The chemical and structural diversity and the extent of post-transcriptional modification of RNA are remarkable! Currently there are more than 163 naturally-occurring modified nucleosides known. Modified nucleosides have also been a target for pharmaceutical application as potential anticancer and antiviral agents such that many more synthetically-modified nucleosides have been studied. While the biochemical and physiological roles of modified nucleosides have been elucidated in some cases, their importance to RNA biochemistry is still largely unknown and underappreciated. Thus, comparative studies of the canonical versus naturally-occurring and synthetically-modified nucleosides to elucidate changes in structure and glycosidic bond stability arising from modifications are important to understanding their mechanisms of action. Synergistic tandem mass spectrometry and computational chemistry approaches are used to characterize the structures and relative glycosidic bond stabilities of the protonated and sodium cationized forms of the canonical DNA and RNA nucleosides and a wide variety of naturally-occurring and synthetic nucleosides. Vibrational frequency-resolved infrared multiple dissociation (IRMPD) action spectroscopy experiments coupled with spectral analyses of the stable low-energy conformers and their predicted linear IR spectra are used to elucidate the gas-phase conformations of these nucleosides. The stable structures of the nucleosides, their relative stabilities, and predicted linear IR spectra are determined using ab initio and density functional theory methods. Energy-resolved collision-induced dissociation (ER-CID) experiments coupled with survival yield analyses are used to elucidate the relative glycosidic bond stabilities of the nucleosides. Differences in structure induced by modifications and trends in the relative glycosidic bond stabilities of the canonical versus modified nucleosides are examined in detail. Implications of nucleoside modifications on structure-function relationships are discussed.