Supporting Information

Tandem Catalysis for the Preparation of Cylindrical Polypeptide Brushes

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Materials and Methods.

Reactions were conducted under an inert atmosphere of N_2, using oven-dried glassware unless otherwise stated. Hexanes and THF were purified by first purging with dry nitrogen, followed by passage through columns of activated alumina.[1] DMF was purified by drying over 4Å molecular sieves followed by vacuum distillation. Infrared spectra were recorded on a Perkin Elmer 1605 FT-IR Spectrophotometer. Deionized water (18 MΩ-cm) was obtained by passing in-house deionized water through a Millipore Milli-Q Biocel A10 purification unit. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a SSI Accuflow Series III liquid chromatograph pump equipped with a Wyatt DAWN EOS light scattering (LS) and Optilab rEX refractive index (RI) detectors. Separations were achieved using 10^5, 10^4, and 10^3Å Phenomenex Phenogel 5 μm columns using 0.10 M LiBr in DMF as the eluent at 60 °C. All GPC/LS samples were prepared at concentrations of 8 mg/mL. ^{1}H NMR spectra were recorded on a DRX500 Bruker spectrometer (500 MHz) and are reported relative to deuterated solvent. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Data for ^{13}C NMR spectra are reported in chemical shift. High-resolution mass spectrometry (HRMS) was performed on a Micromass Quatro-LC Electrospray spectrometer with a pump rate of 20 μL/min using electrospray ionization (ESI).

Table S1. Molecular weight (M_n) of poly(K^{AM}) as a function of monomer to initiator ratio using $(PMe_3)_4Co$ in THF at 20 °C.

M:I[a]	M_n[b]	M_w/M_n[c]	DP	yield (%)[d]
13	8 230	1.23	25	94
25	16 800	1.18	49	98
50	35 500	1.12	103	99
75	78 200	1.28	228	90
100	100 100	1.23	292	92

[a]M:I = monomer to $(PMe_3)_4Co$ initiator ratio, [b] M_n determined by end-capping with PEG-NCO (MW = 2000 Da) and analysis by ^1H NMR. [c]M_w/M_n determined by gel permeation chromatography (GPC/LS), [d]Isolated yields of PEG end-capped polypeptides.

Table S2. End-capping of activated poly(K)-*b*-poly(K^{AM}) chains with PEG-NCO (MW = 350 and 1000 Da) to determine side-chain activation efficiency.

	K Segment	K^{AM} Segment	PEG DP[b]		
entry	DP[a]	DP[a]	Theoretical	Found	% funct.[c]
1	60	15	8	7.7	97
2	60	30	8	7.7	96
3	60	15	23	21	91
4	60	30	23	23	100

[a]Degrees of polymerization (DP) of K and K^{AM} segments in poly(K)-*b*-poly(K^{AM}) block copolymers. [b]Degrees of polymerization (DP) of PEG-NCO chains used for end-capping experiments. [c]PEG end-capping efficiency (% funct.) defined as 100x(number of found PEG chains)/(number of theoretical PEG chains for 100% functionalization).

Table S3. Molecular weight (M_n and DP) data for poly(K)-*b*-poly(K^{MPBLG}) brush copolypeptides.

poly(K)-*b*-poly(K^{AM})			poly(E) segments				
	K Segment[a]	K^{AM} Segment[a]					
entry	DP	DP	M:I[b]	DP[c]	M_w/M_n[c]	M_n[c]	yield (%)[d]
1	85	9	10	12	1.15	2 630	82
2	60	17	10	12	1.25	2 630	98
3	60	30	10	12	1.23	2 630	96
4	63	16	10	11	1.04	2 410	97
5	85	9	15	15	1.10	3 290	91
6	60	17	15	15	1.13	3 290	89
7	60	30	15	16	1.08	3 500	92
8	63	16	15	16	1.02	3 500	98
9	85	9	25	27	1.10	5 910	87
10	60	17	25	28	1.20	6 130	93
11	60	30	25	25	1.18	5 470	93
12	63	16	25	25	1.13	5 470	94

[a]Degrees of polymerization (DP) of K and K^{AM} segments in poly(K)-*b*-poly(K^{AM}) precursor block copolymers. [b]M:I = γ-Benzyl-L-glutamate NCA monomer to nickel activated alloc-

methionyl initiator ratio. [c]Average degree of polymerization (DP), polydispersity index (M_w/M_n) and molecular weight (M_n) of poly(E) chains grown off of poly(K)-*b*-poly(K^{AM}) block copolymers, as determined by ^{1}H NMR and GPC/LS. [d]Isolated yields of poly(K)-*b*-poly(K^{MPBLG}) brush copolypeptides.

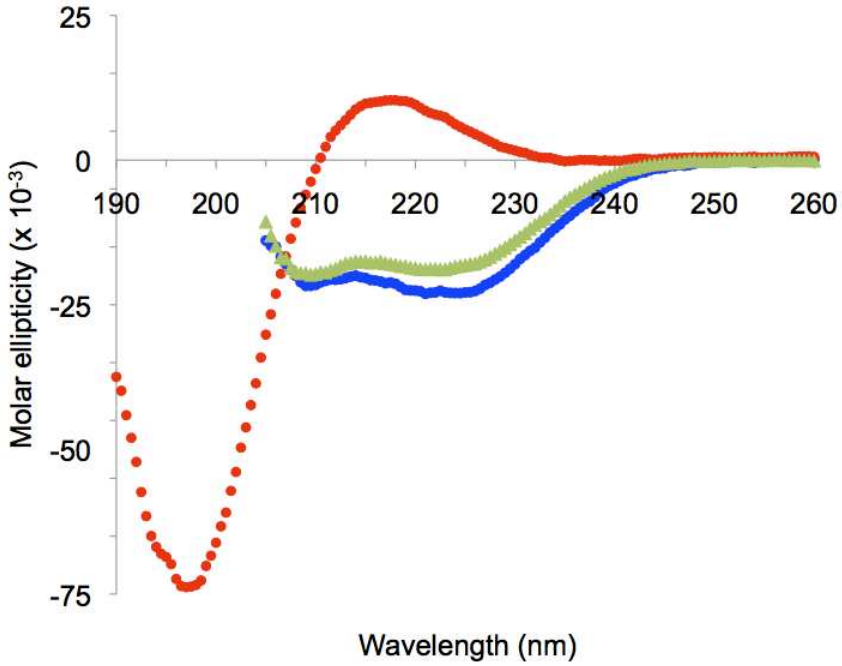
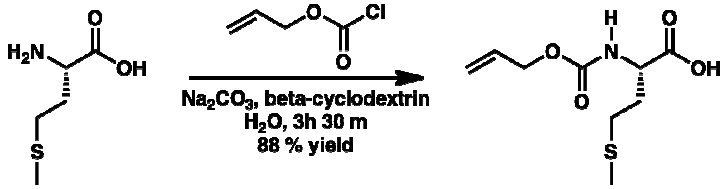


Figure S1. Circular dichroism spectra of poly(K^{AM}) in THF (blue data), protected poly(K)-*b*-poly(K^{MPBLG}) brush copolypeptide in THF (green data), and deprotected poly(K)-*b*-poly(K^{MPGA}) brush copolypeptide in deionized H_2O, pH = 6 (red data). Concentrations of all samples = 0.5 mg/mL, 20 °C.

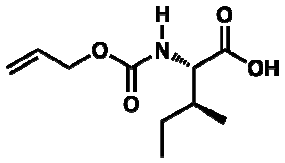
Experimental Procedures

A. Synthesis and polymerization of NCA monomer

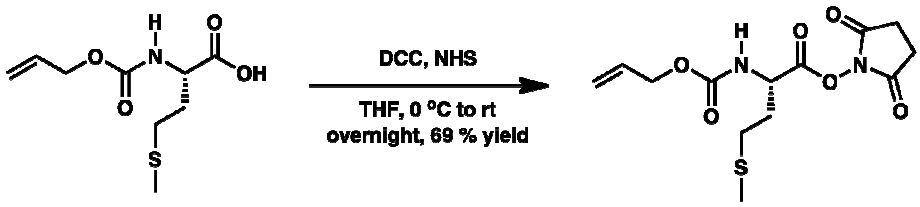


Representative procedure for synthesis of allyloxycarbonyl-L-amino acids: allyloxycarbonyl-L-methionine (1a). L-Methionine (10.0 g, 67.1 mmol) was dissolved in 1:1 THF/H_2O (350 mL total). Sodium carbonate (Na_2CO_3) (14.2 g, 134 mmol) was added followed by the addition of β-cyclodextrin (1.88 g, 1.7 mmol).[2] Allylchloroformate (7.13 mL, 67.1 mmol)

was added and the reaction stirred at 21 °C until completion. THF was removed and the reaction was acidified to pH 2 with 10% aqueous HCl (200 mL). The acidified, aqueous mixture was extracted with EtOAc (3 x 100 mL) and the organic layer was dried over sodium sulfate (Na_2SO_4), filtered and evaporated to dryness. The crude product was a clear, colorless oil. No further purification was necessary. Mass isolated: 13.8 g (88% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.94 (m, 1H), 5.41 (s, 1 H), 5.34-3.32 (d, J = 10, 1 H), 5.25-5.23 (d, J = 8, 1 H) 4.60 (m, 2 H), 4.12 (m, 1 H), 2.61-2.58 (t, J = 3.5, 2 H), 2.21 (m, 2 H), 2.11 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.44, 157.80, 134.09, 119.83, 67.86, 54.71, 33.25, 31.62, 17.08; FT-IR (THF): 1731, 1654, 1539 cm^{-1}, HRMS-ESI (*m/z*) $[2M + 2Na]^+$ Calcd for $C_{18}H_{28}NO_8S_2$: 511.12; found: 511.12.

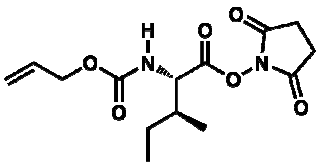


Allyloxycarbonyl-L-isoleucine (1b). No further purification required; 12.6 g isolated as a white solid (77% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.73 (b, 1H), 5.91-5.89 (J = 5.1, 1 H), 5.34-5.31 (d, J = 18, 1 H), 5.24-5.21 (d, J = 9, 1 H), 4.59 (m, 2 H), 4.38 (m, 1 H), 1.96 (m, 1 H), 1.48 (m, 1 H), 1.21 (m, 1 H), 0.99-0.94 (m, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.13, 156.28, 132.65, 118.14, 66.15, 58.36, 37.88, 24.95, 15.63, 11.73. Additional characterization data available in the literature.[3]

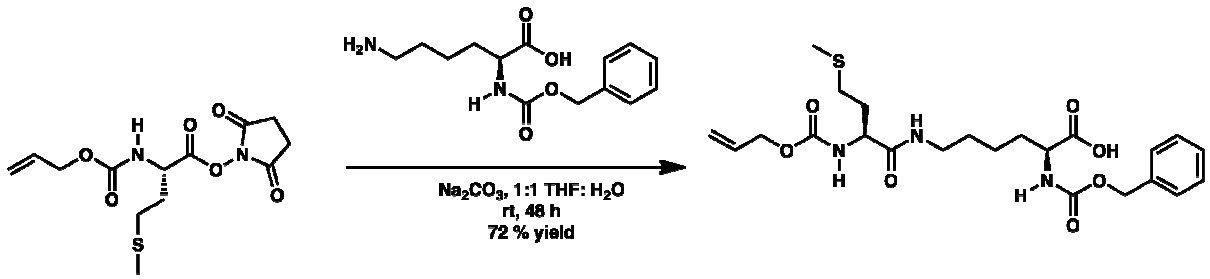


Representative procedure for preparation of allyloxycarbonyl-L-methionine-*N*-hydroxysuccin-imide ester (2a). Under an atmosphere of N_2, allyloxycarbonyl-L-methionine (13.8 g, 59.2 mmol) was dissolved in THF (320 mL). *N*-hydroxysuccinimide (8.18 g, 71.1 mmol) was added to the reaction mixture and cooled to 0 °C. Dicyclohexylcarbodiimide (DCC) (12.8 g, 62.2 mmol) was added and the reaction warmed to 21 °C, with stirring for 1 h. The reaction flask was placed in the freezer overnight where dicyclohexylurea (DCU) precipitated

and was removed by vacuum filtration. The crude product was an opaque colorless oil (13.4 g crude isolated, 69% yield). Residual DCU was separated from the product by flash chromatography (crude product was loaded in EtOAc and eluted in 1:1 Hex: EtOAc). Product R_f = 0.32. 7.30 g purified product was isolated as a clear, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.93 (m, 1 H), 5.66 (s, 1 H), 5.34-5.32 (d, J= 10, 1 H), 5.25-5.23 (d, J= 8, 1 H), 4.9 (s, 1 H), 4.58 (m, 2 H), 2.83 (s, 4 H), 2.65 (m, 2 H), 2.26 (m, 1 H), 2.12 (m, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.48, 167.83, 132.23, 118.04, 66.10, 51.47, 31.82, 29.38, 15.23. FT-IR (THF): 1788, 1741, 1722 cm^{-1}. HRMS-ESI (*m/z*) $[M + Na]^+$ Calcd for $C_{13}H_{18}N_2O_6SNa$: 353.08; found: 353.08.

