Supplementary information to

Simultaneous cyclization and derivatization of peptides using cyclopentenediones

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1- Supplementary figures

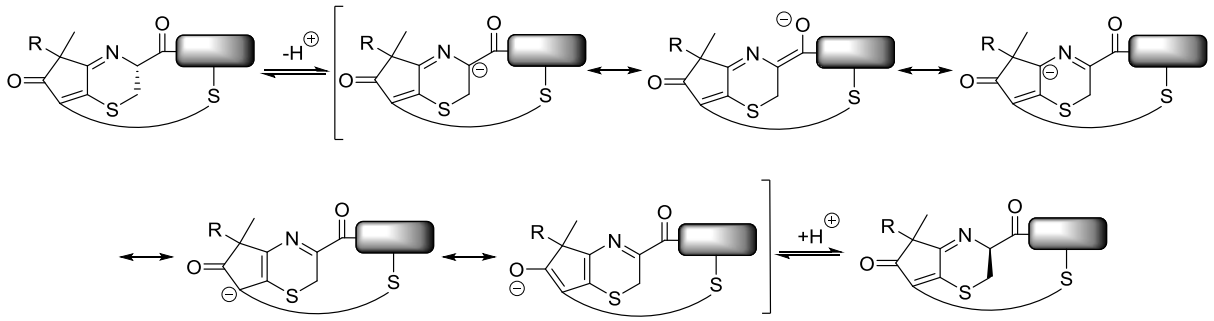


Figure S1. Resonance-stabilization of the plausible intermediate anion in the *N*-terminal Cys epimerization.

2- Materials and methods

DIPC was from Sigma-Aldrich and HOBt·H_2O from Iris Biotech. Fmoc-amino acids were purchased from Novabiochem. Acid-free DCM was obtained by filtration through basic alumina.

Samples were lyophilized in either Labconco or FreezeMobile Virtis instruments. All NMR spectra were recorded on a Brucker 400 MHz spectrometer.

Reversed-phase HPLC analyses and purifications were performed in Shimadzu systems. HPLC/MS analyses were recorded in an Alliance Waters 2690 separation module with a Waters micromass ZQ4000 MS detector.

Procedures for the synthesis and characterization data of the CPDs used in this work can be found in reference 1.

2.1- Peptide synthesis, purification and quantification

Peptides were assembled on a NovaSyn TGR resin (MBHA-PS resin derivatized with PEG chains and a Rink amide linker, Novabiochem), which was washed with DCM (3 ×), DMF (3 ×), MeOH (3 ×), DCM (3 ×). The resin was always allowed to swell in DCM for 2 min before Fmoc removal (all synthesis cycles), which was effected by reaction with 20 % piperidine/DMF (1 × 3 min + 1 × 10 min), followed by washing with DMF (5 ×) and DCM (5 ×). Incorporation of the first amino acid onto the resin and peptide assembly were accomplished by using 3 equiv of both Fmoc-amino acid, HOBt·H_2O and DIPC, all dissolved in the minimal amount of NMP, for 90 min at rt. Coupling was followed by washing with DCM, DMF and MeOH (3 × each). In case the coupling was not complete, as assessed by the Kaiser test,[2] it was repeated using 2 equiv of the reagents. If necessary, unreacted amines were capped by reaction with Ac_2O/2,6-lutidine/DMF (5:6:89 mixture, 2 × 5 min) for 15 min at rt, which was followed by washing with DMF (5 ×) and DCM (5 ×).

Cysteine-containing peptides were cleaved from the resin and deprotected by using a 94:2.5:1:2.5 TFA/H_2O/TIS/EDT mixture. Filtrate and washings (TFA) were collected and concentrated by blowing N_2 over the mixture, and diethyl ether was added to the resulting oil or semi-solid. After precipitating the

peptide, the suspension was centrifuged (7800 rpm, 10 min, 0 ºC) and the diethyl ether supernatant discarded. This procedure was performed three times. The precipitated peptide was dissolved in water and lyophilized before analysis and purification by HPLC.

Pure peptides were quantified by UV/Vis spectroscopy on the basis of their absorbance at 280 nm (Tyr, ε_{280}=1490; Trp, ε_{280}=5500).

2.2- HPLC and HPLC-MS analysis and purification

HPLC:·

· Analysis conditions: Jupiter Proteo column (4 µm, 90 Å, 250 × 4.6 mm) from Phenomenex. Linear gradients of 30 min were always used. Solvent A: 0.045% TFA in water, solvent B: 0.036% TFA in ACN, flow: 1 mL/min, detection wavelength: 200 to 500 nm (diode array detector). Unless otherwise stated, all injections were performed at rt.

· Purification conditions: Jupiter Proteo column (10 µm, 90 Å, 250 × 10.0 mm) from Phenomenex. Linear gradients of 30 min were always used. Solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in ACN, flow: 3 mL/min, detection wavelength: 220 and 260 nm. Unless otherwise stated, all injections were performed at rt.

HPLC-MS:

Jupiter Proteo column (4 µm, 90 Å, 250 × 4.6 mm) from Phenomenex. Linear gradients of 30 min were always used. Solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid in ACN, flow: 1 mL/min, detection wavelength: 210 to 600 nm (diode array detector). Unless otherwise stated, all injections were performed at rt. When samples with salts were injected to the HPLC-MS, the MS detector was disconnected for the first five minutes after the injection to avoid salt deposition on the equipment.

3- Characterization of the linear peptides

	Structure	Characterization
1	H-Cys-Trp-Gly-Arg-Gly-Cys-NH_2	HPLC-MS: 0-30% B, t_R = 8.5 min MS: *m/z* 680.0 $[M+H]^+$, calcd. for $C_{27}H_{41}N_{11}O_6S_2$ 679.3 Purification conditions: 15-30% B, t_R = 8.7 min
2	H-Cys-Ser-Tyr-Ala-Cys-Lys-Tyr-Gly-NH_2	HPLC-MS: 0-30% B, t_R = 12.3 min MS: *m/z* 893.5 $[M+H]^+$, calcd. for $C_{38}H_{56}N_{10}O_{11}S_2$ 892.4 Purification conditions: 15-45% B, t_R = 8.2 min
3	H-Cys-Ser-Tyr-Ala-Cys-NH_2	HPLC-MS: 0-30% B, t_R = 9.8 min MS: *m/z* 544.8 $[M+H]^+$, calcd. for $C_{21}H_{32}N_6O_7S_2$ 544.2 Purification conditions: 15-50% B, t_R = 7.1 min

4	H-Cys-Tyr-Ser-Ala-Cys-NH_2	HPLC-MS: 0-30% B, t_R = 8.5 min MS: *m/z* 544.9 $[M+H]^+$, calcd. for $C_{21}H_{32}N_6O_7S_2$ 544.2 Purification conditions: 15-40% B, t_R = 6.7 min
5	H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2	HPLC-MS: 10-60% B, t_R = 9.6 min MS: *m/z* 1009.2 $[M+H]^+$, calcd. for $C_{43}H_{68}N_{12}O_{12}S_2$ 1008.5 Purification conditions: 25-50% B, t_R = 6.8 min
6	H-Cys-Asn-Trp-Ala-Val-DAla-His-Leu-Cys-NH_2	HPLC-MS: 10-40% B, t_R = 11.5 min MS: *m/z* 1015.5 $[M+H]^+$, calcd. for $C_{44}H_{66}N_{14}O_{10}S_2$ 1014.5 Purification conditions: 25-50% B, t_R = 9.6 min
7	H-DCys-Trp-Gly-Arg-Gly-Cys-NH_2	HPLC-MS: 0-30% B, t_R = 9.5 min MS: *m/z* 680.0 $[M+H]^+$, calcd. for $C_{27}H_{41}N_{11}O_6S_2$ 679.3 Purification conditions: 15-30% B, t_R = 10.2 min
8	H-DCys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2	HPLC-MS: 10-60% B, t_R = 15.0 min MS: *m/z* 1009.2 $[M+H]^+$, calcd. for $C_{43}H_{68}N_{12}O_{12}S_2$ 1008.5 Purification conditions: crude deemed pure enough for use
9	H-DCys-Asn-Trp-Ala-Val-DAla-His-Leu-Cys-NH_2	HPLC-MS: 10-40% B, t_R = 10.8 min MS: *m/z* 1015.5 $[M+H]^+$, calcd. for $C_{44}H_{66}N_{14}O_{10}S_2$ 1014.5 Purification conditions: 25-50% B, t_R = 8.3 min
10	H-DCys-Ser-Lys-Ala-Tyr-Gly-Cys-Tyr-Gly-NH_2	HPLC-MS: 0-50% B, t_R = 9.1 min MS: *m/z* 950.3 $[M+H]^+$, calcd. for $C_{40}H_{59}N_{11}O_{12}S_2$ 949.4 Purification conditions: 0-50% B, t_R = 17.5 min

4- Description of the optimized cyclization methods

· Optimized protocol 1 (no LiCl):

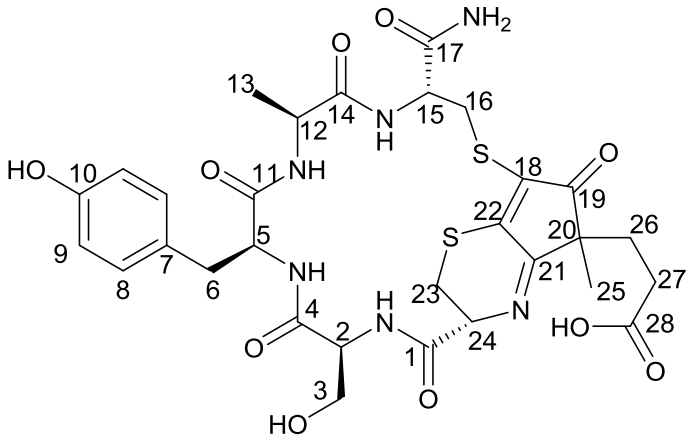
Freeze-dried aliquots of the peptide (typically 100 nmol, but some reactions were carried out at a higher scale) were dissolved in either water or H_2O/MeOH mixtures in an eppendorf tube, and 1.2-1.5 equiv of CPD (from a concentrated solution) were added. The total amount of solvent was such that at this point peptide concentration was 0.5 mM. The reaction was left to proceed at 60 ºC (without any particular measure, no N_2 or Ar atmosphere). HPLC-MS analyses were regularly performed (normally every 45-60 min) until an approximate 1:1 ratio between the **M**-18 and the **M**-20 Da adducts was detected (as determined at 280 nm). At this stage, the reaction temperature was lowered to 37 ºC and 0.2 equiv of TEMPO (from a 2 mM solution in water) were added. 60 min later, new additions of 0.2 equiv of TEMPO were performed every 30 min until completion of the reaction.

· Optimized protocol 2 (presence of LiCl):

Freeze-dried aliquots of the peptide (500 nmol in most cases) were dissolved in either water or H_2O/MeOH mixtures in an eppendorf tube. Then, LiCl (from a 8 M aqueous solution), 1.2-1.5 equiv of CPD (from a concentrated solution) and 0.2 equiv of TEMPO (from a 2 mM aqueous solution) were subsequently added. The total amount of solvent was such that at this point peptide and LiCl concentrations were 0.5 mM and 2 M, respectively. The resulting mixture was allowed to react at 60 ºC (without any particular measure, no N_2 or Ar atmosphere). Additional 0.2 equiv of TEMPO were added at 60 and 90 min reaction times, and the reaction analyzed and purified after 120 min reaction time.

