SUPPORTING INFORMATION

Solid-phase Synthesis and CD-Spectroscopic Investigations of Novel β-peptides from L-Aspartic Acid and β-Amino-L-Alanine

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Table of Contents

Experimental Section

Solvents and reagents. Rink amide MBHA resin (0.58 mmol/g), N-α-Fmoc-L-aspartic acid α-allyl ester, Boc-L-asparagine, mono-trityl 1,4-diaminobutane acetic acid salt, BOP, and HOBt were purchased from NovaBiochem (San Diego, CA). N-methyl morpholine (NMM), and trifluoroacetic acid (TFA) were purchased from Aldrich while piperidine was purchased from Caledon (Canada). All other reagents were purchased from Sigma-Aldrich. All commercial reagents and solvents were used as received.

Column chromatography was performed under slight positive air pressure employing on silica gel 60, 70-230 mesh (Rose Scientific Ltd.); thin-layer chromatography (TLC) was performed using silica-plates (Machery-Nagel Layer: 0.20 mm silica gel 60 with fluorescent indicator UV_{254}).

Equipment. RP-HPLC purification and analysis were carried out on a Waters (625 LC system) HPLC system using Vydac semi-preparative C18 (1 x 25 cm, 5 μm) and analytical C8 (0.46 x 25 cm, 5 μm) columns. Compounds were detected by UV absorption at 220 nm. Mass spectra were recorded on a MALDI Voyager time-of-flight (TOF) spectrometer (Voyager^{TM} Elite) or on a Waters micromass ZQ. NMR experiments were recorded at 25 ºC on a Bruker AM-300 spectrometer or on a Varian INOVA 500 MHz NMR spectrometer equipped with a triple-resonance HCN Cold Probe with z-axis pulsed field gradients.

Solid-Phase Synthesis. Peptide syntheses were performed manually in a glass peptide synthesis vessel with a glass frit at the bottom and screw cap with a septum at the top for addition of reagents. Solvents and soluble reagents were removed by suction. Washing between deprotection and coupling was carried out with DMF (4 x 1 min) and DCM (4 x 1 min) using 4 mL of solvent/0.05 mmol of resin each time. Fmoc group was removed by treatment with 20% piperidine/DMF (1 x 5 mins, 2 x 10 mins). Deprotection of the allyl from carboxyl group (All) and allyloxycarbonyl from amine group (Alloc) was carried out with $Pd(PPh_3)_4$ (0.08 equiv) and $PhSiH_3$ (8 equiv) in DCM/DMF (35 mins x 3) under nitrogen.

The deallylation reaction for the removal of side chain All from the carboxyl group, after coupling first amino acid (N^{α}-Fmoc-L-aspartic acid α-allyl ester) to the resin, was optimized by varying the reaction conditions as shown in Table S1. Greater than 99% deallylation was achieved by carrying out the reaction with 0.08 equiv $Pd(PPh_3)_4$ and repeating the process twice. The reaction was monitored by test cleavage followed by analytical reverse-phased HPLC and mass spectrometric analysis. Similar conditions were required for the complete removal of Alloc protection from an amine group.

Table S1. Deallylation of the All group from side chain carboxylic acid.

Time (min) x the number of times the reaction was repeated	**No of equiv. of $(Pd(PPh_3)_4$**	**Deallylation (%)**
35 x 1	0.16	30
35 x 2	0.16	55
35 x 3	0.16	>99
20 x 3	0.16	45
20 x 4	0.16	65
15 x 3	0.08	30
35 x 3	0.08	>99

β^3-peptide (1, heterooligomer) synthesis. Fmoc/allyl combined solid-phase strategy was used to carry out the synthesis on MBHA resin (172 mg, 0.1 mmol). N-α-Fmoc-L-aspartic acid α-allyl ester (79 mg, 2 equiv) was coupled in the presence of BOP (86 mg, 1.95 equiv), HOBt (27 mg, 2 equiv), and NMM (45 ul, 4.5 equiv) in DMF for 2 h at room temperature. After coupling the resin was washed with DMF and DCM. Coupling was monitored with the Kaiser test. Following deprotection of the side chain All, coupling of the carboxyl was carried out using corresponding amine (RNH_2, 5 equiv) and the same coupling agents as above for 6-8 h at 25 °C. The resin was washed with DMF and DCM followed by removal of the N^{α}-Fmoc group and the sequence of reactions was repeated to complete the synthesis of **1**. β^3-hexapeptide **1** was cleaved from the resin using cleavage reagent (5 mL, 95:2:3,

