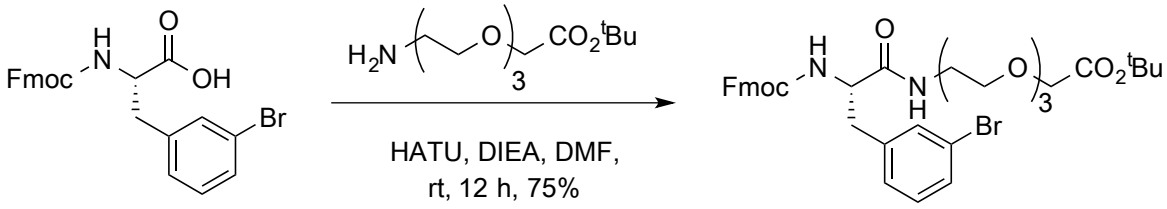
doi:10.1038/nature23652

Supplementary Information

Materials and general methods for synthesis of Cmpd-15PA: All chemicals and solvents were purchased from Sigma-Aldrich, Santa Cruz Biotechnology, Acros Organics, Enamine, Alfa Aesar, or Cambridge Isotope Laboratories, and were used without further purification. Pre-coated silica gel 60 F254 aluminum plates were used for analytical thin layer chromatography (TLC). Course of reactions were followed by visualization under UV (254 nm or 366 nm) and/or using standard staining procedures such as $KMnO_4$. Flash column chromatography (FCC) was performed on Merck silica gel 60 (SiO_2; 230-400 mesh) as a stationary phase under positive pressure of dry liquid nitrogen. Structural characterization of compounds was performed by nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C) and mass spectrometry (MS). ^{1}H NMR and ^{13}C NMR (FT-NMR Bruker Avance Ultra Shield Spectrometer at 400.13 [or 300] and 100.62 [or 75] MHz, respectively) were used to characterize synthesized compounds. Chemicals shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Coupling constants (*J*) values are in Hz, and the splitting patterns are described as follows: singlet (s); doublet (d); triplet (t); quartet (q); multiplet (m). High-resolution mass spectra (HRMS ESI-MS) analyses were conducted on a Waters LCT Premier XE time-of –flight (TOF) using electrospray ionization.

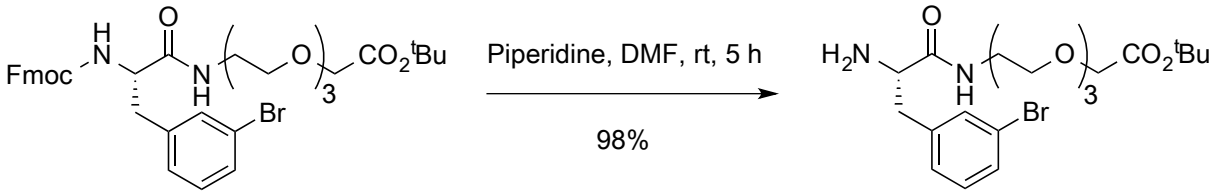
Synthetic route:



1 **2**

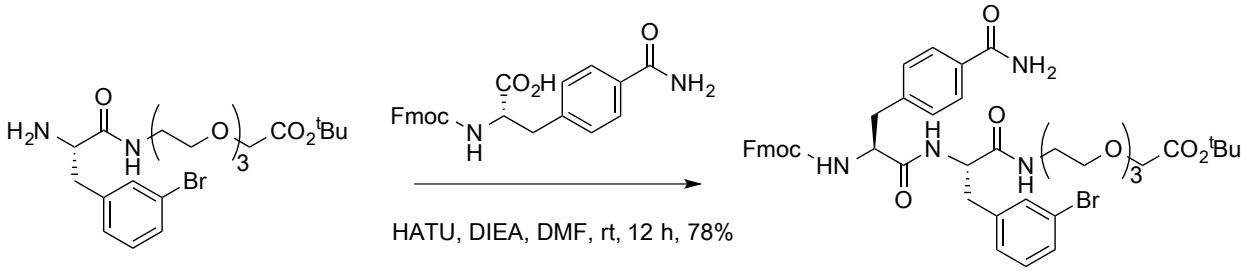
Preparation of *tert*-butyl (*S*)-5-(3-bromobenzyl)-1-(9*H*-fluoren-9-yl)-3,6-dioxo-2,10,13,16-tetraoxa-4,7-diazaoctadecan-18-oate (2): To a stirred solution of Fmoc-3-bromo-L-phenylalanine (**1**, 930 mg, 2 mmol) in DMF (23 mL), HATU (888 mg, 2.3 mmol) and DIEA (336 mg, 2.6 mmol) were added, and the mixture was allowed to stir for at room temperature for 30 min under an argon atmosphere. *tert*-Butyl 2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)acetate (527 mg, 2 mmol) in 4 mL DMF was added, and the

mixture was stirred for 12 h at room temperature. The mixture was then concentrated, diluted with dichloromethane (DCM) (50 mL) and washed with distilled water and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (silica gel, hexane/EtOAc 1:1) gave **2** as white solid (1.09 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 9H), 2.84-3.00 (m, 3H), 3.37 (s, 2H), 3.50-3.53 (m, 5H), 3.58-3.63 (m, 4H), 3.93 (s, 2H), 4.14-4.16 (m, 1H), 4.35-4.38 (m,1H), 4.40-4.41 (m, 2H), 7.09-7.11 (m, 2H), 7.21-7.38 (m, 8H), 7.49-7.53 (m, 2H), 7.70-7.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ28.2, 38.6, 39.5, 47.2, 56.1, 67.0, 69.0, 69.6, 70.2, 70.5, 70.5, 70.7, 81.9, 120.0, 122.6, 125.1, 127.2, 127.8, 128.1, 130.1, 132.5, 139.2, 141.3, 143.8, 143.9, 155.8, 169.8, 170.6; HRMS (TOF-ESI): Calcd for $C_{36}H_{43}BrN_2NaO_8$ $[M+Na]^+$ 733.2100, 735.2080; observed: 733.2099, 735.2086.



