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Synthesis of Side-Chain Modified Polypeptides

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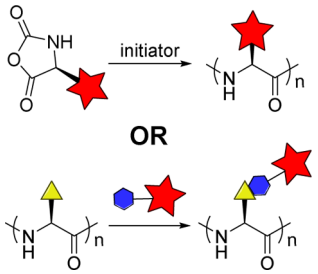

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1. INTRODUCTION

Proteins have long fascinated scientists since their complex sequences and diversity of chemical functionality can lead to structurally defined folded chains with highly specific biological or catalytic activities. Over many years, there has been considerable effort to replicate the structural and functional capabilities of proteins in synthetic systems. In the 1940s, chemists and biochemists from all over the world began research programs on the nascent field of synthetic polypeptides.[1] These seminal efforts energized and solidified this field such that it would remain highly active over the next 3 decades and produce thousands of publications on the synthesis, structures, and biological activities of these materials. Synthetic polypeptides were attractive, and remain so today, since they possess the same backbone repeat as proteins, yet are prepared chemically by the ring-opening polymerization of α-amino acid *N*-carboxyanhydride (NCA) monomers.[2]

The polymerization of NCAs (eq 1) is a highly economical and expedient process for synthesis of long polypeptide chains, especially in comparison to solid-phase peptide synthesis. This method is advantageous in allowing rapid preparation of high molecular weight polypeptides (up to *ca.* 500 kDa) in good yield and at large scale (kilograms) with no detectable amino acid racemization during synthesis. Since it is a chemical process, unnatural amino acid and D-enantiomer NCAs can also be polymerized, allowing exceptional diversity in polypeptide functionality.[2] The disadvantages of NCA polymerization are the polymerization process itself, which can lead to broad chain length distributions, inability to accurately control chain length, as well as poor control of residue sequence in copolymerizations. All of these issues make synthetic polypeptides heterogeneous materials, unlike proteins that can often be obtained as pure molecules. The achievement of living and controlled polymerizations of NCAs within the past 18 years has been a major breakthrough for the synthetic polypeptide field.[2] These methods now permit the synthesis of polypeptides with well-defined chain lengths, and block copolypeptides with controlled compositions and controlled sequences of polypeptide domains, which allows these materials to better mimic the properties of proteins.

The earliest studies on synthetic polypeptides focused primarily on the conformational properties of the chains.[3,4] Structural studies on simple homopolypeptide sequences allowed experimental determination of the α-helix and β-strand secondary structures, as well as elucidation of propensities of different amino acids to form these structures.[3,4] Soon thereafter, many researchers began to also examine the functional properties of synthetic polypeptides, in particular

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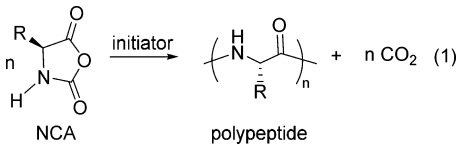

their ability to mimic protein functions and interact with biological systems.[5,6] These studies focused more on the chemical functionality present on the polypeptides, and led to the development of the first side-chain modified (SCM) polypeptides in the mid-1950s. Since this time, there has been ongoing development of a wide variety of SCM polypeptides, especially in recent years, where modifications to polypeptide side-chains have been found to affect both conformational as well as functional properties.[7−11]

There are two fundamental routes for preparation of SCM polypeptides: the functional monomer route, where SCM NCA monomers are polymerized, and the post-polymerization modification (PPM) route, where various functional reagents are chemically conjugated to reactive polypeptide side-chains (Figure 1). Both routes have advantages and disadvantages that

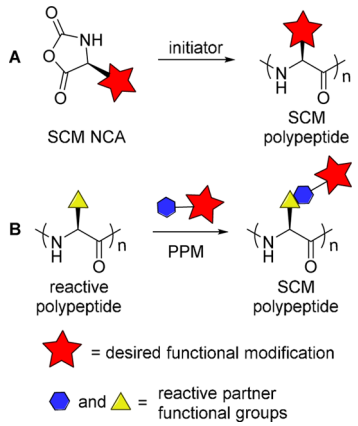

Figure 1. Schematic showing two pathways for synthesis of side-chain modified polypeptides.

warrant consideration. The functionalized monomer route, while requiring upfront investment in synthesis and purification of NCAs, has the distinct advantage of allowing one to produce polypeptides with sequences that are 100 mol % modified, and allows controlled facile incorporation of multiple types of modification into individual chains. On the contrary, PPM can often suffer from incomplete functionalization due to steric crowding and inefficiency in coupling yields. Due to limitations in selectivity and solubility, the complete chemical toolbox available for modification of small molecules includes many reactions that are often not compatible with polypeptides. Therefore, PPM strategies, especially in recent years, often rely on "click" type reactions that can result in high modification efficiencies. Such efficient PPMs have the advantage of avoiding the need to separately prepare and purify many different functional monomers and individually optimize their polymerization conditions. The history and scope of both of these strategies applied to polypeptides are described in detail and discussed in the following sections.

This review covers literature on the synthesis of SCM polypeptides, spanning the years 1954−2015: more than 60 years of functional polypeptide synthesis. The article is divided into two categories: polymerization of SCM NCA monomers, and PPM of synthetic polypeptides. The review is focused on synthesis of these polypeptides, and is not comprehensive in covering properties or applications of these materials. As such, the review excludes literature on preparation of polypeptide bioconjugates, where molecules such as small molecule drugs, polymers, proteins, peptides, oligosaccharides, polysaccharides, or oligonucleotides are conjugated to polypeptides. Furthermore, the review is focused on polypeptides based on α-amino acid residues, and so does not include literature on poly(β-peptides) or *N*-alkylated polypeptides (e.g., polypeptoids). Finally, this review also does not include trivial modifications, such as protecting groups for natural side-chain functionalities that are used during polypeptide synthesis.

2. POLYMERIZATION OF SIDE-CHAIN MODIFIED (SCM) α-AMINO ACID *N*-CARBOXYANHYDRIDE (NCA) MONOMERS

Historically, most SCM NCAs have been derived from natural proteinaceous amino acids that contain chemically reactive side-chain functional groups. More recently, functional monomers have also been constructed using more elaborate synthetic schemes, based on either natural or unnatural amino acid precursors. Consequently, the range of functional groups that have been incorporated into NCA monomers has increased substantially over the past 60 years, and this diversity is expected to increase. Aside from the reasons mentioned above, another key advantage of the SCM NCA route is the ability to incorporate functional groups with exactly the same structures as those that occur naturally in proteins via post-translational modification processes. PPM strategies often lead to incorporation of unnatural linkers that can alter polypeptide structure or functionality. Bearing this in mind, one might reason that the SCM NCA approach is the method of choice for all polypeptide functionalizations. However, the purification of moisture sensitive NCAs, which becomes even more challenging as complex modifications are added, is often the major limitation of this method, and may be prohibitive for practical applications. This concern is especially true when well-defined polypeptides and copolypeptides are desired, and the chemist must weigh these advantages and disadvantages before planning a synthetic strategy. The variety of SCM NCAs reported in the literature is detailed below.

2.1. Alkylated Cysteine and Homocysteine Modified NCAs

Some of the earliest SCM NCAs and polypeptides synthesized were based upon alkylated derivatives of cysteine and homocysteine. These include poly(*S*-allyl-L-cysteine), first reported in 1955,[12] which will be discussed below with alkene modified NCAs in section 2.7. Carboxyalkylated and amino-alkylated cysteine NCAs are the most widely investigated of this monomer type. The resulting polymers were of interest due to the conformational preferences of these residues and their functional similarity to polylysine and polyglutamate.

Initial studies focused on the NCAs of *S*-carbobenzoxymethyl-L-cysteine[13−15] and *S*-carbobenzoxyethyl-L-cysteine[16] (Figure 2A). Analogous amino SCM NCAs were also prepared, namely those of *S*-(2-Cbz-aminoethyl)-L-cysteine, *S*-(3-Cbz-aminopropyl)-L-cysteine, *S*-(L-2-Cbz-aminopropyl)-L-cysteine, and *S*-(D-2-Cbz-aminopropyl)-L-cysteine, as well as L-homocysteine versions of these four derivatives (Figure 2B,C).[17,18] All these monomers were polymerized efficiently in polar organic solvents, yielding polymers that precipitated in less

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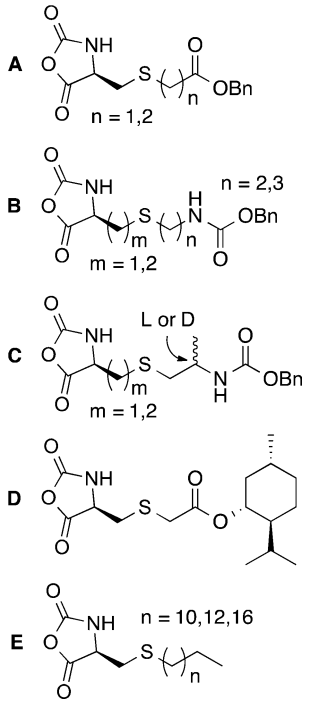

Figure 2. (A–E) Structures of alkylated cysteine and homocysteine modified NCAs.

polar solvents (e.g., dioxane) or gelled in more polar solvents (e.g., nitrobenzene or methylene chloride).

The Cbz-protected amino alkylcysteine polymers described above were found to possess β-sheet conformations in the solid-state or in suspension. Most of these polymers could be dissolved in H-bond disrupting solvents (e.g., dichloroacetic acid), whereupon they were found to adopt disordered conformations. After removal of Cbz groups, the amino alkylcysteine polymers were water-soluble under conditions where the side-chain amino or carboxylate groups were ionized. When the side-chain groups were neutralized by adjustment of solution pH, the polymers adopted β-sheet conformations. An interesting polycysteine derivative that did not form β-sheets was prepared from *S*-(L-menthyloxycarbonylmethyl)-L-cysteine NCA (Figure 2D).[19] This polymer was found to be soluble in many organic solvents (e.g., chloroform, dioxane), and possessed a α-helical conformation. The helical conformation of this polymer, as opposed to β-sheet formation seen with other polycysteine derivatives, is possibly due to preferential packing of the menthyl groups in the α-helical conformation.

Straight-chain hydrocarbon alkylated cysteine NCAs have also been reported. Hayakawa and co-workers prepared lauryl, myristyl, and stearyl derivates of L-cysteine and their corresponding NCAs (Figure 2E).[20] The resulting homopolymers were found to be soluble in chloroform, chlorobenzene, DCA and TFA. In the solid-state and in chloroform, the polymers were found to adopt β-sheet structures, and in acidic solvents, the polymers were found to be in disordered conformations.

2.2. Mesogen Modified NCAs

A limitation of polypeptides as compared to many common synthetic polymers has been the difficulty in using melt processing with these materials. Since polypeptides contain abundant H-bonds and poor chain flexibility, melting of the chains before decomposition is usually not observed. Although solution based methods allow processing of these materials for most applications, melt processing, or even capability for thermal annealing, would greatly expand the utility of polypeptides. Stable polypeptide melts and thermotropic liquid crystalline behavior were first demonstrated in long-chain *n*-alkyl esters of poly(glutamates) by Watanabe's group.[21−23] These materials were all prepared by PPM and are discussed in section 3.2. Peggion reported synthesis of *n*-alkyl-L-lysine NCAs in 1975, with stearyl and pelargonyl groups attached to lysine via amide bonds (Figure 3A), and prepared their corresponding

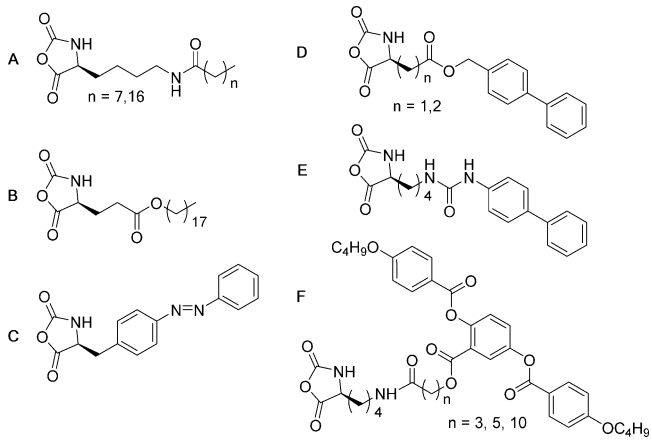

Figure 3. (A–F) Structures of mesogen modified NCAs.

homopolymers.[24] These polymers were hydrocarbon-soluble, and were α-helical in solution but showed only hexagonal packing of the side-chains in the solid-state. In related work, Daly's lab reported the synthesis of γ-stearyl-L-glutamate NCA in 1988 (Figure 3B).[25] Recently, some other *n*-alkyl-L-glutamate NCAs were synthesized,[26] where the longest side-chains were *n*-butyl, yet the resulting polymers were not studied as bulk solids.

The first NCA monomer containing a mesogenic side-chain was *p*-phenylazo-L-phenylalanine NCA, prepared by Goodman's lab (Figure 3C).[27] This monomer was efficiently homopolymerized and also copolymerized with γ-benzyl-L-glutamate NCA in varying ratios. The homopolymer, and copolymers with less than 50 mol % γ-benzyl-L-glutamate content, were insoluble in dioxane, but dissolved in dichloroacetic acid. The dioxane-soluble copolymers were all found to be α-helical, with azoaromatic groups primarily in the *trans* configuration. In related work, biphenyl "end-on" modified lysine, glutamate, and aspartate NCAs were prepared (Figure 3D,E).[28,29] While the modified aspartate polymers were found to possess disordered conformations, poly(*p*-biphenylmethyl-L-glutamate) was found to be soluble, and α-helical in many solvents. Poly(*p*-phenylbenzamido-L-lysine) was soluble in DMF and found to form a layered S_{A2} smectic structure in the solid-state, with the polypeptide backbone in the β-sheet conformation.

