Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for: Tuning Conformation and Properties of Peptidomimetic Backbones through Dual N/C_α-Substitution

Revital Kaminker, ‡[a]* Ilia Kaminker, ‡[b] Will R. Gutekunst,[a] Yingdong Luo,[a] Sang-Ho Lee,[a] Jia Niu,[a] Songi Han,[b]* and Craig J. Hawker[ab]*

[a]Materials Research Laboratory, University of California, Santa Barbara, California 93106, United States
[b]Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, United States

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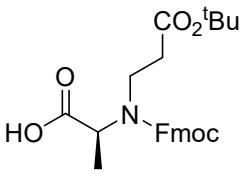
Monomers Synthesis

General Comments. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as the visualizing agent and an acidic mixture of anisaldehyde, ceric ammonium molybdate, or basic aqueous potassium permangante (KMnO4), and heat as developing agents. E. Merck silica gel (60, particle size 0.043–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker Avance 500 MHz and Varian VNMRS 600 MHz instruments and calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$ @ 7.26 ppm ^{1}H NMR, 77.16 ppm ^{13}C NMR; DMSO @ 2.50 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). The following abbreviations (or combinations thereof) were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra (MS) were recorded on a quadrupole/time-of-flight tandem mass spectrometer (ESI), Waters GCT Premier high resolution time-of-flight mass spectrometer (EI, FD) or matrix assisted laser desorption/ionization (MALDI) using a 2,5 dihydroxy benzoic acid (DHB) matrix. Chiral High Performance Liquid Chromatography (HPLC) was carried out on Shimadzu HPLC fitted with a Chiralpak OD column (4.6 mm x 250mm) using hexane/isopropanol (v/v = 9/1) as eluents.

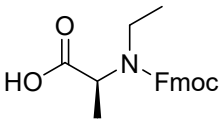


Fmoc-*N*($EtCO_2{}^tBu$)-L-Ala-OH. Alanine (2.00 g, 22.5 mmol) was dissolved in 23 mL of water and sodium hydroxide was added (560 mg, 14.0 mmol, 0.625 eq). *Tert*-butyl acrylate (4.9 mL, 33.8 mmol, 1.5 eq) was added to the aqueous solution and the reaction mixture was stirred vigorously for 20 hours at room temperature. After separation of the excess *tert*-butyl acrylate from the aqueous layer using a separatory funnel, sodium carbonate (2.4 grams, 22.5 mmol) was added to the aqueous layer. A solution of Fmoc-Cl (4.35 g, 16.8 mmol, 0.75 eq) in dioxane (23 mL) was added dropwise and the reaction mixture was vigorous stirred for 1 hour. The reaction mixture was then

diluted with ethyl acetate (50 mL), acidified with 1N aq. HCl (75 mL), partitioned and separated. After additional extraction with ethyl acetate (50 mL), the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by column chromatography (Full removal of Fmoc-Ala byproduct required 3 columns; 2-10% acetone in DCM, 1-5% MeOH in DCM and 2-10% acetone in DCM) to give **Fmoc-*N*(EtCO$_2$^{t}Bu)-L-Ala-OH** (1.25 g, 22% yield) as a white crispy foam.

MS (*m/z*): calcd for $C_{25}H_{29}NO_6$, $[M+]^+$, 439.1995; found, 439.20; **^{1}H NMR (600 MHz, $CDCl_3$)**: 2:1 mixture of rotamers: δ 10.19 (br s, 1H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.5 Hz, 2H), 7.40 – 7.32 (m, 2H), 7.32 – 7.25 (m, 2H), 4.60 – 4.10 (m, 4H), 3.67 – 3.21 (m, 2H), 2.56 (ddt, *J* = 52.0, 15.9, 7.0 Hz, 0.7H (minor)), 2.31 (dtd, *J* = 31.0, 16.2, 6.6 Hz, 1.3H (major)), 1.43 (s, 12H).

^{13}C NMR ($CDCl_3$, 151 MHz): (both observed rotamers) δ 176.93, 171.17, 156.07, 155.75, 143.93, 141.49, 127.83, 127.24, 124.97, 120.09, 81.06, 67.56, 56.43, 55.94, 47.44, 43.39, 42.62, 35.39, 34.97, 28.22, 28.18, 15.68, 15.16.

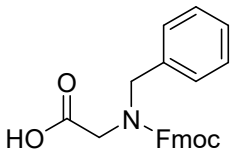


Fmoc-*N*(Et)-L-Ala-OH. In a 200 ml round bottom flask, finely ground (L)-alanine (1.78 g, 20 mmol) was dissolved in 50 ml of methanol under argon, followed by the addition of finely ground sodium hydroxide (0.88 g, 22 mmol). The mixture was sonicated at room temperature to a homogenous solution. The flask was transferred into an ice bath and acetaldehyde (1.76 g, 40 mmol) was added into the solution dropwise. The resulted solution was stirred at room temperature for 30 min. Then the solution was transferred into the ice bath again and NaBH4 (1.85 g, 50 mmol) was added portion wise. The mixture was warmed to room temperature and stirred for 1 hour. The mixture was acidified to isoelectric point with concentrated HCl (12 M) and then concentrated in vacuum. The white powder is triturated with acetone, filtered, dried and used directly in next step.

In a 500 ml round bottom flask, the white powder and sodium carbonate (10.6 g, 100 mmol) were dissolved in a mixture of 150 ml of H_2O and 75 ml of dioxane. A solution of Fmoc-Cl (6.4 g, 25 mmol) in 75 ml of dioxane was added dropwise into the flask at room temperature. The reaction was stirred for another 2 hours before it was acidified by HCl aqueous solution (3 M). The mixture

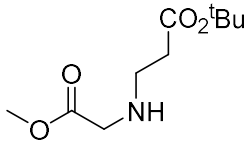
was extracted with 200 ml of dichloromethane for three times. The organic phases were combined, dried by $MgSO_4$ and concentrated by retovap. A flash chromatography (MeOH/dichloromethane = 1/20, R_f = 0.3) was performed to obtain 5.1 g product in 76% yield as white powder.

MS (*m/z*): calcd for $C_{20}H_{21}NO_4Na$, $[M+Na]^+$ 362.137,; found 362.136,; **^{1}H NMR (600 MHz, DMSO)**: 2:1 mixture of rotamers: δ 12.64 (b, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.71 – 7.65 (m, 2H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.40 – 7.32 (m, 2H), 4.49 – 4.22 (m, 4H), 3.39 – 3.29 (m, 0.33H), 3.22 – 3.11 (m, 1H), 3.08 – 2.97 (m, 0.67H), 1.40 – 1.27 (m, 3H), 1.14 – 1.03 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 2H). **^{13}C NMR (DMSO, 151 MHz)**: δ. 173.48, 155.42, 144.42, 144.34, 141.29, 141.15, 128.00, 127.49, 125.26, 120.49, 67.23, 66.83, 56.34, 55.14, 47.18, 41.19, 16.32, 15.64, 14.99, 14.65.



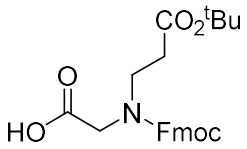
Fmoc-*N*(Bn)-Gly-OH. A mixture of N-Benzylglycine hydrochloride (10 g, 50.0 mmol, 1.00 equiv), dry dichloromethane (150 mL), and TMS-Cl (25.4 mL, 200 mmol, 4.00 equiv) were placed in 500 mL round-bottom flask. The mixture was refluxed for 2 h and then cooled in an ice bath. Diisopropylethylamine (15.1 mL, 86.5 mmol, 1.73 equiv) and Fmoc-Cl (8.67 g, 33.5 mmol, 0.67 equiv) were added to reaction mixture and stirred for 20 min at 0 ˚C. The mixture was warmed to room temperature and then stirred for 3 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in ethylacetate, followed by washing with 1 M HCl solution (two times). The organic layer was washed with brine and dried over Na_2SO_4 for several hours, and the filtered solution was evaporated. The product was purified by column chromatography with dichloromethane and methanol (9:1) and the obtained product was recrystallized with dichloromethane and hexane (9.70 g, 74.7 % yield).

MS (*m/z*): calcd for $C_{24}H_{21}NO_4Na$, $[M+Na]^+$, 410.14; found, 410.16; **^{1}H NMR (600 MHz, $CDCl_3$; rotamer ratio = 1 : 1.23)**: δ 11.03 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.65 – 7.47 (m, 2H), 7.47 – 7.16 (m, 8H), 7.10 (d, *J* = 6.8 Hz, 1H), 4.59 (d, *J* = 5.0 Hz, 3H), 4.52 (s, 1H), 4.28 (dd, *J* = 17.1, 10.5 Hz, 1H), 4.03 (s, 1H), 3.79 (s, 1H). **^{13}C NMR ($CDCl_3$, 101 MHz)**: δ 175.24, 175.15, 156.78, 156.43, 143.86, 143.82, 141.45, 136.47, 136.43, 128.92, 128.84, 128.28, 127.91, 127.88, 127.82, 127.81, 127.69, 127.22, 127.19, 125.02, 124.98, 124.92, 120.11, 120.08, 68.14, 67.88, 51.51, 51.30, 47.87, 47.42, 47.29, 46.96.



