Convergent and modular synthesis of candidate precolibactins. Structural revision of precolibactin A.

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Supporting Information

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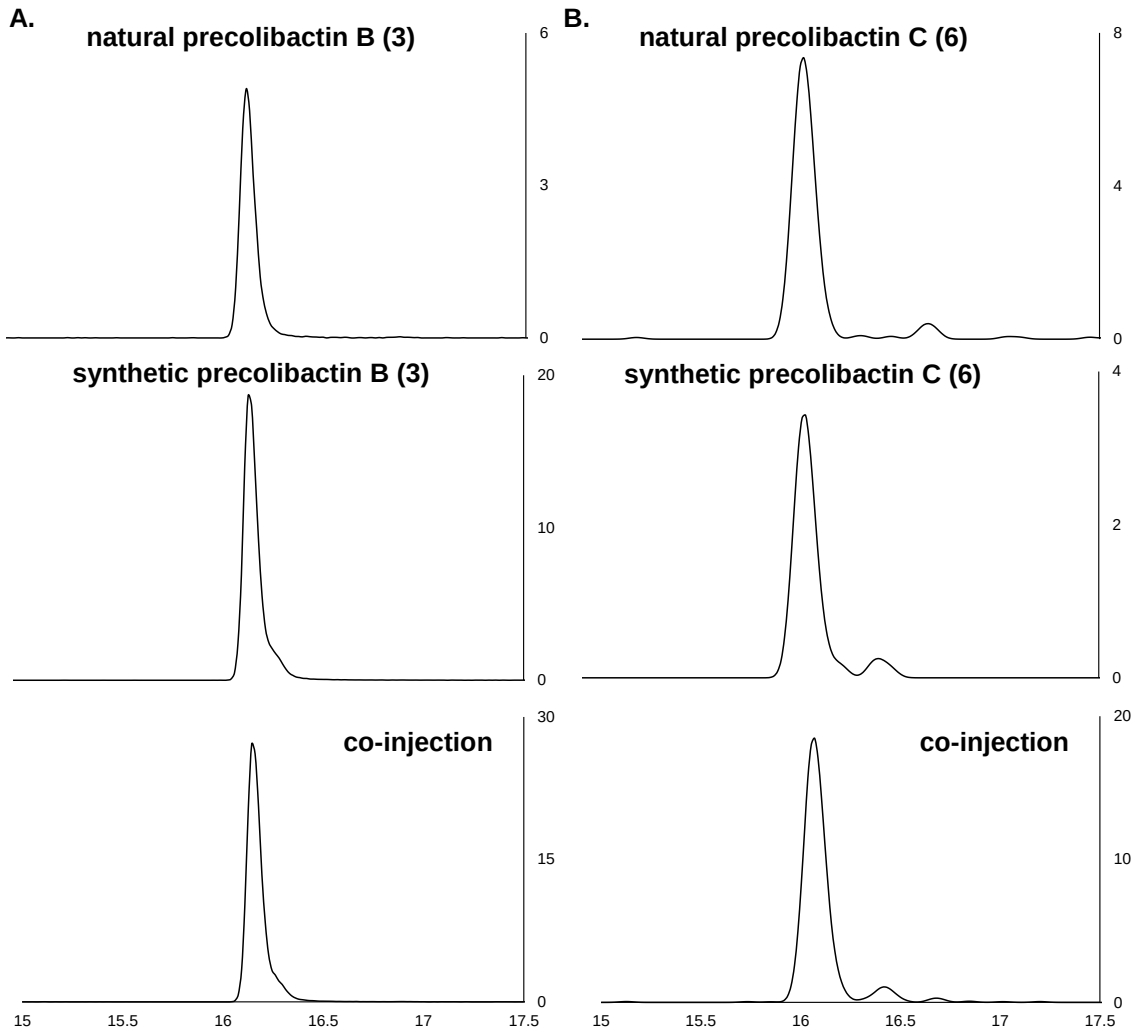


Figure S1. A. Mass-selective (*m/z* = 713.3696) LC/HRMS-QTOF analysis of ethyl acetate extracts of *clb*$^+$ *E. coli* Δ*clbP* (top), synthetic precolibactin B (**3**; middle), and co-injection (bottom). **B.** Mass-selective (*m/z* = 796.3526) LC/HRMS-QTOF analysis of ethyl acetate extracts of *clb*$^+$ *E. coli* Δ*clbP* (top), synthetic precolibactin C (**6**; middle), and co-injection (bottom).

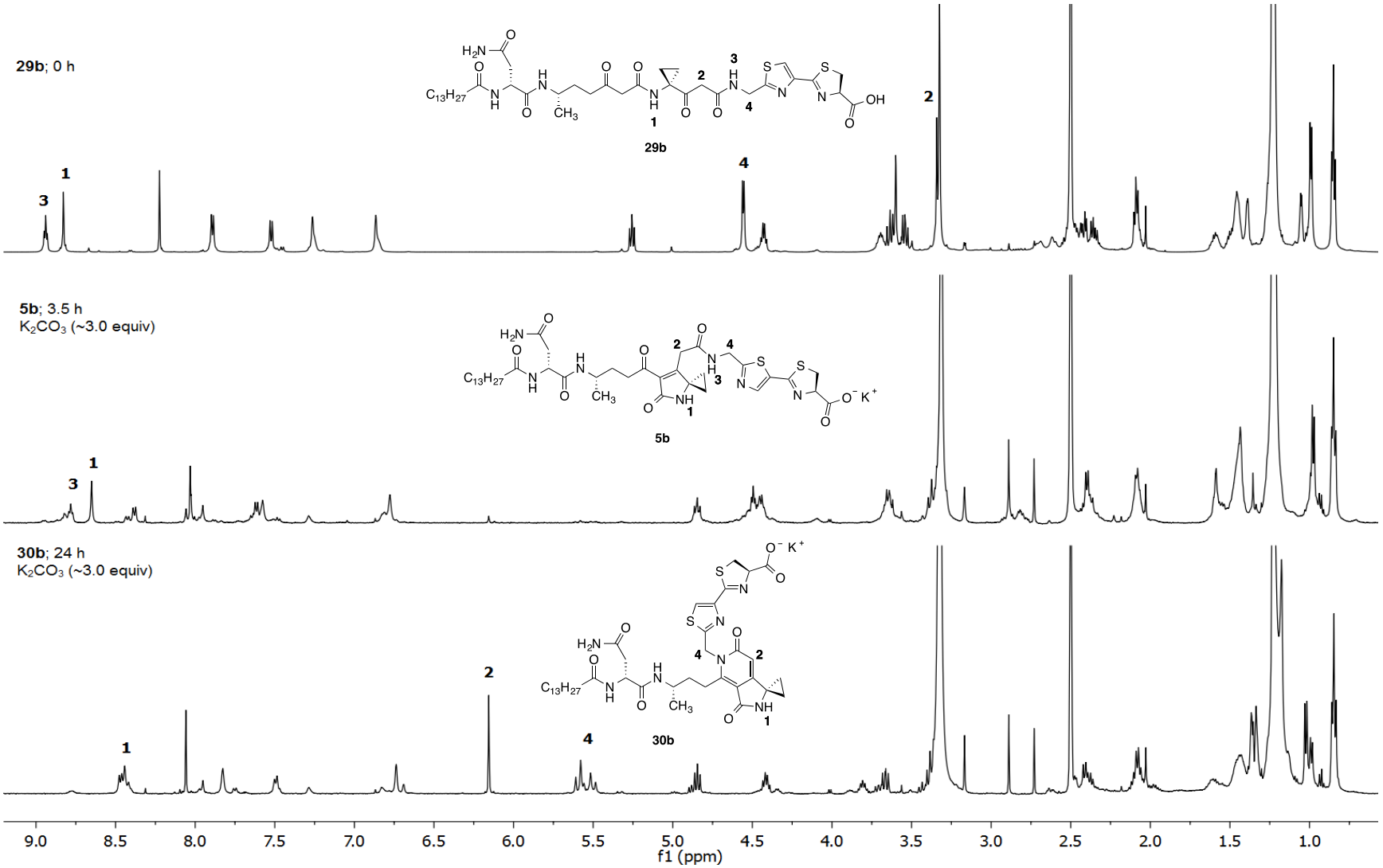
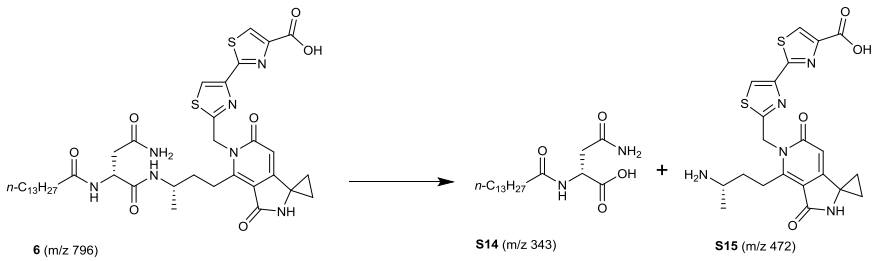


Figure S2. NMR time–course of the cyclodehydration of the linear precursor **29b** to the pyridone **30b** *via* the monocyclized intermediate **5b**. ^{1}H spectroscopic data were recorded in DMSO-d_6 (500 MHz, 23 °C).



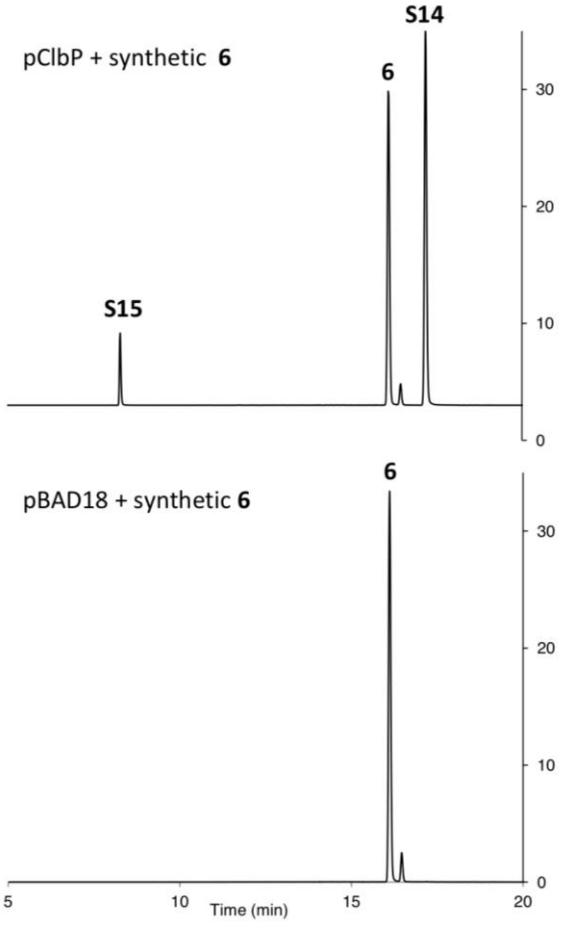
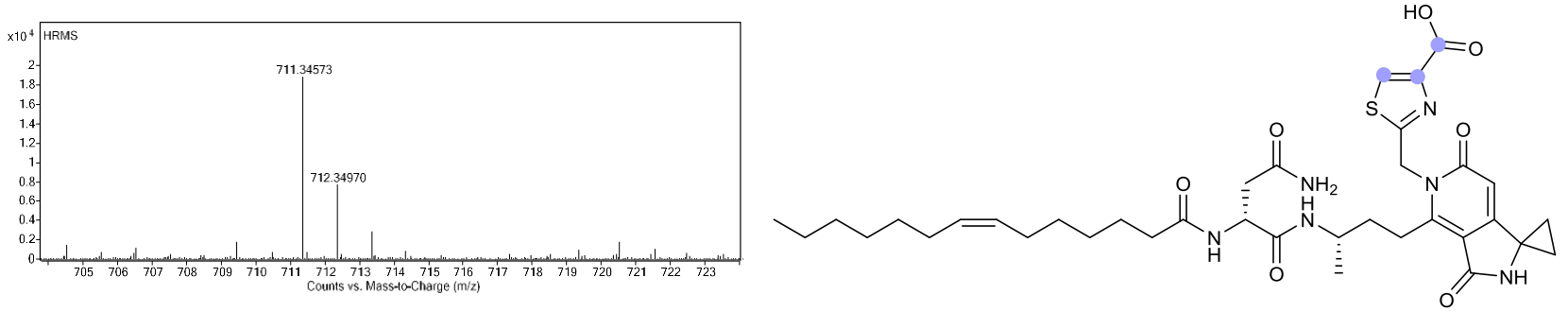
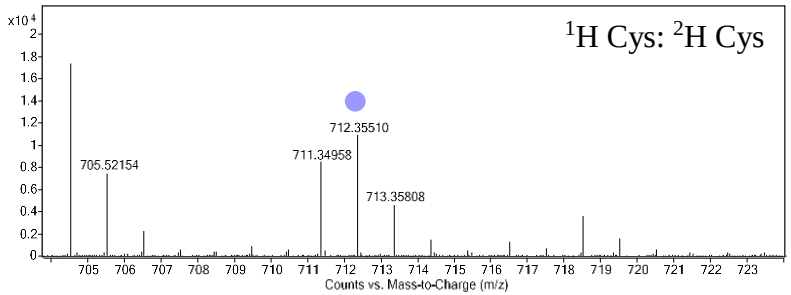


Figure S3. Mass-selective LC/HRMS–QTOF data for the *in vivo* peptidase activity of arabinose-inducible ClbP (pClbP) with synthetic precolibactin C (**6**). Extracted ion chromatograph of starting material with m/z 796 (**6**), and the cleavage products with *m/z* 343 (**S14**), and *m/z* 472 (**S15**) from organic extracts of pClbP (top) and pBAD18 control (bottom). pBAD 18 is the empty plasmid backbone.

A.



B.



C.

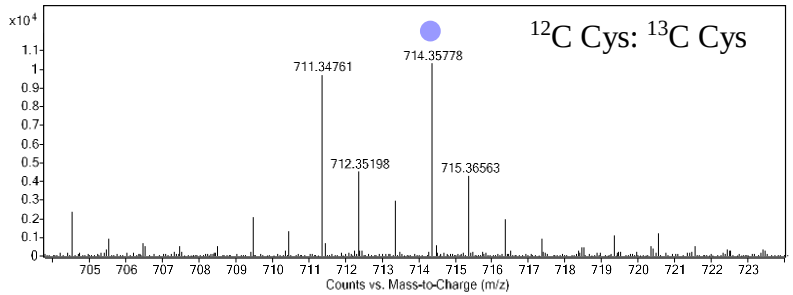
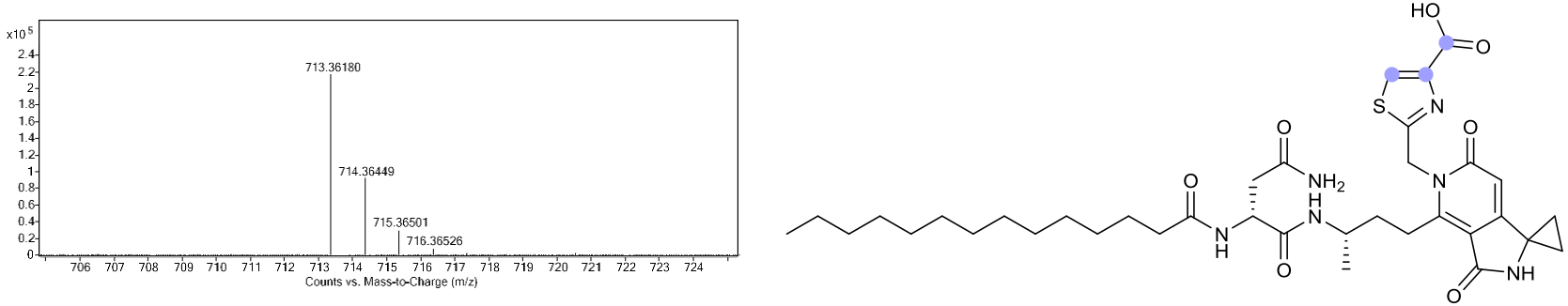


Figure S4. Cysteine isotopic labeling studies. HR-ESI-MS scan of m/z = 711. **A.** 1:1 ratio of L-cysteine:L-[U–^{2}H]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **B.** 1:1 ratio of L -cysteine: L -[U–^{13}C]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **C.** Inset: Predicted structure. ^{13}C-amino acid isotopic labeling studies (asparagine, alanine, methionine, glycine, and cysteine) for this ion were previously reported, and labeling studies were carried out as previously described in Vizcaino, M.I.; Crawford, J.M. *Nat Chem*. **2015**, 7, 411.

A.



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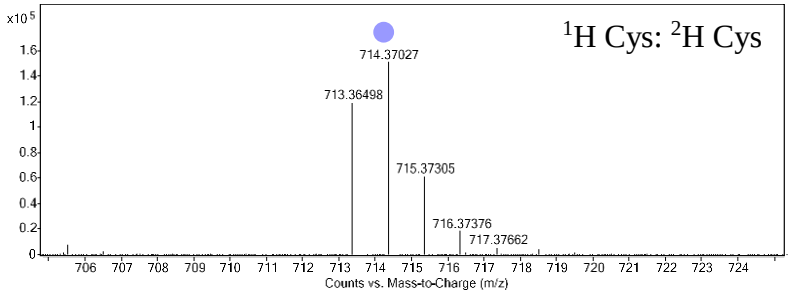


C.

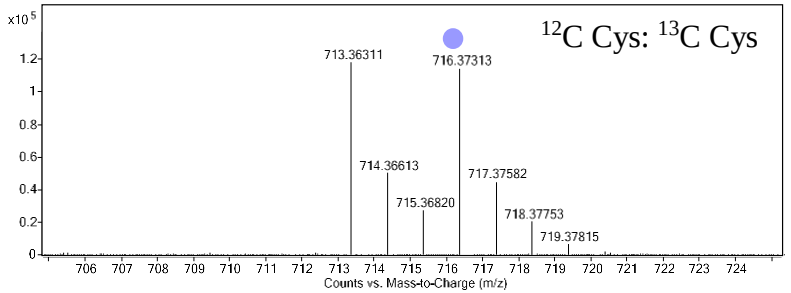


Figure S5. Cysteine isotopic labeling studies. HR-ESI-MS scan of m/z = 713. **A.** 1:1 ratio of L-cysteine: L-[U–^{2}H]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **B.** 1:1 ratio of L-cysteine: L-[U–^{13}C]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **C.** Inset: Structure of metabolite confirmed by synthesis. ^{13}C-amino acid isotopic labeling studies (asparagine, alanine, methionine, glycine, and cysteine) for this ion were previously reported, and labeling studies were carried out as previously described in Vizcaino, M.I.; Crawford, J.M. *Nat Chem*. **2015**, 7, 411.

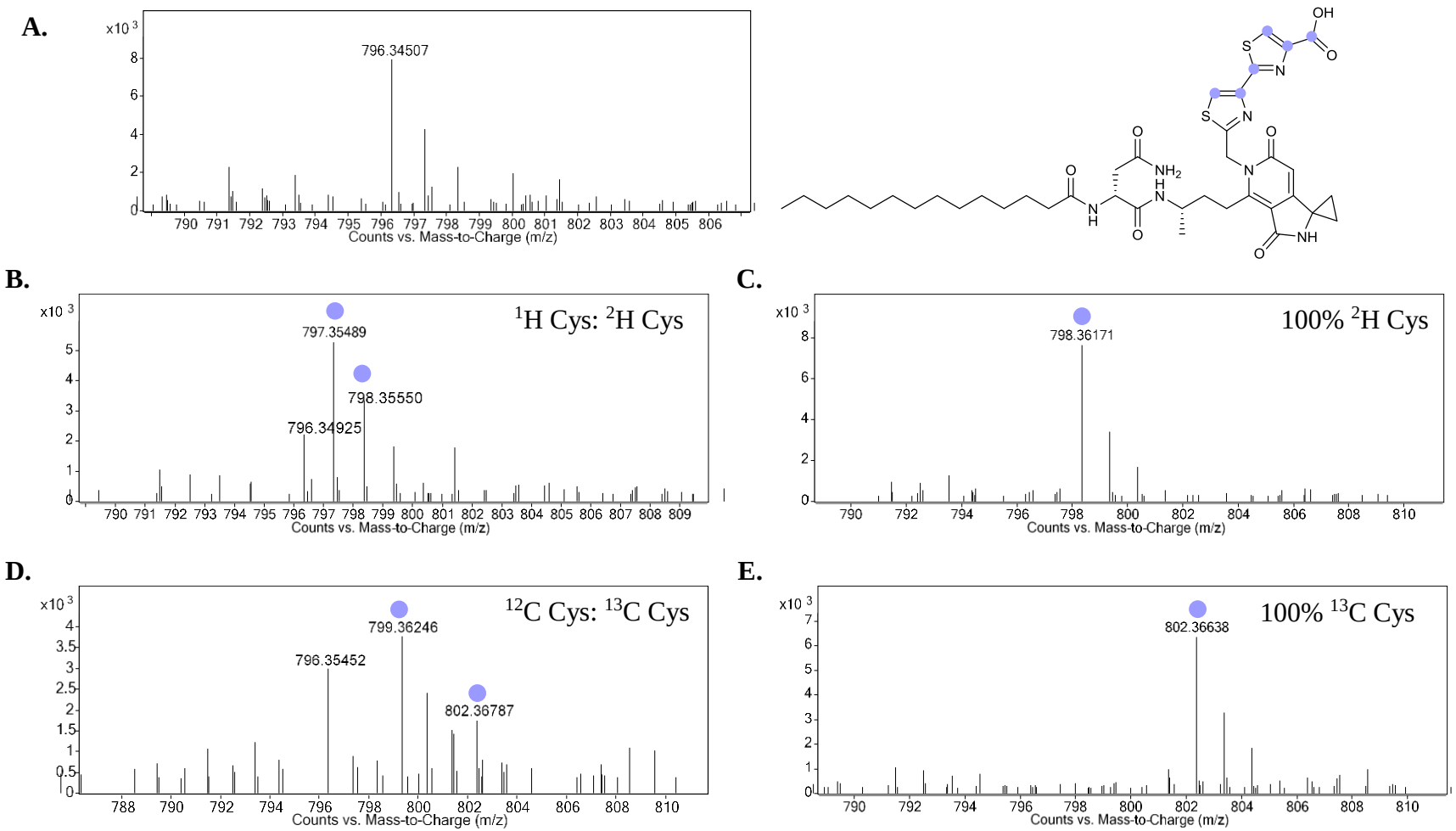
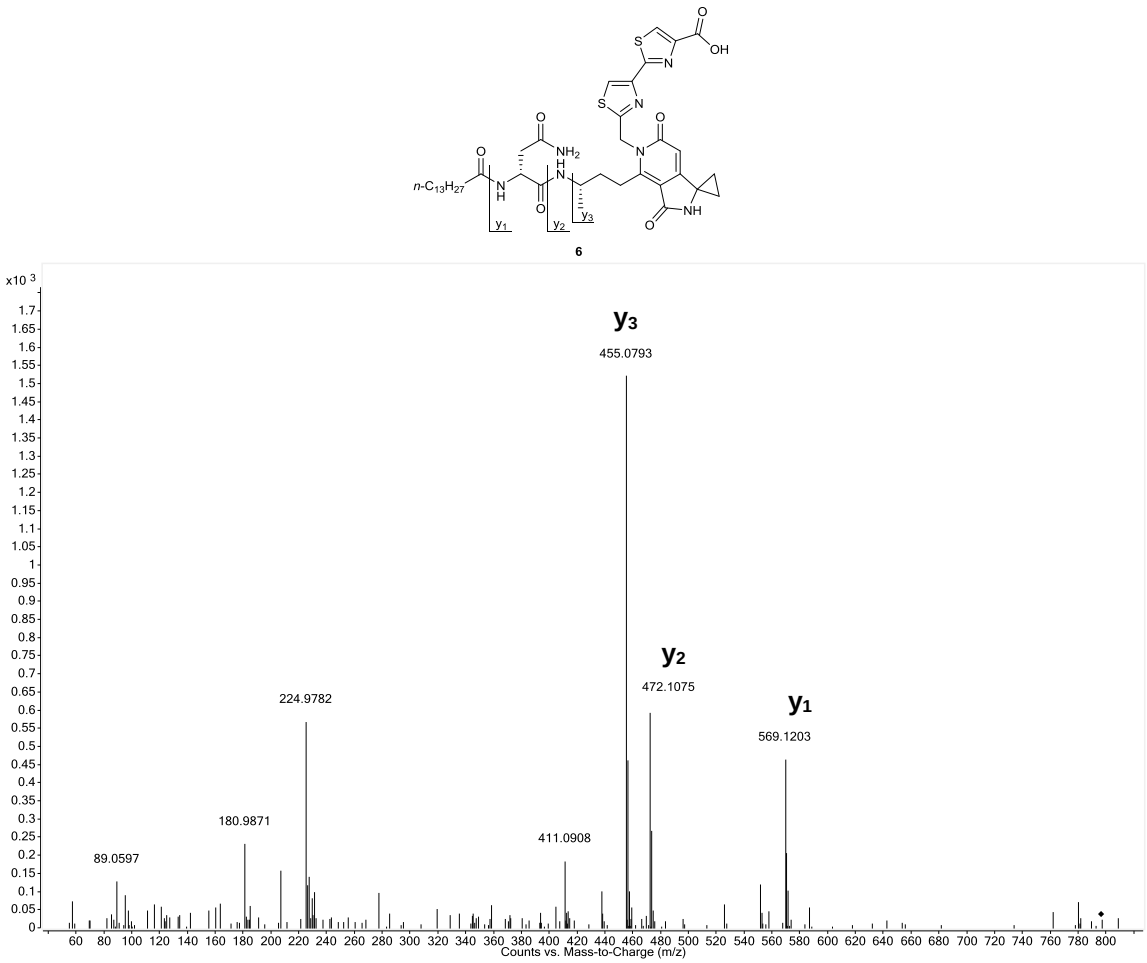
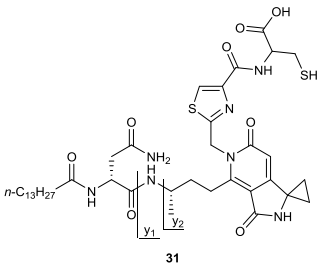


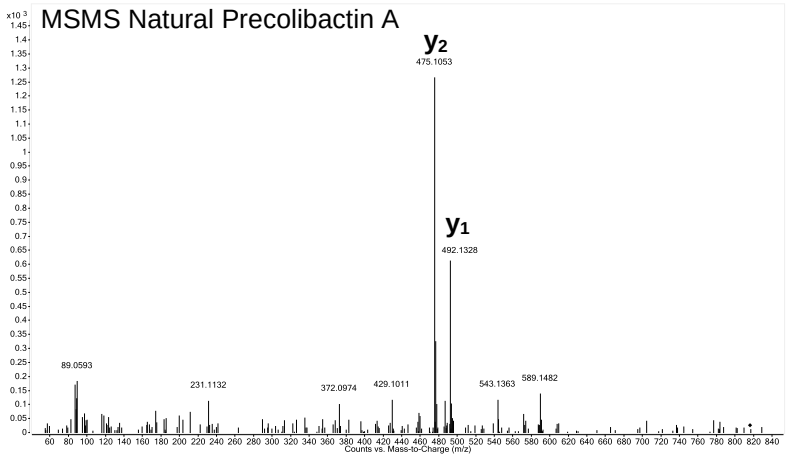
Figure S6. Cysteine isotopic labeling studies. HR-ESI-MS scan of m/z = 796. **A.** 1:1 ratio of L-cysteine: L-[U–^{2}H]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **B.** L-[U–^{2}H]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **C.** 1:1 ratio of L-cysteine: L-[U–^{13}C]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **D.** L-[U–^{13}C]-cysteine fed to an *E. coli* cysteine auxotroph expressing *clb* Δ*clbP*. **E.** Inset: Structure of metabolite confirmed by synthesis. ^{13}C-amino acid isotopic labeling studies were carried out as previously described in Vizcaino, M.I.; Crawford, J.M. *Nat Chem*. **2015**, 7, 411.

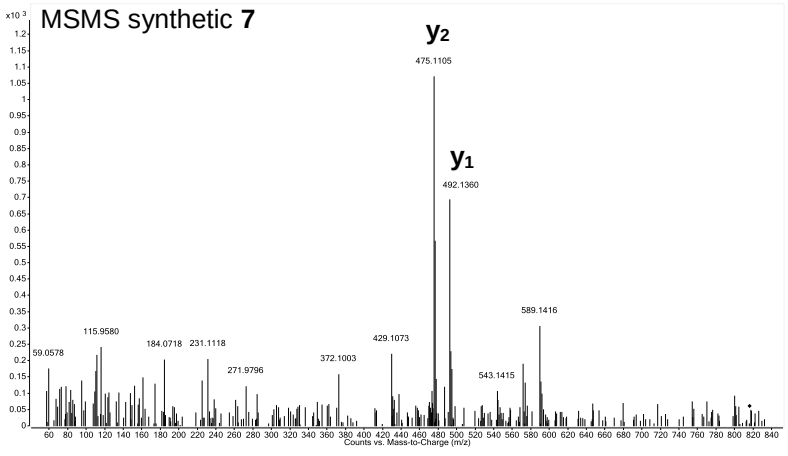


Species	Previously Reported	Obs. Mass	Calc. Mass	Δppm
$[M+H]^+$	**796.3521**[a]	**796.3463**	**796.3526**	**7.9**
y_1	569.142[b]	569.1203	569.1272	8.5
y_2	472.139[b]	472.1075	472.1103	12
y_3	455.112[b]	455.0793	455.0837	9.7

Table S1. MSMS fragmentation comparison of natural precolibactin C (**6**) and synthetic precolibactin C (**6**). Collision energy set at 40 V. [a]HRMS data for natural precolibactin C (**6**) were obtained from the following reference: Zha, L.; Wilson, M. R.; Brotherton, C. A.; Balskus, E. P. *ACS Chem. Biol.* **2016**, [Online early access]. DOI: 10.1021/acschembio.6b00014. Published Online: Feb. 18, 2016. http://pubs.acs.org/doi/abs/10.1021/acschembio.6b00014 (accessed March 28, 2016). [b]MSMS fragmentation data for natural precolibactin C (**6**) were obtained from the following reference: Li, Z. R.; Li, Y.; Lai, J. Y.; Tang, J.; Wang, B.; Lu, L.; Zhu, G.; Wu, X.; Xu, Y.; Qian, P. Y. *Chembiochem* **2015**, *16,* 1715.



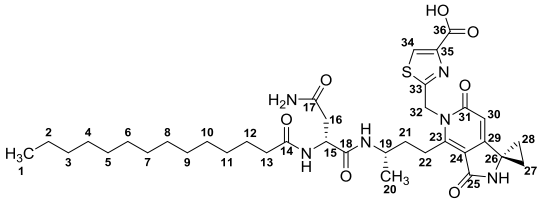




Natural Precolibactin A				Synthetic 7			
Species	**Obs. Mass**	**Calc. Mass**	**Δppm**	**Species**	**Obs. Mass**	**Calc. Mass**	**Δppm**
$[M+H]^+$	**816.3789**	**816.3788**	**0.3**	**$[M+H]^+$**	**816.3787**	**816.3788**	**0.1**
y_1	492.1328	492.1370	8.5	y_1	492.1360	492.1370	2.0
y_2	475.1053	475.1104	10	y_2	475.1105	475.1104	0.1

Table S2. MSMS fragmentation comparison of natural precolibactin A and synthetic **7**. Collision energy set at 40V.

Table S3: Comparison of ^{1}H and ^{13}C NMR Data of Natural and Synthetic Precolibactin B (**3**, DMSO-d_6)



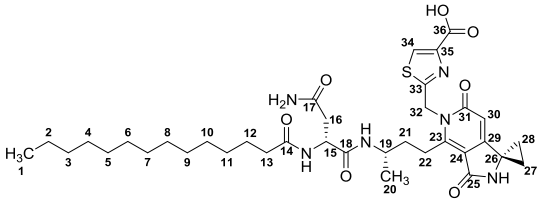
precolibactin B (**3**)

Position	δ_H (Synthetic) [a]	δ_H (Natural)[a,b]	δ_C (Synthetic)[a]	δ_C (Natural)[a,b]
1	0.84 (t, 7.0)	0.84 (t, 7.1)	14.1	14.1
2	1.12−1.27 (m)	1.24 (m)	22.2	22.2
3	1.12−1.27 (m)	1.13−1.22 (m)	31.4	31.4
4–10	1.12−1.27 (m)	1.13−1.22 (m)	28.8, 28.9, 29.1, 29.1, 29.2, 29.2, 29.8	28.7, 28.9, 29.0, 29.1, 29.1, 29.1, 29.4
11	1.12−1.27 (m)	1.13−1.22 (m)	28.8	28.8
12	1.42 (m)	1.42 (m)	25.2	25.2
13	2.03 (m)	2.02 (m) 2.08 (m)	35.4	35.3
14	—	—	172.7	172.2
	7.83 (NH, d, 8.1)	8.41 (NH, bs)		
15	4.52 (q, 7.2)	4.56 (m)	50.5	50.0
16	2.41 (dd, 7.4, 15.2) 2.45 (m)	2.48 (dd, 8.3, 14.6) 2.53 (dd, 5.3, 14.5)	37.4	37.3
17	—	—	172.2	171.7
	6.83 (NH, bs)	6.78 (NH, bs)		
	7.25 (NH, bs)	7.62 (NH, bs)		
18	—	—	170.8	170.6
	7.72 (NH, d, 8.3)	7.83 (NH, bs)		
19	3.87 (m)	3.89 (m)	44.6	44.6
20	1.03 (d, 6.6)	1.03 (d, 6.6)	20.8	20.6
21	1.57 (m) 1.69 (m)	1.47 (m) 1.68 (m)	35.7	35.5
22	3.27 (m) 3.39 (m)	3.23 (m) 3.41 (m)	24.3	24.2
23	—	—	153.2	153.2
24	—	—	109.8	109.7
25	—	—	166.7	166.7
	8.47 (NH, s)	8.44 (NH, s)		
26	—	—	39.8	39.9
27	1.34 (m) 1.37 (m)	1.33 (m) 1.36 (m)	15.3	15.3
28	1.34 (m) 1.37 (m)	1.33 (m) 1.36 (m)	15.3	15.3

29	—	—	159.9	159.9
30	6.16 (s)	6.16 (s)	103.2	103.3
31	—	—	162.0	162.0
32	5.51 (d, 15.8) 5.62 (d, 15.7)	5.48 (d, 15.5) 5.61 (d, 15.2)	44.3	44.3
33	—	—	164.2	165.7
34[c]	8.39 (s)	8.09 (s)	126.9	130.1
35[c]	—	—	151.7	153.2
36[c]	—	—	163.4	161.9

[a]NMR spectra were obtained in DMSO-d_6 at 600 MHz for ^{1}H and 150 MHz for ^{13}C. [b]Data for natural precolibactin B (**3**) were obtained from the following reference: Li, Z. R.; Li, Y.; Lai, J. Y.; Tang, J.; Wang, B.; Lu, L.; Zhu, G.; Wu, X.; Xu, Y.; Qian, P. Y. *Chembiochem* **2015**, *16*, 1715. [c]The discrepencies between synthetic and natural **3** at these positions are attributed to differences in the concentrations of the samples and/or protonation state. The purification conditions used to isolate natural **3** were not specificed, and the NMR spectrum of natural **3** contains a significant amount of residual water (see the reference above).

Table S4: Comparison of ^{1}H and ^{13}C NMR Data of Natural and Synthetic Precolibactin B (**3**; methanol-d_4)



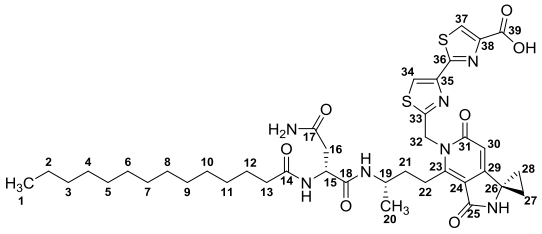
precolibactin B (**3**)

Position	δ_H (Synthetic) [a]	δ_H (Natural)[a,b]	δ_C (Synthetic)[a]	δ_C (Natural)[a,b]
1	0.90 (t, 6.9)	0.90 (t, 6.6)	14.4	14.4
2	1.19−1.34 (m)	1.31−1.33 (m)	23.7	23.7
3	1.19−1.34 (m)	1.28−1.30 (m)	33.0	33.0
4–10	1.19−1.34 (m)	1.22−1.25 (m)	30.4, 30.4, 30.6, 30.7, 30.7, 30.7	30.3, 30.5, 30.5, 30.6, 30.8, 30.8
11	1.19−1.34 (m)	1.22−1.25 (m)	30.3	30.3
12	1.54−1.62 (m)	1.55−1.57 (m)	26.8	26.7
13	2.16−2.30 (m)	2.18−2.21 (m)	37.0	37.0
14	—	—	176.4	176.4
15	4.82 (t, 6.5)	4.80 (dd, 5.4)	52.3	52.1
16	2.72−2.81 (m)	2.74 (dd, 7.8, 15.6) 2.80 (dd, 7.8, 15.6)	37.9	37.8
17	—	—	175.2	175.2
18	—	—	172.9	173.0
19	4.03−4.20 (m)	4.11−4.15 (m)	46.7	46.8
20	1.16 (d, 6.6)	1.16 (d, 6.6)	21.1	21.0
21	1.54−1.62 (m) 1.82−1.99 (m)	1.54−1.56 (m) 1.86−1.91 (m)	36.8	36.8
22	3.42−3.52 (m) 3.53−3.71 (m)	3.46−3.51 (m) 3.61−3.68 (m)	26.0	25.9
23	—	—	155.2	155.2
24	—	—	112.2	112.4
25	—	—	169.2	169.3
26	—	—	41.4	41.4
27	1.47−1.56 (m) 1.35−1.45 (m)	1.50−1.53 (m) 1.40−1.42 (m)	16.3	16.3
28	1.47−1.56 (m) 1.35−1.45 (m)	1.50−1.53 (m) 1.40−1.42 (m)	16.3	16.3
29	—	—	162.4[c]	165.0
30	6.17 (s)	6.17 (s)	104.1	104.1
31	—	—	164.9[c]	162.5
32	5.68 (d, 15.2) 5.82 (d, 15.3)	5.70 (d, 15.6) 5.85 (d, 15.6)	46.0	46.1
33	—	—	166.0	166.3
34	8.17 (s)	8.35 (s)	128.9	131.1

35	—	—	151.5	148.4
36	—	—	166.4	166.8

[a]NMR spectra were obtained in CD_3OD-d_4 at 600 MHz for ^1H and 150 MHz for ^{13}C. [b]Data for natural precolibactin B (**3**) were obtained from the following reference: Zha, L.; Wilson, M. R.; Brotherton, C. A.; Balskus, E. P. *ACS Chem. Biol.* **2016**, [Online early access]. DOI: 10.1021/acschembio.6b00014. Published Online: Feb. 18, 2016. http://pubs.acs.org/doi/abs/10.1021/acschembio.6b00014 (accessed March 28, 2016). [c]The carbon atoms at positions 29 and 31 were assigned by observation of an HMBC correlation from H27/28 to C29. These positions are believed to be misassigned in the reference above.