To encourage parallel orientation of the mesogens and polypeptide backbone, Deming prepared three-ring aromatic ester "side-on" mesogen modified lysine NCAs with spacers of 3, 5, and 10 methylene units between the lysine amino groups and the mesogens (Figure 3F).[30] Mesogen derivatized polypeptides were prepared by ring-opening polymerization of the NCAs using $(PMe_3)_4Co$ initiator in THF solvent.[31] The polypeptides were all soluble in THF, and were found to adopt

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α-helical conformations in solution. The polymer with the longest spacer was found to undergo a transition in bulk to a liquid crystalline mesophase above 105 °C, where a hexatic ordering of the polypeptide α-helices was found to coexist with nematic ordering of the side-chain mesogens.

2.3. Oligoethylene Glycol Modified NCAs

Nonionic, water-soluble polypeptides are desired for many applications since they avoid issues seen with polypeptides such as poly(L-lysine) or poly(L-glutamate), which are polyelectrolytes that display pH dependent solubility and can aggregate with oppositely charged polymers and surfaces.[3] However, aside from short chains (<25 residues), all nonionic homopolypeptides derived from naturally occurring amino acids are notoriously insoluble in water. To address this issue, Deming developed nonionic, water-soluble polypeptides through SCM using well-defined, short oligoethylene glycol segments incorporated into NCA monomers. Deming's lab prepared N_ε-2-[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine and N_ε-2-(2-methoxyethoxy)acetyl-L-lysine NCAs and found that, while both polymerize well, only poly(N_ε-2-[2-(2-methoxyethoxy)-ethoxy]acetyl-L-lysine) gave high molecular mass polymers with high water solubility (Figure 4A).[32] This polymer was also found to form a stable α-helical conformation in water, and is the first example of a nonionic polypeptide that is both α-helical and water-soluble. Similar monomers and polymers were also prepared by Klok using succinate linkages between the oligoethylene glycol segments and lysine (Figure 4B).[33] In these polymers the ester linkages to the oligoethylene glycol segments are potentially degradable in water, and the polymers were found to prevent nonspecific protein adsorption when used to coat surfaces.

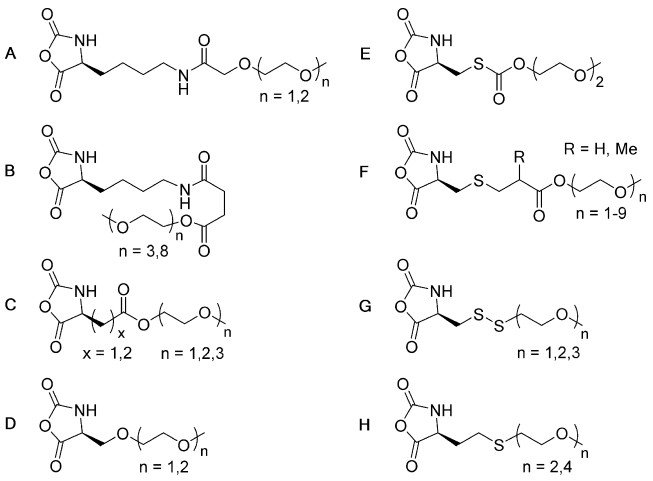

Figure 4. (A–H) Structures of oligoethylene glycol modified NCAs.

In recent years, oligoethylene glycol modified glutamate and aspartate NCAs have also been prepared (Figure 4C). In 2011, Li prepared mono-, di-, and triethylene glycol modified glutamate ester NCAs.[34] The monoethylene glycol poly(L-glutamate) was found to have poor solubility in most solvents, but the di- and triethylene glycol poly(L-glutamate)s were soluble in water, as well as in some organic solvents. The remarkable feature of the di- and triethylene glycol poly(L-glutamate)s was that their water solubility was temperature dependent, where they underwent a solubility transition at elevated temperatures causing polymer precipitation. Only the α-helical modified poly(L-glutamate)s possessed this characteristic, as their racemic counterparts remained water-soluble at elevated temperatures. Li's lab also prepared mono-, di-, and triethylene glycol modified aspartate NCAs.[35] The monoethylene glycol poly(L-aspartate) was found to have poor water solubility, and the di- and triethylene glycol poly(L-aspartate)s were water-soluble, but possessed only disordered conformations and no temperature dependent solubility transitions.

Similar oligoethylene glycol modifications to the β-sheet preferring amino acids L-serine and L-cysteine have been reported. These SCM residues were found in some cases to provide good water solubility to the corresponding polymers, which could then form β-sheet structures upon solvent evaporation or an increase in temperature. The synthesis of oligoethylene glycol modified serine and cysteine NCAs was first reported by Deming's lab (Figure 4D,E).[36] The cysteine derivative, poly(*S*-(2-(2-methoxyethoxy)ethoxy)carbonyl-L-cysteine), was found to form β-sheet structures and possessed poor water solubility. Poly(*O*-(2-(2-methoxyethoxy)ethyl)-L-serine), on the other hand, was found to have high water solubility and a disordered conformation in solution, yet was able to form β-sheet structures in the solid-state. Recently, Li prepared a variety of oligoethylene glycol modified cysteine NCAs, which are based either upon Michael addition of oligoethylene glycol acrylates or methacrylates to cysteine,[37] or disulfide linkage of oligoethylene glycol sulfenyl chlorides to cysteine[38] (Figure 4F,G).

The (meth)acrylate derived monomers were oils that were difficult to purify, especially for the longer side-chains studied (i.e., those with 8–9 ethylene glycol repeats), and consequently difficult to polymerize in a controlled manner. In general, water solubility of the (meth)acrylate derived polymers increased with ethylene glycol repeat length as expected, and all the polymers showed significant β-sheet content in solution and as solids. Polypeptides with ethylene glycol repeats of 3–5 were found to possess temperature dependent water solubility similar to the glutamate derivatives discussed above. The disulfide-linked cysteine NCAs gave oligoethylene glycol modified poly(cysteine)s with properties similar to those described above, except that their transitions from water-soluble to water-insoluble at elevated temperature were found to be irreversible, even though their β-sheet content was found to be low by circular dichroism analysis. The irreversible assembly of the chains was believed to be due to disulfide exchanges that result in interchain covalent cross-links.

To avoid the β-sheet formation seen extensively in cysteine derivatives, Deming prepared oligoethylene glycol modified homocysteine NCAs (Figure 4H),[39] since the extra methylene in the homocysteine side-chain is known to favor α-helical conformations. The monomer with 4 ethylene glycol repeats gave polypeptides with good water solubility and stable α-helical conformations. This polymer was also found to possess temperature dependent water solubility similar to that of the glutamate derivatives discussed above. In addition to this solubility switch, the thioether groups in this polymer's side-chain could also be reversibly alkylated or oxidized to change the conformation from α-helix to coil. The details of these PPM reactions will be discussed in sections 3.1 and 3.6.

2.4. Saccharide Modified NCAs

In seminal work, Sela's and Rüde's laboratories prepared the first saccharide SCM NCAs, O-linked glycosylated serine NCAs (Figure 5A),[40,41] in an effort to study how addition of sugars to polypeptides would affect their immunological properties.

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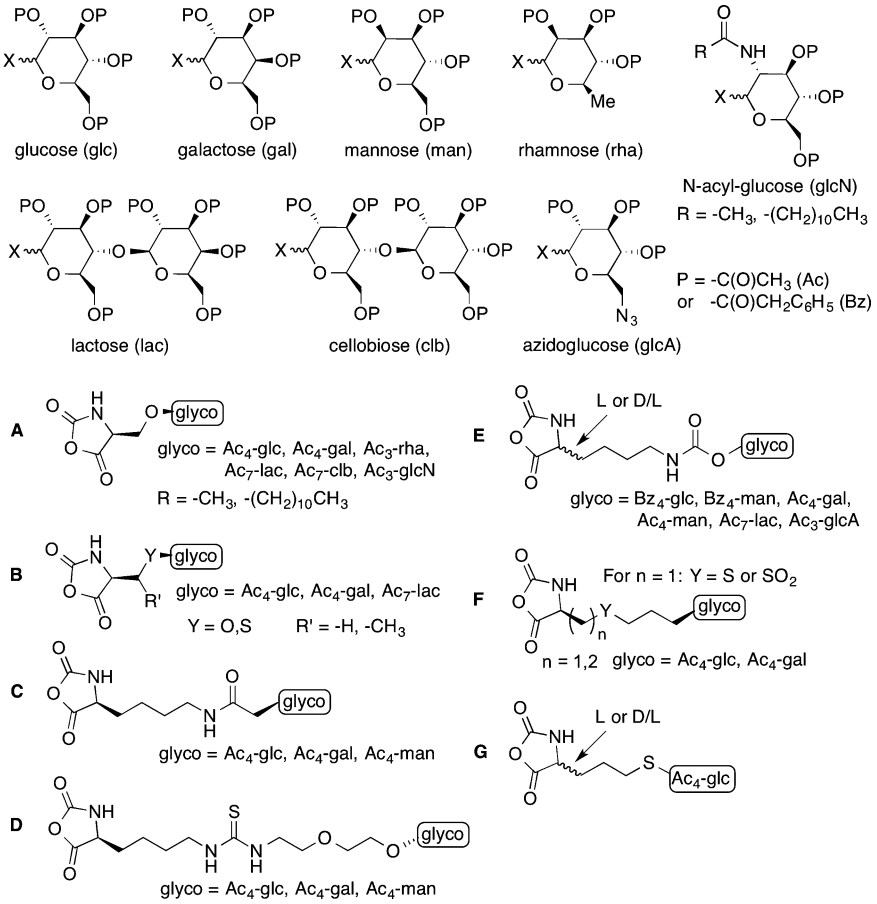

Figure 5. (A–G) Structures of saccharide modified NCAs.

Acetobromoglucose and N-Cbz-L-serine benzyl ester were reacted together in a mercuric cyanide mediated Koenigs–Knorr reaction, followed by deprotection and then the Leuchs method to form the NCA in 15–40% yield. NCA polymerization was initiated by the amine termini of different D/L-Ala, L-Lys, L-Glu, or L-Tyr containing copolypeptides to give "multichain" glycopolypeptides with 6–11% glucose by weight. In a later publication, Rüde and co-workers extended this methodology to include several other mono- and disaccharide modified serine NCAs (Figure 5A).[41] Polymerizations of these NCAs gave mainly short, oligomeric products where chain growth was likely inhibited by impurities in the NCA monomers.

Cameron's lab recently reported an improved synthesis of O-linked glycosylated serine and O-linked glycosylated threonine NCAs (Figure 5B),[42] which eliminated the need for the highly toxic and environmentally damaging mercury salts used by Rüde for the Koenigs–Knorr reaction. Cameron's procedure for preparation of glycosylated serine conjugates employed the reaction of *N*-Boc-L-serine or *N*-Boc-L-threonine with acetobromosugars as glycosyl donors, with iodine as the Lewis acid promoter, and with potassium carbonate in acetonitrile. Conjugates were obtained in 43–59% yield. After removal of the Boc groups, the glycosylated amino acid conjugates were converted to NCAs using triphosgene in 26–51% yield. However, monomer purity remained as an issue as these NCAs were not sufficiently pure to allow polymerization.

To overcome these difficulties in synthesis, purification, and polymerization of glycosylated NCAs, Deming's lab prepared glycosylated L-lysine NCA monomers (Figure 5C)[43] and reported a new general purification method for these noncrystalline NCAs.[44] The glycosylated lysine conjugates employ C-linked sugars and amide linkages for improved stability against deglycosylation compared to the previously described O-linked conjugates. C-Linked glycopeptides are also known to bind targets with nearly equal affinity and conformation as native O-linked analogues and have been widely utilized when stable glycoprotein mimetics are desired. The side-chains of lysine also provide a longer linker to the sugars compared to those of serine and threonine, which can help provide good monomer polymerizability.

Three different monosaccharide bearing glycosylated lysine NCAs, based on D-galactose, D-glucose, or D-mannose (Figure 5C), were synthesized by Leuch's method via treatment with Cl_2CHOCH_3, and then purified by anhydrous flash column chromatography.[43,44] The chromatography technique developed by Deming's lab was found to be a useful general method to obtain highly pure NCAs bearing complex functionalities, and is particularly useful for purification of low melting or difficult to crystallize NCAs. Using transition metal initiation, these high purity glycosylated lysine NCAs were found to undergo the first living polymerizations of glycosylated NCAs, which allowed preparation of well-defined, high molecular weight glycopolypeptides and glycopolypeptide containing block copolymers in excellent yield. Soluble homoglycopolypeptides were prepared with degrees of polymerization greater than 300 residues, significantly larger than the chains prepared from the previously described glycosylated serine NCAs (degree of polymerization <50). The deprotected glycopoly-

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peptides were all found to be highly soluble and α-helical in aqueous media.

Following this work, the laboratories of Sen Gupta and Wenz reported the synthesis and polymerization of additional glycosylated lysine NCAs. Wenz and co-workers attached peracetylated monosaccharides (glucose, mannose, or galactose) to N_α-Cbz-L-lysine via a thiourea linker, followed by conversion to the glycosylated NCAs using Leuch's method (Figure 5D).[45] NCAs were obtained in 76−82% yield after purification by aqueous workup and precipitation. The NCAs were then copolymerized with both oligoethylene glycol modified and N_ε-trifluoroacetyl L-lysine NCAs using either a tertiary amine or nickel initiator. The resulting statistical copolypeptides were obtained in good yield, were water-soluble after full deprotection, and had α-helical secondary structures.

