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Supplementary Information

Single Addition of an Allyl Amine Monomer Enables Access to End-Functionalized RAFT Polymers *via* Native Chemical Ligation

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MATERIALS

(Propanoic acid)yl butyl trithiocarbonate (C4-RAFT) was kindly provided by Dr. Algi Serelis from Dulux Group Australia. 2,2'-Azobis[2-(2-imidzolin-2-yl)propane]dihydrochloride (VA044, 98%) was obtained from Wako Pure Chemical Industries, Ltd. Japan. Tetraethyl orthosilicate (TEOS, 95%) was purchased from Acros Organics. Tyramine (99%), 4-(dimethylamino)pyridine (DMAP), n-hydroxyethyl acrylamide (HEAm, 97%), n-Boc-allyl amine (N-Boc-AA, 98%), 2,2'-azobis(2-methylpropionitrile) (AIBN, 99%), isopropyl amine (99%), α-cyano-4-hydroxycinnamic acid (α-CCA), (3-aminopropyl)triethoxysilane (APTS), hexafluoro-2-propanol (HFIP), ethyl 3-mercaptopropionate, thioanisole, tri*iso*propylsilane (TIPS), guanidine hydrochloride, sodium phosphate dibasic (Na_2HPO_4, 98%), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, 99.5%), *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) and 2,2,2-trifluoroethanethiol (TFET, 95%) were purchased from Sigma Aldrich. Magnesium sulfate ($MgSO_4$), dichloromethane (DCM), trifluoroacetic acid (TFA), ammonia solution (NH_3, 28%), piperidine, acetic anhydride, pyridine, acetone, dimethylformamide (DMF), acetonitrile, hexane, absolute ethanol, tetrahydrofuran (THF, 99%) were all sourced from AJAX Finechem. Dimethyl sulfoxide (DMSO), ethyl acetate (EA, 99.5%), dimethylacetamide (DMAc), 1,2-dimethoxyethane, 4-methylmorpholine (NMM) were purchased from Merck Millipore. Fmoc-protected amino acids, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl), N,N-diisopropylethylamine (DIPEA) were purchased from GL Biochem. (Benzotriazol-1-yl-oxy) tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 2-chlorotrityl chloride resin were purchased from Novabiochem. Diethyl ether, hexane (99%), acetone (99%), methanol (MeOH, 99%) and ethanol (EtOH, 95%) were supplied by Redox Chemicals. Water was purified by a MilliQ system to a specific resistivity of ~18MΩ cm. All chemicals were used as received unless otherwise stated.

ANALYSES

^{1}H-NMR and ^{13}C-NMR were acquired on a Bruker 300 or 200 MHz. $CDCl_3$, D_2O, MeOD, or DMSO-D_6 were used as solvents as indicated. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ (δ 7.26 [^{1}H]), D_2O (δ 4.79 [^{1}H]), MeOD (δ 3.31 [^{1}H]), DMSO (δ 2.70 [^{1}H]). ^{1}H NMR data is reported as chemical shift (δ), multiplicity, relative integral, coupling constant (J Hz) and assignment where possible.

Size exclusion chromatography (SEC) was performed using a Shimadzu CBM-20A liquid chromatography system with a Polymer Laboratories Pl-Gel 5 μM guard column and two Polymer Laboratories Styragel columns using DMAc as the eluent at a flow rate of 1.0 mL min^{-1} at 40 °C. The system was equipped with a Shimadzu RID-10A differential refractive index detector, Wyatt MiniDawn TREOS light scattering and Wyatt Viscostar-II viscometer. Before injection (100 μL), the samples were filtered through a polytetrafluoroethylene (PTFE) membrane with 0.45 μm pores. Narrow polystyrene (PS) standards (Đ < 1.1) were used to calibrate the SEC system.

High resolution MALDI-FTICR mass spectra were measured on a Bruker–Daltonics Apex Ultra 7.0T Fourier transform mass spectrometer (FTICR) using a matrix of 10 mg/mL α-CCA in water/acetonitrile (1:1 v/v).

LC-MS was performed either on a Shimadzu LC-MS 2020 instrument consisting of a LC-M20A pump and a SPD-20A UV/Vis detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode or a Shimadzu UPLC-MS equipped with the same modules as the LC-MS system except for a SPD-M30A diode array detector. Separations were performed on the LC-MS system on a Waters Sunfire 5 μm, 2.1 x 150 mm column (C-18) operating at a flow rate of 0.2 mL min^{-1}. Separations were performed using a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) and a linear gradient of 0-50% B over 30 min.

Analytical HPLC was performed on either a Waters Acquity UPLC system equipped with PDA eλ detector (λ = 210 – 400 nm), Sample Manager FAN and Quaternary Solvent Manager (H-class) modules or a Waters System 2695 separations module with a 2996 photodiode array detector. Peptides were analyzed using a Waters Sunfire 5 μm, 2.1 x 150 mm column (C-18) at a flow rate of 0.2 mL min^{-1} on the HPLC system using a mobile phase composed of 0.1% trifluoroacetic acid in H_2O (Solvent A) and 0.1% trifluoroacetic acid in acetonitrile (Solvent B). The analysis of the chromatograms was conducted using Empower 3 Pro software (2010).

Direct inject analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. Peptides were analyzed using a Waters Sunfire 5 μm, 2.1 x 150 mm column (C-18) at a flow rate of 2 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). Results were analyzed with Waters Empower software.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 230 and 280 nm. Peptides were purified on a Waters Sunfire 5 μm (C-18) preparative column operating at a flow rate of 7 mL min^{-1} using a mobile phase of 0.1% trifluoroacetic acid in water (Solvent A) and 0.1% trifluoroacetic acid in acetonitrile (Solvent B) and a linear gradient of 0-80% B over 40 min.

METHODS

Synthesis of butyl (1-[(4-hydroxyphenethyl)amino]-1-oxopropan-2-yl) carbonotrithioate (Tyramine-C4) (1)

C4-RAFT (2.5 g, 10 mmol) was dissolved in 10 mL of DCM and allowed to stir in an ice bath for 10 min. Separately, EDC·HCl (2.4 g, 12 mmol) and DMAP (0.15 g, 0.12 mmol) were mixed and dissolved in 10 mL of DCM before adding it dropwise into the C4-RAFT in DCM solution to form a red solution. Tyramine (1.7 g, 12 mmol) dissolved in 30 mL DMSO was then dropwise added to the reaction mixture. The reaction was allowed to stir in an ice bath for 1 hour before stirring at room temperature overnight. The reaction was monitored and considered complete by thin layer chromatography with EA as a mobile phase. The crude mixture was then diluted in EA (200 mL) and washed three times with water (200 mL) *via* solvent-solvent extraction and dried with $MgSO_4$. The mixture was then concentrated and purified by flash column chromatography (eluent = EA) to afford **1** as a yellow viscous oil (3.4 g, 89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 6.90-7.15 (m, 2H, OH-**Ar**-CH_2-CH_2-NH), 6.65-6.90 (m, 2H, OH-**Ar**-CH_2-CH_2-NH), 6.25-6.50 (bs, 1H, OH-Ar-CH_2-CH_2-**NH)**, 4.50-4.80 (m, 1H, NH-CO-**CH**-CH_3), 3.40-3.60 (m, 2H, S-**CH_2**-CH_2-CH_2-CH_3), 3.15-3.40 (m, 2H, OH-Ar-CH_2-**CH_2**-NH), 2.55-2.85 (t, 2H, *J*=6.95 Hz, OH-Ar-**CH_2**-CH_2-NH), 1.60-1.85 (m, 2H, S-CH_2-**CH_2**-CH_2-CH_3), 1.50-1.60 (m, 3H, NH-CO-CH-**CH_3**), 1.30-1.50 (m, 2H, S-CH_2-CH_2-**CH_2**-CH_3), 0.55-1.05 (t, 3H, *J*=7.32 Hz, S-CH_2-CH_2-CH_2-**CH_3**). ^{13}C-NMR (300 MHz, $CDCl_3$) δ ppm: 13.5, 22.0, 29.8, 34.4, 37.2, 41.2, 47.8, 115.5, 129.8, 154.7, 170.9 and 223.7. HRMS: ($[C_{16}H_{23}NO_2S_3+Na]^+$): 380.078, found: 380.078.

One-pot synthesis of Tyramine-poly(hydroxyethyl acrylamide)-NBocAA-C4 [to produce (2), (3) and (4)]

Polymerization of hydroxyethyl acrylamide (HEAm) was carried out with VA044 as the initiator and Tyramine-C4 (**1**) as the controlled transfer agent/RAFT agent (Scheme 2). Concentrations of HEAm, VA044 and Tyramine-C4 (see Table S1) were added to a 3:7 mixed solvent ratio of DMF: MilliQ water equipped with a magnetic stirrer in a round bottom flask and sealed with a rubber septum. A sample of the reaction was taken for ^{1}H-NMR at t=0. The reaction was degassed with pure nitrogen for 20 minutes before reacting for 2 hours in a 70 °C oil bath. The reaction was monitored by ^{1}H-NMR for monomer conversion (98% conversion). After completion, polymer solutions were quenched by exposure to air.

2: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 1H, **NH**-CO-C(CH_3)-CH_2-CH(CO-**NH**-CH_2-CH_2-$OH)_{10}$-S), 6.80-7.13 (d, 0.14H, OH-**Ar**-CH_2-CH_2-NH), 6.49-6.80 (d, 0.13H, OH-**Ar**-CH_2-CH_2-NH), 4.08-5.41 (br, 1H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-CH_2-CH_2-**OH**)$_{10}$-S), 3.28-3.74 (br, 4.44H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-**CH_2-CH_2**-$OH)_{10}$-S), 0.53-1.01 (m, 0.47H, S-CH_2-CH_2-CH_2-**CH_3**).

3 and **4** were not isolated but observed in ^{1}H-NMR as crude samples and compared to t=0 samples until 98% conversion was achieved before proceeding to NBocAA addition in a one-pot fashion.

Table S1: Polymerization conditions for Tyr-p(HEAm)n-NBocAA-C4, n=the degree of polymerization

	Concentration (M)					
	1[a]			2*[b]		
Sample	[HEAm]	[Tyramine-C4]	[VA044]	[Tyr-p(HEAm)$_n$-C4]	[NBocAA]	[AIBN]
p(HEAm)$_{10}$-NBocAA-C4 **5**	4	0.396	0.004	0.129	2	0.004
p(HEAm)$_{30}$-NBocAA-C4 **6**	4	0.132	0.001			
p(HEAm)$_{100}$-NBocAA-C4 **7**	4	0.039	0.0004			

[a] Polymerization was carried out at 70 °C for 2 h to achieve 98% conversion

*Reaction was diluted with DMF to a [Tyr-p(HEAm)$_n$-C4] concentration of 0.129 M

[b] Polymerization was carried out at 70 °C for 16 h

Tyr-p(HEAm)$_n$-C4 (**2**, **3** or **4**) from the first step was diluted with DMF to a concentration of 0.13 M before chain extension with 2 M of NBocAA and 4.0 mM of AIBN as the source of radical (Table S1). Under similar set up, the reaction was degassed for 20 minutes with pure nitrogen before allowing it to react overnight in a 70 °C oil bath. The reaction was then quenched by exposure to air and cooled down. Polymers **5**, **6**, and **7** were purified by recrystallization in acetone and dried at 40 °C in a vacuum oven.