5- NMR characterization of the cyclic adduct

Product **3a** was synthesized following the optimized protocol 1, with the only difference that a 2.0 mM peptide concentration was used. 20 parallel reactions at a 1 µmol scale were run to obtain enough product for NMR characterisation. Both isomers were directly subjected to NMR after HPLC purification and lyophilization. Both had small impurities, likely reduced TEMPO and remaining excess of CPD **a** (as observed in the ^{1}H NMR), which did not pose any problems to characterize both isomers of product **3a**. Assignment of each peak was achieved as follows. Each ^{13}C peak was assigned as a primary, secondary, tertiary or quaternary carbon using the DEPT spectrum. Afterwards, HSQC was used to link each of them to its corresponding H atoms. The tyrosine aromatic system was used as a starting point to ensemble the peptide structure due to its ease to be identified. HMBC correlations of the aromatic ring allowed for the identification of its α and β carbons. The carbonyl amide signal correlating with both the α and β hydrogens was that of the own amino acid, while the carbonyl correlating only with the α hydrogens was that of the amino acid at its *N*-terminus. In a third step and from these amide signals, HMBC correlations permitted to identify the α hydrogens of the amino acids located at the tyrosine *C*- and *N*-termini. From these signals, the side-chains of said amino acids could be pinpointed. Expansion of this methodology towards both the *N* and *C*-terminal positions permitted to link each C and H atom of the peptide structure to an NMR signal.

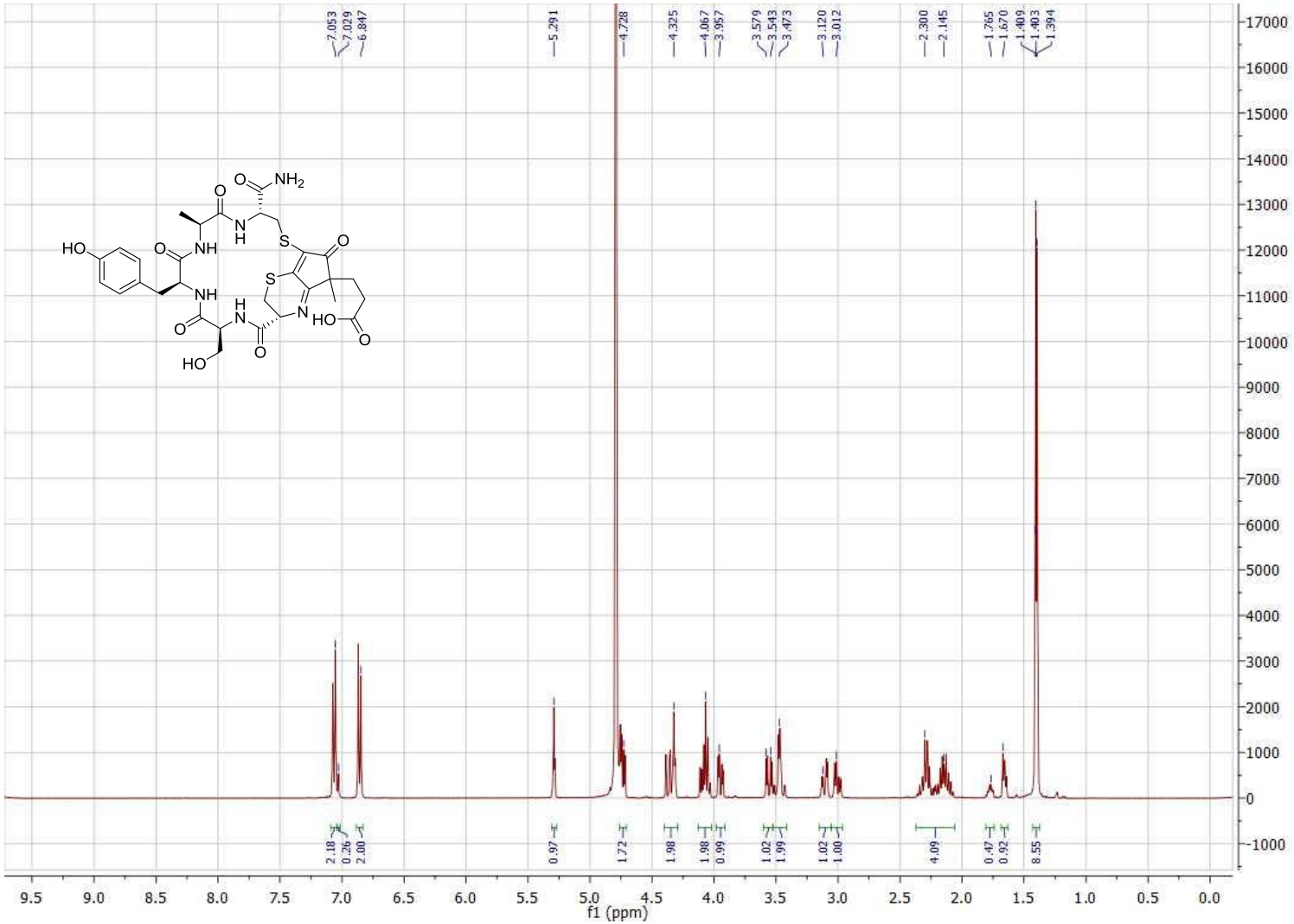


Isomer 1 (t_R = 9.0 min, see section 12)		
Position	C δ (ppm)	H δ (ppm) and multiplicity
1	169.0	-
2	57.2	4.29-4.40 (m)
3	60.1	4.02-4.12 (m) + 3.95 (dd, J_1 = 11.6 Hz, J_2 = 3.6 Hz)
4	169.7	-
5	52.3	4.70-4.77 (m)
6	36.9	3.12 (dd, J_1 = 14.0 Hz, J_2 = 3.6 Hz) + 3.00 (dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz)
7	127.0	-
8	131.7	7.06 (d, J = 8.8 Hz)
9	115.6	6.86 (d, J = 8.8 Hz)
10	154.8	-
11	170.4	-
12	50.3	4.02-4.12 (m)
13	15.4	1.37-1.43 (m)
14	175.0	-
15	52.6	4.70-4.77 (m)
16	30.8	4.29-4.40 (m) + 3.55 (dd, J_1 = 14.0 Hz, J_2 = 4.4 Hz)
17	173.4	-
18	148.1	-
19	198.7	-
20	51.8	-
21	172.5	-
22	143.0	-
23	25.0	3.42-3.52 (m)
24	54.9	5.29 (t, J = 2.8 Hz)
25	19.7	1.37-1.43 (m)
26	31.1	2.06-2.22 (m)
27	29.4	2.22-2.38 (m)
28	176.6	-

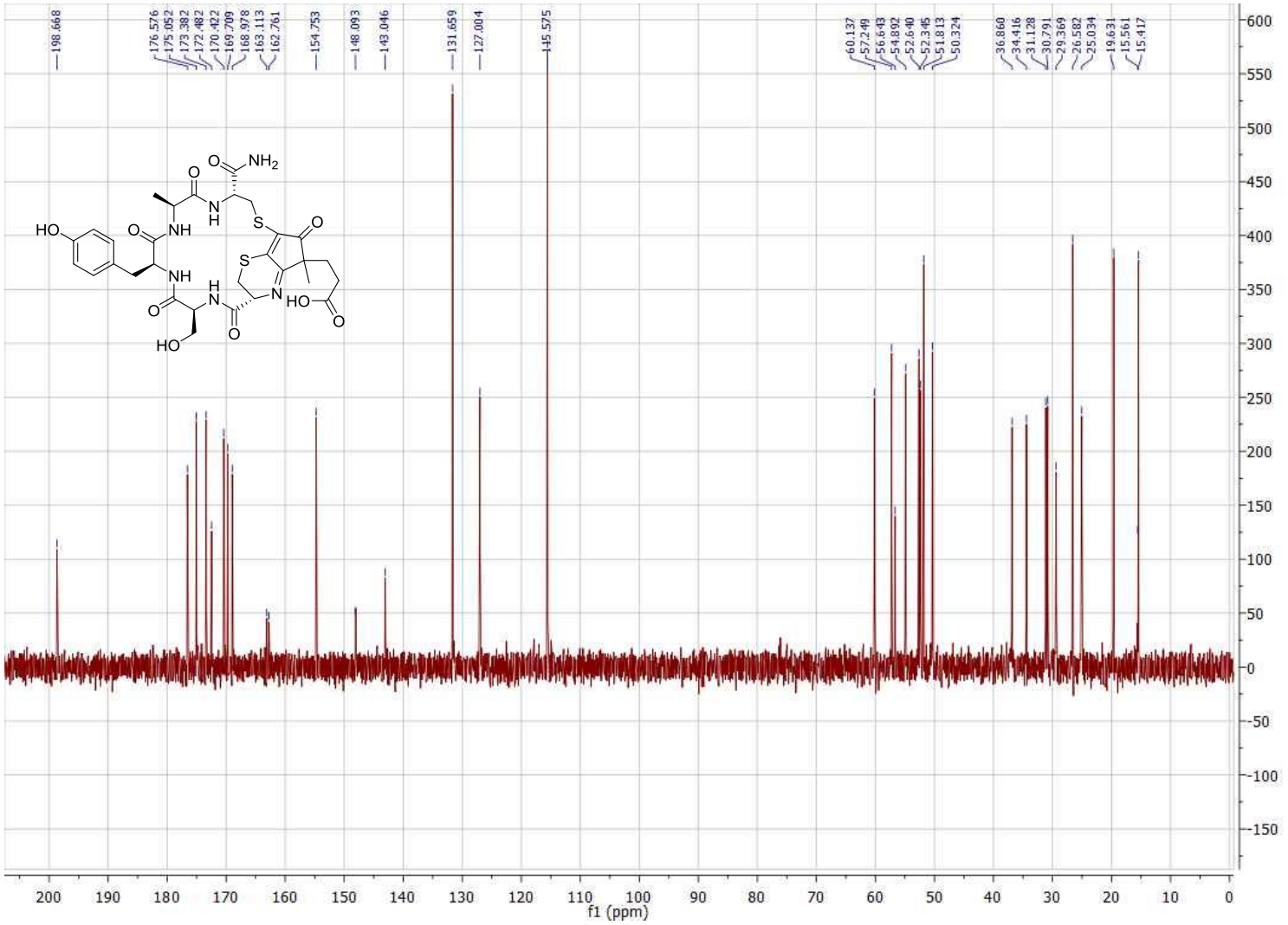
Isomer 2 (t_R = 10.4 min, see section 12)		
Position	C δ (ppm)	H δ (ppm) and multiplicity
1	168.9	-

2	57.2	4.33 (dd, J_1 = 6.0 Hz, J_2 = 3.2 Hz)
3	60.3	4.05-4.12 (m) + 3.93 (dd, J_1 = 12.0 Hz, J_2 = 3.2 Hz)
4	169.5	-
5	52.3	Under the HDO signal
6	37.0	3.11 (dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz) + 3.00 (dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz)
7	127.0	-
8	131.8	7.07 (d, J = 8.8 Hz)
9	115.5	6.86 (d, J = 8.8 Hz)
10	154.9	-
11	170.1	-
12	50.3	4.05-4.12 (m)
13	15.5	1.39 (d, J = 7.2 Hz)
14	175.4	-
15	52.7	Under the HDO signal
16	30.7	4.44 (dd, J_1 = 14.0 Hz, J_2 = 11.2 Hz) + 3.55 (dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz)
17	173.5	-
18	150.0	-
19	198.1	-
20	51.7	-
21	173.0	-
22	143.0	-
23	25.2	3.44-3.54 (m)
24	54.9	5.31 (t, J = 2.8 Hz)
25	20.6	1.37 (s)
26	30.2	2.10-2.34 (m)
27	28.7	2.10-2.34 (m) + 2.46-2.56 (m)
28	176.9	-

