Supporting Information

Synthetic Fermentation of β-Peptide Macrocycles by Thiadiazole-Forming Ring-Closing Reactions

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General Methods

Reactions and Purifications

Reactions were carried out under air unless otherwise stated. Thin layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm) pre-coated with silica gel 60 F254 and visualized by UV quenching and/or staining with potassium permanganate stain and warming with a heat gun. Flash column chromatography was performed under a forced-flow of air using Silicycle SiliaFlash F60 (40-63 mm particle size). Macrocycle mixtures were analyzed and purified by reversed phase high performance liquid chromatography (RP-HPLC) on Jasco analytical and preparative instruments with dual pumps, mixer and in-line degasser, a variable wavelength UV detector (simultaneous monitoring of the eluent at 220 nm, 254 nm, 301 nm) and a Rheodyne 7725i injector fitted with a 20 μL injection loop. The mobile phase for analytical and preparative HPLC were Millipore-H_2O with 0.1% TFA (Buffer A) and HPLC grade CH_3CN with 0.1% TFA (Buffer B). Analytical HPLC was performed on Shiseido C18 (5 μm, 4.6 mm I.D. x 250 mm) column at a flow rate of 1 mL/min. Preparative HPLC was performed on YMC C18 (5 μm, 20 mm I.D. x 250 mm) column at a flow rate of 10 mL/min. LCMS analysis was performed on Dionex UltiMate 3000 RSLC connected to a Surveyor MSQ Plus mass spectrometer; a reversed-phase RESTEK Pinnacle DB C18 (4.6 x 50 mm) column was used, running a gradient of 5 to 100% CH_3CN in H_2O over 6.5 min, 100% CH_3CN for 2.5 min.

Characterization

NMR spectra were recorded on Bruker AV-400 or AV-III-600 instruments. Chemical shifts (δ) are given in ppm relative to residual solvent peaks. Data for 1H NMR are reported as follows: chemical shift (multiplicity, coupling constants where applicable, number of hydrogens). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), dd (doublet of doublet), m (multiplet), br (broad), ABq (AB quartet). IR spectra were recorded on a Jasco FT/IR-4100 spectrometer and major peaks are reported in frequency of absorption (cm^{-1}). Optical rotations were measured on a Jasco P-2000 operating at the sodium D line with a 100 mm path length cell. High-resolution mass spectra were obtained by the mass spectrometry service of the ETH Zürich Laboratorium für Organische Chemie on a Bruker Daltonics maXis ESI-QTOF spectrometer (ESI).

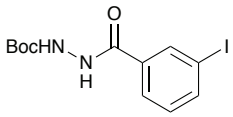
Solvents and Reagents

All organic solvents (CH_3CN, DMF, ^tBuOH, Et_2O, MeOH) were used as supplied (ACS or HPLC grade) unless otherwise stated. THF was purified by distillation over sodium benzophenone ketyl prior to use. CH_2Cl_2 was purified by distillation over calcium hydride. H_2O used for reactions was obtained from a Millipore purification system. All other starting materials were used as supplied by commercial vendors or prepared by the method described in the corresponding reference.

Synthesis of Building Blocks

1.1 Synthesis of Initiator 16

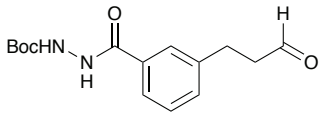
1.1.1 *3-Iodophenylhydrazide* **19**



EDCI·HCl (0.95 g, 4.98 mmol, 1.1 equiv) was added to a suspension of 3-iodobenzoic acid (1.20 g, 4.98 mmol, 1.1 equiv) and HOBt (0.67 g, 4.98 mmol, 1.1 equiv) in CH_2Cl_2 (23 mL) at rt. The mixture was stirred for 10 min to give a yellow solution. *t*-Butylcarbazate (0.60 g, 4.52 mmol, 1.0 equiv) and EtN^iPr_2 (3.2 mL, 18.1 mmol, 4.0 equiv) were added and the mixture was stirred for 16 h. The resulting suspension was cooled to 0 ºC, filtered and washed with cold CH_2Cl_2, to afford the desired product **19** (1.05 g, 64%) as a white solid.

MP 198–199 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.96 (s, 1H), 8.19 (s, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 1.43 (s, 9H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.6, 155.4, 140.3, 135.8, 134.5, 130.7, 126.7, 94.7, 79.3, 28.1; **IR** (u/cm^{-1}, thin film): 3232 (br), 2980, 1718, 1659, 1425, 1394, 1366, 1250, 1152, 1066, 699; **HRMS** (ESI): calculated for $C_{12}H_{15}IN_2NaO_3$ $[M+Na]^+$: 385.0020, found: 385.0025.

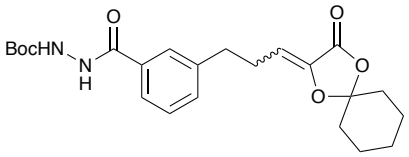
1.1.2 *Aldehyde* **21**



3-Iodophenylhydrazide **19** (1.00 g, 2.76 mmol, 1.0 equiv), $Pd(OAc)_2$ (37 mg, 0.17 mmol, 6 mol%), tetrabutylammonium bromide (0.89 g, 2.76 mmol, 1.0 equiv), $NaHCO_3$ (0.58 g, 6.90 mmol, 2.5 equiv) and MS 4Å were placed under a N_2 atmosphere in a flame dried flask. DMF (8.3 mL) was added, followed by allyl alcohol (0.28 mL, 4.14 mmol, 1.5 equiv) and the mixture was heated to 70 ºC for 4 h. The resulting mixture was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, and concentrated *in vacuo.* The crude mixture was purified by flash chromatography (hexanes/EtOAc, 4:1→1:1) to afford the desired product **21** (0.66 g, 81%) as a white solid.

MP 60–61 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 8.95 (br. s, 1H), 7.67 (s, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.38 – 7.17 (m, 2H), 6.96 (br. s, 1H), 2.91 (t, *J* = 7.5 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 1.48 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 201.3, 166.9, 156.4, 141.1, 132.4, 132.0, 128.9, 127.3, 125.4, 82.2, 45.0, 28.2, 27.9; **IR** (υ/cm^{-1}, thin film): 3281 (br), 2978, 2932, 1713, 1661, 1524, 1480, 1393, 1367, 1252, 1156, 730; **HRMS** (ESI): calculated for $C_{15}H_{20}N_2NaO_4$ $[M+Na]^+$: 315.1315, found: 315.1316.

1.1.3 *Enol ester* **23**

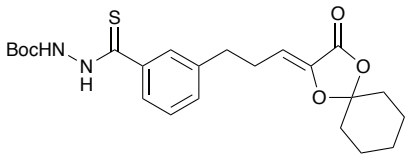


Tetramethylguanidine (0.25 mL, 2.05 mmol, 1.2 equiv) was added dropwise to a mixture of phosphonate **22**[1,2] (0.55 g, 1.88 mmol, 1.1 equiv) and LiCl (86 mg, 2.05 mmol, 1.2 equiv) in THF (9 mL) at –10 °C under an atmosphere of N_2. After stirring for 30 min a solution of aldehyde **21** (0.50 g, 1.71 mmol, 1.0 equiv) in THF (2 mL) was added. The resulting mixture was stirred for 10 min, diluted with sat. aq. NH_4Cl and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo.* The crude mixture was purified

by flash chromatography (hexanes/EtOAc, 4:1→2:1) to afford the desired product **23** (0.53 g, 72%, 2:1 mixture of isomers) as a white solid.

MP 46–48 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.26 (br. s, 1H), 7.68 – 7.58 (m, 2H), 7.36 – 7.28 (m, 2H), 6.80 (br. s, 1H), 5.56* (t, *J* = 7.7 Hz, 0.33H), 5.47 (t, *J* = 8.3 Hz, 0.66H), 2.92 – 2.81 (m, 1.33H), 2.79-2.72 (m, 2H), 2.35 – 2.48* (m, 0.66H), 1.79 – 1.61 (m, 8H), 1.49 – 1.39 (m, 11H). (*signals from minor isomer); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.1, 167.0, 163.0, 162.6, 156.0, 141.7, 141.6, 139.0, 138.1, 132.5, 132.4, 132.0, 128.7, 128.7, 127.6, 127.6, 125.2, 125.1, 113.2, 111.9, 111.0, 108.8, 82.0, 82.0, 36.2, 36.1, 35.7, 34.5, 28.2, 27.0, 25.7, 24.3, 24.3, 22.9, 22.8 (mixture of isomers); **IR** (υ/cm^{-1}, thin film): 3283 (br), 2939, 2855, 1780, 1721, 1667, 1480, 1452, 1368, 1250, 1158, 940, 909, 730; **HRMS** (ESI): calculated for $C_{23}H_{30}N_2NaO_6$ $[M+Na]^+$: 453.1996, found: 453.1996.

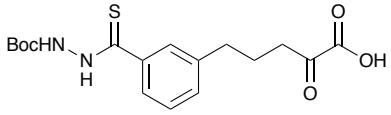
1.1.4 *Thiohydrazide* **24**



A suspension of enol ester **23** (0.28 g, 0.65 mmol, 1.0 equiv) and Lawesson's reagent (0.26 g, 0.65 mmol, 1.0 equiv) in THF (1.2 mL) was stirred at 45 °C for 6 h under an atmosphere of N_2. The mixture was filtered through a silica plug, washing hexanes/EtOAc (4:1), and the filtrate was concentrated *in vacuo*. The crude mixture was purified by flash chromatography (hexanes/EtOAc, 9:1) to afford the desired product **24** (0.29 g, 70%, single isomer) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (br. s, 1H), 8.86 (br. s, 1H), 7.62 (s, 1H), 7.58 – 7.56 (m, 1H), 7.32 – 7.30 (m, 2H), 5.56 (t, *J* = 7.7 Hz, 1H), 2.79 (t, *J* = 7.7 Hz, 2H), 2.52 (dt, *J* = 7.7, 7.7 Hz, 2H), 1.79 – 1.63 (m, 8H), 1.51 (s, 9H), 1.49 – 1.38 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.0, 153.6, 141.9, 139.1, 138.2, 131.9, 128.8, 127.3, 124.7, 111.9, 108.6, 83.5, 36.2, 34.5, 28.3, 27.0, 24.3, 22.9. (C=S carbon not observed); **IR** (υ/cm^{-1}, thin film): 3263 (br), 2938, 2803, 1784, 1719, 1449, 1367, 1264, 1247, 1154, 936, 734, 697; **HRMS** (ESI): calculated for $C_{23}H_{30}N_2NaO_5S$ $[M+Na]^+$: 469.1768, found: 469.1774.

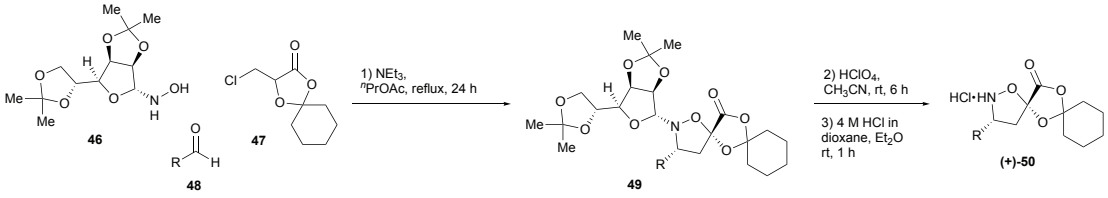
1.1.5 α-*Ketoacid* **16**



Aqueous NaOH (2 M, 0.22 mL, 0.44 mmol, 2.0 equiv) was added to a solution of thiohydrazide **24** (0.10 g, 0.22 mmol, 1.0 equiv) in methanol (1.1 mL) and the reaction was stirred at rt for 15 min. The mixture was diluted with H_2O and washed with Et_2O. The aqueous phase was acidified to pH 1 with 3 M HCl and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired product **16** (80 mg, 99%) as a yellow oil that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (br. s, 1H), 8.85 (br. s, 1H), 7.62 (d, *J* = 7.3 Hz, 1H), 7.53 (s, 1H), 7.35 – 7.27 (m, 2H), 2.89 (t, *J* = 7.2 Hz, 2H), 2.70 (t, *J* = 7.2 Hz, 2H), 2.01 (tt, *J* = 7.2, 7.2 Hz, 2H), 1.53 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 195.5, 160.3, 154.2, 141.5, 138.4, 132.0, 129.1, 127.1, 125.5, 84.0, 36.7, 34.7, 28.3, 24.8. (C=S carbon not observed); **IR** (u/cm^{-1}, thin film): 3258 (br), 2979, 2934, 1713, 1427, 1368, 1250, 1152, 735, 698; **HRMS** (ESI): calculated for $C_{17}H_{22}N_2NaO_5S$ $[M+Na]^+$: 389.1142, found: 389.1144.

1.2 Synthesis of Monomers



Monomers were prepared using a previously described three-step procedure from 2,3:5,6-*O*-diisopropylidene-D-gulose oxime (**46**), 5-chloromethyl-2,2-pentamethylene-1,3-dioxolan-4-one (**47**) and commercially available or known aldehydes **48**.[2,3]

General procedure:

1) A solution of NEt_3 (2.0 equiv) and dioxolanone **47** (1.0 equiv) in ^{n}PrOAc (0.5 M) was heated to reflux for 18 h. Oxime **46** (1.0 equiv) and aldehyde **48** (1.0 equiv) were added and the mixture was heated to reflux for 24 h. The reaction mixture was

diluted with EtOAc, washed with 1 M HCl and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by flash chromatography and/or recrystallization to afford the desired cycloaddition products **49**.

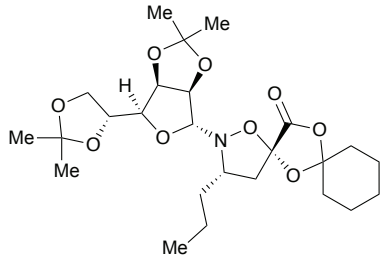
2) $HClO_4$ (70%, 3.0 equiv) was added dropwise to a solution of cycloaddition product **49** (1.0 equiv) in CH_3CN (0.1 M) at rt. The reaction was stirred for 6 h, sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by flash chromatography to afford the unprotected isoxazolidine product.

3) HCl in dioxane (4 M, 1.1 equiv) was added dropwise to a solution of unprotected isoxazolidine (1.0 equiv) in Et_2O (0.1 M). After stirring at rt for 30 min a precipitate was formed. The precipitate was collected by filtration, washed with Et_2O and dried under vacuum to afford the desired monomer HCl salt **50**.

For monomers previously reported (**25, 30** and **37**), the syntheses were performed according to the literature procedures.[2,3]

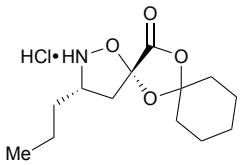
1.2.1 Characterization Data for New Monomers and Related Intermediates

n-Propyl monomer intermediate **51**



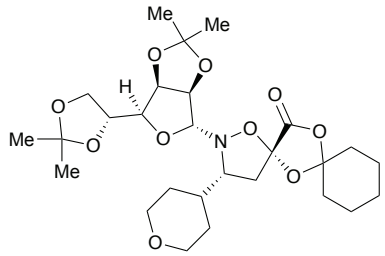
$[\alpha]_D^{28}$ (c = 0.53, $CHCl_3$) = +19.4; **MP** 90–91°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87 (d, *J* = 6.1 Hz, 1H), 4.69 – 4.64 (m, 2H), 4.36 (dt, *J* = 8.5, 6.7 Hz, 1H), 4.19 (dd, *J* = 8.6, 6.8 Hz, 1H), 4.03 (dd, *J* = 8.5, 3.9 Hz, 1H), 3.85 – 3.78 (m, 1H), 3.71 (dd, *J* = 8.6, 6.6 Hz, 1H), 2.92 (dd, *J* = 13.8, 7.7 Hz, 1H), 2.11 (dd, *J* = 13.8, 1.9 Hz, 1H), 1.92 – 1.58 (m, 9H), 1.50 – 1.33 (m, 14H), 1.28 (s, 3H), 0.94 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.6, 113.0, 111.8, 109.9, 105.8, 96.9, 84.5, 84.4, 80.3, 75.7, 66.2, 60.5, 41.1, 37.7, 36.5, 35.6, 26.8, 26.2, 25.4, 25.1, 24.4, 23.1, 23.0, 20.3, 13.9; **IR** (u/cm^{-1}, thin film): 2988, 2939, 1754, 1380, 1372, 1216, 1093, 1060, 1031, 844; **HRMS** (ESI): calculated for $C_{25}H_{40}NO_9$ $[M+H]^+$: 498.2698, found: 498.2701.

n-Propyl monomer **26**



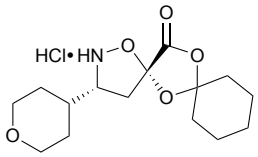
$[\alpha]_D^{28}$ (c = 0.52, MeOH) = +34.9; **MP** 113–114 °C; **^{1}H NMR** (400 MHz, MeOD) δ 4.19 (ddt, *J* = 7.6, 7.6, 7.6 Hz, 1H), 3.14 (dd, *J* = 14.3, 7.6 Hz, 1H), 2.56 (dd, *J* = 14.3, 7.6 Hz, 1H), 1.95 – 1.83 (m, 6H), 1.80 – 1.65 (m, 4H), 1.56 – 1.43 (m, 4H), 1.03 (t, *J* = 7.3 Hz, 3H); **^{13}C NMR** (101 MHz, MeOD) δ 166.4, 114.6, 108.1, 62.9, 41.0, 38.2, 36.6, 32.6, 25.1, 24.0, 23.9, 20.8, 14.0; **IR** (u/cm^{-1}, thin film): 2959, 2859, 1807, 1377, 1292, 1258, 1191, 1139, 915, 718; **HRMS** (ESI): calculated for $C_{13}H_{22}NO_4$ [M-Cl]$^+$ 256.1543, found: 256.1550.

Pyran monomer intermediate **52**



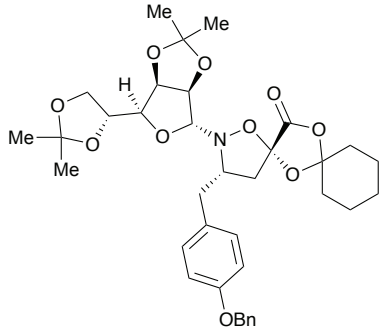
$[\alpha]_D^{25}$ (c = 0.53, $CHCl_3$) = −15.3; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.85 (d, *J* = 6.1 Hz, 1H), 4.69 (s, 1H), 4.65 (dd, *J* = 6.1, 3.9 Hz, 1H), 4.35 (dt, *J* = 8.5, 6.7 Hz, 1H), 4.20 (dd, *J* = 8.6, 6.9 Hz, 1H), 4.02 – 3.94 (m, 3H), 3.72 (dd, *J* = 8.6, 6.5 Hz, 1H), 3.58 – 3.53 (m, 1H), 3.43 – 3.33 (m, 2H), 2.79 (dd, *J* = 14.1, 8.0 Hz, 1H), 2.31 (dd, *J* = 14.1, 1.1 Hz, 1H), 1.92 – 1.56 (m, 11H), 1.47 – 1.25 (m, 16H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.5, 113.1, 111.8, 109.9, 106.0, 96.7, 84.7, 84.3, 80.3, 75.7, 67.8, 67.6, 66.1, 65.4, 37.7, 36.7, 36.6, 36.5, 31.3, 30.2, 26.9, 26.2, 25.3, 25.0, 24.4, 23.1, 23.1; **IR** (u/cm^{-1}, thin film): 2986, 2939, 2856, 1800, 1450, 1372, 1249, 1237, 1210, 1086, 1032, 847, 732; **HRMS** (ESI): calculated for $C_{27}H_{41}NNaO_{10}$ [M+Na]$^+$: 562.2623, found: 562.2620.

Pyran monomer **27**



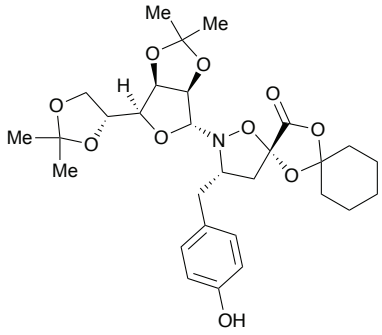
$[\alpha]_D^{28}$ (c = 0.51, MeOH) = +28.1; **MP** 129–132 °C; **^{1}H NMR** (400 MHz, MeOD) δ 4.01 – 3.89 (m, 3H), 3.50 – 3.39 (m, 2H), 3.17 (dd, *J* = 14.4, 7.5 Hz, 1H), 2.60 (dd, *J* = 14.4, 9.9 Hz, 1H), 2.12-2.02 (m, 1H), 1.94 – 1.83 (m, 4H), 1.81 – 1.60 (m, 6H), 1.57 – 1.46 (m, 4H); **^{13}C NMR** (101 MHz, MeOD) δ 165.3, 113.2, 106.9, 66.6, 66.2, 38.2, 36.8, 35.8, 35.3, 30.0, 29.0, 23.7, 22.6, 22.5; **IR** (υ/cm^{-1}, thin film): 2929, 2863, 2834, 1799, 1279, 1250, 1244, 1199, 1088, 943, 711; **HRMS** (ESI): calculated for $C_{15}H_{24}NO_5$ [M-Cl]$^+$: 298.1649, found: 298.1650

4-Benzyloxybenzyl monomer intermediate **53**



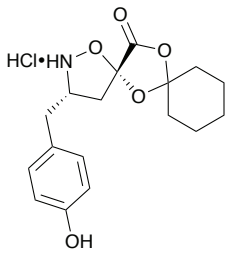
$[\alpha]_D^{26}$ (c = 0.53, $CHCl_3$) = –24.3; **MP** 123–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.30 (m, 5H), 7.13 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.03 (s, 2H), 4.85 (d, *J* = 6.1 Hz, 1H), 4.69 (s, 1H), 4.57 (dd, *J* = 6.0, 4.1 Hz, 1H), 4.32 – 4.26 (m, 1H), 4.13 (dd, *J* = 8.4, 6.6 Hz, 1H), 4.10 – 4.04 (m, 1H), 3.64 (dd, *J* = 8.4, 4.1 Hz, 1H), 3.56 (dd, *J* = 8.4, 6.9 Hz, 1H), 3.01 (dd, *J* = 13.7, 7.7 Hz, 1H), 2.82 – 2.73 (m, 2H), 2.20 (dd, *J* = 14.0, 1.7 Hz, 1H), 1.94 – 1.88 (m, 2H), 1.81 – 1.67 (m, 6H), 1.50 – 1.42 (m, 8H), 1.37 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 157.6, 137.2, 130.9, 130.6, 128.7, 128.1, 127.6, 114.9, 113.0, 111.8, 109.8, 105.9, 96.4, 84.5, 84.1, 80.2, 75.7, 70.1, 66.1, 61.7, 39.8, 38.5, 37.7, 36.5, 27.1, 26.2, 25.5, 25.0, 24.4, 23.2, 23.1; **IR** (υ/cm^{-1}, thin film): 2990, 2940, 1798, 1512, 1376, 1239, 1209, 1156, 1089, 1066, 1037, 847; **HRMS** (ESI): calculated for $C_{36}H_{46}NO_{10}$ [M+H]$^+$: 652.3116, found: 652.3104.

4-Hydroxybenzyl monomer intermediate **54**



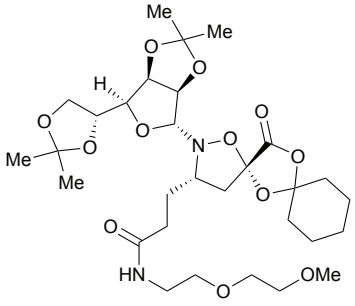
$[\alpha]_D^{28}$ (c = 0.52, $CHCl_3$) = –26.2; **MP** 83–85 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.5 Hz, 2H), 6.75 (d, *J* = 8.5 Hz, 2H), 5.45 (s, 1H), 4.84 (d, *J* = 6.0 Hz, 1H), 4.68 (s, 1H), 4.56 (dd, *J* = 6.1, 4.0 Hz, 1H), 4.32 – 4.27 (m, 1H), 4.13 (dd, *J* = 8.4, 6.6 Hz, 1H), 4.07 – 4.00 (m, 1H), 3.62 – 3.55 (m, 2H), 2.98 (dd, *J* = 13.8, 8.0 Hz, 1H), 2.80 – 2.70 (m, 2H), 2.18 (dd, *J* = 13.9, 1.7 Hz, 1H), 1.93 – 1.87 (m, 2H), 1.82 – 1.65 (m, 6H), 1.48 – 1.40 (m, 8H), 1.37 (s, 3H), 1.27 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.4, 154.4, 130.8, 130.6, 115.5, 113.0, 111.9, 109.8, 106.0, 96.4, 84.5, 84.0, 80.2, 75.7, 66.1, 61.7, 39.8, 38.5, 37.7, 36.5, 26.9, 26.1, 25.3, 24.9, 24.4, 23.2, 23.0; **IR** (u/cm^{-1}, thin film): 2987, 2938, 1799, 1516, 1372, 1228, 1210, 1085, 1066, 1038, 844; **HRMS** (ESI): calculated for $C_{29}H_{40}NO_{10}$ $[M+H]^+$: 562.2647, found: 562.2648.

4-Hydroxybenzyl monomer **28**



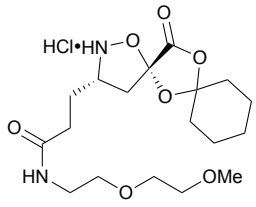
$[\alpha]_D^{28}$ (c = 0.53, MeOH) = +47.9; **MP** 120–122°C; **^{1}H NMR** (400 MHz, MeOD) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 4.43 – 4.35 (m, 1H), 3.12 (d, *J* = 8.5 Hz, 2H), 3.03 (dd, *J* = 14.4, 7.5 Hz, 1H), 2.59 (dd, *J* = 14.4, 6.7 Hz, 1H), 1.95 – 1.87 (m, 4H), 1.82 – 1.65 (m, 4H), 1.57 – 1.48 (m, 2H); **^{13}C NMR** (101 MHz, MeOD) δ 166.4, 158.3, 131.2, 126.9, 117.0, 114.6, 108.0, 64.3, 40.4, 38.3, 36.6, 35.5, 25.1, 24.0, 24.0; **IR** (u/cm^{-1}, thin film): 3318, 2940, 2875, 1807, 1519, 1269, 1213, 1190, 1153, 1116, 920, 872, 839, 720; **HRMS** (ESI): calculated for $C_{17}H_{22}NO_5$ $[M-Cl]^+$: 320.1492, found: 320.1493.

Amide monomer intermediate **55**



$[\alpha]_D^{28}$ (c = 0.52, $CHCl_3$) = +31.6; **MP** 144–145°C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.53 (t, *J* = 5.5 Hz, 1H), 4.87 (d, *J* = 6.0 Hz, 1H), 4.67 – 4.61 (m, 2H), 4.35 (dt, *J* = 8.2, 7.0 Hz, 1H), 4.22 (dd, *J* = 8.6, 6.7 Hz, 1H), 4.09 (dd, *J* = 8.2, 3.7 Hz, 1H), 3.74 – 3.67 (m, 2H), 3.61 – 3.47 (m, 7H), 3.40 – 3.34 (m, 4H), 2.95 (dd, *J* = 13.9, 8.1 Hz, 1H), 2.37 – 2.30 (m, 1H), 2.17 – 2.10 (m, 2H), 2.08 – 1.98 (m, 1H), 1.92 – 1.59 (m, 9H), 1.47 – 1.36 (m, 11H), 1.28 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.6, 169.2, 113.2, 111.9, 110.0, 106.0, 97.5, 84.6, 83.7, 80.3, 75.8, 72.0, 70.3, 69.9, 66.1, 61.1, 59.1, 41.0, 39.5, 37.7, 36.5, 34.5, 30.0, 26.8, 26.2, 25.5, 25.1, 24.4, 23.1, 22.9; **IR** (u/cm^{-1}, thin film): 2988, 2939, 1755, 1379, 1372, 1217, 1060, 1031, 845; **HRMS** (ESI): calculated for $C_{30}H_{49}N_2O_{12}$ $[M+H]^+$: 629.3280, found: 629.3281.

Amide monomer **29**



$[\alpha]_D^{24}$ (c = 0.49, MeOH) = –17.5; **^{1}H NMR** (400 MHz, MeOD) δ 4.33 – 4.26 (m, 1H), 3.62 – 3.60 (m, 2H), 3.57 – 3.54 (m, 4H), 3.41 – 3.37 (m, 5H), 3.15 (dd, *J* = 14.4, 7.9 Hz, 1H), 2.66 – 2.49 (m, 3H), 2.28 – 2.09 (m, 2H), 1.93 – 1.85 (m, 4H), 1.76-1.66 (m, 4H), 1.54 – 1.47 (m, 2H); **^{13}C NMR** (101 MHz, MeOD) δ 175.0, 166.2, 114.5, 107.8, 73.0, 71.0, 70.3, 62.8, 59.2, 41.3, 40.6, 38.2, 36.6, 32.9, 26.4, 25.0, 24.0, 23.9; **IR** (u/cm^{-1}, thin film): 2938, 2857, 1798, 1651, 1269, 1238, 1177, 1139, 1090, 929; **HRMS** (ESI): calculated for $C_{18}H_{31}N_2O_7$ $[M-Cl]^+$: 387.2122, found: 387.2126.

1.3 Synthesis of Thiohydrazides

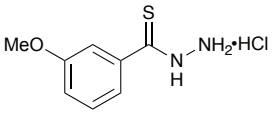
General procedure:

1) EDCI·HCl (1.1 equiv) was added to a suspension of carboxylic acid (1.1 equiv) and HOBt (1.1 equiv) in CH_2Cl_2 (0.2 M) at rt. The mixture was stirred for 10 min, *t*-butylcarbazate (1.0 equiv) and DIPEA (4 equiv) were added and the mixture was stirred for 16 h. The reaction mixture was diluted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$, 1 M HCl and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by flash chromatography (hexanes/EtOAc) to afford the desired hydrazide product.

2) A suspension of hydrazide (1.0 equiv) and Lawesson's reagent (1.0 equiv) in THF (0.5 M) was stirred at 45 °C for 24 h under an atmosphere of N_2. The mixture was filtered through a silica plug, washing with hexanes/EtOAc (2:1), and the filtrate was concentrated *in vacuo*. The crude mixture was purified by flash chromatography (hexanes/EtOAc) to afford the desired Boc-thiohydrazide product.

3) Boc-thiohydrazide (1.0 equiv) was dissolved in HCl in dioxane (4 M, 10 equiv) and the solution was stirred for 1-24 h (depending on the substrate) at rt. The reaction mixture was diluted with Et_2O and the precipitate was collected by filtration, washed with Et_2O and dried under vacuum to afford the desired thiohydrazide-HCl salt.

1.3.1 *3-Methoxyphenyl thiohydrazide* **10**

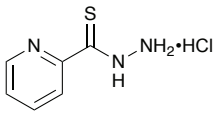


Prepared according to the general procedure. 1) 2.27 mmol scale, quant. yield. 2) 2.03 mmol scale, 68% yield. 3) 1.27 mmol scale, 2 h, 70% yield, white solid.

MP 141–143 °C; **^{1}H NMR** (400 MHz, MeOD) δ 7.40 – 7.37 (m, 3H), 7.17 – 7.13 (m, 1H), 3.86 (s, 3H); **^{13}C NMR** (101 MHz, MeOD) δ 198.0, 159.7, 129.3, 119.3, 117.7, 113.0, 54.6; **IR** (u/cm^{-1}, thin film): 3200-2500 (br), 3129, 3082, 2942, 1607, 1580,

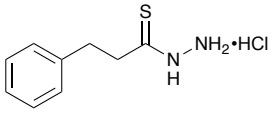
1483, 1459, 1433, 1288, 1147, 1042, 989, 786, 778; **HRMS** (EI): calculated for $C_8H_{10}N_2OS$ [M-HCl]$^+$: 182.0508, found: 182.0509

1.3.2 *Pyridine thiohydrazide* **12**



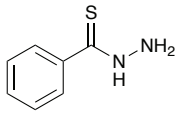
Prepared according to the general procedure. 1) 4.55 mmol scale, 61% yield. 2) 2.53 mmol scale, 22% yield. 3) 0.55 mmol scale, 24 h, 79% yield, yellow solid.
MP 133–134 °C; **^{1}H NMR** (400 MHz, MeOD) δ 8.64 (ddd, *J* = 5.5, 1.6, 1.0 Hz, 1H), 8.56 (ddd, *J* = 7.9, 1.0. 1.0 Hz, 1H), 8.41 (ddd, *J* = 7.9, 7.9, 1.6 Hz, 1H), 7.90 (ddd, *J* = 7.9, 5.5, 1.0 Hz, 1H); **^{13}C NMR** (101 MHz, MeOD) δ 184.4, 150.0, 146.0, 143.7, 128.7, 126.1; **IR** (υ/cm^{-1}, thin film): 3382 (br), 3073, 3038, 2993, 2531(br), 1578, 1557, 1513, 1456, 1351, 1250, 1219, 1081, 979, 942, 773; **HRMS** (ESI): calculated for $C_6H_8N_3S$ [M–Cl]$^+$: 154.0433, found: 154.0437

1.3.3 *Alkyl thiohydrazide* **15**



Prepared according to the general procedure. 1) 4.66 mmol scale, 60% yield. 2) 2.92 mmol scale, 27% yield. 3) 0.80 mmol scale, 1 h, 67% yield, white solid.
MP 121-123 °C; **^{1}H NMR** (400 MHz, MeOD) δ 7.29 – 7.16 (m, 5H), 3.12 (t, *J* = 7.3 Hz, 2H), 2.99 (t, *J* = 7.3 Hz, 2H); **^{13}C NMR** (101 MHz, MeOD) δ 205.0, 141.3, 129.6, 127.5, 45.1, 36.2; **IR** (υ/cm^{-1}, thin film): 3200-2600 (br), 1581, 1548, 1465, 1372, 1209, 1110, 1065, 752, 695; **HRMS** (ESI): calculated for $C_9H_{13}N_2S$ [M-Cl]$^+$: 181.0794, found: 181.0799

1.3.4 *Phenyl thiohydrazide* **2**

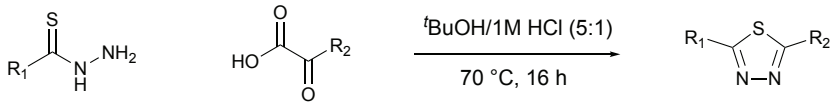


Phenyl thiohydrazide **2** was prepared according to a literature procedure.[4]

S-(thiobenzoyl)-thioglycolic acid (1.30 g, 6.12 mmol, 1.0 equiv) was dissolved in 1 M NaOH (6 mL) and H_2O (6 mL) was added. The solution was cooled to 0 °C and hydrazide hydrate (1.1 mL, 12.2 mmol, 2.0 equiv) was added dropwise. After stirring for 10 min the pH of the reaction mixture was adjusted to pH 5-6 using 1 M HCl and the resulting suspension was stirred for a further 1 h. The precipitate was collected by filtration, washing with cold H_2O, and dried under vacuum. The crude material was recrystallized (CH_2Cl_2/hexanes) to provide desired product **2** (0.61 g, 66%) as a pale yellow solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.72 – 7.69 (m, 2H), 7.47 – 7.38 (m, 3H). The spectroscopic data were in agreement with literature values.[4]

Synthesis of 1,3,4-Thiadiazoles

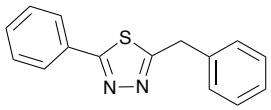
2.1 Intermolecular Reaction of Thiohydrazides and α-Ketoacids



General procedure:

A solution of thiohydrazide (1.5 equiv) in ^{t}BuOH/1M HCl (5:1, 0.1 M) was added to a solution of α-ketoacid (1.0 equiv) in ^{t}BuOH/1M HCl (5:1, 0.1 M) and the mixture was heated at 70 °C for 16 h. The reaction mixture was diluted with water and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by flash chromatography to afford the desired 1,3,4-thiadiazole products.

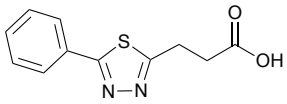
2.1.1 *Phenyl thiadiazole* **5**



Thiadiazole **5** was prepared following the general procedure with thiohydrazide **2** (50 mg, 0.33 mmol, 1.5 equiv) and α-ketoacid **3** (36 mg, 0.22 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 9:1→4:1) as a white solid (39 mg, 71%).

MP 71–72 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.91 – 7.87 (m, 2H), 7.47 – 7.41 (m, 3H), 7.38 – 7.33 (m, 4H), 7.32 – 7.28 (m, 1H), 4.45 (s, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.8, 169.4, 137.3, 131.1, 130.3, 129.2, 129.1, 129.0, 127.9, 127.6, 36.7; **IR** (υ/cm^{-1}, thin film): 3066, 3030, 1494, 1455, 1427, 1225, 1124, 1058, 981, 920, 763, 702, 690; **HRMS** (ESI): calculated for $C_{15}H_{13}N_2S$ $[M+H]^+$: 253.0794, found: 253.0793

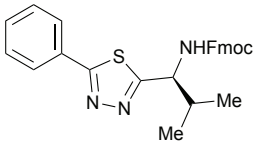
2.1.2 Propanoic acid thiadiazole **7**



Thiadiazole **7** was prepared following the general procedure with thiohydrazide **2** (50 mg, 0.33 mmol, 1.5 equiv) and α-ketoacid **6** (32 mg, 0.22 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 4:1 + 1% formic acid) as a white solid (42 mg, 73%).