Sen Gupta and co-workers prepared glycosylated lysine NCAs by modification of N_ε-Boc-N_α-Cbz-L-lysine benzyl ester via reaction with the propargyl 1,2-orthoester of per-*O*-benzoylated-D-glucose or mannose using $HAuCl_4$ and molecular sieves in CH_2Cl_2 to give conjugates with carbamate linkages in good yield. After removal of the amino acid protecting groups, the conjugates were converted into NCAs using triphosgene and purified by precipitation (Figure 5E).[46] Polymerizations were initiated with primary amines in the presence of 1,8-bis(dimethylamino)naphthalene as a proton sponge to remove residual HCl that had not been removed during NCA purification. Under optimized conditions, homoglycopolypeptides were obtained in 80−95% yield with narrow molecular weight distributions and degrees of polymerization ranging from 22 to 89. It was noted that deprotection of the benzoyl protecting groups on the sugars could not be accomplished without decomposition of the polypeptides.

In a later report, Sen Gupta and co-workers prepared similar carbamate-linked glycosylated lysine NCAs, as well as racemic and lactosylated versions, using acetyl protecting groups instead of the previously reported benzoyl groups (Figure 5E).[47] Polymerizations were initiated with amino terminated PEG chains in the presence of "proton sponge" in dioxane to remove residual HCl present in the monomers. Control over glycopolypeptide length was not demonstrated; however, narrow molecular weight distributions and degrees of polymerization from 35 to 64 were reported. After removal of the acetyl groups, the water-soluble, enantiomerically pure glycosylated poly-L-lysines were found to have conformations ranging from 30 to 70% α-helical depending on the chain length and type of sugar presented. In a recent study, Sen Gupta's lab reported synthesis and polymerization of a structurally similar glycosylated lysine NCA, where the sugar contained a reactive azido group (Figure 5E).[48]

In 2012, Deming's lab also described the preparation of glycosylated cysteine and homocysteine NCA monomers. The monomers were prepared in high yield by coupling of allyl functionalized peracetylated C-glycosides of D-galactose or D-glucose to L-cysteine or L-homocysteine using thiol−ene "click" chemistry. The glycosylated amino acid conjugates were converted to the corresponding glycosylated NCAs by Leuch's method via treatment with Cl_2CHOCH_3 and obtained in high purity after purification by anhydrous flash column chromatography (Figure 5F).[49] These glycosylated thioether-linked NCA monomers were found to polymerize efficiently using $(PMe_3)_4Co$ initiator in THF, giving side-chain-protected glycopolypeptides in excellent yields. Glycopolypeptide lengths increased linearly with monomer to initiator stoichiometry, and low polydispersity indices were observed (<1.2). Soluble, high molecular weight glycopolypeptides with 100% glycosylation were prepared with reproducible and precisely controlled chain lengths up to *ca.* 200 residues long. The resulting glycosylated poly(cysteine) samples were found to be water-soluble and partially α-helical in solution, while the glycosylated poly(homocysteine)s were found to be fully α-helical in water. A sulfone analogue of the galactosylated L-cysteine NCA was also prepared (Figure 5F),[49] and could also be polymerized, yet polymerizations of this monomer proceeded at much slower rates compared to the parent thioether-linked NCAs.

The main challenges in all of the above studies on preparation of glycosylated NCAs have been the multistep, and sometimes complicated, syntheses required to prepare these SCM NCAs, as well as difficulties in obtaining the NCAs in sufficient purity to allow their controlled polymerization. In 2014, Schlaad's lab reported a possible way to streamline this process by forming glycosylated NCAs *in situ*, via the radical catalyzed thiol−ene reaction of allylglycine NCA (to be discussed with alkene modified NCAs in section 2.7) with 1-thio-β-D-glucopyranose-2,3,4,6-tetraacetate (Figure 5G).[50] Allylglycine NCA,[51] although it is based on an expensive unnatural amino acid, is straightforward to prepare and purify, and 1-thio-sugars are readily available. Under optimized conditions, they found the thiol−ene conjugation reactions were able to go to completion without any attack of the thiols on the NCA monomers. The as-formed glycosylated NCAs could then be polymerized directly using an amine initiator to give glycopolypeptides with degrees of polymerization from 24 to 55.

2.5. Phosphate and Phosphonate Modified NCAs

Phosphorylation of serine residues in proteins is a natural post-translational modification. Such phosphorylated residues are believed to be a key component of these proteins that mediates many diverse processes such as protein−protein interactions, protein activation and inhibition, and biomineralization. The synthesis of polypeptide mimics of phosphoproteins may lead to their application as dental adhesives, as adhesion promoters, and in bone regeneration. Yamamoto reported the first synthesis of a phosphate modified NCA in 1999: *O*-diphenylphospho-L-serine NCA (Figure 6A).[52] This NCA was prepared in 88% yield from the modified amino acid, and was purified by recrystallization. The corresponding polypeptide was obtained in good yield after polymerization of the NCA in dioxane using triethylamine as initiator. No control over polypeptide chain length was demonstrated. Poly(*O*-phospho-L-serine) was obtained after removal of the phenyl protecting groups using stoichiometric PtO_2. The polypeptide was found to have good water solubility, and possessed disordered conformations in water between pH 1.3 and 8.1.

Sen Gupta's lab recently reported the synthesis of phosphate and phosphonate modified NCAs derived from modification of L-cysteine (Figure 6B,C).[53] The SCM amino acids were prepared via thiol−ene coupling of alkene functional reagents with L-cysteine, and were subsequently converted to NCAs using triphosgene. After NCA purification using anhydrous flash column chromatography, polymerizations were initiated with different functional amines in either dioxane of DMF. In either solvent, polypeptides were obtained with degrees of polymerization ranging from 25 to 40 and narrow molecular weight distributions. The protected polypeptides were found to possess α-helical conformations in acetonitrile, and the

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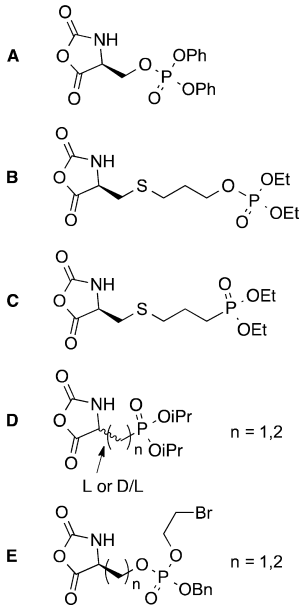

Figure 6. (A–E) Structures of phosphate and phosphonate modified NCAs.

deprotected phosphonate modified polymer possessed a disordered conformation in water at pH 7.2. It was noted that deprotection of the phosphate modified polymer could not be accomplished without loss of phosphate groups from the polypeptides.

Deming's lab also reported the synthesis of phosphonate modified NCAs, which were derived from L-serine and L-homoserine. These protected phosphonoalanine and phosphonohomoalanine NCAs (Figure 6D)[54] are more structurally similar to L-phosphoserine compared to the cysteine derivatives prepared by Sen Gupta's lab, which possessed longer side-chain tethers. The phosphonohomoalanine NCA was obtained in optically pure form; however, the phosphonoalanine NCA was obtained in partially racemized form, possibly due to reversible formation of dehydroalanine intermediates during NCA synthesis. Both monomers, purified by anhydrous flash column chromatography, underwent controlled polymerization using $(PMe_3)_4Co$ initiator in THF, giving soluble, side-chain-protected polypeptides in excellent yields. Polypeptides were obtained with degrees of polymerization ranging from 22 to 168 with narrow molecular weight distributions, and the NCAs could also be incorporated into block copolypeptides. The deprotected poly(L-phosphonohomoalanine) was found to possess a disordered conformation in water at pH 7.4, but adopted a α-helical conformation in water at pH 1.0 due to protonation of the phosphonate groups.

In a subsequent study, Deming's lab reported the synthesis of phosphate modified NCAs, which were also derived from L-serine and L-homoserine. These protected phosphoserine and phosphohomoserine NCAs (Figure 6E)[55] utilized different protecting groups than those employed by Yamamoto, which allowed for selective PPM of the resulting polymers to yield phosphorylcholine modified polypeptides (see section 3.7). Both NCAs were obtained in optically pure form and, after purification by anhydrous flash column chromatography, underwent controlled polymerization using $(PMe_3)_4Co$ initiator in THF, giving soluble, side-chain-protected polypeptides in excellent yields. Polypeptides were obtained with degrees of polymerization ranging from 34 to 130 with narrow molecular weight distributions, and the NCAs could also be incorporated into block copolymers.

2.6. Alkyne Modified NCAs

There has been tremendous interest and activity in recent years in the preparation of SCM NCAs that contain reactive functional groups. In particular, most of these studies have focused on functional groups that undergo "click" types reactions, such as azide–alkyne cycloadditions, thiol–ene, and thiol–yne reactions.[7,8] The alkyne functional group is central to many of these reactions, and as described in sections 3.4 and 3.5, many PPM reactions on polypeptides involve this functional group. The first alkyne modified NCA reported was D/L-propargylglycine NCA prepared by Schlögl in 1960 by phosgenation of the amino acid (Figure 7A).[56] This monomer was polymerized in nitrobenzene using ammonia as initiator and gave a polymer with degree of polymerization of 32.

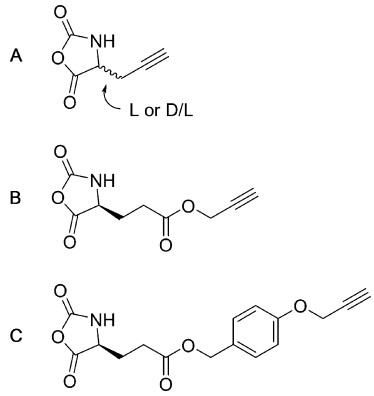

Figure 7. (A–C) Structures of alkyne modified NCAs.

Due to the high cost of the unnatural amino acid propargyl glycine, and the poor solubility of its polymers, the development of other alkyne SCM NCAs has been pursued in recent years. In 2009, Hammond's lab reported the synthesis of γ-propargyl-L-glutamate NCA (Figure 7B),[57] where the precursor glutamate ester, that is readily prepared from the amino acid and propargyl alcohol, was converted to the NCA using triphosgene. This monomer was polymerized in DMF using heptylamine initiator, and gave the corresponding polymer in 54% yield with a degree of polymerization of 40 and polydispersity index of 1.45. Control of chain length was not demonstrated and may have been hampered by monomer impurities. All of the alkyne groups in poly(γ-propargyl-L-glutamate) were found to be available for efficient azide–alkyne cycloadditions.

Yin and Cheng recently reported the synthesis of γ-(4-propargyloxybenzyl)-L-glutamate NCA (Figure 7C),[58] which was polymerized using hexamethyldisilazane in DMF. Although the synthesis of this monomer required additional steps in comparison to γ-propargyl-L-glutamate NCA, the authors proposed that the longer spacer in γ-(4-propargyloxybenzyl)-L-glutamate would help stabilize α-helical conformations in the polymers. A homopolypeptide was prepared with a degree of polymerization of 49 and polydispersity index of 1.05, and the alkyne groups were derivatized with a variety of functional azides.

2.7. Alkene Modified NCAs

A wide variety of alkene modified NCAs have been prepared, including those derived from modification of natural amino

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acids and those that are purely synthetic. D/L-Allylglycine NCA (Figure 8A) was first prepared in 1954 by Schlögl, who was able

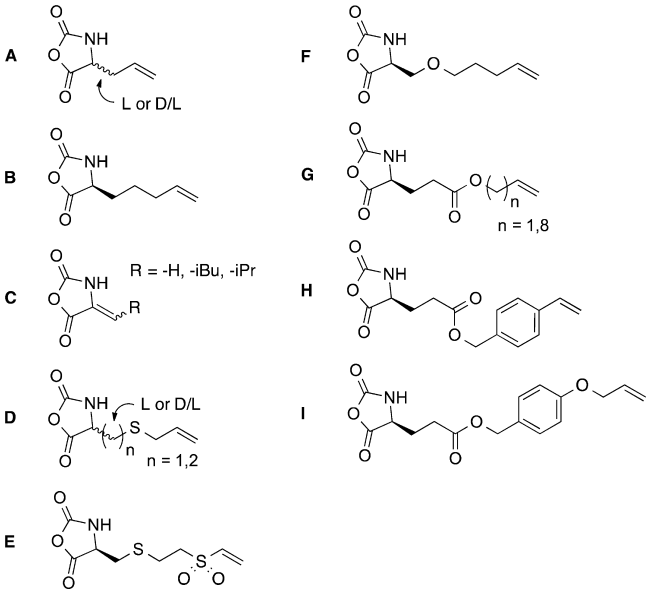

Figure 8. (A–I) Structures of alkene modified NCAs.

to demonstrate its polymerization in high yield to give a polymer with a degree of polymerization of 40.[51] Schlaad's lab reported an improved synthesis of this NCA in 2010,[59] and later the synthesis of L-allylglycine NCA,[60] where they used α-pinene as a sacrificial olefin during NCA synthesis to avoid hydrochlorination of the allyl side-chains. In related studies, Blanch and co-workers prepared L- and D/L-pentenylglycine NCAs (Figure 8B),[61] and polymerized these monomers in dioxane using triethylamine initiator. The poly(L-pentenylglycine) was found to adopt a α-helical conformation in the solid-state.

