Supporting Information for Acid-catalyzed Esterification and Biotin/Fluorescent Labeling of Glutathione Polysulfides Based on Stability under Heating and pH Change

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Supplementary Materials

^{1}H-, ^{13}C-NMR spectra were recorded on a JEOL ECS (400 MHz) or Bruker AVANCE III (600 MHz). ^{13}C-NMR spectra were recorded with acetone as an external standard (δ 30.89). The following abbreviations (or combinations thereof) were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Melting points were determined with a Yanagimoto micro melting point apparatus without correction. Infrared (IR) spectra were recorded on a JASCO FT/IR-410 spectrometer. Mass spectra were measured on a SHIMAZU LCMS-IT-TOF or Agilent 6230 LC/TOF spectrometer. UV-Vis absorption and photoluminescence spectra were measured on SHIMAZU UV-2600i and JASCO FP-6500 spectrometer. X-ray crystallographic data was collected using a Rigaku XtaLAB Synergy R, DW system, HyPix diffractometer.

Materials

Glutathione trisulfide GSSSG **1** and glutathione tetrasulfide GSSSSG **2** were prepared according to the literature procedures and spectral data were confirmed.[S1)]

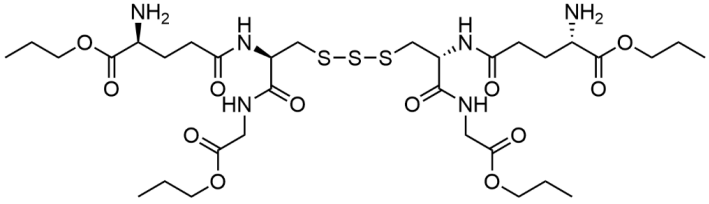
Glutathione trisulfide (1): Colorless solid. M.p. 208.5°C decomposed (MeCN/H_2O = 10/1). $[\alpha]_D^{28}$ −38.1° (c 0.2, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.87-4.82 (2H, m), 3.96 (4H, s), 3.82 (2H, t, *J* = 6.4 Hz), 3.48 (2H, dd, *J* = 14.4, 4.8 Hz), 3.23 (2H, dd, *J* = 14.4, 8.8 Hz), 2.57 (4H, td, *J* = 7.6, 3.2 Hz), 2.22-2.16 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.3, 172.7, 172.3, 171.5, 52.5, 52.3, 41.2, 38.8, 31.0, 25.5. IR (KBr) ν 3394, 1653, 1541, 1416, 1227, 668 cm^{-1}. MS (ESI) HRMS Calcd for $C_{20}H_{33}N_6O_{12}S_3$: 645.1319. Found: 645.1335.

Glutathione tetrasulfide (2): Colorless solid. M.p. 201.5°C decomposed (MeCN/H_2O = 10/1). $[\alpha]_D^{31}$ +51.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.85 (2H, dd, *J* = 9.3, 4.8 Hz), 4.01 (4H, s), 3.86 (2H, t, *J* = 6.4 Hz), 3.56 (2H, dd, *J* = 14.4, 4.8 Hz), 3.30 (2H, dd, *J* = 14.4, 9.3 Hz), 2.65-2.52 (4H, m), 2.25-2.16 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.8, 174.3, 173.7, 172.0, 54.1, 53.0, 42.0, 39.6, 31.6, 26.1. IR (KBr) ν 3057, 1653, 1541, 1397, 1226 cm^{-1}. MS (ESI) HRMS Calcd for $C_{20}H_{33}N_6O_{12}S_4$: 677.1039. Found: 677.0995.

General procedure for synthesis of the dipropyl 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosane-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6a).

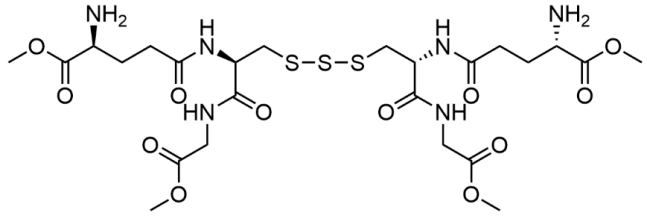
In a two-necked flask were placed glutathione trisulfide (100.0 mg, 0.16 mmol) and 1-propanol (1 mL), and chlorotrimethylsilane (0.32 mL, 2.5 mmol, 16 equiv.) was added dropwise under stirring with a magnetic stirrer. The mixture was stirred for 20 h at room temperature under an argon atmosphere. The solvent was removed under reduced pressure, and the residue was purified by reversed phase column chromatography, giving dipropyl 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosane-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) **6a** (128.8 mg, 99%). **6a**: Colorless solid. M.p. 85.7-86.4°C (MeCN/H_2O = 10). $[\alpha]_D^{29}$ −92.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.84 (2H, dd, *J* = 8.6, 5.2 Hz), 4.26 (4H, t, *J* = 6.6 Hz), 4.21 (2H, t, *J* = 6.8 Hz), 4.16 (2H, t, *J* = 6.6 Hz), 4.10 (2H, d, *J* = 17.7 Hz), 4.04 (2H, d, *J* = 17.7 Hz), 3.45 (2H, dd, *J* = 14.4, 5.2 Hz), 3.24 (2H, dd, *J* = 14.4, 8.6 Hz), 2.68-2.57 (4H, m), 2.34-2.24 (4H, m), 1.74 (4H, sextet, *J* = 7.1 Hz), 1.69 (4H, sextet, *J* = 7.1 Hz), 0.97 (6H, t, *J* = 7.3 Hz), 0.94 (6H, t, *J* = 7.3 Hz). ^{13}C-NMR (100 MHz, D_2O) δ 174.0, 172.3, 171.4, 169.8, 69.1, 68.0, 52.5, 52.3, 41.5,

38.8, 30.9, 25.4, 21.4, 21.3, 9.66, 9.65. IR (KBr) 3646, 2974, 2371, 2319, 1747, 1508, 1338, 1211 cm^{-1}. MS (ESI) HRMS Calcd for $C_{32}H_{57}N_6O_{12}S_3$: 813.3197. Found: 813.3178.



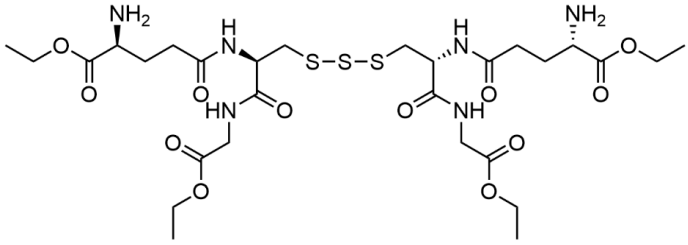
Dimethyl 5,5'-(((7*R*,13*R*)-3,6,14,17-tetraoxo-2,18-dioxa-9,10,11-trithia-5,15-diazanonadecane-7,13-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6b)

Colorless solid. M.p. 94.3-95.1°C (MeCN/H_2O = 9). $[\alpha]_D^{28}$ −63.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.83 (2H, dd, *J* = 8.7, 5.2 Hz), 4.21 (2H, t, *J* = 6.6 Hz), 4.10 (2H, d, *J* = 18.0 Hz), 4.05 (2H, d, *J* = 18.0 Hz), 3.88 (6H, s), 3.78 (6H, s), 3.46 (2H, dd, *J* = 14.4, 5.1 Hz), 3.25 (2H, dd, *J* = 14.4, 8.8 Hz), 2.69-2.56 (4H, m), 2.34-2.22 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 148.3, 139.3, 137.7, 137.6, 134.2, 132.4, 129.1, 127.2, 118.8, 115.8. IR (KBr) 3400, 3269, 3043, 2954, 1746, 1657, 1534, 1440, 1225, 980 cm^{-1}. MS (ESI) HRMS Calcd for $C_{24}H_{41}N_6O_{12}S_3$: 701.1945. Found: 701.1894.



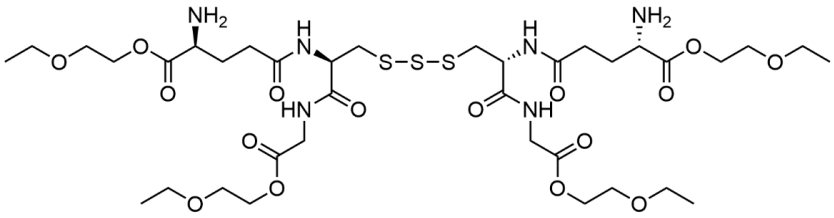
Diethyl 5,5'-(((8*R*,14*R*)-4,7,15,18-tetraoxo-3,19-dioxa-10,11,12-trithia-6,16-diazahenicosane-8,14-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6c)

Colorless solid. M.p. 102.1-102.7°C (MeCN/H_2O = 10). $[\alpha]_D^{31}$ −56.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.83 (2H, dd, *J* = 8.6, 5.2 Hz), 4.33 (4H, dq, *J* = 7.2, 1.2 Hz), 4.23 (4H, q, *J* = 7.2 Hz), 4.18 (2H, t, *J* = 6.7 Hz), 4.07 (2H, d, *J* = 17.7 Hz), 4.02 (2H, d, *J* = 17.7 Hz), 3.45 (2H, dd, *J* = 14.4, 5.2 Hz), 3.24 (2H, dd, *J* = 14.4, 8.8 Hz), 2.69-2.56 (4H, m), 2.34-2.20 (4H, m), 1.33 (6H, t, *J* = 7.1 Hz), 1.28 (6H, t, *J* = 7.1 Hz). ^{13}C-NMR (100 MHz, D_2O) δ 174.1, 172.4, 171.3, 169.7, 63.8, 62.6, 52.6, 52.3, 41.6, 38.8, 30.9, 25.3, 13.3. IR (KBr) 3564, 2989, 2348, 2309, 1744, 1659, 1530, 1375, 1217 cm^{-1}. MS (ESI) HRMS Calcd for $C_{28}H_{50}N_6O_{12}S_3$: 757.2571. Found: 757.2555.



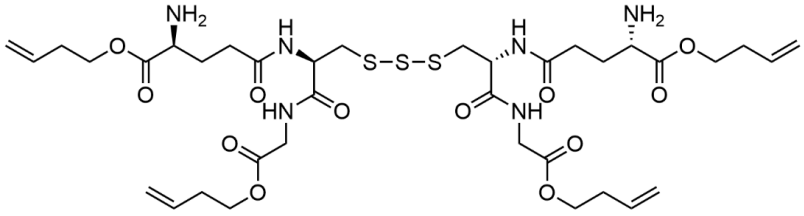
Bis(2-ethoxyethyl) 5,5'-(((11*R*,17*R*)-7,10,18,21-tetraoxo-3,6,22,25-tetraoxa-13,14,15-trithia-9,19-diazaheptacosane-11,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6d)

Colorless solid. M.p. 124.6-125.3°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$ −122.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.86 (2H,dd, *J* = 8.8, 5.2 Hz), 4.53-4.43 (4H, m), 4.37-4.35 (4H, m), 4.27 (2H, t, *J* = 6.6 Hz), 4.15 (2H, d, *J* = 17.8 Hz), 4.09 (2H, d, *J* = 17.8 Hz), 3.84 (4H, t, *J* = 4.5 Hz), 3.81-3.79 (4H, m), 3.67 (4H, q, *J* = 7.1 Hz), 3.66 (4H, q, *J* = 7.1 Hz), 3.48 (2H, dd, *J* = 14.4, 5.0 Hz), 3.26 (2H, dd, *J* = 14.4, 8.8 Hz), 2.72-2.60 (4H, m), 2.38-2.27 (4H, m), 1.27 (6H, t, *J* = 7.1 Hz), 1.235 (6H, t, *J* = 7.1 Hz). ^{13}C-NMR (100 MHz, D_2O) δ 174.1, 172.4, 171.0, 169.5, 67.8, 67.6, 66.78, 66.77, 65.6, 64.8, 52.6, 52.3, 41.4, 38.9, 30.9, 25.5, 14.3. IR (KBr) 3424, 3301, 2974, 1739, 1638, 1530, 1223, 1124, 1037 cm^{-1}. MS (ESI) HRMS Calcd for $C_{36}H_{65}N_6O_{16}S_3$: 933.3619. Found: 933.3615.



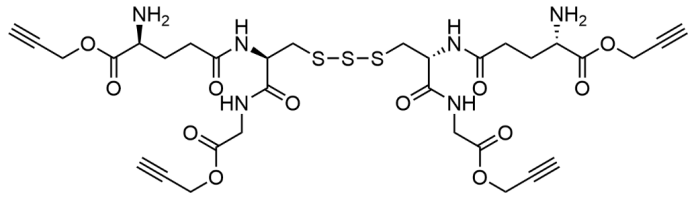
Di(but-3-en-1-yl) 5,5'-(((10*R*,16*R*)-6,9,17,20-tetraoxo-5,21-dioxa-12,13,14-trithia-8,18-diazapentacosa-1,24-diene-10,16-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6e)

Colorless solid. M.p. 184.3°C decomposed (MeCN/H_2O = 9). $[\alpha]_D^{28}$ −54.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 5.93-5.81 (4H, m), 5.22-5.19 (2H, m), 5.18-5.12 (6H, m), 4.82 (2H, dd, *J* = 8.8, 5.2 Hz), 4.41-4.31 (4H, m), 4.26 (4H, t, *J* = 6.4 Hz), 4.18 (2H, t, *J* = 6.4 Hz), 4.08 (2H, d, *J* = 17.7 Hz), 4.02 (2H, d, *J* = 17.7 Hz), 3.44 (2H, dd, *J* = 14.4, 5.2 Hz), 3.22 (2H, dd, *J* = 14.4, 8.8 Hz), 2.68-2.54 (4H, m), 2.51-2.42 (8H, m), 2.28-2.21 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.0, 172.3, 171.2, 169.7, 134.6, 134.4, 117.6, 117.3, 66.1, 65.2, 52.5, 52.2, 41.5, 38.9, 32.31, 3229, 30.9, 25.4. IR (KBr) 3271, 2960, 1743, 1655, 1533, 1205, 1090 cm^{-1}. MS (ESI) HRMS Calcd for $C_{36}H_{56}N_6O_{12}S_3Na$: 883.3016. Found: 883.3001.



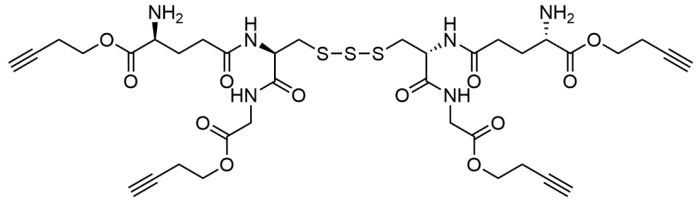
Di(prop-2-yn-1-yl) 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosa-1,22-diyne-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6f)

Colorless solid. M.p. 120.3-121.0°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$−10.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.94 (4H, d, *J* = 2.4 Hz), 4.85 (2H, dd, *J* = 8.6, 5.2 Hz), 4.84 (4H, d, *J* = 2.4 Hz), 4.29 (2H, t, *J* = 6.6 Hz), 4.16 (2H, d, *J* = 17.8 Hz), 4.10 (2H, d, *J* = 17.8 Hz), 3.48 (2H, dd, *J* = 14.4, 5.2 Hz), 3.28 (2H, dd, *J* = 14.4, 8.6 Hz), 3.07 (2H, t, *J* = 2.4 Hz), 3.00 (2H, t, *J* = 2.4 Hz), 2.68-2.64 (4H, m), 2.35-2.29 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.0, 172.5, 170.4, 169.0, 77.5, 77.2, 76.9, 76.5, 54.4, 53.4, 52.6, 52.1, 41.4, 38.9, 30.8, 25.3. IR (KBr) 3280, 3059, 1750, 1659, 1531, 1198 cm^{-1}. MS (ESI) HRMS Calcd for $C_{32}H_{41}N_6O_{12}S_3$: 797.1945. Found: 797.1943.



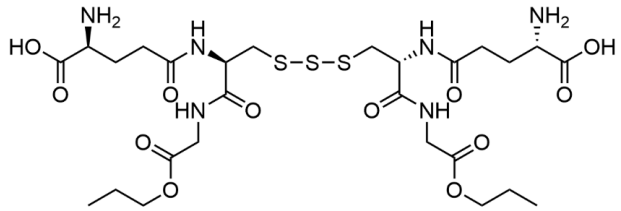
Di(but-3-yn-1-yl) 5,5'-(((10*R*,16*R*)-6,9,17,20-tetraoxo-5,21-dioxa-12,13,14-trithia-8,18-diazapentacosa-1,24-diyne-10,16-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6g)

Colorless solid. M.p. 81.9-82.7°C (MeCN/H_2O = 9). $[\alpha]_D^{27}$ +117.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.84 (2H, dd, *J* = 8.8, 5.2 Hz), 4.47-4.35 (4H, m), 4.30 (4H, t, *J* = 6.4 Hz), 4.25 (2H, t, *J* = 6.4 Hz), 4.16 (2H, d, *J* = 17.8 Hz), 4.08 (2H, d, *J* = 17.8 Hz), 3.47 (2H, dd, *J* = 14.4, 5.2 Hz), 3.25 (2H, dd, *J* = 14.4, 8.8 Hz), 2.71-2.62 (12H, m), 2.48 (2H, t, *J* = 2.6 Hz), 2.45 (2H, t, *J* = 2.6 Hz), 2.33-2.25 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.0, 172.3, 171.0, 169.5, 81.5, 81.2, 71.1, 70.8, 64.6, 63.7, 52.5, 52.2, 41.4, 39.0, 31.0, 25.4, 18.27, 18.25. IR (KBr) 3731, 3625, 3283, 2348, 2308, 1747, 1659, 1530, 1383, 1206, 672, 648 cm^{-1}. MS (ESI) HRMS Calcd for $C_{36}H_{49}N_6O_{12}S_3$: 853.2571. Found: 853.2533.



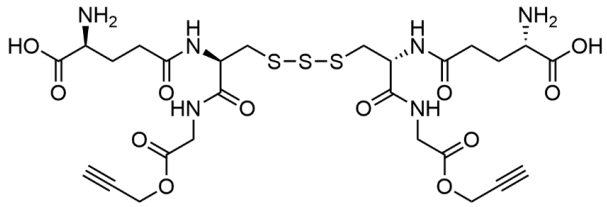
General procedure for synthesis of the (9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-propoxyethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicosan-21-oic acid (5a)

In a two-necked flask were placed glutathione trisulfide (100.0 mg, 0.16 mmol) and 1-propanol (1 mL), and chlorotrimethylsilane (0.32 mL, 2.5 mmol, 16 equiv.) was added dropwise under stirring with a magnetic stirrer. The mixture was stirred for 3 h at room temperature under an argon atmosphere. The solvent was removed under reduced pressure, and the residue was purified by reversed phase column chromatography, giving (9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-propoxyethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicosan-21-oic acid **5a** (84.0 mg, 72%) and **6a** (29.9 mg, 23%). **5a**: Colorless solid. M.p. 185.3-186.1°C (MeCN/H_2O = 9). $[\alpha]_D^{27}$ +122.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.82 (2H, dd, *J* = 9.0, 4.9 Hz), 4.15 (4H, t, *J* = 6.6 Hz), 4.08 (2H, d, *J* = 17.7 Hz), 4.02 (2H, d, *J* = 17.7 Hz), 3.89 (2H, t, *J* = 6.4 Hz), 3.45 (2H, dd, *J* = 14.4, 4.9 Hz), 3.22 (2H, dd, *J* = 14.4, 9.0 Hz), 2.64-2.51 (4H, m), 2.24-2.17 (4H, m), 1.68 (6H, t, *J* = 7.4 Hz), 0.93 (6H, t, *J* = 7.4 Hz). ^{13}C-NMR (100 MHz, D_2O) δ 174.7, 173.2, 172.6, 171.5, 68.0, 53.5, 52.6, 41.6, 38.9, 31.3, 26.0, 21.4, 9.6. IR (KBr) ν 3429, 3308, 3103, 2965, 2477, 2349, 2308, 1728, 1643, 1543, 1402, 1213, 672 cm^{-1}. MS (ESI) HRMS Calcd for $C_{26}H_{45}N_6O_{12}S_3$: 729.2258. Found: 729.2241.



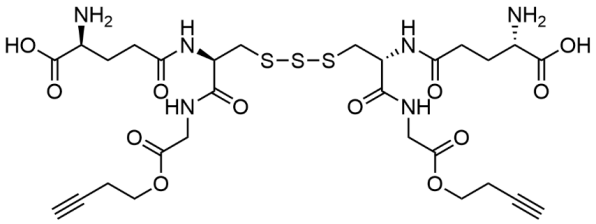
(9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-(prop-2-yn-1-yloxy)ethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicos-1-yn-21-oic acid (5f)

Colorless solid. M.p. 166.0°C decomposed(MeCN/H_2O = 9). $[\alpha]_D^{31}$+60.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.835 (4H, dd, *J* = 9.0, 4.9 Hz), 4.830 (2H, d, *J* = 2.4 Hz), 4.14 (2H, d, *J* = 17.8 Hz), 4.09 (2H, d, *J* = 17.8 Hz), 4.16 (2H, d, *J* = 17.8 Hz), 3.80 (2H, t, *J* = 6.4 Hz), 3.47 (2H, dd, *J* = 14.4, 4.9 Hz), 3.25 (2H, dd, *J* = 14.4, 9.0 Hz), 2.99 (2H, t, *J* = 2.4 Hz), 2.59-2.55 (4H, m), 2.26-2.14 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.9, 173.9, 172.7, 170.4, 77.4, 76.5, 54.1, 53.3, 52.6, 41.4, 39.0, 31.4, 26.2. IR (KBr) ν 3419, 3287, 3071, 1752, 1634, 1548, 1530, 1406, 1185 cm^{-1}. MS (ESI) HRMS Calcd for $C_{26}H_{37}N_6O_{12}S_3$: 721.1632. Found: 721.1584.



