found 503.08369.



Acid 27. Amine **24**[6] was dissolved in CH_2Cl_2 and treated with 1.83mL (1.31 mmol, 1.5 equiv.) Et_3N, followed by dropwise addition of a solution of 2.29g (10.5 mmol, 1.2 equiv.) Boc anhydride in CH_2Cl_2. The resulting reaction was stirred overnight, then quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, dried

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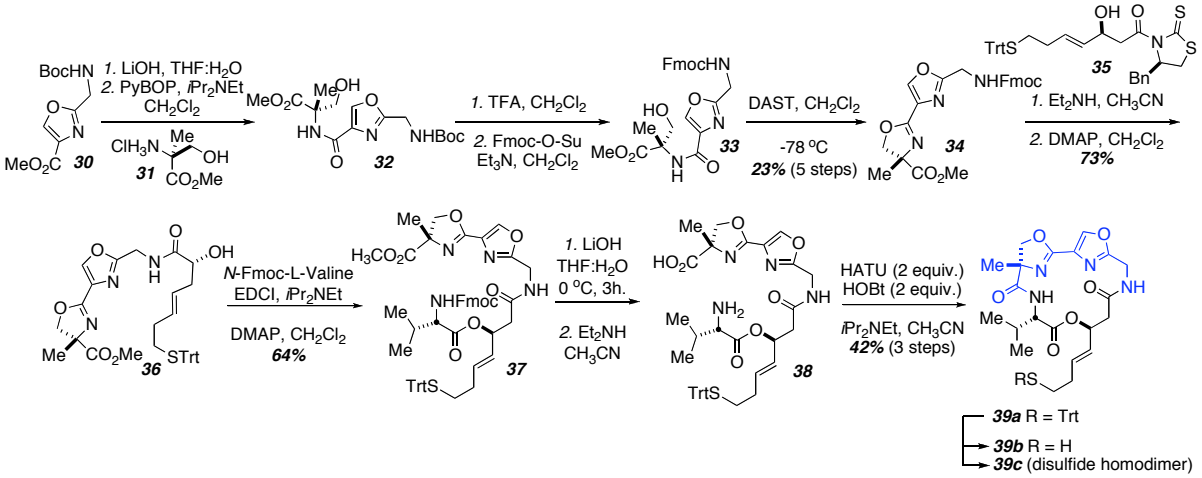
over Na_2SO_3, filtered, and concentrated to give the Boc protected amine as a white solid. This solid was then dissolved in 90 mL CH_3OH together with 1.62g (8.7 mmol, 1.0 equiv.) α-methyl cysteine and 2.44 mL (17.5 mmol, 2.0 equiv.) Et_3N. The resulting solution was heated at reflux overnight. The reaction was subsequently cooled to room temperature and the solvents removed in vaccuo. The crude reaction mixture was then dissolved in saturated aqueous $NaHCO_3$ and washed with diethyl ether. The aqueous layer was then acidified to pH ~3-4 by dropwise addition of 3N HCl and extracted with EtOAc (3x 30mL). The combined organics were dried over Na_2SO_4, filtered, and concentrated to provide 0.500g (1.4 mmol, 30% yield) of acid **27** in spectroscopically pure form. **(27)** $[\alpha]^{24}_D$: +55.2 (c = 2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.67 (s, 3H), 3.28 (d *J* = 11.7 Hz, 1H), 3.81 (d *J* = 11.7 Hz, 1H), 4.47 (d *J* = 5.4 Hz, 2H), 5.53 (s, 1H), 7.37 (d *J* = 7.8 Hz, 1H), 7.74 (t *J* = 7.8 Hz, 1H), 8.00 (d *J* = 7.8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.9, 24.2, 28.6, 36.1, 40.5, 45.5, 47.9, 79.8, 85.1, 120.6, 123.9, 137.4, 149.9, 156.4, 157.4, 157.6, 171.3, 175.8. HRMS (ESI): *m/z* calcd. for $C_{16}H_{20}N_3Na_2O_4S$ $(M-H+2Na)^+$ 396.09699, found 396.09616.

Thiazoline-pyridine acyclic precursor 28. 1.100g (1.3 mmol, 1.0 equiv.) diester **19** and 0.500g (1.4 mmol, 1.1 equiv.) acid **27** were coupled according to the same procedure described above for synthesis of **43**. 0.800g (0.90 mmol, 59% yield) **28** was obtained after column chromatography. **(28):** Clear oil. $[\alpha]^{24}_D$: -22.1 (c = 2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73 (d *J* = 6.9 Hz, 3H), 0.81 (d *J* = 6.9 Hz, 3H), 0.94-0.99 (m, 2H), 1.47 (s, 9H), 1.58 (s, 3H), 2.03-2.19 (m, 5H), 2.55 (dd *J* = 5.7, 15.9 Hz, 1H), 2.69 (dd *J* = 8.1, 15.9 Hz, 1H), 3.31 (d *J* = 11.7 Hz, 1H), 3.69 (d *J* = 11.7 Hz, 1H), 4.13-4.18 (m, 2H), 4.48-4.53 (m, 3H), 5.37 (dd *J* = 7.5, 15.3 Hz, 1H), 5.48-5.52 (m, 1H), 5.61-5.74 (m, 2H), 7.18-7.29 (m, 10H), 7.35-7.40 (m, 7H), 7.68 (t *J* = 7.8 Hz, 1H), 7.98 (d *J* = 7.8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ -1.2, 17.5, 17.6, 19.3, 25.0, 28.7, 31.3, 31.5, 39.9, 40.8, 45.7, 56.9, 63.3, 66.8, 72.0, 79.8, 85.9, 120.2, 124.0, 126.8, 128.0, 128.1, 129.7, 134.2, 137.6, 145.0, 150.1, 156.3, 157.9, 169.9, 170.7, 171.2, 174.7. HRMS (ESI): *m/z* calcd. for $C_{52}H_{66}N_4NaO_7S_2Si$ $(M + Na)^+$ 973.40344, found 973.40443.

***S*-Trityl macrocycle 29a.** According to the general procedure, 0.400g (0.42 mmol) **28** was deprotected and cyclized to provide 0.200g (0.27 mmol, 64% yield) macrocycle **29a** after column chromatography. Eluent: EtOAc. **(29a):** Clear oil. $[\alpha]^{24}_D$: +16.7 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ 0.52 (d *J* = 6.9 Hz, 3H), 0.74 (d *J* = 6.9 Hz, 3H), 1.84 (s, 3H), 2.03-2.17 (m, 5H), 2.64-2.79 (m, 2H), 3.38 (d *J* = 11.4 Hz, 1H), 4.09 (d *J* = 11.4 Hz, 1H), 4.30 (d *J* = 17.7 Hz, 1H), 4.68 (dd *J* = 3.9, 9.9 Hz, 1H), 5.00 (dd *J* = 6.6, 17.7 Hz, 1H), 5.40 (dd *J* = 6.6, 15.6 Hz, 1H), 5.60-5.66 (m, 1H), 5.75 (dt *J* = 7.8, 15.6 Hz, 1H), 7.10-7.37 (m, 18H), 7.64 (d *J* = 7.5 Hz, 1H), 7.80 (t *J* = 7.5 Hz, 1H). ^{13}C NMR (100.6 MHz, 10:1 $CDCl_3$:CD_3OD): δ 16.2, 19.0, 24.5, 31.3, 31.5, 33.5, 38.7, 41.2, 43.4, 43.6, 51.4, 57.4, 66.7, 73.3, 84.3, 123.2, 124.7, 126.7, 128.0, 128.2, 129.7, 133.3, 138.2, 144.9, 148.7, 157.4, 166.0, 169.2, 170.0, 173.3, 173.6. HRMS (ESI): *m/z* calcd. for $C_{42}H_{44}N_4NaO_4S_2$ $(M + Na)^+$ 755.26962, found 755.26961.

Thiol 23b. According to the general procedure, 0.033g **23a** was deprotected to give 0.019g **23b** after preparative thin layer chromatography. **(29b):** Clear oil. $[\alpha]^{24}_{D}$: +3.4 (c = 0.2, $CHCl_3$). ^{1}H NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ 0.54 (d *J* = 6.9 Hz, 3H), 0.75 (d *J* = 6.9 Hz, 3H), 1.43 (t *J* = 7.8 Hz, 1H), 1.89 (s, 3H), 2.04-2.17 (m, 1H), 2.32-2.39 (m, 2H), 2.49-2.57 (m, 2H), 2.69-2.86 (m, 2H), 3.39 (d *J* = 11.4 Hz, 1H), 4.10 (d *J* = 11.4 Hz, 1H), 4.37 (dd *J* = 2.4, 17.7 Hz, 1H), 4.72 (dd *J* = 3.9, 9.9 Hz, 1H), 5.04 (dd *J* = 6.9, 17.7 Hz, 1H), 5.57 (dd *J* = 6.6, 15.6 Hz, 1H), 5.67-5.73 (m, 1H), 5.87 (dt *J* = 7.2, 15.6 Hz, 1H), 7.38 (d *J* = 7.8 Hz, 1H), 7.66 (d *J* = 7.8 Hz, 1H), 7.84 (t *J* = 7.8 Hz, 1H). ^{13}C NMR (100.6 MHz, 10:1 $CDCl_3$:CD_3OD): δ 16.9, 19.2, 24.1, 24.8, 33.8, 36.7, 41.8, 43.8, 44.1, 51.4, 57.7, 72.5, 85.0, 123.5, 124.6, 128.6, 132.7, 138.1, 156.9, 169.0, 169.3, 173.6. HRMS (ESI): *m/z* calcd. for $C_{23}H_{30}N_4NaO_4S_2$ $(M + Na)^+$ 513.16007, found 513.16058.



Oxazoline-oxazole 34. 1.1g (4.3 mmol, 1.0 equiv.) Oxazole **30**[7] was dissolved in 200mL 2:1 THF:H_2O and treated with 0.205g (8.6 mmol, 2.0 equiv.) LiOH. The resulting solution was stirred for ~1 hr., when TLC showed complete disappearance of starting material. The reaction was acidified with 1N HCl and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_3, filtered, and concentrated to give the crude acid, which was taken on without further purification. The acid was taken up in dry CH_2Cl_2. 4.47g (8.6 mmol, 2.0 equiv.) PyBOP was added, followed by 0.874g (5.2 mmol, 1.2 equiv.) α-methyl-serine-methylester-HCL salt[8] and 2.24mL (12.9 mmol, 3.0 equiv.) *i*Pr_2NEt. The resulting reaction was stirred for ~2 hr., then concentrated and passed through a short plug of silica, washing with EtOAc, to give alcohol **32**.

Crude alcohol **32** was dissolved in 100mL CH_2Cl_2 (to ~0.003M), cooled to 0 °C and treated with 14mL TFA (to ~0.3M). The reaction was stirred ~2 hr., when TLC showed complete disappearance of starting material. The reaction mixture was concentrated, the residue dissolved in toluene, and concentrated again. The crude amine salt was dried on an oil pump pump for ~2 hr., then dissolved in 50mL dry CH_2Cl_2, cooled to 0 °C and treated successively with 0.720mL (8.6 mmol, 2.0 equiv.) Et_3N and 1.72g (5.2 mmol, 1.2 equiv.) Fmoc-*O*-

[7] Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; David R. Williams, D. R. *Organic Letters* **2000** *2* (8), 1165-1168.

[8]

succinimide in 10mL CH_2Cl_2. The resulting reaction was allowed to warm to room temperature and stirred overnight. The reaction was then quenched with saturated aqueous $NaHCO_3$, dried over Na_2SO_3, filtered, and concentrated. The residue was passed through a short plug of silica, washing with EtOAc to give alcohol **33**.

Crude alcohol **33** was dissolved in 20mL dry CH_2Cl_2 and cooled to -78 ^oC. 0.690mL (5.6 mmol, 1.2 equiv.) DAST was added dropwise and the reaction was allowed to stir at -78 ^oC for an additional 2 hr. The mixture was then poured onto a saturated aqueous solution of $NaHCO_3$ at 0 ^oC. The organic layer was separated, dried over Na_2SO_3, filtered, and concentrated. The residue was purified by column chromatography. An unidentified by-product elutes first in 1:1 hexanes: EtOAc, followed by the desired oxazoline **34** in 1:2 hexanes:EtOAc (0.455g, 0.99 mmol, 23% yield from **30**). **(34):** $[\alpha]^{24}_D$: +72.7 (c = 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 1.62 (s, 3H), 3.78 (s, 3H), 4.17 (d *J* = 8.7 Hz, 1H), 4.23 (t *J* = 6.9 Hz, 1H), 4.42-4.58 (m, 4H), 4.82 (d *J* = 8.7 Hz, 1H), 5.54-5.57 (m, 1H), 7.31 (t *J* = 7.5 Hz, 2H), 7.40 (t *J* = 7.5 Hz, 2H), 7.59 (d *J* = 7.5Hz, 2H), 7.76 (d *J* = 7.5 Hz, 2H), 8.12 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.1, 38.5, 47.3, 53.1, 67.4, 74.5, 77.6, 120.2, 125.3, 127.3, 127.9, 130.4, 141.5, 142.0, 143.9, 156.5, 158.5, 162.3, 173.4. HRMS (ESI): *m/z* calcd. for $C_{25}H_{23}N_3NaO_6$ $(M + Na)^+$ 484.14791, found 484.14790.

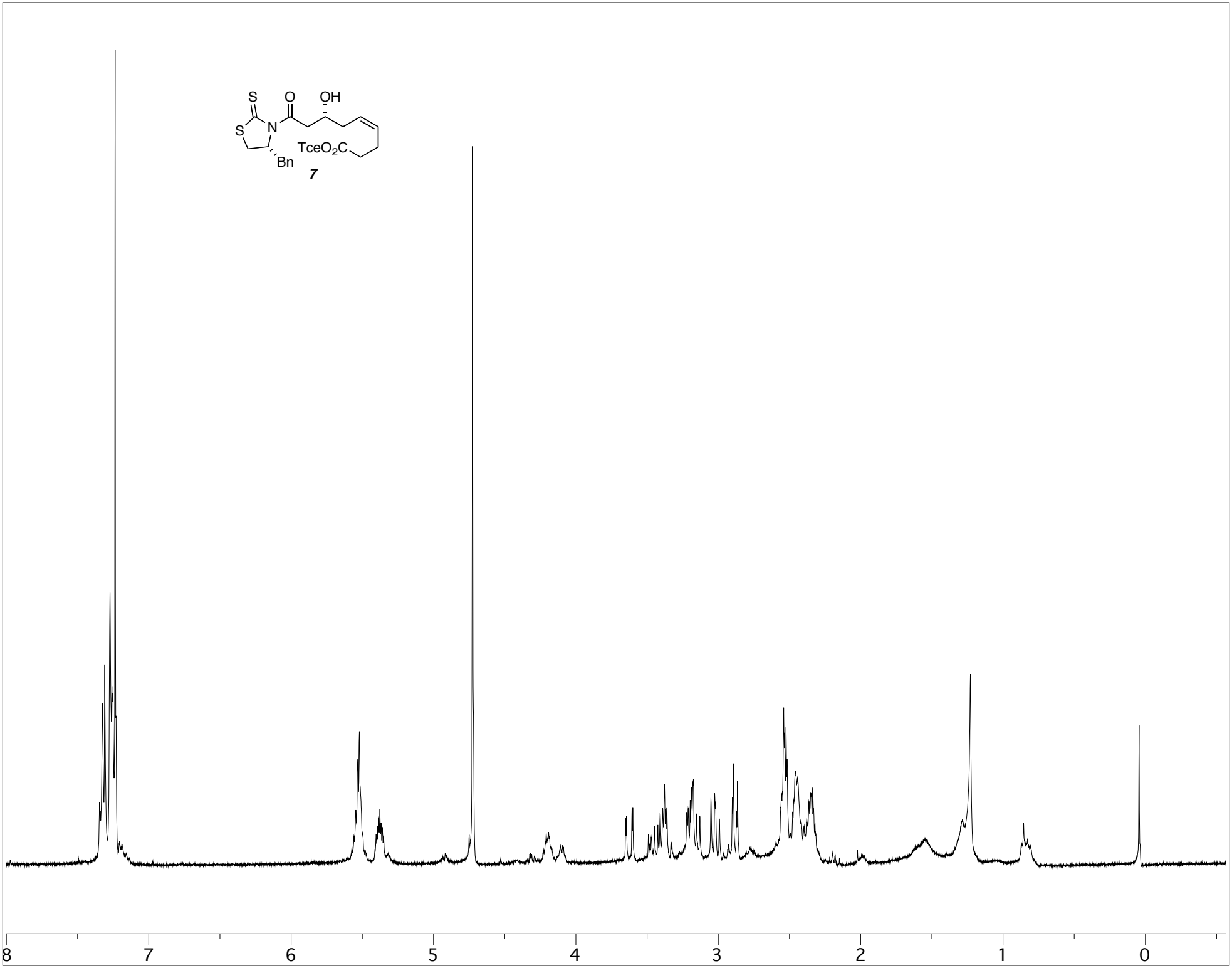
Alcohol 36. 0.250g (0.54 mmol, 1.0 equiv.) **34** was dissolved in 30mL CH_3CN and treated with 3mL Et_2NH. The reaction was allowed to stir for 2 hr. and then concentrated, redissolved in EtOAc and concentrated again. The crude amine, thus obtained was taken up in 5 mL dry CH_2Cl_2 together with 0.010g (0.08 mmol, 0.15 equiv.) DMAP and added dropwise to a solution of 0.495g (0.81 mmol, 1.5 equiv.) **35** in 20mL CH_2Cl_2. The resulting reaction mixture was allowed to stir overnight, then concentrated and submitted immediately to column chromatography. 0.253g (0.39mmol, 73% yield) alcohol **36** eluted in 1:2 hexanes:EtOAc. **(36):** $[\alpha]^{24}_D$: -18.1 (c = 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 1.61 (s, 3H), 2.03-2.10 (m, 2H), 2.16-2.22 (m, 2H), 2.38 (dd *J* = 8.4, 15.3 Hz, 1H), 2.46 (dd *J* = 3.9, 15.3 Hz, 1H), 3.77 (s, 3H), 4.17 (d *J* = 9.0 Hz, 1H), 4.41-4.47 (m, 1H), 4.58 (d *J* = 5.4 Hz, 2H), 4.81 (d *J* = 9.0 Hz, 1H), 5.41 (dd *J* = 6.3, 15.6 Hz, 1H), 5.56 (dt *J* = 6.9, 15.6 Hz, 1H), 6.63-6.66 (m, 1H), 7.17-7.30 (m, 9H), 7.37-7.41 (m, 6H), 8.11 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.2, 31.6, 31.7, 36.7, 43.0, 53.1, 66.8, 69.1, 74.4, 76.4, 126.8, 128.1, 129.8, 129.9, 130.2, 132.7, 142.1, 145.1, 158.6, 162.3, 172.4, 173.4. HRMS (ESI): *m/z* calcd. for $C_{36}H_{37}N_3NaO_6S$ $(M + Na)^+$ 662.22953, found 662.22913.

Oxazoline-oxazole acyclic precursor 37. 0.250g (0.39 mmol) of alcohol **36** and 0.663g (2.0 mmol, 5 equiv.) *N*-Fmoc-L-valine were dissolved in 20 mL dry CH_2Cl_2. The reaction was cooled to 0º C and 0.449g (2.4 mmol, 6 equiv.) EDCI and 0.003g (0.02 mmol, cat.) DMAP were added in ~5 mL CH_2Cl_2, followed by 0.4 mL iPr$_2$NEt. The reaction was allowed to warm to room temperature and stirred overnight, when TLC showed complete disappearance of **36**. The reaction was concentrated and the product (0.240g, 64% yield) purified by silica gel chromatography. Eluent: 1:2 hexanes:EtOAc. **(37):** $[\alpha]^{24}_D$: -2.0 (c = 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 0.83 (d *J* = 6.9 Hz, 3H), 0.90 (d *J* = 6.9 Hz, 3H), 1.58 (s, 3H), 1.64-1.73 (m, 1H), 2.00-2.18 (m, 5H), 2.49-2.62 (m, 2H), 3.76 (s, 3H), 4.12 (d *J* = 8.7 Hz, 1H), 4.10-4.21 (m, 2H), 4.36 (d *J* = 6.9 Hz, 2H), 4.45-4.60 (m, 2H), 4.77

(d J = 8.7 Hz, 1H), 5.34-5.42 (m, 2H), 5.58-5.71 (m, 2H), 6.50 (t J = 5.1 Hz, 1H), 7.17-7.32 (m, 12H), 7.36-7.41 (m, 8H), 7.57 (d J = 7.5 Hz, 2H), 7.75 (d J = 7.5 Hz, 2H), 8.06 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.6, 17.9, 18.8, 19.3, 25.1, 31.2, 31.3, 31.5, 37.0, 41.5, 47.4, 53.1, 53.8, 59.4, 66.8, 67.1, 72.4, 74.5, 76.3, 120.2, 125.3, 126.8, 127.3, 127.9, 128.1, 129.8, 130.3, 134.1, 141.5, 142.0, 144.0, 144.1, 145.0, 156.6, 158.5, 162.0, 169.3, 171.3, 173.4. HRMS (ESI): *m/z* calcd. for $C_{56}H_{56}N_4NaO_9S$ $(M + Na)^+$ 983.36602, found 983.36673.

***S*-Trityl macrocycle 39a.** 0.240g (0.25 mmol, 1.0 equiv.) acyclic precursor **37** was dissolved in 7.5 mL 4:1 THF:H_2O and cooled to 0 ^oC. A 0.5mL of a 0.5M aqueous solution of LiOH was added dropwise and the resulting reaction mixture was allowed to stir for ~3 hr. at 0 ^oC, when TLC demonstrated disappearance of the starting material. The reaction was neutralized by dropwise addition of 1N HCl and extracted with CH_2Cl_2. The combined organics were dried and filtered and solvents evaporated to provide the crude acid. This was immediately dissolved in 25 mL CH_3CN (to ~0.01M) and treated with 1.25mL Et_2NH (to ~0.2M). The reaction was stirred for ~2 hr., when the reaction was assumed to be complete. The resulting solution was concentrated, taken up in EtOAc, and concentrated again. The crude amino acid was dried on the mechanical pump overnight and then submitted to cyclization conditions as described above. Column chromatography provided 0.074g (0.10 mmol, 74% yield) macrocycle **39a** as a clear oil. **(39a):** $[\alpha]^{24}_D$: +42.2 (c = 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.53 (d J = 6.9 Hz, 3H), 0.56 (d J = 6.9 Hz, 3H), 1.58 (s, 3H), 1.89-2.16 (m, 5H), 2.51 (dd J = 1.8, 17.1 Hz, 1H), 2.87 (dd J = 9.9, 17.1 Hz, 1H), 3.82 (dd J = 3.6, 17.7 Hz, 1H), 3.99 (d J = 9.0 Hz, 1H), 4.32-3.36 (m, 1H), 4.62 (d J = 9.0 Hz, 1H), 4.60-4.69 (m, 1H), 5.34-5.49 (m, 2H), 5.58 (dt J = 6.9, 14.7 Hz, 1H), 7.08-7.21 (m, 9H), 7.26-7.31 (m, 7H), 7.62-7.67 (m, 1H), 7.97 (s, 1H). ^{13}C NMR (100.6 MHz, 10:1 $CDCl_3$:CD_3OD): δ 17.1, 18.7, 21.1, 31.4, 34.0, 37.4, 39.9, 58.0, 66.8, 72.7, 73.6, 77.5, 78.8, 126.8, 128.0, 128.4, 129.2, 129.7, 133.2, 141.7, 144.9, 162.2, 164.1, 168.7, 171.9, 174.3. HRMS (ESI): *m/z* calcd. for $C_{40}H_{42}N_4NaO_6S$ $(M + Na)^+$ 729.27173, found 729.27147.