MP 168–170 °C; **^{1}H NMR** (400 MHz, MeOD) δ 7.95-7.91 (m, 2H), 7.55 – 7.49 (m, 3H), 3.42 (t, *J* = 7.0 Hz, 2H), 2.86 (t, *J* = 7.0 Hz, 2H); **^{13}C NMR** (101 MHz, MeOD) δ 177.1, 171.5, 170.7, 132.5, 131.1, 130.4, 128.8, 34.7, 26.6; **IR** (υ/cm^{-1}, thin film): 3376 (br), 3062, 2934, 1693, 1536, 1456, 1419, 1239, 1048, 982, 758, 690; **HRMS** (ESI): calculated for $C_{11}H_{11}N_2O_2S$ $[M+H]^+$: 235.0536, found: 235.0535

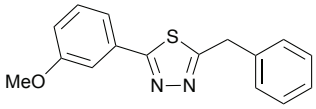
2.1.3 *Fmoc-amine thiadiazole* **9**



Thiadiazole **9** was prepared following the general procedure with thiohydrazide **2** (58 mg, 0.39 mmol, 1.5 equiv) and α-ketoacid **8**[5,6] (94 mg, 0.26 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 4:1) followed by recrystallization (hexanes/EtOAc) as a white solid (64 mg, 54%).

$[\alpha]_D^{24}$ (c = 0.25, $CHCl_3$) = −41.7; **MP** 154-156 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.98 – 7.92 (m, 2H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.64 – 7.58 (m, 2H), 7.52 – 7.46 (m, 3H), 7.42 – 7.36 (m, 2H), 7.34 – 7.27 (m, 2H), 5.67 (d, *J* = 9.1 Hz, 1H), 5.13 – 5.07 (m, 1H), 4.47 (d, *J* = 7.0 Hz, 2H), 4.23 (t, *J* = 7.0 Hz, 1H), 2.48 – 2.37 (m, 1H), 1.03 (d, *J* = 6.8 Hz, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.9, 168.9, 156.2, 143.8, 141.5, 131.3, 130.1, 129.3, 128.1, 127.9, 127.2, 125.2, 120.1, 67.2, 56.5, 47.4, 33.6, 19.4, 18.0; **IR** (υ/cm^{-1}, thin film): 3306, 2968, 2950, 1690, 1532, 1452, 1425, 1301, 1261, 1236, 1021; **HRMS** (ESI): calculated for $C_{27}H_{25}N_3NaO_2S$ $[M+Na]^+$: 478.1560, found: 478.1556

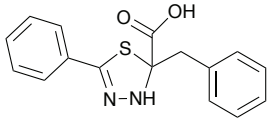
2.1.4 *3-Methoxyphenyl thiadiazole* **11**



Thiadiazole **11** was prepared following the general procedure with thiohydrazide **10** (46 mg, 0.21 mmol, 1.5 equiv) and α-ketoacid **3** (23 mg, 0.14 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 9:1→4:1) as a yellow oil (32 mg, 80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 2.6, 1.6 Hz, 1H), 7.41 – 7.28 (m, 7H), 7.00 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 4.46 (s, 2H), 3.86 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.0, 169.4, 160.2, 137.3, 131.5, 130.3, 129.2, 129.0, 127.7, 120.7, 117.6, 112.2, 55.3, 36.8; **IR** (υ/cm^{-1}, thin film): 3028, 3004, 2935, 1598, 1581, 1455, 1426, 1206, 1043, 1002, 843, 781; **HRMS** (ESI): calculated for $C_{16}H_{15}N_2OS$ $[M+H]^+$: 283.0900, found: 283.0899

2.1.5 *2,3-Dihydrothiadiazole carboxylic acid* **4**

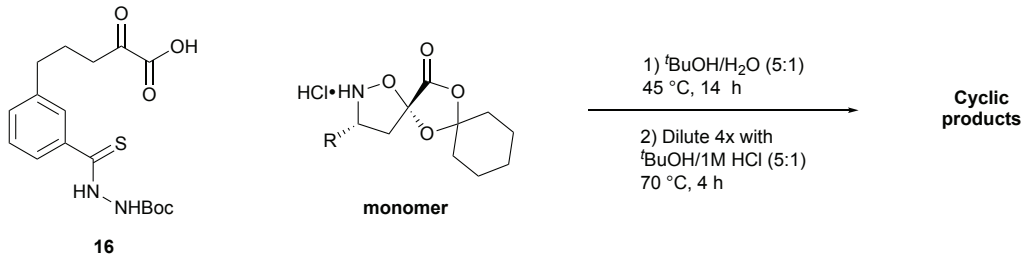


A solution of thiohydrazide **2** (50 mg, 0.33 mmol, 1.0 equiv) and α-ketoacid **3** (54 mg, 0.33 mmol, 1.0 equiv) in ^{t}BuOH/H_2O (5:1, 6.6 mL) was heated at 45 °C for 14 h. The reaction mixture was diluted with water and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by preparative HPLC (35-95% CH_3CN with 0.1% TFA over 28 min) to obtain a sample of desired product **4** for characterization. Retention time: 16.9 min.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.56 (m, 2H), 7.40 – 7.34 (m, 3H), 7.31 – 7.23 (m, 5H), 3.56 (ABq, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.8, 147.4, 134.2, 130.6, 130.3, 130.2, 128.8, 128.8, 127.9, 127.2, 84.1, 44.5: **IR** (u/cm^{-1}, thin film): 3299, 2923, 2853, 1724, 1494, 1447, 1267, 1193, 973, 759, 688; **HRMS** (ESI): calculated for $C_{16}H_{15}N_2O_2S$ $[M+H]^+$: 299.0849, found: 299.0844

Synthesis of β-Peptide Macrocycle Mixtures

3.1 One-Pot Elongation/Macrocyclization with One Monomer



General procedure:

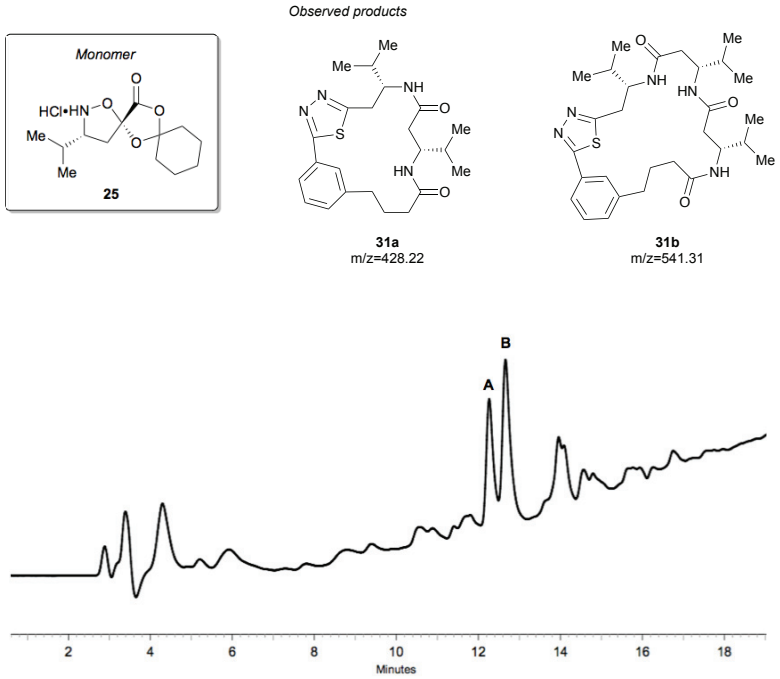
Reactions were prepared using 0.1 M stock solutions of all reagents in ^{t}BuOH/H_2O (5:1).

Solutions of initiator **16** (10 μL, 1.0 equiv) and a monomer (20 μL, 2.0 equiv) were mixed and heated at 45 °C for 14 h. The mixture was diluted with ^{t}BuOH/1M HCl (5:1) (90 μL) and heated to 70 °C for 4 h. The resulting mixture of cyclic compounds was analyzed by HPLC and/or LCMS.

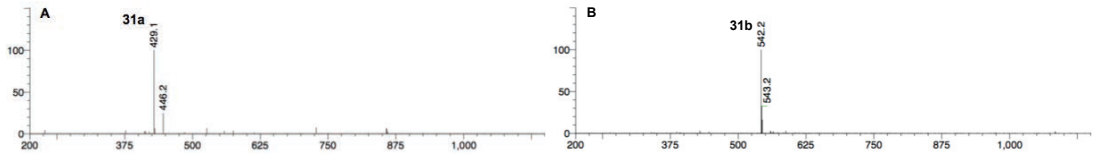
This procedure was performed on a larger scale (e.g. 0.90 mL of **16**, 1.80 mL monomer) for purification by preparative HPLC. For cyclization on larger scale the mixtures were stirred in an oversized flask (e.g. 50 mL flask for 9 mL solvent) to enhance O_2 exchange.

3.1.1 HPLC spectra with mass traces of the major peaks

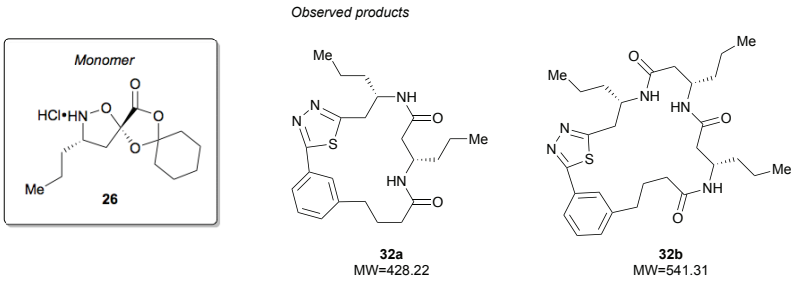
Monomer **25**



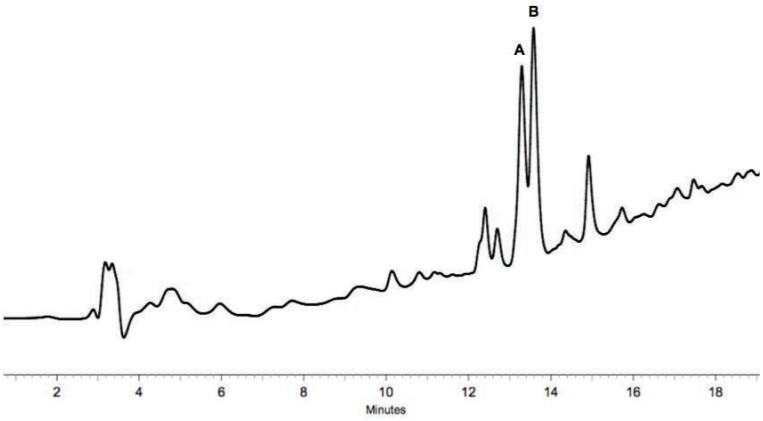
HPLC: Gradient 30 to 90% CH_3CN with 0.1% TFA in 17 min

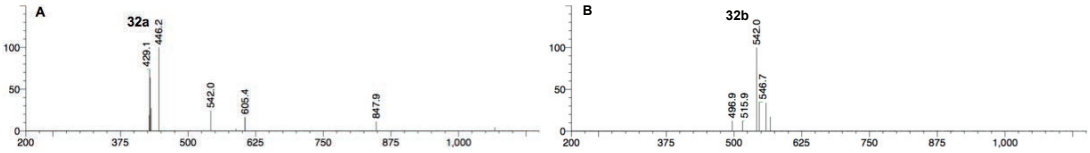


Monomer **26**

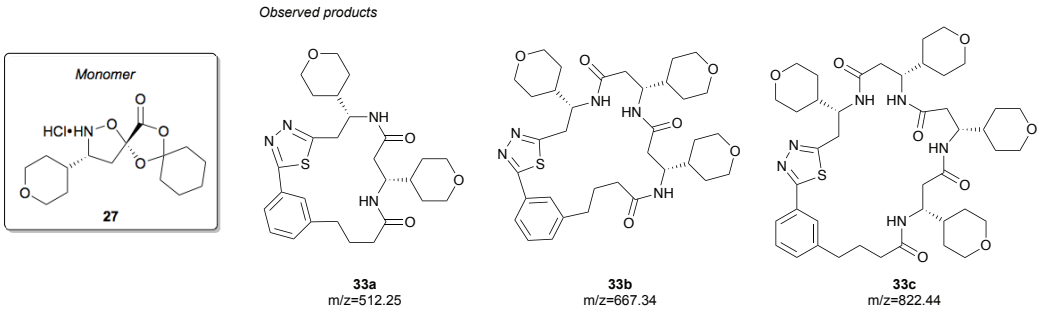


HPLC: Gradient 30 to 90% CH_3CN with 0.1% TFA in 17 min

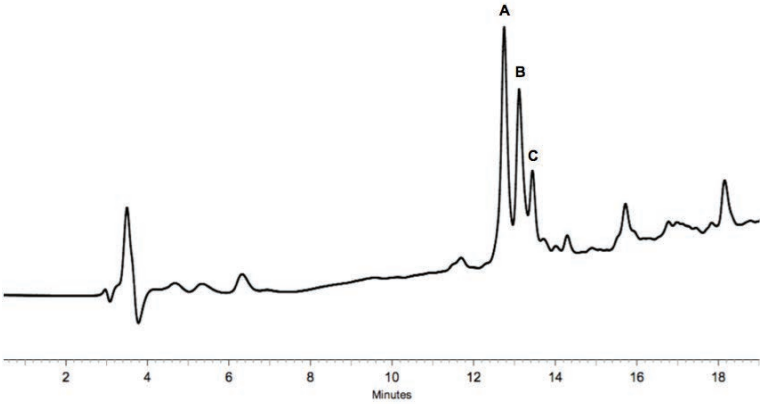


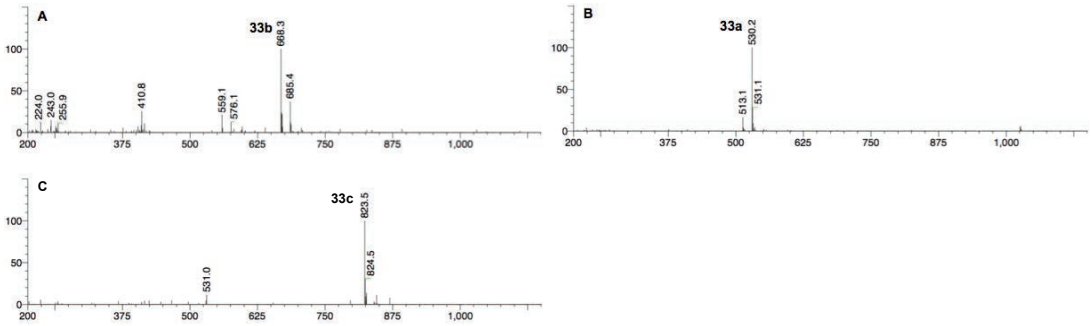


Monomer **27**

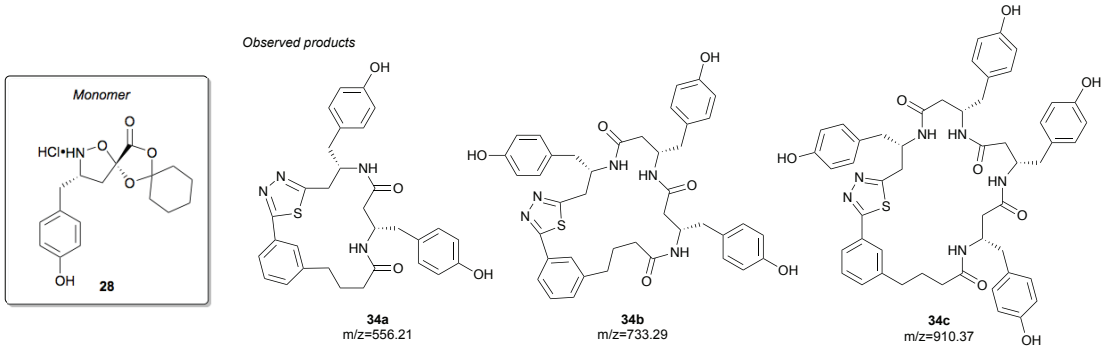


HPLC: Gradient 20 to 70% CH_3CN with 0.1% TFA in 17 min

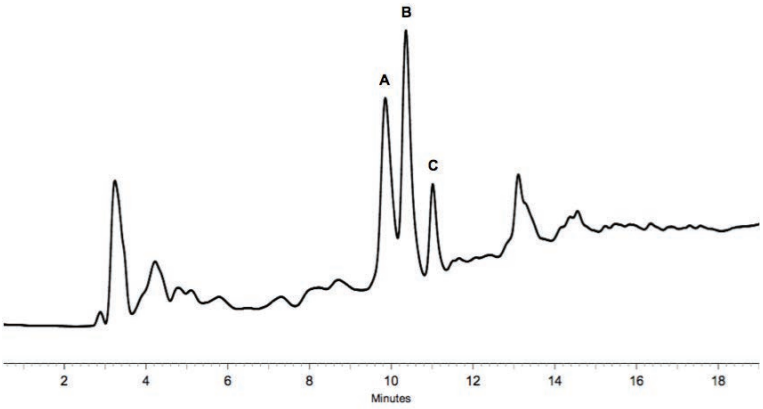


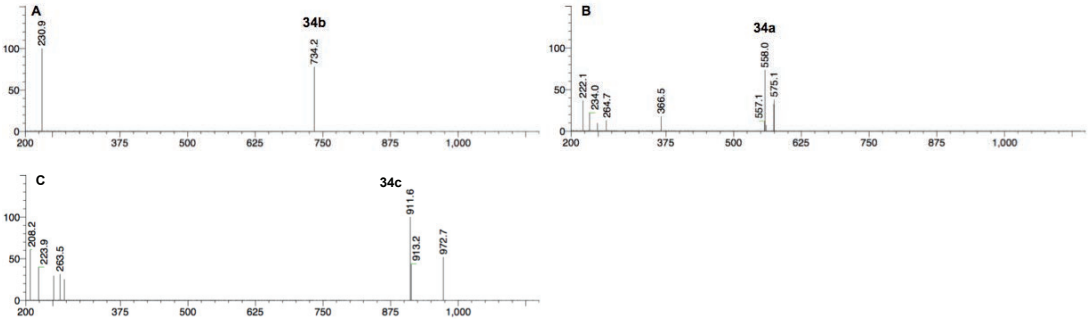


Monomer **28**

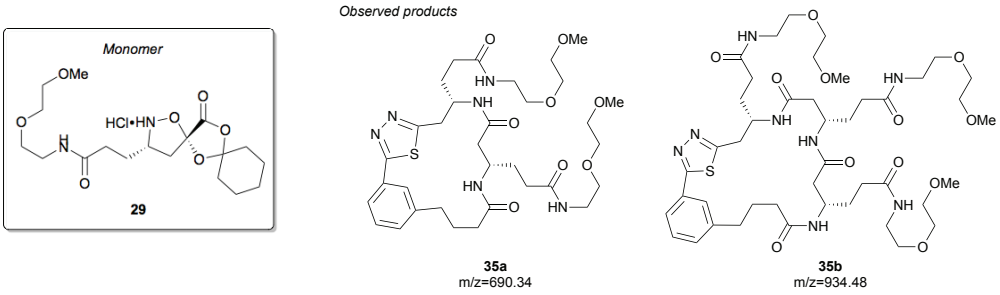


HPLC: Gradient 30 to 90% CH_3CN with 0.1% TFA in 17 min

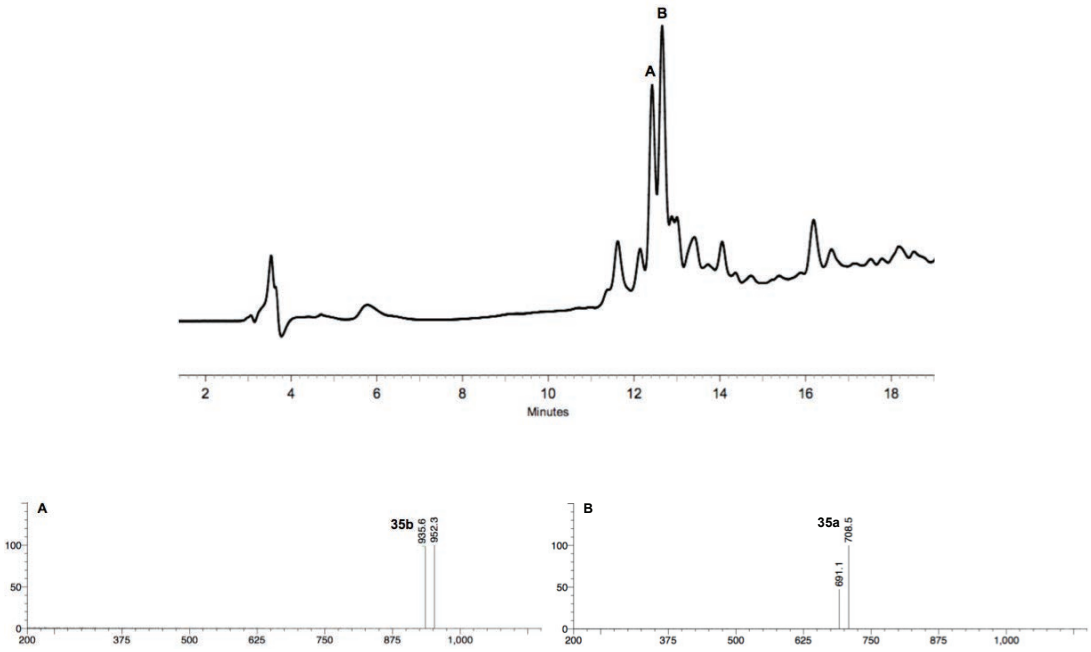




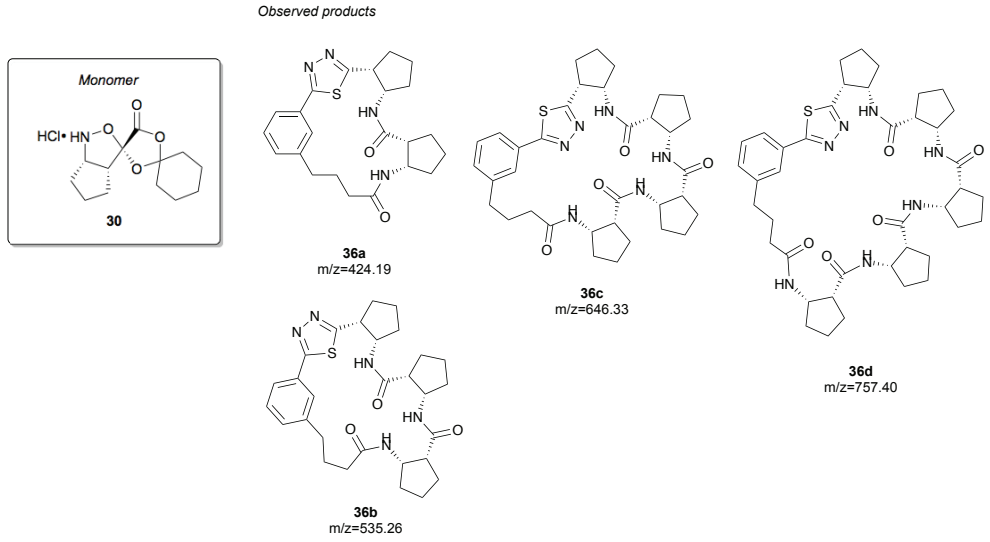
Monomer **29**



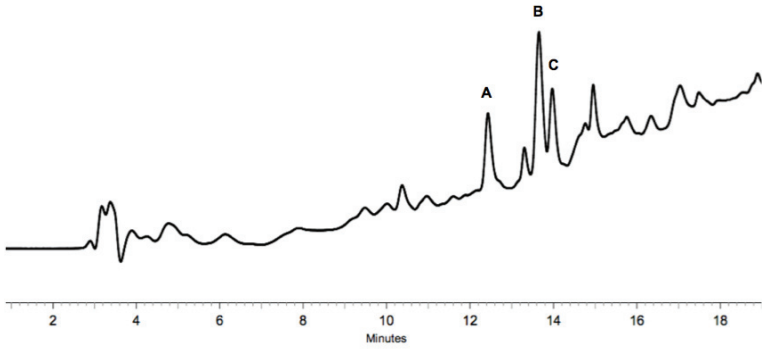
HPLC: Gradient 20 to 70% CH_3CN with 0.1% TFA in 17 min

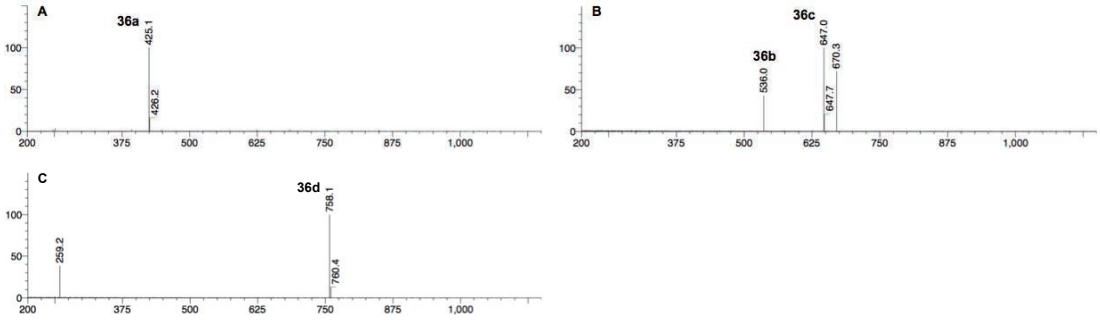


Monomer **30**

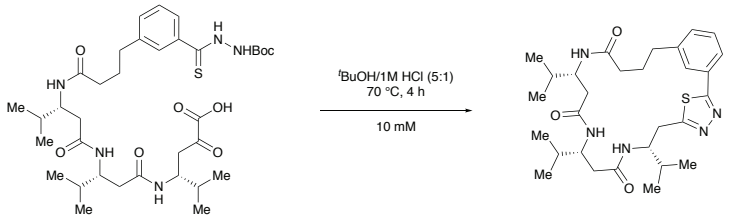


HPLC: Gradient 30 to 90% CH_3CN with 0.1% TFA in 17 min





3.2 Cyclization of Purified Tri-β-Peptide



56
m/z=705.38

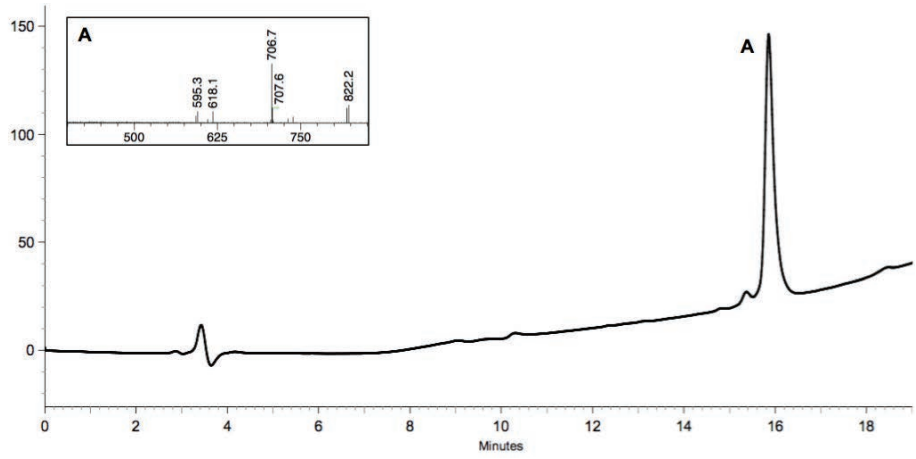
32b
m/z=541.31

A solution of tripeptide **56** (0.26 mg, 0.37 μmol) was dissolved in ^{t}BuOH/1M HCl (5:1, 37 μL) was heated to 70 °C for 4 h. The crude reaction mixture was directly analyzed by HPLC (Gradient 30 to 90% CH_3CN with 0.1% TFA in 17 min).

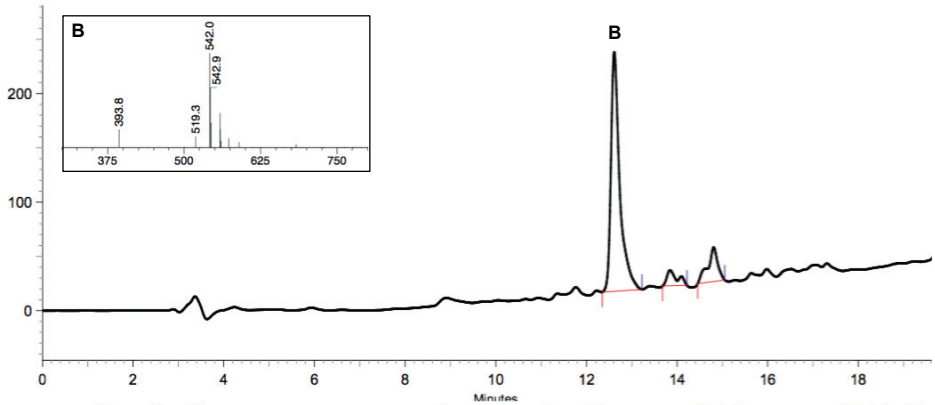
HRMS (ESI) Tripeptide ketoactid **56**: calculated for $C_{35}H_{56}N_5O_8S$ $[M+H]^+$: 706.3844, found: 706.3837. Calculated for $C_{35}H_{54}N_5O_8S$ $[M-H]^-$: 704.3699, found: 704.3696.

HRMS (ESI) Macrocycle **32b**: calculated for $C_{29}H_{44}N_5O_3S$ $[M+H]^+$: 542.3159, found: 542.3154.

HPLC: Before reaction

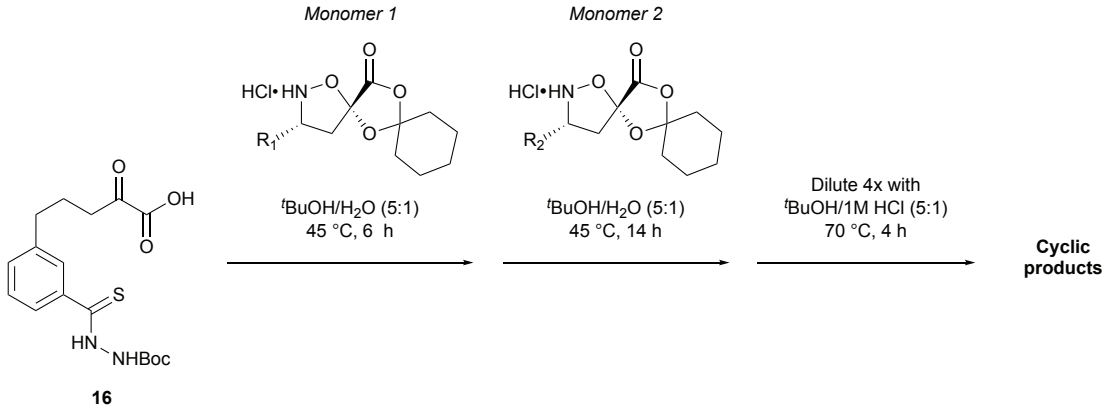


HPLC: Crude reaction mixture



Retention Time	Area	Area %	Height	Height %
12.610	2939287	80.51	220360	82.79
13.843	227011	6.22	14309	5.38
14.807	484463	13.27	31497	11.83

3.3 One-Pot Elongation/Macrocyclization Reaction with Two Monomers



General procedure:

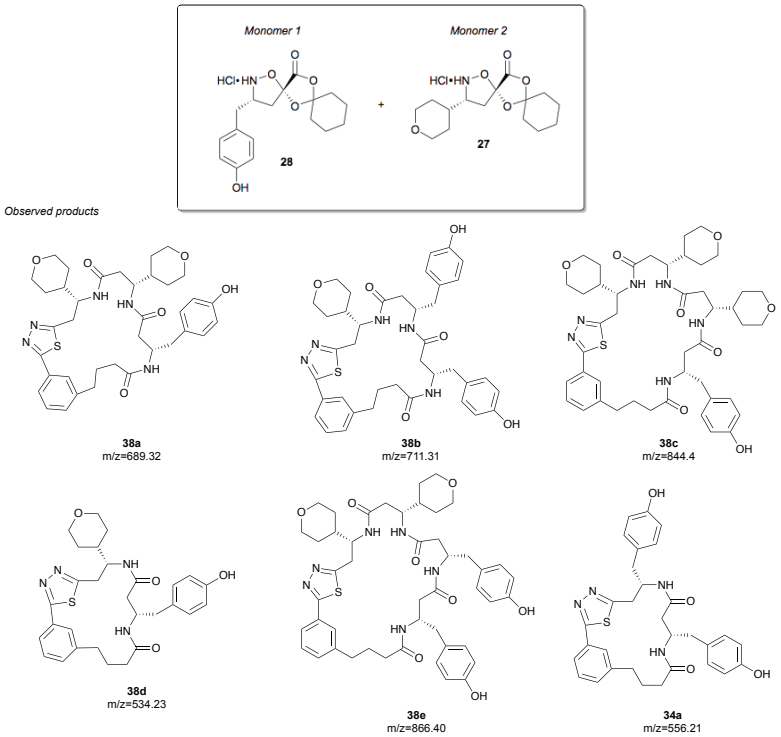
Reactions were prepared using 0.1 M stock solutions of all reagents in ^{t}BuOH/H_2O (5:1).

Solutions of initiator **16** (10 μL, 1.0 equiv) and the first monomer (10 μL, 1.0 equiv) were mixed and heated at 45 °C for 6 h. A solution of the second monomer (10 μL, 1.0 equiv) was added and the mixture heated at 45 °C for 14 h. The reaction mixture were diluted with ^{t}BuOH/1 M HCl (5:1) (90 μL) and heated to 70 °C for 4 h. The resulting mixture of cyclic compounds was analyzed by HPLC and/or LCMS.

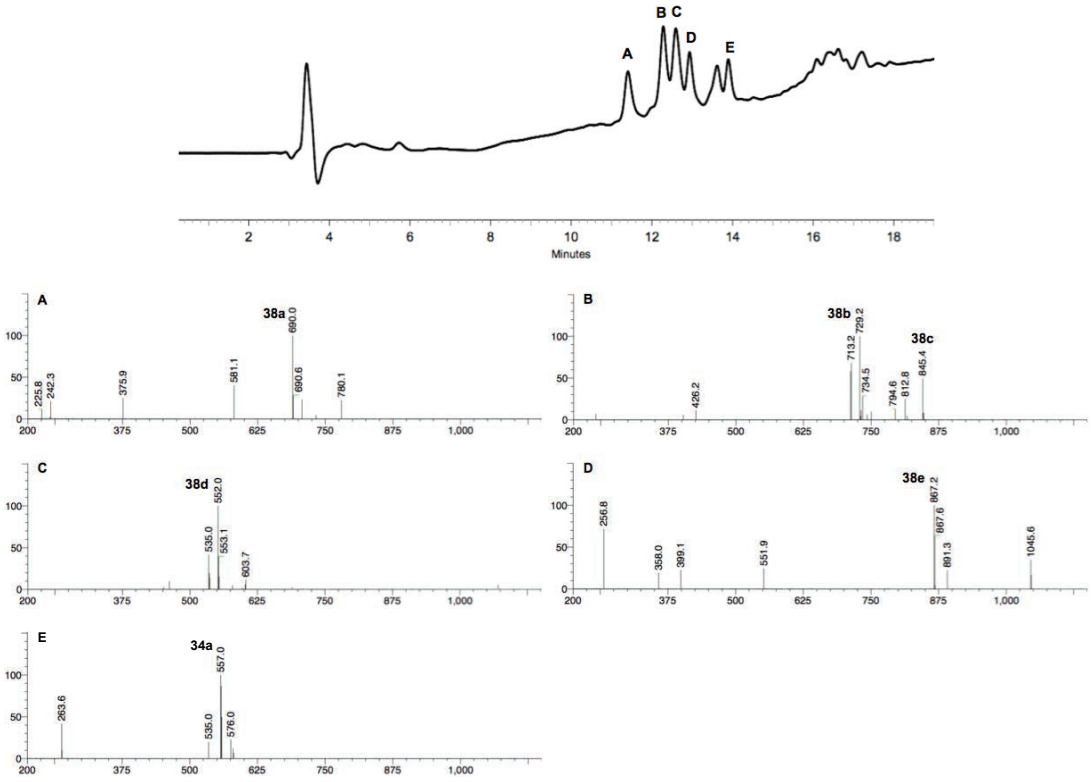
This procedure was performed on a larger scale (e.g. 0.90 mL of **16**, 0.90 mL of each monomer) for purification by preparative HPLC. For large-scale cyclization the mixtures were stirred in an oversized flask (e.g. 50 mL flask for 9 mL solvent) to enhance O_2 exchange.

