

## Supporting information

# Coupling the antimalarial cell penetrating peptide TP10 to classical antimalarial drugs primaquine and chloroquine produces strongly hemolytic conjugates

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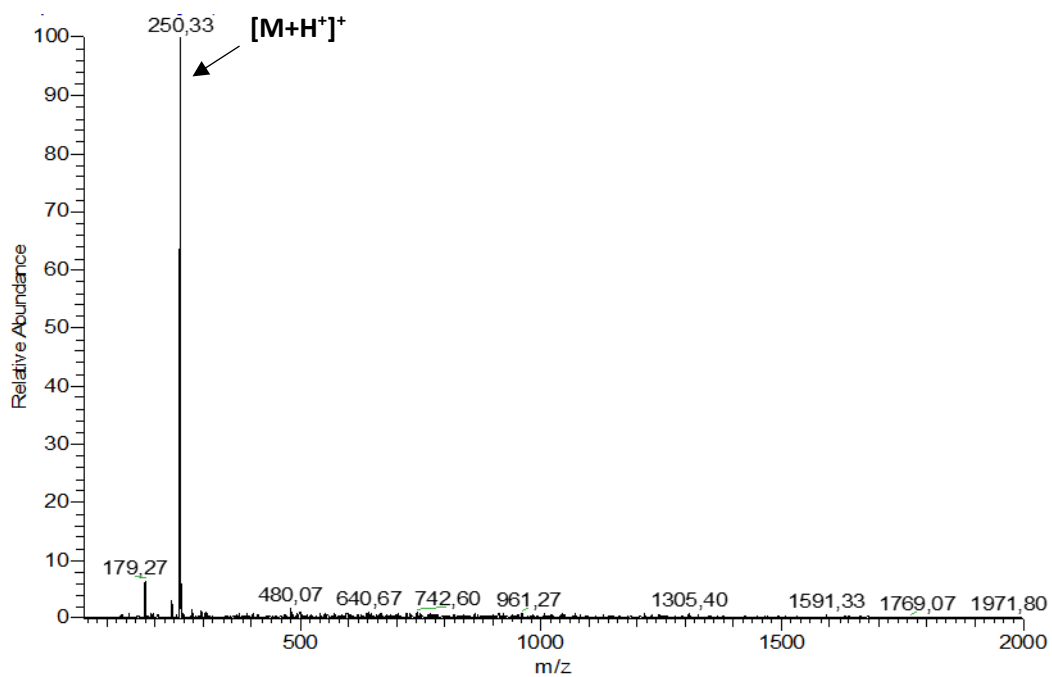
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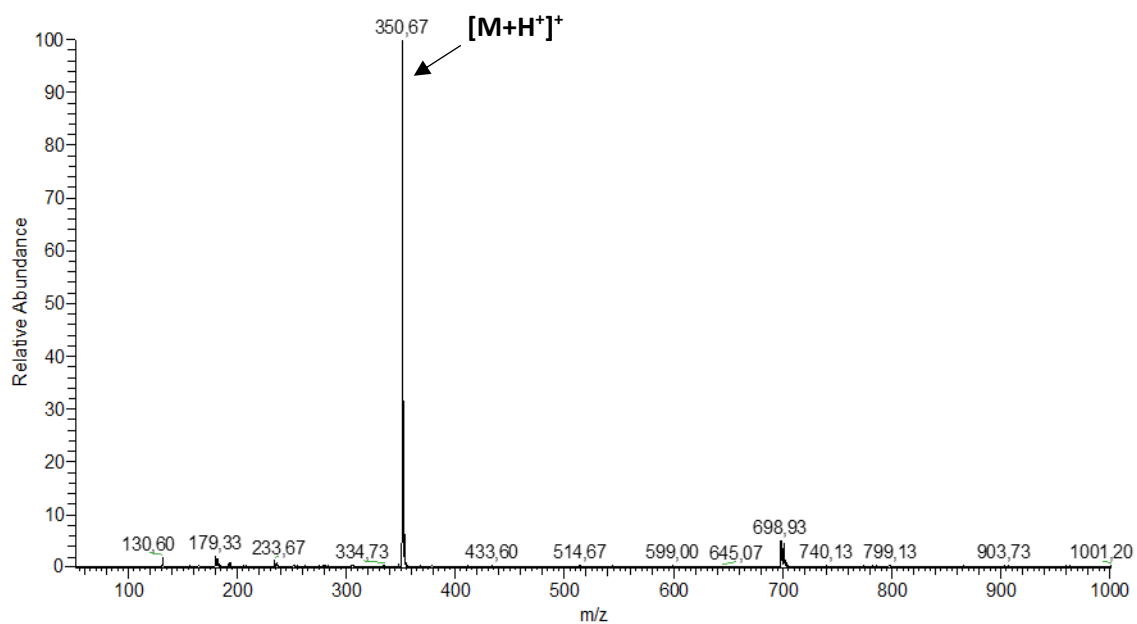
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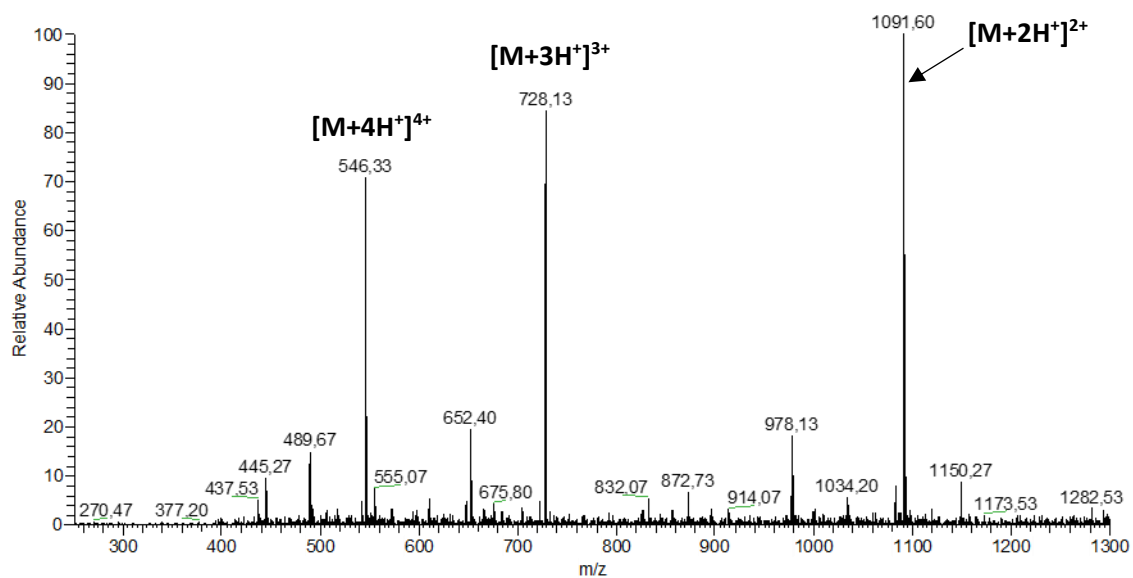
\* Correspondence: pgomes@fc.up.pt



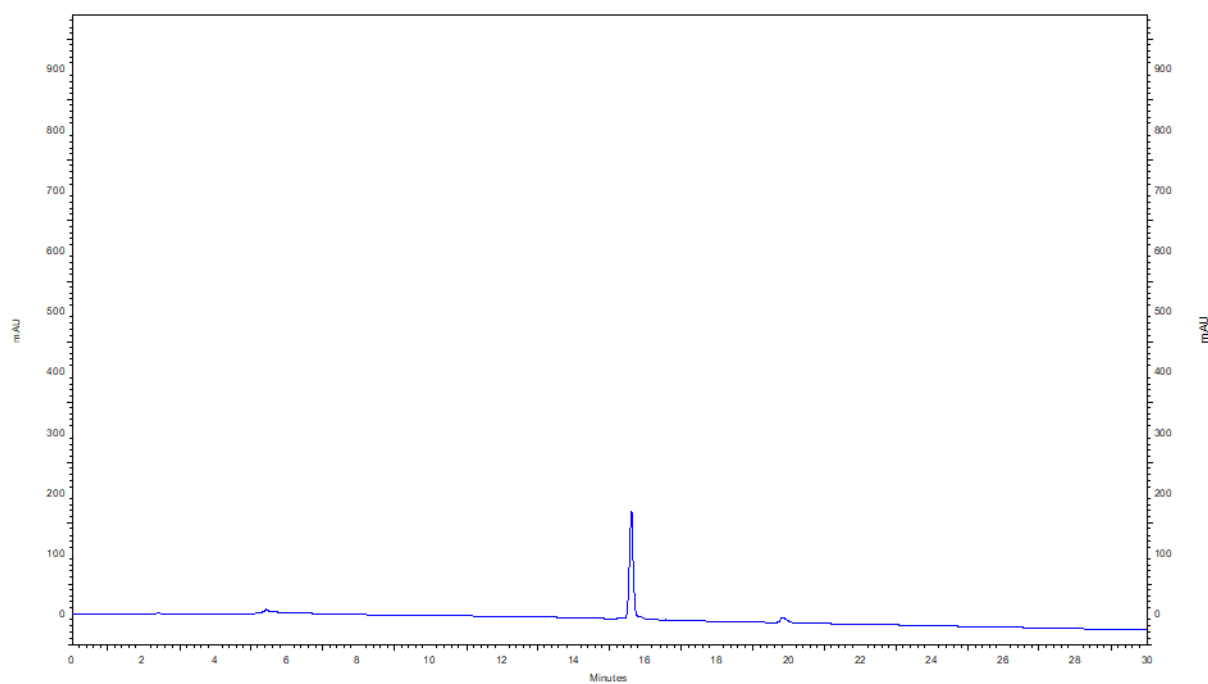
**Figure S1** – ESI-IT MS spectrum (positive mode) of Cq (4)  
 (observed as the quasi-molecular ion)



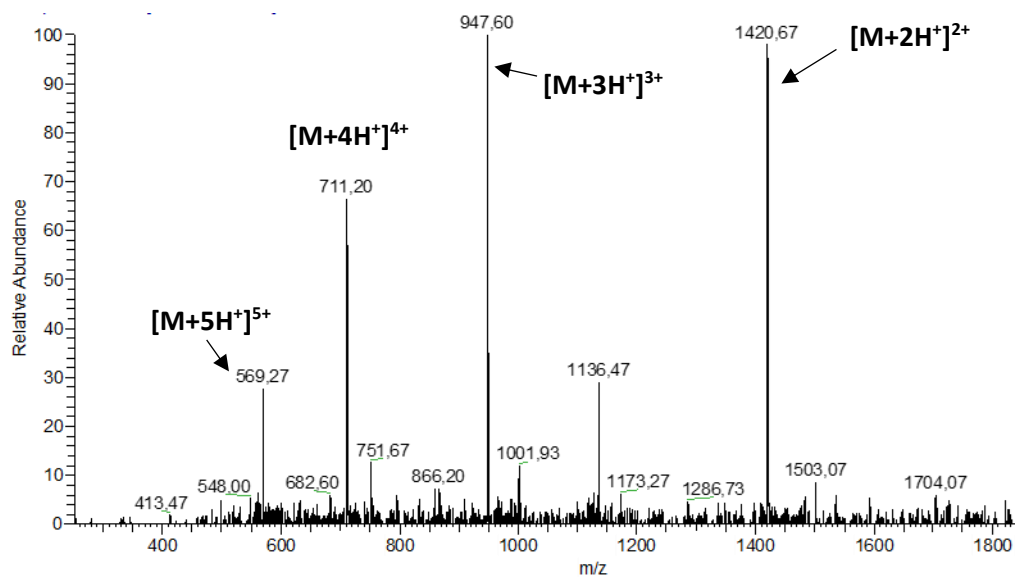
**Figure S2** – ESI-IT MS spectrum (positive mode) of Cq-C4 (6)  
 (observed as the quasi-molecular ion)



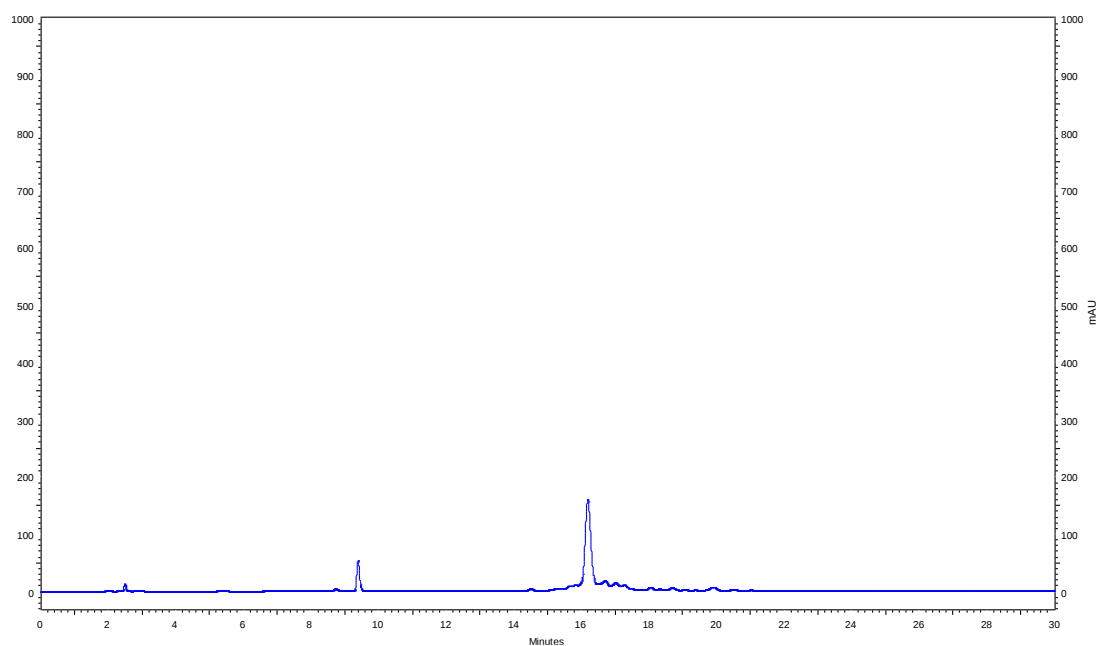
**Figure S3** - ESI-IT MS spectrum (positive mode) of peptide TP10  
(observed as the di-, tri-, and tetra-protonated ions)



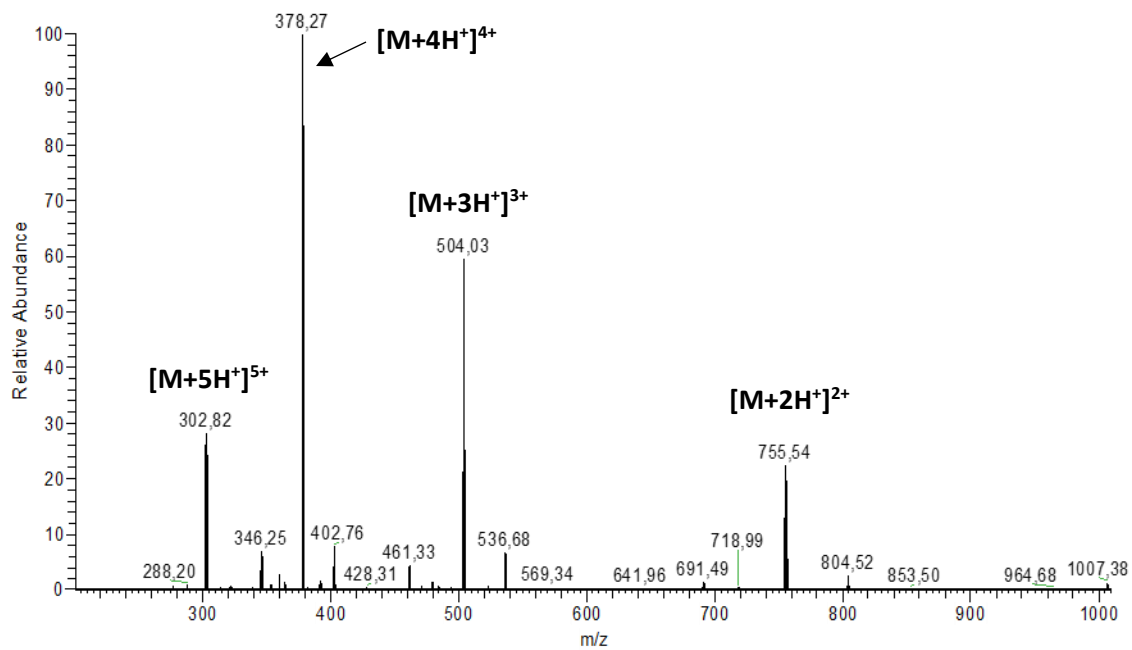
**Figure S4** – Chromatogram obtained for TP10, with a gradient elution of 0-100% ACN in water (0.05% TFA) in a reverse phase C18 column Purospher star RP-C18 of 125 × 4.0 mm with 5 μm pore size, for 30 min and at a flow rate of 1 mL/min, with detection at λ=220 nm.