An unusual alkene containing NCA, dehydroalanine NCA (Figure 8C), was first reported by Sakakibara in 1959, who subsequently reported its polymerization in 1960.[62,63] The NCA was prepared from *N*-Cbz-dehydroalanine by Leuch's method, and was found to be quite stable against hydrolysis. Consequently, it was also found to polymerize much more slowly via amine initiation in dioxane at 30 °C compared to D/L-alanine NCA, reaching only 35% conversion after 24 h. Satisfactory polymerization was obtained using triethylamine initiator in boiling toluene, which gave poly(dehydroalanine) as a brown precipitate. The freshly formed polymer was soluble in water and polar organic solvents, but lost solubility upon removal of solvent to dryness, or after standing for 6 months. There was some evidence that the aged polymers became chemically cross-linked. In a follow-up study, Iwakura's lab prepared dehydroleucine and dehydrovaline NCAs (Figure 8C), but neither of these were found to give polypeptides.[64]

The first alkene containing NCA prepared by modification of a natural amino acid was *S*-allyl-L-cysteine NCA in 1955 (Figure 8D).[12] This NCA was prepared by phosgenation of the modified amino acid, and was polymerized by different methods. No data were reported on molecular weights of the polymers, which exhibited poor solubility in most solvents. The low solubility of these polymers, combined with the poly(cysteine) backbone, suggests the polymer forms β-sheet structures. The homologous *S*-allyl-D/L-homocysteine NCA was also synthesized (Figure 8D).[65] More recently, Zhong's lab synthesized a different alkene modified cysteine NCA that was prepared by reaction of divinyl sulfone with L-cysteine, followed by reaction with triphosgene in the presence of α-pinene. This *S*-(2-vinylsulfonylethyl)-L-cysteine NCA (Figure 8E) was copolymerized with a variety of other NCAs using *N*-trimethylsilyl allylamine as initiator in DMF.[66] The monomer could also be homopolymerized to give poly(*S*-(2-vinylsulfonylethyl)-L-cysteine) with M_n of 16 kDa and polydispersity index of 1.58.

In related work, Cheng's lab reported the synthesis of *O*-pentenyl-L-serine NCA (Figure 8F) by reaction of the modified amino acid with triphosgene.[67] The NCA was purified using anhydrous flash column chromatography and then polymerized using an amine terminated polyethylene glycol initiator in DMF. Homopolypeptides were found to be insoluble in most solvents and adopted β-sheet conformations. In 1997, Daly's lab reported the alkene modified glutamate derivatives γ-allyl-L-glutamate NCA and γ-(9-decenyl)-L-glutamate NCA (Figure 8G),[68] which were prepared in a manner analogous to that used for γ-propargyl-L-glutamate NCA described above. The allyl modified polymer was noted to form gels in THF or chloroform, while the 9-decenyl modified polymer was readily soluble in these solvents. In 2011, Cheng's lab synthesized γ-(4-vinylphenyl)methyl-L-glutamate NCA (Figure 8H)[69] and γ-(4-allyloxybenzyl)-L-glutamate NCA (Figure 8I)[70] which were both used to prepare polypeptides that could undergo a variety of PPM reactions (described in section 3.5).[71] The 4-vinylphenyl SCM NCA was prepared by phosgenation of the modified amino acid and purified by fractional recrystallization in 35% yield. Polymerizations were conducted in DMF using hexamethyldisilazane initiator and including nitrobenzene to inhibit radical reactions of the vinyl groups.

2.8. Azido Modified NCAs

In contrast to the long history of studies on preparation of alkyne and alkene modified NCAs, azide SCM NCAs have only been reported within the past 3 years. As described above, in 2014 Sen Gupta reported synthesis and polymerization of a glycosylated lysine NCA, where the sugar contained a reactive azido group (Figure 5).[48] Previously, in 2012 Deming's lab published the synthesis and polymerization of azide modified NCAs that were derived from L-lysine and L-ornithine using a diazotransfer reaction. These monomers, L-azidonorvaline NCA and L-azidonorleucine NCA (Figure 9),[72] were purified using

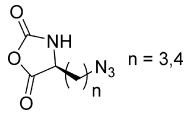

Figure 9. Structures of azido modified NCAs.

anhydrous flash column chromatography, and underwent controlled polymerization using $(PMe_3)_4Co$ initiator in THF, giving the corresponding polypeptides in excellent yields. The monomers could also be incorporated into block copolypeptides with controlled compositions. Poly(L-azidonorleucine) was found to have better solubility in THF and was prepared with controlled degrees of polymerization ranging from 36 to 103 and polydispersity indices of <1.14. This polypeptide was also found to be α-helical in organic solvents.

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2.9. Halogen Modified NCAs

The preparation of halogen modified NCAs is another area that has seen much recent activity. In most cases, the halogen, typically chloride but also bromide, was introduced to enable PPM reactions on the resulting polypeptides. An early study by Hashimoto described the synthesis of γ-4-chlorobenzyl-L-aspartate NCA (Figure 10A),[73] which was prepared to study the conformational stability of poly(L-aspartate esters). More recently, a number of NCAs have been prepared as glutamate esters, due to the ease and versatility of preparing these derivatives. In 2010, Zhang's lab reported the synthesis of γ-3-chloropropyl-L-glutamate NCA (Figure 10B),[74] which was polymerized using hexamethyldisilazane in DMF to yield soluble polymers with controlled length and polydispersity indices of <1.26. No loss of chloroalkyl groups was observed, and these could be converted to azido groups via PPM reaction (see section 3.4). Cheng's lab followed up on this work with preparation of additional chloroalkyl glutamate NCAs, namely, γ-6-chlorohexyl-L-glutamate NCA and γ-6-chlorohexyl-D/L-glutamate NCA (Figure 10B),[75] which could also be polymerized efficiently and the chloroalkyl groups then modified using PPM reactions.

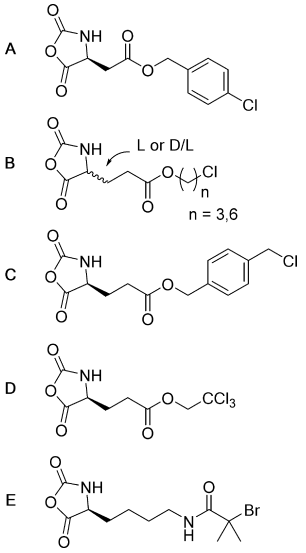

Figure 10. (A–E) Structures of halogen modified NCAs.

Tang's lab recently reported the synthesis of γ-(4-chloromethyl)benzyl-L-glutamate NCA (Figure 10C), which was polymerized using butylamine in DMF.[76] The resulting soluble polymers retained the benzylic chloride groups, which were then used to alkylate substituted imidazoles in PPM reactions (see section 3.7). Endo's lab also reported preparation of a chlorinated glutamate monomer, γ-2,2,2-trichloroethyl-L-glutamate NCA (Figure 10D),[77] but in this case the chlorination served a different purpose. The trichloroethyl ester groups in resulting polymers were used as "active esters" for PPM reactions of the polymers with primary amines (see section 3.3).

As described above, Deming's lab has prepared phosphoserine and phosphohomoserine NCAs that contain bromoethyl groups (Figure 6E).[55] The bromoethyl functionalities were stable during $(PMe_3)_4Co$ mediated polymerization in THF, and

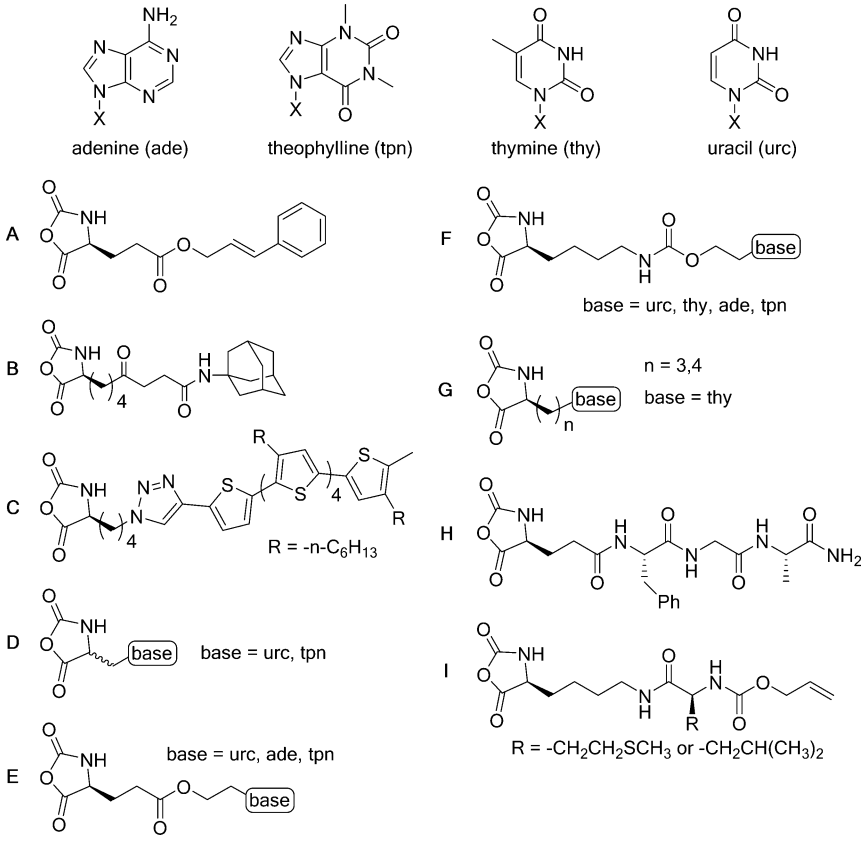

Figure 11. (A–I) Structures of other modified NCAs.

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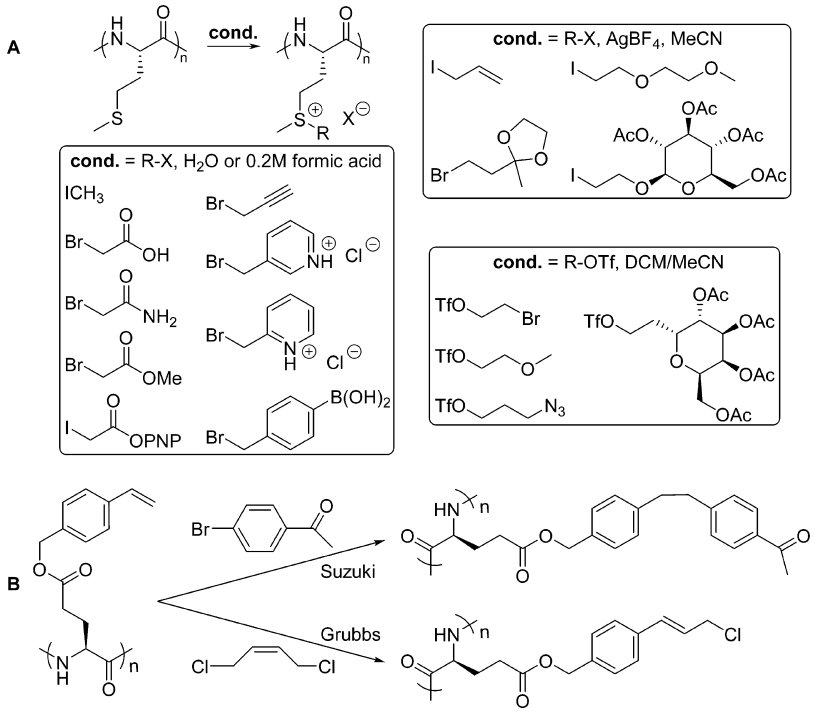

Figure 12. (A, B) Post-polymerization alkylation reactions.

then converted to trimethylammonium groups via PPM reactions. Li also reported synthesis of a bromoalkyl modified monomer, N_ε-bromoisobutryl-L-lysine NCA (Figure 10E), where the bromoalkyl group served as an initiator for ATRP of different vinyl monomers.[78] This NCA was synthesized using Leuch's method, purified using anhydrous flash column chromatography, and underwent controlled polymerization using [1,2-bis(diethylphosphino)ethane]Ni(1,5-cyclooctadiene) initiator in DMF.[79,80] The nickel initiator was used to obtain high molecular weight polymers and to prevent possible side-reactions of the reactive bromoalkyl groups that may occur during amine initiated polymerizations.

2.10. Other Modified NCAs

A variety of other SCM NCAs have been prepared in order to obtain properties for specific applications. Jing's lab reported the preparation of γ-cinnamyl-L-glutamate NCA (Figure 11A),[81] which yielded soluble polypeptides with degrees of polymerization ranging from 9 to 57 and polydispersity indices of <1.15. The cinnamyl groups could then be photodimerized to yield cross-linked polypeptides. Zhang's group recently reported an adamantyl modified L-lysine NCA (Figure 11B),[82] where the adamantyl groups were envisioned to promote assembly by complexing with β-cyclodextrin groups. An oligothiophene modified NCA was reported by Kumar's lab, which was prepared by azide−alkyne conjugation of alkyne terminated sexihexylthiophene with L-azidonorleucine.[83] The product was converted to the NCA (Figure 11C) using triphosgene, but the low solubility of the NCA in organic solvents hindered attempts to purify the monomer. Consequently, this NCA could only be polymerized using high initiator loadings (i.e., 10 mol %), since much of the initiator was consumed by impurities.