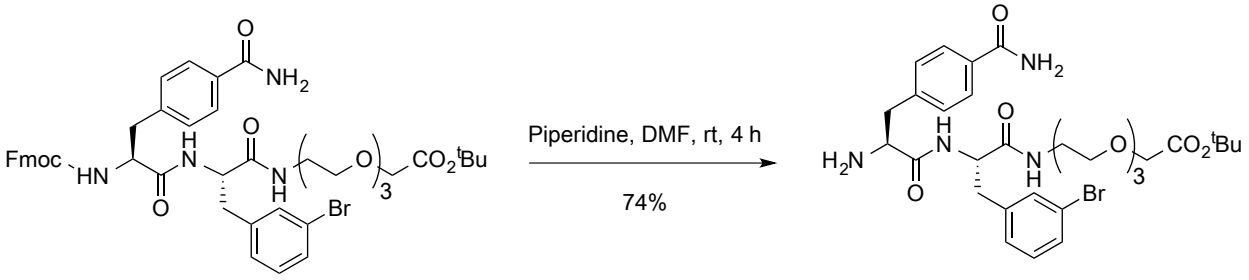
2 **3**

Preparation of *tert*-butyl (*S*)-14-amino-15-(3-bromophenyl)-13-oxo-3,6,9-trioxa- 12-azapentadecanoate (3): To a stirred solution of **2** (*tert*-butyl (S)-5-(3-bromo- benzyl)-1-(9H-fluoren-9-yl)-3,6-dioxo-2,10,13,16-tetraoxa-4,7-diazaoctadecan-18-oate; 1.09 g, 1.54 mmol) in DMF (12 mL) was added piperidine (3 mL) at room temperature. The reaction mixture was stirred at ambient temperature and under nitrogen atmosphere for 5 h or until the starting material completely disappeared, as checked by TLC. After removal of solvent by a rotary evaporator the residue was purified by flash column chromatography (eluting with DCM/MeOH=30:1) to afford light yellow liquid (737 mg, 98% yield). ^{1}H NMR (300 MHz, MeOH-d_4): δ1.49 (s, 9H), 2.83-3.02 (m, 2H), 3.29-3.71 (m, 13H), 4.04 (s, 2H), 7.23-7.27 (m, 2H), 7.39-7.43 (m, 2H); ^{13}C NMR (75 MHz, MeOH-d4): δ 28.4, 40.2, 41.6, 57.4, 69.7, 70.5, 71.2, 71.4, 71.6, 82.8, 123.4, 129.4, 131.0, 131.4, 133.4, 141.3, 171.6, 175.6; HRMS (TOF-ESI): Calcd for $C_{21}H_{34}BrN_2O_6$ $[M+1]^+$ 489.1595，491.1574; observed: 489.1595, 491.1577.



3 4

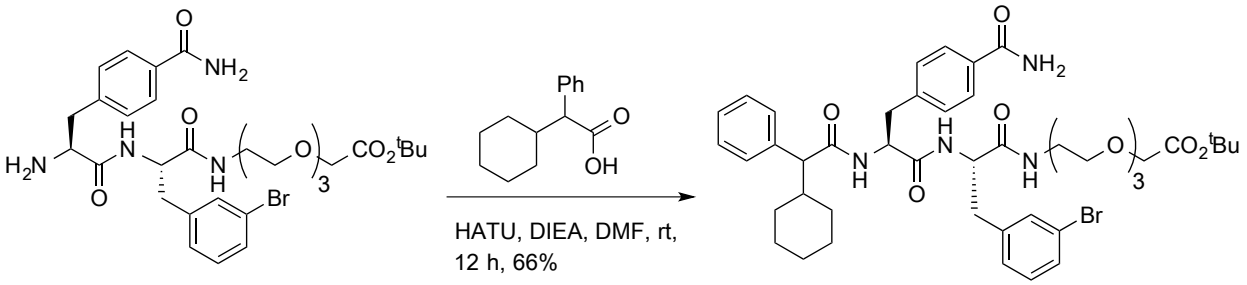
Preparation of *tert*-butyl (5*S*,8*S*)-8-(3-bromobenzyl)-5-(4-carbamoylbenzyl)-1- (9*H*-fluoren-9-yl)-3,6,9-trioxo-2,13,16,19-tetraoxa-4,7,10-triazahenicosan-21-oate (4): To a stirred solution of Fmoc-L-4-carbamoylphenylalanine (228 mg, 0.53 mmol) in DMF (8 mL), HATU (262 mg, 0.69 mmol) and DIEA (89 mg, 0.69 mmol) were added and the mixture was allowed to stir for 30 min under an argon atmosphere. *tert*-Butyl (S)-14-amino-15-(3-bromophenyl)-13-oxo-3,6,9-trioxa-12-azapentadecanoate (**3**) (259 mg, 0.53 mmol) in DMF (4 mL) was added, and the mixture was stirred for 12 h at room temperature. The mixture was then concentrated, diluted with DCM (40 mL) and washed with distilled water and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (silica gel, DCM/MeOH=30:1) gave **4** (370 mg, 78%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ1.08 (s, 9 H), 2.60-3.10 (m, 9 H), 3.15-3.58 (m, 7 H), 3.63 (s, 2 H), 3.81-4.22 (m, 6 H), 6.84-7.09 (m, 10 H), 7.45 (d, *J* = 7.6 Hz, 2 H), 7.54 (d, *J* = 7.6 Hz, 2 H), 7.62-7.88 (m, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ27.5, 37.0, 37.3, 46.3, 53.5, 55.7, 65.5, 67.9, 68.6, 69.4, 69.41, 69.5, 69.9, 80.6, 119.9, 121.2, 124.9, 126.9, 127.1, 128.2, 128.6, 129.1, 131.7, 134.4, 139.3, 140.0, 141.3, 143.3, 143.5, 150.9, 155.5, 167.9, 169.2, 170.4, 171.0; HRMS (TOF-ESI): Calcd for $C_{46}H_{53}BrNaN_4O_{10}$ $[M+Na]^+$ 923.2843，925.2822; observed: 923.2829, 925.2820.