TFA/H_2O/triisopropylsilane) at room temperature for 2 hours and then washing the resin with the cleavage reagent (2 x 2 mins, 3 mL). The cleaved peptide was collected, combined with TFA washes, and concentrated by rotary evaporation. Cold diethyl ether (~ 10 mL) was added to precipitate the crude cleaved peptide. After trituration for 2 mins, the peptide was collected upon centrifugation and decantation of the ether. The crude peptide was reconstituted in 30% CH_3CN and purified on a semipreparative Vydac C18 HPLC column (10 x 250 mm, flow rate = 2 mL/min, monitored at 220 nm) using a gradient of 10-40 % CH_3CN in 0.05%aqueous TFA over a period of 50 min. The identity and purity of the hexapeptide **1** were assessed by analytical HPLC (Figure S2) and MALDI-TOF mass spectrometry. ^1H NMR [CF_3CD_2OH, 500 MHz]: δ 0.83-0.96 (m, 12H, isovaleric methyls), 1.12-1.28 (m, 12H, isopropyl methyls), 1.56-1.69 (m, 8H, two side chain CH_2 linkages to amine, $CH_2\underline{CH_2}\underline{CH_2}CH_2NH_2$), 1.7-1.85 (m, 2H, two isovaleric CH), 2.5-2.85 (m, 12H, backbone CH_2), 2.9-3.1 (m, 4H, two isovaleric CH_2), 2.95-3.05 (m, 4H, two side chain methylenes $CH_2CH_2CH_2C\underline{H_2}NH_2$), 3.15-3.32 (m, 4H, two side chain methylenes $C\underline{H_2}CH_2CH_2CH_2NH_2$), 3.95-4.1 (m, 2H, two isopropyl CH), 4.2-4.28 (m, 1H, backbone CH N-terminal), 4.85-5.25 (m, 5H, backbone CH), 6.67 (s, 1H, carboxamide), 6.84-6.92 (m, 1H, side chain isovaleric NH), 7.22 (d, 1H, J= 7.4 Hz, backbone amide), 7.27-7.42 (m, 3H, one backbone NH, one side chain NH, and one carboxamide NH), 7.45 (d, 1H, J=7.3 Hz, isopropyl side chain NH), 7.8-7.88 (m, 1H, backbone amide), 8.18 (d, 1H, J=7.4 Hz, isopropyl side chain NH), 8.21-8.32 (m, 2H, side chain amides), 8.57 (d, 1H, J= 8.78 Hz, backbone amide), 8.78 (d, 1H, J= 7.7 Hz, backbone amide), 9.06-9.14 (m, 1H, backbone amide). MALDI-TOF calcd for $C_{46}H_{85}N_{15}O_{12}$, $[M + H]^+$ 1040.00; found $[M + H]^+$ 1040.50 and $[M + Na]^+$ 1062.46; overall yield 53%.

β^2-peptide (2, heterooligomer) synthesis. Synthesis of β^2-peptide **2** was achieved by essentially following the same procedure as for β^3-peptide. Briefly, N^{α}-Alloc-N^{β}-Fmoc-L-diaminopropionic acid (41 mg, 2 equiv) was coupled to MBHA resin (86 mg, 0.05 mmol) in the presence of BOP (43.1 mg, 1.95 equiv), HOBt (13.5 mg, 2 equiv), and NMM (25 ul, 4.5 equiv) in DMF for 2.5 h at room temperature. Following deallylation (35 min x 3) of the side chain Alloc, coupling of side chain amino group was carried out using RCOOH (2 equiv), BOP (43 mg, 1.95 equiv), HOBt (13.5 mg, 2 equiv), and NMM (25 ul, 4.5 equiv) in DMF for 3 h at 25 °C. After washing and removal of the Fmoc group, the reactive sequence was

