A Two-Directional Strategy for the Diversity-Oriented Synthesis of Macrocyclic Scaffolds

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1. General experimental details

NMR spectra were recorded on the following instruments: Bruker Avance 300, Bruker Avance 400, Bruker DPX 400, Bruker Avance 500 BB ATM and Bruker Avance 500 Cryo Ultrashield 500 MHz; in deuterochloroform, deuteromethanol, deuterodimethyl sulfoxide or deuterium oxide. ^{1}H NMR spectra were recorded at 300, 400 and 500 MHz and ^{13}C spectra were recorded at 75, 100 and 125 MHz; all spectra were recorded at ambient temperature unless otherwise stated. Chemical shifts are reported in ppm to the nearest 0.01 ppm (^{1}H) or 0.1 ppm (^{13}C) and are quoted relative to the residual non-deuterated solvent peak. Coupling constants (*J*) are quoted in Hertz to the nearest 0.5 Hz. The following abbreviations are used to indicate the multiplicity of the signals: s singlet, d doublet, t triplet, q quartet, quint quintet, sext sextet, m multiplet, br broad and app. apparent. Assignments were determined either by unambiguous chemical shift and splitting pattern, by patterns observed in 2D experiments (DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY) or by analogy to the fully interpreted spectra of related compounds. Non-equivalent geminal protons are assigned H_a and H_b where H_a is at the lower chemical shift. Where NMR spectra are dynamic or rotameric on the NMR timescale resonances are assigned H and H' or

C and C' where appropriate. NMR spectra of non-symmetrical macrocycles or non-equivalent alkyl chains are assigned H and H* or C and C* for the equivalent resonances. The numbering schemes used on selected spectra do not follow the IUPAC naming system and are used for the clear assignment of ^{1}H and ^{13}C spectra.

High resolution mass spectroscopic (HRMS) analyses were measured on a Micromass Q-TOF or micromass LCT Premier spectrometer and values are reported within the error limits of ±5 ppm.

Infrared spectra were recorded on a Perkin-Elmer 1600 FT IR spectrometer. The sample was prepared neat or as thin films in the solvent indicated. Selected absorption maxima are recorded in wavenumbers (cm^{-1}) to the nearest whole number.

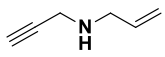
Melting points were determined on a Buchi B-545 melting point apparatus and are uncorrected. The solvent system quoted refers to that used in the final purification step.

Analytical thin layer chromatography (TLC) was carried out on Merck pre-coated 0.23 mm thick plates of Keiselgel 60 F_{254}. Visualisation was achieved by the quenching of UV fluorescence or by staining with potassium permanganate or molecular iodine. Flash column chromatography was carried out using Merck 9385 Keiselgel 60 SiO_2 (230-400 mesh). Automated flash chromatography was performed using a Biotage Isolera system using pre-packed SiO_2 columns. Preparative HPLC purification was performed on an Agilent 1260 Infinity system fitted with a Supercoil ABZ+PLUS column (25 cm x 21.2 mm; 5 μm particle size) at a flow rate of 20 $mLmin^{-1}$.

All reactions were carried out using oven-dried glassware under an atmosphere of nitrogen using freshly distilled solvents. THF was distilled from $LiAlH_4$ with triphenylmethane as indicator. Et_2O was distilled from CaH_2 and $LiAlH_4$. CH_2Cl_2, hexanes, EtOAc, MeOH, toluene and MeCN were distilled from CaH_2. Pet. ether was distilled before use and refers to the 40-60 fraction. *n*-BuLi was titrated with *N*-benzylbenzamide before use. All other reagents were used as obtained from commercial sources.

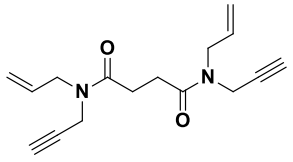
2. Synthesis of linear precursors

Allylpropargylamine (S1)[1]



Propargyl chloride (7 g, 6.8 mL, 95 mmol) was added slowly to allylamine (50 mL, 668 mmol) and the resulting solution was heated to 60 °C for 48 hours. The reaction was then cooled to RT then partitioned between H_2O and Et_2O, the aqueous phase was further extracted with Et_2O (x 2) and the combined extracts dried over $MgSO_4$ then concentrated *in vacuo*. The resulting oil was distilled at atmospheric pressure with the desired product collected between 120-128 °C as a colourless oil (4.18 g, 48%). δ_H (400 MHz, $CDCl_3$) 2.21 (1H, t, *J* = 2.0, C≡C<u>H</u>), 3.32 (2H, dd, *J* = 6.0, 1.0, C<u>H</u>$_2$CH=CH$_2$), 3.40 (2H, app. t, *J* = 2.0, C<u>H</u>$_2$C≡CH), 5.11 (1H, app. d, *J* = 10.0, CH$_2$CH=C<u>H</u>$_2$), 5.20 (1H, dt, *J* = 17.0, 1.0, CH$_2$CH=C<u>H</u>$_2$) 5.83-5.91 (1H, m, CH$_2$C<u>H</u>=CH$_2$). δ_C (100 MHz, $CDCl_3$) 37.6 (C<u>H</u>$_2$CH≡CH$_2$), 51.2 (<u>C</u>H$_2$C=CH), 71.7 (CH$_2$C≡<u>C</u>H), 82.4 (CH$_2$<u>C</u>≡CH), 116.7 (CH$_2$CH=<u>C</u>H$_2$), 136.3 (CH$_2$<u>C</u>H=CH$_2$). υ_{max} (neat) 3298 (C≡C-H stretch), 3079 (C=C-H stretch), 2979, 2921 (C-H stretch), 2105 (C≡C stretch), 1644 (C=C stretch). Spectral data is consistent with previously reported values.[1]

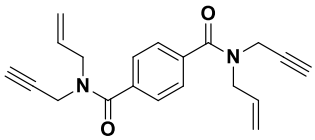
N^1,*N*^{4}-diallyl-*N*^{1},*N*^{4}-di(prop-2-yn-1-yl)succinamide (1a)



Succinic acid (500 mg, 4.2 mmol) was dissolved in CH_2Cl_2 (25 mL). EDC.HCl (1.78 g, 9.3 mmol) was added to the reaction followed by DMAP (103 mg 0.84 mmol) and allylpropargylamine (**54**) (885 mg, 9.3 mmol). The reaction was then allowed to stir at RT overnight. After this time the reaction was washed with $HCl_{(aq)}$ (10% v/v) and the washings were then extracted (x3) with CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was then purified

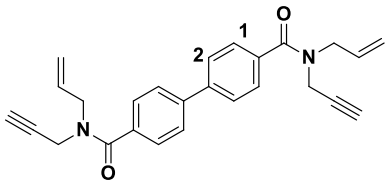
by flash chromatography on silica (3:7 EtOAc:pet. ether) to give the title compound as a yellow solid (954 mg, 84%). R_f = 0.30 (1:1 EtOAc:pet. ether). mp 65-68 °C (EtOAc). δ_H (500 MHz, *d*$_6$-DMSO, 120 °C) 2.66 (4H, s, C$\underline{H}_2$C=O) 2.84 (2H, app s, C≡C$\underline{H}$), 4.05 (4H, d, *J* = 5.5, C$\underline{H}_2$CH=CH$_2$), 4.14 (4H, d, *J* = 2.5, C$\underline{H}_2$C≡CH), 5.17-5.24 (4H, m, CH$_2$CH=C$\underline{H}_2$), 5.82-5.89 (2H, m, CH$_2$C$\underline{H}$=CH$_2$). δ_C (125 MHz, *d*$_6$-DMSO, 120 °C) 27.4 (C$\underline{H}_2$C=O), 34.7 (C$\underline{H}_2$C≡CH), 48.0 (C$\underline{H}_2$CH=CH$_2$) 73.1 (CH$_2$C≡C$\underline{H}$), 79.3 (CH$_2$C$\underline{\equiv}$CH), 116.2 (CH$_2$CH=C$\underline{H}_2$), 132.9 (CH$_2$C$\underline{H}$=CH$_2$) 170.7 (C=O). υ_{max} (neat) 3208 (C≡C-H stretch), 2925 (C-H stretch), 2112 (C≡C stretch), 1631 (C=O stretch). HRMS (ESI) calculated for $C_{16}H_{21}N_2O_2$ (MH^+) 273.1603 found 273.1594.

N^1,*N*^{4}-diallyl-*N*^{1},*N*^{4}-di(prop-2-yn-1-yl)terephthalamide (1b)



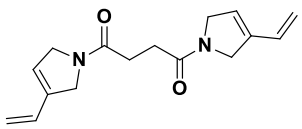
Terephthalic acid (200 mg, 1.2 mmol) was suspended in CH_2Cl_2 (10 mL), to the resulting suspension were added EDC.HCl (506 mg 2.6 mmol), DMAP (29 mg, 0.24 mmol) and allylpropargylamine (251 mg, 2.6 mmol). The reaction was then allowed to stir at RT overnight. After this time the reaction was washed with $HCl_{(aq)}$ (10% v/v) and the washings were then extracted (x3) with CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash chromatography on silica (stepped gradient; 2:8, 3:7 EtOAc:pet. ether) to furnish the desired product as a white crystalline solid (360 mg, 94%). R_f = 0.43 (1:1 EtOAc:pet. ether). mp 108-109 °C (EtOAc). δ_H (500 MHz, *d*$_6$-DMSO, 100 °C) 3.07 (2H, t, *J* = 2.0, CH$_2$C≡C$\underline{H}$), 4.05 (4H, d, *J* = 5.0, C$\underline{H}_2$CH=CH$_2$), 4.15 (4H, app s, C$\underline{H}_2$C≡CH), 5.21-5.24 (4H, m, CH$_2$CH=C$\underline{H}_2$), 5.84-5.91 (2H, m, CH$_2$C$\underline{H}$=CH$_2$), 7.50 (4H, s, ArH). δ_C (125 MHz, *d*$_6$-DMSO, 100 °C) 35.6 (C$\underline{H}_2$C≡CH), 48.7 (C$\underline{H}_2$CH=CH$_2$), 74.0 (CH$_2$C≡C$\underline{H}$), 78.9 (CH$_2$C$\underline{\equiv}$CH), 117.2 (CH$_2$CH=C$\underline{H}_2$), 126.2 (ArCH), 132.6 (CH$_2$C$\underline{H}$=CH$_2$), 136.6 (ArC), 169.2 (C=O). υ_{max} (neat) 3231 (C≡C-H stretch), 2120 (C≡C stretch), 1630 (C=O). HRMS (ESI) calculated for $C_{20}H_{21}N_2O_2$ (MH^+) 321.1603 found 321.1600.

N^4, $N^{4'}$-diallyl-N^4, $N^{4'}$-di(prop-2-yn-1-yl)-[1,1'-biphenyl]-4,4'-dicarboxamide (1c)



4,4'-Biphenylcarboxylic acid (1 g, 4.3 mmol) was suspended in CH_2Cl_2 and to the resulting suspension were added EDC.HCl (1.73 g, 9.1 mmol), DMAP (100 mg, 0.82 mmol) and allylpropargyl amine (860 mg, 9.1 mmol). The reaction was then allowed to stir at RT overnight. After this time the reaction was washed with $HCl_{(aq)}$ (10% v/v) and the washings were then extracted (x3) with CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was then purified by flash chromatography on silica (1:1 EtOAc:pet. ether) to furnish the title compound as a white amorphous solid (836 mg, 51%). R_f = 0.30 (1:1 EtOAc:pet. ether). mp 96-100 °C (EtOAc). δ_H (500 MHz, d_6-DMSO, 100 °C) 3.07 (2H, t, J = 2.0 $CH_2C{\equiv}C\underline{H}$), 4.08 (4H, d, J = 4.5, $C\underline{H}_2CH{=}CH_2$), 4.17 (4H, d, J = 2.5, $C\underline{H}_2C{\equiv}CH$), 5.22-5.26 (4H, m, $CH_2CH{=}C\underline{H}_2$), 5.86-5.93 (2H, m, $CH_2C\underline{H}{=}CH_2$), 7.53 (4H, app dd, J = 6.5, 2.0, H2), 7.77 (4H, app dd, J = 6.5, 2.0, H1). δ_C (125 MHz, d_6-DMSO, 100 °C) 35.6 ($\underline{C}H_2C{\equiv}CH$), 48.7 ($\underline{C}H_2CH{=}CH_2$), 73.9 ($CH_2C{\equiv}\underline{C}H$), 79.0 ($CH_2\underline{C}{\equiv}CH$), 117.1 ($CH_2CH{=}\underline{C}H_2$), 126.3 (ArCH), 126.8 (ArCH), 132.7 ($CH_2\underline{C}H{=}CH_2$), 134.7 (ArC), 140.2 (ArC), 169.5 (C=O). υ_{max} (neat) 3253 (C≡C-H stretch), 2122 (C≡C stretch), 1621 (C=O stretch). HRMS (ESI) calculated for $C_{26}H_{25}N_2O_2$ (MH^+) requires 397.1916 found 397.1916

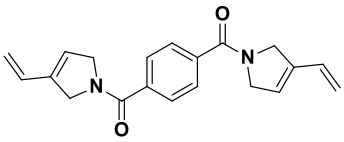
1,4-*bis*(3-vinyl-2,5-dihydro-1H-pyrrol-1-yl)butane-1,4-dione (2a)



Compound **1a** (700 mg, 2.57 mmol) was dissolved in toluene (100 mL). The flask was then backfilled twice with ethylene before Grubbs I (211 mg, 0.257 mmol) was added and the reaction was heated to 70 °C under an ethylene atmosphere for 18

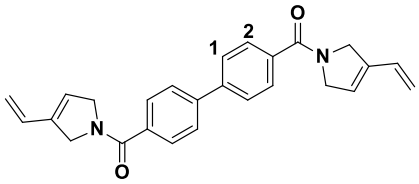
hours. After this time the solvent was removed *in vacuo* and the resulting residue purified by flash chromatography on silica (EtOAc) to furnish the title compound as a grey amorphous solid (504 mg, 72%). R_f = 0.34 (EtOAc). mp 250-260 °C (decomposition) (EtOAc). δ_H (500 MHz, *d*$_6$-DMSO, 90 °C) 2.56 (2H, br s, C$\underline{H}_2$C=O) 2.61 (2H, br s, CH_2C=O), 4.16-4.19 (4H, m, C$\underline{H}_2$N) 4.39-4.43 (4H, m, C$\underline{H}_2$N), 5.16-5.23 (4H, m, CH=C$\underline{H}_2$), 5.92 (2H, s, CH$_2$C$\underline{H}$=C), 6.54 (2H, dd, *J* = 17.5, 11.0 CC$\underline{H}$=CH$_2$). δ_C (125 MHz, *d*$_6$-DMSO, 90 °C) (two resonances observed for some signals due to amide rotamers), 28.8, (C$\underline{H}_2$C=O) 29.1 (C'H_2C=O), 51.8 (C$\underline{H}_2$N), 52.2 (C'H_2N), 53.3(C$\underline{H}_2$N), 53.7 (C'H_2N), 116.5 (CH=C$\underline{H}_2$), 116.7 (CH=C'H_2), 124.7 (C=C$\underline{H}$), 131.1 (C=C$\underline{H}$), 137.7 (C=CH), 137.9 (C'=CH), 170.3 (C=O). υ_{max} 1650 (C=O stretch), 1622 (C=O stretch), 1437 (C=C stretch), 1418 (C=C stretch). HRMS (ESI) calculated for $C_{16}H_{21}N_2O_2$ (MH^+) 273.1603 found 273.1609.

1,4-Phenylene*bis*((3-vinyl-2,5-dihydro-1H-pyrrol-1-yl)methanone) (2b)



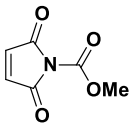
Compound **1b** (1.2 g, 3.75 mmol) was dissolved in toluene (150 mL). The flask was then backfilled twice with ethylene before Grubbs I (308 mg, 0.375 mmol) was added and the reaction was heated to 70 °C for 18 hours under an ethylene atmosphere. After this time the solvent was removed *in vacuo* and the resulting residue purified by flash chromatography on silica (7:3 EtOAc:pet. ether) to furnish the title compound as an off-white amorphous solid (832 mg, 69%). R_f = 0.47 (EtOAc). mp 280-285 °C (decomposition) (EtOAc). δ_H (500 MHz, *d*$_6$-DMSO, 100 °C) 4.35-4.39 (8H, m, C$\underline{H}_2$NC$\underline{H}_2$), 5.14-5.21 (4H, m, CH=C$\underline{H}_2$), 5.93 (2H, s, CH$_2$C$\underline{H}$=C), 6.53 (2H, dd, *J* = 17.5, 11.0 CH$_2$=C$\underline{H}$C), 7.63 (4H, s, ArH). δ_C (125 MHz, *d*$_6$-DMSO, 100 °C). (two resonances are absent from the high temperature NMR corresponding to 2 x N$\underline{C}$H$_2$; at RT two rotameric signals are observed for each) 115.8 (C=C$\underline{H}_2$), 123.7 (C=C$\underline{H}$), 126.5 (Ar$\underline{C}$H), 129.9 (C=C$\underline{H}$), 136.7 (C=$\underline{C}$), 137.5 (Ar$\underline{C}$), 167.5 ($\underline{C}$=O). υ_{max} (neat) 1651 (C=C stretch), 1610 (C=O stretch), 1594 (C=C stretch). HRMS (ESI) calculated for $C_{20}H_{21}N_2O_2$ (MH^+) 321.1603 found 321.1604.

(1,1'-biphenyl)-4,4'-diyl*bis*[(3-vinyl-2, 5-dihydro-1H-pyrrol-1-yl)methanone) (2c)



Compound **1c** (500 mg, 1.26 mmol) was dissolved in toluene (50 mL). The flask was then backfilled twice with ethylene before Grubbs I (308 mg, 0.375 mmol) was added and the reaction was heated to 70 °C for 18 hours under an ethylene atmosphere. After this time the solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography on silica (7:3 EtOAc: pet. ether) to furnish the title compound as a grey amorphous solid (285 mg, 57%). R_f = 0.23 (EtOAc). mp >300 °C (EtOAc). δ_H (500 MHz, *d*$_6$-DMSO, 90 °C) 4.39-4.43 (8H, m, C$\underline{H}_2$NC$\underline{H}_2$), 5.21-5.23 (4H, br m, CH=C$\underline{H}_2$), 5.95 (2H, br s, $CH_2C\underline{H}$=C), 6.55 (2H, dd, *J* = 17.5, 11.0 CC$\underline{H}$=CH_2), 7.70 (4H, d, *J* = 8.5, H1), 7.80 (4H, d, *J* = 8.5 H2). δ_C (125 MHz, *d*$_6$-DMSO, 90 °C) (two resonances are absent from the high temperature NMR corresponding to 2 x NCH_2) 116.8 (C=CH_2), 124.7 (C=$\underline{C}$H), 127.1 (Ar$\underline{C}$H), 128.2 (Ar$\underline{C}$H), 130.9 (C=$\underline{C}$H), 136.5 (C=$\underline{C}$), 137.6 (Ar$\underline{C}$), 141.2 (Ar$\underline{C}$), 168.8 ($\underline{C}$=O) . υ_{max} (neat) 2855 (C-H stretch), 1603 (C=O stretch), 1425 (C=C stretch). HRMS (ESI) calculated for $C_{26}H_{25}N_2O_2$ (MH^+) 397.1916 found 397.1903.

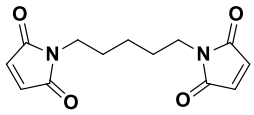
N-Methoxycarbonyl maleimide (S2)[2]



Maleimide (2 g, 20.6 mmol) was dissolved in EtOAc (70 mL) and the resulting solution was cooled to 0 °C. *N*-methylmorpholine (3.12g, 3.40 mL, 30.9 mmol) was added and the reaction was stirred at 0 °C for 10 mins before methylchloroformate (2.9g 2.38 mL, 30.9 mmol) was added as a solution in EtOAc (10 mL). The reaction was then allowed to warm to RT over 2 hours before being further diluted with EtOAc (50 mL). The reaction was then washed sequentially with sat. $NaHCO_{3(aq)}$,

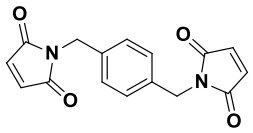
H_2O and brine. The organic layer was then dried over $MgSO_4$ and the solvent removed *in vacuo*. The resulting oil was purified by flash chromatography on silica (3:7 EtOAc:pet. ether) to give the title compound as a white crystalline solid (1.65g, 52%). R_f = 0.38 (1:1 EtOAc:pet. ether). mp 135 °C (EtOAc). δ_H (400 MHz, $CDCl_3$) 3.94 (3H, s, OC<u>H</u>$_3$), 6.83 (2H, s, <u>H</u>C=C<u>H</u>). δ_C (100 MHz, $CDCl_3$) 54.2 (O<u>C</u>H$_3$) 135.2 (H<u>C</u>=<u>C</u>H), 148.0 (N<u>C</u>O$_2$Me), 165.6 (N<u>C</u>OCH). υ_{max} (neat) 1759 (C=O stretch), 1709 (C=O stretch). Spectral data is consistent with previously reported values.[2]

1,1'-(pentane-1,5-diyl)*bis*(1H-pyrrole-2,5-dione) (3a)



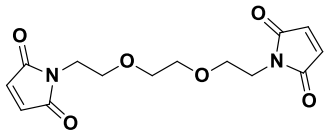
1,5-diaminopentane (408 mg, 467 µL, 4 mmol) was dissolved in $NaHCO_{3(aq)}$ (1M, 15 mL) and the resulting solution was cooled to 0 °C. Methoxycarbonyl maleimide (1.36 g, 8.8 mmol) was added and the reaction was stirred at 0 °C for 30 mins before being further diluted with the addition of H_2O (30 mL) and MeCN (15 mL). The reaction was then allowed to warm to RT over 2 hours. After this time CH_2Cl_2 was added to the reaction and the resulting layers were separated. The aqueous phase was extracted with CH_2Cl_2 (x3) then the combined organic layers were dried over $MgSO_4$ and the solvent was removed *in vacuo.* The resulting oil was then purified by flash chromatography on silica (stepped gradient; 2:8, 3:7 EtOAc:pet. ether) to furnish the title compound as a white crystalline solid (613mg, 58%). R_f = 0.54 (1:1 EtOAc: pet ether). mp 105-108 °C (EtOAc). δ_H (400 MHz, $CDCl_3$) 1.21-1.28 (2H, m, C<u>H</u>$_2$CH$_2$CH$_2$N) 1.58 (4H, quint., J = 7.5, CH$_2$C<u>H</u>$_2$CH$_2$N) 3.47 (4H, t, J = 7.0, CH$_2$CH$_2$C<u>H</u>$_2$N), 6.65 (4H, s, <u>H</u>C=C<u>H</u>). δ_C (100 MHz, $CDCl_3$) 23.8 (<u>C</u>H$_2$CH$_2$CH$_2$N), 28.0 (CH$_2$<u>C</u>H$_2$CH$_2$N), 37.4 (CH$_2$CH$_2$<u>C</u>H$_2$N), 134.0 (H<u>C</u>=<u>C</u>H), 170.6 (C=O). υ_{max} (neat) 2940 (C-H stretch), 1695 (C=O stretch), 1582 (C=C stretch). Spectral data is consistent with previously reported values.[3]

1,1'-(1,4-phenylene*bis*(methylene))*bis*(1H-pyrrole-2,5-dione) (3b)



p-Xylene diamine (136 mg, 1 mmol) was dissolved in $NaHCO_{3(aq)}$ (1 M, 10 mL). Yhe resulting solution was cooled to 0 °C before *N*-methoxycarbonyl maleimide (**S2**) (372 mg, 2.4 mmol) was added in one portion. The reaction was stirred at 0 °C for 10 mins then diluted with H_2O (20 mL) and MeCN (5mL). The reaction was then allowed to warm to RT over 1.5 hours. After this time the reaction was worked up with the addition of CH_2Cl_2, the layers were separated and the aqueous phase extracted (x3) with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvent evaporated to leave the title compound as a white crystalline solid (210 mg, 71%). R_f = 0.20 (1:1 EtOAc:pet. ether). mp 249-252 °C (decomposition); literature mp >250 °C.[4] δ_H (500 MHz, d_6-DMSO) 4.55 (4H, s, ArC$\underline{H}_2$N), 7.06 (4H, s, =CH), 7.18 (4H, s, ArH). δ_C (125 MHz, d_6-DMSO) 40.3 (Ar$\underline{C}H_2$N), 127.6 (Ar CH), 134.7 (=CH), 136.0 (Ar C), 170.9 (C=O). υ_{max} (neat) 1703 (C=O stretch). Spectral data is consistent with previously reported values.[4]

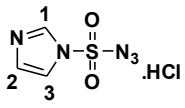
1,1'-((ethane-1,2-diyl*bis*(oxy))*bis*(ethane-2, 1-diyl))*bis*(1H-pyrrole-2,5-dione) (3c)



2,2'-(Ethylenedioxy)*bis*-(ethylamine) (400 mg, 2.7 mmol) was dissolved in $NaHCO_{3(aq)}$ (1M, 20mL) and the resulting solution was cooled to 0 °C. Methoxycarbonyl maleimide (**S2**) was added and the reaction was stirred at 0 °C for 30 mins before being further diluted with H_2O (40 mL) and MeCN (20 mL). The reaction was allowed to warm to RT over 2 hours then extracted (x3) with CH_2Cl_2, the combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash chromatography on silica (1:1 EtOAc:pet. ether) to furnish the title compound as a white amorphous solid (248 mg 34%). R_f =

0.54 (1: 1 EtOAc: pet ether). mp 100-102 °C (EtOAc); literature mp 92-94 °C.[5] δ_H (500 MHz, $CDCl_3$) 3.53 (4H, s, $OC\underline{H}_2C\underline{H}_2O$), 3.59 (4H, t, J = 6.0, $NC\underline{H}_2CH_2O$), 3.69 (4H, t, J = 6.0, $NCH_2C\underline{H}_2O$), 6.69 (4H, s, $\underline{H}C{=}C\underline{H}$). δ_C (125 MHz, $CDCl_3$) 37.1 (CH_2N), 67.8 (CH_2O), 70.0 (CH_2O), 134.1 (HC=CH), 170.6 (C=O). υ_{max} (neat) 2881 (C-H stretch), 1697 (C=O stretch). Spectral data is consistent with previously reported values.[5]

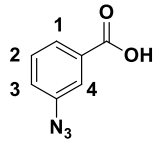
1H-Imidazole-1-sulfonyl azide hydrochloride (S3)[6]



Sodium azide (6g, 92.3 mmol) was suspended in acetonitrile (90 mL) and the resulting suspension was cooled to 0 °C. Sulfuryl chloride (12.46 g, 7.48 mL, 92.3 mmol) was added at 0 °C then the reaction was allowed to warm to RT overnight. After stirring overnight imidazole (12.55g, 184.6 mmol) was added in two portions and the reaction was stirred at RT for a further 3 hours. The reaction was then diluted with EtOAc and extracted with H_2O (x2) and 1M $NaHCO_{3(aq)}$ then dried over $MgSO_4$ and filtered. A solution of HCl in EtOH was then prepared by adding acetyl chloride (10.8 g, 9.78 mL, 138.4 mmol) to EtOH (40 mL), the resulting solution was then added to the filtrate. The reaction was stirred at RT for around 20 min until a significant amount of white precipitate had formed then cooled to 0 °C and filtered to furnish the title compound as a white crystalline solid (12.84 g, 66%). δ_H (300 MHz, D_2O) 7.63 (1H, app. t, J = 1.5, H2), 8.03 (1H, app. t, J = 1.5, H3), 9.46 (1H, app. t, J = 1.5, H1). δ_C (75 MHz, D_2O) 120.7 (C3), 123.1 (C2), 138.1 (C1). υ_{max} 2168 (azide stretch), 1581 (Ar C=C stretch), 1322 (O=S=O), 1158 (O=S=O). Spectral data is consistent with previously reported values.[6]

