Supporting information for

Orthogonal strategy for the synthesis of dual-functionalised β^3-peptide based hydrogels

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Contents

General

Unless otherwise noted, all reactions were carried out using oven-dried glassware under an atmosphere of nitrogen. Air- and moisture-sensitive reagents were transferred using oven dried, gas-tight glass syringes or PTFE cannulae through rubber septa.

Melting points were obtained on a Stuart Scientific SMP3 melting point apparatus (Stuart Scientific, Stone, Staffordshire, UK), and are reported uncorrected to the nearest degree Celsius.

Optical rotations were obtained using a PolAAR 2001 automatic polarimeter (Optical Activity Ltd., Huntingdon, Cambridgeshire, United Kingdom), using a 10 cm cell, at a wavelength of 589 nm (sodium D line), and are quoted as $[\alpha]_D$, concentration *c* (g/100mL).

^{1}H NMR spectra were obtained at 400 MHz on Bruker Avance III 400 spectrometer; or 600 MHz on a Bruker Avance III 600 spectrometer (Bruker BioSpin Corporation, Billerica, Massachusetts, USA and Varian Inc., Palo Alto, California, USA). The residual solvent peak (7.26 ppm for $CDCl_3$ or 1.94 ppm for CD_3CN) was used as an internal reference. Resonances are reported as follows: chemical shift in parts per million, multiplicity, coupling constant, number of protons. Multiplicity is denoted as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) or broad (br), or appropriate combinations of these. All integrals are rounded to the nearest integer.

^{13}C NMR spectra were recorded at 100 MHz on a Bruker Avance III 400; or 150 MHz on a Bruker Avance III 600 spectrometer, with the solvent peak (77.16 ppm for $CDCl_3$ or 1.32 ppm and 118.26 ppm for CD_3CN) used as an internal reference. Resonances are reported as: chemical shift in parts per million.

Mass spectra were acquired using an Agilent 1100 MSD SL ion trap mass spectrometer (Agilent Technologies, Santa Clara, California, USA). High resolution mass spectra were recorded using an Agilent G1969A LC-TOF system (Agilent Technologies, Santa Clara, California, USA), with reference mass correction and a capillary voltage of 4000V for ESI; and an Agilent 7200 Q-TOF GCMS system.

Absorption infra-red (IR) spectra were obtained using a Bruker Equinox IFS55 FTIR spectrometer (Bruker Optics Inc., Billerica, Massachusetts, USA), fitted with a mercury-cadmium-telluride detector and a Specac Golden Gate single reflection diamond ATR window, using 50 scans at a resolution of 4 cm^{-1}. Absorption bands are reported as: peak wavenumber (cm^{-1}), intensity. Intensity is described as strong (s), medium (m) or weak (w).

Analytical thin layer chromatography was carried out using Merck Silica Gel 60 F254 aluminium-backed plates. Visualisation was achieved using ultraviolet light or by staining with alkaline $KMnO_4$. Flash chromatography was performed using Grace Davison Davisil® silica gel 40–63 μm (W. R. Grace & Co., Columbia, Maryland, USA) or Silicycle SiliaFlash® P60, 230-400 mesh (Silicycle Inc., Quebec City, Quebec).

Commercially available chemicals were purchased from Sigma-Aldrich Co. Milwaukee, Wisconsin, USA), AK Scientific Inc. (Union City, California, USA), Merck KGaA (Darmstadt, Hesse, Germany), Chem-Supply Pty. Ltd. (Gillman, South Australia, Australia), Oakwood Products, Inc. (West Columbia, South Carolina, USA), Strem Chemicals Inc. (Newburyport, Massachusetts, USA), GL Biochem (Shanghai, China) or Iris Biotech Gmbh (Marktredwitz, Germany).

Anhydrous solvents were prepared by the following standard procedures: $^{i}Pr_2EtN$ and CH_2Cl_2 were distilled from CaH; THF was distilled from sodium; DMF was stored over 4 Å molecular sieves. $CDCl_3$ used for NMR spectroscopy was stored over silver and anhydrous K_2CO_3.

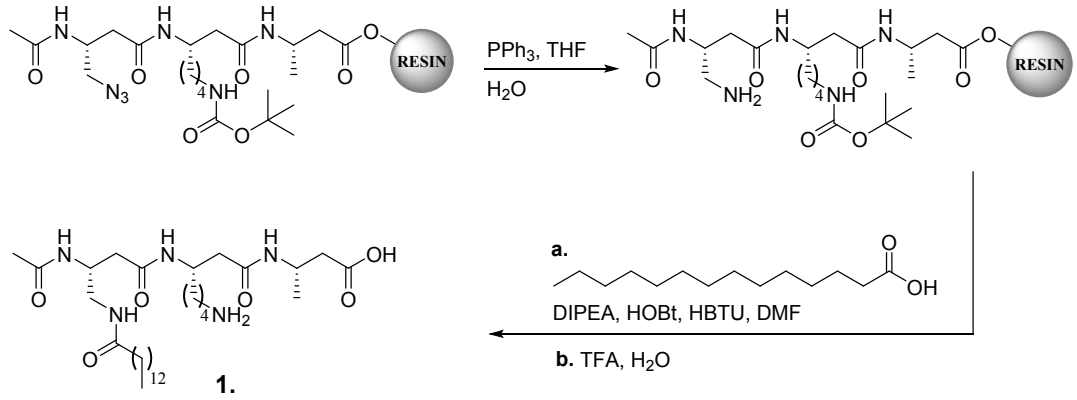
Preparative High Performance Liquid Chromatography (HPLC) was performed using a Hewlett-Packard 1200 series HPLC system (Agilent Technologies, CA), fitted with an Agilent 1100/1200 multiple wavelength UV detector. Samples were injected onto a reverse-phase preparative (C18, 300 Å, 5 μm, 10 mm x 250 mm) column (W. R. Grace & Co., Columbia, Maryland, USA) and detected at wavelengths of 214 nm and 280 nm. Analytical HPLC was performed using a Hewlett-Packard 1100 series HPLC system (Agilent Technologies, CA), fitted with an Agilent 1100 variable wavelength UV detector. The samples were injected onto a reverse-phase Vydac™ (W. R. Grace & Co., Columbia, Maryland, USA) analytical (C18, 300 Å, 5 μm, 4.6 mm x 150 mm) column and detected at a wavelength of 214 nm.

Transmission electron microscopy (TEM) was performed on a Hitachi H-7500 TEM (Hitachi High-Technologies, Tokyo, Japan) or a Tecnai TEM (FEI Hillsboro, Oregon, USA) operating at 100keV and 120Kev respectively. Sample solution (~2 μL) was deposited on a carbon-coated copper grid for 30 minutes. The excess solution was then removed with a piece of filter paper, and the sample was subsequently left to dry under ambient conditions.

