Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Peptide Based Folding and Function of Single Polymer Chains

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List of abbreviations

AIBN:	Azobisisobutyronitrile
PEGMA:	Poly(ethyleneglycole)methylmethermethacrylate (M_n=300 g/mol)
DIPEA:	N,N-Diisoproylethylamine
COMU:	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
DCM:	Dichloromethane
DMF:	Dimethylformamide
DMAC:	N,N-Dimethylacetamide
HEMA:	2-Hydroxyethylmethacrylate
THF:	Tetrahydrofuran
TFA:	Trifluoroacetic acid
TES:	Triethylsilane
HBTU:	*N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate
DBU:	1,8-Diazabicyclo[5.4.0.]undec-7-ene
Fmoc-Asp(OtBu):	Fmoc-L-aspartic acid 4-*tert*-butyl ester
Fmoc-His(Trt)-OH:	N_{α}-Fmoc-$N_{(im)}$-trityl-L-histidine
Fmoc-Ser(tBu)-OH:	Fmoc-*O*-*tert*-butyl-L-serine
Boc-Cys(Trt)-OH:	*N*-(*tert*-Butoxycarbonyl)-*S*-trityl-L-cysteine

1 Experimental details

1.1 DMAC-SEC

The SEC measurements were conducted on a *PSS* SECurity^2 system consisting of a *PSS* SECurity Degasser, *PSS* SECurity TCC6000 Column Oven (60 °C), *PSS* GRAM Column Set (8x150 mm 10 µm Precolumn, 8x300 mm 10 µm Analytical Columns, 1000 Å, 1000 Å and 30 Å) and an *Agilent* 1260 Infinity Isocratic Pump, *Agilent* 1260 Infinity Standard Autosampler, *Agilent* 1260 Infinity Diode Array and Multiple Wavelength Detector (A: 254 nm, B: 360 nm), *Agilent* 1260 Infinity Refractive Index Detector (35 °C). HPLC grade DMAC, 0.01 M LiBr, is used as eluent at a flow rate of 1 mL·min^{-1}. Narrow disperse linear poly(styrene) (M_n: 266 g·mol^{-1} to 2.52x10^6 g·mol^{-1}) and poly(methyl methacrylate) (M_n: 202 g·mol^{-1} to 2.2x10^6 g·mol^{-1}) standards (*PSS* ReadyCal) were used as calibrants. All samples were passed over 0.22 µm PTFE membrane filters. Molecular weight and dispersity analysis was performed in *PSS* WinGPC UniChrom software (version 8.2).

1.2 NMR-Measurements

^{1}H NMR spectra were recorded on a *Bruker* System 600 Ascend LH, equipped with a BBO-Probe (5 mm) with z-gradient (^{1}H: 600.13 MHz) or a Bruker 400 UltraShield spectrometer equipped with a Quattro Nucleus Probe (QNP) with an operating frequency of 400 MHz (^{1}H). All measurements were carried out in deuterated solvents. The chemical shift (δ) is recorded in parts per million (ppm) and relative to the residual solvent protons.[1] The measured coupling constants were calculated in Hertz (Hz). To analyze the spectra the software MESTRENOVA 11.0 was used. The signals were quoted as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet and m = multiplet.

1.3 UV/VIS-Measurements

UV/vis spectra were recorded on a *Shimadzu* UV-2700 spectrophotometer equipped with a CPS-100 electronic temperature control cell positioner. Samples were prepared in phosphate buffered aqueous solutions (50 mM, pH=7) and measured in *Hellma Analytics* quartz high precision cells with a path length of 10 mm at ambient temperature. The solution concentrations were 120 µM (substrate) and 260 nM (peptide).

2 Synthetic procedures

2.1 Materials

Unless stated otherwise, all chemicals and solvents were used as received from the supplier without further purification.

N-(*tert*-Butoxycarbonyl)-*S*-trityl-L-cysteine (Combi-Blocks) (Boc-Cys(Trt)-OH), 2-Hydroxyethylmethacrylate (Sigma Aldrich) (HEMA), N,N-Diisopropylethylamine (Sigma Aldrich) (DIPEA), (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (Combi-Blocks) (COMU), Tetrahydrofuran (Sigma Aldrich) (THF) (after drying and purification with SP-1 Stand Alone Solvent Purification System LC Technology Solutions Inc.), Dichloromethane (DCM) (Fisher Chemical), Poly(ethyleneglycole)methylmethermethacrylate (PEGMA, M_n=300 g/mol) (Sigma Aldrich) (purified through an aluminum oxide column), Methylmethacrylate (MMA), Azobisisobutyronitrile (AIBN) (recrystallized), 2-Cyanopropan-2-yl-benzodithioate (Sigma Aldrich), 1,4-Dioxane (Sigma Aldrich) (purified through an aluminum oxide column), hexane (Ajax Finechem), Trifluoroacetic acid (Alfa Aesar) (TFA), Triethylsilane (Sigma Aldrich) (TES), Diethylether (Ajax Finechem), Dimethylformamide (DMF) (Ajax Finechem), *N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (Mimotopes) (HBTU), 1,8-Diazabicyclo[5.4.0.]undec-7-ene (DBU) (Sigma Aldrich), Fmoc-L-aspartic acid 4-*tert*-butyl ester (Fmoc-Asp(OtBu)) (Mimotopes), N_{α}-Fmoc-$N_{(im)}$-trityl-L-histidine (Fmoc-His(Trt)-OH) (Mimotopes), Fmoc-*O*-*tert*-butyl-L-serine (Fmoc-Ser(tBu)-OH) (Mimotopes), glycine preloaded 2CT resin (Mimotopes).

2.2 Synthesis of M1

A dry Schlenk flask was evacuated and purged with nitrogen several times before 30 mL dry THF from the solvent purification system were added to the flask. After connecting the flask to a Schlenk line, evacuating and purging the connecting lines several times with argon, *N*-(*tert*-butoxycarbonyl)-*S*-trityl-L-cysteine (1.00 g, 2.16 mmol, 1.0 eq.), 2-hydroxyethylmethacrylate (2.81 g, 21.57 mmol, 10.0 eq.), DIPEA (0.75 mL, 4.31 mmol, 2.0 eq.) and COMU (1.39 g, 3.24 mmol, 1.5 eq.) were added to the flask under a constant Argon stream. The mixture was stirred for 24 hours at room temperature under Argon.

The product was concentrated under reduced pressure and purified by column chromatography (SiO_2, dichloromethane).

Isolated yield: 482.4 mg (39%)

^{1}H-NMR: ^{1}H NMR (600 MHz, CD_3CN) δ 7.41 – 7.36 (m, 6H), 7.34 – 7.29 (m, 6H), 7.28 – 7.21 (m, 3H), 5.99 (dq, *J* = 2.1, 1.0 Hz, 1H), 5.55 (m, 1H), 5.48 (d, *J* = 8.5 Hz, 1H), 4.37 – 4.17 (m, 4H), 3.98 – 3.80 (m, 1H), 2.56 (dd, *J* = 12.9, 8.6 Hz, 1H), 2.48 (dd, *J* = 12.8, 4.8 Hz, 1H), 1.84 (dd, *J* = 1.6, 1.0 Hz, 3H), 1.39 (s, 9H).

LC-MS: Calculated for $C_{33}H_{37}NO_6SNa^+$: 598.2234; found: 598.2242. Calculated for $C_{33}H_{37}NO_6SNH_4^+$: 593.7585; found: 593.2690

