

Supporting Information

Isolation, Structure Elucidation, Biosynthesis, and Synthesis of Antalid, a Secondary Metabolite from *Polyangium species*

Thomas Tautz,^a Judith Hoffmann,^b Thomas Hoffmann,^b Heinrich Steinmetz,^c Peter Washausen,^c Brigitte Kunze,^c Volker Huch,^d Andreas Kitsche,^e Hans Reichenbach,^c Gerhard Höfle,^c Rolf Müller,^{b,*} and Markus Kalesse^{a,c,*}

^a Institute for Organic Chemistry, Leibniz Universität Hannover, Schneiderberg 1B, D-30167 Hannover, Germany,

^b Helmholtz Institute for Pharmaceutical Research Saarland, Helmholtz Centre for Infection Research and Department of Pharmaceutical Biotechnology, Saarland University, Building E8.1, D-66123 Saarbrücken, Germany

^c Helmholtz Centre for Infection Research (HZI), Inhoffenstr. 7, D-38124 Braunschweig, Germany

^d Institute for Inorganic Chemistry, Saarland University, Building B2.2, D-66123 Saarbrücken, Germany

^e Institute for Biostatistics, Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419 Hannover

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1 Isolation and Structure Elucidation

1.1 Fermentation and Isolation Procedure

Producing organism and Cultivation

The myxobacterium *Polyangium sp.*, strain Pl 4620, was isolated from a soil sample taken in the near of Antalya, Turkey. Antalid was isolated from cultures of this strain whereas numerous alternative myxobacterial producers have been identified by conducting the in-house screening platform of the Helmholtz-Institute for Pharmaceutical Research Saarland.

Fermentation and Isolation

For the isolation of antalid, strain Pl 4620 was cultivated in a 150 L bioreactor equipped with a flat-blade turbine stirrer (Bioengineering, Wald, Switzerland) and containing 90 L of probion liquid medium consisting of 0.3% Probion (single cell protein, prepared from *Methylomonas clarae*, Hoechst), 0.3% soluble starch, 0.2 % MgCl₂•7H₂O, 0.05 % CaCl₂•2H₂O, HEPES buffer 10 mM (2.38 g/L), 50 mL of a standard vitamin solution, and to which 1% (v/v) of the adsorber resin Amberlite XAD 16 (Rohm and Haas, Frankfurt, Germany) was added before sterilization. The pH was adjusted to 7.0 with 1 N KOH. Foam formation was blocked by the addition of a 30% antifoam emulsion (AS-DC-AF, Dow Corning, USA). The fermenter was inoculated with 12x400 mL (4.8 L) cultures in 1 L Erlenmeyer flasks grown for 4 days in the same medium (HEPES 50 mM) on a gyratory shaker at 160 rpm and collected after growth in a sterile 5-Liter flask. The bioreactor was kept at 30 °C, aerated at 0.05 vvm per minute and agitated at 100 rpm for 5 days. At the end of fermentation the XAD resin and the cells were collected by centrifugation.

After removal of the cells from the XAD 16 resin, the compounds were eluted with methanol (7.5 L). The organic solvent was evaporated and the aqueous layer extracted with ethyl acetate, dried with sodium sulfate, filtered and concentrated in vacuo to yield 14.7g crude extract. The residue was solved in 600 mL methanol and extracted with n-heptane three times. The methanol layer contains the antalids, which were further purified by a silicagel filtration with a dichloromethane/methanol gradient as solvent. After evaporation of the enriched compound (8.6g) the fraction was separated by RP-MPLC in two portions [column 300 x 60 mm,

(Kronlab); HD-Sil RP-18, 20-45 μ m 60 A°; solvent methanol water gradient from 50% methanol to 100% methanol in 180 min.; flow: 20 mL/min.; detection 226 nm]. After evaporation of the antalid-containing fraction, the yield of pure compound was 30 mg.

Optical rotation of antalid (**1**) was measured on a Jasco P-2000 polarimeter at 20 °C at a wavelength of λ 589 nm (sodium D line) in a 0.1 mL quartz cell (0.1 dm length). The concentration is given in g/100 mL. $[\alpha]_D$ (20 °C): −14.5 (c = 1.0, CHCl₃).

1.2 NMR data

¹H NMR, ¹³C NMR and 2D spectra were recorded with a Bruker AV-500 [500 MHz (¹H) and 125 MHz (¹³C)] spectrometer in DMSO-d₆. Chemical shifts are reported in ppm relative to TMS. The solvent was used as the internal standard. See Chapter 1.7 for the spectra.

Table 1: NMR-data (500 MHz, DMSO-d₆) for compound antalid (**1**).

position	δ_H , mult (J in Hz)	δ_C	COSY	HMBC
1	0.79, d (7.4)	9.6	2	2, 3
2	1.29, m	30.3	1, 3	1, 3, 4
3	3.41, m	68.6	2, 3-OH, 4	1, 2, 4, 5
3-OH	4.43, d (5.7)	-	3	2, 3, 4
4	1.63, m	37.6	3, 5	2, 3, 5, 6
5	4.76, m	77.3	4, 6	3, 4, 6, 7, 8, 20
6	2.87, m	35.1	5, 7, 8	7, 8, 9, 10, 11
7	1.12, d (7.0)	12.7	6	5, 6, 8
8	6.60, dd (8.5, 1.3)	136.3	6, 10	5, 6, 7, 9, 10, 11
9	-	132.5	-	-
10	1.79, d (1.0)	13.0	8	6, 8, 9, 11
11	-	171.6	-	-
12	5.23, dd (9.2, 3.7)	56.5	12-NH, 13	11, 13, 14, 15, 17
12-NH	7.96, d (9.3)	-	12	11, 12
13	2.27, m	33.6	12, 14	12, 14, 15
14a	0.77, d (6.8)	16.7	13	12, 13
14b	1.04, d (6.6)	20.2	13	12, 13, 15

15	-	171.4	-	-
16	8.34, s	124.7	-	12, 15, 17, 18
17	-	147.0	-	-
18	-	159.9	-	-
19	4.57, m	57.1	19-NH, 21	5, 18, 20, 21, 22
19-NH	8.25	-	19	17, 18, 19, 20, 21
20	-	168.4	-	-
21a	3.01, dd (<i>13.3, 10.0</i>)	37.9	19, 21b	19, 20, 22, 23, 24
21b	3.23, dd (<i>13.3, 5.6</i>)		19, 21a	19, 20, 22, 23, 24
22	-	136.2	-	-
23	7.30, m	128.5	24	22
24	7.24, m	120.1	23, 25	21
25	7.24, m	126.9	24	24

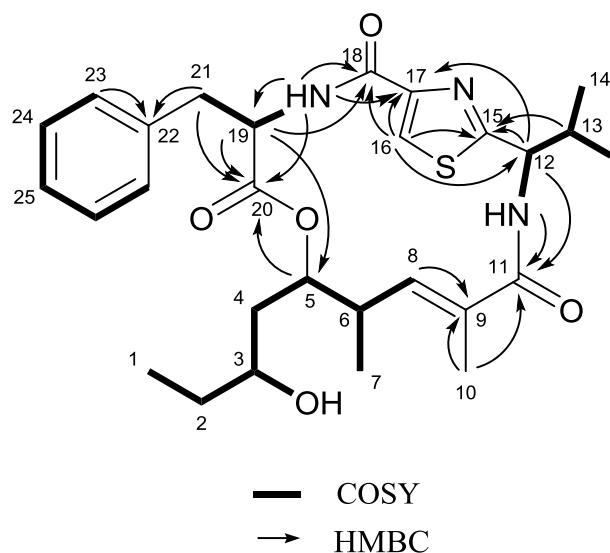


Figure 1: Key 2D NMR correlations for antalid (1)

1.3 MS data

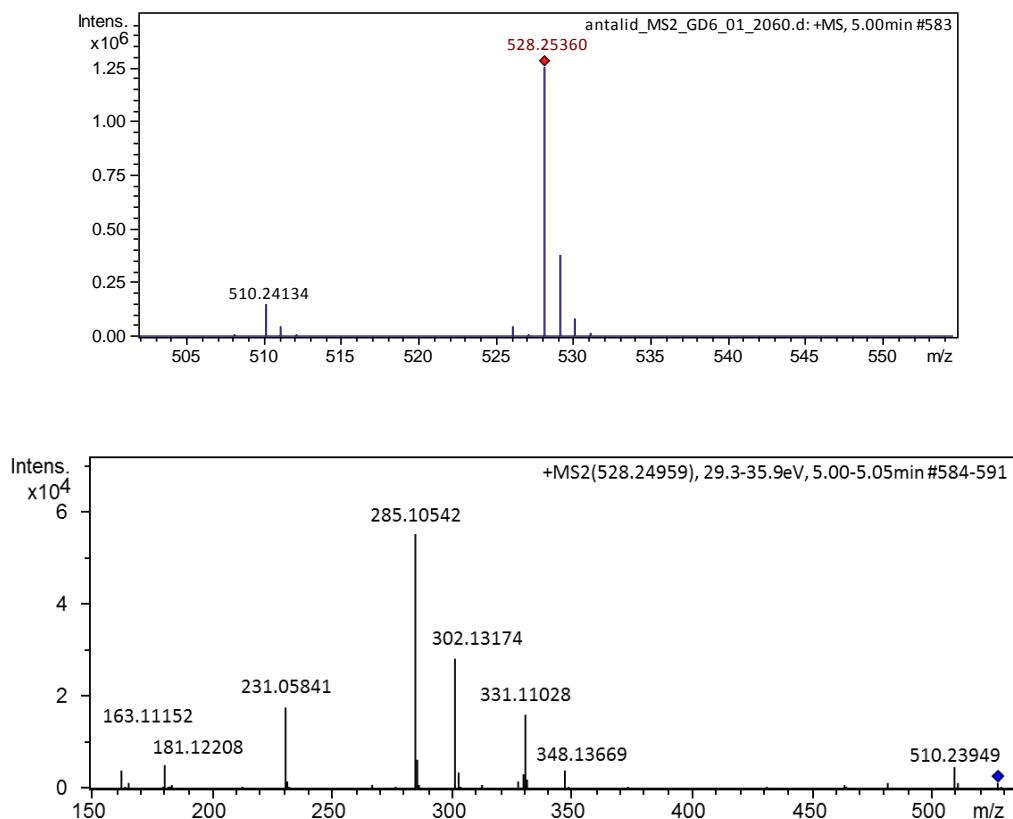


Figure 2: ESI-MS and ESI-MS/MS analysis of antalid, with $[M+H]^+$ = 528.2536 m/z (calc. for $C_{28}H_{38}N_3O_5S^+$: 528.2527, Δ = 1.77 ppm). Measured on a Bruker maXis 4G UHR-qToF.

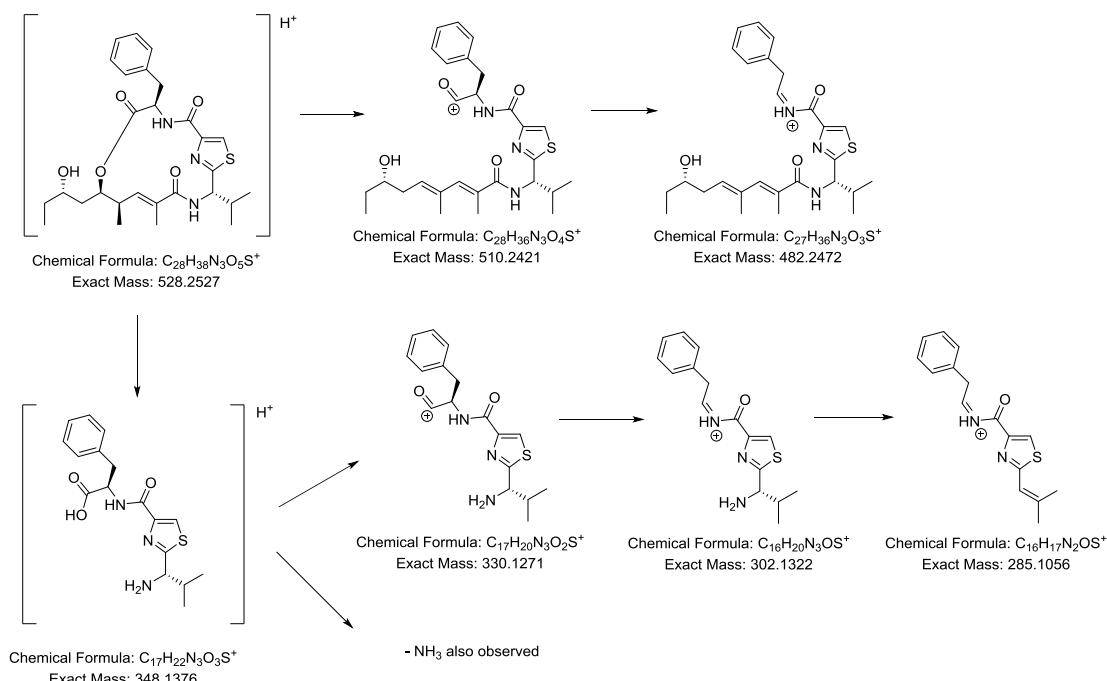


Figure 3: Tentative structures that would fit the m/z values observed for a CID-based fragmentation of antalid.

1.4 Crystal structure

Crystals suitable for single-crystal x-ray analysis were obtained from DMSO-d₆ /MeOH mixture (1:1) at -80 °C, 3 weeks. The data were collected at 152 K on a BrukerAXS X8Apex CCD diffractometer operating with graphite-monochromatized Mo K α radiation. Frames of 0.5° oscillation were exposed; deriving 10532 unique reflections ($R_{\text{int}} = 0.02$) in the θ range of 2 to 31° with a completeness of ~99%. Structure solution and full least-squares refinement with anisotropic thermal parameters of all non-hydrogen atoms and rigid group refinement of the hydrogen were performed using SHELX^[1]. The final refinement results in $R1 = 0.028$ and the absolute configuration could be determined by anomalous-dispersion (Flack = 0.02(4)).

Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers CCDC 1463242 (www.ccdc.cam.ac.uk/data_request/cif).

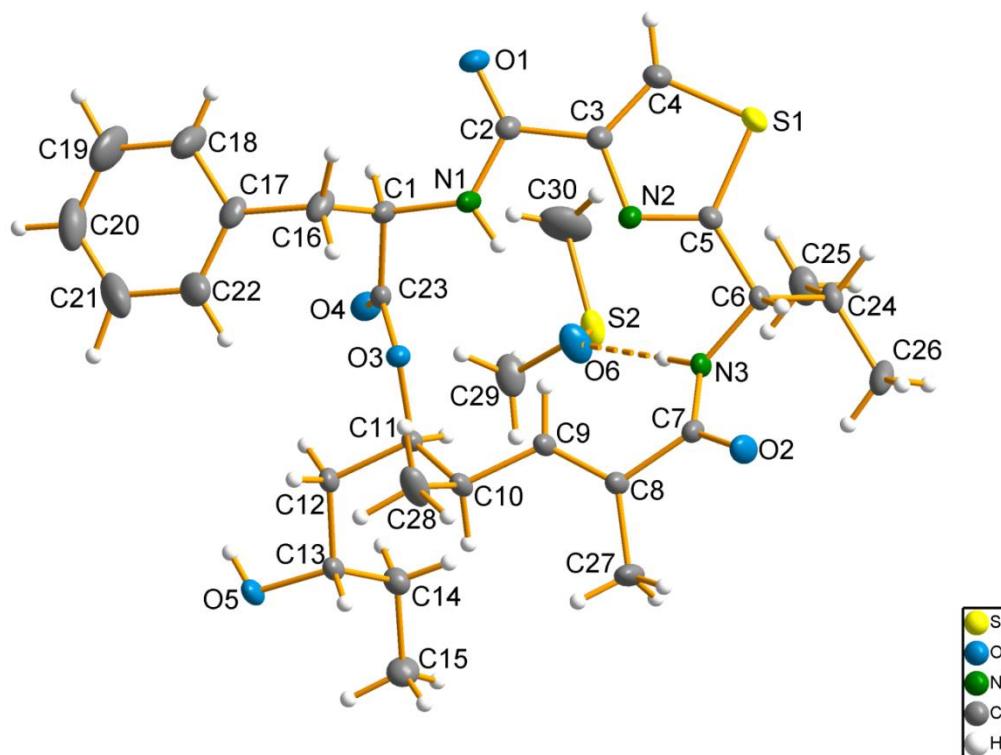


Figure 4: Crystal structure of antalid

Table 2: Crystal data and structure refinement for antalid (=sh3571)

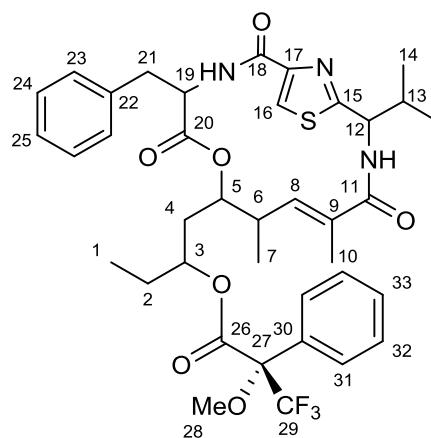
Identification code	sh3571
Empirical formula	C ₃₀ H ₄₃ N ₃ O ₆ S ₂
Formula weight	605.79

Temperature	152(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 ₁
Unit cell dimensions	a = 9.7001(3) Å b = 16.4251(6) Å c = 10.6115(3) Å
	α= 90°. β= 111.8116(14)°. γ = 90°.
Volume	1569.64(9) Å ³
Z	2
Density (calculated)	1.282 Mg/m ³
Absorption coefficient	0.215 mm ⁻¹
F(000)	648
Crystal size	0.926 x 0.611 x 0.112 mm ³
Theta range for data collection	2.067 to 31.671°.
Index ranges	-13<=h<=14, -24<=k<=24, -15<=l<=15
Reflections collected	49059
Independent reflections	10532 [R(int) = 0.0232]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7462 and 0.7016
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	10532 / 1 / 542
Goodness-of-fit on F ²	1.038
Final R indices [I>2sigma(I)]	R1 = 0.0281, wR2 = 0.0685
R indices (all data)	R1 = 0.0312, wR2 = 0.0704
Absolute structure parameter	0.002(10)
Extinction coefficient	n/a
Largest diff. peak and hole	0.283 and -0.202 e.Å ⁻³

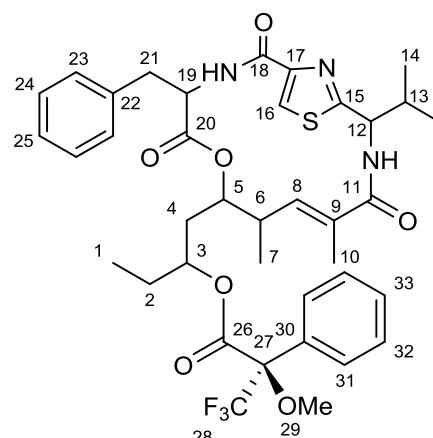
1.5 Preparation and Analysis of (*R*)- and (*S*)-Mosher esters

Reaction control *via* LC/MS was performed on a Dionex UltiMate 3000 system (Autosampler, Column Compartment, Pump, UV-Detector, column: Acquity UPLC BEH C18 1.7 μ m d_p, 2.1 x 50 mm, Waters) and a Bruker amaZon speed mass spectrometer.

Semipreparative HPLC: Dionex UltiMate 3000 system (Autosampler, Pump, UV-Detector, Fraction Collector, external column oven), column: Luna C18-RP, 250 x 4.6 mm, 5 µm d_p, Phenomenex.



(S)-Mosher ester (**1-a**)



(R)-Mosher ester (**1-b**)

Figure 5: Structure of the two Mosher ester of antalid.

Preparation protocol:

First, four stock solutions were prepared:

A – (S)-Mosher acid¹: 2.5 mg (10.68 µmol) in 50 µl CH₂Cl₂ *abs.* (0.2136 mol L⁻¹)

B – (*R*)-Mosher acid¹: 2.5 mg (10.68 µmol) in 50 µl CH₂Cl₂ *abs.* (0.2136 mol L⁻¹)

C – DIC (*N,N'*-diisopropylcarbodiimide): 5.4 μ l in 100 μ l CH_2Cl_2 *abs.* (0.3509 mol L⁻¹)

D – DMAP (*N,N'*-dimethylaminopyridine): 8.6 mg in 100 μ l CH₂Cl₂ *abs.* (0.7039 mol L⁻¹)

(S)-Mosher ester (**1-a**):

To a solution of antalid (**1**, 0.6 mg, 1.14 µmol, 1.0 eq) in CH₂Cl₂ *abs.* (50 µL) were added stock solution **A** ((S)-Mosher acid, 17 µL, 3.63 µmol, 3.1 eq), stock solution **C** (DIC, 10 µL, 3.51 µmol, 3.1 eq) and stock solution **D** (DMAP, 5 µL, 3.51 µmol, 3.1 eq) and the mixture was stirred at rt. Reaction control (LC/MS) after 20 h showed significant conversion. The reaction mixture was diluted with MeCN (ca. 1:1) and purified using semipreparative HPLC (gradient: H₂O:MeOH 95:5 → 5:95 in 20 min, then 6 min at 95 % MeOH, both solvents without

¹ Mosher acid: α -methoxy- α -trifluoromethylphenylacetic acid (MTPA)

additives), affording 0.7 mg (0.94 μ mol, 83 %) of (*S*)-Mosher ester **1-a** as a colorless solid. ^1H NMR (500 MHz, DMSO-*d*₆): δ = 0.68 (t, *J* = 7.4 Hz, 3H, 1-H), 0.78 (d, *J* = 6.8 Hz, 3H, 14-H_a), 1.06 (d, *J* = 6.7 Hz, 3H, 14-H_b), 1.12 (d, *J* = 7.0 Hz, 3H, 7-H), 1.52 (m, 2H, 2-H), 1.68 (s, 3H, 10-H), 1.80 (ddd, *J* = 14.6, 5.0, 5.0 Hz, 1H, 4-H_a), 1.98 (ddd, *J* = 14.6, 7.4, 7.4 Hz, 1H, 4-H_b), 2.28 (m, 1H, 13-H), 2.72 (m, 1H, 6-H), 3.03 (dd, *J* = 13.4, 9.8 Hz, 1H, 21-H_a), 3.30 (m, 1H, 21-H_b), 4.63 (m, 1H, 19-H), 4.67 (m, 1H, 5-H), 5.17 (m, 1H, 3-H), 5.24 (dd, *J* = 91.1, 3.7 Hz, 1H, 12-H), 6.62 (d, *J* = 7.4 Hz, 1H, 8-H), 7.21–7.29 (m, 5H, 23-H, 24-H, 25-H), 7.43–7.48 (m, 5H, 31-H, 32-H, 33-H), 7.96 (d, *J* = 9.1 Hz, 1H, 12-NH), 8.36 (s, 1H, 16-H), 8.36 (d, *J* = 7.4 Hz, 1H, 19-NH).

(*R*)-Mosher ester (**1-b**):

To a solution of antalid (**1**, 0.7 mg, 1.33 μ mol, 1.0 eq) in CH₂Cl₂ *abs.* (50 μ L) were added stock solution **B** ((*R*)-Mosher acid, 19 μ L, 4.06 μ mol, 3.1 eq), stock solution **C** (DIC, 12 μ L, 4.21 μ mol, 3.1 eq) and stock solution **D** (DMAP, 6 μ L, 4.22 μ mol, 3.1 eq) and the mixture was stirred at rt. Reaction control (LC/MS) after 20 h showed significant conversion. The reaction mixture was diluted with MeCN (ca. 1:1) and purified using semipreparative HPLC (gradient: H₂O:MeOH 95:5 → 5:95 in 20 min, then 6 min at 95 % MeOH, both solvents without additives), affording 0.8 mg (1.08 μ mol, 81 %) of (*R*)-Mosher ester **1-b** as a colorless solid. ^1H NMR (500 MHz, DMSO-*d*₆): δ = 0.79 (d, *J* = 6.8 Hz, 3H, 14-H_a), 0.80 (t, *J* = 7.4 Hz, 3H, 1-H), 1.06 (d, *J* = 7.0 Hz, 3H, 7-H), 1.07 (d, *J* = 6.5 Hz, 3H, 14-H_b), 1.59 (m, 2H, 2-H), 1.65 (s, 3H, 10-H), 1.75 (ddd, *J* = 14.7, 5.1, 5.1 Hz, 1H, 4-H_a), 1.92 (ddd, *J* = 14.8, 7.3, 7.3 Hz, 1H, 4-H_b), 2.28 (m, 1H, 13-H), 2.56 (m, 1H, 6-H), 3.02 (dd, *J* = 13.4, 9.7 Hz, 1H, 21-H_a), 3.27 (m, 1H, 21-H_b), 4.63 (ddd, *J* = 9.7, 7.4, 5.9 Hz, 1H, 19-H), 4.58 (m, 1H, 5-H), 5.21 (m, 1H, 3-H), 5.23 (m, 1H, 12-H), 6.57 (d, *J* = 7.0 Hz, 1H, 8-H), 7.20–7.28 (m, 5H, 23-H, 24-H, 25-H), 7.45–7.49 (m, 5H, 31-H, 32-H, 33-H), 7.88 (d, *J* = 9.3 Hz, 1H, 12-NH), 8.36 (s, 1H, 16-H), 8.31 (d, *J* = 7.4 Hz, 1H, 19-NH).

Analysis

For both Mosher esters (**1-a** and **1-b**), ^1H NMR spectra were recorded in DMSO-d₆. All signals were assigned to the corresponding protons for both diastereomers **1-a** and **1-b**. Analysis of the differences $\Delta\delta$ ($=\delta_S - \delta_R$) of the chemical shifts for all protons was performed as described in the literature (T. R. Hoye *et al.*^{17b}). Results are summarized in Table 2 and Figure 6, ^1H NMR spectra for **1-a** and **1-b** are depicted in Figure 7.

Table 2: $\Delta\delta$ ($=\delta_S - \delta_R$)-data for the (S)- and (R)-antalid-Mosher esters **1-a** and **1-b**.

	δ S-ester [ppm]	δ R-ester [ppm]	ppm	$\Delta\delta^{\text{SR}}$ ($=\delta_S - \delta_R$) Hz (500 MHz)
1	0.6833	0.8041	-0.1208	-60.4
2	1.5180	1.5870	-0.0690	-34.5
3	5.1687	5.2119	-0.0432	-21.6
4a	1.7960	1.7545	0.0415	20.8
4b	1.9803	1.9150	0.0653	32.7
5	4.6725	4.5758	0.0967	48.4
6	2.7226	2.5591	0.1635	81.8
7	1.1242	1.0583	0.0659	33.0
8	6.6154	6.5721	0.0433	21.7
10	1.6776	1.6497	0.0279	14.0
12	5.2434	5.2337	0.0097	4.9
12-NH	7.9560	7.8845	0.0715	35.8
13	2.2765	2.2846	-0.0081	-4.1
14a	0.7768	0.7857	-0.0089	-4.4
14b	1.0633	1.0726	-0.0093	-4.7
16	8.3597	8.3604	-0.0007	-0.4
19	4.6346	4.6328	0.0018	0.9
19-NH	8.3594	8.3143	0.0451	22.6
21a	3.0326	3.0245	0.0081	4.0
21b	3.2970	3.2725	0.0245	12.3

$\Delta\delta^{\text{SR}}$ ($=\delta_S - \delta_R$):
negative $\rightarrow R^2$

$\Delta\delta^{\text{SR}}$ ($=\delta_S - \delta_R$):
positive $\rightarrow R^1$

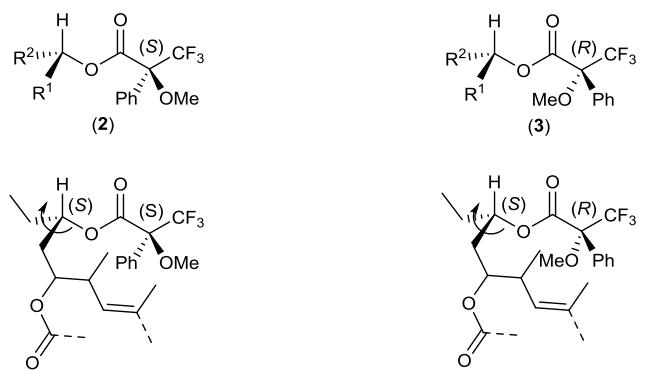
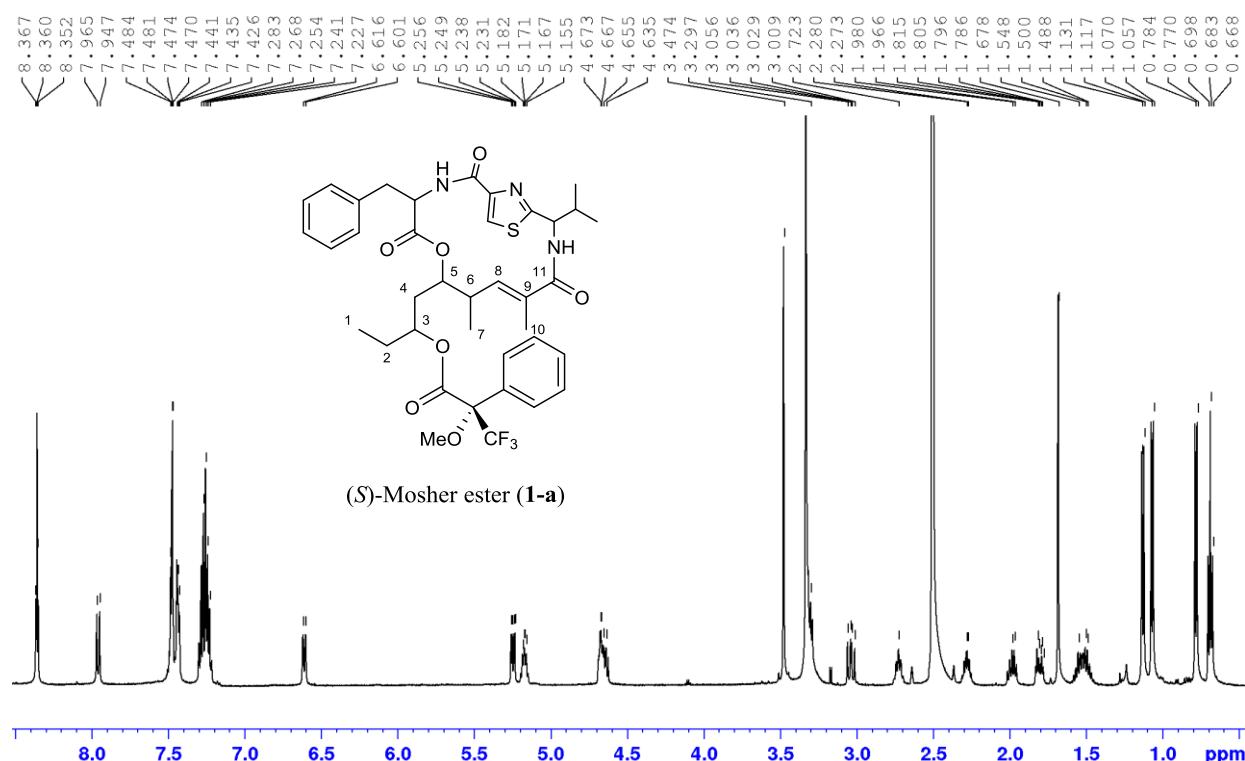


Figure 6: Determination of the absolute stereochemistry for antalid (**1**) via Mosher ester analysis. Positive $\Delta\delta^{\text{SR}}$ values mean that the corresponding protons are located in R^1 , negative $\Delta\delta^{\text{SR}}$ values belong to R^2 protons.



*Figure 7: ^1H NMR spectra for Mosher ester **1-a**.*

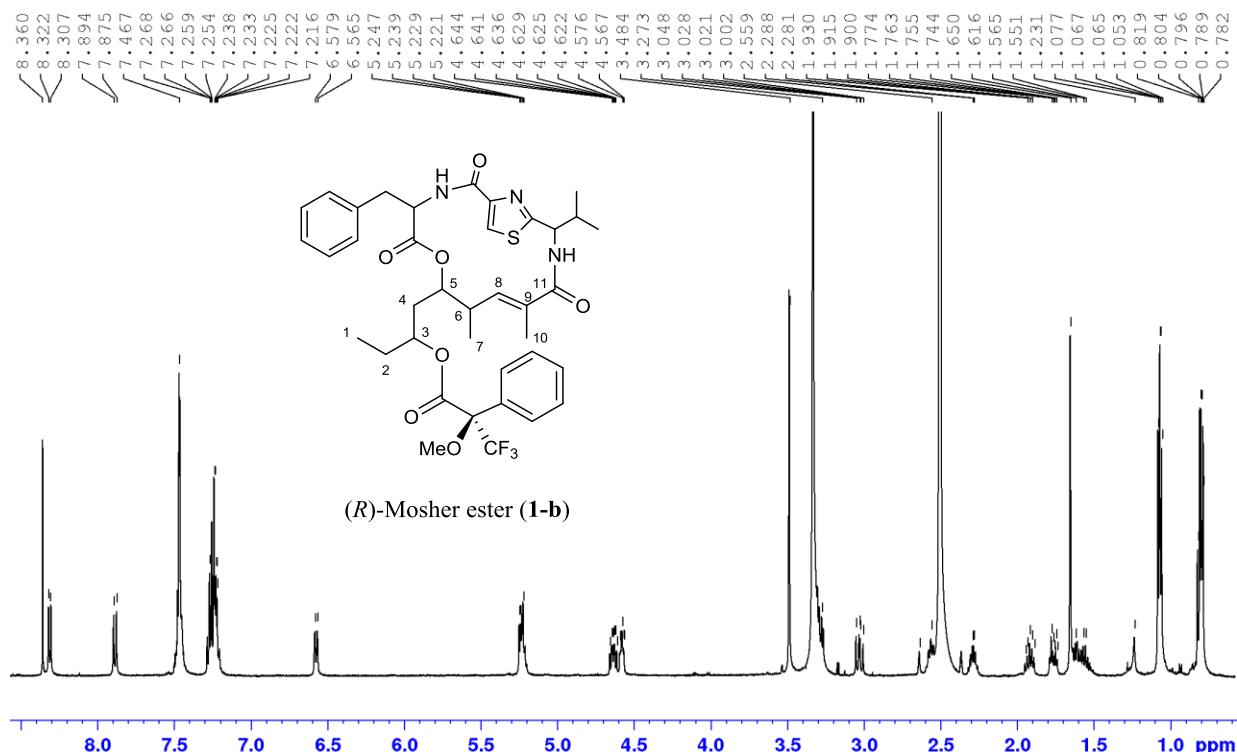


Figure 8: ^1H NMR spectra for Mosher ester **1-b**.

1.6 Absolute Stereochemistry of antalid

The stereochemistry could be completely elucidated by the combination of the results from the crystal structure and the Mosher ester analysis. The Carbon atom number labeling of the NMR data does not correspond to that of the crystal structure.

Table 3: Absolute stereochemistry of antalid.

$\text{C}_3(\text{OH})$	$\text{C}_5(\text{OR})$	$\text{C}_6(\text{Me})$	$\text{C}_{12}(\text{Val})$	$\text{C}_{19}(\text{Phe})$	
S	R	R	S	R	correct
R	S	S	R	S	

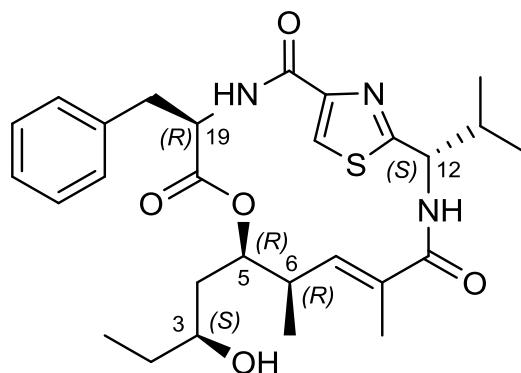


Figure 9: Absolute stereochemistry of antalid.

1.7 Copies of NMR spectra

^1H -NMR spectrum (500 MHz, DMSO- d_6) of Antalid

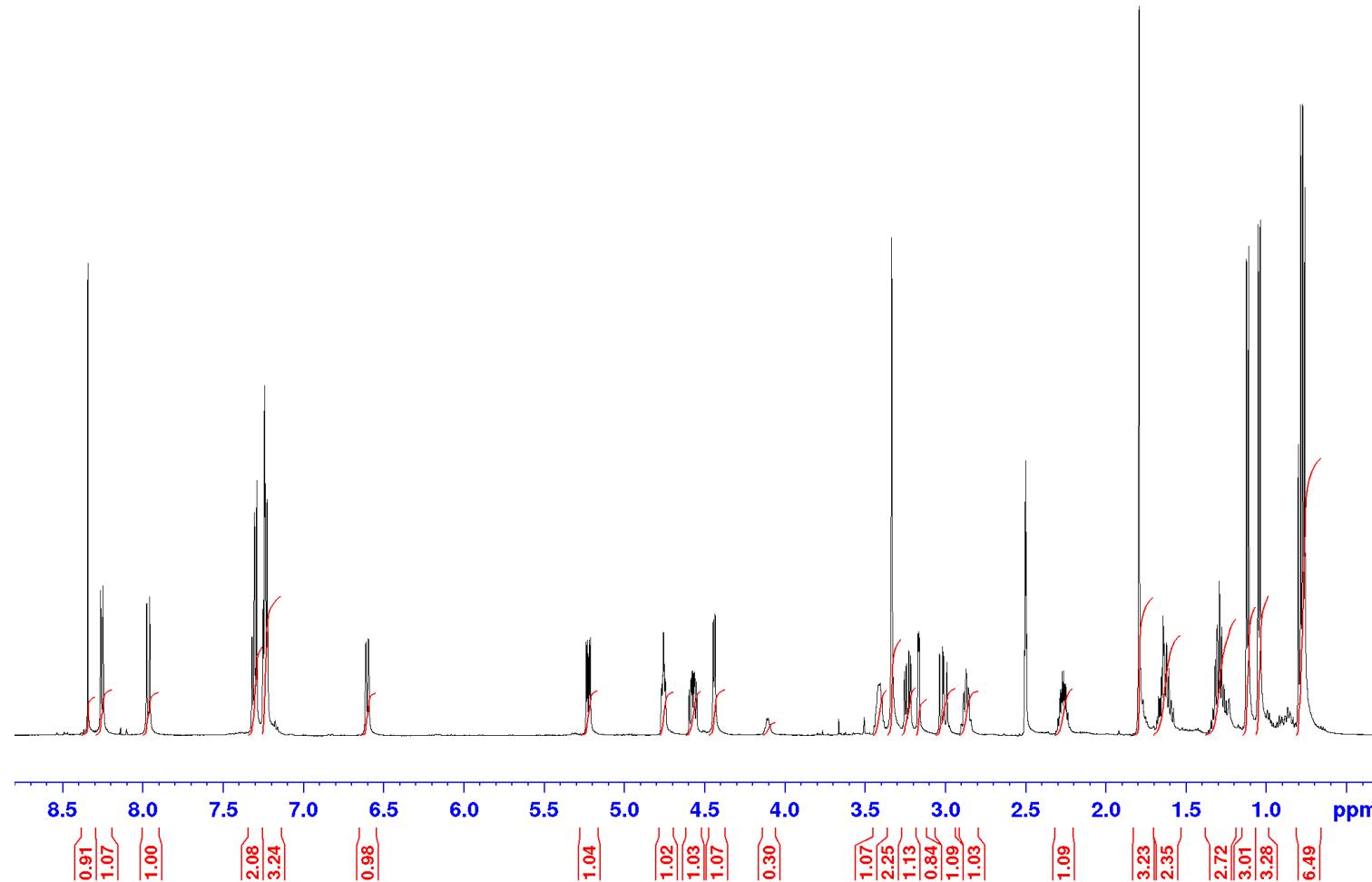


Figure 10: ^1H -NMR spectrum (500 MHz, DMSO- d_6) of antalid.

^{13}C -NMR spectrum (500 MHz, DMSO-d₆) Antalid

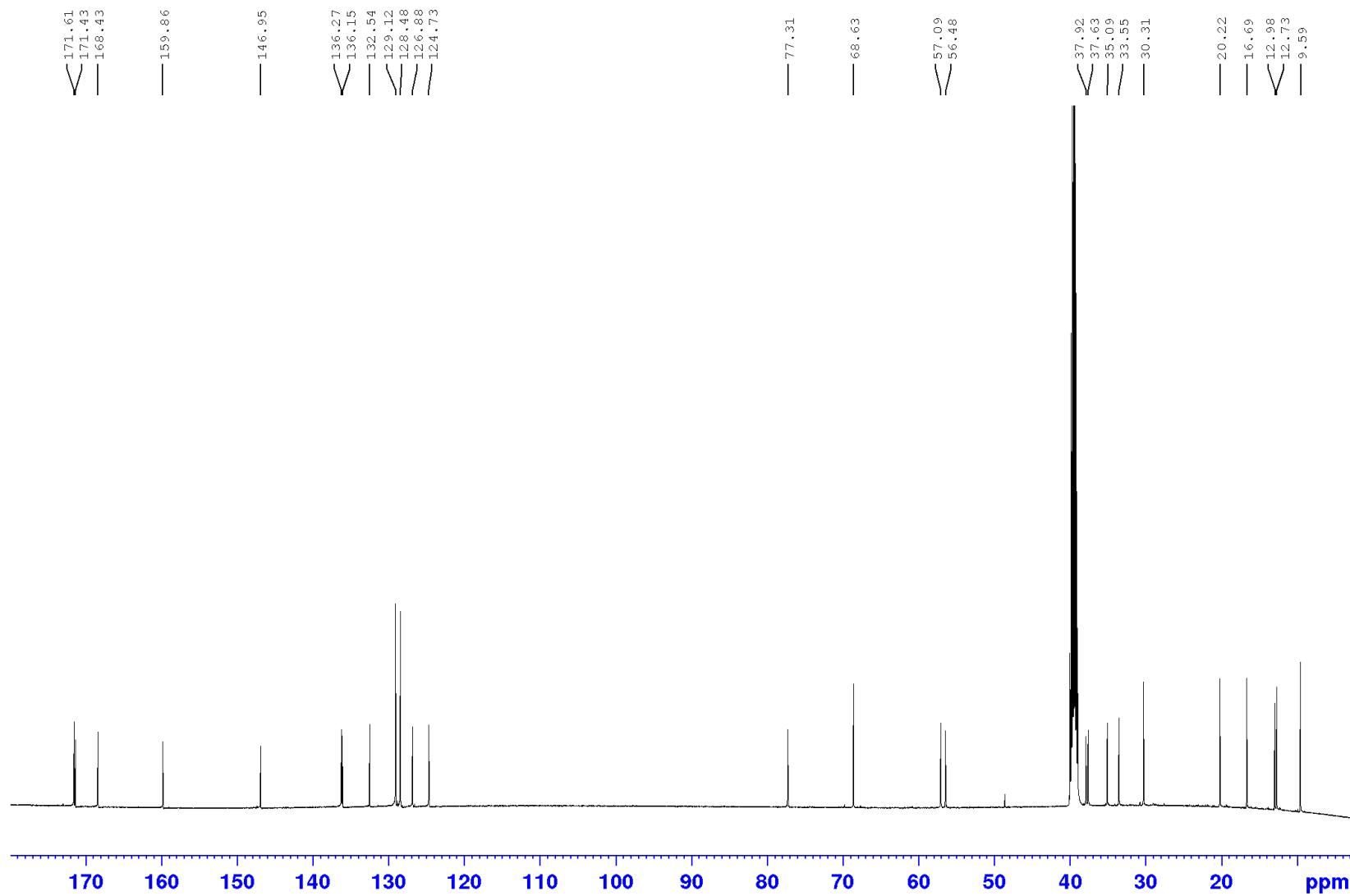


Figure 11: ^{13}C -NMR spectrum (500 MHz, DMSO-d₆) of antalid.

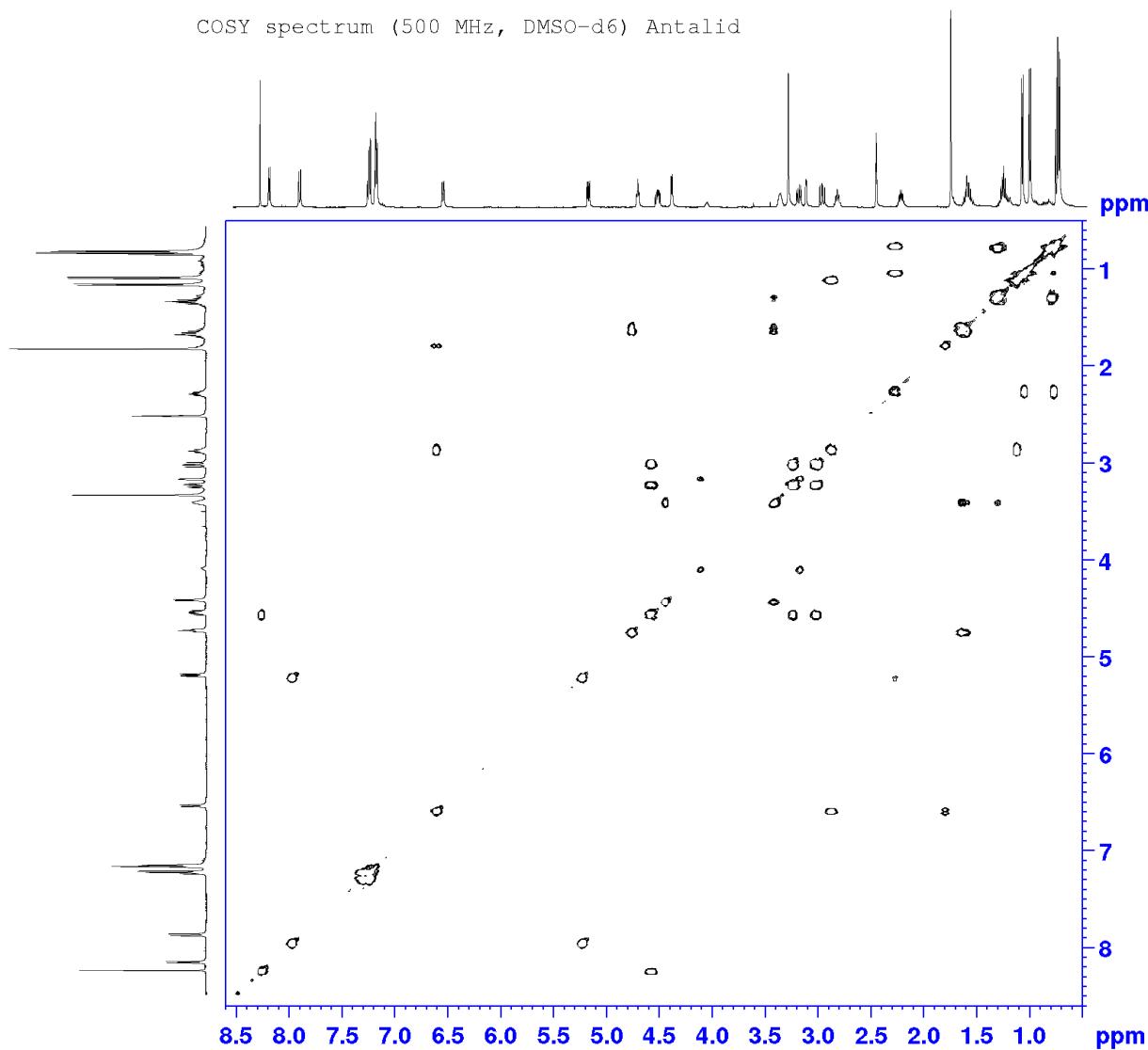


Figure 12: COSY spectrum (500 MHz, DMSO-d₆) of antalid.

