Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Facile Synthesis of GalNAc Monomers and Block Polycations for Hepatocyte Gene Delivery

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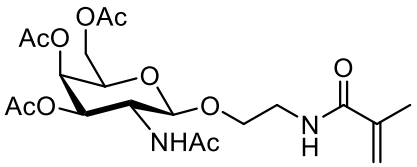
Experimental Section

Materials and Reagents:

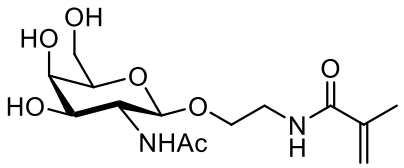
Chemicals and solvents were purchased from Acros Organics, Alfa Aesar, Carbosynth and Sigma-Aldrich and were used as received. The monomer 2-aminoethylmethacrylamide hydrochloride (AEMA) was purchased from Polysciences (Warrington, PA) and used directly. The chain transfer agent (CTA) 4-cyano-4-(propylsulfanylthiocarbonyl)-sulfanylpentanoic acid (CPP) was synthesized as previously reported.[1] *N*-(hydroxyethyl)methacrylamide (HEMAm) was synthesized as previously reported.[2] All reactions were performed under an inert atmosphere of dry nitrogen gas (N_2) in oven-dried (180 °C) glassware. TLC analyses were performed on TLC silica gel 60F254 plates from EMD Chemical Inc. and were visualized with UV light and $KMnO_4$ stain. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26), water (4.79) or dimethyl sulfoxide (2.50); carbon chemical shifts are reported in ppm from an internal standard of residual chloroform (77.15) or dimethyl sulfoxide (39.5). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, m = multiplet, br = broad), coupling constant(s), integration. High-resolution mass spectra were obtained on an LTQ Orbitrap Velos instrument (Thermo Scientific, Waltham, MA).

Synthetic Procedures:



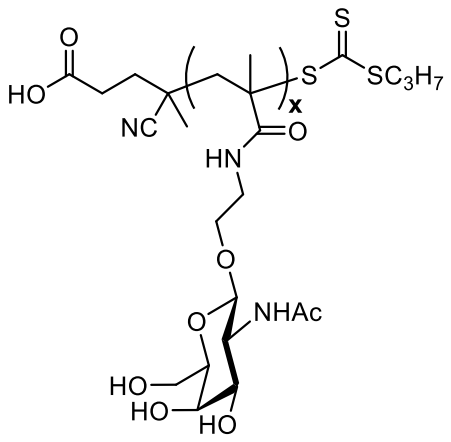
2

***N*-[2-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyloxy)ethyl]methacrylamide (2)**. To a suspension of 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose (**1**, 3.0 g, 7.70 mmol, 1.0 equiv) in 1,2-dichloroethane (60 mL) was added trimethylsilyl trifluoromethanesulfonate (1.68 mL, 10.0 mmol, 1.3 equiv) dropwise at 23 °C. The reaction was then heated to 50 °C and stirred at this temperature for 5 h during which the reaction turned translucent amber. To this solution was then added a solution of *N*-(2-hydroxyethyl)methacrylamide (2.20 g, 16.9 mmol, 2.2 equiv) in 1,2-dichloroethane (5 mL) dropwise in one portion. The reaction was then stirred at 50 °C overnight. After 16 h, the reaction was cooled to 23 °C, quenched with triethylamine (2 mL) and diluted with CH_2Cl_2 (100 mL). The diluted mixture was washed with saturated aqueous $NaHCO_3$ (200 mL), saturated aqueous NaCl (200 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford an orange oil. Purification by flash chromatography on silica gel (5% MeOH–CH_2Cl_2) afforded the title compound (3.41 g, 97 %) as a white foam: R_f = 0.25 (5% MeOH–CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (br s, 1H), 5.74 (s, 1H), 5.60 (d, J = 8.8 Hz, 1H), 5.35 (s, J = 2.9, 1.4 Hz, 2H), 5.12 (dd, J = 11.3, 3.4 Hz, 1H), 4.62 (d, J = 8.4 Hz, 1H), 4.36–4.02 (m, 3H), 4.01–3.81 (m, 2H), 3.78–3.59 (m, 2H), 3.52–3.34 (m, 1H), 2.15 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.63, 170.56, 170.3, 168.7, 140.1, 119.9, 101.3, 71.0, 70.4, 68.4, 66.8, 61.6, 51.3, 39.2, 23.6, 20.84, 20.82, 18.8; HRMS (ESI+) calcd for $C_{20}H_{30}N_2NaO_{10}^+$ $[M + Na]^+$ 481.1793, found 481.2423.



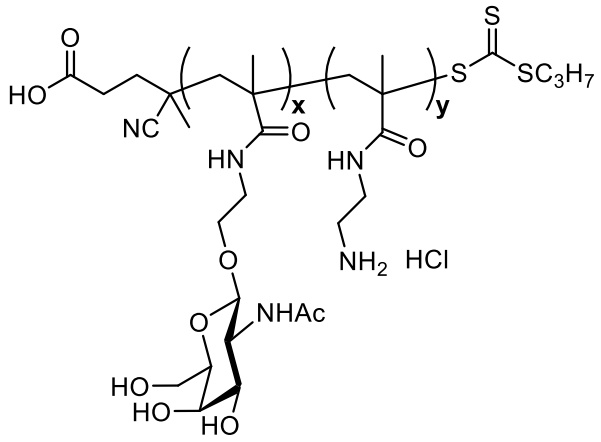
3

***N*-(2-[2-Acetamido-2-deoxy-β-D-galactopyranosyloxy]ethyl)methacrylamide/MAGalNAc (3)**. To a solution of **2** (2.70 g, 5.89 mmol, 1.0 equiv) in MeOH (degassed, 60 mL), was added sodium methoxide (0.98 g, 18.3 mmol, 3.1 equiv) until the reaction reached pH ~9. The reaction was stirred at 23 °C and monitored by TLC. After 1.5 h, the reaction was neutralized (pH 7) with DOWEX 50WX8 hydrogen form resin (ca. 2.0 g) and filtered. The filtrate was concentrated *in vacuo* to afford an amber oily residue, which was re-dissolved in H_2O (20 mL) and lyophilized to complete dryness, to afford the title compound (1.81 g, 94%) as an off-white foam: ^{1}H NMR (400 MHz, D_2O) δ 5.72 (s, 1H), 5.49 (s, 1H), 4.46 (d, J = 8.4 Hz, 1H), 4.03–3.64 (m, 9H), 3.57–3.38 (m, 2H), 2.79 (s, 3H), 2.98 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.3, 171.1, 170.5, 141.2, 120.7, 103.1, 76.5, 73.0, 69.7, 68.9, 62.4, 53.8, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 40.6, 23.1, 18.8; HRMS (ESI+) calcd for $C_{14}H_{24}N_2NaO_7^+$ $[M + Na]^+$ 355.1476, found 355.1605.



PGx

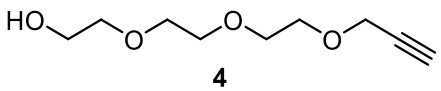
General Procedure of MAGalNAc polymerization (PGx). A Schlenk flask (10 mL) affixed to a vacuum manifold was charged with a magnetic stir bar, **3** (500 mg, 1.50 mmol), 4-cyano-4-(propylsulfanylthiocarbonyl)-sulfanylpentanoic acid (CPP), 4,4′-azobis(4-cyanovaleric acid) (V-501) and a 2:1 (v:v) mixture of water and ethanol (1.50 mL). The monomer: CTA: initiator molar ratios for each polymer are depicted in Table S1. The solution was de-gassed via three freeze-pump-thaw cycles and purged with N_2 (g) before stirring at 70 °C. After 16 h, the reaction was quenched by N_2 (*l*) submersion and opening the reaction to air. The polymer was diluted with H_2O (10 mL) and then purified by dialysis against pure H_2O with either a 1 or 3.5 kDa molecular weight cutoff (MWCO) membrane tubing for 4 days (replacing H_2O twice per day) and dried via lyophilization to afford the polymer as an off-white foam.



PGxAy

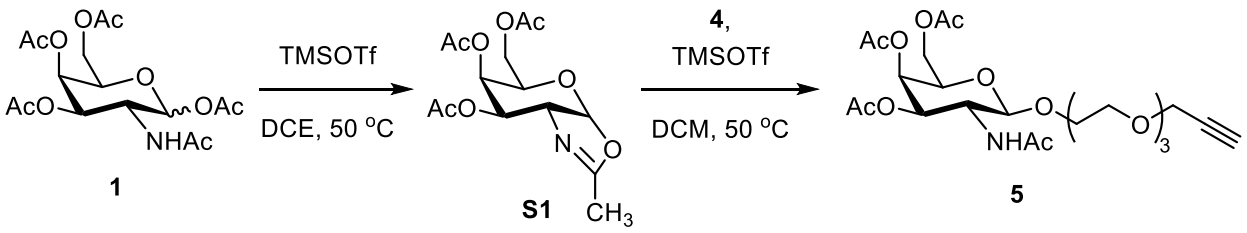
General Procedure of P(MAGalNAc-*b*-AEMA) synthesis (PGxAy). A Schlenk flask (10 mL) affixed to a vacuum manifold was charged with a magnetic stir bar, 2-aminoethylmethacrylamide hydrochloride, **PGx** (1 equiv.), 4,4′-azobis(4-

cyanovaleric acid) (V-501) and a 9:1 (v:v) mixture of 1 M sodium acetate/acetic acid buffer (pH 4.5) and ethanol (1 M). The monomer: CTA: initiator molar ratios for each polymer are depicted in Table S1. The solution was de-gassed via three freeze-pump-thaw cycles and purged with N_2 (g) before stirring at 70 °C. After 8-16 h, the reaction was quenched by N_2 (*l*) submersion and opening the reaction to air. The polymer was diluted with H_2O (10 mL) and then purified by dialysis against acidified H_2O (pH ~ 2) with a 3.5 kDa molecular weight cutoff (MWCO) membrane tubing for 3 days (replacing acidified H_2O once per day) and dried via lyophilization to afford the polymer as an off-white foam.



4

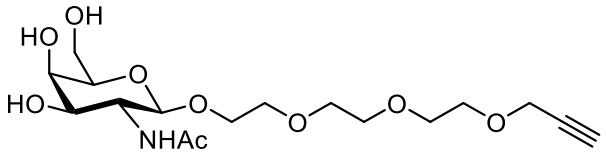
2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-ol (4). To a solution of triethylene glycol (3.0 g, 20.0 mmol, 2.0 equiv) in anhydrous THF (50 mL) was added potassium *tert*-butoxide (1.13 g, 10.1 mmol, 1.01 equiv) slowly at 0 °C. After 30 min, the reaction became a white slurry. To this suspension was added propargyl bromide (1.07 mL, 10.0 mmol, 1.0 equiv, 80 % [w/v] in toluene) dropwise. The reaction was stirred at 23 °C and monitored by TLC. After 21 h, the reaction was filtered through Celite and washed with CH_2Cl_2 (10 mL). The filtrate was concentrated *in vacuo* to afford a brown oil. Purification by flash chromatography on silica gel (60% EtOAc/hexanes) afforded the title compound (1.72 g, 91%) as a yellow oil: R_f = 0.20 (60% EtOAc–hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (d, J = 2.4 Hz, 2H), 3.83–3.63 (m, 10H), 3.63–3.56 (m, 2H), 2.43 (t, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 79.7, 74.7, 72.6, 70.8, 70.49, 70.45, 69.2, 61.9, 58.5; HRMS (ESI+) calcd for $C_9H_{16}NaO_4^+$ $[M + Na]^+$ 211.0941, found 211.0915.



2-{2-[2-(prop-2-yn-1-yloxy)ethoxy]ethoxy}ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (5). To a solution of **1** (7.0 g, 18.0 mmol, 1.0 equiv) in 1,2-dichloroethane (180 mL) was added trimethylsilyl trifluoromethanesulfonate (3.9 mL, 23.4 mmol, 1.3 equiv) dropwise at 23 °C. The reaction was then heated to 50 °C and monitored by TLC. After 5 h, the reaction was cooled and quenched with triethylamine (4 mL) over ice and diluted with CH_2Cl_2 (100 mL). The diluted mixture was washed with saturated aqueous $NaHCO_3$ (200 mL), saturated aqueous NaCl (200 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford **S1** as an amber oil (6.0 g, >100 %), which is used in the next step directly without further purification:^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (d, J = 6.8 Hz, 1H), 5.58 – 5.35 (m, 1H), 5.00–4.78 (m, 1H), 4.27–4.15 (m, 2H), 4.13–4.07 (m, 1H), 4.02–3.96 (m, 1H), 2.11 (s, 3H), 2.06 (s, 6H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.3, 170.0, 165.6, 101.6, 72.0, 69.7, 65.4, 63.7, 61.7, 53.6, 20.94, 20.85, 20.7, 14.6; HRMS (ESI+) calcd for $C_{14}H_{19}NNaO_8^+$ $[M + Na]^+$ 352.1003, found 352.1007.

To a solution of **S1** (2.10 g, 6.38 mmol, 1.2 equiv) and **4** (1.00 g, 5.31 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL) was added 4 Å molecular sieves (powdered, 100 mg / mmol **S1**) at 23 °C. To this suspension was added trimethylsilyl trifluoromethanesulfonate (0.27 mL, 1.59 mmol, 0.3 equiv) dropwise at 23 °C. The reaction was heated to 50 °C and monitored by TLC. After 17 h, the reaction was filtered over Celite and the filtrate was diluted with additional CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ (100 mL), saturated aqueous NaCl (100 mL), dried

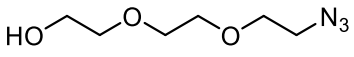
($MgSO_4$) and concentrated *in vacuo* to afford an amber oil. Purification by flash chromatography on silica gel (5% MeOH–CH_2Cl_2) afforded the title compound (1.98 g, 72%) as a yellow oil: R_f = 0.32 (5% MeOH–CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (d, *J* = 9.5 Hz, 1H), 5.30 (dd, *J* = 3.4, 1.1 Hz, 1H), 4.96 (dd, *J* = 11.2, 3.4 Hz, 1H), 4.78 (d, J = 8.6 Hz, 1H), 4.35 – 4.04 (m, 5H), 3.95–3.79 (m, 2H), 3.80–3.54 (m, 10H), 2.43 (dt, *J* = 6.0, 2.4 Hz, 1H), 2.14 (s, 3H), 2.09–1.88 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.7, 170.61, 170.58, 102.6, 79.6, 75.0, 71.8, 71.4, 70.8, 70.6, 70.4, 69.2, 68.7, 66.8, 61.8, 58.4, 50.5, 23.4, 20.89, 20.87, 20.83, 20.75; HRMS (ESI+) calcd for $C_{23}H_{35}NNaO_{12}^+$ $[M + Na]^+$ 540.2051, found 583.1895.



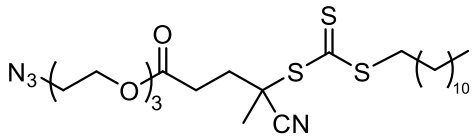
6

2-{2-[2-(prop-2-yn-1-yloxy)ethoxy]ethoxy}ethyl 2-acetamido

-2-deoxy-β-D-galactopyranoside (6). To a solution of **5** (1.00 g, 1.93 mmol, 1.0 equiv) in MeOH (20 mL) was added sodium methoxide (0.44 g, 8.13 mmol, 4.2 equiv) until the reaction reached a pH of 9. The reaction was stirred at 23 °C and monitored by TLC. After 3 h, the reaction was neutralized with DOWEX 50WX8 hydrogen form resin (approximately 1.0 g) and filtered. The filtrate was concentrated *in vacuo* to afford a brown oil. Purification by flash chromatography on silica gel (20% MeOH–CH_2Cl_2) afforded the title compound (0.36 g, 47%) as a white solid: R_f = 0.31 (20% MeOH–CH_2Cl_2); ^{1}H NMR (400 MHz, D_2O) δ 4.52 (d, J = 8.6 Hz, 1H), 4.26 (s, 2H), 4.02 (ddd, J = 11.6, 5.5, 3.0 Hz, 1H), 3.97–3.88 (m, 2H), 3.88–3.65 (m, 15H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 101.5, 75.1, 71.1, 69.73, 69.67, 69.61, 69.4, 68.8, 68.7, 67.8, 61.0, 57.9, 52.4, 22.2; HRMS (ESI+) calcd for $C_{17}H_{29}NNaO_9^+$ $[M + Na]^+$ 414.1735, found 414.1776.

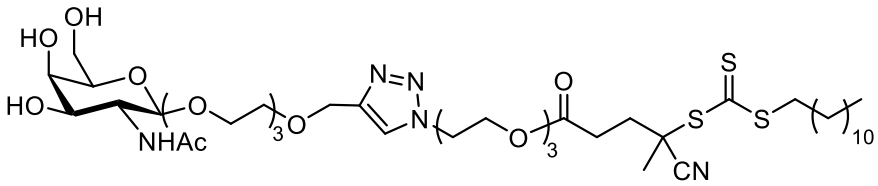


2-[2-(2-azidoethoxy)ethoxy]ethanol. To a solution of 2-[2-(2-chloroethoxy)ethoxy]ethanol (10.0 g, 59.3 mmol, 1.0 equiv) in H_2O (200 mL) was added sodium azide (7.71 g, 119 mmol, 2.0 equiv) in one portion. The reaction was then heated to reflux (100 °C) and monitored by TLC. After 48 h, the reaction was cooled to 23 °C, and the product was extracted with CH_2Cl_2 (2 × 200 mL). The combined organic extracts were washed with saturated aqueous NaCl (300 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the title compound (10.3 g, 99 %) as a colorless oil which was used without further purification: ^{1}H NMR, ^{13}C NMR and HRMS matched reported spectra.[3]



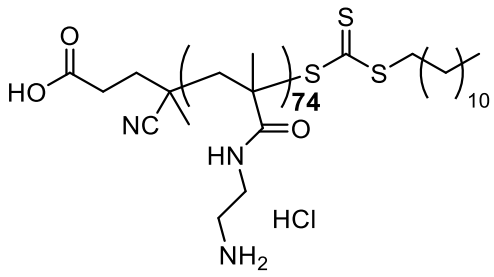
7

2-[2-(2-azidoethoxy)ethoxy]ethyl 4-cyano-4-{[(dodecylthio)carbonothioyl]thio}pentanoate (7). To a solution of 4-cyano-4-{[(dodecylthio)carbonothioyl]thio}pentanoic acid (**7**, CDP, 1.00 g, 2.48 mmol, 1.0 equiv), *N*,*N*′-dicyclohexylcarbodiimide (0.61 g, 2.97 mmol, 1.2 equiv), 4-dimethylaminopyridine (28 mg, 0.25 mmol, 0.1 equiv) in CH_2Cl_2 (20 mL) was added 2-[2-(2-azidoethoxy)ethoxy]ethanol (0.52 g, 2.97 mmol, 1.2 equiv) at 23 °C. The reaction was stirred at 23 °C and monitored by TLC. After 14 h, the reaction was cooled to 0 °C and filtered. The filtrate was concentrated *in vacuo* to afford a deep yellow oil. Purification by flash chromatography on silica gel (35% EtOAc–hexanes) afforded the title compound (1.08 g, 78 %) as a yellow oil: R_f = 0.60 (35% EtOAc–hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36–4.16 (m, 2H), 3.75–3.66 (m, 8H), 3.39 (t, J = 5.0 Hz, 2H), 3.36–3.28 (t, J = 7.4 Hz, 2H), 2.74–2.60 (m, 2H), 2.60–2.45 (m, 1H), 2.43–2.42 (m, 1H), 1.87 (s, 3H), 1.76–1.60 (m, 2H), 1.49–1.18 (m, 18H), 0.95–0.82 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.7, 171.5, 119.0, 70.7, 70.7, 70.2, 69.1, 64.1, 50.7, 46.4, 37.1, 33.8, 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.1, 28.9, 27.7, 24.9, 22.7, 14.1; HRMS (ESI+) calcd for $C_{25}H_{44}N_4NaO_4S_3^+$ $[M + Na]^+$ 583.2417, found 583.2437.