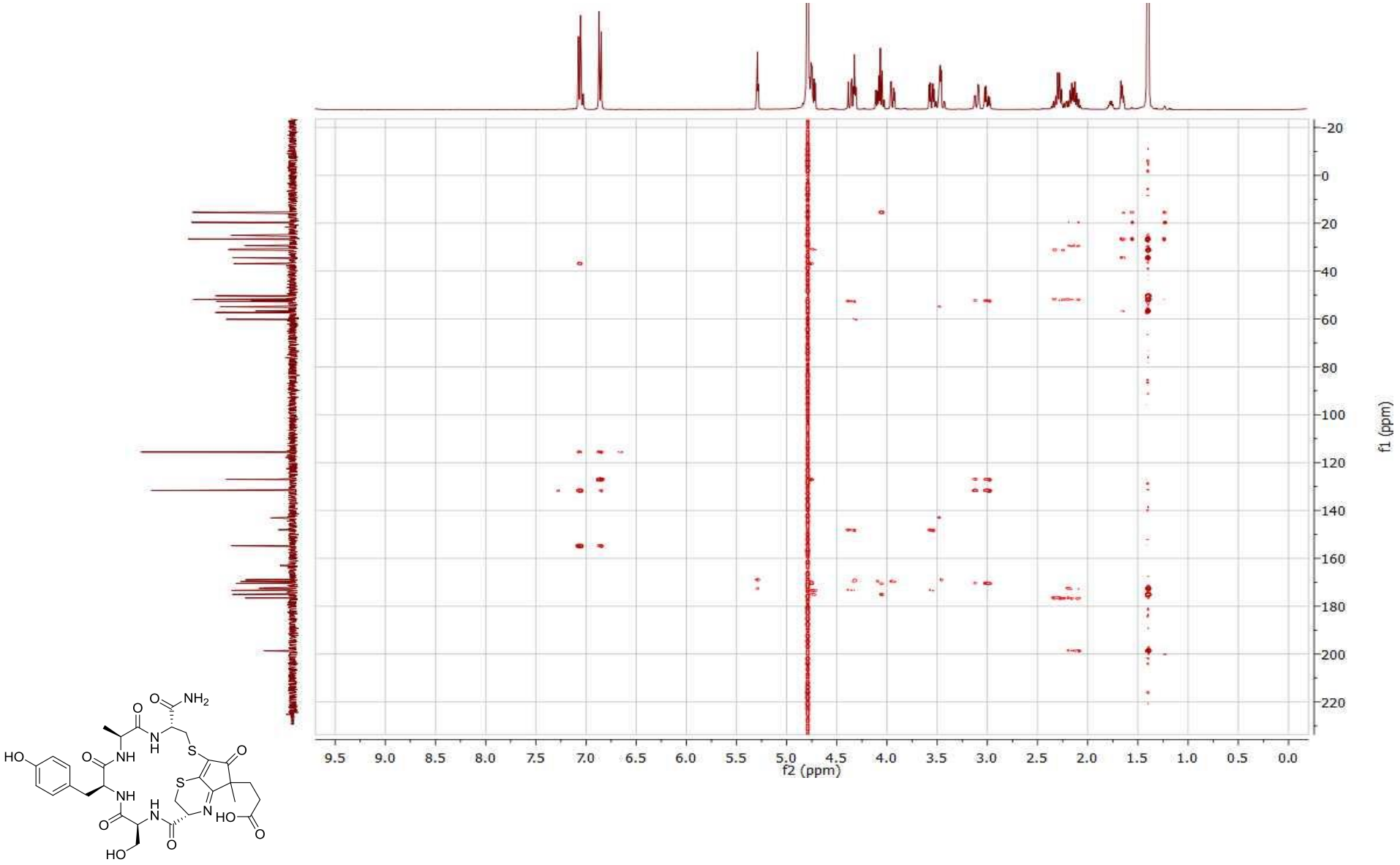
^{1}H NMR of Isomer 1



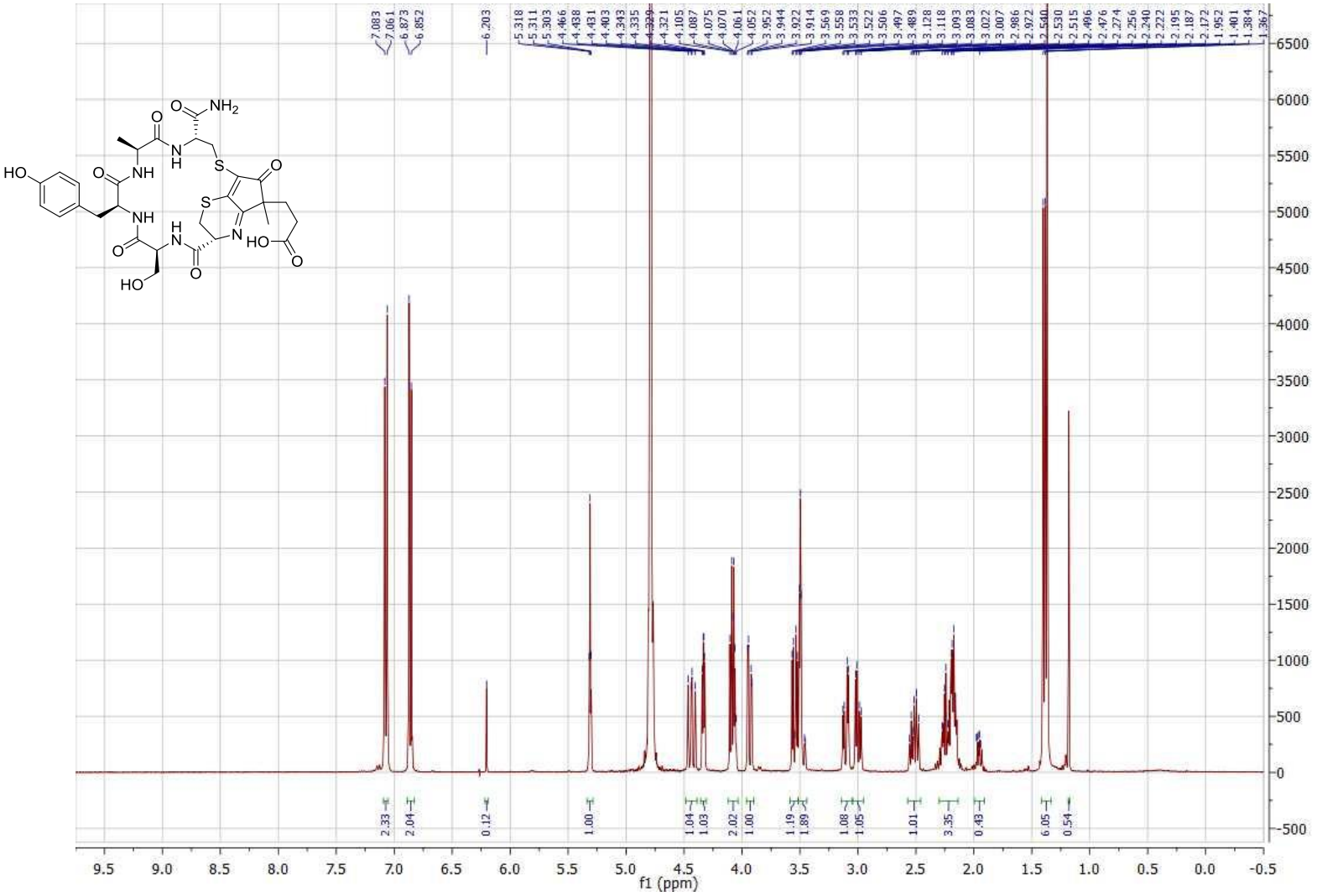
^{13}C NMR of Isomer 1



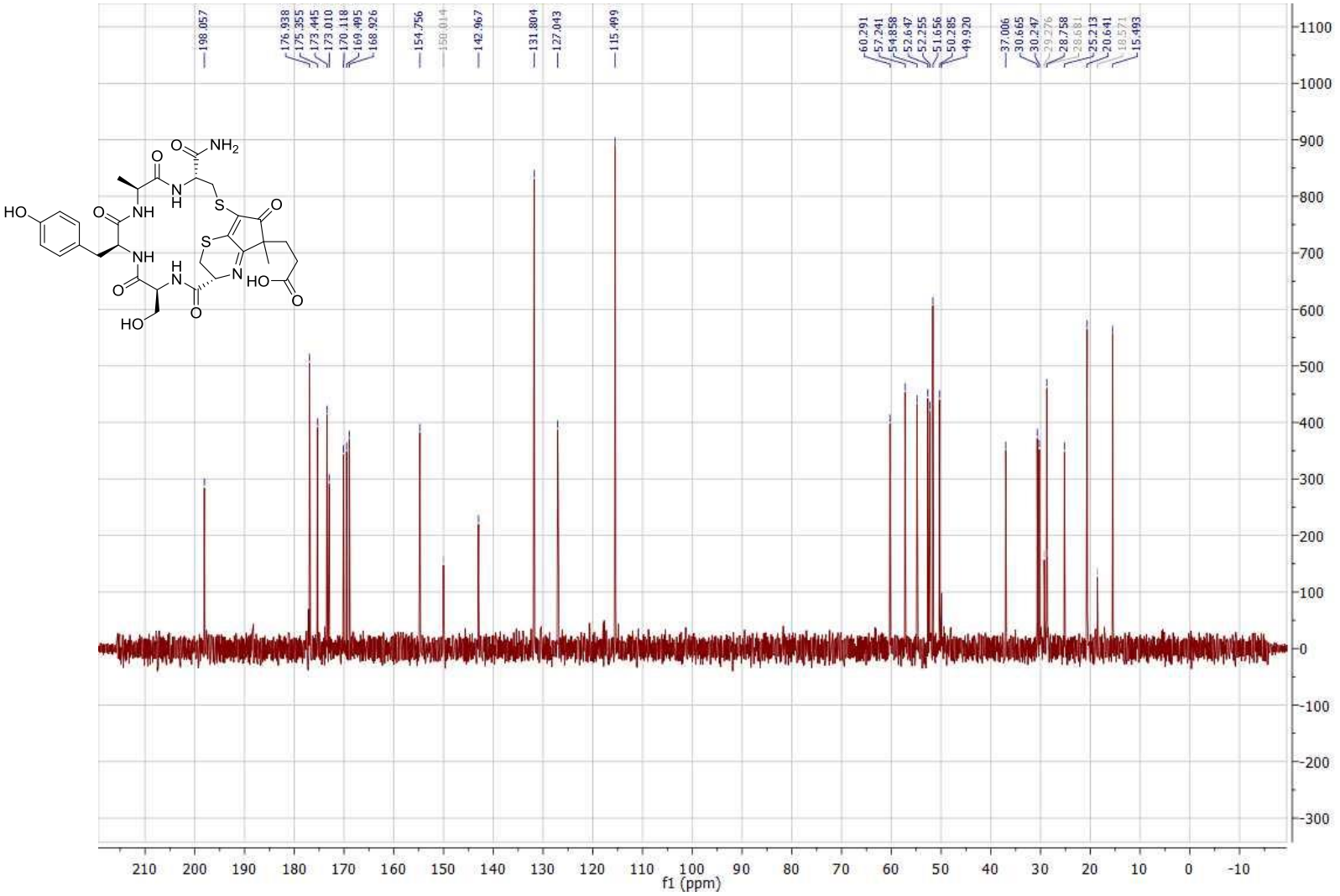
HMBC NMR of Isomer 1



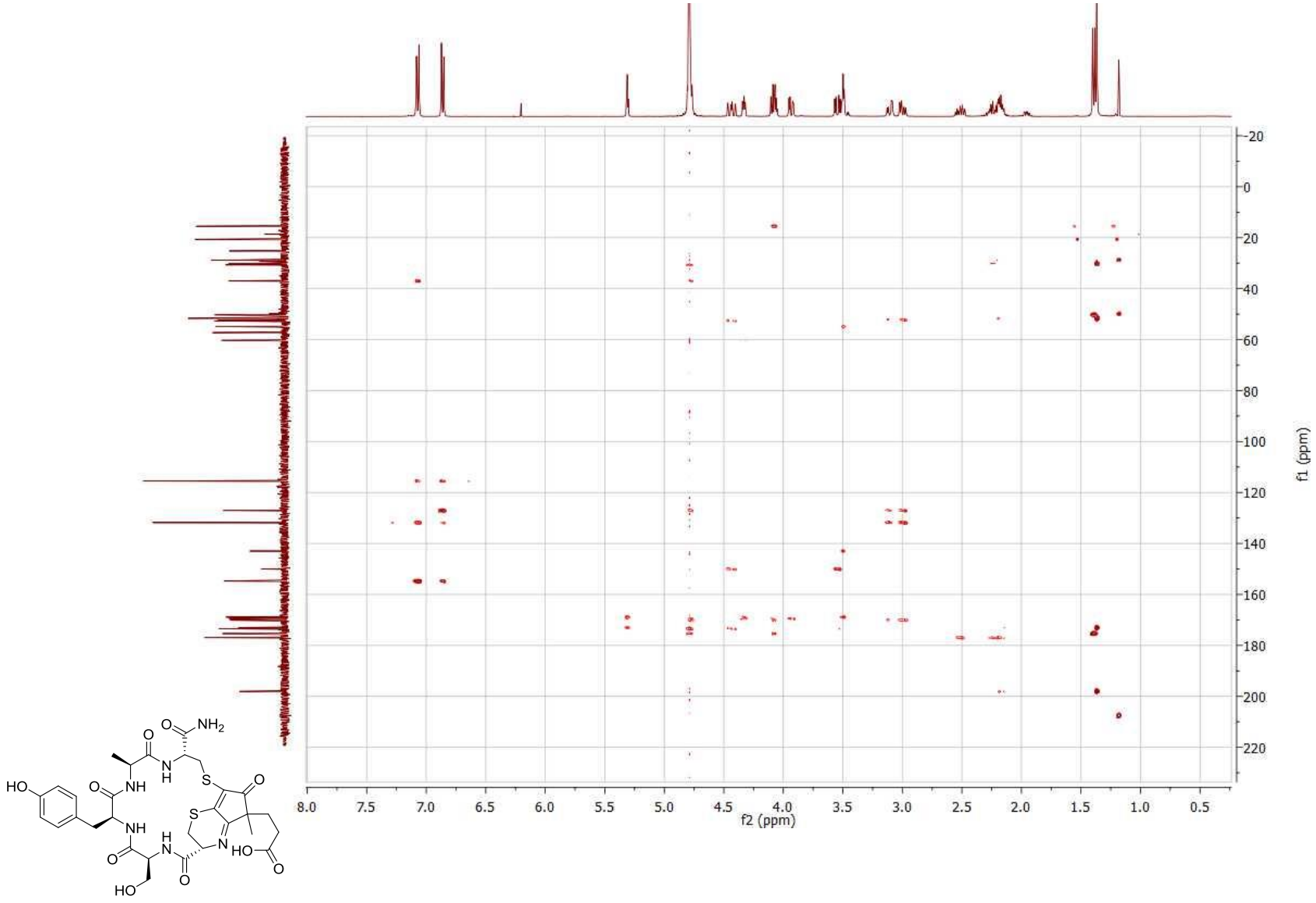
^{1}H NMR of Isomer 2



^{13}C NMR of Isomer 2

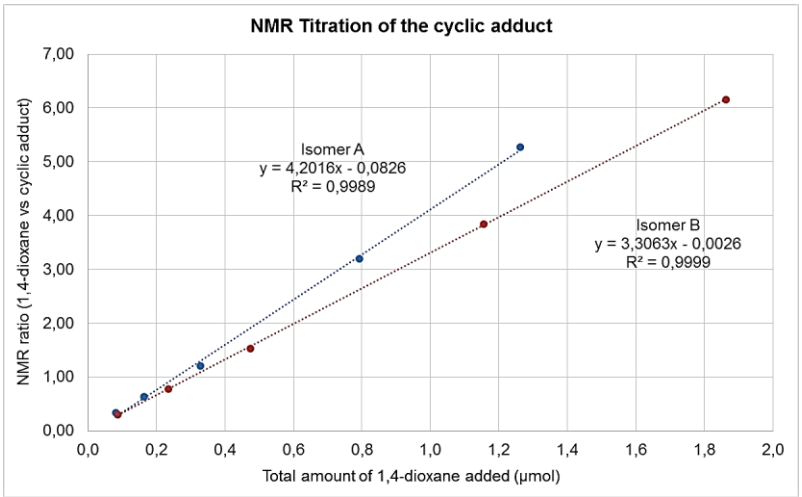


HMBC NMR of Isomer 2



6- Determination of the molar absorption coefficient

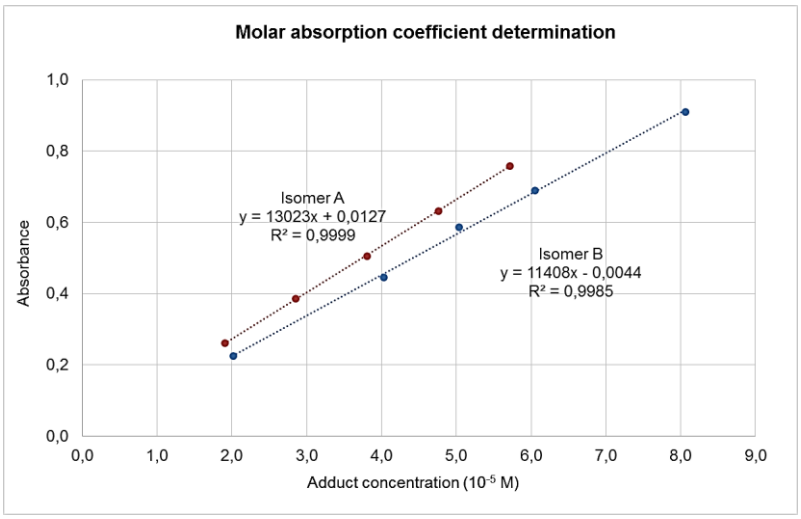
The already purified and characterized isomers 1 and 2 of **3a** were dissolved in an approximately 1:1 (v/v) d_6-DMSO/D_2O mixture and titrated by NMR. 1,4-Dioxane (extra pure quality, 99+ %) was used as titrating agent. This was passed through a basic alumina column to remove peroxides and stored over calcium hydride before use. For the titration process, carefully weighed amounts of a 1,4-dioxane solution in D_2O were sequentially added to each of the isomers' solutions. NMR spectra were recorded (the relaxation delay was set to 35 s to allow for the complete relaxation of all the H nuclei present in the sample) and the integration of both the cyclic adduct and the titrating agent was measured.
Data obtained are shown below. This allowed to quantify the amount of cyclic adduct in the solution as follows:



Amount of cyclic adduct:

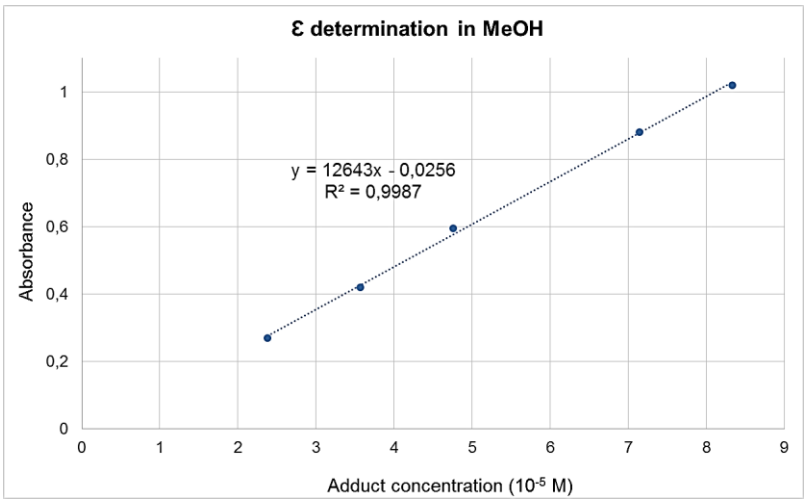
Isomer A: $\frac{8}{1}$ x $\frac{1}{4.202} = 1.904\ \mu mol$ Isomer B: $\frac{8}{1}$ x $\frac{1}{3.306} = 2.419\ \mu mol$