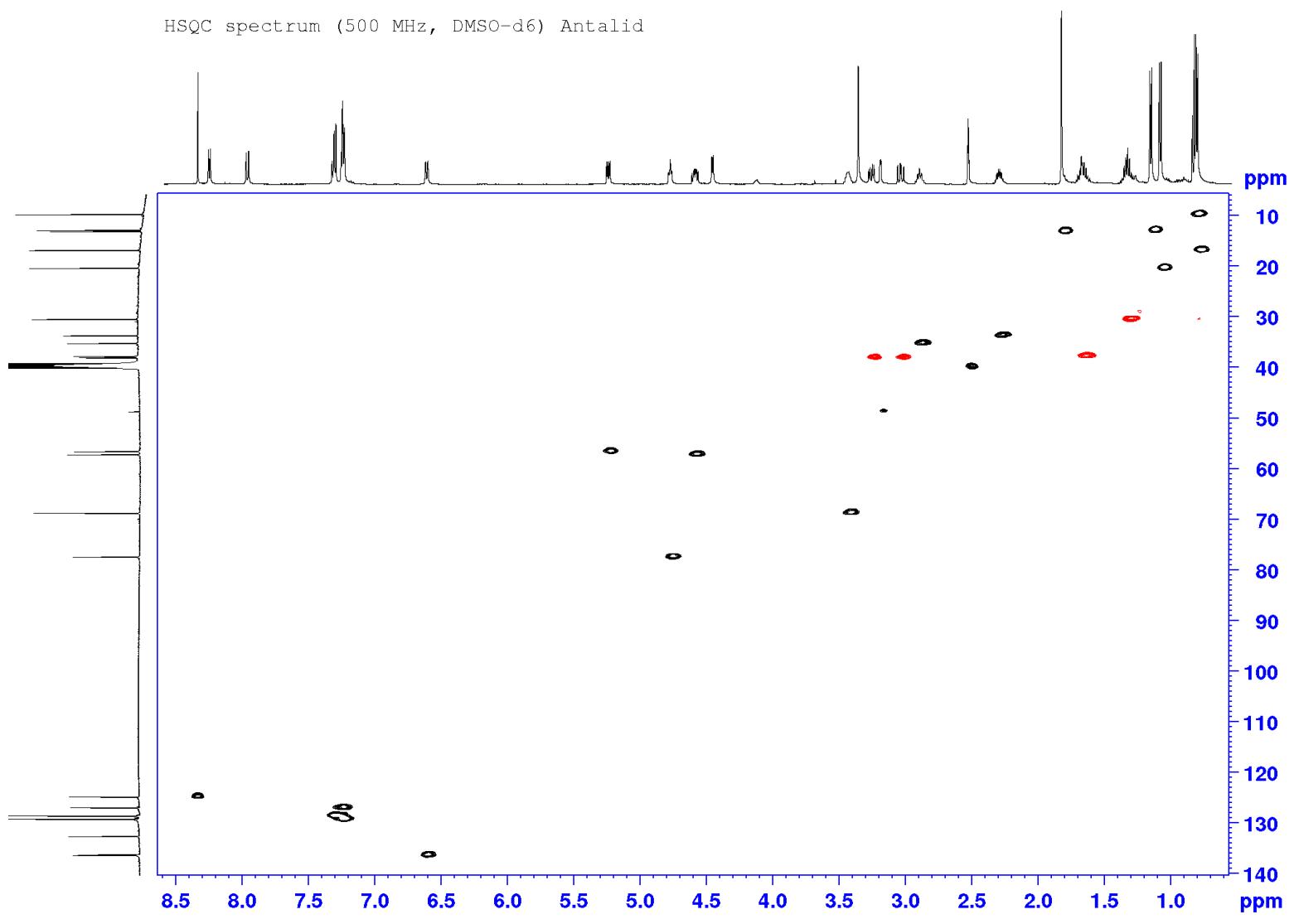


Figure 13: HSQC spectrum (500 MHz, DMSO-d₆) of antalid.

HMBC spectrum (500 MHz, DMSO-d₆) Antalid

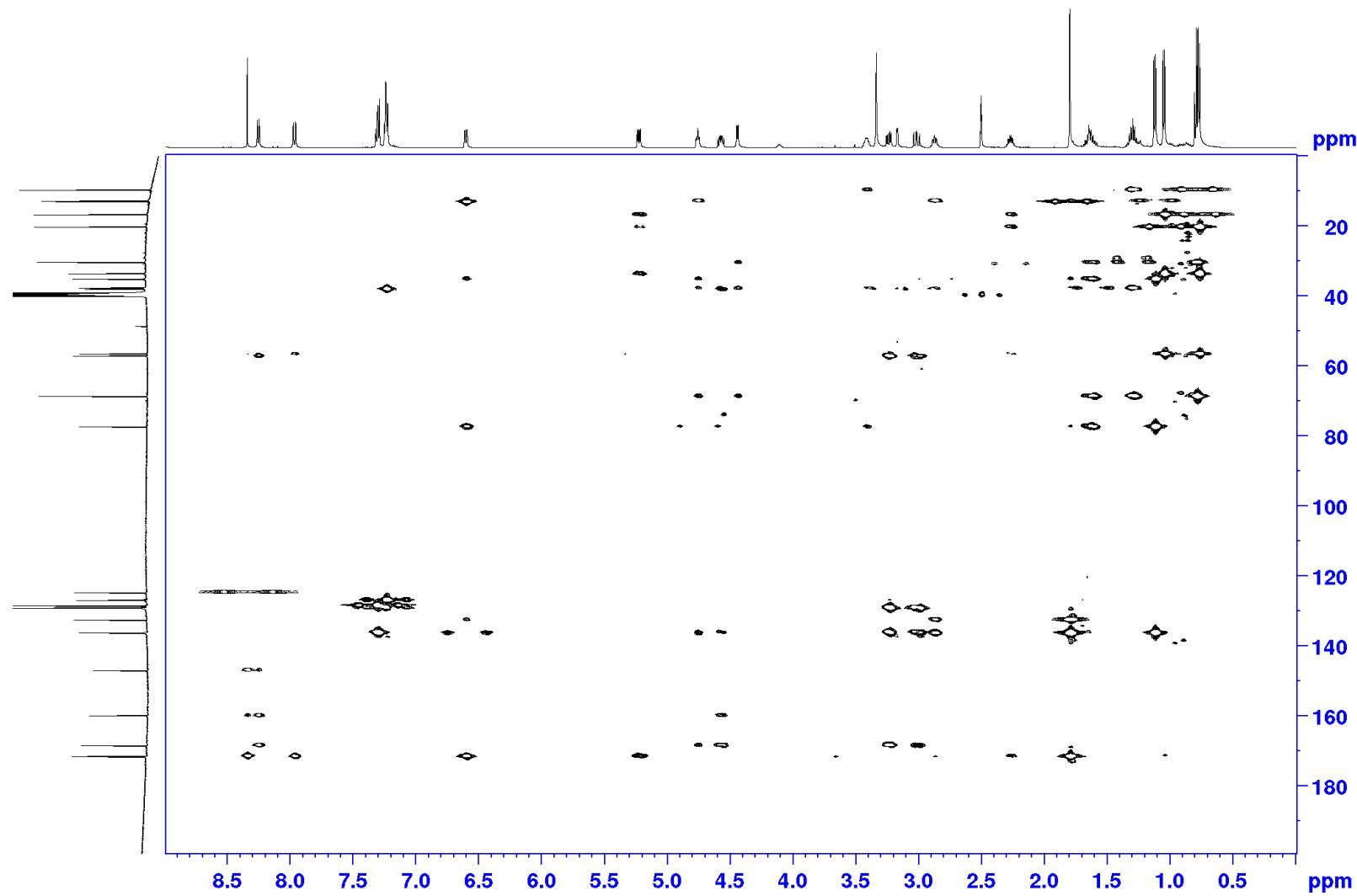


Figure 14: HMBC spectrum (500 MHz, DMSO-d₆) of antalid.

2 Bioactivity screening

Results from the bioactivity screening (performed by Jennifer Herrmann and Viktoria Schmitt) were summarized in Table 3. Antalid showed no antibacterial activity and no significant activity against CHO-K1 cell line.

Table 4: Bioactivity of antalid against different bacteria and cell lines

Test Strain	MIC [$\mu\text{g mL}^{-1}$]
<i>B. subtilis</i> DSM-10	> 64
<i>M. luteus</i> DSM-1790	> 64
<i>S. aureus</i> Newman	> 64
<i>M. smegmatis</i> mc ² 155	> 64
<i>E. coli</i> (TolC-deficient)	> 64
<i>E. coli</i> DSM-1116	> 64
<i>P. aeruginosa</i> PA14	> 64
<i>C. violaceum</i> DSM-30191	> 64
<i>P. anomala</i> DSM-6766	> 64
<i>C. albicans</i> DSM-1665	> 64
<i>M. hiemalis</i> DSM-2656	> 64
	IC ₅₀ [$\mu\text{g mL}^{-1}$]
CHO-K1 (Chinese hamster ovary cell line)	32.8

2.1 Biological Activity of antalid

The antibiotic spectrum of antalid was determined by an agar diffusion test using paper discs. Antalid showed no inhibitory effect against bacteria, yeasts and fungi as inferred from the results in the table above.

No Cytotoxicity against L929 mouse fibroblasts

In addition, antalid was tested in biofilms of *Streptococcus mutans* and *Gardnerella vaginalis* as well as in a homoserine-lactone (AHL)-based Quorum sensing assay. Antalid showed no noteworthy activity in those test systems.

Table 5: Antimicrobial spectrum of antalid.

Test organism*	Diameter of inhibition zone** (mm)
<i>Escherichia coli</i>	0
<i>Pseudomonas aeruginosa</i>	0
<i>Salmonella sp.</i>	0
<i>Bacillus subtilis</i>	0
<i>Brevibacterium ammoniagenes</i>	not tested
<i>Corynebacterium fascians</i> ***	0
<i>Mycobacterium phlei</i>	0
<i>Staphylococcus aureus</i>	0
<i>Streptococcus faecalis</i>	0
<i>Micrococcus luteus</i> ***	0
<i>Candida albicans</i>	0
<i>Debaryomyces</i>	not tested
<i>Rhodotorula glutinis</i>	not tested
<i>Saccharomyces cerevisiae Myc.</i>	0
<i>Saccharomyces cerevisiae N3</i>	0
<i>Schizosaccharomyces pombe</i> ***	0
<i>Mucor hiemalis</i> ***	0
<i>Botrytis cinerea</i> ***	0
<i>Trichoderma koningii</i>	not tested
<i>Giberella fujikuroi</i>	not tested
<i>Rhizopus arrhizus</i>	not tested
<i>Phythium debaryanum</i>	not tested
<i>Ustilago maydis</i>	not tested
<i>Aspergillus fumigatus</i>	not tested

*Bacteria were tested on nutrient agar, fungi on malt extract peptone agar.

** Determined by the agar diffusion test with 20 µg antalid per 6-mm paper discs

***Also no activity on Minimal agar tested

3 Biosynthesis

Anaylsis of the biosynthetic gene cluster is based on the strain *Polyangium spumosum* MSr6761 which is an alternative myxobacterial producer of antalid.

3.1 Terminal C domain

The antalid biosynthetic gene cluster ends with a C domain. There is no thioesterase (TE) domain which is usually responsible for compound release. Special C_T domains which are able to cyclize NRPS-based compounds from the assembly line are known from several fungal gene clusters.¹ For PKS-based compounds, only FK520 and rapamycin biosynthesis feature a terminal C domain that are likely involved in cyclization of the natural products.¹ However, in both natural products the terminal amino acid is an uncommon pipecolate which may have caused yet another special type of C_T domain. Aside of a fast phylogenetic analysis of C domains using the NaPDoS web tool (see main text's figure for the result), we set out to do the alignment from scratch using a set of 8 known C_T domains alongside with 41 myxobacterial C domains and the NaPDoS repository (185 domains, downloaded 13th November 2015).²

Table 6: Terminal C_T domains which catalyze the release of the biosynthetic product similar to a typical thioesterase domain. The listed C_T domains were used as input sequences for a phylogenetic analysis of C domains.

Name	Description	Organism
Aba1_CT	Aureobasidin A1	<i>Aureobasidium pullulans</i>
Aps1_CT	Apicidin	<i>Fusarium incarnatum</i>
Enn_CT	Enniatin	<i>Fusarium oxysporum f. sp. cubense race 1</i>
Fer3_CT	Ferrichrome A, putative peptide synthetase	<i>Ustilago maydis</i> 521
FkbP_C2_CT	FK520, terminal CT domain	<i>Streptomyces hygroscopicus</i> subsp. <i>ascomyceticus</i>
RapP_C2_CT	Rapamycin terminal C domain	<i>Streptomyces rapamycinicus</i>
SimA_CT	Cyclosporine	<i>Toxopodium inflatum</i>
TqaA_CT	Tryptophalalanine	<i>Penicillium aethiopicum</i>

Four domains from the NaPDoS list were deleted as they messed up the MUSCLE alignment³ and prevented PhyML3.0 from execution as there were too many gaps: hctox4_C3_dual, hctox4_C2_dual, Stro1024_1, SP01_3144. After MUSCLE alignment, the resulting alignment file was forwarded to the PhyML3.0 pipeline for refinement (JTT amino acid substitution model, bootstrap = 100).⁴ The resulting tree is visualized as unrooted tree with transformed branches (cladogram) using Geneious 9.0.4 (Biomatters).

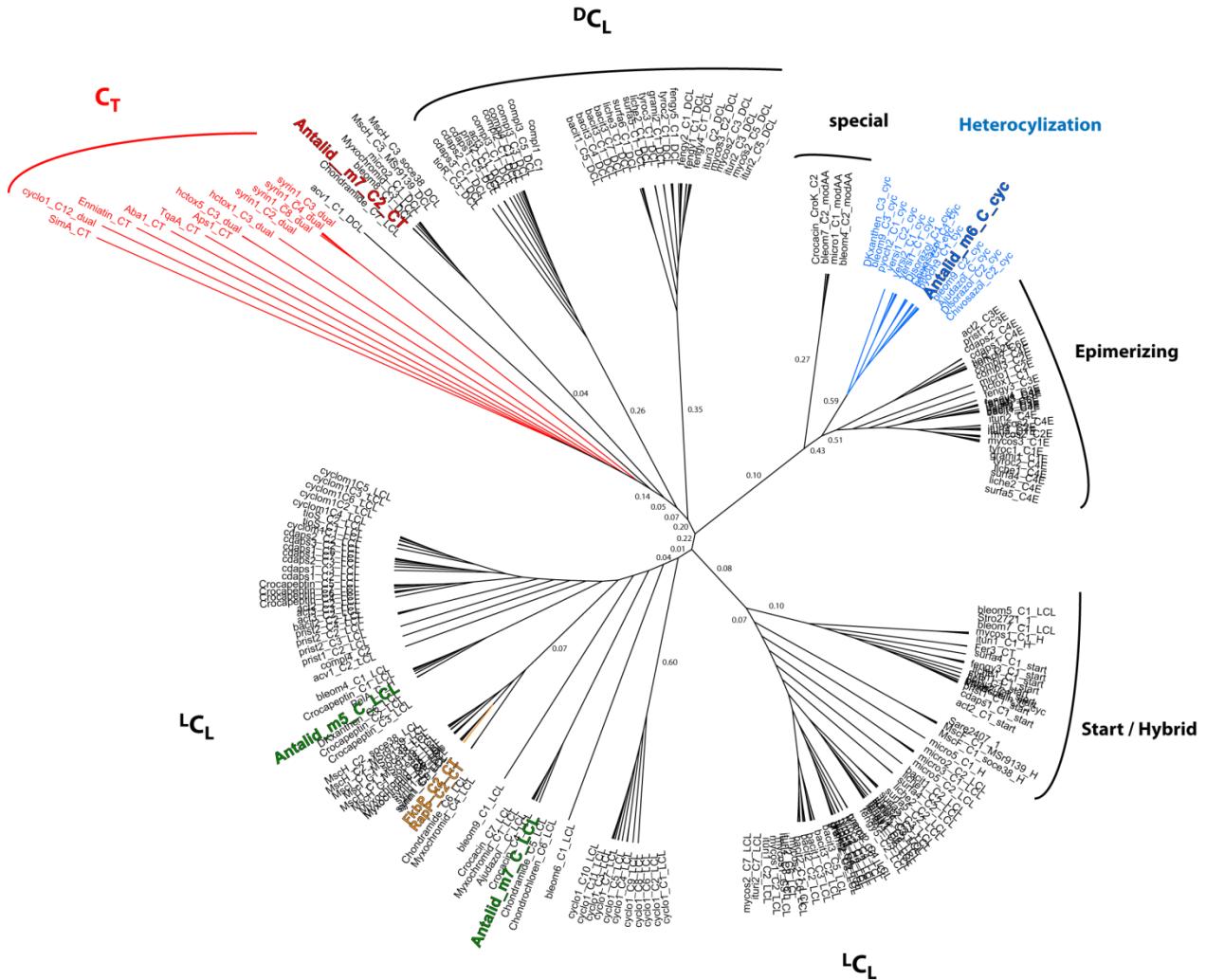


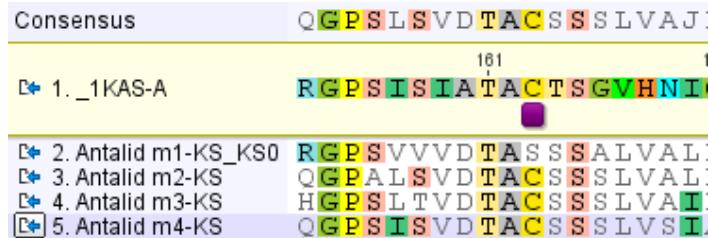
Figure 15: Phylogenetic tree of C-domain amino acid sequence alignment using MUSCLE for alignment and PhyML3.0 for tree creation and refinement. (JTT amino acid substitution model, bootstrap = 100). The terminal antalid C domain, ant-m7-C2 groups close to the known CT domains from fungal NRPS.

The terminal antalid domain, ant-m7-C2 still groups close with the known fungal CT domains derived from NRPS. We can thus conclude that this particular antalid C domain is releasing antalid by means of intramolecular cyclization and thereby replaces a typical TE domain. It should be noted that the two terminal C domains of FK520 and rapamycin biosynthesis group within a ^LC_L clade and are apparently highly similar to “normal” C domains. This strengthens the notion that FkbP_{CT} and RapP_{CT} are special owing to their unusual pipeolate substrate and are thus different from the fungal CT domains known to date.

3.2 KS domains

Analyzing the amino acid sequence of antalid’s KS domains directly shows that the first KS, ant-m1-KS, lacks the active site cysteine (Cys163) which is essential for catalyzing the chain

elongation reaction. Thus, we assume that this first KS catalyzes the decarboxylation of a loaded extender unit.



*Figure 16: Alignment of the antalid KS domains to the reference *E.coli* 1KAS (PDB 1KAS). The first domain ant-m1-KS lacks the active site cysteine at position 163.*

Note that this KS domain lacks the cys to glu replacement which is typical for KS^Q domains found in loading modules of modular PKS but rather features the cys to ser replacement often found in trans-AT-related KS0 domains.⁵⁻⁷ However, a KS domain alignment and subsequent calculation of a phylogenetic tree revealed that AntA-KS1 is closer to KS^Q than to KS0 domains which is in line with the modular PKSI architecture of the antalid cluster. Consequently, we classified the respective domain as KS^Q.

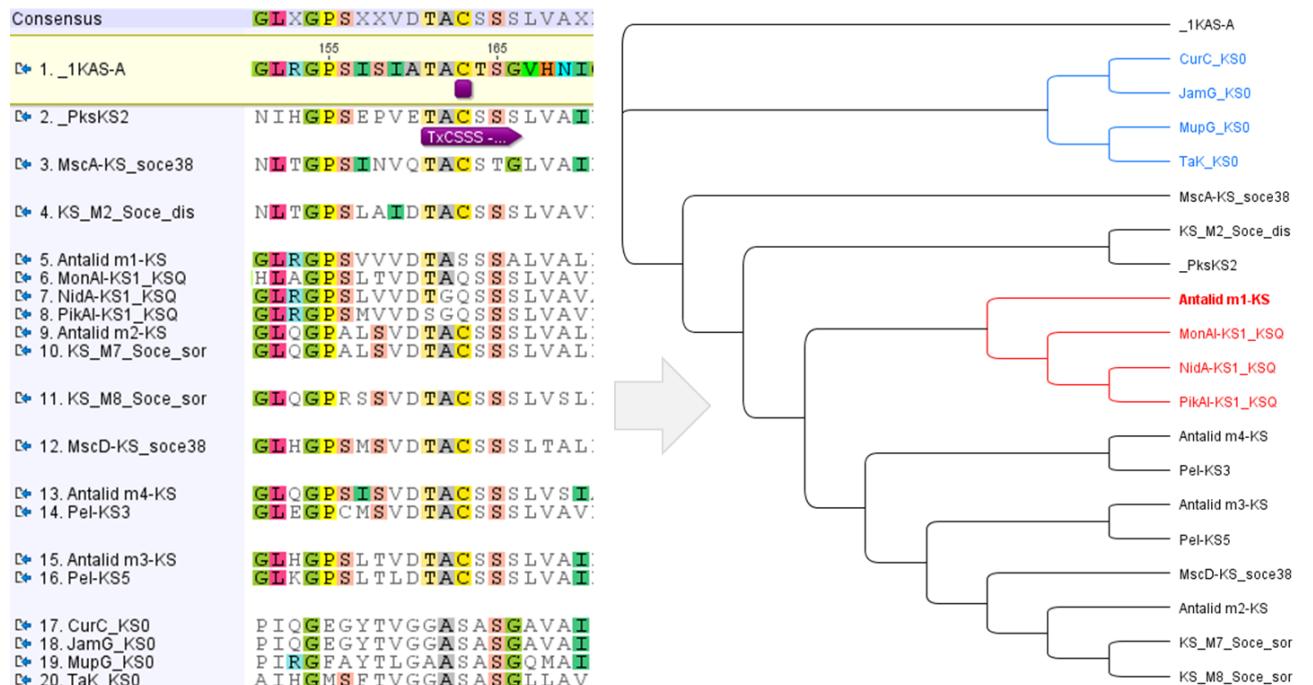


Figure 17: The active site motif TxCSSS with the active cysteine is rather conserved for typical KS and KSQ domains whereas KS0 domains deviate significantly. Accordingly, ant-m1-KS falls in a clade with known KSQ domains while all KS0 domains group separately.

3.3 AT domains

Prediction of the acyl transferase (AT) substrate specificity was done by conserved motif analyses based on the alignment of all antalid AT domains to the reference AT from *E.coli* FAS (PDB 1MLA, UniProtKB P0AAI9). The catalytic motif GxSxG is found in all antalid AT domains. Position 200 is a good indicator for whether mal-CoA or mmal-CoA is loaded.⁸ According to this, m1-AT2, m3-AT and m4-AT are specific for mmal-CoA whereas m2-AT is specific for mal-CoA. The prediction is in agreement with the observed structure of antalid considering that the methylmalonyl loaded onto m1-AT2 is decarboxylated by the KS^Q of module 1. The very first AT domain of module 1 is likely a remnant of an old version of the cluster where the KS^Q was still able to catalyze the condensation reaction. We assume that m1-AT is of no use in the present cluster.

Table 7: Prediction of AT substrate specificity by alignment to 1MLA from E.coli (PDB 1MLA). Amino acid numbering is according to the 1MLA reference sequence.

Domain	observed	predicted	11	63	90	91	92	93	94	117	200	201	231	250	255	15	58	59	60	61	62	70	72	197	198	199
1MLA	Mal	Mal	Q	Q	G	H	S	L	G	R	S	H	N	Q	V	T	K	T	W	Q	T	S	A	S	V	P
m1-AT	-	Prop	L	Q	G	H	S	A	G	Y	G	H	T	N	V	W	D	A	V	F	V	Q	A	D	V	A
m1-AT2	Prop	mmal	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	W	R	V	D	V	V	M	S	D	Y	A
m2-AT	Mal	Mal	Q	Q	G	H	S	I	G	R	F	H	N	H	V	R	E	T	S	Y	T	E	A	S	H	A
m3-AT	mmal	mmal	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	W	R	V	D	V	V	M	G	D	Y	A
m4-AT	mmal	mmal	Q	Q	G	H	S	M	G	R	S	H	T	N	V	W	Q	I	D	V	V	Q	G	D	V	A

200F malonate

200S methyl malonate

others unusual substrates

3.4 KR domains

An alignment of the antalid KR domains shows a functional cofactor binding motif GxGxxGxxxA for each domain.

Table 8: Alignment of the co-factor binding motif region indicates a functional motif GxGxxGxxxA for all antalid ketoreductases.

KR domain	co-factor binding consensus									
	G	x	G	x	x	G	x	x	x	A
Ery_KR1_2S-3R	G	T	G	G	V	G	G	Q	I	A
Tyl_KR1_2R-3R	G	M	G	A	I	G	R	R	L	A
Antalid-m2-KR	G	T	G	A	L	G	G	L	L	A
Antalid-m3-KR	G	L	G	G	L	G	L	V	L	A
Antalid-m4-KR	G	L	G	G	L	G	L	S	V	A

Attempts to predict the stereochemical outcome of the catalyzed reduction turned out to be ambiguous using the motifs described by Keatinge-Clay.⁹ The typical signature amino acids were not indicative for ant-m2-KR and ant-m3-KR.

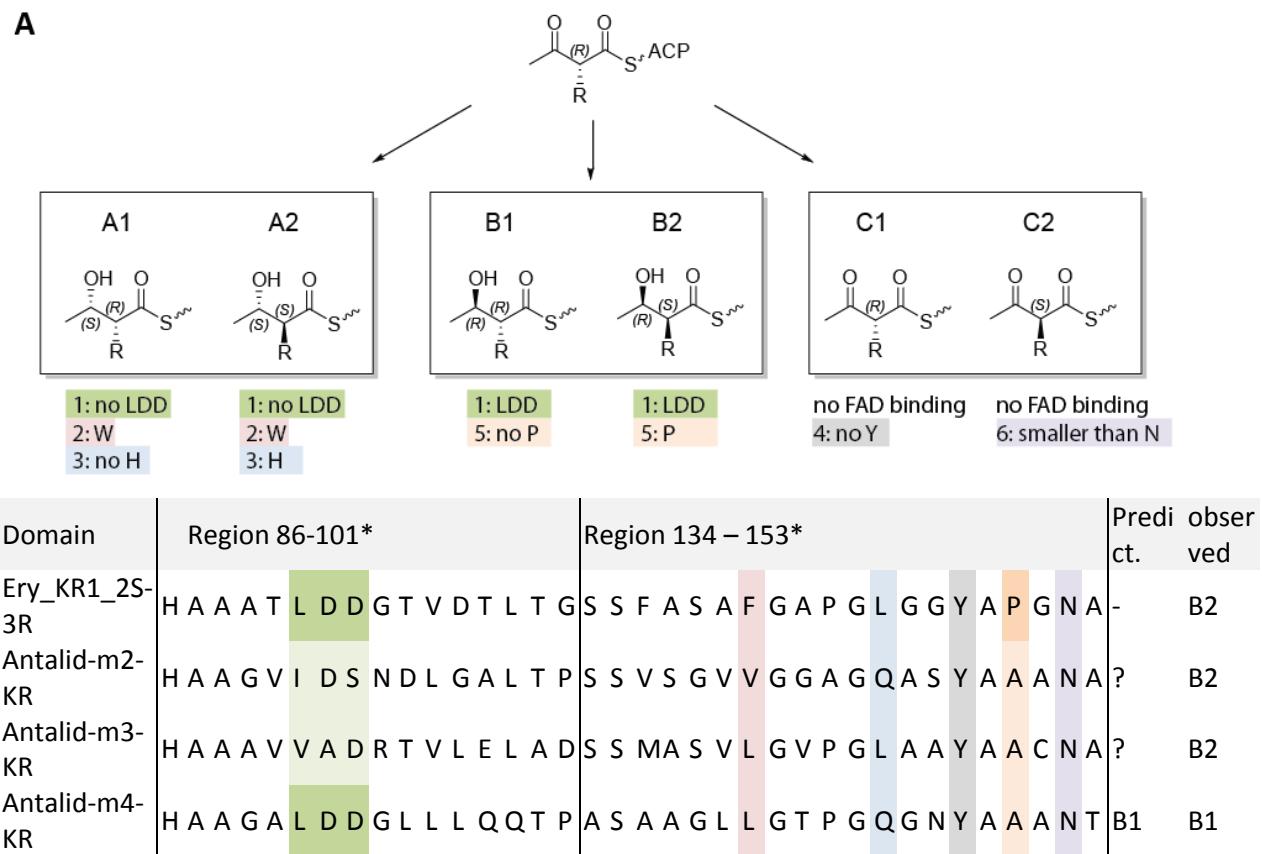


Figure 18: Indicative motifs as highlighted by Keatinge-Clay and their impact on the observed stereochemistry after ketoreduction.⁹ Motifs are highlighted for the antalid KR domains based on an alignment with a reference sequence (Erythromycin Ery-KR1, PDB 2FR0).

As a consequence to the ambiguous prediction with the Keatinge-Clay model a rather new HMM model-based prediction was tested.¹⁰ The web tool by A. Kitsche is at the time of this publication available under https://akitsche.shinyapps.io/profileHMM_App. Each of the three KR domains is a B-type KR according to this model. Moreover, the classification of m3-KR and m4-KR as B2 and B1, respectively, is unambiguous while the KR-type prediction for m2-KR scores very low and it thus less trustworthy.

Table 9: KR domain analysis using a HMM model. Discriminatory values are good if large, i.e. the closer to 0 the worse the prediction.

Domain	ScoreDiff value		predicted	Comment
	OH configuration	Me configuration		
Ant-m2-KR	9.81 for 3R	1.31 for 2R	B1	bad prediction (too close to 0)
Ant-m3-KR	53.36 for 3R	-24.72 for 2S	B2	good prediction
Ant-m4-KR	34.86 for 3R	12.00 for 2R	B1	moderate prediction

In summary, in silico analyses indicated B-type KR domains for antalid biosynthesis. Full stereochemical assignment of the isolated compound using crystallographic data revealed that solely the prediction for ant-m2-KR was wrong as it is a B2-type KR domain. m3-KR and m4-KR were both in agreement with the real structure. For m4-KR which is accompanied by a DH domain, the biosynthetic outcome is a (E)-double bond which is usually derived when a B-type KR domain is acting within the module.⁹ For this particular gene cluster the HMM model-based prediction seems more reliable compared to the strictly motif-based prediction.

3.5 DH domains

As inferred from the structure of antalid, one DH domain is inactive. However, a DH motif alignment did not result in a clear indication that Ant-m3-DH is inactive. We do currently not know why the real structure is not reduced during biosynthesis. The alignment is referred to Ery-DH4 from the erythromycin pathway (PDB 3EL6).¹¹

Table 10: DH alignment highlighting the consensus motifs found in the aa sequence. There is no obvious deviation which could mark ant-m3 as inactive.

Domains	Consensus motifs of DH domains																							
	H	X	X	X	G	X	X	X	X	P	G	Y	X	Y	G	P	X	F	D	X	X	X	(Q/H)	
Ery_DH4	H	V	V	G	G	R	T	L	V	P	G	Y	E	Y	G	P	S	F	D	A	V	A	Q	
Ant-m3-DH	H	R	L	G	G	E	A	I	L	P	G	V	A	Y	G	P	R	L	D	G	C	F	Q	
Ant-m4-DH	H	R	I	H	G	V	A	V	F	P	G	I	Q	Y	G	P	A	F	D	A	C	L	Q	

4 Total Synthesis

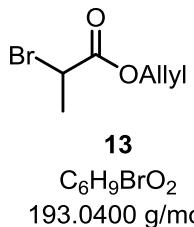
4.1 General Methods

All reactions were performed under inert atmosphere (N₂ or Ar) and stirred magnetically with the help of a Teflon coated stir bar. The used glassware was heated under high vacuum and flushed with inert gas (N₂ or Ar)) prior to use. Dry solvents were obtained by heating under reflux over CaH₂ and subsequent distillation (methylene chloride), by filtration through drying columns on a M. Braun solvent purification system (DMF) or by heating under reflux over sodium and subsequent distillation (THF). Petroleum ether and ethyl acetate for flash column chromatography were distilled before use.

Flash chromatography was performed on Macherey-Nagel silica 60 M (grain size 40-63 µm). Thin layer chromatography was performed on Macherey-Nagel silica gel coated aluminium sheets Alugram® Xtra SIL G/UV₂₅₄ (0.20 mm layer). Indication was achieved with UV light (λ 254 nm) or common dip stains (cerium, potassium permanganate or vanillin). ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker DPX-200 or DPX-400 instruments with the residual proton signals of the solvents given as internal standard (in ppm: CDCl₃ 7.26 for ¹H and 77.16 for ¹³C, DMSO 2.50 for ¹H and 39.52 for ¹³C). Chemical shifts are given in ppm, coupling constants *J* are given in Hz. The used abbreviations for the multiplicities are as follows: s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet. Electron spray ionisation mass spectra (ESI-HRMS) were obtained from a Waters Micromass LCT instrument. The probes were injected in a loops mode of a Waters Alliance 2695 HPLC system. Optical rotations were measured on a polarimeter Perkin-Elmer 341 at 20 °C at a wavelength of λ 589.3 nm (sodium D line) in a 1 mL quartz cell (1 dm length). The concentration is given in g/100 mL.

4.2 Experimental Procedures and Characterizations

allyl 2-bromopropanoate (13)¹³



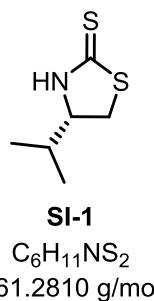
To a stirred solution of allylic alcohol (3.0 g, 3.6 mL, 51.2 mmol, 1.0 equiv) in CH₂Cl₂ (200 mL) at 0 °C was added slowly Et₃N (5.3 g, 7.3 mL, 52.2 mmol, 1.0 equiv) followed by dropwise addition of 2-bromopropionyl bromide (16.9 g, 8.2 mL, 78.3 mmol, 1.5 equiv). Afterwards the mixture was allowed to warm to room temperature and stirred there for 3.5 h. Subsequently the reaction mixture was washed with H₂O (3 × 50 mL), brine (50 mL), dried over anhydrous MgSO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (pentane:Et₂O, 10:1) to afford **13** (9.8 g, 51.2 mmol, 100%) as colorless oil.

R_f = 0.73 (PE:EtOAc, 10:1);

¹H-NMR (400 MHz, CDCl₃): δ 5.93 (ddt, *J* = 17.1, 10.5, 5.7 Hz, 1H), 5.42 – 5.34 (m, 1H), 5.31 – 5.25 (m, 1H), 4.71 – 4.62 (m, 2H), 4.40 (*q*, *J* = 6.9 Hz, 1H), 1.84 (d, *J* = 6.9 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 170.06, 131.41, 119.05, 66.53, 40.07, 21.79 ppm.

(S)-4-isopropylthiazolidine-2-thione (SI-1)¹⁴



To a stirred solution of L-Valine (25.0 g, 212.7 mmol, 1.0 equiv) in THF (450 mL) at 0 °C was added slowly NaBH₄ (19.3 g, 510.6 mmol, 2.4 equiv) followed by dropwise addition of a solution of I₂ (54.0 g, 212.7 mmol, 1.0 equiv) in THF (150 mL). Subsequently the flask was equipped with a reflux condenser and the reaction mixture was stirred under reflux for 24 h. It was cooled again to 0 °C and MeOH (150 mL) was added carefully. The mixture was allowed to stir at 0 °C for 5 min and concentrated afterwards under reduced pressure. The residue was treated with 20% aq KOH (400 mL) and stirred for 4 h at room temperature. The aqueous phase was extracted with CH₂Cl₂ (3 × 50 mL) and the combined organic extracts were washed with brine (50 mL), dried over anhydrous MgSO₄, filtrated and concentrated to provide the crude alcohol **SI-2**.

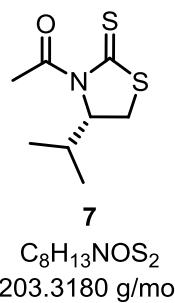
This crude alcohol **SI-2** was dissolved in EtOH (64 mL) and stirred at room temperature. Subsequent addition of CS₂ (43.7 g, 34.7 mL, 574.3 mmol, 2.7 equiv) and a solution of KOH (32.2 g, 574.3 mmol, 2.7 equiv) in H₂O:EtOH (1:1, 252 mL) was followed by heating at reflux for 96 h. The mixture was cooled to room temperature and extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were dried over anhydrous MgSO₄, filtrated and concentrated. The residue was recrystallized from PE/EtOAc to afford (S)-Nagao auxiliary **SI-1** (17.7 g, 110.1 mmol, 52%) as white needles.

R_f = 0.55 (PE:EtOAc, 3:1); **melting point:** 64 °C; **[α]_D** (20 °C): -35.9 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.30 (s, 1H), 4.12 – 3.99 (m, 1H), 3.49 (dd, *J* = 11.1, 8.3 Hz, 1H), 3.30 (dd, *J* = 11.1, 8.2 Hz, 1H), 2.06 – 1.90 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 201.06, 70.15, 35.93, 32.08, 18.88, 18.28 ppm.

(S)-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (7)



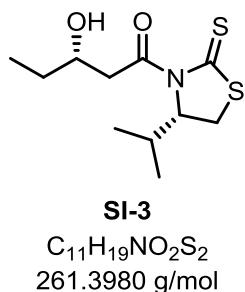
To a stirred solution of NaH (60% dispersion in mineral oil, 1.9 g, 47.1 mmol, 1.1 equiv) in THF (62 mL) at 0 °C was added dropwise a solution of auxiliary **SI-1** (6.9 g, 42.8 mmol, 1.0 equiv) in THF (24 mL). The mixture was allowed to stir for 10 min at the same temperature before dropwise addition of acetyl chloride (3.7 g, 3.4 mL, 47.1 mmol, 1.1 equiv). After stirring further 10 min at 0 °C the reaction mixture was allowed to warm to room temperature and stirred there for 18 h. Subsequently the reaction was quenched by addition of 2 M aq HCl (40 mL). The aqueous phase was extracted with EtOAc (3×30 mL) and the combined organic extracts were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 3:1) to afford **7** (7.8 g, 38.1 mmol, 89%) as yellow oil.

$R_f = 0.77$ (PE:EtOAc, 3:1); $[\alpha]_D$ (20 °C): +442.1 (c = 1.0, $CHCl_3$);

1H -NMR (400 MHz, $CDCl_3$): δ 5.15 (ddd, $J = 7.7, 6.2, 1.1$ Hz, 1H), 3.50 (dd, $J = 11.5, 8.0$ Hz, 1H), 3.02 (dd, $J = 11.5, 1.2$ Hz, 1H), 2.77 (s, 3H), 2.44 – 2.29 (m, 1H), 1.06 (d, $J = 6.8$ Hz, 3H), 0.97 (d, $J = 6.9$ Hz, 3H) ppm;

^{13}C -NMR (100 MHz, $CDCl_3$): δ 203.34, 170.85, 71.40, 30.91, 30.53, 27.07, 19.19, 17.89 ppm.

(S)-3-hydroxy-1-((S)-4-isopropyl-2-thioxothiazolidin-3-yl)pentan-1-one (SI-3)¹⁵



To a stirred solution of **7** (7.8 g, 38.4 mmol, 1.0 equiv) in CH₂Cl₂ (192 mL) at 0 °C was added slowly TiCl₄ (1 M in CH₂Cl₂, 42.3 mL, 42.3 mmol, 1.1 equiv). The mixture was allowed to stir for 5 min at the same temperature before cooling to −78 °C. Diisopropylethylamine (5.5 g, 7.2 mL, 42.3 mmol, 1.1 equiv) was added dropwise and the resultant solution was stirred at −78 °C for 2 h. After the addition of propionaldehyde (3.3 g, 4.2 mL, 57.6 mmol, 1.5 equiv) in CH₂Cl₂ (144 mL) the solution was stirred at the same temperature for further 3 h prior to addition of satd aq NH₄Cl (200 mL). The reaction mixture was allowed to warm to room temperature and the aqueous phase was extracted with CH₂Cl₂ (3 × 70 mL) and the combined organic extracts were dried over anhydrous Na₂SO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 3:1 to 2:1) to afford alcohol **SI-3** (8.2 g, 31.5 mmol, 82%) as yellow oil.

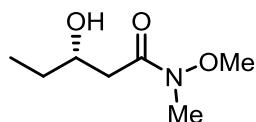
R_f = 0.29 (PE:EtOAc, 3:1); [α]_D (20 °C): +406.5 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 5.24 – 5.10 (m, 1H), 4.14 – 3.98 (m, 1H), 3.65 (dd, J = 17.7, 2.4 Hz, 1H), 3.52 (dd, J = 11.5, 8.0 Hz, 1H), 3.11 (dd, J = 17.7, 9.4 Hz, 1H), 3.03 (dd, J = 11.5, 0.9 Hz, 1H), 2.79 (s, 1H), 2.44 – 2.30 (m, 1H), 1.61 – 1.52 (m, 2H), 1.07 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 5.8 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H) ppm.

¹³C-NMR (100 MHz, CDCl₃): δ 203.21, 173.46, 71.52, 69.46, 45.23, 31.00, 30.73, 29.37, 19.23, 17.97, 10.09 ppm;

HRMS (ESI) = calculated for C₁₁H₁₉NO₂S₂Na [M+Na⁺]: 284.0755, found: 284.0758.

(S)-3-hydroxy-N-methoxy-N-methylpentanamide (SI-4)¹⁶



SI-4

C₇H₁₅NO₃

161.2010 g/mol

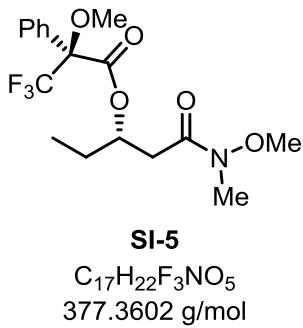
To a stirred suspension of *N,O*-dimethylhydroxylamine hydrochloride (4.7 g, 47.8 mmol, 2.9 equiv) in CH₂Cl₂ (191 mL) at 0 °C was added slowly Me₃Al (2 M in heptane, 23.9 mL, 47.8 mmol, 2.9 equiv). The mixture was allowed to stir for 30 min at the same temperature before cooling to –20 °C. A solution of alcohol **SI-3** (4.3 g, 16.5 mmol, 1.0 equiv) in CH₂Cl₂ (47 mL) was added and the resultant solution was stirred at –20 °C for 1 h prior to warming to room temperature. After 5 h the reaction mixture was cooled to 0 °C and 0.5 M aq HCl (80 mL) was added. The mixture was allowed to warm to room temperature and the aqueous phase was extracted with CH₂Cl₂ (3 × 70 mL). The combined organic extracts were washed with brine (100 mL), dried over anhydrous Na₂SO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 1:3) to afford alcohol **SI-4** (2.7 g, 16.5 mmol, 100%) as yellow oil.

R_f = 0.31 (PE:EtOAc, 1:3); [α]_D (20 °C): +70.6 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 4.00 – 3.90 (m, 1H), 3.78 (s, 1H), 3.69 (s, 3H), 3.19 (s, 3H), 2.67 (d, J = 16.7 Hz, 1H), 2.43 (dd, J = 16.7, 9.7 Hz, 1H), 1.63 – 1.44 (m, 2H), 0.98 (t, J = 7.5 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 174.13, 69.41, 61.37, 37.86, 31.98, 29.52, 10.07 ppm.

(S)-1-(methoxy(methyl)amino)-1-oxopentan-3-yl (S)-3,3,3-trifluoro-2-methoxy-2-phenyl-propanoate (SI-5)¹⁷



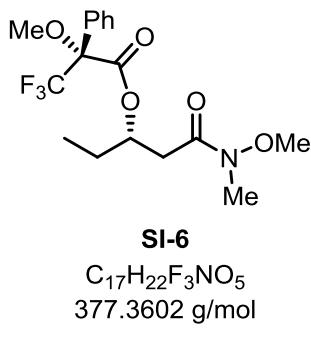
To a stirred solution of alcohol **SI-4** (15.0 mg, 93.1 μ mol, 1.0 equiv) in CH_2Cl_2 (3.1 mL) at 0 °C were added Et_3N (75.2 mg, 103.0 μ L, 743.1 μ mol, 8.0 equiv), 4-DMAP (15.9 mg, 130.1 μ mol, 1.4 equiv) and (*R*)-(—)- α -methoxy- α -trifluoromethylphenylacetyl chloride (94.1 mg, 69.7 μ L, 372.4 μ mol, 4.0 equiv) subsequently. After being stirred at the same temperature for 5 min the mixture was allowed to warm to room temperature and stirred there for further 8.5 h before being diluted with EtOAc (15 mL). The reaction mixture was then sequentially washed with 1.0 M aq $NaHSO_4$ (10 mL), 1.0 M aq $NaOH$ (10 mL) and satd aq $NaHCO_3$ (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 1:1) to afford (*S*)-Mosher-ester **SI-5** (26.6 mg, 70.5 μ mol, 76%) as colorless oil.

R_f = 0.62 (PE:EtOAc, 1:1); $[\alpha]_D$ (20 °C): -42.3 (c = 1.0, $CHCl_3$);

1H -NMR (400 MHz, $CDCl_3$): δ 7.58 – 7.51 (m, 2H), 7.44 – 7.38 (m, 3H), 5.61 – 5.52 (m, 1H), 3.63 (s, 3H), 3.58 – 3.52 (m, 3H), 3.18 (s, 3H), 2.94 (dd, J = 15.9, 8.7 Hz, 1H), 2.60 (dd, J = 16.0, 4.4 Hz, 1H), 1.80 – 1.70 (m, 2H), 0.89 (t, J = 7.5 Hz, 3H) ppm;

HRMS (ESI) = calculated for $C_{17}H_{22}NO_5F_3Na$ [$M+Na^+$]: 400.1348, found: 400.1348.

(S)-1-(methoxy(methyl)amino)-1-oxopentan-3-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenyl-propanoate (SI-6)¹⁷



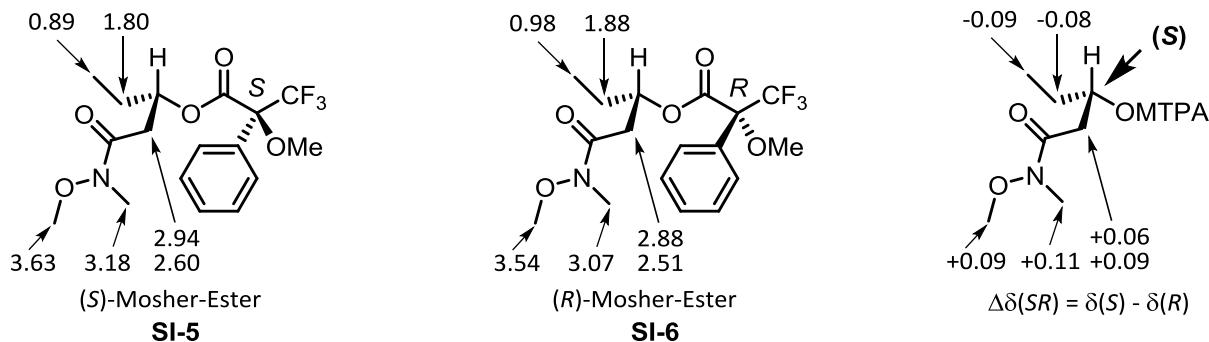
To a stirred solution of alcohol **SI-4** (15.0 mg, 93.1 μmol , 1.0 equiv) in CH_2Cl_2 (3.1 mL) at 0 °C were added Et_3N (75.2 mg, 103.0 μL , 743.1 μmol , 8.0 equiv), 4-DMAP (15.9 mg, 130.1 μmol , 1.4 equiv) and (*S*)-(+)- α -methoxy- α -trifluoromethylphenylacetyl chloride (94.1 mg, 69.7 μL , 372.4 μmol , 4.0 equiv) subsequently. After being stirred at the same temperature for 5 min the mixture was allowed to warm to room temperature and stirred there for further 8.5 h before being diluted with EtOAc (15 mL). The reaction mixture was then sequentially washed with 1.0 M aq NaHSO_4 (10 mL), 1.0 M aq NaOH (10 mL) and satd aq NaHCO_3 (10 mL). The organic phase was dried over anhydrous MgSO_4 , filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE: EtOAc , 1:1) to afford (*R*)-Mosher-ester **SI-6** (25.8 mg, 68.4 μmol , 73%) as colorless oil.

R_f = 0.62 (PE:EtOAc, 1:1); [α]_D (20 °C): +26.3 (c = 1.0, CHCl₃);

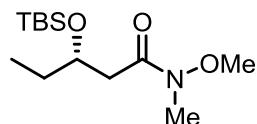
¹H-NMR (400 MHz, CDCl₃): δ 7.55 – 7.49 (m, 2H), 7.41 – 7.35 (m, 3H), 5.60 – 5.50 (m, 1H), 3.56 – 3.54 (m, 3H), 3.54 (s, 3H), 3.07 (s, 3H), 2.88 (dd, *J* = 15.8, 8.3 Hz, 1H), 2.51 (dd, *J* = 15.9, 4.9 Hz, 1H), 1.88 – 1.74 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H) ppm;

HRMS (ESI) = calculated for C₁₇H₂₂NO₅F₃Na [M+Na⁺]: 400.1348, found: 400.1348.

Mosher-Ester Analysis:



(S)-3-((*tert*-butyldimethylsilyl)oxy)-*N*-methoxy-*N*-methylpentanamide (8**)¹⁶**



8

C₁₃H₂₉NO₃Si
275.4640 g/mol

To a stirred solution of alcohol **SI-4** (2.6 g, 16.1 mmol, 1.0 equiv) in CH₂Cl₂ (32.3 mL) at 0 °C were added 2,6-lutidine (5.2 g, 5.6 mL, 48.4 mmol, 3.0 equiv) and TBSOTf (6.4 g, 5.6 mL, 24.2 mmol, 1.5 equiv) subsequently. After being stirred at the same temperature for 10 min the mixture was allowed to warm to room temperature and stirred there for further 3 h before being diluted with CH₂Cl₂ (40 mL). Satd aq NH₄Cl (40 mL) was added and after separation of phases the aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic extracts were washed with 1.0 M aq NaHSO₄ (50 mL), dried over anhydrous MgSO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 3:1) to afford **8** (4.3 g, 15.6 mmol, 97%) as colorless oil.