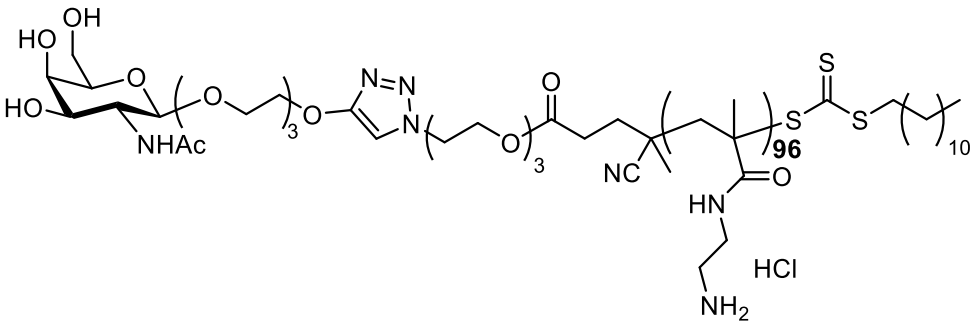
9

2-{2-(2-[4-{(2-[2-{2-(2-Acetamido-2-deoxy-β-D-galactopyranosyloxy)ethoxy}ethoxy]ethoxy)methyl}-1H-1,2,3-triazol-1-yl]ethoxy)ethoxy}ethyl 4-cyano-4-([{dodecylthio}carbonothioyl]thio)pentanoate (9). To a solution of **6** (100 mg, 0.255 mmol, 1.0 equiv) and **8** (157 mg, 0.281, 1.1 equiv) in $CHCl_3$/EtOH/H_2O (4:1:1, 6 mL) was added $CuSO_4 \cdot 5H_2O$ (13 mg, 0.0510 mmol, 0.2 equiv) and sodium ascorbate (20 mg, 0.102 mmol, 0.4 equiv) at 23 °C. The reaction was stirred at 23 °C and monitored by TLC. After 24 h, the reaction was concentrated *in vacuo*. Purification by flash chromatography on silica gel (5 to 20% MeOH–CH_2Cl_2) afforded the title compound (120 mg, 50%) as a yellow solid: R_f = 0.60 (20% MeOH–CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 4.71–4.61 (m, 2H), 4.61–4.52 (m, 2H), 4.36–4.17 (m, 2H), 4.06 – 3.76 (m, 7H), 3.76–3.45 (m, 16H), 3.32 (t, J = 7.4 Hz, 2H), 2.76–2.61(m, 2H), 2.59–2.45 (m, 1H), 2.44–2.28 (m, 1H), 1.98 (s, 3H), 1.88 (s, 3H), 1.69 (p, J = 7.3 Hz, 2H), 1.46–1.16 (m, 18H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.1, 174.0, 171.6, 119.2, 101.9, 74.5, 74.1, 71.2, 70.7, 70.6, 70.5, 69.7, 69.5, 69.1, 68.9, 68.7, 64.2, 62.7, 55.0, 51.0, 50.5, 46.5, 37.2, 34.0, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.2, 29.1, 27.8, 25.0, 23.1, 22.8, 14.3; HRMS (ESI+) calcd for $C_{42}H_{73}N_5NaO_{13}S_3^+$ [M + Na]$^+$ 974.4259, found 974.4333.



AEMA homopolymerization with CDP. A Schlenk flask (10 mL) affixed to a vacuum manifold was charged with a magnetic stir bar, 2-aminoethylmethacrylamide hydrochloride (300 mg, 1.82 mmol, 50 equiv.), 4-cyano-4-(dodecylsulfanylthiocarbonyl)-sulfanylpentanoic acid (**6**, CDP, 14.7 mg, 0.0364 mmol, 1 equiv.), 4,4′-azobis(4-

cyanovaleric acid) (V-501, 10.0 mg, 0.00364 mmol, 0.1 equiv.) and a 9:1 (v:v) mixture of 1 M sodium acetate/acetic acid buffer (pH 4.5) and ethanol (3.0 mL total, 0.61 M). The solution was de-gassed via three freeze-pump-thaw cycles and purged with N_2 (g) before stirring at 70 °C. After 18 h, the reaction was quenched by N_2 (*l*) submersion and opening the reaction to air. The polymer was diluted with H_2O (10 mL) and then purified by dialysis against acidified H_2O (pH ~ 2) with a 1 kDa molecular weight cutoff (MWCO) membrane tubing for 3 days (replacing acidified H_2O once per day) and dried via lyophilization to afford the polymer (191 mg, 64 %) as an off-white foam.



AEMA homopolymerization with 8. A Schlenk flask (10 mL) affixed to a vacuum manifold was charged with a magnetic stir bar, 2-aminoethylmethacrylamide hydrochloride (300 mg, 1.82 mmol, 50 equiv.), **8** (34.7 mg, 0.0364 mmol, 1 equiv.), 4,4′-azobis(4-

cyanovaleric acid) (V-501, 10.0 mg, 0.00364 mmol, 0.1 equiv.) and a 2:1 (v:v) mixture of 1 M sodium acetate/acetic acid buffer (pH 4.5) and ethanol (2.4 mL total, 0.80 M). The solution was de-gassed via three freeze-pump-thaw cycles and purged with N_2 (g) before stirring at 70 °C. After 18 h, the reaction was quenched by N_2 (*l*) submersion and opening the reaction to air. The polymer was diluted with H_2O (10 mL) and then purified by dialysis against acidified H_2O (pH ~ 2) with a 1 kDa molecular weight cutoff (MWCO) membrane tubing for 3 days (replacing acidified H_2O once per day) and dried via lyophilization to afford the polymer (260 mg, 78 %) as an off-white foam.

Table S1: Molar ratios of monomer, CTA and initiator, molecular weights and dispersities of all polymers synthesized.

Polymer	**CTA**	**Monomer : CTA : Initiator**	M_n **(kDa)**	M_w **(kDa)**	$Đ$
$P(MAGalNAc)_{26}$	CPP	50 : 1 : 0.2	8.6	8.8	1.01
$P(MAGalNAc)_{31}$	CPP	46 : 1 : 0.1	10.5	10.8	1.04
$P(MAGalNAc)_{40}$	CPP	50 : 1 : 0.1	13.6	15.0	1.10
$P(MAGalNAc)_{102}$	CPP	160 : 1 : 0.1	34.3	40.3	1.17
PG26A30	$P(MAGalNAc)_{26}$	40 : 1 : 0.2	13.9	15.5	1.11
PG26A41	$P(MAGalNAc)_{26}$	60 : 1 : 0.2	15.4	18.2	1.18
PG31A55	$P(MAGalNAc)_{31}$	80 : 1 : 0.2	19.5	25.7	1.32
PG31A76	$P(MAGalNAc)_{31}$	160 : 1 : 0.2	23.1	31.2	1.35
PG40A54	$P(MAGalNAc)_{40}$	80 : 1 : 0.1	22.5	33.3	1.45
PG102A12	$P(MAGalNAc)_{102}$	25 : 1 : 0.2	36.3	44.2	1.22
PG102A60	$P(MAGalNAc)_{102}$	50 : 1 : 0.2	44.2	54.4	1.23
$P(AEMA)_{74}$	CDP	50 : 1 : 0.1	12.7	17.5	1.38
$P(AEMA)_{96}$-Gal	**9**	50 : 1 : 0.1	16.8	22.5	1.34

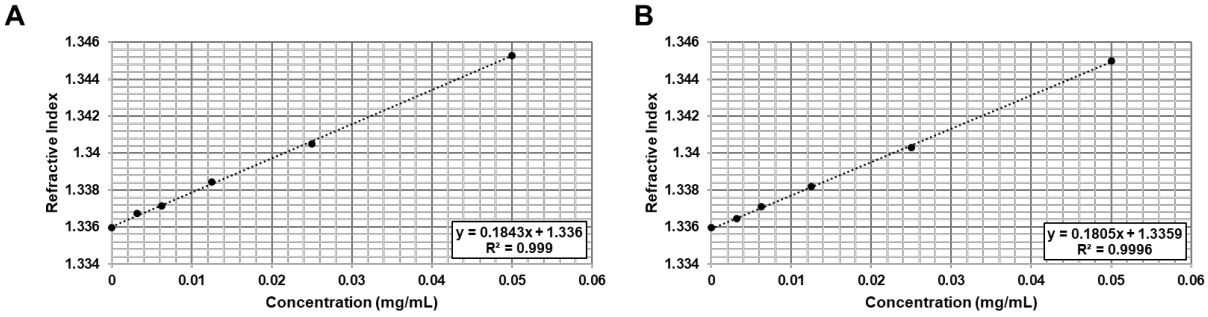


Figure S1: Determination of d*n*/d*c* by refractometry in 1.0 (wt) % acetic acid/0.1 M Na_2SO_4 of polymers A) $P(AEMA)_{74}$ and B) $P(AEMA)_{96}$-Gal. The d*n*/d*c* is represented as the slope of the linear fit. Each data point was run in duplicate.

Polymer Characterization:

Polymer molecular weight (number-average, M_n and weight-average, M_w) and dispersity ($Đ$) were determined by size exclusion chromatography (SEC) partnered with multi-angle light scattering (MALS), employing an aqueous eluent of 1.0 (wt) % acetic acid/0.1 M Na_2SO_4. A flow rate of 0.4 mL/min, Eprogen (Downers Grove, IL) columns [CATSEC1000 (7 μm, 50 × 4.6), CATSEC100 (5 μm, 250 × 4.6), CATSEC300 (5 μm, 250 × 4.6), and CATSEC1000 (7 μm, 250 × 4.6)], a Wyatt HELEOS II light scattering detector (λ = 662 nm) and an Optilab rEX refractometer (λ = 658 nm) were used. Astra V (version 5.3.4.18, Wyatt Technologies, CA) was utilized for the determination of M_n, $Đ$, and d*n*/d*c* (previously measured[4]) of the (co)polymers (**PGxAy**). The d*n*/d*c* of $P(AEMA)_{74}$ and $P(AEMA)_{96}$-Gal were determined by refractometry as described above (Figure S1). ^1H NMR measurements were performed with a temperature-controlled Varian 400-MR (Palo Alto, CA) spectrometer operating at a frequency of 399.7 MHz in D_2O (HDO internal standard). Block polymer compositions were determined by comparing resonance of the MAGalNAc block with those associated with the AEMA block.

Polyplex Formulation and DNA Binding Studies by Gel Electrophoresis. All polyplex solutions were prepared in DNase/RNase-free water, unless otherwise specified. Stock solutions of polymer were prepared in water at a concentration of 15 mM ionizable amines. The stock solutions were diluted in water to appropriate concentrations necessary for the desired N/P ratios. Polyplexes were formulated by adding an equal volume of polymer solution to pDNA in water. For DNA-binding studies by gel electrophoresis, aqueous polymer solutions (10 μL) at appropriate concentrations were added to 10 μL of pDNA solution (50 ng/μL) to make polyplexes at various N/P values. For example, to achieve N/P = 5, the stock polymer solution was diluted to 0.75 mM of ionizable amines before adding 10 μL of it to pDNA solution at 50 ng/μL (0.15 mM phosphates).

Polyplex formulations were kept at room temperature to equilibrate for 1 h before running them on a 0.6% agarose gel containing 0.3 μg/mL ethidium bromide. Gel electrophoresis was carried out at 100 V for 10 min followed by 200 V for 30 min. The binding of pDNA to cationic polymers resulted in its retardation on the gel which was visualized and imaged (Figure S4) with a Fotodyne FOTO/Analyst Luminary/FX workstation from Fotodyne (Hartland,WI).

Particle Size and Zeta Potential Measurements. Zeta potential measurements were carried out by using the Zetasizer Nano-ZS from Malvern Instruments Ltd. (Malvern, U.K.). For zeta potential measurements, 150 μL of aqueous polymer solution was added to 150 μL pDNA (20 ng/μL in water) to achieve N/P values of 2.5, 5 and 10. After equilibration at room temperature for 1 hour, 600 μL of water was added to each sample. The zeta potential was measured at a detection angle of 17°.

Particle size measurements were carried out by using dynamic light scattering with a Dynapro Plate Reader III (Wyatt Technologies, CA) and analyzed via Dynamics software (version 7.10.21). For particle size measurements, polyplexes were prepared by adding 25 μL of aqueous polymer solutions to 25 μL of pDNA (20 ng/μL in water). The polymer concentrations were adjusted to achieve N/P ratios of 2.5, 5, and 10, as described earlier. The mixture was incubated for an hour before adding 100 μL of Opti-MEM. The sample was analyzed immediately to determine the particle size at 0 h. Additional measurements were performed after 1, 2 and 4 h to study the stability of polyplexes over time. Z-average radii calculated by the instrument at 0, 1, 2, and 4 h time points are reported.

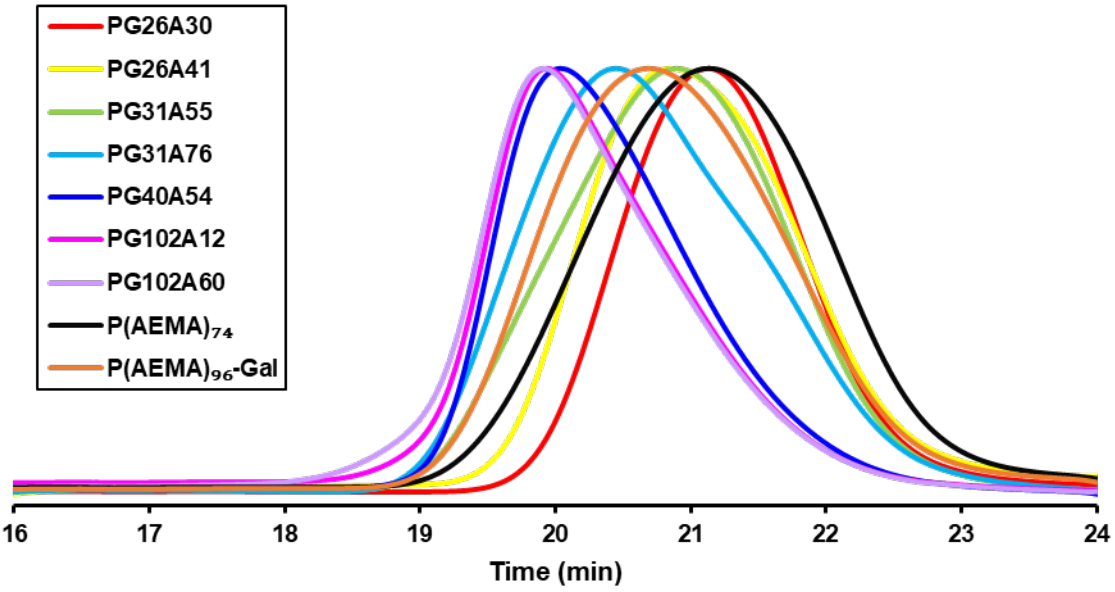


Figure S2: dRI-SEC traces of polymers synthesized for this study.

Table S2: Polydispersity Indexes (PDI) of Polyplexes.

	N/P		
	2.5	**5**	**10**
PG26A30	0.309	0.189	0.207
PG26A41	0.484	0.378	0.149
PG31A55	0.297	0.155	0.162
PG31A76	0.273	0.173	0.223
PG40A54	0.392	0.213	0.168
PG102A12	0.329	0.432	Multimodal
PG102A60	0.537	0.350	0.398
P(AEMA)$_{74}$	–[a]	–	–
P(AEMA)$_{96}$-Gal	–	–	–

[a]Polymers without a PDI value showed aggregation.

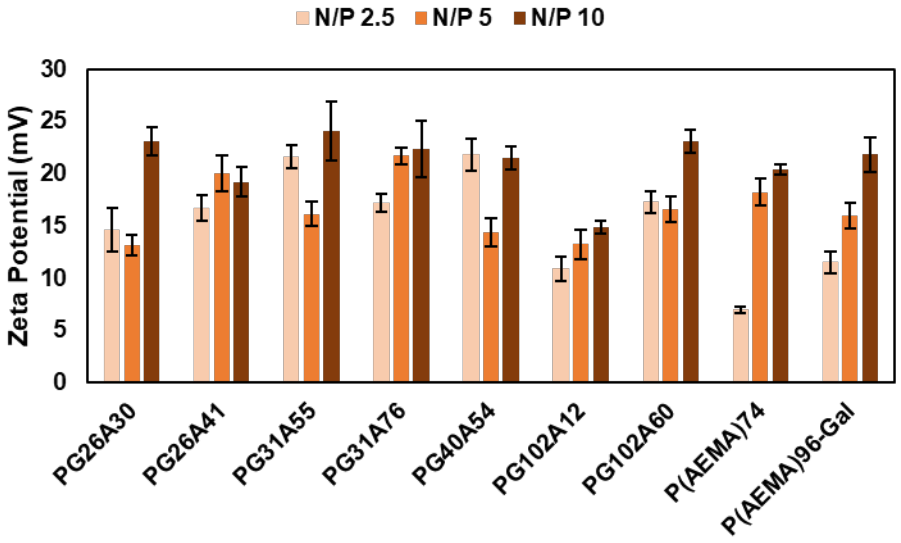


Figure S3: Zeta potentials of polyplexes formulated in water.

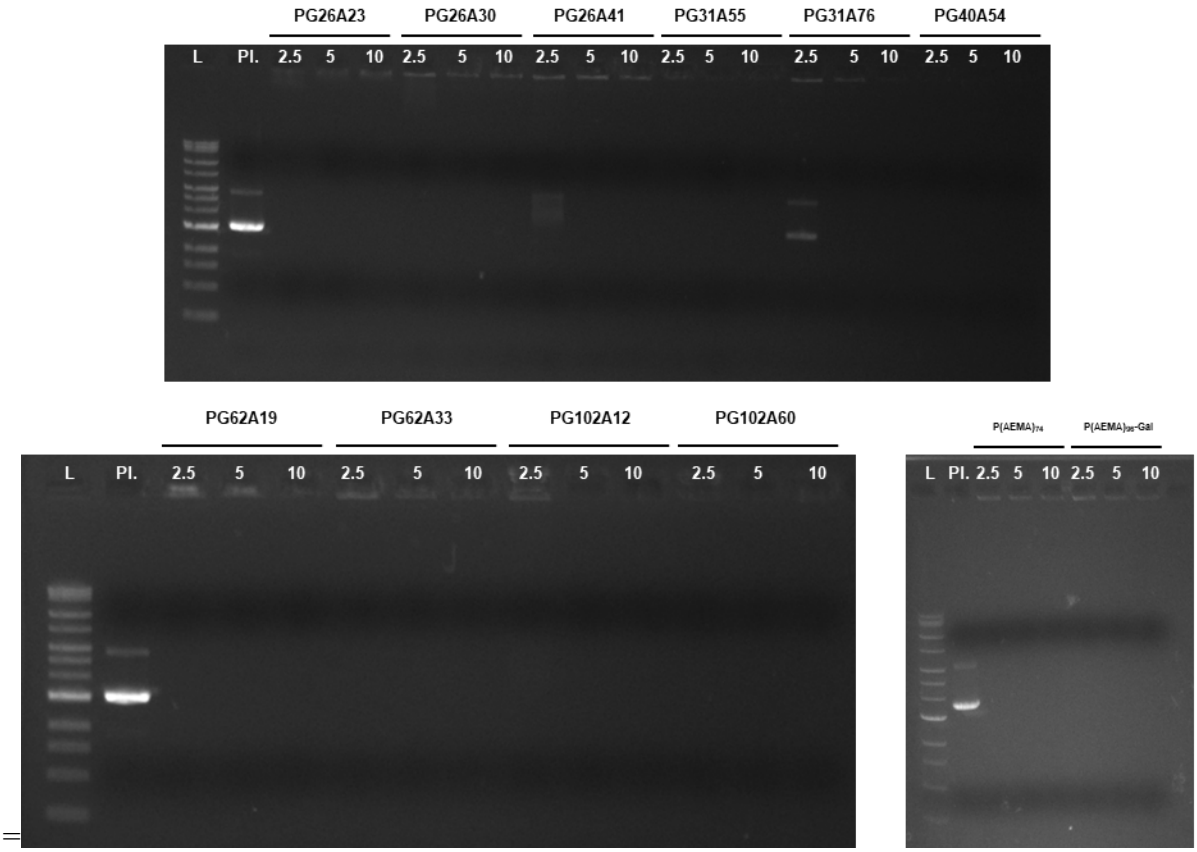


Figure S4: Agarose gel migration assay via gel electrophoresis of pDNA polyplexes. The numbers marking each land represent the N/P value for each formulation. Pl. = Plasmid only; L = DNA ladder (1 kbp). Polymer labeled as "PG26A23" was not used in this study.

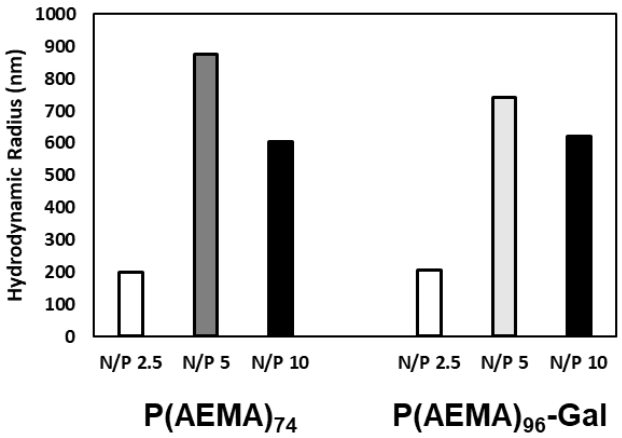


Figure S5: Hydrodynamic radii of polyplexes as measured by dynamic light scattering (DLS). Polyplexes were formed by mixing equal volumes of pDNA and polymer solutions as N/P ratios of 2.5, 5, and 10. The polyplexes were incubated at room temperature for 1 h before diluting with OptiMEM. These polymers were measured after 1 hour.

