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Supporting Information

Differential Peptide Multi-Macrocyclizations at the Surface of a Helical Foldamer Template

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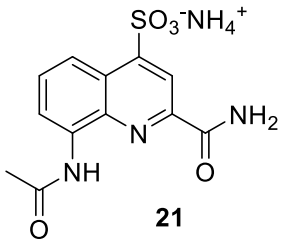
Table of Content

1. Supporting Figures and Tables

1.1. RP-HPLC and LC-MS monitoring of reactions and product analysis



21

Figure S1 - Ac-Q^{Sul}-NH$_2$ monomer (**21**) used as an internal standard for RP-HPLC analyses

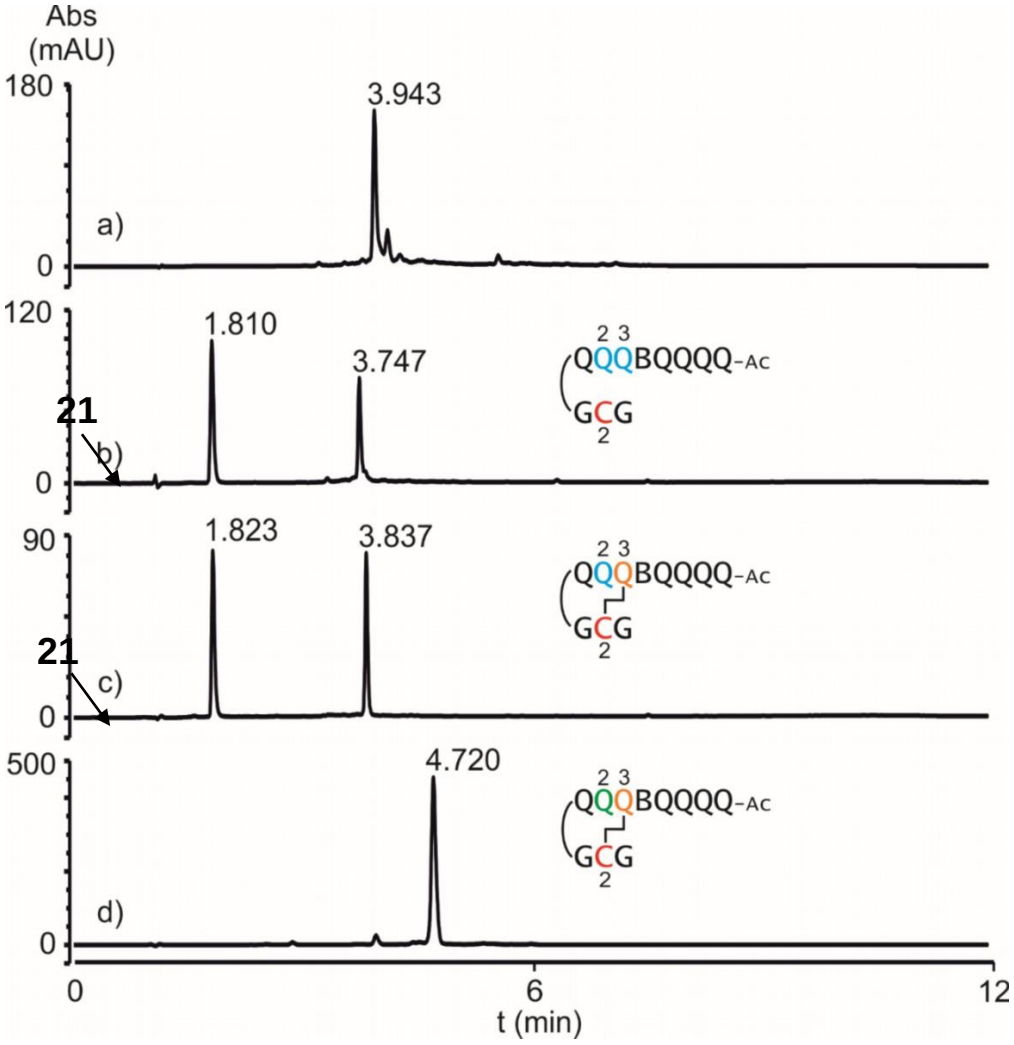


Figure S2 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 1. Overlay of RP-HPLC profiles measured from each synthetic step of compound **1Q** synthesized from compound **1** measured with standard HPLC conditions. Compound **1** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21**(b), after cyclization and co-injected with **21** (**1C**, c) and after benzyl mercaptan installation (**1Q**, d). Chromatograms a), c) and d) obtained after the reaction without prior purification. a) and d) recorded at 300 nm, b) and c) at 375 nm.

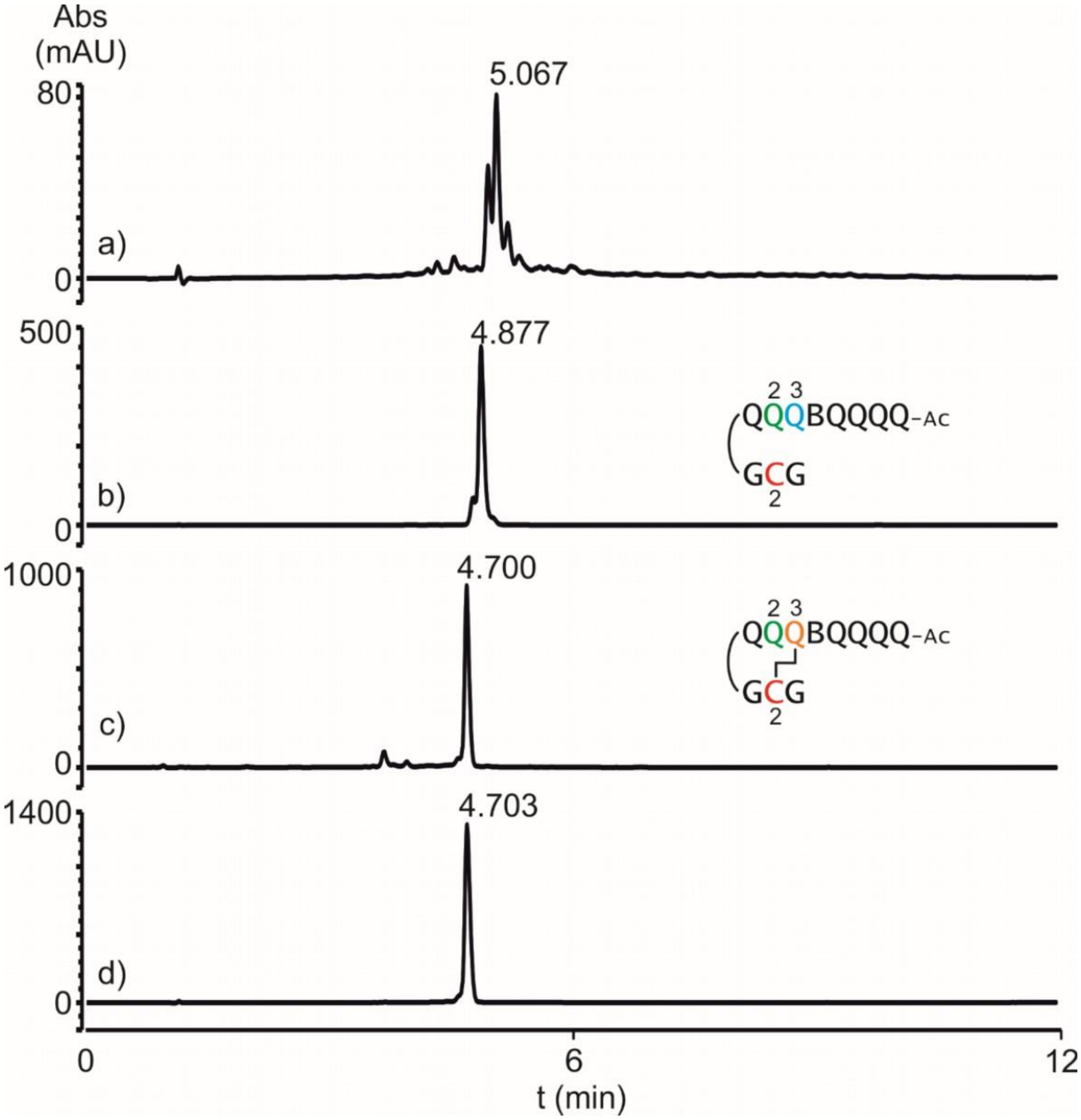


Figure S3 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence R1: Overlay of RP-HPLC profiles measured from each synthetic step of compound **1Q** synthesized from compound **R1** measured with standard HPLC conditions. Compound **R1** after SPS/SPFS as a crude (a), after purification (b), after cyclization (**1Q**, c) and after final purification d). Chromatograms a), and c) obtained after the reaction without prior purification. All chromatograms were recorded at 300 nm.

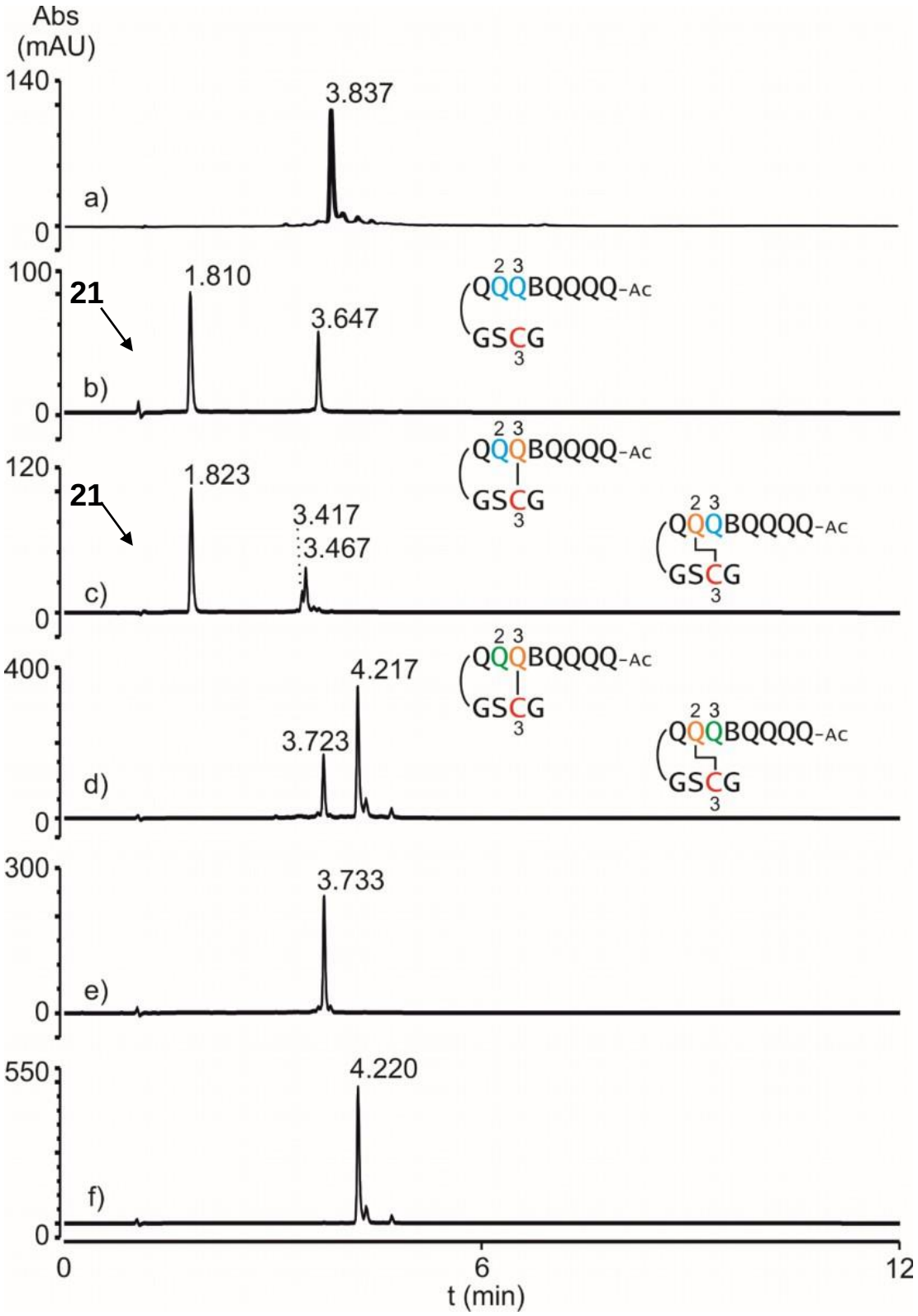


Figure S4 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 2. Overlay of RP-HPLC profiles measured from each synthetic step of compound **2Q** measured with standard HPLC conditions. Compound **2** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**2C**, c), after benzyl mercaptan installation (**2Q**, d) and final purification (**2Q**-a, e and **2Q**-b, f). Chromatograms a), c) and d) obtained after the reaction without prior purification. a) recorded at 300 nm and b)-f) at 375 nm.

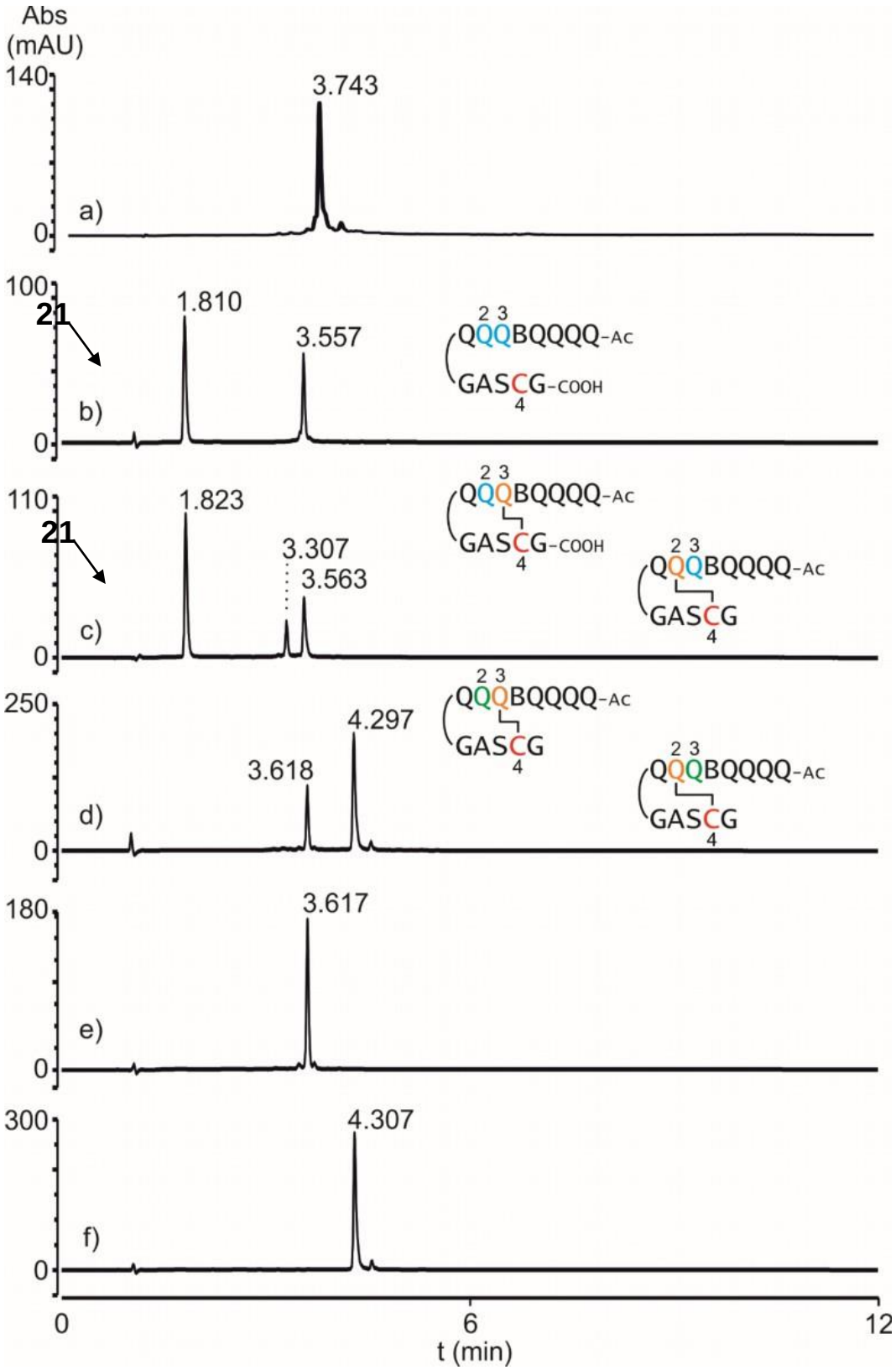


Figure S5 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 3. Overlay of RP-HPLC profiles measured from each synthetic step of compound **3Q** measured with standard HPLC conditions. Compound **3** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**3C**, c), after benzyl mercaptan installation d) and final purification (**3Q**-a, e and **3Q**-b, f). Chromatograms a), c) and d) obtained after the reaction without prior purification. a) recorded at 300 nm and b)-f) at 375 nm.

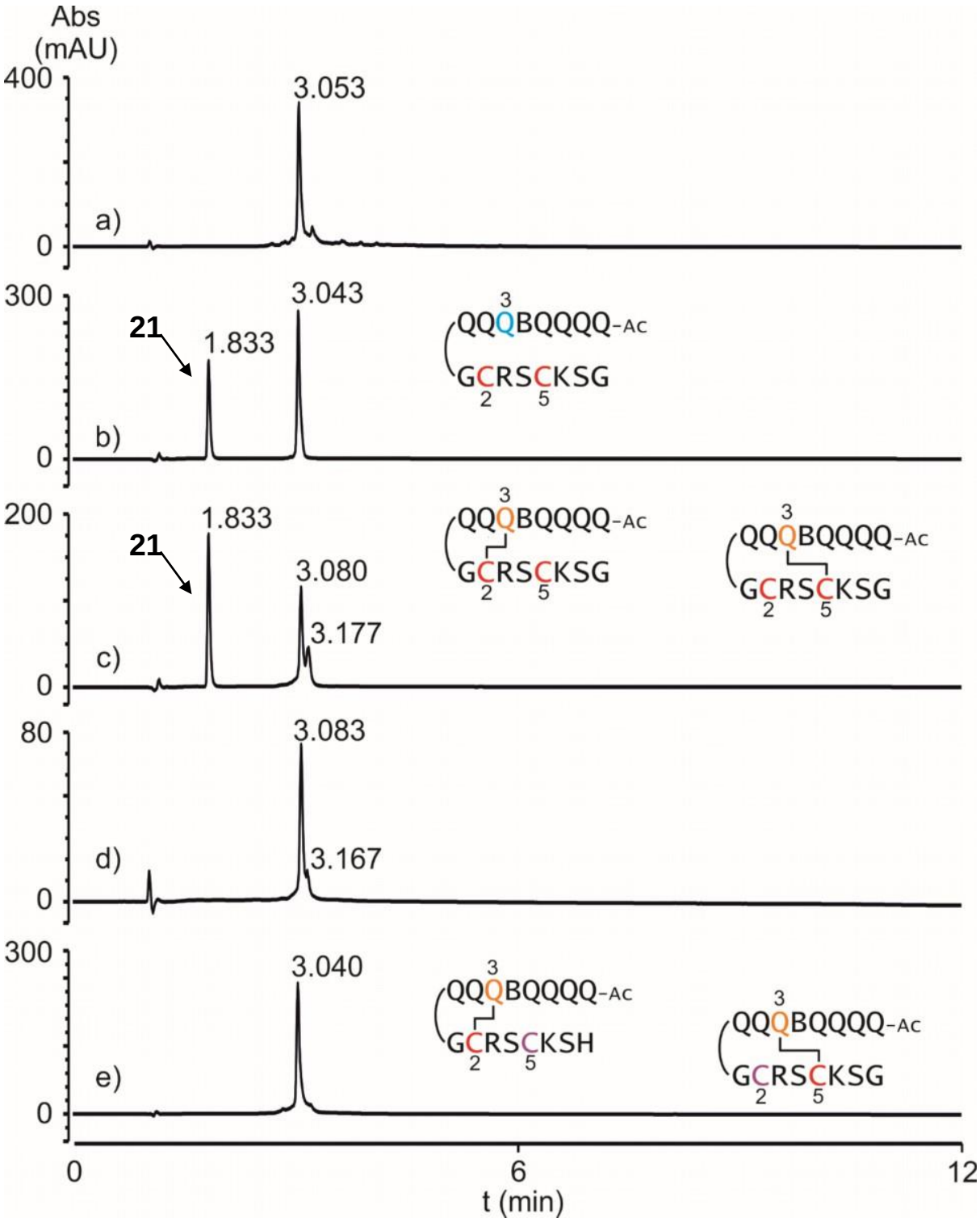


Figure S6 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 4. Overlay of RP-HPLC profiles measured from each synthetic step of compound **4Q** measured with standard HPLC conditions. Compound **4** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**4C**, c), after reduction of the disulfide inpurity with TCEP (disappearance of disulfide proven by LC-MS) d) and acetamide installation (**4Q**, e). Chromatograms a), c), d) and e) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

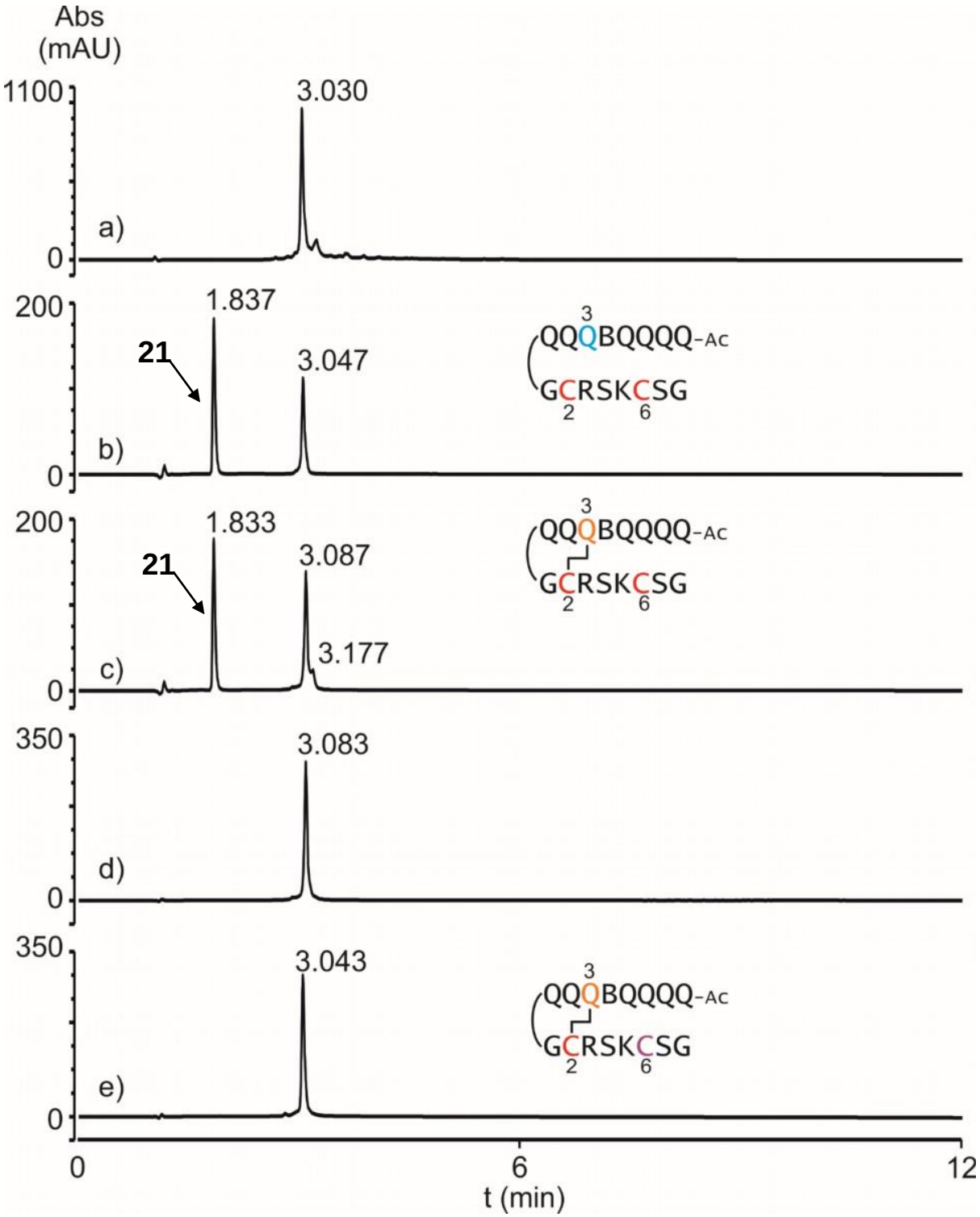


Figure S7 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 5. Overlay of RP-HPLC profiles measured from each synthetic step of compound **5Q** measured with standard HPLC conditions. Compound **5** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**5C**, c), after reduction of the disulfide inpurity with TCEP (disappearance of disulfide proven by LC-MS) d) and acetamide installation (**5Q**, e). Chromatograms a), c), d) and e) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

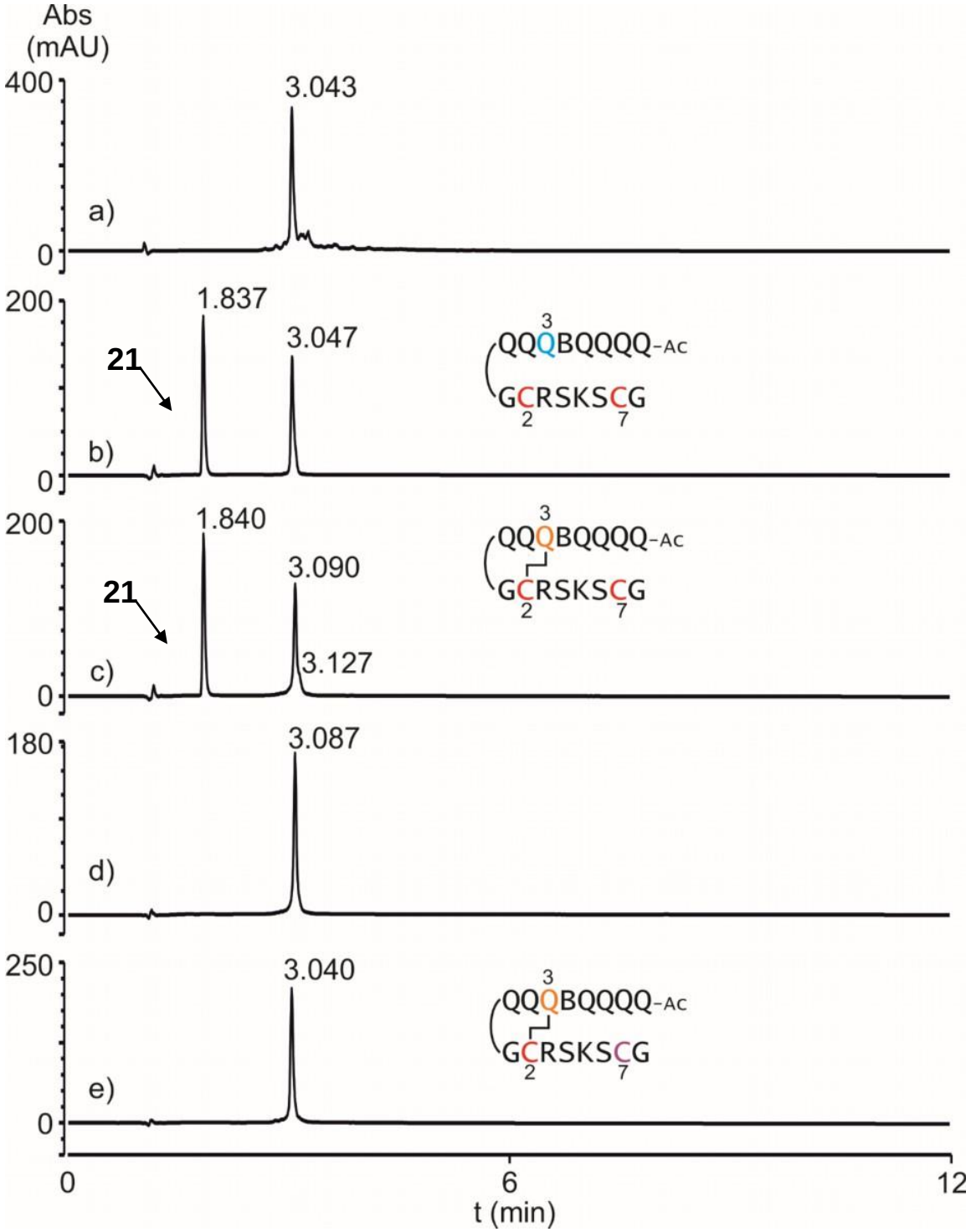


Figure S8 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 6. Overlay of RP-HPLC profiles measured from each synthetic step of compound **6** measured with standard HPLC conditions. Compound **6** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**6C**, c), after reduction of the disulfide inpurity with TCEP (disappearance of disulfide proven by LC-MS, d) and acetamide installation (**6Q**, e). Chromatograms a), c), d) and e) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

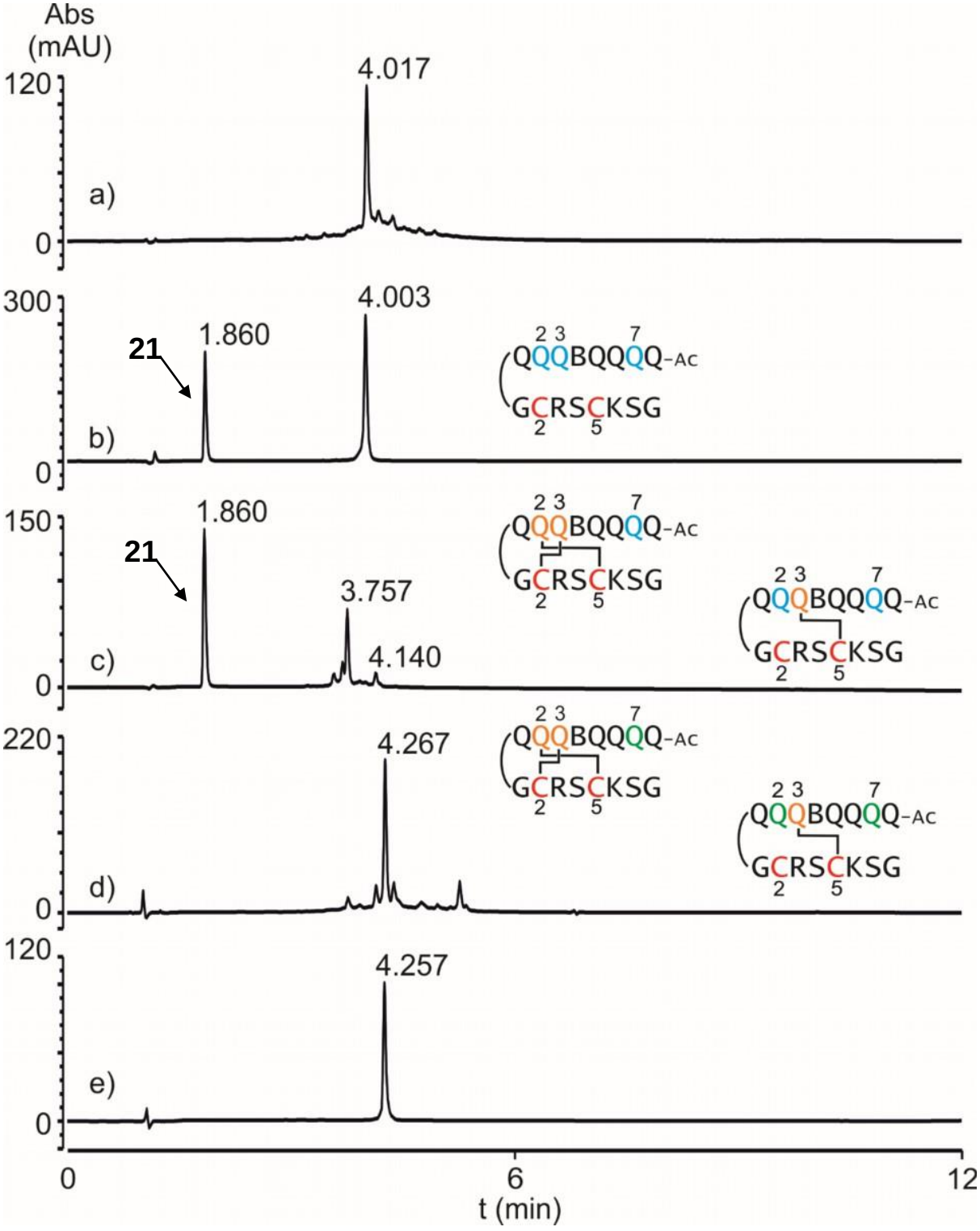


Figure S9 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 7. Overlay of RP-HPLC profiles measured from each synthetic step of compound **7Q** measured with standard HPLC conditions. Compound **7** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**7C**, c), after benzyl mercaptan installation d) and final purification (**7Q**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

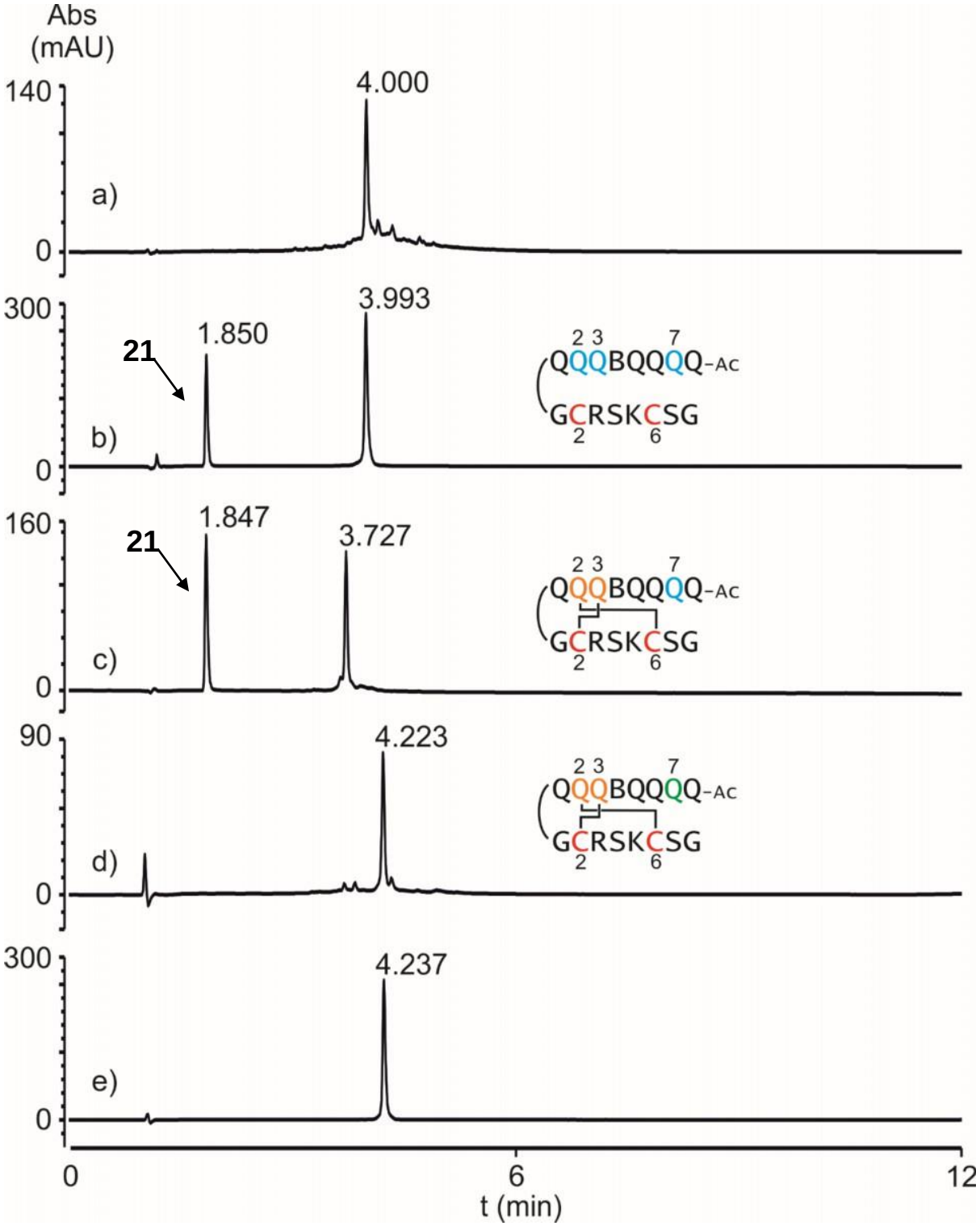


Figure S10 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 8. Overlay of RP-HPLC profiles measured from each synthetic step of compound **8Q** measured with standard HPLC conditions. Compound **8** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**8C**, c), after benzyl mercaptan installation d) and final purification (**8Q**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

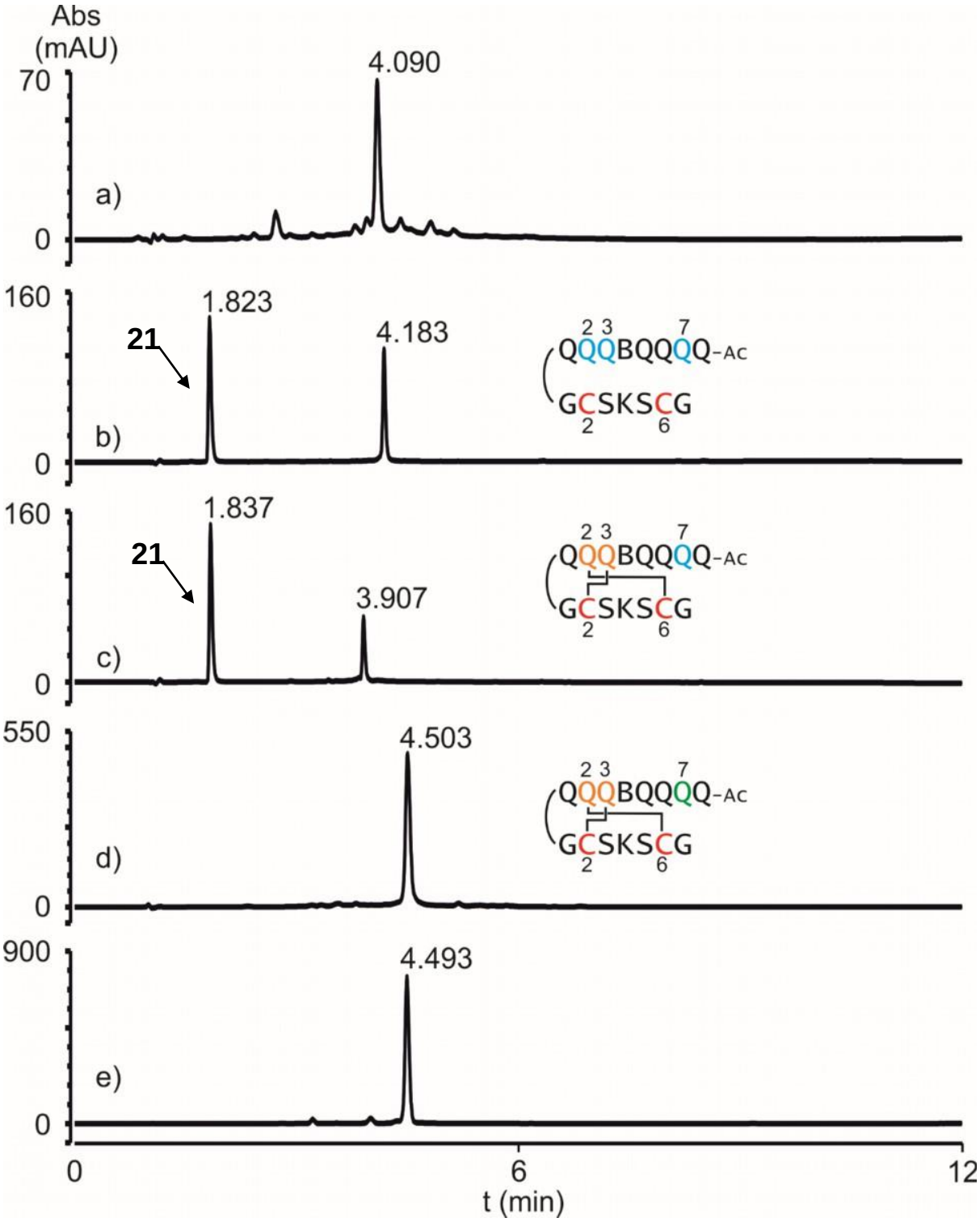


Figure S11 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 9. Overlay of RP-HPLC profiles measured from each synthetic step of compound **9Q** measured with standard HPLC conditions. Compound **9** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (9C, c), after benzyl mercaptan installation d) and final purification (**9Q**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. a), d) and e) were recorded at 300 nm and b-c) at 375 nm.

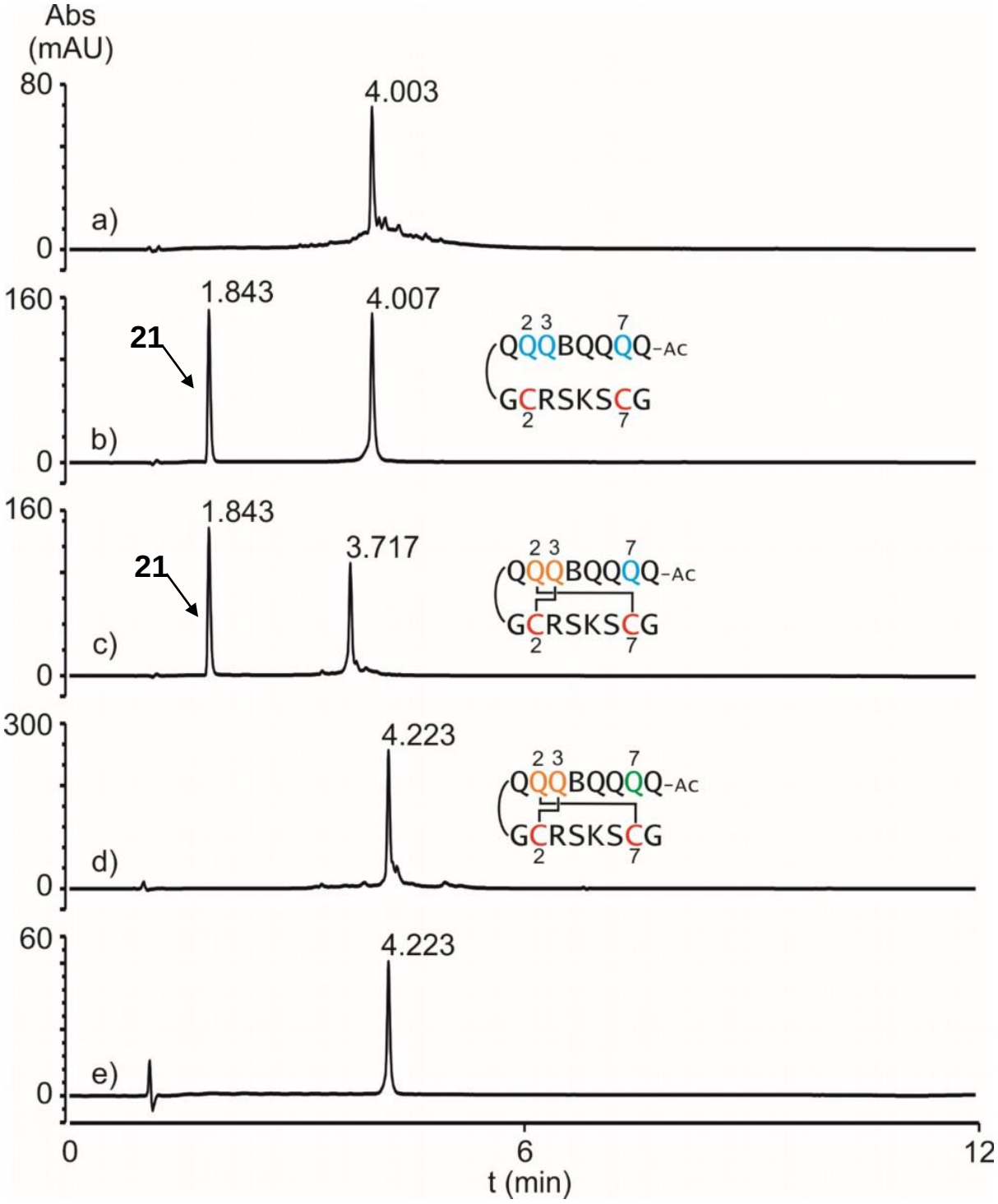


Figure S12 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 10. Overlay of RP-HPLC profiles measured from each synthetic step of compound **10Q** measured with standard HPLC conditions. Compound **10** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**10C**, c), after benzyl mercaptan installation d) and final purification (**10Q**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

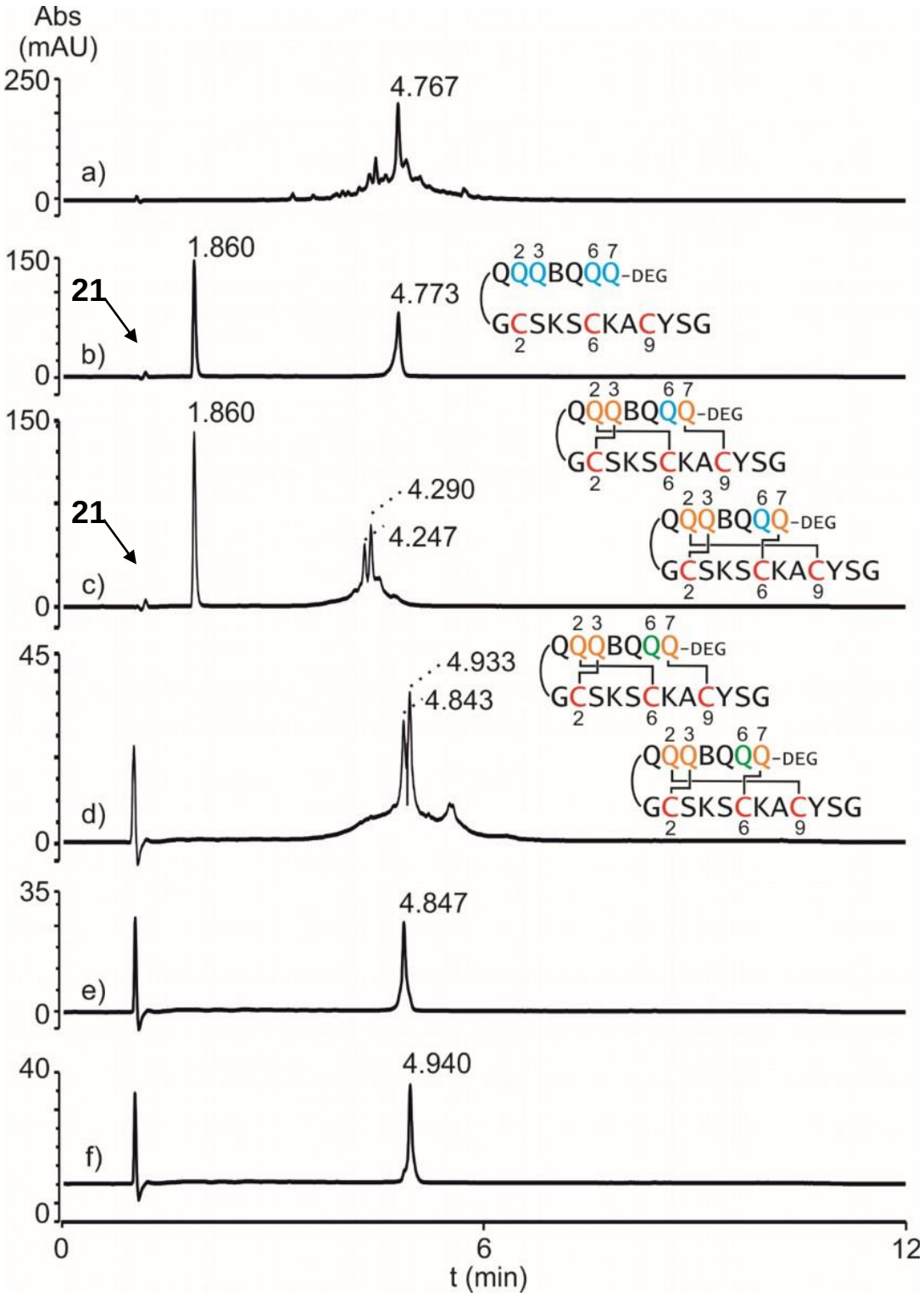


Figure S13 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 11. Overlay of RP-HPLC profiles measured from each synthetic step of compound **11Q** synthesized from compound **11** measured with standard HPLC conditions. Compound **11** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**11C**, c), after benzyl mercaptan installation d) and final purification (**11Q-a**, e and **11Q-b**, f). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

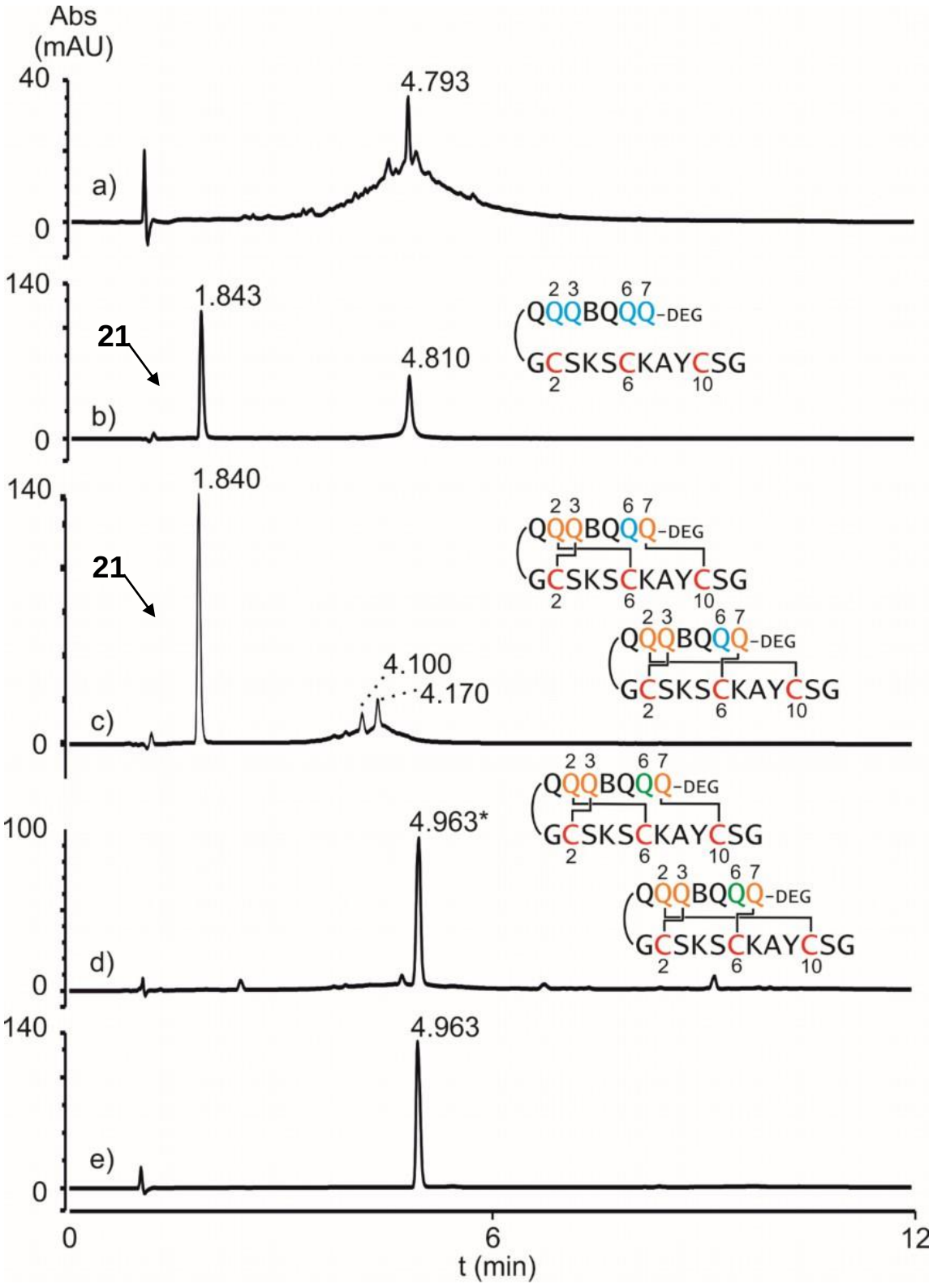


Figure S14 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 12. Overlay of RP-HPLC profiles measured from each synthetic step of compound **12Q** synthesized from compound **12** measured with standard HPLC conditions. Compound **12** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**12C**, c), after benzyl mercaptan installation d) and final purification (**12Q-a**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. Single peak marked with * interpreted as co-elution of both conformers**.** All chromatograms were recorded at 375 nm.