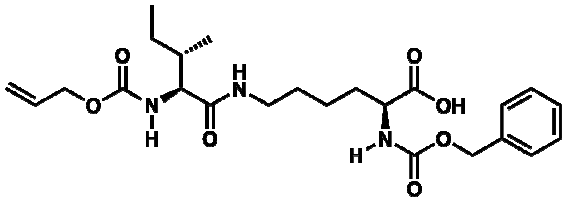


Allyloxycarbonyl-L-isoleucine-*N*-hydroxysuccinimide ester (2b). Purified by flash column chromatography starting with 1:1 Hex: EtOAc (product R_f = 0.43), 3.95 g isolated as a colorless oil (94% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.91-5.89 (m, 1 H), 5.34-5.33 (d, J = 5, 1 H), 5.25-5.23 (d, J = 8, 1 H), 4.70 (m, 1 H), 4.59 (m, 2 H), 2.84 (s, 4 H), 1.96 (m, 1 H), 1.61-1.58 (m, 1 H), 1.29-1.25 (m, 1 H), 1.06-1.05 (d, J = 2, 3 H), 0.99-0.97 (t, J = 7, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.68, 168.84, 156.66, 133.54, 119.32, 67.34, 58.00, 39.45, 26.73, 25.84, 16.25, 12.65. FT-IR (THF): 2973, 2861, 1816, 1787, and 1745 cm^{-1}. HRMS-ESI (*m/z*) $[M + Na]^+$ Calcd for $C_{14}H_{20}N_2NaO_6$: 335.12; found: 335.12.

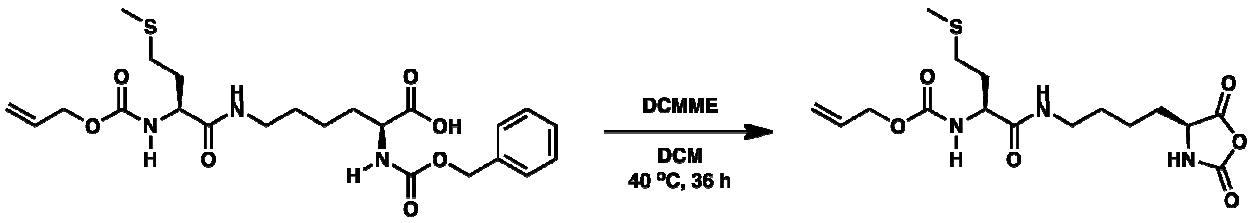


N_ε-(allyloxycarbonyl-L-methionyl)-N_α-benzyloxycarbonyl-L-lysine (3a). Allyloxycarbonyl-L-methionine-*N*-hydroxysuccinimide ester (7.30 g, 22.1 mmol) was dissolved in THF (73 mL) in a 250 mL round bottom flask equipped with a Teflon stir bar. In a separate 250 mL round

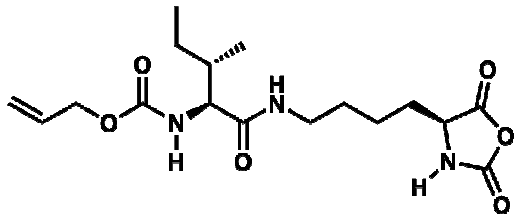
bottom flask Na_2CO_3 (2.56 g, 24.2 mmol) was dissolved in water (73 mL). N_α-benzyloxycarbonyl-L-Lysine (6.78 g, 24.2 mmol) was also dissolved in the aqueous mixture. The allyloxycarbonyl-L-methionine-*N*-hydroxysuccinimide ester solution was transferred to the aqueous reaction flask and the resulting mixture allowed stirred for 2 days. THF was removed under reduced pressure and the aqueous layer was acidified to pH 2 and extracted with EtOAc (3 x 50 mL). The organic layer was separated, dried using Na_2SO_4, filtered and concentrated. The resulting oil was again dissolved in minimal EtOAc and precipitated into hexanes yielding a white solid. Mass isolated prior to precipitation: 8.41 g. Mass isolated post-precipitation: 7.93 g (72 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33-7.32 (m, 5 H), 6.91 (s, 1H), 6.09 (s, 1H), 5.88-5.82 (2 H), 5.31-5.30 (d, *J* = 10, 1H), 5.19-5.11 (d, *J* = 8, 1H), 5.09 (s, 1H), 4.52 (m, 2H), 4.34 (m, 2H), 3.24-3.17 (m, 2 H) 2.48 (m, 2 H), 2.04 (s, 3H), 1.91-1.89 (m, 2 H), 1.78-1.66 (m, 2 H), 1.49-1.26 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$): 175.00, 172.19, 156.43, 136.07, 132.32, 128.43, 117.88, 66.98, 65.9 9, 53.86, 53.52, 39.00, 31.45, 29.99, 28.48, 25.32, 22.12, 15.13, 14.01, 11.33. FT-IR (THF): 2922, 1704, 1658, 1528 cm^{-1}. HRMS-ESI (*m/z*) $[M + H]^+$ Calcd for $C_{23}H_{34}N_3O_7S$: 496.21; found: 496.21.



***N*ε-(allyloxycarbonyl-L-isoleucyl)-*N*α-benzyloxycarbonyl-L-lysine (3b)**. Purified by flash column chromatography using 1:1 Hex:EtOAc; 3.60 g white solid isolated (80% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.45 (b, 1 H), 7.85 (s, 1 H), 7.26-7.12 (m, 5 H), 7.10-7.08 (s, 1 H), 5.80-5.77 (m, 1 H), 5.30-5.29(d, J = 10, 1 H), 5.25-5.24(d, J = 7, 1 H), 5.18 (m, 2 H), 4.36 (s 2 H), 3.81-3.79 (m, 1 H), 3.68 (m, 1 H), 3.22 (m, 2 H), 1.85 (m, 1 H), 1.76 (m, 2 H), 1.51-1.23 (m, 5 H), 1.08 (m, 1 H), 0.89-0.87 (m, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.17, 172.52, 156.87, 156.39, 136.31, 132.60, 128.64, 128.31, 118.06, 67.19, 66.16, 59.94, 53.73, 39.02, 36.99, 31.74, 28.75, 24.85, 22.29, 15.57, 22.08. FT-IR (THF): 2958, 2873, 1722, 1691, 1642, 1533 cm^{-1}. HRMS-ESI (*m/z*) [M + H]: Calcd for $C_{24}H_{36}N_3O_7$: 478.26; found: 478.26.



***N*ε-(allyloxycarbonyl-L-methionyl)-L-lysine-*N*-carboxyanhydride (K^{AM} NCA) (4a).** NCA precursor **3a** (1.0 g, 2.02 mmol) was added to a 125 mL Schlenk flask equipped with a Teflon stir bar and then dissolved in anhydrous DCM (70 mL) under N_2. α,α-Dichloromethylmethyl ether (460 μL, 5.0 mmol) was added via syringe to the stirring solution under N_2. The reaction was refluxed for 36 h. The solution was evaporated to dryness under reduced pressure and transferred to a glovebox (N_2 atmosphere) for purification. The crude residue was purified by anhydrous flash chromatography[4] (10% to 75% THF in hexanes) and collected in 15 x 10 mL fractions. K^{AM} NCA gave an R_f = 0.464 in 1:1 THF:Hex. 547 mg of NCA was isolated as a clear oil after evaporation of the THF and hexanes from the combined fractions (70% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (s, 1 H), 6.50 (s, 1 H), 5.92-5.89 (m, 1 H), 5.78-5.69 (m, 1 H), 5.34 (dd, J= 1.5, 9.5, 1 H), 5.23 (dd, J =5.5, 1.5, 1 H), 4.31 (s, 2 H), 4.31-4.17 (m, 2 H), 3.64-3.59 (m, 1 H), 3.35 (m, 2 H), 2.57 (m, 1 H), 2.13 (m, 1 H), 2.00 (m, 2 H), 1.76 (m, 2 H), 1.59 (m, 2 H), 1. 48 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 131.38, 65.54, 55.72, 37.42, 29.74, 27.46, 14.45. FT-IR (THF): 1853, 1783, 1713, 1622, 1528 cm^{-1}. HRMS-ESI (*m/z*) $[M + H]^+$ Calcd for $C_{16}H_{25}N_3O_6S$: 388.16; found: 388.15.



***N*ε-(allyloxycarbonyl-L-isoleucyl)-L-lysine-*N*-carboxyanhydride (4b).** Purified by anhydrous flash column chromatography using gradient elution (10:1 Hex:THF to 1:1 Hex:THF to 2:3 Hex:THF to 1:3 Hex:THF to THF); 240 mg white solid isolated (63 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49 (s, 1 H), 6.17 (s, 1H), 5.92 (m, 1 H), 5.34-5.25 (m, 2 H), 5.25(dd, J = 10, J = 1.3, 1 H), 4.61 (s, 2 H), 4.29-4.27 (dd, J = 15, J= 1.2, 1H), 3.90 (m, 1 H), 3.36-3.34 (m, 1 H), 3.30-3.29 (m, 1 H), 2.05 (m, 1H), 1.66 (m, 1 H), 1.48-1.15 (m, 6 H), 1.18-1.15 (m, 1H), 0.97-

0.93 (m, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.22, 170.38, 156.83, 152.43, 132.44, 118.09, 66.37 60.53, 57.43, 38.67, 36.81, 31.28, 28.51, 24.99, 21.84, 15.74, 11.30. FT-IR (THF): 1856, 1789, 1723, 1679, 1652 cm^{-1}. HRMS-ESI (*m/z*) $[M + Na]^+$ Calcd for $C_{17}H_{27}N_3NaO_6$: 392.18; found: 392.18.

General procedure for the polymerization of K^{AM} NCA. All polymerization reactions were performed in a dinitrogen filled glove box. To a solution of K^{AM} NCA (15 mg, 38.7 μmol) in dry THF (375 μL) was rapidly added, via syringe, a solution of $(PMe_3)_4Co$ in dry THF (28 μL, 1.55 μmol). The reaction was stirred at room temperature and polymerization progress was monitored by removing small aliquots for analysis by FTIR. Polymerization reactions were generally complete within 1 hour. Reactions were removed from the drybox, all THF was removed, and the polypeptide was washed with 100 mM HCl (2 x 15 mL), centrifuged for 5 minutes at 3000 rpm and the supernatent was removed. The white polypeptide was washed with 15 mL water and then lyophilized to yield poly(K^{AM}) as a fluffy white solid (12 mg, 94 % yield). ^{1}H NMR (500 MHz, TFA-d): δ 5.87 (s, 1 H), 5.27 (s, 2H), 4.60 (s, 3 H),3.36 (s, 2 H), 2.70 (s, 2 H), 2.16 (s, 3 H), 1.87-1.63 (s, 2 H), 1.62- 1.48 (s, 3 H). FT-IR (THF): 3095, 2922 ,1650,1622, 1541, 1528 cm^{-1}.

General Procedure for Endcapping of poly(K^{AM}) with poly(ethylene glycol) and Molecular Weight Determination by Endgroup Analysis. The general procedure for polymerization of K^{AM} NCA was followed. Upon completion of the reaction, as confirmed by FTIR, a solution of α-methoxy-ω-isocyanoethyl-poly(ethylene glycol), PEG-NCO (MW = 2000 Da), in THF (5 equiv per $(PMe_3)_4Co$) was added to the polymerization reaction in a dinitrogen filled glove box. The reaction was stirred overnight at room temperature and then removed from the drybox, all THF was removed, and the polypeptide was washed with 100 mM HCl (2 x 15 mL) to remove unconjugated PEG, centrifuged for 5 minutes at 3000 rpm and the supernatent was removed. The white polypeptide was washed with 15 mL water and then lyophilized to yield PEG-endcapped poly(K^{AM}) as a fluffy white solid (91 % yield). To determine poly(K^{AM}) molecular weights (M_n), ^{1}H NMR spectra were obtained in deuterated trifluoroacetic acid (TFA-*d*). Since it has been shown that end-capping is quantitative for $(PMe_3)_4Co$ initiated NCA polymerizations when

excess isocyanate is used,[5] integrations of methioninyl methyl and methylene resonances versus the polyethylene glycol resonance at δ 3.64 could be used to obtain poly(K^{AM}) lengths (see example in spectral data section).

