Supporting Information for

Solid Phase-Based Total Synthesis and Stereochemical Assignment of the Cryptic Natural Product Aurantizolicin

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1 General Methods

1.1 Reagents, solvents and reaction conditions

All reagents were purchased from ACROS CHEMICALS, ALFA AESAR, APOLLO SCIENTIFIC, ABCR, CARBOLUTION CHEMICALS, CARBOSYNTH, GRÜSSING, MANCHESTER ORGANICS, MERCK, NOVABIOCHEM, SIGMA-ALDRICH, TCI EUROPE and VWR. All solvents, if not purchased in suitable purity or dryness, were distilled. Deionized water was used for all experiments. Buffer (pH 3) was prepared by dissolving $NaH_2PO_4 \cdot 2H_2O$ (246.5 g, 1.58 mol) and 85% H_3PO_4 (62.2 mL, 0.92 mol) in water to a total volume of 1 L. CH_2Cl_2 was refluxed with CaH_2, distilled and stored under N_2 atmosphere. THF was treated with Na/benzophenone under reflux, distilled and stored under N_2 atmosphere. DBU and 2.6-lutidine were purified according to literature procedures.[1] All reactions were performed under protective atmosphere (N_2 or Ar) if not stated otherwise.

1.2 Thin Layer Chromatography

Merck precoated silica gel plates (60F-254) were used. Compounds were visualized using ultraviolet light irradiation at 254 nm and 366 nm or by using the following staining agents (dip, dry and heat development).

Staining solution A: $KMnO_4$ (1.00 g), K_2CO_3 (6.60 g), 5% NaOH (1.70 mL) in H_2O (90 mL).

Staining solution B: Ninhydrin (1 g) in EtOH/HOAc (97:3, 100 mL).

1.3 Silica Gel Flash Chromatography

Purifications were performed using silica gel from MACHEREY & NAGEL (particle size 40-60 µm) under approximately 0.3 bar pressure.

1.4 NMR Spectroscopy

^{1}H- and ^{13}C-NMR spectra were recorded using a BRUKER *Avance I 250*, *Fourier 300*, *Avance III 400, Avance III HD 500 or Avance III 600* system. Chemical shifts are given in ppm relative to the external standard Me_4Si. Residual peaks of the particular solvent were used as an internal standard ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.16 ppm; methanol-d_4: δ_H = 3.31 ppm, δ_C = 49.00 ppm, dioxane-d_8: δ_H = 3.53 ppm, δ_C = 66.66 ppm; DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.52 ppm).[2]

1.5 Mass Spectrometry

High resolution mass spectrometry (HR-MS) measurements for sum formula confirmation were performed on a LC-coupled *MAXIS Impact ESI-TOF* spectrometer (BRUKER DALTRONICS, Bremen, Germany). Calculated masses were obtained using the software *ChemDraw Ultra* (CAMBRIDGESOFT CORPORATION) or *Xcalibur*.

1.6 Analytical Reversed-Phase High Performance Liquid Chromatography (RP-HPLC)

Analyses were performed on a SHIMADZU system consisting of a system controller (SLC-10AVP), a column oven (CTO-10ACVP), an auto-injector (SIL-10ADVP), a degasser (DGU-14A), three pumps (LC-10ATVP), a fluorescence detector (RF-10AXL), a diode array detector (SPD-M20A) and a UV-vis-detector (SPD-10AVP). Eluents used: CH_3CN (A), water (B) and 2% TFA in water (C).

System A: NUCLEODUR C18 Gravity 5 µm. Gradient: 10 min 10-95% acetonitrile, 10 min 95% acetonitrile, 5 min 10% acetonitrile. 5% C. Flow rate: 1 mL/min. detection by diode array 190-600 nm.

System B: NUCLEODUR C18 Gravity 5 µm. Gradient: 10 min 30-95% acetonitrile, 10 min 95% acetonitrile, 5 min 30% acetonitrile. 5% C. Flow rate: 1 mL/min. detection by diode array 190-600 nm.

1.7 Fourier-Transform Infrared Spectroscopy (FTIR)

IR spectra were measured on SHIMADZU *IRAffinity 1* filled with an ATR unit. Wavenumbers are given in cm^{-1}. Abbreviations for relative intensities of absorptions: s (strong), m (medium), w (weak).

1.8 Specific Rotation

Optical Rotations were recorded on a JASCO *P-2000* polarimeter at 589 nm. Concentration *c* is given in g/100 mL. Pass lengths of cuvettes were *d* = 10 mm or *d* = 100 mm.

1.9 Melting Points

Melting points were measured with a BÜCHI *B-540* melting point apparatus in open capillaries (uncorrected).

2 NMR Comparison of Cyclopeptides 1, 26 and 27

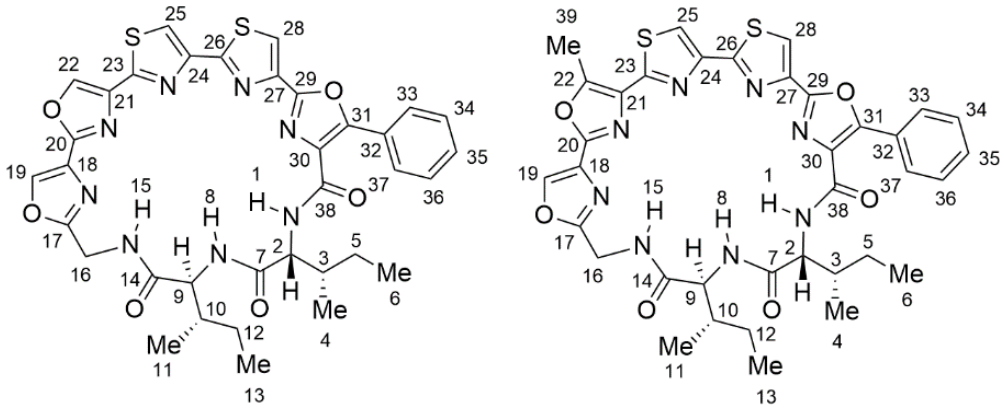


Figure S1. Numbering scheme of cyclopeptides **1** and **2** (numbering follows the isolation article of aurantizolicin[3]).

Table S1. Comparison of the chemical shifts of the natural product aurantizolicin (**1**), the synthetic sample and the closely related natural product curacozole (**2**). ^{1}H NMR spectra were recorded in DMSO-d_6.

Position	σ_H / ppm isolated aurantizolicin[3] (1)	σ_H / ppm (*J* = Hz) synthetic sample	σ_H /ppm (*J* / Hz) isolated curacozole[4] (2)
1	8.13 (d)	8.12 (d, 7.7)	8.10 (d, 7.7)
2	4.82 (m)	4.81 (dd, 7.6, 4.0)	4.78 (dd, 7.7, 4.2)
3	1.98 (ov.)	1.96 (m)	1.94 (m)
4	0.91 (ov.)	0.81-0.94	0.87 (ov.)
5	1.62, 1.05 (ov.)	1.60 (m), 1.05 (m)	1.57 (m), 1.03 (m)
6	0.87 (ov.)	0.81-0.94	0.87 (ov.)
7	-	-	-
8	8.60 (d)	8.57 (d, 8.7)	8.53 (d, 8.5)
9	4.39 (t)	4.39 (dd, 8.5, 6.5)	4.32 (dd, 8.5, 6.5)
10	1.88 (ov.)	1.87 (m)	1.84 (m)
11	0.90 (ov.)	0.81-0.94	0.86 (ov.)
12	1.52, 1.27 (ov.)	1.51 (m), 1.26 (m)	1.49 (m), 1.23 (m)
13	0.86 (ov.)	0.81-0.94	0.81 (t, 7.3)
14	-	-	-
15	8.80 (d)	8.74 (dd, 9.0, 1.5)	8.71 (dd, 9.1, 2.5)
16	5.02, 4.17 (m)	5.03 (dd, 16.5, 9.0), 4.15 (dd, 16.3, 2.0)	4.99 (dd, 16.6, 9.1), 4.12 (dd, 16.6, 2.3)
17	-	-	-
18	-	-	-
19	8.90 (s)	8.90 (s)	8.79 (s)
20	**-**	**-**	**-**
21	-	-	-
22	9.08 (s)	9.08 (s)	-
23	-	-	-
24	-	-	-
25	8.60 (s)	8.59 (s)	8.60 (s)
26	-	-	-
27	-	-	-
28	8.69 (s)	8.69 (s)	8.65 (s)
29	-	-	-
30	-	-	-
31	-	-	-
32	-	-	-
33	8.35 (d)	8.36 (m)	8.33 (d, 7.5)
34	7.57 (t)	7.57 (m)	7.55 (t, 7.5)
35	7.51 (t)	7.52 (m)	7.50 (t, 7.5)
36	7.57 (t)	7.57 (m)	7.55 (t, 7.5)
37	8.35 (d)	8.36 (m)	8.33 (d, 7.5)
38	-	-	-

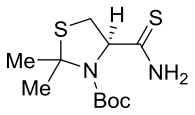
Table S2. Comparison of the chemical shifts of the natural product aurantizolicin (**1**), the synthetic sample and the closely related natural product curacozole (**2**). ^{13}C NMR spectra were recorded in DMSO-d_6.(* new assignment by HSQC correlation)

Position	σ_C (ppm) isolated aurantizolicin[3] (1)	σ_C (ppm) synthetic sample	σ_C (ppm) isolated curacozole[4] (2)
1	-	-	-
2	56.11	56.41	56.5
3	38.66	39.11 (via HSQC correlation)	39.2
4	-	14.70	14.8
5	25.34	25.63	25.8
6	-	12.10	12.2
7	-	170.00	170.2
8	-	-	-
9	56.88	57.09	57.4
10	36.75	37.03	37.0
11	-	15.90	16.0
12	28.21	24.14*	24.3
13	-	10.90	11.0
14	-	171.00	171.2
15	-	-	-
16	34.85	35.17	35.9
17	162.78	162.90	162.9
18	-	129.40	-
19	139.48	139.97	139.5
20	155.30	155.32	153.4
21	-	135.80	131.4
22	139.00	139.51	149.1
23	157.06	157.15	157.6
24	147.79	147.96	148.0
25	120.70	120.98	120.8
26	161.11	161.23	161.5
27	141.93	141.92	142.1
28	122.59	123.00	123.1
29	-	154.20	154.3
30	-	130.60	130.6
31	150.87	150.74	150.9
32	126.66	126.72	126.7
33	127.37	127.67	127.8
34	128.37	128.67	128.8
35	129.84	130.12	130.3
36	128.37	128.67	128.8
37	127.37	127.67	127.8
38	-	160.20	160.3

3 Synthetic Procedures and Physical Data

3.1 Advanced Building Blocks

The following compound was prepared according to literature procedures.



13 (ref. 5)

3.2 General Procedures for Solid-Phase-Peptide-Synthesis

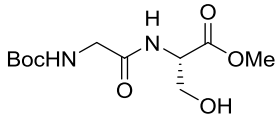
General Procedure A: Fmoc-Deprotection on solid support

The resin (250-280 mg dry weight) was swollen in CH_2Cl_2 (3 mL) for 15 min and treated with piperidine (3 mL, 20 vol% in DMF) for 5 min and washed with CH_2Cl_2 (3 × 3 mL) and DMF (3 mL). After a second treatment with piperidine (3 mL, 20 vol% in DMF) for 15 min the resin was washed with CH_2Cl_2 (3 × 3 mL) and DMF (3 mL).

General Procedure B: Amide-coupling on solid support

A solution of the respective amino acid, HBTU, HOBt and EtN*i*Pr$_2$ in CH_2Cl_2/DMF (3 mL, 1:1 v/v) was added to the resin and the mixture was shaken at rt for 2-3 h. The solvent was removed and the resin was washed with DMF (3 × 3 mL) and CH_2Cl_2 (3 × 3 mL). After evaporation of the solvents, the resin was stored at –25 °C under N_2-atmosphere.

3.3 Dipeptide 21



21

At 0 °C EtN*i*Pr$_2$ (16.3 mL, 93.3 mmol) was added to a suspension of Boc-Gly-OH (8.17 g, 46.7 mmol), Ser-OMe×HCl (7.26 g, 46.7 mmol), EDC×HCl (10.7 g, 56.0 mmol) and HOBt (7.57 g, 56.0 mmol) in CH_2Cl_2 (100 mL). The resulting solution was stirred at room temperature for 16 h. Saturated NH_4Cl solution (50 mL) was added, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with 1 N HCl, saturated Na_2CO_3 solution, and saturated NaCl solution (50 mL each). The

organic layer was treated with Na_2SO_4 and evaporated under reduced pressure to afford dipeptide **21** as a slightly yellow oil (12.8 g, 42.1 mmol, 90%).

TLC: R_f = 0.21 (petroleum ether/EtOAc, 1:1).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.0 K): δ = 1.39 (s, 9 H), 3.72 (s, 3 H), 3.79 (d, J = 5.7 Hz, 2 H), 3.91 (s, 1 H), 4.60 (m, 1 H), 5.31 (s, 1 H), 7.06 (d, J = 7.3 Hz, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 75.48 MHz, 298.1 K): δ = 28.2, 52.7, 54.7, 62.7, 156.2, 169.8, 170.7 ppm.

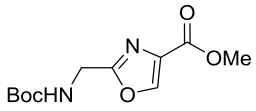
HPLC: t_R = 7.381 min (system A).

HRMS (ESI-TOF) calculated for $C_{11}H_{20}N_2O_6$ $[M+Na]^+$ 299.1214; found 299.1219.

$[\alpha]_D$ = 23.9 (THF, c = 0.1, 25.5 °C).

IR (ATR): $\tilde{\nu}$ = 3314 (w), 2974 (w), 2936 (w), 1740 (m), 1667 (s), 1512 (s), 1439 (m), 1366 (s), 1277 (m), 1215 (s), 1161 (s), 1053 (s), 1030 (s), 941 (m), 860 (m), 733 (m) cm^{-1}.

3.4 Oxazole 22



22

At –68 °C DAST (1.98 mL, 15.0 mmol) was added to a solution of dipeptide **21** (4.34 g, 14.3 mmol) in CH_2Cl_2 (45 mL). The solution was stirred for 2 h at this temperature. K_2CO_3 (3.84 g, 28.5 mmol) was added and the mixture was slowly warmed to 0 °C in 3 h. Saturated $NaHCO_3$ solution (50 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were treated with Na_2SO_4 and concentrated. The residue was dissolved in CH_2Cl_2 (45 mL) and at –70 °C DBU (5.75 mL, 38.5 mmol) and $BrCCl_3$ (2.27 mL, 22.8 mmol) were added. The solution was stirred for 3 h at that temperature. Then phosphate buffer (pH 3, 50 mL) was added. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic extracts were treated with Na_2SO_4 and evaporated under reduced pressure. Flash chromatography of the residue (200 g silica, petroleum ether:EtOAc = 1:1) afforded oxazole **22** as a colorless solid (2.54 g, 9.92 mmol, 69%).

TLC: R_f = 0.27 (petroleum ether/EtOAc, 1:1).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.1 K): δ = 1.44 (s, 9 H), 3.90 (s, 3 H), 4.48 (d, J = 5.8 Hz, 2 H), 5.23 (s, 1 H), 8.19 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 75.48 MHz, 298.1 K): δ = 28.3, 37.9, 52.3, 80.4, 133.3, 144.3, 155.5, 161.3, 162.3 ppm.

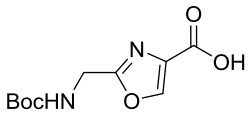
HPLC: t_R = 5.003 min (system B).

HRMS (ESI-TOF) calculated for $C_{11}H_{16}N_2O_5$ $[M+H]^+$ 257.1132; found 257.1140.

Melting point: T_m = 55 °C

IR (ATR): $\tilde{\nu}$ = 3372 (w), 3144 (w), 2970 (w), 1701 (s), 1558 (w), 1520 (m), 1450 (m), 1350 (m), 1312 (m), 1246 (m), 1173 (m), 1146 (m), 1111 (m), 1030 (m), 1007 (m), 984 (m), 914 (m), 872 (m), 783 (m), 710 (m) cm^{-1}.

3.5 Carboxylic acid 23



23

LiOH (201 mg, 8.39 mmol) in H_2O (20 mL) was added to a solution of oxazole **22** (1.79 g, 6.99 mmol) in THF (20 mL) and the yellow solution was stirred 2 h at room temperature. The mixture was evaporated to dryness under reduced pressure and phosphate buffer (pH 3, 20 mL) was added. 1 N HCl (aq.) was added to reach pH 2 and the aqueous layer was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were treated with Na_2SO_4 and then evaporated to afford carboxylic acid **23** as a colorless solid (1.36 g, 5.63 mmol, 81%).

TLC: R_f = 0.29 (CH_2Cl_2/MeOH/HCO_2H, 9:1:0.5%).

^{1}H-NMR (dioxane-d_8, 300.19 MHz, 299.9 K): δ = 1.43 (s, 9 H), 4.39 (d, J = 5.9 Hz, 2 H), 6.49 (s, 1 H), 8.38 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR (dioxane-d_8, 75.48 MHz, 300.2 K): δ = 28.6, 38.3, 79.7, 134.4, 145.6, 156.3, 162.1, 163.6 ppm.

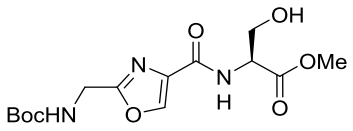
HPLC: t_R = 8.299 min (system A).

HRMS (ESI-TOF) calculated for $C_{10}H_{14}N_2O_5$ $[M+H]^+$ 243.0975; found 243.0978.

Melting point: Decomposition >156 °C

IR (ATR): $\tilde{\nu}$ = 3433 (m), 2920 (m), 2851 (w), 1713 (s), 1574 (m), 1497 (s), 1366 (m), 1308 (m), 1269 (m), 1238 (s), 1219 (m), 1161 (s), 1146 (s), 1111 (s), 910 (m), 860 (m), 741 (m) cm^{-1}.

3.6 Peptide 24



24

At 0 °C HBTU (2.72 g, 7.18 mmol) and EtN*i*Pr_2 (1.89 mL, 11.1 mmol) were added to a solution of carboxylic acid **23** (1.34 g, 5.52 mmol) in CH_2Cl_2/DMF (27 mL, 1:1 v/v). After 10 min, Ser-OMe·HCl (1.03 g, 6.63 mmol) was added and the solution was stirred at room temperature for 24 h. Phosphate buffer (pH 3, 30 mL) was added and the aqueous layer was extracted with ethyl acetate (5 × 50 mL). The combined organic extracts were treated with Na_2SO_4 and concentrated. Flash chromatography of the residue (150 g silica, petroleum ether:EtOAc = 1:5) afforded peptide **24** as a colorless oil (1.50 g, 4.36 mmol, 79%).

TLC: R_f = 0.31 (petroleum ether/EtOAc, 1:5).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.1 K): δ = 1.45 (s, 9 H), 3.05 (s, 1 H), 3.79 (s, 3 H), 4.04 (ddd, *J* = 24.7, 11.4, 3.7 Hz, 2 H), 4.42 (d, *J* = 5.6 Hz, 2 H), 4.80 (dt, *J* = 7.7, 3.7 Hz, 1 H), 5.55 (s, 1 H), 7.79 (d, *J* = 7.8 Hz, 1 H), 8.11 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 75.48 MHz, 298.2 K): δ = 28.4, 37.9, 52.8, 54.5, 63.0, 80.6, 135.6, 141.9, 155.7, 160.7, 161.6, 170.6 ppm.

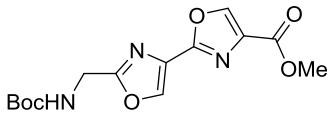
HPLC: t_R = 2.912 min (system B).

HRMS (ESI-TOF) calculated for $C_{14}H_{21}N_3O_7$ $[M+H]^+$ 344.1452; found 344.1455.

$[\alpha]_D$ = 20.5 (THF, c = 0.1, 25.5 °C).

IR (ATR): $\tilde{\nu}$ = 3321 (w), 2874 (w), 2936 (w), 1701 (m), 1659 (s), 1601 (s), 1512 (s), 1439 (m), 1366 (m), 1323 (m), 1277 (m), 1250 (s), 1211 (s),1161 (s), 1107 (s), 1076 (m), 1034 (m), 991 (m), 914 (m), 860 (m), 760 (m), 729 (m) cm^{-1}.

3.7 2-5´-bioxazole 25



25

At –65 °C DAST (614 µL, 4.65 mmol) was added to a solution of peptide **24** (1.45 g, 4.23 mmol) in CH_2Cl_2 (25 mL). After 30 min, K_2CO_3 (1.17 g, 8.46 mmol) was added and the mixture was stirred 2 h to reach –5 °C. Saturated $NaHCO_3$ solution (10 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 × 25 mL). The combined organic extracts were treated with Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (25 mL) and at –72 °C DBU (739 µL, 4.95 mmol) and $BrCCl_3$ (488 µL, 4.95 mmol) were added. The solution was stirred 24 h at –25 °C. All volatiles were evaporated. Flash chromatography of the residue (75 g silica, petroleum ether:acetone = 2:1 → 1:1) afforded bioxazole **25** as a slightly yellow solid (895 mg, 2.77 mmol, 65%).

TLC: R_f = 0.29 (petroleum ether/acetone, 2:1).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.0 K): δ = 1.45 (s, 9 H), 3.94 (s, 3 H), 4.53 (d, J = 5.8 Hz, 2 H), 5.23 (s, 1 H), 8.29 (s, 1 H), 8.32 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 100.63 MHz, 297.0 K): δ = 28.3, 38.0, 52.3, 80.4, 129.8, 134.4, 139.8, 143.8, 144.8, 155.6, 161.3, 162.8 ppm.

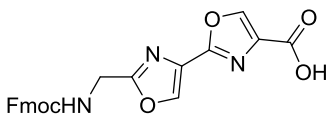
HPLC: t_R = 6.251 min (system B).

HRMS (ESI-TOF) calculated for $C_{14}H_{17}N_3O_6$ $[M+H]^+$ 324.1190; found 324.1194.

Melting point: T_m = 185 °C.

IR (ATR): $\tilde{\nu}$ = 3379 (w), 3144 (w), 2978 (w), 2932 (w), 1705 (s), 1647 (m), 1520 (m), 1451 (m), 1350 (m), 1335 (m), 1246 (m), 1165 (m), 1150 (m), 1088 (m), 1049 (m), 1030 (m), 999 (m), 961 (m), 922 (m), 872 (m), 802 (m), 783 (m), 733 (m) cm^{-1}.

3.8 Carboxylic acid 11



11

LiOH (79.6 mg, 3.32 mmol) in H_2O (14 mL) was added to a solution of bioxazole **25** (895 mg, 2.77 mmol) in THF (14 mL) and the yellow solution was stirred 2 h at room temperature. The mixture was evaporated to dryness under reduced pressure and phosphate buffer (pH 3, 10 mL) was added to the residue. 1 N HCl (aq.) was added to reach pH 2 and the aqueous layer was extracted with ethyl acetate (4 × 250 mL). The combined organic layers were treated with Na_2SO_4 and then evaporated under reduced pressure. The residue was stirred in TFA/H_2O (30 mL, 1:1 v/v) for 3 h. All volatiles were removed in vacuo and the residue was dissolved in MeCN/H_2O (50 mL, 3:2 v/v). At 0 °C Fmoc-OSu (1.03 g, 3.05 mmol) and Na_2CO_3 (880 mg, 8.30 mmol) were added and the mixture was stirred 14 h at room temperature. The mixture was evaporated under reduced pressure and phosphate buffer (pH 3, 30 mL) was added. After treatment with 1 N HCl to reach pH 2, the aqueous layer was extracted with ethyl acetate (4 × 100 mL). The combined organic layers were treated with Na_2SO_4 and evaporated under reduced pressure. Flash chromatography of the residue (100 g silica, CH_2Cl_2:MeOH:HCO_2H = 95:5:0.5%) afforded carboxylic acid **11** as a colorless solid (1.05 g, 2.44 mmol, 88%).

TLC: R_f = 0.38 (CH_2Cl_2/MeOH/HCO_2H, 95:5:0.5%).

^{1}H-NMR (DMSO-d_6, 300.19 MHz, 298.2 K): δ = 4.26 (d, J = 6.4 Hz, 1 H), 4.35 (d, J = 6.7 Hz, 2 H), 4.40 (d, J = 5.7 Hz, 2 H), 7.33 (t, J = 7.2 Hz, 2 H), 7.41 (t, J = 7.3 Hz, 2 H), 7.71 (d, J = 7.2 Hz, 2 H), 7.89 (d, J = 7.4 Hz, 2 H), 8.13 (m, 1 H), 8.82 (s, 1 H), 8.88 (s, 1 H), 13.30 (sb, 1 H) ppm.

^{13}C{^{1}H}-NMR (DMSO-d_6, 100.63 MHz, 298.2 K): δ = 46.6, 65.8, 79.2, 120.1, 125.2, 127.1, 127.7, 129.1, 134.5, 140.7, 143.8, 145.0, 153.8, 154.9, 156.3, 161.9, 163.0 ppm.

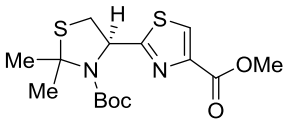
HPLC: t_R = 10.752 min (system A).

HRMS (ESI-TOF) calculated for $C_{23}H_{17}N_3O_6$ $[M+H]^+$ 454.1015; found 454.1011.

Melting point: Decomposition >229 °C.

IR (ATR): $\tilde{\nu}$ = 3426 (w), 3375 (w), 3136 (w), 1734 (m), 1686 (s), 1516 (m), 1435 (m), 1234 (m), 1173 (m), 1088 (m), 1049 (m), 984 (m), 957 (m), 910 (m), 756 (m), 733 (s) cm^{-1}.

3.9 Thiazole 14



14

At 0 °C methyl bromopyruvate (4.18 mL, 48.7 mmol) was added to a solution of thiocarboxamide **13** (4.49 g, 16.2 mmol) and $KHCO_3$ (13.8 g, 138 mmol) in THF (120 mL). The mixture was stirred 22 h at room temperature. Ethyl acetate (200 mL) was added and the organic layer was washed with water and saturated NaCl solution (each 100 mL). The combined aqueous layer was extracted with ethyl acetate (2 × 200 mL) and the combined organic extracts were treated with Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in THF/pyridine (68 mL; 3:1 v/v) and TFAA (6.77 mL, 48.7 mmol) was added at 0 °C. The solution was stirred for 2.5 h and then ethyl acetate (300 mL) was added. The organic layer was washed with 1 N HCl and saturated $NaHCO_3$ solution (100 mL each). The combined aqueous layer was extracted with ethyl acetate (2 × 200 mL) and the combined organic extracts were treated with Na_2SO_4 and evaporated under reduced pressure. Flash chromatography of the residue (300 g silica, petroleum ether:EtOAc = 2:1) afforded thiazole **14** as a slightly yellow solid (4.99 g, 13.9 mmol, 86%).

TLC: R_f = 0.51 (petroleum ether/EtOAc, 2:1).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.1 K): δ = 1.28 (s, 6.5 H), 1.50 (s, 2.5 H), 1.81 (s, 3 H), 1.98 (s, 3 H), 3.17 (m, 1 H), 3.53 (dd, J = 12.4, 6.5 Hz, 1 H), 3.94 (s, 3 H), 5.66 (m, 1 H), 8.11 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 100.63 MHz, 297.0 K): δ = 28.4, 28.9, 30.2, 34.0, 34.4, 52.6, 65.5, 66.3, 72.3, 81.3, 127.6, 146.5, 151.9, 162.0, 176.2 ppm.

HPLC: t_R = 11.093 min (system B).

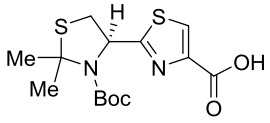
HRMS (ESI-TOF) calculated for $C_{15}H_{22}N_2O_4S_2$ $[M+H]^+$ 359.1094; found 359.1101.

Melting point: T_m = 122 °C.

$[\alpha]_D$ = –96.2 (THF, c = 0.1, 25.3 °C).

IR (ATR): $\tilde{\nu}$ = 2978 (w), 2936 (w), 1697 (s), 1485 (w), 1439 (w), 1346 (s), 1308 (m), 1234 (s), 1219 (s), 1165 (s), 1107 (m), 1069 (s), 984 (m), 918 (w), 860 (m), 826 (w), 768 (s), 625 (w) cm^{-1}.

3.10 Carboxylic acid 15



15

LiOH (142 mg, 5.92 mmol) was added to a solution of methyl ester **14** (1.93 g, 5.38 mmol) in THF/H_2O (20 mL, 1:1 v/v) and the solution was stirred 3 h. All volatile components were removed in vacuum and phosphate buffer (pH 3, 20 mL) was added. The aqueous layer was adjusted with 1 N HCl (aq.) to pH 2 and then extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were treated with Na_2SO_4 and then evaporated under reduced pressure to afford carboxylic acid **15** as a colorless solid (1.80 g, 5.24 mmol, 97%).

TLC: R_f = 0.40 (CH_2Cl_2/MeOH/FA, 95:5:0.5%).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.6 K): δ = 1.30 (s, 5.5 H), 1.51 (s, 3.5 H), 1.83 (s, 3 H), 1.98 (s, 3 H), 3.18 (m, 1 H), 3.56 (dd, J = 12.4, 6.5 Hz, 1 H), 5.70 (m, 1 H), 8.23 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 62.90 MHz, 296.9 K): δ = 28.4, 28.9, 34.3, 65.4, 66.0, 81.5, 117.6, 129.0, 145.8, 164.1, 176.5 ppm.

HPLC: t_R = 11.030 min (system A).

