Evidence of Diketopiperazine and Oxazolone Structures for HA b_2^+ Ion

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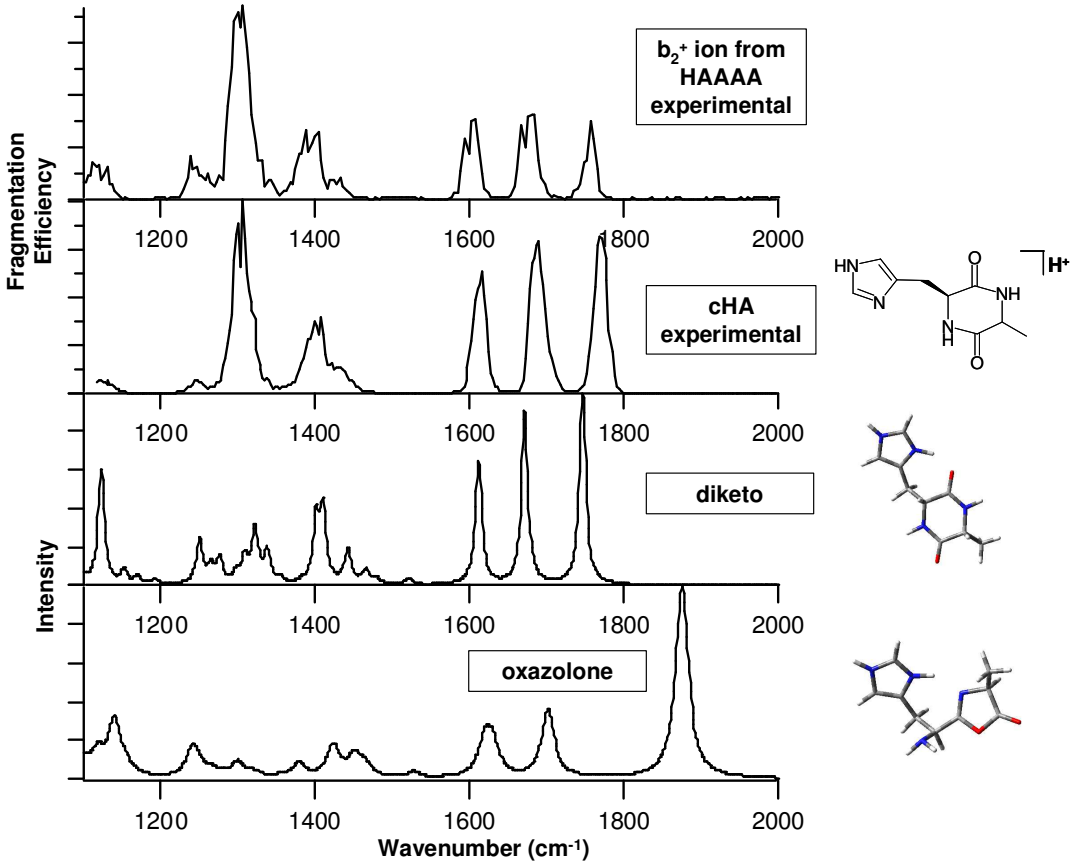


Figure S1: FT-IRMPD action spectra of a) the b_2^+ ion produced by QCID fragmentation of protonated HA_4 and b) the protonated cyclic dipeptide HA plotted as fragmentation efficiency for formation of the fragment ions vs wavenumber. Calculated spectra for c) diketopiperazine and d) oxazolone structures. Because the percent conversion of precursor to fragments was kept low in these experiments and the overlap of the ion cloud with the laser beam is poorer, the oxazolone peak at 1875 cm^{-1} that is seen in the ion trap (Figure 1) was not detected.

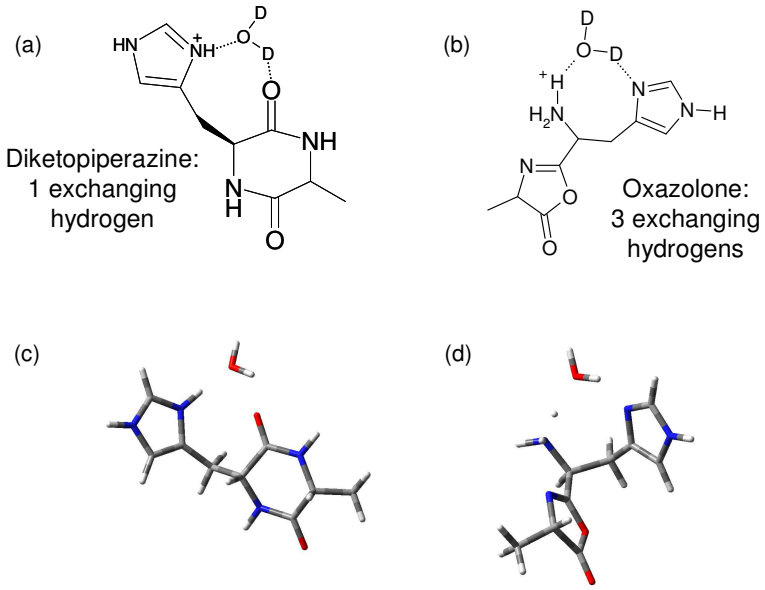


FIGURE S2: Relay mechanism for a) diketopiperazine and b) oxazolone with corresponding optimized structures. (c,d)