Table S5: Comparison of ^{1}H and ^{13}C NMR Data of Natural and Synthetic Precolibactin C (**6**; methanol-d_4)



precolibactin C (**6**)

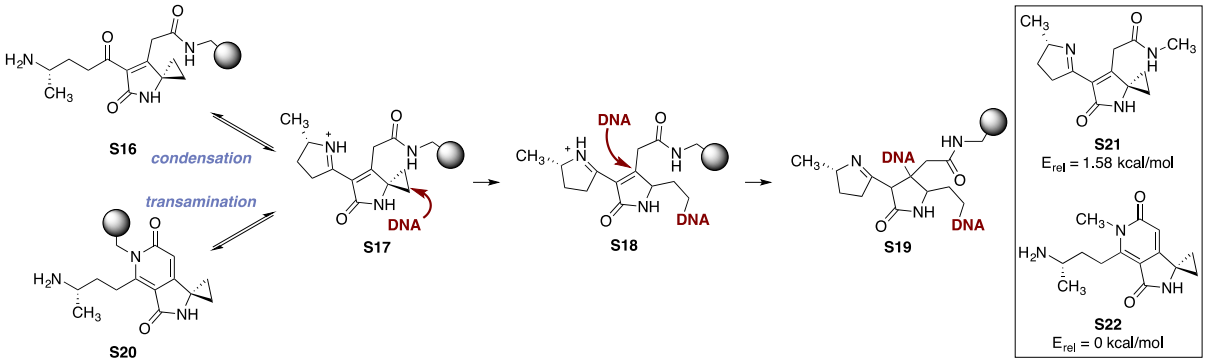
Position	δ_H (Synthetic) [a]	δ_H (Natural)[a,d]	δ_C (Synthetic)[a,b]	δ_C (Natural)[a,c,d]
1	0.89 (t, 7.0)	0.89 (t, 7.8)	14.1	14.1
2	1.20−1.34 (m)	1.29−1.35 (m)	23.4	23.4
3	1.20−1.34 (m)	1.28−1.32 (m)	32.7	32.7
4–11	1.20−1.34 (m)	1.20−1.31 (m)	29.9, 30.1, 30.1, 30.3, 30.4, 30.4, 30.4	30.3, 30.3
12	1.53−1.66 (m)	1.59−1.62 (m)	26.4	26.5
13	2.23 (t, 7.4)	2.22−2.24 (m)	36.6	36.6
14	—	—	175.9	NR
15	4.73 (t, 6.5)	4.73 (dd, 6.0, 6.6)	51.6	51.6
16	2.71 (t, 6.1)	2.69 (dd, 6.0, 15.6) 2.72 (dd, 6.0,15.6)	37.6	37.7
17	—	—	174.6	NR
18	—	—	172.5	NR
19	3.99−4.12 (m)	4.03−4.08 (m)	46.6	46.5
20	1.18 (d, 6.6)	1.17 (d, 6.6)	20.5	20.4
21	1.68−1.81 (m) 1.86−1.98 (m)	1.72−1.76 (m) 1.90−1.94 (m)	36.4	36.5
22	3.42−3.55 (m) 3.57−3.71 (m)	3.47−3.52 (m) 3.60−3.65 (m)	25.6	25.5
23	—	—	154.8	NR
24	—	—	111.8	NR
25	—	—	168.9	NR
26	—	—	41.1	NR
27	1.50−1.54 (m) 1.38−1.47 (m)	1.51−1.56 (m) 1.41−1.43 (m)	15.9	15.9
28	1.50−1.54 (m) 1.38−1.47 (m)	1.51−1.56 (m) 1.41−1.43 (m)	15.9	15.9
29	—	—	162.1	NR
30	6.16 (s)	6.16 (s)	103.9	103.9
31	—	—	164.5	NR
32	5.70 (d, 15.7) 5.75 (d, 15.7)	5.69 (d, 15.6) 5.74 (d, 15.6)	45.6	45.7
33	—	—	166.8	NR

34	8.28 (s)	8.16 (s)[e]	119.8	NR
35	—	—	147.7	NR
36	—	—	163.0	NR
37	8.16 (s)	8.28 (s)[e]	126.5	120.2
38	—	—	148.9	NR
39	—	—	163.2	NR

[a]NMR spectra were obtained in CD_3OD-d_4 at 600 MHz for ^1H and 150 MHz for ^{13}C. [b]Chemical shifts for synthetic precolibactin C (**6**) are referenced to the carbon resonances of the solvent (CD_3OD, δ 48.6). [c]^{13}C NMR data for natural precolibactin C (**6**) were determined from gHSQC NMR data. [d]Data for natural precolibactin C (**6**) were obtained from the following reference: Zha, L.; Wilson, M. R.; Brotherton, C. A.; Balskus, E. P. *ACS Chem. Biol.* **2016**, [Online early access]. DOI: 10.1021/acschembio.6b00014. Published Online: Feb. 18, 2016. http://pubs.acs.org/doi/abs/10.1021/acschembio.6b00014 (accessed March 28, 2016). [e] The positions 34 and 37 were assigned by observation of a mutual HMBC correlation from H34 and H32 to C33. These resonances are believed to be missassigned in the reference above. NR = Not reported.

Discussion of the Colibactin Mechanism of Action.

It was proposed[1,2] that the deacylated colibactin cytotoxins (**S16**) undergo cyclization to unsaturated iminium ions such as **S17**, which then form DNA adducts, including cross-links[1] (**S17**→**S19**, Scheme S1). If this mechanism is correct, the presence of the pyridone structure in precolibactins A (**7**), B (**3**), and C (**6**) would require a transamination reaction instead (**S20**→**S17**). DFT calculations [B3LYP 6–31G(d,p)+] suggest that the unsaturated imine **S21** is 1.58 kcal/mol higher in energy than the corresponding pyridone **S22** (although the energies of **S21** and **S22** may be modulated on binding DNA) and the electron-deficient nature of the pyridone may lower the kinetic barrier to transamination. We also cannot exclude the possibility that the pyridone itself (**S20**) behaves as an electrophile.



Scheme S1. Previously postulated (**S16**→**S17**) and additional (**S20**→**S17**) mechanism for formation of DNA adducts.

General Experimental Methods.

Cysteine incorporation based on carbon and deuterium labeling. Nonlabeled control culture conditions, *L-[U-^{13}C]*-Cys isotope culture conditions, *and L-[2,3,3-D]*-Cys isotope culture conditions were employed as previously reported.[1]

LC-HRMS analysis for the *in vivo* cleavage of synthetic 6 by ClbP. *E. coli* DH10B pClbP (pPEB018) and the bacteria harboring the empty vector pBAD18 (as previously reported[3]) were grown in LB medium supplemented with 100 µg/mL ampicillin. The next morning, 50 µL of these saturated cultures were used to inoculate 2.5 mL of LB medium containg 100 µg/mL ampicillin. Cultures were incubated at 37 °C with shaking at 250 rpm. At an OD_{600} of 0.4–0.5, cultures were induced with L-arabinose and left to grow for another 30 min. Then, the synthetic **6** substrate (10 mM stock solution in DMSO) or DMSO (vector control) was added to each culture to a final concentration of 50 µM, and incubated at 37°C for 24 h. At the designated time point, the cultures were extracted with organic solvent and analyzed by LC-HRMS on a Phenomenex C18-A column (150 x 4.6 mm, 180Å, 5 µm particle size, Agilent) with a water:acetonitrile (ACN) gradient containing 0.1% formic acid 0.7 mL/min: 1–2 min, 5% ACN; ramp to 98% ACN over 18 min; hold for 5 min at 100% ACN.

Extraction of naturally-produced advanced precolibactins. *Escherichia coli* DH10B pBAC Δ*clbP*, generated by nonpolar gene deletion of *clbP*,[3] was used in co-injection experiments as natural compound comparison with synthetic compounds. A single colony of the Δ*clbP* strain was grown in M9 media as previously reported.[1] At the designated time point, the culture was extracted with ethyl acetate (EtOAc), and the re-constituted organic extract was utilized in comparison studies with synthetic precolibactins.

HRMS, and MS/MS data acquisition. All liquid chromatography high-resolution mass spectrometry (LC-HRMS) data described for this paper was collected on an Agilent iFunnel 6550 Quadrupole time-of-flight (QTOF) mass spectrometer equipped with an electrospray ionization (ESI) source coupled to an Agilent Infinity 1290 UHPLC scanning from *m/z* 50-1200. Data was acquired using MassHunter Workstation Software LC/MS Data Acquisition (Version B.05.01, Agilent Technologies) and processed with Qualitative Analysis (Version B.06.00). Co-injection experiments were analyzed on a C18 Kinetex column (2.5 × 100 mm, 1.7 µm) using water (0.1% formic acid) as mobile phase A and acetonitrile (0.1% formic acid) as mobile phase B. Gradient conditions were as follow: 0–2 min, 5% B; ramp to 75% B over 20 min; wash at 98% B for 6 min, and equilibrate at 5% B for 8 min. Flow rate was set at 0.3 mL/min, and injection volume at 5 µL. MS data was collected in ESI+ mode with source gas temp at 225°C, drying gas at 15 l/min, nebulizer at 35 psig, Vcap set at 4000V, Nozzle Voltage at 1000 V. Acquisition rate was 1 spectra/s. MSMS fragmentation was acquired with three collision energies (40, 60, 90) with an unbiased isotope model.

DFT calculations. Lowest-energy conformations of **40** and **41** were obtained by molecular mechanics optimization (500 starting conformers) using BOSS.[4] The corresponding lowest-energy conformers were then subjected to DFT optimization in Gaussian 09 [B3LYP 6-31G(d,p+] in a water environment.[5]

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of nitrogen unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula, or were handled in a nitrogen-filled drybox (working oxygen level <10 ppm). Organic solutions were concentrated by rotary evaporation at 28–32 °C. Flash-column chromatography was performed as described by Still et al.,[6] employing silica gel (60 Å, 40–63 μm particle size) purchased from Sorbent Technologies (Atlanta, GA). Anion-exchange chromatography was performed as described by Béland et al.,[7] employing trimethylamine acetate-functionalized silica gel (SiliaBond® TMA Acetate). Analytical thin-layered chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV).

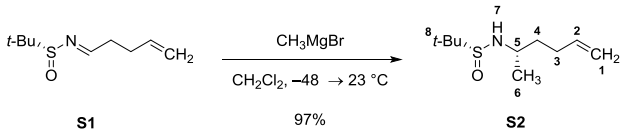
Materials. Commercial solvents and reagents were used as received with the following exceptions. Dichloromethane, diethyl ether and *N*,*N*-dimethylformamide were purified according to the method of Pangborn et al.[8] Triethylamine was distilled from calcium hydride under an atmosphere of argon immediately before use. Di-*iso*-propylamine was distilled from calcium hydride and was stored under nitrogen. Methanol was distilled from magnesium turnings under an atmosphere of nitrogen immediately before use. Tetrahydrofuran was distilled from sodium–benzophenone under an atmosphere of nitrogen immediately before use. Trifluoroacetic anhydride was fractionally-distilled before use. Molecular sieves were activated by heating to 200 °C under vacuum (<1 Torr) for 12 h, and were stored in an oven at >160 °C. Propylsulfonic acid-functionalized silica gel (SiliaBond® SCX-2) and trimethylamine acetate-functionalized silica gel (SiliaBond® TMA Acetate) were purchased from SiliCycle (Quebec City, CA). (–)-(R_S)-2-methyl-*N*-(pent-4-en-1-ylidene)propane-2-sulfinamide (**S1**)[9], 3-(*tert*-butylthio)-3-oxopropanoic acid (**S7**)[10] and 2-(((tert-butoxycarbonyl)amino)methyl)thiazole-4-carboxylic acid (**S12**)[11] were prepared according to published procedures.

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400, 500, or 600 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CDCl_3$, δ 7.26; CD_3OD, δ 3.31; C_2D_6OS, δ 2.50). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quarter, m = multiplet and/or multiple resonances, br = broad, app = apparent), coupling constant in Hertz, integration, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 100, 125 or 150 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent ($CDCl_3$, δ 77.0; CD_3OD, δ 49.0; C_2D_6OS, δ 39.5). Signals of protons and carbons were assigned, as far as possible, by using the following two dimensional NMR spectroscopy techniques: [1H, 1H] COSY (Correlation Spectroscopy), [1H, 13C] HSQC (Heteronuclear Single Quantum Coherence) and long range [1H, 13C] HMBC (Heteronuclear Multiple Bond Connectivity). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were obtained using a Thermo Electron Corporation Nicolet 6700 FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Analytical ultra high-performance liquid chromatography/mass spectrometry (UPLC/MS) was performed on a Waters UPLC/MS instrument equipped with a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm), dual atmospheric pressure chemical ionization (API)/electrospray (ESI)

mass spectrometry detector, and photodiode array detector. Samples were eluted with a linear gradient of 5% acetonitrile–water containing 0.1% formic acid→100% acetonitrile containing 0.1% formic acid over 0.75 min, followed by 100% acetonitrile containing 0.1% formic acid for 0.75 min, at a flow rate of 800 μL/min. High-resolution mass spectrometry (HRMS) were obtained on a Waters UPLC/HRMS instrument equipped with a dual API/ESI high-resolution mass spectrometry detector and photodiode array detector. Unless otherwise noted, samples were eluted over a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm) with a linear gradient of 5% acetonitrile–water containing 0.1% formic acid→95% acetonitrile–water containing 0.1% formic acid for 1 min, at a flow rate of 600 μL/min. Optical rotations were measured on a Perkin Elmer polarimeter equipped with a sodium (589 nm, D) lamp. Optical rotation data are represented as follows: specific rotation ($[\alpha]_\lambda^T$), concentration (g/100 mL), and solvent.

Synthetic Procedures.

*Synthesis of the sulfinamine **S2**:*



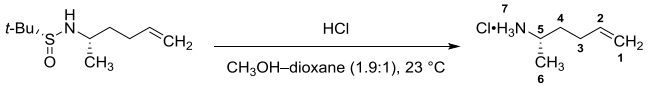
S1 **S2**

A solution of methylmagnesium bromide in ether (3.0 N, 3.56 mL, 10.7 mmol, 2.00 equiv) was added dropwise via syringe pump over 30 min to a solution of the sulfinimine **S1** (1.00 g, 5.34 mmol, 1 equiv) in dichloromethane (35 mL) at –48 °C. The resulting mixture was allowed to warm over 30 min to –30 °C. The reaction mixture was stirred for 4 h at –30 °C. The reaction mixture was then allowed to warm over 30 min to 23 °C. The product mixture was diluted sequentially with saturated aqueous ammonium chloride solution (20 mL) and ethyl acetate (20 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 30 mL). The organic layers were combined and the combined organic layers were washed with saturated aqueous sodium chloride solution (30 mL). The washed organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the unpurified sulfinamine **S2** as a yellow oil (1.05 g, 97%).

The product **S2** obtained in this way was estimated to be of >95% purity and 88% de by ^{1}H NMR analysis (see accompanying spectrum) and was used without further purification. The configuration of the newly-formed stereocenter was assigned by analogy to related addition products.[12,13]

^{1}H NMR (400 MHz MHz, $CDCl_3$): δ 5.79 (ddt, *J* = 16.9, 10.1, 6.5 Hz, 1H, H_2), 5.08 – 4.87 (m, 2H, H_1), 3.37 (app hept, *J* = 6.8 Hz, 1H, H_5), 2.86 (d, *J* = 7.2 Hz, 1H, H_7), 2.20 – 2.04 (m, 2H, H_3), 1.66 – 1.42 (m, 2H, H_4), 1.28 (d, *J* = 6.5 Hz, 3H, H_6), 1.20 (s, 9H, H_8). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.2 (CH), 115.1 (CH_2), 55.8 (C), 52.3 (CH), 37.5 (CH_2), 30.2 (CH_2), 23.4 (CH_3), 22.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 2972 (w), 1642 (s), 1457 (m), 1364 (s), 1050 (s), 910 (s). HRMS-CI (m/z): $[M + Na]^+$ calcd for $C_{10}H_{21}NNaOS$, 226.1242; found, 226.1294. $[\alpha]_D^{20}$ −21.2 (*c* 1.0, CH_3OH).

*Synthesis of the amine **8**:*



S2 >99% **8**

A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 2.58 mL, 10.3 mmol, 2.00 equiv) was added dropwise via syringe pump over 30 min to a solution of the sulfinamine **S2** (1.05 g, 5.16 mmol, 1 equiv) in methanol (5.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The product mixture was concentrated to dryness. The residue obtained was suspended in ether (10 mL) and the resulting suspension was concentrated to dryness. This process was repeated to provide the amine **8** as white solid (700 mg, >99%, *CAUTION:* hygroscopic).

The product **8** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

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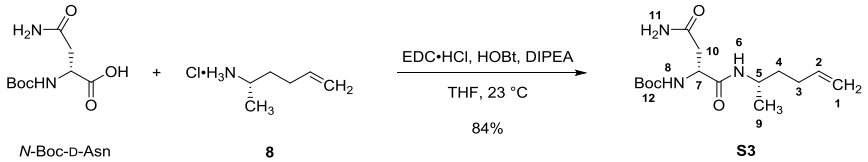
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (bs, 3H, H_7), 5.75 (ddt, J = 17.0, 10.3, 6.6 Hz, 1H, H_2), 5.11 (dd, J = 17.0, 1.9 Hz, 1H, H_1), 5.02 (dd, J = 10.3, 1.9 Hz, 1H, H_1), 3.46 – 3.03 (m, 1H, H_5), 2.34 – 2.09 (m, 2H, H_3), 1.98 – 1.87 (m, 1H, H_4), 1.76 – 1.67 (m, 1H, H_4), 1.42 (d, J = 6.3 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 136.4 (CH), 116.5 (CH_2), 48.0 (CH), 34.1 (CH_2), 29.7 (CH_2), 18.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 2915 (w), 1645 (s), 1612 (s), 1510 (s), 1454 (s), 1390 (s), 1198 (s), 996 (s), 910 (s). $[\alpha]_D^{20}$ +3.5 (*c* 2.0, CH_3OH).

*Synthesis of the alkene **S3**:*



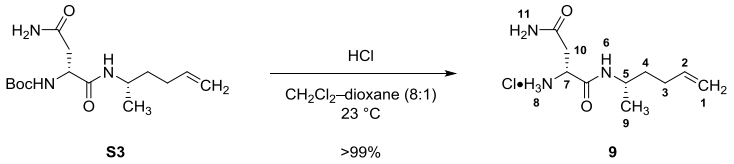
N-Boc-D-Asn **8** **S3**

N_α-(*tert*-butoxycarbonyl)-D-asparagine (880 mg, 3.79 mmol, 1 equiv), 1-hydroxybenzotriazole hydrate (HOBt, 638 mg, 4.17 mmol, 1.10 equiv), *N*,*N*-diisopropylethylamine (1.45 mL, 8.34 mmol, 2.20 equiv), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrogenchloride (EDC•HCl, 726 mg, 3.79 mmol, 1.00 equiv) were added in sequence to a solution of the amine **8** (514 mg, 3.79 mmol, 1.00 equiv) in tetrahydrofuran (40 mL) at 23 °C. The reaction mixture was stirred for 4 h at 23 °C. The heterogeneous product mixture was concentrated and the residue obtained was diluted with saturated aqueous ammonium chloride solution (60 mL). The resulting mixture was extracted with ethyl acetate (5 × 30 mL) and the organic layers were combined. The combined organic layers were washed sequentially with water (30 mL) and saturated aqueous sodium chloride solution (30 mL). The washed organic layer was dried over magnesium sulfate and the dried solution was filtered. The filtrate was concentrated to provide the alkene **S3** as a white solid (995 mg, 84%).

The product **S3** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 8.5 Hz, 1H, H_6), 7.25 (bs, 1H, H_{11}), 6.87 (bs, 1H, H_{11}), 6.84 (d, J = 8.1 Hz, 1H, H_8), 5.77 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, H_2), 4.98 (dd, J = 17.2, 2.0 Hz, 1H, H_1), 4.92 (d, J = 10.2 Hz, 1H, H_1), 4.14 (td, J = 8.0, 5.6 Hz, 1H, H_7), 3.83 – 3.54 (m, 1H, H_5), 2.43 – 2.27 (m, 2H, H_{10}), 2.10 – 1.90 (m, 2H, H_3), 1.53 – 1.37 (m, 2H, H_4), 1.37 (s, 9H, H_{12}), 1.00 (d, J = 6.5 Hz, 3H, H_9). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5 (C), 170.5 (C), 155.1 (C), 138.5 (CH), 114.7 (CH_2), 78.1 (C), 51.5 (CH), 43.8 (CH), 37.3 (CH_2), 35.2 (CH_2), 29.8 (CH_2), 28.2 (CH_3), 20.7 (CH_3). IR (ATR-FTIR), cm^{-1}: 3393 (br), 3298 (br), 2977 (w), 2930 (w), 1688 (m), 1635 (s), 1552 (m), 1519 (m), 1171 (s), 1054 (m), 610 (br). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{15}H_{27}N_3O_4$, 314.2080; found, 314.2001. $[\alpha]_D^{20}$ +15.4 (*c* 0.9, CH_3OH).

*Synthesis of the amine **9**:*

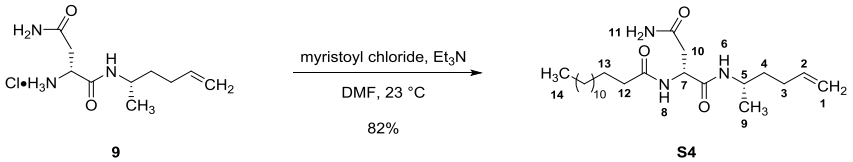


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 4.31 mL, 17.2 mmol, 6.00 equiv) was added dropwise via syringe pump over 20 min to a solution of the alkene **S3** (900 mg, 2.87 mmol, 1 equiv) in dichloromethane (30 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The product mixture was concentrated to provide the amine **9** as a white solid (717 mg, >99%).

The product **9** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.34 (d, J = 8.0 Hz, 1H, H_6), 8.19 (bs, 3H, H_8), 7.72 (bs, 1H, H_{11}), 7.22 (bs, 1H, H_{11}), 5.79 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, H_2), 5.02 (dd, J = 17.2, 1.9 Hz, 1H, H_1), 4.95 (dt, J = 10.2, 1.7 Hz, 1H, H_1), 4.05 – 3.85 (m, 1H, H_7), 3.84 – 3.64 (m, 1H, H_5), 2.67 (dd, J = 16.5, 5.1 Hz, 1H, H_{10}), 2.60 (dd, J = 16.5, 8.1 Hz, 1H, H_{10}), 2.13 – 1.89 (m, 2H, H_3), 1.59 – 1.41 (m, 2H, H_4), 1.04 (d, J = 6.6 Hz, 3H, H_9). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.6 (C), 167.0 (C), 138.3 (CH), 114.9 (CH_2), 49.3 (CH), 44.5 (CH), 35.6 (CH_2), 34.8 (CH_2), 29.8 (CH_2), 20.4 (CH_3). IR (ATR-FTIR), cm^{-1}: 2932 (br), 1655 (s), 1548 (m), 1452 (m), 1427 (m), 906 (m), 812 (br). HRMS-CI (m/z): [M + Na]$^{+}$ calcd for $C_{10}H_{19}N_3NaO_2$, 236.1375; found, 236.1361. $[\alpha]_D^{20}$ −3.9 (*c* 1.0, CH_3OH).

*Synthesis of the alkene **S4**:*



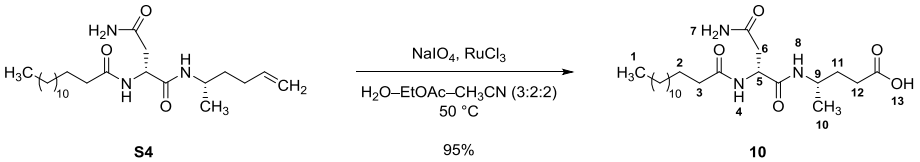
9 **S4**

Triethylamine (977 µL, 7.01 mmol, 2.50 equiv) and myristoyl chloride (990 µL, 3.64 mmol, 1.30 equiv) were added in sequence to a solution of the amine **9** (700 mg, 2.80 mmol, 1 equiv) in *N*,*N*-dimethylformamide (35 mL) at 23 °C. The reaction mixture was stirred for 4 h at 23 °C. The heterogeneous product mixture was diluted with aqueous hydrogen chloride solution (1.0 N, 50 mL). The precipitate that formed was isolated by filtration and the isolated precipitate was washed sequentially with aqueous hydrogen chloride solution (1.0 N, 20 mL) and water (20 mL). The resulting solid was triturated with dichloromethane (20 mL) to afford the alkene **S4** as a white solid (974 mg, 82%).

The product **S4** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (d, J = 8.1 Hz, 1H, H_8), 7.44 (d, J = 8.4 Hz, 1H, H_6), 7.24 (bs, 1H, H_{11}), 6.83 (bs, 1H, H_{11}), 5.77 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, H_2), 4.98 (dd, J = 17.2, 2.0 Hz, 1H, H_1), 4.92 (dd, J = 10.2, 2.0 Hz, 1H, H_1), 4.52 – 4.40 (m, 1H, H_7), 3.80 – 3.59 (m, 1H, H_5), 2.44 (dd, J = 15.2, 6.2 Hz, 1H, H_{10}), 2.32 (dd, J = 15.2, 7.7 Hz, 1H, H_{10}), 2.08 (t, J = 7.5 Hz, 2H, H_{12}), 2.03 – 1.89 (m, 2H, H_3), 1.51 – 1.34 (m, 4H, H_4, H_{13}), 1.23 (s, 20H, CH_2), 1.00 (d, J = 6.6 Hz, 3H, H_9), 0.85 (t, J = 6.9 Hz, 3H, H_{14}). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.1 (C), 171.4 (C), 170.3 (C), 138.5 (CH), 114.8 (CH_2), 49.8 (CH), 43.8 (CH), 37.4 (CH_2), 35.2 (2 × CH_2), 31.4 (CH_2), 29.9 (CH_2), 29.1 (3 × CH_2), 29.0 (2 × CH_2), 28.9 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 25.3 (CH_2), 22.2 (CH_2), 20.6 (CH_3), 14.1 (CH_3). IR (ATR-FTIR), cm^{-1}: 3297 (w), 2916 (w), 2850 (s), 1662 (s), 1638 (s), 1540 (s), 1394 (m), 909 (s). HRMS-CI (m/z): [M + H]$^+$ calcd for $C_{24}H_{46}N_3O_3$, 424.3539; found, 424.3516. $[\alpha]_D^{20}$ +23.7 (*c* 0.5, CH_3OH−DMF (1:1)).

*Synthesis of the carboxylic acid **10**:*

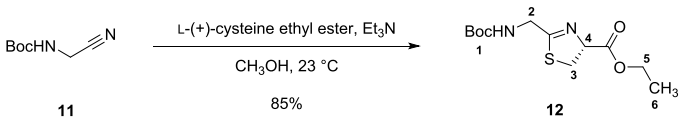


Water (12 mL), sodium periodate (611 mg, 2.86 mmol, 4.10 equiv), and ruthenium chloride (3.9 mg, 17.0 μmol, 0.025 equiv) were added in sequence to a suspension of the alkene **S4** (295 mg, 696 μmol, 1 equiv) in ethyl acetate (8.0 mL) and acetonitrile (8.0 mL) at 23 °C. The reaction vessel was placed in an oil bath that had been preheated to 50 °C. The reaction mixture was stirred vigorously for 2 h at 50 °C. The heterogeneous product mixture was partially concentrated to remove ethyl acetate and acetonitrile. The partially concentrated solution was diluted with aqueous hydrogen chloride solution (1.0 N, 50 mL). The precipitate that formed was isolated by filtration and the isolated precipitate was dissolved in ethyl acetate–methanol (5:1 v/v, 60 mL). Activated charcoal (4.5 g) was added and the resulting heterogeneous mixture was stirred for 3 h at 23 °C. The stirred heterogeneous mixture was filtered and the filtrate was concentrated to provide the carboxylic acid **10** as a white solid (292 mg, 95%).

The product **10** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (d, J = 8.0 Hz, 1H, H_4), 7.48 (d, J = 8.4 Hz, 1H, H_8), 7.25 (bs, 1H, H_7), 6.84 (bs, 1H, H_7), 4.46 (td, J = 7.8, 6.0 Hz, 1H, H_5), 3.77 – 3.64 (m, 1H, H_9), 2.43 (dd, J = 15.2, 6.0 Hz, 1H, H_6), 2.32 (dd, J = 15.2, 7.7 Hz, 1H, H_6), 2.24 – 2.09 (m, 2H, H_{12}), 2.08 (t, J = 7.5 Hz, 2H, H_3), 1.65 – 1.51 (m, 2H, H_{11}), 1.51 – 1.36 (m, 2H, H_2), 1.23 (bs, 20H, CH_2), 1.00 (d, J = 6.6 Hz, 3H, H_{10}), 0.85 (t, J = 7.0 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.4 (C), 172.1 (C), 171.4 (C), 170.4 (C), 49.8 (CH), 43.9 (CH), 37.4 (CH_2), 35.2 (CH_2), 31.3 (CH_2), 31.2 (CH_2), 30.5 (CH_2), 29.09 (CH_2), 29.07 (2 × CH_2), 29.04 (CH_2), 28.97 (CH_2), 28.86 (CH_2), 28.73 (CH_2), 28.65 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 20.5 (CH_3), 14.0 (CH_3). IR (ATR-FTIR), cm^{-1}: 3295 (w), 2920 (w), 2851 (s), 1666 (s), 1631 (s), 1541 (s), 1340 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{23}H_{44}N_3O_5$, 442.3281; found, 442.3251. $[\alpha]_D^{20}$ +16.6 (*c* 0.9, CH_3OH).

*Synthesis of the thiazoline **12**:*



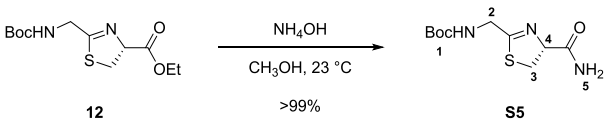
Triethylamine (540 μL, 3.84 mmol, 0.20 equiv) was added to a deoxygenated solution of *N*-(*tert*-butoxycarbonyl)-2-aminoacetonitrile (**11**, 3.00 g, 19.2 mmol, 1 equiv) and L-(+)-cysteine ethyl ester hydrogenchloride (5.35 g, 28.8 mmol, 1.50 equiv) in methanol (35 mL) at 23 °C. The resulting mixture was stirred for 14 h at 23 °C. The product mixture was concentrated and the residue obtained was purified by flash-column chromatography (eluting with 20% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes, linear gradient) to provide the thiazoline **12** as a colorless oil (4.71 g, 85%).

The product **12** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H and ^{13}C NMR data for the thiazoline **12** prepared in this way were in agreement with the literature.[14]

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.46 (t, J = 6.3 Hz, 1H), 5.12 (app t, J = 9.3 Hz, 1H, H_4), 4.23 – 4.06 (m, 2H, H_5), 3.94 – 3.90 (m, 2H, H_2), 3.53 (app t, J = 10.5 Hz, 1H, H_3), 3.39 (dd, J = 11.3, 8.9 Hz, 1H, H_3), 1.39 (s, 9H, H_1), 1.22 (t, J = 7.1 Hz, 3H, H_6). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.8 (C), 170.4 (C), 155.6 (C), 78.4 (C), 78.0 (CH), 61.0 (CH_2), 42.3 (CH_2), 33.9 (CH_2), 28.2 (CH_3), 14.0 (CH_2).

*Synthesis of the amide **S5**:*

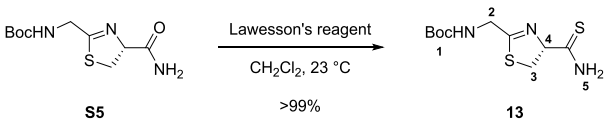


Aqueous ammonium hydroxide solution (28% w/v, 50 mL) was added to a solution of the ester **12** (3.86 g, 13.5 mmol, 1 equiv) in methanol (100 mL) at 23 °C. The resulting mixture was stirred for 16 h at 23 °C. The product mixture was concentrated and the residue obtained was dried by azeotropic distillation from toluene (2 × 30 mL) to provide the amide **S5** as a white solid (3.49 g, >99%).

The product **S5** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.45 (t, J = 6.1 Hz, 1H), 7.33 (bs, 1H, H_5), 7.14 (bs, 1H, H_5), 4.99 – 4.91 (m, 1H, H_4), 4.02 – 3.82 (m, 2H, H_2), 3.54 – 3.39 (m, 2H, H_3), 1.39 (s, 9H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.5 (C), 172.3 (C), 155.7 (C), 78.5 (CH), 78.4 (C), 42.5 (CH_2), 34.6 (CH_2), 28.2 (CH_3). IR (ATR-FTIR), cm^{-1}: 3321 (w), 1684 (s), 1615 (m), 1503 (s), 1251 (s), 1156 (w). ***m/z*** (ES+) 260.15 ([M+H]+, 100 %). $[\alpha]_D^{20}$ +6.5 (*c* 0.8, CH_3OH).

*Synthesis of the thioamide **13**:*



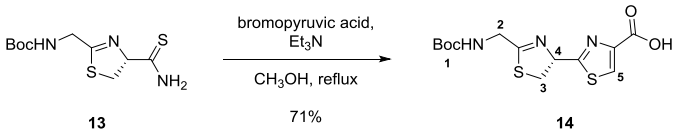
S5 **13**

Lawesson's reagent (4.03 g, 9.96 mmol, 0.75 equiv) was added to a solution of the amide **S5** (3.44 g, 13.3 mmol, 1 equiv) in dichloromethane (80 mL) at 23 °C. The resulting mixture was stirred for 16 h at 23 °C. The product mixture was filtered through a pad of celite (2.5 × 4.5 cm). The filter cake was washed with dichloromethane (20 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was dissolved in ethyl acetate (50 mL) and the resulting solution was washed sequentially with saturated aqueous sodium bicarbonate solution (2 × 30 mL) and saturated aqueous sodium chloride solution (30 mL). The washed organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the thioamide **13** as a pale yellow solid (3.66 g, >99%).

The thioamide **13** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (bs, 1H, H_5), 7.74 (bs, 1H, H_5), 5.35 (t, J = 9.1 Hz, 1H, H_4), 5.29 – 5.12 (m, 1H), 4.18 – 4.05 (m, 2H, H_2), 3.90 – 3.83 (m, 1H, H_3), 3.90 – 3.83 (m, 1H, H_3), 1.45 (s, 9H, H_1). ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.7 (C), 174.5 (C), 156.2 (C), 84.3 (CH), 80.7 (C), 43.6 (CH_2), 39.3 (CH_2), 28.4 (CH_3). IR (ATR-FTIR), cm^{-1}: 3300 (w), 1688 (s), 1596 (s), 1501 (s), 1248 (s), 1157 (w). HRMS-CI (m/z): $[M + Na]^+$ calcd for $C_{10}H_{17}N_3NaO_2S_2$, 298.0660; found, 298.0621. $[\alpha]_D^{20}$ −1.4 (*c* 1.0, CH_3OH).

*Synthesis of the thiazoline–thiazole **14**:*

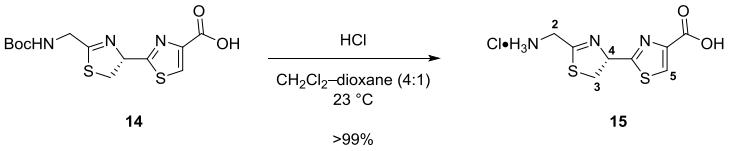


13 14

Triethylamine (759 μL, 5.48 mmol, 3.00 equiv) was added dropwise via syringe to a solution of the thioamide **13** (500 mg, 1.82 mmol, 1 equiv) and bromopyruvic acid (364 mg, 2.18 mmol, 1.20 equiv) in methanol (13 mL) at 23 °C. The reaction vessel was fitted with a reflux condenser and then was placed in an oil bath that had been preheated to 72 °C. The reaction mixture was stirred and heated for 3 h at 72 °C. The product mixture was concentrated and the residue obtained was purified using trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol) to provide the thiazoline–thiazole **14** as a white solid (440 mg, 71%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (s, 1H, H_6), 5.89 (app t, J = 8.6 Hz, 1H, H_4), 5.35 (t, J = 5.7 Hz, 1H), 4.30 – 4.16 (m, 2H, H_2), 3.89 (dd, J = 11.4, 9.2 Hz, 1H, H_3), 3.64 (dd, J = 11.4, 7.6 Hz, 1H, H_3), 1.46 (s, 9H, H_1). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.7 (C), 172.4 (C), 164.2 (C), 155.7 (C), 147.0 (C), 129.0 (CH), 80.5 (C), 77.0 (CH), 43.3 (CH_2), 39.1 (CH_2), 28.5 (CH_3). IR (ATR-FTIR), cm^{-1}: 1696 (w), 1507 (s), 1248 (s), 1156 (s). HRMS-CI (m/z): [M + Na]$^+$ calcd for $C_{13}H_{17}N_3NaO_4S_2$, 366.0558; found, 366.0508. $[\alpha]_D^{20}$ +6.6 (*c* 2.7, CH_3OH).

*Synthesis of the amine **15**:*



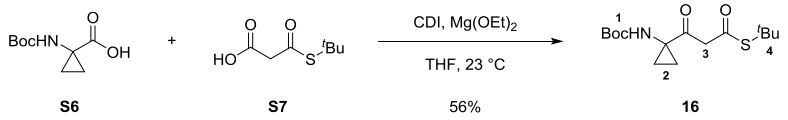
14 **15**

A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 3.50 mL, 14.0 mmol, 16.7 equiv) was added dropwise via syringe pump over 20 min to a solution of the thiazoline–thiazole **14** (287 mg, 836 µmol, 1 equiv) in dichloromethane (14 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The product mixture was concentrated to provide the amine **15** as a white solid (234 mg, >99%).

The product **15** obtained in this way was used directly in the following step without further purification.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 – 8.54 (bs, 3H), 8.47 (s, 1H, H_5), 6.02 – 5.83 (m, 1H, H_4), 4.05 (s, 2H, H_2), 4.04 – 4.00 (m, 1H, H_3), 3.70 (dd, J = 11.3, 8.5 Hz, 1H, H_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.3 (C), 167.9 (C), 162.0 (C), 147.1 (C), 129.3 (CH), 76.3 (CH), 40.2 (CH_2), 38.9 (CH_2).

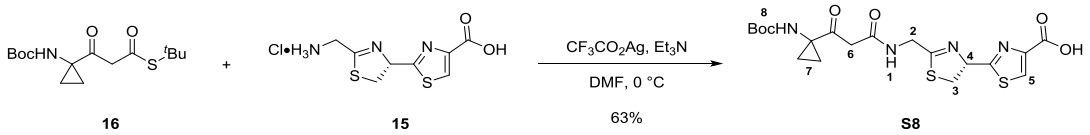
*Synthesis of the β-ketothioester **16**:*



1,1′-Carbonyldiimidazole (846 mg, 5.22 mmol, 1.50 equiv) was added to a solution of *N*-(*tert*-butoxycarbonyl)-1-amino-1-cyclopropane carboxylic acid (**S6**, 700 mg, 3.48 mmol, 1 equiv) in tetrahydrofuran (18 mL) at 23 °C. The resulting mixture was stirred for 6 h at 23 °C. In a second round-bottomed flask, magnesium ethoxide (299 mg, 2.61 mmol, 0.75 equiv) was added to a solution of 3-(*tert*-butylthio)-3-oxopropanoic acid (**S7**, 920 mg, 5.22 mmol, 1.50 equiv) in tetrahydrofuran (9.0 mL) at 23 °C. The resulting mixture was stirred for 6 h at 23 °C, and then was concentrated to dryness. The activated carboxylic acid prepared in the first flask was transferred via cannula to the dried magnesium salt prepared in the second flask. The resulting mixture was stirred for 14 h at 23 °C. The product mixture was diluted sequentially with saturated aqueous ammonium chloride solution (20 mL) and ethyl acetate (30 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 30 mL). The organic layers were combined and the combined organic layers were washed with saturated aqueous sodium chloride solution (30 mL). The washed organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with hexanes initially, grading to 10% ethyl acetate–hexanes, linear gradient) to provide the β-ketothioester **16** as a colorless oil (614 mg, 56%).