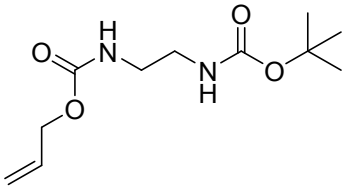
Atomic force microscopy (AFM) was performed on a Nanoscope IV AFM with a Multimode head (Veeco, Santa Barbara, CA, USA) using a vertical engage 'E' scanner. 2 μL of peptide solution in water (0.25mg mL^{-1}) was placed on a clean mica surface. The sample was incubated under a petri dish for 30 minutes and then dried with gentle stream of N_2 gas Images were obtained in H_2O *via* tapping mode with NSC-15 'B' silicon cantilevers (Micromasch, Tallinn, Estonia) with a nominal force constant of 40 N/m. Topographic, phase and amplitude images at a resolution of 512 x 512 were simultaneously obtained using scan frequency of 1 Hz with typical scan sizes of 5 μm x 5 μm and 2 μm x 2 μm. Images were processed with a sequence of plane fitting and offset flattening using Gwyddion 2.29 (www.gwyddion.net) software.

Rheological studies were conducted using Anton Paar rheometer (Physica MCR 501) with an 8 mm parallel plate and a plate gap of 0.1 mm. The temperature was maintained at 35 °C for all the experiments. For the hydrogel formation and frequency sweep experiments, the strain was set constant (1%). The storage (elastic) and loss (viscous) moduli were reported as a function of time. When the hydrogel reached its stable state, its response to frequency variation was examined. All of the experiments were repeated three times and the average values reported. To investigate the self-healing properties of the hydrogel, its network was exposed to a high strain value of 100% for 1 min and the hydrogel recovery properties was studied by decreasing the strain value back to 1%. The step strain experiment was performed for four times, each time after 10 minutes.

Scheme S1. Synthesis of C_{14} acylated *N*-acetyl β^3-tripeptide **1**



Synthesis of *N*-Boc *N*-Alloc ethylenediamine **s1**



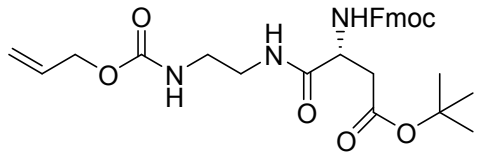
Allylchloroformate (5.1 mL, 48 mmol) was added dropwise to a solution of *N*-Boc ethylenediamine **2** (3.1 g, 19.4 mmol) in a mixture of anhydrous THF (15 mL) and pyridine (15 mL) at 0 ºC and the suspension stirred at room temperature for 18 hours. The reaction mixture was filtered and concentrated under vacuum. The recovered solution was then diluted with CH_2Cl_2 (150 mL) and washed with saturated aqueous solution of NH_4Cl (2 x 100 mL), saturated aqueous solution of $NaHCO_3$ (2 x 100 mL), and H_2O (100 mL) and further concentrated under vacuum to afford **s1** as an off-white solid (4.25 g, 90 % yield). Analytical data was in accordance with the literature values.[1]

m.p. 110 – 112 ˚C (lit. 111 – 112 ˚C)[1]

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 – 5.85 (m, 1H); 5.30 (dd, *J* 17.2 Hz, 1.5 Hz, 1H); 5.21 (dd, *J* 10.4 Hz, 1.2 Hz, 1H); 5.11 (brs, 1H); 4.82 (brs, 1H); 4.56 (d, *J* 5.3 Hz, 2H); 3.34 – 3.20 (m, 4H); 1.44 (s, 9H).

[1] K. B. Jensen, T. M. Braxmeier, M. Demarcus, J. G. Frey, and J. D. Kilburn, *Chem. Eur. J.*, 2002, **8**, 1300-1309.

Synthesis of (*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide β-*tert*-butyl ester **s2**



To a solution of *N*-Boc *N*-Alloc ethylenediamine **s1** (4.2 g, 17.2 mmol) in CH_2Cl_2 (120 mL) was slowly added CF_3COOH (50 mL) and stirred for 2 hours at room temperature. Solvent was then evaporated under reduced pressure. Residual CF_3COOH was azeotroped with toluene (2 x 50 mL) to give thick orange foam. The viscous residue was redissolved in EtOAc (150 mL) and washed with saturated aqueous $NaHCO_3$ (25 mL). Solid $NaHCO_3$ was then added to the residual aqueous layer to create a suspension which was extracted with EtOAc (2 x 50 mL). The organic layer was concentrated under reduced pressure to afford pale yellow viscous oil *N*-Alloc ethylenediamine **3** (2.4 g, 97 % yield).

To a solution of Fmoc-D-Asp(O^{t}Bu)-OH **4** (6.0 g, 14.6 mmol) in CH_2Cl_2 (120 mL) were added *N*-Alloc ethylenediamine **3** (2.4 g, 16.65 mmol), HOBt hydrate (2.5 g, ~16 mmol), DIPEA (3.2 mL, 18.4 mmol) under an atmosphere of N_2 at 0 °C. The mixture was stirred for 10 minutes and EDCI・HCl (3.5 g, 18.3 mmol) was added in one portion. The reaction was stirred at room temperature for 12 h. Solvent was evaporated under reduced pressure and the residue was diluted with EtOAc (400 ml). The ensuing solution was washed successively with saturated aqueous NH_4Cl (2 x 100 mL), saturated aqueous $NaHCO_3$ (2 x 100 mL), and H_2O (2 x 100 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was resuspended in EtOAc (250 ml) and the suspension filtered. The filtrate was dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (EtOAc–hexane 3:2) to yield **s2** as a white solid (4.2 g, 54 %).

m.p. 152 – 154 °C

Specific rotation $[\alpha]_D^{25}$ -21 (*c* 1 in $CHCl_3$)

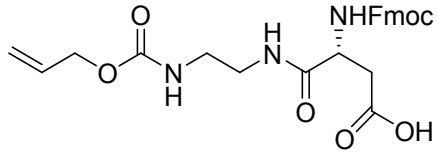
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, *J* 7.5 Hz, 2H); 7.62 – 7.53 (m, 2H); 7.39 (t, *J* 7.5 Hz, 2H); 7.29 (t, *J* 7.5 Hz, 2H); 7.05 (s, 1H); 6.10 (d, *J* 8.2 Hz, 1H); 5.90 – 5.81 (m, 1H); 5.58 (s, 1H); 5.25 (d, *J* 17.2 Hz, 1H); 5.15 (d, *J* 10.4 Hz, 1H); 4.56 – 4.48 (m, 3H); 4.46 – 4.36 (m, 2H); 4.20 (t, *J* 6.6 Hz, 1H); 3.45 – 3.34 (m, 2H); 3.34 – 3.24 (m, 2H); 2.87 (dd, *J* 16.2 Hz, 3.6 Hz, 1H); 2.69 (dd, *J* 16.3 Hz, 5.1 Hz, 1H); 1.44 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 170.9, 156.8, 156.2, 143.7, 141.3, 132.9, 127.8, 127.1, 125.1, 120.0, 117.6, 81.8, 67.2, 65.6, 51.4, 47.1, 40.5, 40.1, 37.6, 28.0, 27.9.