repeated to obtain **2**. Both the backbone elongation and the side chain coupling were monitored by the Kaiser test as well as test cleavage. After cleavage from the resin, the crude peptide was reconstituted in 30% CH_3CN and purified on a semipreparative Vydac C18 HPLC column (10 x 250 mm, flowrate = 2 mL/min, monitored at 220 nm) using a gradient of 10-35 % CH_3CN in 0.05%aqueous TFA over a period of 1 h. The identity and purity of the hexapeptide **2** were assessed by analytical HPLC (Figure S3) and MALDI-TOF mass spectrometry. ^{1}H NMR [CF_3CD_2OH, 500 MHz]: δ 0.85-0.1 (m, 12H, isovaleric methyls), 1.05-1.2 (m, 12H, isopropyl methyls), 1.62-1.7 (m, 8H, two side chain CH_2 linkages to amine, $CH_2\underline{CH_2}\underline{CH_2}CH_2NH_2$), 2.02-2.1 (m, 2H, two isovaleric CH), 2.11-2.2 (m, 4H, two isovaleric CH_2), 2.31-2.40 (m, 4H, two side chain methylenes $C\underline{H_2}CH_2CH_2CH_2NH_2$), 2.5-2.59 (m, 2H, two isopropyl CH), 3.0-3.09 (m, 4H, two side chain methylenes $CH_2CH_2CH_2C\underline{H_2}NH_2$), 3.4-3.8 (m, 12H, backbone CH_2), 4.4-4.9 (m, 6H, backbone CH), 7.15-7.98 (m, 12H, backbone and side chain amides, overlapping peaks could not be resolved in 1D NMR). MALDI-TOF calcd for $C_{46}H_{85}N_{15}O_{12}$, $[M + H]^+$ 1040.00; found $[M + H]^+$ 1040.20 and $[M + Na]^+$ 1060.00 found $[M + H]^+$ 1060.20; overall yield 39%.

β^2-peptide (3, homooligomer) synthesis. Synthesis of β^2-peptide **3** was carried out on MBHA resin (86 mg, 0.05 mmol) as mentioned above. N^{α}-Alloc-N^{β}-Fmoc-L-diaminopropionic acid (41 mg, 2 equiv) was coupled to the resin in the presence of BOP (43.1 mg, 1.95 equiv), HOBt (13.5 mg), and NMM (25 ul, 4.5 equiv) in DMF for 2.5 h at room temperature. This was followed by Fmoc deprotection and the procedure was repeated for six times. After stepwise coupling of the amino acids, deallylation was carried out in the presence of $Pd(PPh_3)_4$ (0.48 equiv, 0.08 equiv for each allyl group) and $PhSiH_3$ (48 equiv) in DCM/DMF (4 x 35 mins) under nitrogen to obtain **3**. After cleavage from the resin, the crude peptide was reconstituted in 10% CH_3CN and purified on a semipreparative Vydac C18 HPLC column (10 x 250 mm, flowrate = 2 mL/min, monitored at 220 nm) using a gradient of 8-40 % MeCN in 0.05%aqueous TFA over a period of 1 h. The identity and purity of the hexapeptide **3** were assessed by analytical HPLC (Figure S4) and mass spectrometry. ^{1}H NMR [CF_3CD_2OH, 500 MHz]: δ 3.35-3.8 (m, 12H, backbone CH_2), 4.02-4.31 (m, 6H, backbone CH), 7.5-7.7 (m, 2H, carboxamide), 8.3-8.85 (m, 5H backbone amides). ESI mass

calcd for $C_{18}H_{39}N_{13}O_6$, $[M + H]^+$ 534.00; found $[M + H]^+$ 534.05 and $[M + Na]^+$ 556.00; found $[M + Na]^+$ 556.23; overall yield 92 %.

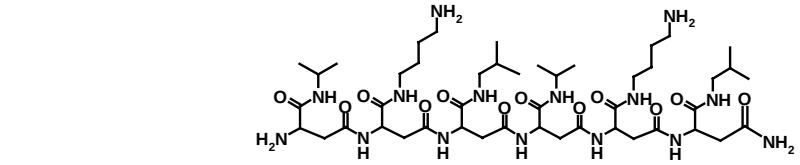
Solution Phase Synthesis of N^{α}-Alloc-N^{β}-Fmoc-L-diaminopropionic acid (4).