R_f = 0.36 (20% ethyl acetate-hexanes; UV). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.25 (bs, 1H), 3.80 (s, 2H, H_3), 1.62 (q, J = 4.5 Hz, 2H, H_2), 1.49 (s, 9H, H_4), 1.47 (s, 9H, H_1), 1.22 – 1.15 (m, 2H, H_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 203.7 (C), 193.9 (C), 155.8 (C), 80.7 (C), 55.3 (CH_2), 49.1 (C), 41.6 (C), 29.8 (CH_3), 28.5 (CH_3), 21.8 (CH_2). IR (ATR-FTIR), cm^{-1}: 3382 (m), 3333 (m), 2969 (br), 1699 (s), 1658 (m), 1597 (m), 1487 (s), 1249 (s), 1161 (s), 1065 (s). HRMS-CI (m/z): $[M + Na]^+$ calcd for $C_{15}H_{25}NNaO_4S$, 338.1402; found, 338.1384.

*Synthesis of the β-ketoamide **S8**:*

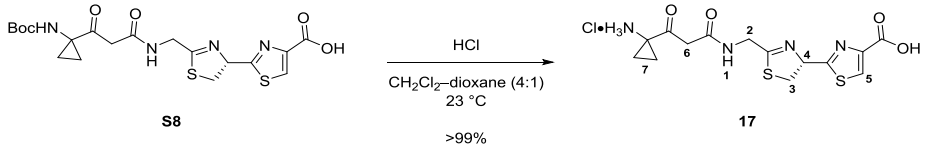


16 **15** **S8**

Three equal portions of silver trifluoroacetate (26.8 mg, 122 µmol, 0.40 equiv each) were added over 1 h to a solution of triethylamine (170 µL, 1.22 mmol, 4.00 equiv), **15** (85.0 mg, 304 µmol, 1 equiv), and **16** (115 mg, 365 µmol, 1.20 equiv) in *N*,*N*-dimethylformamide (3.5 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The product mixture was directly applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated. The residue obtained was further purified by automated flash-column chromatography (eluting with 2% acetic acid–dichloromethane initially, grading to 2% acetic acid–6% methanol–dichloromethane, linear gradient) to afford the β-ketoamide **S8** as a white solid (90.0 mg, 63%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 12.99 (bs, 1H), 8.67 (t, J = 6.0 Hz, 1H), 8.40 (s, 1H, H_5), 7.73 (bs, 1H), 5.91 (t, J = 8.7 Hz, 1H, H_4), 4.31 – 4.08 (m, 2H, H_2), 3.86 (dd, J = 11.3, 9.3 Hz, 1H, H_3), 3.55 – 3.49 (m, 3H, H_6, H_3), 1.40 (s, 9H, H_8), 1.35 (d, J = 3.1 Hz, 2H, H_7), 1.06 (d, J = 3.6 Hz, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.6 (C), 173.9 (C), 171.5 (C), 166.6 (C), 162.2 (C), 156.3 (C), 147.1 (C), 128.9 (CH), 78.6 (C), 76.9 (CH), 45.9 (CH_2), 41.2 (C), 41.1 (CH_2), 37.7 (CH_2), 28.2 (CH_3), 19.5 (CH_2). IR (ATR-FTIR), cm^{-1}: 3000 (br), 1702 (s), 1507 (m), 1249 (m), 1164 (3), 1068 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{19}H_{25}N_4O_6S_2$, 469.1216; found, 469.1257. $[\alpha]_D^{20}$ +3.0 (*c* 2.0, CH_3OH).

*Synthesis of the amine **17**:*

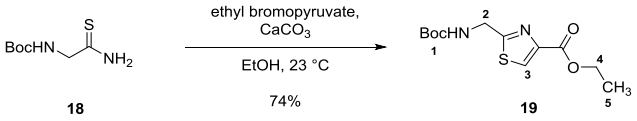


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 1.00 mL, 4.00 mmol, 22.6 equiv) was added dropwise via syringe pump over 20 min to a solution of the β-ketoamide **S8** (83.0 mg, 177 μmol, 1 equiv) in dichloromethane (4.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **17** as a white solid (71.7 mg, >99%).

The product **17** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.87 (t, J = 6.0 Hz, 1H, H_1), 8.81 (s, 3H), 8.41 (s, 1H, H_5), 5.96 – 5.86 (m, 1H, H_4), 4.25 – 4.01 (m, 2H, H_2), 3.87 (dd, J = 11.3, 9.2 Hz, 1H, H_3), 3.54 (dd, J = 11.8, 8.2 Hz, 1H, H_3), 3.35 (s, 2H, H_6), 1.86 – 1.67 (m, 2H, H_7), 1.56 – 1.37 (m, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 199.2 (C), 173.4 (C), 171.3 (C), 165.7 (C), 162.0 (C), 147.1 (C), 129.0 (CH), 76.8 (CH), 42.3 (CH_2), 42.0 (C), 41.2 (CH_2), 37.8 (CH_2), 13.1 (CH_2).

*Synthesis of the thiazole **19**:*

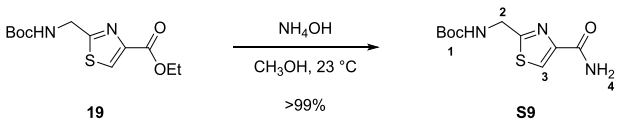


Ethyl bromopyruvate (2.37 mL, 18.9 mmol, 1.20 equiv) and calcium carbonate (1.58 g, 15.8 mmol, 1.00 equiv) were added in sequence to a solution of *tert*-butyl 2-amino-2-thioxoethylcarbamate (**18**, 3.00 g, 15.8 mmol, 1 equiv) in ethanol (60 mL) at 23 °C. The reaction mixture was stirred for 6 h at 23 °C. The product mixture was concentrated and the residue obtained was purified by flash-column chromatography (eluting with 10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes, linear gradient) to furnish the thiazole **19** as a white solid (3.34 g, 74%).

^{1}H and ^{13}C NMR data for thiazole **19** prepared in this way were in agreement with the literature.[15]

R_f = 0.39 (40% ethyl acetate-hexanes; UV). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 (s, 1H, H_3), 4.65 (d, J = 6.4 Hz, 2H, H_2), 4.42 (q, J = 7.1 Hz, 2H, H_4), 1.46 (s, 9H, H_1), 1.40 (t, J = 7.1 Hz, 3H, H_5). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.1 (C), 161.4 (C), 155.8 (C), 147.1 (C), 128.1 (CH), 80.7 (C), 61.7 (CH_2), 42.5 (CH_2), 28.5 (CH_3), 14.5 (CH_3).

*Synthesis of the amide **S9**:*



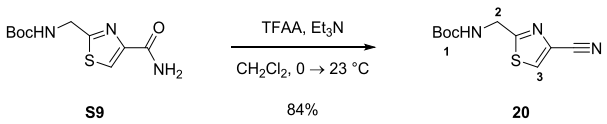
A solution of aqueous ammonia (28% w/v, 42 mL) was added to a solution of the thiazole **19** (2.38 g, 8.29 mmol, 1 equiv) in anhydrous methanol (84 mL) at 23 °C. The resulting mixture was stirred for 16 h at 23 °C. The product mixture was concentrated and the residue obtained was dried by azeotropic distillation from toluene (2 × 30 mL) to afford the product **S9** as a yellow solid (2.13 g, >99%).

The amide **S9** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H and ^{13}C NMR data for amide **S9** prepared in this way were in agreement with the literature.[16]

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 (s, 1H, H_3), 7.12 (bs, 1H, H_4), 5.83 (bs, 1H, H_4), 5.30 (t, *J* = 6.2 Hz, 1H), 4.60 (d, *J* = 6.2 Hz, 2H, H_2), 1.47 (s, 9H, H_1). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.5 (C), 163.0 (C), 155.7 (C), 149.3 (C), 124.8 (CH), 80.7 (C), 42.5 (CH_2), 28.5 (CH_3).

*Synthesis of the nitrile **20**:*



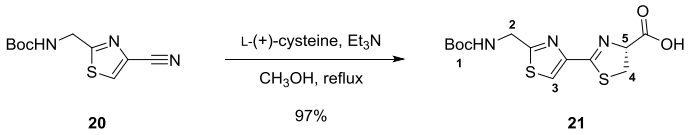
S9 **20**

Trifluoroacetic anhydride (1.24 mL, 8.94 mmol, 1.10 equiv) was added dropwise via syringe pump over 20 min to a solution of the amide **S9** (2.09 g, 8.12 mmol, 1 equiv) and triethylamine (2.49 mL, 17.9 mmol, 2.20 equiv) in dichloromethane (120 mL) at 0 °C. The resulting mixture was stirred for 30 min at 0 °C. The reaction mixture was then allowed to warm over 30 min to 23 °C. The warmed reaction mixture was stirred for 2 h at 23 °C. The product mixture was concentrated and the residue obtained was purified by flash-column chromatography (eluting with hexanes initially, grading to 20% ethyl acetate–hexanes, linear gradient) to furnish the nitrile **20** as a white solid (1.63 g, 84%).

^{1}H and ^{13}C NMR data for nitrile **20** prepared in this way were in agreement with the literature.[16]

R_f = 0.51 (40% ethyl acetate-hexanes; UV). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (s, 1H, H_3), 5.31 (t, J = 6.4 Hz, 1H), 4.62 (d, J = 6.4 Hz, 2H, H_2), 1.47 (s, 9H, H_1). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.5 (C), 155.8 (C), 131.0 (CH), 126.6 (C), 113.9 (C), 81.0 (C), 42.5 (CH_2), 28.4 (CH_3). IR (ATR-FTIR), cm^{-1}: 3334 (w), 3073 (w), 1684 (s), 1520 (s), 1297 (s), 1161 (m), 618 (m).

*Synthesis of the thiazole–thiazoline **21**:*



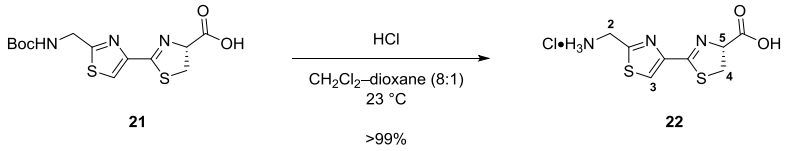
Triethylamine (1.01 mL, 7.21 mmol, 1.10 equiv) was added dropwise via syringe to a solution of the nitrile **20** (1.57 g, 6.55 mmol, 1 equiv) and L-cysteine (870 mg, 7.21 mmol, 1.10 equiv) in methanol (60 mL) at 23 °C. The reaction vessel was fitted with a reflux condenser and then placed in an oil bath that had been preheated to 73 °C. The reaction mixture was stirred and heated for 3 h at reflux. The product mixture was cooled to 23 °C and the cooled product mixture was concentrated. The residue obtained was dissolved in saturated aqueous sodium bicarbonate solution (40 mL) and the resulting solution was washed with ether (30 mL). The aqueous layer was acidified to pH ~3–4 by the dropwise addition of 3.0 N aqueous hydrochloric acid solution. The resulting mixture was extracted with ethyl acetate (3 × 30 mL) and the organic layers were combined. The combined organic layers were dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated to provide the thiazole–thiazoline **21** as a white solid (2.18 g, 97%).

The thiazole–thiazoline **21** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H and ^{13}C NMR data for thiazole–thiazoline **21** prepared in this way were in agreement with the literature.[17]

^{1}H NMR (600 MHz, CD_3OD) δ 8.16 (s, 1H, H_3), 5.30 (t, J = 9.1 Hz, 1H, H_5), 4.52 (s, 2H, H_2), 3.85 – 3.51 (m, 2H, H_4), 1.47 (s, 9H, H_1). ^{13}C NMR (151 MHz, CD_3OD) δ 173.8 (C), 173.3 (C), 167.9 (C), 158.3 (C), 149.3 (C), 123.0 (CH), 81.0 (C), 79.1 (CH), 43.1 (CH_2), 35.6 (CH_2), 28.7 (CH_3). IR (ATR-FTIR), cm^{-1}: 3351 (w), 2978 (w), 1701 (w), 1519 (w), 1250 (s), 1165 (s).

*Synthesis of the amine **22**:*

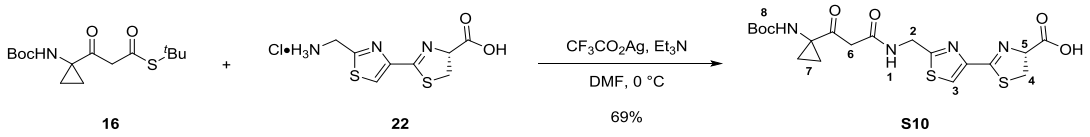


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 1.5 mL, 6.00 mmol, 6.90 equiv) was added dropwise via syringe pump over 20 min to a solution of the thiazole–thiazoline **21** (300 mg, 870 μmol, 1 equiv) in dichloromethane (12 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **22** as a white solid (244 mg, >99%).

The product **22** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, CD_3OD) δ 9.02 (s, 1H, H_3), 5.66 (dd, *J* = 10.5, 5.8 Hz, 1H, H_5), 4.64 (s, 2H, H_2), 4.17 (dd, *J* = 12.1, 10.5 Hz, 1H, H_4), 4.10 (dd, *J* = 12.1, 5.8 Hz, 1H, H_4). ^{13}C NMR (151 MHz, CD_3OD) δ 180.3 (C), 170.3 (C), 166.0 (C), 143.5 (C), 134.2 (CH), 69.9 (CH), 40.9 (CH_2), 35.7 (CH_2).

*Synthesis of the β-ketoamide **S10**:*

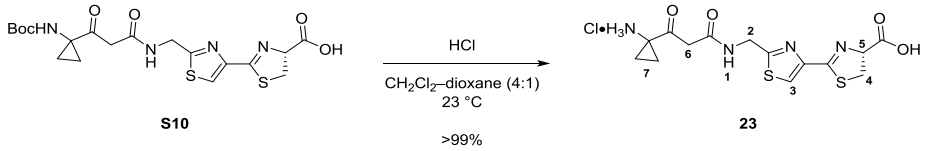


16 **22** **S10**

Three equal portions of silver trifluoroacetate (31.6 mg, 14.3 μmol, 0.40 equiv each) were added over 1 h to a solution of triethylamine (199 μl, 1.43 mmol, 4.00 equiv), the β-ketothioester **16** (135 mg, 42.9 μmol, 1.20 equiv), and the amine **22** (100 mg, 35.7 μmol, 1 equiv) in *N*,*N*-dimethylformamide (3.5 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The product mixture was directly applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated. The residue obtained was further purified by automated flash-column chromatography (eluting with 2% acetic acid–dichloromethane initially, grading to 2% acetic acid–6% methanol–dichloromethane, linear gradient). The fractions containing the product **S10** were collected, combined, and concentrated to provide the β-ketoamide **S10** as a white solid (115 mg, 69%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.95 (t, J = 6.0 Hz, 1H, H_1), 8.23 (s, 1H, H_3), 5.26 (dd, J = 9.7, 8.2 Hz, 1H, H_5), 4.55 (d, J = 4.5 Hz, 1H, H_2), 3.63 (dd, J = 11.3, 9.7 Hz, 1H, H_4), 3.56 (s, 2H, H_6), 3.56 – 3.51 (m, 1H, H_4), 1.41 (s, 9H, H_8), 1.40 – 1.31 (m, 2H, H_7), 1.07 (q, J = 4.3 Hz, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.7 (C), 171.8 (C), 170.1 (C), 166.8 (C), 163.3 (C), 156.0 (C), 147.4 (C), 122.1 (CH), 78.6 (C), 78.3 (CH), 46.1 (CH_2), 41.1 (C), 40.3 (CH_2), 34.4 (CH_2), 28.2 (CH_3), 19.5 (CH_2). IR (ATR-FTIR), cm^{-1}: 3317 (br), 1702 (s), 1506 (m), 1248 (m), 1162 (s), 1063 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{19}H_{25}N_4O_6S_2$, 469.1216; found, 469.1257. $[\alpha]_D^{20}$ +5.6 (*c* 2.2, CH_3OH).

*Synthesis of the amine **23**:*

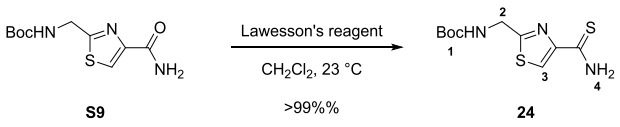


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 1.0 mL, 4.00 mmol, 21.5 equiv) was added dropwise via syringe pump over 20 min to a solution of the β-ketoamide **S10** (87.0 mg, 186 µmol, 1 equiv) in dichloromethane (4.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **23** as a white solid (75.2 mg, >99%).

The product **23** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.17 (t, J = 6.0 Hz, 1H, H_1), 8.83 (bs, 3H), 8.30 (s, 1H, H_3), 5.28 (dd, J = 9.7, 8.1 Hz, 1H, H_5), 4.58 (d, J = 5.9 Hz, 2H, H_2), 3.66 (dd, J = 11.2, 9.9 Hz, 1H, H_4), 3.60 – 3.51 (m, 1H, H_4), 3.38 (s, 2H, H_6), 1.81 – 1.72 (m, 2H, H_7), 1.57 – 1.49 (m, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 199.39 (C), 171.66 (C), 169.76 (C), 165.92 (C), 164.04 (C), 147.09 (C), 122.66 (CH), 77.72 (CH), 42.38 (CH_2), 42.00 (C), 40.43 (CH_2), 34.40 (CH_2), 13.12 (CH_2).

*Synthesis of the thioamide **24**:*



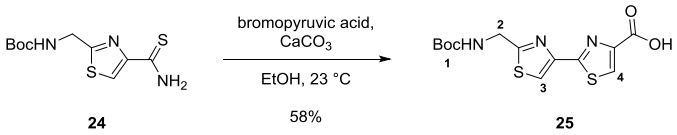
Lawesson's reagent (1.51 g, 7.73 mmol, 0.75 equiv) was added to a solution of the amide **S9** (1.28 g, 4.98 mmol, 1 equiv) in dichloromethane (30 mL) at 23 °C. The resulting mixture was stirred for 16 h at 23 °C. The product mixture was filtered through a pad of celite (2.5 × 4.5 cm). The filter cake was washed with dichloromethane (10 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was dissolved in ethyl acetate (40 mL) and the resulting solution was washed sequentially with saturated aqueous sodium bicarbonate solution (2 × 20 mL) and saturated aqueous sodium chloride solution (20 mL). The washed organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the thioamide **24** as a pale yellow solid (1.36 g, >99%).

The thioamide **24** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification.

^{1}H and ^{13}C NMR data for the thioamide **24** prepared in this way were in agreement with the literature.[17]

R_f = 0.37 (2% methanol-CH_2Cl_2; UV). ^{1}H NMR (600 MHz, $(CD_3)CO_2$) δ 9.04 (bs, 1H, H_4), 8.38 (s, 1H, H_3), 6.96 (t, *J* = 6.3 Hz, 1H), 4.55 (d, *J* = 6.1 Hz, 2H, H_2), 1.44 (s, 9H, H_1). ^{13}C NMR (151 MHz, $(CD_3)CO_2$) δ 191.3 (C), 172.0 (C), 156.7 (C), 154.6 (C), 127.5 (CH), 79.8 (C), 43.2 (CH_2), 28.5 (CH_3).

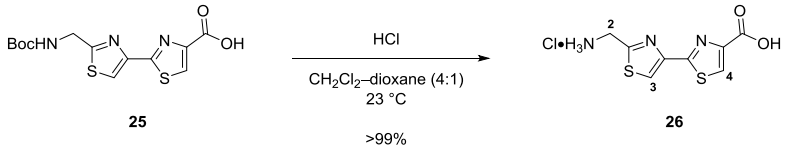
*Synthesis of the bithiazole **25**:*



Bromopyruvic acid (170 mg, 1.02 mmol, 1.50 equiv) and calcium carbonate (136 mg, 1.36 mmol, 2.00 equiv) were added in sequence to a solution of the thioamide **24** (186 mg, 680 µmol, 1 equiv) in ethanol (6.0 mL) at 23 °C. The reaction mixture was stirred for 16 h at 23 °C. The product mixture was concentrated and the residue obtained was applied to a trimethylamine acetate-functionalized silica column (S Si-TMA acetate; eluting with 2% acetic acid–methanol) to provide the bithiazole **25** as a white solid (135 mg, 58%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.42 (s, 1H, H_4), 8.22 (s, 1H, H_3), 7.87 (t, J = 6.1 Hz, 1H), 4.45 (d, J = 6.1 Hz, 2H, H_2), 1.42 (s, 9H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.82 (C), 162.25 (C), 161.92 (C), 155.80 (C), 149.09 (C), 147.34 (C), 128.29 (CH), 117.78 (CH), 78.72 (C), 41.93 (CH_2), 28.16 (CH_3). IR (ATR-FTIR), cm^{-1}: 3322 (w), 3128 (w), 1685 (s), 1534 (m), 1290 (m), 1233 (m), 1168 (m), 772 (m), 751 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{13}H_{16}N_3O_4S_2$, 342.0582; found, 342.0577.

*Synthesis of the amine **26**:*



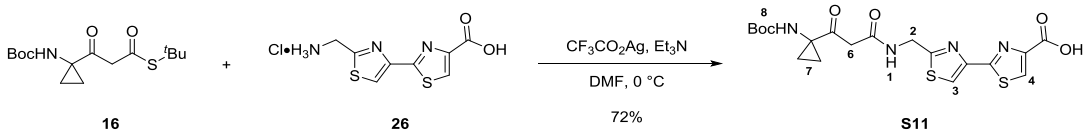
25 **26**

A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 1.5 mL, 6.00 mmol, 16.8 equiv) was added dropwise via syringe pump over 20 min to a solution of the bithiazole **25** (122 mg, 357 μmol, 1 equiv) in dichloromethane (6.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **26** as a white solid (99.3 mg, >99%).

The product **26** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.57 (bs, 3H), 9.33 (s, 1H, H_4), 9.24 (s, 1H, H_3), 5.32 (s, 2H, H_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.2 (C), 162.4 (C), 162.1 (C), 148.6 (C), 147.7 (C), 129.7 (CH), 120.7 (CH), 39.7 (CH_2).

*Synthesis of the β-ketoamide **S11**:*

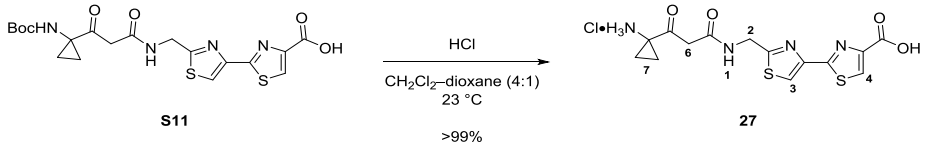


16 **26** **S11**

Three equal portions of silver trifluoroacetate (27.0 mg, 12.2 μmol, 0.40 equiv) were added over 1 h to a solution of triethylamine (171 μL, 1.22 mmol, 4.00 equiv), the β-ketothioester **16** (116 mg, 36.7 μmol, 1.20 equiv), and the amine **26** (85.0 mg, 30.6 μmol, 1 equiv) in *N*,*N*-dimethylformamide (3.50 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The product mixture was directly applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated. The residue obtained was further purified by automated flash-column chromatography (eluting with 2% acetic acid–dichloromethane initially, grading to 2% acetic acid–6% dichloromethane–methanol, linear gradient). The fractions containing the product **S11** were collected, combined, and concentrated to provide the β-ketoamide **S11** as a white solid (102 mg, 72%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 13.14 (bs, 1H), 8.98 (t, J = 6.0 Hz, 1H, H_1), 8.47 (s, 1H, H_4), 8.25 (s, 1H, H_3), 7.77 (s, 1H), 4.60 (d, J = 6.0 Hz, 2H, H_2), 3.57 (s, 2H, H_6), 1.41 (s, 9H, H_8), 1.40 – 1.36 (m, 2H, H_7), 1.08 (q, J = 4.6 Hz, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.7 (C), 171.3 (C), 166.9 (C), 162.1 (C), 162.0 (C), 156.1 (C), 148.2 (C), 147.1 (C), 128.9 (CH), 118.4 (CH), 78.6 (C), 46.1 (CH_2), 41.2 (C), 40.5 (CH_2), 28.2 (CH_3), 19.6 (CH_2). IR (ATR-FTIR), cm^{-1}: 3322 (m), 2931 (br), 1709 (s), 1686 (s), 1511 (m), 1282 (m), 1237 (m), 1164 (m), 758 (m). HRMS-CI (m/z): $[M + Na]^+$ calcd for $C_{19}H_{22}N_4NaO_6S_2$, 489.0878; found, 489.0794.

Synthesis of the amine ***27:***

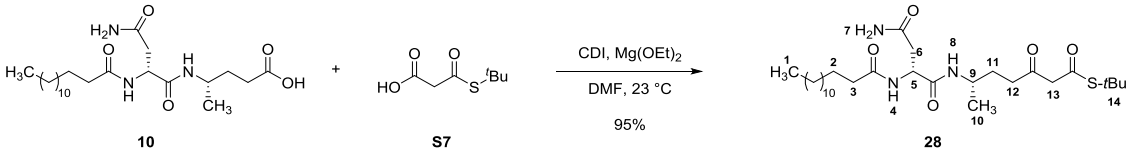


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 0.5 mL, 2.00 mmol, 33.3 equiv) was added dropwise via syringe pump over 20 min to a solution of the β-ketoamide **S11** (28.0 mg, 60.0 µmol, 1 equiv) in dichloromethane (2.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **27** as a white solid (24.2 mg, >99%).

The product **27** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 13.15 (bs, 1H), 9.19 (t, J = 6.0 Hz, 1H, H_1), 8.79 (bs, 3H), 8.49 (s, 1H, H_4), 8.28 (s, 1H, H_3), 4.63 (d, J = 5.9 Hz, 2H, H_2), 3.40 (s, 2H, H_6), 1.78 (q, J = 5.9 Hz, 2H, H_7), 1.53 (q, J = 6.0 Hz, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 199.4 (C), 170.7 (C), 166.0 (C), 162.1 (C), 162.0 (C), 148.1 (C), 147.2 (C), 129.0 (CH), 118.4 (CH), 42.4 (CH_2), 42.0 (C), 40.5 (CH_2), 13.1 (CH_2).

*Synthesis of the thioester **28**:*

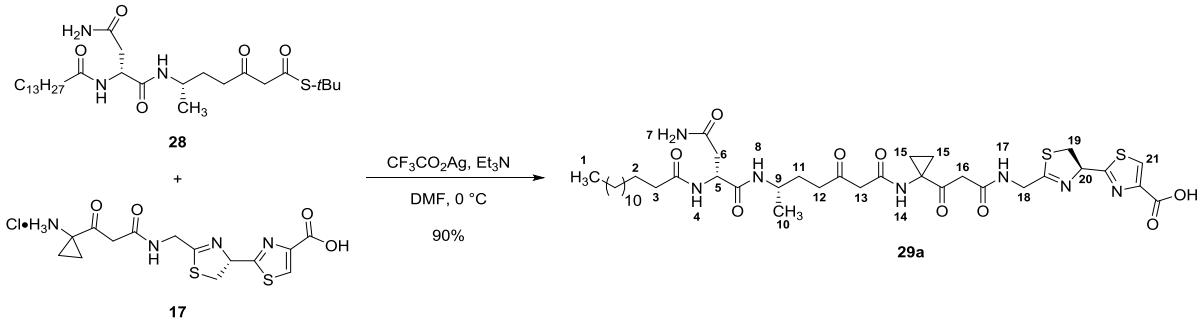


10 S7 28

1,1′-Carbonyldiimidazole (CDI; 165 mg, 1.02 mmol, 1.50 equiv) was added to a solution of the carboxylic acid **10** (300 mg, 679 µmol, 1 equiv) in *N*,*N*-dimethylformamide (7.0 mL). The resulting solution was stirred for 8 h at 23 °C. In a second round-bottomed flask, magnesium ethoxide (77.7 mg, 679 µmol, 1.00 equiv) was added to a solution of 3-(*tert*-butylthio)-3-oxopropanoic acid (**S7**, 238 mg, 1.36 mmol, 2.00 equiv) in tetrahydrofunan (3.0 mL). The resulting mixture was stirred for 10 h at 23 °C. The reaction mixture was concentrated to provide the magnesium salt of the β-ketothioester **S7** (271 mg, >99%) as a colorless solid. A solution of the magnesium salt of the β-ketothioester **S7** in *N*,*N*-dimethylformamide (1.0 mL) was transferred via cannula to the activated carboxylic acid and the resulting mixture was stirred for 16 h at 23 °C. The product mixture was diluted with aqueous hydrogen chloride solution (1.0 N, 20 mL). The resulting solid precipitate was isolated by filtration and the isolated filtrate was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with methanol) to provide the β-ketothioester **28** as a white solid (359 mg, 95%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (d, J = 7.8 Hz, 1H, H_4), 7.50 (d, J = 8.6 Hz, 1H, H_8), 7.25 (bs, 1H, H_7), 6.85 (bs, 1H, H_7), 4.41 (q, J = 7.4 Hz, 1H, H_5), 3.74 – 3.59 (m, 3H, H_5, H_{13}), 2.50 – 2.42 (m, 2H, H_{12}), 2.41 (dd, J = 14.1, 5.0 Hz, 1H, H_6), 2.33 (dd, J = 15.1, 7.7 Hz, 1H, H_6), 2.09 (dd, J = 9.4, 6.7 Hz, 2H, H_3), 1.66 – 1.52 (m, 1H, H_{11}), 1.54 – 1.42 (m, 3H, H_{11}, H_2), 1.41 (s, 9H, H_{14}), 1.23 (bs, 20H, 10 × CH_2), 0.98 (d, J = 6.6 Hz, 3H, H_{10}), 0.85 (t, J = 6.5 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 202.5 (C), 192.8 (C), 172.1 (C), 171.4 (C), 170.5 (C), 57.5 (CH_2), 49.9 (CH), 48.1 (C), 43.5 (CH), 39.0 (CH_2), 37.3 (CH_2), 35.2 (CH_2), 31.3 (CH_2), 29.6 (CH_2), 29.2 (CH_3), 29.06 (CH_3), 29.04 (2 × CH_2), 29.01 (CH_2), 28.95 (CH_2), 28.87 (CH_2), 28.84 (CH_2), 28.7 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 20.5 (CH_3), 14.0 (CH_3). IR (ATR-FTIR), cm^{-1}: 3297 (m), 2955 (m), 1660 (s), 1633 (s), 1542 (m), 1121 (br), 1029 (m), 587 (m). HRMS-CI (m/z): [M + H]$^+$ calcd for $C_{29}H_{54}N_3O_5S$, 556.3784; found, 556.3759. $[\alpha]_D^{20}$ +4.8 (*c* 1.7, CH_3OH).

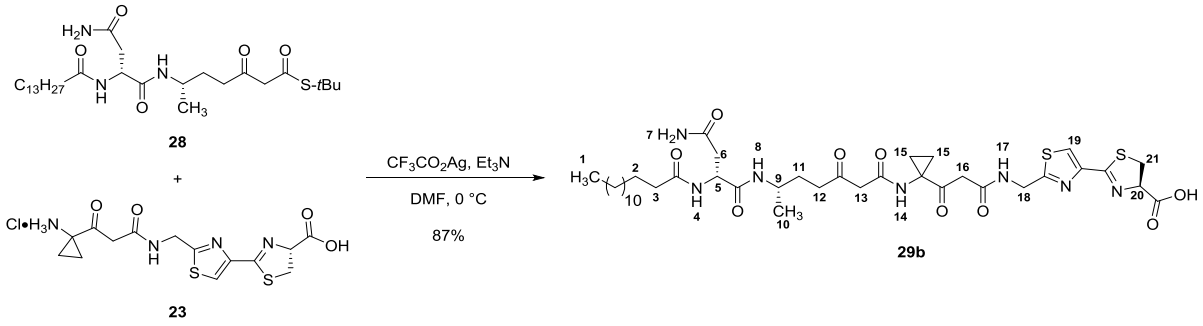
*Synthesis of the linear precursor **29a**:*



Silver trifluoroacetate (18.3 mg, 83.0 μmol, 2.00 equiv) was added to a solution of triethylamine (23.0 μL, 166 μmol, 4.00 equiv), the β-ketothioester **28** (23.0 mg, 41.0 μmol, 1 equiv), and the amine **17** (20.1 mg, 50.0 μmol, 1.20 equiv) in *N*,*N*-dimethylformamide (0.8 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was diluted with aqueous citric acid solution (5%, 4.0 mL). The resulting precipitate was isolated by filtration. The solid was dried *in vacuo* to provide the linear precursor **29a** as a white solid (31.0 mg, 90%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 1H, H_{14}), 8.67 (t, J = 6.1 Hz, 1H, H_{17}), 8.37 (s, 1H, H_{21}), 7.90 (bs, 1H, H_4), 7.52 (d, J = 8.5 Hz, 1H, H_8), 7.28 (bs, 1H, H_7), 6.86 (bs, 1H, H_7), 5.91 (t, J = 8.6 Hz, 1H, H_{20}), 4.43 (q, J = 7.2 Hz, 1H, H_5), 4.17 (d, J = 5.5 Hz, 2H, H_{18}), 3.86 (t, J = 10.2 Hz, 1H, H_{19}), 3.75 – 3.63 (m, 1H, H_9), 3.55 (s, 2H, H_{16}), 3.52 (dd, J = 11.5, 8.4 Hz, 1H, H_{19}), 3.34 (s, 1H, H_{13}), 2.55 – 2.43 (m, 2H, H_{12}), 2.42 (dd, J = 15.2, 6.1 Hz, 1H, H_6), 2.36 (dd, J = 15.5, 7.9 Hz, 1H, H_6), 2.13 – 2.03 (m, 2H, H_3), 1.67 – 1.53 (m, 1H, H_{11}), 1.54 – 1.47 (m, 1H, H_{11}), 1.50 – 1.40 (m, 2H, H_2), 1.38 (d, J = 4.4 Hz, 2H, H_{15}), 1.22 (bs, 20H, 10 × CH_2), 1.05 – 1.01 (m, 2H, H_{15}), 0.99 (d, J = 6.6 Hz, 3H, H_{10}), 0.85 (t, J = 6.9 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.8 (C), 204.0 (C), 173.8 (C), 172.2 (C), 172.1 (C), 171.4 (C), 171.3 (C), 170.5 (C), 168.0 (C), 166.7 (C), 162.2 (C), 128.4 (CH), 76.9 (CH), 50.2 (CH_2), 50.0 (CH), 46.4 (CH_2), 43.6 (CH), 41.1 (CH_2), 40.5 (C), 39.1 (CH_2), 37.7 (CH_2), 37.4 (CH_2), 35.2 (CH_2), 31.3 (CH_2), 29.7 (CH_2), 29.09 (CH_2), 29.07 (2 × CH_2), 29.03 (CH_2), 28.97 (CH_2), 28.87 (CH_2), 28.73 (CH_2), 28.67 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 20.6 (CH_3), 19.4 (CH_2), 14.0 (CH_3). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{60}N_7O_9S_2$, 834.3894; found, 834.3833. $[\alpha]_D^{20}$ −17.0 (*c* 0.8, DMSO).

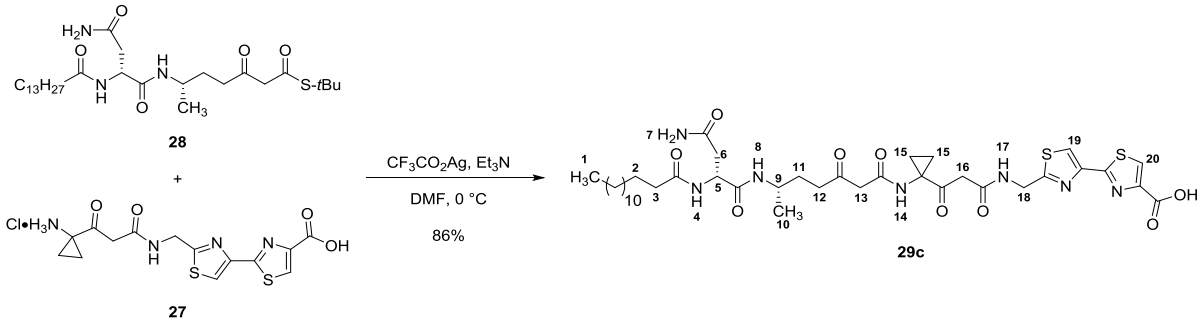
*Synthesis of the linear precursor **29b**:*



Silver trifluoroacetate (23.8 mg, 0.11 mmol, 2.00 equiv) was added to a solution of triethylamine (30.0 µL, 0.22 mmol, 4.00 equiv), the β-ketothioester **28** (30.0 mg, 54.0 µmol, 1 equiv), and the amine **23** (26.2 mg, 65.0 µmol, 1.20 equiv) in *N*,*N*-dimethylformamide (2.0 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was diluted with aqueous citric acid solution (5%, 10 mL). The resulting precipitate was isolated by filtration. The solid was dried *in vacuo* to provide the linear precursor **29b** as a white solid (39.0 mg, 87%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.94 (t, J = 6.0 Hz, 1H, H_{17}), 8.83 (s, 1H, H_{14}), 8.22 (s, 1H, H_{19}), 7.89 (d, J = 7.7 Hz, 1H, H_4), 7.52 (d, J = 8.5 Hz, 1H, H_8), 7.26 (bs, 1H, H_7), 6.87 (bs, 1H, H_7), 5.26 (t, J = 9.0 Hz, 1H, H_{20}), 4.56 (d, J = 6.0 Hz, 2H, H_{18}), 4.43 (q, J = 7.2 Hz, 1H, H_5), 3.70 (td, J = 8.6, 4.4 Hz, 1H, H_9), 3.67 – 3.60 (m, 1H, H_{21}), 3.60 (s, 2H, H_{16}), 3.54 (dd, J = 11.3, 8.2 Hz, 1H, H_{21}), 3.34 (s, 2H, H_{13}), 2.56 – 2.44 (m, 2H, H_{12}), 2.42 (dd, J = 15.4, 6.0 Hz, 1H, H_6), 2.35 (dd, J = 15.4, 7.7 Hz, 1H, H_6), 2.09 (t, J = 7.5 Hz, 2H, H_3), 1.68 – 1.53 (m, 1H, H_{11}), 1.55 – 1.47 (m, 1H, H_{11}), 1.49 – 1.41 (m, 2H, H_2) 1.42 – 1.31 (m, 2H, H_{15}), 1.23 (bs, 20H, 10 × CH_2), 1.05 (q, J = 3.3 Hz, 2H, H_{15}), 0.99 (d, J = 6.7 Hz, 3H, H_{10}), 0.85 (t, J = 6.9 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.8 (C), 204.1 (C), 172.2 (C), 171.8 (C), 171.3 (C), 170.5 (C), 170.2 (C), 168.1 (C), 167.0 (C), 163.3 (C), 147.3 (C), 122.2 (CH), 78.2 (CH), 50.2 (CH_2), 49.9 (CH), 46.5 (CH_2), 43.6 (CH), 40.5 (CH_2), 40.5 (C), 39.1 (CH_2), 37.4 (CH_2), 35.2 (CH_2), 34.4 (CH_2), 31.3 (CH_2), 29.7 (CH_2), 29.09 (CH_2), 29.07 (2 × CH_2), 29.03 (CH_2), 28.97 (CH_2), 28.87 (CH_2), 28.73 (CH_2), 28.67 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 20.6 (CH_3), 19.4 (CH_2), 14.0 (CH_3). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{60}N_7O_9S_2$, 834.3894; found, 834.3835. $[\alpha]_D^{20}$ −10.0 (*c* 0.8, DMSO).