HRMS calculated for ($C_{29}H_{35}N_3O_7Na^+$) *m/z* = 560.2367, found 560.2371.

IR (ATR, neat) ν_{max} 3294 s, 3067 w, 2978 w, 2939 w, 1724 m, 1690 s, 1654 s, 1532 s, 1449 w, 1392 w, 1367 w, 1233 m, 1147 m, 1084 w, 1034 w, 992 w, 936 w, 846 w, 757 w, 737 w, 645 w, cm^{-1}.

Synthesis of (*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide **5**



(*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide β-*tert*-butyl ester **s2** (3.00 g, 5.6 mmol) was dissolved in a CH_2Cl_2 (20 mL). The reaction was cooled to 0 °C and CF_3COOH (10 mL) was added slowly over a period of 10 minutes. The reaction solution was then warmed to room temperature and stirred for 2 hours. The reaction was diluted with CH_2Cl_2 (20 mL) and evaporated in vacuo. The viscous residue was co-evaporated with CH_2Cl_2 (3 x 30 mL) to remove any trace CF_3COOH. The product was precipitated as a white solid by diluting with hexanes (150 mL), filtered, and air dried. The white solid was resuspended in 1:1 EtOAc-MeOH (200 mL), the suspension warmed to 50-60 °C, and followed by hot filtration. The solvent was removed under reduced pressure to afford **5** as a white solid (2.5 g, 93 % yield).

m.p. 156 – 158 °C

Specific rotation $[\alpha]_D^{25}$ +17 (*c* 1 in DMF)

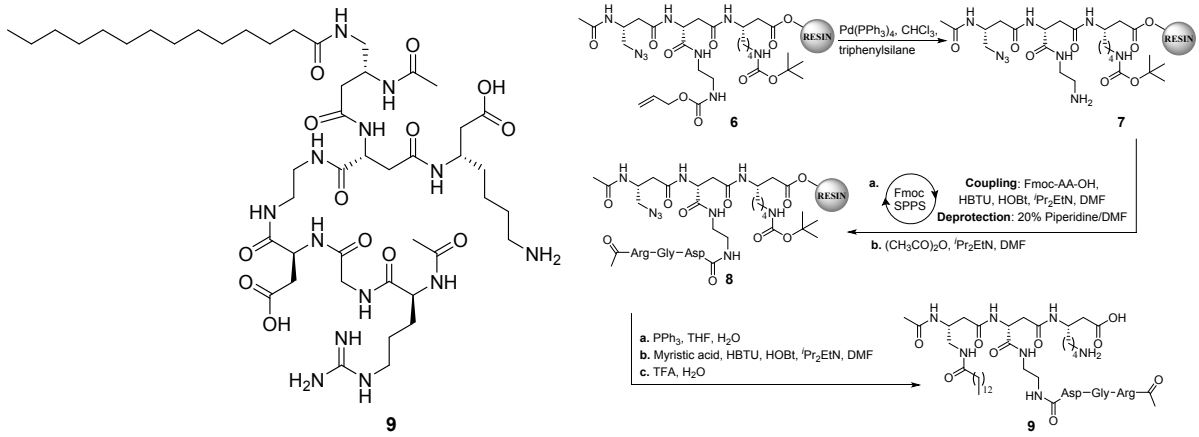
^{1}H NMR (600 MHz, CD_3CN) δ 7.84 (d, *J* 7.6 Hz, 2H); 7.67 (d, *J* 7.3 Hz, 2H); 7.43 (t, *J* 7.4 Hz, 2H); 7.35 (t, *J* 7.4 Hz, 2H); 6.96 (s, 1H); 6.12 (s, 1H); 5.95 – 5.84 (m, 1H); 5.68 (s, 1H); 5.25 (d, *J* 17.2 Hz, 1H); 5.14 (d, *J* 10.4 Hz, 1H); 4.51 – 4.44 (m, 2H); 4.41 (dd, *J* 10.6 Hz, 7.1 Hz, 1H); 4.40 – 4.32 (m, 2H); 4.25 (t, *J* 6.9 Hz, 1H); 3.30 – 3.19 (m, 2H); 3.17 (q, *J* 5.7 Hz, 1H); 2.78 (d, *J* 13.2 Hz,1H); 2.68 (d, *J* 10.7 Hz, 1H).

^{13}C NMR (150 MHz, CD_3CN) δ 172.7, 172.2, 157.7, 157.2, 145.2, 142.3, 134.7, 128.8, 128.2, 126.3, 121.1, 117.5, 67.7, 66.0, 52.7, 48.2, 41.3, 40.6, 36.9.

HRMS calculated for ($C_{25}H_{27}N_3O_7Na^+$) *m/z* = 504.1741, found 504.1740.

IR (ATR, neat) ν_{max} 3298 s, 3066 w, 2950 w, 1688 s, 1651 s, 1533 s, 1449 w, 1276 m, 1236 m, 1148 w, 1105w, 1084 w, 994 w, 934 w, 776 w, 756 w, 738 w, 643 w, cm^{-1}.

Peptide synthesis



The peptide was synthesised on a 0.3 mmol scale using standard Fmoc chemistry on Wang resin (0.1 mmol/g loading). The resin was swollen in DMF (4 mL) and then soaked in Fmoc-protected β amino acid (2.1 eq. to resin loading), dissolved in DMF (4 mL) along with HBTU (2 eq. to resin loading), HOBt (2 eq. to resin loading), DMAP (10 mol %) and DIPEA (3 eq. to resin loading), overnight with gentle agitation. The resin was thoroughly washed with DMF (3 x 4 mL) and the Fmoc protecting group on the amino acid was removed by soaking the resin twice in 20 % piperidine, with 0.1 M HOBt, in DMF (4 mL) for 15 minutes each. The resin was washed with DMF (3 x 5 mL), soaked in Fmoc-protected amino acid (2.1 eq. to resin loading), dissolved in DMF (4 mL) along with HBTU (2 eq. to resin loading), HOBt (2 eq. to resin loading) and DIPEA (3 eq. to resin loading), for 2 hours. β Peptide elongation cycle was then repeated until the sequence was complete. After removing the terminal Fmoc protecting group on the peptide, the resin was treated with a solution of 10 % v/v acetic anhydride and 2.5 % v/v DIPEA in DMF (4 mL) for 30 minutes to afford an acetyl-capped N-terminus. The resin was washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), Et_2O (2 x 4 mL), air dried for 10 minutes, and transferred to a 50 mL vial for further manipulation.