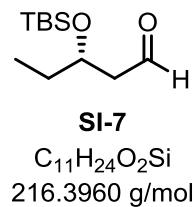
R_f = 0.68 (PE:EtOAc, 3:1); [α]_D (20 °C): +20.5 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 4.24 – 4.12 (m, 1H), 3.69 (s, 3H), 3.17 (s, 3H), 2.71 (dd, J = 14.3, 7.3 Hz, 1H), 2.38 (dd, J = 14.6, 5.3 Hz, 1H), 1.59 – 1.47 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.03 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 70.66, 61.45, 39.21, 32.11, 30.64, 26.00, 18.21, 9.49, –4.53, –4.58 ppm, C=O was not detected;

HRMS (ESI) = calculated for C₁₃H₂₉NO₃Si [M+H⁺]: 276.1995, found: 276.1993.

(S)-3-((*tert*-butyldimethylsilyl)oxy)pentanal (SI-7**)¹⁶**



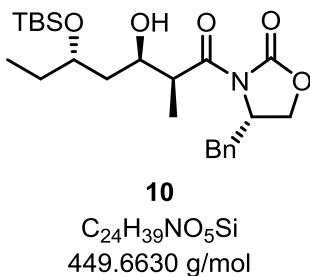
To a stirred solution of amide **8** (2.6 g, 9.4 mmol, 1.0 equiv) in THF (94.5 mL) at -78°C was added DiBAL-H (1.0 M in CH₂Cl₂, 37.8 mL, 37.8 mmol, 4.0 equiv) slowly over 1 h. After being stirred at the same temperature for further 1.5 h acetone (10 mL) was added carefully and stirring at -78°C was continued for 10 min prior to the addition of satd aq potassium sodium tartrate (100 mL). The reaction mixture was allowed to warm to room temperature and stirred there for 1.5 h at which two clear phases formed. The aqueous phase was extracted with CH₂Cl₂ (3 \times 40 mL) and the combined organic extracts were washed with brine, dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (pentane:Et₂O, 10:1) to afford **SI-7** (2.0 g, 9.4 mmol, 100%) as colorless oil.

R_f = 0.78 (PE:EtOAc, 10:1); [α]_D (20 °C): +1.6 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 9.81 (t, J = 2.5 Hz, 1H), 4.21 – 4.06 (m, 1H), 2.55 – 2.47 (m, 2H), 1.59 – 1.54 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 202.64, 69.48, 50.50, 30.70, 25.91, 18.16, 9.56, -4.28, -4.58 ppm.

(S)-4-benzyl-3-((2*S*,3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-2-methylheptanoyl)-oxazolidin-2-one (10**)**



To a stirred solution of (*S*)-4-benzyl-3-propionyloxazolidin-2-one (**9**) (2.8 g, 11.8 mmol, 1.25 equiv) in CH₂Cl₂ (47.2 mL) at -78 °C was added diisopropylethylamine (1.8 g, 2.3 mL, 13.7 mmol, 1.45 equiv) followed by dropwise addition of *n*Bu₂BOTf (1.0 M in CH₂Cl₂, 12.3 mL, 12.3 mmol, 1.3 equiv). Subsequently the reaction mixture was allowed to warm to 0 °C and stirred there for 45 min before it was recooled to -78 °C. Then a solution of aldehyde **SI-7** (2.0 g, 9.4 mmol, 1.0 equiv) in CH₂Cl₂ (47.2 mL) was added dropwise. Afterwards was stirred for 1 h at the same temperature before warming to 0 °C. After stirring for 2 h at 0 °C the mixture was recooled to -78 °C where subsequently aq pH 7 buffer (30 mL) and MeOH (30 mL) were added carefully. It was allowed to warm to 0 °C and a solution of H₂O₂ (30 wt.% in H₂O, 20 mL) in MeOH (40 mL) was added. After stirring for 10 h at room temperature the mixture was concentrated under reduced pressure. The residue was treated with H₂O (50 mL) and extracted with CH₂Cl₂ (3 × 30 mL). The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 3:1) to afford alcohol **10** (3.2 g, 7.1 mmol, 75%) as colorless oil.

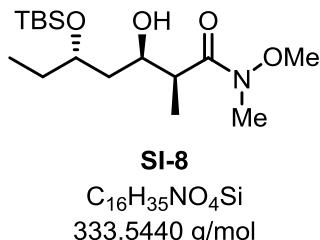
R_f = 0.54 (PE:EtOAc, 3:1); [α]_D (20 °C): +40.9 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 7.37 – 7.26 (m, 3H), 7.25 – 7.18 (m, 2H), 4.76 – 4.65 (m, 1H), 4.29 – 4.23 (m, 1H), 4.24 – 4.15 (m, 2H), 3.97 – 3.88 (m, 1H), 3.81 – 3.71 (m, 1H), 3.59 (d, *J* = 1.7 Hz, 1H), 3.28 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.77 (dd, *J* = 13.4, 9.6 Hz, 1H), 1.73 – 1.49 (m, 4H), 1.28 (d, *J* = 7.0 Hz, 3H), 0.89 (s, 9H), 0.88 (t, *J* = 7.3 Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 176.58, 153.28, 135.36, 129.59, 129.10, 127.52, 72.13, 68.56, 66.23, 55.44, 43.31, 38.55, 37.97, 29.48, 26.02, 18.17, 11.45, 9.88, -4.40, -4.62 ppm;

HRMS (ESI) = calculated for C₂₄H₄₀NO₅Si [M+H⁺]: 450.2676, found: 450.2670.

(2*S*,3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-*N*-methoxy-*N*,2-dimethylheptanamide (SI-8)



To a stirred suspension of *N,O*-dimethylhydroxylamine hydrochloride (1.9 g, 19.4 mmol, 2.9 equiv) in CH₂Cl₂ (77.5 mL) at 0 °C was added slowly Me₃Al (2 M in heptane, 9.7 mL, 19.4 mmol, 2.9 equiv). The mixture was allowed to stir for 30 min at the same temperature before cooling to –20 °C. A solution of alcohol **10** (3.0 g, 6.7 mmol, 1.0 equiv) in CH₂Cl₂ (19.1 mL) was added and the resultant solution was stirred at –20 °C for 1 h prior to warming to room temperature. After 13 h the reaction mixture was cooled to 0 °C, diluted with CH₂Cl₂ (60 mL) and 0.5 M aq HCl (60 mL) was added carefully. The mixture was allowed to warm to room temperature and the aqueous phase was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with brine (80 mL), dried over anhydrous MgSO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 1:3) to afford alcohol **SI-8** (2.2 g, 6.7 mmol, 100%) as colorless oil.

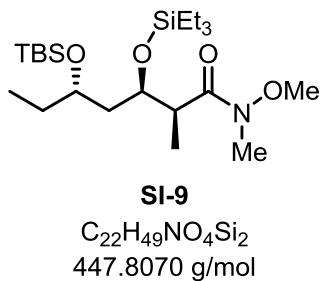
R_f = 0.44 (PE:EtOAc, 3:1); [α]_D (20 °C): +23.0 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 4.14 – 4.04 (m, 1H), 3.99 (bs, 1H), 3.95 – 3.87 (m, 1H), 3.69 (s, 3H), 3.19 (s, 3H), 2.86 (bs, 1H), 1.66 – 1.54 (m, 3H), 1.45 (ddd, *J* = 14.1, 6.9, 2.0 Hz, 1H), 1.20 (d, *J* = 7.0 Hz, 3H), 0.89 (s, 9H), 0.86 (t, *J* = 6.5 Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 71.84, 68.88, 61.67, 40.58, 39.53, 32.10, 29.91, 26.05, 18.20, 11.91, 9.71, –4.36, –4.57 ppm, C=O was not detected;

HRMS (ESI) = calculated for C₁₆H₃₅NO₄Si [M+H⁺]: 334.2414, found: 334.2412.

(2S,3R,5S)-5-((*tert*-butyldimethylsilyl)oxy)-N-methoxy-N,2-dimethyl-3-((triethylsilyl)oxy)-heptanamide (SI-9)



To a stirred solution of alcohol **SI-8** (820.0 mg, 2.5 mmol, 1.0 equiv) in CH₂Cl₂ (4.9 mL) at 0 °C were added 2,6-lutidine (793.0 mg, 861.9 μL, 7.4 mmol, 3.0 equiv) and TESOTf (978.1 mg, 835.9 μL, 3.7 mmol, 1.5 equiv) subsequently. After being stirred at the same temperature for 10 min the mixture was allowed to warm to room temperature and stirred there for further 5 h before being diluted with CH₂Cl₂ (10 mL). Satd aq NH₄Cl (10 mL) was added and after separation of phases the aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL). The combined organic extracts were washed with 1.0 M aq NaHSO₄ (20 mL), dried over anhydrous MgSO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 5:1) to afford **SI-9** (1.1 g, 2.4 mmol, 96%) as colorless oil.

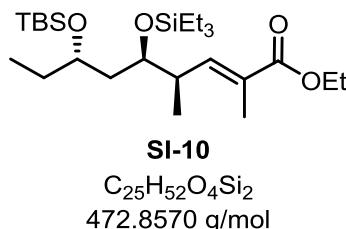
R_f = 0.37 (PE:EtOAc, 10:1); [α]_D (20 °C): −10.7 (c = 1.0, CHCl₃);

¹H-NMR (200 MHz, CDCl₃): δ 4.12 – 3.95 (m, 1H), 3.66 (s, 3H), 3.62 – 3.50 (m, 1H), 3.17 (s, 3H), 2.96 – 2.76 (m, 1H), 1.70 – 1.38 (m, 4H), 1.13 (d, J = 6.9 Hz, 3H), 0.95 (t, J = 7.8 Hz, 9H), 0.88 (s, 9H), 0.89 – 0.82 (m, 3H), 0.65 – 0.52 (m, 6H), 0.06 (s, 3H), 0.05 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 71.19, 71.04, 61.24, 44.21, 41.57, 32.36, 30.19, 26.05, 18.26, 12.44, 9.74, 7.12, 5.39, −3.85, −4.03 ppm;

HRMS (ESI) = calculated for C₂₂H₄₉NO₄Si₂ [M+H⁺]: 448.3278, found: 448.3279.

ethyl (4*R*,5*R*,7*S*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-5-((triethylsilyl)oxy)non-2-enoate (SI-10)



To a stirred solution of amide **SI-9** (620.0 mg, 1.4 mmol, 1.0 equiv) in THF (13.4 mL) at -78°C was added DiBAL-H (1.0 M in CH₂Cl₂, 5.5 mL, 5.5 mmol, 4.0 equiv) slowly over 1 h. After being stirred at the same temperature for further 1.5 h acetone (3 mL) was added carefully and stirring at -78°C was continued for 10 min prior to the addition of satd aq potassium sodium tartrate (20 mL). The reaction mixture was allowed to warm to room temperature and stirred there for 1 h at which two clear phases formed. The aqueous phase was extracted with CH₂Cl₂ (3×10 mL) and the combined organic extracts were washed with brine, dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure to provide the crude aldehyde **11**

This crude aldehyde **11** was dissolved in toluene (6.9 mL) and stirred at room temperature. Ethyl 2-(triphenylphosphoranylidene)propionate (2.0 g, 5.5 mmol, 4.0 equiv) was added and the reaction mixture afterwards heated to 60°C where it was stirred for 18 h. It was cooled to room temperature, diluted with MTB-ether (15 mL) and washed subsequently with H₂O (2×20 mL) and brine (20 mL). The organic phase was dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure. The crude material was purified by flash column chromatography (PE:EtOAc, 30:1) to afford ester **SI-10** (587.0 mg, 1.2 mmol, 89%) as colorless oil.

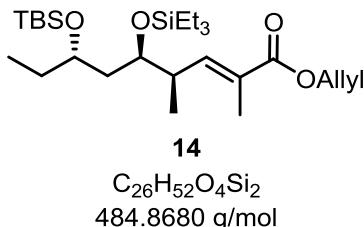
R_f = 0.54 (PE:EtOAc, 30:1); **[α]_D** (20 °C): +9.9 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 6.73 (dq, *J* = 2.7, 1.3 Hz, 1H), 4.25 – 4.11 (m, 2H), 3.77 – 3.69 (m, 1H), 3.69 – 3.62 (m, 1H), 2.56 – 2.49 (m, 1H), 1.84 (d, *J* = 1.4 Hz, 3H), 1.67 – 1.56 (m, 2H), 1.51 – 1.43 (m, 2H), 1.29 (t, *J* = 7.1 Hz, 3H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.95 (t, *J* = 7.9 Hz, 9H), 0.88 (s, 9H), 0.88 (t, *J* = 7.3 Hz, 3H), 0.63 – 0.57 (m, 6H), 0.06 (s, 3H), 0.05 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 168.44, 145.50, 126.86, 73.04, 71.37, 60.54, 42.95, 39.18, 30.54, 26.06, 18.26, 14.42, 14.02, 12.78, 9.46, 7.13, 5.44, -3.66, -4.06 ppm;

HRMS (ESI) = calculated for C₂₅H₅₂O₄Si₂Na [M+Na⁺]: 495.3302, found: 495.3298.

allyl (*4R,5R,7S,E*)-7-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-5-((triethylsilyl)oxy)non-2-enoate (14)



To a stirred solution of allylester **13** (944.0 mg, 4.9 mmol, 4.0 equiv) in toluene (4.9 mL) at room temperature was added *n*Bu₃P (1.0 g, 1.2 mL, 4.9 mmol, 4.0 equiv). After being stirred at the same temperature for 24 h the reaction mixture was diluted with CH₂Cl₂ (30 mL) and washed with 2.0 M aq NaOH (40 mL). The organic phase was dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure to provide crude allyl 2-(tri-*n*-butylphosphoranylidene)propionate (**12**).

In the meantime DiBAL-H (1.0 M in CH₂Cl₂, 4.9 mL, 4.9 mmol, 4.0 equiv) was added slowly over 1 h to a stirred solution of amide **SI-9** (550.0 mg, 1.2 mmol, 1.0 equiv) in THF (12.3 mL) at -78 °C. After being stirred at the same temperature for further 1.5 h acetone (3 mL) was added carefully and stirring at -78 °C was continued for 10 min prior to the addition of satd aq potassium sodium tartrate (20 mL). The reaction mixture was allowed to warm to room temperature and stirred there for 1 h at which two clear phases formed. The aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL) and the combined organic extracts were washed with brine, dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure to provide the crude aldehyde **11**.

This crude aldehyde **11** was dissolved in CH₂Cl₂ (3.1 mL) and added to a stirred solution of above prepared crude allyl 2-(tri-*n*-butylphosphoranylidene)propionate (**12**) in CH₂Cl₂ (3.3 mL) at room temperature. After stirring for 42 h at the same temperature the reaction mixture was diluted with MTB-ether (15 mL) and washed subsequently with H₂O (2 × 20 mL) and brine (20 mL). The organic phase was dried over anhydrous MgSO₄, filtrated and concentrated. The crude material was purified by flash column chromatography (PE to PE:EtOAc, 20:1) to afford ester **14** (577.0 mg, 1.2 mmol, 97%) as colorless oil.

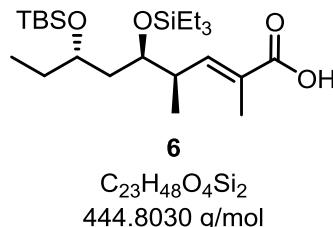
R_f = 0.24 (PE); [α]_D (20 °C): +9.8 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 6.81 – 6.73 (m, 1H), 5.95 (ddt, *J* = 17.2, 10.4, 5.7 Hz, 1H), 5.38 – 5.28 (m, 1H), 5.26 – 5.19 (m, 1H), 4.66 – 4.61 (m, 2H), 3.78 – 3.71 (m, 1H), 3.68 – 3.65 (m, 1H), 2.58 – 2.51 (m, 1H), 1.85 (d, *J* = 1.4 Hz, 3H), 1.66 – 1.57 (m, 2H), 1.50 – 1.45 (m, 2H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.95 (t, *J* = 7.9 Hz, 9H), 0.88 (s, 9H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.62 – 0.57 (m, 6H), 0.06 (s, 3H), 0.06 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 168.02, 146.02, 132.72, 126.68, 117.95, 73.07, 71.37, 65.34, 42.84, 39.28, 30.56, 26.06, 18.25, 14.12, 12.81, 9.44, 7.12, 5.44, -3.64, -4.07 ppm;

HRMS (ESI) = calculated for C₂₆H₅₃O₄Si₂ [M+H⁺]: 485.3482, found: 485.3481.

(4*R*,5*R*,7*S*,*E*)-7-((tert-butyldimethylsilyl)oxy)-2,4-dimethyl-5-((triethylsilyl)oxy)non-2-enoic acid (6)



To a stirred solution of allyl ester **14** (390.0 mg, 0.81 mmol, 1.0 equiv) in THF (54 mL) at room temperature was added subsequently *N*-methylaniline (215.7 mg, 217.9 μ L, 2.01 mmol, 2.5 equiv) and Pd(PPh₃)₄ (93.0 mg, 80.5 μ mol, 0.1 equiv). The reaction mixture was stirred for 40 h at the same temperature and then diluted with EtOAc (50 mL) before 10% aq citric acid (50 mL) was added. The aqueous phase was extracted with EtOAc (3×30 mL) and the combined organic extracts were washed with brine (50 mL), dried over anhydrous MgSO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (PE:EtOAc, 20:1 to 3:1) to afford acid **6** (338.0 mg, 0.76 mmol, 94%) as pale yellow oil.

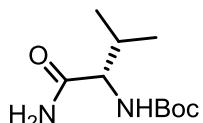
R_f = 0.15 (PE:EtOAc, 20:1); [α]_D (20 °C): +13.3 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 6.91 – 6.86 (m, 1H), 3.79 – 3.72 (m, 1H), 3.71 – 3.64 (m, 1H), 2.65 – 2.51 (m, 1H), 1.85 (d, *J* = 1.4 Hz, 3H), 1.65 – 1.56 (m, 2H), 1.51 – 1.44 (m, 2H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.95 (t, *J* = 8.0 Hz, 9H), 0.88 (s, 9H), 0.88 (t, *J* = 7.4 Hz, 3H), 0.63 – 0.57 (m, 6H), 0.06 (s, 3H), 0.06 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 173.28, 148.22, 126.23, 73.04, 71.39, 42.65, 39.55, 30.62, 26.04, 18.25, 14.16, 12.45, 9.42, 7.11, 5.43, –3.64, –4.09 ppm;

HRMS (ESI) = calculated for C₂₃H₄₇O₄Si₂ [M–H⁺]: 443.3013, found: 443.3015.

tert-butyl (S)-(1-amino-3-methyl-1-oxobutan-2-yl)carbamate (SI-11)



SI-11

C₁₀H₂₀N₂O₃

216.2810 g/mol

To a stirred solution of Boc-L-Valine (2.0 g, 9.2 mmol, 1.0 equiv) in CH₂Cl₂ (115 mL) at 0 °C was added subsequently HOBt·xH₂O (12 wt.% H₂O, 1.4 g, 9.2 mmol, 1.0 equiv) and DCC (2.5 g, 12.0 mmol, 1.3 equiv). After being stirred at the same temperature for 5 min the reaction mixture was allowed to warm to room temperature where it was stirred for further 1 h. The mixture was recooled to 0 °C and 25% aq NH₃ (2.3 g, 2.3 mL, 33.1 mmol, 3.6 equiv) was added. Stirring at 0 °C was continued for 1 h before filtering through celite® which was afterwards rinsed several times with CH₂Cl₂. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂:MeOH, 15:1) to afford amide **SI-11** (1.6 g, 7.4 mmol, 80%) as white solid.

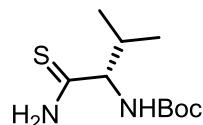
R_f = 0.33 (CH₂Cl₂:MeOH, 15:1); **melting point:** 145 °C; [α]_D (20 °C): +21.4 (c = 1.0, acetone);

¹H-NMR (400 MHz, DMSO): δ 7.25 (bs, 1H), 6.99 (bs, 1H), 6.50 (d, J = 9.1 Hz, 1H), 3.72 (dd, J = 8.9, 6.8 Hz, 1H), 1.94 – 1.86 (m, 1H), 1.38 (s, 9H), 0.85 (d, J = 6.8 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H) ppm;

¹³C-NMR (100 MHz, DMSO): δ 173.37, 155.40, 77.89, 59.48, 30.25, 28.18, 19.32, 17.96 ppm;

HRMS (ESI) = calculated for C₁₀H₂₀N₂O₃Na [M+Na⁺]: 239.1372, found: 239.1370.

tert-butyl (S)-(1-amino-3-methyl-1-thioxobutan-2-yl)carbamate (SI-12)¹⁸



SI-12

C₁₀H₂₀N₂O₂S
232.3420 g/mol

To a stirred solution of amide **SI-11** (1.0 g, 4.6 mmol, 1.0 equiv) in THF (11.5 mL) at room temperature was added Lawesson's reagent (3.7 g, 9.3 mmol, 2.0 equiv). The reaction mixture was heated to 50 °C where it was stirred for 4 h. Afterwards it was cooled to 0 °C and satd aq NaHCO₃ (20 mL) was added carefully before diluting with EtOAc (20 mL). The mixture was allowed to warm to room temperature and stirred there for 30 min before the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (30 mL), dried over anhydrous Na₂SO₄, filtrated and concentrated to provide the crude material, which was purified by flash column chromatography (CH₂Cl₂:MeOH, 30:1) to afford **SI-12** (948.0 mg, 4.1 mmol, 89%) as white foam.

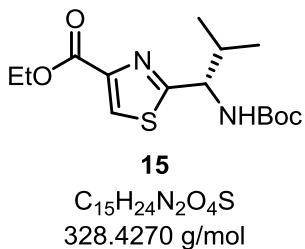
R_f = 0.60 (CH₂Cl₂:MeOH, 20:1); **melting point:** 46.5 °C; [α]_D (20 °C): -37.6 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.29 (bs, 1H), 7.79 (bs, 1H), 5.36 (d, J = 8.9 Hz, 1H), 4.27 – 4.16 (m, 1H), 2.21 – 2.02 (m, 1H), 1.42 (s, 9H), 0.98 (s, 3H), 0.97 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 209.57, 156.12, 80.41, 65.32, 33.46, 28.48, 19.67, 18.38 ppm;

HRMS (ESI) = calculated for C₁₀H₂₀N₂O₂SNa [M+Na⁺]: 255.1143, found: 255.1146.

ethyl (S)-2-((*tert*-butoxycarbonyl)amino)-2-methylpropylthiazole-4-carboxylate (15)¹⁸



To a stirred solution of thioamide **SI-12** (500.0 mg, 2.2 mmol, 1.0 equiv) in fresh distilled DME (13.5 mL) at -15°C was added KHCO₃ (1.9 g, 19.0 mmol, 8.7 equiv). It was stirred at the same temperature for 20 min prior to the addition of ethyl bromopyruvate (1.4 g, 892.6 μL , 7.1 mmol, 3.3 equiv). Afterwards stirring at -15°C was continued for 15 min before warming to room temperature where it was stirred for 30 min. Then the reaction mixture was recooled to -15°C and a solution of 2,6-lutidine (2.1 g, 2.3 mL, 20.0 mmol, 9.3 equiv) and TFAA (2.0 g, 1.3 mL, 9.5 mmol, 4.4 equiv) in fresh distilled DME (3.7 mL) was added dropwise. After stirring for further 30 min at the same temperature the mixture was allowed to warm to room temperature where stirring was continued for 13 h. It was concentrated under reduced pressure prior to the addition of H₂O (20 mL). The aqueous phase was extracted with CH₂Cl₂ (3 \times 10 mL) and the combined organic extracts were washed with 1.0 M aq NaHSO₄ (20 mL), dried over anhydrous Na₂SO₄, filtrated and concentrated under reduced pressure. The crude material was purified by flash column chromatography (PE:EtOAc, 3:1) to afford thiazole **15** (705.0 mg, 2.1 mmol, 100%) as pale yellow solid.

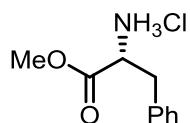
R_f = 0.72 (PE:EtOAc, 3:1); **melting point:** 102 °C; [α]_D (20 °C): -38.5 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.06 (s, 1H), 5.33 – 5.23 (m, 1H), 4.93 – 4.86 (m, 1H), 4.41 (q, J = 7.1 Hz, 2H), 2.51 – 2.40 (m, 1H), 1.43 (s, 9H), 1.39 (t, J = 7.0 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 173.36, 161.50, 155.58, 147.56, 126.89, 80.23, 61.54, 58.19, 33.43, 28.45, 19.58, 17.40, 14.52 ppm;

HRMS (ESI) = calculated for C₁₅H₂₅N₂O₄S [M+H⁺]: 329.1535, found: 329.1534.

methyl D-phenylalaninate hydrochloride (SI-13)



SI-13

C₁₀H₁₄CINO₂
215.6770 g/mol

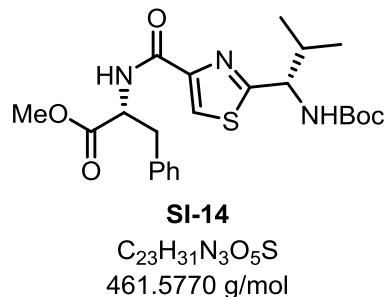
Under stirring acetyl chloride (13.8 mL, 15.2 g, 193.7 mmol, 3.2 equiv) was added dropwise to MeOH (75 mL) at 0 °C. After 10 min at the same temperature D-phenylalanine (10.0 g, 60.5 mmol, 1.0 equiv) was added. Stirring was continued at the same temperature for 2 h prior to warming to room temperature where the reaction mixture was stirred for further 14 h. It was concentrated under reduced pressure to afford ester **SI-13** (13.0 g, 60.3 mmol, 100%) as white solid.

melting point: 143 °C; [α]_D (20 °C): −17.3 (c = 1.0, MeOH);

¹H-NMR (400 MHz, DMSO): δ 8.63 (bs, 3H), 7.36 – 7.22 (m, 5H), 4.27 (dd, *J* = 7.1, 6.1 Hz, 1H), 3.66 (s, 3H), 3.20 – 3.05 (m, 2H) ppm;

¹³C-NMR (100 MHz, DMSO): δ 169.37, 134.61, 129.38, 128.60, 127.28, 53.20, 52.58, 35.88 ppm.

methyl ((2-((S)-1-((tert-butoxycarbonyl)amino)-2-methylpropyl)thiazole-4-carbonyl)-D-phenylalaninate (SI-14)



To a stirred solution of thiazole **15** (1.0 g, 3.1 mmol, 1.0 equiv) in THF (33.9 mL) at room temperature was added subsequently MeOH (8.7 mL), H₂O (17.9 mL) and 0.5 M aq LiOH (7.3 mL). After being stirred at the same temperature for 3.5 h the reaction mixture was poured in 0.1 M aq HCl (400 mL). The aqueous phase was extracted with CH₂Cl₂ (3 × 30 mL) and the combined organic extracts were dried over anhydrous Na₂SO₄, filtrated and concentrated under reduced pressure to provide the crude acid **SI-15**.

This crude acid **SI-15** was dissolved in CH₂Cl₂ (6.1 mL) and cooled to 0 °C. Amine **SI-13** (1.3 g, 6.1 mmol, 2.0 equiv) and HOBr·xH₂O (12 wt.% H₂O, 1.4 g, 9.1 mmol, 3.0 equiv) were added subsequently and it was stirred at the same temperature for 5 min. Then EDC·HCl (1.3 g, 6.7 mmol, 2.2 equiv) and diisopropylethylamine (1.1 g, 1.4 mL, 8.2 mmol, 2.7 equiv) were added and the reaction mixture was prior to warming to room temperature stirred further 5 min at 0 °C. After 16 h at room temperature the mixture was diluted with CH₂Cl₂ (20 mL) and 1.0 M aq NaHSO₄ (20 mL) was added. The aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL) and the combined organic extracts were washed with satd aq NH₄Cl (20 mL), dried over anhydrous Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 3:1 to 2:1) to afford peptide **SI-14** (1.1 g, 4.4 mmol, 78%) as colorless oil.

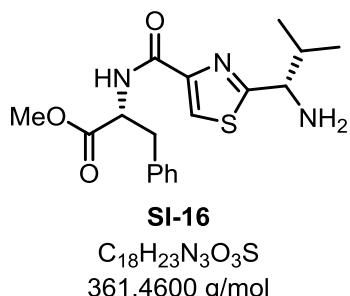
R_f = 0.27 (PE:EtOAc, 3:1); [α]_D (20 °C): −59.1 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.00 (s, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.30 – 7.24 (m, 3H), 7.18 – 7.14 (m, 2H), 5.13 (d, *J* = 8.5 Hz, 1H), 5.03 (dt, *J* = 8.3, 6.0 Hz, 1H), 4.91 – 4.80 (m, 1H), 3.73 (s, 3H), 3.22 (d, *J* = 6.0 Hz, 2H), 2.39 – 2.23 (m, 1H), 1.47 (s, 9H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 171.82, 160.62, 149.53, 136.02, 129.48, 128.71, 127.30, 123.24, 80.39, 57.99, 53.24, 52.49, 38.32, 33.36, 28.49, 19.34, 17.62 ppm, two C=O were not detected;

HRMS (ESI) = calculated for C₂₃H₃₂N₃O₅S [M+H⁺]: 462.2063, found: 462.2063.

methyl (2-((S)-1-amino-2-methylpropyl)thiazole-4-carbonyl)-D-phenylalaninate (SI-16)



To a stirred solution of peptide **SI-14** (1.0 g, 2.2 mmol, 1.0 equiv) in CH_2Cl_2 (43.5 mL) at 0 °C was added TFA (25.1 g, 16.9 mL, 220.0 mmol, 100.0 equiv) dropwise. After being stirred at the same temperature for 5 min the reaction mixture was allowed to warm to room temperature and stirred there for further 2 h. Then toluene (40 mL) was added and the volatiles were removed under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2 :MeOH, 20:1 to 15:1) to afford amine **SI-16** (790.0 mg, 2.2 mmol, 99%) as pale yellow solid.

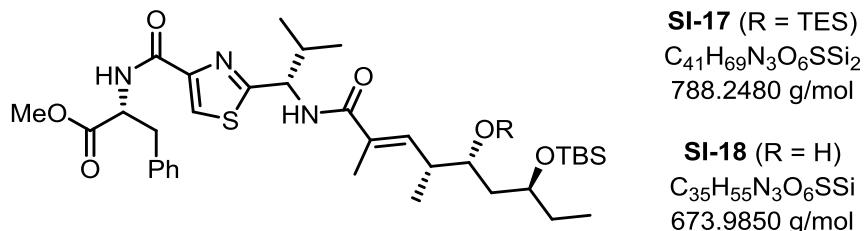
$R_f = 0.39$ (CH_2Cl_2 :MeOH, 20:1); **melting point:** 62–64 °C; $[\alpha]_D$ (20 °C): −7.2 (c = 1.0, $CHCl_3$);

1H -NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.78 (d, $J = 7.5$ Hz, 1H), 7.27 – 7.22 (m, 3H), 7.18 – 7.12 (m, 2H), 5.09 – 4.98 (m, 1H), 4.09 (d, $J = 4.9$ Hz, 1H), 3.73 (s, 3H), 3.22 (d, $J = 6.1$ Hz, 2H), 2.28 – 2.17 (m, 1H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.93 (d, $J = 6.8$ Hz, 3H) ppm;

^{13}C -NMR (100 MHz, $CDCl_3$): δ 173.14, 164.44, 160.64, 148.98, 136.45, 129.25, 128.66, 127.10, 125.53, 58.31, 54.37, 52.84, 37.57, 32.30, 18.34, 18.10 ppm;

HRMS (ESI) = calculated for $C_{18}H_{24}N_3O_3S$ [$M+H^+$]: 362.1538, found: 362.1539.

methyl (2-((S)-1-((4R,5R,7S,E)-7-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-5-((triethylsilyl)oxy)non-2-enamido)-2-methylpropyl)thiazole-4-carbonyl)-D-phenylalaninate (SI-17) and **methyl (2-((S)-1-((4R,5R,7S,E)-7-((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-2,4-dimethylnon-2-enamido)-2-methylpropyl)thiazole-4-carbonyl)-D-phenylalaninate (SI-18)**



To a stirred solution of acid **6** (270.0 mg, 0.61 mmol, 1.3 equiv) in CH_2Cl_2 (4.7 mL) at 0 °C were added amine **SI-16** (170.0 mg, 0.47 mmol, 1.0 equiv) and HOBt·xH₂O (12 wt.% H₂O, 215.0 mg, 1.4 mmol, 3.0 equiv) subsequently. It was stirred at the same temperature for 5 min. Then EDC·HCl (197.0 mg, 1.0 mmol, 2.2 equiv) and diisopropylethylamine (164.0 mg, 215.8 μL, 1.3 mmol, 2.7 equiv) were added and the reaction mixture was prior to warming to room temperature stirred further 5 min at 0 °C. After 16 h at room temperature the mixture was diluted with CH_2Cl_2 (10 mL) and 1.0 M aq NaHSO₄ (20 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic extracts were washed with satd aq NH₄Cl (20 mL), dried over anhydrous Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 3:1 to 1:1) to afford TES-ether **SI-17** (222.0 mg, 0.28 mmol, 60%) and alcohol **SI-18** (88.0 mg, 0.13 mmol, 28%) both as colorless oils.

TES-ether **SI-17**:

$R_f = 0.29$ (PE:EtOAc, 3:1); $[\alpha]_D$ (20 °C): -20.4 (c = 1.0, CHCl₃);

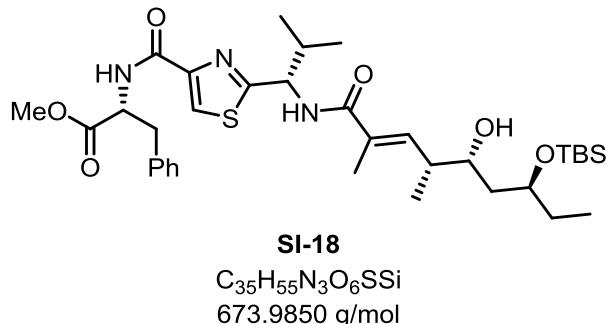
¹H-NMR (400 MHz, CDCl₃): δ 8.00 (s, 1H), 7.67 (d, $J = 8.1$ Hz, 1H), 7.26 – 7.22 (m, 3H), 7.17 – 7.13 (m, 2H), 6.39 – 6.32 (m, 1H), 6.22 (d, $J = 8.9$ Hz, 1H), 5.25 (dd, $J = 8.8, 5.8$ Hz, 1H), 5.02 (dt, $J = 8.1, 6.1$ Hz, 1H), 3.80 – 3.74 (m, 1H), 3.73 (s, 3H), 3.70 – 3.63 (m, 1H), 3.26 – 3.18 (m, 2H), 2.63 – 2.50 (m, 1H), 2.47 – 2.36 (m, 1H), 1.89 (d, $J = 1.3$ Hz, 3H), 1.66 – 1.59 (m, 2H), 1.52 – 1.44 (m, 2H), 1.02 (d, $J = 6.8$ Hz, 3H), 0.98 (d, $J = 2.1$ Hz, 3H), 0.96 (d, $J = 2.9$ Hz, 3H), 0.95 (t, $J = 7.9$ Hz, 9H), 0.90 – 0.86 (m, 12H), 0.63 – 0.56 (m, 6H), 0.07 (s, 3H), 0.06 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 171.97, 171.82, 169.09, 160.59, 149.50, 140.03, 136.06, 130.08, 129.43, 128.67, 127.25, 123.39, 73.08, 71.26, 56.30, 53.38, 52.50, 42.60, 38.75, 38.33, 33.15, 30.50, 26.06, 19.48, 18.25, 17.93, 14.21, 13.28, 9.41, 7.18, 5.54, -3.66, -4.07 ppm;

HRMS (ESI) = calculated for C₄₁H₆₉N₃O₆Si₂Na [M+Na⁺]: 810.4343, found: 810.4338.

Analytical data for alcohol **SI-18** see procedure below.

methyl (2-((S)-1-((4R,5R,7S,E)-7-((tert-butyldimethylsilyl)oxy)-5-hydroxy-2,4-dimethylnon-2-enamido)-2-methylpropyl)thiazole-4-carbonyl)-D-phenylalaninate (SI-18)



To a stirred solution of TES-ether **SI-17** (20.0 mg, 25.4 μ mol, 1.0 equiv) in THF (440.0 μ L) at room temperature were added MeOH (2.0 mL) and PPTs (11.5 mg, 45.8 μ mol, 1.8 equiv). It was stirred for 2.5 h at the same temperature before satd aq NaHCO₃ (5 mL) was added. The aqueous phase was extracted with CH₂Cl₂ (3 \times 5 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 1:1 to EtOAc) to afford alcohol **SI-18** (12.9 mg, 19.2 μ mol, 75%) as colorless oil.

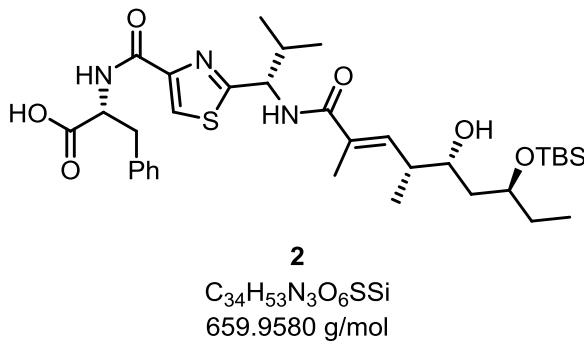
R_f = 0.61 (PE:EtOAc, 1:1); $[\alpha]_D$ (20 °C): -31.8 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.01 (s, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.26 – 7.23 (m, 3H), 7.17 – 7.13 (m, 2H), 6.34 – 6.26 (m, 2H), 5.24 (dd, J = 8.8, 6.0 Hz, 1H), 5.02 (dt, J = 8.0, 6.0 Hz, 1H), 3.94 – 3.88 (m, 1H), 3.88 – 3.83 (m, 1H), 3.78 (bs, 1H), 3.74 (s, 3H), 3.25 – 3.19 (m, 2H), 2.59 – 2.47 (m, 1H), 2.42 – 2.32 (m, 1H), 1.91 (d, J = 1.1 Hz, 3H), 1.64 – 1.57 (m, 4H), 1.09 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 2.3 Hz, 3H), 0.95 (d, J = 2.3 Hz, 3H), 0.89 (s, 9H), 0.83 (t, J = 7.5 Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 171.86, 171.52, 168.76, 160.53, 149.43, 139.45, 136.03, 130.38, 129.43, 128.68, 127.27, 123.47, 73.57, 71.87, 56.31, 53.38, 52.52, 39.93, 38.30, 38.27, 33.46, 28.64, 25.95, 19.49, 18.07, 15.80, 13.32, 10.49, -4.47, -4.66 ppm;

HRMS (ESI) = calculated for C₃₅H₅₆N₃O₆SSi [M+H⁺]: 674.3659, found: 674.3658.

(2-((S)-1-((4*R*,5*R*,7*S*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-2,4-dimethylnon-2-enamido)-2-methylpropyl)thiazole-4-carbonyl)-D-phenylalanine (2)



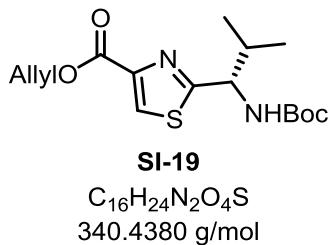
To a stirred solution of ester **SI-18** (70.0 mg, 0.1 mmol, 1.0 equiv) in THF (1.2 mL) at room temperature was added subsequently MeOH (300.0 μ L), H₂O (612.0 μ L) and 0.5 M aq LiOH (1.0 mL). After being stirred at the same temperature for 3 h the reaction mixture was poured into 10% aq citric acid (10 mL). The aqueous phase was extracted with EtOAc (3×10 mL) and the combined organic extracts were washed with brine (20 mL), dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂:MeOH, 10:1) to afford acid **2** (68.2 mg, 0.1 mmol, 99%) as colorless oil.

R_f = 0.34 (CH₂Cl₂:MeOH, 10:1);

¹H-NMR (400 MHz, CDCl₃): δ 8.02 (s, 1H), 7.78 (d, *J* = 7.5 Hz, 1H), 7.24 – 7.17 (m, 5H), 6.46 (d, *J* = 8.5 Hz, 1H), 6.31 – 6.24 (m, 1H), 5.26 – 5.17 (m, 1H), 5.03 – 4.93 (m, 1H), 3.93 – 3.87 (m, 2H), 3.35 – 3.18 (m, 2H), 2.57 – 2.48 (m, 1H), 2.38 – 2.28 (m, 1H), 1.90 (s, 3H), 1.66 – 1.57 (m, 4H), 1.06 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 7.1 Hz, 3H), 0.91 (d, *J* = 7.3 Hz, 3H), 0.88 (s, 9H), 0.83 (t, *J* = 7.5 Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 171.47, 169.04, 161.03, 149.02, 139.20, 136.10, 130.52, 129.55, 128.64, 127.18, 123.84, 73.57, 71.93, 56.42, 53.68, 39.70, 38.07, 37.74, 33.45, 28.61, 25.93, 19.48, 18.09, 18.06, 15.39, 13.33, 10.46, -4.47, -4.66 ppm, one C=O was not detected.

allyl (S)-2-((*tert*-butoxycarbonyl)amino)-2-methylpropyl)thiazole-4-carboxylate (SI-19)



To a stirred solution of thiazole **15** (1.7 g, 5.2 mmol, 1.0 equiv) in THF (58 mL) at room temperature was added subsequently MeOH (15 mL), H₂O (31 mL) and 0.5 M aq LiOH (12.5 mL). After being stirred at the same temperature for 3 h the reaction mixture was poured in 0.1 M aq HCl (200 mL). The aqueous phase was extracted with CH₂Cl₂ (3 × 40 mL) and the combined organic extracts were dried over anhydrous Na₂SO₄, filtrated and concentrated under reduced pressure to provide the crude acid **SI-15**.

This crude acid **SI-15** was dissolved in CH₂Cl₂ (52 mL) at room temperature. 4-DMAP (127.0 mg, 1.0 mmol, 0.2 equiv), allyl alcohol (453.0 mg, 533.0 μL, 7.8 mmol, 1.5 equiv) and DCC (1.6 g, 7.8 mmol, 1.5 equiv) were added subsequently and it was stirred at the same temperature for 18 h. The reaction mixture was then filtered through celite® which was afterwards rinsed several times with CH₂Cl₂. Satd aq NaHCO₃ (40 mL) was added to the filtrate. The aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 3:1) to afford allyl ester **SI-19** (1.6 g, 4.8 mmol, 92%) as yellow solid.

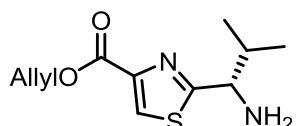
R_f = 0.57 (PE:EtOAc, 3:1); **melting point:** 100 °C; **[α]_D** (20 °C): −31.1 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.10 (s, 1H), 6.04 (ddt, *J* = 16.2, 10.4, 5.8 Hz, 1H), 5.46 – 5.22 (m, 3H), 4.95 – 4.86 (m, 1H), 4.86 – 4.83 (m, 2H), 2.51 – 2.38 (m, 1H), 1.45 (s, 9H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 173.44, 161.11, 155.57, 147.17, 132.05, 127.22, 119.00, 80.25, 66.07, 58.19, 33.43, 28.45, 19.56, 17.41 ppm;

HRMS (ESI) = calculated for C₁₆H₂₄N₂O₄SNa [M+Na⁺]: 363.1354, found: 363.1353.

allyl (S)-2-(1-amino-2-methylpropyl)thiazole-4-carboxylate (5)



5

C₁₁H₁₆N₂O₂S

240.3210 g/mol

To a stirred solution of allyl ester **SI-19** (1.9 g, 5.6 mmol, 1.0 equiv) in CH₂Cl₂ (112 mL) at 0 °C was added TFA (31.9 g, 21.6 mL, 280.0 mmol, 50.0 equiv) dropwise. After being stirred at the same temperature for 5 min the reaction mixture was allowed to warm to room temperature and stirred there for further 2 h. Then toluene (40 mL) was added and the volatiles were removed under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂:MeOH, 15:1) to afford amine **5** (1.3 g, 5.5 mmol, 99%) as pale yellow oil.

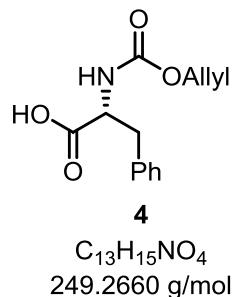
R_f = 0.61 (CH₂Cl₂:MeOH, 10:1); [α]_D (20 °C): -2.1 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.20 (s, 1H), 5.99 (ddt, J = 16.2, 10.4, 5.9 Hz, 1H), 5.47 – 5.26 (m, 2H), 4.81 (d, J = 5.7 Hz, 2H), 4.60 (d, J = 6.7 Hz, 1H), 2.52 – 2.36 (m, 1H), 1.13 (d, J = 6.8 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 165.92, 161.39, 146.25, 131.43, 129.18, 119.53, 66.73, 58.39, 32.68, 18.49, 18.26 ppm;

HRMS (ESI) = calculated for C₁₁H₁₆N₂O₂SNa [M+Na⁺]: 263.0830, found: 263.0826.

((allyloxy)carbonyl)-D-phenylalanine (4)¹⁹



To a stirred solution of D-phenylalanine (5.0 g, 30.3 mmol, 1.0 equiv) in THF (15.9 mL) at room temperature was added H₂O (33.6 mL). Subsequently a solution of allyl chloroformate (3.7 g, 3.2 mL, 30.3 mmol, 1.0 equiv) and 2.0 M aq NaOH (30.3 mL, 60.5 mmol, 2.0 equiv) were added. After stirring at the same temperature for 2 h the reaction mixture was concentrated under reduced pressure to a volume of 30 mL and then the pH value was adjusted to 2 with 2.0 M aq HCl. The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure to afford carbamate **4** (7.5 g, 30.1 mmol, 99%) as colorless oil.

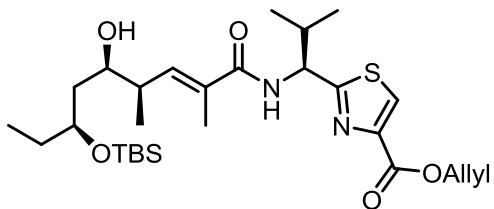
[*a*]D (20 °C): -29.1 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 9.60 (bs, 1H), 7.33 – 7.27 (m, 3H), 7.19 – 7.17 (m, 2H), 5.95 – 5.82 (m, 1H), 5.28 (d, *J* = 17.2 Hz, 1H), 5.23 – 5.18 (m, 2H), 4.73 – 4.65 (m, 1H), 4.56 (d, *J* = 5.5 Hz, 2H), 3.24 – 3.09 (m, 2H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 176.39, 155.90, 135.63, 132.55, 129.46, 128.82, 127.39, 118.13, 66.16, 54.67, 37.88 ppm;

HRMS (ESI) = calculated for C₁₃H₁₄NO₄ [M-H⁺]: 248.0923, found: 248.0922.

allyl 2-((S)-1-((4*R*,5*R*,7*S*,*E*)-7-((tert-butyldimethylsilyl)oxy)-5-hydroxy-2,4-dimethylnon-2-enamido)-2-methylpropylthiazole-4-carboxylate (16)



16

C₂₈H₄₈N₂O₅SSi
552.8460 g/mol

To a stirred solution of acid **6** (650.0 mg, 1.46 mmol, 1.0 equiv) in DMF (14.6 mL) at 0 °C were added amine **5** (1.1 g, 4.39 mmol, 3.0 equiv) and HOEt·xH₂O (12 wt.% H₂O, 1.3 g, 8.78 mmol, 6.0 equiv) subsequently. It was stirred at the same temperature for 5 min. Then EDC·HCl (1.2 g, 6.44 mmol, 4.4 equiv) and diisopropylethylamine (1.0 g, 1.3 mL, 7.90 mmol, 5.4 equiv) were added and the reaction mixture was prior to warming to room temperature stirred further 5 min at 0 °C. After 48 h at room temperature the mixture was diluted with CH₂Cl₂ (30 mL) and 1.0 M aq NaHSO₄ (30 mL) was added. The aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL) and the combined organic extracts were washed with satd aq NH₄Cl (30 mL), dried over anhydrous Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 3:1 to 2:1) to afford alcohol **16** (645.0 mg, 1.17 mmol, 80%) as pale yellow oil.