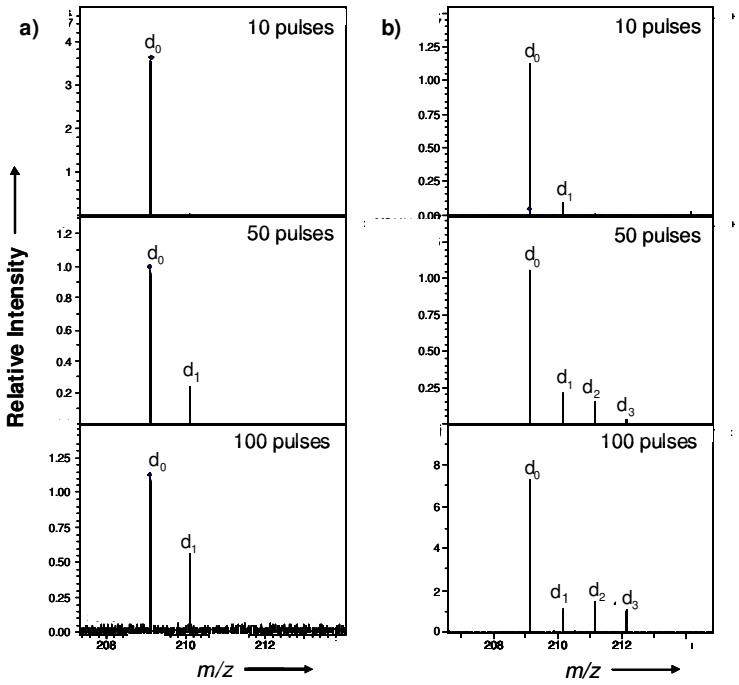
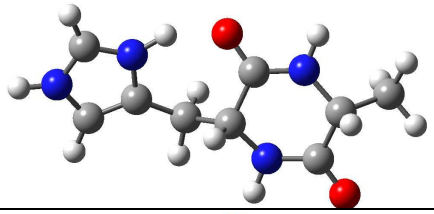
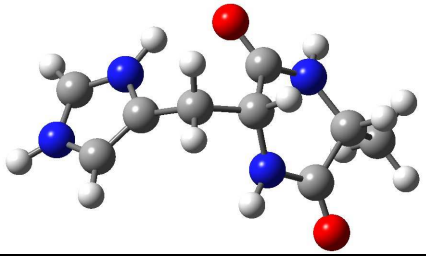
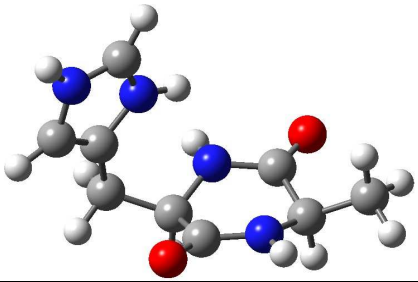
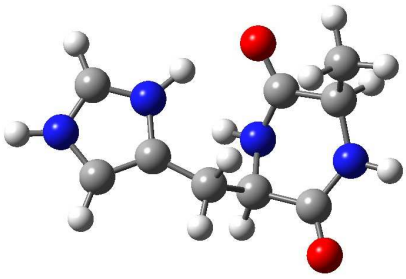
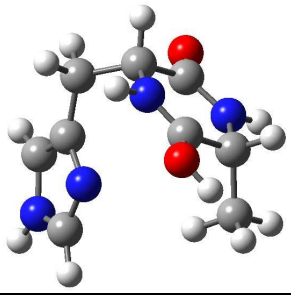