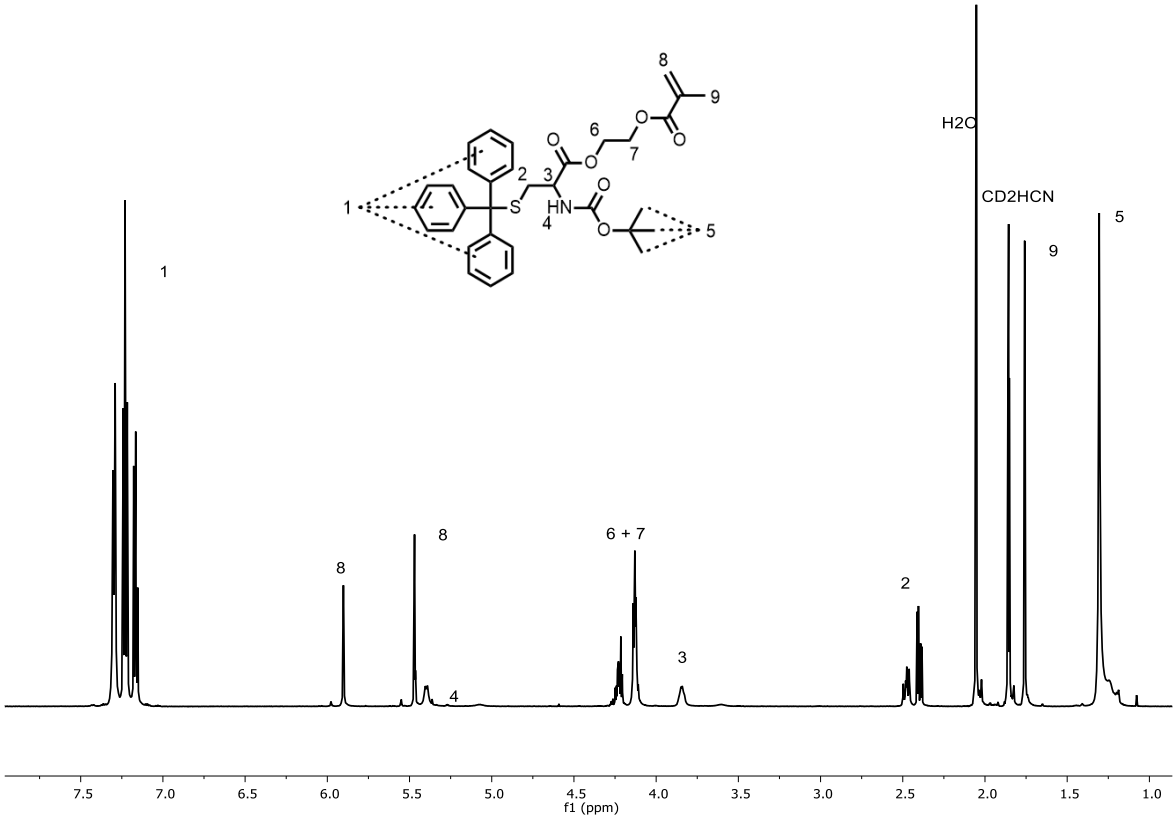

Figure S1: 1H-NMR of M1 in Acetonitrile-d3

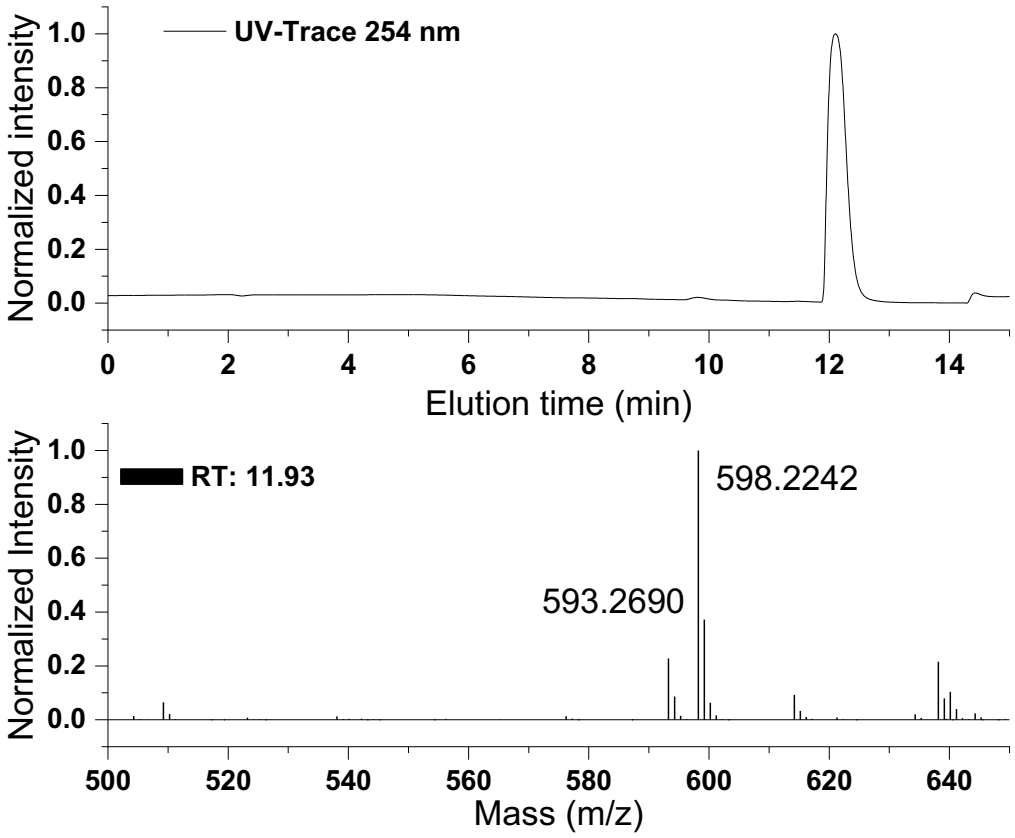

Figure S2: LC-MS of M1.

2.3 Synthesis of M2

The same procedure was used to synthesize M2 (compare M1). The following amounts were used: Peptide sequence (Cys-Ser-His-Asp-Gly) (788.1 mg, 649 µmol, 1.0 eq), HEMA (844.5 mg, 6.49 mmol, 10 eq.), DIPEA (115 µL, 1.3 mmol, 2 eq.), COMU (417 mg, 973 mmol, 1.5 eq.). Column chromatography was carried out with DCM (45 %), Ethylacetate (45 %) and Methanol (10 %).

After the purification by column chromatography, the fractions containing the product were still impure and therefore combined and the solvent was removed under reduced pressure. The remaining solid was dissolved in 5 mL Methanol. The mixture was dropped into water and centrifuged for 15 minutes. The product was freeze dried over night.

Isolated yield: 370 mg (43.0 %)

^{1}H-NMR: ^{1}H NMR (600 MHz, DMSO) δ 9.25 (s, 1H), 8.42 (d, *J* = 8.3 Hz, 1H), 7.91 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.38 – 7.04 (m, 33H), 6.00 – 5.97 (m, 1H), 5.64 (p, *J* = 1.6 Hz, 1H), 4.72 – 4.65 (m, 1H), 4.50 – 4.45 (m, 1H), 4.28 – 4.16 (m, 5H), 3.90 – 3.70 (m, 3H), 2.84 (d, *J* = 5.4 Hz, 2H), 2.73 (dd, *J* = 16.0, 5.5 Hz, 1H), 2.43 – 2.29 (m, 3H), 2.02 – 1.79 (m, 3H), 1.40 – 1.20 (m, 20 H), 1.02 (s, 9H).

LC-MS: Calculated for $C_{75}H_{87}N_7O_{13}SH^+$: 1326.6155; found: 1326.6144.

Calculated for $C_{75}H_{87}N_7O_{13}SNa^+$: 1348.5975; found: 1348.5954.

Calculated for $C_{75}H_{87}N_7O_{13}S^-$: 1324.6009; found: 1324.6004.

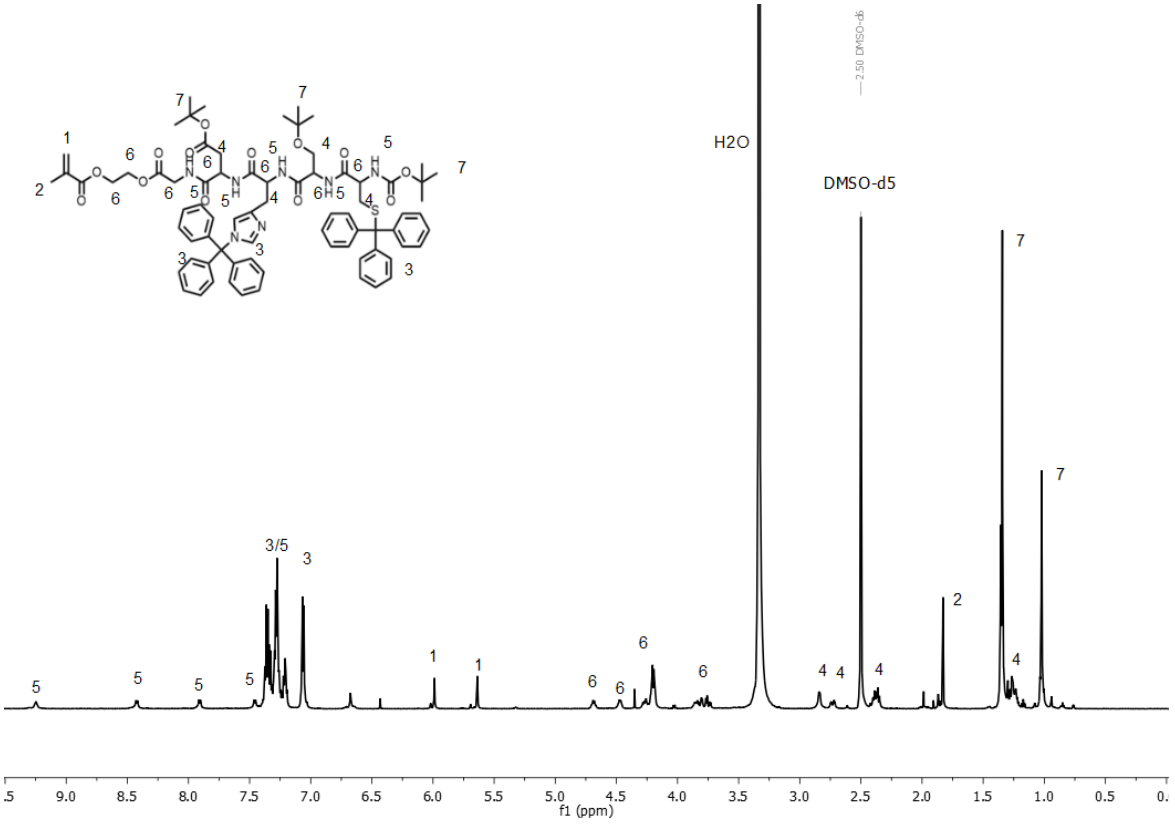

Figure S3: 1H-NMR of M2 in Dimethylsulfoxide-d6.