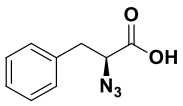
In a recent publication, safety concerns regarding the diazo transfer reagent **S3** have been raised. Though we never observed any incident using **S3**, we would recommend to consider the usage of alternative diazo transfer reagents introduced in that publication.[12]

3-Azidobenzoic acid (6i)



3-Aminobenzoic acid (810 mg, 6 mmol) was dissolved in MeOH (15 mL) and to the resulting solution were added 1H-imidazole-1-sulfonyl azide hydrochloride (**S3**) (1.51 g, 7.2 mmol), K_2CO_3 (2.03g, 14.7 mmol) and $CuSO_4.5H_2O$ (15 mg, 0.06 mmol). The reaction was then stirred at RT for 18 hours. After this time the MeOH was removed *in vacuo* and the resulting residue redissolved in H_2O. The resulting solution was cooled to 0 °C and carefully acidified with the addition of 2M $HCl_{(aq)}$ then extracted (x3) with EtOAc. The combined extracts were dried over $MgSO_4$ and the EtOAc removed *in vacuo*. The crude residue was then purified by automated flash chromatography (Isolera, 0-60% EtOAc in hexanes) to furnish the desired product as a yellow amorphous solid (789 mg, 80%). R_f = 0.31 (1:1 EtOAc:hexanes). mp 163-165 °C (EtOAc); literature mp 160 °C.[7] δ_H (300 MHz, d_6-DMSO) 7.38 (1H, ddd, J = 8.0, 2.5, 1.0 H3), 7.52-7.58 (2H, m, H2, H4), 7.75 (1H, dt, J = 8.0, 1.0, H1) 13.2 (1H, br. s, COOH). δ_C (75 MHz, d_6-DMSO) 119.8 (ArCH), 123.8 (ArCH), 126.2 (ArCH), 130.7 (ArCH), 132.9 (ArC), 140.3 (ArC), 166.8 (C=O). υ_{max} 2825, 2656 (C-H stretch), 2129 (azide stretch), 1681 (C=O stretch), 1581 (Ar C=C stretch). Spectral data is consistent with previously reported values.[8]

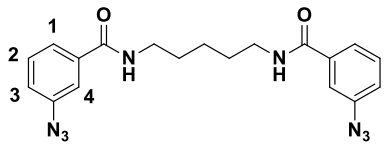
L-Azidophenylalanine (6ii)



L-Phenylalanine (500 mg, 3.03 mmol) was dissolved in MeOH (20 mL) and to the resulting solution were added 1H-imidazole-1-sulfonyl azide hydrochloride (**S3**) (764 mg, 3.63 mmol), K_2CO_3 (1.03 g, 7.42 mmol) and $CuSO_4.5H_2O$ (7.5 mg, 0.03 mmol). The reaction was then stirred at RT for 18 hours. After this time the MeOH was removed *in vacuo* and the resulting residue redissolved in H_2O. The resulting solution was cooled to 0 °C and carefully acidified with the addition of 2M $HCl_{(aq)}$ then extracted (x3) with EtOAc. The combined extracts were dried over $MgSO_4$ and the EtOAc removed *in vacuo*. The crude residue was then purified by flash

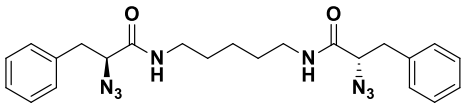
chromatography on silica (1:9 EtOAc:pet. ether + 1% AcOH) to furnish the title compound as a yellow oil (477 mg, 82%). R_f = 0.23 (1:9 EtOAc:pet. ether). $[\alpha]_D^{25}$-61.2 (c = 1, $CHCl_3$) (literature rotation: $[\alpha]_D^{30}$-70.9 (c = 1, $CHCl_3$)[9]. δ_H (400 MHz, $CDCl_3$) 3.03 (1H, dd, *J* = 14.0, 9.0, $ArC\underline{H}_2CHN_3$ H_a), 3.23 (1H, dd, J = 14.0, 5.0 $ArC\underline{H}_2CHN_3$ H_b), 4.15 (1H, dd, J = 9.0, 5.0, $ArCH_2C\underline{H}N_3$), 7.24-7.36 (5H, m, 5xArH) 9.80 (1H, br. s, COOH). δ_C (100 MHz, $CDCl_3$) 37.5 ($Ar\underline{C}H_2CHN_3$), 63.2 ($ArCH_2\underline{C}HN_3$), 127.4 (ArCH), 128.8 (2x ArCH), 129.2 (2x ArCH), 135.6 (ArC), 175.6 (C=O). υ_{max} 3032 (C-H stretch), 2106 (azide), 1715 (C=O stretch), 1604 (Ar C=C stretch). Spectral data is consistent with previously reported values.[10]

N, *N*'-(Pentane-1,5-diyl)*bis*(3-azidobenzamide) (4ai)



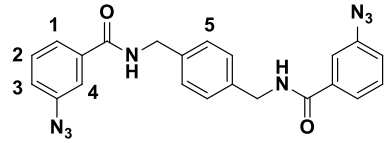
3-Azidobenzoic acid (**6i**) (100 mg, 0.61 mmol) was dissolved in DMF (5 mL), to the resulting solution were added DIPEA (172 mg, 231 µL, 1.33 mmol) and HATU (253 mg, 0.67 mmol) followed by 1,5 diaminopentane (30.9mg, 35 µL, 0.30 mmol). The reaction was then stirred at RT overnight. After this time the DMF was removed *in vacuo* and the resulting residue partitioned between EtOAc and sat. $NaHCO_{3(aq)}$. The aqueous phase was extracted (x3) with EtOAc and the combined extracts dried over $MgSO_4$ then concentrated *in vacuo*. The crude product was purified by flash chromatography on silica (stepped gradient; 1:1, 7:3 EtOAc:pet ether) to furnish the title compound as a white amorphous solid (103 mg, 86%). R_f = 0.53 (EtOAc). mp 175-180 °C (decomposition) (EtOAc). δ_H (300 MHz, d_6-DMSO) 1.32-1.40 (2H, m, $C\underline{H}_2CH_2CH_2N$), 1.57 (4H, quint, *J* = 7.0 $CH_2C\underline{H}_2CH_2N$), 3.26 (4H, q, *J* = 6.5, $CH_2CH_2C\underline{H}_2N$), 7.25 (2H, ddd, *J* = 8.0, 2.0, 1.0, H3), 7.48 (2H, t, *J* = 8.0, H2), 7.55 (2H, t, *J* = 2.0, H4), 7.65 (2H, t, *J* = 8.0, H1), 8.52 (2H, t, *J* = 5.5, NH). δ_C (75 MHz, d_6-DMSO) 24.3 ($\underline{C}H_2CH_2CH_2N$), 29.1 ($CH_2\underline{C}H_2CH_2N$), 39.6 ($CH_2CH_2\underline{C}H_2N$), 118.0 (ArCH), 122.0 (ArCH), 124.3 (ArCH), 130.3 (ArCH), 136.7 (ArC), 139.9 (ArC), 165.4 (C=O). υ_{max} (neat) 3320 (N-H stretch), 2113 (azide stretch), 1638 (amide I), 1544 (amide II). HRMS (ESI) calculated for $C_{19}H_{21}N_8O_2$ (MH^+) 393.1774 found 393.1782.

(2S, 2'S)-*N, N'*-(pentane-1,5-diyl)*bis*(2-azido-3-phenylpropanamide) (4aii)



L-Azidophenylalanine (**6ii**) (150 mg, 0.78 mmol) was dissolved in DMF (5 mL), to the resulting solution were added DIPEA (223 mg, 300 μL, 1.73 mmol) and HATU (328 mg, 0.86 mmol) followed by 1, 5-diaminopentane (40 mg, 46 μL, 0.39 mmol). The reaction was then allowed to stir at RT overnight. After this time the DMF was removed *in vacuo* and the resulting residue partitioned between EtOAc and sat. $NaHCO_{3(aq)}$. The aqueous phase was extracted (x3) with EtOAc and the combined extracts dried over $MgSO_4$ then concentrated *in vacuo*. The crude product was purified by flash chromatography on silica (stepped gradient; 3:7, 1:1 EtOAc:pet. ether) to furnish the title compound as a light yellow amorphous solid (126 mg, 72%). R_f= 0.5 (1:1 EtOAc: pet. ether). mp 70-74 °C (EtOAc) $[\alpha]_D^{25}$ +36.2 (c = 0.5, DMSO). δ_H (500 MHz, $CDCl_3$) 1.11-1.17 (2H, m, C$\underline{H}_2$CH_2CH_2N), 1.41 (4H, quint, *J* = 7.5, CH_2C$\underline{H}_2$CH_2N), 3.02 (2H, dd, *J* = 14.0, 8.0, ArC$\underline{H}_2$CHN$_3$ H_a), 3.13-3.22 (4H, m, CH_2CH_2C$\underline{H}_2$N), 3.31 (2H, dd, *J* = 14.0, 4.0, ArC$\underline{H}_2$CHN$_3$ H_b), 4.17 (2H, dd, *J* = 8.0, 4.5, ArCH_2C$\underline{H}$N$_3$), 6.22 (2H, br s, NH), 7.21-7.32 (10H, m, ArH). δ_C (125 MHz, $CDCl_3$) 23.8 (C$\underline{H}_2$CH_2CH_2N), 28.9 (CH_2C$\underline{H}_2$CH_2N), 38.5 (CH_2CH_2C$\underline{H}_2$N), 39.1 (Ar$\underline{C}$H$_2$CHN$_3$), 65.5 ArCH_2C$\underline{H}$N$_3$), 127.2 (ArCH), 128.6 (ArCH), 129.5 (ArCH), 136.1 (ArC), 168.5 (C=O). υ_{max} 3308 (N-H stretch), 2110 (azide stretch), 1651 (Amide I), 1531 (Amide II). HRMS (ESI) calculated for $C_{23}H_{29}N_8O_2$ (MH^+) 449.2413 found 449.2417.

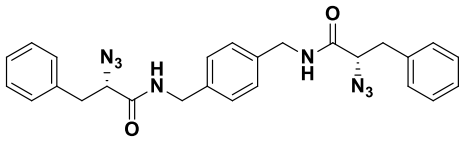
N, N'-(1,4-Phenylene*bis*(methylene))*bis*(3-azidobenzamide) (4bi)



3-Azidobenzoic acid (**6i**) (300 mg, 1.82 mmol) was suspended in CH_2Cl_2 (5mL) and cooled to 0 °C before oxalyl chloride (2M in CH_2Cl_2 1.365 mL, 2.73 mmol) and DMF

(0.05 mL) were added. The reaction was allowed to warm to RT over 2 hours then the reaction mixture was added to a solution of *p*-xylenediamine (111 mg, 0.82 mmol) and Na_2CO_3 (346 mg, 1.8 mmol) in H_2O (5 mL). The biphasic reaction was then stirred at RT overnight. After this time the reaction was diluted with additional CH_2Cl_2, the layers separated and the organic layer washed with sat. $Na_2CO_{3(aq)}$ and brine then dried over $MgSO_4$. The CH_2Cl_2 was then removed *in vacuo* to furnish the title compound as an off-white amorphous solid (144 mg, 41%). mp 190-195 °C (decomposition) (CH_2Cl_2). δ_H (400 MHz, d_6-DMSO) 4.41 (4H, d, J = 6.0 ArC$\underline{H}_2$NH), 7.22-7.25 (6H, m, H3, H5), 7.47 (2H, t, J = 8.0, H2), 7.60 (2H, t, J = 1.5, H4), 7.70 (2H, d, J = 7.5, H1), 9.20 (2H, t, J = 6.0, NH). δ_C (100 MHz, d_6-DMSO) 42.5 (Ar$\underline{C}$H$_2$NH), 117.9 (ArCH), 121.9 (ArCH), 124.1 (ArCH), 127.4 (ArCH), 130.1 (ArCH), 136.1 (ArC), 138.1 (ArC), 139.7 (ArC), 165.2 (C=O). υ_{max} 3264 (N-H stretch), 2100 (azide), 1634 (amide I), 1583 (Ar C=C), 1536 (amide II). HRMS (ESI) calculated for $C_{22}H_{19}N_8O_2$ (MH^+) 427.1625 found 427.1621.

(2S, 2'S)-*N*, *N*'-(1,4-phenylene*bis*(methylene))*bis*(2-azido-3-phenylpropanamide) (4bii)

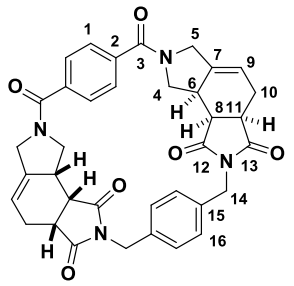


L-Azidophenylalanine (**6ii**) (250 mg, 1.31 mmol) was dissolved in CH_2Cl_2 (5 mL) and the resulting solution was cooled to 0 °C before oxalyl chloride (2M in CH_2Cl_2, 0.98 mL, 1.96 mmol) and DMF (0.05 mL) were added. The reaction was allowed to warm to RT over 2 hours then the reaction mixture was added to a solution of *p*-xylenediamine (80.1 mg, 0.59 mmol) and Na_2CO_3 (250 mg, 2.35 mmol) in H_2O (5mL). The reaction was then allowed to stir at RT overnight. After this time the reaction was diluted with additional CH_2Cl_2, the layers separated and the organic layer washed with sat. $Na_2CO_{3(aq)}$ and brine then dried over $MgSO_4$. The CH_2Cl_2 was then removed *in vacuo* to furnish the title compound as an off-white amorphous solid (185 mg, 65 %). mp 139-140 °C (CH_2Cl_2). $[\alpha]_D^{25}$ +56.1 (c = 1.1, DMSO). δ_H (500 MHz, d_6-DMSO) 2.96 (2H, dd, J = 13.5, 8.5, ArC$\underline{H}_2$CHN$_3$ H$_a$), 3.09 (2H, dd, J = 13.5, 6.5, ArC$\underline{H}_2$CHN$_3$ H$_b$), 4.01 (2H, dd, J = 8.0, 6.5 ArCH$_2$C$\underline{H}$N$_3$), 4.18 (2H, dd, J =

15.0, 5.5 ArC$\underline{H}_2$NH H_a) 4.26 (2H, dd, *J* = 15.0, 6.0 ArC$\underline{H}_2$NH H_b), 7.01 (4H, s, Ar$\underline{H}$CH_2NH) 7.23-7.30 (10H, m, ArH), 8.65 (2H, t, *J* = 6.0 NH). δ_C (125 MHz, *d_6*-DMSO) 37.4 (Ar$\underline{C}H_2$CHN$_3$), 42.3 (Ar$\underline{C}H_2$NH), 63.0 (ArCH$_2\underline{C}$HN$_3$), 127.2 (ArCH), 127.6 (ArCH), 128.8 (ArCH), 129.6 (ArCH), 137.1 (ArC), 137.7 (ArC), 169.1 (C=O). υ_{max} 3279 (N-H stretch), 3064, 3030, 2929 (C-H stretch), 2094 (azide), 1646 (amide I), 1540 (amide II). HRMS (ESI) calculated for $C_{26}H_{27}N_8O_2$ (MH^+) 483.2251 found 483.2245.

3. Synthesis of macrocyclic library compounds

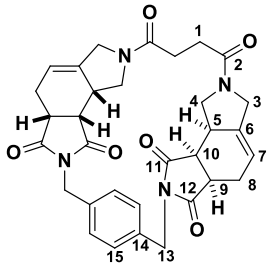
(+/-)-8



Bis-diene **2b** (50 mg, 0.156 mmol) and *bis*-maleimde **3b** (46 mg, 0.156 mmol) were dissolved in 1,2-DCE (17 mL) and the reaction was heated to 120 °C in a sealed tube overnight. The reaction was then cooled to RT and the solvent removed *in vacuo*. The resulting residue was purified by flash chromatography on silica (stepped gradient; 1-2-3-4-5% MeOH in CH_2Cl_2) to furnish the title compound as a light yellow amorphous solid (32 mg, 33%). R_f = 0.38 (5:95 MeOH:CH_2Cl_2). mp >300 °C (MeOH). δ_H (500 MHz, $CDCl_3$) 2.17-2.19 (2H, m, H10$_a$) 2.72-2.81 (4H, m, H10$_b$ and H6) 3.18 (2H, t, *J* = 8.0 , H11), 3.37 (2H, t, *J* = 7.5, H8), 3.62 (2H, dd, *J* = 12.5, 4.5, H4$_a$), 3.85 (2H, d, J = 15.5 H5$_a$), 4.11 (2H, d, *J* = 15.5, H5$_b$) 4.47 (2H, d, *J* = 15, H14$_a$), 4.65 (2H, d, *J* = 15 Hz, H14$_b$), 5.01 (2H, d, *J* = 12.5, H4$_b$), 5.54-5.56 (2H, m, H9) 7.03 (4H, s, H16), 7.17 (4H, s, H1). δ_C (125MHz, $CDCl_3$) 26.0 (C10), 37.5 (C6), 39.4 (C11), 41.9 (C14), 42.4 (C8), 46.9 (C4), 51.8 (C5), 116.9 (C9), 127.5 (C1), 127.7 (C16), 135.3 (C15), 137.3 (C2), 142.0 (C7), 168.3 (C3), 176.6 (C12), 179.4 (C13). υ_{max} (neat) 1694 (C=O), 1619 (C=C). HRMS (ESI) calculated for $C_{36}H_{33}N_4O_6$ (MH^+) 617.2400 found 617.2397.

Crystallisation and X-ray crystallography of 8: X-Ray quality crystals of **8** were obtained by vapour diffusion of petroleum ether (30-40 fraction) into chloroform leading to the formation of colourless needle-shaped crystals containing two molecules of chloroform within the crystal packing. **Relevant crystal data:** Molecular formula $C_{36}H_{32}N_4O_6.2CHCl_3$, M = 855.39, monoclinic, a = 15.1710(2) Å, b = 17.9068(3) Å, c = 140740(2) Å, α = 90º, β = 90.417(1)º, γ = 90º, V = 3823.3(1) Å^3, T = 180(2) K, space group $P2(1)/c$, Z = 4, 38105 reflections measured, 8725 independent reflections (R_{int} = 0.0688). The final R_1 values were 0.0559 ($I > 2\sigma(I)$). The final w$R(F^2)$ values were 0.1152 ($I > 2\sigma(I)$). The final R_1 values were 0.0956 (all data). The final w$R(F^2)$ values were 0.1322 (all data).

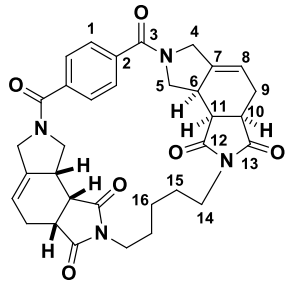
(+/-)-9



Bis-diene **2a** (42.5 mg, 0.156 mmol) and *bis*-maleimide **3b** (46 mg, 0.156 mmol) were dissolved in 1,2-DCE (17 mL) and heated to 120 °C overnight in a sealed tube. The reaction was then allowed to cool to RT and the solvent was removed *in vacuo*. The resulting residue was then purified sequentially by flash chromatography on silica (stepped gradient; 1-2-3-4-5% MeOH in CH_2Cl_2) and HPLC (0-30% MeCN in H_2O) to furnish the title compound as a yellow oil (14.6 mg, 17%). R_f = 0.12 (5:95 MeOH:CH_2Cl_2). δ_H (500 MHz, $CDCl_3$, two conformers observed, major conformer peaks listed) 2.03-2.18 (2H, m, H8$_a$), 2.44-2.50 (2H, m, H1$_a$), 2.63-2.68 (2H, m, H1$_b$), 2.76-2.79 (2H, m, H8$_b$), 2.86-2.93 (2H, m, H5), 3.15-3.19 (2H, m, H9), 3.26-3.65 (2H, m, H10), 3.67-3.72 (2H, m, H4$_a$), 3.85-3.95 (2H, m, H3$_a$), 4.11 (2H, d, J = 15.5, H3$_b$), 4.52 (2H, d, J = 15.5, H13$_a$), 4.61-4.65 (2H, m, H13$_b$), 4.92 (2H, dd, J = 13.0, 3.0, H4$_b$), 5.62-5.66 (2H, m, H7), 7.03 (4H, s, H15). δ_C (125 MHz, $CDCl_3$, major conformer peaks listed) 25.7 (C8), 30.2 (C1), 37.1 (C5), 39.4 (C9), 41.5 (C10), 41.8 (C13), 46.6 (C4), 50.1 (C3), 118.7 (C7), 127.5 (C15), 135.6 (C14), 140.6 (C6), 171.5 (C2), 177.0 (C11), 178.8 (C12). υ_{max} (thin film CH_2Cl_2) 1695 (C=O stretch), 1615

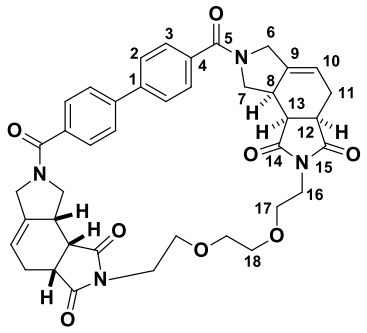
(C=C stretch). HRMS (ESI) calculated for $C_{32}H_{32}N_4O_6$ (MH^+) 569.2400 found 569.2400.

(+/-)-10



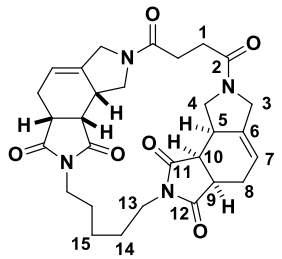
Bis-diene **2b** (50 mg, 0.156 mmol), and *bis*-maleimide **3a** (41 mg, 0.156 mmol) were dissolved in 1,2-DCE (17 mL) and the reaction was heated to 120 °C in a sealed tube overnight. The reaction was then allowed to cool to RT and the solvent was removed *in vacuo*. The resulting residue was then purified sequentially by flash chromatography on silica (stepped gradient; 1-2-3-4-5% MeOH in CH_2Cl_2) and HPLC (20-40 % MeCN in H_2O) to furnish the title compound as a yellow gum (24 mg, 26%). R_f = 0.24 (5:95 MeOH:CH_2Cl_2). δ_H (500 MHz, $CDCl_3$), 0.91-1.16 (6H, m, H15, H16), 2.86 (2H, d, J = 13.5, $H9_a$), 2.78-2.82 (4H, m, H6, $H9_b$), 3.07 (2H, t, J = 7.0, H10), 3.24 (2H, t, J = 8.0, H11), 3.28-3.38 (4H, m, H14), 3.63 (2H, dd, J = 17.5, 9.0, $H5_a$), 3.85 (2H, d, J = 16.0, $H4_a$), 4.08 (2H, d, J = 16.0, $H4_b$), 5.03-5.05 (2H, m, $H5_b$), 5.67-5.69 (2H, m, H8), 7.42 (2H, s, H1). δ_C (125 MHz, $CDCl_3$) 24.4 (C16), 25.7 (C9), 28.6 (C15), 37.8 (C6), 38.4 (C14), 39.7 (C10), 41.3 (C11), 46.8 (C4), 51.5 (C5), 117.8 (C8), 126.7 (C1), 137.9 (C2), 141.3 (C7), 168.3 (C3), 176.2 (C12), 178.9 (C13). υ_{max} (neat) 2938 2865 (C-H stretch), 1770 (C=O stretch), 1692 (C=O stretch), 1631 (C=C stretch). HRMS (ESI) calculated for $C_{33}H_{35}N_4O_6$ (MH^+) 583.2557 found 583.2547

(+/-)-11



Bis-diene **2c** (61.8 mg, 0.156 mmol) and *bis*-maleimide **3c** (48.2 mg, 0.156 mmol) were dissolved in 1,2-DCE (17 mL) and the reaction was heated to 120 °C in a sealed tube overnight. After this time the reaction was allowed to cool to RT and the solvent was removed *in vacuo.* The resulting material was purified by flash chromatography on silica (1-2-3-5% MeOH in CH_2Cl_2) to furnish the title compound as a yellow oil (33.0 mg, 30 %). R_f= 0.44 (5:95 MeOH:CH_2Cl_2). δ_H (500 MHz, $CDCl_3$) 2.12 (2H, br s, $H11_a$), 2.82 (4H, br s, H8, $H11_b$), 3.05-3.17 (4H, br m, H12, H13), 3.31-3.82 (12H, m, H16, H17, H18), 4.11-4.43 (8H, m, H6, H7), 5.78 (2H, br s, H10), 7.51-7.62 (8H, m, H2, H3). δ_C (125 MHz, $CDCl_3$) 24.8 (C11), 38.1 (C16), 38.5 (C8), 39.5 (C12), 40.8 (C13), 48.8 (C6), 49.9 (C7), 66.9 (C17), 69.8 (C18), 117.3 (C10), 127.0 (C3), 127.9 (C2), 135.8 (C4), 139.9 (C9), 141.7 (C1), 169.6 (C5), 177.1 (C14), 179.1 (C15)**.** υ_{max} (thin film CH_2Cl_2) 1695 (C=O stretch), 1609 (C=O stretch). HRMS (ESI) $C_{40}H_{41}N_4O_8$ (MH^+) 705.2924 found 705.2902.

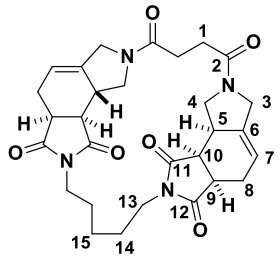
(+/-)-12a



Bis-diene **2a** (42.5 mg, 0.156 mmol) and *bis*-maleimide **3a** (41 mg, 0.156 mmol) were dissolved in 1,2-DCE (17 mL) and heated to 120 °C overnight in a sealed tube. The reaction was then allowed to cool to RT and the solvent was removed *in vacuo*. The

resulting residue was then purified sequentially by flash chromatography on silica (stepped gradient, 1-2-3-4-5% MeOH in CH_2Cl_2) and HPLC (18-22% MeCN in H_2O) to furnish the title compound as a yellow oil (13.5 mg, 16 %). R_f = 0.15 (5:95 MeOH:CH_2Cl_2). δ_H (500 MHz, $CDCl_3$, two conformers observable by NMR, major conformer peaks listed) 0.94-1.05 (2H, m, H15), 1.39-1.58 (4H, m, H14), 2.15-2.21 (2H, br m, $H8_a$), 2.59-2.62 (2H, m, $H1_a$), 2.74 (2H, dd, J = 15.5, 7.5 $H8_b$), 2.92-2.94 (2H, m, H5), 3.09 (2H, t, J = 8.5, H9), 3.19-3.28 (4H, m, $H1_b$, H10), 3.36-3.40 (4H, m, H13), 4.16 (2H, t, J = 11.0, $H4_a$), 4.14 (2H, d, J =17.5 $H3_a$), 4.53 (2H, d, J = 17.5, $H3_b$), 4.68 (2H, dd, J = 11.5, 5.5 $H4_b$), 5.76 (2H, br s, H7). δ_C (125 MHz, $CDCl_3$, major conformer peaks listed) 22.7 (C15), 25.0 (C8), 27.0 (C14), 30.9 (C1), 37.8 (C5), 38.2 (C13), 39.1 (C9), 41.1 (C10), 48.8 (C4), 49.5 (C3), 117.1 (C7), 140.0 (C6), 172.3 (C2), 177.4 (C11), 179.3 (C12). υ_{max} (thin film CH_2Cl_2) 2948 (C-H stretch), 1689 (C=O stretch), 1626 (C=O stretch). HRMS (ESI) calculated for $C_{29}H_{35}N_4O_6$ (MH^+) 535.2557 found 535.2561.

