Electronic Supplementary Information

Total chemical synthesis of monoglycosylated GM2 ganglioside activator using a novel cysteine surrogate

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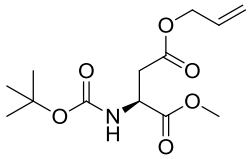
General methods

All reactions except for peptide synthesis were carried out under a positive pressure of argon. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. Mass spectra were recorded on a Waters MICROMASS® LCT PREMIER™ (ESI-TOF). NMR spectra were recorded using a Bruker AV400N at 400 MHz frequency for ^{1}H, and JEOL JNM-AL300 at 75 MHz frequency for ^{13}C. For HPLC separations, a Cosmosil 5C_{18}-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL min^{-1}), a Cosmosil Protein-R analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL min^{-1}), a Cosmosil Protein-R semi-preparative column (Nacalai Tesque, 10 × 250 mm, flow rate 3.0 mL min^{-1}) or a Cosmosil 5C_{18}-AR-II preparative column (Nacalai Tesque, 20 × 250 mm, flow rate 10 mL min^{-1}) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA aqueous solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) was used for HPLC elution. Optical rotations were measured using a JASCO P-2200 polarimeter (concentration in g dL^{-1}). Measurement of absorbance at 280 nm was performed using a Thermo Scientific™ NanoDrop™ 8000. CD spectrum was recorded with a JASCO J1500 spectropolarimeter.

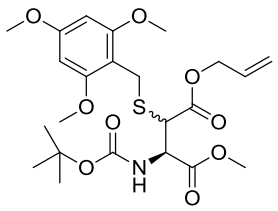
Synthesis of N-glycosylated asparagine derivative 1



8

Protected aspartate **8** was synthesized similarly to its enantiomer.[S1] To ice-cold allyl alcohol (50.0 mL) was added acetyl chloride (7.10 mL, 100 mmol). The reaction mixture was stirred at the same temperature for 15 min followed by at room temperature for 1 hour, and then L-aspartic acid (3.33 g, 25.0 mmol) was added. The resulting mixture was stirred at room temperature for 23 hours and was poured into ice-cold Et_2O (250 mL) to give precipitate. After being stirred at 0 °C for 1 hour, the precipitate was collected by filtration and was washed with Et_2O to give the aspartic acid β-allyl ester as a white powder (4.05 g, 77%). To the solution of the allyl ester (3.50 g, 16.7 mmol) in water (25.0 mL) were added Boc_2O (4.37 g, 20.0 mmol) in dioxane (25.0 mL) and Et_3N (6.50 mL, 46.8 mmol), and then the solution was stirred at room temperature for 3 hours. After the reaction mixture was washed with Et_2O, the aqueous phase was acidified by 10% (w/v) citric acid aq. to pH 4–5 and then was extracted into EtOAc. The organic

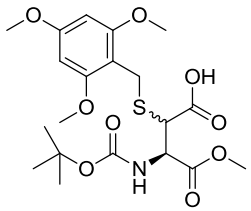
phase was successively washed with 10% (w/v) citric acid aq. and brine, and then dried over Na_2SO_4. Filtration of the mixture followed by concentration under reduced pressure gave the *N*-Boc-aspartate as a colorless oil (4.53 g, 99%). To the solution of the resulting compound (4.00 g, 14.6 mmol) in DMF (50.0 mL) was added K_2CO_3 (6.07 g, 43.9 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Then iodomethane (2.73 mL, 43.9 mmol) was added and the resultant mixture was stirred for additional 12 hours. After DMF was evaporated, the residue was dissolved in EtOAc. The organic phase was washed with 1 M HCl aq., saturated $NaHCO_3$ aq., 5% (w/v) $Na_2S_2O_3$ aq. and brine, and then dried over Na_2SO_4. After filtration and subsequent removal of the solvent under reduced pressure, chromatographic purification on silica gel (*n*-hexane/EtOAc = 5:1, (v/v)) gave the fully protected aspartate **8** in 86% isolated yield (3.62 g) as a colorless oil. The NMR spectrum was identical to that of the enantiomer: $[\alpha]^{18}_{D}$ -14.3 (*c* 0.96, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.45 (9H, s), 2.85 (1H, dd, 17.0 and 4.9 Hz), 3.03 (1H, dd, 17.0 and 4.6 Hz), 3.76 (3H, s), 4.60 (2H, ddd, 5.8, 1.3 and 1.4 Hz), 4.52–4.67 (1H, m), 5.25 (1H, ddt, 10.4, 1.4 and 1.3 Hz), 5.32 (1H, ddt, 17.2, 1.4 and 1.4 Hz), 5.49 (1H, br d, 7.7 Hz), 5.90 (1H, ddt, 17.2, 10.4 and 5.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.2, 36.7, 49.9, 52.5, 65.5, 79.8, 118.4, 131.7, 155.2, 170.5, 171.4.



10

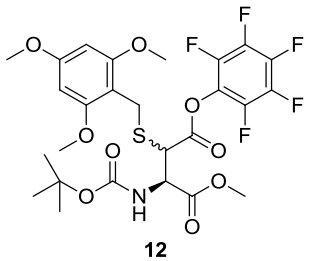
To a solution of **8** (3.26 g, 11.4 mmol) in THF (120 mL) at -78 °C was added LiHMDS (1 M in THF, 25.0 mL, 25.0 mmol) and the reaction mixture was stirred at the same temperature for 2 hours. Then a solution of *S*-(2,4,6-trimethoxybenzyl)toluenethiosulfonate **9**[S2] (5.88 g, 16.0 mmol) in THF (60.0 mL) was added and the resultant was stirred for additional 3 hours at -78 °C. After quenching with saturated NH_4Cl aq., THF was removed under reduced pressure, and the residue was extracted with EtOAc. The organic phase was successively washed with saturated NH_4Cl aq. and brine, and then dried over Na_2SO_4. After filtration followed by removal of the solvent *in vacuo*, Et_2O was added to give precipitate. The precipitate was filtrated off and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1 to 3:1, (v/v)) to

give a diastereomeric mixture of **10** (7:2) as a pale yellow oil (4.64 g, 81%). The major diastereomer was isolated for characterization: $[\alpha]^{19}_{D}$ 57.7 (*c* 0.96, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.44 (9H, s), 3.70 (3H, s), 3.81 (9H, s), 3.90 (1H, br d, 12.8 Hz), 3.99 (1H, br d, 12.8 Hz), 4.12 (1H, br d, 4.3 Hz), 4.59 (2H, br d, 5.6 Hz), 4.82 (1H, br dd, 9.6 and 4.3 Hz), 5.23 (1H, dq, 10.6 and 1.1 Hz), 5.33 (1H, bq, 17.3 and 1.4 Hz), 5.66 (1H, br d, 9.6 Hz), 5.87 (1H, ddt, 17.3, 10.6 and 5.6 Hz), 6.11 (2H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 25.0, 28.4, 48.5, 52.6, 55.0, 55.4, 55.8, 55.8, 66.1, 80.0, 90.6, 90.6, 106.9, 118.4, 118.5, 131.7, 155.9, 159.0, 159.0, 160.7, 171.0, 171.5; HRMS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{33}NNaO_9S$ ($[M + Na]^+$): 522.1774, found 522.1780.