Orthogonally protected β-amino-L-alanine (L-diaminopropionic acid) was synthesized in four steps following the sequence shown in Scheme 2. First, N^{α}-Boc-2,3-diaminopropionic acid (N^{α}-Boc-Dap) was prepared by the reaction of Boc-L-asparagine (3.75 g, 16.1 mmol) with iodobenzene diacetate (4.16 g, 19.4 mmol) in a mixture of solvents (45 mL, EtOAc:MeCN:H_2O, 2:2:1, v/v) at 0 °C following the literature procedure.[1,2] After work up, the aqueous layer was evaporated to dryness to yield 2.52 g of pure N^{α}-Boc-Dap (76.6% yield). MS calcd for $C_8H_{16}N_2O_4$, $[M + H]^+$ 205.23; HR-ESI found $[M + H]^+$ 204.92, $[M + Na]^+$ 226.92. The free amino group in N^{α}-Boc-Dap was protected with Fmoc group followed by removal of N^{α}-Boc following the reported procedure.[1,3]

Finally, Alloc protection of the free amino group in N^{β}-Fmoc-Dap was carried out by dropwise addition of allyloxycarbonyl chloride (17.6 μL, 1.5 equiv) to a mixture of N^{β}-Fmoc-Dap (36.2 mg, 1 equiv) and $NaHCO_3$ (44.8 mg) in dioxane/water (1:1, 7 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 40 min followed by acidification (10% HCl) and extraction with DCM. The crude product was purified using column chromatography to give 40 mg pure **4** (88.3% yield). The purity of **4** was assessed using analytical HPLC (Figure S5), ^{1}H NMR (Figure S6), ^{13}C NMR (Figure S7), and mass spectrometry. ^{1}H NMR [CD_3OD, 300 MHz]: δ 3.4-3.65 (m, 2H, CHC$\underline{H}_2$NH), 4.2 (m, 1H, C$\underline{H}$CH_2O), 4.25-4.4 (m, 3H, CHC$\underline{H}_2$O & $CH_2C\underline{H}CO$), 4.5 (d, 2H, CHC$\underline{H}_2$O), 5.15 (d, J = 10.3 Hz, 1H, cisC$\underline{H}_2$=CH), 5.3 (d, J = 17 Hz, 1H, transC$\underline{H}_2$=CH), 5.8-6.0 (m, 1H, CH_2=C$\underline{H}$), 7.2-7.8 (m, 8H, aryl$\underline{H}$). ^{13}C NMR [CD_3OD, 75 MHz]: δ 43.1, 48.4, 55.7, 66.9, 68.0, 117.6, 120.8, 126.2, 128.1, 128.7, 134.1, 142.4, 145.2, 158.3, 159.0, 173.5. MS calcd for $C_{22}H_{22}N_2O_6$, $[M + H]^+$ 411.43; HR-ESI found $[M + H]^+$ 410.92, $[M + Na]^+$ 432.92.

Circular Dichroism. All CD measurements were made on an Olis CD spectrometer (Georgia, USA) at 25 °C in a thermally controlled quartz cell over 190-260 nm. All samples

were dissolved in appropriate amount of the different solvents, namely, water, phosphate buffer (1 mM, pH 7.4), methanol, and TFE. The final concentrations for CD measurements were 100, 250, and 500 μM for all the peptides. The length of the cuvette was 0.02 cm and number of scans was set to 10. Smoothing and correction of the background spectra was performed afterwards. The CD data are normalized and are expressed in terms of mean residue ellipticity (deg cm^2 $dmol^{-1}$).