B. Sample diblock copolypeptide syntheses

Poly(γ-benzyloxycarbonyl-L-Lysine)-*block*-poly(N_ε-(allyloxycarbonyl-L-methionyl)-L-lysine), Poly(K)-*b*-poly(K^{AM}). All polymerization reactions were performed in a dinitrogen filled glove box. A solution of $(PMe_3)_4Co$ in THF (71 μL, 3.91 μmol) was added to a solution of N_ε-benzyloxycarbonyl-L-Lysine NCA (K NCA) (20 mg, 0.0652 mmol) in THF (1.0 mL). After 1 h, the polymerization reaction was complete as determined by FTIR. An aliquot of poly(Z-L-lysine) was removed and analyzed by GPC/LS (M_n = 18700, M_w/M_n = 1.18, DP = 71). K^{AM} NCA (23 mg, 0.059 mmol) was then added to the stirring solution of poly(K). The reaction was stirred for an additional 1 h after which all NCA was consumed. An aliquot of poly(K)-b-poly(K^{AM}) was removed from the reaction and analyzed by GPC/LS (M_n = 43000, M_w/M_n = 1.12, DP = 142). The reaction mixture was concentrated to dryness and the copolypeptide was removed from the glovebox. The copolypeptide was dissolved in minimal THF (250 μL), and precipitated by the slow addition of 100 mM aqueous HCl (3 x 15 mL) followed by centrifugation (3000 rpm) for 5 minutes. The supernatants were discarded and the residue was lyophilized to remove remaining water, giving the product as a white fluffy solid. (35.2 mg, 99 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24 (m, 5 H), 5.85 (bs, 1 H), 5.28 (m, 2 H), 5.11 (s, 2 H), 4.58 (m, 4 H), 3.61 (s, 1 H), 3.34 (bs, 2 H), 3.14 (bs, 2 H), 2.70 (bs, 2 H), 2.20 (m, 4 H), 1.77-1.35 (m, 11 H) . FT-IR (THF): 3095, 2922, 1741, 1651, 1540 cm^{-1}.

Poly(N_ε-(allyloxycarbonyl-L-methionyl)-L-lysine)-*block*-poly(γ-benzyloxycarbonyl-L-lysine), Poly(K^{AM})-*b*-poly(K). A solution of $(PMe_3)_4Co$ in THF (55 μL, 3.1 μmol) was added to a solution of K^{AM} NCA (60 mg, 0.15 mmol) in THF (1.5 mL). After 1 h, the polymerization reaction was complete as determined by FTIR. The reaction mixture was divided into 6 equal portions (0.025 mmol each) and two aliquots of poly(K^{AM}) were reacted with excess PEG-NCO (MW = 2000 Da) and analyzed by ^{1}H NMR and GPC/LS (M_n = 31200, M_w/M_n = 1.11, DP = 91). K NCA (8 mg, 0.025 mmol) was then added to a stirring solution of poly(K^{AM}) (0.025 mmol, 1

aliquot). The reaction was stirred for an additional 1 h after which all NCA was consumed and analyzed by GPC/LS (M_n = 55000, M_w/M_n = 1.12, DP = 184). The reaction mixture was concentrated to dryness and the copolypeptide was removed from the glovebox. The polymer was then dissolved in minimal THF (250 μL), and precipitated by the slow addition of 100 mM aqueous HCl (3 x 15 mL) followed by centrifugation (3000 rpm) for 5 minutes. The supernatants were discarded and the residue was lyophilized to remove remaining water, giving the product as a white fluffy solid. (15 mg, 100 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31 (m, 5 H), 5.92 (bs, 1 H), 5.43-5.19 (m, 3 H), 4.64 (m, 4 H), 3.47 (m, 2.4 H), 3.01 (bs, 2 H), 2.91 (bs, 2 H), 2.23 (bs, 4 H), 1.87 (bs, 4 H), 1.55 (bs, 3 H). FT-IR (THF): 3095, 2922, 1741, 1651, 1540 cm^{-1}.

C. Activation and reactivity of poly(K^{AM})

General procedure for activating poly(K^{AM}) using Ni(0).[6] In the glove box, poly(K^{AM}) (0.077 mmol of K^{AM} residues) was freshly prepared, most of the THF then removed under vacuum, and the residue dissolved in DMF (5 mL). In a separate vial $Ni(COD)_2$ (21 mg, 0.077 mmol, 1.0 eq. per K^{AM} residue) was dissolved in minimal THF (500 μL). 1,2-Bis(dimethylphosphino)ethane (dmpe) (26 μL, 0.15 mmol, 2.0 eq. per Ni) was then added to the $Ni(COD)_2$ solution followed by stirring for 10 minutes. The poly(K^{AM}) solution was then combined with the Ni(0) solution, changing it from a yellow to deep orange color. This solution was transferred to a thick walled glass tube that was sealed with a teflon cap and heated overnight (15 h) at 80 ^oC. The resulting activated poly(K^{AM}) solution was cooled to ambient temperature and then used directly for further reactions and polymerizations.

Quenching of activated poly(K^{AM}) with HCl. Excess aqueous 4.0 M HCl (4 mL) was added to a THF (500 μL) and DMF(2.0 mL) solution of activated poly(K^{AM}) (0.103 mmol). The reaction was stirred for 2 h after which the solution was dialyzed (MWCO = 2000) against water and EDTA (0.01 M). After several water changes, the polymer precipitate was dialyzed against HCl (0.02 M), after which the polymer product became fully water soluble. After several water changes the product was dialyzed against pure deionized water. The sample was lyophilized and the product analyzed using ^{1}H NMR, which showed that the resonances from the

allyloxycarbonyl groups of poly(K^{AM}) had essentially disappeared, indicating near complete activation of the allyloxycarbonyl side-chains (Figure 3). ^{1}H NMR (500 MHz, D_2O): δ 4.11 (2 H), 3.35 (2 H), 2.59-2.50 (2 H), 2.19 (1 H), 2.14 (3 H), 1.94 (2 H), 1.59 (2 H), 1.43 (2 H).

Stability of allyloxycarbonyl groups to 4.0 M HCl. To ensure any unreacted allyloxycarbonyl groups in the poly(K^{AM}) sample above are stable against hydrolysis by 4.0 M HCl alone, N_ε-(allyloxycarbonyl-L-methionyl)-N_α-benzyloxycarbonyl-L-lysine (**3a**) (100 mg, 0.20 mmol) was dissolved in 5.0 mL THF. Excess 4.0 M HCl (5.0 mL) was added and the reaction stirred at 21 ^{o}C for 2 h. THF was removed under vacuum and the aqueous layer was then extracted with EtOAc and dried with $MgSO_4$, filtered, and concentrated. ^{1}H NMR analysis showed that resonances of the allyloxycarbonyl group remained intact: 5.88-5.83 ppm (1 H, m) and 5.29-5.10 ppm (2H, dd).

General procedure for quenching of activated poly(K^{AM}) with PEG-NCO (MW = 350 or 1000 Da). PEG-NCO (MW = 1000) (76.0 mg, 0.076 mmol) in DMF (3.0 mL) was added to a stirring solution of activated poly$(K)_{60}$-*b*-poly$(K^{AM})_{17}$ (0.427 mL, 0.015 mmol of activated alloc groups) in DMF. The resulting solution was stirred overnight at room temperature. The reaction mixture was concentrated under vacuum, and the polymer residue was dissolved in Millipore water and dialyzed (MWCO = 6,000-8,000) against aqueous EDTA (0.01 M), followed by Millipore water for 5 days, changing water twice a day. The sample was then lyophilized to give the product as a white solid (32 mg, 98 % yield). ^{1}H NMR analysis of the sample was used to determine the degree of PEG end-capping of activated alloc groups. ^{1}H NMR (500 MHz, d-TFA): δ 7.26 (br s, 5 H), 5.13 (br s, 2 H), 4.55 (br s, 2 H), 3.88 (br s, 59 H), 3.16 (br s, 2 H), 2.14 (br s) 1.79-1.72 (br m), 1.49-1.34 (br m).

D. Growth of cylindrical brush copolypeptides from activated poly(K)-*b*-poly(K^{AM})

Preparation of activated poly$(K)_{60}$-*b*-poly$(K^{AM})_{17}$ for cylindrical brush growth. All polymerization reactions were performed in a dinitrogen filled glove box. A solution of $(PMe_3)_4Co$ in THF (297 μL, 16.1 μmol) was added to a solution of K NCA (100 mg, 0.33 mmol) in THF (2 mL). After 1 h, the polymerization reaction was complete as determined by FTIR. An

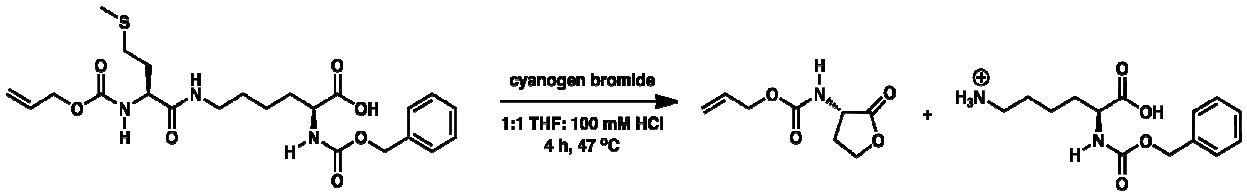
aliquot of poly(K) was removed and analyzed by GPC/LS (M_n = 15650, M_w/M_n = 1.11, DP = 60). K^{AM} NCA (32 mg, 0.083 mmol) in THF (530 μL) was then added to the stirring solution of poly(K). The reaction was stirred for an additional 1 h after which all NCA was consumed. An aliquot of poly(K)$_{60}$-*b*-poly(K^{AM})$_{17}$ was removed for analysis by GPC/LS and ^{1}H NMR (K^{AM} segment: M_n = 5800, M_w/M_n = 1.09, DP = 17). Poly(K)$_{60}$-*b*-poly(K^{AM})$_{17}$ (0.077 mmol of K^{AM} residues) in THF was then concentrated under vacuum and then diluted in DMF (2.0 mL). In a separate vial, Ni(COD)$_2$ (21 mg, 0.077 mmol, 1 eq. per K^{AM} residue) was dissolved in minimal THF (500 μL) and dmpe (26 μL, 0.15 mmol, 2 eq. per Ni) was then added. The poly(K)$_{60}$-*b*-poly(K^{AM})$_{17}$ solution was then combined with the Ni(0) solution, changing it from a yellow to deep orange color. This solution was transferred to a thick walled glass tube that was sealed with a teflon cap and heated overnight (15 h) at 80 ^{o}C. The resulting activated poly(K)$_{60}$-*b*-poly(K^{AM})$_{17}$ solution was cooled to ambient temperature and then used directly for polymerizations.

Preparation of cylindrical brush polypeptides: Poly(γ-benzyloxycarbonyl-L-Lysine)$_{60}$-*block*-poly(N_ε-(poly(γ-benzyl-L-glutamate)-*graft*-L-methionyl)-L-lysine)$_{17}$, Poly(K)$_{60}$-*b*-poly(K^{MPBLG})$_{17}$. γ-Benzyl-L-glutamate NCA, E NCA, (68.0 mg, 0.258 mmol) in DMF (1.7 mL) was added to activated poly(K)$_{60}$-*b*-poly(K^{AM})$_{17}$ solution (0.0258 mmol *of activated nickel amidoamidate groups*) to grow the poly(E) chains. The solution was then stirred overnight (15 h) at 21 ^{o}C to ensure reaction completion. The sample was concentrated under reduced pressure and precipitated by addition to 100 mM HCl (2 x 15 mL) followed by centrifugation. The sample was washed with water (1 x 15 mL), centrifuged and the product isolated by lyophilization as a white solid (75.6 mg, 87 % yield). ^{1}H NMR (500 MHz, d-TFA): δ 7.20 (m), 5.11 (m), 4.65 (bs), 6.43 (m), 3.15 (bs), 2.43 (bs), 2.14 (bs), 1.93 (bs), 1.47 (bs), 1.33 (bs).

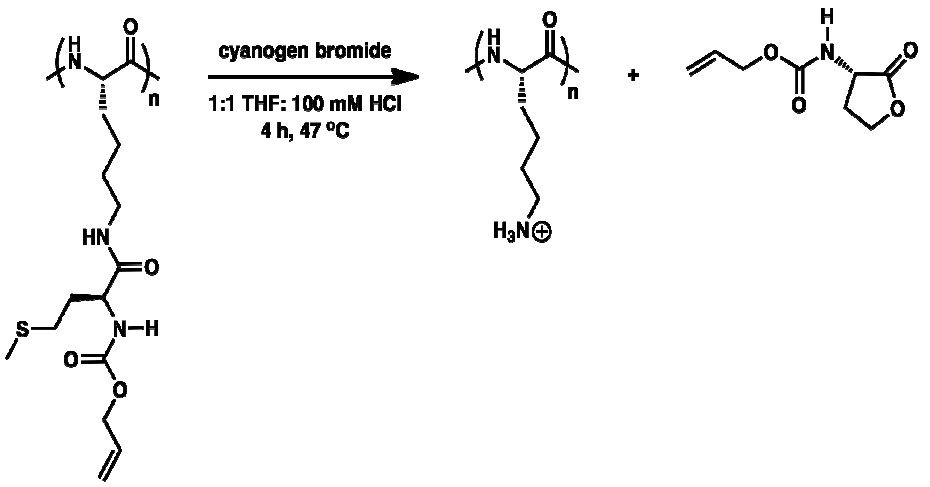
Deprotection of cylindrical brush copolypeptides using TMSI. Poly(N_ε-(poly(γ-benzyl-L-glutamate)$_{44}$-*graft*-L-methionyl)-L-lysine)$_{12}$, poly(K^{MPBLG}), brush copolypeptide (80 mg, 0.673 μmol) was dissolved in 10 mL DCM. An excess of TMSI (222 μL, 1.64 mmol) was added and the reaction was refluxed for 16 h. The deprotected polypeptide was precipitated with 20 mL hexanes and the product was redissolved in water and dialyzed (2000 MWCO dialysis tubing) against EDTA (0.01 M), followed by basic water (NaOH, pH = 8) then Millipore water for 4

days. The product poly(N_ε-(poly(L-glutamatic acid)$_{44}$-*graft*-L-methionyl)-L-lysine)$_{12}$, poly(K^{MPGA}), was isolated by lyophilization as a white solid (31 mg, 65 % yield). ^{1}H NMR (500 MHz, D_2O): δ 4.18-4.17 (m, 1 H), 2.14-2.05 (m, 2 H), 1.95-1.89 (bs, 1 H), 1.79-1.73 (bs, 1 H).

