Spectroscopic characterization of an extensive set of *c*-type peptide fragment ions formed by Electron Transfer Dissociation suggests exclusive formation of amide isomers

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Supplementary Information

Additional figures showing a comparison between the c_3-ions of doubly protonated KAHA and KAHAAAA (S1), and the calculated spectra of alternative enol-imine structures in which the proton has transferred from the Lys or His side chain to the imine nitrogen atom (S2). Table S1 lists the calculated relative Gibbs energies for all presented *c*-ion structures at three different levels of theory.
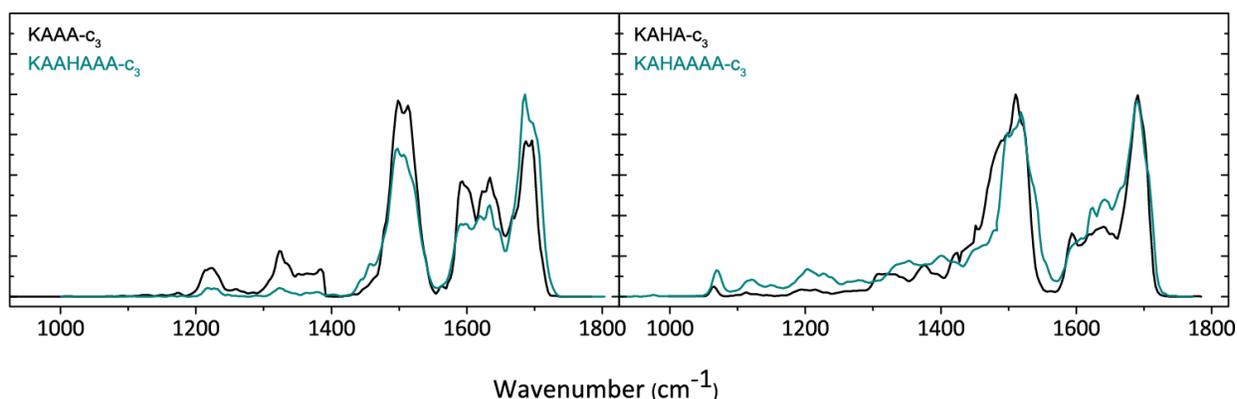
Figure S1. *Overlay of the experimental IRMPD spectra of the c_3-ions of doubly protonated KAAA and KAAHAA (left panel), and between the c_3-ions of KAHA and KAHAAAA (right panel). Ignoring a small apparent frequency miscalibration between 1300 and 1600 cm^{-1} in the right panel, the two spectra are nearly identical, which we take as evidence for the two pairs of c_3-ions to possess identical structures. We conclude that the different neutral loss fragments do not influence the structure of the c-ions formed.*
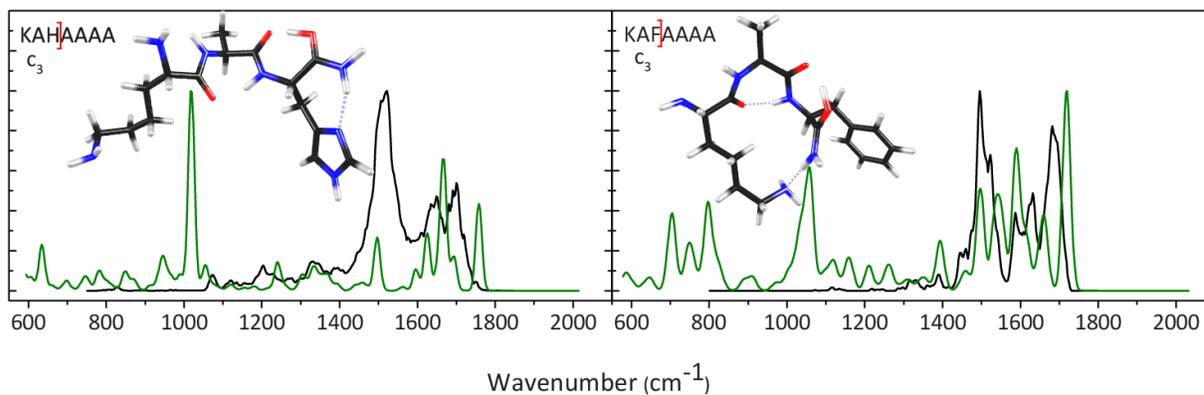

Figure S2. *The experimental spectra of the c_3-ions of KAHAAAA and KAFAAAA compared with calculated spectra of structures that have formed by proton transfer from the Lys or His side chain to the enol-imine nitrogen atom. The calculated spectra for these tautomers do not possess the typical NH-bending mode around 1100 cm^{-1}. Overall, we conclude that the match with the experimental spectra is poor and we discard these structures*

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Table S1. *Relative Gibbs energies of the amide and enol-imine structures. In cases where the best matching IR spectrum belongs to a higher-energy conformer, the energy difference to the lowest energy conformer is presented as an additional entry. The second column indicates the number of conformations investigated for each isomeric structure. Relative free energies are computed at the B3LYP/6-31++G(d,p) level of theory (3rd column). Single-point electronic energies calculated at the MP2/6-311+G(2d,2p) level using the B3LYP/6-31++G(d,p) optimized structure are presented in the 4th columns.*

		B3LYP/6-31++G(d,p)	B3LYP/6-31++G(d,p) //MP2/6-311+G(2d,2p)
KAAA	c_3		
Amide	25	0	0
		+ 16	+ 18
Enol	28	+ 68	+ 60
KAHA	c_3		
Amide	22	0	0
Enol	22	+ 68	+ 67
KAAAAAA	c_4		
Amide	13	0	0
		+ 20	+ 25
Enol	20	+ 78	+ 70
KAAHAAA	c_3		
Amide	26	0	0
Enol	15	+ 68	+ 60
KAAHAAA	c_4		
Amide	25	0	0
		+ 33	+ 33
Enol	23	+ 102	+ 100
KAHAAAA	c_3		
Amide	29	0	0
Enol	16	+68	+ 67
KAHAAAA	c_4		
Amide	30	0	0
Enol	21	+ 76	+ 68
KAFAAAA	c_3		
Amide	16	+ 0.5	0
Enol	12	+ 79	+ 51
KAFAAAA	c_4		
Amide	14	0	0
Enol	14	+ 60	+ 57