H*N*(EtCO$_2$^{t}Bu)-Gly-OMe. Glycine methyl ester hydrochloride (10.0 g, 79.6 mmol) was added to methanol (80 mL), followed by triethylamine (11.1 mL, 79.6 mmol, 1 eq) and *tert*-butyl acrylate (9.25 mL, 63.7 mmol, 0.8 eq). The reaction mixture was stirred at room temperature for 20 hours and concentrated *in vacuo*. The residue was partitioned between brine (100 mL) and ethyl acetate (100 mL), separated and the organic layer dried over Na_2SO_4. After concentration, the residue was purified by column chromatography (25% to 100% EtOAc in hexanes) to give the aza-Michael intermediate (**HN(EtCO$_2$^{t}Bu)-Gly-OMe**) (10.9 g, 79% yield) as a colorless oil.

MS (*m/z*): calcd for $C_{10}H_{19}NO_4$, $[M]^+$, 217.1314; found, 217.13; **^{1}H NMR (500 MHz, CDCl$_3$)**: (500 MHz, Chloroform-*d*) δ 3.67 (s, 3H), 3.37 (s, 2H), 2.79 (t, *J* = 6.6 Hz, 2H), 2.37 (t, *J* = 6.6 Hz, 2H), 1.85 (s, 1H), 1.40 (s, 9H). **^{13}C NMR (CDCl$_3$, 126 MHz)**: δ 172.73, 171.79, 80.61, 51.77, 50.79, 45.04, 36.05, 28.15.



Fmoc-*N*(EtCO$_2$^{t}Bu)-Gly-OH. Intermediate **H*N*(EtCO$_2$^{t}Bu)-Gly-OMe** (10.9 g, 50.3 mmol) was dissolved in methanol (65 mL) and 1.43 N aq. NaOH (35 mL, 50 mmol, 1 eq) was added. The reaction mixture was warmed to 40 °C and stirred for 14 hours. The reaction mixture was cooled to room temperature and the methanol was removed *in vacuo*. To this crude sodium carboxylate solution was added sodium bicarbonate (6.34 g, 75.5 mmol, 1.5 eq) followed by Fmoc-Cl (13.0 g, 50.3 mmol, 1 eq) dissolved in dioxane (125 mL). The reaction mixture was stirred vigorously for 3 hours at room temperature and then acidified with 1 N HCl (150 mL). The solution was extracted with EtOAc (100x 3), washed with brine and dried over Na_2SO_4. After concentration *in vacuo*, the residue was purified by column chromatography (DCM to 7% MeOH in DCM) to give **Fmoc-*N*(EtCO$_2$^{t}Bu)-Gly-OH** (10.1 g, 47% yield).

MS (*m/z*): calcd for $C_{24}H_{27}NO_6$, $[M]^+$, 425.1838; found, 425.18; **^{1}H NMR (500 MHz, CDCl$_3$)**: ^{1}H NMR (500 MHz, Chloroform-*d*) 1:1 mixture of rotamers. Pairs of rotamer chemical shifts listed in brackets. δ 9.92 (br s, 1H), [7.75 (d, *J* = 7.5 Hz), 7.71 (d, *J* = 7.6 Hz,) 2H], [7.58 (d, *J* = 7.4 Hz), 7.52 (d, *J* = 7.5 Hz) 2H], 7.42 – 7.24 (m, 4H), [4.52 (d, *J* = 6.2 Hz), 4.42 (d, *J* = 6.5 Hz) 2H], [4.26 (t, *J* = 6.2 Hz), 4.18 (t, *J* = 6.5 Hz) 1H], [4.11 (s),

4.04 (s) 2H], [3.57 (t, *J* = 6.5 Hz), 3.44 (t, *J* = 6.8 Hz) 2H], [2.57 (t, *J* = 6.5 Hz), 2.31 (t, *J* = 6.7 Hz) 2H], [1.44 (s), 1.43 (s) 9H]. **^{13}C NMR ($CDCl_3$, 151 MHz)**: (both observed rotamers) δ 175.12, 174.96, 171.83, 171.32, 156.51, 155.85, 143.90, 143.89, 141.49, 141.42, 127.87, 127.80, 127.27, 127.17, 124.96, 124.92, 120.11, 120.07, 81.15, 81.13, 67.84, 50.25, 49.95, 47.36, 47.28, 45.38, 44.67, 34.92, 34.72, 28.20, 28.16.

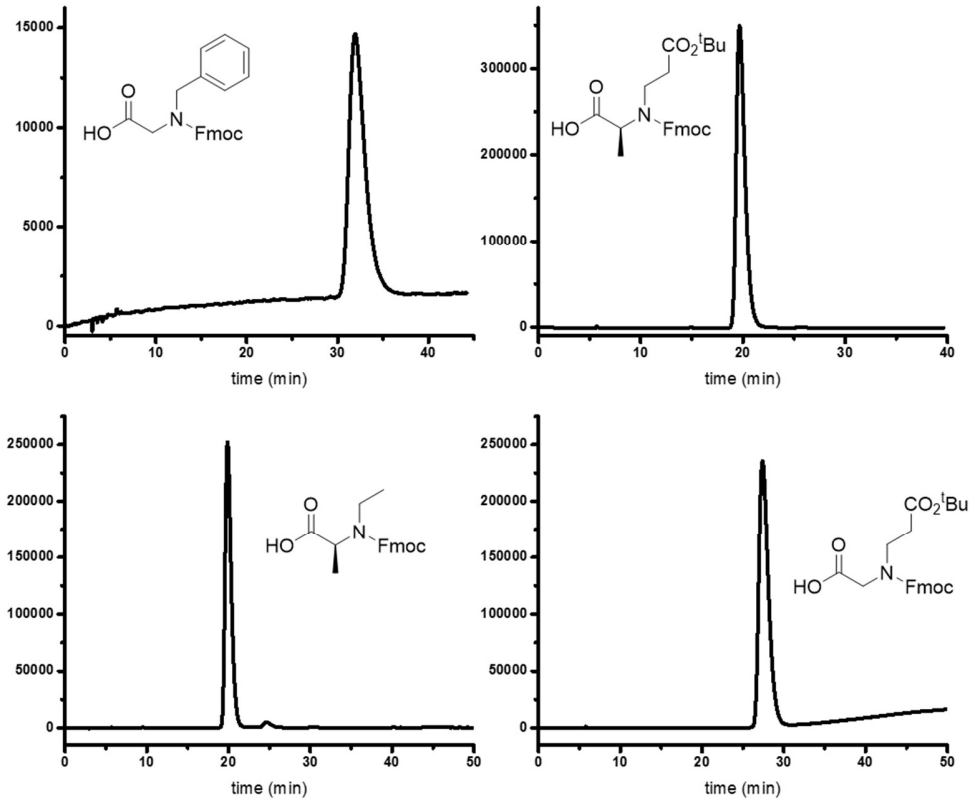


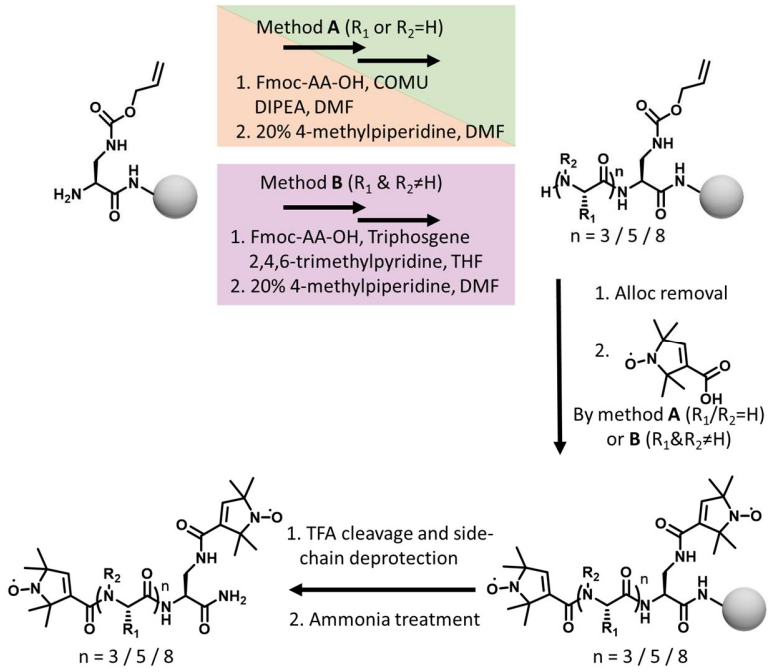
Figure S1. Chiral High Performance Liquid Chromatograms (HPLC) of the synthesized monomers