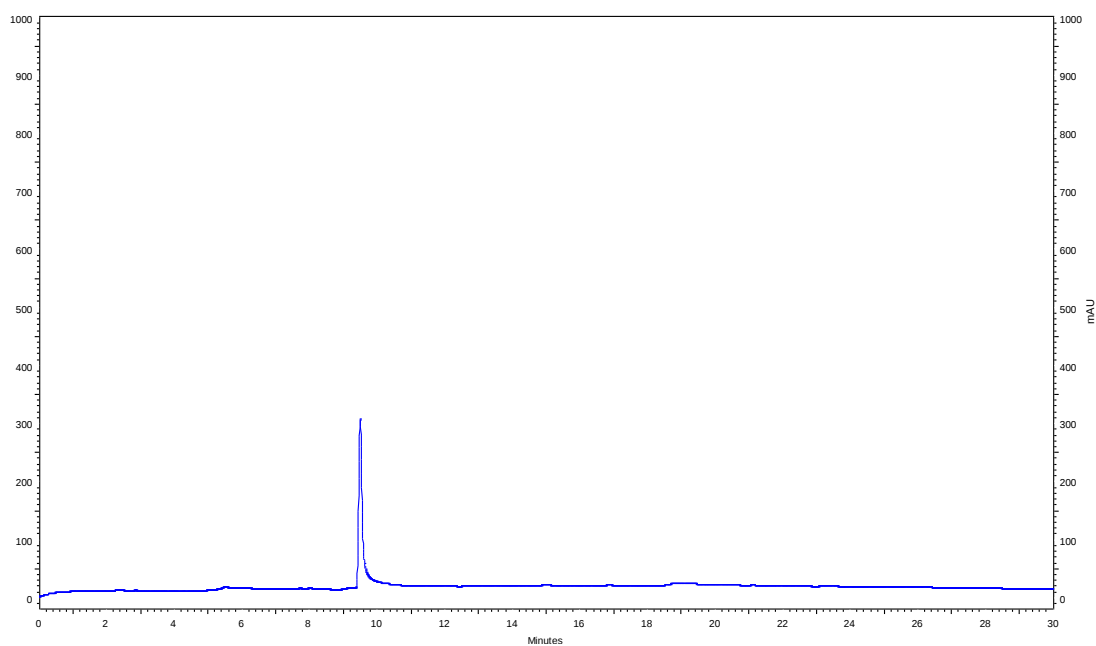
**Figure S5** - ESI-IT MS spectrum (positive mode) of peptide Transportan  
(observed as the di-, tri-, tetra-, and penta-protonated ions)



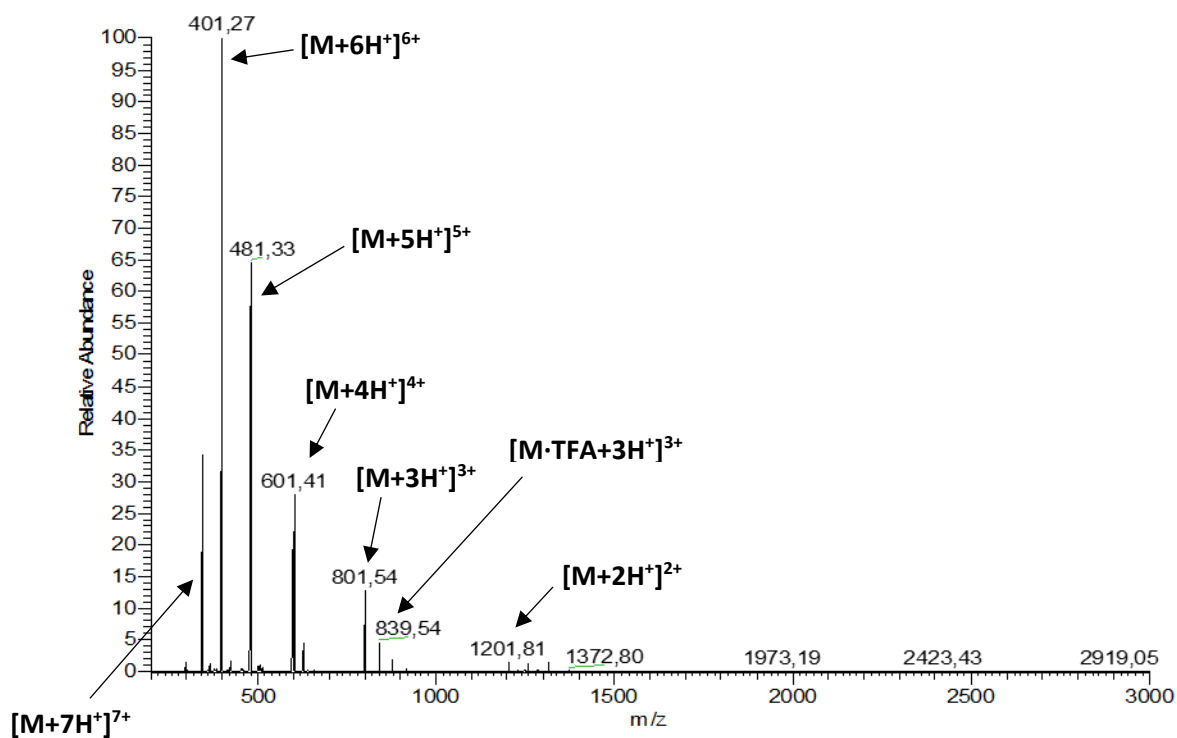
**Figure S6** – Chromatogram obtained for Transportan, with a gradient elution of 0-100% ACN in water (0.05% TFA) in a reverse phase C18 column Purospher star RP-C18 of 125 × 4.0 mm with 5 μm pore size, for 30 min and at a flow rate of 1 mL/min, with detection at λ=220 nm.



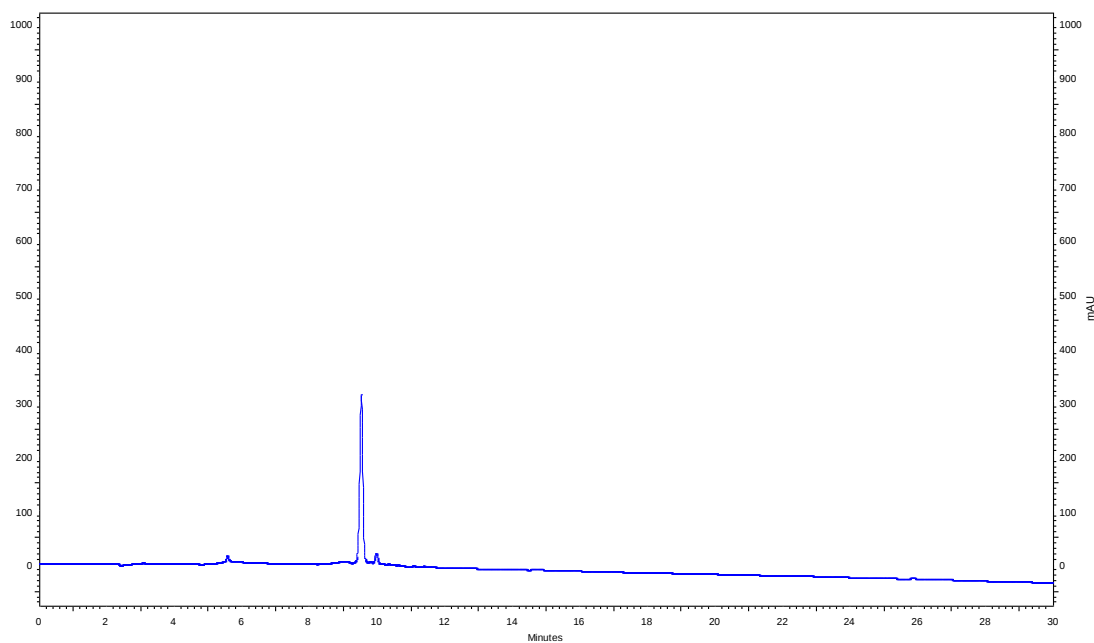
**Figure S7** - ESI-IT MS spectrum (positive mode) of peptide DPT-sh1  
 (observed as the di-, tri-, tetra-, and penta-protonated ions)



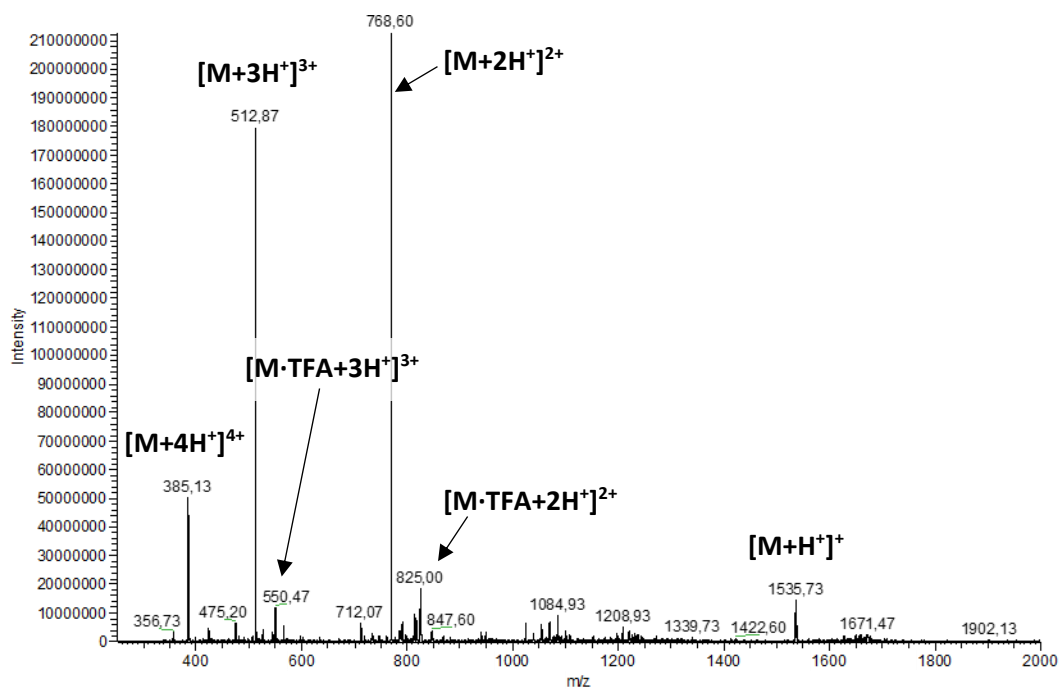
**Figure S8** – Chromatogram obtained for DPT-sh1, with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5 μm), for 30 min and a flow rate of 1 ml/min, with detection at λ=220 nm.



**Figure S9** - ESI-IT MS spectrum (positive mode) of peptide DPT-sh2  
 (observed as the di-, tri-, tetra-, penta-, hexa-, and hepta-protonated ions;  
 TFA adducts at different protonation states also observed)



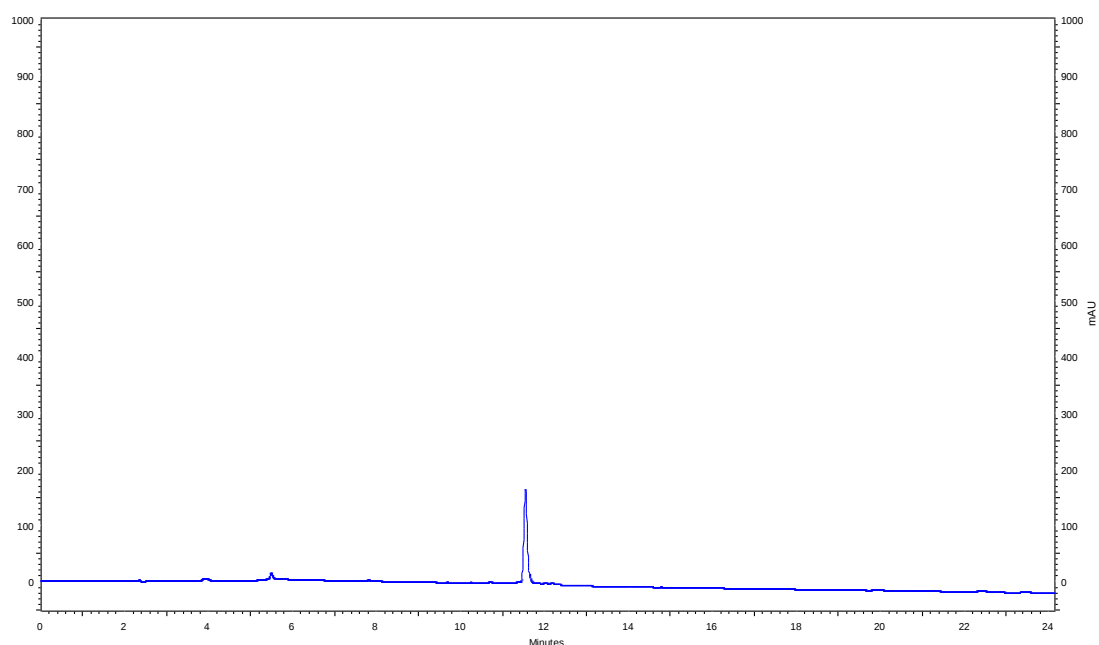
**Figure S10** – Chromatogram obtained for DPT-sh2, with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda$ =220 nm.



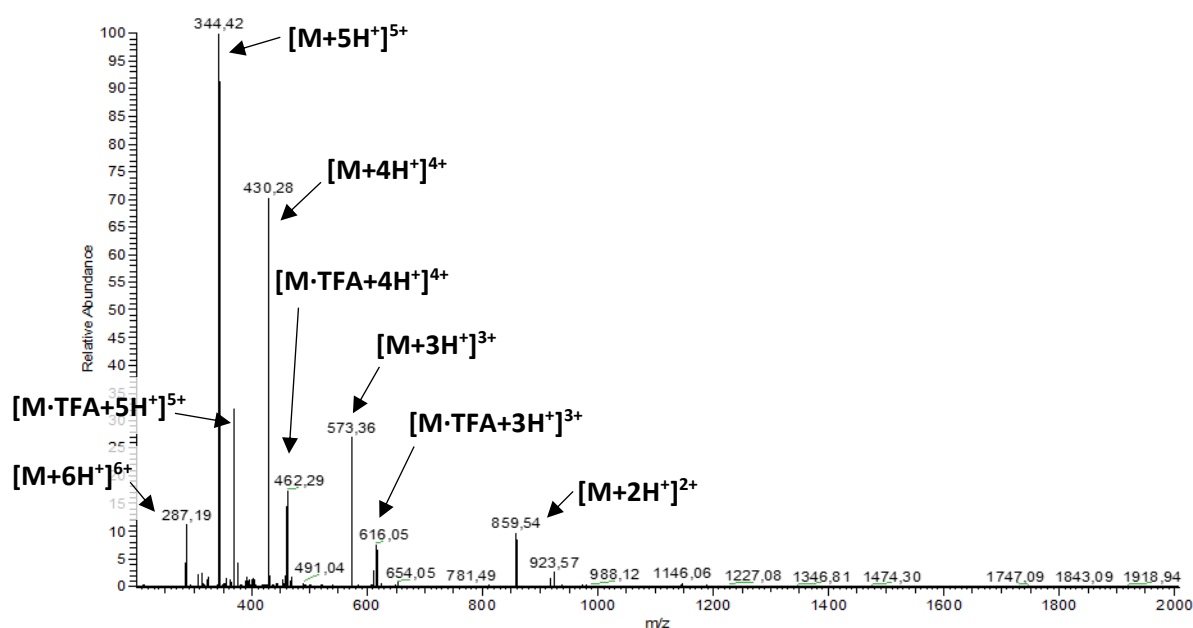
**Figure S11** - ESI-IT MS spectrum (positive mode) of peptide IDR-1018

(observed as the quasi-molecular ion, and also as the di-, tri-, and tetra-protonated ions;

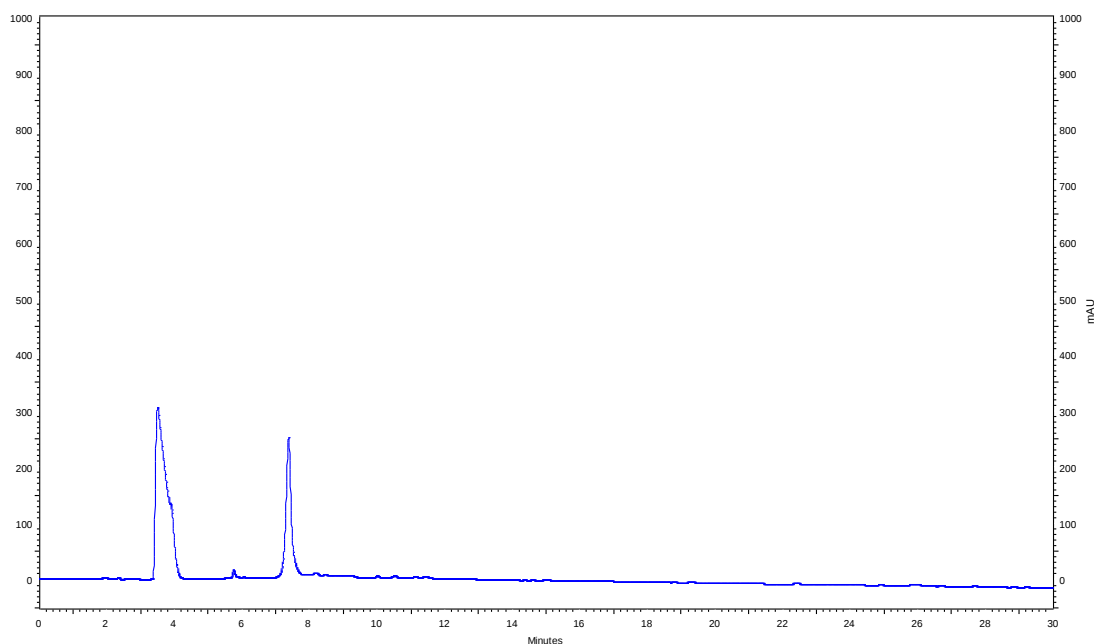
TFA adducts at different protonation states also observed)