3.3.1 HPLC spectra with mass traces of the major peaks

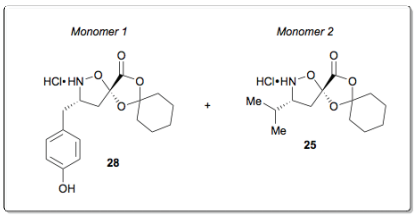
Monomers **28** *and* **27**



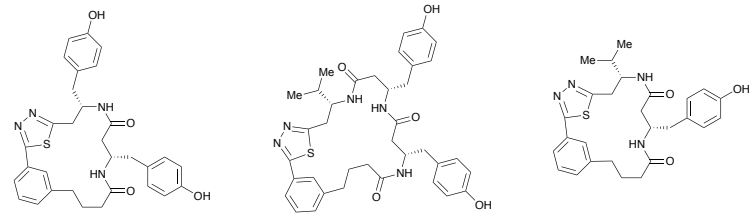
HPLC: 25 to 70% CH_3CN with 0.1% TFA over 17 min

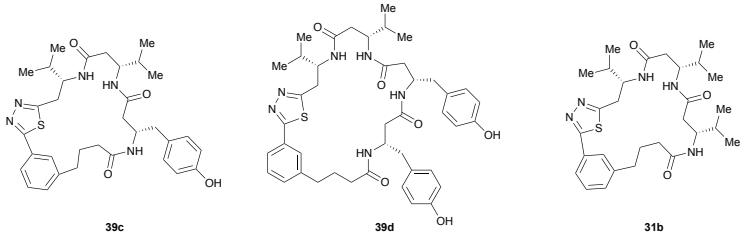


Monomers **28** *and* **25**

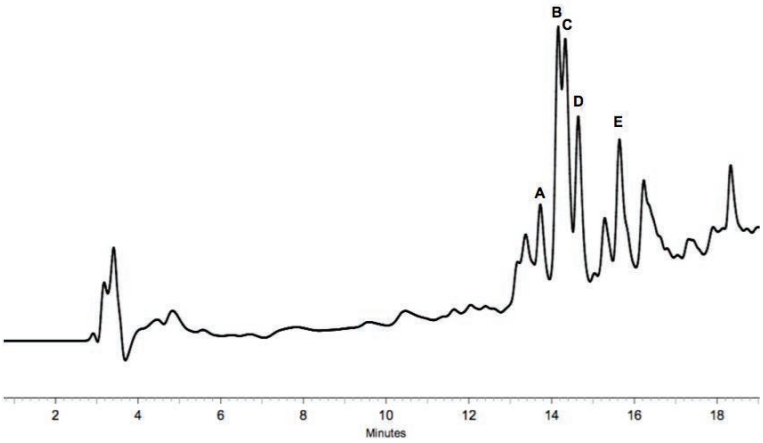


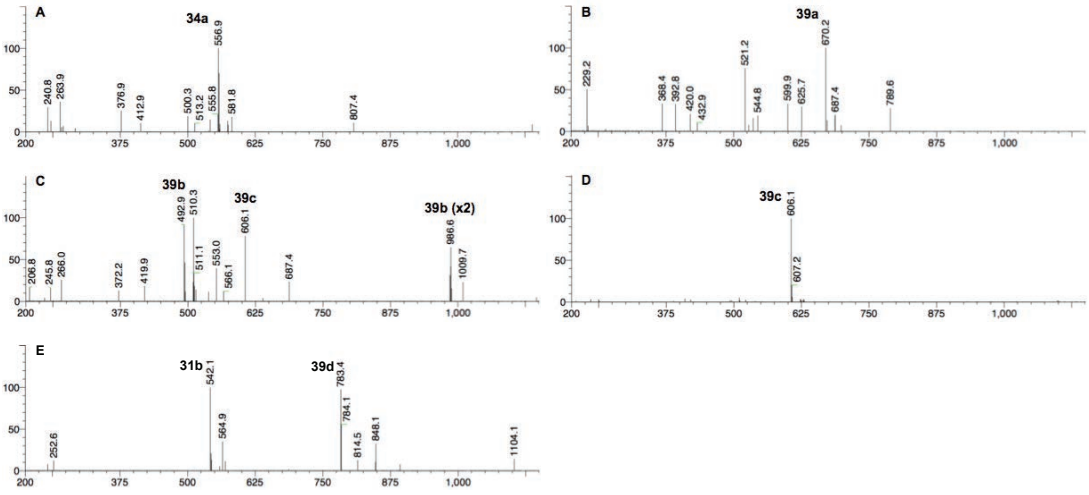
Observed products



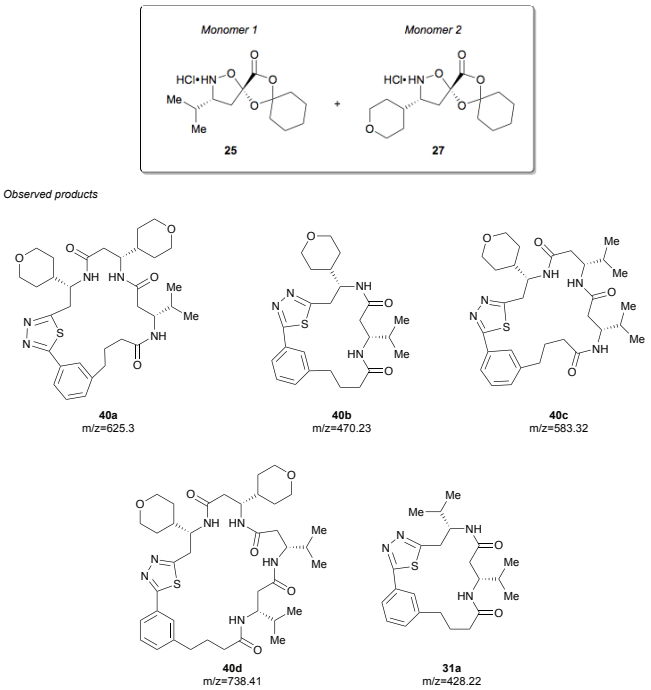


HPLC: 25 to 70% CH_3CN with 0.1% TFA over 17 min

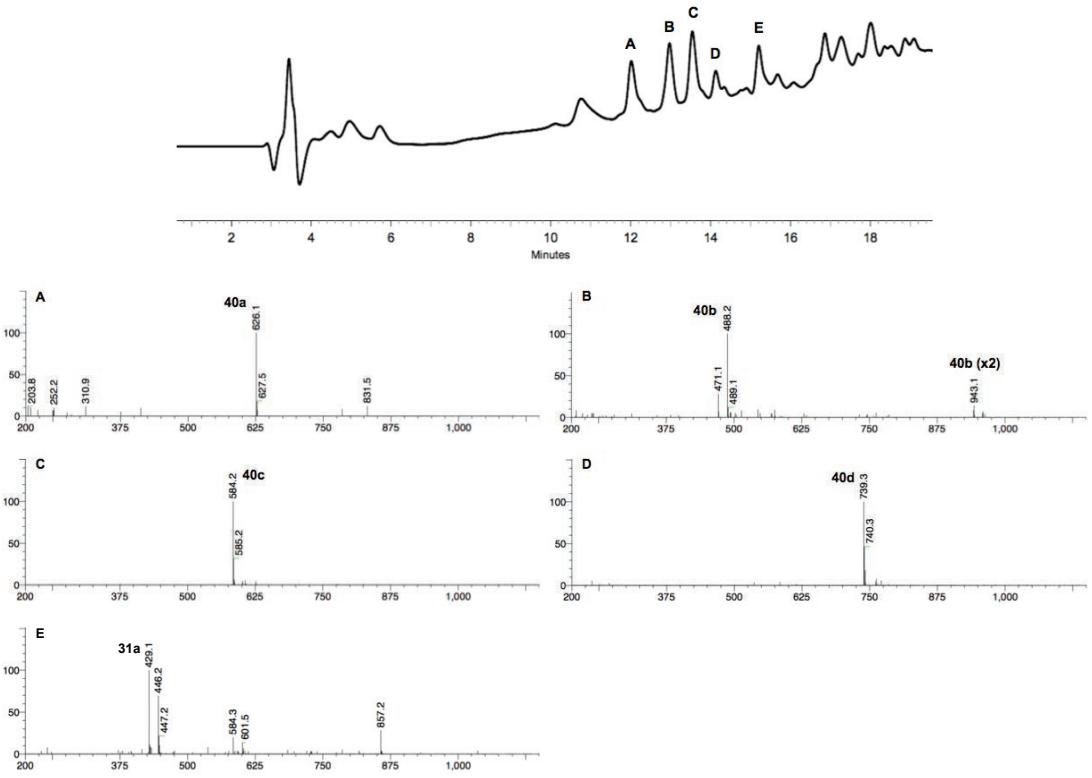




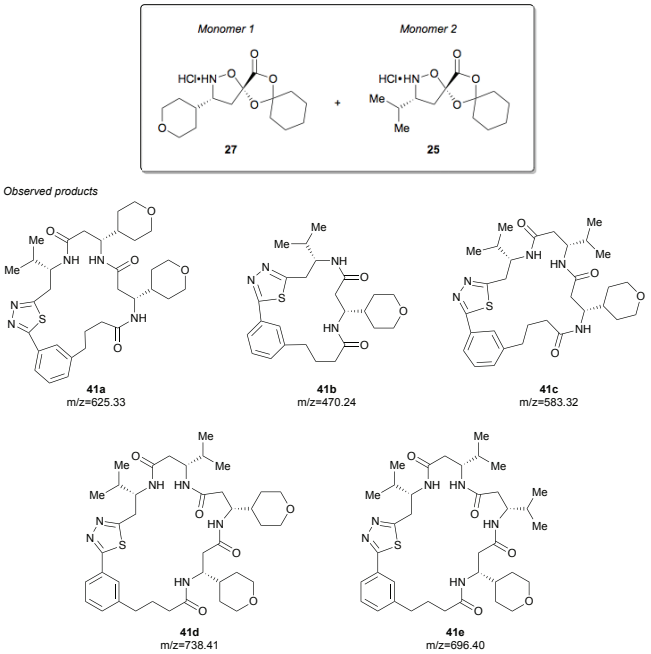
Monomers **25** *and* **27**



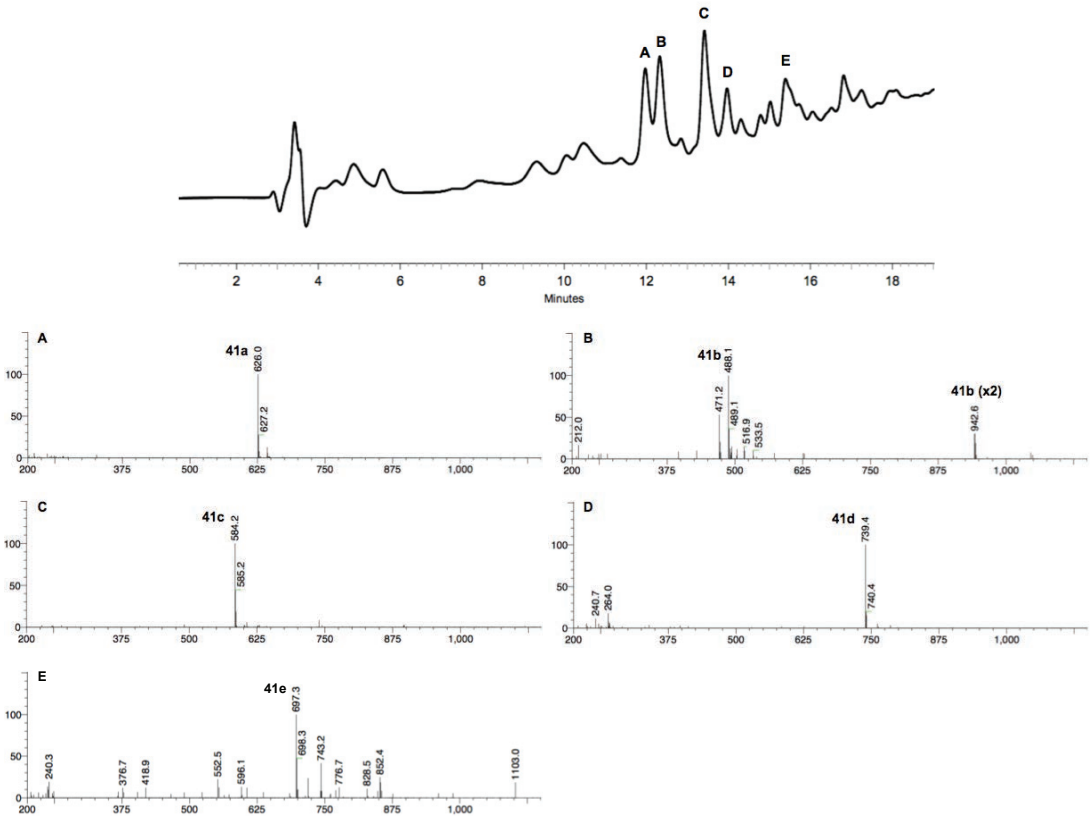
HPLC: 25 to 70% CH_3CN with 0.1% TFA over 17 min



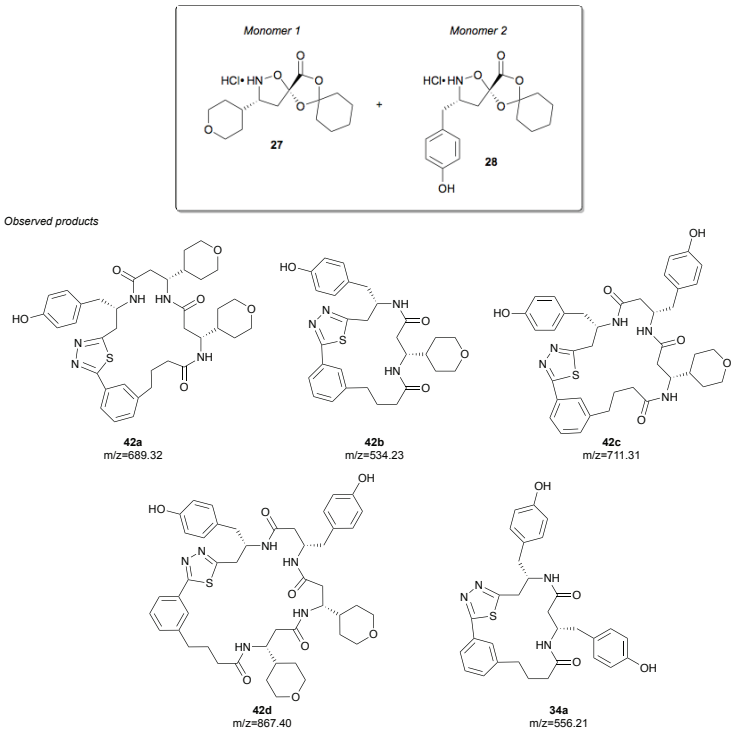
Monomers **27** *and* **25**



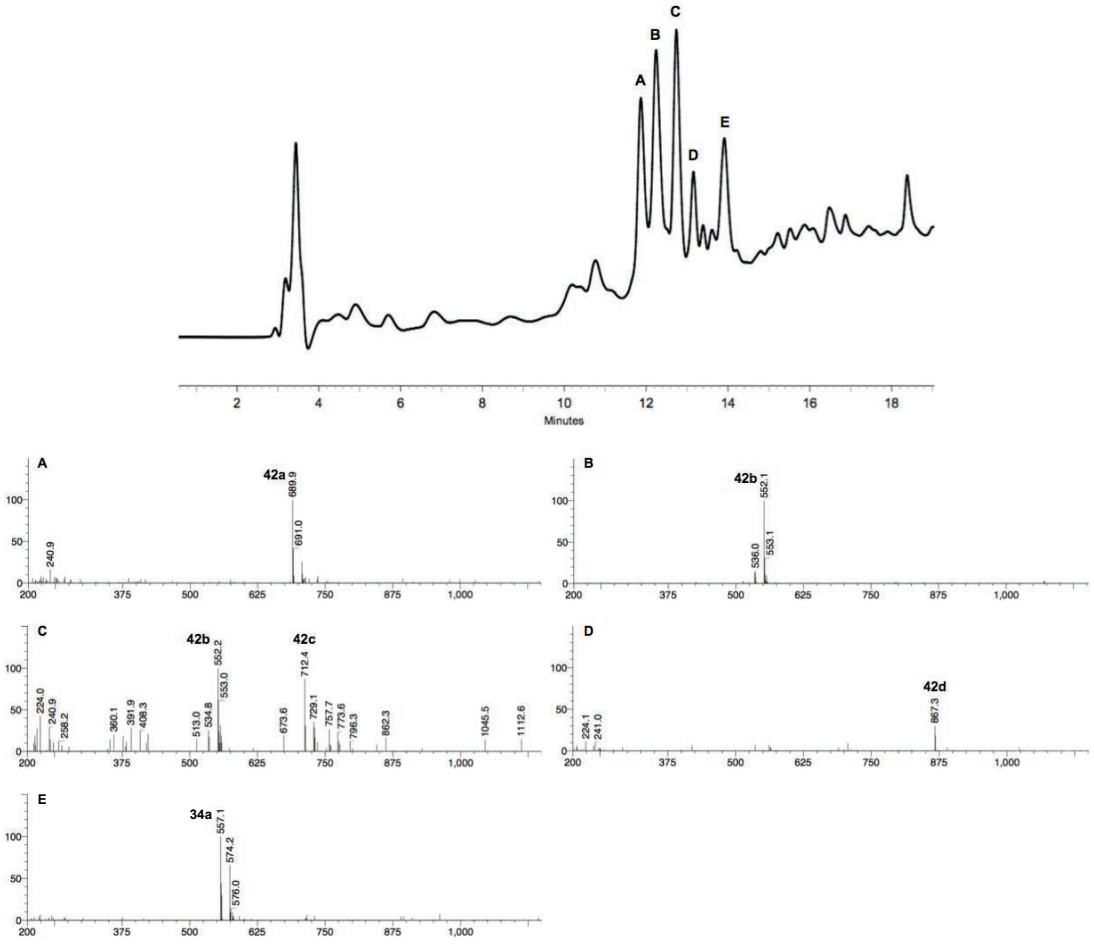
HPLC: 25 to 70% CH_3CN with 0.1% TFA over 17 min



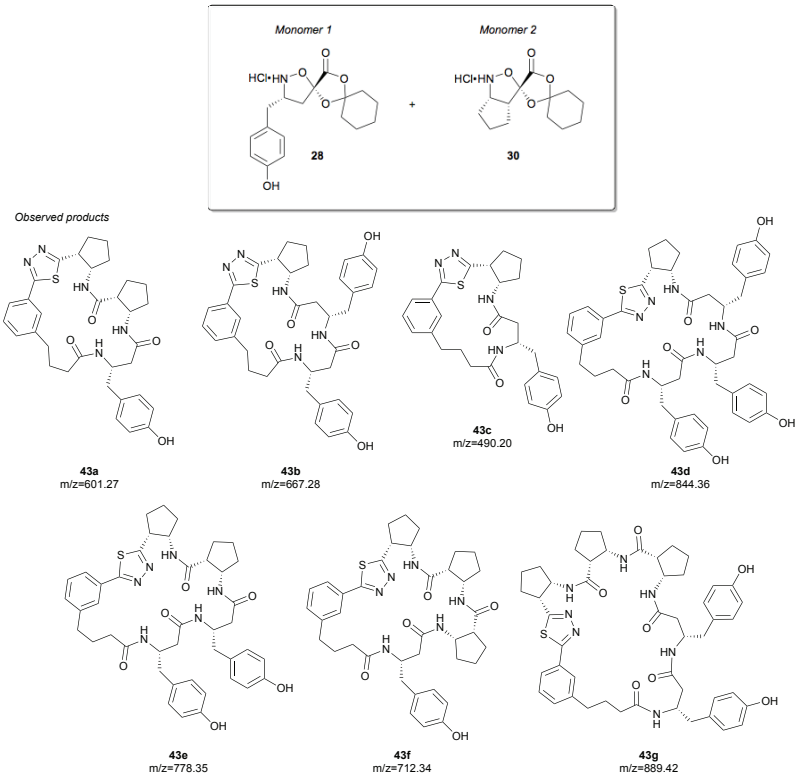
Monomers **27** *and* **28**



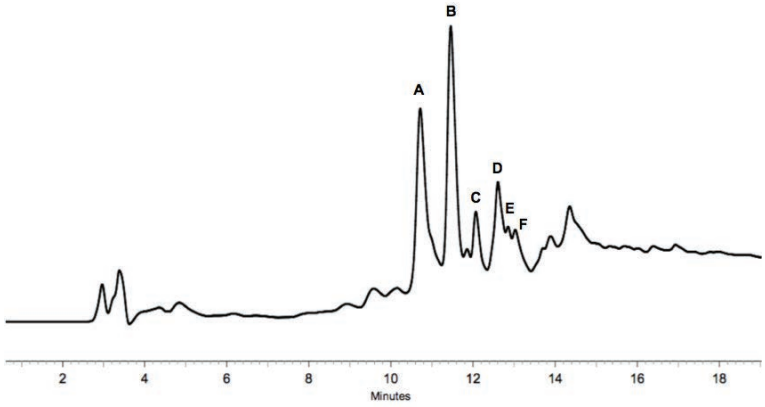
HPLC: 25 to 70% CH_3CN with 0.1% TFA over 17 min

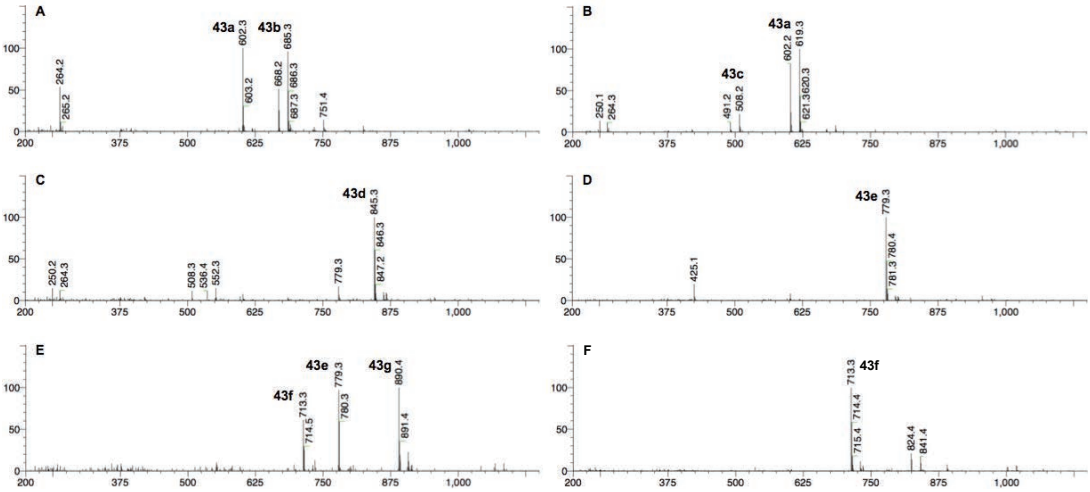


Monomers **28** *and* **30**

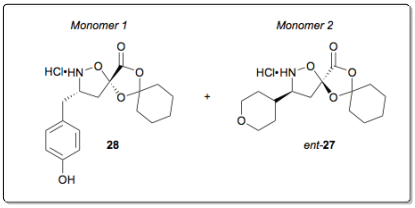


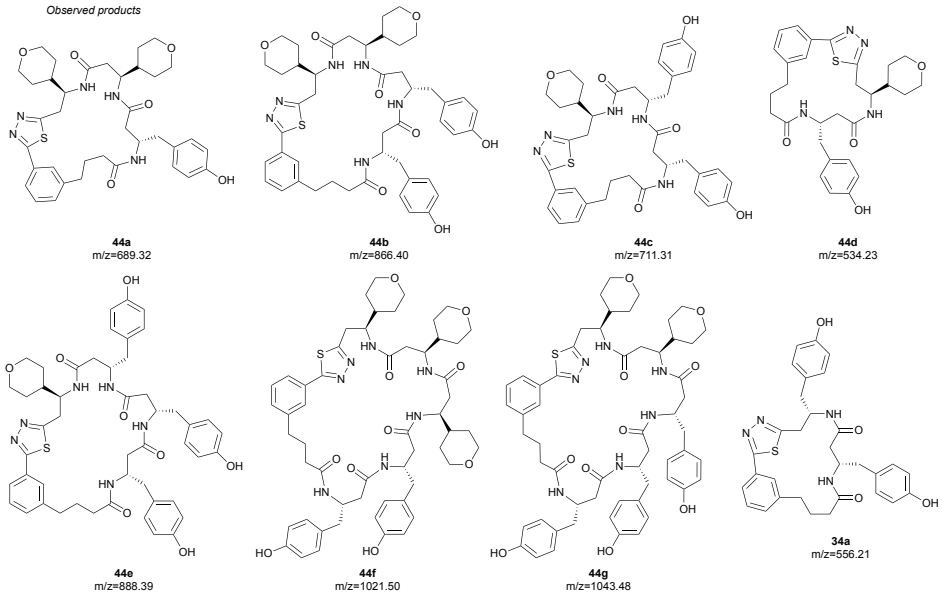
HPLC: 30 to 90% CH_3CN with 0.1% TFA over 17 min



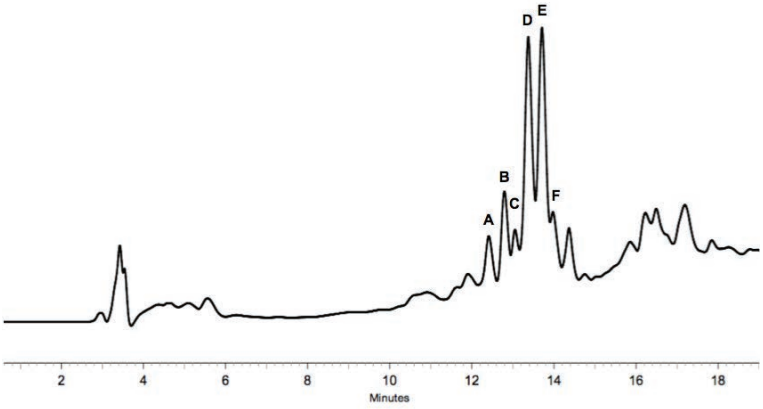


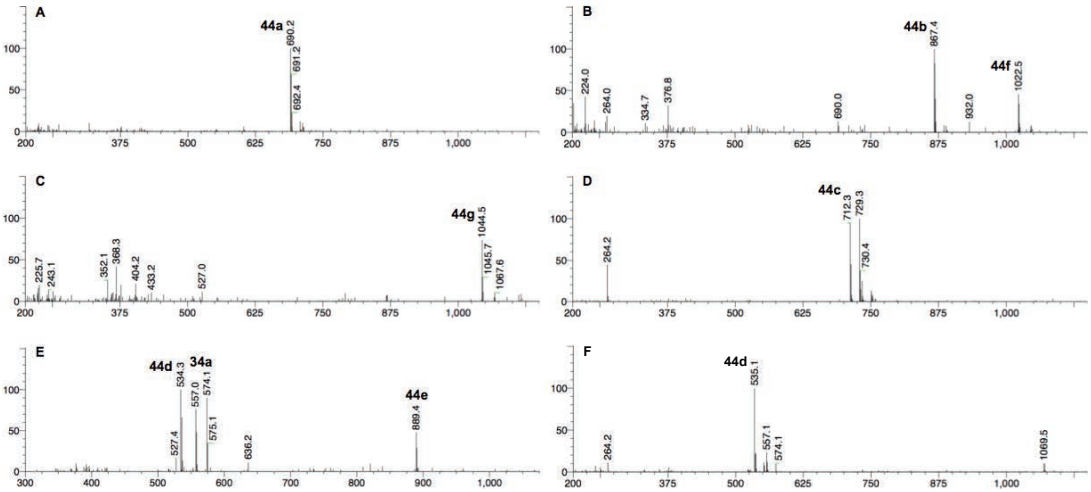
Monomers **28** *and* ***ent*-27**



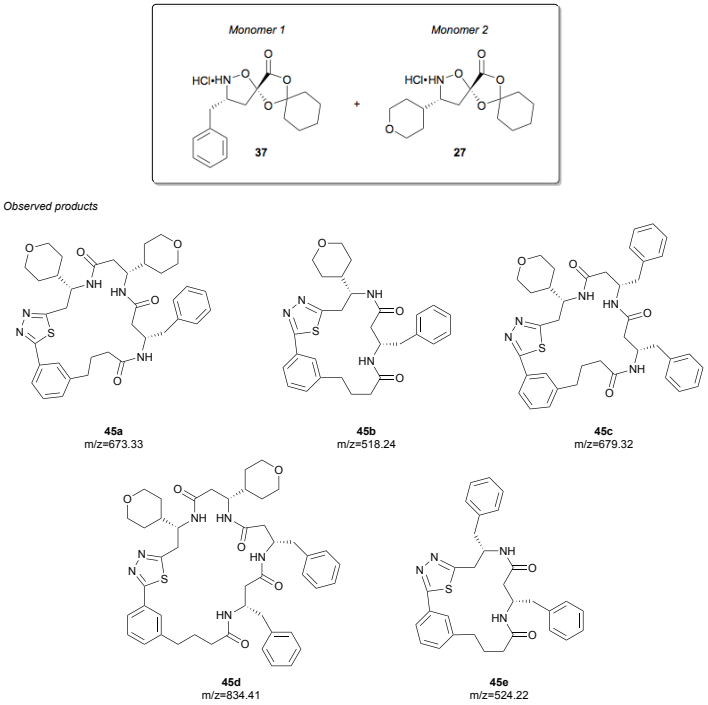


HPLC: 25 to 70% CH_3CN with 0.1% TFA over 17 min

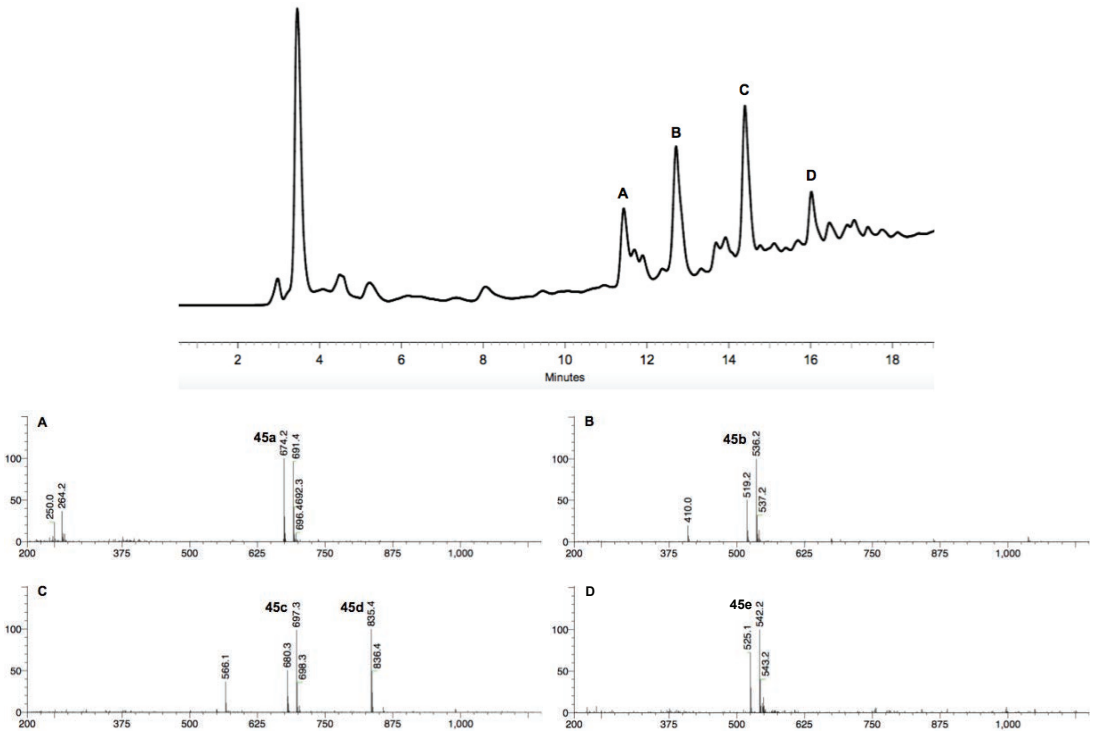




Monomers **37** *and* **27**

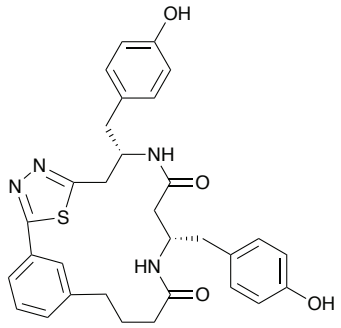


HPLC: 30 to 90% CH_3CN with 0.1% TFA over 17 min



3.4 Isolation and Characterization of Macrocyclic Compounds

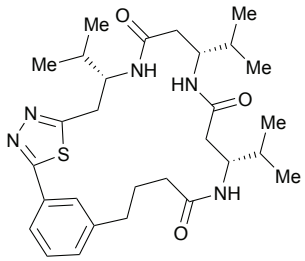
Macrocycle **34a**



Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator **16** (0.09 mmol, 1.0 equiv) and monomer **28** (0.18 mmol, 2.0 equiv). The crude reaction mixture was diluted with CH_3CN:H_2O (1:1) and purified directly by preparative HPLC (20 to 95% CH_3CN with 0.1% TFA over 28 min). Macrocycle **34a** was isolated as a white powder after lyophilization. Retention time: 15.8 minutes.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.22 (s, 1H), 9.17 (s, 1H), 8.02 (d, *J* = 9.5 Hz, 1H), 7.83 – 7.72 (m, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.37 – 7.29 (m, 1H), 7.15 (s, 1H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 8.5 Hz, 2H), 6.69 – 6.66 (m, 4H), 4.34 – 4.30 (m, 1H), 4.21-4.15 (m, 1H), 3.25 (dd, *J* = 15.8, 2.6 Hz, 1H), 2.95 (dd, *J* = 15.8, 11.9 Hz, 1H), 2.81 – 2.75 (m, 1H), 2.74 – 2.61 (m, 3H), 2.48 – 2.43 (m, 1H), 2.26 (dd, *J* = 16.5, 10.1 Hz, 1H), 2.15 (dd, *J* = 16.5, 2.2 Hz, 1H), 2.09 – 1.95 (m, 3H), 1.72 – 1.65 (m, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.1, 169.9, 168.5, 167.4, 155.7, 155.6, 141.5, 131.2, 130.3, 130.2, 130.0, 129.5, 129.3, 128.6, 128.0, 122.3, 115.0, 114.9, 49.9, 47.0, 40.7, 39.3, 37.7, 33.7, 31.4, 31.4, 22.6; **HRMS** (ESI): calculated for $C_{31}H_{32}N_4NaO_4S$ $[M+Na]^+$: 579.2036, found: 579.2039.

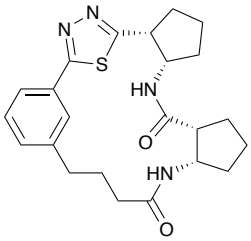
Macrocycle **31b**



Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator **16** (0.10 mmol, 1.0 equiv) and monomer **25** (0.20 mmol, 2.0 equiv). The crude reaction mixture was diluted with CH_3CN:H_2O (1:1) and purified directly by preparative HPLC (30 to 65% CH_3CN with 0.1% TFA over 28 min). Macrocycle **31b** was isolated as a white powder after lyophilization. Retention time: 17.0 minutes.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (d, *J* = 9.3 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.63 (s, 1H), 7.48 – 7.45 (m, 3H), 7.39 (d, *J* = 7.7 Hz, 1H), 4.16 – 4.11 (m, 1H), 3.91 – 3.82 (m, 2H), 3.37 (dd, *J* = 14.8, 3.0 Hz, 1H), 3.03 (dd, *J* = 14.8, 11.4 Hz, 1H), 2.76 – 2.71 (m, 1H), 2.67 – 2.63 (m, 1H), 2.30 (dd, *J* = 14.1, 9.7 Hz, 1H), 2.22 (dd, *J* = 14.0, 4.0 Hz, 1H), 2.13 – 1.98 (m, 4H), 1.85 – 1.75 (m, 3H), 1.73 – 1.65 (m, 2H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.82 – 0.80 (m, 6H), 0.77 (d, *J* = 6.8 Hz, 3H), 0.69 (d, *J* = 6.9 Hz, 3H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.0, 170.2, 169.9, 168.3, 167.2, 143.1, 131.2, 129.5, 129.4, 129.2, 123.4, 53.4, 51.3, 49.7, 39.9, 39.8, 39.7, 39.5, 39.4, 39.2, 39.1, 37.3, 34.8, 34.0, 32.3, 32.3, 31.8, 30.0, 27.1, 19.5, 19.2, 18.8, 18.3, 18.2, 16.4; **HRMS** (ESI): calculated for $C_{29}H_{44}N_5O_3S$ $[M+H]^+$: 542.3159, found: 542.3154.

Macrocycle **36a**

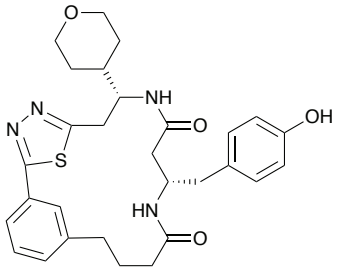


Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator **16** (0.08 mmol, 1.0 equiv) and monomer **30** (0.12 mmol, 1.5 equiv). The crude reaction mixture was diluted with CH_3CN:H_2O (1:1) and purified

directly by preparative HPLC (25 to 55% CH_3CN with 0.1% TFA over 38 min). Macrocycle **36a** was isolated as a white powder after lyophilization. Retention time: 22.5 minutes.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (d, *J* = 9.4 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.22 (s, 1H), 4.22 – 4.14 (m, 2H), 3.46 – 3.39 (m, 1H), 2.81 – 2.75 (m, 1H), 2.74 – 2.68 (m, 1H), 2.53 – 2.51 (m, 1H), 2.29 – 2.24 (m, 2H), 2.20 – 2.16 (m, 1H), 2.12 – 2.02 (m, 3H), 1.95 – 1.64 (m, 8H), 1.62 – 1.53 (m, 2H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 172.1, 171.0, 170.1, 168.2, 141.6, 131.3, 131.2, 129.9, 129.2, 122.4, 56.7, 53.0, 47.0, 45.7, 31.8, 31.7, 31.2, 30.7, 27.3, 24.3, 24.2, 21.2, 20.7; **HRMS** (ESI): calculated for $C_{23}H_{28}N_4NaO_2S$ $[M+Na]^+$: 477.1825, found: 447.1831.

Macrocycle **38d**

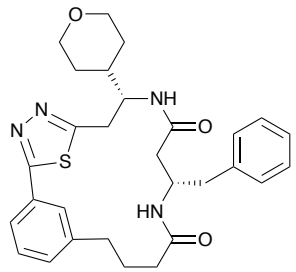


Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator **16** (0.09 mmol, 1.0 equiv), monomer **28** (0.09 mmol, 1.0 equiv) and monomer **27** (0.09 mmol, 1.0 equiv). The crude reaction mixture was diluted with CH_3CN:H_2O (1:1) and purified directly by preparative HPLC (25 to 60% CH_3CN with 0.1% TFA over 40 min). Macrocycle **38d** was isolated as a white powder after lyophilization. Retention time: 32.5 minutes.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.17 (s, 1H), 7.89 (d, *J* = 9.7 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.16 (s, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 4.42 – 4.36 (m, 1H), 3.99 – 3.91 (m, 1H), 3.88 – 3.83 (m, 2H), 3.40 – 3.35 (m, 1H), 3.26 – 3.19 (m, 2H), 2.97 (dd, *J* = 15.9, 12.2 Hz, 1H), 2.80 – 2.71 (m, 2H), 2.67 (dd, *J* = 13.3, 4.3 Hz, 1H), 2.47 (dd, *J* = 12.3, 7.0 Hz, 2H), 2.28 – 2.25 (m, 2H), 2.06 – 1.98 (m, 3H), 1.71 – 1.60 (m, 3H), 1.51 – 1.42 (m, 1H), 1.28 – 1.19 (m, 2H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.3, 170.6, 168.6, 168.1, 155.7, 141.6, 131.3, 130.5, 130.3, 129.6, 129.4, 128.7, 122.3, 115.0, 67.0, 66.7, 52.2, 47.0, 40.0, 39.3, 37.6, 31.9, 31.5, 31.5, 29.5,

28.3, 22.7; **HRMS** (ESI): calculated for $C_{29}H_{35}N_4O_4S$ $[M+H]^+$: 535.2374, found: 535.2381.