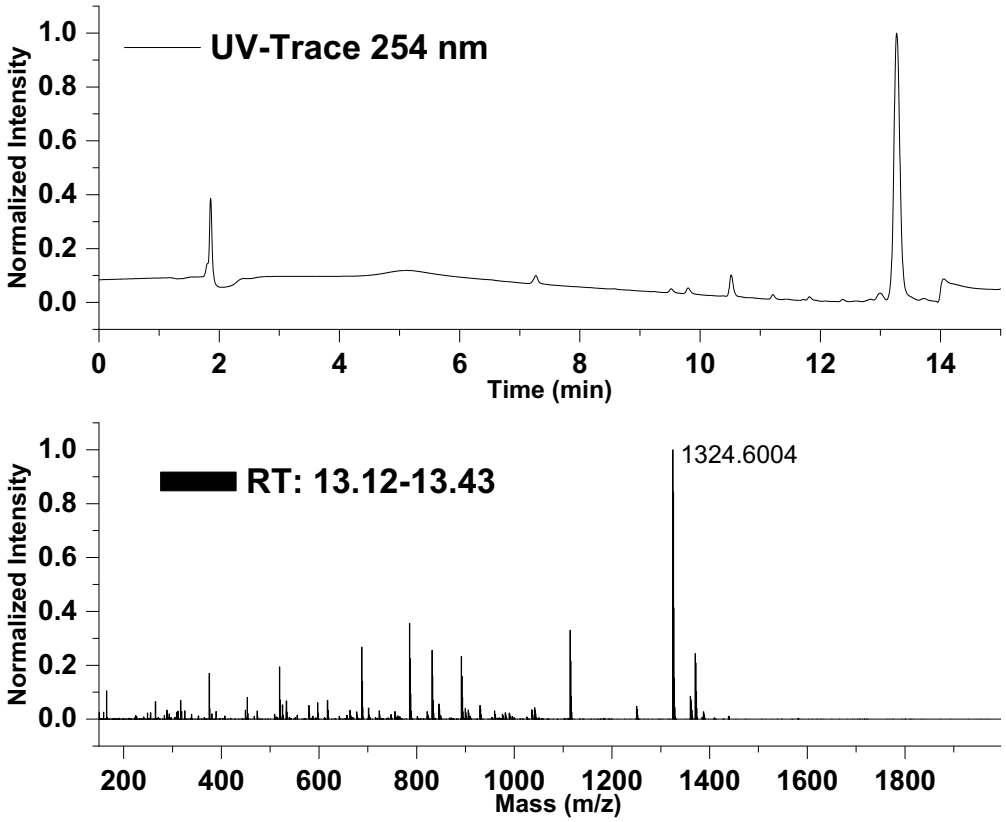

Figure S4: LC-MS (negative) of M2.

2.4 Synthesis of P1

M1 (83.00 mg, 0.145 mmol, 0.25 eq.), poly(ethyleneglycole)methylethermethacrylat (PEGMA) (M_n=300 g/mol; 160.00 mg, 0.579 mmol, 1.00 eq.), AIBN (0.95 mg; 5.8 µmol, 0.01 eq.) and 2-cyanopropan-2-yl-benzodithioate (6.40 mg, 29 µmol, 0.05 eq.) were dissolved in 0.25 mL 1,4-dioxane and added to a cramp vial. The solution was purged with argon for 15 min before the polymerization was started at 80 °C. After 2.5 h (P1) the polymerization was stopped by floating the vial with air to obtain a pink, highly viscous liquid. The mixture was diluted in 1.5mL 1,4-Dioxane and precipitated out of cold hexane. The polymer was obtained as a pink sticky solid after centrifugation (20 min, 5000 RPM). The product was dried in the vacuum oven at 40°C.

Isolated yield: 239.8 mg (47.7 %).

SEC (PMMA cal.): M_n= 7,000 g/mol, $Đ$ = 1.2.

^{1}H-NMR: 1H NMR (600 MHz, CD3CN) δ 7.46 – 7.20 (m, 15H), 6.12 – 5.65 (m, 1H), 4.05 (s, 12H), 3.57 – 3.50 (m, 62H), 3.33 – 3.20 (m, 12H), 1.46 – 1.19 (m, 9H).

2.5 Synthesis of P2

For P2, M2 (20 mg, 15 µmol, 10.00 eq.), PEGMA (28 mg, 100 µmol, 66.00 eq), MMA (21 mg, 202 µmol, 134.00 eq.), AIBN (50 µg, 0.3 µmol, 0.20 eq.) and 2-cyanopropan-2-yl-benzodithioate (0.34 mg, 1.5 µmol, 1.00 eq.) were dissolved in 0.07 mL 1,4-dioxane and added to a cramp vial. The solution was purged with argon for 15 min before the polymerization was started at 80 °C. After 15 h, the polymerization was stopped by floating the vial with air to obtain a pink, highly viscous liquid. Half of the reaction mixture was purified via precipitated out of cold hexane. The polymer was obtained as a pink sticky solid after centrifugation (20 min, 5000 RPM). The product was dried in the vacuum oven at 40°C.

Isolated yield: 17.7 mg.

SEC (PMMA cal.): M_n= 11,600 g/mol, Đ= 1.2.

^{1}H-NMR: 1H NMR (600 MHz, CD3CN) δ 7.35 – 6.97 (m, 32H), 3.98 (s, 18H), 3.52 – 3.43 (m, 112H), 3.39 (s, 21H), 3.21 (d, 24H), 1.76 – 1.63 (m, 9H), 1.29 (m, 18H).

2.6 Synthesis of P3

60 mg M2 (45 µmol, 10.00 eq.), 83 mg PEGMA (299 µmol, 66.00 eq.), 61 mg MMA (606 µmol, 134.00 eq.), 149 µg AIBN (0.9 µmol, 0.20 eq.) and 2-cyanopropan-2-yl-benzodithioate (1.00 mg, 4.5 µmol, 1.00 eq.) were dissolved in 0.21 mL 1,4-dioxane and added to a crimp vial. The solution was purged with argon for 15 min before the polymerization was started at 80 °C. After 3 h, the polymerization was stopped by floating the vial with air to obtain a pink, highly viscous liquid. Half of the reaction mixture was purified via precipitation out of cold hexane. The polymer was obtained as a pink sticky solid after centrifugation (20 min, 5000 RPM). The product was dried in the vacuum oven at 40°C.

Isolated yield: 122.6 mg.

SEC (PMMA cal.): M_n= 19,700 g/mol, Đ= 1.1.

^{1}H-NMR: (600 MHz, CD_3CN) δ 7.45 – 6.82 (m, 32H), 3.98 (s, 18H), 3.49 (s, 113H), 3.40 (s, 14H), 3.23 (s, 18H).

The polymerization of P3 was tracked via ^{1}H-NMR to compare the reactivities of the different monomers. The following table shows the incorporation ratio of all monomers in relation to the peptide-monomer:

	Integral			Ratio
t [h]	**MMA**	**PEGMA**	**Peptide**	**MMA/PEGMA/Peptide**
0	0	0	0	0/0/0
1	18.67	96.91	32	6.2/6.1/1.0
1.5	16.41	97.02	32	5.5/6.1/1.0
3	20.45	134.46	32	6.8/8.4/1.0

Table S1: Tracking of P3 over time. The feed ratio was 134/66/10.

The calculations are based on the integrals of the resonances 1 (peptide), 3 (PEGMA) and 5 (MMA) in the following assigned spectrum:

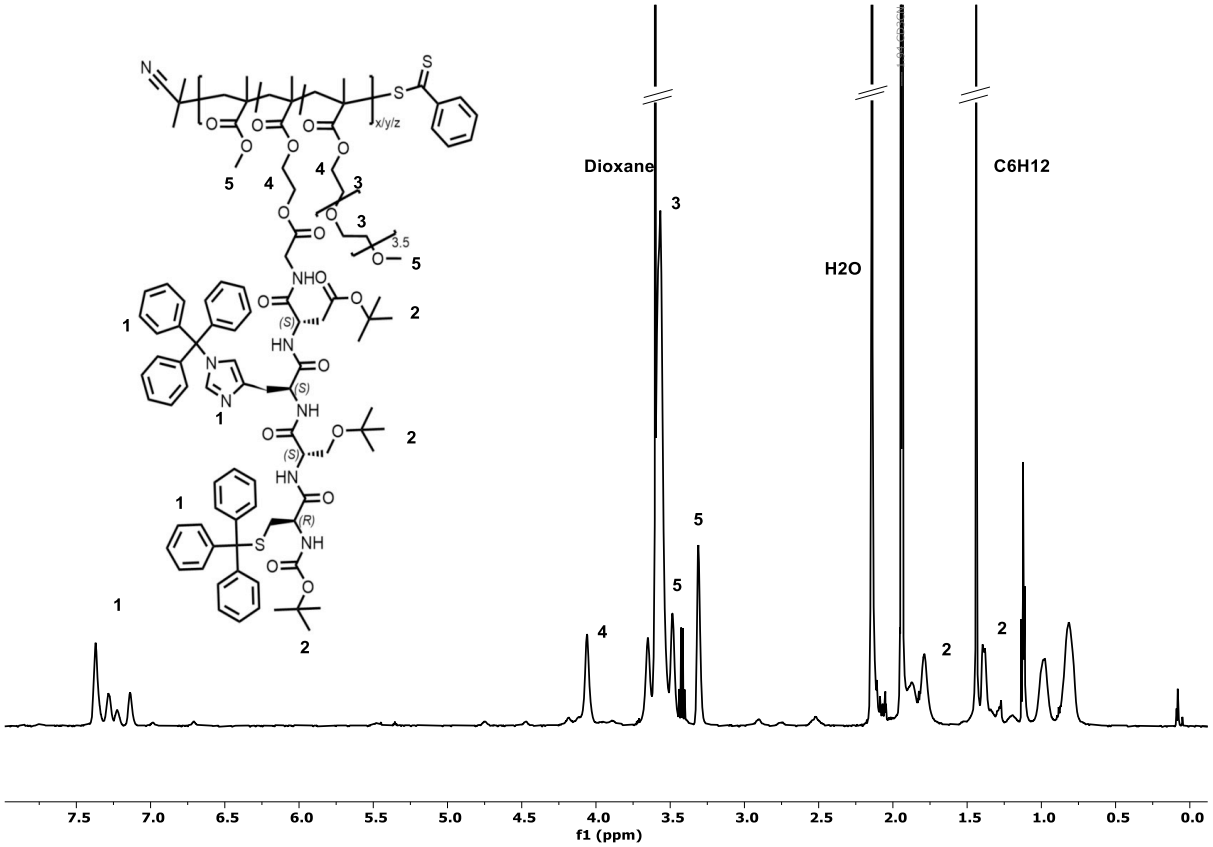

Figure S5: ^{1}H-NMR of P3 in Acetonitrile-d_3

2.7 Synthesis of SCNP1

50 mg of P1 (7.1 µmol) were dissolved in a mixture of 0.2 mL triethylsilane and 1.8 mL trifluoroacetic acid and stirred for 30 min at room temperature. The mixture was afterwards precipitated out of cold hexane and diethylether (1:1, 30 mL) and centrifuged (20 min, 5000 RPM) to obtain a pink solid, which was dried in a vacuum oven at 40 °C.

SEC (PMMA cal.): SEC: M_n= 6,600 g/mol, Đ= 1.2.

1H-NMR: 1H NMR (600 MHz, CD3CN) δ 4.08 (s, 12H), 3.59 (s, 62H), 3.32 (s, 12H).

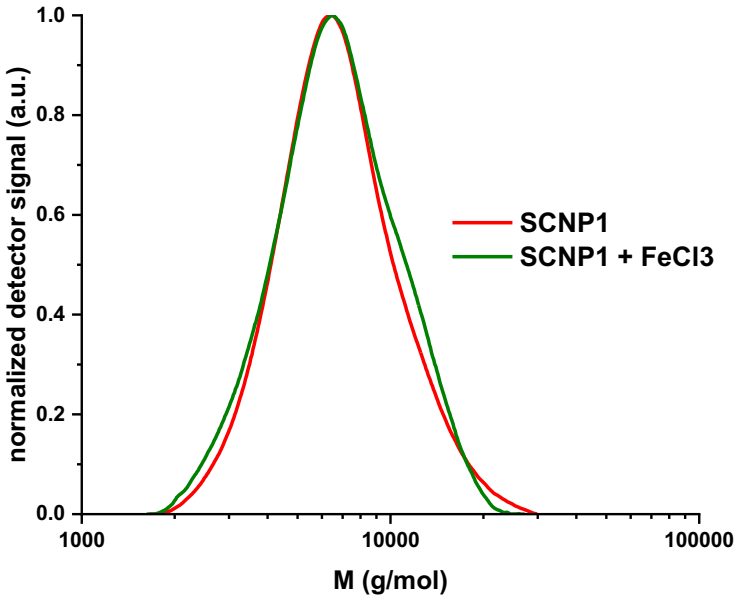

Figure S6: SEC-Overlay of SCNP1 and SNCP1 + FeCl3.

A spatula tip of Iron(III)chloride Hexahydrate was added to a solution of SCNP1 in DMAC in order to obtain the SEC graph in Figure S6.

The ^{1}H-NMR of SCNP1 shows aromatic resonances which indicate an incomplete deprotection (close to 90%). To complete the deprotection, the deprotection reaction was repeated with P1 as the starting material and an extended reaction time (60 min instead of 30 min). The following ^{1}H-NMR shows the afterwards completed deprotection of the obtained SNCP1a:

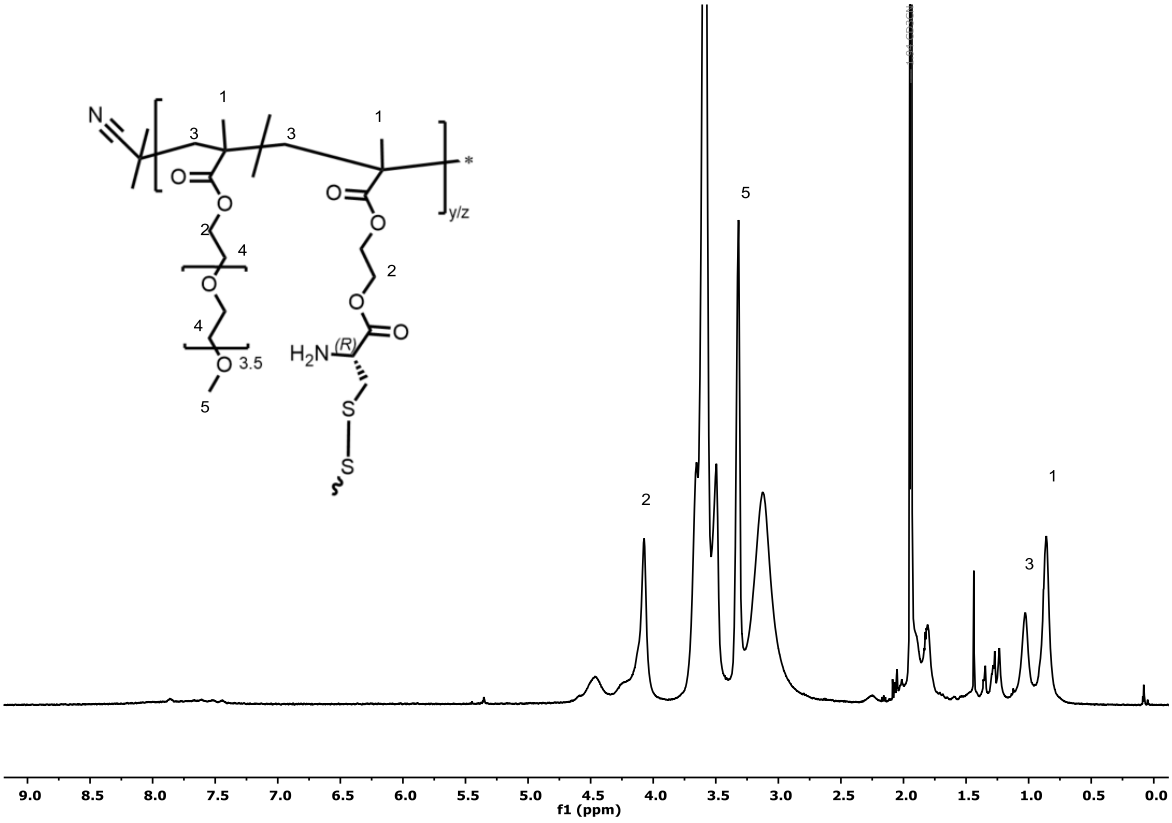

Figure S7: ^{1}H-NMR of SCNP1a in Acetonitrile-d_3.

However, the extended reaction time leads not only to a complete deprotection, but also to intermolecular crosslinking hence the incomplete deprotection was favoured. The following SEC trace shows the broadening of the molecular weight distribution:

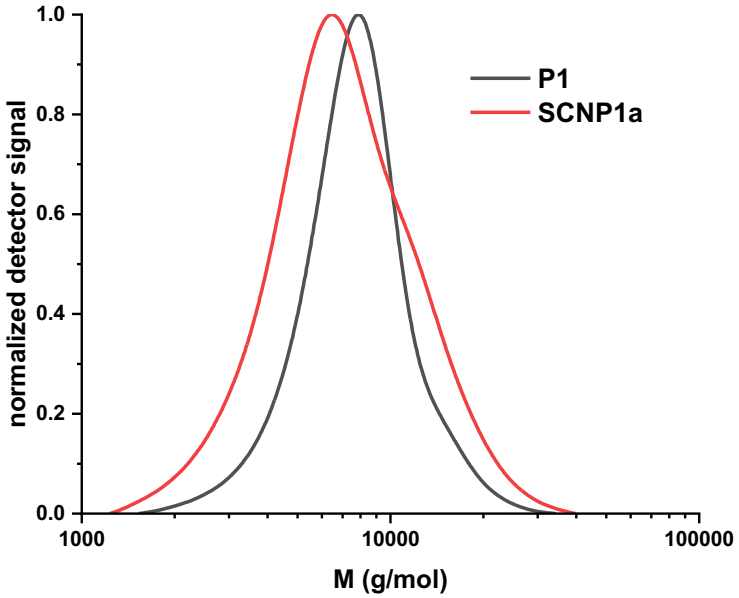

Figure 8: SEC-Overlay of P1 and SCNP1a obtained through an extended reaction time

Lowering the polymer concentration in the folding reaction did not result in a complete disappearance of the high molecular weight shoulder as the following SEC trace shows:

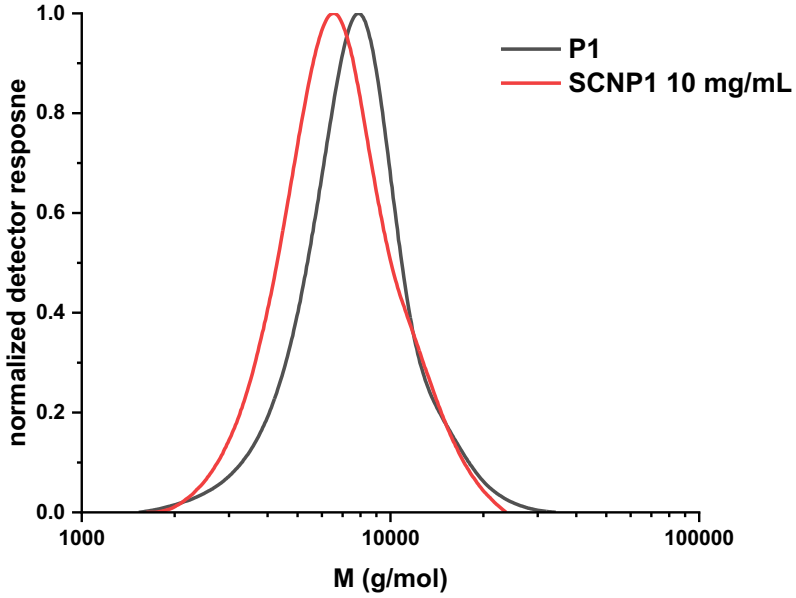

Figure S9: SEC-Overlay of P1 and SCNP1 obtained through folding at 10 mg/mL.

2.8 Synthesis of SCNP2

15 mg of P2 were dissolved in a mixture of 1.6 mL trifluoroacetic acid and 0.4 mL triethylsilane and stirred at room temperature for 30 minutes. The mixture was afterwards precipitated out of cold hexane and diethylether (1:1, 30 mL) and centrifuged (20 min, 5000 RPM) to obtain a pink solid, which was dried in a vacuum oven at 40 °C.

SEC (PMMA cal.): SEC: M_n= 10,800 g/mol, Đ= 1.2.

^{1}H-NMR: 1H NMR (600 MHz, CD3CN) δ 4.07 (s, 18H), 3.58 (m, 112H), 3.49 (s, 21H), 3.31 (d, 24H).

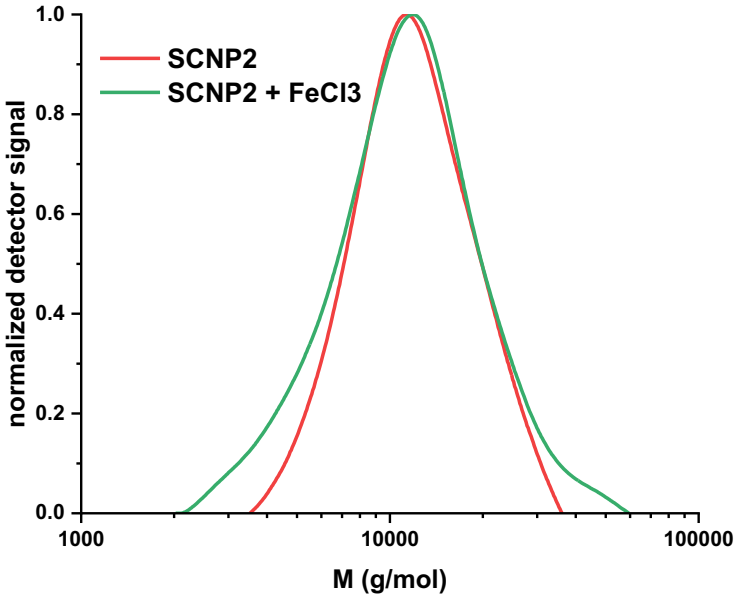

Figure S10: SEC-Overlay of SCNP2 and SCNP2 + FeCl3

A spatula tip of Iron(III)chloride Hexahydrate was added to a solution of SCNP2 in DMAC in order to obtain the SEC graph in Figure S10.

2.9 Synthesis of SCNP3

16 mg of P3 were dissolved in a mixture of 2.4 mL trifluoroacetic acid and 0.6 mL triethylsilane and stirred at room temperature for 30 minutes. The mixture was afterwards precipitated out of cold hexane and diethylether (1:1, 30 mL) and centrifuged (20 min, 5000 RPM) to obtain a pink solid, which was dried in a vacuum oven at 40 °C.

SEC (PMMA cal.): SEC: M_n= 16,900 g/mol, Đ= 1.2.

^{1}H-NMR: 1H NMR (600 MHz, CD3CN) δ 4.07 (s, 2H), 3.58 (d, J = 15.1 Hz, 19H), 3.50 (s, 3H), 3.32 (s, 3H).

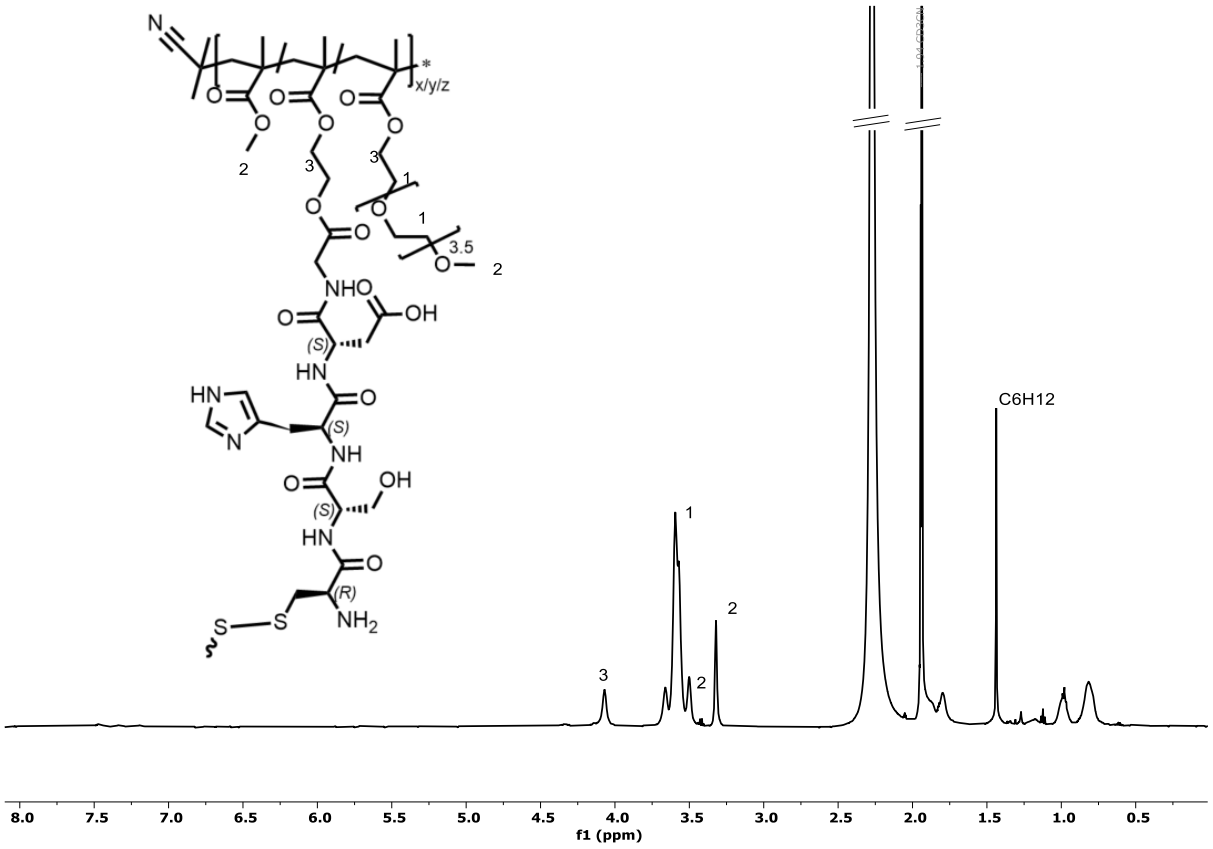

Figure S11: ^{1}H-NMR of SCNP3 in Acetonitrile-d_3

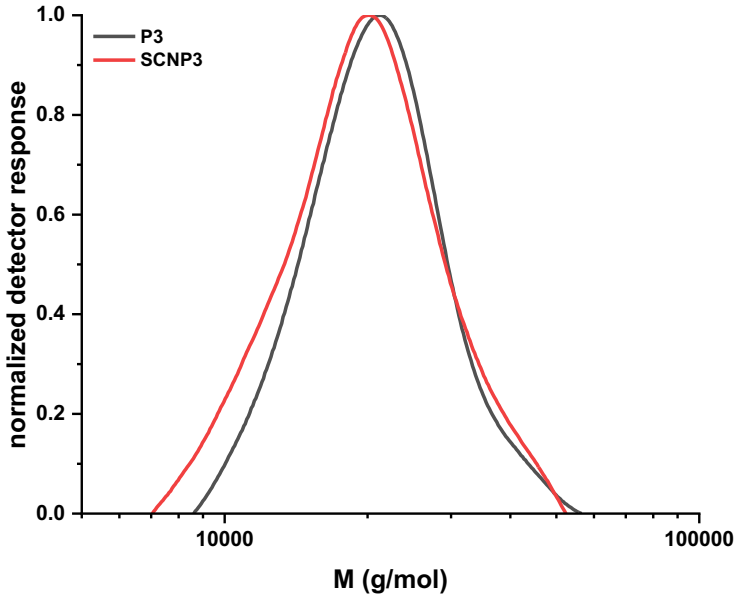

Figure S12: SEC-Overlay of P3 and SCNP3.