Double-Labelled Oligomers Synthesis

General Comments. TentaGel® S NH2 resin (90 µm, 0.26 mmol/g) was purchased from Rapp Polymere Gmbh. Rink amide linker was purchased from AnaSpec. tetrakis(triphenylphosphine)palladium [99.5%] and N_{α}-Fmoc-N_{β}-Alloc-L-2,3-diaminopropionic acid (Fmoc-L-Dap(Alloc)-OH) [9%] were purchased from Chem-Impex. Fmoc-L-Abu-OH (EMD Millipore) [98%] was purchased from Fisher Scientific. Fmoc-*N*(Et)-Gly-OH [96%] was purchased from Aurum Pharmatech. The free radical probe 3-carboxy-prollyl [99%] was obtained from Acros Organics. The following chemicals were purchased from Sigma Aldrich: COMU®,97% (1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate, triphosgene [98%], N,N-Diisopropylethylamine (DIPEA) [99.5%], biotech grade, 2,4,6-Trimethylpyridine (collidine) [99%], N,N-Dimethylformamide (DMF) [99.9%], Trifluoroacetic acid (TFA), CHROMASOLV® for HPLC [99.0%], Ammonia solution (7 N in methanol), Fmoc-L-Phe-OH [98%], and Fmoc-L-Glu(O^{t}Bu)-OH [98%]. The solvents dichloromethane (DCM) [99.9%], methanol (MeOH) [99.9%], and Tetrahydrofuran (THF) [99.9%] were purchased from Fisher Scientific. DCM and MeOH were used without further purification whereas THF was degassed and passed through activated alumina column. Disposable polypropylene columns (1.2 mL, Bio-Rad laboratories; 5 mL and 10 mL, Thermo Scientific) were used as reaction vessels for solid phase synthesis. Vessels containing reaction mixtures were rotated gently while fixed into Thermo Scientific Labquake Tube Shaker/Rotators. Wash volumes were approximately 3 mL of solvent per 100 mg of resin. Analytical High Performance Liquid Chromatography (HPLC) was carried out on reversed-phase ZORBAX Eclipse Plus C18 column (5 µm, 95 Å, 4.6x250 mm) and preparative HPLC was performed using a ZORBAX Eclipse XDB-C18 column (5 µm, 80 Å, 9.4x250 mm) on an Agilent 1200 series instrument (Agilent Technologies). A linear gradient of 10–100% ACN in water (0.1% TFA) over 30 min was used at a flow rate of 1 mL/min and 4 mL/min, respectively. Mass spectra (MS) were recorded on a time-of-flight (TOF) mass spectrometer using matrix assisted laser desorption ionization (MALDI) with α-cyano-4-hydroxycinnamic acid (CHCA) and on a high resolution TOF

mass spectrometer using electro spray ionization (ESI) method, Micromass Q-TOF-2 (Micromass UK Limited).

Solid Phase Peptide/Peptoid Synthesis. In a polypropylene column equipped with a porous polyethylene disc, a stopper, and an end cap, TentaGel resin (600 mg, 0.26 mmol/gr) was loaded and pre-swollen in DMF for 1.5 h. Rink amide cleavable linker (252 mg, 0.47 mmol, 3 equiv) and COMU (200 mg, 0.47 mmol, 3 equiv) were dissolved in 6 mL of DMF. DIPEA (164 uL, 0.94 mmol, 6 equiv) was added and after 3 min of pre-activation the solution was poured to the DMF-drained resin. The reaction mixture was allowed to slowly rotate for 2 h. The resin was washed with DMF (x7) and deproteced by 20 % 4-methylpiperidine in DMF for 20 min. The resin was washed again with DMF (x2), DCM (x2), and DMF (x2) and then coupled with Fmoc-L-Dap(Alloc)-OH. Cycles of coupling and Fmoc deprotection were repeated in this fashion on smaller scales (normally ~50 mg resin) until the desired trimers, pentamers, or octamers sequences were achieved. Coupling conditions were remained the same for both peptide and peptoid systems. The progress of the coupling reactions was monitored by a qualitative chloranil color test.



Scheme S1. Solid phase strategy for the preparation of dual-labeled peptide, peptoid, and DS-peptoid oligomers. Method **A** is optimal when R_1/R_2=H (peptides and peptoids monomers,

orange and green, respectively), and method **B** when at least one monomer fulfills the condition R_1&R_2≠H (DS-peptoid monomers, purple). The first unit, Fmoc-L-Dap(Alloc)-OH, was coupled according to method **A** for all sequences.

Solid Phase Synthesis of N/C_α-Disubstituted (DS) Peptoids. In the case of DS peptoid oligomers stronger reaction conditions were used. After coupling the first amino acid Fmoc-L-Dap(Alloc)-OH and its sequential Fmoc deprotection step, the resin was pre-swollen in $THF_{(anhyd)}$ for 15 min. The next Fmoc monomer (0.26 mmol, 5 equiv) and triphosgene (0.086 mmol, 1.66 equiv) were dissolved in 3 mL of $THF_{(anhyd)}$ to afford concentration of 0.1 M. The mixture was cooled to 0 ˚C before 2,4,6-trimethylpyridine (0.73 mmol, 14 equiv) was added. The obtained white suspension was mixed at 0 ˚C with a polypropylene transfer pipette for 1 min and then added to the solvent-drained resin. The reaction mixture was allowed to slowly rotate for 2 h. After draining the reaction solution, the resin was washed with MeOH (x2), DCM (x2), MeOH (x2), DCM (x2), DMF (x2), and DCM (x2). [*Caution: triphosgene is a hazardous material that can be decomposed to phosgene, a highly toxic gas. Therefore, it has to be handled in well-ventilated hood.*] The resin was deprotected by 20 % 4-methylpiperidine in DMF for 20 min and then washed again with DMF (x2), DCM (x2), DMF (x2). Coupling and deprotection steps were repeated to obtain the trimer, pentamer, and octamer of the DS peptoids bound to small portions of resin (normally ~50 mg). The progress of the coupling reactions was monitored by a qualitative chloranil color test.

Removal of Alloc Protecting Group. [Note: in the case of mono labelled trimer this step was avoided.] Prior treatment the resin was pre-swollen in DCM for 5 min. The solvent was then drained, and phenylsilane (24 equiv) in DCM was added to the resin followed by tetrakis(triphenylphosphine)palladium (0.25 equiv) in DCM after 1 min. (The total amount of DCM solvent was 100 uL per 10 mg resin) The reaction mixture was allowed to slowly rotate for 1 h before the solution was drained and the resin was washed with DCM (x5).

Spin Labelling of Diamino Trimers, Pentamers, and Octamers. In the case of peptides or peptoids, 3-carboxy-prollyl (10 equiv) and COMU (10 equiv) were dissolved in DMF (100 uL per 10 mg resin). DIPEA (20 equiv) was added, and after 3 min of pre-activation the solution was

poured to the resin-bound diamino oligomer (2 equiv). The reaction mixture was allowed to slowly rotate for 2 h. The resin was washed with DMF (x5) and DCM (x1).

In the case of the DS peptoids, the resin was pre-swollen in $THF_{(anhyd)}$ for 15 min. The 3-carboxy-prollyl (10 equiv) and triphosgene (3.3 equiv) were dissolved in $THF_{(anhyd)}$ to afford concentration of 0.1 M. The mixture was cooled to 0 ˚C before 2,4,6-trimethylpyridine (14 equiv) was added. The obtained white suspension was mixed with a polypropylene transfer pipette for 1 min and then added to the solvent-drained resin (The resin-immobilized diamino oligomers was considered as 2 equiv). The reaction mixture was allowed to slowly rotate for 2 h. After draining the reaction solution, the resin was washed with MeOH (x2), DCM (x2), MeOH (x2), DCM (x2), DMF (x2), and DCM (x2). Chloranil color test was used in order to qualitatively follow conversions.

The double-labelled oligomers were cleaved in a pre-prepared mixture of TFA/H_2O/triisopropylsilane; 95/2.5/2.5 for 1.5 h. The solutions were collected by filtration into polypropylene vials and after evaporated the oily crude products were washed with cold ether resulting in a white precipitation. To regenerate the prollyl radicals the compounds were subjected to ammonia treatment (7 N in MeOH/water; 90/10) for 3 h of vigorous shaking in a polypropylene vial. The solvent was then evaporated, and the compounds were resolvated in 4 mL of water/acetonitrile; 1/1 and lyophilized. The compounds were purified by high performance liquid chromatography (HPLC) using analytical C18 column (5 µm, 4.6x250 mm) or semi-prep C18 column (5 µm, 9.4x250 mm). Compounds were eluted with a linear gradient of 10–100% ACN in water (0.1% TFA) over 30 min at a flow rate of 1 or 5 ml/min, respectively. The compounds were collected and reinjected to an analytical HPLC column and their molecular weights were confirmed by TOF- MS MALDI and ESI-MS.

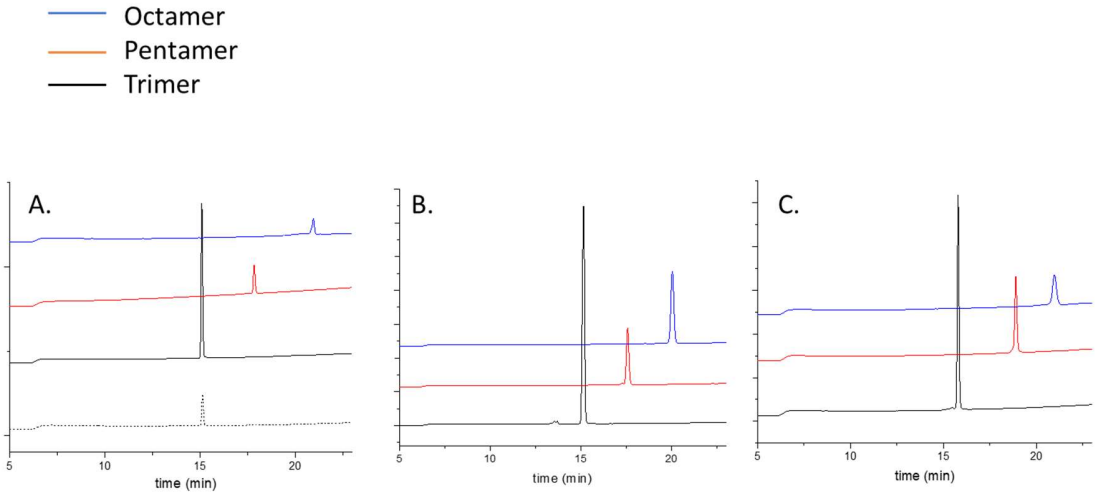


Figure S2. HPLC traces at 254nm for the (A) peptide (B) peptoid, and (C) DS peptoid oligomers. Color legend: dashed black; mono-labelled trimer, black; trimers, red; pentamers, and blue; octamers.