Disulfide dimer 39c. To a vigorously stirring solution of 0.319g (1.3 mmol, 12 equiv.) I_2 in 300 mL 10% MeOH/CH_2Cl_2 was added 0.074g (0.10mmol, 1.0 equiv.) protected thiol **39a** in 60mL 10% MeOH/ CH_2Cl_2 dropwise over 10 minutes. The resulting mixture was stirred for a further 10 minutes. 250mL 0.01 N $Na_2S_2O_3$ was added and the organic phase extracted with CH_2Cl_2 the combined organic extract washed with brine, dried over Na_2SO_3, filtered, and the solvent removed. The residue was purified by flash chromatography, washing first with EtOAC, then 10:1 CH_2Cl_2:CH_3OH. **(39c):** Clear oil. $[\alpha]^{24}_D$: +19.0 (c = 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) d 0.67-0.70 (m, 6H), 1.66 (s, 3H), 2.12-2.18 (m, 1H), 2.40-2.47 (m, 2H), 2.71-2.80 (m, 3H), 3.02 (dd J = 9.6, 16.5 Hz, 1H), 3.96 (dd J = 4.5, 17.4 Hz, 1H), 4.05 (d J = 9.0 Hz, 1H), 4.51 (dd J = 3.6, 8.7 Hz, 1H), 4.73 (d J = 9.0 Hz, 1H), 4.85 (dd J = 9.0, 17.4 Hz, 1H), 5.54-5.69 (m, 2H), 5.90 (dt J = 6.9, 15.6 Hz, 1H), 6.89-6.94 (m, 1H), 8.01 (s, 1H). ^{13}C NMR (100.6 MHz, 10:1 $CDCl_3$:CD_3OD): δ 17.4, 18.7, 21.4, 29.8, 31.9, 34.1, 37.6, 38.0, 40.3, 51.4, 58.1, 72.4, 74.0, 78.9, 128.5, 129.8, 133.7, 141.2, 161.0, 164.2, 168.7, 170.8, 174.1. HRMS (ESI): *m/z* calcd. for $C_{42}H_{54}N_8NaO_{12}S_2$ $(M + Na)^+$ 949.31948, found 949.32045.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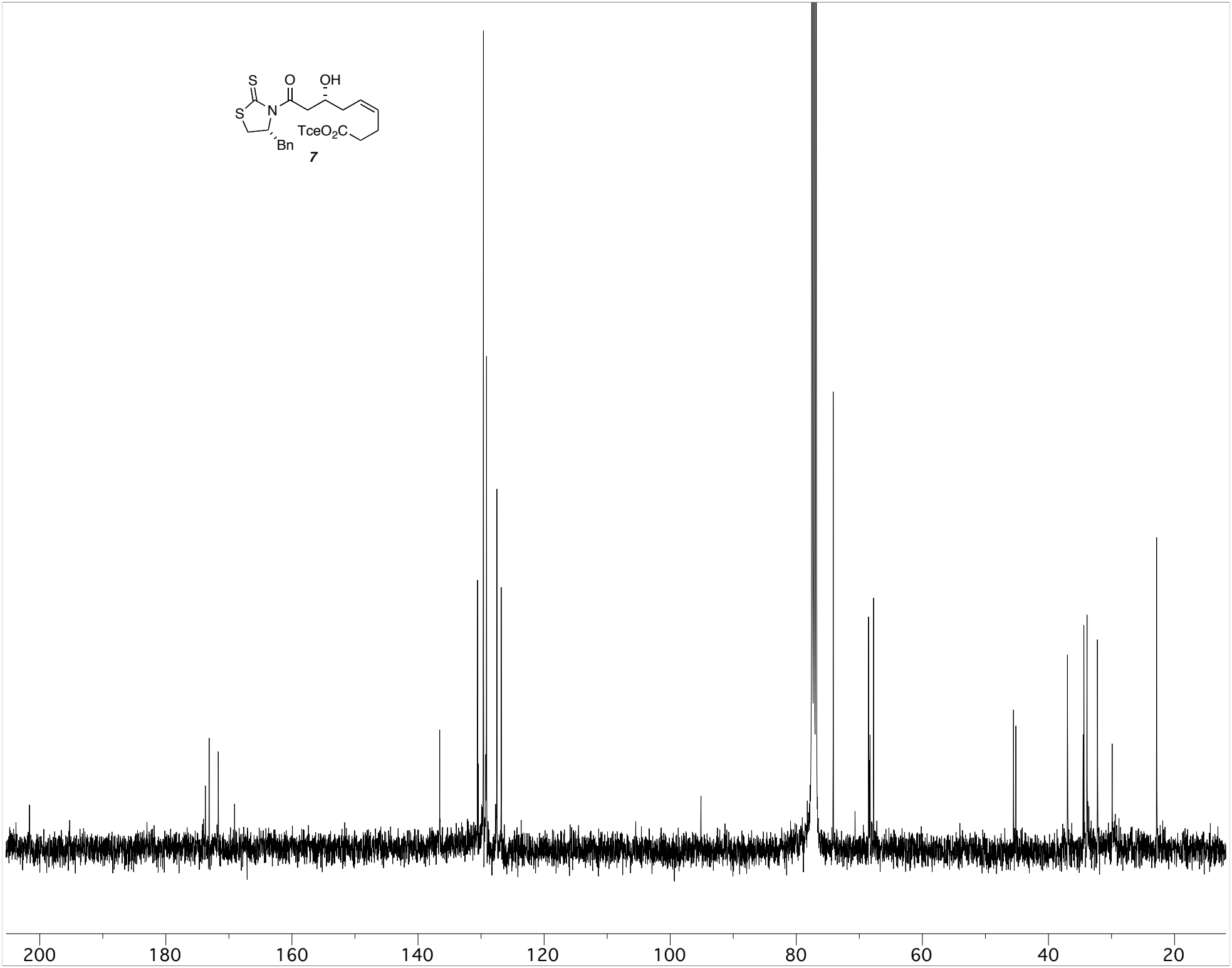
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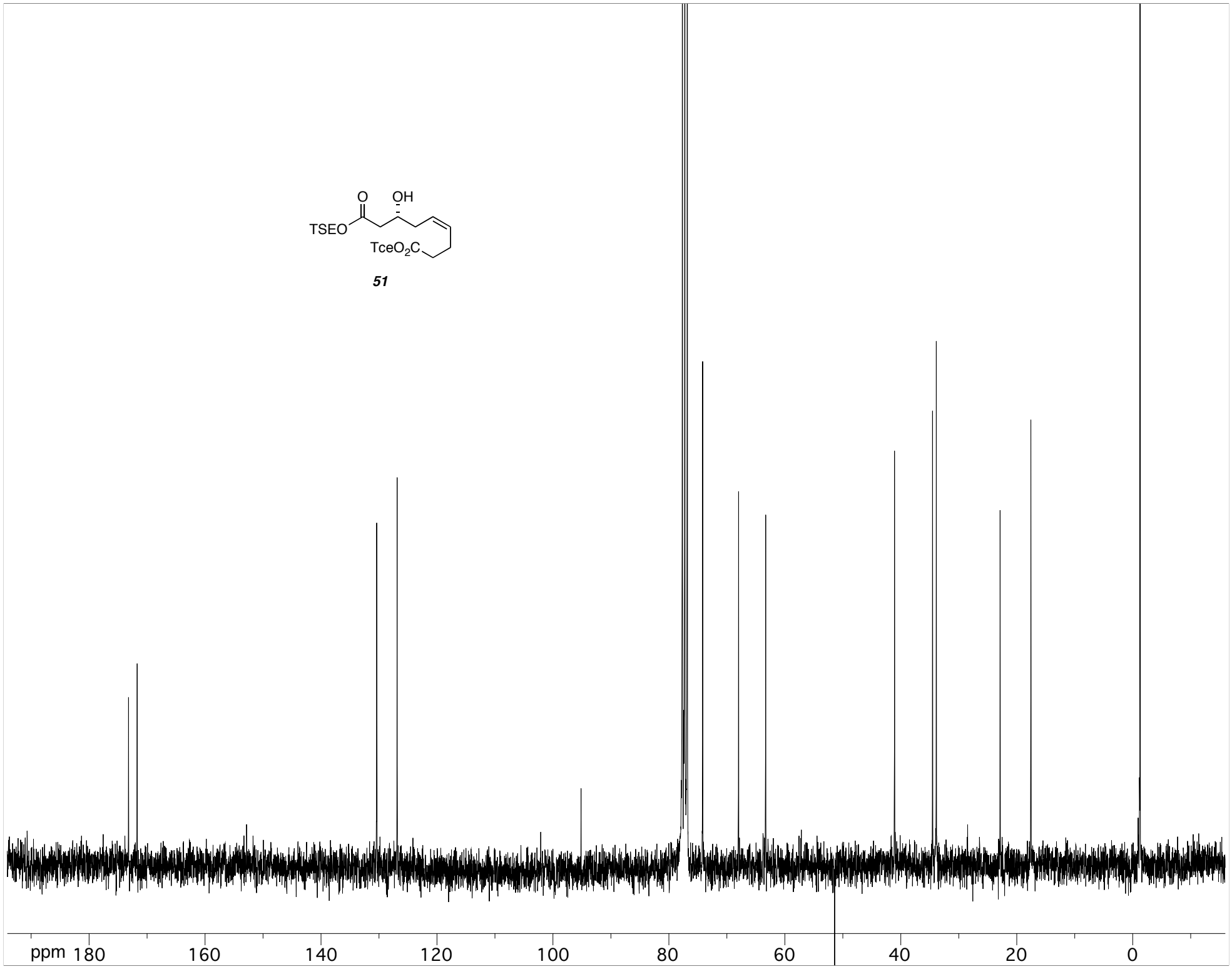
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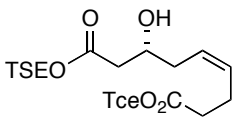

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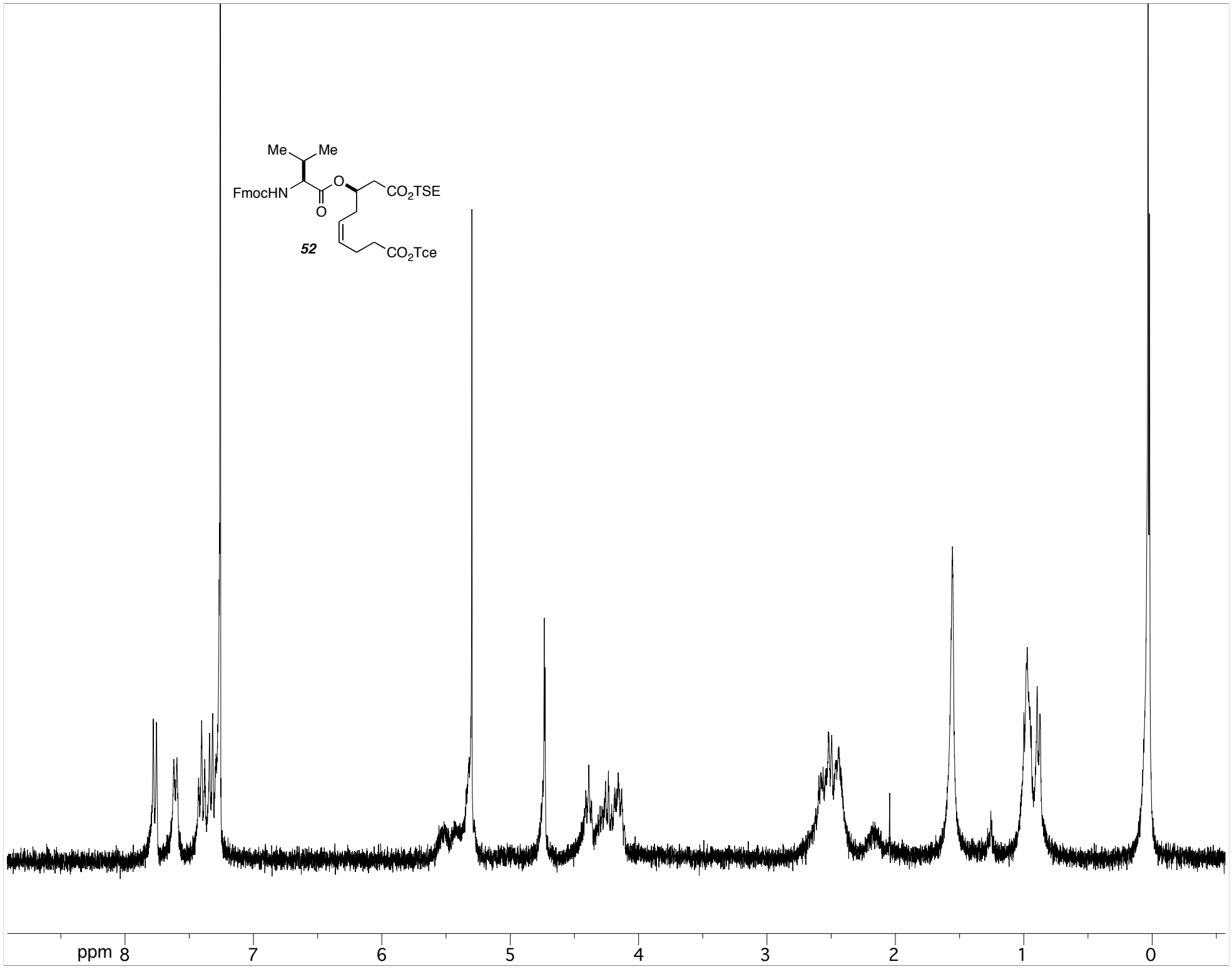
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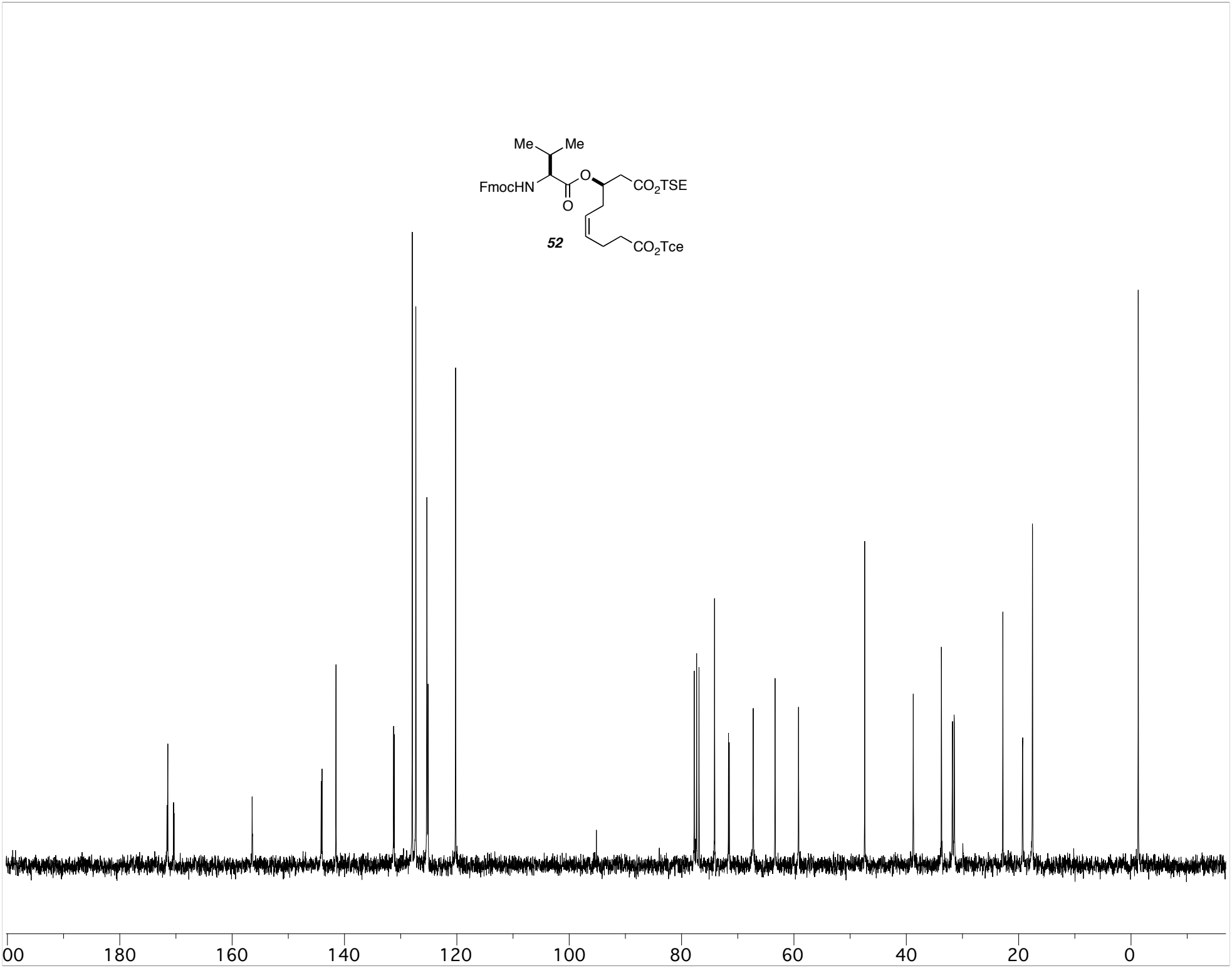
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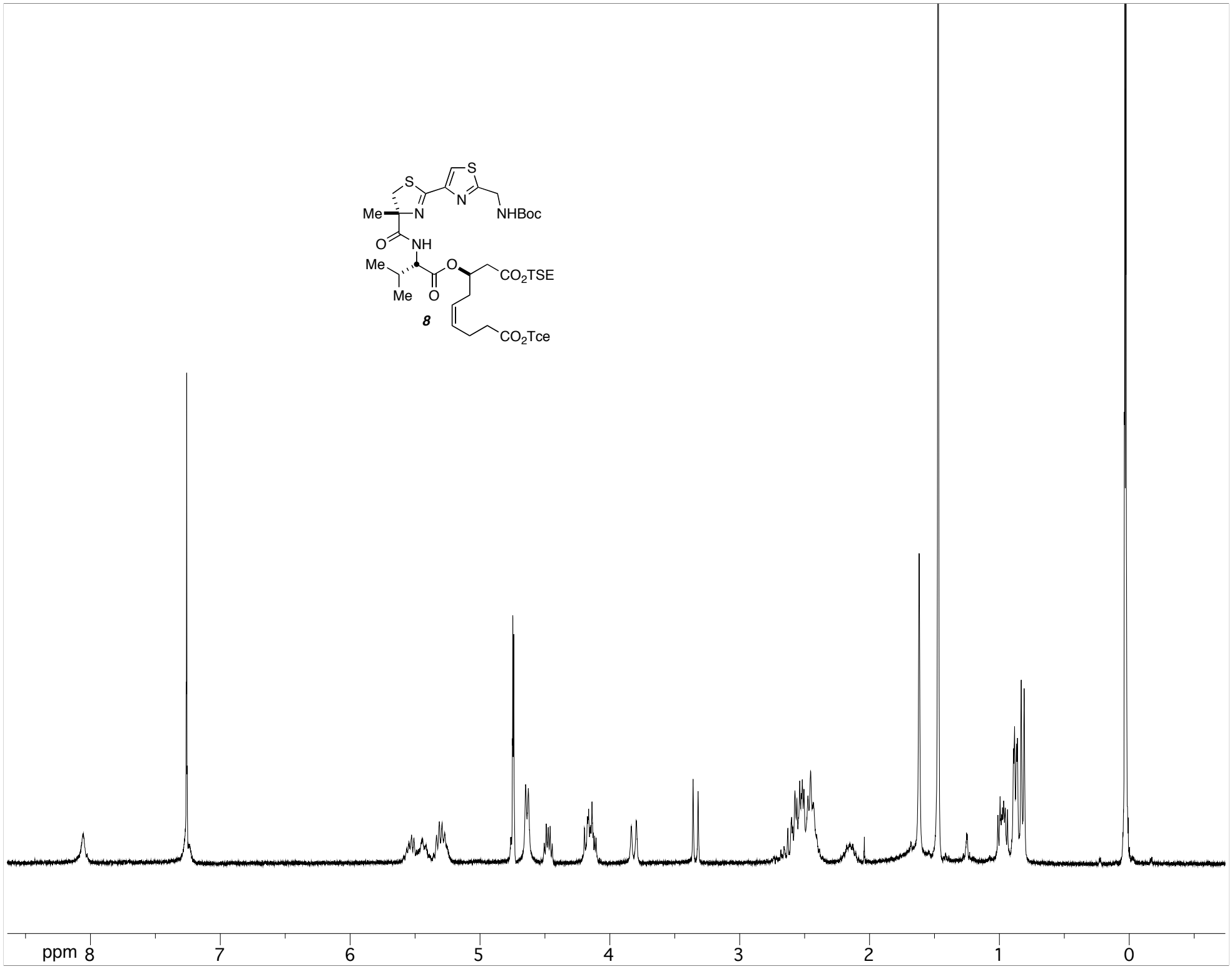
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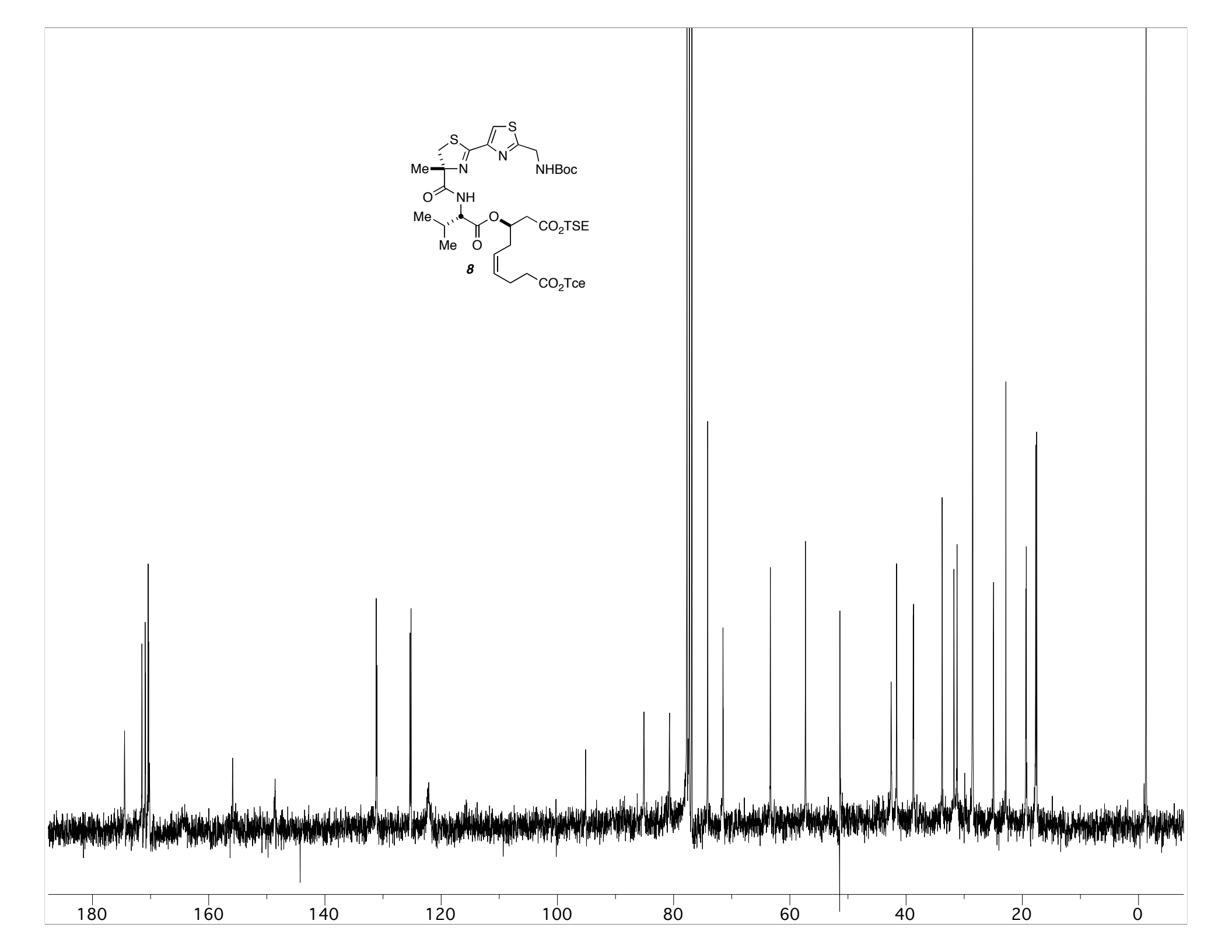
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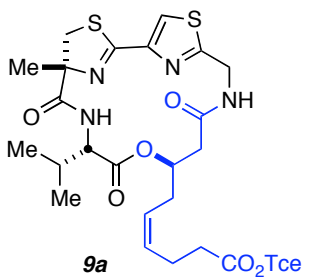