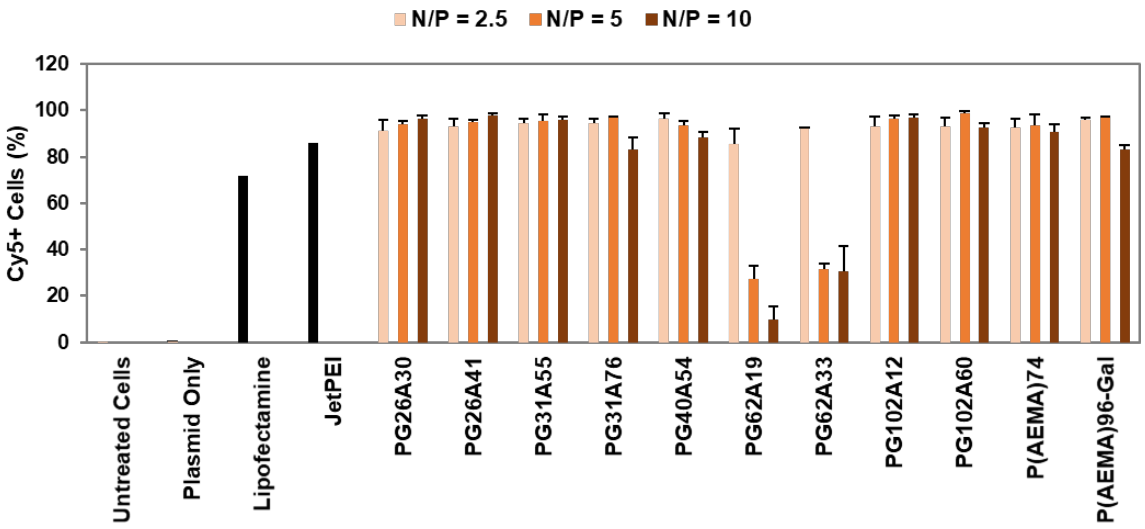


Figure S6. HepG2 cellular internalization of polyplexes formed with Cy5-labeled pDNA at various N/P ratios (2.5, light orange; 5, orange; 10, brown). Data was obtained 24 h after transfection and are presented as mean ± standard deviation (n = 3).

Table S3: Numerical representation of the data presented in Figure S6.

Polymer	**Cy5+ Cells (%)**		
Untreated Cells	0.06 ± 0.05		
Plasmid Only	0.4 ± 0.2		
Lipofectamine	71.5 ± 4.2		
JetPEI	85.6 ± 2.6		
	N/P		
	2.5	**5**	**10**
PG26A30	91.0 ± 4.8	94.0 ± 1.5	94.2 ± 1.5
PG26A41	92.9 ± 3.4	94.8 ± 1.2	97.8 ± 0.7
PG31A55	94.7 ± 1.4	95.6 ± 2.7	95.8 ± 1.3
PG31A76	94.4 ± 1.7	96.6 ± 0.9	83.0 ± 5.3
PG40A54	96.2 ± 2.6	93.4 ± 2.0	88.2 ± 2.3
PG102A12	93.0 ± 4.5	96.6 ± 1.2	96.7 ± 1.7
PG102A60	92.9 ± 3.8	98.6 ± 0.9	92.4 ± 2.2
P(AEMA)$_{74}$	92.7 ± 3.7	93.6 ± 4.6	90.5 ± 3.3
P(AEMA)$_{96}$-Gal	95.8 ± 1.1	97.0 ± 0.4	83.0 ± 1.9

Biological Data:

General Methods:

All cell culture media used in this study were purchased from Life Technologies (Grand Island, NY): minimum essential medium (MEM), reduced-serum medium (Opti-MEM), heat inactivated fetal bovine serum (HI FBS), phosphate-buffered saline (PBS) pH = 7.4, trypsin-EDTA (0.25%), and antibiotic–antimycotic (100×). The cell counting kit (CCK)-8 was purchased from Millipore Sigma (Burlington, MA). The luciferase assay system was purchased from Promega (Madison, WI) and the reagents were prepared as described. Human hepatocellular carcinoma (HepG2, ATCC HB-8065) cell lines were purchased from ATCC (Manassas, VA).

pCAG-FLuc plasmid DNA, used for the luciferase assay, were obtained from Limelight Bio (Philadelphia, PA), respectively. Cy5-labeled plasmid DNA, used for the uptake experiments, was obtained via a Label-IT Cy5 DNA labeling kit (Label IT® Nucleic Acid Labeling Kit Cy-3, Mirus, Madison, WI) and purified by ethanol precipitation. The concentration of purity of the pDNA was determined by UV-Vis spectrophotometry (Nanodrop, Thermo-Fisher, Waltham, MA). Commercially available transfection reagents were used as positive controls in this study. JetPEI (Polyplus-Transfection Inc., Illkirch, France), and Lipofectamine 2000 (Invitrogen, Waltham, MA) were used as standards and positive controls for transfections. Unless specified otherwise, all biological experiments were performed in triplicate, and the mean and standard deviation of data are reported in all figures and tables.

Cell Culture. HepG2 cells were cultured in MEM containing 10% FBS in 75 cm^2 flasks at 37 °C under 5% CO_2 atmosphere to maintain physiological pH. The culture medium was supplemented with Antibiotic−Antimycotic solution from Life Technologies (Grand Island, NY) at a final concentration of 10 μg/ mL penicillin, 10 μg/mL streptomycin, and 25 ng/mL Fungizone.

Cells were monitored for confluency regularly and passaged as necessary. For plating, cells were trypsinized and suspended in MEM containing 10% FBS. A hemocytometer was used to count cells prior to plating. Trypan blue was used to distinguish between viable and dead cells. For CCK-8 and luciferase assays: 50,000 viable cells in 1 mL of MEM containing 10% FBS were plated per well in 24-well plates.

Transfection of HepG2 Cells. HepG2 cells were transfected in 24-well plates at a density of 50,000 cells/well in 1 mL of MEM containing 10% FBS. Transfections were then carried out 24 h after plating. Polyplexes were prepared 1 h before transfection by adding 175 μL of aqueous polymer solutions at appropriate concentrations to 175 μL of pDNA or Cy5-labeled pDNA (20 ng/μL in water) to achieve desired N/P values (2.5, 5 and 10), as described above (330 μL total volume). JetPEI and Lipfectamine 2000 control reagents were prepared as described. The polymer−pDNA mixtures were vortexed and kept at 23 °C for 1 h. MEM was removed via aspiration from each well and cells were washed with 1× PBS. Polyplexes were diluted with reduced-serum Opti-MEM (660 μL; 990 μL total volume). The diluted solution was then added to each well (300 μL/well, n = 3). Four hours after transfection, MEM containing 10% FBS was added to each well (1 mL). A total of 24 h after transfection, the culture medium was replaced with fresh MEM containing 10% FBS.

Luciferase Assay. A total of 48 h after transfection, the culture medium was removed via aspiration and the cells were washed with 1× PBS. An aliquot (100 μL) of 1× lysis buffer was added to each well and kept at room temperature for a minimum of 15 min to allow cell lysis to occur. Cell lysate (5 μL) was then pipetted into an opaque white 96-well plate. After addition of 95 μL of luciferase substrate, the luminescence was measured using a TECAN GENios Pro

microplate reader (Tecan, Männedorf, Switzerland). Protein concentration in each sample was measured using Pierce™ BCA Protein Assay Kit from Thermo Fisher Scientific (Waltham, MA).

Cell Viability Assay. HepG2 cells were transfected as described above in 24 well plates. A total of 48 h after transfection, the culture medium was removed via aspiration and the cells were washed with 1× PBS. Trypsin-EDTA (0.25%, 150 μL) was added to each well to detach cells, and incubated at 37 °C and 5% CO_2 for 5 min. The cell samples were diluted with DMEM containing 10% FBS (350 μL) and resuspended via pipetting. An aliquot of each cell suspension (60 μL) was transferred to a 96-well plate and to each sample was added CCK-8 solution (50 μL). The cells were incubated for 4 h at 37 °C and 5% CO_2. Cell viability was then determined by measuring absorbance at 450 and 650 nm using Biotek Synergy H1 plate reader.

Cellular Uptake of Polyplexes. HepG2 cells were transfected with polyplexes made from Cy5-labeled pDNA, as described above in 24 well plates. At 4 h after transfection, cell culture media and polyplexes were removed and cells were washed once with PBS (1 mL). Trypsin (0.5 mL) was then added to each well to detach the cells from the bottom of the well. Ten minutes after adding trypsin, MEM (0.5 mL) was added to each well and the cell suspensions were transferred to conical-bottomed 96-well plates. Samples were centrifuged at 1100 x g for 10 min at 4 °C. The supernatant was carefully removed via aspiration. Cell pellets were then resuspended in PBS (0.6 mL) to further remove serum proteins and re-centrifuged. The supernatant was again removed, and cells were exposed to Cell-Scrub (0.4 mL) for 10 min at room temperature, followed by quenching with PBS (0.8 mL). Cells were re-pelleted and the supernatant was removed. Propidium iodide solutions were added to each well, and samples were transferred to 96-well round-bottomed plates. Samples were analyzed on a Bio-Rad ZE5 Cell Analyzer flow cytometer (Hercules, CA). A helium−neon laser was used to excite Cy5 (633 nm). Ten thousand events were

collected for each falcon tube. The percentages of Cy5 positive cells were determined by < 0.1% positive population appeared in untreated cells (negative control) sample. The geometric mean of the Cy5 fluorescence intensities were computed and used for subsequent statistical analyses.

Statistical Analysis:

Table S4. ANOVA for cellular uptake.[1]

Source	Sequential Sum of Squares (10^6)	Degrees of Freedom (DF)	Mean Squares (10^6)	*F*-statistic	Probability > *F*
Model	7.4	20	0.37	8.6	$< 10^{-4}$
GalNAc length (A)	0.45	2	0.225	5.3	0.007
AEMA length (B)	1.1	2	0.55	19.9	$< 10^{-4}$
N/P ratio (C)	2.3	2	1.15	27	$< 10^{-4}$
AB	0.17	2	0.085	2	0.15
AC	2.15	4	0.54	12.6	$< 10^{-4}$
BC	0.74	4	0.19	4.3	0.003
ABC	0.46	4	0.12	2.7	0.04
Residuals	3.3	78	0.04	-	-
Total	10.7	98	0.109	-	-

[1]RMSE: 206.7; $R^2 = 0.69$, Adjusted $R^2 = 0.61$

Table S5. ANOVA for transfection.[1]

Source	Sequential Sum of Squares (10^6)	Degrees of Freedom (DF)	Mean Squares (10^6)	*F*-statistic	Probability > *F*
Model	121	20	6.1	15	$< 10^{-4}$
GalNAc length (A)	59.3	2	29.7	73.5	$< 10^{-4}$
AEMA length (B)	11.9	2	6.0	14.8	$< 10^{-4}$
N/P ratio (C)	6.2	2	3.1	7.7	0.0009
AB	9.5	2	4.8	11.7	$< 10^{-4}$
AC	17.3	4	4.3	10.7	$< 10^{-4}$
BC	11.4	4	2.9	7	0.0001
ABC	5.3	4	1.3	3.3	0.015
Residuals	31.5	78	0.4	-	-
Total	152.4	98	1.6	-	-

[1]RMSE: 63526.1; $R^2 = 0.80$, Adjusted $R^2 = 0.74$

Table S6. ANOVA for toxicity.[1]

Source	Sequential Sum of Squares (10^6)	Degrees of Freedom (DF)	Mean Squares (10^6)	*F*-statistic	Probability > *F*
Model	66097.6	20	3304.9	15.9	$< 10^{-4}$
GalNAc length (A)	6711.4	2	3355.7	16.1	$< 10^{-4}$
AEMA length (B)	3765.2	2	1882.6	9	0.0003
N/P ratio (C)	43254.6	2	21627.3	103.8	$< 10^{-4}$
AB	70.2	2	35.1	0.2	0.85
AC	2554.0	4	638.5	3.1	0.02
BC	5692.4	4	1423.1	6.8	0.0001
ABC	4049.7	4	1012.4	4.9	0.0015
Residuals	16249.8	78	208.3	-	-
Total	82347.4	98	840.3	-	-

[1]RMSE: 14.4; $R^2 = 0.80$, Adjusted $R^2 = 0.75$

References:

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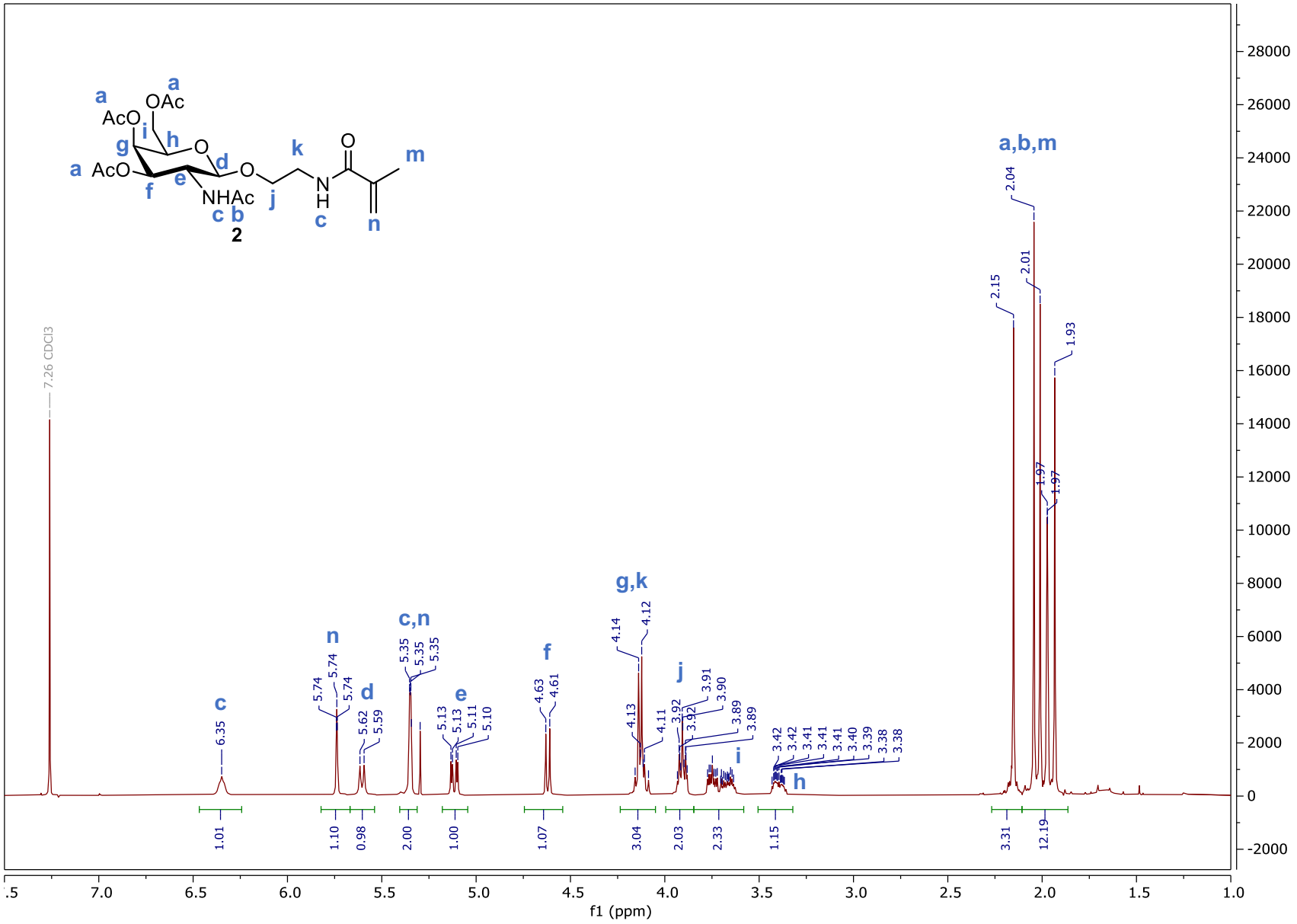
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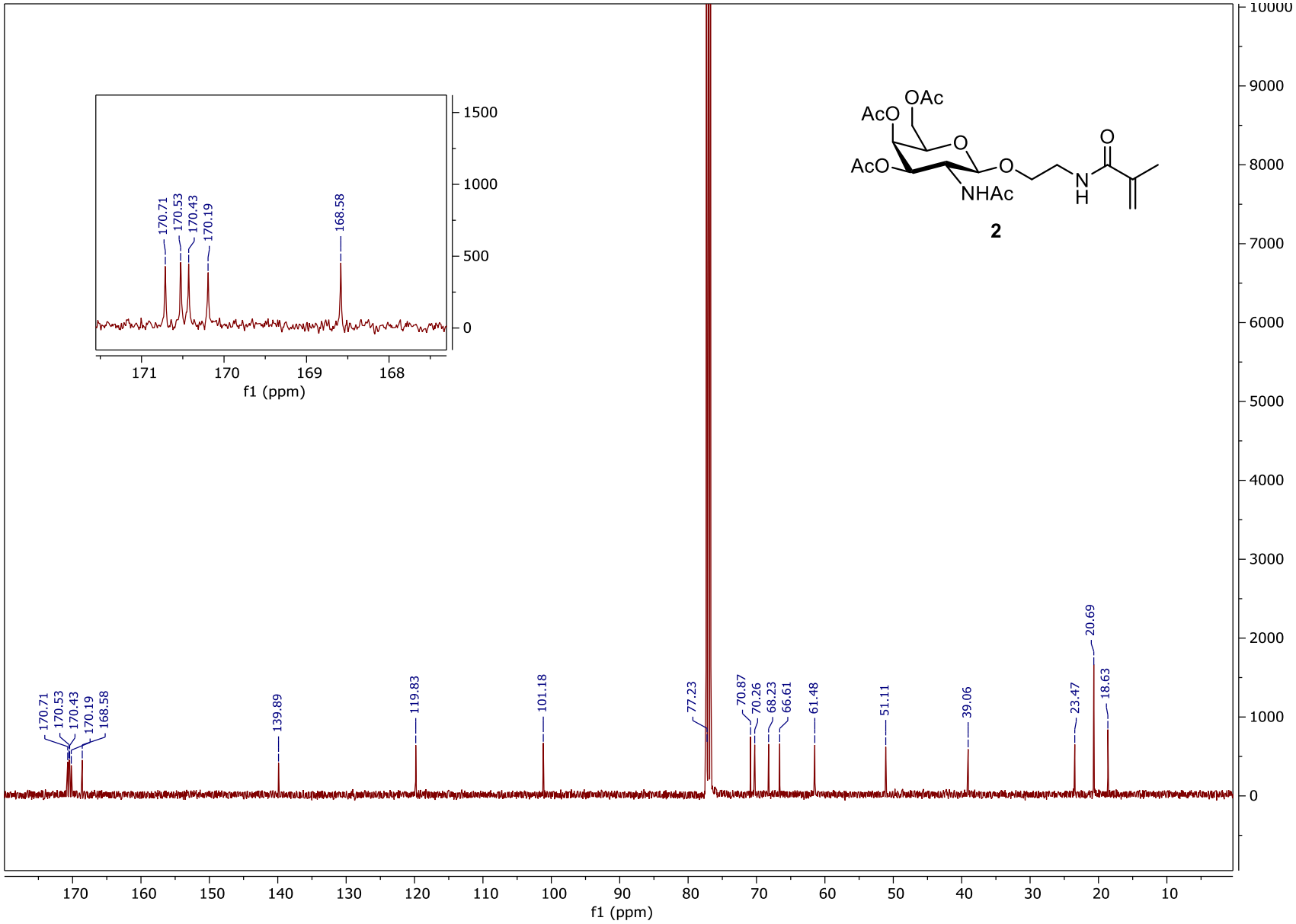
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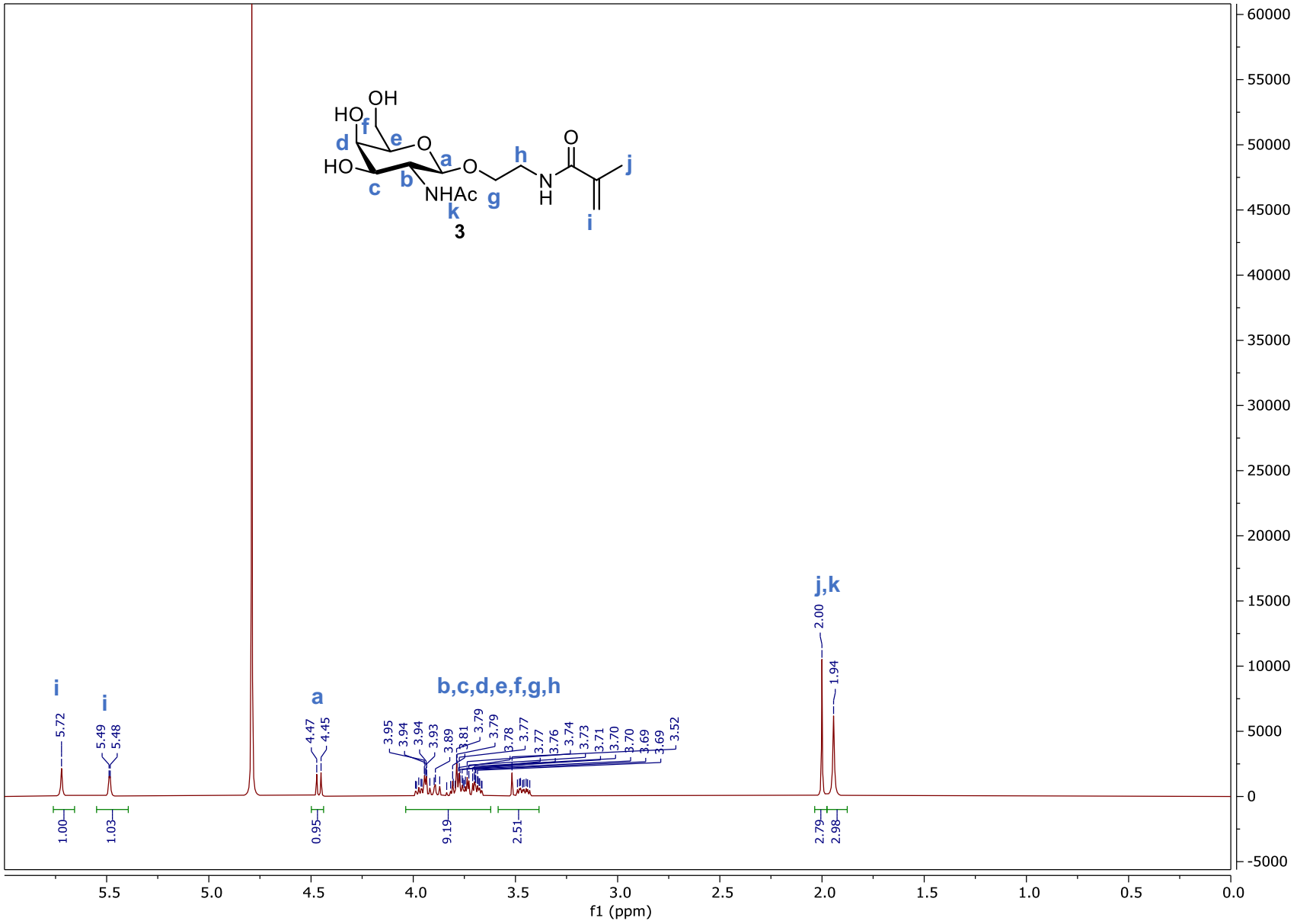
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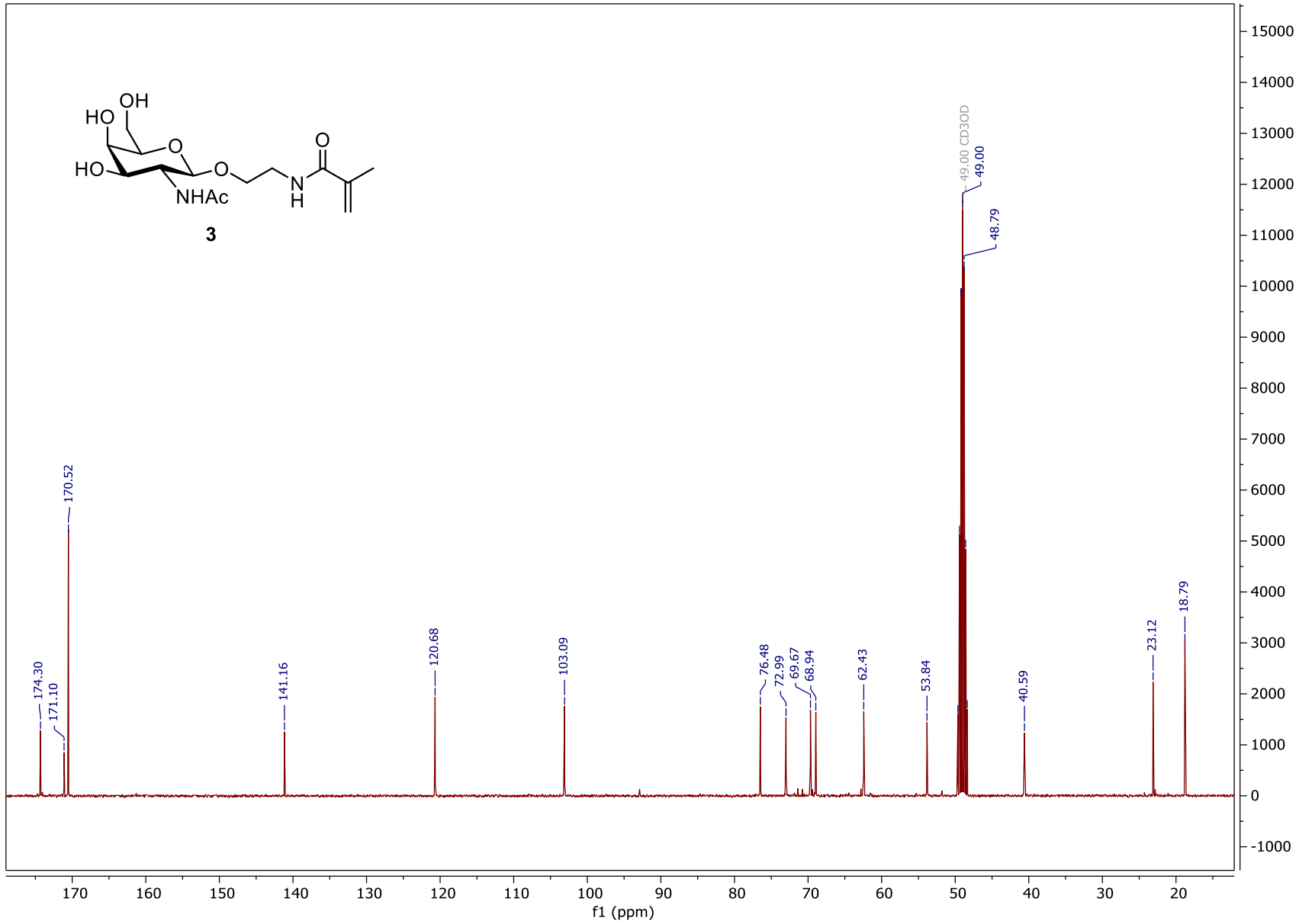
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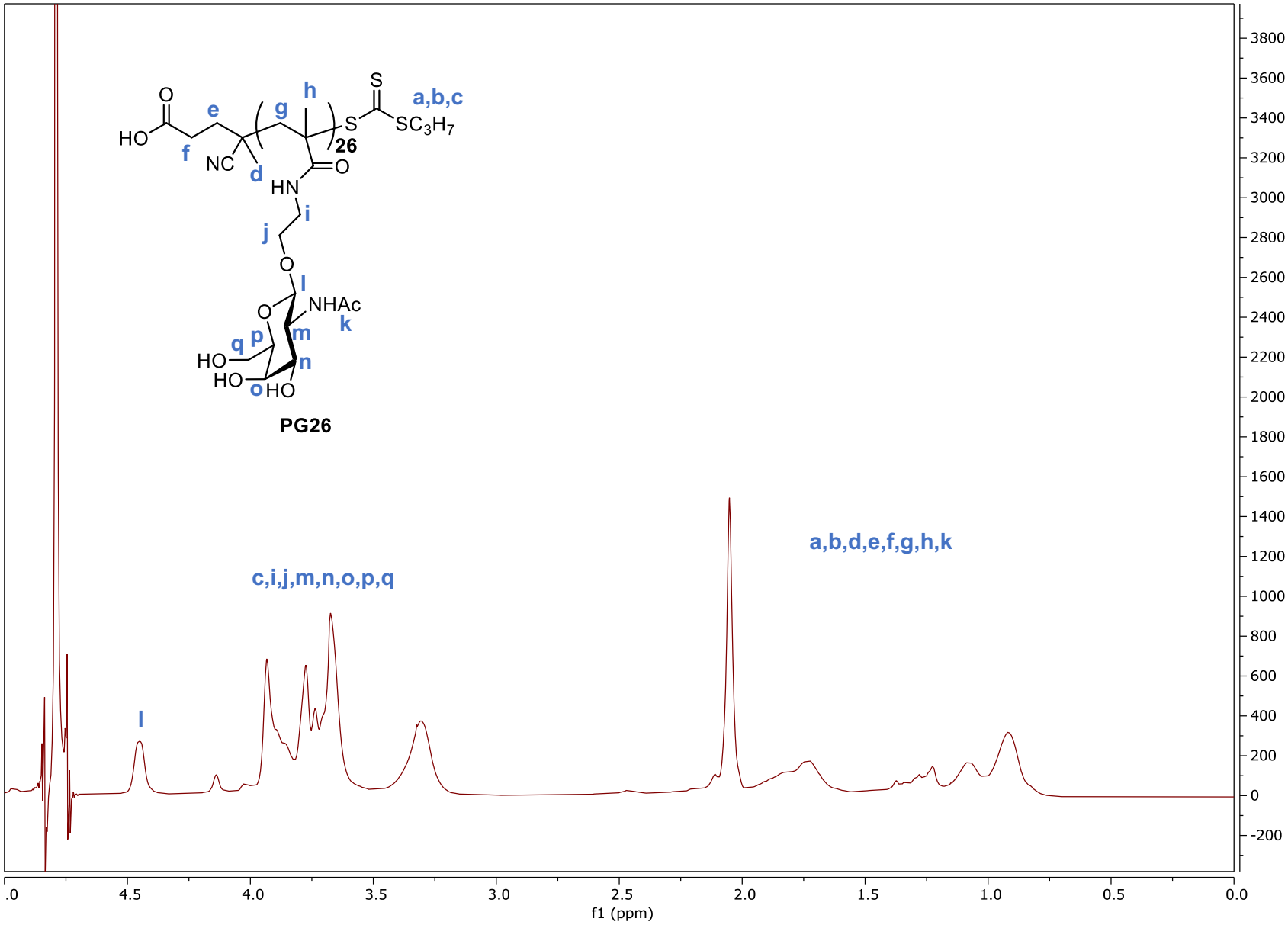
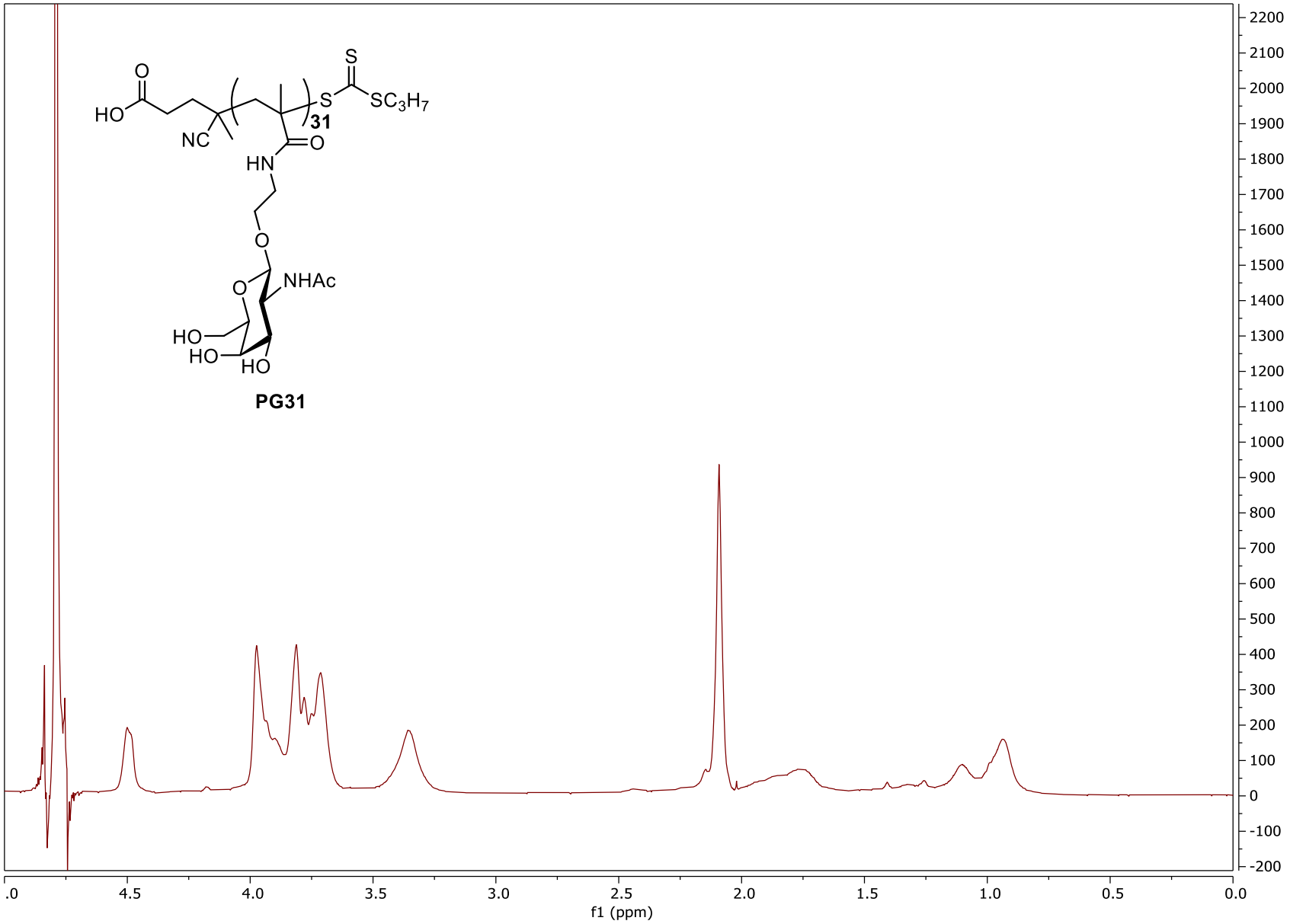
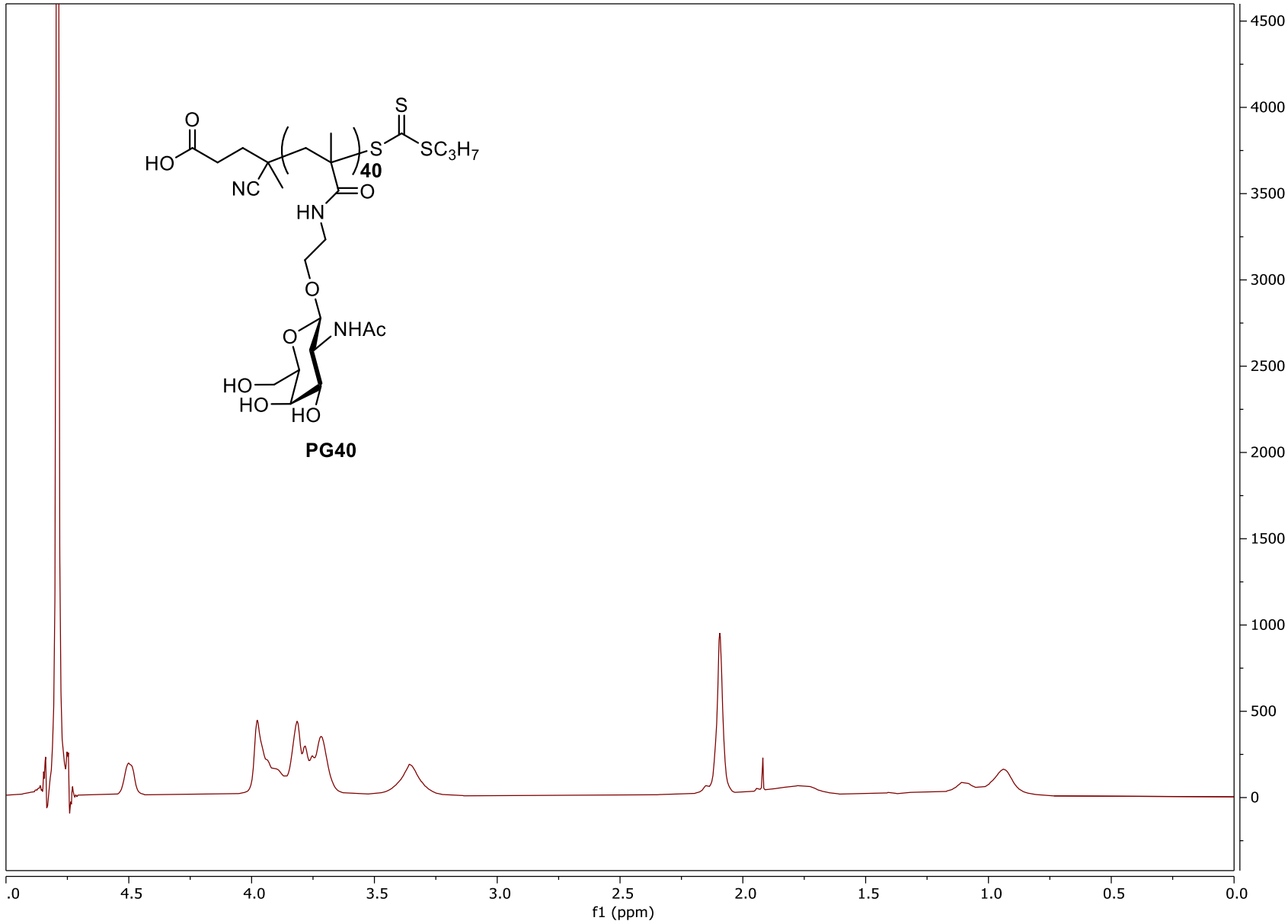
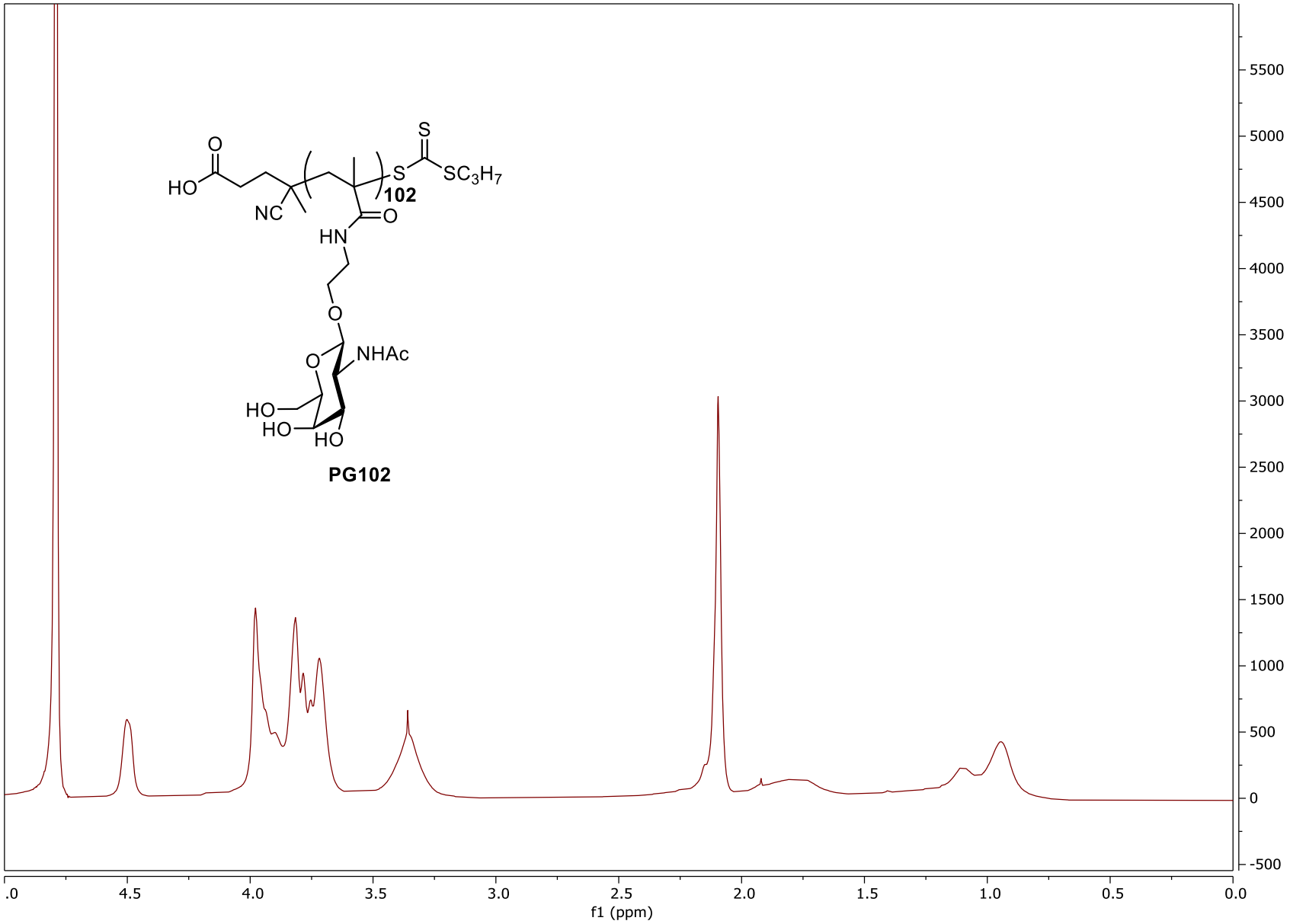
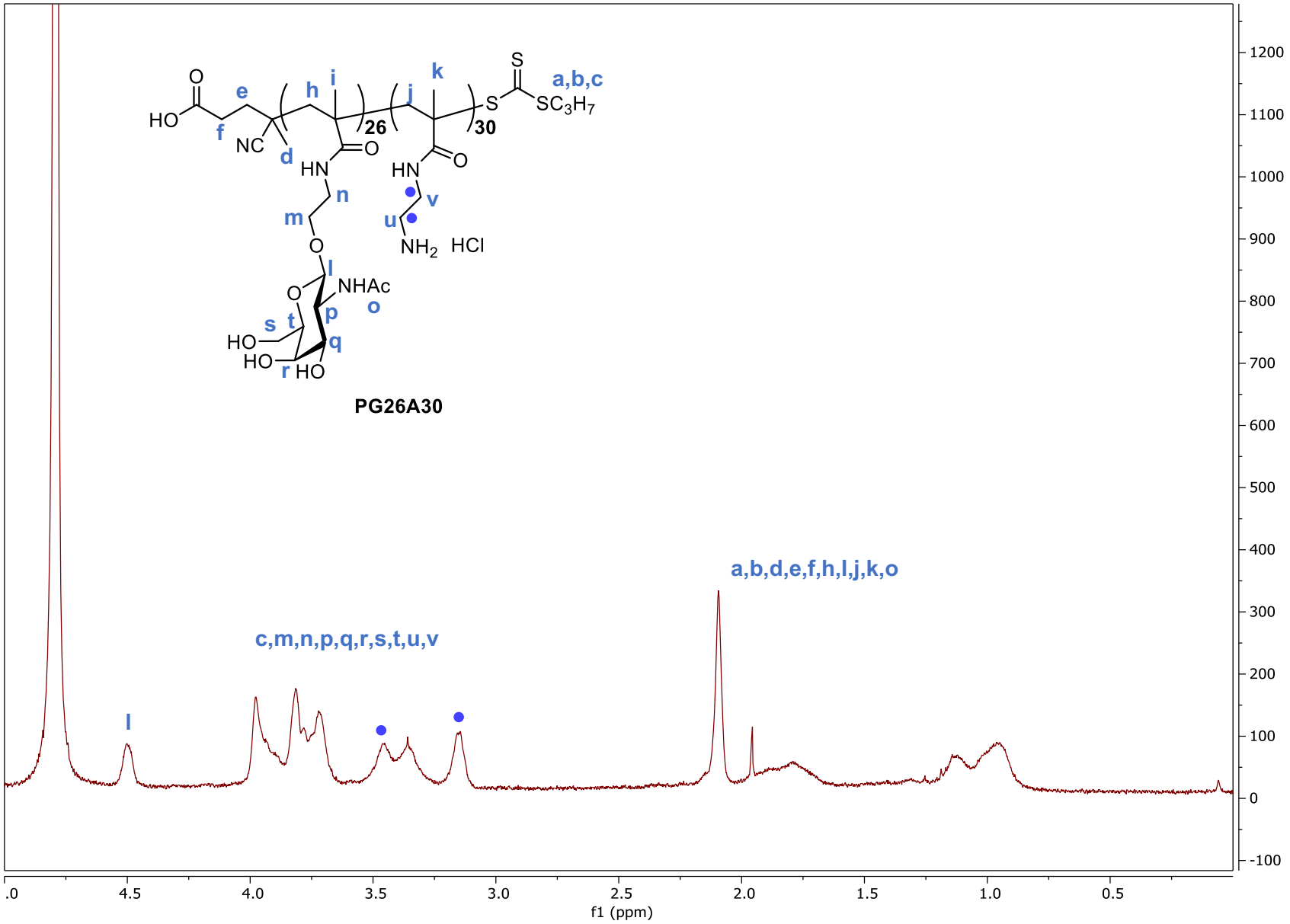
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