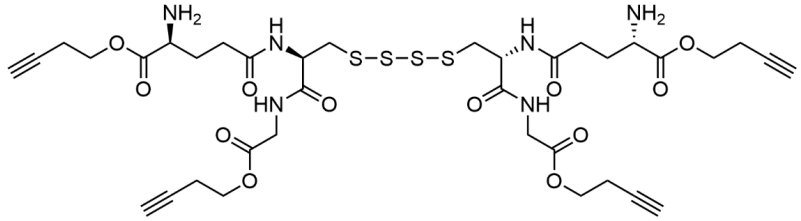
(10*R*,16*R*,21*S*)-21-amino-10-((*S*)-4-amino-4-carboxybutanamido)-16-((2-(but-3-yn-1-yloxy)-2-oxoethyl)carbamoyl)-6,9,18-trioxo-5-oxa-12,13,14-trithia-8,17-diazadocos-1-yn-22-oic acid (5g)

Colorless solid. M.p. 130.8-131.7°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$−54.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.83 (2H, dd, *J* = 9.0, 4.7 Hz), 4.30 (4H, t, *J* = 6.2 Hz), 4.13 (2H, d, *J* = 17.8 Hz), 4.07 (2H, d, *J* = 17.8 Hz), 3.79 (2H, t, *J* = 6.4 Hz), 3.48 (2H, dd, *J* = 14.5, 4.7 Hz), 3.24 (2H, dd, *J* = 14.5, 9.0 Hz), 2.63 (4H, td, *J* = 6.2 , 2.6 Hz), 2.59-2.54 (4H, m), 2.44 (2H, t, *J* = 2.6 Hz), 2.21-2.15 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.9, 173.9, 172.6, 171.0, 81.5, 70.7, 63.7, 54.1, 52.6, 41.4, 39.0, 31.4, 26.2, 18.2. IR (KBr) ν 3286, 3065, 1741, 1652, 1530, 1406, 1201, 647 cm^{-1}. MS (ESI) HRMS Calcd for $C_{28}H_{41}N_6O_{12}S_3$: 749.1945. Found: 749.1928.



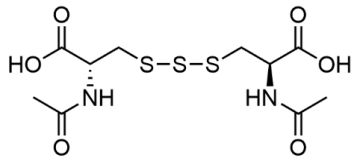
General procedure for synthesis of the di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (7g).

In a two-necked flask were placed glutathione tetrasulfide (52.5 mg, 0.078 mmol) and 3-butyn-1-ol (0.5 mL), and chlorotrimethylsilane (0.16 mL, 1.3 mmol, 16 equiv.) was added dropwise under stirring with a magnetic stirrer. The mixture was stirred for 21 h at room temperature under an argon atmosphere. The solvent was removed under reduced pressure, and the residue was purified by reversed phase column chromatography, giving di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) **7g** (64.5 mg, 94%). **7g**: Colorless solid. M.p. 131.1-131.4°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$−55.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.86 (2H, dd, *J* = 9.2, 4.8 Hz), 4.48-4.36 (4H, m), 4.31 (4H, t, *J* = 6.3 Hz), 4.26 (2H, t, *J* = 6.6 Hz), 4.14 (2H, d, *J* = 17.8 Hz), 4.08 (2H, d, *J* = 17.8 Hz), 3.56 (2H, dd, *J* = 14.4, 4.8 Hz), 3.25 (2H, dd, *J* = 14.4, 9.2 Hz), 2.72-2.62 (12H, m), 2.48 (2H, t, *J* = 2.6 Hz), 2.45 (2H, t, *J* = 2.6 Hz), 2.34-2.27 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 174.1, 172.3, 171.0, 169.5, 81.5, 81.3, 71.1, 70.8, 64.6, 63.8, 52.8, 52.2, 41.4, 39.6, 30.9, 25.5, 18.26, 18.25. IR (KBr) ν 3281, 2976, 1743, 1653, 1531, 1203, 647 cm^{-1}. MS (ESI) HRMS Calcd for $C_{36}H_{49}N_6O_{12}S_4$: 885.2291. Found: 885.2282.



General procedure for synthesis of (2*R*,2'*R*)-3,3'-trisulfanediylbis(2-acetamidopropanoic acid) (8). [S2)]

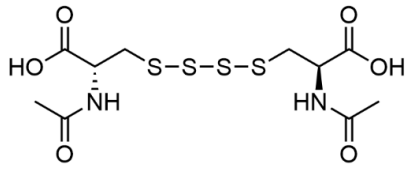
In a two-necked flask were placed $RhH(PPh_3)_4$ (178.7 mg, 5 mol%), cis-1,2- bis(diphenylphosphino)ethylene (dppv, 122.8 mg, 10 mol%), *N*-acetyl cystine (1.0 g, 3.1 mmol), and sulfur (99.2 mg, 1/8 eq.) in THF (21.7 mL) and H_2O (9.3 mL) under an argon atmosphere and the mixture was stirred for 24 h at 40 °C. The solvent was removed under reduced pressure, and the residue was purified by reversed phase column chromatography, giving (2*R*,2'*R*)-3,3'-trisulfanediylbis(2-acetamidopropanoic acid) **8** (375.7 mg, 34%) and (2*R*,2'*R*)-3,3'-tetrasulfanediylbis(2-acetamidopropanoic acid) (122.8 mg, 10%). **8**: Colorless solid. M.p. 76.2-77.2°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$ −27.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.82-4.79 (2H, m), 3.52 (2H, dd, *J* = 14.5, 4.4 Hz), 3.28 (2H, dd, *J* = 14.5, 8.8 Hz), 2.08 (6H, s). ^{13}C-NMR (100 MHz, D_2O) δ 174.3, 173.7, 52.0, 39.1, 21.9. IR (KBr) ν 3273, 2826, 1714, 1647, 1540, 1297, 1224, 1047, 917, 762 cm^{-1}. MS (ESI) HRMS Calcd for $C_{10}H_{17}N_2O_6S_3$: 357.0249. Found: 357.0223.



(2*R*,2'*R*)-3,3'-tetrasulfanediylbis(2-acetamidopropanoic acid) [S3)]

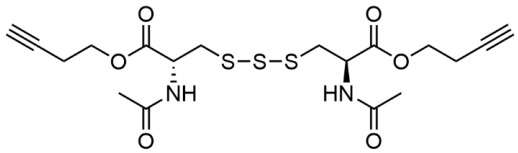
Pale yellow solid. M.p. 105.6-106.5°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$ −69.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 4.82 (2H, dd, *J* = 8.9, 4.4 Hz), 3.60 (2H, dd, *J* = 14.4, 4.4 Hz), 3.35 (2H, dd, *J* = 14.4, 8.9 Hz), 2.09 (6H, s). ^{13}C-NMR (100 MHz, D_2O) δ 174.3, 173.8, 52.3, 39.4, 21.8. IR (KBr) ν 3389, 1647, 1540, 1290, 1224,

1047, 916, 767 cm^{-1}. MS (ESI) HRMS Calcd for $C_{10}H_{17}N_2O_6S_4$: 388.9969. Found: 388.9941.



General procedure for synthesis of the di(but-3-yn-1-yl) 3,3'-trisulfanediyl(2*R*,2'*R*)-bis(2-acetamidopropanoate) (9g).

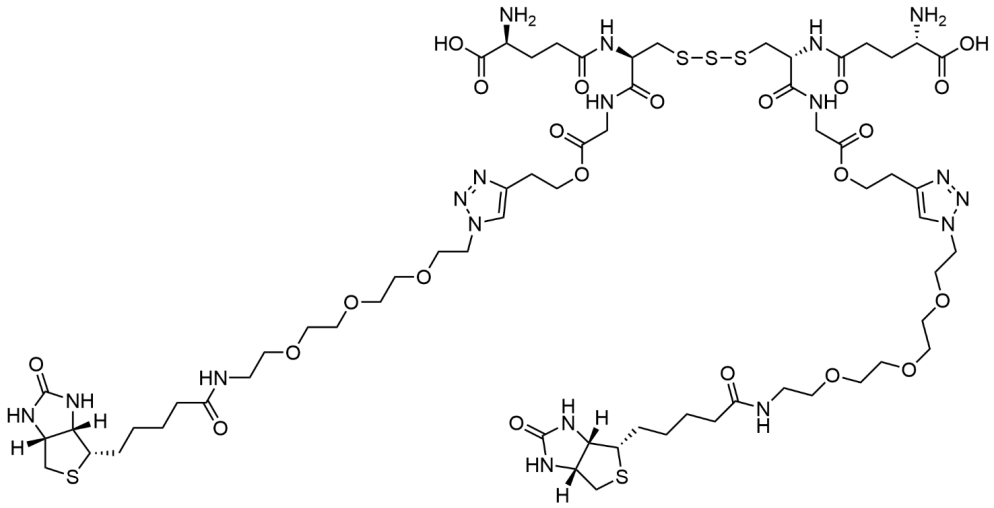
In a two-necked flask were placed (2*R*,2'*R*)-3,3'-trisulfanediylbis(2-acetamidopropanoic acid) (64.9 mg, 0.2 mmol) and 3-butyn-1-ol (0.5 mL), and chlorotrimethylsilane (0.2 mL, 1.6 mmol, 8 equiv.) was added dropwise under stirring with a magnetic stirrer. The mixture was stirred for 22 h at room temperature under an argon atmosphere. The solvent was removed under reduced pressure, and the residue was washed with H_2O, MeCN, and hexane, giving di(but-3-yn-1-yl) 3,3'-trisulfanediyl(2*R*,2'*R*)-bis(2-acetamidopropanoate) **9g** (87.5 mg, 95%). **9g**: Colorless solid. M.p. 145.7-146.0°C (AcOEt). $[\alpha]_D^{32}$−89.0° (c 0.1, CH_3Cl). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.59 (2H, d, *J* = 7.6 Hz), 4.96 (2H, dt, *J* = 7.7, 4.9 Hz), 4.29 (4H, t, *J* = 6.5 Hz), 3.45 (4H, d, *J* = 4.9 Hz), 2.58 (4H, td, *J* = 6.6, 2.6 Hz), 2.08 (6H, s), 2.04 (2H, t, *J* = 2.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.01, 169.95, 79.4, 70.4, 63.5, 51.8, 41.5, 23.1, 18.9. IR (KBr) ν 3276, 3081, 2351, 2301, 1726, 1648, 1547, 1374, 1338, 1244, 1036 cm^{-1}. MS (ESI) HRMS Calcd for $C_{18}H_{25}N_2O_6S_3$: 461.0875. Found: 461.0870.



General procedure for synthesis of the di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (10g).

In a two-necked flask were placed (10*R*,16*R*,21*S*)-21-amino-10-((*S*)-4-amino-4-carboxybutanamido)-16-((2-(but-3-yn-1-yloxy)-2-oxoethyl)carbamoyl)-6,9,18-trioxo-5-oxa-12,13,14-trithia-8,17-diazadocos-1-yn-22-oic acid (18.7 mg, 0.025 mmol), biotin-PEG3-azide (22.2 mg, 0.05 mmol, 2 equiv.), $CuSO_4$ (2.0 mg, 0.0125 mmol, 50 mol%), and sodium L-ascorbate (5.0 mg, 0.025 mmol, 1 equiv.) in H_2O (0.2 mL) and THF (0.3 mL) under an argon atmosphere and the mixture was stirred for 7 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified by reversed phase column chromatography, giving (8*R*,14*R*,19*S*)-19-amino-8-((*S*)-4-amino-4-carboxybutanamido)-4,7,16-trioxo-1-(1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1*H*-1,2,3-triazol-4-yl)-14-((2-oxo-2-(2-(1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1*H*-1,2,3-triazol-4-yl)ethoxy)ethyl)carbamoyl)-3-oxa-10,11,12-trithia-6,15-diazaicosan-20-oic acid **10g** (40.5 mg, 99%). **10g**: Colorless solid. M.p. 107.5-108.0°C (MeCN/H_2O = 9). $[\alpha]_D^{32}$+51.0° (c 0.1, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ 7.95 (2H, s), 4.84 (4H, dd, *J* = Hz), 4.65-4.61 (6H,

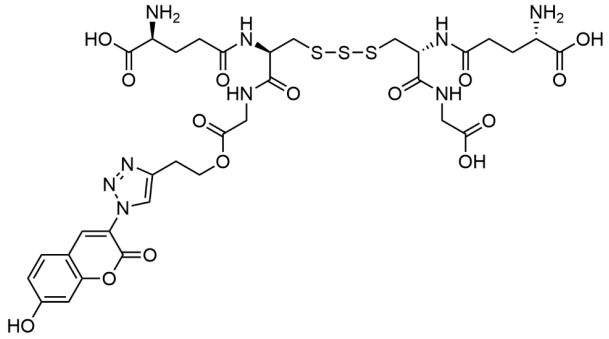
m), 4.48-4.42 (6H, m), 4.10-3.98 (8H, m), 3.79 (2H, t, *J* = 6.3 Hz), 3.67-3.61 (22H, m), 3.40 (4H, t, *J* = 5.2 Hz), 3.56-3.31 (4H, m), 3.19-3.11 (6H, m), 3.00 (2H, dd, *J* = 13.0, 4.9 Hz), 2.79 (2H, d, *J* = 13.0 Hz), 2.57-2.53 (2H, m), 2.28 (4H, t, *J* = 7.2 Hz), 1.79-1.54 (10H, m), 1.45-1.38 (4H, m). ^{13}C-NMR (100 MHz, D_2O) δ 176.9, 174.8, 173.9, 172.4, 171.0, 165.3, 144.4, 124.3, 69.8, 69.7, 69.6, 69.5, 68.94, 68.89, 64.5, 62.1, 60.3, 55.4, 54.1, 52.6, 50.1, 41.4, 39.8, 39.0, 38.9, 35.5, 31.4, 28.0, 27.8, 26.2, 25.2, 24.5. IR (KBr) ν 3282, 3075, 2934, 1680, 1549, 1454, 1412, 1360, 1202, 1116, 689 cm^{-1}. MS (ESI) HRMS Calcd for $C_{64}H_{105}N_{18}O_{22}S_5$:



1637.6254. Found: 1637.6236.

General procedure for synthesis of the N^5-((*R*)-3-(((*R*)-2-((*S*)-4-amino-4-carboxybutanamido)-3-((2-(2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethoxy)-2-oxoethyl)amino)-3-oxopropyl)trisulfaneyl)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-*L*-glutamine (12g).

N^5-((*R*)-3-(((*R*)-2-((*S*)-4-amino-4-carboxybutanamido)-3-((2-(2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethoxy)-2-oxoethyl)amino)-3-oxopropyl)trisulfaneyl)-1-((2-(but-3-yn-1-yloxy)-2-oxoethyl)amino)-1-oxopropan-2-yl)-*L*-glutamine **11g** (3.0 mg, 3.2 μmol) was dissolved PBS buffer (pH 7, 0.1 mL) in an Eppendorf tube. A suspension of porcine liver esterase in ammonium sulfate solution (Sigma-Aldrich, E2884, 300 units) was added, and the mixture was incubated at 37°C for 1 h. After incubation, the reaction mixture was purified by reversed-phase column chromatography, giving N^5-((*R*)-3-(((*R*)-2-((*S*)-4-amino-4-carboxybutanamido)-3-((2-(2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethoxy)-2-oxoethyl)amino)-3-oxopropyl)trisulfaneyl)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-*L*-glutamine **7** (2.2 mg, 2.4 μmol, 78%) with the recovery of **6** (0.5 mg, 0.53 μmol, 17%). **12g**: Green solid. M.p. 208.2°C decomposed (MeCN/H_2O = 10). $[\alpha]_D^{32}$−511.0° (c 0.1, DMSO). ^{1}H-NMR (400 MHz, D_2O) δ 8.42 (1H, s), 8.33 (1H, s), 7.68 (1H, d, *J* = 8.6 Hz), 6.97 (1H, d, *J* = 8.6 Hz), 6.93 (1H, s), 4.67 (1H, dd, *J* = 9.4, 4.4 Hz), 4.62 (1H, dd, *J* = 9.1, 4.1 Hz), 4.51 (2H, t, *J* = 5.0 Hz), 4.03 (1H, d, *J* = 17.7 Hz), 3.95 (1H, d, *J* = 17.7 Hz), 3.78-3.68 (6H, m), 3.26 (1H, dd, *J* = 14.5, 4.1 Hz), 3.17-3.11 (1H, m), 3.01 (1H, dd, *J* = 14.5, 9.1 Hz), 2.93 (1H, dd, *J* = 14.5, 9.4 Hz), 2.51-2.44 (4H, m), 2.14-2.09 (4H, m). ^{13}C-NMR (150 MHz, D_2O) δ 174.81, 174.75, 173.83, 173.79, 173.74, 172.3, 171.4, 171.0, 161.8, 158.8, 154.8, 144.6, 138.5, 131.3, 124.8, 119.2, 114.9, 111.2, 102.8, 69.6, 64.2, 54.2, 52.5, 52.4, 42.3, 41.5, 38.9, 38.7, 31.7, 31.3, 26.2, 26.1, 24.5. IR (KBr) ν 3418, 2923, 1731, 1614, 1556, 1394, 1232, 1121 cm^{-1}. MS (ESI) HRMS Calcd for $C_{33}H_{42}N_9O_{15}S_3$: 900.1962. Found: 900.1996.



UV-Vis and photoluminescence spectroscopic analysis of 12g

(a) (b)

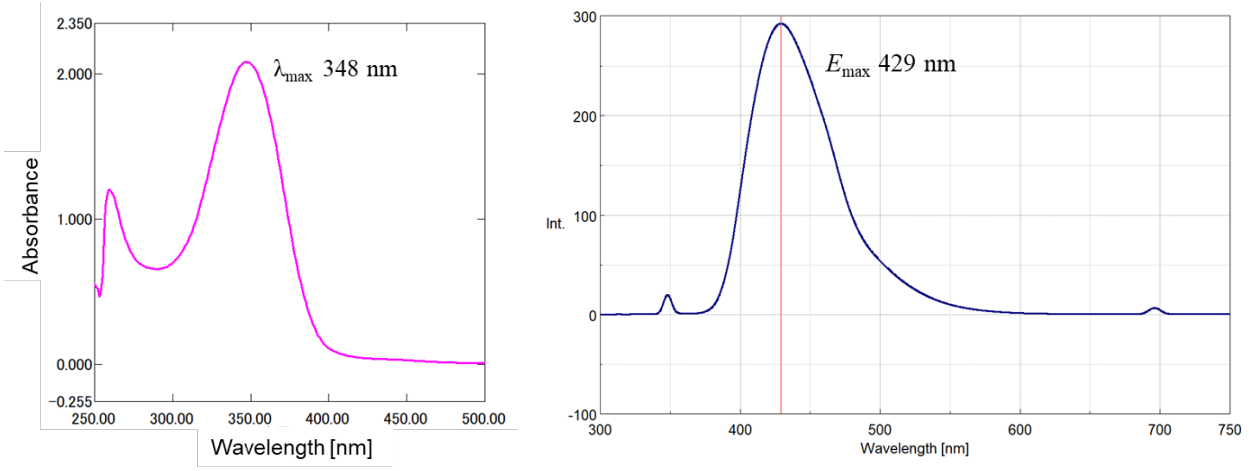


Figure S1. (a) UV-Vis and (b) photoluminescence (Excitation at 348 nm) spectra of **12g** in DMSO (1.9 μM) at room temperature.

Crystal data

Crystal data of 1

Single colorless plate-shaped crystals of GSSSG **1** were obtained by recrystallization from H_2O. The structure of **1** was determined by X-ray crystal structure analysis. X-Ray crystallography: CCDC 2481054 contains the supplementary crystallographic data for this paper. This data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

A suitable crystal 0.08×0.04×0.03 mm^3 was selected and mounted on a suitable support on an XtaLAB Synergy R, DW system, HyPix diffractometer. The crystal was kept at a steady T = 99.99(10) K during data collection. The structure was solved with the ShelXT (Sheldrick, 2015) structure solution program using the Intrinsic Phasing solution method and by using Olex2 (Dolomanov et al., 2009) as the graphical interface. The model was refined with version 2018/3 of ShelXL 2018/3 (Sheldrick, 2015) using Least Squares minimisation.