Derivatisation of the *N*-acetyl β^3-tripeptide **7**, on solid support, was preceded by the selective cleavage of the allyloxycarbonyl substituent. The resin (0.3 mmol) was thoroughly dried under vacuum for 1 hour at 40 °C. To a 50 mL vial was added $CHCl_3$ (12 mL) which was rigorously degassed by bubbling a stream of argon. A portion of the degassed $CHCl_3$ (~2 mL) was then used to swell the resin, contained in a separate 50 mL vial pre-purged of air with argon. $PhSiH_3$ (700 µL) was added to the remaining $CHCl_3$ (~10 mL) whilst still bubbling with a stream of argon. $Pd(PPh_3)_4$ (650 mg, 0.56 mmol) was then added and the mixture was shaken gently until a homogeneous solution was achieved. The resin was then soaked in the $Pd(PPh_3)_4$ solution for 2 hours, with gentle agitation, filtered through a sintered glass funnel, and washed with CH_2Cl_2 (3 x 4 mL) and DMF (3 x 4 mL) to remove the catalyst.

The resin was soaked in Fmoc-protected α amino acid (3.1 eq. to resin loading), dissolved in DMF (4 mL) along with HBTU (3 eq. to resin loading), HOBt (3 eq. to resin loading) and DIPEA (4.5 eq. to resin loading), for an hour. The resin was thoroughly washed with DMF (3 x 4 mL) and the Fmoc protecting group on the amino acid was removed by soaking the resin twice in 20 % piperidine, with 0.1 M HOBt, in DMF (4 mL) for 15 minutes each. α-Peptide elongation cycle was then repeated until the sequence was complete. After removing the terminal Fmoc protecting group on the peptide, the resin was treated with a solution of 10 % v/v acetic anhydride and 2.5 % v/v DIPEA in DMF (4 mL) for 30 minutes to afford an acetyl-capped N-terminus. The resin was washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), Et_2O (2 x 4 mL), air dried for 10 minutes, split in half, and transferred to a 15 mL vial for further manipulation.

To facilitate attachment of the desired aliphatic chain, reduction of the azido-alanine residue on the β^3-peptide **8** was effected on solid support. The resin (0.15 mmol) was swollen in THF (1 mL) and then soaked in a solution of PPh_3 (4 eq. to resin loading) in THF (2.5 mL) and H_2O (50 µL), sealed in a capped vial and heated to 65 °C, for 4 hours. The resin was filtered through a sintered glass funnel and washed with THF (2 x 4 mL) and DMF (2 x 4 mL). The resin was then soaked in myristic acid (3.1 eq. to resin loading), dissolved in DMF (4 mL) along with HBTU (3 eq. to resin loading), HOBt (3 eq. to resin loading) and DIPEA (4.5 eq. to resin loading), for 2 hours. The resin was subsequently washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), Et_2O (2 x 4 mL), air dried for 10 minutes,

Cleavage was performed on the resin (0.15 mmol), by treating the resin with a cleavage solution (10 mL) comprising of H_2O (2.5 % v/v), triisopropylsilane (2.5 % v/v) and ethanedithiol (0.5 % v/v) in CF_3COOH, for 3 hours. CF_3COOH was then evaporated under a stream of N_2 and the peptide was precipitated by addition of Et_2O (50 mL). The precipitate was filtered and redissolved in 50 % aqueous CH_3CN for lyophilisation. The peptide was redissolved in 60 % aqueous CH_3CN (5 mL) and purified by injecting the sample onto a reverse-phase preparative column, eluted over a 60 min gradient from 10 to 70 % solvent B, (solvent A: 0.1 % TFA/H_2O; solvent B: 0.1 % TFA/CH_3CN) with a flow rate of 6 mL/min. The fractions were collected and analysed for purity by injecting the samples onto a reverse-phase analytical column, eluted over a 45 min gradient from 0 to 75 % solvent B, (solvent A: 0.1 % TFA/H_2O; solvent B: 0.1 % TFA/CH_3CN) with a flow rate of 1 mL/min. Pure fractions were pooled to afford peptide **9** (31 mg) in 20 % yield. ($C_{47}H_{87}N_{13}O_{13}^{2+}$) *m/z* = 520.8, found 520.7; ($C_{47}H_{84}N_{13}O_{13}^{-}$) *m/z* = 1038.6, found 1038.4.

High resolution microscopy

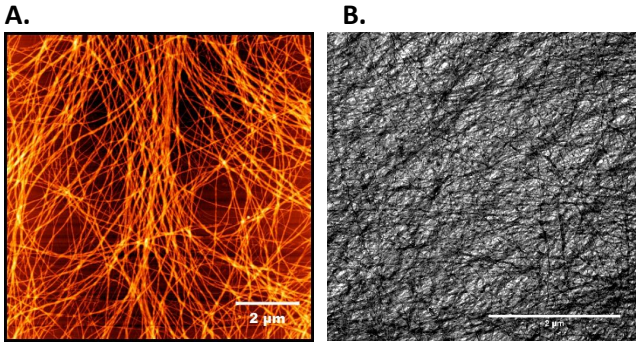


Figure S1. High resolution microscopy of peptide **9** observed using **A)** AFM and **B)** TEM. The scale bar indicates 2 µm.

Rheology characterization

Rheological studies of peptide **9** hydrogel

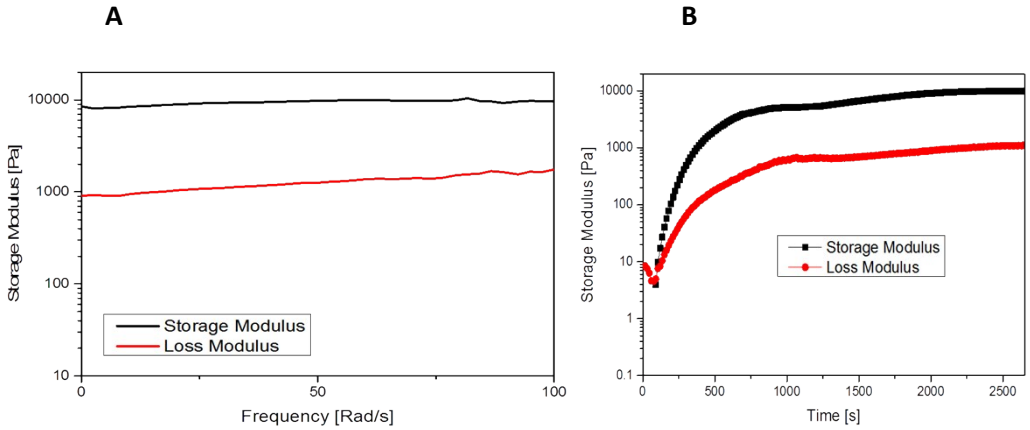


Figure S2. A) Frequency dependent oscillatory shear rheology of peptide **9** hydrogel, formed at a concentration of 9.6 mM (10 mg/mL) in PBS, performed using a dynamic frequency sweep from 0 Rad/s to 100 Rad/s (strain=1 %; time=20 min. 35 °C) **B**) Oscillatory rheological properties of the hydrogel, 10 mg/mL in PBS, at 35 °C (with 1 % strain at 1 Hz).

