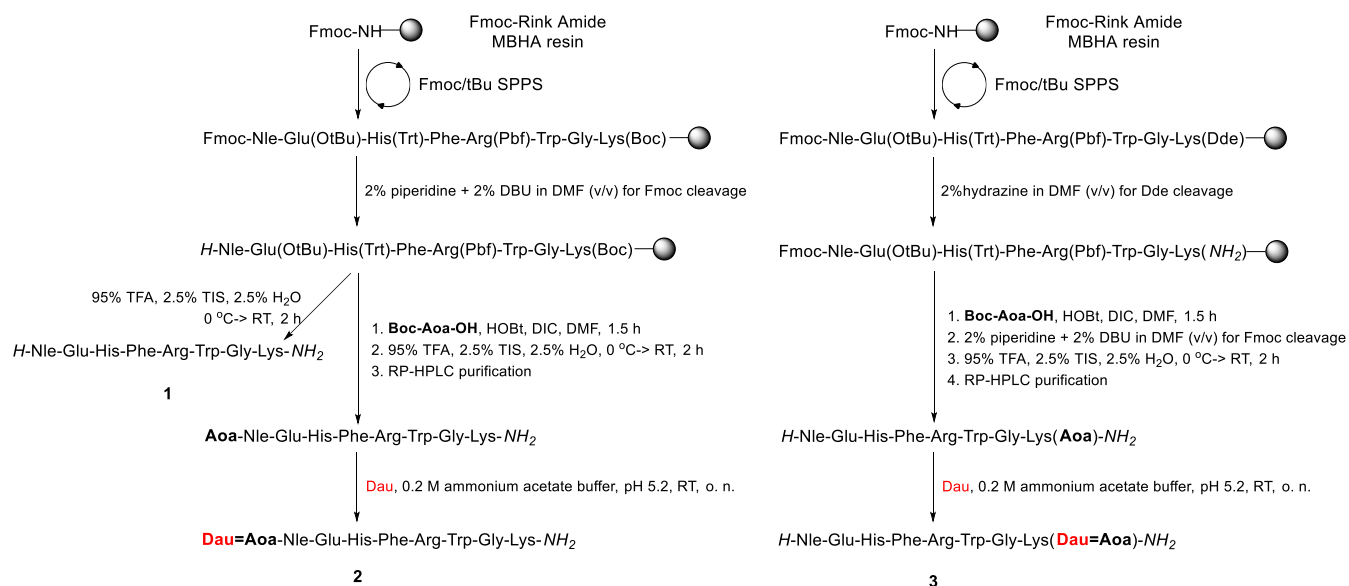
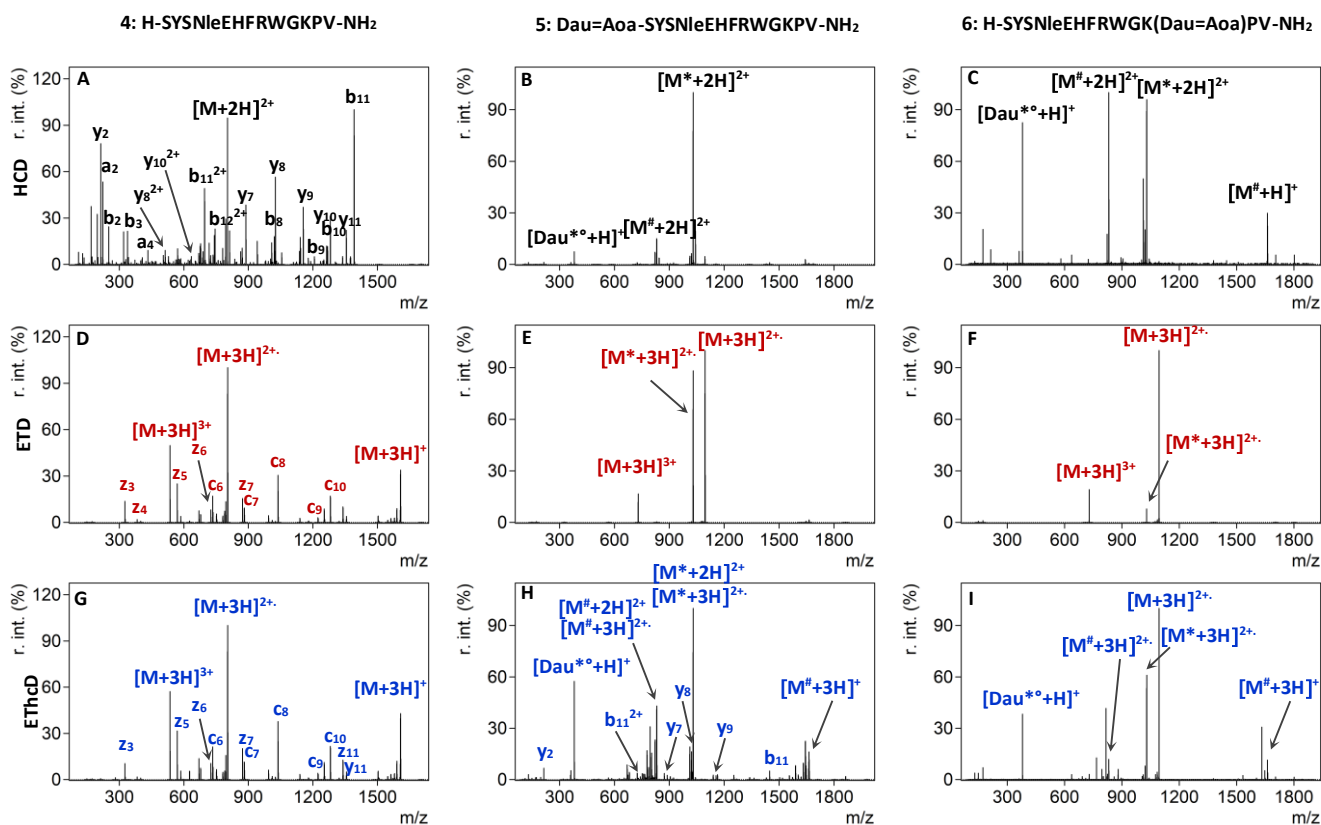