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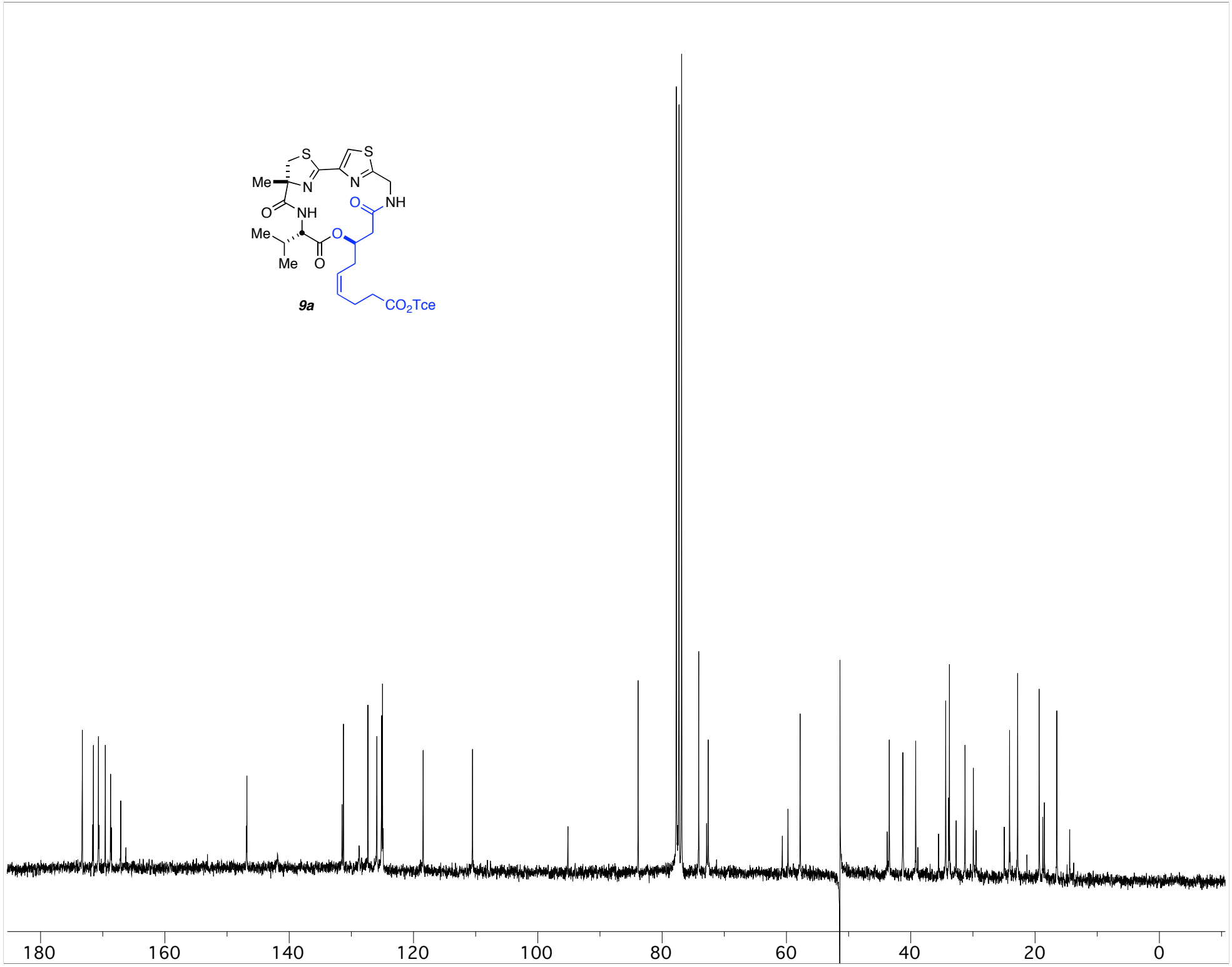
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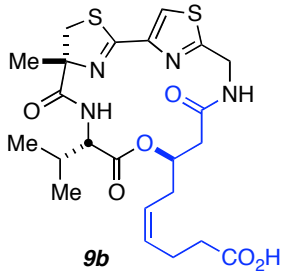

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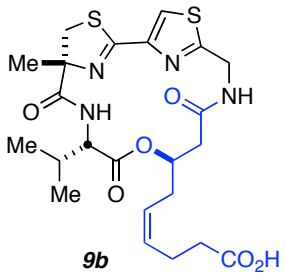

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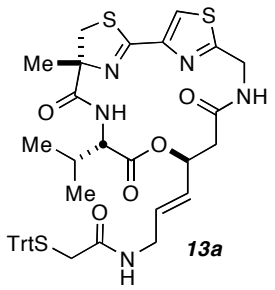

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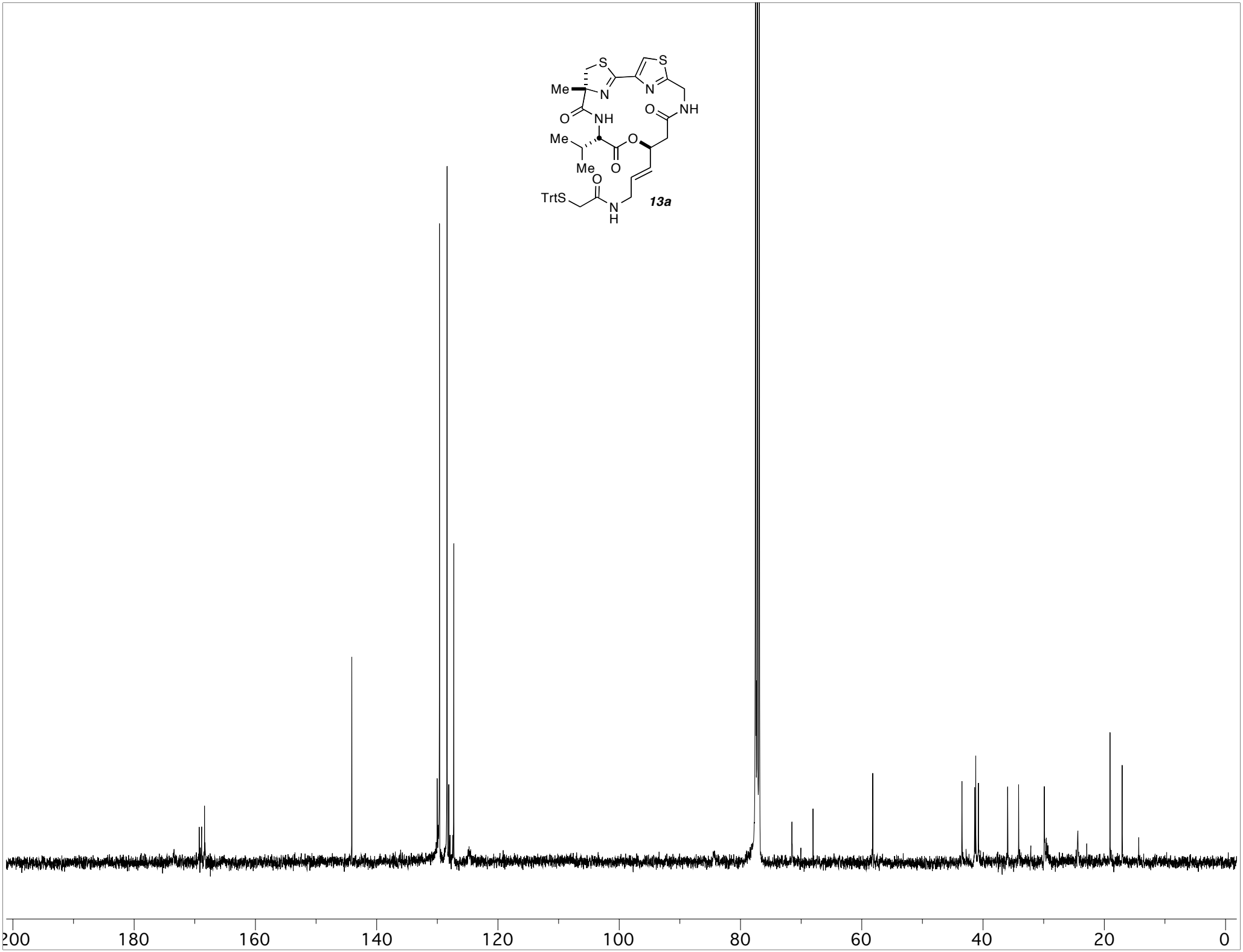
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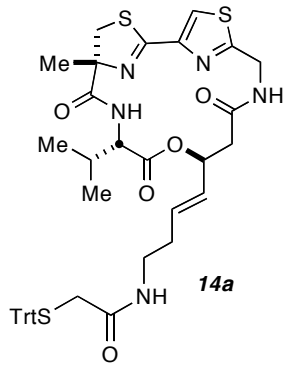

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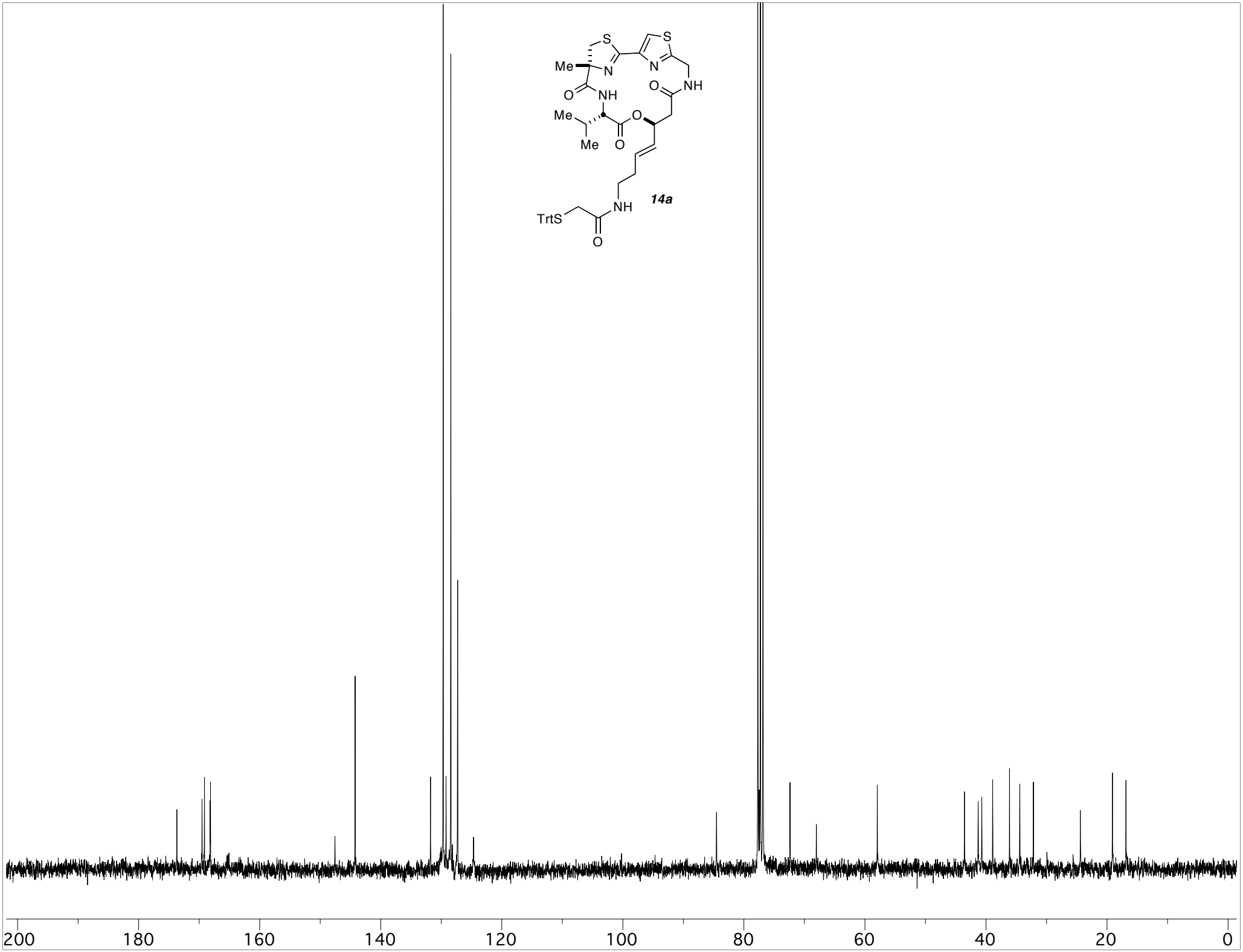
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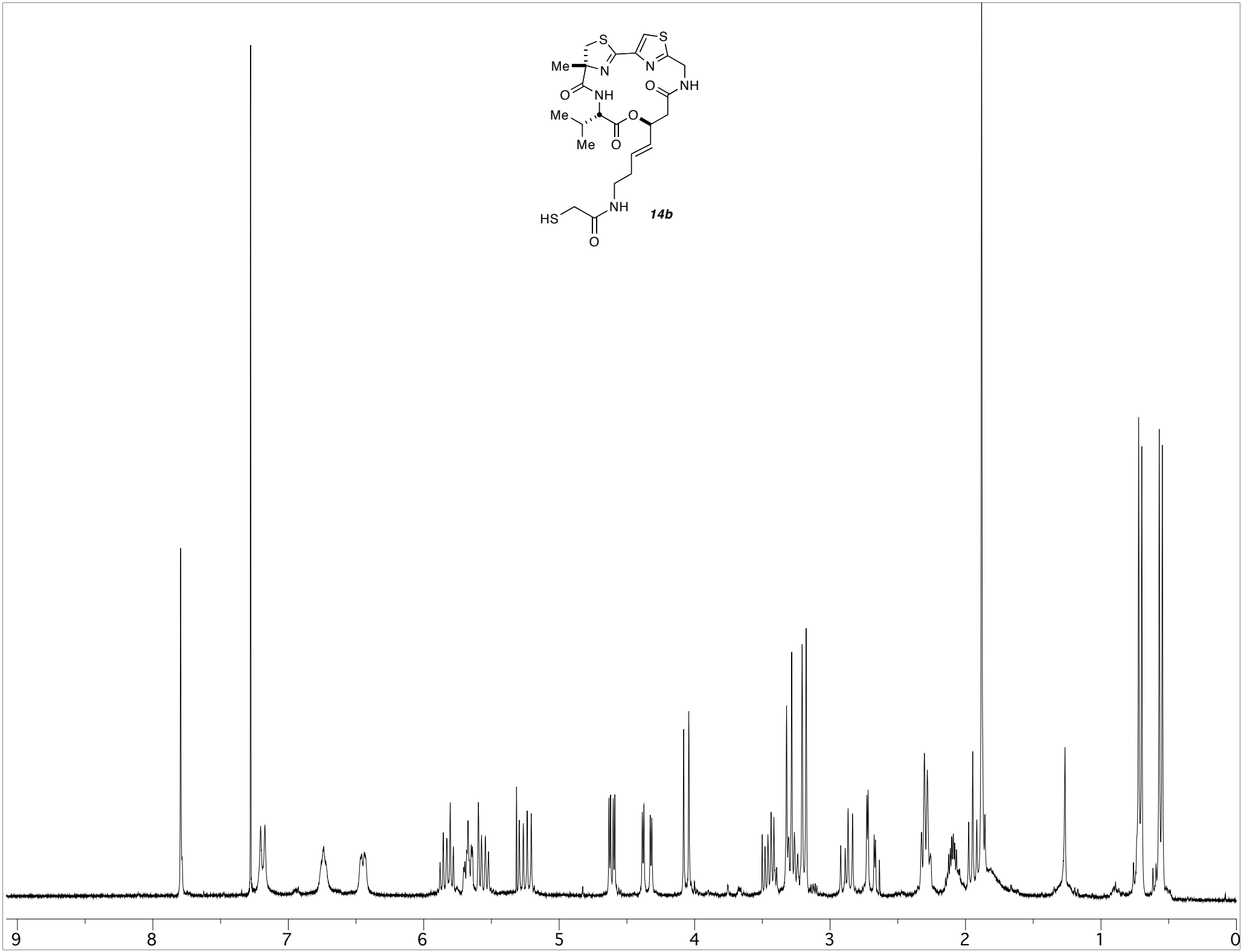
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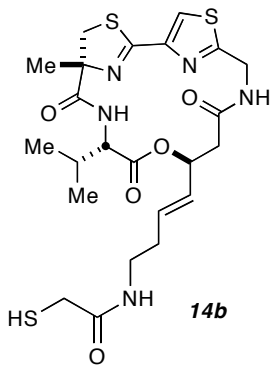
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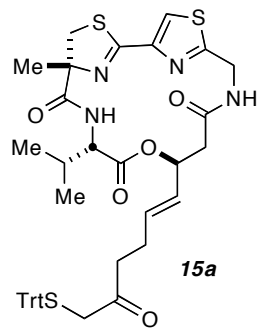

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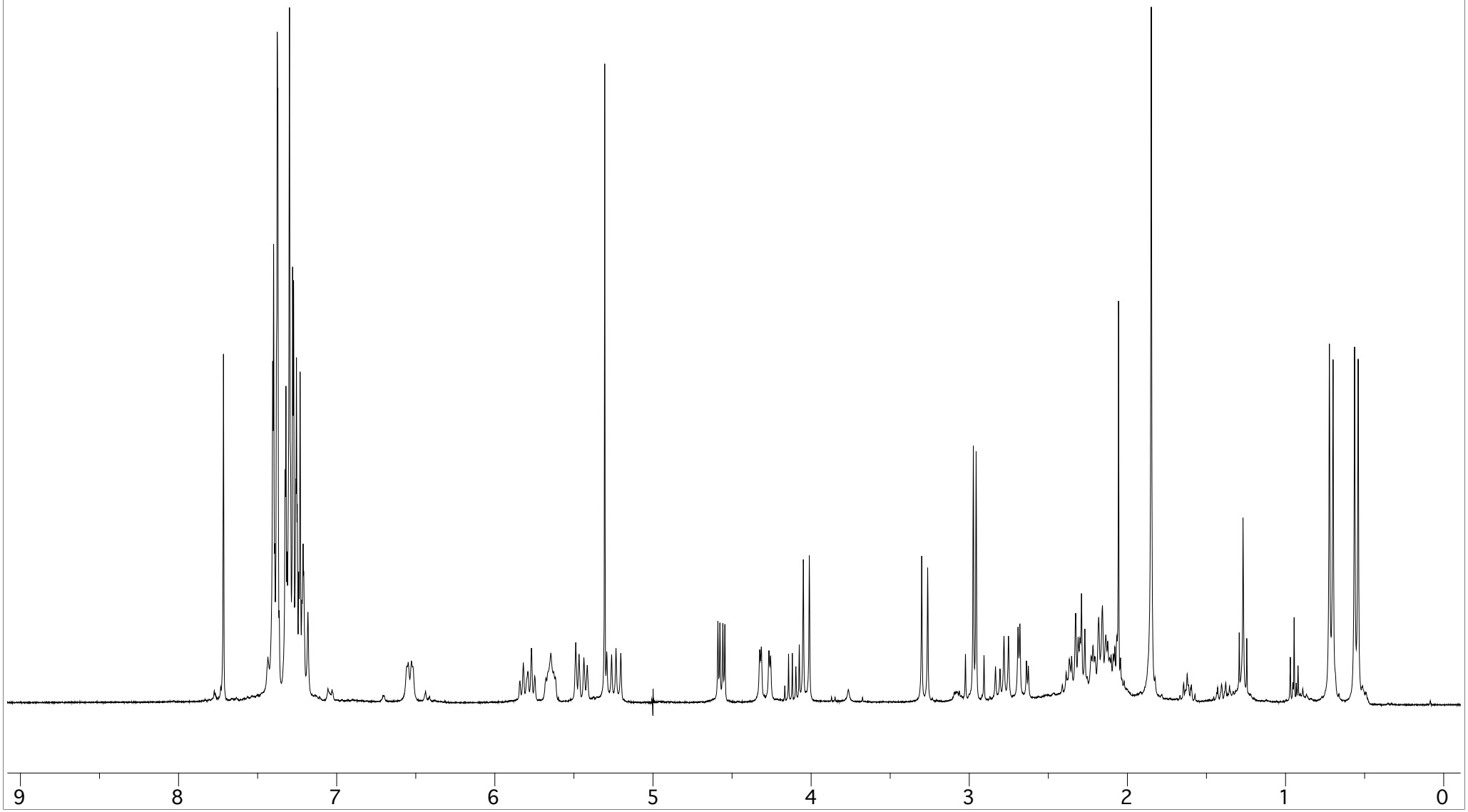