Both isomers were, after the titration process, quantitatively recovered from the NMR tube and lyophilized. Once this had been done, each isomer was dissolved in DMSO and diluted with water to obtain different solutions of known concentration, reaching a maximum DMSO value of 2 % (v/v), and the absorbance of these solutions was measured. Molar absorption coefficients in water were obtained from the slope of the line of best fit obtained when representing the absorbance of the different solutions versus their concentration (the cuvette pathlength was 1 cm). The mean value, which was rounded to the closest five or ten, was calculated from the two independently obtained molar absorption coefficients. Data obtained are shown below.



$\varepsilon = 12215\ M^{-1}\ cm^{-1}$

To determine the molar absorption value in methanol only isomer A was used (the sample of isomer B was lost during its manipulation). This isomer was dissolved in methanol and the absorbance of different dilutions (in methanol) measured. Data obtained can be seen below. The slope of the line of best fit was, again, the molar absorption value, which was rounded to the closest five or ten.



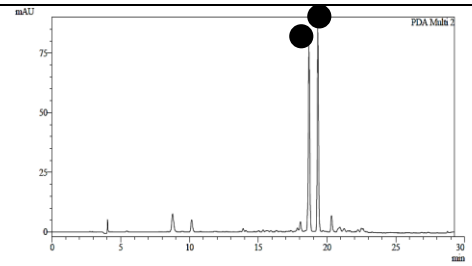
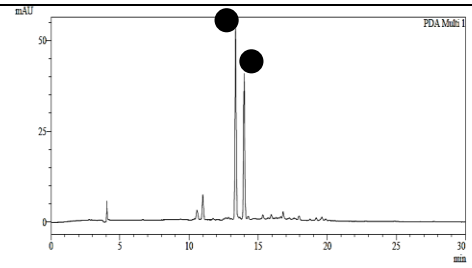
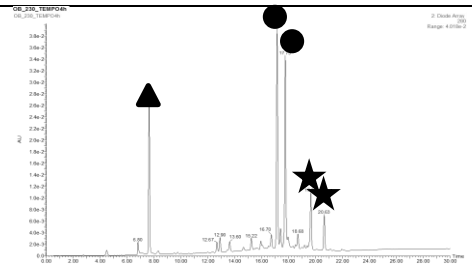
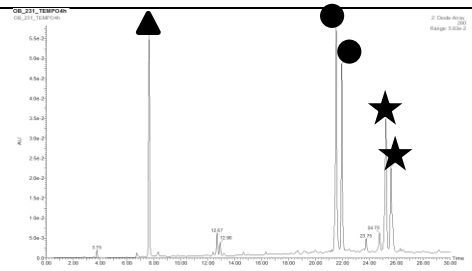
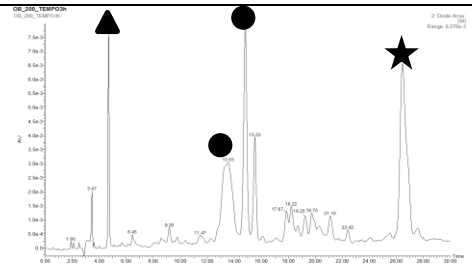
$\varepsilon = 12645\ M^{-1}\ cm^{-1}$

7- Reactions appearing in Table 1

All reactions were carried out at the 100 nmol scale and following the optimized protocol 1. Water was used as solvent in all cases except in the **1** + **c** reaction, in which a 1:1 water/methanol mixture was used. Peptide sequences are shown in section 3.