E. Cleavage of polypeptides at methionine residues using cyanogen bromide



Cleavage of N_ε-(allyloxycarbonyl-L-methionyl)-N_α-benzyloxycarbonyl-L-lysine using cyanogen bromide. N_ε-(allyloxycarbonyl-L-methionyl)-N_α-benzyloxycarbonyl-L-lysine (114 mg, 0.23 mmol) was dissolved in THF (2.0 mL). Excess cyanogen bromide (6.0 mL, 0.25 M) was added to the solution and the resulting mixture was heated at 47 ^{o}C for 4 h. Next, all THF was removed under reduced pressure and the product homoserine lactone was isolated by extraction of the aqueous layer with EtOAc (3 x 10 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated (42 mg, 99 % yield). The homoserine lactone was purified by flash column chromatography in 1:2 Hex:EtOAc with an R_f = 0.44. After removal of solvents, the homoserine lactone isolated as a white solid (19 mg, 45% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.91-5.82 (m, 1H), 5.38-5.34 (s, 1H), 5.31-5.25 (d, J = 1.5, 9.5, 1 H), 5.25-5.22 (d, J = 5.5, 1.5, 1 H), 4.61 (d, J = 5.5, 2 H), 4.46 (m, 2 H), 4.27 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.10, 156.10, 132.403, 118.31, 66.31, 65.91, 50.60, 30.53. FT-IR (film in THF): 2924, 1777, 1699, 1527 cm^{-1}. No N_ε-(allyloxycarbonyl-L-methionyl)-N_α-benzyloxycarbonyl-L-lysine was observed by TLC or ^{1}H NMR indicating all of the starting material was consumed.



Cleavage of poly(K^{AM}) side-chains using cyanogen bromide. A sample of poly(K^{AM}) (0.145 mmol) was prepared as described above and the resulting polymer solution was further diluted in THF (to a final of volume of 3.5 mL) and removed from the glovebox. Aqueous HCl (7.0 mL, 100 mM) was added to the polymer solution resulting in precipitation of the polypeptide. Cyanogen bromide solution in THF (4.31 mL , 0.25 M) was added to the polypeptide suspension, which was then heated to 47 ^{o}C for 18 h. After 3 h the initially cloudy reaction mixture became completely clear and colorless. The THF was then removed under reduced pressure. Additional aqueous HCl (10 mL, 100 mM) was added to the reaction mixture followed by EtOAc (7.0 mL). Upon addition of EtOAc, the cloudy aqueous mixture become clear. The homoserine lactone byproduct was isolated and purified using flash column chromatography in 1:2 Hex:EtOAc with an R_f = 0.32 in 1:1 Hex:EtOAc (20 mg, 75 % yield). No poly(K^{AM}) was observed by ^{1}H NMR ($CDCl_3$).

Cleavage of deprotected cylindrical brush polypeptides, poly(K^{MPGA}). A sample of poly(K^{MPGA}) (9.3 mg, 1.1 μmol) was dissolved in 1.90 mL 70% formic acid (aqueous). Cyanogen bromide (88 μL, 0.022 mmol) in 70 % formic acid (0.25 M) was added to the polypeptide solution. The reaction mixture was stirred at 21 ^{o}C for 24 h. Formic acid was removed under reduced pressure and the polypeptide residue was dissolved in freshly prepared PBS buffer (10 mg/ml). The sample, composed primarily of cleaved poly(L-glutamic acid) (PGA) segments, was filtered through a 0.2 μm PTFE filter and analyzed using SDS-PAGE as described below.

Analysis of cleaved PGA segment chain length distributions using SDS-PAGE. Two PGA standards (M_n = 5120, M_w/M_n = 1.05 and M_n = 18900, M_w/M_n = 1.13) were prepared using nickel amidoamidate initiator in DMF, and deprotected using TMSI following procedures as described above. PGA segments (M_n = 3820, determined by ^{1}H NMR and GPC/LS as described above) cleaved from a brush copolymer were obtained as described above. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a Mini-PROTEAN Tetra cell electrophoresis system using a 16.5% polyacrylamide Mini-PROTEAN Tris-Tricine Precast Gel to maximize separation of small molecular weight PGA (see Figure 4b) in Tris/Tricine/SDS buffer (100mM Tris, 100mM tricine, 0.1% SDS, pH 8.3, Bio Rad). The PGA samples were visualized using silver staining.

Rebenzylation of cleaved PGA Segments.[7] Under an atmosphere of N_2, A samples of cleaved PGA segment (4.5 mg, 0.033 mmol) was dissolved in minimal benzene. Phenyldiazolmethane (1.5 mL, 0.13 mmol) was added to the PGA solution and stirred for 48 h at 21 ^{o}C. A 250 μL aliquot was taken directly from the reaction and analyzed by GPC.

Preparation of Phenyldiazomethane.[7] Benzaldehyde tosyl hydrazone (530 mg, 1.93 mmol) was dissolved in benzene (28 mL). Benzyltriethylammonium chloride (43 mg, 0.193 mmol) was added to 14 % w/w NaOH (53 mL) and this solution was added to the benzaldehyde tosyl hydrazone solution. The reaction mixture was protected from light and refluxed at 70 ºC for 5 h. Phenyldiazomethane in benzene was separated from the aqueous layer and washed with water (3 x 50 mL). The orange organic layer was again separated and dried over Na_2SO_4, filtered and used directly.

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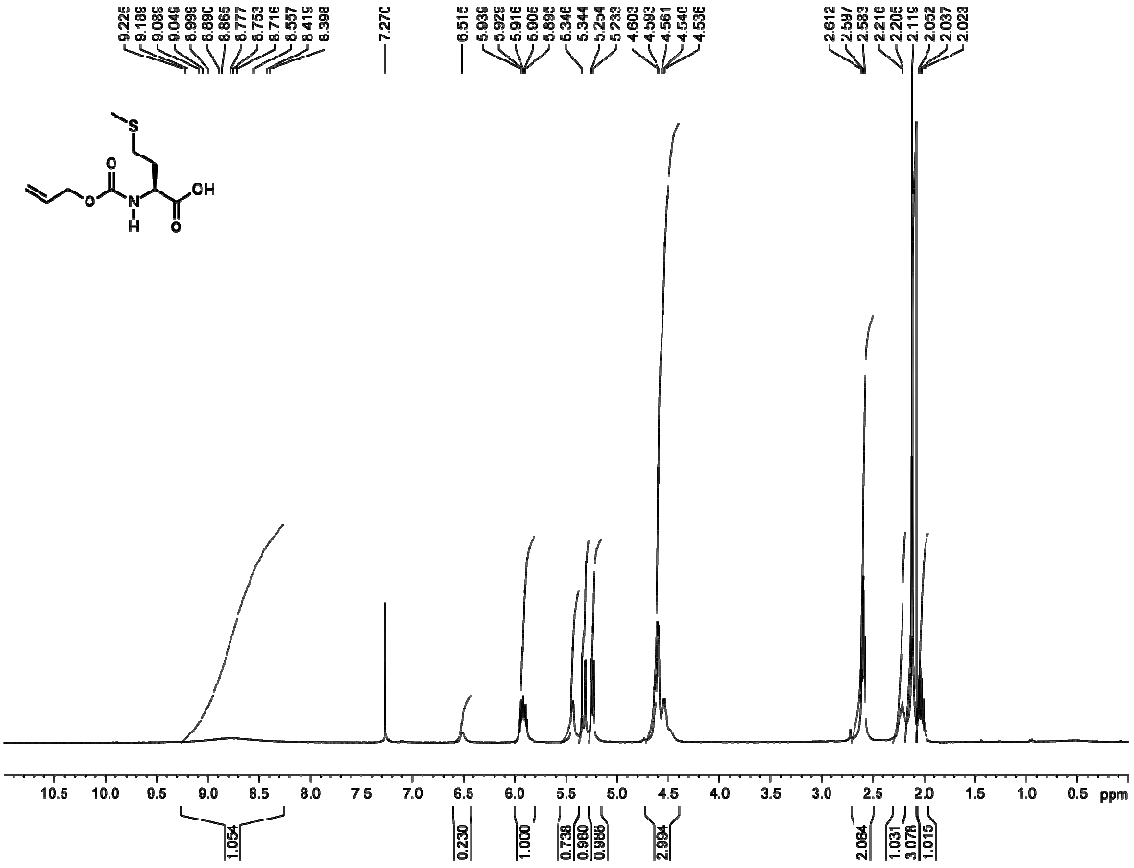