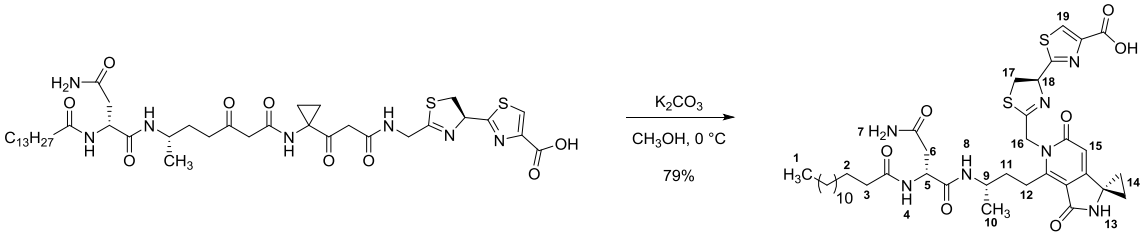
*Synthesis of the linear precursor **29c**:*



Silver trifluoroacetate (19.1 mg, 86.0 µmol, 2.00 equiv) was added to a solution of triethylamine (24.0 µL, 0.17 mmol, 4.00 equiv), the β-ketothioester **28** (24.0 mg, 43.0 µmol, 1 equiv), and the amine **27** (21.0 mg, 52.0 µmol, 1.20 equiv) in *N*,*N*-dimethylformamide (0.8 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was diluted with aqueous citric acid solution (5%, 8.0 mL). The resulting precipitate was isolated by filtration. The solid was dried *in vacuo* to provide the linear precursor **29c** as a white solid (31.0 mg, 86%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (t, J = 6.0 Hz, 1H, H_{17}), 8.84 (s, 1H, H_{14}), 8.47 (s, 1H, H_{20}), 8.25 (s, 1H, H_{19}), 7.90 (d, J = 7.7 Hz, 1H, H_4), 7.52 (d, J = 8.6 Hz, 1H, H_8), 7.27 (bs, 1H, H_7), 6.87 (bs, 1H, H_7), 4.60 (d, J = 6.0 Hz, 2H, H_{18}), 4.43 (q, J = 7.2 Hz, 1H, H_5), 3.76 – 3.64 (m, 1H, H_9), 3.62 (s, 2H, H_{16}), 3.35 (s, 2H, H_{13}), 2.55 – 2.44 (m, 2H, H_{12}), 2.42 (dd, J = 15.3, 5.8 Hz, 1H, H_6), 2.35 (dd, J = 15.3, 7.8 Hz, 1H, H_6), 2.14 – 1.94 (m, 2H, H_3), 1.67 – 1.53 (m, 1H, H_{11}), 1.54 – 1.46 (m, 1H, H_{11}), 1.48 – 1.42 (m, 2H, H_2), 1.40 (q, J = 3.2 Hz, 2H, H_{15}), 1.31 – 1.13 (m, 20H, 10 × CH_2), 1.06 (q, J = 3.5 Hz, 2H, H_{15}), 0.99 (d, J = 6.6 Hz, 3H, H_{10}), 0.84 (t, J = 6.9 Hz, 3H, H_1). ^{13}C NMR (151 MHz, Chloroform-d) δ 204.8 (C), 204.1 (C), 172.2 (C), 171.4 (C), 171.3 (C), 170.5 (C), 168.1 (C), 167.0 (C), 162.1 (C), 162.0 (C), 148.1 (C), 147.1 (C), 129.0 (CH), 118.3 (CH), 50.2 (CH_2), 50.0 (CH), 46.5 (CH_2), 43.6 (CH), 40.6 (CH_2), 40.5 (C), 39.1 (CH_2), 37.4 (CH_2), 35.2 (CH_2), 31.3 (CH_2), 29.7 (CH_2), 29.08 (CH_2), 29.07 (2 × CH_2), 29.03 (CH_2), 28.97 (CH_2), 28.87 (CH_2), 28.73 (CH_2), 28.67 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 20.6 (CH_3), 19.4 (CH_2), 14.0 (CH_3). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{58}N_7O_9S_2$, 832.3737; found, 832.3693. $[\alpha]_D^{20}$ +28.0 (*c* 0.8, DMSO).

*Synthesis of the pyridone **30a**:*



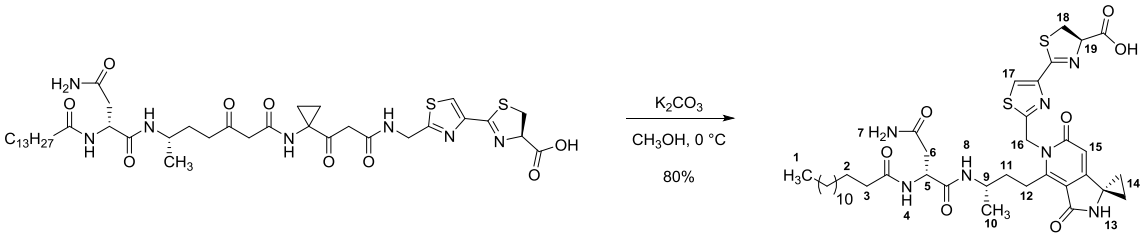
29a **30a**

Potassium carbonate (9.94 mg, 72.0 µmol, 3.00 equiv) was added to a solution of the linear precursor **29a** (20.0 mg, 24.0 µmol, 1 equiv) in methanol (1.5 mL) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C. The heterogeneous product mixture was filtered through a plug of propylsulfonic acid functionalized silica gel. The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing the product **30a** were collected, combined, and concentrated to provide the pyridone **30a** as a white solid (15.1 mg, 79%).

^{1}H NMR (500 MHz, DMSO-d_6–CD_3OD (3:1)) δ 8.19* (bs, 1H, H_{13}), 8.17 (s, 1H, H_{19}), 7.83* (app t, J = 7.7 Hz, 1H, H_4), 7.64* (app dd, J = 8.0, 5.2 Hz, 1H, H_8), 7.29* (bs, 1H, H_7), 6.79* (bs, 1H, H_7), 6.07 (s, 1H, H_{15}), 5.86 (app t, J = 8.4 Hz, 1H, H_{18}), 5.24 – 5.06 (m, 2H, H_{16}), 4.58 – 4.46 (m, 1H, H_5), 3.92 (app t, J = 10.3 Hz, 1H, H_{17}), 3.89 – 3.77 (m, 1H, H_9), 3.68 – 3.57 (m, 1H, H_{17}), 3.34 – 3.16 (m, 2H, H_{12}), 2.49 – 2.37 (m, 2H, H_6), 2.16 – 2.02 (m, 2H, H_3), 1.80 – 1.68 (m, 1H, H_3), 1.68 – 1.57 (m, 1H, H_3), 1.51 – 1.41 (m, 2H, H_2), 1.39 (q, J = 4.8, 3.9 Hz, 2H, H_{14}), 1.32 (app t, J = 3.5 Hz, 2H, H_{14}), 1.28 – 1.12 (m, 20H, 10 × CH_2), 1.05 (app dd, J = 9.0, 6.5 Hz, 3H, H_{10}), 0.83 (t, J = 6.9 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6–CD_3OD (3:1)) δ 172.7 (C), 172.1 (C), 171.1 (C), 170.8 (C), 167.2 (C), 164.1 (C), 163.1 (C), 162.1 (C), 160.0 (C), 153.6 (C), 149.7 (C), 127.4 (CH), 109.5 (C), 103.2 (CH), 76.7 (CH), 50.2 (CH), 45.1 (CH), 45.0 (CH_2), 40.1 (C), 39.0 (CH_2), 37.6 (CH_2), 35.6 (CH_2), 35.5 (CH_2), 31.7 (CH_2), 29.45 (CH_2), 29.43 (2 × CH_2), 29.41 (CH_2), 29.34 (CH_2), 29.25 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 25.6 (CH_2), 24.8 (CH_2), 22.5 (CH_2), 20.3 (CH_3), 15.4 (CH_2), 14.1 (CH_3). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{56}N_7O_7S_2$, 798.3683; found, 798.3623. $[\alpha]_D^{20}$ −5.0 (*c* 0.8, DMSO).

*H-D exchange occurred slowly in solution.

*Synthesis of the pyridone **30b**:*



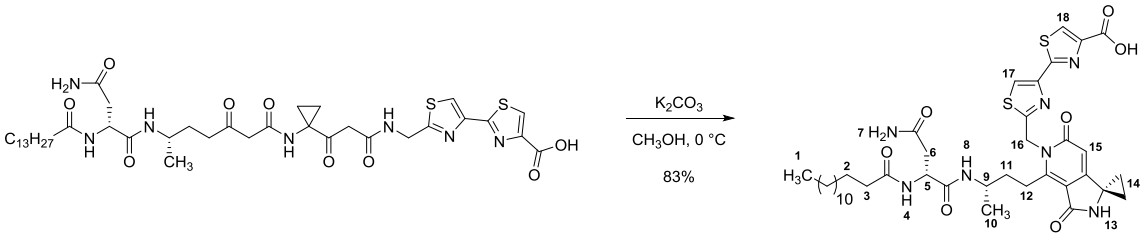
29b **30b**

Potassium carbonate (4.97 mg, 36.0 µmol, 3.00 equiv) was added to a solution of the linear precursor **29b** (10.0 mg, 12.0 µmol, 1 equiv) in methanol (800 µL) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C. The heterogeneous product mixture was filtered through a plug of propylsulfonic acid functionalized silica gel. The filter cake was washed with methanol (8.0 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing the product **30b** were collected, combined, and concentrated to provide the pyridone **30b** as a white solid (7.7 mg, 80%).

^{1}H NMR (400 MHz, DMSO-d_6–CD_3OD (3:1)) δ 8.17 (bs, 2H, H_{13}*, H_{17}), 7.89* (d, J = 7.9 Hz, 1H, H_4), 7.65* (d, J = 7.9 Hz, 1H, H_8), 7.30* (bs, 1H, H_7), 6.77* (bs, 1H, H_7), 6.11 (s, 1H, H_{15}), 5.70 – 5.39 (m, 2H, H_{16}), 5.24 – 4.98 (m, 1H, H_{19}), 4.53 (app q, J = 7.3 Hz, 1H, H_5), 3.97 – 3.77 (m, 1H, H_9), 3.64 – 3.45 (m, 2H, H_{18}), 3.44 – 3.26 (m, 2H, H_{12}), 2.53 (dd, J = 15.5, 6.3 Hz, 1H, H_6), 2.43 (dd, J = 15.2, 7.4 Hz, 1H, H_6), 2.23 – 1.98 (m, 2H, H_3), 1.82 – 1.66 (m, 1H, H_{11}), 1.66 – 1.55 (m, 1H, H_{11}), 1.50 – 1.41 (m, 2H, H_2), 1.39 (q, J = 5.1, 4.0 Hz, 2H, H_{14}), 1.32 (q, J = 6.1, 5.0 Hz, 2H, H_{14}), 1.28 – 1.11 (m, 20H, 10 × CH_2), 1.07 (d, J = 6.5 Hz, 3H, H_{10}), 0.82 (t, J = 6.7 Hz, 3H, H_1). ^{13}C NMR (DMSO-d_6–CD_3OD (3:1)) δ 173.2 (C), 172.5 (C), 172.4 (C), 171.1 (C), 167.3 (C), 166.1 (C), 163.4 (C), 162.5 (C), 160.5 (C), 153.7 (C), 147.8 (C), 123.7 (CH), 110.2 (C), 103.6 (CH), 79.6 (CH), 50.5 (CH), 45.2 (CH), 44.8 (CH_2), 40.1 (C), 37.5 (CH_2), 35.9 (CH_2), 35.8 (CH_2), 35.2 (CH_2), 31.9 (CH_2), 29.61 (2 × CH_2), 29.58 (CH_2), 29.56 (CH_2), 29.50 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 25.7 (CH_2), 24.7 (CH_2), 22.6 (CH_2), 20.5 (CH_3), 15.5 (CH_2), 14.1 (CH_3). HRMS-CI (m/z): [M + H]$^+$ calcd for $C_{39}H_{56}N_7O_7S_2$, 798.3683; found, 798.3625. $[\alpha]_D^{20}$ +39.0 (*c* 0.8, DMSO).

*H-D exchange occurred slowly in solution.

*Synthesis of precolibactin C (**6**):*



29c precolibactin C (**6**)

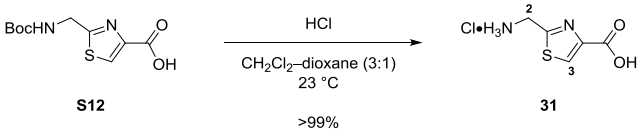
Potassium carbonate (7.48 mg, 54.0 µmol, 3.00 equiv) was added to a solution of the linear precursor **29c** (15.0 mg, 18.0 µmol, 1 equiv) in methanol (1.0 mL) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C. The heterogeneous product mixture was filtered through a plug of propylsulfonic acid functionalized silica gel (1.0 × 0.5 cm). The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing the product **6** were collected, combined, and concentrated to provide precolibactin C (**6**) as a white solid (11.9 mg, 83%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.51 (s, 1H, H_{13}), 8.24 (s, 1H, H_{17}), 8.18 (s, 1H, H_{18}), 7.99 (d, J = 7.8 Hz, 1H, H_4), 7.72 (d, J = 8.0 Hz, 1H, H_8), 7.29 (bs, 1H, H_7), 6.82 (bs, 1H, H_7), 6.16 (s, 1H, H_{15}), 5.57 (q, J = 15.9 Hz, 2H, H_{16}), 4.48 (q, J = 7.2 Hz, 1H, H_5), 3.88 (dq, J = 13.8, 6.8 Hz, 1H, H_9), 3.50 – 3.31 (m, 2H, H_{12}), 2.47 (dd, J = 15.2, 6.2 Hz, 1H, H_6), 2.37 (dd, J = 15.0, 7.4 Hz, 1H, H_6), 2.11 – 1.98 (m, 2H, H_3), 1.78 – 1.64 (m, 2H, H_{11}), 1.44 – 1.35 (m, 4H, H_2, H_{14}), 1.36 – 1.31 (m, 2H, H_{14}), 1.26 – 1.11 (m, 20H, 10 × CH_2), 1.06 (d, J = 6.5 Hz, 3H, H_{10}), 0.83 (t, J = 7.0 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.2 (C), 171.6 (C), 170.5 (C), 166.7 (C), 166.5 (C), 164.0 (C), 163.2 (C), 161.8 (C), 161.0 (C), 159.8 (C), 153.1 (C), 147.2 (C), 126.1 (CH), 118.6 (CH), 109.6 (C), 103.3 (CH), 50.1 (CH), 44.7 (CH), 44.4 (CH_2), 39.7 (C), 37.4 (CH_2), 35.4 (CH_2), 35.2 (CH_2), 31.3 (CH_2), 29.07 (CH_2), 29.04 (2 × CH_2), 29.02 (CH_2), 28.96 (CH_2), 28.87 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 25.2 (CH_2), 24.1 (CH_2), 22.1 (CH_2), 20.3 (CH_3), 15.2 (CH_2), 14.0 (CH_3).

^{1}H NMR (500 MHz, CD_3OD) δ 8.28 (s, 1H, H_{17}), 8.16 (s, 1H, H_{18}), 6.16 (s, 1H, H_{15}), 5.75 (d, J = 15.7 Hz, 1H, H_{16}), 5.70 (d, J = 15.7 Hz, 1H, H_{16}), 4.73 (t, J = 6.5 Hz, 1H, H_5), 4.12 – 3.99 (m, 1H, H_9), 3.71 – 3.57 (m, 1H, H_{12}), 3.55 – 3.42 (m, 1H, H_{12}), 2.71 (t, J = 6.1 Hz, 2H, H_6), 2.23 (t, J = 7.4 Hz, 2H, H_3), 1.98 – 1.86 (m, 1H, H_{11}), 1.81 – 1.68 (m, 1H, H_{11}), 1.66 – 1.53 (m, 2H, H_2), 1.54 – 1.50 (m, 2H, H_{14}), 1.47 – 1.38 (m, 2H, H_{14}), 1.32 – 1.20 (m, 20H, 10 × CH_2), 1.18 (d, J = 6.6 Hz, 3H, H_{10}), 0.89 (t, J = 7.0 Hz, 3H, H_1). ^{13}C NMR (126 MHz, Methanol-d4) δ 176.3 (C), 175.0 (C), 172.9 (C), 169.3 (C), 167.2 (C), 164.9 (C), 163.6 (C), 163.4 (C), 162.5 (C), 155.2 (C), 149.3 (C), 148.1 (C), 126.9 (CH), 120.2 (CH), 112.2 (C), 104.2 (CH), 52.0 (CH), 47.0 (CH), 46.0 (CH_2), 41.5 (C), 38.0 (CH_2), 37.0 (CH_2), 36.8 (CH_2), 33.1 (CH_2), 30.80 (CH_2), 30.77 (CH_2), 30.76 (2 × CH_2), 30.65 (CH_2), 30.51 (CH_2), 30.48 (CH_2), 30.3 (CH_2), 26.8 (CH_2), 26.0 (CH_2), 23.7 (CH_2), 20.8 (CH_3), 16.3 (CH_2), 14.4 (CH_3).

HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{54}N_7O_7S_2$, 796.3526; found, 796.3466. $[\alpha]_D^{20}$ −21.0 (*c* 0.8, DMSO).

*Synthesis of the amine **31**:*



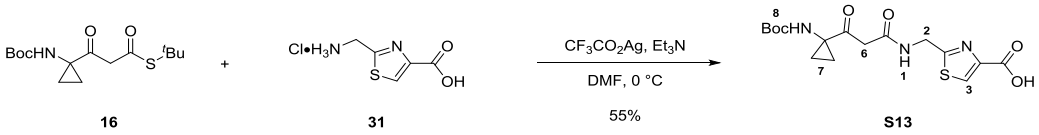
S12 >99% **31**

A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 6.0 mL, 24.0 mmol, 13.3 equiv) was added dropwise via syringe pump over 20 min to a solution of the thiazole **S12** (467 mg, 1.81 mmol, 1 equiv) in dichloromethane (18.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **31** as a white solid (352 mg, >99%).

The product **31** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (bs, 3H), 8.52 (s, 1H, H_3), 4.44 (q, J = 5.8 Hz, 2H, H_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.8 (C), 161.8 (C), 146.8 (C), 130.6 (CH), 39.5 (CH_2).

*Synthesis of the β-ketoamide **S13**:*

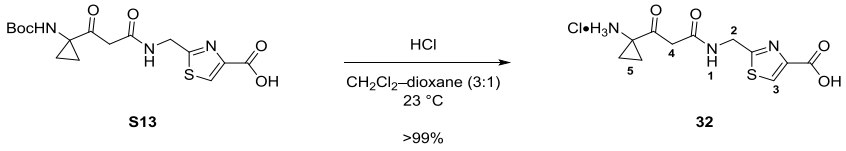


16 **31** **S13**

Three equal portions of silver trifluoroacetate (72.6 mg, 328 μmol, 0.40 equiv) were added over 1 h to a solution of triethylamine (459 μL, 3.29 mmol, 4.00 equiv), the β-ketothioester **16** (311 mg, 986 μmol, 1.20 equiv), and the amine **31** (160 mg, 822 μmol, 1 equiv) in *N*,*N*-dimethylformamide (9.0 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The product mixture was directly applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated. The residue obtained was further purified by automated flash-column chromatography (eluting with 2% acetic acid–dichloromethane initially, grading to 2% acetic acid–10% dichloromethane–methanol, linear gradient). The fractions containing the product **S13** were collected, combined, and concentrated to provide the β-ketoamide **S13** as a white solid (173 mg, 55%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 12.95 (bs, 1H), 8.93 (t, J = 6.0 Hz, 1H, H_1), 8.36 (s, 1H, H_3), 7.76 (s, 1H), 4.54 (d, J = 6.0 Hz, 2H, H_2), 3.55 (s, 2H, H_6), 1.40 (s, 9H, H_8), 1.38 – 1.33 (m, 2H, H_7), 1.07 (q, J = 4.2 Hz, 2H, H_7). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.7 (C), 169.8 (C), 166.8 (C), 162.0 (C), 156.0 (C), 146.6 (C), 128.9 (CH), 78.6 (C), 46.1 (CH_2), 41.2 (C), 40.5 (CH_2), 28.2 (CH_3), 19.5 (CH_2). IR (ATR-FTIR), cm^{-1}: 3316 (br), 2977 (w), 1701 (s), 1684 (s), 1509 (s), 1249 (s), 1161 (s), 1069 (s), 751 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{16}H_{22}N_3O_6S$, 384.1229; found, 384.1224.

Synthesis of the amine ***32:***

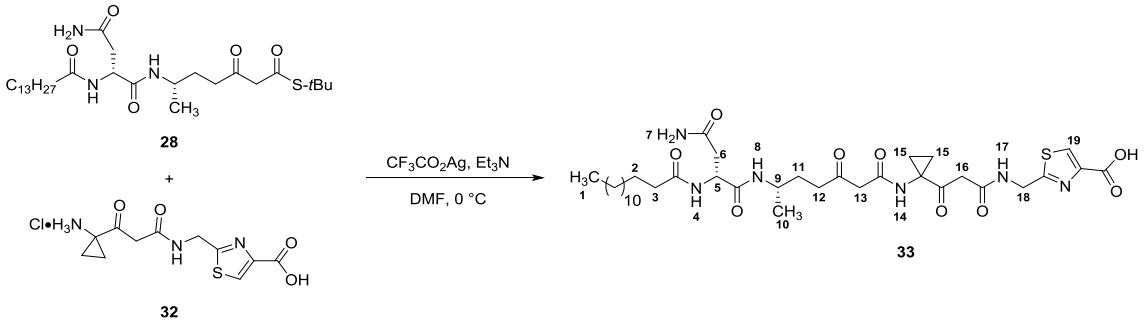


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 2.0 mL, 8.0 mmol, 55.9 equiv) was added dropwise via syringe pump over 20 min to a solution of the β-ketoamide **S13** (55.0 mg, 143 µmol, 1 equiv) in dichloromethane (6.0 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The reaction mixture was concentrated to provide the amine **32** as a white solid (45.9 mg, >99%).

The product **32** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.13 (t, J = 6.0 Hz, 1H, H_1), 8.79 (bs, 3H), 8.38 (s, 1H, H_3), 4.57 (d, J = 5.9 Hz, 2H, H_2), 3.38 (s, 2H, H_4), 1.83 – 1.68 (m, 2H, H_5), 1.57 – 1.26 (m, 2H, H_5). ^{13}C NMR (151 MHz, DMSO-d_6) δ 199.40 (C), 169.29 (C), 165.86 (C), 161.99 (C), 146.67 (C), 128.94 (CH), 42.35 (CH_2), 42.01 (C), 40.51 (CH_2), 13.10 (CH_2).

*Synthesis of the linear precursor **33**:*

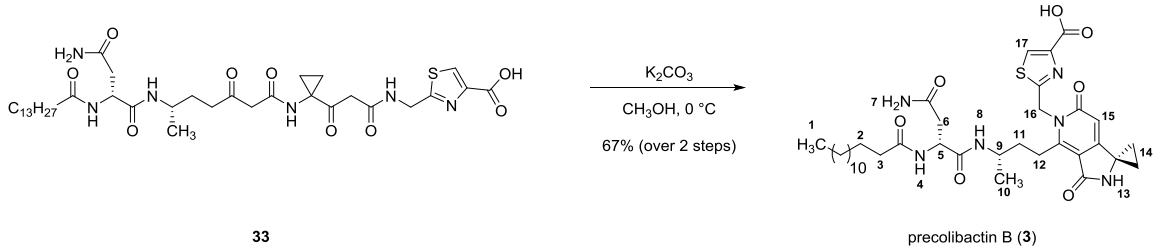


Silver trifluoroacetate (33.4 mg, 151 µmol, 2.00 equiv) was added to a solution of triethylamine (42.1 µL, 302 µmol, 4.00 equiv), the β-ketothioester **28** (42.0 mg, 75.6 µmol, 1 equiv), and the amine **32** (24.2 mg, 75.6 µmol, 1.00 equiv) in *N*,*N*-dimethylformamide (1.5 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was diluted with aqueous citric acid solution (5%, 12 mL). The resulting precipitate was isolated by filtration and was dried in vacuo to provide **33**.

The product **33** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (t, J = 6.0 Hz, 1H, H_{17}), 8.83 (s, 1H, H_{14}), 8.36 (s, 1H, H_{19}), 7.89 (d, J = 8.0 Hz, 1H, H_4), 7.52 (d, J = 8.6 Hz, 1H, H_8), 7.29 – 7.18 (m, 1H, H_7), 6.91 – 6.78 (m, 1H, H_7), 4.55 (d, J = 5.9 Hz, 2H, H_{18}), 4.49 – 4.36 (m, 1H, H_5), 3.78 – 3.62 (m, 1H, H_9), 3.59 (s, 2H, H_{16}), 3.34 (s, 2H, H_1), 2.56 – 2.45 (m, 2H, H_{12}), 2.48 – 2.37 (m, 1H, H_6), 2.39 – 2.27 (m, 1H, H_6), 2.08 (q, J = 7.7 Hz, 2H, H_3), 1.64 – 1.53 (m, 1H, H_{11}), 1.51 – 1.43 (m, 3H, $H_{11,}$ H_2), 1.39 (q, J = 3.4 Hz, 2H, H_{15}), 1.29 – 1.18 (m, 20H, 10 × CH_2), 1.05 (q, J = 3.6 Hz, 2H, H_{15}), 1.04 – 0.93 (m, 3H, H_{10}), 0.85 (t, J = 7.0 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.8 (C), 204.1 (C), 172.2 (C), 171.4 (C), 171.3 (C), 170.5 (C), 168.1 (C), 166.9 (C), 162.0 (C), 146.6 (C), 128.9 (CH), 50.2 (CH_2), 49.9 (CH), 46.6 (CH_2), 43.6 (CH), 40.53 (CH_2), 40.50 (C), 39.1 (CH_2), 37.4 (CH_2), 35.2 (CH_2), 31.3 (CH_2), 29.7 (CH_2), 29.08 (CH_2), 29.07 (2 × CH_2), 29.03 (CH_2), 28.97 (CH_2), 28.87 (CH_2), 28.73 (CH_2), 28.67 (CH_2), 25.2 (CH_2), 22.1 (CH_2), 20.6 (CH_3), 19.4 (CH_2), 14.0 (CH_3).

*Synthesis of precolibactin B (**3**):*



33 precolibactin B (**3**)

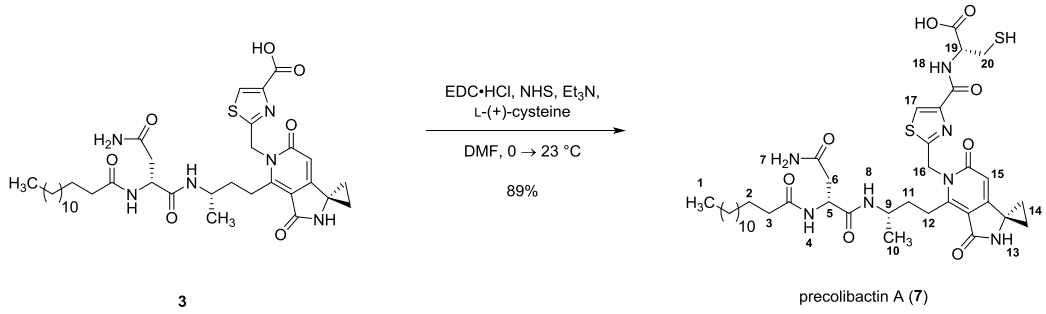
Potassium carbonate (31.3 mg, 227 µmol, 3.00 equiv) was added to a solution of the unpurified linear precursor **33** (nominally 56.6 mg, 75.6 µmol, 1 equiv) in methanol (3.5 mL) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C. The heterogeneous product mixture was filtered through a pad of propylsulfonic acid functionalized silica gel (1.0 × 0.5 cm). The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing the product **3** were collected, combined, and concentrated to provide precolibactin B (**3**) as a white solid (36.0 mg, 67% over 2 steps).

^{1}H NMR (600 MHz, DMSO-d_6) δ 13.09 (bs, 1H), 8.47 (s, 1H, H_{13}), 8.39 (s, 1H, H_{17}), 7.83 (d, J = 8.1 Hz, 1H, H_4), 7.72 (d, J = 8.3 Hz, 1H, H_8), 7.25 (bs, 1H, H_7), 6.83 (bs, 1H, H_7), 6.16 (s, 1H, H_{15}), 5.62 (d, J = 15.7 Hz, 1H, H_{16}), 5.51 (d, J = 15.8 Hz, 1H, H_{16}), 4.52 (q, J = 7.2 Hz, 1H, H_5), 3.93 – 3.79 (m, 1H, H_9), 3.44 – 3.35 (m, 1H, H_{12}), 3.33 – 3.22 (m, 1H, H_{12}), 2.50 – 2.45 (m, 1H, H_6), 2.41 (dd, J = 15.2, 7.4 Hz, 1H, H_6), 2.07 – 1.97 (m, 2H, H_3), 1.77 – 1.61 (m, 1H, H_{11}), 1.62 – 1.51 (m, 1H, H_{11}), 1.48 – 1.39 (m, 2H, H_2), 1.39 – 1.30 (m, 4H, H_{14}), 1.27 – 1.12 (m, 20H, 10 × CH_2), 1.03 (d, J = 6.6 Hz, 3H, H_{10}), 0.84 (t, J = 7.0 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.2 (C), 171.7 (C), 170.6 (C), 166.7 (C), 165.7 (C), 162.0 (C), 161.9 (C), 159.9 (C), 153.2 (C), 146.3 (C), 130.1 (CH), 109.7 (C), 103.3 (CH), 50.0 (CH), 44.6 (CH), 44.3 (CH_2), 39.9 (C), 37.3 (CH_2), 35.5 (CH_2), 35.3 (CH_2), 31.4 (CH_2), 29.4 (CH_2), 29.13 (2 × CH_2), 29.11 (CH_2), 29.04 (CH_2), 28.9 (CH_2), 28.81 (CH_2), 28.7 (CH_2), 25.2 (CH_2), 24.2 (CH_2), 22.2 (CH_2), 20.6 (CH_3), 15.3 (CH_2), 14.1 (CH_3).

^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (s, 1H, H_{17}), 6.17 (s, 1H, H_{15}), 5.82 (d, J = 15.3 Hz, 1H, H_{16}), 5.68 (d, J = 15.2 Hz, 1H, H_{16}), 4.82 (t, J = 6.5 Hz, 1H, H_5), 4.20 – 4.03 (m, 1H, H_9), 3.71 – 3.53 (m, 1H, H_{12}), 3.52 – 3.42 (m, 1H, H_{12}), 2.81 – 2.72 (m, 2H, H_6), 2.30 – 2.16 (m, 2H, H_3), 1.99 – 1.82 (m, 1H, H_{11}), 1.62 – 1.54 (m, 3H, $H_{11,}$ H_2), 1.56 – 1.47 (m, 2H, H_{14}), 1.45 – 1.35 (m, 2H, H_{14}), 1.34 – 1.19 (m, 20H, 10 × CH_2), 1.16 (d, J = 6.6 Hz, 3H, H_{10}), 0.90 (t, J = 6.9 Hz, 3H, H_1). ^{13}C NMR (126 MHz, CD_3OD) δ 176.4 (C), 175.2 (C), 172.9 (C), 169.2 (C), 166.4 (C), 166.0 (C), 164.9 (C), 162.4 (C), 155.2 (C), 151.5 (C), 128.9 (CH), 112.2 (C), 104.1 (CH), 52.3 (CH), 46.7 (CH), 46.0 (CH_2), 41.4 (C), 37.9 (CH_2), 37.0 (CH_2), 36.8 (CH_2), 33.0 (CH_2), 30.74 (CH_2), 30.71 (3 × CH_2), 30.6 (CH_2), 30.4 (2 × CH_2), 30.3 (CH_2), 26.8 (CH_2), 26.0 (CH_2), 23.7 (CH_2), 21.1 (CH_3), 16.3 (CH_2), 14.4 (CH_3).

HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{36}H_{53}N_6O_7S$, 713.3696; found, 713.3689. $[\alpha]_D^{20}$ −15.0 (*c* 0.7, CH_3OH).

Synthesis of precolibactin A (7):



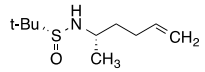
3 precolibactin A (**7**)

N-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrogen chloride (EDC•HCl, 2.6 mg, 13.5 µmol, 1.20 equiv) was added to a degassed solution of the pyridone **3** (8.00 mg, 11.2 µmol, 1 equiv) and *N*-hydroxysuccinimide (NHS, 1.81 mg, 15.7 µmmol, 1.40 equiv) in *N*,*N*-dimethylformamide (300 µL) at 0 °C. After 30 min the reaction mixture was warmed to 23 °C and stirred for 8 h. L-cysteine (2.72 mg, 22.4 µmol, 2.00 equiv) and triethylamine (9.26 µL, 44.9 µmol, 4.00 equiv) were added to the reaction mixture. The reaction mixture was stirred for a further 14 h at 23 °C. The product mixture was filtered through a plug of propylsulfonic acid functionalized silica gel (1.0 × 0.5 cm) under an atmosphere of dinitrogen. The filter cake was washed with methanol (5.0 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was diluted with water (10 mL) and the resulting precipitate was isolated by filtration through a plug of propylsulfonic acid functionalized silica gel (2.0 × 1.0 cm) under a N_2 atmosphere. The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated to provide precolibactin A (**7**) as a white solid (8.2 mg, 89%).

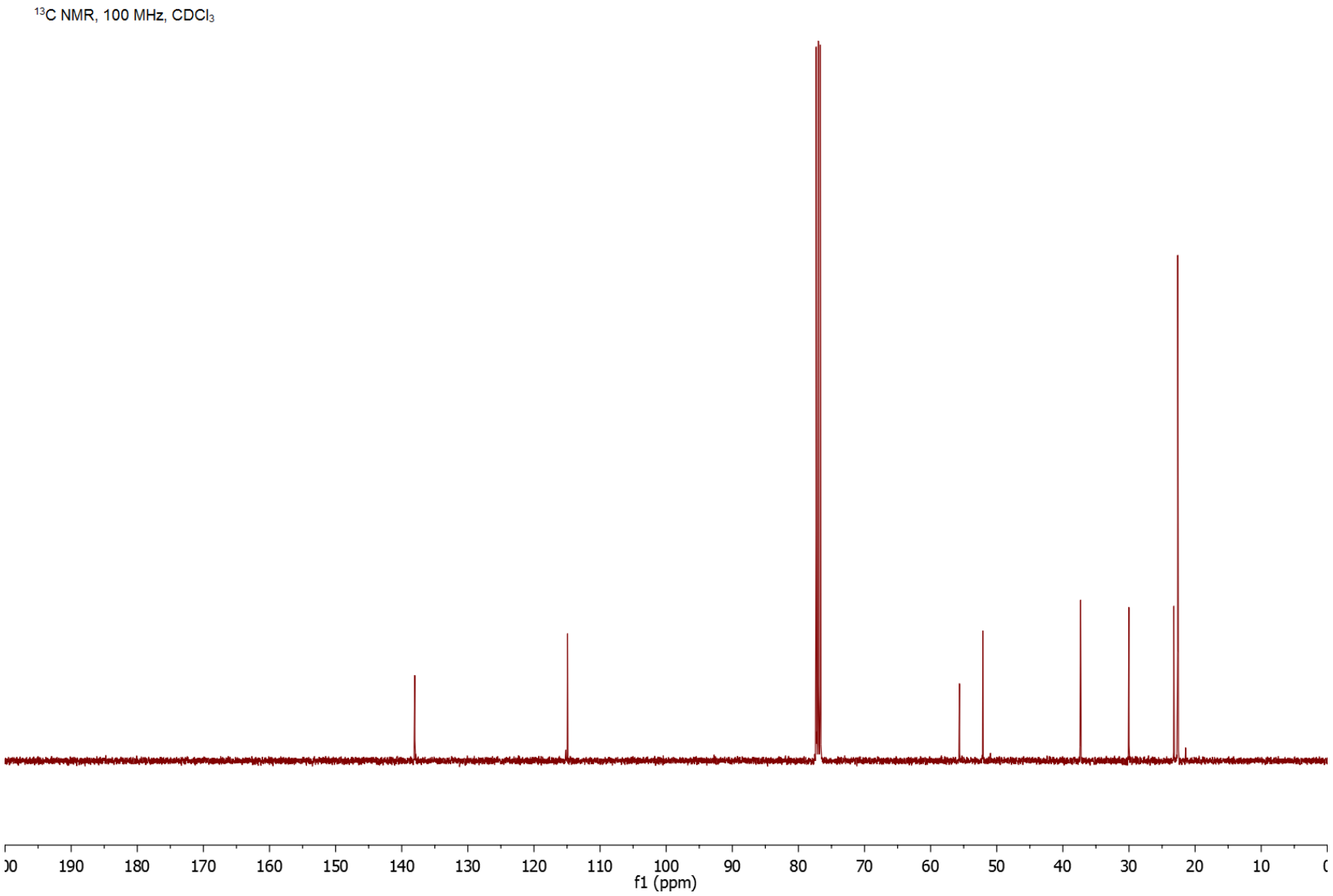
^{1}H NMR (600 MHz, CD_3OD) δ 8.23 (s, 1H, H_{17}), 6.16 (s, 1H, H_{15}), 5.70 (s, 2H, H_{16}), 4.79 (t, J = 5.2 Hz, 1H, H_{19}), 4.73 (t, J = 6.5 Hz, 1H, H_5), 4.10 – 4.02 (m, 1H, H_9), 3.70 – 3.63 (m, 1H, H_{12}), 3.48 – 3.41 (m, 1H, H_{12}), 3.11 (d, J = 5.3 Hz, 2H, H_{20}), 2.70 (app t, J = 6.2 Hz, 2H, H_6), 2.23 (t, J = 7.4 Hz, 2H, H_3), 1.99 – 1.90 (m, 1H, H_{11}), 1.77 – 1.67 (m, 1H, H_{11}), 1.62 – 1.54 (m, 2H, H_2), 1.54 – 1.49 (m, 2H, H_{14}), 1.44 – 1.38 (m, 2H, H_{14}), 1.36 – 1.20 (m, 20H, 10 × CH_2), 1.21 (d, J = 6.9 Hz, 3H, H_{10}), 0.89 (t, J = 7.1 Hz, 3H, H_1). ^{13}C NMR (151 MHz, CD_3OD) δ 176.3 (C), 175.1 (C), 172.83 (C), 172.81 (C), 169.2 (C), 166.8 (C), 164.9 (C), 162.7 (C), 162.6 (C), 155.2 (C), 149.6 (C), 127.2 (CH), 112.2 (C), 104.2 (CH), 55.6 (CH), 51.9 (CH), 46.9 (CH), 46.0 (CH_2), 41.5 (C), 38.1 (CH_2), 37.0 (CH_2), 36.7 (CH_2), 33.1 (CH_2), 30.81 (CH_2), 30.79 (CH_2), 30.77 (2 × CH_2), 30.66 (CH_2), 30.51 (CH_2), 30.49 (CH_2), 30.3 (CH_2), 26.83 (CH_2), 26.79 (CH_2), 25.8 (CH_2), 23.8 (CH_2), 20.7 (CH_3), 16.3 (CH_2), 14.5 (CH_3). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{58}N_7O_8S_2$, 816.3788; found, 816.3787. $[\alpha]_D^{20}$ −1.0 (*c* 1.0, CH_3OH).

