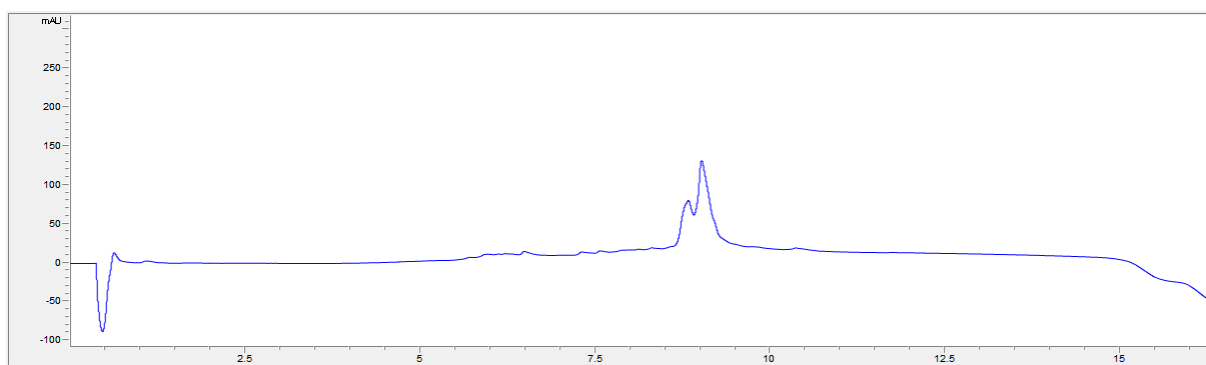


## Peptide Analysis

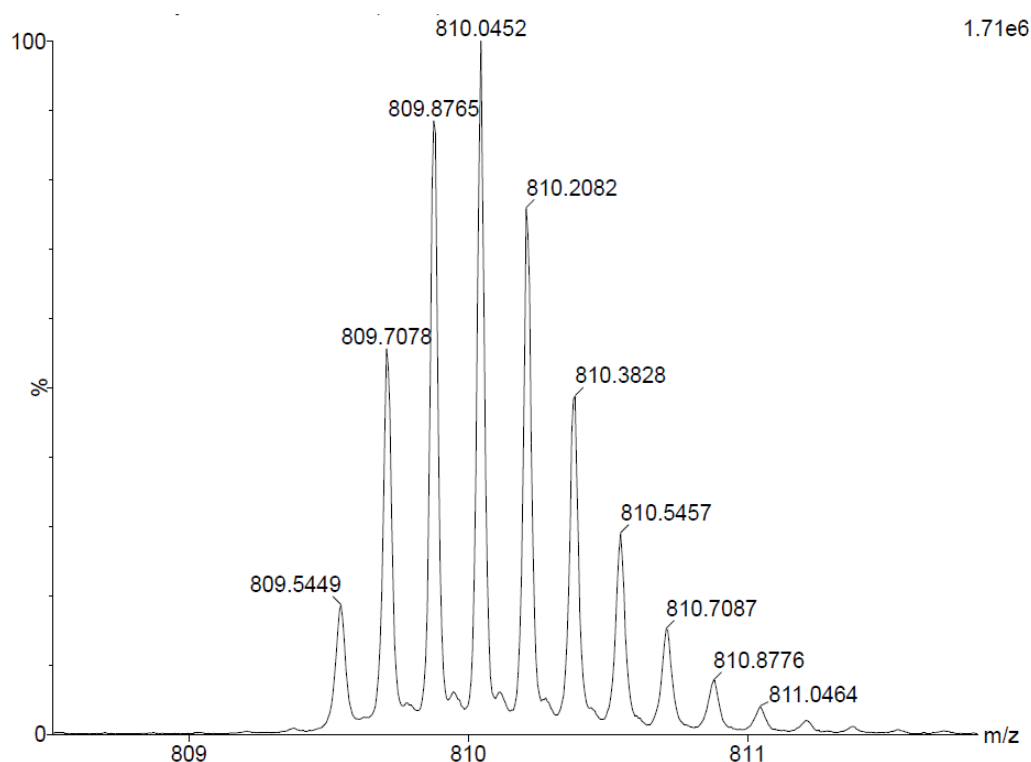
**Superimposition of RP-HPLC of crude precursor and analysis of the folding for BeKm-1 analogues and synthetic native BeKm-1 with HRMS analysis (ESI/QTOF):****Peptide 1: Cy5-PEG3-linker4-BeKm1**

Analysis on C18 Agilent AdvanceBio Peptide mapping column, run: 5-65%B in 12 min.  
Solvent system: A: H<sub>2</sub>O 0.1% TFA, B: MeCN 0.1% TFA.