4 5

Preparation of *tert*-butyl (14*S*,17*S*)-17-amino-14-(3-bromobenzyl)-18-(4-

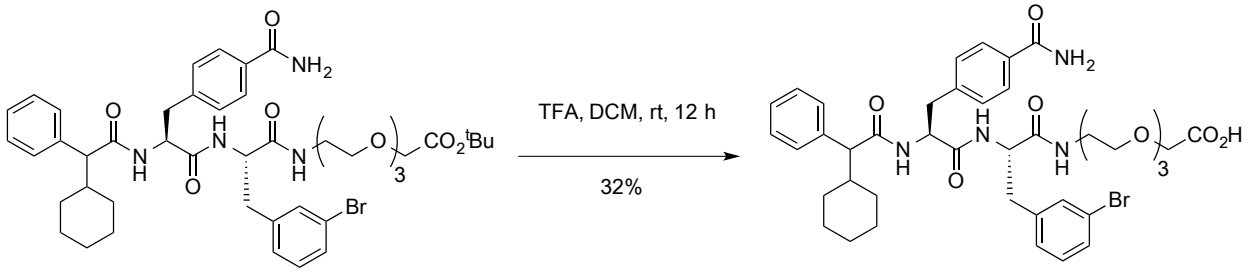
carbamoylphenyl)- 13,16-dioxo-3,6,9-trioxa-12,15-diazaoctadecanoate (5): To a stirred solution of **4** ((*tert*-butyl (5*S*,8*S*)-8-(3-bromobenzyl)-5-(4-carbamoylbenzyl)- 1-(9*H*-fluoren-9-yl)-3,6,9-trioxo-2,13,16,19-tetraoxa-4,7,10-triazahenicosan-21-oate**;** 1.06 g, 1.2 mmol) in DMF (10 mL) was added piperidine (2 mL) at room temperature. The reaction mixture was stirred at ambient temperature and under nitrogen atmosphere for 4 h or until the starting material completely disappeared, as checked by TLC. After removal of solvent by a rotary evaporator, the residue was purified by flash column chromatography (eluting with DCM/MeOH=30:1) to afford **5** (591 mg, 74% yield) as white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ1.47 (s, 9 H), 2.75-2.82 (m, 1 H), 2.86-3.07 (m, 4 H), 3.28-3.68 (m, 11 H), 4.01 (s, 2 H), 4.56-4.60 (m, 1H), 7.18-7.21 (m, 2 H), 7.28 (d, *J* = 7.5 Hz, 2 H), 7.36-7.41 (m, 2 H), 7.79 (d, *J* = 7.5 Hz, 2 H); ^{13}C NMR (75 MHz, MeOH-d_4): δ 28.4, 39.0, 40.4, 41.8, 55.5, 57.2, 69.7, 70.4, 71.3, 71.5, 71.5, 71.7, 82.8, 123.3, 129.0, 129.4, 130.0, 131.0, 131.3, 133.3, 133.5, 140.9, 143.1, 171.6, 172.0, 172.7, 176.0; HRMS (TOF-ESI): Calcd for $C_{31}H_{43}BrN_4NaO_8$ $[M+Na]^+$ 701.2162，703.2142; observed: 701.2156，703.2143.



5 **6**

Preparation of *tert*-butyl (4*S*,7*S*)-7-(3-bromobenzyl)-4-(4-carbamoylbenzyl)- 1-cyclohexyl-2,5,8-trioxo-1-phenyl-12,15,18-trioxa-3,6,9-triazaicosan-20-oate (6; Cmpd-15 PEG$_3$-CO$_2$Bu^t): To a stirred solution of 2-cyclohexyl-2-phenylacetic acid (191 mg, 0.87 mmol) in DMF (10 mL), HATU (430 mg, 1.13 mmol) and DIEA (146 mg, 1.13 mmol) were added and the mixture was allowed to stir for 30 min under an argon atmosphere. **5** [*tert*-butyl (14*S*,17*S*)-17-amino-14-(3-bromobenzyl)-18-(4-carbamoyl-phenyl)-13,16-dioxo-3,6,9-trioxa-12,15-diazaoctadecanoate] (591 mg, 0.87 mmol) in DMF (5 mL) was added and the mixture was stirred for 12 h at room temperature. The mixture was then concentrated, diluted with DCM (20 mL) and washed with distilled