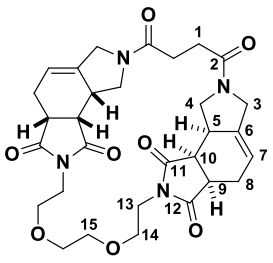
(+/-)-12b



Procedure as for **12a**, title compound obtained as a yellow oil (10 mg, 12 %). R_f = 0.15 (5:95 MeOH:CH_2Cl_2). δ_H (500 MHz, $CDCl_3$) 0.90-0.99 (2H, m, H15), 1.32-1.41 (2H, m, $H14_a$), 1.55-1.60 (2H, m, $H14_b$), 2.12-2.19 (2H, m, $H8_a$), 2.53 (1H, dt, J = 14.5, 9.0, $H1_a$), 2.67 (1H, dt, J = 17.0, 8.5, $H1^*_a$), 2.78 (2H, dd, J = 15.0, 7.5, $H8_b$), 2.89-2.96 (3H, m, $H1^*_b$, H5), 3.09-3.18 (3H, m, $H1_b$, H9), 3.24-3.44 (6H, m, H10, H13), 3.81-3.87 (2H, m, $H4_a$, $H3_a$), 3.95 (1H, dd, J = 12.5, 4.5, $H4^*_a$), 4.20 (1H, d, J = 16.0, $H3^*_a$), 4.37-4.46 (2H, m, $H3_b$, $H3^*_b$), 4.61 (1H, dd, J = 13.0, 5.5, $H4^*_b$), 4.77 (1H, dd, J = 11.5, 3.5. $H4_b$), 5.74-5.77 (2H, m, H7). δ_C (125MHz, $CDCl_3$), 23.2 (C15), 25.0 (C8), 25.7 (C8*), 26.5 (C14), 27.3 (C14*), 28.7 (C1), 30.0 (C1*), 37.3 (C5), 37.9 (C5*), 38.0 (C13), 38.7 (C13*), 38.9 (C9), 39.6 (C9*), 40.8 (C10), 41.6 (C10*), 47.3 (C4), 48.1 (C4*), 49.0 (C3), 49.8 (C3*), 117.6 (C7), 118.0 (C7*), 139.6 (C6), 141.4

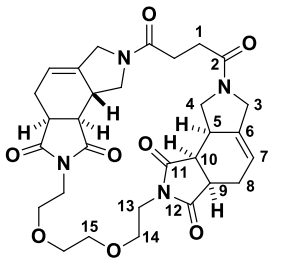
(C6*), 171.6 (C2), 173.1 (C2*), 177.2 (C11), 177.7 (C11*), 179.1 (C12), 179.4 (C12*). υ_{max} (thin film; CH_2Cl_2), 2942 (C-H stretch), 1687 (C=O stretch), 1625 (C=C stretch). HRMS (ESI) calculated for $C_{29}H_{35}N_4O_6$ (MH^+) 535.2557 found 535.2563.

(+/-)-13a



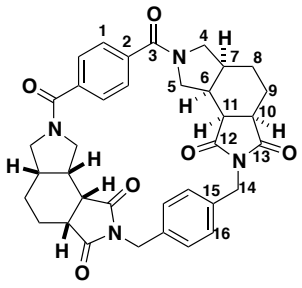
Bis-diene **2a** (42.5 mg, 0.156 mmol) and *bis*-maleimide **3c** (48.2 mg, 0.156 mmol) were dissolved in 1,2-DCE (17 mL) and the reaction was heated to 120 °C in a sealed tube overnight. The reaction was then allowed to cool to RT and the solvent was removed *in vacuo*. The resulting residue was then purified sequentially by flash chromatography on silica (stepped gradient, 1-2-3-4-5% MeOH in CH_2Cl_2) and HPLC (15-30% MeCN in H_2O) to furnish the title compound as a yellow gum (23.7 mg, 26%). δ_H (500 MHz, $CDCl_3$, two conformers observable, major conformer listed) 2.12-2.15 (2H, m, $H8_a$), 2.60-2.65 (2H, m, $H1_a$), 2.79-2.95 (4H, m, H5, $H8_b$), 3.13-3.18 (4H, $H1_b$, H9), 3.28 (2H, app quint, J = 8.0, H10), 3.37-3.69 (13H, m, $H4_a$, H13, H14, H15), 3.81-4.03 (2H, m, $H4_b$, $H3_a$), 4.20-4.35 (2H, m, $H3_b$), 4.55-4.71 (2H, m, $H4_b$), 5.76-5.80 (2H, m, H7). δ_C (125 MHz, $CDCl_3$, major conformer listed) 24.7 (C8), 29.2 (C1), 37.8 (C6), 38.2 (C13), 39.3 (C9), 41.1 (C10), 48.1 (C4), 49.2 (C3), 67.6 (C14), 69.5 (C15), 118.1 (C7), 139.1 (C5), 172.2 (C2), 177.0 (C11), 179.3 (C12). υ_{max} (thin film; CH_2Cl_2) 1694 (C=O stretch), 1630 (C=C stretch). HRMS (ESI) $C_{30}H_{37}N_4O_6$ (MH^+) 581.2611 found 581.2629.

(+/-)-13b



Procedure as for **13a,** the title compound was obtained as a yellow gum (16 mg, 18%). δ_H (500 MHz, $CDCl_3$) 2.13-2.17 (2H, m, $H8_a$), 2.59-2.61 (2H, m, $H1_a$), 2.81-2.98 (6H, m, $H1_b$, H5, $H8_b$), 3.13 (2H, t, *J* = 8.0 H9), 3.27-3.30 (2H, m, H10), 3.37-3.63 (12H, m, H13, H14, H15), 3.64-4.02 (3H, $H4_a$, $H4^*_a$, $H3_a$), 4.02 (1H, d, *J* = 15.5, $H3^*_a$), 4.30-4.34 (3H, $H3_b$, $H3^*_b$, $H4^*_b$), 4.58 (1H, dd, *J* = 12.0, 4.0, $H4_b$), 5.77-5.82 (2H, m, H7). δ_C (125 MHz, $CDCl_3$) 24.5 (C8), 24.8 (C8*), 29.4 (C1), 29.6 (C1*), 36.3 (C6), 37.9 (C6*), 38.1 (C13), 38.5 (C13*), 39.3 (C9), 39.6 (C9*), 40.9 (C10), 41.1 (C10*), 47.3 (C4), 48.0 (C4*), 49.0 (C3), 50.0 (C3*), 67.0 (C14), 67.5 (C14*), 69.3 (C15), 69.8 (C15*), 118.0 (C7), 118.1 (C7*), 139.2 (C5), 139.8 (C5*), 171.8 (C2), 172.0 (C2*), 177.0 (C11), 177.2 (C11*), 179.1 (C12), 179.1 (C12*). υ_{max} (thin film CH_2Cl_2) 2937 (C-H stretch) 1693 (C=O stretch) 1628 (C=C stretch). HRMS (ESI) calculated for $C_{30}H_{37}N_4O_8$ (MH^+) 581.2611 found 581.2603.

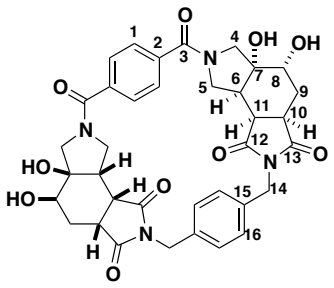
(+/-)-14



Macrocycle **8** (20 mg, 0.032 mmol) was dissolved in MeOH (2 mL), palladium on charcoal, (10 % w/w, 6.8 mg, 0.0064 mmol) was added and the reaction was stirred at RT for two days under a H_2 atmosphere. The reaction mixture was then filtered through Celite and concentrated *in vacuo*. The resulting oil was purified by HPLC

(20-25% MeCN in H_2O) to furnish the title compound as a white crystalline solid (10.2 mg, 51%). mp 275-280 °C (decomposition) (H_2O/MeCN). δ_H (500 MHz, *d*$_6$-DMSO, molecule appears dynamic on NMR timescale) 1.43-1.77 (8H, m, H8, H9), 2.34-2.35 (1H, m, H6), 2.43-2.57 (4H, m, $H4_a$, H6' H7), 3.02-3.10 (2H, m, H10, H11), 3.15-3.67 (7H, m, $H4_b$, $H4'_a$, $H5_a$, $H5_b$, $H5'_a$, H10', H11'), 3.84 (1H, dd, *J* = 12.0, 7.0, $H4'_b$), 4.11 (1H, d, *J* = 12.5 $H5'_b$), 4.32 (1H, d, *J* = 16.0, $H14_a$), 4.42 (1H, d, *J* = 14.0, $H14'_a$), 4.64 (1H, d, *J* = 16.0, $H14_b$), 4.71 (1H, d, *J* = 14.0, $H14'_b$), 7.01 (2H, d, *J* = 8.0, H16), 7.36 (4H, app d, *J* = 8.0, H1, H16'), 7.63 (1H, d, *J* = 8.0, H1'). δ_C (125 MHz, *d*$_6$-DMSO) two resonances are observed for each signal due to the dynamic nature of the molecule, 19.5 (C9), 21.3 (C9'), 22.6 (C8), 24.6 (C8'), 33.2 (C6), 33.6 (C6'), 35.9 (C7), 36.4 (C7'), 37.4 (C10), 3.7.6 (C10'), 40.4 (C11), 40.5 (C11'), 41.4 (C14), 42.2 (C14'), 49.1 (C5), 49.6 (C5'), 53.5 (C4), 55.0 (C4'), 125.8 (C16), 126.0 (C16'), 127.7 (C1), 129.3 (C1'), 135.2 (C15), 136.1 (C15'), 137.4 (C2), 139.8 (C2'), 167.7 (C3), 168.0 (C3'), 179.1 (C12), 179.2 (C12'), 179.9 (C13), 180.2 (C13'). υ_{max} (neat) 2940 2877 (C-H stretch), 1690 (C=O stretch). HRMS (ESI) calculated for $C_{36}H_{37}N_4O_6$ (MH^+) 621.2713 found 621.2728.

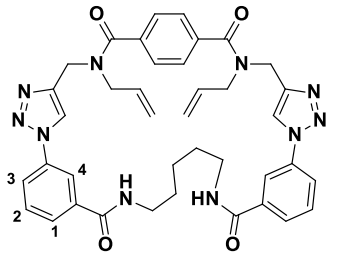
(+/-)-15



Macrocycle **8** (30 mg, 0.0487 mmol) was dissolved in 2:1 acetone:H_2O (1.5 mL). To the resulting solution was added NMO (45.6 mg, 0.39 mmol) and OsO_4 (2.5% solution in *tert*-BuOH, 100 µL) and the reaction was stirred at RT overnight. After this time the reaction was purified by HPLC (20-40% MeCN in H_2O) to furnish the title compound as a white crystalline solid (7.6 mg, 23%). mp >300 °C (H_2O/MeCN). δ_H (500 MHz, *d*$_6$-DMSO) 1.53 (1H, d, *J* = 13.0, $H9_a$), 1.77-1.83 (1H, m, $H9_b$), 1.95-2.01 (1H, m, $H9'_a$), 2.14-2.16 (1H, m, $H9'_b$), 2.44-2.47 (1H, m, H6), 2.65 (1H, br d, *J* = 12.0, H8), 2.74 (1H, d, *J* = 11.5, $H5_a$), 2.81-2.87 (1H, m, H6'), 3.04-3.11 (1H, m,

H10), 3.19-3.52 (7H, m, $H4_a$, $H5_b$, $H5'_{a+b}$, H10', H11, H11'), 3.71-3.75 (3H, m, $H4_b$, $H4'_a$, H8'), 4.15 (1H, d, J = 12.0, $H4'_b$), 4.34 (1H, d, J = 16.0, $H14_a$), 4.41 (1H, d, J = 14.0, $H14'_a$), 4.66 (1H, d, J = 16.0, $H14_b$), 4.72 (1H, d, J = 14.0, $H14'_b$), 4.92 (2H, br s, OH), 5.25 (2H, br s, OH), 7.06 (2H, d, J = 8.0, H16), 7.34-7.38 (4H, m, H1, H16'), 7.67 (2H, d, J = 8.0, H1'). δ_C (125 MHz, d_6-DMSO) two resonances are observed for each signal due to the dynamic nature of the molecule; 24.1 (C9), 29.6 (C9'), 36.0 (C10), 37.2 (C10'), 38.9 (C11), 39.4 (C11'), 41.4 (C14), 42.5 (C6), 42.6 (C14'), 43.8 (C6'), 48.1 (C4), 49.6 (C5), 54.6 (C4'), 61.2 (C5'), 65.4 (C8), 66.8 (C8'), 75.1 (C7), 79.6 (C7'), 125.7 (C1), 126.4 (C16), 128.0 (C1'), 129.0 (C16'), 135.2 (C15), 136.1 (C15'), 136.7 (C2), 139.9 (C2'), 167.8 (C3), 168.0 (C3'), 178.0 (C12), 178.9 (C12'), 179.5 (C13), 180.0 (C13'). υ_{max} (neat) 3352 (O-H stretch), 1694 (C=O stretch). HRMS (ESI) calculated for $C_{36}H_{37}N_4O_{10}$ (MH^+) 685.2510 found 685.2502.

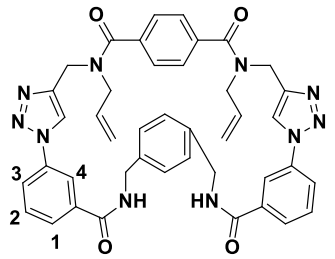
16



Bis-eneyne **1b** (40.4 mg, 0.12 mmol) and *bis*-azide **4ai** (50 mg, 0.12 mmol) were dissolved in THF (3.6 mL). CuI (48 mg, 0.25 mmol) and DIPEA (65 mg, 88 µL, 0.51 mmol) were added and the reaction was heated to 80 °C overnight in a sealed tube. After this time the reaction was concentrated *in vacuo* and the resulting residue was purified by flash chromatography on silica (stepped gradient; 3-4-5% MeOH in CH_2Cl_2) to furnish the title compound as a white amorphous solid (19.2 mg, 22.5 %). R_f = 0.47 (1:9 MeOH:CH_2Cl_2). mp 293-295 °C (decomposition) (DMSO; freeze dried). δ_H (500 MHz, d_6-DMSO, 90 °C) 1.42 (2H, quint, J = 7.5, $C\underline{H}_2CH_2CH_2N$), 1.65 (4H, quint, J = 7.0, $CH_2C\underline{H}_2CH_2N$), 3.35 (4H, q, J = 6.5, $CH_2CH_2C\underline{H}_2N$), 4.06 (4H, br s, $C\underline{H}_2CH{=}CH_2$), 4.68 (4H, br s, $NC\underline{H}_2$triazole), 5.21-5.24 (4H, m, $CH_2CH{=}C\underline{H}_2$), 5.85-5.93 (2H, m, $CH_2C\underline{H}{=}CH_2$), 7.57 (4H, s, 4 x ArH), 7.64 (2H, t, J = 8.0, H2), 7.91 (2H, d, J = 7.5, H1/3), 8.00 (2H, d, J = 8.0 Hz, H1/3) 8.23 (2H, t, J = 2.0 Hz, H4), 8.35 (2H, br s, N$\underline{H}$), 8.60 (2H, s, triazole$\underline{H}$). δ_C (125 MHz, d_6-DMSO, 90 °C)

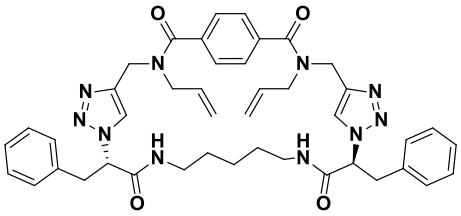
(two resonances are absent from the spectrum corresponding to 2 x NCH_2) 23.1 (CH_2) 28.2 (CH_2), 38.7 (NCH_2), 117.0 (=CH_2), 118.8 (ArCH), 120.9 (ArCH), 122.4 (ArCH), 126.1 (ArCH), 126.7 (ArCH), 129.2 (ArCH), 132.7 (=CH), 136.1 (ArC), 136.2 (ArC), 136.8 (ArC), 144.2 (ArC), 164.7 (C=O), 169.6 (C=O). υ_{max} (neat) 3302 (N-H stretch), 1633 (C=O stretch) 1588 (C=C stretch). HRMS (ESI) calculated for $C_{39}H_{40}N_{10}O_4Na$ (MNa^+) 735.3132 found 735.3102

17



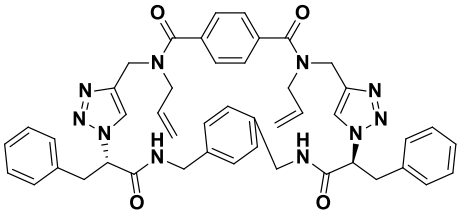
Bis-enyne **1b** (30 mg, 0.070 mmol) and *bis*-azide **4bi** (22.5 mg, 0.070 mmol) were dissolved in THF (2.1 mL). CuI (26 mg, 0.141 mmol) and DIPEA (36 mg, 49 μL, 0.282 mmol) were added and the reaction was heated to 80 °C overnight in a sealed tube. After this time the reaction was concentrated *in vacuo* and the resulting residue was purified by flash chromatography on silica (stepped gradient; 2-3-4-5 % MeOH in CH_2Cl_2) to furnish the title compound as a white amorphous solid (3.7 mg, 7%). R_f = 0.52 (1:9 MeOH:CH_2Cl_2). mp 185-186 °C (MeOH). δ_H (500 MHZ, *d*$_6$-DMSO, 90 °C) 4.03 (4H, br s, C$\underline{H}_2$CH=CH_2), 4.52 (4H, d, *J* = 6.0, ArC$\underline{H}_2$NH), 4.66 (4H, s, C$\underline{H}_2$triazole), 5.19-5.22 (4H, m , CH_2CH=C$\underline{H}_2$), 5.84-5.91 (2H, m, CH_2C$\underline{H}$=CH_2), 7.34 (4H, s, Ar$\underline{H}$), 7.54 (4H, s, Ar$\underline{H}$), 7.69 (2H, t, *J* = 8.0 H2), 8.01-8.04 (4H, m, H1+H3), 8.32 (2H, s, H4), 8.67 (2H, s, triazole$\underline{H}$), 8.98 (2H, br t, *J* = 5.5, N$\underline{H}$). δ_C (125 MHz, *d*$_6$-DMSO, 90 °C) (two resonances are absent from the spectrum, corresponding to 2 x NCH_2) 43.3 (CH_2), 118.0 (=CH_2), 119.7 (ArCH), 122.1 (ArCH), 123.4 (ArCH), 127.1 (ArCH), 128.0 (ArCH), 128.0 (ArCH), 130.3 (ArCH), 133.7 (=CH), 136.7 (ArC), 137.3 (ArC), 137.7 (ArC), 138.6 (ArC), 145.1 (ArC), 165.6 (C=O), 170.6 (C=O). υ_{max} (thin film CH_2Cl_2) 3337 (N-H stretch), 1639 (C=O stretch), 1542 (C=C stretch). HRMS (ESI) calculated for $C_{42}H_{39}N_{10}O_4$ (MH^+) 747.3156 found 747.3184.

18



Bis-enyne **1b** (35.7 mg, 0.112 mmol) and *bis*-azide **4aii** (50 mg, 0.112 mmol) were dissolved in THF (2.24 mL). CuI (42.4 mg, 0.223 mmol) and DIPEA (57.5 mg, 77.6 μL, 0.446 mmol) were added and the reaction was heated to 60 °C overnight in a sealed tube. After this time the reaction was concentrated *in vacuo* and the resulting residue was purified by flash chromatography on silica (stepped gradient; 1-2-3-4-5% MeOH in CH_2Cl_2) to furnish the title compound as a white amorphous solid (14.7 mg, 17%). R_f = 0.57 (1:9 MeOH:CH_2Cl_2). mp 158-159 °C (MeOH). $[\alpha]_D^{25}$ +12.5 (c = 0.2, DMSO). δ_H (500 MHZ, *d*$_6$-DMSO, 90 °C) 1.16 (2H, quint, *J* = 7.0, C$\underline{H}_2$CH$_2$CH$_2$N) 1.29-1.37 (4H, m, CH$_2$C$\underline{H}_2$CH$_2$N), 2.88-2.94 (2H, m, CH$_2$CH$_2$C$\underline{H}_2$N, H_a), 3.07-3.18 (2H, m, CH$_2$CH$_2$C$\underline{H}_2$N, H_b), 3.37 (2H, dd, *J* = 14.5, 9.0, ArC$\underline{H}_2$CHN, H_a), 3.44 (2H, dd, *J* = 14.0, 7.0, ArC$\underline{H}_2$CHN, H_b), 3.97 (4H, br s, NC$\underline{H}_2$CH=CH$_2$), 4.45 (2H, d, *J* = 15.5, NC$\underline{H}_2$triazole, H_a), 4.53 (2H, br d, *J* = 17.0, NC$\underline{H}_2$triazole, H_b), 5.12-5.16 (4H, m, CH$_2$CH=C$\underline{H}_2$), 5.48 (2H, dd, *J* = 8.5, 6.5, ArCH$_2$C$\underline{H}$N), 5.58-5.83 (2H, m, CH$_2$C$\underline{H}$=CH$_2$), 7.16-7.25 (10H, m, CH$_2$Ar$\underline{H}$), 7.37 (4H, s, $\underline{H}$ArC=O), 7.93 (2H, s, triazole$\underline{H}$), 8.00 (2H, br t, N$\underline{H}$). δ_C (125 MHZ, *d*$_6$-DMSO, 90 °C) (One resonance is absent from the spectrum, corresponding to NCH$_2$) 23.6 (CH$_2$), 28.6 (CH$_2$), 38.0 (NCH$_2$), 39.1 (NCH$_2$), 53.0 (CH$_2$), 64.8 (CH), 117.8 (=CH$_2$), 122.8 (ArCH), 126.9 (ArCH), 127.1 (ArCH) 128.6 (ArCH), 129.3 (ArCH), 133.7 (=CH), 136.9 (ArC), 137.8 (ArC), 143.7 (ArC), 167.5 (C=O), 170.5 (C=O). υ_{max} (thin film EtOH) 3282 (N-H stretch), 1677 (C=O stretch), 1629 (C=O stretch), 1555 (C=C stretch). HRMS (ESI) calculated for $C_{43}H_{49}N_{10}O_4$ (MH^+) 769.3938 found 769.3944.

19



Bis-enyne **1b** (19.9 mg, 0.06 mmol) *bis*-azide **4bii** (30 mg, 0.06 mmol) were dissolved in THF (1.8 mL). CuI (23.7 mg, 0.12 mmol) and DIPEA (32 mg, 43 µL, 0.25 mmol) were added and the reaction was heated to 80 °C overnight in a sealed tube. After this time the reaction was concentrated *in vacuo* and the resulting residue was purified by flash chromatography on silica (stepped gradient; 2-3-4-5% MeOH in CH_2Cl_2) to furnish the title compound as a white amorphous solid (10.1 mg, 21%). R_f = 0.54 (1:9 MeOH:CH_2Cl_2). mp 260-265 °C (decomposition) (d_6-DMSO; freeze dried). $[\alpha]_D^{25}$ +63.0 (c = 0.1, DMSO). δ_H (500 MHz, d_6-DMSO, 90 °C) 3.41 (2H, dd, *J* = 14.0, 9.5 ArC$\underline{H}_2$CHN, H_a), 3.49 (2H, dd, *J* = 14.0, 6.0, ArC$\underline{H}_2$CHN, H_a), 3.89 (4H, br s, C$\underline{H}_2$CH=CH_2), 4.01 (2H, dd, *J* = 10.0, 5.0, ArC$\underline{H}_2$N H_b), 4.45-4.49 (6H, m, CH_2triazole, ArC$\underline{H}_2$N, H_b), 5.08-5.14 (4H, m, CH_2CH=C$\underline{H}_2$), 5.57 (2H, dd, *J* = 9.5, 6.5, ArCH_2C$\underline{H}$N), 5.73-5.81 (2H, m, CH_2C$\underline{H}$=CH_2), 7.08 (4H, s, ArH), 7.15-7.25 (10H, m, CH_2Ar$\underline{H}$), 7.46 (4H, s, Ar$\underline{H}$), 7.97 (2H, s, triazoleH), 8.55 (2H, br t, *J* = 6.0 N$\underline{H}$). δ_C (125 MHz, d_6-DMSO, 90 °C) (One CH_2 resonance is absent from the spectrum, corresponding to NCH_2) 38.2 (CH_2), 43.0 (CH_2), 54.3 (CH_2), 64.7 (CH), 117.8 (=CH_2), 123.5 (ArCH), 127.1 (ArCH), 127.2 (ArCH), 128.7 (ArCH), 129.3 (ArCH), 132.5 (ArC), 133.6 (=CH), 136.8 (ArC), 138.0 (ArC), 141.7 (ArC), 167.7 (C=O), 170.4 (C=O). υ_{max} (neat) 3282 (N-H stretch), 1664 (C=O stretch), 1615 (C=O stretch), 1549 (C=C stretch). HRMS (ESI) calculated for $C_{46}H_{47}N_{10}O_4$ (MH^+) 803.3782 found 803.3755.

4. Creation of Diversity Plot:

The 14 DOS compounds described in this work were combined with 656 drugs obtained from DrugBank with a molecular weight below 1000, as well as 95 macrocycles from the the same source and the same molecular weight cut-off and the additional requirement of having at least one ring containing at least 12 atoms. All following operations were performed in MOE 2008.10. Counterions and solvents of all structures were removed using the 'Wash' option and protonation states were assinged according to neutral pH. Subsequently, force field partial charges were assigned to all structures by checking the 'Calculate Forcefield Partial Charges' option in the 'Database Minimize' function. All 2D molecular descriptors available were calculated, followed by a Principal Components Analysis, requiring 100% variance to be explained in the resulting components. The first two principle components have been visualized in the main text, explaining 38.75% and 9.40% of variance (a total of 48.15%), respectively.

5. Additional Biological data and Biological Materials and Methods:

The library compounds screened in a phenotypic assay for their ability to arrest U2OS cells in mitosis using a high content screening approach (HCA). None of the compounds displayed an increase in mitotic cells; however, compound **19** showed clear signs of cytotoxicity, with the cells displaying condensed and fragmented nuclei: characteristic indicators of apoptosis (**Figure S1**).

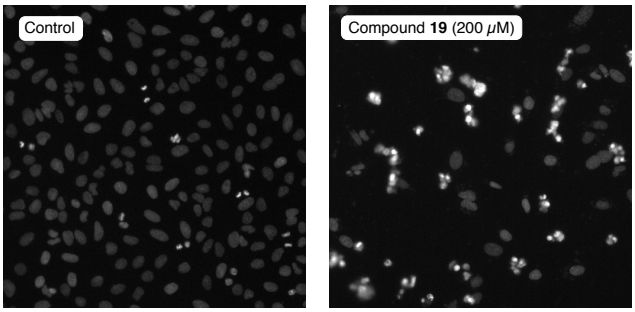


Figure S1 U2OS cells stained with Hoechst after 24 hours: left, control; right, treated with 200 μM **19**. Treated cells show characteristic signs of cell death by apoptosis

Reagents:

All compounds assayed were synthesized in our labs according to the procedures specified. Hoechst was purchased from Invitrogen (H3570) and used at 1:2500. Anti phospho-histone H3 (S10) was purchased from Abcam (ab5176) and used at 1:2000. AlexaFluor 488 goat anti-rabbit IgG was purchased from Invitrogen (A11034) and used at 1:500.