Due to the crash of one of our computers, the data related to Cy5-PEG3-linker4-BeKm-1 synthetic intermediates are no longer available. We deeply apologize for this inconvenience.

HRMS analysis (ESI/QTOF) of Cy5-PEG3-linker4-BeKm1:  $[M+5H]^{6+} = 809.54$  (4852.19 Da versus theoretical mass of 4852.72 Da)

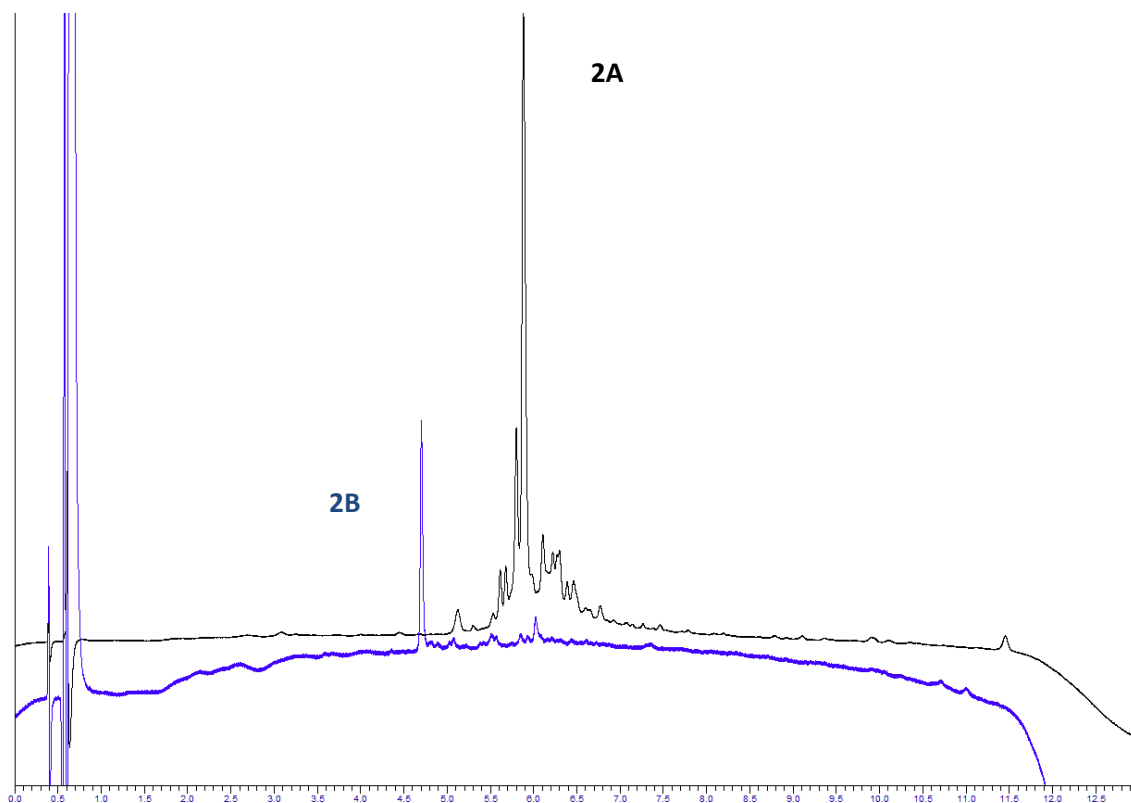


Peptide **2**: Cy5-PEG5-BeKm1-Lys<sup>27</sup>

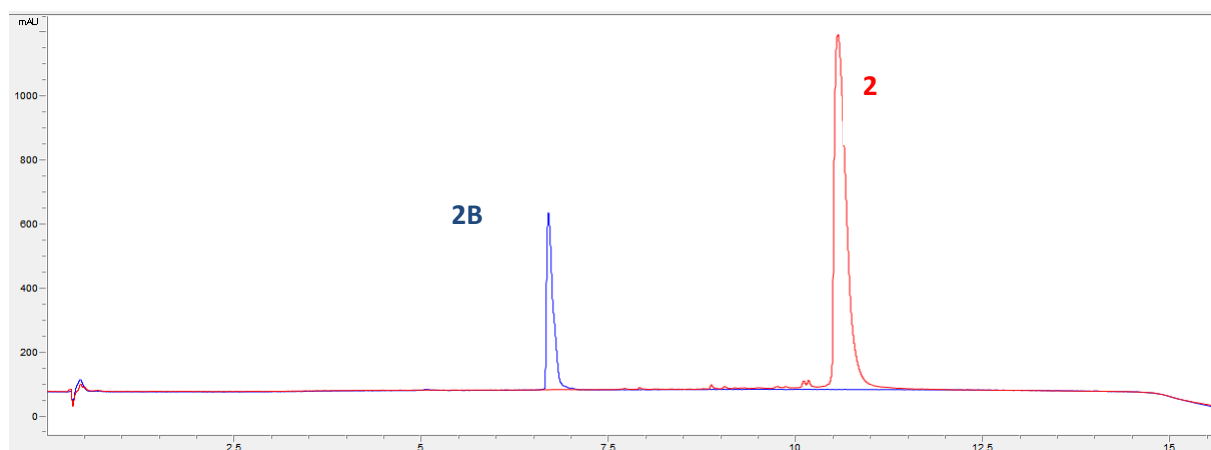
Precursor **2A** Linear (reduced) BeKm1 R27K(pentynoic acid)