# Structural Characterization of Daunomycin-peptide Conjugates by Various Tandem Mass Spectrometric Techniques

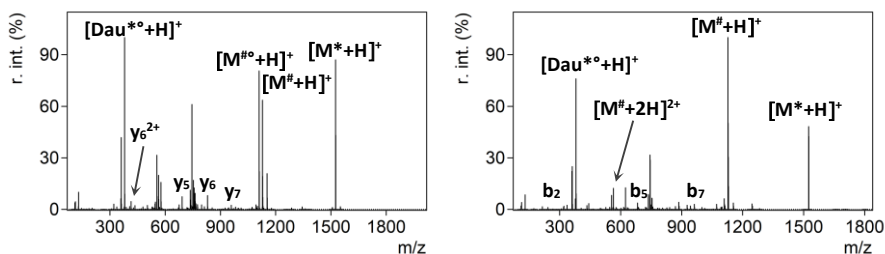
Adina Borbély<sup>1</sup>, Lilla Pethő<sup>2</sup>, Ildikó Szabó<sup>2</sup>, Mohammed Al-Majidi<sup>1,3</sup>, Arnold Steckel<sup>1,3</sup>, Tibor Nagy<sup>4</sup>, Sándor Kéki<sup>4</sup>, Gergő Kalló<sup>5</sup>, Éva Csósz<sup>5</sup>, Gábor Mező<sup>2,6</sup> and Gitta Schlosser<sup>1,\*</sup>



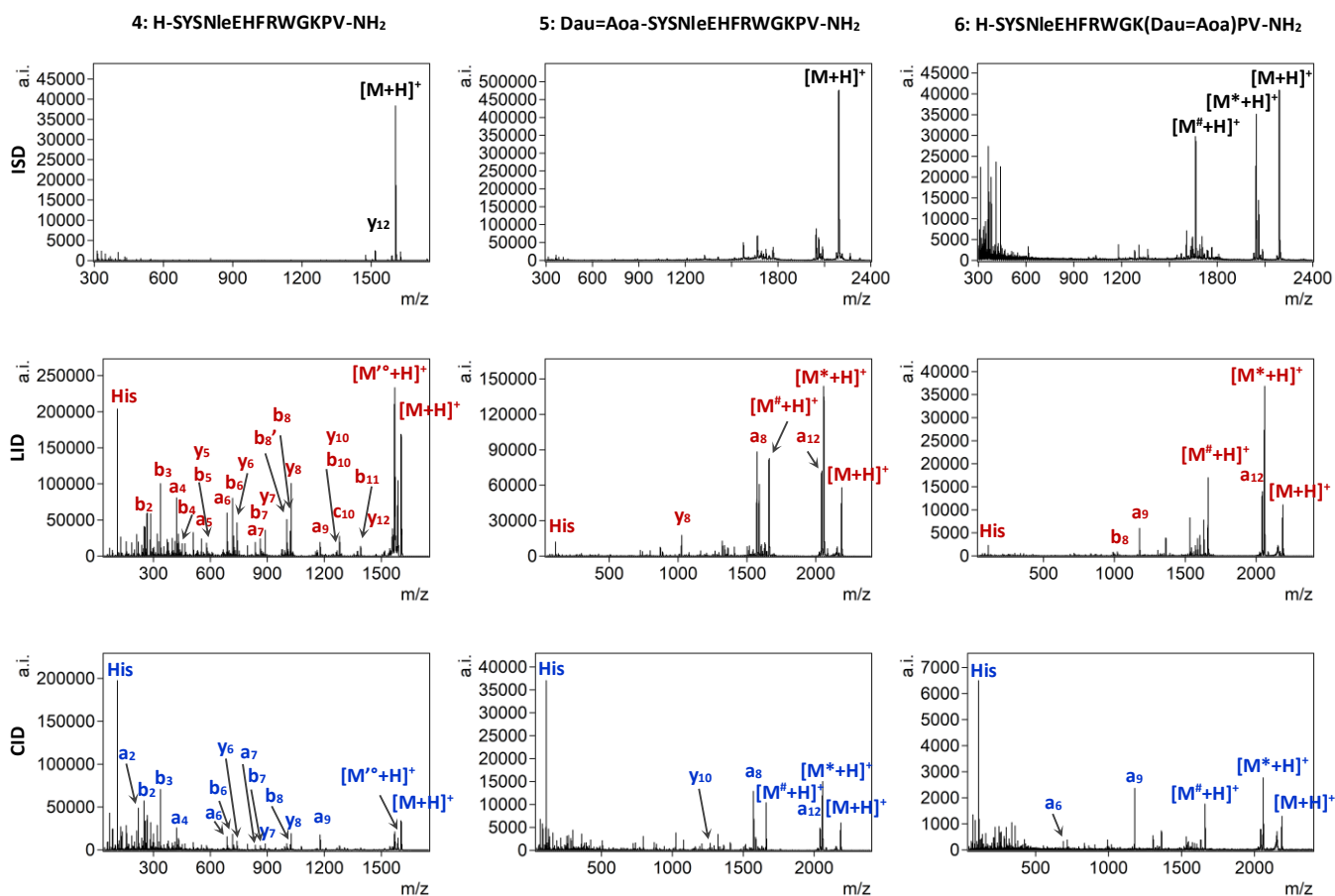
**Scheme S1:** Synthesis of the peptide **1** and Daunomycin- $\alpha$ -MSH conjugates **2** and **3**.