water and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (silica gel, DCM/MeOH=50:1 to 30:1) gave **6** (506 mg, 66% yield) as white solid. ^{1}H NMR (400 MHz, MeOH-d_4): δ 1.09-1.30 (m, 8 H), 1.44 (s, 9 H), 1.44-1.99 (m, 8 H), 2.78-3.09 (m, 5 H), 3.18-3.38 (m, 3 H), 3.36-3.63 (m, 7 H), 3.98 (s, 2 H), 4.38-4.55 (m, 2 H), 6.94-6.99 (m, 2 H), 7.15-7.50 (m, 7 H), 7.50 (d, *J* = 7.5 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2 H); ^{13}C NMR (100 MHz, MeOH-d_4): δ 26.9, 27.2, 27.5, 28.4, 31.7, 32.6, 33.0, 38.6，39.0, 40.4, 55.2, 55.7, 60.6, 70.3, 71.3, 82.8, 122.1, 123.2, 128.0, 128.6, 129.3, 130.2, 130.9，131.2, 132.9, 133.3, 140.6, 142.3, 143.0，152.0, 171.6, 171.7, 172.0, 172.5，175.8; HRMS (TOF-ESI): Calcd for $C_{45}H_{59}BrN_4NaO_9$ $[M+Na]^+$ 901.3363，903.3343; observed: 901.3345，903.3337.



6 Compound 15-PEG$_3$-CO$_2$H (Cmpd-15PA)

Preparation of ((4*S*,7*S*)-7-(3-bromobenzyl)-4-(4-carbamoylbenzyl)-1-cyclohexyl-2,5,8-trioxo-1-phenyl-12,15,18-trioxa-3,6,9-triazaicosan-20-oic acid (Compound 15-PEG$_3$-CO$_2$H; Cmpd-15PA). To a solution of **6** [*tert*-butyl (4*S*,7*S*)-7-(3- bromobenzyl)-4-(4-carbamoylbenzyl)-1-cyclohexyl-2,5,8-trioxo-1-phenyl-12,15,18- trioxa-3,6,9-triazaicosan-20-oate, 15-PEG$_3$-CO$_2^t$Bu] (470 mg, 0.53 mmol) in DCM (2 mL) at 0°C was added trifluoroacetic acid (TFA, 3 mL). The reaction mixture was stirred at room temperature for 12 h, after which time the solvents were removed by rotary evaporator. The crude material was diluted with EtOAc (30 mL) and filtered, and the resulting solid (330 mg) was further purified by recrystallization from DCM/MeOH (18 mL, 5:1 v/v) to give the target compound (142 mg, 32% yield) as white solid. ^{1}H NMR (400 MHz, MeOH-d_4): δ0.65-1.23 (m, 8 H), 1.43-1.98 (m, 7 H), 2.70-3.10 (m，5 H)，3.12-3.63 (m, 12H), 4.10 (s, 2H), 4.40-4.87 (m, 2H), 6.94-6.99 (m, 2 H), 7.12-7.52 (m, 7 H), 7.50 (d, *J* = 7.5 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 2 H); ^{13}C NMR (100 MHz, MeOH-d_4): δ26.9，27.1，27.5，30.0，31.7, 32.6， 38.6, 39.0，40.3，40.4，41.5，48.1, 55.0，55.7，58.6，

68.7，70.1，71.7，123.1，127.9，128.3，128.9，130.0，130.9，131.2，132.8，133.5，139.8，140.5，140.7，141.6，143.0，171.9，172.9，173.9，175.8，175.9; HRMS (TOF-ESI): Calcd for $C_{41}H_{50}BrN_4O_9$ $[M-H]^+$ 821.2761，823.2741; observed: 821.2764，823.2754.