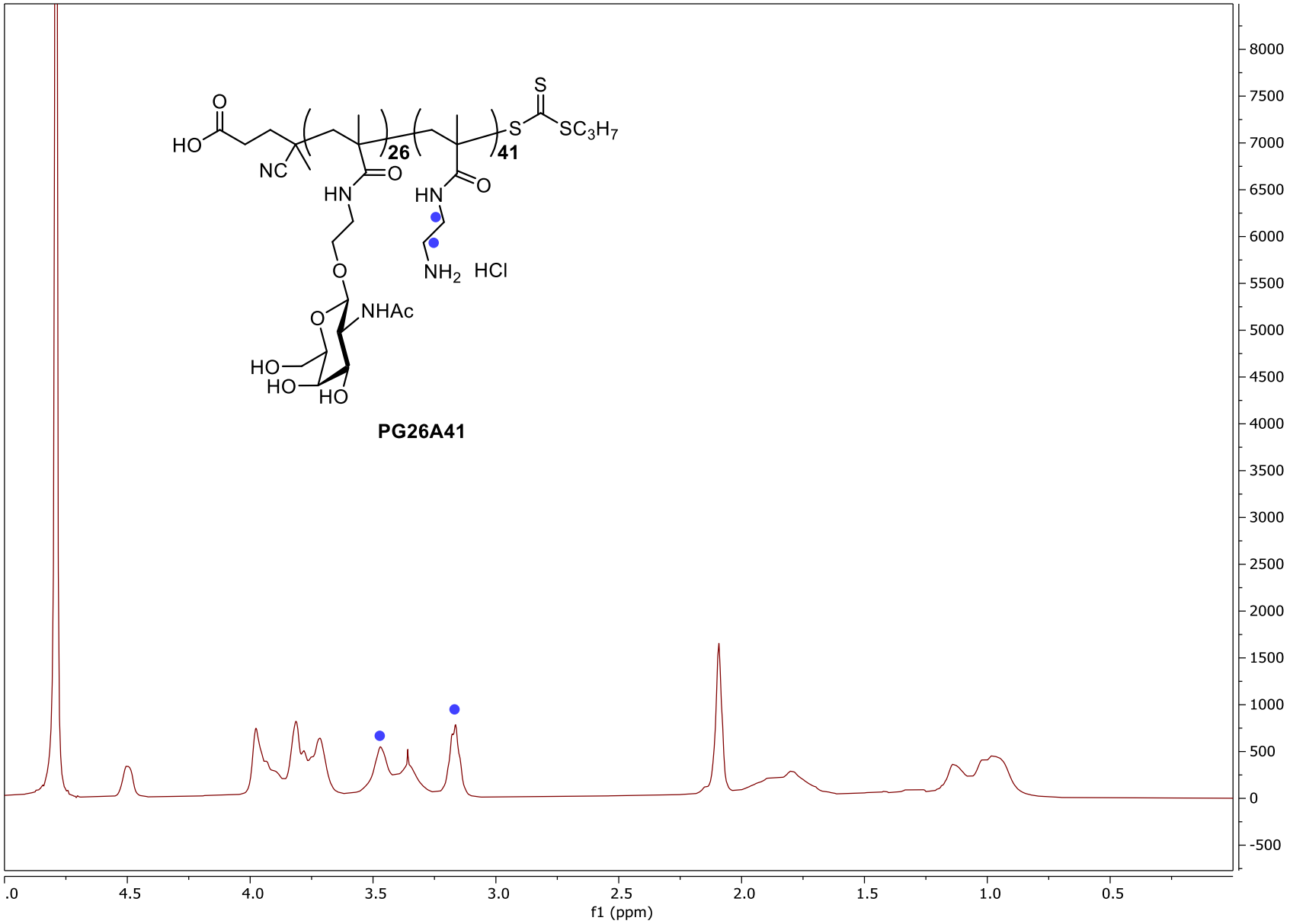

PG26
a,b,c
26
NHAc
l
c,i,j,m,n,o,p,q
a,b,d,e,f,g,h,k
f1 (ppm)

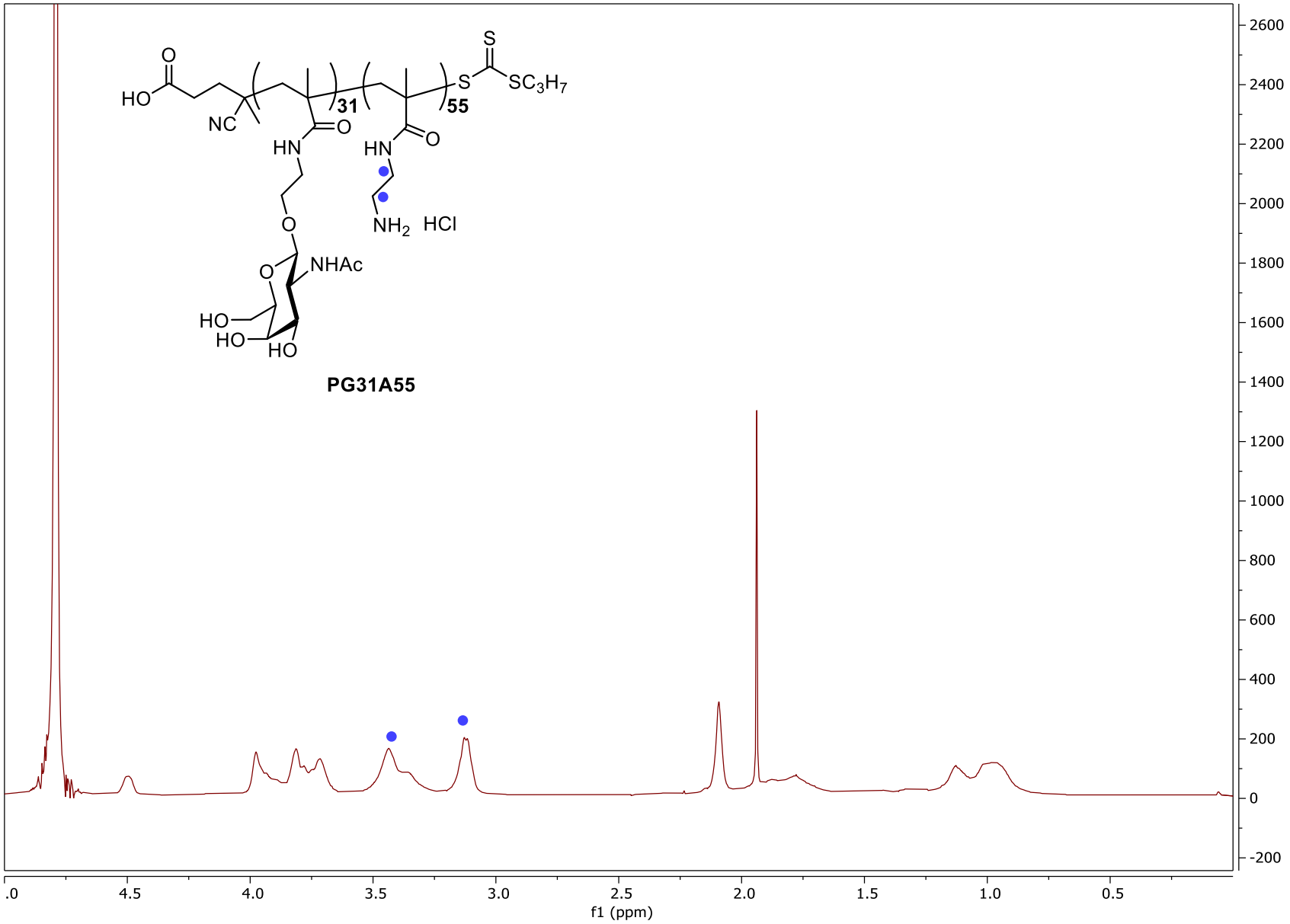

HO
O
NC
S
S
SC3H7
31
O
HN
O
NHAc
HO
HO
HO
PG31
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

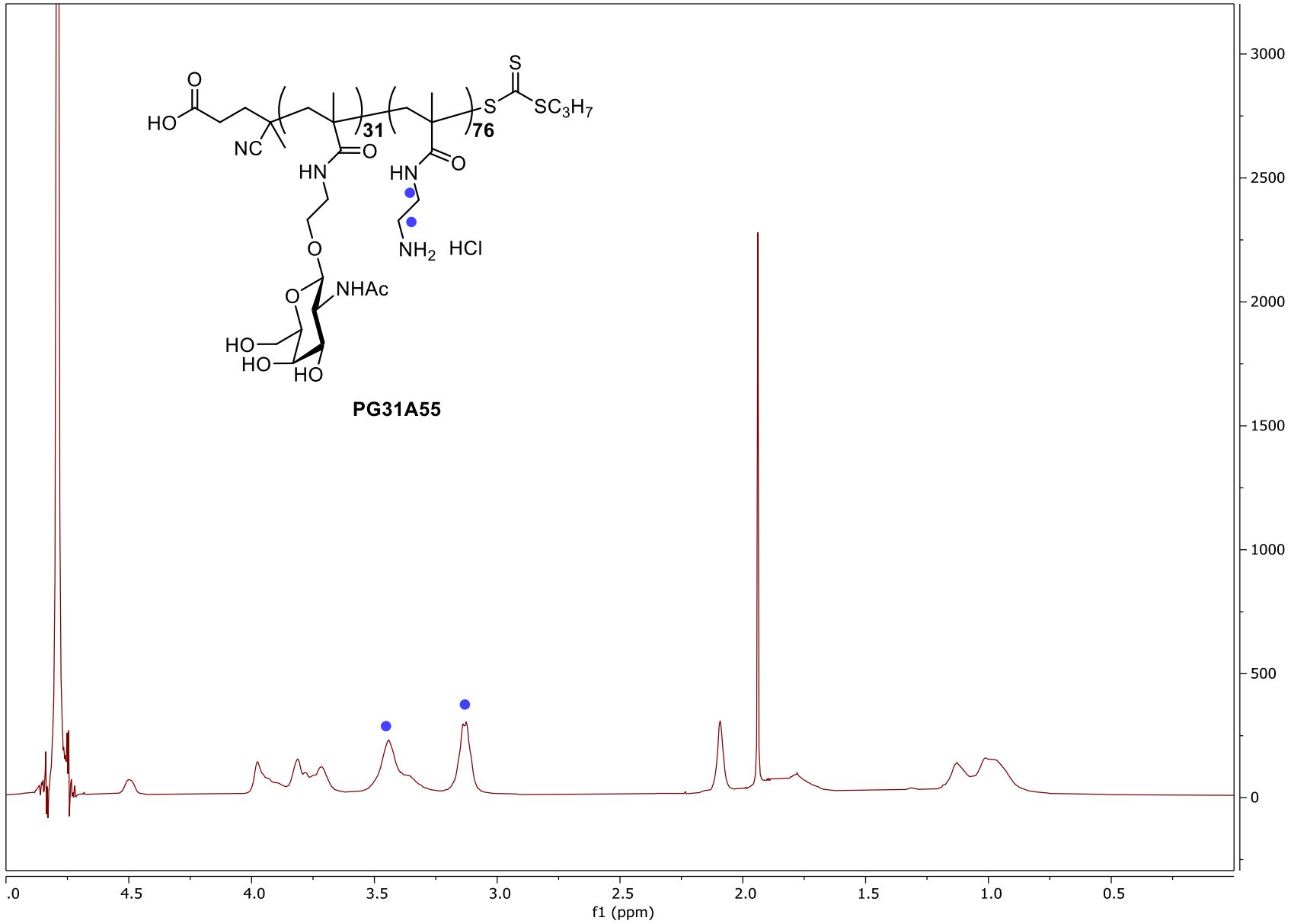

HO
O
NC
S
S
SC3H7
40
O
HN
O
NHAc
HO
HO
HO
PG40
4500
4000
3500
3000
2500
2000
1500
1000
500
0
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

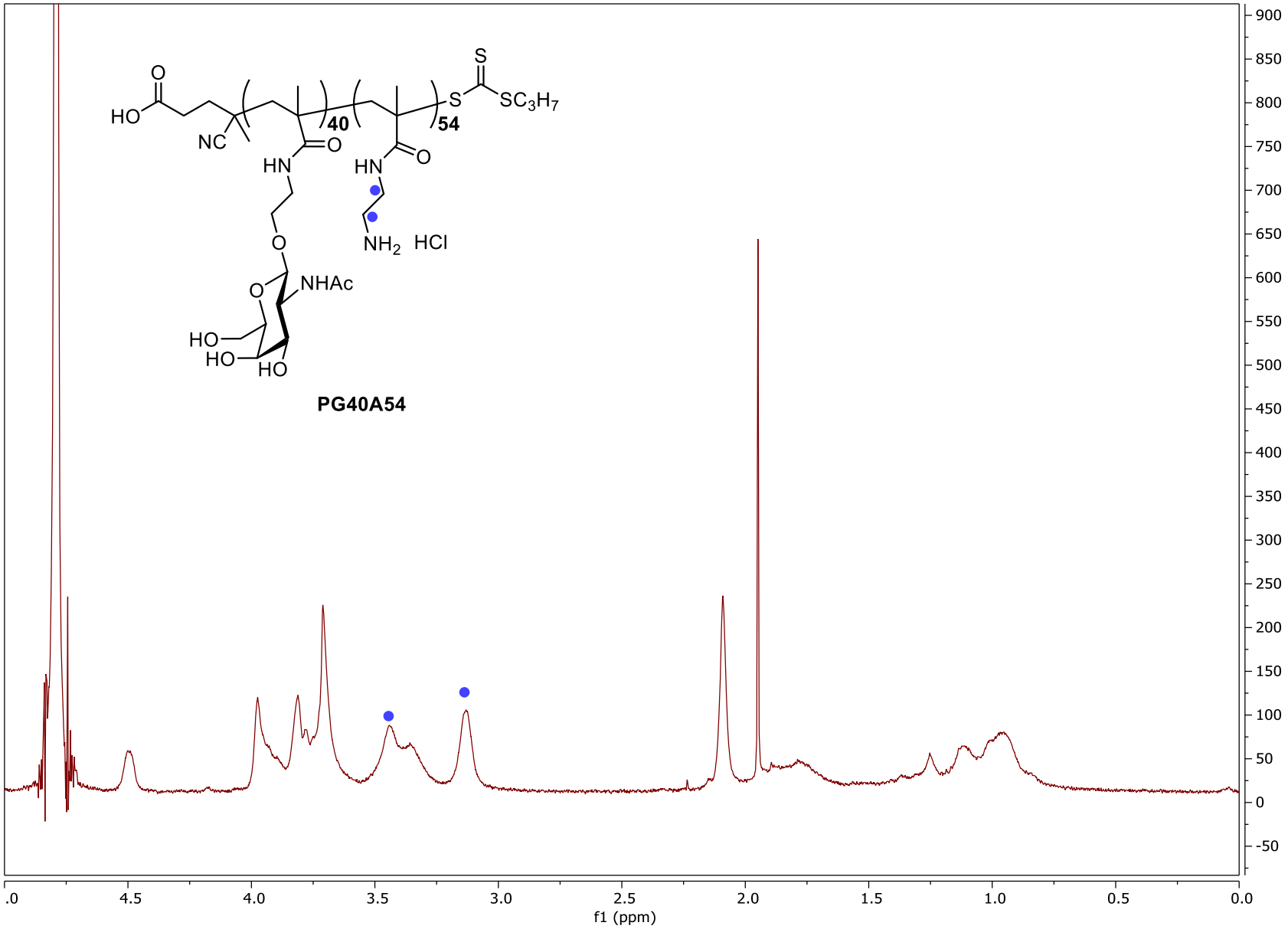

HO
O
NC
S
S
SC3H7
102
O
HN
O
NHAc
O
HO
HO
HO
PG102
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

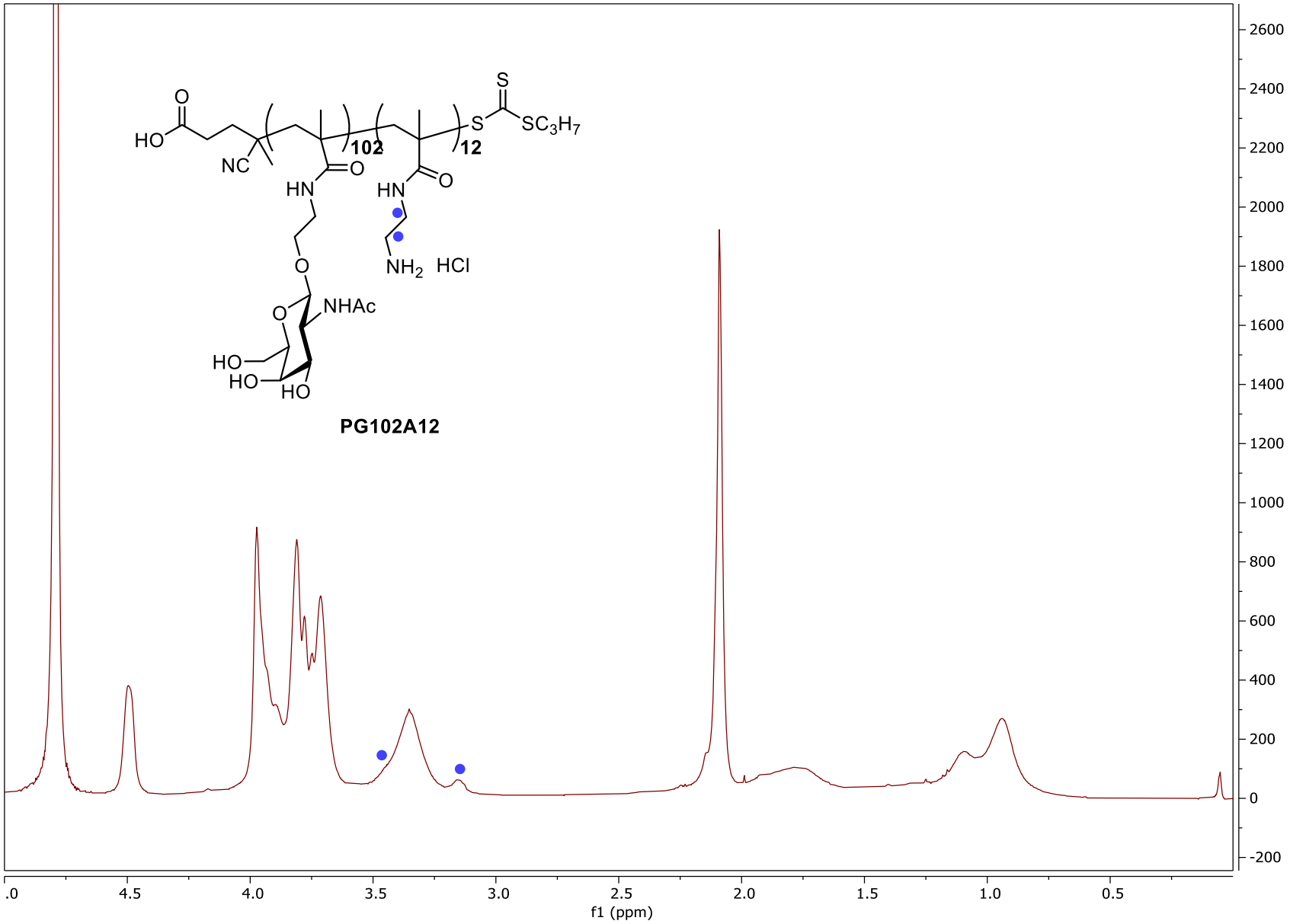

PG26A30
a,b,c
26
30
NHAc
NH2 HCl
a,b,d,e,f,h,l,j,k,o
c,m,n,p,q,r,s,t,u,v
l
f1 (ppm)