**Figure S1:** HCD MS/MS spectra of doubly protonated **4** (A) at 30% NCE, **5** (B) and **6** (C) at 20% NCE. ETD MS/MS spectra of triply protonated **4** (D), **5** (E) and **6** (F). EThcD MS/MS spectra of triply protonated **4** (G) at 10% NCE, **5** (H) at 40% NCE and **6** (I) at 30% NCE. Neutral losses are depicted as follows: °=H<sub>2</sub>O, \*=sugar, #=Dau+sugar. For the ease of labeling, neutral losses of water or ammonia from precursors are not depicted.



**Figure S2:** HCD MS/MS spectra of triply protonated **2** (A) and **3** (B) at 25% NCE.



**Figure S3:** MALDI MS/MS spectra of the singly protonated compounds **4**, **5**, and **6**. A, B, C: MALDI-ISD spectra, D, E, F: MALDI-TOF/TOF LID spectra, G, H, I: MALDI-TOF/TOF CID spectra. Neutral losses are depicted as follows: °=sugar, °'=NH<sub>3</sub>, °°=H<sub>2</sub>O, #=Dau+sugar, x=Dau+sugar+Aoa.

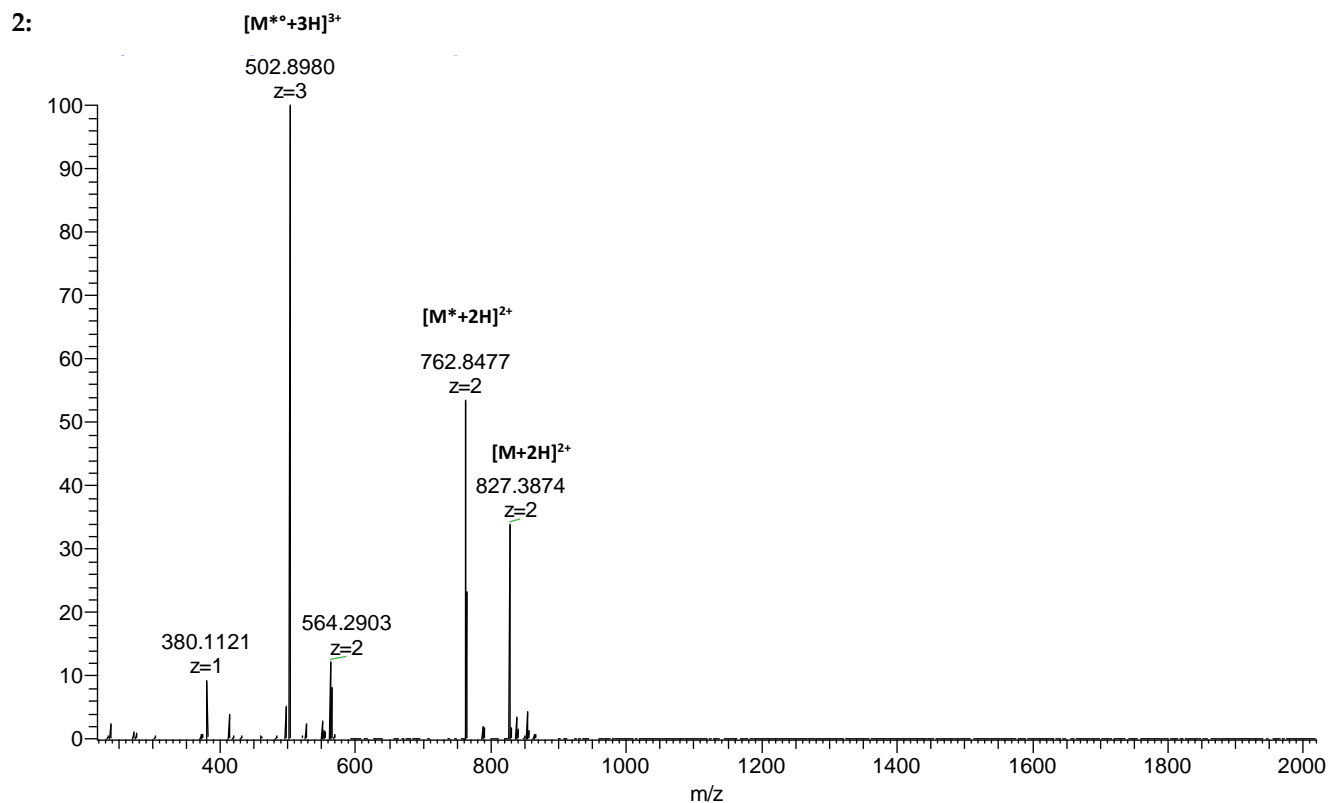
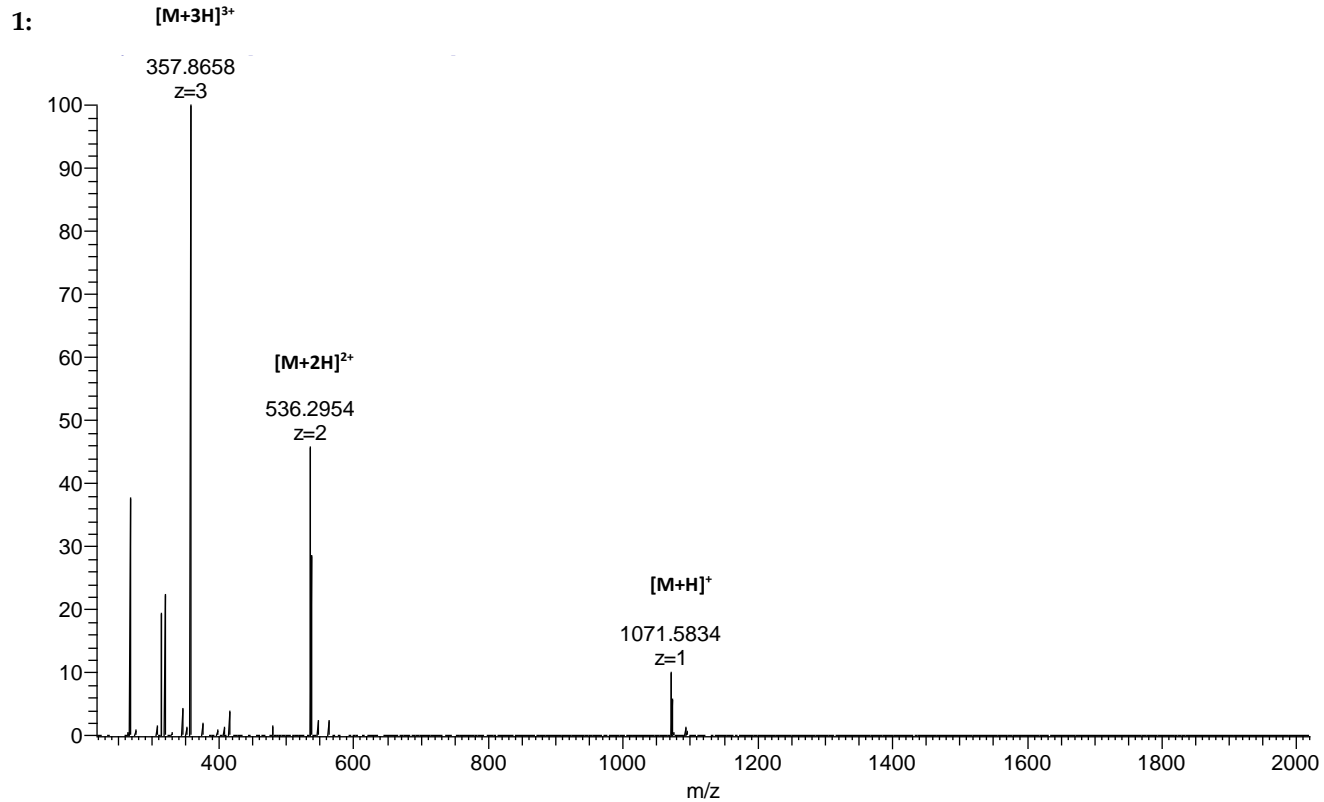
**Table S1:** Structural and analytical properties of the Angiopep-2 and Angiopep-2-bioconjugates.