Rheological studies on hybrid peptide hydrogel of **1** with 5 wt % peptide **9**

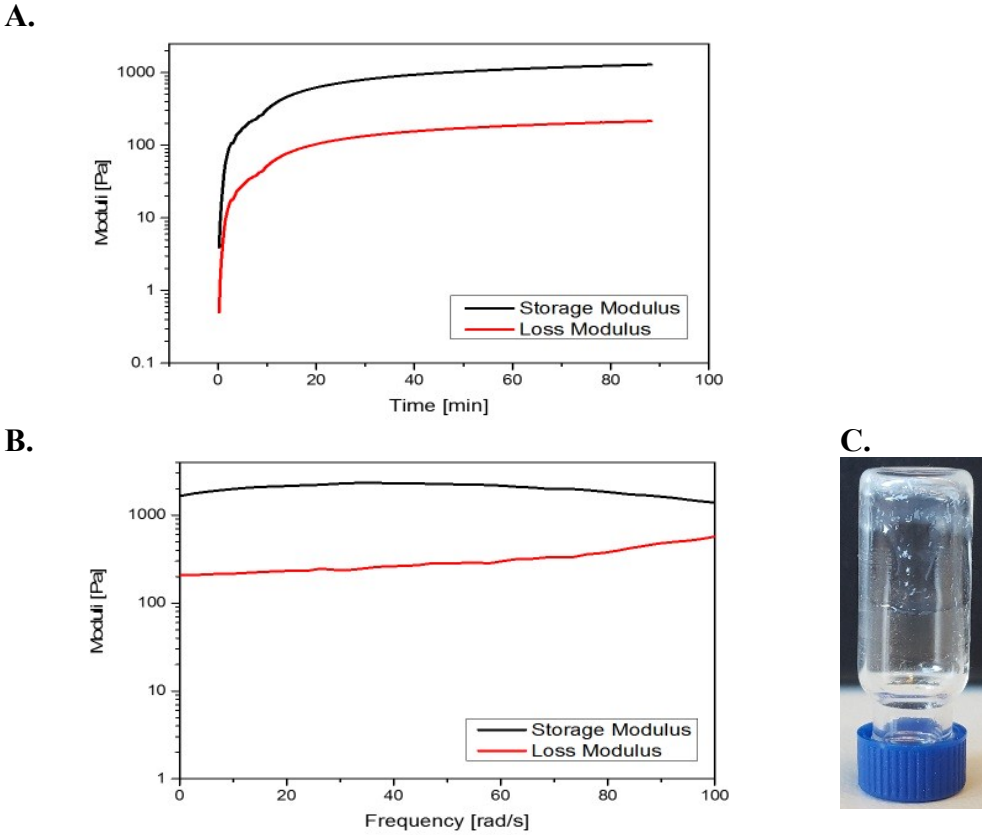


Figure S3. Rheological properties of hybrid hydrogel made from a mixture of 5% RGD peptide **9** and 95% C_{14} peptide **1**. **A)** Elastic modulus of 5 to 7 times higher than viscous modulus showed a completely solid-like hydrogel. The hydrogel reached a value of 1.3kPa after 40 minutes, Freq. = 1Hz, Strain = 1%; **B)** Viscoelastic

behaviour of the hydrogel over the entire range of frequencies (1-100 rad s^{-1}). Strain = 1%; Temperature = 35°C. **C)** Inversion test showing the self-supporting hydrogel.

Cell Viability Analysis

L929, mouse fibroblastic cell line, was used to examine the viability of cells on the hydrogel. All experiments were performed in triplicates. The hybrid hydrogel was formed by adding 2, 5, 8, 10, 15, 25, 50 and 75 wt % of peptide **9** to peptide **1** and dissolved in PBS to reach the concentration of 10 mg mL^{-1} and were added to each well of a 96-well plate to completely cover the bottom of the well. The formed hydrogel was then incubated overnight. Control experiments were performed by adding the same population of cells on top of hydrogels composed only of peptide **1** or peptide **9** or into empty wells. To extract residual TFA, the formed hydrogels were equilibrated with PBS for three hours and the PBS was then extracted from the top of the hydrogel. Three wells were prepared for each condition. The cells were trypsinized to give a cell suspension and approximately 3,000 cells were added on top of each hydrogel. To determine cell viability, cells were stained with calcein AM (live cells) and ethidium homodimer (dead cells) after three days. Live and dead cells of each well were counted and the average ratio of live cells for each condition were compared to each other and TCPS.

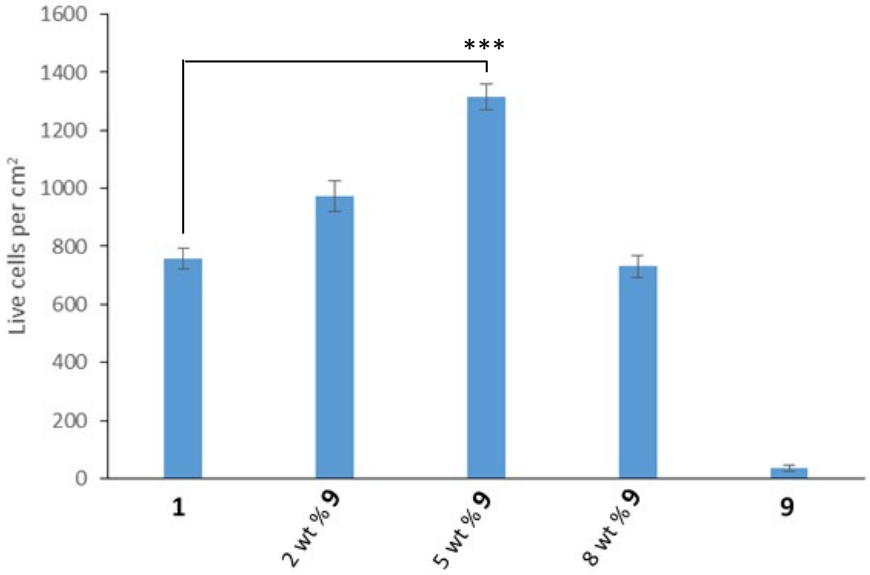


Figure S4. The number of live cells after 3 days culture. (*** means P≤.001)

Statistical Analysis

The number of viable cells on top of the hydrogels with different concentrations of RGD peptide **9** was counted and the number of live cells per cm^2 was reported as mean ± standard

deviation. To determine statistical significance, first equal variances in different groups were confirmed by Levene's Median Test and then the groups were compared using one-way ANOVA with Turkey's post hoc testing (GraphPad Prism Version 6.01).