5: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 1.02H, **NH**-CO-C(CH$_3$)-CH$_2$-CH(CO-**NH**-CH$_2$-CH$_2$-OH)$_{10}$-NBocAA-S), 6.80-7.13 (m, 0.14H, OH-**Ar**-CH$_2$-CH$_2$-NH), 6.49-6.80 (m, 0.13H, OH-**Ar**-CH$_2$-CH$_2$-NH), 4.08-5.41 (br, 1H, NH-CO-C(CH$_3$)-CH$_2$-CH(CO-NH-CH$_2$-CH$_2$-**OH**)$_{10}$-NBocAA-S), 3.28-3.74 (br, 4.44H, NH-CO-C(CH$_3$)-CH$_2$-CH(CO-NH-**CH$_2$-CH$_2$**-OH)$_{10}$-NBocAA-S), 1.21-1.45 (m, 1.84H, NH-CO-C(CH$_3$)-CH$_2$-CH(CO-NH-CH$_2$-CH$_2$-OH)$_{10}$-N**Boc**AA-S), 0.53-1.01 (m, 0.4H, S-CH$_2$-CH$_2$-CH$_2$-**CH$_3$**).

6: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 3.26H, **NH**-CO-C(CH$_3$)-CH$_2$-CH(CO-**NH**-CH$_2$-CH$_2$-OH)$_{30}$-NBocAA-S), 6.80-7.13 (m, 0.10H, OH-**Ar**-CH$_2$-CH$_2$-NH), 6.49-6.80 (m, 0.10H, OH-**Ar**-CH$_2$-CH$_2$-NH), 4.50-5.16 (br, 3.45H, NH-CO-C(CH$_3$)-CH$_2$-CH(CO-NH-CH$_2$-CH$_2$-**OH**)$_{30}$-NBocAA-S), 2.84-3.35 (br, 8.12H, NH-CO-C(CH$_3$)-CH$_2$-CH(CO-NH-**CH$_2$-CH$_2$**-OH)$_{30}$-NBocAA-S), 1.32-1.40 (m, 1.78H, NH-CO-C(CH$_3$)-CH$_2$-CH(CO-NH-CH$_2$-CH$_2$-OH)$_{30}$-N**Boc**AA-S), 0.61-1.00 (m, 0.38H, S-CH$_2$-CH$_2$-CH$_2$-**CH$_3$**).

7: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 9.11H, **NH**-CO-C(CH_3)-CH_2-CH(CO-**NH**-CH_2-CH_2-OH)$_{10}$-NBocAA-S), 6.80-7.13 (m, 0.10H, OH-**Ar**-CH_2-CH_2-NH), 6.49-6.80 (m, 0.17H, OH-**Ar**-CH_2-CH_2-NH), 4.08-5.41 (br, 10.24H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-CH_2-CH_2-**OH**)$_{10}$-NBocAA-S), 3.28-3.74 (br, 19.23H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-**CH_2-CH_2**-OH)$_{10}$-NBocAA-S), 1.33-1.38 (m, 2.07H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-CH_2-CH_2-OH)$_{10}$-N**Boc**AA-S), 0.77-1.03 (m, 0.45H, S-CH_2-CH_2-CH_2-**CH_3**).

Post modification of p(HEAm) for end-functionalised pseudo-cysteine functional groups

0.1 M concentration of Tyr-p(HEAm)$_n$-NBocAA-C4 (**5**, **6** or **7**) in water was aminolysed with 10 eq. of isopropyl amine. The aminolysis was left to stir at room temperature for 16 h. The product **8**, **9**, and **10** was then recrystallized from acetone and dried at 40 °C in a vacuum oven.

0.1 M concentration of aminolysed polymer (**8**, **9** and **10**) in water were Boc-deprotected with 50 eq. of TFA and allowed to stir at room temperature for 16 h before recrystallization from acetone. Final polymers **11**, **12**, and **13** were redissolved in water and lyophilized to afford a white powder.

11: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 1.02H, **NH**-CO-C(CH_3)-CH_2-CH(CO-**NH**-CH_2-CH_2-OH)$_{10}$-AA-SH), 6.80-7.13 (m, 0.14H, OH-**Ar**-CH_2-CH_2-NH), 6.49-6.80 (m, 0.13H, OH-**Ar**-CH_2-CH_2-NH), 4.08-5.41 (br, 1H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-CH_2-CH_2-**OH**)$_{10}$-AA-SH), 3.28-3.74 (br, 4.44H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-**CH_2-CH_2**-OH)$_{10}$-AA-SH).

12: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 2.30H, **NH**-CO-C(CH_3)-CH_2-CH(CO-**NH**-CH_2-CH_2-OH)$_{30}$-AA-SH), 6.80-7.13 (m, 0.10H, OH-**Ar**-CH_2-CH_2-NH), 6.49-6.80 (m, 0.10H, OH-**Ar**-CH_2-CH_2-NH), 3.56-4.23 (br, 7.88H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-**CH_2-CH_2**-OH)$_{30}$-AA-SH).

13: ^{1}H-NMR (300 MHz, DMSO) δ ppm (polymer backbone): 7.21-7.84 (br, 4.86H, **NH**-CO-C(CH_3)-CH_2-CH(CO-**NH**-CH_2-CH_2-OH)$_{100}$-AA-SH), 6.80-7.13 (m, 0.08H, OH-**Ar**-CH_2-CH_2-NH), 6.49-6.80 (m, 0.10H, OH-**Ar**-CH_2-CH_2-NH), 3.52-4.02 (br, 17.19H, NH-CO-C(CH_3)-CH_2-CH(CO-NH-**CH_2-CH_2**-OH)$_{100}$-AA-SH).

Characterization of butyl (1-[(4-hydroxyphenethyl)amino]-1-oxopropan-2-yl) carbonotrithioate *(Tyramine-C4*, 1*)*