Table S1. Crystal data for **1**

Formula	$C_{20}H_{36}N_6O_{14}S_3$
Formula Weight	680.73
Crystal System	triclinic
Space Group	P1
$D_{calc.}$/ g cm^{-3}	1.539
μ/mm^{-1}	2.998
Colour	colourless
Shape	plate
Size/mm^3	0.08×0.04×0.03
T/K	99.99(10)
Flack Parameter	-0.017(10)
Hooft Parameter	-0.019(8)
Space Group	*P*1
a/Å	5.34785(12)
b/Å	9.40623(18)
c/Å	14.69319(20)
α/°	83.7804(13)
β/°	88.7700(14)
γ/°	89.4031(17)
V/Å^3	734.57(2)
Z	1
Z'	1
Wavelength/Å	1.54184
Radiation type	Cu K_α
Θ_{min}/°	3.026
Θ_{max}/°	74.480
Measured Refl.	19904
Independent Refl.	5503
Reflections with I > 2(I)	5296
R_{int}	0.0512
Parameters	404
Restraints	9
Largest Peak	0.336
Deepest Hole	-0.335
GooF	1.060
wR_2 (all data)	0.0829
wR_2	0.0820
R_1 (all data)	0.0341

R_1	0.0326

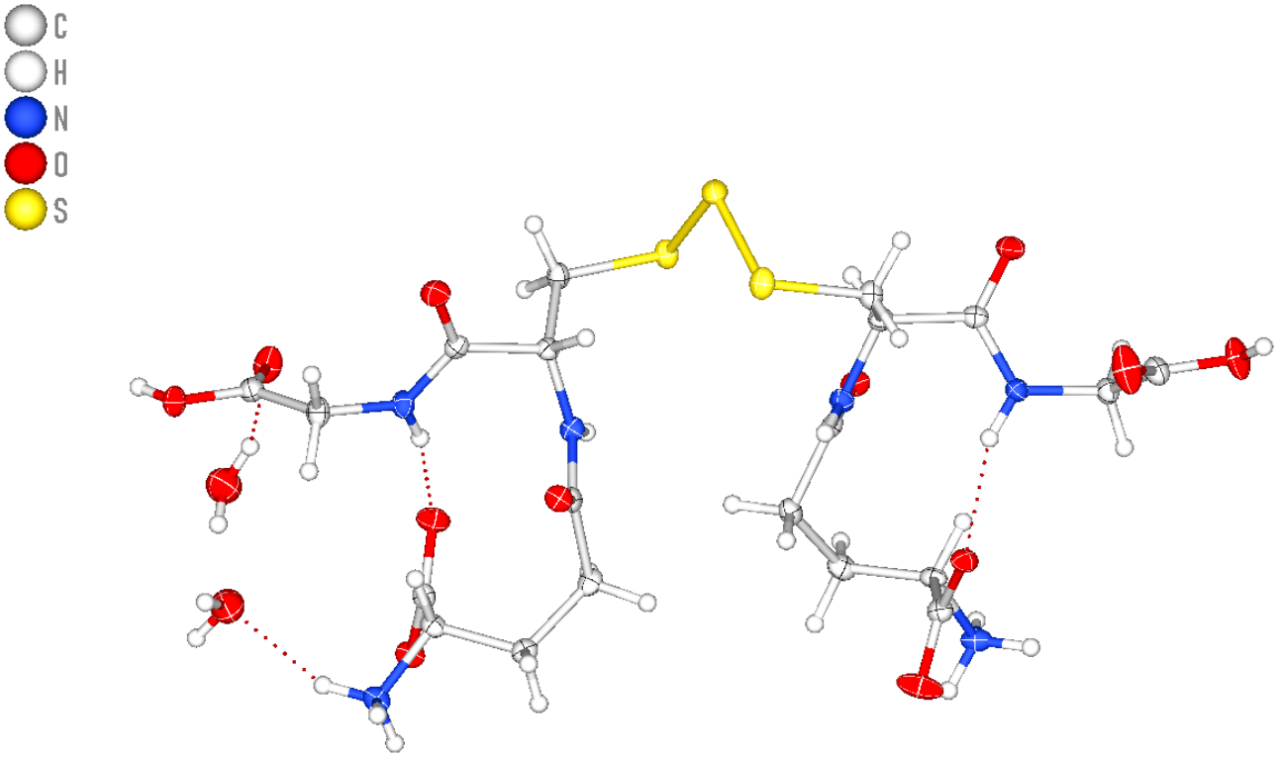


Figure S2. ORTEP view of **1** with thermal ellipsoids drawn at the 50% probability level.

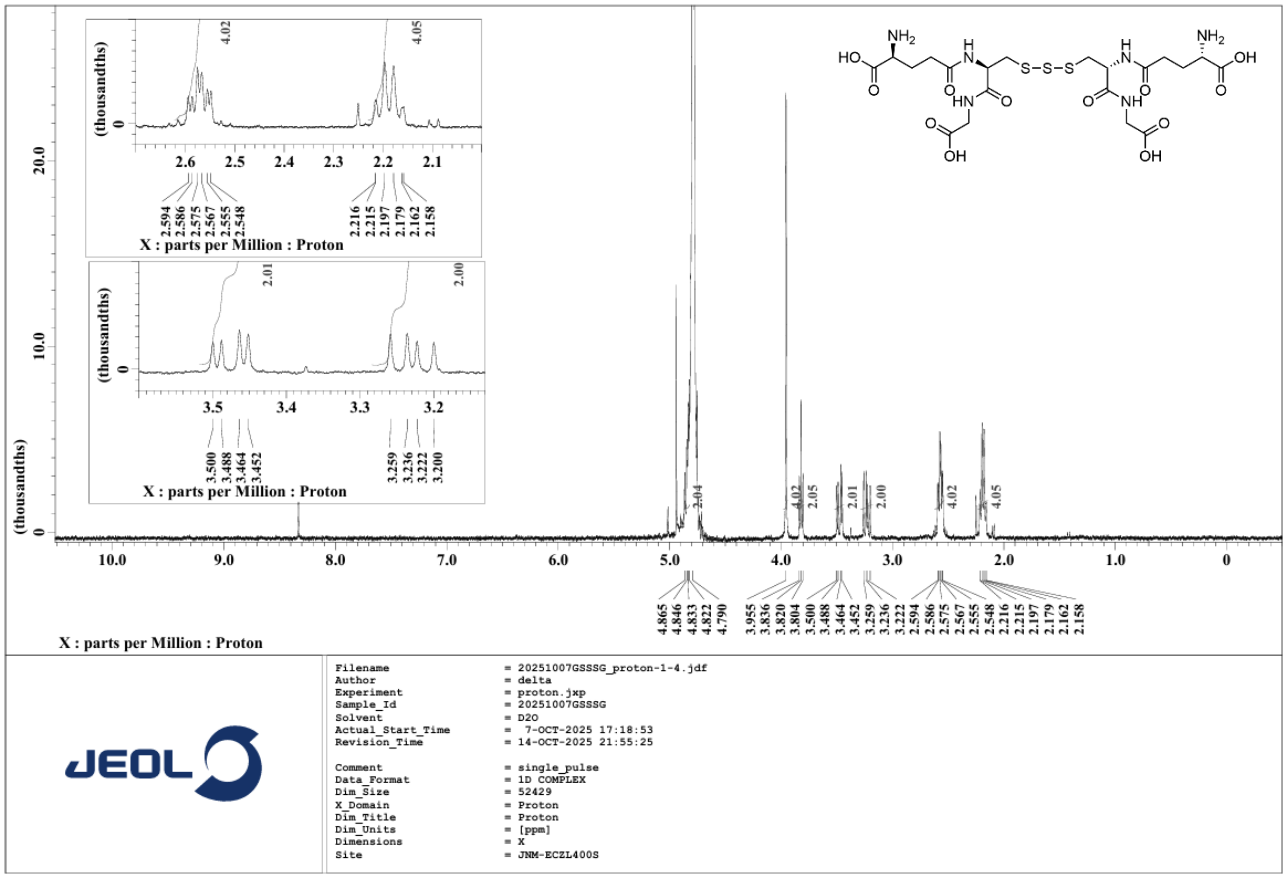
Reference

[S1] K. Fukumoto, M. Yazaki and M. Arisawa, Rhodium-Catalyzed Synthesis of Peptide Polysulfides by Insertion of Sulfur into Unprotected Peptide Disulfides. *Org. Lett.,* 2022, **24**, 8176−8179.

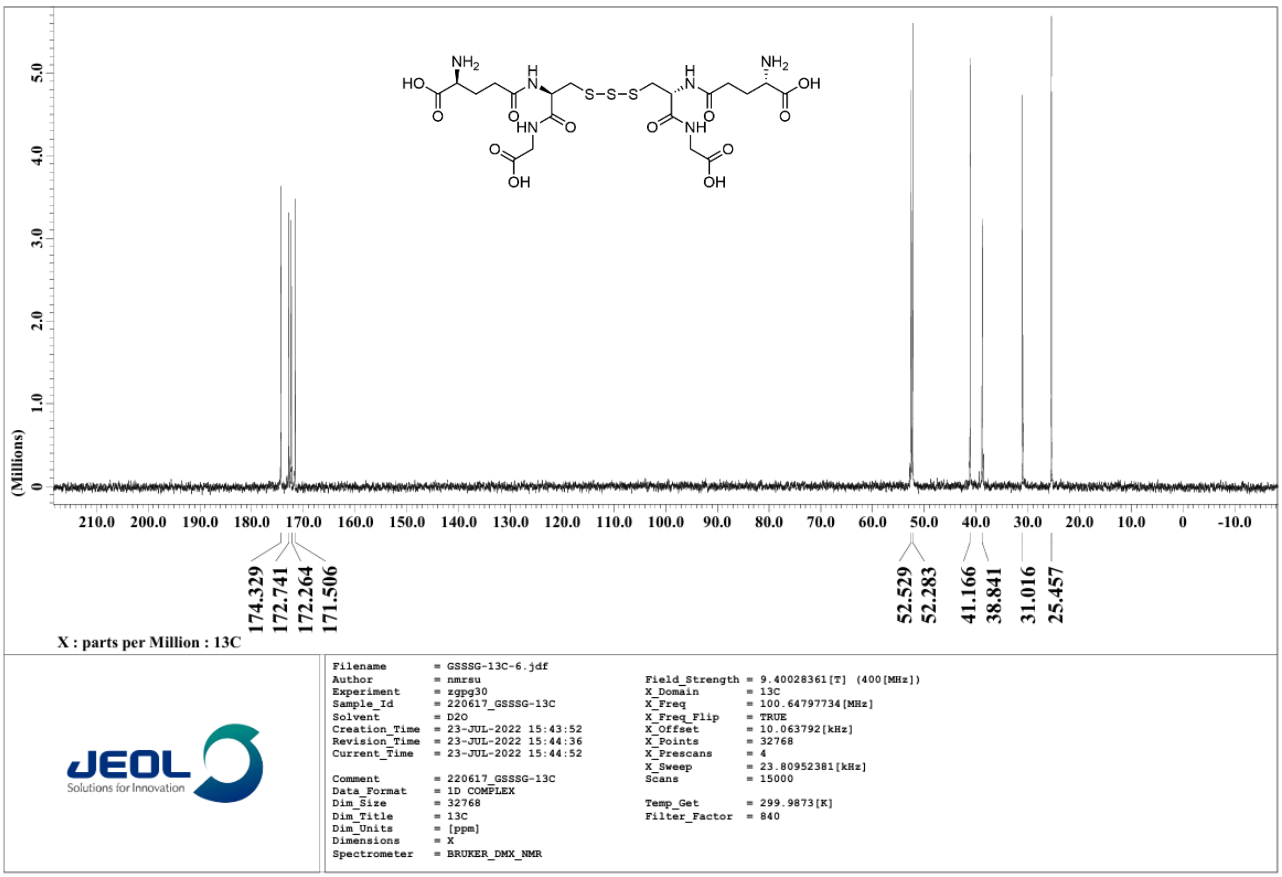
[S2] E. M. Brown and N. B. Bowden, Stabilities of Three Key Biological Trisulfides with Implications for Their Roles in the Release of Hydrogen Sulfide and Bioaccumulation of Sulfane Sulfur. *ACS Omega*, 2022, **7**, 11440−11451.

[S3] M. M. Cerda, M. D. Hammers, M. S. Earp, L. N. Zakharov and M. D. Pluth, Applications of Synthetic Organic Tetrasulfides as H_2S Donors. *Org. Lett.*, 2017, **19**, 2314−2317.