Macrocycle **45b**



Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator **16** (0.08 mmol, 1.0 equiv), monomer **38** (0.07 mmol, 0.9 equiv) and monomer **27** (0.07 mmol, 0.9 equiv). The crude reaction mixture was diluted with CH_3CN:H_2O (1:1) and purified directly by preparative HPLC (30 to 60% CH_3CN with 0.1% TFA over 38 min). Macrocycle **45b** was isolated as a white powder after lyophilization. Retention time: 18.5 minutes.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (d, *J* = 9.7 Hz, 1H), 7.81 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.34 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.29-7.27 (m, 2H), 7.22 – 7.13 (m, 4H), 4.49 – 4.44 (m, 1H), 3.99 – 3.92 (m, 1H), 3.87-3.83 (m, 2H), 3.40 – 3.36 (m, 1H), 3.28 – 3.19 (m, 2H), 2.97 (dd, *J* = 15.9, 12.3 Hz, 1H), 2.81 – 2.71 (m, 3H), 2.62 (dd, *J* = 13.2, 7.8 Hz, 1H), 2.31 – 2.29 (m, 2H), 2.07 – 1.99 (m, 3H), 1.70 – 1.64 (m, 2H), 1.64 – 1.58 (m, 1H), 1.51 – 1.46 (m, 1H), 1.27 – 1.20 (m, 2H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.4, 170.5, 168.6, 168.1, 141.6, 138.6, 131.3, 130.5, 129.6, 129.5, 129.4, 128.2, 126.1, 122.3, 66.9, 66.7, 52.2, 46.7, 40.1, 40.0 37.7, 31.9, 31.5, 31.4, 29.6, 28.3, 22.7; **HRMS** (ESI): calculated for $C_{29}H_{34}N_4NaO_3S$ $[M+Na]^+$: 541.2244, found: 541.2237.

References

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2 T. Gerfaud, Y.-L. Chiang, I. Kreituss, J. A. Russak and J. W. Bode, *Org. Process Res. Dev.*, 2012, **16**, 687–696.
3 Y.-L. Huang and J. W. Bode, *Nat. Chem.*, 2014, **6**, 877–884.
4 D. S. Kalinowski, P. C. Sharpe, P. V. Bernhardt and D. R. Richardson, *J. Med. Chem.*, 2007, **50**, 6212–6225.
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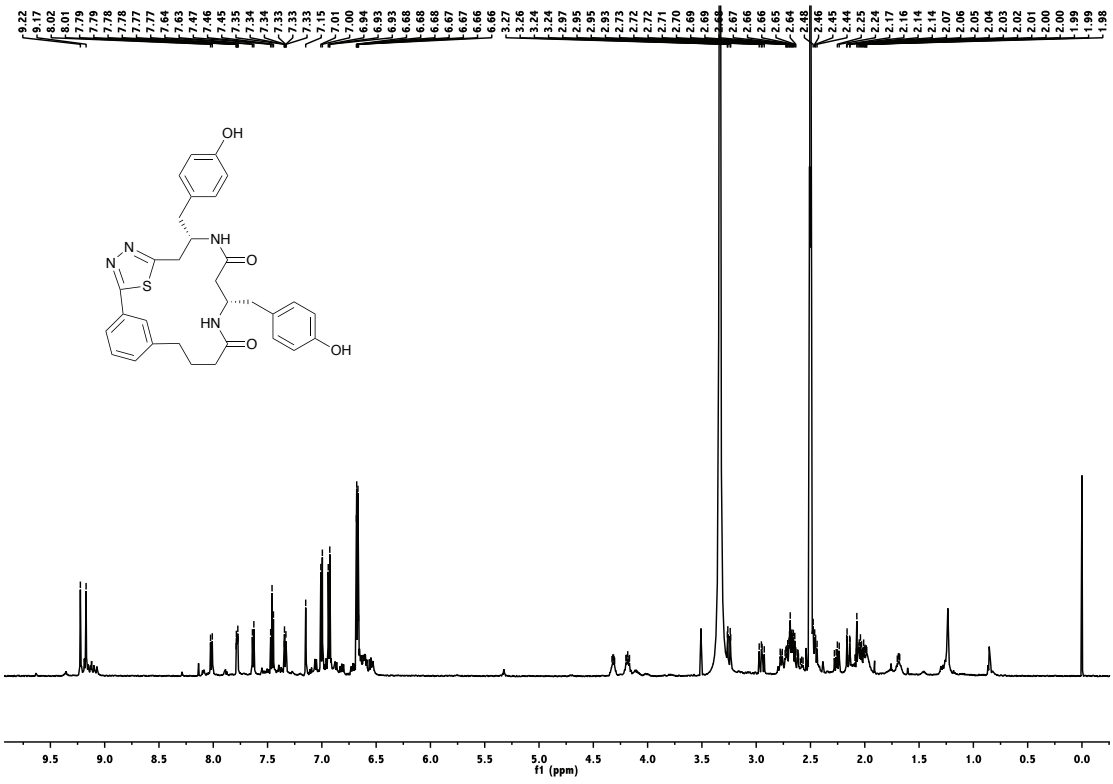
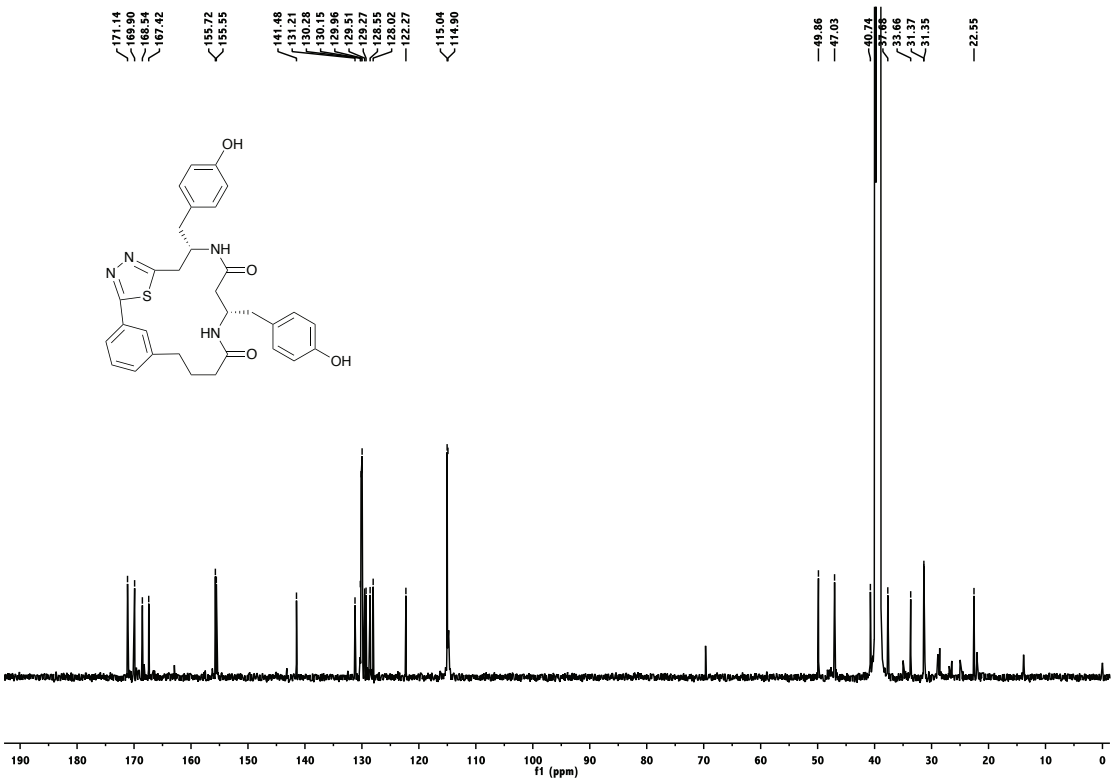
NMR spectra
Macrocycle 34a

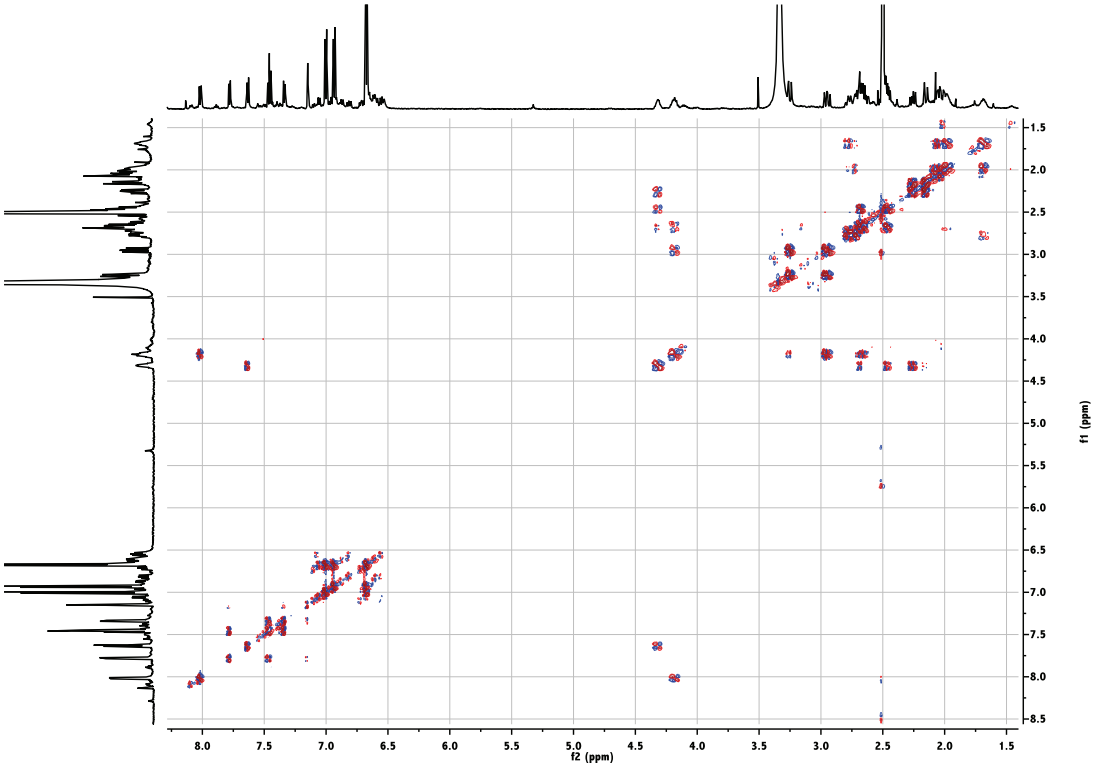
^{1}H NMR (600 MHz, DMSO-d_6)



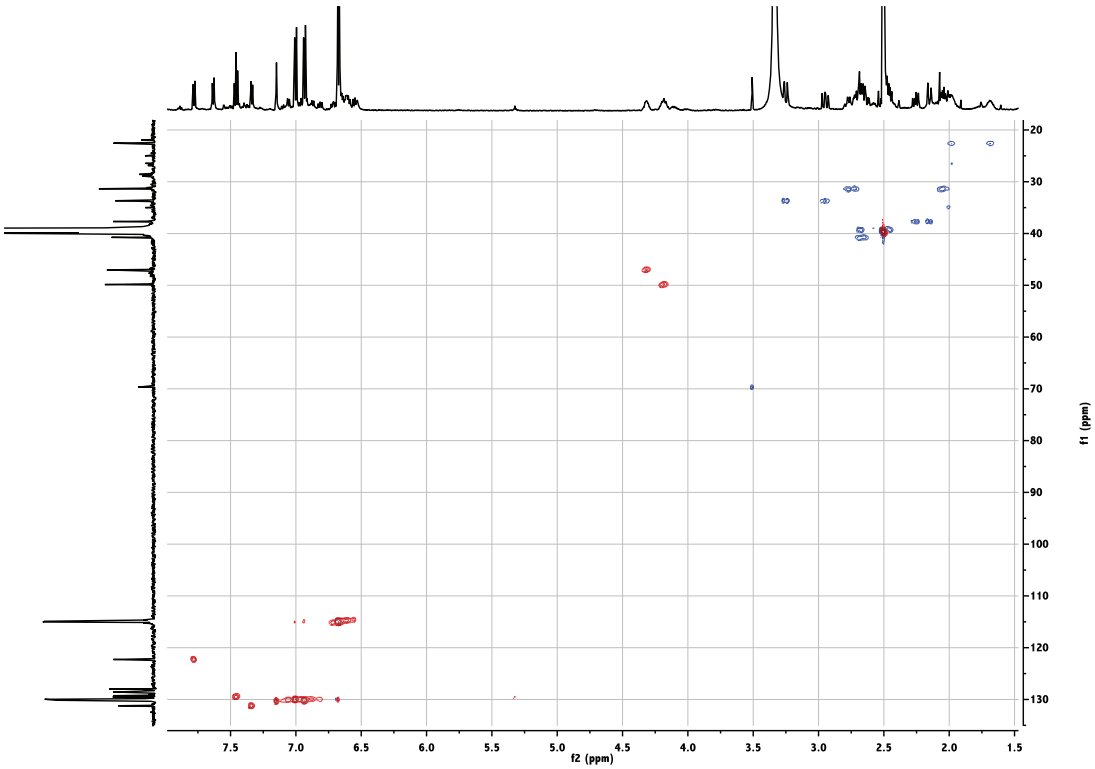
^{13}C NMR (150 MHz, DMSO-d_6)



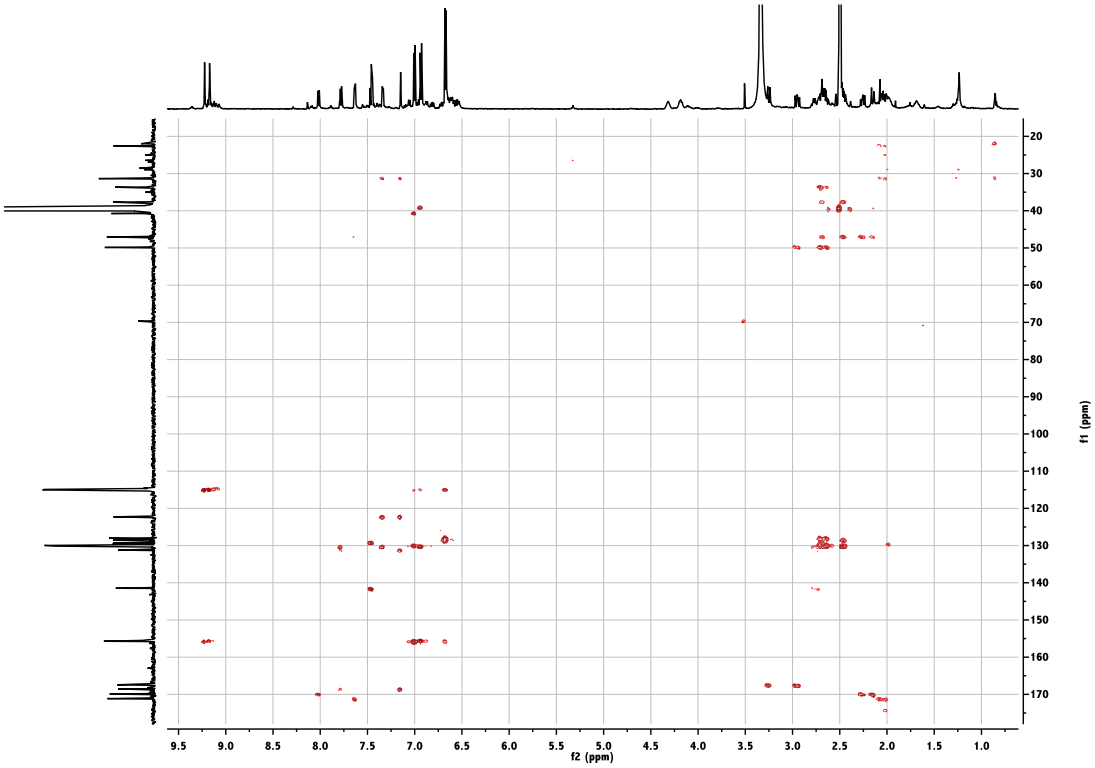
^{1}H-^{1}H DQF-COSY (600 MHz, DMSO-d_6)



^{1}H-^{13}C HSQC (600 MHz, DMSO-d_6)

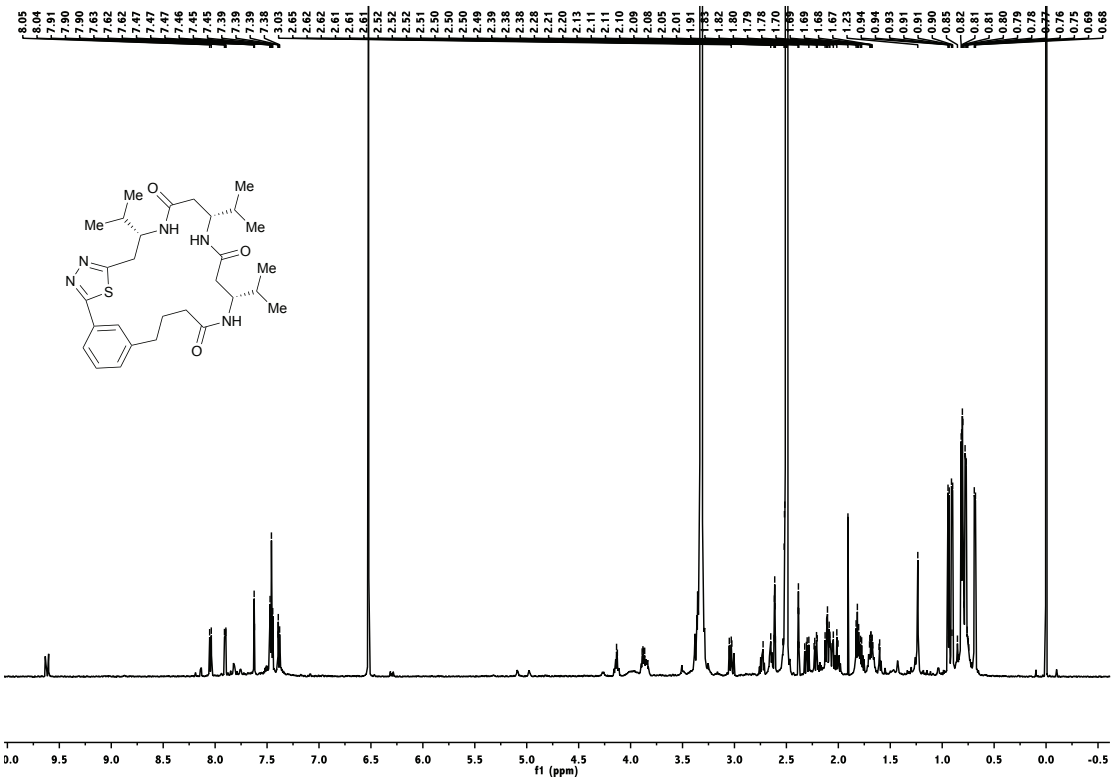


^{1}H-^{13}C HMBC (600 MHz, DMSO-d_6)

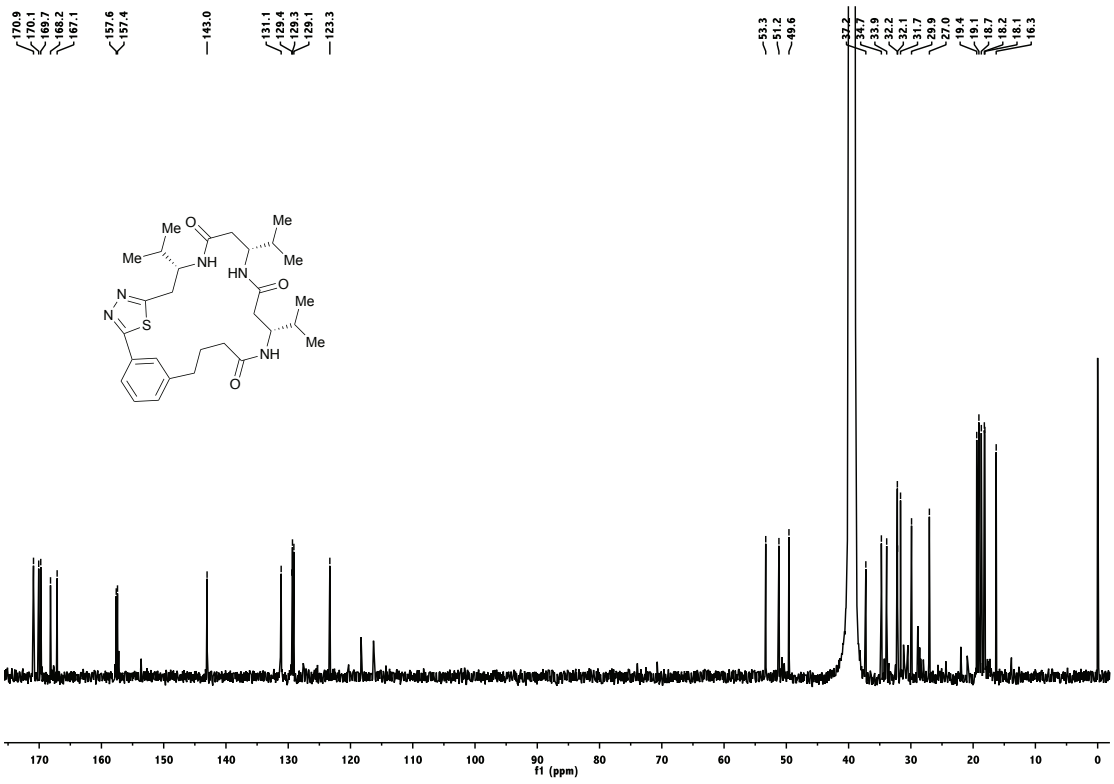


Macrocycle 31b

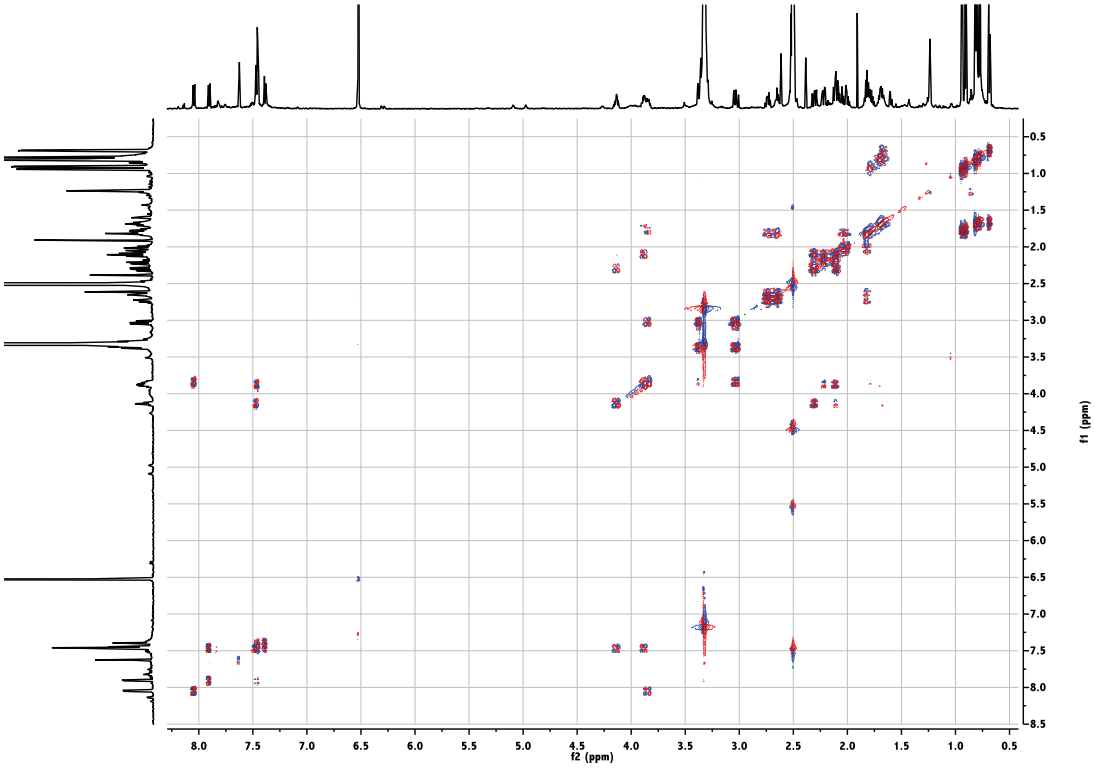
^{1}H NMR (600 MHz, DMSO-d_6)



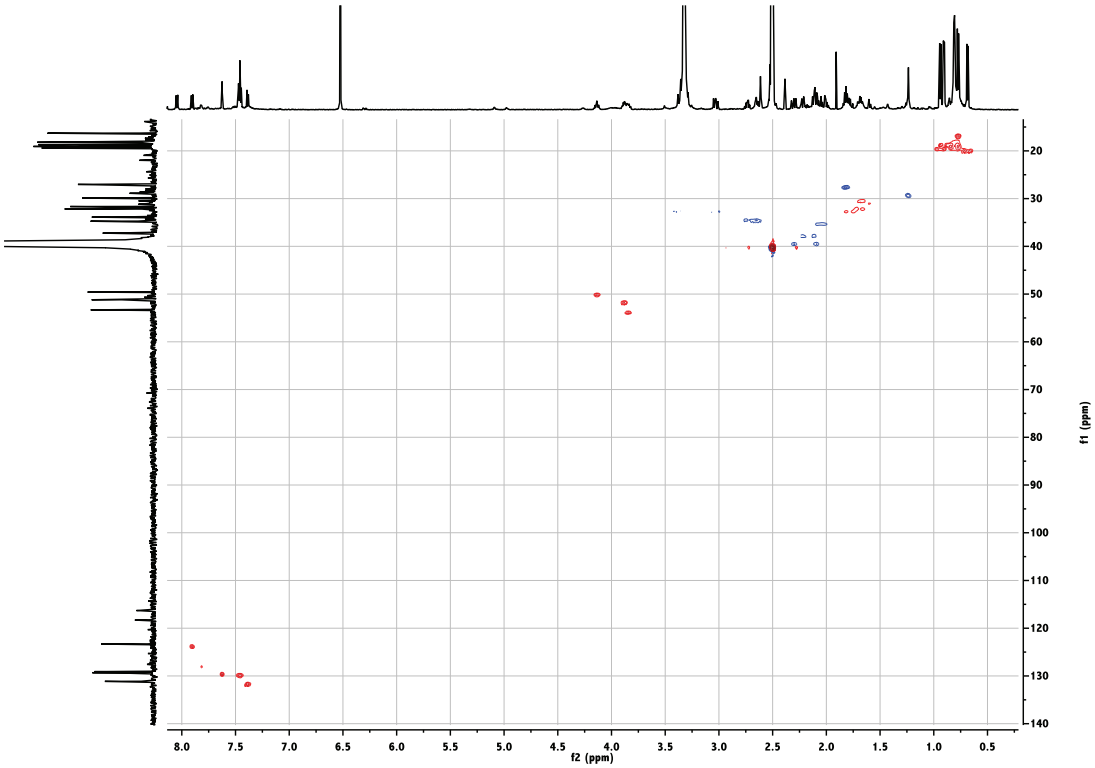
^{13}C NMR (150 MHz, DMSO-d_6)



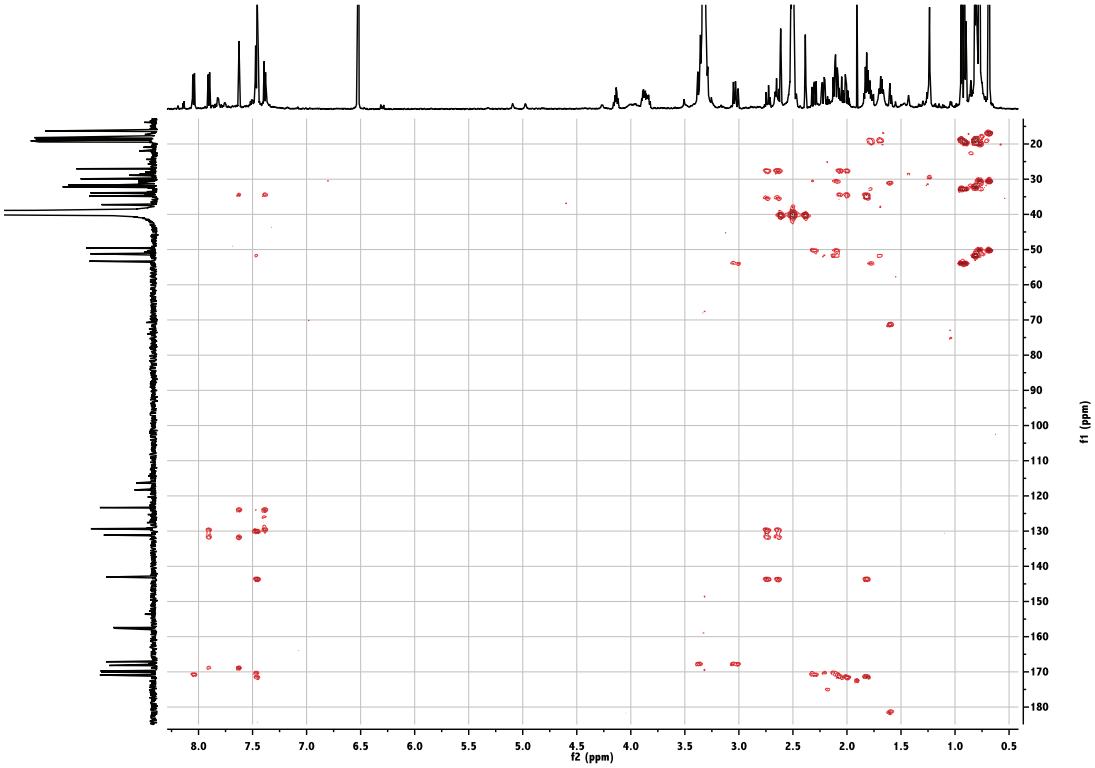
^{1}H-^{1}H DQF-COSY (600 MHz, DMSO-d_6)



^{1}H-^{13}C HSQC (600 MHz, DMSO-d_6)

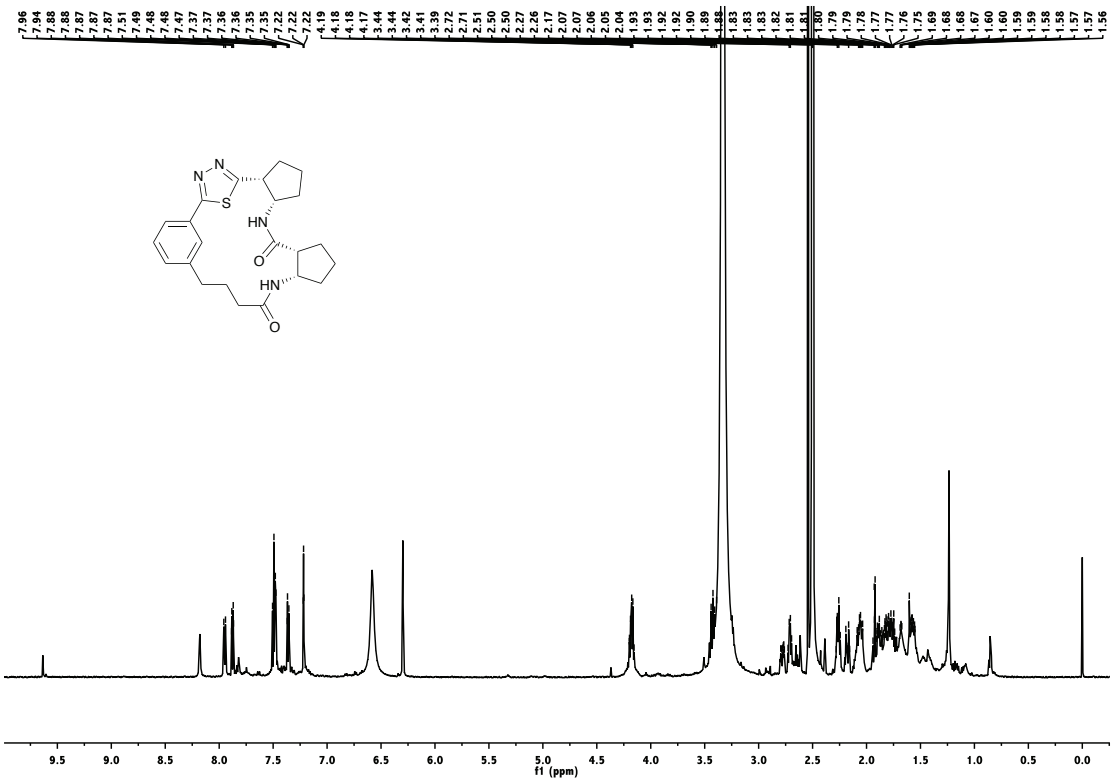


^{1}H-^{13}C HMBC (600 MHz, DMSO-d_6)

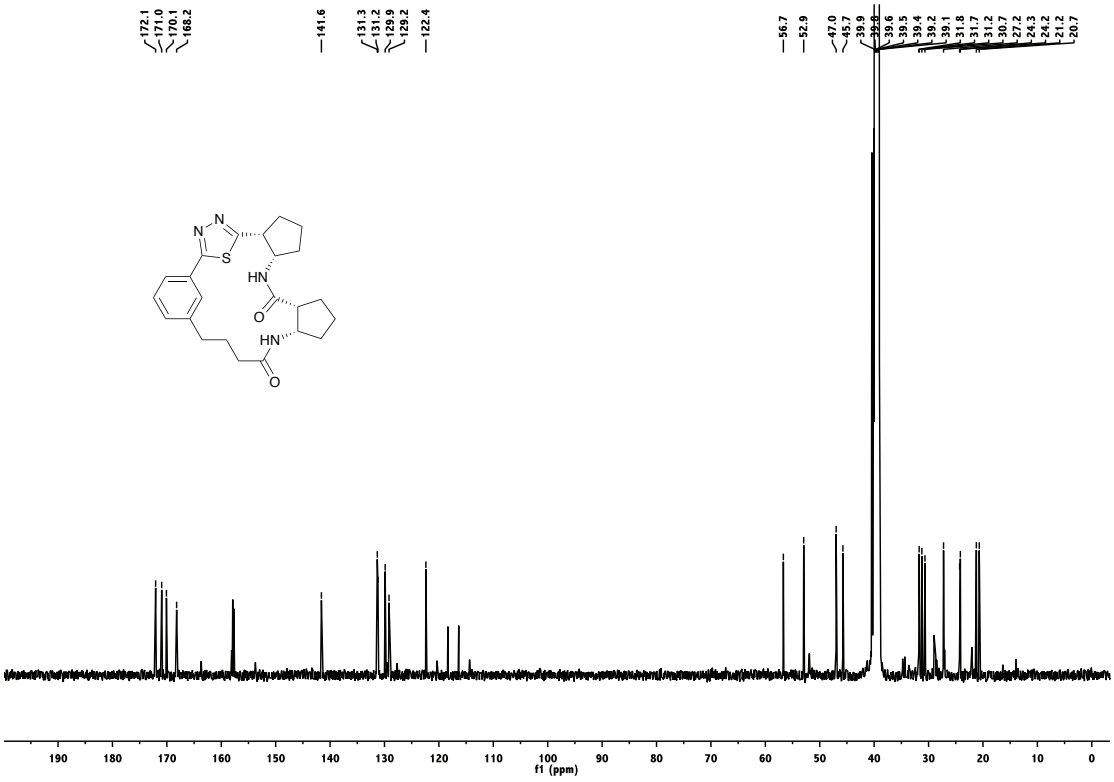


Macrocycle 36a

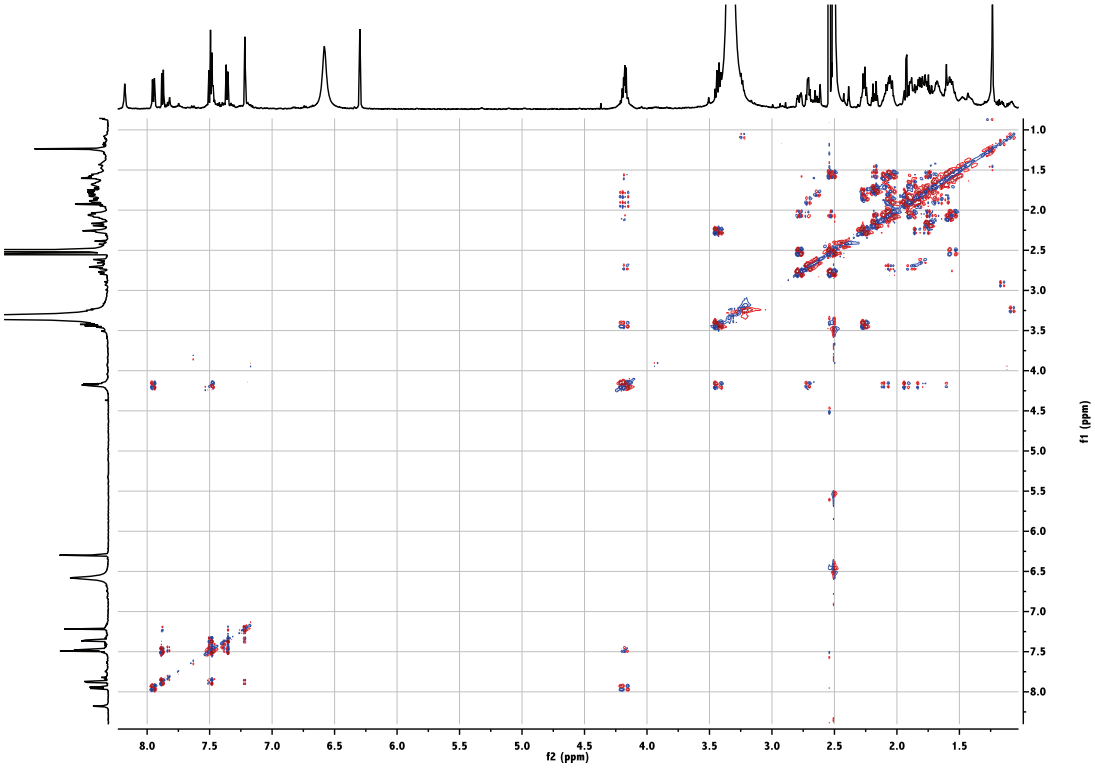
^{1}H NMR (600 MHz, DMSO-d_6)