Additionally, P3 was deprotected with an extended reaction time (over night). The following NMR clearly shows the removal of protecting groups and the SEC trace shows the increased occurrence of intermolecular crosslinking for extended reaction times:

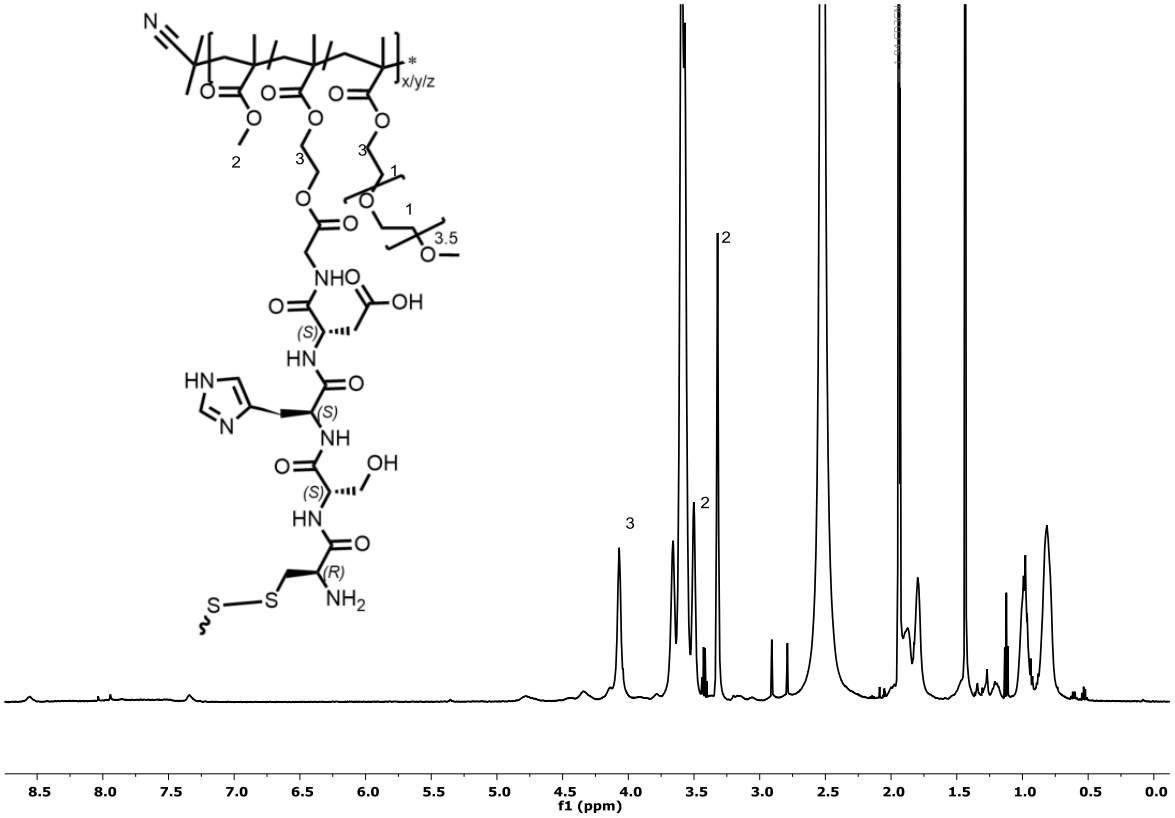

Figure S13: ^{1}H-NMR of SCNP3a in Acetonitrile-d_3

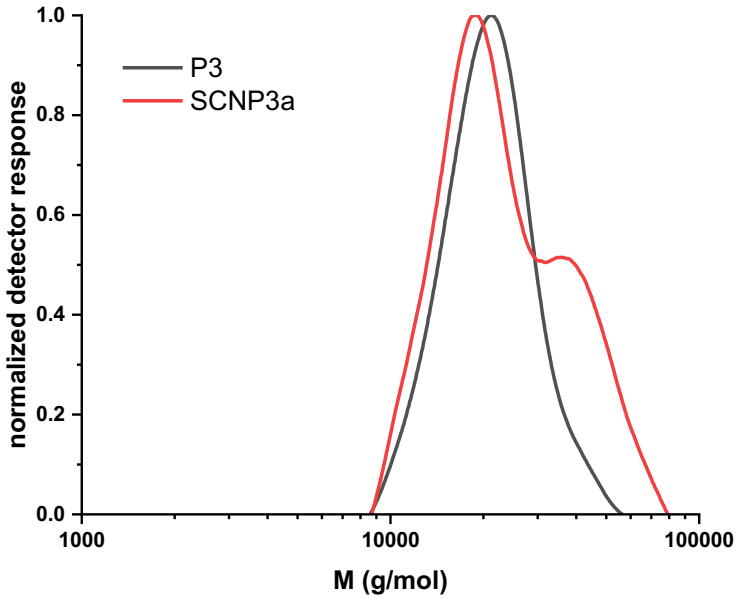

Figure S14: SEC Overlay of P3 and SCNP3a obtained after deprotection overnight.

2.10 Synthesis of the peptide sequence

The peptide sequence was synthesized on solid phase using a glycine loaded resin.
1.00 g of the loaded resin were used (1.25 mmol, 1 eq.) initially. Before every coupling reaction, the amino acid on the resin was deprotected first. Therefore, 2x10 mL of 5 % DBU in DMF were added to the resin and the mixture was agitated for 10 minutes. Afterwards, the resin was washed 3x with DMF and 3x with DCM. For each coupling step, the coupling solution containing HBTU (5.00 mmol, 4 eq., 1.90 g), DIPEA (5.00 mmol, 4 eq., 0.92 mL) and the respective amino acid (5.00 mmol, 4 eq.) was added to the resin and the mixture was agitated for 1 h. Afterwards, the resin was washed again 3x with DMF and 3x with DCM. Those steps are repetitive for each coupling and deprotection.

Amino Acid	**m [g]**
Fmoc-Asp(OtBu)	2.06
Fmoc-His(Trt)-OH	3.10
Fmoc-Ser(tBu)-OH	1.92
Boc-Cys(Trt)-OH	2.32

Table S2: Amounts of Amino Acids used for the coupling steps.

After the coupling of cysteine, the peptide was cleaved from the resin with 3x 1 % TFA in DCM. The mixture was agitated for 45 min each time.

LC-MS: Calculated for $C_{69}H_{79}N_7O_{11}SH^+$: 1214.5631, found: 1214.5657

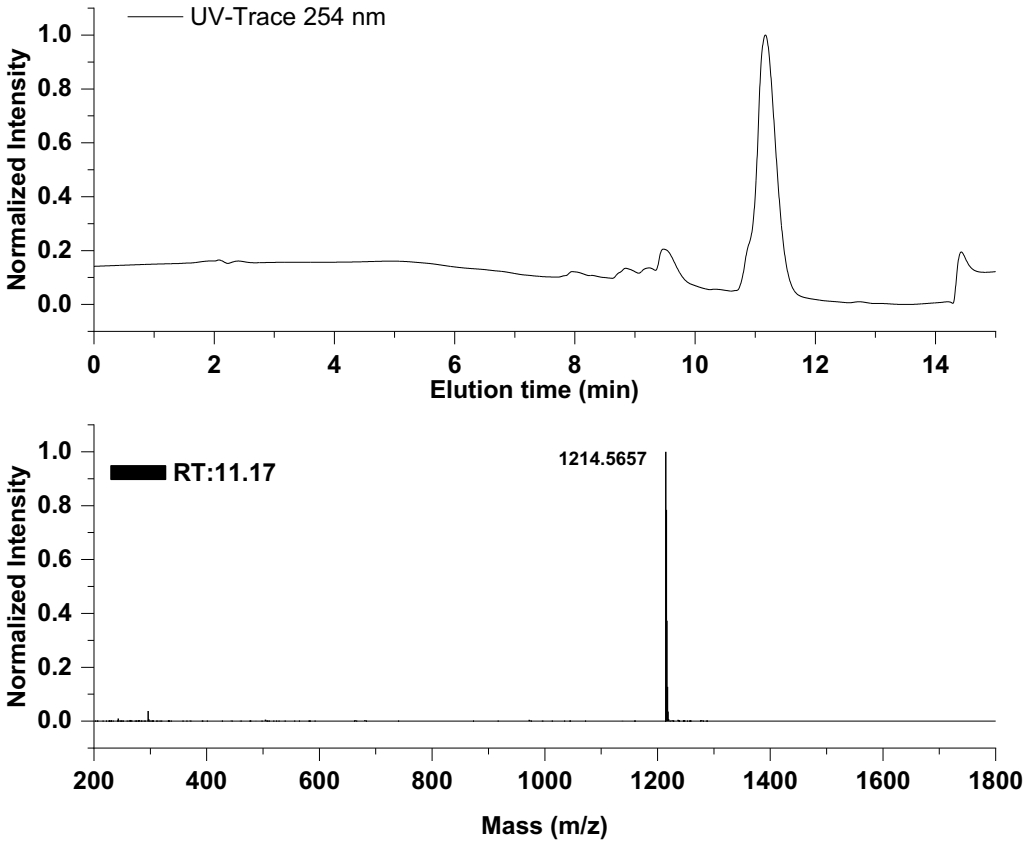

Figure S15: LC-MS spectra of the peptide sequence after the solid phase peptide synthesis.