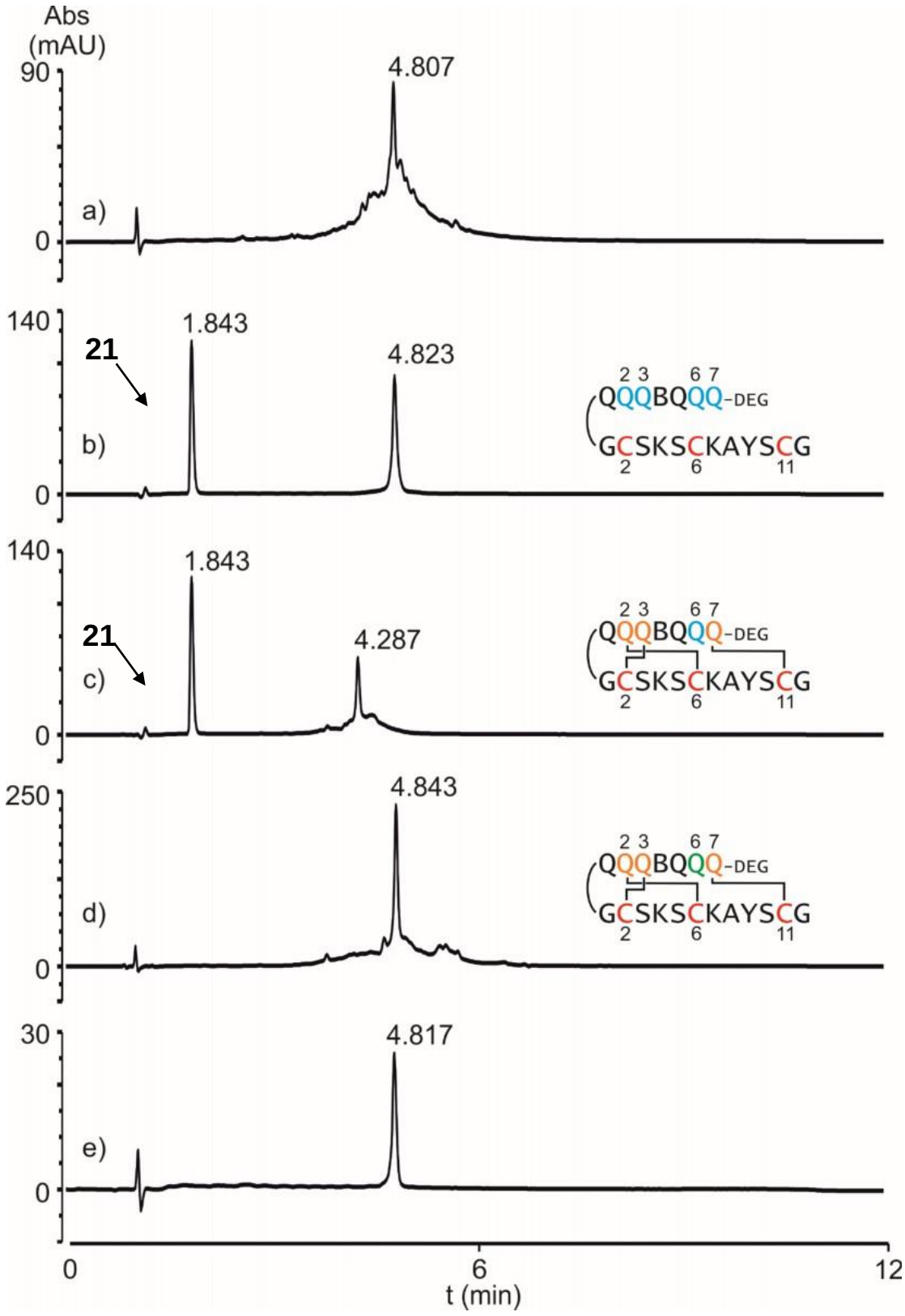


Figure S15 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 13. Overlay of RP-HPLC profiles of each synthetic step of compound **13Q** which were measured with standard HPLC conditions. Compound **13** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**13C**, c), after benzyl mercaptan installation d) and final purification (**13Q**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

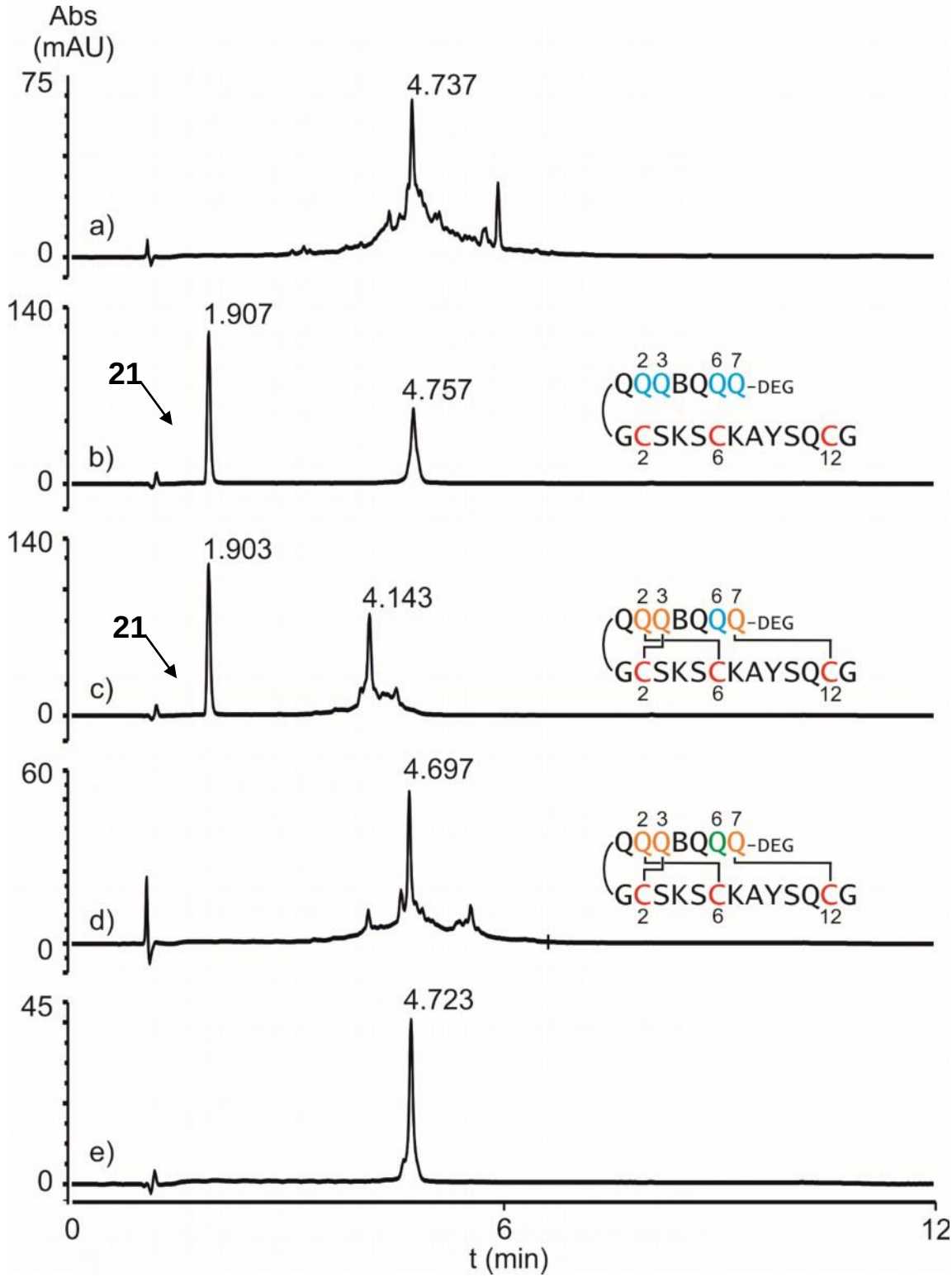


Figure S16 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 14. Overlay of RP-HPLC profiles from each synthetic step of compound **14Q** synthesized from **14** measured with standard HPLC conditions. Compound **14** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**14C**, c), after benzyl mercaptan installation d) and final purification (**14Q**, e). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

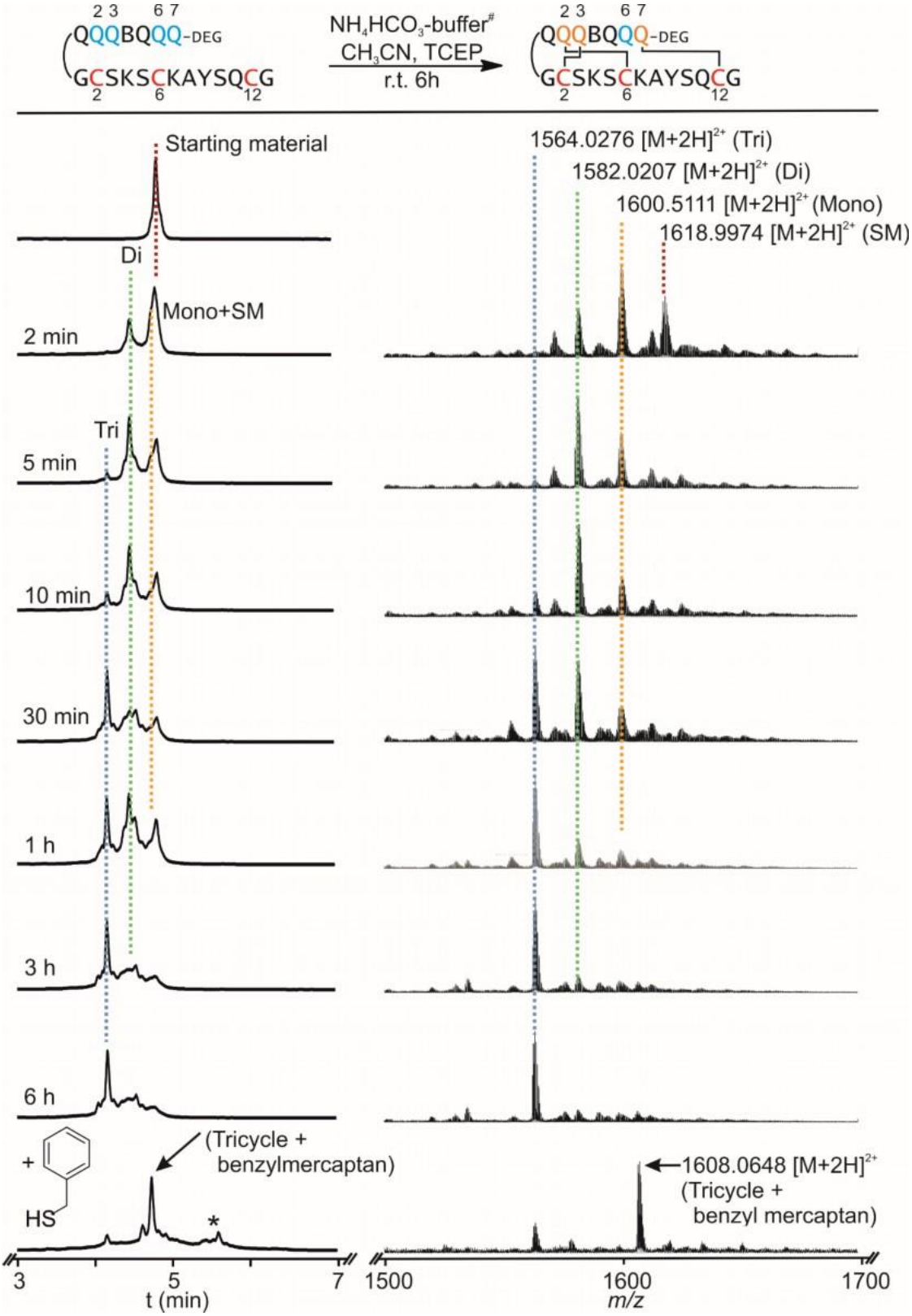


Figure S17 - Excerpts of the RP-HPLC and ESI-MS traces of the macrocyclization progression of 14C with final termination of the unreacted chloroacetamide group with benzyl mercaptan (*). The ESI-MS spectra reported correspond to the time segment 2.5 to 5 min. The resolution of the LC traces was not good enough to distinguish each peak intermediate on the base peak chromatogram (BPC) of the TOF detector. Sodium hydrogen carbonate buffer pH 8.5, 60 mM (#). Chromatograms were measured with standard HPLC conditions and recorded at 375 nm.

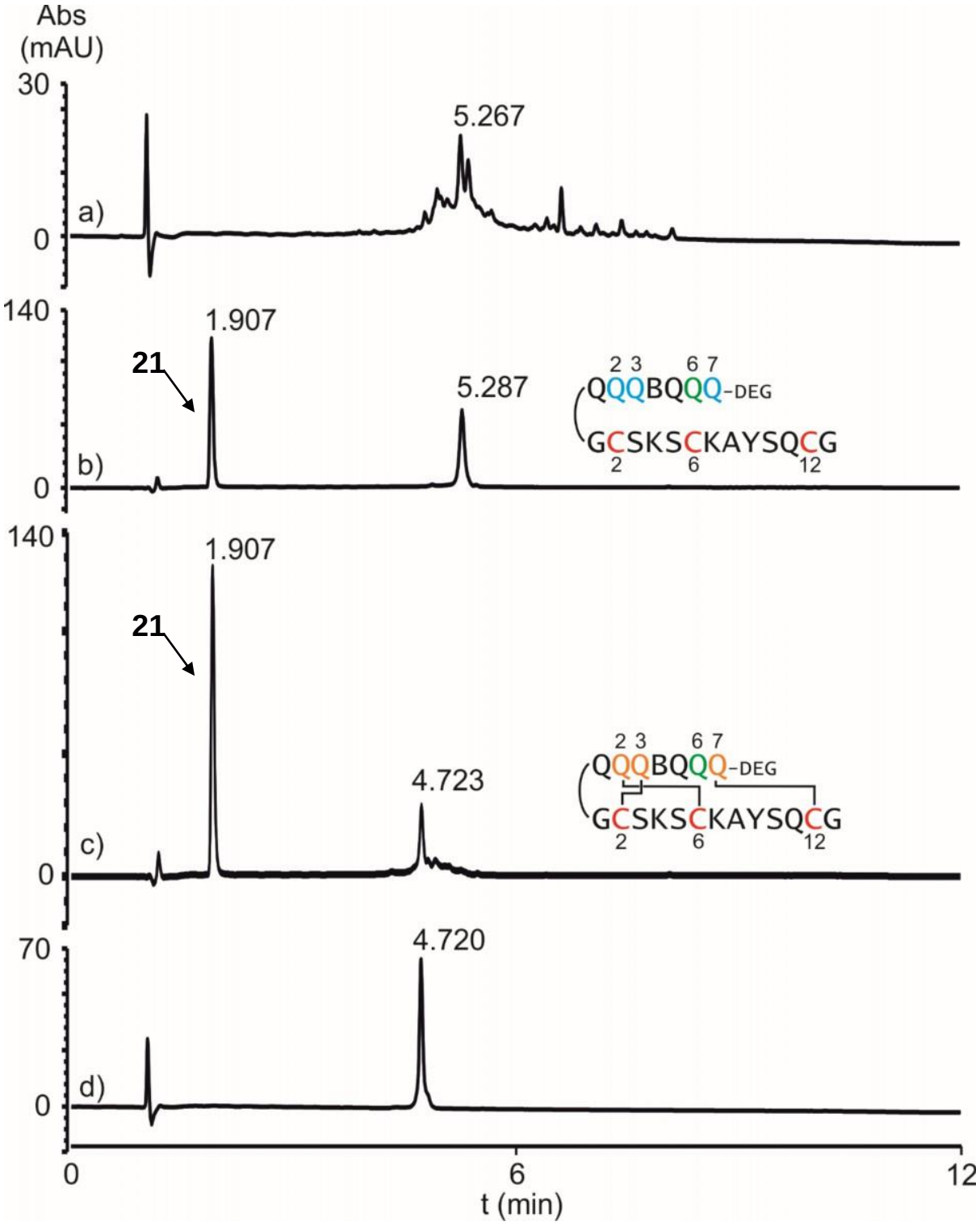


Figure S18 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 14. Overlay of RP-HPLC profiles from each synthetic step of compound **14Q** synthesized from **R14** measured with standard HPLC conditions. Compound **R14** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**14Q**, c), and final purification (**14Q**, d). Chromatograms a), and c) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

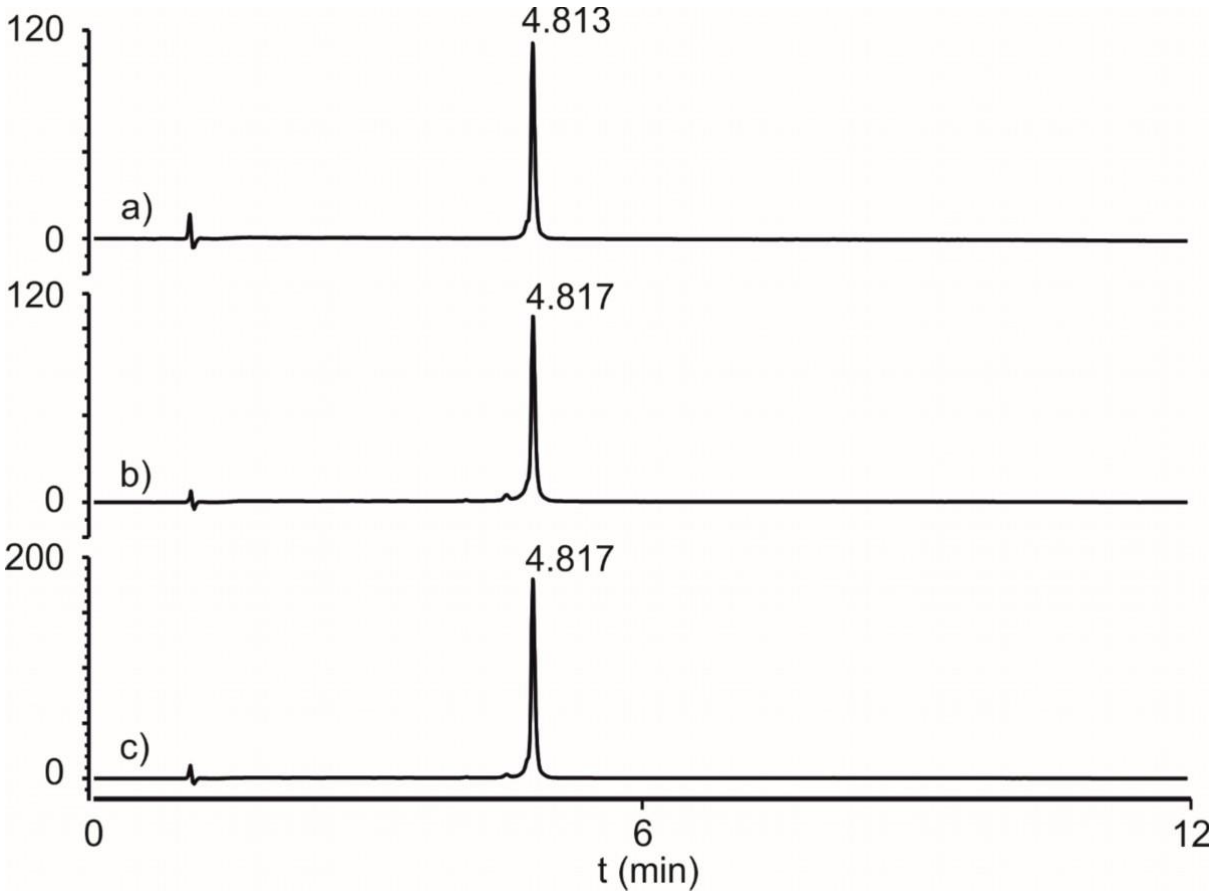


Figure S19 - RP-HPLC analysis of sequence 14. Overlay of RP-HPLC profiles from compound **14** obtained from the competition experiment (a), synthesized from the reference experiment (b) and as a co-injection (c). Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

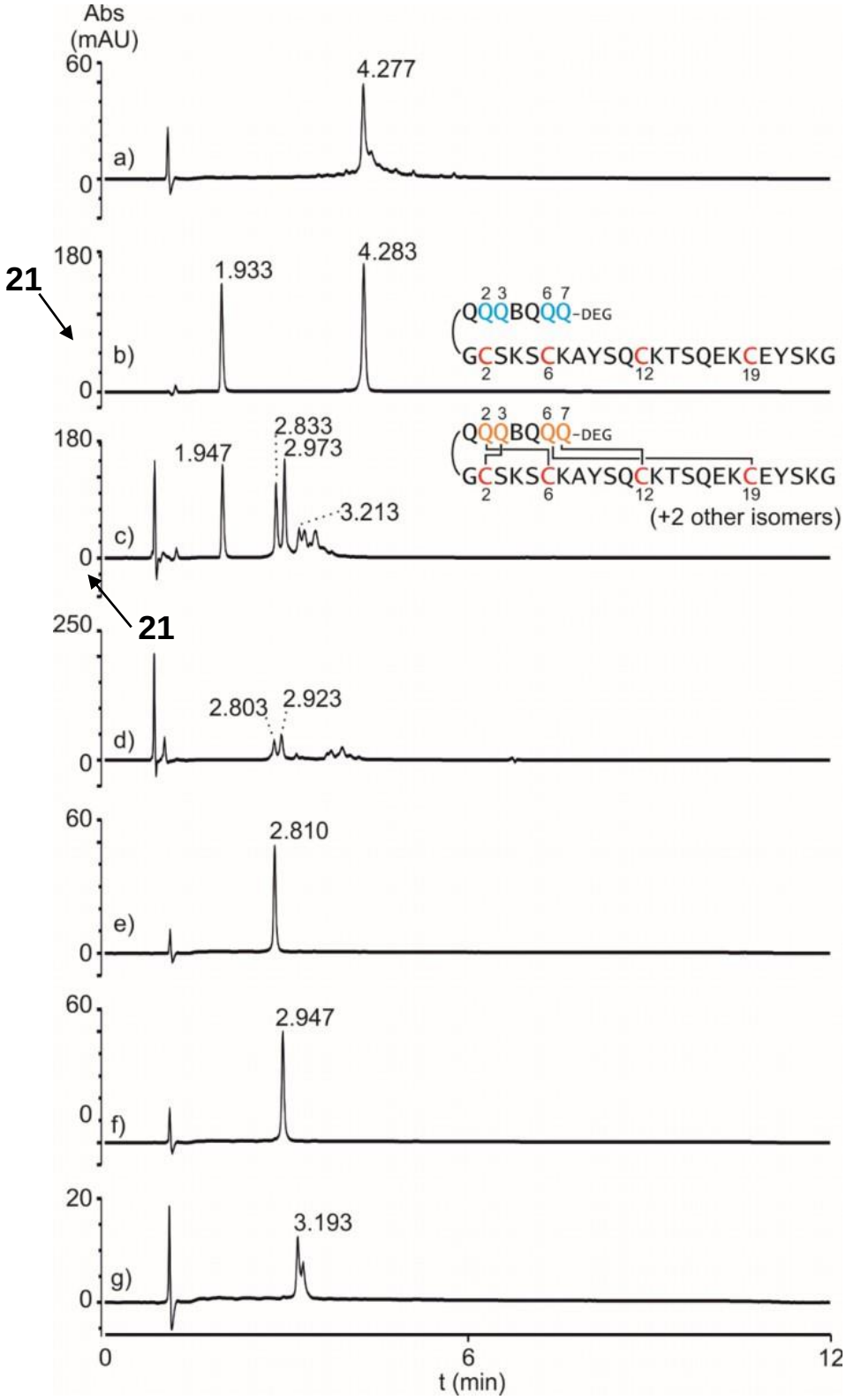


Figure S20 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 15. Overlay of RP-HPLC profiles from each synthetic step of compound **15C** synthesized from **15** measured with standard HPLC conditions. Compound **15** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**15C**, c), and after addition of benzylmercaptan (d). The three isomers (**15C-a**, **15C-b** and **15C-c** were separated in e) f) and g). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

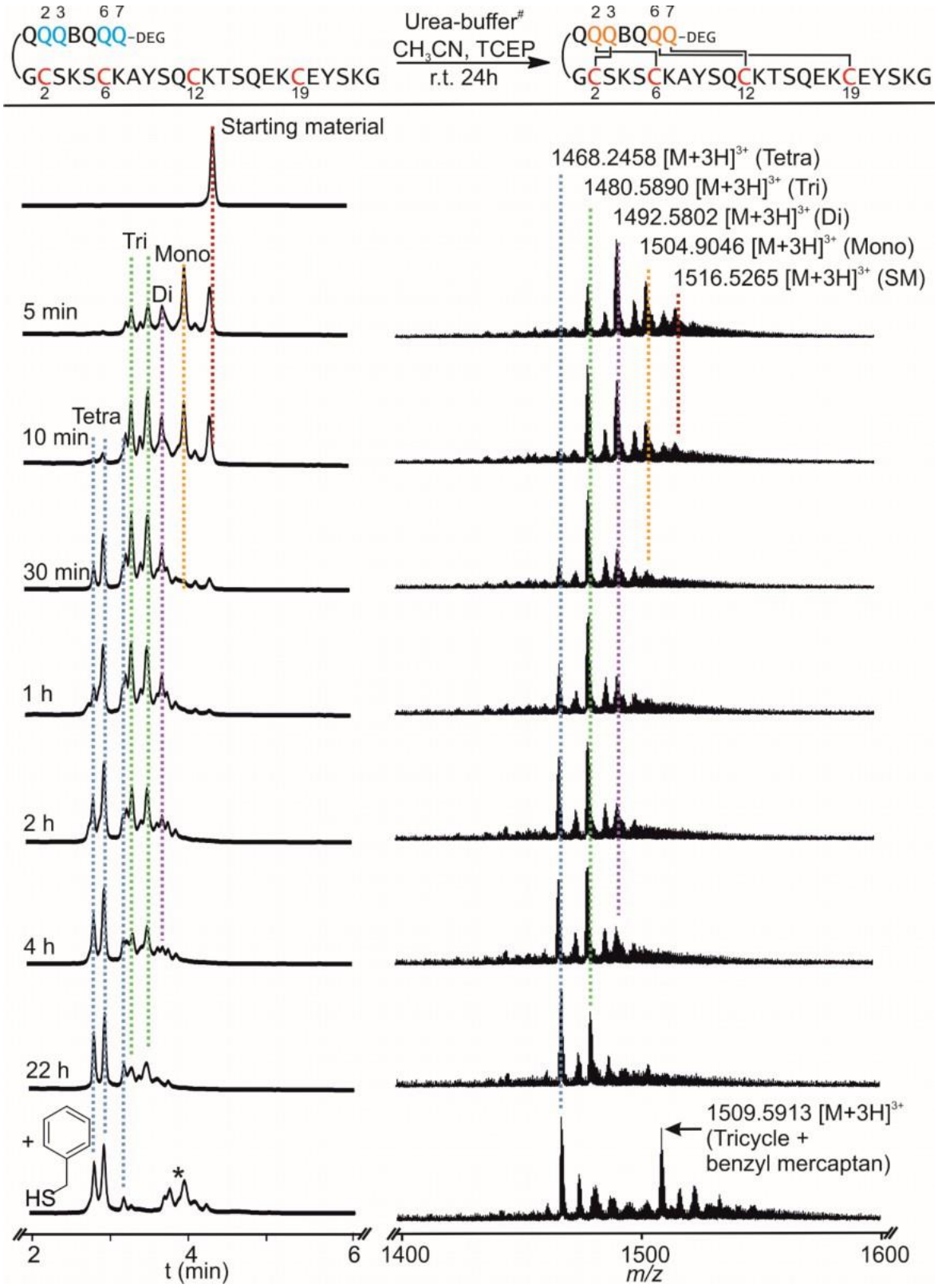


Figure S21 - Excerpts of the RP-HPLC and ESI-MS traces of the macrocyclization progression of 15C with final termination of the unreacted chloroacetamide group with benzyl mercaptan (*). The ESI-MS spectra reported correspond to the time segment 2.5 to 4.5 min. The resolution of the LC traces was not good enough to distinguish each peak intermediate on the base peak chromatogram (BPC) of the TOF detector. Urea buffer pH 8.5, 8 M (#). Chromatograms were measured with standard HPLC conditions and recorded at 375 nm.

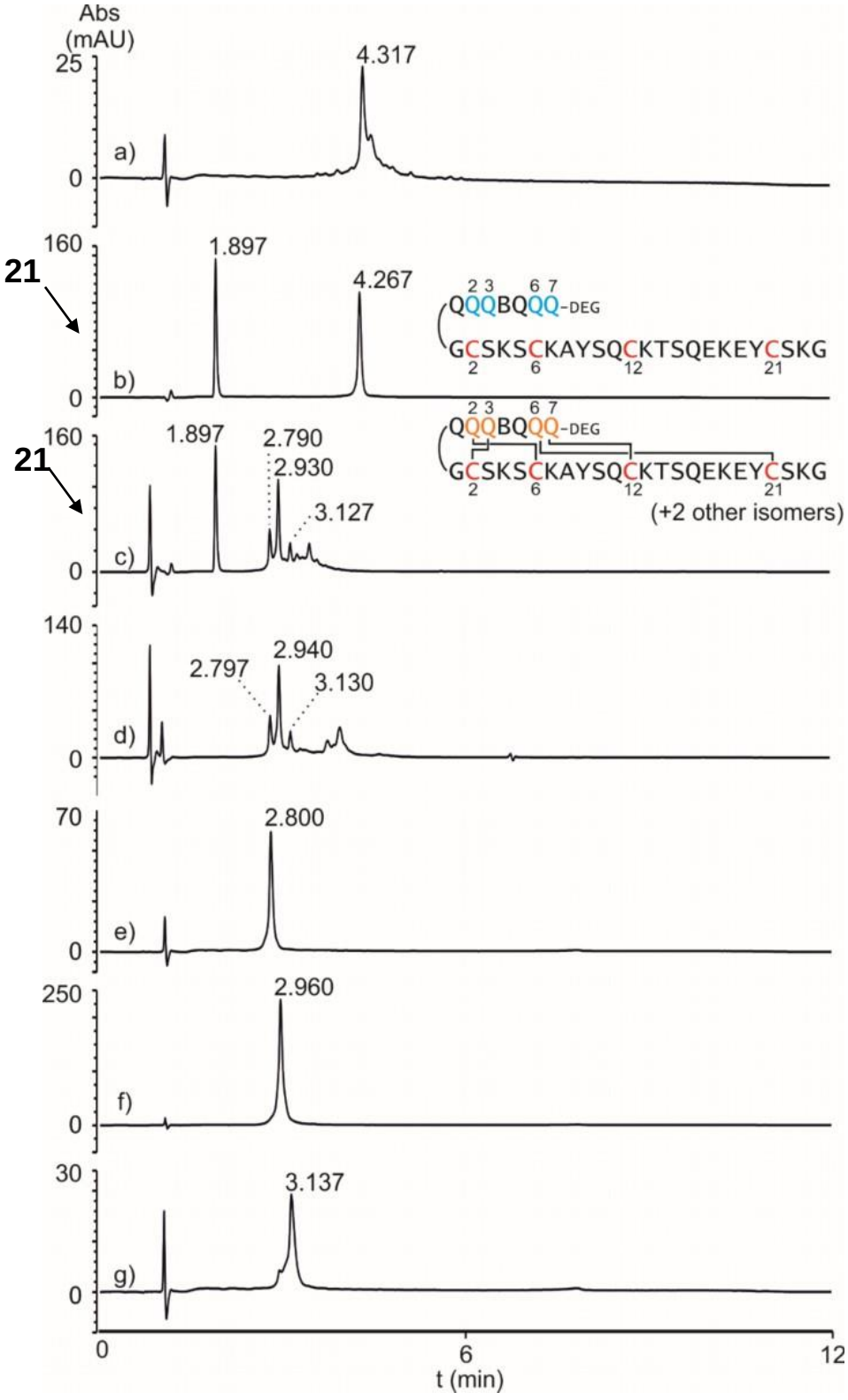


Figure S22 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 16. Overlay of RP-HPLC profiles from each synthetic step of compound **16C** synthesized from **16** measured with standard HPLC conditions. Compound **16** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**16C**, c), and after addition of benzylmercaptan (d). The three main isomers (**16C-a**, **16C-b** and **16C-c** were separated in e) f) and g). Chromatograms a), c) and d) obtained after the reaction without prior purification. All chromatograms were recorded at 375 nm.

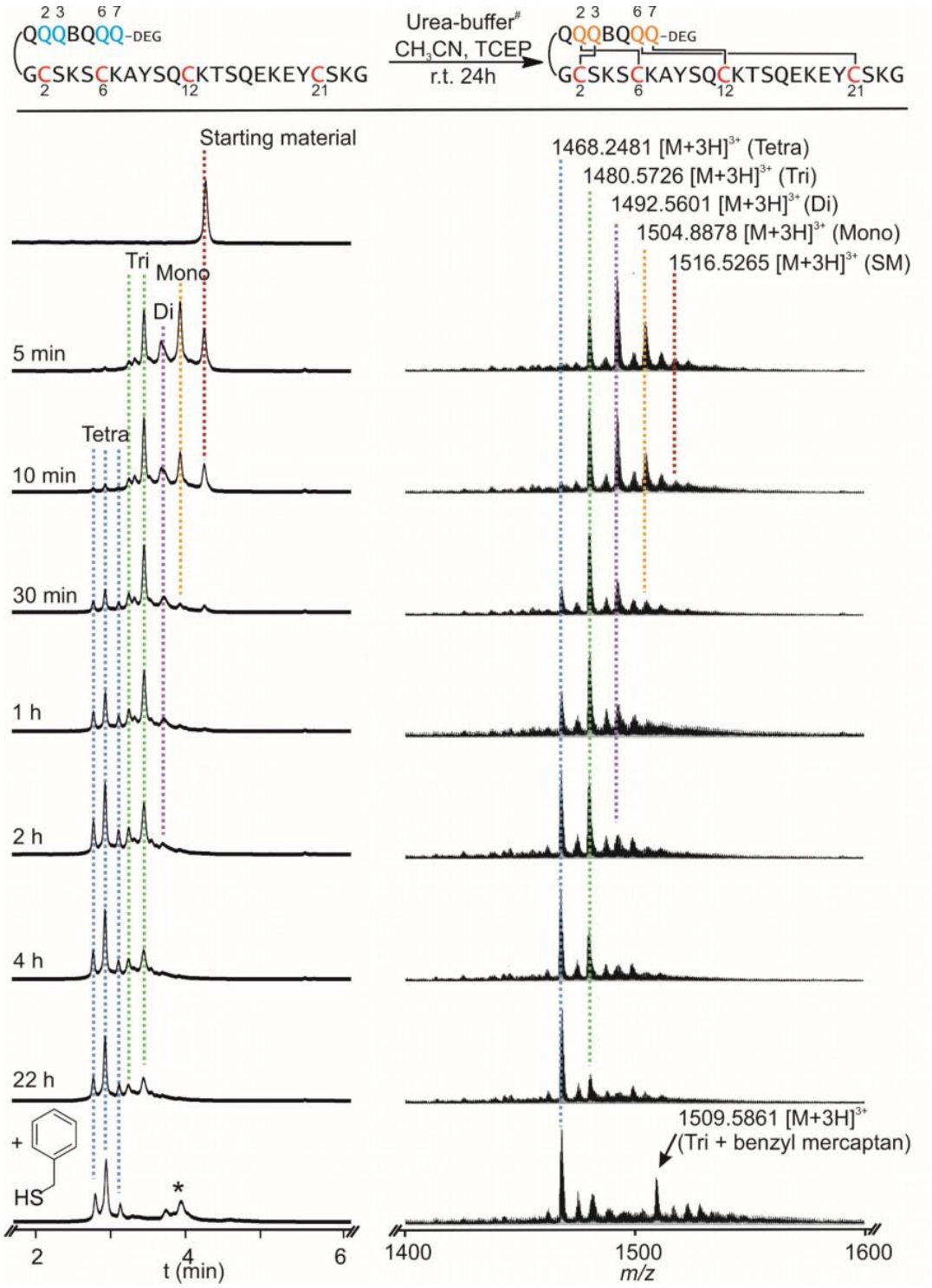


Figure S23 - Excerpts of the RP-HPLC and ESI-MS traces of the macrocyclization progression of 16C with final termination of the unreacted chloroacetamide group with benzyl mercaptan (*). The ESI-MS spectra reported correspond to the time segment 2.5 to 4.5 min. The resolution of the LC traces was not good enough to distinguish each peak intermediate on the base peak chromatogram (BPC) of the TOF detector. Urea buffer pH 8.5, 8 M (#). Chromatograms were measured with standard HPLC conditions and recorded at 375 nm.

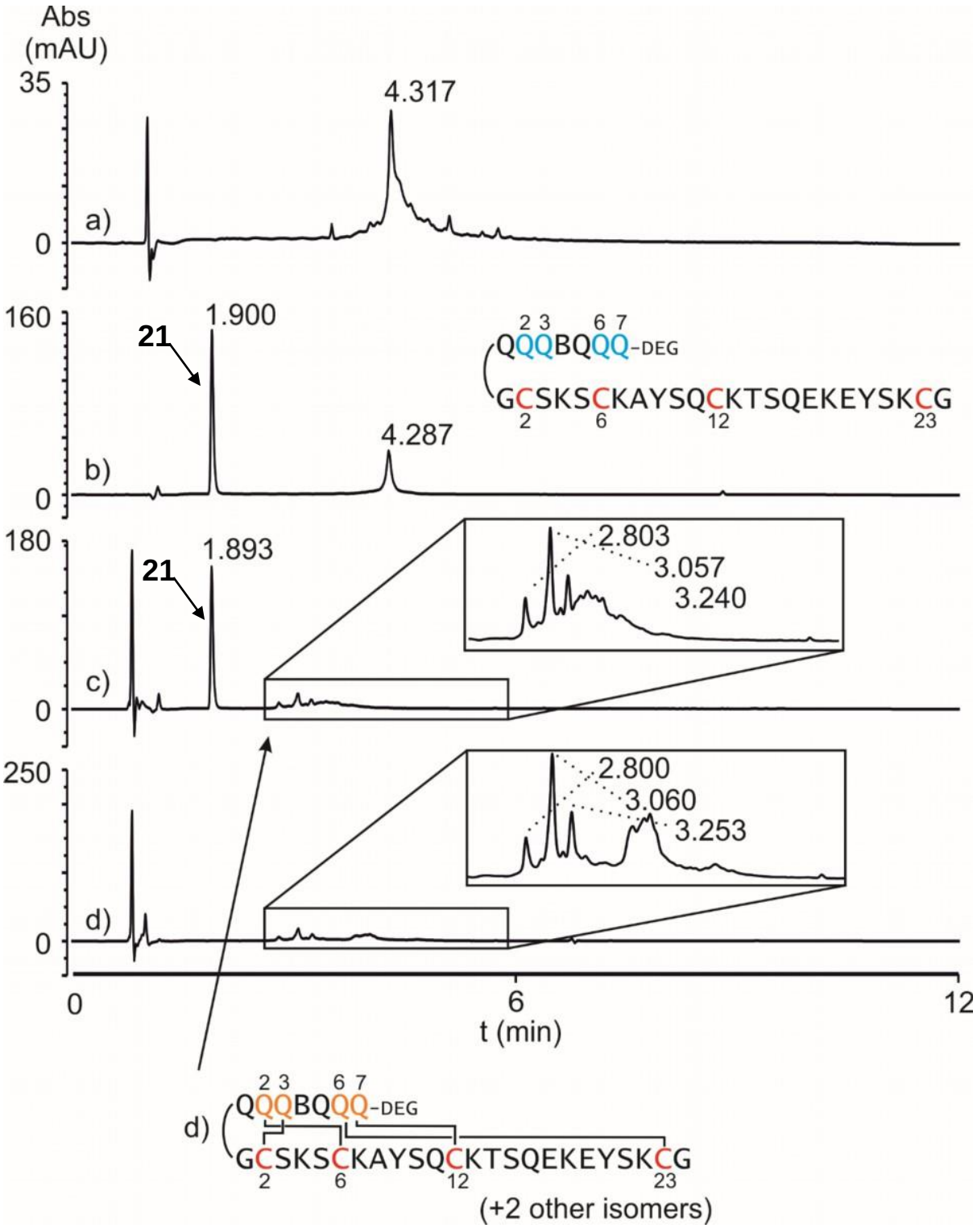


Figure S24 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 17. Overlay of RP-HPLC profiles from each synthetic step of compound **17C** synthesized from **17**. Compound **17** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with internal standard **21** (**17C**, c), and after addition of benzylmercaptan (d). Chromatograms a), c) and d) obtained after the reaction without prior purification. Due low amounts, no final purification was carried out. Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

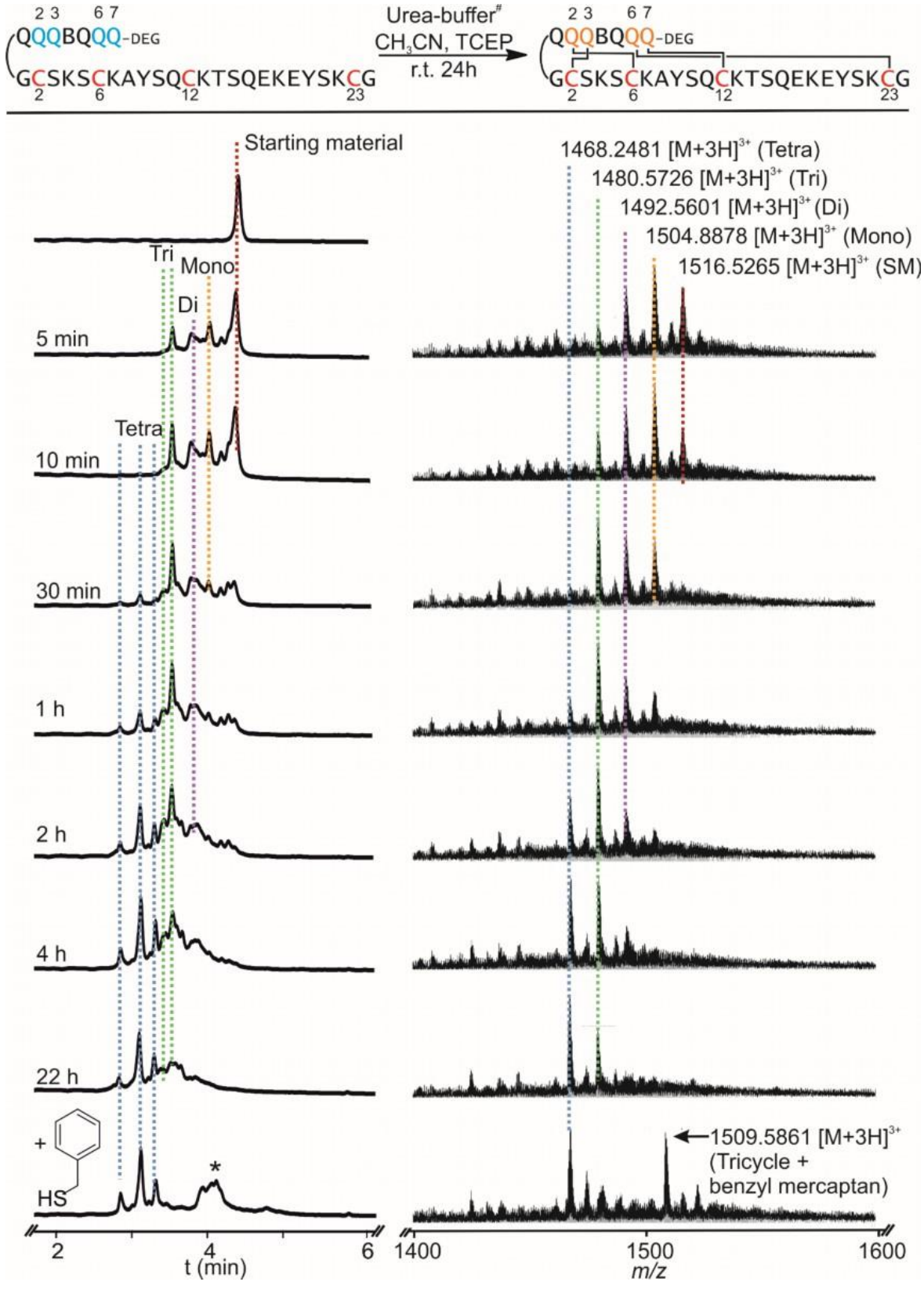


Figure 25 - Excerpts of the RP-HPLC and ESI-MS traces of the macrocyclization progression of 17C with final termination of the unreacted chloroacetamide group with benzyl mercaptan (*). The ESI-MS spectra reported correspond to the time segment 2.5 to 4.5 min. The resolution of the LC traces was not good enough to distinguish each peak intermediate on the base peak chromatogram (BPC) of the TOF detector. Urea buffer pH 8.5, 8 M (#). Chromatograms were measured with standard HPLC conditions and recorded at 375 nm.