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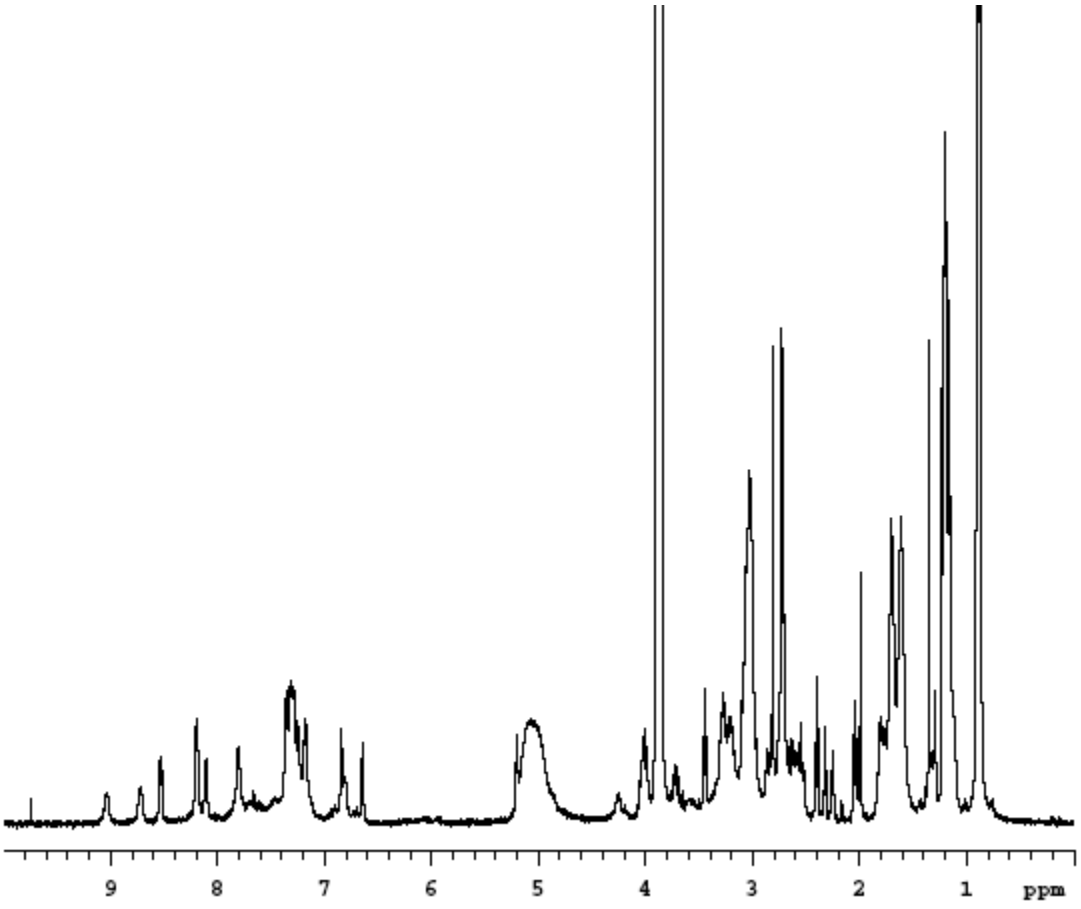