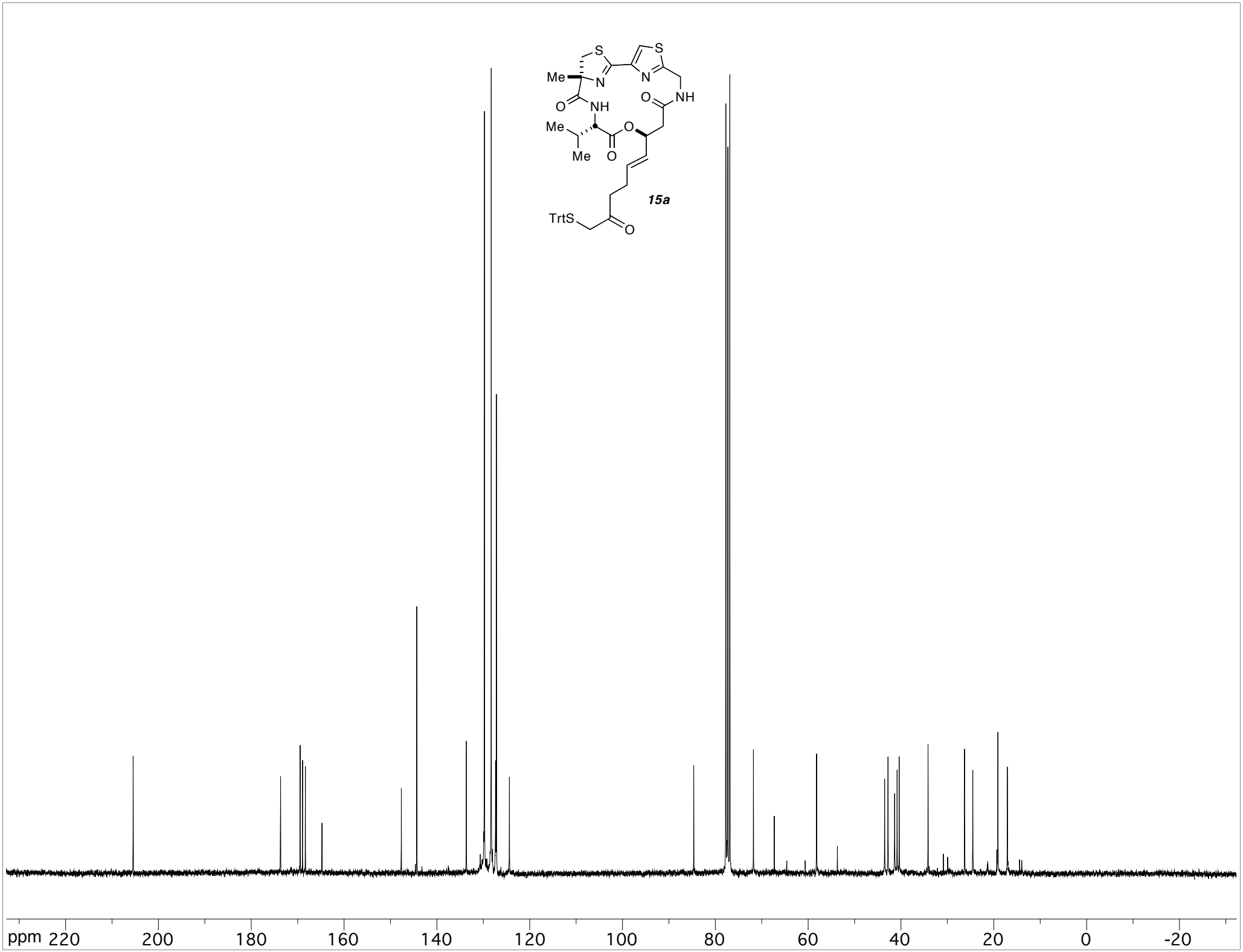
Me
N
S
S
N
O
NH
O
NH
Me
Me
O
O
O
TrtS
O
15a
ppm
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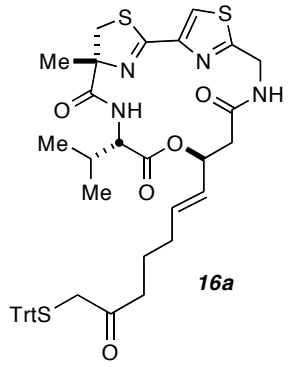
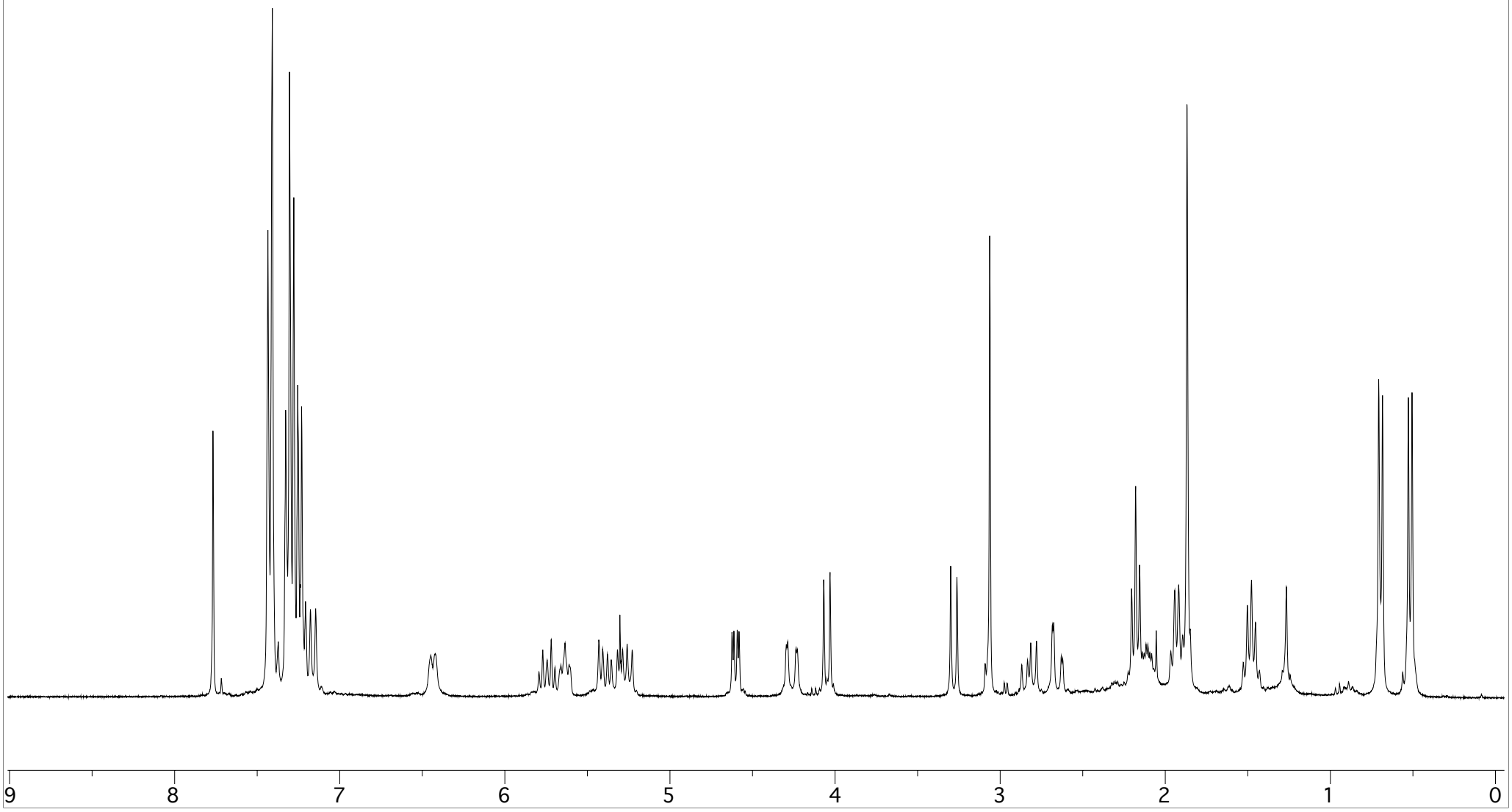
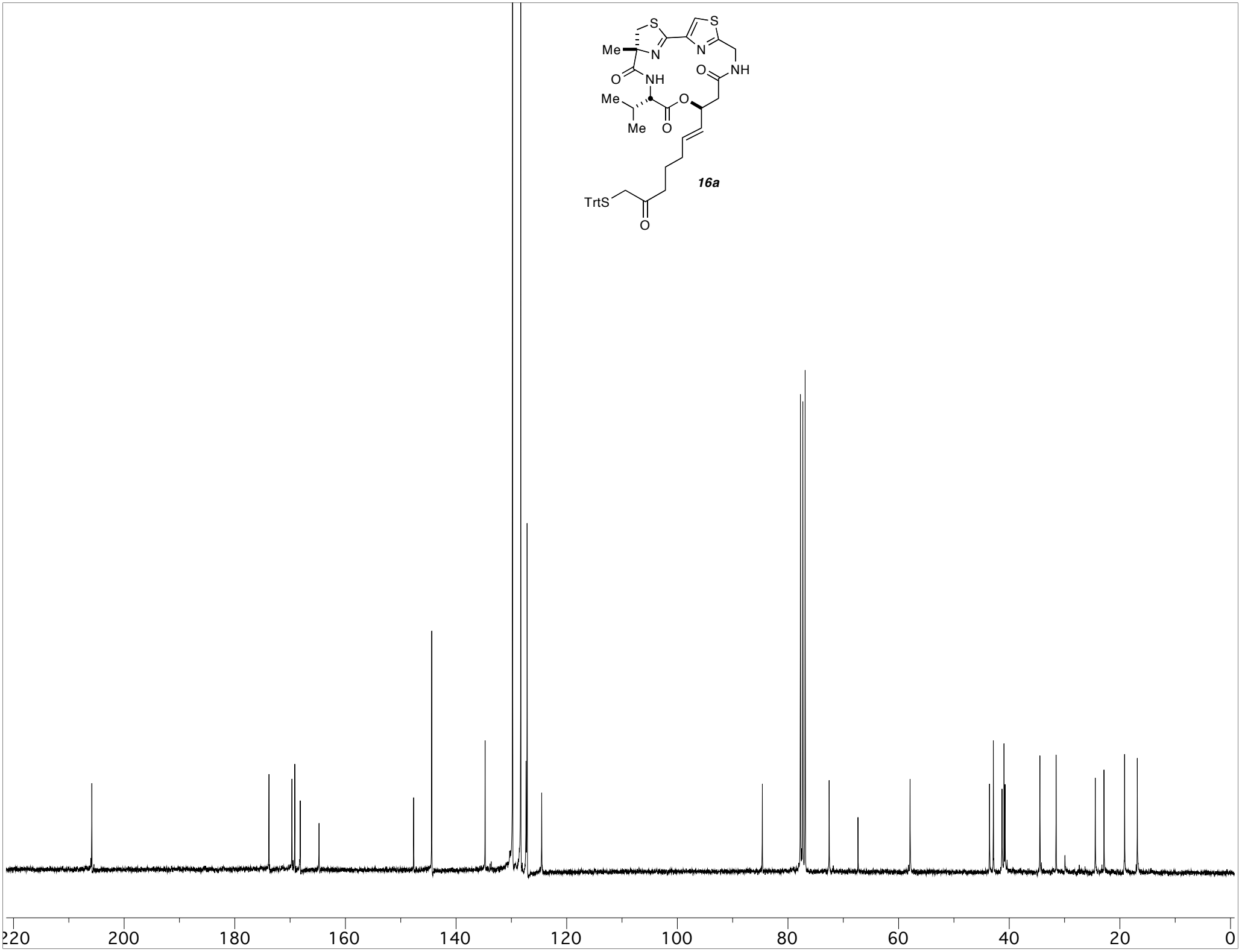
0
-20



16a




Me
N
S
S
N
O
NH
O
NH
Me
Me
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O
TrtS
O
16a
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140
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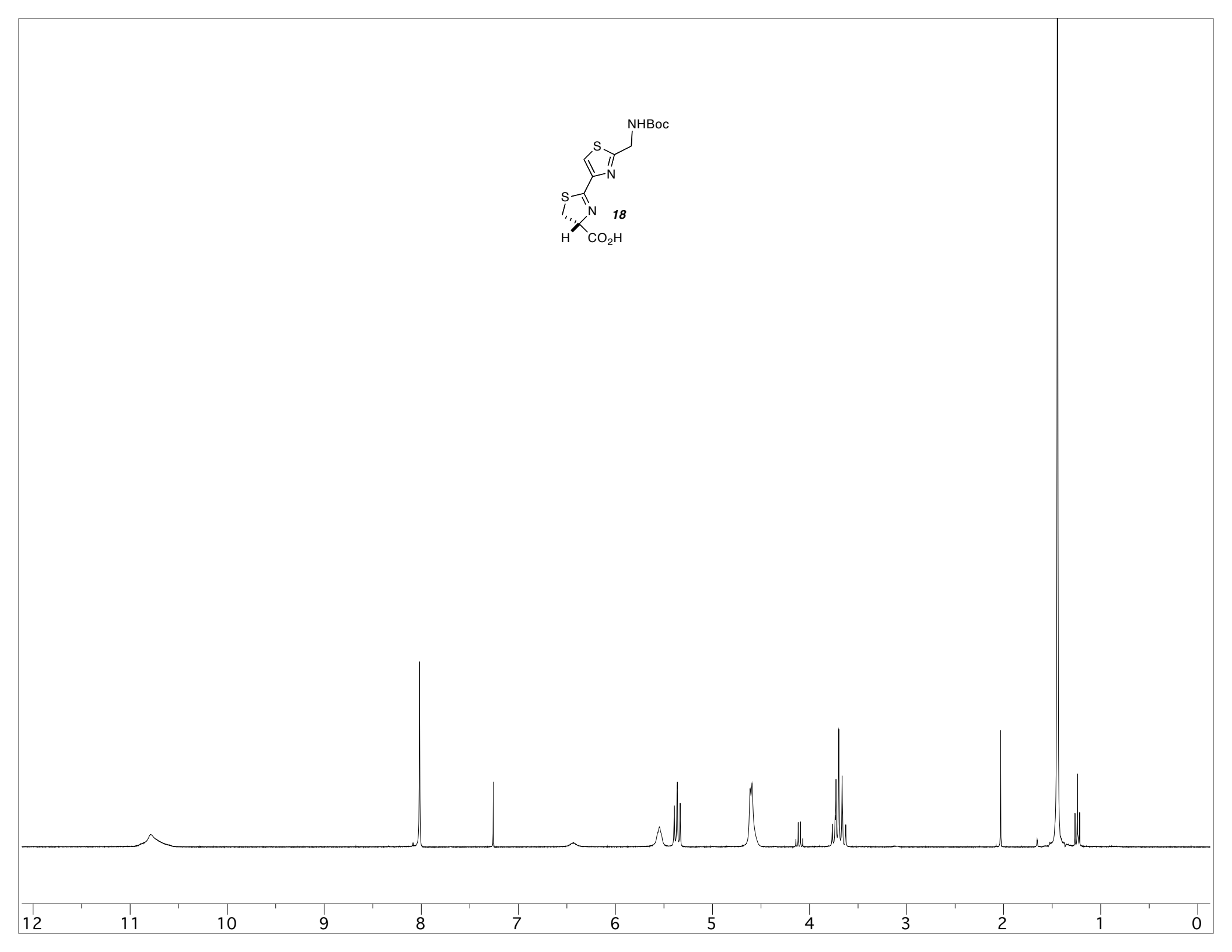
NHBoc
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7
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3
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1
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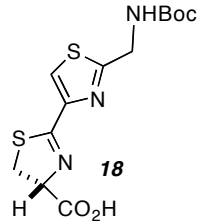

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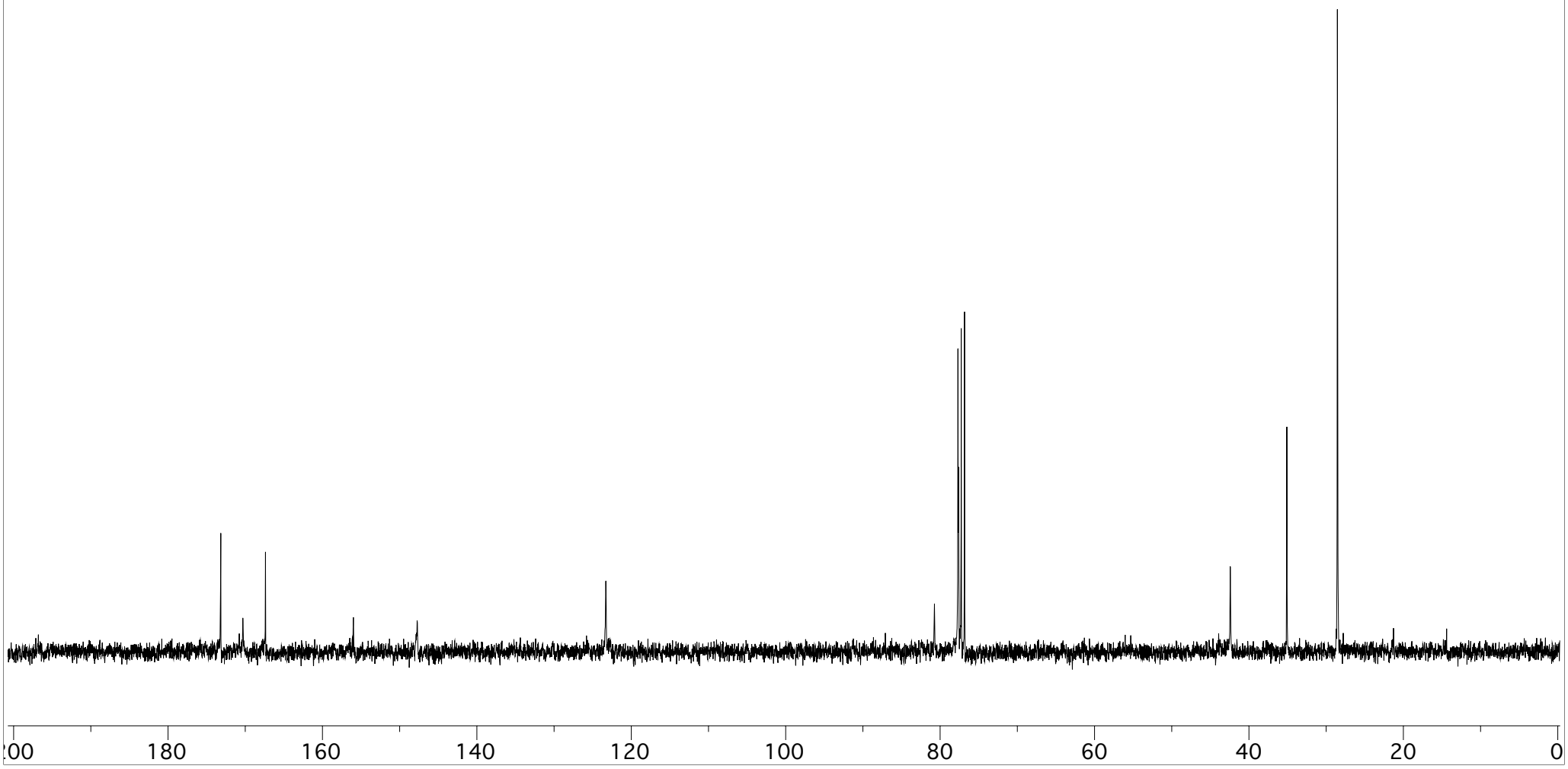

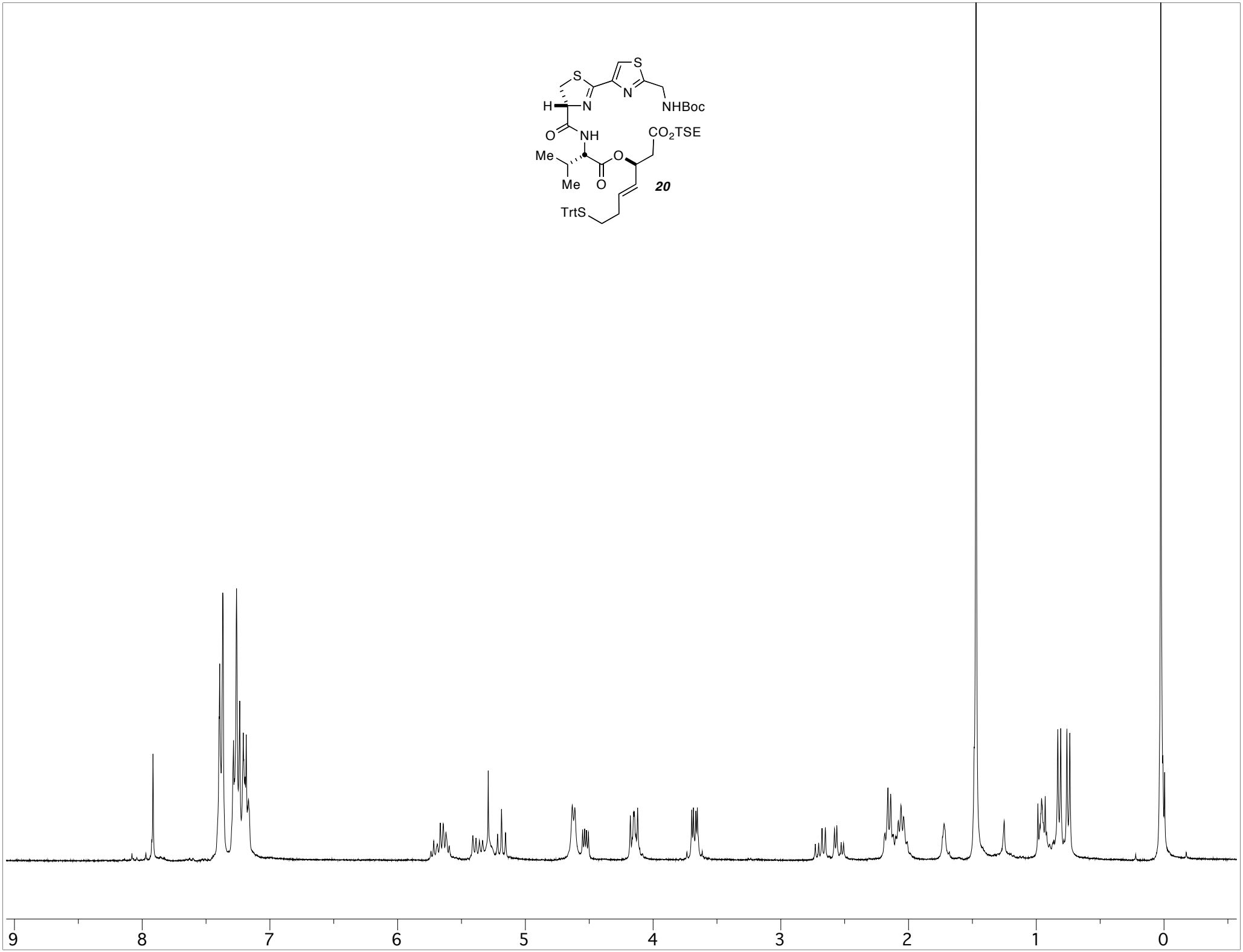
S
S
N
H
N
NHBoc
O
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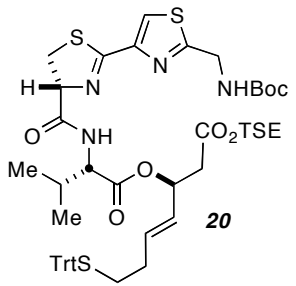
CO2TSE
Me
O
Me
O
20
TrtS
9
8
7
6
5
4
3
2
1
0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N
H
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NHBoc
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NH
CO_2TSE
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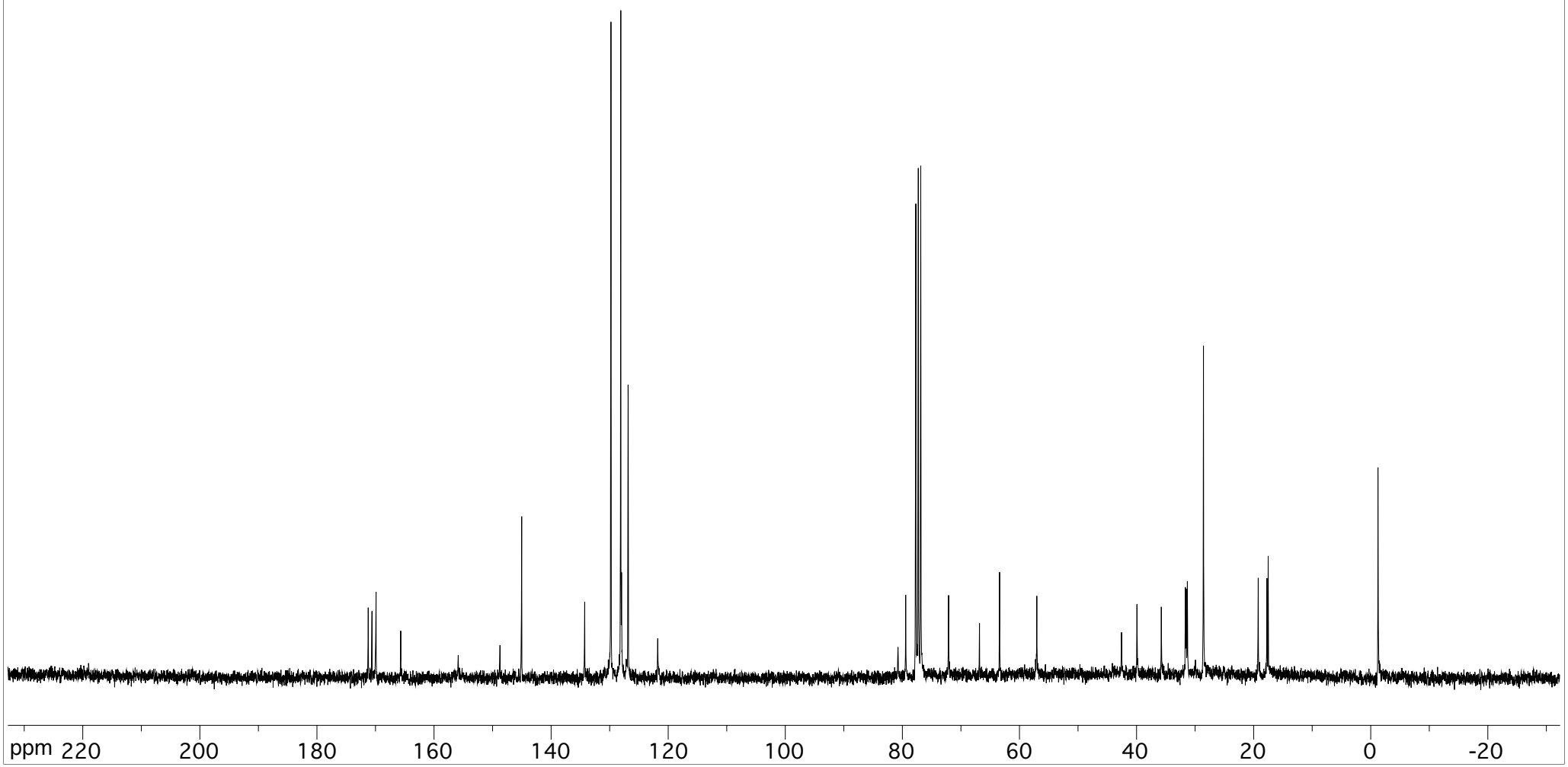
Me
O
TrtS