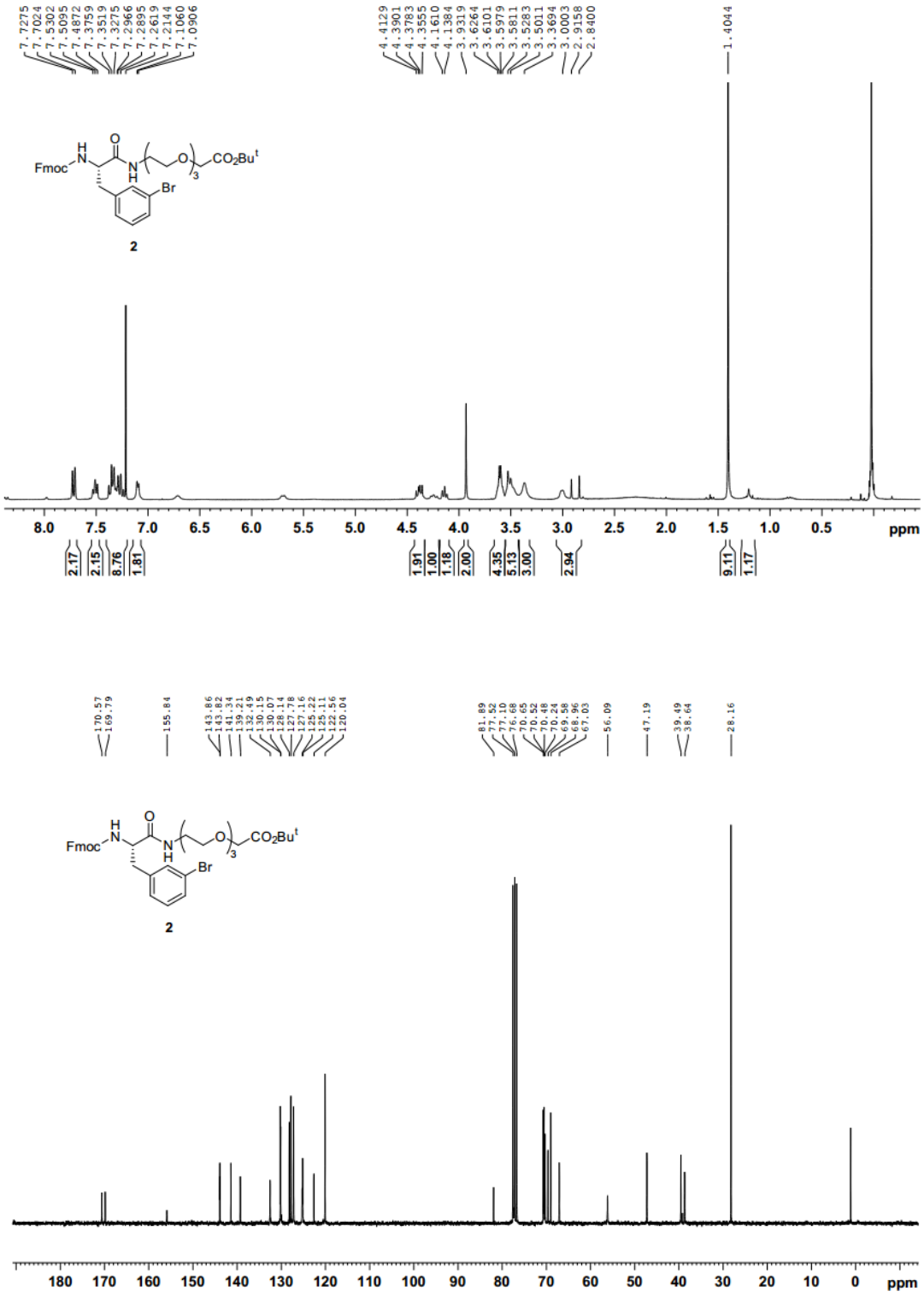


Figure S1: ^{1}H NMR and ^{13}C NMR spectra for compound **2**

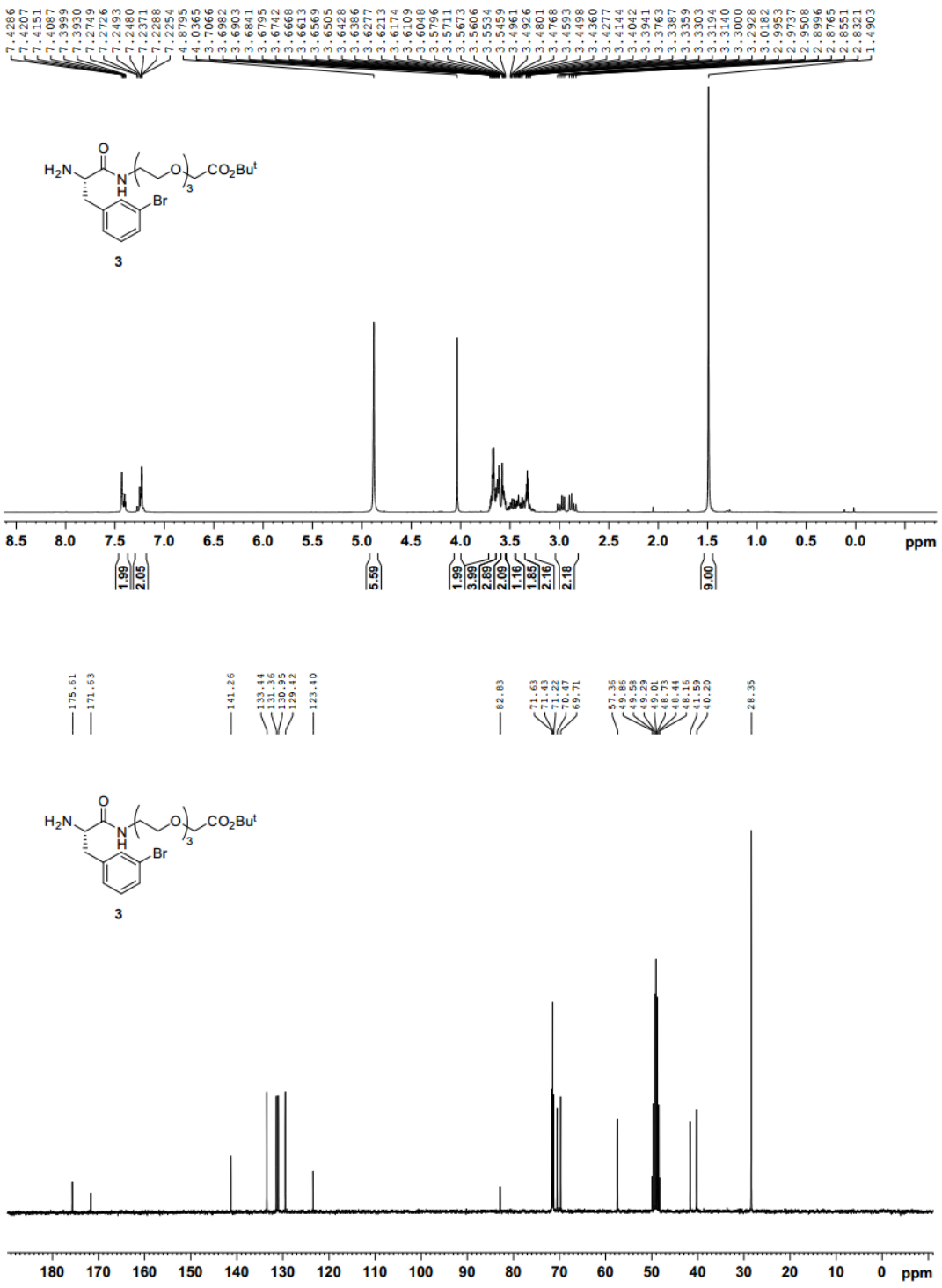