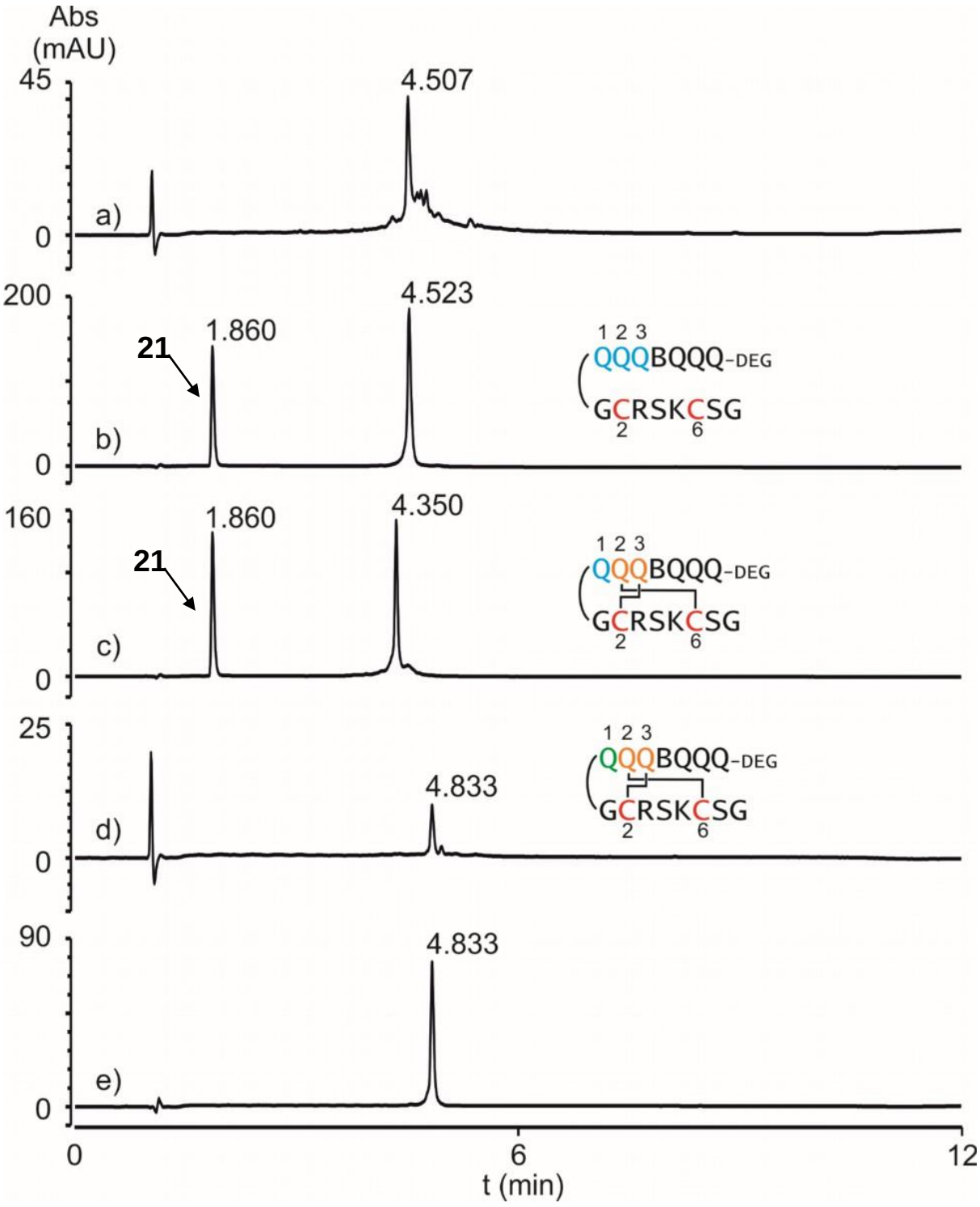


Figure S26 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 18. Overlay of RP-HPLC profiles from each synthetic step of compound **18Q** synthesized from **18**. Compound **18** after SPS/SPFS as a crude (a), after purification co-injected with internal standard 21 (b), after cyclization co-injected with internal standard **21** (**18C**, c), after benzyl mercaptan installation (**18Q**, d) and final purification (**18Q**, e). Chromatograms a), and c) obtained after the reaction without prior purification. Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

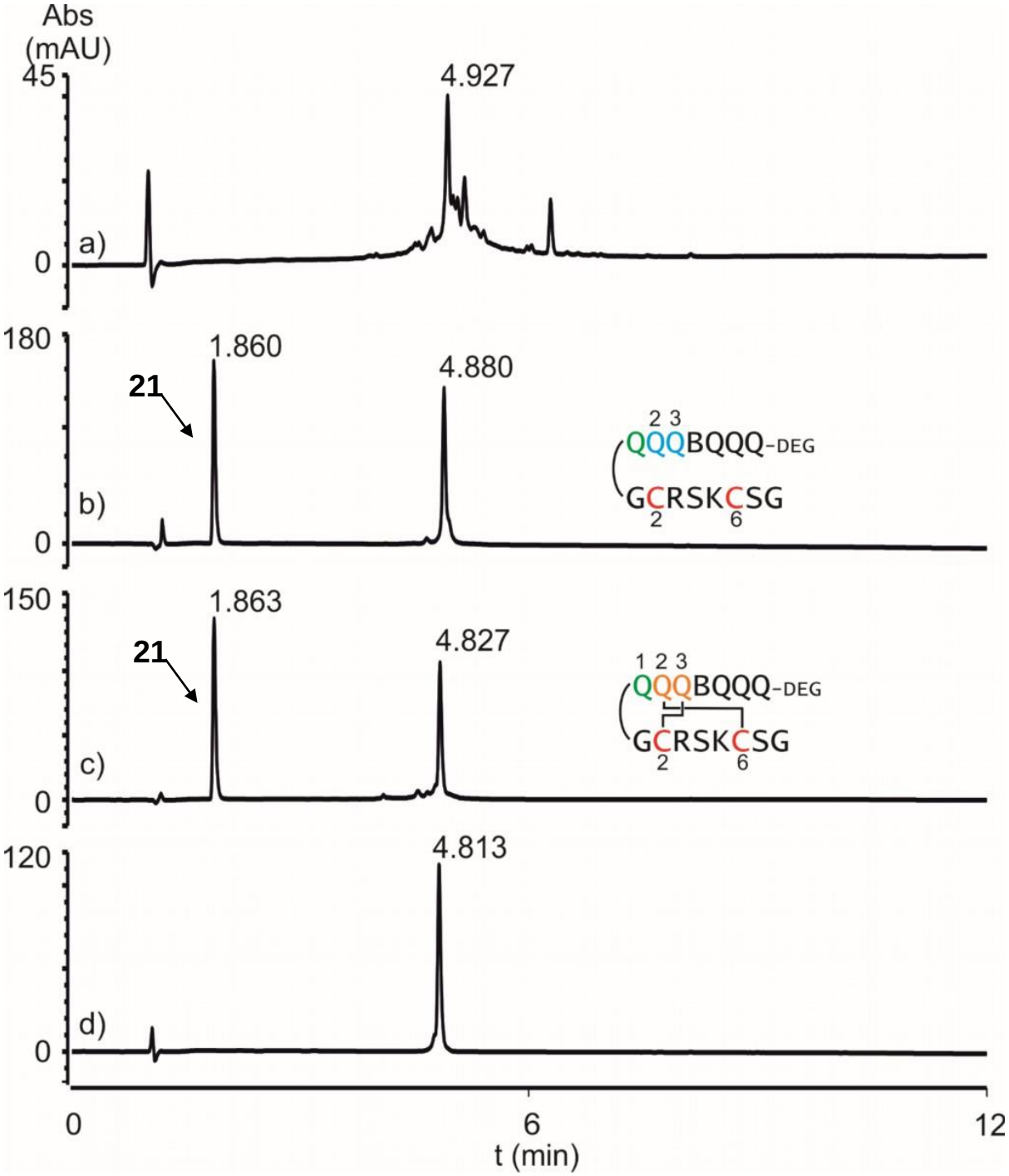


Figure S27 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence R18. Overlay of RP-HPLC profiles from each synthetic step of compound **18Q** synthesized from **R18**. Compound **R18** after SPS/SPFS as a crude (a), after purification co-injected with internal standard 21 (b), after cyclization co-injected with internal standard **21** (**18Q**, c) and final purification (**18Q**, d). Chromatograms a), and c) obtained after the reaction without prior purification. Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

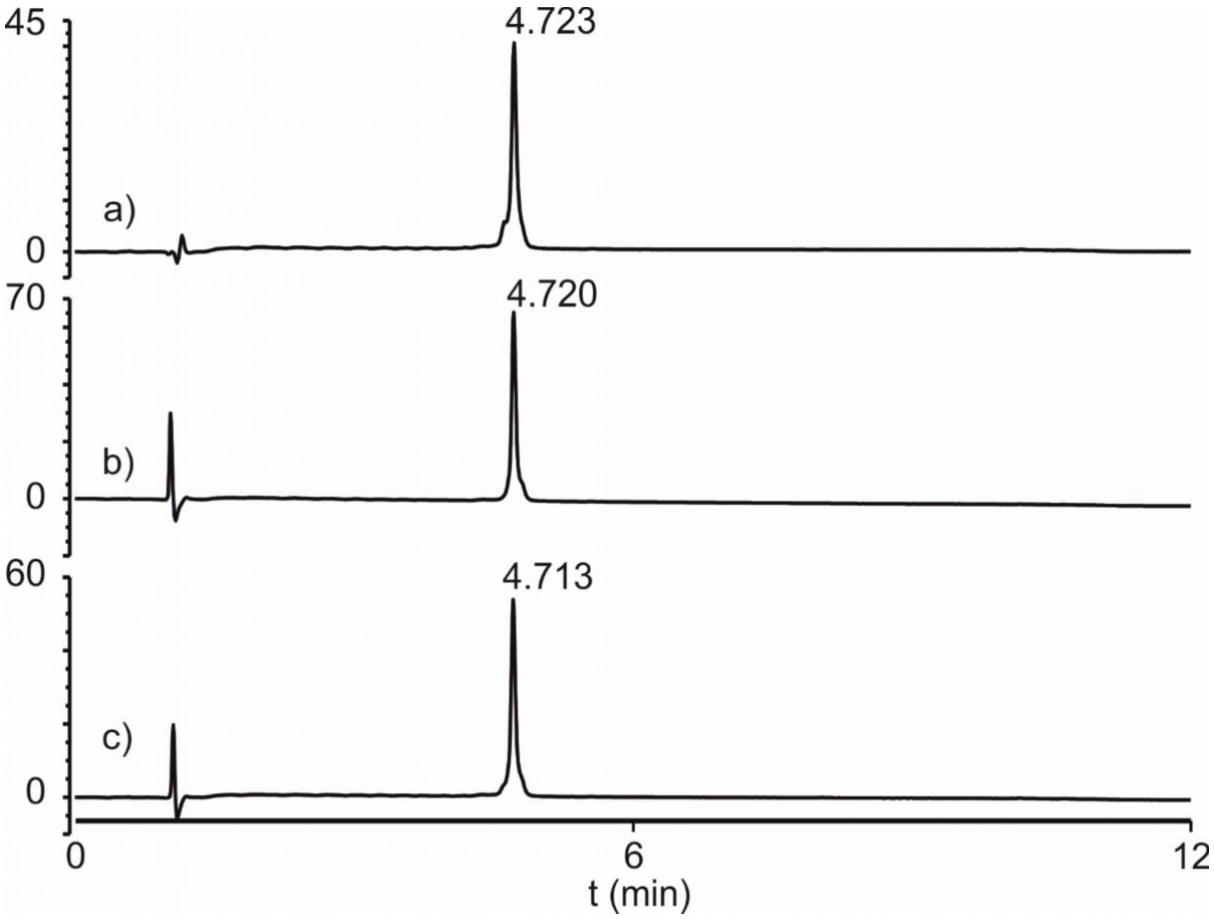


Figure S28 - RP-HPLC analysis of sequences 18. Overlay of RP-HPLC profiles measured from compound **18** obtained from the competition experiment (a), synthesized from the reference experiment (b) and as a co-injection (c). Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

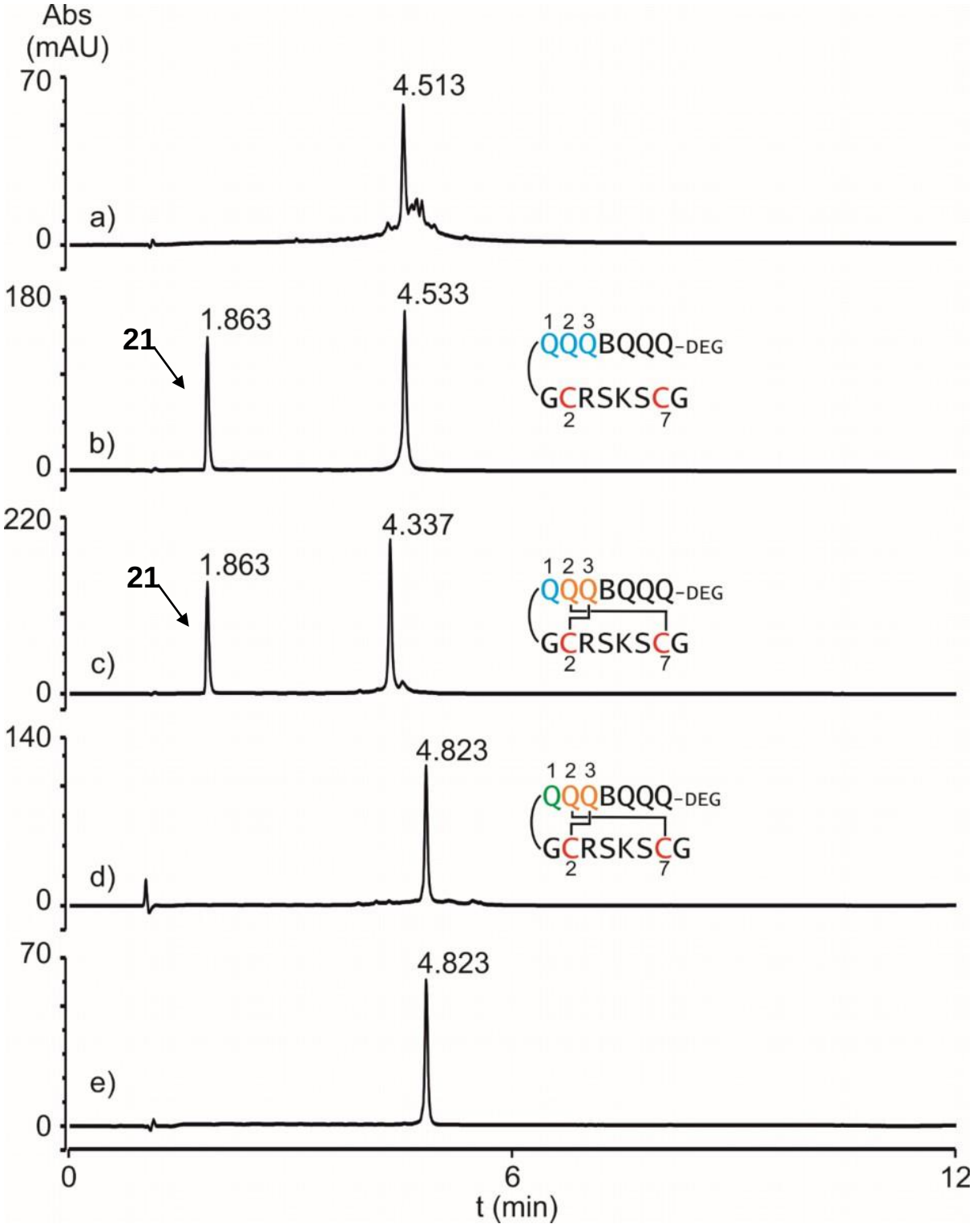


Figure S29 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 19. Overlay of RP-HPLC profiles measured from each synthetic step of compound **19Q**. Compound **19** after SPS/SPFS as a crude (a), after purification co-injected with internal standard **21** (b), after cyclization co-injected with **21** (**19C,** c), after benzyl mercaptan installation d) and final purification (**19Q**, e). Chromatograms a), c) and d) were recorded after the reaction without prior purification. Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

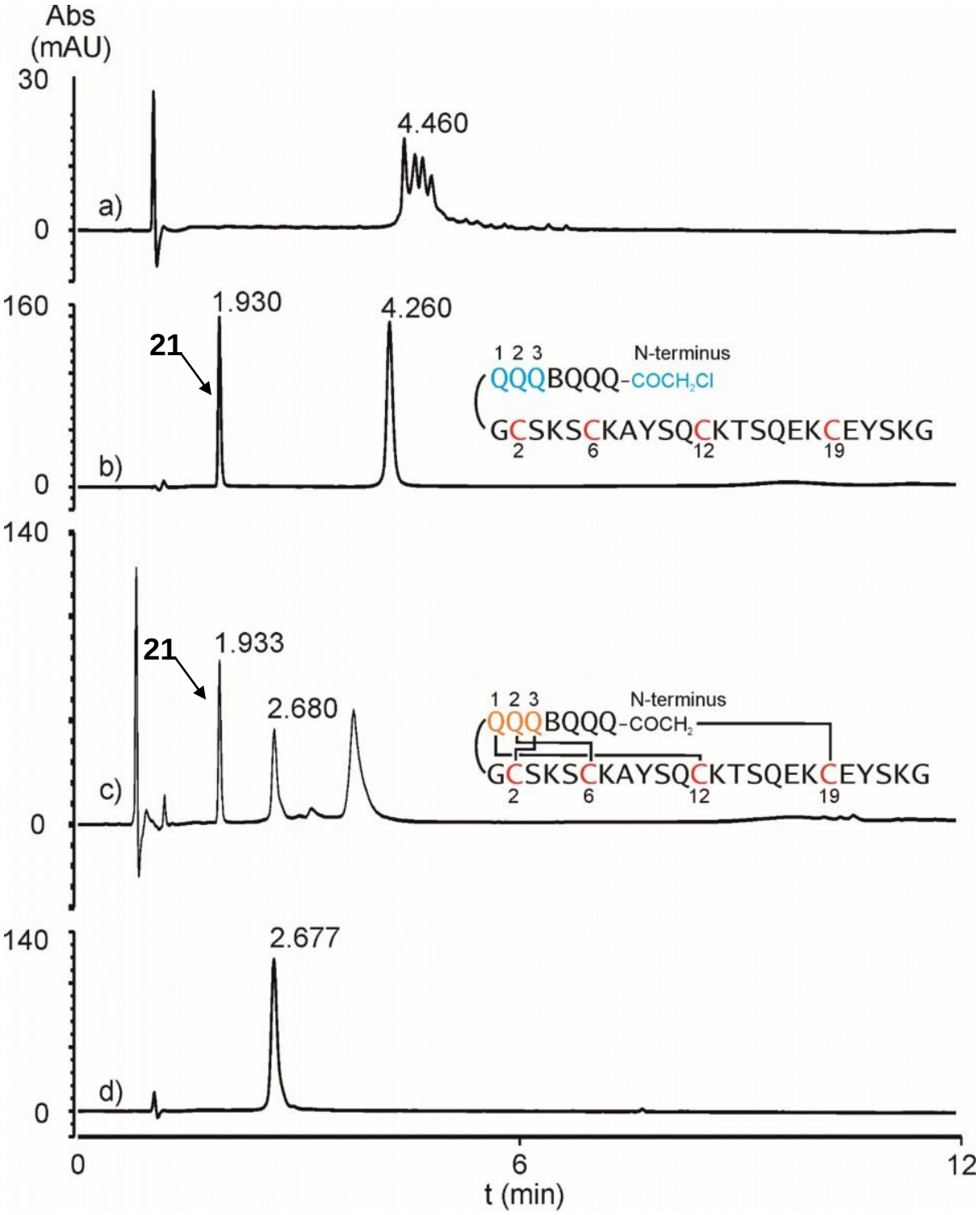


Figure S30 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 20. Overlay of RP-HPLC profiles measured from each synthetic step of compound **20Q**. Compound **20** after SPS/SPFS as a crude (a), after purification co-injected with internal standard b(b), after cyclization co-injected with **20** (**20C**, c), and final purification (**20C,** d). Chromatograms a), and c) were recorded after the reaction without prior purification. Chromatograms were measured with standard HPLC conditions. All chromatograms were recorded at 375 nm.

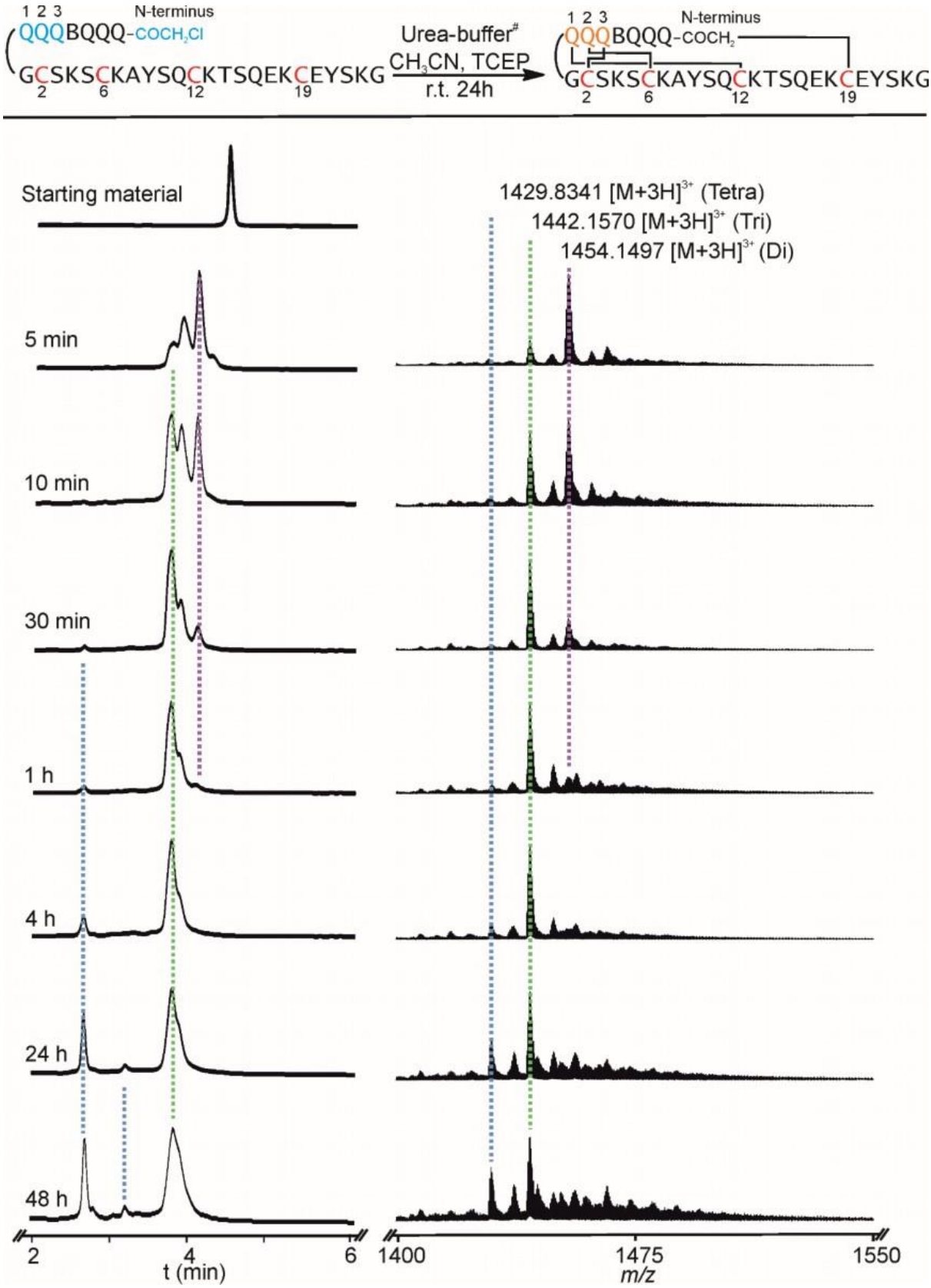


Figure S31 - Excerpts of the RP-HPLC and ESI-MS traces of the macrocyclization progression of 20C. The ESI-MS spectra reported correspond to the time segment 2.0 to 4.5 min. The resolution of the LC traces was not good enough to distinguish each peak intermediate on the base peak chromatogram (BPC) of the TOF detector**.** Urea buffer pH 8.5, 8 M (#). Chromatograms were measured with standard HPLC conditions and recorded at 375 nm.

1.2. NMR identification of products and structural study of **9Q**

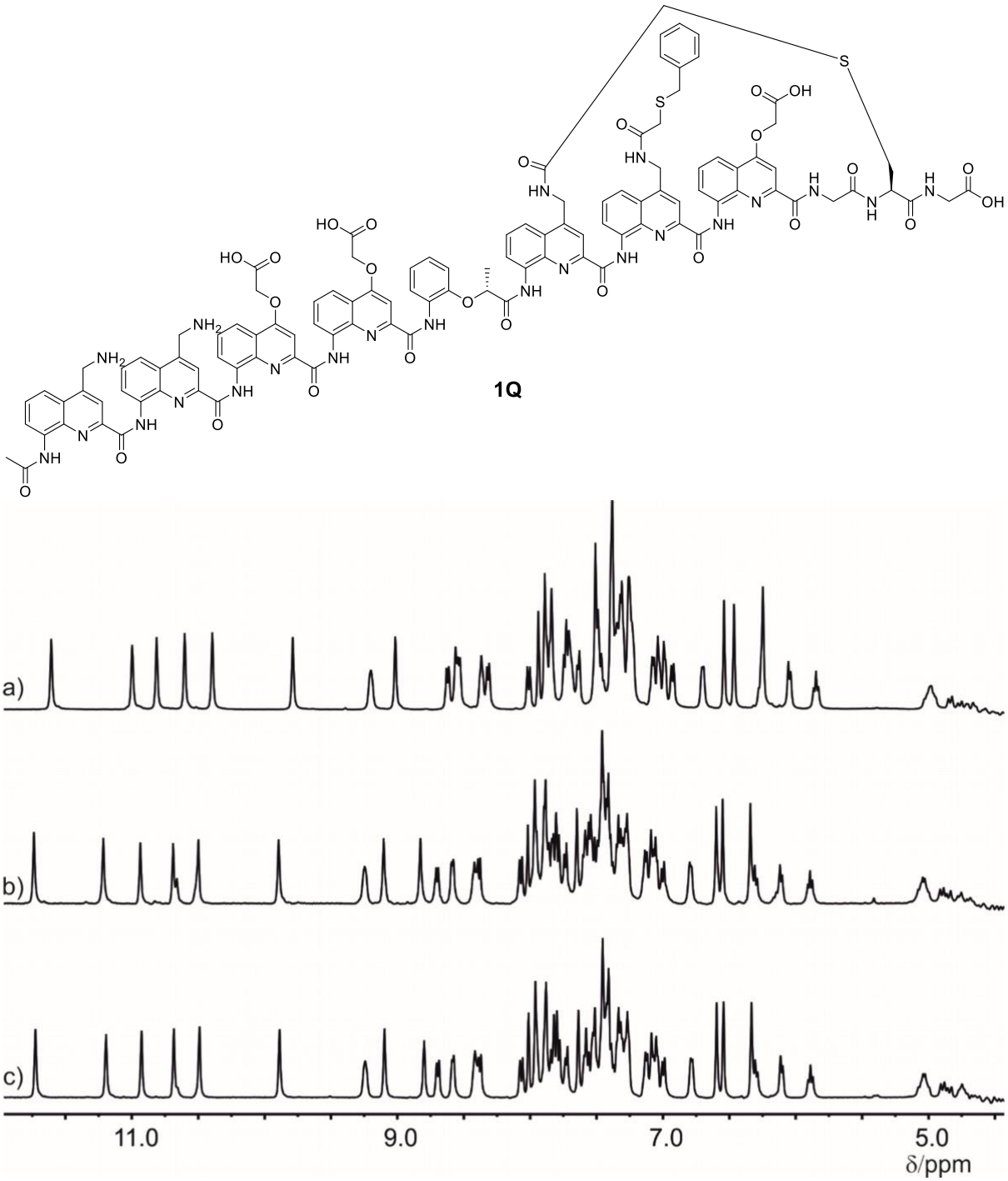
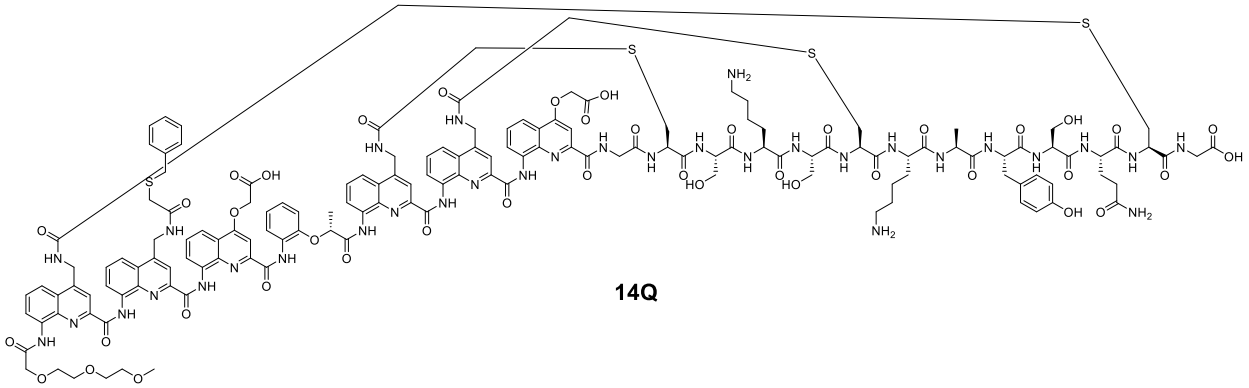


Figure S32 - NH and aromatic region of the 1H NMR overlaid spectra of 1Q synthesized by two different routes. ^{1}H NMR spectra recorded in a solvent mixture of NH_4OAc 3mM pH 8.5 in CD_3CN (50:50, v/v) (500 MHz, 25°C) with TMSP (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt) of **1Q** synthesized from compound **1** *(a)*; synthesized from compound **R1** *(b)* and the 1:1 mixture of the two products synthesized via (a) or (b) route *(c)*.



14Q

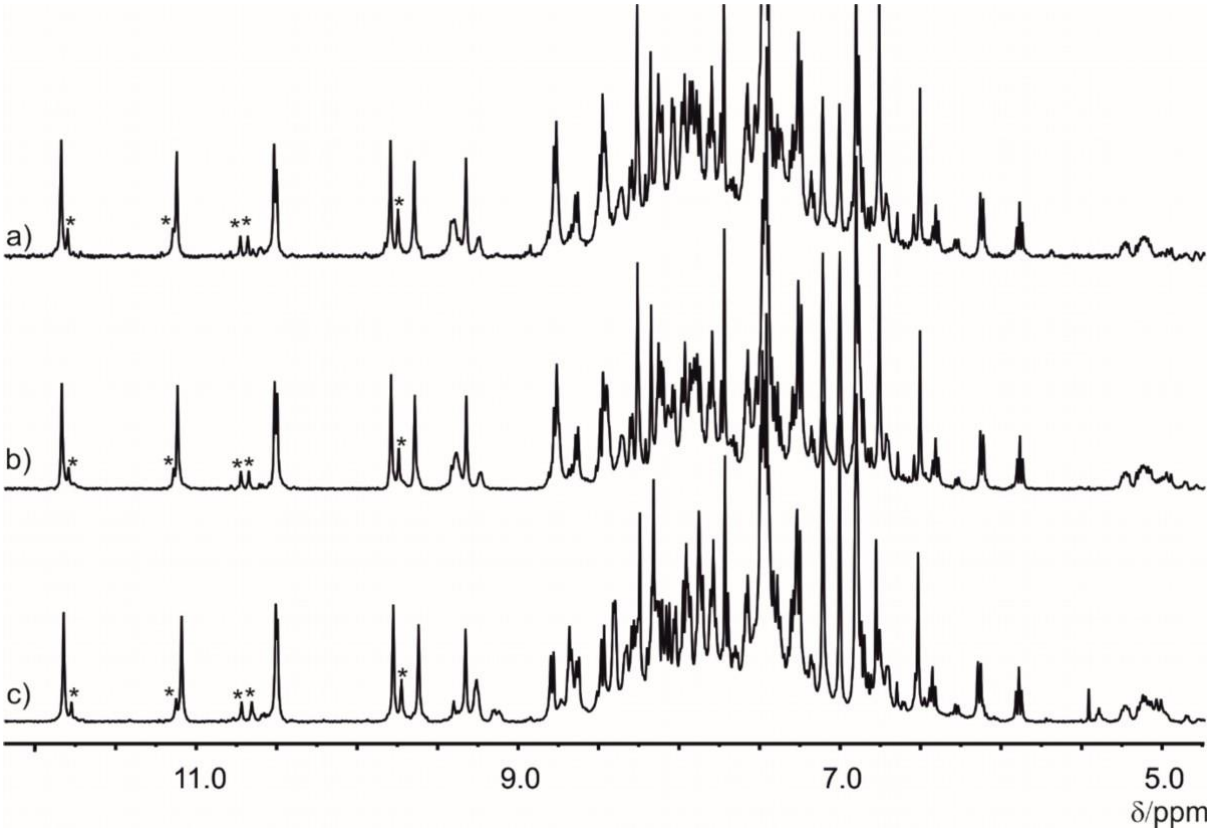


Figure S33 - NH and aromatic region of the 1H NMR overlaid spectra of 14Q synthesized by two different routes. 1H NMR spectra recorded in a solvent mixture of NH_4OAc 3mM pH 8.5 in CD_3CN (50:50, v/v) (500 MHz, 25°C) with TMSP of **14Q** synthesized from compound **14** *(a)*; from compound **R14** *(b)* and the 1:1 mixture of the two products synthesized via (a) or (b) route *(c)*. Signals assigned with * are considered as a synthetic impurity (over insertion of a Ser residue was detected by LC-MS analysis). Yet the presence of a different conformer cannot be excluded.

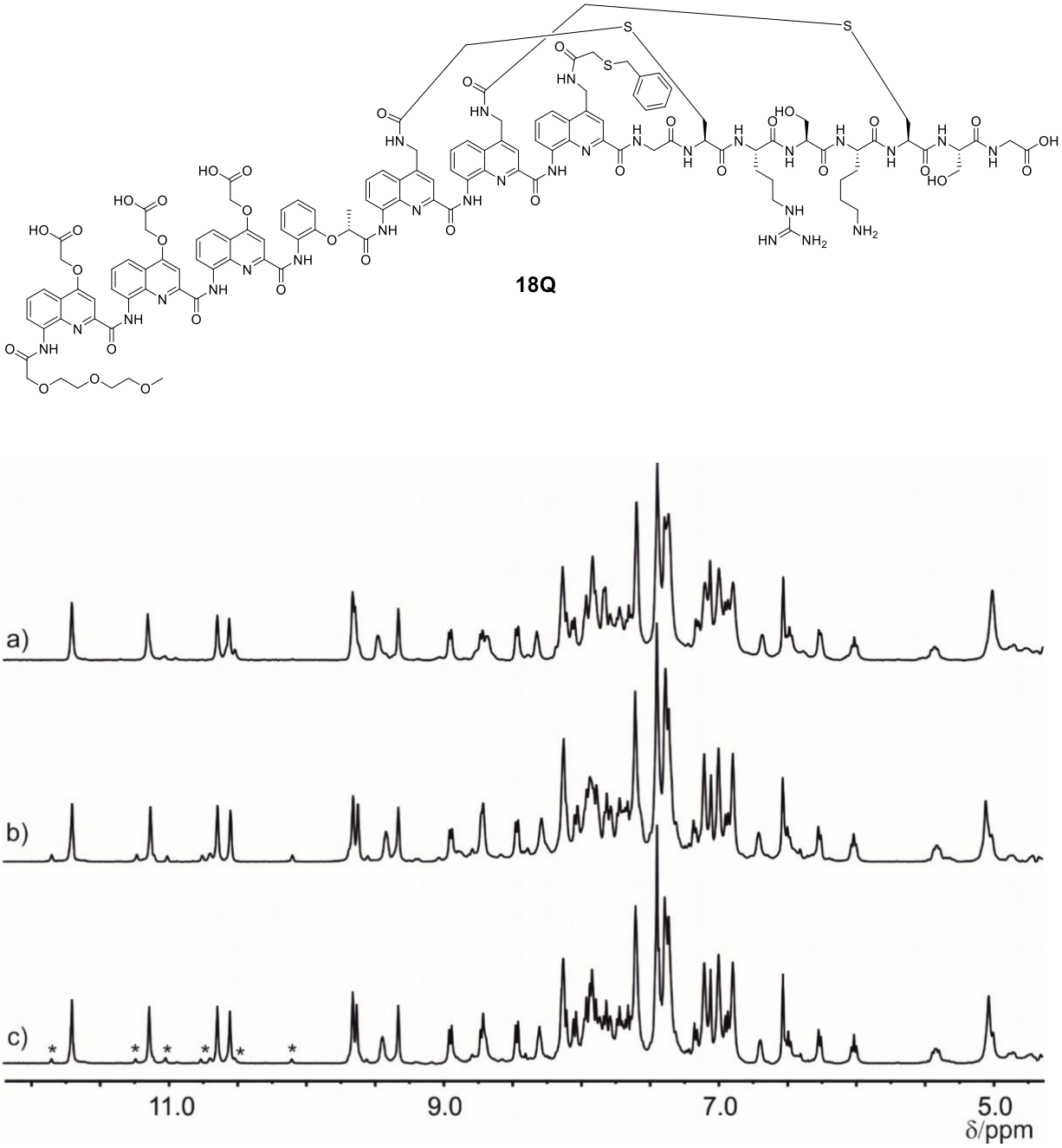


Figure S34 - NH and aromatic region of the 1H NMR overlaid spectra of 18Q synthesized by two different routes. ^{1}H NMR spectra recorded in a solvent mixture of NH_4OAc 3mM pH 8.5 in CD_3CN (50:50, v/v) (500 MHz, 25°C) with TMSP of **18Q** synthesized from compound **18** *(a)*; from compound **R18** *(b)* and the 1:1 mixture of the two products synthesized via (a) or (b) route *(c)*. Signals assigned with * are considered as a synthetic impurity.

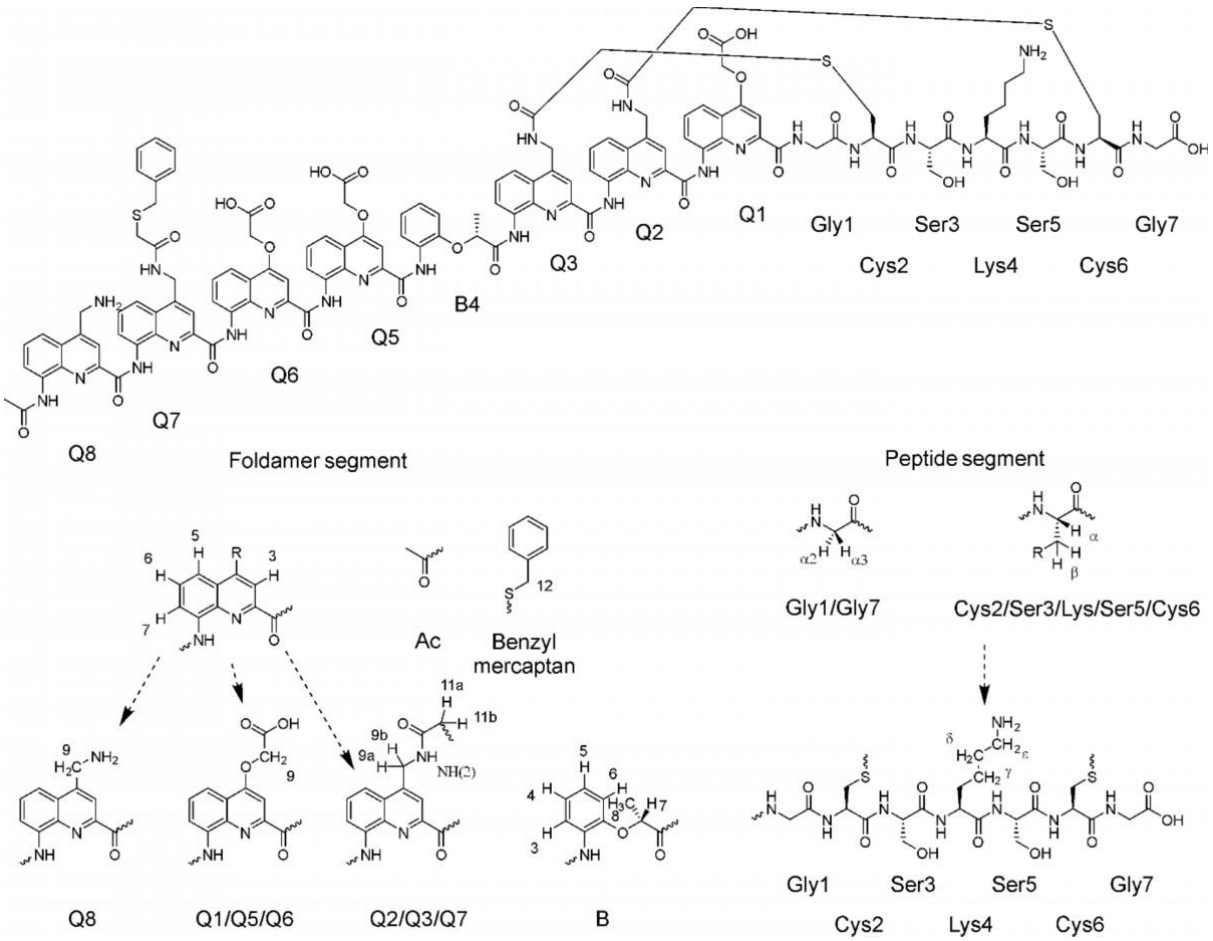


Figure S35 - Residue numbering of 9Q for full ^{1}H NMR assignment

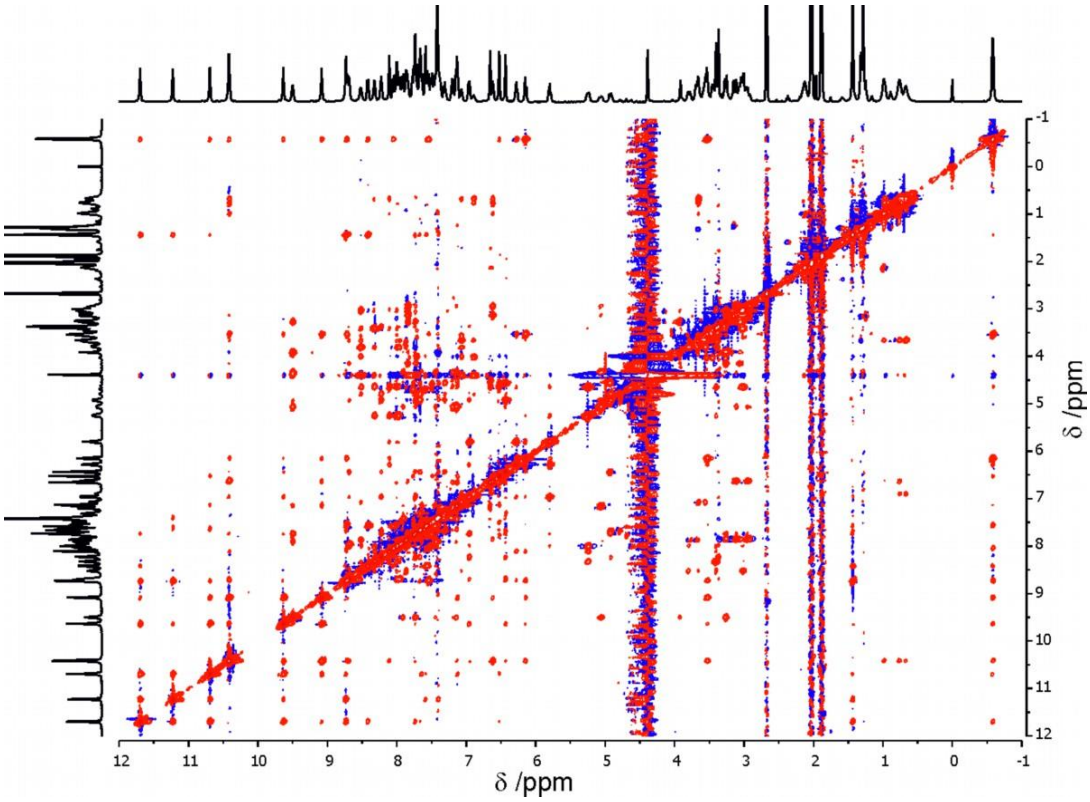


Figure S36 - ^{1}H NOESY spectrum of 9Q. NMR solvent mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C) with TMSP.

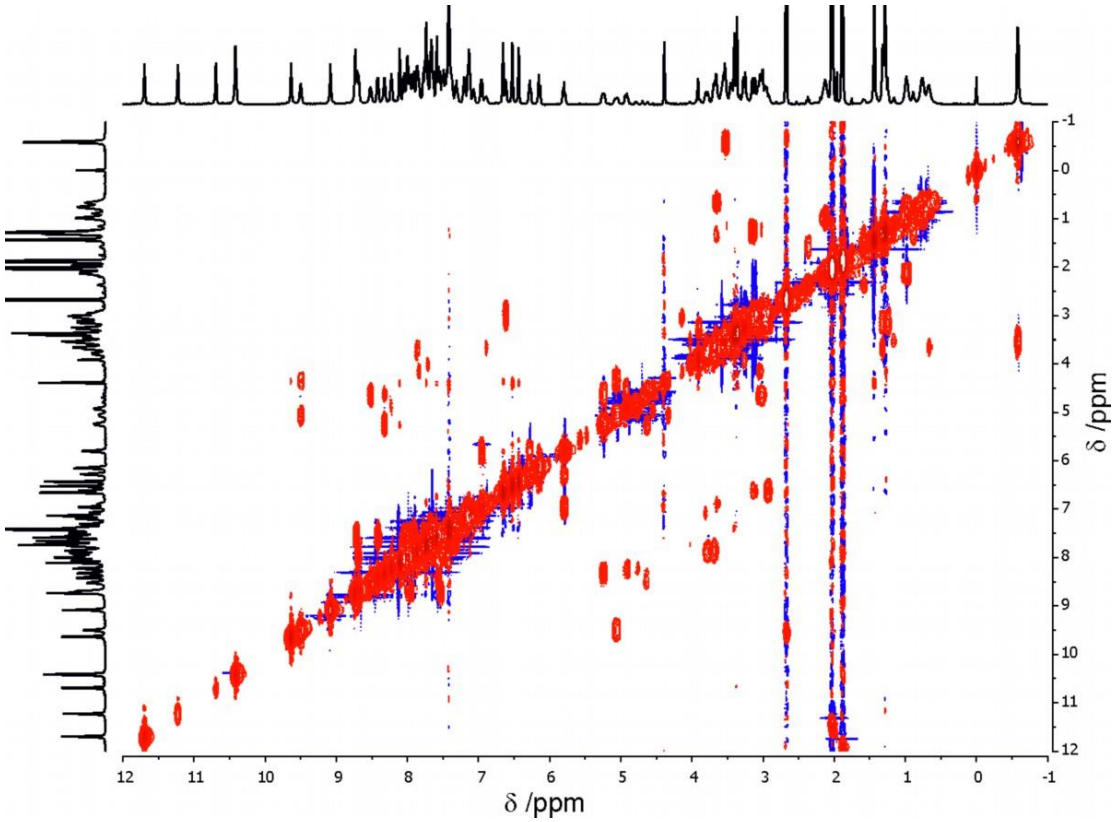


Figure S37 - ^{1}H COSY spectrum of 9Q. NMR solvent mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C) with TMSP.