	Compound	<i>m/z</i> calc. for [M+3H] <sup>3+</sup>	<i>m/z</i> meas. <sup>1</sup> for [M+3H] <sup>3+</sup>	Deviation (ppm)	R <sub>t</sub> (min) <sup>2</sup>
7	H-TFFYGGSRGKRNNFKTEEY-OH	767.7064	767.7057	0.9	6.2
8	Dau=Aoa-TFFYGGSRGKRNNFKTEEY-OH	961.7681	961.7666	1.6	8.3
9	H-TFFYGGSRGK(Dau=Aoa)RNNFKTEEY-OH	961.7681	961.7665	1.7	7.6
10	H-TFFYGGSRGKRNNFK(Dau=Aoa)TEEY-OH	961.7681	961.7674	0.7	7.9

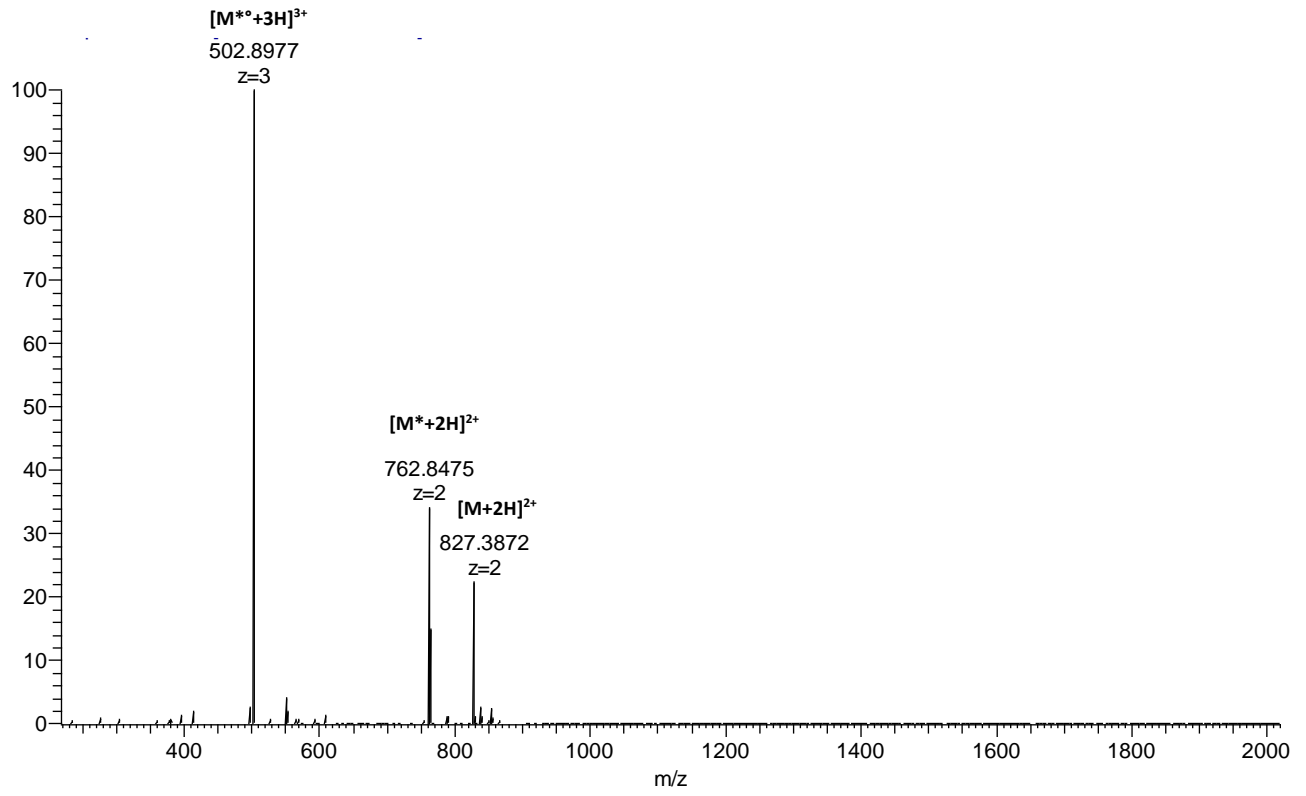
<sup>1</sup> ESI-MS data measured on a Thermo Scientific Q Exactive Focus mass