Precursor **2B** Folded (oxidized) BeKm1 R27K(pentynoic acid)

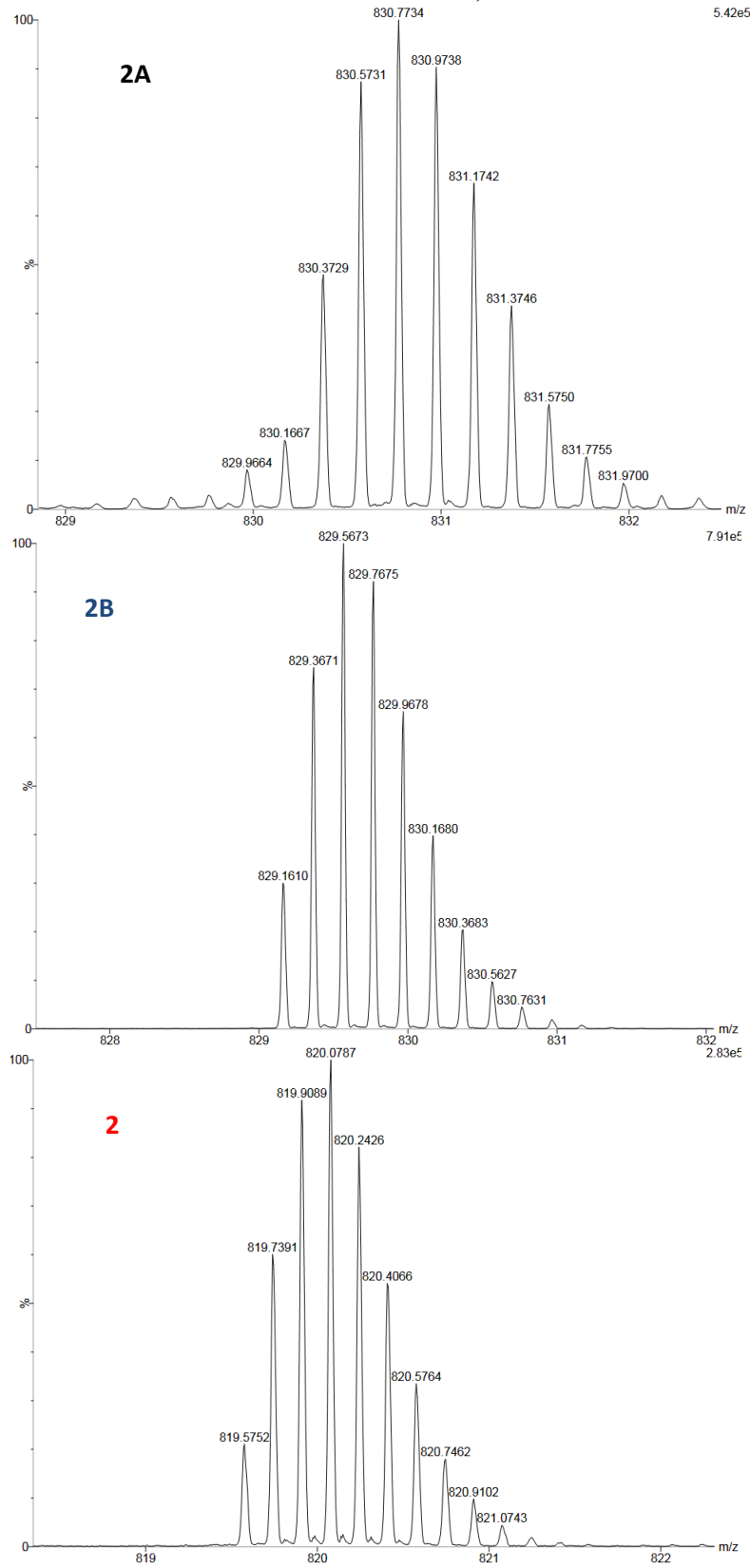
Superimposition of RP-HPLC of crude precursor **2A** and analysis of the folding bath after 24h reaction time showing **2B** as major product. Analysis on Chromolith® RP-18e column, run: 5-65%B in 8 min, 3 ml/min. Solvent system: A: H<sub>2</sub>O 0.1%TFA, B: H<sub>2</sub>O/MeCN 9:1 0.1%TFA.