20


ppm
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120
100
80
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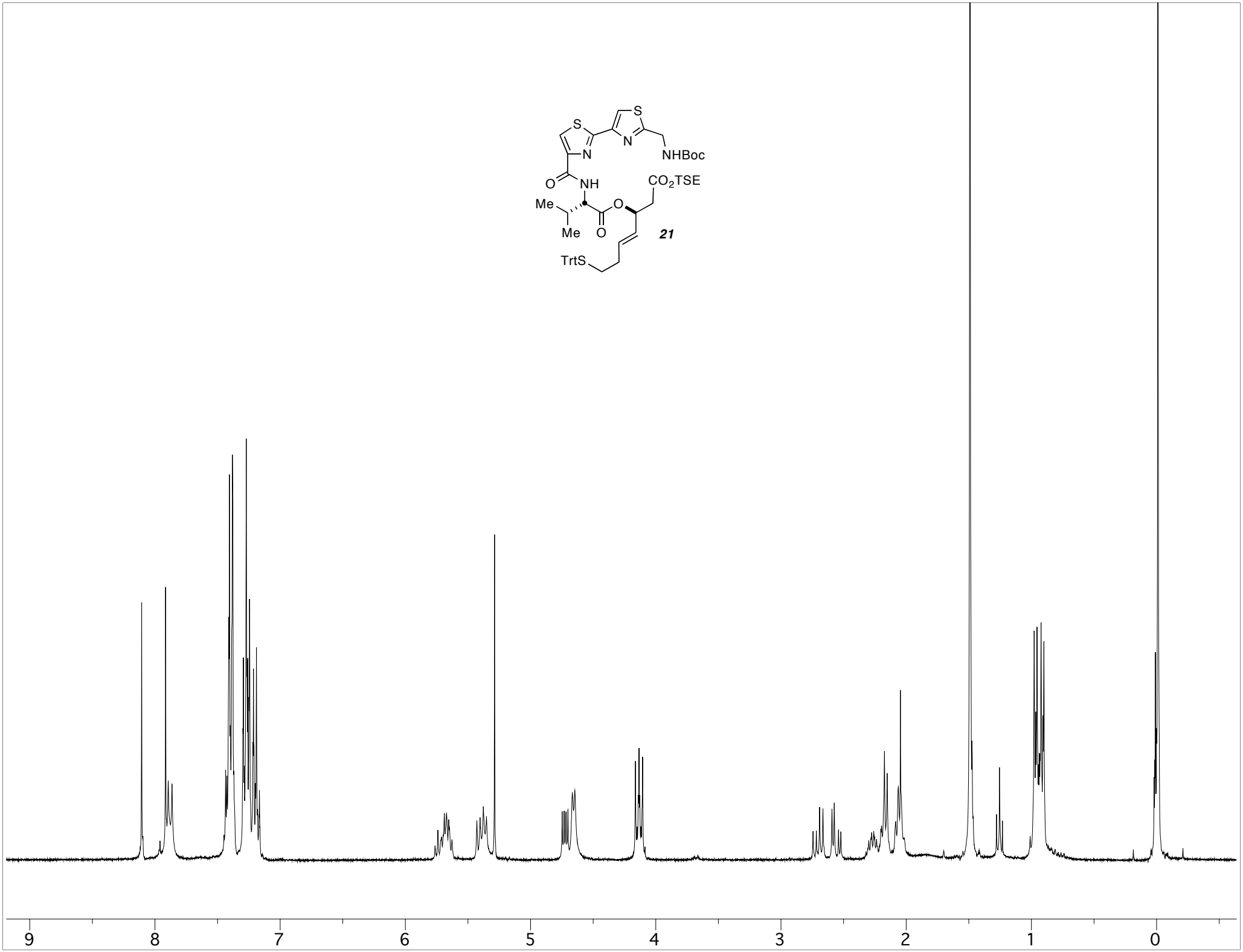
20
0
-20


S
S
N
N
NHBoc
O
NH
CO2TSE
Me
O
Me
O
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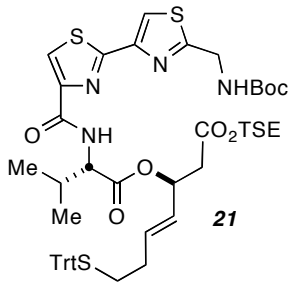
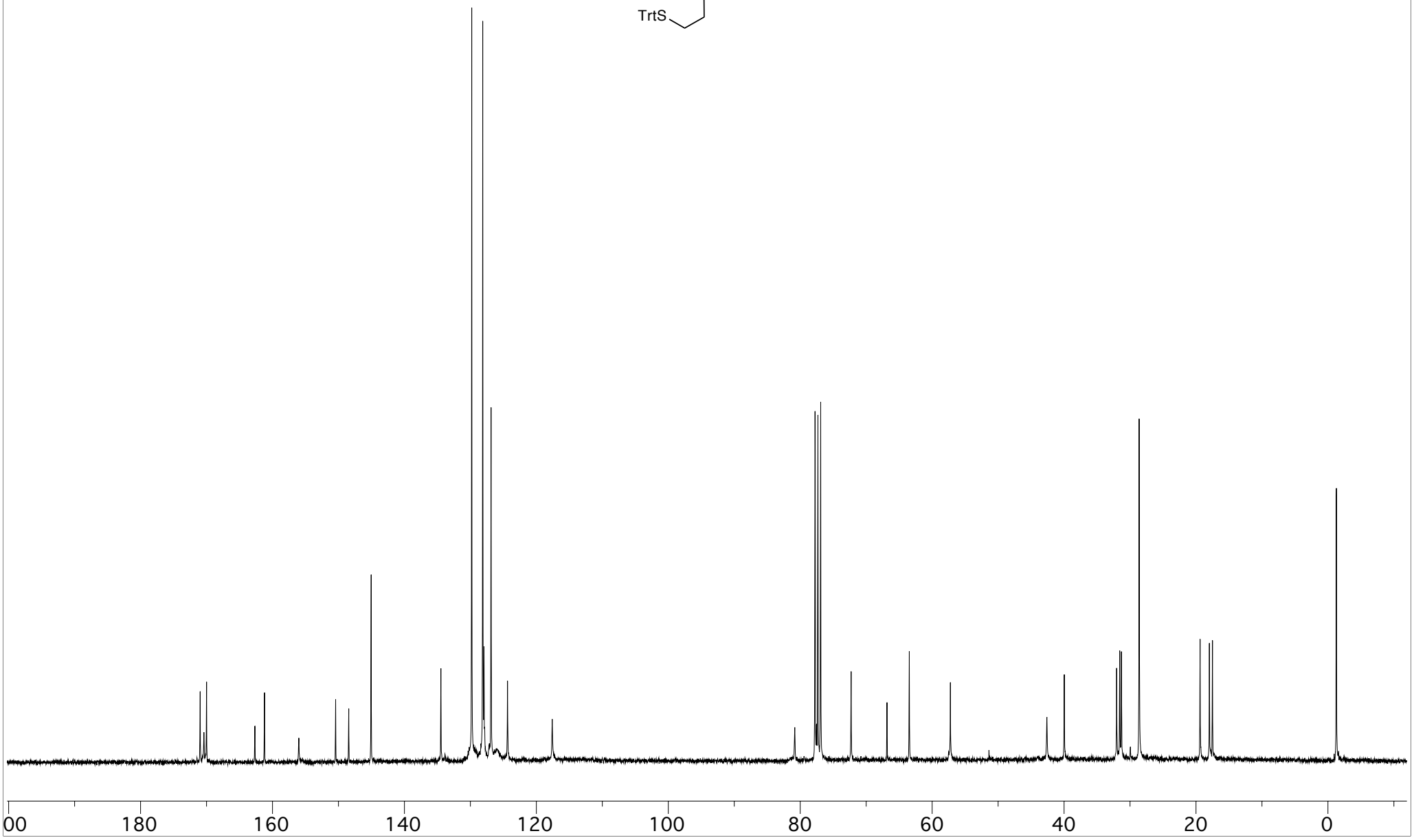
TrtS
9
8
7
6
5
4
3
2
1
0


S
S
N
N
NHBoc
O
NH
CO2TSE
Me
O
Me
O
TrtS

21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100
80
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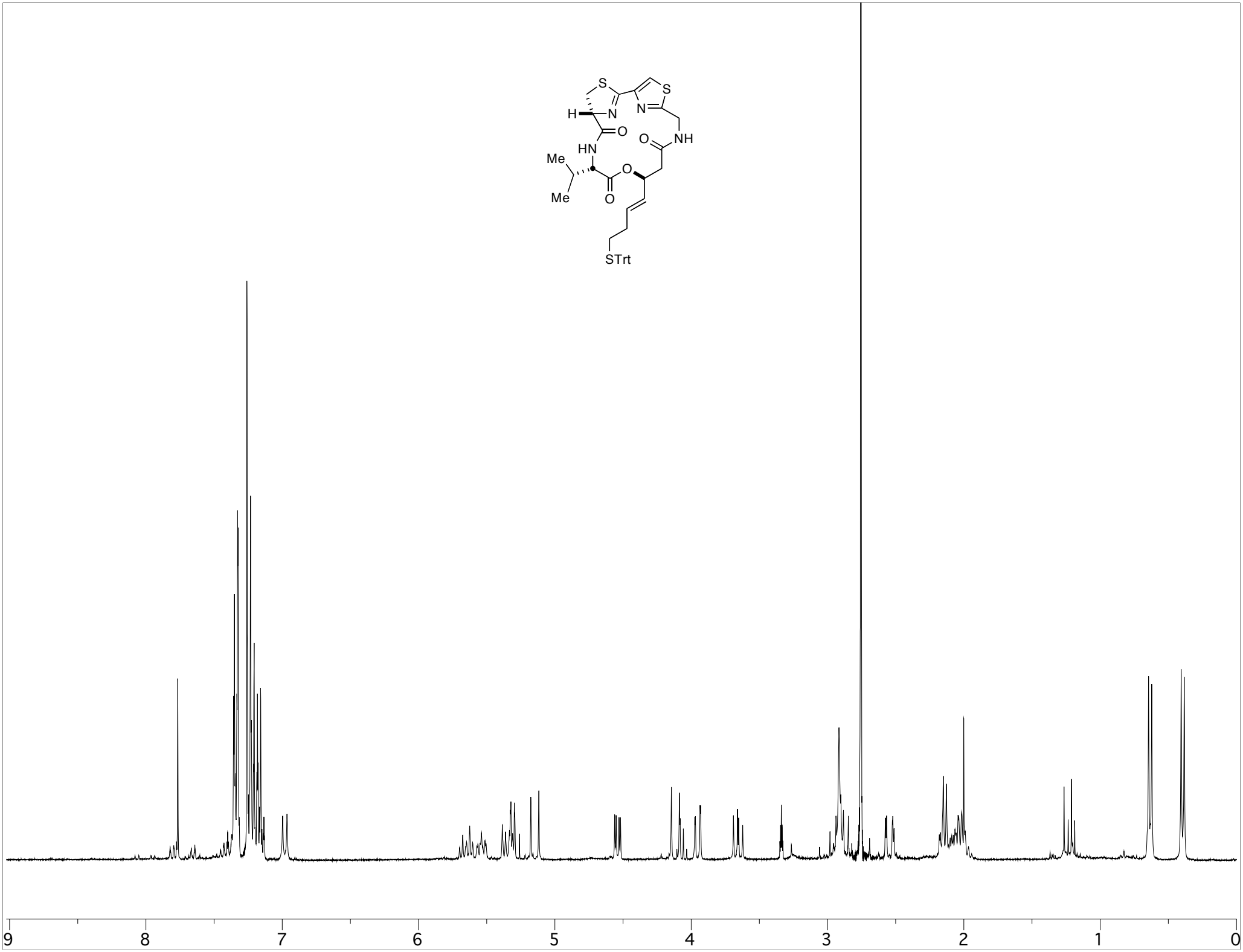
40
20
0


S
S
H
N
N
O
HN
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NH
Me
O
Me
O
STrt
9
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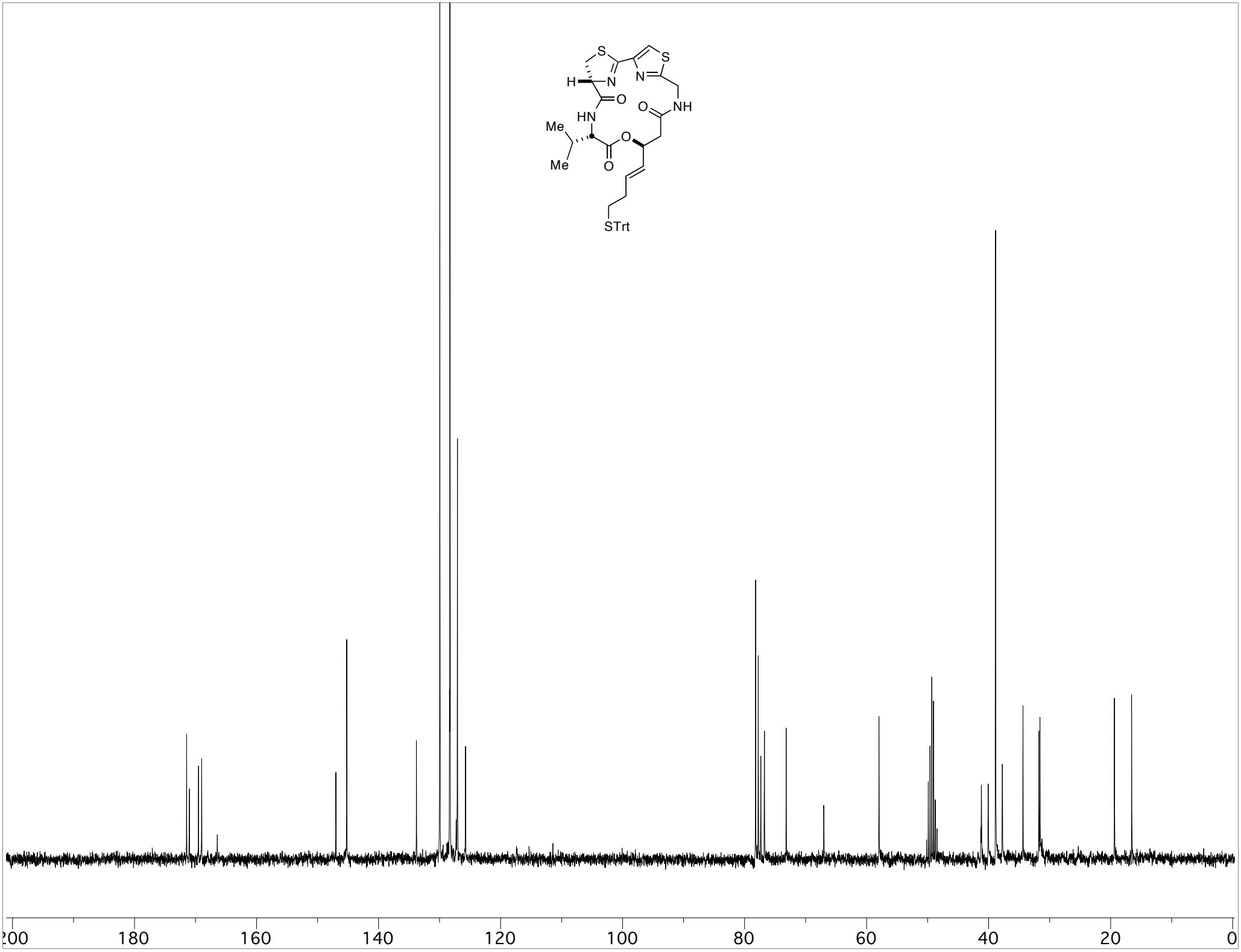
7
6
5
4
3
2
1
0


S
S
H
N
N
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Me
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Me
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STrt
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60
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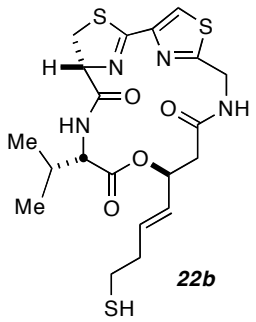
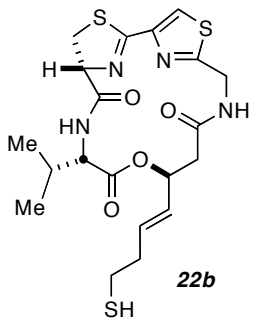
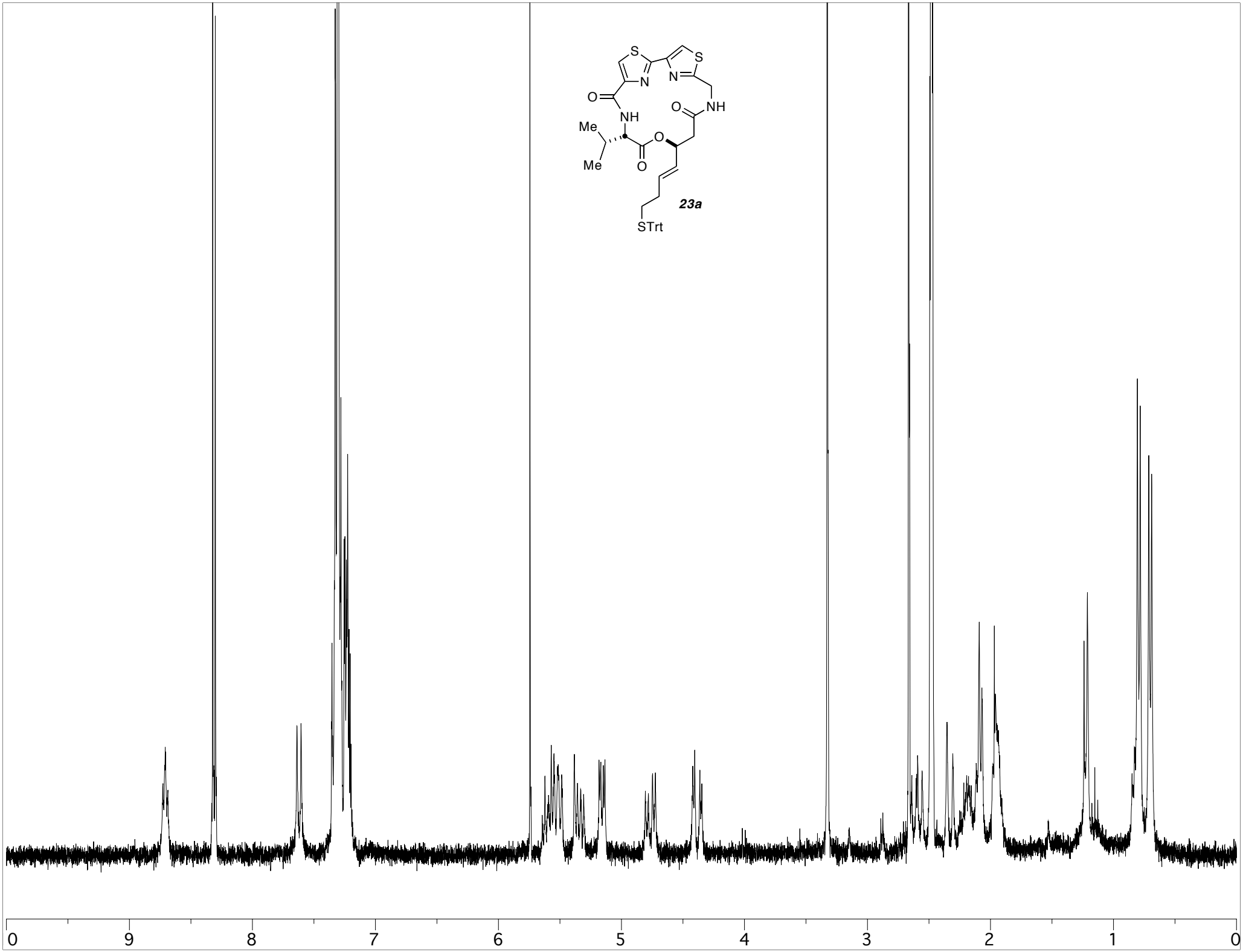
20
0