Product code:

●: Desired cyclic adduct ▲: Cyclic disulfide ★: Thiol oxidation by-product

Reaction	Reaction time	HPLC-MS Yield (at 280 nm)	HPLC or HPLC-MS trace (280 nm)[1]
1 + **a**	2.75 h	81%	
2 + **a**	2.25 h	84%	
1 + **b**	5.5 h	64%	
1 + **c**	6.5 h	49%	
5 + **a**	4 h[2]	> 30%	

[1] **1** + **a** and **2** + **a**: HPLC. Other entries: HPLC-MS.

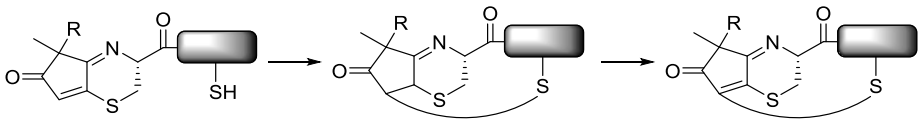
[2]Reaction not allowed to evolve further due to the large amount of side-products.

8- 3 + a and 4 + a cyclizations

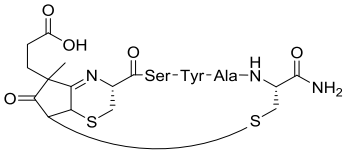
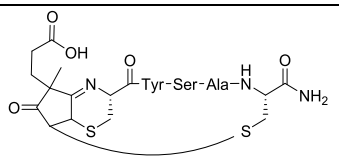
Peptides **3** and **4** (100 nmol) were independently dissolved in water and incubated with 1.3 equiv of CPD **a** (previously dissolved in water as well) at 37 ºC (after the two solutions were mixed, peptide concentration was 0.5 mM). 0.20 equiv of TEMPO (from a 2 mM aqueous solution) were added to each reaction mixture at t = 0, 60, 90, 120 and 150 min. HPLC/MS analyses were performed after 30, 60, 120, 180 and 240 min reaction times. These experiments were run in duplicate, and in one of the experiments involving peptide **4**, a further addition of TEMPO at 240 min and an HPLC/MS analysis after a total of 300 min were performed.

A third experiment (in the same conditions) was run to monitor the progression of the early stages of the reaction (0-120 min). Peaks corresponding to the **M**-18 adduct, **M**-20 adduct, cyclized but unoxidized intermediate, final cyclic peptide and thiol oxidation by-product were integrated at 280 nm to evaluate and compare both reactions. Due to co-elution problems, the ratio between the **M**-18 and the **M**-20 Da adducts could not be determined for the **3** + **a** reaction.

In these experiments, an intermediate adduct, with a mass of **M**-22 Da and showing no absorbance above 300 nm was detected and assigned to the cyclic, non-oxydized product:



· Characterization of the intermediate products:

Reaction	Structure	Characterization
3 + **a**		HPLC-MS: 0-50% B, t_R = 17.6 min MS: *m/z* 707.2 $[M+H]^+$, calcd. for $C_{30}H_{38}N_6O_{10}S_2$ 706.2
4 + **a**		HPLC-MS: 0-50% B, t_R = 17.2 min MS: *m/z* 707.2 $[M+H]^+$, calcd. for $C_{30}H_{38}N_6O_{10}S_2$ 706.2

Data obtained are depicted in the following table. Reproducibility problems were encountered at those stages of the reaction when the cyclic adduct started to be formed, likely due to the large amount of processes taking place simultaneously. Despite this, general trends could be inferred and clear conclusions drawn at long reaction times (after 3 h).

	Product percentages							
	Reactions of peptide **3**			Reactions of peptide **4**				
Time	**M**-18 + **M**-20 Da	Intermediate	Cyclic adduct	**M**-18 + **M**-20 Da	**M**-18/**M**-20 Da	Intermediate	Cyclic adduct	Thiol oxidation by-product
30min	79	14	7	91	38/55	5	2	n.d
	38	17	45	92	41/51	5	3	n.d
	90	7	3	91	35/56	6	3	n.d
1 h	43	21	36	91	31/60	5	4	n.d
	42	20	38	92	31/61	5	3	n.d
	56	24	20	91	28/63	6	3	n.d
2 h	19	10	71	66	0/66	7	27	n.d
	17	10	73	47	0/47	1	52	n.d
	18	12	70	57	0/57	5	38	n.d
3 h	5	3	92	22	0/22	0	68	10
	8	6	86	17	0/17	0	69	14
4 h	0	0	100	19	0/19	0	70	11
	0	0	100	15	0/15	0	70	15
5 h	n.d.	n.d.	n.d.	0	0/0	0	77	23

Note: Due to co-elution problems, the ratio between the **M**-18 and the **M**-20 Da adducts could not be determined for the **3** + **a** reaction.

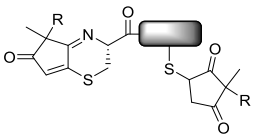
9- Effect of LiCl in the reaction mixture

Peptide **5** (500 nmol, 0.5 mM concentration after addition of **a**) and 2 equiv of **a** were reacted for 45 min at 60 ºC, at which time the **M**-18/**M**-20 Da adducts ratio was almost 1:1 (as seen by HPLC/MS, data not shown). After this, the temperature was lowered to 37 ºC and the reaction was split into four aliquots. Solid lithium chloride was added to three of them to reach the following final concentrations: 5 molal (aliquot A), 2 molal concentration (aliquot B) and 1 molal concentration (aliquot C). No LiCl was added to the fourth aliquot (aliquot D) to serve as control. At the same time, 0.20 equiv of TEMPO were added to each of the aliquots (also to aliquot D) prior to be left shaking at 37 ºC.

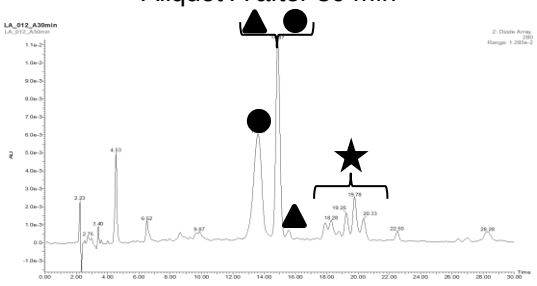
HPLC/MS details: 25-35 % B, traces shown at 280 nm

Product code:

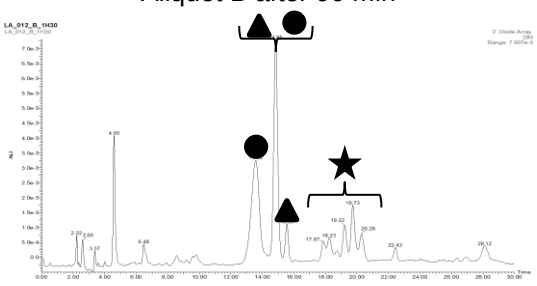
●: Cyclic adduct; ▲: **M**-20 Da adduct, ★: Double addition products:



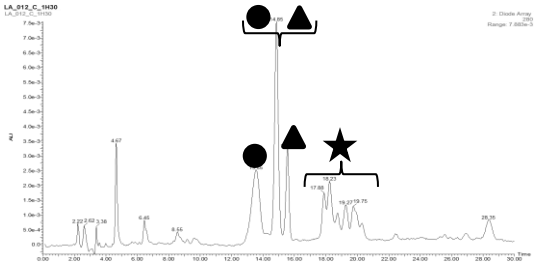
Aliquot A after 30 min



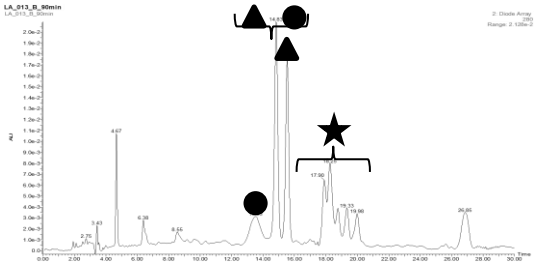
Aliquot B after 90 min



Aliquot C after 90 min



Aliquot D after 90 min



Aliquot:	A[a] (5 molal LiCl)	B[b] (2 molal LiCl)	C[b] (1 molal LiCl)	D[b] (no LiCl)
5a/**M**-20 Da ratio:	97:3	88:12	70:30	35:65