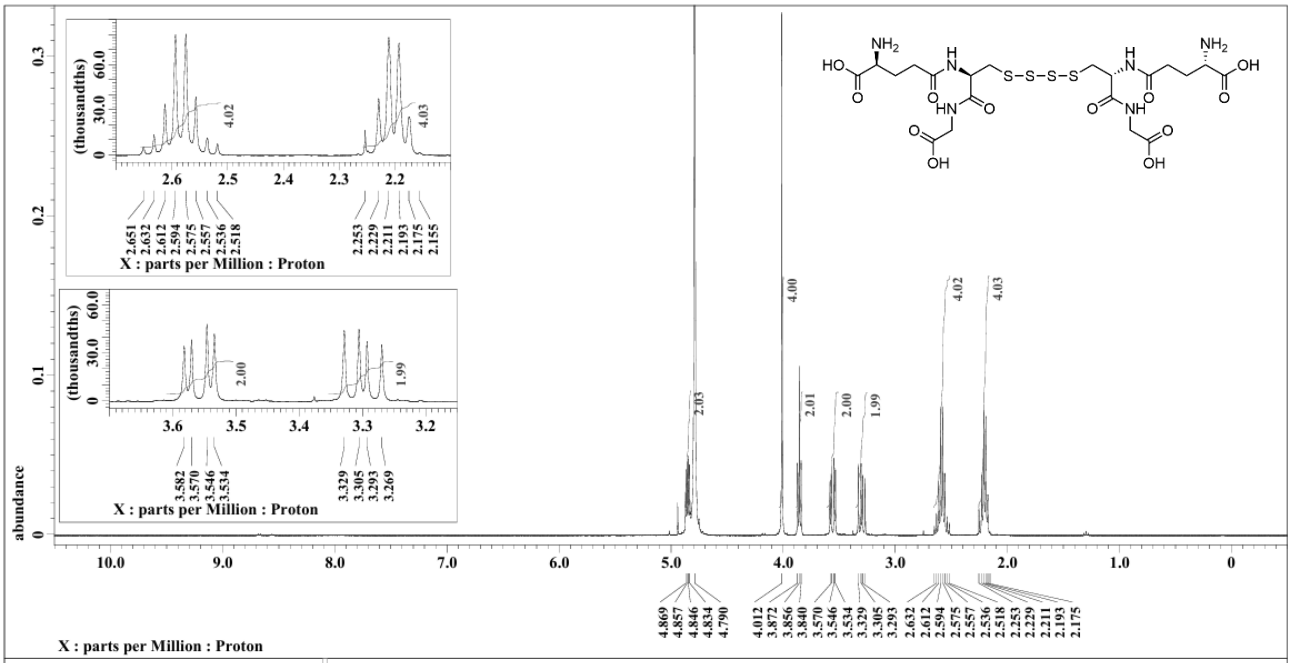
Glutathione trisulfide (1) ^{1}H-NMR



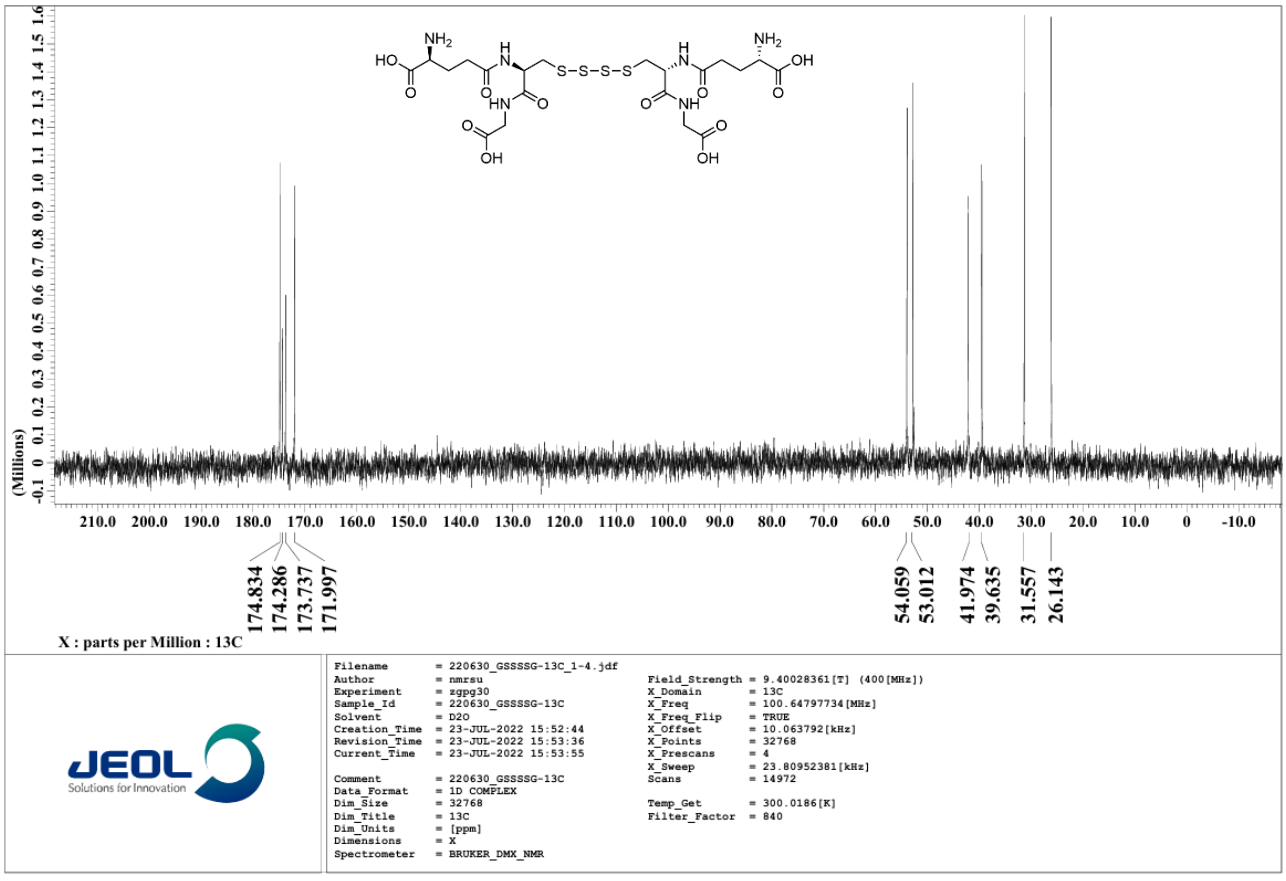
Glutathione trisulfide (1) ^{13}C-NMR



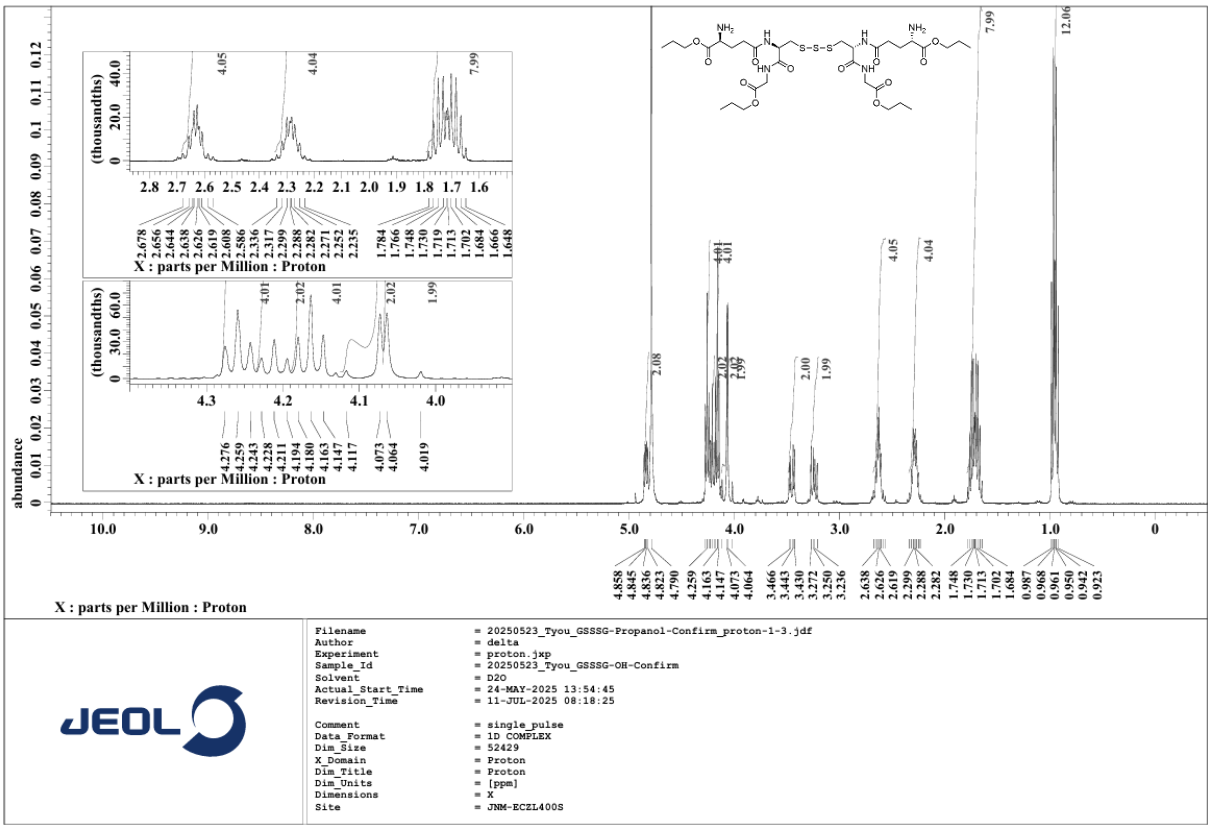
Glutathione tetrasulfide (2) ^{1}H-NMR



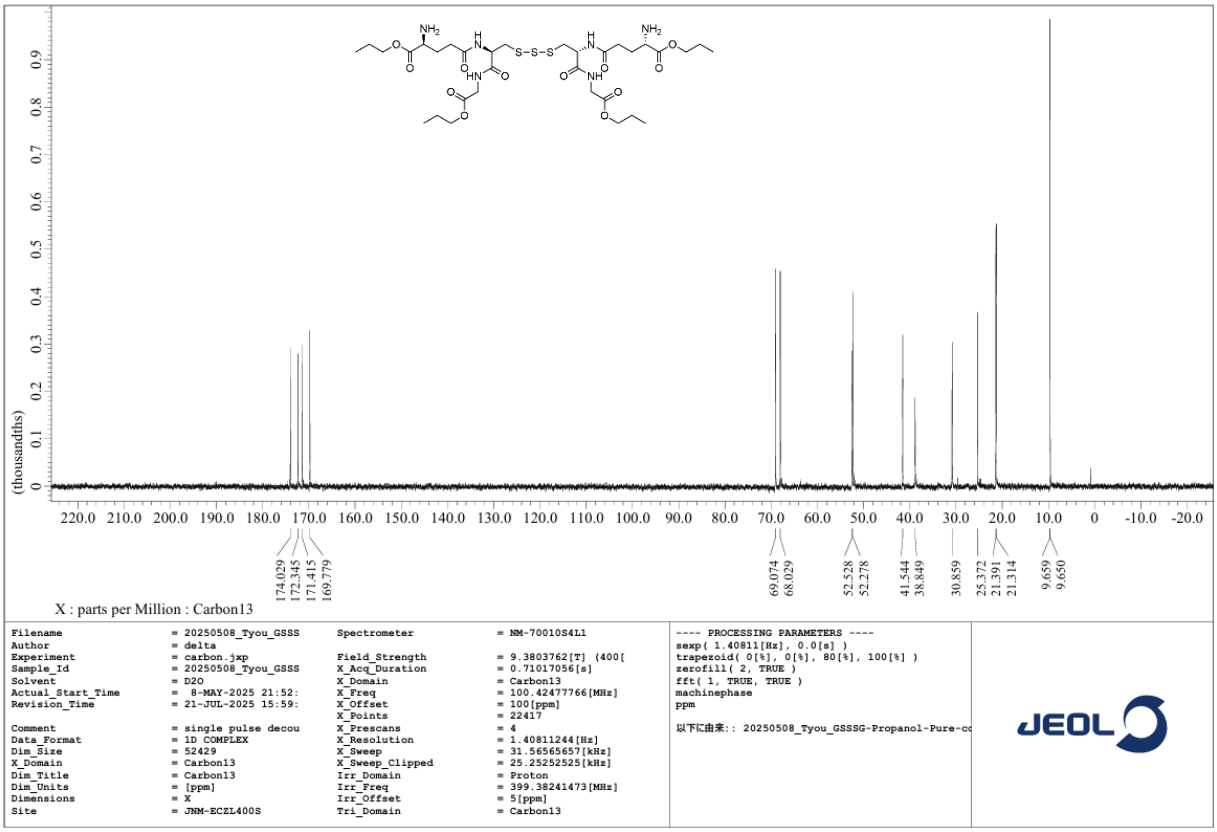
Glutathione tetrasulfide (2) ^{13}C-NMR



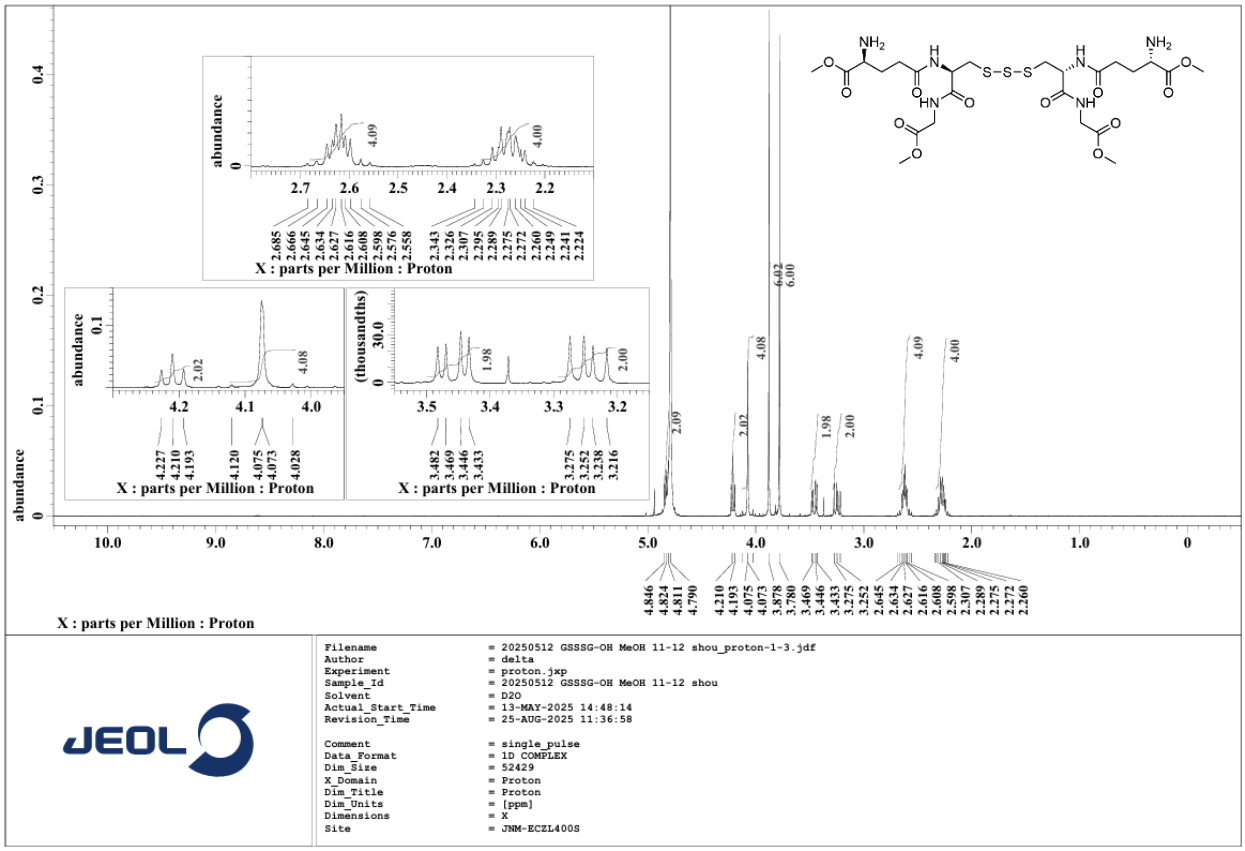
Dipropyl 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosane-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6a) ^{1}H-NMR



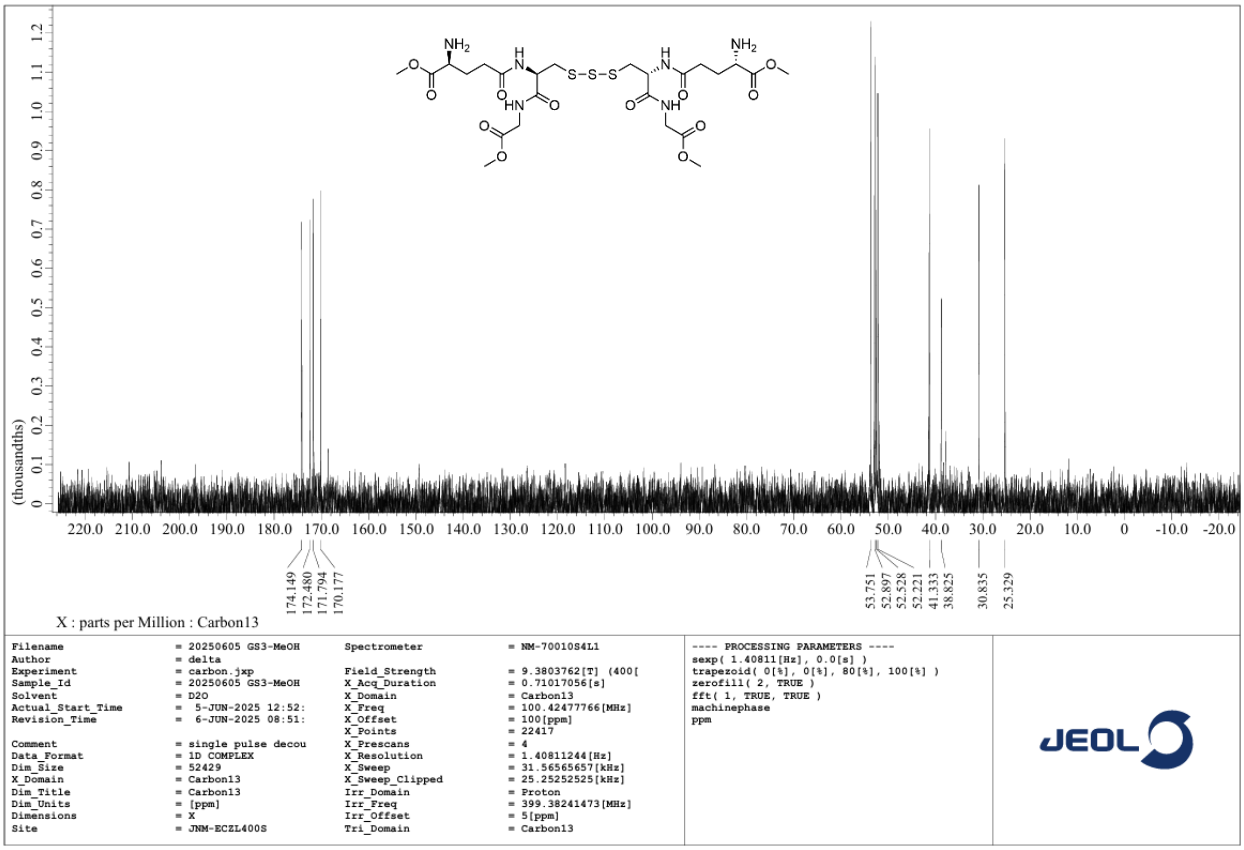
Dipropyl 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosane-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6a) ^{13}C-NMR



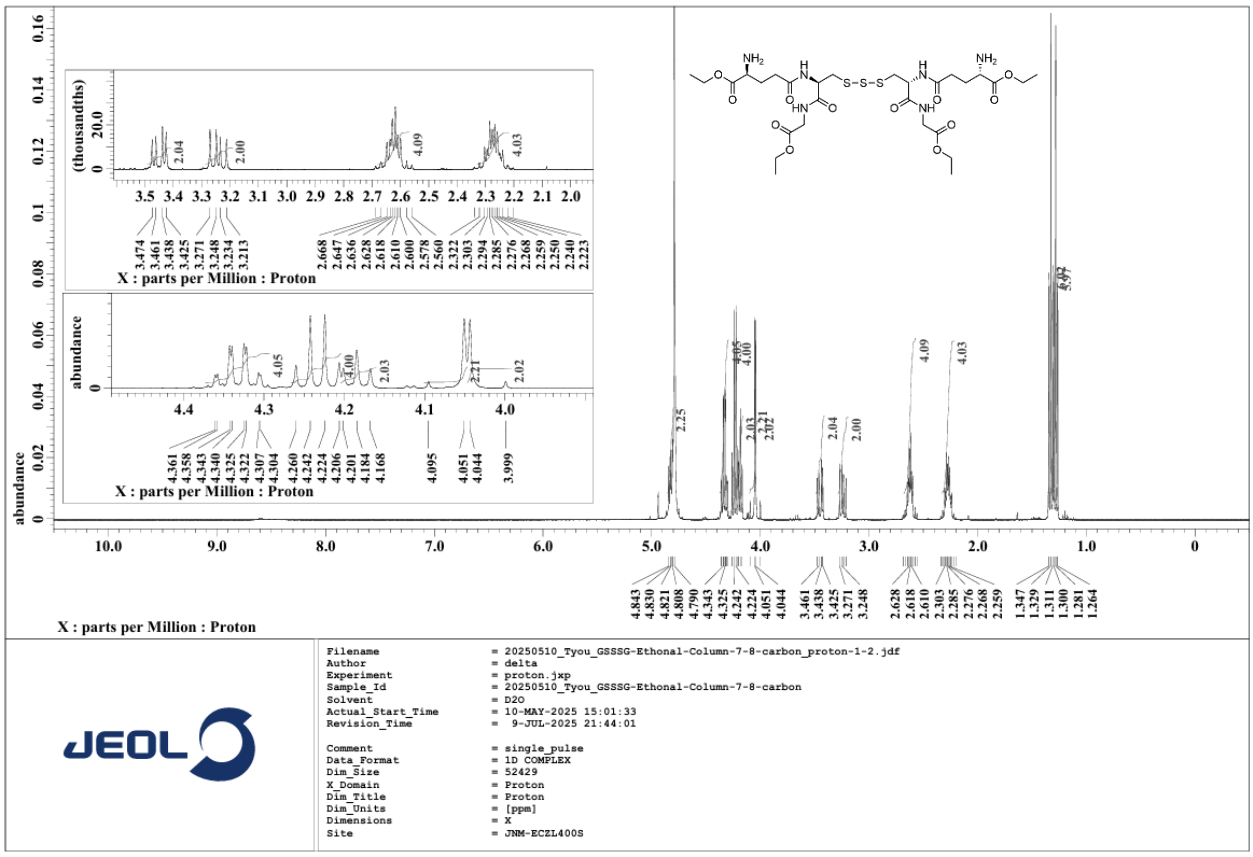
Dimethyl 5,5'-(((7*R*,13*R*)-3,6,14,17-tetraoxo-2,18-dioxa-9,10,11-trithia-5,15-diazanonadecane-7,13-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6b) ^{1}H-NMR



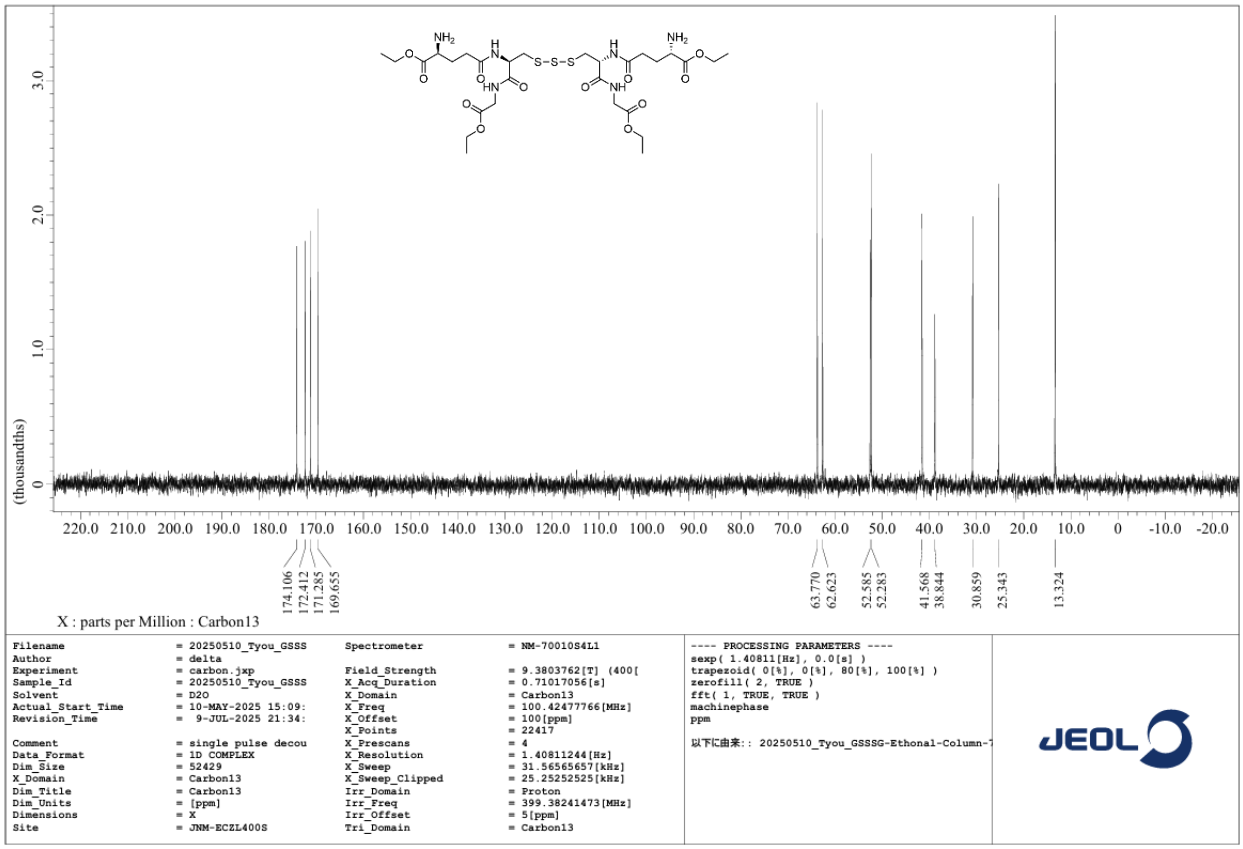
Dimethyl 5,5'-(((7*R*,13*R*)-3,6,14,17-tetraoxo-2,18-dioxa-9,10,11-trithia-5,15-diazanonadecane-7,13-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6b) ^{13}C-NMR



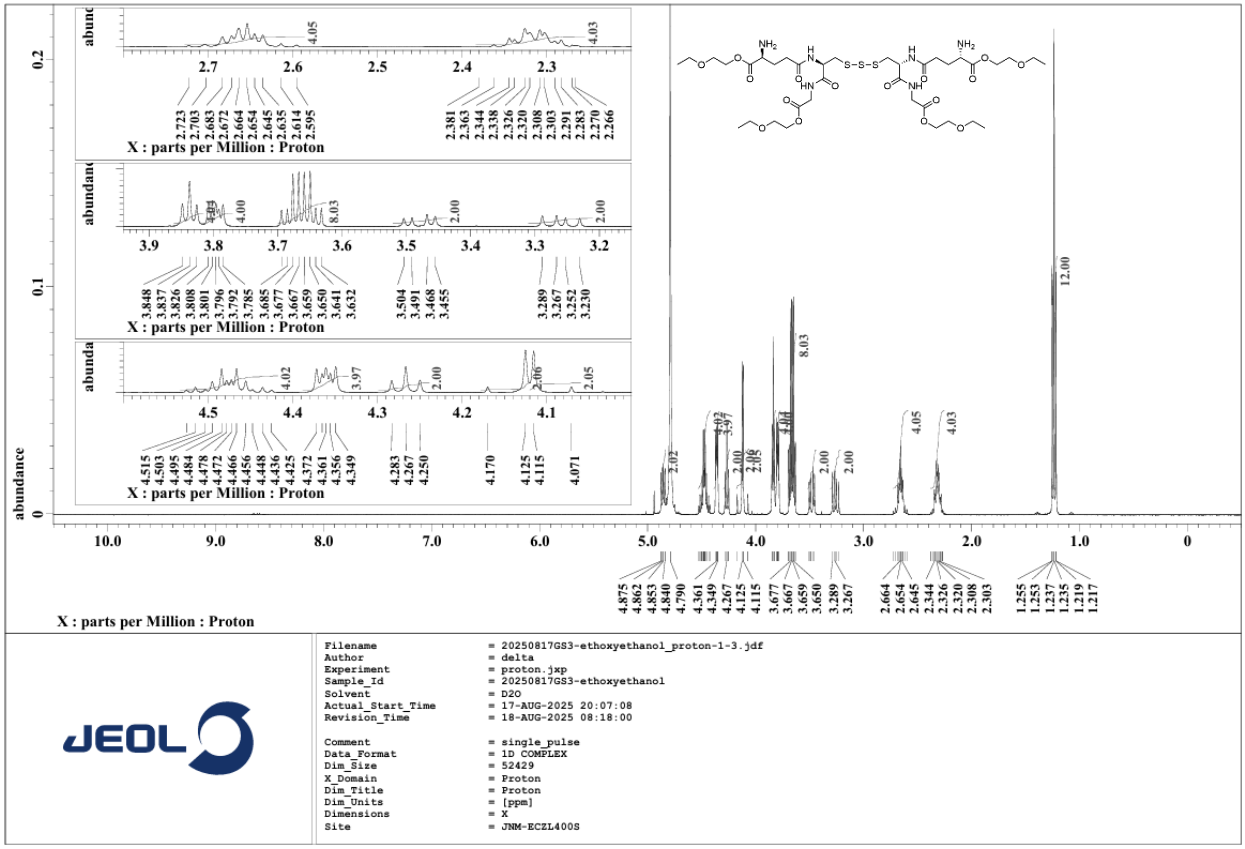
Diethyl 5,5'-(((8*R*,14*R*)-4,7,15,18-tetraoxo-3,19-dioxa-10,11,12-trithia-6,16-diazahenicosane-8,14-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6c) ^{1}H-NMR



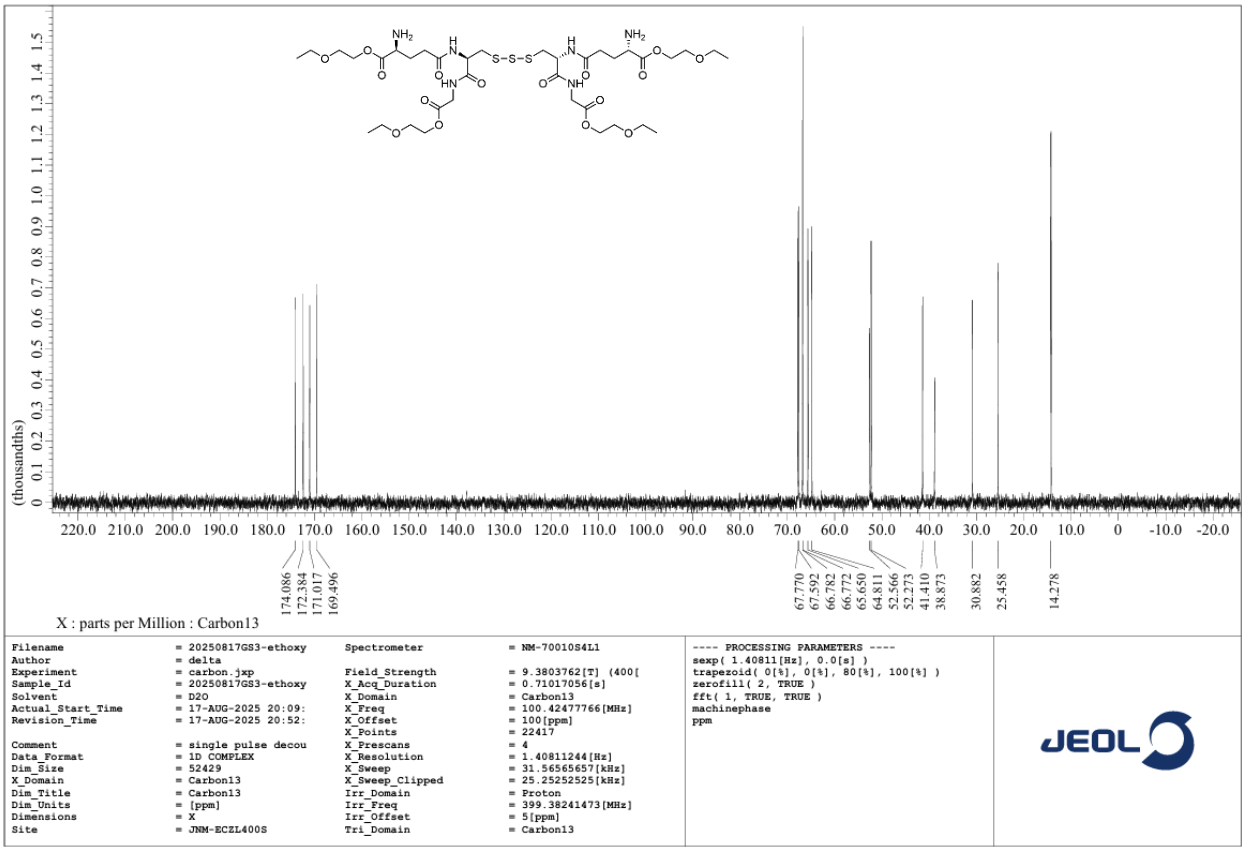
Diethyl 5,5'-(((8*R*,14*R*)-4,7,15,18-tetraoxo-3,19-dioxa-10,11,12-trithia-6,16-diazahenicosane-8,14-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6c) ^{13}C-NMR