In 1973, Takemoto and co-workers reported the synthesis of 7-theophillinyl-D/L-alanine NCA and 2,4-dioxo-1-pyrimidinyl-D/L-alanine NCA (Figure 11D),[84] which could be considered as analogues of D/L-phenylalanine where the phenyl group is replaced with a nucleobase. Polymerization of these monomers with either tertiary or primary amines in DMF gave only oligomers with degrees of polymerization of less than 5. Takemoto followed this work with the synthesis of γ-2-(7-theophillinyl)ethyl-L-glutamate NCA and nucleobase modified L-lysine NCAs (Figure 11E,F),[85,86] perhaps reasoning that the longer side-chain spacer would improve the polymerization, yet only oligomers were formed. In 1982, Kricheldorf also reported the synthesis of a series of pyrimidine nucleobase modified L-lysine and L-ornithine NCAs (Figure 11G).[87] These monomers were polymerized in DMF or pyridine and yielded the functional polypeptides with degrees of polymerization of less than 30. It is possible that the nucleobase side-chains interfere with the polymerization process and thus limit chain growth.

In addition to nucleobases, there have also been reports of amino acid and peptide modified NCAs. Using solid-phase methods, Amblard and co-workers reported the synthesis of a alanine-glycine-phenylalanine tripeptide modified glutamate NCA (Figure 11H).[88] The phenylalanine N-terminal amine group was conjugated to the side-chain carboxylate group of glutamic acid, and the alanine C-terminal group was converted to an amide. The NCA was prepared on the solid support using Leuch's method with cyanuric chloride as the cyclizing reagent, followed by cleavage from the resin using anhydrous TFA. Although polymerization was inefficient using primary amine initiation, possibly due to residual acid in the NCA, use of diethylamine initiator was able to give peptide brush polypeptides in up to 75% yield with degrees of polymerization of up to 30.

Deming's lab reported a slightly different class of monomers, where *N*-allyloxycarbonyl-protected amino acids were used to modify lysine residues (Figure 11I).[89] With the protecting groups present, these monomers possessed better solubility in organic solvents compared to the peptide modified monomer described above, and so allowed for better purification. These monomers underwent controlled polymerization using

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$(PMe_3)_4Co$ initiator in THF, giving the corresponding polypeptides in excellent yields with degrees of polymerization up to 292. The monomers could also be incorporated into block copolypeptides with controlled compositions. The *N*-allyloxycarbonyl groups could be removed post-polymerization, and were also used in a PPM reaction to generate nickelacycle initiators for growth of block copolypeptide brushes in a "grafting from" process.

3. SIDE-CHAIN MODIFIED POLYPEPTIDES VIA POST-POLYMERIZATION REACTIONS

As noted in the previous section, many SCM NCAs incorporate chemically reactive side-chain functional groups that are inert during polypeptide synthesis, yet can then undergo PPM reactions. Use of such PPM reactions as a means to introduce functional groups onto polypeptides has many potential advantages. Some functional groups are difficult to incorporate into SCM NCAs, or will interfere with their polymerization. Also, the synthesis of side-chain reactive polypeptides can be synthetically simpler and more cost-effective compared to the many reactions and tedious purifications involved in preparation of some SCM NCAs. However, the key to success in all PPM strategies is efficient and straightforward coupling of functional molecules to side-chain reactive groups. In addition to these reactions occurring in high yield, they may also need to occur using stoichiometric reagents, or be chemoselective in not reacting with other polypeptide functional groups. Multiple functionalizations, where all side-chains on a polypeptide contain a reactive group for PPM, can also be low yielding when sterically demanding reactive molecules are used. The use of "click" type reactions for PPM of polypeptides has overcome many of the potential limitations described above, but there are many examples where problems still occur. The variety of PPM modifications of polypeptide side-chains reported in the literature is detailed below.

3.1. Alkylation Reactions

Due to its role in biology, the alkylation of thioether groups in methionine has been studied for some time. The majority of work in this area has employed simple alkylating agents, such as iodomethane to give the naturally occurring, dietary supplement *S*-methyl-methionine, as well as iodoacetic acid to form soluble derivatives used to probe active sites of proteins. Berger and Katchalski were the first to alkylate poly(methionine) in a PPM reaction, preparing both methyl and carboxymethyl sulfonium derivatives from the corresponding alkyl bromides and poly(methionine) in neat formic acid (Figure 12A).[90,91] Contrary to hydrophobic poly(methionine), these polysulfoniums were found to be water-soluble, and were also stable and possessed disordered conformations in water.

Realizing methionine alkylation has the potential to be much broader in scope as a PPM reaction, Deming's lab developed and expanded this alkylation chemistry as a means to introduce a wide range of functional groups onto polypeptides. They showed that poly(L-methionine) segments can be prepared with controlled lengths and are readily incorporated into block copolymers using cobalt initiators.[44] Using well-defined poly(L-methionine), Deming showed that methionine residues can be modified directly with activated alkyl halides or alkyl triflates.[92] Alkylations with unactivated alkyl halides proceeded upon addition of silver tetrafluoroborate (Figure 12A). Follow-up work by Deming's lab described the reversible PPM of polypeptides via chemoselective dealkylation of methionine sulfonium residues.[93] These PPM reactions were found to be compatible with deprotection of other functional groups, and use an inexpensive, natural amino acid that is readily polymerized and that requires no protecting groups, which makes this methodology both cost-effective and scalable.

Cheng's lab has also reported PPM alkylation reactions on polypeptides. The polymer from γ-(4-vinylphenyl)methyl-L-glutamate NCA described above (Figure 8H) was modified by reacting the pendant alkene with 4-bromoacetophenone in a Suzuki reaction, and with *cis*-1,4-dichlorobutene in a cross metathesis reaction catalyzed using Grubb's second generation catalyst (Figure 12B).[71] While these are not strictly alkylation reactions, they do showcase the addition of hydrocarbon groups to polypeptides. There are also many examples of PPM alkylation reactions of thiol groups in polypeptides, but these are described later in section 3.5 on thiol−ene and thiol−yne reactions.

3.2. Esterification Reactions

Watanabe's group reported pioneering studies on thermotropic polypeptides, where poly(glutamates) were derivatized in PPM reactions with either *n*-alkyl chains or by end-on attachment of biphenyl mesogens. In these studies poly(γ-methyl-L-glutamate) of varying length was reacted with excess quantities of various *n*-alkyl alcohols (C_5 to C_{18}) or 4-butoxy-4′-((ω-hydroxyhexyl)oxy)biphenyl in dichloroethane at 60 °C using *p*-toluenesulfonic acid as catalyst (Figure 13A).[21−23] After

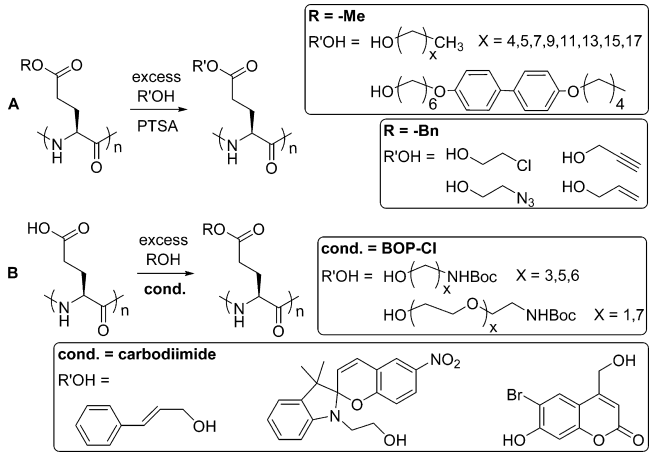

Figure 13. (A, B) Post-polymerization esterification reactions.

reaction times up to 10 days, complete conversion of methyl esters to the modified esters was observed. In related work, poly(β-benzyl-L-aspartate) was modified in a similar manner using stearyl alcohol, where the degree of transesterification was varied from 8 to 71 mol %.[94]

The modified polyglutamates with *n*-alkyl side-chains greater than 10 carbons long were thermotropic and gave melting transitions from −26 to 54 °C. These samples formed cholesteric liquid crystalline phases above the melting transition, but formed layered structures at low temperatures driven by crystallization of the side-chains. Furthermore, poly(γ-octadecyl-L-glutamate) was found to form a columnar hexagonal phase at temperatures above 200 °C, where the rod-like helices make up the 2D lattice. When the biphenyl mesogen was used to modify poly(glutamate) side-chains, layered structures were observed in the crystalline and liquid

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crystalline states, followed by transition into a cholesteric structure at higher temperatures.

Huang's group reported additional transesterification reactions, where poly(γ-benzyl-L-glutamate) was reacted with excess quantities of various functional alcohols in dichloroethane at 55 °C using *p*-toluenesulfonic acid as catalyst (Figure 13A).[95] To avoid benzyl ester hydrolysis, large excesses of functional alcohols (>5 equiv) per each benzyl ester were employed. By variation of reaction conditions, degrees of PPM using different functional alcohols were found to range from 12 to 56 mol %. Cheng also reported modification of poly(L-glutamic acid) by esterification using Boc-protected α,ω-aminoalcohols containing different alkyl spacers (Figure 13B).[69] They reported modification efficiencies of 90 mol % or higher, and the modified, deprotected polypeptides with longer alkyl spacers were found to adopt stable α-helical conformations in water.

The use of PPM reactions that form glutamate esters to give photoresponsive polymers has also been explored. In 1989, Ciardelli reported esterification of poly(L-glutamic acid) with an alcohol derivative that contained a spiropyran group using carbodiimide coupling in DMF (Figure 13B).[96] The resulting polymer contained *ca.* 41 mol % spiropyran groups based on total available glutamate residues, and was soluble in HFIP. Photoisomerization between the colorless closed spiro structure and the colored ring-opened merocyanine structure was found to correlate, respectively, with a conformational transition between α-helical and coil-like conformations of the polypeptide. Mezzenga recently reported adaptation of this chemistry to polypeptide containing block copolymers that can reversibly associate or dissociate in water upon treatment with light.[97]

In another modification to introduce a photoreactive groups via a PPM process, Chen reported esterification of poly(L-glutamic acid) with cinnamyl alcohol using carbodiimide coupling in DMSO, to yield copolymers containing up to 36 mol % cinnamyl groups (Figure 13B).[98] The cinnamyl groups in the modified polyglutamate segments were used to form photoactivated cross-links between the polyglutamate chains. In a similar process, Zhao reported esterification of poly(L-glutamic acid) with a coumarin derivative to yield copolymers containing up to 38 mol % coumarin groups (Figure 13B).[99] These copolymers formed micelles in water that could be disrupted by irradiation using near-infrared light, which cleaved the coumarin ester bonds.

3.3. Amidation Reactions

There have been many examples of PPM reactions on poly(lysine) or poly(glutamate/aspartate) side-chains to add functionality via formation of amide bonds. Often these reactions have been used to add alkyl chains, drug molecules, or fluorescent probes for applications such as therapeutic delivery, which are not covered in this review. In general, amidation modifications take advantage of the many efficient methods to form amide bonds, as well as the ease of incorporating amine and carboxylic acid/carboxylic ester functionalities into polypeptides. Many of these modifications have been reported as means to introduce photoreactive groups into polypeptides, similar to some of the esterification modifications described above for polyglutamate chains.

Modification by amidation is a natural choice for functionalization of amine containing polylysines and polyornithines. In 1986, Yamamoto's lab reported the PPM reaction of poly(L-lysine) with *p*-(phenylazo)benzoic acid *p*-nitrophenyl ester in aqueous DMF to give the 95 mol % derivatized polymer in good yield (Figure 14A).[100] Similar to other polymers of this type, the product was only soluble in HFIP,

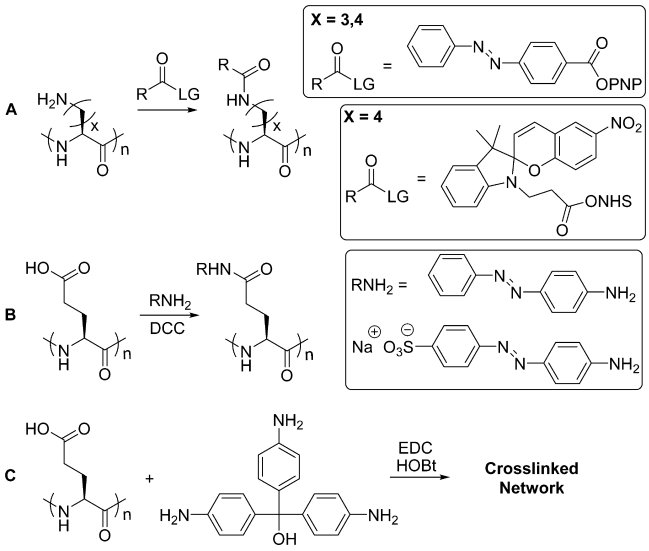

Figure 14. (A–C) Post-polymerization amidation reactions.

and the azobenzene side-chains were found to switch reversibly between *cis* and *trans* forms upon exposure to light and dark, respectively. This same PPM of poly(L-lysine) was reinvestigated by Ciardelli in 1987,[101] who examined different methods for conjugation of the azobenzene group, and prepared polylysines with degrees of modification that varied from 5 to 44 mol %. Ciardelli found that the *cis*/*trans* photoisomerization of the azobenzene groups did not significantly alter the α-helical conformation of the polypeptide. In 1990, Yamamoto also reported the synthesis of *p*-(phenylazo)benzoyl modified poly(L-ornithine) (Figure 14A),[102] which possessed properties similar to those of the lysine derivative. In 1995, Ciardelli and co-workers reported the PPM modification of poly(L-lysine) with spiropyran groups (Figure 14A),[103] with degrees of modification that varied from 25 to 46 mol %. In certain solvent mixtures, photoisomerization of the spiropyran groups was able to effect reversible α-helix–coil transitions in the polypeptide backbone.