Oligomer class	Oligomer	Yield	TOF-ESI [(M+Na)$^+$]		
			Chemical Formula	MS (calculated)	MS (found)
peptide	Mono-labelled trimer	88%	$C_{30}H_{41}N_6NaO_9^{\bullet}$	652.3	652.3
	trimer	80%	$C_{35}H_{49}N_7NaO_9^{2\bullet}$	734.4	734.4
	pentamer	54%	$C_{48}H_{65}N_9NaO_{11}^{2\bullet}$	966.5	966.5
	octamer	49%	$C_{71}H_{90}N_{12}NaO_{16}^{2\bullet}$	1389.7	1389.7
peptoid	trimer	62%	$C_{35}H_{49}N_7NaO_9^{2\bullet}$	734.4	734.4
	pentamer	41%	$C_{48}H_{65}N_9NaO_{11}^{2\bullet}$	966.5	966.5
	octamer	37%	$C_{71}H_{90}N_{12}NaO_{16}^{2\bullet}$	1389.7	1389.7
DS peptoid	trimer	36%	$C_{36}H_{51}N_7NaO_9^{2\bullet}$	748.4	748.4
	pentamer	14%	$C_{50}H_{69}N_9NaO_{11}^{2\bullet}$	994.5	994.5
	octamer	8%	$C_{74}H_{96}N_{12}NaO_{16}^{2\bullet}$	1431.7	1431.7

Table S1. Yields and monoisotopic mass values for the examined oligomers obtained by analytical RP-HPLC and ESI-MS, respectively.

EPR Measurements

Sample preparation. For DEER and low temperature CW EPR measurements doubly lbelled oligomers were dissolved in 1:1 H_2O / DMSO v/v to obtain a solution of 0.3—0.5 mM. The solution was transferred into 3mm OD EPR tubes, flash frozen in liquid nitrogen and kept frozen until measured.

CW EPR measurements. Low temperature CW EPR spectra were acquired at 150K using one of the two Bruker EMX CW EPR spectrometers; one installed in the Laboratory of Songi Han and second one installed in the shared experimental facility of the Material Research Laboratory at UCSB. Both were equipped with standard variable temperature accessory. Experimental parameters were: mw power 0.6 mW; conversion time 40 ms; time constant 20 ms; modulation amplitude 0.1 mT; multiple scans were accumulated until satisfactory signal to noise ratio was achieved.

Second moment analysis of trimer CW EPR spectra.

The simplest way to convert dipolar broadening in CW EPR spectra of solids to distance is by using a second moment analysis.[18,19]

Second moment of CW EPR spectra was calculated according to:

$$\mu_2 = \sum (B - \overline{B})^2 s$$

Where B is a value of a magnetic field, s is signal intensity at this magnetic field and $\overline{B}$ is the mean magnetic field in the spectrum. Second moment of the dipolar interaction μ_{2d} is given by the difference of the second moments of the dipolar broadened and reference (unbroadened) spectra:

$$\mu_{2d} = \mu_2^{sample} - \mu_2^{reference}$$

Where $\mu_2^{reference}$ and μ_2^{sample} are second moments of the mono-labelled and double labelled peptides respectively.

Assuming a well-defined interspin distance *r,* a second moment of the dipolar interaction can be calculated using the Van Vleck formula:[1]

$$\mu_{2d} = \frac{3}{5} \frac{\mu_\beta^4 g^4 \mu_0^2}{(4\pi)^2 h^2} S(S+1) \frac{1}{r^6}$$

which allows to solve for *r* and arrive at the interspin distance for a given value of μ_{2d} .

This analysis results in the distances of 7.0, 7.2 and 7.5 Å for peptoid, DS-peptoid and peptide trimers, respectively. The second moment formalism assumes a single distance, which is clearly not the case here. Consequently those numbers should not be taken as absolute values, but rather interpreted as a trend showcasing.

In similar analysis performed for the octamer series we found $\mu_{2d} \approx 0$ indicating absence of short distances < 15 Å .

DEER measurements. DEER measurements were performed on a commercial Bruker Elexsys 580 pulsed EPR spectrometer at 85K using CF935 flow cryostat (Oxford Instruments). The 4-pulse DEER sequence $\pi_{obs}/2 - \tau - \pi_{obs} - t - \pi_{pump} - (T-t) - \pi_{obs} - (T-\tau)$ – echo DEER pulse sequence is shown on Figure S3. The echo intensity was acquired as function of the delay time *t* incremented in 8 ns steps. Bruker ER 4118X-MS3 resonator was tuned to ν_{pump}, overcoupled to Q ~ 100, and the magnetic field was set such that the pump frequency corresponded to the maximum of the nitroxide EPR spectrum. Other parameters: $\nu_{observe} = \nu_{pump}$ +65MHz; π_{obs} = 32 ns; π_{pump} = 12 ns; τ = 250 ns;

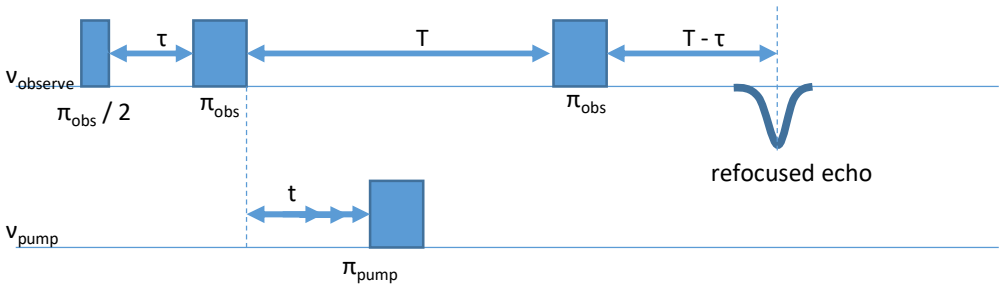


Figure S3. Pulse sequence of the 4-pulse DEER experiment.

The DEER data was processed using standard procedure using the Deer Analysis software package.[2] Intermolecular background was calculated assuming homogeneous three dimensional distribution and subtracted from the raw data. Next, distance distributions were reconstructed using Tikhonov regularization procedure using the excitation bandwidth corrected kernel as implemented in the software. The optimal regularization parameter value α = 100 was determined using the L-curve criteria.

Raw time domain DEER data for the octamer distance distributions presented in figure 2 in the main text is presented in figure S4. The ~15MHz modulation visible in the time domain trace is due to ^{1}H Electron Spin Echo Envelope Modulation (ESEEM). This high frequency modulation does not affect the reconstructed distance distributions.

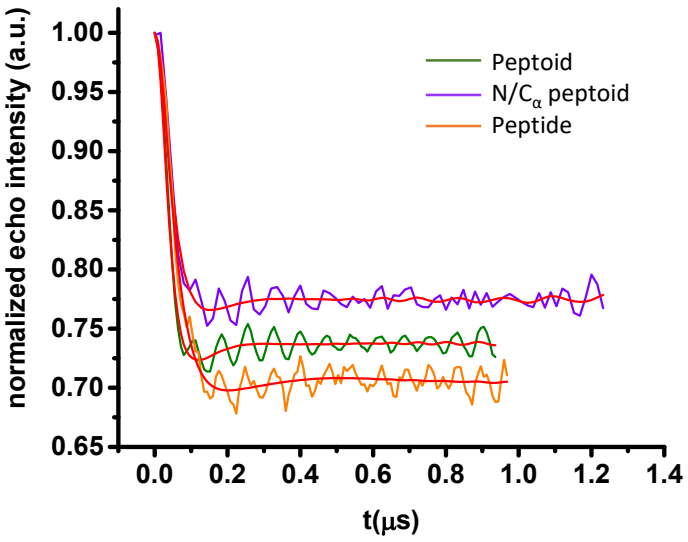


Figure S4. Time domain DEER traces after background subtraction for octamer peptoid (green), DS-peptoid (purple) and peptide (orange). The corresponding fits are shown in red.

Raw time domain DEER data for the pentamers and the corresponding distance distributions are presented in figure S5 in (A) and (B) respectively. For the pump pulse bandwidth used in our experiments, when short interspin distances < 2 nm are present in the sample DEER modulation

depth λ (Figure S5-A) strongly depends on the shape of the distance distribution[3] and decreases with the increase in the amount of short distances. Note that the modulation depth for the pentamer peptoid (Fig S5-B) is significantly smaller λ = 0.12 than for the other two pentamers λ ~ 0.22. This confirms our conclusion drawn from CW EPR that the pentamer peptoid forms the most compact ensemble of the three pentamers. Consequently we conclude that shorter distances present in the pentamer peptoid sample are not resolved in the reconstructed distance distributions. We therefore refrain from analyzing them in the context of oligomer conformations. In addition we always find that the modulation depth λ is smaller for the pentamer (Fig S5-A) than for the corresponding octamer (Fig S4) which is consistent with the octamers forming ensembles with on average longer interspin distances.