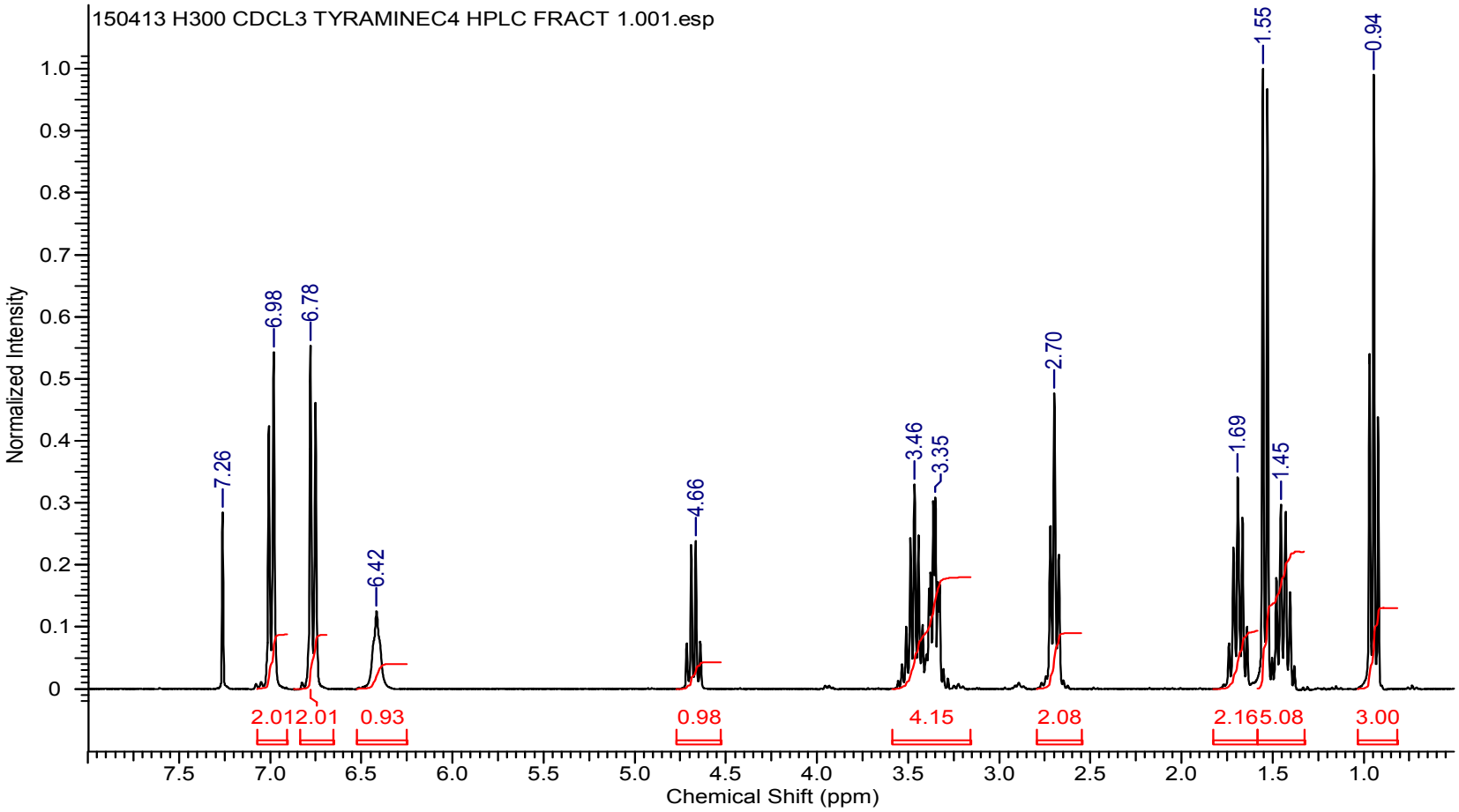

Figure S1: ^{1}H-NMR spectrum of tyramine-C4 **1**

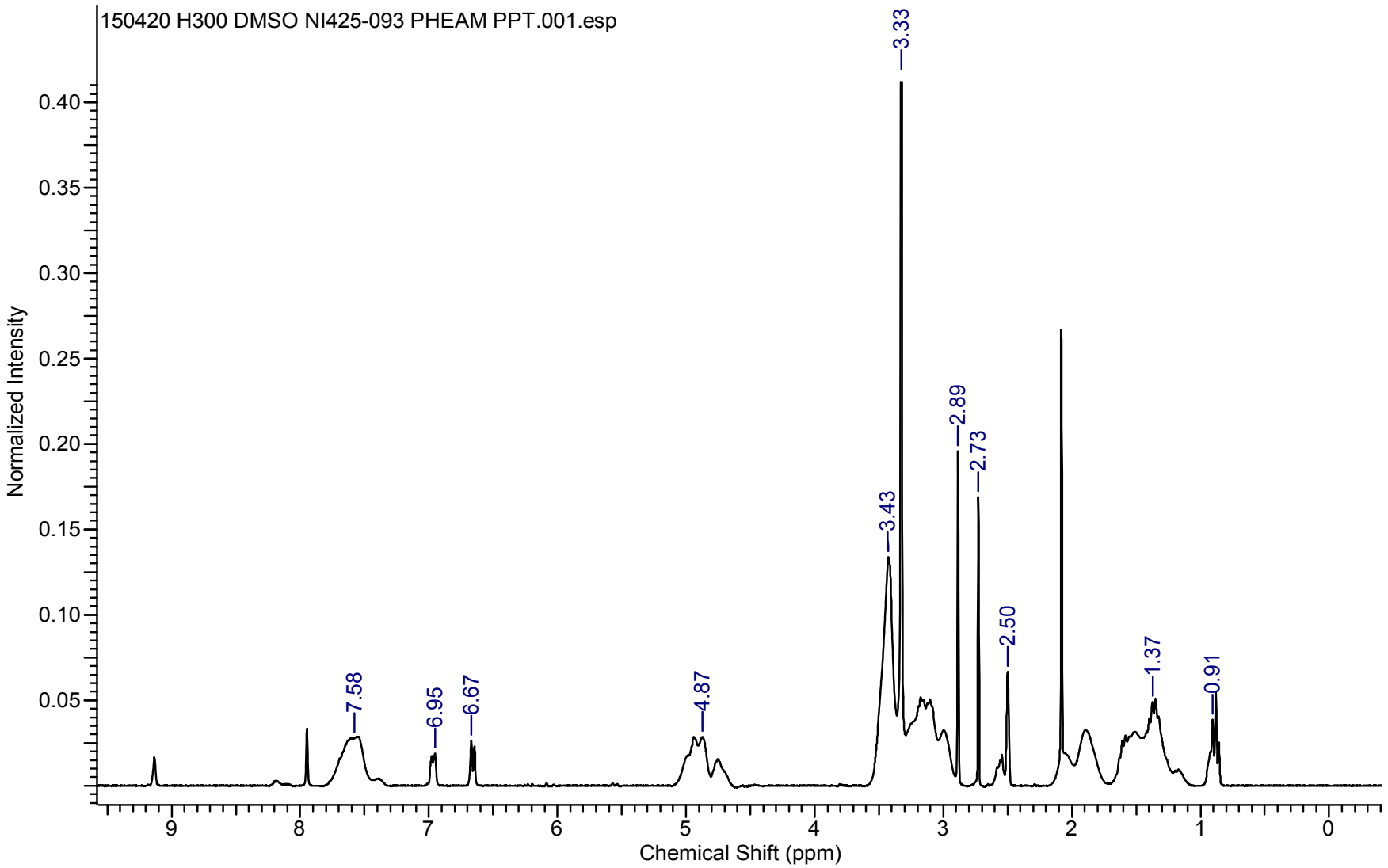

Figure S2: ^{1}H-NMR spectrum of p(HEAm)$_{10}$ **2**

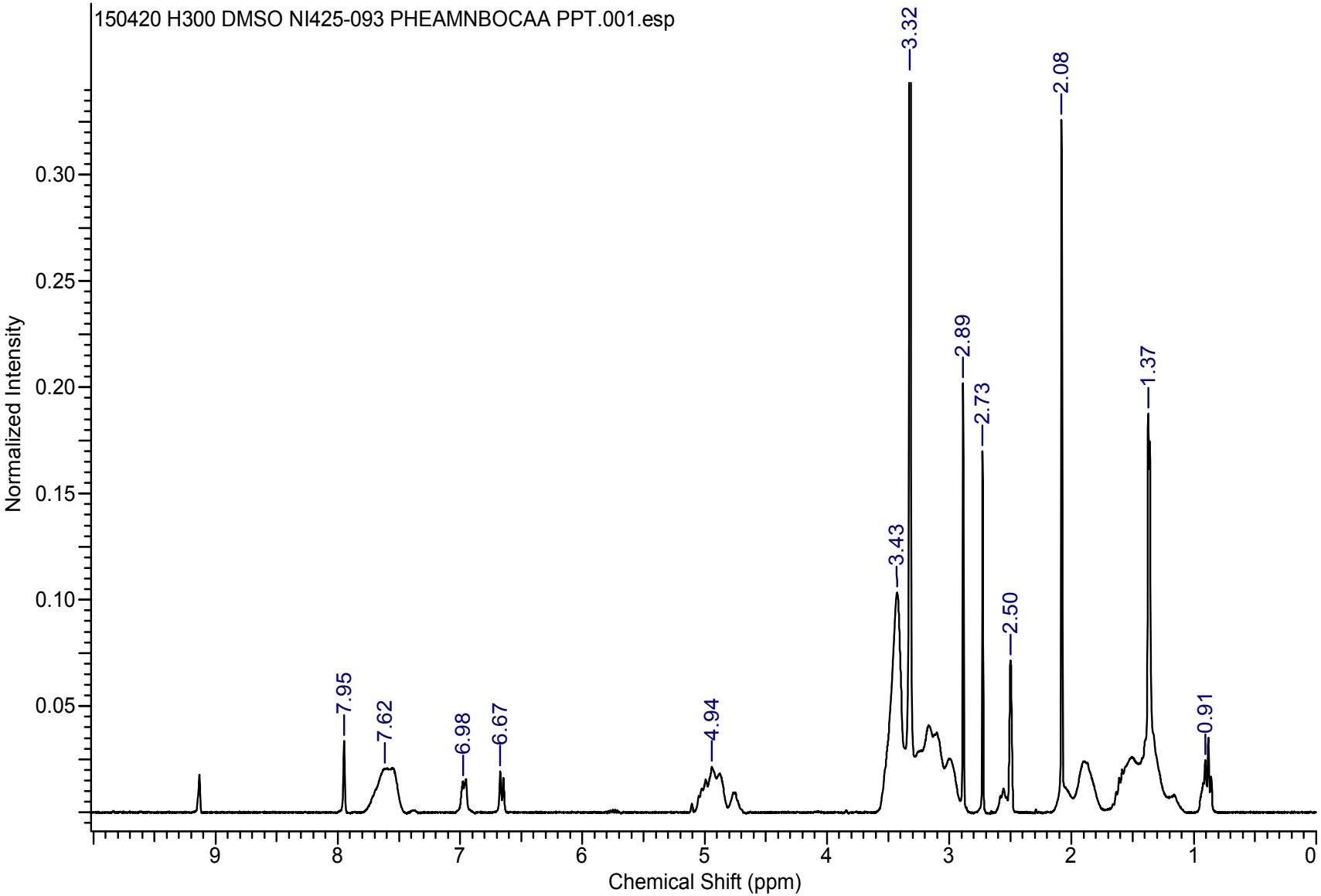

Figure S3: ^{1}H-NMR spectrum of $(pHEAm)_{10}$-NBocAA-C4 **5**

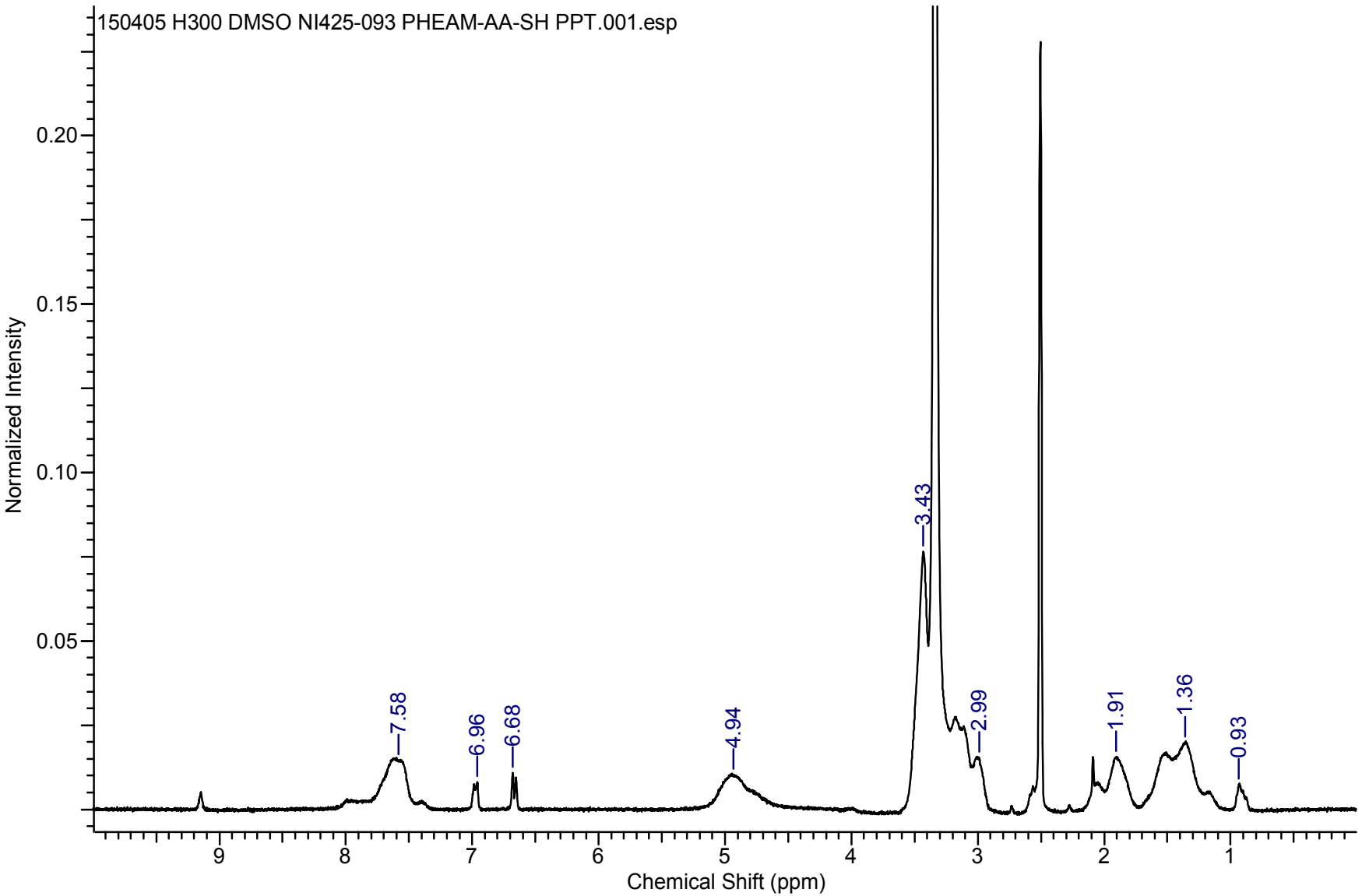