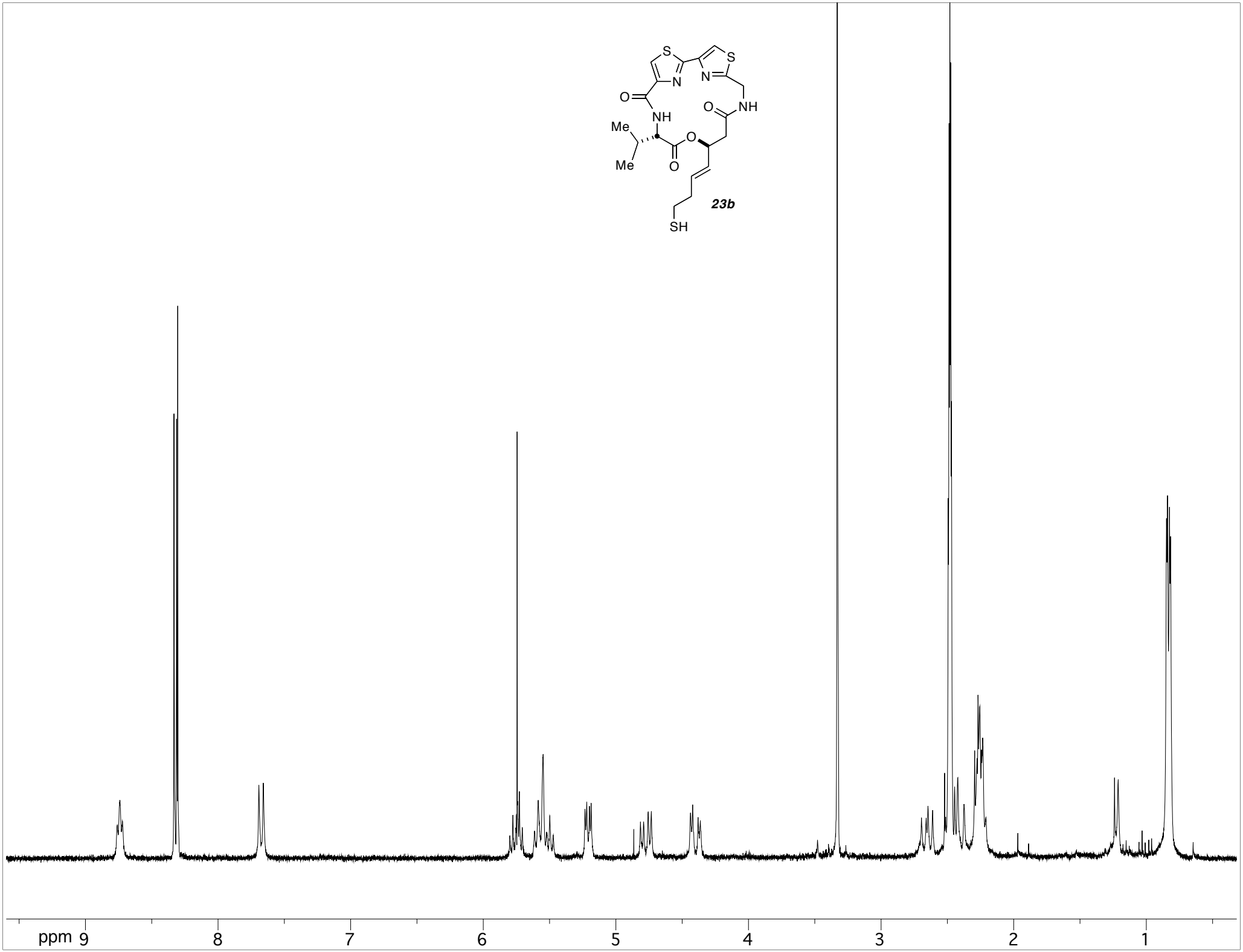


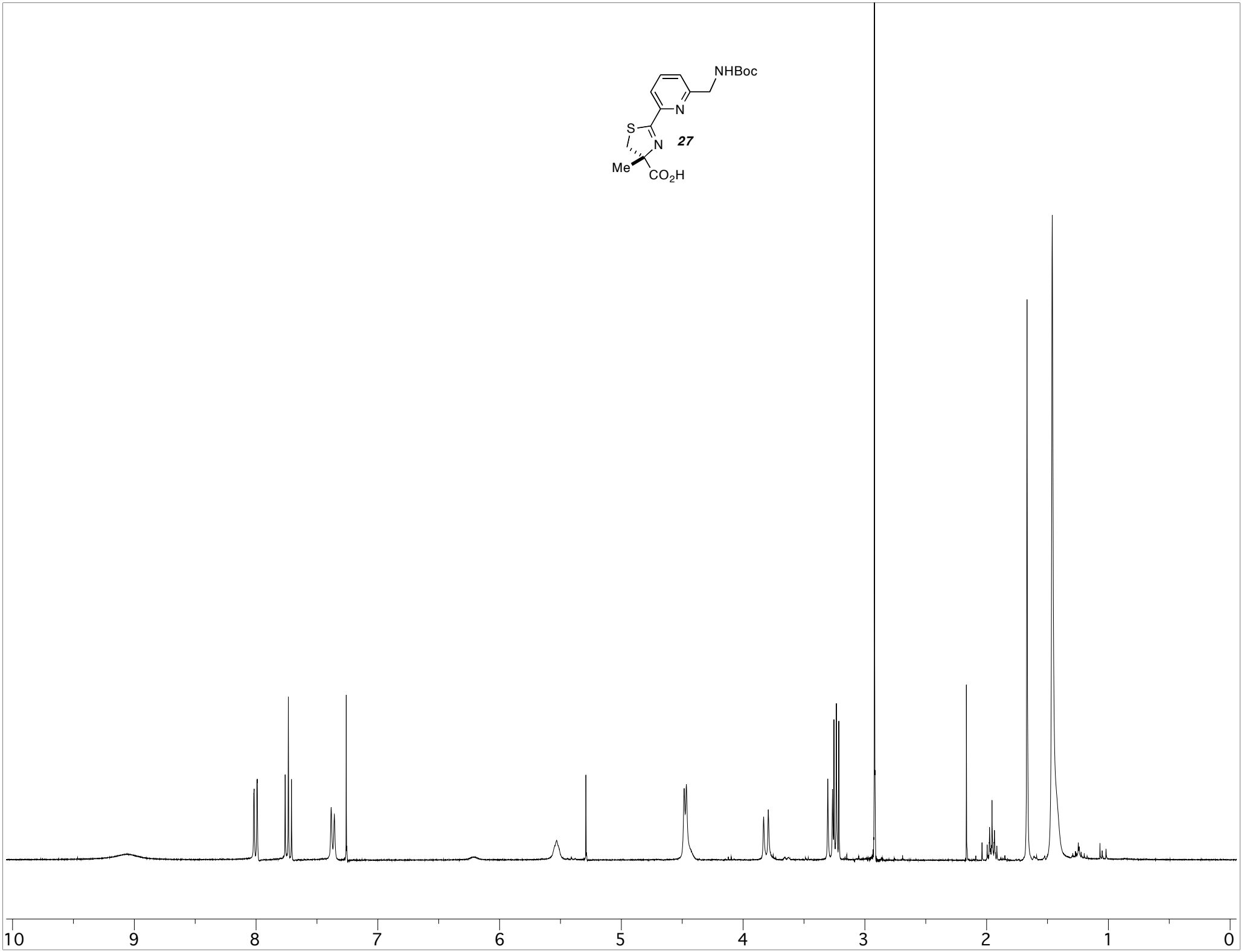
22b

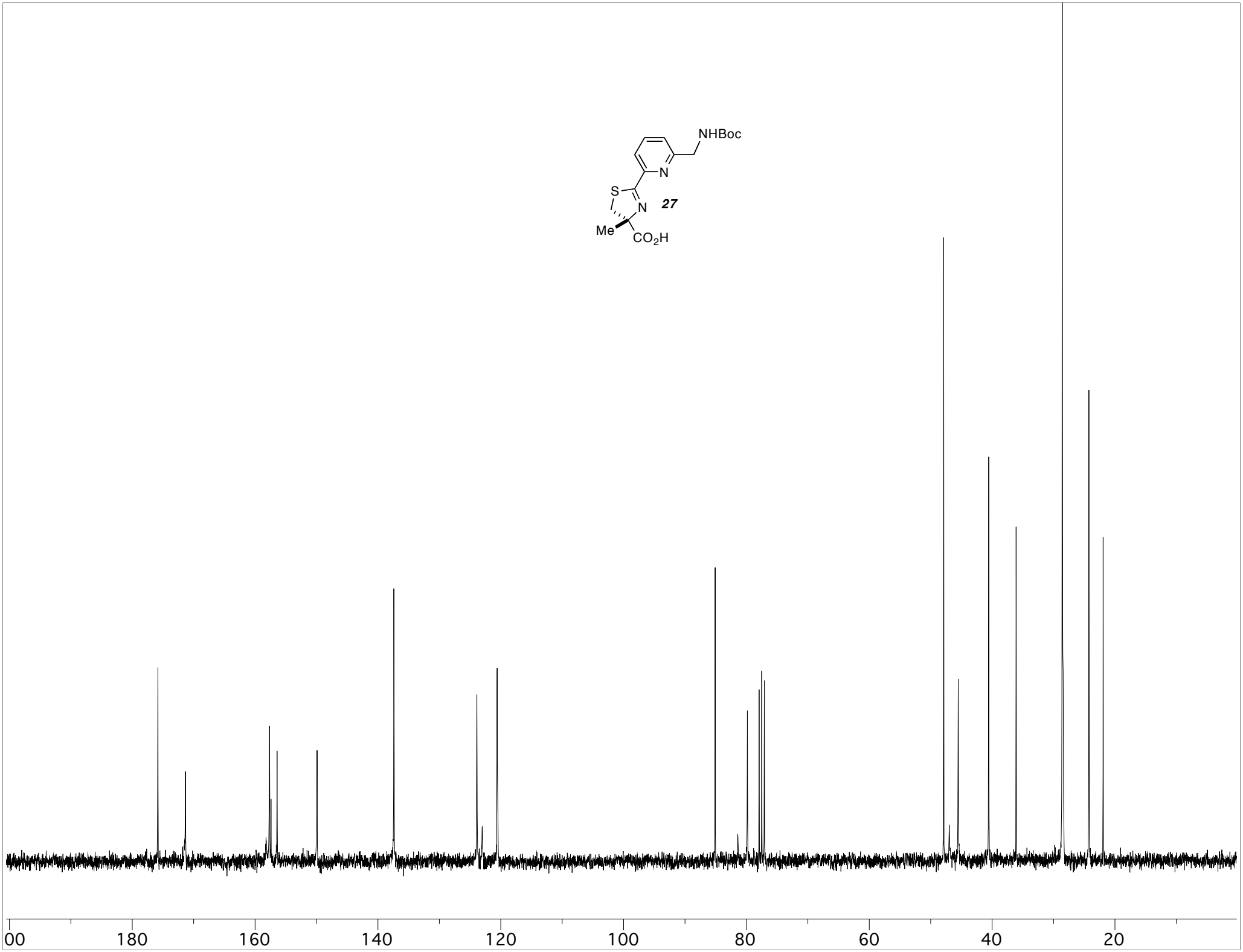


22b


S
S
N
N
O
NH
O
NH
Me
Me
O
O
STrt
23a
0
9
8
7
6
5
4
3
2
1
0


S
S
N
N
O
NH
O
NH
Me
Me
O
O
SH
23b
ppm
9
8
7
6
5
4
3
2
1


NHBoc
N
S
N
27
Me
CO2H
10
9
8
7
6
5
4
3
2
1
0


NHBoc
N
S
N
27
Me
CO2H
00
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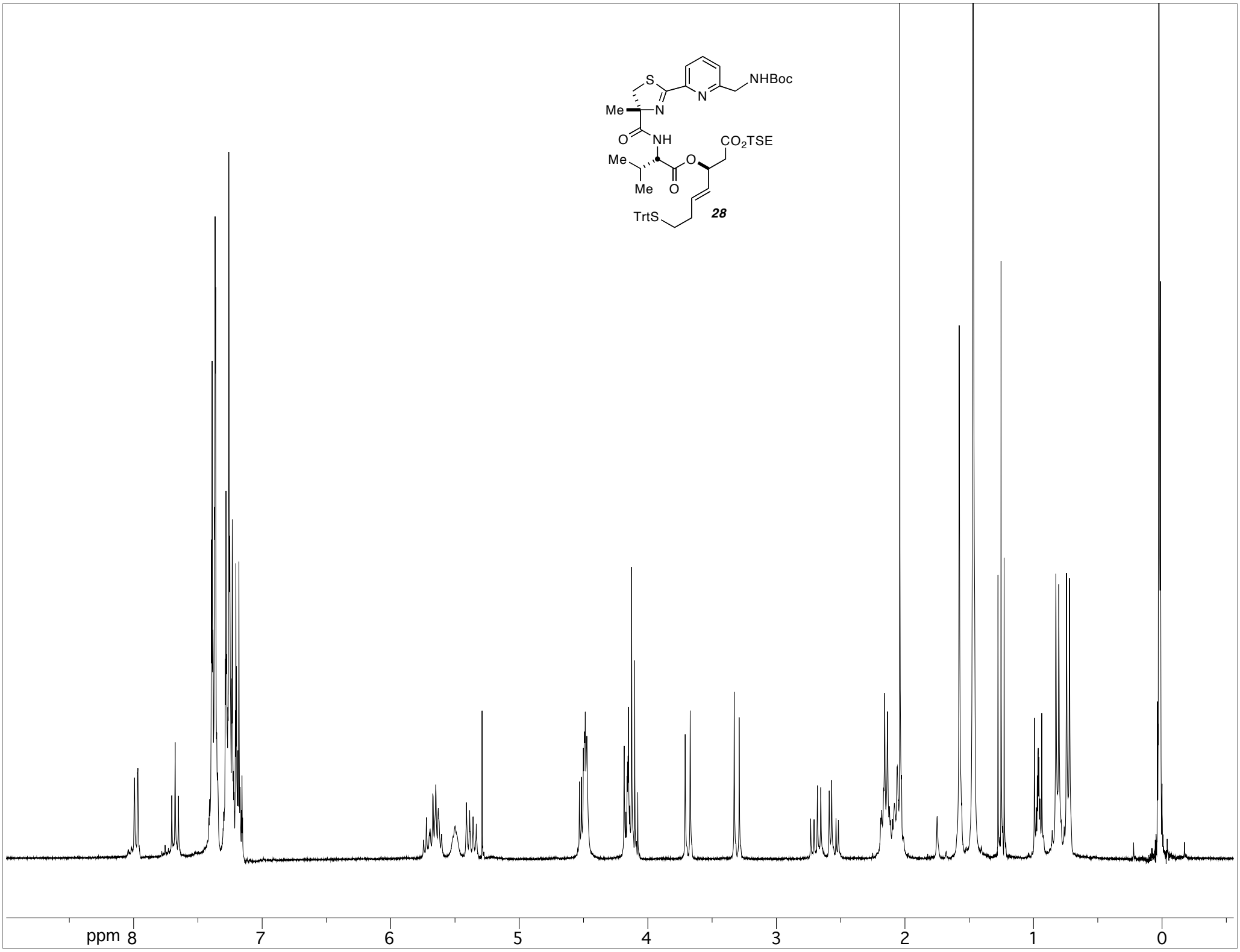
160
140
120
100
80
60
40
20


S
N
NHBoc
Me
N
O
NH
CO2TSE
Me
O
Me
O
TrtS
28
ppm
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7
6
5
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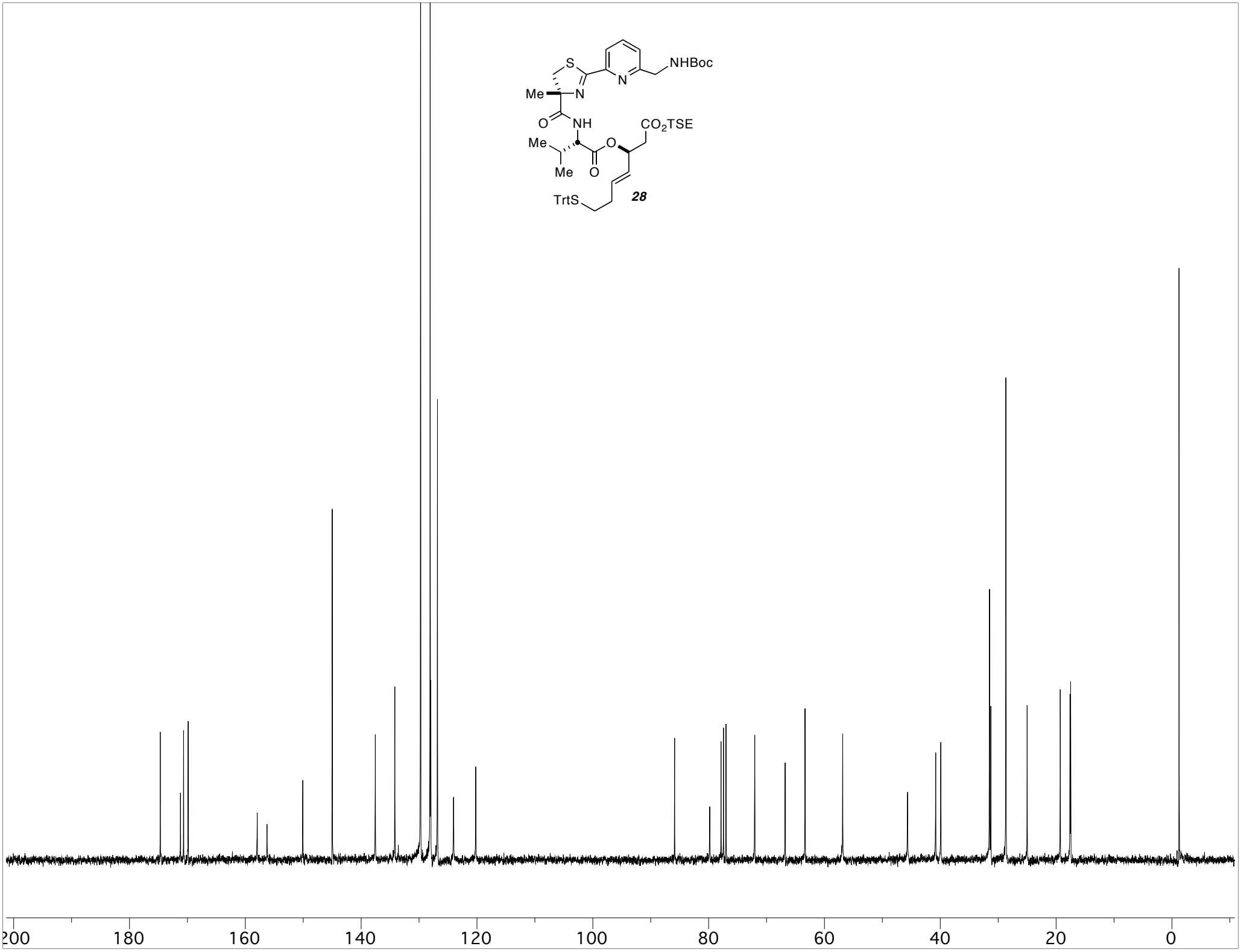
3
2
1
0


S
N
NHBoc
Me
N
O
NH
CO2TSE
Me
O
Me
O
TrtS
28
200
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140
120
100
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60
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20
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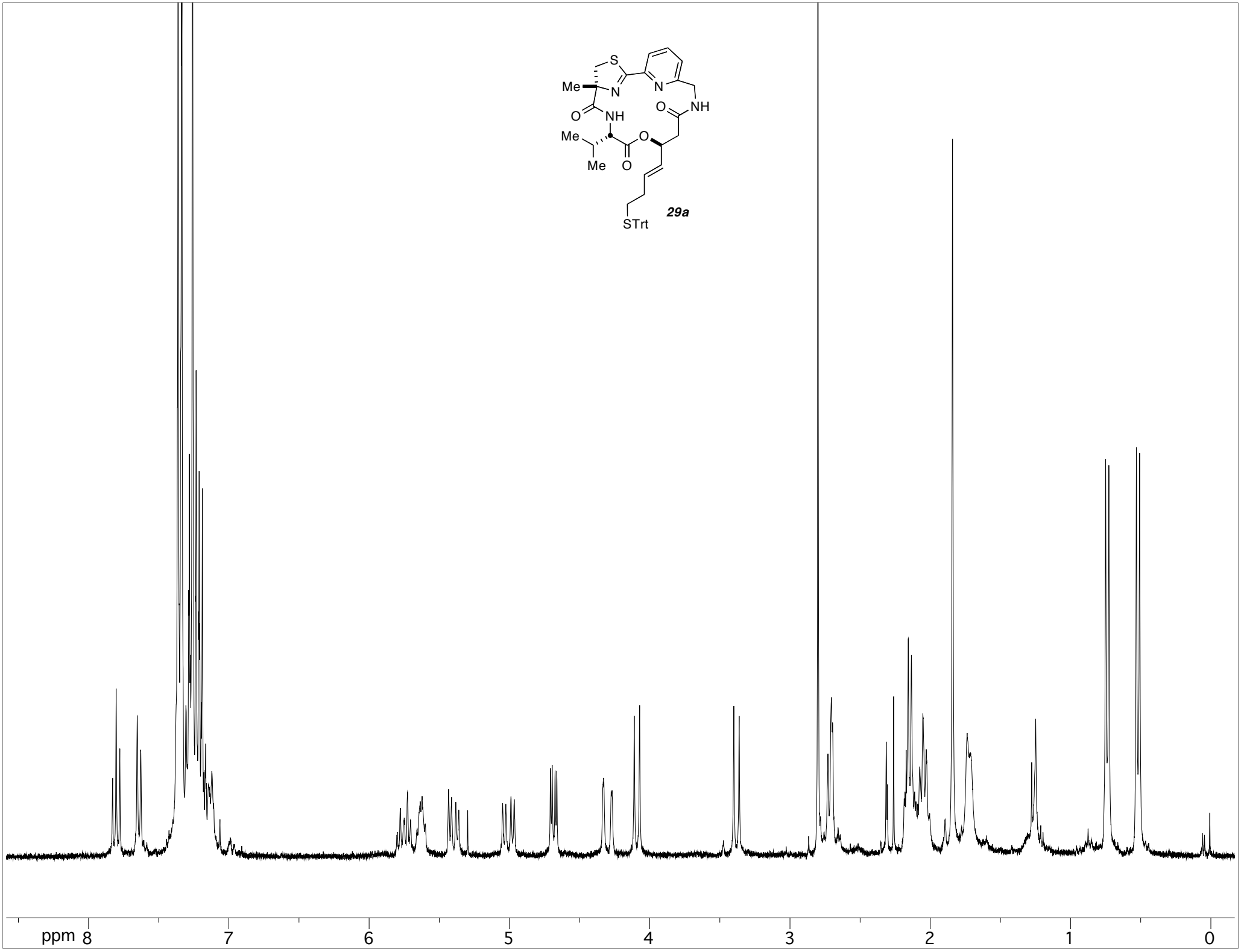
S
Me
N
N
O
NH
O
NH
Me
O
Me
O
STrt
29a
ppm
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7
6
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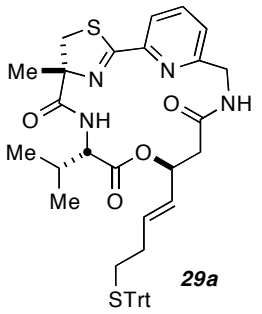
3
2
1
0


S
Me
N
N
O
NH
O
NH
Me
O
Me
O
STrt

29a

190
180
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150
140
130
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100
90
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70
60
50
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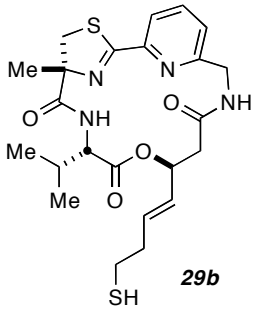
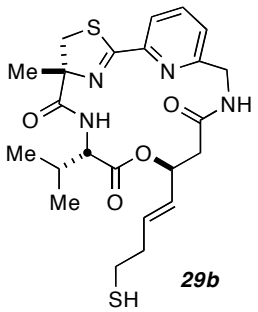
30
20
10
0