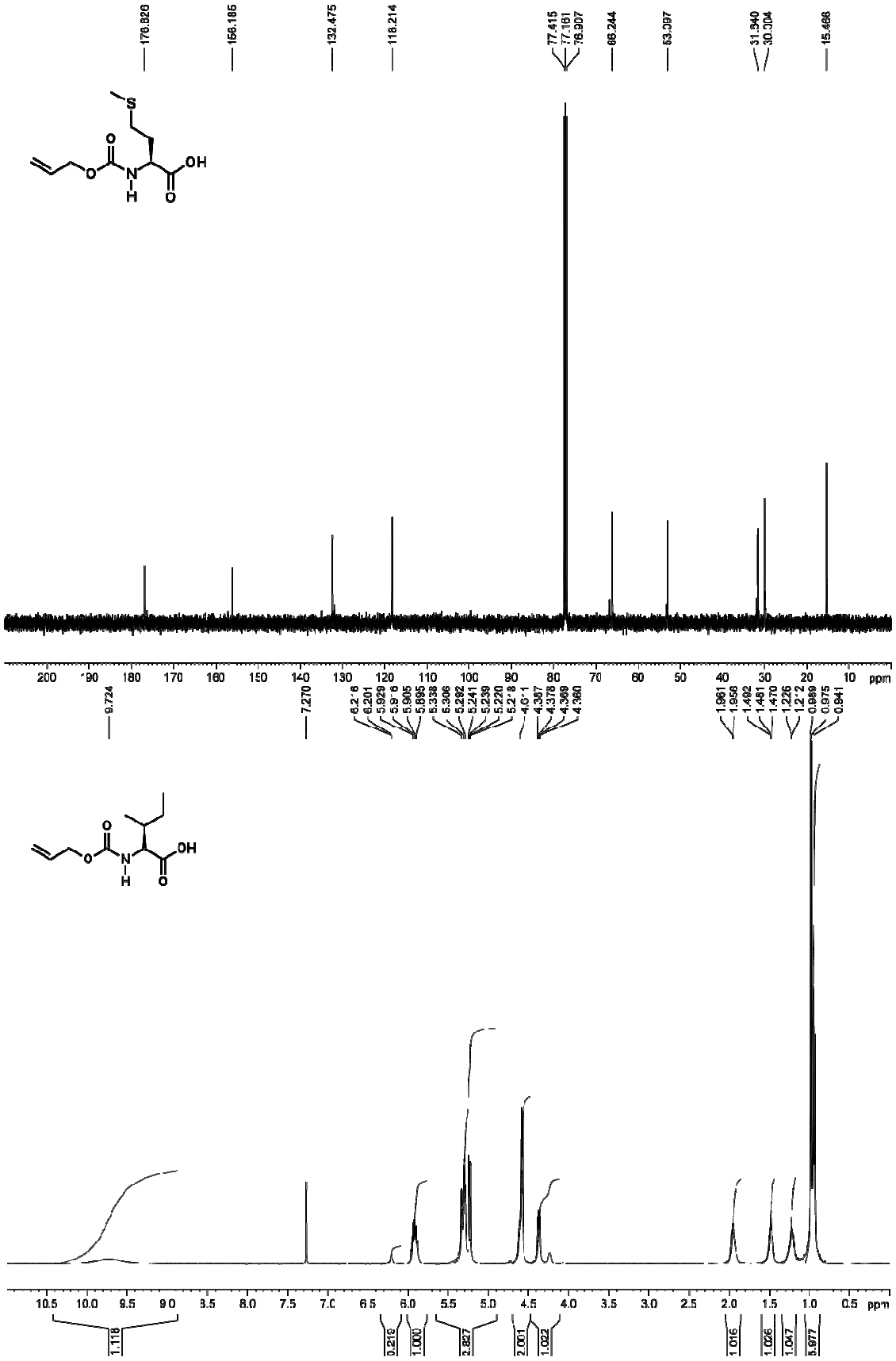
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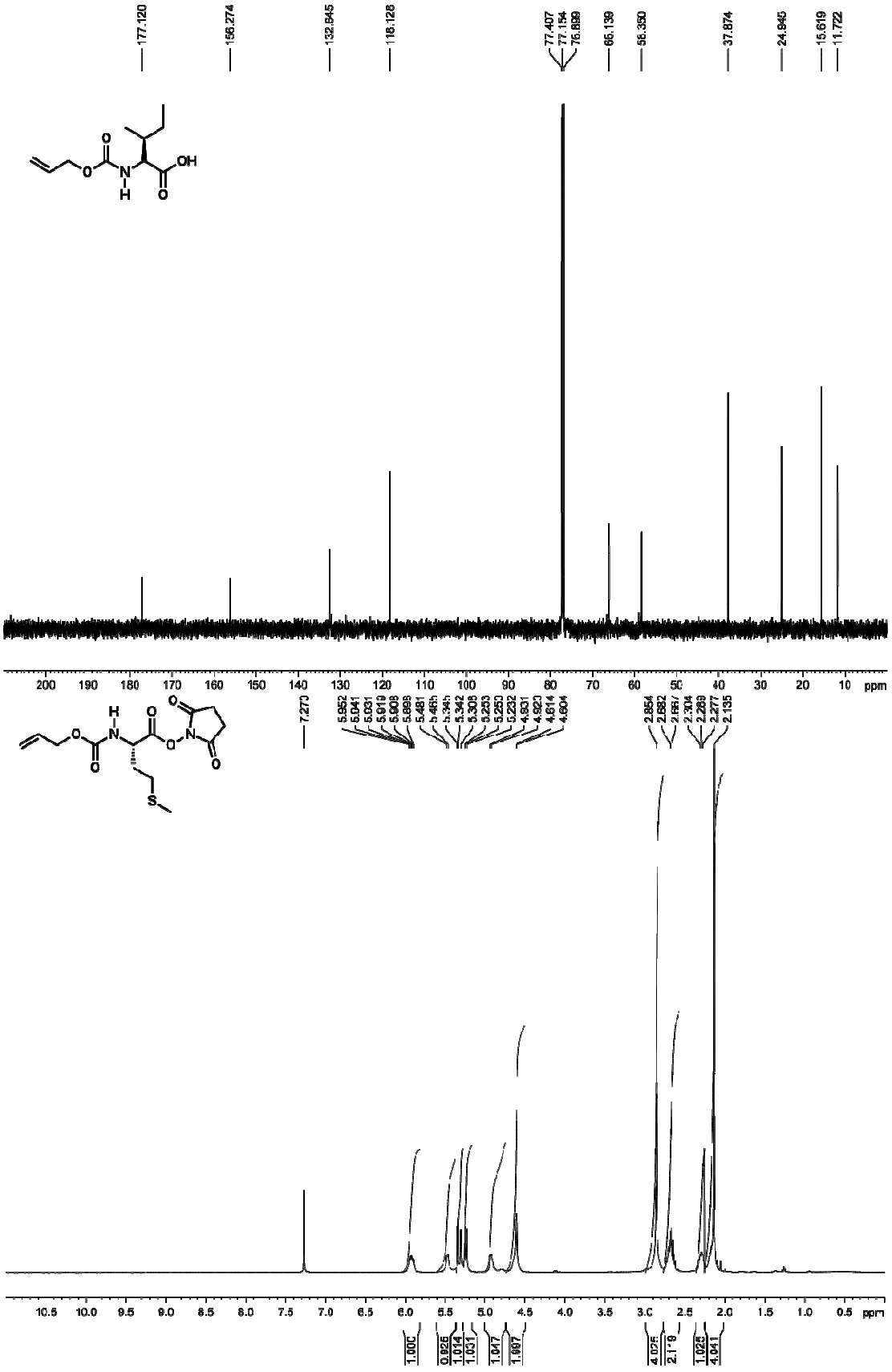
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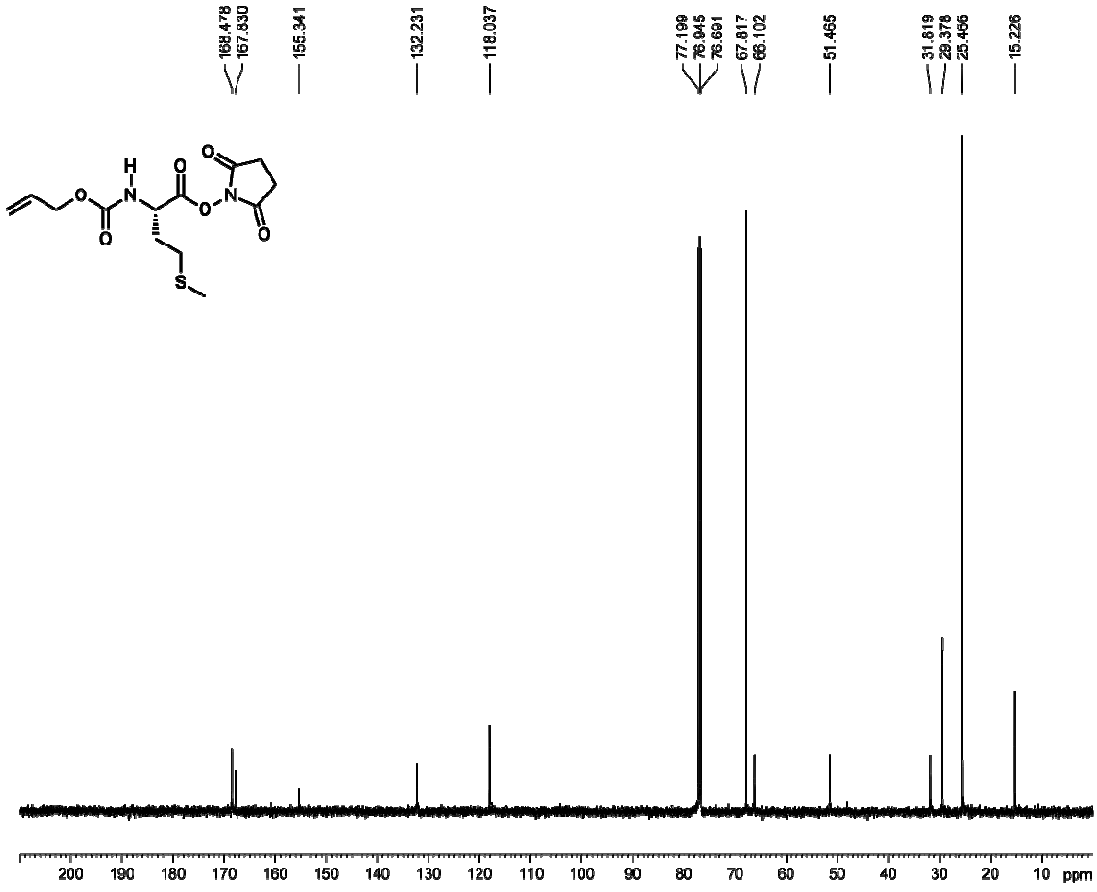
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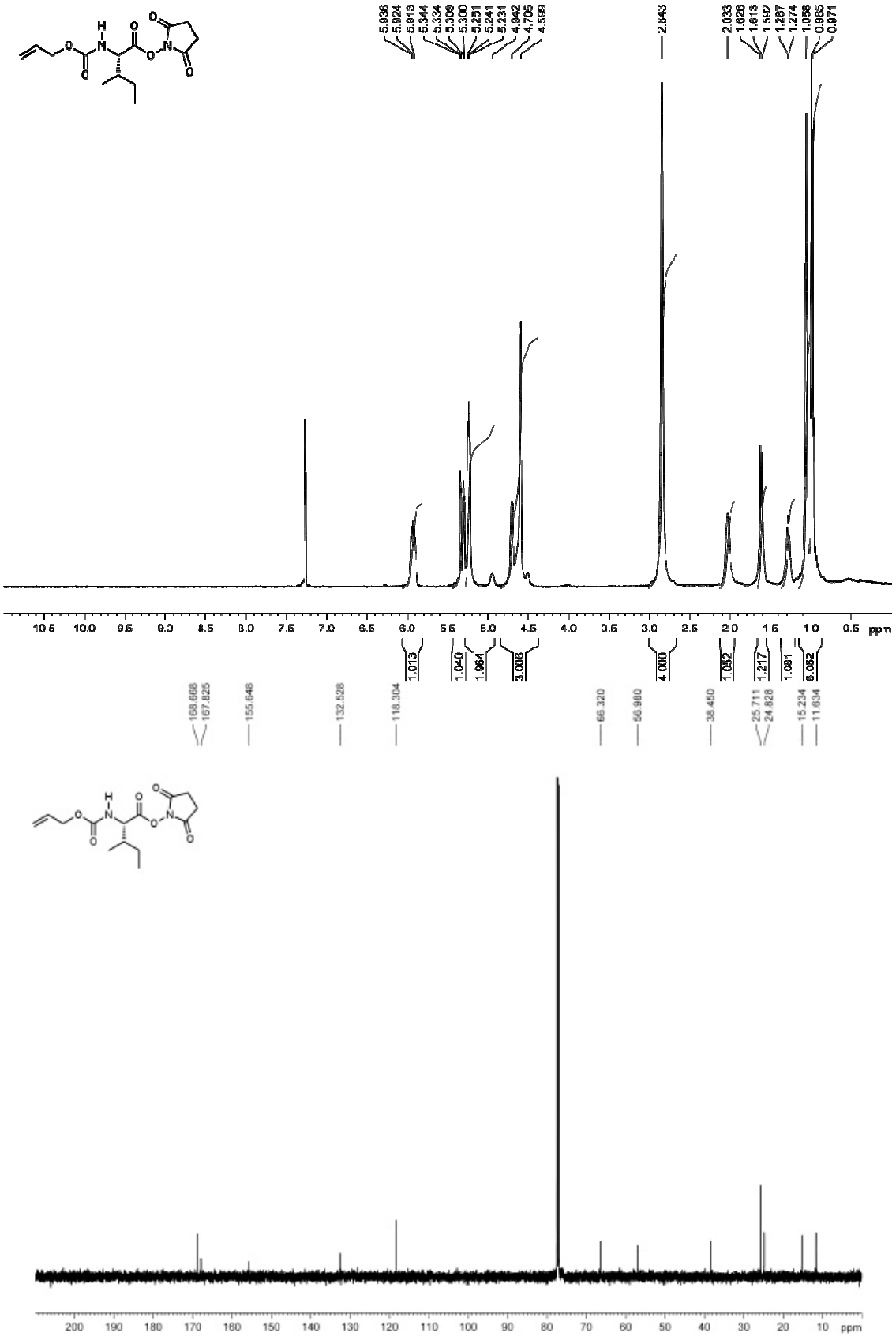
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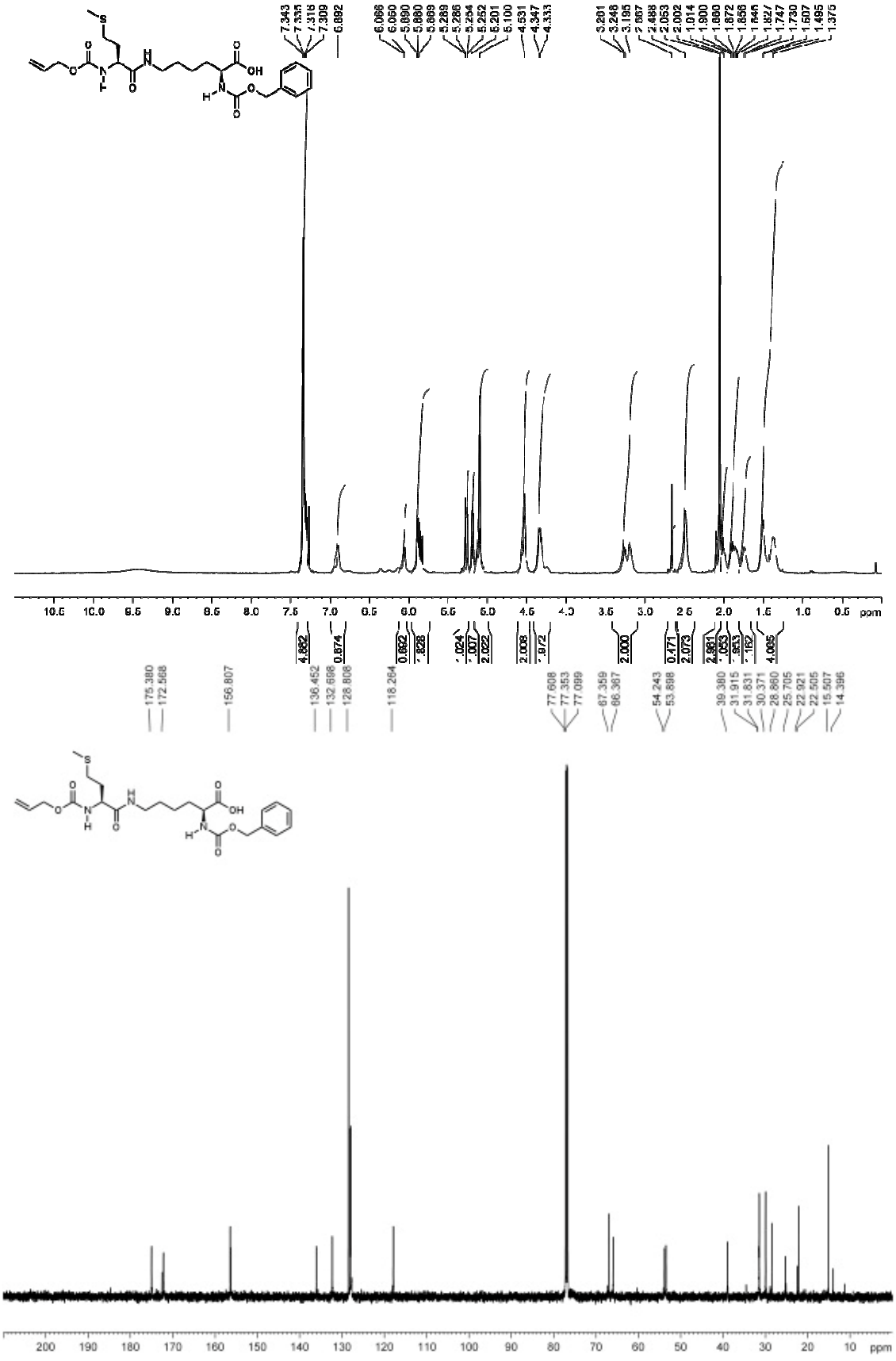