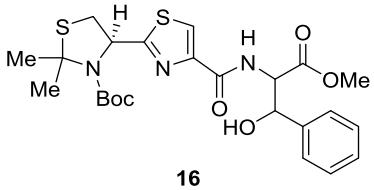
HRMS (ESI-TOF) calculated for $C_{14}H_{20}N_2O_4S_2$ $[M+H]^+$ 345.0937; found 345.0943.

Melting point: Decomposition >189 °C.

$[\alpha]_D$ = –103.1 (THF, c = 0.1, 25.3 °C).

IR (ATR): $\tilde{\nu}$ = 3105 (w), 2978 (w), 2932 (w), 1682 (s), 1484 (m), 1366 (m), 1339 (s), 1315 (m), 1223 (s), 1161 (s), 1069 (s), 903 (m), 833 (w), 772 (m), 737 (s), 621 (w) cm^{-1}.

3.11 Peptide 16



16

At 0 °C HBTU (1.32 g, 5.63 mmol) and Et*N*iPr_2 (1.20 mL, 8.66 mmol) were added to a solution of carboxylic acid **15** (1.49 g, 4.33 mmol) in CH_2Cl_2/DMF (43 mL, 1:1 v/v). 10 min later *β*-hydroxy phenylalanine methyl ester hydrochloride (1.30 g, 5.63 mmol) was added and the solution was stirred for 5 h at room temperature. Phosphate buffer (pH 3, 30 mL) was added and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 50 mL). The combined organic layer was treated with Na_2SO_4 and then evaporated under reduced pressure. Flash chromatography of the residue (150 g silica, petroleum ether:EtOAc = 1:1) afforded peptide **16** as yellow oil (mixture of stereoisomers, 1.89 g, 3.63 mmol, 84%).

TLC: R_f = 0.37 (petroleum ether/EtOAc, 1:1).

^{1}H-NMR ($CDCl_3$, 300.19 MHz, 298.0 K): δ = 1.30 (s, 5.5 H), 1.51 (s, 3.5 H), 1.83 (s, 3 H), 1.95 (s, 3 H), 3.17 (m, 1 H), 3.51 (dd, *J* = 12.1, 5.7 Hz, 1 H), 3.70-3.82 (m, 3 H), 4.23 (d, *J* = 5.3 Hz, 0.3 H), 5.01 (m, 0.7 H), 5.17 (dd, *J* = 7.5, 3.8 Hz, 0.2 H), 5.36 (m, 0.8 H), 5.59 (m, 1 H), 7.28 (m, 3 H), 7.37 (m, 2 H), 7.96 (m, 1 H), 8.02 (m, 1 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 100.63 MHz, 297.0 K): δ = 27.4, 28.3, 29.0, 29.8, 30.4, 34.1, 41.2, 52.5, 52.7, 52.76, 52.80, 58.4, 58.5, 59.0, 59.1, 62.3, 66.0, 70.9, 72.2, 73.9, 74.1, 75.0, 81.3, 81.9, 124.0, 124.1, 124.2, 124.6, 126.05, 126.09, 128.45, 128.52, 129.7, 139.2, 139.8, 148.5, 151.8, 154.7, 161.40, 161.43, 161.5, 170.2, 170.8, 170.9 ppm.

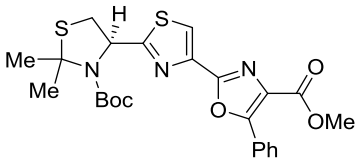
HPLC: t_R = 11.381 min (system B).

HRMS (ESI-TOF) calculated for $C_{24}H_{31}N_3O_6S_2$ $[M+H]^+$ 522.1727; found 522.1734.

$[\alpha]_D$ = –53.8 (THF, c = 0.1, 25.4 °C).

IR (ATR): $\tilde{\nu}$ = 3399 (w), 2974 (w), 2932 (w), 1736 (m), 1697 (m), 1543 (m), 1493 (m), 1342 (s), 1215 (m), 1165 (s), 1065 (m), 910 (m), 729 (s) cm^{-1}.

3.12 Biazole 17



17

At –78 °C DAST (773 µL, 5.85 mmol) was added to a solution of peptide **16** (2.54 g, 4.87 mmol) in THF (50 mL). The solution was stirred 3 h to reach –30 °C. After cooling to –78 °C, pyridine

(785 µL, 9.75 mmol) was added and the solution was stirred for 1 h. Saturated $NaHCO_3$ solution (50 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic extracts were treated with Na_2SO_4 and then evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and at –70 °C DBU (816 µL, 5.36 mmol) and $BrCCl_3$ (532 µL, 5.36 mmol) were added. The solution was stirred for 5 h to reach 0 °C. Acetone (10 mL) was added and all volatile components were removed in vacuum. Flash chromatography of the residue (150 g silica, petroleum ether:EtOAc = 2:1) afforded biazole **17** as a yellow oil (1.64 g, 3.26 mmol, 67%).

TLC: R_f = 0.34 (petroleum ether/EtOAc, 2:1).

^{1}H-NMR ($CDCl_3$, 400.21 MHz, 297.0 K): δ = 1.31 (s, 6 H), 1.52 (s, 3 H), 1.84 (s, 3 H), 1.97 (sb, 3 H), 3.32 (m, 1 H), 3.56 (dd, J = 12.3, 6.4 Hz, 1 H), 3.95 (s, 3 H), 5.72 (s, 0.65 H), 5.84 (s, 0.35 H), 7.50 (m, 3 H), 8.08 (s, 1 H), 8.13 (m, 2 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 100.63 MHz, 297.0 K): δ = 28.4, 28.9, 30.4, 33.9, 34.4, 52.5, 52.9, 65.5, 66.3, 72.3, 81.4, 121.3, 124.0, 124.8, 126.8, 127.9, 128.6, 128.8, 129.6, 129.9, 130.6, 134.0, 142.4, 151.9, 155.4, 155.6, 159.3, 162.7 ppm.

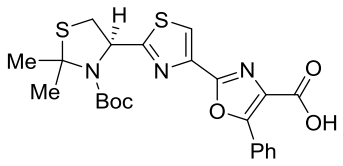
HPLC: t_R = 13.547 min (system B).

HRMS (ESI-TOF) calculated for $C_{24}H_{27}N_3O_5S_2$ $[M+H]^+$ 502.1465; found 502.1468.

$[\alpha]_D$ = –72.8 (THF, c = 0.1, 25.4 °C).

IR (ATR): $\tilde{\nu}$ = 2974 (w), 2932 (w), 1697 (m), 1589 (w), 1450 (w), 1435 (w), 1342 (s), 1281 (m), 1211 (m), 1165 (m), 1092 (m), 1069 (m), 910 (m), 845 (w), 768 (m), 725 (s), 691 (m), 644 (m) cm^{-1}.

3.13 Carboxylic acid 18



18

LiOH (81.1 mg, 3.38 mmol) was added to a solution of methyl ester **17** (849 mg, 1.69 mmol) in THF/H_2O (17 mL, 1:1 v/v). The solution was stirred 5 h at room temperature. The solvent was removed under reduced pressure and phosphate buffer (pH 3, 20 mL) and 1 N HCl (aq.) were

added to reach pH 2. The aqueous layer was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were treated with Na_2SO_4 and evaporated in vacuum to afford carboxylic acid **18** as a colorless foam (816 mg, 1.67 mmol, 99%).

TLC: R_f = 0.43 (CH_2Cl_2/MeOH/HCO_2H, 95:5:0.5%).

^{1}H-NMR (dioxane-d_8, 400.21 MHz, 300.0 K): δ = 1.39 (m, 9 H), 1.81 (s, 3 H), 1.95 (s, 3 H), 3.27 (m, 1 H), 3.58 (m, 1 H), 5.68 (m, 1 H), 7.47 (m, 3 H), 8.23 (m, 3 H) ppm.

^{13}C{^{1}H}-NMR (dioxane-d_8, 100.63 MHz, 300.1 K): δ = 28.5, 28.9, 34.4, 49.9, 72.8, 81.3, 121.9, 128.1, 129.0, 129.1, 129.3, 130.9, 135.5, 143.4, 152.4, 155.3, 155.8, 163.3 ppm.

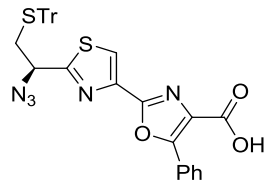
HPLC: t_R = 13.035 min (system A).

HRMS (ESI-TOF) calculated for $C_{23}H_{25}N_3O_5S_2$ $[M+Na]^+$ 510.1128; found 510.1140.

$[\alpha]_D$ = –55.0 (MeOH, c = 1.0, 22.5 °C).

IR (ATR): $\tilde{\nu}$ = 3102 (w), 2978 (w), 2932 (w), 1697 (s), 1589 (w), 1338 (s), 1165 (s), 1072 (m), 1003 (m), 849 (m), 787 (m), 625 (w) cm^{-1}.

3.14 Azide 9



9

Biazole **18** (249 mg, 511 µmol) was stirred in CH_2Cl_2/TFA (5 mL, 1:1 v/v) at room temperature for 14 h. Toluene (3 mL) was added and all volatile components were removed in vacuo. EtOH (3 mL) was added and the solution was concentrated in vacuo. The residue was dissolved in TFA (2.4 mL) and TrCl (149 mg, 536 µmol) was added. The solution was stirred 30 min at room temperature. CH_2Cl_2 (3 mL) was added and all volatile components were removed in vacuum to yield a yellow foam that was used in the subsequent diazo transfer without further purification. At 0 °C Tf_2O (258 µL, 1.53 mmol) was added to NaN_3 (199 mg, 3.06 mmol) in CH_2Cl_2/H_2O (2 mL, 1:1 v/v) and the mixture was stirred 2 h at 0 °C. Saturated $NaHCO_3$ solution (1.5 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (2 × 1.5 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (2 × 1.5 mL) and were then used in the

diazo transfer reaction.

At 0 °C $ZnSO_4$ (4.1 mg, 25.6 µmol) and NEt_3 (214 µL, 1.53 mmol) were added to a suspension of the Trityl protected amino acid in $MeOH/H_2O$ (8.2 mL, 5:1 v/v). Then, the freshly prepared solution of TfN_3 in CH_2Cl_2 (4 mL) was added and the solution was stirred 2 h at 0 °C. Phosphate buffer (pH 3, 10 mL) and 1 N HCl (aq.) were added to reach pH 2. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were treated with Na_2SO_4 and evaporated. Flash chromatography of the residue (25 g silica, petroleum ether:acetone:HCO_2H = 2:1:0.5%) afforded azide **9** as a colorless foam (208 mg, 338 µmol, 66%).[6]

TLC: R_f = 0.29 (petroleum ether/acetone/HCO_2H, 3:2:0.5%).

^{1}H-NMR ($CDCl_3$ 400.21 MHz, 297.0 K): δ = 2.87 (dd, *J* = 13.5, 7.4 Hz, 1 H), 3.00 (dd, *J* = 13.4, 5.8 Hz, 1 H), 4.20 (m, 1 H), 7.23 (t, *J* = 7.2 Hz, 3 H), 7.31 (t, *J* = 7.6 Hz, 6 H), 7.46 (m, 6 H), 7.51 (m, 3 H), 8.11 (s, 1 H), 8.25 (m, 2 H) ppm.

^{13}C{^{1}H}-NMR ($CDCl_3$, 100.63 MHz, 297.0 K): δ = 37.1, 61.6, 67.5, 122.5, 126.1, 126.99, 127.04, 128.2, 128.637, 128.644, 129.6, 131.0, 142.5, 144.2, 154.5, 155.7, 163.3, 169.7 ppm.

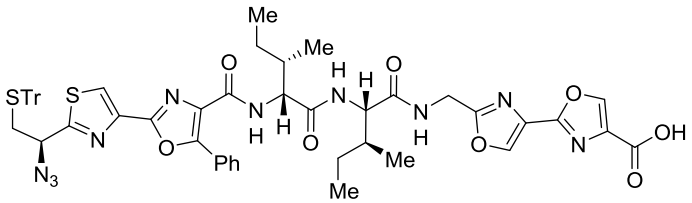
HPLC: t_R = 14.769 min (system A).

HRMS (ESI-TOF) calculated for $C_{34}H_{25}N_5O_3S_2$ $[M+Na]^+$ 638.1291; found 638.1291.

$[\alpha]_D$ = 7.44 (THF, c = 1.0, 21.3 °C).

IR (ATR): $\tilde{\nu}$ = 2959 (w), 2878 (w), 2110 (s), 1717 (m), 1593 (w), 1492 (m), 1446 (m), 1315 (m), 1292 (m), 1254 (m), 1211 (m), 1069 (m), 1034 (m), 1007 (m), 926 (m), 844 (m) cm^{-1}.

3.15 Polyazole peptide 8



8

2-chlorotrityl chloride resin (1.12 g, 673 µmol) was washed with CH_2Cl_2 (2 × 10 mL), swollen in CH_2Cl_2 (10 mL) and then shaken with carboxylic acid **11** (290 mg, 673 µmol) and $EtN(iPr)_2$ (576 µL, 3.36 mmol) in CH_2Cl_2/DMF (10 mL, 1:1 v/v) for 6 h. The resin was washed with CH_2Cl_2 (3 × 10 mL) and *tert*-butylmethylether (10 mL) and then treated with a solution of

CH_2Cl_2/MeOH/EtN*i*Pr_2 (10 mL, 17:2:1 v/v/v) for 1 h. The solvent was removed and the resin was washed with CH_2Cl_2 (3 × 10 mL) and MeOH (10 mL) and then dried in vacuum. The loading was determined to be 0.37 mmol/g by a test cleavage and measurement of the absorption of the formed piperidine-fulvene adduct.[7]

Loaded resin (255 mg, 97.3 µmol) was swollen in CH_2Cl_2 (3 mL) and the Fmoc group was cleaved (GP A). Fmoc-Ile-OH (133 mg, 377 µmol), HBTU (143 mg, 377 µmol), HOBt (56.7 mg, 377 µmol) and EtN*i*Pr_2 (129 µL, 754 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 2 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The Fmoc group was cleaved (GP A). Fmoc-D-*allo*-Ile (133 mg, 377 µmol), HBTU (143 mg, 377 µmol), HOBt (56.7 mg, 377 µmol) and EtN*i*Pr_2 (129 µL, 754 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 2 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The Fmoc group was cleaved (GP A). Biazole **9** (72.5 mg, 118 µmol), HBTU (44.7 mg, 118 µmol), HOBt (18.0 mg, 118 µmol) and EtN*i*Pr_2 (40.3 µL, 235 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 14 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The cleavage of the peptide was performed by shaking the resin with 1.1.1.6.6.6-hexafluoro isopropanol (HFIP, 30 vol% in CH_2Cl_2, each 3 mL; 3 × 30 min). After each treatment, the resin was extracted with CH_2Cl_2 (3 × 3 mL) and CH_2Cl_2/MeOH (2 × 3 mL, 1:1 v/v). Toluene (20 mL) was added to the combined organic eluents and all volatiles were removed in vacuo. Purification of the residue by flash chromatography (25 g silica, CH_2Cl_2/*i*PrOH/HCO_2H = 9:1:0.25%) afforded peptide **8** as a slightly yellow glass (68.5 mg, 68.5 µmol, 70%).

TLC: R_f = 0.29 (CH_2Cl_2/*i*PrOH/HCO_2H, 9:1:0.25%).

^{1}H-NMR (dioxane-d_8, 300.19 MHz, 298.0 K): δ = 0.92 (m, 12 H), 1.19 (m, 2 H), 1.56 (sb, 2 H), 1.95 (sb, 2 H), 2.94 (m, 2 H), 4.40 (m, 2 H), 4.52 (s, 2 H), 4.63 (m, 1 H), 7.20-7.31 (m, 10 H), 7.42 (m, 9 H), 7.79 (s, 1 H), 7.93 (d, *J* = 5.5 Hz, 1 H), 8.28 (s, 1 H), 8.34 (d, *J* = 5.8 Hz, 2 H), 8.43 (m, 2 H) ppm.

^{13}C{^{1}H}-NMR (dioxane-d_8, 100.63 MHz, 300.1 K): δ = 11.2, 12.1, 15.0, 16.1, 25.5, 27.2, 36.7, 37.3, 37.6, 39.3, 57.5, 57.9, 62.6, 68.1, 123.0, 127.7, 128.2, 128.9, 129.1, 129.2, 130.4, 130.7, 130.9, 131.2, 135.3, 140.9, 144.0, 145.3, 145.5, 152.9, 154.9, 156.4, 161.6, 162.1, 163.6, 170.2, 172.0, 172.1 ppm.

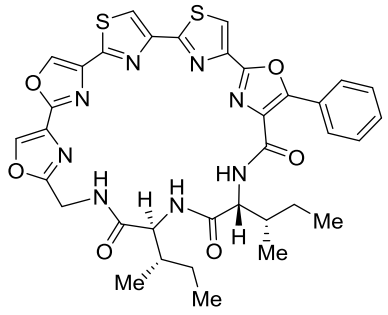
HPLC: t_R = 14.915 min (system B).

HRMS (ESI-TOF) calculated for $C_{54}H_{52}N_{10}O_8S_2$ $[M+H]^+$ 1033.3484; found 1033.3487.

$[\alpha]_D$ = –4.5 (THF, c = 0.1, 25.3 °C).

IR (ATR): $\tilde{\nu}$ = 3283 (w), 2967 (w), 2932 (w), 2878 (w), 2095 (m), 1717 (s), 1636 (m), 1508 (m), 1447 (m), 1373 (m), 1315 (m), 1215 (s), 1184 (s), 1103 (s), 1037 (s), 891 (m), 733 (s), 698 (s) cm^{-1}.

3.16 Aurantizolicin (1)



1

TFA (800 µL) and $EtSiH_3$ (1 mL) were added to a solution of carboxylic acid **8** (62.5 mg, 60.5 µmol) in CH_2Cl_2 (10 mL) and the solution was stirred 2 h at room temperature. Toluene (5 mL) was added and all volatile components were removed in vacuum to afford the *ω*-mercapto carboxylic acid. Solutions of PyBOP (37.8 mg, 72.6 µmol) and the *ω*-mercapto carboxylic acid in CH_2Cl_2/DMF (each 12.3 mL, 4.4:1 v/v) were given to a solution of EtN*i*Pr_2 (20.7 µL, 121 µmol) in CH_2Cl_2 (13.1 mL) over 4 h via a syringe pump. The solution was stirred 48 h at room temperature. Toluene (10 mL) was added and the solvents were removed in vacuum. Flash chromatography of the residue (20 g silica, CH_2Cl_2:*i*PrOH = 95:5) afforded the corresponding macrothiolactone as a colorless solid (37.3 mg, 80%) that was used in the next step without any characterization. PPh_3 (19.0 mg, 72.4 µmol) was added to a solution of macrothiolactone (37.3 mg, 48.3 µmol) in 2.6-lutidine (1 mL) and the yellow solution was stirred 7 h at 60 °C. The solvent was removed in vacuum under inert conditions and the residue was dissolved in CH_2Cl_2 (1.5 mL). At –70 °C DBU (10.8 µL, 72.4 µmol) and $BrCCl_3$ (7.1 µL, 72.4 µmol) were added and the solution was stirred 12 h at –25 °C. Flash chromatography of the reaction mixture (20 g silica, CH_2Cl_2:MeOH = 95:5) afforded aurantizolicin (**1**) as a colorless solid (16.9 mg, 23.3 µmol, 39% over 4 steps).

TLC: R_f = 0.42 (CH_2Cl_2/MeOH, 90:10).

^{1}H-NMR (DMSO-d_6, 500.30 MHz, 297.0 K): δ = 0.83 (t, *J* = 7.4 Hz, 3 H), 0.86-0.94 (m, 9 H), 1.05 (m, 1 H), 1.26 (m, 1 H), 1.51 (m, 1 H), 1.60 (m, 1 H), 1.85 (m, 1 H), 1.96 (m, 1 H), 4.15 (dd, *J* = 16.5, 1.5 Hz, 1 H), 4.39 (dd, *J* = 8.5, 6.5 Hz, 1 H), 4.81 (dd, *J* = 7.6, 4.0 Hz, 1 H), 5.03 (dd, *J* = 16.5, 9.0 Hz, 1 H), 7.52 (m, 1 H), 7.57 (m, 2 H), 8.12 (d, *J* = 7.7 Hz, 1 H), 8.36 (m, 2 H), 8.57 (d, *J* = 8.7 Hz, 1 H), 8.59 (s, 1 H), 8.69 (s, 1 H), 8.74 (dd, *J* = 9.0, 1.5 Hz, 1 H), 8.90 (s, 1 H), 9.08 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR (DMSO-d_6, 125.80 MHz, 297.0 K): δ = 10.9, 12.1, 14.7, 15.9, 24.1, 25.6, 35.2, 37.0, 39.1, 56.4, 57.1, 121.0, 123.0, 126.7, 127.7, 128.7, 129.4, 130.1, 130.6, 135.8, 139.5, 140.0, 141.9, 148.0, 150.7, 154.2, 155.3, 157.2, 160.2, 161.2, 162.9, 170.0, 171.0 ppm.

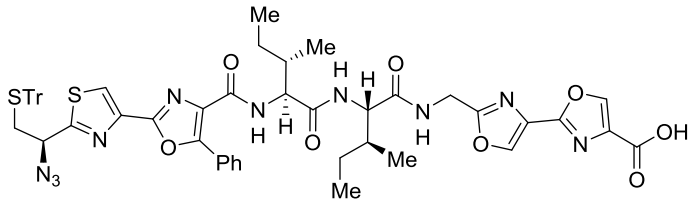
HPLC: t_R = 11.658 min (system B).

HRMS (ESI-TOF) calculated for $C_{35}H_{34}N_8O_6S_2$ $[M+H]^+$ 727.2115; found 727.2132.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}$ = 68.4 (MeCN, c = 0.1, 21.1 °C).

IR (ATR): $\tilde{\nu}$ = 2963 (m), 2932 (m), 1655 (s), 1504 (s), 1458 (m), 1427 (m), 1258 (w), 1103 (m), 1053 (m), 768 (m) cm^{-1}.

3.17 Peptide 28 – Gly-Ile-Ile



28

2-chlorotrityl chloride resin (897 mg, 1.44 mmol) was washed with CH_2Cl_2 (2 × 10 mL) and swollen in CH_2Cl_2 (10 mL) and then shaken with carboxylic acid **11** (232 mg, 538 µmol) and Et*i*Pr$_2$N (461 µL, 2.69 mmol) in CH_2Cl_2/DMF (10 mL, 1:1 v/v) for 6 h. The resin was washed with CH_2Cl_2 (3 × 10 mL) and *tert*-butylmethylether (10 mL) and then treated with a solution of CH_2Cl_2/MeOH/Et*i*Pr$_2$N (10 mL, 17:2:1 v/v/v) for 1 h. The solvent was removed and the resin was washed with CH_2Cl_2 (3 × 10 mL) and MeOH (10 mL) and then dried in vacuum. The loading was determined to be 0.35 mmol/g by a test cleavage and measurement of the absorption of the formed piperidine-fulvene adduct.

The loaded resin (282 mg, 98.6 µmol) was swollen in CH_2Cl_2 (3 mL) and the Fmoc group was cleaved (GP A). Fmoc-Ile-OH (139 mg, 395 µmol), HBTU (150 mg, 395 µmol), HOBt (60.0 mg, 395 µmol) and EtN*i*Pr$_2$ (135 µL, 789 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 2 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The Fmoc group was cleaved (GP A). Fmoc-Ile-OH (139 mg, 395 µmol), HBTU (150 mg, 395 µmol), HOBt (60.0 mg, 395 µmol) and EtN*i*Pr$_2$ (135 µL, 789 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 2 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The Fmoc group was cleaved (GP A). Biazole **9** (76.0 mg, 123 µmol), HBTU (46.8 mg, 123 µmol), HOBt (18.9 mg, 123 µmol) and EtN*i*Pr$_2$ (42.2 µL, 246 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 24 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The cleavage of the peptide was performed by shaking the resin with 1.1.1.6.6.6-hexafluoro isopropanol (HFIP, 30 vol% in CH_2Cl_2, each 3 mL; 3 × 30 min). After each treatment, the resin was extracted with CH_2Cl_2 (3 × 3 mL) and CH_2Cl_2/MeOH (2 × 3 mL, 1:1 v/v). Toluene (20 mL) was added to the combined organic eluents. The solution was evaporated in vacuum. Purification of the residue by flash chromatography (25 g silica, CH_2Cl_2/*i*PrOH/HCO_2H = 9:1:0.5%) afforded peptide **28** as a colorless solid (61.5 mg, 59.5 µmol, 60%).

TLC: R_f = 0.31 (CH_2Cl_2/*i*PrOH/HCO_2H, 9:1:0.5%).

^{1}H-NMR (dioxane-d_8, 400.21 MHz, 297.0 K): δ = 0.83 (t, *J* = 7.4 Hz, 3 H), 0.89 (m, 9 H), 1.16 (m, 2 H), 1.58 (m, 2 H), 1.83 (m, 1 H), 1.91 (m, 1 H), 2.88 (dd, *J* = 13.1, 7.1 Hz, 1 H), 2.97 (dd, *J* = 13.1, 5.7 Hz, 1 H), 4.27 (t, *J* = 8.9 Hz, 1 H), 4.47 (m, 3 H), 4.63 (dd, *J* = 16.4, 6.0 Hz, 1 H), 7.20 (t, *J* = 7.2 Hz, 3 H), 7.29 (t, *J* = 7.6 Hz, 6 H), 7.45 (m, 9 H), 7.61 (d, *J* = 9.1 Hz, 1 H), 7.76 (t, *J* = 5.6 Hz, 1 H), 7.88 (d, *J* = 9.1 Hz, 1 H), 8.27 (s, 1 H), 8.37 (m, 2 H), 8.48 (s, 1 H), 8.48 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR (dioxane-d_8, 100.61 MHz, 297.0 K): δ = 11.3, 11.4, 15.9, 25.6, 25.9, 26.0, 30.4, 36.7, 37.6, 38.4, 57.9, 62.6, 68.1, 122.9, 127.7, 128.9, 129.0, 129.2, 130.4, 130.6, 131.0, 131.3, 135.4, 140.9, 144.0, 145.3, 145.5, 152.8, 154.9, 156.3, 161.3, 162.0, 163.6, 170.1, 171.9, 172.1, 172.2 ppm.

HPLC: t_R = 14.595 min (system B).

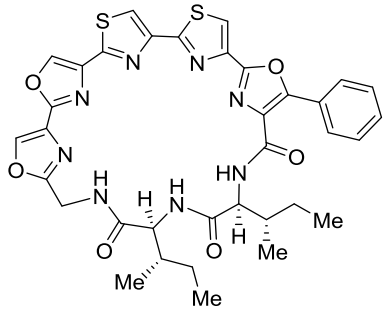
HRMS (ESI-TOF) calculated for $C_{54}H_{52}N_{10}O_8S_2$ $[M+H]^+$ 1033.3484; found 1033.3484.

Melting point: Decomposition >118 °C.

$[\alpha]_D$ = –31.7 (THF, c = 0.1, 21.4 °C).

IR (ATR): $\tilde{\nu}$ = 3275 (w), 2963 (w), 2936 (w), 2878 (w), 2110 (m), 1721 (m), 1639 (m), 1493 (m), 1447 (m), 1427 (m), 1377 (w), 1215 (w), 1069 (m), 1034 (m), 745 (m), 702 (m) cm^{-1}.

3.18 Polyazole cyclopeptide 26 – Gly-Ile-Ile



26

TFA (800 µL) and $EtSiH_3$ (1 mL) were added to a solution of carboxylic acid **28** (38.2 mg, 32.3 µmol) in CH_2Cl_2 (10 mL) and the solution was stirred 2 h at room temperature. Toluene (5 mL) was added and all volatile components were removed in vacuum to afford the *ω*-mercapto carboxylic acid. Solutions of PyBOP (20.2 mg, 38.8 µmol) and the *ω*-mercapto carboxylic acid in CH_2Cl_2/DMF (each 6.5 mL, 4.4:1 v/v) were given to a solution of Et*i*Pr_2 (11.1 µL, 64.6 µmol) in CH_2Cl_2 (7.0 mL) over 4 h via a syringe pump. The solution was stirred 38 h at room temperature. Toluene (5 mL) was added and the solvents were removed in vacuum. Flash chromatography of the residue (20 g silica, CH_2Cl_2:*i*PrOH = 95:5) afforded the corresponding macrothiolactone as a colorless solid (13.5 mg, 45%) that was used in the next step without any characterization. PPh_3 (6.9 mg, 26.2 µmol) was added to a solution of macrothiolactone (13.5 mg, 17.5 µmol) in 2.6-lutidine (1 mL) and the yellow solution was stirred 7 h at 60 °C. The solvent was removed in vacuum under inert conditions and the residue was dissolved in CH_2Cl_2 (1 mL). At –70 °C DBU (3.9 µL, 26.2 µmol) and $BrCCl_3$ (2.6 µL, 26.2 µmol) were added and the solution was stirred 12 h at –25 °C. Flash chromatography of the reaction mixture (20 g silica, CH_2Cl_2:MeOH = 95:5) afforded cyclopeptide **26** as a slightly yellow solid (8.5 mg, 11.7 µmol, 36% over 4 steps).

TLC: R_f = 0.17 (CH_2Cl_2/MeOH, 90:10).