spectrometer. <sup>2</sup> Analytical UPLC was performed using a Waters Acquity UPLC BEH C18 1.7 μm column (2.1 × 50 mm), at 40 °C; eluent A: water + 0.1% formic acid, eluent B: 80% acetonitrile + 0.1% formic acid. The following gradient was used: 0 min 2% B, 1 min 2% B, 17 min 100% B, 17.5 min 100% B, 18 min 2% B, 21 min 2% B. The flow rate was 300 μL min<sup>-1</sup>.

## MS spectra of the compounds 1-10:

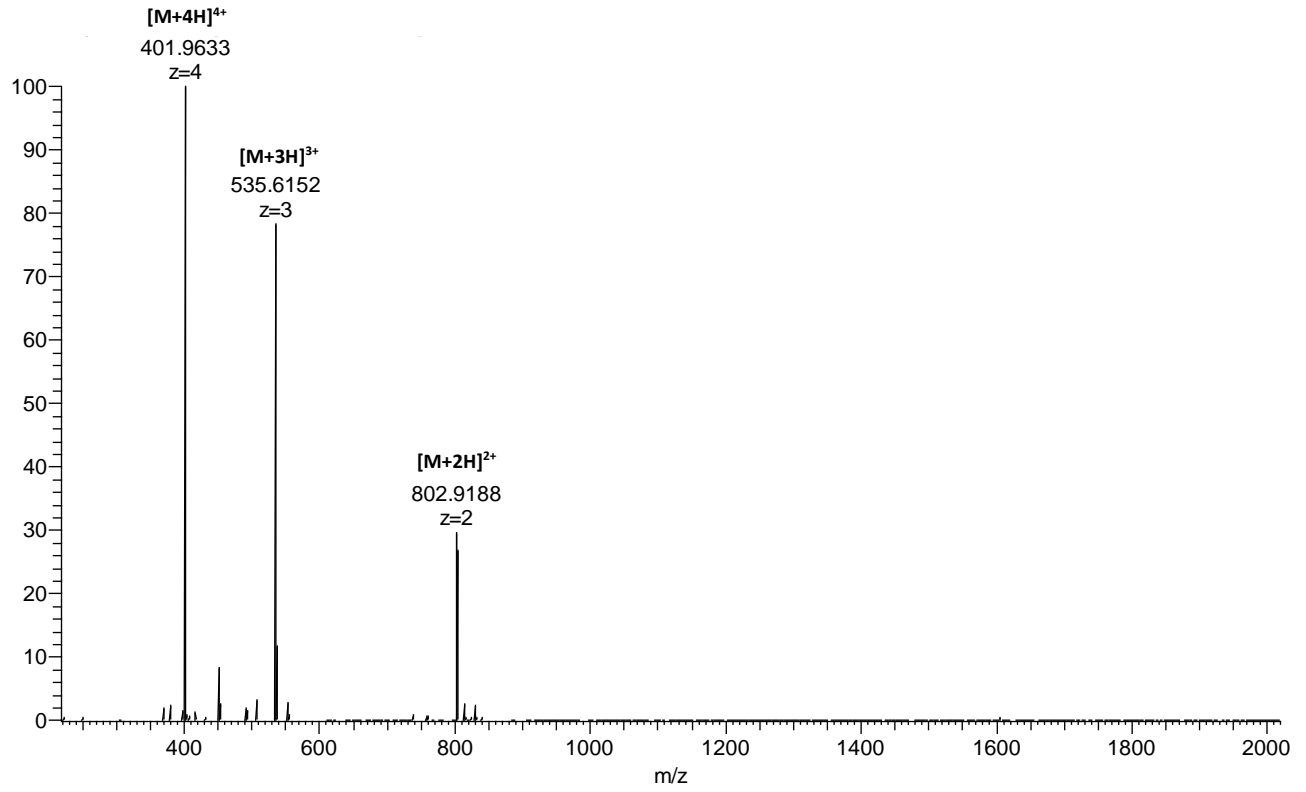
Neutral losses are depicted as follows: \* = sugar, ° = H<sub>2</sub>O.