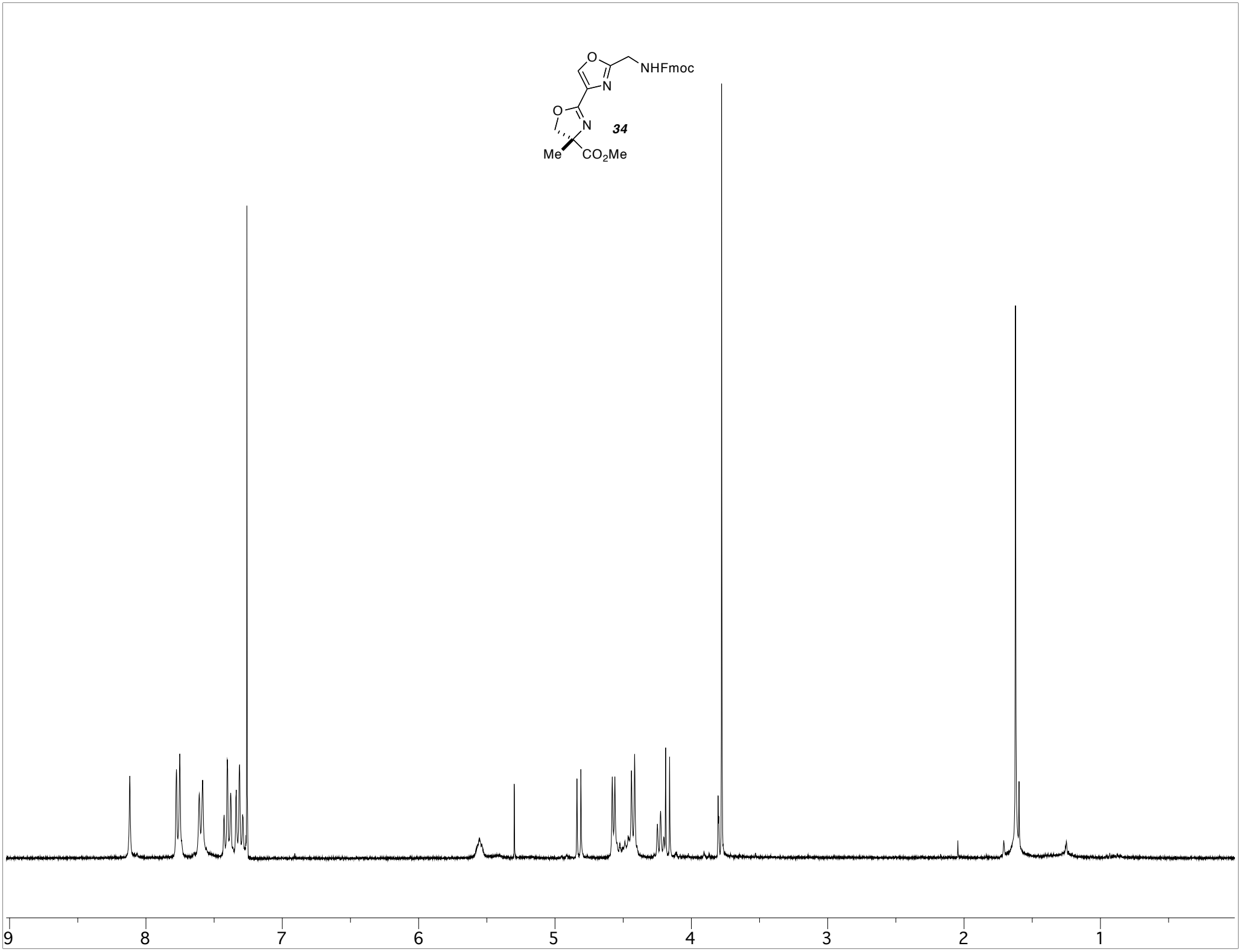


29b


S
Me
N
N
O
NH
O
NH
Me
O
Me
O
SH

29b

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


NHFmoc
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N
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Me
CO2Me
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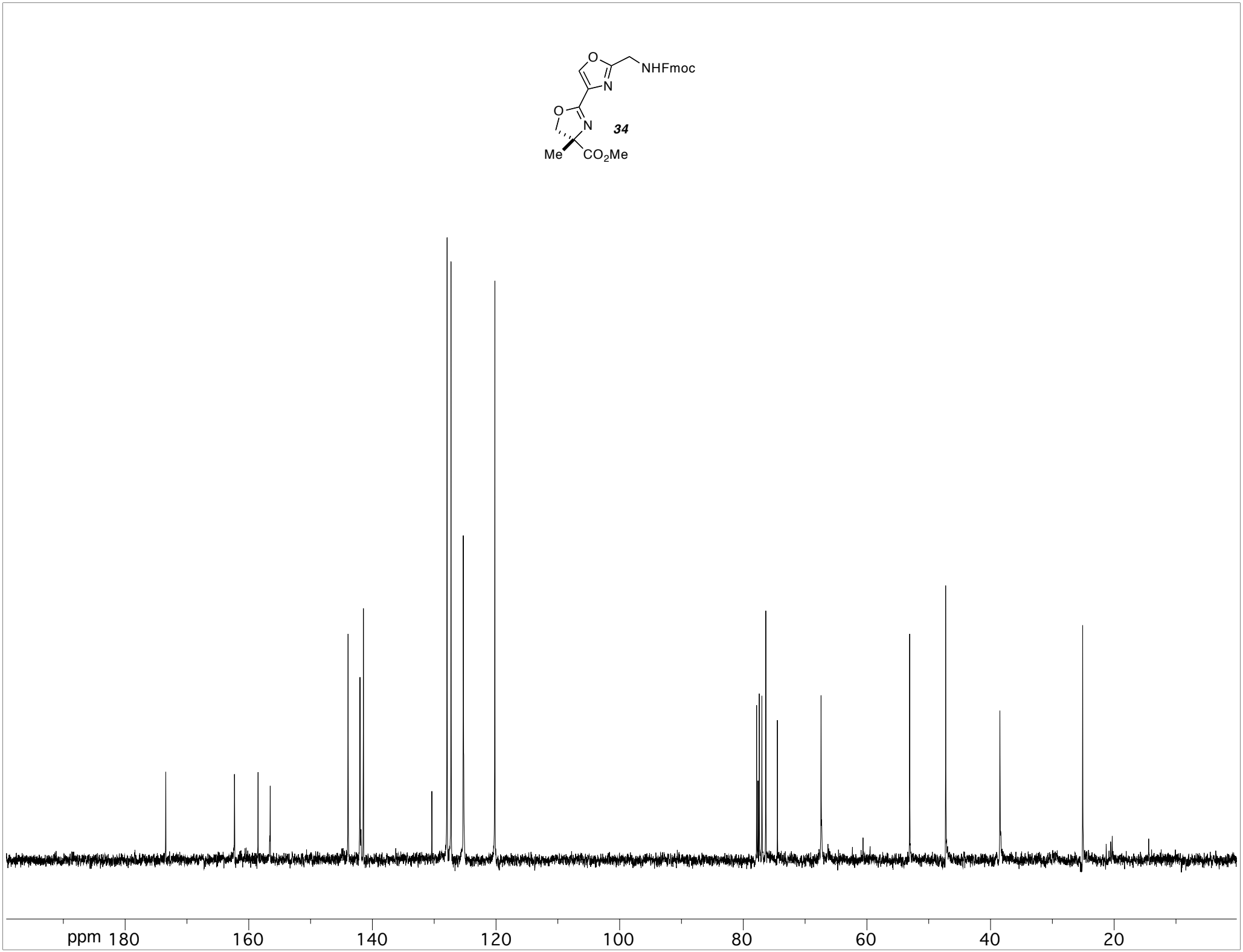
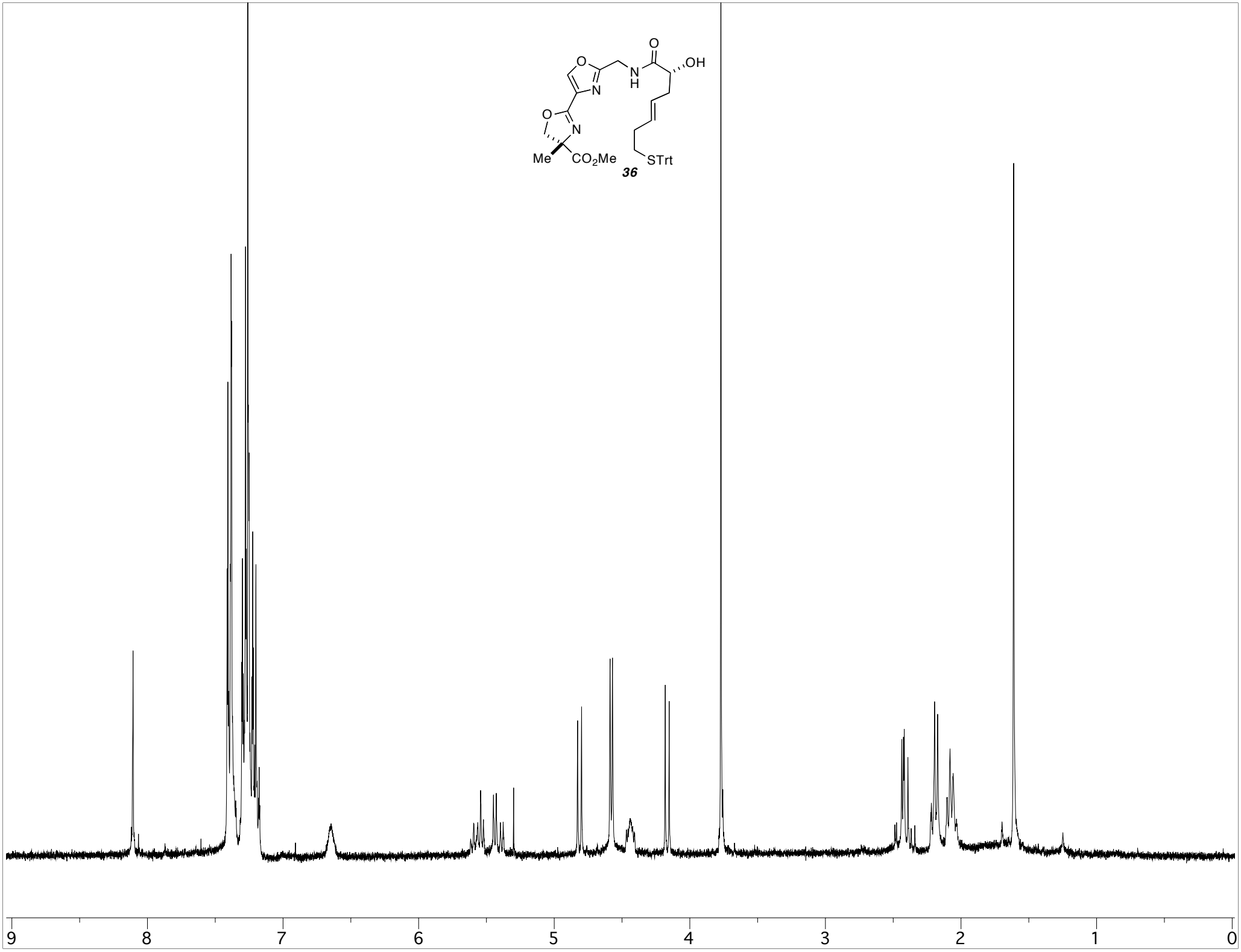
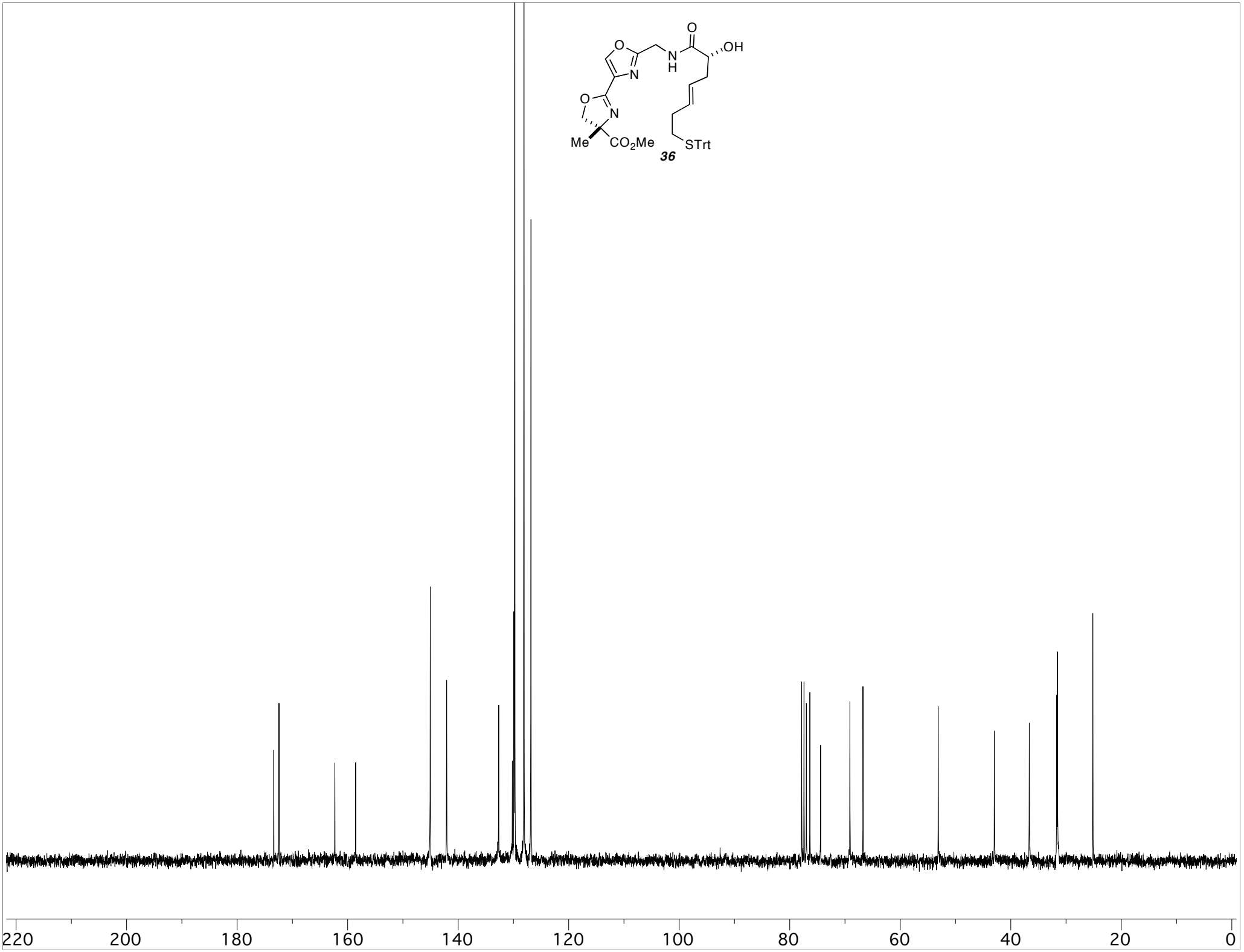
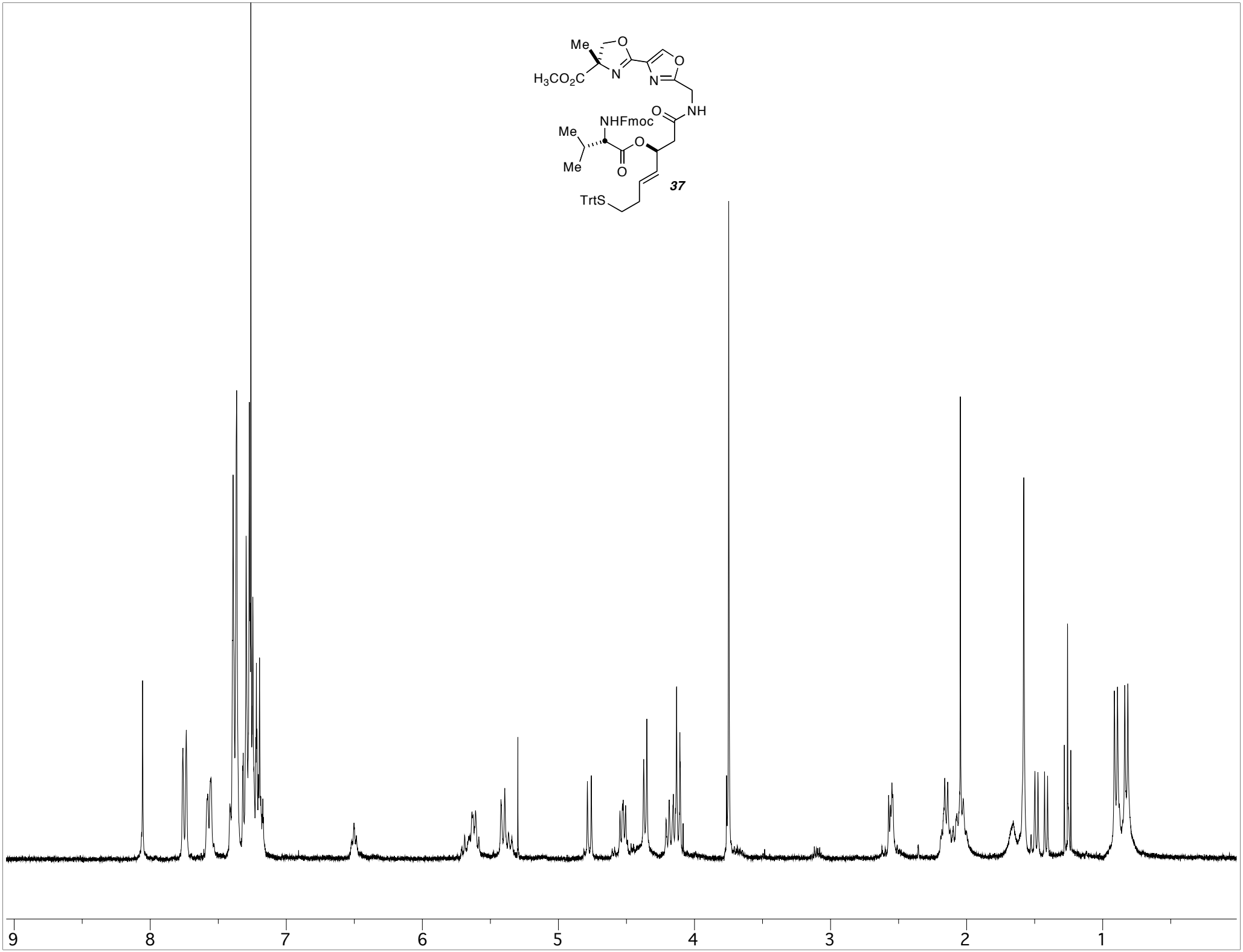
8
7
6
5
4
3
2
1

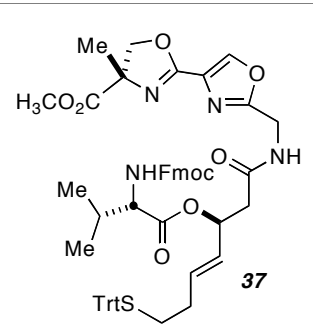

NHFmoc
O
N
O
N
34
Me
CO2Me
ppm
180
160
140
120
100
80
60
40
20


O
O
N
H
OH
N
O
N
Me
CO2Me
STrt
36
9
8
7
6
5
4
3
2
1
0


O
N
H
OH
O
N
O
N
Me
CO2Me
STrt
220
200
180
160
140
120
100
80
60
40
20
0

36


Me
O
O
H_3CO_2C
N
N
O
NH
NHFmoc
Me
O
Me
O
TrtS
37
9
8
7
6
5
4
3
2
1


Me
O
O
H_3CO_2C
N
N
O
NH
NHFmoc
Me
Me
O
O
TrtS

37

180
170
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150
140
130
120
110
100
90
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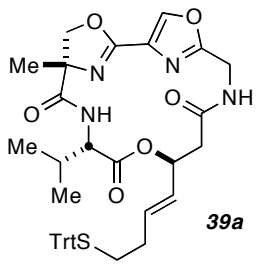
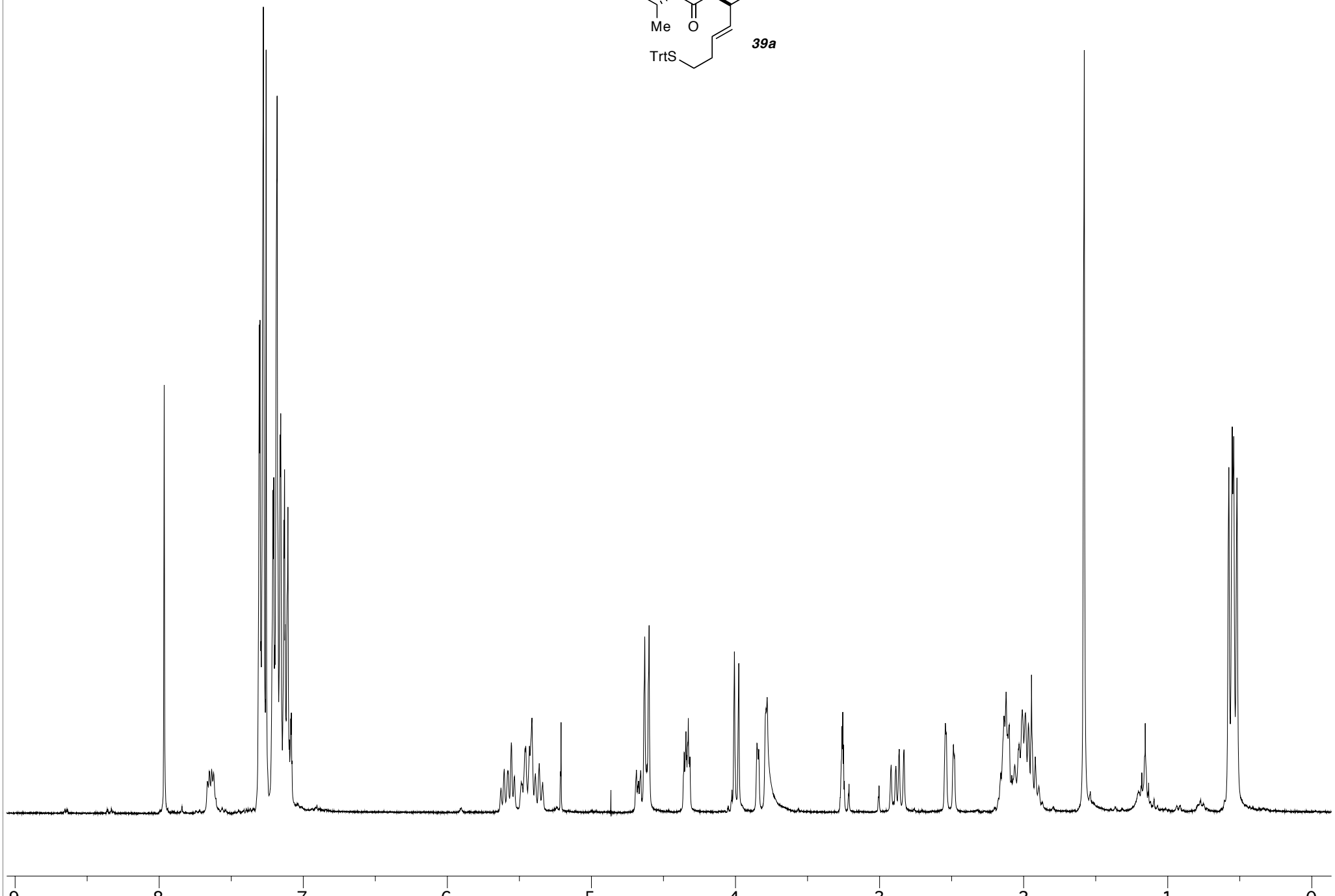
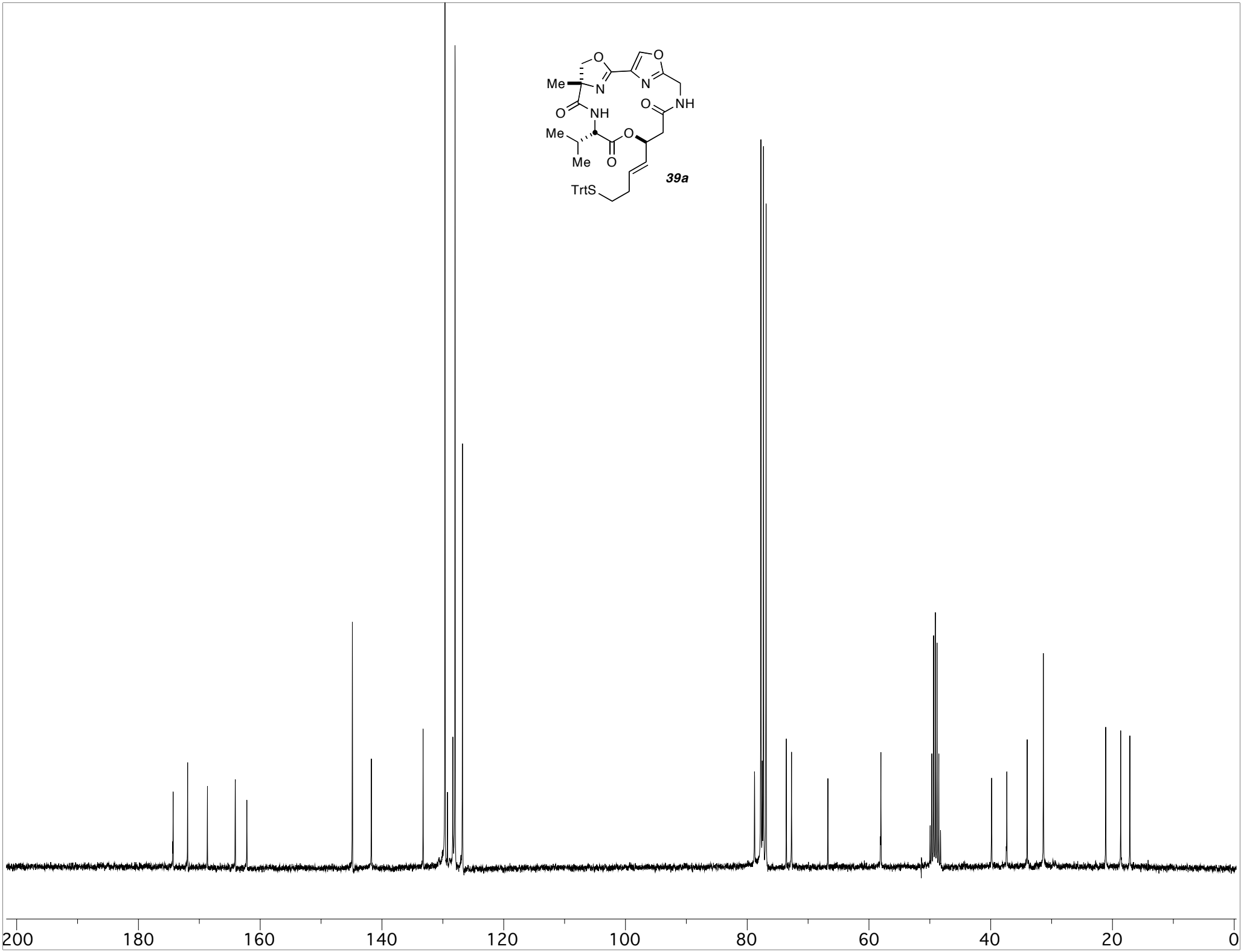
70
60
50
40
30
20
10
0



39a




Me
O
N
O
N
O
NH
O
NH
Me
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Me
O
TrtS
39a
200
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140
120
100
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60
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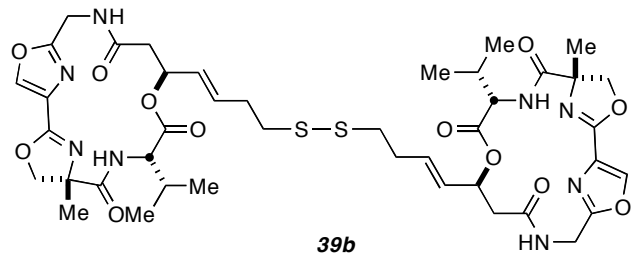
20
0


NH
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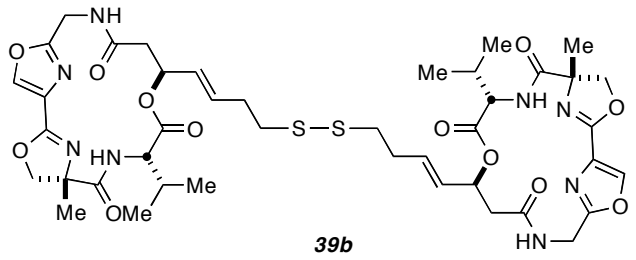
N
O
O
O
O
N
HN
Me
Me
OMe
S
S
MeO
Me
Me
NH
N
O
O
O
O
N
O
O
HN