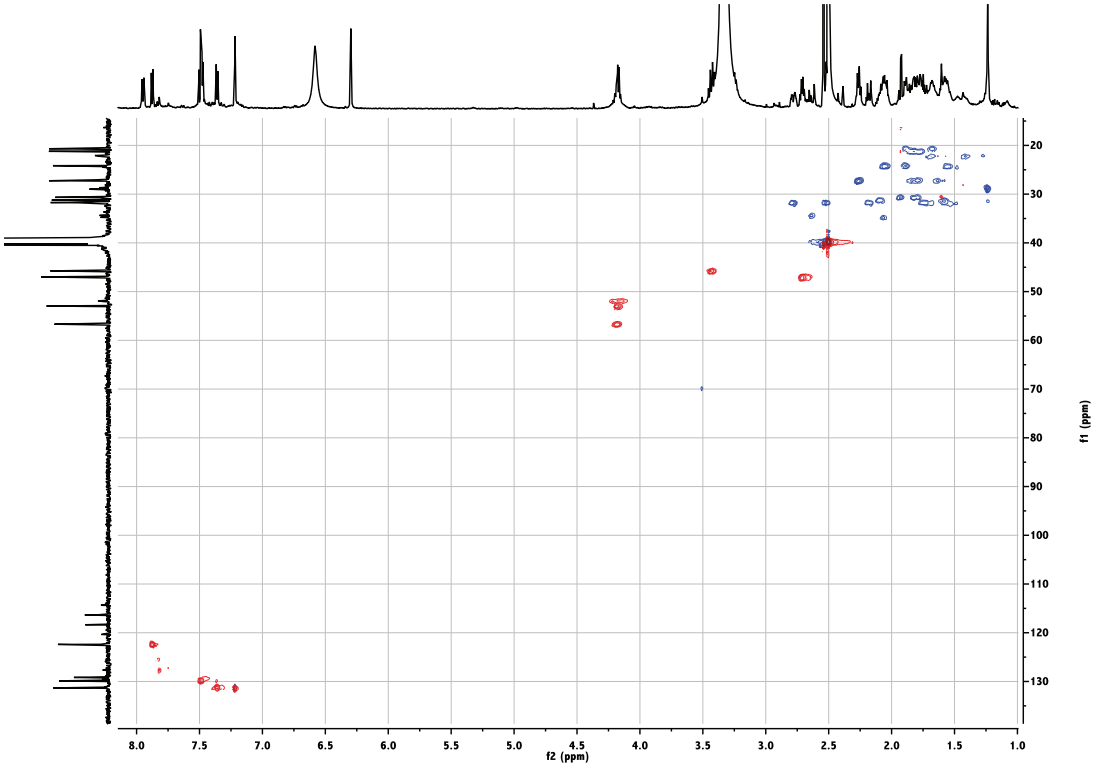
^{13}C NMR (150 MHz, DMSO-d_6)



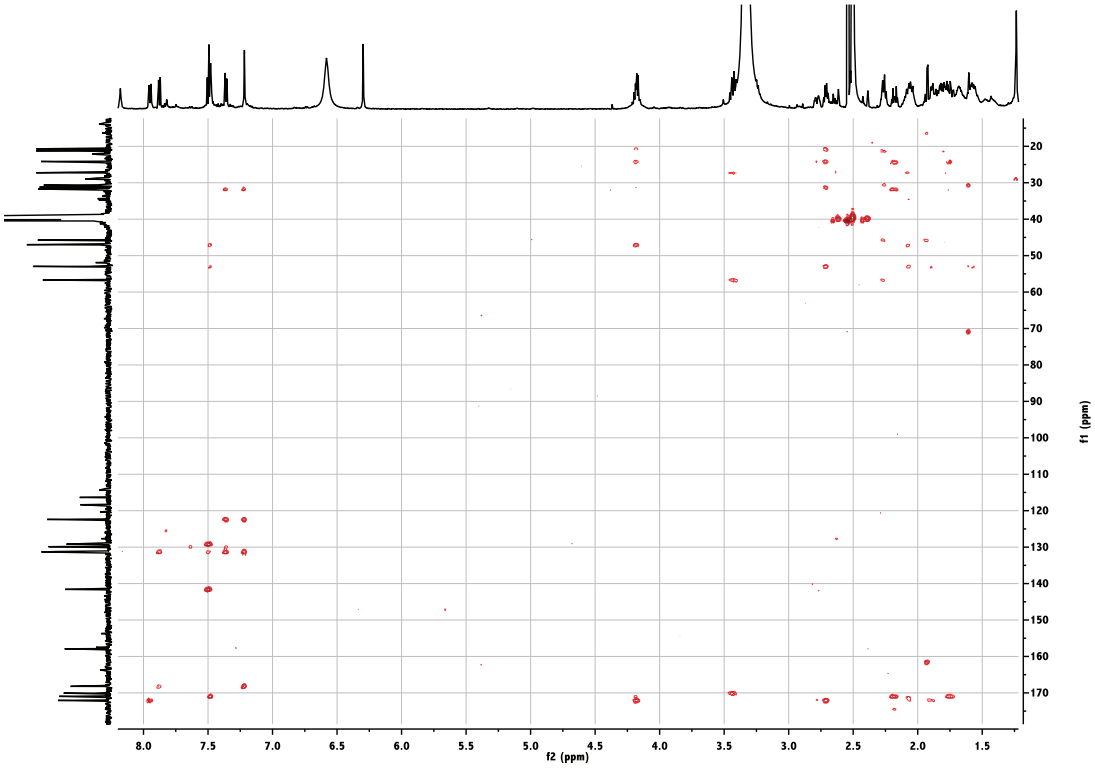
^{1}H-^{1}H DQF-COSY (600 MHz, DMSO-d_6)



^{1}H-^{13}C HSQC (600 MHz, DMSO-d_6)

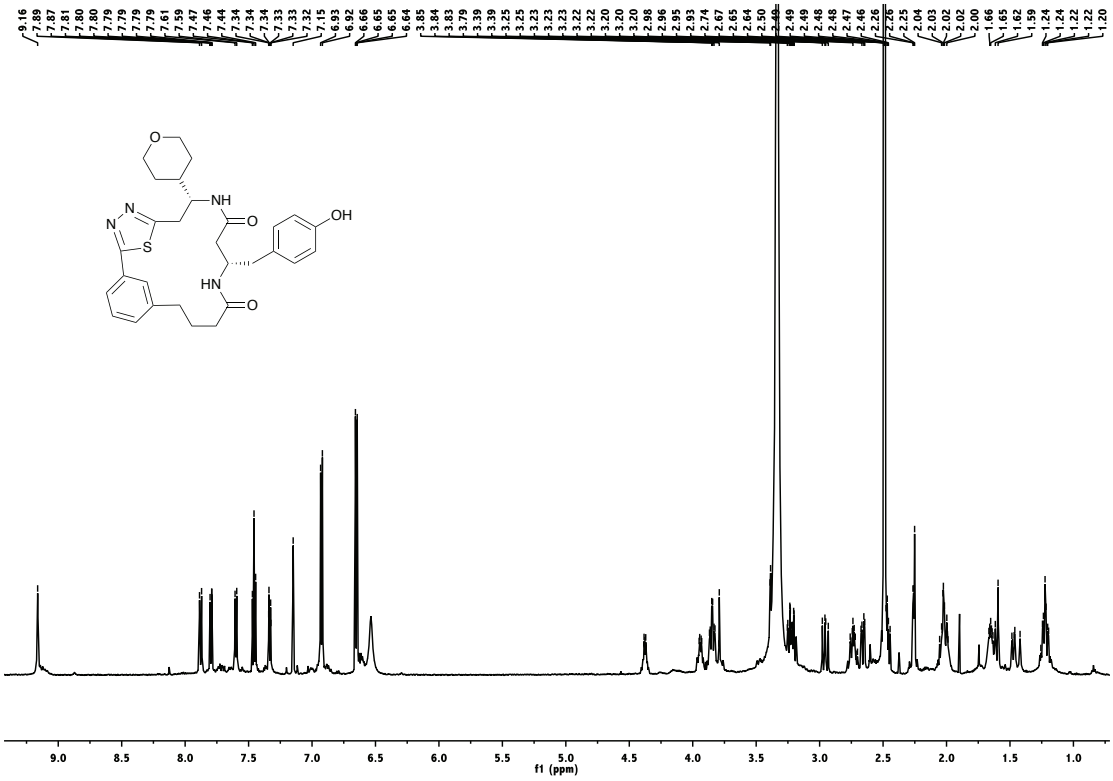


^{1}H-^{13}C HMBC (600 MHz, DMSO-d_6)

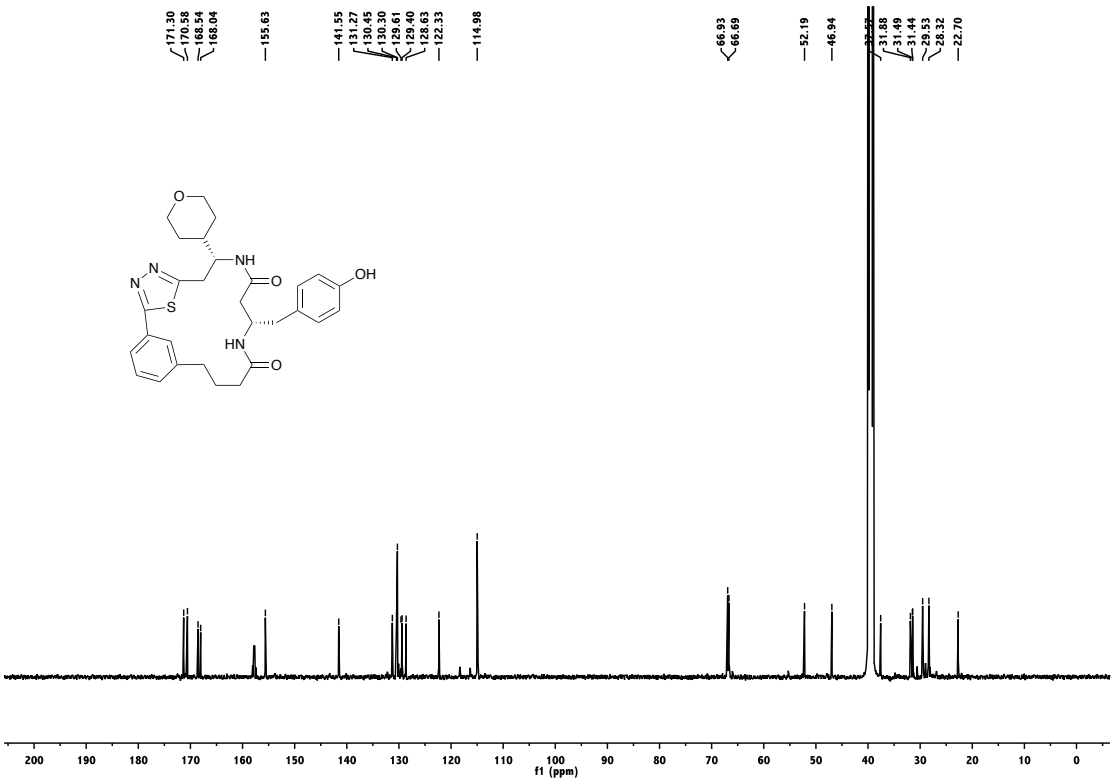


Macrocycle 38d

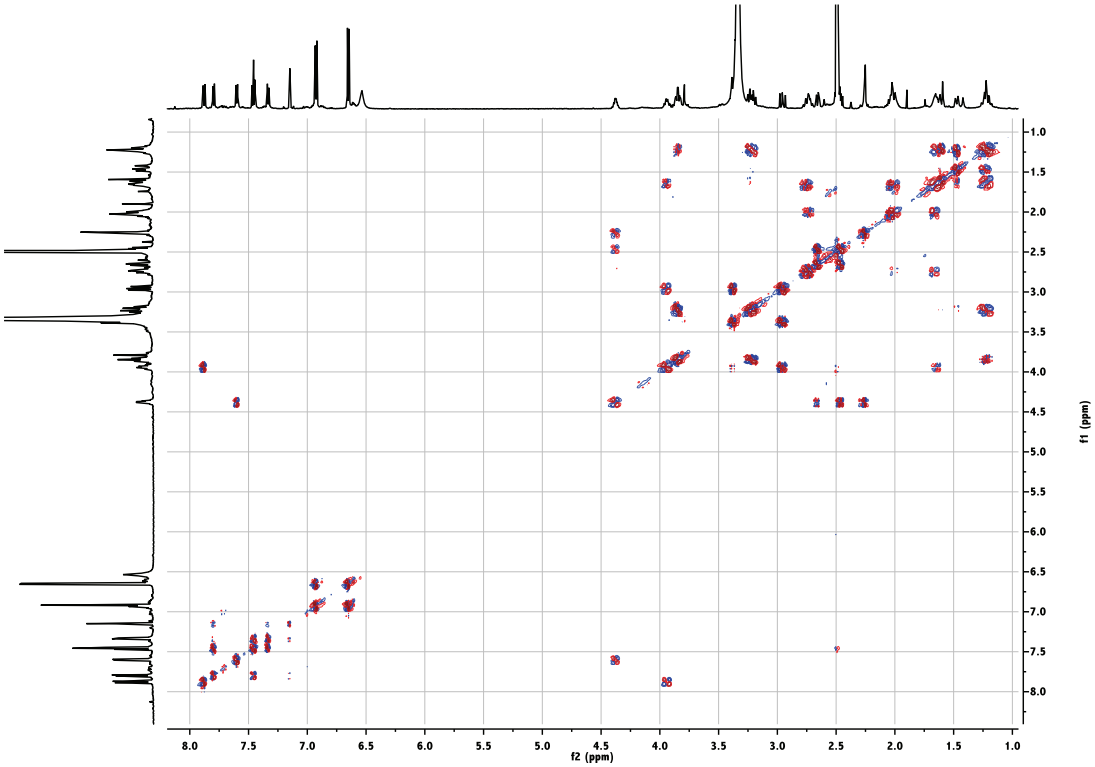
^{1}H NMR (600 MHz, DMSO-d_6)



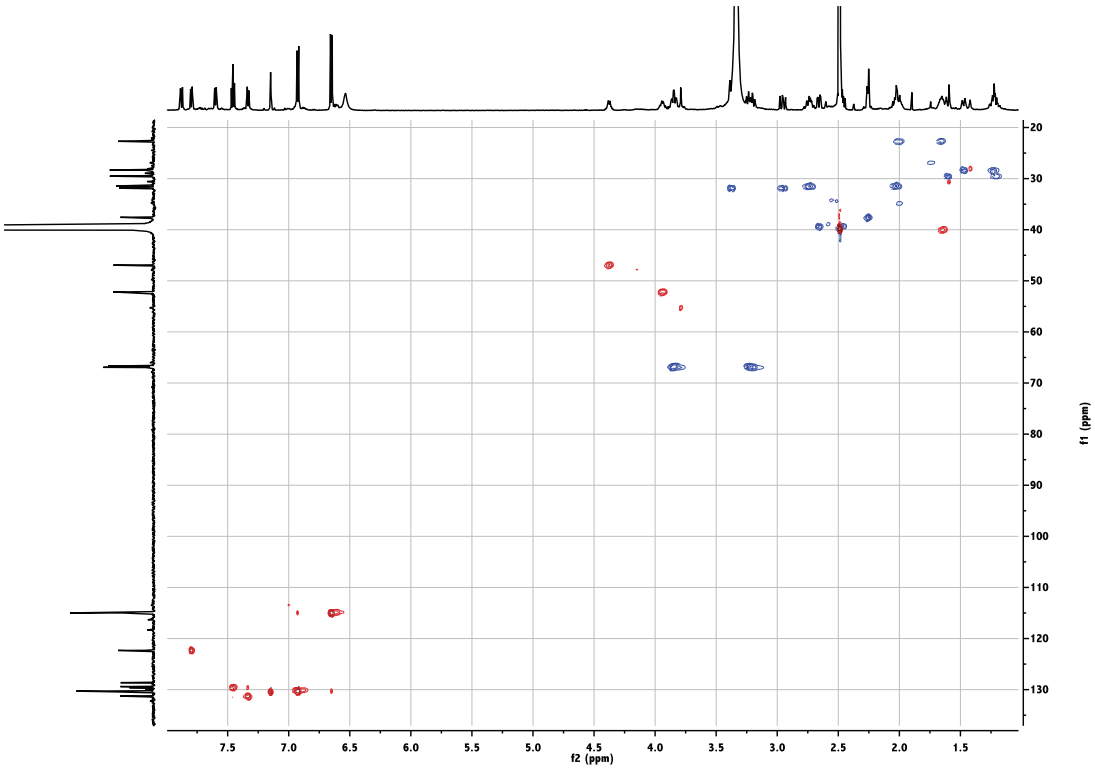
^{13}C NMR (150 MHz, DMSO-d_6)



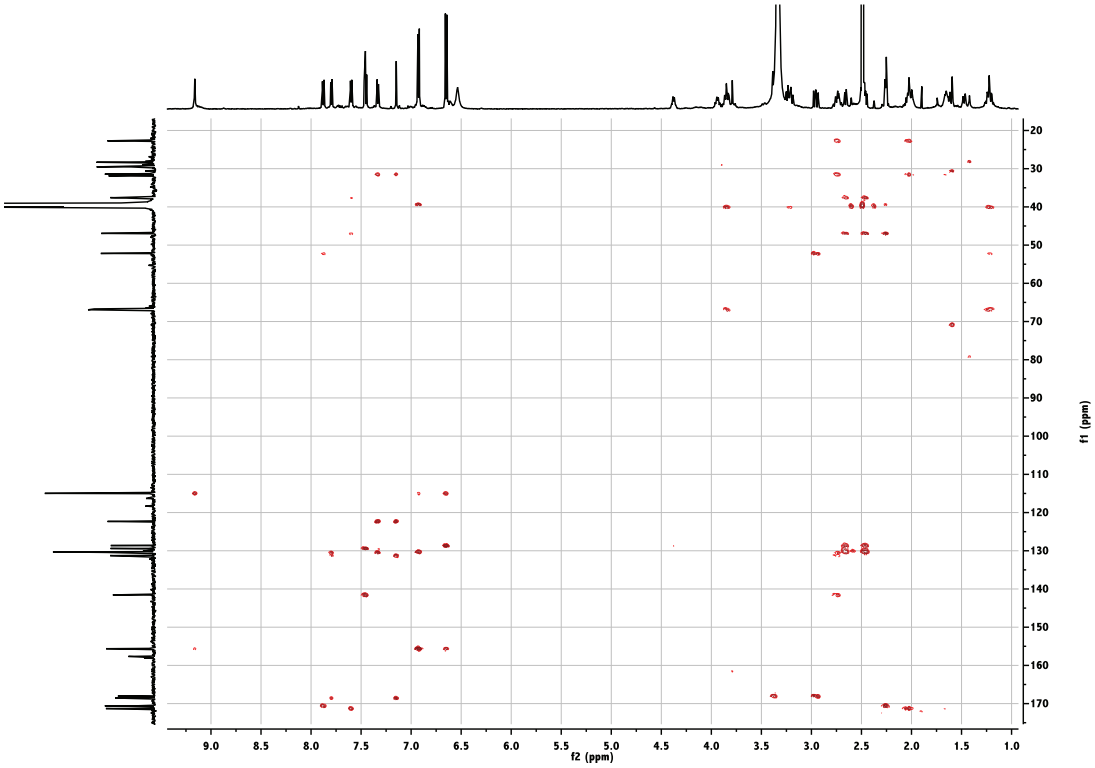
^{1}H-^{1}H DQF-COSY (600 MHz, DMSO-d_6)



^{1}H-^{13}C HSQC (600 MHz, DMSO-d_6)

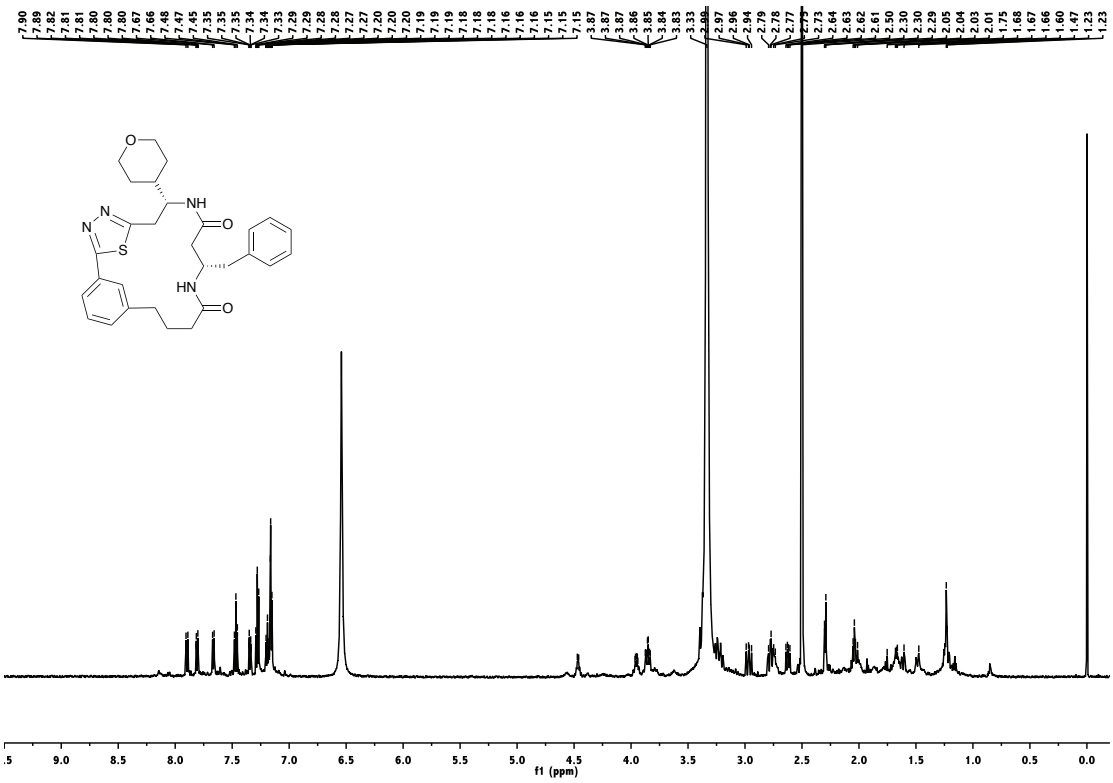


^{1}H-^{13}C HMBC (600 MHz, DMSO-d_6)

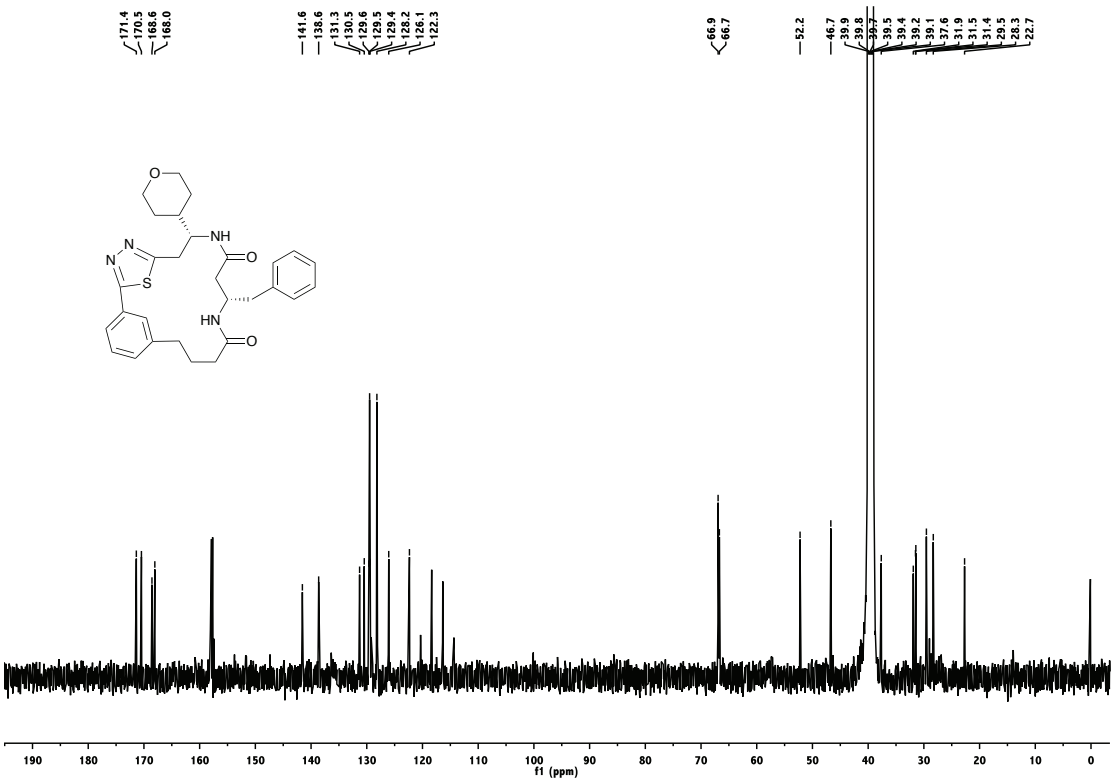


Macrocycle 45b

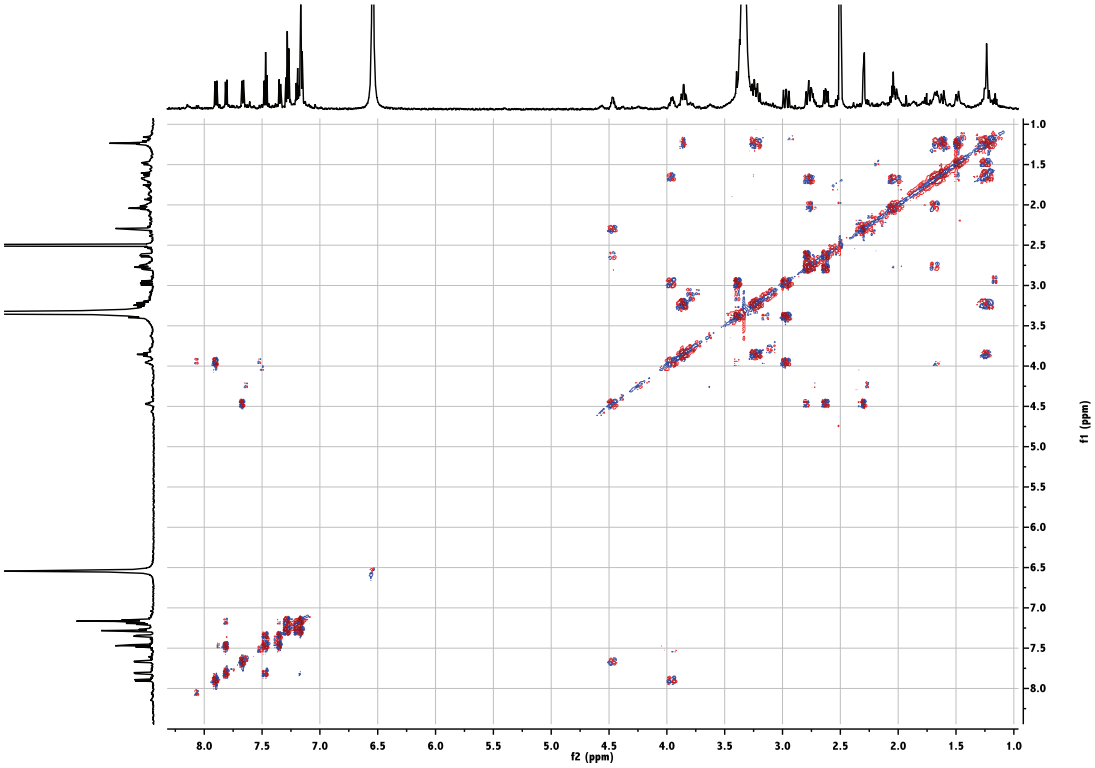
^{1}H NMR (600 MHz, DMSO-d_6)



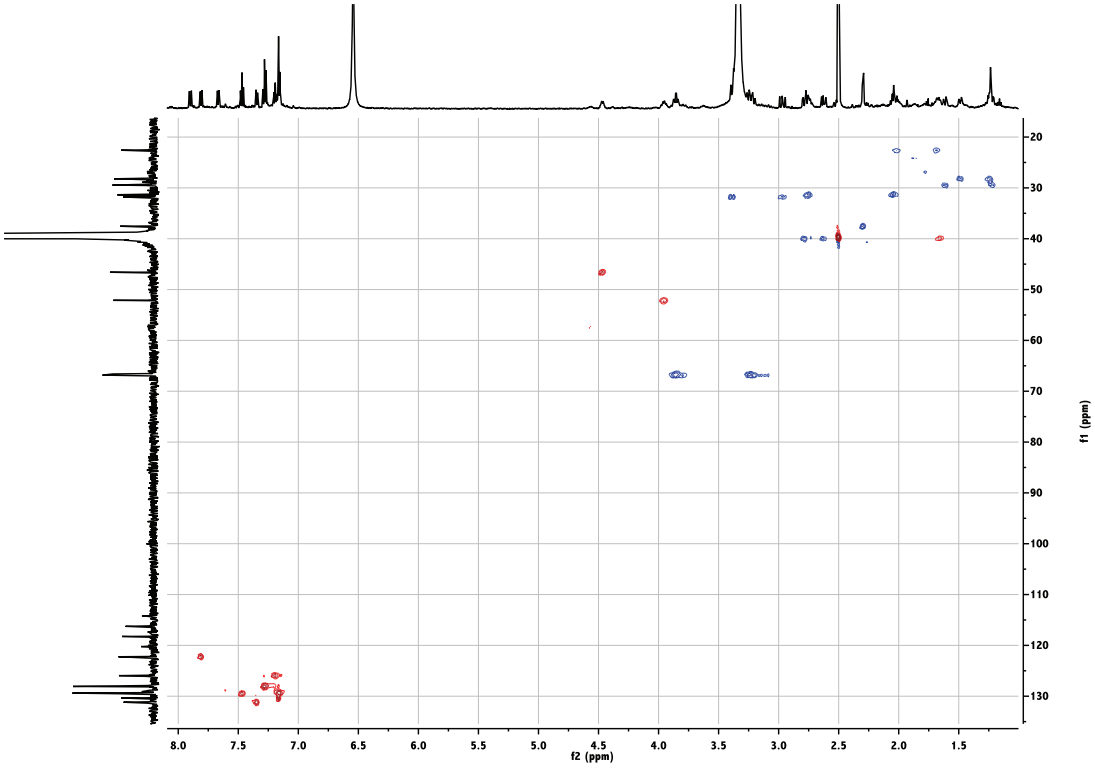
^{13}C NMR (150 MHz, DMSO-d_6)



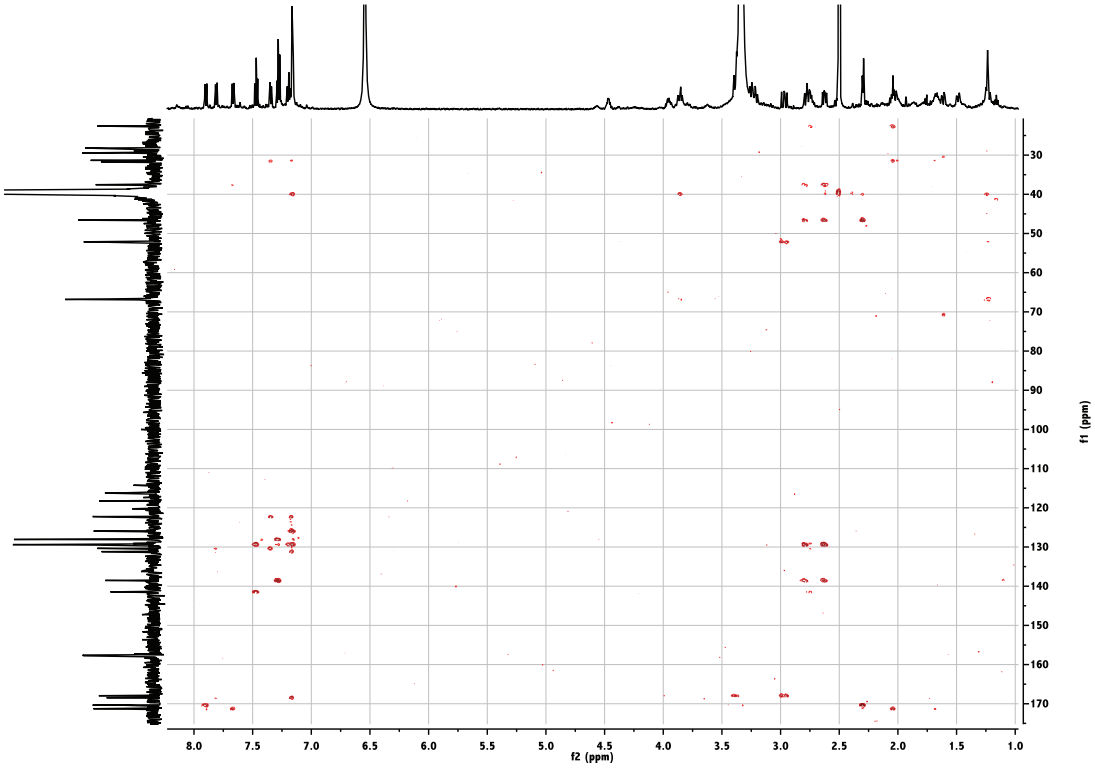
^{1}H-^{1}H DQF-COSY (600 MHz, DMSO-d_6)



^{1}H-^{13}C HSQC (600 MHz, DMSO-d_6)

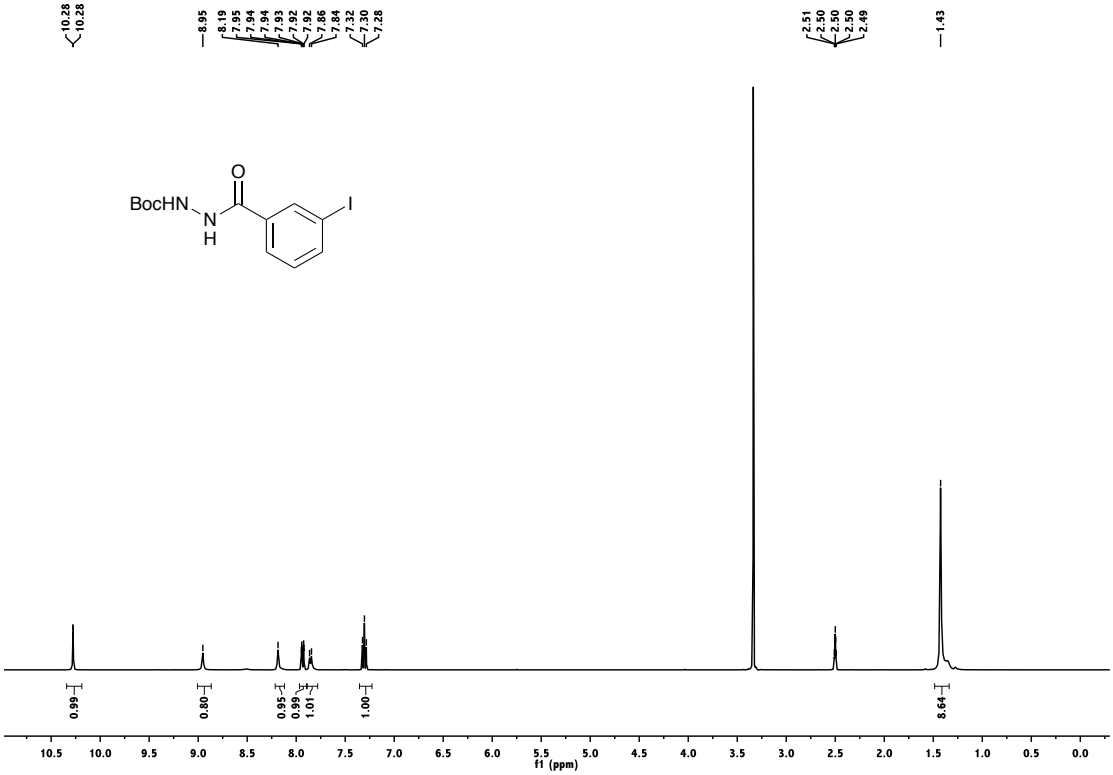


^{1}H-^{13}C HMBC (600 MHz, DMSO-d_6)