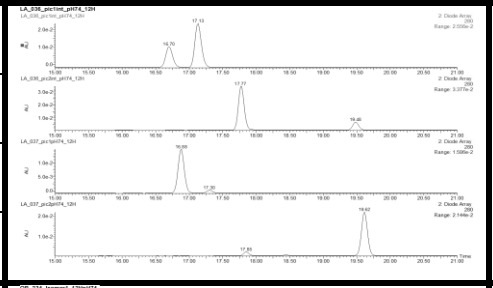
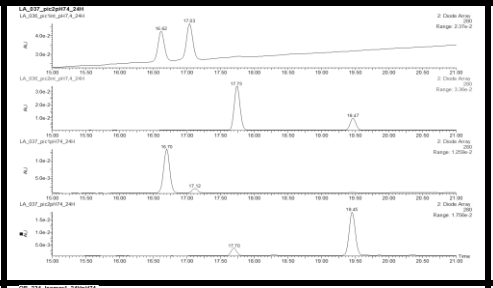
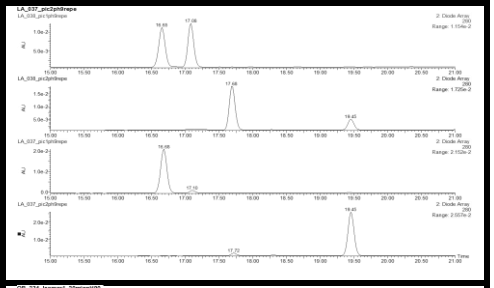
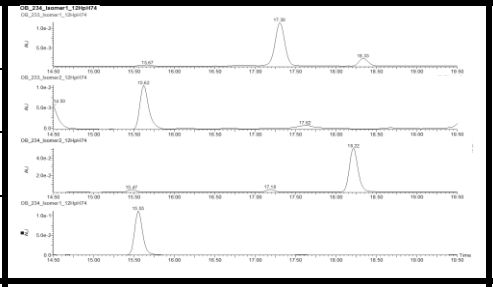
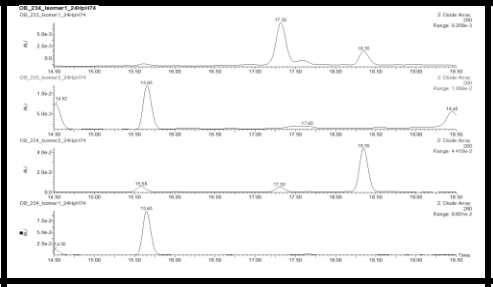
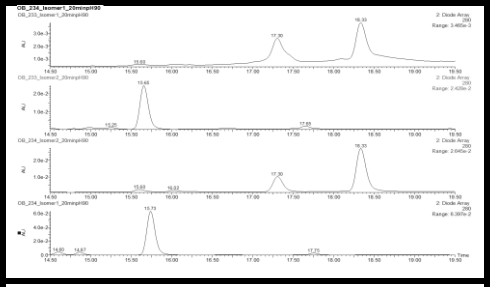
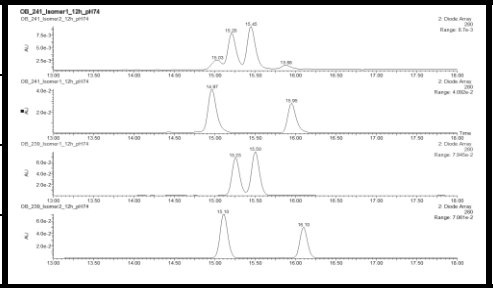
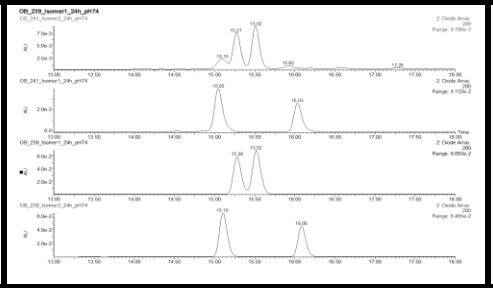
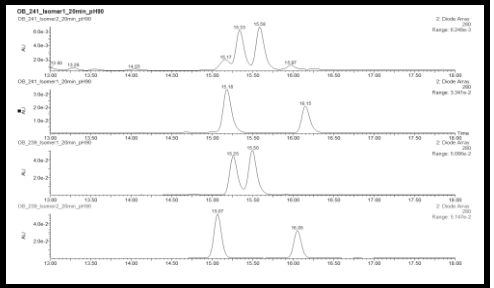
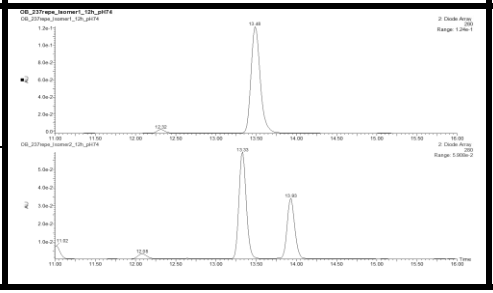
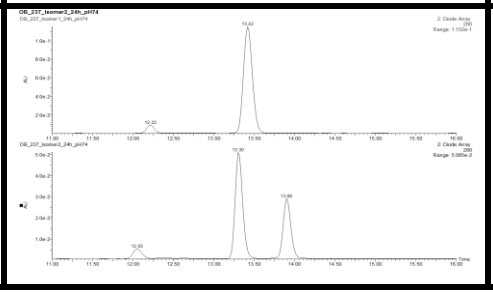
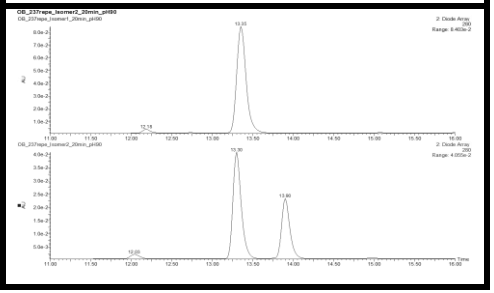
Relative **5a**/**M**-20 Da ratios at different reaction times were determined by HPLC/MS (280 nm) on the basis of the integrations of the **5a** and **M**-20 Da adduct peaks that did not co-elute, *in lieu* of using the integration of the two HPLC peaks of **5a** and the two of the **M**-20 Da adduct. [a]Analyzed 30 min after the addition of TEMPO. [b]Analyzed 90 min after the addition of TEMPO.

10- Stabilities of different *N*-terminal epimers

Both isomers of each cyclic peptide, independently isolated and just collected from the HPLC, were brought to pH 7.4 (PBS buffer) and pH 9.0 (Tris·HCl buffer), and their integrity was checked by HPLC/MS after 12 and 24 h at pH 7.4 (phosphate buffer), and after 20 min at pH 9.0 (NaOH solution). Stability to epimerization (% of remaining non-epimerized product) and the HPLC-MS traces of all experiments are shown below. Isomers are numbered according to corresponding epimers rather than elution time (the first-eluting isomer of the L-peptide is not necessarily transformed into the first-eluting isomer of the D-peptide, and the same applies to the other isomer).

	Non-epimerized product after incubation in the basic media (%)					
	pH 7.4, 12 h		pH 7.4, 24 h		pH 9.0, 20 min	
Product	Isomer 1	Isomer 2	Isomer 1	Isomer 2	Isomer 1	Isomer 2
1b	64	83	54	79	50	80
7b	95	93	91	87	95	95
5a	87	6	77	5	45	5
8a	94	99	89	96	73	96
6a	45	59	45	60	47	62
9a	54	40	55	40	55	39
9b	100	36	100	36	100	36

Note: Stability of product **6b** could not be determined due to the complex crude obtained, which precluded isolation of the pure isomers.

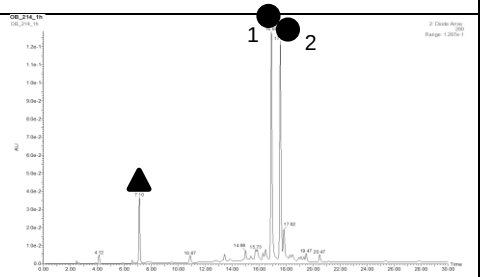
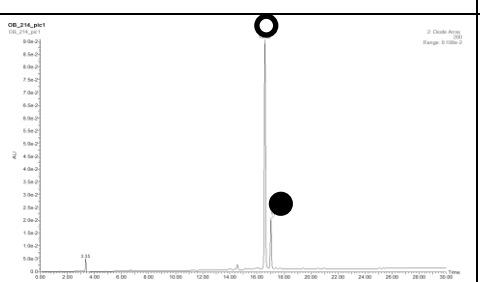
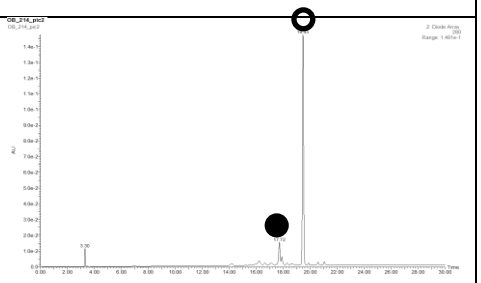
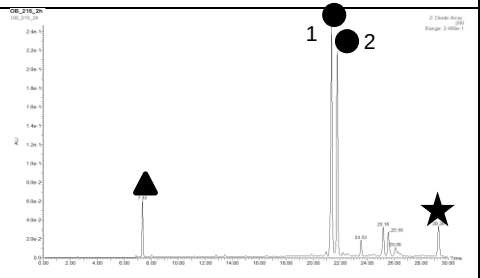
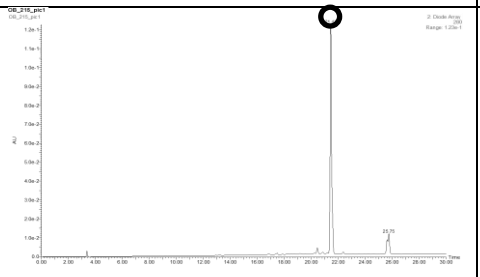
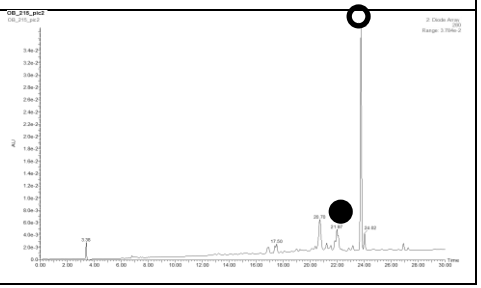
Products	pH 7.4 12 h	pH 7.4 24 h	pH 9.0 20 min
1b isomer 1			
1b isomer 2			
7b isomer 1			
7b isomer 2			
5a isomer 1			
5a isomer 2			
8a isomer 1			
8a isomer 2			
6a isomer 1			
6a isomer 2			
9a isomer 1			
9a isomer 2			
9b isomer 1			
9b isomer 2			

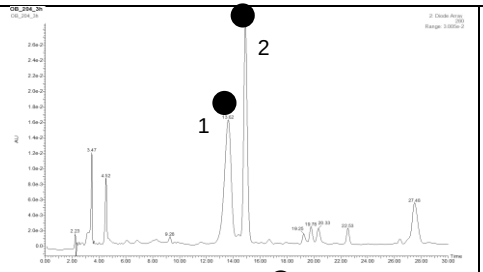
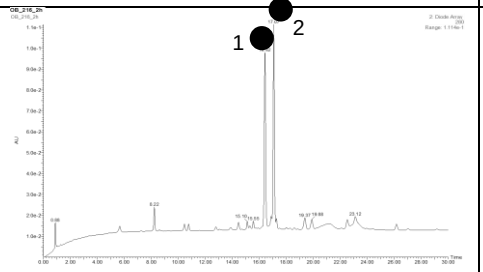
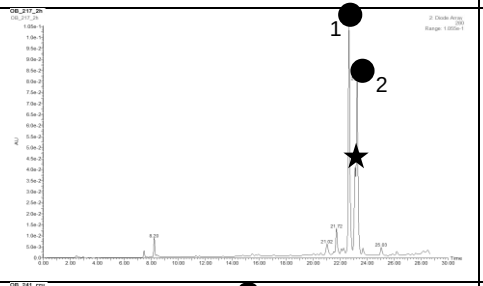
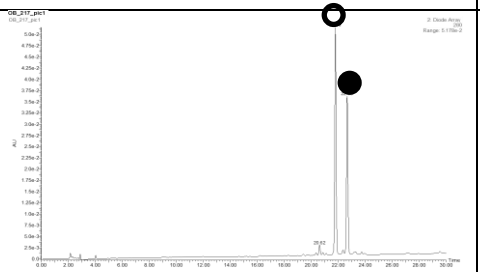
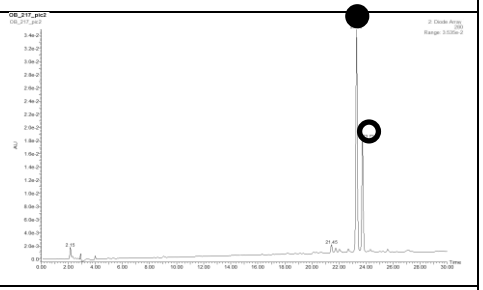
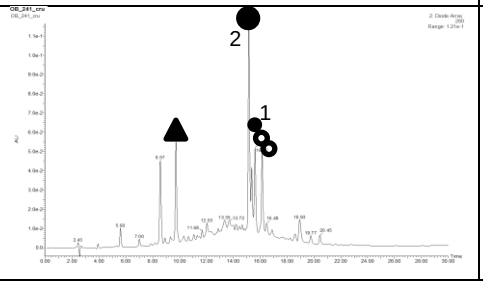
11- Reactions appearing in Table 2