^{1}H-NMR (DMSO-d_6, 400.21 MHz, 297.0 K): δ = 0.85 (m, 6 H), 0.92 (d, *J* = 6.8 Hz, 3 H), 1.00 (d, *J* = 6.7 Hz, 3 H), 1.09 (m, 1 H), 1.26 (m, 1 H), 1.55 (m, 2 H), 1.81 (m, 1 H), 1.92 (m, 1 H), 4.09 (t, *J* = 6.0 Hz, 1 H), 4.15 (d, *J* = 15.7 Hz, 1 H), 4.63 (dd, *J* = 8.6, 6.0 Hz, 1 H), 5.08 (dd, *J* = 17.4, 8.8 Hz, 1 H), 7.55 (m, 3 H), 8.08 (d, *J* = 5.1 Hz, 1 H), 8.39 (d, *J* = 7.4 Hz, 1 H), 8.45 (m, 2 H), 8.48 (d, *J* = 8.7 Hz, 1 H), 8.62 (s, 1 H), 8.71 (s, 1 H), 8.92 (s, 1 H), 9.10 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR (DMSO-d_6, 100.63 MHz, 297.0 K): δ = 11.2, 11.5, 15.4, 15.6, 240., 24.6, 35.55, 35.63, 56.0, 58.8, 121.3, 123.0, 126.7, 128.0, 128.5, 129.2, 130.1, 130.2, 135.8, 139.6, 140.3, 141.8, 148.0, 151.2, 153.9, 155.4, 157.4, 159.7, 161.4, 162.9, 170.35, 170.36 ppm.

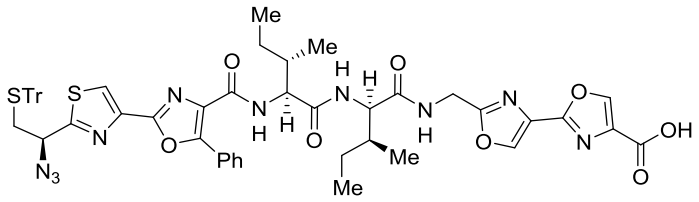
HPLC: t_R = 11.123 min (system B).

HRMS (ESI-TOF) calculated for $C_{35}H_{34}N_8O_6S_2$ $[M+Na]^+$ 749.1935; found 749.1934.

$[\alpha]_D$ = 9.80 (MeCN, c = 0.1, 21.1 °C).

IR (ATR): $\tilde{\nu}$ = 3271 (m), 2967 (w), 2928 (w), 1627 (s), 1508 (m), 1458 (m), 1196 (m), 1142 (m), 1115 (m), 1057 (w) cm^{-1}.

3.19 Peptide 29 – Gly-D-*allo*-Ile-Ile



29

The loaded resin (0.35 mmol/g, 278 mg, 97.4 µmol) was swollen in CH_2Cl_2 (3 mL), the Fmoc group was cleaved (GP A). Fmoc-D-*allo*-Ile-OH (138 mg, 389 µmol), HBTU (148 mg, 389 µmol), HOBt (59.6 mg, 389 µmol) and EtN*i*Pr$_2$ (133 µL, 778 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 2 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The Fmoc group was cleaved (GP A). Fmoc-Ile-OH (138 mg, 389 µmol), HBTU (148 mg, 389 µmol), HOBt (59.6 mg, 389 µmol) and EtN*i*Pr$_2$ (133 µL, 778 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 2 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The Fmoc group was cleaved (GP A). Biazole **9** (75.0 mg, 122 µmol), HBTU (46.1 mg, 122 µmol), HOBt (18.6 mg, 122 µmol) and EtN*i*Pr$_2$ (41.7 µL, 244 µmol) were dissolved in CH_2Cl_2/DMF (3 mL, 1:1 v/v), added to the swollen resin and shaken for 24 h (GP 2). The resin was washed with CH_2Cl_2 (3 × 3 mL).

The cleavage of the peptide was performed by shaking the resin with 1.1.1.6.6.6-hexafluoro isopropanol (HFIP, 30 vol% in CH_2Cl_2, each 3 mL; 3 × 30 min). After each treatment, the resin was extracted with CH_2Cl_2 (3 × 3 mL) and CH_2Cl_2/MeOH (2 × 3 mL, 1:1 v/v). Toluene (20 mL) was added to the combined organic solution. The solution was evaporated in vacuum. Purification of the residue by flash chromatography (25 g silica, CH_2Cl_2/*i*PrOH/HCO_2H = 9:1:0.5%) afforded peptide **29** as a slightly yellow glass (60.8 mg, 58.8 µmol, 60%).

TLC: R_f = 0.55 (CH_2Cl_2/*i*PrOH/HCO_2H, 9:1:0.5%).

^{1}H-NMR (dioxane-d_8, 400.21 MHz, 296.9 K): δ = 0.93 (m, 9 H), 1.00 (d, *J* = 6.7 Hz, 3 H), 1.20 (m, 2 H), 1.43 (m, 1 H), 1.66 (m, 1 H), 2.02 (m, 2 H), 2.87 (dd, *J* = 13.1, 7.0 Hz, 1 H), 2.96 (dd, *J* = 13.1, 5.7 Hz, 1 H), 4.32 (dd, *J* = 16.5, 5.8 Hz, 1 H), 4.50 (m, 4 H), 7.20 (t, *J* = 7.2 Hz, 3 H), 7.29 (t, *J* = 7.6 Hz, 6 H), 7.41 (m, 9 H), 7.84 (t, *J* = 5.7 Hz, 1 H), 8.02 (d, *J* = 8.0 Hz, 1 H), 8.25 (s, 1 H), 8.30 (m, 2 H), 8.39 (s, 1 H), 8.43 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR (dioxane-d_8, 100.63 MHz, 297.0 K): δ = 11.4, 12.1, 15.2, 16.2, 25.9, 27.3, 36.7, 37.5, 37.6, 37.9, 56.7, 58.9, 62.5, 68.1, 123.0, 127.7, 128.1, 128.9, 129.0, 129.2, 130.4, 130.7, 131.1, 135.3, 140.8, 143.9, 145.3, 145.5, 153.0, 155.0, 156.4, 162.0, 162.1, 163.7, 170.3, 172.0, 172.3 ppm.

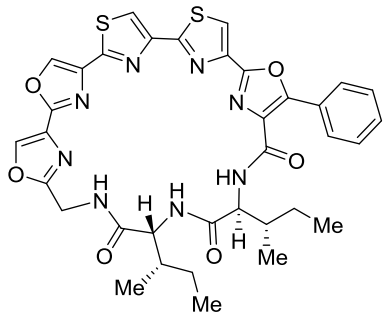
HPLC: t_R = 14.837 min (system B).

HRMS (ESI-TOF) calculated for $C_{54}H_{52}N_{10}O_8S_2$ $[M+H]^+$ 1033.3484; found 1033.3461.

$[\alpha]_D$ = 33.7 (THF, c = 0.2, 21.4 °C).

IR (ATR): $\tilde{\nu}$ = 2959 (m), 2924 (m), 2870 (w), 2114 (m), 1721 (m), 1655 (m), 1493 (m), 1446 (s), 1427 (s), 1377 (m), 1215 (m), 1096 (m), 1034 (m), 748 (w) cm^{-1}.

3.20 Cyclopeptide 27 – Gly-D-*allo*-Ile-Ile



27

TFA (800 µL) and $EtSiH_3$ (1 mL) were added to a solution of carboxylic acid **29** (48.8 mg, 47.2 µmol) in CH_2Cl_2 (10 mL) and the solution was stirred 2 h at room temperature. Toluene (5 mL) was added and all volatile components were removed in vacuum to afford the *ω*-mercapto carboxylic acid. Solutions of PyBOP (29.5 mg, 56.7 µmol) and the *ω*-mercapto carboxylic acid in CH_2Cl_2/DMF (each 9.6 mL, 4.4:1 v/v) were given to a solution of $EtN(iPr)_2$ (16.2 µL, 94.5 µmol) in CH_2Cl_2 (10.2 mL) over 4 h via a syringe pump. The solution was stirred 24 h at room temperature. Toluene (5 mL) was added and the solvents were removed in vacuum. Flash chromatography of the residue (20 g silica, CH_2Cl_2:*i*PrOH = 95:5) afforded the corresponding macrothiolactone as a colorless solid (21.7 mg, 59%) that was used in the next step without any characterization. PPh_3 (11.1 mg, 42.1 µmol) was added to a solution of macrothiolactone (21.7 mg, 28.1 µmol) in 2.6-lutidine (1.5 mL) and the yellow solution was stirred 7 h at 60 °C. The solvent was removed in vacuum under inert conditions and the residue was dissolved in CH_2Cl_2 (1.5 mL). At –70 °C DBU (6.3 µL, 42.1 µmol) and $BrCCl_3$ (4.2 µL, 42.1 µmol) were added and the solution was stirred 14 h at –25 °C. Flash chromatography of the reaction mixture (20 g silica, CH_2Cl_2:MeOH = 95:5) afforded cyclopeptide **27** as a slightly yellow solid (10.7 mg, 14.7 µmol, 31% over 4 steps).

TLC: R_f = 0.42 (CH_2Cl_2/MeOH, 90:10).

^{1}H-NMR (DMSO-d_6, 400.21 MHz, 297.0 K): δ = 0.88 (m, 12 H), 1.08 (m, 1 H), 1.25 (m, 3 H), 1.61 (m, 1 H), 1.90 (m, 1 H), 2.00 (m, 1 H), 4.16 (d, *J* = 14.3 Hz, 1 H), 4.63 (dd, *J* = 9.0, 4.0 Hz, 1 H), 4.81 (dd, *J* = 7.3, 4.4 Hz, 1 H), 5.03 (dd, *J* = 16.5, 9.0 Hz, 1 H), 7.52 (m, 1 H), 7.57 (t, *J* = 7.3 Hz, 2 H), 8.19 (d, *J* = 7.4 Hz, 1 H), 8.38 (m, 2 H), 8.43 (d, *J* = 9.1 Hz, 1 H), 8.59 (s, 1 H), 8.69 (s, 1 H), 8.71 (d, *J* = 9.0 Hz, 1 H), 8.90 (s, 1 H), 9.07 (s, 1 H) ppm.

^{13}C{^{1}H}-NMR (DMSO-d_6, 100.63 MHz, 297.0 K): δ = 11.7, 11.9, 14.9, 15.1, 24.8, 26.4, 35.1, 37.5, 38.5, 55.0, 56.9, 121.0, 123.0, 126.7, 127.7, 128.6, 129.4, 130.1, 130.6, 135.8, 139.5, 139.9, 141.9, 148.0, 150.8, 154.1, 155.3, 157.2, 160.0, 161.2, 163.1, 170.0, 171.1 ppm.

HPLC: t_R = 11.598 min (system B).

HRMS (ESI-TOF) calculated for $C_{35}H_{34}N_8O_6S_2$ $[M+H]^+$ 727.2115; found 727.2115.

$[\alpha]_D$ = –55.8 (MeCN, c = 0.1, 21.1 °C).

IR (ATR): $\tilde{\nu}$ = 3387 (w), 3279 (w), 2963 (w), 2928 (w), 1654 (s), 1508 (m), 1458 (m), 1257 (w), 1107 (m), 1053 (w), 826 (w) cm^{-1}.

3.21 Chromatographic comparison of cyclopeptides 1, 26 and 27

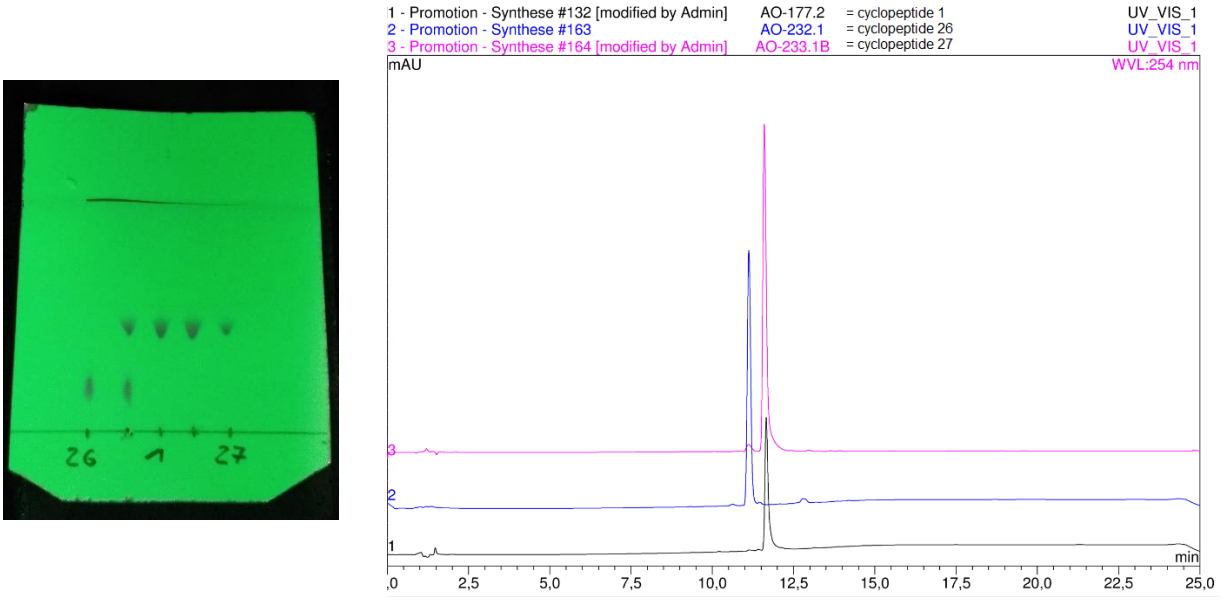


Figure S2. TLC plate using CH_2Cl_2/MeOH 9:1 as eluant (left) under UV irradiation (254 nM) and HPLC traces using system B (right) of cyclopeptides **1**, **26** and **27**.

4 Cultivation of *S. aurantiacus* JA 4570 and Detection of Aurantizolicin

Liquid Chromatography Coupled with Mass Spectrometric Detection (LC-MS)

LC-MS analyses of isaolated materials were performed on a SHIMADZU LCMS-2020 system equipped with single quadrupole mass spectrometer using a Kinetex C18 column (50 x 2.1 mm, particle size 1.7 µm, pore diameter 100 Å, PHENOMENEX). Column oven was set to 40 °C; scan range of MS was set to m/z 150 to 2,000 with a scan speed of 10,000 u/s and event time of 0.25 s under positive and negative mode. Desolvation line temperature was set to 250 °C with an interface temperature of 350 °C and a heat block temperature of 400 °C. The nebulizing gas flow was set to 1.5 L/min and dry gas flow to 15 L/min. Method: flow rate = 0.7 mL/min; 0 – 0.5 min: 10% (v/v) MeCN in water containing 0.1% formic acid; 0.5 – 8.5 min: linear gradient 10 – 100% MeCN in water containing 0.1% formic acid; 8.5 – 11.5 min: 100% MeCN containing 0.1% formic acid.

Analytical High-Performance Liquid Chromatography

Analytical HPLC analyses were performed on a Shimadzu HPLC system (LC-20AD, SPD-M20A) using a Luna® Phenyl-Hexyl column (250 × 4.6 mm, 5 µm, 100 Å, PHENOMENEX). An isocratic flow of 50% (v/v) MeCN in water containing 0.1% formic acid was used as mobile phase.

Cultivation and Natural Product Extraction

Streptomyces aurantiacus JA 4570 was obtained from the Hans Knöll Institute (IMET 43917). It was cultivated on Difco™ ISP medium 4 (BD, Sparks, USA) at 28 °C. After 14 days, the agar was extracted twice with acetone, filtered, and the organic solvent removed in vacuo. The residue was dissolved in a small amount of MeOH, loaded on an HM-N column (BIOTAGE, Hengoed, UK) and fractionated using a BIOTAGE flash purification system equipped with a SNAP C18 cartridge (30 g, BIOTAGE; flow rate 30 mL/min, method: 0 – 18 min: linear gradient 10 – 100% (v/v) MeOH in water; 18 – 25 min 100% MeOH). Aurantizolicin containing fractions were obtained between 17.5 – 22.5 min, as verified by LC-MS.

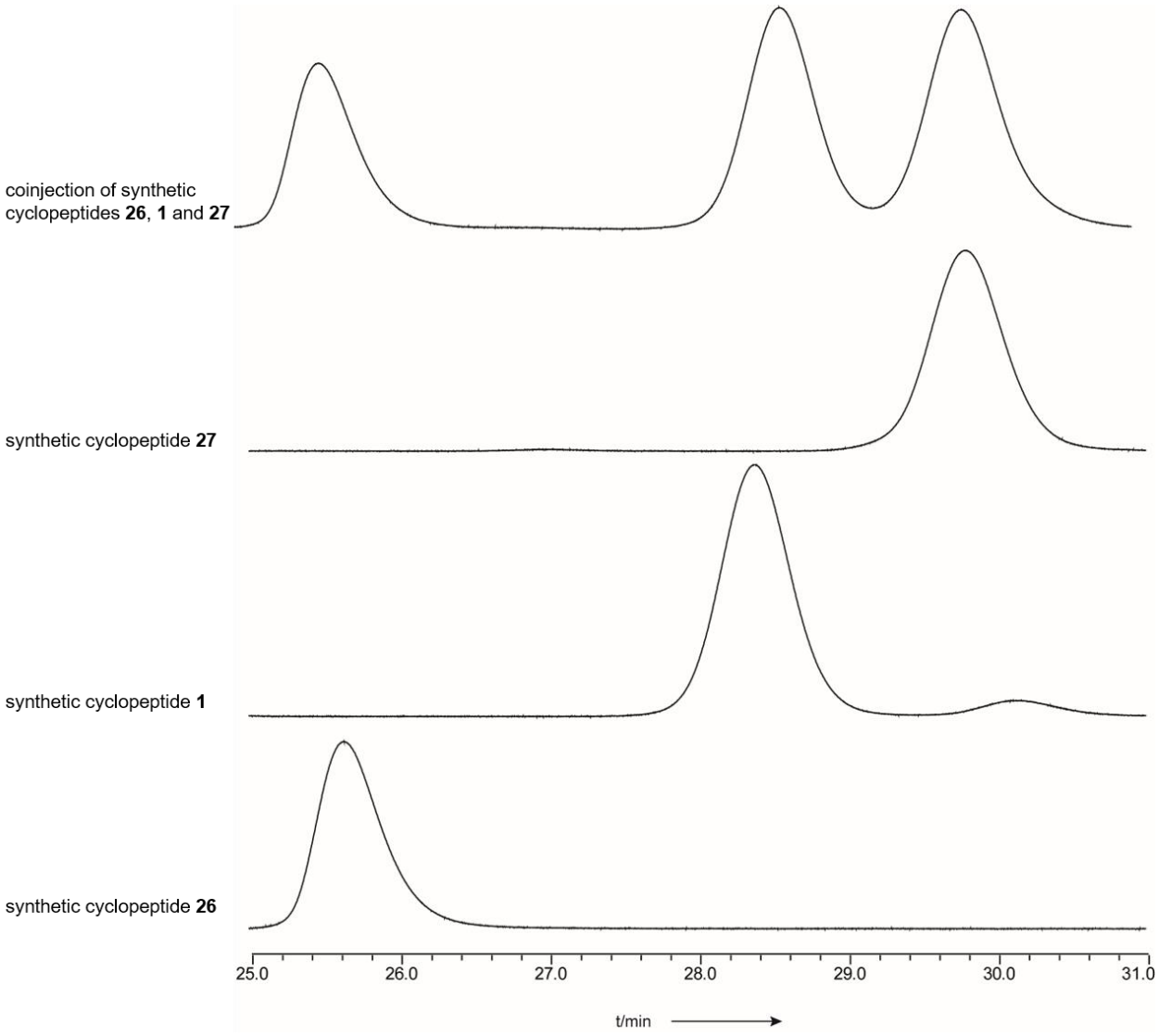


Figure S3. HPLC-traces (absorbance at 300 nm) of synthetic cyclopeptides **1**, **26**, **27** and the coinjection of all three peptides.

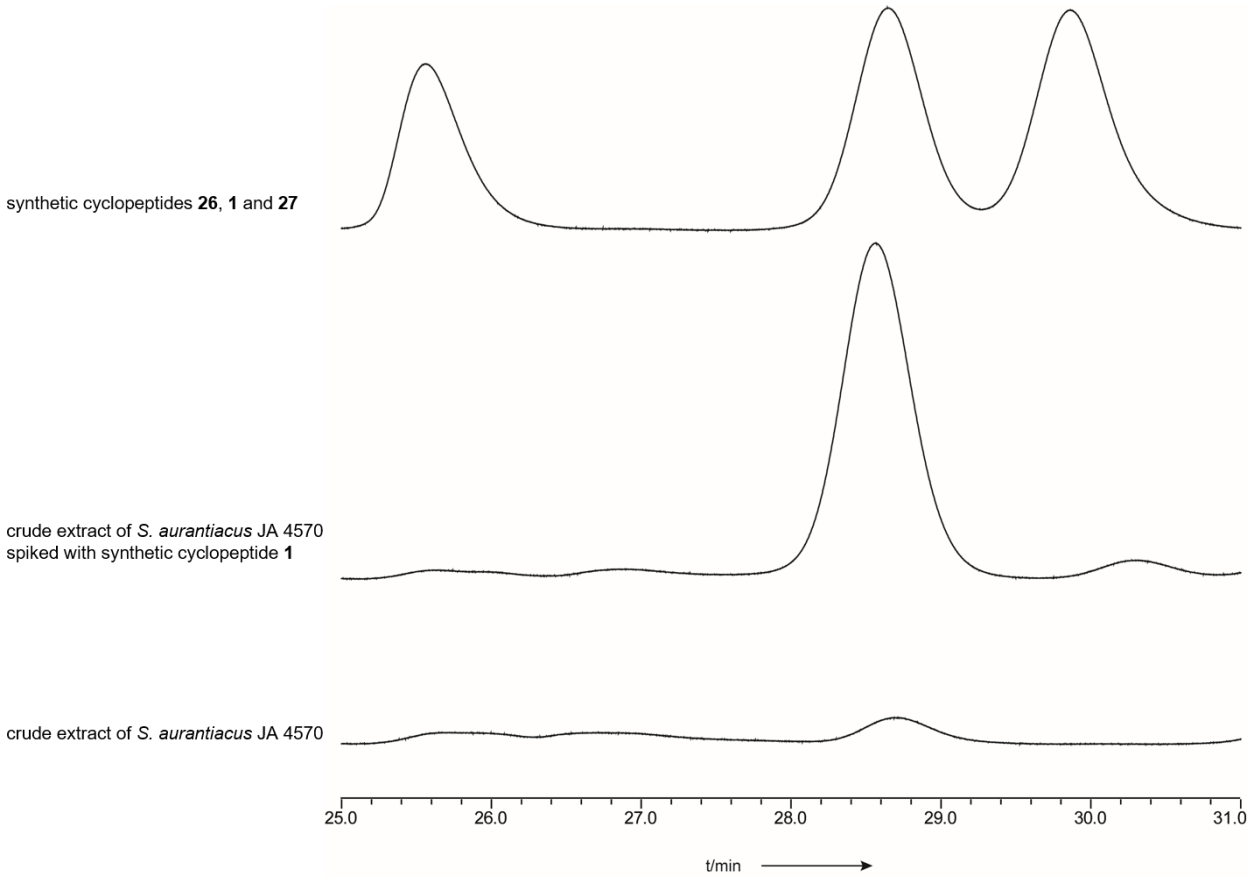


Figure S4. HPLC-traces (absorbance at 300 nm) of the crude extract of *S. aurantiacus* JA 4570, the crude extract spiked with synthetic peptide **1** and the synthetic cyclopeptides **1**, **26** and **27**.

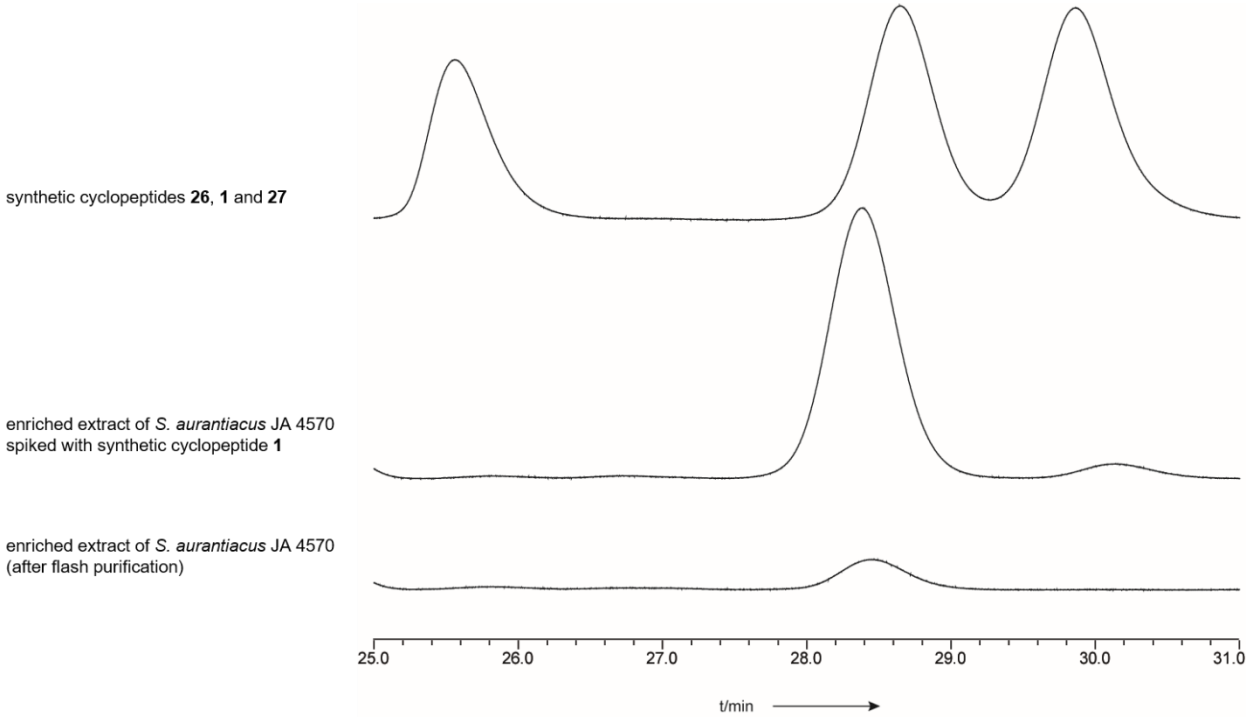


Figure S5. HPLC-traces (absorbance at 300 nm) of the enriched extract of *S. aurantiacus* JA 4570 after flash purification, the enriched extract spiked with synthetic peptide **1** and the synthetic cyclopeptides **1**, **26** and **27**.

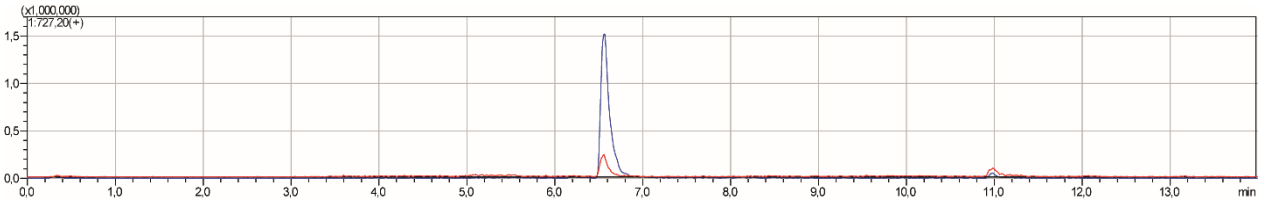


Figure S6. Extracted-ion chromatogram (*m/z* = 727.2) of the crude extract of *S. aurantiacus* JA 4570 (red) and the crude extract spiked with synthetic peptide **1** (blue).

4.1 Validation of HPLC analyses

HPLC experiments were repeated on a different system in order to reproduce the previous findings. Validation was performed by using a LC-coupled *MAXIS Impact ESI-TOF* spectrometer (BRUKER DALTRONICS, Bremen, Germany) using a Gemini column (150 × 2 mm, 3 µm, 110 Å, PHENOMENEX). An isocratic flow (0.5 mL/min) of 53% (v/v) MeCN in water containing 0.1% formic acid was used as the mobile phase.

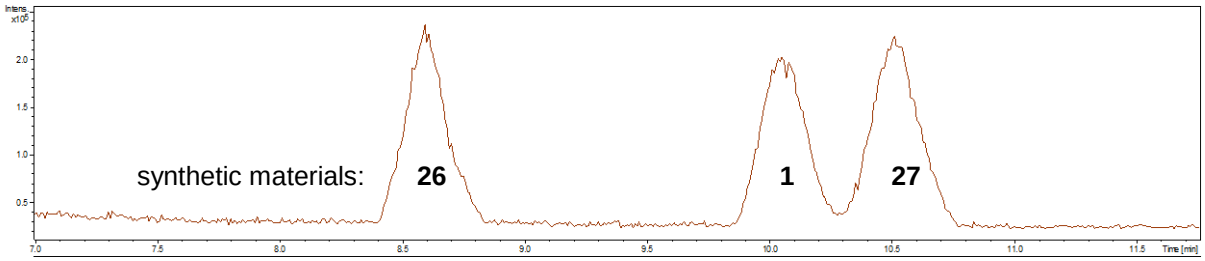


Figure S7. Extracted-ion chromatogram (*m/z* = 727.2) of the coinjected synthetic cyclopeptides **26**, **1** and **27**.