High Content Analysis:

HCA was performed using an Arrayscan II HCS reader and integrated software from Cellomics. U2OS osteosarcoma cells were seeded in a NUNC clear flat bottomed 96-well plate at 10,000/well in a total of 100 µL. They were incubated at 37 ºC overnight. Cells were then treated with compounds (25 µL) at a top concentration of 200 µM, diluting serially 1:2. Cells were then incubated at 37 ºC for 72 h. The medium was gently removed from all the wells and 50 µL 12.5 % formaldehyde was added to each well. This was incubated at RT for 10 min, before the formaldehyde was removed. To the wells was then added 100 µL/well permeabilization buffer (PB, contains PBS + 0.1% Triton X-100), incubating for 10 min. PB was removed and wells washed with 100 µL/well blocking buffer (BB, contains PBS + 1% BSA). BB was removed and 50 µL/well of primary antibody solution (anti-PH3 (S10), 1:800) was added. Plates were incubated for 1 h at room temperature. The antibody was removed and wells washed with 2 x 100 µL/well BB. BB was removed and 50 µL/well of secondary antibody solution containing Hoechst (1:2500) and AlexaFluor 546 Goat anti-mouse IgG (1:500) was added. Plates were incubated at RT for 1 h in the dark. Secondary antibody solution was removed and plates washed with 2 x 100 µL BB. The BB was then removed and 100 µL PBS/well were added. The plates were sealed with opaque film and images taken on a 20x 0.4 NA objective. Data was analysed on Cellomics Arrayscan software using the Target Activation v4 protocol. Critical output features are: ValidObjectCount and %Responder_AvgIntenCh2. IC50 data was calculated using Prism (Graphpad) and is an average of two independent experiments conducted in triplicate.

Sulforhodamine B colorimetric assay for cytotoxicity screening

This assay was performed according to the procedure of Vichai *et al.*[11]

U2OS cells were seeded at 4000 cells/well in 180 µL and A549 cells at 3000 cells/well and compounds were added after 24 h at a final top concentration of 200 µM from a dilution plate. After incubation for 72 h at 37 °C the medium was removed by aspiration, and 100 µL of 1% TCA solution was added. This was incubated for 1 h and then removed. The plates were washed 4 x with tap water and the plates were allowed to air dry at RT. 100 µL of a 0.057 % wt/vol solution of Sulforhodamine B were added to each well, incubating for 30 min. The plates were then washed quickly with 4 x 100 µL of 1% acetic acid solution and then airdried. 200 µL of 10 mM TRIS pH 10.5 was added to each well to resolubilise the dye. The plates were then read at 510 nm on a TECAN UV spectrophotometer. IC50 data was calculated using Prism (Graphpad) and is an average of two independent experiments conducted in triplicate.

6. References

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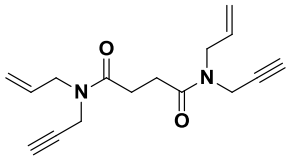
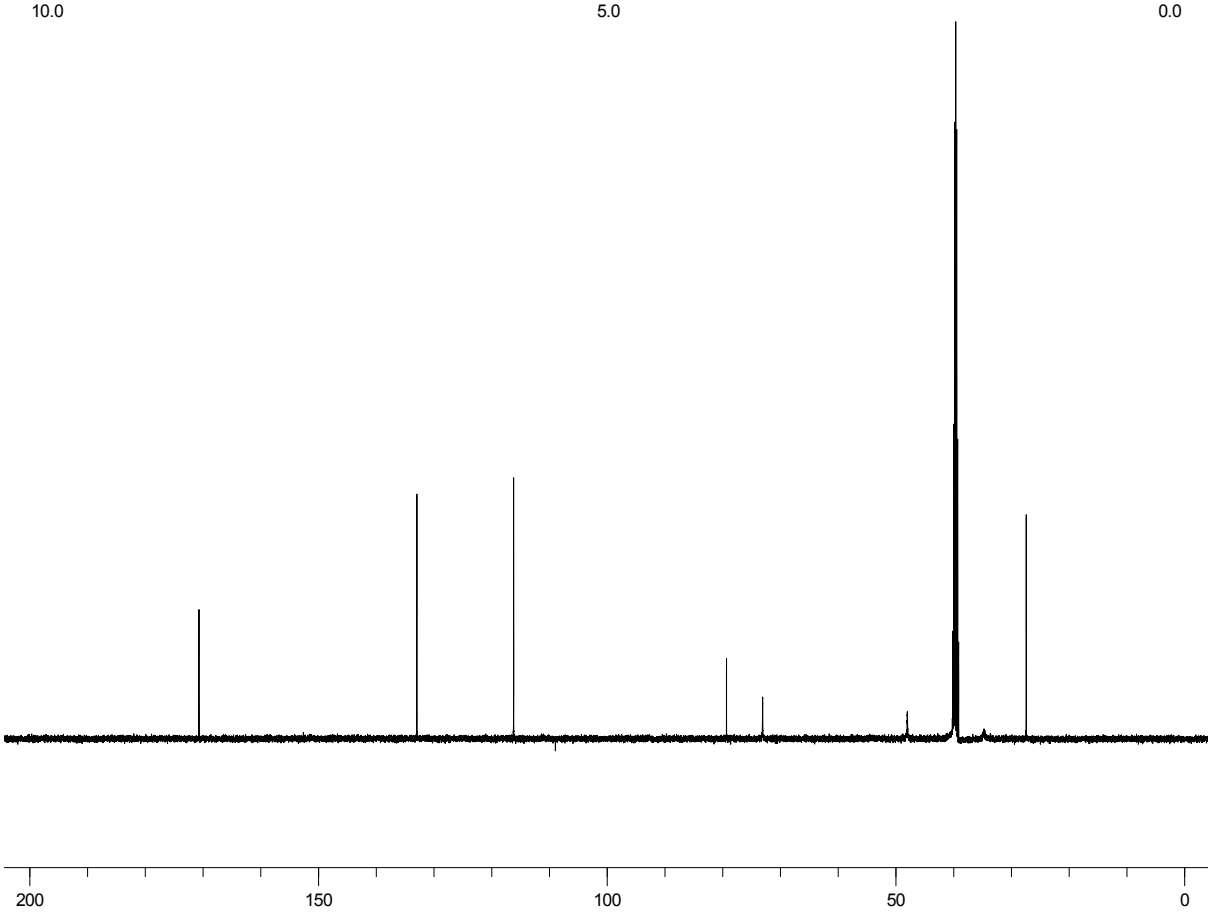
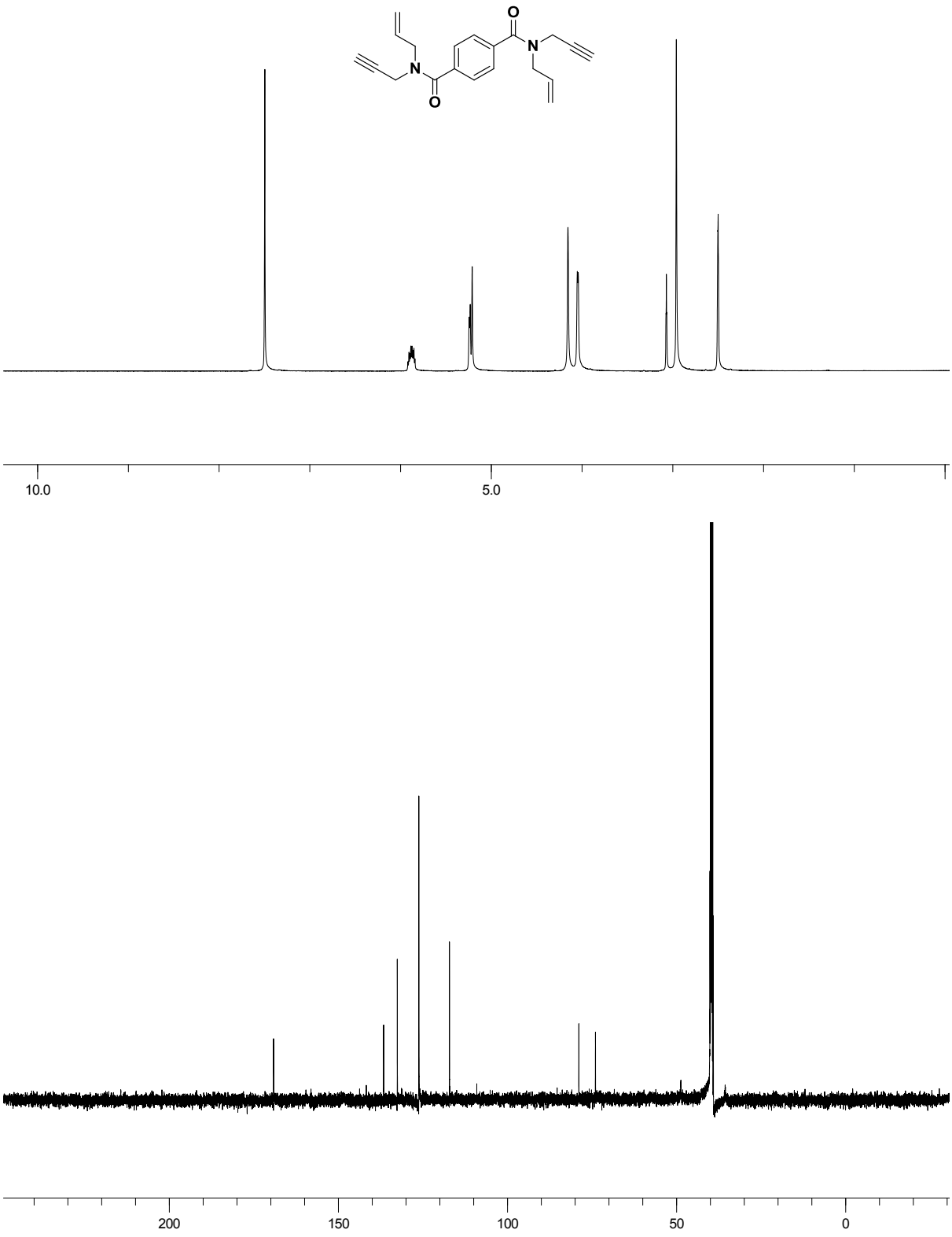
NMR Spectra of novel compounds

N^1,N^4-diallyl-N^1,N^4-di(prop-2-yn-1-yl)succinamide (1a)

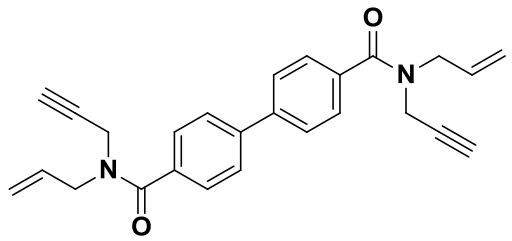


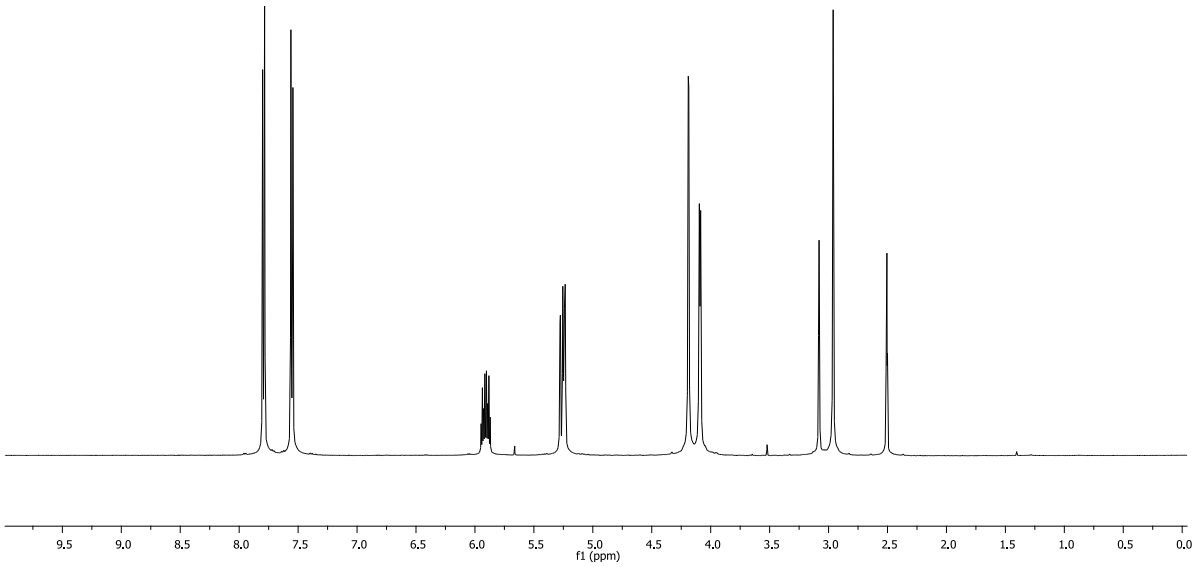


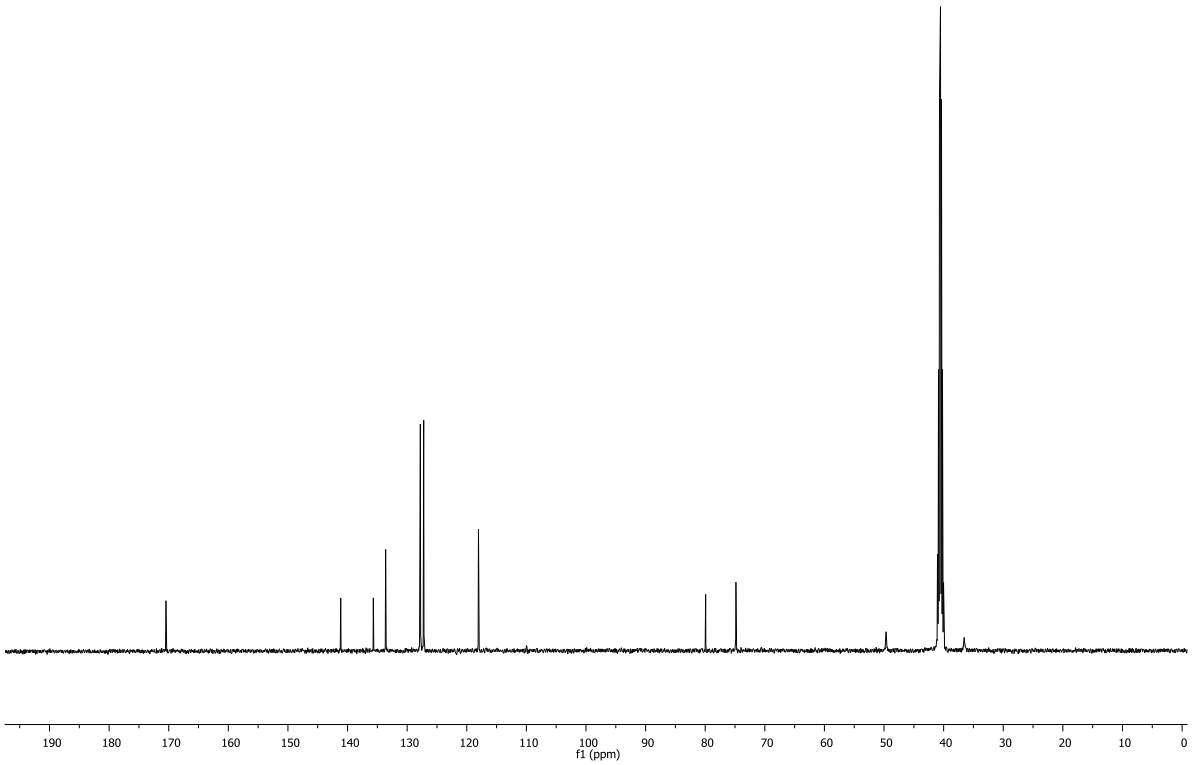
N^1,N^4-diallyl-N^1,N^4-di(prop-2-yn-1-yl)terephthalamide (1b)



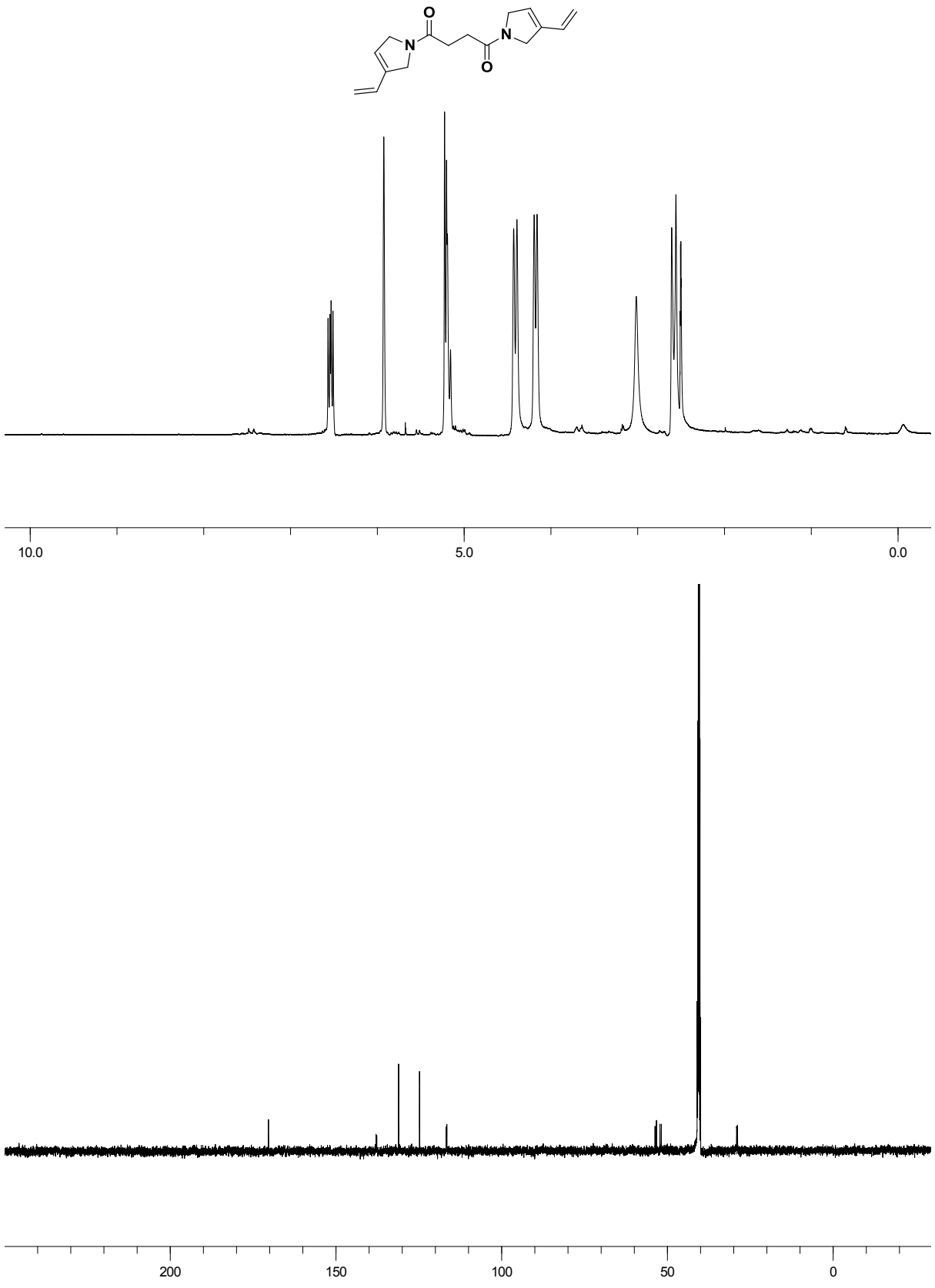
N^4,$N^{4'}$-diallyl-N^4,$N^{4'}$-di(prop-2-yn-1-yl)-[1,1'-biphenyl]-4,4'-dicarboxamide (1c)



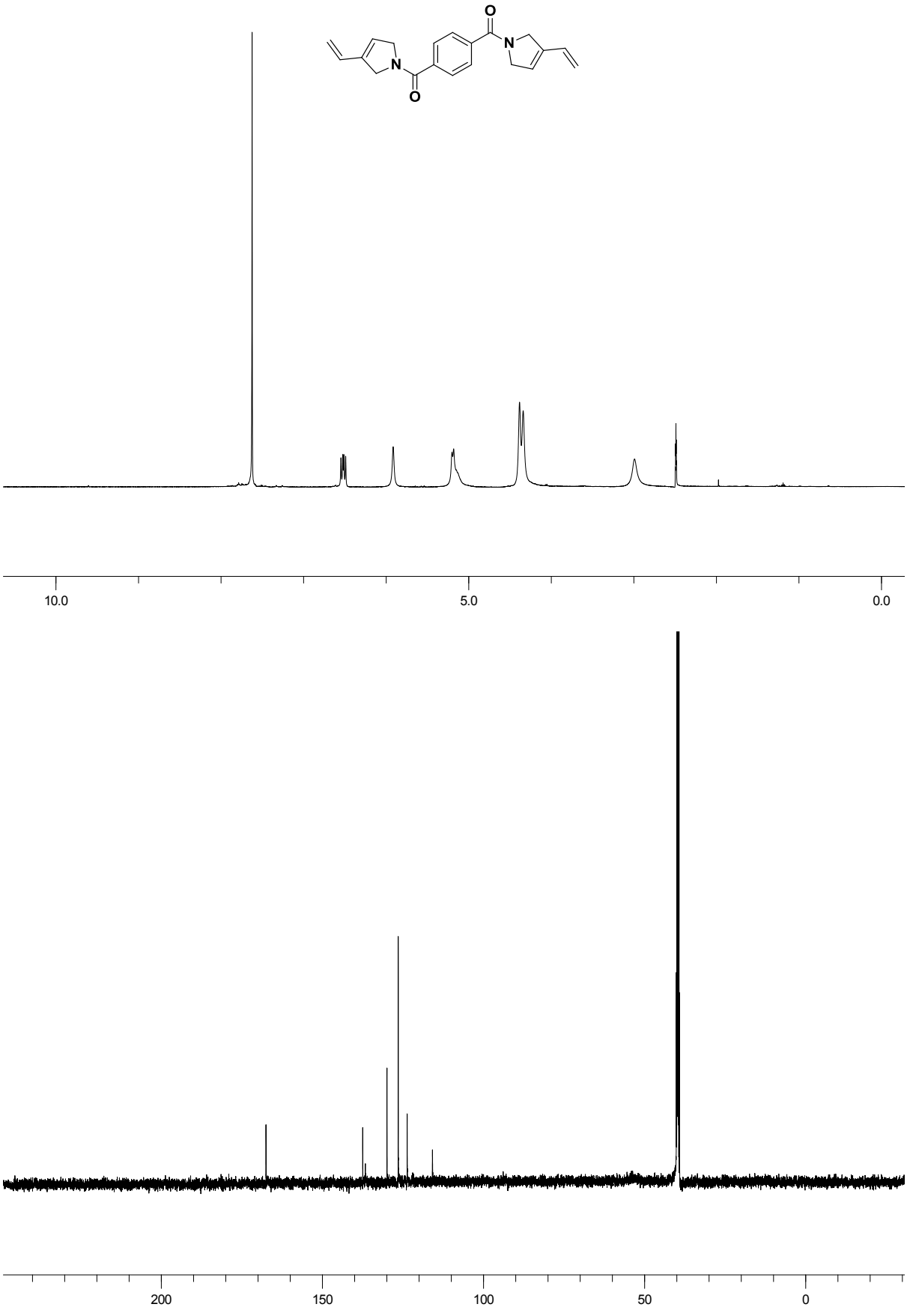




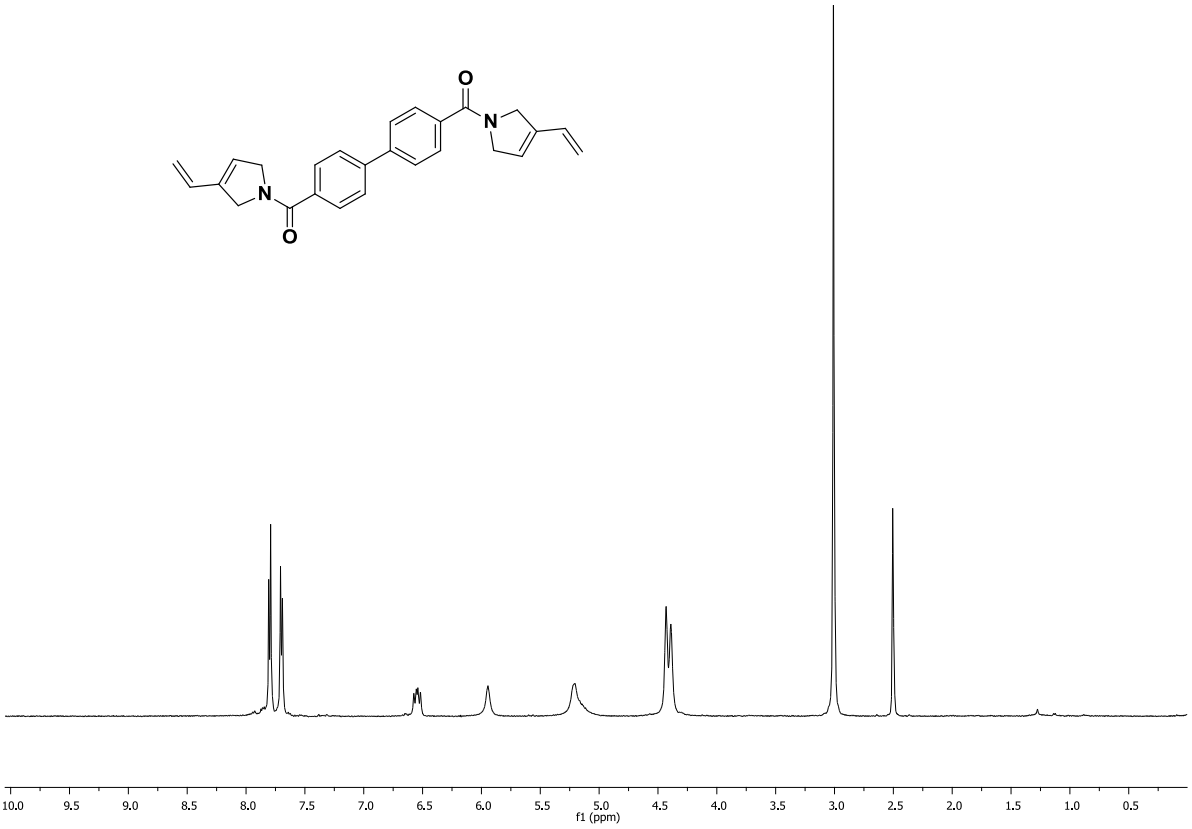
1,4-bis(3-vinyl-2,5-dihydro-1H-pyrrol-1-yl)butane-1,4-dione (2a)