Catalog of Nuclear Magnetic Resonance Spectra

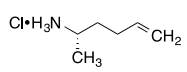
^{1}H NMR, 400 MHz, $CDCl_3$



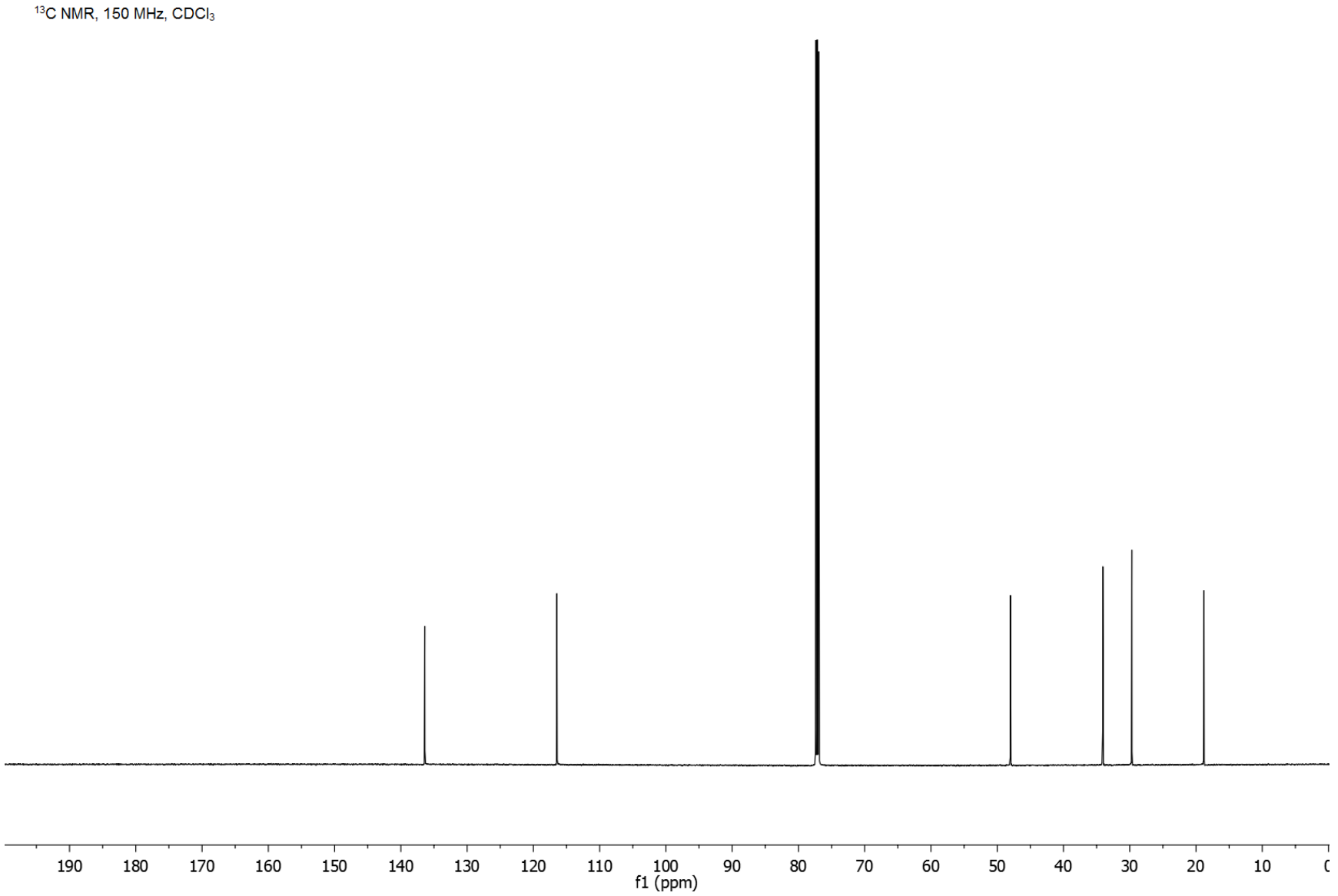
S2


^{13}C NMR, 100 MHz, $CDCl_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

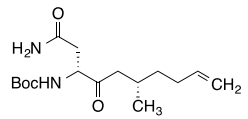
^{1}H NMR, 600 MHz, $CDCl_3$



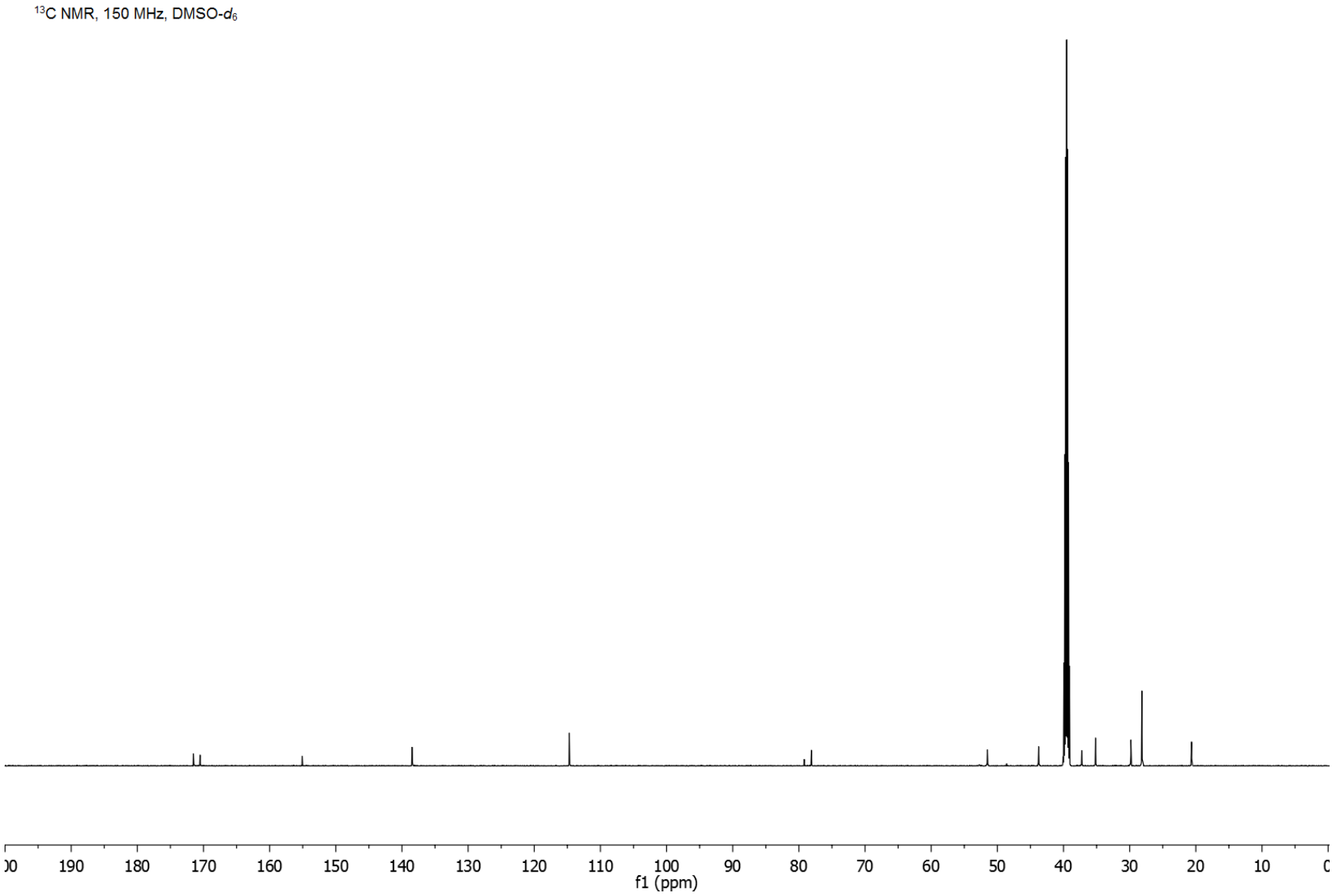
8


^{13}C NMR, 150 MHz, $CDCl_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

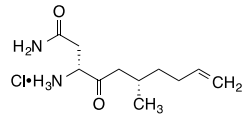
^{1}H NMR, 400 MHz, DMSO-d_6



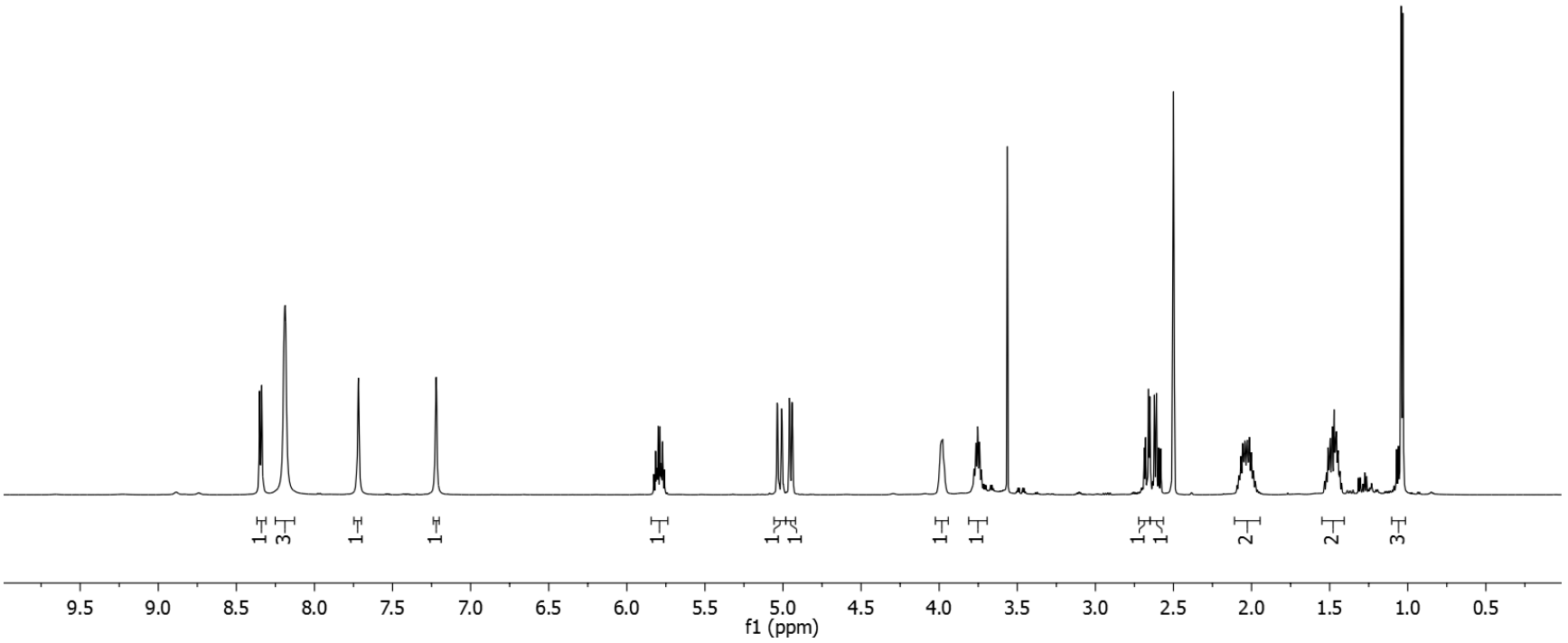
S3

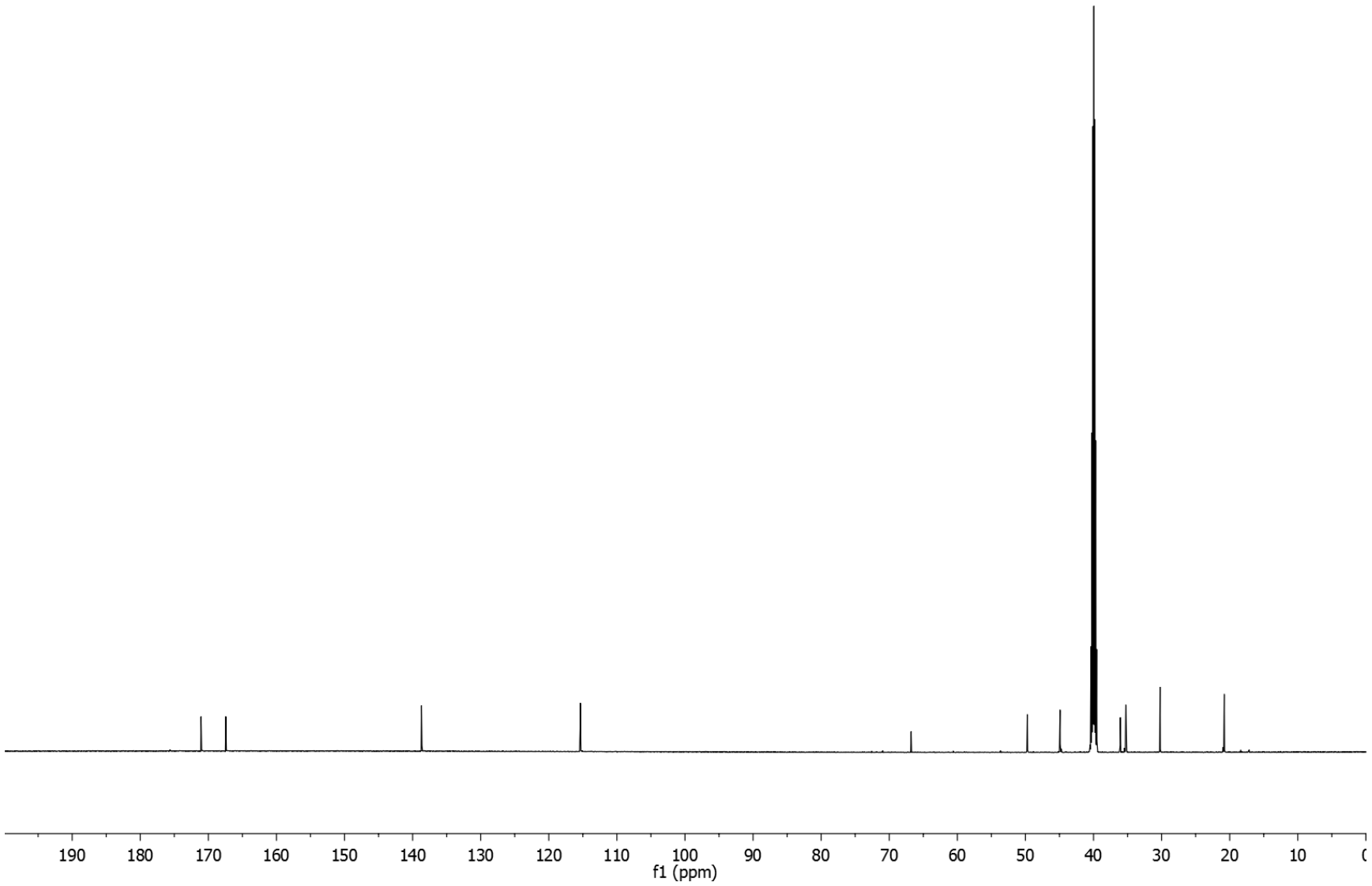

^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO

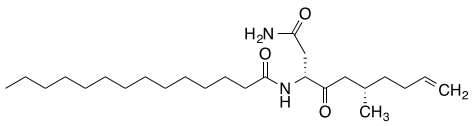


9

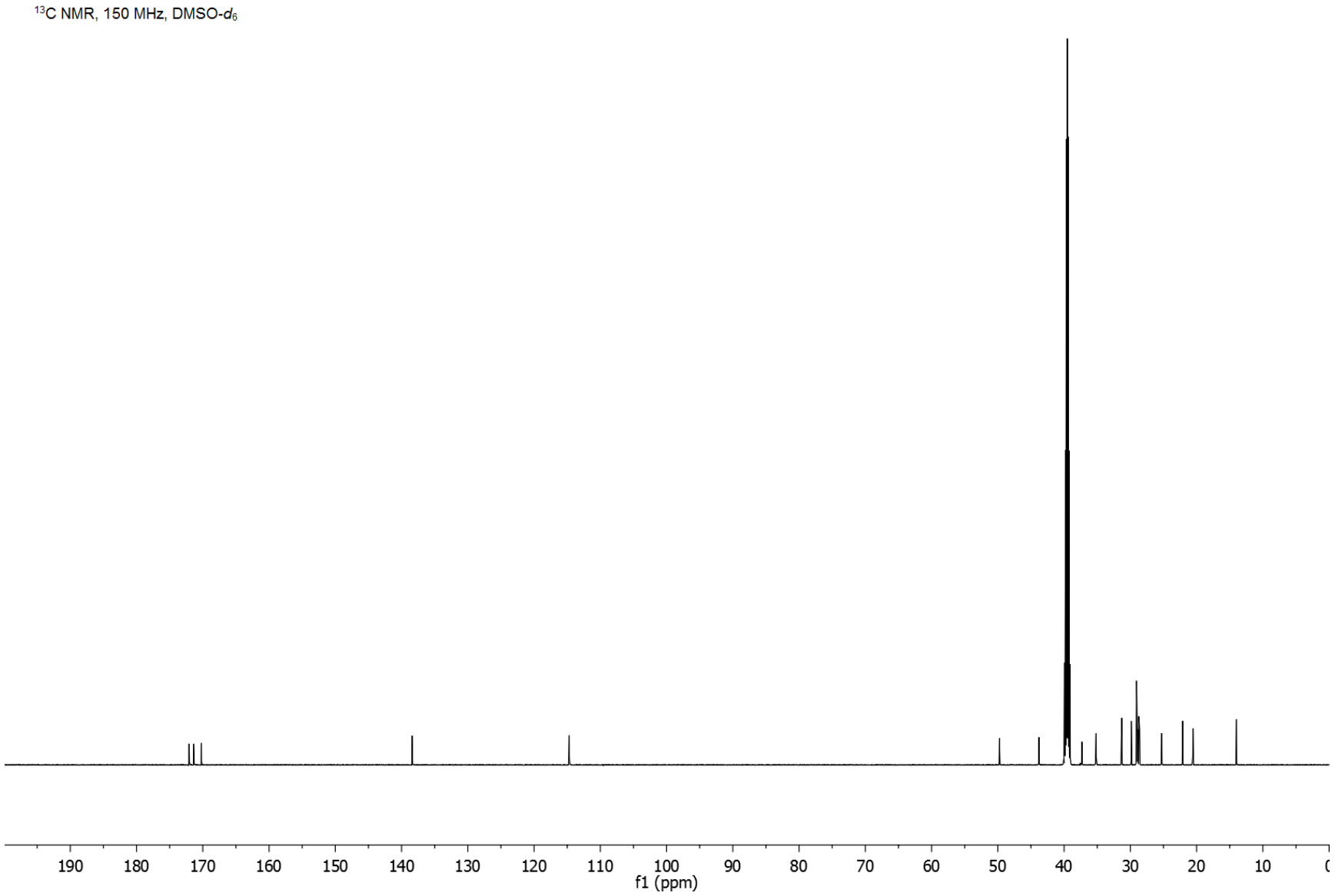



^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

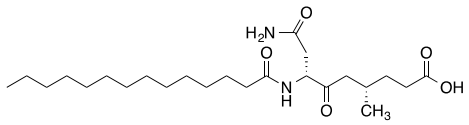
^{1}H NMR, 600 MHz, DMSO-d_6



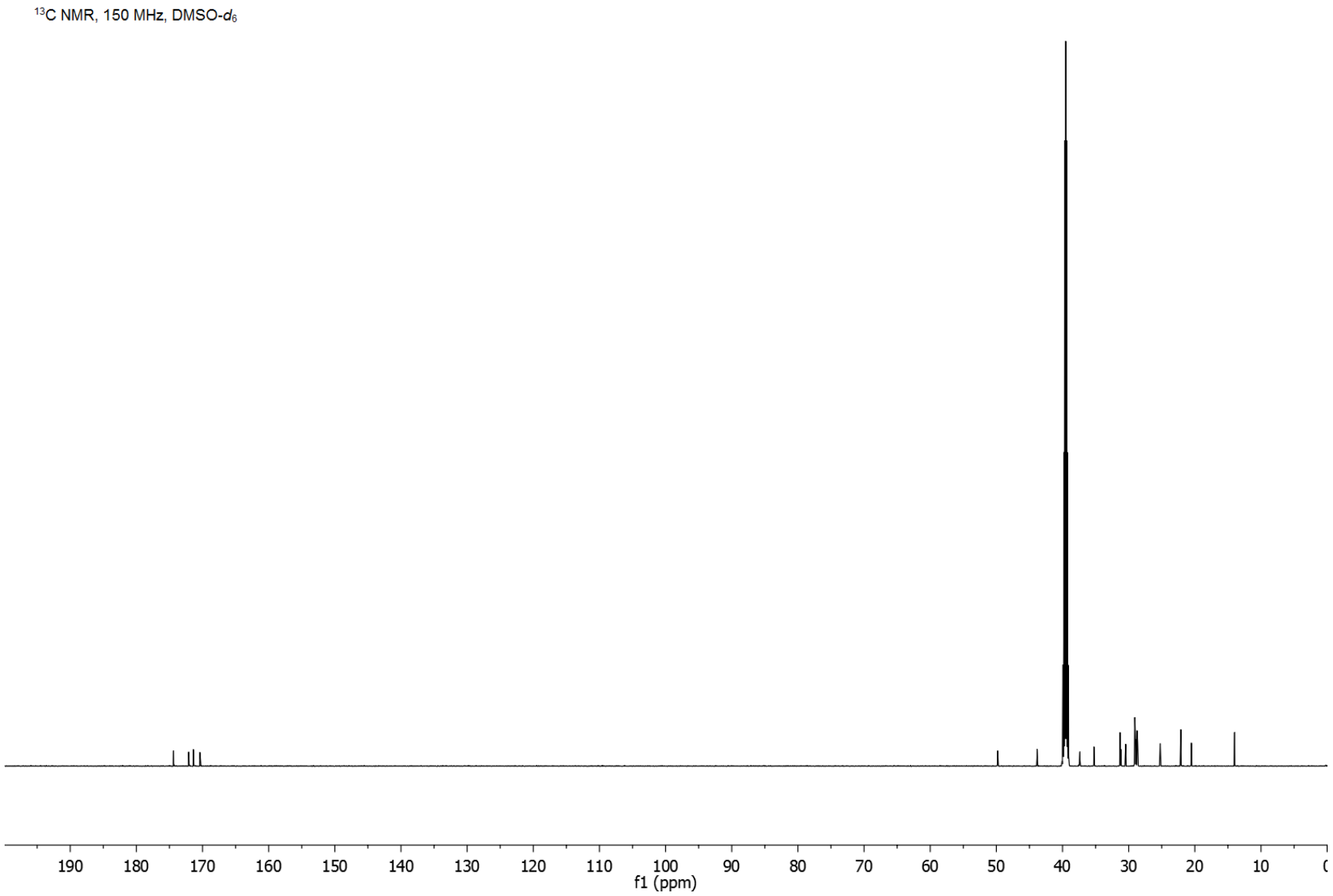
S4


^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

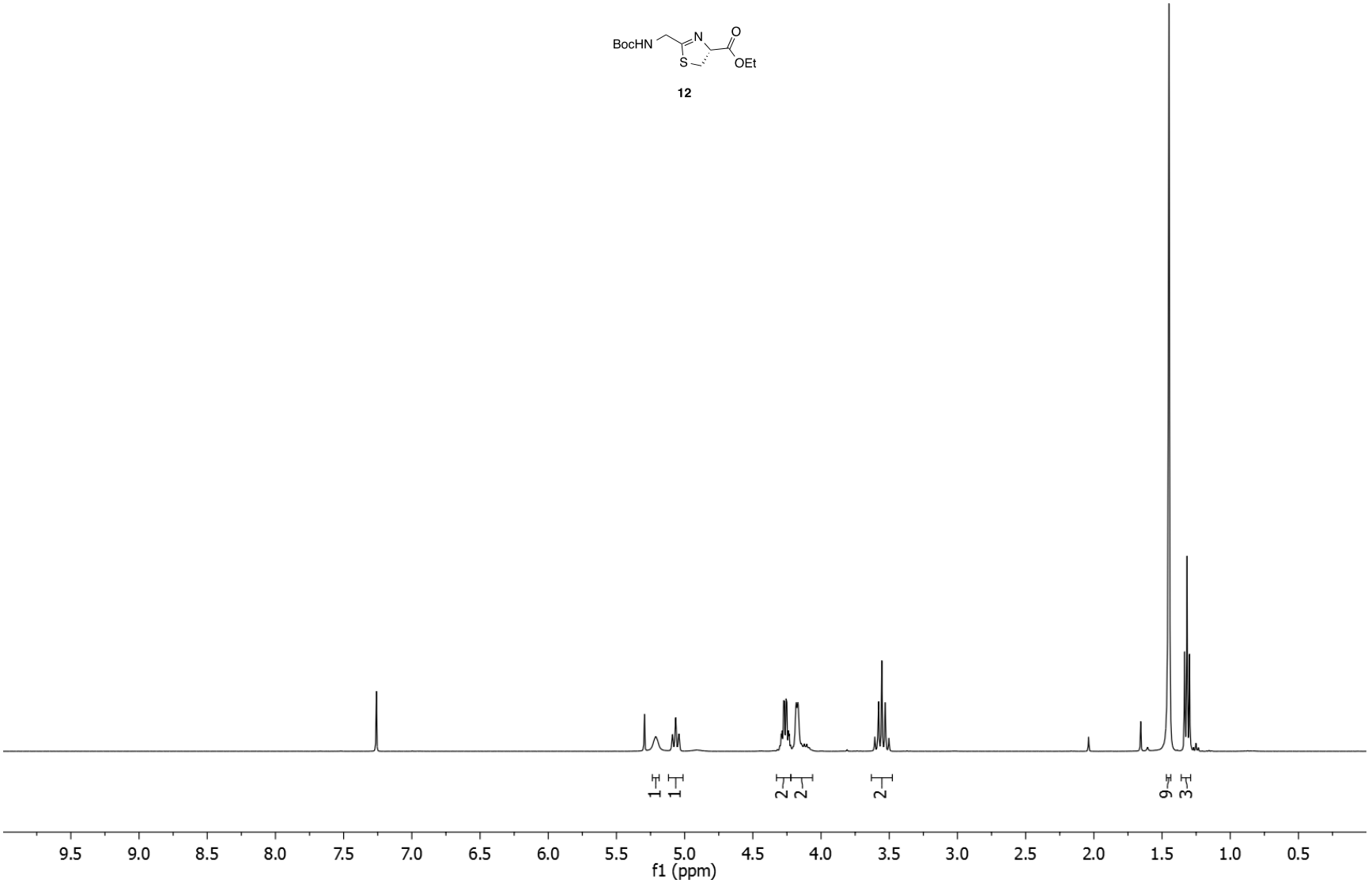
^{1}H NMR, 600 MHz, DMSO-d_6



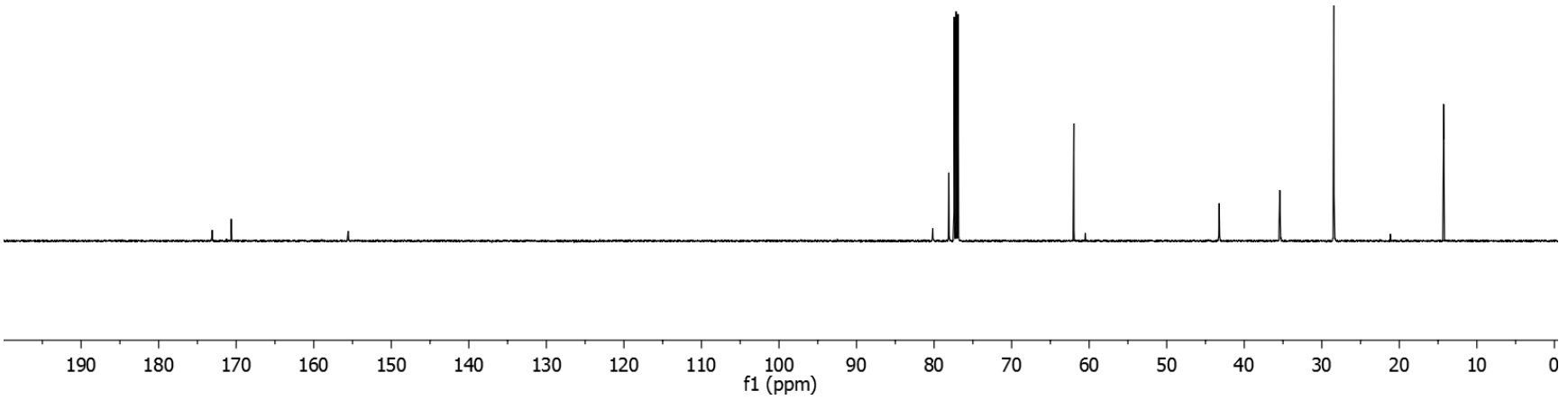
10


^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

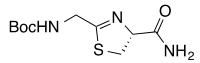
^{1}H NMR, 400 MHz, DMSO-d_6



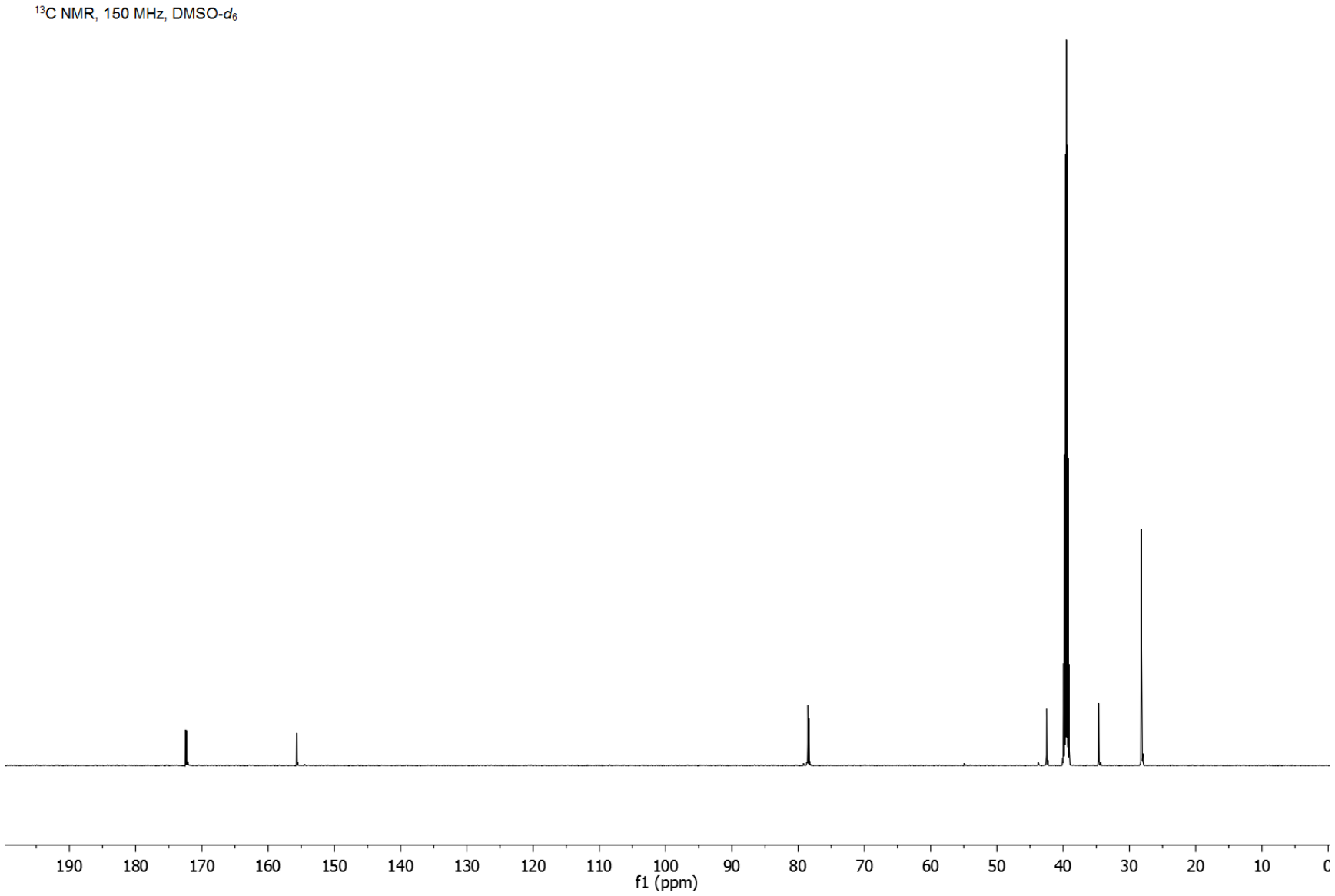
^{13}C NMR, 125 MHz, DMSO-d_6



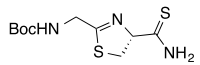
^{1}H NMR, 600 MHz, DMSO-d_6



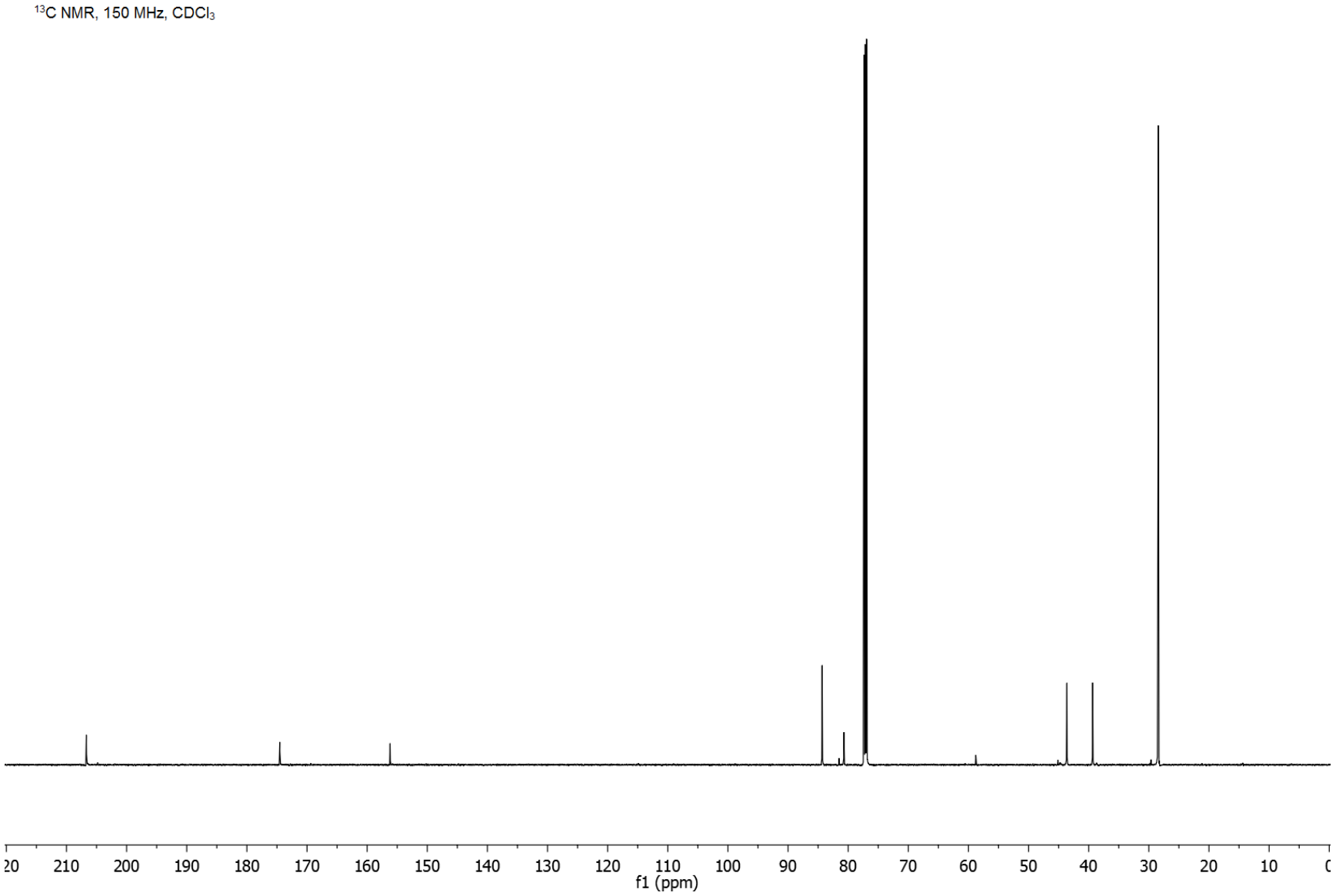
S5


^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

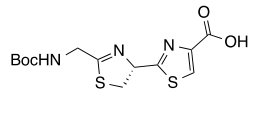
^{1}H NMR, 600 MHz, $CDCl_3$



13


^{13}C NMR, 150 MHz, $CDCl_3$
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