11

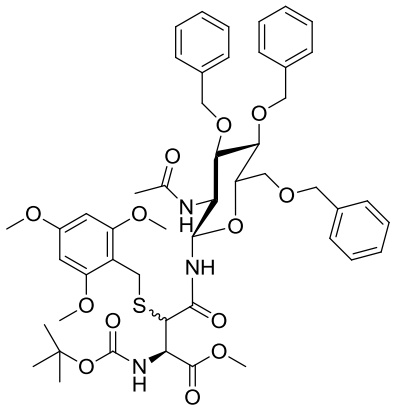
Allyl ester **10** (4.00 g, 8.01 mmol, diastereomeric mixture) in THF (30.0 mL) was treated with *N*-methylaniline (8.71 mL, 80.1 mmol) and $Pd(PPh_3)_4$ (185 mg, 0.160 mmol). After being stirred at room temperature for 2 hours, the reaction mixture was concentrated *in vacuo* and the product was purified by silica gel column chromatography (*n*-hexane/EtOAc/AcOH = 2:1:0.05 to 3:2:0.05, (v/v)) to give a diastereomeric mixture of **11** (7:2) as a pale yellow amorphousness (3.31 g, 90%). The major diastereomer was isolated for characterization: $[\alpha]^{19}_{D}$ 41.6 (*c* 0.98, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.45 (9H, s), 3.72 (3H, s), 3.81 (3H, s), 3.82 (6H, s), 3.95 (2H, s), 4.12 (1H, br d, 3.7 Hz), 4.89 (1H, br dd, 9.9 and 3.7 Hz), 5.52 (1H, brd, 9.9 Hz), 6.13 (2H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 25.3, 28.4, 52.9, 55.0, 55.5, 55.9, 55.9, 80.3, 90.7, 106.6, 155.9, 159.0, 160.9, 160.9, 170.8, 170.8; HRMS (ESI-TOF) *m*/*z* calcd for $C_{20}H_{29}NNaO_9S$ ($[M + Na]^+$): 482.1461, found 482.1460.



12

Compound **11** (2.01 g, 4.37 mmol, diastereomeric mixture) in CH_2Cl_2 (15.0 mL) was

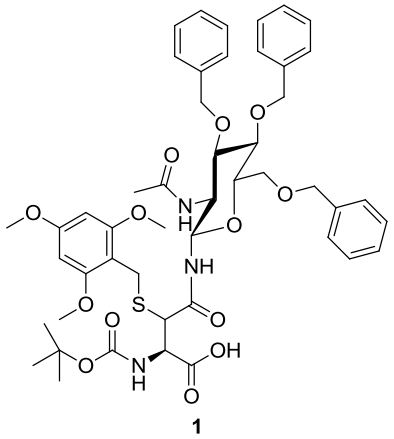
treated with pentafluorophenol (687 μL, 6.56 mmol) and EDC·HCl (1.01 g, 5.24 mmol). After being stirred at room temperature for 1 hour, the mixture was concentrated *in vacuo*. EtOAc was added to the residue and the obtained solution was washed with water, saturated $NaHCO_3$ aq., saturated NH_4Cl aq. and brine. After drying over Na_2SO_4 followed by filtration, the organic layer was concentrated under reduced pressure. The obtained residue was subjected to chromatographic purification on silica gel (*n*-hexane/EtOAc = 3:1, (v/v)) to give a diastereomeric mixture of **12** (1:1) in 81% isolated yield (2.21 g) as a colorless amorphousness. Less polar diastereomer was isolated for characterization: $[\alpha]^{18}_{D}$ 108.4 (*c* 0.93, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.44 (9H, s), 3.74 (3H, s), 3.80 (3H, s), 3.83 (6H, s), 4.02 (2H, s), 4.39 (1H, br d, 4.8 Hz), 4.98 (1H, br dd, 10.1 and 4.8 Hz), 5.49 (1H, br d, 10.1 Hz), 6.12 (2H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 25.5, 28.4, 48.3, 52.9, 55.0, 55.4, 55.8, 55.8, 80.4, 90.6, 90.6, 106.5, 124.9, 138.0 (d, 246.6 Hz), 139.7 (d, 251.6 Hz), 141.3 (d, 246.6 Hz), 155.6, 159.1, 159.1, 160.9, 168.2, 170.2; HRMS (ESI-TOF) *m*/*z* calcd for $C_{26}F_5H_{28}NNaO_9S$ ($[M + Na]^+$): 648.1303, found 648.1292.



14

Tributylphosphine (1.40 mL, 5.60 mmol) was added to a stirred solution of pentafluorophenyl ester **12** (1.75 g, 2.80 mmol, diastereomeric mixture), 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide **13** (2.17 g, 4.20 mmol, commercially available), and 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt, 456 mg, 2.80 mmol) in THF containing 2% (v/v) H_2O (28.0 mL) at 0 °C. The mixture was stirred at room temperature for 16 hours, and then the solvent was evaporated *in vacuo*. MeOH/CH_2Cl_2 (9:1, (v/v)) was added to the residue and the solution was washed with water and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by silica gel column chromatography ($CHCl_3$) followed by reprecipitation from *n*-hexane/EtOAc to afford a

5:1 of diastereomeric mixture of **12** as a white amorphousness (1.26 g, 48%). Major diastereomer was isolated for characterization: $[\alpha]^{18}_{D}$ -6.7 (*c* 0.82, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.39 (9H, s), 1.89 (3H, s), 3.53–3.58 (1H, m), 3.59 (3H, s), 3.62–3.71 (2H, m), 3.71–3.77 (4H, m), 3.78 (9H, s), 3.87 (1H, d, 12.4 Hz), 4.00 (1H, t, 9.8 Hz), 4.51 (1H, d, 11.9 Hz), 4.57 (1H, d, 11.9 Hz), 4.61 (1H, d, 11.2 Hz), 4.67 (1H, brs), 4.70 (1H, d, 11.3 Hz), 4.77 (1H, d,11.2 Hz), 4.80 (1H, d, 11.3 Hz), 4.97, (1H, d, 9.8 Hz), 6.19 (1H, s), 7.16–7.42 (15H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 23.1, 26.0, 28.7, 51.7, 52.9, 55.2, 55.8, 56.3, 56.3, 56.8, 69.8, 74.4, 75.9, 76.2, 77.9, 79.4, 80.5, 84.7, 91.8, 91.8, 106.7, 128.7, 128.7, 128.7, 128.8, 129.0, 129.2, 129.4, 129.4, 139.4, 139.6, 139.9, 160.2, 160.2, 162.5, 172.2, 172.8, 174.0; HRMS (ESI-TOF) *m*/*z* calcd for $C_{49}H_{61}N_3NaO_{13}S$ ($[M + Na]^+$): 954.3823, found 954.3787.



1

Methyl ester **14** (0.30 g, 0.32 mmol) in 1,2-dichloroethane (3.0 mL) was treated with trimethyltin hydroxide (0.18 g, 0.97 mmol). After 10 hours reaction at 50 °C, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with 5% (w/v) $KHSO_4$ aq. and brine. After drying over Na_2SO_4 followed by filtration, the organic layer was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (EtOAc/MeOH/AcOH = 95:5:0.5, (v/v)) afforded **1** (dr = 5:1) in 69% isolated yield (0.20 g) as a colorless amorphousness. The obtained diastereomeric mixture was used to a subsequent reaction without separation: ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.48–1.49 (9H), 1.89 (3H, s), 3.48–4.01 (17H), 4.51 (1H, d, 11.8Hz), 4.59, (1H, d, 11.8 Hz), 4.61 (1H, d, 11.0 Hz), 4.65–4.83 (1H, m), 4.69 (1H, d, 11.3 Hz), 4.77 (1H, d, 10.5 Hz), 4.80 (1H, d, 11.0 Hz), 4.91–5.01 (1H, m), 6.18–6.22 (2 H, s), 7.10–7.45 (15H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 23.1, 26.3, 28.7, 52.1, 55.2, 55.8, 56.3, 56.3, 56.8, 69.8, 74.4, 75.9, 76.2, 77.9, 79.4, 80.6, 84.6, 91.9, 91.9, 106.8, 128.7, 128.7, 128.7, 129.0, 129.1, 129.4, 129.4, 129.4, 139.4,

139.6, 139.9, 160.2, 160.2, 162.4, 173.0, 173.4, 174.0; HRMS (ESI-TOF) *m*/*z* calcd for $C_{48}H_{59}N_3NaO_{13}S$ ($[M + Na]^+$): 940.3666, found 940.3653.