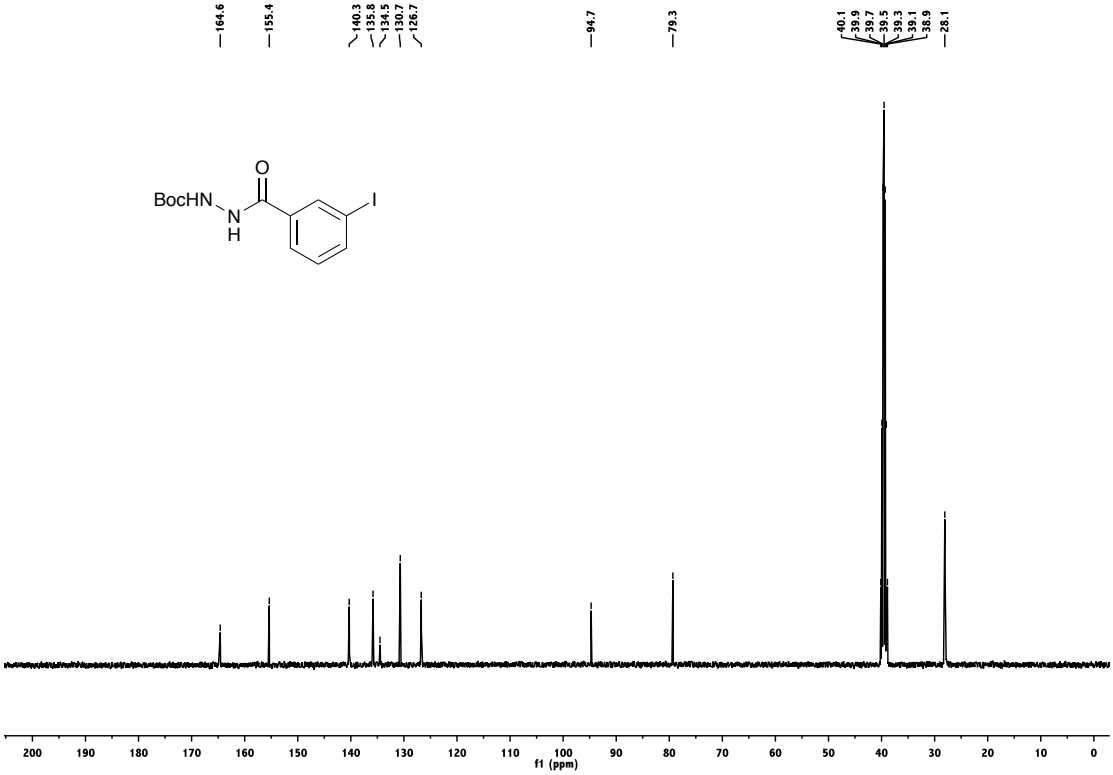


3-Iodophenylhydrazide 19

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

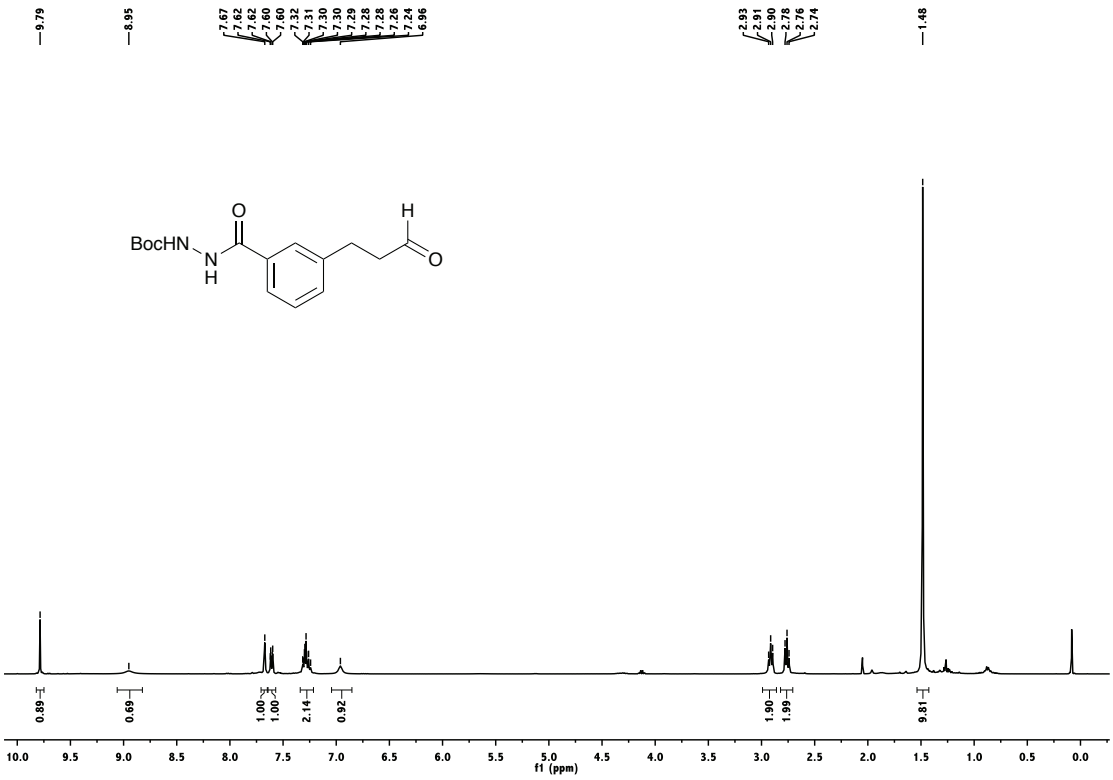


^{13}C NMR (101 MHz, DMSO-d_6)

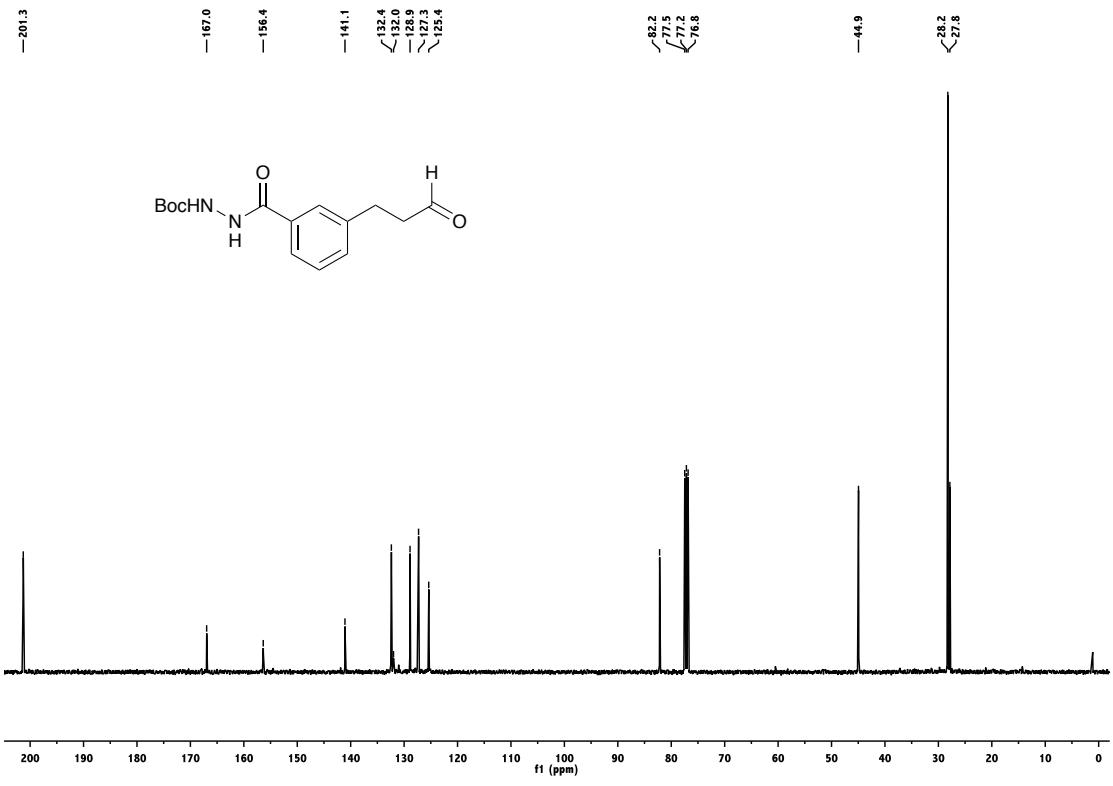


Aldehyde 21

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

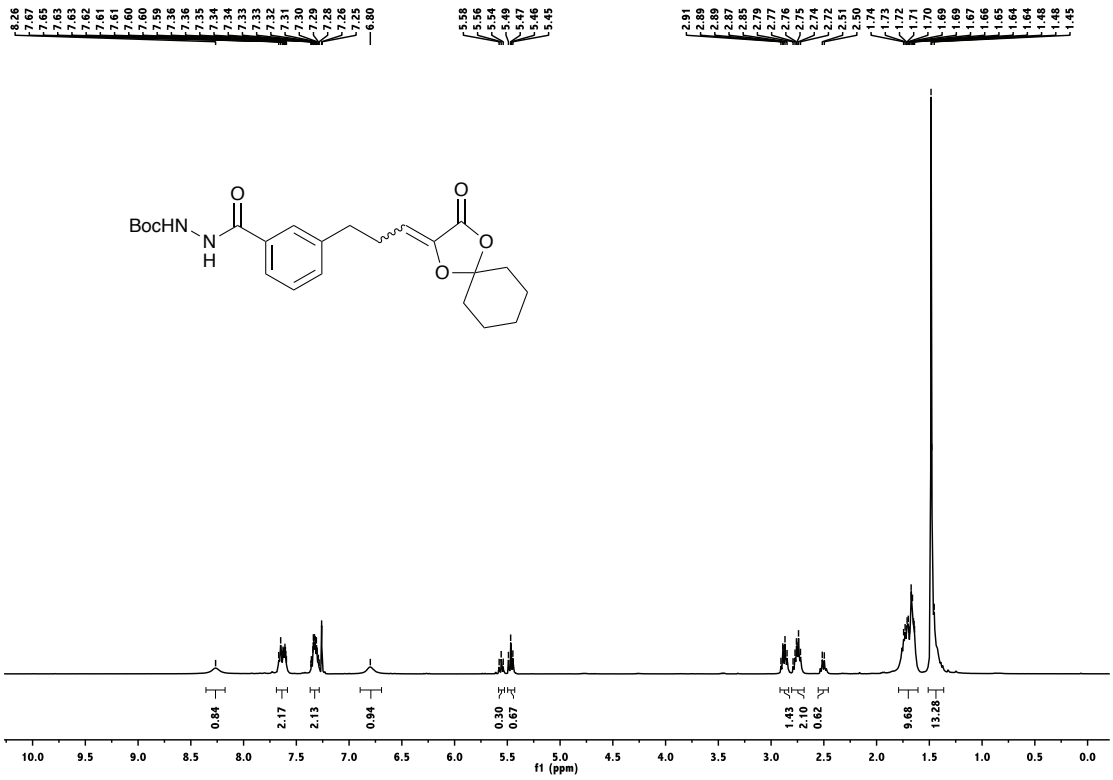


^{13}C NMR (101 MHz, $CDCl_3$)

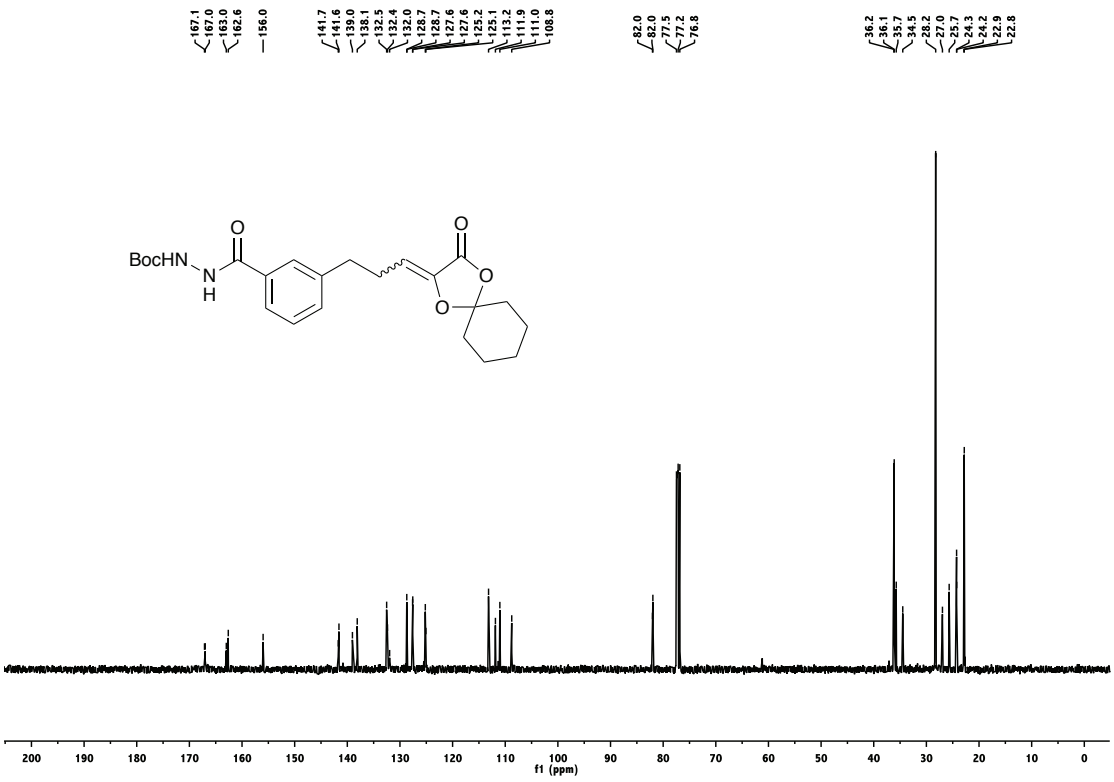


Enol ester 23

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

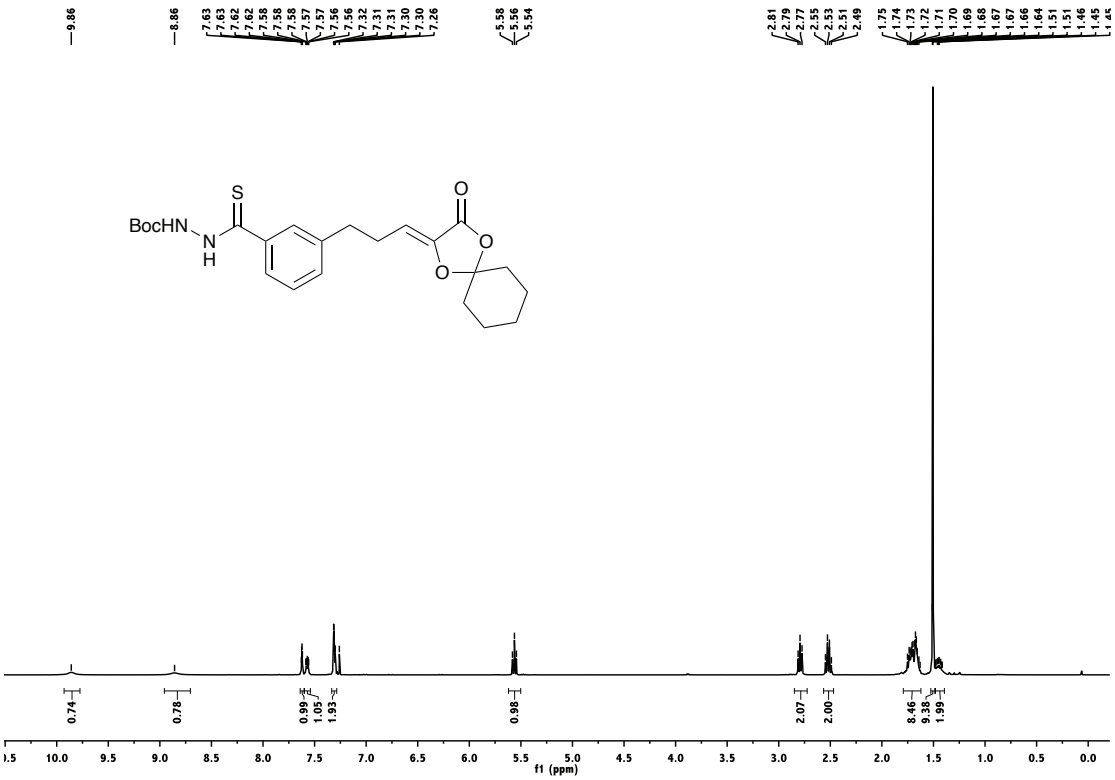


^{13}C NMR (101 MHz, $CDCl_3$)

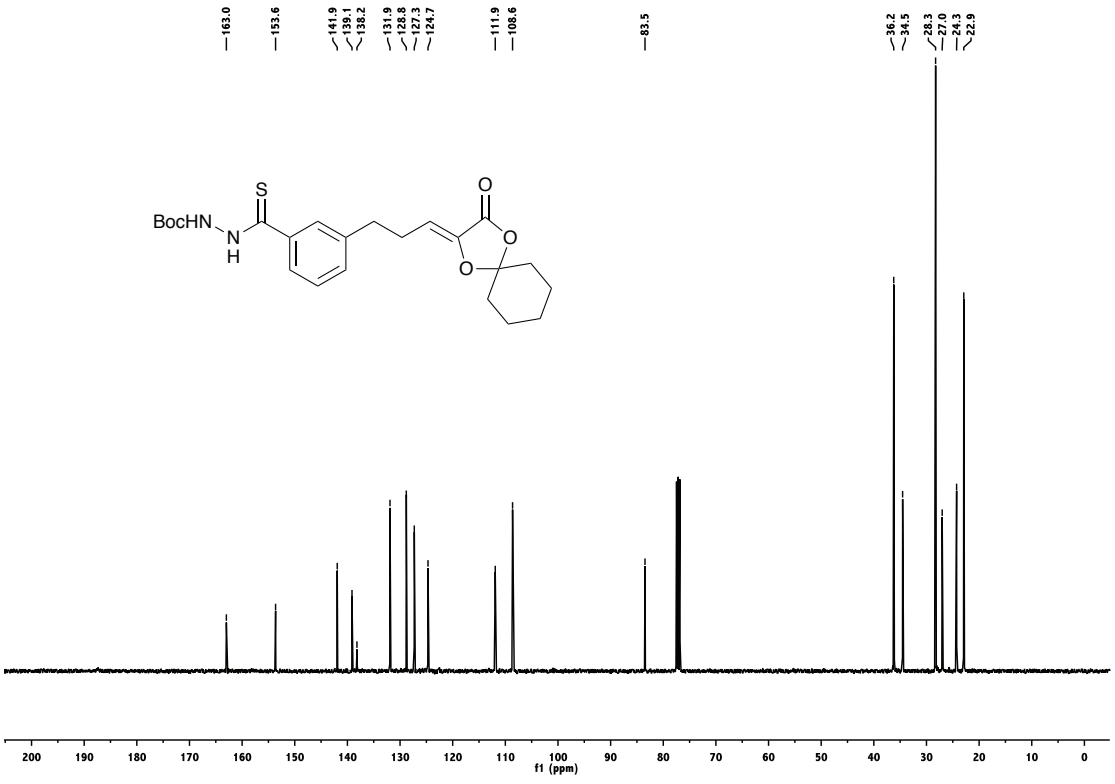


Thiohydrazide 24

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

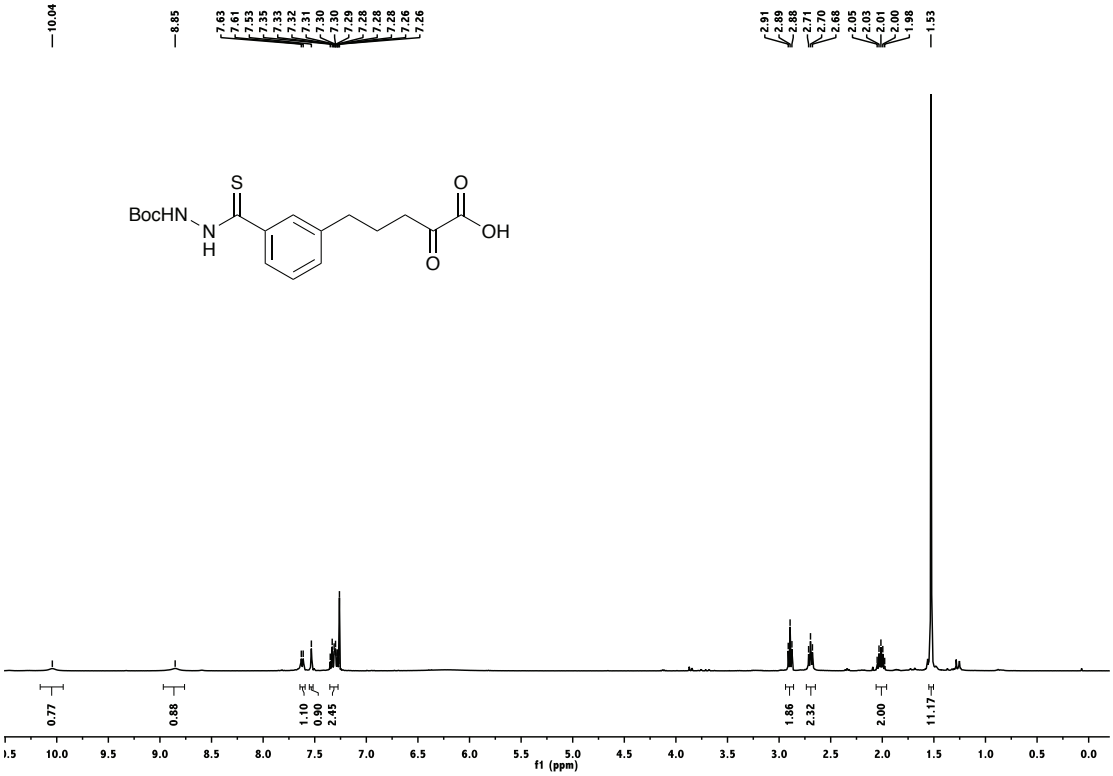


^{13}C NMR (101 MHz, $CDCl_3$)

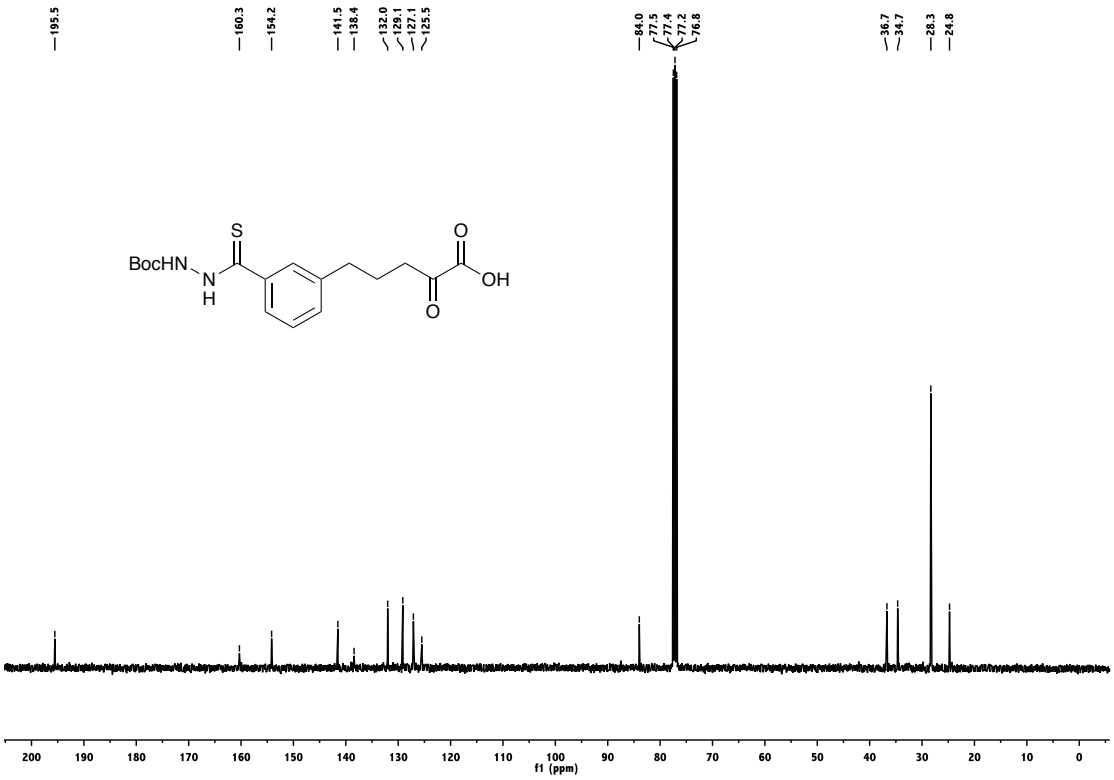


Ketoacid 16

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

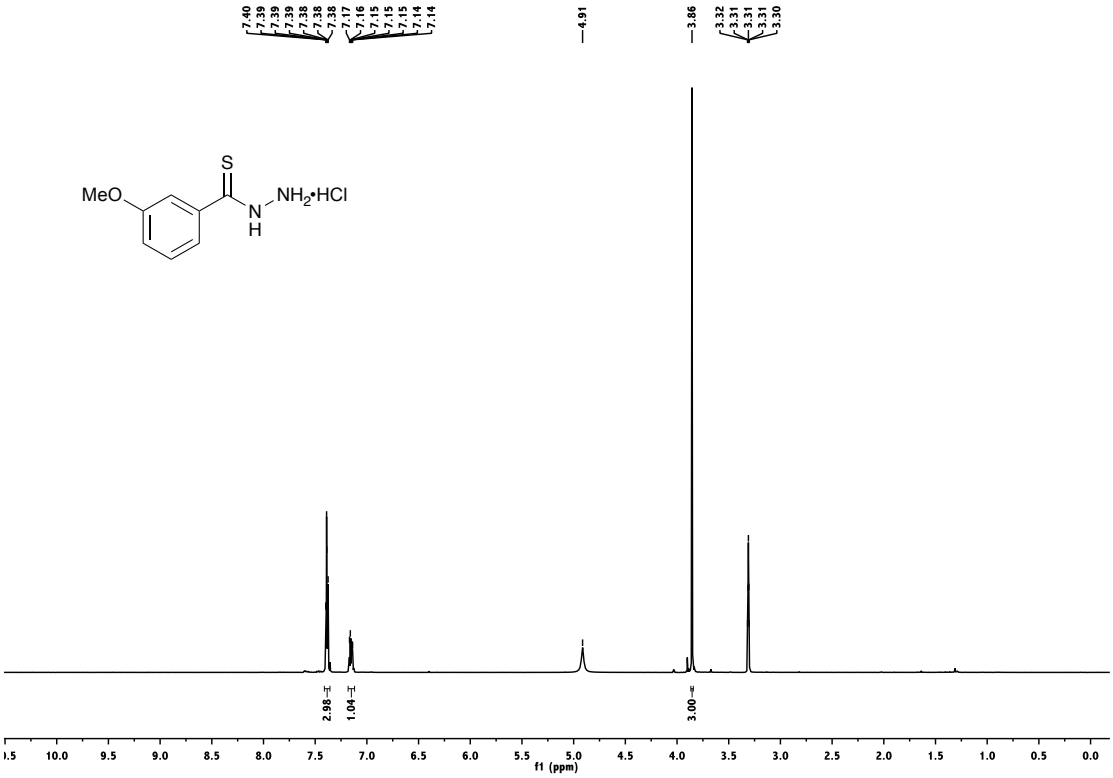


^{13}C NMR (101 MHz, $CDCl_3$)

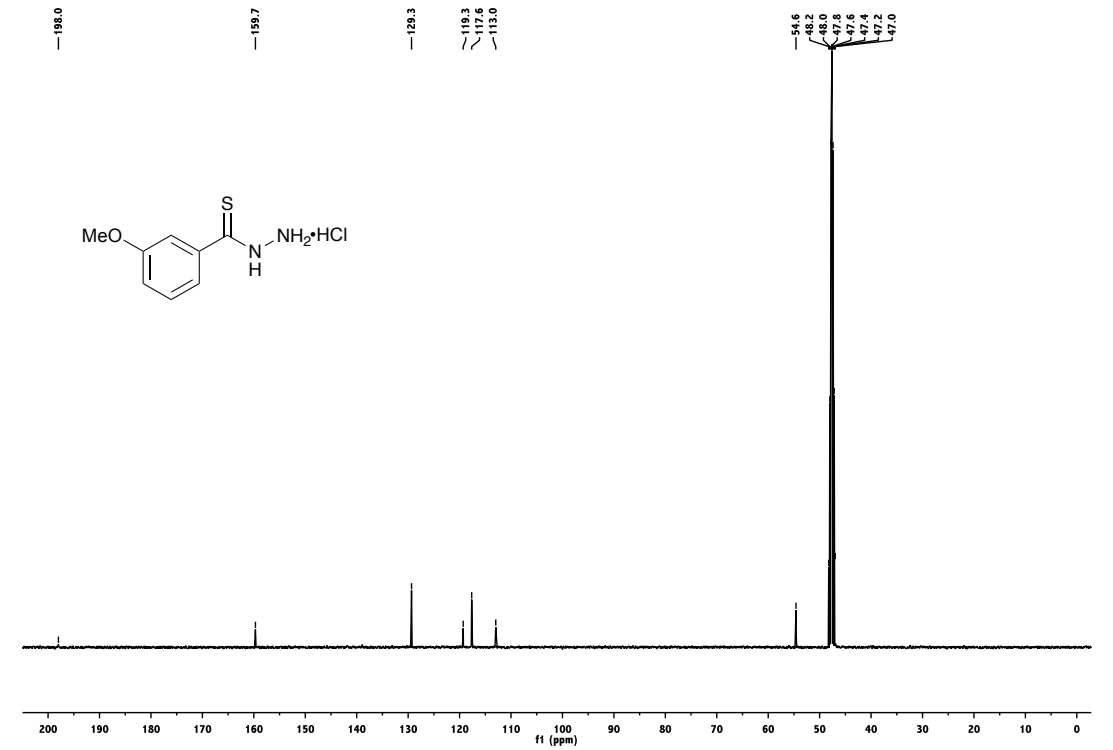


3-Methoxyphenyl thiohydrazide 10

^{1}H NMR (400 MHz, MeOD)

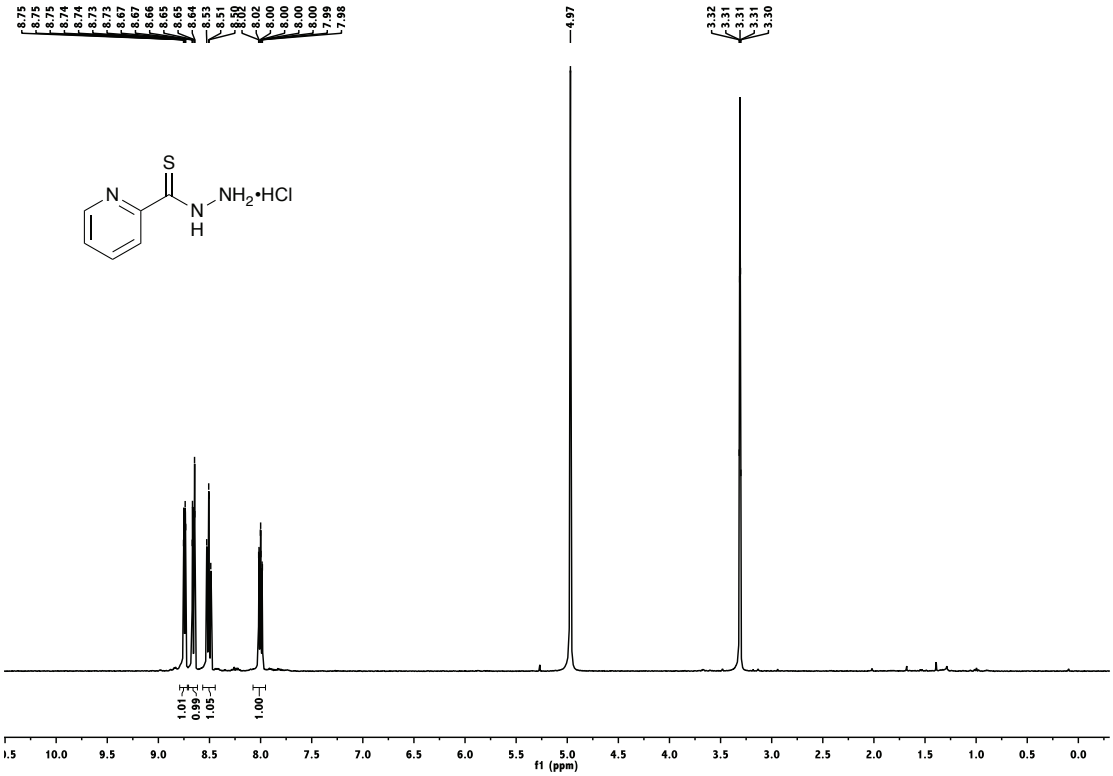


^{13}C NMR (101 MHz, MeOD)

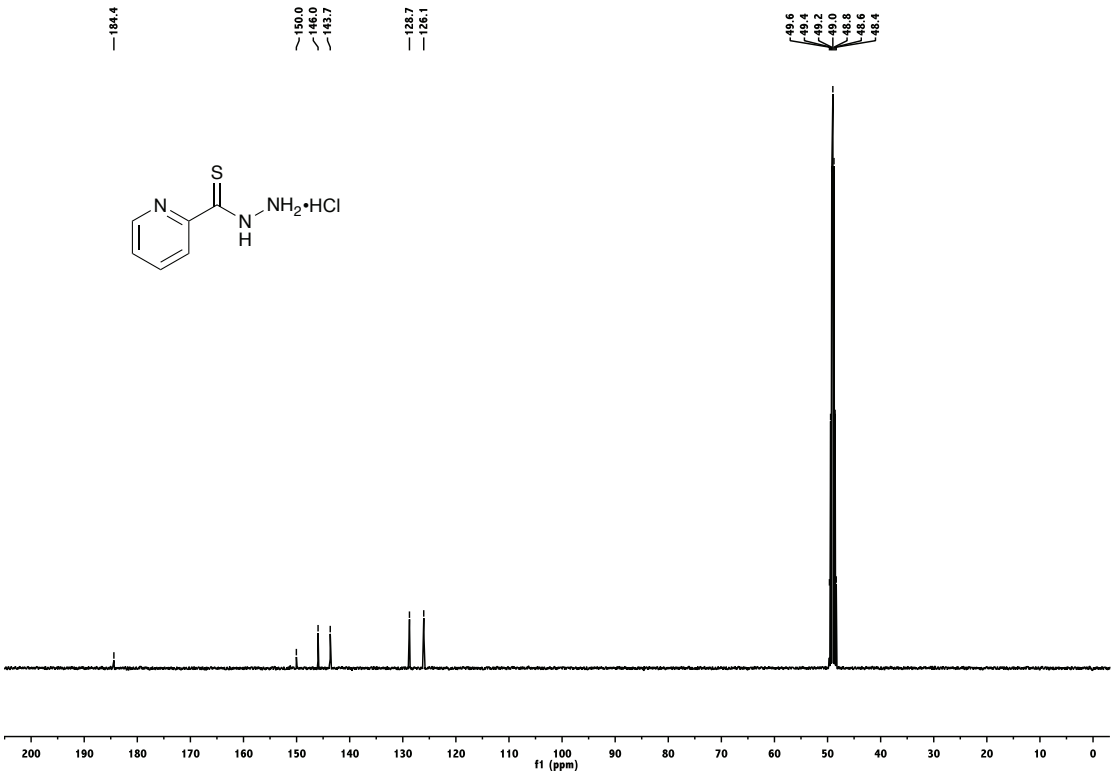


Pyridine thiohydrazide 12

^{1}H NMR (400 MHz, MeOD)

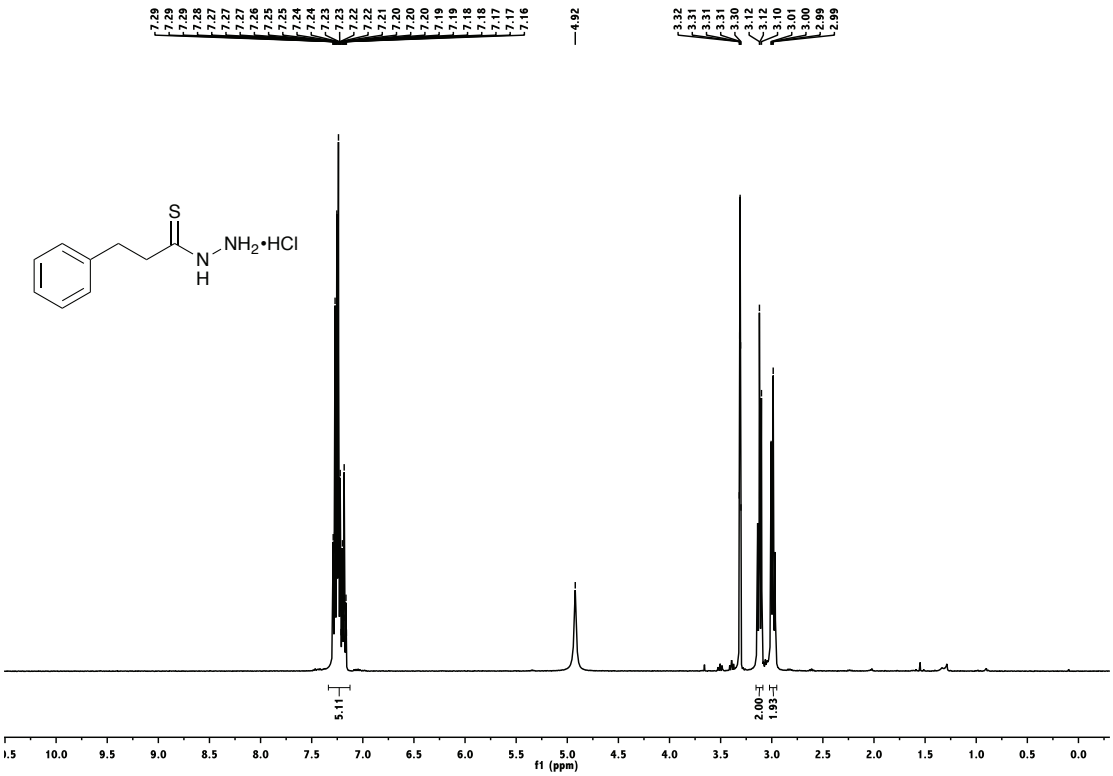


^{13}C NMR (101 MHz, MeOD)