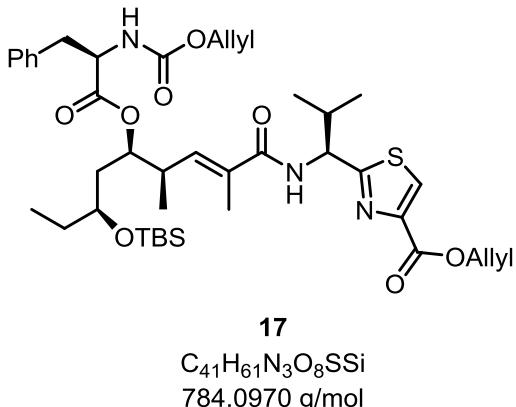
R_f = 0.33 (PE:EtOAc, 3:1); [α]_D (20 °C): −21.6 (c = 1.0, CHCl₃);

¹H-NMR (400 MHz, CDCl₃): δ 8.09 (s, 1H), 6.52 (d, J = 8.8 Hz, 1H), 6.23 – 6.19 (m, 1H), 6.07 – 5.97 (m, 1H), 5.44 – 5.28 (m, 2H), 5.22 (dd, J = 8.8, 7.1 Hz, 1H), 4.86 – 4.83 (m, 2H), 3.91 – 3.85 (m, 1H), 3.84 – 3.79 (m, 1H), 2.53 – 2.42 (m, 2H), 1.90 (d, J = 1.3 Hz, 3H), 1.64 – 1.55 (m, 4H), 1.08 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.88 (s, 9H), 0.82 (t, J = 7.5 Hz, 3H), 0.08 (s, 3H), 0.06 (s, 3H) ppm;

¹³C-NMR (100 MHz, CDCl₃): δ 171.72, 169.00, 161.00, 147.01, 139.12, 131.99, 130.67, 127.39, 119.02, 73.50, 71.94, 66.09, 56.54, 39.97, 38.34, 33.56, 28.68, 25.95, 19.74, 18.37, 18.07, 15.90, 13.36, 10.43, −4.47, −4.66 ppm;

HRMS (ESI) = calculated for C₂₈H₄₈N₂O₅SSiNa [M+Na⁺]: 575.2951, found: 575.2946.

allyl 2-((7*R*,10*R*,11*R*,16*S*,*E*)-7-benzyl-10-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-11,13,17-trimethyl-5,8,14-trioxo-4,9-dioxa-6,15-diazaoctadeca-1,12-dien-16-yl)thiazole-4-carboxylate (17)



To a stirred solution of alcohol **16** (110.0 mg, 0.20 mmol, 1.0 equiv) in CH_2Cl_2 (2.2 mL) at room temperature were added acid **4** (99.3 mg, 0.40 mmol, 2.0 equiv), 4-DMAP (4.9 mg, 0.04 mmol, 0.2 equiv) and DCC (86.3 mg, 0.42 mmol, 2.1 equiv) subsequently. It was stirred at the same temperature for 18 h and then filtered through celite® which was afterwards rinsed several times with CH_2Cl_2 . Satd aq $NaHCO_3$ (10 mL) was added to the filtrate and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 3:1) to afford ester **17** (129.1 mg, 0.16 mmol, 83%) as colorless oil.

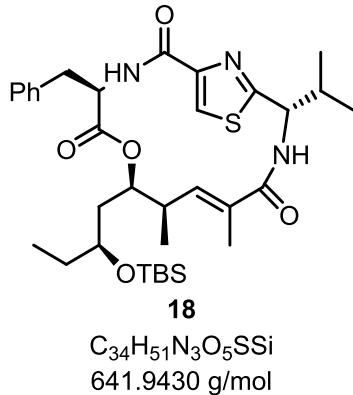
$R_f = 0.49$ (PE:EtOAc, 3:1); $[\alpha]_D$ (20 °C): +14.4 (c = 1.0, $CHCl_3$);

1H -NMR (400 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.29 – 7.24 (m, 4H), 7.17 – 7.14 (m, 2H), 6.78 (d, $J = 8.6$ Hz, 1H), 6.13 (d, $J = 9.6$ Hz, 1H), 6.01 (ddt, $J = 16.2, 10.5, 5.8$ Hz, 1H), 5.89 – 5.78 (m, 1H), 5.42 – 5.27 (m, 2H), 5.24 – 5.14 (m, 3H), 5.10 – 5.04 (m, 1H), 4.81 (d, $J = 5.8$ Hz, 2H), 4.59 – 4.53 (m, 1H), 4.50 (d, $J = 4.6$ Hz, 2H), 3.62 – 3.53 (m, 1H), 3.15 (dd, $J = 14.1, 5.4$ Hz, 1H), 2.94 (dd, $J = 14.1, 8.1$ Hz, 1H), 2.80 – 2.71 (m, 1H), 2.54 – 2.45 (m, 1H), 1.86 (d, $J = 1.2$ Hz, 3H), 1.69 – 1.59 (m, 2H), 1.49 – 1.43 (m, 2H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.97 – 0.93 (m, 6H), 0.87 (s, 9H), 0.84 (t, $J = 7.2$ Hz, 3H), 0.02 (s, 3H), 0.02 (s, 3H) ppm;

^{13}C -NMR (100 MHz, $CDCl_3$): δ 172.38, 171.81, 169.30, 161.00, 155.80, 146.99, 136.92, 136.02, 132.69, 132.03, 131.71, 129.28, 128.82, 127.34, 127.28, 118.95, 117.98, 76.30, 69.96, 66.03, 66.00, 56.82, 55.28, 38.63, 38.03, 37.00, 33.19, 30.53, 26.06, 19.75, 18.29, 18.18, 14.88, 13.36, 8.78, -3.95, -4.66 ppm;

HRMS (ESI) = calculated for $C_{41}H_{61}N_3O_8SSiNa$ [$M+Na^+$]: 806.3846, found: 806.3849.

($1^2Z,4R,7R,8R,9E,13S$)-4-benzyl-7-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-13-isopropyl-8,10-dimethyl-6-oxa-3,12-diaza-1(4,2)-thiazolacyclotridecaphan-9-ene-2,5,11-trione (18)



To a stirred solution of bis-protected linear precursor **17** (120.0 mg, 0.15 mmol, 1.0 equiv) in CH_2Cl_2 (3.1 mL) at room temperature were added subsequently phenylsilane (66.3 mg, 75.3 μ L, 0.61 mmol, 4.0 equiv) and $Pd(PPh_3)_4$ (17.7 mg, 15.3 μ mol, 0.1 equiv). The reaction mixture was stirred for 3 h at the same temperature and then diluted with CH_2Cl_2 (10 mL) before it was poured into 1.0 M aq $NaHSO_4$ (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3×10 mL) and the combined organic extracts were dried over anhydrous $MgSO_4$, filtrated and concentrated to provide the crude amino acid **SI-20**.

This crude amino acid **SI-20** was dissolved in DMF (5.1 mL) and diisopropylethylamine (79.2 mg, 107.0 μ L, 0.61 mmol, 4.0 equiv) was added under stirring at room temperature. Subsequently a solution of HATU (61.2 mg, 0.16 mmol, 1.05 equiv) in DMF (4.0 mL) was added over 1 h through a syringe pump and the reaction mixture was stirred for further 22 h. Afterwards it was diluted with EtOAc (20 mL) and 1.0 M aq $NaHSO_4$ (30 mL) was added. The aqueous phase was extracted with EtOAc (3×10 mL) and the combined organic extracts were washed with H_2O (30 mL) and brine (30 mL) subsequently, dried over anhydrous Na_2SO_4 , filtrated and concentrated. The residue was purified by column chromatography (PE:EtOAc, 2:1) to afford macrolactone **18** (62.1 mg, 96.8 μ mol, 63%) as colorless oil.

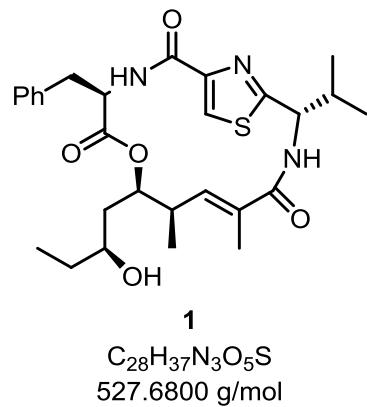
$R_f = 0.46$ (PE:EtOAc, 2:1); $[\alpha]_D$ (20 °C): -21.9 ($c = 1.0, CHCl_3$);

1H -NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.86 (d, $J = 7.4$ Hz, 1H), 7.33 – 7.29 (m, 2H), 7.27 – 7.23 (m, 3H), 6.66 – 6.60 (m, 1H), 6.35 (d, $J = 9.4$ Hz, 1H), 5.39 (dd, $J = 9.4, 3.0$ Hz, 1H), 4.84 (ddd, $J = 9.8, 7.4, 5.1$ Hz, 1H), 4.79 – 4.71 (m, 1H), 3.86 – 3.78 (m, 1H), 3.42 (dd, $J = 13.5, 5.1$ Hz, 1H), 3.04 (dd, $J = 13.5, 9.9$ Hz, 1H), 2.92 – 2.82 (m, 1H), 2.36 – 2.27 (m, 1H), 1.96 (d, $J = 1.2$ Hz, 3H), 1.77 – 1.72 (m, 2H), 1.49 – 1.42 (m, 2H), 1.19 (d, $J = 6.7$ Hz, 3H), 1.13 (d, $J = 7.0$ Hz, 3H), 0.91 (s, 9H), 0.83 (t, $J = 7.4$ Hz, 3H), 0.83 (d, $J = 5.1$ Hz, 3H), 0.08 (s, 3H), 0.06 (s, 3H) ppm;

^{13}C -NMR (100 MHz, $CDCl_3$): δ 171.85, 171.58, 168.60, 160.41, 147.97, 135.83, 135.49, 134.87, 129.35, 128.78, 127.30, 124.11, 77.94, 71.28, 57.49, 57.31, 39.21, 37.61, 36.21, 34.92, 30.47, 26.01, 20.08, 18.16, 16.12, 13.48, 13.28, 8.82, $-3.96, -4.33$ ppm;

HRMS (ESI) = calculated for $C_{34}H_{51}N_3O_5SSiNa$ [$M+Na^+$]: 664.3216, found: 664.3224.

Antalid (**1**)



To a stirred solution of TBS-ether **18** (17.0 mg, 26.5 μ mol, 1.0 equiv) in THF (1.3 mL) at 0 °C was added HF-pyridine (~70% hydrogen fluoride, 270.0 μ L) dropwise. After being stirred at the same temperature for 1 h the reaction mixture was allowed to warm to room temperature where stirring was continued for 4 h. Then the mixture was recooled to 0 °C and THF (10 mL) and satd aq NaHCO₃ (10 mL) were added subsequently. The aqueous phase was extracted with EtOAc (3 \times 10 mL) and the combined organic extracts were washed with aq pH 7 buffer (20 mL), dried over anhydrous MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography (PE:EtOAc, 1:2) to afford antalid (**1**) (12.6 mg, 23.9 μ mol, 90%) as white solid.

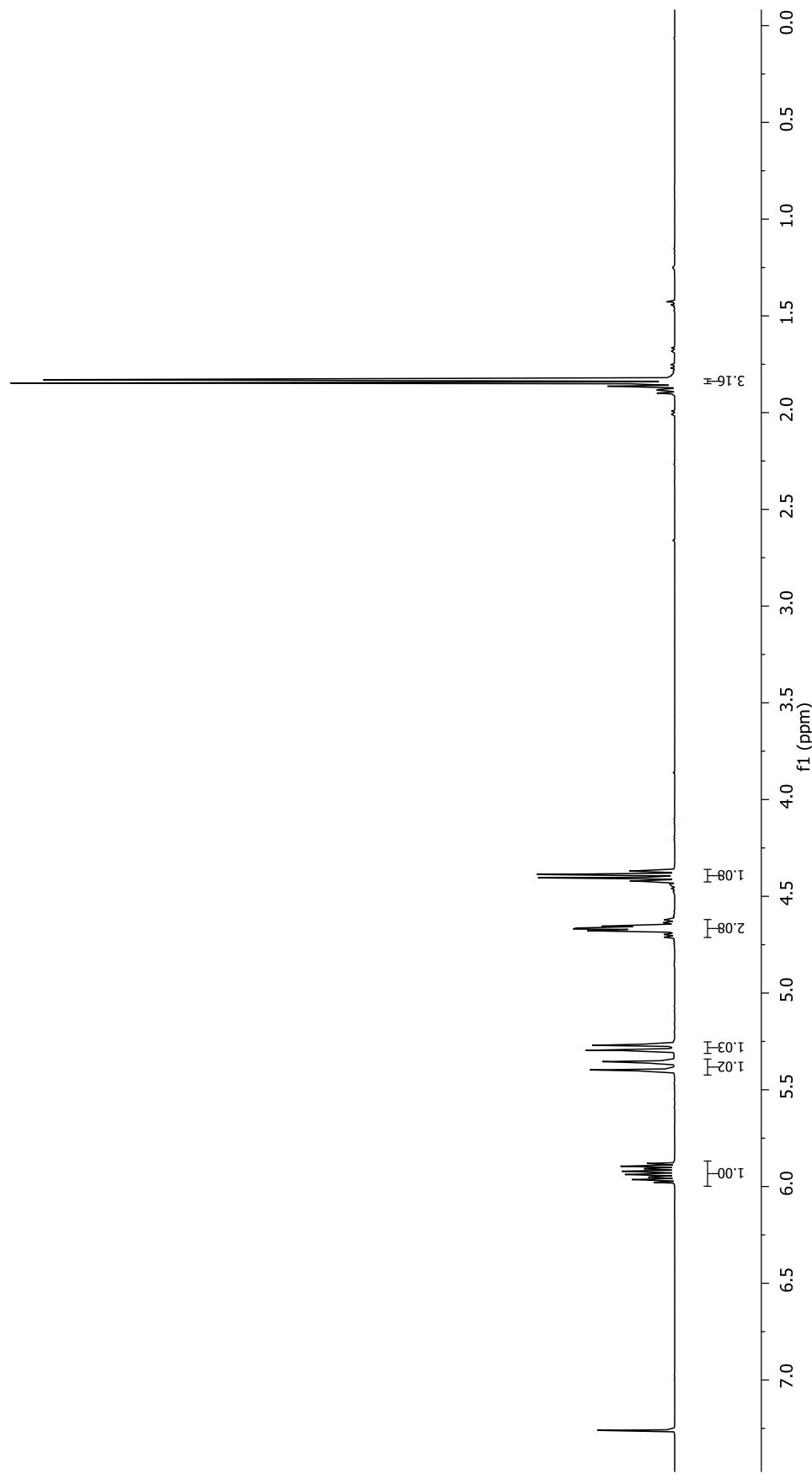
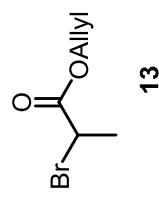
R_f = 0.24 (PE:EtOAc, 1:2); [α]_D (20 °C): -19.1 (c = 1.0, CHCl₃);

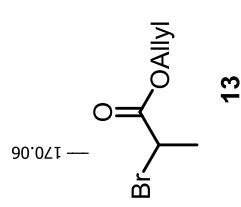
¹H-NMR (400 MHz, DMSO): δ 8.34 (s, 1H), 8.25 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.26 – 7.22 (m, 3H), 6.60 (dd, J = 8.4, 1.1 Hz, 1H), 5.22 (dd, J = 9.2, 3.7 Hz, 1H), 4.79 – 4.73 (m, 1H), 4.57 (ddd, J = 9.9, 7.6, 5.8 Hz, 1H), 4.43 (d, J = 5.7 Hz, 1H), 3.46 – 3.37 (m, 1H), 3.24 (dd, J = 13.3, 5.6 Hz, 1H), 3.01 (dd, J = 13.3, 10.0 Hz, 1H), 2.92 – 2.82 (m, 1H), 2.31 – 2.22 (m, 1H), 1.79 (d, J = 0.7 Hz, 3H), 1.68 – 1.57 (m, 2H), 1.33 – 1.26 (m, 2H), 1.12 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 6.7 Hz, 3H), 0.79 (t, J = 7.4 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H) ppm;

¹³C-NMR (100 MHz, DMSO): δ 171.58, 171.39, 168.40, 159.84, 146.93, 136.23, 136.13, 132.53, 129.10, 128.45, 126.86, 124.69, 77.30, 68.61, 57.08, 56.48, 37.91, 37.62, 35.08, 33.52, 30.30, 20.21, 16.69, 12.96, 12.72, 9.57 ppm;

HRMS (ESI) = calculated for C₂₈H₃₇N₃O₅SNa [M+Na⁺]: 550.2352, found: 550.2353.

4.3 NMR spectra





— 21.79

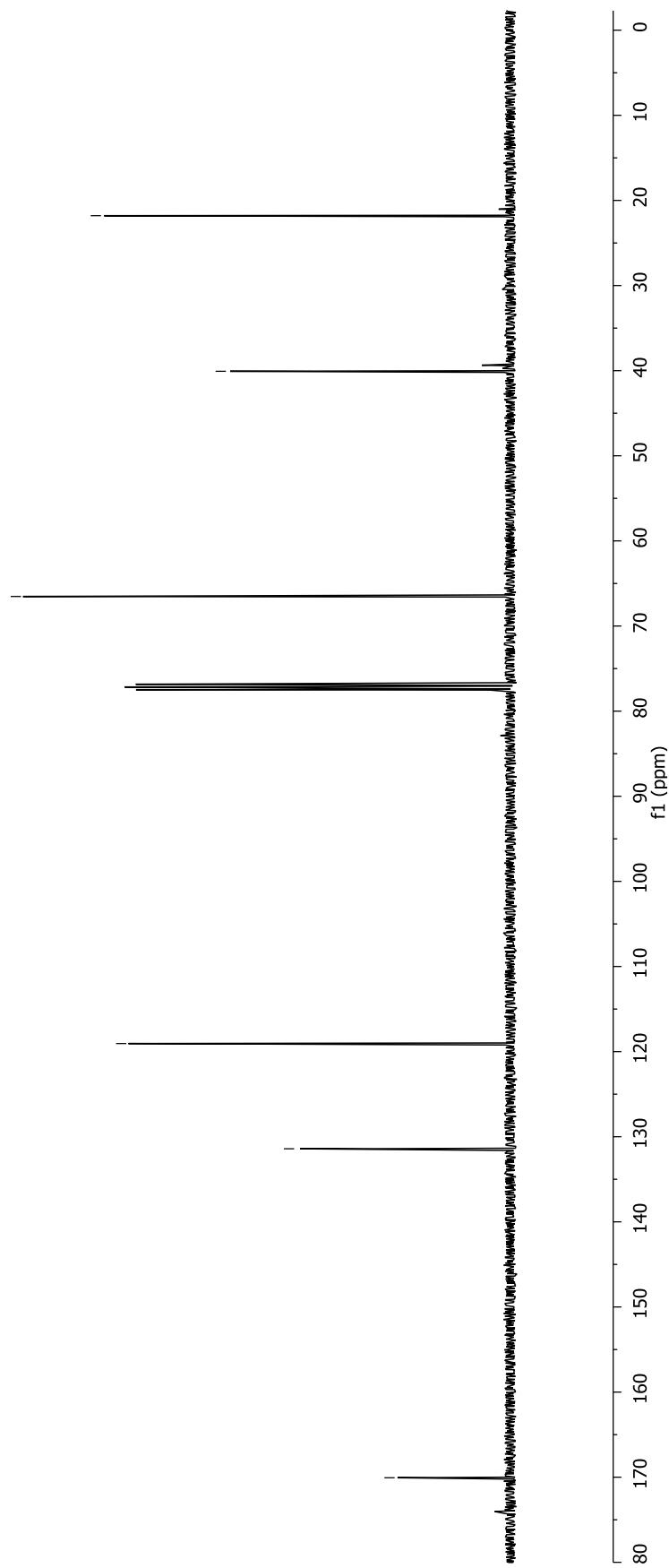
— 40.07

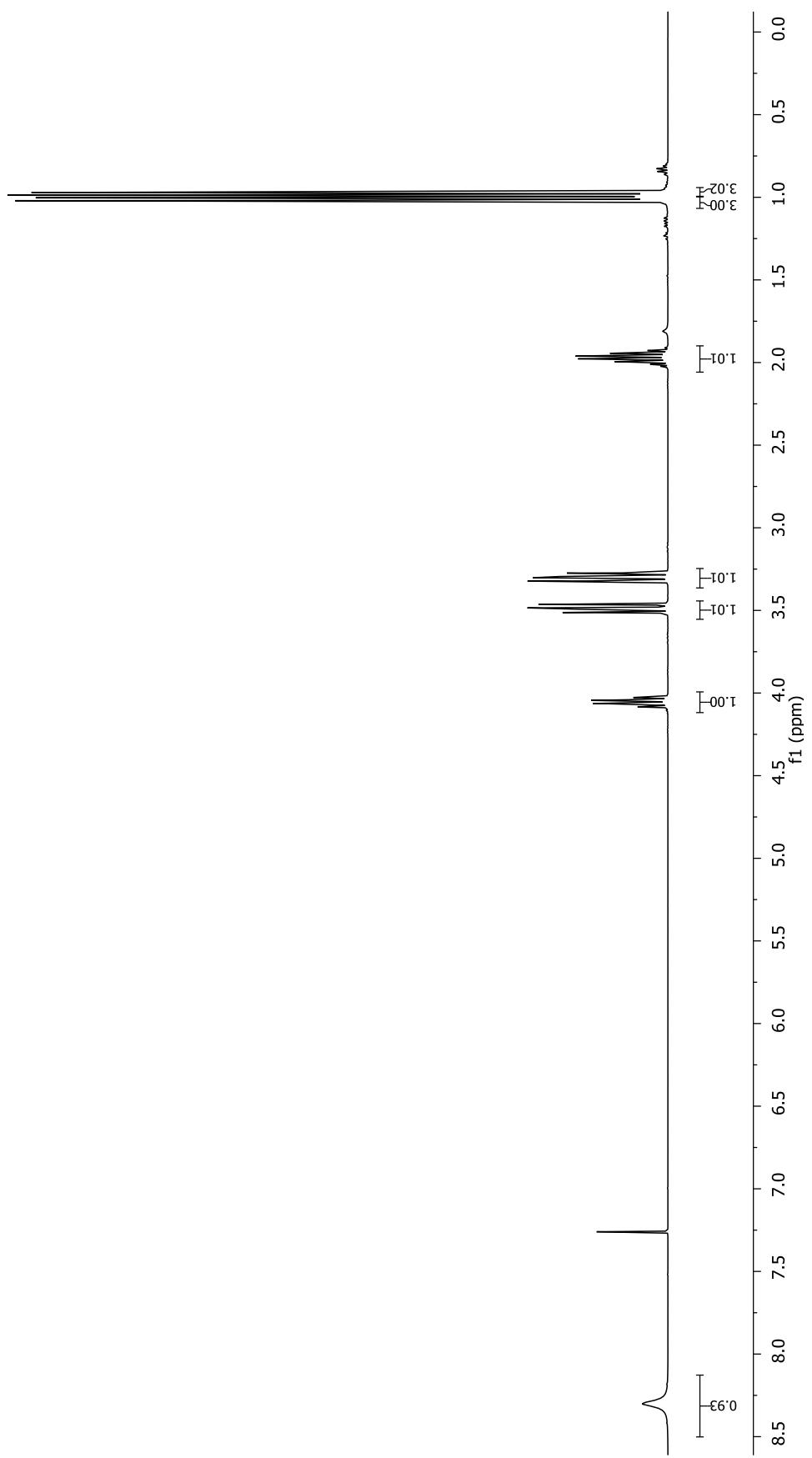
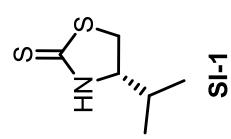
— 66.53

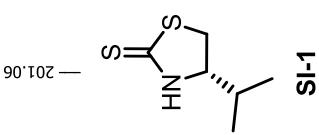
— 119.05

— 131.41

— 170.06



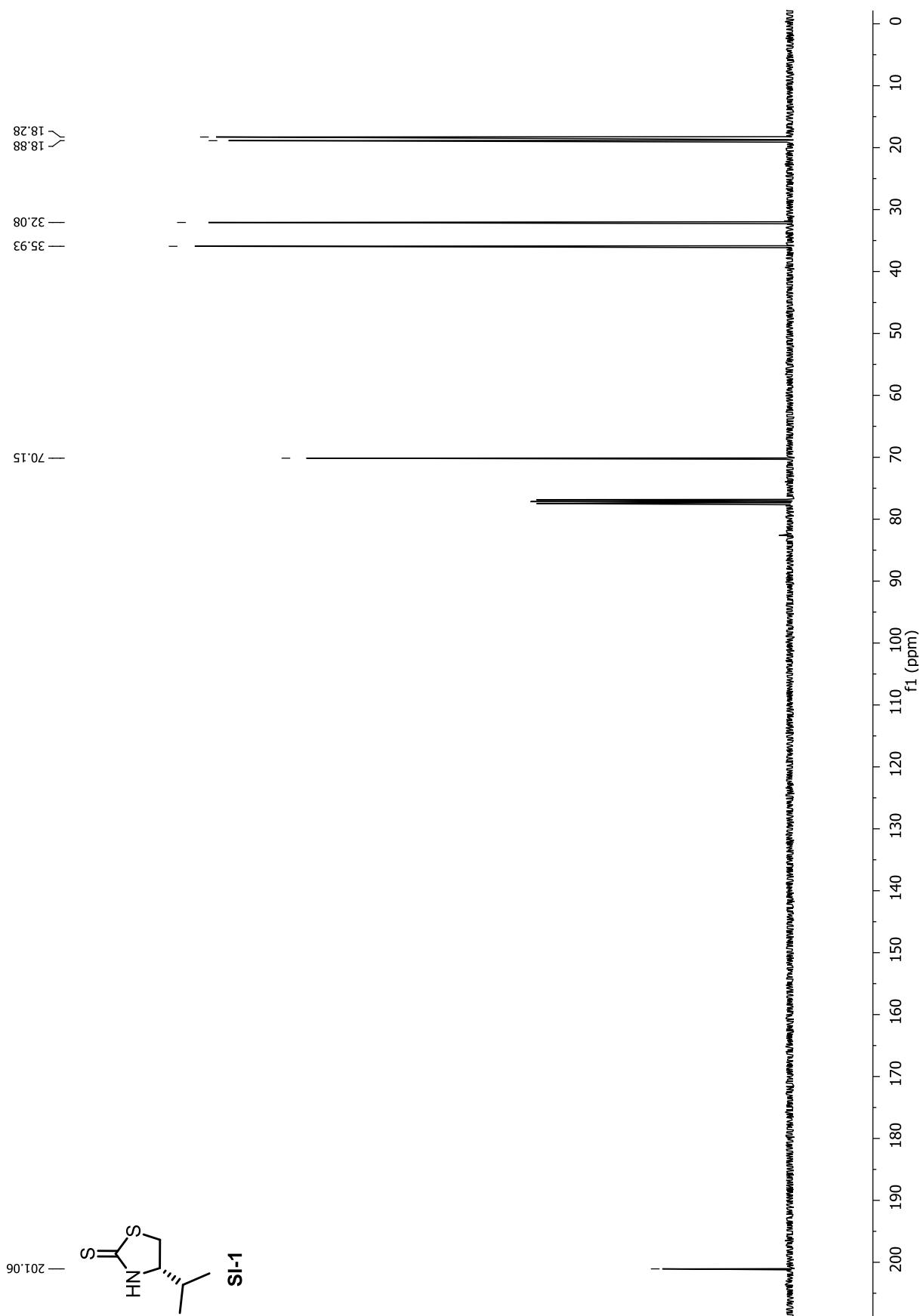


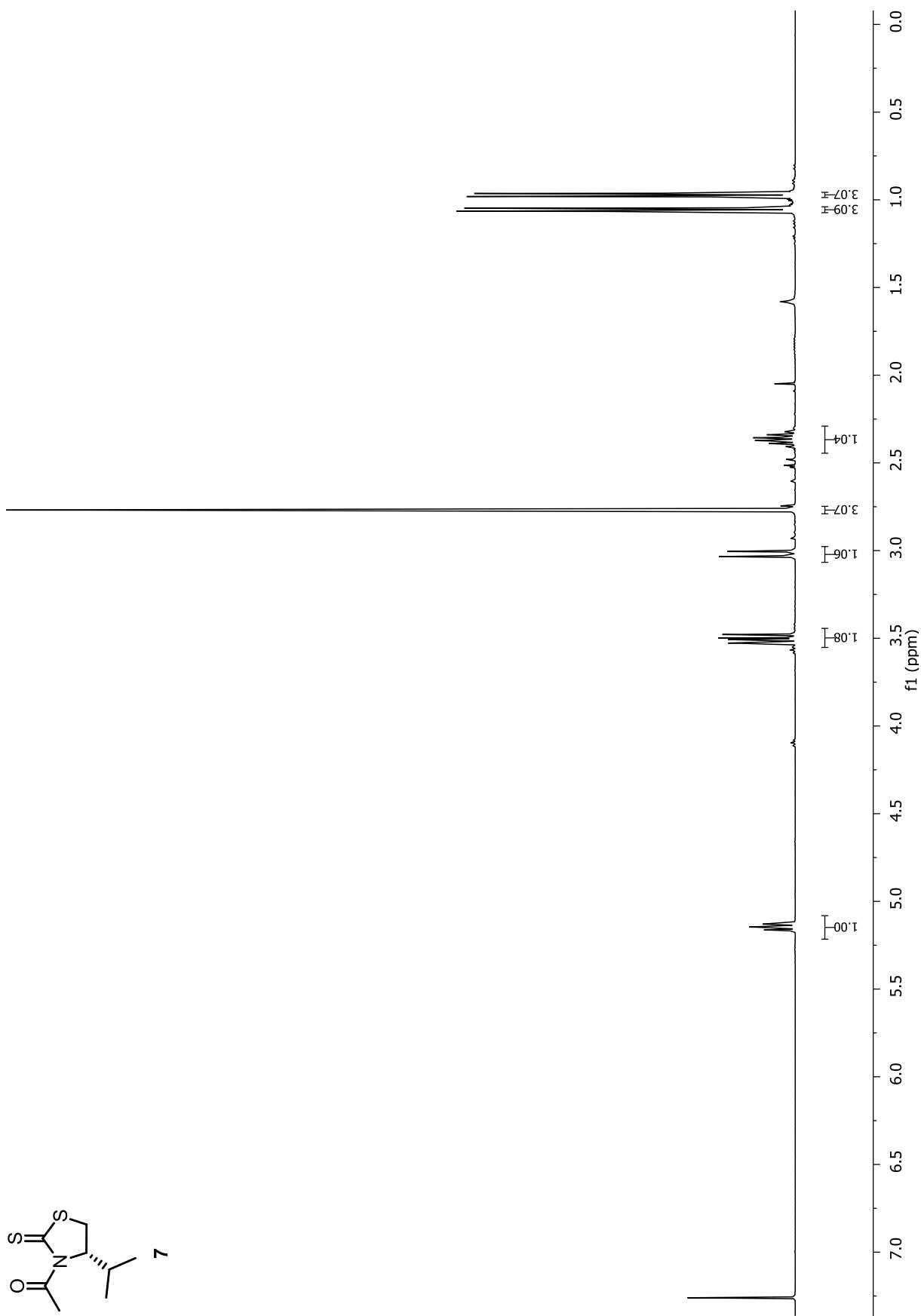


— 18.88

— 32.08
— 35.93

— 70.15

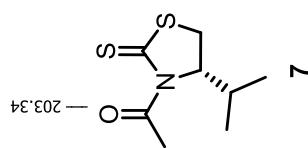
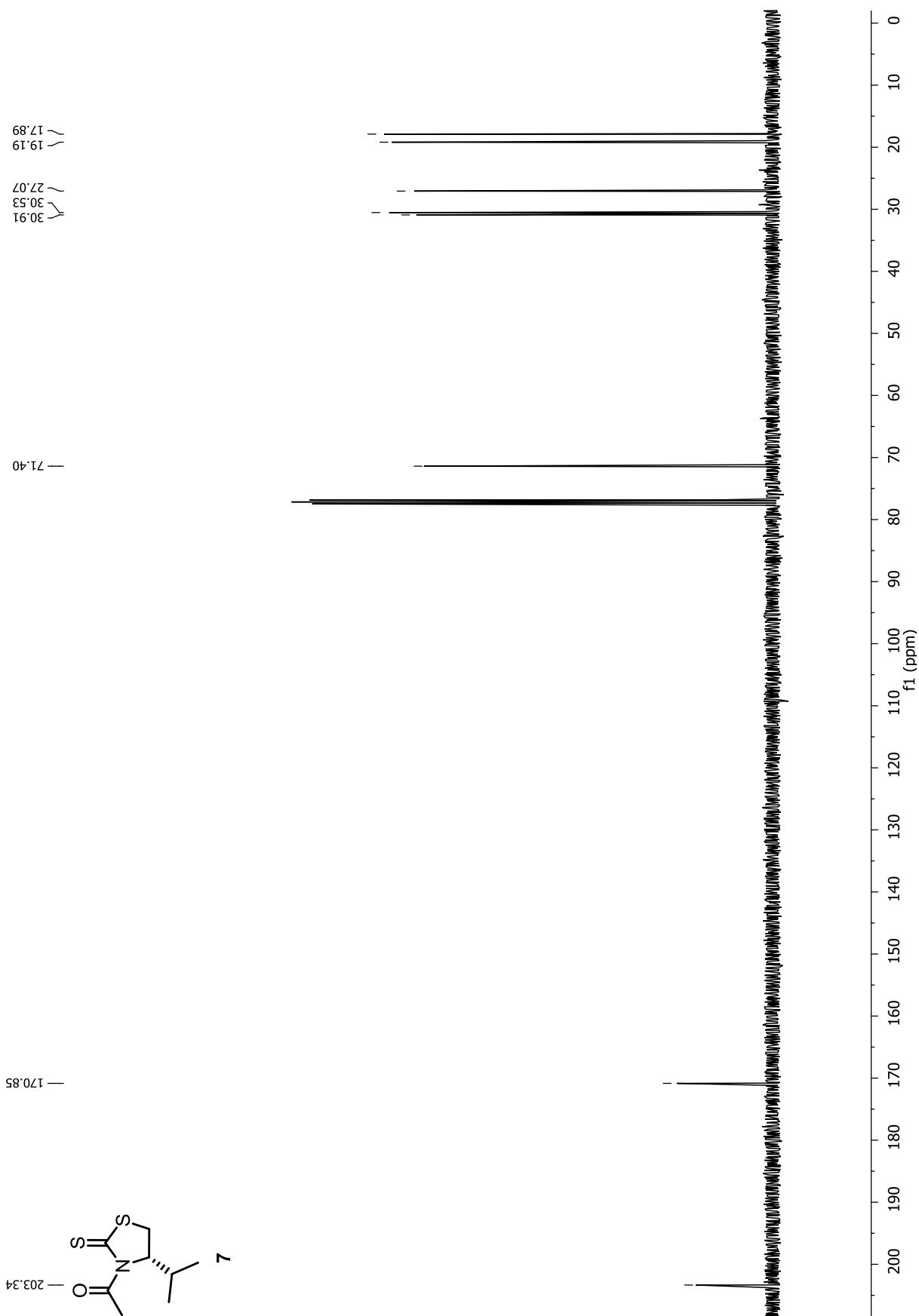


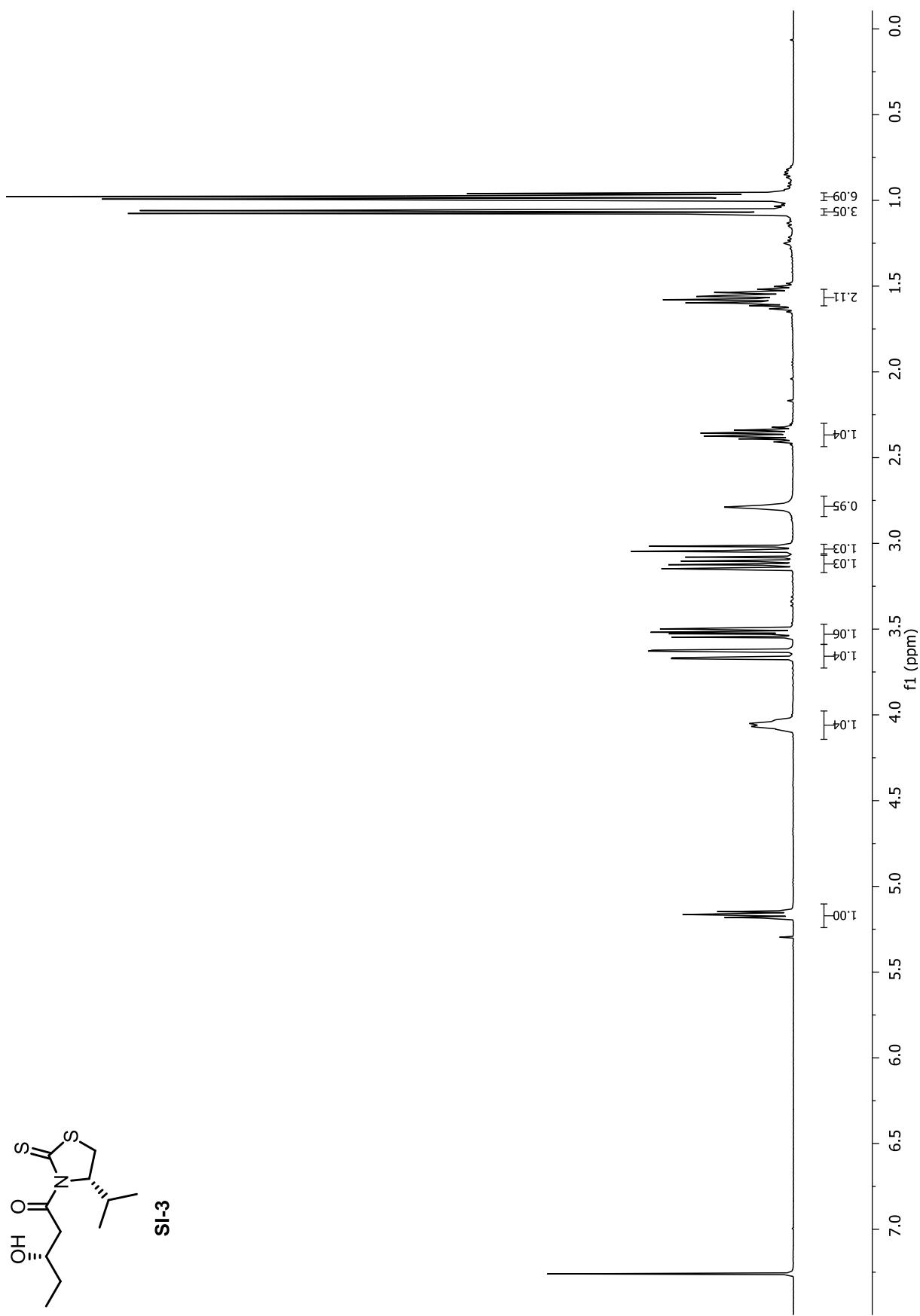
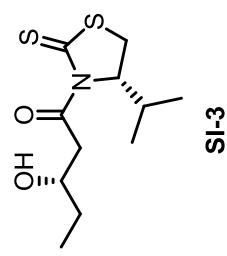


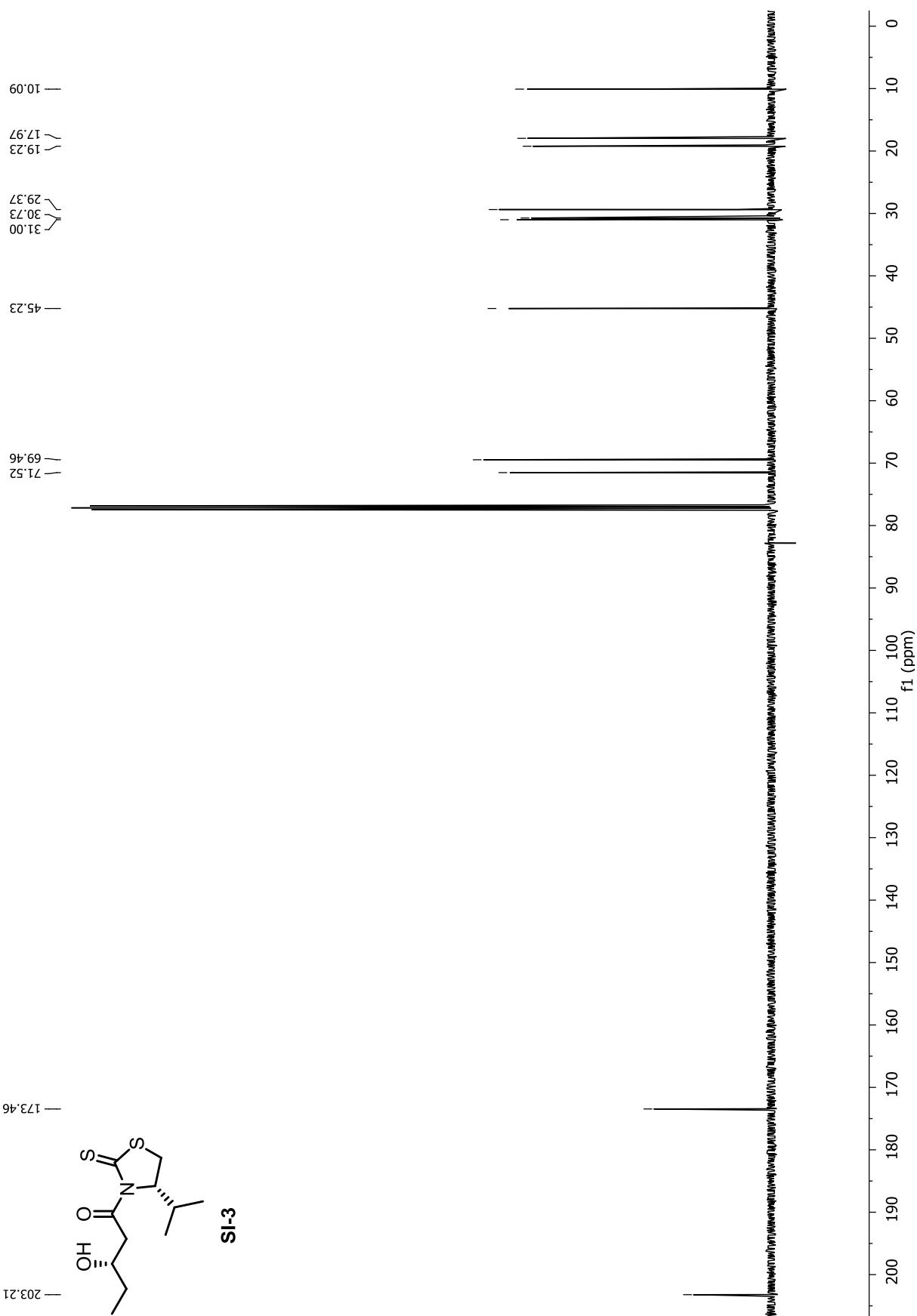
— 170.85

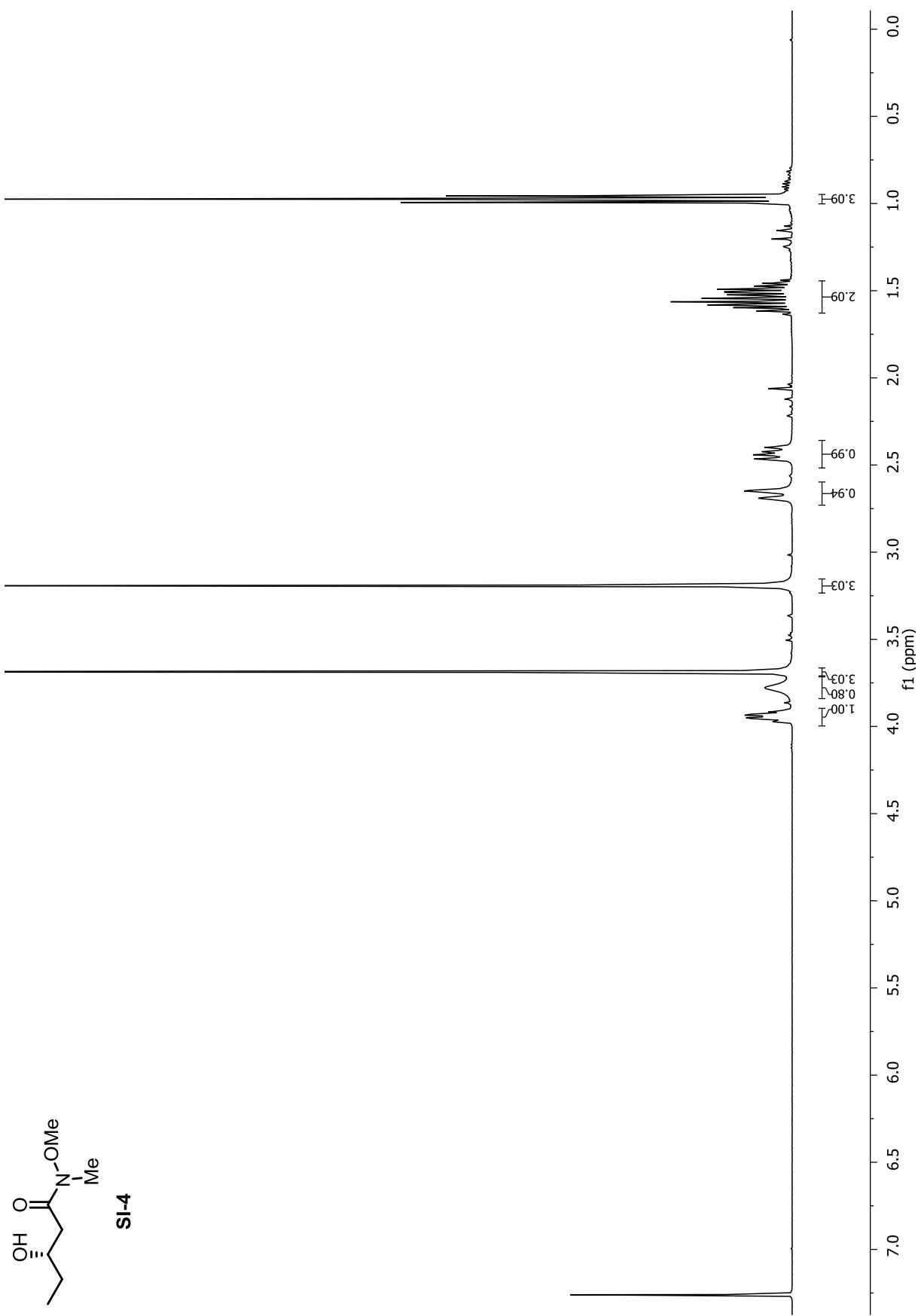
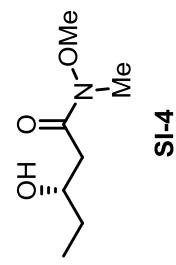
— 203.34