Preparation of SEAlide peptide 2

On Rink amide ChemMatrix resin (0.54 mmol amine/g, 0.46 g, 0.25 mmol) was coupled Fmoc-Leu-OH (4.0 equiv) with the aid of *N*,*N'*-diisopropylcarbodiimide (DIPCDI, 4.0 equiv) and HOBt·H_2O (4.0 equiv) in DMF at room temperature for 2 hours. Then Fmoc removal was performed with 20% (v/v) piperidine in DMF to give a Leu-incorporated resin. The resulting resin was treated with an Fmoc-Ser(^{t}Bu)-incorporating *N*-sulfanylethylaniline(SAcm) linker[S3] (2.0 equiv), DIPCDI (2.0 equiv), and HOBt·H_2O (2.0 equiv) to yield the SEAlide-linked resin. On this resin, peptide was elongated using standard Fmoc-based protocols (4.0 equiv each of amino acid using DIPCDI (4.0 equiv) and HOBt·H_2O (4.0 equiv) in DMF (2 hours) and Fmoc removal with 20% piperidine in DMF (10 min)). On the N-terminus of the resin, **1** (2.0 equiv) was coupled with the aid of PyBop (2.1 equiv) and DIEA (4.0 equiv) in DMF at room temperature for 3 hours. A portion of the resulting resin (500 mg) was treated with 1 M TMSBr-thioanisole in TFA (50 μL/1 mg resin)/*m*-cresol/EDT (100/5/5, (v/v)) at 4 °C for 2 hours. The resin in the reaction mixture was filtrated off. To the resulting filtrate was added cooled Et_2O to give precipitate. The formed precipitate was collected by centrifugation and thoroughly washed with Et_2O to afford crude SEAlide peptide **2**. The peptide was purified by preparative HPLC (Cosmosil 5C_{18}-AR-II preparative column with a linear gradient of solvent B in solvent A over 30 min: 34% to 40%) to afford purified SEAlide peptide **2** (16.3 mg from 500 mg of the protected resin).

Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 30% to 50% over 30 min, retention time = 15.8 min, MS (ESI-TOF) *m*/*z* calcd ($[M + 4H]^{4+}$) 1307.7, found 1307.4.

One-pot ligation/desulfurization between SEAlide peptide 2 and peptide thioester 3

Peptide thioester **3** (11 mg, 2.7 μmol) and *N*-glycosylated asparagine derivative-incorporating SEAlide peptide **2** (15 mg, 2.7 μmol) were dissolved in 2.7 mL of 6 M Gn·HCl-0.1 M Na phosphate, 3% (v/v) TFET, pH 7.3, and the solution was incubated at 37 °C. The reaction was completed within 8 hours. After treatment of the

reaction mixture with nitrogen stream, to the reaction mixture were added 2.7 mL of aqueous solution of Gn·HCl (6 M) and TCEP·HCl (0.4 M), pH 7.0, reduced form glutathione (in solid form, 40 mM) and VA-044 (in solid form, 20 mM). After incubation at 37 °C for additional 18 hours, the crude product was purified by semi-preparative HPLC (Cosmosil Protein-R semi-preparative column with a linear gradient of solvent B in solvent A over 30 min: 38% to 41%) to give purified Acm-protected SEAlide peptide **5** (13 mg, 1.4 μmol, 53%).

Analytical HPLC conditions: Cosmosil Protein-R analytical column with a linear gradient of solvent B in solvent A, 30% to 50% over 30 min, retention time = 20.0 min, MS (ESI-TOF) *m/z* calcd ($[M + 8H]^{8+}$) 1076.6, found 1076.6.

Acm removal for preparation of native N-half fragment 6

The Acm-protected peptide **5** (13 mg, 1.4 μmol) was treated with AgOTf (36 mg, 0.14 mmol) and anisole (15 μL, 0.14 mmol) in TFA (340 μL, 4.0 mM peptide) at 4 °C. After 13 hours, to the reaction mixture was added cooled Et_2O to give precipitate. The formed precipitate was collected by centrifugation and thoroughly washed with Et_2O. The resulting crude peptide was dissolved in 4 mL solution of 6 M Gn·HCl-0.2 M HEPPS buffer (pH 6.1) containing 5% (w/v) dithiothreitol. The suspension was incubated at 37 °C for 30 min and then 4 mL of 6 M Gn·HCl aq. was added. After filtration, the filtrate was purified by semi-preparative HPLC (Cosmosil Protein-R semi-preparative column with a linear gradient of solvent B in solvent A over 30 min: 40% to 43%) to afford the purified **6** (7.0 mg, 0.77 μmol, 54%).

Analytical HPLC conditions: Cosmosil Protein-R analytical column with a linear gradient of solvent B in solvent A, 30% to 50% over 30 min, retention time = 23.6 min, MS (ESI-TOF) *m/z* calcd ($[M + 8H]^{8+}$) 1049.9, found 1049.7.

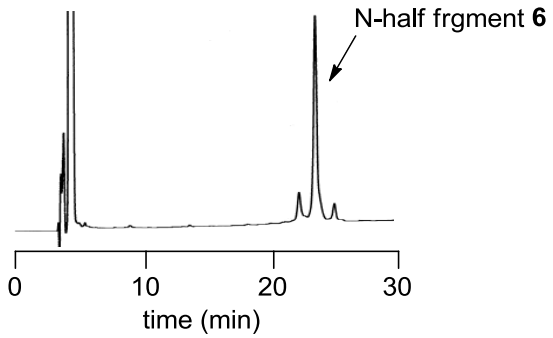


Fig. S1 HPLC analysis of Acm-removed crude materials after 13 hours reaction followed by dithiothreitol treatment.

Convergent assembly of native N-half fragment 6 and C-half fragment 7

The native N-half fragment **6** (3.7 mg, 0.34 μmol) and the C-half fragment **7**[S3] (3.1 mg, 0.34 μmol) were dissolved in 6 M Gn·HCl-0.5 M Na phosphate buffer containing 50 mM TCEP·HCl and 50 mM MPAA (pH 6.1, 0.68 mL) and the mixture was incubated at 37 °C. After 30 hours, the reaction mixture was diluted with 3 mL of 6 M Gn·HCl aq. with 0.5% (w/v) TCEP·HCl, and then the crude peptide was purified by semi-preparative HPLC (Cosmosil Protein-R semi-preparative column with a linear gradient of solvent B in solvent A over 30 min: 43% to 46% over 30 min) to give purified reduced form of native GM2AP **15** (2.6 mg, 0.14 μmol, 40%).

Analytical HPLC conditions: Cosmosil Protein-R analytical column with a linear gradient of solvent B in solvent A, 35% to 55% over 30 min, retention time = 20.9 min, MS (ESI-TOF) calcd (average isotopes) 17792.4, found 17793.4.

Folding for preparation of monoglycosylated GMAP

The reduced form GM2AP **15** (1.0 mg) was dissolved in DMSO (0.50 mL). The resulting solution was added to 50 mM 3-[4-(2-hydroxyethyl)piperazin-1-yl]propane-1-sulfonic acid (HEPPS) buffer containing 1.0 mM reduced form glutathione, 0.10 mM oxidized form glutathione and 1.0 mM EDTA (pH 8.0, 9.5 mL, final concentration of protein 0.10 mg mL^{-1}). After being stored at 37 °C for 24 hours, the folded protein was purified by semi-preparative HPLC (Cosmosil Protein-R semi-preparative column with a linear gradient of solvent B in solvent A over 30 min: 35% to 55% over 30 min) to give purified monoglycosylated native GM2AP (0.52 mg, 52%, determined by measurement of absorbance at 280 nm (ε_{280} = 22960, calculated as previously reported[S4])).

Analytical HPLC conditions: Cosmosil Protein-R analytical column with a linear gradient of solvent B in solvent A, 35% to 55% over 30 min, retention time = 17.0 min, MS (ESI-TOF) calcd (average isotopes) 17784.4, found 17783.7.