Superimposition of RP-HPLC of crude precursor **2B** and Cy5-peptide conjugate **2** Analysis on C18 Agilent AdvanceBio Peptide mapping column, run: 5-65%B in 12 min. Solvent system: A: H<sub>2</sub>O 0.1%TFA, B: MeCN 0.1%TFA.



HRMS analysis (ESI/QTOF) of Cy5-PEG5-BeKm1-Lys<sup>27</sup>:  $[M+5H]^{6+} = 819.58$  (4912.43 Da versus theoretical mass of 4912.27 Da).

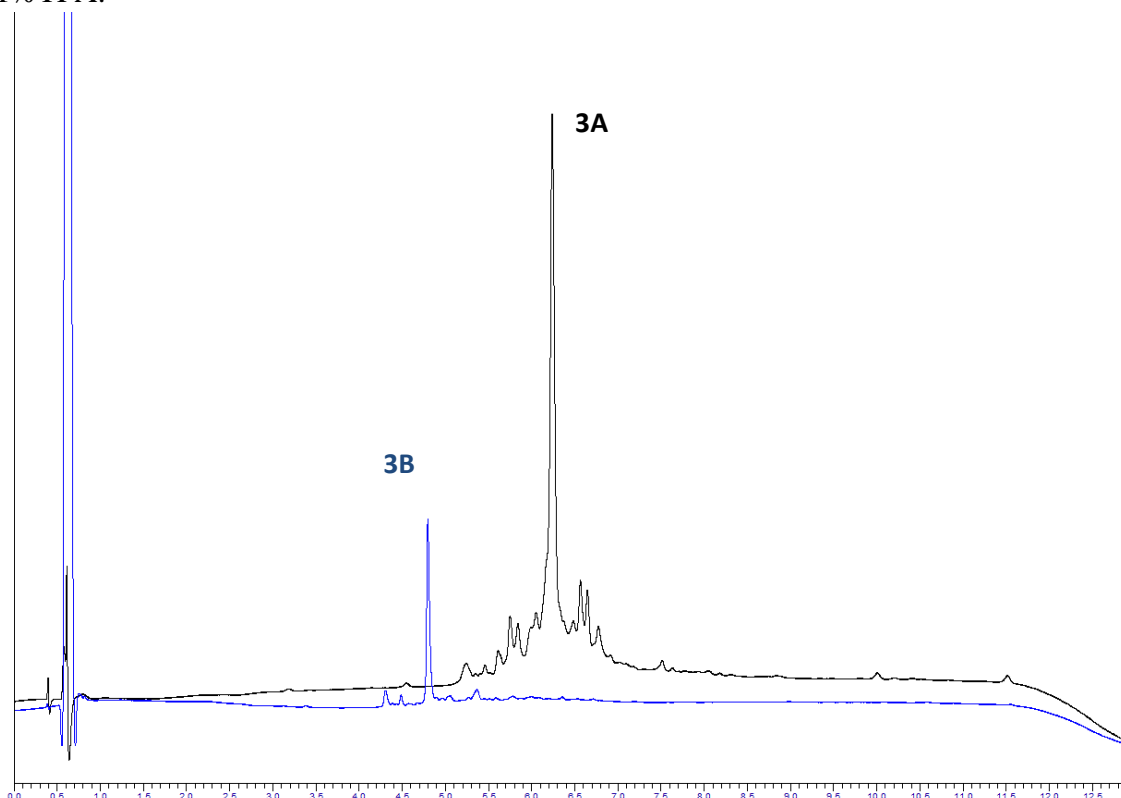


**Peptide 3: Cy5-spacerGS-linker6-BeKm1**

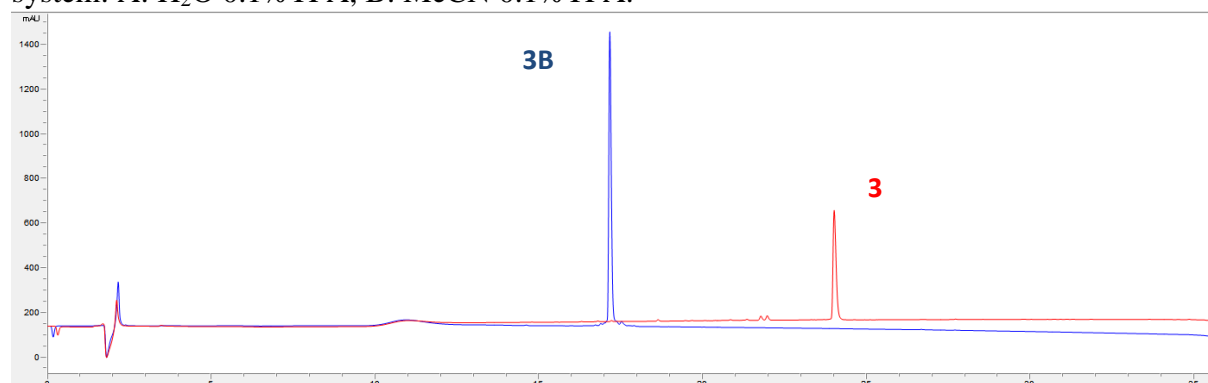