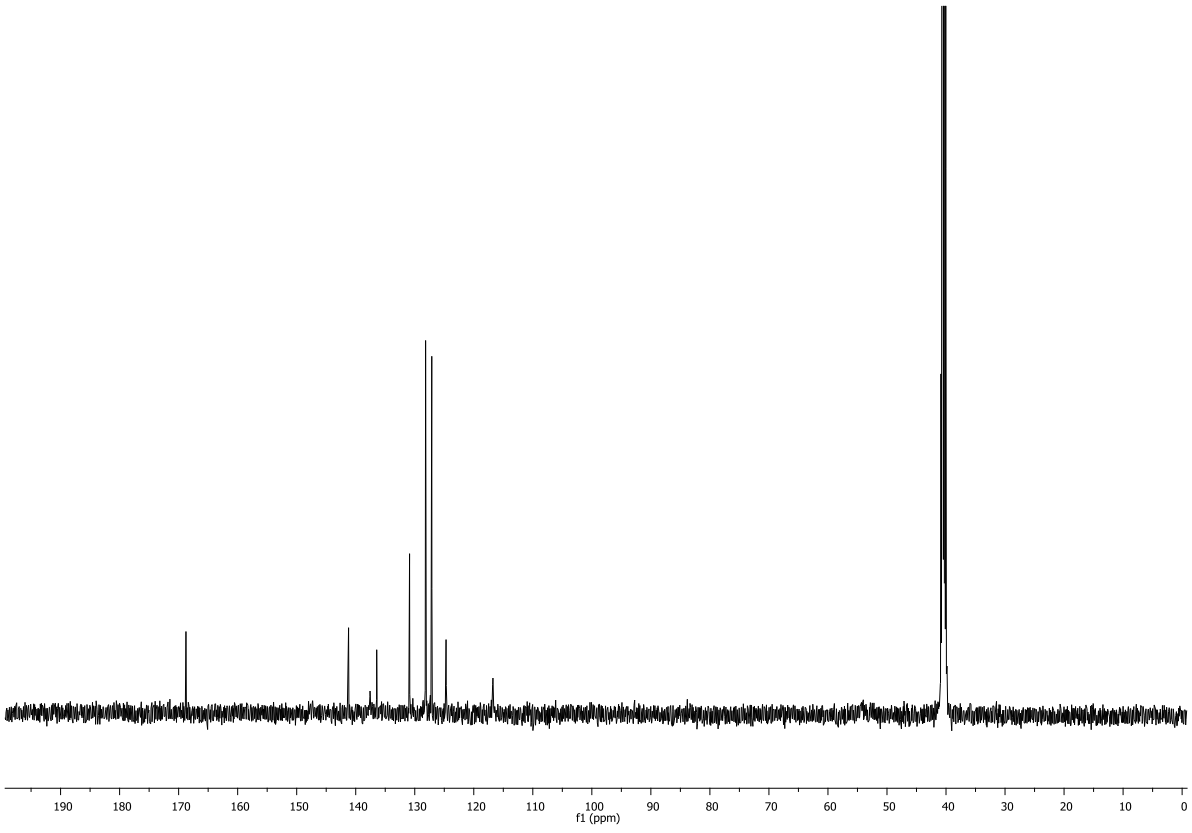


1,4-Phenylenebis((3-vinyl-2,5-dihydro-1H-pyrrol-1-yl)methanone) (2b)

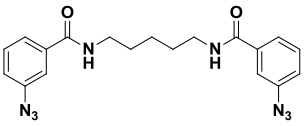


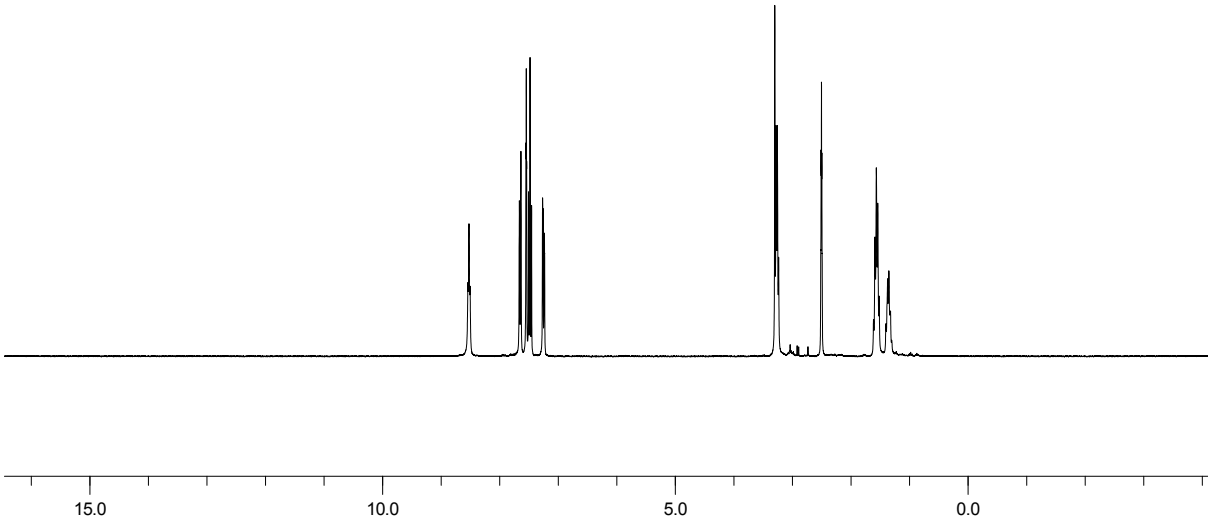
[1,1'-biphenyl]-4,4'-diylbis((3-vinyl-2,5-dihydro-1H-pyrrol-1-yl)methanone) (2c)

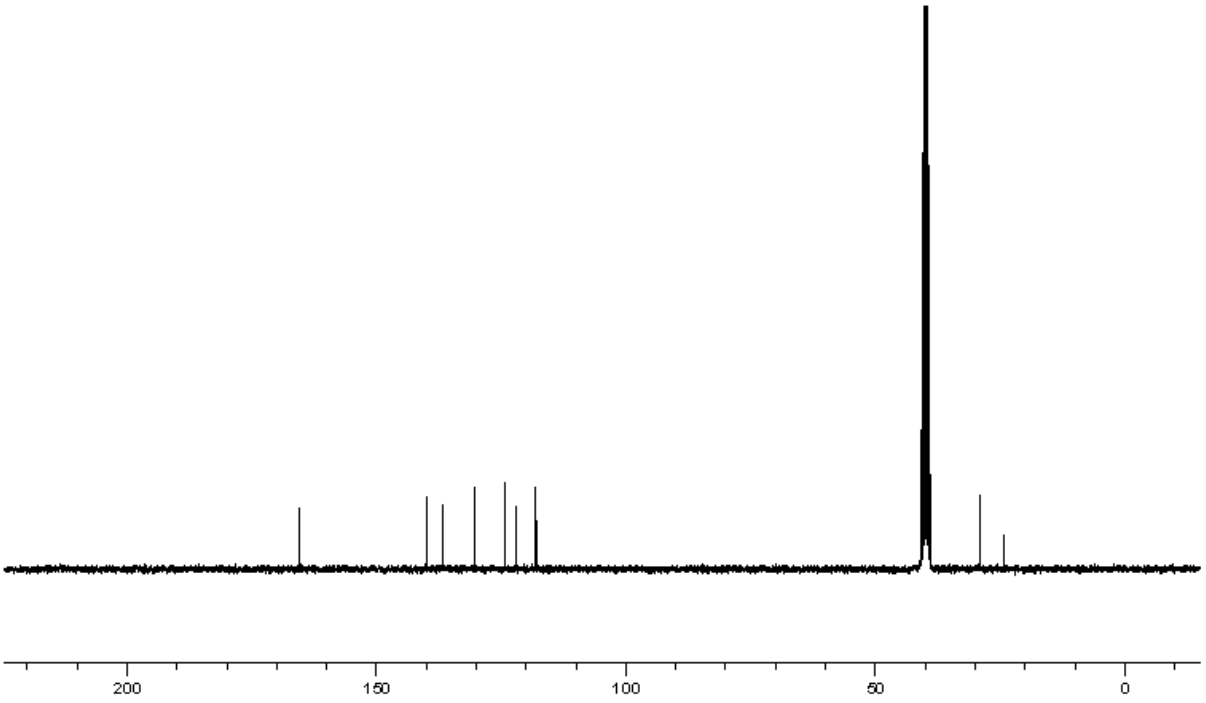




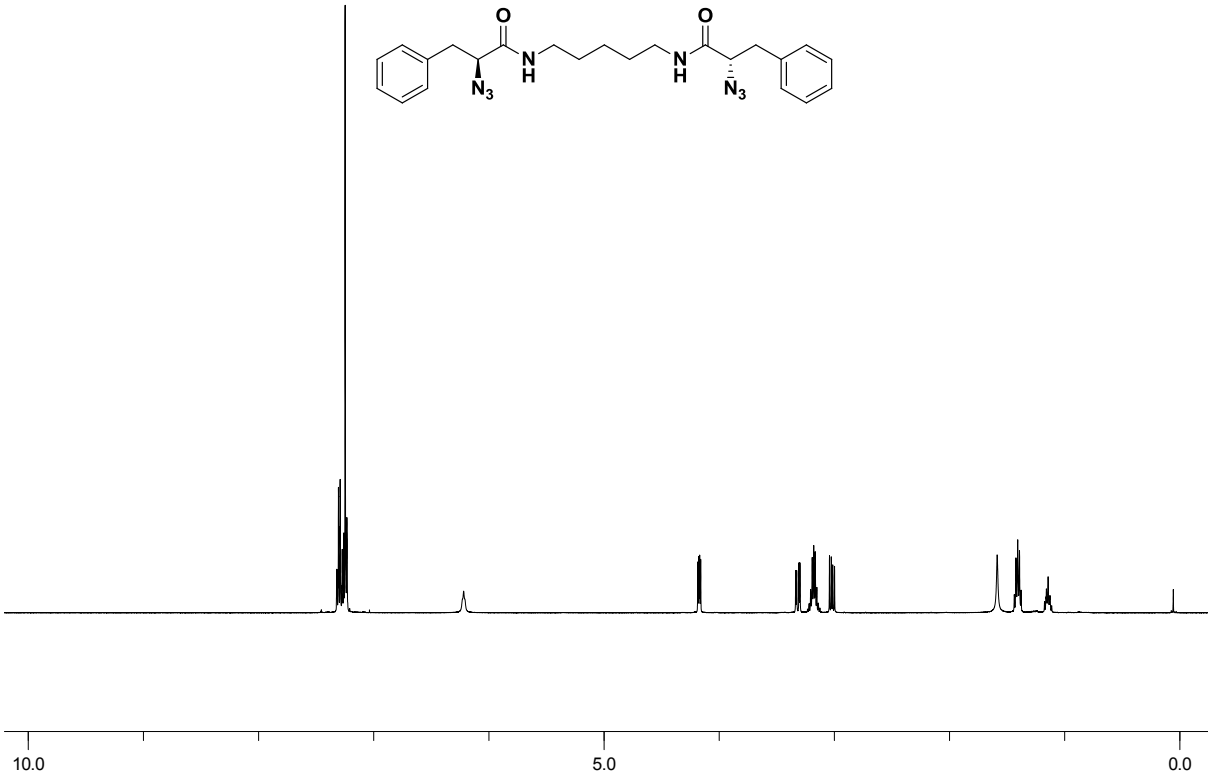
***N, N'*-(Pentane-1,5-diyl)bis(3-azidobenzamide) (4ai)**

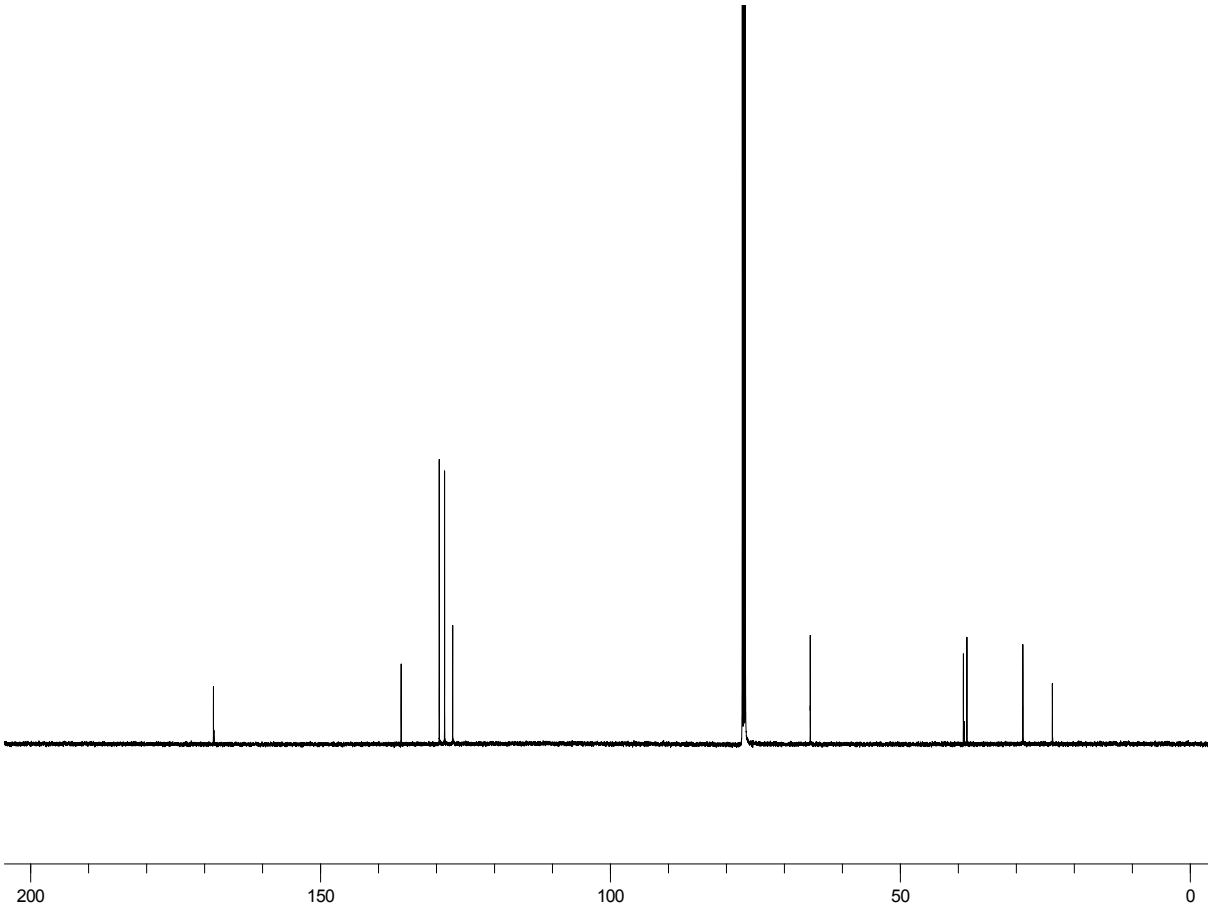




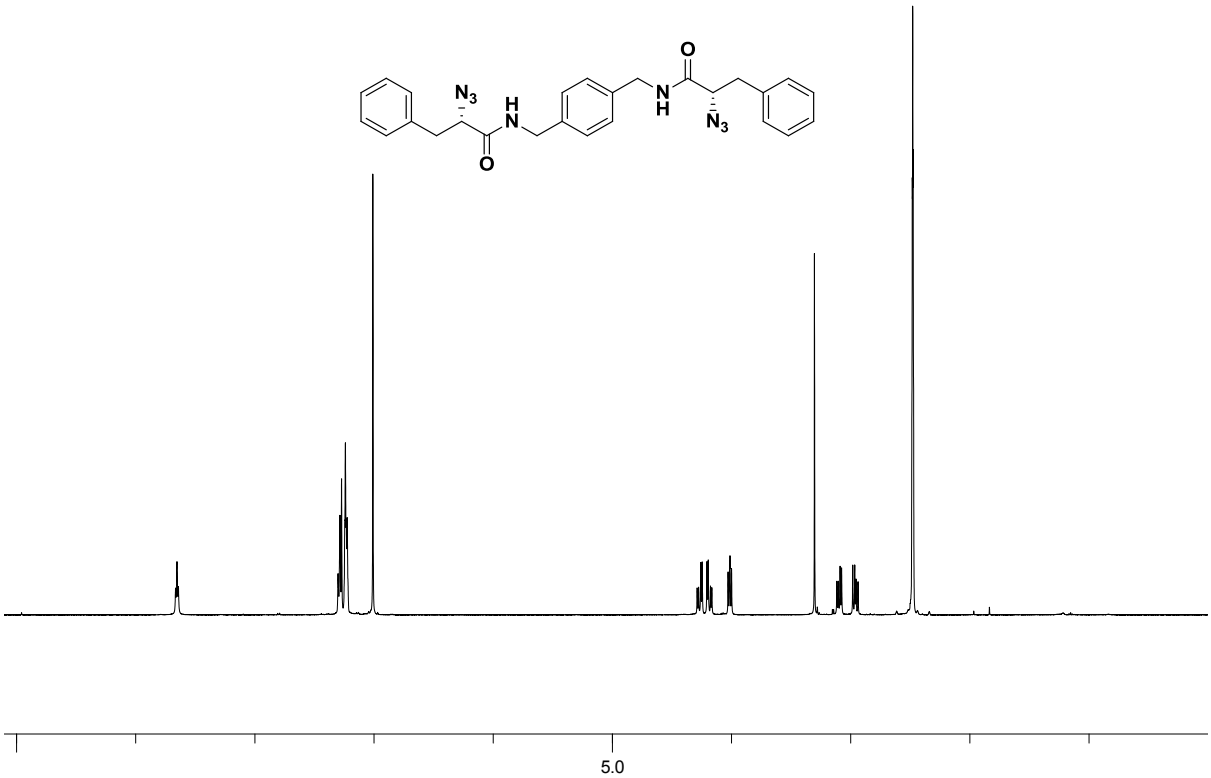


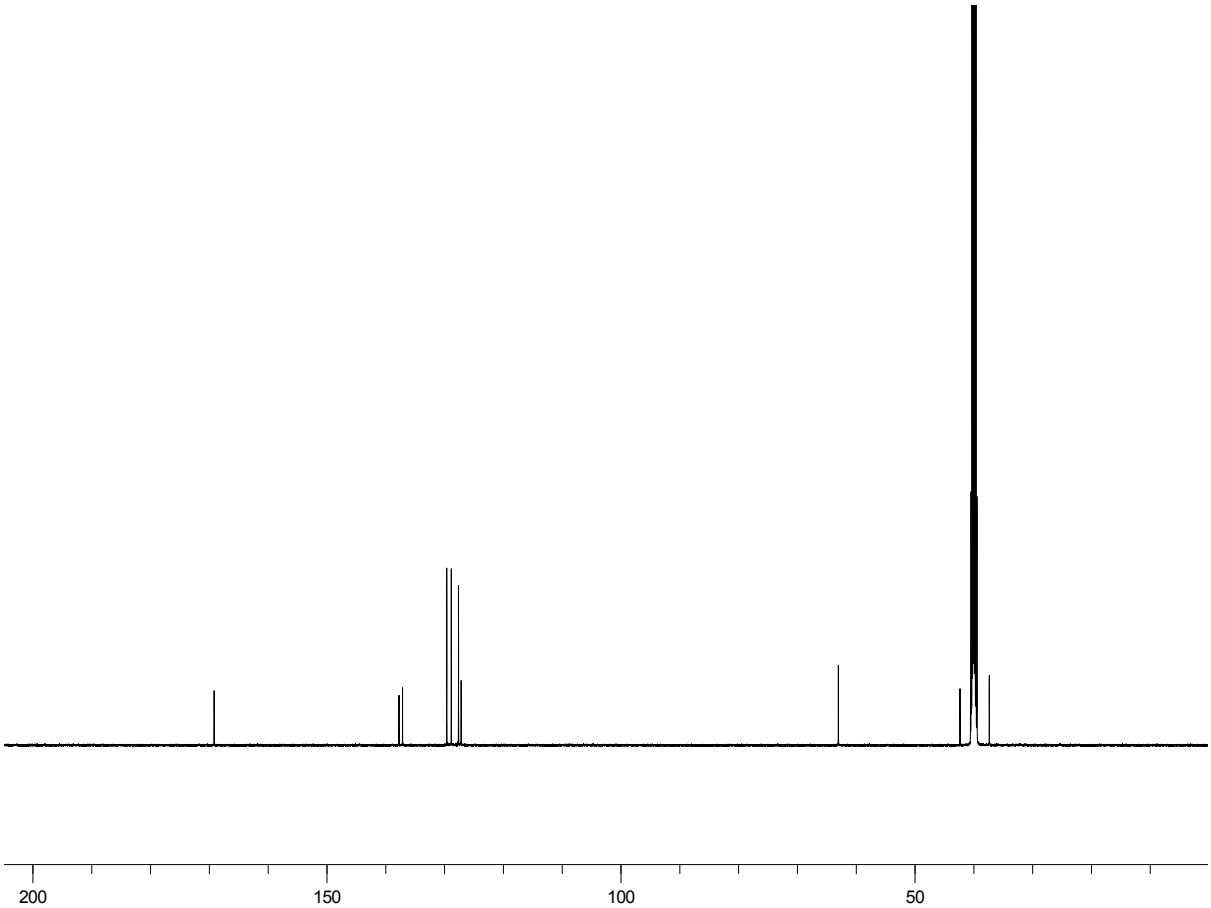
(2S, 2'S)-*N*, *N'*-(pentane-1,5-diyl)bis(2-azido-3-phenylpropanamide) (4aii)





(2S, 2'S)-*N*, *N*'-(1,4-phenylenebis(methylene))bis(2-azido-3-phenylpropanamide) (4bii)





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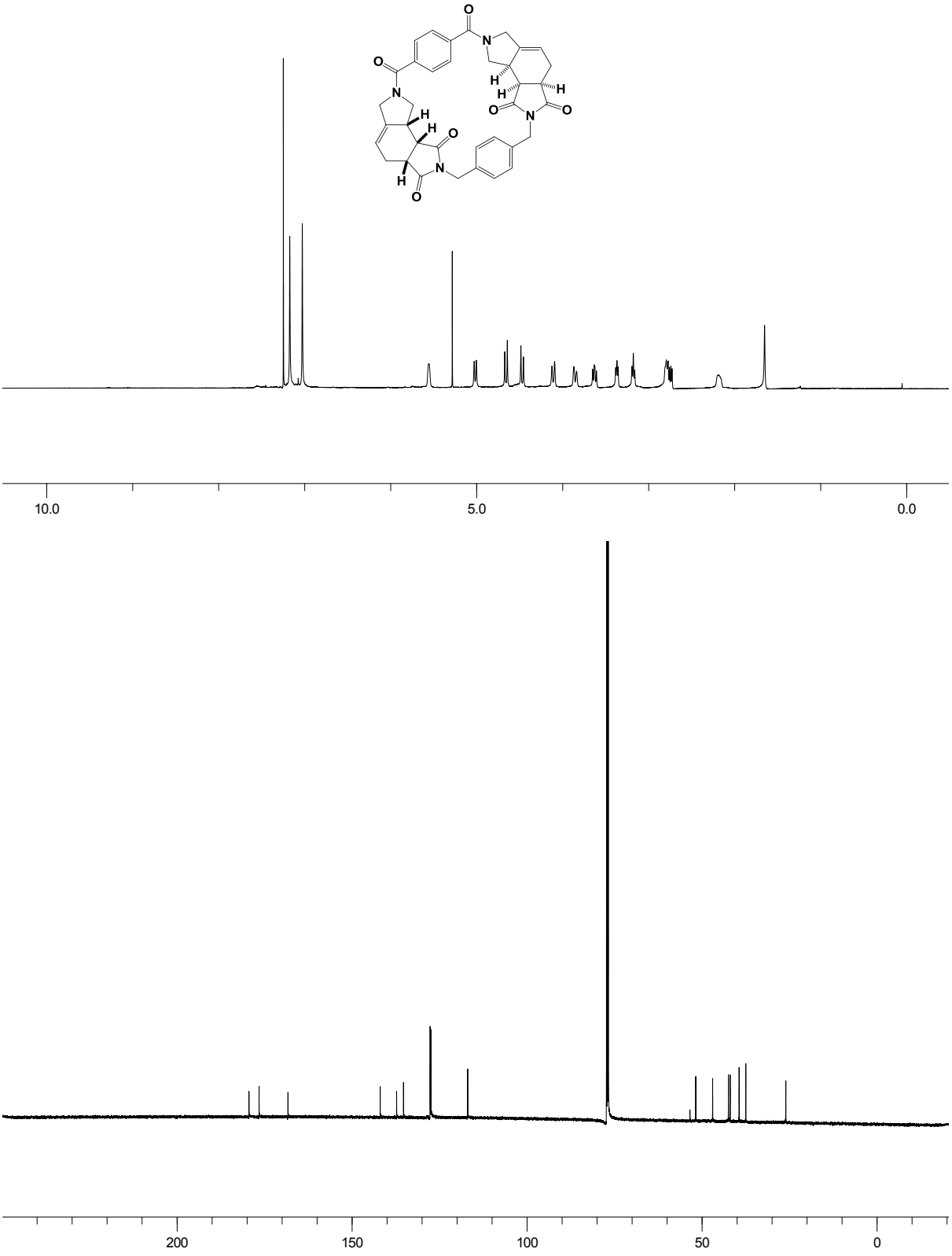
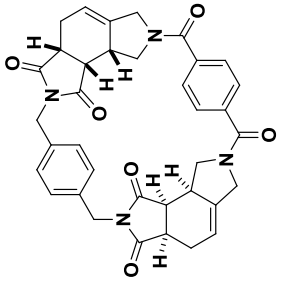
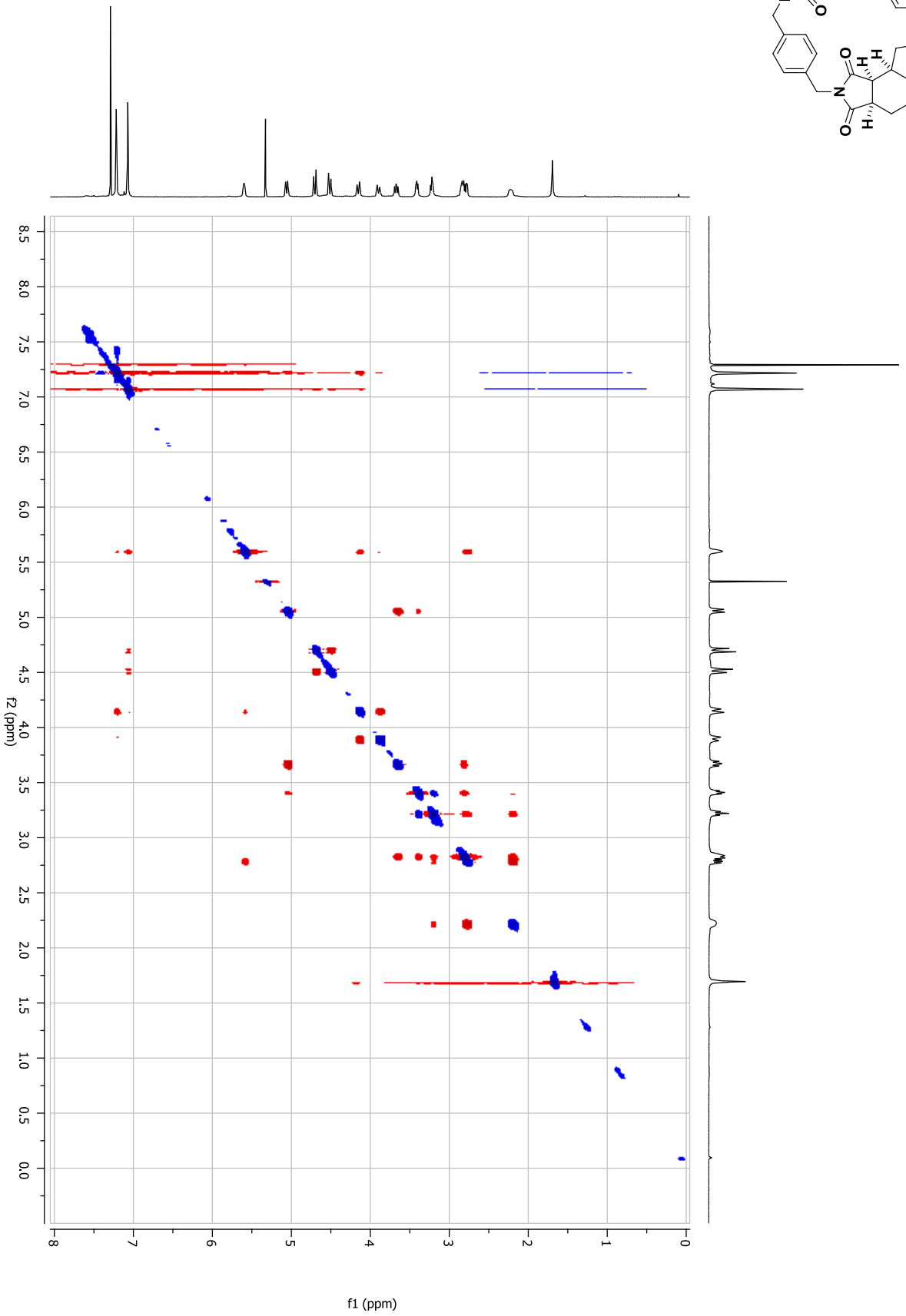