Figure S4: ^{1}H-NMR of p(HEAm)$_{10}$-AA-SH **11**

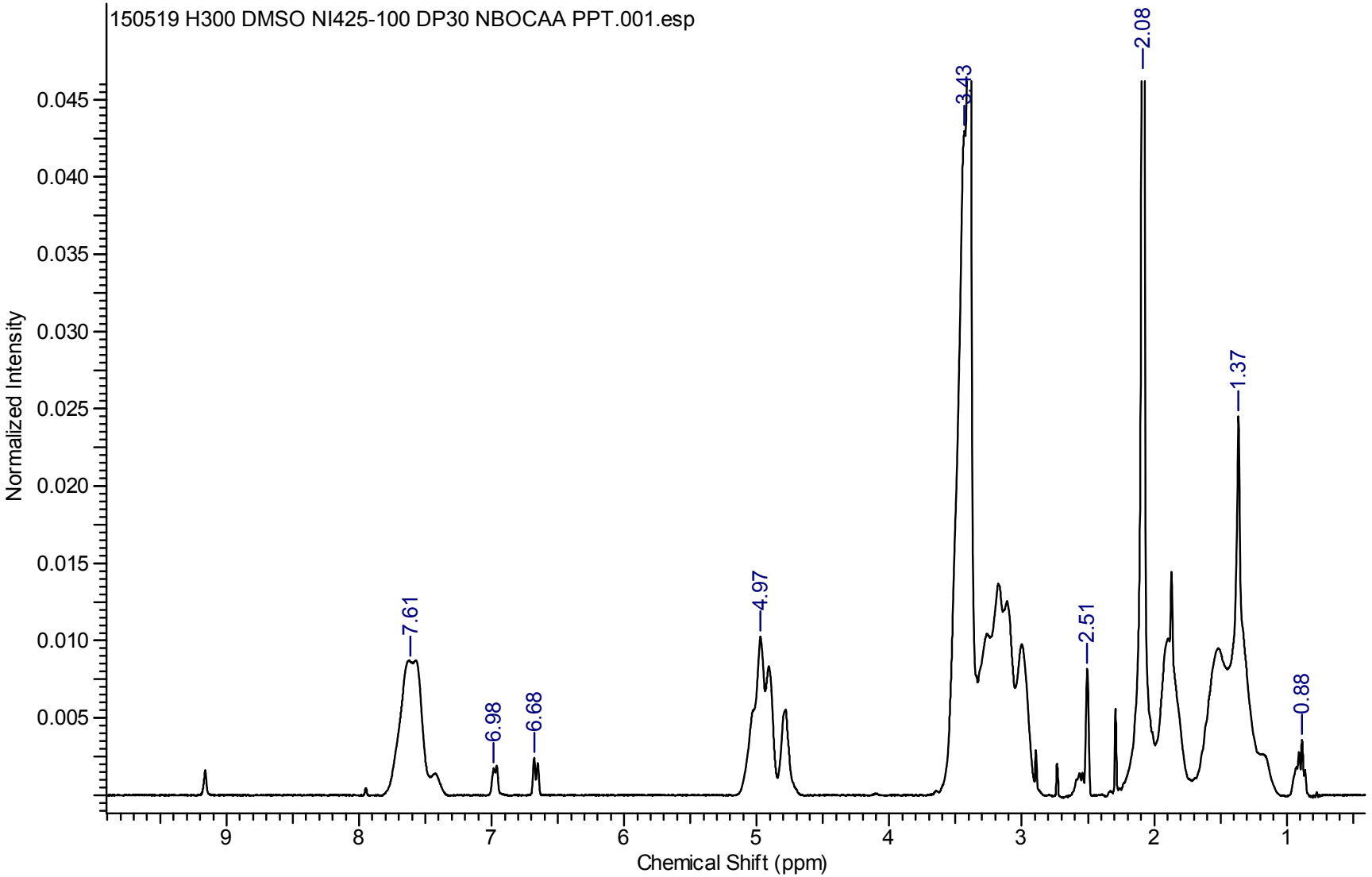

Figure S5: ^{1}H-NMR spectrum of $(pHEAm)_{30}$-NBocAA-C4 **6**

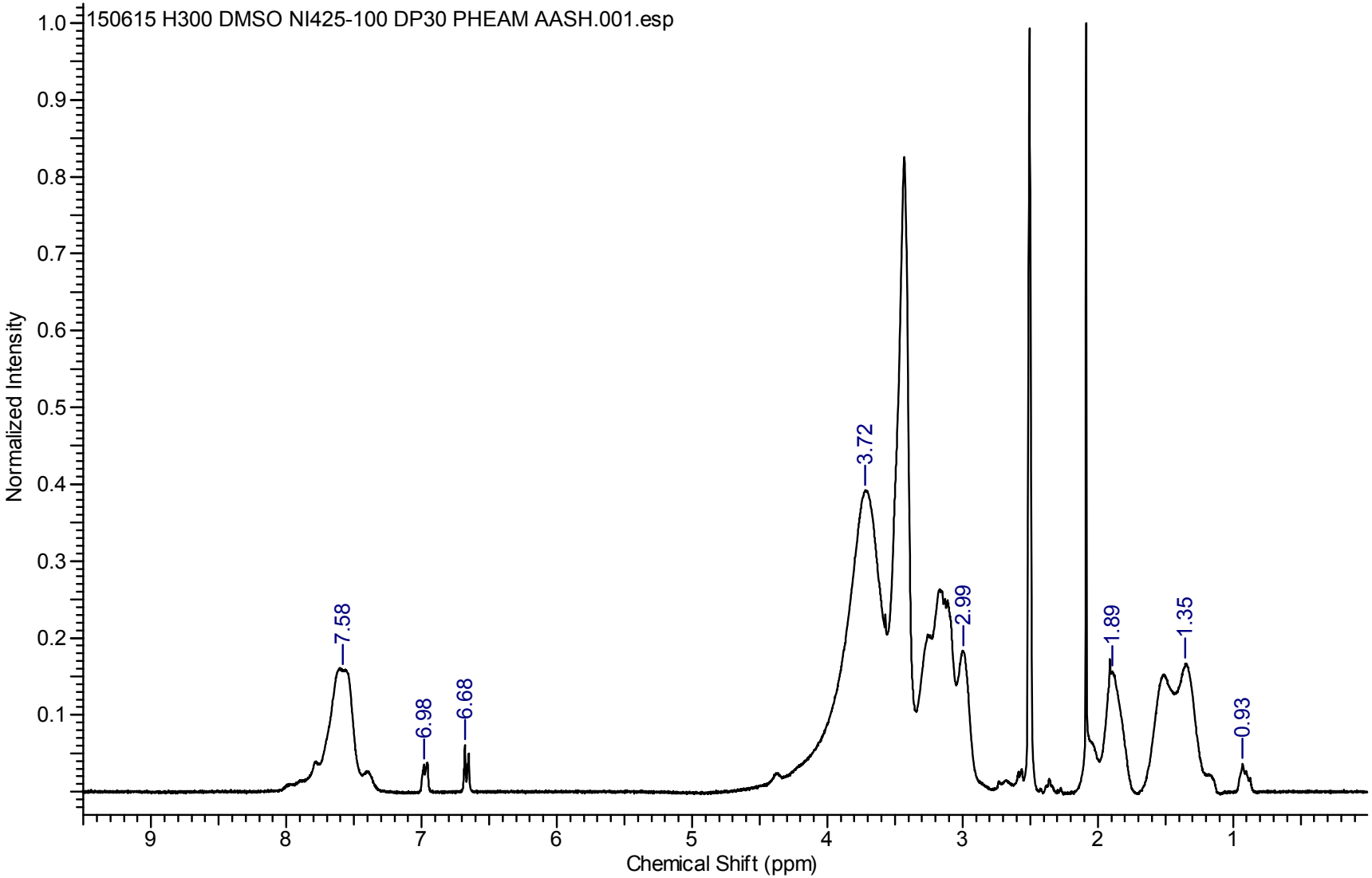

Figure S6: ^{1}H-NMR spectrum of $(pHEAm)_{30}$-AA-SH **12**

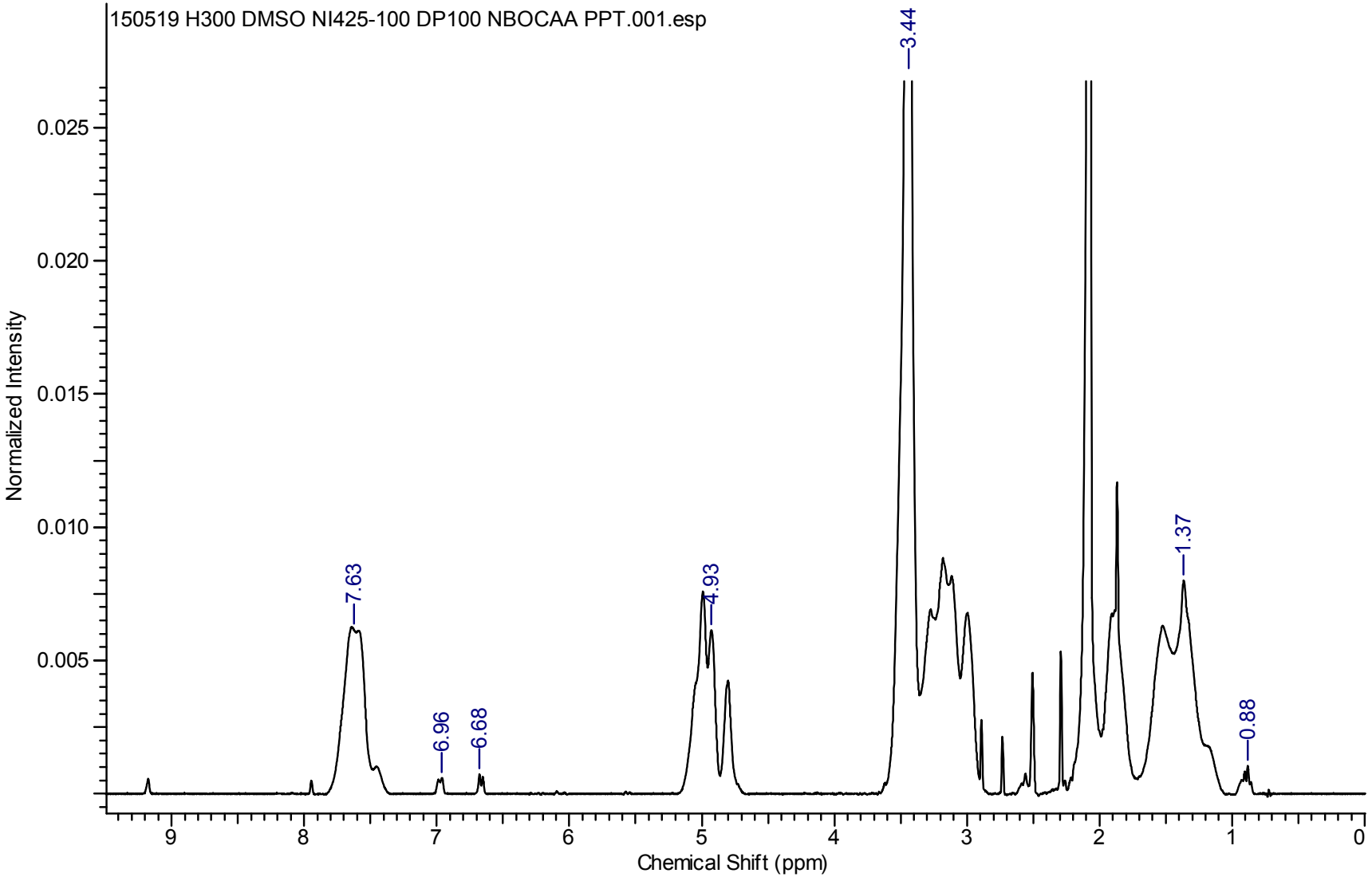

Figure S7: ^{1}H-NMR spectrum of $(pHEAm)_{100}$-NBocAA-C4 **7**