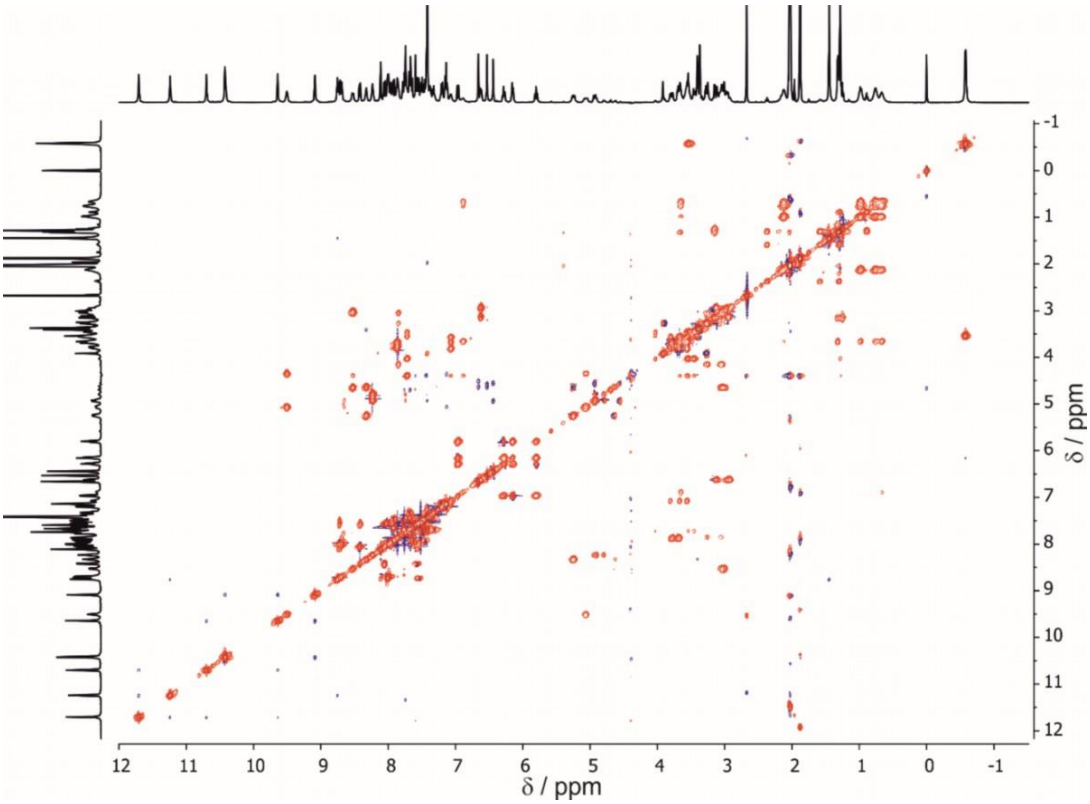


Figure S38 - ^{1}H TOCSY spectrum of 9Q. NMR solvent mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C) with the addition of TMSP.

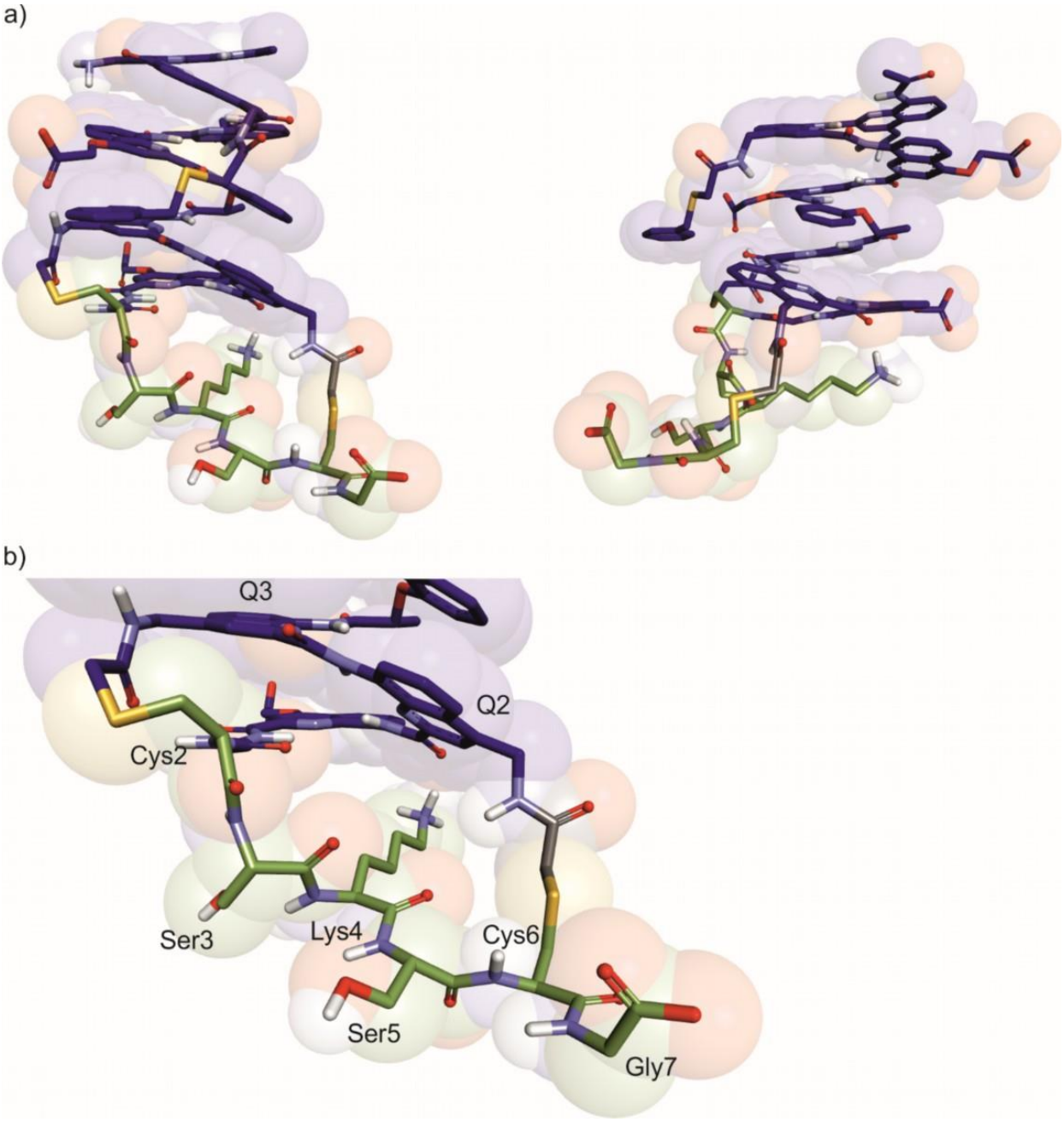


Figure S39 – Molecular models of 9Q. Molecular models obtained from MacroModel in the Maestro platform (force field potential: MMFFs, solvent: water, mini. method PRCG) of **9Q** a) from two different perspectives and b) zoom to the peptide region. Non-polar hydrogens are omitted for clarity. Foldamer carbon atoms are dyed in dark blue, peptide backbone carbon atoms in green.

Res.	H3	H4	H5	H6	H7	H8	H9	H11	*N*H	*N*H(2)	Ac	H12	Benz.
Q8	7.58		7.76	7.50	7.67		4.69		8.74		1.43		
Q7	7.74		7.68	7.32	7.40		4.92 (a), 4.79 (b)	3.40	11.22	8.23		3.92	7.45-7.39
Q6	6.52		8.05	7.55	8.42		4.58		11.69				
Q5	6.43		7.92	7.45	7.64		4.56		10.69				
B	6.96	5.80	6.28	6.15	3.53	-0.59			9.63				
Q3	7.74		7.14	7.20	7.13		5.05 (a), 4.34 (b)	3.92 (a), 3.27 (b)	9.08	9.50			
Q2	8.11		8.00	7.96	8.68		5.25 (a), 4.64 (b)	3.41 (a), 3.04 (b)	10.42	8.32			
Q1	6.65		8.01	7.54	8.72		4.51		10.41				

Table S1. ^{1}H chemical shift values in ppm for the foldamer segment of compound **9Q**

	α	β	γ	δ	ε	*N*H
Gly1	3.13 (2), 2.95 (3)					6.62
Cys2	4.14	3.05, 3.27				7.84
Ser3	4.03	3.45, 3.55				7.71
Lys4	3.66	0.67	0.76	0.99	2.12	6.90
Ser5	4.00	3.70				7.95
Cys6	4.64	3.04				8.52
Gly7	3.78 (2), 3.68 (3)					7.87

Table S2. ^{1}H chemical shift values in ppm for the peptide segment of compound **9Q**

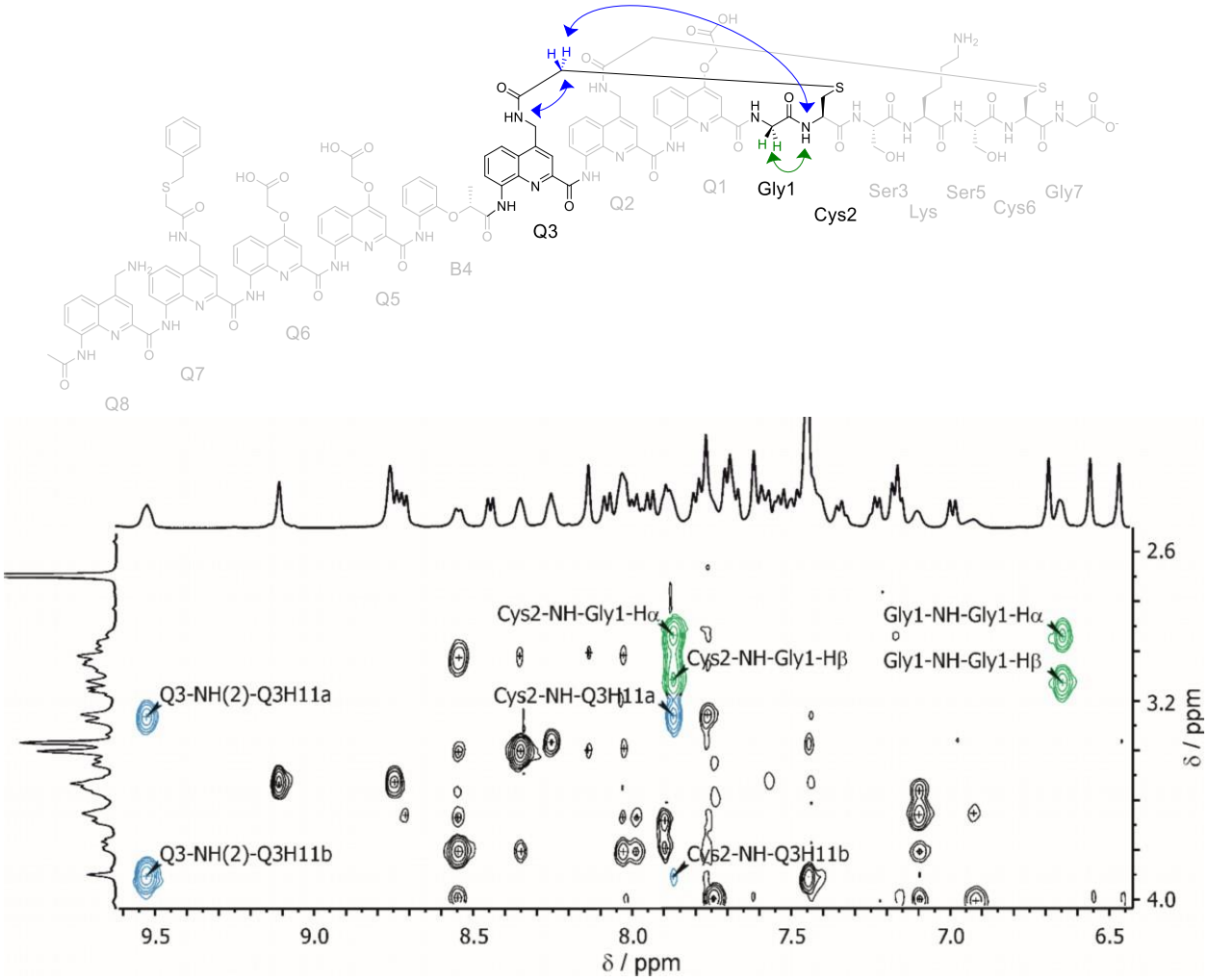


Figure S40 – Q3-macrocycle ^{1}H NOESY correlations. Excerpt of ^{1}H NOESY spectrum of **9Q** in a solvent mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C). Colored cross peaks relate to the correlation between Gly1 and Q3 side chain as part of a macrocycle.

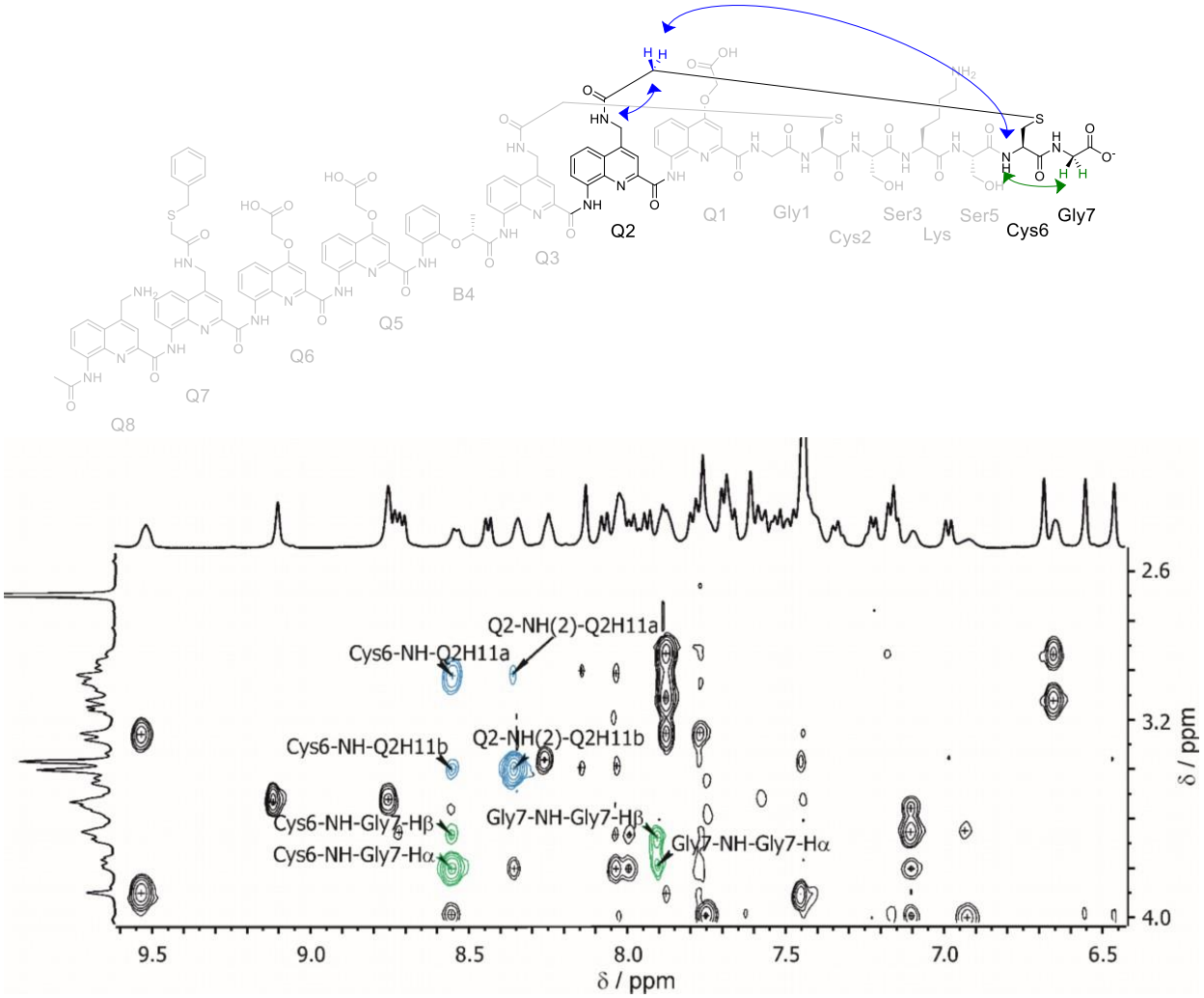


Figure S41 – Q2-macrocycle ^{1}H NOESY correlations. Excerpt of ^{1}H NOESY spectrum of **9Q** in a mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C). Colored cross peaks relate to the correlation between Gly7 and Q2 sidechain as part of a macrocycle.

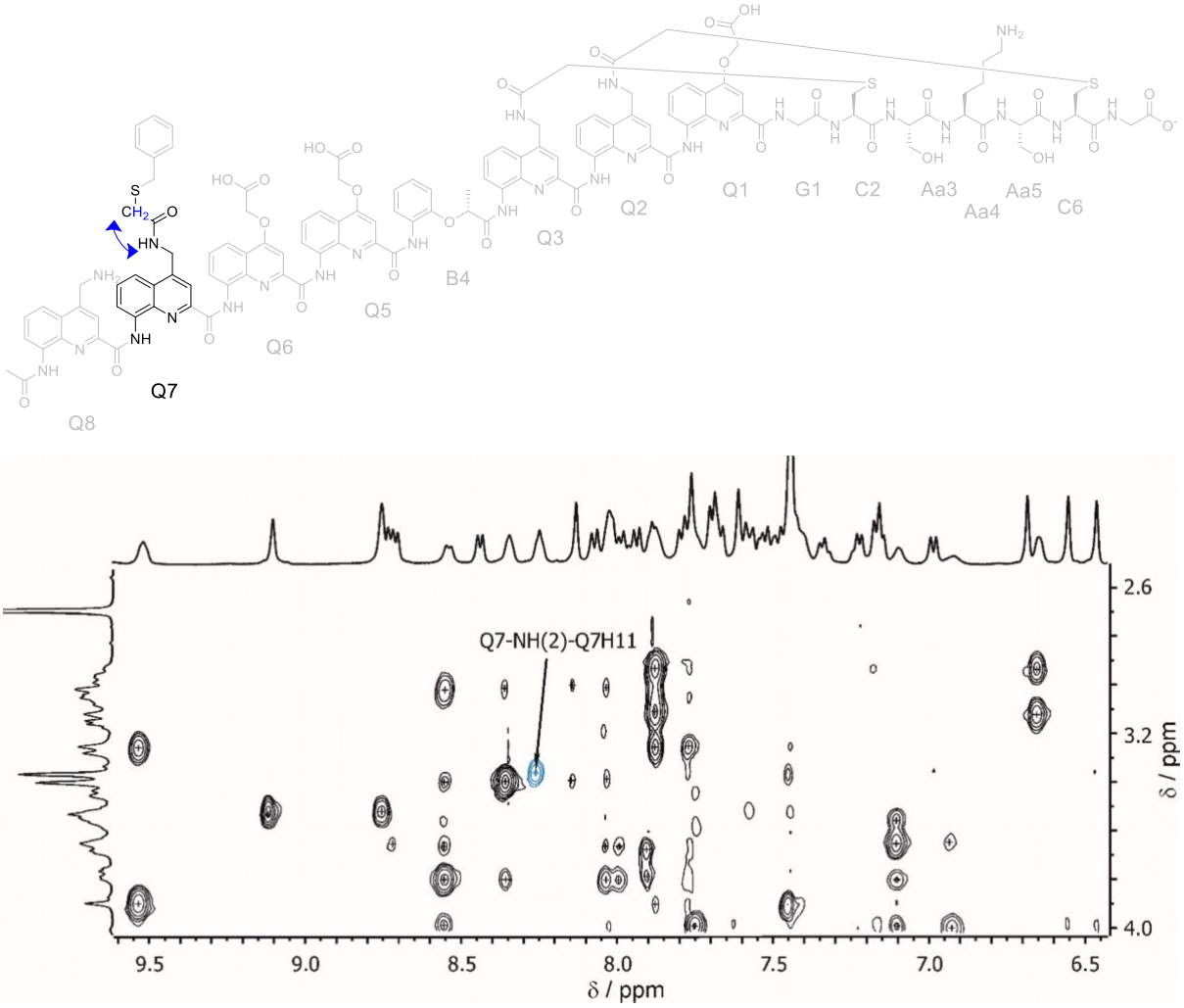


Figure S42 – Q7 NOESY correlation. Excerpt of ^{1}H NOESY spectrum of **9Q** in a mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C). Cross peaks correlation on Q7 side chain is depicted in blue.

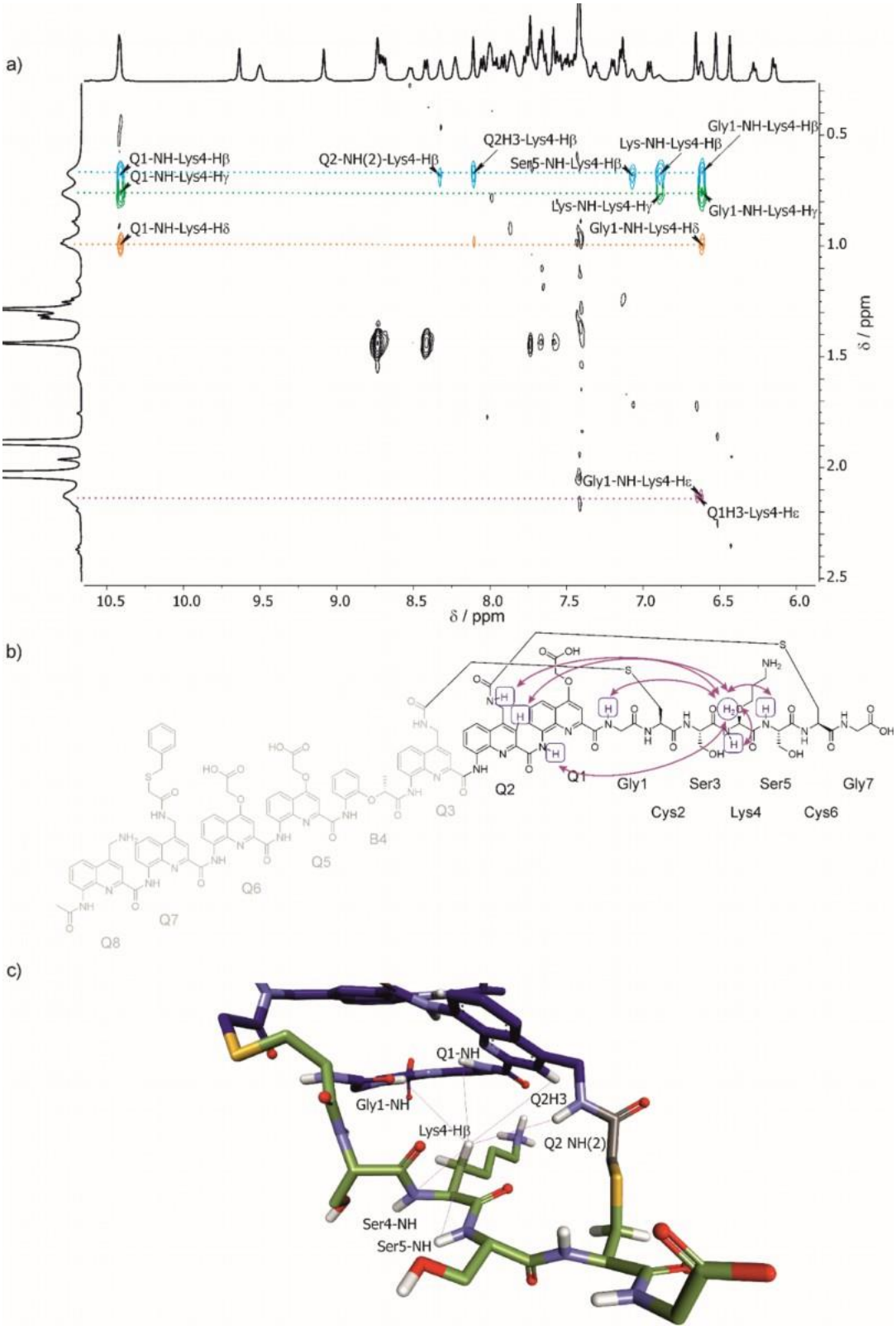


Figure S43 – Lys4 ^{1}H-NOESY correlations. a) Excerpt of ^{1}H NOESY spectrum of **9Q** in a solvent mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C). Colored cross peaks relate to the correlation between Lys4-Hβ (blue), Lys4-Hγ (green), Lys4-Hδ, and Lys4-Hε and neighboring aromatic and amide protons. b) Chemical structure of **9Q** and Lys4-Hβ correlations. c) Model of **9Q** and their correlations of signals shown for Lys4-Hβ exemplarily. NOE signals are depicted in purple lines.

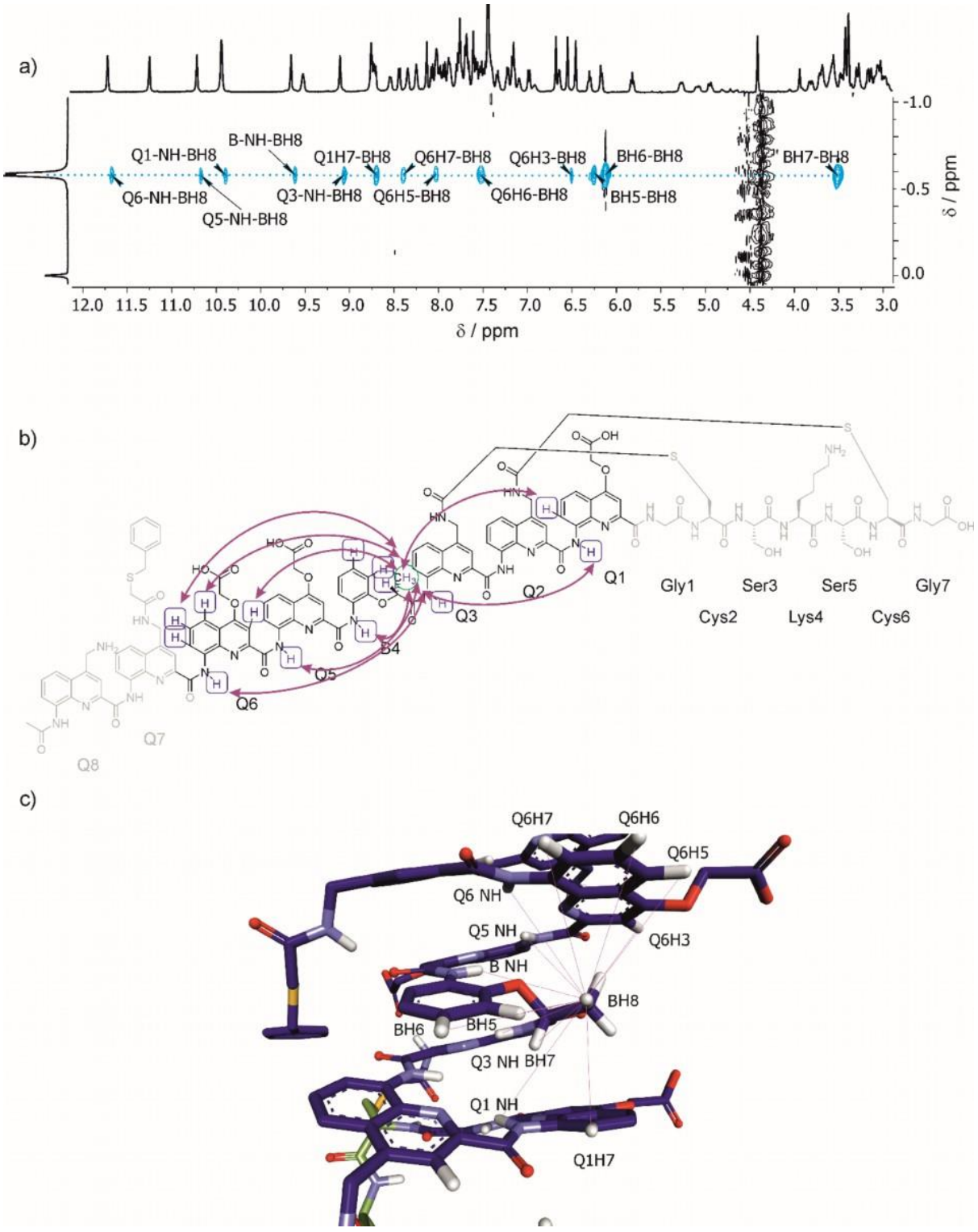


Figure S44 – BH8 ^{1}H-NOESY correlations. a) Excerpt of ^{1}H NOESY spectrum of **9Q** in a solvent mixture of NH_4OAc (5mM pH 8.5) in CD_3CN (50:50, v/v) (500 MHz, 25°C). Marked cross peaks relate to the correlation between BH8 and neighboring protons. Chemical structure of **9Q** and BH8 correlations. c) Model of **9Q** and their correlations of BH8 signals. NOE signals indicated by purple lines.

1.3. Reaction trail analysis of **16**

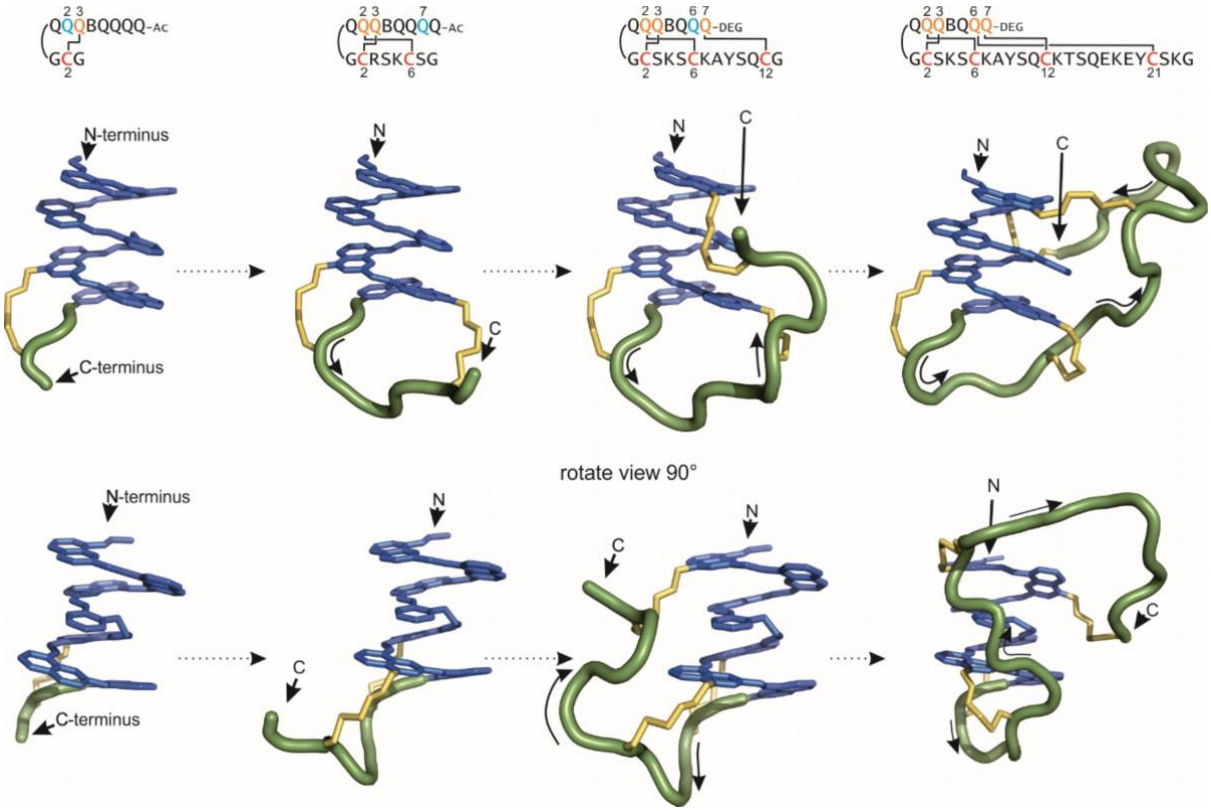


Figure S45 - Molecular models which illustrate the cyclization trail of the mono-, di-, tri- and tetracycle. Foldamer shown in blue, peptide loops in green, thioether linker in yellow. Arrows indicate N- to C-terminus.

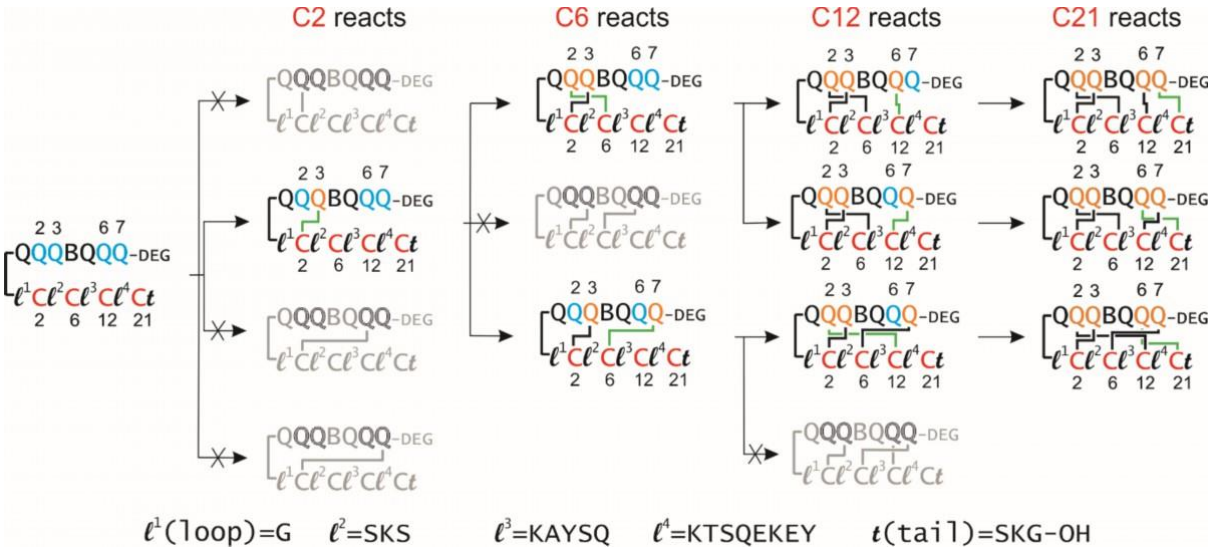


Figure S46 - Scheme of the cyclization trail of 16C. Colored schemes are plausible cyclization trails.

1.4. Synthetic schemes

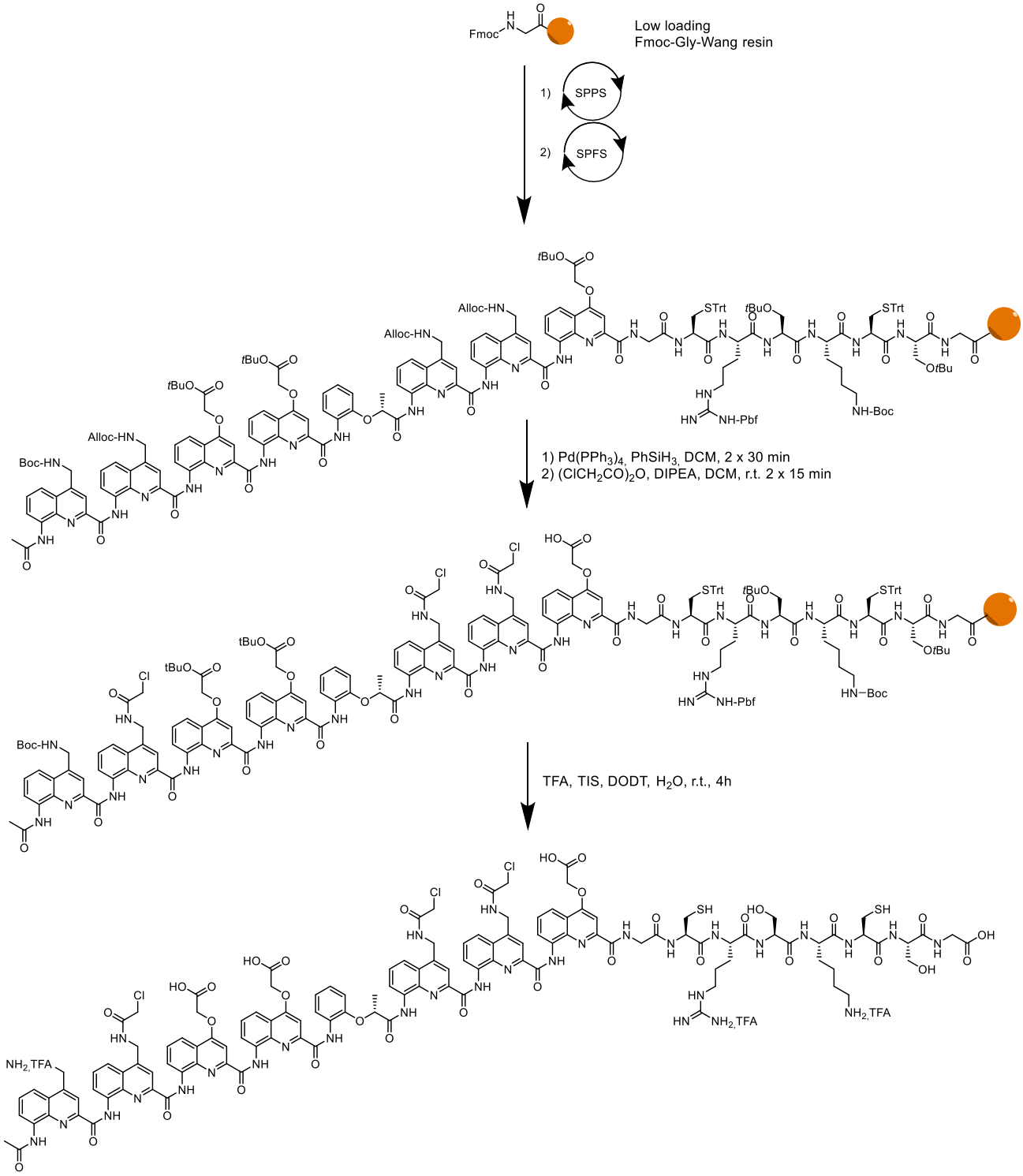


Figure S47 - General synthetic scheme exemplarily shown of linear Foldamer-peptide-hybrid 8 on solid support.

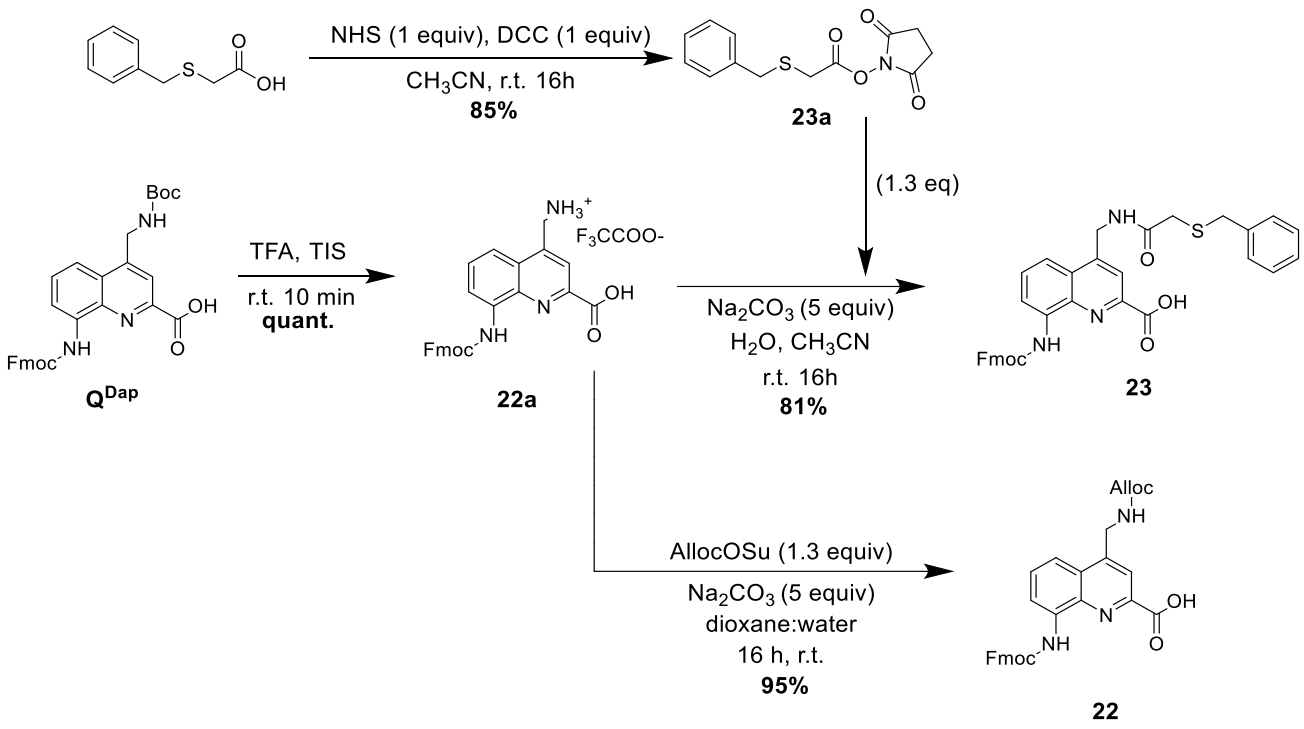


Figure S48 - Synthetic routes for the monomer Fmoc-Q(Dap-Alloc)-OH (22) and Fmoc-Q(SBn)-OH (23)

1.5. Circular dichroism (CD) spectra

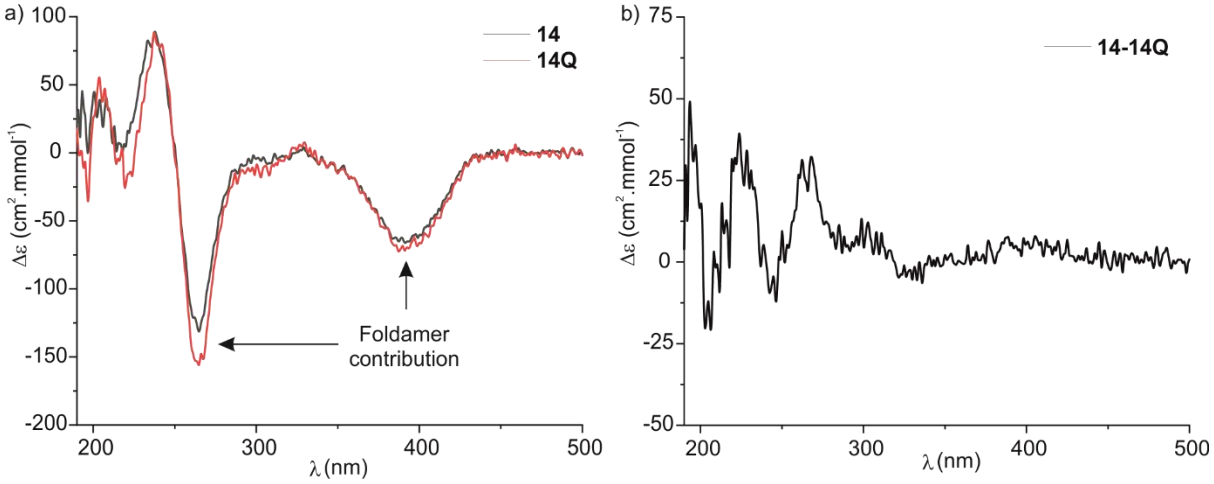


Figure S49 – CD spectrum from 14 and 14Q. a) Individual CD spectra for compound **14** (linear) and **14Q** (cyclic) and CD spectra obtained after substracting the individual spectra of **14**-**14Q**.

2. Material and Methods

2.1. RP-HPLC analyses

Analytical RP-HPLC analyses, were performed on an Ultimate 3000 RP-HPLC System (ThermoFisher Scientific). For analytical analysis, a Nucleodur C18 Gravity column (4 x 100 mm, 5 µm, Macherey-Nagel) was used at a flow rate of 1 ml/min. Semi-preparative purification was performed on a Waters system equipped with a 2545 Quaternary Gradient Module with automated fraction collector system on a Nucleodur C18 Htec 5 µm 125/21 column at a flow rate of 25 ml/min. When using acidic conditions 0.1 % TFA was added to the aqueous mobile phase (referred to as mobile phase A) and to acetonitrile (referred to as mobile phase B). The column eluent was monitored by UV detection at 214, 254, and 300 nm with a diode array detector.

For the quantification of the macrocyclization reaction, a solution of internal standard **21** (0.70 – 0.77 mM, 0.1 % TFA in H_2O/CH_3CN (90:10, v/v)) and a foldamer solution (1.5 – 0.5 mM, 0.1 % TFA in H_2O/CH_3CN (90:10, v/v)) was prepared and the concentration of each solution was determined by measuring the UV absorbance at 375 nm with the NanoDrop UV-spectrometer and a 1 cm cuvette. Prior to RP-HPLC analysis, both solutions were mixed in a 1:1 ratio. The peak areas of standard and analysis compounds were determined separately by integration and the correction factor (KF) was calculated from the purified non-cyclic foldamer-peptide hybrid compound. Identical analysis and re-processing were carried out after the macrocyclization step. The area of the macrocycle was determined by integration and the % of macrocyclization conversion was calculated via the previously determined KF value.

$$KF = \frac{F_A * C_{IS}}{F_{IS} * C_I}$$

$C_{IS} = Concentration\ of\ the\ internal\ standard\ (IS)$

$C_I = Concentration\ of\ the\ analyte\ (A)$

$F_{IS} = Area\ of\ the\ IS$

$F_A = Area\ of\ A$

If not otherwise specified following RP-HPLC profiles were recorded by using a gradient from 10 to 100 % CH_3CN (0.1% TFA) at 50°C. The concentrations of internal standards **21** and analytes were determined by UV-VIS spectroscopy before RP-HPLC analysis.

2.2. LCMS analyses

LCMS spectra were recorded on a Bruker microTOF II in either positive or negative ionization mode. The instrument was calibrated in positive and negative mode by direct infusion of a calibration solution (Agilent Technologies ESI-L Low Concentration Tuning Mix). The HPLC line was an Ultimate 3000 RP-HPLC System (ThermoFisher Scientific) equipped with a Nucleodur C18 Gravity column (2 x 50 mm, 1.8 µm, Macherey-Nagel) at a flow rate of 0.33 ml/min. 0.1 % formic acid was added to the aqueous mobile phase (solvent A) and to acetonitrile (solvent B). The column eluent was monitored by UV detection at 214, 254, and 300 nm with a diode array detector.

2.3. NMR analyses

^{1}H-NMR spectra were recorded on Avance III HD 400 MHz Bruker BioSpin and Avance III HD 500 MHz Bruker BioSpin spectrometers. All chemical shifts (δ) are reported in ppm and calibrated against residual solvent signals of DMSO-d_6 (δ 2.50 ppm) and $CDCl_3$ (δ 7.16 ppm). In the case of ^{1}H-NMR spectra recorded in H_2O/CH_3CN or aqueous salt buffered systems, 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) was added to the medium and calibrated against δ 0.00 ppm. Coupling constants (*J*) are reported in Hz. Signal multiplicities were abbreviated as *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet, and *m*, multiplet.

2.4. Molecular modeling

Models were simulated by using Maestro version 11.5 (Schrödinger Inc.). Energy minimized structures were obtained using MacroModel energy minimization with the following parameters: force field: MMFFs; Solvent: Water; Electrostatic treatment: Constant dielectric; Dielectric constant: 1.0; Charges from: Force field; Cutoff: Extended; Van der Waals: 8.0; Electrostatic: 20.0; H-bond: 4.0; Mini Method: TNCG; Maximum iterations: 2500; converge on: Gradient; Convergence threshold: 0.05. No other extended options.