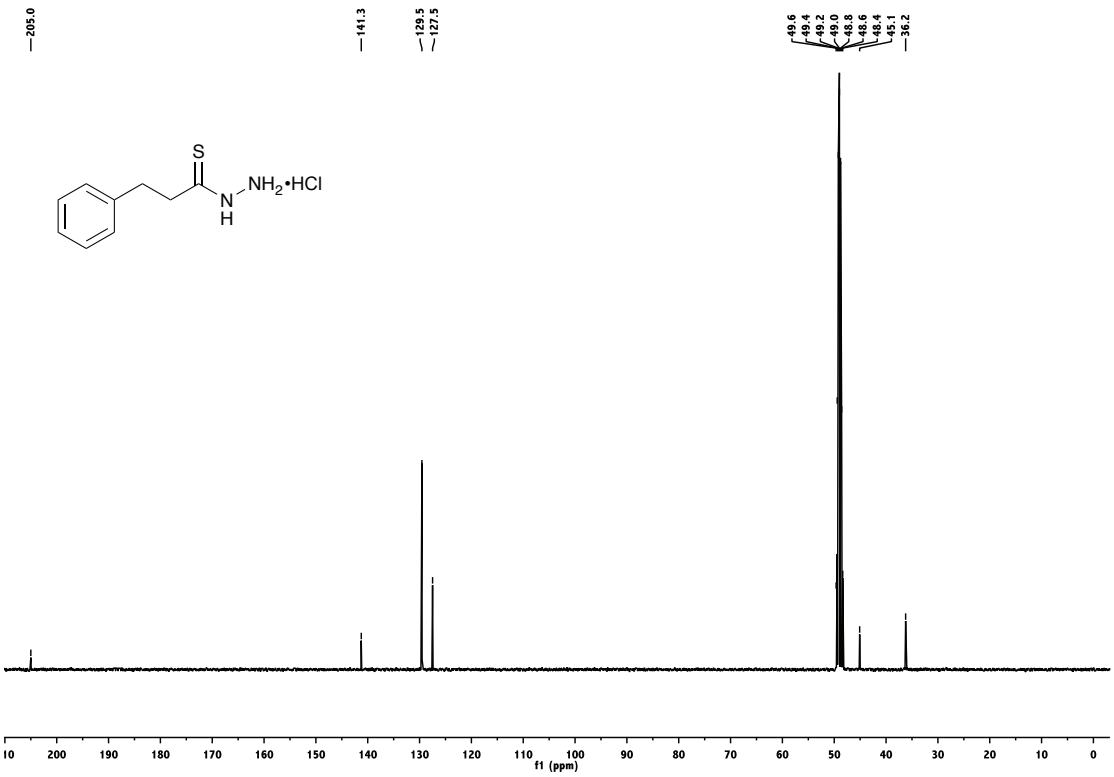


Alkyl thiohydrazide 15

^{1}H NMR (400 MHz, MeOD)

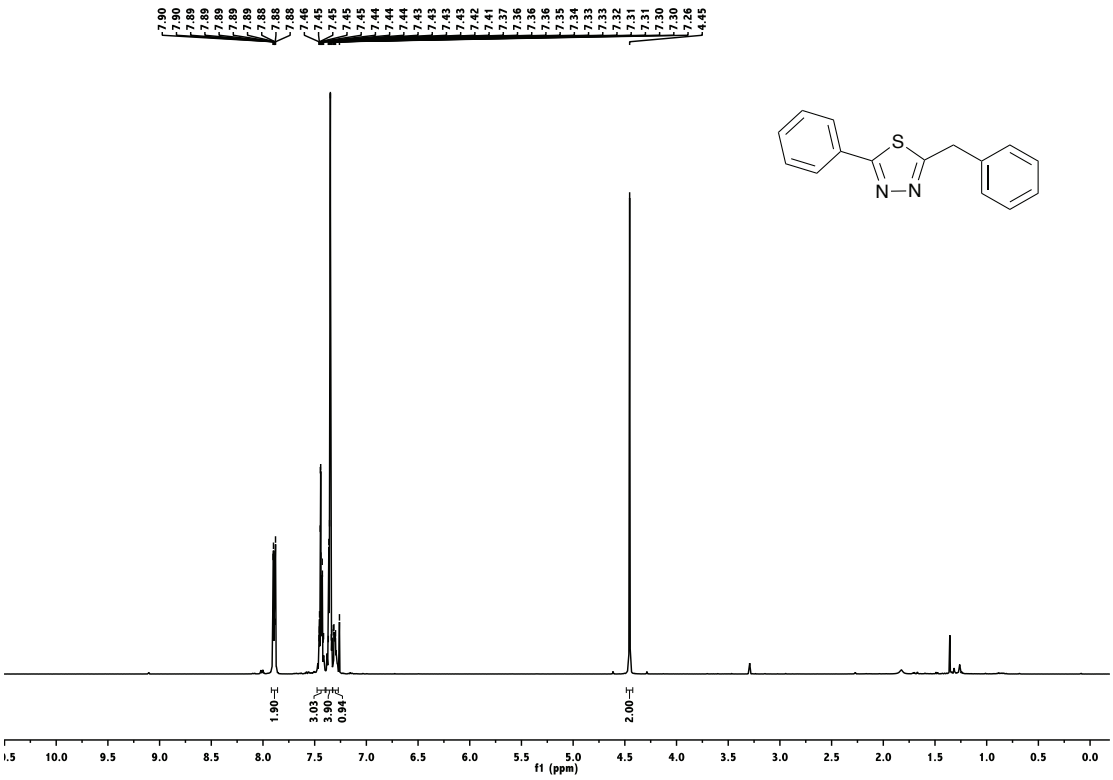


^{13}C NMR (101 MHz, MeOD)

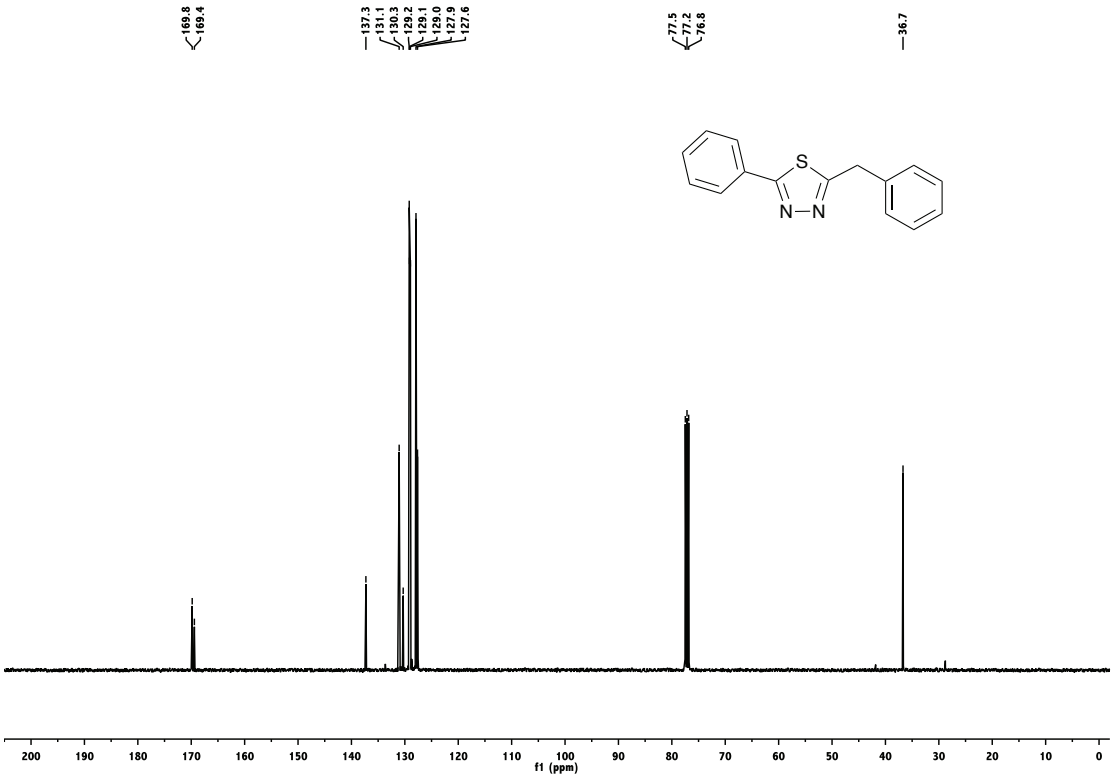


Phenyl thiadiazole 5

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

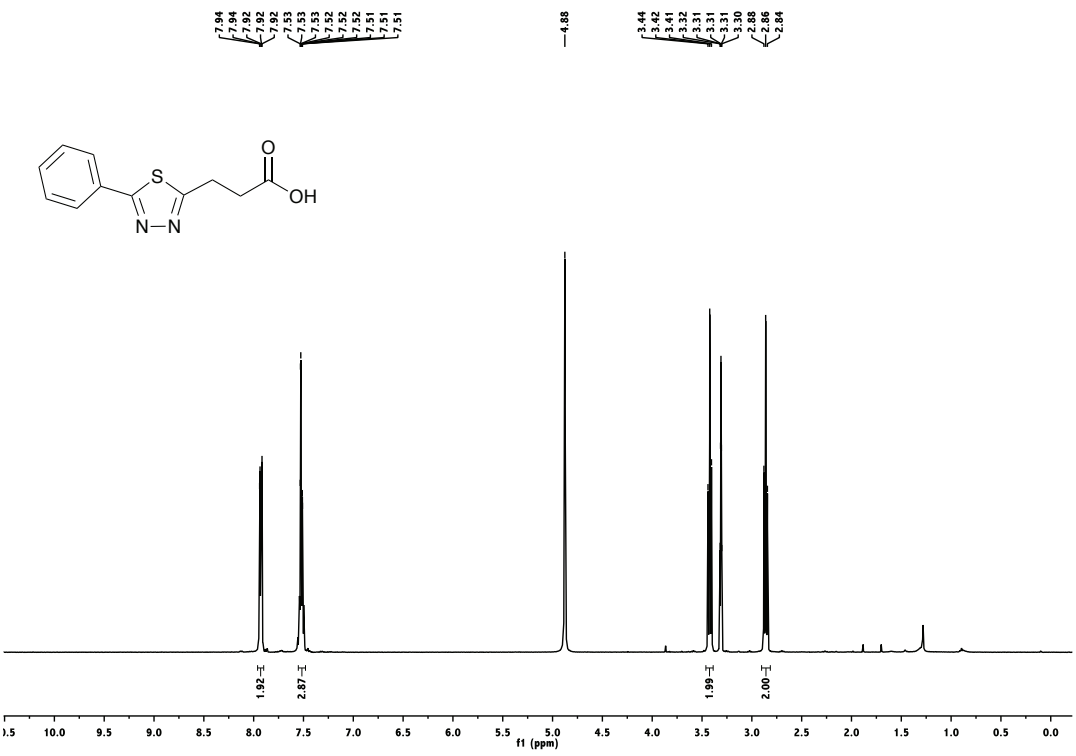


^{13}C NMR (101 MHz, $CDCl_3$)

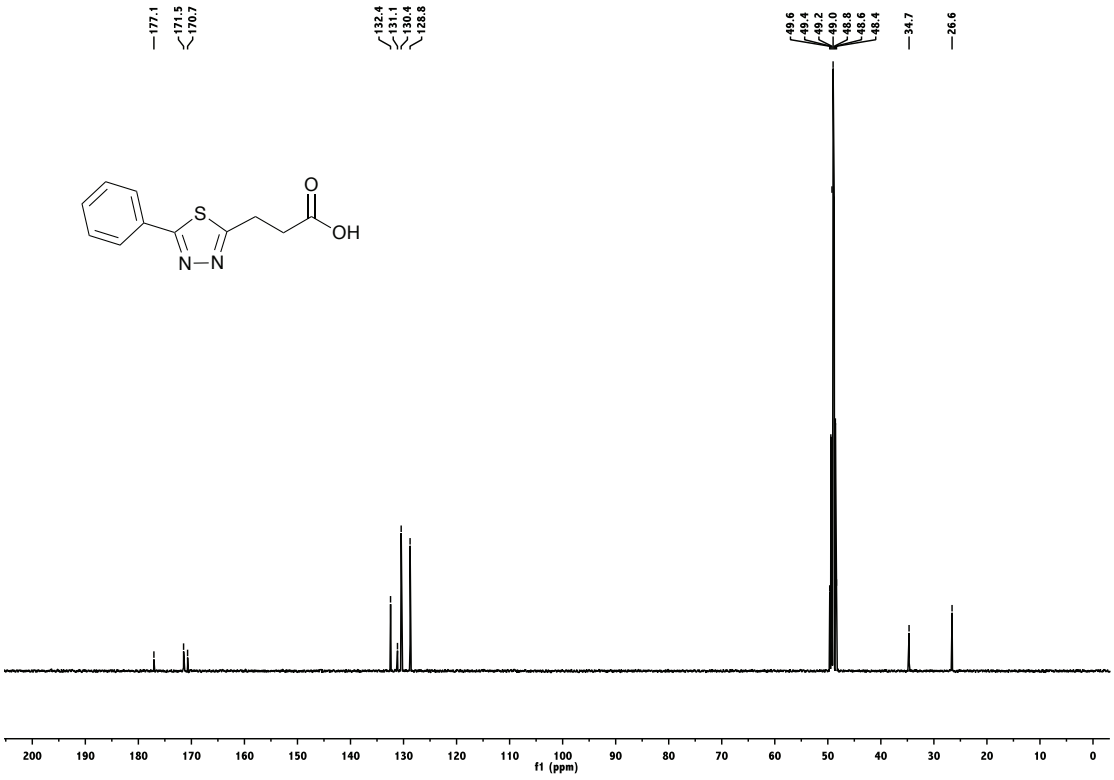


Propanoic acid thiadiazole 7

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

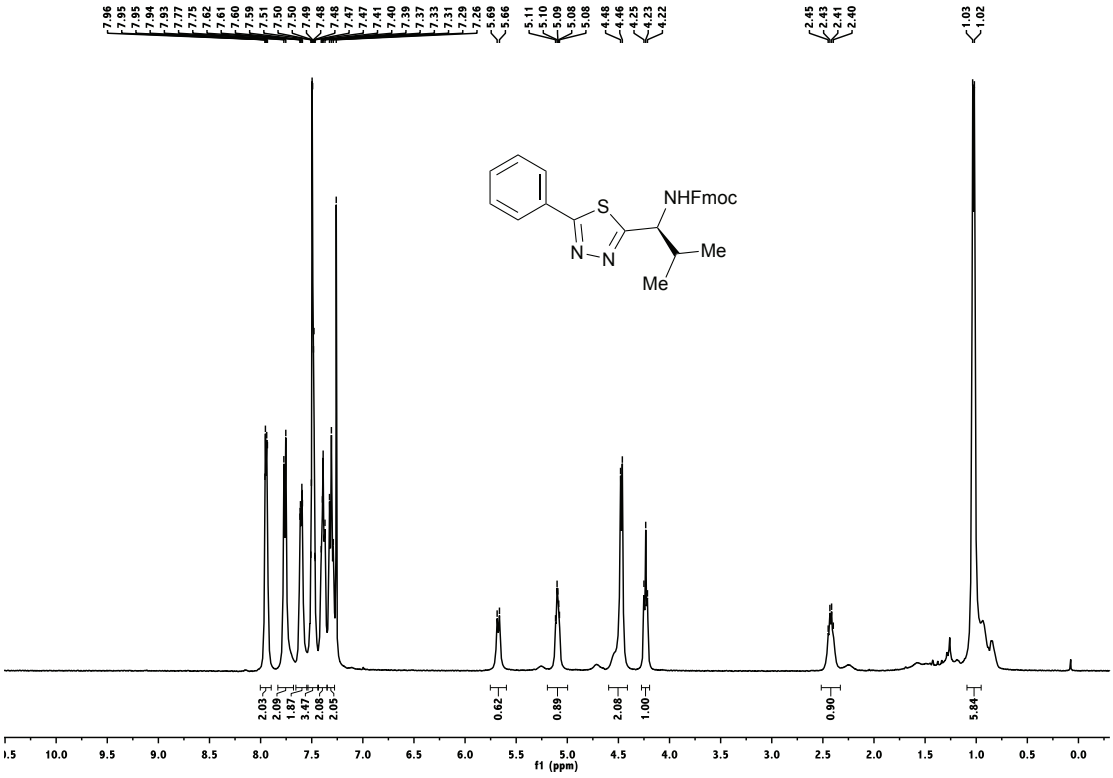


^{13}C NMR (101 MHz, $CDCl_3$)

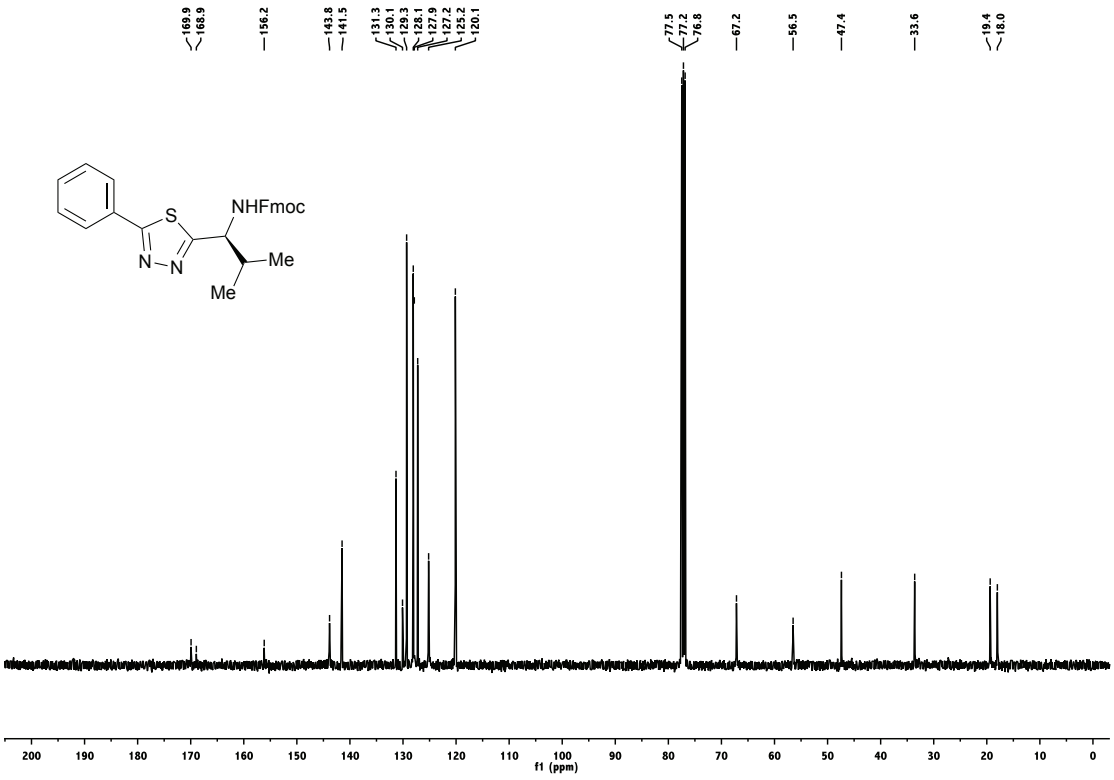


Fmoc-aminothiadiazole 9

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

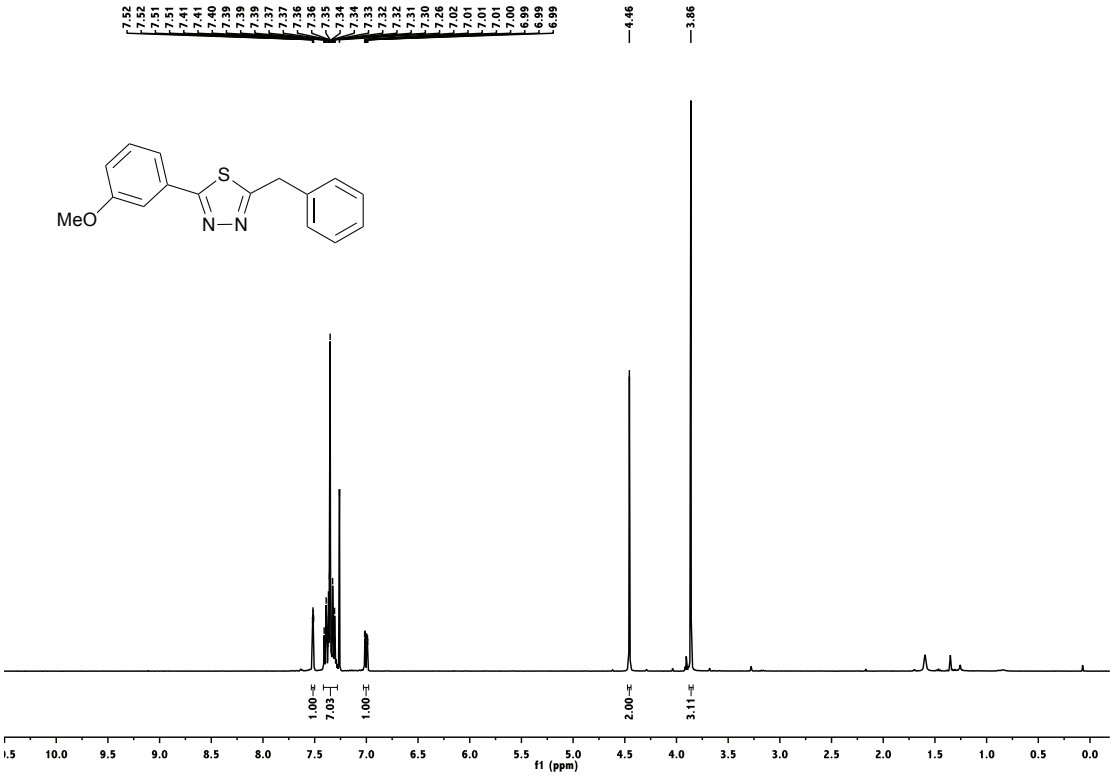


^{13}C NMR (101 MHz, $CDCl_3$)

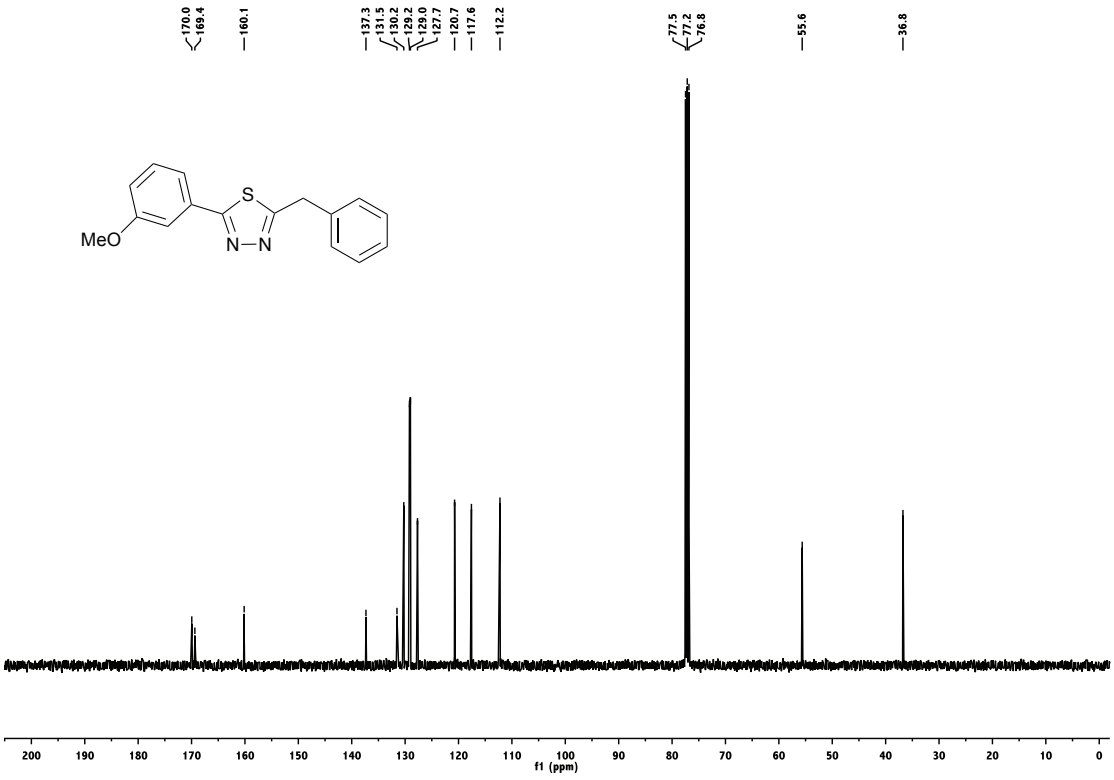


3-Methoxyphenyl thiadiazole 11

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

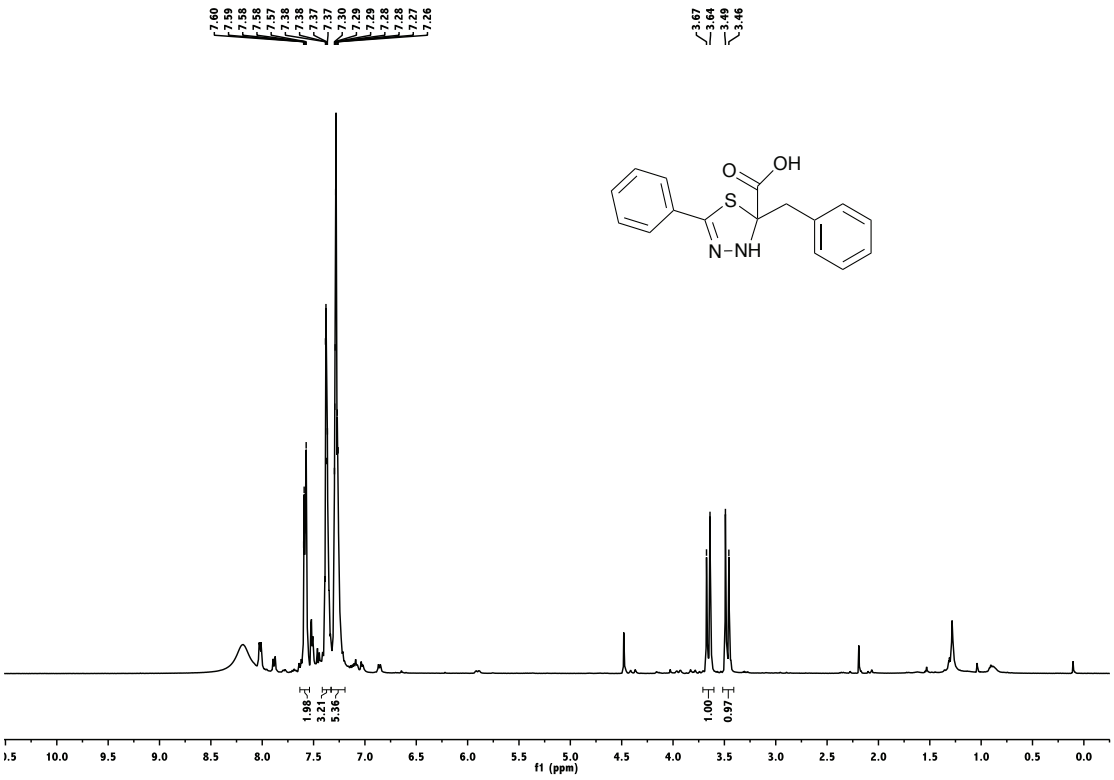


^{13}C NMR (101 MHz, $CDCl_3$)

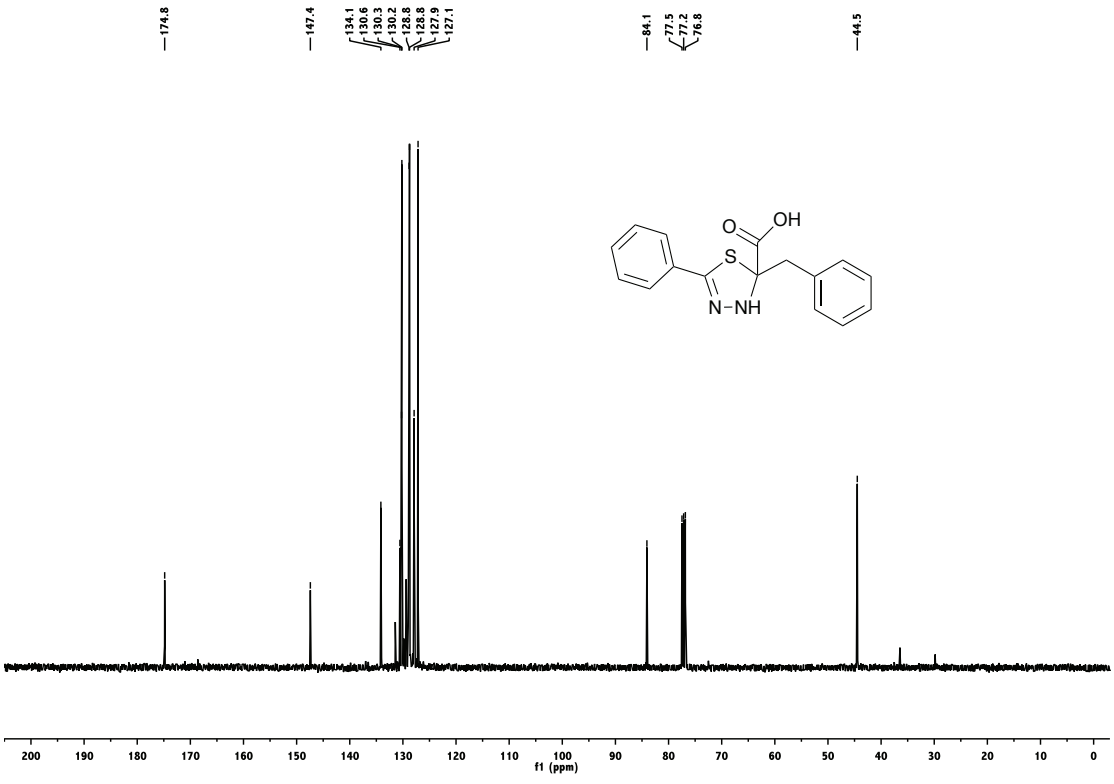


2,3-Dihydrothiadiazole carboxylic acid 4

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

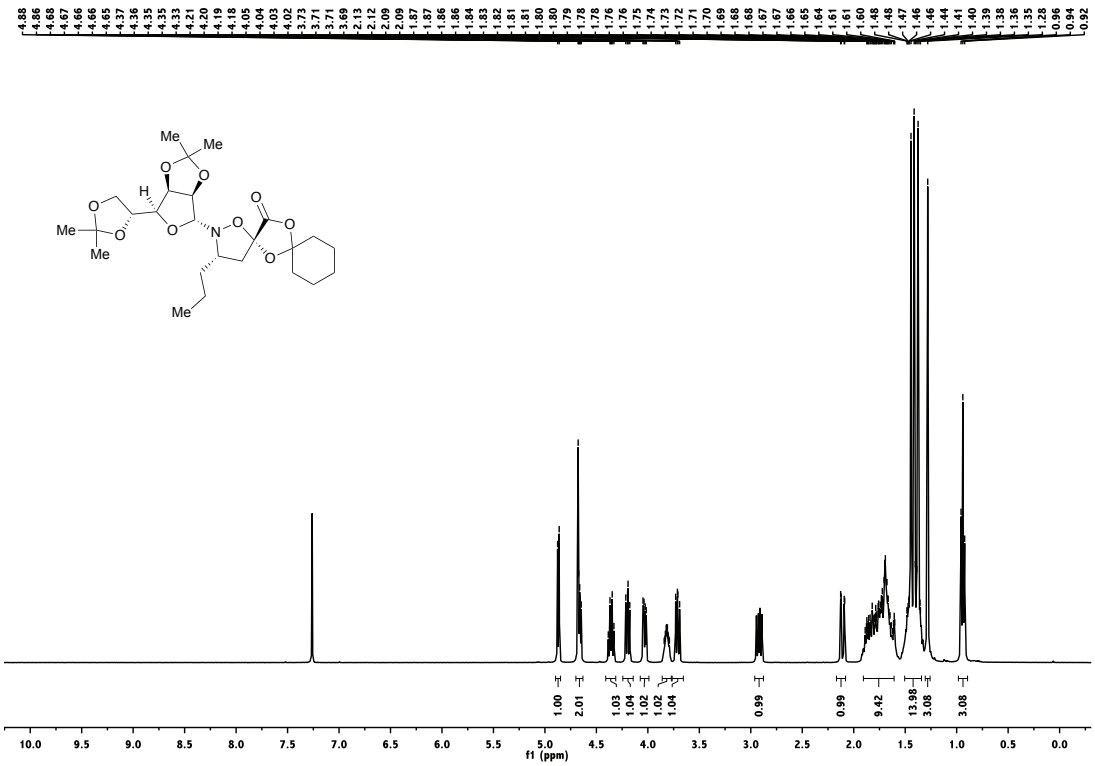


^{13}C NMR (101 MHz, $CDCl_3$)

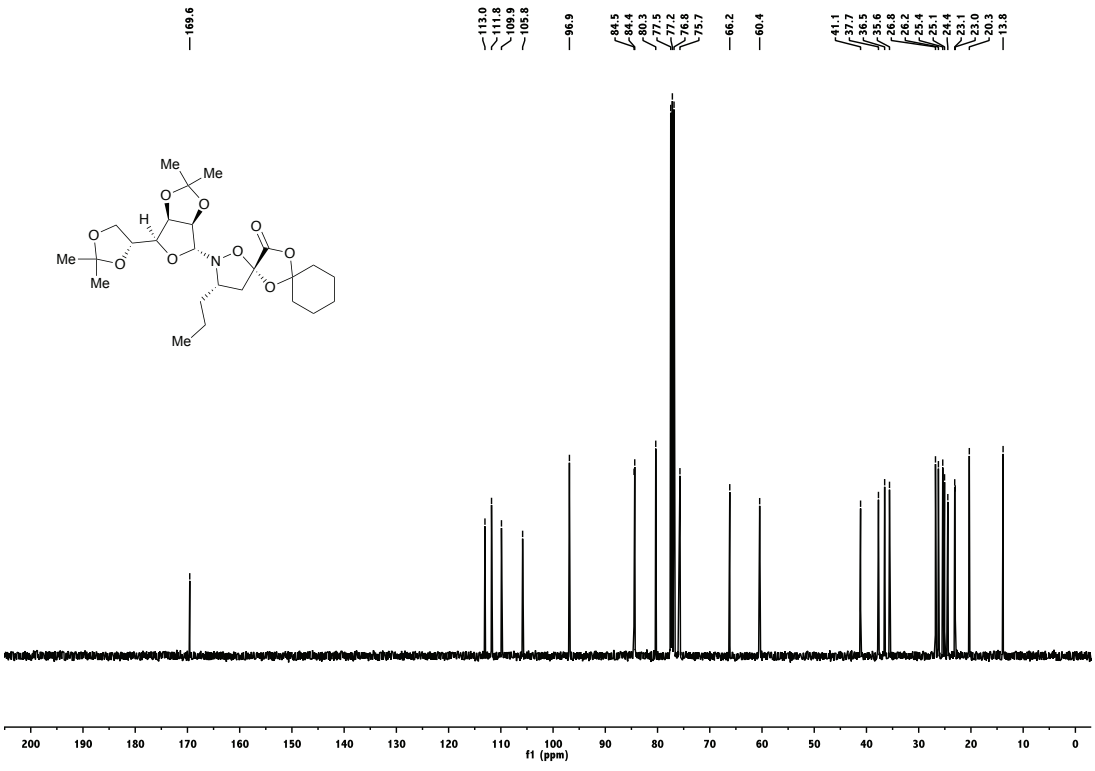


n-Propyl monomer intermediate 51

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

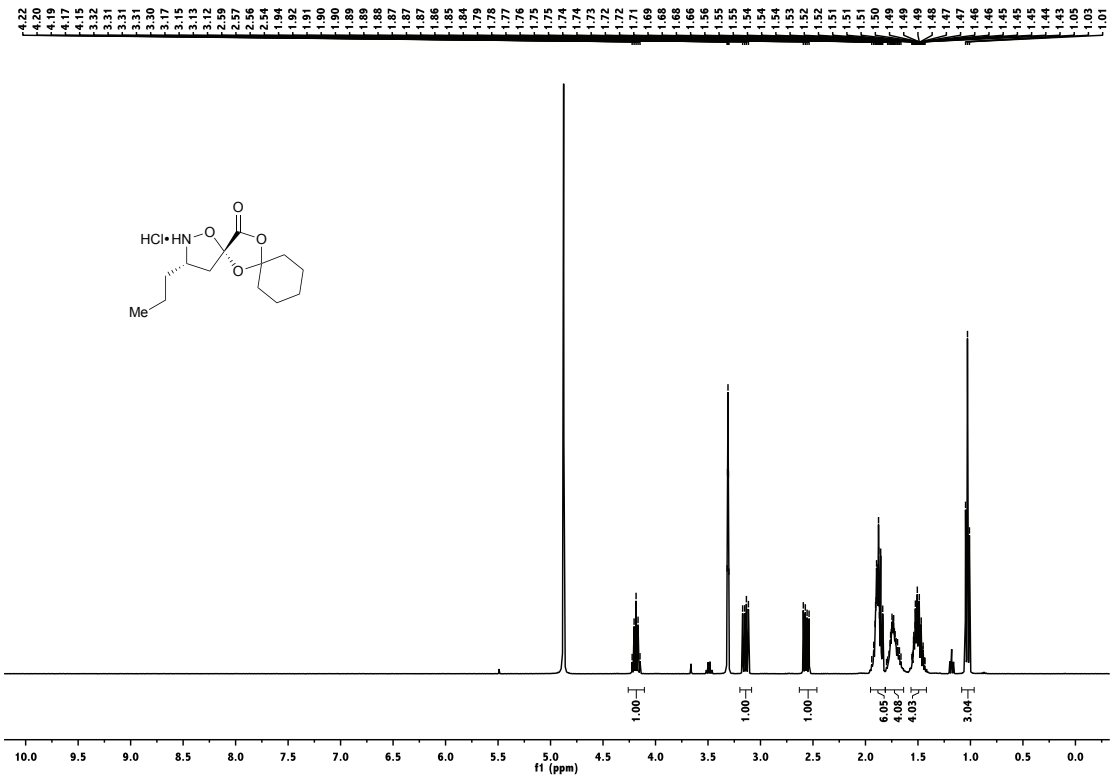


^{13}C NMR (101 MHz, $CDCl_3$)