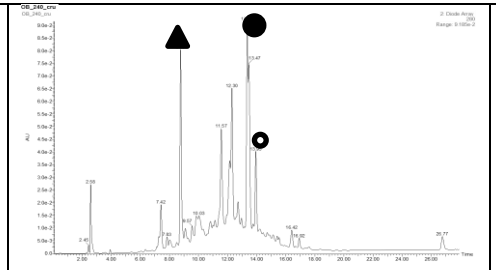
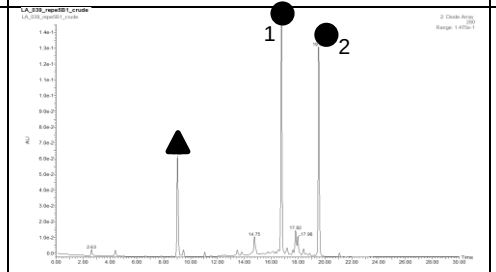
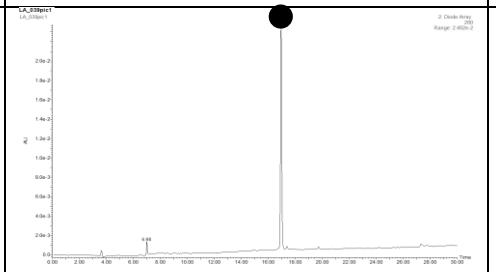
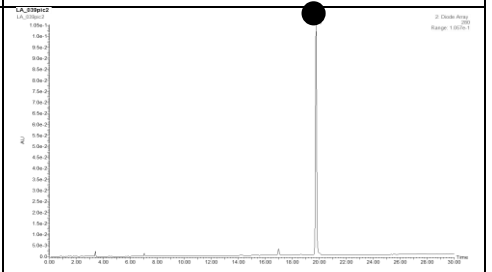
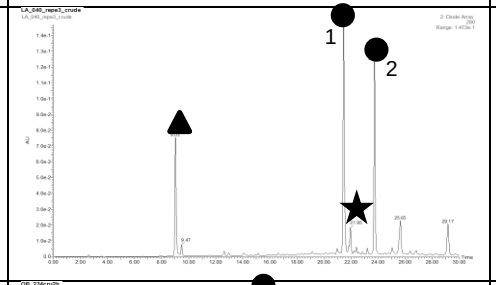
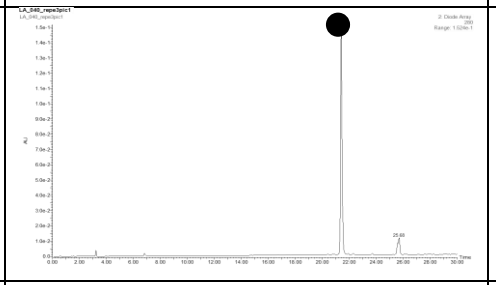
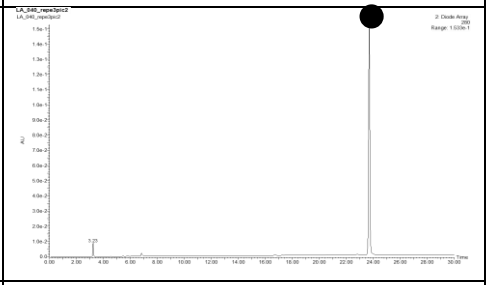
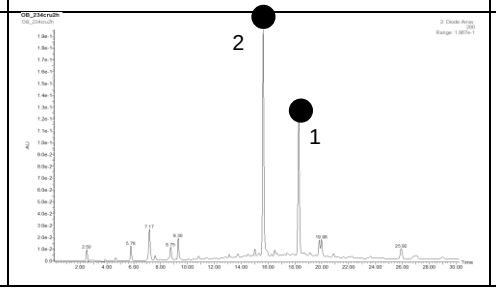
All reactions were carried out following optimized method 2 at the 500 nmol scale (2 h reaction time except for peptide **10**, whose cyclization required). A 1:1 water/methanol mixture was used when working with CPD **c** and for the **5** + **b** and **8** + **b** reactions. In the other cases pure water was used as solvent.

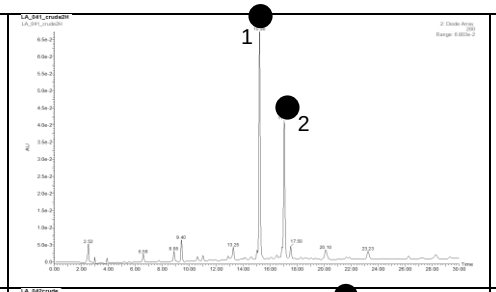
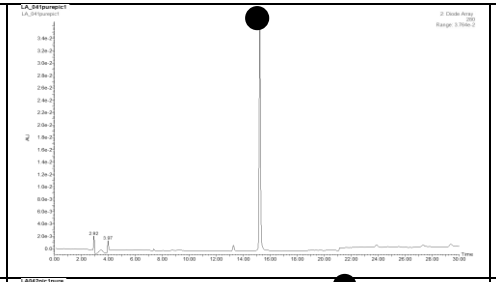
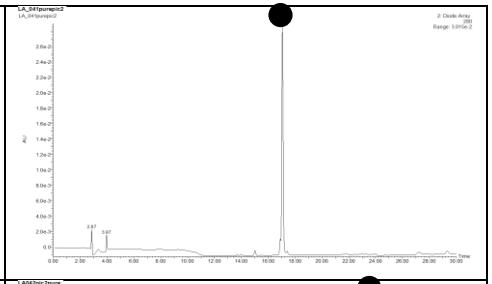
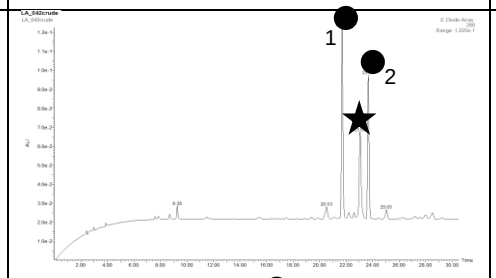
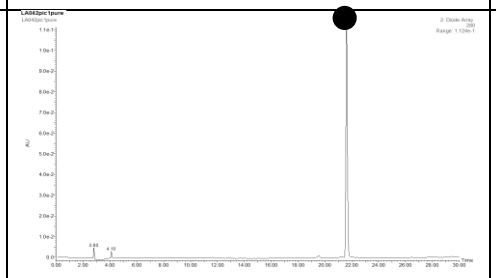
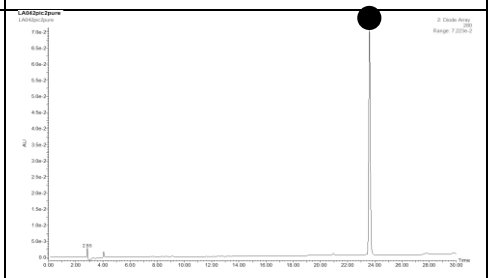
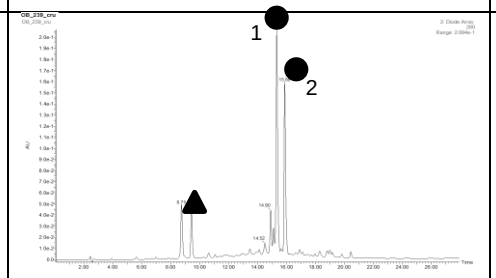
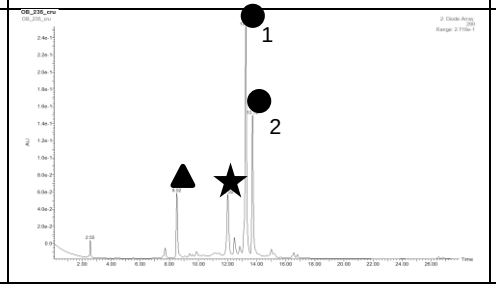
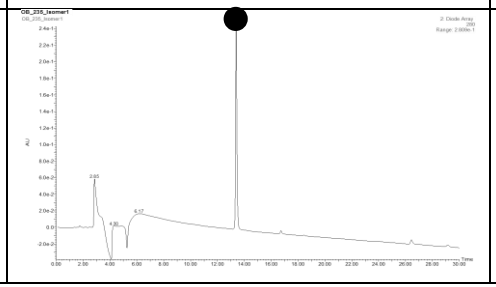
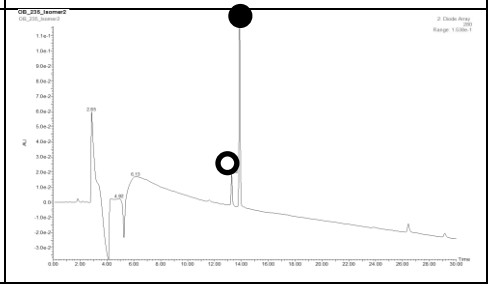
Product code:

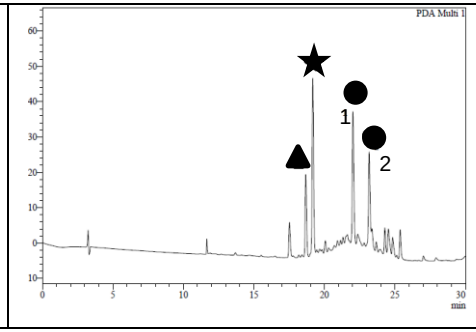
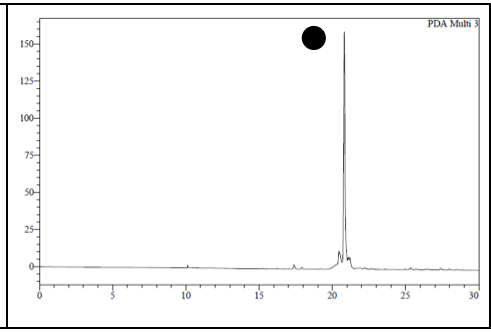
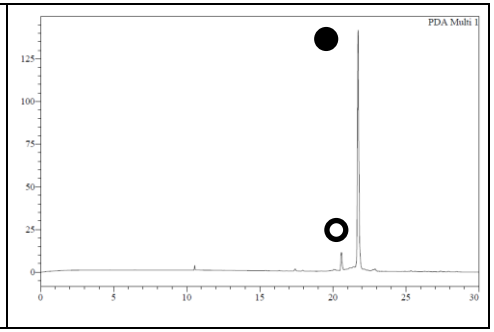
●: Desired cyclic product ○: Product with the opposite *N*-terminal Cys configuration ▲: Cyclic disulfide ★: excess CPD

	Overall yield (2 isomers)				
Reaction	HPLC-MS (280 nm)	Isolated[1]	Crude (Isomers numbered)	Isomer 1 after purification	Isomer 2 after purification
1 + **b**	76%	34%[2]			
1 + **c**	75%	37%[2]			

5 + **a**[3]	80%	-		-	-
5 + **b**	65%	-		-	-
5 + **c**	84%	33%[2]			
6 + **a**	36%	-		-	-

6 + **b**[4]	25%	-		-	-
7 + **b**	76%	40%			
7 + **c**	66%	40%			
8 + **a**	72%	-		-	-

8 + **b**	77%	33%			
8 + **c**	82%	45%			
9 + **a**	63%	-		-	-
9 + **b**	74%	22%[5]			

10 + a	68%	47%			

[1]The isolation yield is the cyclization/derivatization + purification yield. It was calculated as the ratio of the amount of purified peptide (sum of the amounts of the two independently collected isomers) to the amount of linear precursor.