^{1}H NMR, 600 MHz, $CDCl_3$



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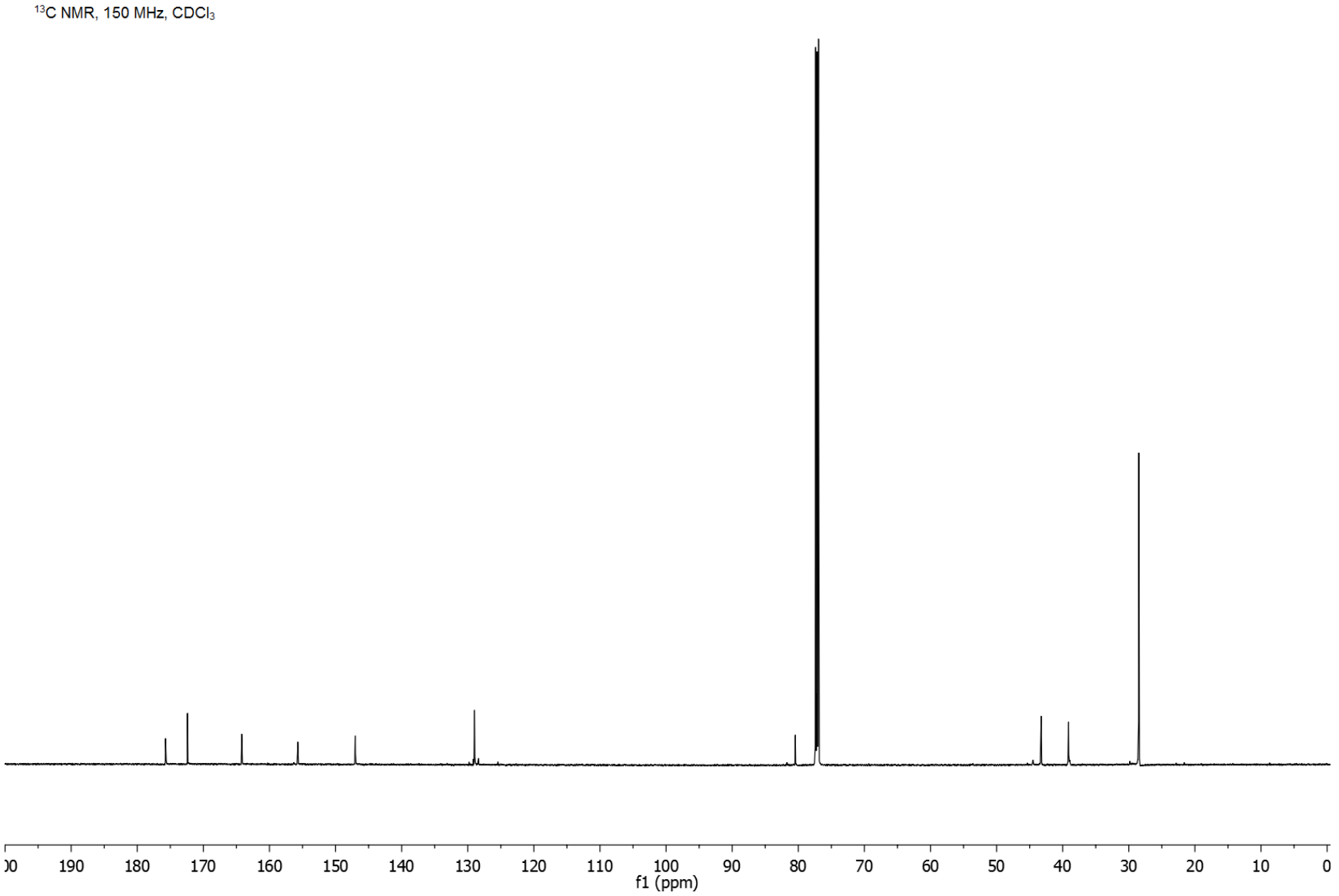
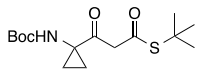
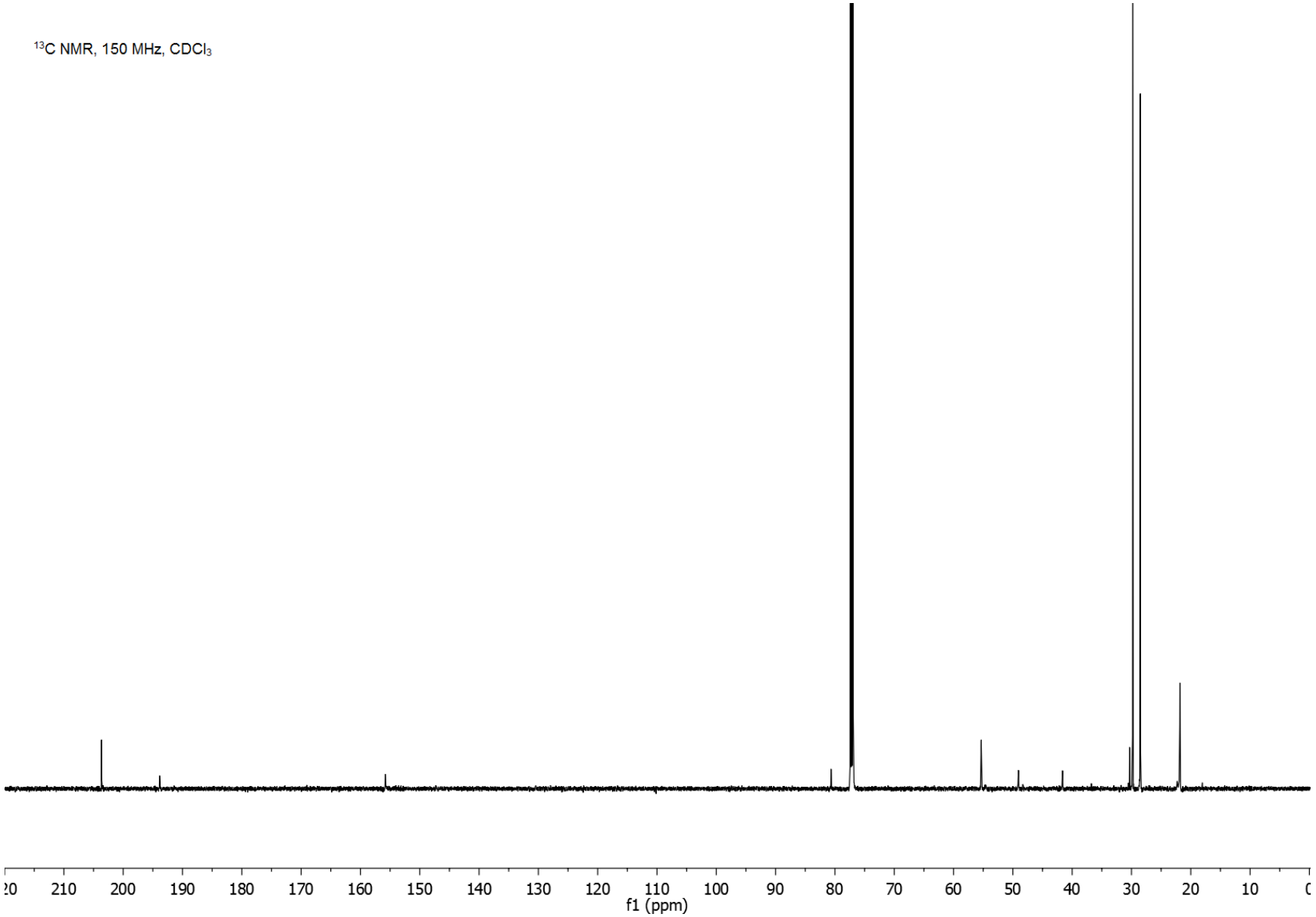

^{13}C NMR, 150 MHz, $CDCl_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

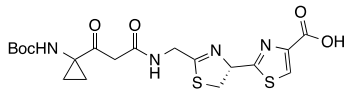
^{1}H NMR, 600 MHz, $CDCl_3$



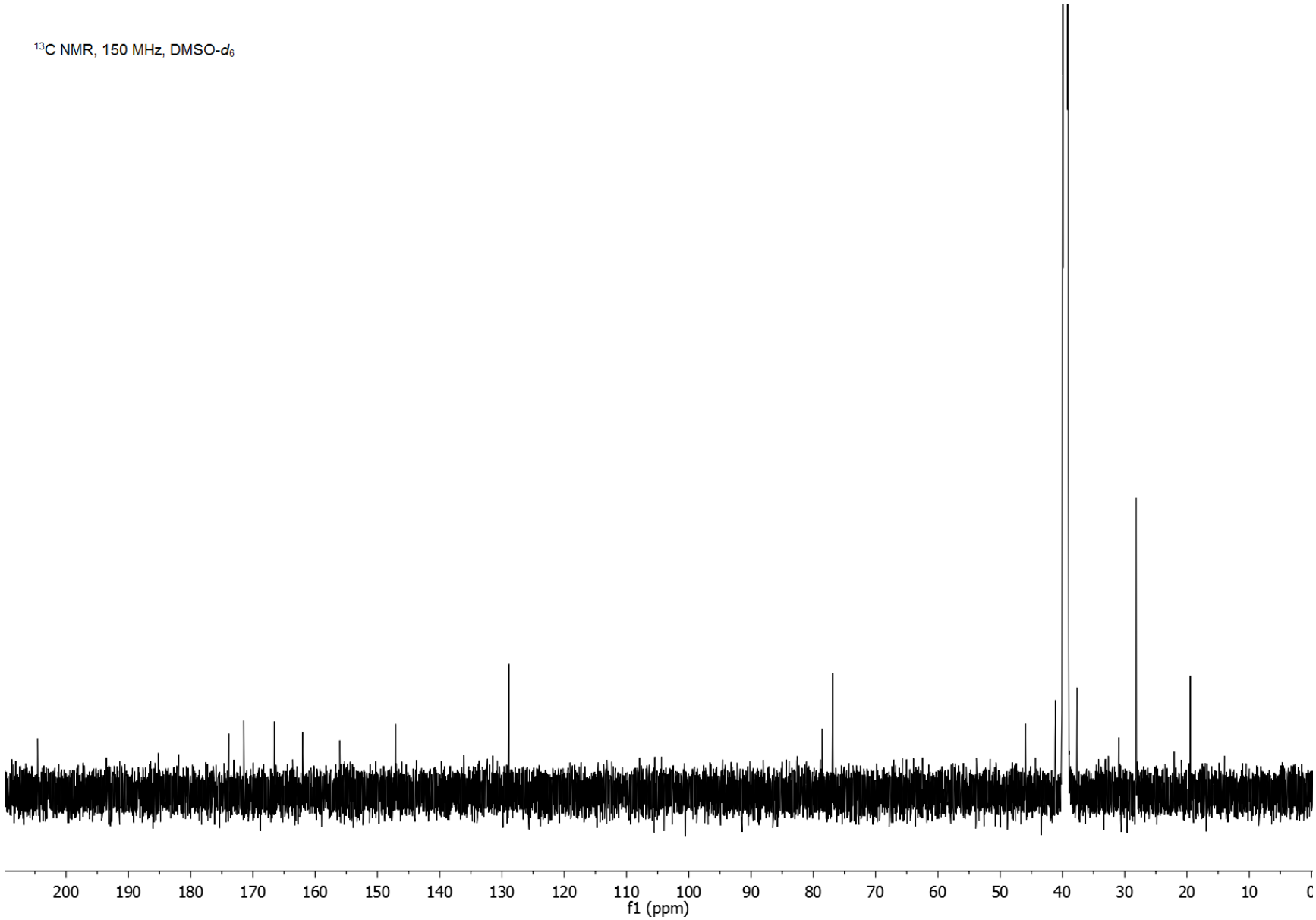
16


^{13}C NMR, 150 MHz, $CDCl_3$
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

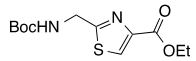
^{1}H NMR, 600 MHz, DMSO-d_6



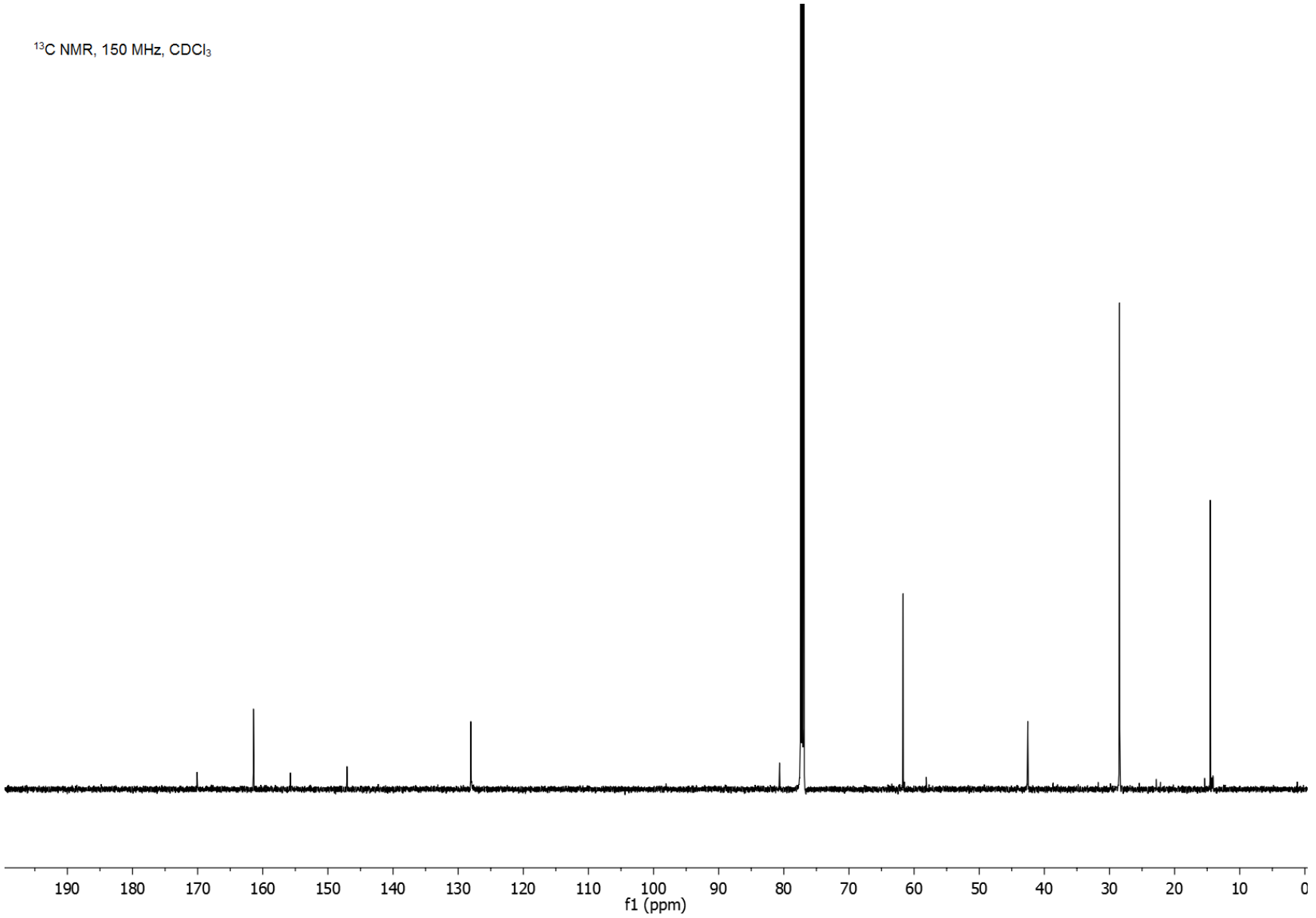
S8


^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

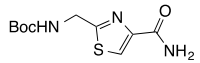
^{1}H NMR, 400 MHz, $CDCl_3$



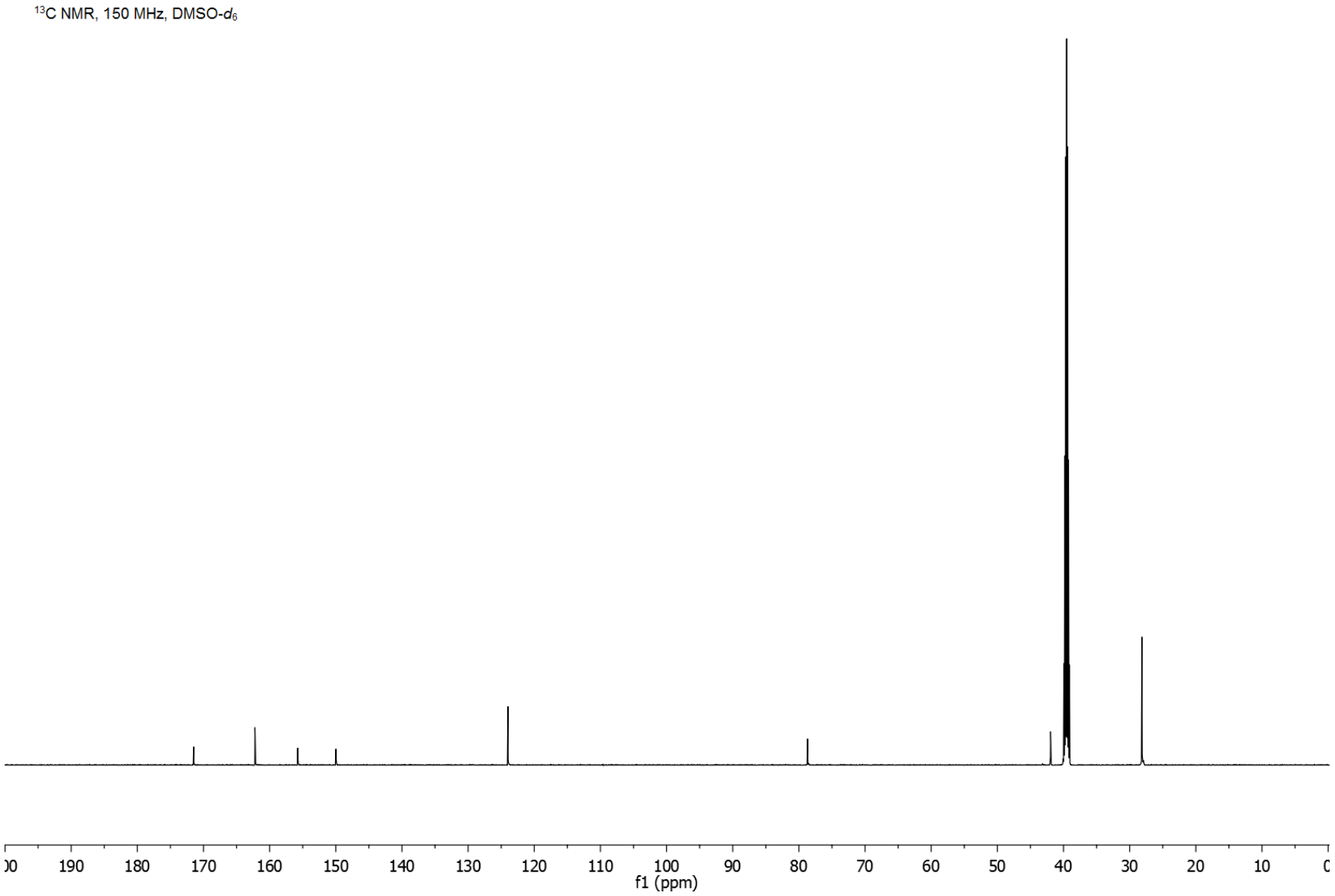
19


^{13}C NMR, 150 MHz, $CDCl_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

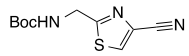
^{1}H NMR, 600 MHz, DMSO-d_6



S9


^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 400 MHz, $CDCl_3$



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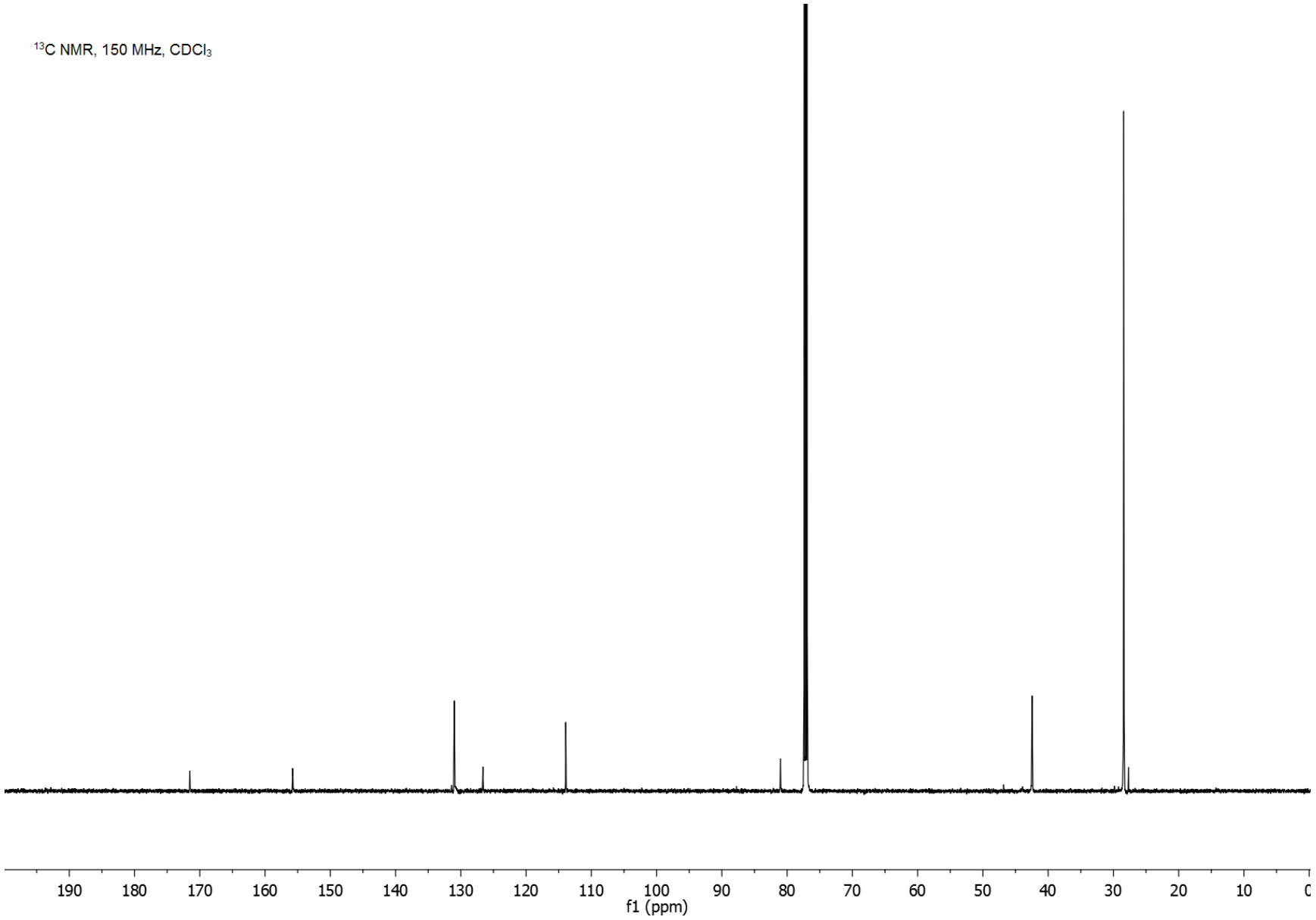
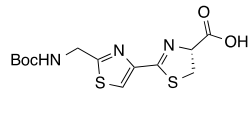
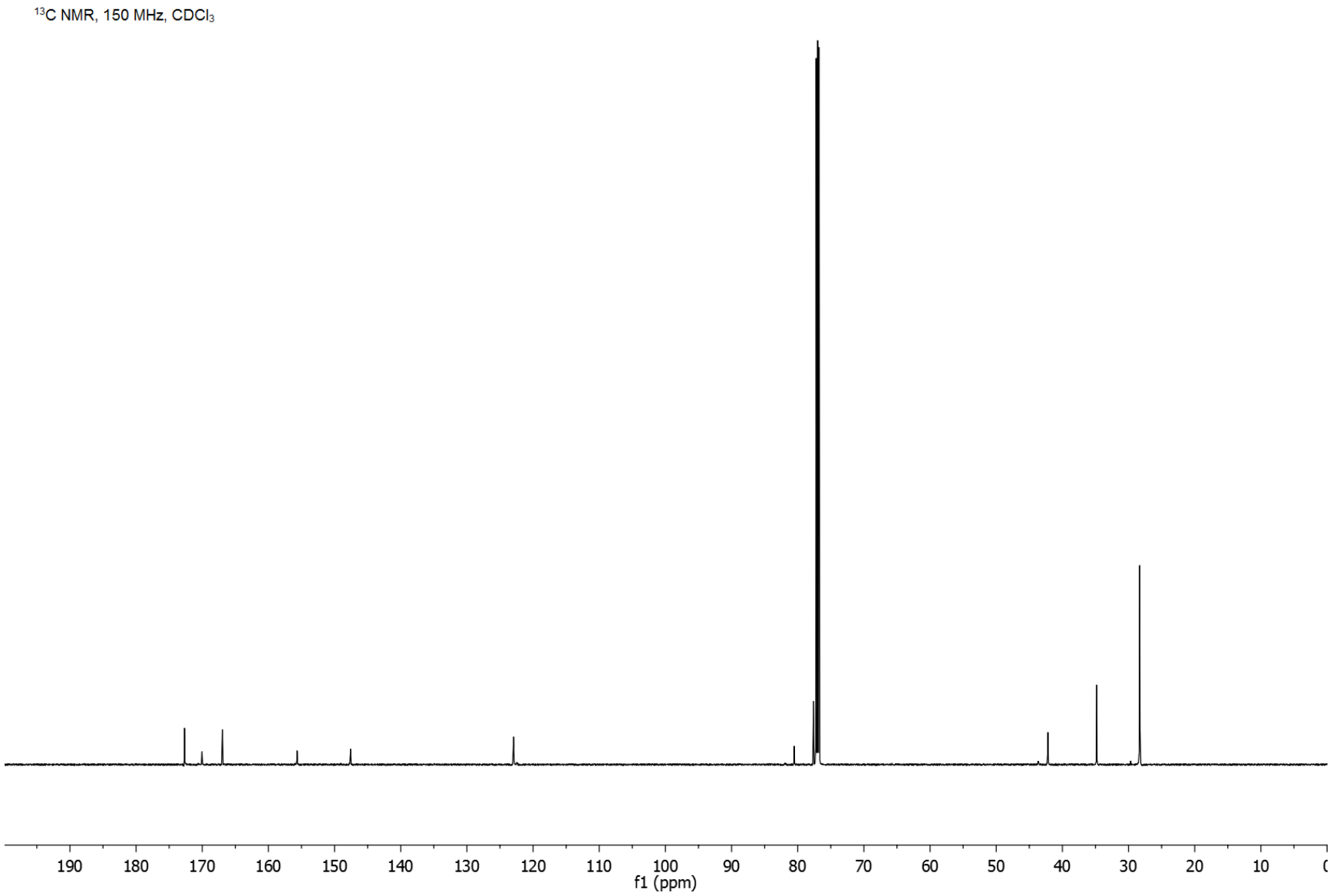

^{13}C NMR, 150 MHz, $CDCl_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

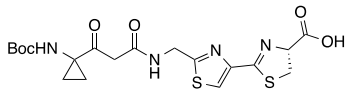
^{1}H NMR, 600 MHz, $CDCl_3$



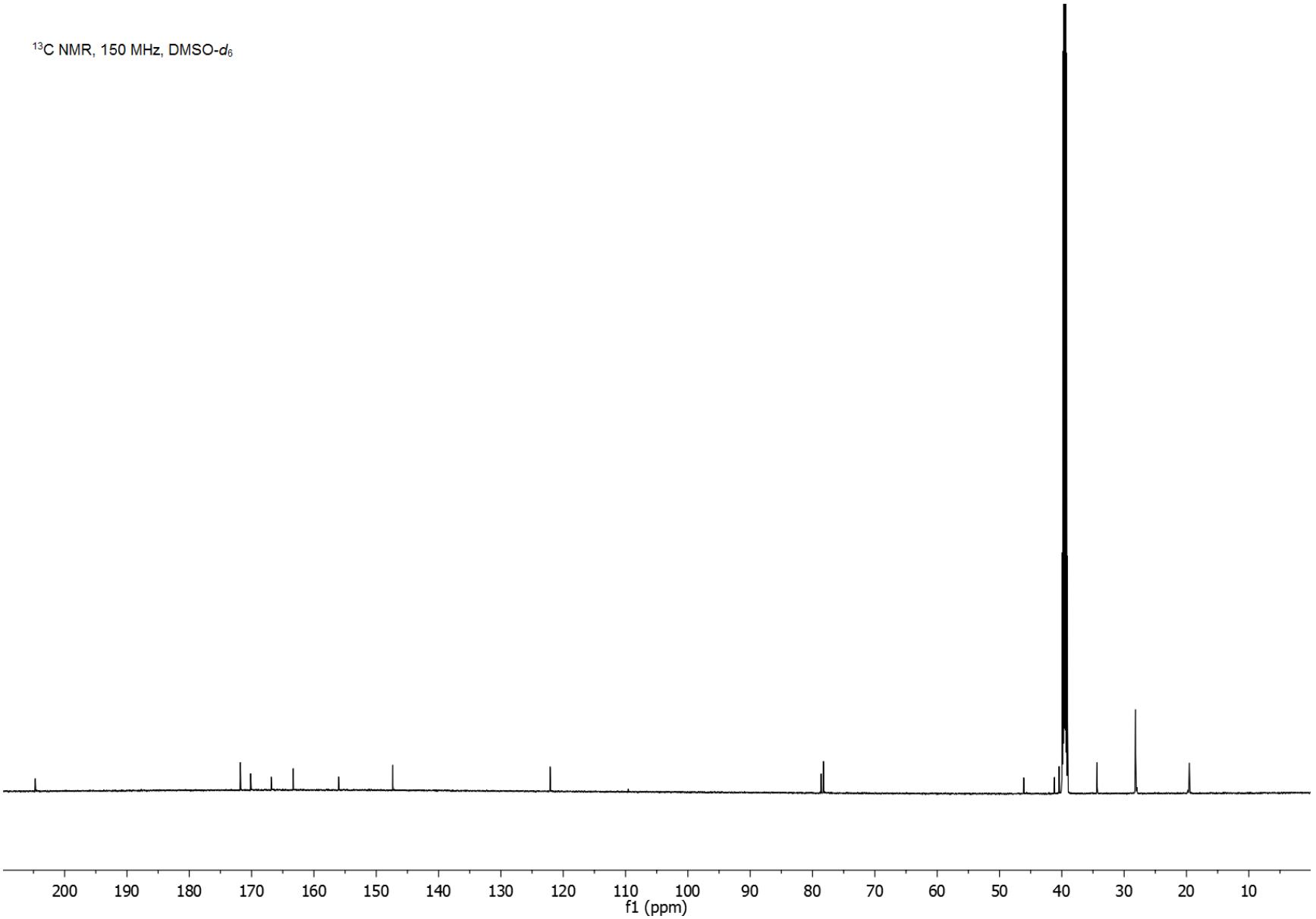
21


^{13}C NMR, 150 MHz, $CDCl_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

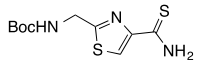
^{1}H NMR, 600 MHz, DMSO-d_6



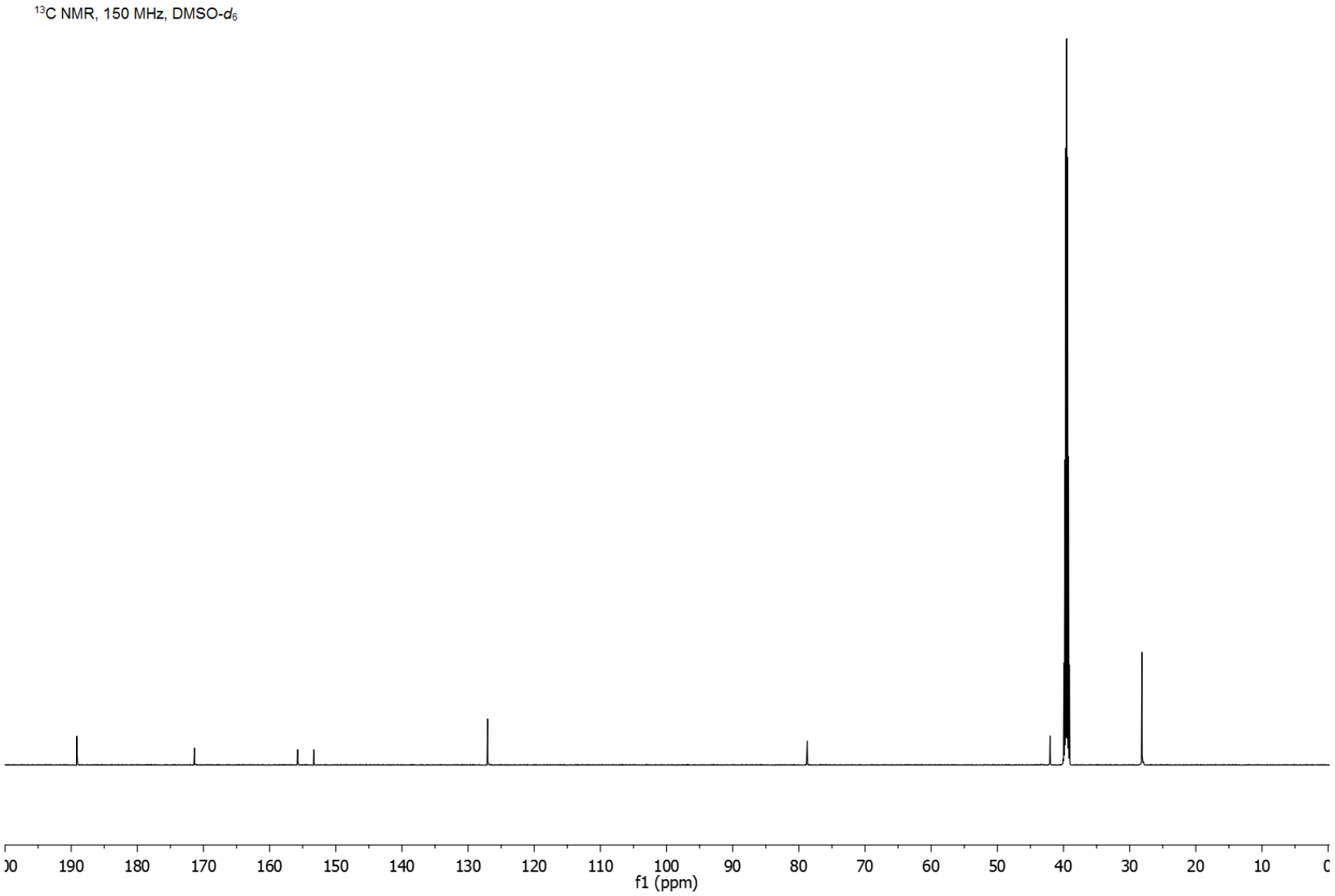
S10


^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

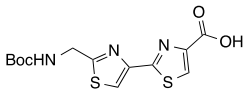
^{1}H NMR, 600 MHz, DMSO-d_6



24


^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6



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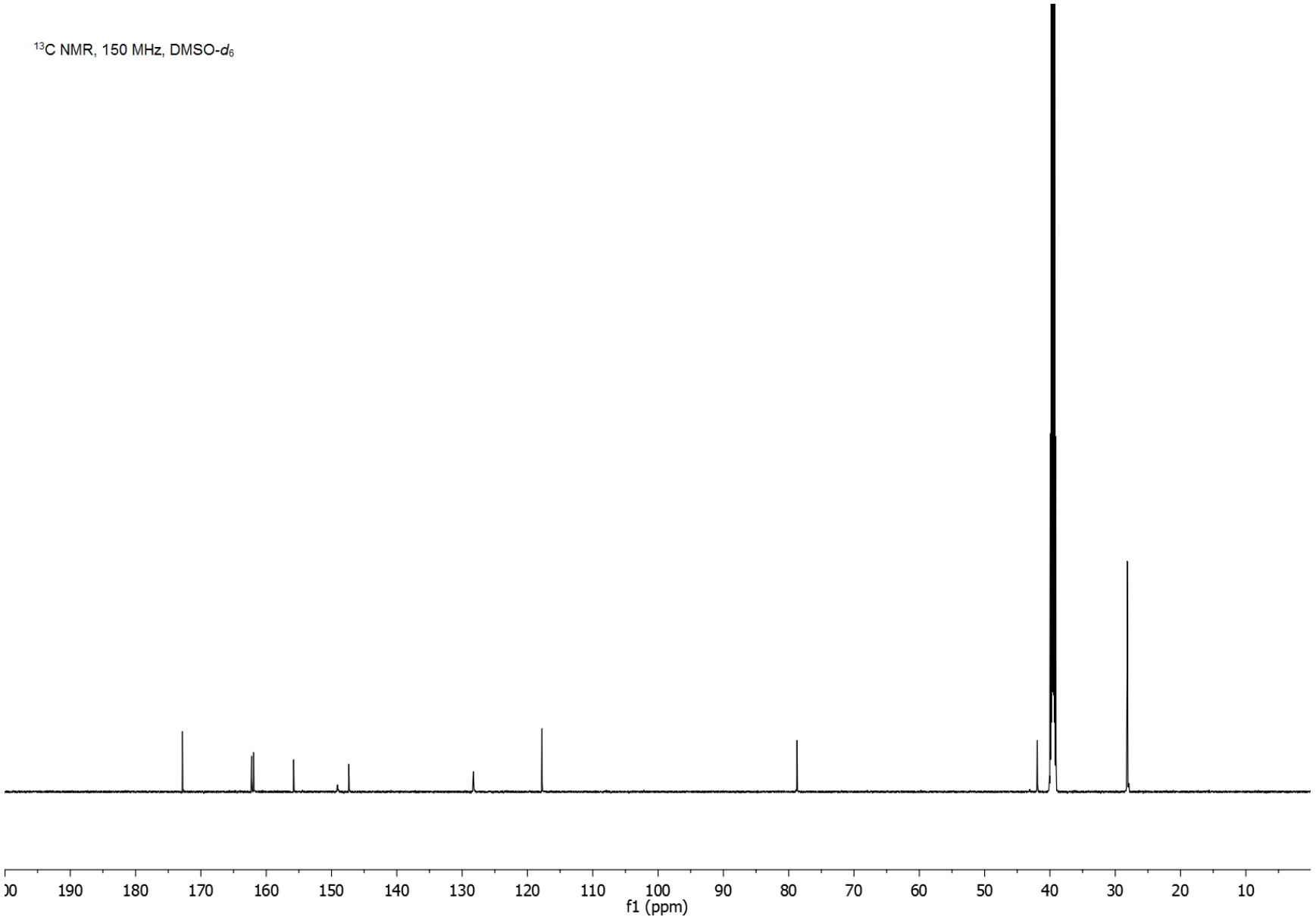
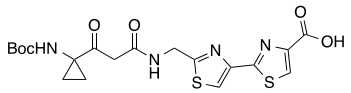
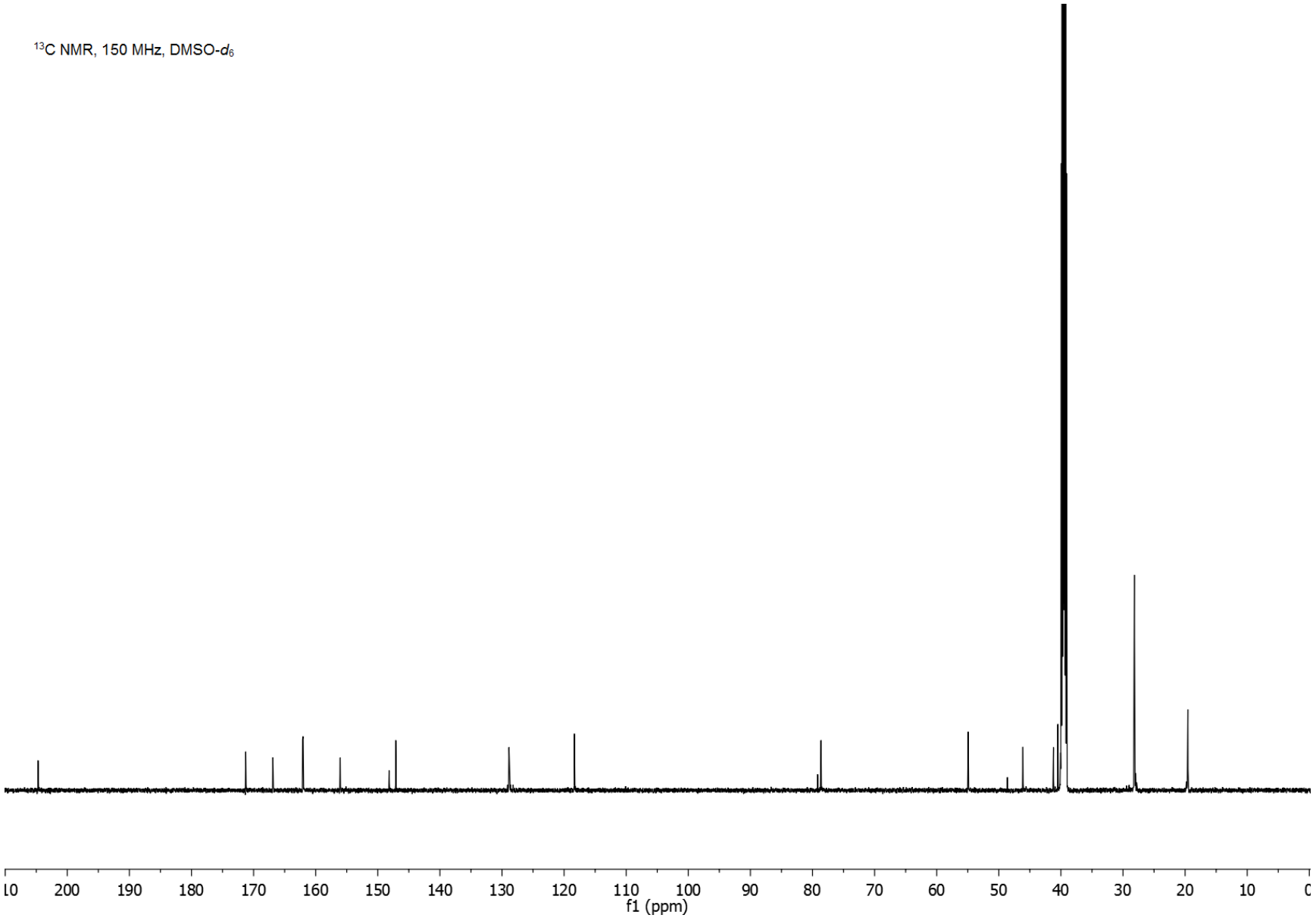