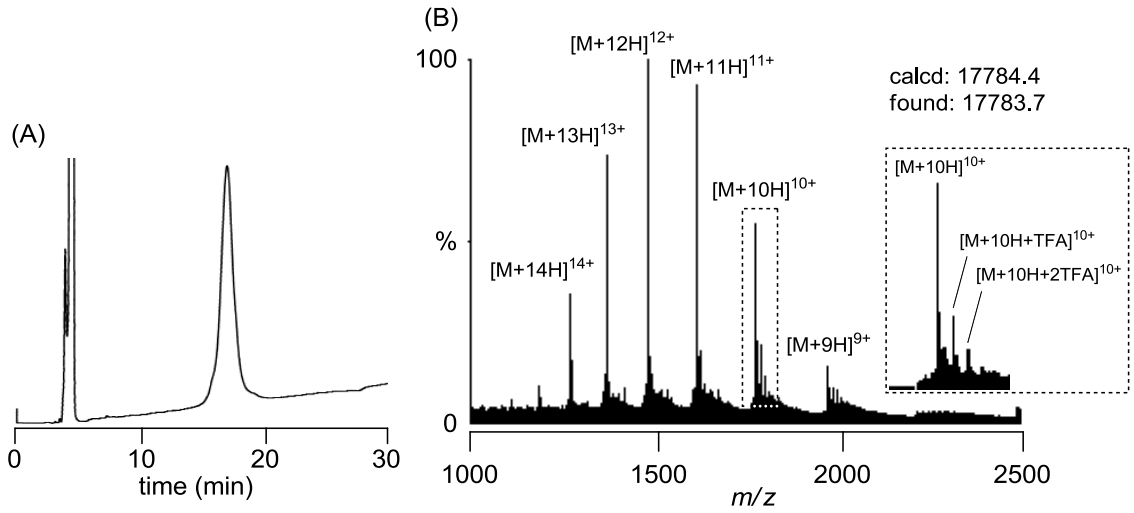


Fig. S2 (A) HPLC analysis of purified monoglycosylated GM2AP. (B) Mass spectrum of synthesized GM2AP.

CD measurement of monoglycosylated GM2AP

CD spectrum of the GM2AP (10 mM citrate buffer, pH 4.5, 0.067 mg mL^{-1} (determined by Bio-Rad DC protein assay)) in the range of 200 to 260 nm was recorded at 25 °C in a 0.2 mm-path quartz cuvette. Molar mean-residue ellipticity [θ] (deg cm^2 $dmol^{-1}$) was calculated as [θ] = θ·100/(l·c·A). The θ is the observed ellipticity (mdeg) and the l is the length of the optical path (cm). The c is the protein concentration (mmol L^{-1}) and the A is the number of amino acids contained in the protein.

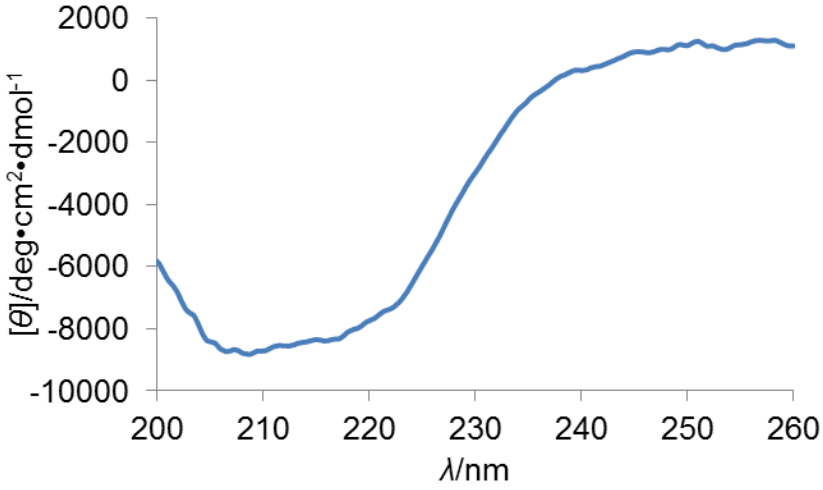


Fig. S3 CD spectrum of synthesized monoglycosylated GM2AP.

In vitro GM2-degradation assay of synthesized GM2AP

In vitro GM2-degradation assay was performed as described previously.[S5] Briefly, the GM2 ganglioside was incubated with recombinant human HexA (2000 nmol h^{-1} 4-methylumbellifery-6-sulfo-β-D-glucosaminide potassium salt (4-MUGS)-degrading activity) in the presence or absence of 5 μg of synthesized GM2AP in 10 mM sodium citrate buffer (pH 4.5) containing 0.1% bovine serum albumin (BSA) at 37 °C for 22 hours. After the incubation, the reaction was stopped by heating the tube with boiling water for 3 min, and then glycosphingolipids (GSLs) were isolated using a C18 Sep-Pak Cartridge. Aliquots of samples were spotted on a silica gel plate and developed with $CHCl_3$/MeOH/0.2% (w/v) $CaCl_2$ aq. (60:40:9, (v/v)). To reveal GSLs, the thin-layer chromatography plate was sprayed with orcinol reagent and heated at 120 °C for 5 min.

References for electronic supplementary information

[S1] K. L. Webster, A. B. Maude, M. E. O'Donnell, A. P. Mehrotra and D. Gani, *J. Chem. Soc. Perkin Trans. 1*, 2001, 1673.

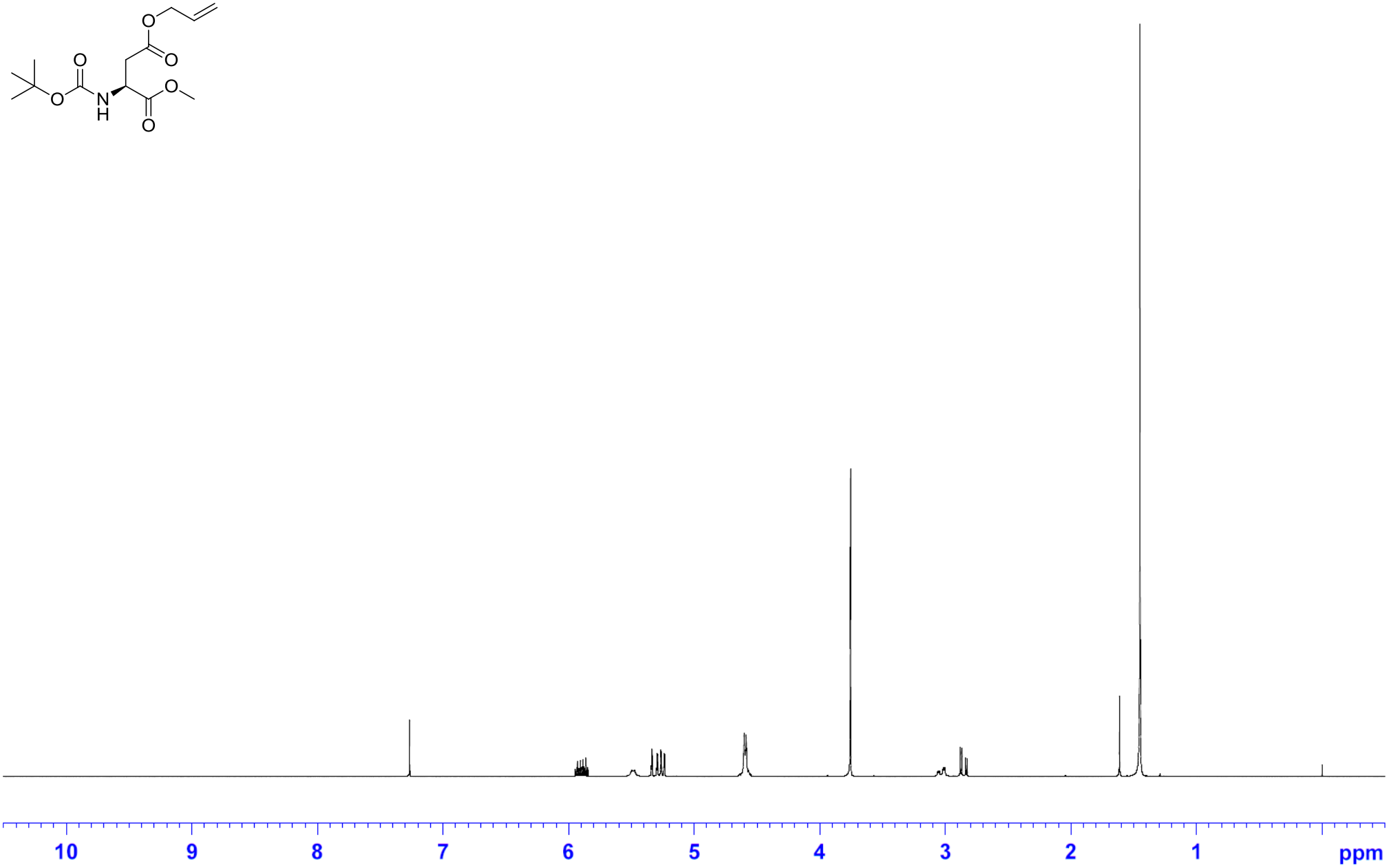
[S2] R. E. Thompson, B. Chan, L. Radom, K. A. Jolliffe and R. J. Payne, *Angew. Chem. Int. Ed.*, 2013, **52**, 9723.