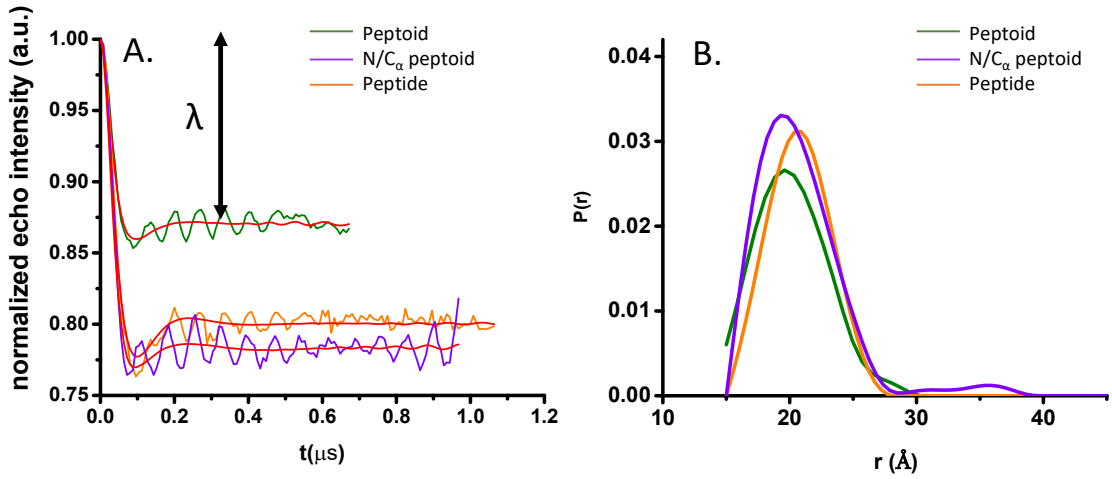


Figure S5. Time domain DEER traces after background subtraction with corresponding fits (red) (A) and corresponding distance distributions (B) for pentamer peptoid (green), DS-peptoid (purple) and peptide (orange).

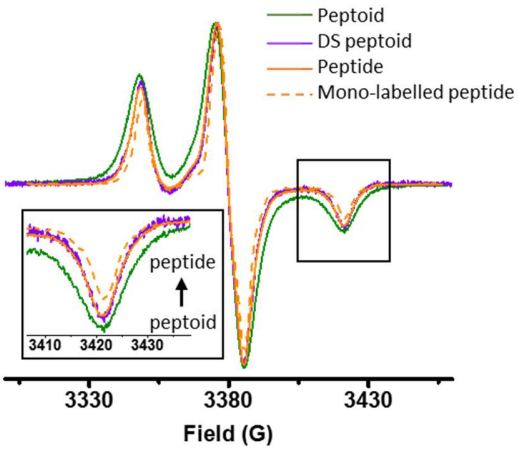


Figure S6. Low temperature CW EPR of the pentamers series overlaid with the mono-labeled peptide and 3CP free radical. Insets: Zoom in of high-field region

Oligomers Interactions with Gold Nanoparticles (AuNPs)

General Comments. Sodium tetrachloroaurate (III) dehydrate and sodium citrate were purchased from Fisher Scientific. All glassware were cleaned by a piranha solution (H_2SO_4 / 30% H_2O_2; 7/3) followed by extensive deionized (DI) and Milli-Q water. Transmission electron microscopy (TEM) imaging were performed with a Tecnai FEI T20 at 200 kV, equipped with a Gatan UltraScan 1000P CCD camera (2k×2k). TEM samples were prepared by placing a 5 µL drop of the aqueous AuNPs solution on a carbon/Cu grid (200 mesh) and blotting after 10 sec. UV-Vis spectra were recorded with a UV-1800 Shimadzu spectrophotometer in the absorption mode upon dilution of solution mixtures (x20). Due to oligomers small amounts, concentrations of the oligomers solutions were determined by CW EPR measurements and calibrated against 1 mM acetonitrile solutions TEMPOL or 3-carboxy-prollyl free radical.

Synthesis of Gold Nanoparticles (AuNPs).[4] Sodium citrate (100 mg, 0.34 mmol) was added to a refluxing, vigorously stirred solution of sodium tetrachloroaurate dihydrate (50 mg, 0.13 mmol) in Milli-Q water (250 mL). The mixture was vigorously stirred under reflux for 15 min before it was allowed to cool to room temperature. The initially yellow solution changed to deep red and was stored in a glass bottle protected from light. The AuNPs formation was confirmed by UV-Vis spectroscopy, which showed a peak at λ_{max}=518 nm and by TEM, which showed an average nanoparticle diameter of 12.1±1.1 nm.

Interactions of double-labelled oligomers with AuNPs. Double-labelled trimer or pentamer oligomers series in acetonitrile (40 uL, 50 uM) were added into 200 uL of AuNPs aqueous solutions in order to obtain a distinct reactivity contrast between the different classes. In the case of the octamers, a lower concentration acetonitrile solutions (40 uL, 15 uM) were added into 200 uL of AuNPs aqueous solutions due to lower solubility of those oligomers as compared to their trimer and pentamer counterparts. Control experiments were performed when only acetonitrile solvent (40 uL) or a mono-labelled peptide trimer acetonitrile solution (40 uL, 50 uM) were added into 200 uL of AuNPs aqueous solutions. UV-vis measurements were performed on diluted samples (x20).

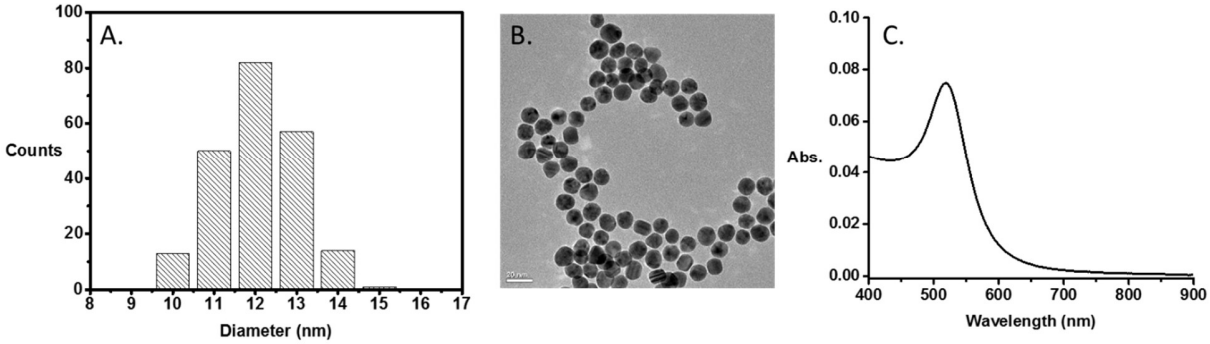
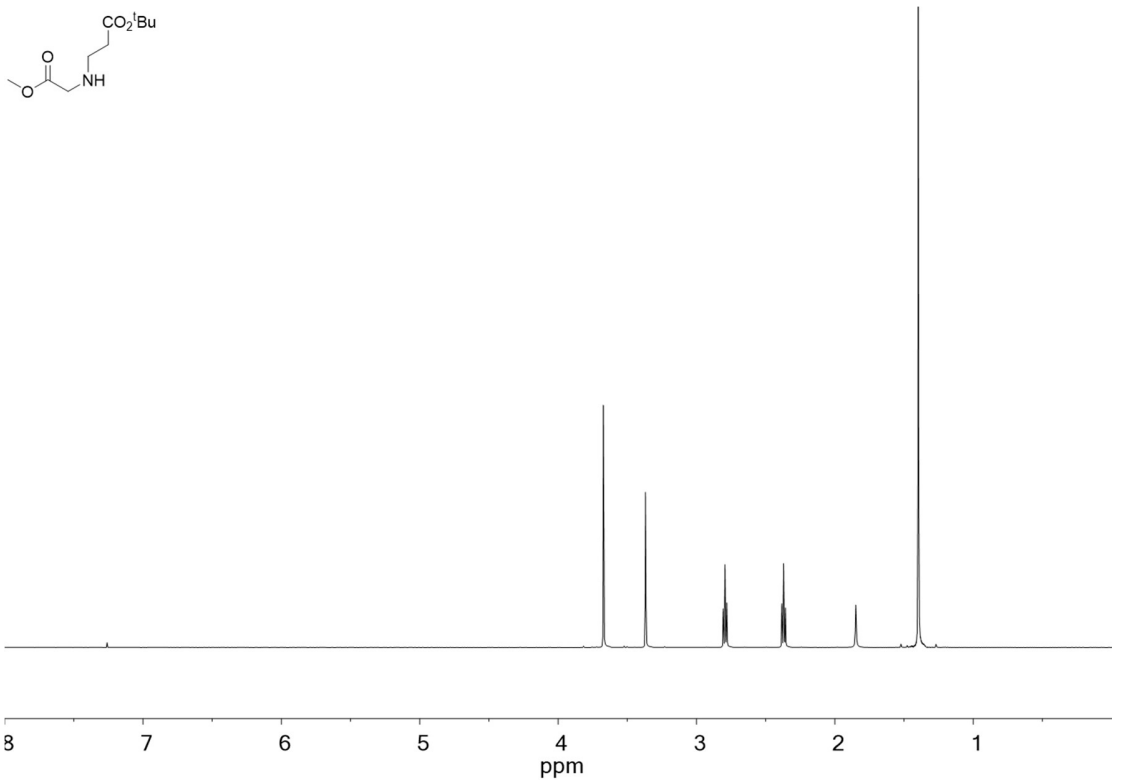


Figure S7. (A) Size distribution histogram of gold nanoparticles (AuNPs) measured by transmission electron microscopy (TEM) showing an average diameter value of 12.1 ± 1.1 nm. (B) Representative TEM micrograph. (C) UV-vis absorption spectrum of AuNPs aqueous solution showing a peak at λ_{max}=518nm.