MTT assay

To analyse the cell viability on top of our hydrogel quantitatively, the same set of samples as described in Cell Viability Analysis section was prepared and the calorimetric assay of reducing 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) to formazan blue by viable cells was performed. After three days of culture, the culture media was exchanged with culture media containing 10% MTT dye (5mg mL-1 in PBS) and incubated for four hours without agitation at 37˚C. The reaction was stopped by removing the media containing dye and adding dimethyl sulfoxide (DMSO). The formazan crystals were solubilized by shaking the plate for 5 min and the absorbance of each well was determined using plate reader at 570nm and 630nm. Optical density (OD) was calculated as the difference of absorbance at 570nm and 630nm. The cell viability was then calculated as a percentage of peptide 1 (control group as 100%) [1, 2].

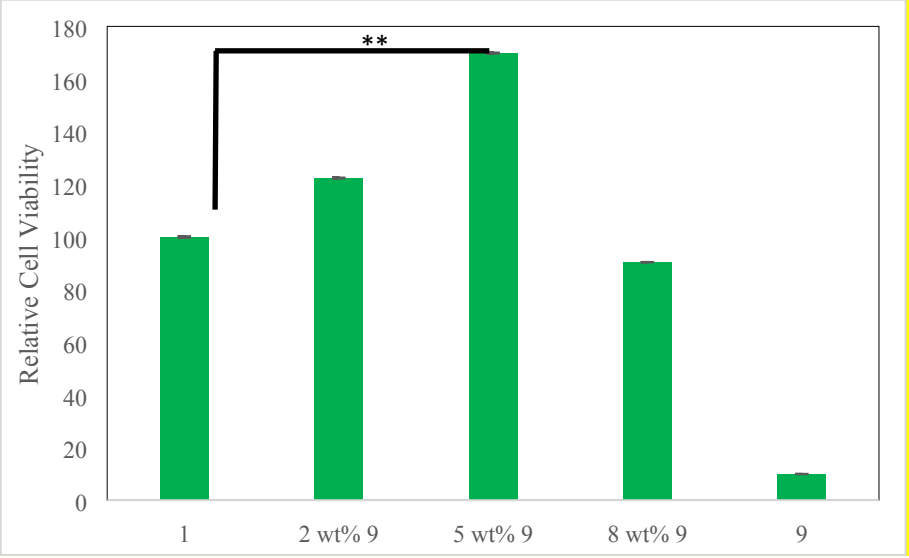


Figure S5. Relative cell viability on hybrid hydrogels with different concentrations of peptide 9 in comparison to peptide 1 after 3 days culture. (** means P≤0.01)

Statistical Analysis

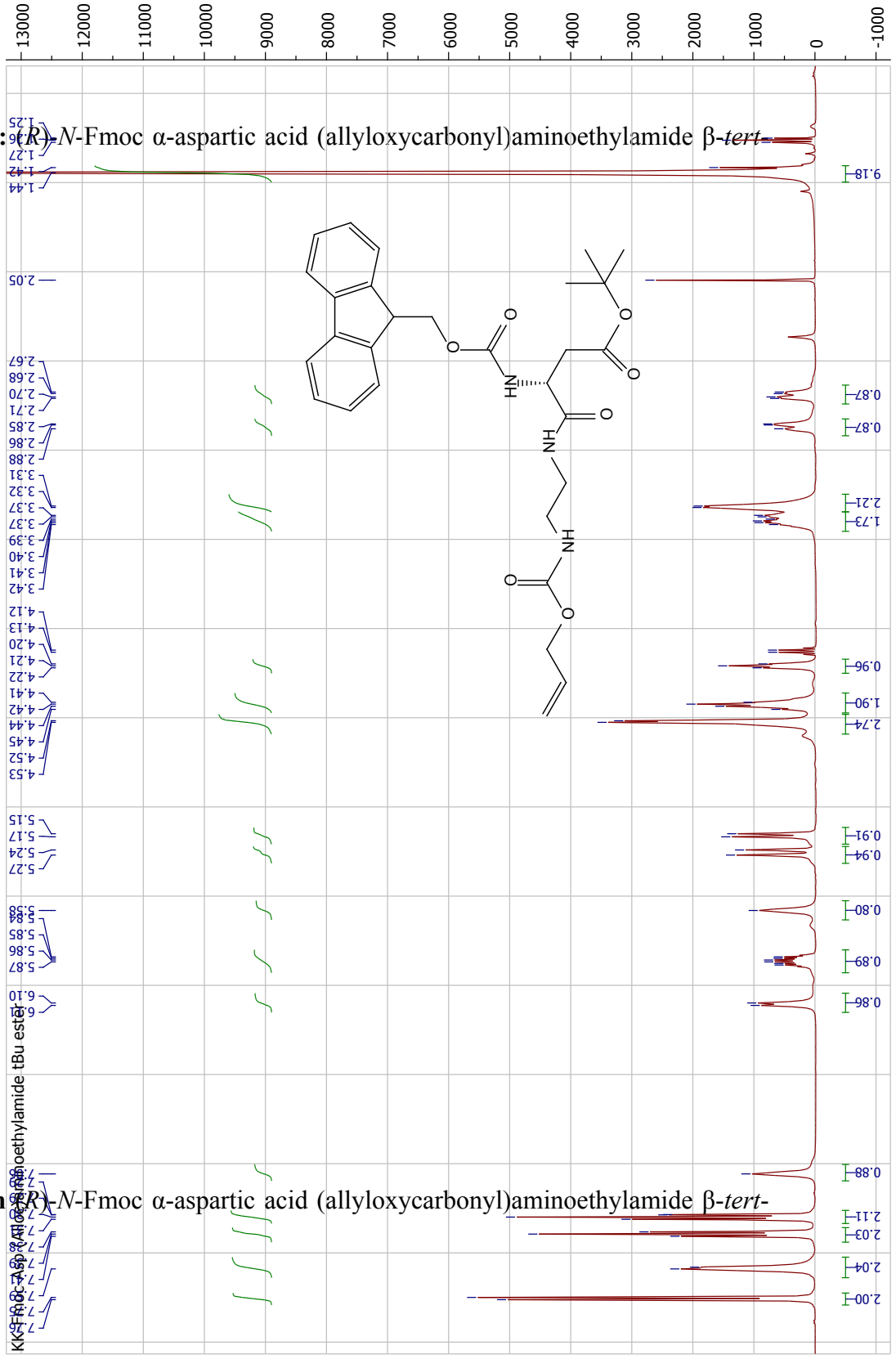
The OD number of cells was reported as mean ± standard deviation. To determine statistical significance, first equal variances in different groups were confirmed by Levene's Median Test and then the groups were compared using one-way ANOVA with Turkey's post hoc testing (GraphPad Prism Version 6.01).