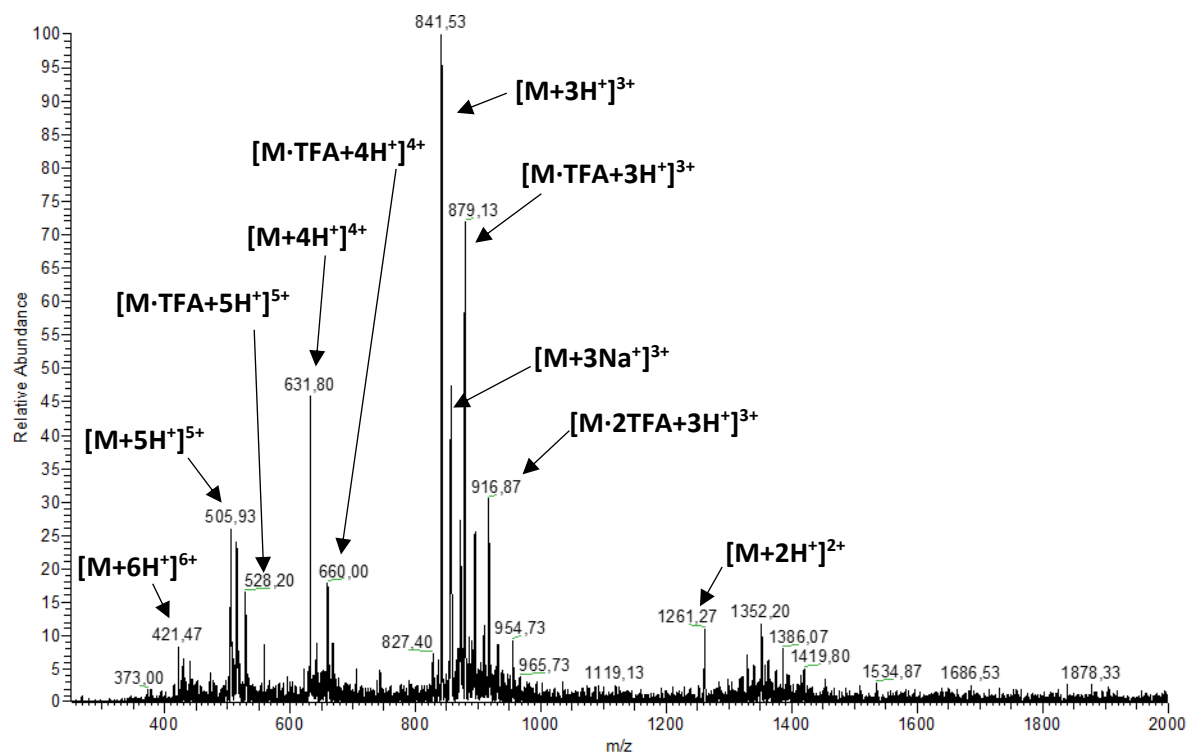
**Figure S12** – Chromatogram obtained for IDR-1018, with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



**Figure S13** - ESI-IT MS spectrum (positive mode) of peptide TAT  
 (observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
 TFA adducts at different protonation states also observed)

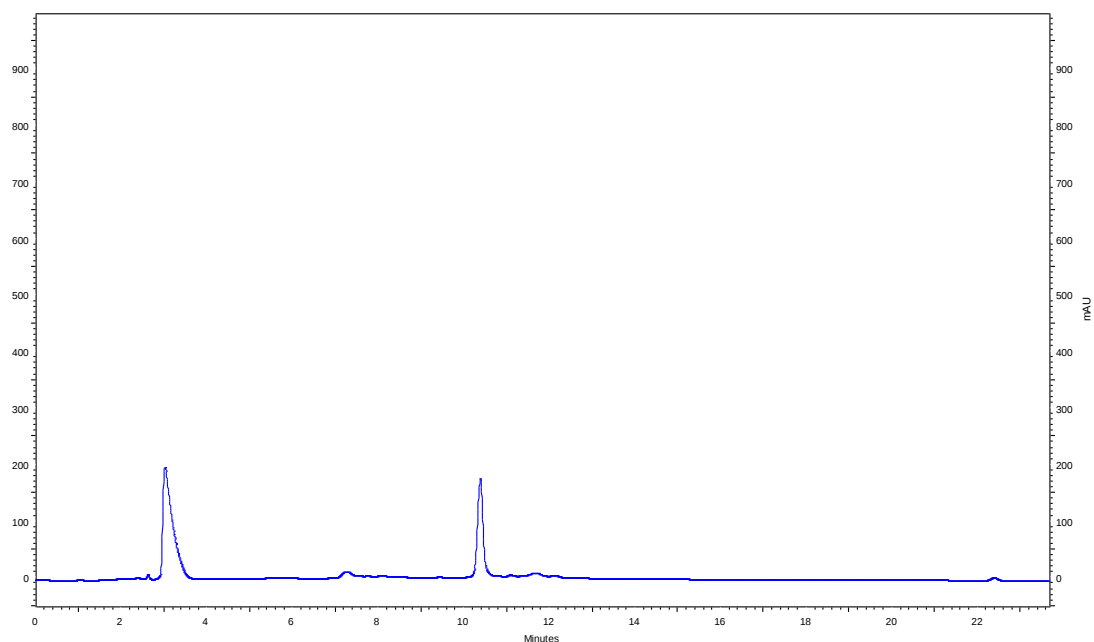


**Figure S14** – Chromatogram obtained for TAT, with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda$ =220 nm.  
 The peak at RT  $\approx$  3-4 min is due to acetic acid, added to the samples for peptide solubilization.



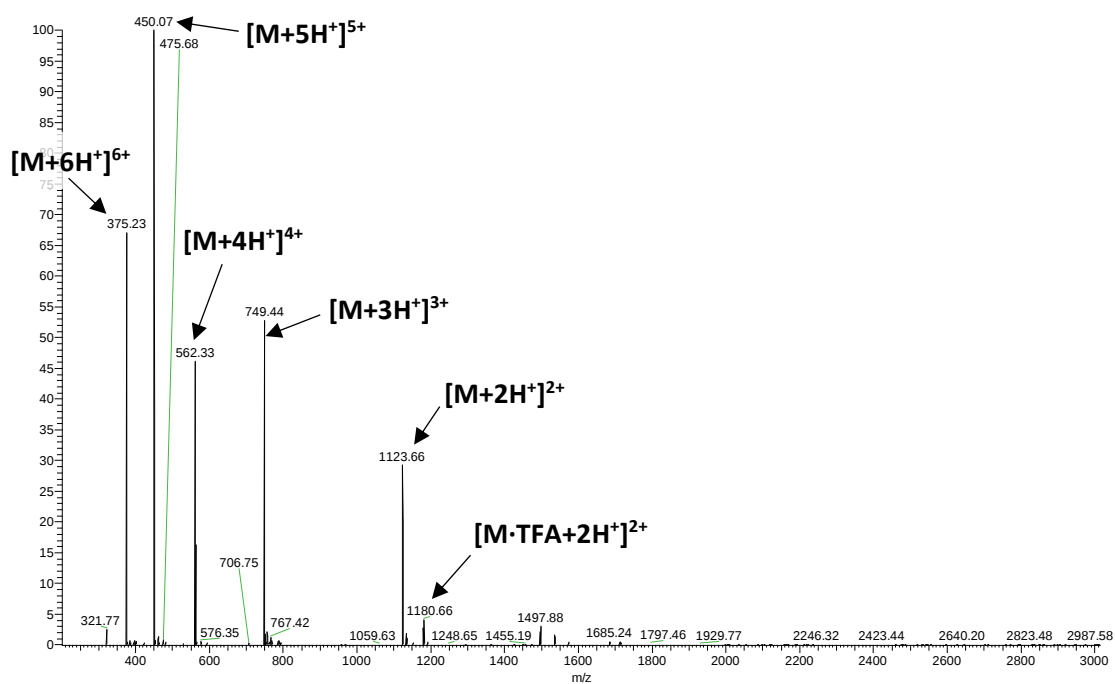
**Figure S15** - ESI-IT MS spectrum (positive mode) of peptide PasTAT

(observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
sodium and TFA adducts at different protonation states also observed)

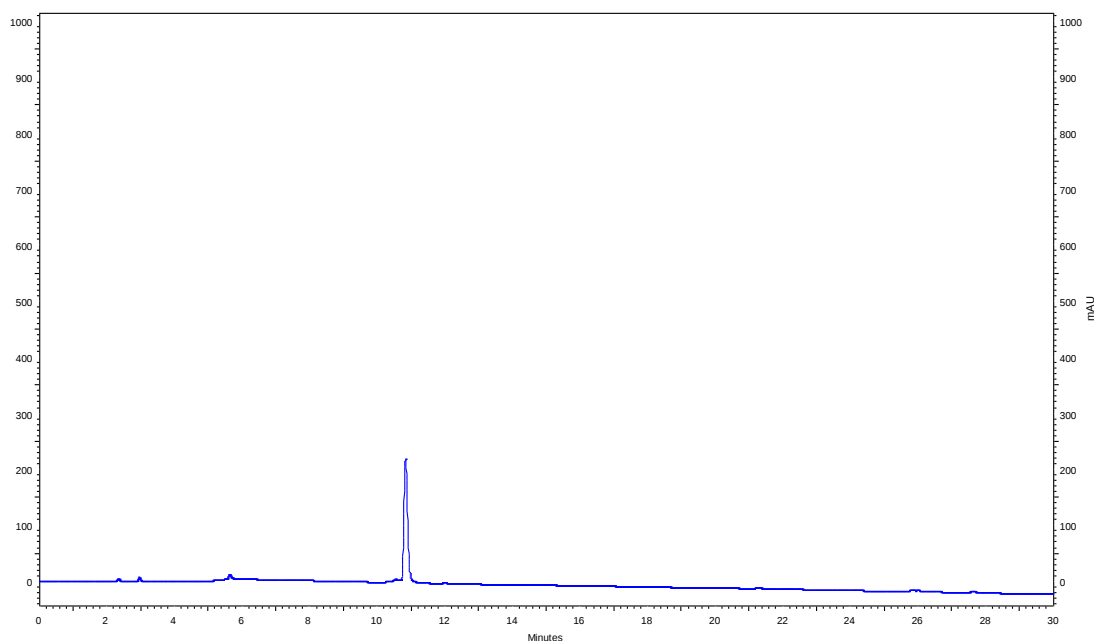


**Figure S16** – Chromatogram obtained for PasTAT, with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.

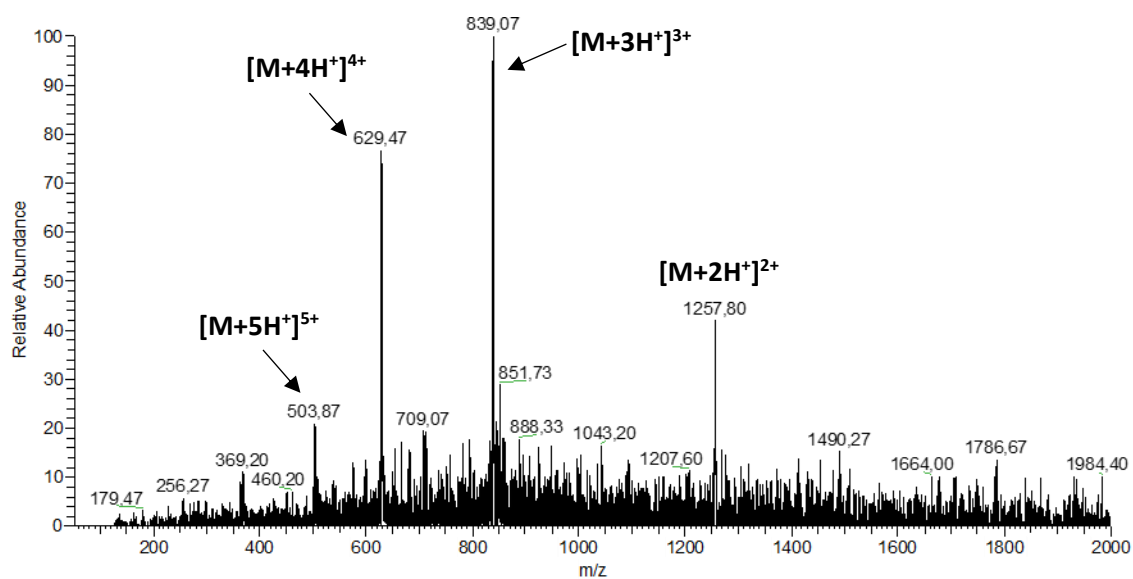
The peak at RT  $\approx$  3 min is due to acetic acid, added to the samples for peptide solubilization.



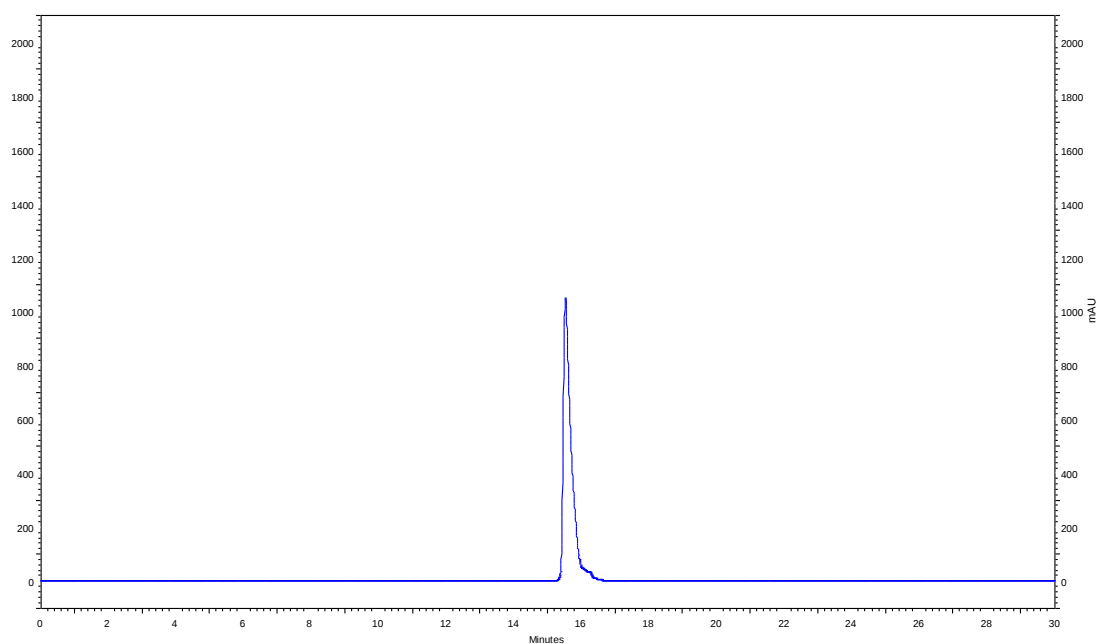
**Figure S17** - ESI-IT MS spectrum (positive mode) of peptide Penetratin  
 (observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
 TFA adducts at different protonation states also observed)



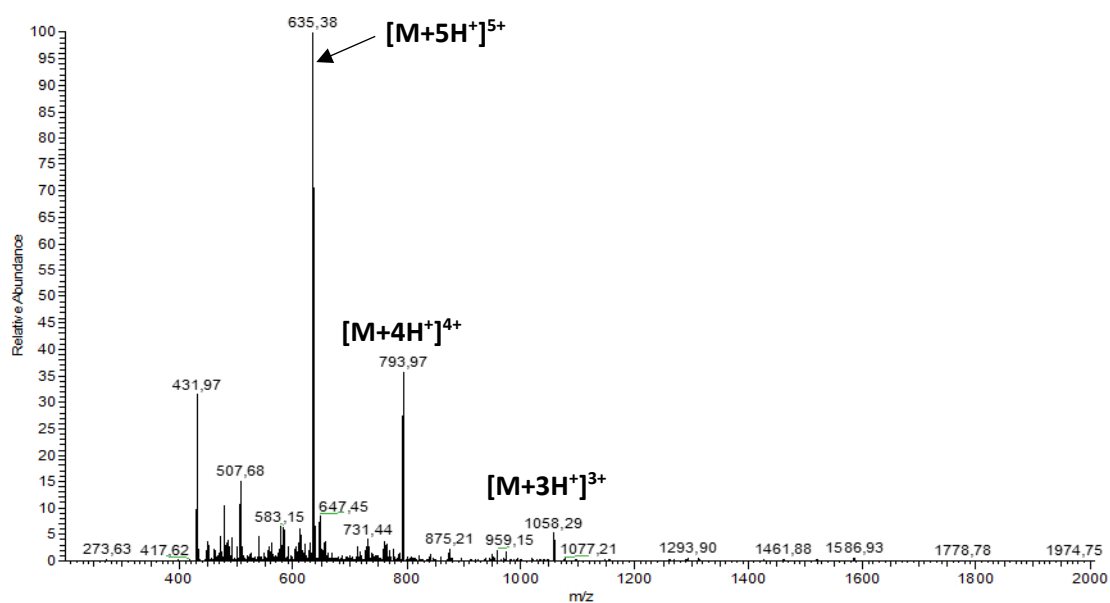
**Figure S18** - Chromatogram obtained for Penetratin, with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