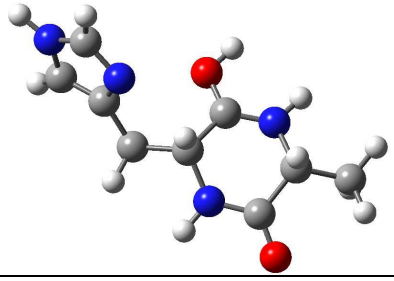
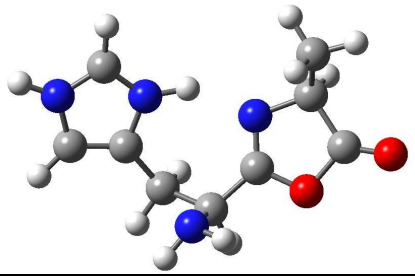
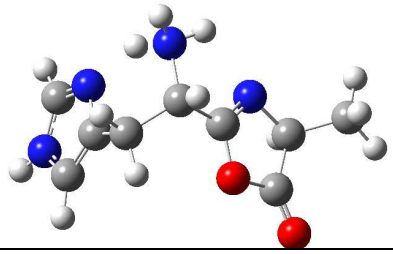
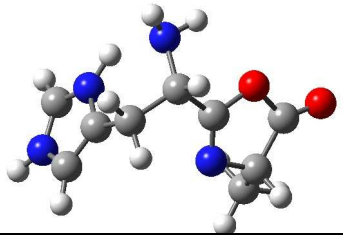
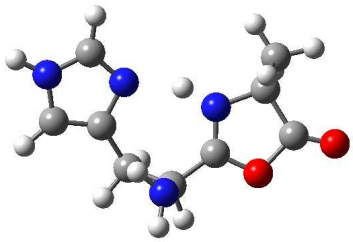
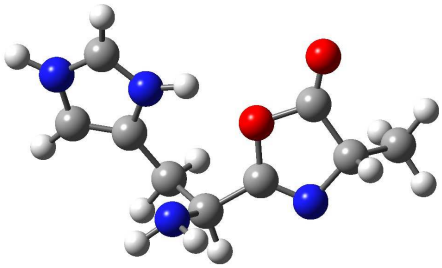
Figure S3: H/D exchange of a) protonated cyclo-His-Ala, produced in the ion source, and b) HA b_2^+ for 10 pulses, 50 pulses, and 100 pulses using a 9.4 T Bruker Apex Qh FT-ICR fitted with a pulse-leak valve. The b_2^+ ions were generated by CID in a hexapole collision cell, transferred to the ICR cell, and exchanged with CD_3OD for various exchange times.

Figure S3a shows the H/D exchange for the protonated cHA at increasing exchange times with CD_3OD. As expected, even at extended exchange times (bottom), the cHA diketopiperazine structure has only exchanged one hydrogen. The b_2^+ ion from protonated HA_4 (Figure S3b) shows a population that incorporates up to three deuterium atoms, indicating the presence of the oxazolone and a clear difference from the cHA exchange, but a larger number of pulses of CD_3OD, would be necessary to unambiguously show the two distinct exchanging populations that are seen in the IonSpec exchange. In the current HDX setup the deuteration agent (CD_3OD) is introduced by a pulsed valve to the ICR cell. The pulse length is variable but in the current

experiments 50 ms pulses were used (i.e., this is the time of leaking CD_3OD into the ICR cell for each pulse). These pulses are separated by 1s during which the pressure does not change significantly and partial pressure of CD_3OD can be built up by applying multiple pulses (e.g., 10, 50, and 100 as for data shown here). With this instrument configuration the pressure fluctuates slightly but the instrumental conditions can be kept the same from sample to sample. The HDX reactions have been performed sequentially for the projectiles investigated and have been repeated many times to check reproducibility. Because we are using relative HDX kinetics data under the same experimental conditions, we believe that the described method is adequate to recognize the presence or absence of multiple structures in isobaric ion populations.

Table S1. Structures, descriptions, and relative energies (kJ/mol) of b_2^+ ion isomers with diketopiperazine and oxazolone structures. Protonated isomer structure descriptions indicate either pillar hetero atoms that bridge the proton or the protonation site.

		$\Delta\Delta H_{298.15}$	$\Delta\Delta G_{298.15}$
	diketo His N_π – $CO_{(His)}$	0	0
	diketoHis N_π – $CO_{(His)}$	8.8	11.0
	diketoHis N_π – $N_{(His)}$	34.4	33.9
	diketoHis N_π – $CO_{(Ala)}$	51.5	54.7
	diketo $CO_{(Ala)}$	100.1	98.0

	diketo $CO_{(His)}$	124.0	119.5
	oxaz His N_{π} – ring $N_{(oxaz)}$	86.2	85.4
	oxaz His N_{π} – $N_{(amino)}$	87.6	87.7
	oxaz His N_{π} – $N_{(amino)}$	88.7	87.9
	oxaz His N_{π} – ring $N_{(oxaz)}$	94.8	93.7
	oxaz His N_{π} – ring $O_{(oxaz)}$	101.6	99.2

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