[S3] K. Sato, A. Shigenaga, K. Kitakaze, K. Sakamoto, D. Tsuji, K. Itoh and A. Otaka, *Angew. Chem. Int. Ed.*, 2013, **52**, 7855.

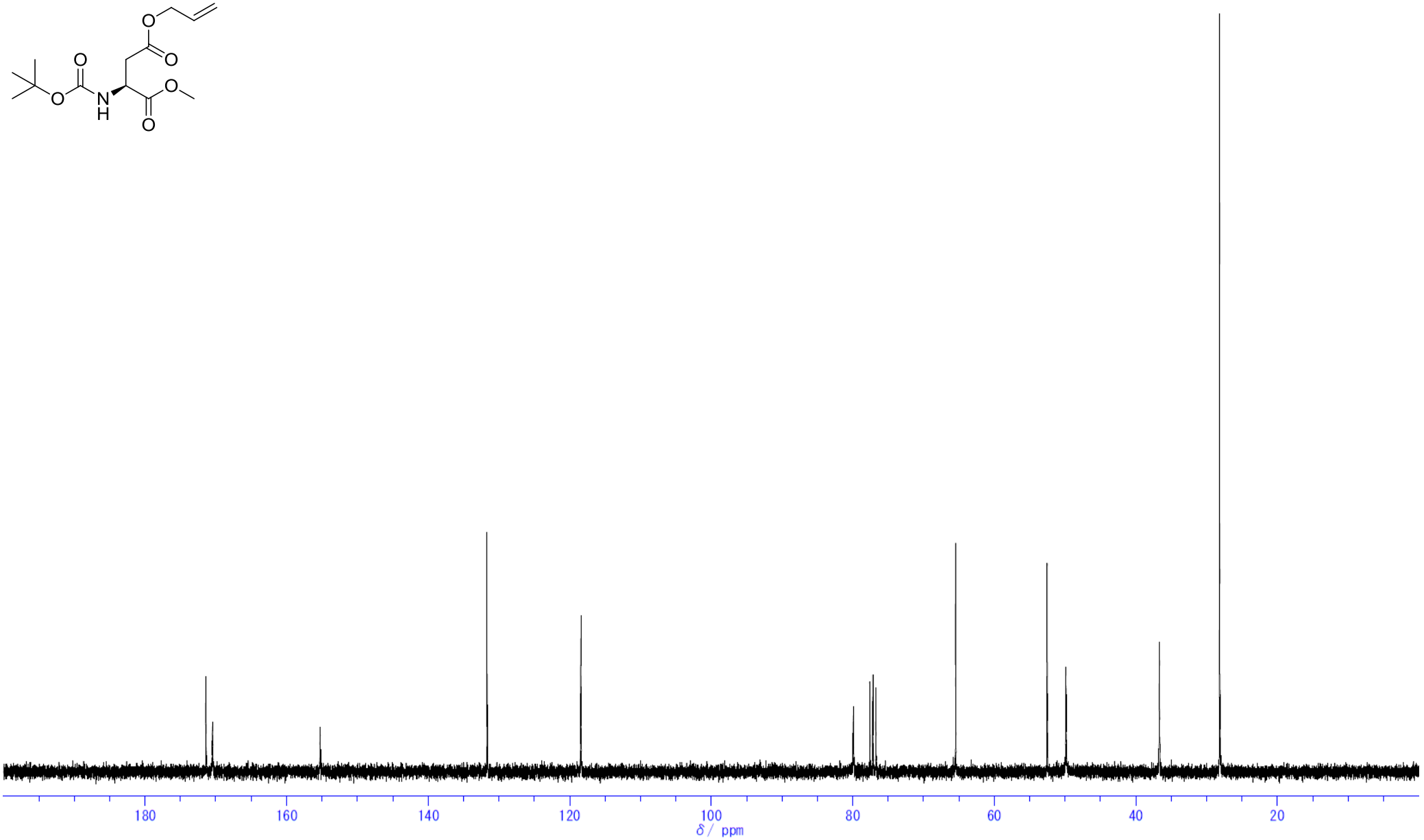
[S4] C. N. Pace, F. Vajdos, L. Fee, G. Grimsley and T. Gray, *Protein Sci.* 1995, **4**, 2411.

[S5] K. Matsuoka, T. Tamura, D. Tsuji, Y. Dohzono, K. Kitakaze, K. Ohno, S. Saito, H. Sakuraba and K. Itoh, *Mol. Ther.* 2011, **19**, 1017.