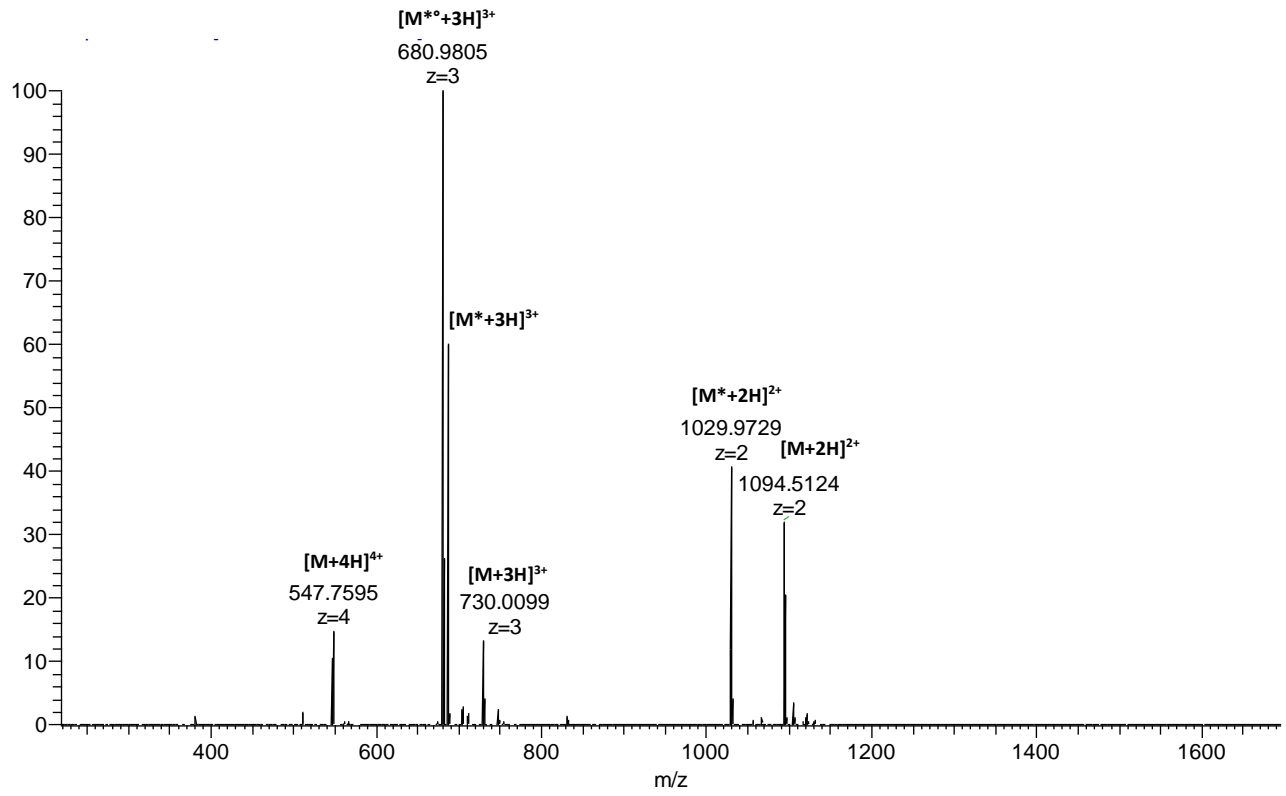
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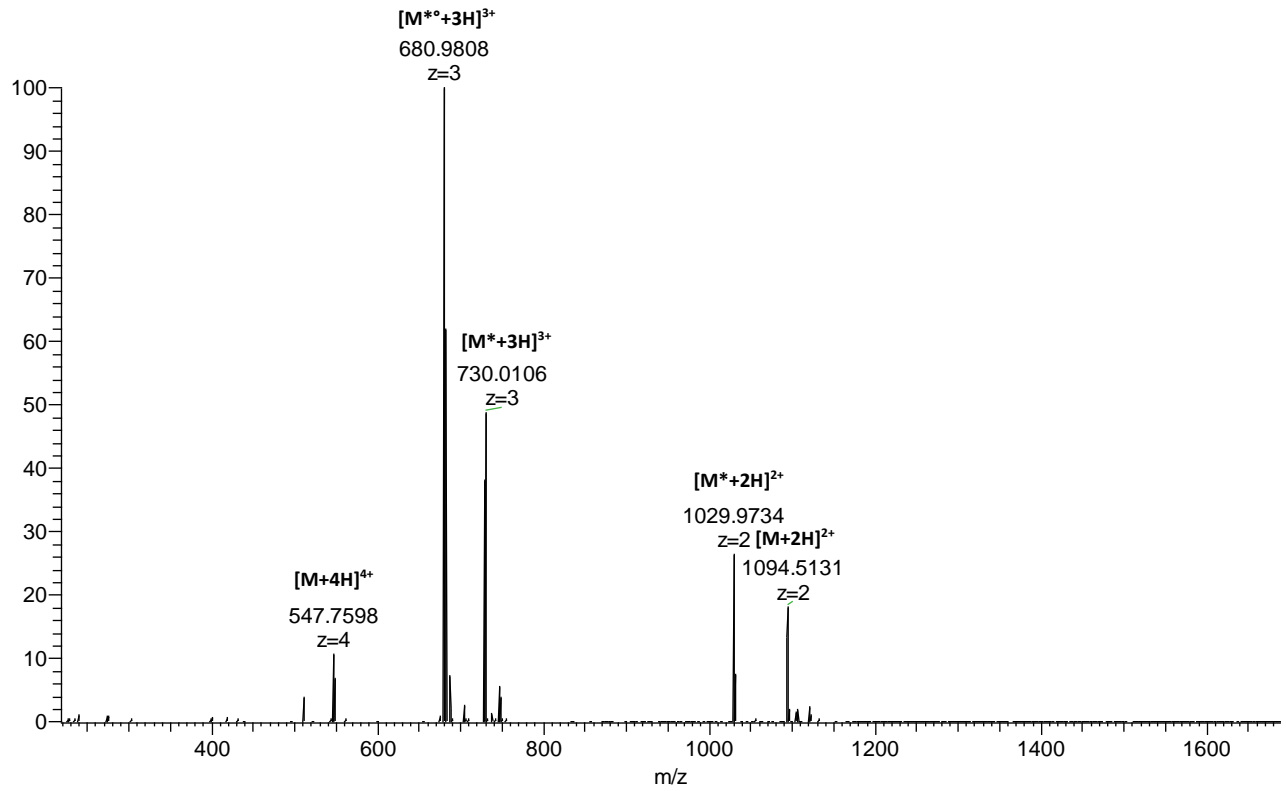
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