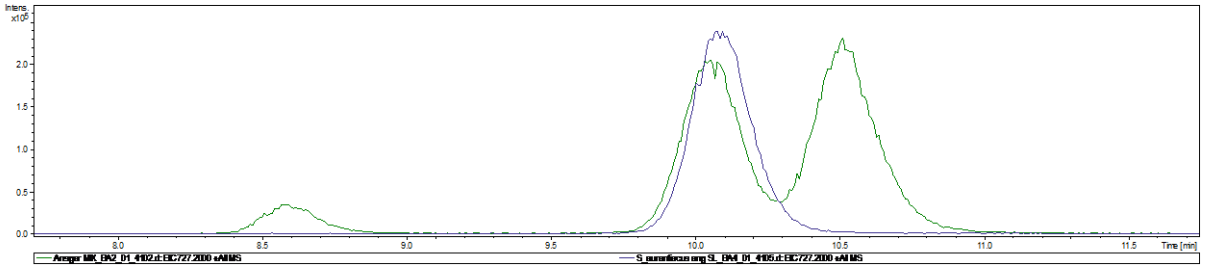


Figure S8. Extracted-ion chromatograms (*m/z* = 727.2) of the enriched extract of *S. aurantiacus* JA 4570 (blue) and the coinjected synthetic cyclopeptides **26**, **1** and **27** (green).

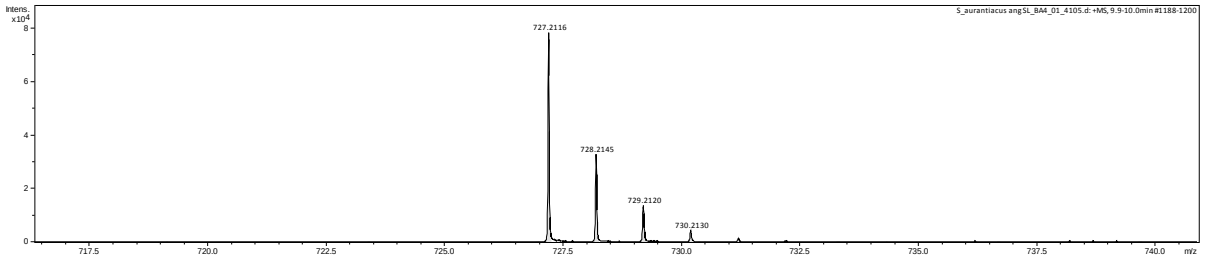
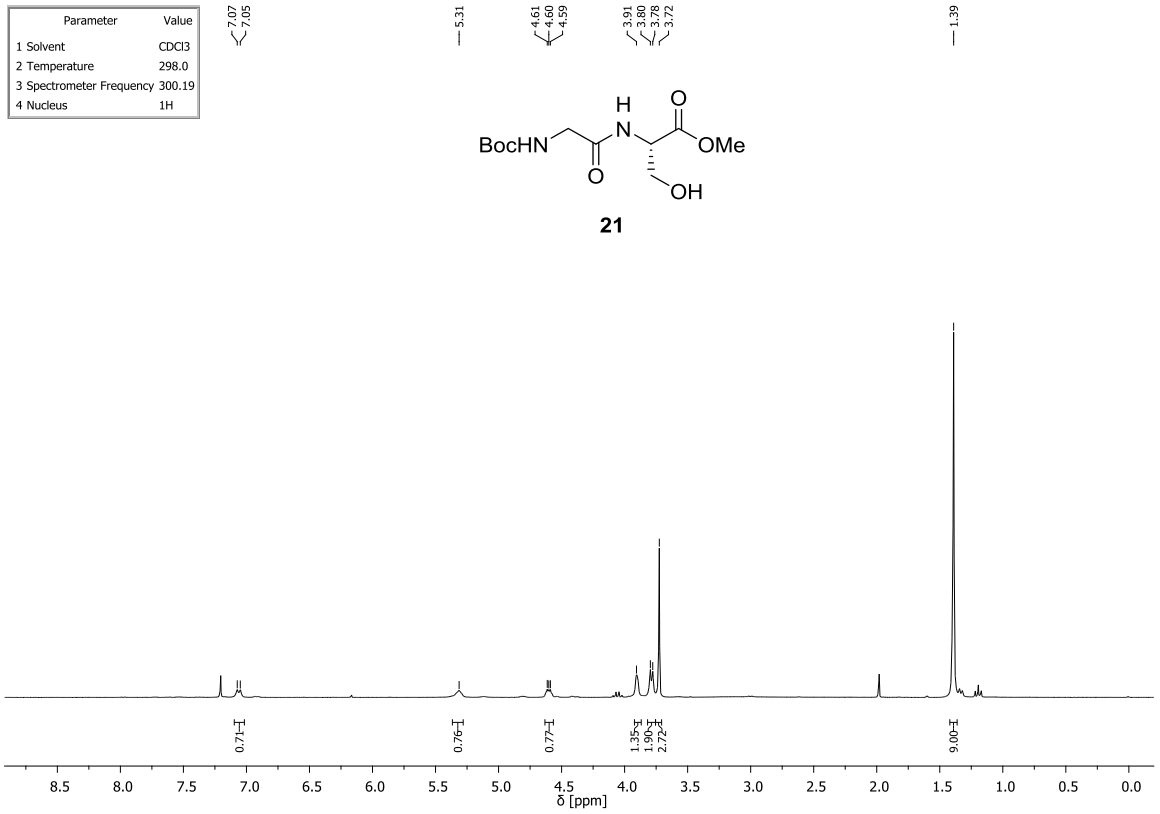


Figure S9. Corresponding high-resolution mass spectrum of the peak at 10 min. Calculated for $[M+H]^+$ 727.2115.

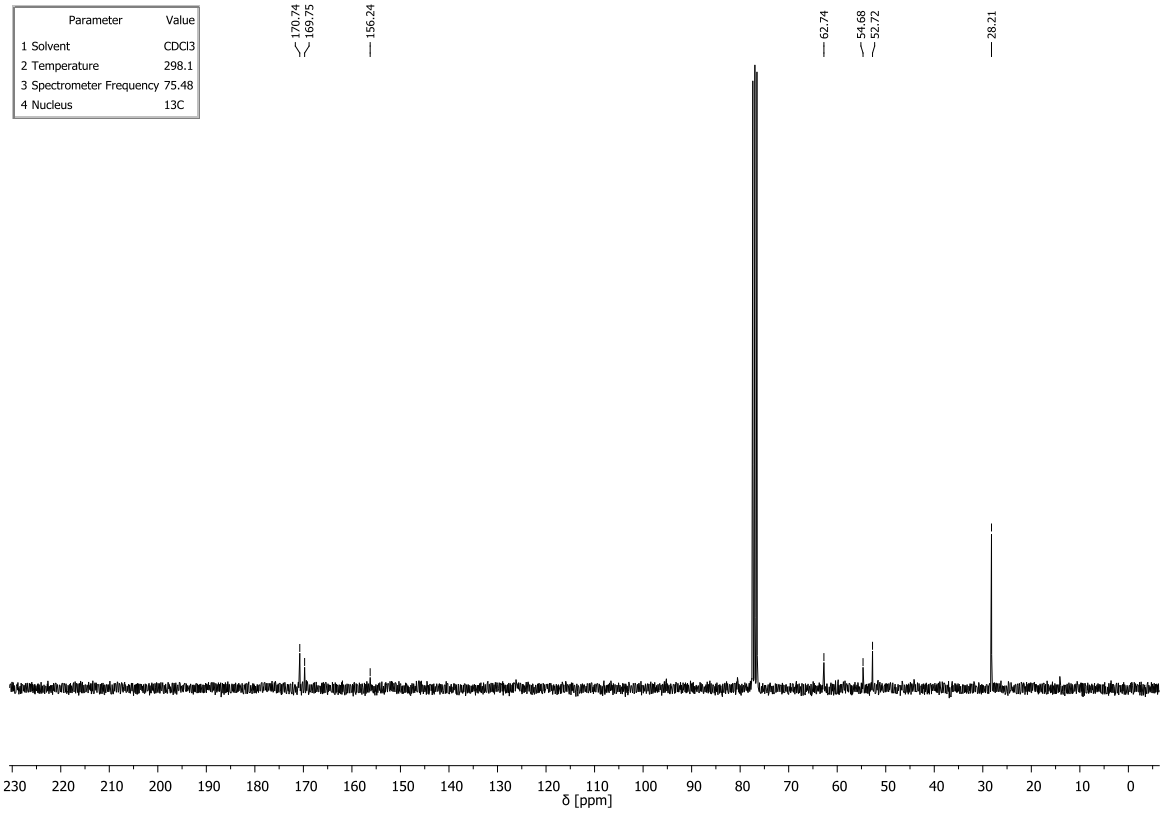
5 NMR spectra

Parameter	Value
1 Solvent	CDCl3
2 Temperature	298.0
3 Spectrometer Frequency	300.19
4 Nucleus	1H

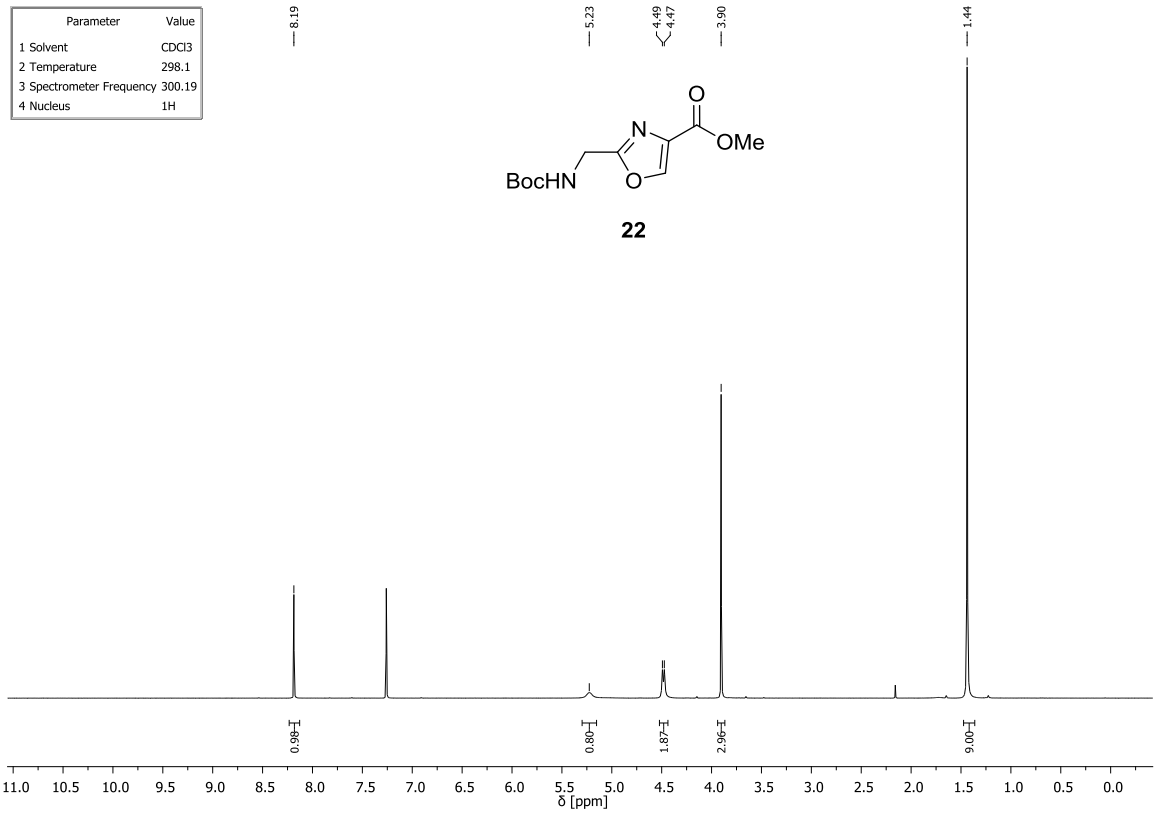


21

Parameter	Value
1 Solvent	CDCl3
2 Temperature	298.1
3 Spectrometer Frequency	75.48
4 Nucleus	13C

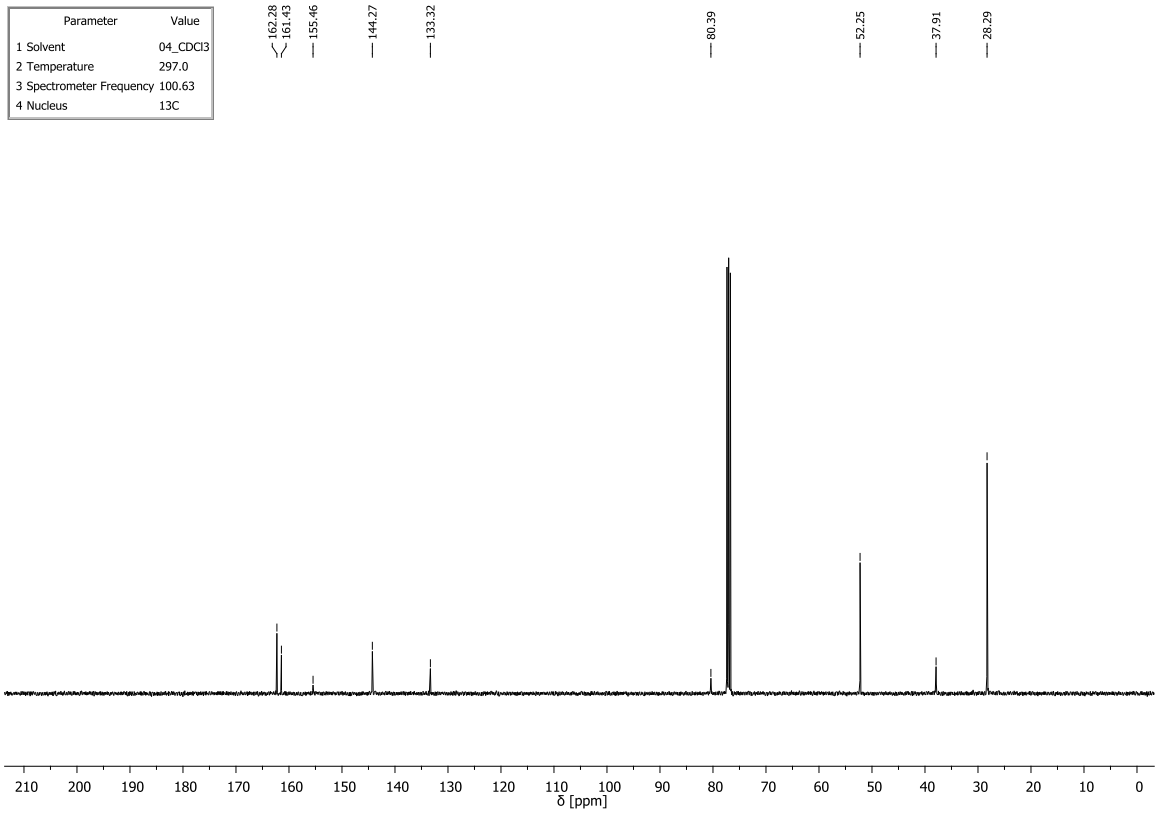


Parameter	Value
1 Solvent	CDCl3
2 Temperature	298.1
3 Spectrometer Frequency	300.19
4 Nucleus	1H

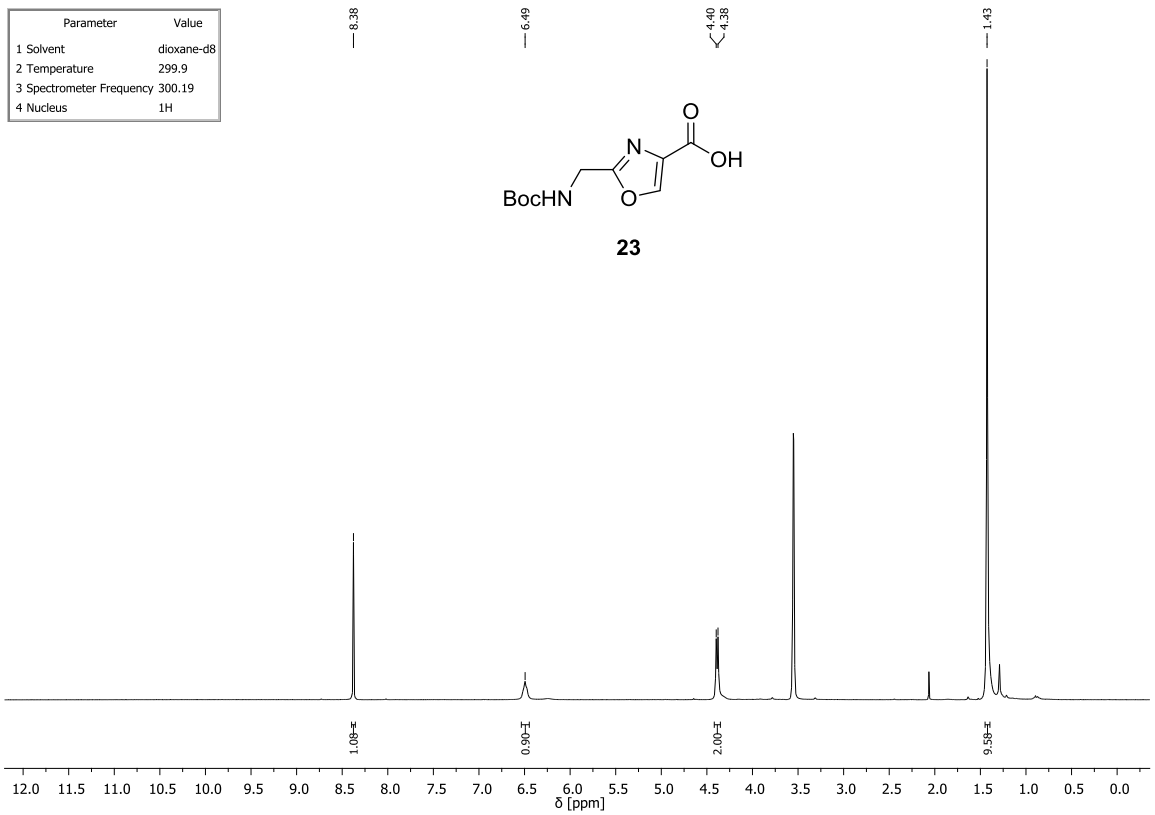


22

Parameter	Value
1 Solvent	04_CDCl3
2 Temperature	297.0
3 Spectrometer Frequency	100.63
4 Nucleus	13C

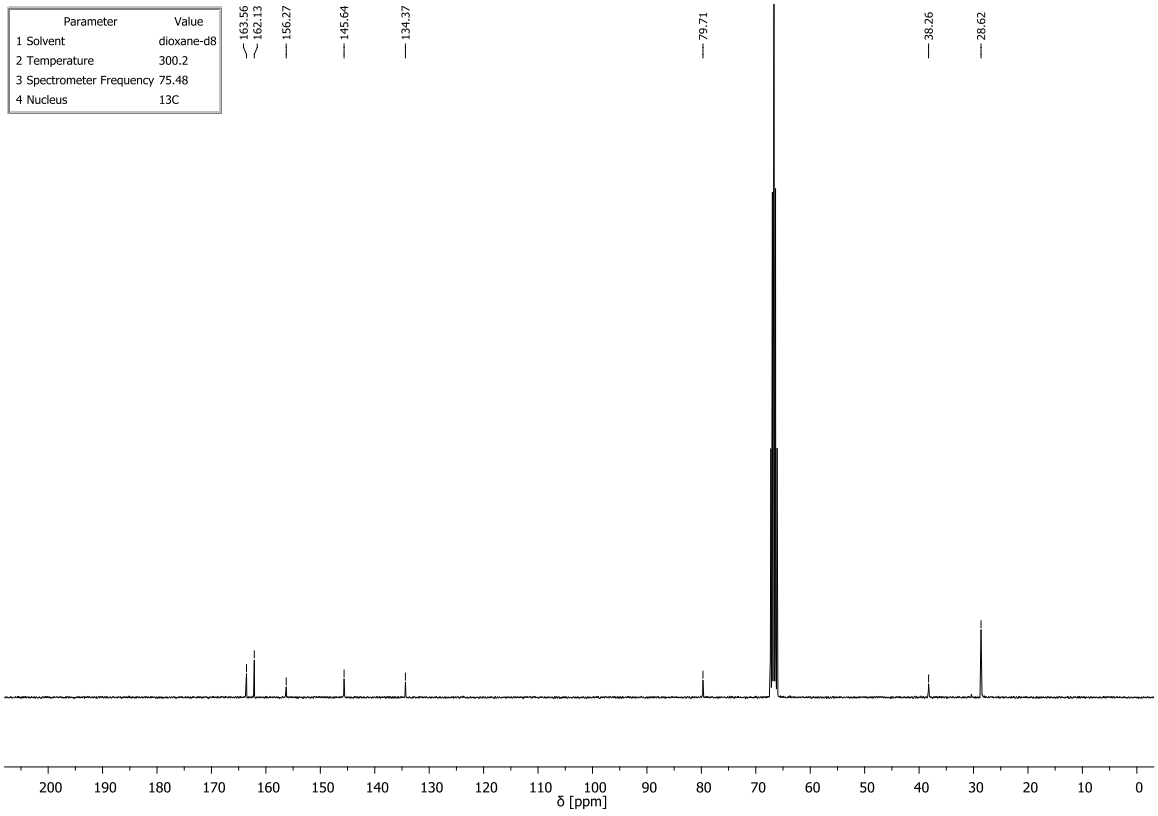


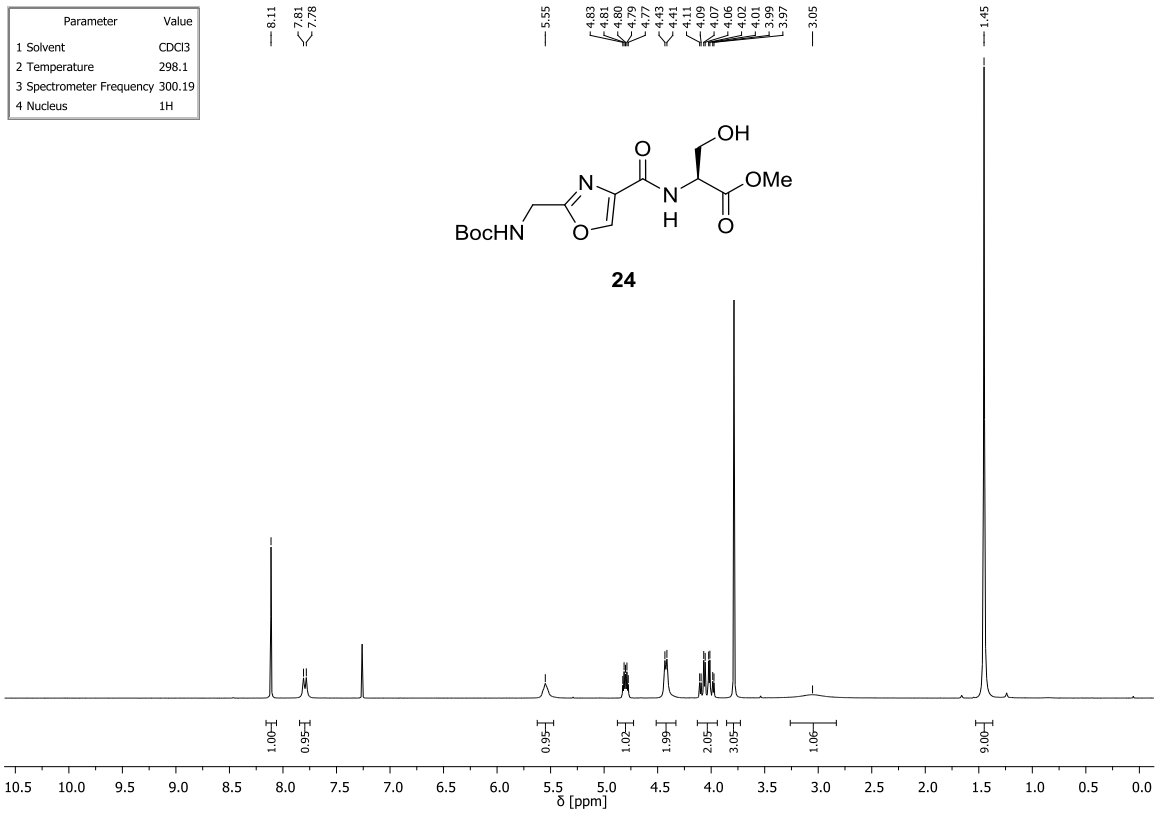
Parameter	Value
1 Solvent	dioxane-d8
2 Temperature	299.9
3 Spectrometer Frequency	300.19
4 Nucleus	1H



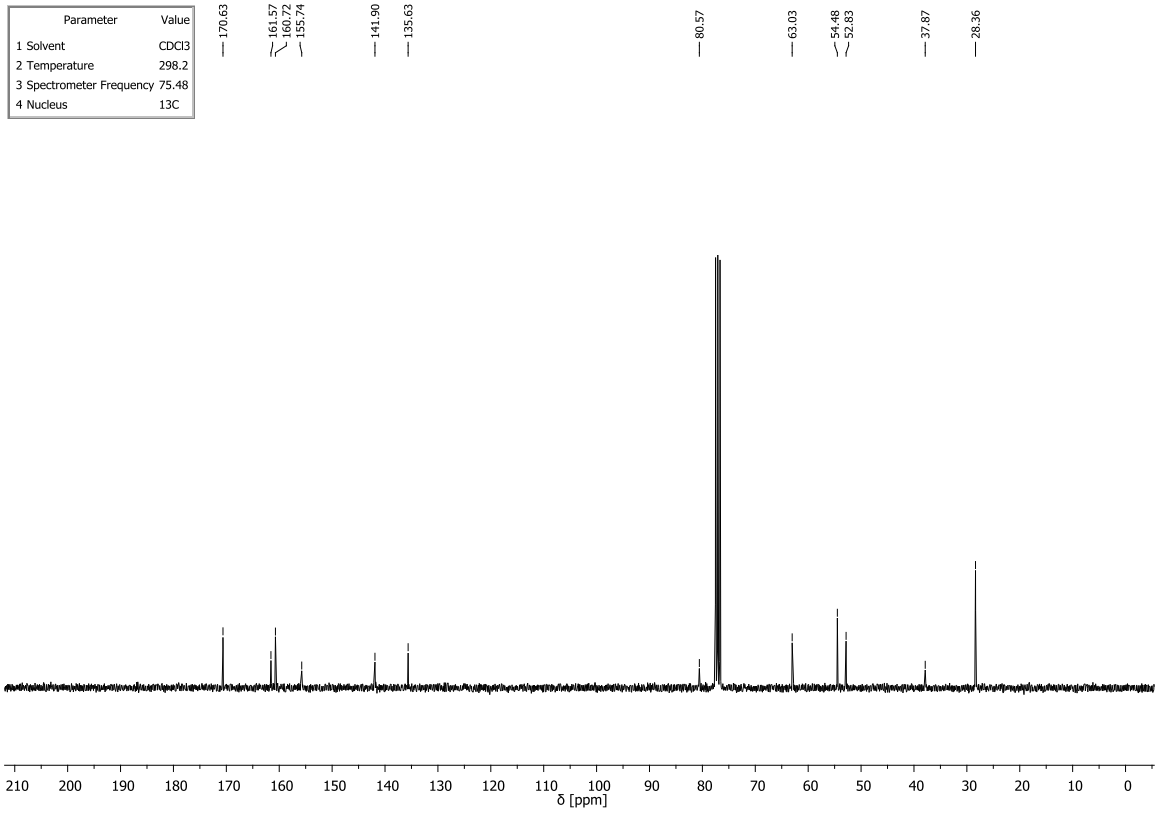
23

Parameter	Value
1 Solvent	dioxane-d8
2 Temperature	300.2
3 Spectrometer Frequency	75.48
4 Nucleus	13C

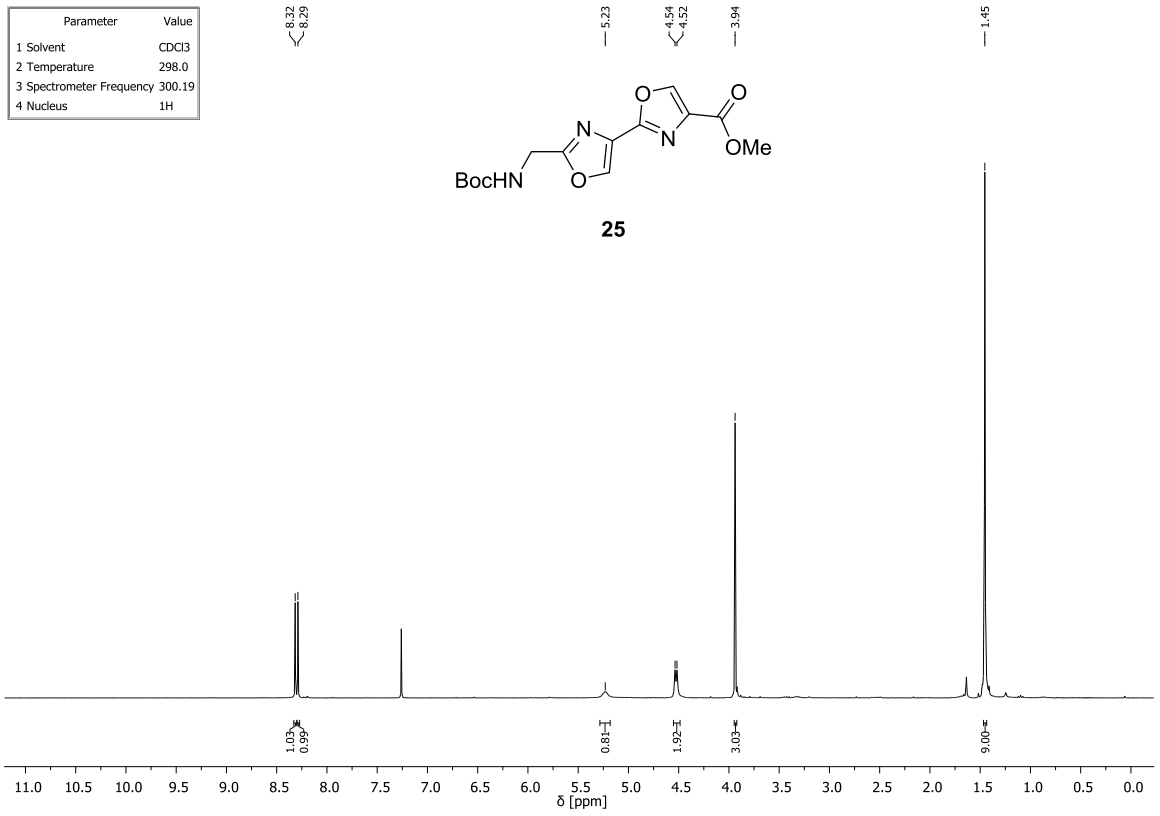



Parameter Value
1 Solvent CDCl3
2 Temperature 298.1
3 Spectrometer Frequency 300.19
4 Nucleus 1H
8.11
7.81
7.78
5.55
4.83
4.81
4.80
4.79
4.77
4.43
4.41
4.11
4.09
4.07
4.06
4.02
4.01
3.99
3.97
3.05
1.45
OH
O
N
OMe
N
H
BocHN
O
O
1.00
0.95
0.95
1.02
1.99
2.05
3.05
1.06
9.00
δ [ppm]