Precursor **3A** Linear (reduced) BeKm1 Nter azidohexanoic acid

Precursor **3B** Folded (oxidized) BeKm1 Nter azidohexanoic acid

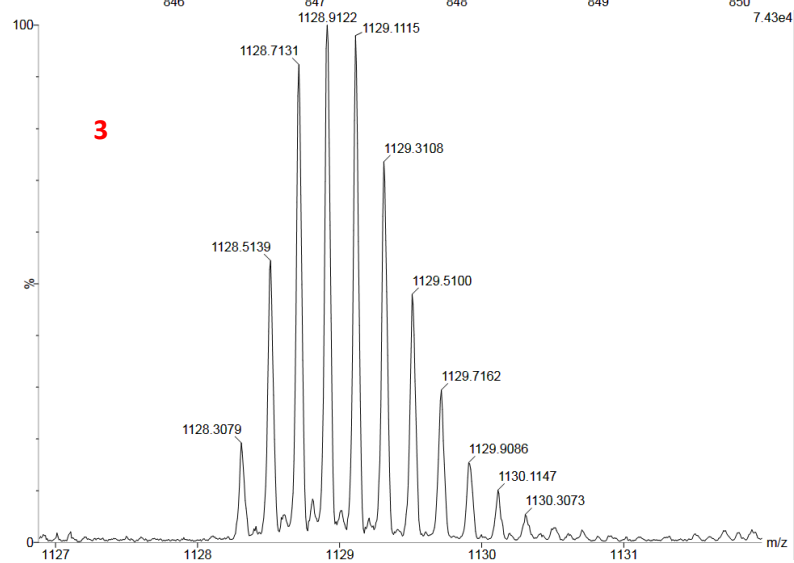
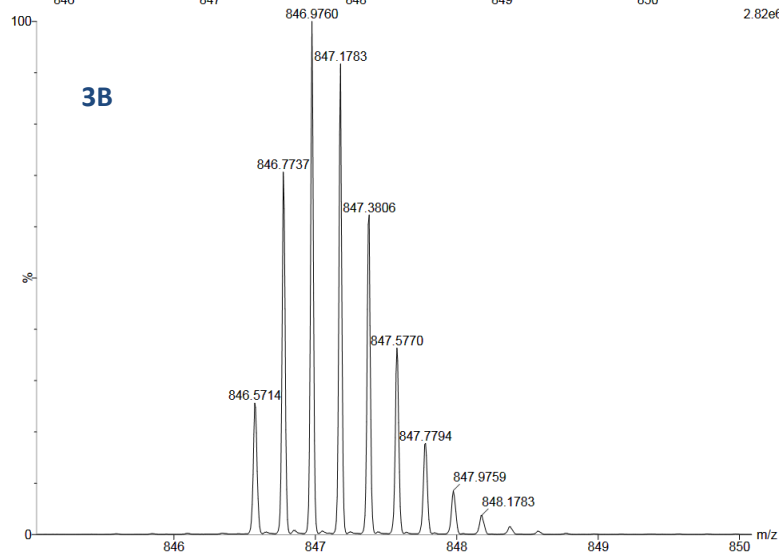
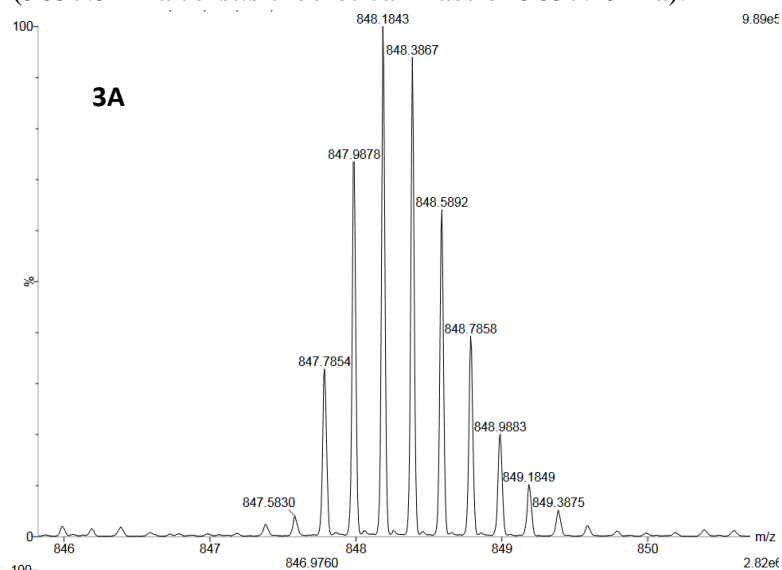
Superimposition of RP-HPLC of crude precursor 3A and analysis of the folding bath after 24h reaction time showing 3B as major product. Analysis on Chromolith® RP-18e column, run: 5-65%B in 8 min, 3 ml/min. Solvent system: A: H<sub>2</sub>O 0.1%TFA, B: H<sub>2</sub>O/MeCN 9:1 0.1%TFA.