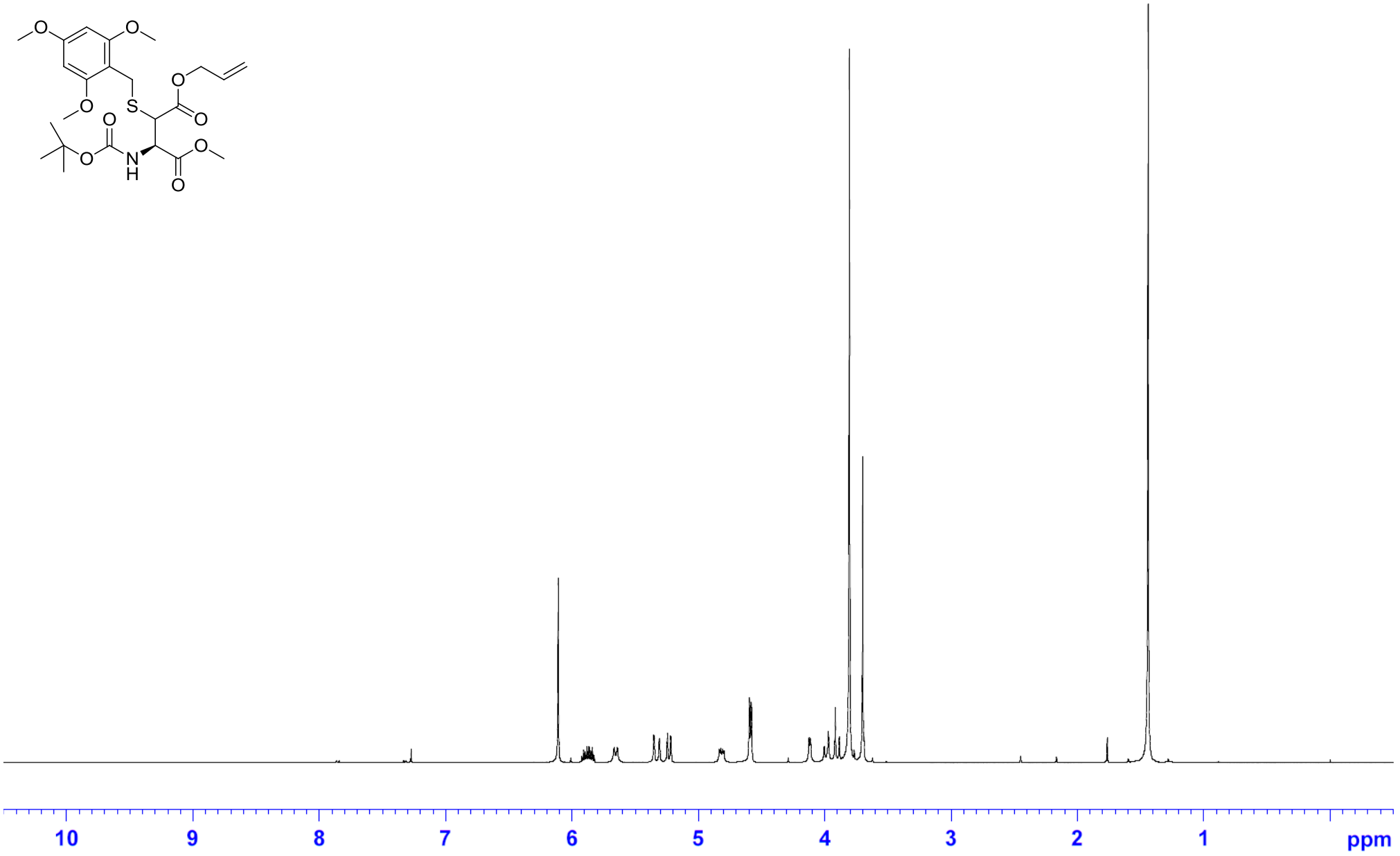
^{1}H NMR spectrum of **8**



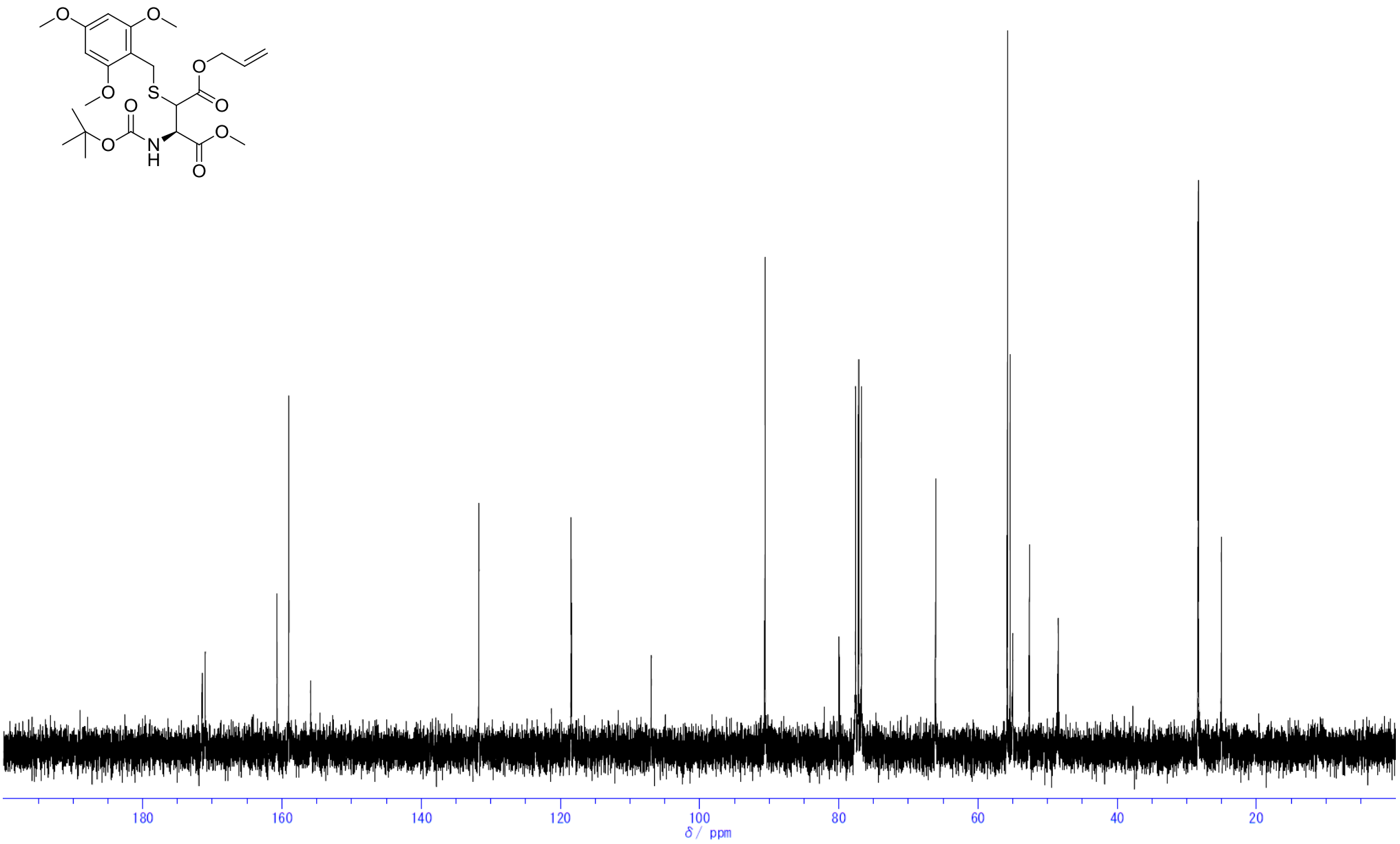
^{13}C NMR spectrum of **8**



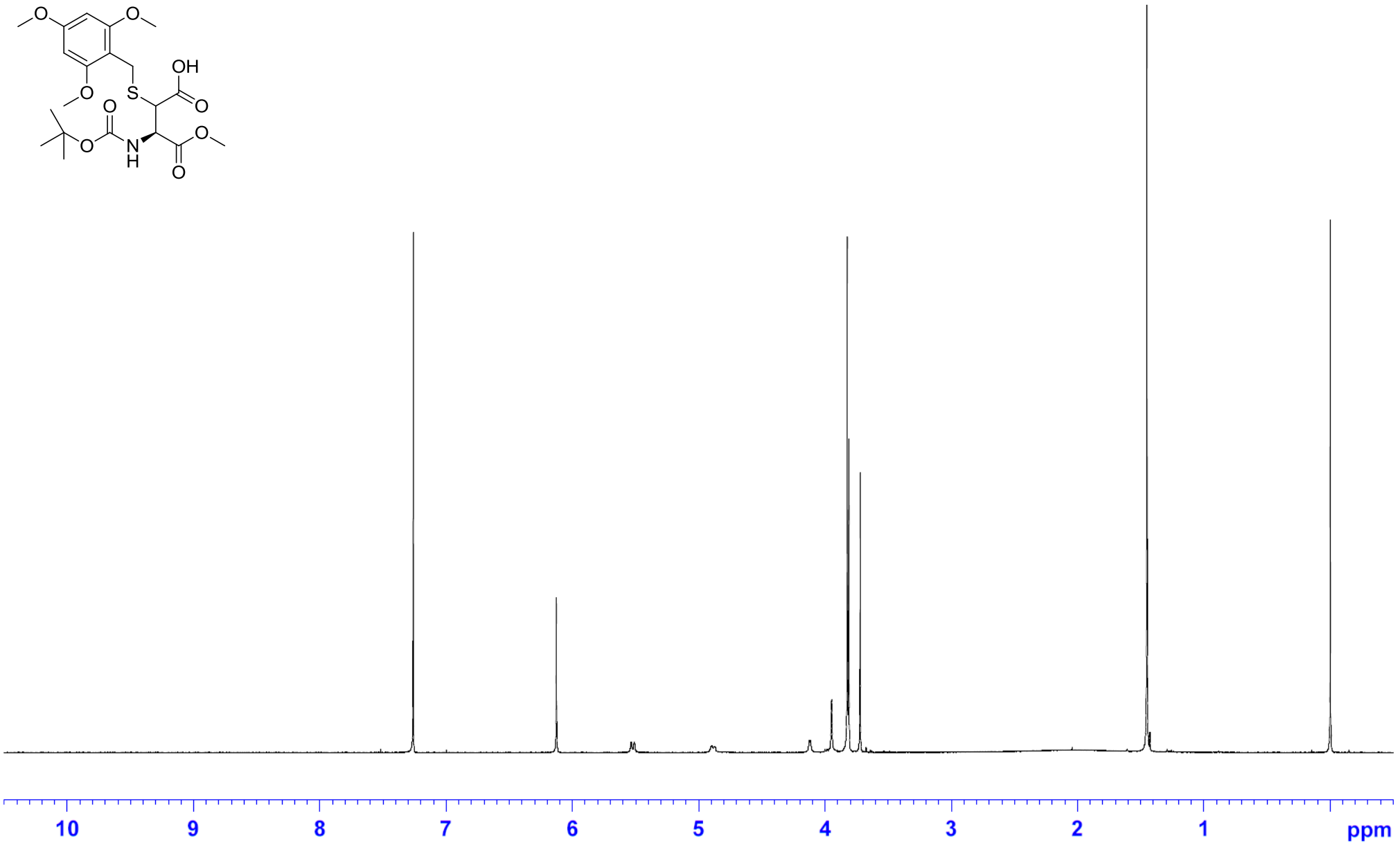
^{1}H NMR spectrum of **10**



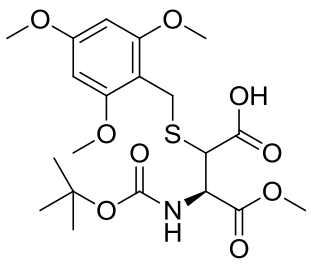
^{13}C NMR spectrum of **10**



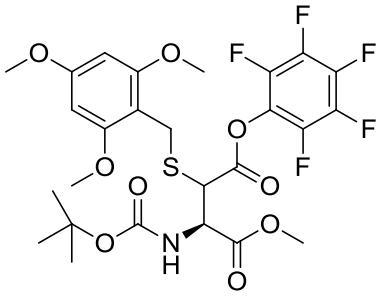
^{1}H NMR spectrum of **11**



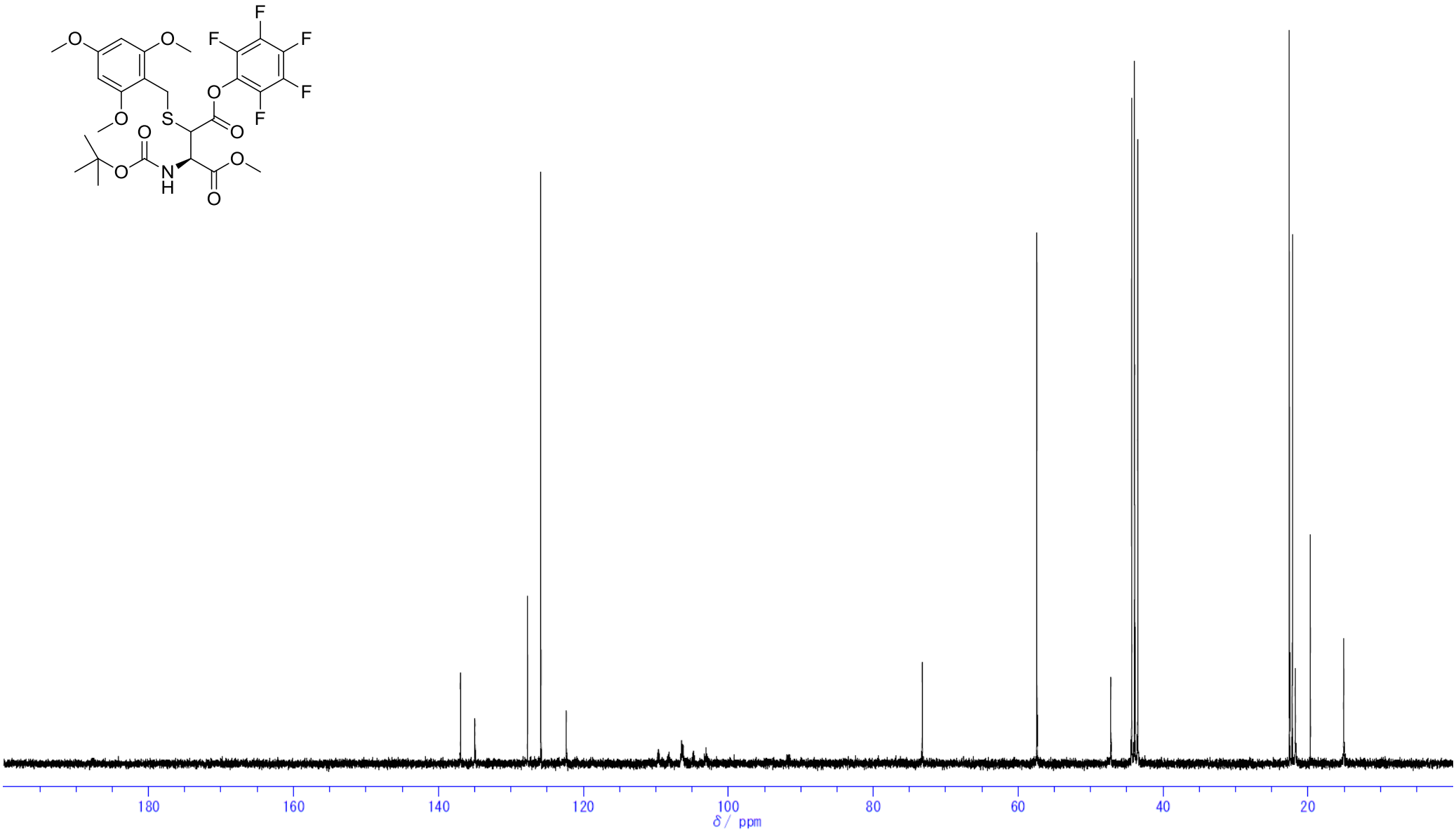
^{13}C NMR spectrum of **11**



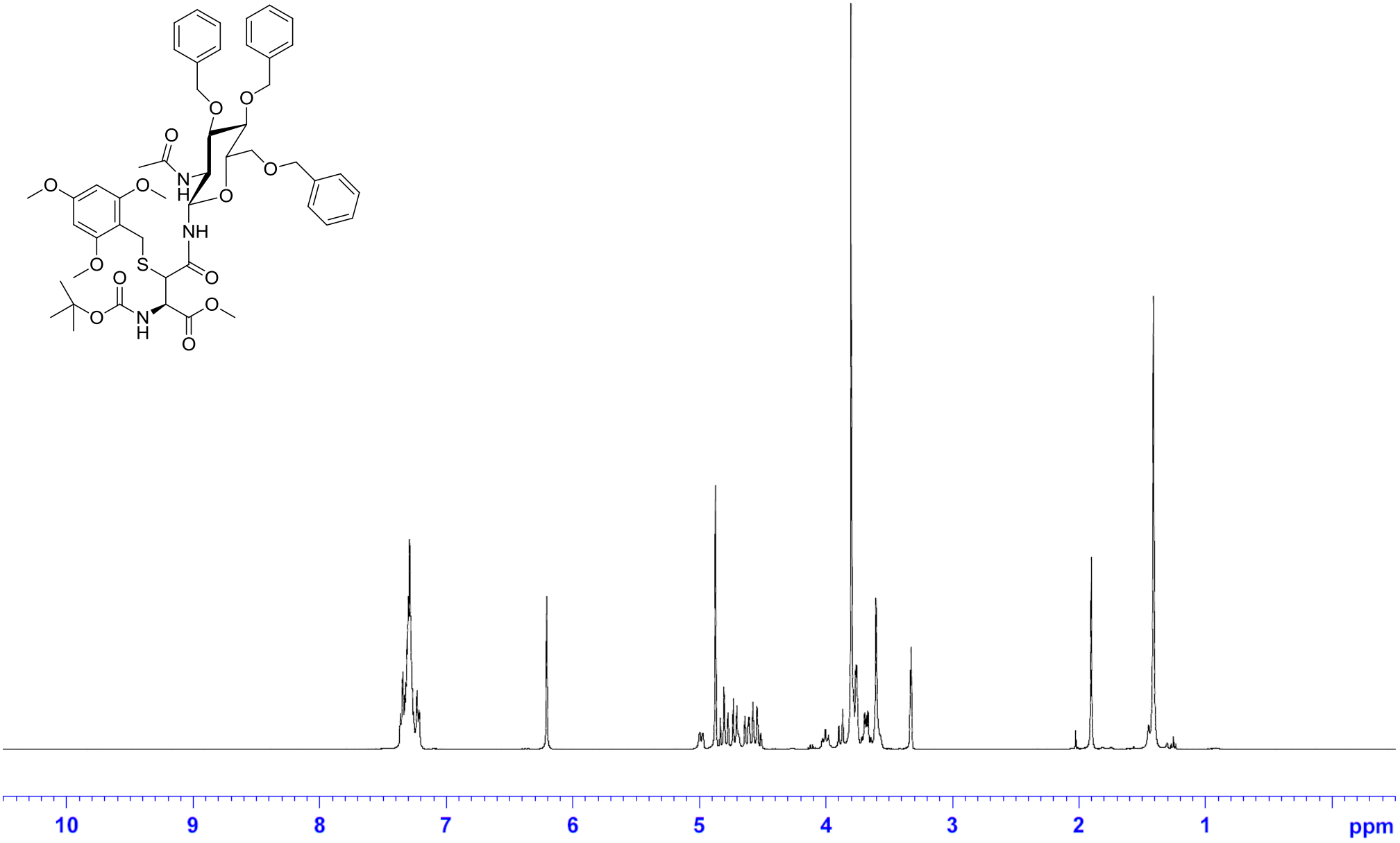
^{1}H NMR spectrum of **12**