References

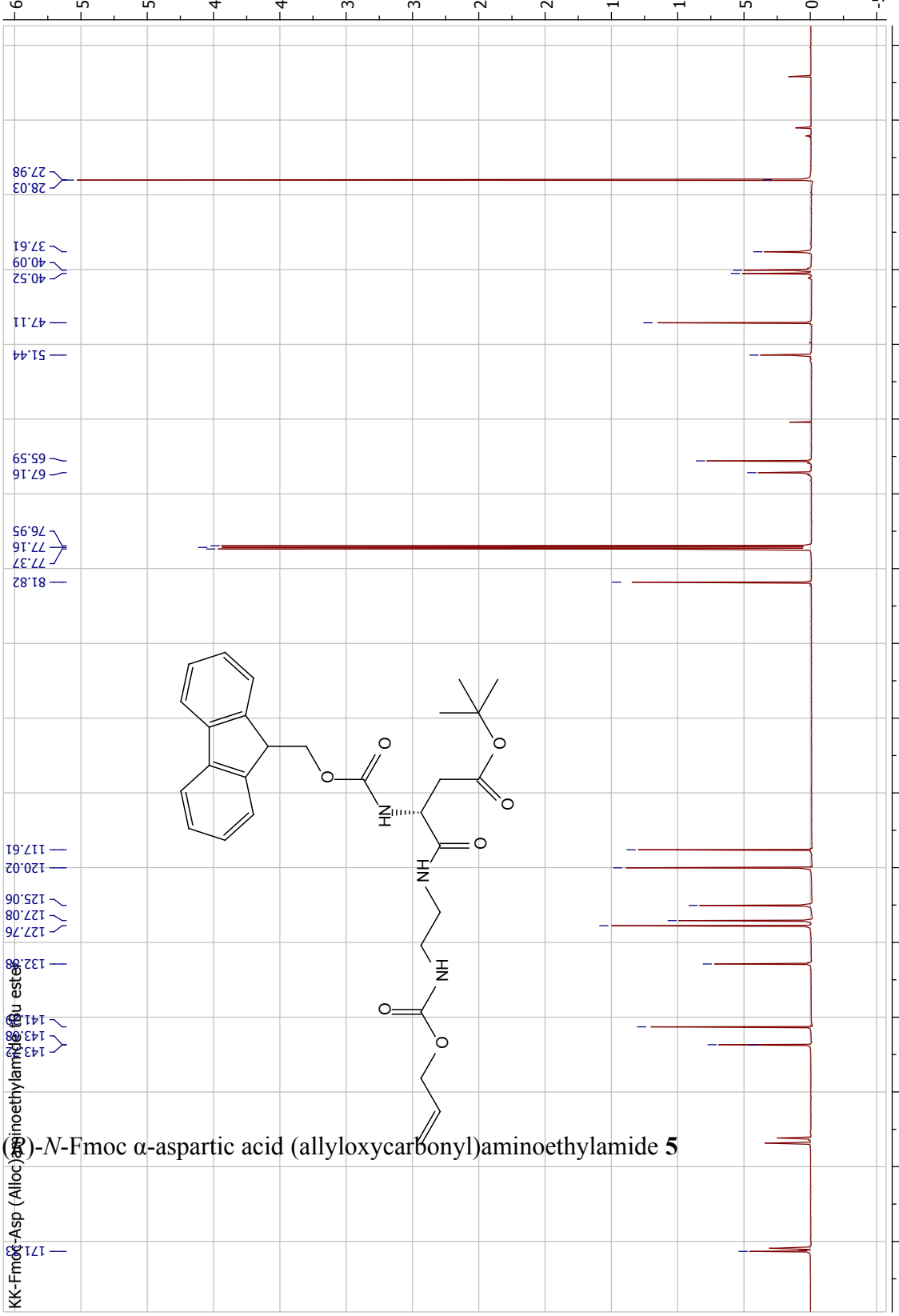
1. Mosmann, T., *Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.* J Immunol Methods, 1983. **65**(1-2): p. 55-63.
2. Poskus, L.T., et al., *Influence of post-cure treatments on hardness and marginal adaptation of composite resin inlay restorations: an in vitro study.* J Appl Oral Sci, 2009. **17**(6): p. 617-22.

NMR Spectra

^{1}H NMR spectrum: (*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide β-*tert*-butyl ester **s2**

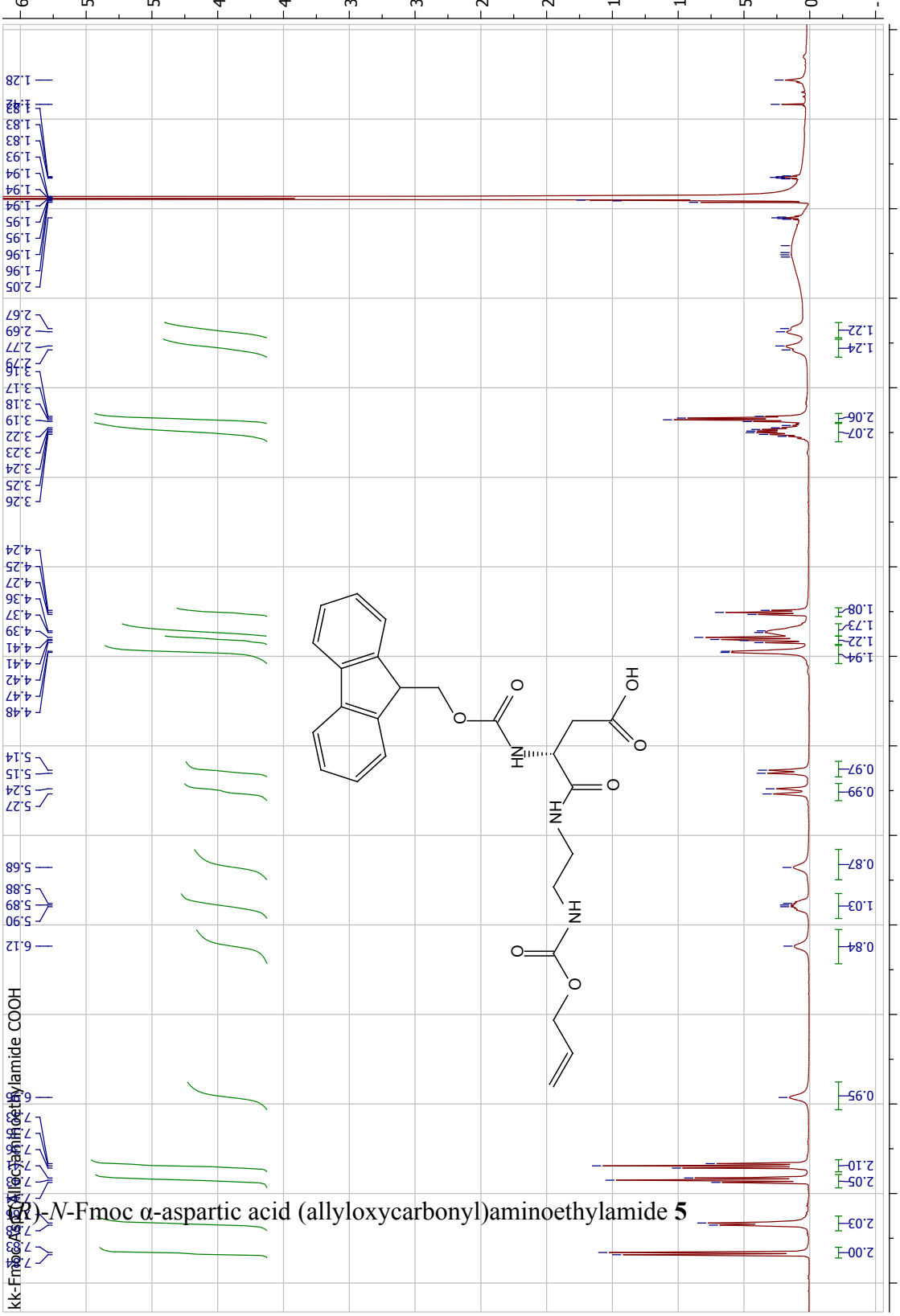


^{13}C NMR spectrum (*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide β-*tert*-butyl ester **s2**