Superimposition of RP-HPLC of crude precursor **3B** and Cy5-peptide conjugate **3** Analysis on C18 Agilent AdvanceBio Peptide mapping column, run: 15-65%B in 25 min. Solvent system: A: H<sub>2</sub>O 0.1%TFA, B: MeCN 0.1%TFA.



HRMS analysis (ESI/QTOF) of Cy5-spacerGS-linker6-BeKm1 :  $[M+4H]^{5+} = 1128.31$   
(5637.51 Da versus theoretical mass of 5637.49 Da).

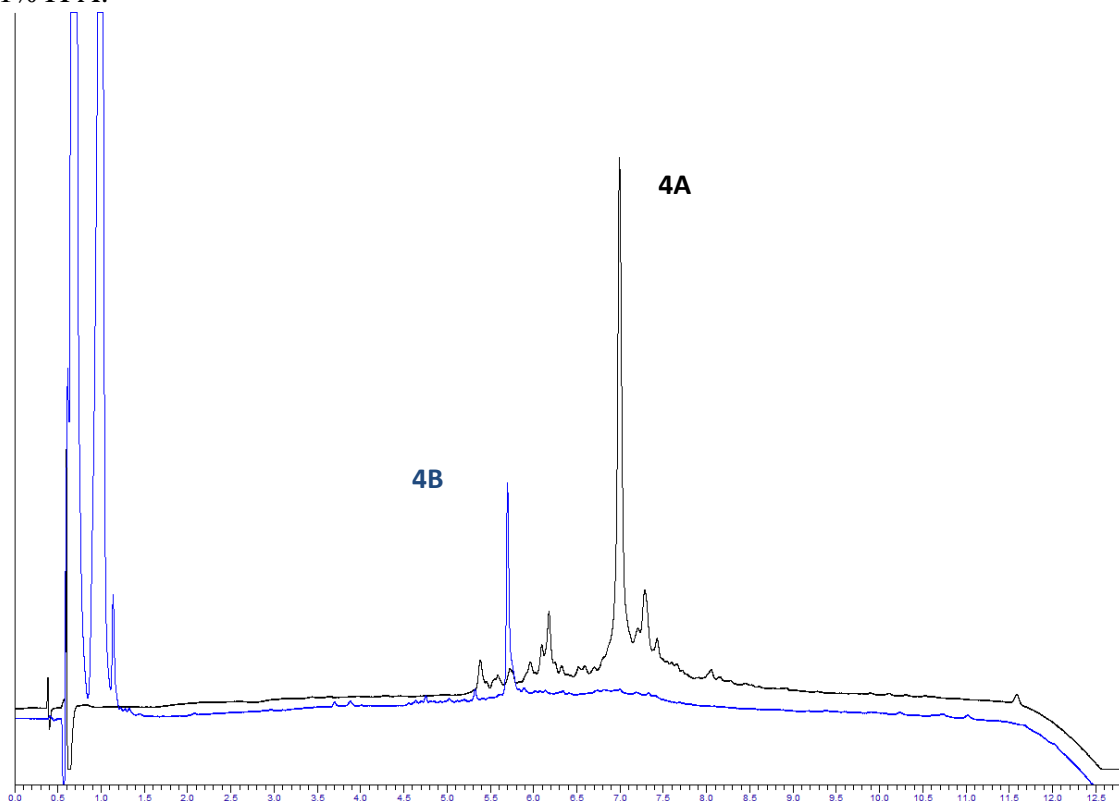


**Peptide 4: Cy5-PEG5-linker10-BeKm1**

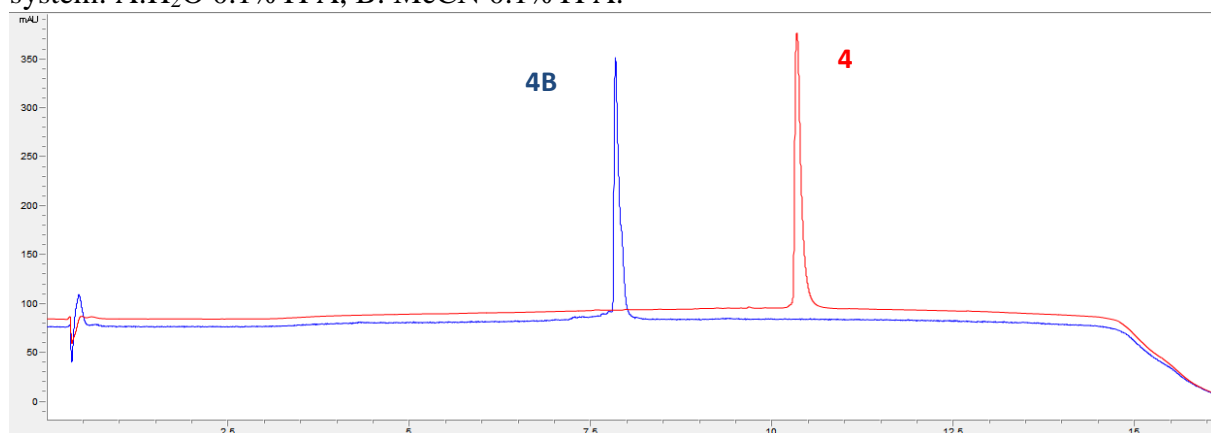