24

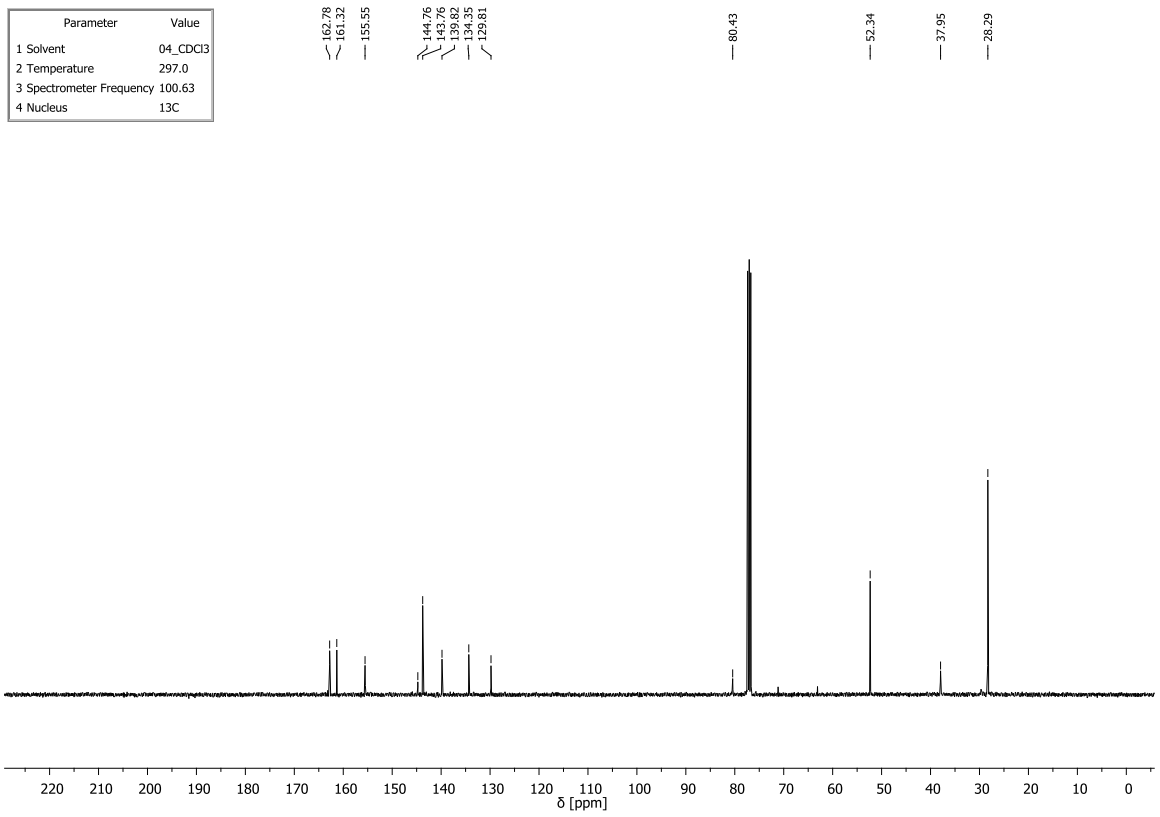

Parameter Value
1 Solvent CDCl3
2 Temperature 298.2
3 Spectrometer Frequency 75.48
4 Nucleus 13C
170.63
161.57
160.72
155.74
141.90
135.63
80.57
63.03
54.48
52.83
37.87
28.36
δ [ppm]

Parameter	Value
1 Solvent	CDCl3
2 Temperature	298.0
3 Spectrometer Frequency	300.19
4 Nucleus	1H

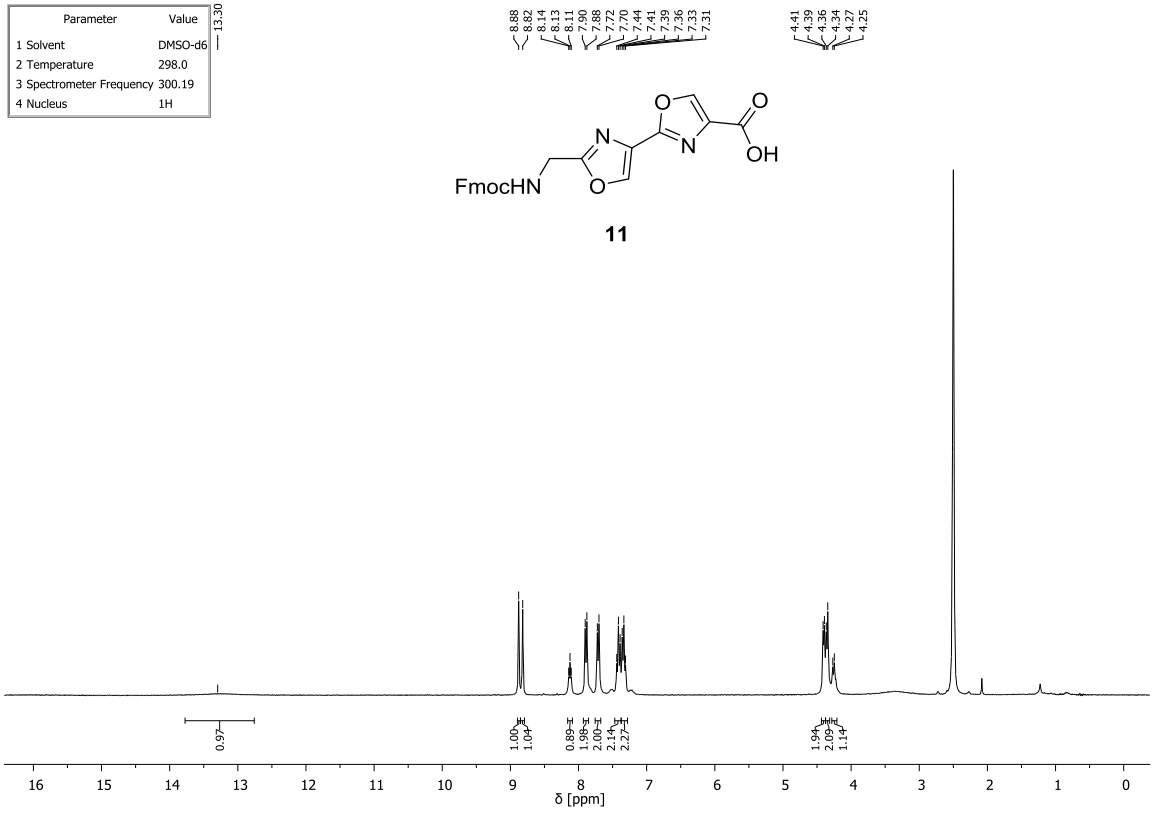


25

Parameter	Value
1 Solvent	04_CDCl3
2 Temperature	297.0
3 Spectrometer Frequency	100.63
4 Nucleus	13C

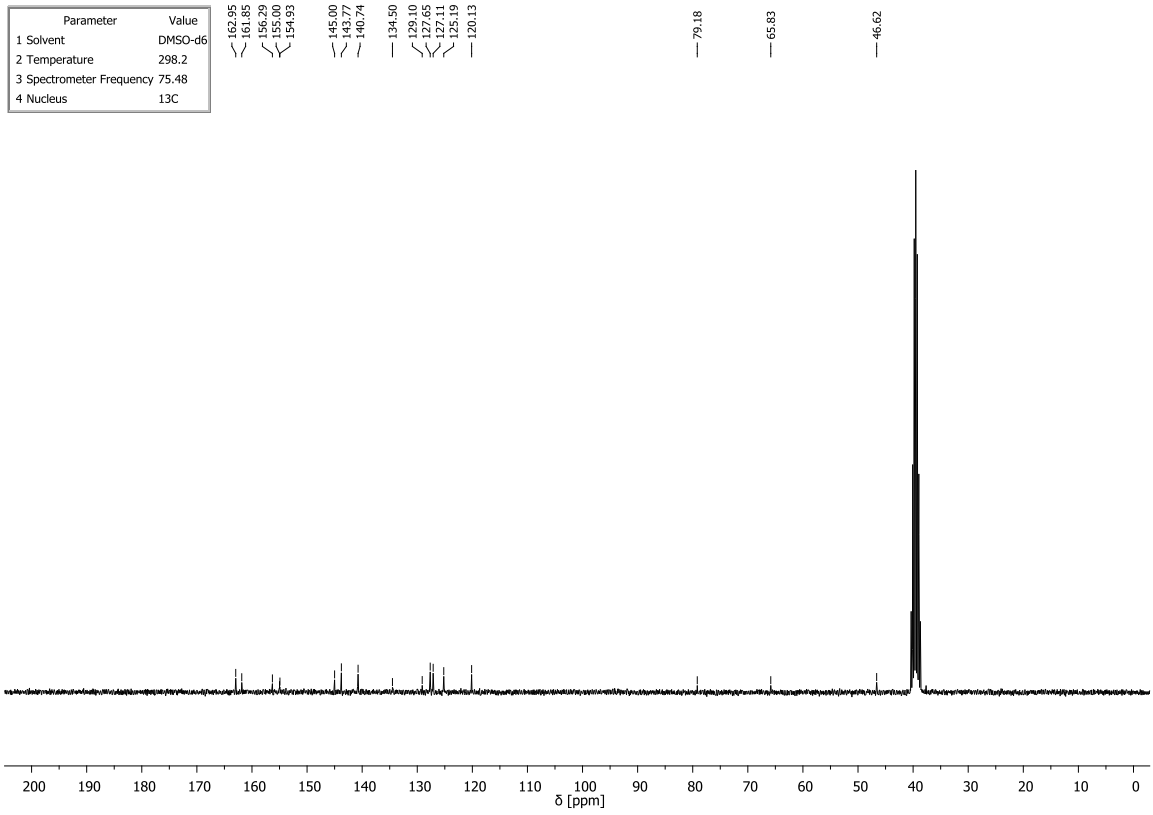


Parameter	Value
1 Solvent	DMSO-d6
2 Temperature	298.0
3 Spectrometer Frequency	300.19
4 Nucleus	1H

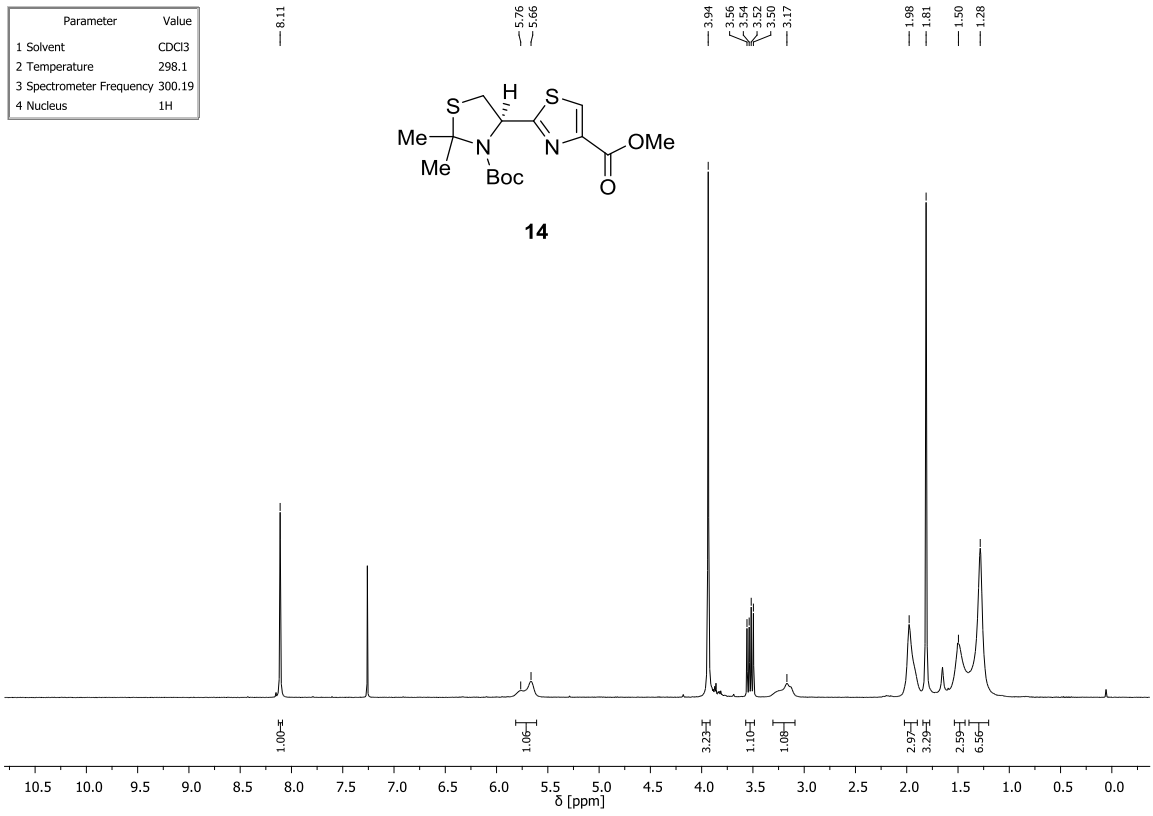


11

Parameter	Value
1 Solvent	DMSO-d6
2 Temperature	298.2
3 Spectrometer Frequency	75.48
4 Nucleus	13C

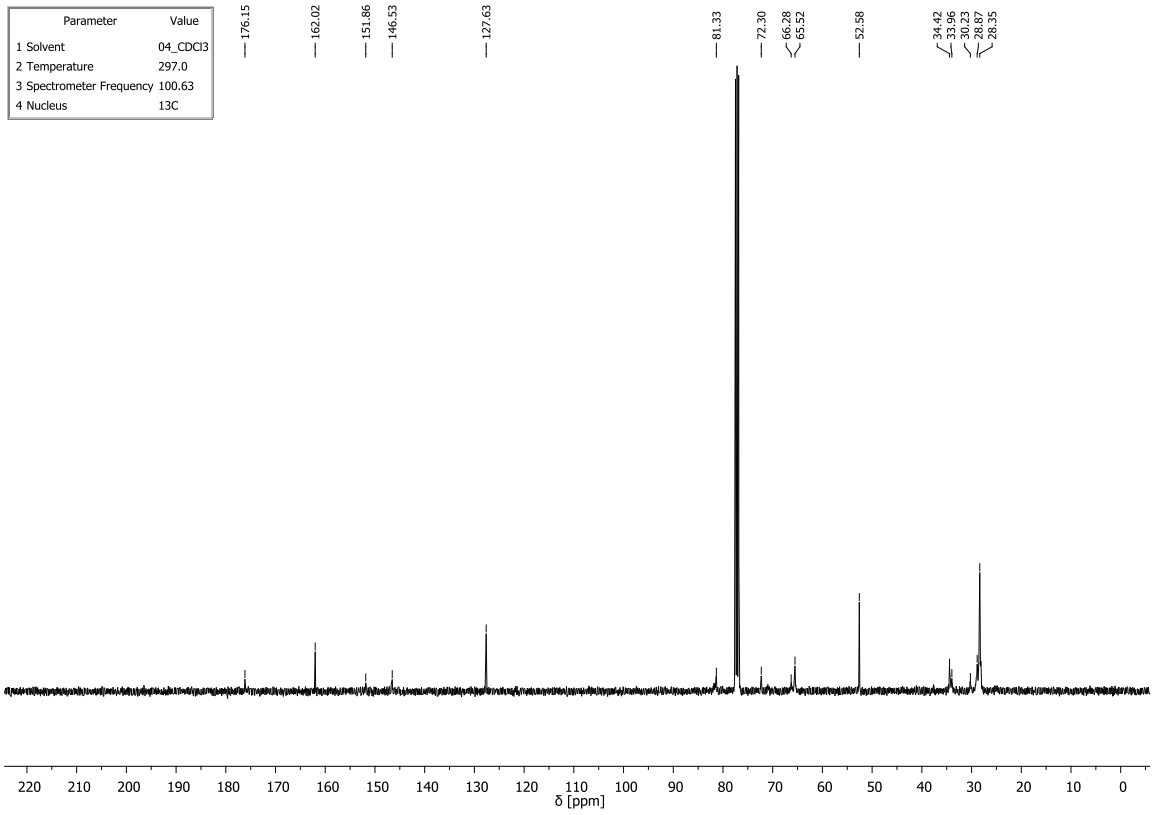


Parameter	Value
1 Solvent	CDCl3
2 Temperature	298.1
3 Spectrometer Frequency	300.19
4 Nucleus	1H

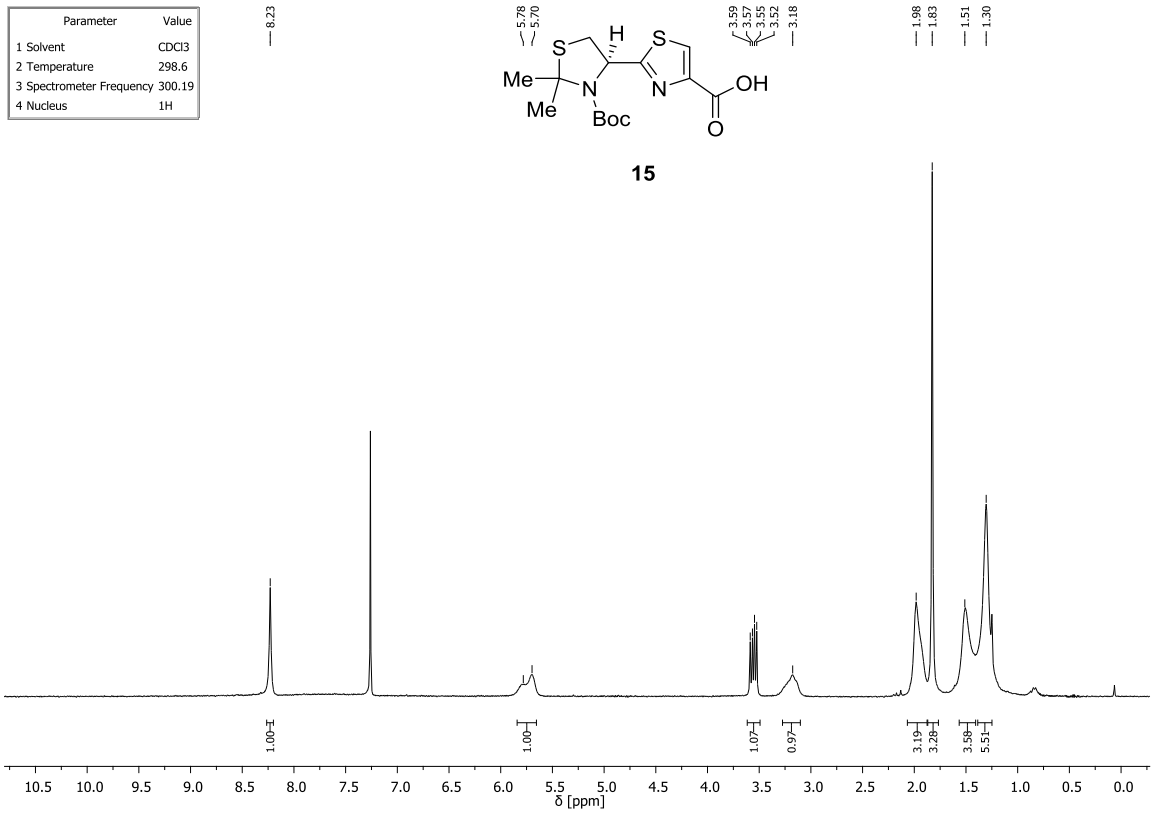


14

Parameter	Value
1 Solvent	04_CDCl3
2 Temperature	297.0
3 Spectrometer Frequency	100.63
4 Nucleus	13C

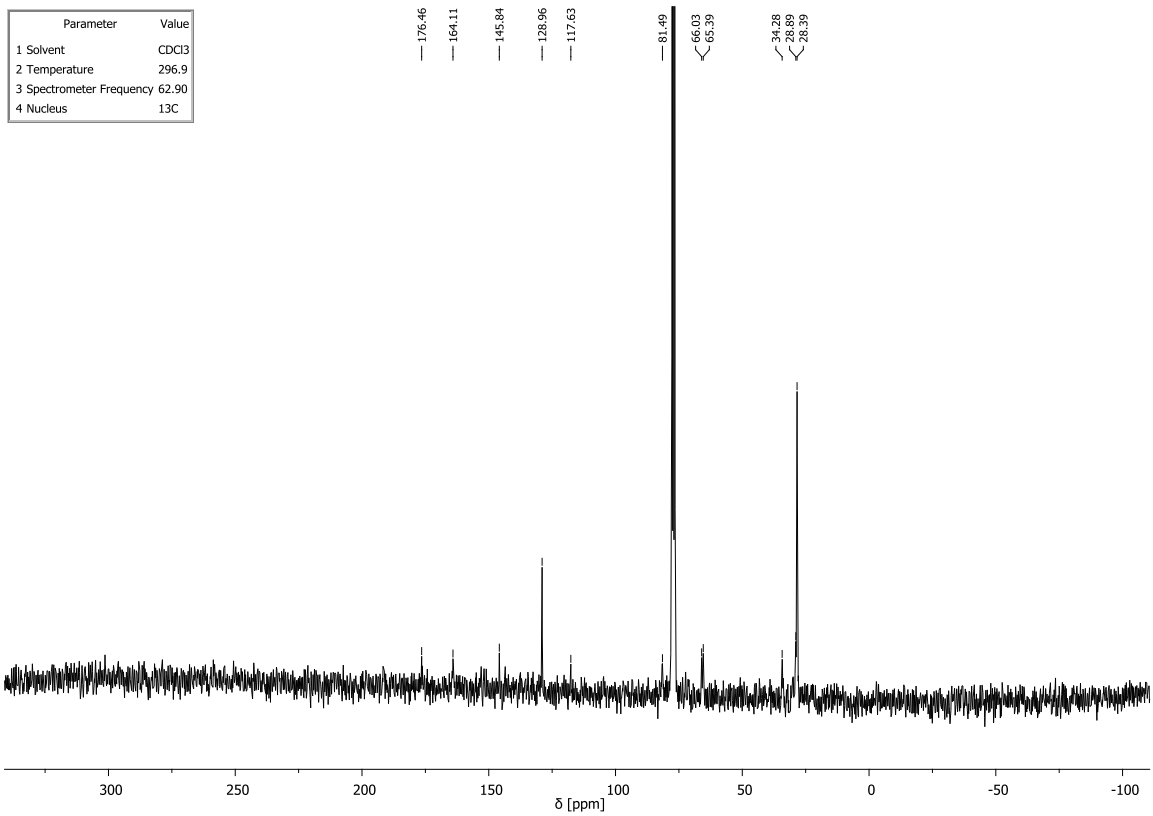


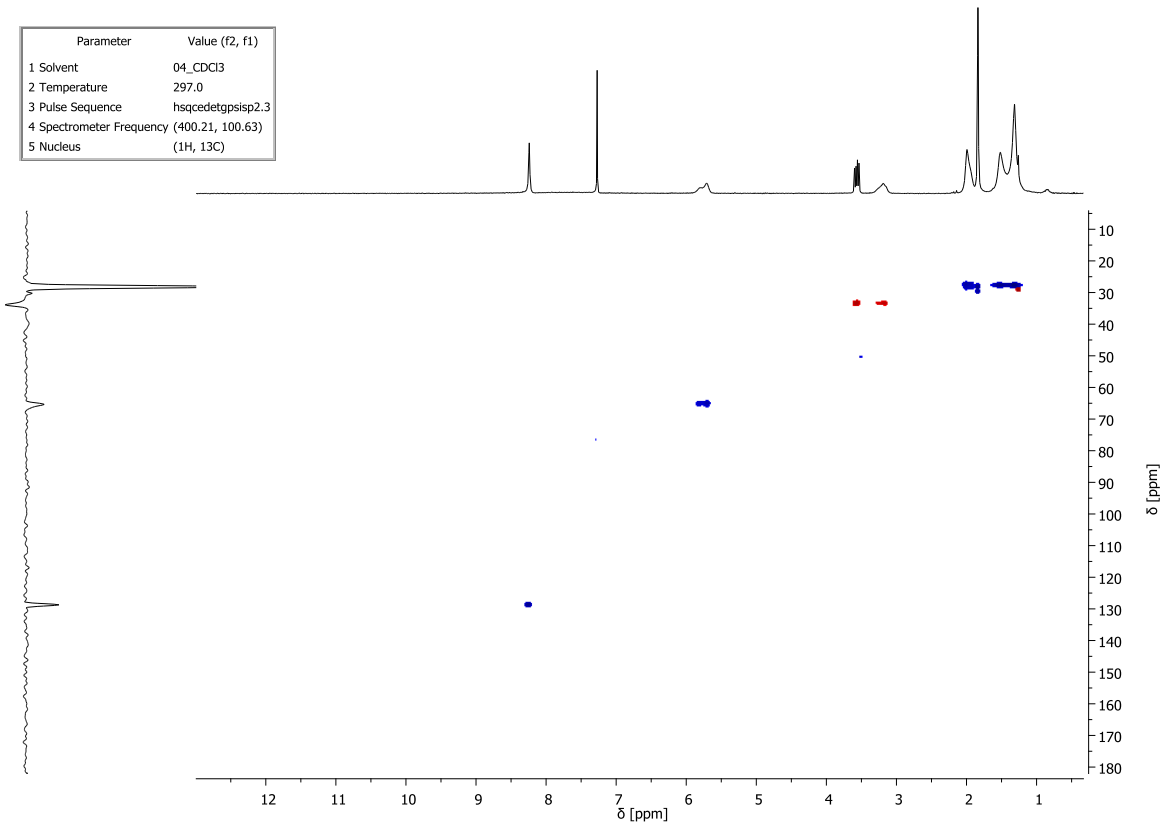
Parameter	Value
1 Solvent	CDCl3
2 Temperature	298.6
3 Spectrometer Frequency	300.19
4 Nucleus	1H

