a prodrug approach where the fully active protein or peptide is slowly released from a systemic inactive deposition that can then be tailored to specific release profiles. This technology is still in the early stages of development and so far no pharmaceuticals using this technology have been approved.

9.2.1.2 Polypeptide Modification

Other attractive polymer conjugation and fusion technologies are available that are either based on random polypeptides or polymers of carbohydrates. The polypeptide approach was based on the design of a random coil structure composed of a minor set of small polar amino acids. The size of the polypeptide impacts the half-life by reduction of renal clearance. Although the polymers are stable in plasma and nonimmunogenic, they are degraded in endosomes liberating only endogenous amino acids, that is in contrast to PEGylation which is not degradable. In addition, the polypeptides are all of a defined size in contrast to the polydisperse nature of PEG making the analysis of the drug protein much easier. Two types of polypeptides, the XTEN and the PASylation technologies, have attracted attention in the recent years. The XTEN technology developed by Amunix is a polypeptide comprising of Ala, Ser, Thr, Glu, and Gly in more or less random sequence. This ensures a highly hydrophilic and flexible polymer that offers both low immunogenicity and is also stable against plasma degradation. The PASylation technology developed by XL-Protein is based on the same principle but uses only three amino acids: Pro, Ala, and Ser (PAS). Both technologies offer the opportunity to express the drug target as a fusion partner, or, chemically link the drug target to the polymer via suitable and site-specific linkages. In the latter case, not just one but several drug molecules may be attached to the polymer raising the avidity of the active molecule. An example of this technology is a once-monthly growth hormone developed by Versatis and Amunix which is currently entering phase II clinical trials.

9.2.1.3 Carbohydrate-Based Polymer Modification

Initially used as a plasma expander, *dextran* (Figure 9.1b) is one of the most studied classes of carbohydrate-based polymers conjugated to proteins. It is produced by bacteria and consists of repeating units of glucose monomers linked by α-1,6-glucosidic linkages. The polymer also displays varying degrees of branching via 1,3-glucosidic linking. Coupling of dextran is accomplished by oxidation with periodate yielding aldehyde groups that react with lysine amino groups or the *N*-terminal of the protein. This procedure results in a relatively inhomogeneous conjugate since the aldehyde groups are randomly distributed along the polymer and the attachment to the protein is nonspecific.

Dextrin (Figure 9.1c) is a similar polysaccharide to dextran and is composed of D-glucose units linked by a 1,4-glucoside linkage that forms a linear polymer with branches via 1,6-glucosidic linkages. This biodegradable polymer is produced by hydrolysis of starch and degraded in vivo by α-amylase which has hampered its use. *Hydroxyethyl starch* (*HES*) (Figure 9.1d) represents a class of polysaccharides that have been engineered to be more stable against α-amylase by the chemical modification of the hydroxy groups to hydroxyethyl. By this chemical modification, the rate of degradation can be modulated by controlling the extent of hydroxyethylation. This polymer is approved as a blood expander and site-specific conjugation has been achieved by the reductive amination of one distal aldehyde in the polysaccharide to the *N*-terminal of the protein target. *Polysialic acid* (*PSA*) (Figure 9.1e) is a linear polymer chain which is linked together by *N*-acetylneuraminic acid (sialic acid) acid. Sialic acid is found on the external membrane of a number of cell types in the body, and polymers of sialic acids are widely expressed on the external membrane on a number of bacterial types. When used for therapeutic protein and peptide drug delivery, PSA provides an increased size which seems to provide a shielding effect similar to the one obtained by PEGylation. Comparable to the HESylation method, PSA can be conjugated using the distal three vicinal hydroxyl groups, which after oxidation to aldehyde by periodate can be coupled to the protein, or to a bifunctional handle, to allow other types of coupling chemistries. PSAs have been chemically conjugated to a few clinically relevant therapeutic proteins like interferon and shown to improve their circulating