Figure S1. ^{1}H-NMR (500 MHz) spectrum of β^{3}-hexapeptide 1 in CF_3CD_2OH

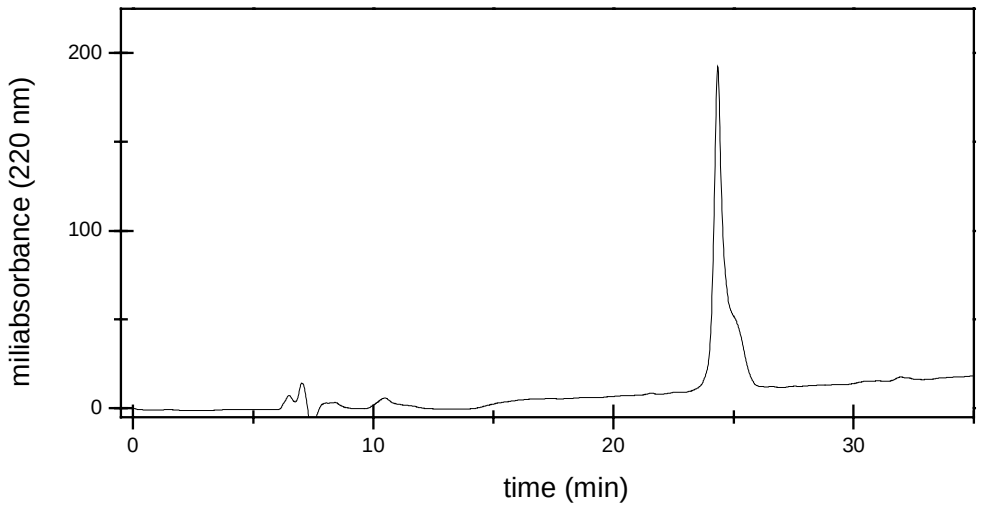


Figure S2. RP-HPLC chromatogram of β^3-hexapeptide 1

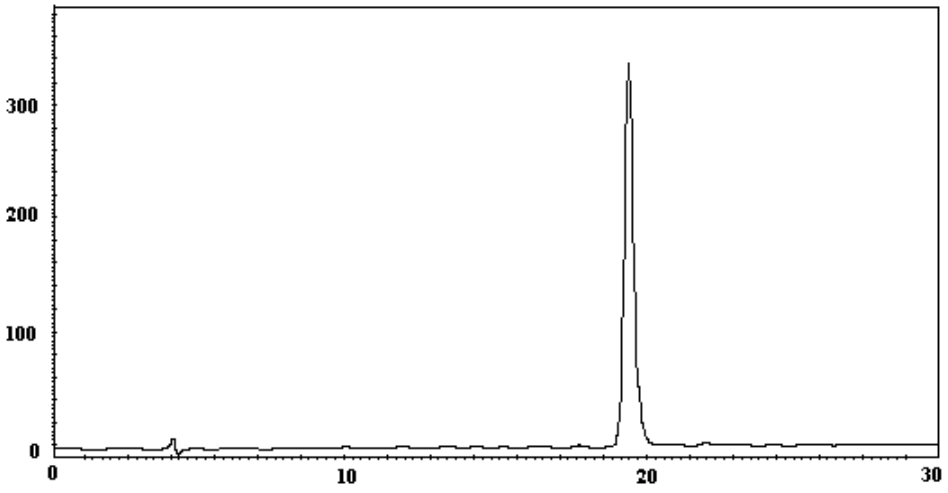


Figure S3. RP-HPLC chromatogram of β^2-hexapeptide 2

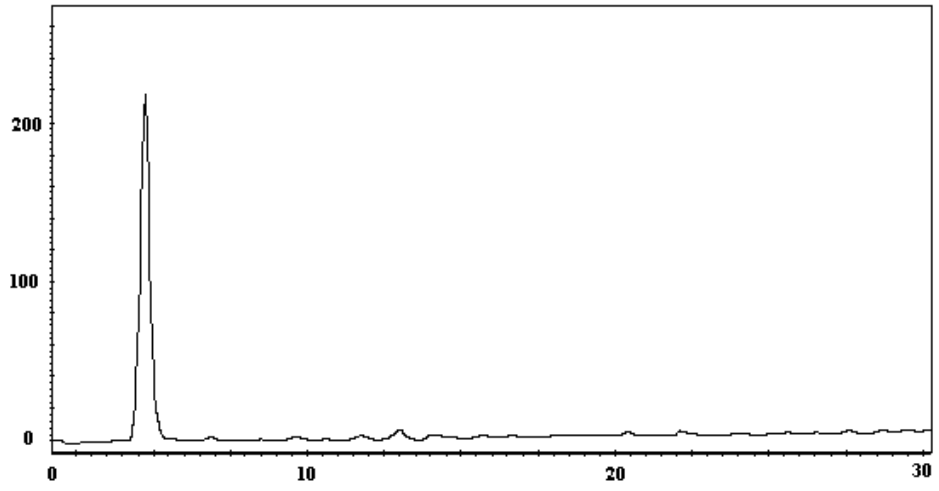


Figure S4. RP-HPLC chromatogram of β^2-hexapeptide 3

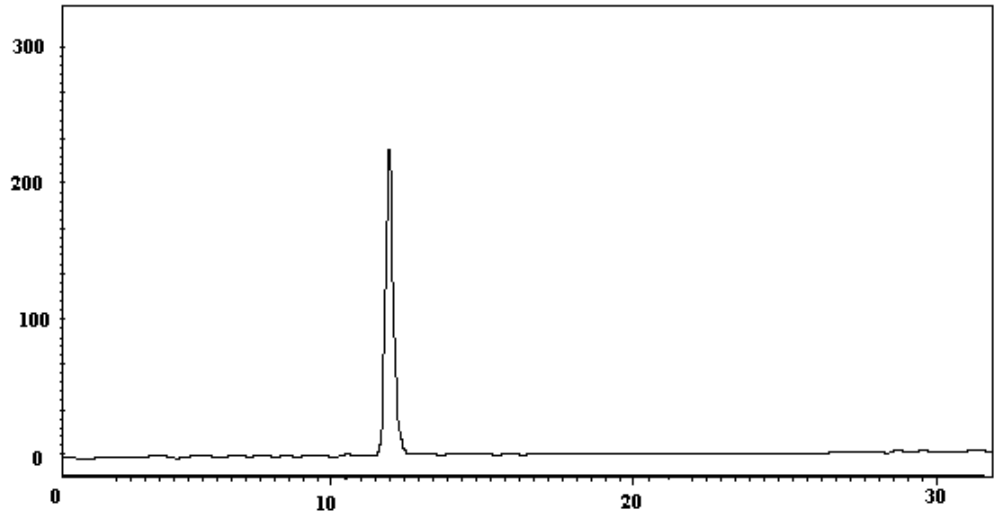
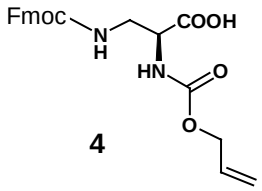