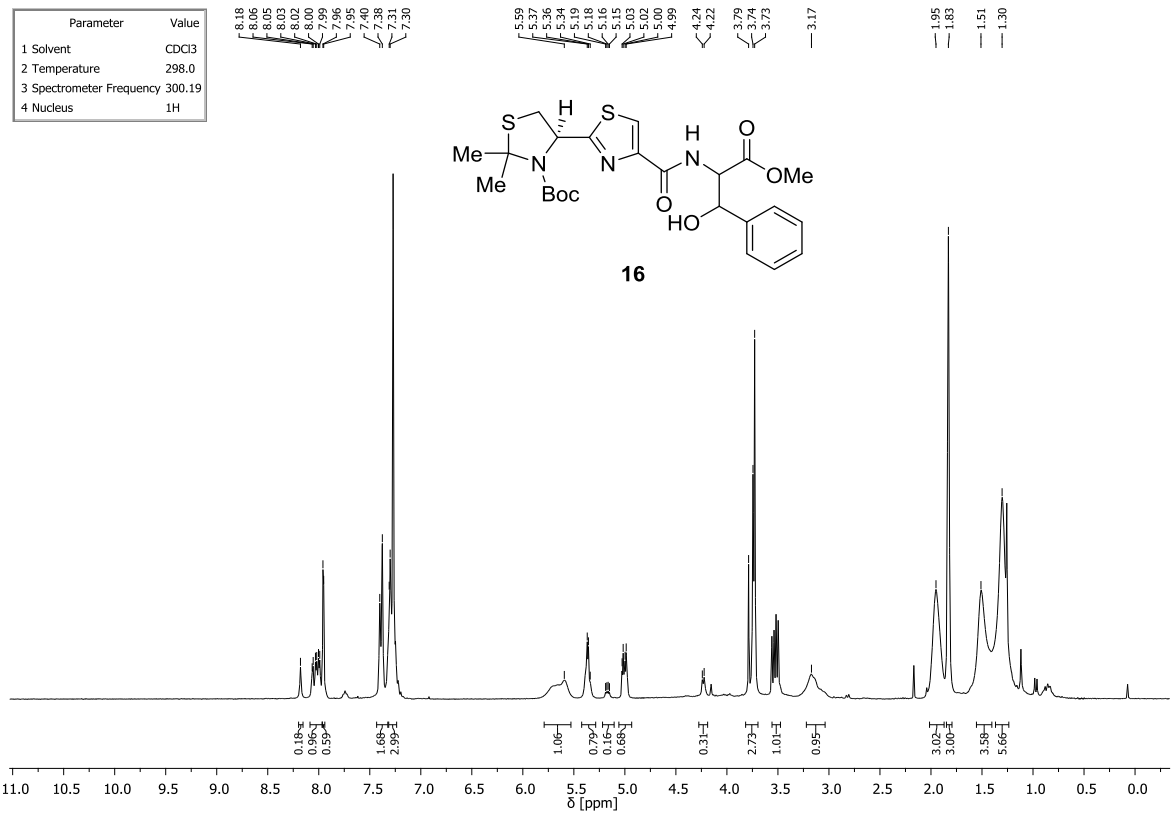


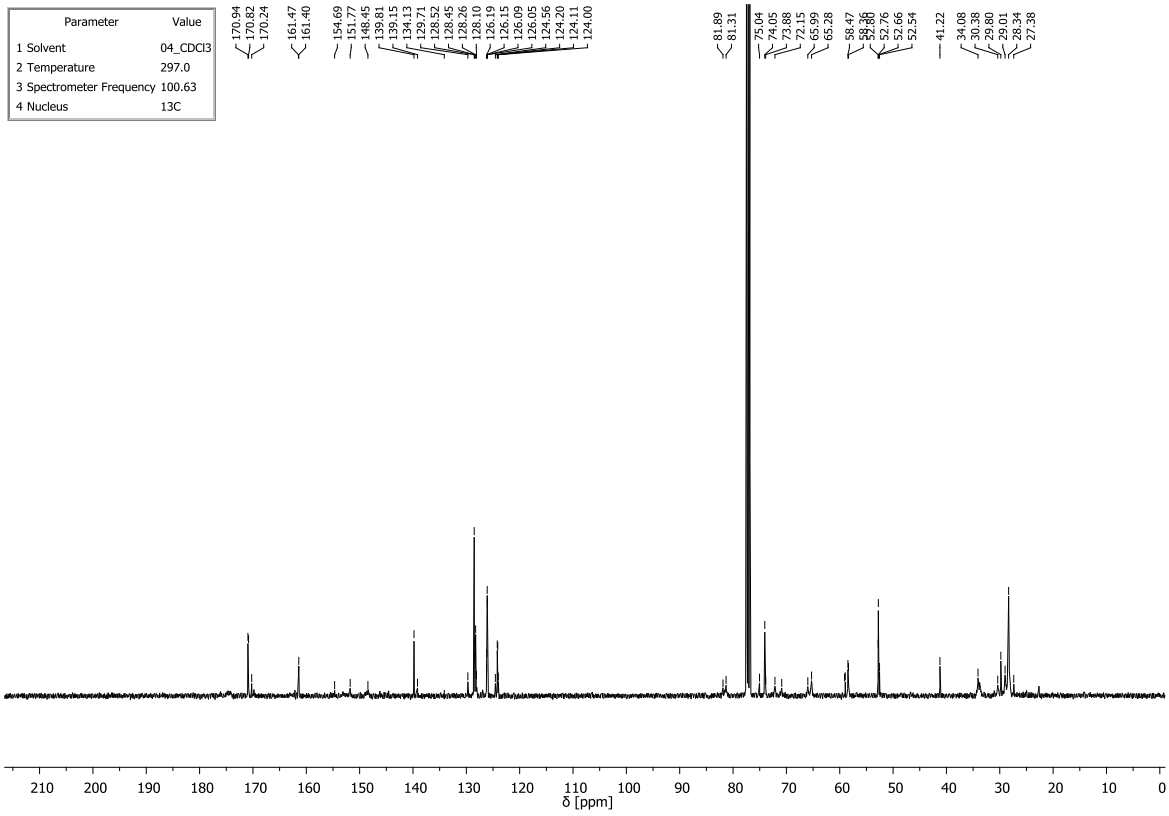
15

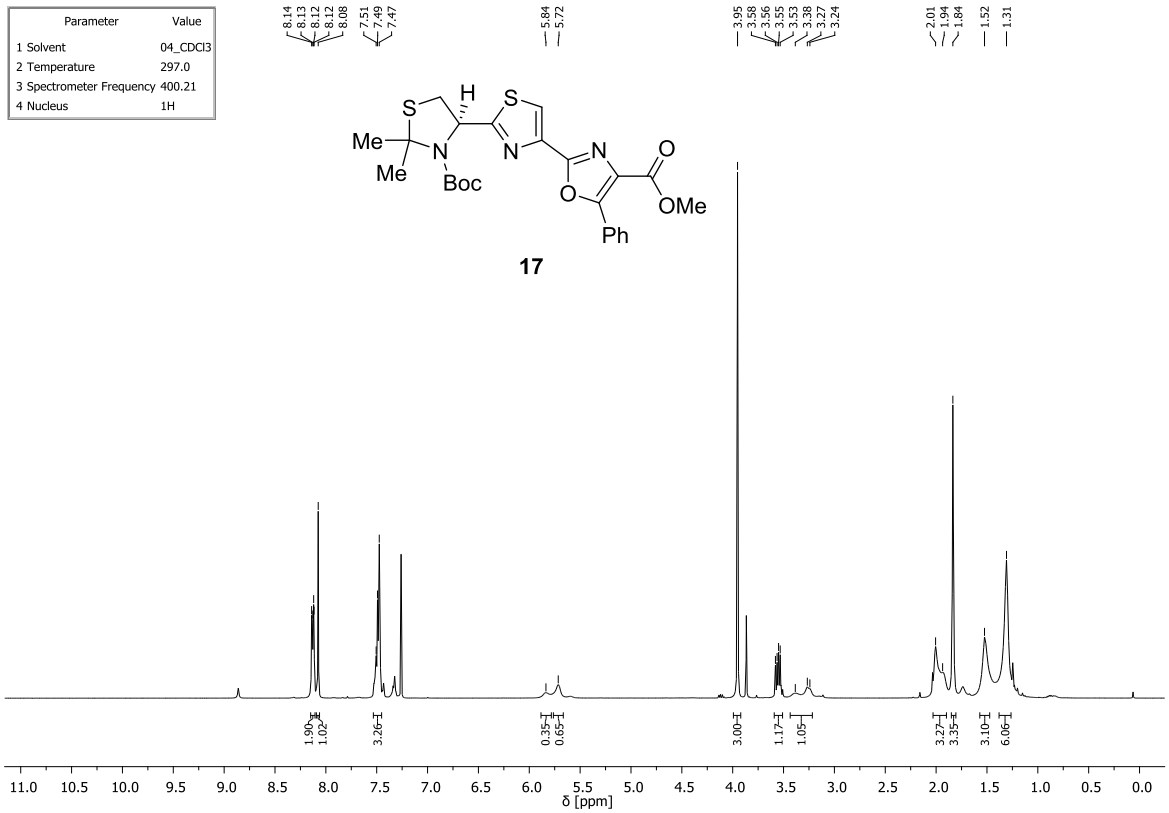
Parameter	Value
1 Solvent	CDCl3
2 Temperature	296.9
3 Spectrometer Frequency	62.90
4 Nucleus	13C

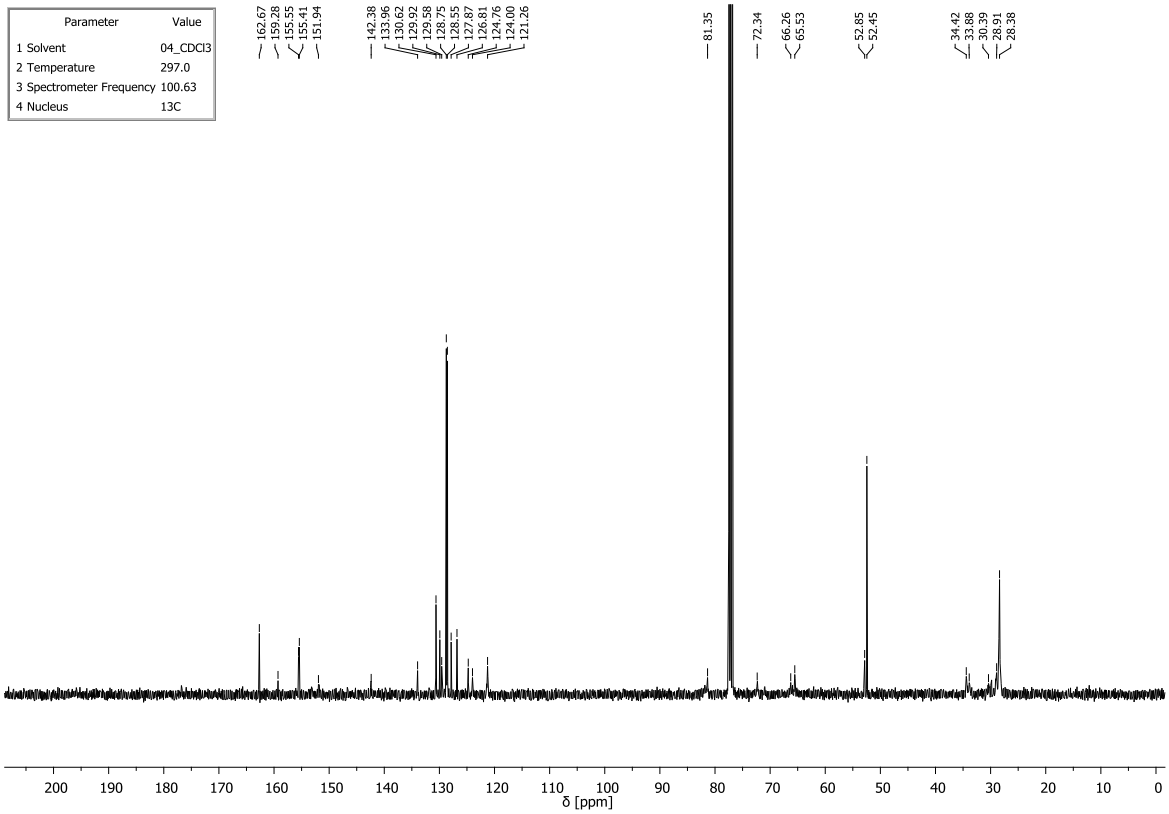



Parameter Value (f2, f1)
1 Solvent 04_CDCl3
2 Temperature 297.0
3 Pulse Sequence hsqcedetgpsisp2.3
4 Spectrometer Frequency (400.21, 100.63)
5 Nucleus (1H, 13C)
δ [ppm]
δ [ppm]

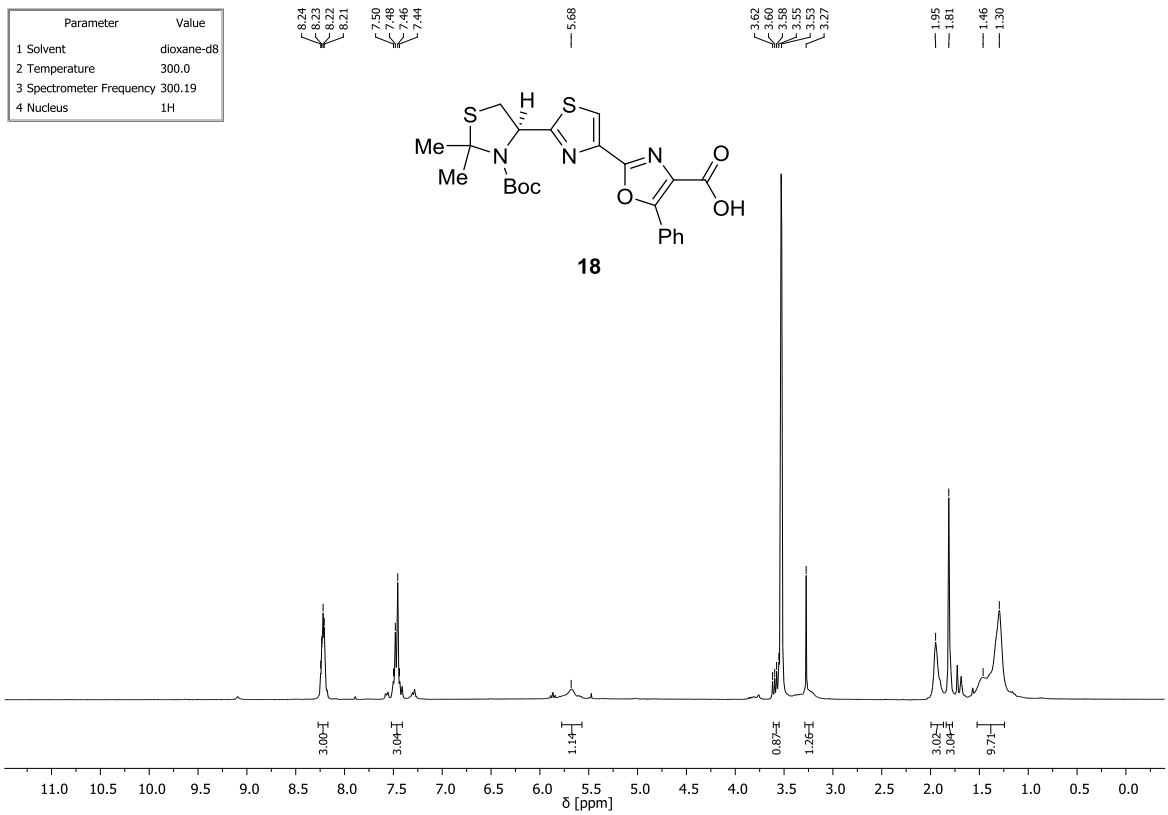

Parameter Value
1 Solvent CDCl3
2 Temperature 298.0
3 Spectrometer Frequency 300.19
4 Nucleus 1H
8.18 8.06 8.05 8.03 8.02 8.00 7.99 7.96 7.95 7.40 7.38 7.31 7.30
5.59 5.37 5.36 5.34 5.19 5.18 5.16 5.15 5.03 5.02 5.00 4.99
4.24 4.22
3.79 3.74 3.73
3.17
1.95 1.83
1.51
1.30
S
H
S
Me
N
N
Me
Boc
H
N
O
OMe
O
HO
16
0.18 0.96 0.59 1.69 2.99 1.06 0.79 0.16 0.68 0.31 2.73 1.01 0.95 3.02 3.00 3.59 5.66
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ [ppm]


Parameter Value
1 Solvent 04_CDCl3
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
170.94 170.82 170.24
161.47 161.40
154.69 151.77 148.45 139.81 139.15 134.13 123.71 128.52 128.45 128.26 128.10 126.19 126.15 126.09 126.05 124.56 124.20 124.11 124.00
81.89 81.31
75.04 74.05 73.88 72.15 65.99 65.28
58.47 58.36 52.80 52.76 52.66 52.54
41.22
34.08 30.38 29.80 29.01 28.34 27.38
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
δ [ppm]


Parameter Value
1 Solvent 04_CDCl3
2 Temperature 297.0
3 Spectrometer Frequency 400.21
4 Nucleus 1H
8.14 8.13 8.12 8.12 8.08
7.51 7.49 7.47
5.84 5.72
3.95 3.58 3.56 3.55 3.53 3.38 3.27 3.24
2.01 1.94 1.84 1.52 1.31
S H S
Me N N N O
Me Boc O OMe
Ph
17
1.90 1.02 3.26 0.35 0.65 3.00 1.17 1.05 3.27 3.33 3.10 6.06
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ [ppm]

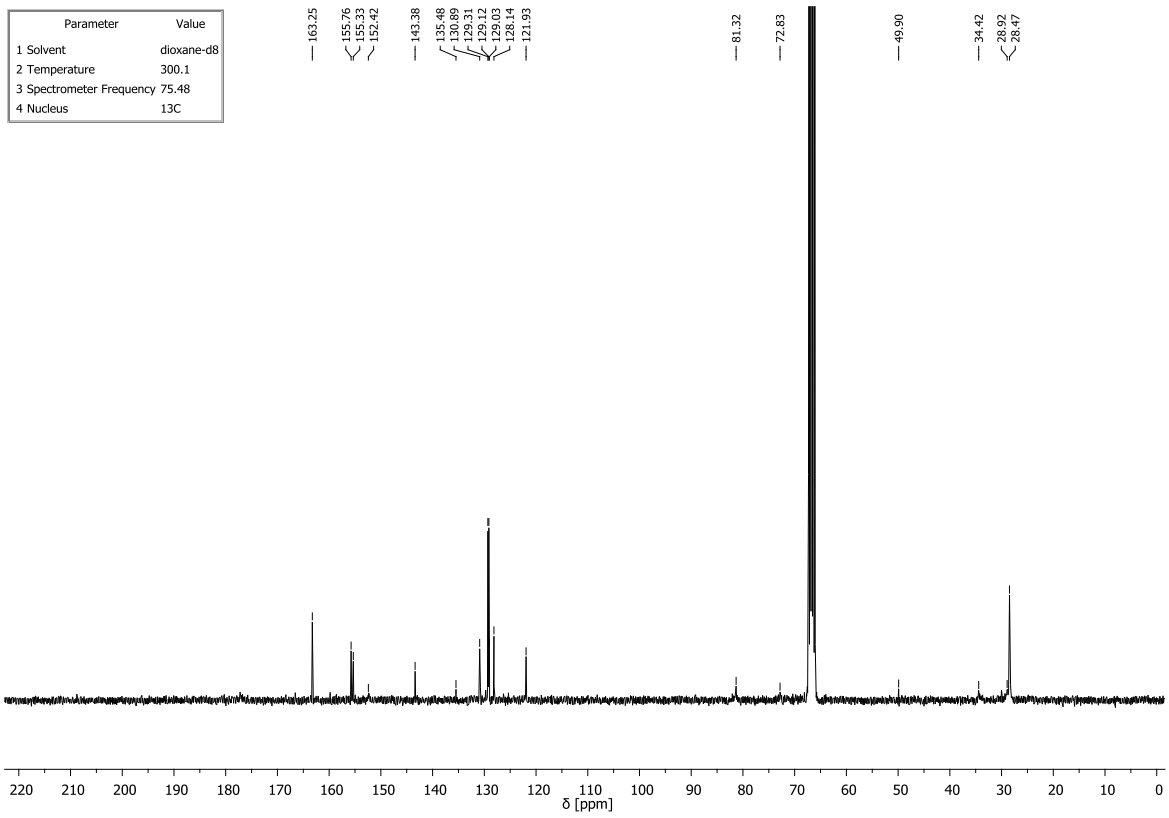

Parameter Value
1 Solvent 04_CDCl3
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
162.67 159.28 155.55 155.41 151.94
142.38 133.96 130.62 129.92 129.58 128.75 128.55 127.87 126.81 124.76 124.00 121.26
81.35
72.34
66.26 65.53
52.85 52.45
34.42 33.88 30.39 28.91 28.38
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
δ [ppm]

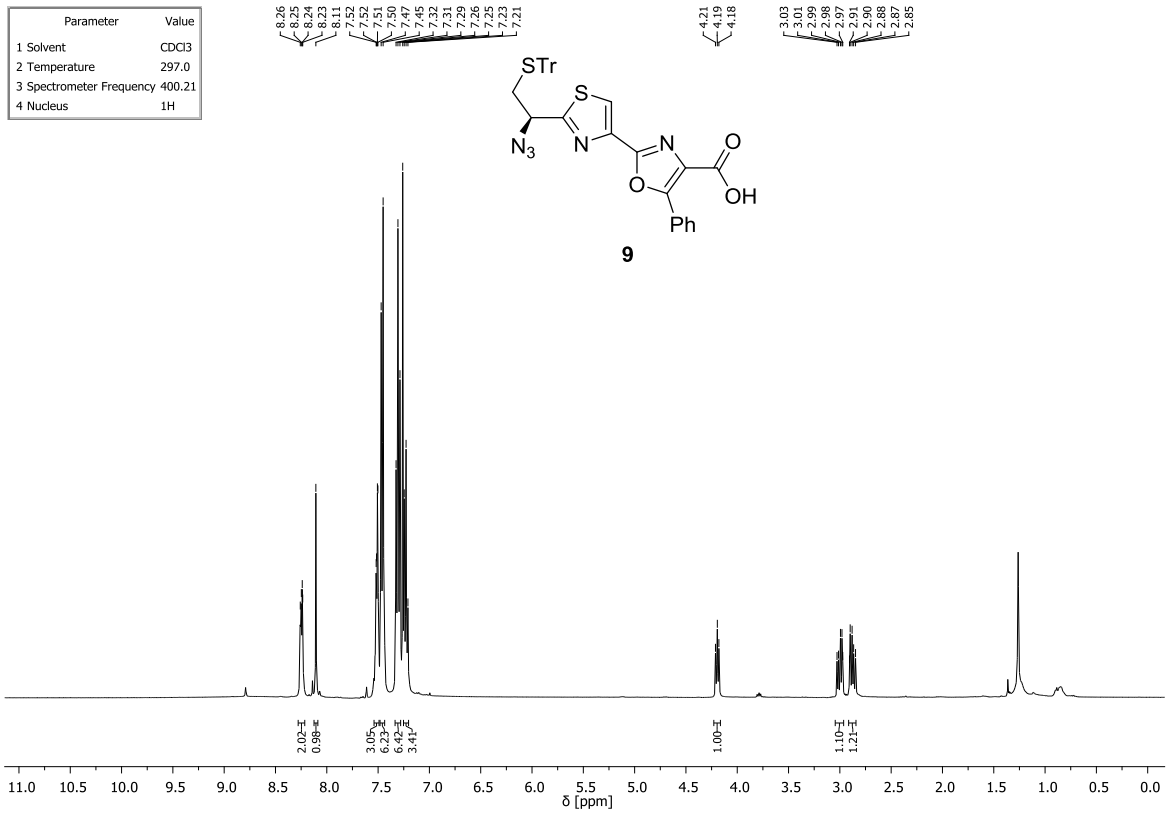
Parameter	Value
1 Solvent	dioxane-d8
2 Temperature	300.0
3 Spectrometer Frequency	300.19
4 Nucleus	1H

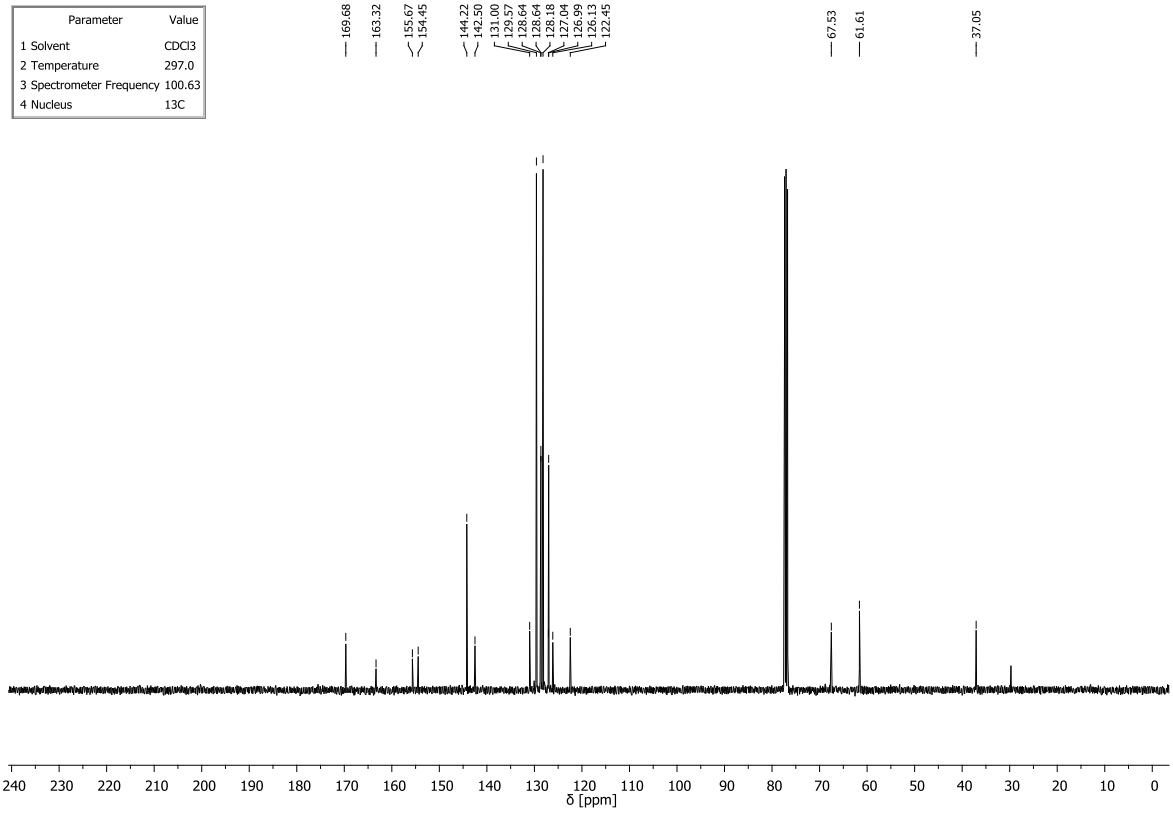


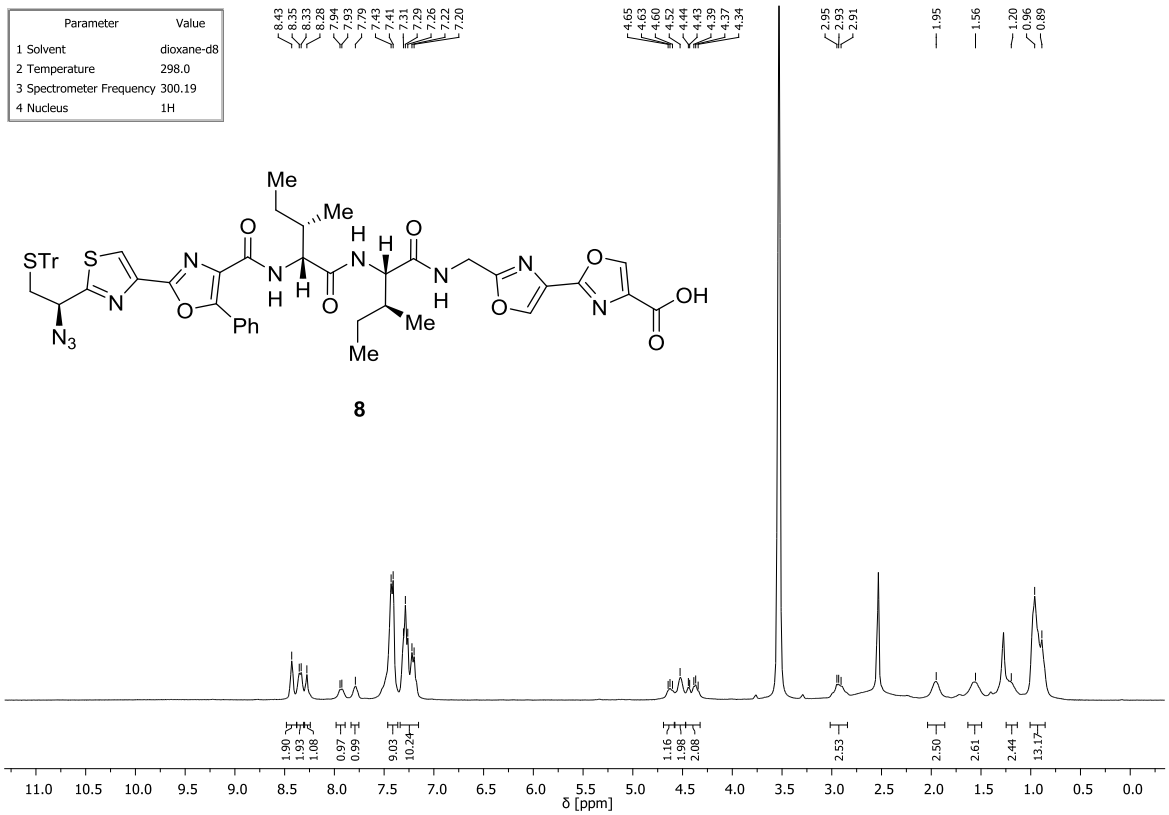
18

Parameter	Value
1 Solvent	dioxane-d8
2 Temperature	300.1
3 Spectrometer Frequency	75.48
4 Nucleus	13C