In 1980, Ciardelli's lab also reported the PPM reaction of poly(L-glutamate) with *p*-aminoazobenzene in DMF to give polymers with degrees of modification that varied from 13 to 56 mol % (Figure 14B).[104] Similar to lysine based polymers described above, the azobenzene side-chains of the product in trimethylphosphate solvent were found to switch reversibly between *cis* and *trans* forms upon exposure to light and dark. In later studies in aqueous solution at pH 5.6, Ciardelli found that a sample containing 21 mol % azobenzene groups was soluble and underwent a conformational change from weakly α-helical to highly α-helical after exposure to light.[105] Kinoshita followed this work by preparing azobenzenesulfonate modified poly(L-glutamic acid)s in a PPM process with degrees of modification that varied from 2 to 46 mol % (Figure 14B).[106] The addition of charged sulfonate groups allowed the products with higher azobenzene content to retain water solubility. Kinoshita and Takizawa also reported a PPM reaction of poly(L-glutamic acid) with pararosaniline to generate cross-linked membranes (Figure 14C), where the polypeptide chain conformations can be switched using light.[107]

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A different approach to polypeptide amidation, the aminolysis of polyglutamate esters, has also been well-utilized. Blout, in 1962, reported the aminolysis of poly(γ-benzyl-L-glutamate) or poly(γ-methyl-L-glutamate) by PPM reaction with 2-(*N*-morpholinyl)ethylamine in dichloroacetic acid at 70 °C (Figure 15A).[108] The resulting polymers were fully modified

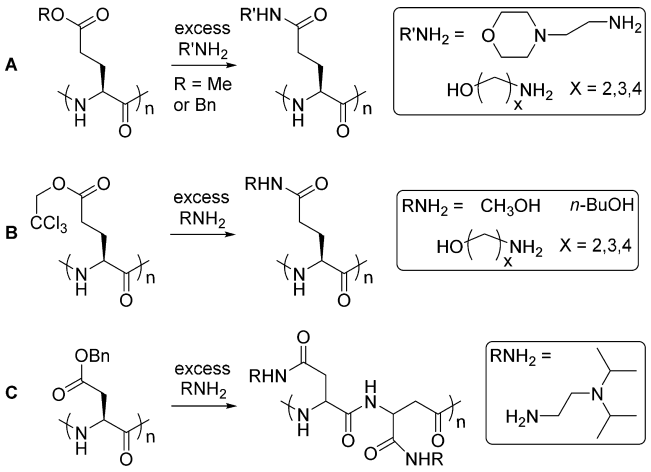

Figure 15. (A–C) Post-polymerization amidation reactions.

and found to possess good water solubility over a pH range of 1.5–13. It was noted that if the reaction was run at higher temperatures, or in a sealed tube, cleavage of the polypeptide chains was observed. Following this work, in 1965 Lupu-Lotan and co-workers reported the PPM reaction of poly(γ-benzyl-L-glutamate) with 3-amino-1-propanol in dioxane at 60 °C to give the fully modified, nonionic poly(L-glutamine) derivative in good yield, yet with some chain degradation (Figure 15A).[109] This modified polymer was found to be water-soluble and possessed a partially α-helical conformation at 20 °C. Later studies by the same lab reported the preparation of the homologous poly(γ-hydroxyethyl-L-glutamine) and poly(γ-hydroxybutyl-L-glutamine) derivatives (Figure 15A),[110] where α-helicity increased and water solubility decreased as the alkyl linker length increased.

In order to prevent the chain-cleavage observed in PPM aminolysis of poly(γ-benzyl-L-glutamate), Endo's lab performed aminolysis reactions on poly(γ-2,2,2-trichloroethyl-L-glutamate) (Figure 15B), which has a better leaving group in the ester functionality.[77,111,112] With this more reactive precursor polymer, 97 mol % functionalization by aminolysis with 3 equiv of 3-amino-1-propanol was obtained in DMF at 10 °C. Other, unhindered primary amines were also found to give high yields of functionalized polypeptides, but more sterically demanding amines gave poor functionalization. Kataoka's lab studied similar reactions, but using poly(β-benzyl-L-aspartate) as the platform for PPM reactions instead of poly(γ-benzyl-L-glutamate) (Figure 15C).[113] In this system, it was observed that chain cleavage did not occur, and quantitative functionalization by aminolysis was achieved using stoichiometric amine at 35 °C in anhydrous polar solvents. The accelerated aminolysis rate here, as compared to that observed for poly(γ-benzyl-L-glutamate), was proposed to be due to rapid imide formation in the polypeptide backbone, which is not favored for glutamate polymers. The only negative consequence of this process is the conversion of the polymer backbone to a mixture of α- and β-peptide linkages (Figure 15C).

In other work aimed at introducing functionality by amidation, Shinkai's lab reported PPM reactions on poly(L-

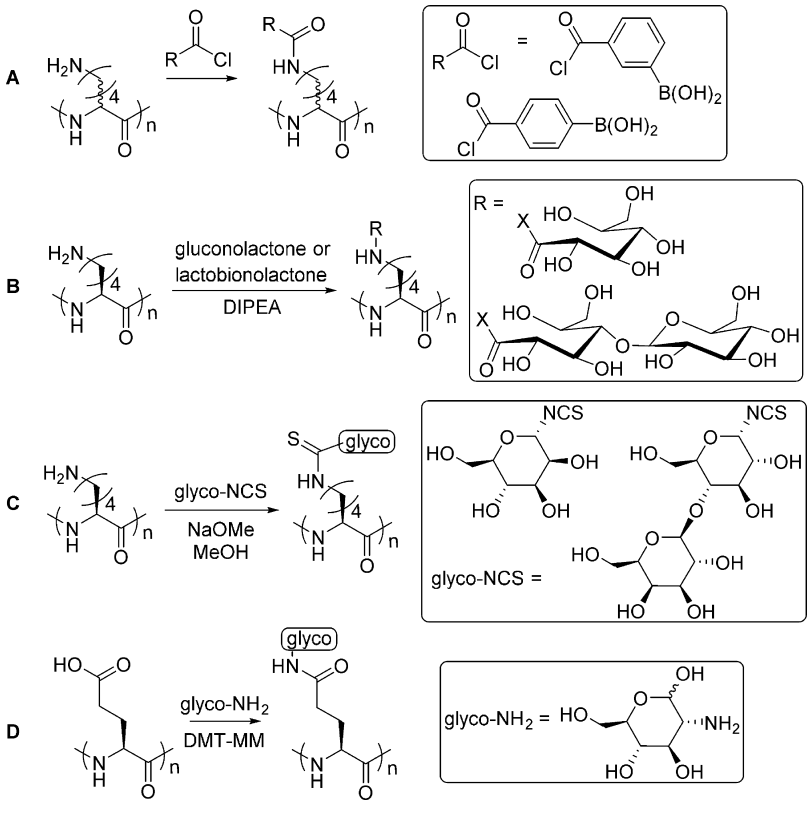

Figure 16. (A–D) Post-polymerization amidation reactions.

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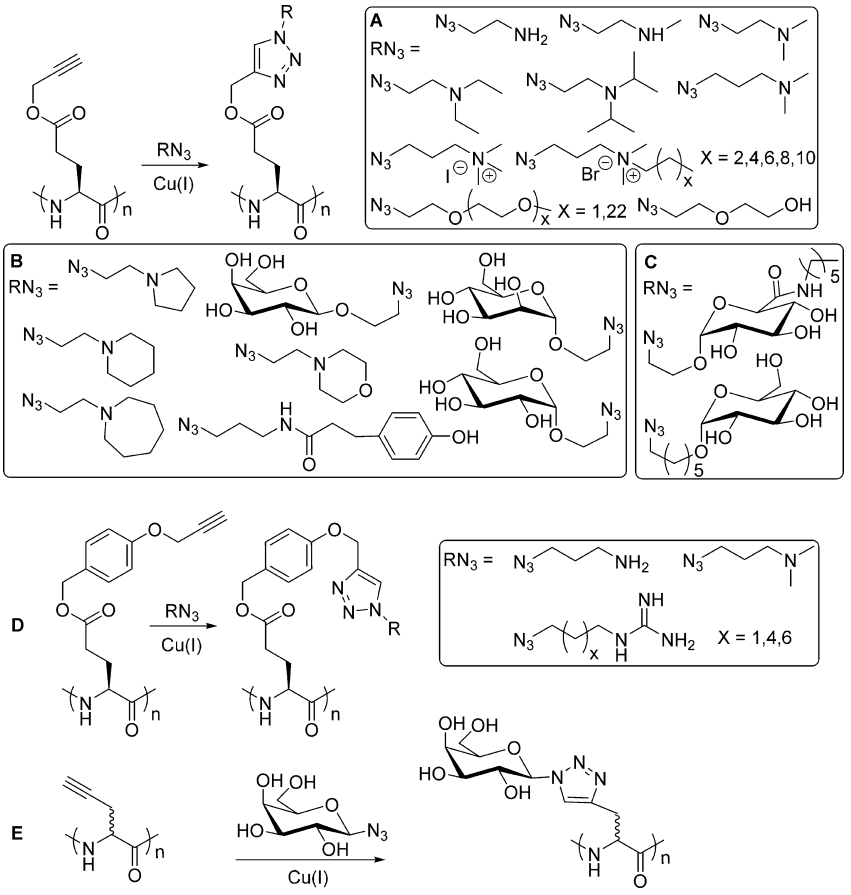

Figure 17. (A–E) Post-polymerization azide–alkyne cycloaddition reactions.

or D-lysine) to prepare *p*- and *m*-phenylboronic acid derivatives (Figure 16A).[114] The authors reported 99 mol % functionalization with phenylboronic acid groups, giving polymers that were water-soluble above pH 8, with 78% α-helical content at pH 9. Complexation of this polymer with D-fructose also gave α-helical polypeptides, with maximum helical content at pH 7. A number of researchers from the 1990s up to the present also conjugated sugars (mannose, galactose, or lactose) to poly(L-lysine) using a variety of coupling chemistries.[115−122] For most of these studies, the focus was on bioconjugate applications, which are not covered in this review, and only fractional glycosylation of the polypeptides was most often desired.

In more recent work, Feng and co-workers prepared glyconamidated polypeptides by PPM of an amphiphilic ABA triblock copolymer scaffold (Figure 16B).[123] The lysine residues of the triblock copolymer were glycosylated by reaction of D-gluconolactone or lactobionolactone under basic conditions to attach ring-opened sugars via amide linkages. Different glycosyl lactone reagent feed ratios gave glycosylation densities ranging from 47% to 75%. Higher substitution densities could not be reached even with 3-fold excess of glycosylating reagent, presumably due to steric crowding of the bulky sugar groups.

In 2010, Li and co-workers used a similar approach to obtain partially glycosylated poly(L-lysine) (Figure 16C).[124] Poly(L-lysine) was treated in a PPM reaction with an isothiocyanate functionalized mannose derivative to give sugar attachment via thiourea linkages and glycosylation densities of 16 and 23 mol %. A different approach to prepare amide-linked glycopolypeptides was taken recently by Menzel, who used poly(L-glutamic acid) as the polypeptide precursor and formed glycopolypeptides by PPM reactions with amino sugars (Figure 16D).[125] The coupling reactions were promoted using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), which is a mild and very efficient amide coupling agent compatible with the reagents used. Conjugation of glucosamine units to the polypeptide was found to be highly efficient (90 mol %) at low loading ratios (less than 40 mol % amino sugar per carboxylate), but decreased at higher sugar loadings, with the maximum loading of sugars being approximately 80 mol %.

3.4. Azide–Alkyne Cycloaddition Reactions

The PPM of polypeptides using azide–alkyne reactions has seen much recent activity due to the high efficiency and broad functional group compatibility of these modifications. Hammond and co-workers published the first example of an azide–alkyne PPM on polypeptides in 2009. Using copper catalysis, 2 equiv of PEG-azide was coupled to alkyne functional poly(γ-propargyl-L-glutamate) to yield PEG-grafted polypeptide brushes with near quantitative modification of alkyne groups (Figure 17A).[57] In subsequent studies, Hammond's lab reacted poly(γ-propargyl-L-glutamate) in a similar manner with a variety of azido containing amines, and azido containing oligoethylene glycol derivatives (Figure 17A), which gave polypeptides with both pH and thermoresponsive properties in aqueous solution.[126−129]

Chen and co-workers also used poly(γ-propargyl-L-glutamate) to introduce different functionalities onto polypeptides using PPM azide–alkyne reactions. They first reported

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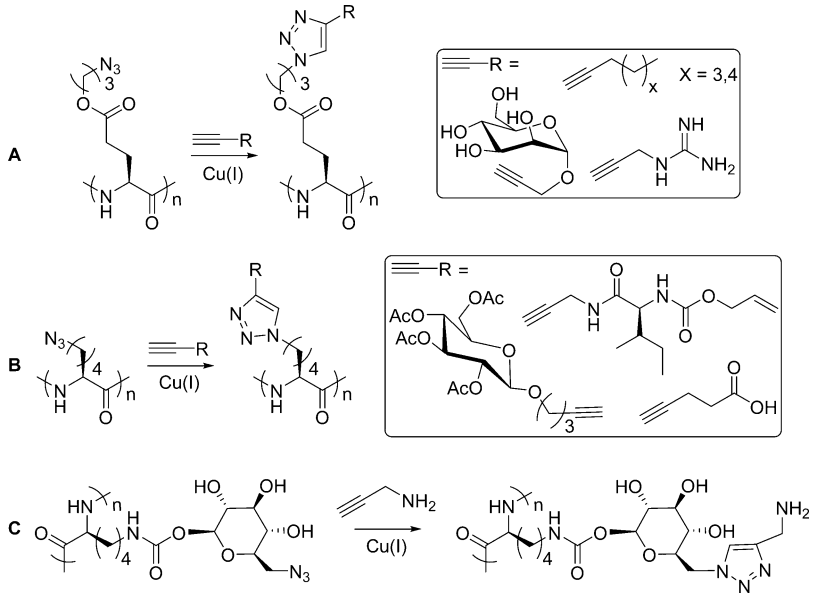

Figure 18. (A–C) Post-polymerization azide–alkyne cycloaddition reactions.