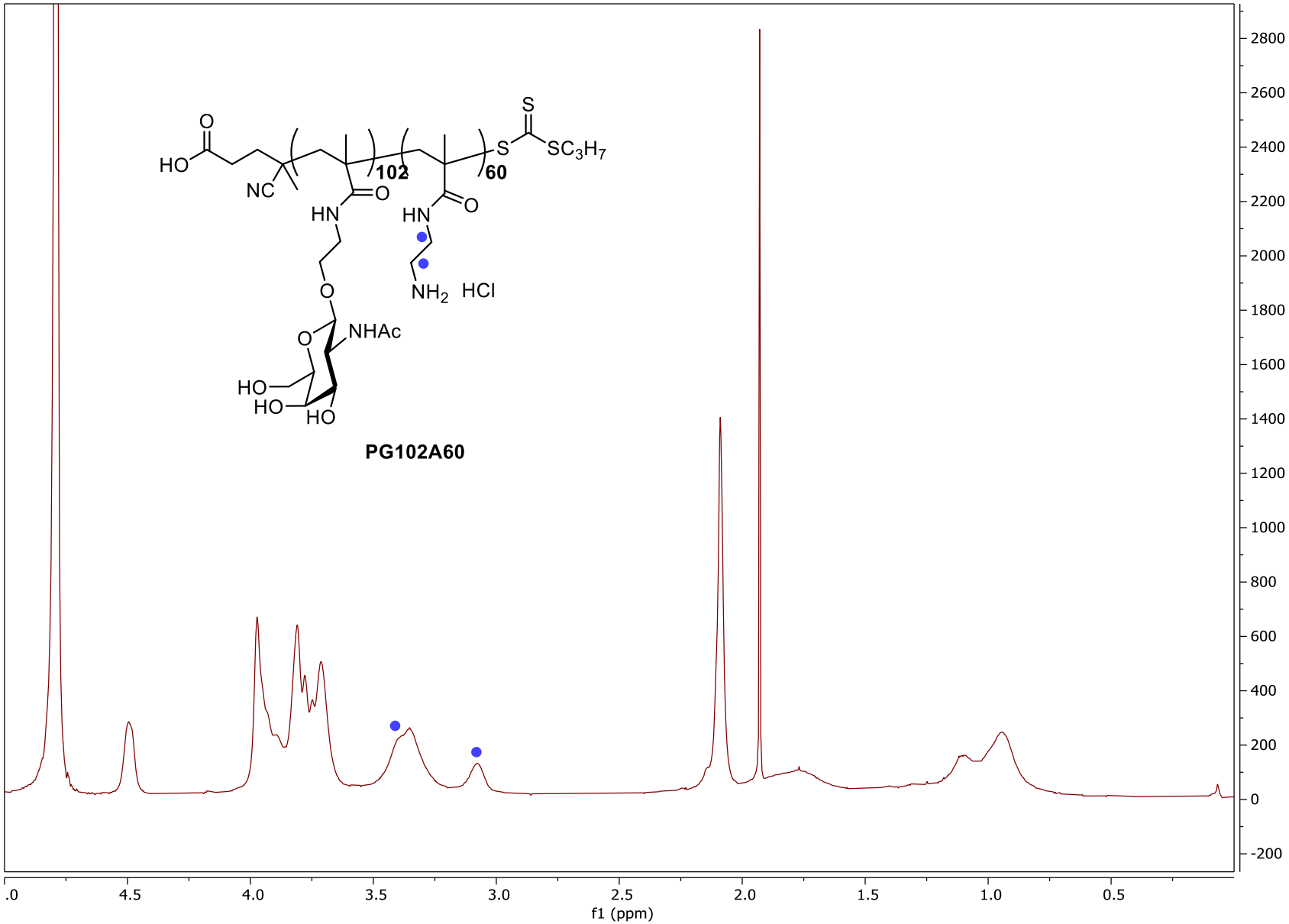

HO
O
NC
26
41
S
SC3H7
HN
HN
NH2 HCl
O
NHAc
HO
HO
HO
PG26A41
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

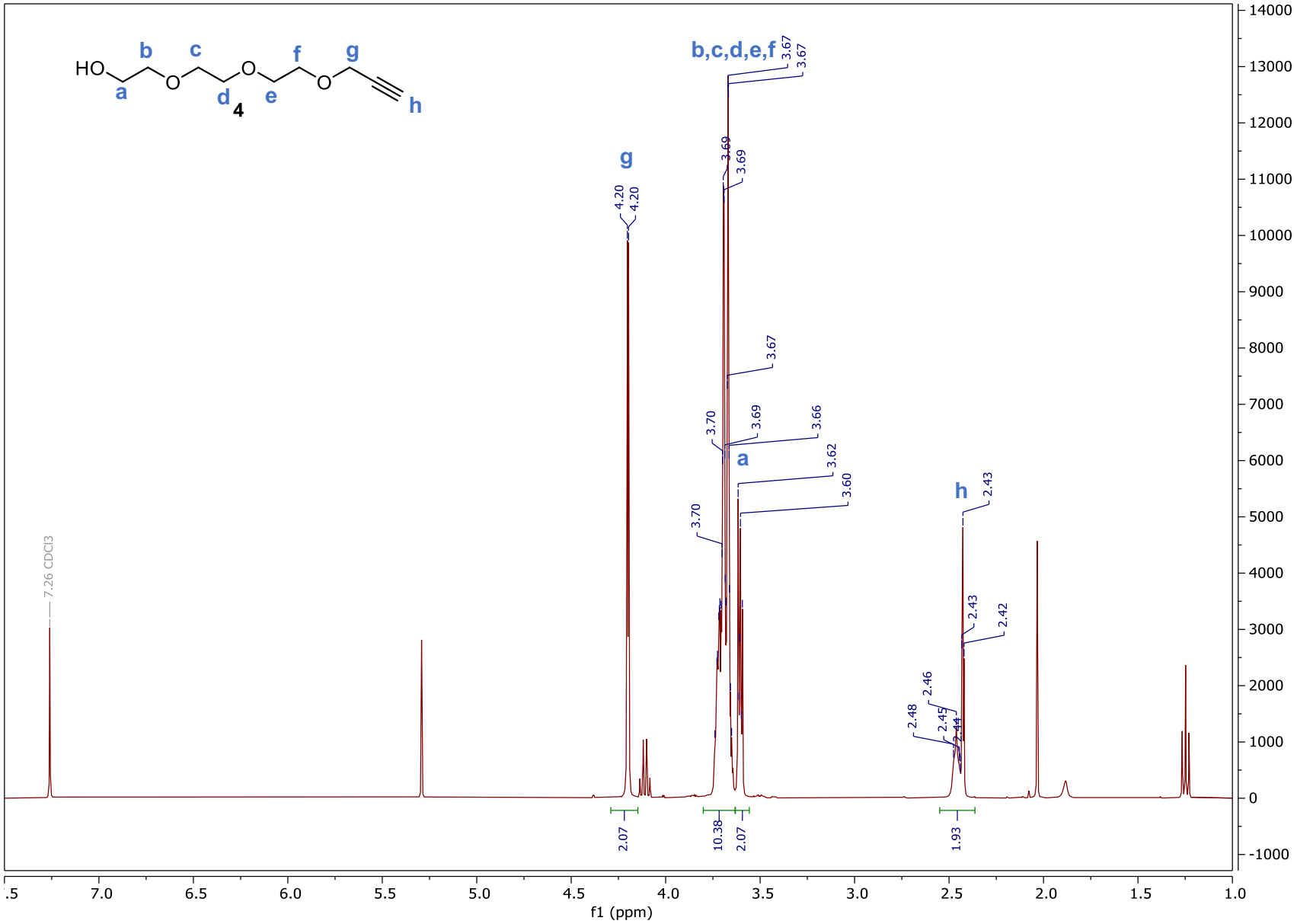

HO
O
NC
31
55
S
SC3H7
HN
O
NH2
HCl
NHAc
HO
HO
HO
PG31A55
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

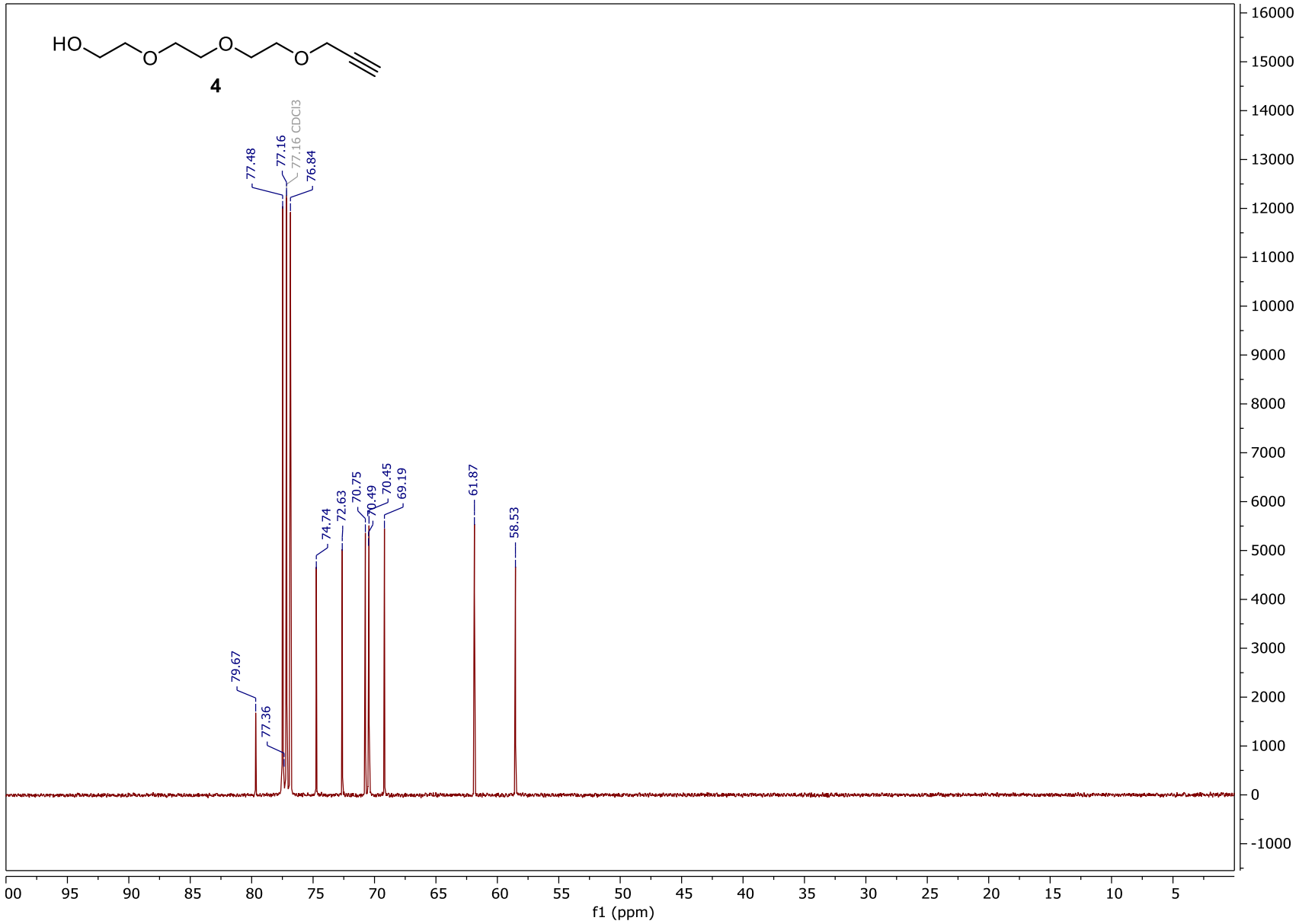

HO
O
NC
31
76
S
SC3H7
HN
HN
NH2 HCl
O
NHAc
HO
HO
HO
PG31A55
3000
2500
2000
1500
1000
500
0
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

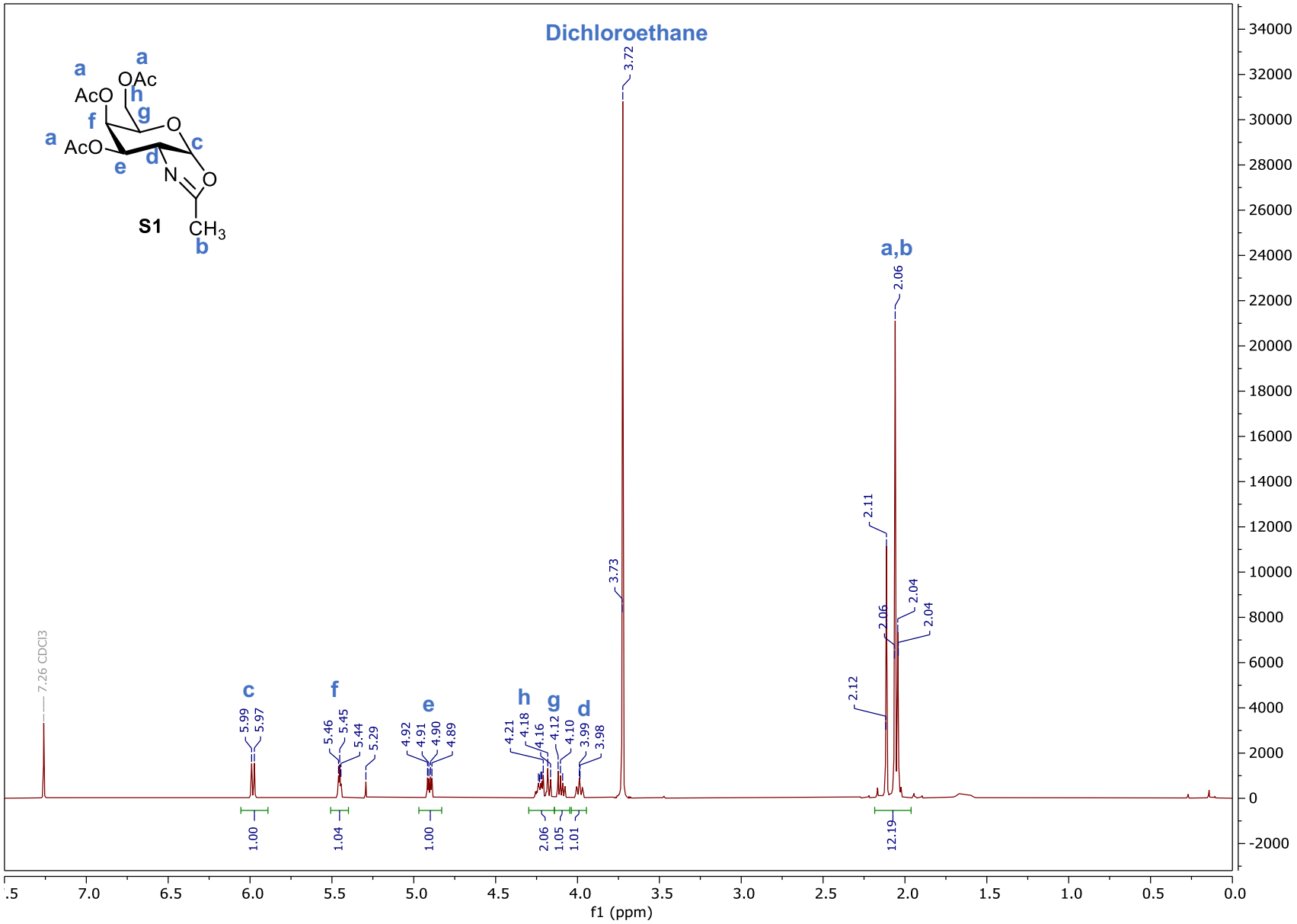

HO
O
NC
40
54
S
SC3H7
HN
HN
NH2 HCl
O
NHAc
HO
HO
HO
PG40A54
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

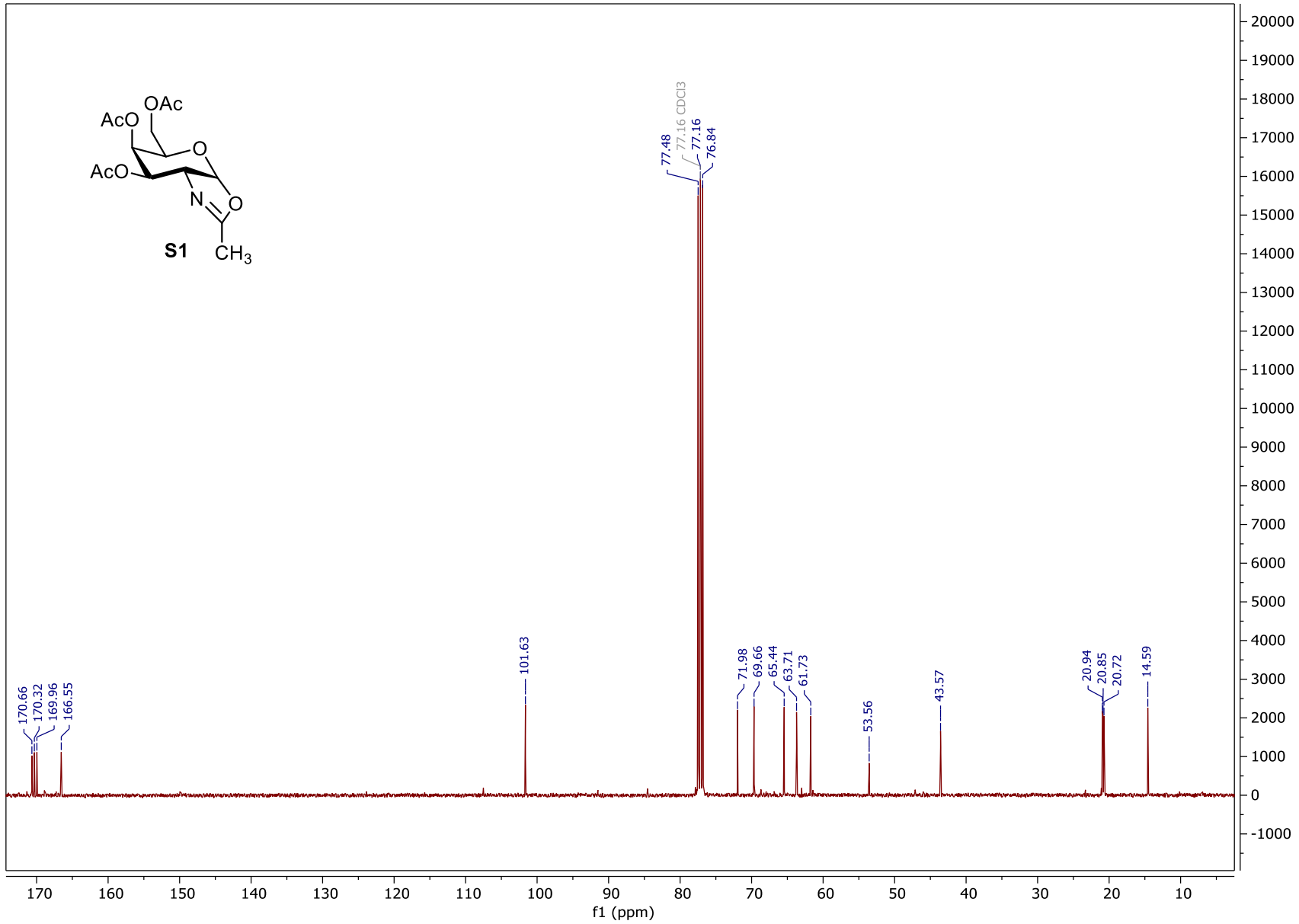

HO
O
NC
102
12
S
SC3H7
HN
HN
NH2 HCl
NHAc
HO
HO
HO
PG102A12
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