Figure S5. RP-HPLC chromatogram of N^{α}-Alloc-N^{β}-Fmoc-L-diaminopropionic acid 4



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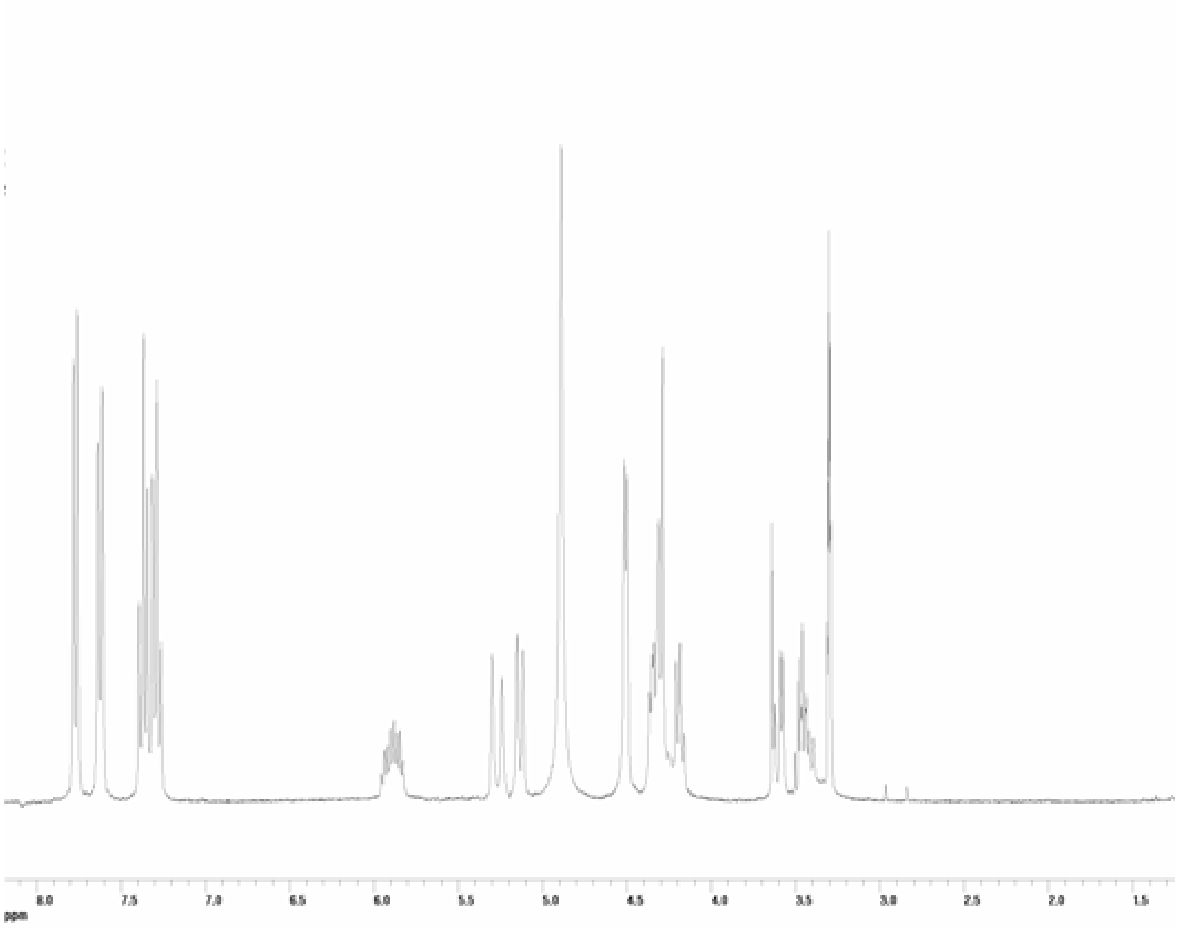