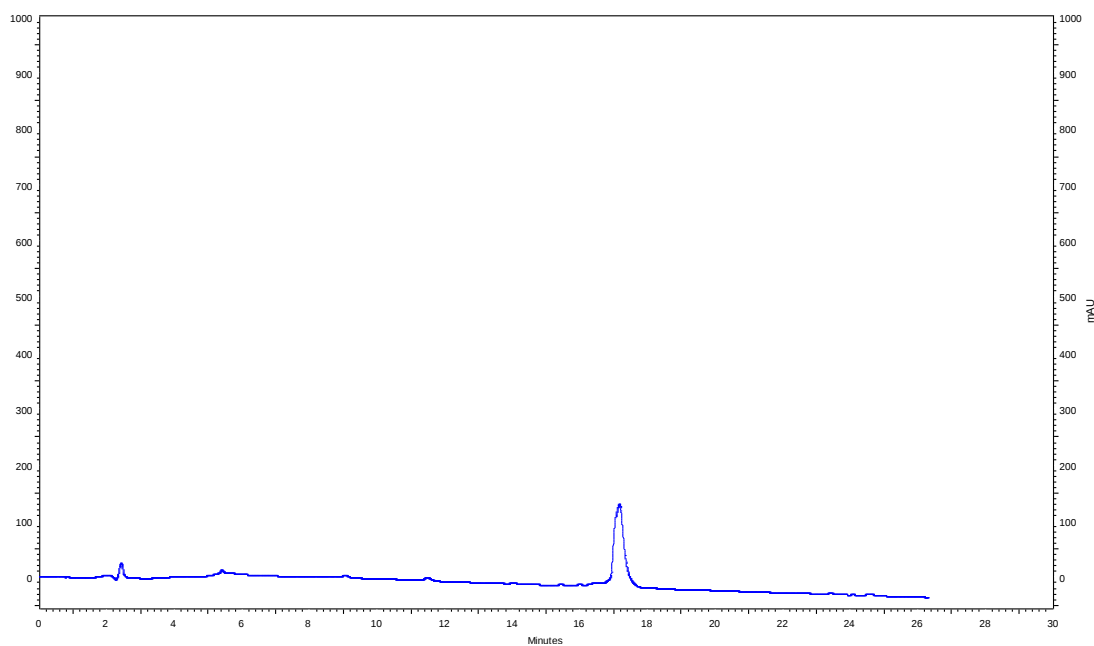
**Figure S19** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-TP10 (**5a**)  
 (observed as the di-, tri-, tetra-, and penta-protonated ions)



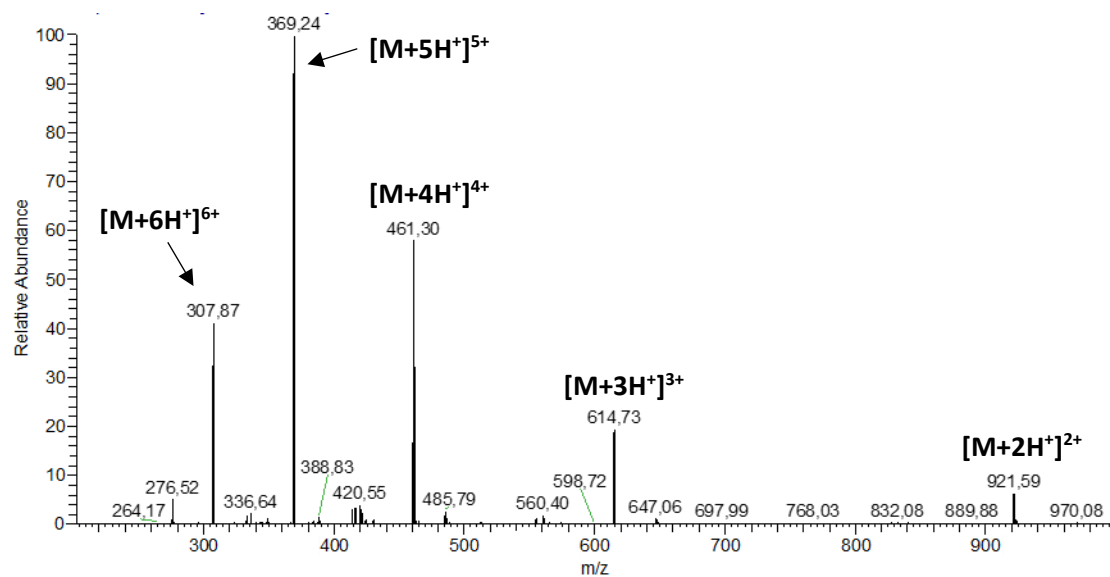
**Figure S20** – Chromatogram obtained for Cq-C4-TP10 (**5a**), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



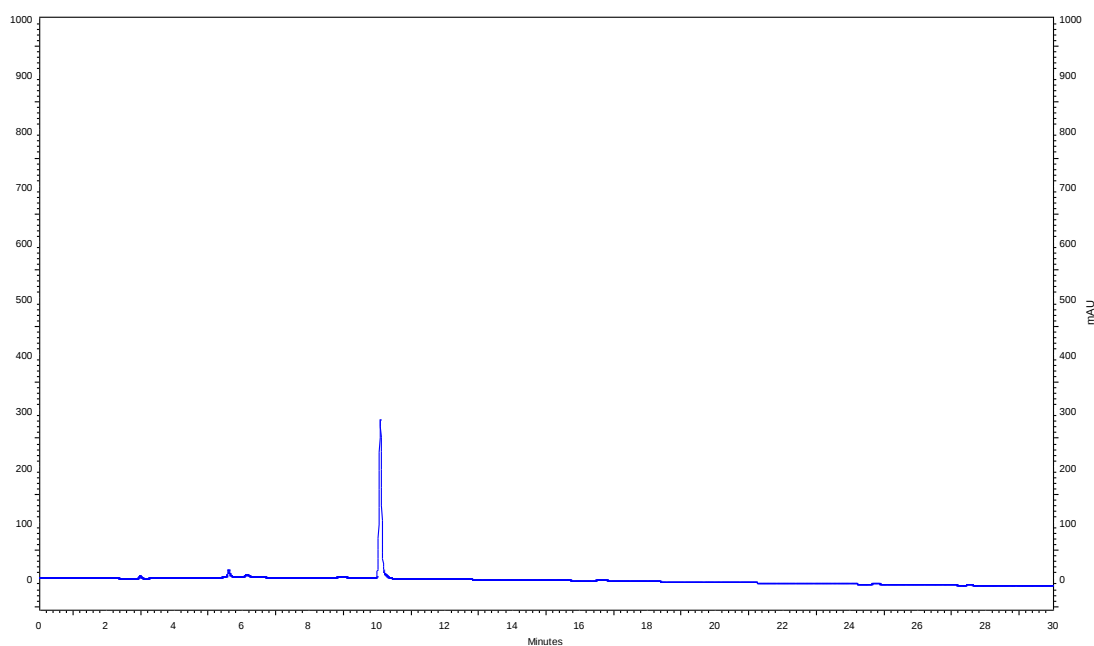
**Figure S21** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-Transportan (**5b**)  
(observed as the tri-, tetra-, and penta-protonated ions)



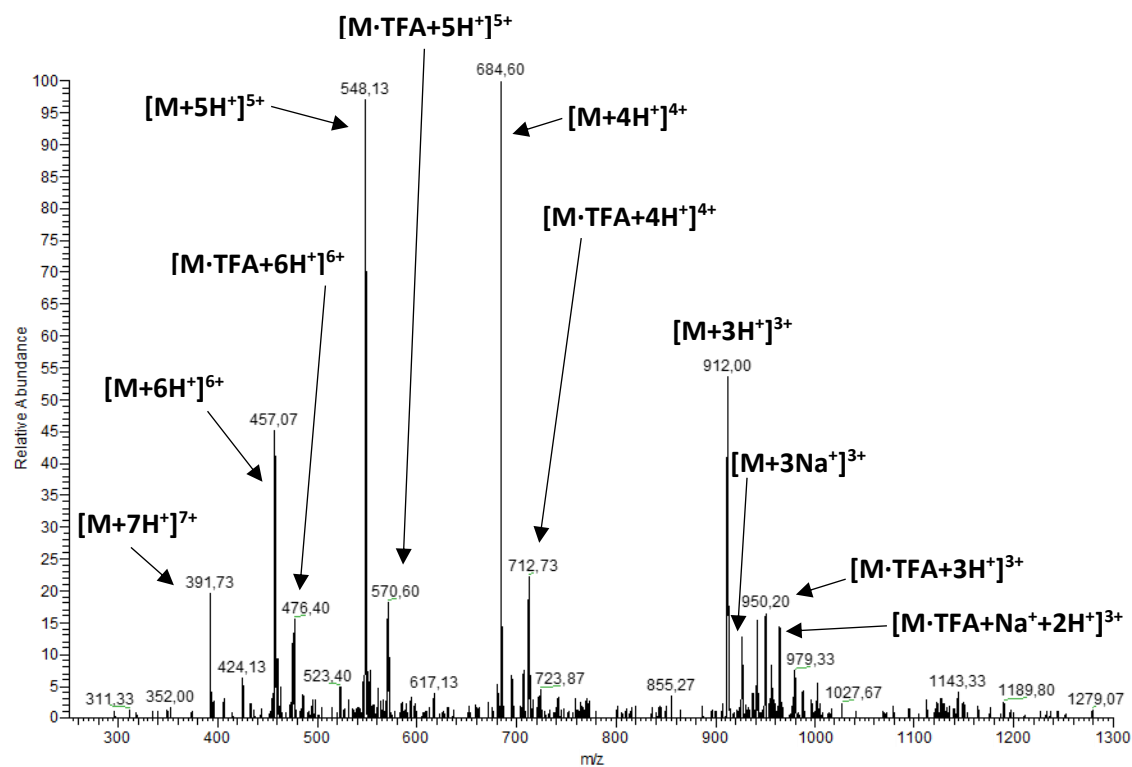
**Figure S22** – Chromatogram obtained for Cq-C4-Transportan (**5b**), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



**Figure S23** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-DPT-sh1 (**5c**)  
(observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions)

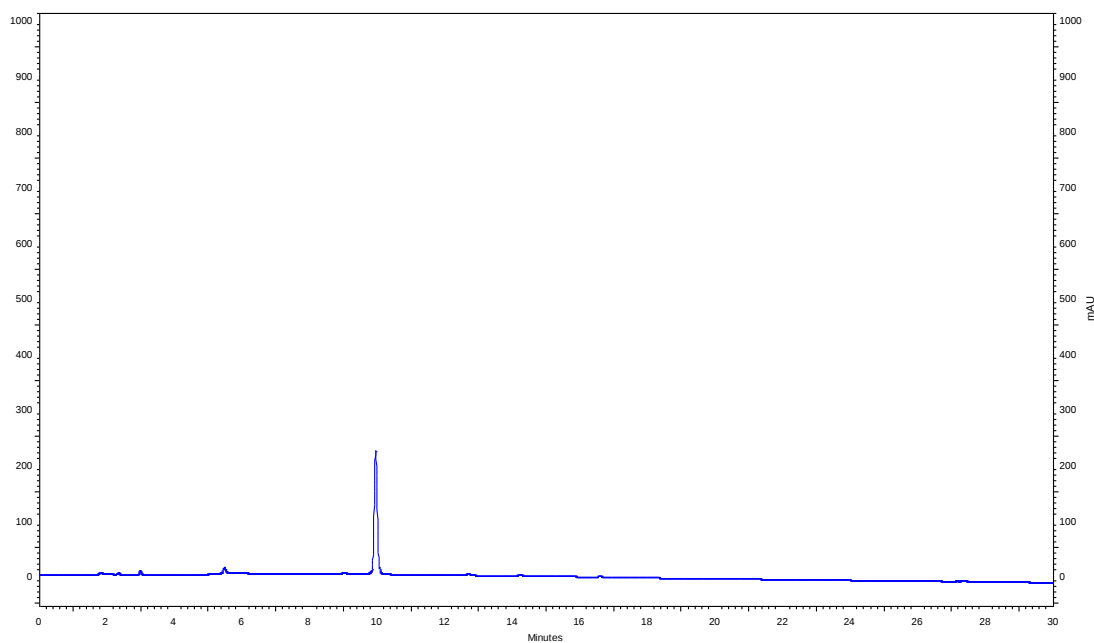


**Figure S24**– Chromatogram obtained for Cq-C4-DPT-sh1 (**5c**), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5 µm), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



**Figure S25** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-DPT-sh2 (**5d**)

(observed as the tri-, tetra-, penta-, hexa-, and hepta-protonated ions;  
sodium and TFA adducts at different protonation states also observed)



**Figure S26** – Chromatogram obtained for Cq-C4-DPT-sh2 (**5d**), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.

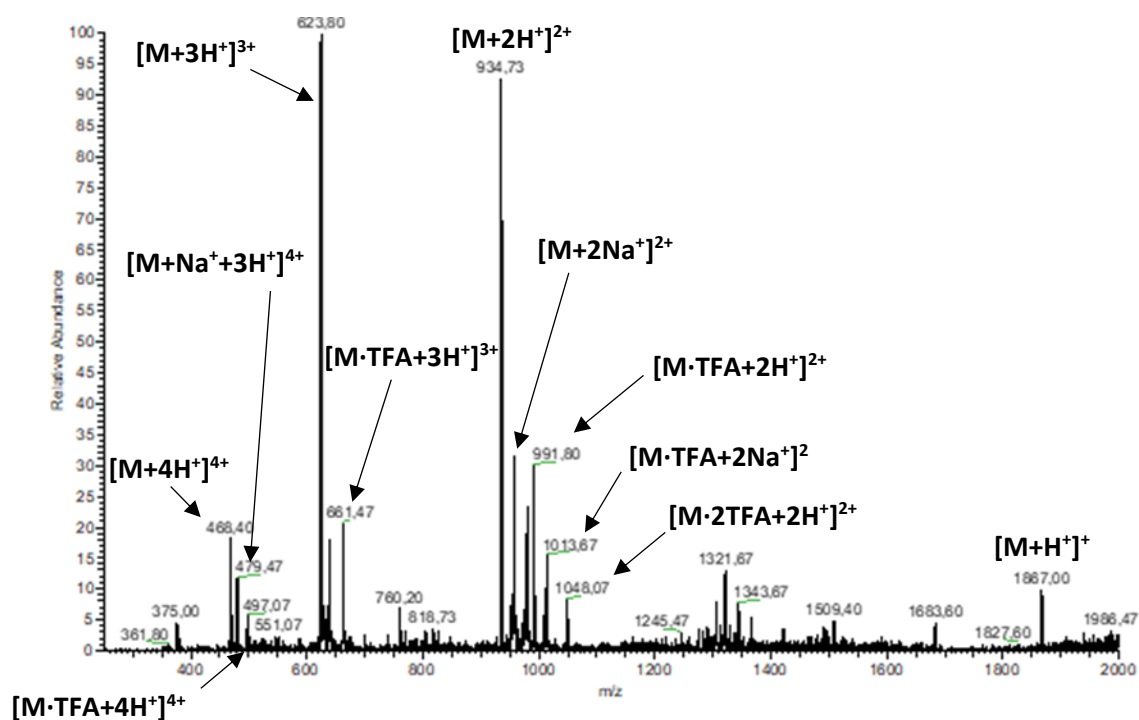


Figure S27 - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-IDR-1018 (5e)  
 (observed as the quasi-molecular ion, and also as the di-, tri-, and tetra-protonated ions;  
 sodium and TFA adducts at different protonation states also observed)

