In addition, many of the linked amino acids can have modifications attached. These attachments can be small (only a few atoms) or very large (similar in size to the rest of the protein). One commonly observed attachment is the addition of complex groups of sugar molecules, called oligosaccharides. Attachments occur at very specific locations on the protein and, like folding, can have a great impact on the therapeutic function of the protein. A protein can thus be represented as a long chain with 20 different types of links with different possible attachments on the links.

To further complicate matters, biological drugs are not composed of structurally identical units. Instead, they are a mixture of products with slightly different features. This is referred to as microheterogeneity and can be represented as a mixture of very similar chains that differ in a few links or in a few of the attachments. The protein chains themselves can then be linked or aggregated (i.e., clumped). It is a challenge to analyze and characterize the composition of such a mixture. Even with currently available analytical technologies, some uncertainty regarding the actual structure of a biologic usually remains. Simple measurements of biological activity, such as enzyme activity, may provide additional information about a product. But there is currently no way to, a priori, understand how the product will perform in patients (e.g., distribution in the body, immune responses against the product). As a result, nonclinical or clinical studies are necessary to assess the safety and the effectiveness of the product.

3.13.2 Potential benefits of improved analytical methods

Advances in analytical tests during the last two decades have driven progress in biopharmaceutical manufacturing, but there is still room for significant improvement. New or enhanced analytical technologies and measurement systems and standards that can more accurately and precisely assess the HOS and the attachments of biological drugs would provide additional assurance of the quality of biological drugs in at least three specific ways:

- Improved analytical methods would enable quicker and more confident assessments of the potential effects of changes in the manufacturing process, the equipment, or the raw materials.
- At present, the manufacturers and the FDA are hampered by the inability to measure fully structural differences that could be caused by changes in the manufacturing process. Since these unknown structural differences could change the properties of the product, the FDA might only approve a manufacturing change after seeing the results of studies of the product in animals or humans. This can significantly slow the implementation of innovative process improvements and impede the