8-NOESY





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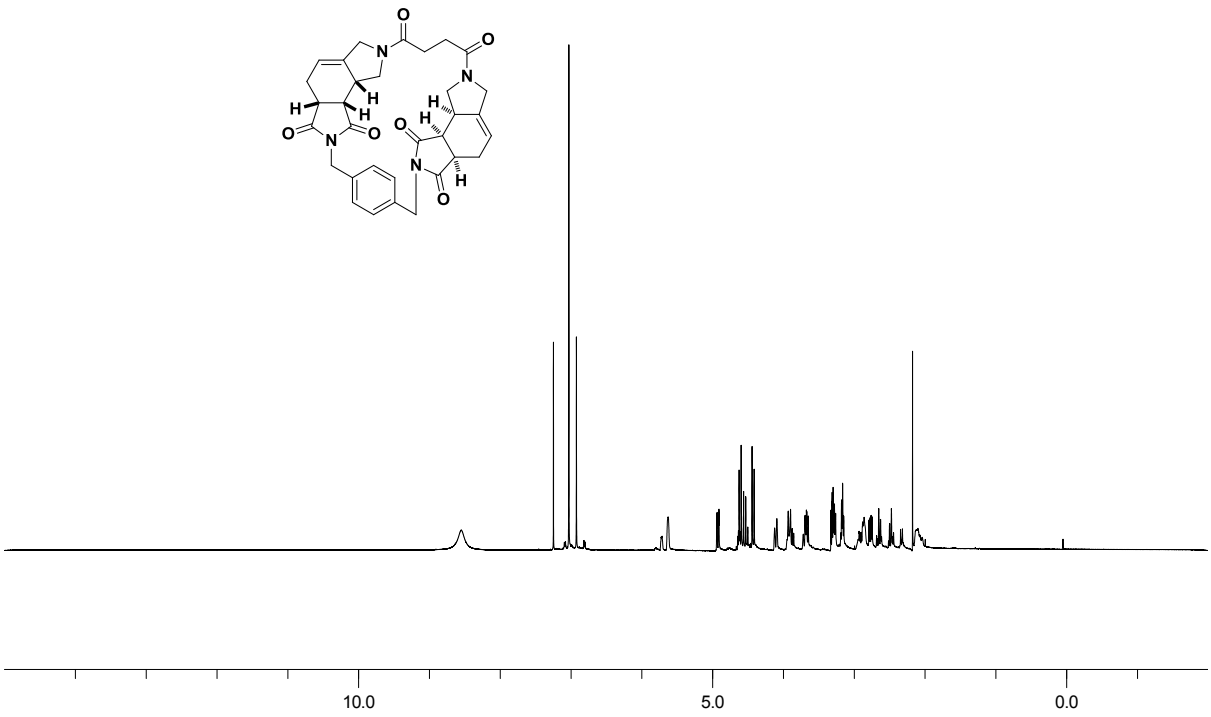
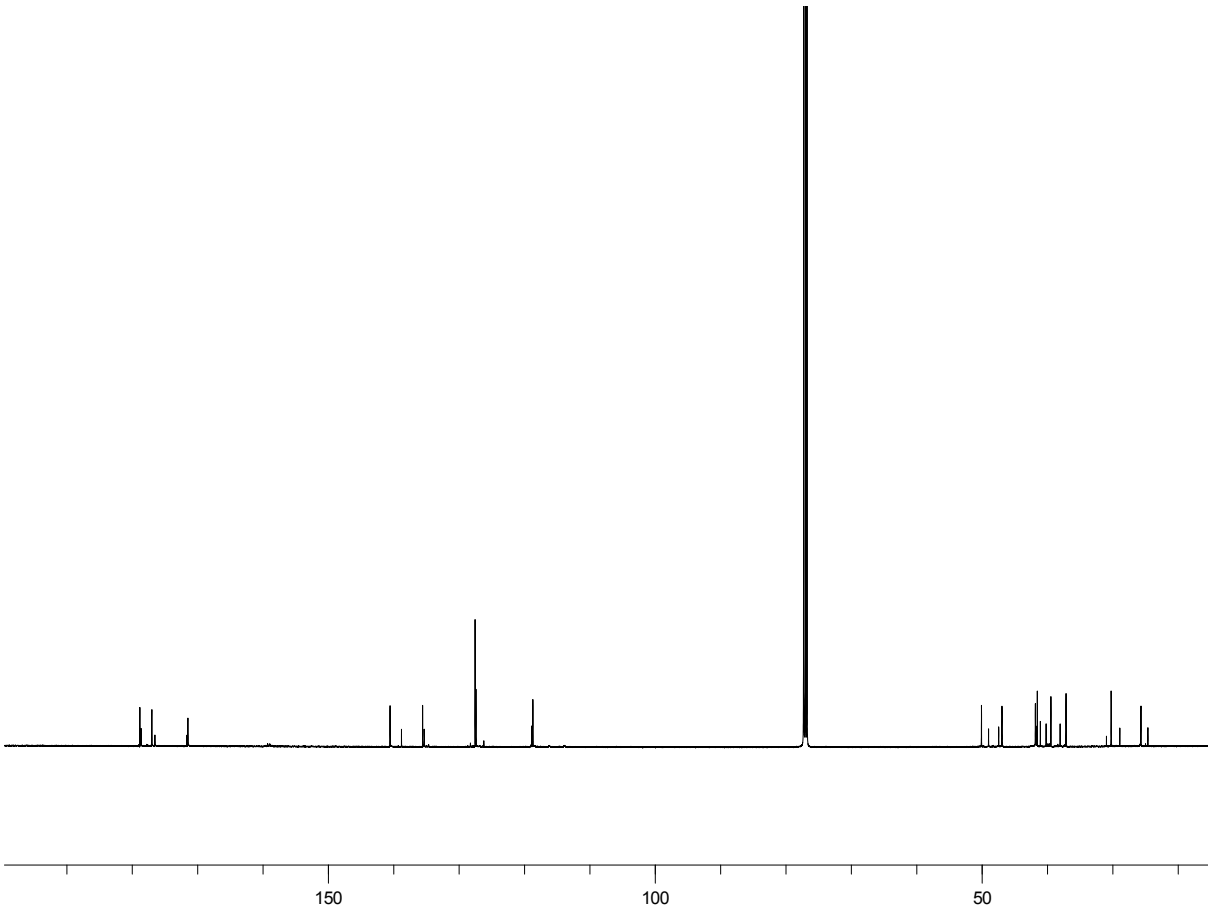
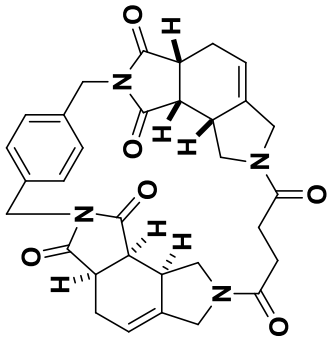
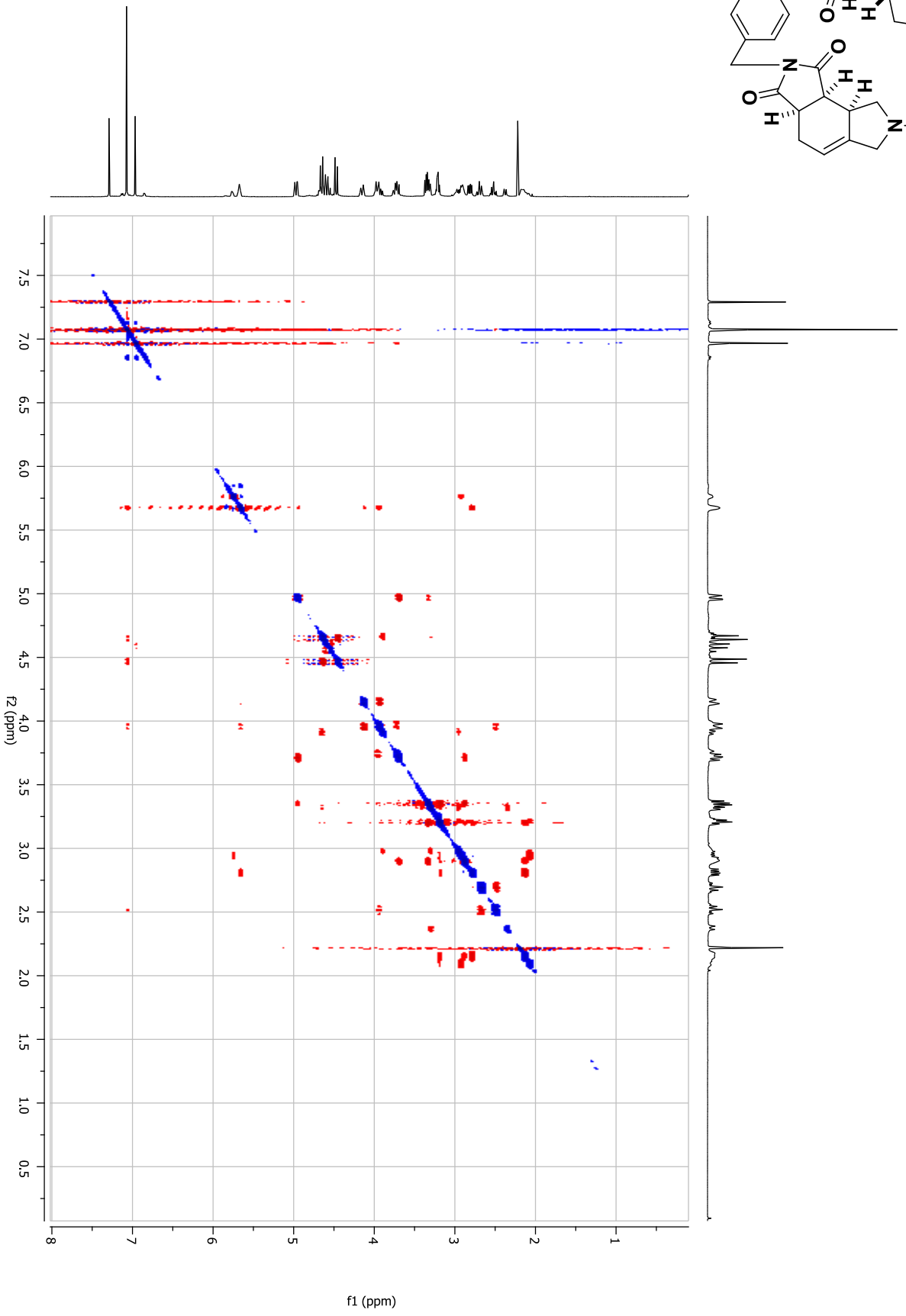


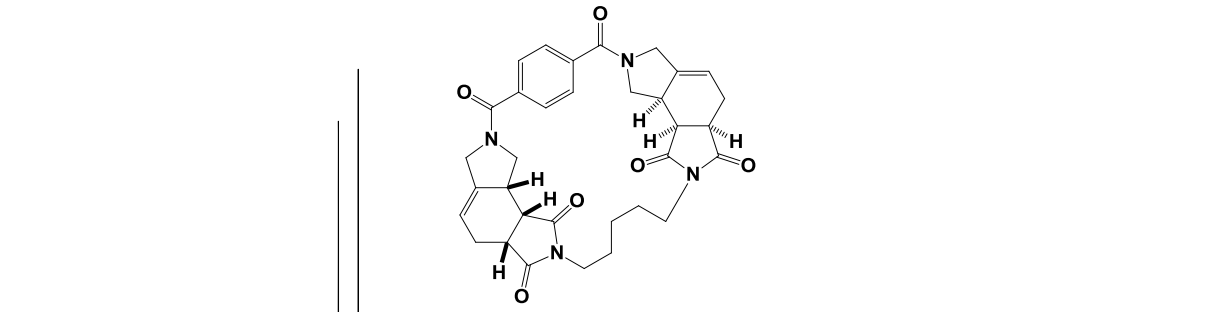


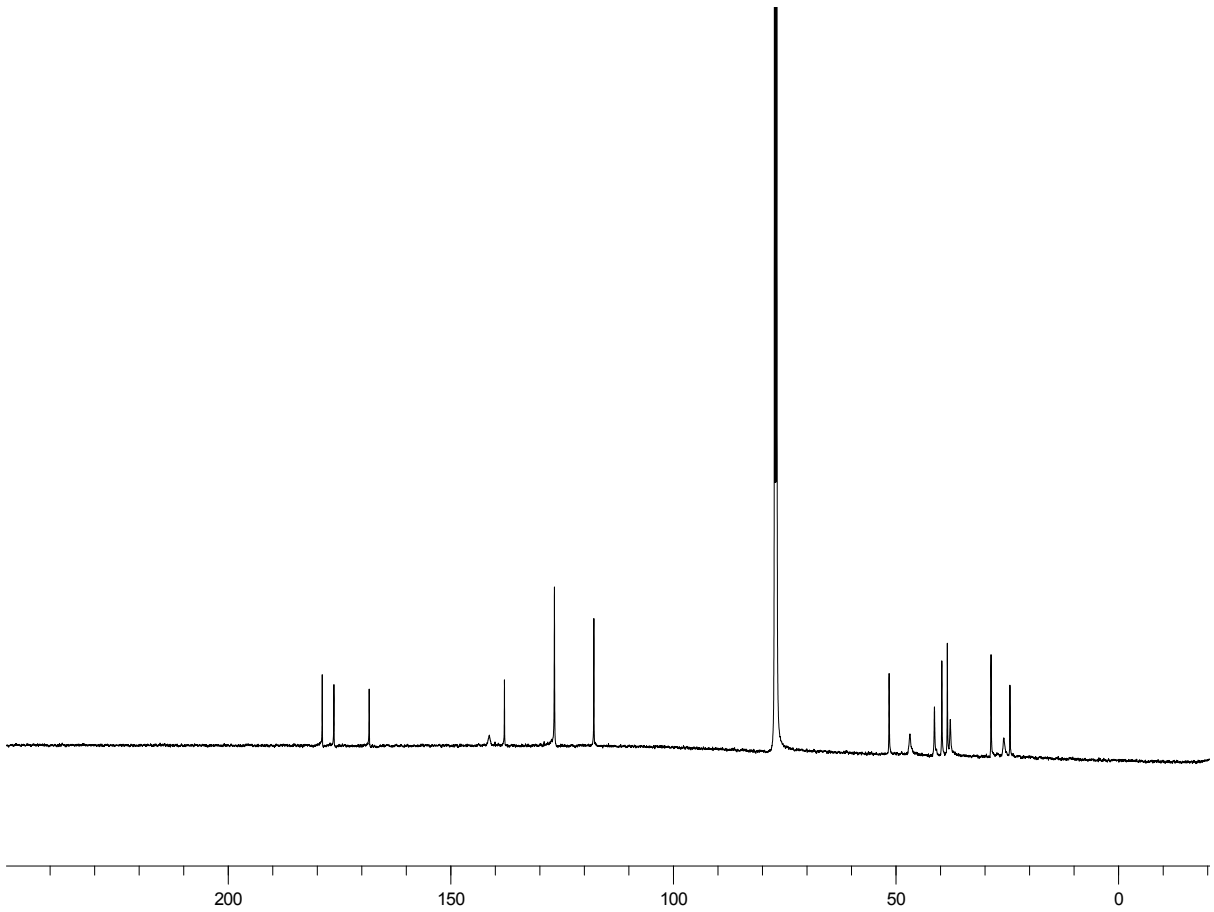
9-NOESY



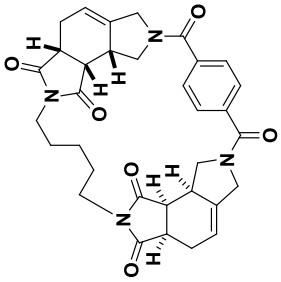


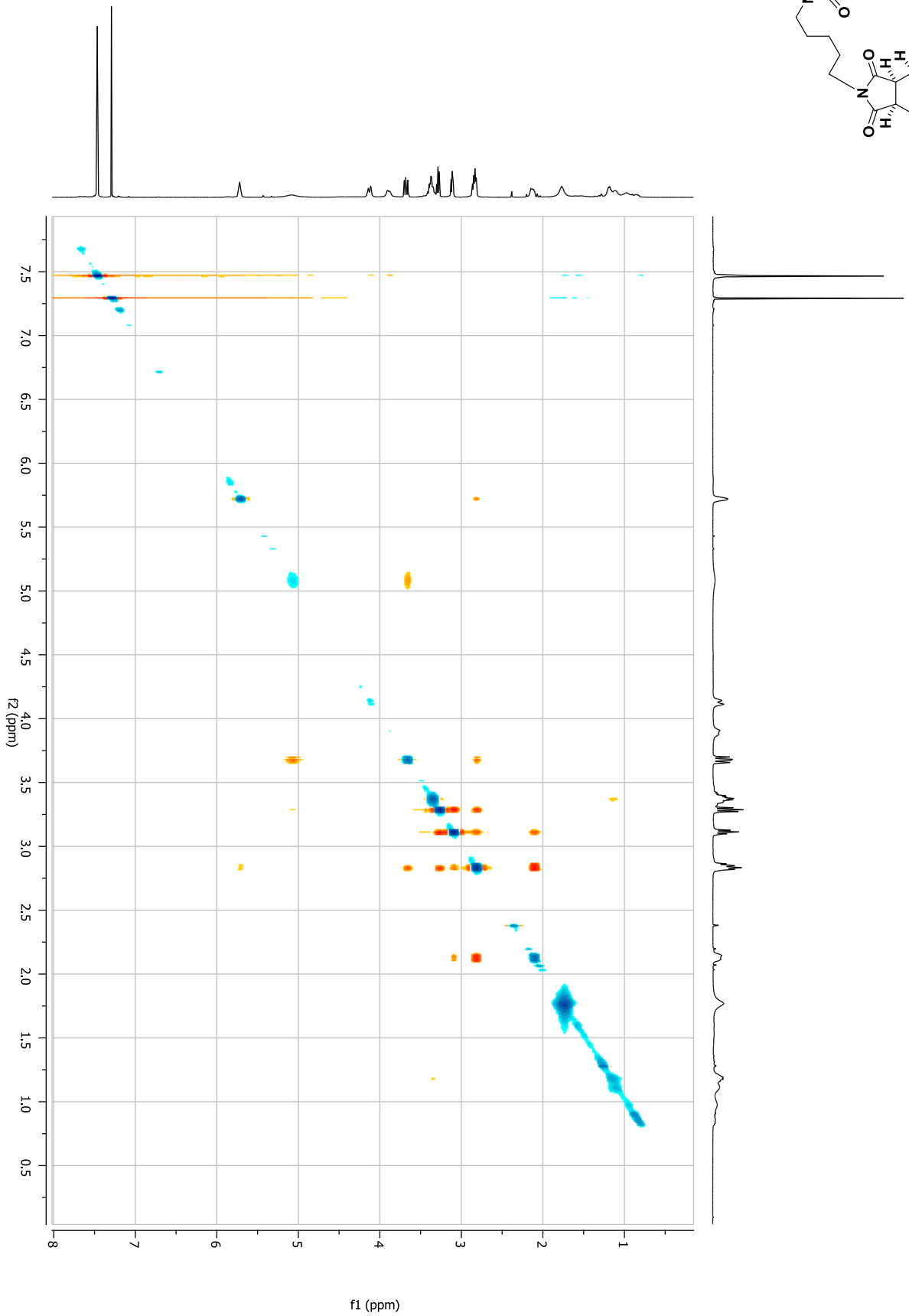
(+/-)-10



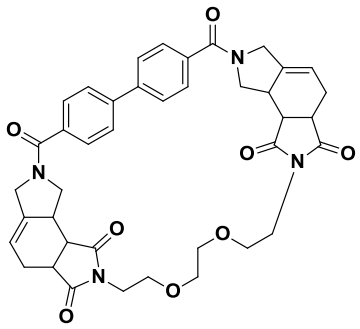


10-NOESY





(+/-)-11


O
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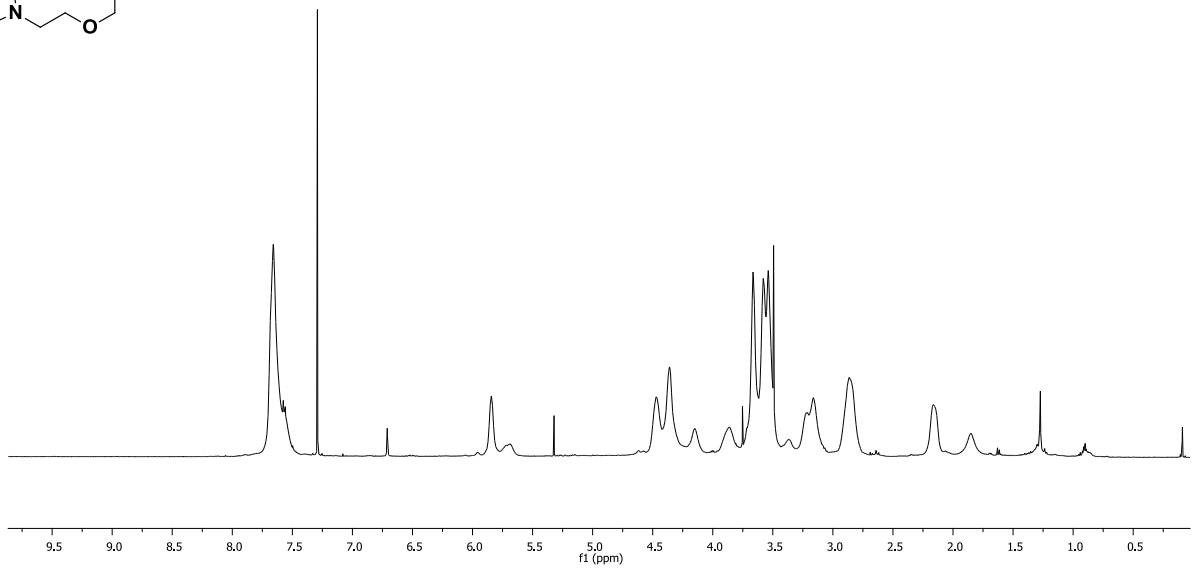
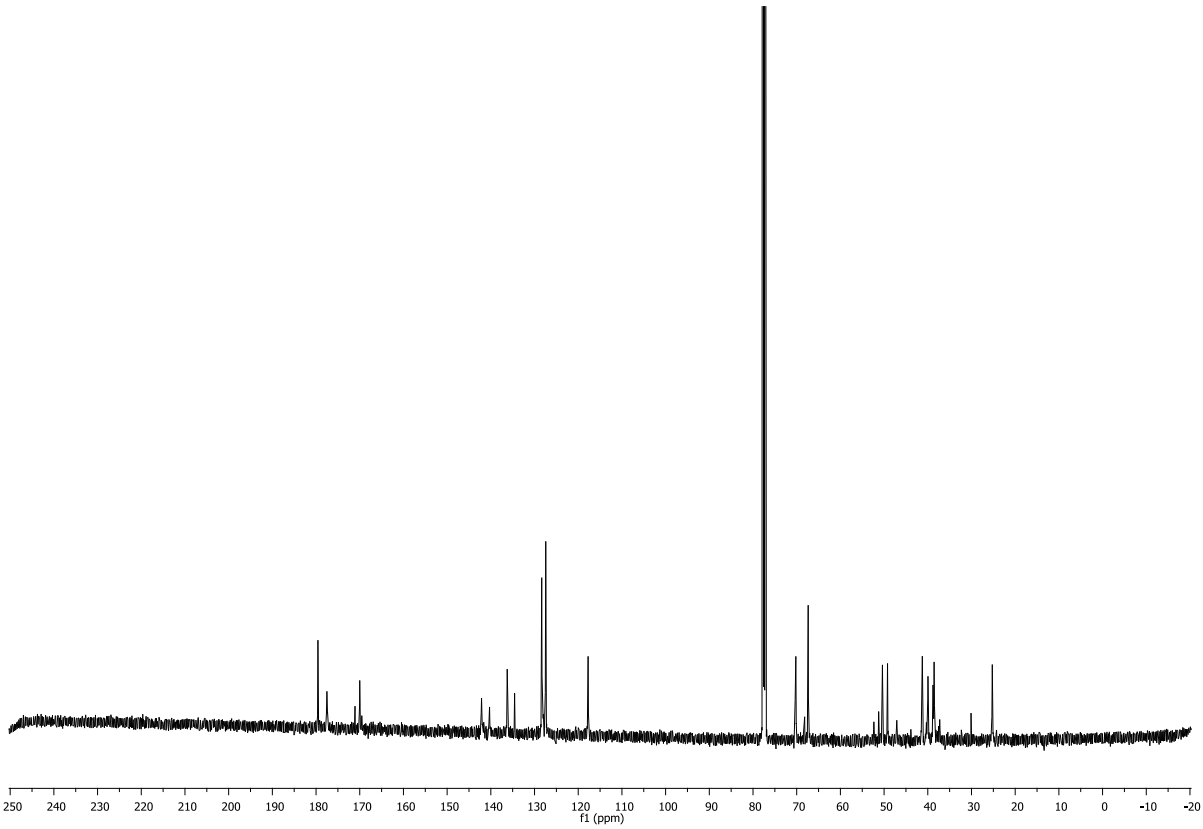
N
O
O
N
O
O
N
O
O
O


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
f1 (ppm)