Precursor **4A** Linear (reduced) BeKm1 Nter undecynoic acid

Precursor **4B** Folded (oxidized) BeKm1 Nter undecynoic acid

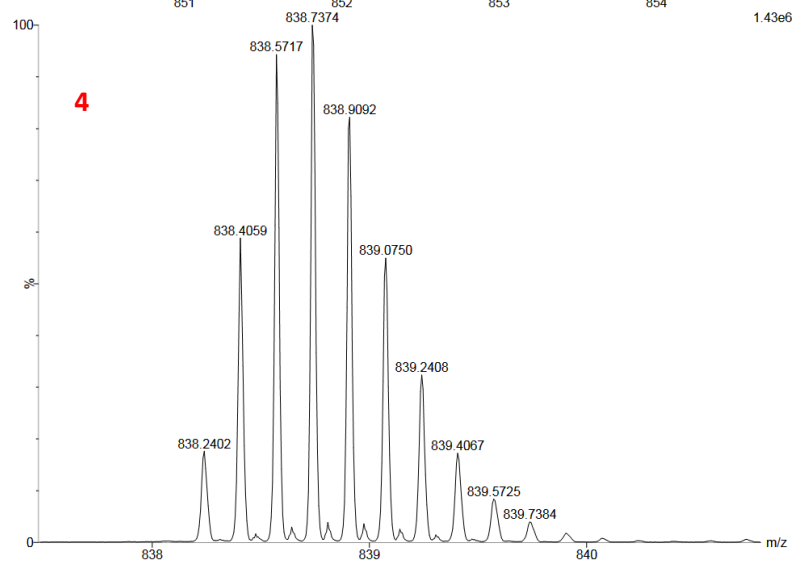
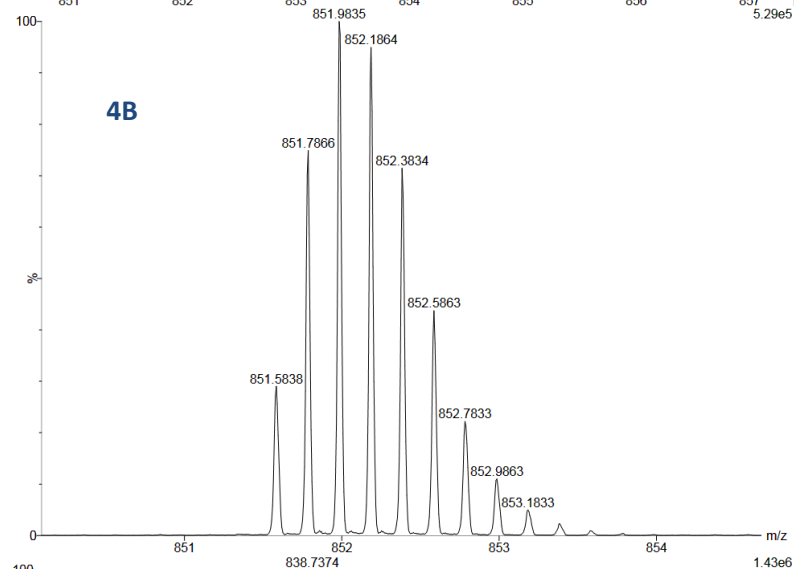
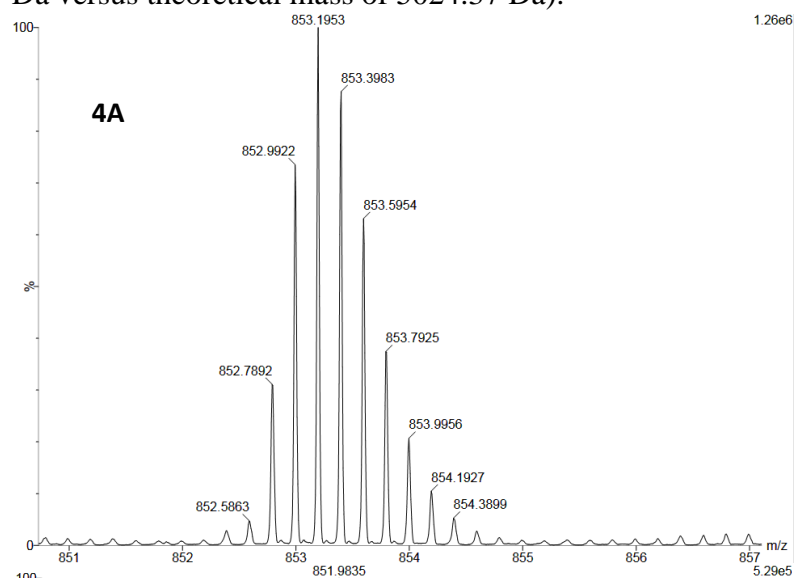
Superimposition of RP-HPLC of crude precursor **4A** and analysis of the folding bath after 24h reaction time showing **4B** as major product. Analysis on Chromolith® RP-18e column, run: 5-65%B in 8 min, 3 ml/min. Solvent system: A: H<sub>2</sub>O 0.1%TFA, B: H<sub>2</sub>O/MeCN 9:1 0.1%TFA.