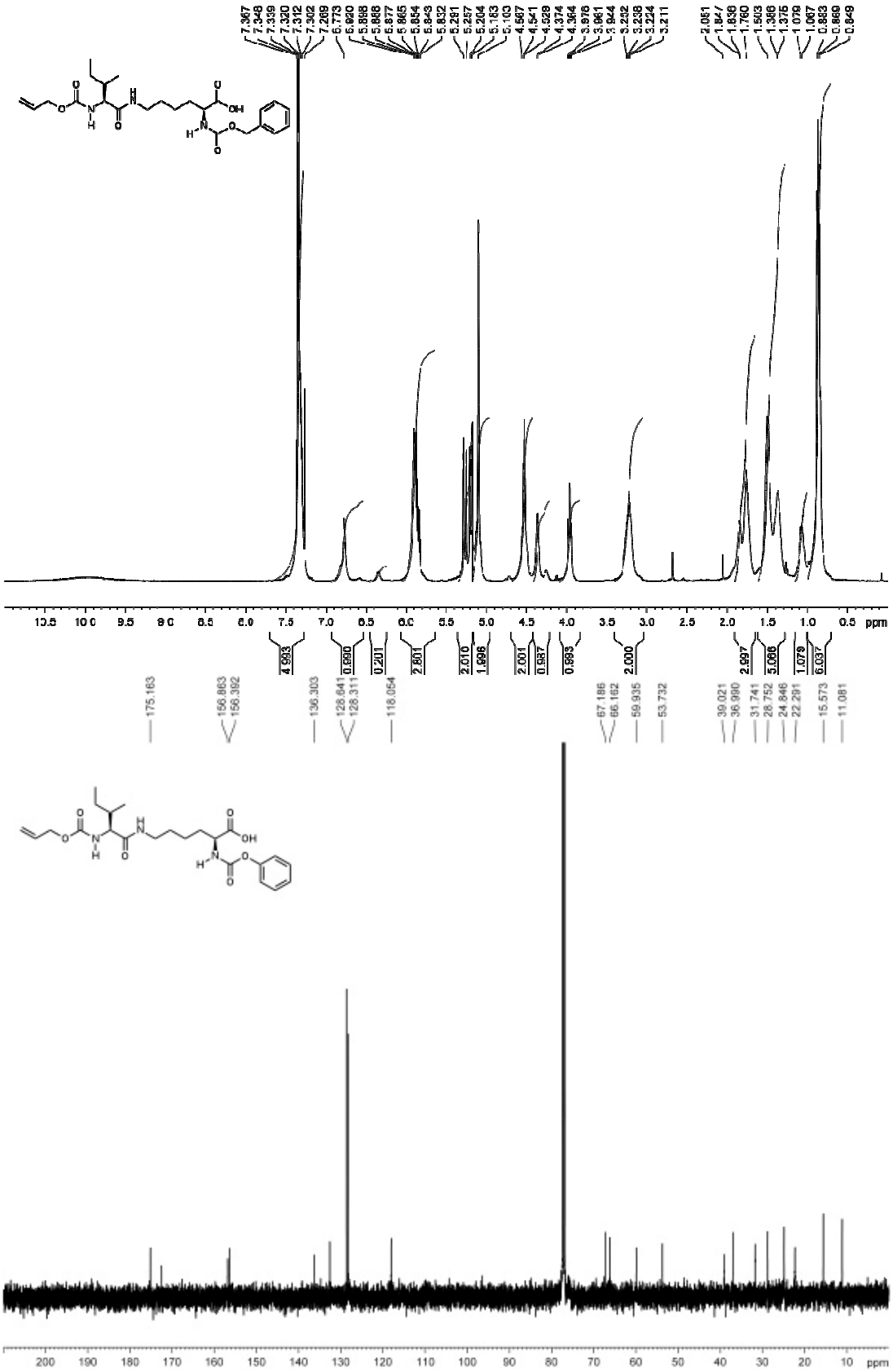
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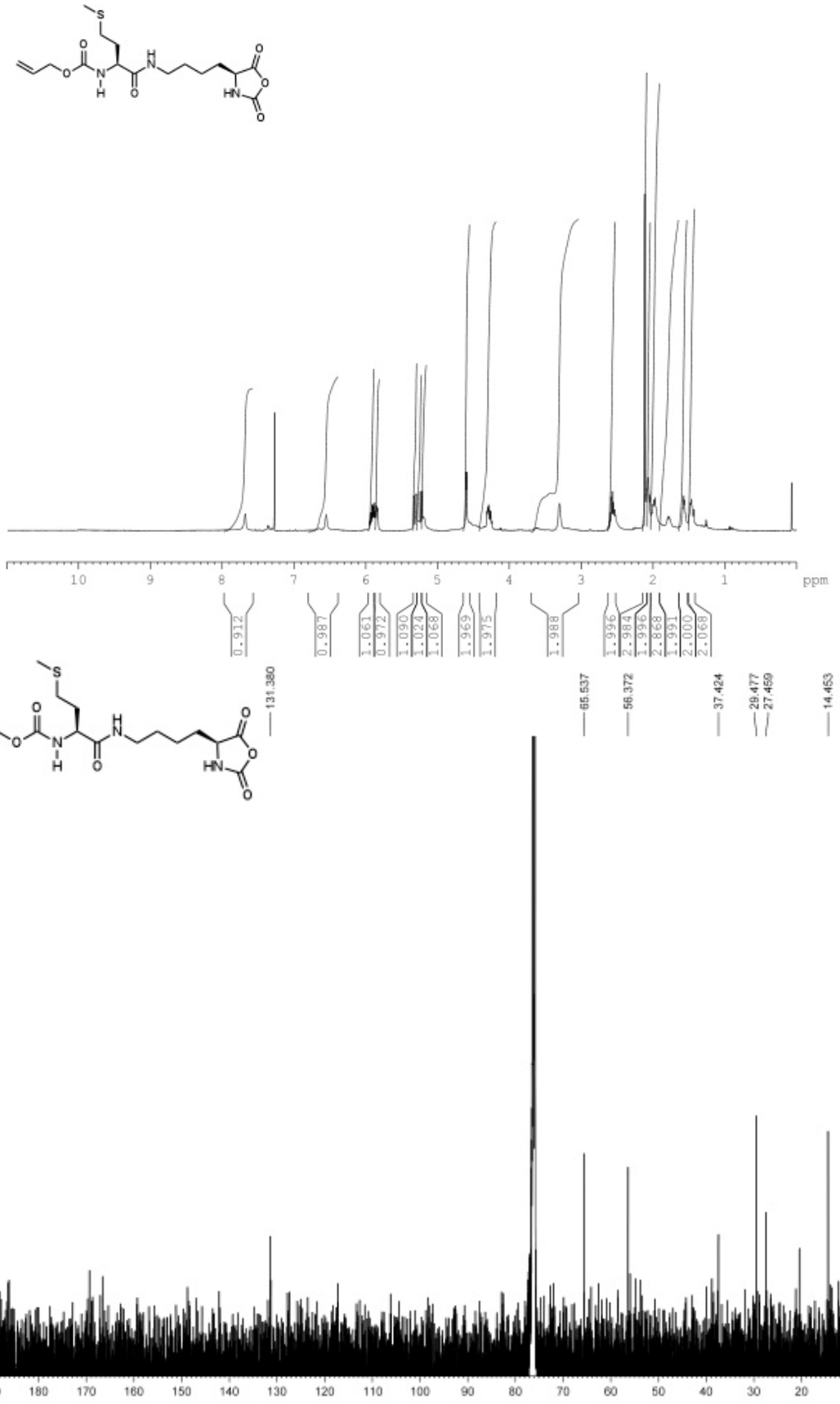
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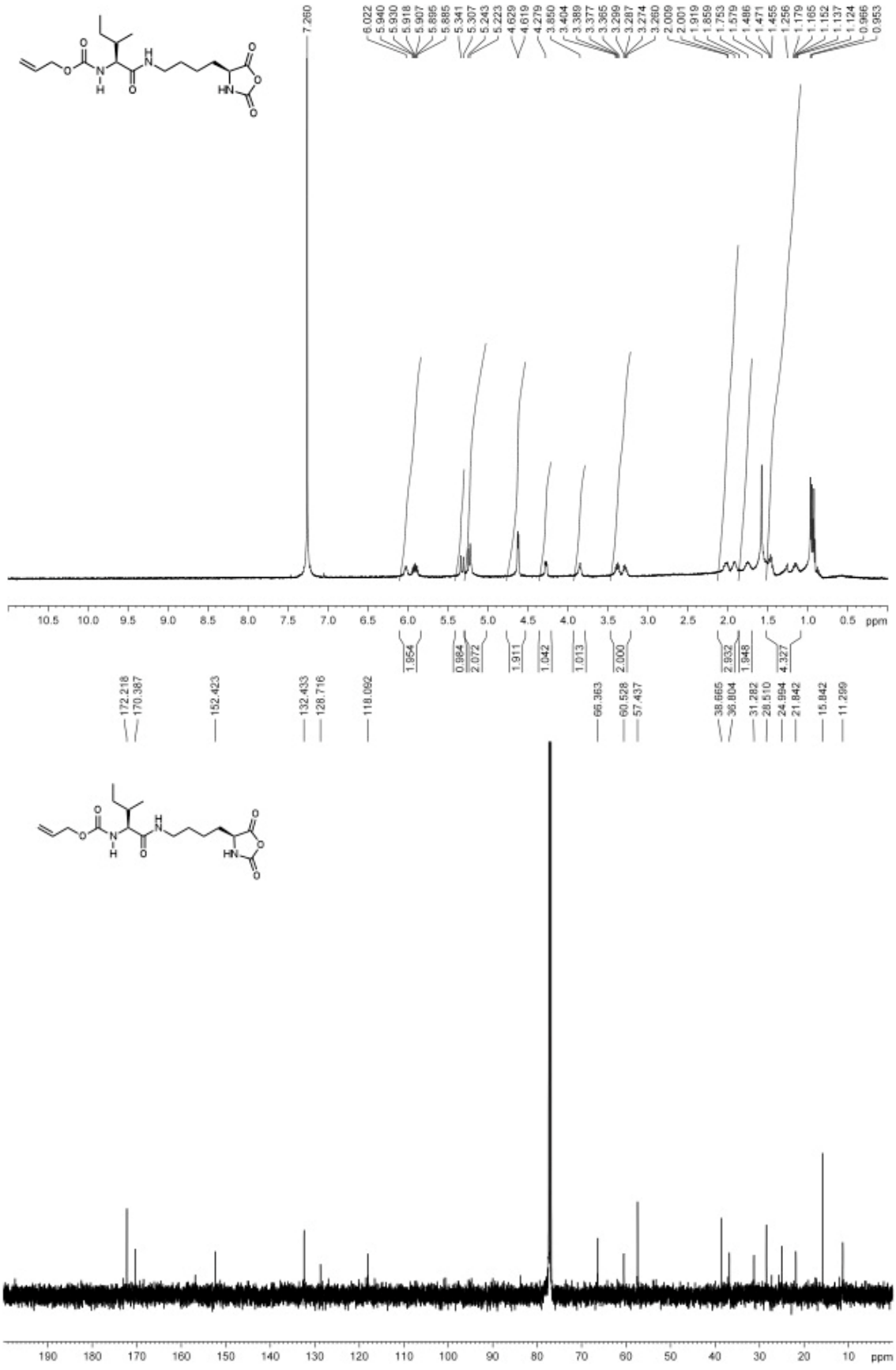
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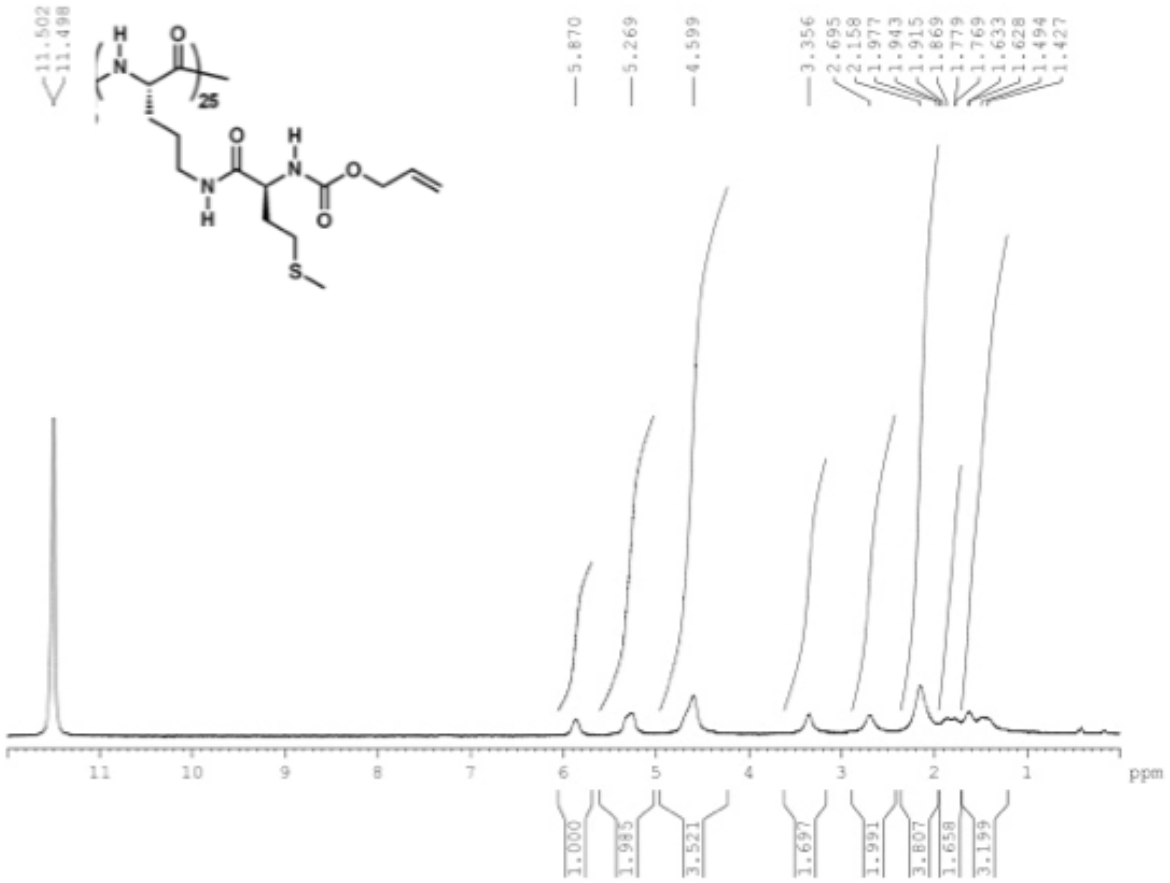