250
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190
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170
160
150
140
130
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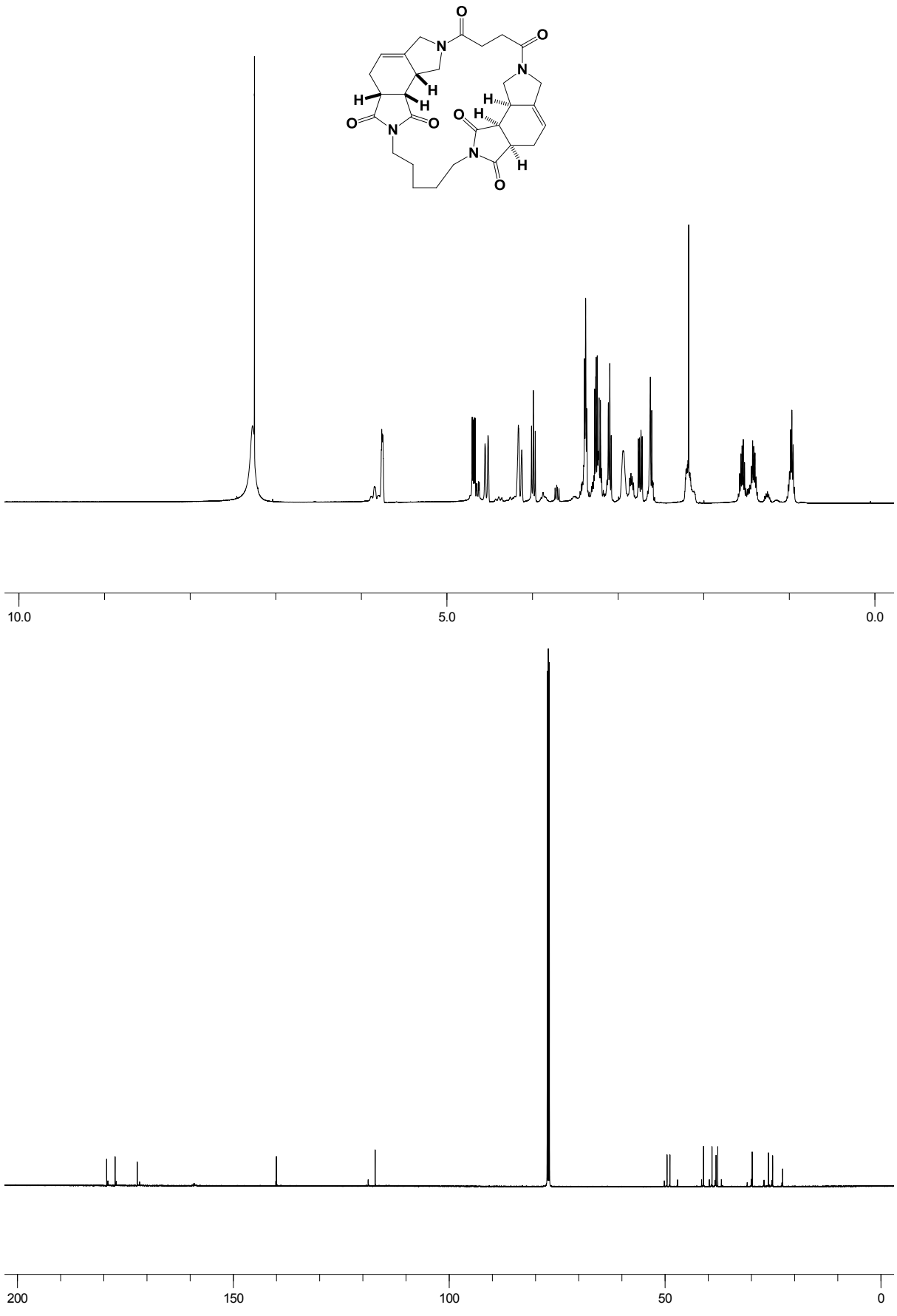
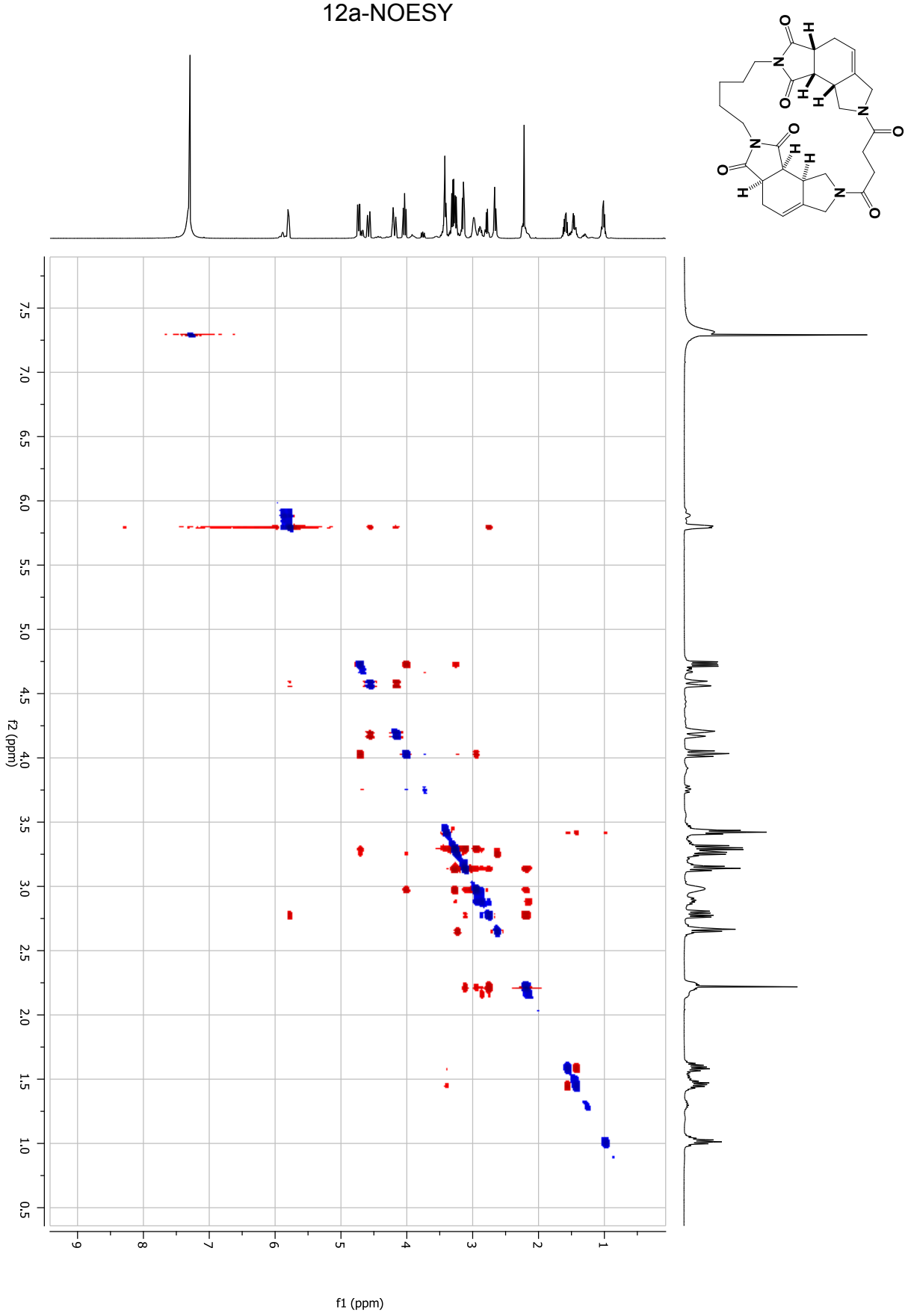
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