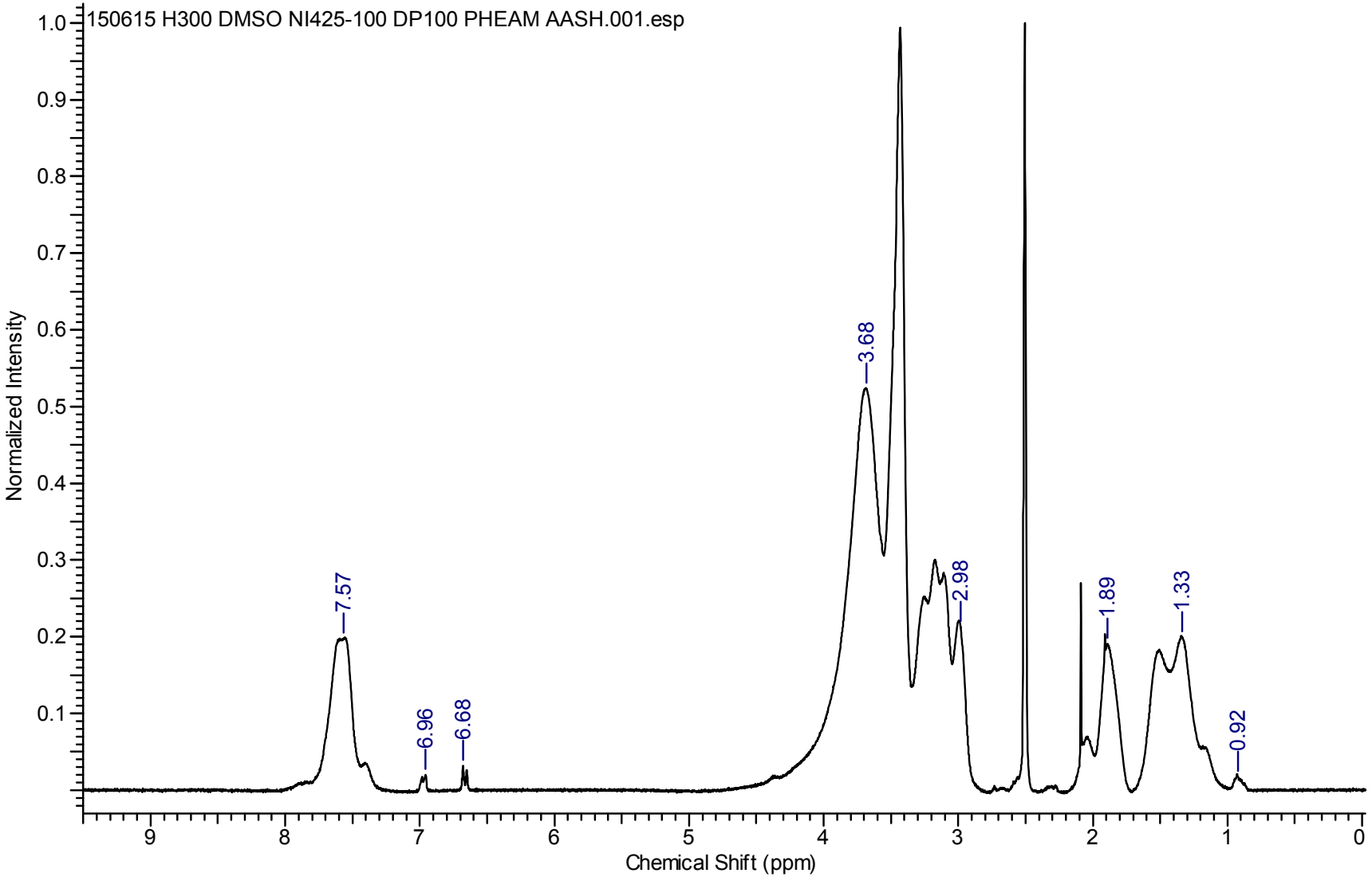

Figure S8: ^{1}H-NMR spectrum of $(pHEAm)_{100}$-AA-SH **13**

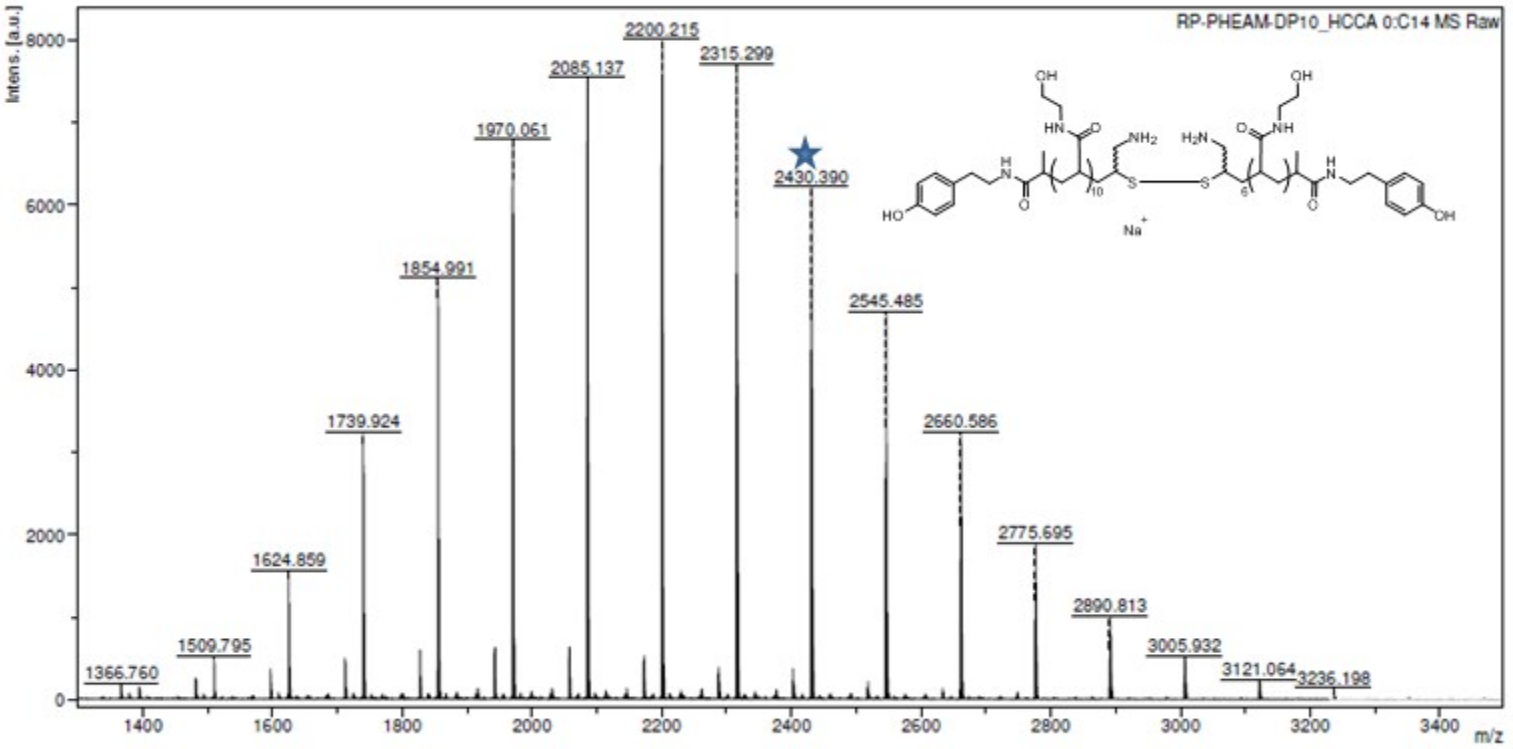

Figure S9: MALDI –ToF spectrum of p(HEAM)$_{10}$-AA-SH **11** in α-CCA matrix in reflectron mode

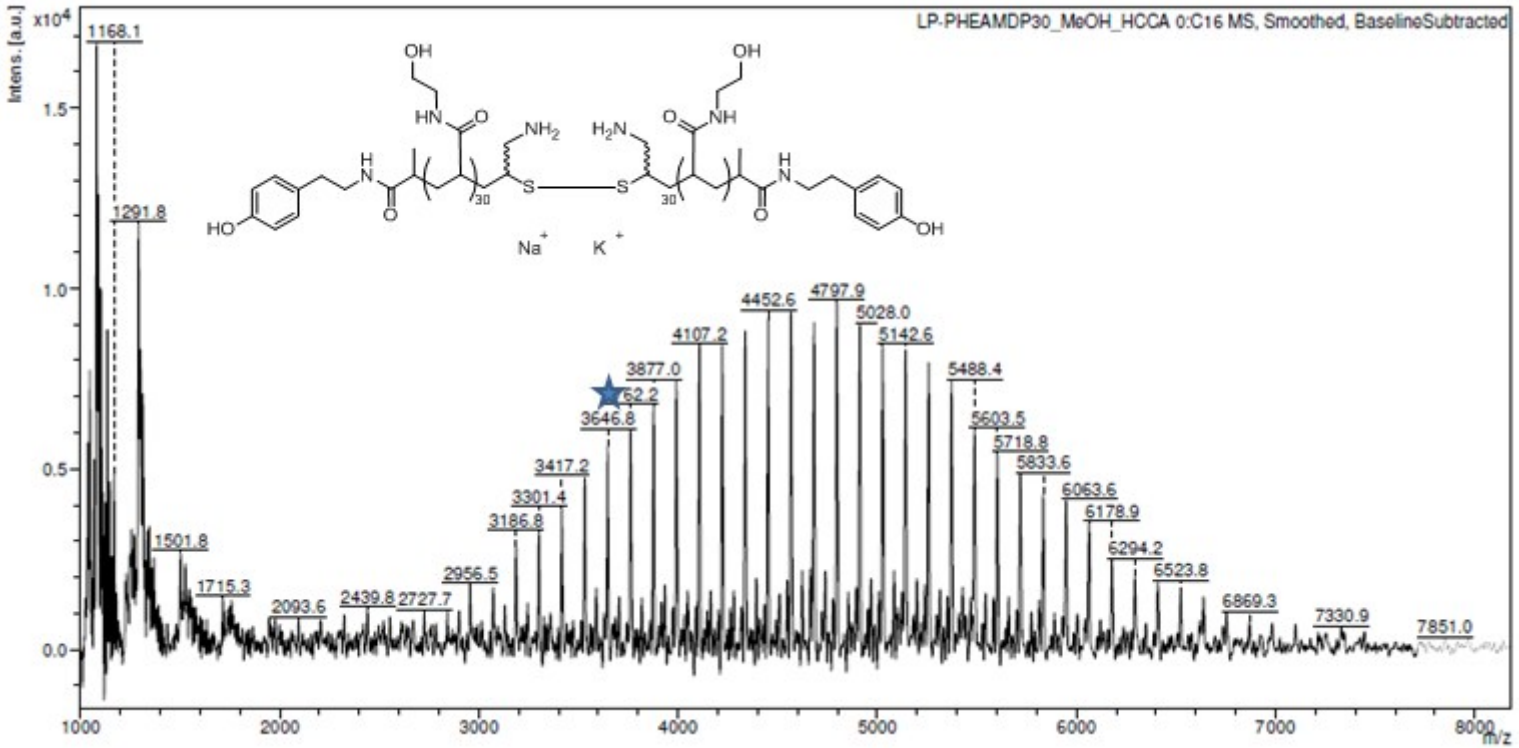

Figure S10: MALDI –ToF spectrum of p(HEAM)$_{30}$-AA-SH **12** in α-CCA matrix in linear mode