2.11 Deprotection of the peptide sequence

To use the peptide sequence as a reference experiment for the catalysis studies, the protected sequence was treated with TFA/TES (8:2) to obtain the deprotected peptide sequence. The success of the reaction was confirmed via ^{1}H-NMR:

^{1}H NMR (600 MHz, D_2O) δ 8.62 (d, *J* = 1.4 Hz, 1H), 7.32 (d, *J* = 1.4 Hz, 1H), 4.48 (t, *J* = 5.6 Hz, 1H), 4.29 (t, *J* = 5.4 Hz, 1H), 4.01 (s, 2H), 3.85 (dd, *J* = 5.7, 3.8 Hz, 2H), 3.34 – 2.77 (m, 8H).

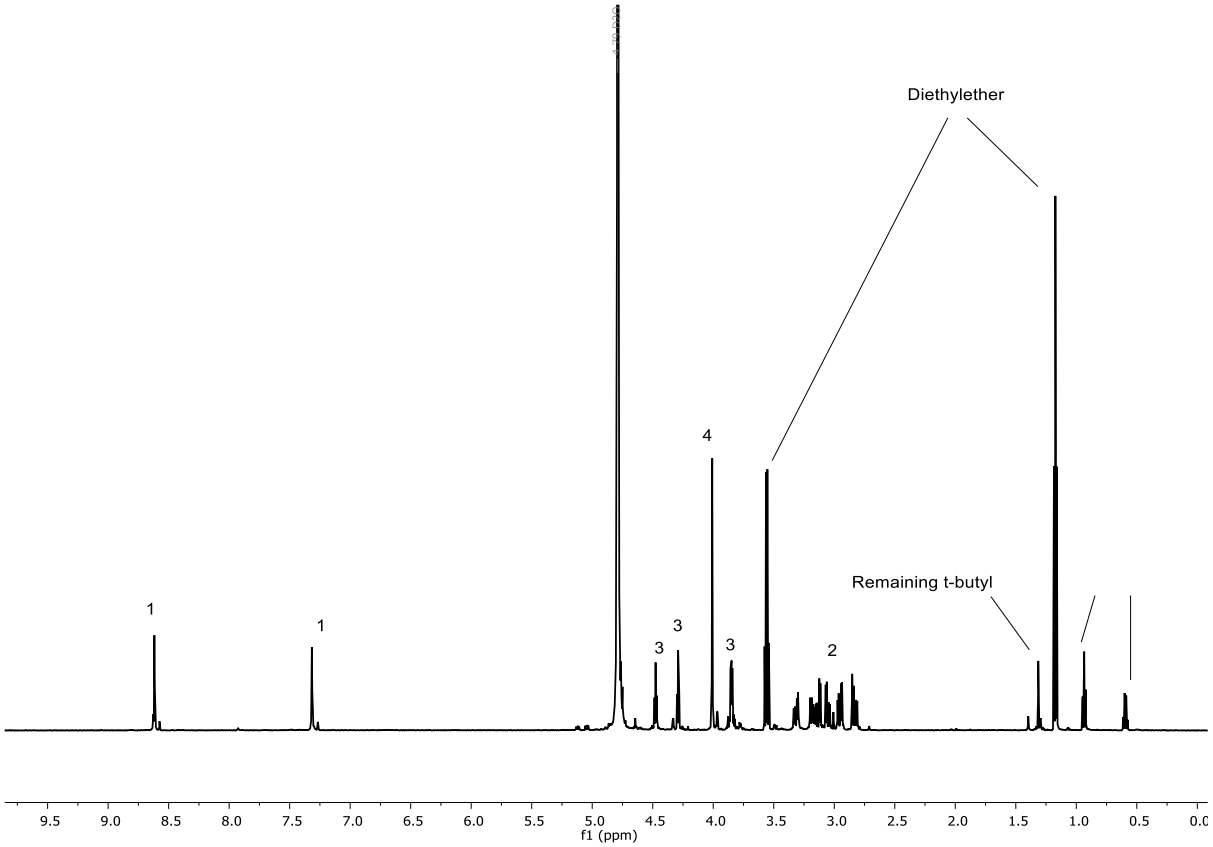

Figure S16: ^{1}H-NMR of GDHSC in D_2O.

3 Calculation of monomers per chain of P1-P3

The average number of monomer units **(M1, M2, MMA, PEGMA)** were calculated using the percentage of the respective monomer in polymer **P1 and P2** obtained from the ^{1}H-NMR spectra and the $\bar{M}_n$ obtained from the SEC:

$$n(M) = n(monomers) \bullet X(M)$$

$$n(MMA) = n(monomers) \bullet X(MMA)$$

With the average number of monomers per polymer chain $n(monomers)$:

$$n(monomers) = \frac{\bar{M}_n\ (Polymer) - M(RAFT\text{- }group)}{M_{Average}(monomer)}$$

With the average monomer mass ($M_{Average}$):

$$M_{Average} = X(M) \bullet M(M) + X(MMA) \bullet M(MMA) + X(PEGMA) \bullet M(PEGMA)$$

Resulting in the following formula:

$$n(M) = \left(\frac{\bar{M}_n\ (Polymer) - M(RAFT\text{- }group)}{X(M) \bullet M(M) + X(MMA) \bullet M(MMA) + X(PEGMA) \bullet M(PEGMA)}\right) \bullet X(M)$$

For **P1** this leads to:

$$n(M1) = \left(\frac{7{,}000\ \ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.20 \bullet 576\ \ g\ mol^{-1} + 0 \bullet 100\ \ g\ mol^{-1} + 0.80 \bullet 300\ \ g\ mol^{-1}}\right) \bullet 0.20 = 4$$

$$n(PEGMA) = \left(\frac{7{,}000\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.20 \bullet 576\ g\ mol^{-1} + 0 \bullet 100\ g\ mol^{-1} + 0.80 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.80 = 15$$

The following signals were used for the calculations:

M1: aromatic signals of the protecting group (δ = 7.46 – 7.20 ppm).

PEGMA: signal of the methoxy group (δ = 4.05 ppm).

For **P2** this leads to:

$$n(M2) = \left(\frac{11{,}600\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.06 \bullet 1327\ g\ mol^{-1} + 0.50 \bullet 100\ g\ mol^{-1} + 0.44 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.06 = 3$$

$$n(PEGMA) = \left(\frac{11{,}600\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.06 \bullet 1327\ g\ mol^{-1} + 0.50 \bullet 100\ g\ mol^{-1} + 0.44 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.44 = 19$$

$$n(MMA) = \left(\frac{11{,}600\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.06 \bullet 1327\ g\ mol^{-1} + 0.50 \bullet 100\ g\ mol^{-1} + 0.44 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.50 = 22$$

The following signals were used for the calculations:

M2: aromatic signals of protecting groups and histidine (δ = 7.35 – 6.97 ppm).

PEGMA: signal of -CH_2- next to ester moiety. (δ = 3.98 ppm).

MMA: signal of the methoxy group (δ = 3.21 ppm).

For **P3** this leads to:

$$n(M2) = \left(\frac{19{,}700\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.06 \bullet 1327\ g\ mol^{-1} + 0.50 \bullet 100\ g\ mol^{-1} + 0.44 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.06 = 5$$

$$n(PEGMA) = \left(\frac{19{,}700\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.06 \bullet 1327\ g\ mol^{-1} + 0.50 \bullet 100\ g\ mol^{-1} + 0.44 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.44 = 33$$

$$n(MMA) = \left(\frac{19{,}700\ g\ mol^{-1} - 221\ g\ mol^{-1}}{0.06 \bullet 1327\ g\ mol^{-1} + 0.50 \bullet 100\ g\ mol^{-1} + 0.44 \bullet 300\ g\ mol^{-1}}\right) \bullet 0.50 = 37$$

The following signals were used for the calculations:

M2: aromatic signals of protecting groups and histidine (δ = 7.35 – 6.97 ppm).

PEGMA: signal of -CH_2- next to ester moiety. (δ = 3.98 ppm).

MMA: signal of the methoxy group (δ = 3.21 ppm).

4 Cysteinemethylester disulphide formation

To investigate the folding reaction in detail, small molecule studies were performed. Cysteinemethylester (44 mg) was dissolved in 4.5 mL TFA and 0.5 mL TES and stirred for 3 hours at room temperature. After precipitation out of hexane/diethylether (30mL, 1:1, V:V) and centrifugation, the solid residual was dried in a vacuum oven at 40°C.

The product was analysed via ^{1}H-NMR.