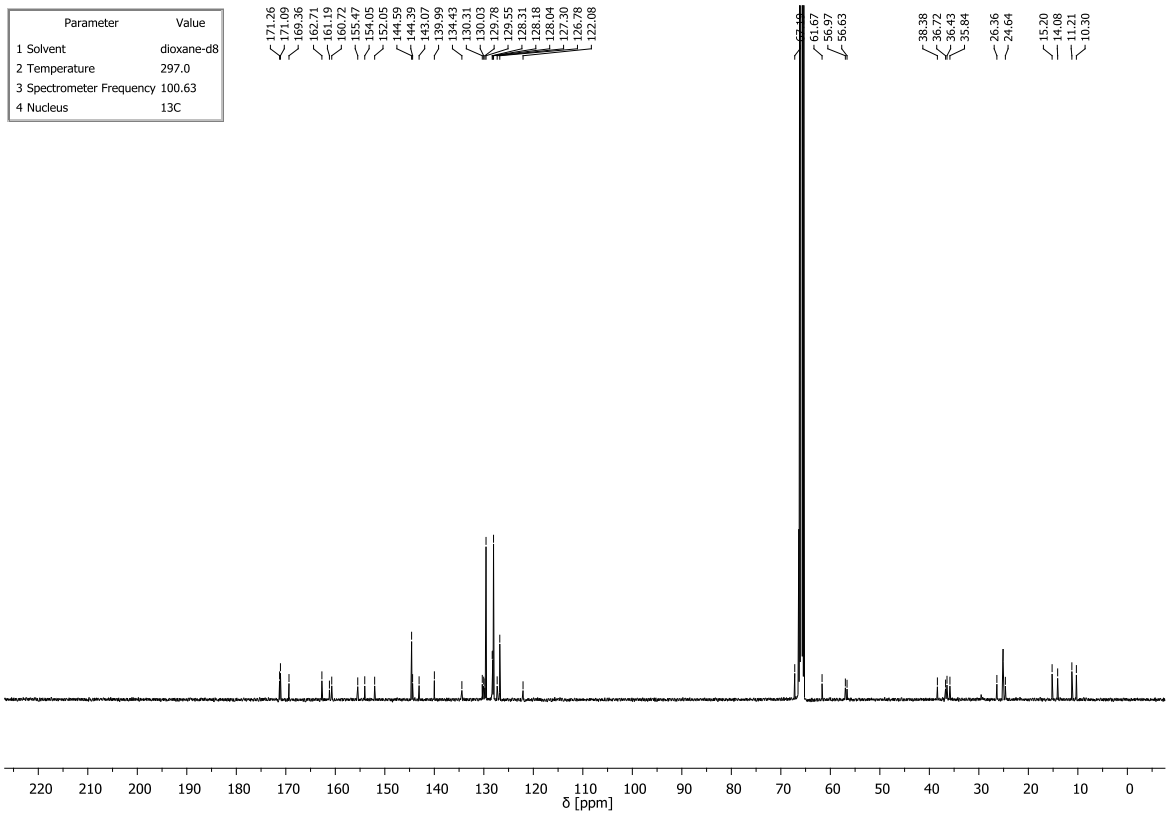


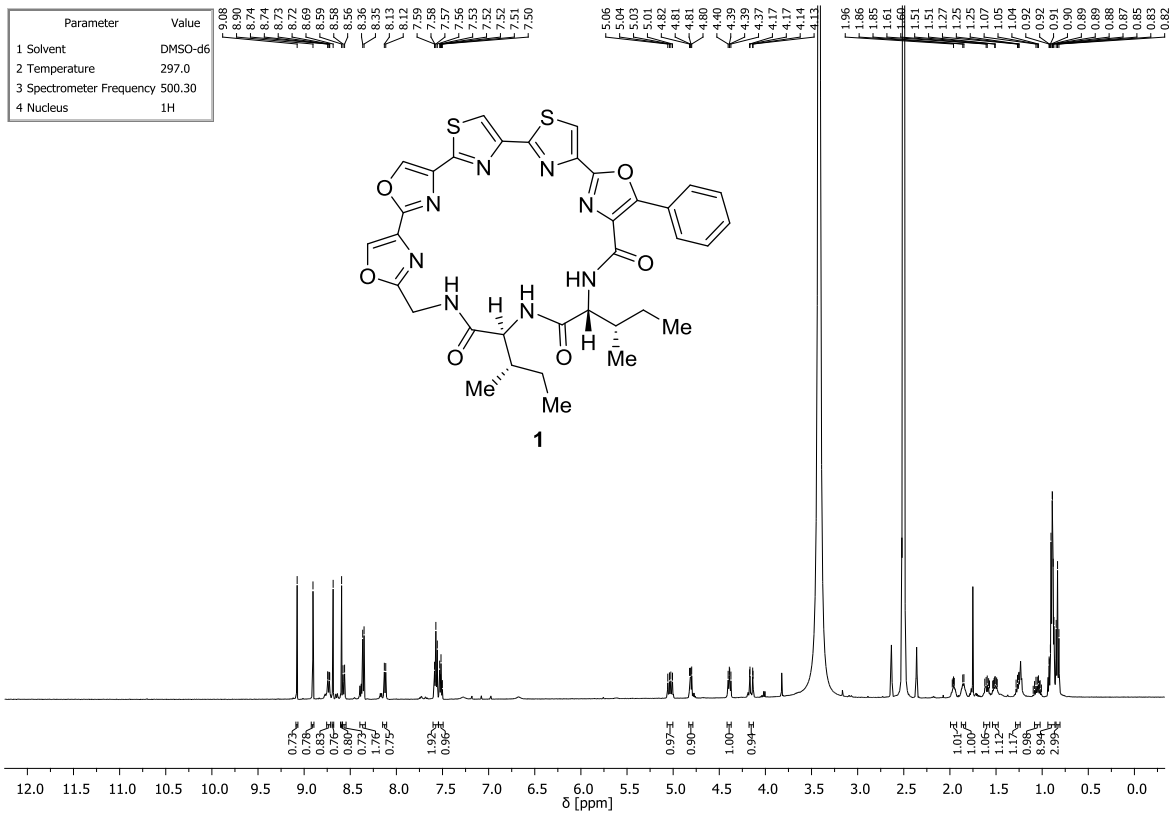

Parameter Value
1 Solvent CDCl3
2 Temperature 297.0
3 Spectrometer Frequency 400.21
4 Nucleus 1H
STr
N3
S
N
O
OH
Ph
9
δ [ppm]


Parameter Value
1 Solvent CDCl3
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
δ [ppm]

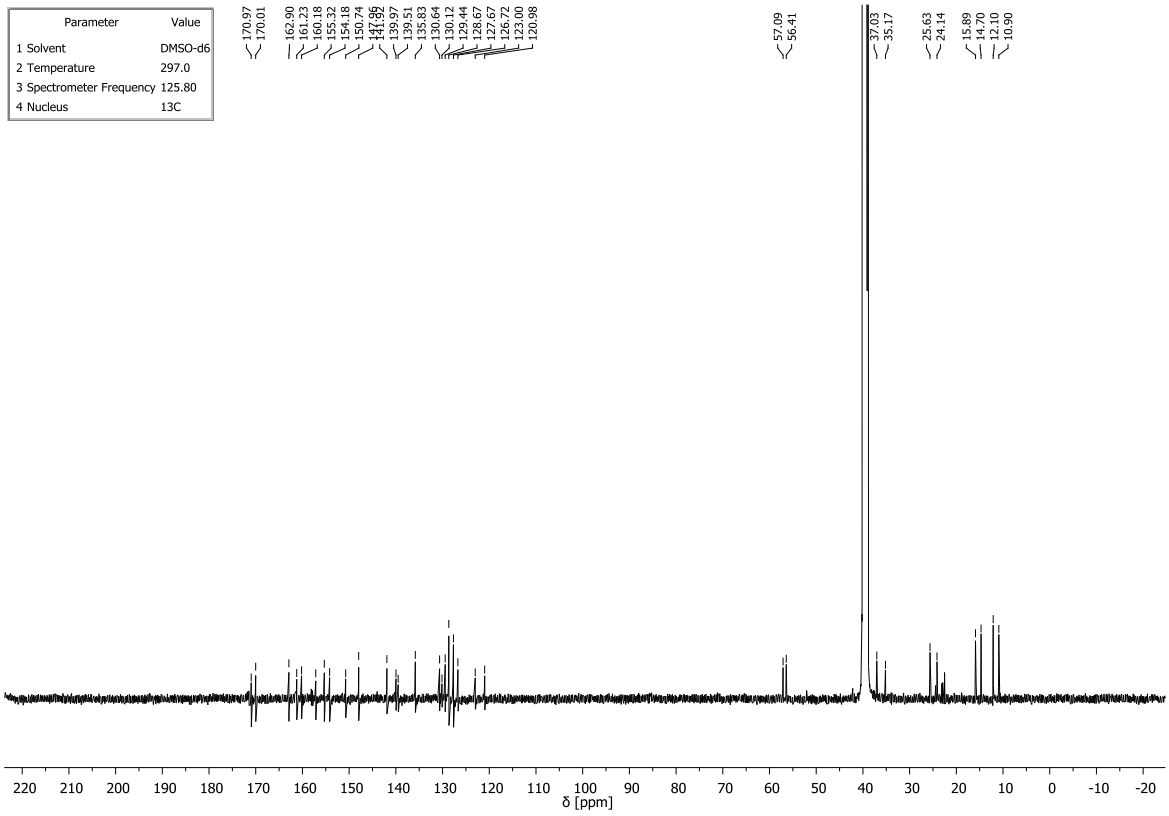

Parameter Value
1 Solvent dioxane-d8
2 Temperature 298.0
3 Spectrometer Frequency 300.19
4 Nucleus 1H
8.43 8.35 8.33 8.28 7.94 7.93 7.79 7.43 7.41 7.31 7.29 7.26 7.22 7.20
4.65 4.63 4.60 4.52 4.44 4.43 4.39 4.37 4.34
2.95 2.93 2.91
1.95 1.56 1.20 0.96 0.89
STr
Ph
OH
Me
1.90 1.93 1.08 0.97 0.99 9.03 10.24 1.16 1.98 2.08 2.53 2.50 2.61 2.44 13.17
δ [ppm]

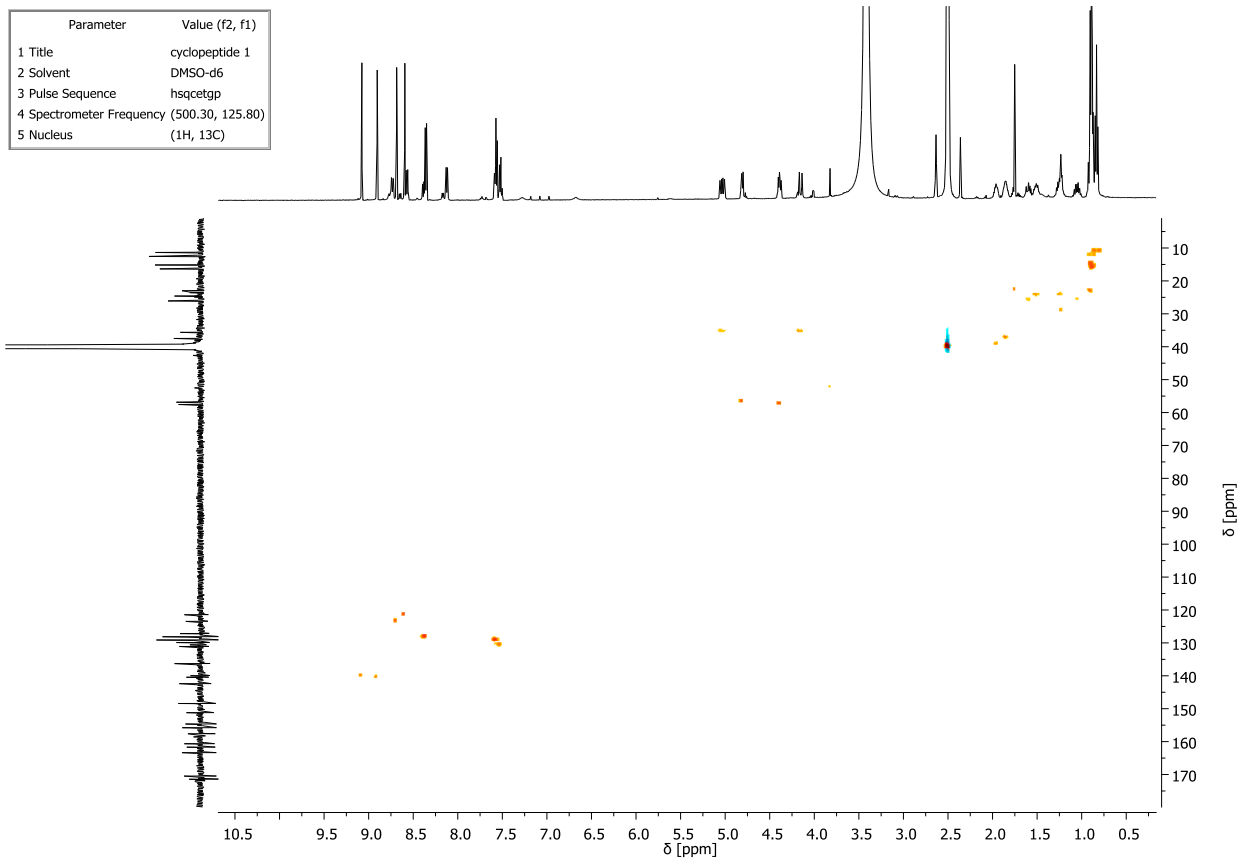
8

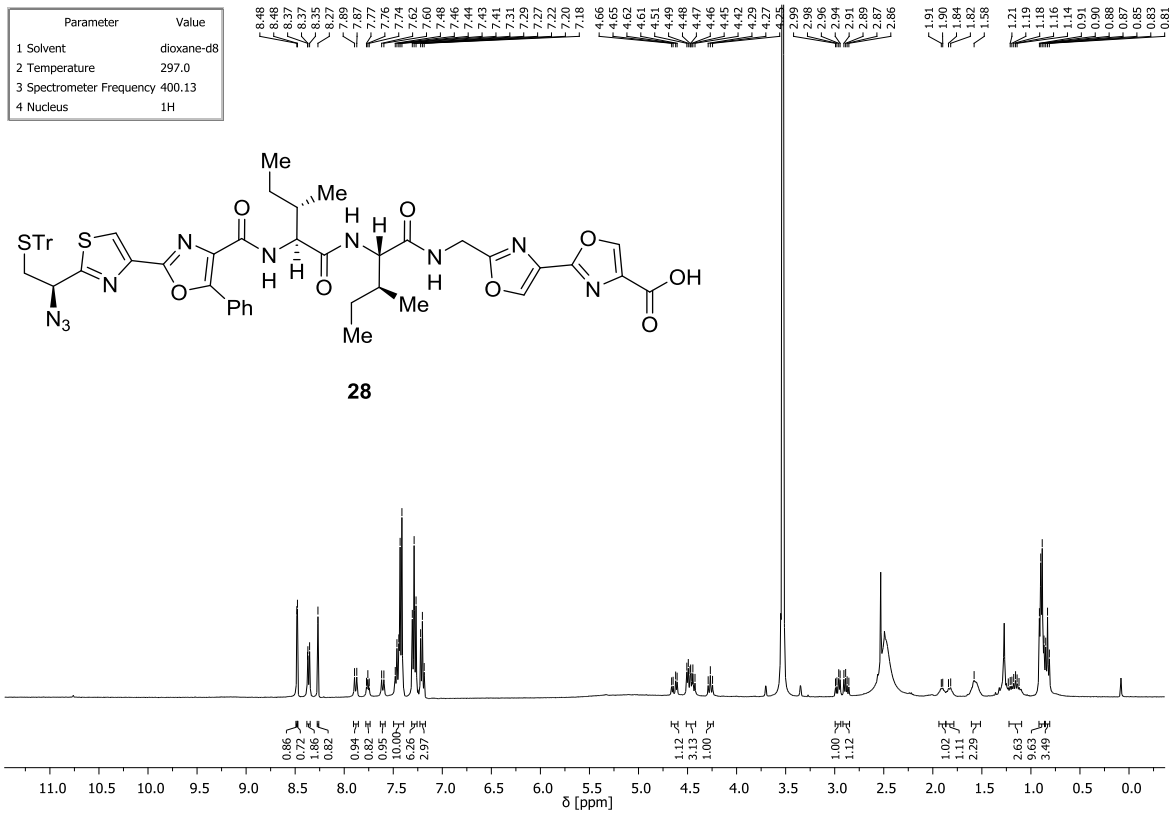

Parameter Value
1 Solvent dioxane-d8
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
171.26 171.09 169.36 162.71 161.19 160.72 155.47 154.05 152.05 144.59 144.39 143.07 139.99 134.43 130.31 130.03 129.78 129.55 128.31 128.18 128.04 127.30 126.78 122.08
61.67 56.97 56.63
38.38 36.72 36.43 35.84 26.36 24.64 15.20 14.08 11.21 10.30
δ [ppm]


Parameter Value
1 Solvent DMSO-d6
2 Temperature 297.0
3 Spectrometer Frequency 500.30
4 Nucleus 1H
δ [ppm]

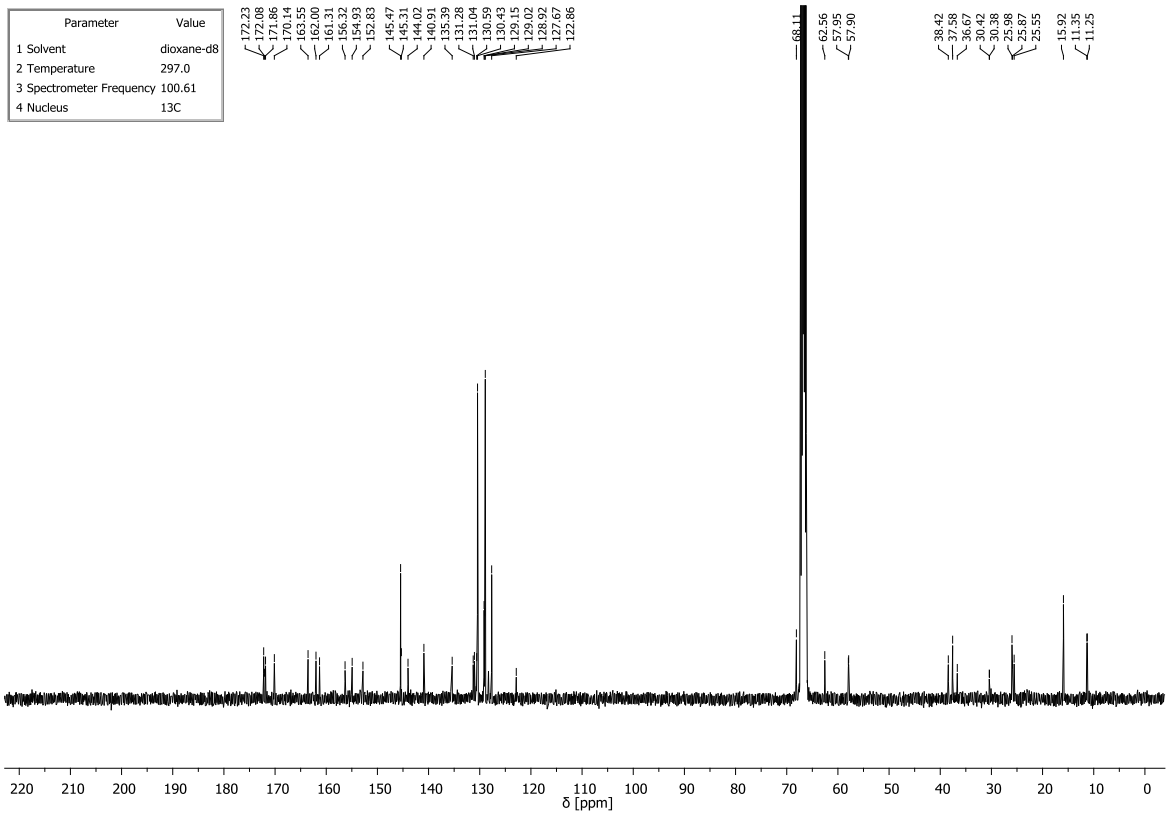
1

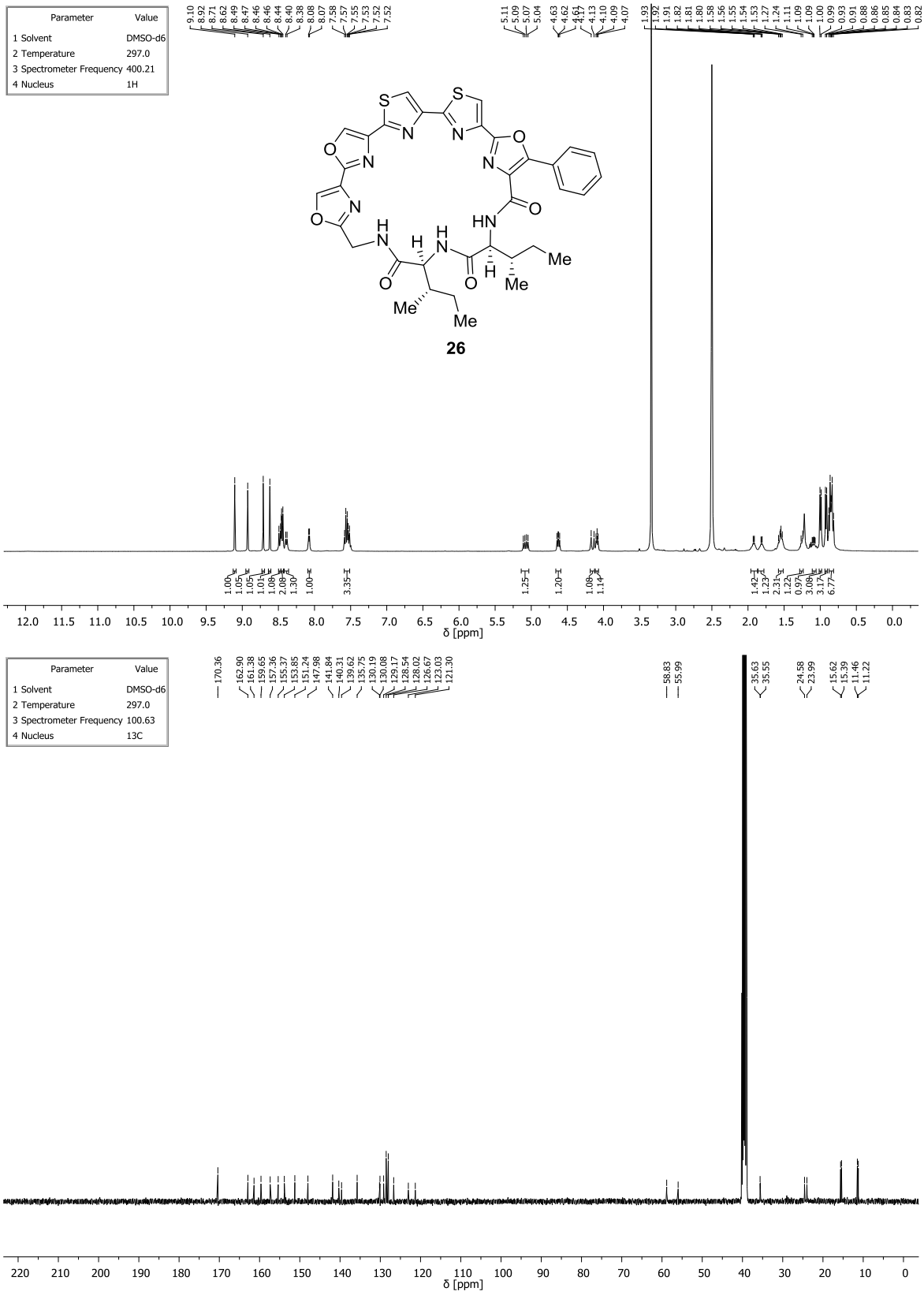

Parameter Value
1 Solvent DMSO-d6
2 Temperature 297.0
3 Spectrometer Frequency 125.80
4 Nucleus 13C
δ [ppm]

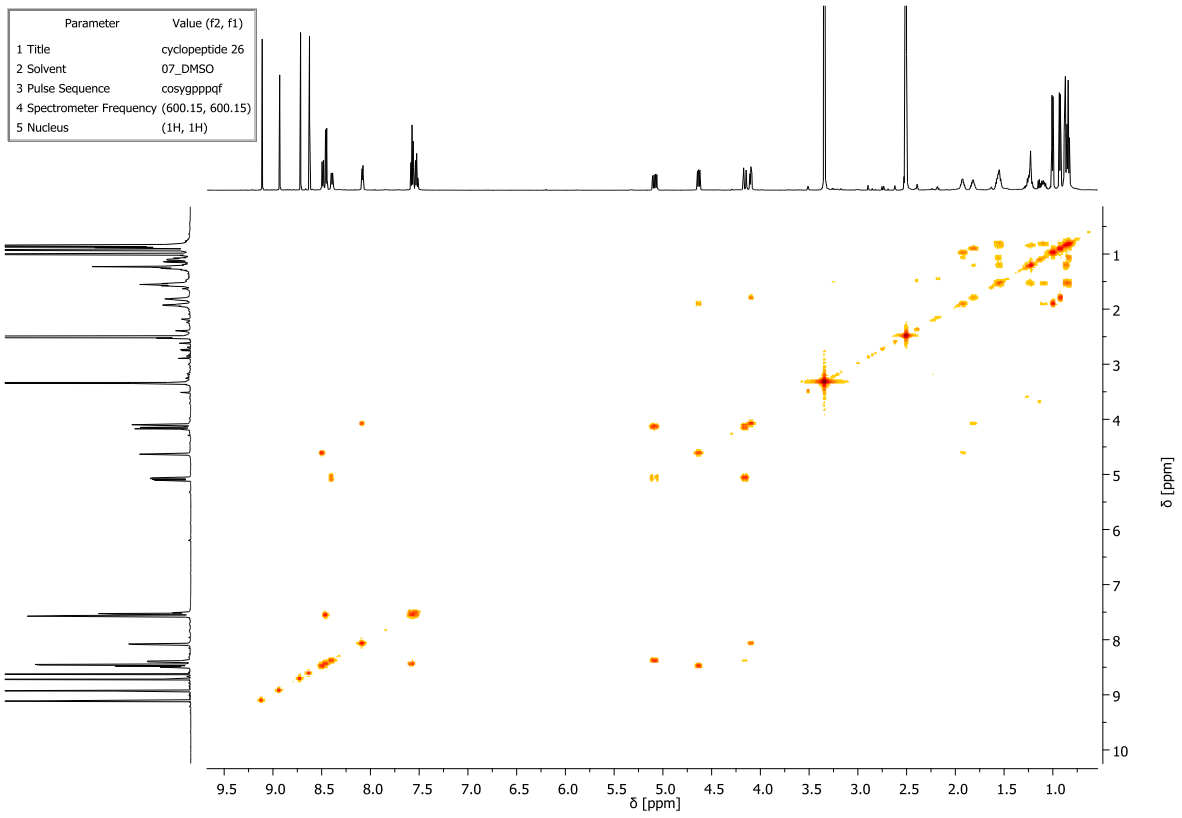

Parameter Value (f2, f1)
1 Title cyclopeptide 1
2 Solvent DMSO-d6
3 Pulse Sequence hsqcetgp
4 Spectrometer Frequency (500.30, 125.80)
5 Nucleus (1H, 13C)
δ [ppm]
δ [ppm]

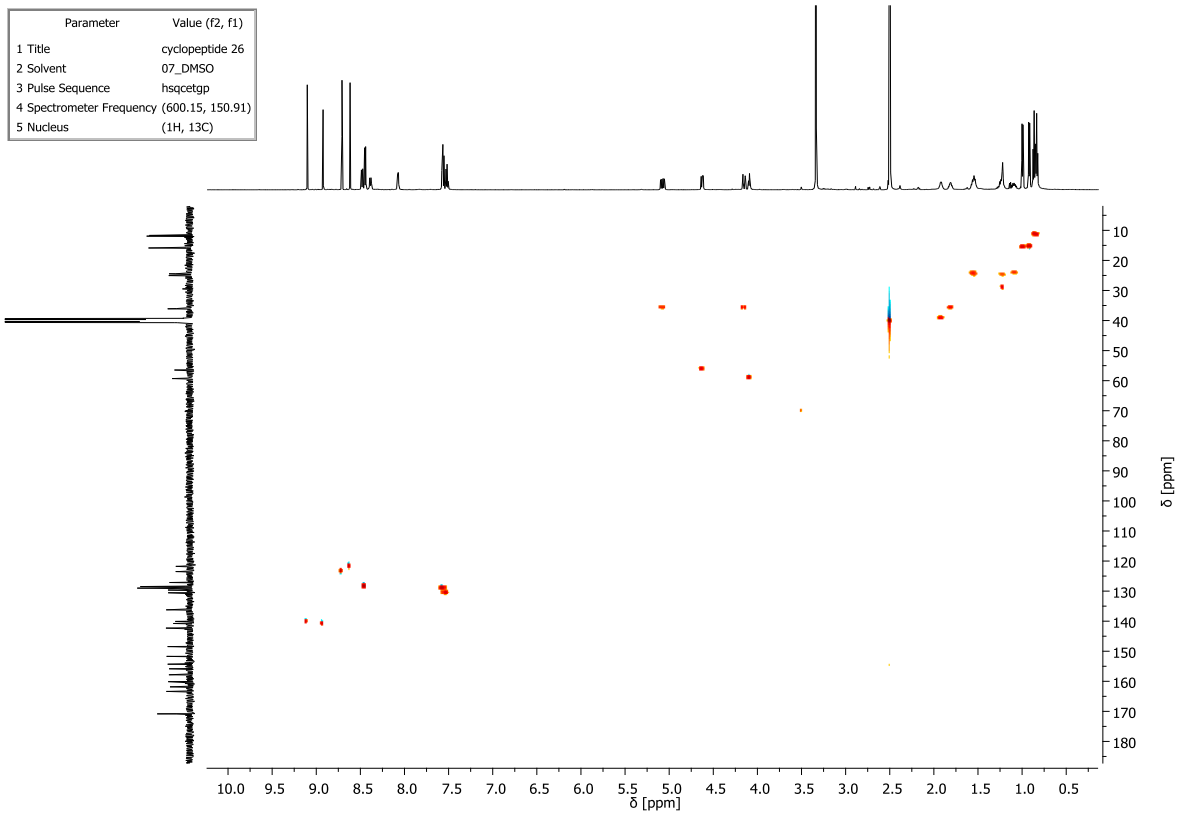

Parameter Value
1 Solvent dioxane-d8
2 Temperature 297.0
3 Spectrometer Frequency 400.13
4 Nucleus 1H
δ [ppm]