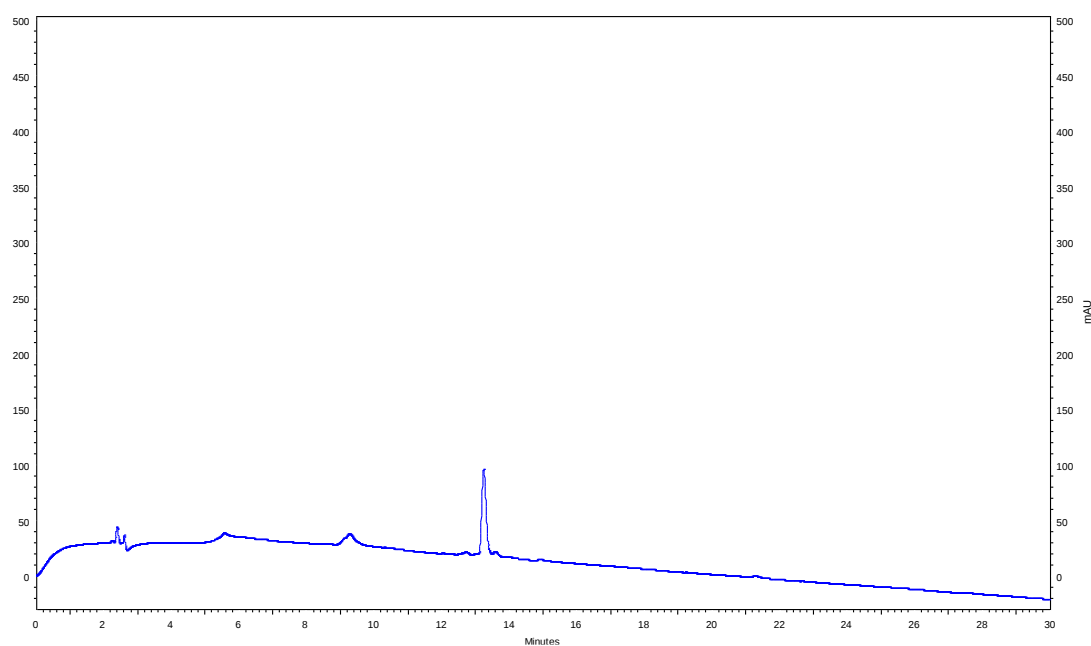
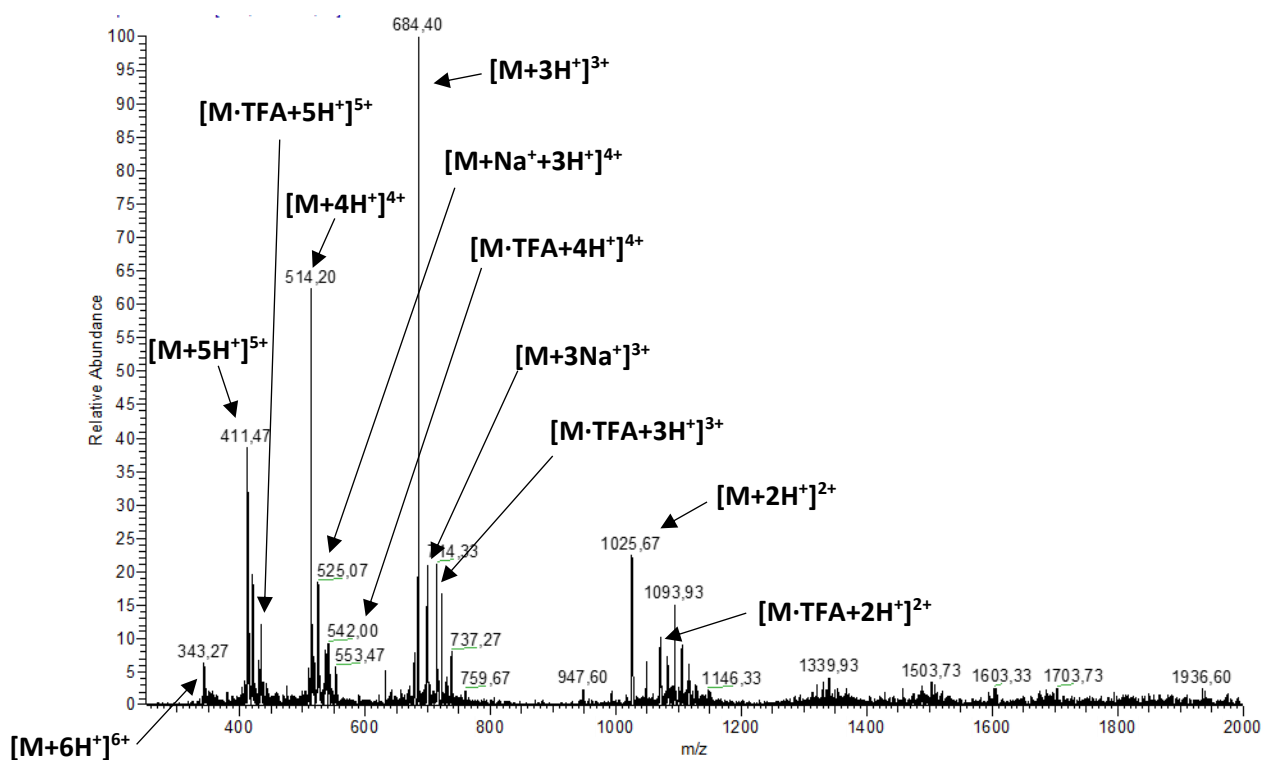
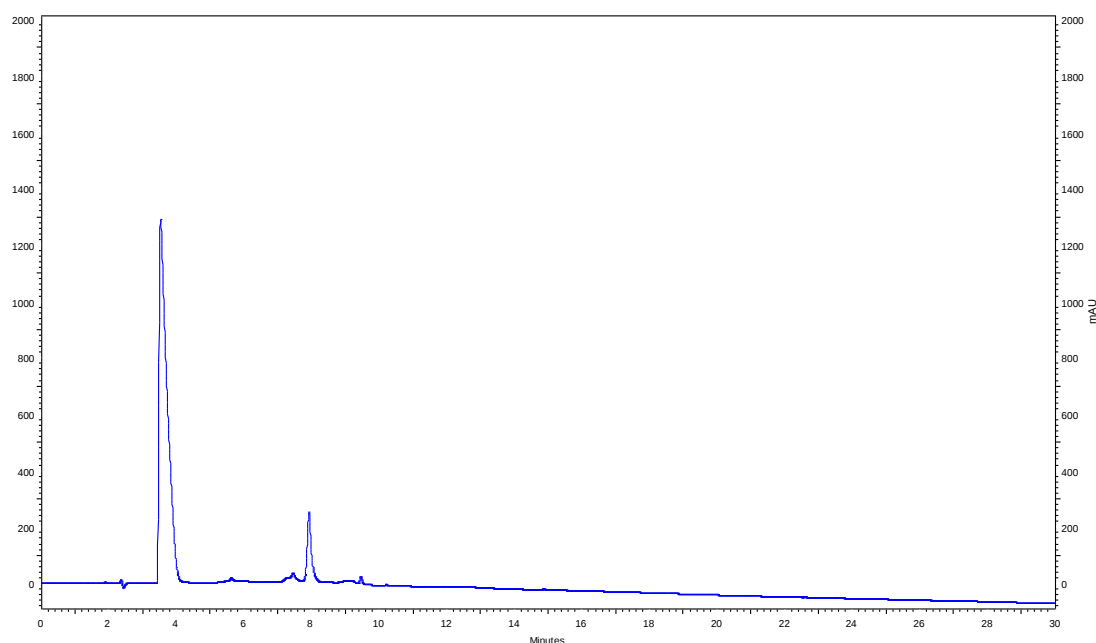


Figure S28 – Chromatogram obtained for Cq-C4-IDR-1018 (5e), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda$ =220 nm.

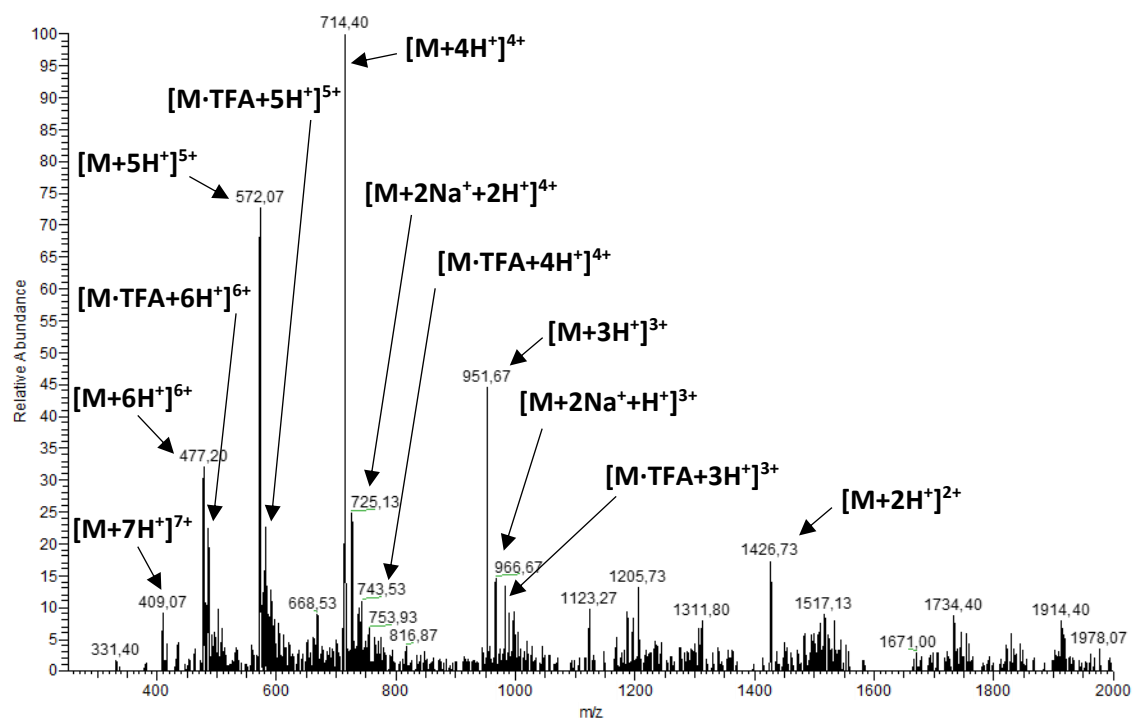


**Figure S29** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-TAT (**5f**)

(observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
sodium and TFA adducts at different protonation states also observed)

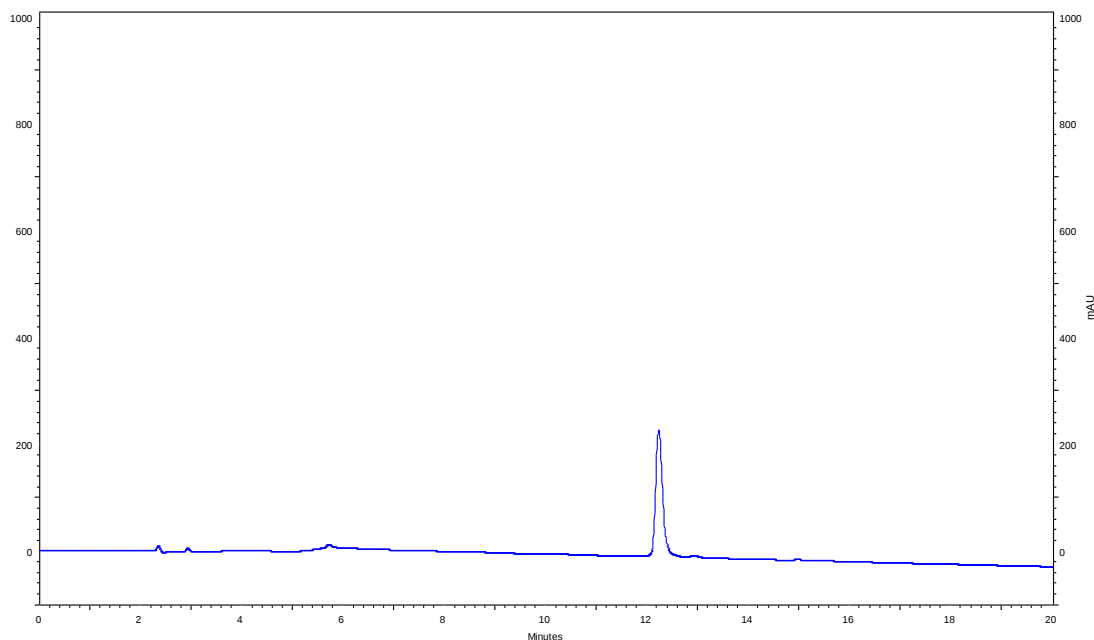


**Figure S30** – Chromatogram obtained for Cq-C4-TAT (**5f**), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm. The peak at RT  $\approx$  4 min is due to acetic acid, added to the samples for complete peptide solubilization.

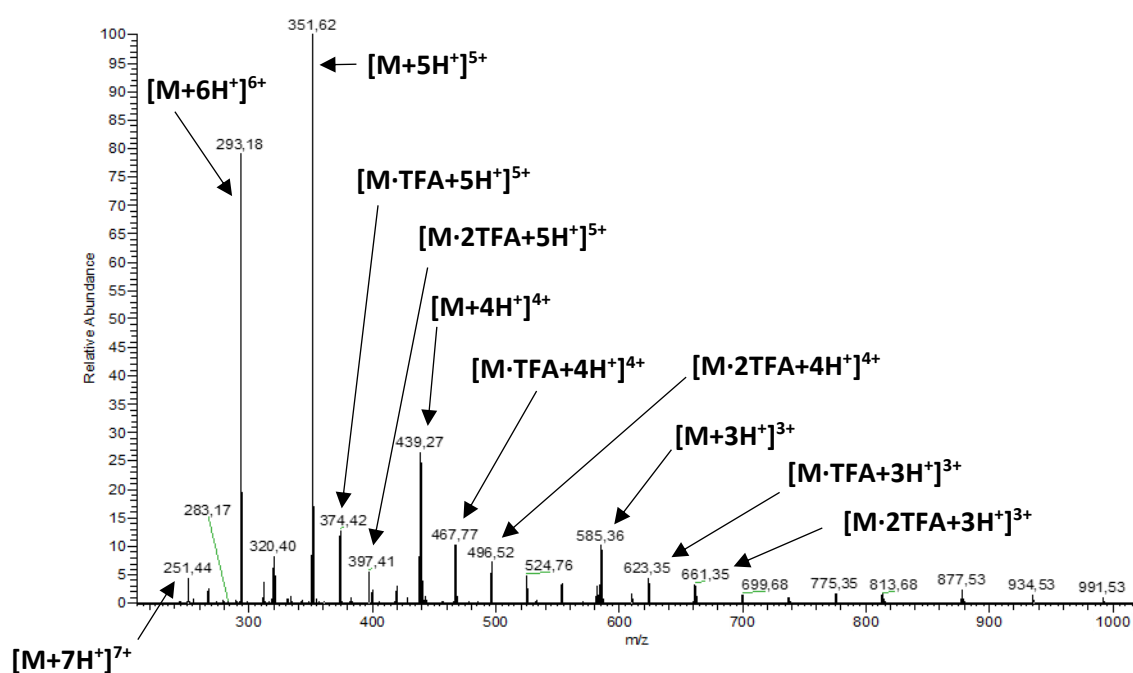


**Figure S31** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-PasTAT (**5g**)

(observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
sodium and TFA adducts at different protonation states also observed)



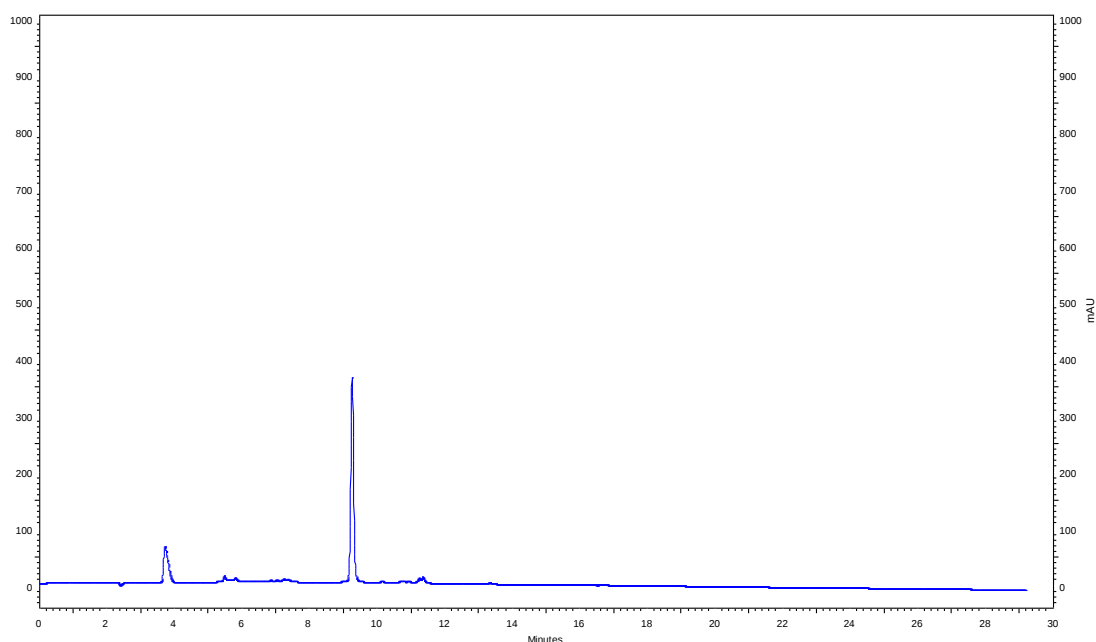
**Figure S32** – Chromatogram obtained for Cq-C4-PasTAT (**5g**), with a gradient elution of 0-66% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 20 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