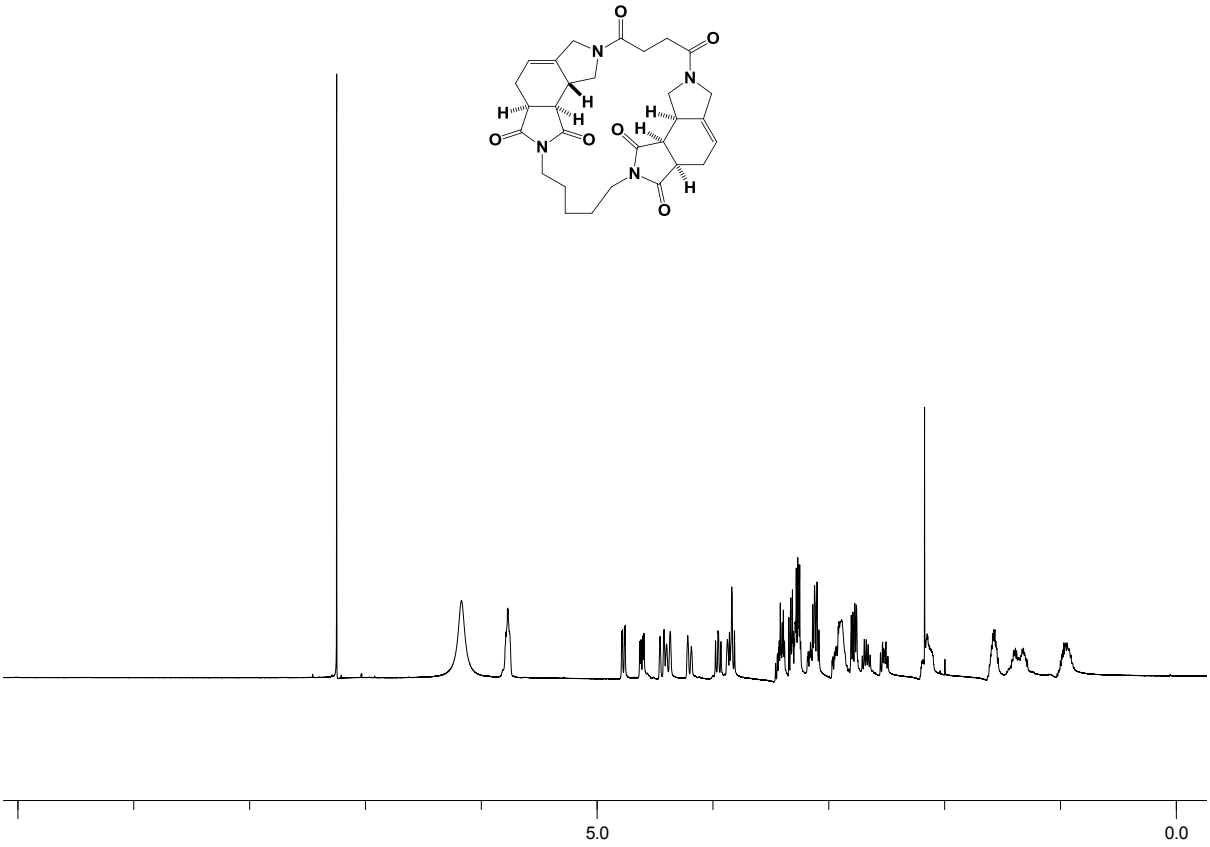
(+/-)-12a

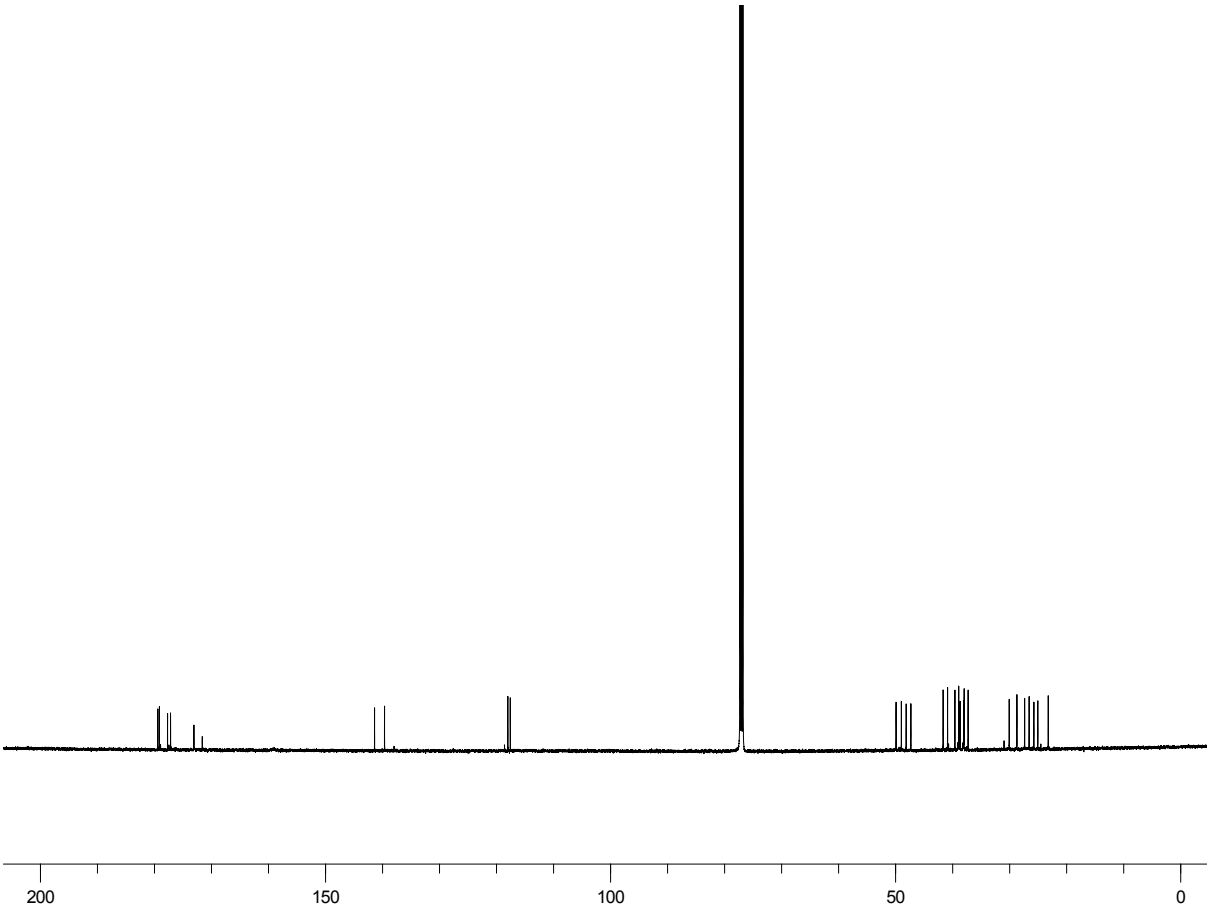


12a-NOESY

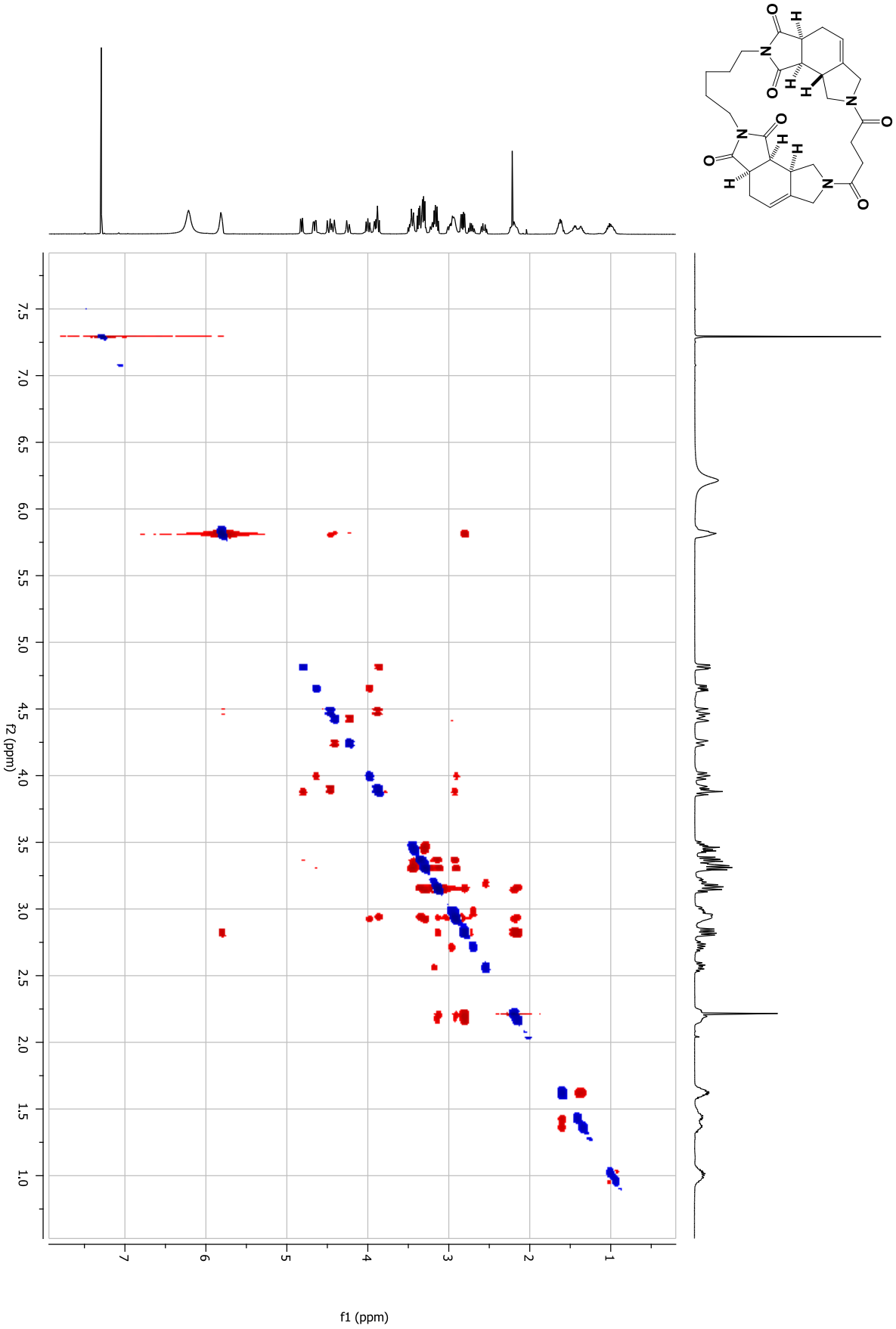


(+/-)-12b

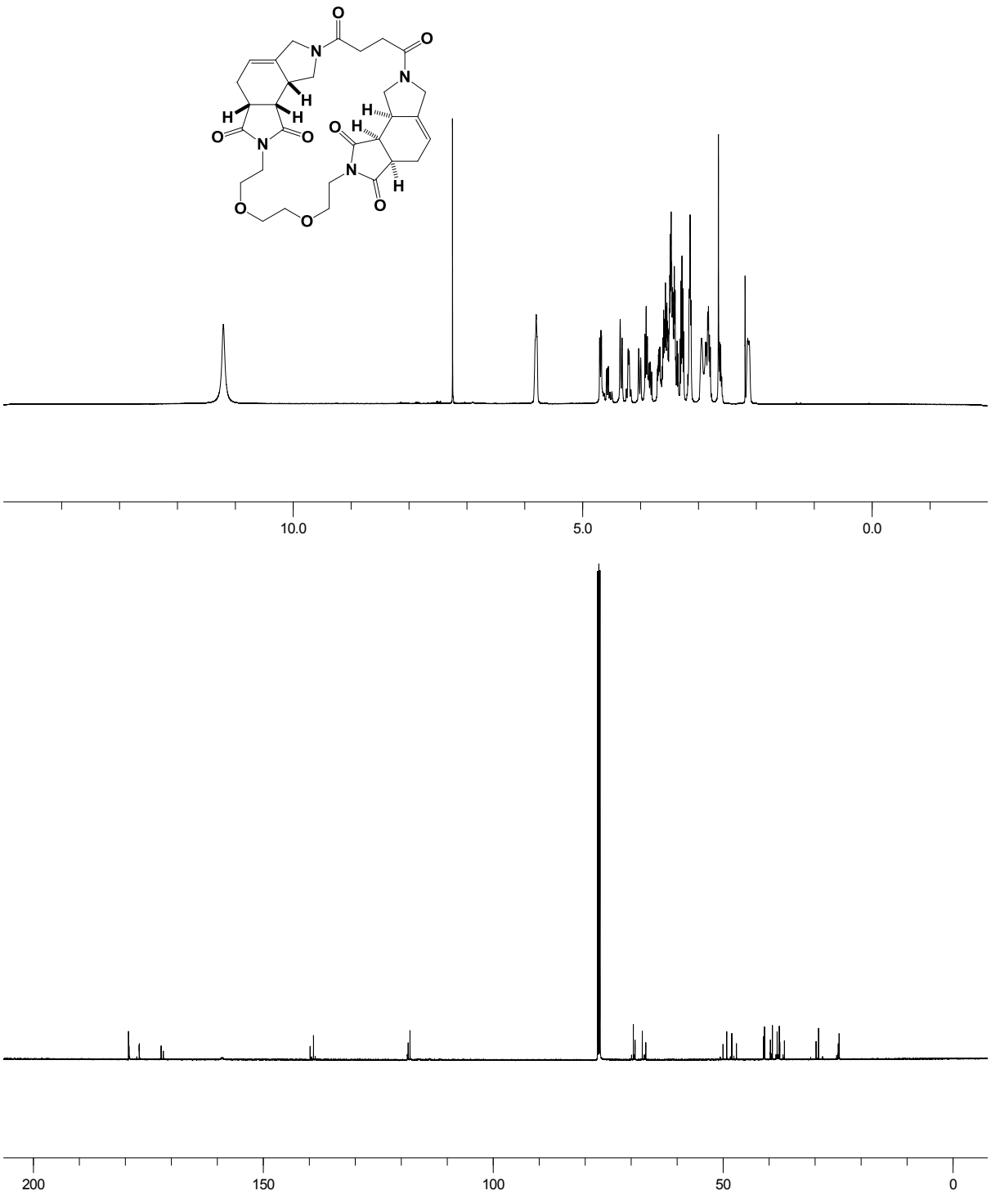




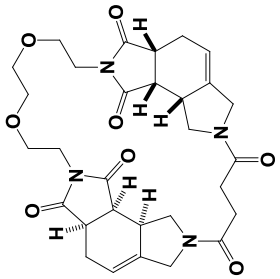
12b-NOESY



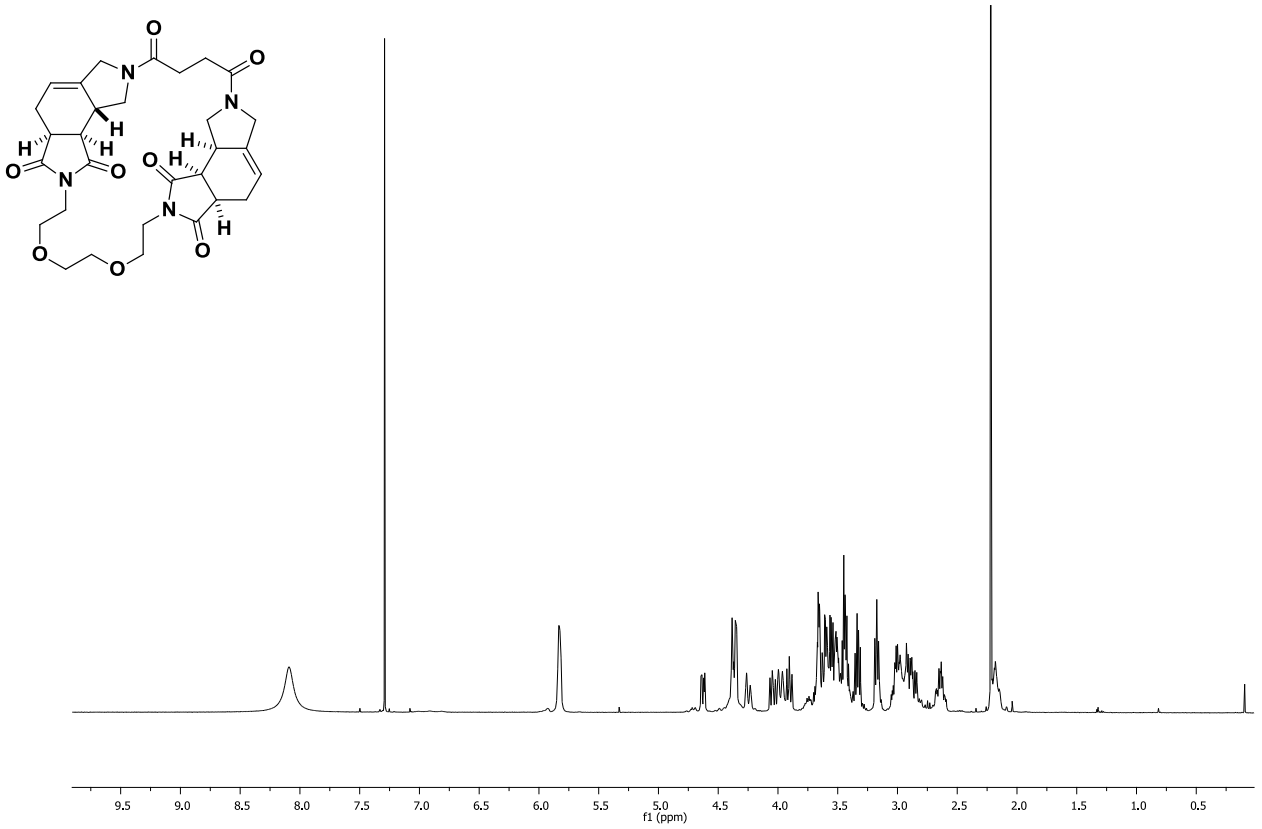
(+/-)-13a

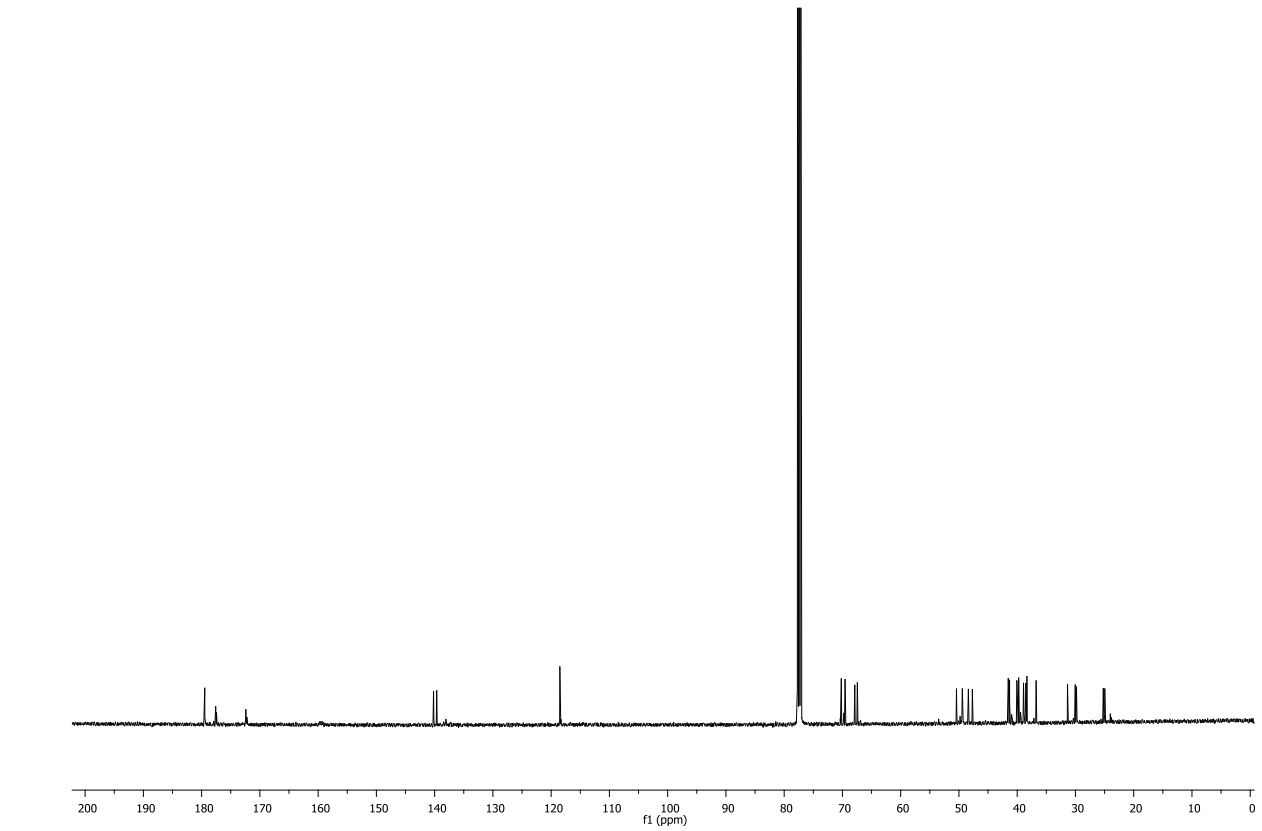


13a-NOESY



(+/-)-13b


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
f1 (ppm)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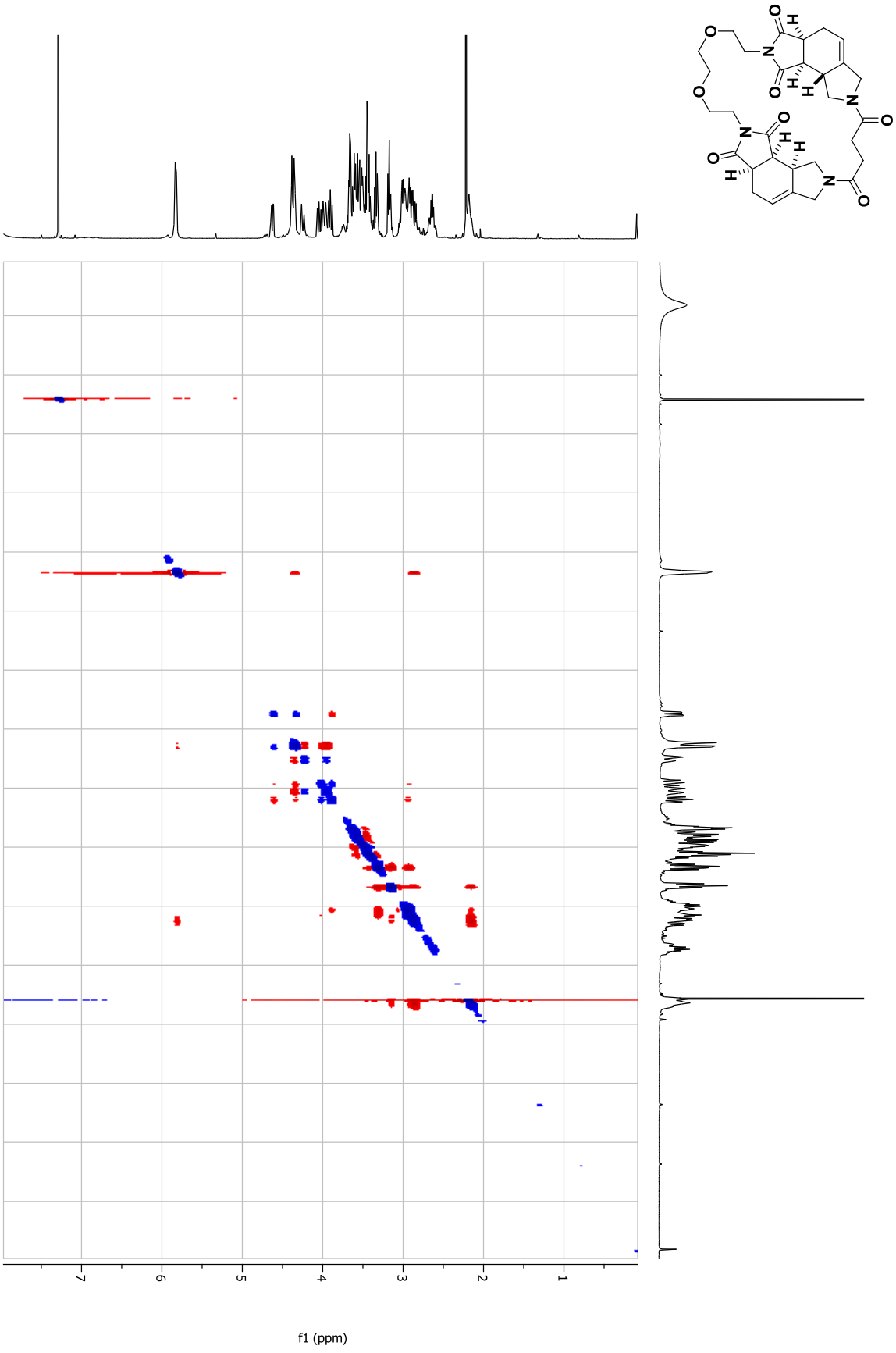
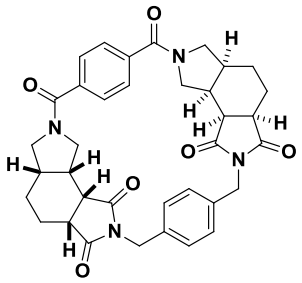
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

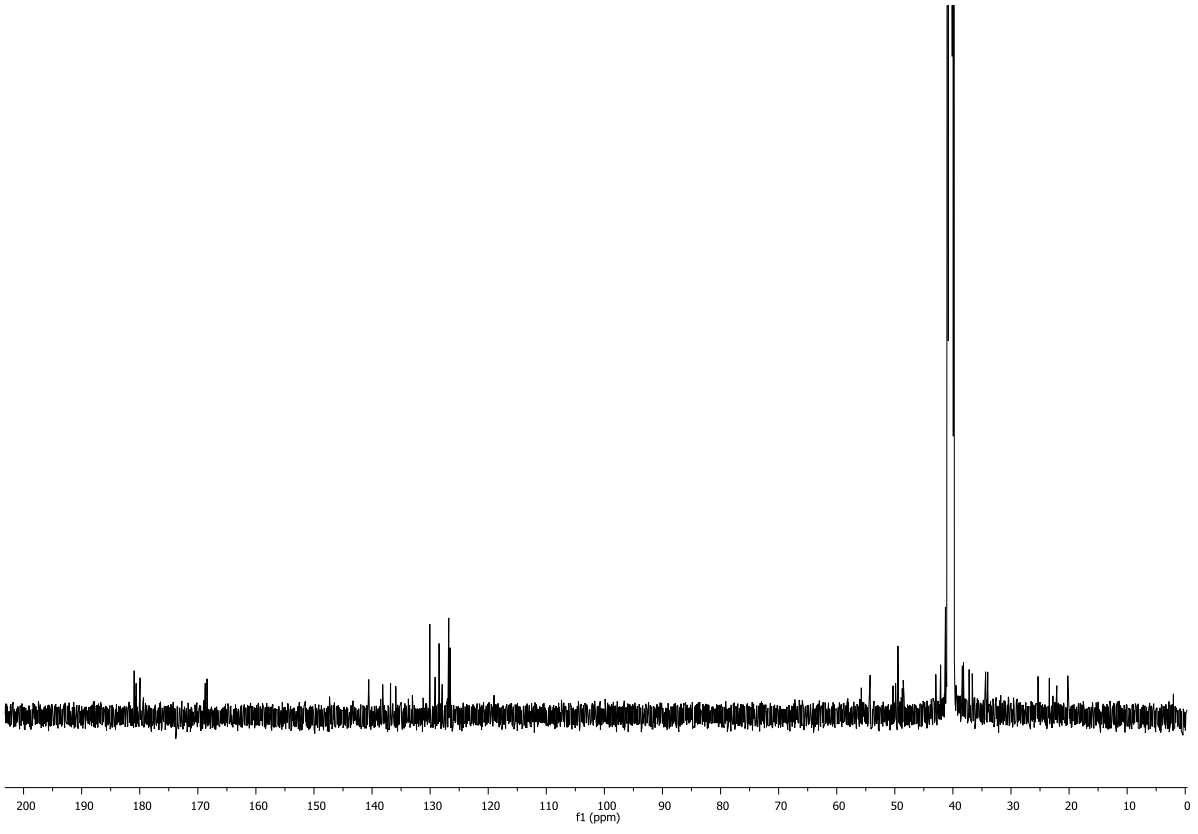
13b-NOESY



(+/-)-14


O
N
H
O
H
H
H
O
N
O
N
H
H
O
H
N
O
O

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
f1 (ppm)


200
190
180
170
160
150
140
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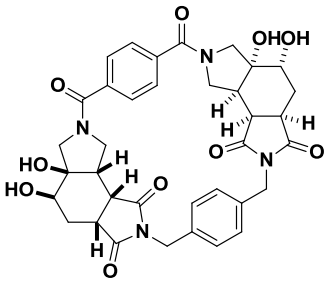
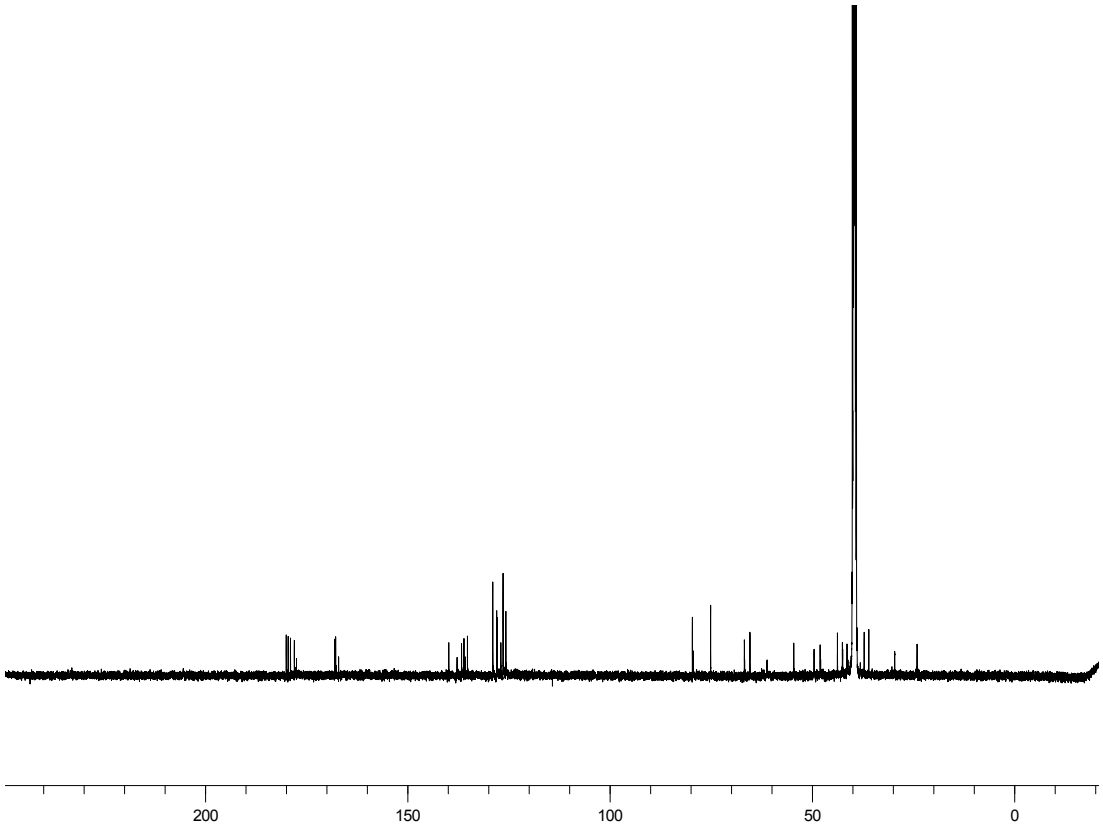
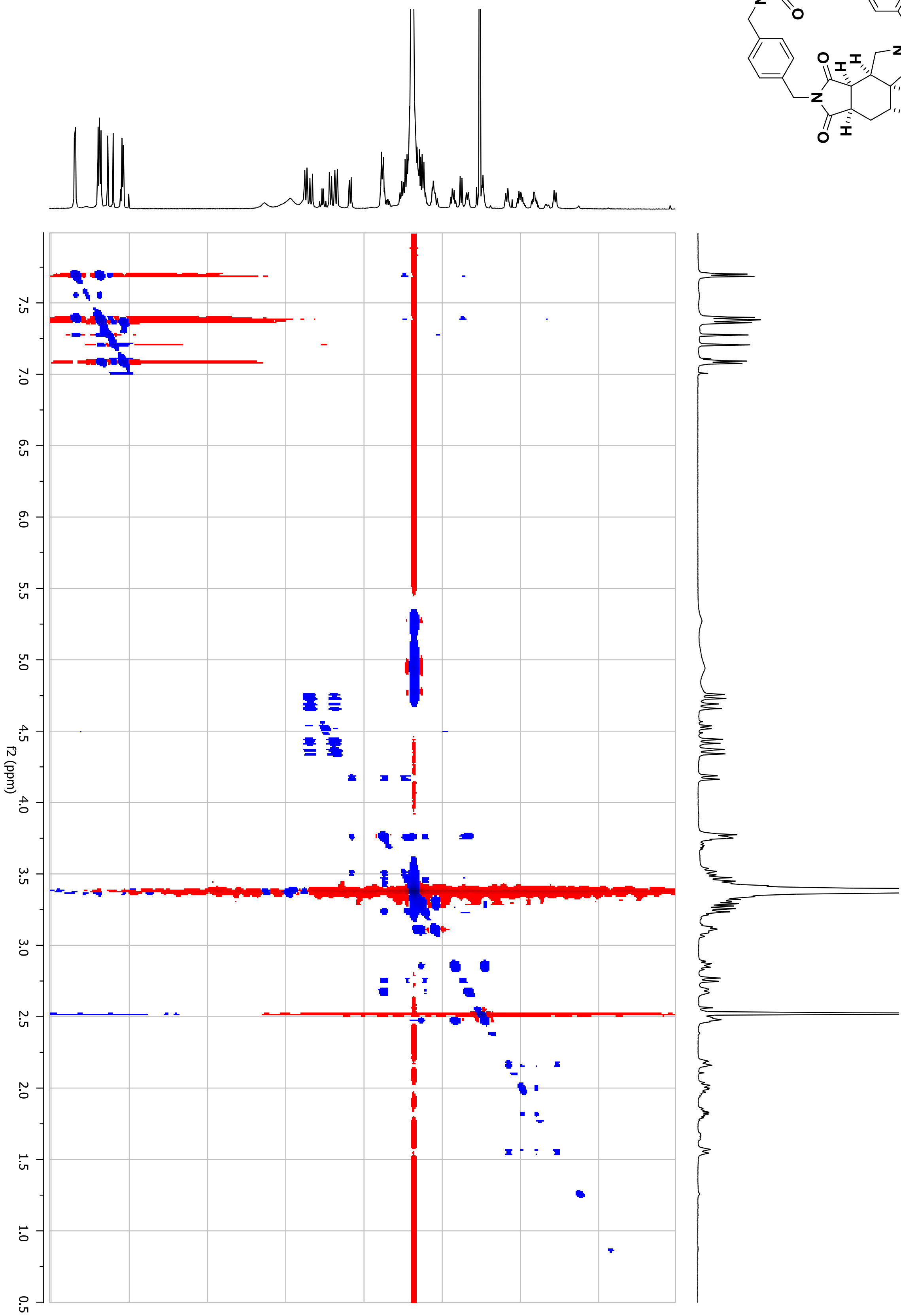
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

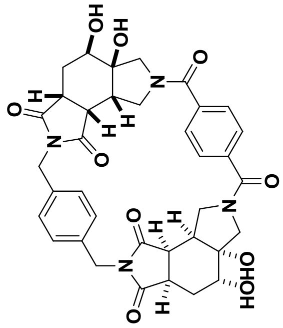
(+/-)-15



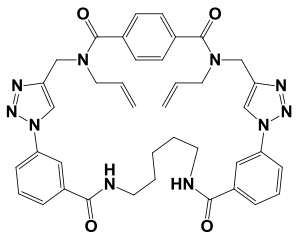


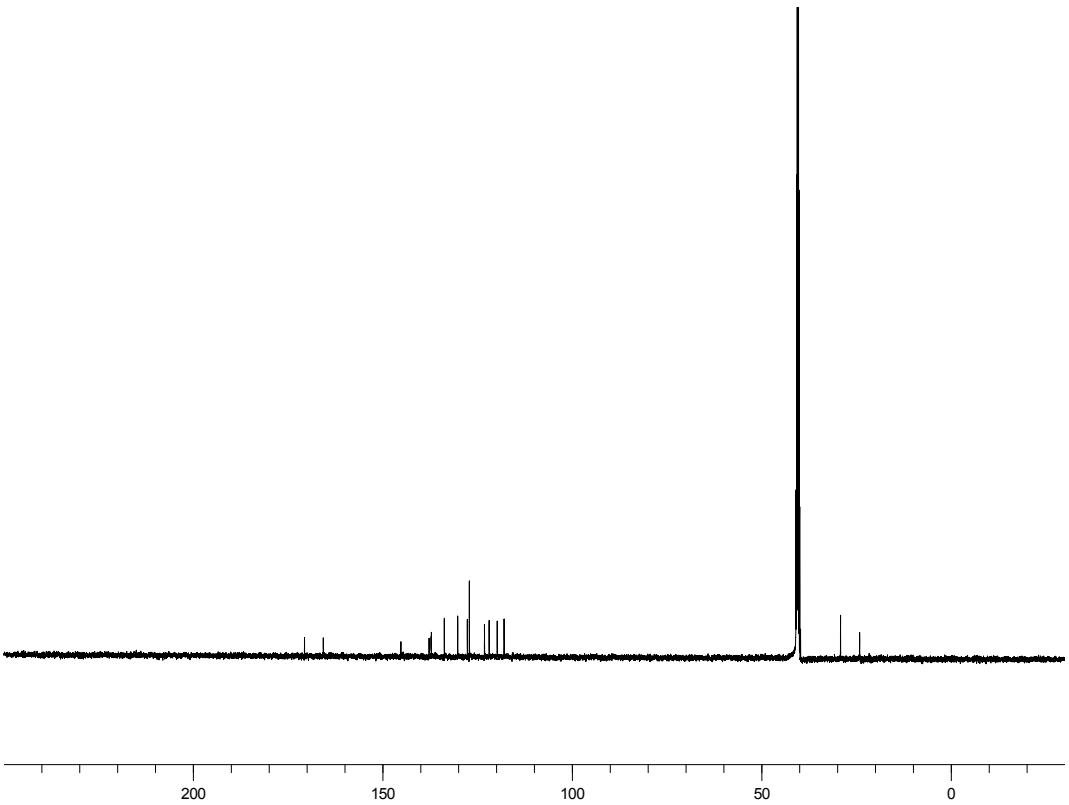
15-NOESY



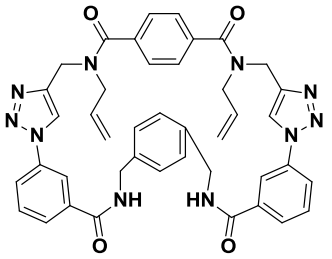


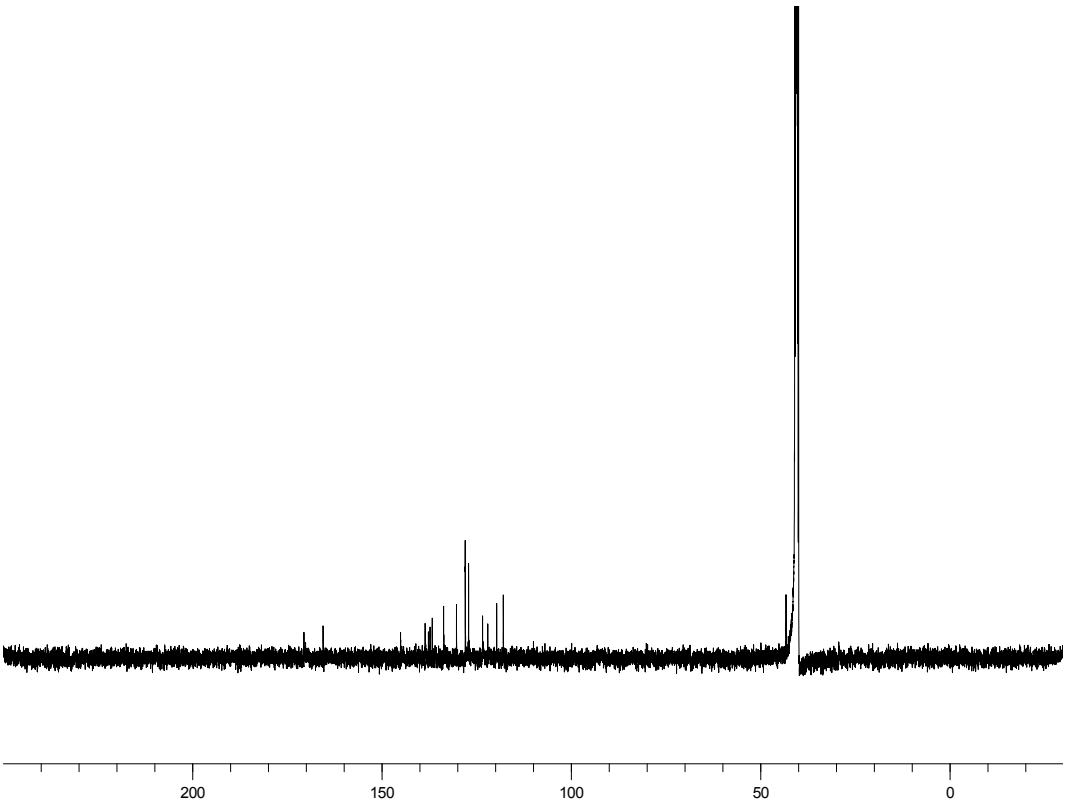
16





17





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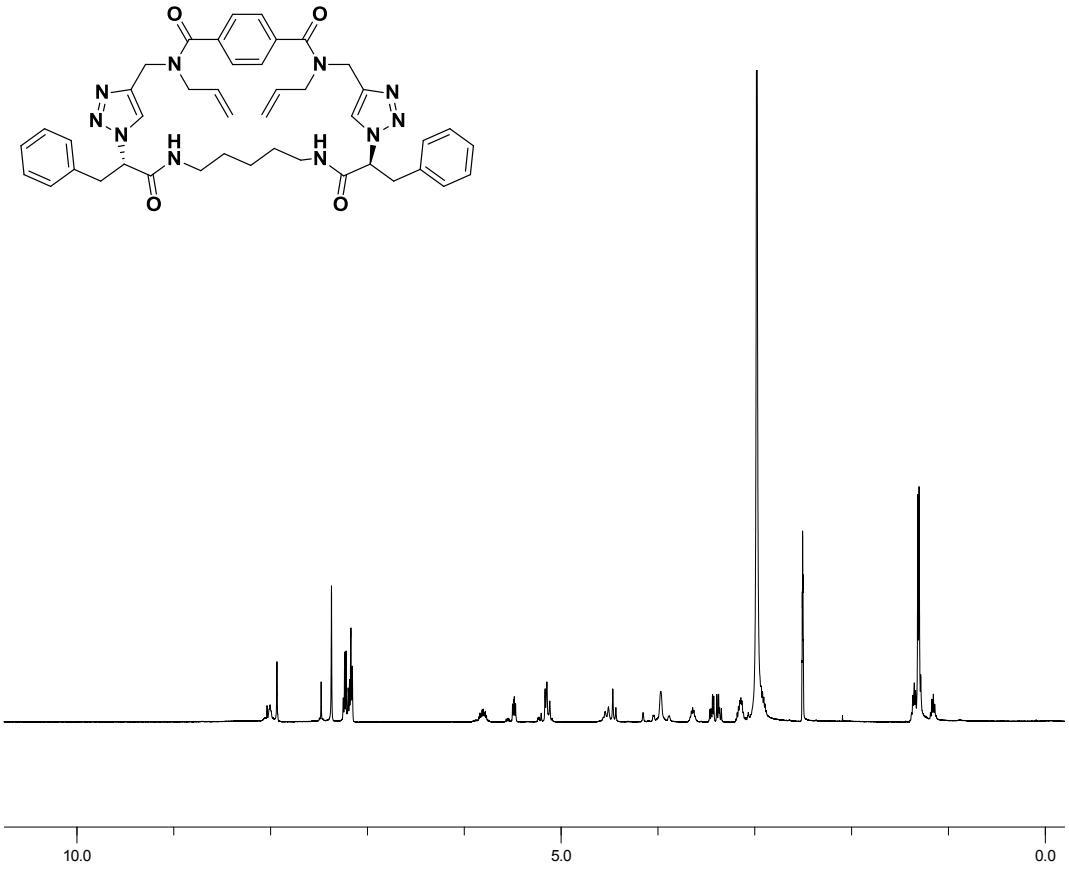

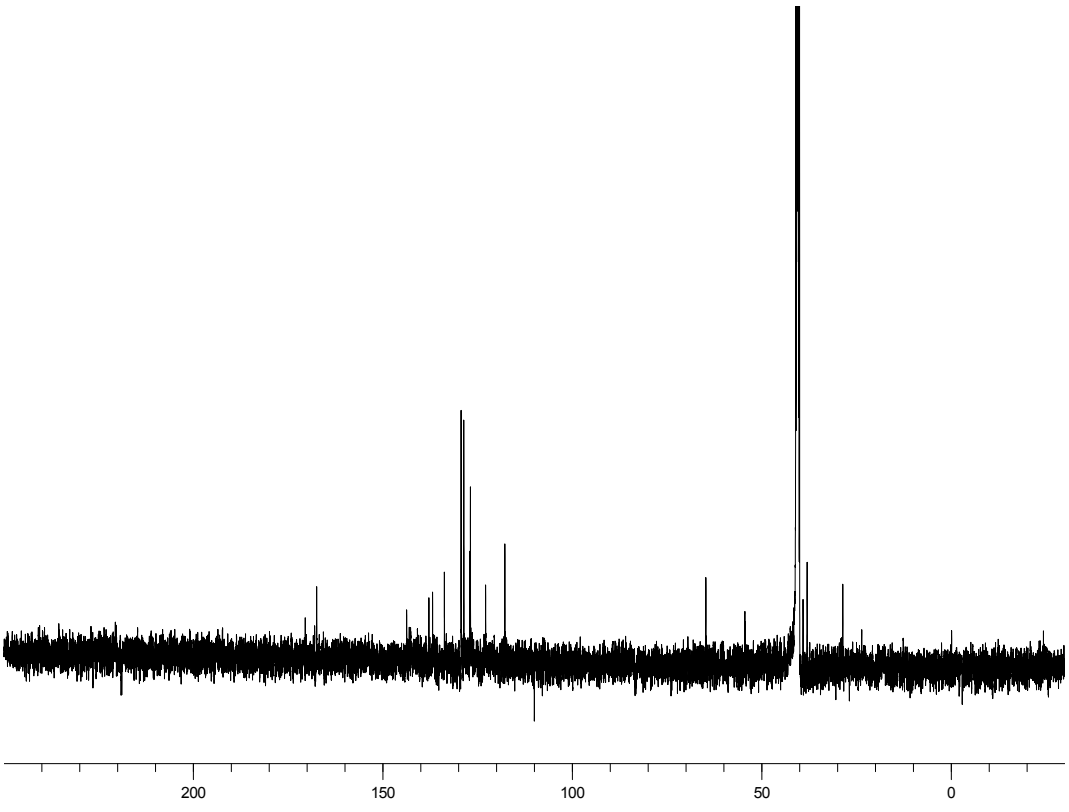

19