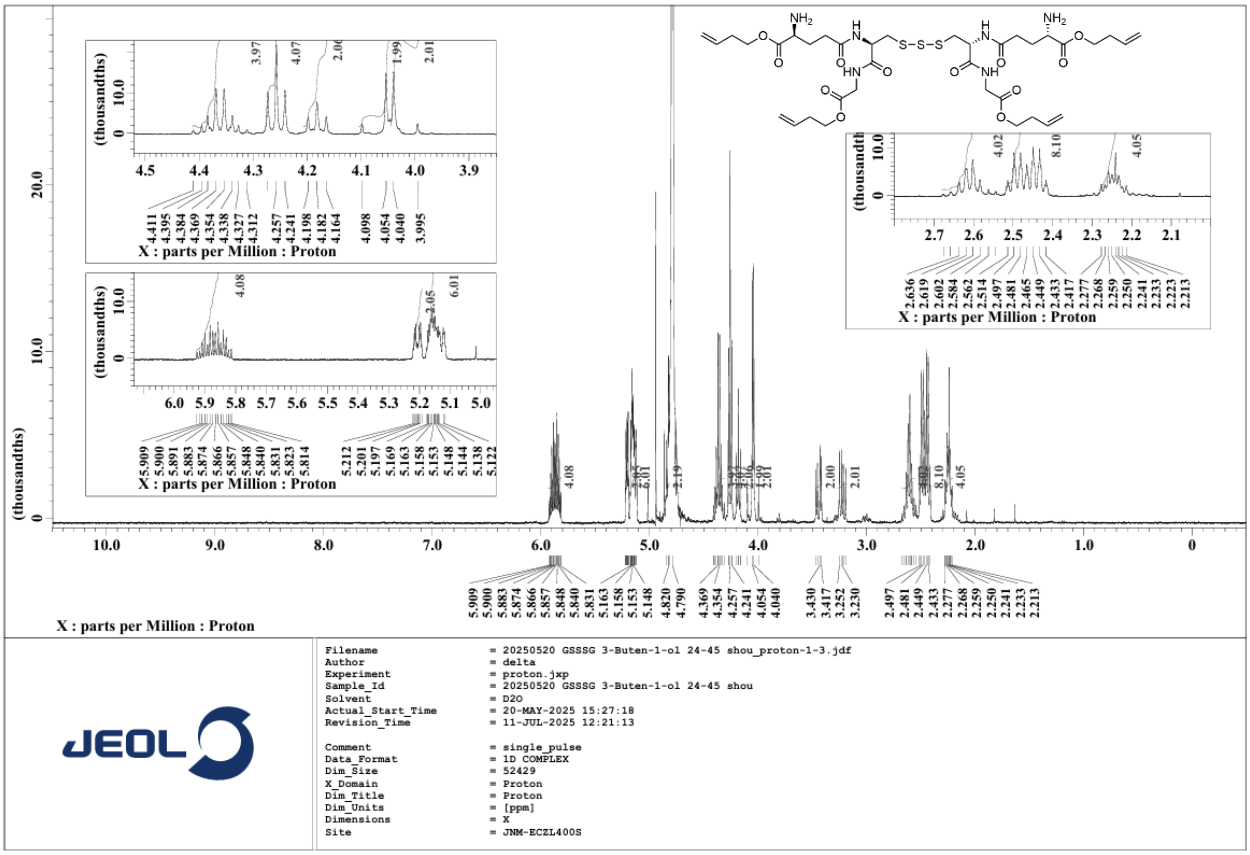
Bis(2-ethoxyethyl) 5,5'-(((11*R*,17*R*)-7,10,18,21-tetraoxo-3,6,22,25-tetraoxa-13,14,15-trithia-9,19-diazaheptacosane-11,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6d) ^{1}H-NMR



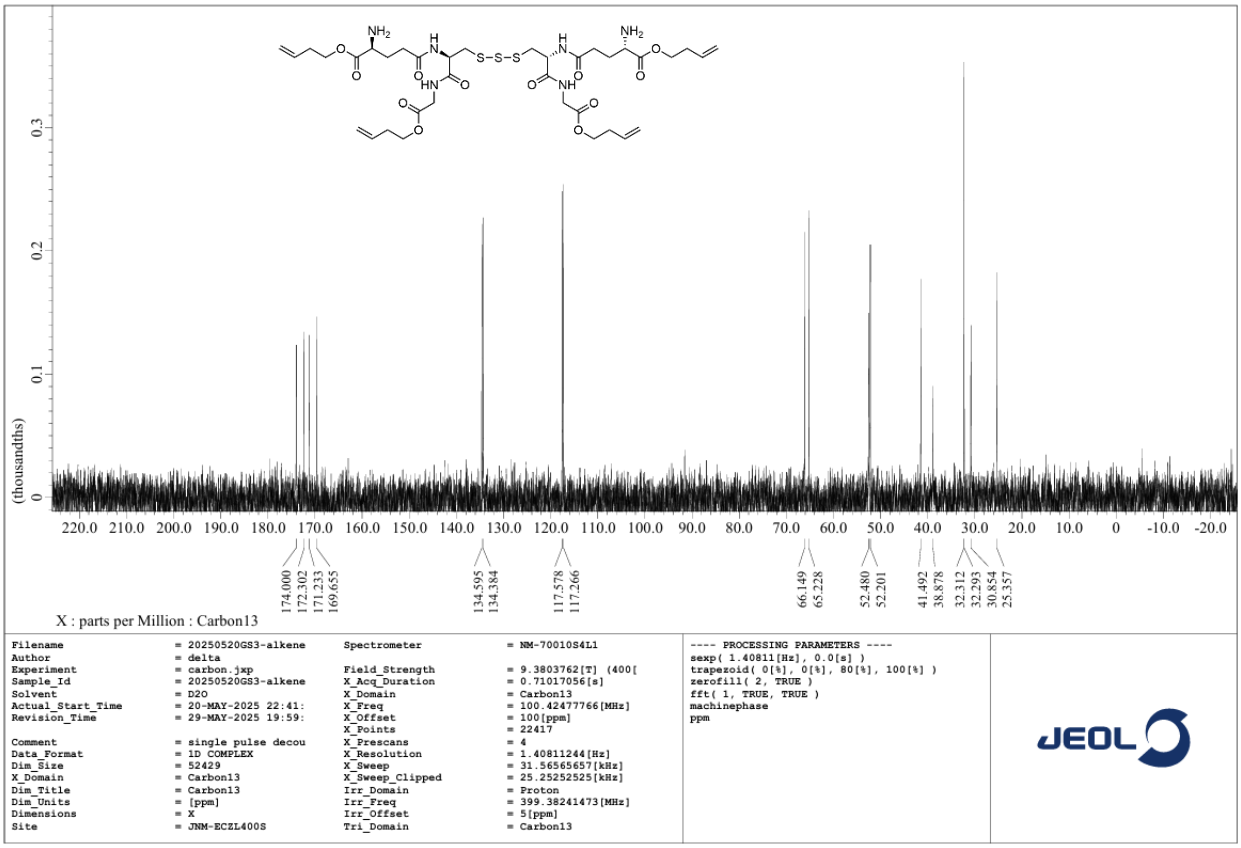
Bis(2-ethoxyethyl) 5,5'-(((11*R*,17*R*)-7,10,18,21-tetraoxo-3,6,22,25-tetraoxa-13,14,15-trithia-9,19-diazaheptacosane-11,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6d) ^{13}C-NMR



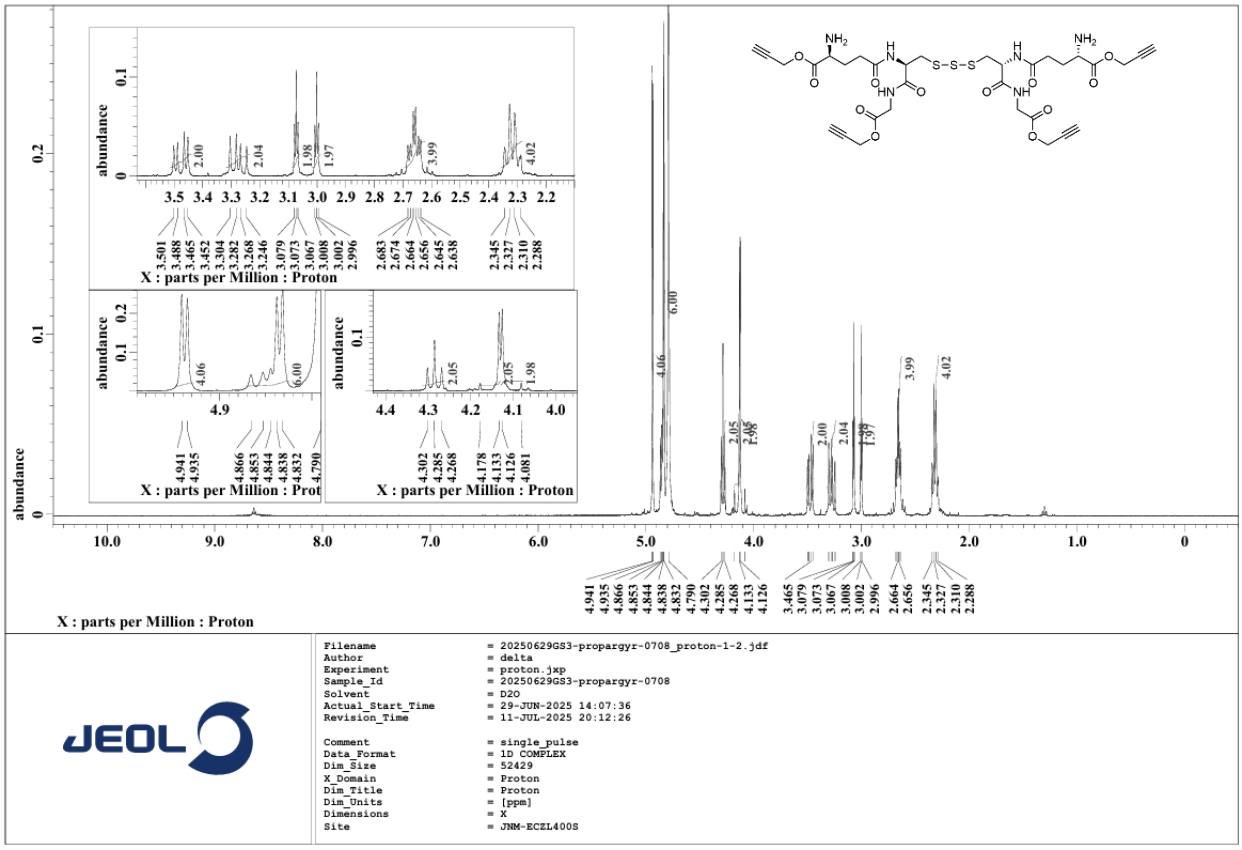
Di(but-3-en-1-yl) 5,5'-(((10*R*,16*R*)-6,9,17,20-tetraoxo-5,21-dioxa-12,13,14-trithia-8,18-diazapentacosa-1,24-diene-10,16-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6e) ^{1}H-NMR



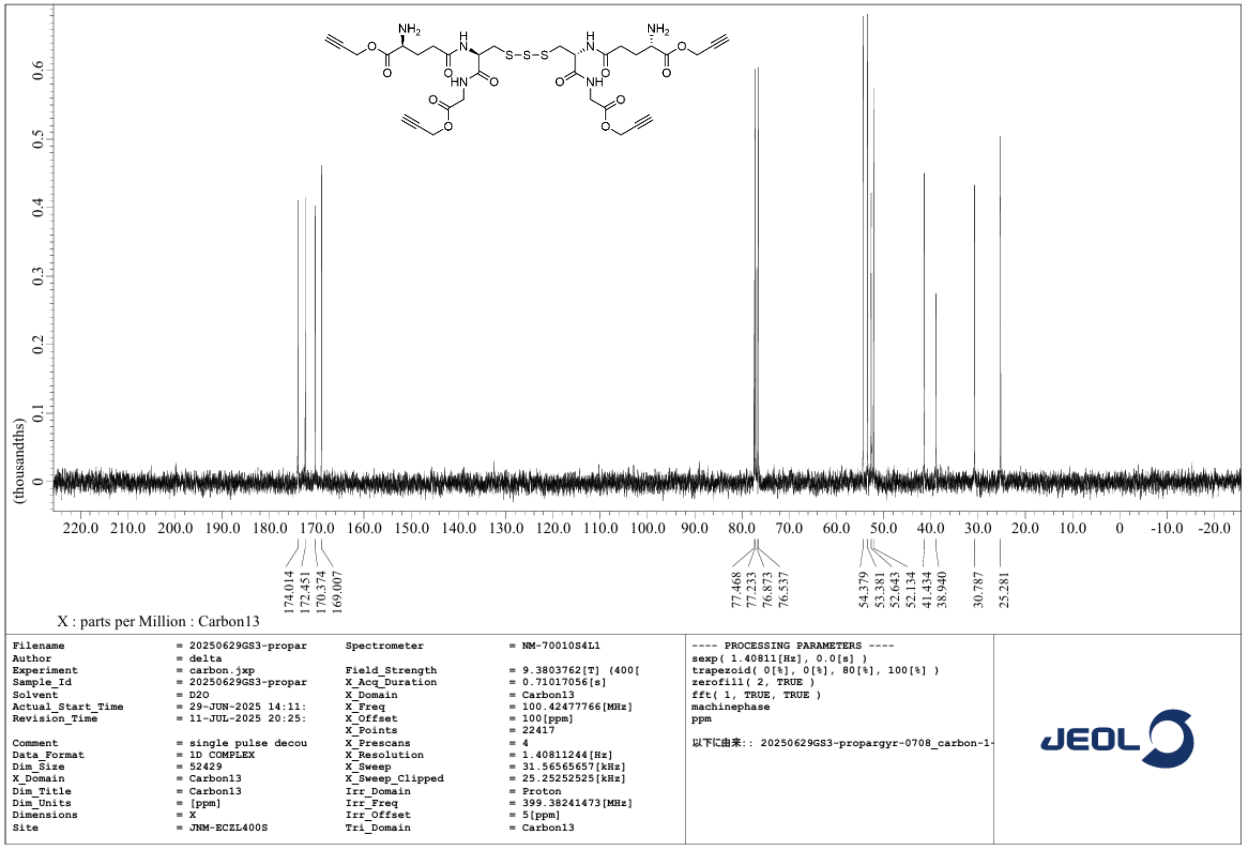
Di(but-3-en-1-yl) 5,5'-(((10*R*,16*R*)-6,9,17,20-tetraoxo-5,21-dioxa-12,13,14-trithia-8,18-diazapentacosa-1,24-diene-10,16-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6e) ^{13}C-NMR



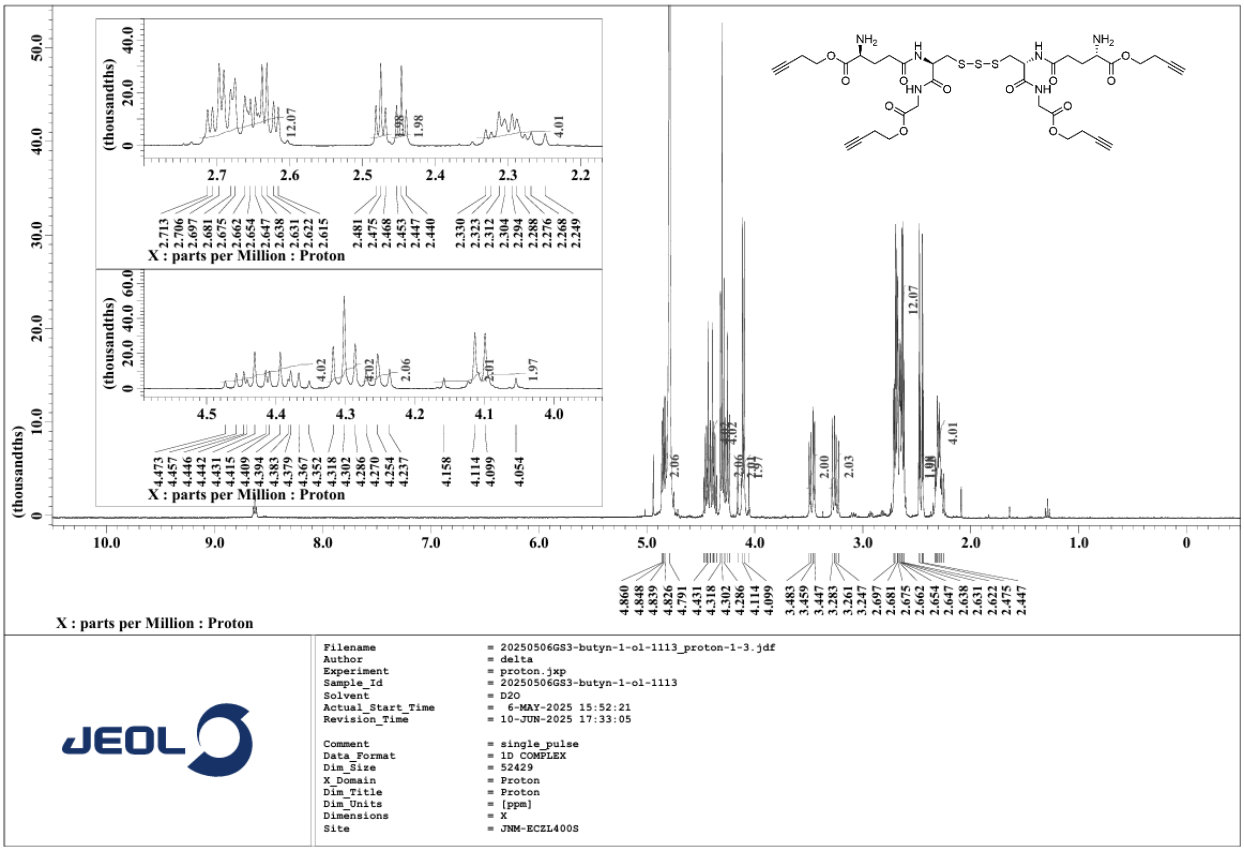
Di(prop-2-yn-1-yl) 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosa-1,22-diyne-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6f) ^{1}H-NMR



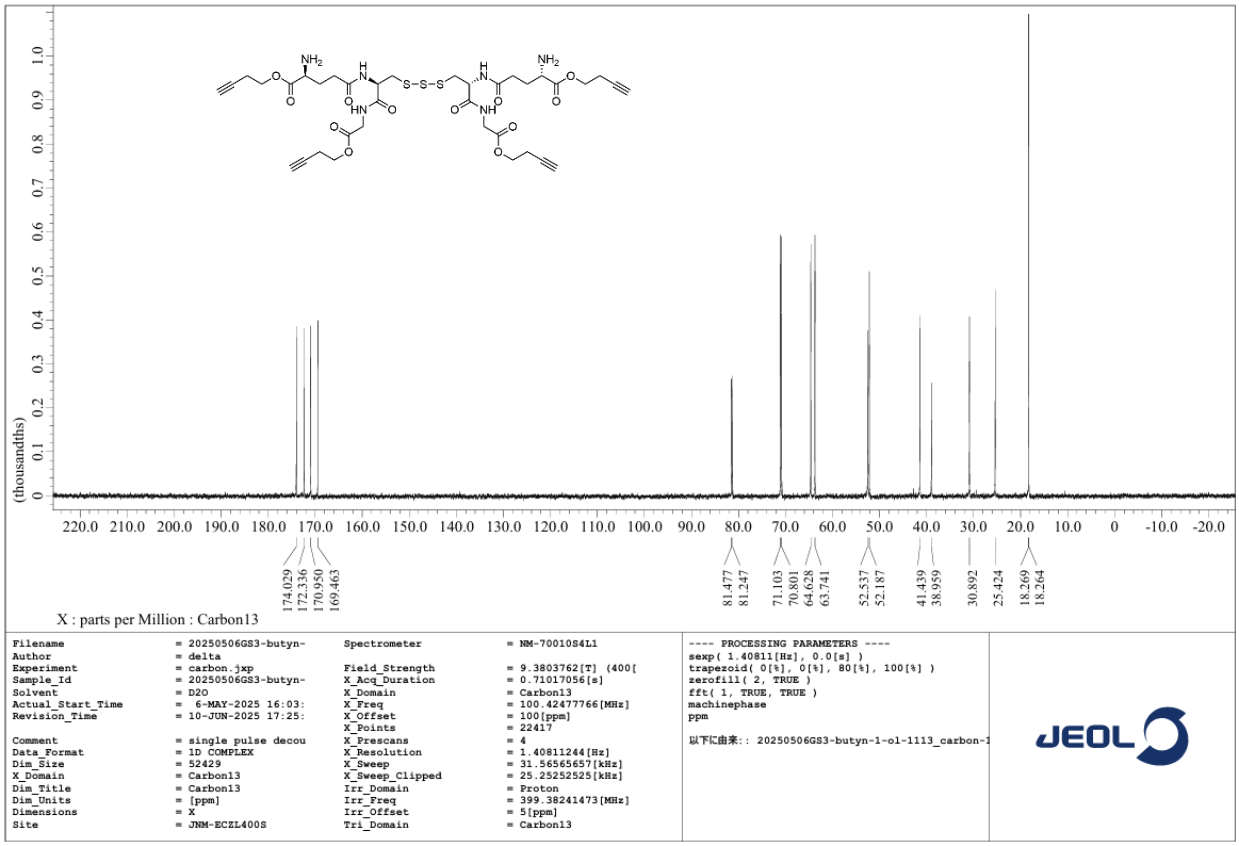
Di(prop-2-yn-1-yl) 5,5'-(((9*R*,15*R*)-5,8,16,19-tetraoxo-4,20-dioxa-11,12,13-trithia-7,17-diazatricosa-1,22-diyne-9,15-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6f) ^{13}C-NMR