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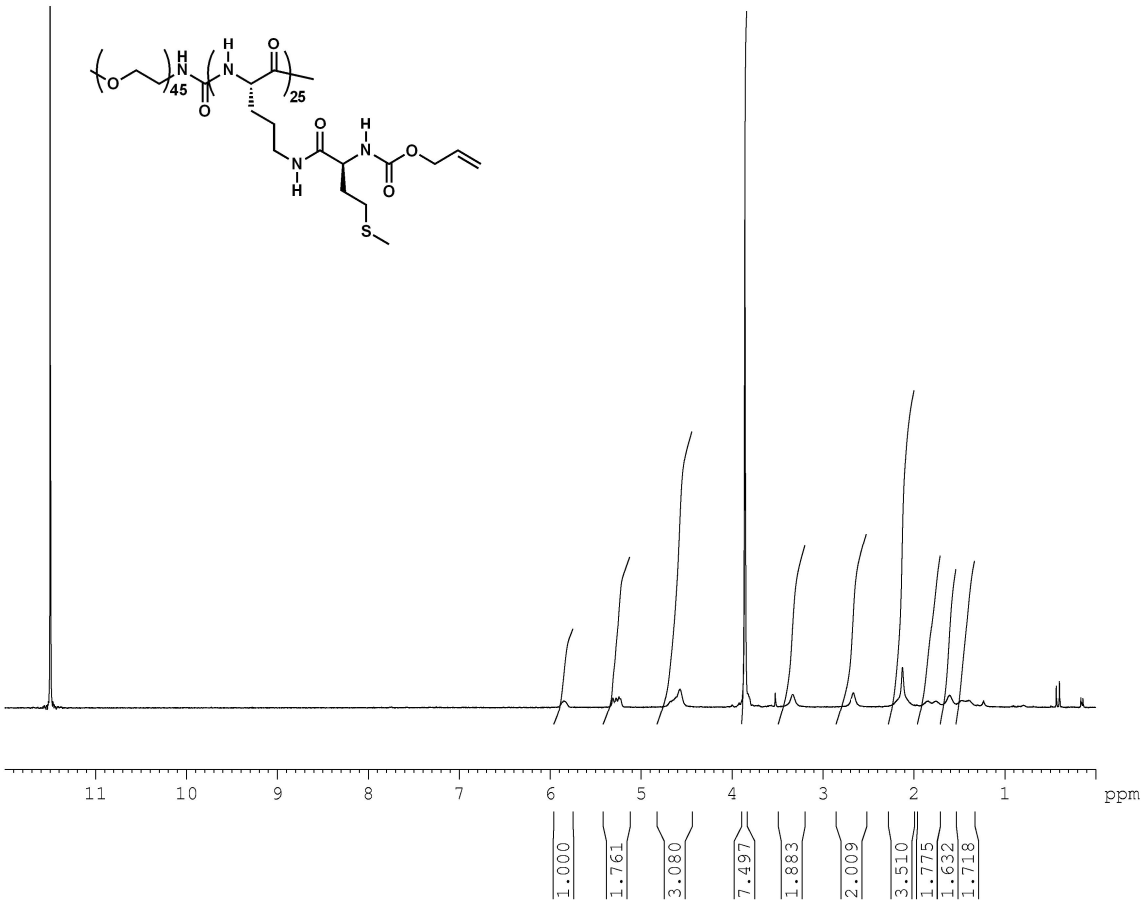
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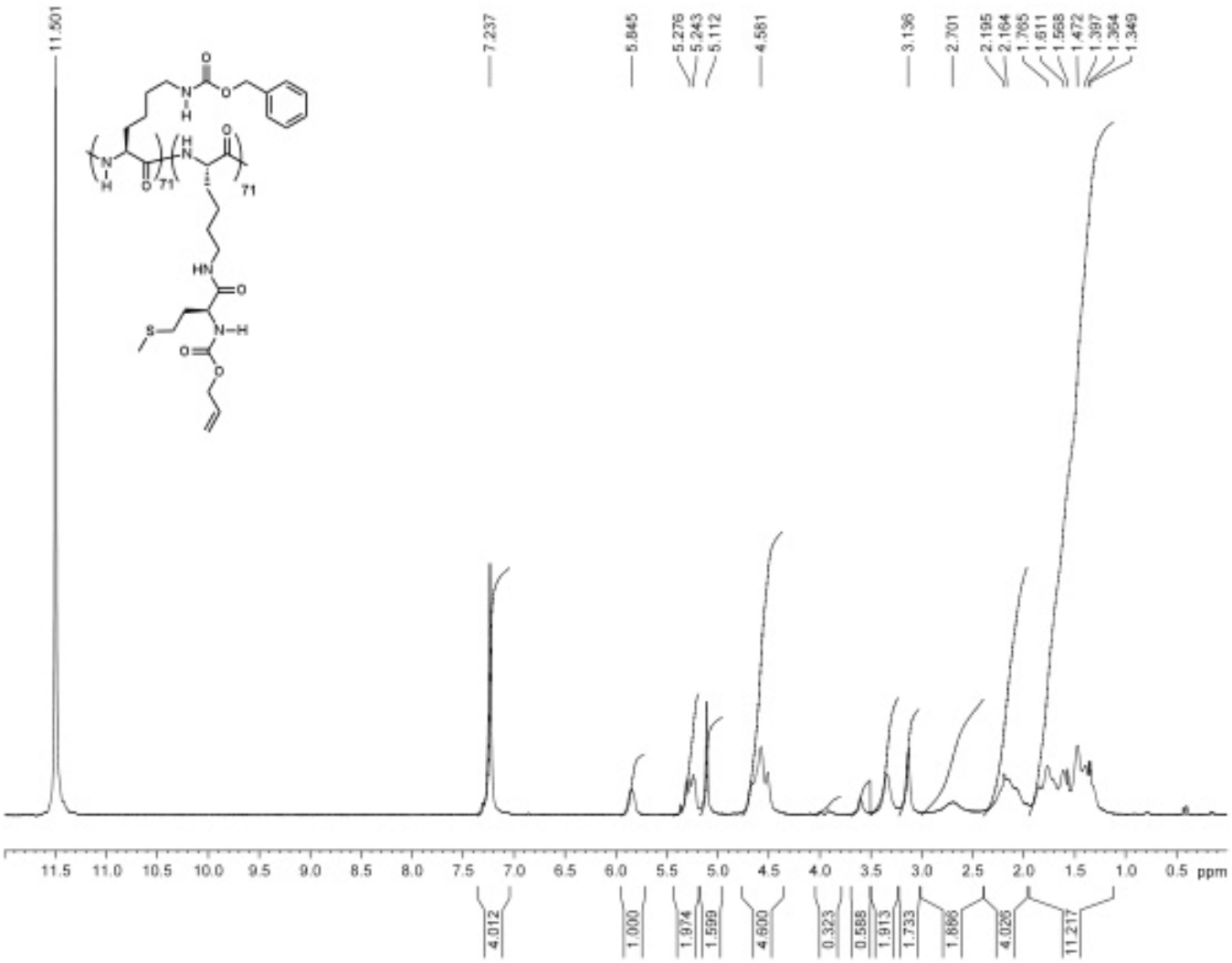
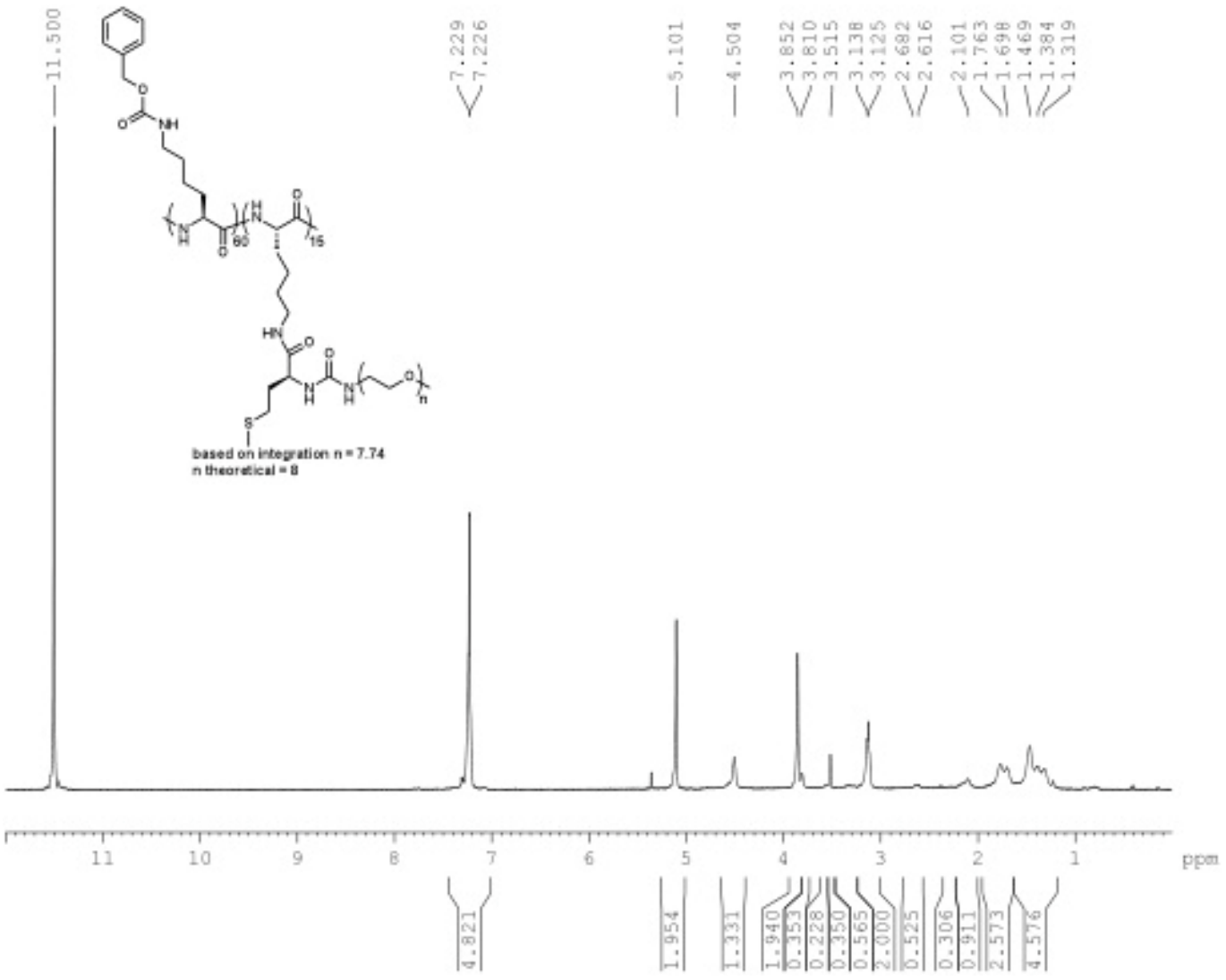
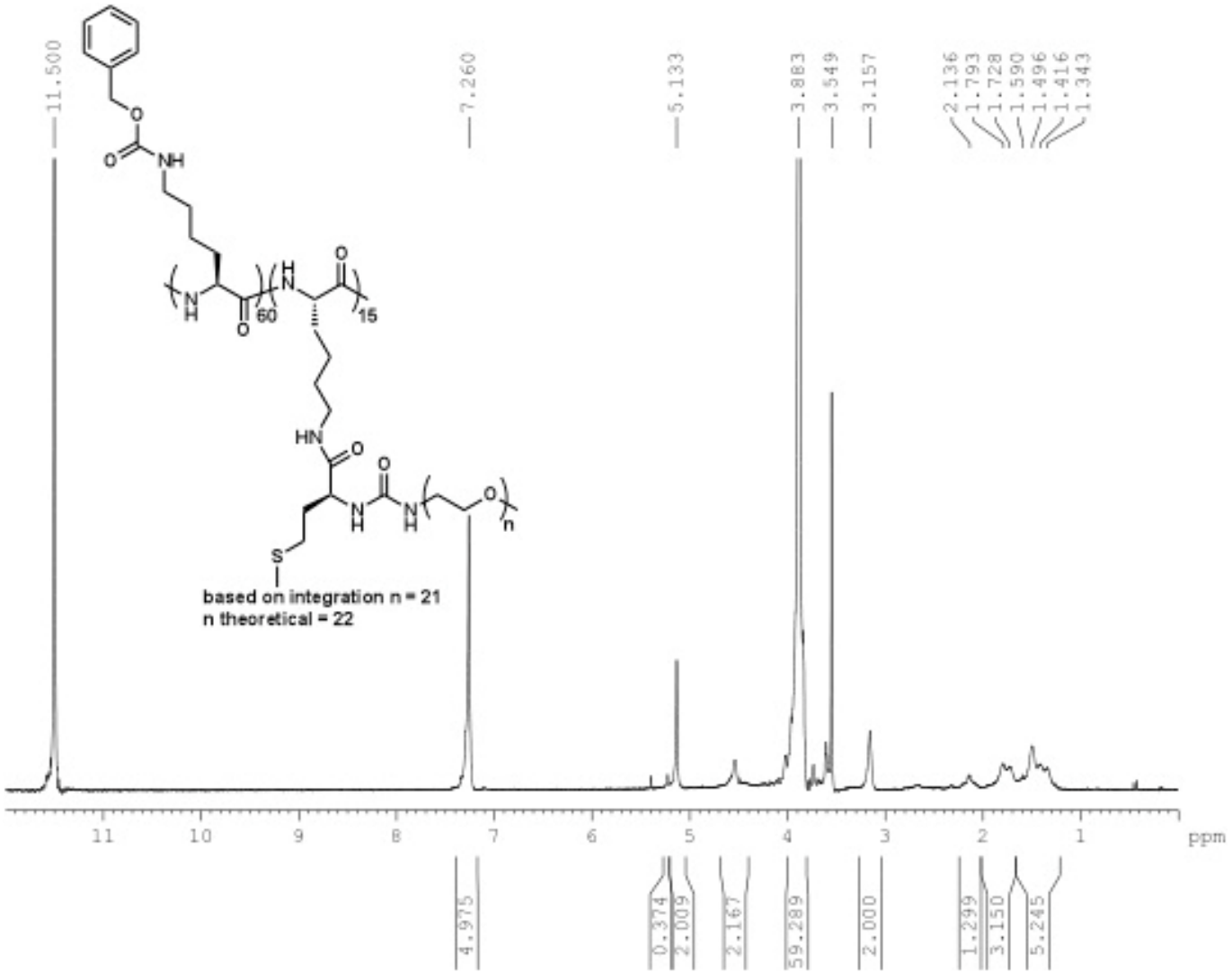
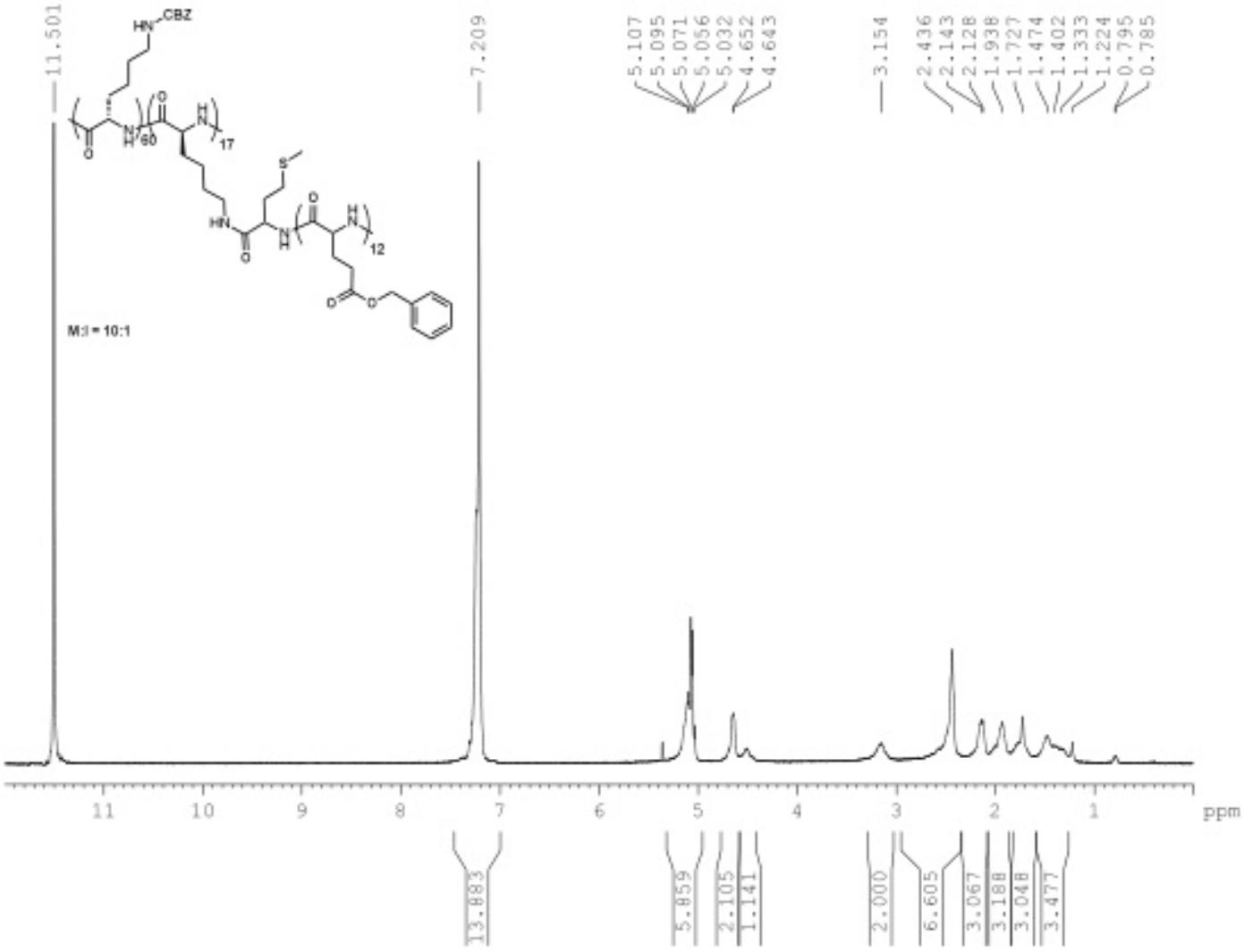
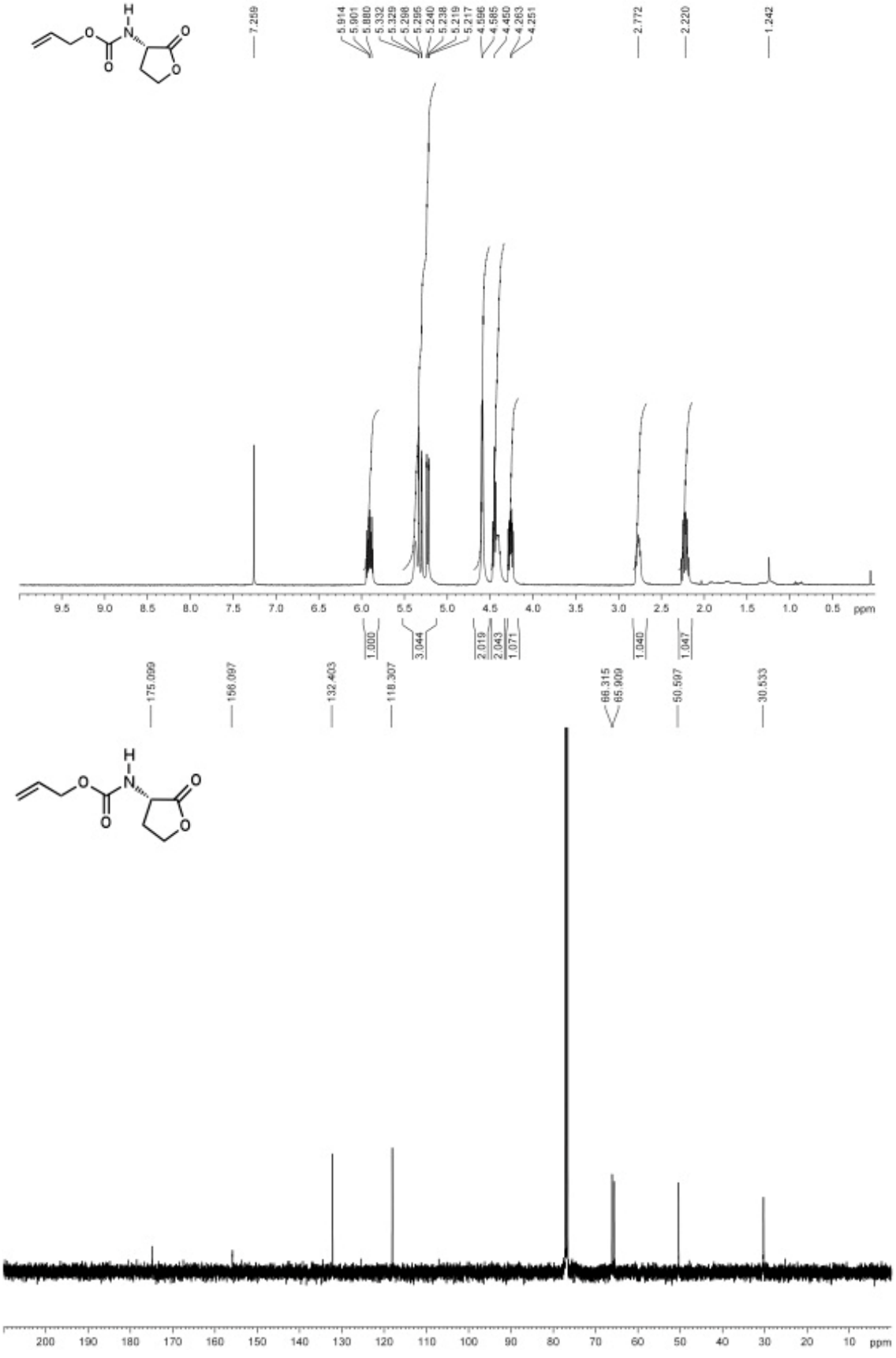
1.718