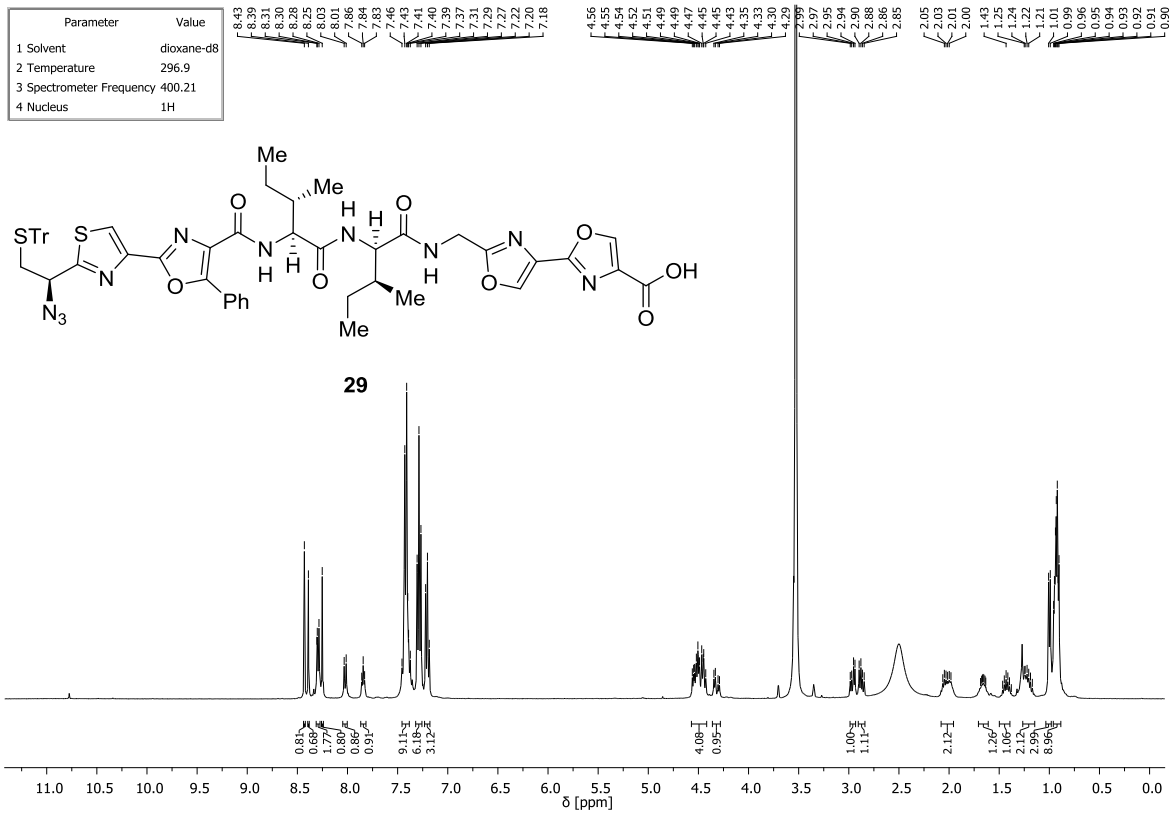
28

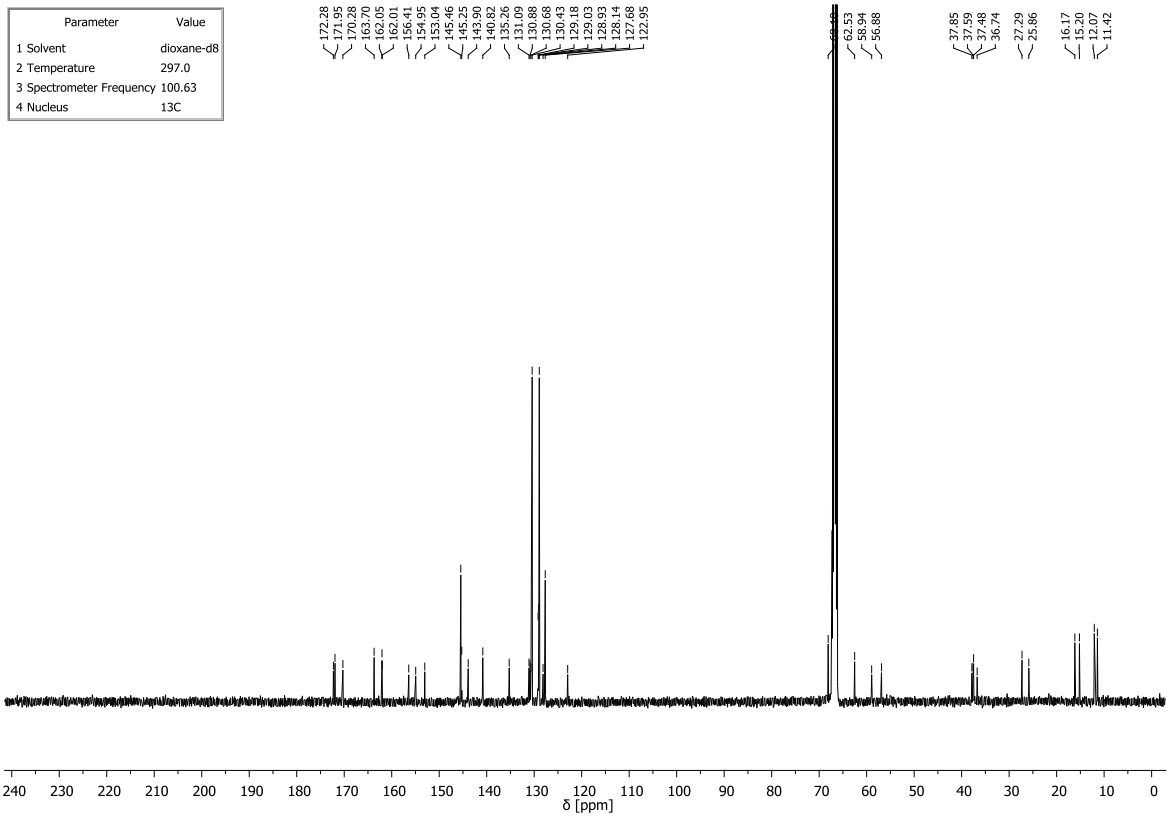

Parameter Value
1 Solvent dioxane-d8
2 Temperature 297.0
3 Spectrometer Frequency 100.61
4 Nucleus 13C
δ [ppm]

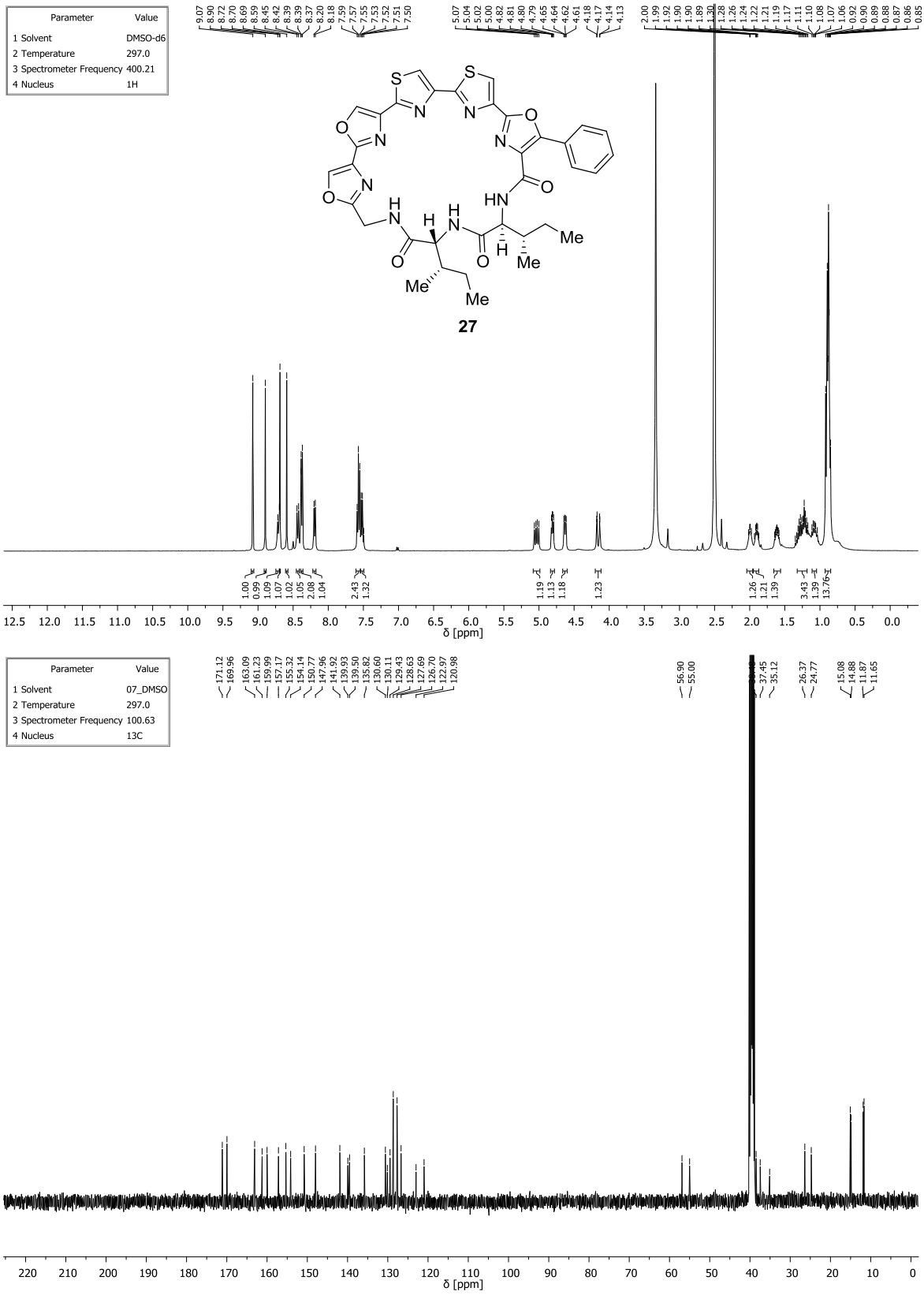

Parameter Value
1 Solvent DMSO-d6
2 Temperature 297.0
3 Spectrometer Frequency 400.21
4 Nucleus 1H
9.10 8.92 8.71 8.62 8.49 8.47 8.46 8.46 8.44 8.40 8.38 8.08 8.07 7.58 7.57 7.55 7.53 7.52 7.52
5.11 5.09 5.07 5.04 4.63 4.62 4.61 4.61 4.13 4.10 4.09 4.07
1.93 1.92 1.91 1.82 1.81 1.80 1.58 1.56 1.55 1.54 1.53 1.27 1.24 1.11 1.09 1.09 1.00 0.99 0.93 0.91 0.88 0.86 0.85 0.84 0.83 0.82
1.00 1.05 1.05 1.01 1.08 2.08 1.30 1.00 3.35 1.25 1.20 1.08 1.14 1.42 1.23 2.31 1.22 0.97 3.08 3.17 6.77
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ [ppm]
26
Parameter Value
1 Solvent DMSO-d6
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
170.36 162.90 161.38 159.65 157.36 155.37 153.85 151.24 147.98 141.84 140.31 139.62 135.75 130.19 130.08 129.17 128.54 128.02 126.67 123.03 121.30
58.83 55.99
35.63 35.55 24.58 23.99 15.62 15.39 11.46 11.22
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
δ [ppm]

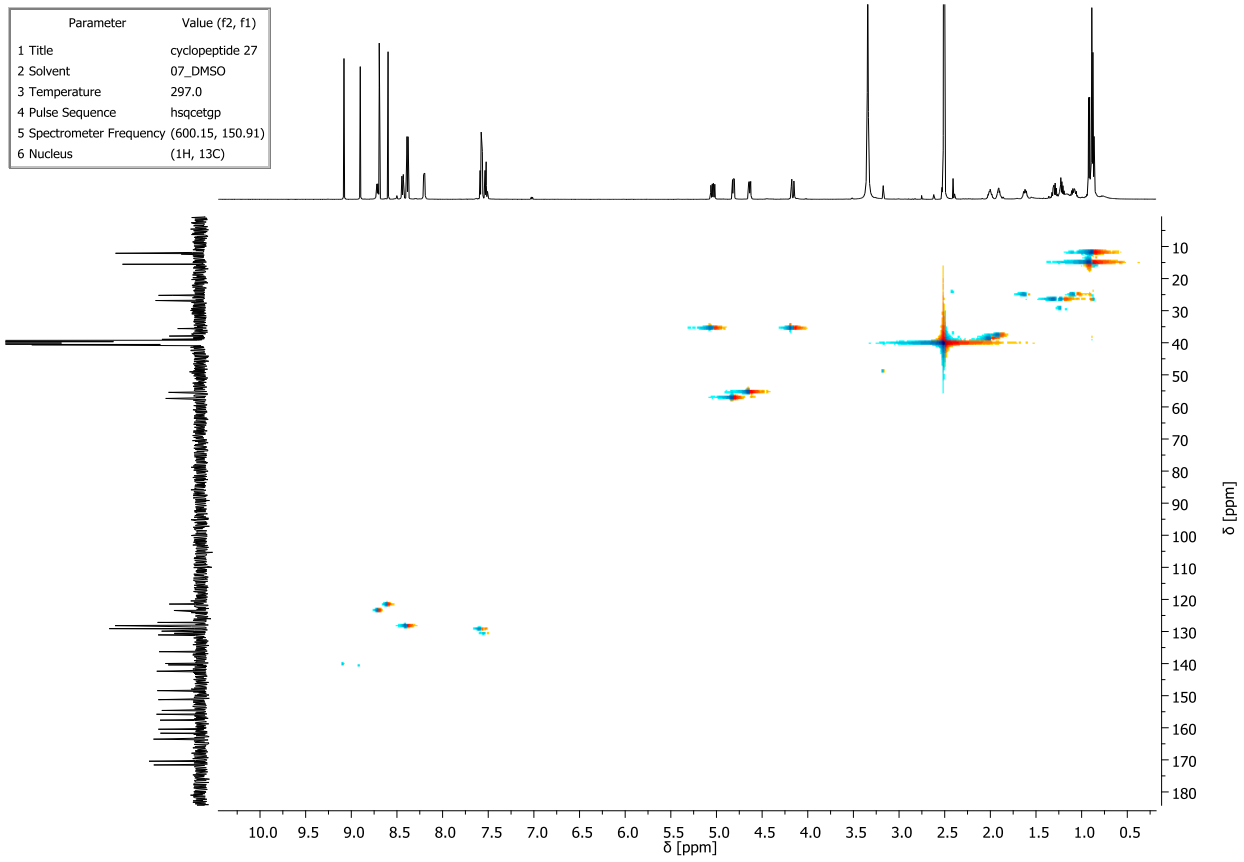

Parameter Value (f2, f1)
1 Title cyclopeptide 26
2 Solvent 07_DMSO
3 Pulse Sequence cosygpppqf
4 Spectrometer Frequency (600.15, 600.15)
5 Nucleus (1H, 1H)
δ [ppm]
δ [ppm]

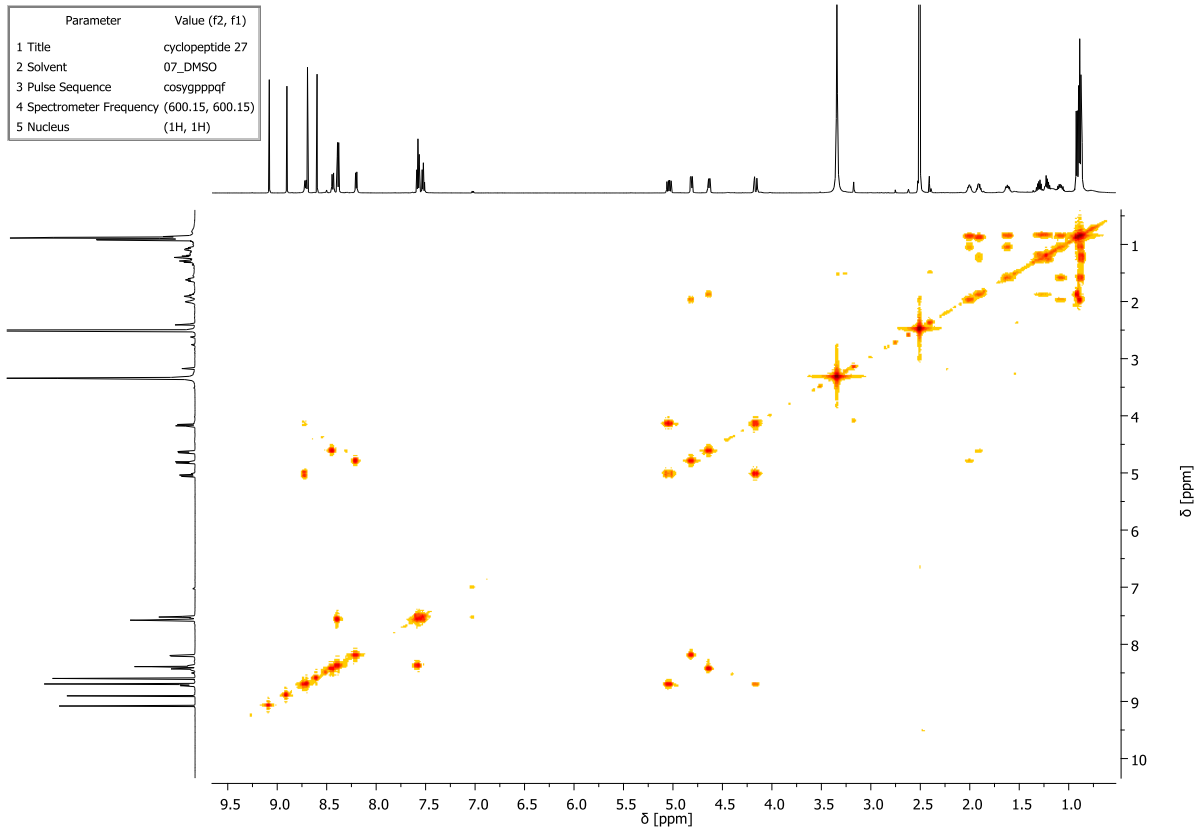

Parameter Value (f2, f1)
1 Title cyclopeptide 26
2 Solvent 07_DMSO
3 Pulse Sequence hsqcetgp
4 Spectrometer Frequency (600.15, 150.91)
5 Nucleus (1H, 13C)
δ [ppm]
δ [ppm]

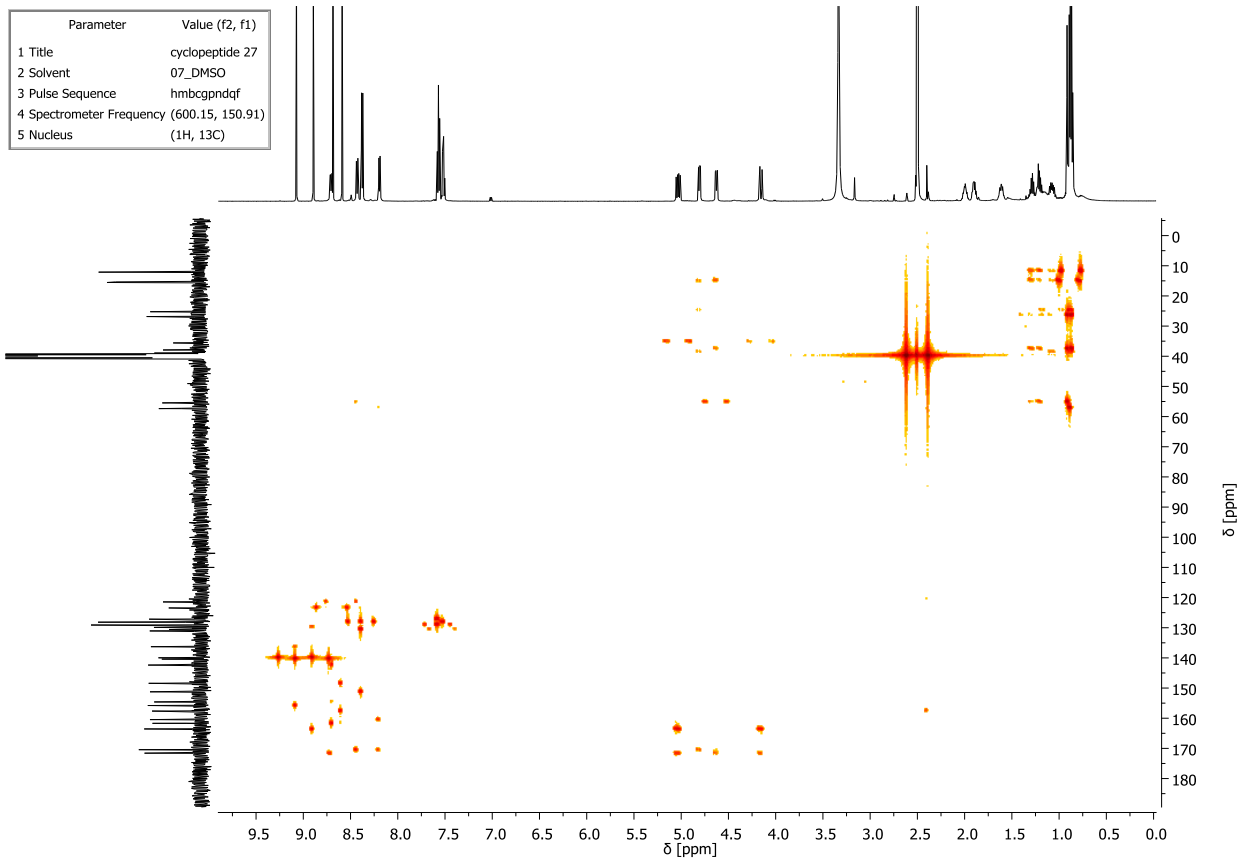

Parameter Value
1 Solvent dioxane-d8
2 Temperature 296.9
3 Spectrometer Frequency 400.21
4 Nucleus 1H
29
δ [ppm]

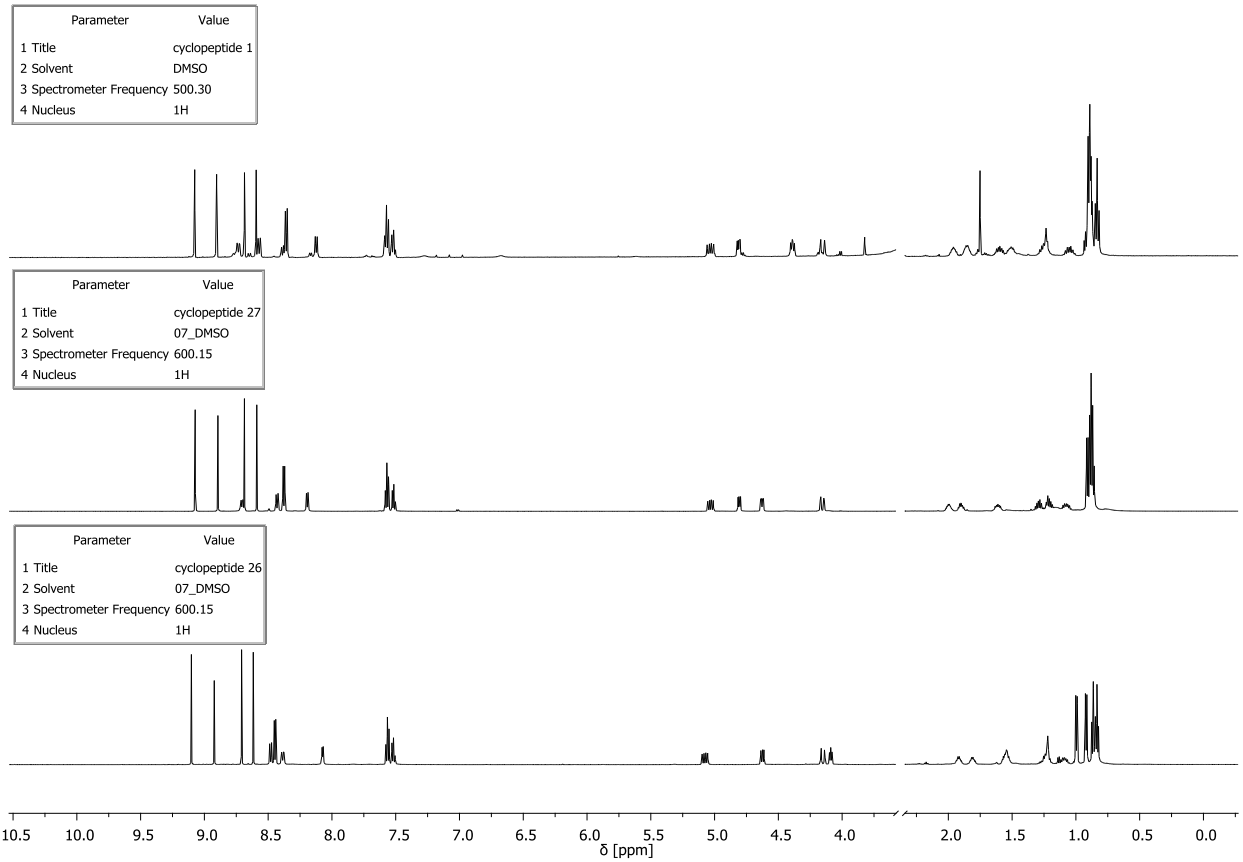

Parameter Value
1 Solvent dioxane-d8
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
δ [ppm]

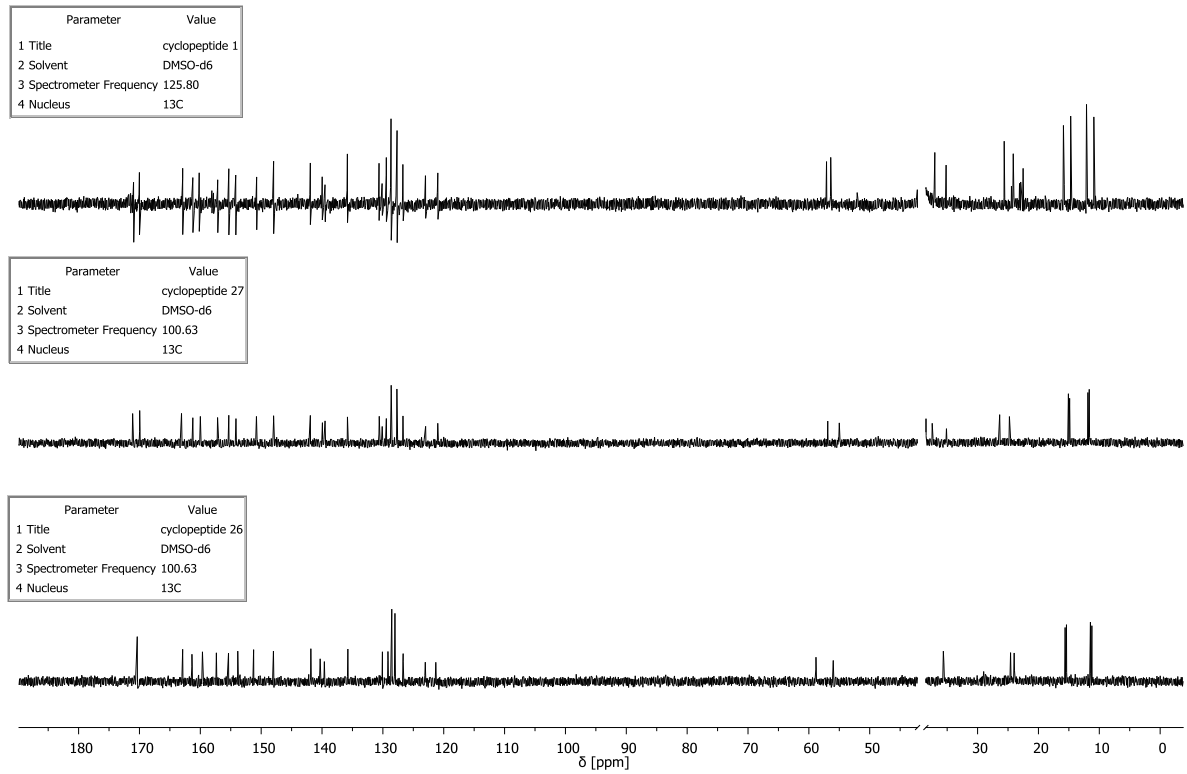

Parameter Value
1 Solvent DMSO-d6
2 Temperature 297.0
3 Spectrometer Frequency 400.21
4 Nucleus 1H
27
δ [ppm]
Parameter Value
1 Solvent 07_DMSO
2 Temperature 297.0
3 Spectrometer Frequency 100.63
4 Nucleus 13C
δ [ppm]


Parameter Value (f2, f1)
1 Title cyclopeptide 27
2 Solvent 07_DMSO
3 Temperature 297.0
4 Pulse Sequence hsqcetgp
5 Spectrometer Frequency (600.15, 150.91)
6 Nucleus (1H, 13C)
δ [ppm]
δ [ppm]


Parameter Value (f2, f1)
1 Title cyclopeptide 27
2 Solvent 07_DMSO
3 Pulse Sequence cosygpppqf
4 Spectrometer Frequency (600.15, 600.15)
5 Nucleus (1H, 1H)
δ [ppm]
δ [ppm]


Parameter Value (f2, f1)
1 Title cyclopeptide 27
2 Solvent 07_DMSO
3 Pulse Sequence hmbcgpndqf
4 Spectrometer Frequency (600.15, 150.91)
5 Nucleus (1H, 13C)
δ [ppm]
δ [ppm]


Parameter Value
1 Title cyclopeptide 1
2 Solvent DMSO
3 Spectrometer Frequency 500.30
4 Nucleus 1H
Parameter Value
1 Title cyclopeptide 27
2 Solvent 07_DMSO
3 Spectrometer Frequency 600.15
4 Nucleus 1H
Parameter Value
1 Title cyclopeptide 26
2 Solvent 07_DMSO
3 Spectrometer Frequency 600.15
4 Nucleus 1H
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 2.0 1.5 1.0 0.5 0.0
δ [ppm]


Parameter Value
1 Title cyclopeptide 1
2 Solvent DMSO-d6
3 Spectrometer Frequency 125.80
4 Nucleus 13C
Parameter Value
1 Title cyclopeptide 27
2 Solvent DMSO-d6
3 Spectrometer Frequency 100.63
4 Nucleus 13C
Parameter Value
1 Title cyclopeptide 26
2 Solvent DMSO-d6
3 Spectrometer Frequency 100.63
4 Nucleus 13C
180 170 160 150 140 130 120 110 100 90 80 70 60 50 30 20 10 0
δ [ppm]

6 References

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