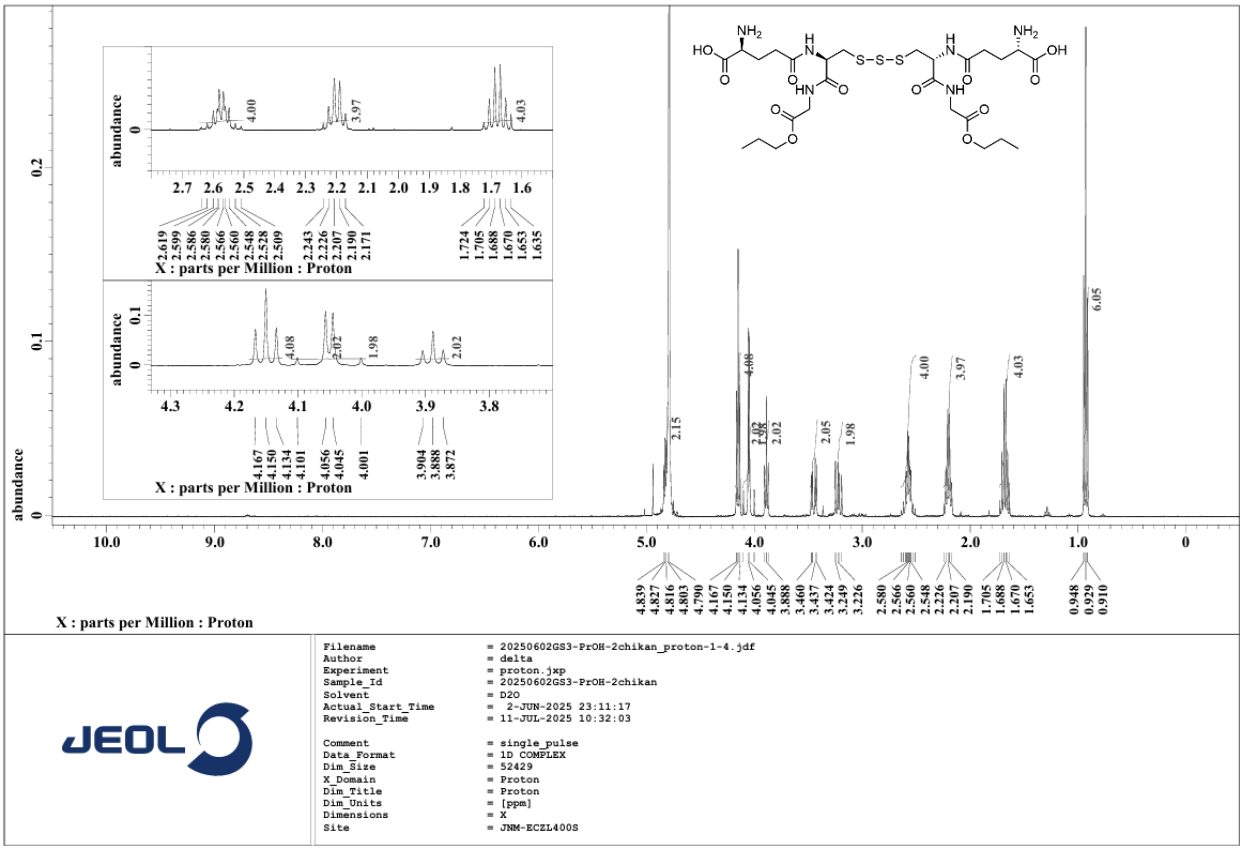
Di(but-3-yn-1-yl) 5,5'-(((10*R*,16*R*)-6,9,17,20-tetraoxo-5,21-dioxa-12,13,14-trithia-8,18-diazapentacosa-1,24-diyne-10,16-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6g) ^{1}H-NMR



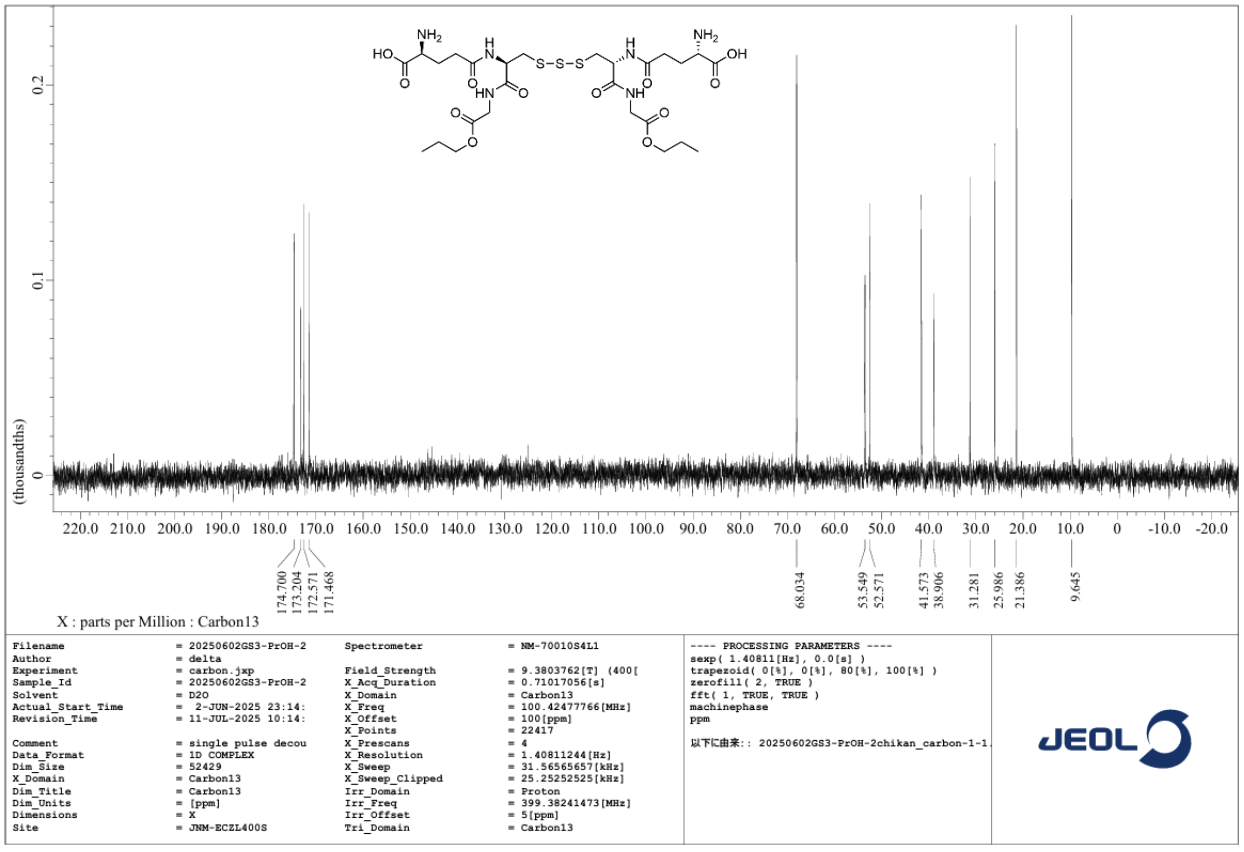
Di(but-3-yn-1-yl) 5,5'-(((10*R*,16*R*)-6,9,17,20-tetraoxo-5,21-dioxa-12,13,14-trithia-8,18-diazapentacosa-1,24-diyne-10,16-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (6g) ^{13}C-NMR



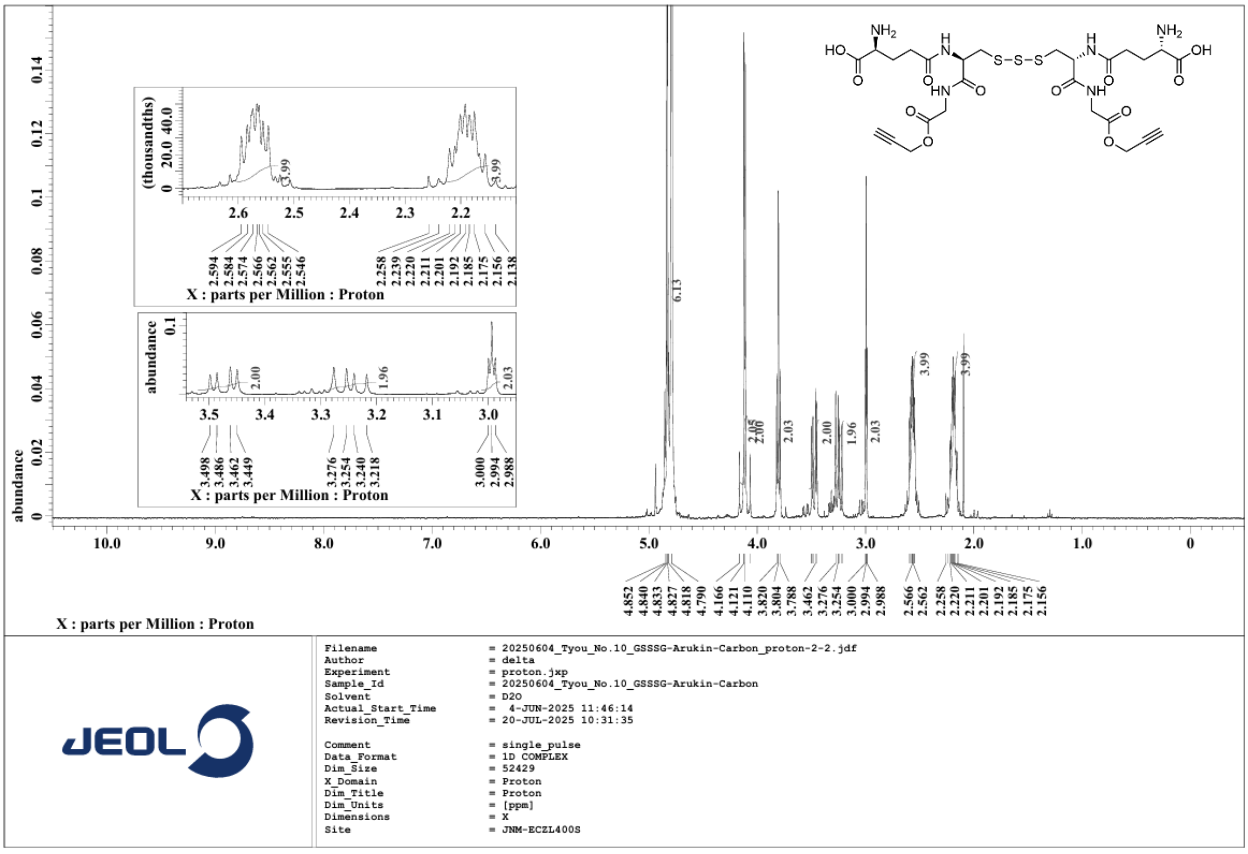
(9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-propoxyethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicosan-21-oic acid (5a) ^{1}H-NMR



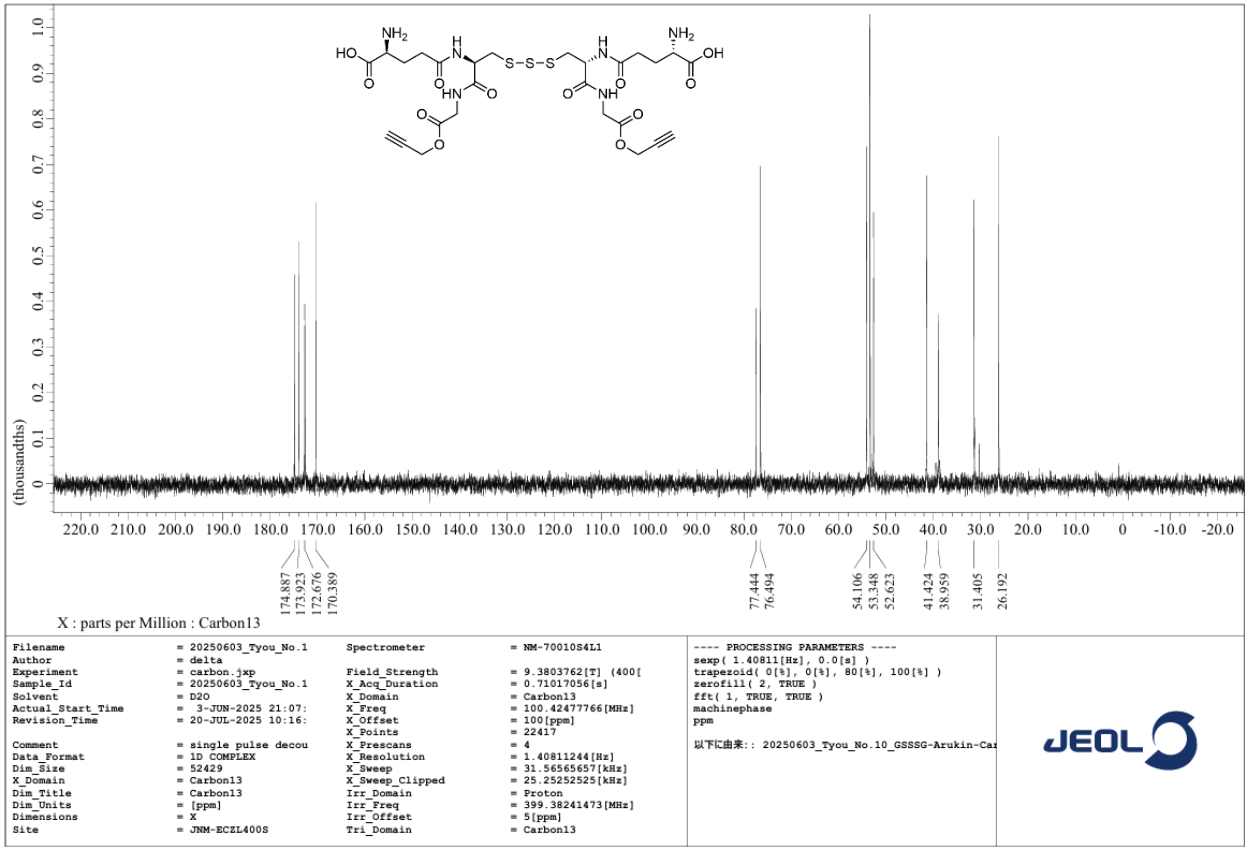
(9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-propoxyethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicosan-21-oic acid (5a) ^{13}C-NMR



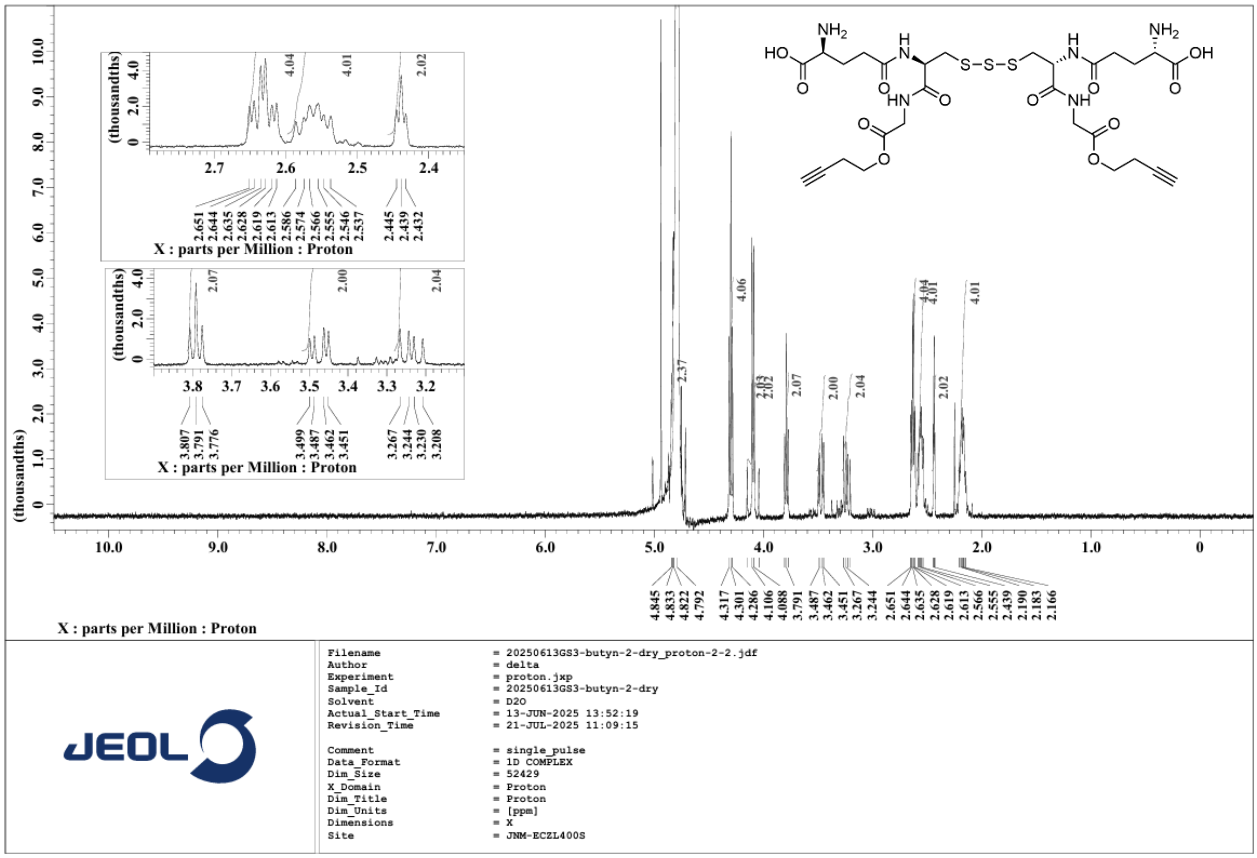
(9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-(prop-2-yn-1-yloxy)ethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicos-1-yn-21-oic acid (5f) ^{1}H-NMR



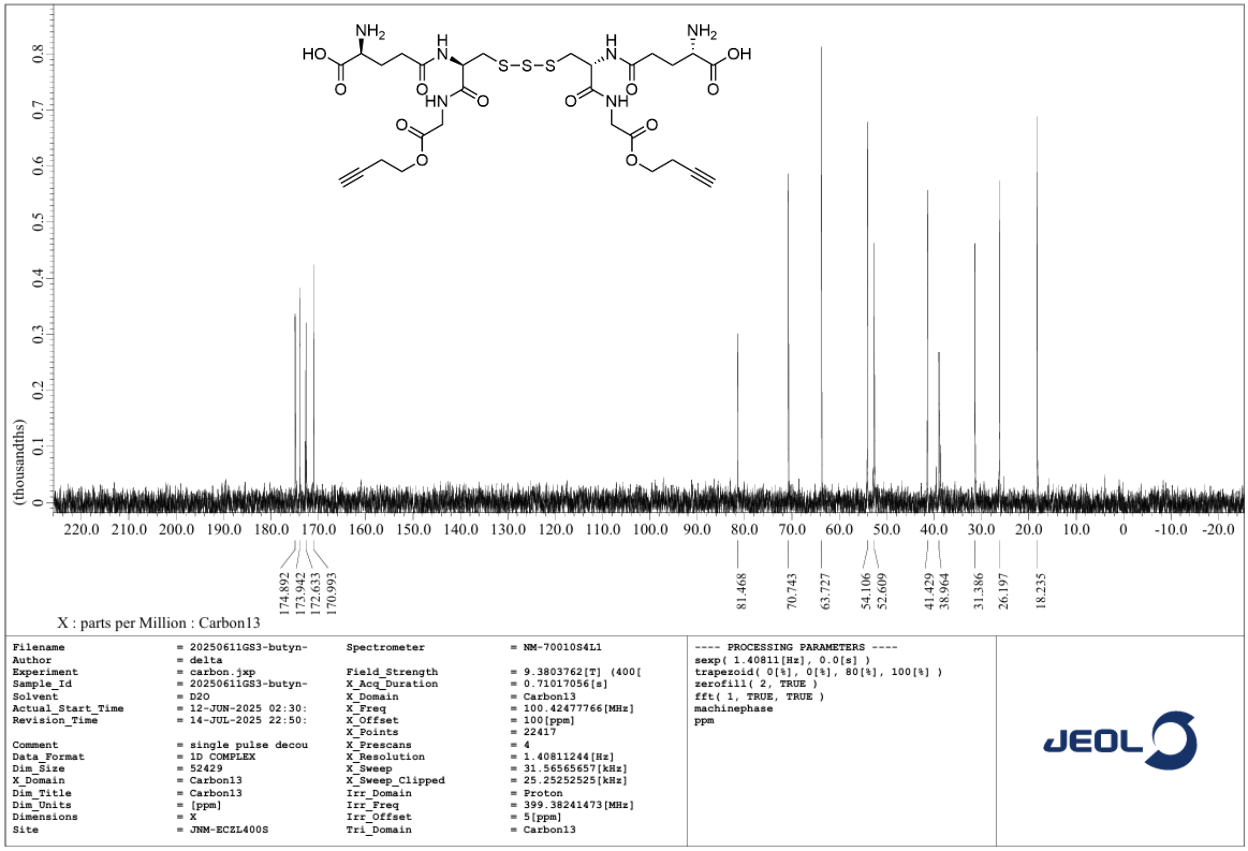
(9*R*,15*R*,20*S*)-20-amino-9-((*S*)-4-amino-4-carboxybutanamido)-5,8,17-trioxo-15-((2-oxo-2-(prop-2-yn-1-yloxy)ethyl)carbamoyl)-4-oxa-11,12,13-trithia-7,16-diazahenicos-1-yn-21-oic acid (5f) ^{13}C-NMR