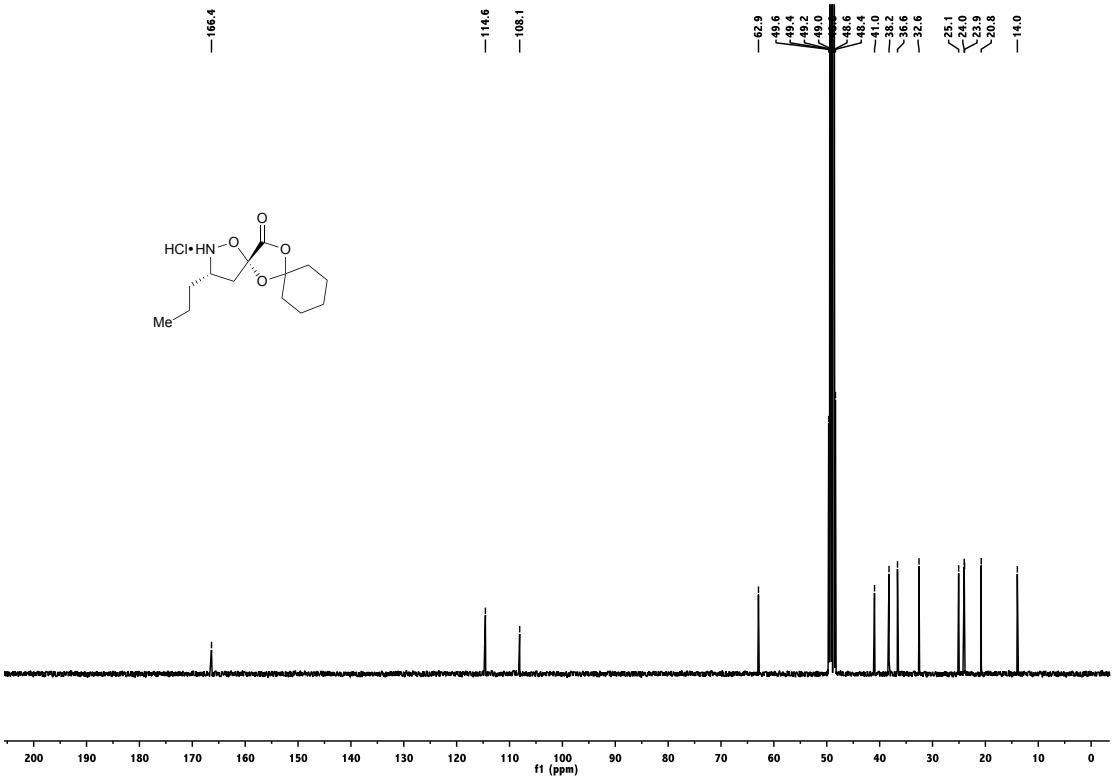


n-Propyl monomer 26

^{1}H NMR (400 MHz, MeOD)

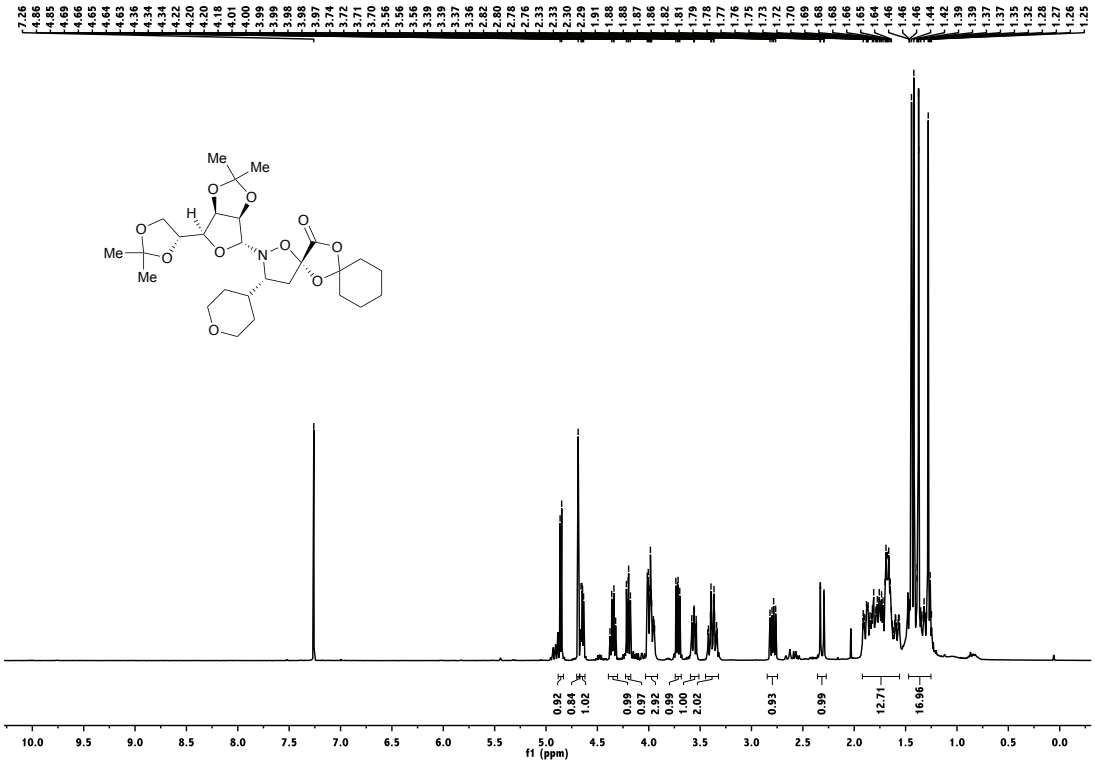


^{13}C NMR (101 MHz, MeOD)

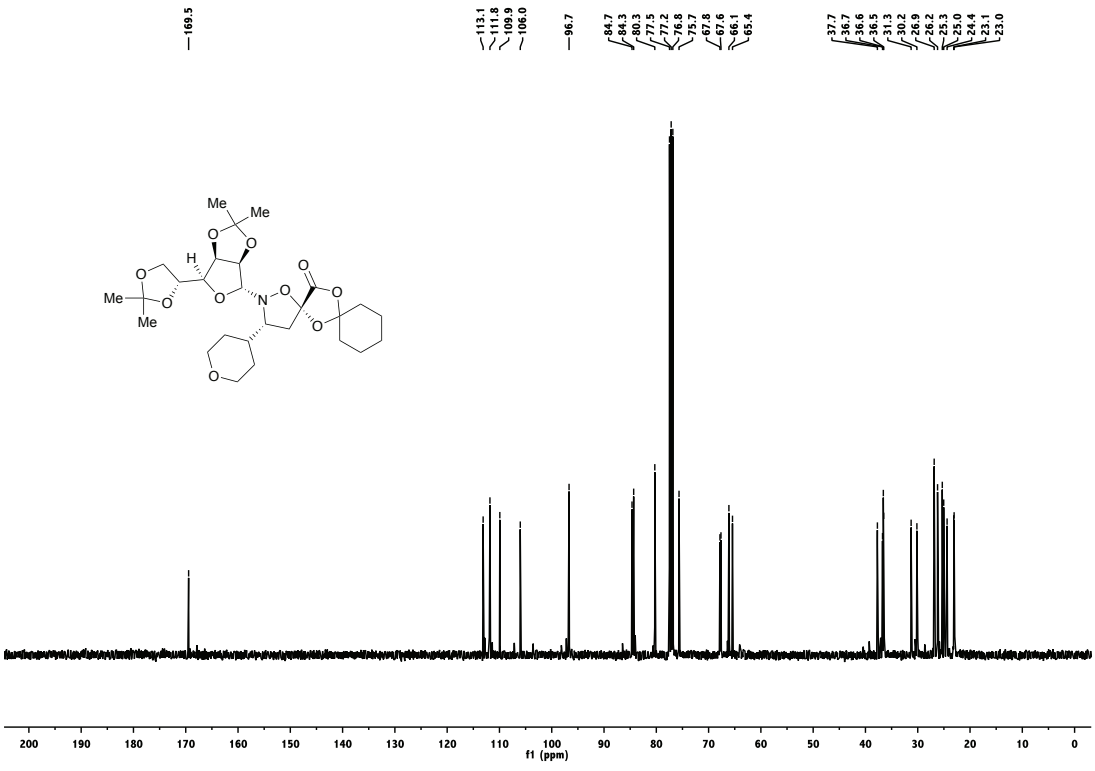


Pyran monomer intermediate 52

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

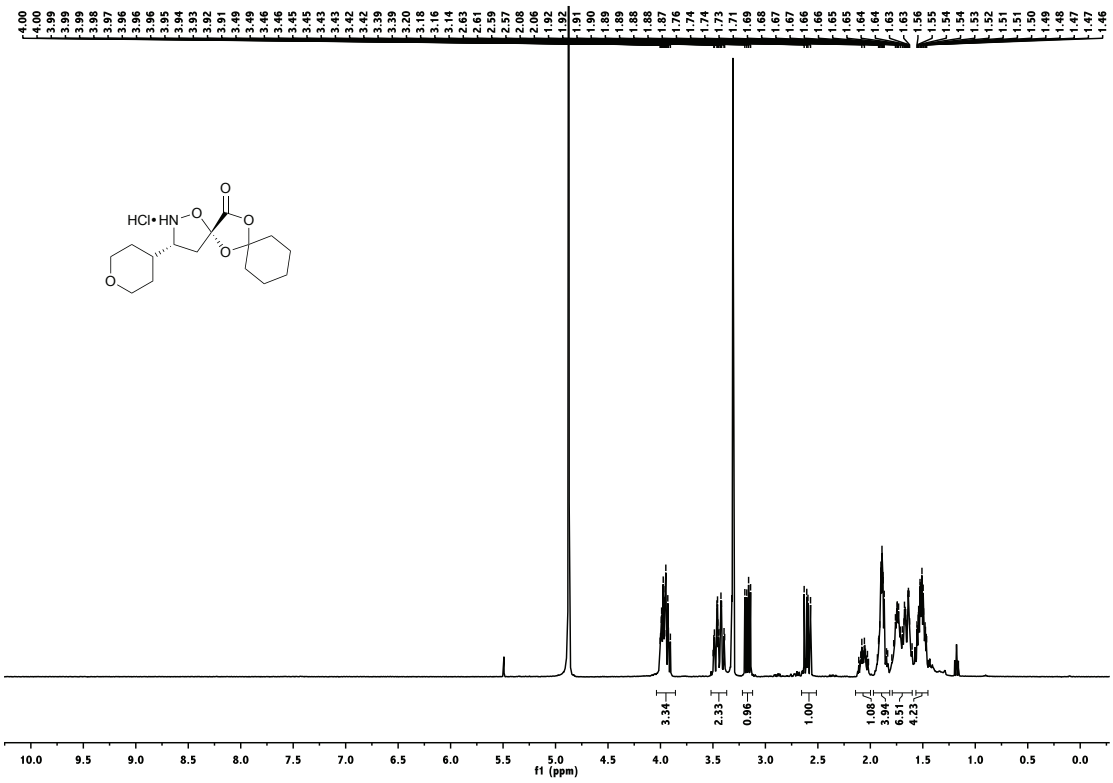


^{13}C NMR (101 MHz, $CDCl_3$)

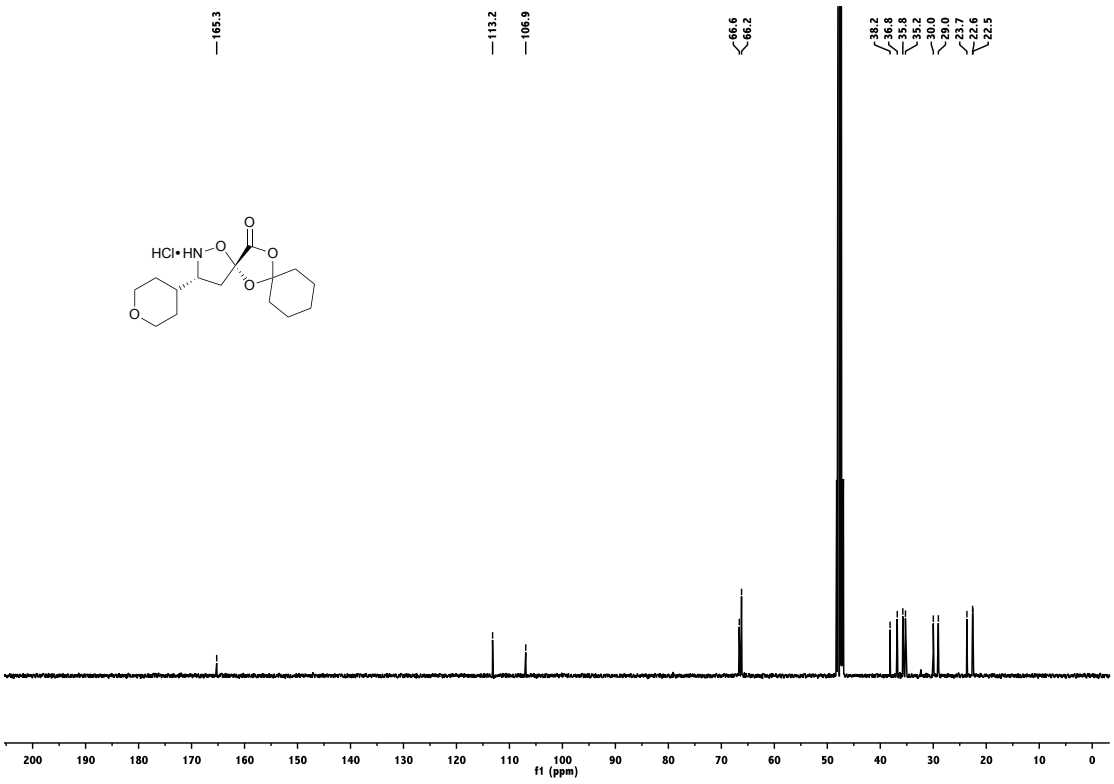


Pyran monomer 27

^{1}H NMR (400 MHz, MeOD)

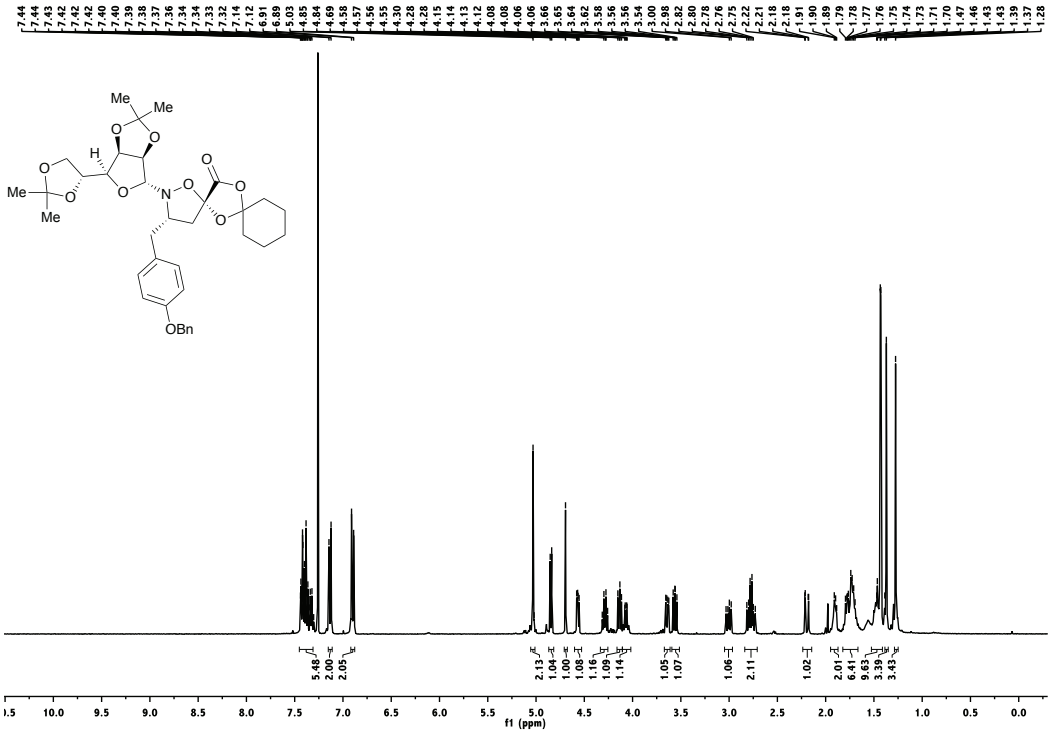


^{13}C NMR (101 MHz, MeOD)

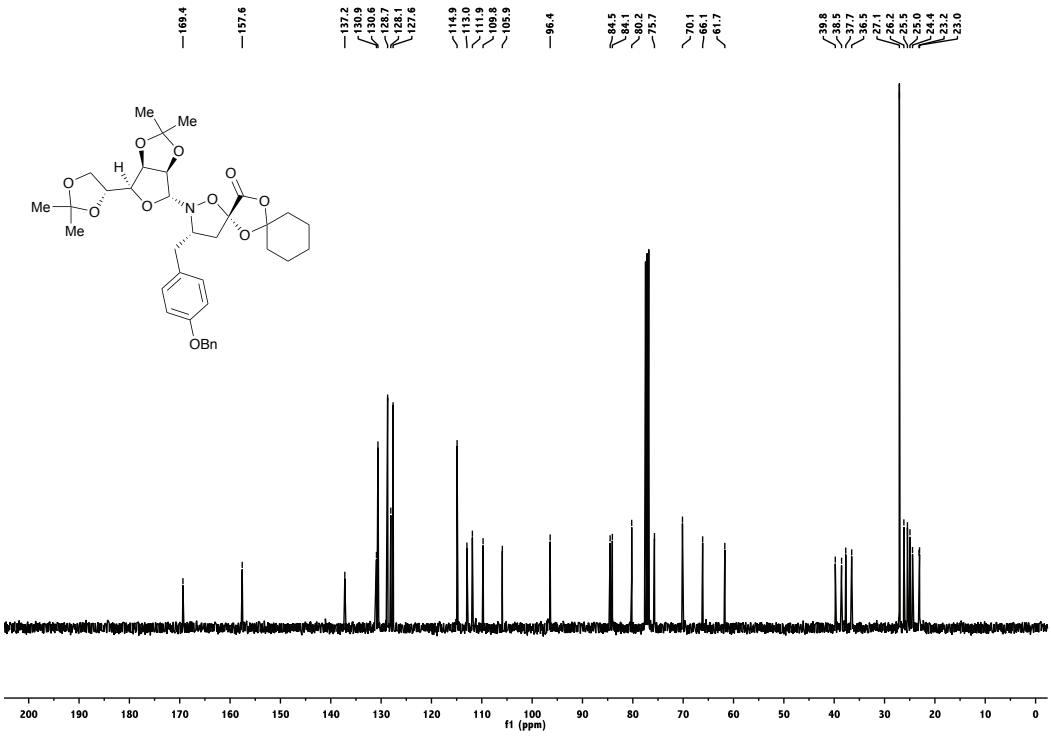


4-Benzyloxybenzyl intermediate 53

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

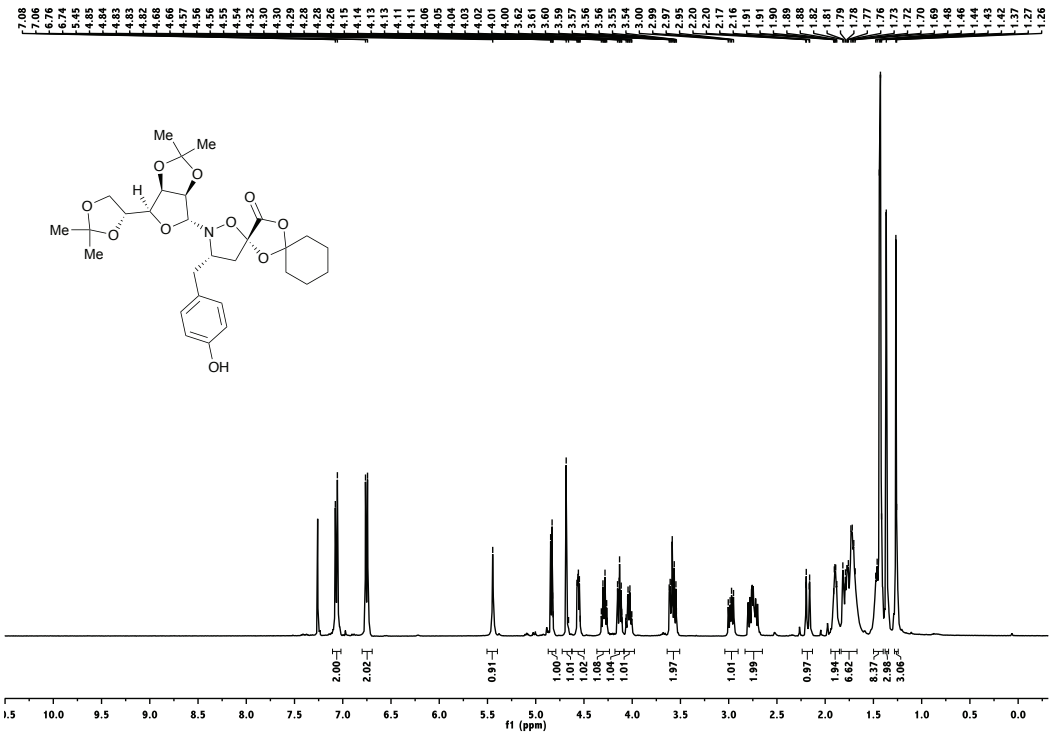


^{13}C NMR (101 MHz, $CDCl_3$)

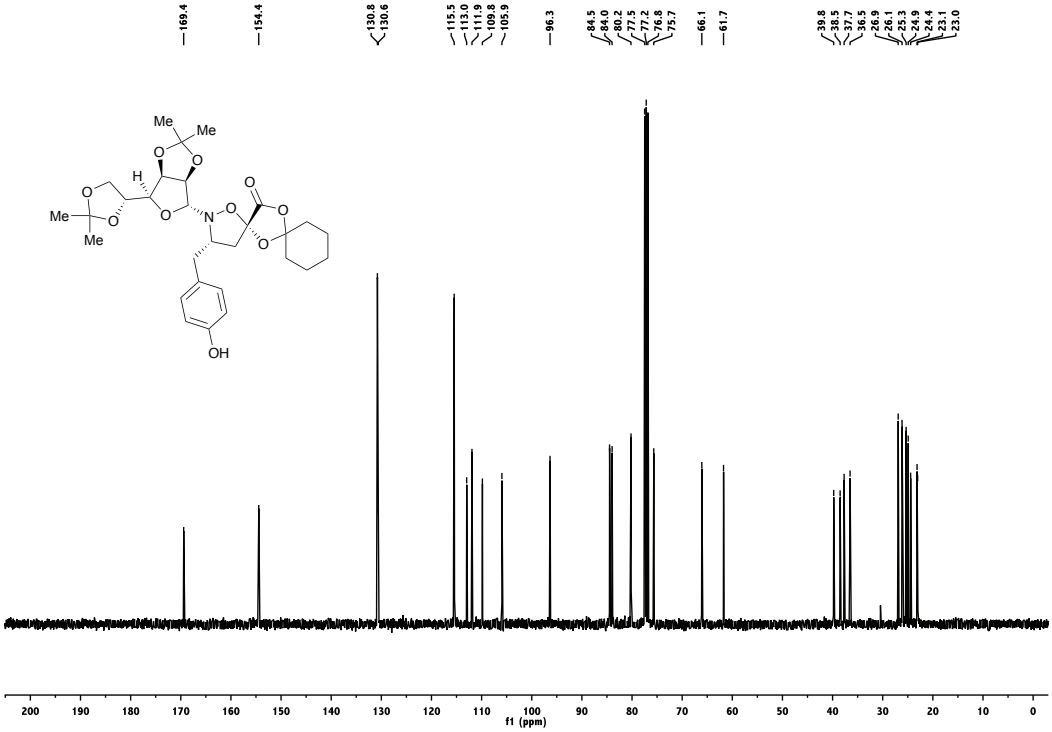


4-Hydroxybenzyl monomer intermediate 54

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

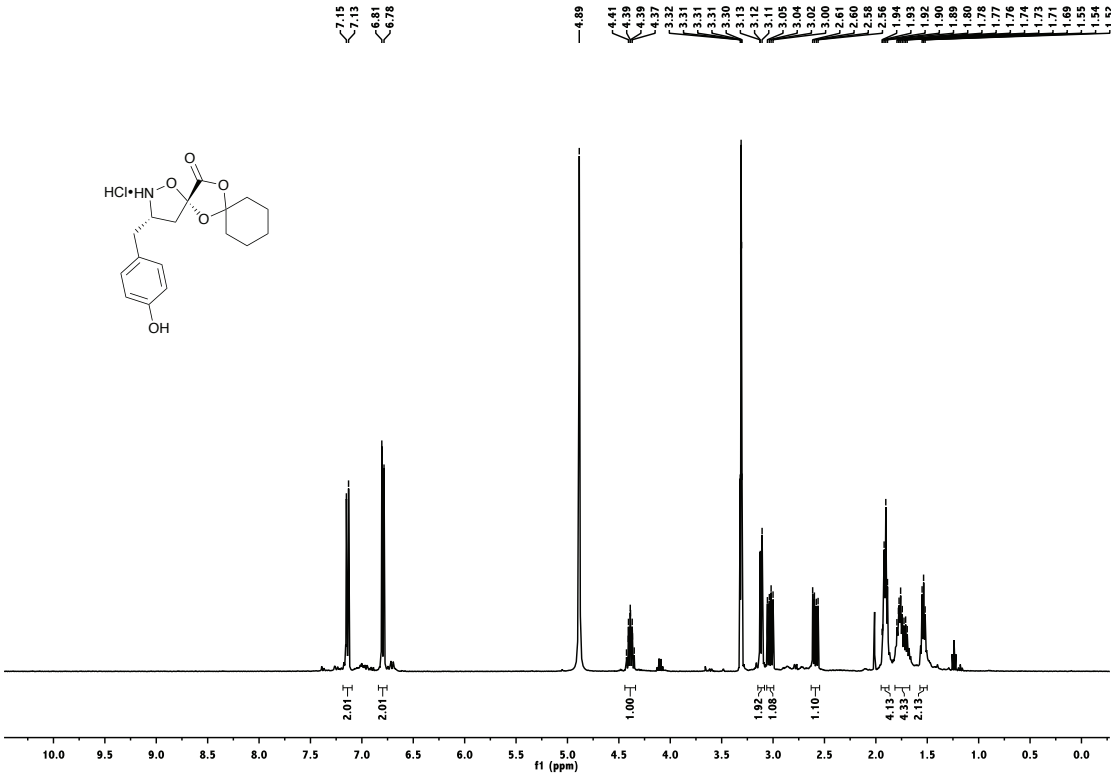


^{13}C NMR (101 MHz, $CDCl_3$)

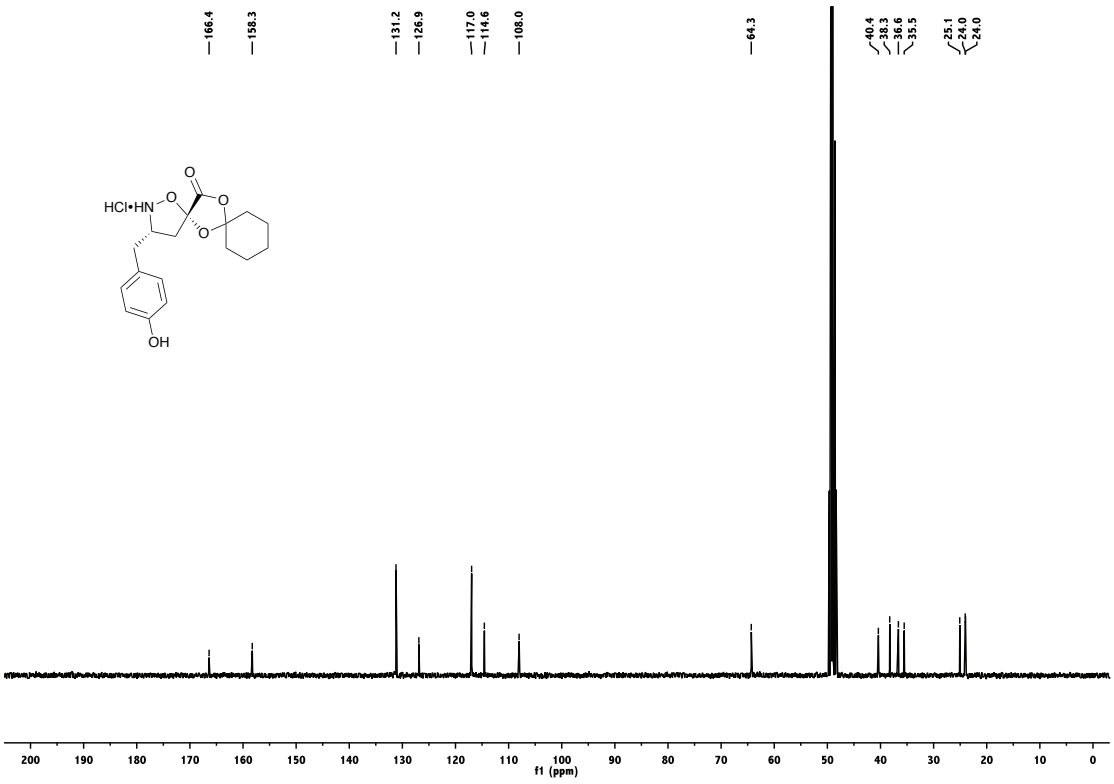


4-Hydroxybenzyl monomer 28

^{1}H NMR (400 MHz, MeOD)

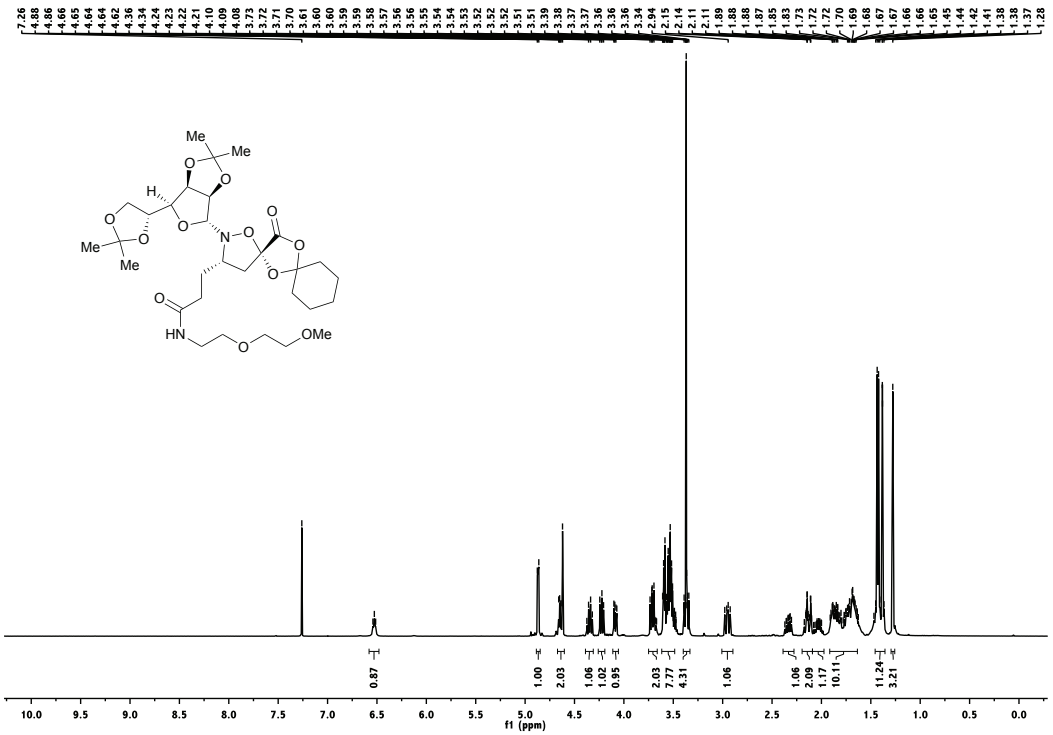


^{13}C NMR (101 MHz, MeOD)

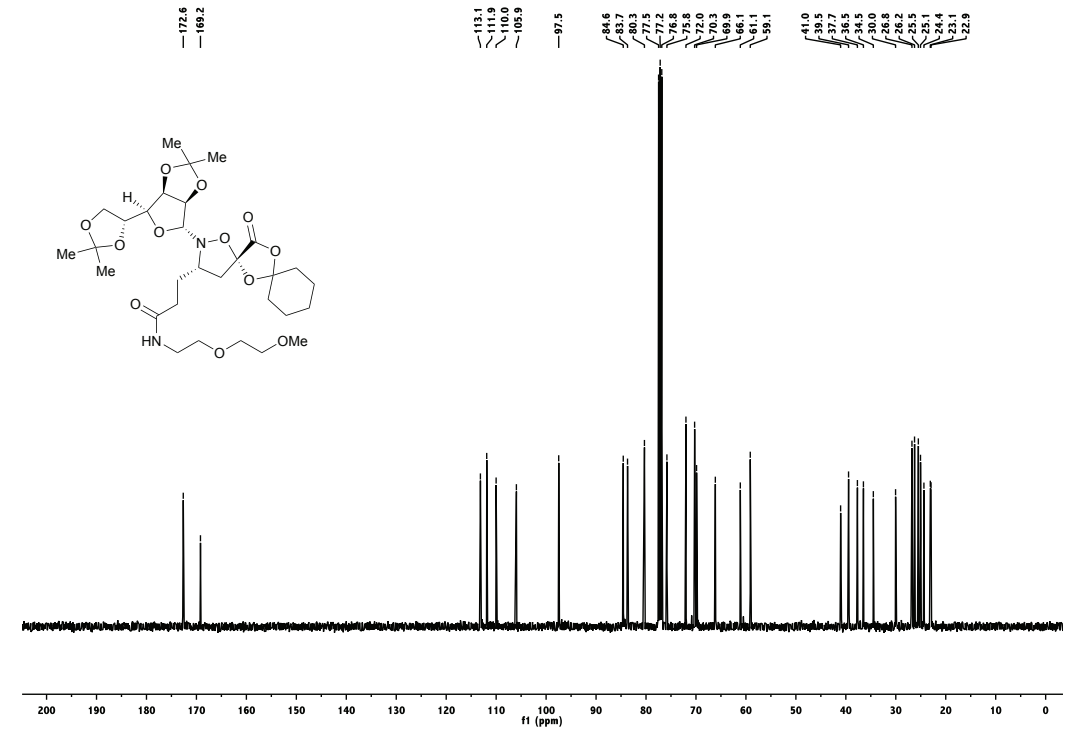


Amide monomer intermediate 55

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

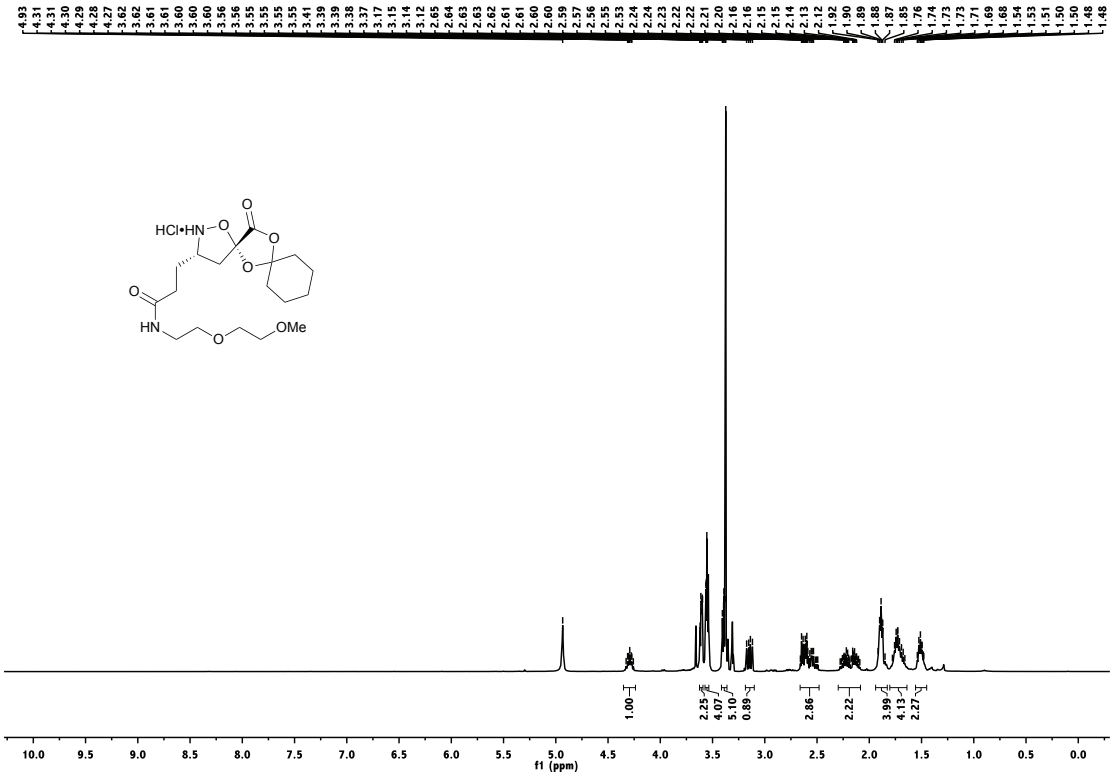


^{13}C NMR (101 MHz, $CDCl_3$)



Amide monomer 29

^{1}H NMR (400 MHz, MeOD)



^{13}C NMR (101 MHz, MeOD)