fragmentation and accurate experimental MW information obtained inside the mass spectrometer, coupled again with prior knowledge concerning the known MW of the monosaccharides, the various known observed oligosaccharide structures, and the known various adducts found on monosaccharides (Moremen et al., 2012).

However, when it comes to the task of linking the diversity of monosaccharide and oligosaccharide structures to biopharmaceutical function, much remains to be learned (Moremen et al., 2012). Nevertheless, some aspects of glycosylation functionality have already been realized (e.g., its role in the drug clearance; Solá and Griebenow, 2010), binding to certain biological receptors (Arnold et al., 2007; Shibata-Koyama et al., 2009), along with its structural impact on the HOS of biopharmaceuticals (Solá and Griebenow, 2009; Solá et al., 2007). As a result, the detailed characterization of the glycosylated structures on biopharmaceuticals will continue to receive a great deal of attention, especially when assessing biosimilarity.

2.6.1.3 Structural Analysis of Cysteine and Cystine

The presence of the amino acid cysteine [the most reactive of all the common amino acids found in proteins (Walsh, 2007)] and its chemical status in the linear sequence of amino acids of a biopharmaceutical can play a particularly important role in the structure of these drug molecules. This unique role of cysteine is due to the high reactivity of its side chain sulfhydryl group, which can readily react with another cysteine sulfhydryl group that might be present in the same or different polypeptide chain of a biopharmaceutical to form a cystine residue via an intrachain or interchain disulfide bond, respectively. This cross-linking reaction between two cysteine residues in the same biopharmaceutical molecule allows the polypeptide chain to take on a unique stable folded structure that will significantly influence the biopharmaceutical's HOS and stability. In addition, the presence of more than one cystine or the presence of an additional free cysteine in the same biopharmaceutical also offers the opportunity for the miscoupling of these disulfides or the scrambling of the different cysteines involved in a disulfide bond. As a result, different cystine cross links may arise due to the differences in the actual two cysteines involved in forming the disulfide bond to make a cystine (see Figure 2.5E).

The result of such miscoupling or scrambling events will create variants or subpopulations of a biopharmaceutical with different HOS(s). Hence, an important task in the primary structure (and HOS) assessment between the biosimilar and its RP, as well as in the structural consistency assessments between lots of RP and lots of biosimilar, not only involves assessing the agreement in the number of free cysteine and cystine residues that are present, but also in the case of cystine demonstrating that the correct specific two cysteine residues are cross-linked in forming each cystine in a biopharmaceutical. Such analysis typically involves conducting nonreducing and reducing peptide mapping using MS detection via a collection of LC-MS techniques. Although this task can be very challenging, a number of approaches to tackle this problem have been documented in the literature to help facilitate this work (Wang et al., 2011; Wiesner et al., 2015).

Another point of concern associated with the chemical status of cysteine and cystine residues in the RP and biosimilar is related to the possibility of intermolecular cross-linking between two or more independent monomeric biopharmaceutical molecules, resulting in the formation of covalent aggregates (Costantino et al., 1994; Lu and May,