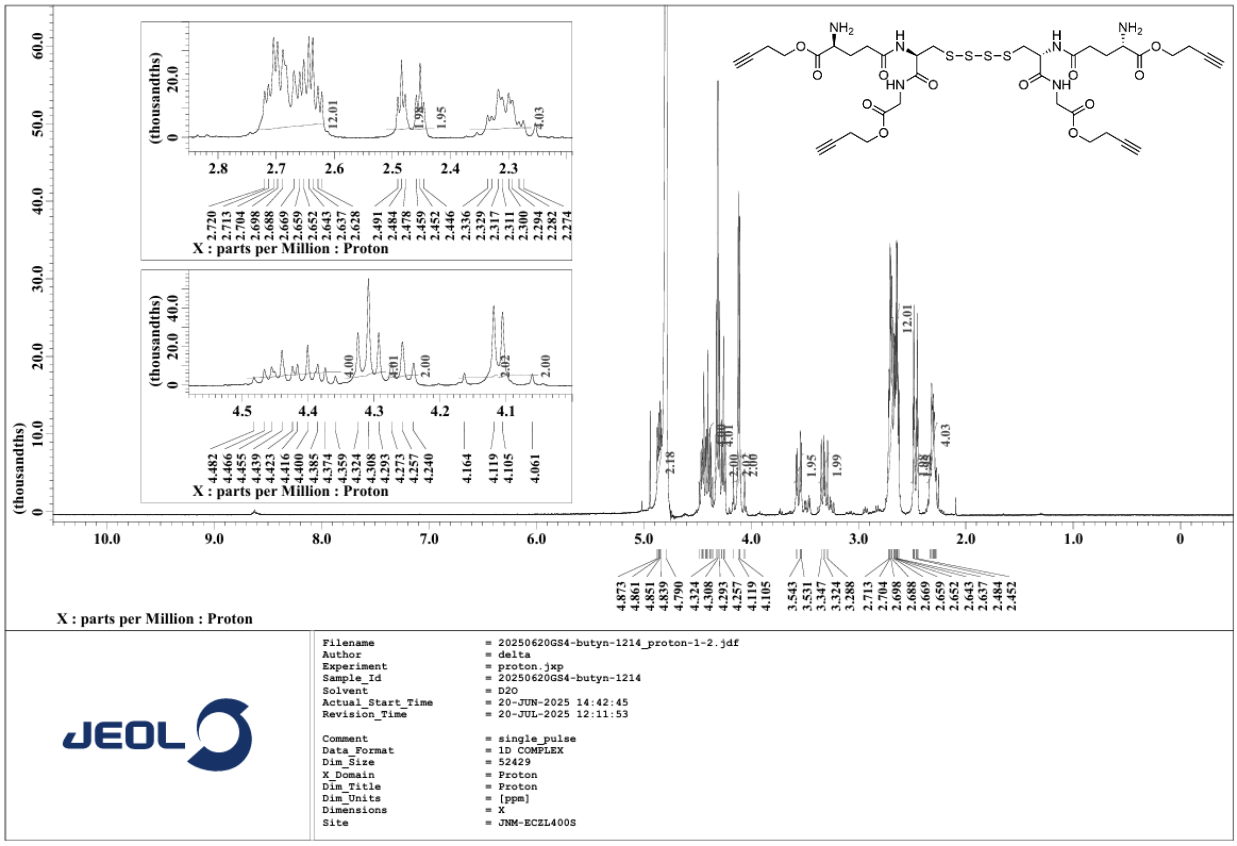
(10*R*,16*R*,21*S*)-21-amino-10-((*S*)-4-amino-4-carboxybutanamido)-16-((2-(but-3-yn-1-yloxy)-2-oxoethyl)carbamoyl)-6,9,18-trioxo-5-oxa-12,13,14-trithia-8,17-diazadocos-1-yn-22-oic acid (5g) ^{1}H-NMR



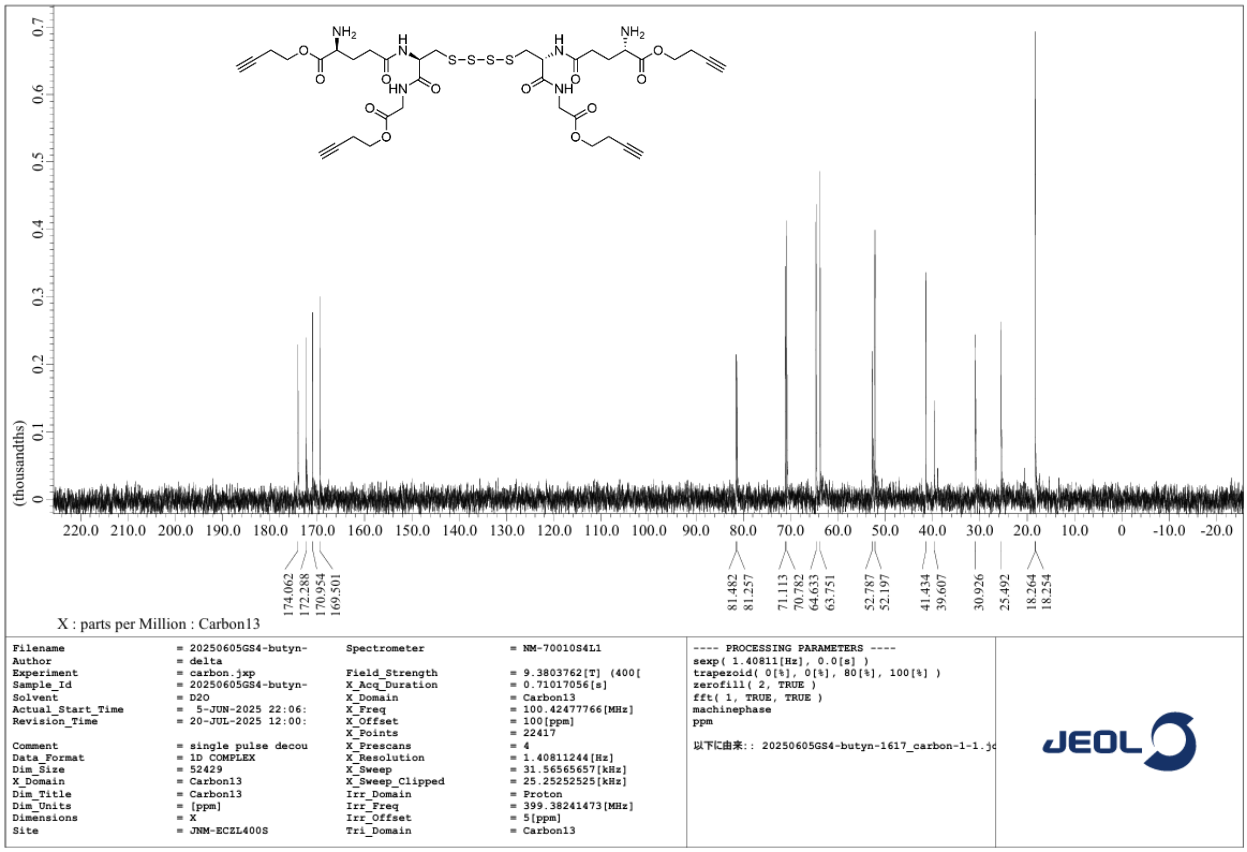
(10*R*,16*R*,21*S*)-21-amino-10-((*S*)-4-amino-4-carboxybutanamido)-16-((2-(but-3-yn-1-yloxy)-2-oxoethyl)carbamoyl)-6,9,18-trioxo-5-oxa-12,13,14-trithia-8,17-diazadocos-1-yn-22-oic acid (5g) ^{13}C-NMR



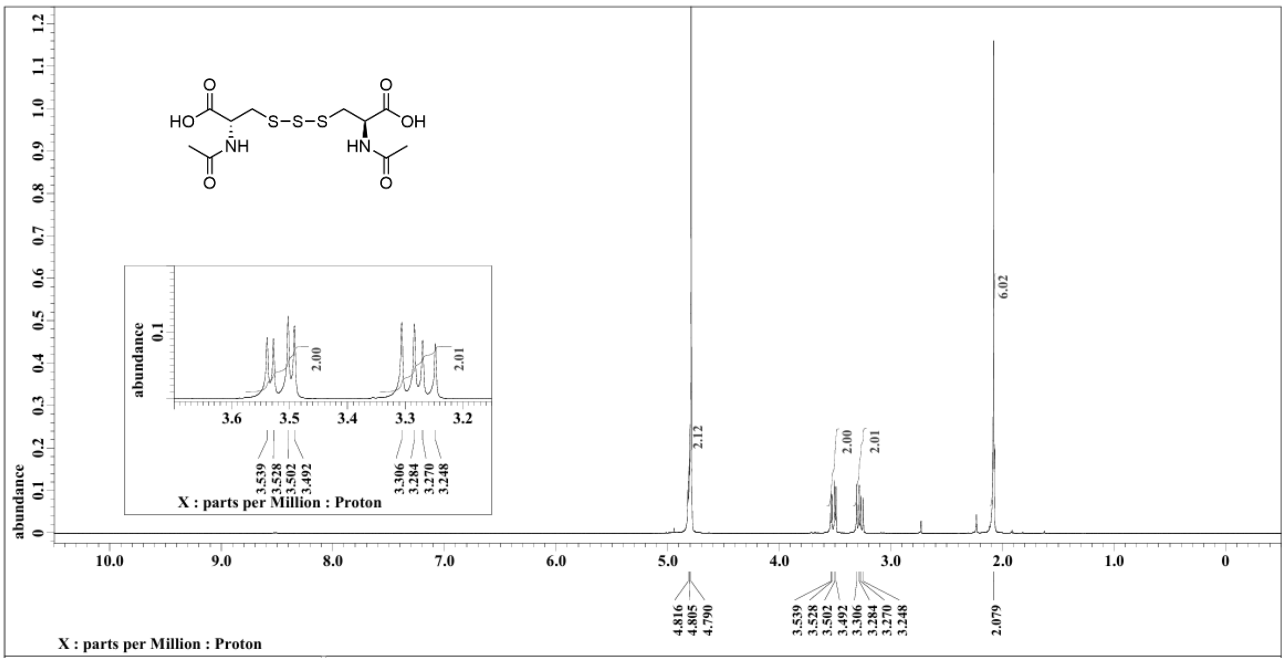
Di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (7g) ^{1}H-NMR



Di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (7g) ^{13}C-NMR

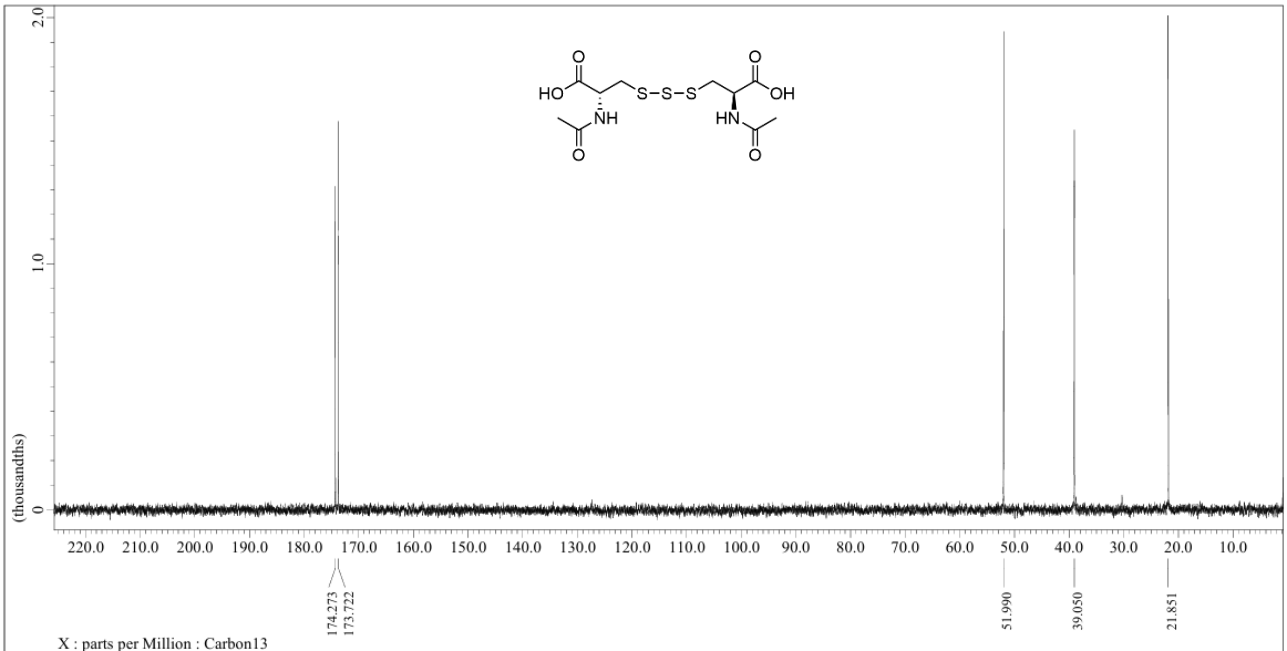


(2*R*,2'*R*)-3,3'-trisulfanediylbis(2-acetamidopropanoic acid) (8) ^{1}H-NMR



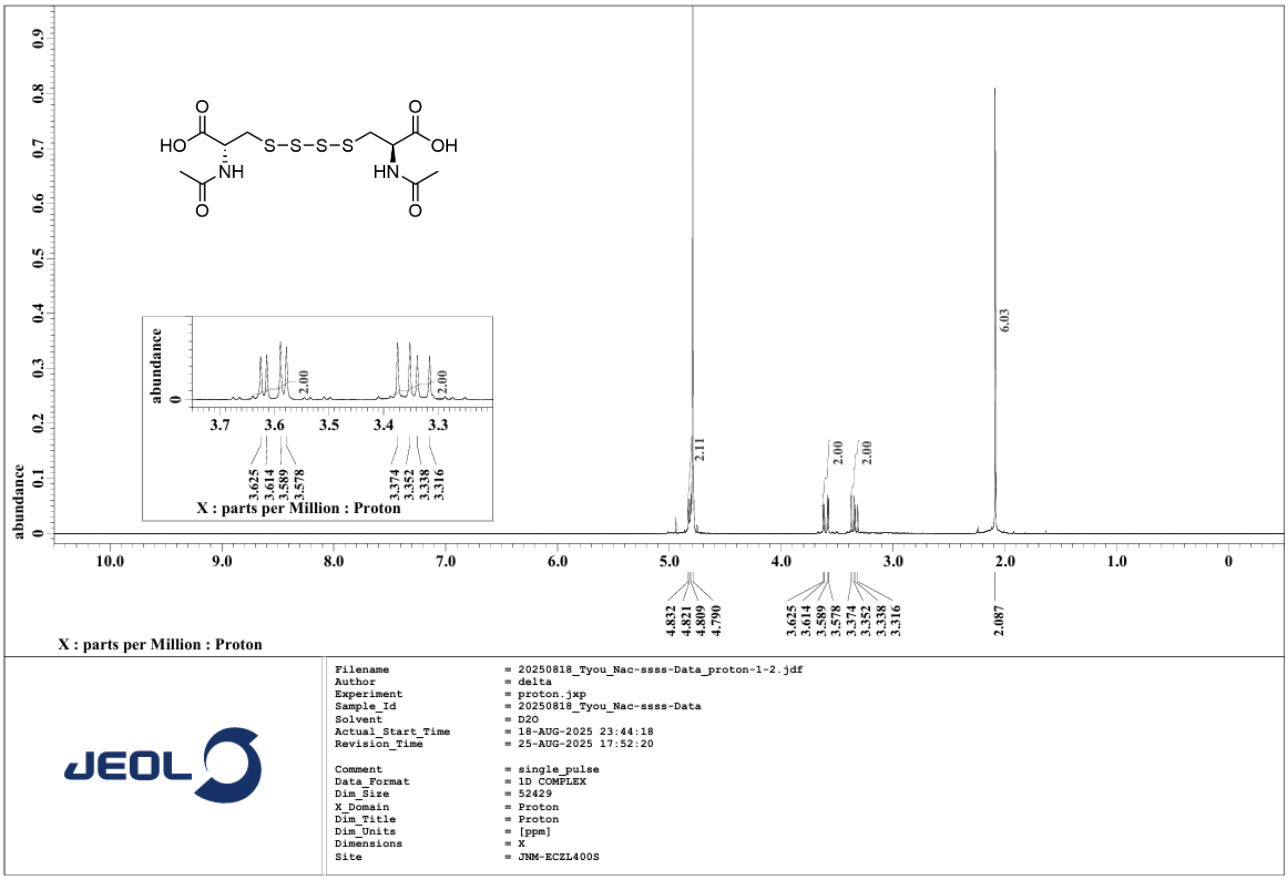
Filename	= 20250818_Tyou_Nac-SSS-Data_proton-1-2.jdf
Author	= delta
Experiment	= proton.jxp
Sample_Id	= 20250818_Tyou_Nac-SSS-Data
Solvent	= D2O
Actual_Start_Time	= 18-AUG-2025 21:43:49
Revision_Time	= 25-AUG-2025 16:53:16
Comment	= single_pulse
Data_Format	= 1D COMPLEX
Dim_Size	= 52429
X_Domain	= Proton
Dim_Title	= Proton
Dim_Units	= [ppm]
Dimensions	= X
Site	= JNM-ECZL400S

(2*R*,2'*R*)-3,3'-trisulfanediylbis(2-acetamidopropanoic acid) (8) ^{13}C-NMR



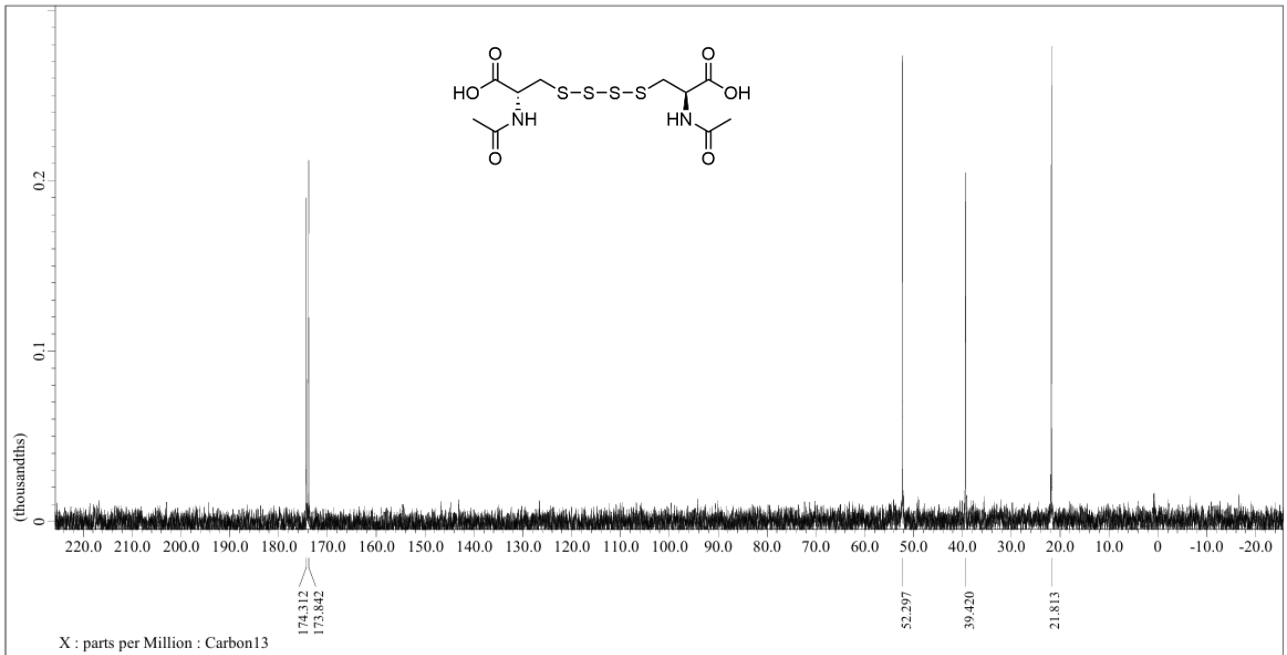
Filename	= 20250818_Tyou_Nac-	Spectrometer	= NM-70010S4L1	---- PROCESSING PARAMETERS ----	
Author	= delta			sexp(1.40811[Hz], 0.0[s])	
Experiment	= carbon.jxp	Field_Strength	= 9.3803762[T] (400[	trapezoid(0[%], 0[%], 80[%], 100[%])	
Sample_Id	= 20250818_Tyou_Nac-	X_Acq_Duration	= 0.71017056[s]	zerofill(2, TRUE)	
Solvent	= D2O	X_Domain	= Carbon13	fft(1, TRUE, TRUE)	
Actual_Start_Time	= 18-AUG-2025 21:48:	X_Freq	= 100.42477766[MHz]	machinephase	
Revision_Time	= 25-AUG-2025 17:12:	X_Offset	= 100[ppm]	ppm	
		X_Points	= 22417		
Comment	= single pulse decou	X_Prescans	= 4	以下に由来:: 20250818_Tyou_Nac-SSS-Data_carbon-1-	
Data_Format	= 1D COMPLEX	X_Resolution	= 1.40811244[Hz]		
Dim_Size	= 52429	X_Sweep	= 31.56565657[kHz]		
X_Domain	= Carbon13	X_Sweep_Clipped	= 25.25252525[kHz]		
Dim_Title	= Carbon13	Irr_Domain	= Proton		
Dim_Units	= [ppm]	Irr_Freq	= 399.38241473[MHz]		
Dimensions	= X	Irr_Offset	= 5[ppm]		
Site	= JNM-ECZL400S	Tri_Domain	= Carbon13		

(2*R*,2'*R*)-3,3'-tetrasulfanediylbis(2-acetamidopropanoic acid) ^{1}H-NMR



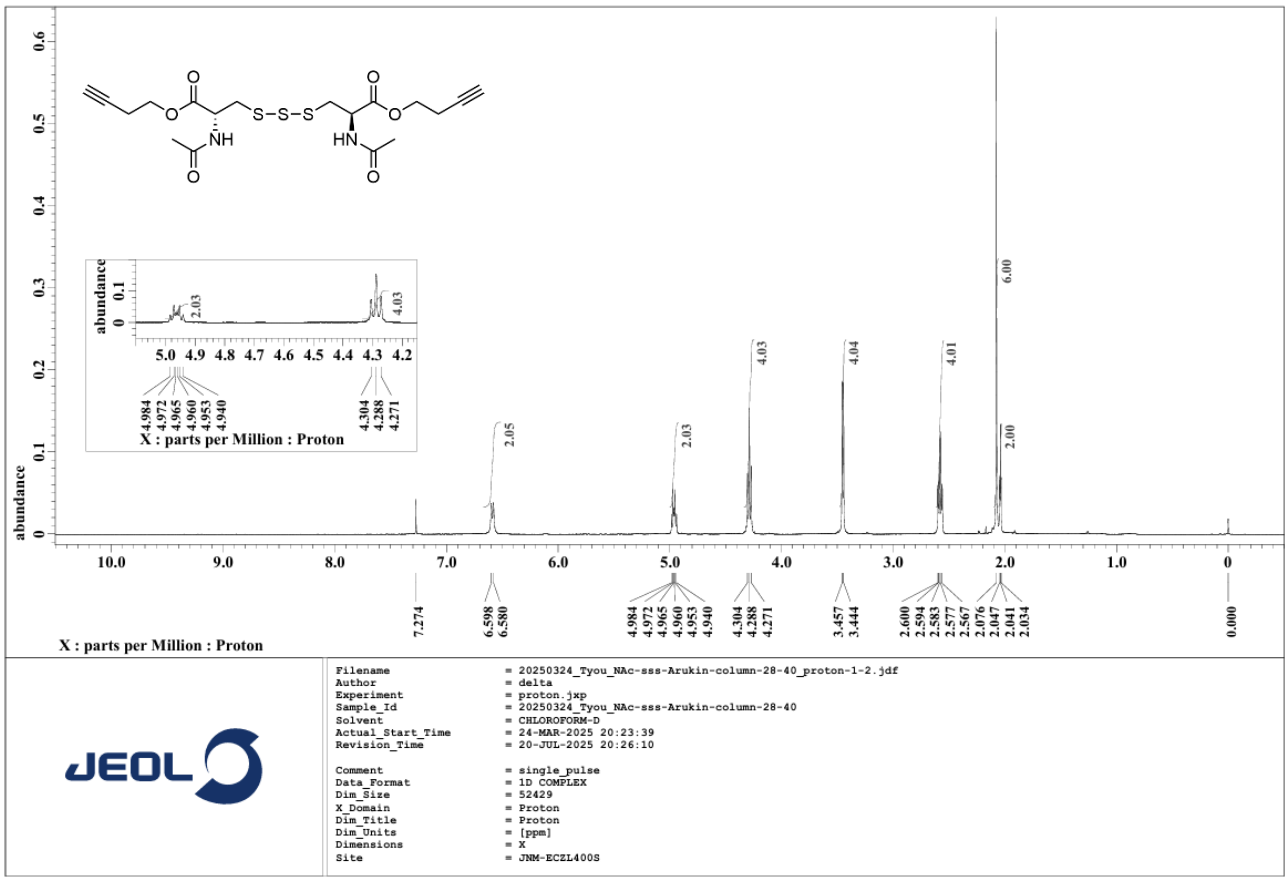
Filename	= 20250818_Tyou_Nac-ssss-Data_proton-1-2.jdf
Author	= delta
Experiment	= proton.jxp
Sample_Id	= 20250818_Tyou_Nac-ssss-Data
Solvent	= D2O
Actual_Start_Time	= 18-AUG-2025 23:44:18
Revision_Time	= 25-AUG-2025 17:52:20
Comment	= single_pulse
Data_Format	= 1D COMPLEX
Dim_Size	= 52429
X_Domain	= Proton
Dim_Title	= Proton
Dim_Units	= [ppm]
Dimensions	= X
Site	= JNM-ECZL400S

(2*R*,2'*R*)-3,3'-tetrasulfanediylbis(2-acetamidopropanoic acid) ^{13}C-NMR

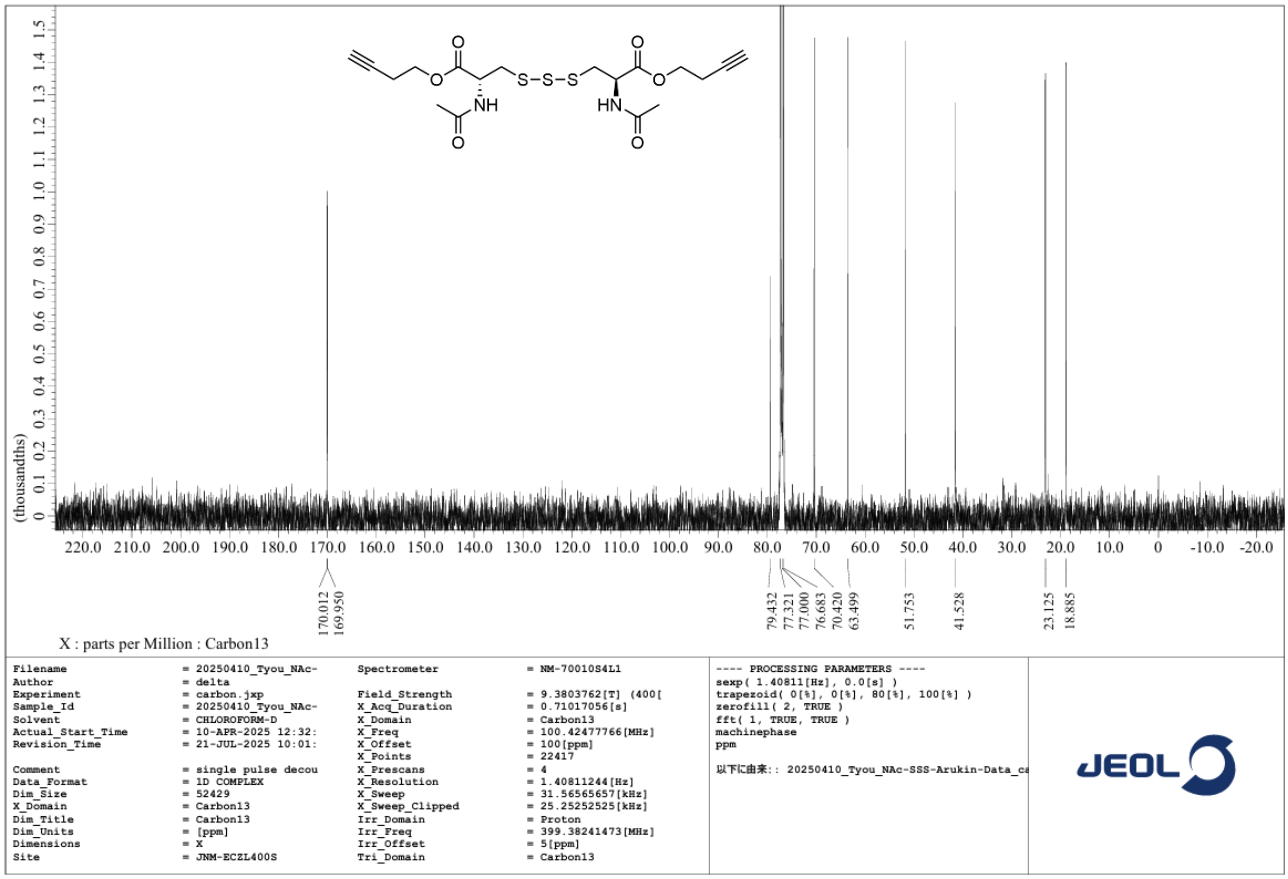


Filename	= 20250818_Tyou_Nac-	Spectrometer	= NM-70010S4L1	---- PROCESSING PARAMETERS ----
Author	= delta			sexp(1.40811[Hz], 0.0[s])
Experiment	= carbon.jxp	Field_Strength	= 9.3803762[T] (400[	trapezoid(0[%], 0[%], 80[%], 100[%])
Sample_Id	= 20250818_Tyou_Nac-	X_Acq_Duration	= 0.71017056[s]	zerofill(2, TRUE)
Solvent	= D2O	X_Domain	= Carbon13	fft(1, TRUE, TRUE)
Actual_Start_Time	= 18-AUG-2025 23:46:	X_Freq	= 100.42477766[MHz]	machinephase
Revision_Time	= 25-AUG-2025 17:40:	X_Offset	= 100[ppm]	ppm
		X_Points	= 22417	
Comment	= single pulse decou	X_Prescans	= 4	以下に由来:: 20250818_Tyou_Nac-ssss-Data_carbon-1
Data_Format	= 1D COMPLEX	X_Resolution	= 1.40811244[Hz]	
Dim_Size	= 52429	X_Sweep	= 31.56565657[kHz]	
X_Domain	= Carbon13	X_Sweep_Clipped	= 25.25252525[kHz]	
Dim_Title	= Carbon13	Irr_Domain	= Proton	
Dim_Units	= [ppm]	Irr_Freq	= 399.38241473[MHz]	
Dimensions	= X	Irr_Offset	= 5[ppm]	
Site	= JNM-ECZL400S	Tri_Domain	= Carbon13	

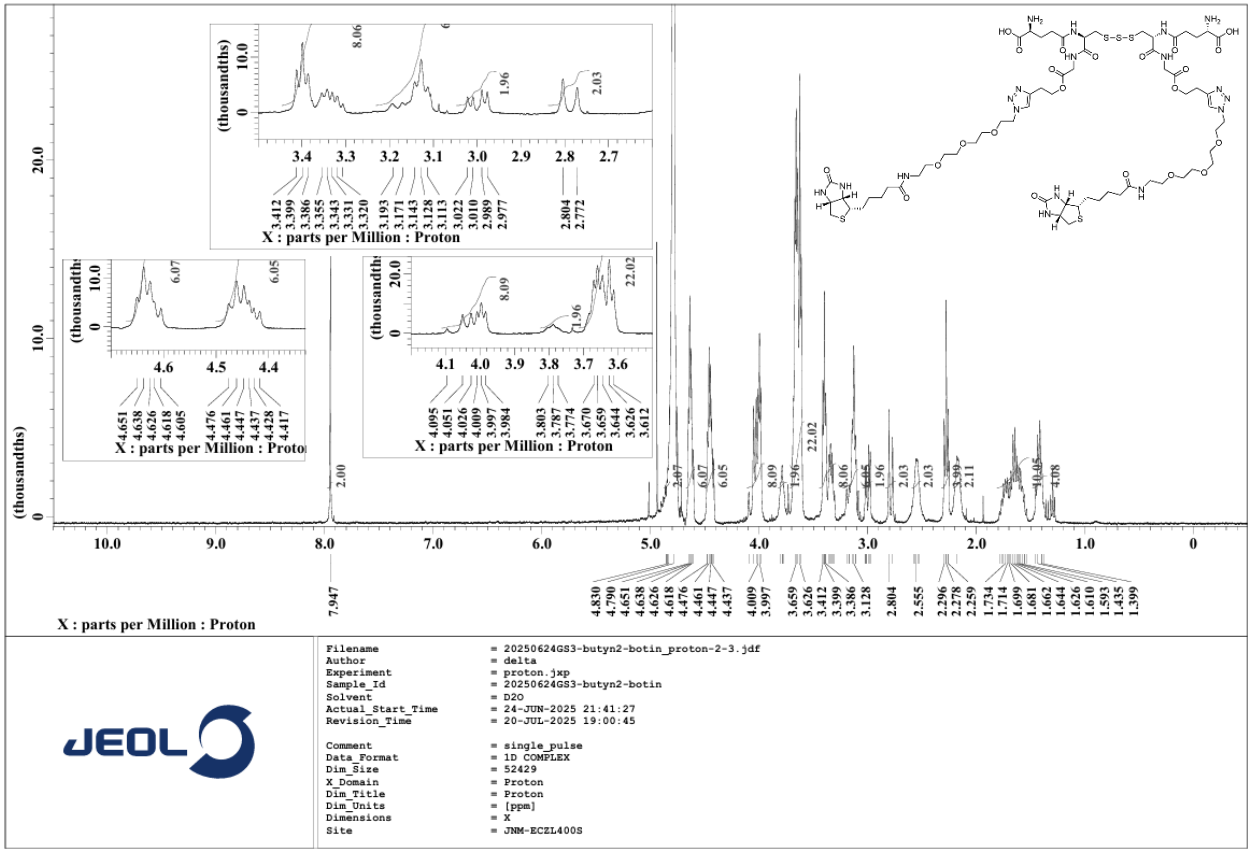
Di(but-3-yn-1-yl) 3,3'-trisulfanediyl(2*R*,2'*R*)-bis(2-acetamidopropanoate) (9g) ^{1}H-NMR



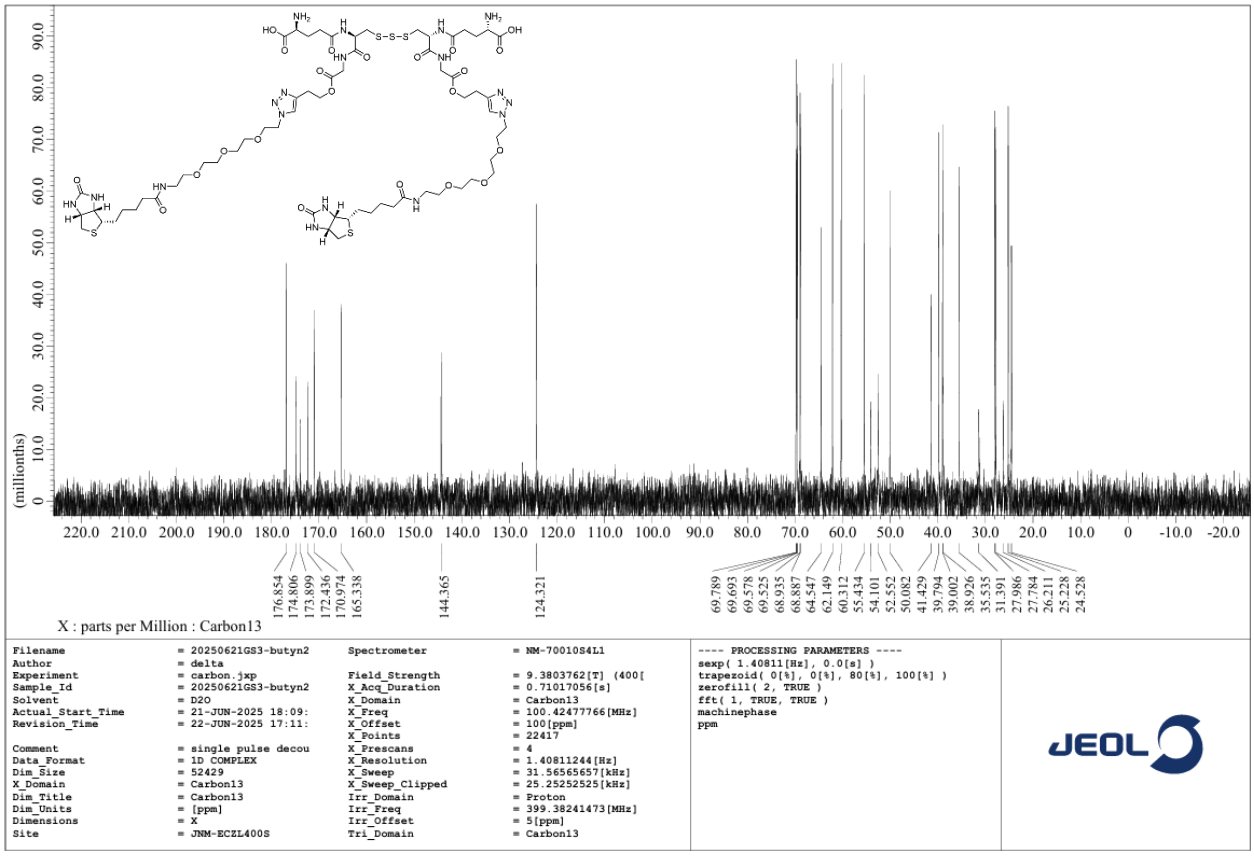
Di(but-3-yn-1-yl) 3,3'-trisulfanediyl(2*R*,2'*R*)-bis(2-acetamidopropanoate) (9g) ^{13}C-NMR



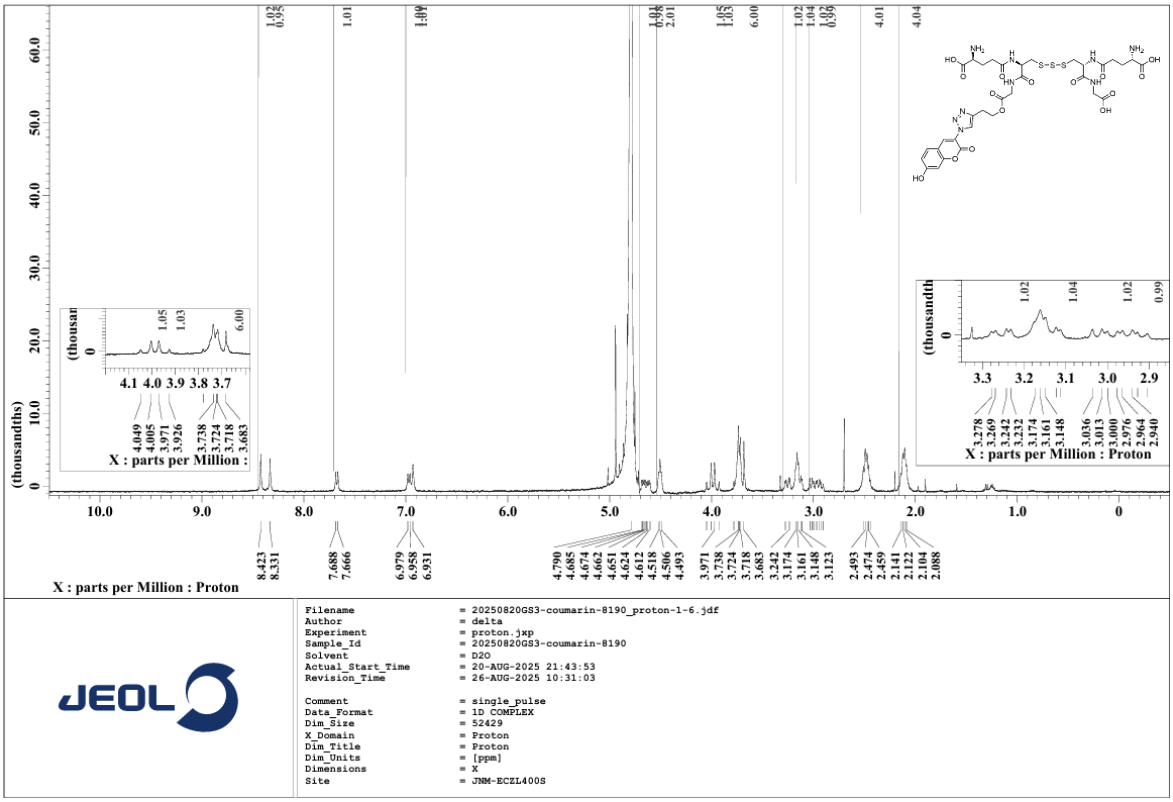
Di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (10g) ^{1}H-NMR



Di(but-3-yn-1-yl) 5,5'-(((10*R*,17*R*)-6,9,18,21-tetraoxo-5,22-dioxa-12,13,14,15-tetrathia-8,19-diazahexacosa-1,25-diyne-10,17-diyl)bis(azanediyl))(2*S*,2'*S*)-bis(2-amino-5-oxopentanoate) (10g) ^{13}C-NMR



N^5-((*R*)-3-(((*R*)-2-((*S*)-4-amino-4-carboxybutanamido)-3-((2-(2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethoxy)-2-oxoethyl)amino)-3-oxopropyl)trisulfaneyl)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-*L*-glutamine (12g) ^{1}H-NMR



N^5-((*R*)-3-(((*R*)-2-((*S*)-4-amino-4-carboxybutanamido)-3-((2-(2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethoxy)-2-oxoethyl)amino)-3-oxopropyl)trisulfaneyl)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-*L*-glutamine (12g) ^{13}C-NMR