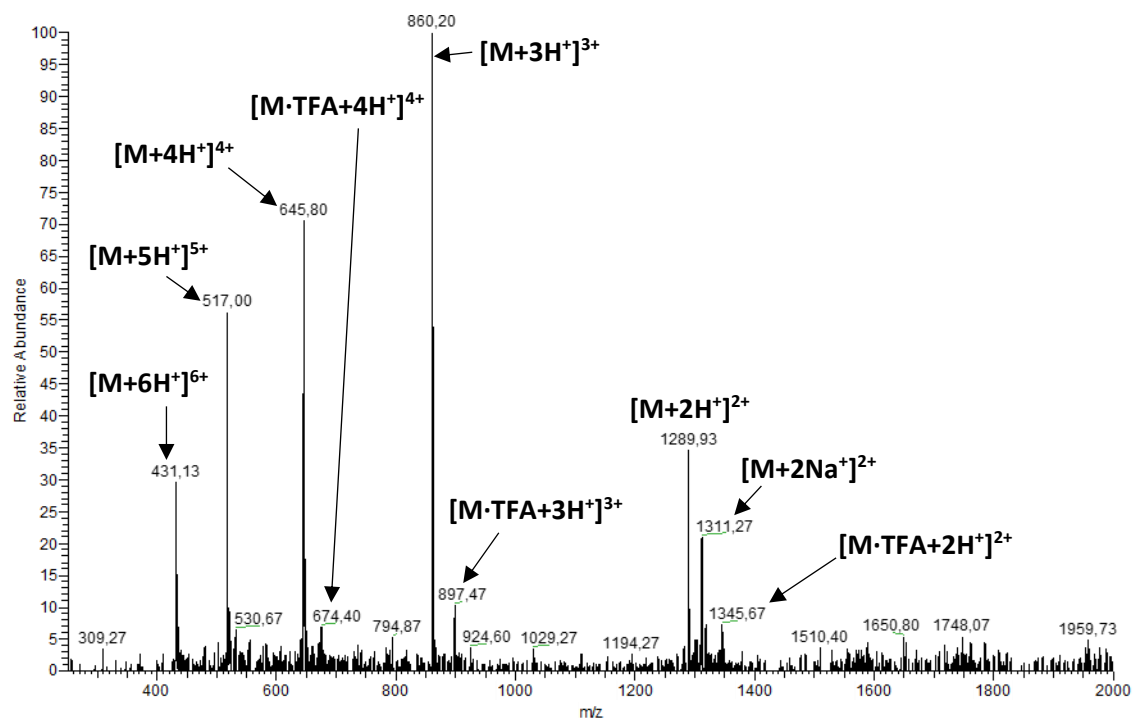
**Figure S33** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-R9 (**5h**)

(observed as tri-, tetra-, penta-, hexa-, and hepta-protonated ions;

TFA adducts at different protonation states also observed)

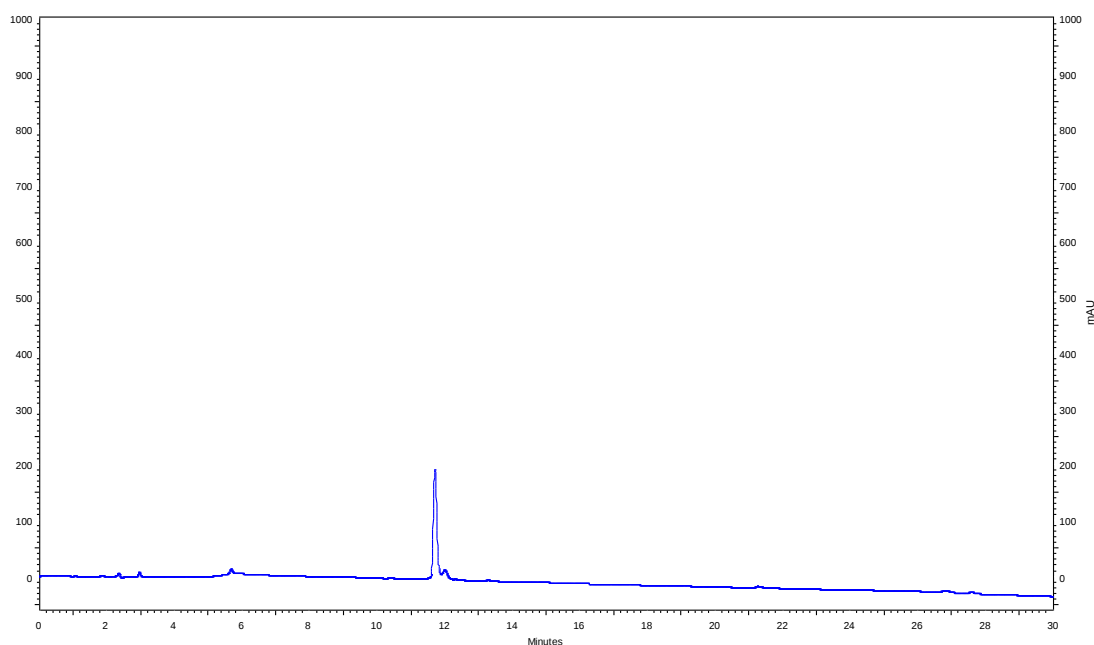


**Figure S34** – Chromatogram obtained for Cq-C4-R9 (**5h**) after purification, with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda$ =220 nm.

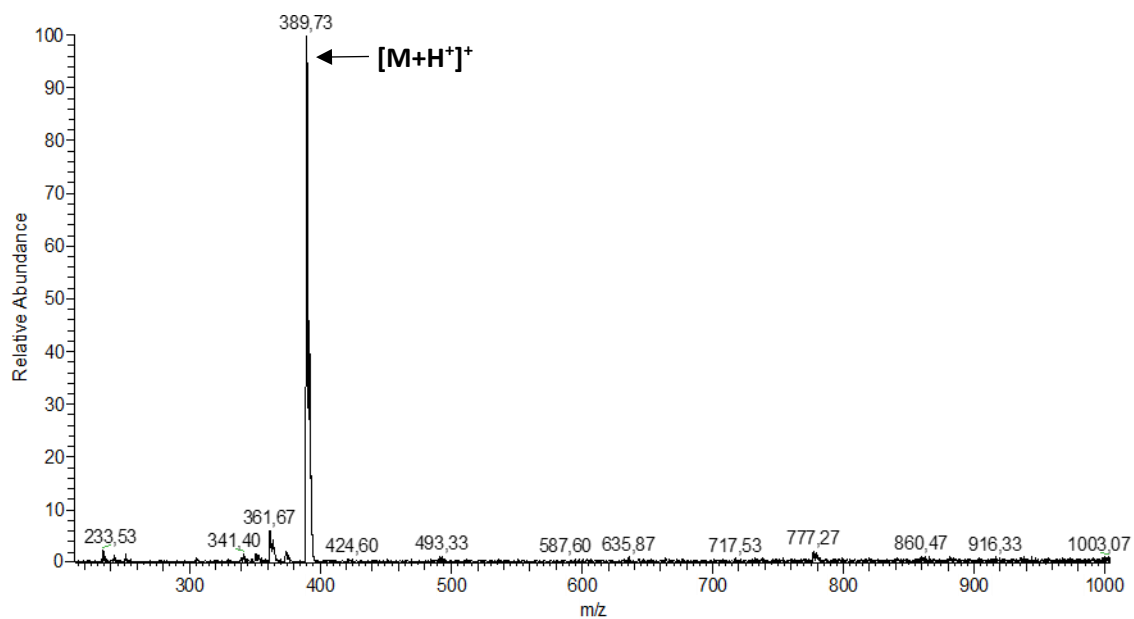


**Figure S35** - ESI-IT MS spectrum (positive mode) of conjugate Cq-C4-Penetratin (**5i**)

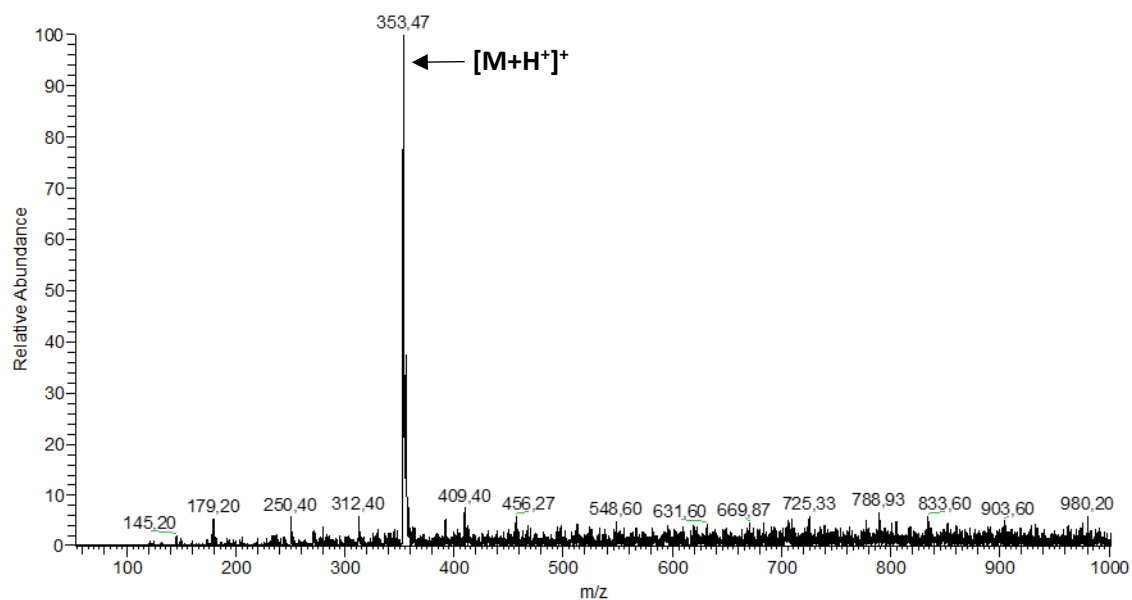
(observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
sodium and TFA adducts at different protonation states also observed)



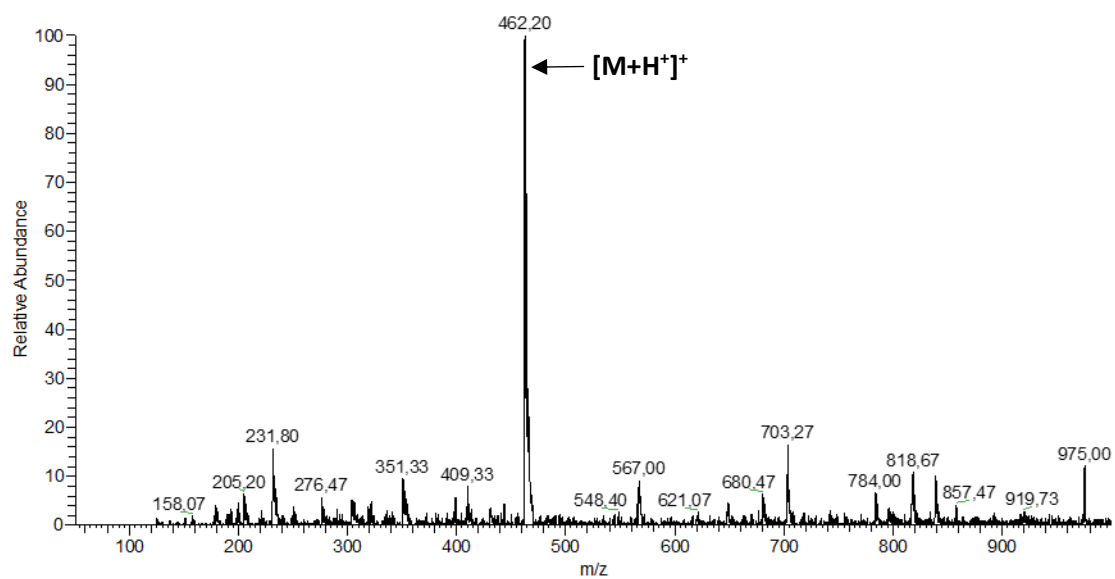
**Figure S36** – Chromatogram obtained for Cq-C4-Penetratin (**5i**), with a gradient elution of 0-100% ACN in water (0,05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



**Figure S37** - ESI-IT MS spectrum (positive mode) of Cq-C6-N<sub>3</sub> (7)  
(observed as the quasi-molecular ion)

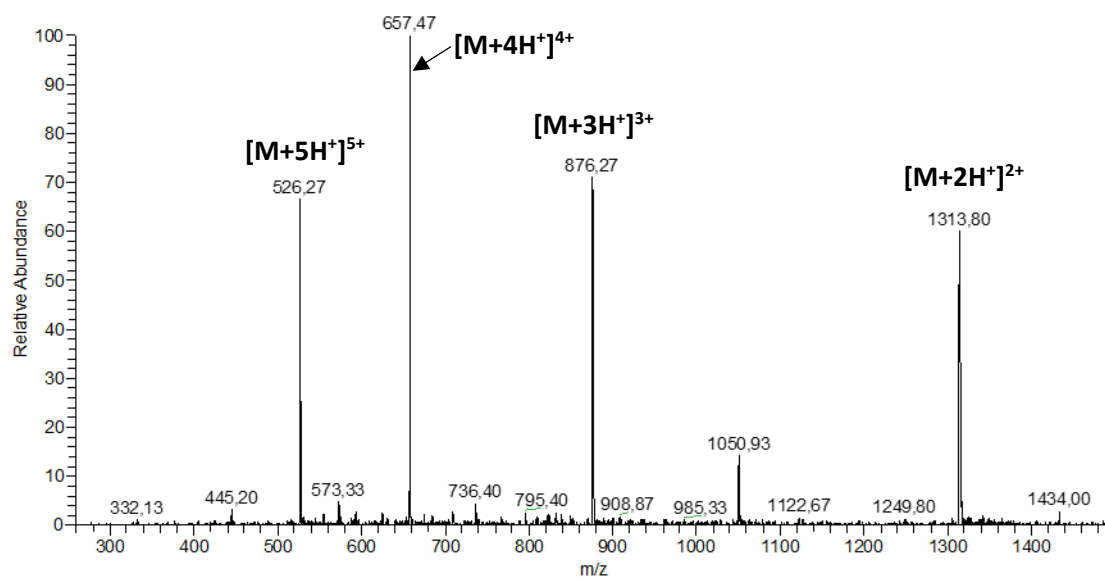


**Figure S38** - ESI-IT MS spectrum (positive mode) of Cq-Cys (8)  
(observed as the quasi-molecular ion)

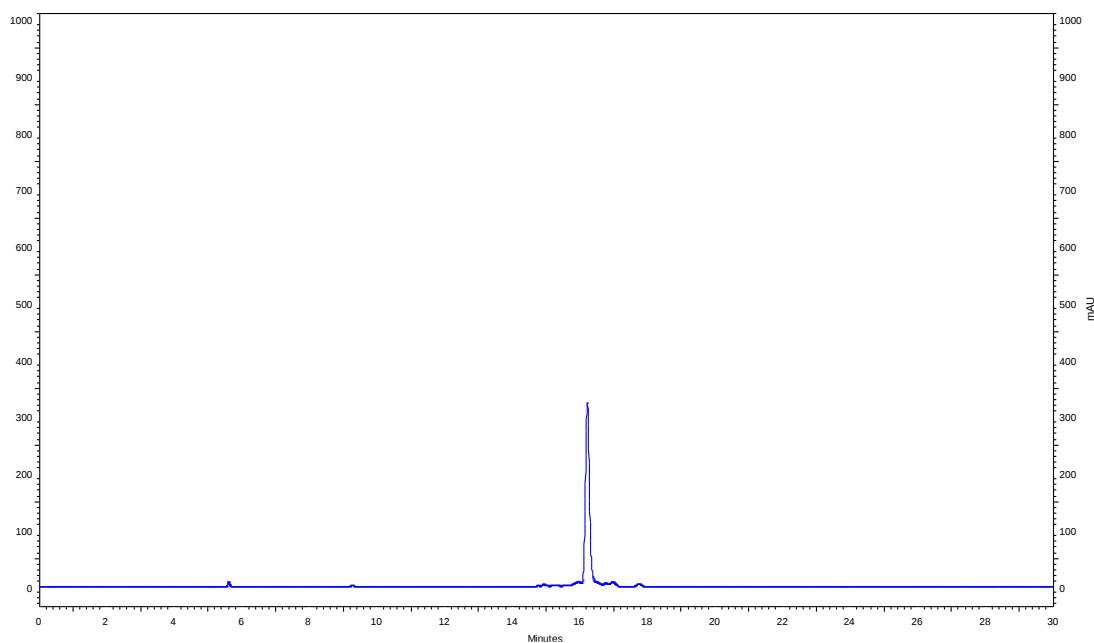


**Figure S39** - ESI-IT MS spectrum (positive mode) of Cq-Cys(2-PDS) (9)

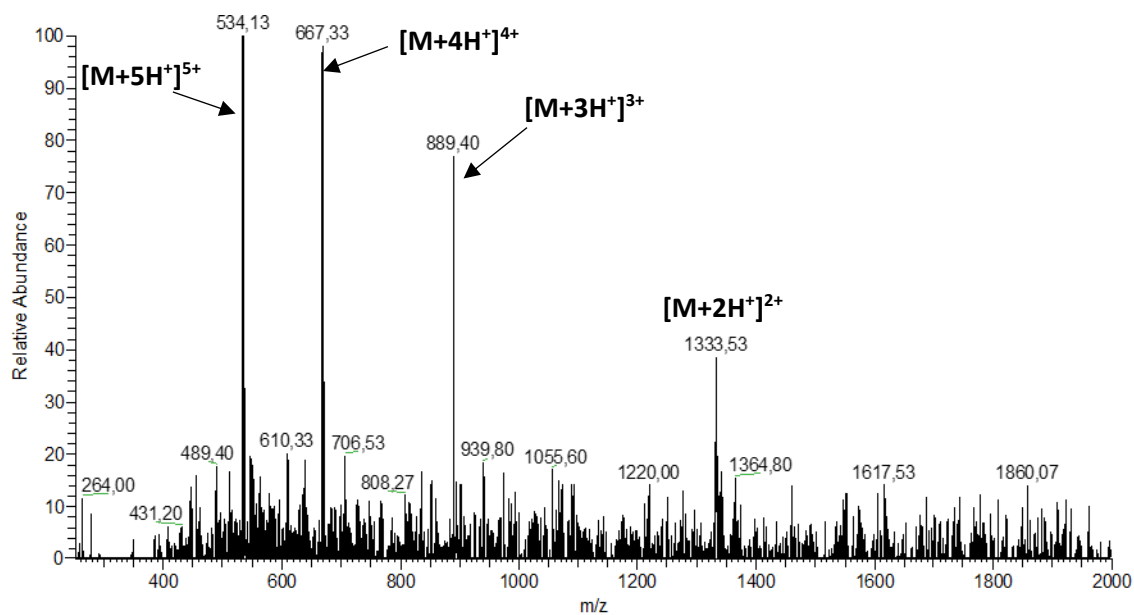
(observed as the quasi-molecular ion)