Figure S2: ^{1}H NMR and ^{13}C NMR spectra for compound **3**

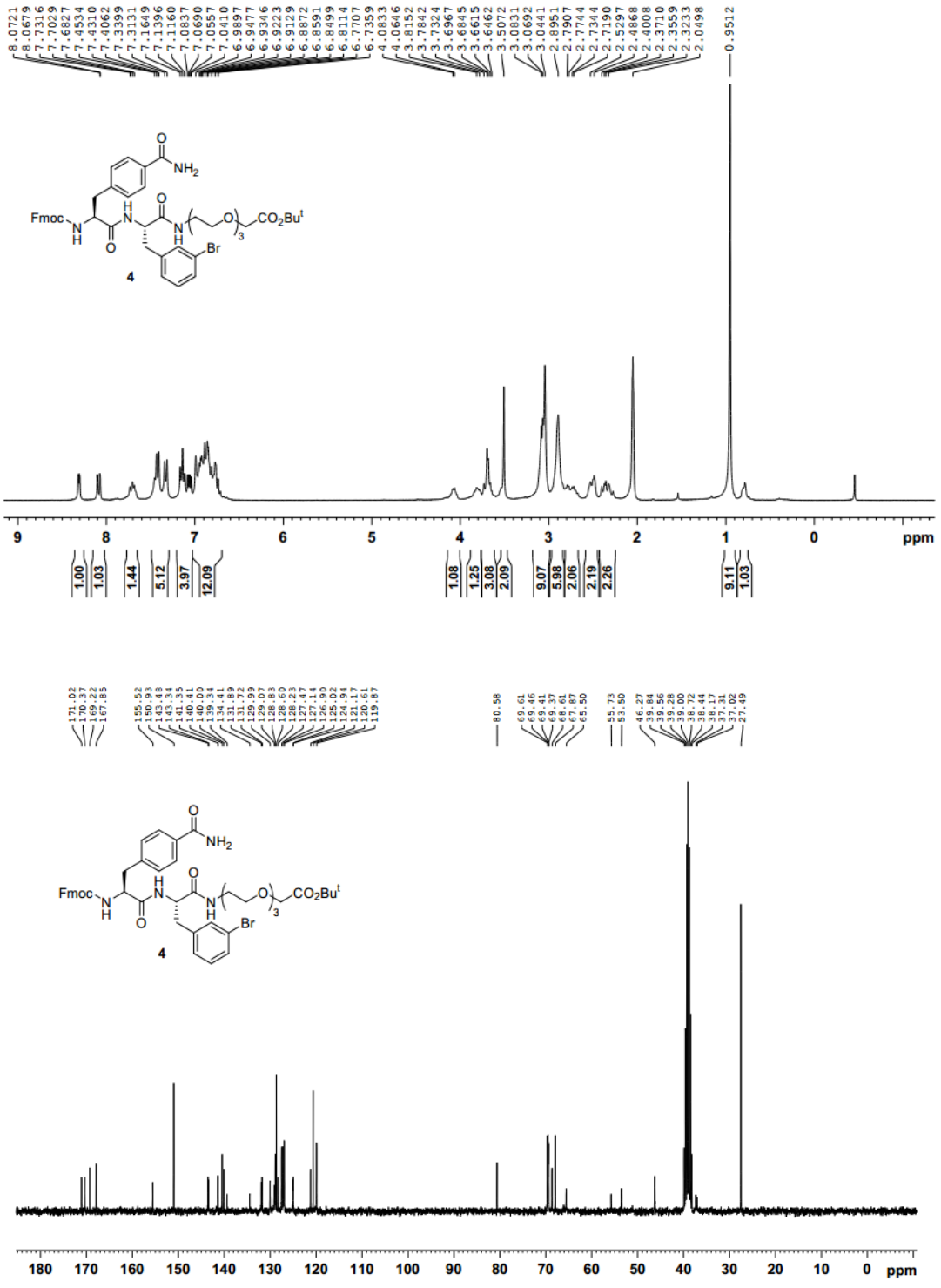


Figure S3: ^{1}H NMR and ^{13}C NMR spectra for compound **4**