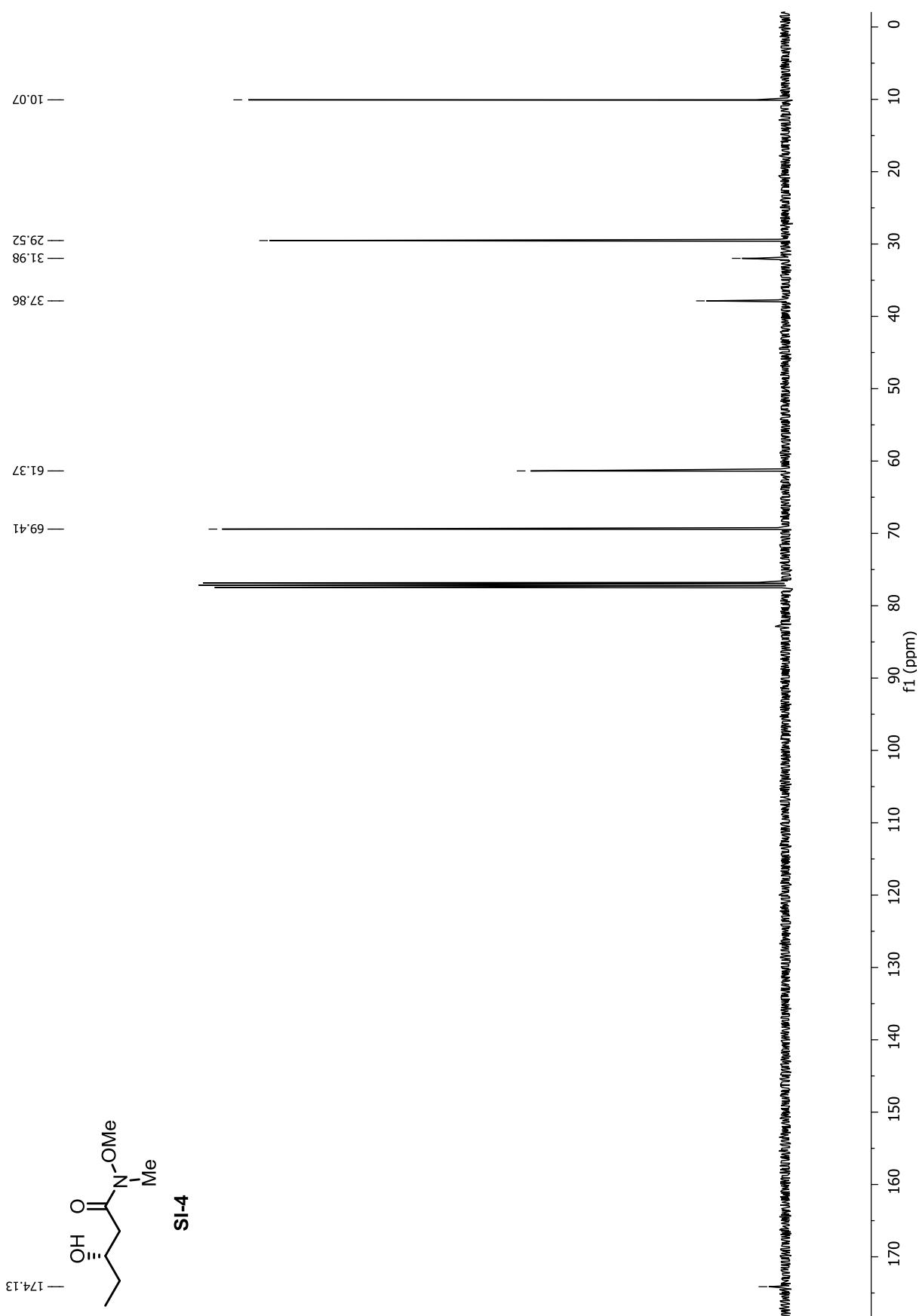
— 71.40

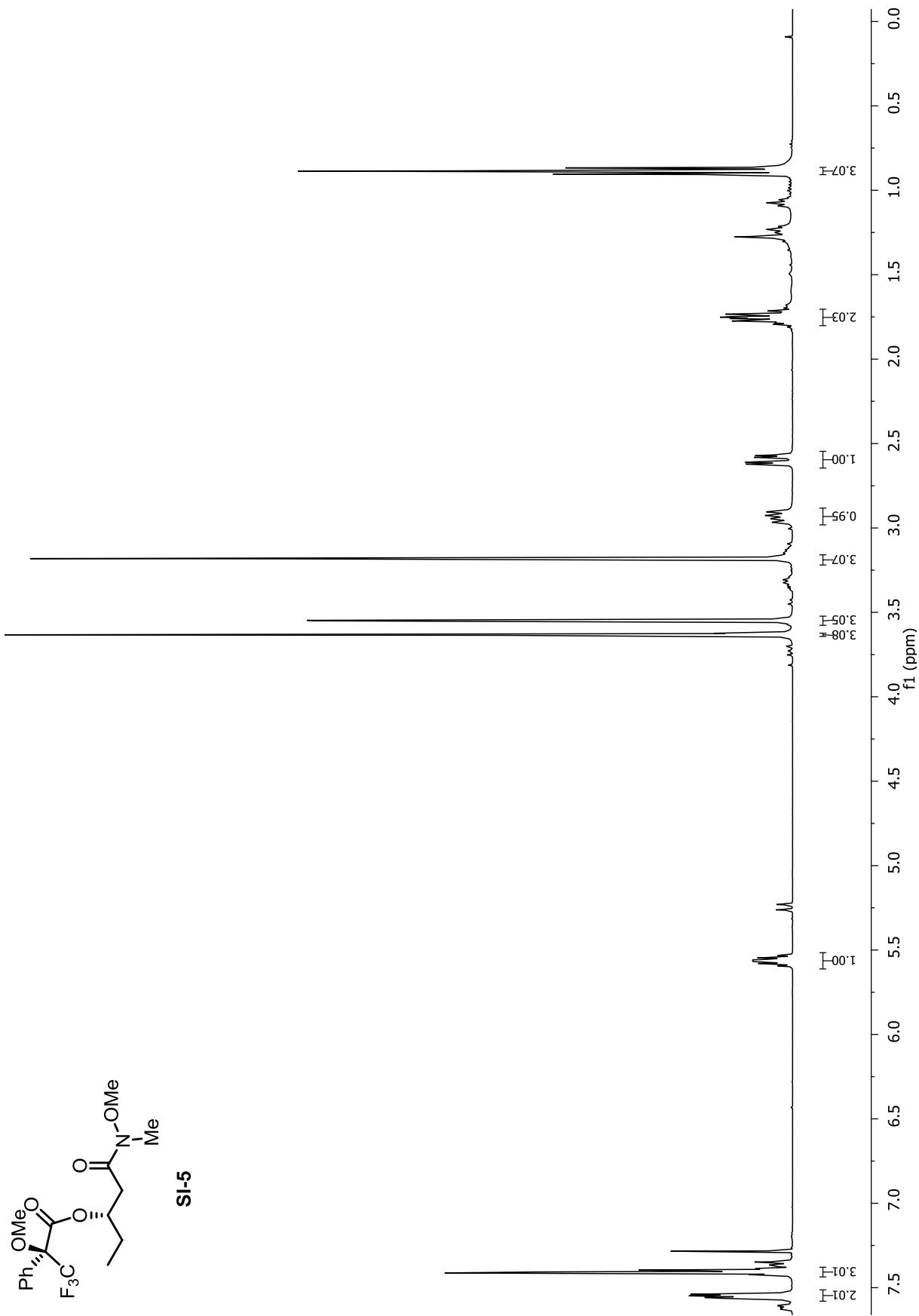
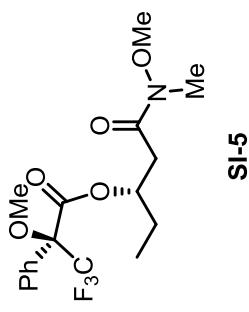


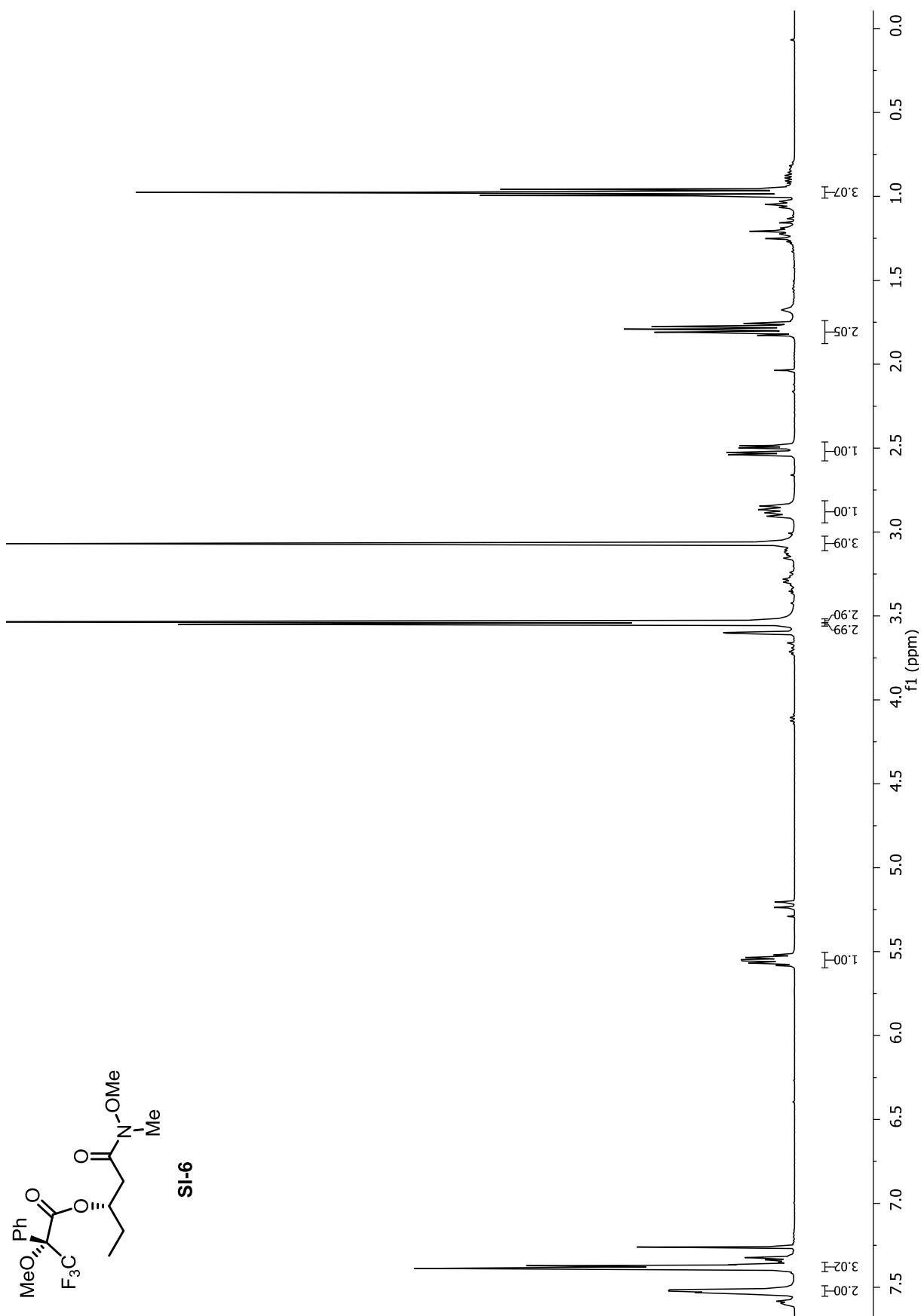


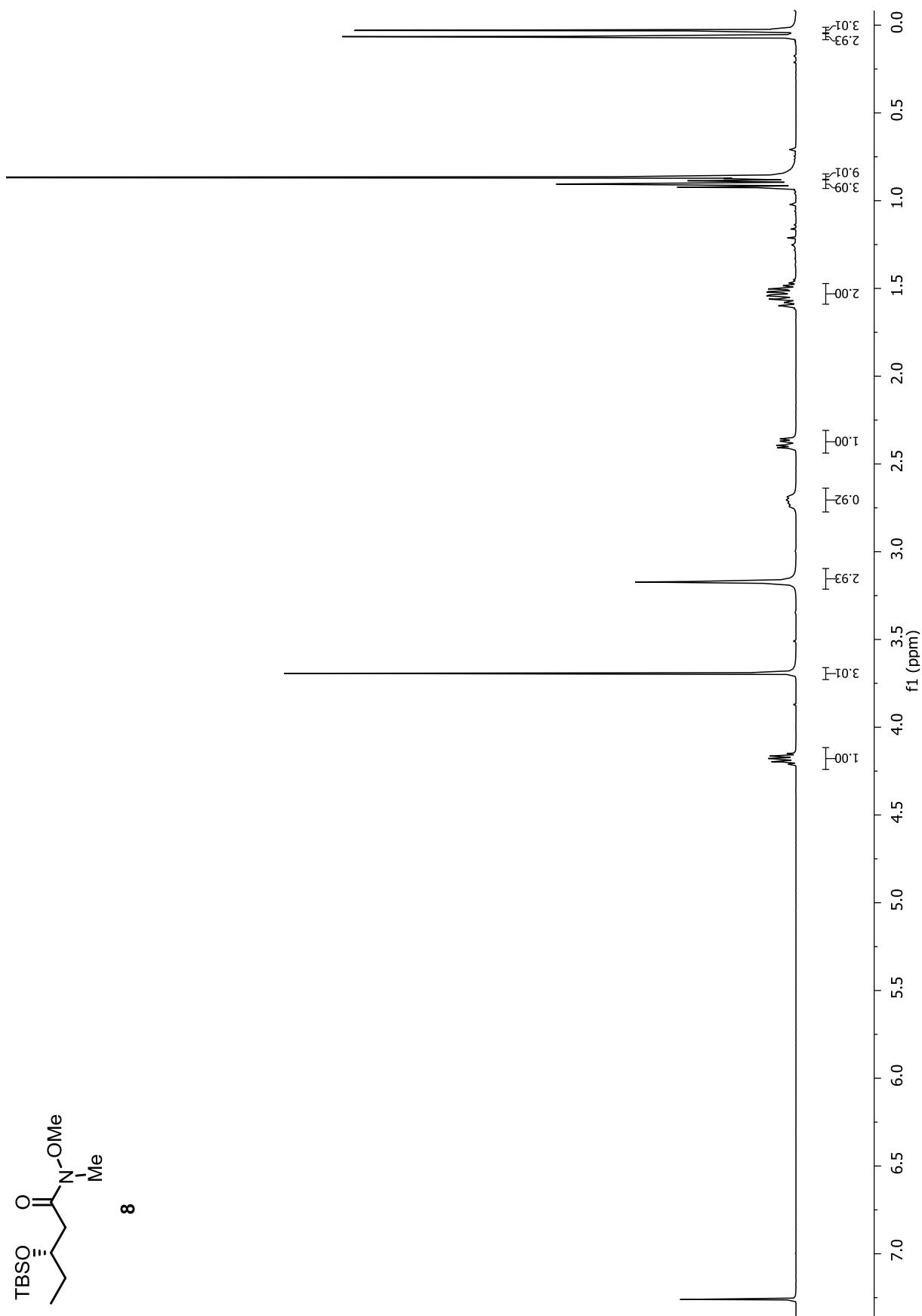


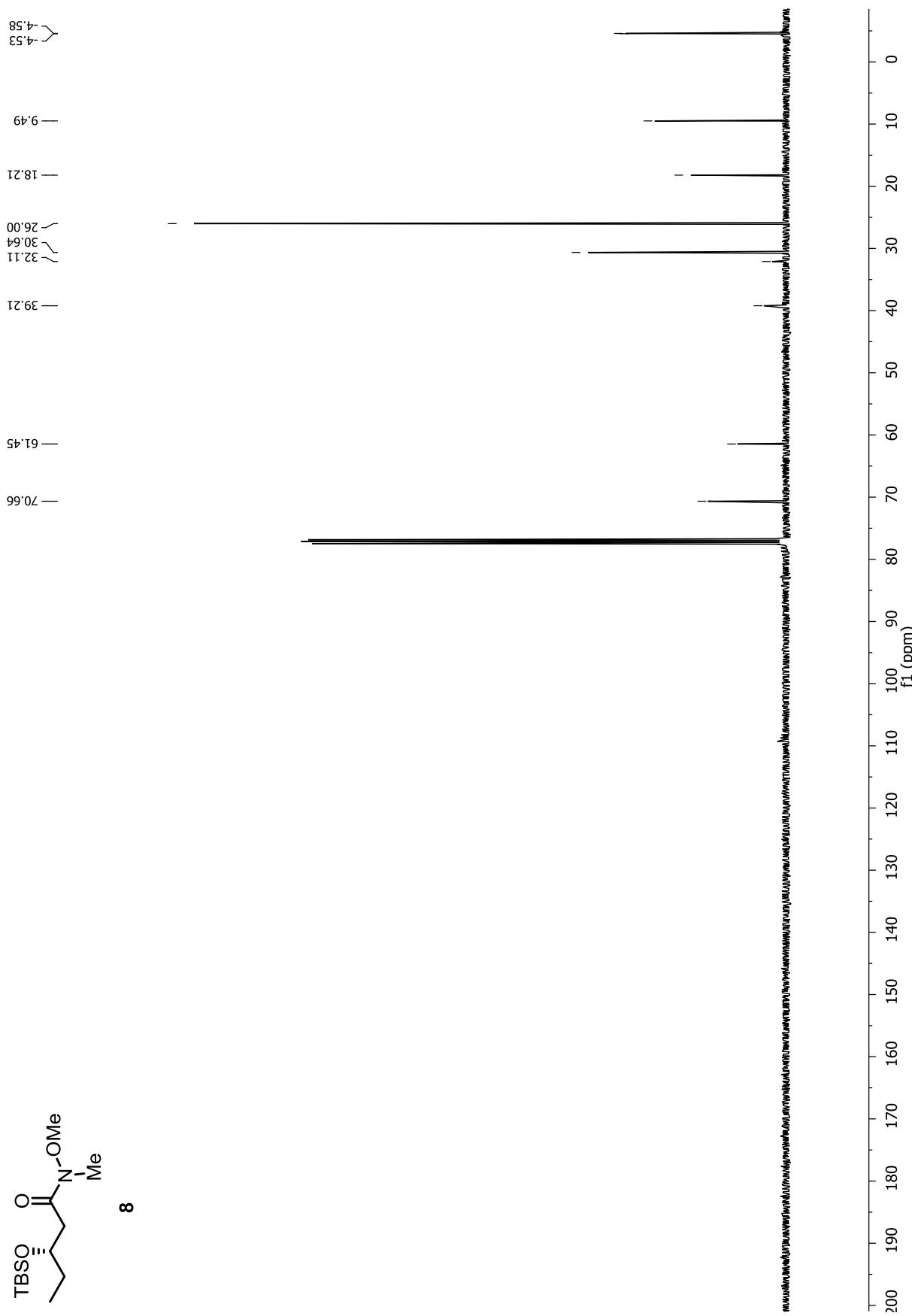
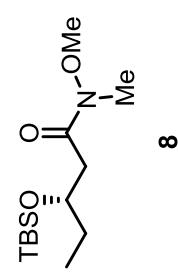


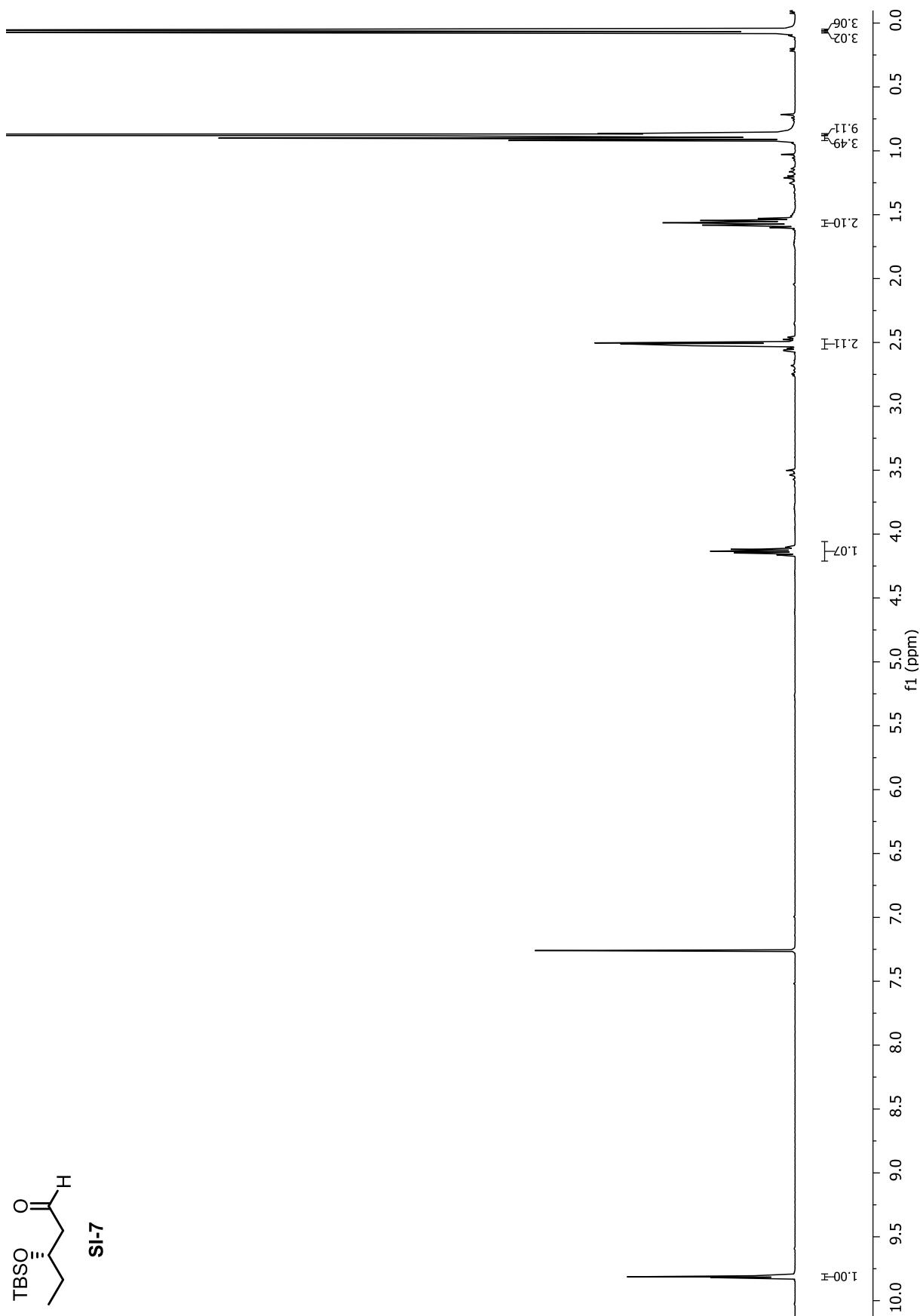
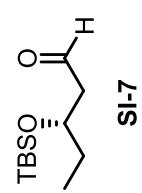


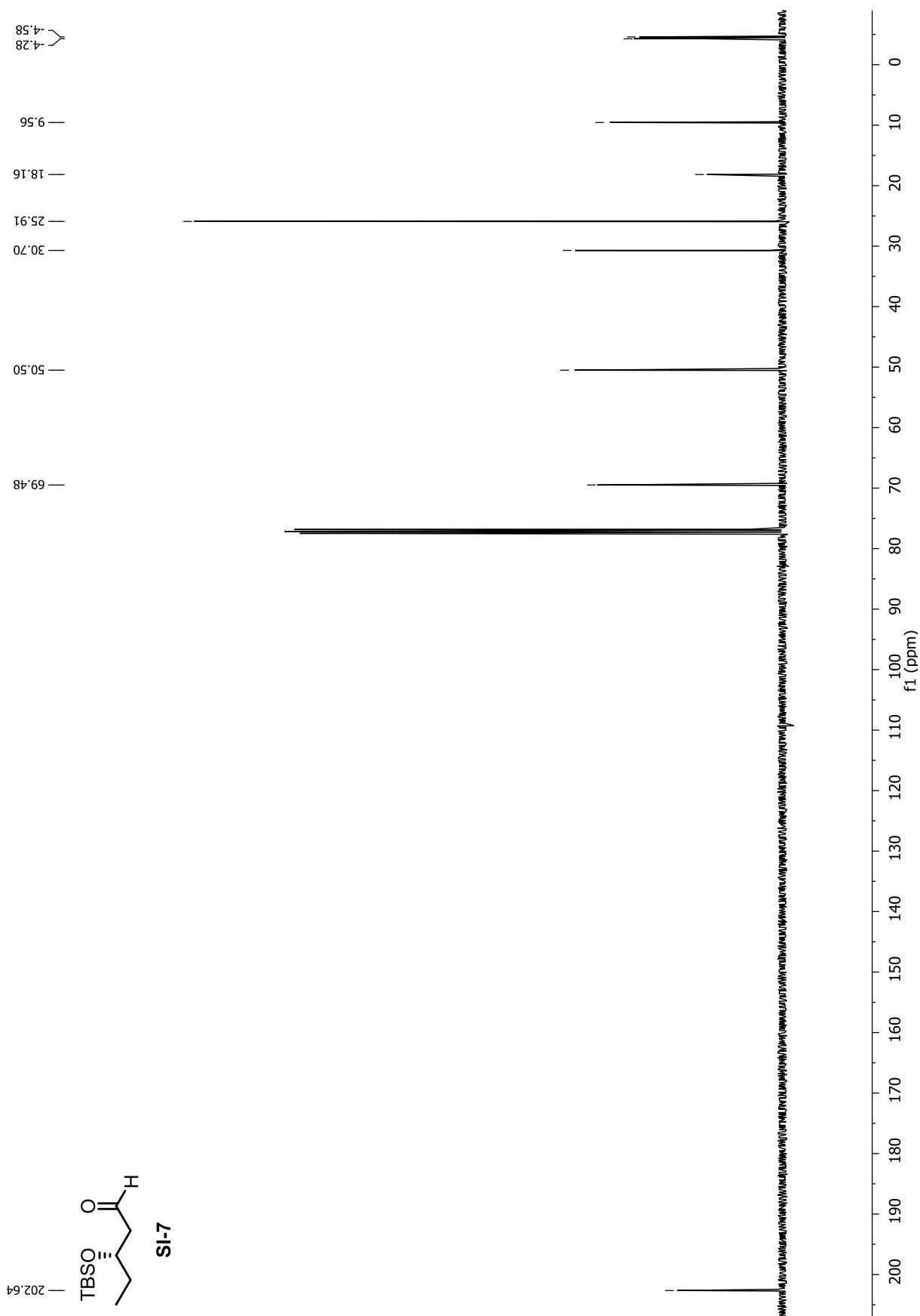


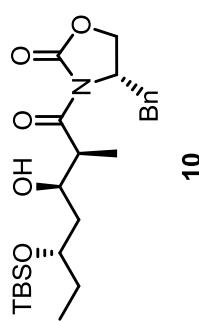
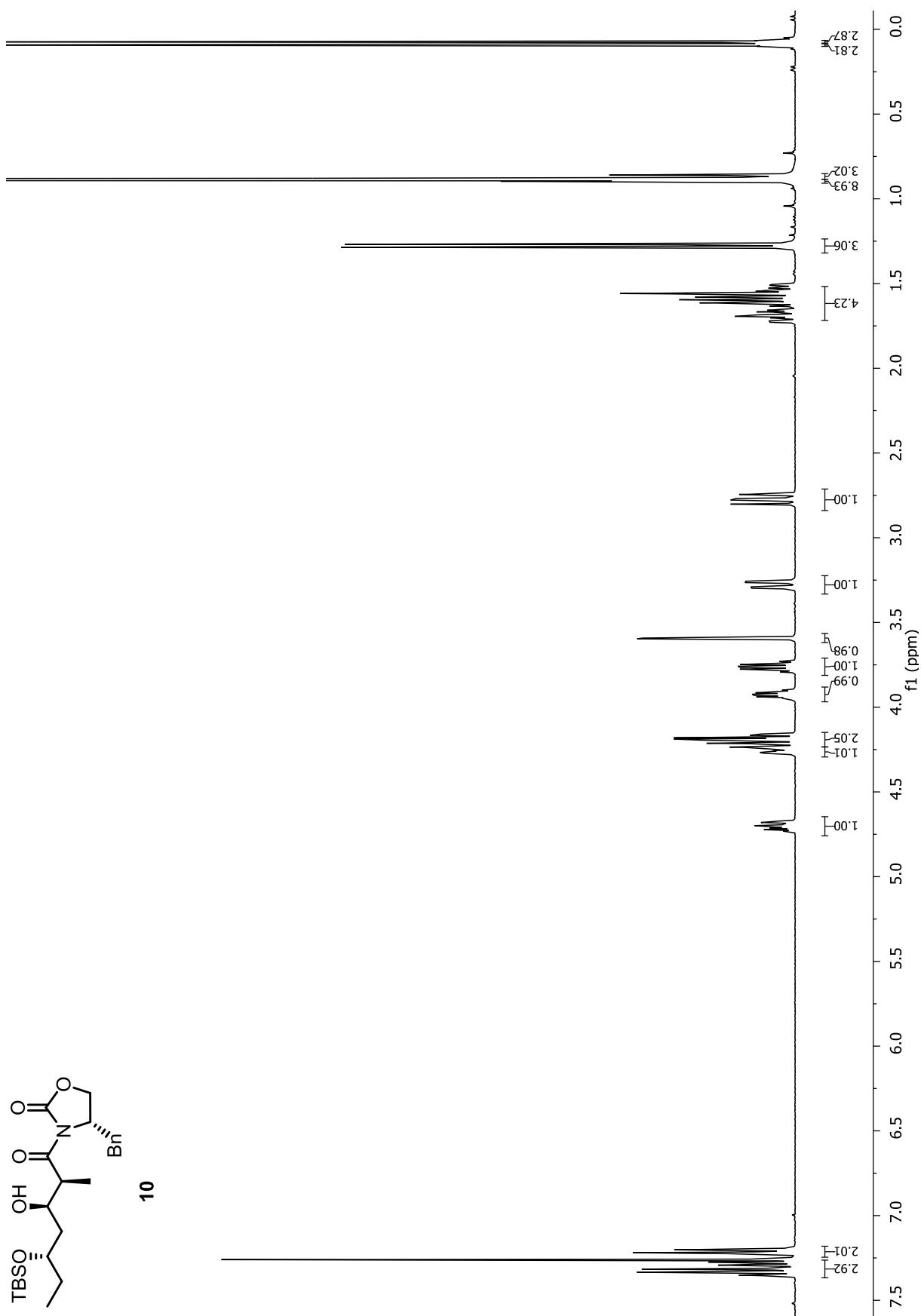


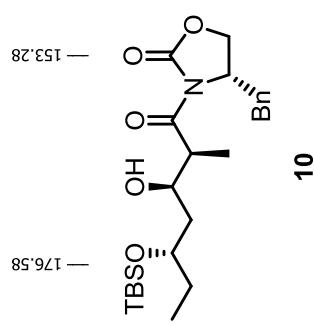
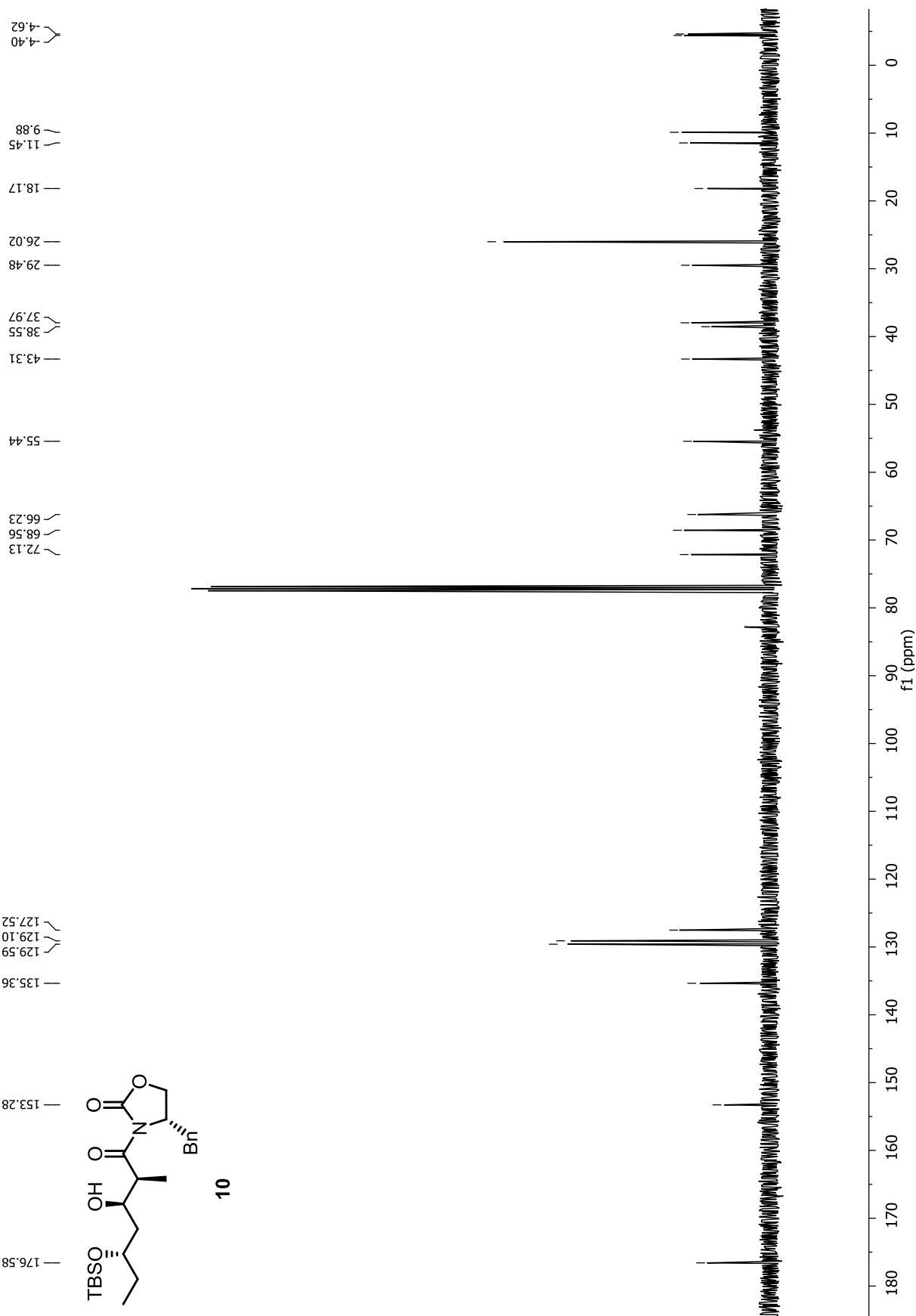


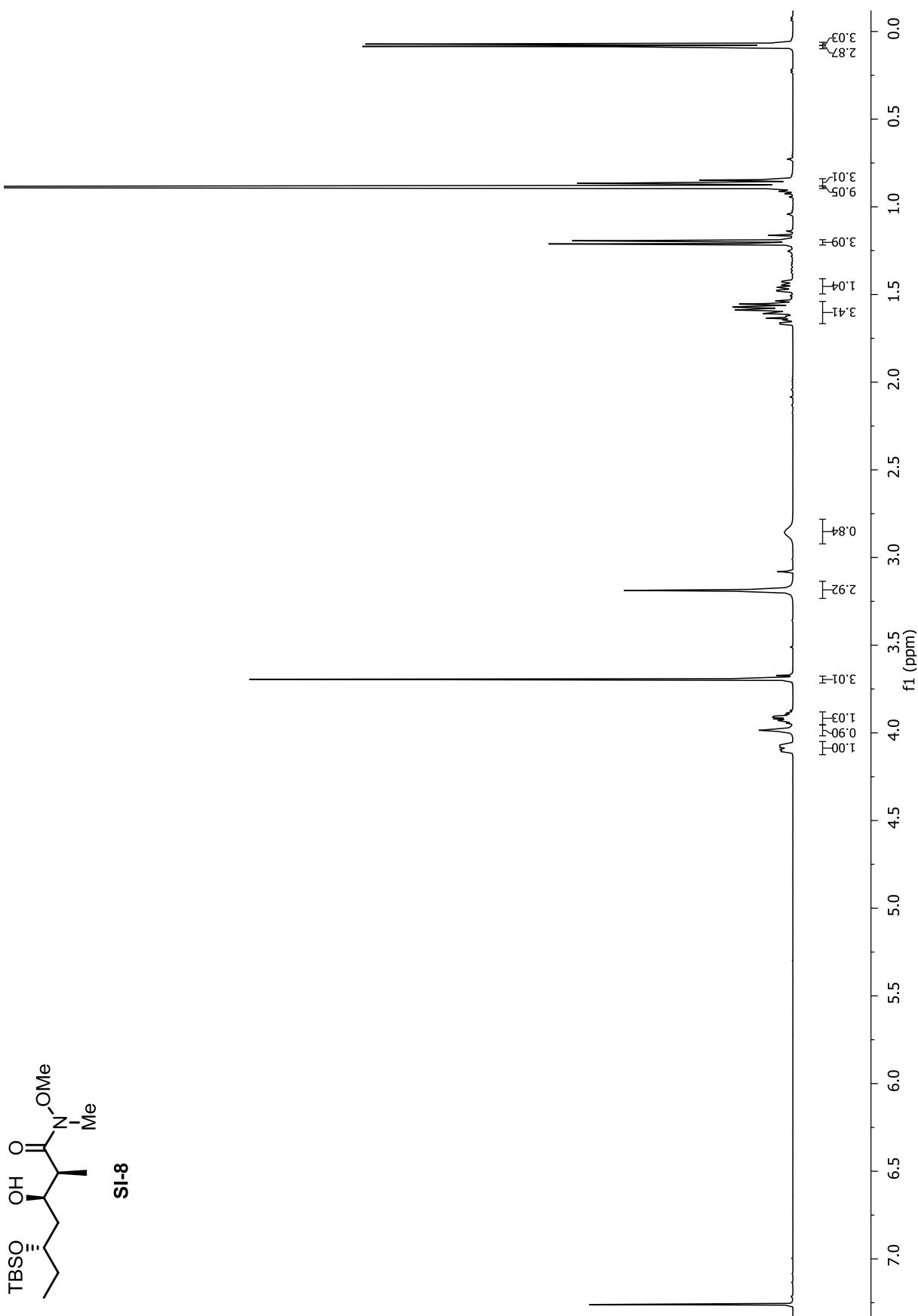
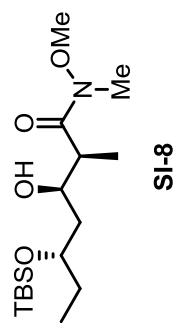


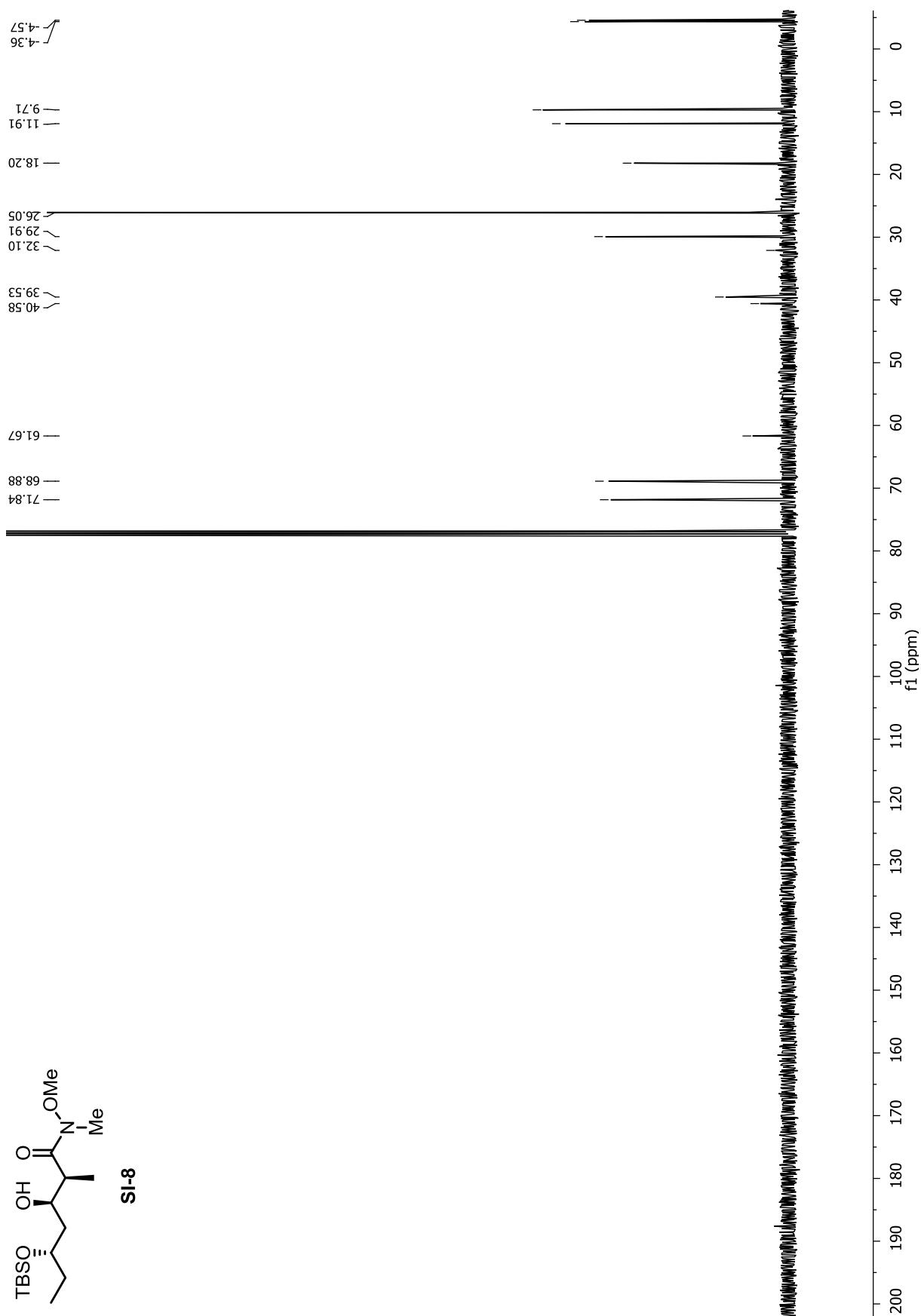


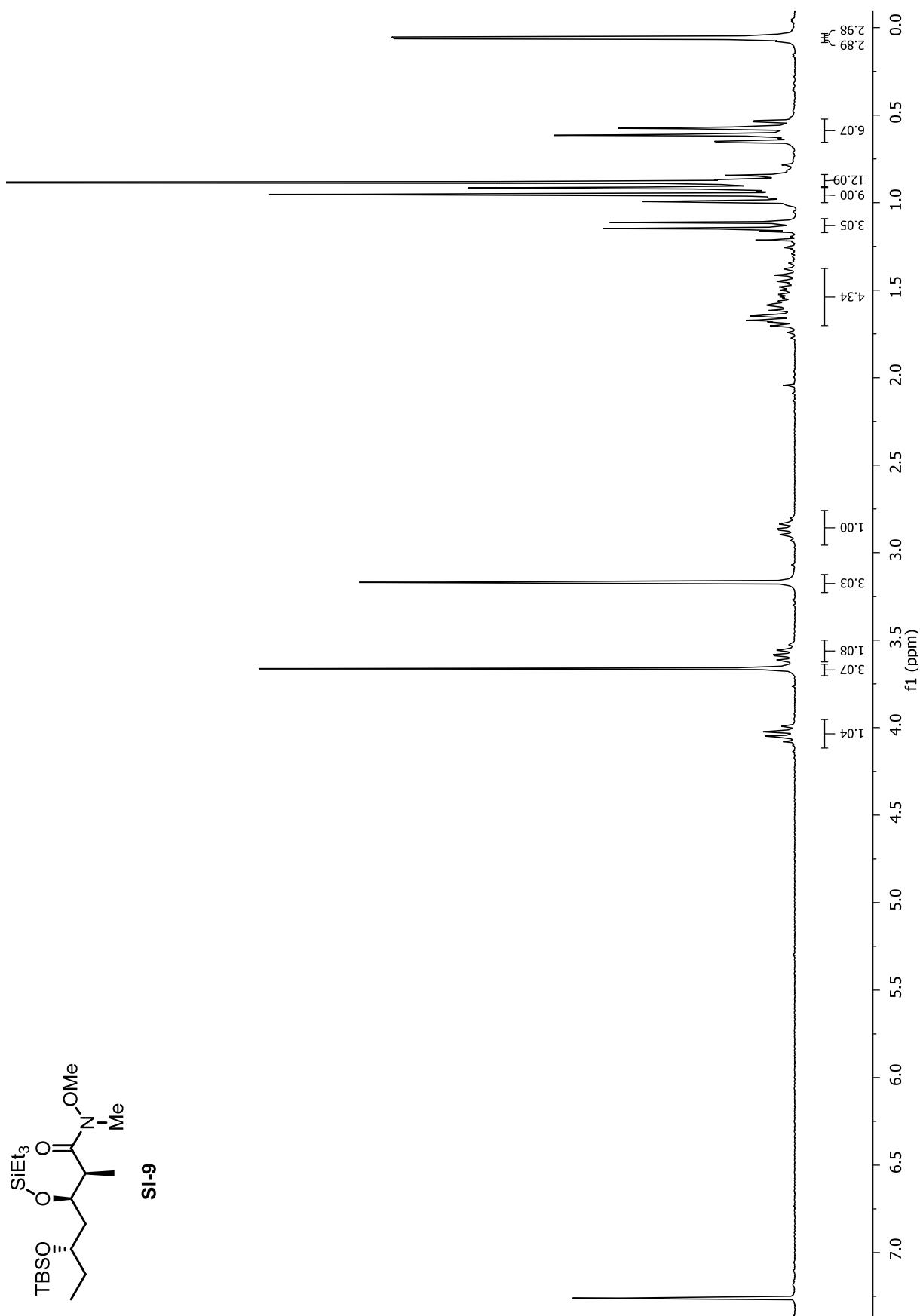
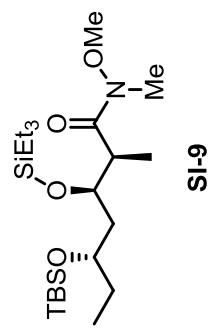


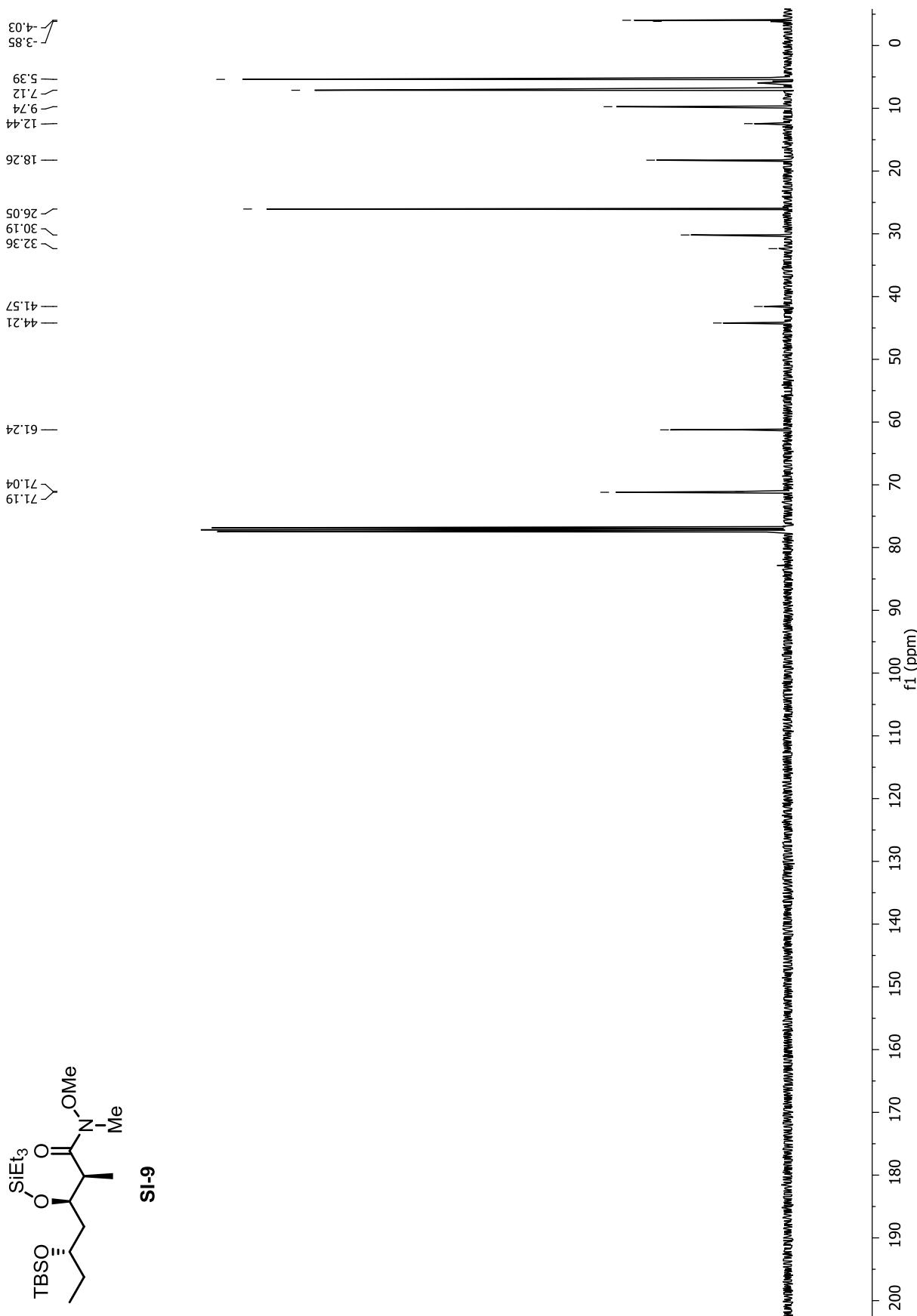
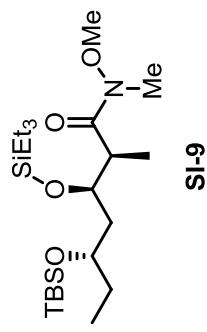