As a starting point, the coordinates of the crystal structure of a previously described peptide-foldamer hybrid macrocycle (CCDC entry # 2010131) were imported. From the imported structure, the peptide was deleted and the helical foldamer part was modified to match the sequence included in the molecules synthesized in this study (*i.e.* DEG- or Ac-QQQBQQQ-OH). The C-terminal peptide was built on the foldamer as a growing alanine chain with cysteine residues at positions consistent with the sequences from the syntheses. Other amino acids than alanine and cysteine were not used. The initial non cyclic model was energy minimized. Acetamidomethyl-containing side chains were introduced at positions of interest on the foldamer helix. The cysteine thiol and acetamide were then joined by deleting the sulfide hydrogen and one acetyl hydrogen atom and replacing them by a sulfur-carbon single bond, thus creating a macrocycle. The initial length of this bond may be very long and can eventually be somewhat reduced by adjusting bond rotations in the peptide chain prior to creating the bond. Energy minimization was started again leading to a quick adjustment of the bond length and translating into conformational changes of the peptide and possibly of the helix. Observation of unfavorable conformational patterns in the energy minimized structure, and in particular, even slight

distortions of the foldamer conformation were interpreted as strain that would make the spontaneous formation of this cycle unfavorable. The process was repeated, placing the cysteine residue at different positions in the peptide sequence and the acetamidomethyl group on different quinoline rings of the foldamer. When a cysteine residue could cyclize without creating strain with different side chains, the nearest reaction site was considered to be favored. These iterations were repeated for bi-, tri- and tetra-cycles. The xyz coordinates of representative multicycles are provided as an extended data set excel file.

2.5. CD analysis

All CD curves were recorded on a Jasco J-810 spectrometer with 10 mm quartz cuvette. Following parameters were used: Wavelength range from 650 to 180 nm. Scan speed: 50 nm/min, accumulation: 2, response time: 1.0 s, bandwidth: 2, temperature: 25 °C, sensitivity: standard, data pitch: 1 nm, nitrogen gas flow rate: 500L/h. The sample solution was prepared in degassed ultrapure water/acetonitrile solvent mixture 90:10 (v/v). $\Delta\varepsilon$ values were obtained by using the formula: $\Delta\varepsilon = m°/(C.l.32980)$; $\Delta\varepsilon = cm^2.mmol^{-1}$, m°= CD value in milli degrees, l = cuvette pathlength in cm, C = sample concentration in mol/L.

2.6. Chemical Synthesis

2.6.1. General

Fmoc-$Q^{Asp(OtBu)}$-OH, Fmoc-$Q^{Dap(Boc)}$-OH and Fmoc-B^{Rme}-OH monomers were prepared by following the reported synthetic protocols.[1] If not otherwise mentioned, chemical reagents were purchased from Sigma-Aldrich, and solvents from Fisher Scientific and used without further purification. Anhydrous tetrahydrofuran (THF) and anhydrous dichloromethane (DCM) were obtained from MBRAUN SPS-800 solvent purification system. Anhydrous chloroform ($CHCl_3$) and N,N-diisopropylethylamine (DIPEA) were distilled over CaH_2 prior to use. Exclusively ultrapure water was used. DMF and NMP (peptide grade) were purchased from Carlo Erba. Rink amide MBHA, Cl-MPA ProTide®, and low-loading preloaded Fmoc-Gly-Wang resins were purchased from CEM. Fmoc-Gly-SASRIN resin was purchased from Novabiochem. Fmoc-N-protected amino acids, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophos-phate (BOP) and 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium-hexafluorophosphate (HBTU) were purchased from IRIS Biotech. 2-(benzylthio)acetic acid was purchased from ABCR. Silica column chromatography purifications were performed on silica gel (230-400 mesh, 40-63 µm, Merck) and thin-layer chromatography was performed on silica gel 60-F254 plates (Merck).

2.6.2. General protocol for SPPS

The peptide segments were assembled by using a Liberty Blue CEM® synthesizer at a minimum scale of 50 µmol. Fmoc deprotection was performed twice with 20% piperidine in DMF at 75°C (1 × 30 sec. and 1 × 180 sec.).The coupling cycles of the peptide segments in sequences **1-14** contained two coupling steps under microwave irradiation at 50°C for 10 min with N-Fmoc-α-AA-OH (6 equiv. relative to the resin loading), PyBOP (6 equiv.), and DIPEA (12 equiv.) in DMF. In the case of the peptide segments present in sequences **15, 16, 17, 20** and **21**, the coupling step were performed once at RT for 15 min with N-Fmoc-α-amino acid (10 equiv. relative to the resin loading), PyBOP (10 equiv.), and DIPEA (20 equiv.) in DMF. After Fmoc deprotection step, the resin was washed with DMF (2 × 2 ml) step and following the coupling step, one time with DMF (3 ml). When ProTide® resin was used, due to its high swelling property, after Fmoc deprotection the resin was extensively washed with DMF (4 × 4 ml) and following the coupling step one time with 4 ml DMF.

2.6.3. General protocol for SPFS

The microwave-assisted solid-phase synthesis (SPS) of foldamer-peptide hybrids was carried out on a Discover-Bio CEM® microwave oven in an open vessel mode. The temperature of the reaction mixture within the reactor vessel was monitored with an optical fiber probe.

*2.6.3.1. Solid-phase foldamer synthesis (SPFS) for compounds **1–14** and **16**-**19**.*

SPFS for compounds **1**–**14**, **16**-**19** was undertaken according to reported protocols:

- ✓ Fmoc Deprotection. The resin was suspended at room temperature in a solution of 20 % piperidine in DMF for 1 × 3 min and 1 × 7 min with one DMF washing in between the two cycles. The resin was next washed once with DMF (3 mL) and with dry THF (3 × 3 mL) prior to perform the in situ coupling.
- ✓ Iterative in situ coupling of Fmoc-Q-OH on resin-bound peptide. To the pre-swollen resin in dry THF (1 ml) was added 2,4,6-Collidine (9.0 equiv. relative to the resin loading). Concurrently, the Fmoc-Q-OH was suspended in 1.25 ml anhydrous $CHCl_3$ together with triphenylphosphine (8 equiv). Trichloroacetonitrile (TCAN) was quickly added and the reaction mixture was vigorously mixed before to be added to the suspended resin within 60 sec. The reaction vessel was then placed under microwave irradiation (25 W, ramp to 50°C over 5 min, then hold at 50°C for 15 min). The resin was filtered off and washed with anhydrous THF (2 × 3 mL). The coupling step was repeated once. The resin was then filtered off and washed with THF (3 × 3 mL) and DMF (2 × 3 mL).

*2.6.3.2. Solid-phase foldamer synthesis (SPFS) of **F15** and **F20***

The SPFS of foldamer fragment of **15** (**F10**) and **20** (**F20**) was carried out on a Discover-Bio CEM® microwave oven in open vessel mode as previously described[2] on Fmoc-Gly-SASRIN resin by using the in situ activation procedure (see above) on a 200 µmol scale.

*2.6.3.3. Fragment condensation to assemble hybrid **15** and **20** on solid support (exemplified with compound **15**)*

F15 was next coupled via a fragment condensation approach on the resin-bound peptide **P15** on a 50 μmol scale. To remove any remaining moisture, **F15** was lyophilized prior to coupling. **F15** (50 mg, 47 μmol, 0.94 eq.) was then dissolved in dry NMP (0.4 mL) and dry THF (1.0 mL) together with DIPEA (35 μL, 0.2 mmol, 4 eq.) and BOP (44 mg, 100 μmol, 2 eq.). After pre-activation for 3 min, the coupling solution was added to the resin-bound H-Phe-peptide **P10** under N_2 atmosphere. The mixture was stirred for 24 h at r.t. by monitoring the progress of the reaction via HPLC analysis. The resin solution was filtered off and washed with DMF (3 x 3 mL). To facilitate HPLC purification, remaining unreacted resin-bound H-Phe-peptide **P10** was acetylated (Method 3.2.5).

2.6.3.4. General protocol for N-terminal acetylation

Before performing the N-terminal acetylation step on the resin-bound NH_2-oligomer, the resin was transferred to a 5 ml syringe equipped with a filter and washed with DCM (3 × 3 ml). The resin was next treated with a solution of acetic anhydride in DCM (50:50, v/v) for 16 h at RT. The resin was then filtered off and washed with DCM (3 × 3 ml).

2.6.3.5. General protocol for Alloc deprotection

The resin was incubated with a solution of $Pd(PPh_3)_4$ (0.1 equiv. relative to the number of Alloc protecting group), phenylsilane (20 equiv.), and dry DCM (1.5 ml) for 30 min at RT. This deprotection step was repeated once without any washing in between. The resin was then filtered off and washed with DCM (3 × 3 ml).

2.6.3.6. *On resin Q^{Dap} side-chain chloroacetylation, resin cleavage and purification*

The chloroacetylation step, resin cleavage and purification of crude foldamer-peptide-hybrids were carried out by following the reported procedures.[2]

2.6.4. Monomer syntheses

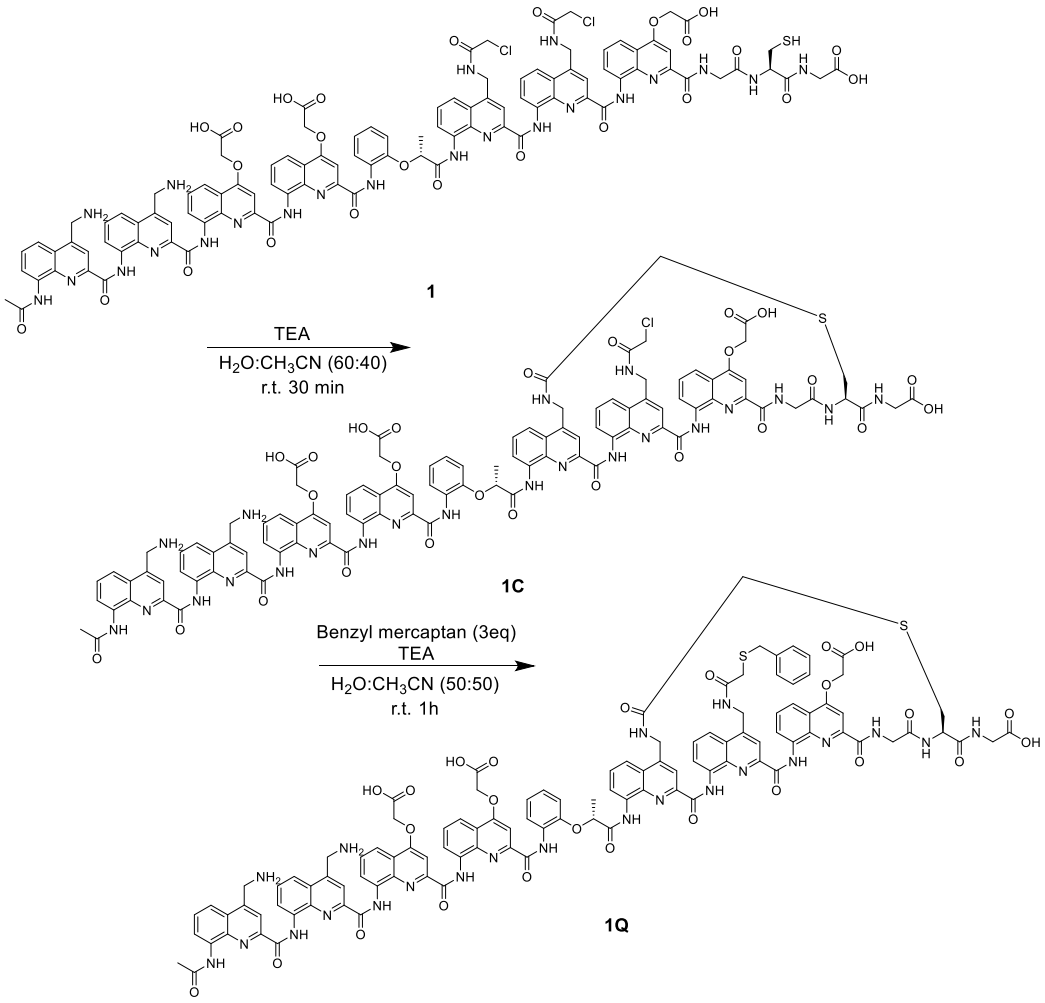
Compound 22a: Fmoc-Q(Dap(Boc))-OH was prepared by established protocol.[1a] It (0.27 mol, 2 g) was dissolved in 60 ml TFA together with 600 µl TIS and stirred at RT for 10 min. The solvent was narrowed down to 5 ml TFA by rotary evaporation and the solution was next precipitated in 120 ml cold diethylether. The suspension was centrifuged and the supernatant discarded. Compound **22a** was obtained as a colorless powder in quantitative yield and used without further purification. ^{1}H NMR (500 MHz, DMSO-d6) δ 13.77 (s, 1H), 10.51 (s, 1H), 8.58 (s, 3H), 8.35 (s, 1H), 7.94 (dt, J = 7.6, 0.9 Hz, 2H), 7.85 (dd, J = 8.5, 1.3 Hz, 1H), 7.78 (m, 3H), 7.44 (tt, J = 7.5, 0.9 Hz, 2H), 7.37 (td, J = 7.5, 1.2 Hz, 2H), 4.65 (d, J = 6.8 Hz, 2H), 4.46 (t, J = 6.7 Hz, 1H). 13C NMR (126 MHz, DMSO) δ 165.24, 153.49, 144.85, 143.69, 142.51, 140.84, 136.51, 136.37, 130.11, 127.81, 127.22, 126.91, 125.12, 120.28, 119.17, 116.75, 116.52, 66.45, 46.59. 38.75. HRMS (ESI^+) *m/z* calcd for $[M+1H]^{+1}$ $C_{26}H_{21}N_3O_4$ 440.1605 found 440.1438.

Compound 22: Compound **22a** (0.27 mmol) was dissolved in 54 ml dioxane and Na_2CO_3 (18 mmol, 1.9 g, 5 equiv.) was added together with 18 ml water to reach a concentration of 50 mM. N-(Allyloxycarbonyloxy)succinimide (4.7 mmol, 720 µl) was added dropwise to the reaction mixture. The reaction mixture was stirred at RT for 16h and acidified afterward with an aqueous solution of 5% HCl. The mixture was extracted with DCM (1 x 40ml, 2 x 20ml), the organic layers were combined, dried over $MgSO_4$, filtered through a cotton plug and evaporated to dryness. The remaining oily liquid was co-evaporated with toluene (3 × 5 ml) and further dried under high vacuum overnight. Compound **22** was obtained in 95 % yield (1.8 g) as a light yellow solid and used without further purification. ^{1}H NMR (500 MHz, DMSO-d6) δ 13.63 (s, 1H), 10.47 (s, 1H), 8.37 (s, 1H), 8.11 (d, J = 6.3 Hz, 2H), 7.93 (dt, J = 7.6, 0.9 Hz, 2H), 7.87 – 7.82 (m, 1H), 7.78 (dd, J = 7.5, 1.0 Hz, 2H), 7.70 (t, J = 8.2 Hz, 1H), 7.44 (tt, J = 7.5, 0.8 Hz, 2H), 7.36 (td, J = 7.4, 1.2 Hz, 2H), 5.95 (ddt, J = 17.2, 10.5, 5.3 Hz, 1H), 5.33 (dq, J = 17.2, 1.7 Hz, 1H), 5.21 (dq, J = 10.4, 1.5 Hz, 1H), 4.78 (d, J = 6.1 Hz, 2H), 4.62 (d, J = 6.9 Hz, 2H), 4.55 (dt, J = 5.3, 1.6 Hz, 2H), 4.45 (t, J = 6.8 Hz, 1H).13C NMR (126 MHz, DMSO) δ 165.24, 153.49, 144.85, 143.69, 142.51, 140.84, 136.51, 136.37, 130.11, 127.81, 127.22, 126.91, 125.12, 120.28, 119.17, 116.75, 116.52, 66.45, 46.59, 41.14. HRMS (ESI^-) *m/z* calcd for $[M-1H]^{-1}$ $C_{30}H_{25}N_3O_6$ 522.1671 found 522.1995.

Compound 23a: For the synthesis of compound **23a**, 2-(benzylthio)acetic acid (700 mg, 3.8 mmol) and N-hydroxysuccinimide (442 mg, 3.8 mmol) were dissolved in acetonitrile (17 ml) and a solution of dicyclohexyl-carbodiimide (783 mg, 3.8 mmol) in acetonitrile (7 ml) was added within 5 minutes. After stirring at RT for 16 h under nitrogen atmosphere, solid dicyclohexylurea was removed, by filtration and the supernatant was evaporated to dryness. The compound was purified by silica gel flash column chromatography with 25% EtOAc in cyclohexane and obtained as a colorless solid after evaporation to dryness (900 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 4H), 7.30 – 7.27 (m, 1H), 3.92 (s, 2H), 3.30 (s, 2H), 2.88 (s, 4H).

Compound 23: Compound **23** was synthesized by slowly adding compound **23a** (348 mg, 1.2 mmol) dissolved in 10 ml acetonitrile to a solution of **23** (556 mg, 1 mmol) together with Na_2CO_3 (530 mg, 5 mmol) in water (10 ml). After stirring at RT for 16 h the reaction was acidified with an aqueous solution of 5% HCl and the target compound **23** was recovered after filtration through a glass filter as a light yellow powder (490 mg, 81%). ^{1}H NMR (500 MHz, DMSO-d6) δ 13.61 (s, 1H), 10.49 (s, 1H), 8.82 (t, J = 6.0 Hz, 1H), 8.37 (s, 1H), 8.17 (s, 1H), 7.93 (dt, J = 7.6, 1.0 Hz, 2H), 7.87 (dd, J = 8.4, 1.2 Hz, 1H), 7.78 (dd, J = 7.5, 1.0 Hz, 2H), 7.72 (t, J = 8.2 Hz, 1H), 7.44 (tt, J = 7.5, 0.8 Hz, 2H), 7.36 (td, J = 7.4, 1.2 Hz, 2H), 7.31 (d, J = 4.2 Hz, 5H), 4.86 (d, J = 5.8 Hz, 2H), 4.63 (d, J = 6.9 Hz, 2H), 4.46 (t, J = 6.8 Hz, 1H), 3.82 (s, 2H), 3.18 (s, 2H). 13C NMR (126 MHz, DMSO-d6) δ 169.82, 165.91, 153.98, 148.09, 145.49, 144.19, 141.30, 138.36, 137.08, 136.75, 130.08, 129.47, 128.84, 128.27, 127.84, 127.71, 127.40, 125.63, 120.74, 118.71, 117.20, 116.60, 66.91, 47.07, 40.13, 36.10, 34.37. HRMS (ESI^+) *m/z* calcd for $[M+H]^{+1}$ $C_{35}H_{30}N_3O_5S$ 604.1901 found 604.1876.

2.6.5. Hybrid sequence synthesis and macrocyclizations reactions



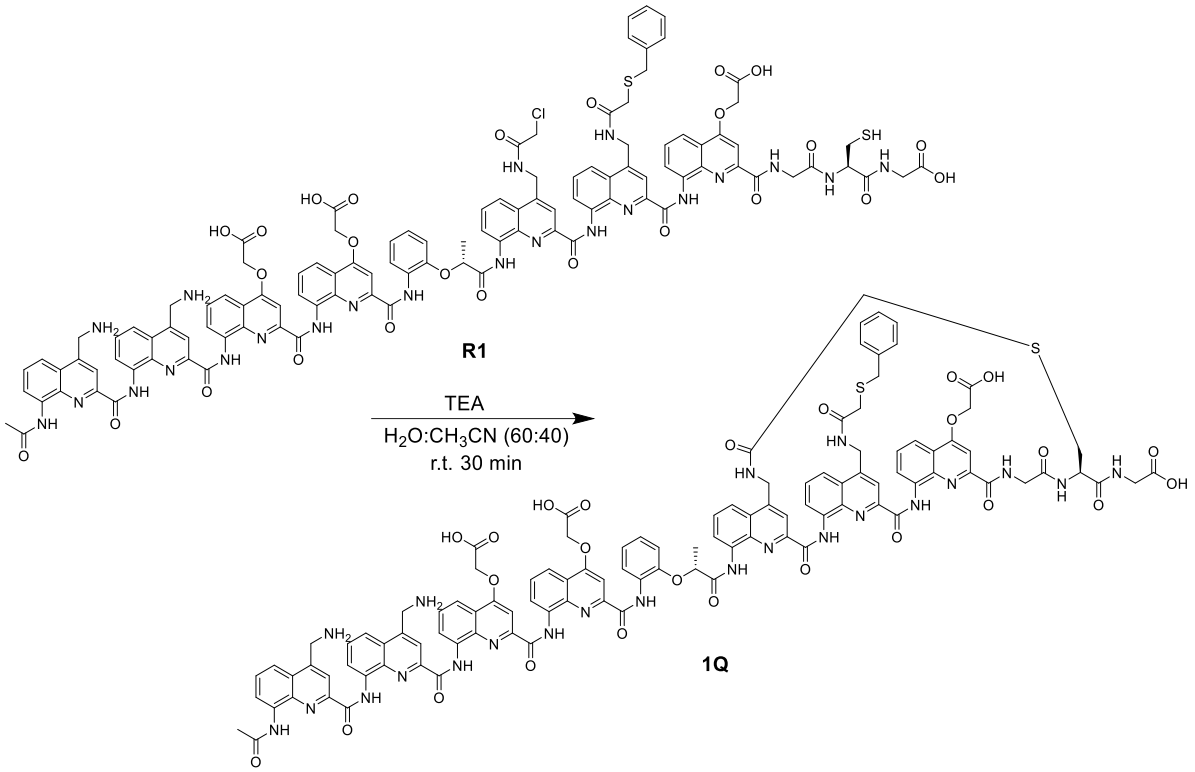
Compound 1: Foldamer-peptide **1** was built on a 25 µmol scale using Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting groups were removed, and directly followed by the chloroacetylation step. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (30 mg, 51%, Figure S2a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 40% solvent B over 15 minutes at 25°C) to give **1** in 14% yield (

Figure S2 – RP-HPLC analysis of the synthesis, purification and cyclization of sequence 1. Overlay of RP-HPLC profiles measured from each synthetic step of compound 1Q synthesized from compound 1 measured with standard HPLC conditions. Compound 1 after SPS/SPFS as a crude (a), after purification co-injected with internal standard 21(b), after cyclization and co-injected with 21 (1C, c) and after benzyl mercaptan installation (1Q, d). Chromatograms a), c) and d) obtained after the reaction without prior purification. a) and d) recorded at 300 nm, b) and c) at 375 nm**.** HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{102}H_{88}Cl_2N_{22}O_{25}S$ $[M+2H]^{2+}$ 1062.2692 found 1062.2833

Compound 1C: After lyophilization, compound **1** (8 mg) was dissolved in a 1:1 mixture of acetonitrile in water (38 ml total) to reach a 0.1 mM concentration and the solvent was freed from oxygen. Freshly

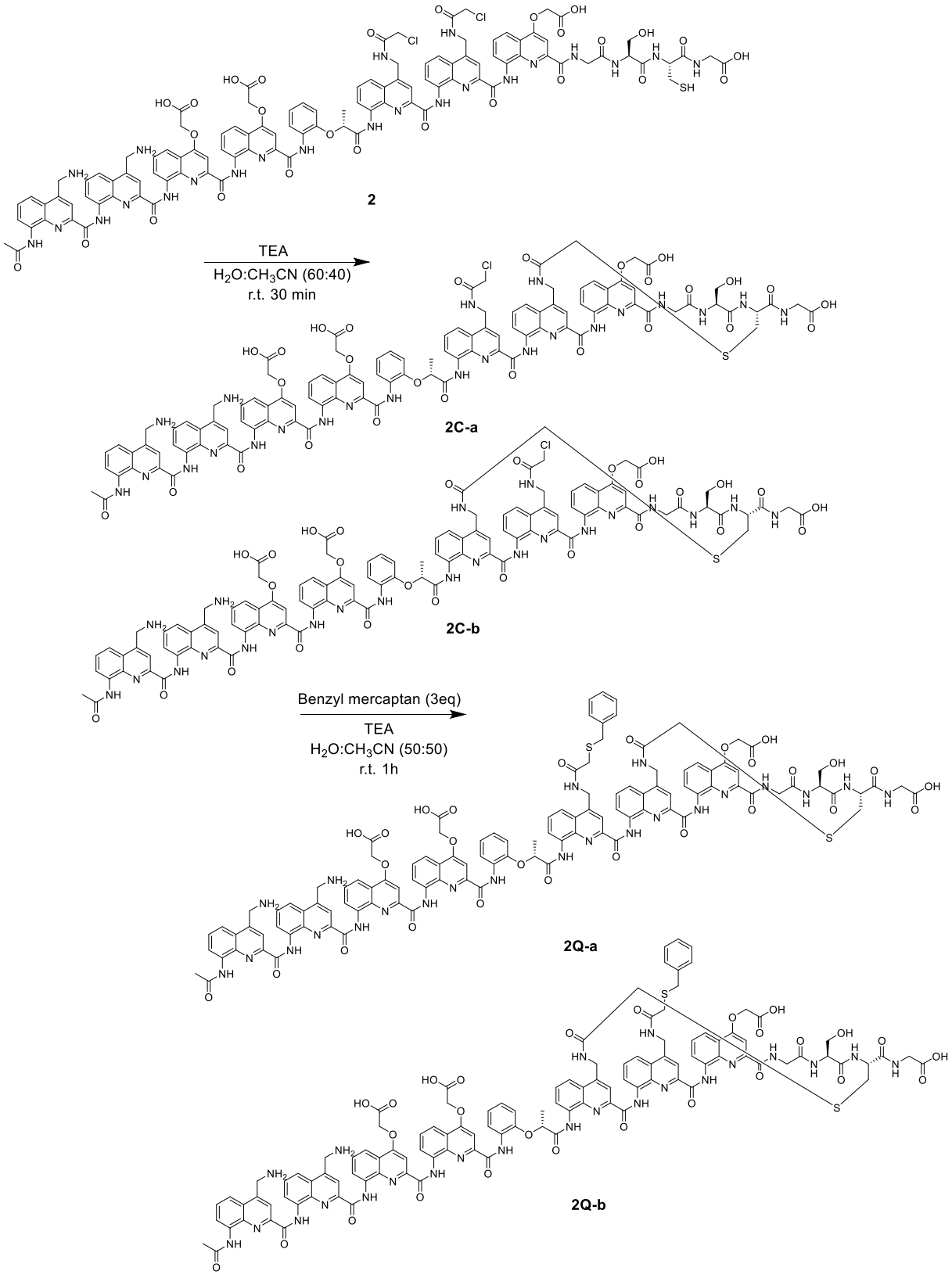
distilled TEA (1.32 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 30 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S2c). $C_{102}H_{86}ClN_{22}O_{25}S$ $[M+H]^+$ 2086.5564 found 2086.5457

Compound 1Q: Compound **1C** (7 mg) was dissolved in a 1:1 mixture of acetonitrile in water (0.1 ml total) to reach a 50 mM concentration and TEA (2.8 µl, 6 equiv.) was added. While stirring, benzyl mercaptan (1.2 µl, 3 equiv.) was added and the reaction mixture was stirred at RT for 60 minutes. Solvents were removed, by lyophilization and the colorless product **1Q** (1.6 mg, Figure S2d) was obtained after semi-preparative RP-RP-HPLC purification using a gradient from 15% to 30% solvent D. ^{1}H NMR (500 MHz,CD_3CN) δ 11.67 (s, 1H), 11.10 (s, 1H), 10.88 (s, 1H), 10.64 (s, 1H), 10.44 (s, 1H), 9.85 (s, 1H), 9.24 (d, *J* = 6.9 Hz, 1H), 9.06 (s, 1H), 8.67 (d, *J* = 9.9 Hz, 2H), 8.56 (d, *J* = 7.0 Hz, 1H), 8.36 (q, *J* = 8.1, 7.2 Hz, 2H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.98 (s, 1H), 7.92 (d, *J* = 8.9 Hz, 2H), 7.87 (d, *J* = 6.8 Hz, 2H), 7.76 (t, *J* = 9.2 Hz, 4H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.58 (s, 1H), 7.54 (t, *J* = 6.1 Hz, 2H), 7.49 (t, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 8.7 Hz, 5H), 7.35 (dd, *J* = 16.5, 7.7 Hz, 6H), 7.26 (dd, *J* = 11.8, 6.8 Hz, 2H), 7.11 (t, *J* = 7.7 Hz, 1H), 7.04 (dd, *J* = 16.8, 7.8 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 1H), 6.74 (d, *J* = 7.4 Hz, 1H), 6.57 (s, 1H), 6.51 (s, 1H), 6.28 (d, *J* = 12.0 Hz, 2H), 6.08 (d, *J* = 8.4 Hz, 1H), 5.87 (t, *J* = 8.5 Hz, 1H), 5.01 (td, *J* = 18.2, 17.1, 7.8 Hz, 1H), 4.90 – 4.56 (m, 2H), 3.67 – 3.49 (m, 1H), 3.45 – 3.20 (m, 7H), 3.13 (q, *J* = 8.0 Hz, 1H), 1.41 (s, 4H), 1.35 – 1.19 (m, 3H), -0.60 (d, *J* = 6.7 Hz, 3H). HRMS (ESI^-) *m/z* calcd (most abundant mass peak) for $C_{109}H_{91}N_{22}O_{25}S_2$ $[M-H]^-$ 2172.5992 found 2172.6627.



Compound R1: Foldamer-peptide **R1** was on a 25 µmol scale using the low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting group was removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (61 mg, 99%, Figure S3a) was purified by semi-preparative RP-HPLC (gradient: from 30% to 50% solvent B over 15 minutes at 25°C, Figure S4b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{109}H_{94}ClN_{22}O_{25}S_2$ $[M+H]^+$ 2210.5918 found 2210.5231.

Compound 1Q: After lyophilization, compound **R1** (13 mg) was dissolved in a 1:1 mixture of acetonitrile in water (54 ml total) to a reach 0.1 mM concentration and the solvent was freed from oxygen. TEA (1.9 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 20min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. 7.3 mg light yellow product was obtained after purification by semi-preparative RP-HPLC (gradient: from 20% to 80% solvent B over 15 minutes at 25°C, Figure S3d). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{109}H_{94}N_{22}O_{25}S_2$ $[M+2H]^{2+}$ 1087.8112 found 1087.8214.

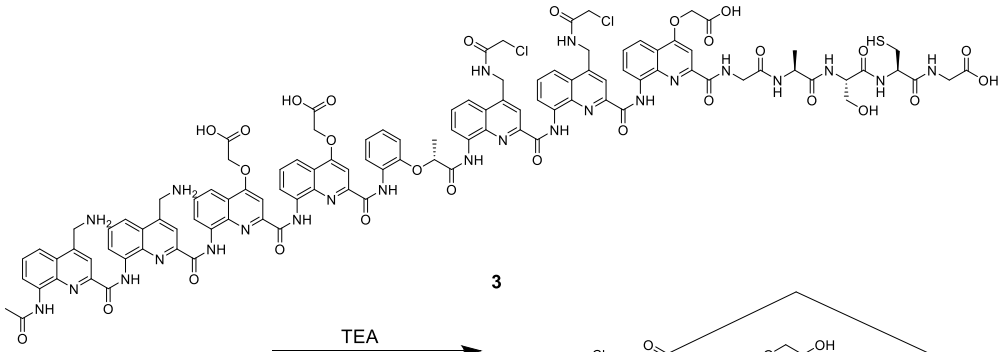


Compound 2: Foldamer-peptide **2** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting group was removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT

(92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (50 mg, 82%, see Figure S4a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 40% solvent B over 15 minutes at 25°C, see Figure S4b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{105}H_{92}Cl_2N_{23}O_{27}S$ $[M+H]^+$ 2210.5676 found 2210.4959

Compound 2C: After lyophilization, compound **2** (14 mg) was dissolved in a mixture of acetonitrile (25 ml) in water (38 ml) to reach a 0.1 mM concentration and the solvent was freed from oxygen. TEA (2.1 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 30 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S4c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{105}H_{91}ClN_{23}O_{27}S$ $[M+H]^+$ 2173.6137 found 2173.5875

Compound 2Q: Compound **2C** (14 mg) was dissolved in a 1:1 mixture of acetonitrile in water (0.13 ml total) to reach 50 mM concentration and TEA (5 µl, 6 equiv.) was added. While stirring, benzyl mercaptan (2.0 µl, 3 equiv.) was added and the reaction mixture was stirred at RT for 60 minutes. Solvents were removed, by lyophilization and the two conformational isomers were isolated by semi-preparative RP-RP-HPLC with a gradient from 15% to 35% solvent D (see Figure S4e and S4f). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{112}H_{98}N_{23}O_{27}S_2$ $[M+H]^+$ 2261.6490 found 2261.7231 for isomer (**2Q-a**) and 2261.7280 for isomer (**2Q-b**).



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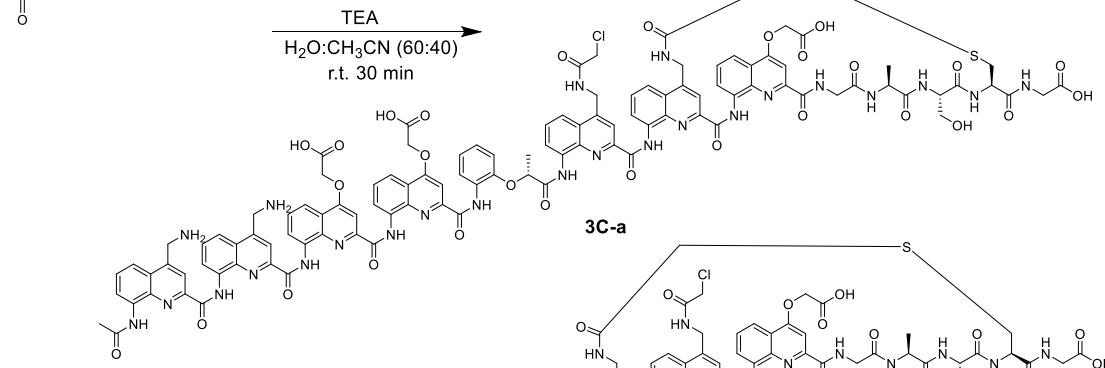
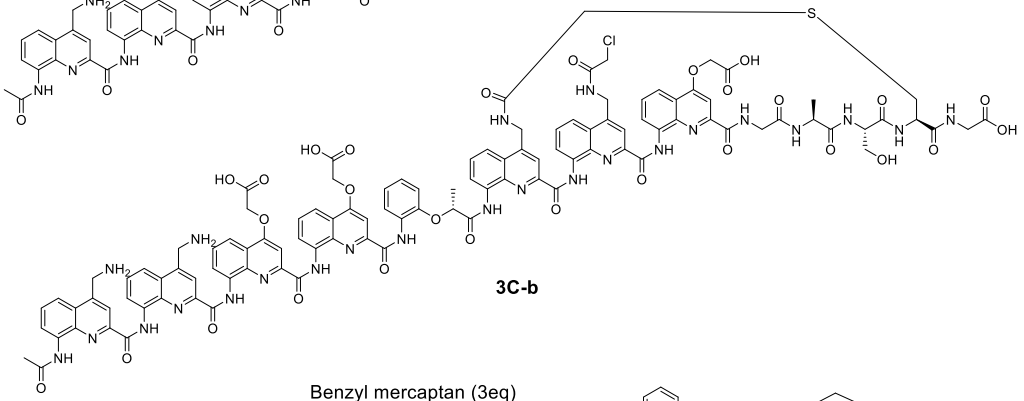
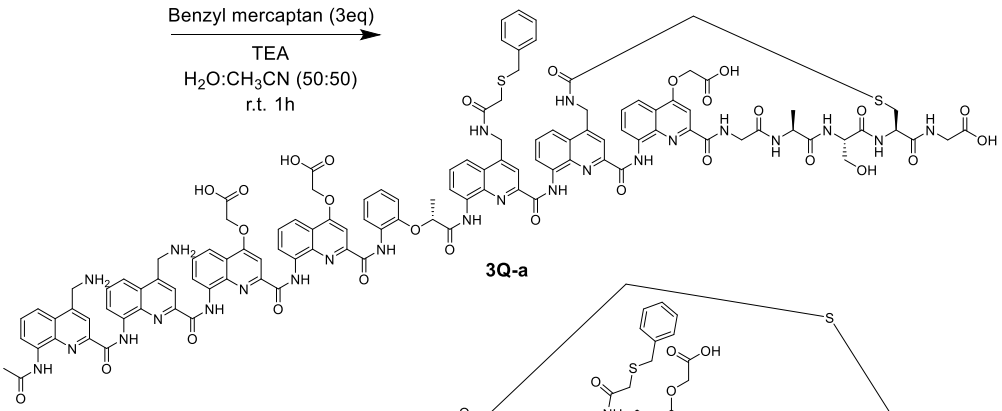


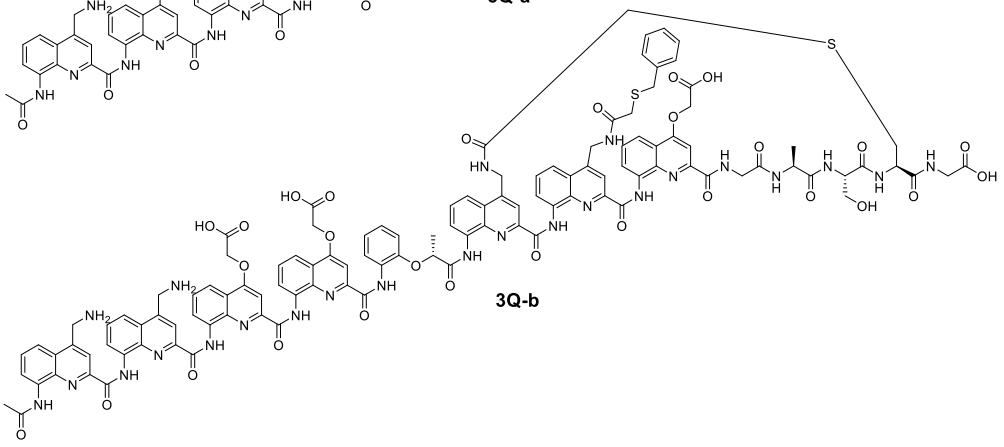
3C-a



3C-b



3Q-a

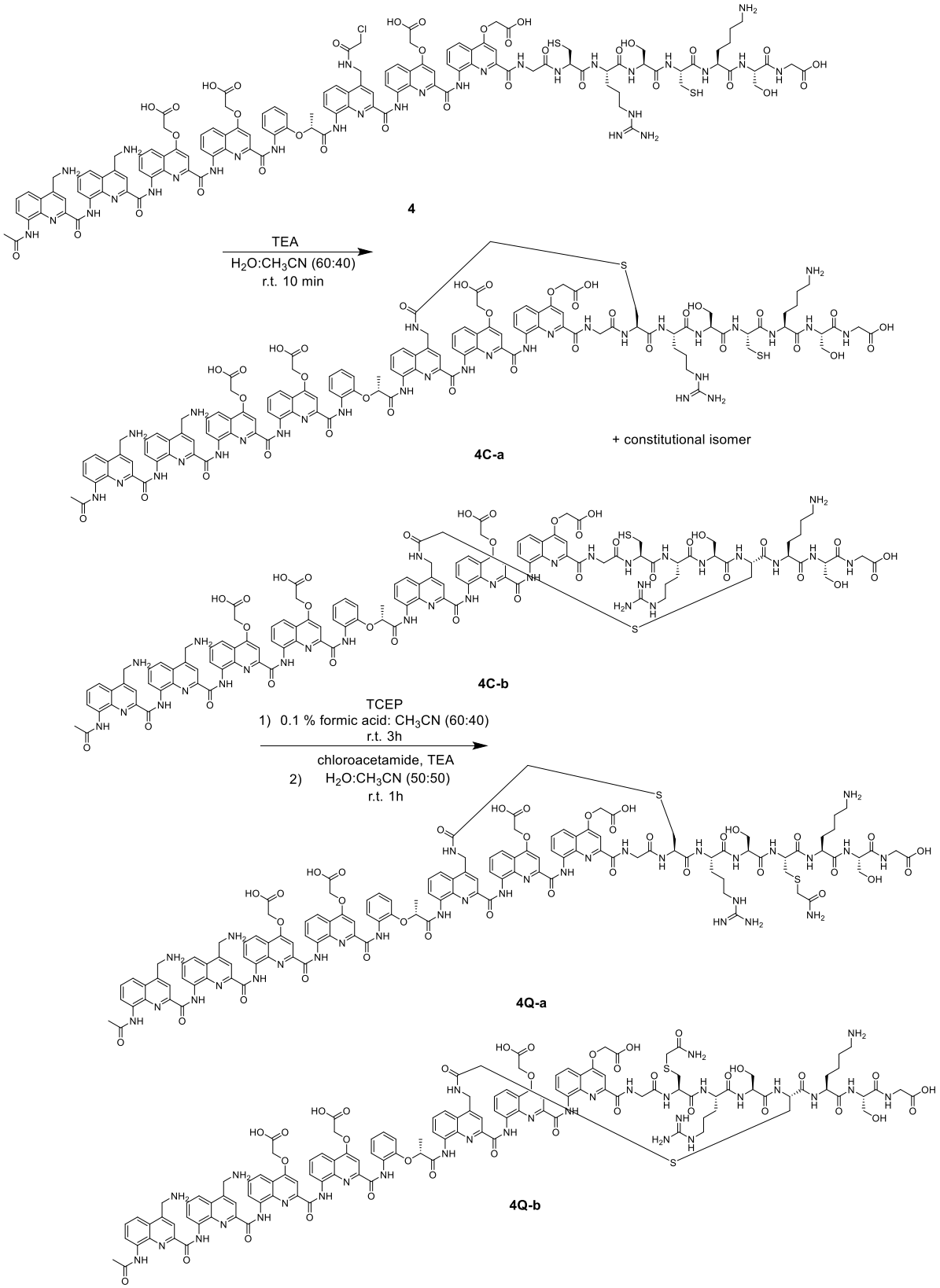


3Q-b

Compound 3: Foldamer-peptide **3** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting group was removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (52 mg, 85%, Figure S5a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 40% solvent B over 15 minutes at 25°C, Figure S5b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{108}H_{97}Cl_2N_{24}O_{28}S$ $[M+H]^+$ 2281.5348 found 2281.6053.

Compound **3C:** After lyophilization, compound **3** (13 mg) was dissolved in mixture of acetonitrile (34 ml) in water (22 ml) to reach 0.1 mM concentration and the reaction mixture was freed from oxygen. TEA (1.9 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 30 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S5c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{108}H_{96}ClN_{24}O_{28}S$ $[M+H]^+$ 2244.6490 found 2244.6337.

Compound **3Q:** Compound **3C** (14 mg) was dissolved in a 1:1 mixture of acetonitrile in water (0.12 ml total) to reach 50 mM concentration and TEA (5 µl, 6 equiv.) was added. While stirring, benzyl mercaptan (2.0 µl, 3 equiv.) was added and the reaction mixture was stirred at RT for 60 minutes. Solvents were removed, by lyophilization and the two conformational isomers were isolated separately after using semi-preparative RP-RP-HPLC with a gradient from 15% to 35% solvent D (Figure S5e and S5f). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{115}H_{103}N_{24}O_{28}S_2$ $[M+H]^+$ 2332.6907 found 2332.7634 for isomer (**3Q-a**) and 2332.7661 for isomer (**3Q-b**).

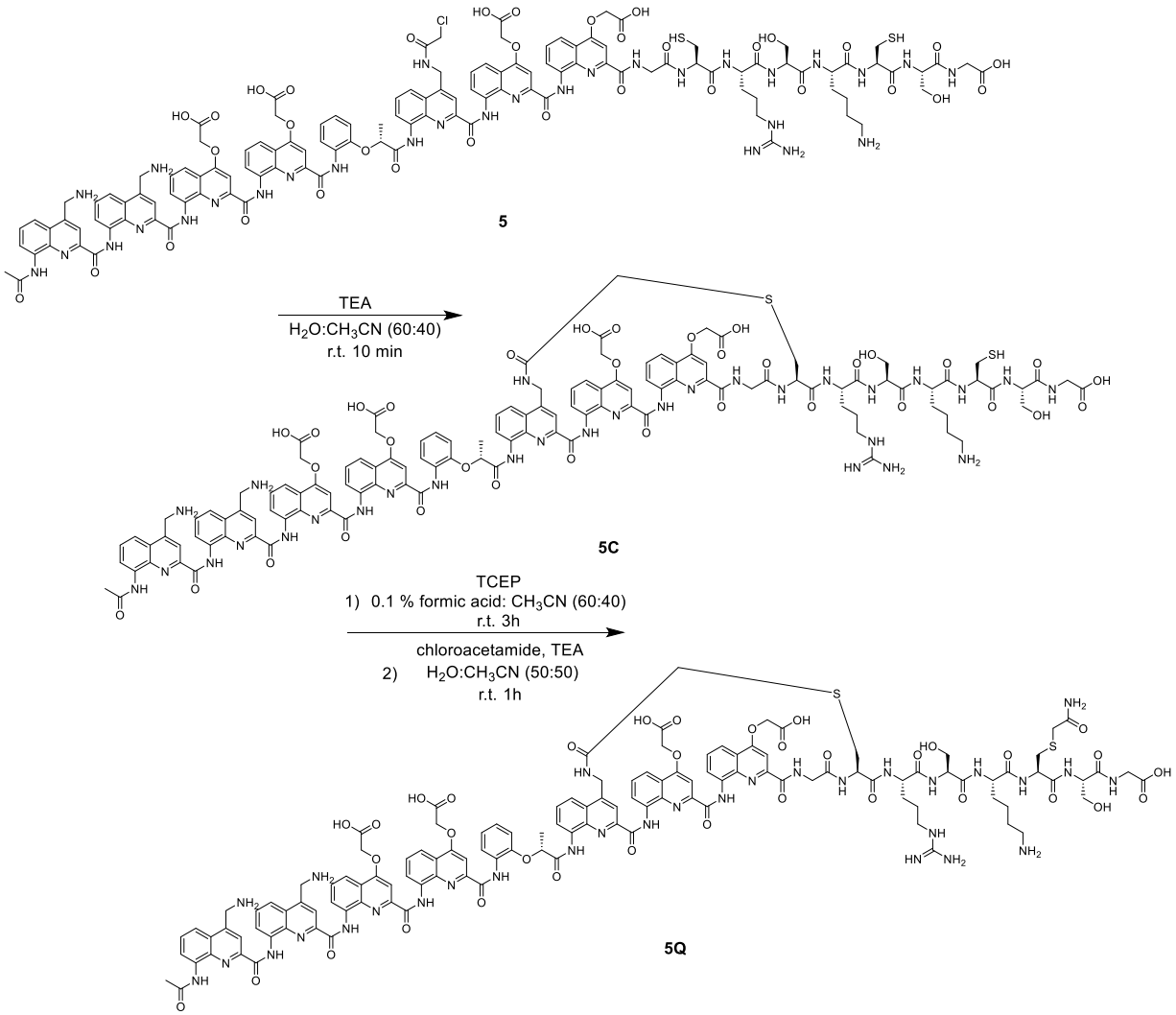


Compound 4: Foldamer-peptide **4** was built on a 25 µmol scale using a Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting group was removed, directly

followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (68 mg, 87%, Figure S6a) was purified by semi-preparative RP-HPLC (gradient: from 15% to 30% solvent B over 8 minutes at 25°C, Figure S6b) to afford 28 mg of pure compound. HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{122}H_{124}ClN_{30}O_{34}S_2$ $[M+H]^+$ 2652.8055 found 2652.8290

Compound 4C: After lyophilization, compound **4** (12 mg) was dissolved in a mixture of acetonitrile (18 ml) in water (27ml) to a reach 0.1 mM concentration and the solvent was freed from oxygen by Freeze-Pump-Thaw. TEA (1.5 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 60 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S6c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{122}H_{123}N_{30}O_{34}S_2$ $[M+H]^+$ 2616.8288 found 2616.8594

Compound 4Q: To reduce inter molecular disulfides, compound **4C** (3.7 mg) was dissolved in a mixture of acetonitrile (0.28 ml) in water (0.42 ml, 0.1% formic acid) to reach a 2 mM concentration and TCEP (141 µl, 10mM, 1 equiv.) was added. The reaction mixture was stirred at RT and the progress of the reaction was monitored by RP-HPLC. The reaction was completed after 3h, chloroacetamide (280 µl, 10 mM, 2 equiv.) was added and the solution was basified with TEA (10 µl). The progression was monitored by RP-HPLC and completed after 6h (Figure S6d and S6e). Solvents were removed, by lyophilization. HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{124}H_{127}N_{31}O_{35}S_2$ $[M+2H]^{2+}$ 1337.4288 found 1337.4685.