^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

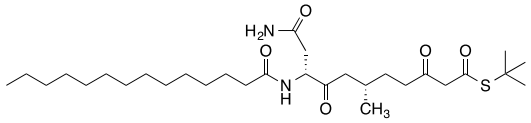
^{1}H NMR, 500 MHz, DMSO-d_6



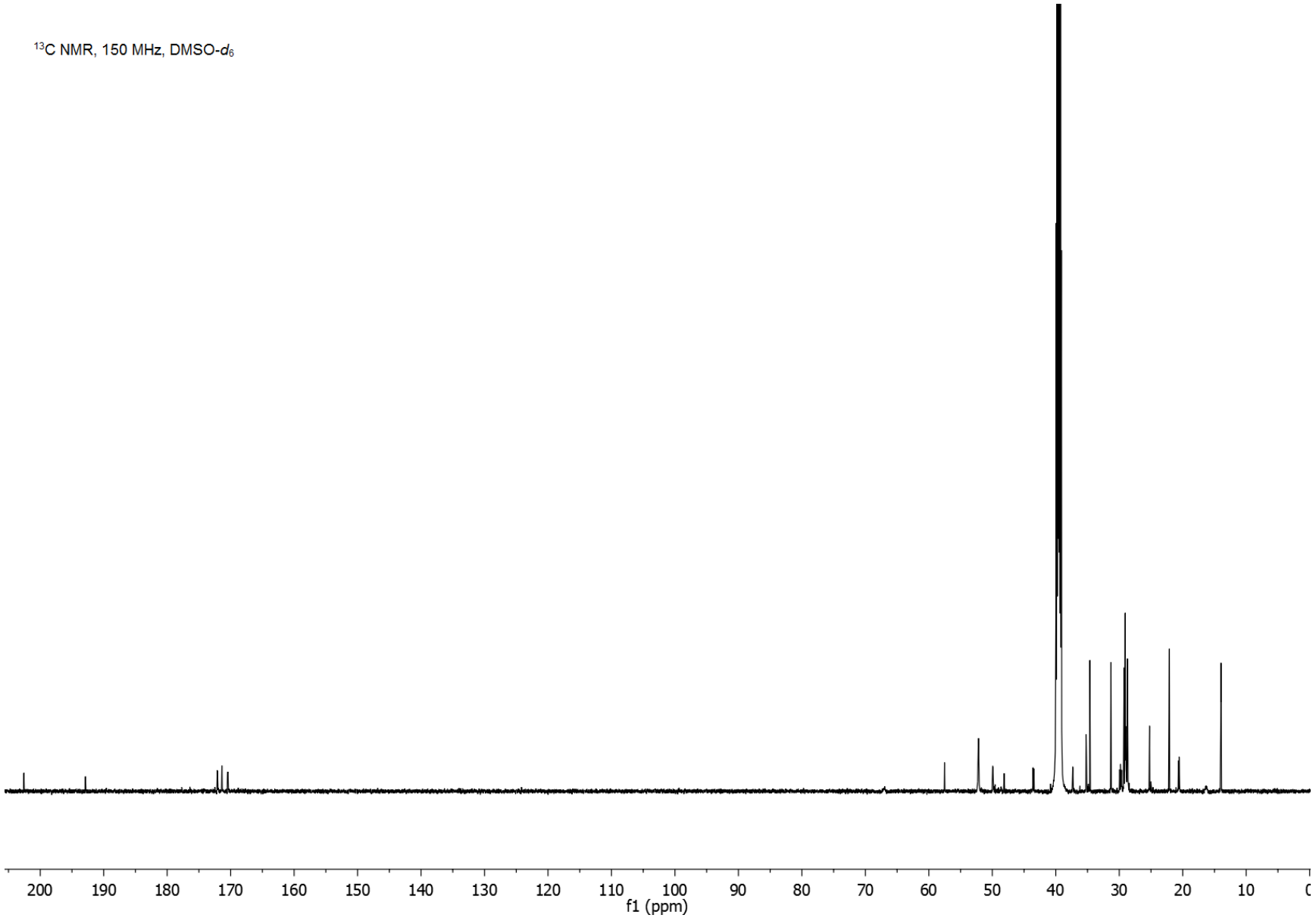
S11


^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

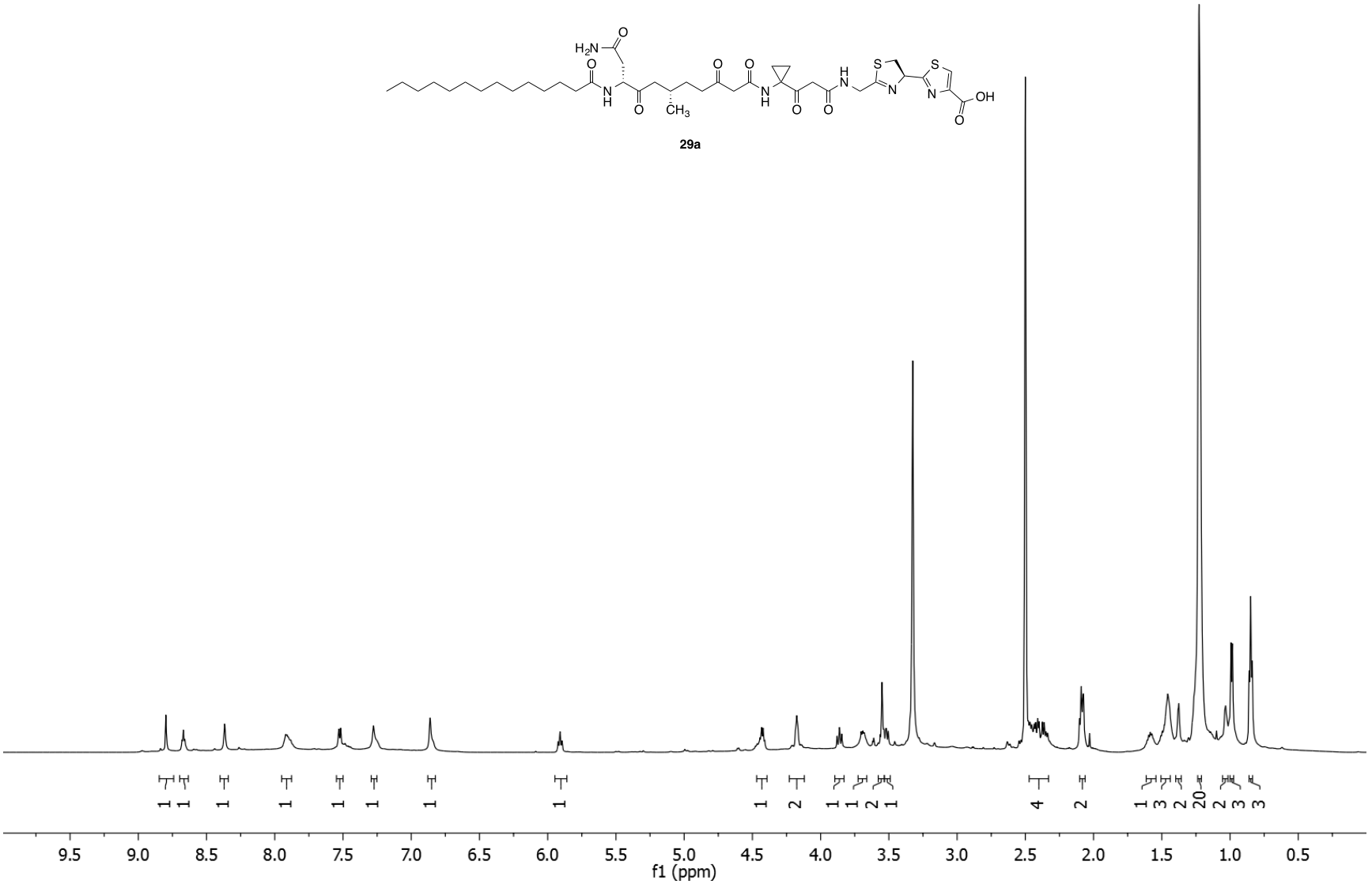
^{1}H NMR, 400 MHz, DMSO-d_6

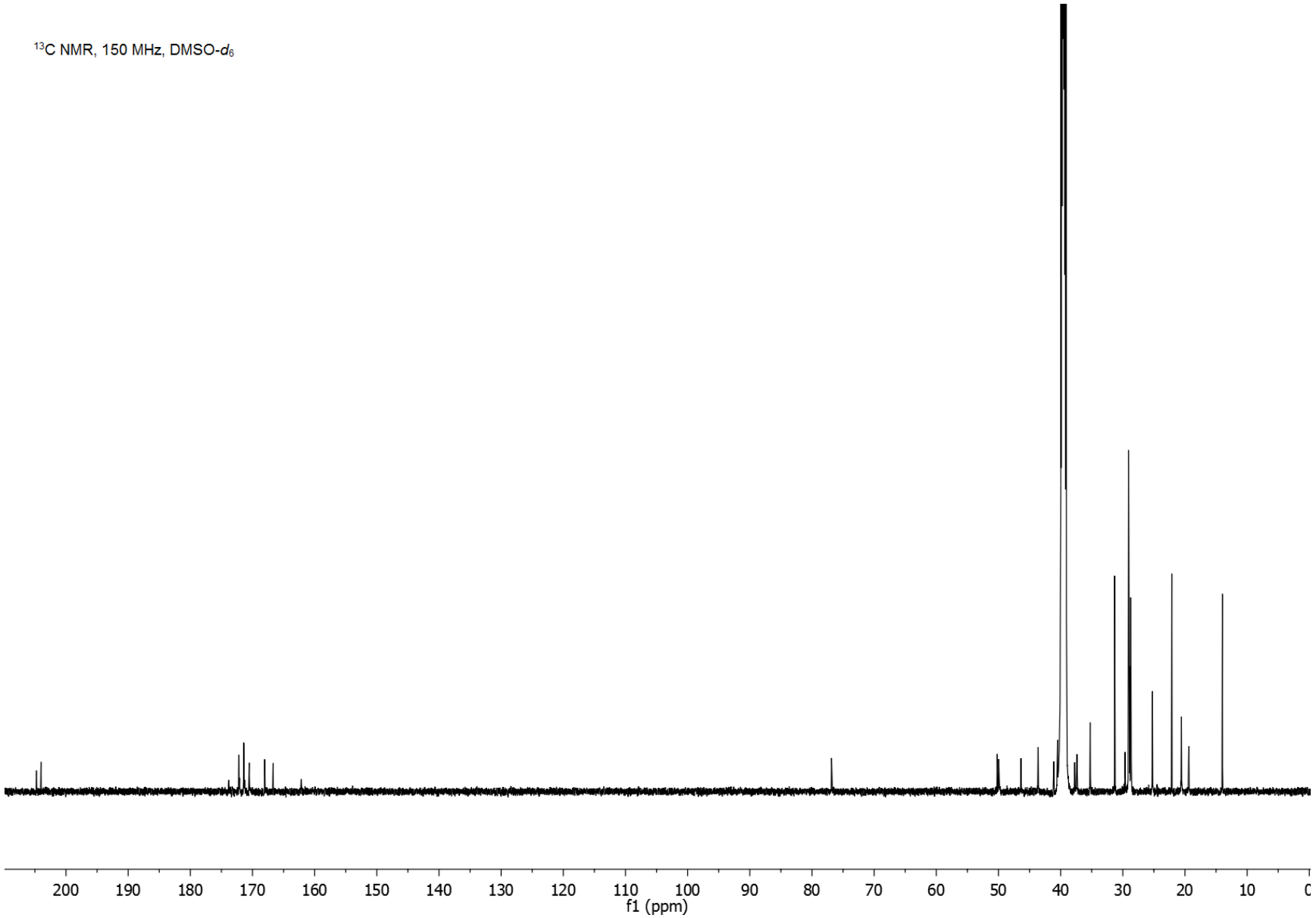


28

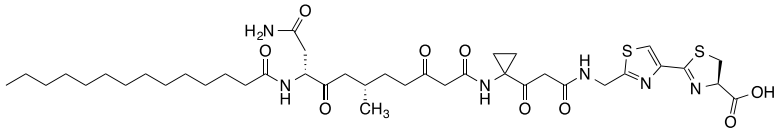

^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6

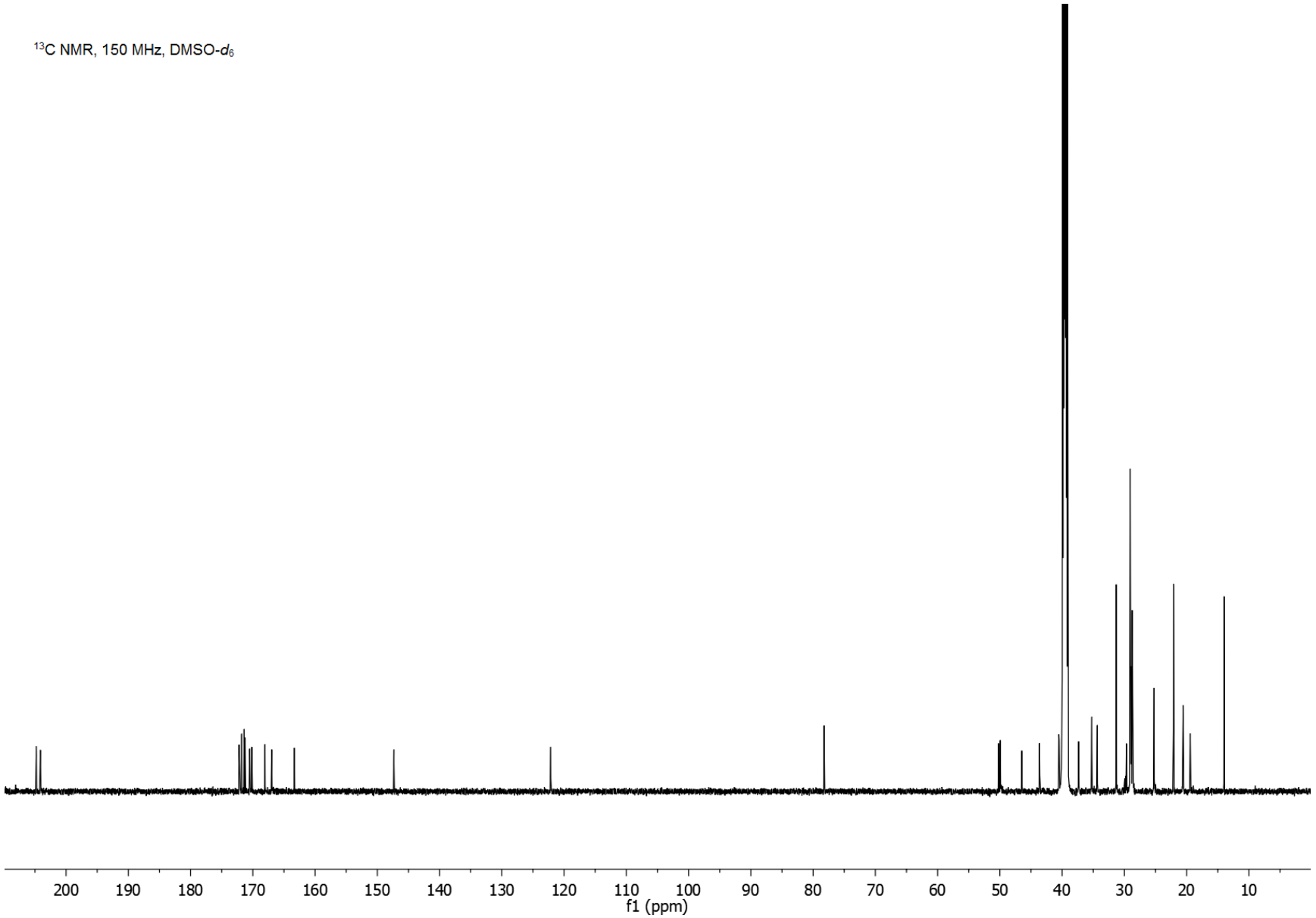



^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

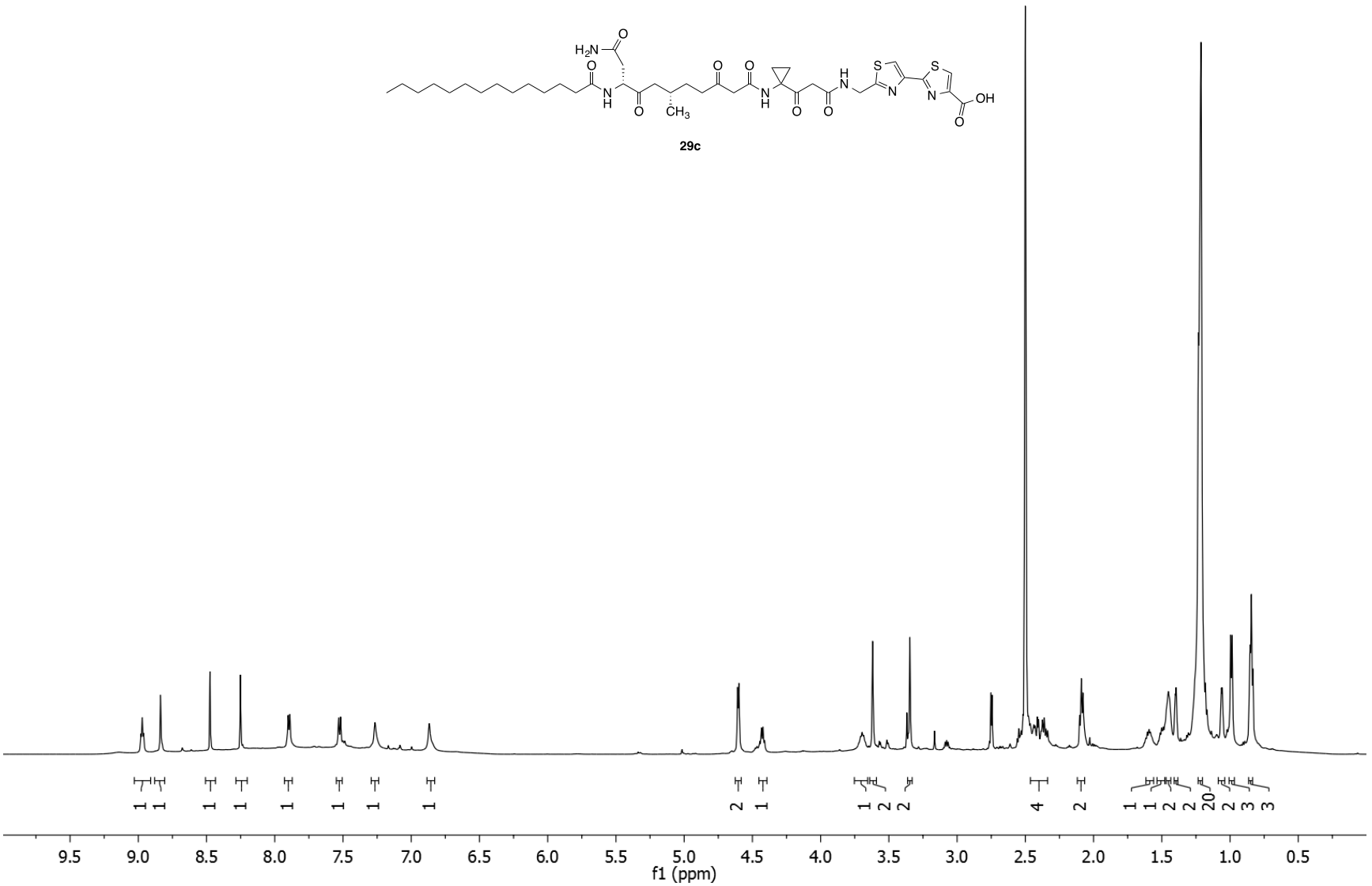
^{1}H NMR, 600 MHz, DMSO-d_6

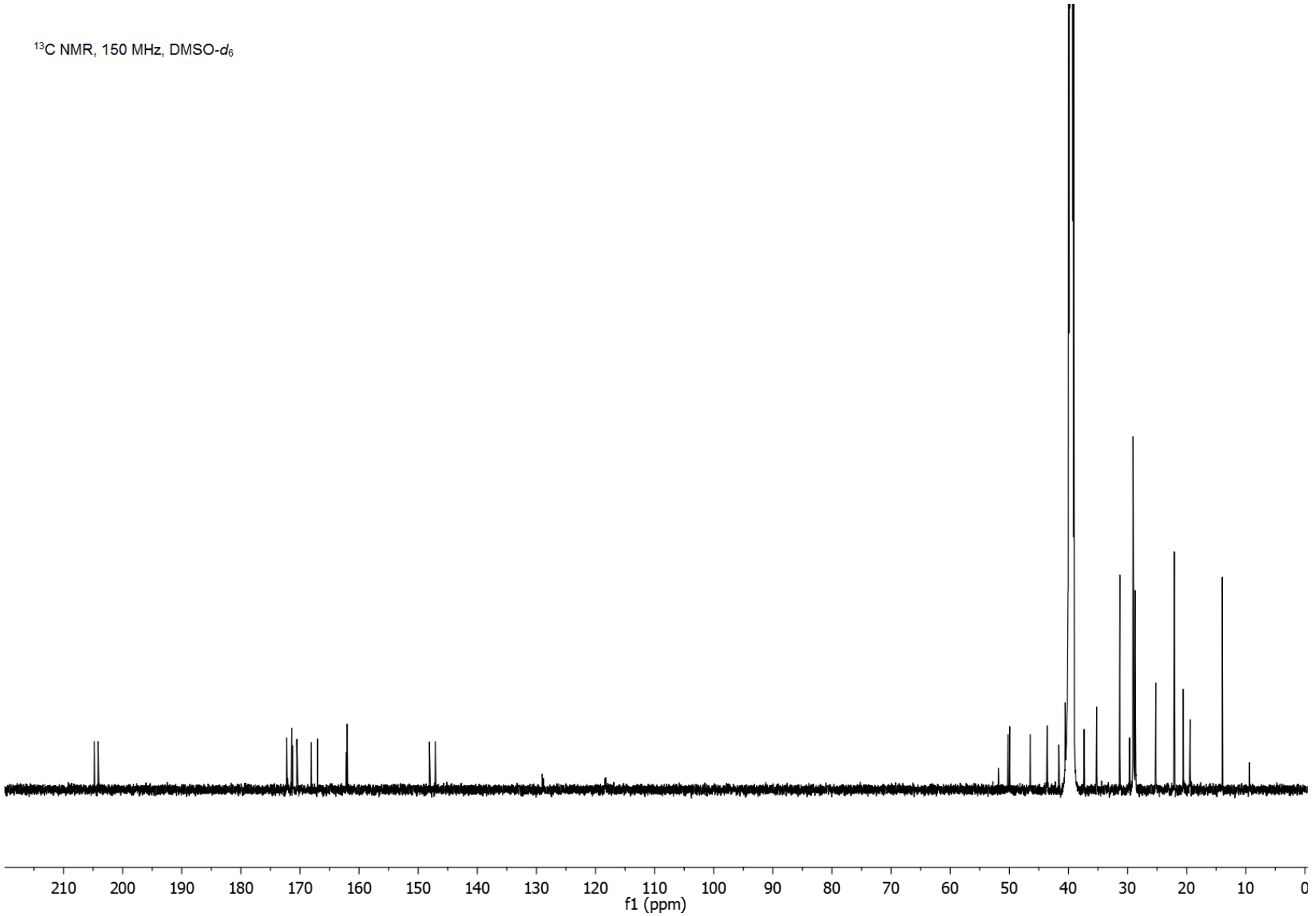


29b

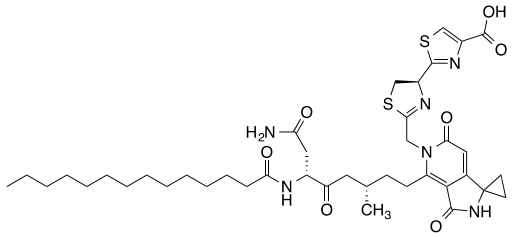

^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6

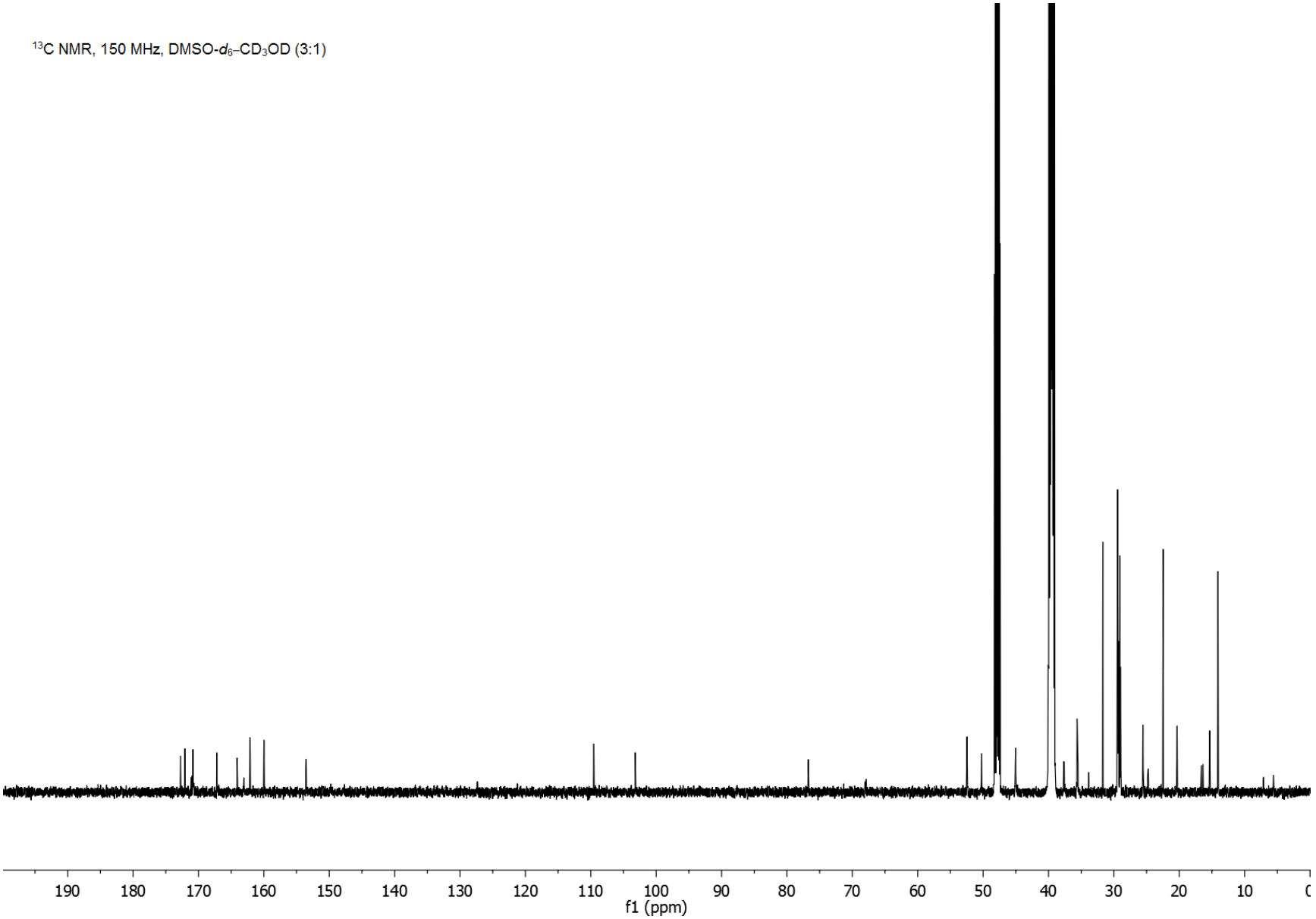



^{13}C NMR, 150 MHz, DMSO-d_6
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

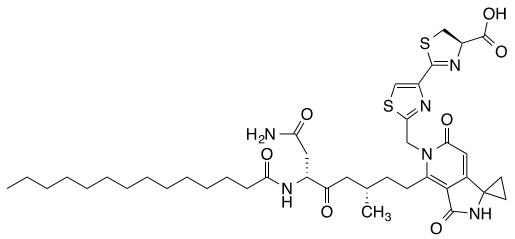
^{1}H NMR, 500 MHz, DMSO-d_6–CD_3OD (3:1)



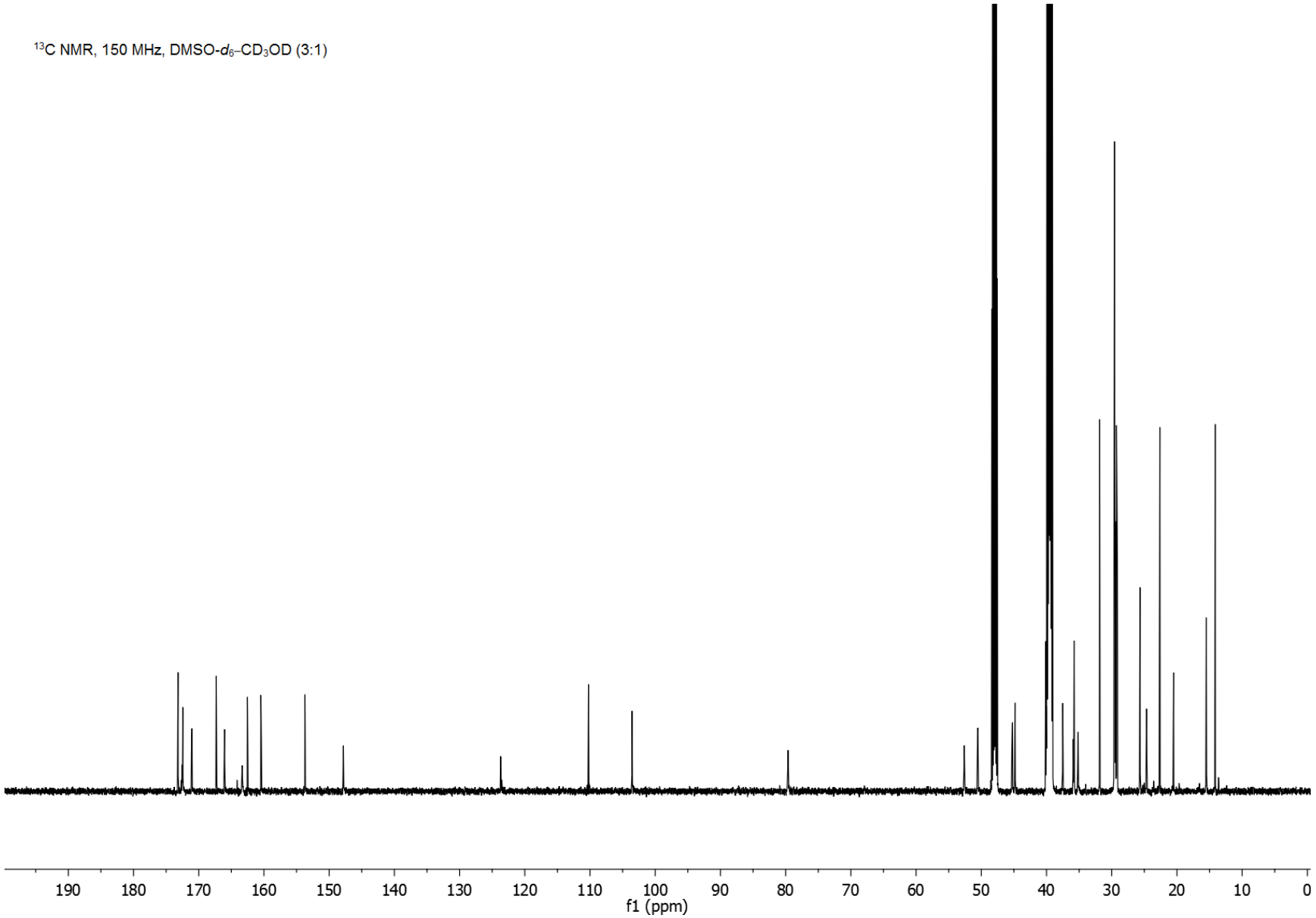
30a


^{13}C NMR, 150 MHz, DMSO-d_6–CD_3OD (3:1)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

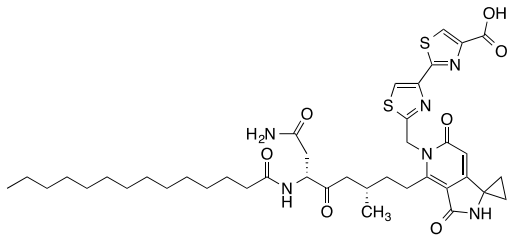
^{1}H NMR, 400 MHz, DMSO-d_6–CD_3OD (3:1)



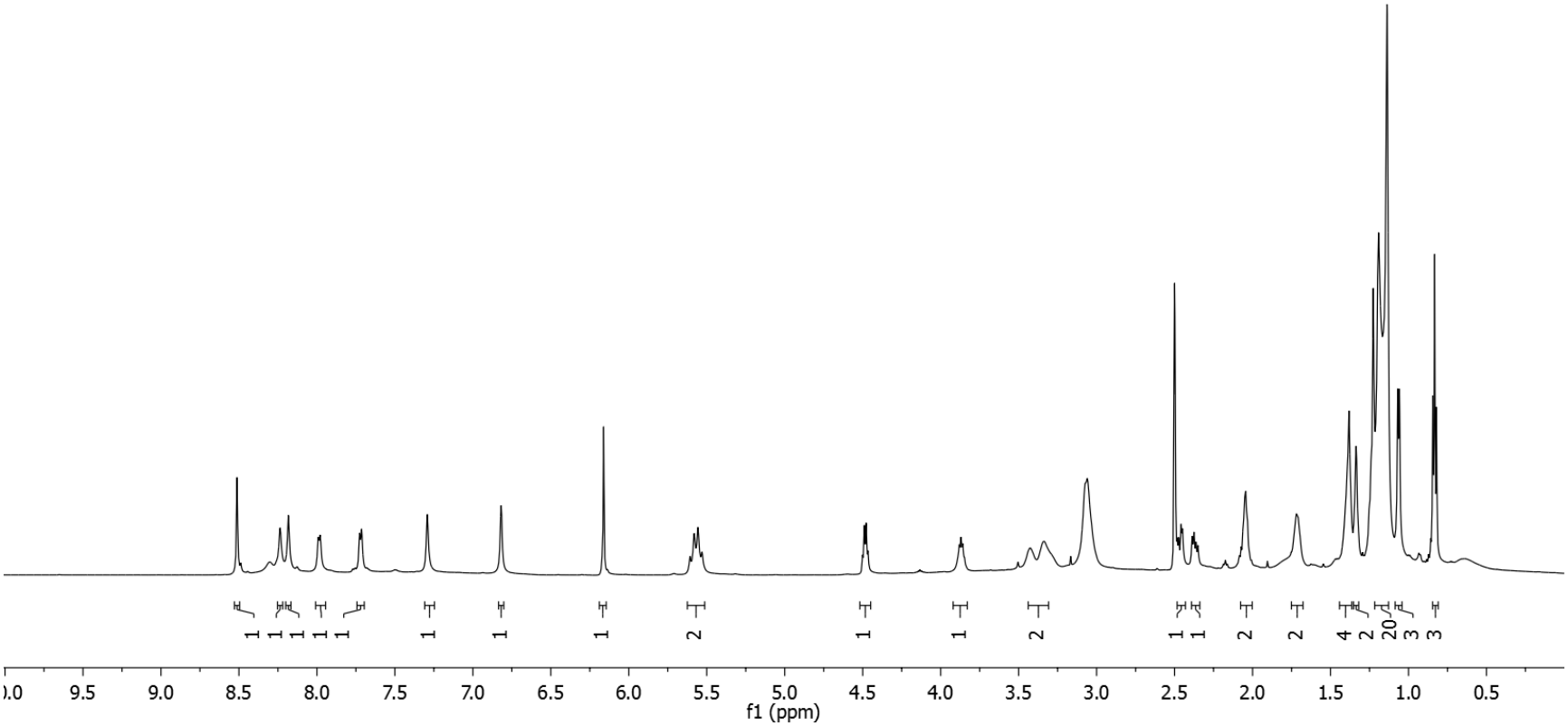
30b

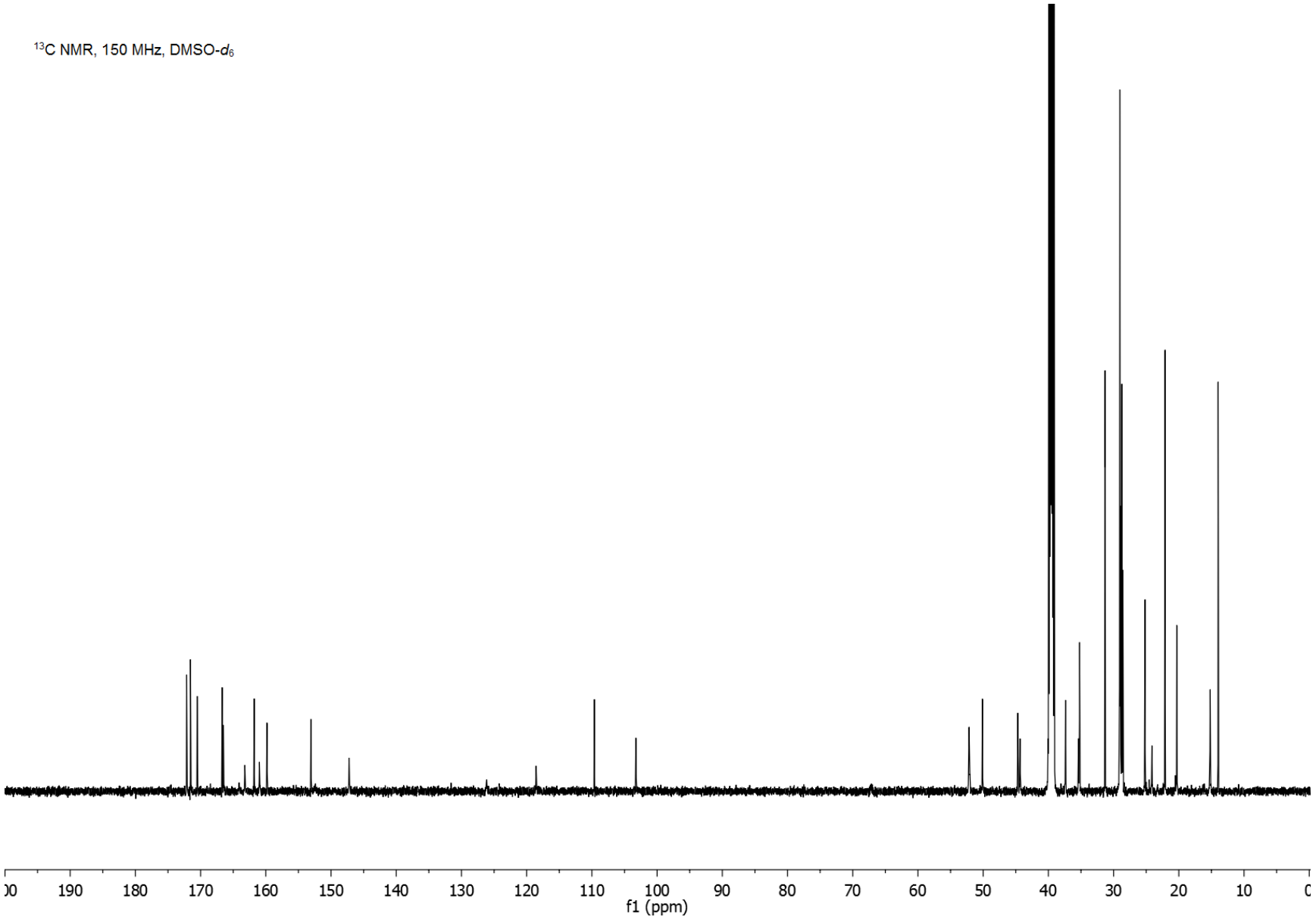

^{13}C NMR, 150 MHz, DMSO-d_6–CD_3OD (3:1)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