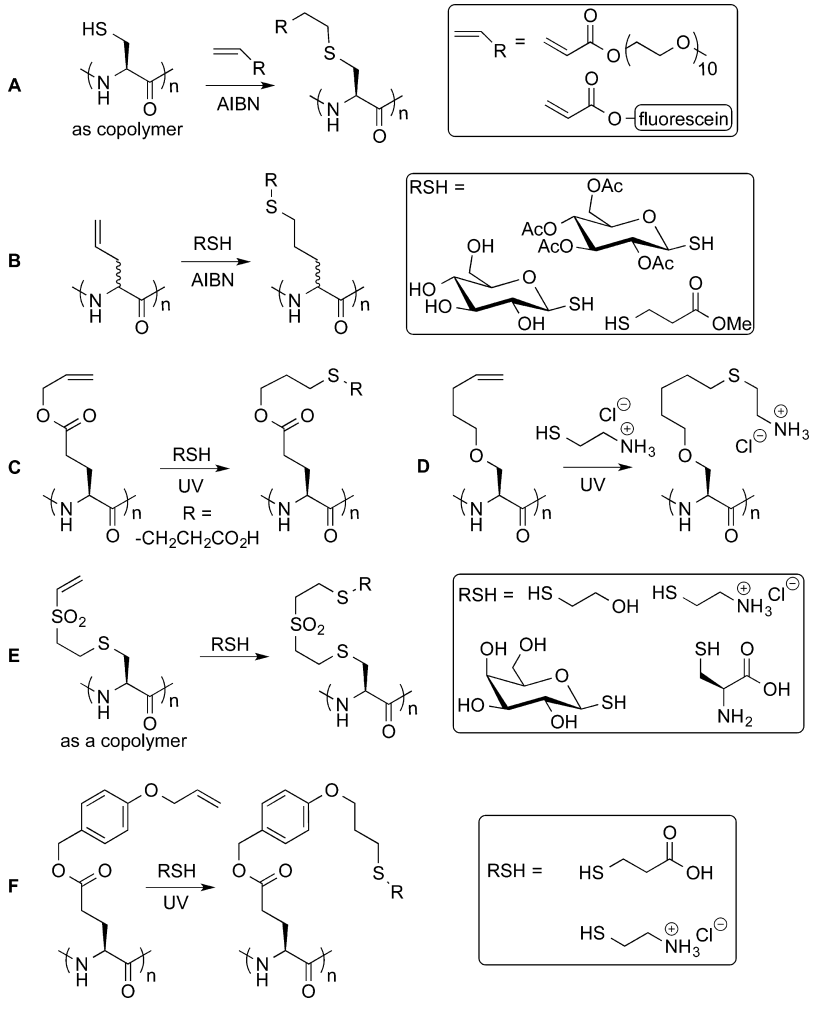

Figure 19. (A–F) Post-polymerization thiol–ene reactions.

modification of poly(γ-propargyl-L-glutamate) using three different azide functionalized monosaccharides using copper catalysis in DMSO (Figure 17B).[130] The modifications were found to proceed to near complete conversion, yielding polypeptides with high glycan densities. The glycopolypeptides were soluble in water after removal of protecting groups, and all

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were found to be α-helical regardless of glycan composition. Chen's lab later used similar methodology to react poly(γ-propargyl-L-glutamate) with other azido containing molecules, such as derivatives of biotin, oligoethylene glycols, amines, and phenol (Figure 17B).[131−134] In related studies, Sen Gupta's lab reported the PPM addition of other azido containing monosaccharides to poly(γ-propargyl-L-glutamate) (Figure 17C), including one that contained a lipophilic group. Cheng's lab also reported a different polypeptide substrate, poly(γ-(4-propargyloxybenzyl)-L-glutamate), which was also found to undergo efficient PPM reactions with a variety of azido derivatives of amines and guanidines (Figure 17D).[58]

While the PPM reactions described above gave high degrees of functionalization, a potential limitation of this strategy may be the hydrolytic instability of the glutamate ester linkages. Ester hydrolysis in aqueous environments can occur, and the ester linkages may limit the possibility of designing more complex systems as they are incompatible with many common deprotection techniques used in synthetic polypeptide chemistry. In 2010, using a permanent side-chain linkage, Heise and co-workers reported the PPM modification of poly(D/L-propargylglycine) using azido functionalized galactose (Figure 17E),[135] which proceeded in high yield using copper catalysis in DMSO. The main limitations of this method are the high cost of this unnatural amino acid, and the poor solubility of the polypeptide at longer chain lengths. The galactosylated homoglycopolypeptide was reported to be water-soluble, but adopted a β-sheet conformation and formed nonuniform aggregates in water.

The obvious alternative to azide−alkyne PPM reactions on alkyne functional polypeptides is the reaction of alkynes with azido functional polypeptides. Zhang reported the synthesis of poly(γ-3-chloropropyl-L-glutamate), which was used as a precursor to poly(γ-3-azidopropyl-L-glutamate) via PPM reaction with NaN_3 (Figure 18A).[74,136] The conversion of chloro to azido groups was near quantitative, and the product azides were then coupled to alkyne functionalized D-mannose using copper catalysis in DMF. This PPM reaction proceeded with high conversions yielding water-soluble, α-helical mannosylated polyglutamate derivatives. Cheng's lab later reported a similar procedure, where the alkyl spacer between the chloro/azido groups and the glutamate esters was varied, and the resulting polypeptides underwent PPM reactions with alkynes containing hydrophobic and guanidinium groups (Figure 18A).[137]

In 2013, Deming's lab reported synthesis of the hydrolytically stable azide containing polypeptides poly(L-azidonorvaline) and poly(L-azidonorleucine), which could be prepared as homo or diblock copolypeptides with controlled segment lengths.[72] These polymers were subsequently modified by copper catalyzed reaction with alkynes bearing a monosaccharide, carboxylate, or amino acid derivative (Figure 18B). The azide groups were found to be quantitatively converted to the corresponding triazole derivatives. Sen Gupta's lab also reported a azidosaccharide functionalized poly(L-lysine) that underwent a PPM reaction with propargyl amine to give a cationic glycopolypeptide (Figure 18C).[48]

3.5. Thiol−Ene and Thiol−Yne Reactions

Similar to the azide−alkyne PPM reactions described above, thiol−ene, and, more recently, thiol−yne PPM reactions have received much attention for the synthesis of functional polypeptides. In 2009, Heise's lab was the first to report such PPM reactions, where he modified copolypeptides containing L-cysteine residues and reacted these with acrylate derivatives containing oligoethylene glycol or a fluorescent probe (Figure 19A).[138] The thiol−ene reactions were catalyzed by AIBN at 70 °C, and while functionalization was obtained, the fraction of functionalized cysteine residues was not determined. Although this method is attractive since it uses a natural amino acid for the PPM reaction, it is limited by the generally poor solubility of polycysteines.

Schlaad's lab followed this work with a number of studies of thiol−ene PPM reactions on poly(allylglycine)s.[59] This polymer also exhibits poor solubility at longer chain lengths, and so low molecular weight oligomers (M_n < 2000 Da), or copolymers with helicogenic amino acids were employed. Radical addition of a variety of thiols, catalyzed by AIBN at elevated temperature using 2 equiv of thiol per alkene, gave polymers with degrees of alkene functionalization that varied greatly with reaction conditions (Figure 19B). Using this methodology, thiol derivatives containing either ester or monosaccharide functionality were added to poly(D/L-allylglycine) segments. In later studies, Schlaad and co-workers achieved higher molecular weight glycosylated copolypeptides (degrees of polymerization from 53 to 60) with defined secondary structures via use of copolypeptides composed of γ-benzyl-L-glutamate and either L-allylglycine or D/L-allylglycine, and reaction with commercially available 1-thio-β-D-glucopyranose.[60,139] Mole fractions of allyl units in the copolypeptides ranged from 9 to 17 mol %, and their conversion to sugar bearing thioethers proceeded with high efficiency. The addition of glucose to these copolypeptides was found to enhance α-helical stability and solubility at low pH compared to the parent copolypeptide before glycosylation.

Following these studies, other laboratories developed complementary polypeptide precursors that could either avoid use of expensive allylglycine, or possess better solubility to allow for more efficient PPM reactions. In 2011, Zhang's lab reported thiol−ene PPM reactions on copolypeptides containing γ-allyl-L-glutamate residues.[136] The allyl groups were reacted with 10 equiv of 3-mercaptopropionic acid using UV light as initiator (Figure 19C), which gave high yields of modified polypeptide. Cheng's lab prepared poly(O-(4-pentenyl)-L-serine) and showed that this underwent UV light catalyzed thiol−ene PPM reaction with mercaptoethylamine hydrochloride in high yield to give cationic polypeptides (Figure 19D).[67] Recently, Zhong's lab also reported copolypeptides containing vinyl sulfone functionalized L-cysteine residues, which underwent PPM Michael reactions with a variety of thiol containing molecules (Figure 19E).[66] Due to the reactivity of the vinyl sulfone group, no catalyst was required. However, a potential drawback of this strategy is the poor solubility of functional polycysteines, necessitating the use of copolymers. Cheng's lab also reported a different polypeptide substrate, poly(γ-(4-allyloxybenzyl)-L-glutamate), which was also found to undergo efficient PPM reactions with thiol derivatives containing amine and carboxylate functionalities (Figure 19F).[70]

In addition to thiol−ene reactions, PPM thiol−yne reactions on polypeptide have also been explored. Schlaad and co-workers prepared copolypeptides composed of γ-benzyl-L-glutamate and D/L-propargylglycine, where the alkyne group theoretically allows addition of two thiol units via thiol−yne chemistry (Figure 20A).[139] Reaction of the copolymer with commercially available 1-thio-β-D-glucopyranose gave only a

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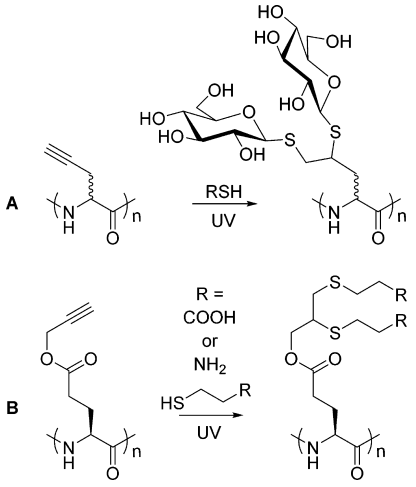

Figure 20. (A, B) Post-polymerization thiol−yne reactions.

maximum of 1.4 glucose additions per propargyl group, even with addition of excess thioglucose. It was observed that the first thiol−yne addition occurred with greater than 90 mol % conversion, but addition of the second thioglucose was inhibited, likely due to steric constraints. Zhu's lab reported the use of poly(γ-propargyl-L-glutamate) segments in thiol−yne PPM reactions,[140] followed more recently by similar work from Wooley's lab.[141] The poly(γ-propargyl-L-glutamate) segments were reacted with either mercaptoethylamine hydrochloride or 3-mercaptopropionic acid using a UV active radical initiator, and 2 equiv of each reagents was able to add to each alkyne in high yield (Figure 20B). In this system, it was likely the combination of use of smaller thiols and γ-propargyl-L-glutamate, with its longer side-chain tether, that facilitated the improved yield of double addition products.

3.6. Oxidation Reactions

As a means to more economical, more flexible, and simpler routes to functional polypeptides, there have been many recent investigations into the post-polymerization oxidation chemistry of thioether functional group in polypeptides. Oxidation can typically occur under mild conditions (i.e., H_2O_2 in aqueous media), can lead to polypeptides with improved water solubility, and can enable a conformational switch. Furthermore, thioether groups in proteins, e.g., methionine residues, are often oxidized in biology, and so are natural modifications.

The PPM oxidation of poly(L-methionine) was first reported in 1978 by Nakagawa and co-workers.[142] They immersed films of α-helical poly(L-methionine) in aqueous H_2O_2 and observed increased permeability and eventual dissolution of the films due to oxidation of methionine thioether groups to more polar sulfoxide and sulfone groups (Figure 21A). Aiba later reported studies on poly(L-methionine sulfoxide), which was found to be water-soluble and adopt a disordered conformation in water.[143] Poly(L-methionine sulfoxide) was also found to be nontoxic when given intravenously up to 2 g/kg in rats.[144] Recently, Deming's lab prepared the fully oxidized derivative, poly(L-methionine sulfone) (Figure 21A), which, unlike disordered poly(L-methionine sulfoxide), was found to be highly α-helical in water and formed aggregates leading to poor solubility.[145] Deming's lab also recently reported the controlled synthesis of poly(L-methionine), leading to well-defined chain lengths and incorporation of these residues into block copolymers.[44,92] PPM oxidation of the block copolymers led to conversion of hydrophobic methionine to hydrophilic methionine sulfoxide segments in a new, practical approach to prepare amphiphilic block copolypeptides.

