

Figure S1: Experimental IR spectra (blue) of a) His-flp-Ala and b) His-Flp-Ala compared to the theoretical diketopiperazine and oxazolone structures (red) of each. The relative energies of each pair of calculated structures as well as ball and stick models of each structure are shown in the insets of each calculated spectrum. The major stretching regions for the imidazole protonated diketopiperazines of His-Flp and His-flp are highlighted in blue boxes for comparison to the experimental spectra.

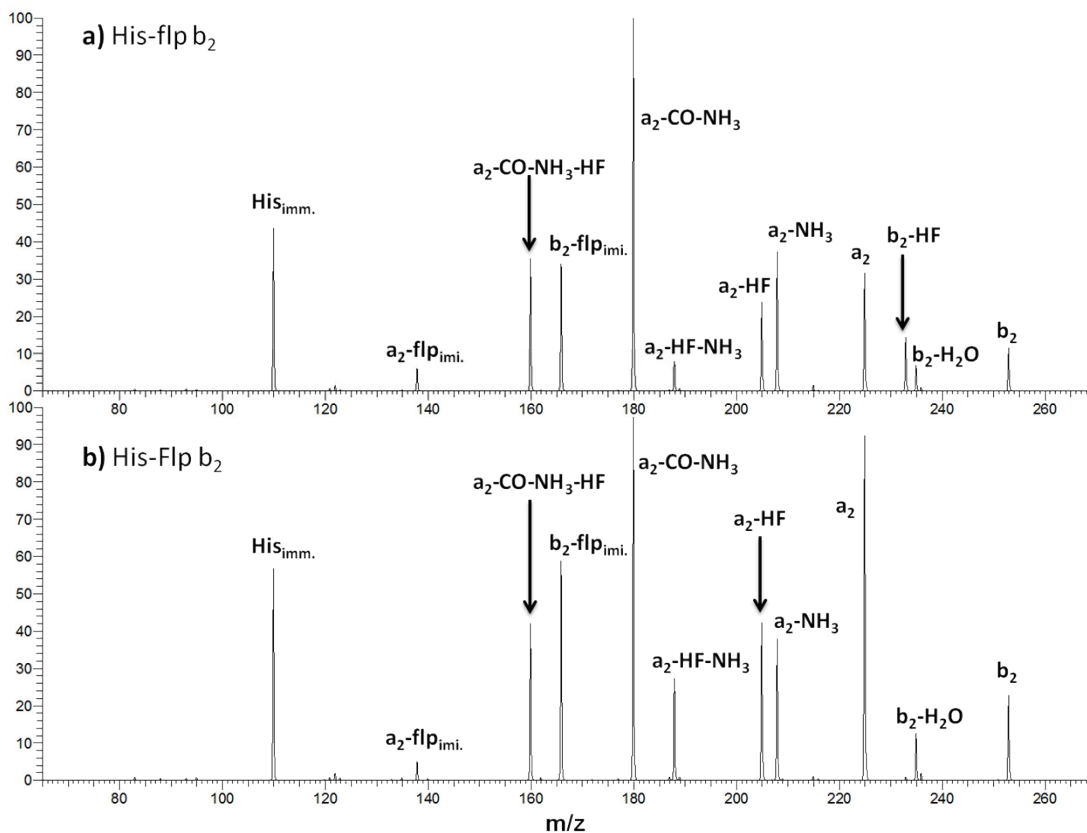


Figure S2: MS³ of b₂ ions of **a)** His-flp-Ala and **b)** His-Flp-Ala performed on a Velos Pro linear ion trap. Precursor tripeptides were isolated at one m/z windows and fragmented at 20% CID collision energy followed by isolation and fragmentation of each b₂ at m/z 253 at the same isolation window and 30% CID collision energy. The -flp_{imi.} and -Flp_{imi.} labels indicate losses of the *S*-fluoroproline, and *R*-Fluoroproline imines while His_{imm.} indicates the Histidine iminium ion.