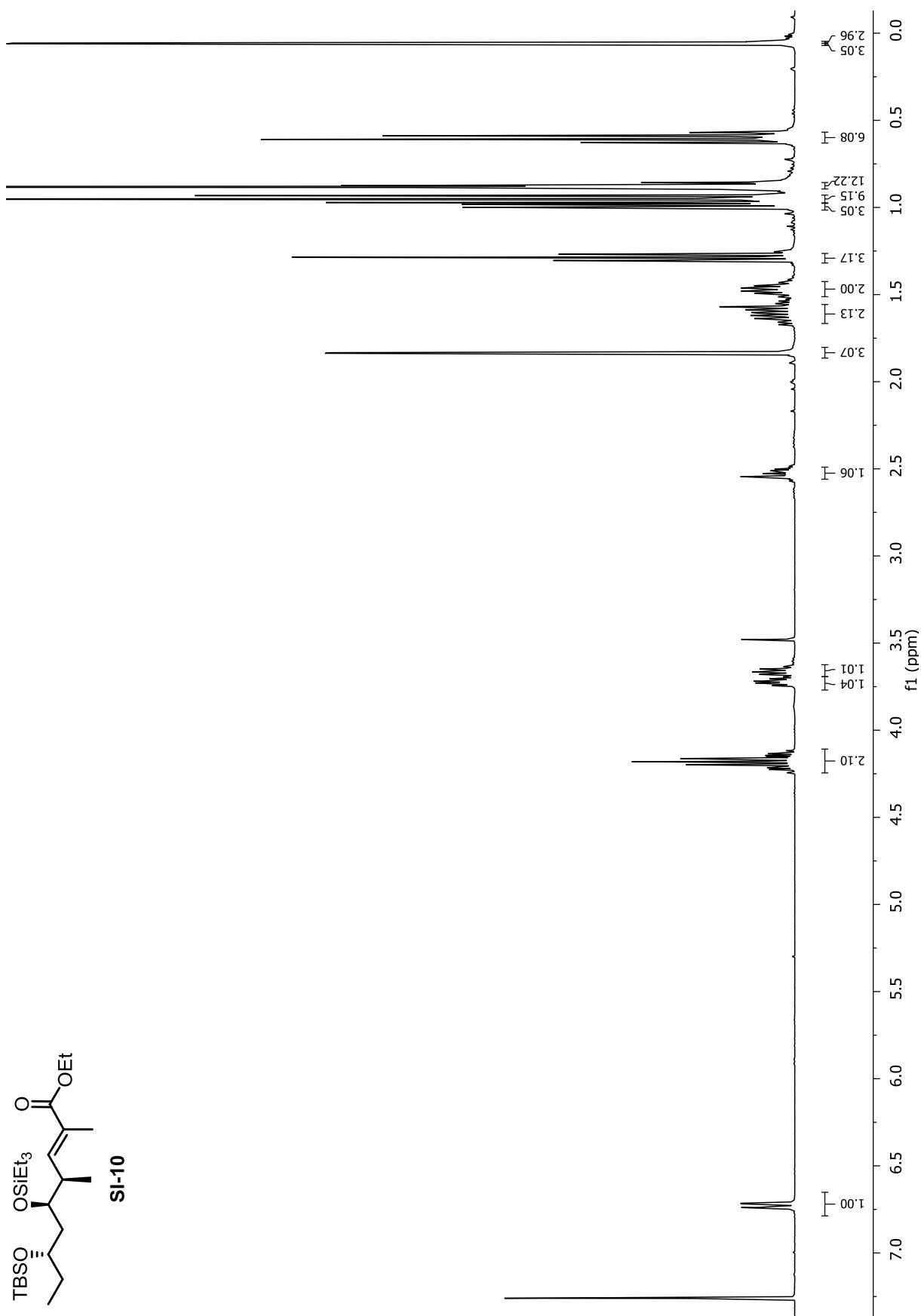


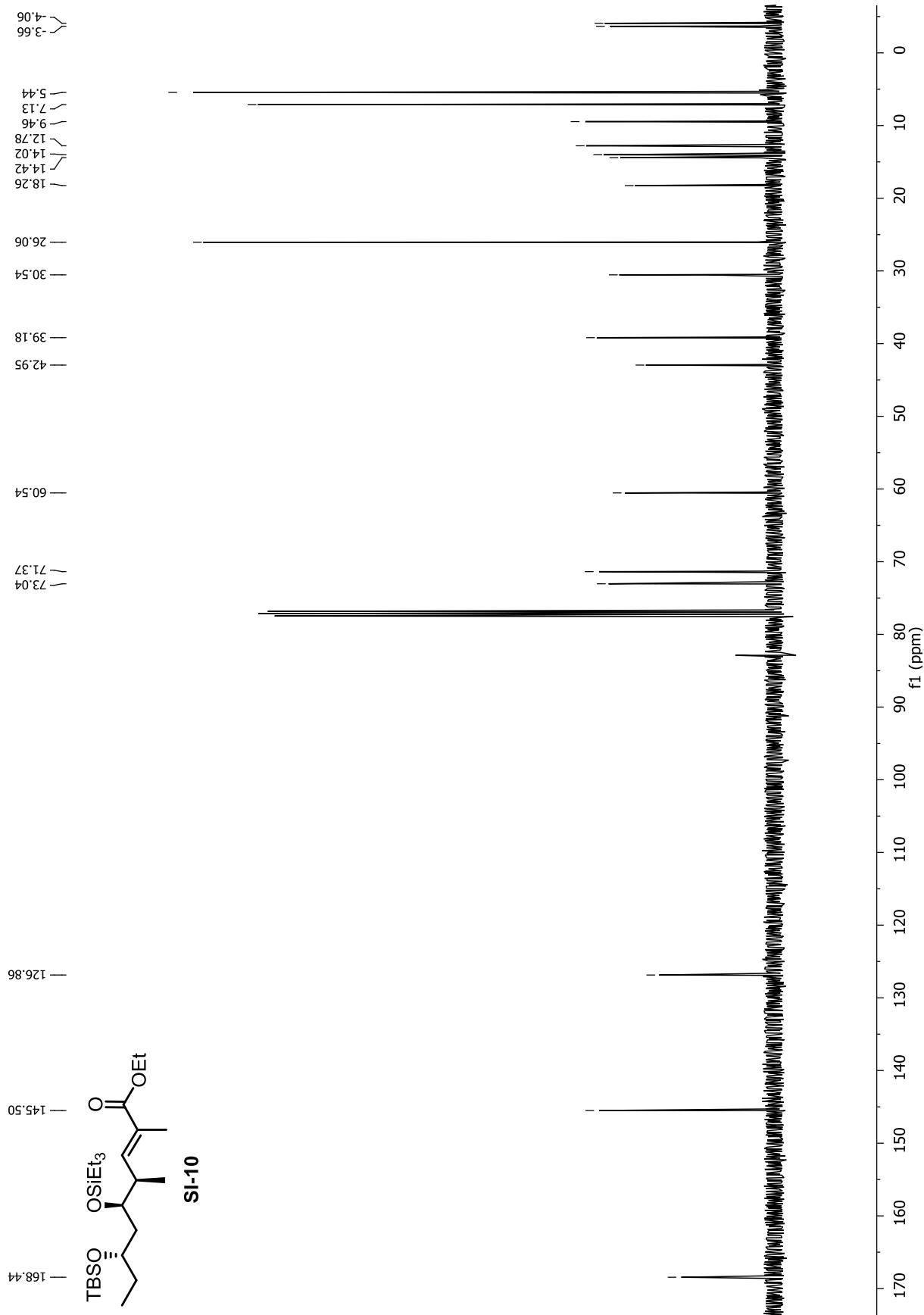


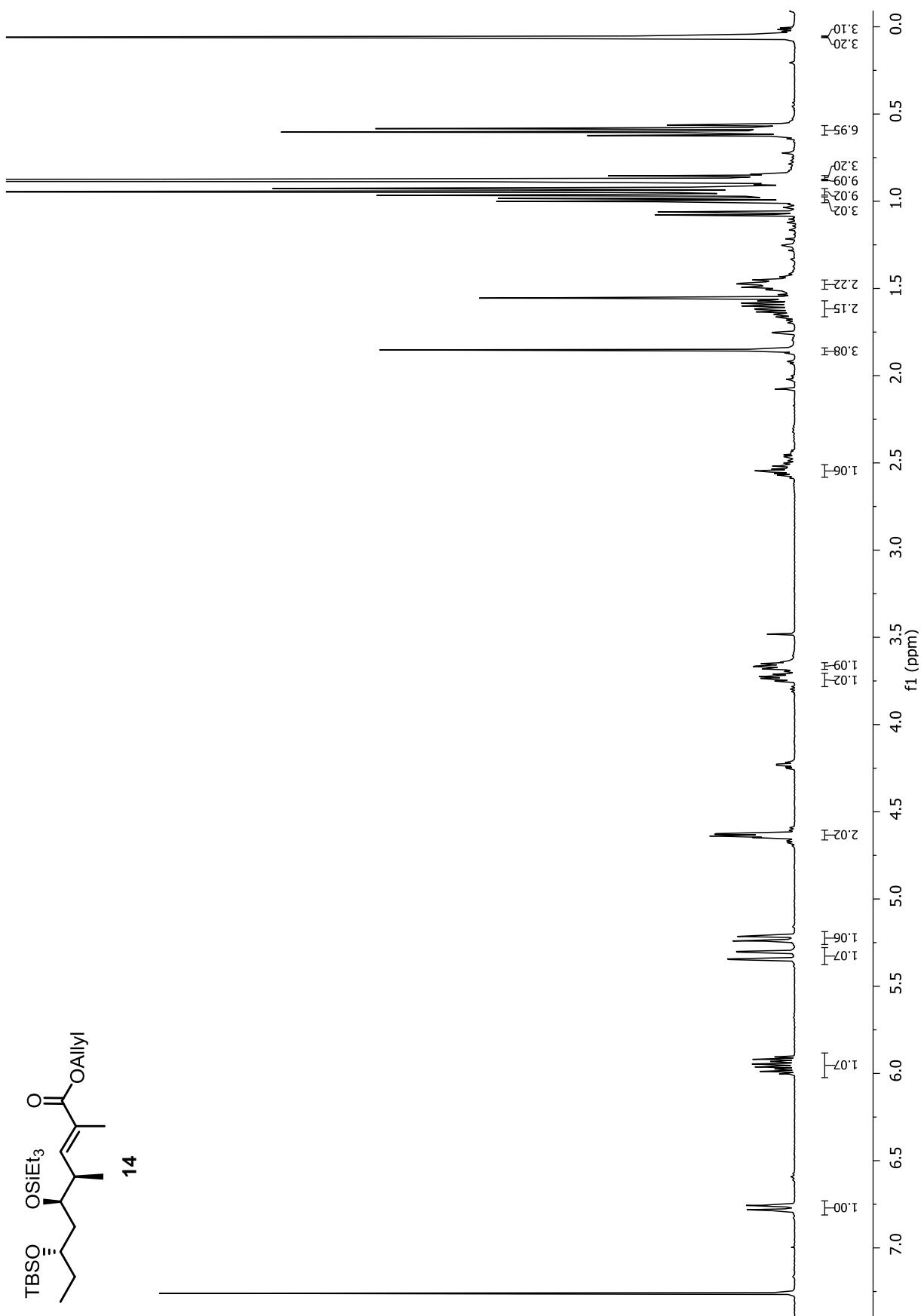
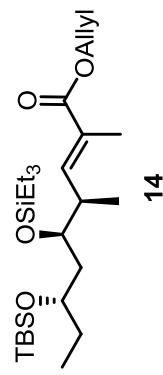


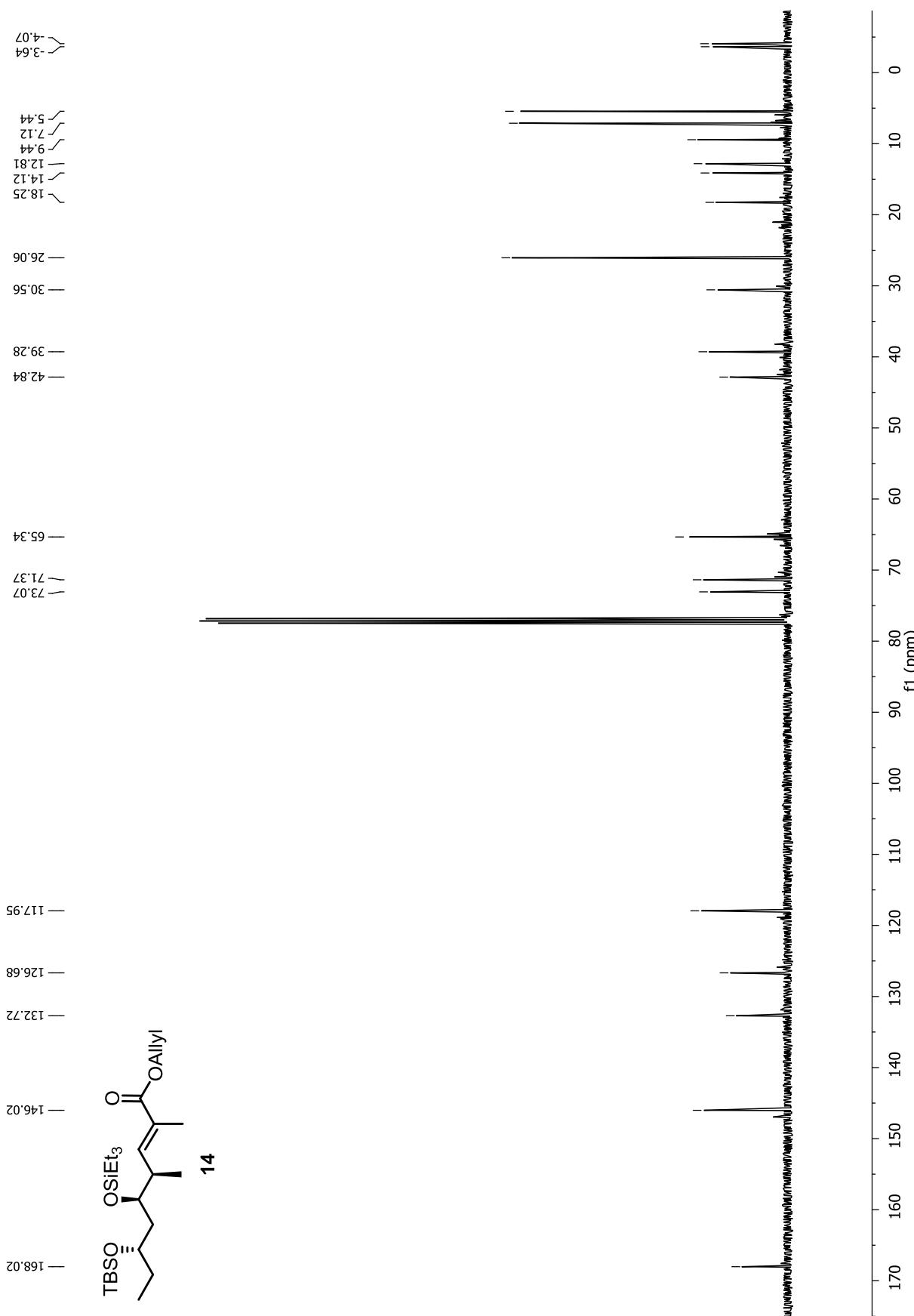


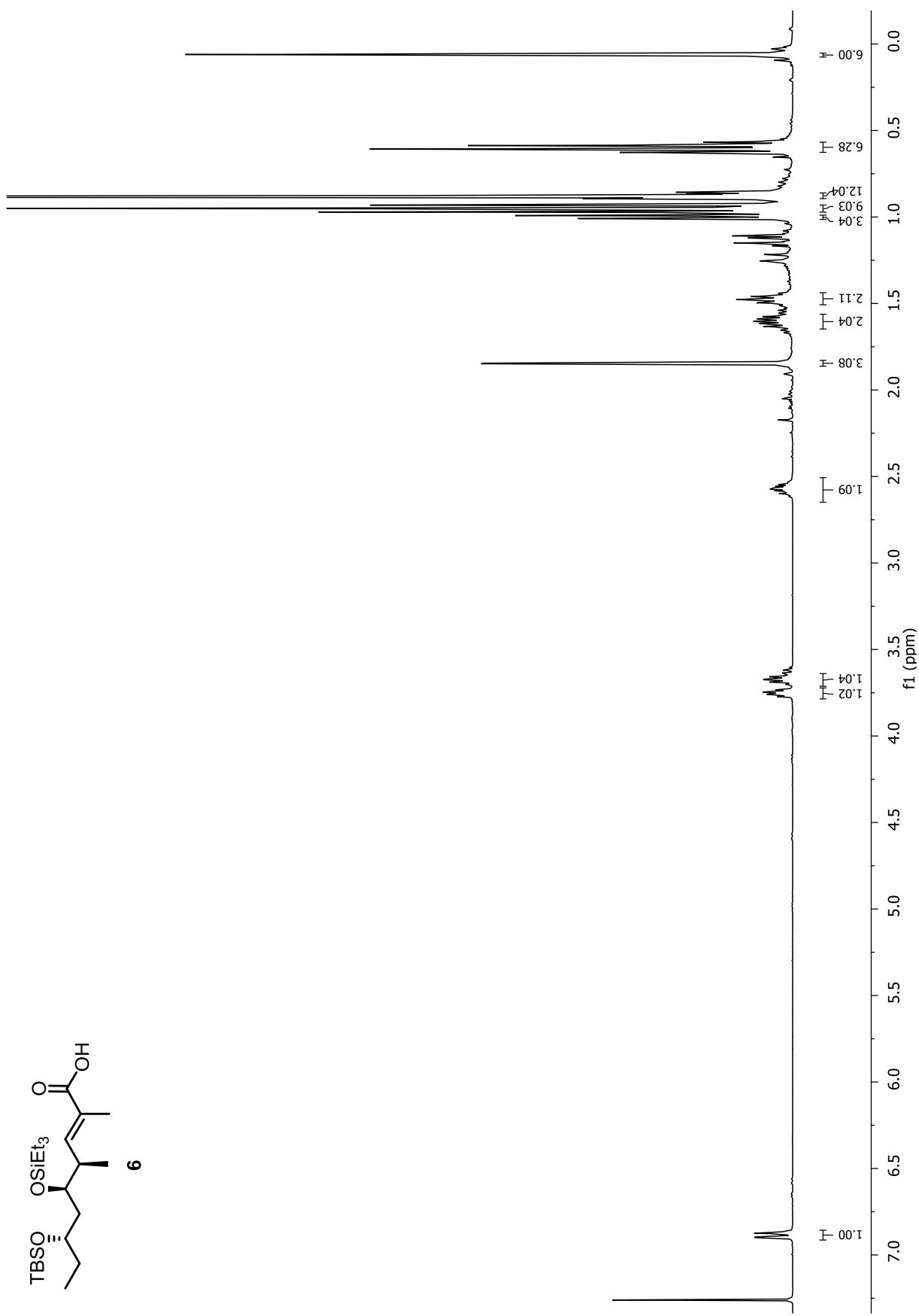
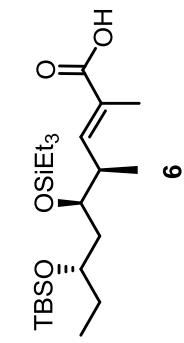


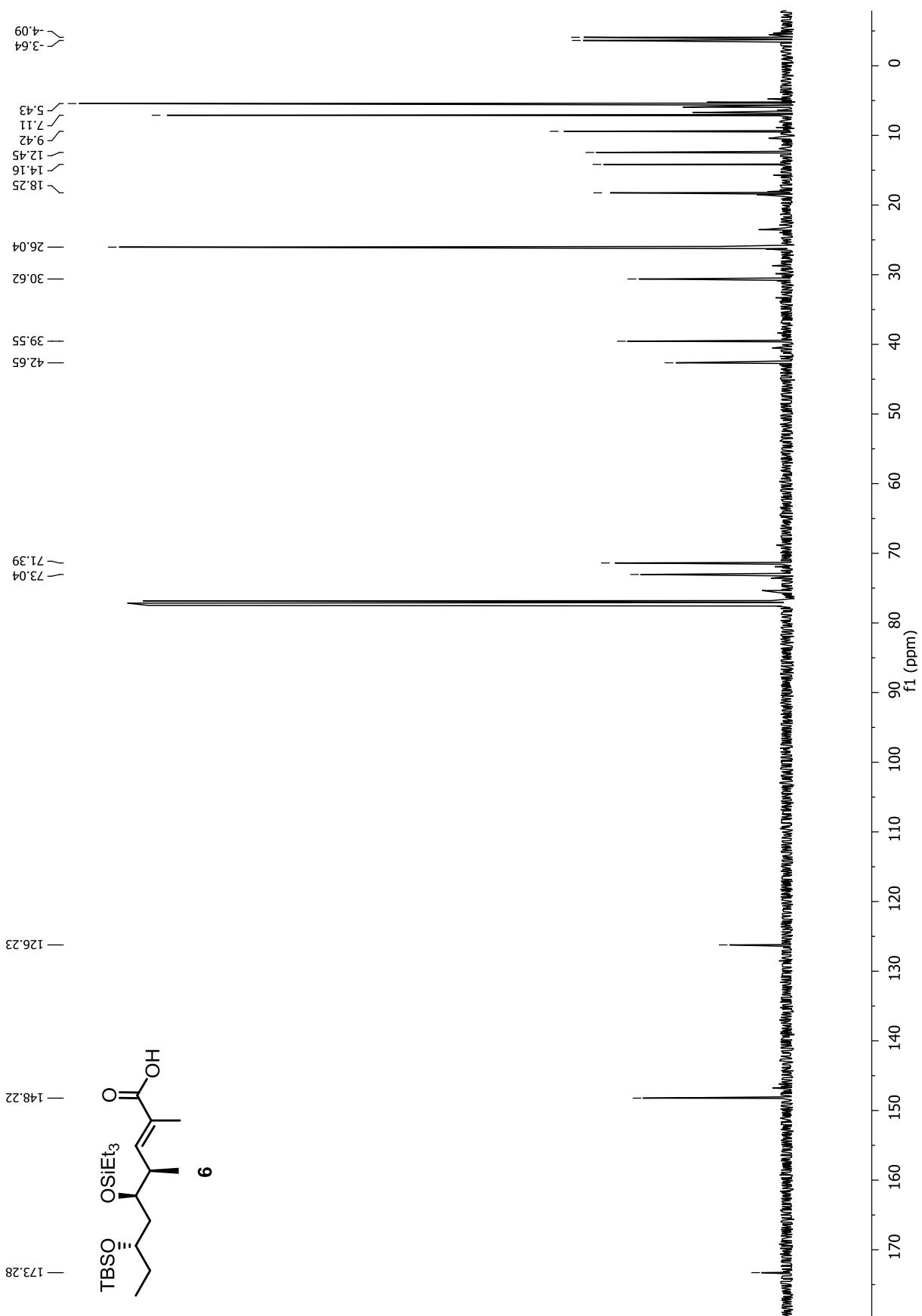


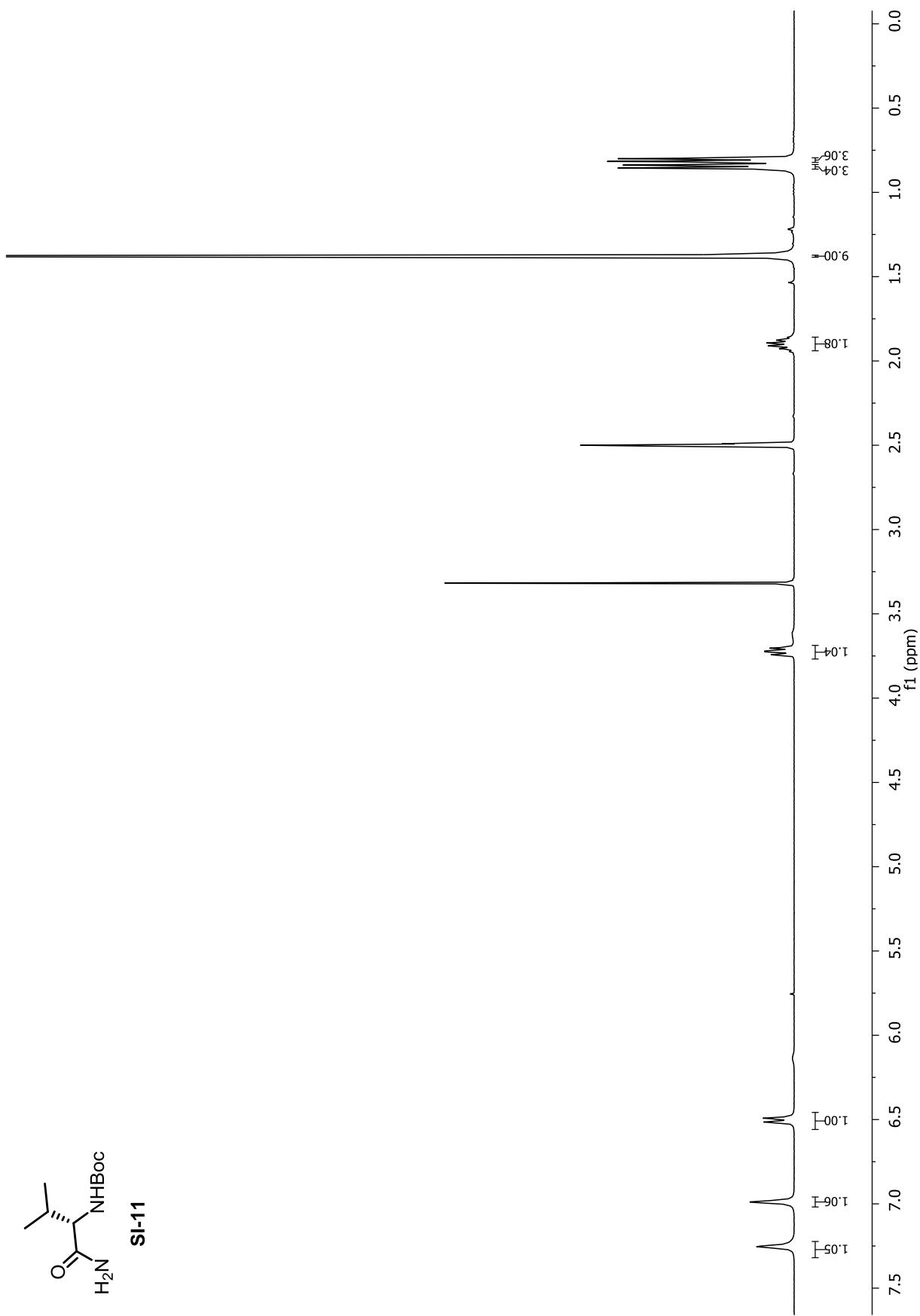
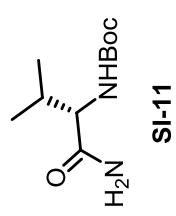


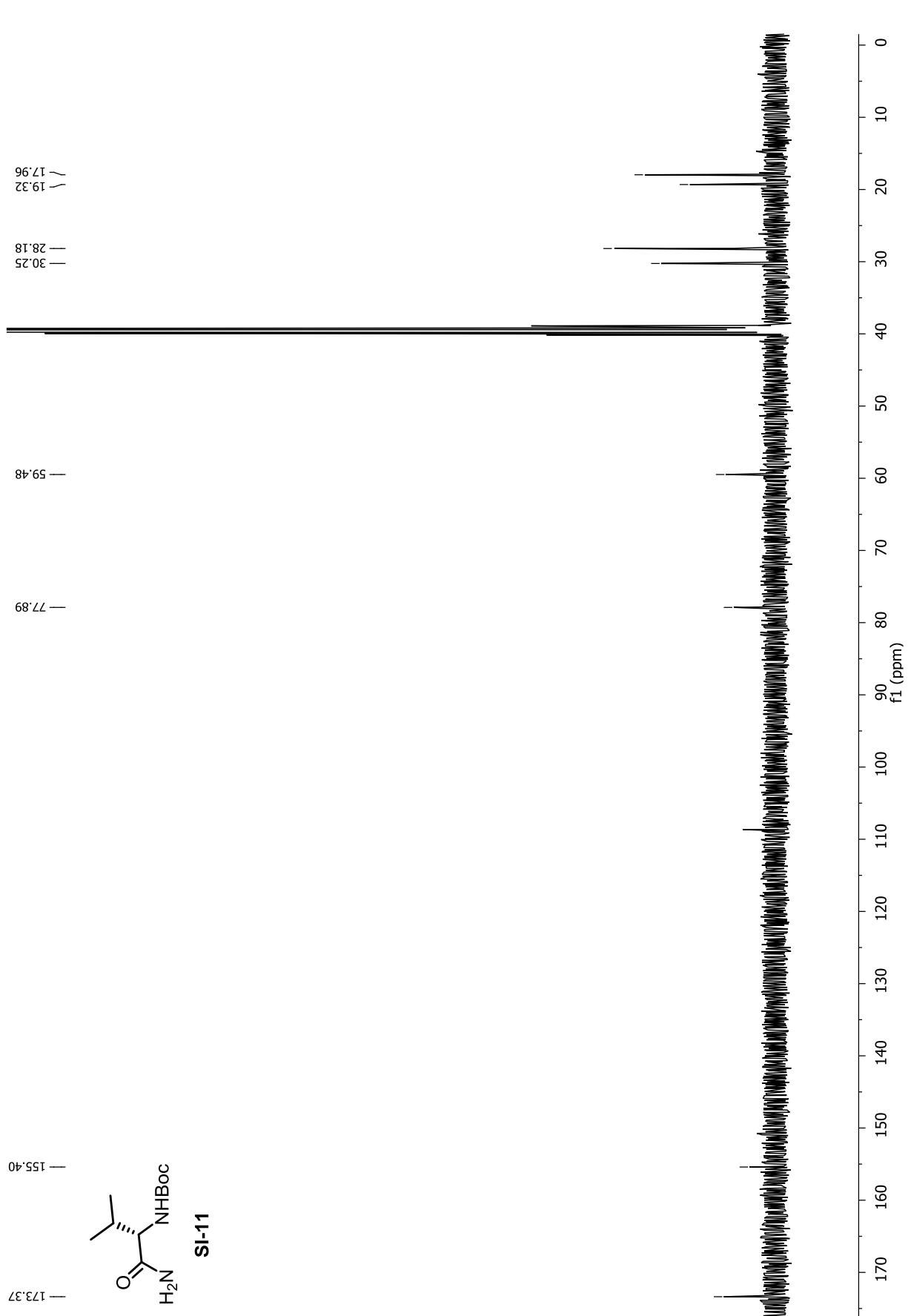


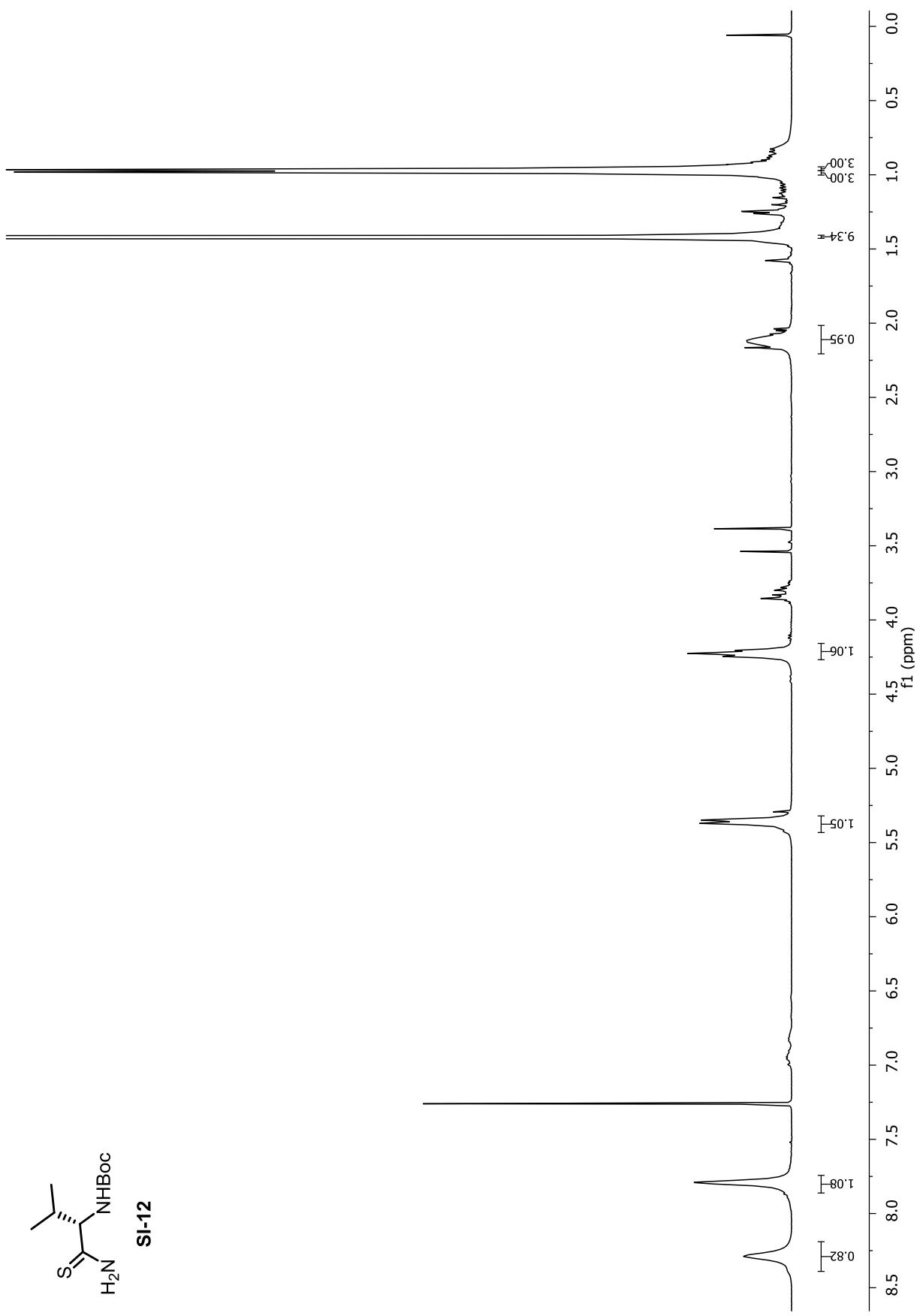
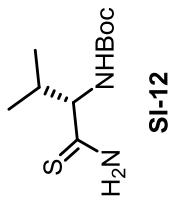


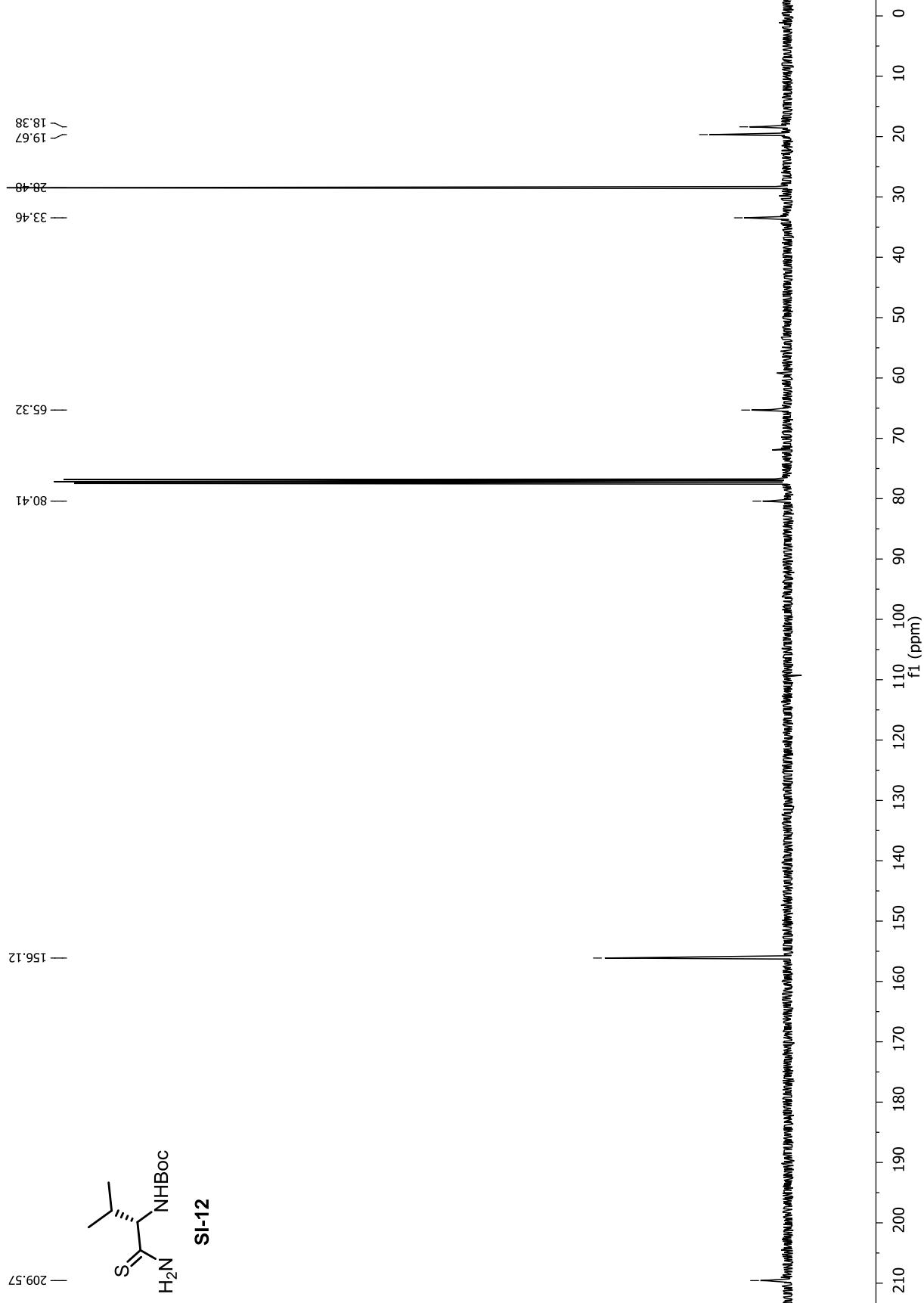
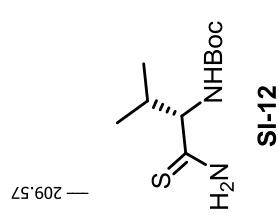


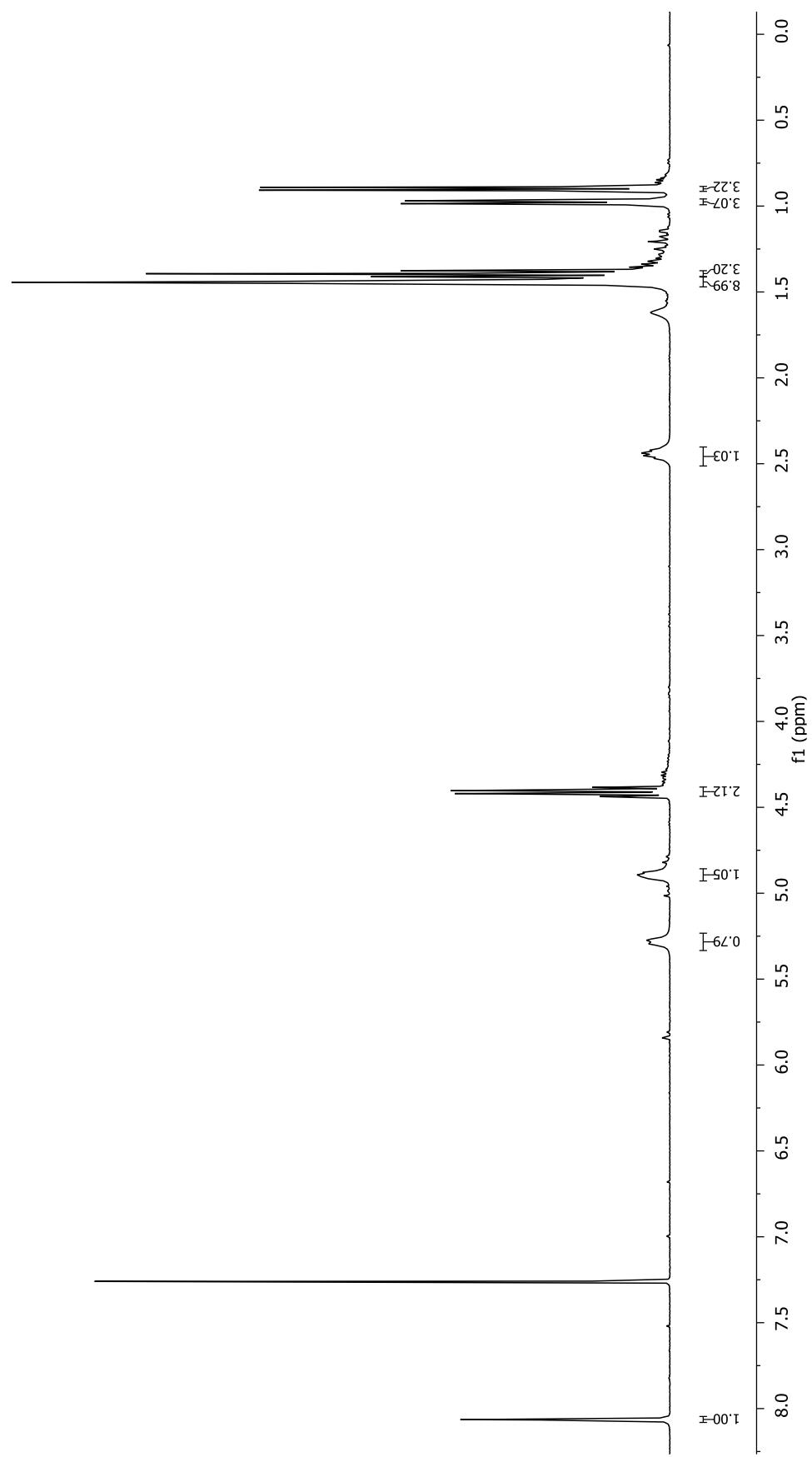
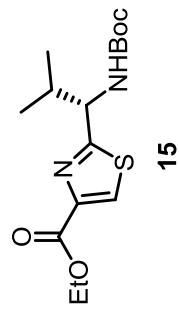


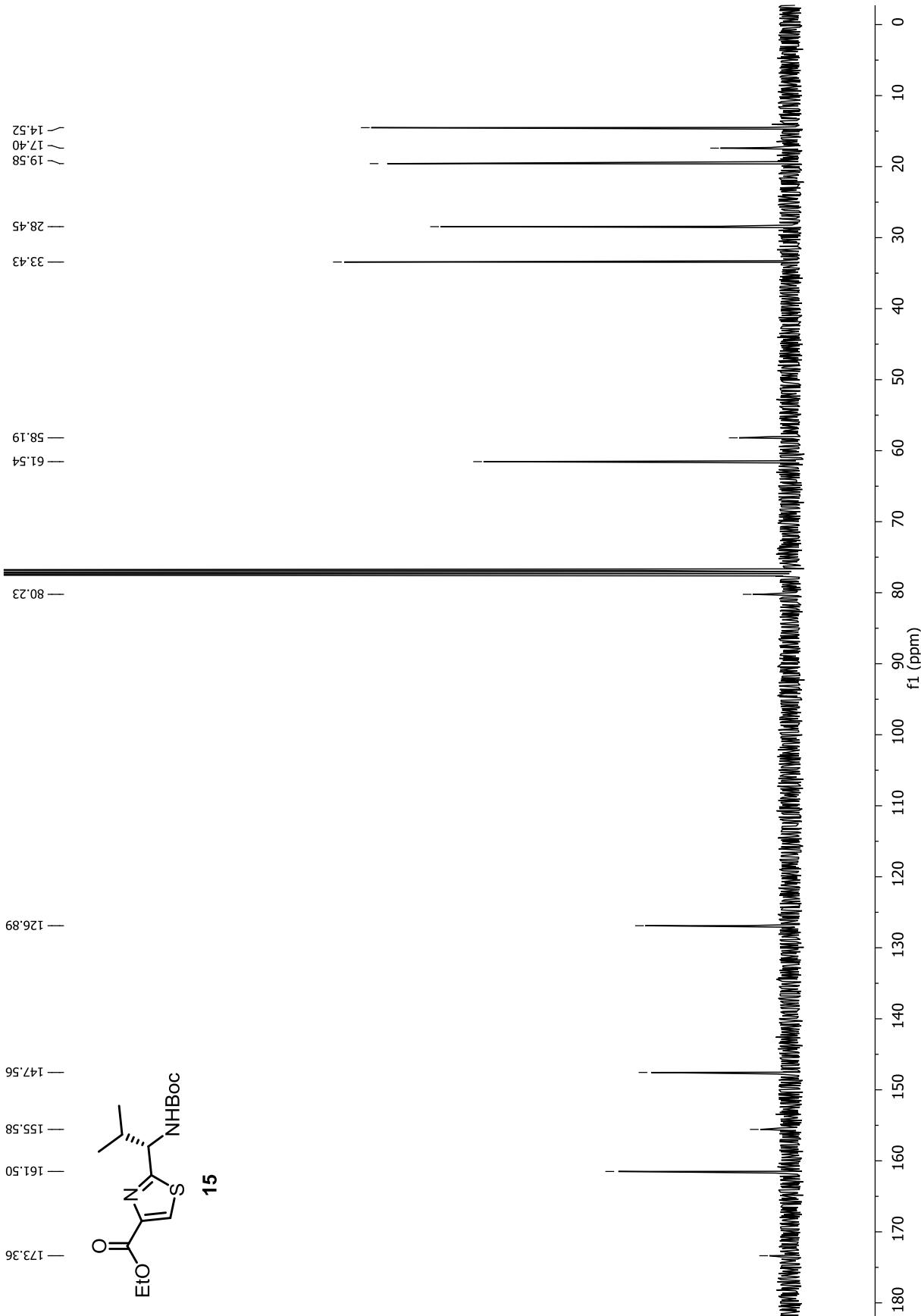


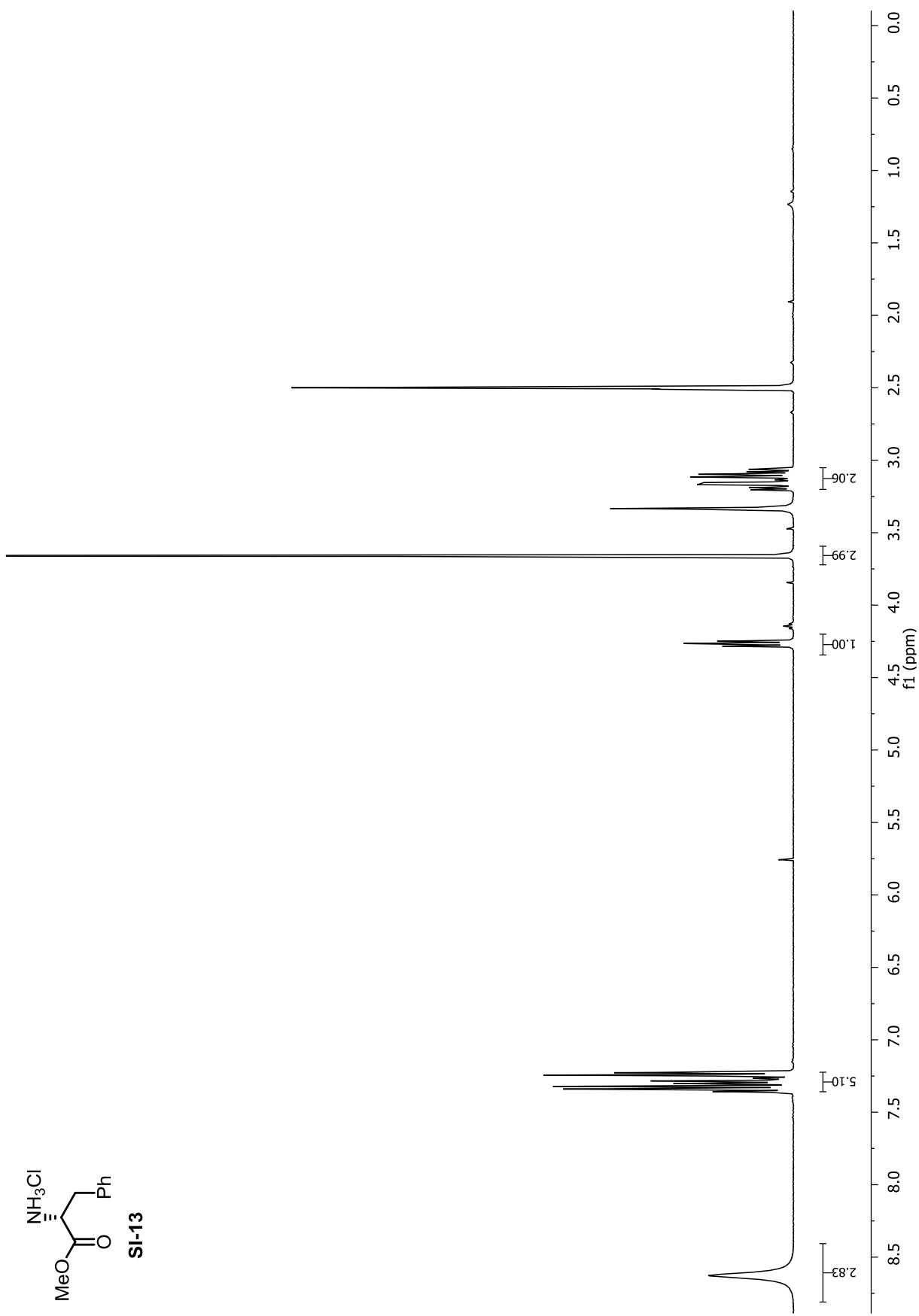
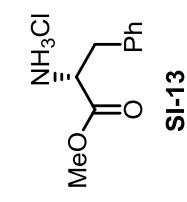