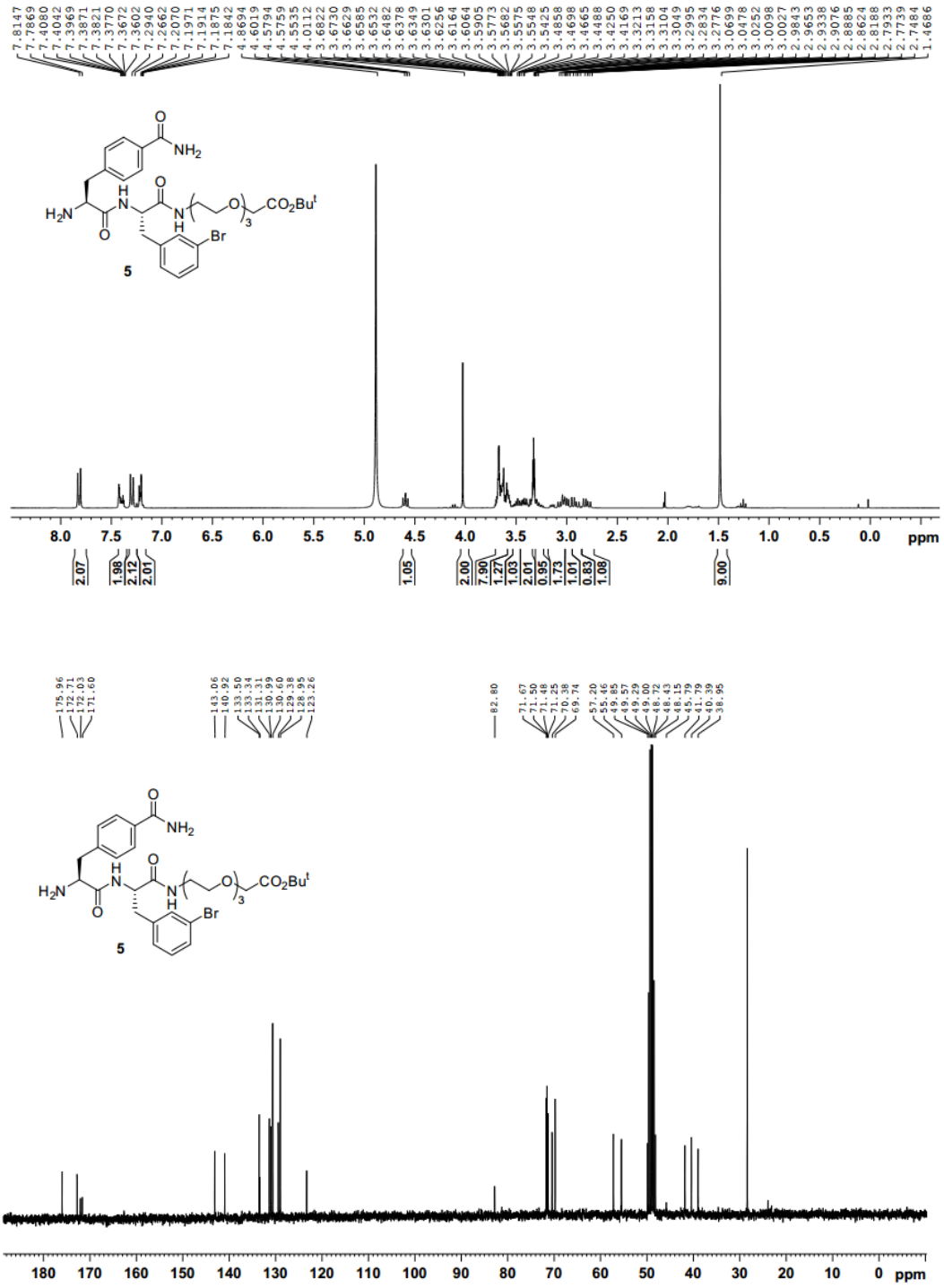


Figure S4: ^{1}H NMR and ^{13}C NMR spectra for compound **5**

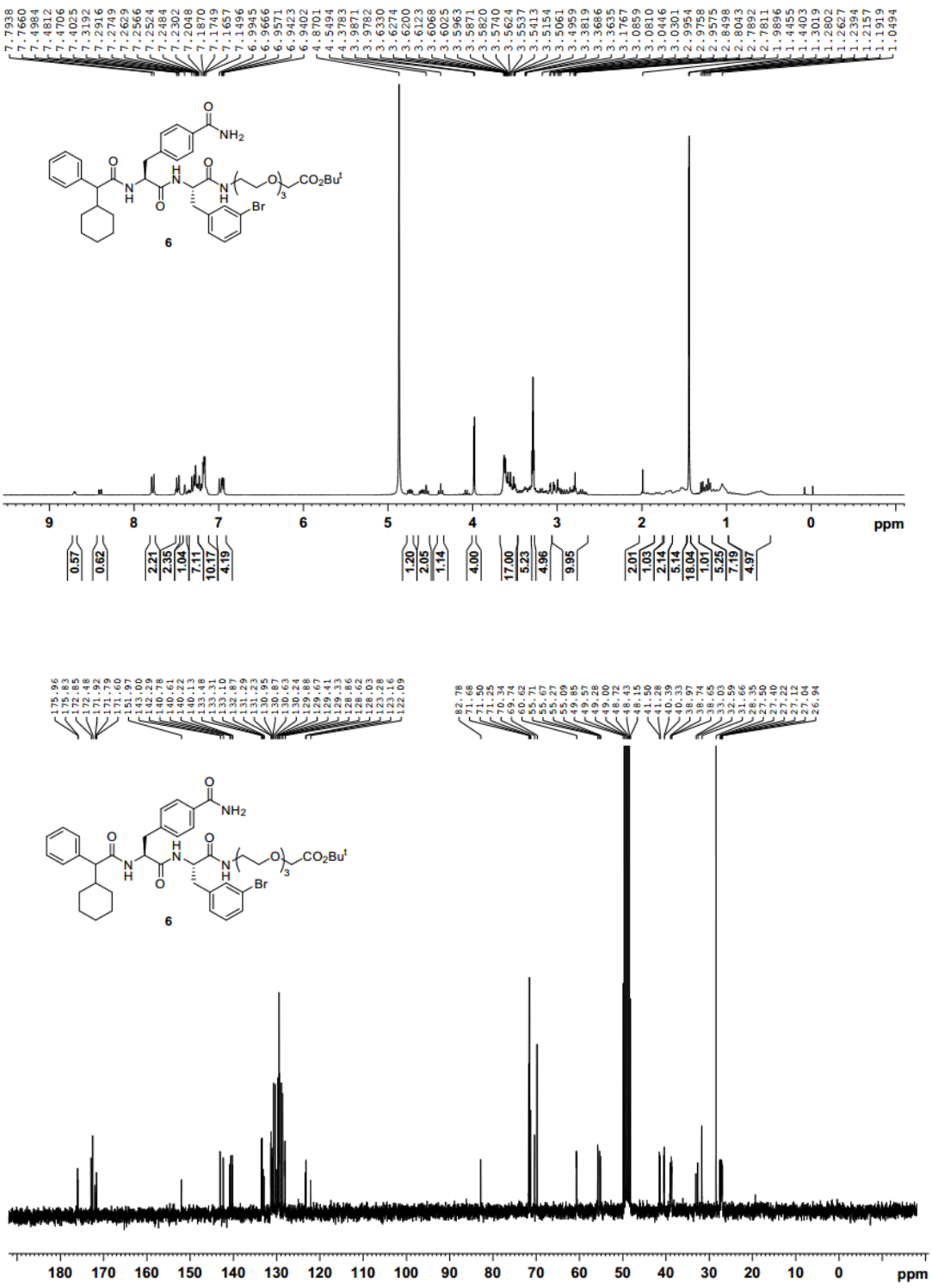