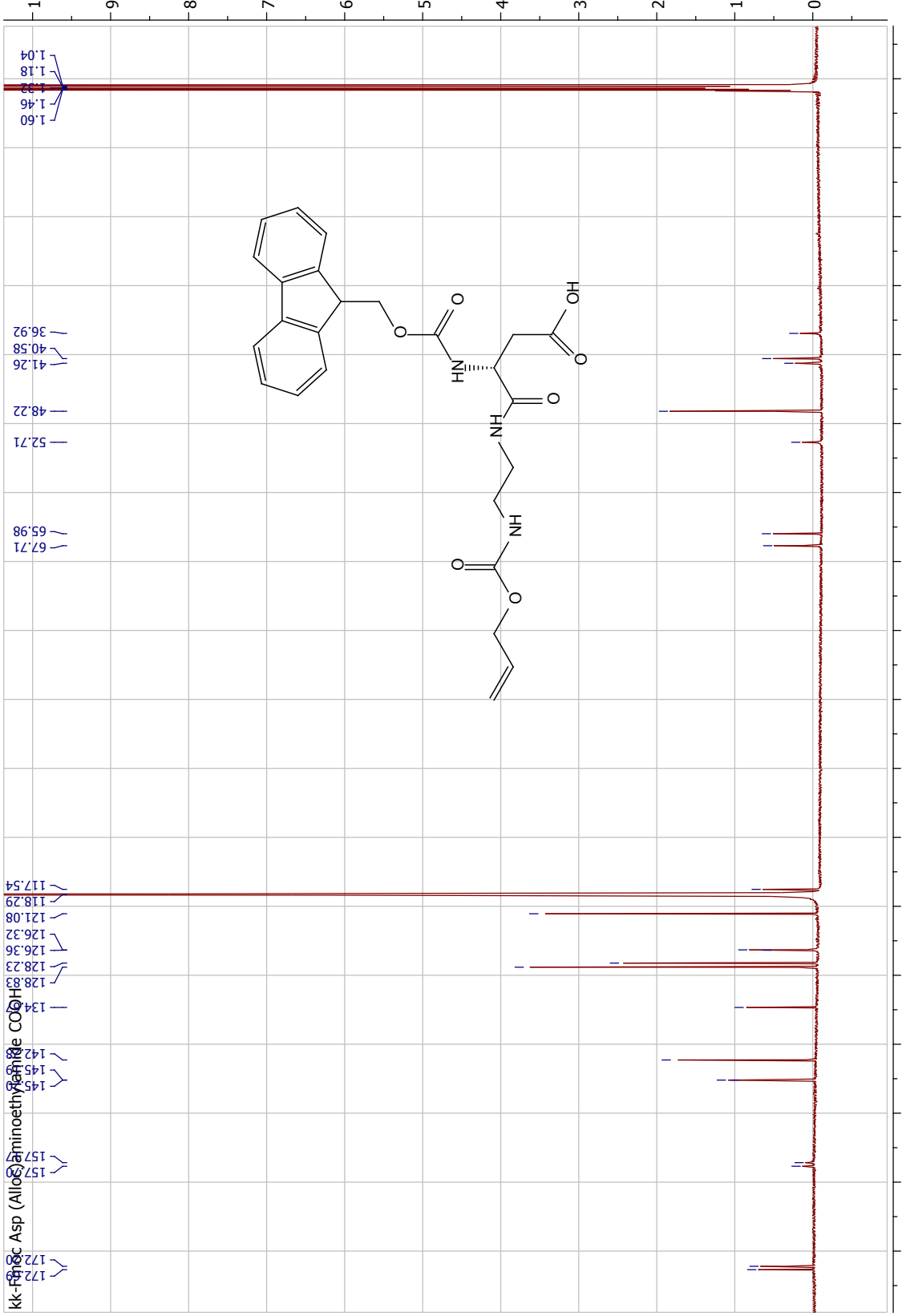


[1]H NMR spectrum: (*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide **5**

^{13}C NMR spectrum: (*R*)-*N*-Fmoc α-aspartic acid (allyloxycarbonyl)aminoethylamide **5**



Peptide Analysis



HPLC trace for peptide **9**

```
======================================================================
Injection Date  : 10/07/2015 10:15:05 AM         Seq. Line :   1
Sample Name     : MYRRGDK                         Location : Vial 1
Acq. Operator   : Ketav                              Inj :   1
Acq. Instrument : Instrument 1                   Inj Volume : 100 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 25 µl
Method          : C:\HPCHEM\1\METHODS\KETAV.M
Last changed    : 6/07/2015 1:19:38 PM by Ketav
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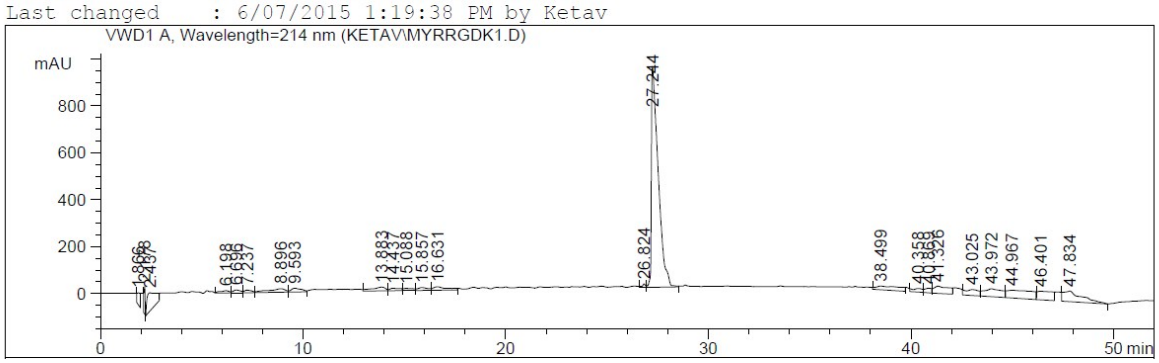

Mass spectrum for peptide **9**