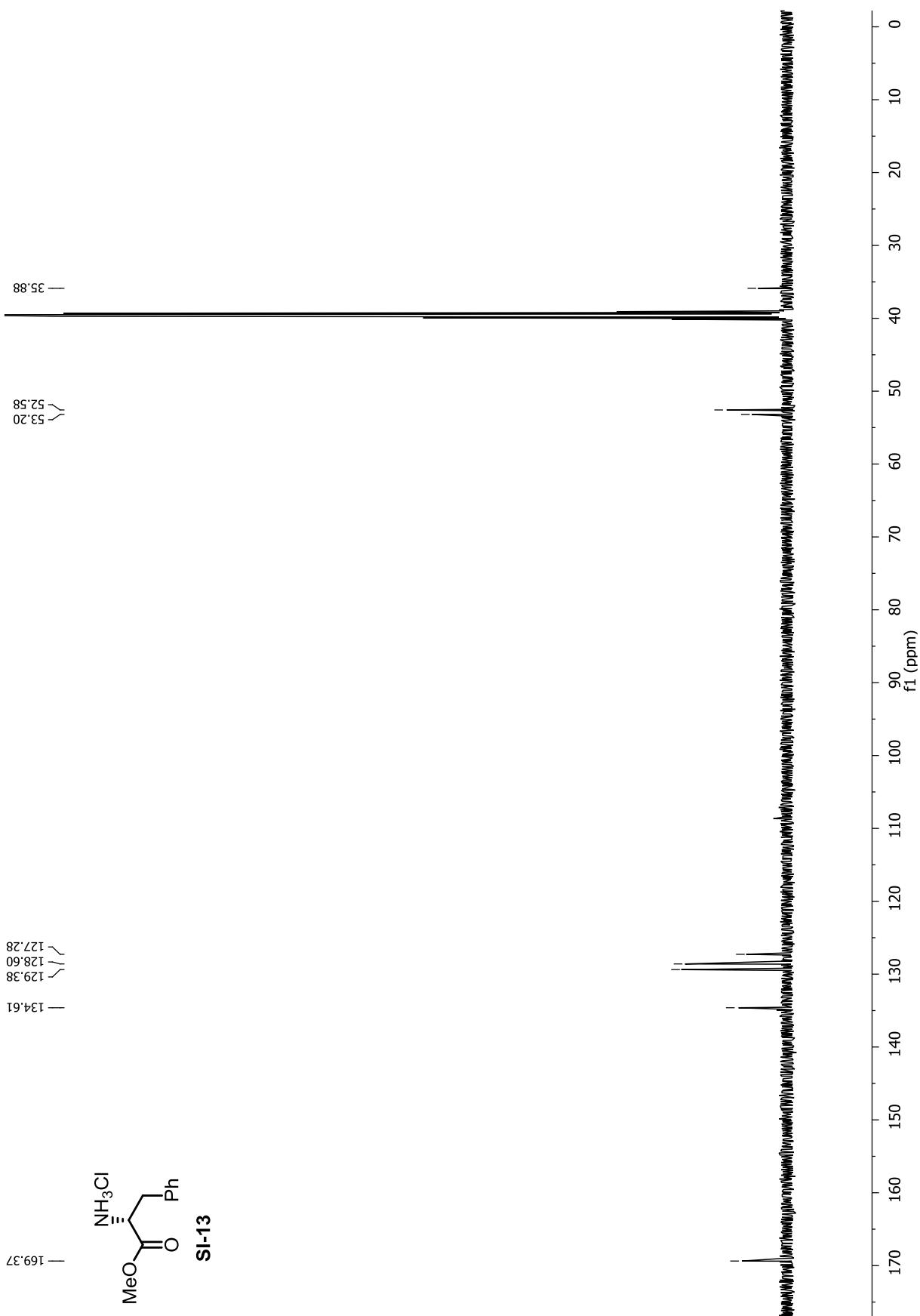


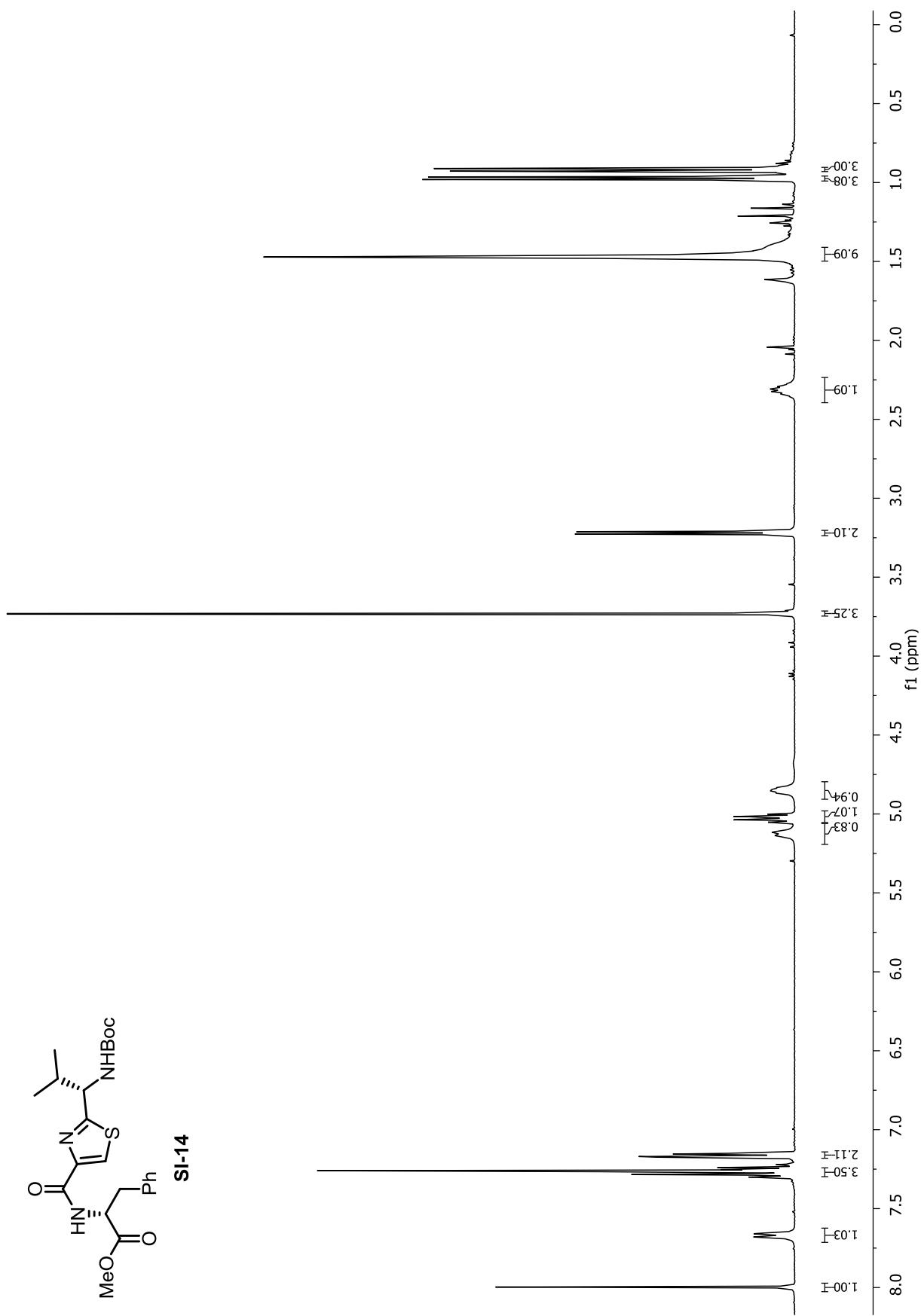
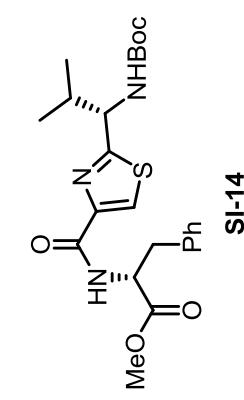


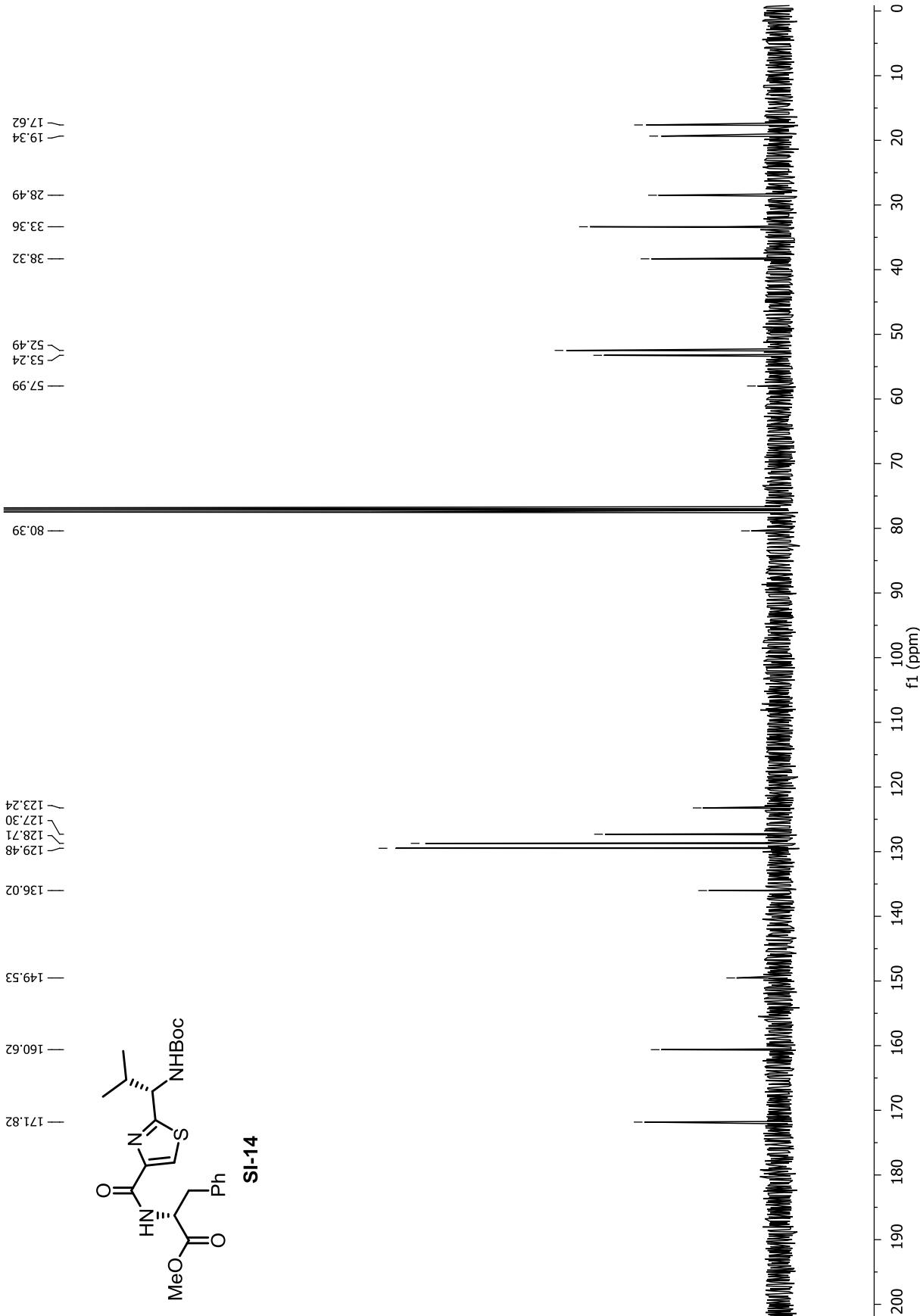


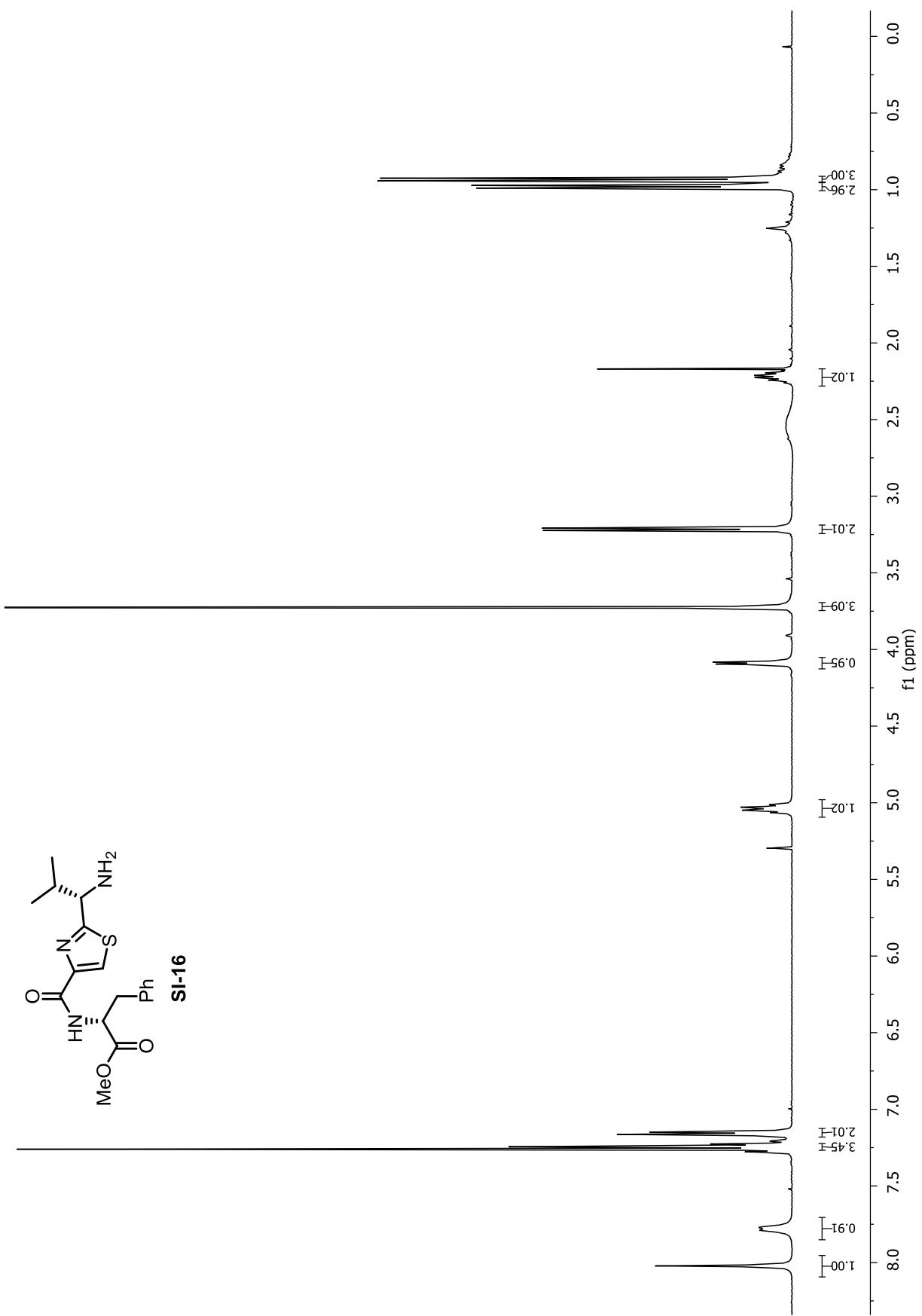


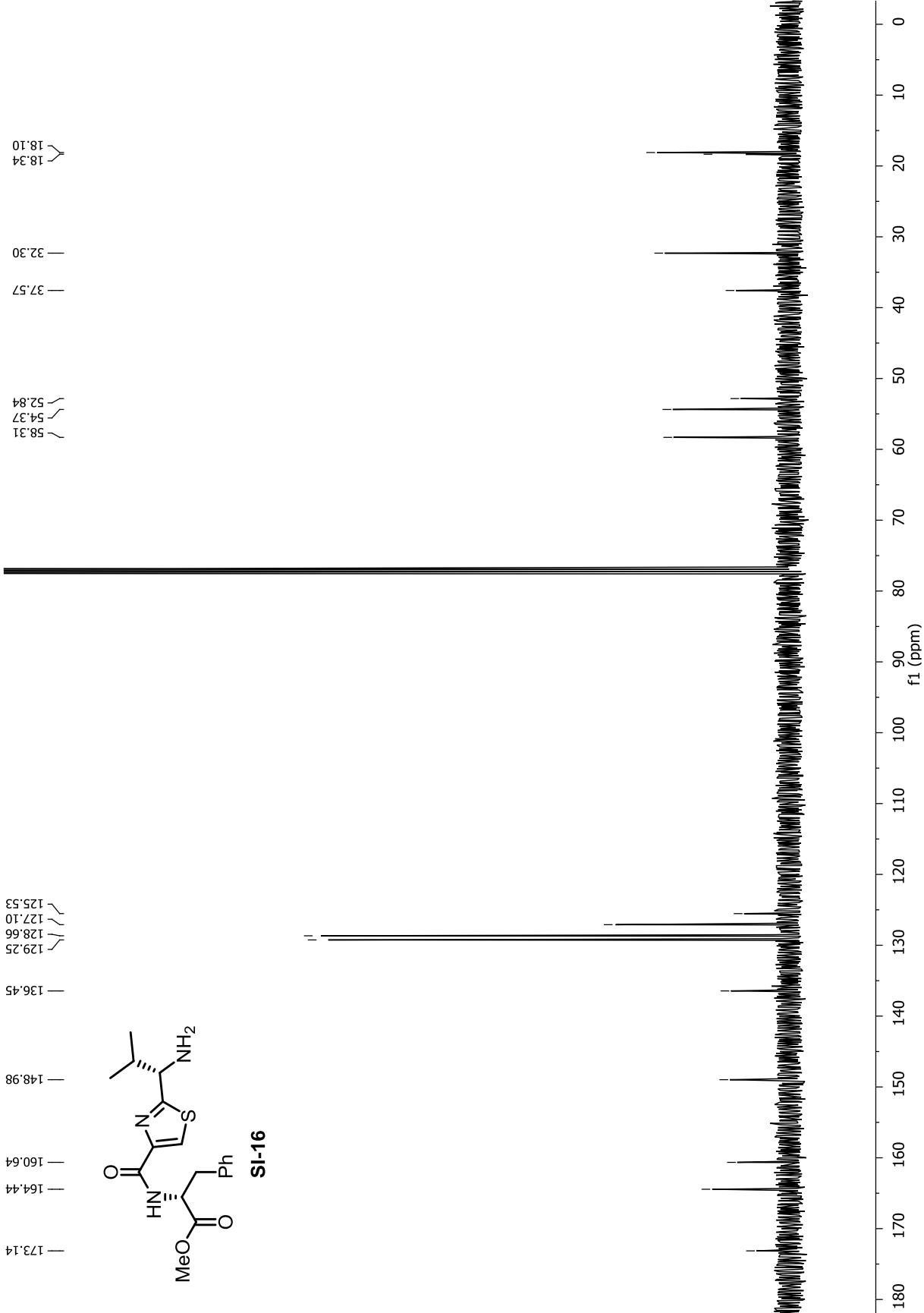
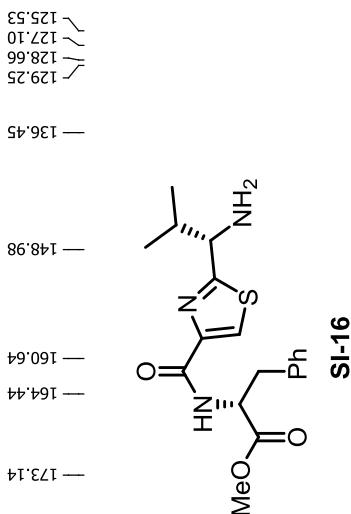


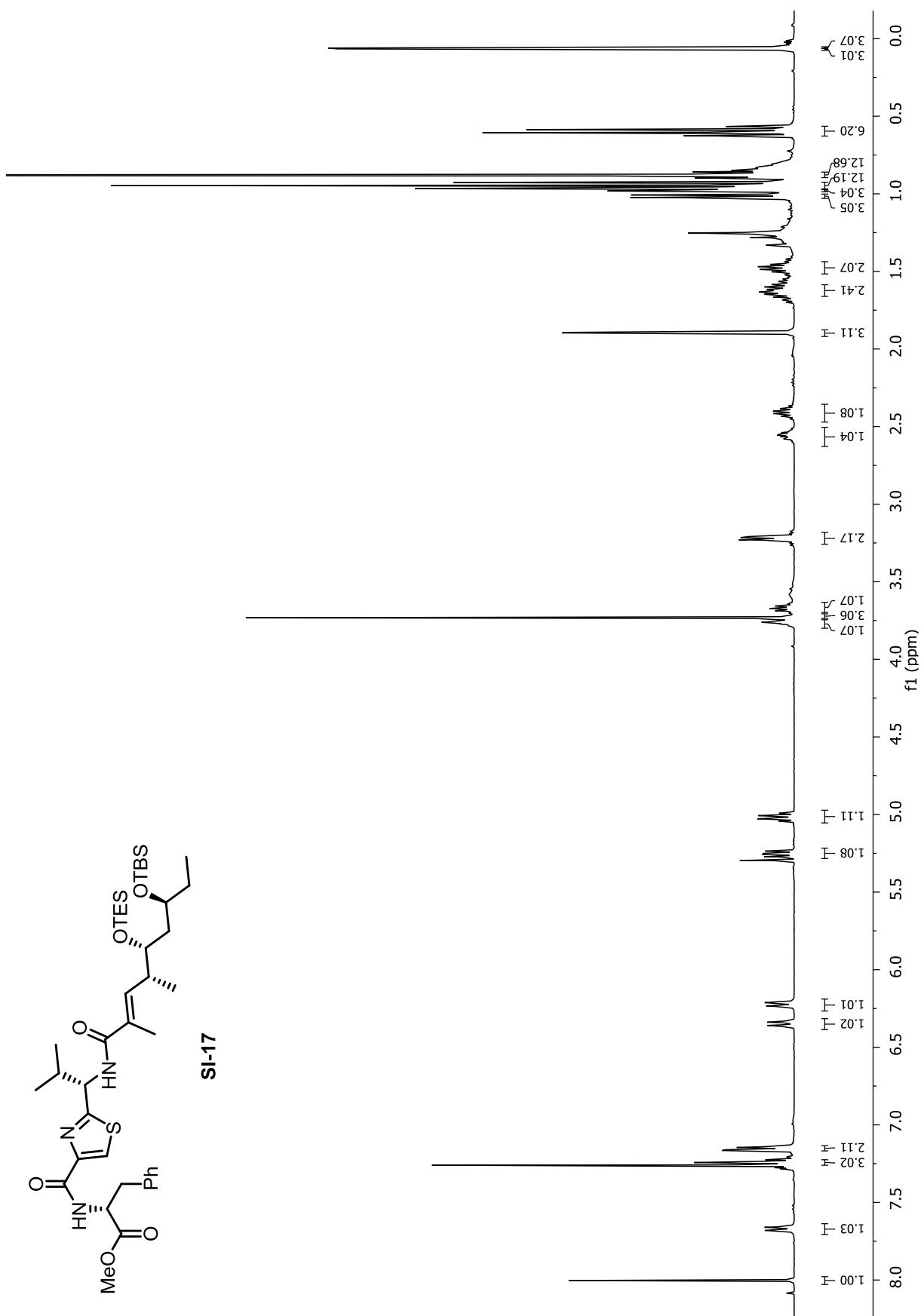
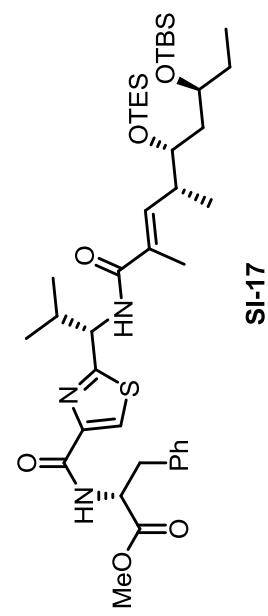


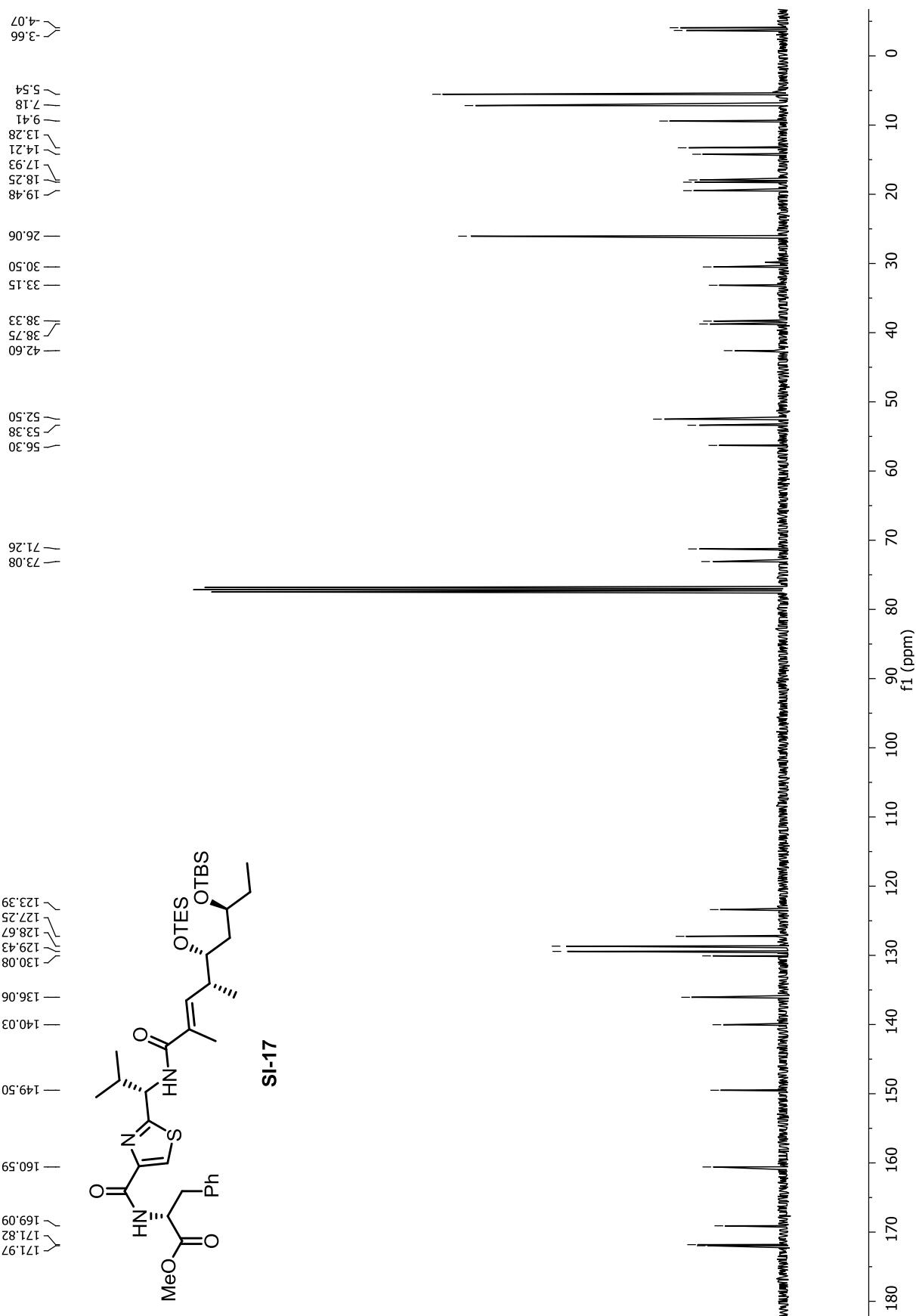


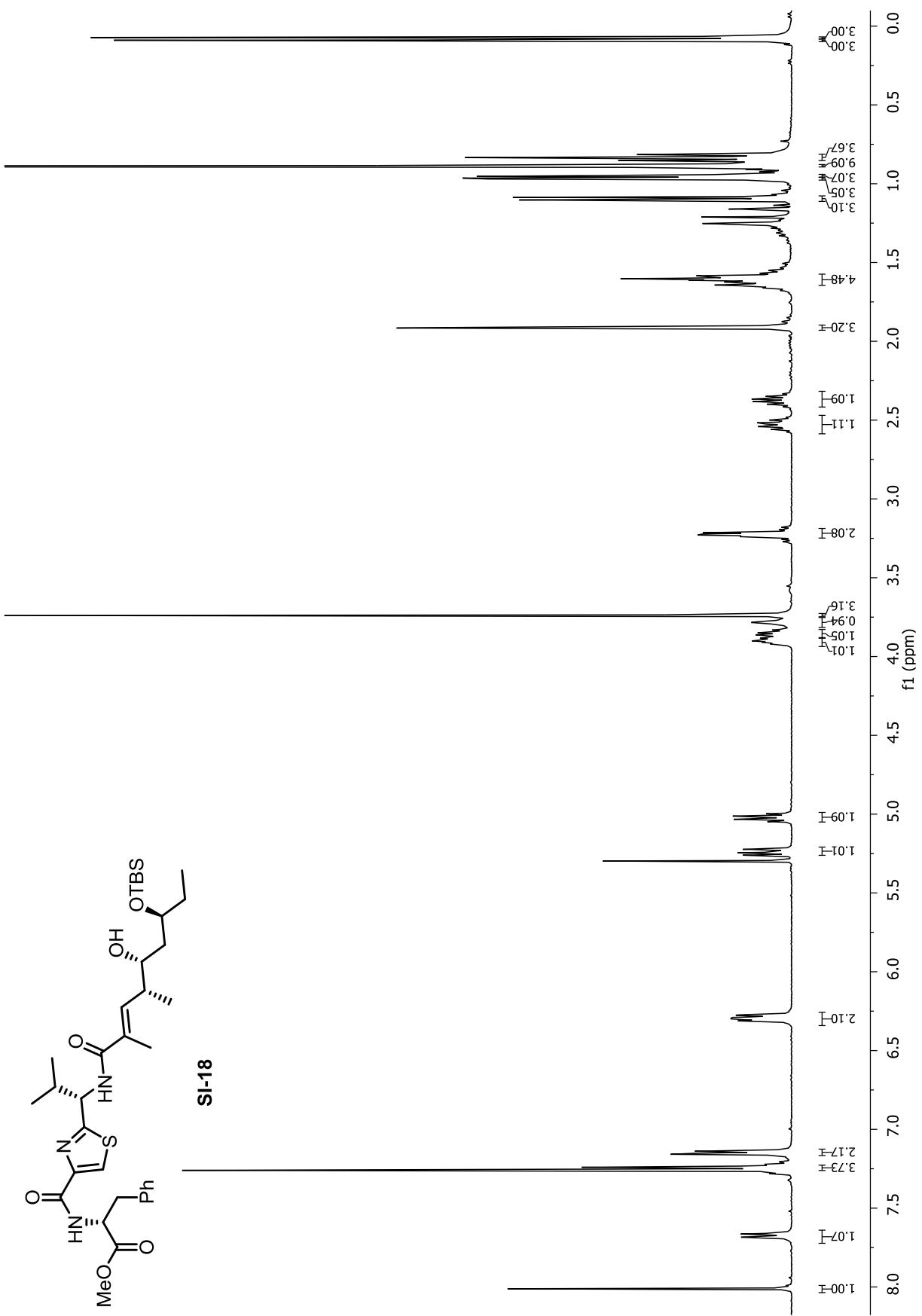


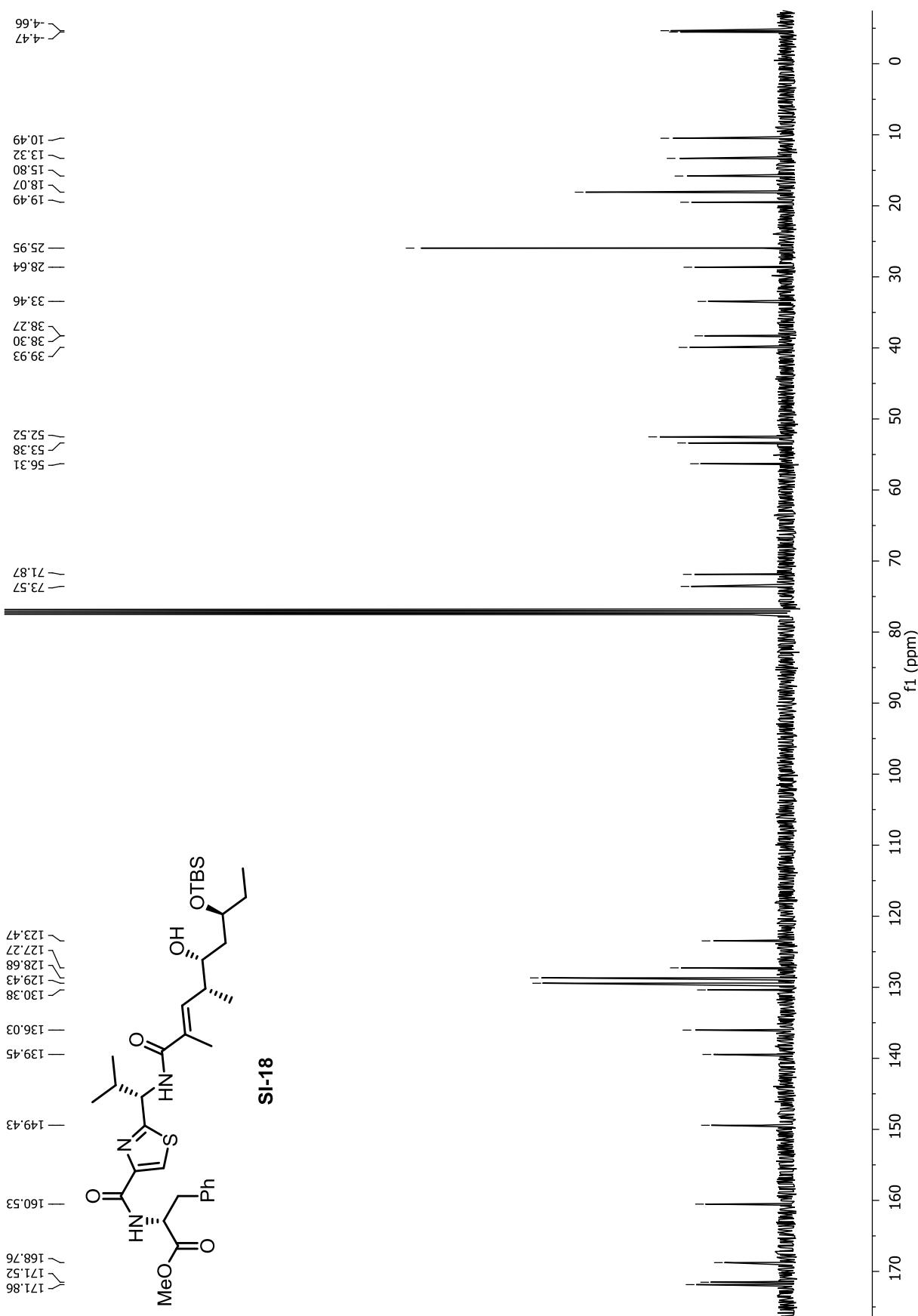


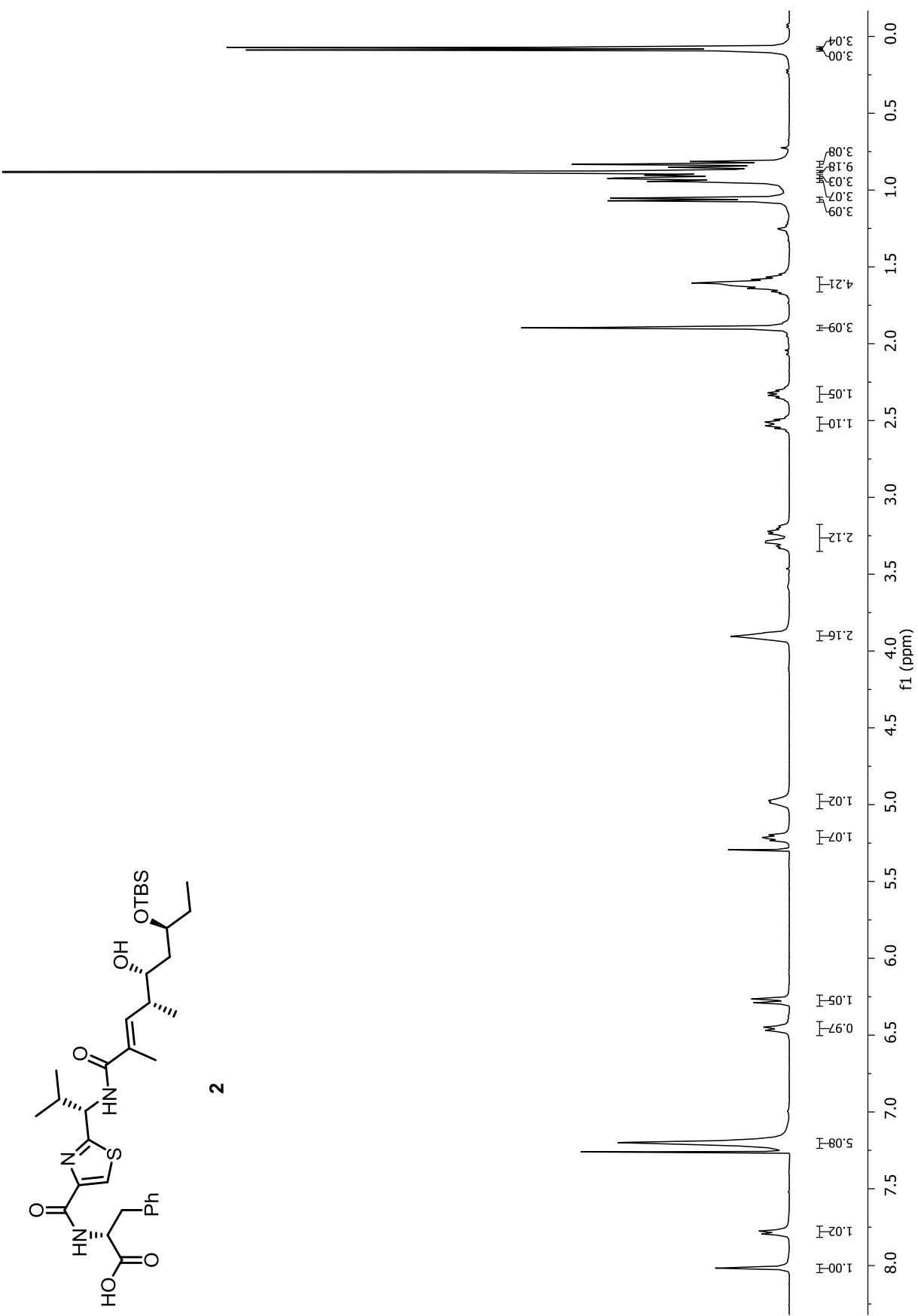


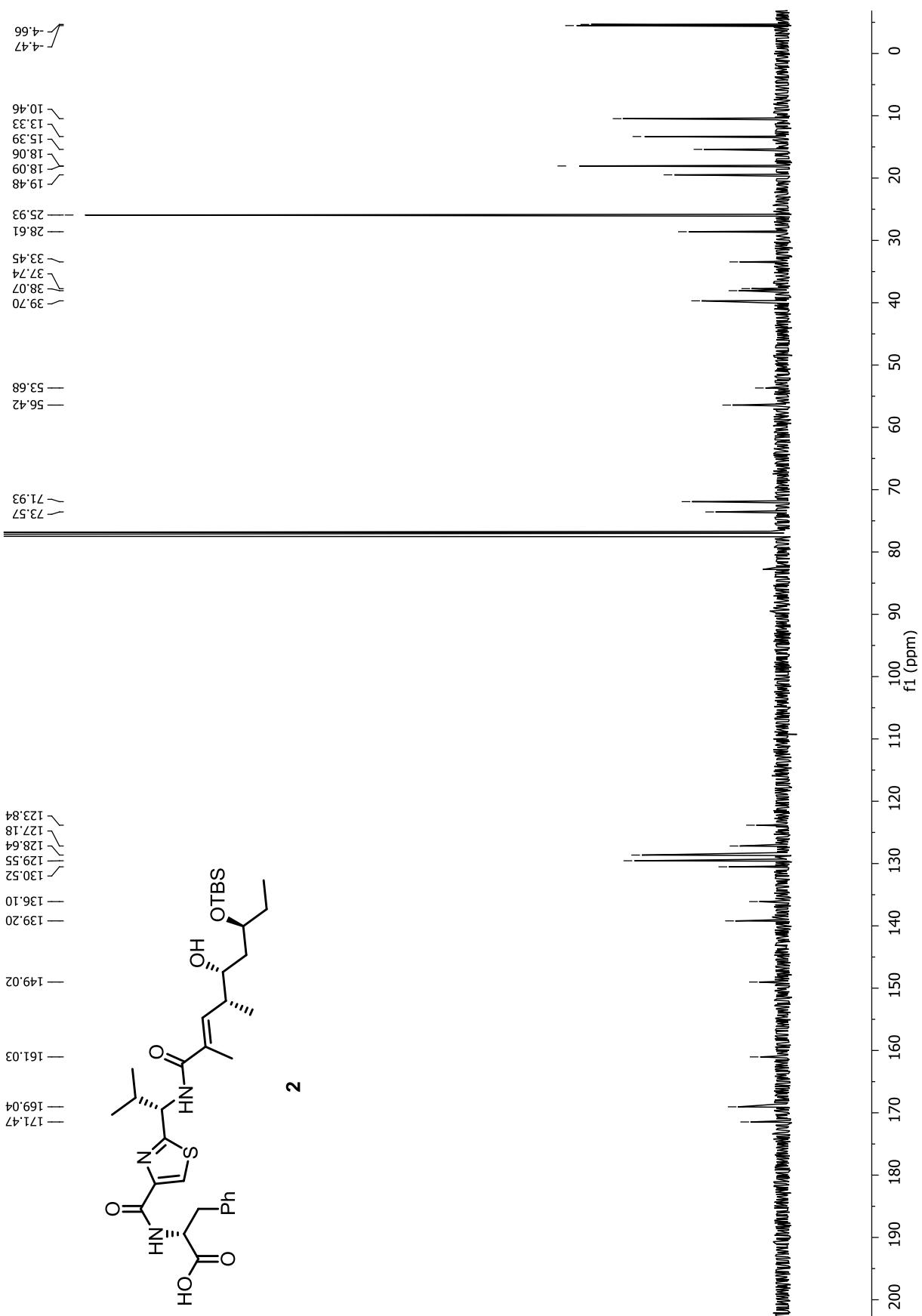


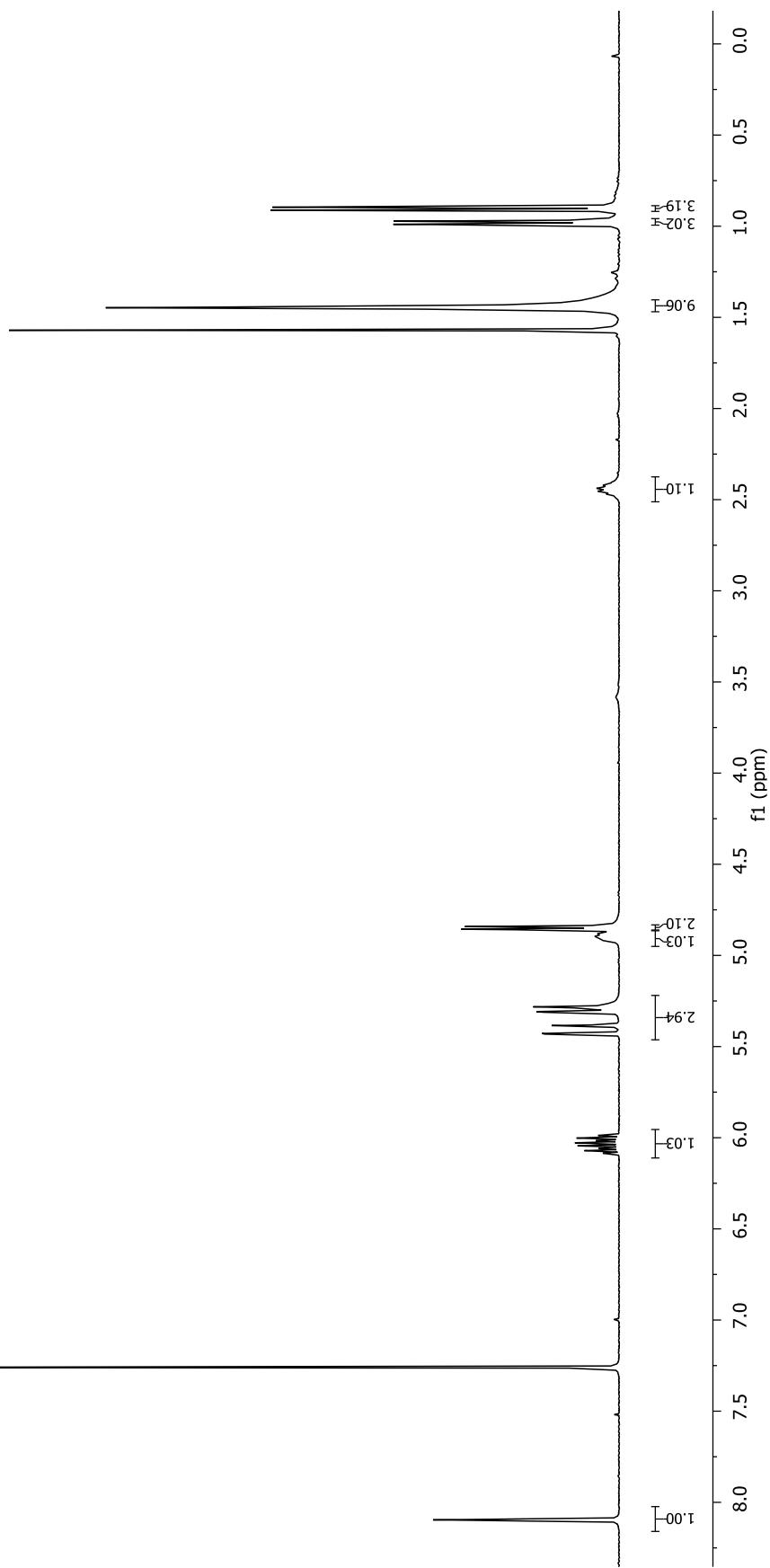
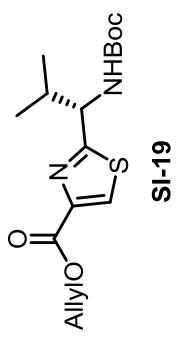