Superimposition of RP-HPLC of crude precursor 4B and Cy5-peptide conjugate 4 Analysis on C18 Agilent AdvanceBio Peptide mapping column, run: 5-65%B in 12 min. Solvent system: A:H<sub>2</sub>O 0.1%TFA, B: MeCN 0.1%TFA.

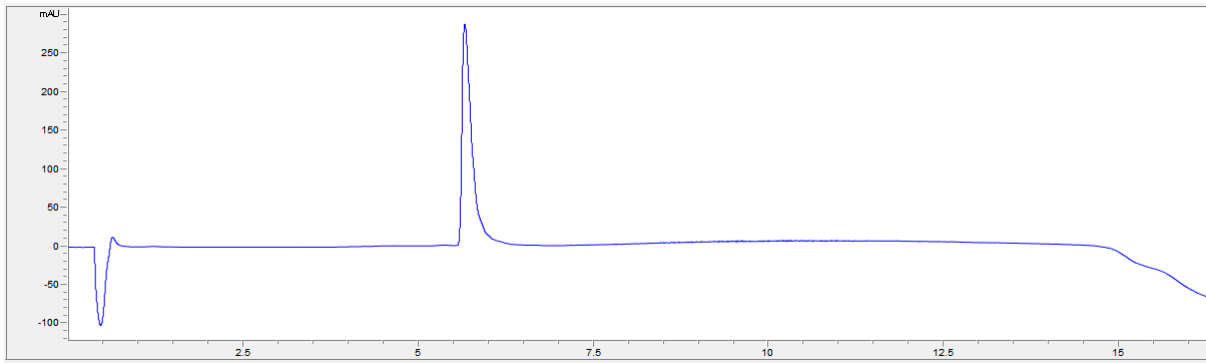


HRMS analysis (ESI/QTOF) of Cy5-PEG5-linker10-BeKm1:  $[M+5H]^{6+} = 838.24$  (5024.39 Da versus theoretical mass of 5024.37 Da).



## Synthetic native Bekm1 toxin

Analysis on C18 Agilent AdvanceBio Peptide mapping column, run: 5-65%B in 12 min.  
Solvent system: A: H<sub>2</sub>O 0.1% TFA, B: MeCN 0.1% TFA.



HRMS analysis (ESI/QTOF) of synthetic toxin:  $[M+3H]^{3+} = 1364.07$  (4089.18 Da *versus* theoretical mass of 4088.78 Da)