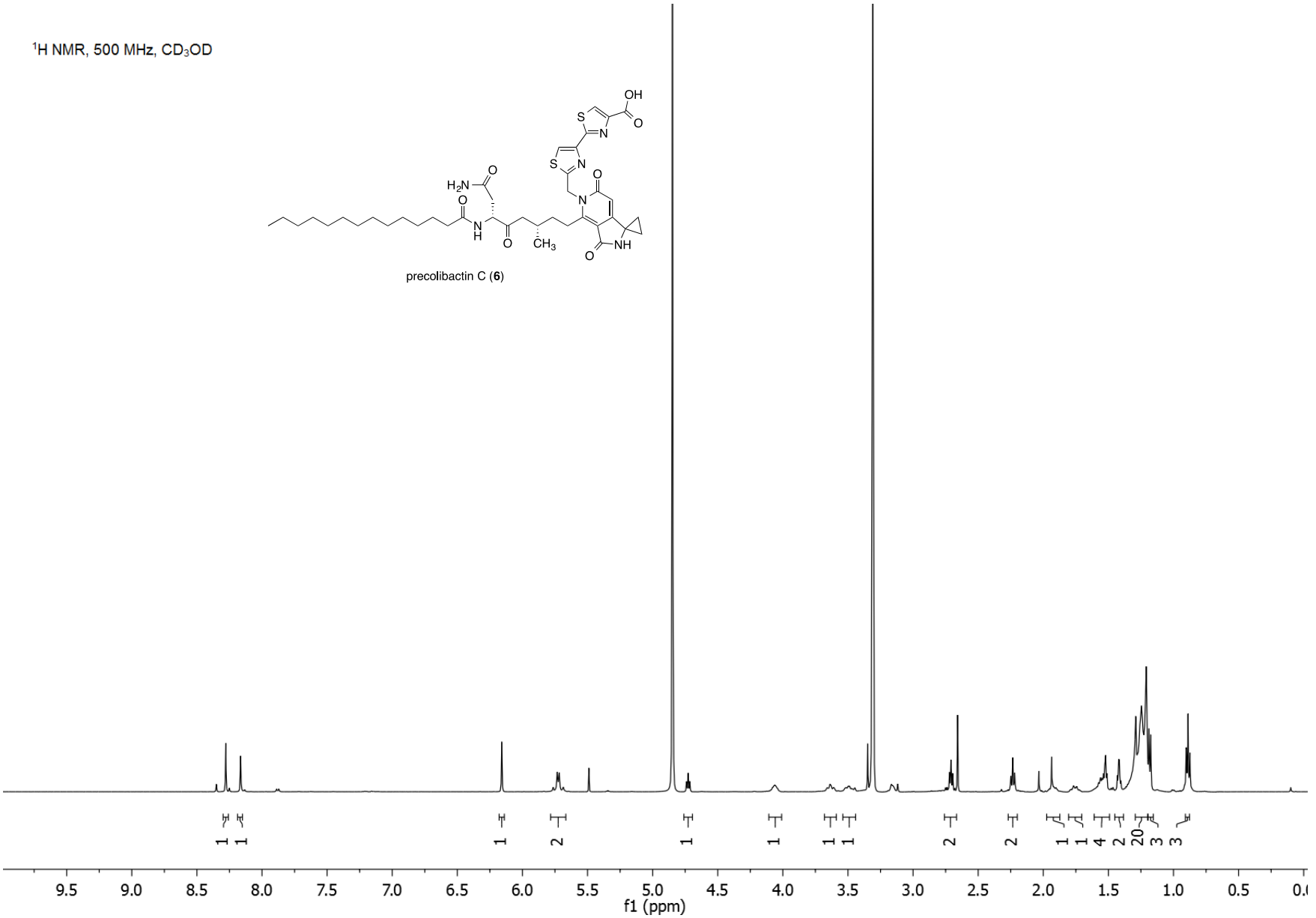
^{1}H NMR, 600 MHz, DMSO-d_6

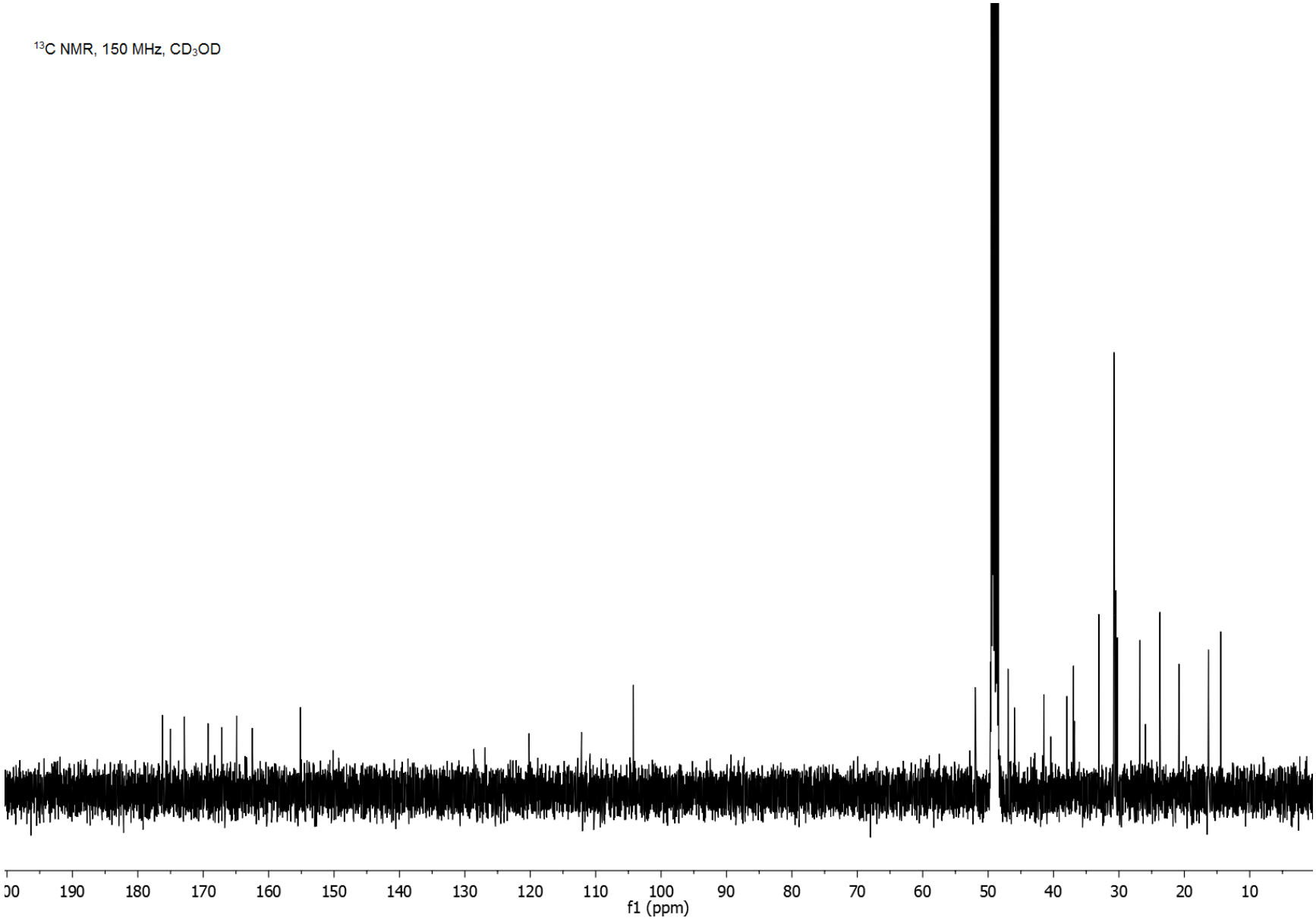


precolibactin C (**6**)

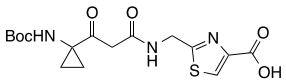



^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

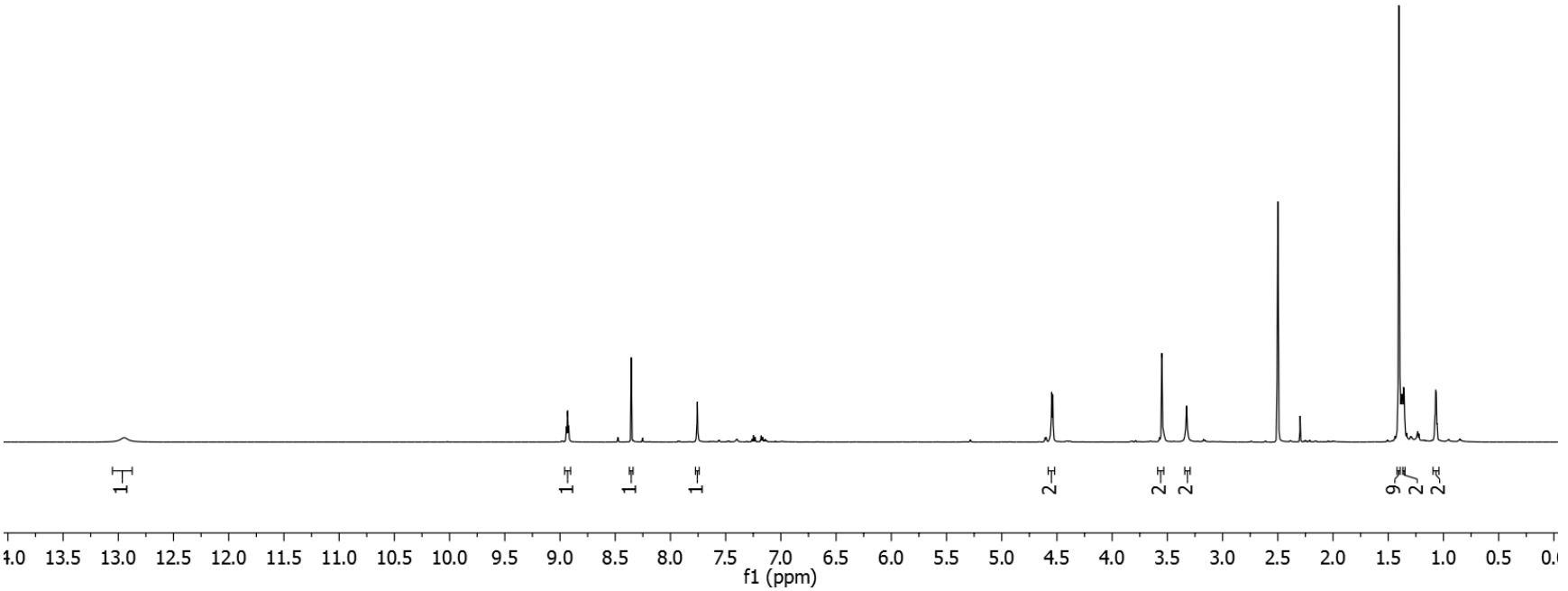

^{1}H NMR, 500 MHz, CD_3OD
precolibactin C (**6**)
f1 (ppm)

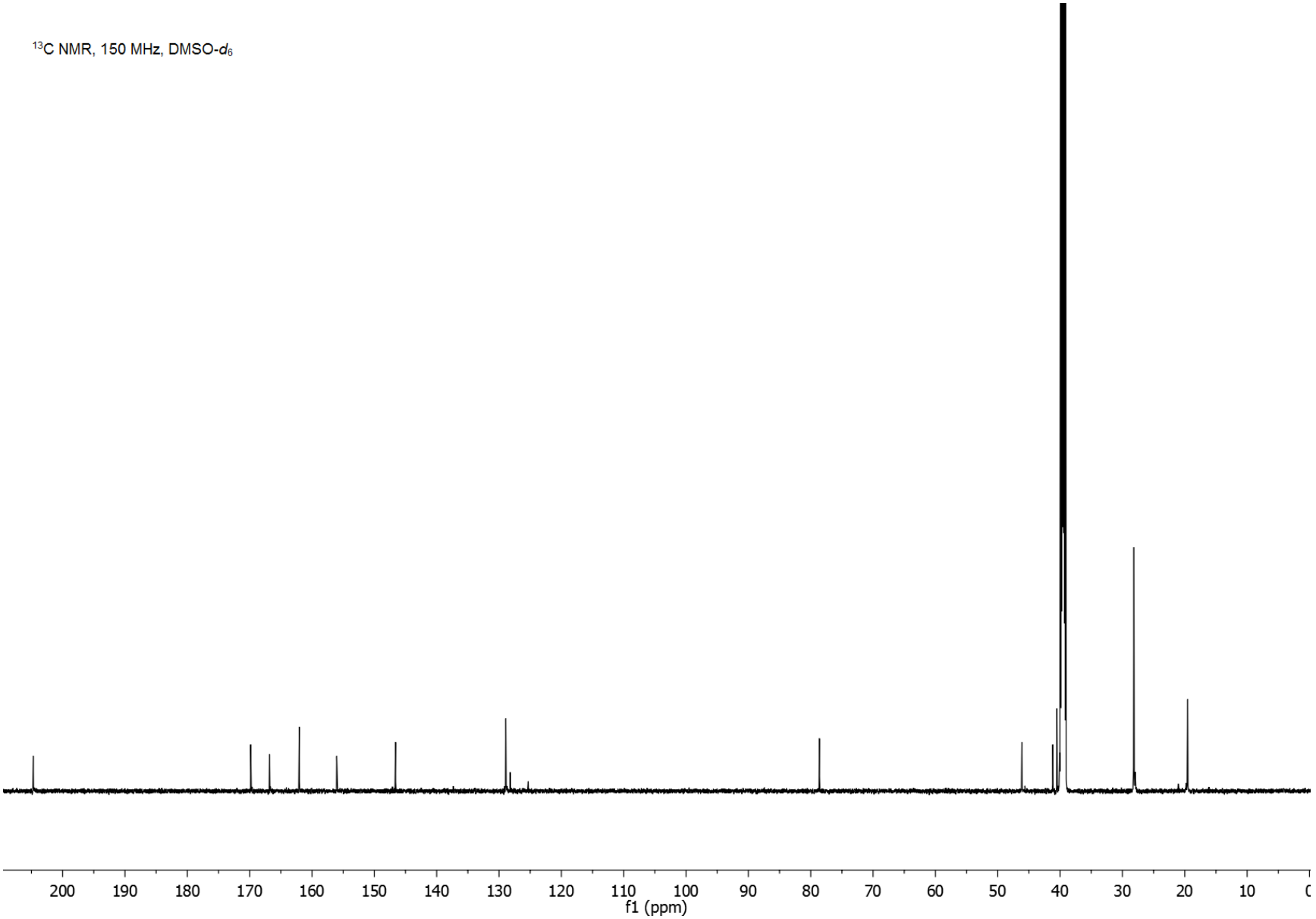

^{13}C NMR, 150 MHz, CD_3OD
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6

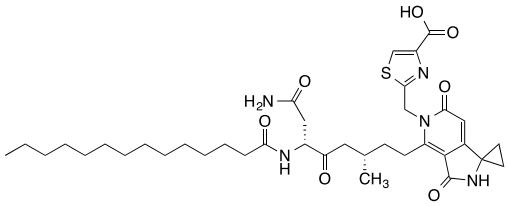


S13

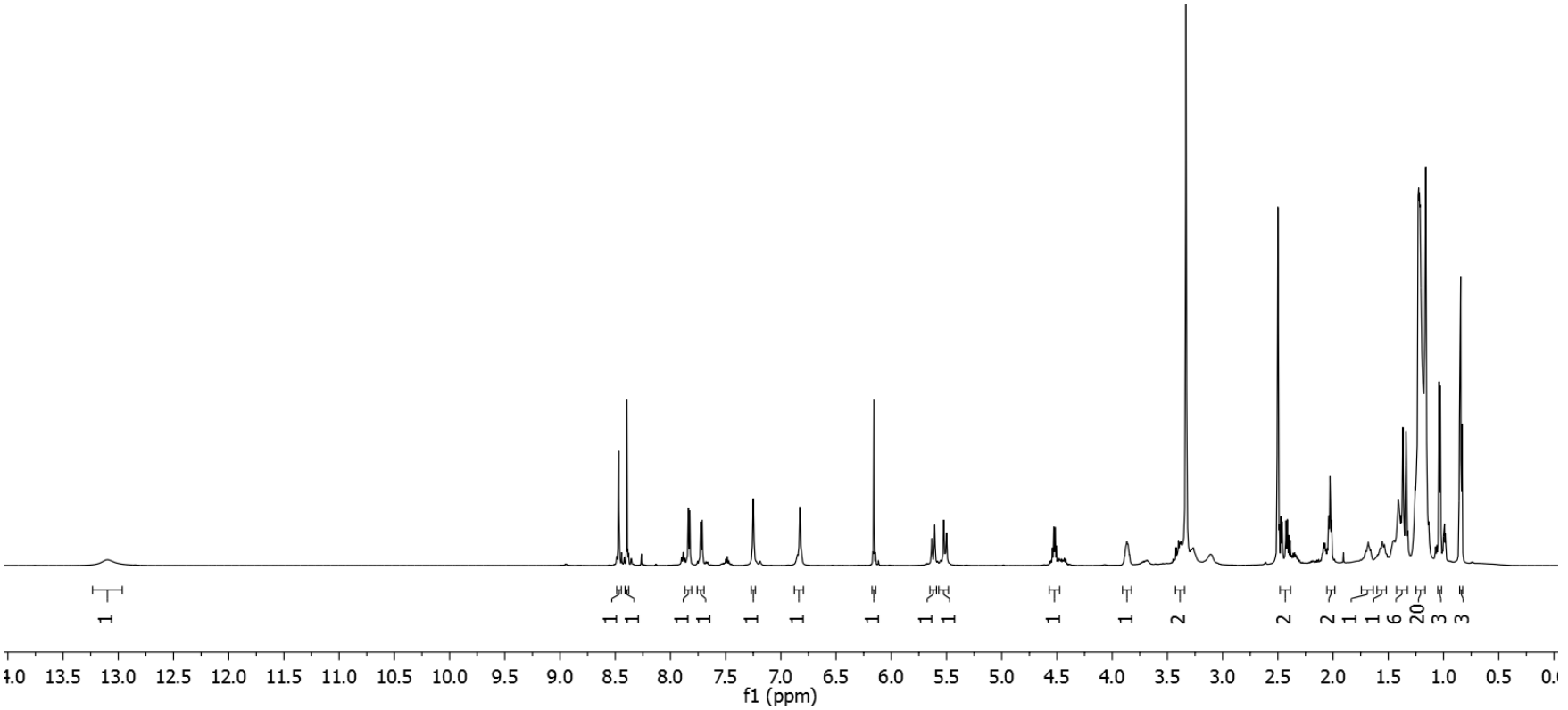


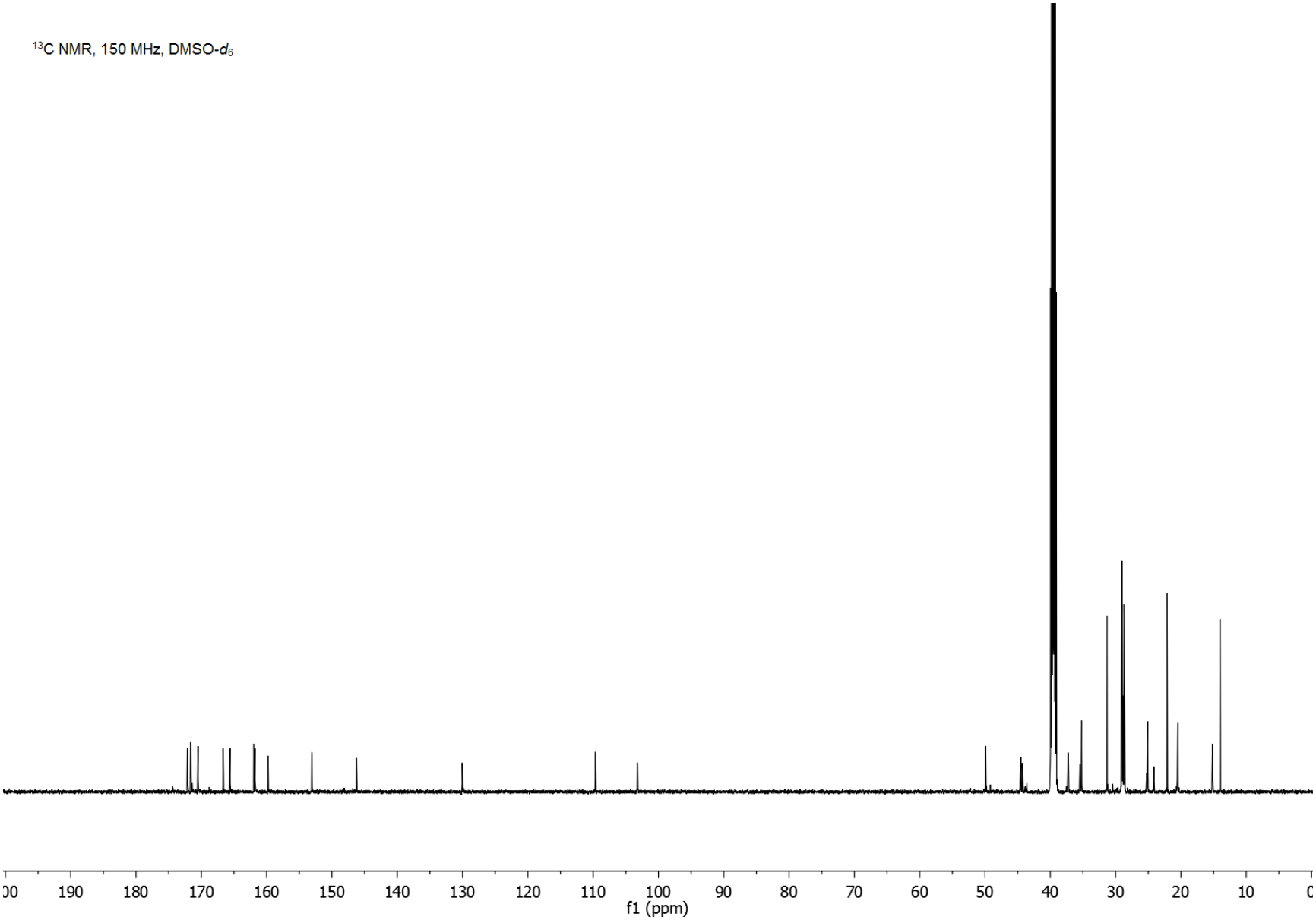

^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

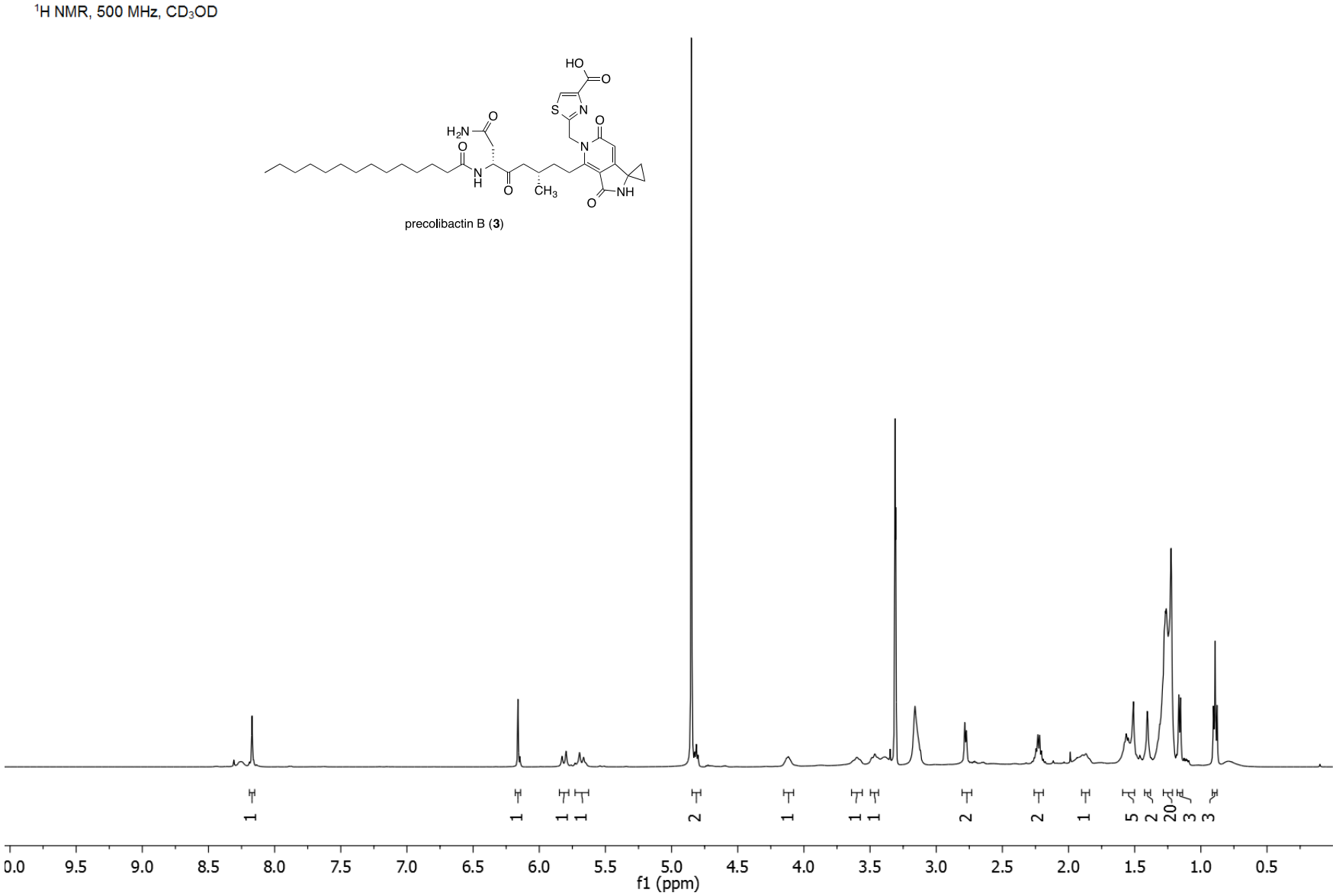
^{1}H NMR, 600 MHz, DMSO-d_6



precolibactin B (**3**)




^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



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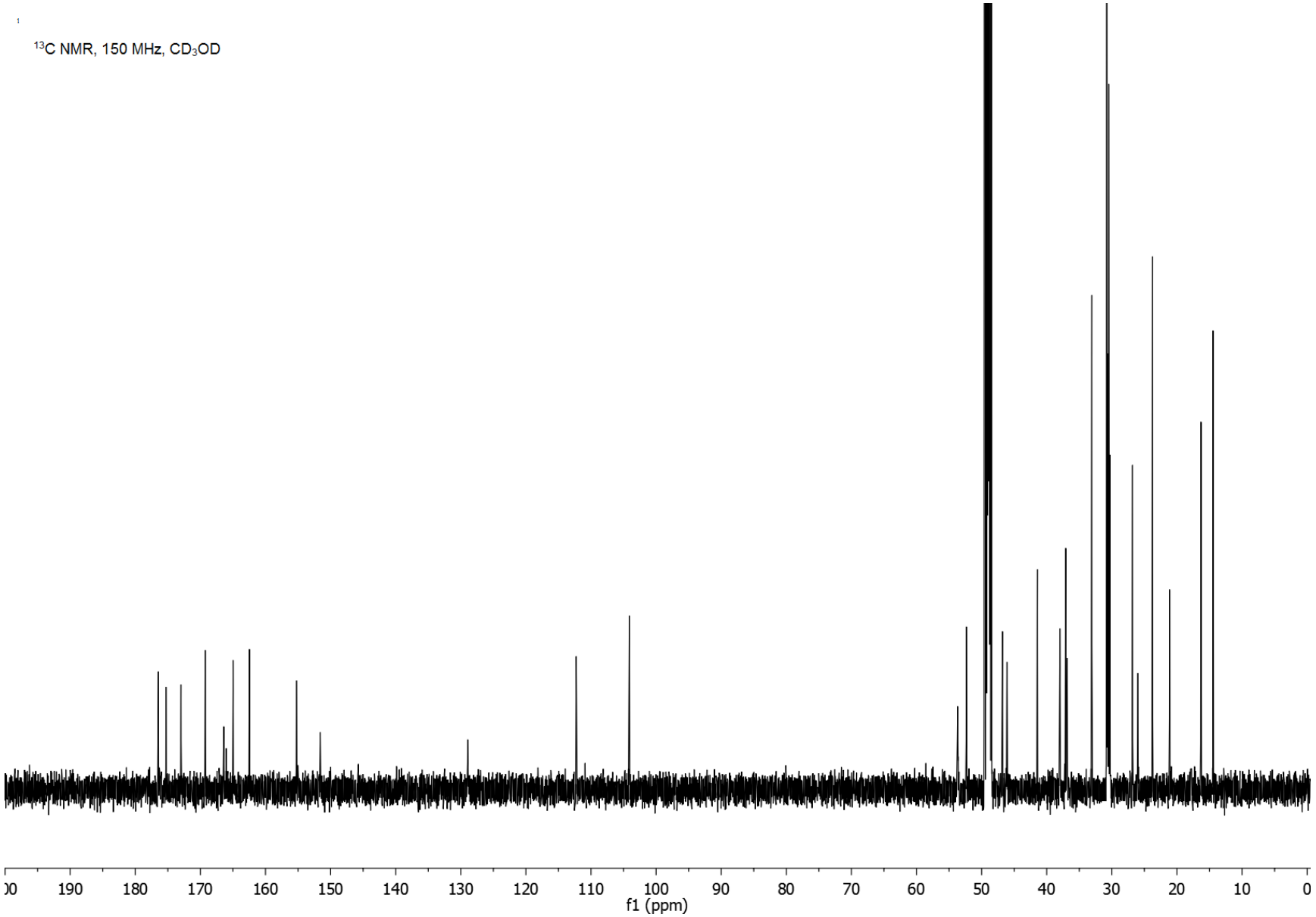
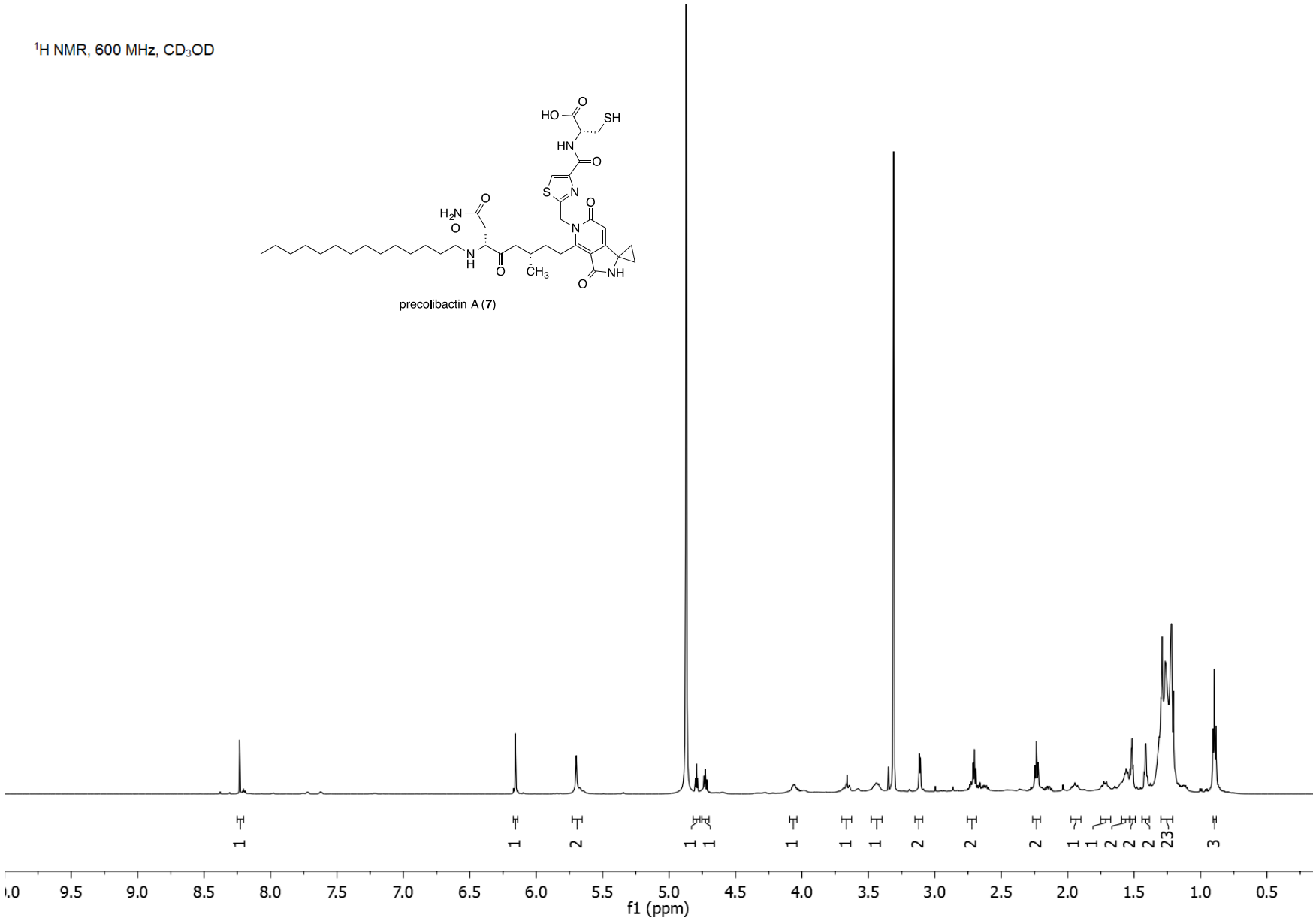
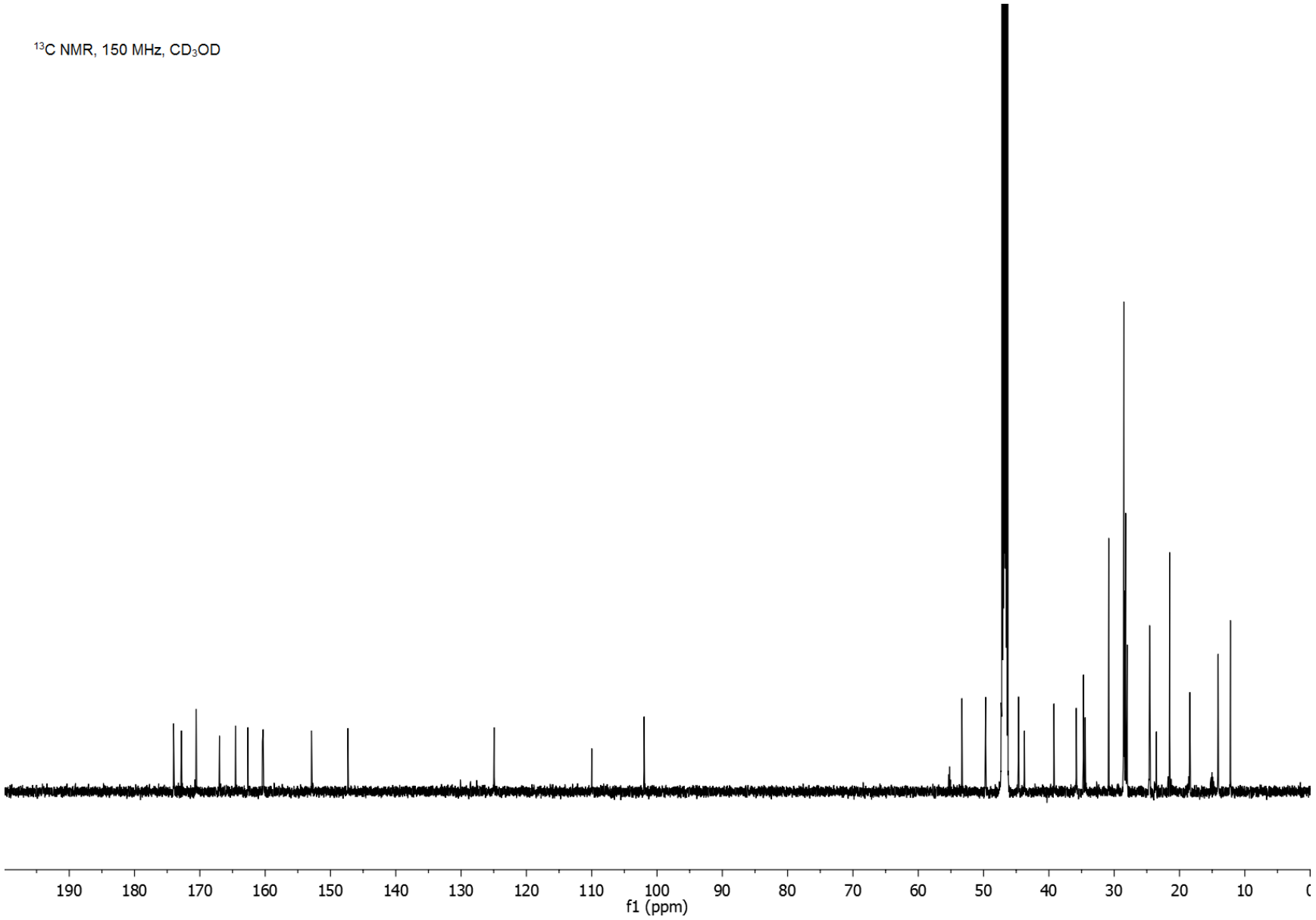

^{13}C NMR, 150 MHz, CD_3OD
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


^{1}H NMR, 600 MHz, CD_3OD
precolibactin A (**7**)
f1 (ppm)


^{13}C NMR, 150 MHz, CD_3OD
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

Bibliography

(1) Vizcaino, M. I.; Crawford, J. M. *Nat. Chem.* **2015**, *7*, 411.

(2) Brotherton, C. A.; Wilson, M.; Byrd, G.; Balskus, E. P. *Org. Lett.* **2015**, *17*, 1545.

(3) Vizcaino, M. I.; Engel, P.; Trautman, E.; Crawford, J. M. *J. Am. Chem. Soc.* **2014**, *136*, 9244.

(4) Jorgensen, W. L.; Tirado–Rives, J. *J. Comput. Chem.* **2005**, *26*, 1689.

(5) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J.; Gaussian, Inc. %! Gaussian 09: Wallingford, CT, USA, 2009.

(6) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(7) *SiliaBond® TMA Acetate: a NEW strong anion exchange sorbent and its applications in SPE format*; François Béland, N. H., Steeves Potvin and Lynda Tremblay, Ed.; SiliCycle, 2009.

(8) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.

(9) Babij, N. R.; Wolfe, J. P. *Angew. Chem., Int. Ed.* **2012**, *51*, 4128.

(10) Bae, H. Y.; Sim, J. H.; Lee, J.-W.; List, B.; Song, C. E. *Angew. Chem., Int. Ed.* **2013**, *52*, 12143.

(11) Videnov, G.; Kaiser, D.; Kempter, C.; Jung, G. *Angew. Chem., Int. Ed.* **1996**, *35*, 1503.

(12) Fustero, S.; Monteagudo, S.; Sanchez-Rosello, M.; Flores, S.; Barrio, P.; del Pozo, C. *Chem.–Eur. J.* **2010**, *16*, 9835.

(13) Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913.

(14) Kaiser, F.; Koerber, K.; Von Deyn, W.; Deshmukh, P.; Narine, A.; Dickhaut, J.; Bandur, N. G.; Langewald, J.; Culbertson, D. L.; Anspaugh, D. D.; BASF SE, Germany . 2012, p 291pp.

(15) Thompson, R. E.; Jolliffe, K. A.; Payne, R. J. *Org. Lett.* **2011**, *13*, 680.

(16) Bhansali, P.; Hanigan, C. L.; Casero, R. A.; Tillekeratne, L. M. V. *J. Med. Chem.* **2011**, *54*, 7453.

(17) Souto, J. A.; Vaz, E.; Lepore, I.; Pöppler, A.-C.; Franci, G.; Álvarez, R.; Altucci, L.; de Lera, Á. R. *J. Med. Chem.* **2010**, *53*, 4654.