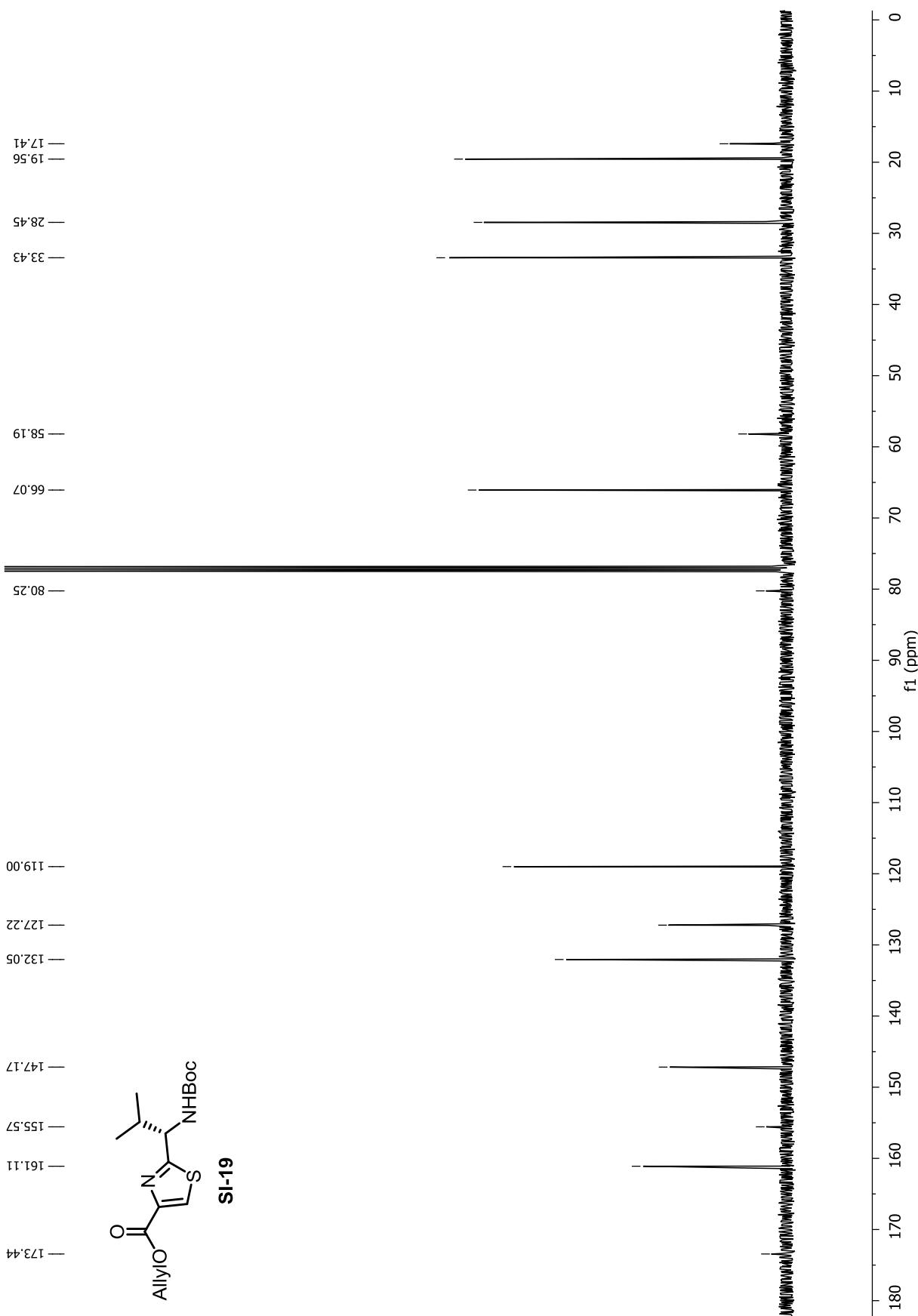


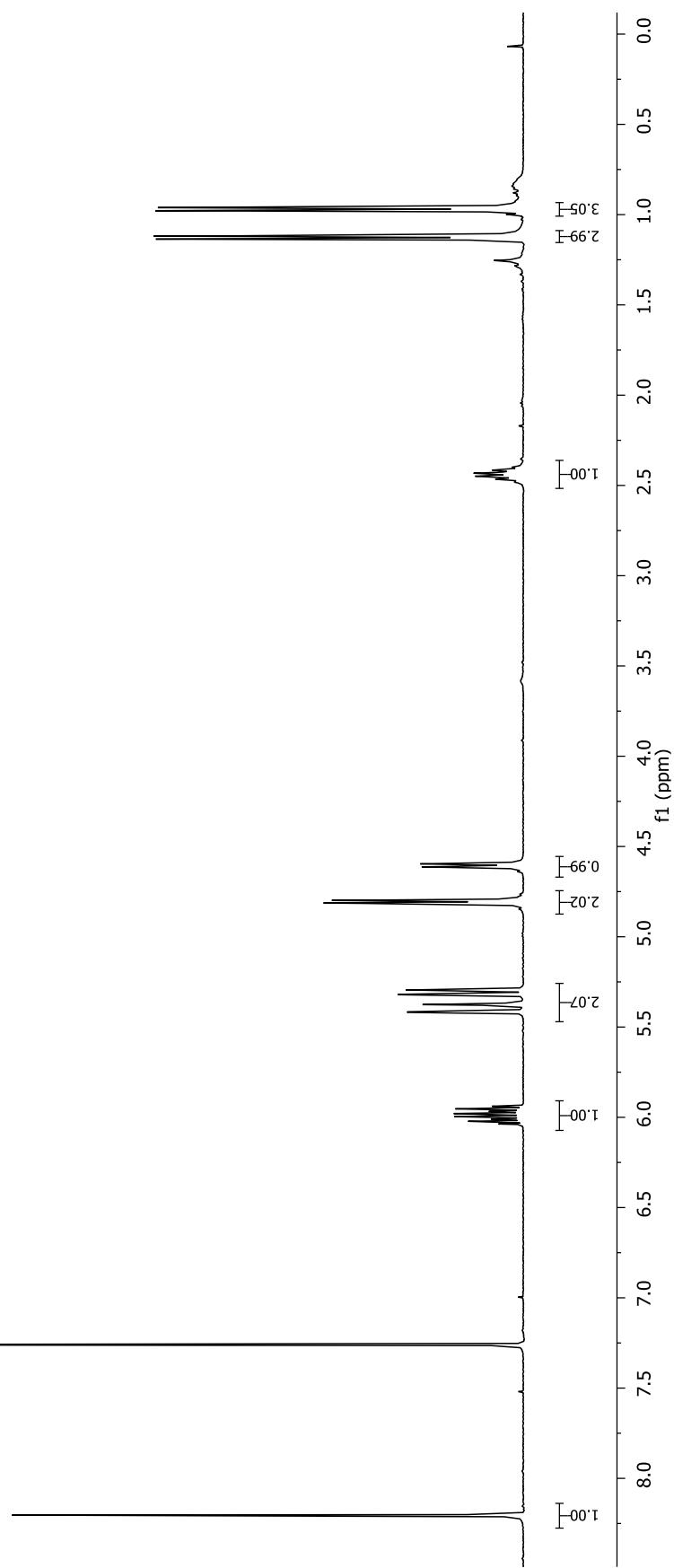
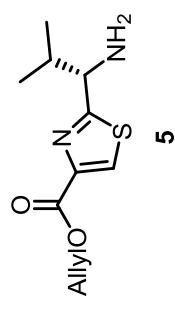












— 119.53 —

— 131.43
— 129.18 —

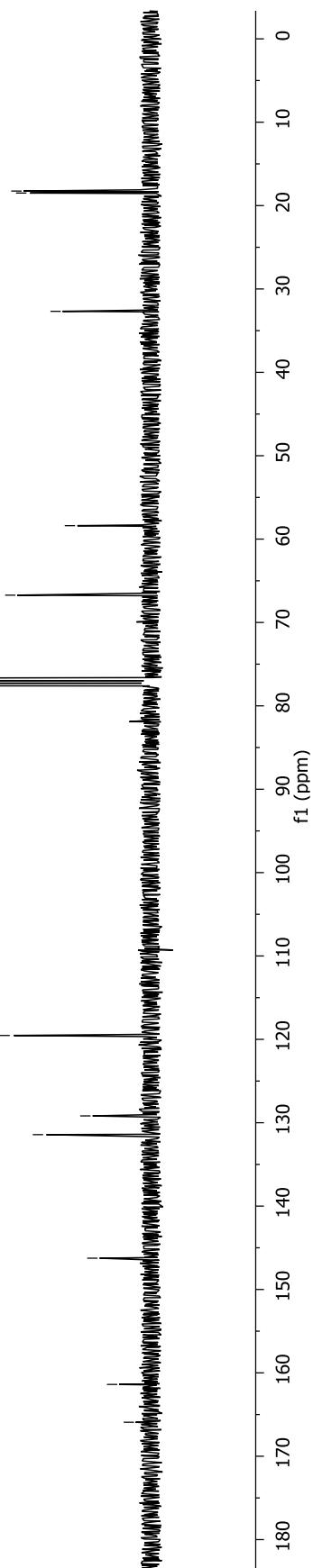
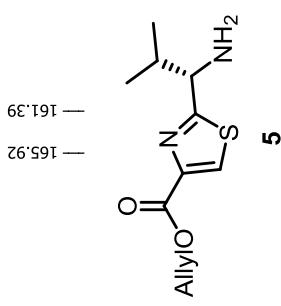
— 146.25 —

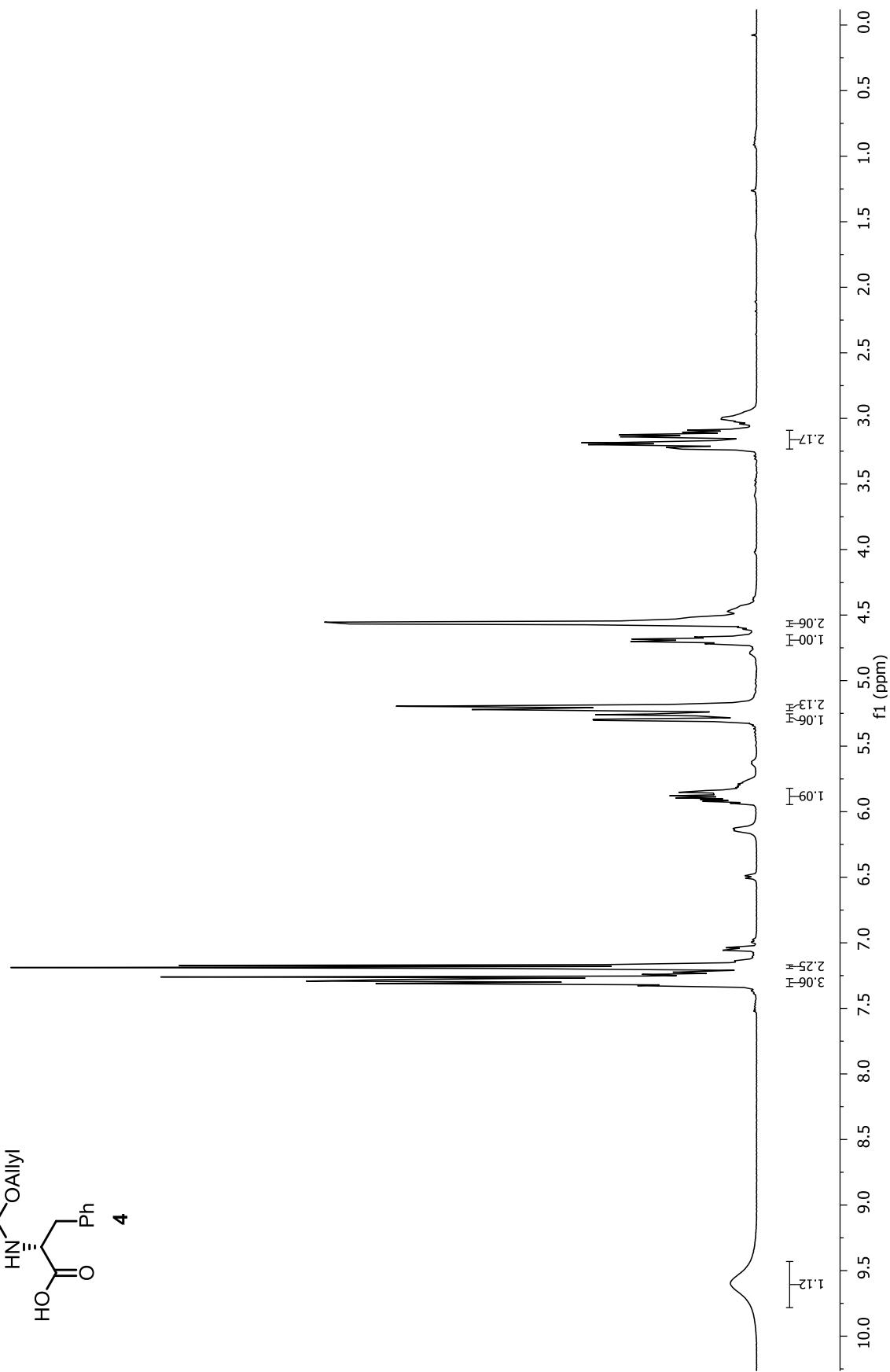
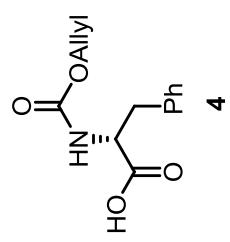
— 32.68 —

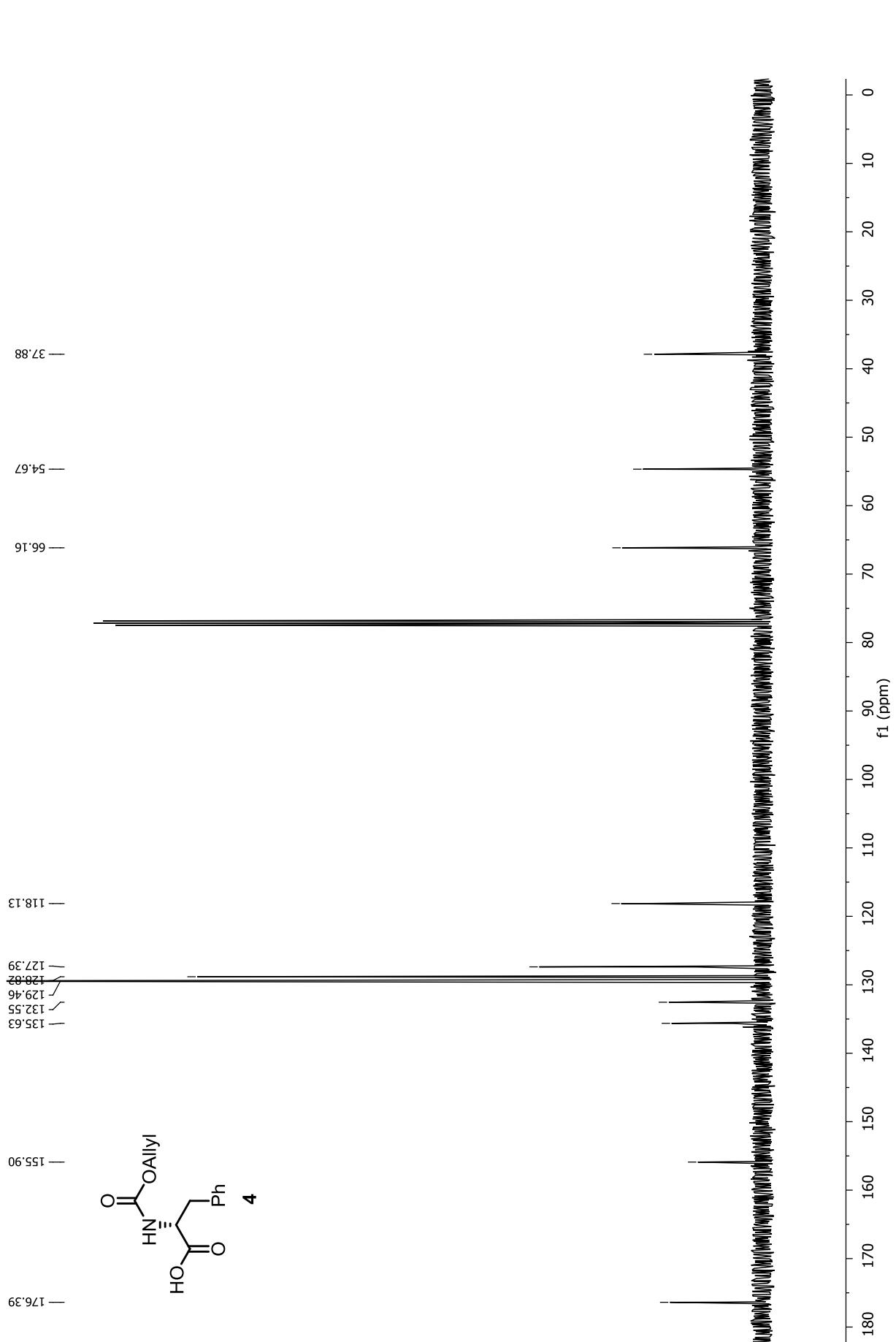
— 18.49
— 18.26 —

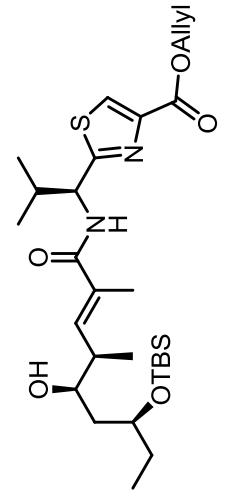
— 58.39 —

— 66.73 —

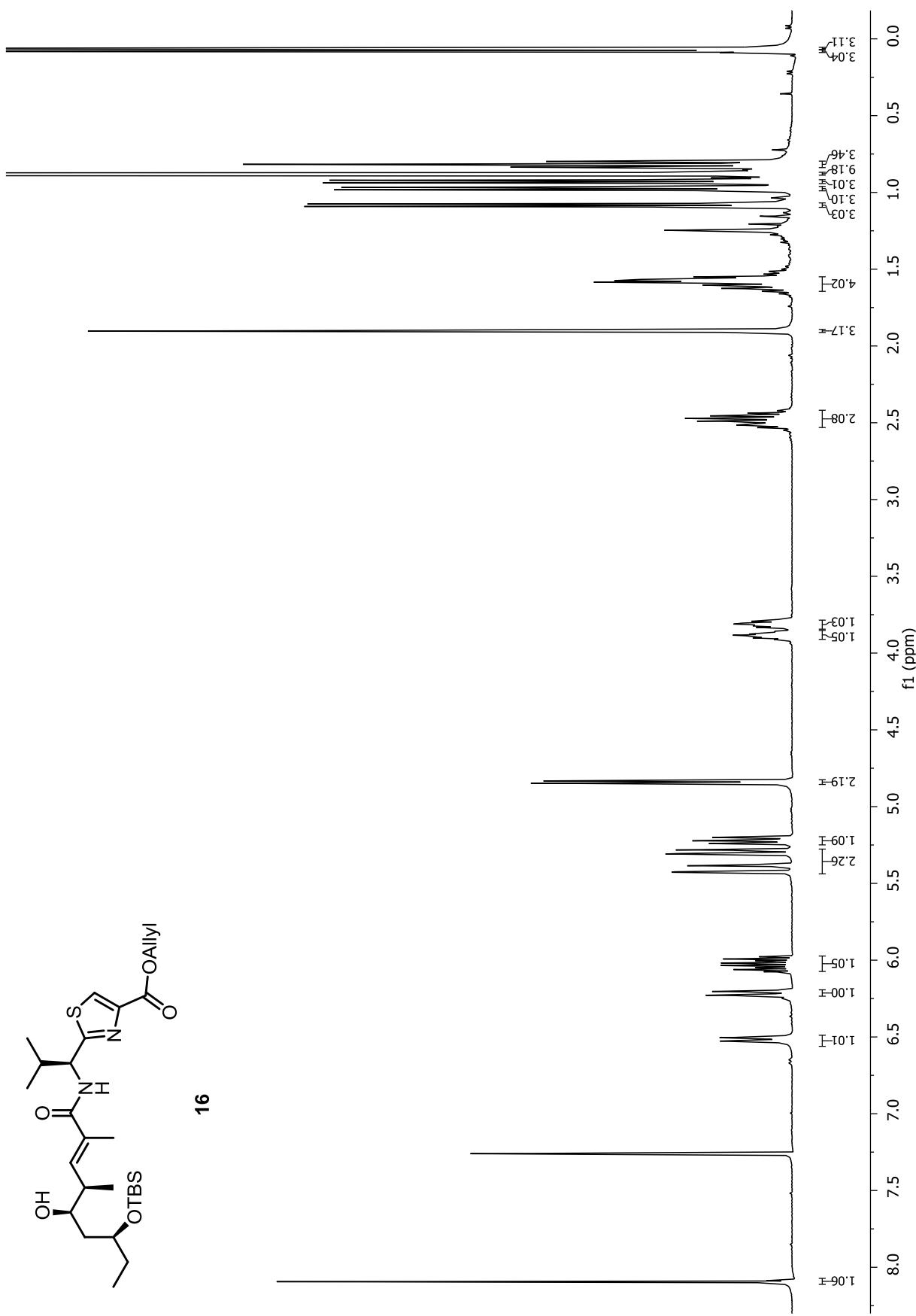


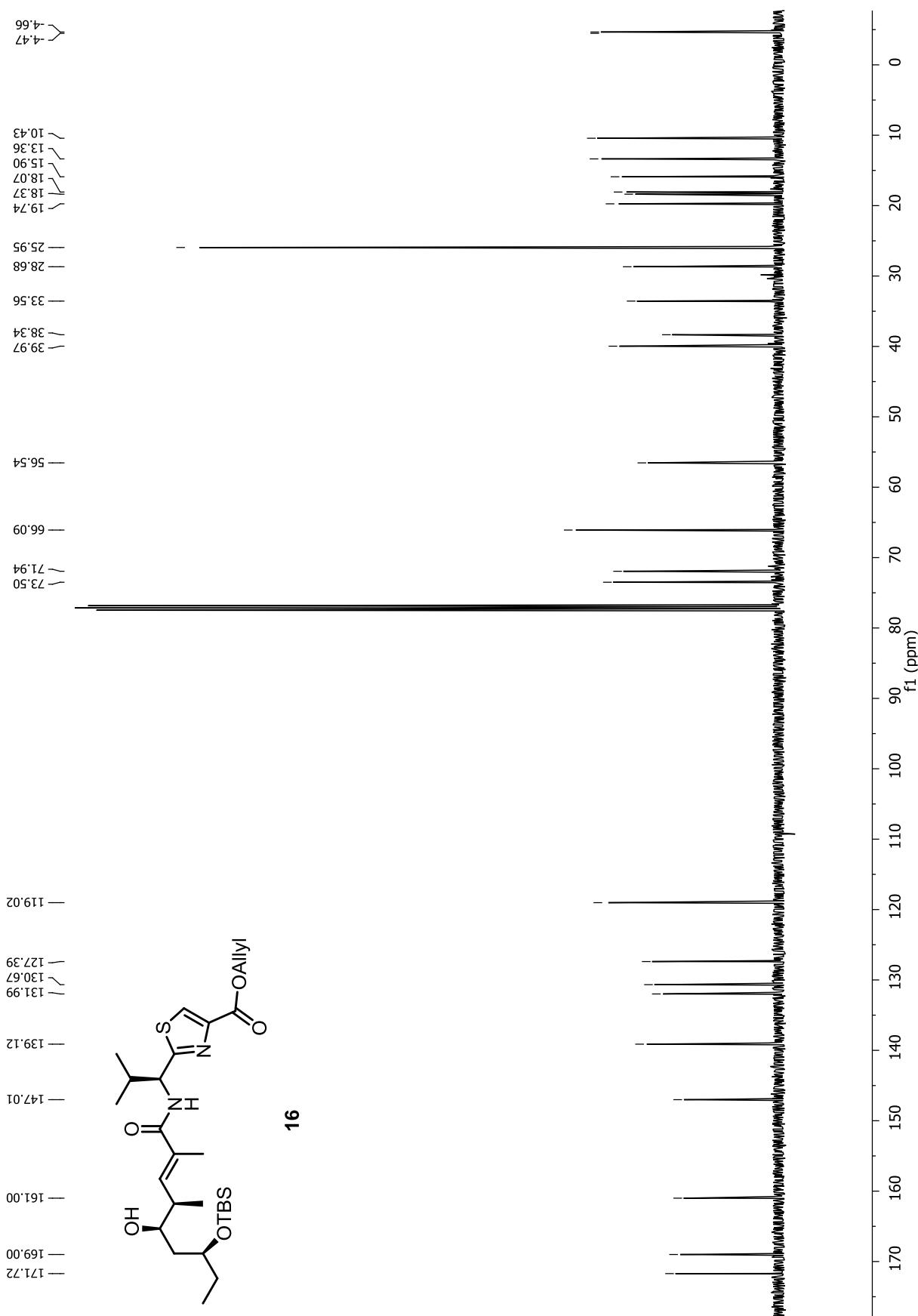


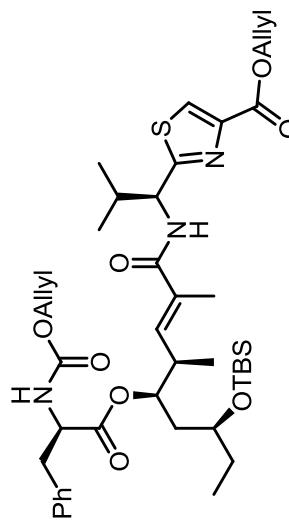




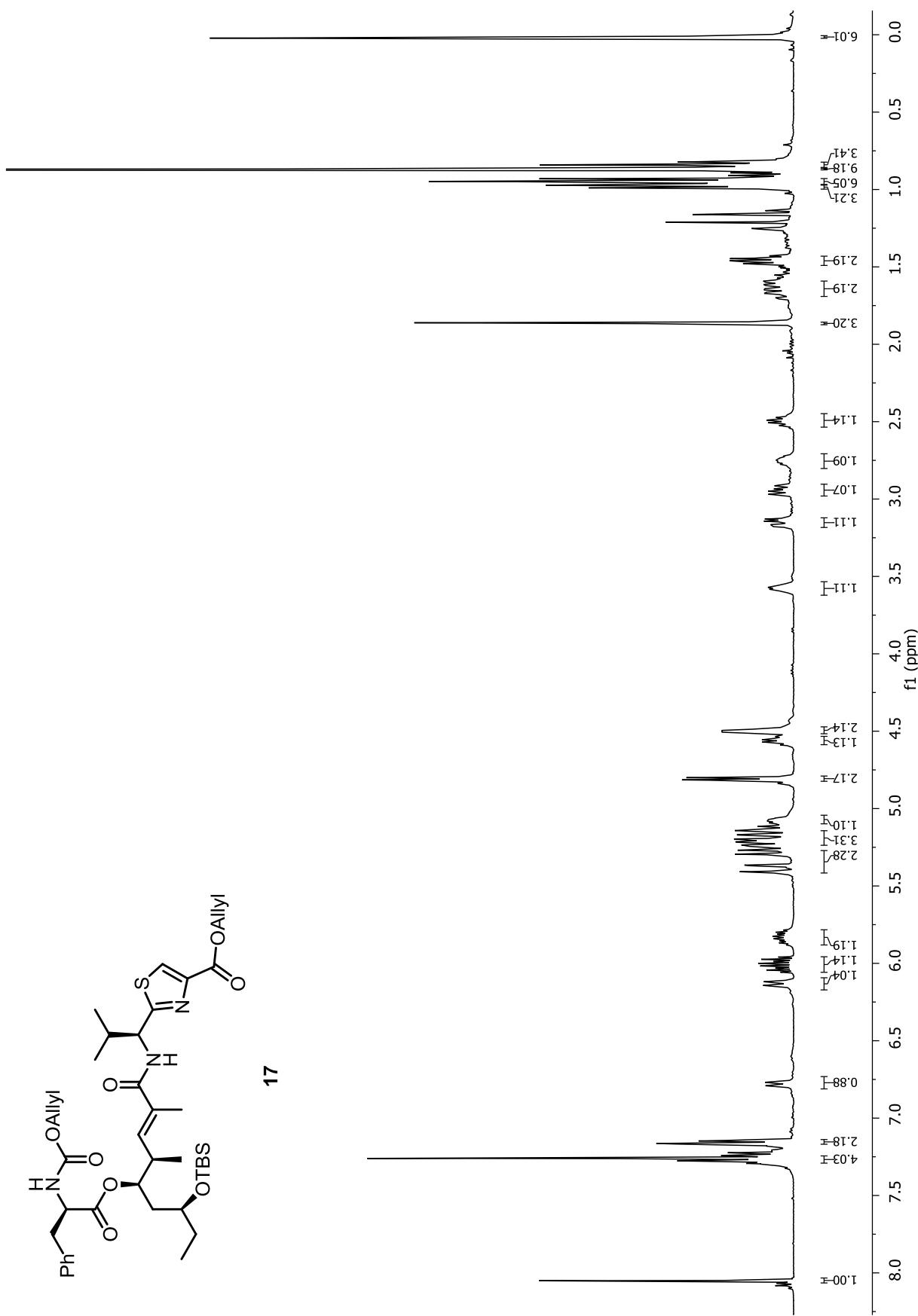
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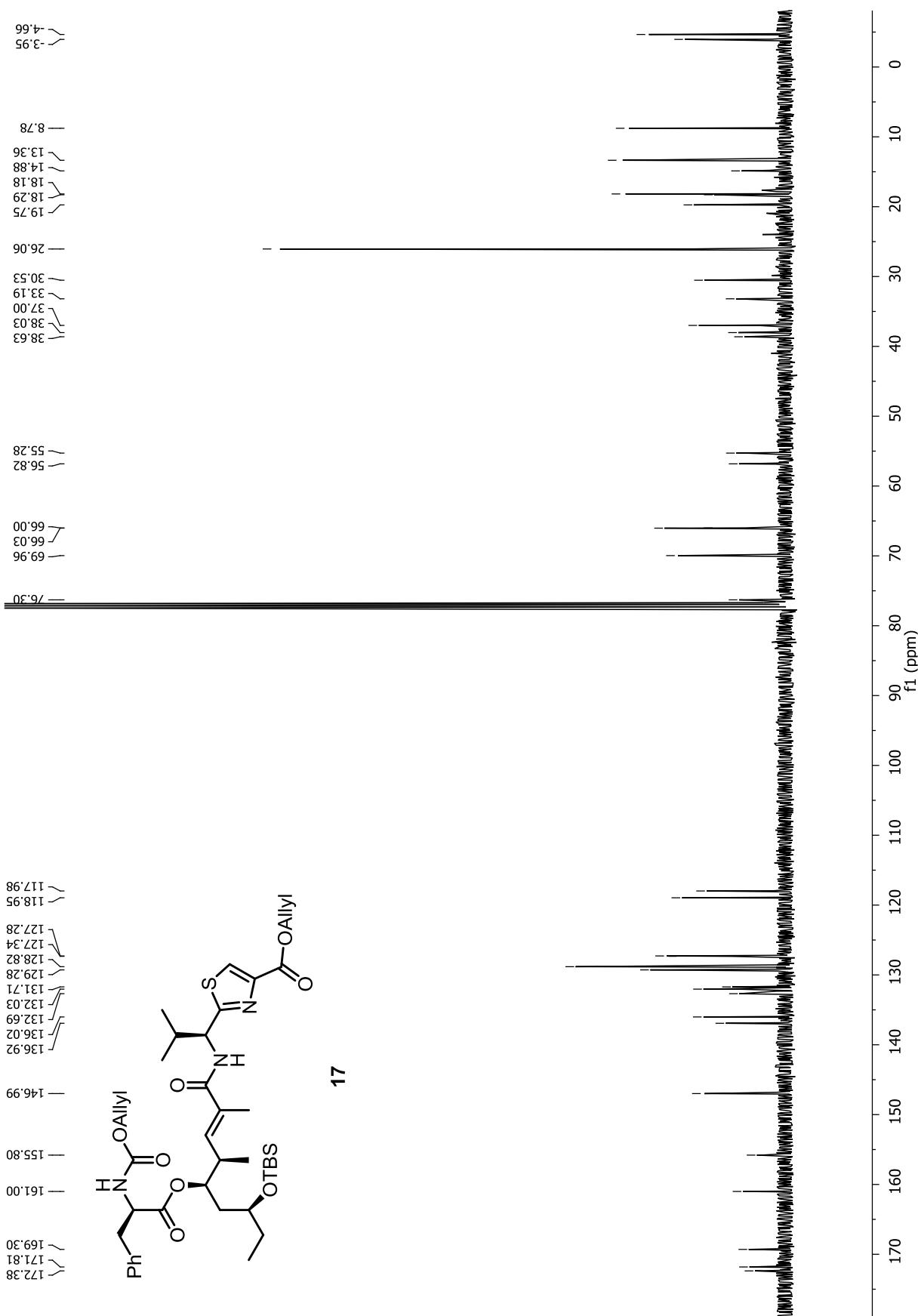


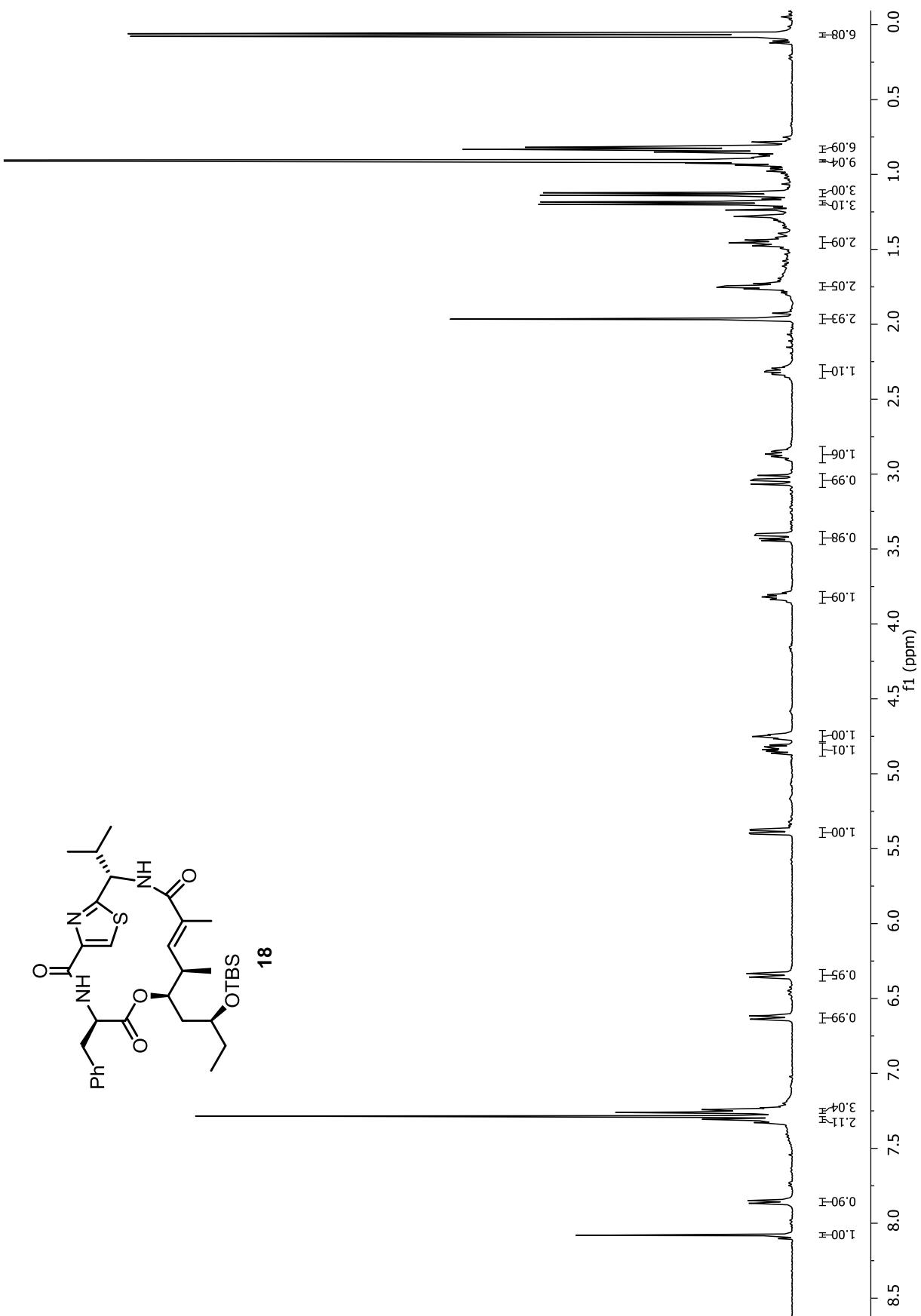


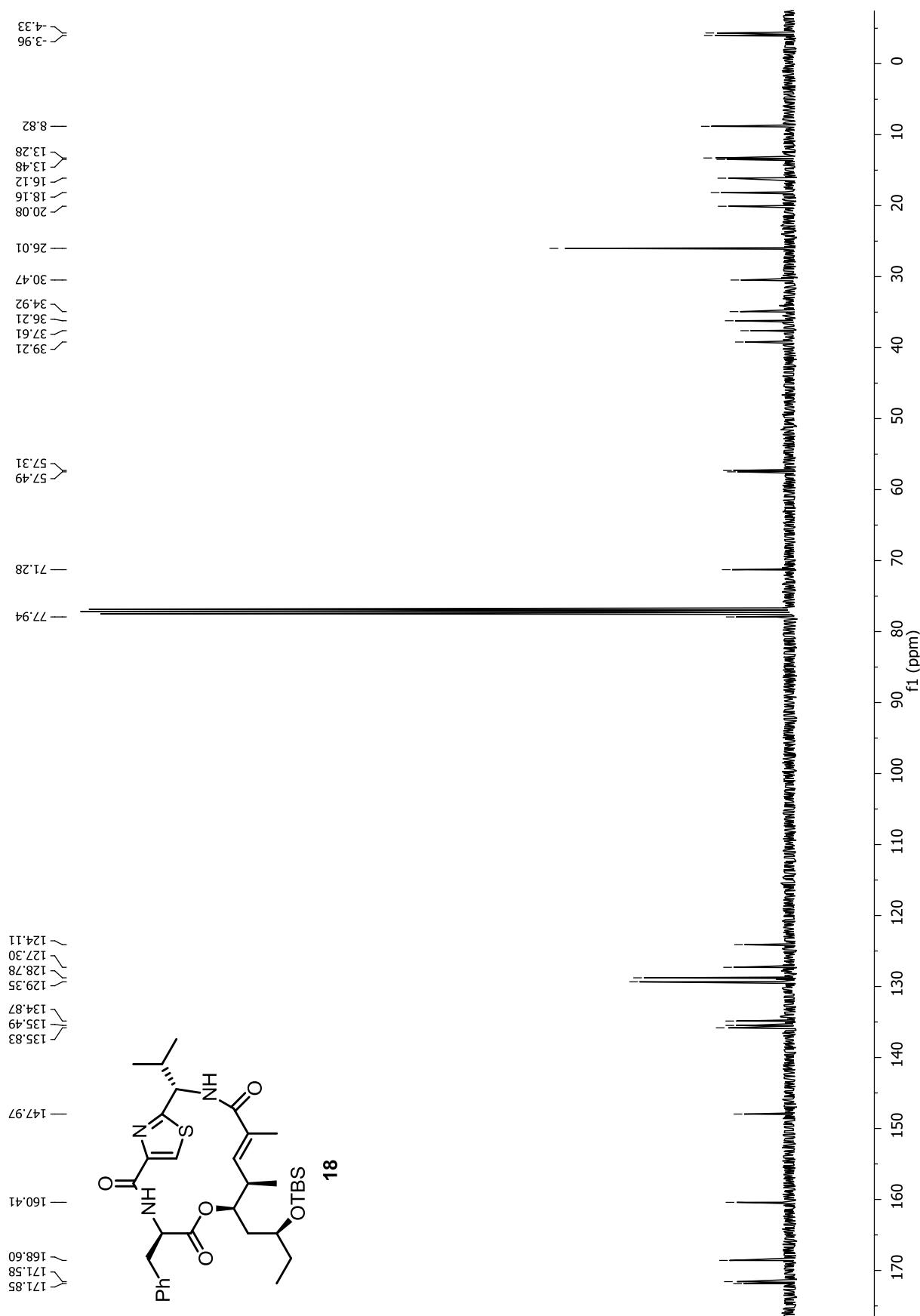


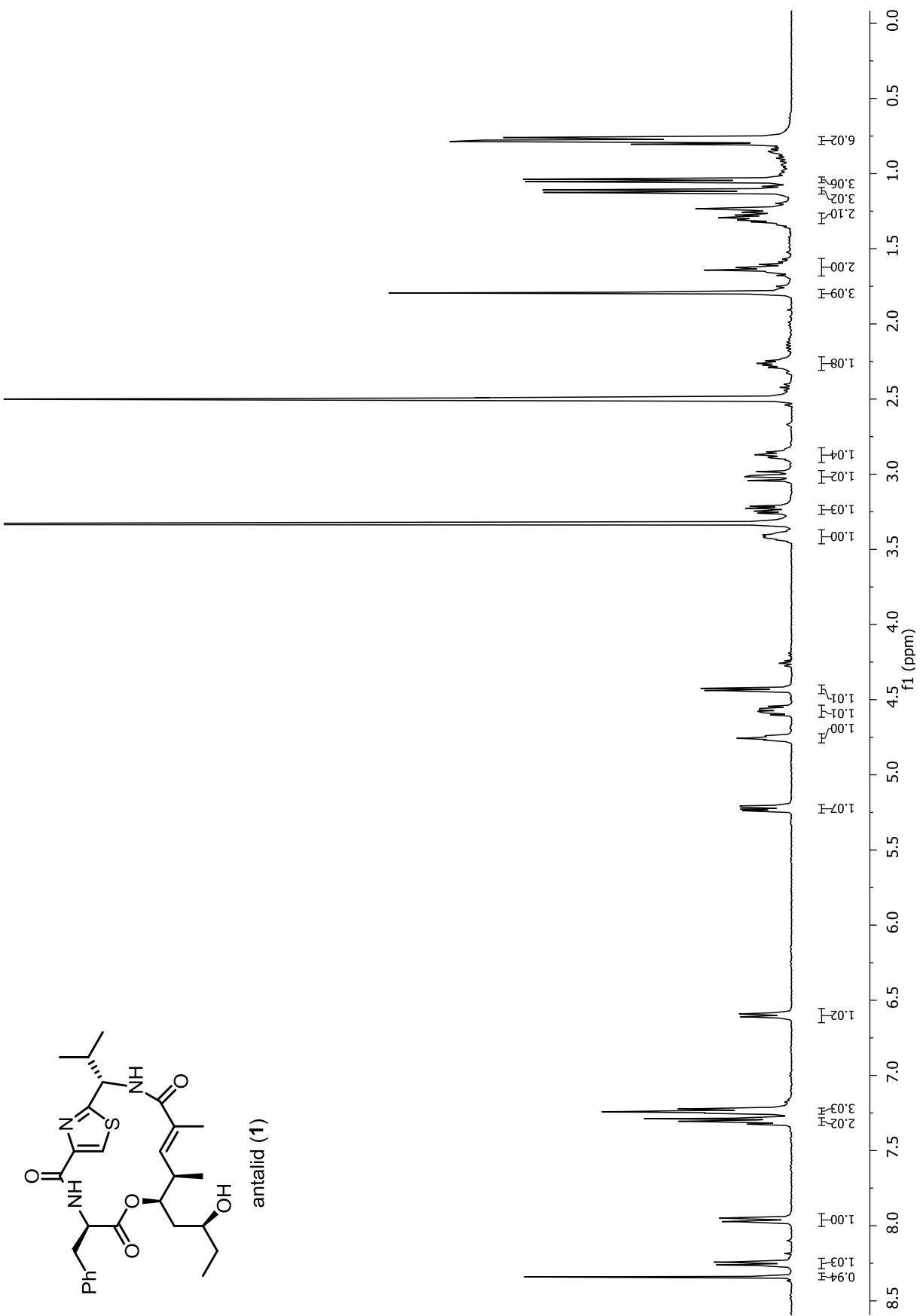
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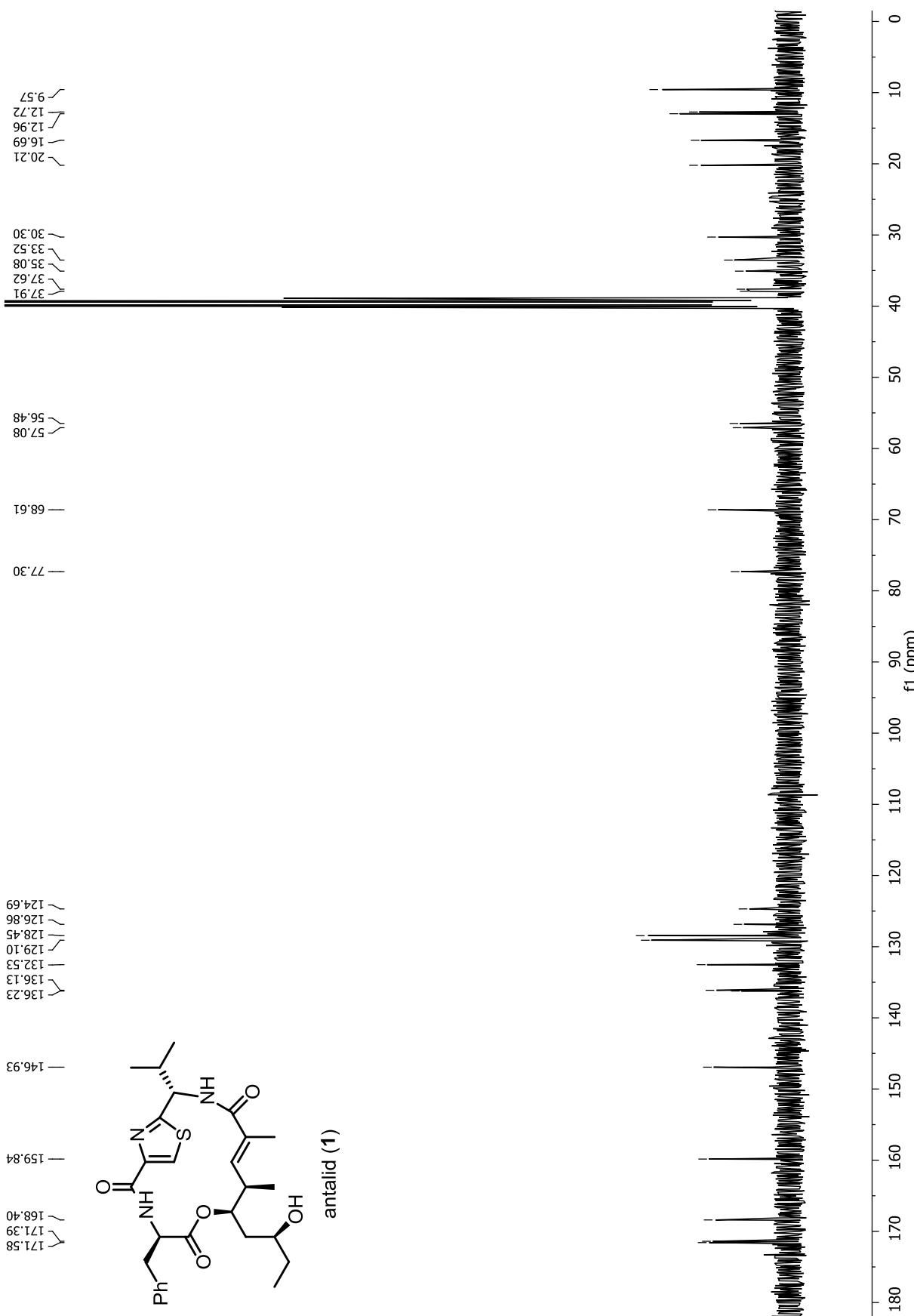


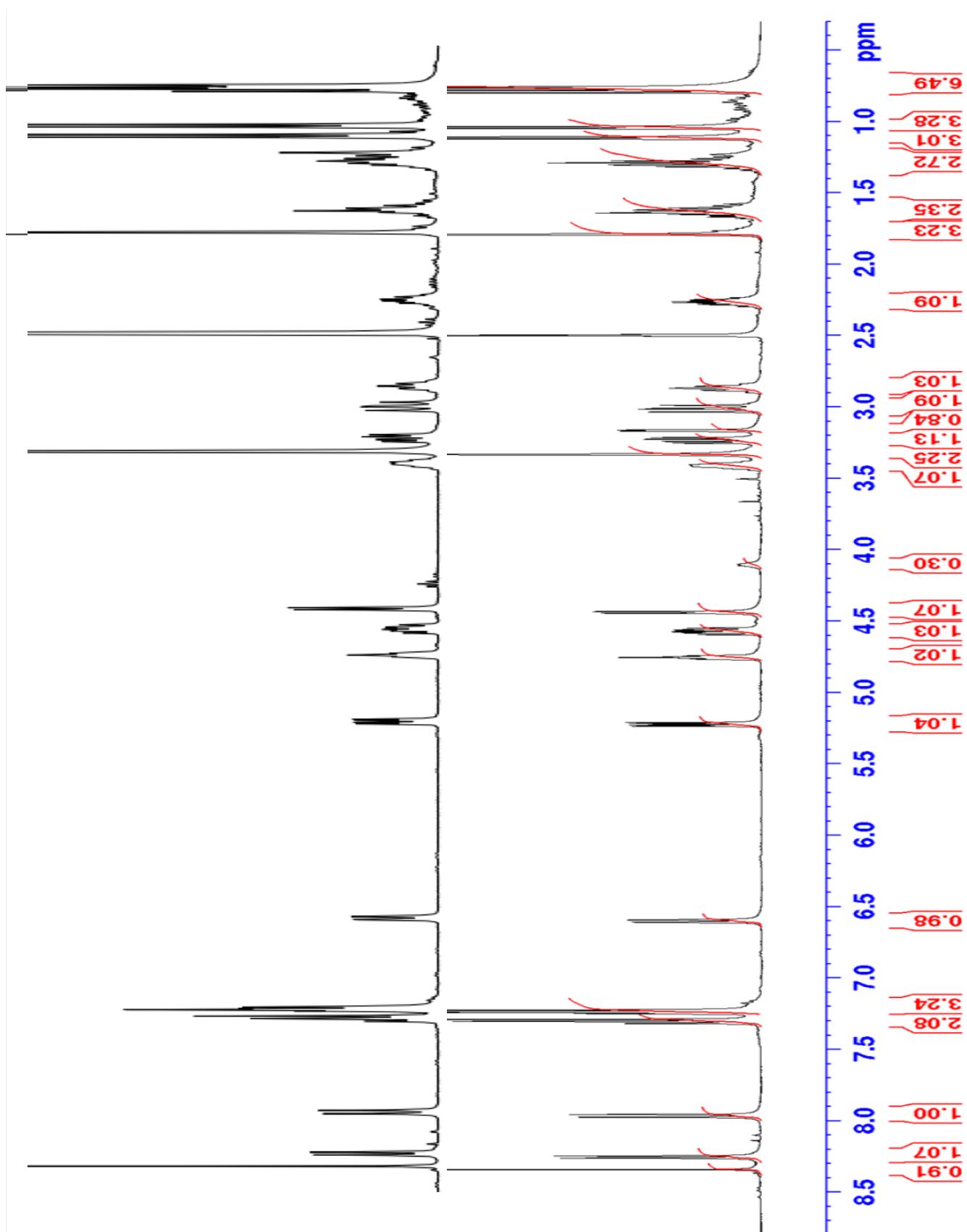












Spectral overlay of synthetic (left, 400 MHz) and authentic (right, 500 MHz) antalid.

5 Literature

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