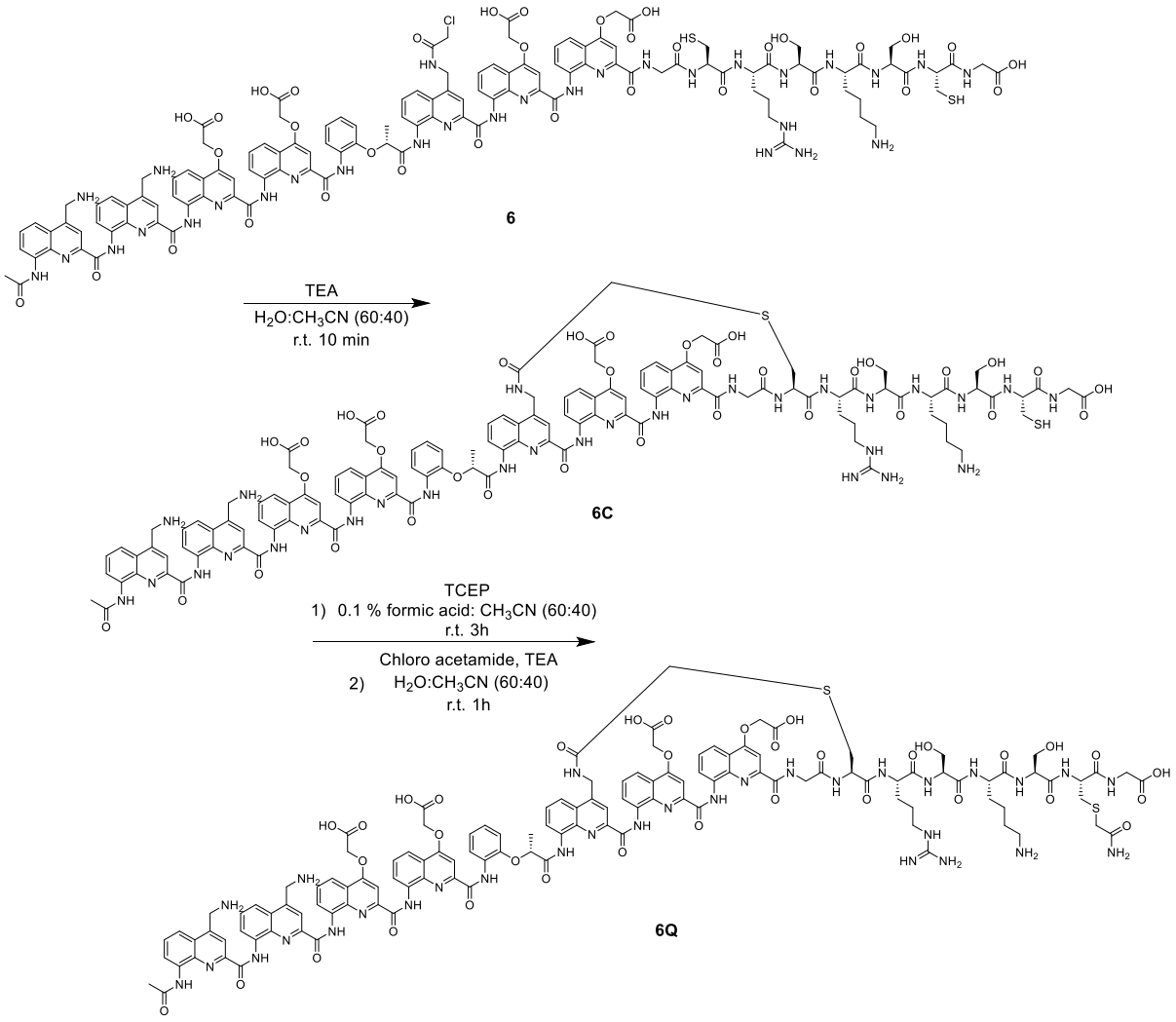


Compound 5: Foldamer-peptide **5** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting group was removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (70 mg, 90%, Figure S7a) was purified by semi-preparative RP-HPLC (gradient: from 15% to 30% solvent B over 8 minutes at 25°C, Figure S7b) to afford 18 mg of pure compound. HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{122}H_{124}ClN_{30}O_{34}S_2$ $[M+H]^+$ 2652.8055 found 2652.8274.

Compound 5C: After lyophilization, compound **5** (9 mg) was dissolved in a mixture of acetonitrile (14 ml) in water (20 ml) to reach a 0.1 mM concentration and the solvent mixture was freed from oxygen. TEA (1.2 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 30 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S7c).

HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{122}H_{123}N_{30}O_{34}S_2$ $[M+H]^+$ 2616.8288 found 2616.8458.

Compound **5Q:** To reduce inter molecular disulfides, compound **5C** (1.3 mg) was dissolved in a mixture of acetonitrile (0.2 ml) in water (0.5 ml, 0.1% formic acid) to reach a 0.5 mM concentration and TCEP (38 µl, 10mM, 1 equiv.) was added. The reaction mixture was stirred at RT and the progress of the reaction was monitored by RP-HPLC. The reaction was completed after 3h, chloroacetamide (45 µl, 10 mM, 2 eq) was added and the solution was basified with TEA (10 µl). The progression was monitored by RP-HPLC and was finished after 3h. Solvents were removed, by lyophilisation (Figure S7e). HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{124}H_{127}N_{31}O_{35}S_2$ $[M+2H]^{2+}$ 1337.4288 found 1337.4722.

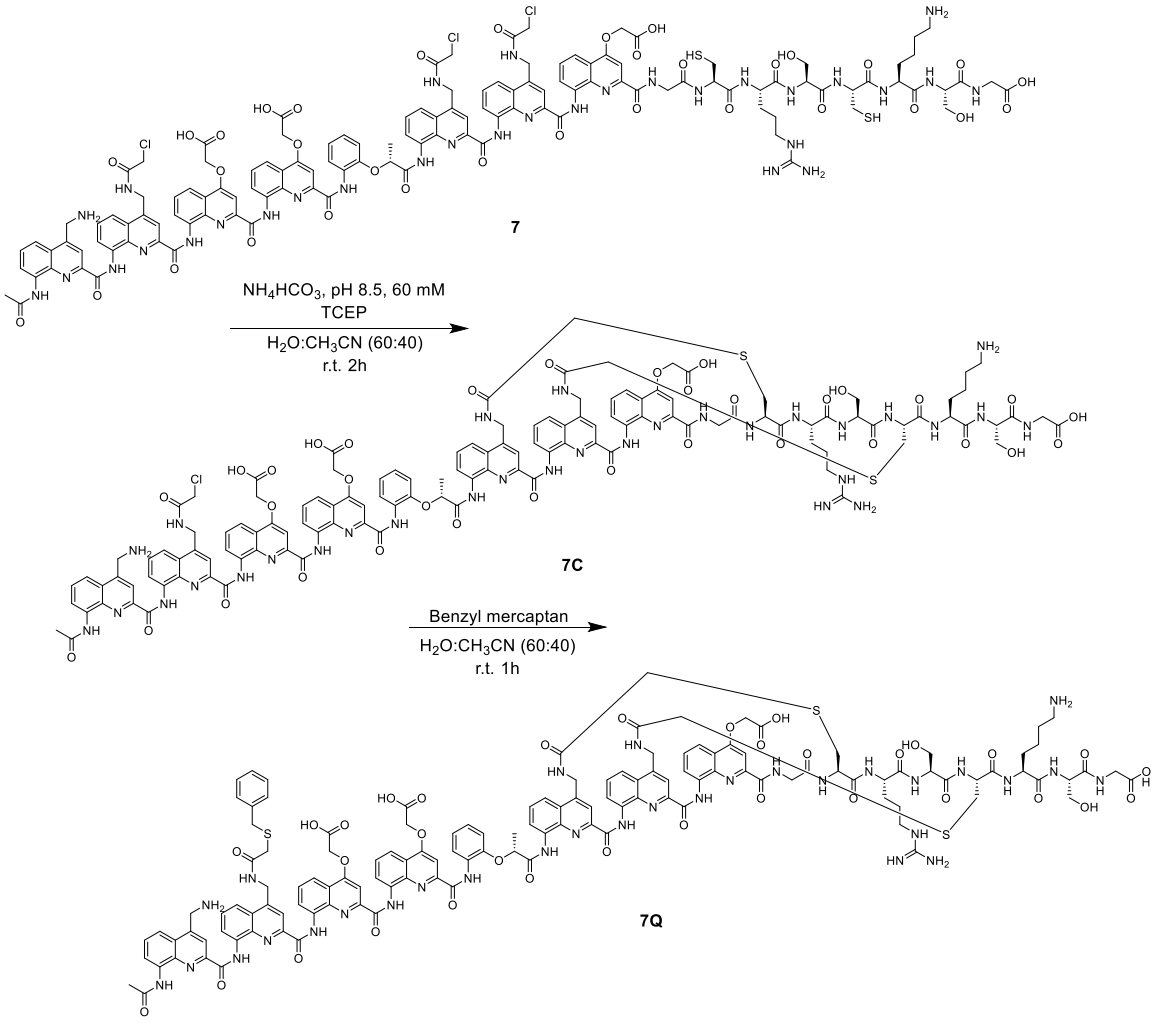


Compound 6: Foldamer-peptide **6** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting group was removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (69 mg, 88%, Figure S8a) was purified by semi-

preparative RP-HPLC (gradient: from 15% to 30% solvent B over 8 minutes at 25°C) to afford 21 mg of pure compound (Figure S8b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{122}H_{124}ClN_{30}O_{34}S_2$ $[M+H]^+$ 2652.8055 found 2652.8218

Compound 6C: After lyophilization, compound **6** (13.5 mg) was dissolved in a mixture of acetonitrile (21 ml) in water (30 ml) to reach a 0.1 mM concentration and the solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Freshly distilled TEA (1.8 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 30 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S8c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{122}H_{123}N_{30}O_{34}S_2$ $[M+H]^+$ 2616.8288 found 2616.8203.

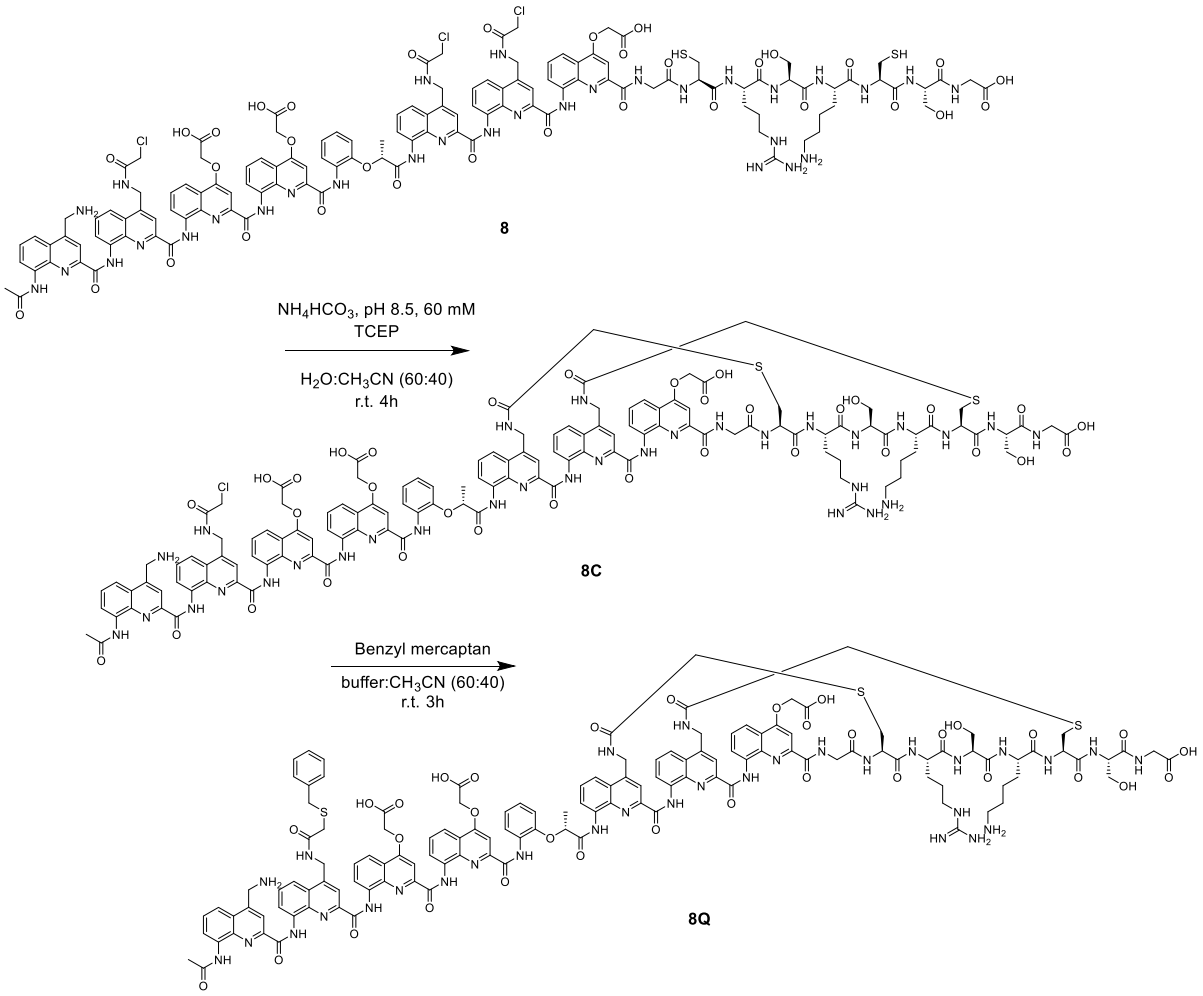
Compound 6Q: To reduce intermolecular disulfides, compound **6C** (3.2 mg) was dissolved in a mixture of acetonitrile (0.24 ml) in water (0.37 ml, 0.1% formic acid) to reach a 2 mM concentration and TCEP (122 µl, 10mM, 1 equiv.) was added. The reaction mixture was stirred at RT and the progress of the reaction was monitored by RP-HPLC. The reaction was completed after 3h, chloroacetamide (120 µl, 10 mM, 2 eq) was added and the solution was basified with TEA (10 µl). The progression was monitored by RP-HPLC and was completed after 3h. Solvents were removed, by lyophilisation (Figure S8e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{124}H_{127}N_{31}O_{35}S_2$ $[M+2H]^{2+}$ 1337.4288 found 1337.4499.



Compound 7: Foldamer-peptide **7** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (64 mg, 82%, Figure S9a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 8 minutes at 25°C) to afford 6 mg of pure compound (Figure S9b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{125}H_{127}Cl_3N_{31}O_{33}S_2$ $[M+H]^+$ 2760.7752 found 2760.7947

Compound 7C: After lyophilization, compound **7** (1 mg) was dissolved in a mixture of acetonitrile (2.4 ml) in water (1.6 ml) to reach a 0.1 mM concentration and TCEP stock solution (4 µl, 10 mM, 1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solvent mixture of NH_4HCO_3 (2.4 ml, 60 mM, pH 8.5) in acetonitrile(1.6 ml) was prepared and oxygen removed, by Freeze-Pump-Thaw. The Foldamer solution was added via syringe to NH_4HCO_3 solution under N_2 atmosphere. The mixture was quickly stirred and left without stirring for 2 h at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 2 h, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S9c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{125}H_{126}ClN_{31}O_{33}S_2$ $[M+2H]^{2+}$ 1344.9144 found 1344.9769.

Compound 7Q: Compound **7C** (1 mg) was dissolved in a 1:1 mixture of NH_4HCO_3 (60 mM, pH 8.5) in acetonitrile (1 ml total) and benzylmercaptan (10.0 µl) was added. The reaction mixture was stirred at RT for 3 h. Solvents were removed by lyophilization. Pure compound was isolated after semi-preparative RP-RP-HPLC purification with a gradient from 25% to 45% solvent B over 10 min at RT (Figure S9e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{131}H_{131}N_{31}O_{33}S_3$ $[M+2H]^{2+}$ 1388.4431 found 1388.5030.

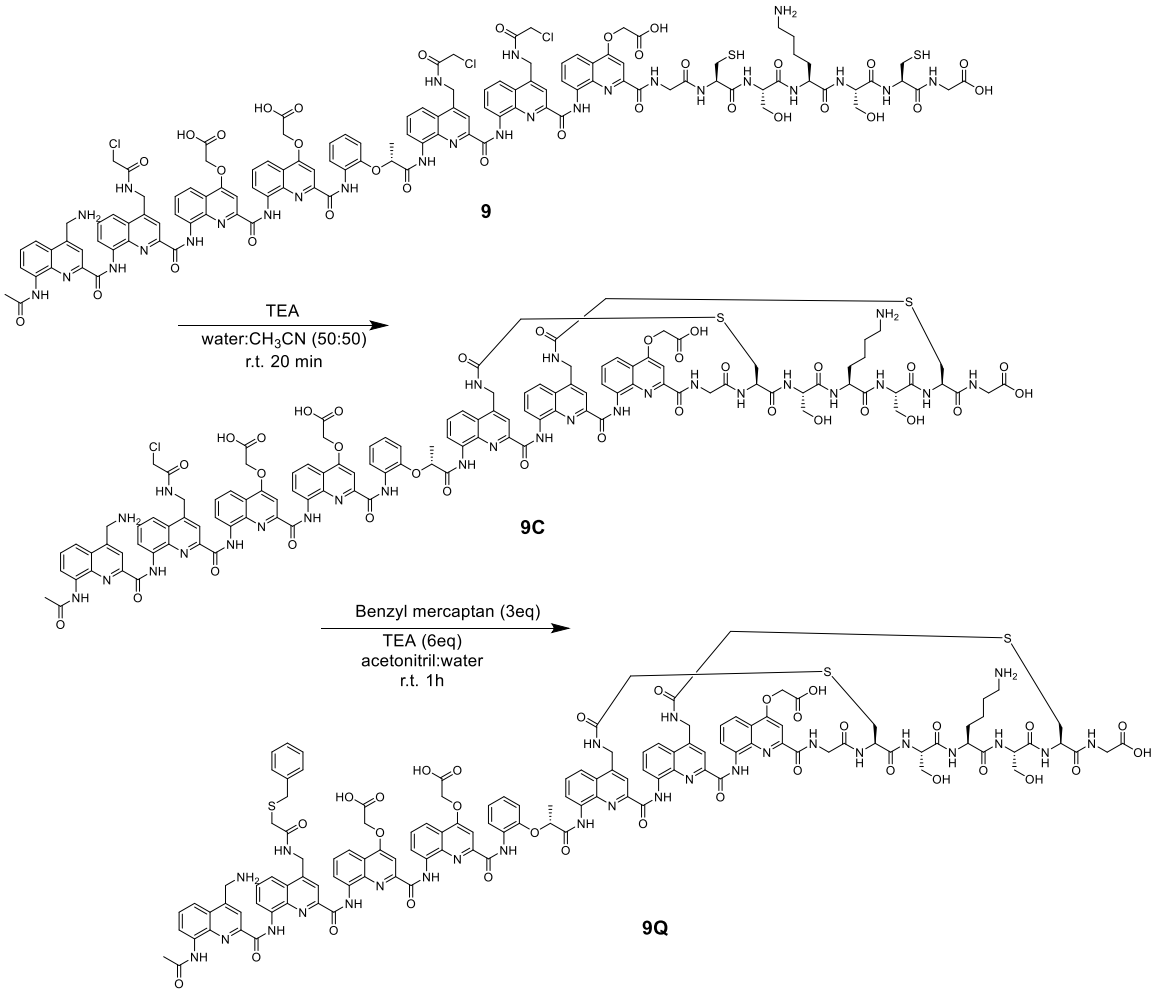


Compound 8: Foldamer-peptide **8** was built on a 25 µmol scale using a Low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (60 mg, 77%, Figure S10a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 8 minutes at 25°C) to afford 6 mg of pure compound (Figure S10b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{125}H_{127}Cl_3N_{31}O_{33}S_2$ $[M+H]^+$ 2760.7752 found 2760.8162.

Compound 8C: After lyophilization, compound **8** (3 mg) was dissolved in a mixture of acetonitrile (4 ml) in water (6.0 ml) to reach a 0.1 mM concentration and TCEP stock solution (10 µl, 10 mM, 1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw.

Independently, a solution of NH_4HCO_3 (6 ml, 60 mM, pH 8.5) in acetonitrile (4 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 4 h at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 4 h, the reaction mixture was quenched with TFA and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S10c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{125}H_{126}ClN_{31}O_{33}S_2$ $[M+2H]^{2+}$ 1344.9144 found 1345.0008.

Compound 8Q: Compound **8C** (3 mg) was dissolved in mixture of NH_4HCO_3 buffer (0.15 ml, 60 mM, pH 8.5) in acetonitrile (0.1 ml) and benzyl mercaptan (1 µl) was added. The reaction mixture was stirred at RT for 3 h. Solvents were removed, by lyophilization. Pure compound was isolated after using semi-preparative RP-RP-HPLC with a gradient from 25% to 45% solvent B over 10 min at RT (Figure S10e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{131}H_{131}N_{31}O_{33}S_3$ $[M+2H]^{2+}$ 1388.4431 found 1388.5030.

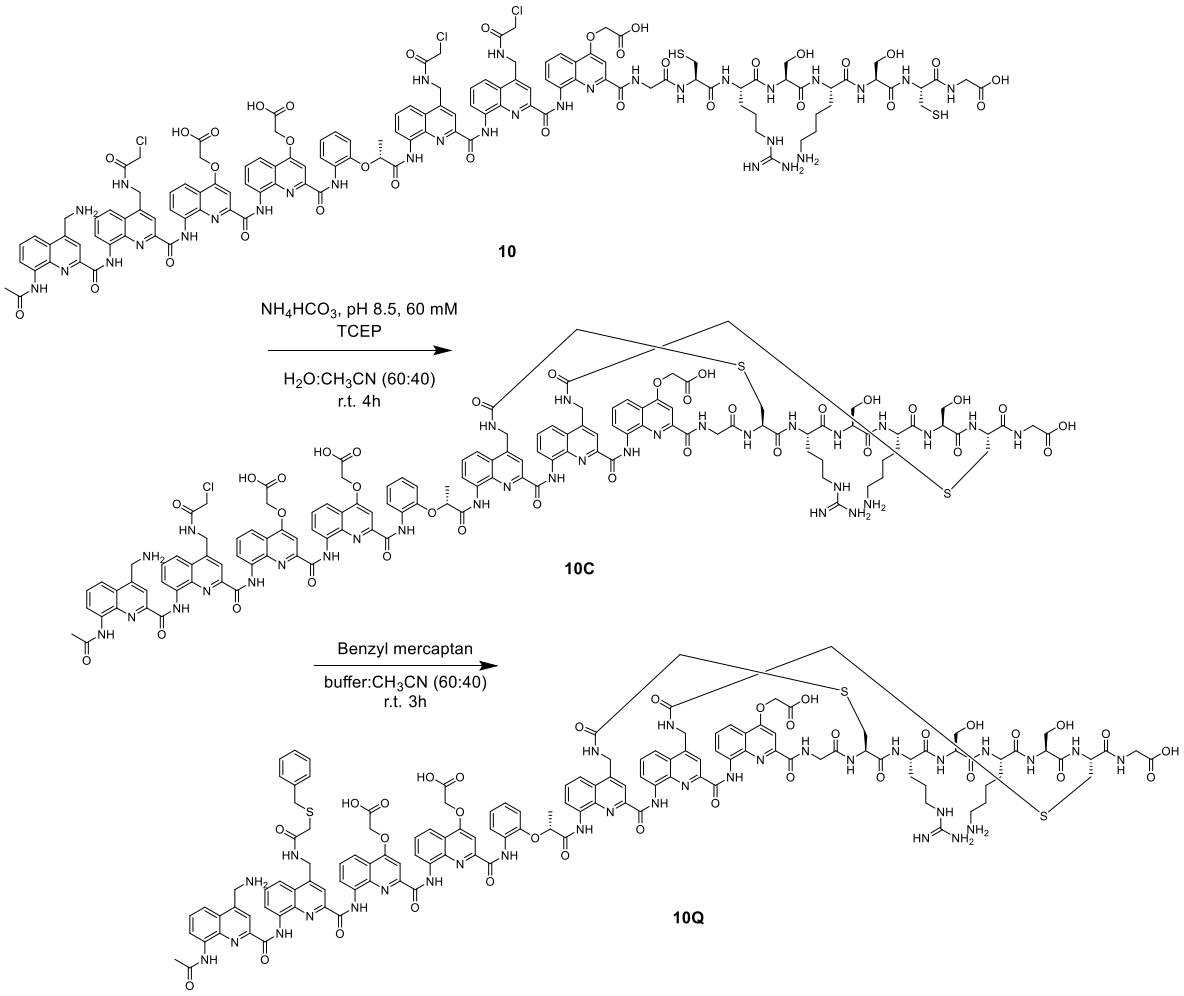


Compound 9: Foldamer-peptide **9** was built by relying on the CEM Liberty Blue microwave peptide synthesizer for the peptide segment and on the Liberty Bio for the stepwise assembly of the foldamer part on a 25 µmol scale using a Low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting groups were removed, directly followed by chloroacetylation.

The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (38 mg, 50%, Figure S11a) was purified by semi-preparative RP-HPLC (gradient: from 35% to 45% solvent B over 15 minutes at 25°C (Figure S11b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{119}H_{115}Cl_3N_{27}O_{32}S_2$ $[M+H]^+$ 2604.6709 found 2604.7563

Compound 9C: After lyophilization, compound **9** (8 mg) was dissolved in a 1:1 mixture of acetonitrile in water (30 ml total) to reach a 0.1 mM concentration and the solvent mixture was freed from oxygen. TEA (1.0 ml, 0.25 mM) was added and the reaction was kept under nitrogen atmosphere without stirring. The progression of the macrocyclization was monitored by RP-HPLC. After 30 min, the reaction mixture was concentrated under reduced pressure and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S11c). $C_{119}H_{113}ClN_{27}O_{32}S_2$ $[M+H]^+$ 2531.7202 found 2531.7897.

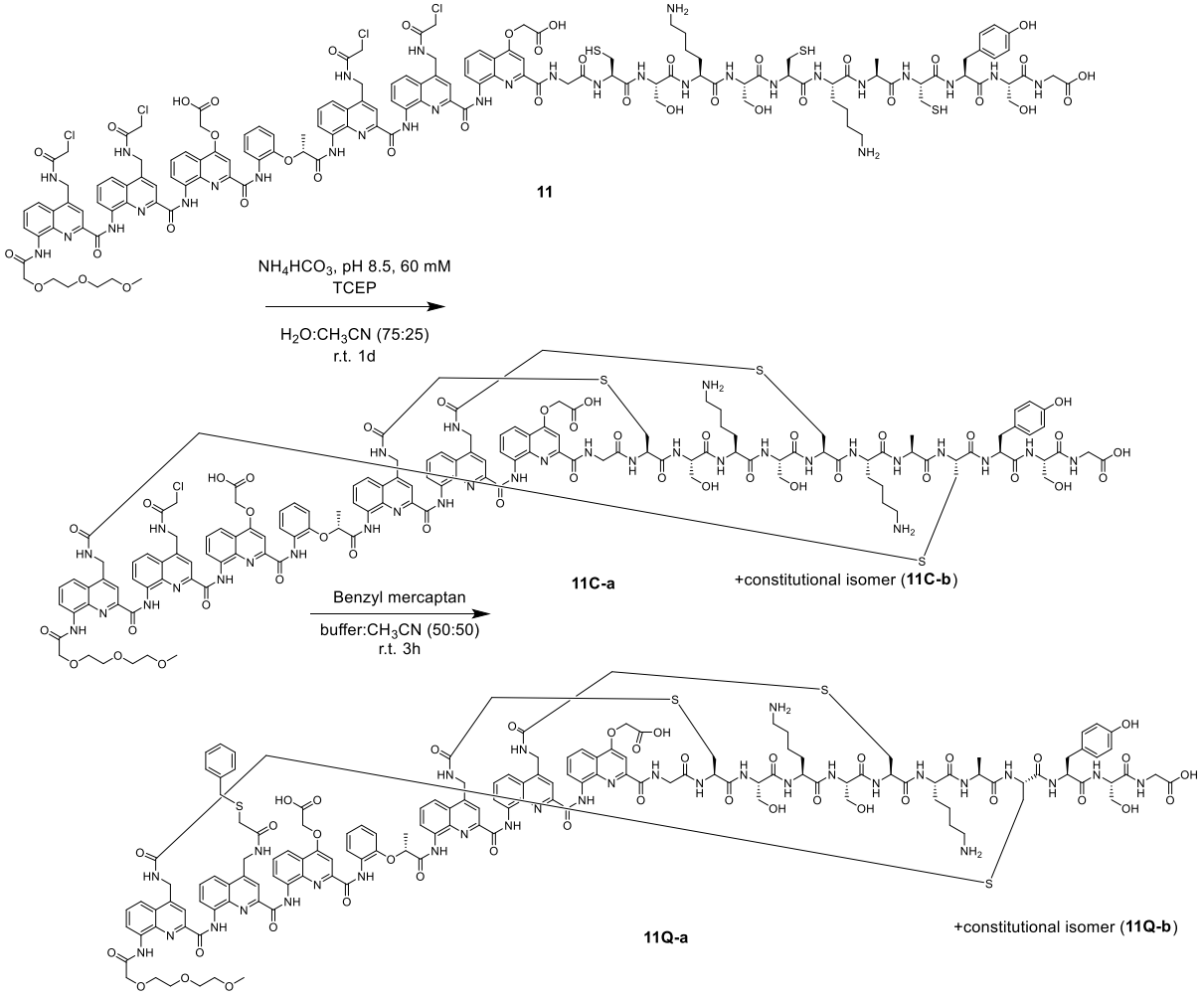
Compound 9Q: 9C (7 mg) was dissolved in 1:1 mixture of acetonitrile in water (0.15 ml total) to reach a 50 mM concentration and TEA (3 µl, 6 equiv.) was added. While stirring, benzylmercaptan (1.0 µl, 3 equiv.) was added and the reaction mixture was stirred at RT for 60 minutes. Solvents were removed, by lyophilization and the colorless product (3.0 mg) was obtained after semi-preparative RP-HPLC purification with a gradient from 15% to 30% solvent D (Figure S11e). ^1H NMR (500 MHz, Acetonitrile-d_3) δ 11.70 (s, 1H), 11.23 (s, 1H), 10.69 (s, 1H), 10.42 (d, *J* = 5.2 Hz, 2H), 9.63 (s, 1H), 9.50 (s, 1H), 9.08 (s, 1H), 8.73 (s, 1H), 8.73 – 8.66 (m, 2H), 8.52 (d, *J* = 8.6 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 8.32 (s, 1H), 8.23 (d, *J* = 5.8 Hz, 1H), 8.11 (s, 1H), 8.05 (d, *J* = 9.1 Hz, 1H), 8.03 – 7.98 (m, 2H), 7.96 (t, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 9.1 Hz, 1H), 7.89 – 7.83 (m, 2H), 7.77 (d, *J* = 8.9 Hz, 1H), 7.74 (s, 2H), 7.71 (s, 1H), 7.66 (dd, *J* = 13.3, 8.1 Hz, 4H), 7.59 (s, 2H), 7.55 (d, *J* = 11.6 Hz, 2H), 7.53 – 7.45 (m, 3H), 7.42 (d, *J* = 4.9 Hz, 6H), 7.38 (s, 1H), 7.31 (t, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 2H), 7.13 (t, *J* = 8.3 Hz, 2H), 7.07 (s, 1H), 6.96 (d, *J* = 9.0 Hz, 1H), 6.90 (s, 1H), 6.66 (s, 1H), 6.62 (s, 1H), 6.53 (s, 1H), 6.43 (s, 1H), 6.28 (t, *J* = 8.0 Hz, 1H), 6.15 (d, *J* = 8.3 Hz, 1H), 5.80 (t, *J* = 8.2 Hz, 1H), 5.24 (t, *J* = 10.4 Hz, 1H), 5.06 (dd, *J* = 17.3, 7.9 Hz, 1H), 4.93 (d, *J* = 13.0 Hz, 1H), 4.8-4.0 (water suppression region), 3.68 (d, *J* = 15.2 Hz, 2H), 3.54 (td, *J* = 15.1, 13.5, 7.6 Hz, 4H), 3.48 – 3.42 (m, 1H), 3.40 (s, 2H), 3.37 (d, *J* = 3.3 Hz, 2H), 3.26 (d, *J* = 13.9 Hz, 2H), 3.20 – 2.88 (m, 7H), 2.13 (s, 2H), 1.44 (s, 3H), 1.06 – 0.92 (m, 2H), 0.76 (s, 2H), 0.67 (s, 2H), -0.58 (d, *J* = 6.9 Hz, 4H). HRMS (ESI^-) *m/z* calcd (most abundant mass peak) for $C_{126}H_{118}N_{27}O_{32}S_3$ $[M-H]^-$ 2617.7637 found 2617.8408.



Compound 10: Foldamer-peptide **10** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal acetylation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (56 mg, 72%, Figure S12a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 8 minutes at 25°C, Figure S12b) to afford 4 mg of pure compound. HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{125}H_{127}Cl_3N_{31}O_{33}S_2$ $[M+H]^+$ 2760.7752 found 2760.8156

Compound 10C: After lyophilization, compound **10** (1.5 mg) was dissolved in a mixture of acetonitrile (2.4 ml) in water (3.6 ml) to reach a 0.1 mM concentration and TCEP stock solution (6 µl, 10 mM, 1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (3.6 ml, 60 mM, pH 8.5) in acetonitrile (2.4 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 4 h at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 4 h, the reaction mixture was quenched with TFA and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S12b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{125}H_{126}ClN_{31}O_{33}S_2$ $[M+2H]^{2+}$ 1344.9144 found 1344.9144

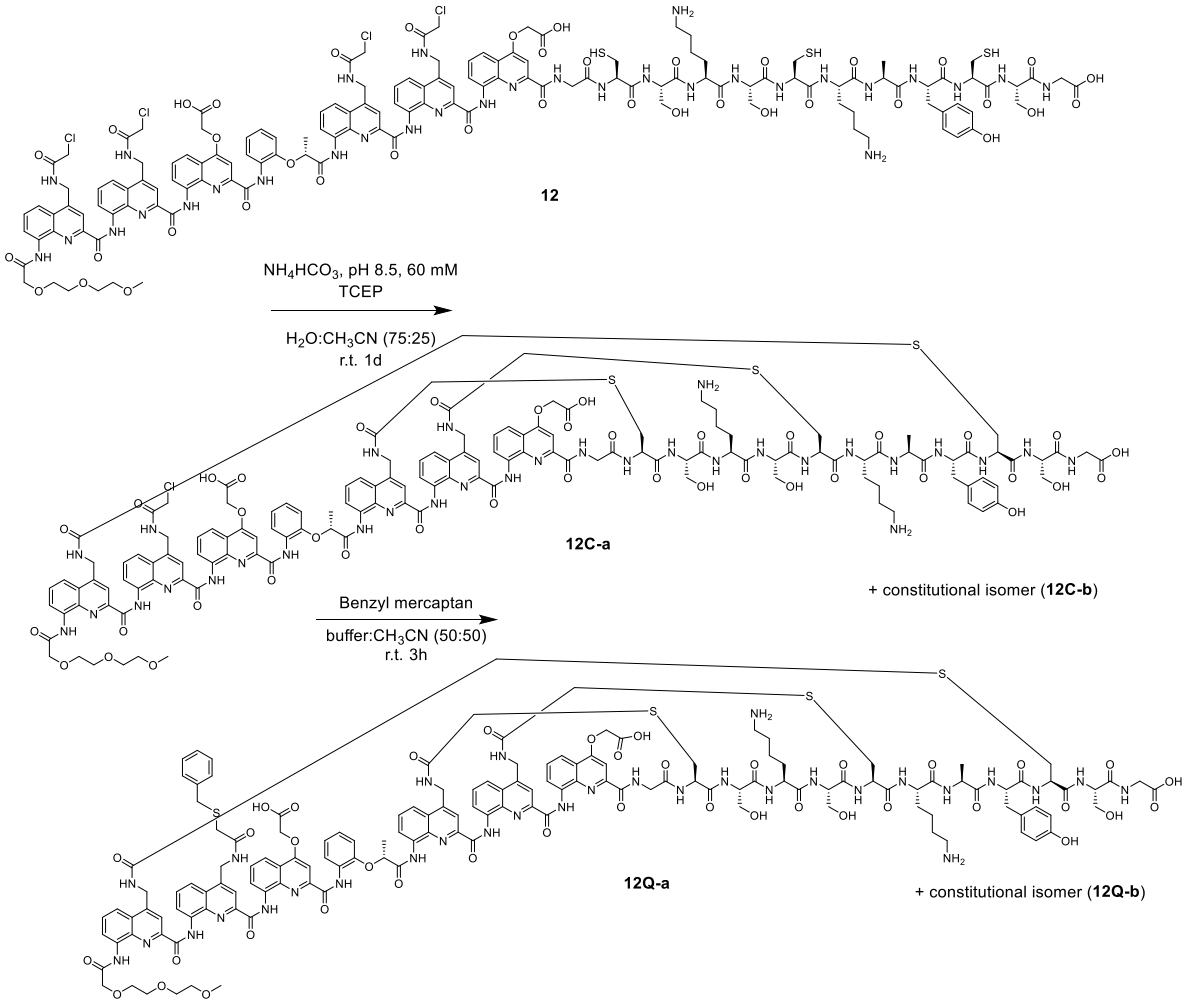
Compound 10Q: Compound **10C** (1.5 mg) was dissolved in a mixture of NH_4HCO_3 buffer (150 µl, 60 mM, pH 8.5) in acetonitrile (100 µl) and benzyl mercaptan (1 µl) was added. The reaction mixture was stirred at RT for 3 h. Solvents were removed, by lyophilization. Pure compound was isolated after semi-preparative RP-HPLC purification with a gradient from 25% to 45% solvent B over 10 min at RT (Figure S12e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{131}H_{131}N_{31}O_{33}S_3$ $[M+2H]^{2+}$ 1388.4431 found 1388.5001.



Compound 11: Foldamer-peptide **11** was built on a 25 µmol scale using a Low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (83 mg, 81%, Figure S13a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 10 minutes at 25°C) to afford 4 mg of pure compound (Figure S14b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{138}H_{153}Cl_4N_{31}O_{39}S_3$ $[M+2H]^{2+}$ 1554.9509 found 1555.0461.

Compound 11C: After lyophilization, compound **11** (3.0 mg) was dissolved in a mixture of acetonitrile (5 ml) in water (15 ml) to reach a 0.05 mM concentration and TCEP stock solution (10 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (15 ml, 60 mM, pH 8.5) in acetonitrile (5 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 1d at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 1d, the reaction mixture was quenched with formic acid and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S13c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{138}H_{150}ClN_{31}O_{39}S_3$ $[M+2H]^{2+}$ 1499.9869 found 1500.0687 (**11C-a**) and 1500.0675 (**11C-b**).

Compound 11Q: Compound **11C** (1.5 mg) was dissolved in a 1:1 mixture of NH_4HCO_3 buffer (60 mM, pH 8.5) in acetonitrile (0.3 ml total) and benzyl mercaptan (4 µl) was added. The reaction mixture was stirred at RT for 3 h. Solvents were removed, by lyophilization. Pure compounds (two constitutional isomers) were isolated after semi-preparative RP-RP-HPLC purification with a gradient from 30% to 50% solvent B over 10 min at RT (Figure S13e and S13f). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{145}H_{157}N_{31}O_{39}S_4$ $[M+2H]^{2+}$ 1543.5156 found 1543.5291 (**11Q-a**) and 1543.5275 (**11Q-b**).

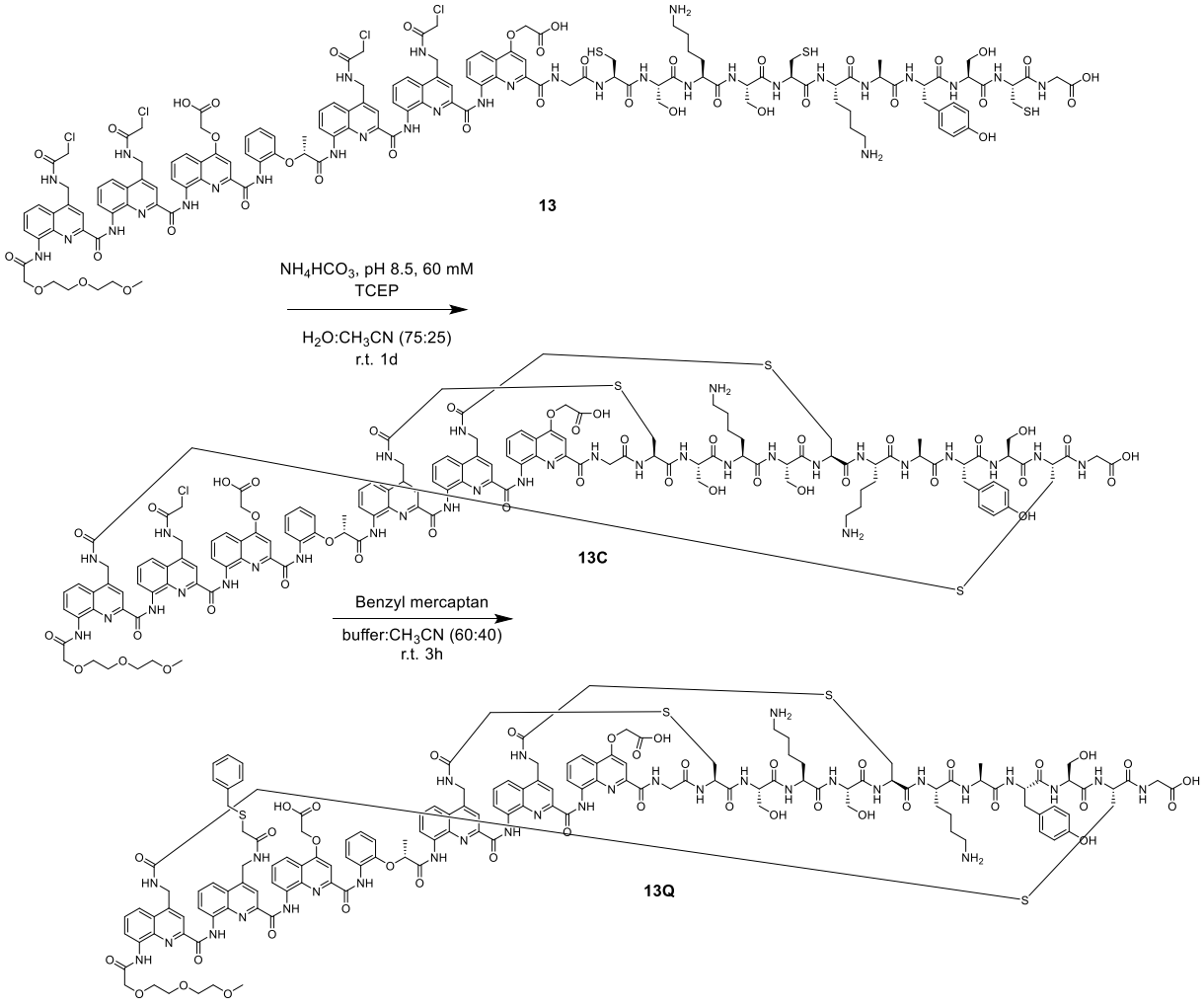


Compound 12: Foldamer-peptide **12** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (70 mg, 68%, Figure S15a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 10 minutes at 25°C) to afford 2 mg of pure compound (Figure S15b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{138}H_{153}Cl_4N_{31}O_{39}S_3$ $[M+2H]^{2+}$ 1554.9509 found 1554.4308

Compound 12C: After lyophilization, compound **12** (1.0 mg) was dissolved in a mixture of acetonitrile (2 ml) in water (8 ml) to reach a 0.05 mM concentration and TCEP stock solution (4 µl 10 mM, 0.1 equiv.) was added .The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (8 ml, 60 mM, pH 8.5) in acetonitrile (2 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 1d at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 1d, the reaction mixture was neutralized with formic acid and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S15c). HRMS (ESI^+) *m/z* calcd (most

abundant mass peak) for $C_{138}H_{150}ClN_{31}O_{39}S_3$ $[M+2H]^{2+}$ 1499.9869 found 1500.0080 (**12C-a**) and 1500.0078 (**12C-b**)

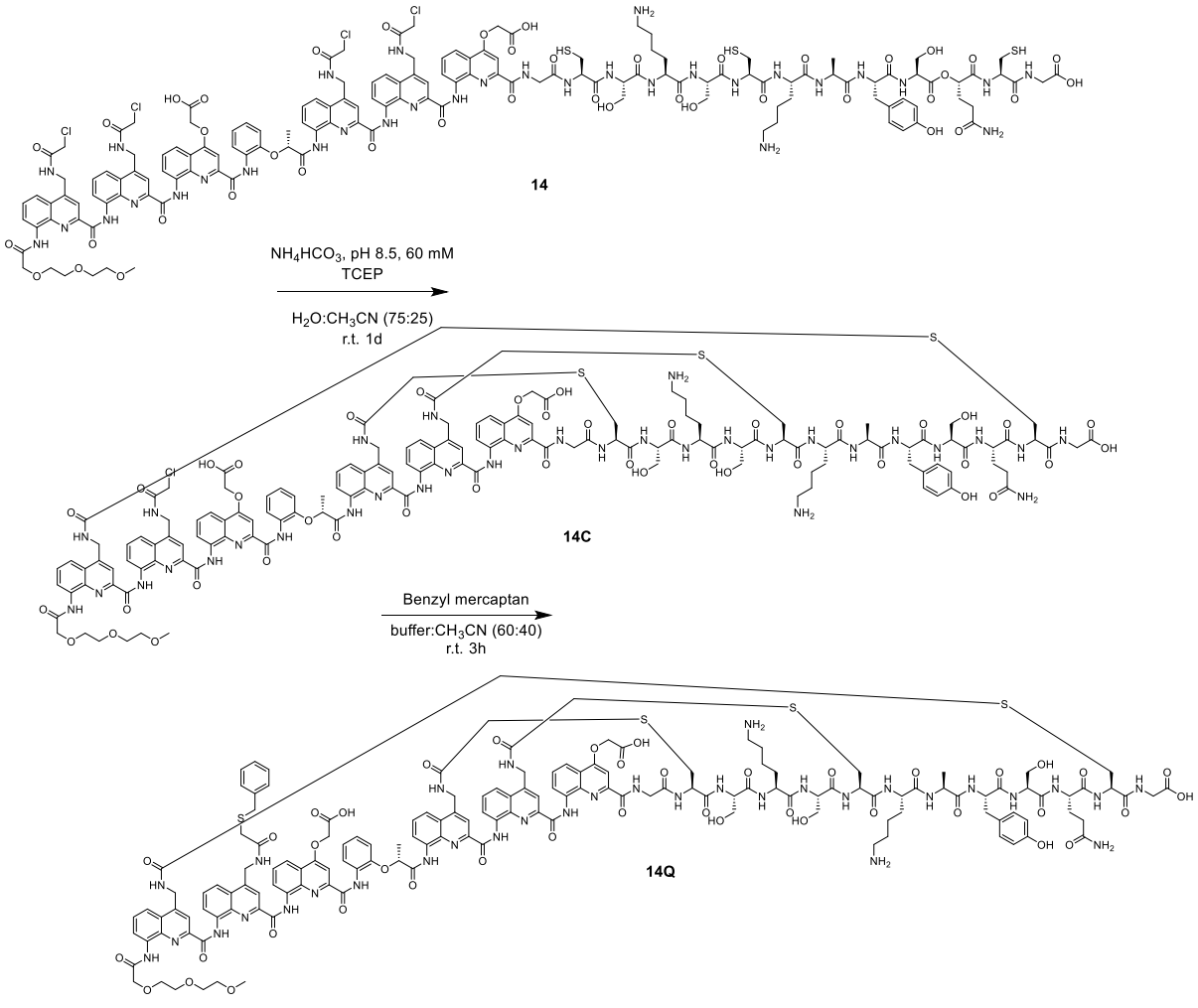
Compound 12Q: Compound **12C** (1.0 mg) was dissolved in 1:1 NH_4HCO_3 buffer (60 mM, pH 8.5) in acetonitrile (1.5 ml total) and benzyl mercaptan (20 µl) was added. The reaction mixture was stirred at RT for 3 h. Solvents were removed, by lyophilization. Pure compounds (two constitutional isomers) were isolated after semi-preparative RP-HPLC purification with a gradient from 30% to 50% solvent B over 10 min at RT (Figure S15e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{145}H_{157}N_{31}O_{39}S_4$ $[M+2H]^{2+}$ 1544.0165 found 1543.9963. Both isomers most likely co-eluted.