11.501
7.237
5.845
5.276
5.243
5.112
4.581
3.136
2.701
2.195
2.164
1.765
1.611
1.568
1.472
1.397
1.364
1.349
71
71
HN
N–H
S
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
4.012
1.000
1.974
1.599
4.600
0.323
0.588
1.913
1.733
1.866
4.026
11.217


11.500
7.229
7.226
5.101
4.504
3.852
3.810
3.515
3.138
3.125
2.682
2.616
2.101
1.763
1.698
1.469
1.384
1.319
based on integration n = 7.74
n theoretical = 8
ppm
4.821
1.954
1.331
1.940
0.353
0.228
0.350
0.565
2.000
0.525
0.306
0.911
2.573
4.576


11.500
7.260
5.133
3.883
3.549
3.157
2.136
1.793
1.728
1.590
1.496
1.416
1.343
based on integration n = 21
n theoretical = 22
ppm
4.975
0.374
2.009
2.167
59.289
2.000
1.299
3.150
5.245


11.501
7.209
5.107
5.095
5.071
5.056
5.032
4.652
4.643
3.154
2.436
2.143
2.128
1.938
1.727
1.474
1.402
1.333
1.224
0.795
0.785
HN
CBZ
M:I = 10:1
ppm
13.883
5.859
2.105
1.141
2.000
6.605
3.067
3.188
3.048
3.477


7.259
5.914
5.901
5.880
5.332
5.329
5.298
5.295
5.240
5.238
5.219
5.217
4.596
4.585
4.450
4.263
4.251
2.772
2.220
1.242
ppm
1.000
3.044
2.019
2.043
1.071
1.040
1.047
175.099
156.097
132.403
118.307
66.315
65.909
50.597
30.533
ppm