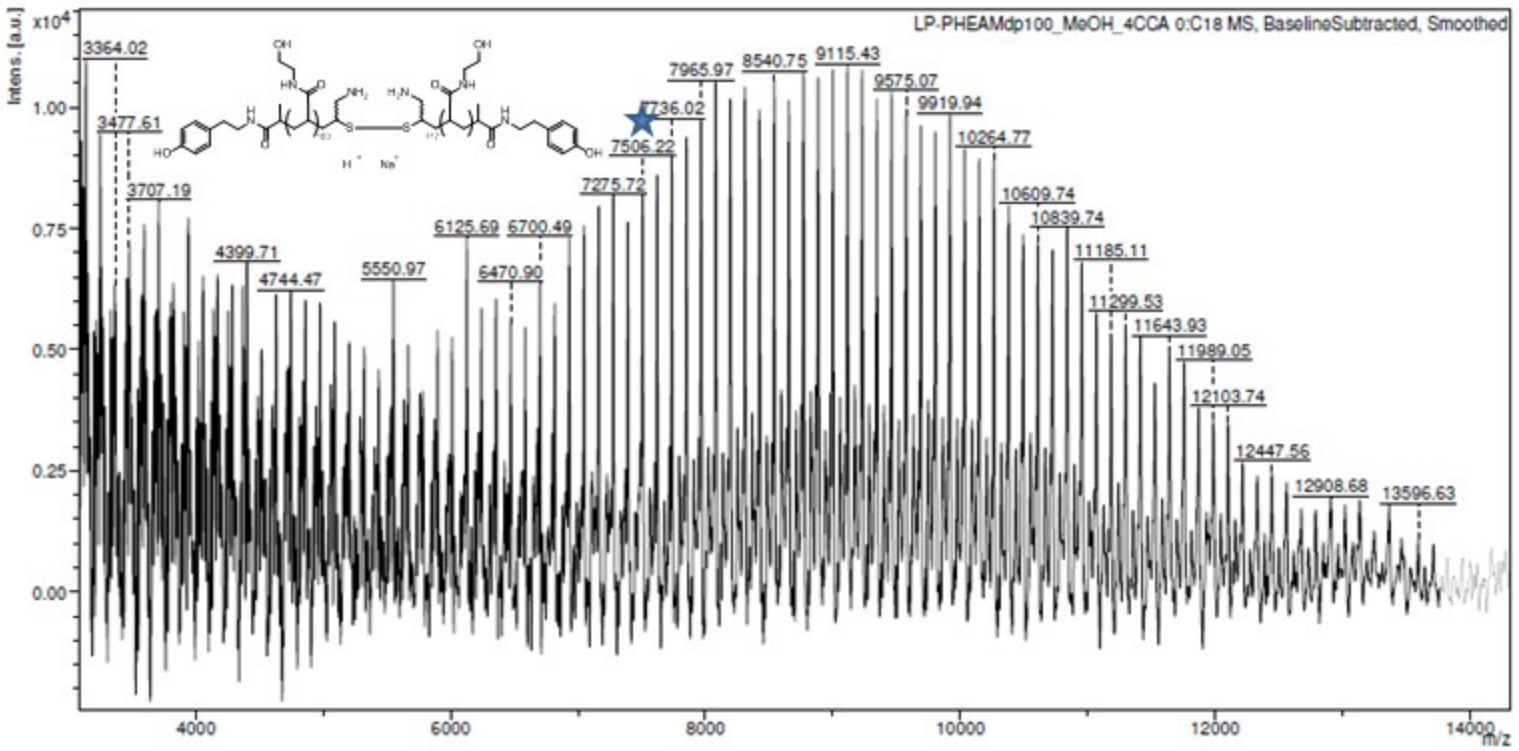

Figure S11: MALDI –ToF spectrum of p(HEAM)$_{100}$-AA-SH **13** in α-CCA matrix in linear mode

Table S2: Summary of Characterization of RAFT polymers

	Size Exclusion Chromatography [a]			MALDI ToF [b]			
Samples	M_n	M_w	$Đ$	M_n	M_w	M_z	$Đ$
$p(HEAm)_{10}$-NBoc AA- C4 **(5)**	6700	7500	1.12				
$p(HEAm)_{10}$-AA-SH **(11)**	8100	8800	1.08	2100*	2200*	2200*	1.02*
$p(HEAm)_{30}$-NBoc AA- C4 **(6)**	14800	15700	1.06				
$p(HEAm)_{30}$-AA-SH **(12)**	14600	16200	1.10	*4800*	*4900*	*5100*	*1.04*
$p(HEAm)_{100}$-NBoc AA- C4 **(7)**	26900	28100	1.05				
$p(HEAm)_{100}$-AA-SH **(13)**	26400	28000	1.06	*9800*	*10000*	*10301*	*1.03*

[a] Size Exclusion Chromatography in DMAc solvent calibrated to PS standards
[b] MALDI-ToF in α-CCA matrix under *reflectron mode and *linear mode*

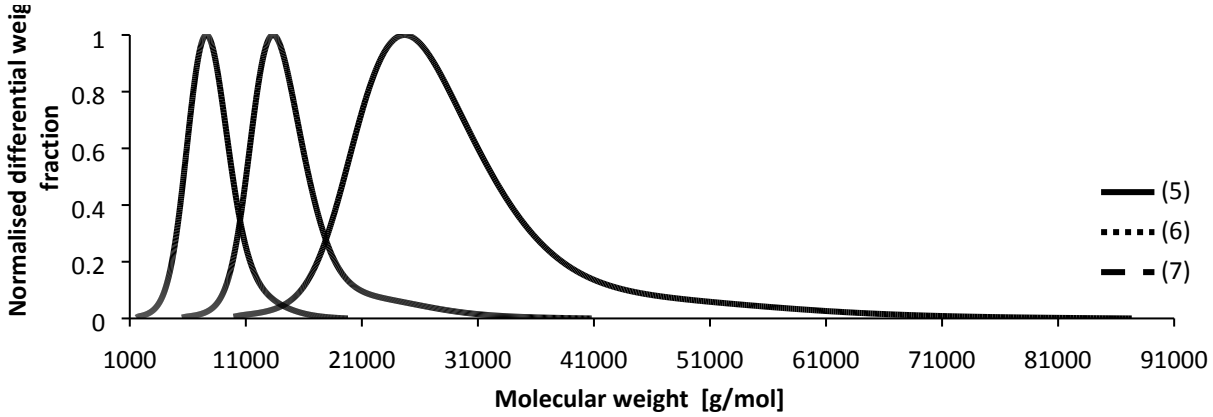

Figure S12: Size exclusion chromatography trace in DMAc solvent system analysed with respect to PS standards for **5**, **6** and **7**.

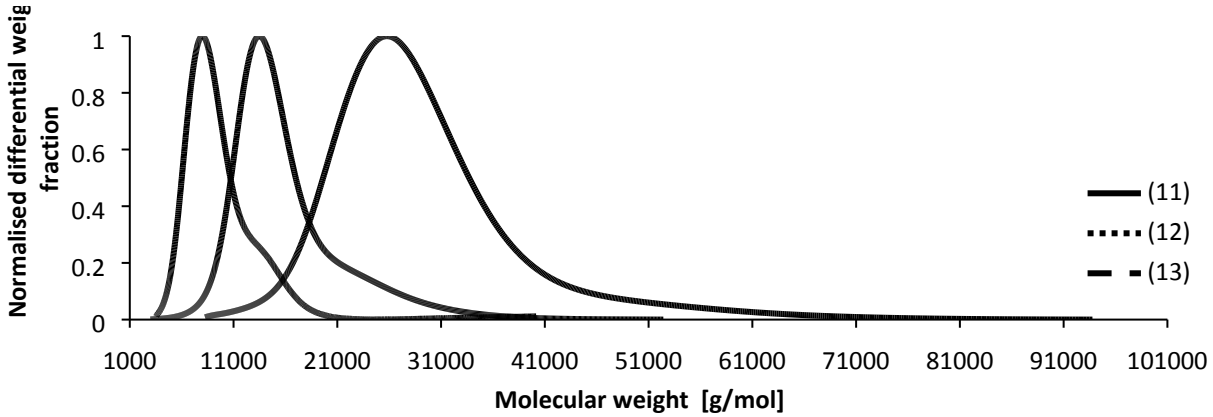

Figure S13: Size exclusion chromatography trace in DMAc solvent system analysed with respect to PS standards for **11**, **12** and **13**.

General procedures for SPPS of peptides following the Fmoc strategy (Iterative peptide assembly)

Preloading 2-chloro-trityl chloride resin: 2-Chlorotrityl chloride resin (1.22 mmol/g loading) was swollen in dry DCM for 30 min then washed with DCM (5 × 3 mL). A solution of Fmoc-AA-OH (4 eq. relative to resin functionalization) and DIPEA (8 eq. relative to resin functionalization) in DMF (final concentration 0.1 M of amino acid) was added and the resin shaken at rt for 2 h. The resin was washed with DMF (5 × 3 mL) and DCM (5 × 3 mL). The resin was treated with a solution of DCM/CH_3OH/DIPEA (17:2:1 v/v/v, 3 mL) for 1 h and washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL). The resin was subsequently submitted to iterative peptide assembly (Fmoc-SPPS).

Estimation of amino acid loading: The resin was treated with 20% piperidine/DMF (2 x 3 mL, 3 min) and 50 μL of the combined deprotection solution was diluted to 10 mL using 20% piperidine/DMF in a volumetric flask. The UV absorbance of the resulting piperidine-fulvene adduct was measured (λ = 301 nm, ε = 7800 M^{-1} cm^{-1}) to estimate the amount of amino acid loaded onto the resin.

General amino acid coupling: A solution of protected amino acid (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After 1 h, the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

Fmoc-Deprotection: The resin was treated with 20% piperidine/DMF (2 x 3 mL, 3 min) and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

Capping**:** Acetic anhydride/pyridine (1:9 v/v) was added to the resin (3 mL). After 3 min the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

Cleavage**:** A mixture of HFIP/DCM (3:7 v/v) was added to the resin and allowed to shake for 2 h at rt. The solution with the cleaved peptide was collected and concentrated under a stream of nitrogen.

Thioesterification: Concentrated peptide was dissolved in DMF (final concentration 0.1 M with respect to the peptide) and cooled to -30 ^oC. Ethyl 3-mercaptopropionate (30 eq. relative to the peptide) was added dropwise, followed by DIPEA (5 eq. relative to the peptide) and then PyBOP (5 eq. relative to the peptide). The solution was stirred at -30 ^oC for 3 h. The solution was concentrated under a nitrogen stream. A mixture of TFA, thioanisole, tri*iso*propylsilane (TIPS) and water (85:5:5:5 v/v/v/v) was then added to the solution and the reaction mixture was stirred at rt for 2 h.

***Work-up*:** The peptide solution was concentrated under a stream of nitrogen to < 5 mL, then precipitated with 30 mL of diethyl ether. The suspension was centrifuged and the resulting pellet was redissolved in water containing 0.1% TFA, filtered and purified by preparative HPLC and analyzed by LC-MS.

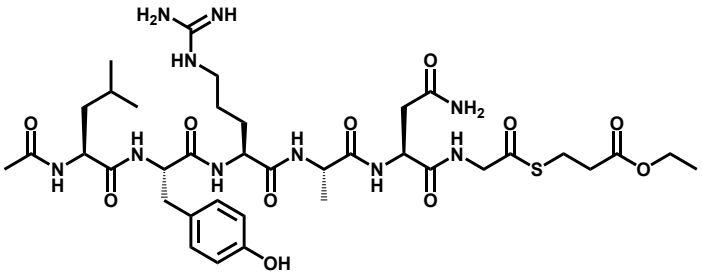

Figure S14: Ac-LYRANG-S$(CH_2)_2CO_2Et$

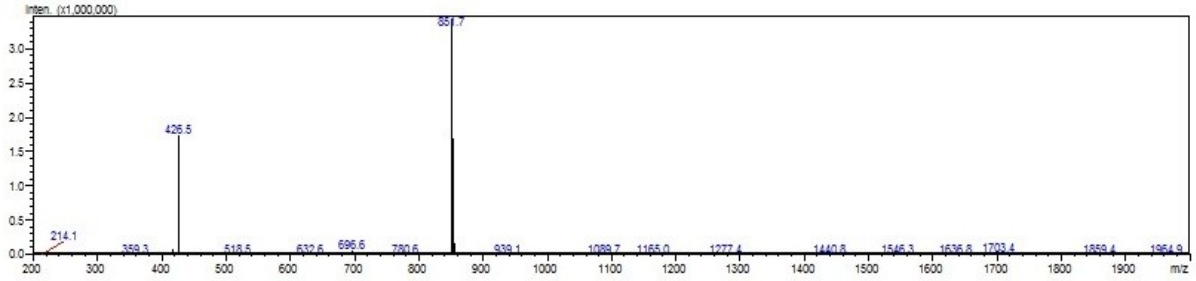

Figure S15: Mass spectra of Ac-LYRANG-S**$(CH_2)_2CO_2Et$.** LRMS-ESI: m/z $[M+2H^+]$ calcd for $C_{37}H_{60}N_{10}O_{11}S$: 852.415; found: 851.7.