^{1}H-NMR and ^{13}C-NMR Spectra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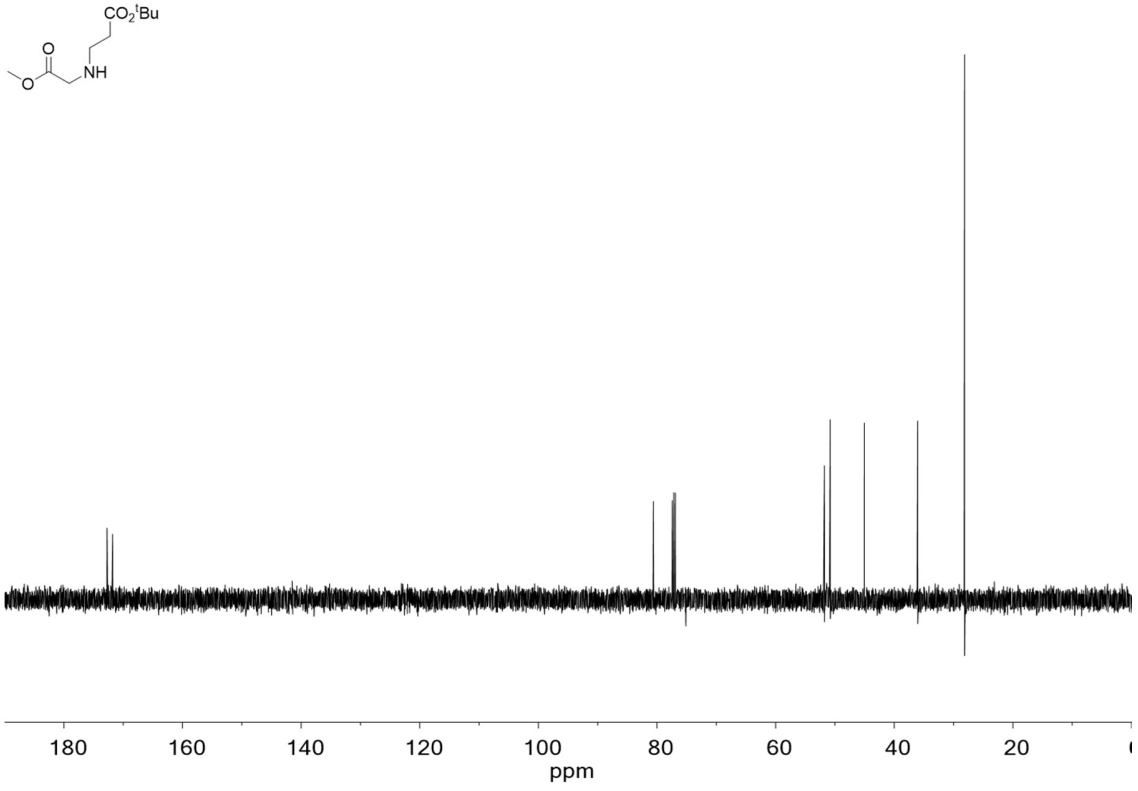
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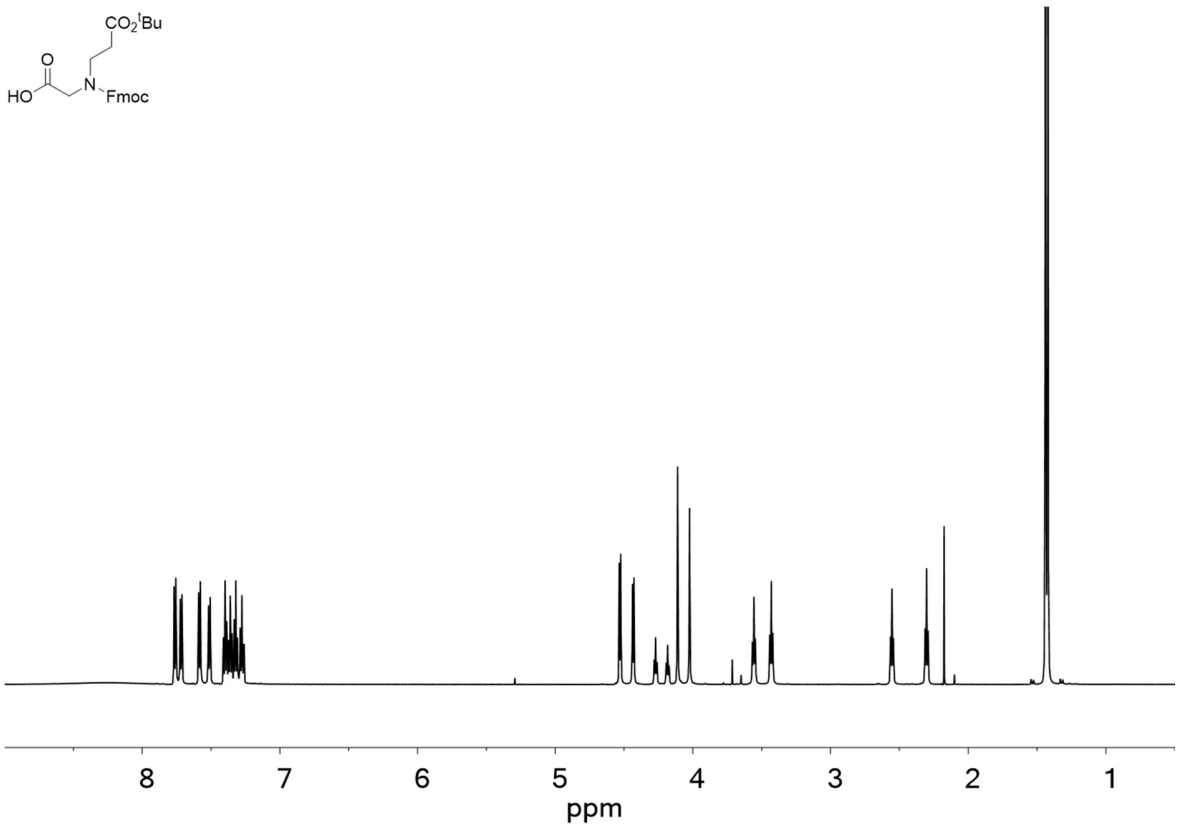
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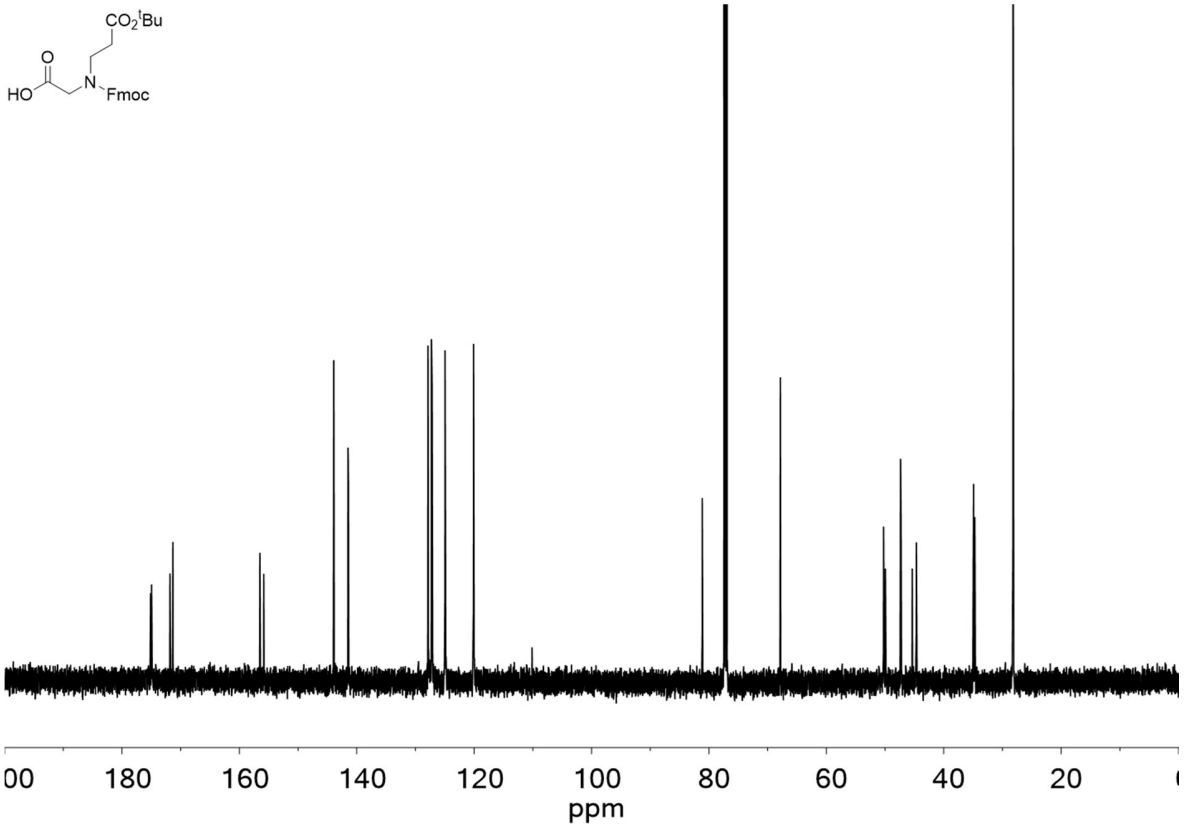
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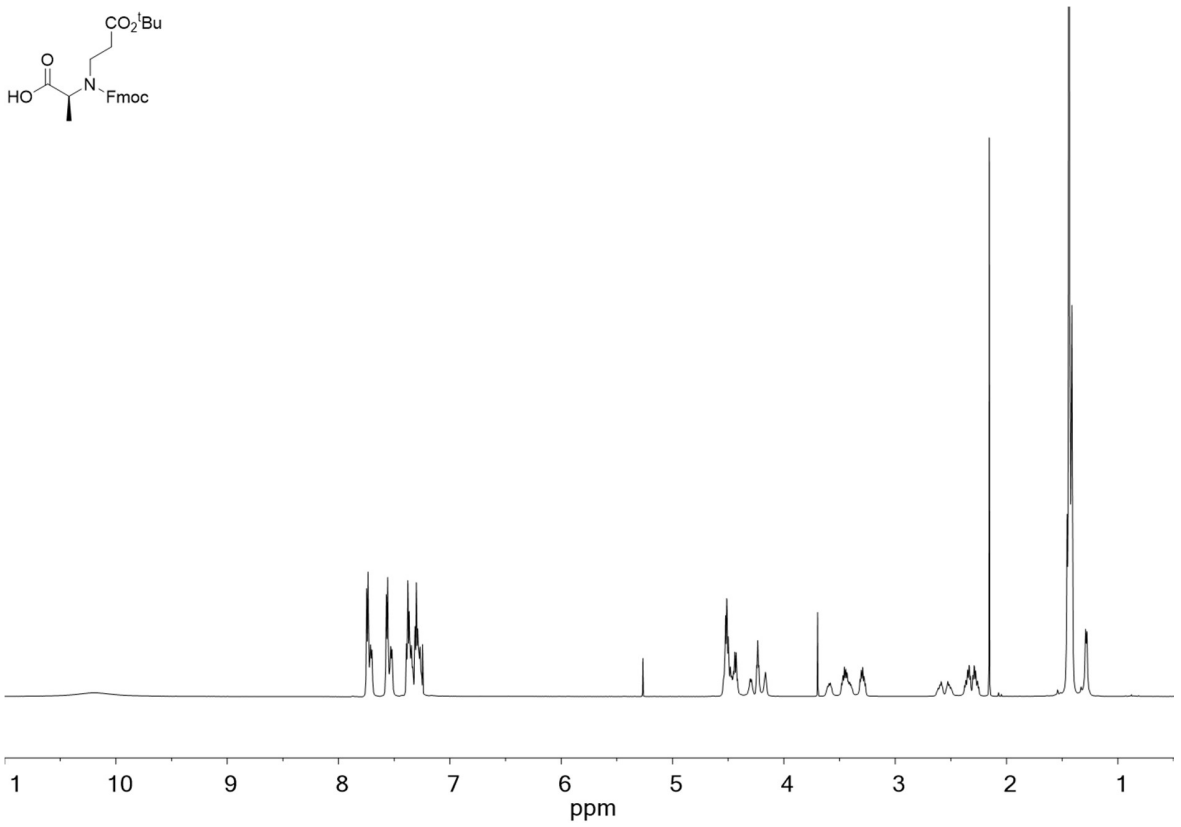
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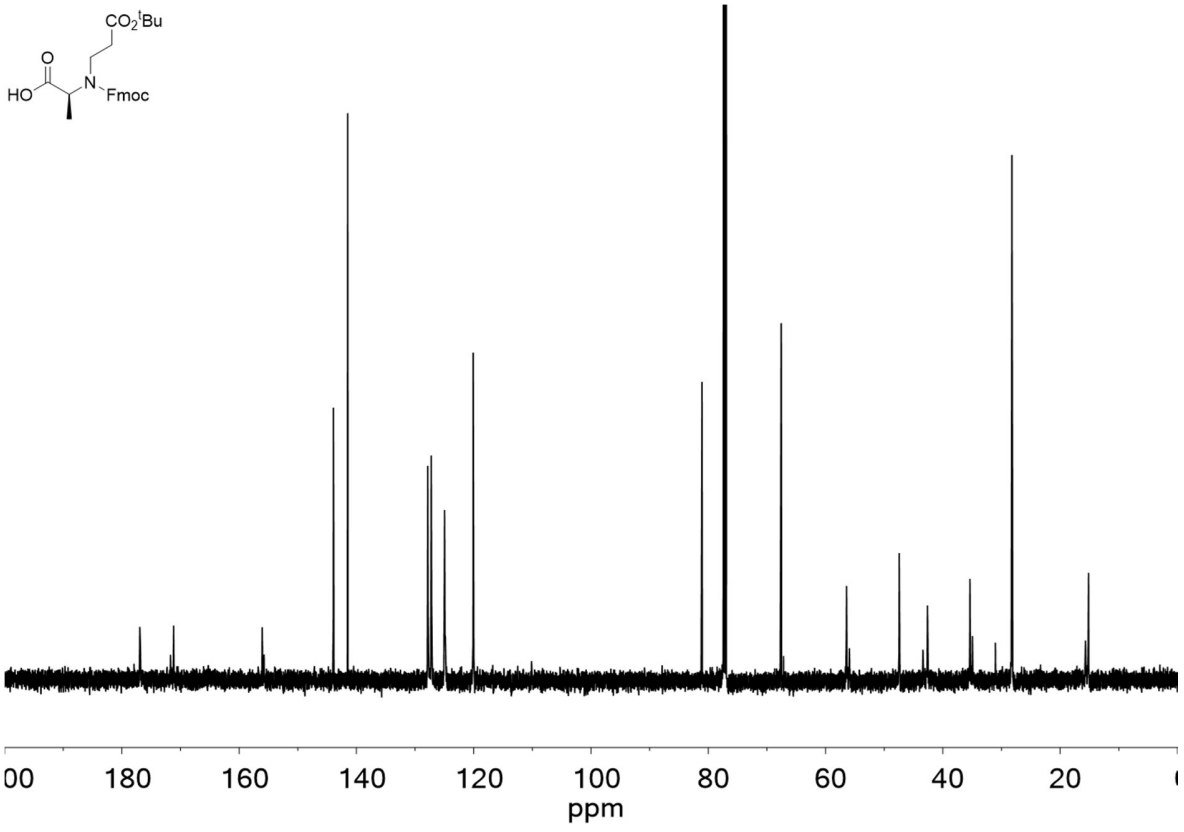