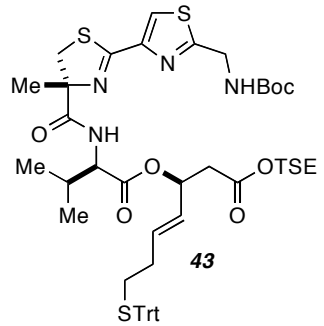
39b

ppm
8
7
6
5
4
3
2
1
0

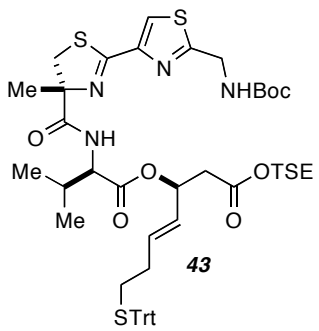

NH
O
N
O
O
O
O
N
HN
Me
Me
OMe
S
S
MeO
Me
Me
NH
N
O
O
O
O
N
O
HN

39b

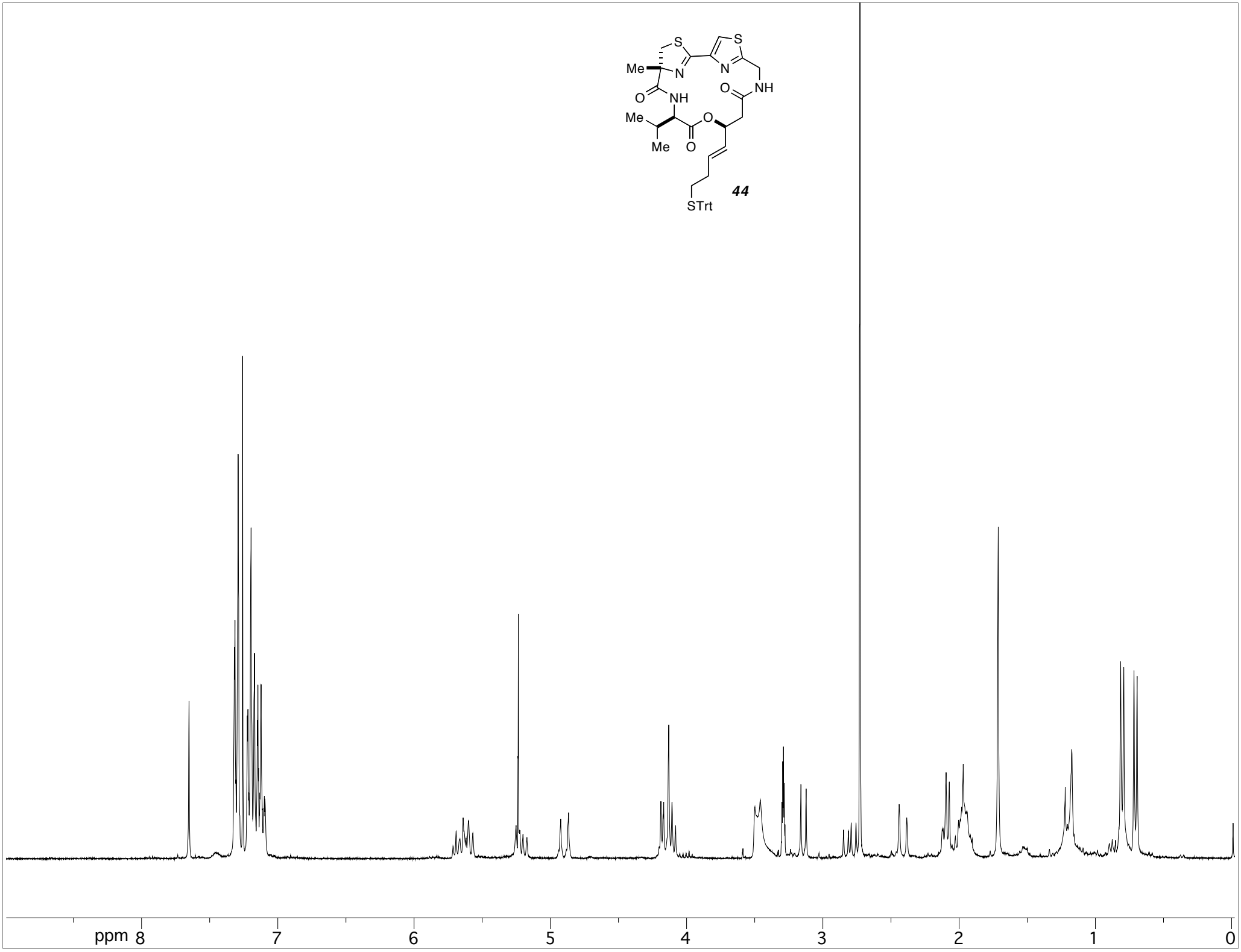
ppm
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

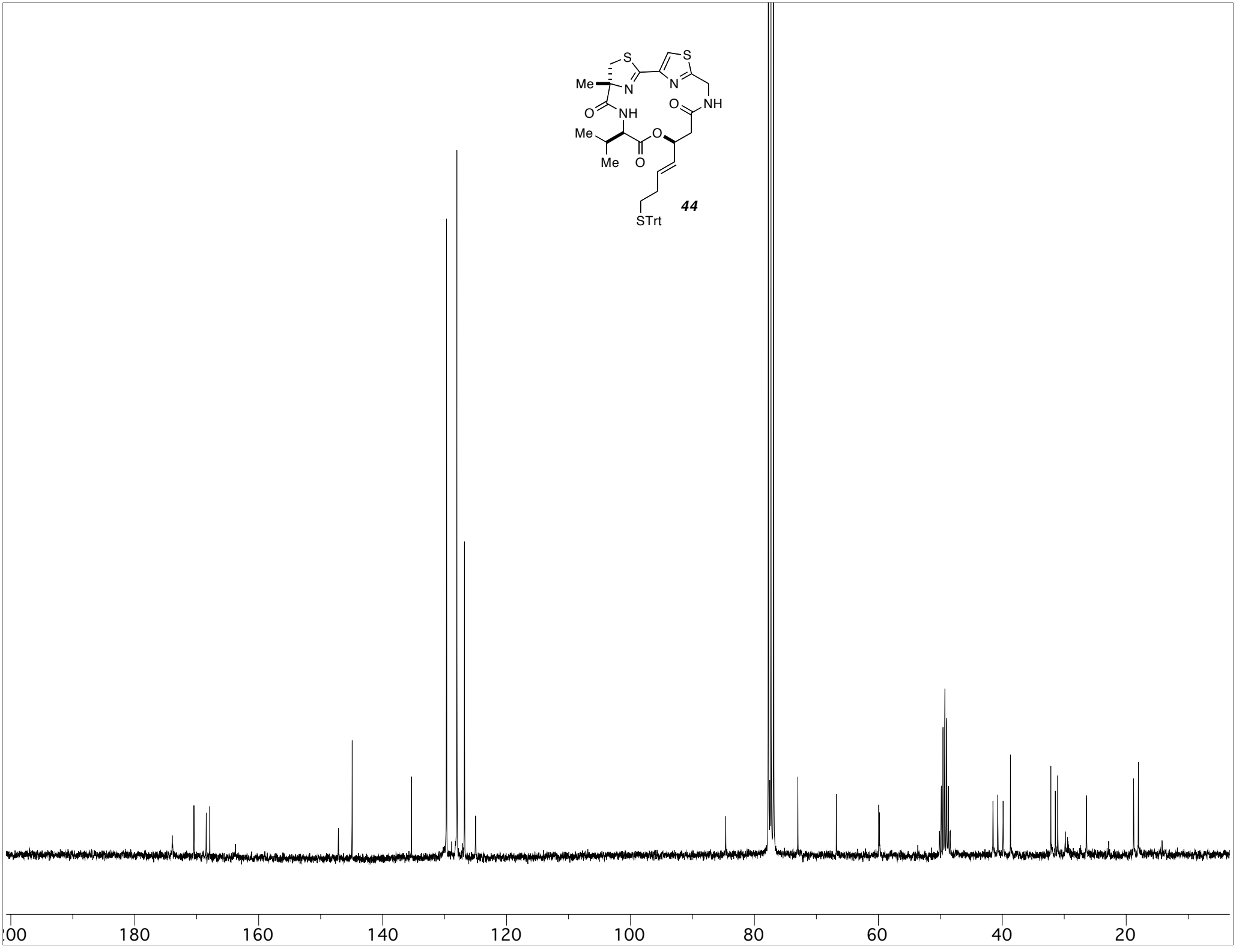


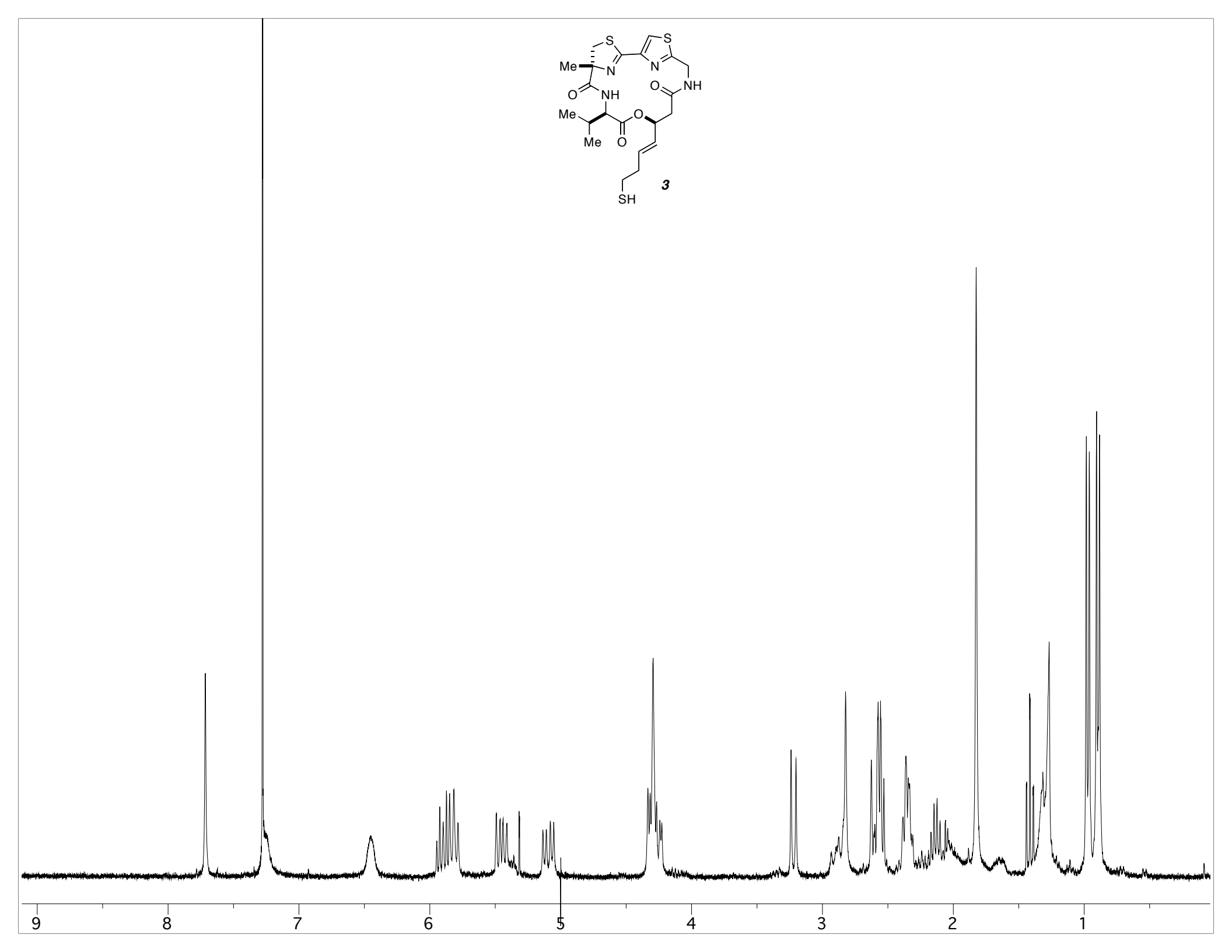
43


S
S
N
Me
N
NHBoc
O
NH
Me
O
OTSE
Me
O
O
43
STrt

200
180
160
140
120
100
80
60
40
20
0


S
S
Me
N
N
O
NH
O
NH
Me
O
Me
O
STrt
44
ppm
8
7
6
5
4
3
2
1
0


Me
S
N
S
N
O
NH
O
NH
Me
O
Me
O
STrt
44
200
180
160
140
120
100
80
60
40
20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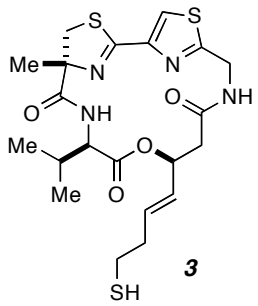
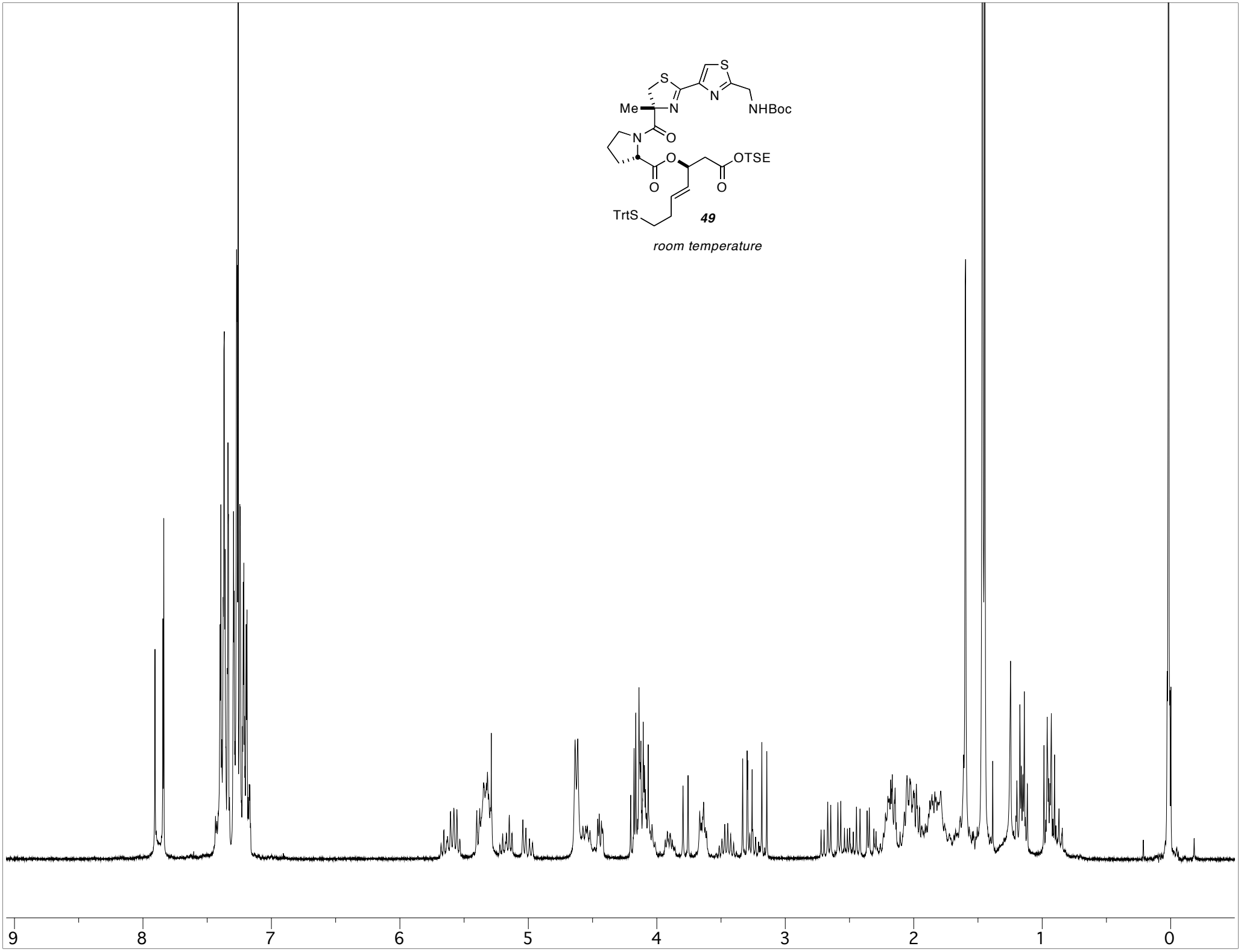
S
Me
N
N
O
NH
O
NH
Me
O
Me
O
SH
3
9
8
7
6
5
4
3
2
1


S
S
Me
N
N
O
NH
O
NH
Me
O
Me
O
SH

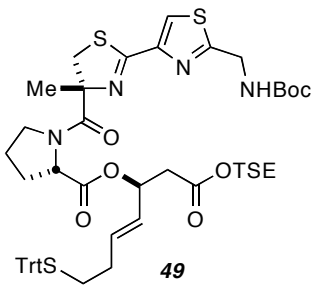
3

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



49

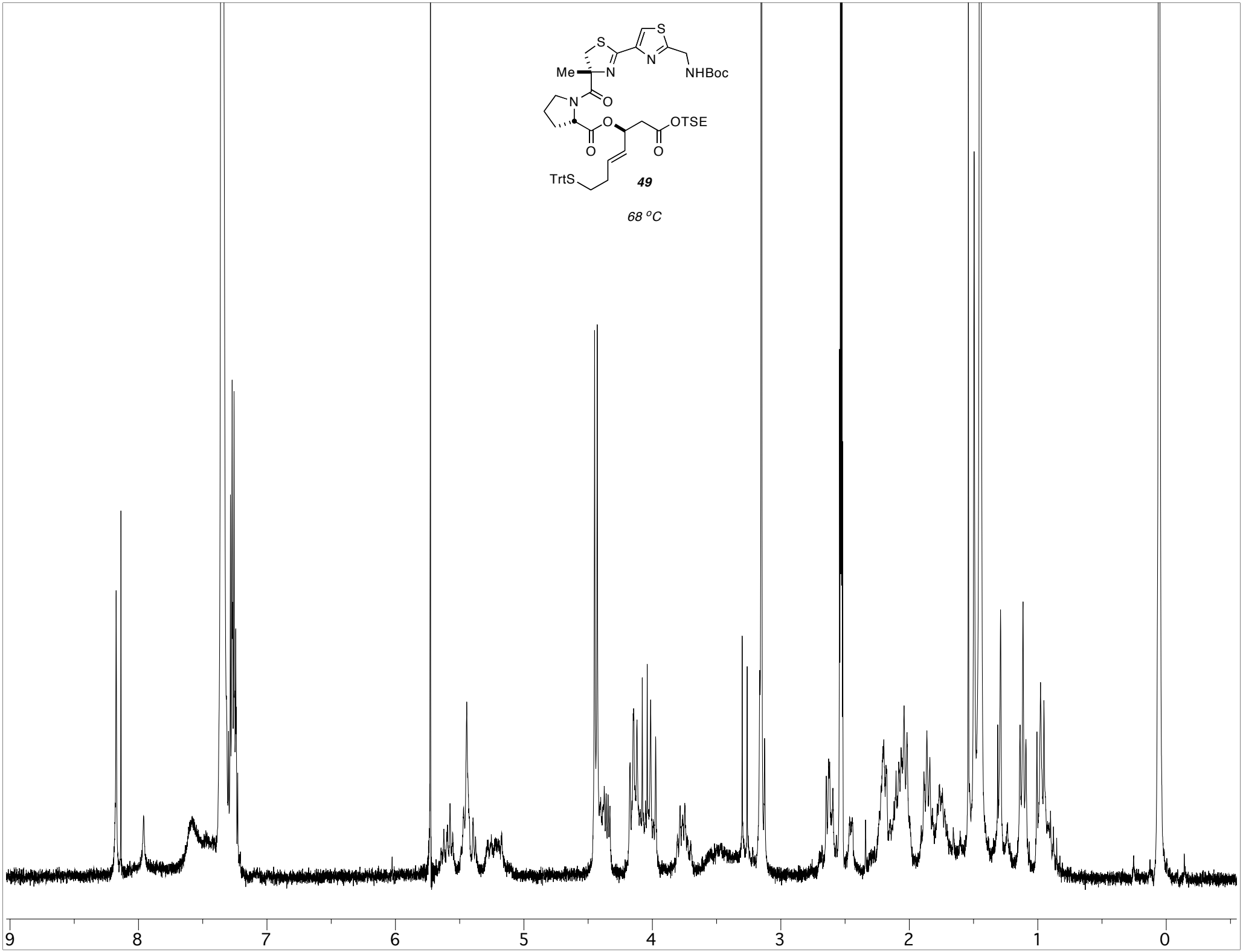
room temperature

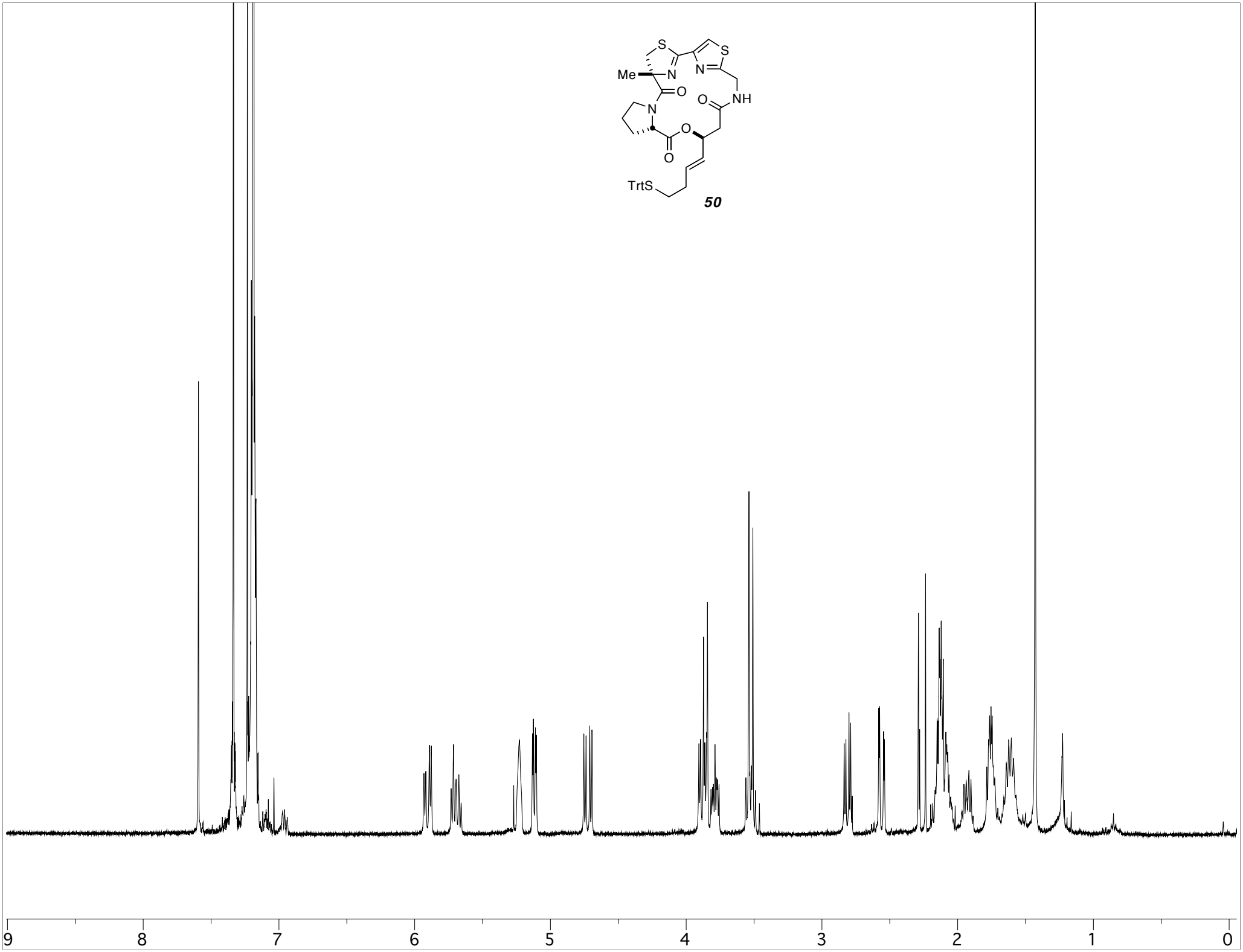


49

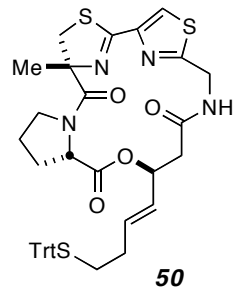
50 °C

9 8 7 6 5 4 3 2 1 0


Me
N
S
S
N
NHBoc
N
O
O
O
O
O
OTSE
TrtS
49
68 °C
9
8
7
6
5
4
3
2
1
0



50



50

HDAC Biochemical Assay

The inhibitory effect of compounds on deacetylase isoenzyme function was determined *in vitro* using an optimized homogenous assay performed in 384-well plate format. In this assay, In this assay, recombinant, full-length HDAC protein (HDAC1 3.33 ng/μL, HDAC2 1 ng/μL, HDAC3/NCor2 0.17 ng/μL, HDAC6 1.3 ng/μL; BPS Biosciences) is incubated with a commercially-available fluorophore conjugated substrate at a concentration equivalent to the substrate K_m (Upstate 17-372; 6 μM for HDAC1, 3 μM for HDAC2, 6 μM for HDAC3 and 16 μM for HDAC6). Reactions are performed in assay buffer (50 mM HEPES, 100 mM KCl, 0.001% Tween-20, 0.05% BSA, 200 μM TCEP, pH 7.4) and followed for fluorigenic release of 7-amino-4-methylcoumarin from substrate upon deacetylase and trypsin enzymatic activity. Fluorescence measurements are obtained approximately every five minutes using a multilabel plate reader and plate-stacker (Envision; Perkin-Elmer). Data are analyzed on a plate-by-plate basis for the linear range of fluorescence over time. Data from the plate capture corresponding to the mid-linear range is imported into analytical software and annotated with well identity and compound concentration (Spotfire DecisionSite). Replicate experimental data from incubations with inhibitor are normalized to control, solvent-only wells and IC-50 is deteremined by logistic regression.