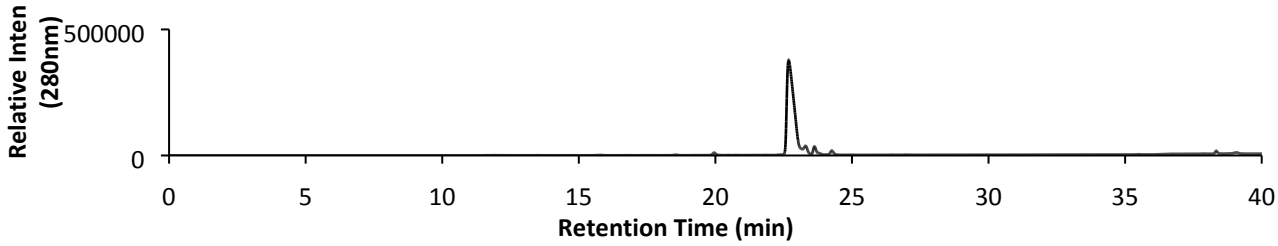

Figure S16: LC trace of **Ac-LYRANG-S**$(CH_2)_2CO_2Et$ at 280 nm after HPLC purification.

NATIVE CHEMICAL LIGATION TO FORM PEPTIDE-POLYMER CONJUGATES

p(HEAm) modified polymers (**11-13**) (5 mg were dissolved in 6 M Gn•HCl, 100 mM Na_2HPO_4 buffer solution with 50 mM TCEP to a final concentration of 5 mM. The solution was adjusted to pH 7 before degassing the reaction with argon for 5 min. The degassed solution was added to the peptide alkyl thioester **14** at polymer:peptide ratios ranging from 0.3-3.0. The pH of the ligation reactions were adjusted (when required) to pH 7. TFET (2% v/v) was added to the mixture and the reaction was gently agitated. The solution was incubated at 37 °C and the reactions monitored by HPLC-MS. Following completion of the reactions, quenching was performed by adding an equal volume of 1% TFA in water. The integrated yields of the reactions were calculated at t =120 h from the UV chromatogram at λ = 280 nm (measurement of the phenolic side chain of Tyr present in both the polymer and the peptide). Reported yields represent an average of two independent experiments.

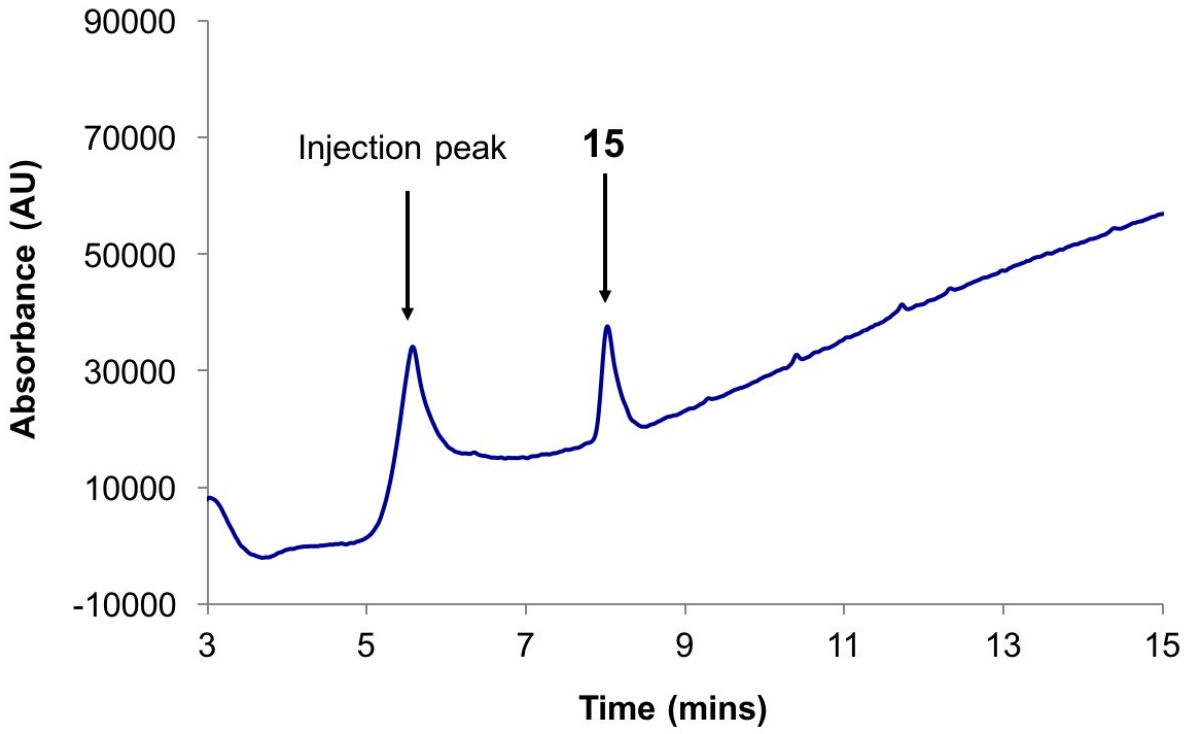

Figure S18: LC trace at 280 nm of HPLC purified peptide-p(HEAM)$_{10}$-AA-SH conjugate (**15**)

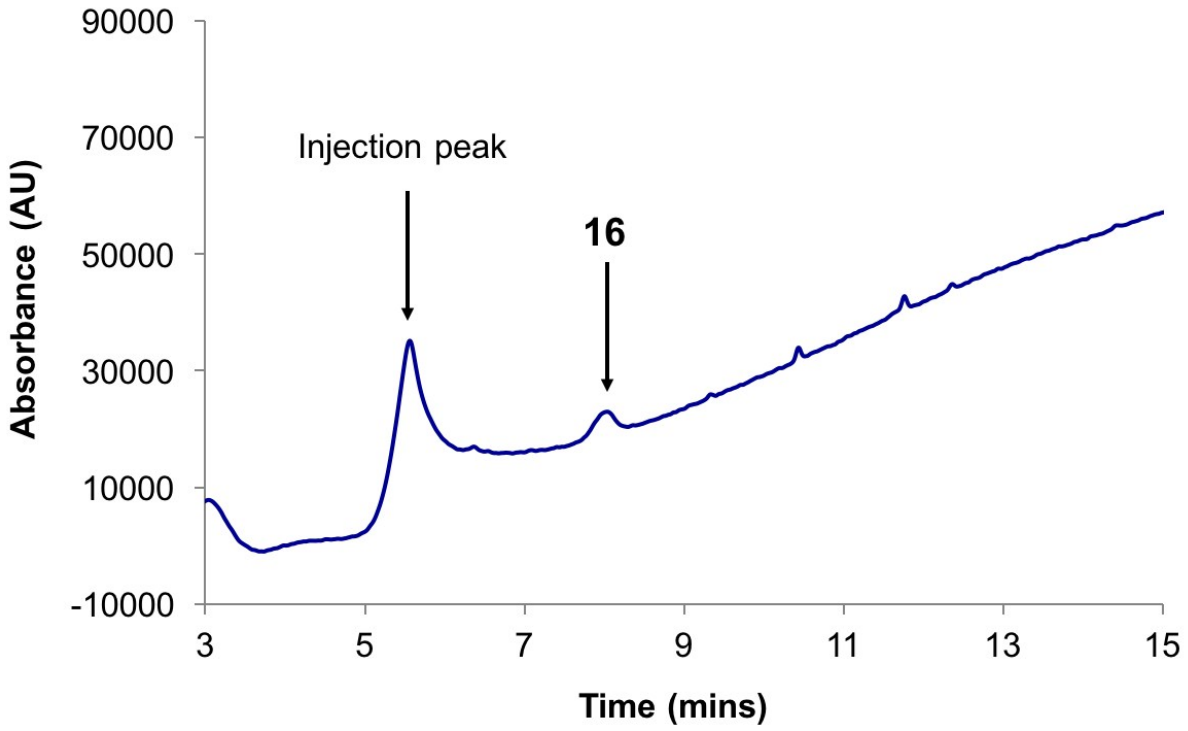

Figure S19: LC trace at 280 nm of HPLC purified peptide-p(HEAM)$_{30}$-AA-SH conjugate (16)

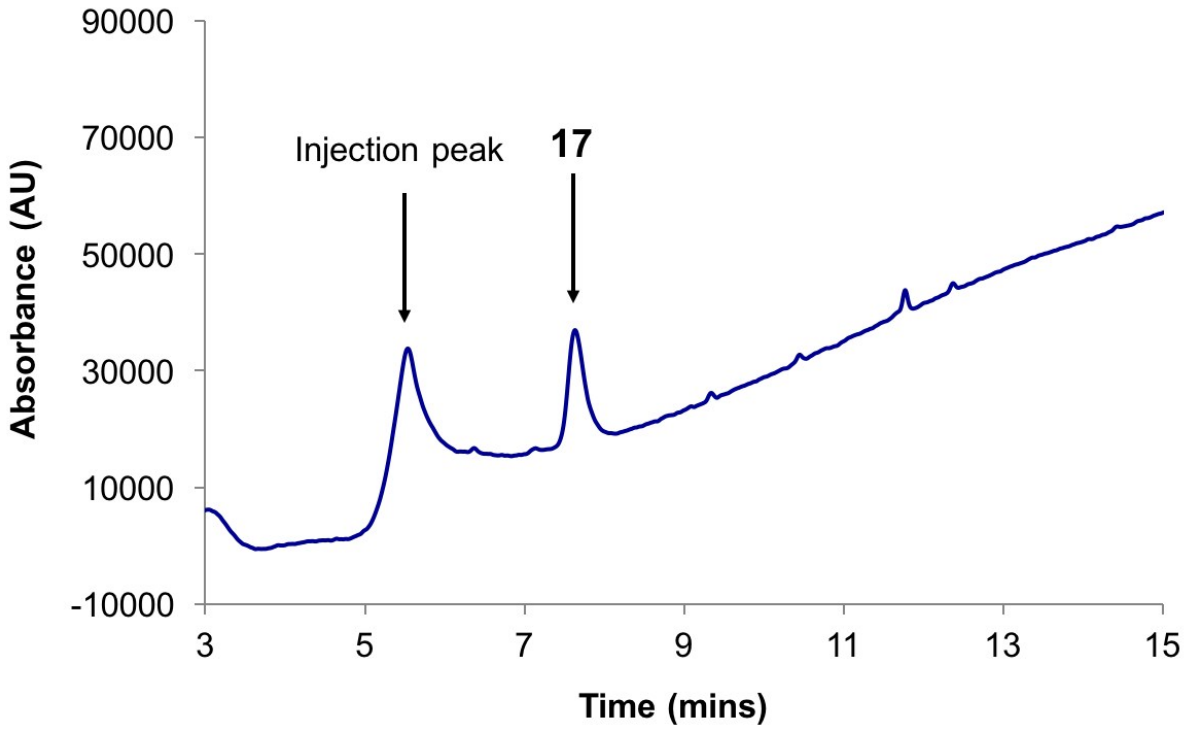

Figure S20: LC trace at 280 nm of HPLC purified peptide-p(HEAM)$_{100}$-AA-SH conjugate (**17**)