Thioether groups in polypeptides derived from cysteine or homocysteine residues can also be subjected to PPM oxidation reactions. In 2012, Deming's lab reported that the thioether groups in glycosylated cysteine or homocysteine polypeptides could be oxidized to either sulfoxide or sulfone groups (Figure 21B).[49] The oxidations proceeded in high yield, and while the sulfoxide derivative of the cysteine based polypeptide retained the α-helical conformation of the parent polymer, the sulfone derivative of the cysteine based polypeptide was found to adopt a disordered conformation in water. Remarkably, the sulfone derivative of the homocysteine based polypeptide was found to remain α-helical in water. The oxidation of the cysteine based glycopolypeptide and resultant change from α-helical to disordered in water was the first example of such a

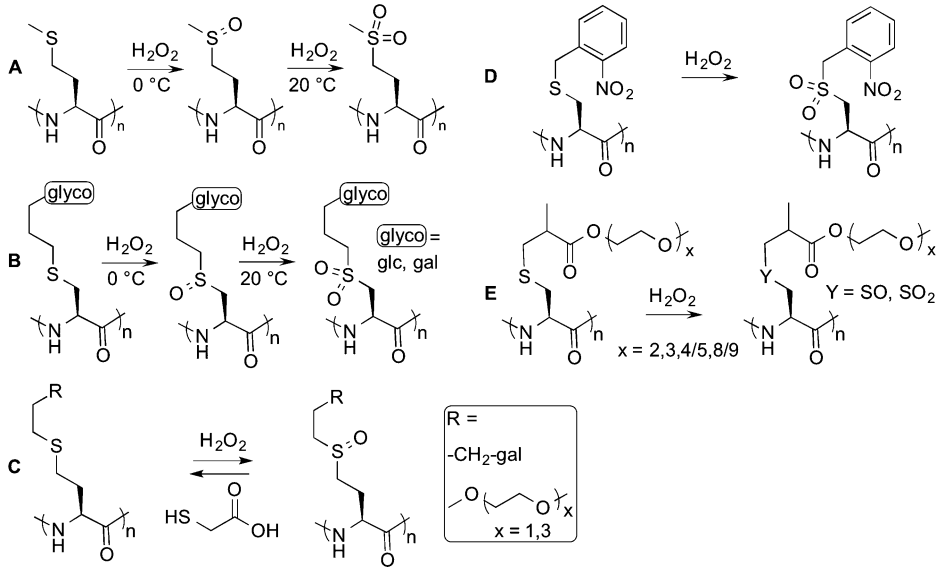

Figure 21. (A−E) Post-polymerization oxidation reactions.

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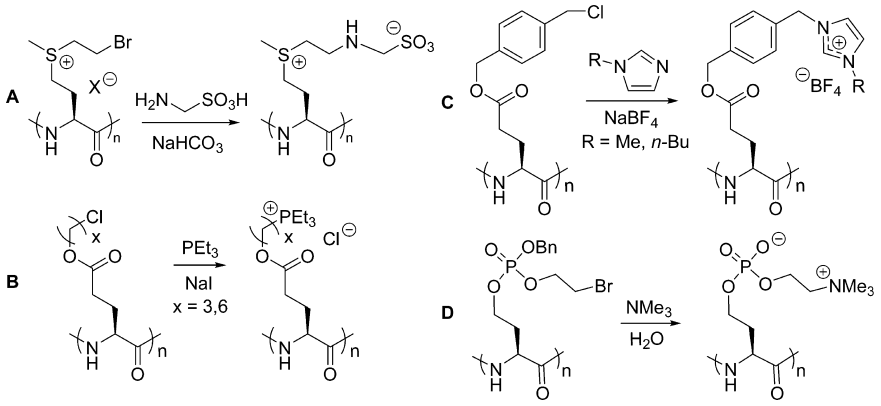

Figure 22. (A–D) Post-polymerization reactions of alkyl halides with N or P atoms.

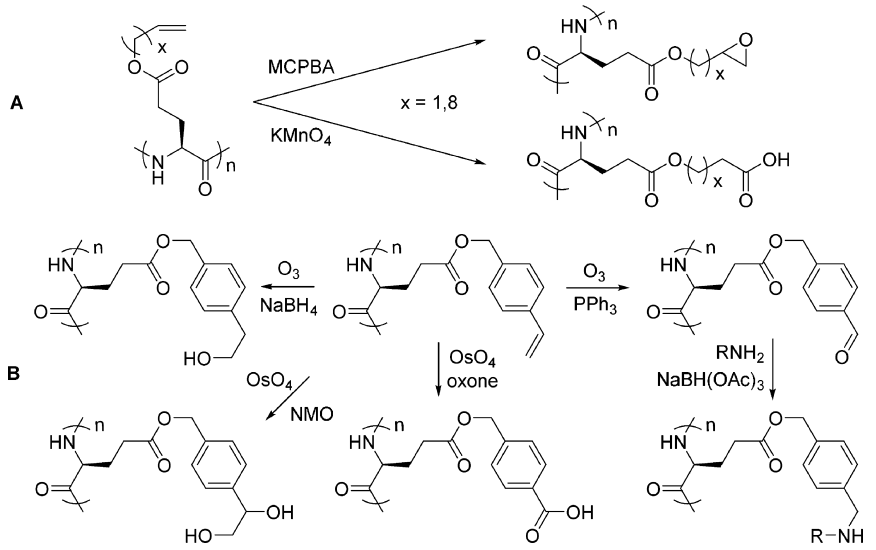

Figure 23. (A, B) Other post-polymerization reactions with alkenes.

conformational switch where polymer in both states maintains good water solubilty.[49]

In a later study, Deming showed that glycosylated or oligoethylene glycol modified poly(L-homocysteines) could undergo reversible, conformational changes in a fully water-soluble system (Figure 21C).[39] This was achieved with the homocysteine backbone, where the sulfoxide derivatives were disordered in water, and the sulfone derivatives were α-helical in water, similar to the conformational changes observed upon PPM oxidation of poly(L-methionine), which shares the same side-chain core structure. An interesting observation is that the conformational changes observed with stepwise oxidation of homocysteine derivatives are opposite to those observed for cysteine derivatives, showing that the specific placement of the sulfur atoms in the side-chain of these polypeptides plays a critical role in determining chain conformation. In a practical sense, since sulfoxide formation is reversible, while sulfone formation is not, the homocysteine polymers can undergo reversible conformational changes with PPM oxidation reactions.

Oxidations of thioether groups in other alkylated cysteine polypeptide have also been reported. Dong reported PPM oxidation of *S*-(*O*-nitrobenzyl)-L-cysteine segments in block copolymer assemblies with H_2O_2 to give the sulfone derivatives (Figure 21D).[146] As both the parent and oxidized polypeptide segments had low water solubility, only small changes in the assemblies were observed. In 2014, Li also reported the PPM oxidation of oligoethylene glycol SCM poly(L-cysteine) with H_2O_2 to give the corresponding sulfoxide and sulfone derivatives (Figure 21E).[147] While both the parent polymers and the sulfoxide derivatives adopted β-sheet conformations, the sulfone polymers were found to switch to disordered conformations with improved water solubility.

3.7. Other Reactions

There are a number of PPM reactions on polypeptides that do not fit into any of the categories described above. Some of these are important reactions, but have only been explored within the past year or two, such as reactions of alkyl halide containing polypeptides with phosphine or amine nucleophiles. In 2012, Deming's lab reported the PPM reaction of alkyl bromide groups that were installed into a poly(L-methionine sulfonium) with a primary amine nucleophile to generate a zwitterionic polypeptide product (Figure 22A).[92] Later, Cheng used alkyl chloride modified polyglutamates, used previously as precursors of azido functionalized polypeptides (see Figure 18A), in PPM reactions with triethylphosphine under iodide catalysis to generate fully phosphonium modified polypeptides (Figure 22B).[75] Tang's lab reported a similar PPM reaction where 1-alkylimidazoles were reacted with a different alkyl chloride modified polyglutamate to generate 1-alkylimidazolium func-

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tionalized polypeptides, which were found to possess temperature dependent solubility in water (Figure 22C).[76] Deming's lab also used alkyl bromide functionalized phosphoryl serine and homoserine polymers in a PPM reaction with trimethyl amine to generate the first example of phosphorylcholine modified polypeptides (Figure 22D).[55] These polypeptides possess side-chain groups that mimic the functionalities of phosphorylcholine containing lipids. Due to its zwitterionic nature, this polypeptide was found to possess a disordered conformation in water at pH ranging from 3.0 to 11.

Alkene functional groups can undergo a wide variety of chemical reactions, and some of these have been explored on alkene functionalized polypeptides. Daly's lab prepared alkene functionalized poly(L-glutamates) and also explored their PPM reactivity.[68] Reactions of these polymers with excess MCPBA in $CHCl_3$ resulted in full conversion of the alkenes to epoxide groups, and oxidation of the polymers with $KMnO_4$ gave 60–70 mol % conversion of alkenes to carboxylic acid groups (Figure 23A). More recently, Cheng's lab reported multiple PPM reactions on the alkene groups of poly(γ-(4-vinylphenyl)-methyl-L-glutamate), where most studies focused on reductive amination reactions using primary amines (Figure 23B).[71] This amination reaction has been used to prepare a diverse array of functional polypeptides, which typically adopt stable α-helical conformations.

Some final PPM reactions on polypeptides were used to prepare mimics of natural biopolymers. In 1967, Katchalski reported the PPM reaction of benzylphosphoric acid with poly(D/L-serine) using DCC as a coupling agent (Figure 24A).[148] Following removal of the benzyl protecting groups,

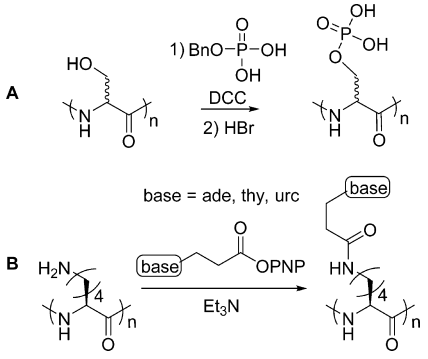

Figure 24. (A, B) Other post-polymerization reactions.

poly(D/L-phosphoserine) was obtained with approximately 50 mol % of the serine hydroxyl groups phosphorylated. The polymer was quickly decomposed, with loss of phosphate groups, in water at basic pH. For preparation of polypeptide mimics of oligonucleotides, Takemoto reported the PPM reaction of poly(L-lysine) with *p*-nitrophenol esters of adenine, thymine, and uracil derivatives in DMSO with triethylamine (Figure 24B).[149] The thymine and uracil groups were conjugated to the poly(L-lysine) at 97 mol % functionalization, but the adenine could only be attached at levels up to 74 mol %, likely due to polymer insolubility at high levels of conjugation. The other, fully functionalized samples were found to be soluble in DMF, DMSO, and 6 N HCl.

4. CONCLUDING REMARKS

As shown in this review, there is tremendous diversity in the type and means of presentation of different functional groups that can be introduced into the side-chains of polypeptides. In addition, there are two general methods for side-chain modification: the polymerization of SCM NCA monomers, or the PPM of reactive precursor polypeptides. As detailed above, there are many cases where both methods can be used to obtain a desired functionality. Thus, options are present to select a method that will be best compatible with the overall synthetic scheme and desired application. Also notable in this review is the substantial increase in research activity for the preparation of side-chain functional polypeptides in recent years. This may be partly due to the availability of newer conjugation chemistries that provide improved compatibility toward different functional groups, but is also the result of the growing interest in the development and use of polypeptides as structure and function rich materials for a range of applications.

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Notes

The authors declare no competing financial interest.

Biography

Timothy J. Deming grew up in Lake Forest, California. Tim received his degrees in Chemistry: B.S. from the University of California, Irvine, 1989, and Ph.D. from the University of California, Berkeley, 1993. After an NIH postdoctoral fellowship at the University of Massachusetts, Amherst, he joined the faculty in the Materials Department at the University of California, Santa Barbara, in 1995. Here, Tim held a joint appointment in the Materials and Chemistry Departments where he was promoted to Associate Professor in 1999 and Full Professor in 2003. In 2004 Tim moved to UCLA where he is now as Professor in the Bioengineering Department and Professor of Chemistry and Biochemistry. Tim's research interests are in polymer and materials synthesis, block copolymer self-assembly, and biomedical applications of polypeptide materials. Research in his lab is focused around new, practical chemical routes for the synthesis of biological and biomimetic polypeptide based materials.

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ABBREVIATIONS

Ac	acetyl
ade	adenine

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AIBN	azobisisobutrylnitrile
ATRP	atom transfer radical polymerization
base	nucleobase group
Bn	benzyl
Boc	*t*-butoxycarbonyl
BOP-Cl	phosphoric acid bis(2-oxooxazolidide) chloride
Cbz	benzyloxycarbonyl
clb	cellobiose
DCA	dichloroacetic acid
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMT-MM	4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
EDC	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide
gal	D-galactose
glc	D-glucose
glcA	D-azidoglucose
glcN	*N*-acyl-D-glucose
glyco	saccharide group
HFIP	hexafluoroisopropanol
HOBt	hydroxybenzotriazole
lac	lactose
man	D-mannose
MCPBA	*m*-chloroperbenzoic acid
NCA	α-amino acid-*N*-carboxyanhydride
NHS	*N*-hydroxysuccinimidyl
NMO	*N*-methyl morpholine *N*-oxide
oxone	potassium peroxymonosulfate
PEG	poly(ethylene glycol)
PMe_3	trimethylphosphine
PNP	*p*-nitrophenyl
PPM	post-polymerization modification
PTSA	*p*-toluenesulfonic acid
rha	D-rhamnose
SCM	side-chain modified
TFA	trifluoroacetic acid
thy	thymine
tpn	theophylline
THF	tetrahydrofuran
urc	uracil
UV	ultraviolet light

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