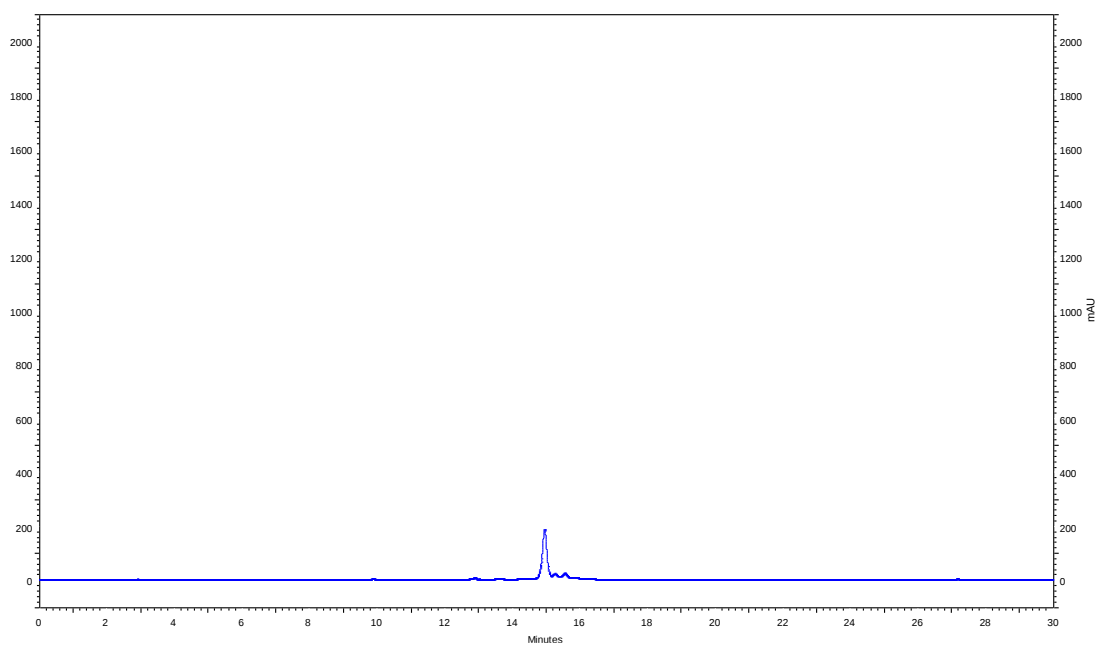
**Figure S40** - ESI-IT MS spectrum (positive mode) of Cq-C10-TP10 (**10**)  
 (observed as the di-, tri-, tetra-, and penta-protonated ions)



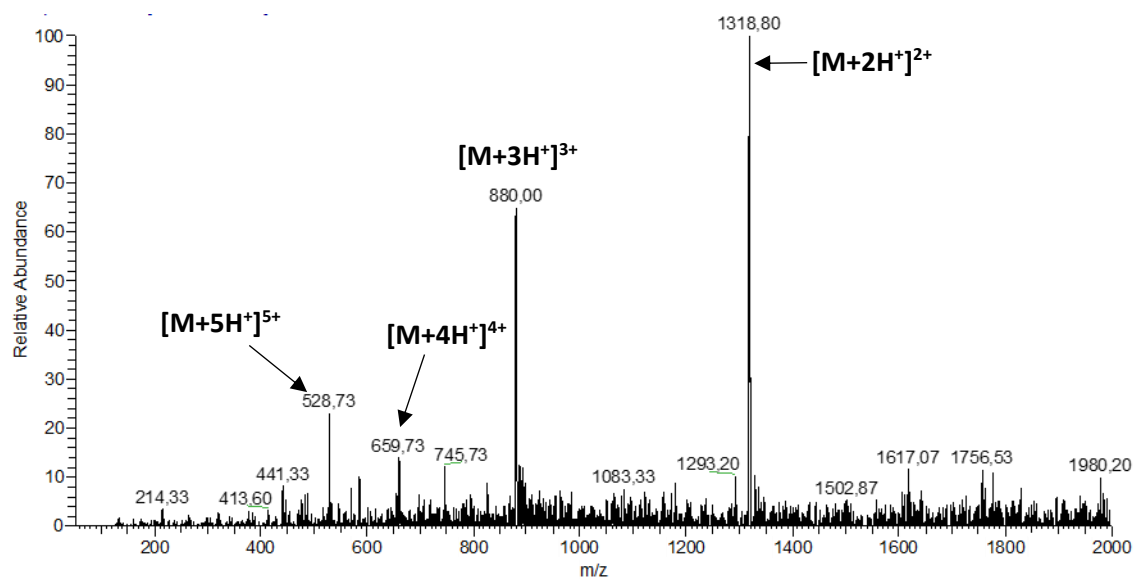
**Figure S41** – Chromatogram obtained for Cq-C10-TP10 (**10**), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda$ =220 nm.



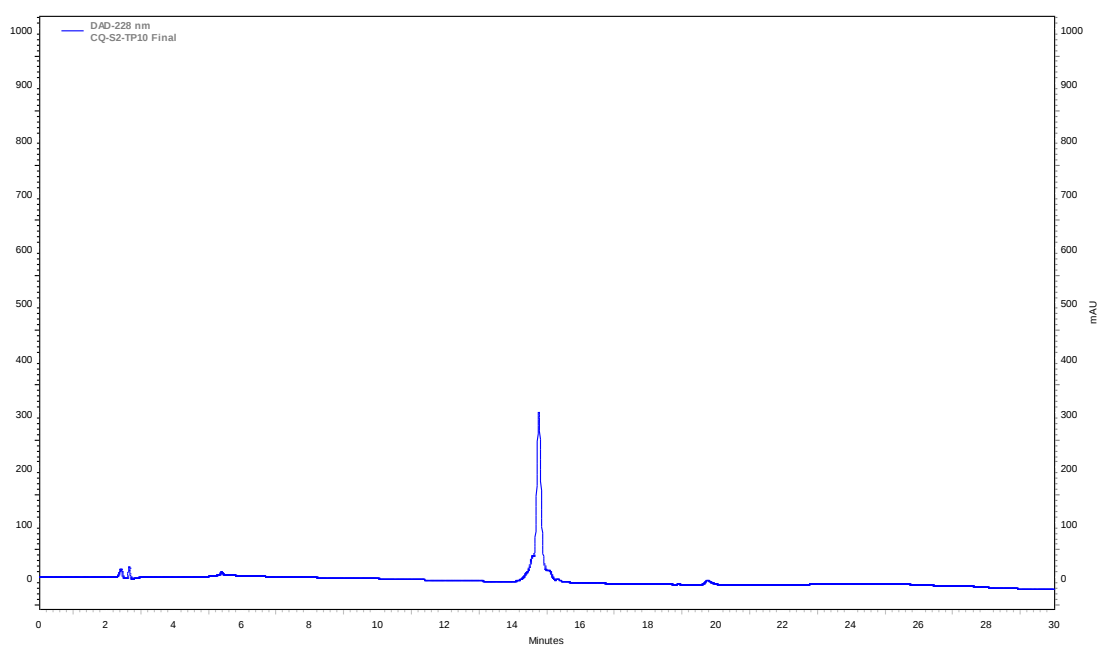
**Figure S42** - ESI-IT MS spectrum (positive mode) obtained for Cq-TR-TP10 (**11**)  
 (observed as the di-, tri, tetra-, and penta-protonated ions)



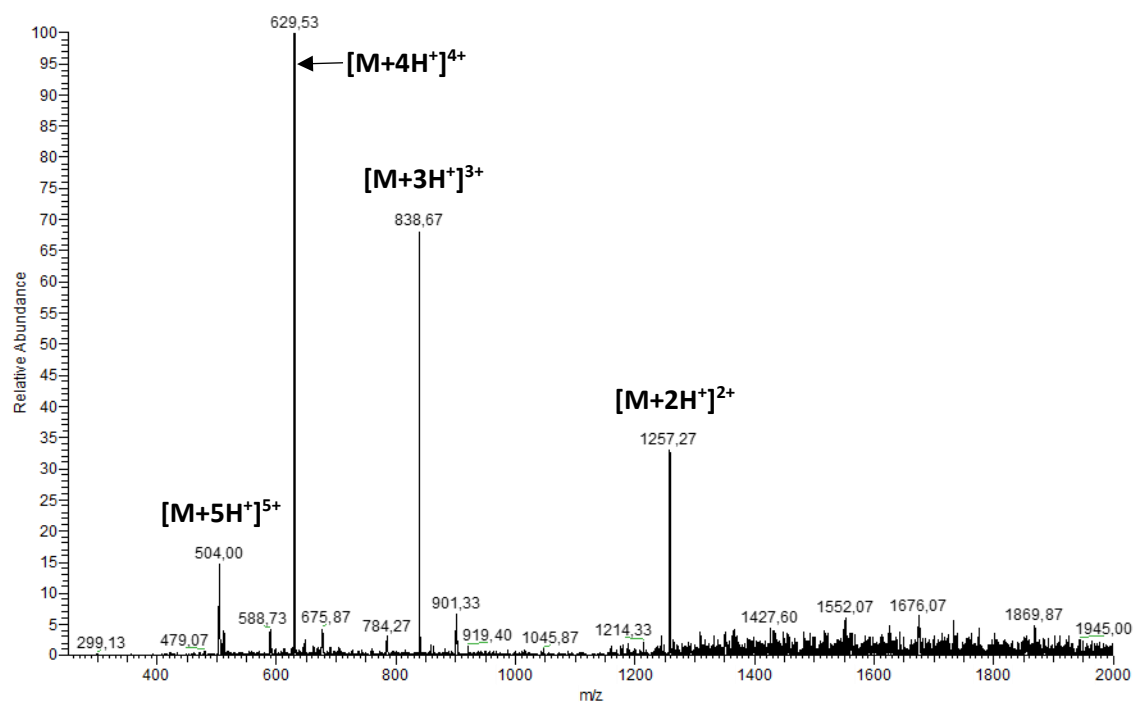
**Figure S43** – Chromatogram obtained for Cq-TR-TP10 (**11**), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



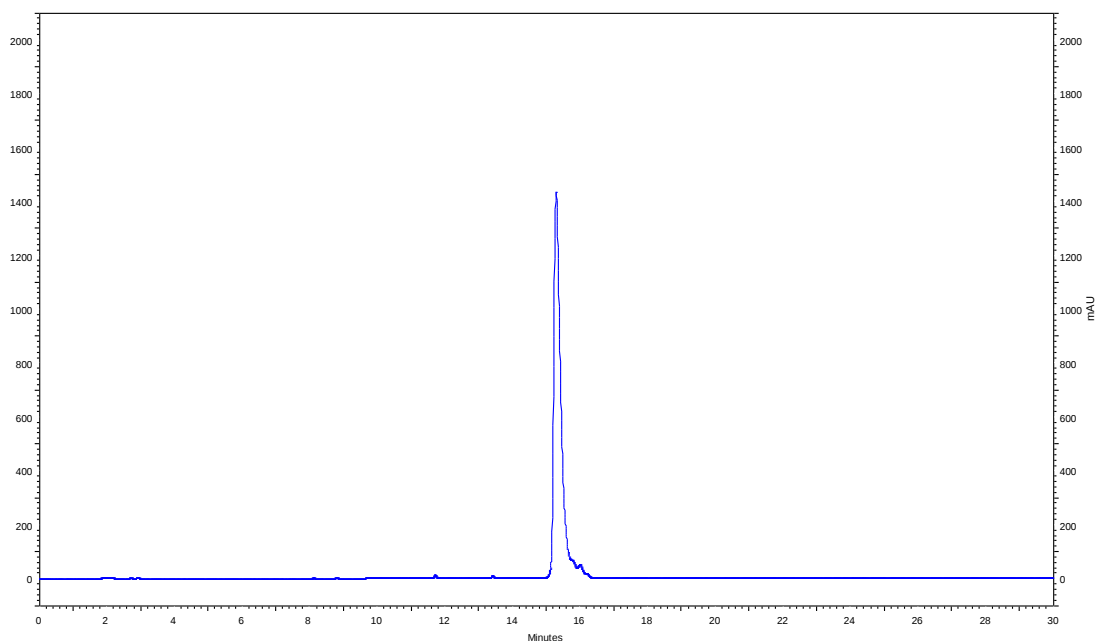
**Figure S44** - ESI-IT MS spectrum (positive mode) obtained for Cq-S-S-TP10 (**12**)  
 (observed as the di-, tri-, tetra-, and penta-protonated ions)



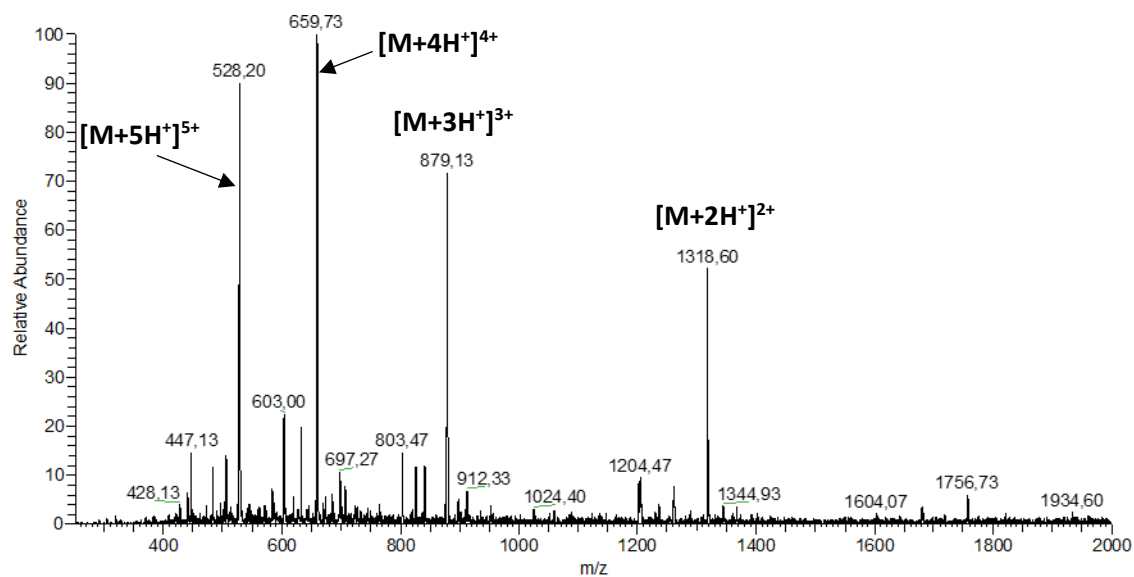
**Figure S45** – Chromatogram obtained for CQn-S-S-TP10 (**12**), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



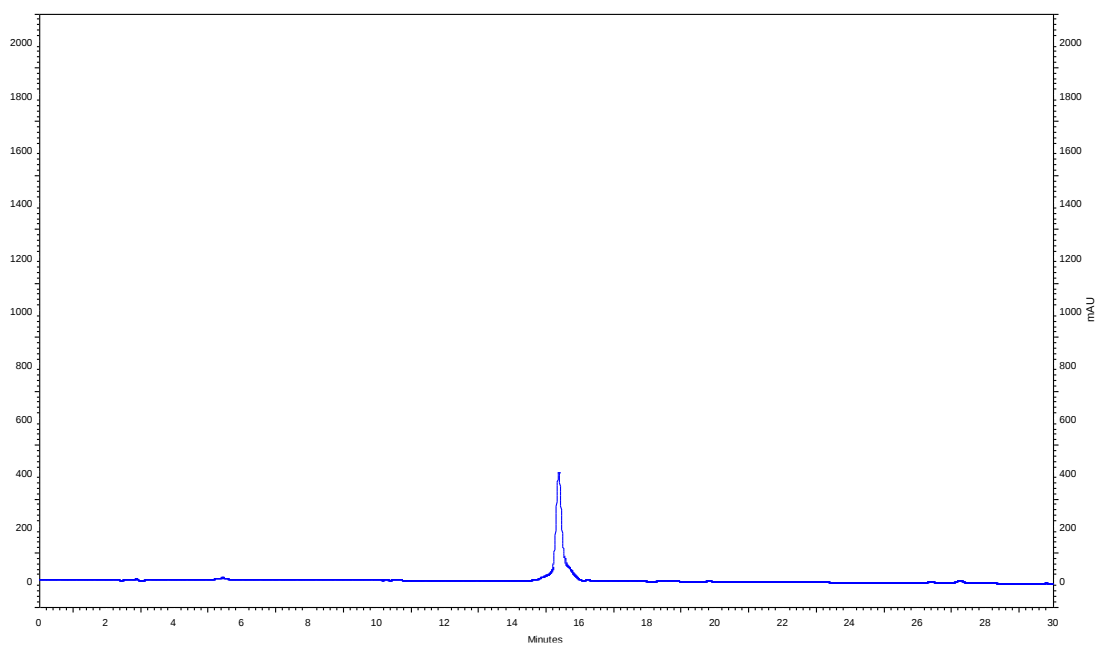
**Figure S46** - ESI-IT MS spectrum (positive mode) of TP10-C4-Cq (**13**)  
 (observed as the di-, tri-, tetra-, and penta-protonated ions)