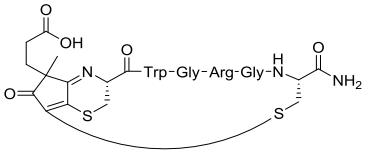
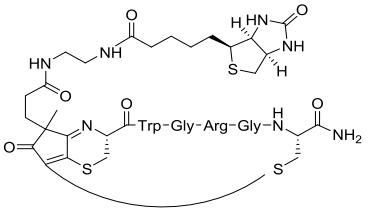
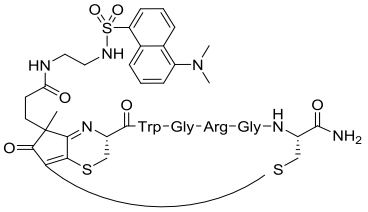
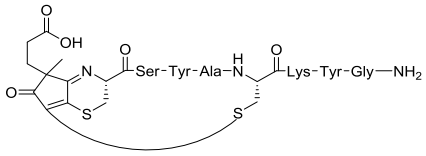
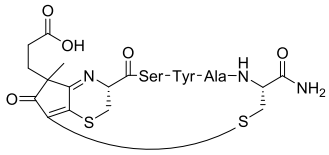
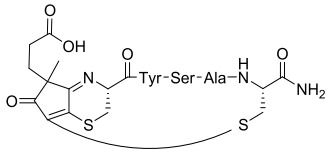
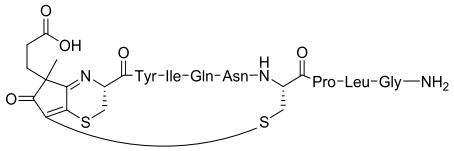
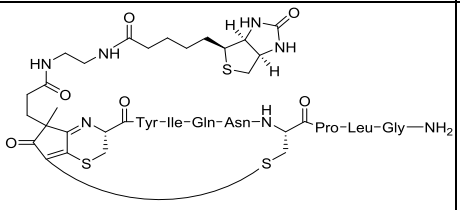
[2]Isomers could not be obtained as the desired enantiomerically pure compounds.

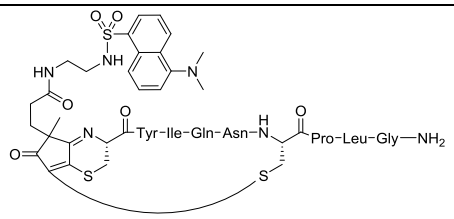
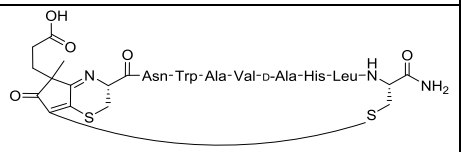
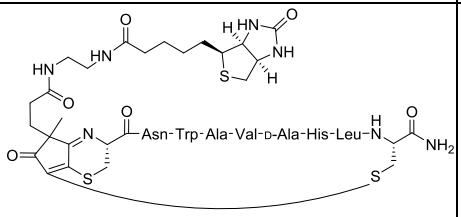
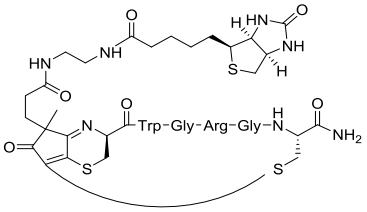
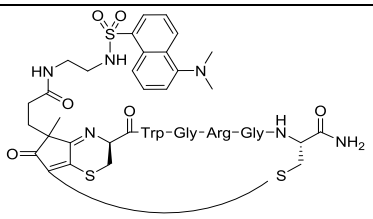
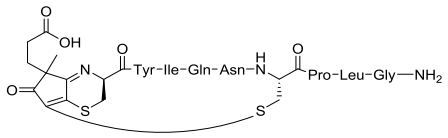
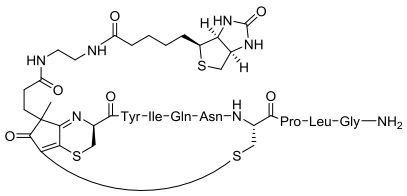
[3]HPLC-MS analysis conditions were 25-35% B, t_R= 13.6 + 14.9 min (at room temperature) instead of the ones optimized and described in section 12.

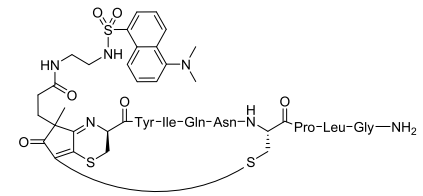
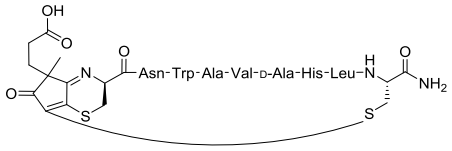
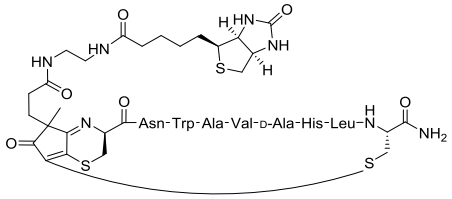
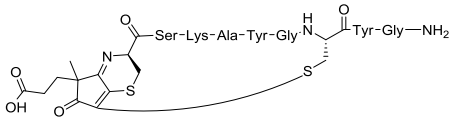
[4]Only one isomer was detected, likely due to the high instability of the other isomer (see section 10).

[5]Small amounts of the *N*-terminal epimer were detected after isolation of isomer 2.

12- Characterization of the cyclic adducts

	Structure	Characterization
1a		HPLC-MS: 0-50% B, t_R = 16.3 + 17.0 min MS: *m/z* 840.0 $[M+H]^+$, calcd. for $C_{36}H_{45}N_{11}O_9S_2$ 839.3 Purification conditions: -[a]
1b		HPLC-MS: 0-50% B, t_R = 16.9 + 17.5 min MS: *m/z* 1108.7 $[M+H]^+$, calcd. for $C_{48}H_{65}N_{15}O_{10}S_3$ 1107.4 Purification conditions: 20-50% B, t_R = 13.6 + 14.2 min
1c		HPLC-MS: 0-50% B, t_R = 21.5 + 21.9 min MS: *m/z* 1115.3 $[M+H]^+$, calcd. for $C_{50}H_{62}N_{14}O_{10}S_3$ 1114.4 Purification conditions: 25-35% B, t_R = 15.0 + 16.2 min
2a		HPLC-MS: 0-50% B, t_R = 13.1 + 13.8 min MS: *m/z* 1053.6 $[M+H]^+$, calcd. for $C_{47}H_{60}N_{10}O_{14}S_2$ 1052.4 Purification conditions: -[a]
3a		HPLC-MS: 0-50% B, t_R = 15.3 + 16.3 min MS: *m/z* 705.0 $[M+H]^+$, calcd. for $C_{30}H_{36}N_6O_{10}S_2$ 704.2 Purification conditions: 15-30% B, t_R = 9.0 + 10.4 min
4a		HPLC-MS: 0-50% B, t_R = 16.6 + 17.9 min MS: *m/z* 705.0 $[M+H]^+$, calcd. for $C_{30}H_{36}N_6O_{10}S_2$ 704.2 Purification conditions: -[a]
5a		HPLC-MS: 25-35% B, t_R = 13.6 + 14.9 min MS: *m/z* 1169.7 $[M+H]^+$, calcd. for $C_{52}H_{72}N_{12}O_{15}S_2$ 1168.5 Purification conditions: 25-45% B (60 ºC), t_R = 13.5 + 14.4 min
5b		HPLC-MS: 10-60% B (60 ºC), t_R = 16.4 + 17.1 min MS: *m/z* 1437.8 $[M+H]^+$, calcd. for $C_{64}H_{92}N_{16}O_{16}S_3$ 1436.6 Purification conditions: 25-45% B (60 ºC), t_R = 12.7 + 14.2 min

5c		HPLC-MS: 10-60% B (60 ºC), t_R = 22.6 + 23.2 min MS: *m/z* 1444.6 $[M+H]^+$, calcd. for $C_{66}H_{89}N_{15}O_{16}S_3$ 1443.6 Purification conditions: 25-45% B (60 ºC), t_R = 17.9 + 18.6 min
6a		HPLC-MS: 10-50% B, t_R = 15.2 + 15.4 min MS: *m/z* 1175.6 $[M+H]^+$, calcd. for $C_{53}H_{70}N_{14}O_{13}S_2$ 1174.5 Purification conditions: 25-50% B, t_R =14.0 + 15.0 min
6b		HPLC-MS: 10-60% B, t_R = 13.4 + n.d. MS: *m/z* 1143.9 $[M+H]^+$, calcd. for $C_{65}H_{90}N_{18}O_{14}S_3$ 1442.6 Purification conditions: -[a]
7b		HPLC-MS: 0-50% B, t_R = 16.8 + 19.5 min MS: *m/z* 1108.7 $[M+H]^+$, calcd. for $C_{48}H_{65}N_{15}O_{10}S_3$ 1107.4 Purification conditions: 20-50% B, t_R = 13.0 + 17.6 min
7c		HPLC-MS: 0-50% B, t_R = 21.4 + 23.7 min MS: *m/z* 1115.5 $[M+H]^+$, calcd. for $C_{50}H_{62}N_{14}O_{10}S_3$ 1114.4 Purification conditions: 30-40% B, t_R = 7.5 + 13.5 min
8a		HPLC-MS: 10-60% B (60 ºC), t_R = 17.3 + 17.7 min MS: *m/z* 1169.7 $[M+H]^+$, calcd. for $C_{52}H_{72}N_{12}O_{15}S_2$ 1168.5 Purification conditions: 25-45% B (60 ºC), t_R = 13.5 + 14.4 min
8b		HPLC-MS: 10-60% B (60 ºC), t_R = 15.2 + 17.0 min MS: *m/z* 1437.4 $[M+H]^+$, calcd. for $C_{64}H_{92}N_{16}O_{16}S_3$ 1436.6 Purification conditions: 25-45% B (60 ºC), t_R = 11.0 + 14.7 min

8c		HPLC-MS: 10-60% B (60 ºC), t_R = 21.7 + 23.7 min MS: *m/z* 1444.5 $[M+H]^+$, calcd. for $C_{66}H_{89}N_{15}O_{16}S_3$ 1443.6 Purification conditions: 25-45% B (60 ºC), t_R = 16.0 + 20.1 min
9a		HPLC-MS: 10-50% B, t_R = 15.3 + 15.9 min MS: *m/z* 1175.6 $[M+H]^+$, calcd. for $C_{53}H_{70}N_{14}O_{13}S_2$ 1174.5 Purification conditions: 25-50% B, t_R = 14.3 + 15.3 min
9b		HPLC-MS: 10-60% B, t_R = 13.4 + 13.8 min MS: *m/z* 1143.9 $[M+H]^+$, calcd. for $C_{65}H_{90}N_{18}O_{14}S_3$ 1442.6 Purification conditions: 25-50% B, t_R =13.6 + 14.8 min
10a		HPLC-MS: 0-50% B, t_R = 14.3 + 15.6 min MS: *m/z* 1110.3 $[M+H]^+$, calcd. for $C_{49}H_{63}N_{11}O_{15}S_3$ 1109.4 Purification conditions: 0-50% B, t_R = 20.6 + 21.9 min

[a]The two isomers of the cyclic peptide were not isolated.

13- References

(1) Brun, O.; Agramunt, J.; Raich, L.; Rovira, C.; Pedroso, E.; Grandas, A. *Org. Lett.* **2016**, *18*, 4836.
(2) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.