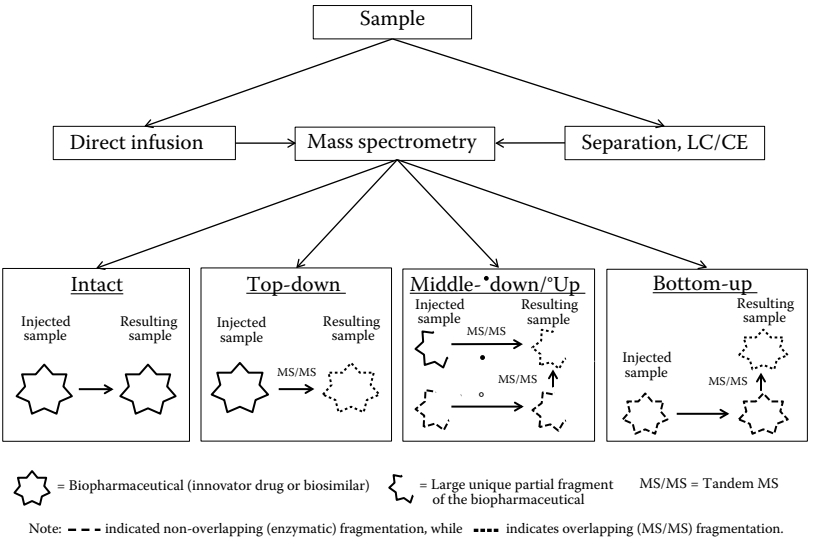

FIGURE 2.8 A pictorial scheme highlighting MS approaches commonly used to extract primary structure information about a biopharmaceutical. They include the following: *intact MS*, which involves the direct injection of the intact biopharmaceutical into the mass spectrometer, *top-down MS* (via tandem MS, MS/MS), which also involves the direct injection of the intact biopharmaceutical into the mass spectrometer where it is then randomly fragmented into overlapping peptides, *middle-down MS* (via MS/MS), which involves the injection of a specific large partial fragment of the biopharmaceutical (generated enzymatically outside the mass spectrometer) into the mass spectrometer where it is then randomly fragmented into overlapping peptide fragments, *middle-up MS*, where the specific large partial fragment of the biopharmaceutical used in middle-down MS is digested into a complex mixture of unique nonoverlapping peptides (using a specific enzyme) that is then injected into the mass spectrometer (*note*: these specific nonoverlapping peptides can be further characterized in a down mode via MS/MS), and *bottom-up MS*, where the intact biopharmaceutical is digested into a complex mixture of unique nonoverlapping peptides (using a specific enzyme) that is then injected into the mass spectrometer (*note*: these specific nonoverlapping peptides can be further characterized in a down mode via MS/MS). The implementation of these MS approaches can be carried out via the simple direct infusion of the biopharmaceutical samples into the mass spectrometer or, alternatively, via the application of a prior online separation (using LC or CE) to significantly improve the mass spectrometer's ability to better characterize a biopharmaceutical's heterogeneity. Note that all samples entering a mass spectrometer must have their noncompatible MS buffer components either adequately minimized or removed via appropriate dilution or solid-phase extraction. In the case of online LC and CE, the mobile phase or electrophoretic buffer system must be MS compatible.

2.6.1.1 MS: A Key Primary Structure Tool for Assessing Biopharmaceutical Consistency, Comparability, and Biosimilarity

Since the successful development of methods for getting proteins into mass spectrometers using the soft ionization modes [such as electrospray ionization (ESI) (Meng et al., 1988) and matrix-assisted laser desorption/ionization (MALDI) (Karas and Hillenkamp, 1988)] in the late 1980s, the role of MS in characterizing