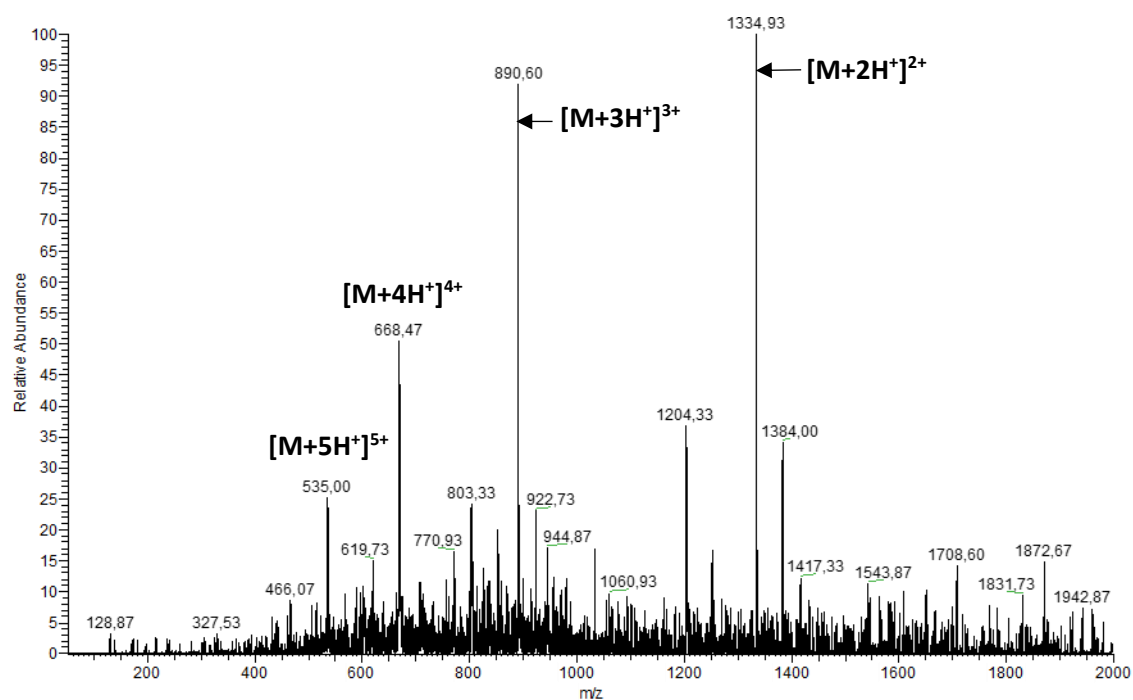
**Figure S47** - Chromatogram obtained for TP10-C4-Cq (**13**), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



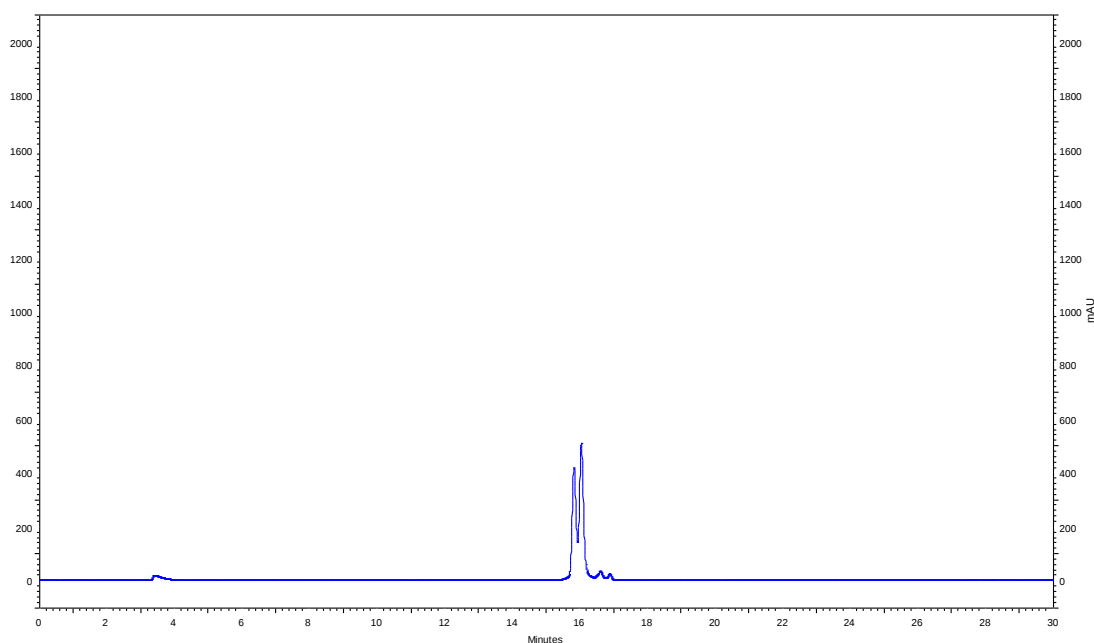
**Figure S48** - ESI-IT MS spectrum (positive mode) of TP10-S-S-Cq (**14**)  
 (observed as the di-, tri-, tetra-, and penta-protonated ions)



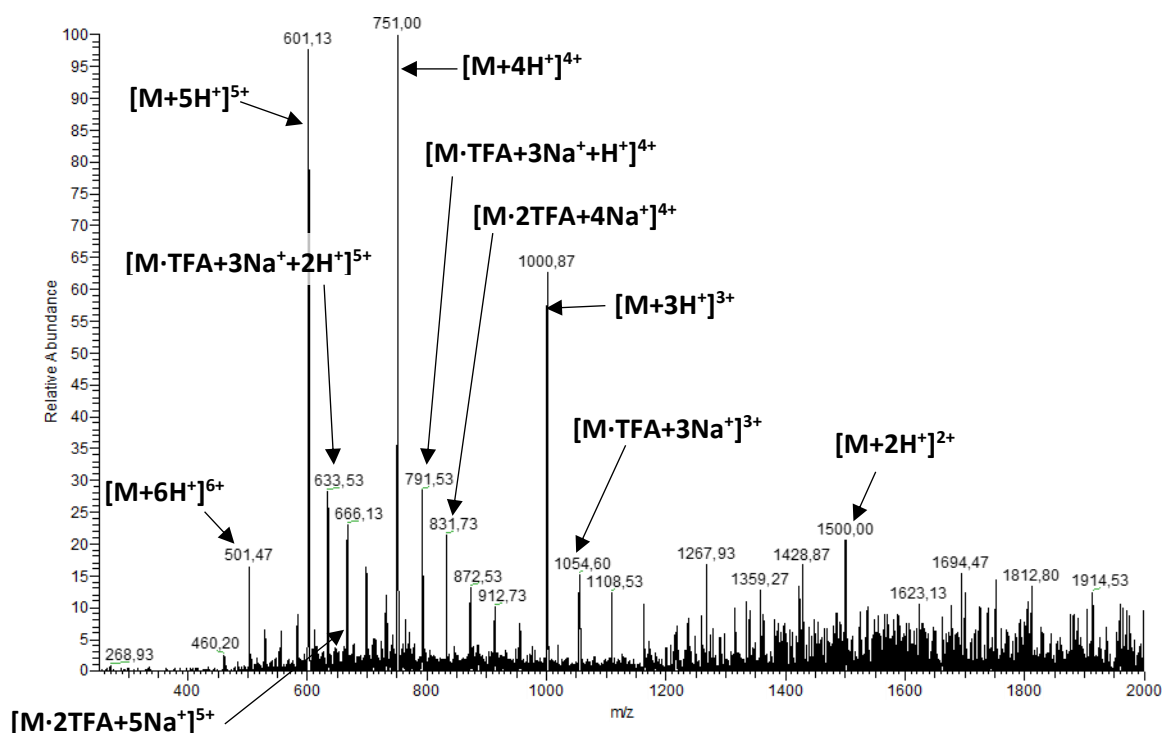
**Figure S49** - Chromatogram obtained for TP10-S-S-Cq (**14**), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm.



**Figure S50** - ESI-IT MS spectrum (positive mode) of TP10-K(CF) (**15**)  
(observed as the di-, tri-, tetra-, and penta-protonated ions)

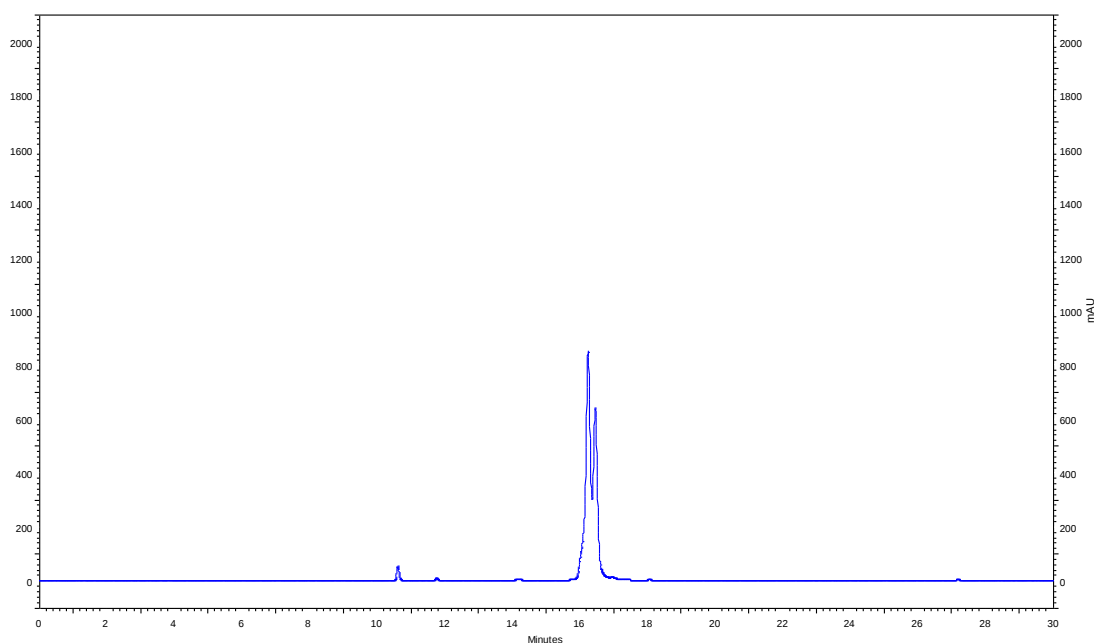


**Figure S51** – Chromatogram obtained for TP10-K(CF) (**15**), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda=220$  nm. Note: a mixture of 5- and 6-carboxyfluorescein was used to label the peptide, hence the two peaks observed in the HPLC trace.



**Figure S52** - ESI-IT MS spectrum (positive mode) of Cq-C4-TP10-K(CF) (16)

(observed as the di-, tri-, tetra-, penta-, and hexa-protonated ions;  
sodium and TFA adducts at different protonation states also observed)

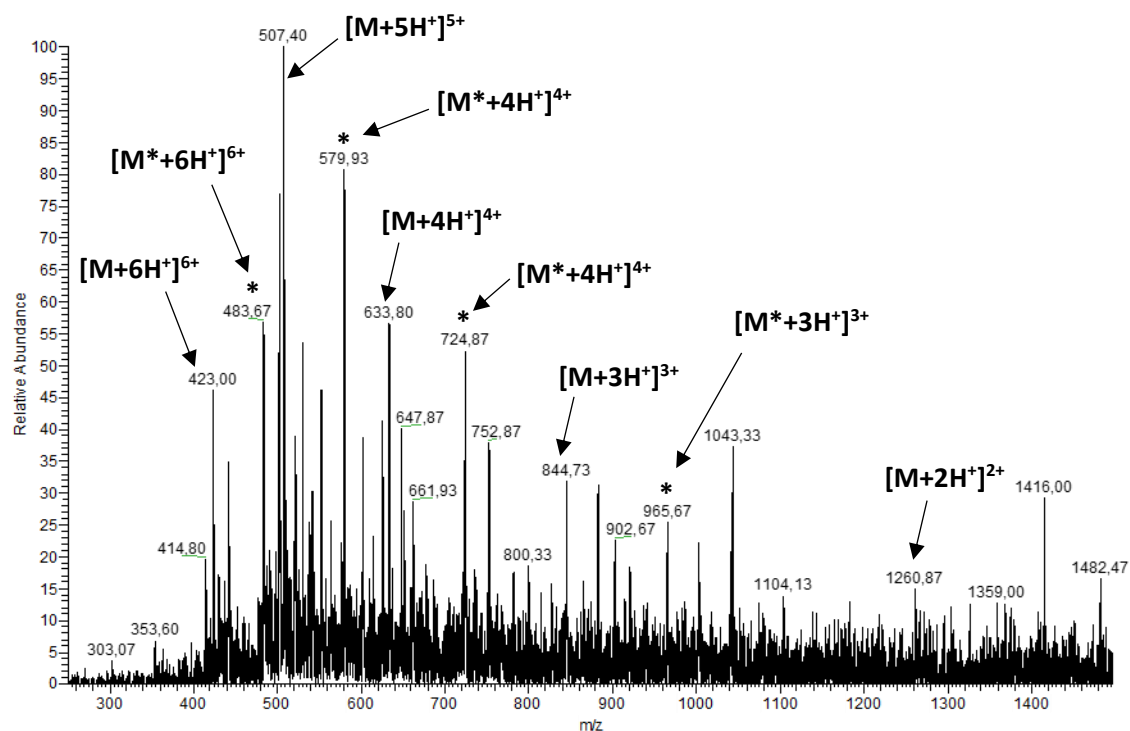


**Figure S53** – Chromatogram for Cq-C4-TP10-K(CF) (16), with a gradient elution of 0-100% ACN in

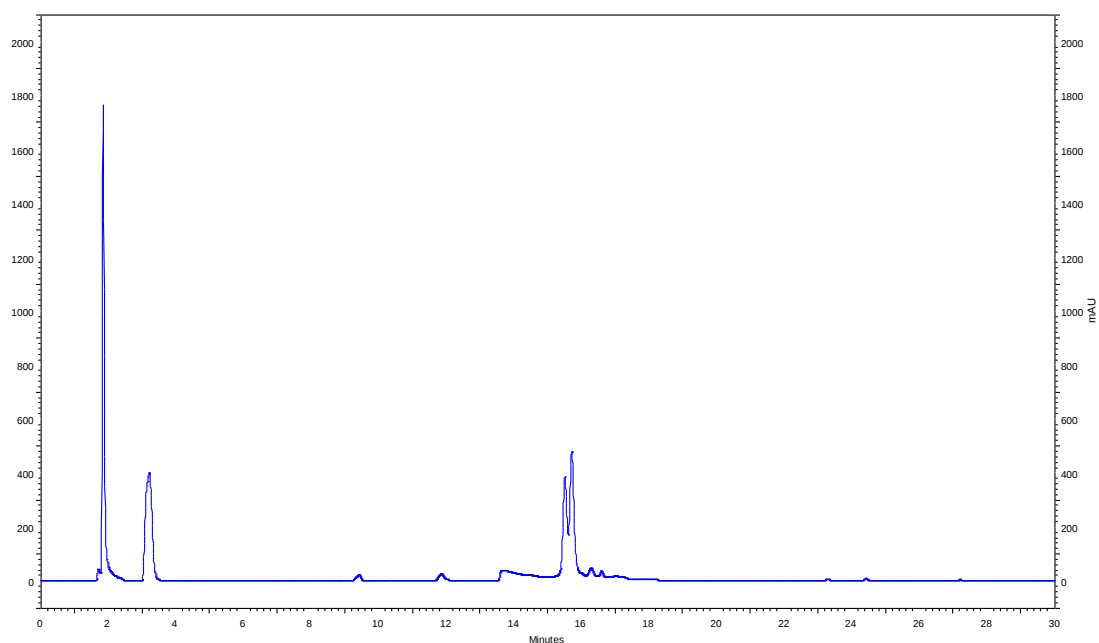
water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at

$\lambda=220$  nm. Note: a mixture of 5- and 6-carboxyfluorescein was used to label the peptide, hence the

two peaks observed in the HPLC trace.



**Figure S54** - ESI-IT MS spectrum (positive mode) obtained for crude Cq-C4-TAT-K(CF) (17) (observed as the di-, tri-, tetra-, penta-, and hexa-protonated ion; peaks labeled with an asterisk are due to a doubly CF-labeled conjugate that was removed upon purification, see HPLC trace below)



**Figure S55** – Chromatogram obtained for Cq-C4-TAT-K(CF) (17), with a gradient elution of 0-100% ACN in water (0.05% TFA) in a RP-18E (5  $\mu$ m), for 30 min and a flow rate of 1 ml/min, with detection at  $\lambda$ =220 nm. The peak at RT  $\approx$  3 min is due to acetic acid, added to the samples for complete peptide

solubilization. Note: a mixture of 5- and 6-carboxyfluorescein was used to label the peptide, hence the two peaks observed in the HPLC trace.