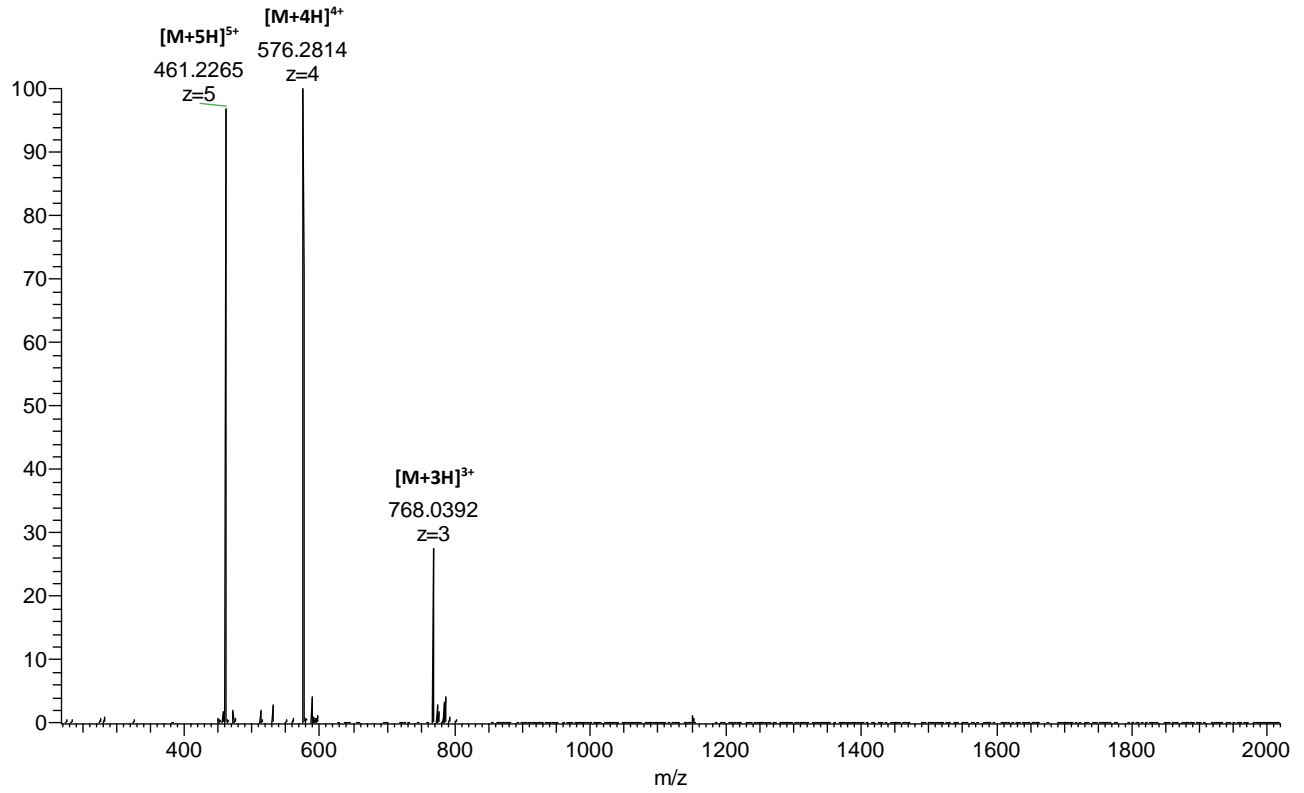
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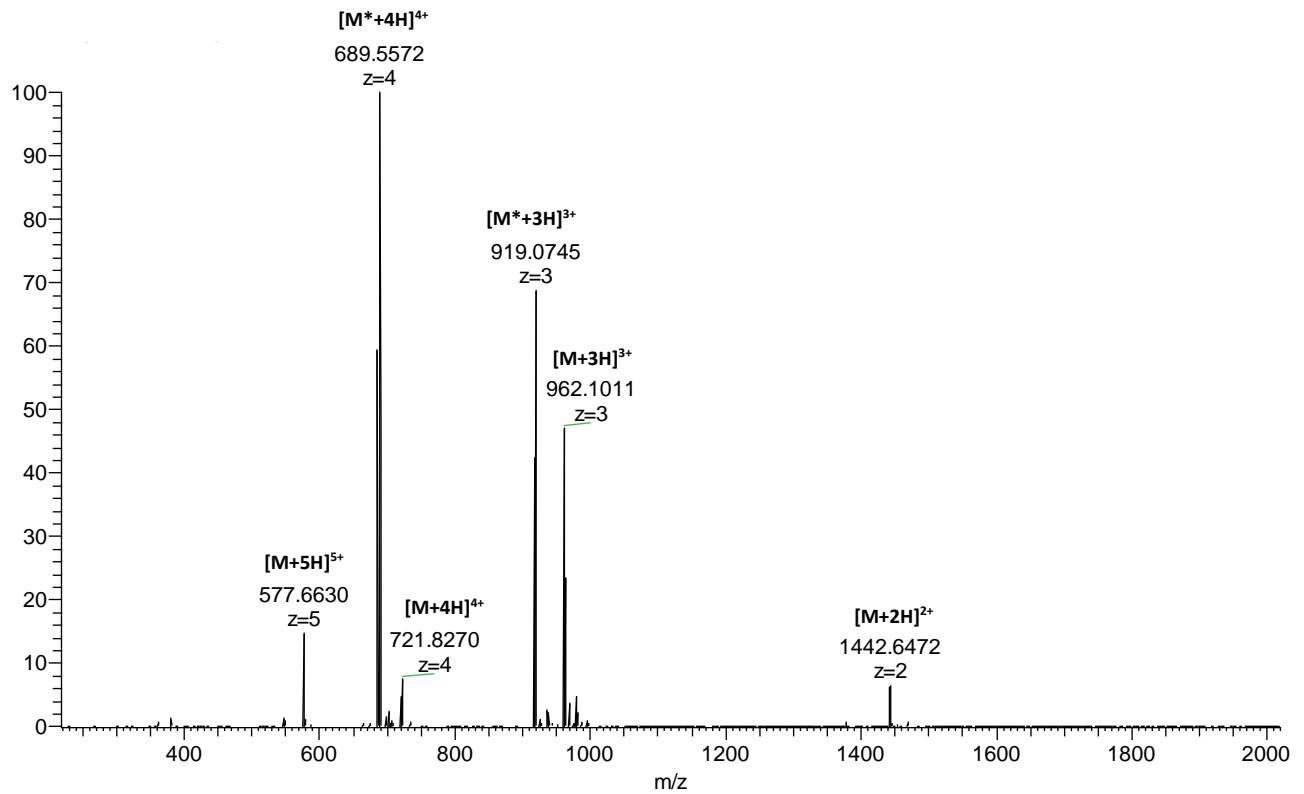
6:



7:

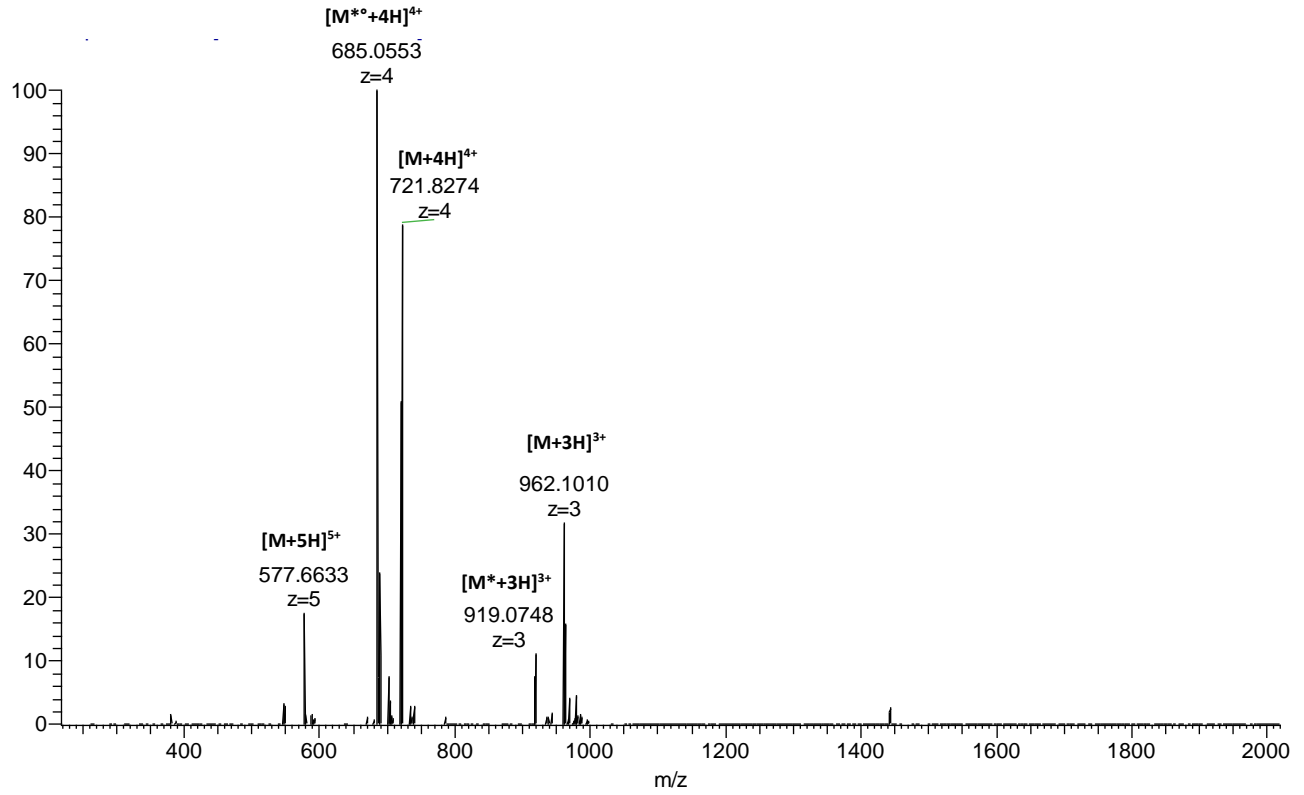


8:





9:



10:

