The conformational preferences of peptide backbones and the resulting hydrogen bonding patterns provide critical biochemical information regarding the structure-function relationship of peptides and proteins. The spectroscopic study of cryogenically-cooled peptide ions in a mass spectrometer probes these H-bonding arrangements and provides information regarding the influence of a charge site. Leucine enkephalin, a biologically active endogenous opioid peptide, has been extensively studied as a model peptide in mass spectrometry. This talk will present a study of the UV and IR spectroscopy of protonated leucine enkephalin \([\text{YGGFL}+\text{H}]^+\) and two of its analogues: the sodiated \([\text{YGGFL}+\text{Na}]^+\) and C-terminally methyl esterified \([\text{YGGFL}-\text{OMe}+\text{H}]^+\) forms. All experiments were performed in a recently completed multi-stage mass spectrometer outfitted with a cryocooled ion trap. Ions are generated via nano-electrospray ionization and the analyte of interest is isolated in a linear ion trap. The analyte ions are trapped in a 22-pole ion trap held at 5 K by a closed cycle helium cryostat and interrogated via UV and IR lasers. Photofragments are trapped and isolated in a second LIT and mass analyzed. Double-resonance UV and IR methods were used to assign the conformation of \([\text{YGGFL}+\text{H}]^+\), using the NH/OH stretch, Amide I, and Amide II regions of the infrared spectrum. The assigned structure contains a single backbone conformation at vibrational/rotational temperatures of 10 K held together with multiple H-bonds that self-solvate the NH$_3^+$ site. A “proton wire” between the N and C termini reinforces the H-bonding activity of the COO-H group to the F-L peptide bond, whose cleavage results in formation of the b$_4$ ion, which is a prevalent, low-energy fragmentation pathway for \([\text{YGGFL}+\text{H}]^+\). The reinforced H-bonding network in conjunction with the mobile proton theory may help explain the prevalence of the b$_4$ pathway. In order to elucidate structural changes caused by modifying this H-bonding activity, structural analogues were investigated. Determining the \([\text{YGGFL}+\text{Na}]^+\) structure will lend insight as to the impact of the ammonium group and methyl esterification of the C-terminus eliminates the carboxy proton. The talk will also report on high resolution, cold UV spectra, non-conformation specific IR gain spectra and conformation specific IR dip spectra for the analogues.