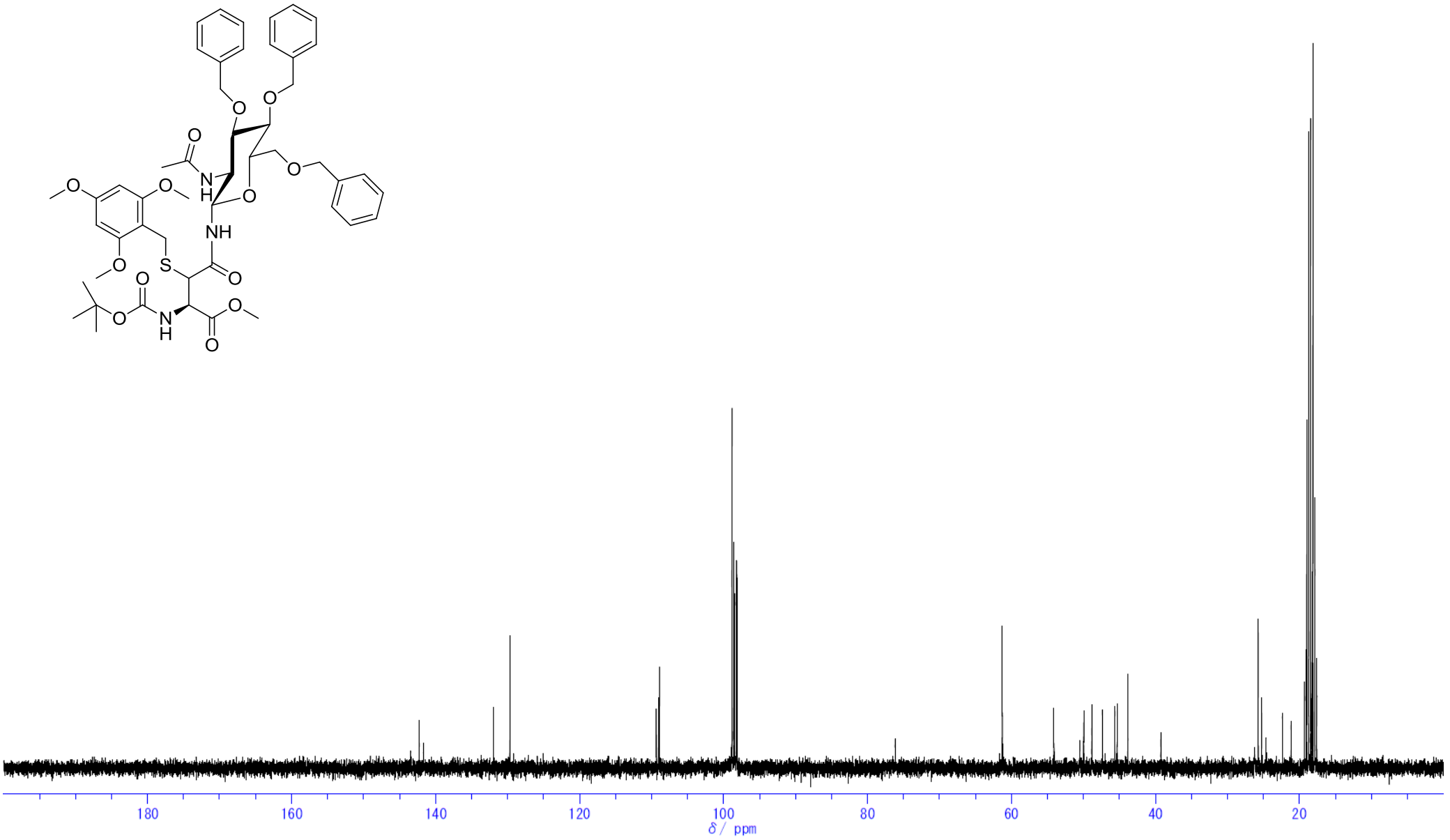
^{13}C NMR spectrum of **12**



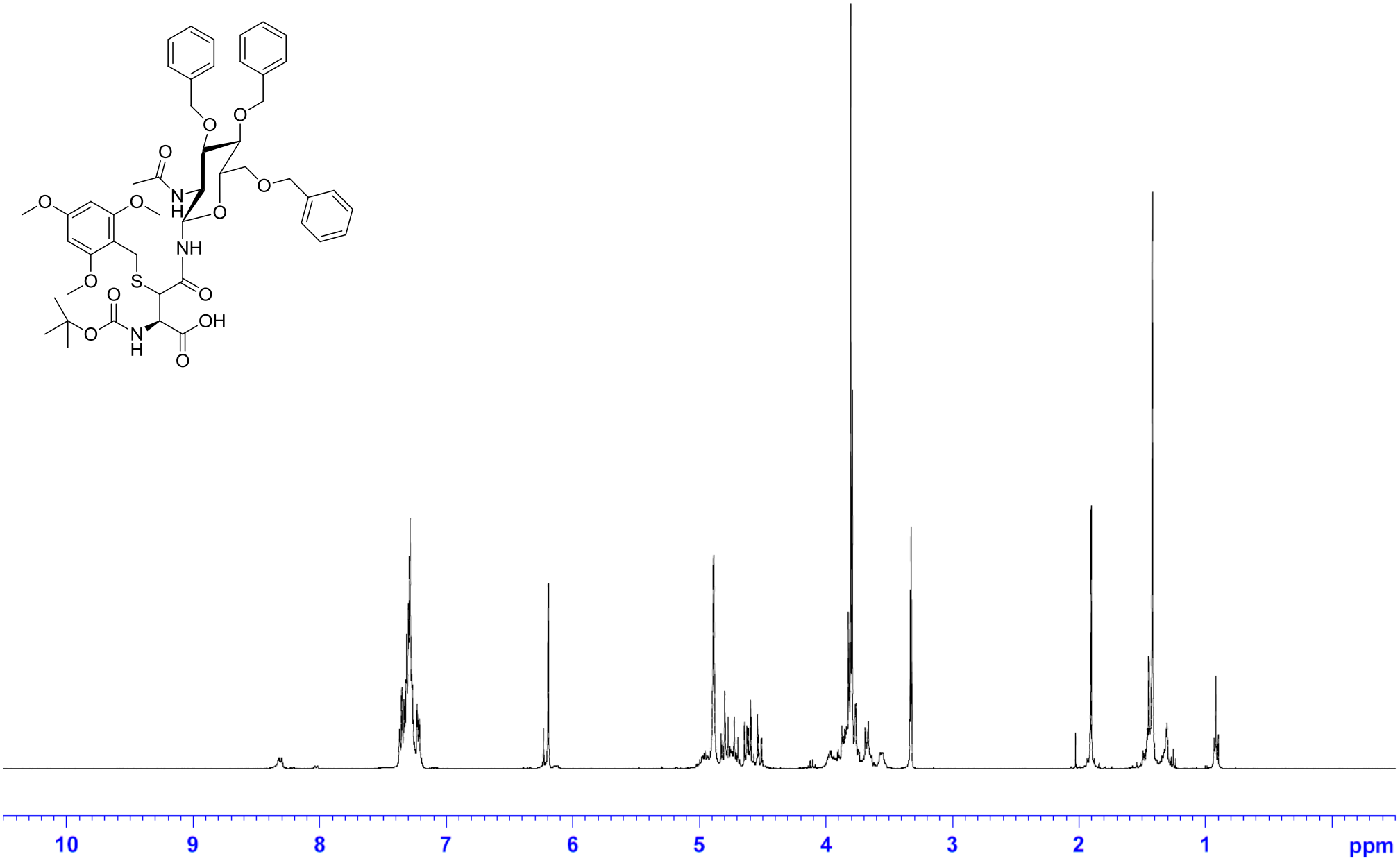
^{1}H NMR spectrum of **14**



^{1}H NMR spectrum of **14**



^{1}H NMR spectrum of **1**



^{13}C NMR spectrum of **1**