Figure S5: ^{1}H NMR and ^{13}C NMR spectra for compound **6**

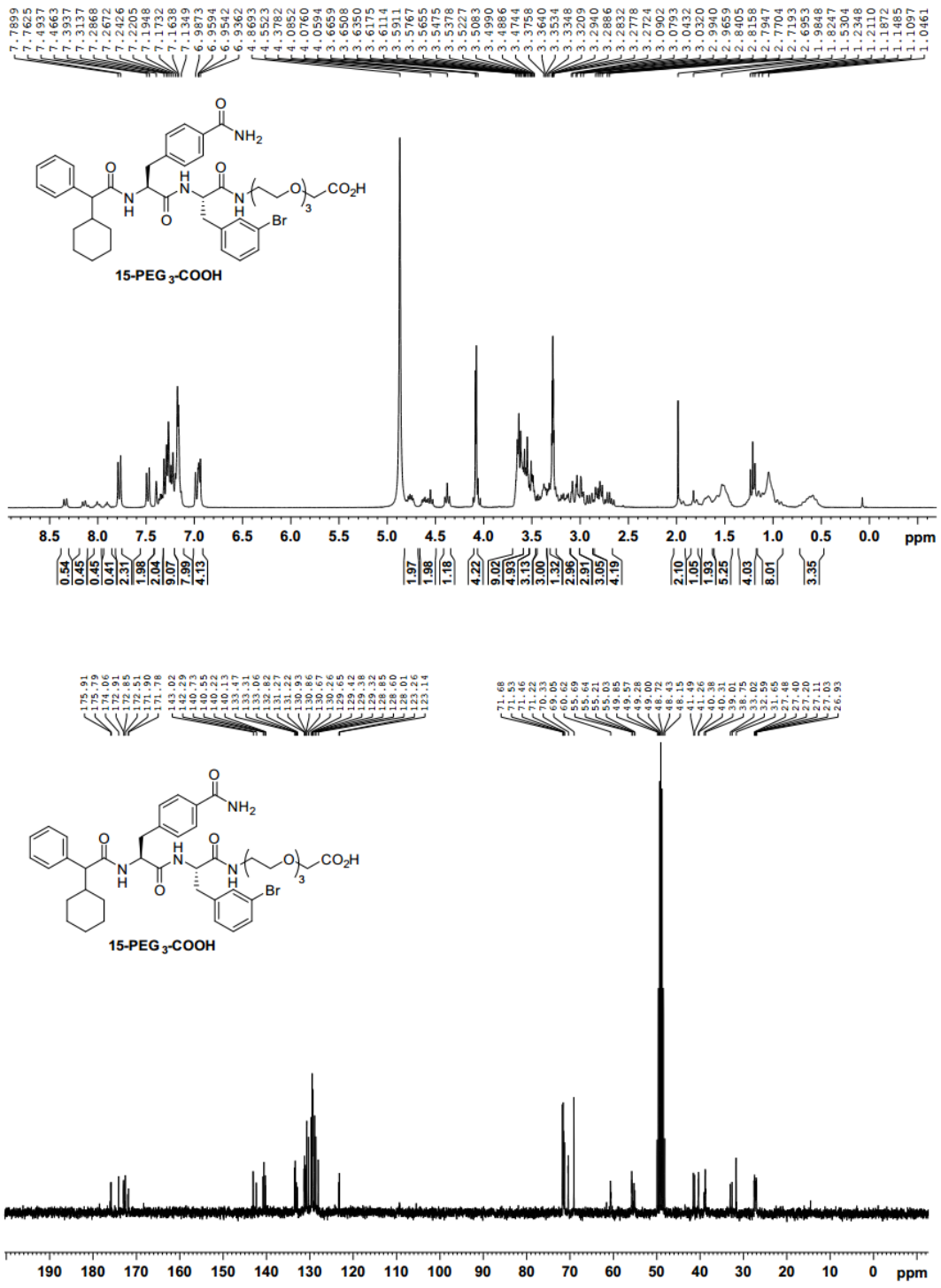


Figure S6: ^{1}H NMR and ^{13}C NMR spectra of compound 15-PEG_3-COOH (**15PA**)