Figure S6. ^{1}H-NMR spectrum of N$^{\alpha}$-Alloc-N$^{\beta}$-Fmoc-L-diaminopropionic acid 4

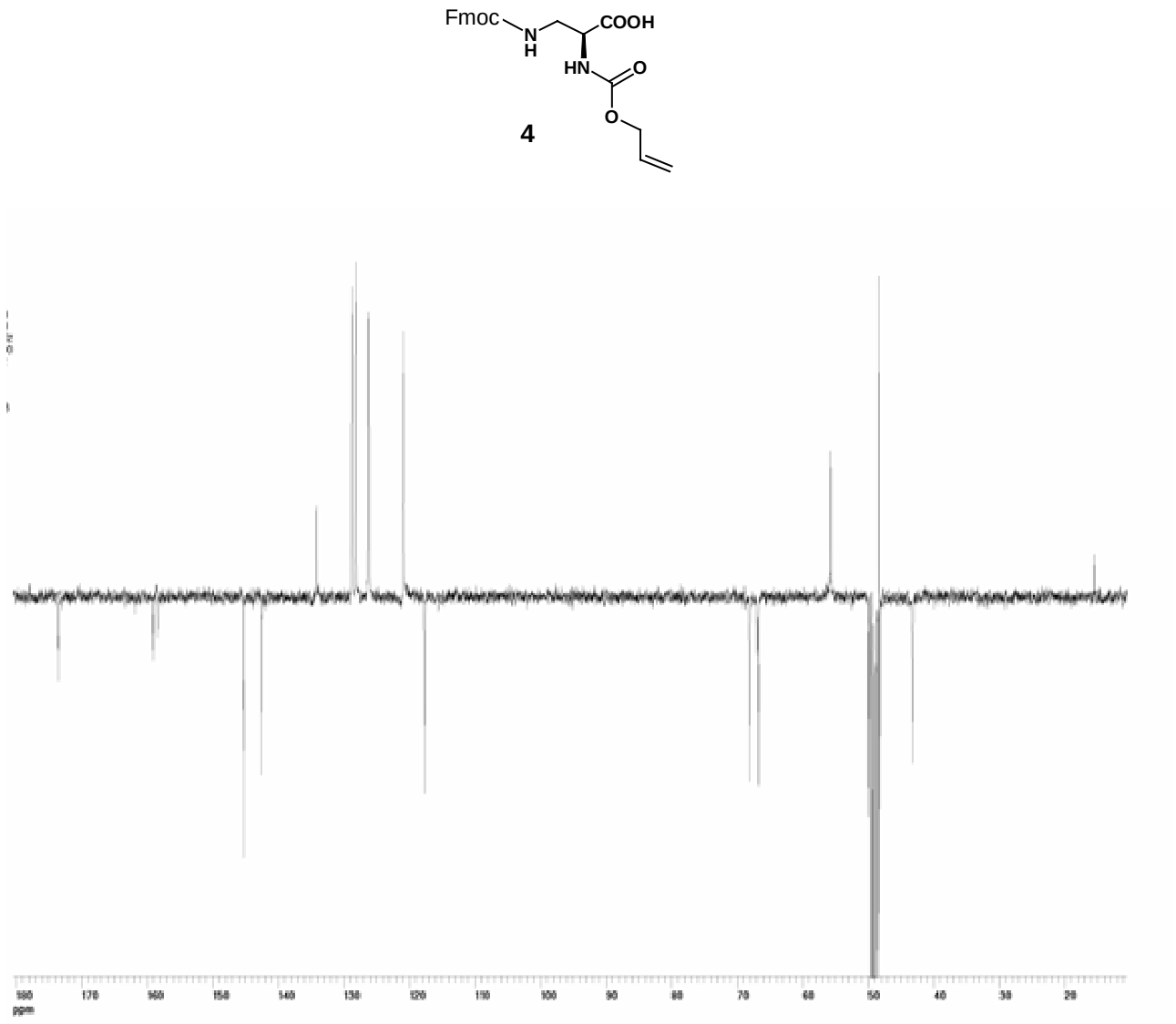

Figure S7. ^{13}C-NMR spectrum of N$^{\alpha}$-Alloc-N$^{\beta}$-Fmoc-L-diaminopropionic acid 4

References

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