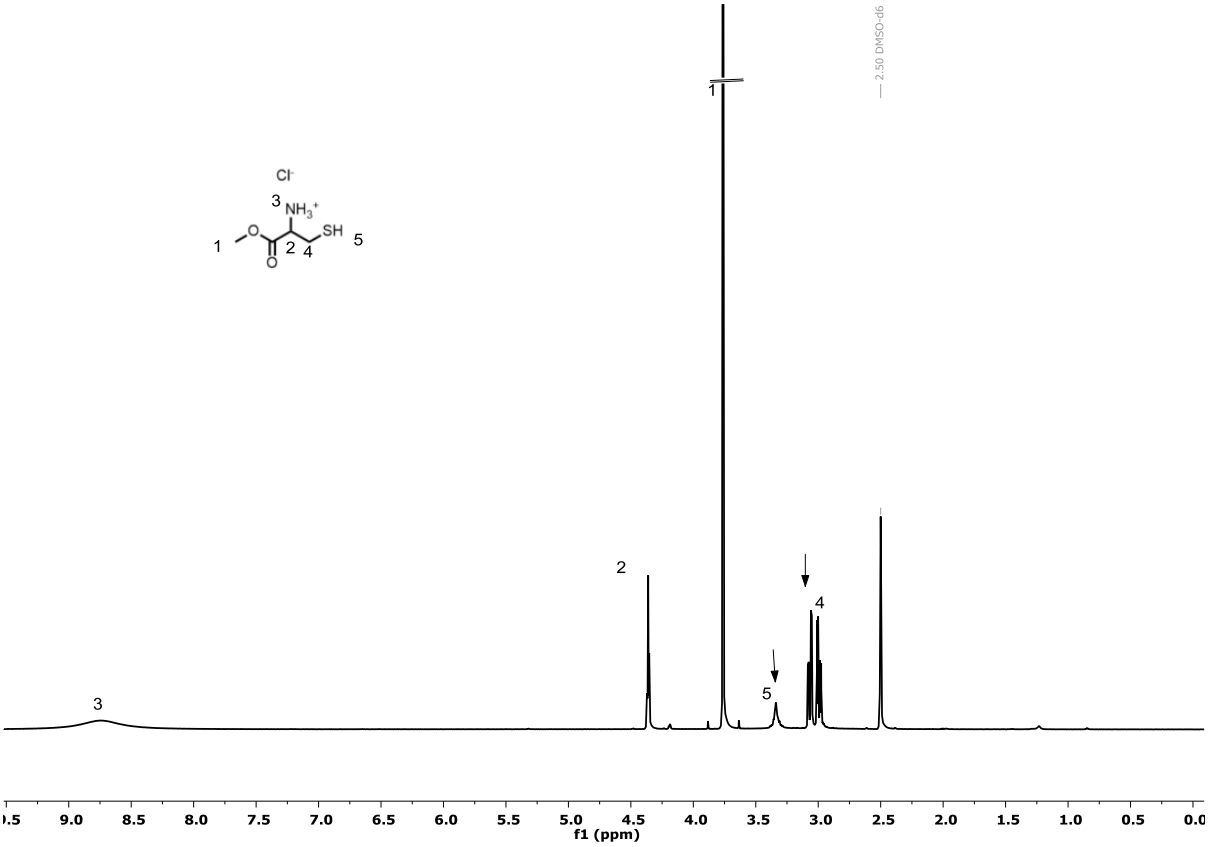

Figure S17: ^{1}H-NMR of Cysteinemethylester (starting material) in Dimethylsulfoxide-d_6

The formation of the disulphide bridges can be monitored via the 2 distinct resonances in the NMR spectrum through the disappearance of resonance **5** and a change in the splitting pattern of resonance **4** when measured in dry DMSO.

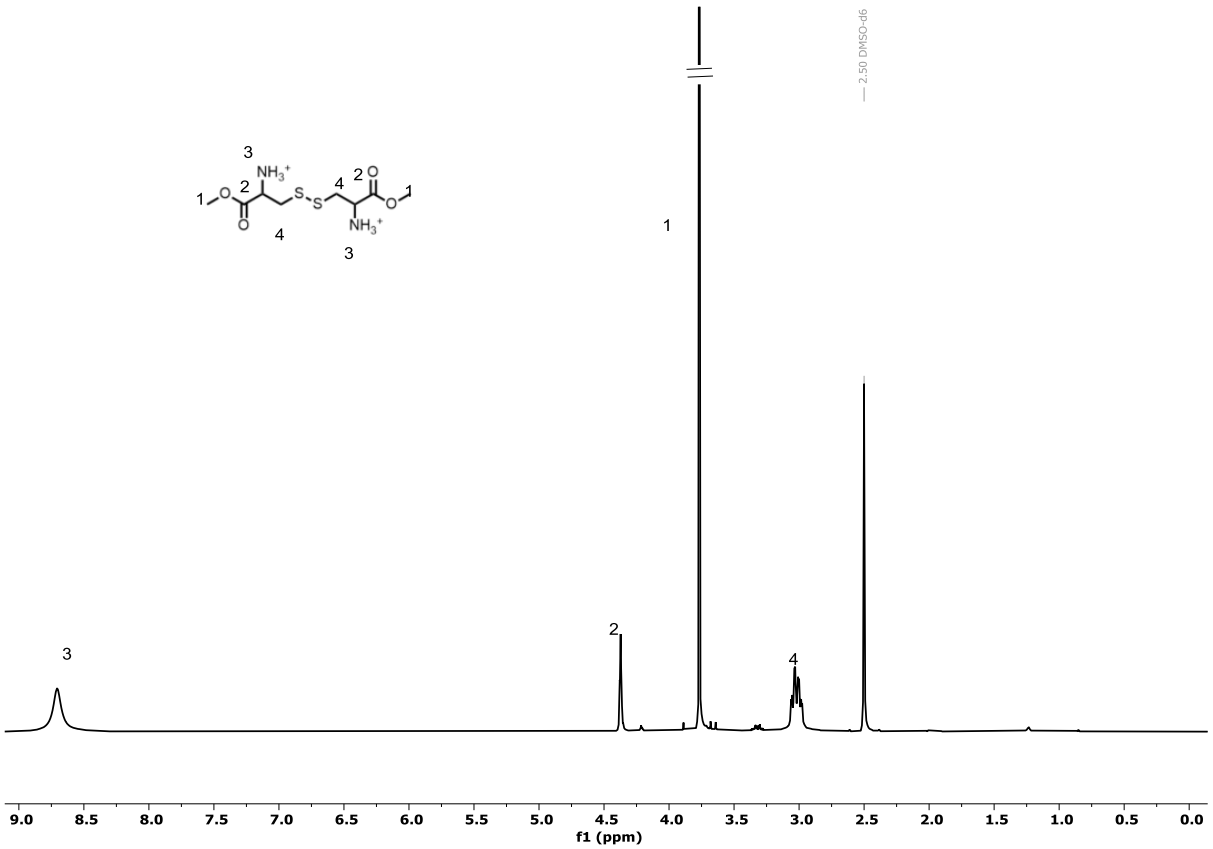

Figure S18: ^{1}H-NMR of the small-molecule studies product in Dimethylsulfoxide-d_6

5 Catalytic activity towards ester degradation

The catalytic activity of the free GDHSC sequence was investigated further after measuring very little activity during the main experiments. Therefore, the peptide sequence was added to the buffered p-NPA solution at different concentrations and monitored via UV/VIS for 120 minutes. The following figure shows the absorbance at 405 nm over time:

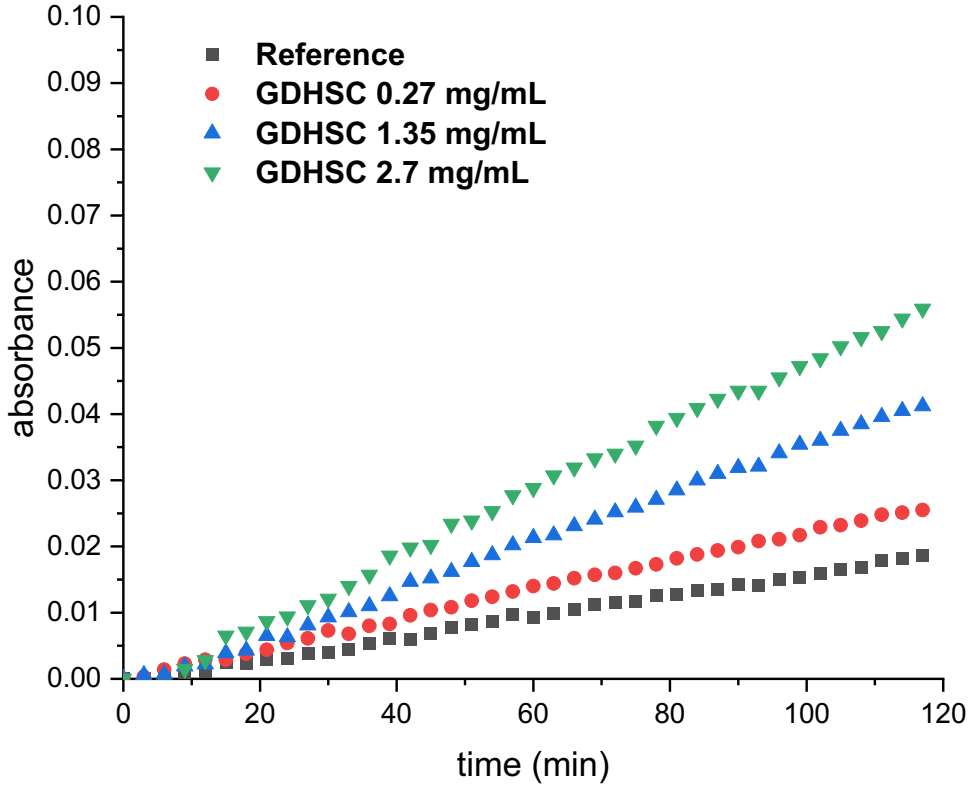

Figure S19: Absorbance at 405 nm vs. time using different GDHSC concentrations.

The initially used concentration of 0.27 mg/mL showed almost no activity in this experiment, increasing the peptide concentration, however, leads to faster hydrolysis rates.