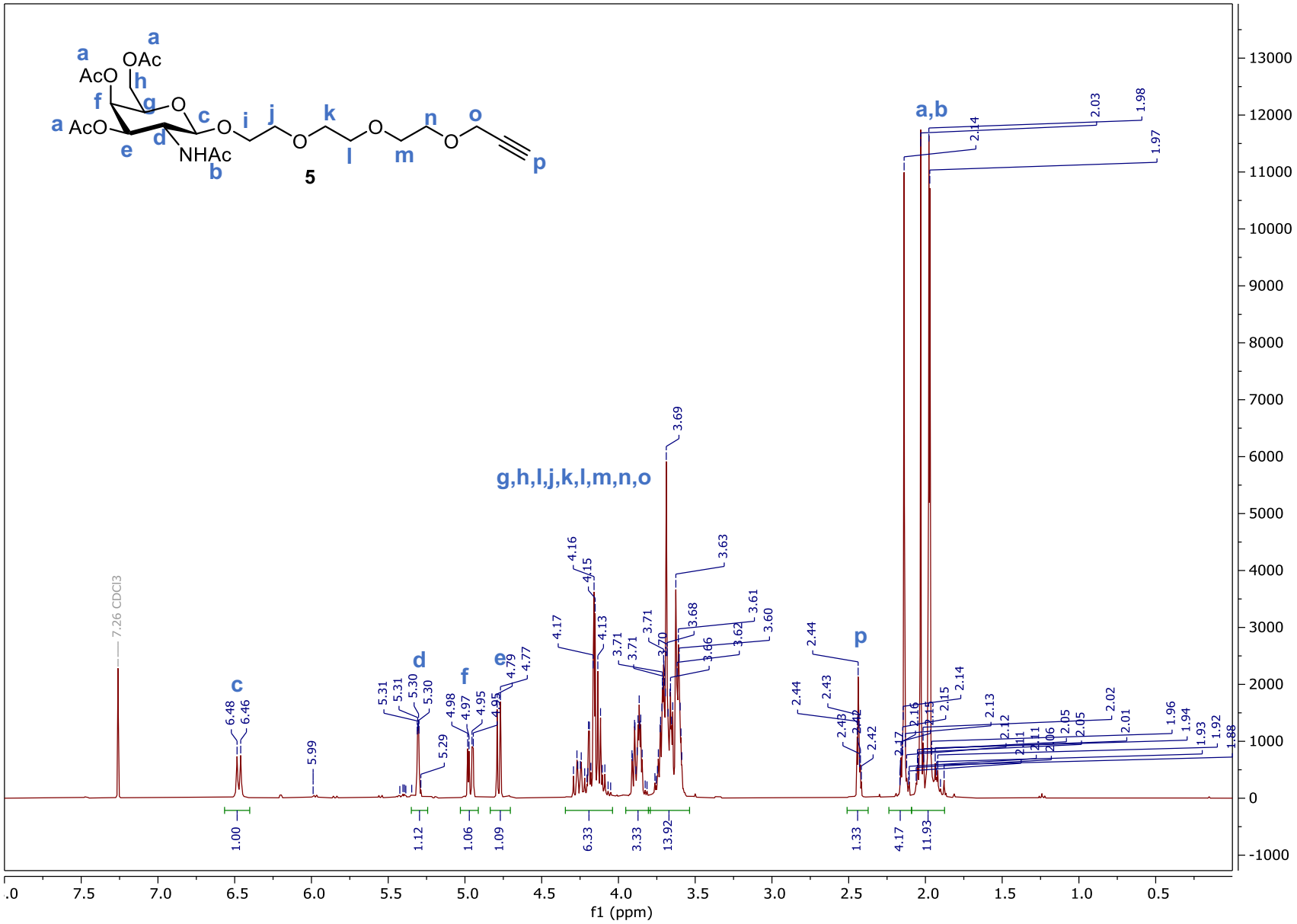

HO
O
NC
102
60
S
SC3H7
HN
HN
NH2
HCl
NHAc
HO
HO
HO
PG102A60
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

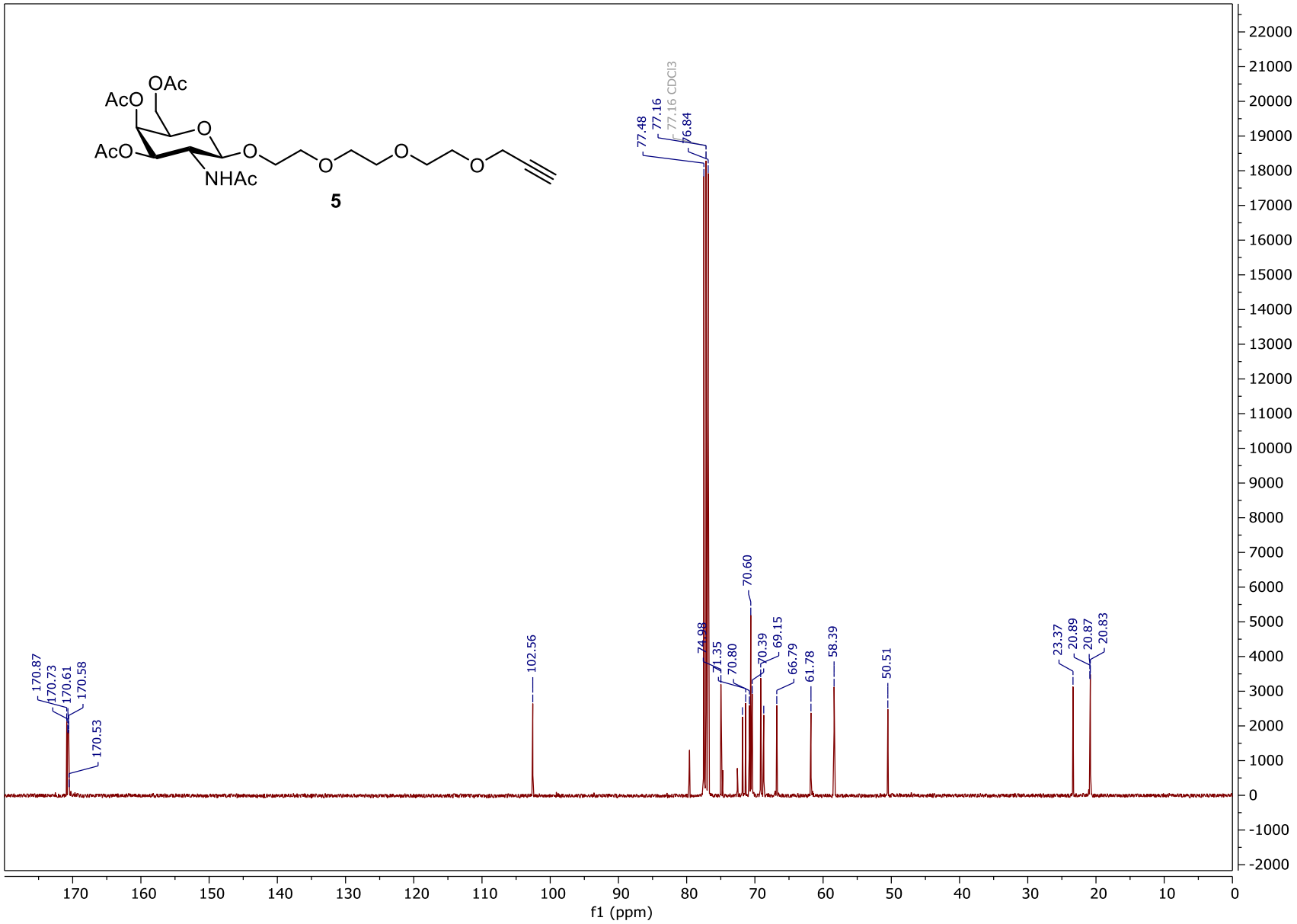

HO
a
b
c
d
e
f
g
h
4
b,c,d,e,f
3.67
3.67
g
4.20
4.20
3.69
3.69
3.67
3.70
3.69
3.66
a
3.62
3.60
3.70
h
2.43
2.43
2.42
2.48
2.46
2.45
2.44
7.26 CDCl3
2.07
10.38
2.07
1.93
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

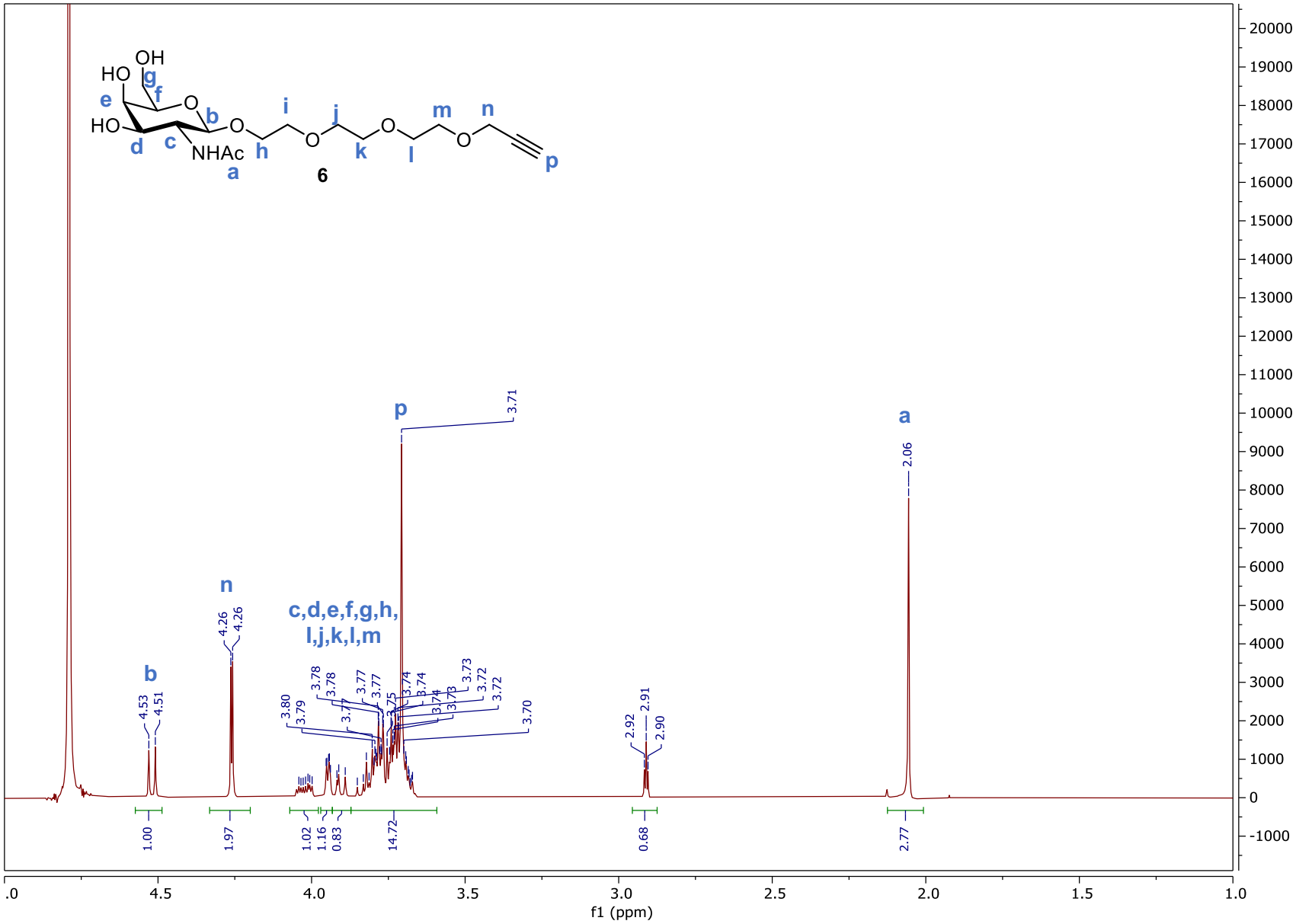

HO
O
O
O
4
77.48
77.16
77.16 CDCl3
76.84
74.74
72.63
70.75
70.49
70.45
69.19
61.87
58.53
79.67
77.36
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
00
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (ppm)

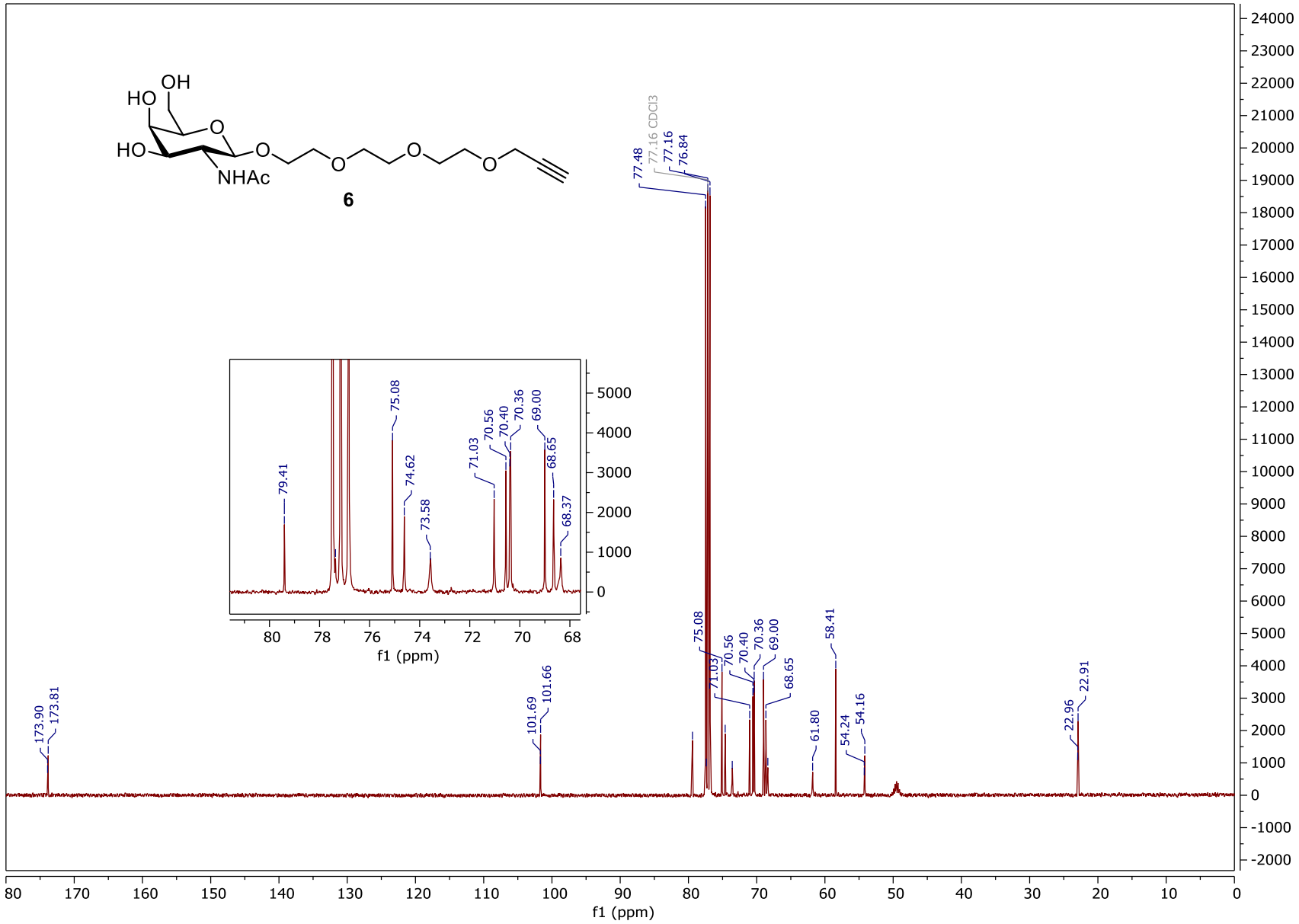

Dichloroethane
S1
a,b
f1 (ppm)

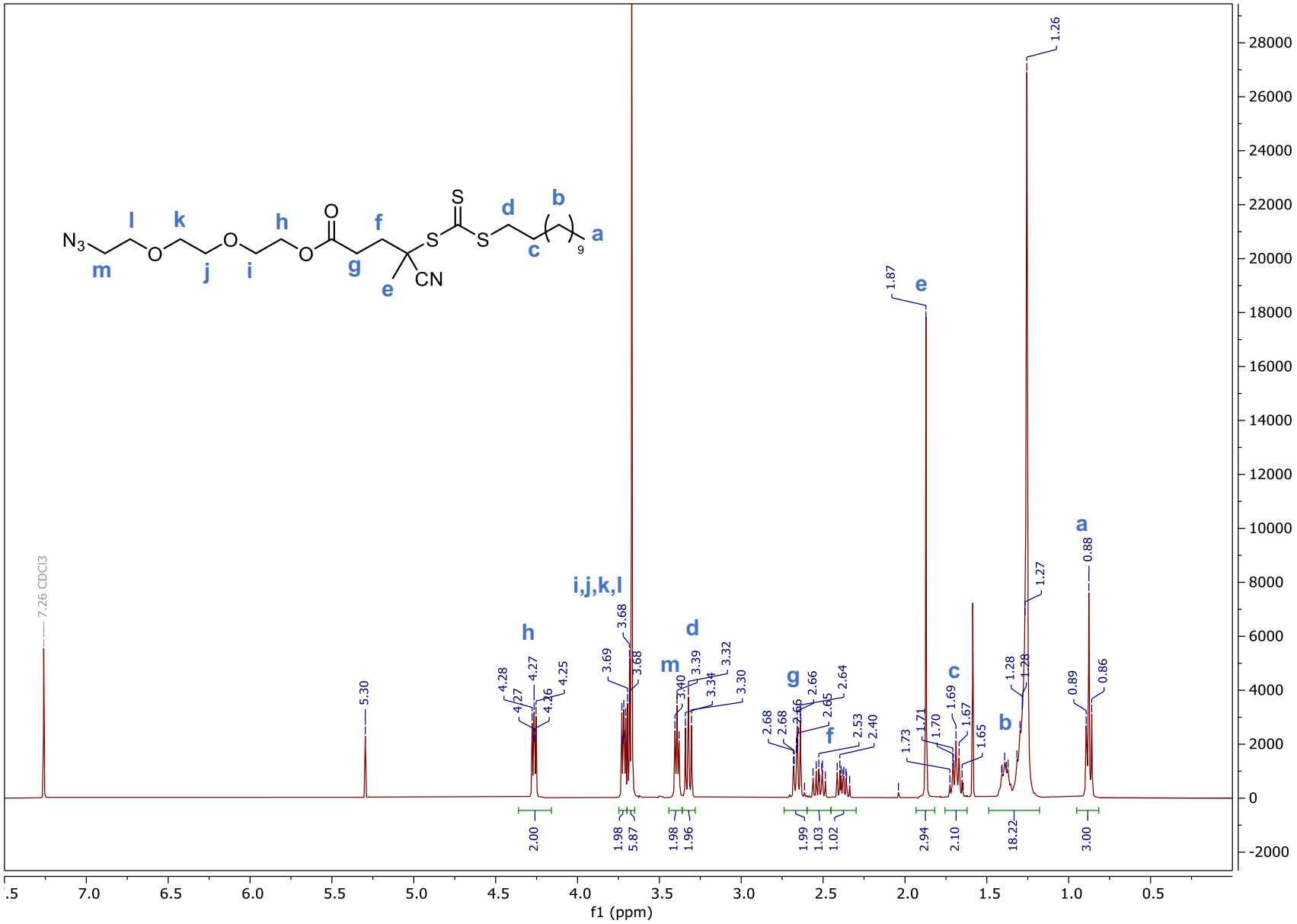

OAc
AcO
AcO
O
N
O
S1
CH3
77.48
77.16 CDCl3
77.16
76.84
170.66
170.32
169.96
166.55
101.63
71.98
69.66
65.44
63.71
61.73
53.56
43.57
20.94
20.85
20.72
14.59
f1 (ppm)