Compound 13: Foldamer-peptide **13** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (65 mg, 63%, Figure S16a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 10 minutes at 25°C) to afford 3 mg of pure compound (Figure S16b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{138}H_{153}Cl_4N_{31}O_{39}S_3$ $[M+2H]^{2+}$ 1554.9509 found 1554.9518

Compound 13Q: After lyophilization, compound **13** (1.8 mg) was dissolved in a mixture of acetonitrile (3 ml) in water (9 ml) to reach a 0.05 mM concentration and TCEP stock solution (4 µl, 10 mM, 0.1 equiv.) and the solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (9 ml, 60 mM, pH 8.5) in acetonitrile (3 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 1d at RT. The progression of the macrocyclization was monitored by RP-HPLC. The reaction mixture was quenched with formic acid and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S16c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{138}H_{150}ClN_{31}O_{39}S_3$ $[M+2H]^{2+}$ 1499.9869 found 1499.9807.

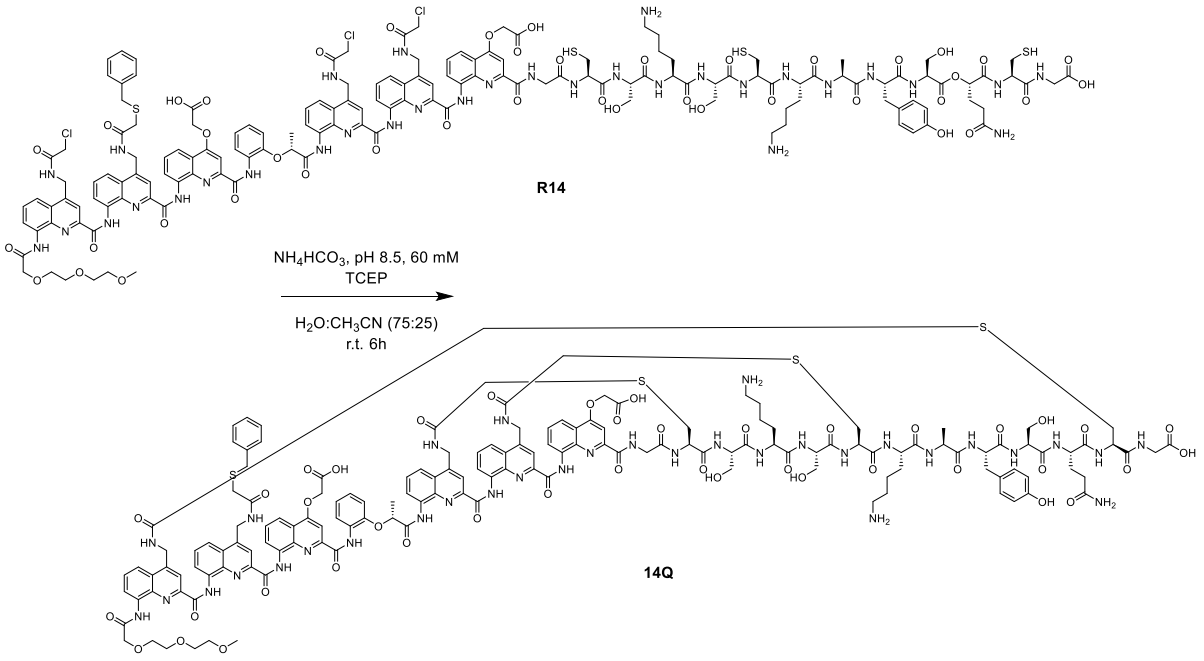
Compound 13Q: Compound **13C** was dissolved in 1:1 mixture of NH_4HCO_3 buffer (60 mM, pH 8.5) in acetonitrile (1.5 ml) and benzyl mercaptan (20 µl) was added. The reaction mixture was stirred at RT for 3 h. Solvents were removed, by lyophilization. Pure compound was isolated after semi-preparative RP-RP-HPLC purification with a gradient from 30% to 50% solvent B over 10 min at RT (Figure S16e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{145}H_{157}N_{31}O_{39}S_4$ $[M+2H]^{2+}$ 1543.5156 found 1544.0290.



Compound 14: Foldamer-peptide **14** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (68 mg, 64%, Figure S17a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 10 minutes at 25°C) to afford 5 mg of pure compound (Figure S17b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{143}H_{161}Cl_4N_{33}O_{41}S_3$ $[M+2H]^{2+}$ 1618.4806 found 1618.4738.

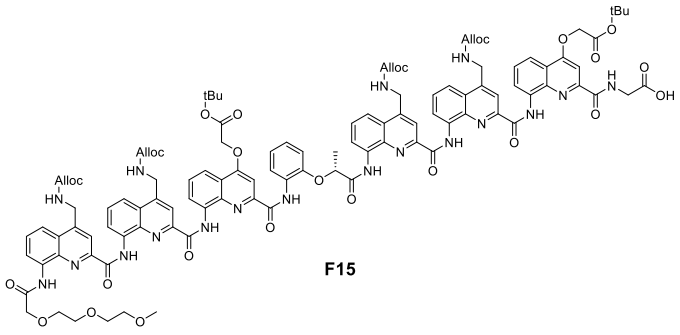
Compound 14C: After lyophilization, compound **14** (2.0 mg) was dissolved in a mixture of acetonitrile (3 ml) in water (9 ml) to reach a 0.05 mM concentration and TCEP stock solution (4 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (9 ml, 60 mM, pH 8.5) in acetonitrile (3 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert N_2 atmosphere. The mixture was quickly stirred and left without stirring for 1d at RT. The progression of the macrocyclization was monitored by RP-HPLC. The reaction mixture was quenched with formic acid and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S17c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{143}H_{158}ClN_{33}O_{41}S_3$ $[M+2H]^{2+}$ 1564.0163 found 1564.0276.

Compound 14Q: Compound **14C** was dissolved in 1:1 mixtture of NH_4HCO_3 buffer (60 mM, pH 8.5) in acetonitrile (0.6 ml total), benzyl mercaptan (8 µl) was added and the reaction mixture was stirred at RT for 5 h. Solvents were removed, by lyophilization. Pure compound were isolated after semi-preparative RP-HPLC purification with a gradient from 30% to 50% solvent B over 10 min at RT (Figure S17e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{150}H_{165}N_{33}O_{41}S_4$ $[M+2H]^{2+}$ 1608.0458 found 1608.0641.



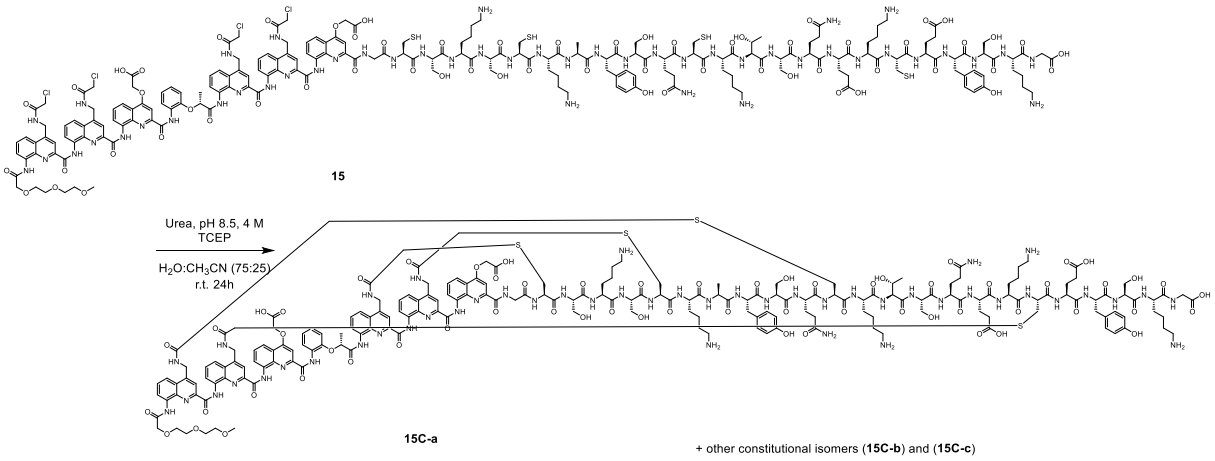
Compound R14: Foldamer-peptide **R14** was built on a 33 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:2.5, v:v:v:v) and the crude product (73 mg, 66%, Figure S18a) was purified by semi-preparative RP-HPLC (gradient: from 33% to 40% solvent B over 10 minutes at 25°C) to afford 3 mg of pure compound (Figure S18b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{150}H_{168}Cl_3N_{33}O_{41}S_4$ $[M+2H]^{2+}$ 1662.5087 found 1662.5291

Compound 14Q: After lyophilization, compound **R14** (2.0 mg) was dissolved in a mixture of acetonitrile (3 ml) in water (9 ml) to reach a 0.05 mM concentration and TCEP stock solution (4 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (9 ml, 60 mM, pH 8.5) in acetonitrile (3 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 6h at RT. The progression of the macrocyclization was monitored by RP-HPLC. The reaction mixture was quenched with formic acid and the remaining solvent lyophilized. The obtained crude product was purified by semi-preparative RP-HPLC (gradient: from 30% to 50% solvent B over 10 minutes at 25°C) to afford 1 mg of pure compound (Figure S18d). ^1H NMR (500 MHz, CD_3CN) δ 11.84 (s, 1H), 11.12 (s, 1H), 10.51 (d, *J* = 8.2 Hz, 2H), 9.79 (s, 1H), 9.65 (s, 1H), 9.41 (d, *J* = 14.9 Hz, 1H), 9.33 (s, 1H), 8.77 (d, *J* = 9.4 Hz, 3H), 8.66 (dd, *J* = 20.3, 8.3 Hz, 2H), 8.49 (dd, *J* = 20.3, 11.5 Hz, 5H), 8.36 (s, 2H), 8.32 – 8.21 (m, 5H), 8.21 – 8.00 (m, 11H), 8.00 – 7.84 (m, 11H), 7.81 (q, *J* = 8.4, 6.4 Hz, 5H), 7.75 – 7.62 (m, 6H), 7.62 – 7.34 (m, 23H), 7.27 (dd, *J* = 18.8, 8.6 Hz, 7H), 7.11 (s, 4H), 7.01 (s, 4H), 6.96 – 6.76 (m, 10H), 6.74 (d, *J* = 24.5 Hz, 5H), 6.53 (d, *J* = 18.6 Hz, 2H), 6.41 (t, *J* = 8.7 Hz, 1H), 6.13 (d, *J* = 9.4 Hz, 1H), 5.89 (t, *J* = 8.5 Hz, 1H), 5.23 (s, 1H), 5.14 (d, *J* = 22.7 Hz, 1H), 5.0-4.1 (water suppression region), 3.89 (q, *J* = 12.4, 11.8 Hz, 7H), 3.62 (d, *J* = 47.7 Hz, 5H), 3.57 – 3.21 (m, 24H), 3.21 – 2.73 (m, 29H), 2.73 – 2.14 (m, 20H), 1.71 (q, *J* = 8.4 Hz, 6H), 1.56 – 1.07 (m, 21H), 0.90 (d, *J* = 35.1 Hz, 4H), -0.52 (d, *J* = 7.2 Hz, 3H). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{150}H_{165}N_{33}O_{41}S_4$ $[M+2H]^{2+}$ 1608.0468 found 1608.0455.



Foldamer fragment F15: **F15** was synthesized on a preloaded Fmoc-Gly-SASRIN resin (0.72 mmol/g) using general SPFS procedure on a 100 µmol scale. Crude **F15** was recovered in 70% yield (150 mg) after cleavage using a mixture of HFIP in DCM (6 ml, 40:60, v/v) for 1h at RT. The obtained

crude product was purified by semi-preparative RP-HPLC (gradient: from 72% to 87% solvent B over 10 minutes at 25°C) to afford 80 mg of pure compound. HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{110}H_{110}N_{18}O_{28}$ $[M+H]^+$ 2132.8064 found 2132.7844.



Compound 15: Foldamer-peptide **15** was built by relying on the CEM Liberty Blue microwave peptide synthesizer for the resin-bound Fmoc-Cys(Trt)-Ser(*t*Bu)-Lys(Boc)-Ser(*t*Bu)-Cys(Trt)-Lys(Boc)-Ala-Tyr(*t*Bu)-Ser(*t*Bu)-Gln(Trt)-Cys(Trt)-Lys(Boc)-Thr(*t*Bu)-Ser(*t*Bu)-Gln(Trt)-Glu(*t*Bu)-Lys(Boc)-Cys(Trt)-Glu(*t*Bu)-Tyr(*t*Bu)-Ser(*t*Bu)-Lys(Boc)-Gly peptide on a 100 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.27 mmol/g). Fragment condensation with foldamer fragment **F15** was next performed on a 25 µmol scale of resin-bound free amine peptide. Therefore **F15** (30 mg, 14 µmol) was dissolved in mixture of DCM/THF (1.4 ml, 1:1, v:v) together with BOP (12 mg, 28 µmol) and DIPEA (7µl, 42 µmol). After incubating the mixture for 5 min at RT, it was added to the resin and mechanical shaking was maintained for 16h at RT. The resin was washed with DCM and the remaining resin-bound peptide free amine groups were capped with 1:1 mixture of acetic anhydride in DCM (2 ml).

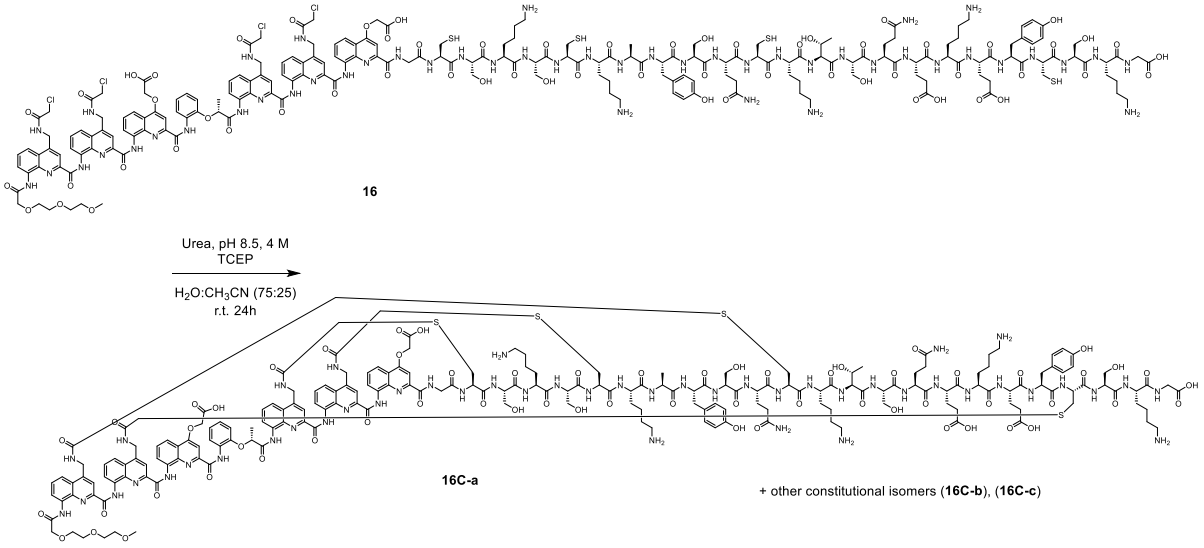
Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:5 v:v:v:v) and the crude product (89 mg, 90%; Figure S20a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 35% solvent B over 10 minutes at 25°C) to afford 14 mg of pure compound (Figure S20b). HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{198}H_{253}Cl_4N_{48}O_{61}S_4$ $[M+3H]^{3+}$ 1516.8609 found 1516.8608.

Compound 15C: After lyophilization, compound **15** (7 mg) was dissolved in mixture of acetonitrile (8ml) in water (24 ml) to reach a 0.05 mM concentration and TCEP stock solution (60 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (24 ml, 60 mM, pH 8.5) in acetonitrile (8 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 24h at RT. The progression of the macrocyclization was monitored by RP-HPLC and used in the next reaction step without further

purification (Figure S20c). Benzyl mercaptan (1.5 ml) was added to block unreacted chloroacetamide groups and the reaction mixture was stirred at RT for 5 h. After neutralizing the reaction with HCl (0.1 M), the solution was diluted with water (25ml) and the excess of benzyl mercaptan was removed by separation in an extraction funnel. Solvents were removed, by lyophilization. The obtained crude product was purified by semi-preparative RP-HPLC (hold 100% solvent A for 5 min then gradient from 10% to 35% solvent B over 15 minutes at 25°C) to afford 1 mg (**15C-a**), 1 mg (**15C-b**) and 0.3 mg (**15C-c**) as pure compounds (Figure S20e, S20f and S20g). ^{1}H NMR (500 MHz, CD_3CN) for **15C-a**: δ 11.85 (s, 1H), 11.14 (s, 1H), 10.64 (d, *J* = 5.6 Hz, 2H), 9.82 (s, 1H), 9.61 (s, 1H), 9.45 (s, 1H), 9.32 (s, 1H), 9.14 (d, *J* = 10.8 Hz, 1H), 8.74 (d, *J* = 8.6 Hz, 1H), 8.54 (d, *J* = 8.9 Hz, 3H), 8.34 (s, 1H), 8.22 – 7.19 (m, 53H), 7.17 – 7.01 (m, 4H), 6.90 (dd, *J* = 21.2, 8.7 Hz, 4H), 6.72 (dd, *J* = 31.9, 7.9 Hz, 7H), 6.51 (d, *J* = 10.3 Hz, 3H), 6.29 (t, *J* = 7.9 Hz, 1H), 6.07 (d, *J* = 8.6 Hz, 1H), 5.91 – 5.84 (m, 1H), 5.5-4.0 (water suppression region), 3.84 – 3.19 (m, 100H), 3.19 – 2.81 (m, 54H), 2.63 – 2.44 (m, 13H), 1.77 – 1.25 (m, 68H), 1.20 – 0.79 (m, 23H), -0.49 (d, *J* = 6.8 Hz, 3H).

^{1}H NMR (500 MHz, CD_3CN)for **15C-b:** ^{1}H NMR (500 MHz, Acetonitrile-d_3) δ 11.79 (s, 1H), 11.11 (s, 1H), 10.53 (s, 2H), 9.79 (s, 1H), 9.56 (s, 1H), 9.39 (s, 1H), 9.30 (s, 1H), 8.87 – 8.77 (m, 2H), 8.65 – 6.69 (m, 100H), 6.51 (d, *J* = 22.7 Hz, 2H), 6.36 (d, *J* = 8.5 Hz, 1H), 6.22 (d, *J* = 8.3 Hz, 1H), 5.86 (t, *J* = 8.4 Hz, 1H), 5.57 (s, 13H), 5.35 (s, 1H), 5.21 – 4.88 (m, 3H), 4.8-4.0 (water suppression region), 3.92 – 3.53 (m, 10H), 3.53 – 2.66 (m, 67H), 2.65 – 2.14 (m, 25H), 1.84 (s, 12H), 1.76 – 1.57 (m, 21H), 1.44 (dd, *J* = 20.6, 7.4 Hz, 22H), 1.28 (d, *J* = 7.3 Hz, 12H), 1.13 (t, *J* = 6.5 Hz, 9H), -0.44 (d, *J* = 6.8 Hz, 3H).

HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{198}H_{249}N_{48}O_{61}S_4$ $[M+3H]^{3+}$ 1468.2258 found 1468.2256 (**15C-a**), 1468.2227 (**15C-b**) and 1468.2196 (**15C-c**).

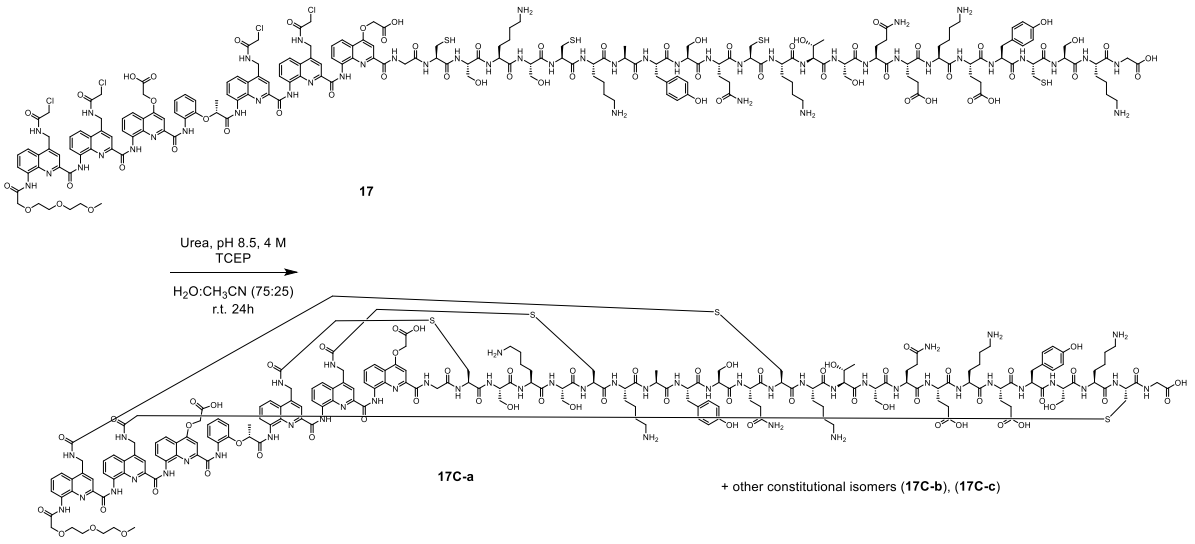


Compound 16: Foldamer-peptide **16** was built by relying on the CEM Liberty Blue microwave peptide synthesizer for the peptide segment Fmoc-Cys(Trt)-Ser(*t*Bu)-Lys(Boc)-Ser(*t*Bu)-Cys(Trt)-Lys(Boc)-

Ala-Tyr(*t*Bu)-Ser(*t*Bu)-Gln(Trt)-Cys(Trt)-Lys(Boc)-Thr(*t*Bu)-Ser(*t*Bu)-Gln(Trt)-Glu(tBu)-Lys(Boc)-Glu(*t*Bu)-Tyr(*t*Bu)-Cys(Trt)-Ser(*t*Bu)-Lys(Boc)-Gly- on a 100 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.27 mmol/g). Fragment condensation with foldamer fragment **F15** was next carried out in the same way as for the synthesis of foldamer-peptide **15** (see above).

Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:5, v/v/v/v) and the crude product (120 mg, 71%, Figure S22a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 35% solvent B over 10 minutes at 25°C) to afford 30 mg of pure compound (Figure S22b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{198}H_{253}Cl_4N_{48}O_{61}S_4$ $[M+3H]^{3+}$ 1516.8609 found 1516.8180.

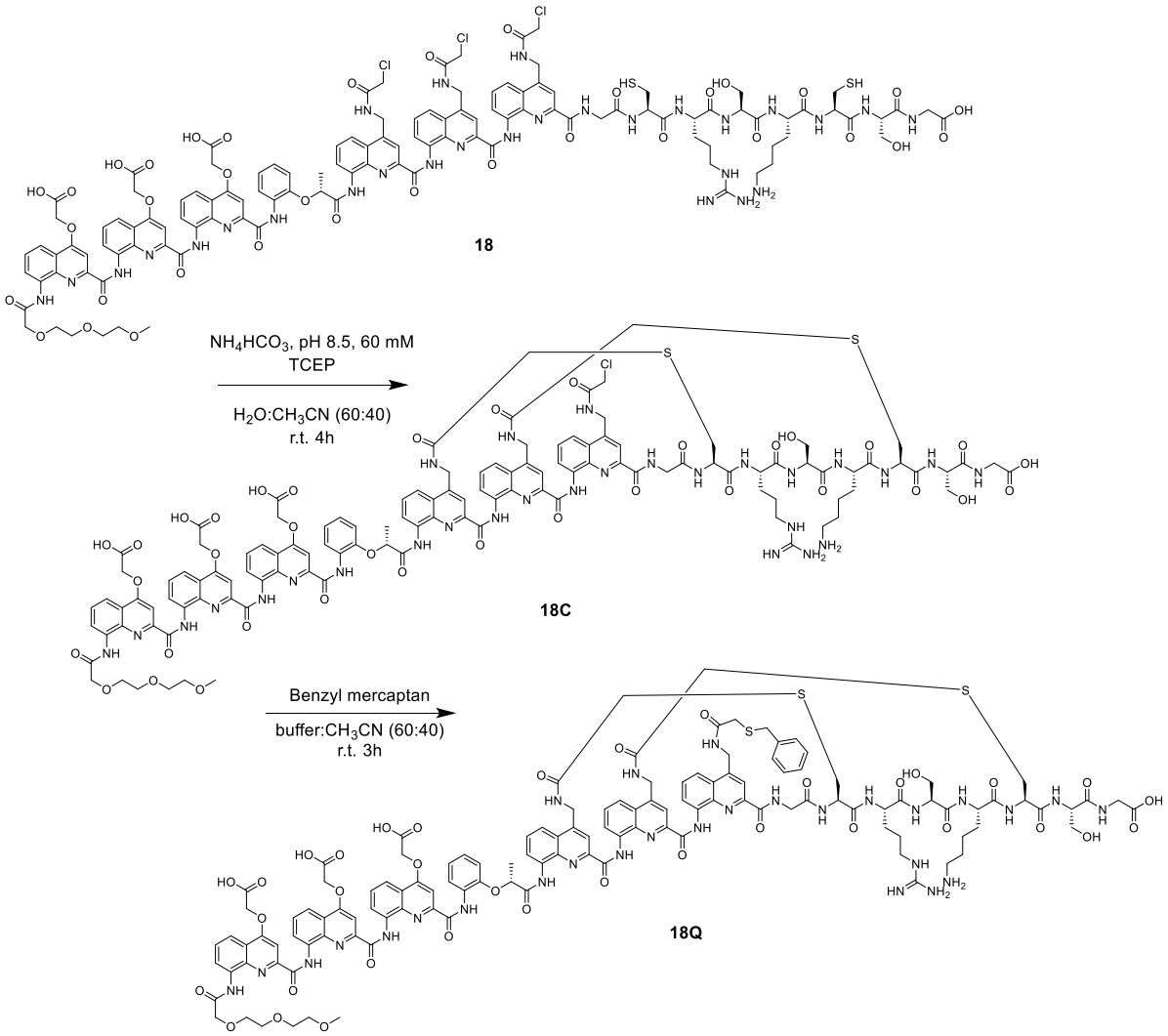
Compound 16C: After lyophilization, compound **16** (5 mg) was dissolved in mixture of acetonitrile (6 ml) in water (16 ml) to reach a 0.05 mM concentration and TCEP stock solution (40 µl, 10 mM, 0.1 equiv.). The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution mixture of urea-buffer (16 ml, 8M, pH 8.5 with 0.1 M NaH_2PO_4) in acetonitrile (6 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 24h at RT. The progression of the macrocyclization was monitored by RP-HPLC and used in the next reaction step without further purification (Figure 22c). Benzyl mercaptan (1 ml) was added to block unreacted chloroacetamide groups and the reaction mixture and was stirred at RT for 5 h. After neutralizing the reaction with HCl (0.1 M), the solution was diluted with water (30ml) and excess benzyl mercaptan was removed by separation in an extraction funnel. Solvents were removed, by lyophilisation (Figure S22d). The obtained crude product was purified by semi-preparative RP-HPLC (hold 100% solvent A for 5 min than gradient from 10% to 35% solvent B over 15 minutes at 25°C) to afford 1 mg ofnn pure compound (**16C-a**), 2 mg of pure compound (**16C-b**), and 1mg of pure compound (**16C-c**) (Figure S22d, S22e, S22f). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{198}H_{249}N_{48}O_{61}S_4$ $[M+3H]^{3+}$ 1468.2258 found 1468.1650 (**16C-a**), 1468.1661 (**16C-b**) and 1468.1640 (**16C-c**).



Compound 17: Foldamer-peptide **17** was built by relying on the CEM Liberty Blue microwave peptide synthesizer for the peptide segment Cys(Trt)-Ser(tBu)-Lys(Boc)-Ser(tBu)-Cys(Trt)-Lys(Boc)-Ala-Tyr(tBu)-Ser(tBu)-Gln(Trt)-Cys(Trt)-Lys(Boc)-Thr(tBu)-Ser(tBu)-Gln(Trt)-Glu(tBu)-Lys(Boc)-Glu(tBu)-Tyr(tBu)-Ser(tBu)-Lys(Boc)-Cys(Trt)-Gly- on a 100 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.27 mmol/g). Fragment condensation with foldamer fragment **F15** was next carried out in the same way as for the synthesis of foldamer-peptide **15** (see above).

Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:5, v/v/v/v) and the crude product (56 mg, Figure S24a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 35% solvent B over 10 minutes at 25°C) to afford 1 mg of pure compound (Figure S24b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{198}H_{253}Cl_4N_{48}O_{61}S_4$ $[M+3H]^{3+}$ 1516.8609 found 1516.8877.

Compound 17C: After lyophilization, compound **17** (0.5 mg) was dissolved in a mixture of acetonitrile (0.6 ml) in water (1.6 ml) to reach a 0.05 mM concentration and TCEP stock solution (4 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution mixture of urea-buffer (1.6 ml, 8M, pH 8.5 with 0.1 M NaH_2PO_4) in acetonitrile (0.6 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 24h at RT. The progression of the macrocyclization was monitored by RP-HPLC. Benzyl mercaptan (12 µl) was added to block unreacted chloroacetamide groups and the reaction mixture and was stirred at RT for 5 h. After neutralizing the reaction with HCl (0.1 M) solvents were removed, by lyophilisation (Figure S24c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{198}H_{249}N_{48}O_{61}S_4$ $[M+3H]^{3+}$ 1468.2258 found 1468.2336. No final purification was performed.

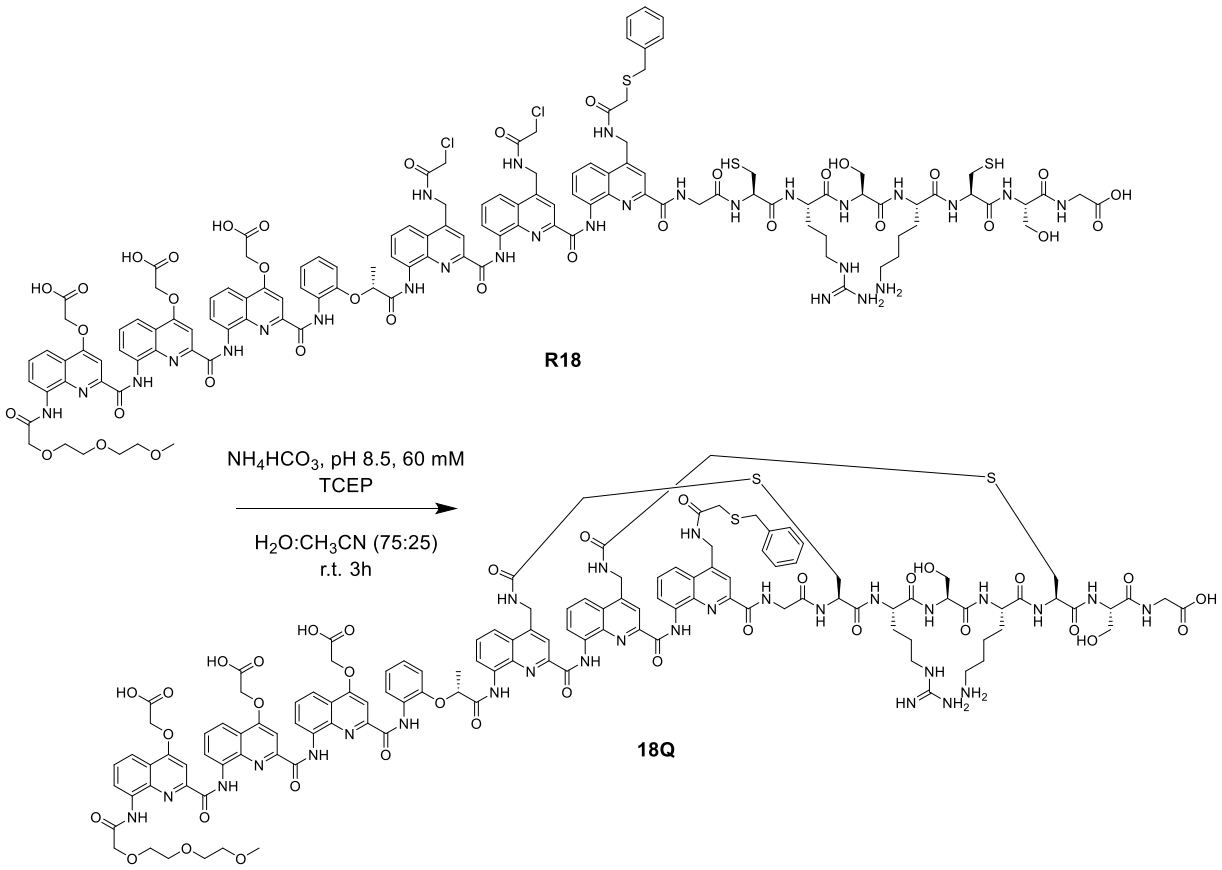


Compound 18: Foldamer-peptide **18** was built on a 25 µmol scale using a Low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (57 mg, 85%, Figure S26a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 8 minutes at 25°C) to afford 8 mg of pure compound (Figure 26b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{119}H_{127}Cl_3N_{28}O_{35}S_2$ $[M+H]^+$ 2679.7612 found 2679.8652

Compound 18C: After lyophilization, compound **18** (7 mg) was dissolved in a mixture of acetonitrile (6 ml) in water (19 ml) to reach a 0.1 mM concentration and TCEP stock solution (25 µl, 10 mM, 0.1 equiv.). The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (37 ml, 60 mM, pH 8.5) in acetonitrile (13 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 3 h at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 3 h, the reaction mixture was quenched with TFA and the remaining solvent lyophilized. The obtained crude product was used in the subsequent

reaction without further purification (Figure S26c). HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{119}H_{125}ClN_{28}O_{35}S_2$ $[M+2H]^{2+}$ 1304.4085 found 1304.4665.

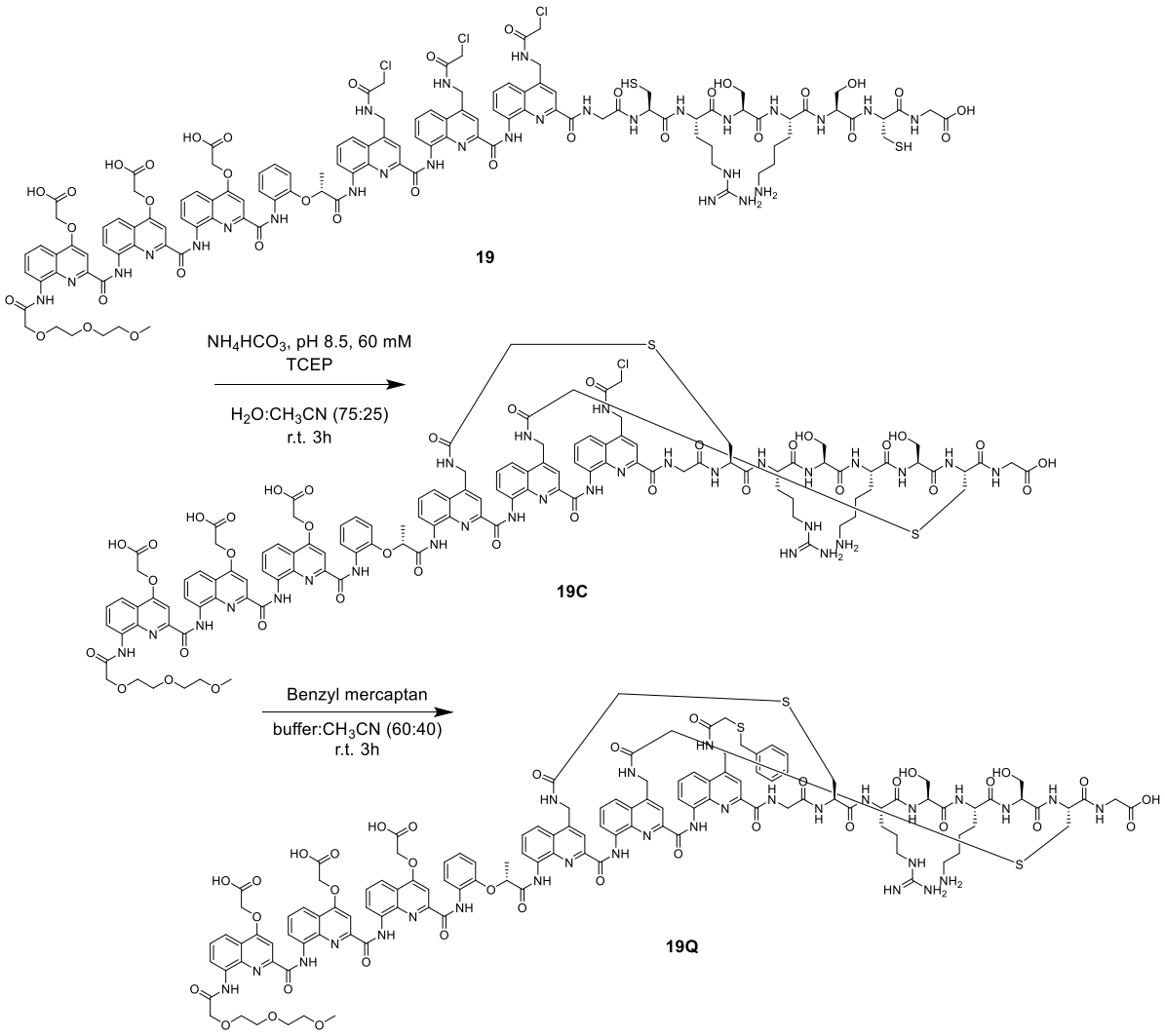
Compound 18Q: Compound **18Q** (6.8 mg) was dissolved in 1:1 mixture of NH_4HCO_3 buffer (60 mM, pH 8.5) in acetonitrile (0.4 ml total), benzyl mercaptan (4 µl) was added and the reaction mixture was stirred at RT for 16 h. Solvents were removed, by lyophilization. Pure compound was isolated after semi-preparative RP-HPLC purification with a gradient from 25% to 45% solvent B over 10 min at RT (Figure S26e). HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{126}H_{132}N_{28}O_{35}S_3$ $[M+2H]^{2+}$ 1347.9373 found 1348.0021.



Compound R18: Foldamer-peptide **R18** was built on a 25 µmol scale using a Low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (50 mg, 72%, Figure S27a) was purified by semi-preparative RP-HPLC (gradient: from 30% to 50% solvent B over 10 minutes at 25°C) to afford 5 mg of pure compound (Figure S27b). HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{126}H_{136}Cl_2N_{28}O_{35}S_2$ $[M+2H]^{2+}$ 1384.4136 found 1384.4135.

Compound 18Q: After lyophilization, compound **R18** (5 mg) was dissolved in a mixture of acetonitrile (6 ml) in water (12 ml) to reach a 0.1 mM concentration and TCEP stock solution (18 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw.

Independently, a solution of NH_4HCO_3 (24 ml, 60 mM, pH 8.5) in acetonitrile (12 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 2 h at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 2 h, the reaction mixture was quenched with TFA and the remaining solvent lyophilized (Figure S27c). The obtained crude product was purified by semi-preparative RP-HPLC with a gradient from 25% to 45% solvent B over 10 min at RT (Figure S27d). ^{1}H NMR (500 MHz, CD_3CN) δ 11.69 (s, 1H), 11.12 (s, 1H), 10.63 (s, 1H), 10.54 (s, 1H), 9.63 (d, *J* = 17.9 Hz, 2H), 9.41 (s, 1H), 9.32 (s, 1H), 8.94 (d, *J* = 9.3 Hz, 1H), 8.71 (d, *J* = 8.8 Hz, 2H), 8.46 (d, *J* = 9.3 Hz, 1H), 8.28 (s, 2H), 8.21 – 7.47 (m, 27H), 7.52 – 7.24 (m, 16H), 7.24 – 6.77 (m, 13H), 6.70 (s, 1H), 6.60 – 6.31 (m, 3H), 6.25 (d, *J* = 9.5 Hz, 1H), 6.01 (t, *J* = 9.1 Hz, 1H), 5.50 – 5.25 (m, 1H), 5.02 (d, *J* = 23.3 Hz, 2H), 4.8-3.9 (water suppression region) 3.58 (d, *J* = 17.0 Hz, 2H), 3.47 – 2.88 (m, 19H), 2.88 – 2.65 (m, 9H), 2.65 – 2.33 (m, 5H), 1.55 (d, *J* = 69.2 Hz, 5H), 1.45 – 1.10 (m, 5H), 1.10 – 0.69 (m, 2H), -0.32 (d, *J* = 7.0 Hz, 3H). HRMS (ESI$^+$) *m/z* calcd (most abundant mass peak) for $C_{126}H_{134}N_{28}O_{35}S_3$ $[M+2H]^{2+}$ 1347.9376 found 1347.9450.

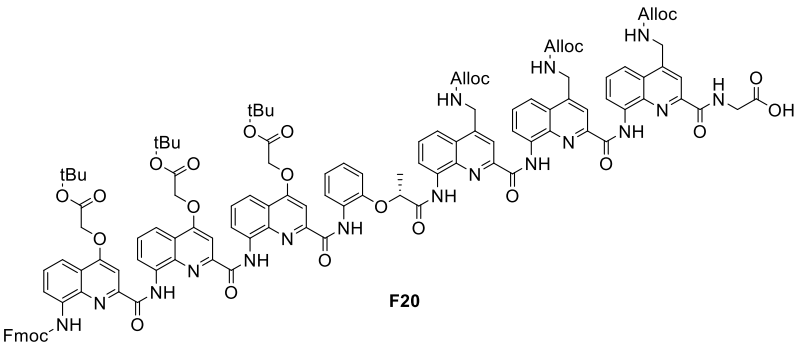


Compound 19: Foldamer-peptide **19** was built on a 25 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.33 mmol/g). After terminal DEG installation, the Alloc protecting groups were removed, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/DODT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (50 mg, 75%, Figure S29a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 45% solvent B over 8 minutes at 25°C) to afford 5 mg of pure compound (Figure S29b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{119}H_{127}Cl_3N_{28}O_{35}S_2$ $[M+H]^+$ 2679.7612 found 2679.8642.

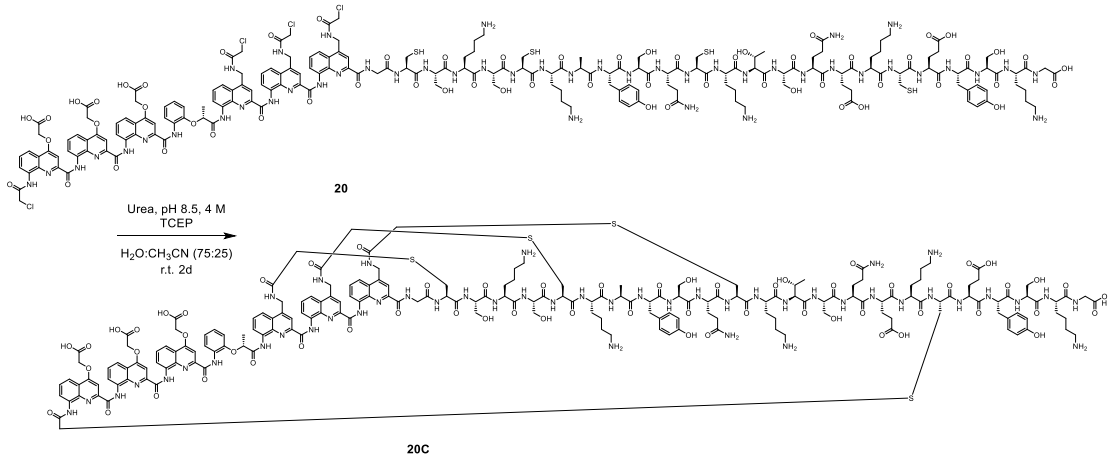
Compound 19C: After lyophilization, compound **19** (2.7 mg) was dissolved in a mixture of acetonitrile (2.5 ml) in water (7.5 ml) to reach a 0.1 mM concentration and TCEP stock solution (10 µl, 10 mM, 0.1 equiv.) was added. The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution of NH_4HCO_3 (15 ml, 60 mM, pH 8.5) in acetonitrile (5 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the Foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 3 h at RT. The progression of the macrocyclization was monitored by RP-HPLC. After 3 h, the reaction mixture was neutralized with

formic acid and the remaining solvent lyophilized. The obtained crude product was used in the subsequent reaction without further purification (Figure S29c). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{119}H_{125}ClN_{28}O_{35}S_2$ $[M+2H]^{2+}$ 1304.4085 found 1304.4509.

Compound 19Q: Compound **19C** (2.0 mg) was dissolved in 1:1 mixture of NH_4HCO_3 buffer (60 mM, pH 8.5) in acetonitrile (0.4 ml total), benzyl mercaptan (4 µl) was added and the reaction mixture was stirred at RT for 8 h. Solvents were removed, by lyophilization. Pure compound was isolated after semi-preparative RP-HPLC purification with a gradient from 25% to 45% solvent B over 10 min at RT (Figure S29e). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{126}H_{134}N_{28}O_{35}S_3$ $[M+2H]^{2+}$ 1347.9878 found 1347.9450.



Foldamer fragment F20: **F20** was synthesized on a preloaded Fmoc-Gly-SASRIN resin (0.72 mmol/g) using general SPFS procedure on a 50 µmol scale. Crude **F20** was recovered in 83% yield (90 mg) after cleavage using a mixture of HFIP in DCM (6 ml, 80:20, v/v) 2 × 30 min at r.t. The obtained crude product was purified by semi-preparative HPLC (gradient: from 90% to 100% solvent B over 10 minutes at 25°C) to achieve 51 mg purified compound. HRMS (ESI^-) *m/z* calcd for $C_{111}H_{111}N_{17}O_{27}$ $[M+2H]^{2+}$ 1106.4007 found 1106.4142.