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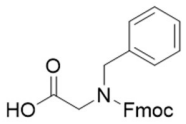
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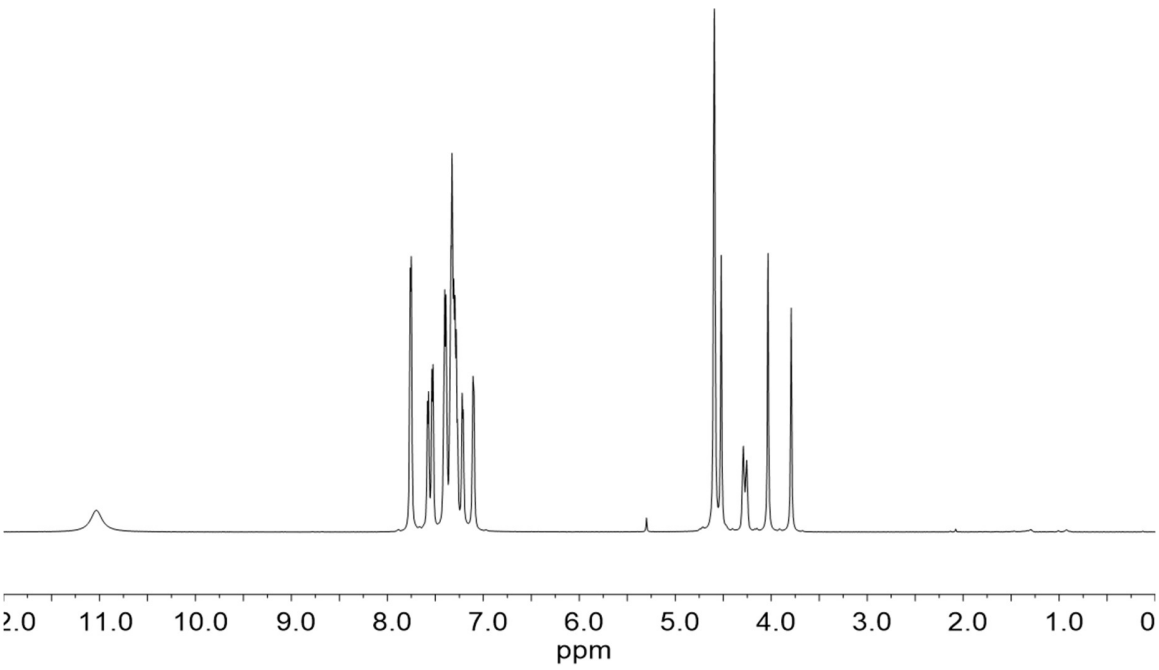
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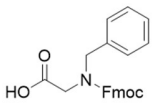
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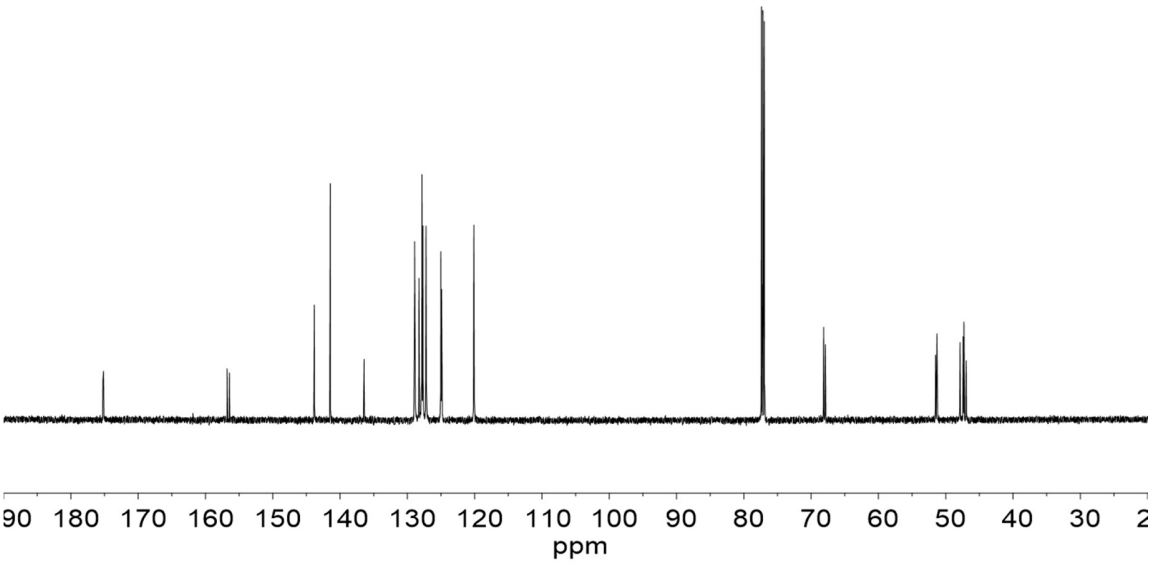
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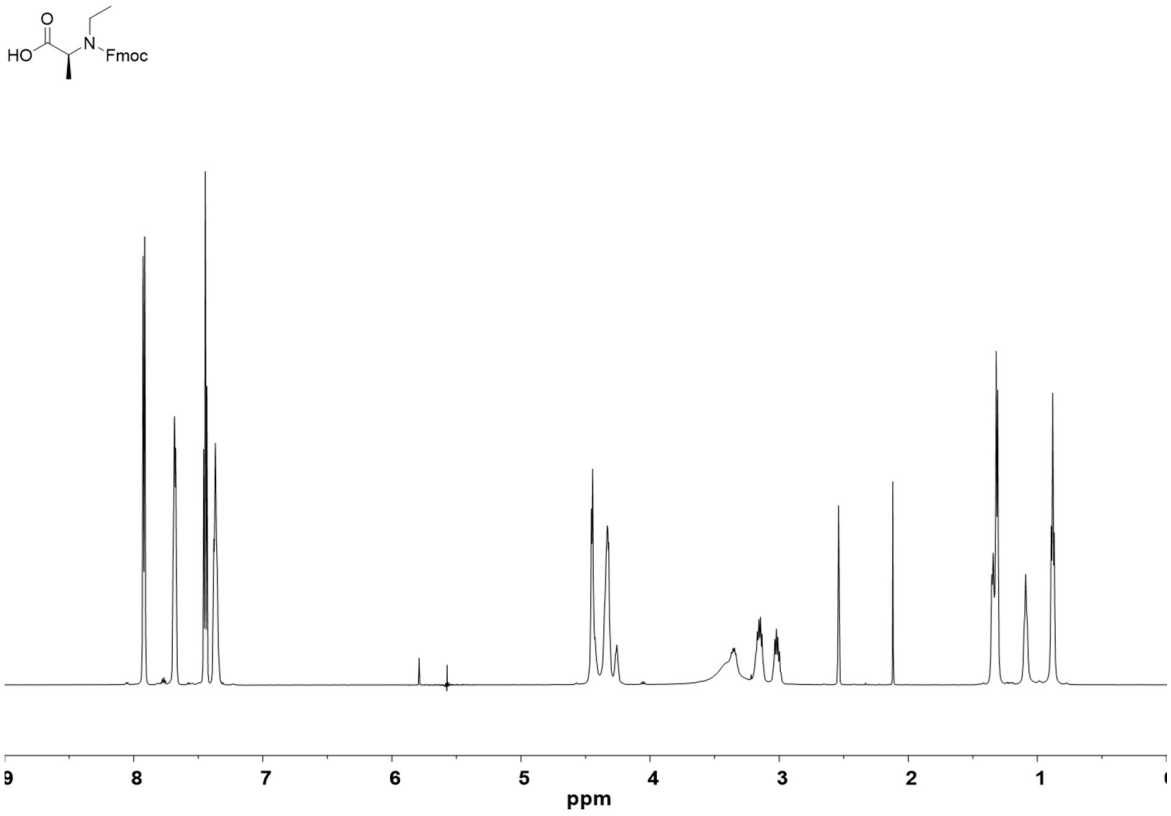
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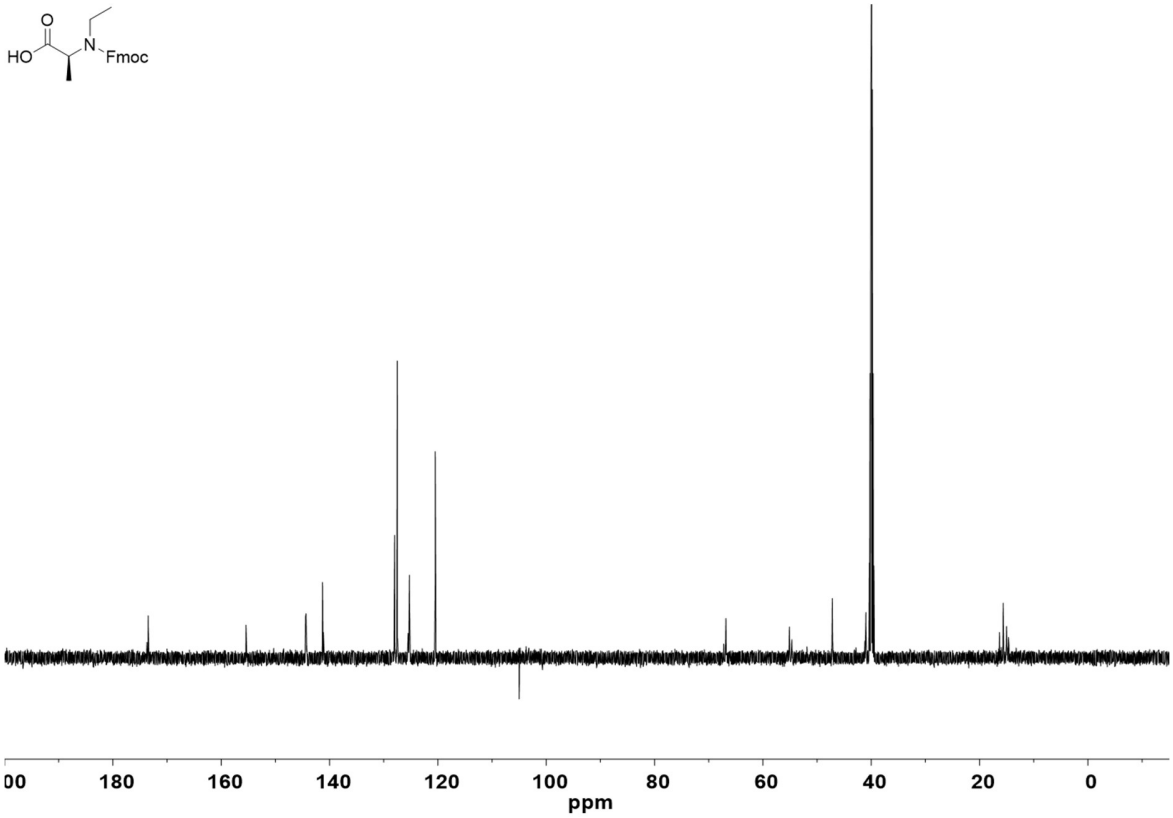
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References

(1) Van Vleck, J. H. *Phys. Rev.* **1948**, *74* (9), 1168.
(2) Jeschke, G.; Chechik, V.; Ionita, P.; Godt, A.; Zimmermann, H.; Banham, J.; Timmel, C. R.; Hilger, D.; Jung, H. *Appl. Magn. Reson.* **2006**, *30* (3-4), 473.
(3) Banham, J. E.; Baker, C. M.; Ceola, S.; Day, I. J.; Grant, G. H.; Groenen, E. J. J.; Rodgers, C. T.; Jeschke, G.; Timmel, C. R. *J. Magn. Reson.* **2008**, *191* (2), 202.
(4) Turkevich, J.; Stevenson, P. C.; Hillier, J. *Discuss. Faraday Soc.* **1951**, *11* (0), 55.