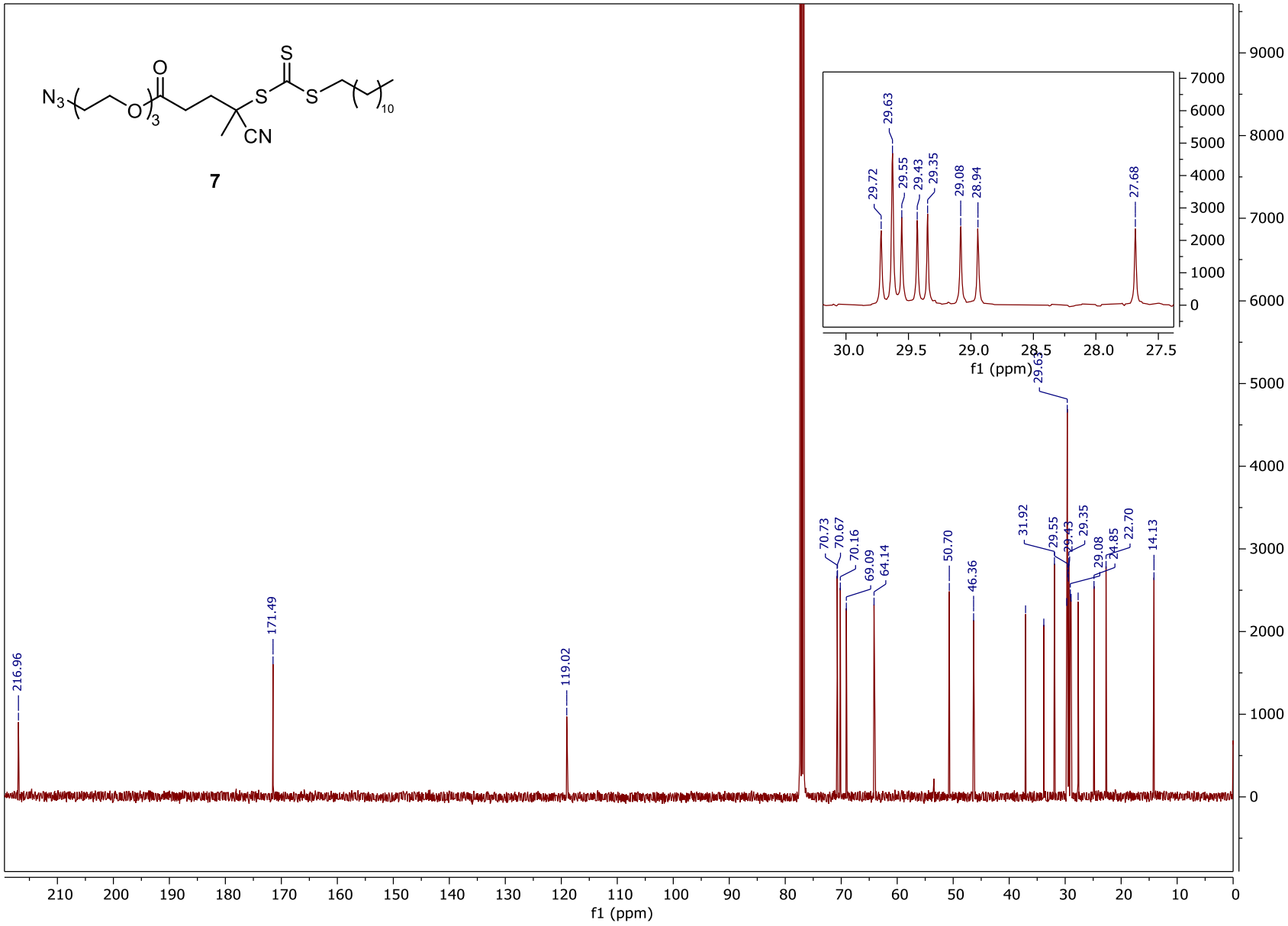

5
a,b
g,h,l,j,k,l,m,n,o
c
d
e
f
p
7.26 CDCl3
f1 (ppm)

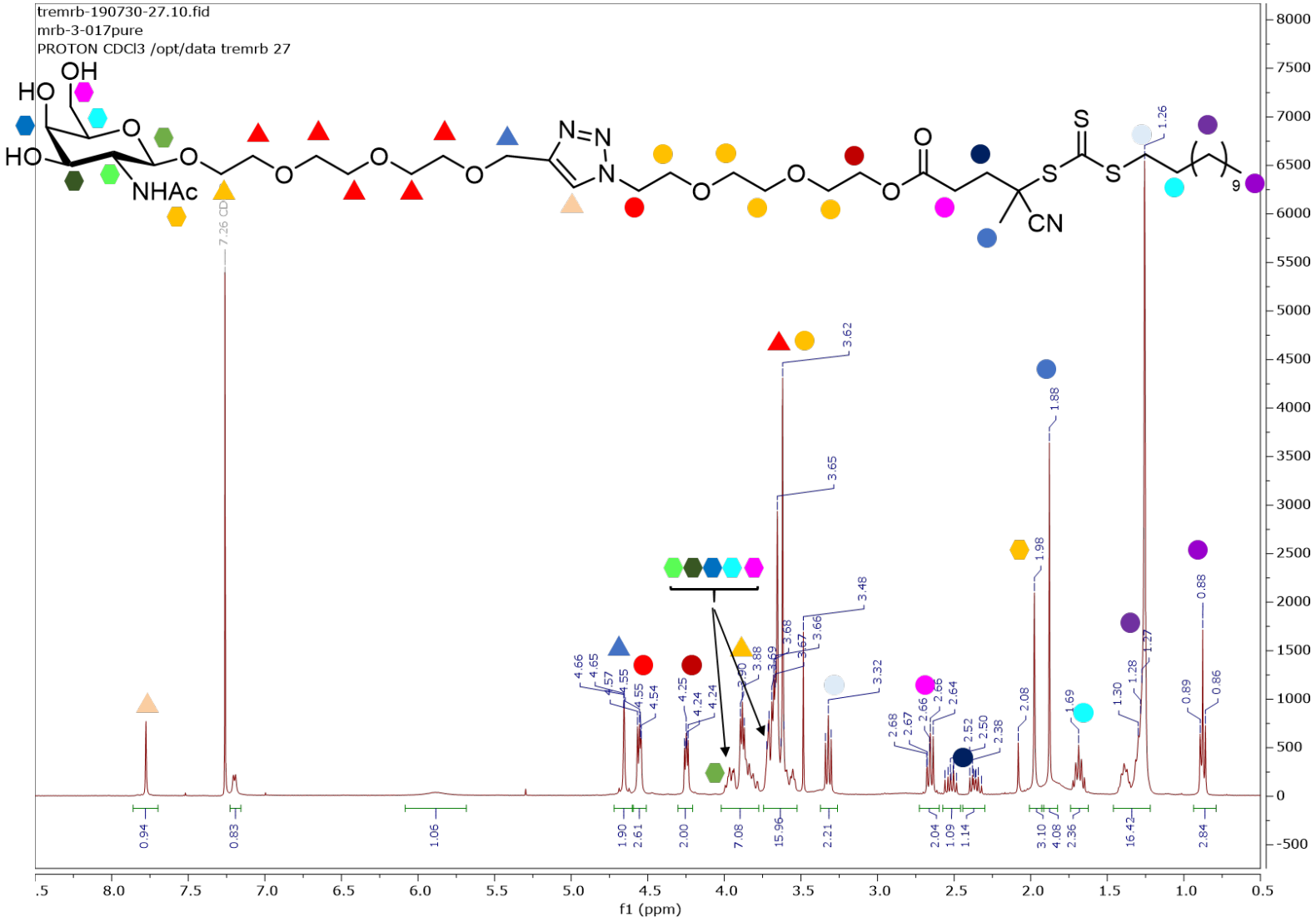

OAc
AcO
AcO
O
O
O
O
O
NHAc
5
77.48
77.16
77.16 CDCl3
76.84
170.87
170.73
170.61
170.58
170.53
102.56
74.98
71.35
70.80
70.60
70.39
69.15
66.79
61.78
58.39
50.51
23.37
20.89
20.87
20.83
f1 (ppm)

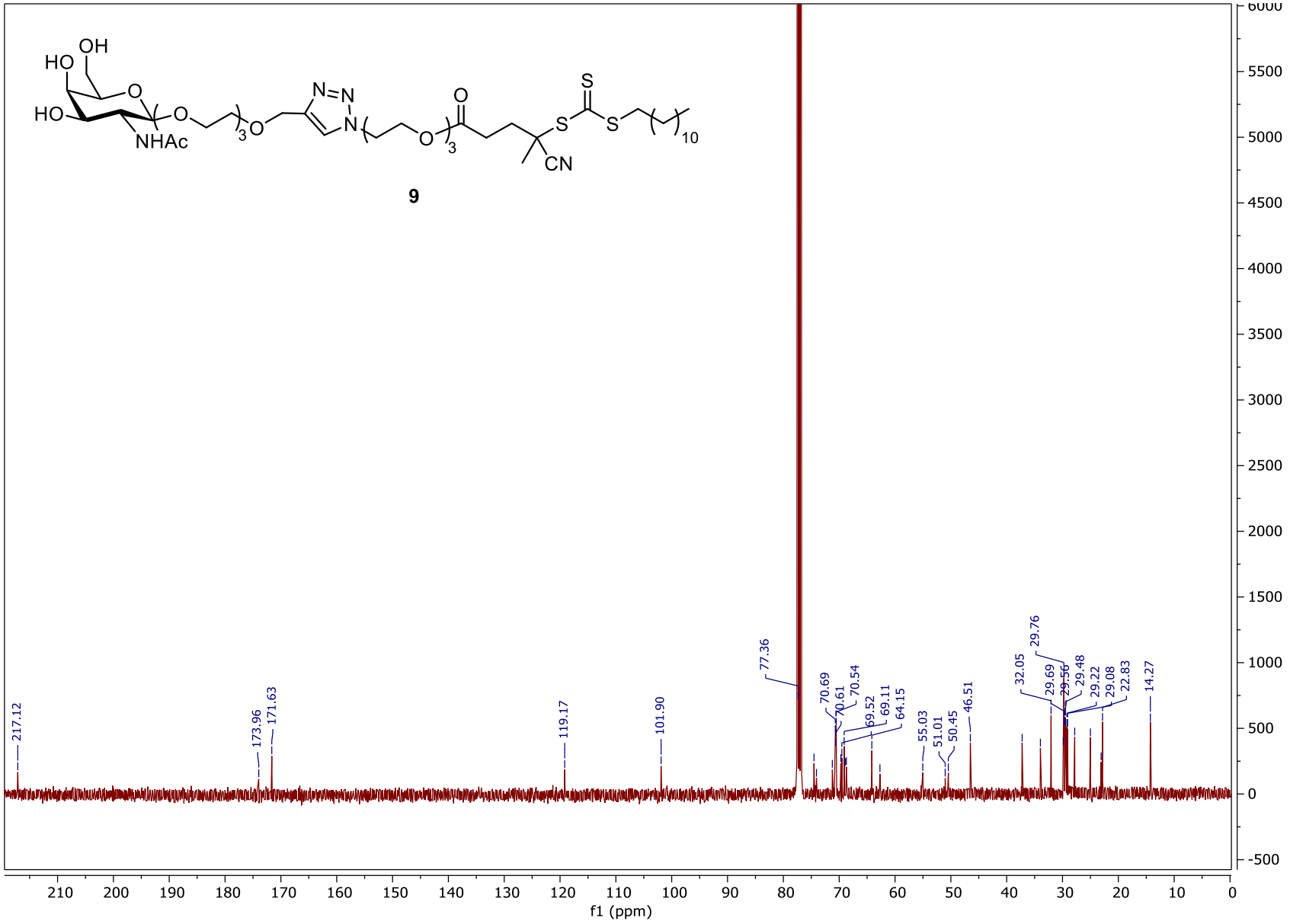

OH
HO
g
f
e
O
b
i
j
m
n
HO
O
O
O
O
d
c
NHAc
h
k
l
p
a
6
p
3.71
a
2.06
n
4.26
4.26
c,d,e,f,g,h,
l,j,k,l,m
b
4.53
4.51
3.80
3.79
3.78
3.78
3.77
3.77
3.77
3.75
3.74
3.74
3.74
3.73
3.73
3.72
3.72
3.70
2.92
2.91
2.90
1.00
1.97
1.02
1.16
0.83
14.72
0.68
2.77
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

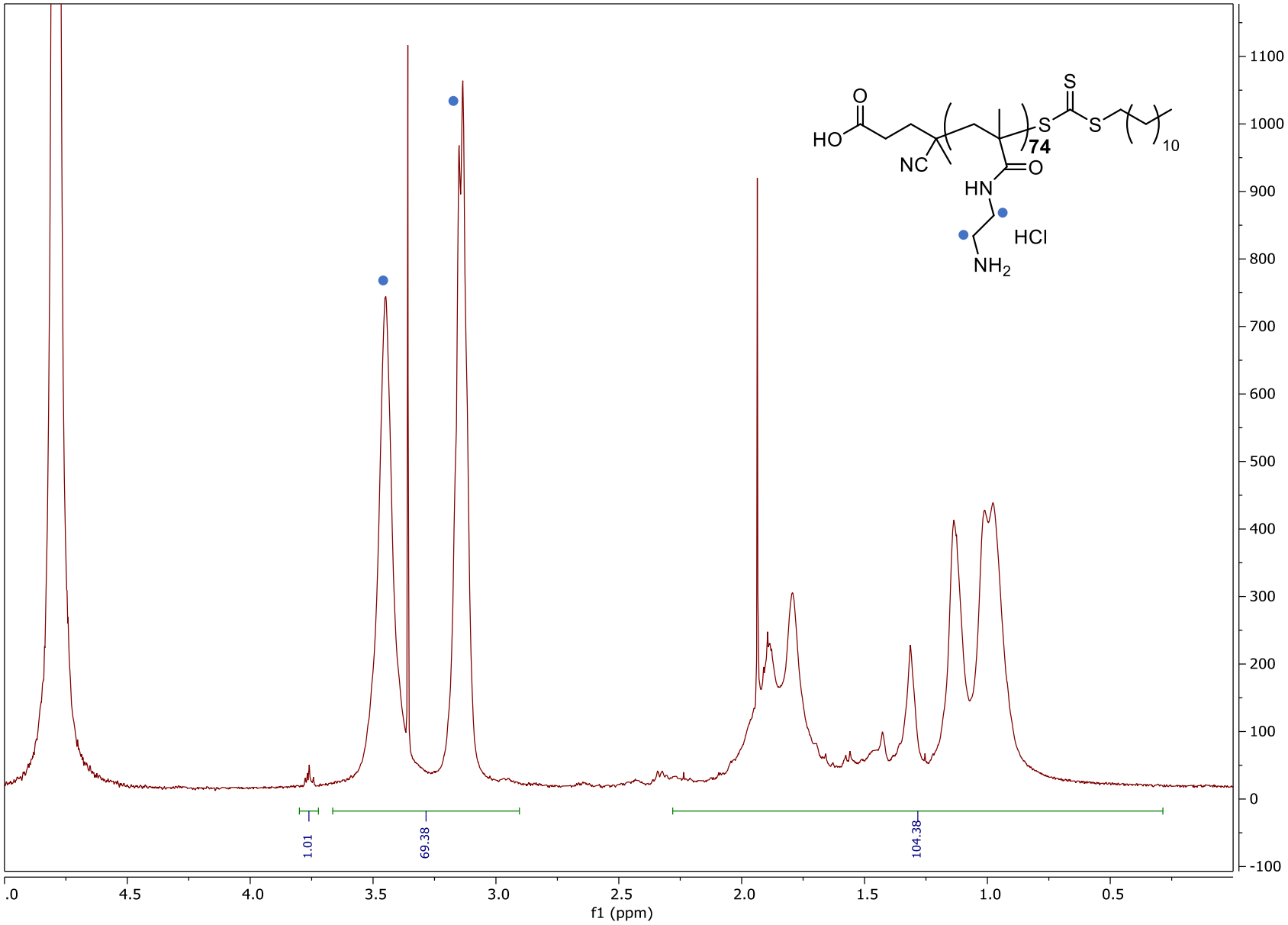

OH
HO
O
HO
O
O
O
O
NHAc
6
77.48
77.16 CDCl3
77.16
76.84
79.41
75.08
74.62
73.58
71.03
70.56
70.40
70.36
69.00
68.65
68.37
f1 (ppm)
173.90
173.81
101.69
101.66
75.08
71.03
70.56
70.40
70.36
69.00
68.65
61.80
58.41
54.24
54.16
22.96
22.91
f1 (ppm)

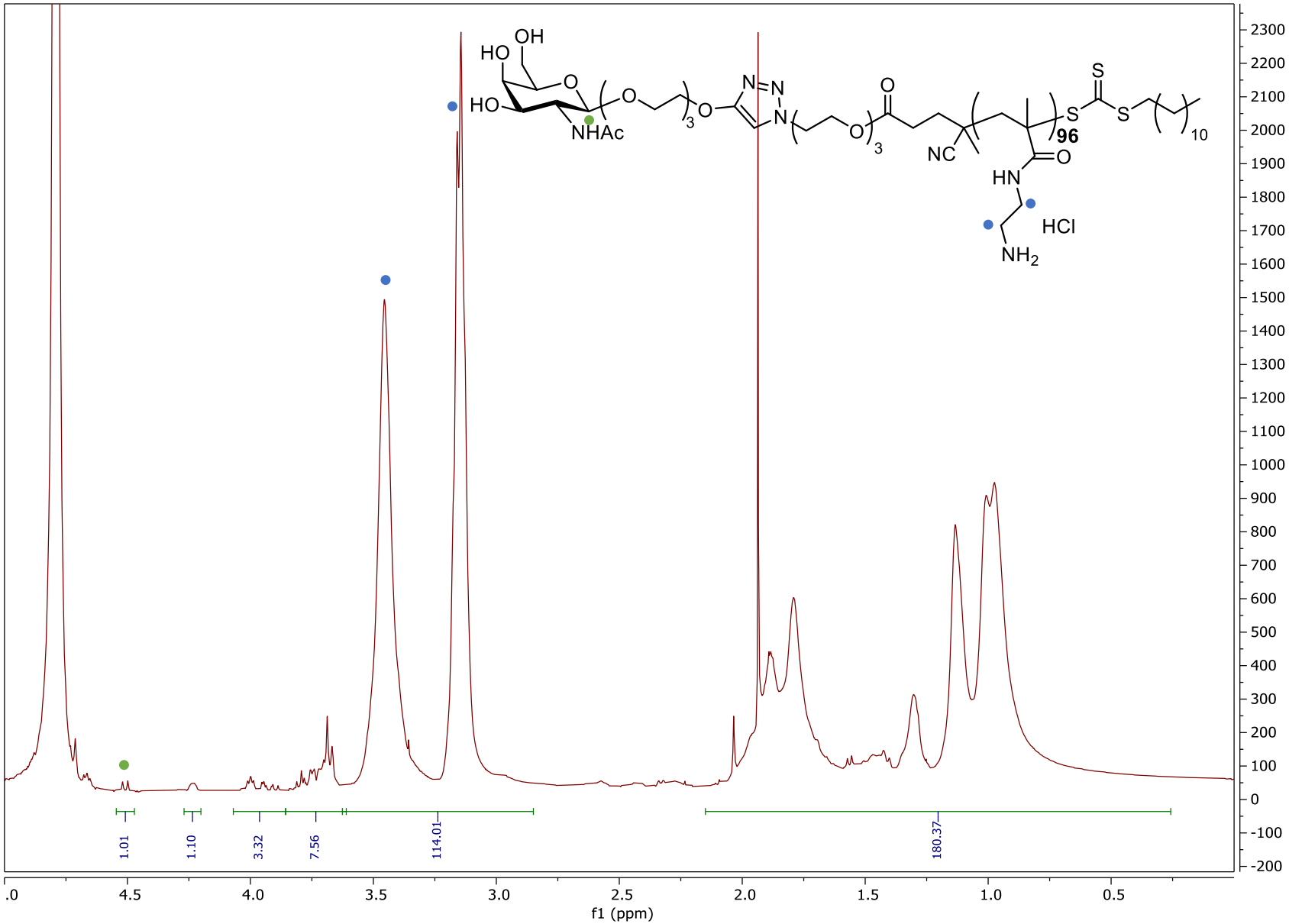

N3
m
l
k
j
i
h
O
g
f
e
CN
S
S
S
d
b
c
9
a
7.26 CDCl3
5.30
h
4.28
4.27
4.27
4.26
4.25
i,j,k,l
3.69
3.68
3.68
d
3.39
3.34
3.32
3.30
m
3.40
g
2.68
2.68
2.66
2.66
2.65
2.64
f
2.53
2.40
1.87
e
1.73
1.71
1.70
1.69
1.67
1.65
c
b
1.28
1.28
1.27
1.26
a
0.89
0.88
0.86
2.00
1.98
5.87
1.98
1.96
1.99
1.03
1.02
2.94
2.10
18.22
3.00
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
.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)


7
216.96
171.49
119.02
70.73
70.67
70.16
69.09
64.14
50.70
46.36
31.92
29.63
29.55
29.43
29.35
29.08
24.85
22.70
14.13
29.72
29.63
29.55
29.43
29.35
29.08
28.94
27.68
f1 (ppm)
f1 (ppm)


tremrb-190730-27.10.fid
mrb-3-017pure
PROTON CDCl3 /opt/data tremrb 27
f1 (ppm)


OH
HO
HO
O
NHAc
O
3
O
N=N
N
O
O
3
O
S
S
S
CN
10
9
217.12
173.96
171.63
119.17
101.90
77.36
70.69
70.61
70.54
69.52
69.11
64.15
55.03
51.01
50.45
46.51
32.05
29.76
29.69
29.56
29.48
29.22
29.08
22.83
14.27
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


HO
O
NC
74
S
S
S
10
HN
O
HCl
NH2
1.01
69.38
104.38
f1 (ppm)


OH
HO
HO
O
NHAc
O
3
O
N
N
N
O
3
O
NC
96
S
S
S
10
HN
HCl
NH2
1.01
1.10
3.32
7.56
114.01
180.37
f1 (ppm)