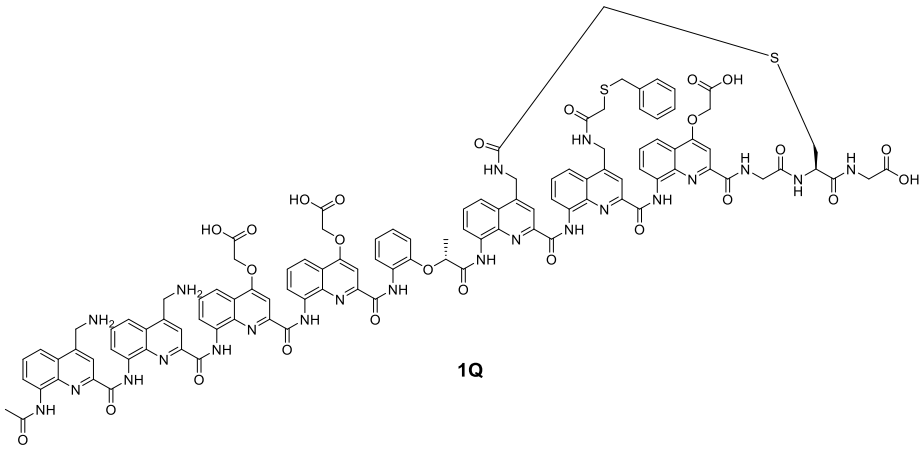


Compound 20: Foldamer-peptide **20** was built by relying on the CEM Liberty Blue microwave peptide synthesizer for the protected resin-bound Fmoc-Cys(Trt)-Ser(*t*Bu)-Lys(Boc)-Ser(*t*Bu)-Cys(Trt)-Lys(Boc)-Ala-Tyr(*t*Bu)-Ser(*t*Bu)-Gln(Trt)-Cys(Trt)-Lys(Boc)-Thr(*t*Bu)-Ser(*t*Bu)-Gln(Trt)-Glu(*t*Bu)-

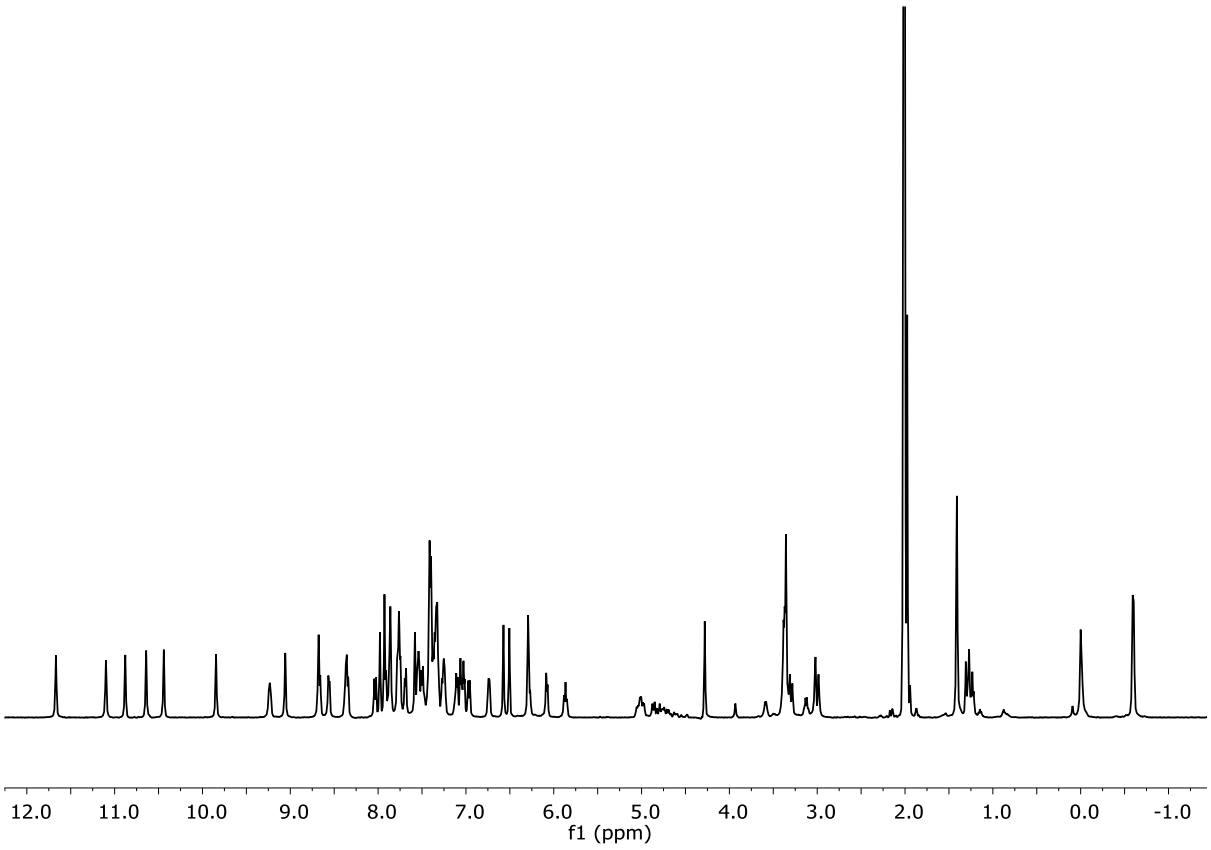
Lys(Boc)-Cys(Trt)-Glu(*t*Bu)-Tyr(*t*Bu)-Ser(*t*Bu)-Lys(Boc)-Gly- peptide on a 100 µmol scale using a low loading Fmoc-Gly-preloaded Wang resin (0.27 mmol/g). Fragment condensation with foldamer fragment **F20** was next performed on a 25 µmol scale of resin-bound free amine peptide. Fragment condensation with foldamer fragment **F20** was next carried out in the same way as for the synthesis of foldamer-peptide **20** (see above). Fmoc was deprotected followed by the removal of Alloc protecting groups, directly followed by chloroacetylation. The resin was cleaved with TFA/TIS/H_2O/EDT (92.5:2.5:2.5:2.5, v/v/v/v) and the crude product (50 mg, Figure S30a) was purified by semi-preparative RP-HPLC (gradient: from 25% to 35% solvent B over 10 minutes at 25°C) to afford 3 mg of pure compound (Figure S30b). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{192}H_{237}Cl_4N_{47}O_{60}S_4$ $[M+3H]^{3+}$ 1478.4944 found 1478.5140.

Compound 20C: After lyophilization, compound **20** (1.5 mg) was dissolved in a mixture of acetonitrile (1 ml) in water (3 ml) to reach a 0.05 mM concentration and TCEP stock solution (10 µl, 10 mM, 0.1 equiv.). The solvent mixture was freed from oxygen by Freeze-Pump-Thaw. Independently, a solution mixture of urea-buffer (3 ml, 8M, pH 8.5 with 0.1 M NaH_2PO_4) in acetonitrile (1 ml) was prepared, oxygen removed, by Freeze-Pump-Thaw and the foldamer solution added via syringe under inert gas atmosphere. The mixture was quickly stirred and left without stirring for 2d at RT. The progression of the macrocyclization was monitored by RP-HPLC. After neutralizing the reaction with HCl (0.1 M), the solvents were removed, by lyophilization. The obtained crude product was purified by semi-preparative RP-HPLC (hold 100% solvent A for 5 min then gradient from 10% to 35% solvent B over 15 minutes at 25°C) to afford 0.2 mg (Figure S30d). ^1H NMR (500 MHz, CD_3CN) δ 11.76 (s, 1H), 11.32 (s, 1H), 10.61 (s, 1H), 10.55 (s, 1H), 9.70 (s, 1H), 9.61 (s, 1H), 9.42 (s, 1H), 9.31 (s, 1H), 8.79 (d, *J* = 8.4 Hz, 1H), 8.50 (d, *J* = 7.6 Hz, 4H), 8.39 (d, *J* = 8.0 Hz, 2H), 8.28 (d, *J* = 20.8 Hz, 4H), 8.15 (d, *J* = 8.9 Hz, 4H), 8.08 (d, *J* = 8.2 Hz, 2H), 8.01 (d, *J* = 13.8 Hz, 3H), 7.94 (d, *J* = 7.4 Hz, 4H), 7.90 – 7.79 (m, 4H), 7.72 (s, 2H), 7.69 – 7.51 (m, 11H), 7.51 – 7.21 (m, 28H), 7.17 (d, *J* = 36.0 Hz, 4H), 7.09 – 6.91 (m, 5H), 6.73 – 6.30 (m, 11H), 6.30 – 6.05 (m, 5H), 5.94 (t, *J* = 8.2 Hz, 1H), 5.54 (d, *J* = 49.4 Hz, 10H), 5.05 (d, *J* = 30.5 Hz, 8H), 3.89 – 3.37 (m, 36H), 3.37 – 3.09 (m, 19H), 3.09 – 2.46 (m, 41H), 1.64 (d, *J* = 99.7 Hz, 31H), 1.39 – 1.23 (m, 34H), 1.23 – 0.78 (m, 21H), -0.34 (d, *J* = 6.6 Hz, 3H). HRMS (ESI^+) *m/z* calcd (most abundant mass peak) for $C_{192}H_{233}N_{47}O_{60}S_4$ $[M+2H]^{2+}$ 2144.2816 found 2144.2216.

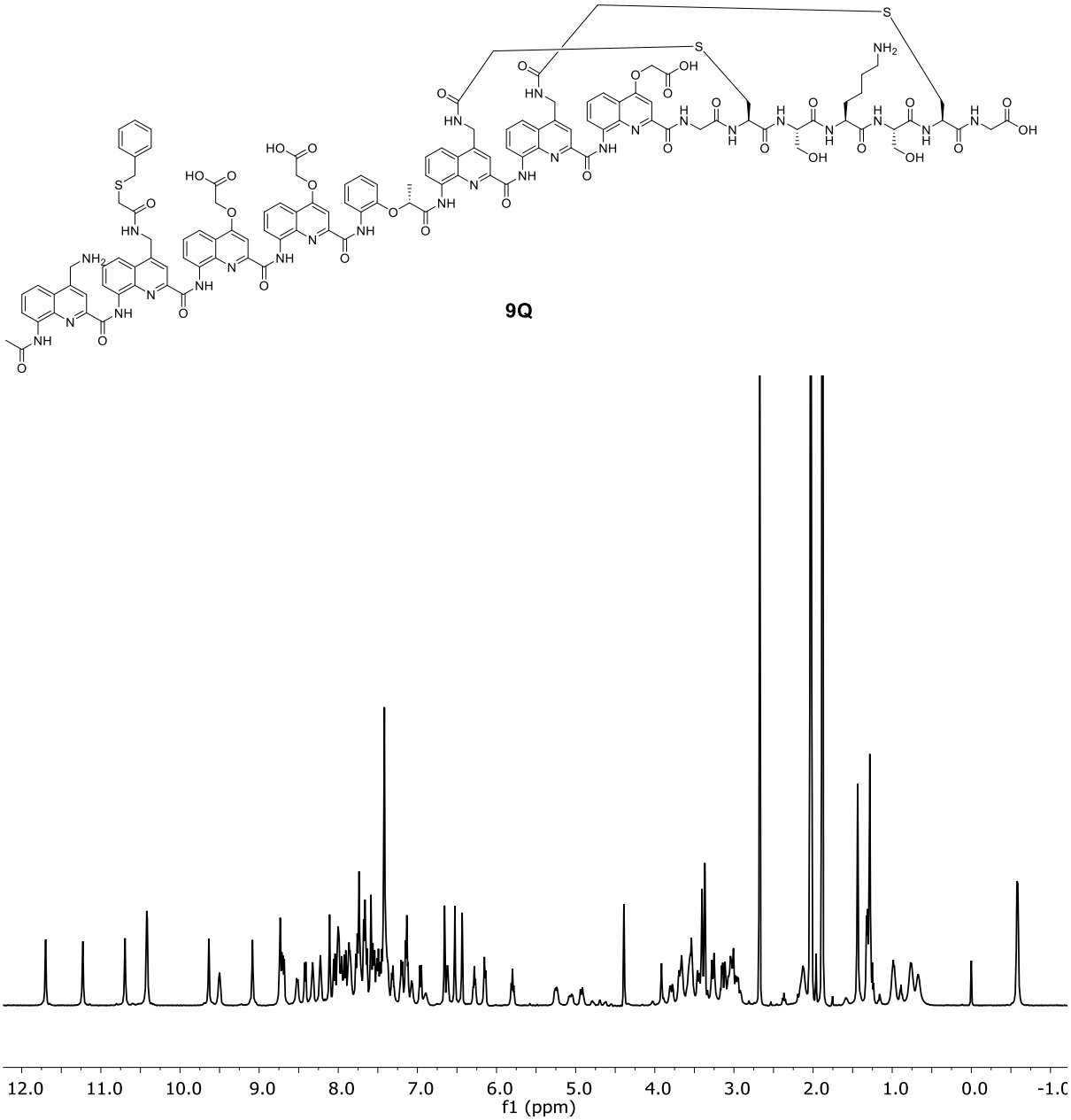
3. NMR spectra of new monomers and isolated macrocyles



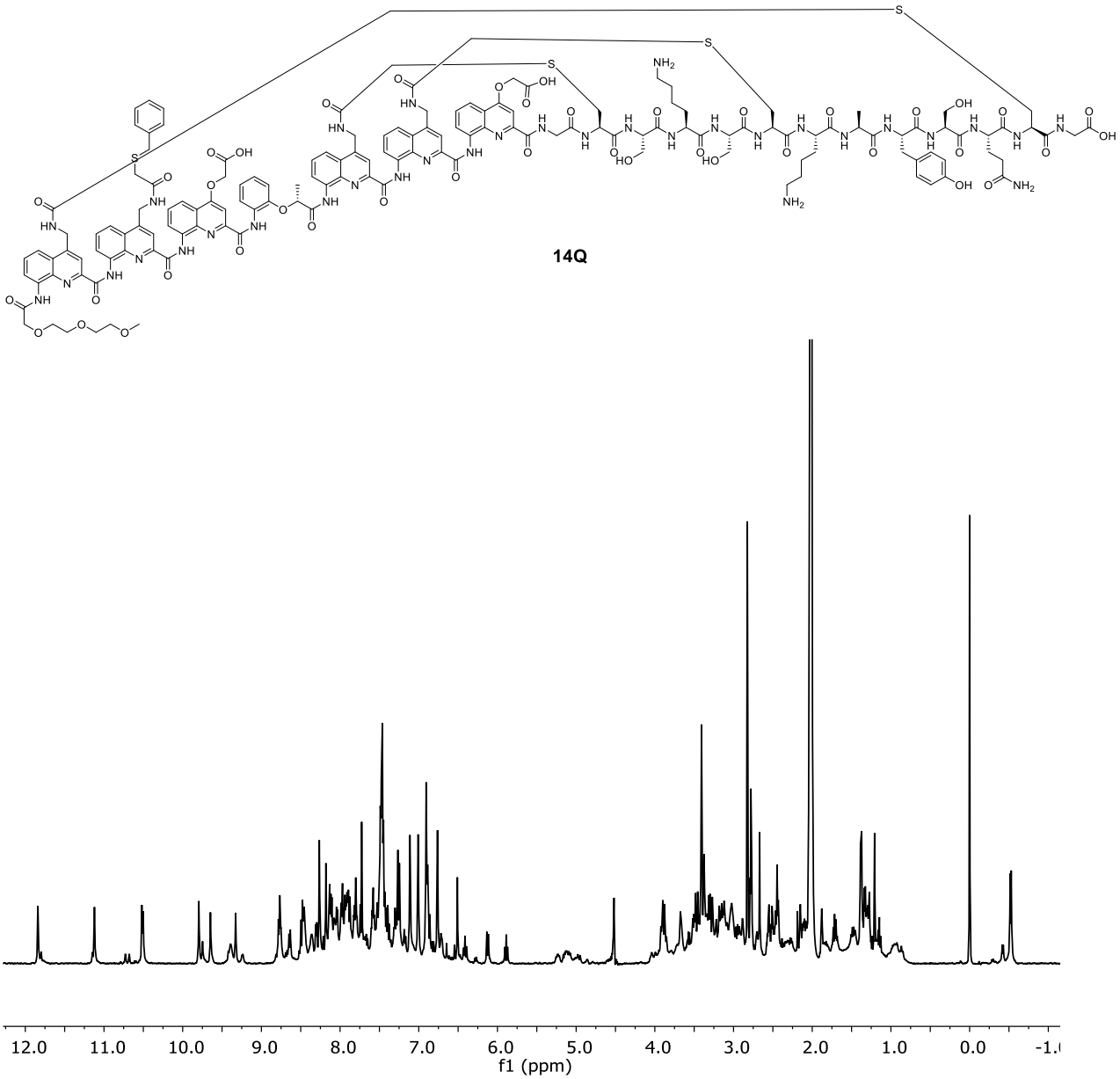
1Q



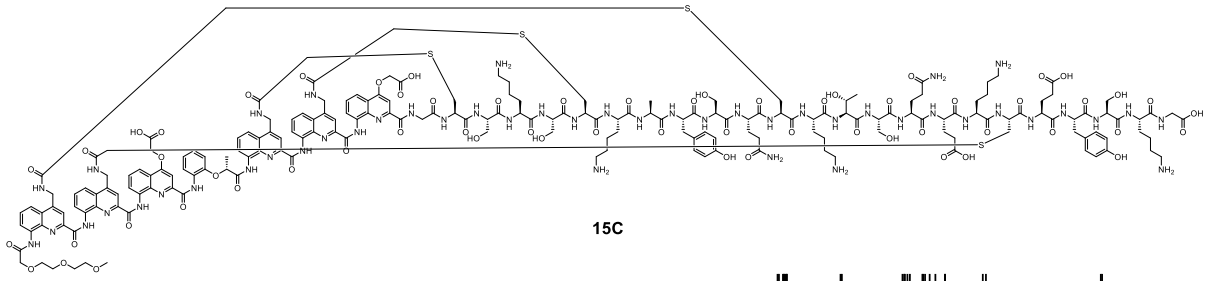
1H NMR Spectrum of **1Q** recorded in a mixture of NH_4OAc (3mM, pH 8.5) in CD_3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C).



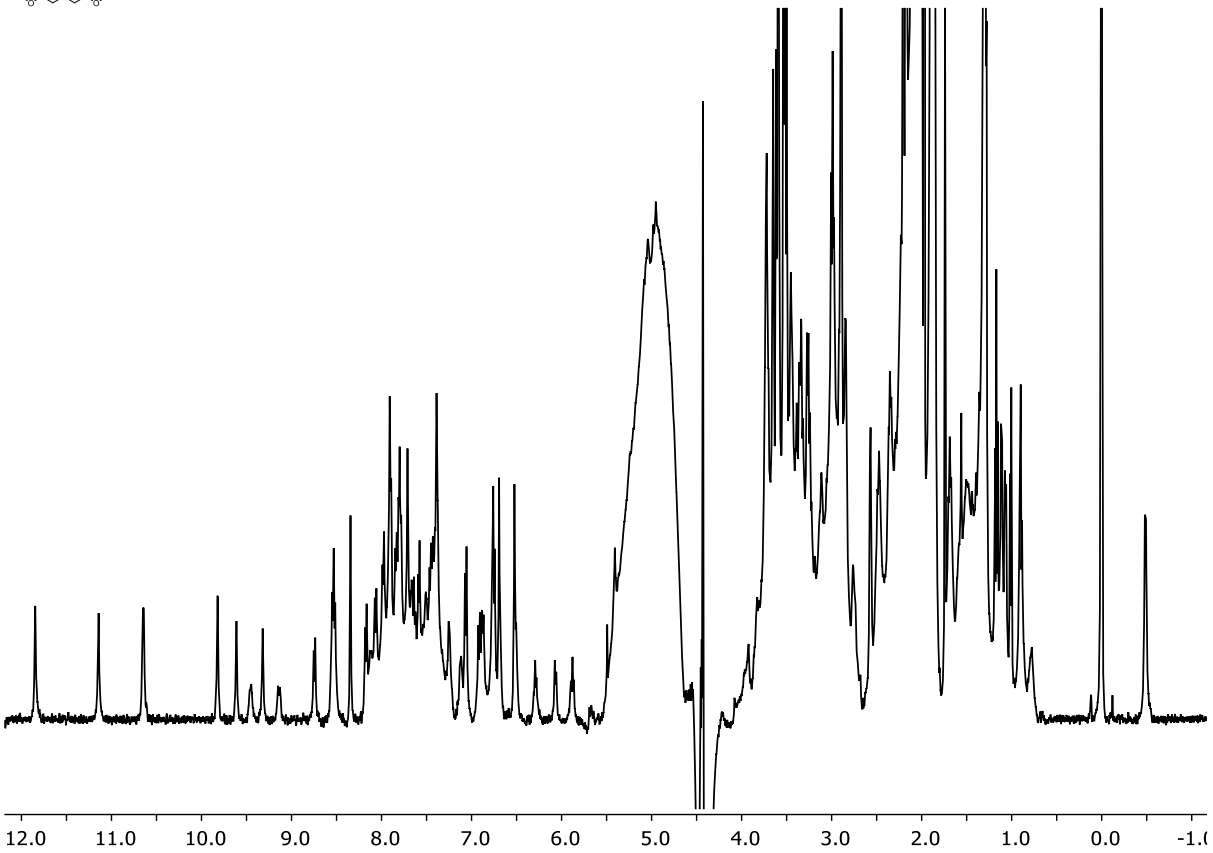
1H NMR Spectrum of **9Q** recorded in a mixture of NH_4OAc (3mM pH 8.5) in CD_3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C).



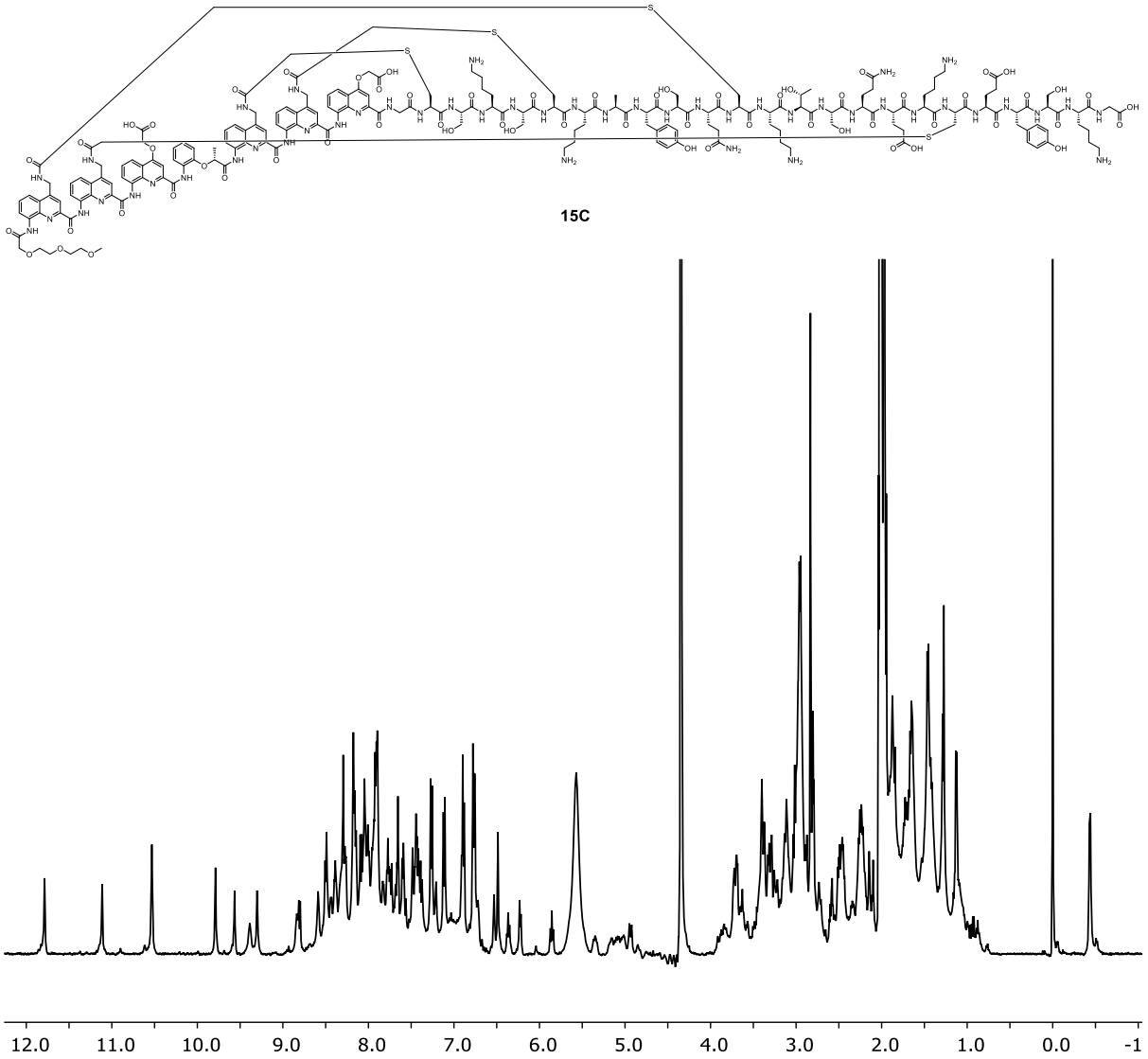
1H NMR Spectrum of **14Q** recorded in a mixture of NH_4OAc (3mM pH 8.5) in CD_3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C).



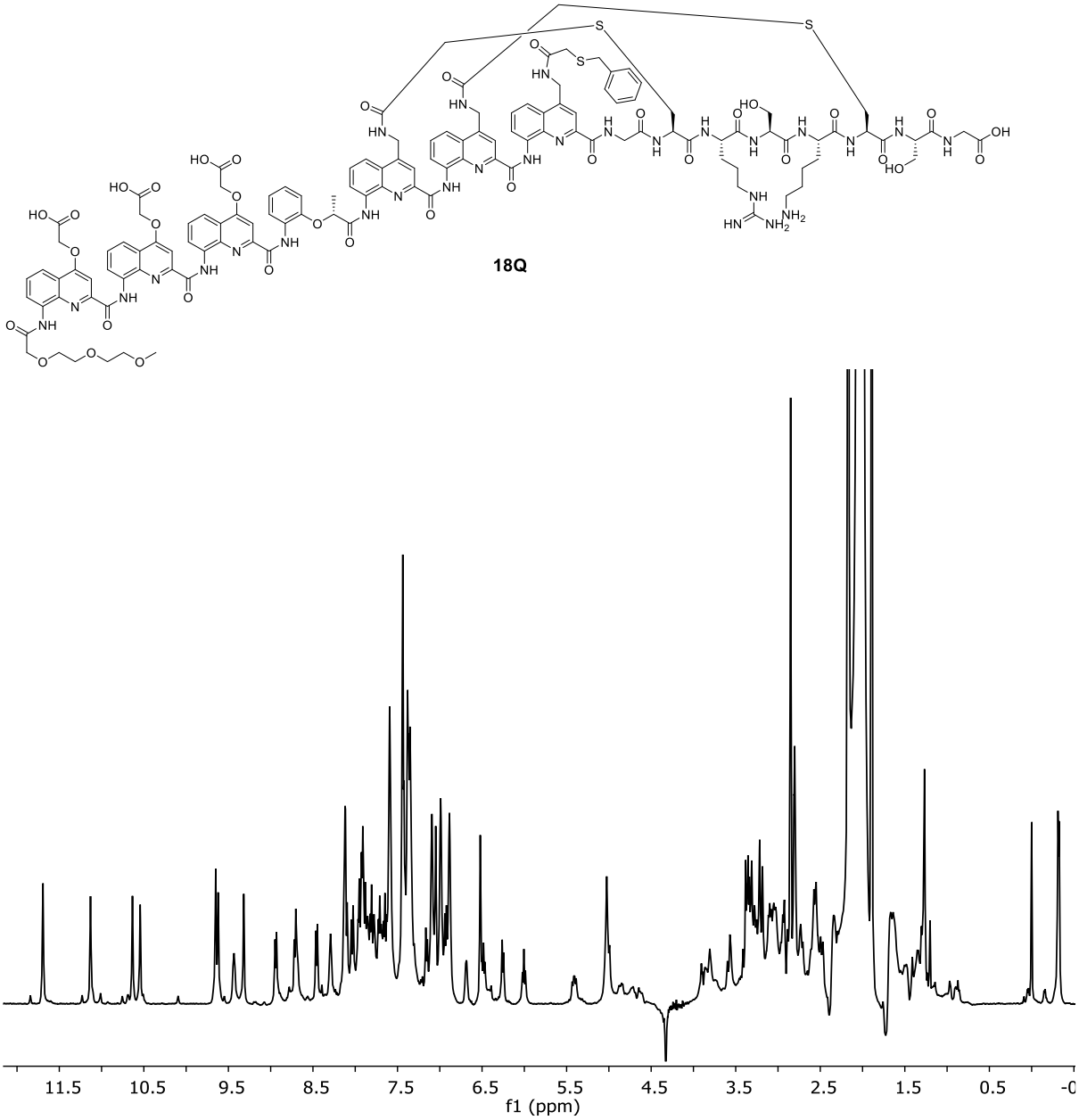
15C



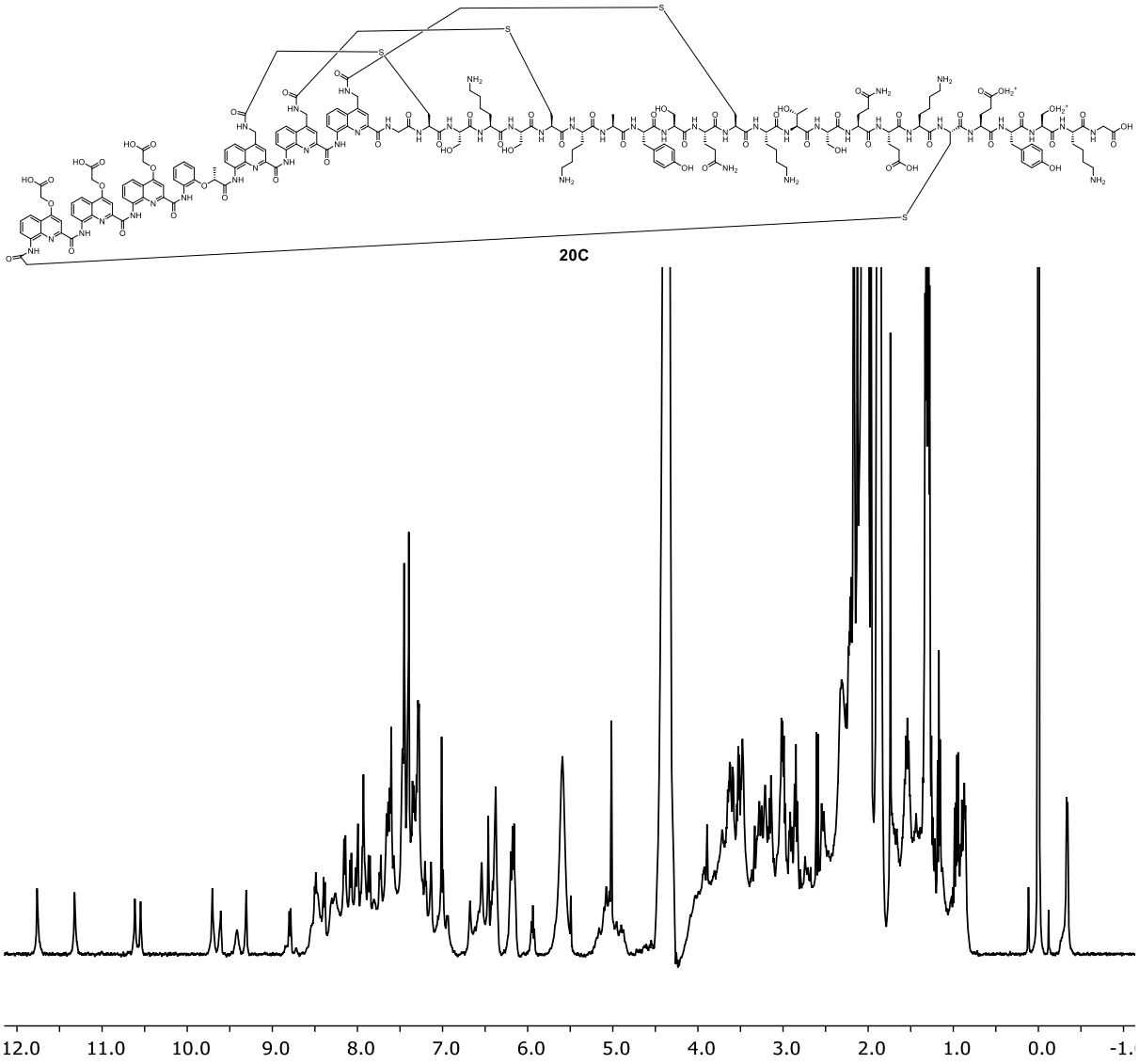
1H NMR Spectrum of **15C-a** recorded in a mixture of NH_4OAc (3mM, pH 8.5) in CD3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C). Similiar NMR signal patterns of **15C-a** and **15C-b** indicate that helices are similarly folded.



1H NMR Spectrum of **15C-b** recorded in a mixture of NH_4OAc (3mM pH 8.5) in CD3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C). Similiar NMR signal patterns of **15C-a** and **15C-b** indicate that helices are similarly folded.

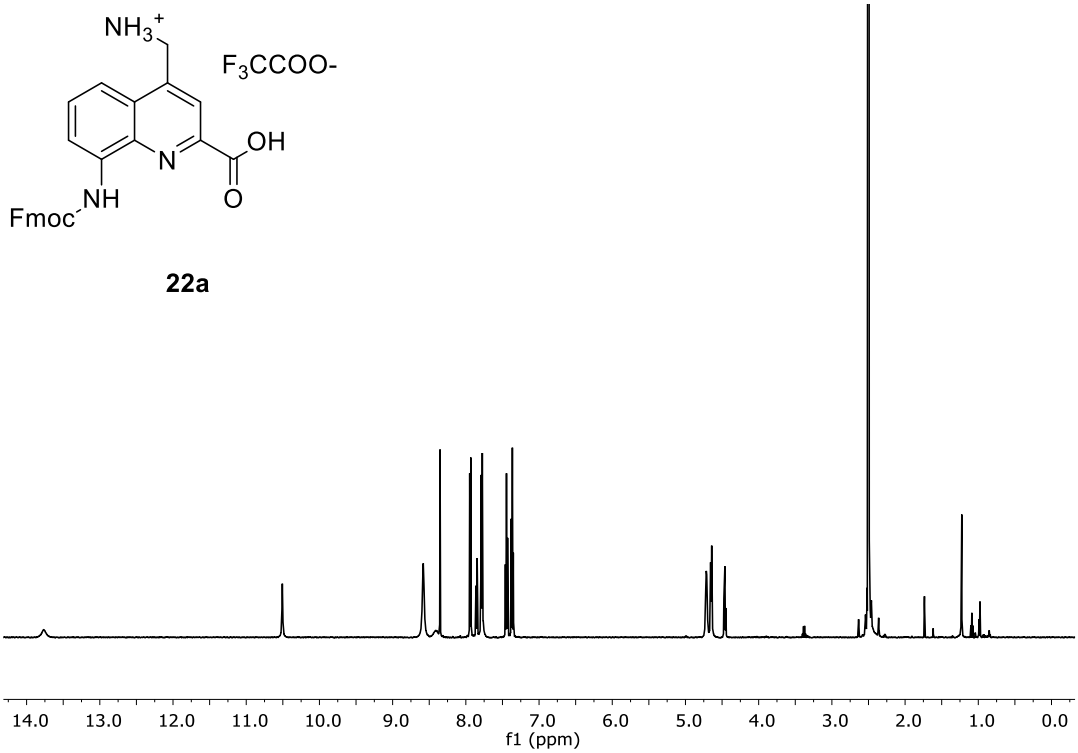


1H NMR Spectrum of **18Q** recorded in a mixture of NH_4OAc (3mM pH 8.5) in CD3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C).



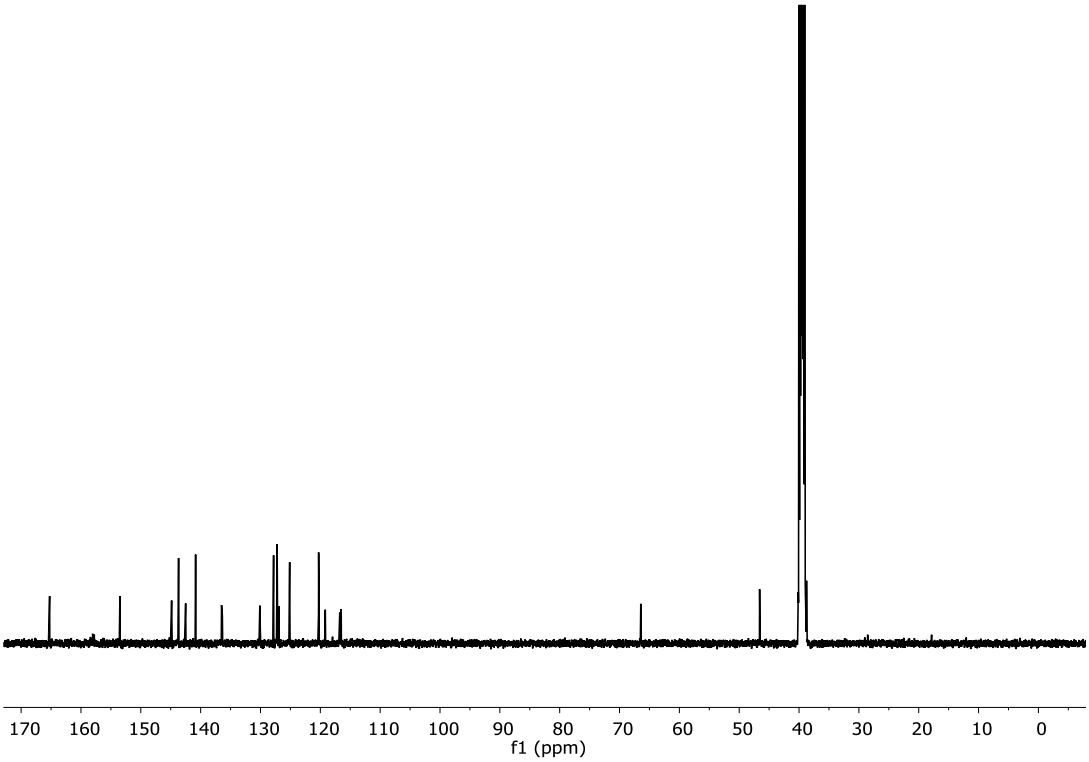
1H NMR Spectrum of **20C** recorded in a mixture of NH_4OAc (3mM pH 8.5) in CD3CN (50:50, v/v) with the addition of TMSP (500 MHz, 25°C).

Compound **22a**



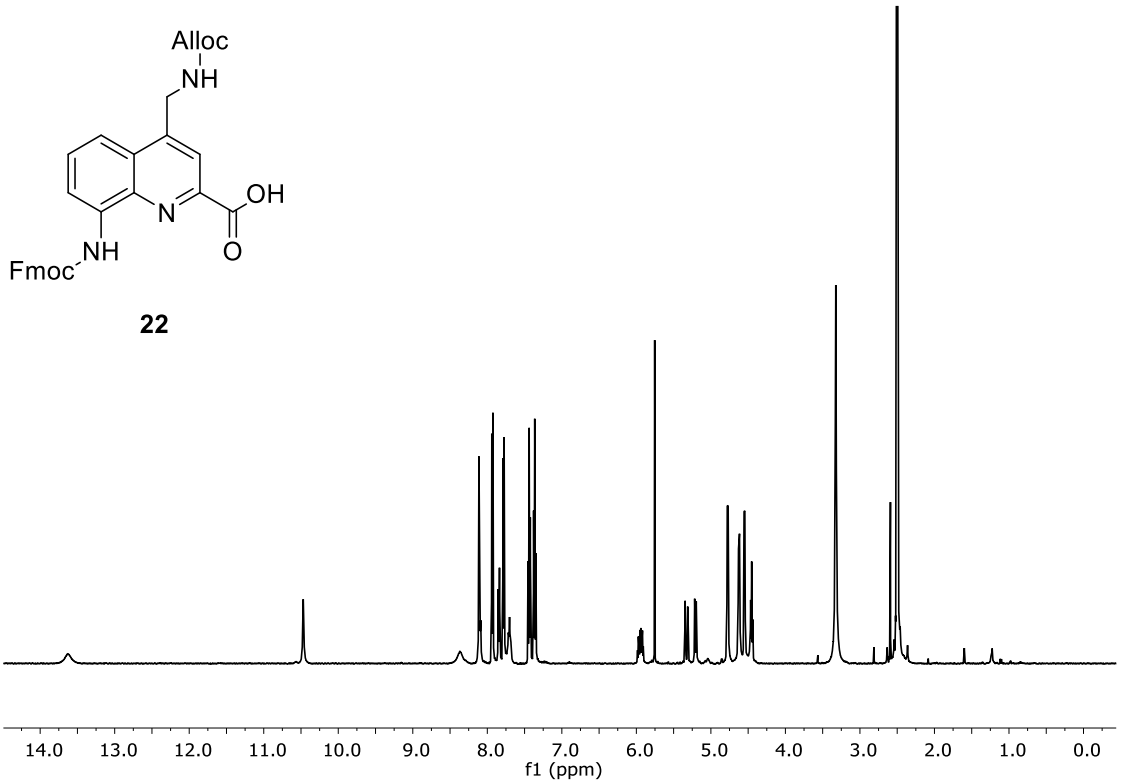
22a

1H NMR Spectrum of **22a** recorded in DMSO-d_6 (500 MHz, 25 °C)



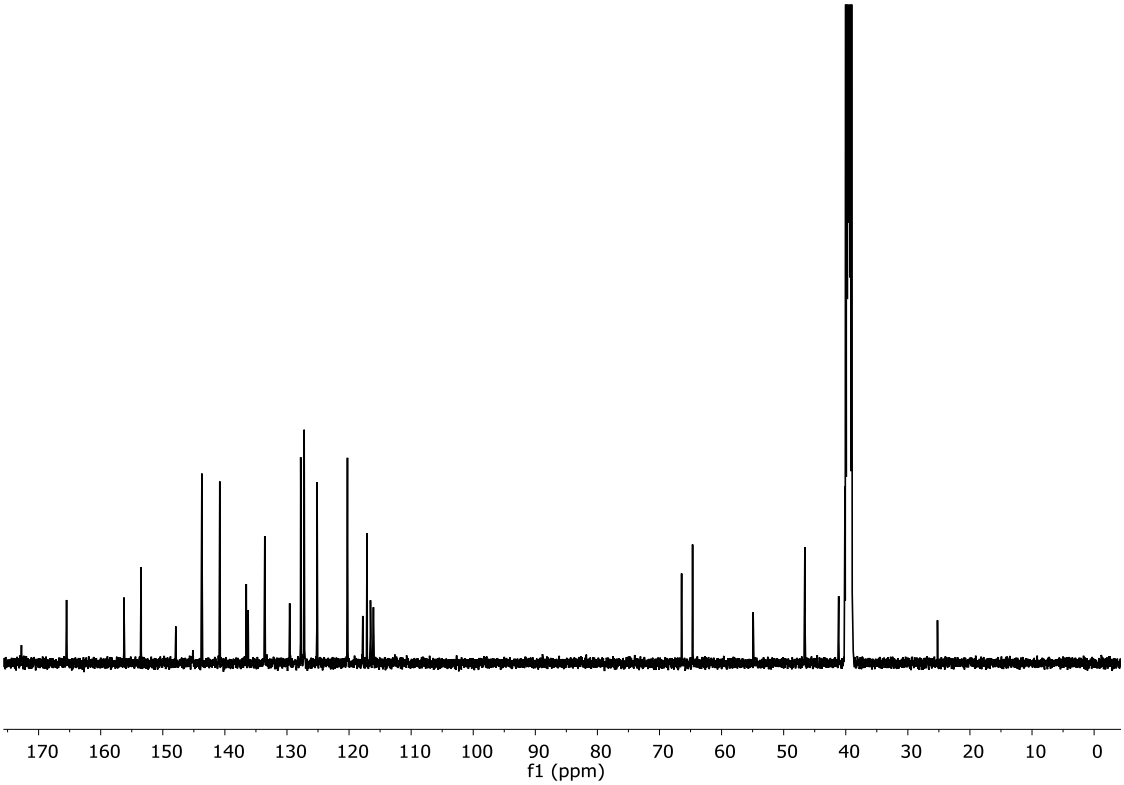
^{13}C NMR Spectrum of **22a** recorded in DMSO-d_6 (126 MHz, 25 °C).

Compound **22**



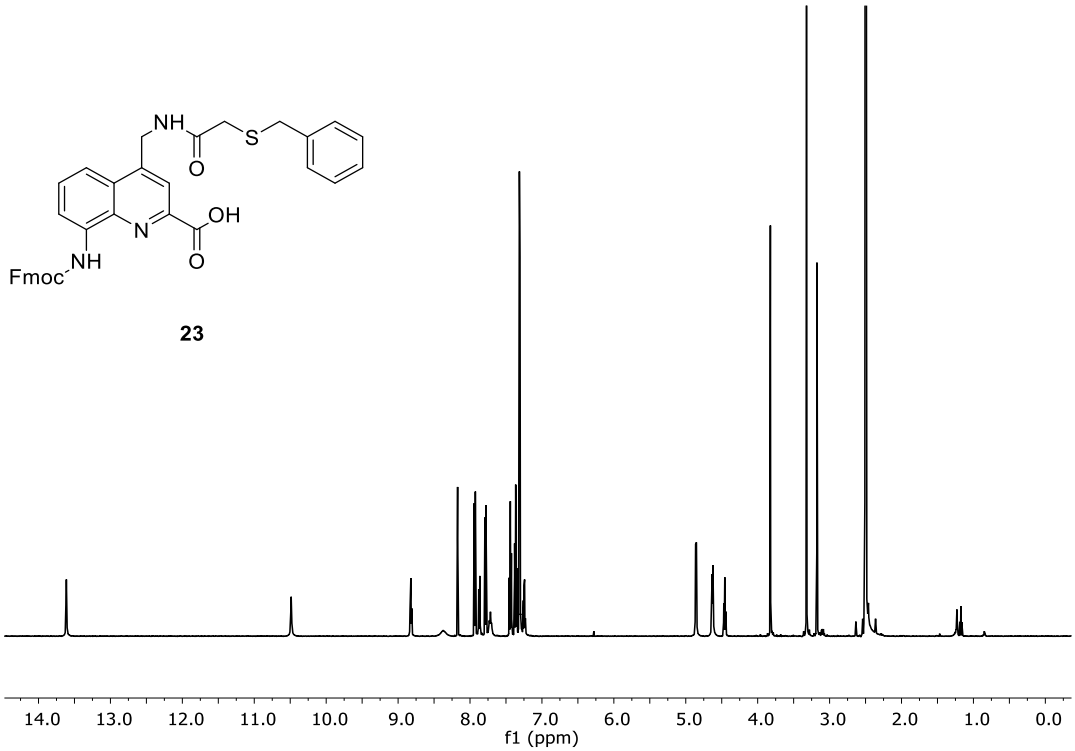
22

^{1}H NMR Spectrum of **22** recorded in DMSO-d_6 (500 MHz, 25 °C).



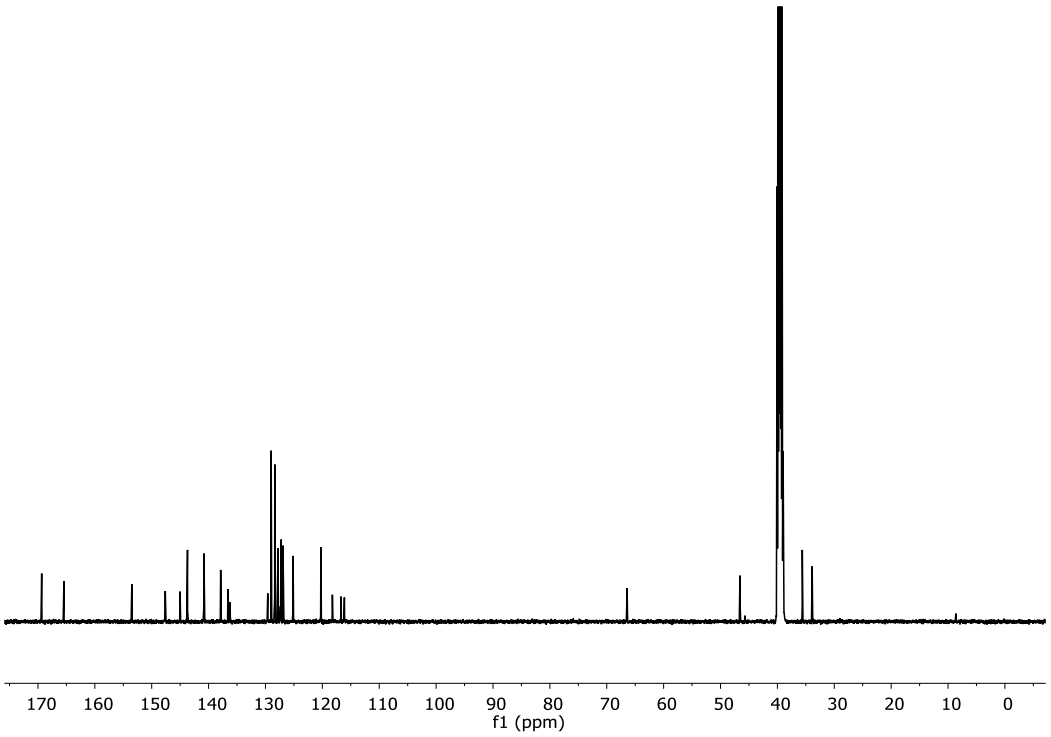
^{13}C NMR Spectrum of **22** recorded in DMSO-d_6 (126 MHz, 25 °C).

Compound **23**



23

^{1}H NMR Spectrum of **23** recorded in DMSO-d_6 (500 MHz, 25 °C).



^{13}C NMR Spectrum of **23** recorded in DMSO-d_6 (126 MHz, 25 °C).

4. References

1. a) M. Vallade, P. Sai Reddy, L. Fischer, I. Huc, Eur. *J. Org. Chem.,* **2018**, *2018*, 5489-5498. b) X. Hu, S. J. Dawson, P. K. Mandal, X. de Hatten, B. Baptiste, I. Huc, *Chem. Sci.*, **2017**, *8*, 3741-3749. c) D. Bindl, E. Heinemann, P. K. Mandal, I. Huc, *Chem. Commun.*, **2021**, *57*, 5662-5665.
2. S. Dengler, P. K. Mandal, L. Allmendinger, C. Douat, I. Huc. *Chem. Sci.,* **2021**,*12,* 11004-11012.