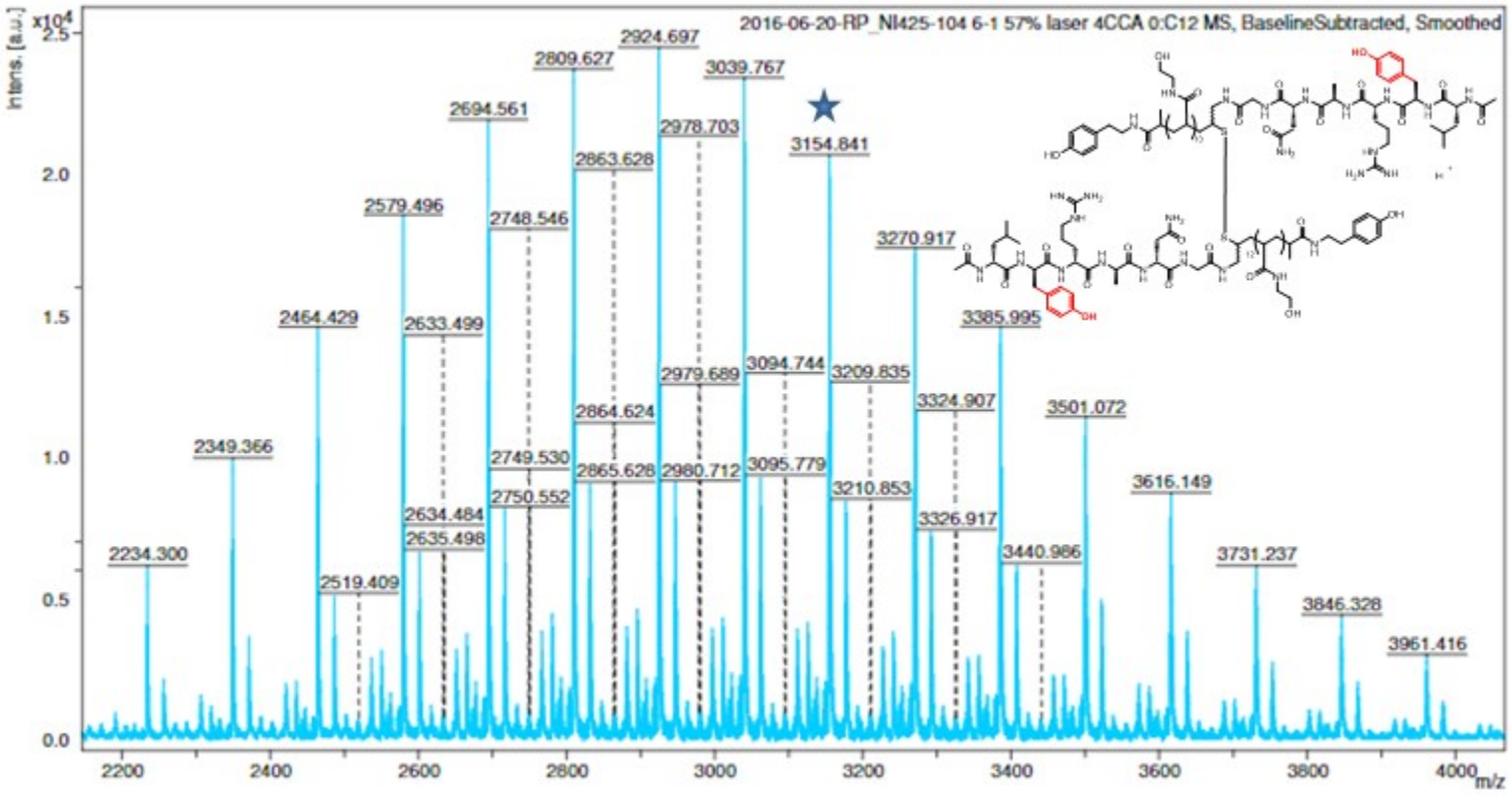

Figure S21: MALDI –ToF spectrum of HPLC purified peptide-p(HEAM)$_{10}$-AA-SH conjugate**15** i n α-CCA matrix in reflectron mode. NB: the oxidized (disulfide) product was the predominant species in the MALDI-ToF spectrum of **15**.

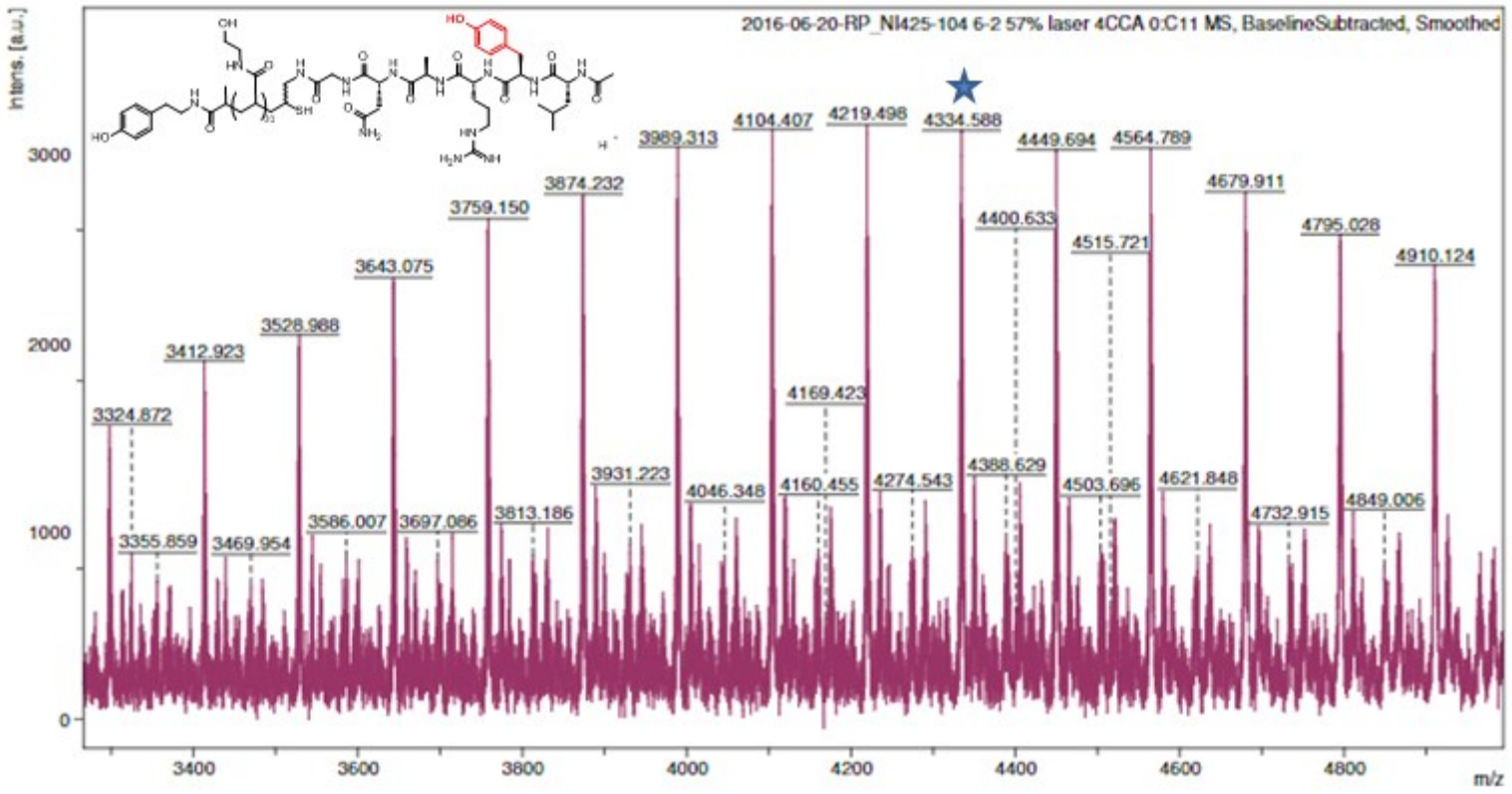

Figure S22: MALDI –ToF spectrum of HPLC purified peptide-p(HEAM)$_{30}$-AA-SH conjugate **16** in α-CCA matrix in linear mode.

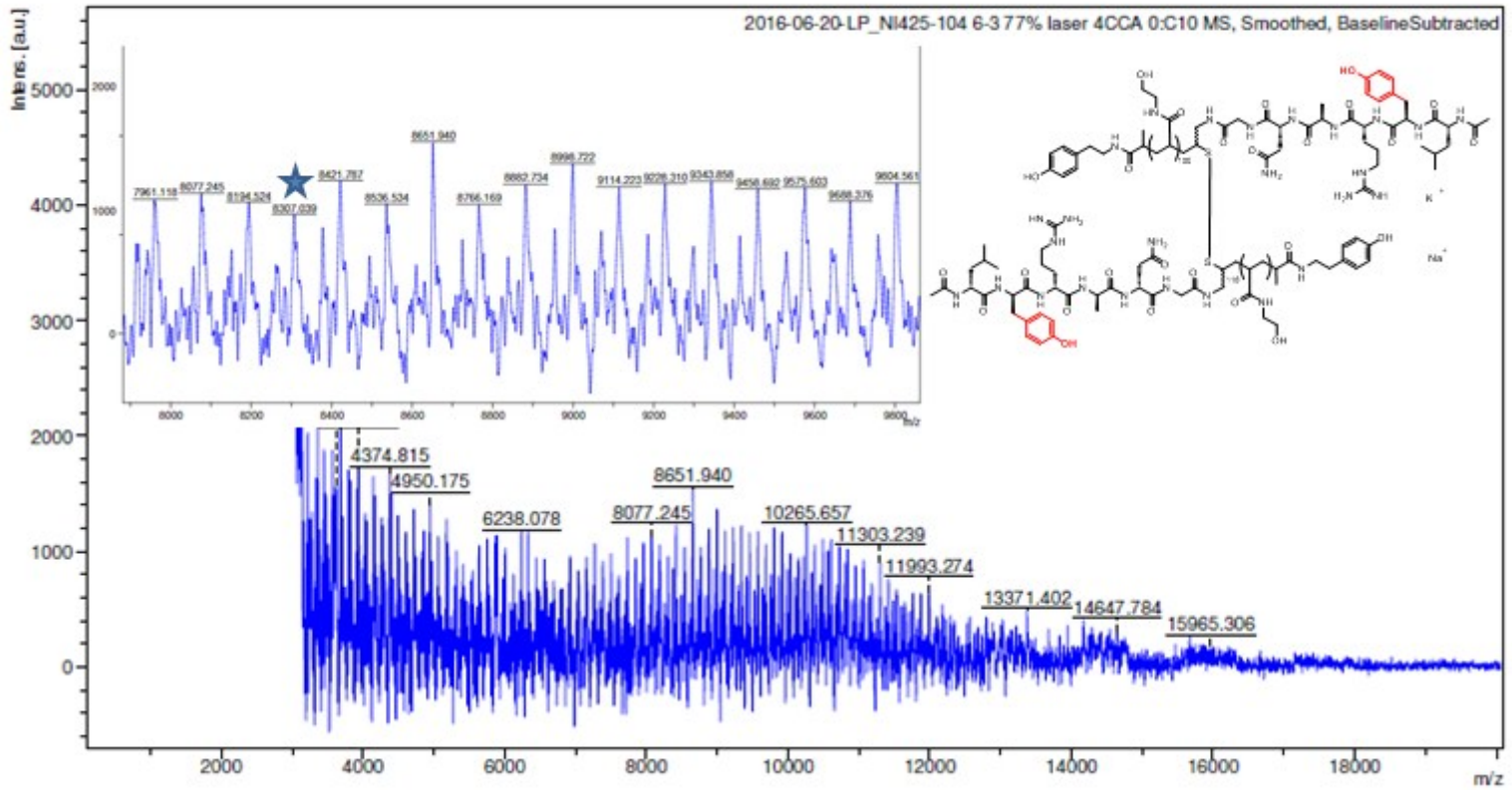

Figure S23: MALDI –ToF spectrum of HPLC purified peptide-p(HEAM)$_{100}$-AA-SH conjugate **17** in α-CCA matrix in reflectron mode. NB: the oxidized (disulfide) product was the predominant species in the MALDI-ToF spectrum of **17**.