Synthesis of Well-Defined Polypeptide-Based Materials via the Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides

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Contents

1. Introduction

Since 1906, when Leuchs synthesized the first α-amino acid *N*-carboxyanhydrides (NCAs),[1] later referred to as Leuchs' anhydrides, a great number of publications dealing with the ring-opening polymerization (ROP) of these monomers (Scheme 1) has accumulated. This interest stems from the wide variety of polypeptides that this polymerization can generate.

The synthetic polypeptides produced from the NCAs, although far from being monodisperse or constructed from a precise sequence and composition of α-amino acid residues, possess the ability, as their natural relative-proteins, to form α-helix and β-sheet motifs. These secondary structures contribute significantly to the self-assembling character of polypeptide chains, leading to novel supramolecular structures with potential biomedical and pharmaceutical applications.[2]

As for their natural counterparts, it is important for such synthetic polypeptides to be well-defined with high molecular and structural homogeneity in order to favor their self-assembly into precisely defined nanostructures, a requirement for appropriate functionality.

It was not until 1997, when Deming[3] reported the first living initiating system for the ROP of NCAs, that the synthesis of well-defined polypeptides was achieved. Following this first report, other alternative living initiating systems or methods have also been developed. These living systems lead to well-defined homo-/copolypeptides and hybrids, with high molecular weight and structural homogeneity. Nevertheless, the earlier studies served as the springboard for developments in the whole area of polypeptide synthesis.

Several excellent reviews[4] have been dedicated to the ROP of NCAs, elucidating the mechanistic aspects of this polymerization. However, only a few have addressed the synthesis of polypeptide-based materials with different macromolecular architectures.[4c,5,6]

This review is divided into three parts. The first highlights the mechanistic developments of the ROP of NCAs from the conventional to the living initiating systems/methods; the second is dedicated to the synthesis of polypeptides and polypeptide hybrids with different macromolecular architectures; and the third deals with surface-bound polypeptides. Surface-bound polypeptides were incorporated in the review due to the great interest in biologically active surfaces for medical diagnostics and sensors.[7]

2. Ring-Opening Polymerization (ROP) of α-Amino Acid N-Carboxyanhydrides (NCAs)

Until the early 1980s many publications were dedicated to the mechanistic study of the ROP of NCAs. The huge amount of data is often confusing and sometimes contradic-

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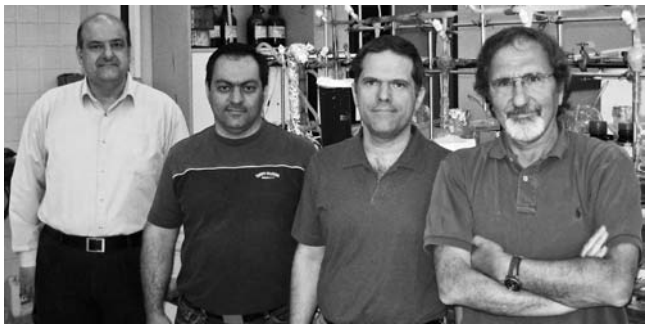


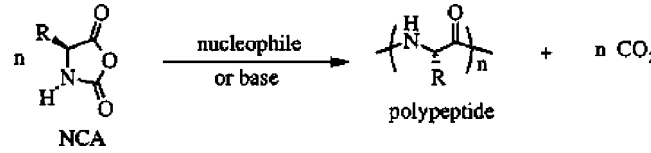
Nikos Hadjichristidis (far right) received his B.Sc. from the University of Athens, Greece (1966); his Ph.D. from the University of Liège, Belgium (1971, advisor, Professor V. Desreux); and his D.Sc. from the University of Athens, Greece (1978), and he did Postdoctoral research at the University of Liège with Professor V. Desreux (1971−1972) and also at the National Research Council of Canada with Dr. J. Roovers (1972−1973). He then became Lecturer (1973), Assistant Professor (1982), Associate Professor (1985), Full Professor (1988), Director of Industrial Chemistry Laboratory (since 1994), and Chairman of the Chemistry Department (1991−1995, 1999−2003, and since 2007) of the University of Athens; Visiting Scientist at the University of Liège (Summers 1974, 1975); Visiting Research officer at NRC of Canada (Summer 1976); Visiting Scientist, University of Akron, at Dr. L. Fetters' Laboratory (Summers 1977 through 1982); Distinguished Visiting Scientist, NRC of Canada (1983); and Visiting Professor, Exxon Research and Engineering Co, NJ, since 1984, every year for 1 or 2 months. He is also President of the European Polymer Federation (1995−1996); a Member of the National Advisory Research Council (1994−2007); President of the State Highest Chemical Board (1995−2005); and Director of the Institute of "Organic and Pharmaceutical Chemistry" of the National Hellenic Research Foundation (2000−2001). He has received the following awards: Academy of Athens Award for Chemistry (1989); Empirikion Award for Sciences (1994) and Greek Chemists Association Award (2000); ACS PMSE A. K. Doolittle Award (2003); and the International Award of the Society of Polymer Science, Japan (2007). He was elected as a PMSE Fellow for 2004 and was the "Ralph Milkovich" Memorial Lecturer for 2006 at the University of Akron. He has dedicated his career primarily to the synthesis of model polymers and has published more than 350 papers in refereed scientific journals.

Hermis Iatrou (second from right) received his B.Sc. (1989) and Ph.D. (1993, advisor, Professor Nikos Hadjichristidis) from the University of Athens, Greece. He did postdoctoral research at the Institute of Material Science in the Center of Nuclear Science in Juelich, Germany, with Professor Dieter Richter (1994−1995), and at the University of Alabama at Birmingham, with Professor Jimmy Mays (August 1997−February 1998). He was a Research Associate at the University of Athens (1998−2002) and Assistant Professor (2002) and was promoted to Associate Professor (2009). He has been a Visiting Scientist at the National Recearch Council of Canada (September 1992), Forschungszentrum Juelich (2001, 2003, 2006), and at the University of Tennessee at Knoxville (August 2006). His research interests are the synthesis and characterization of complex macromolecular architectures by anionic methods; polymerization, synthesis, and characterization of well defined polypeptides with ring opening polymerization of N-carboxy anhydrides; the self-assembly behavior of biomaterials; and their medical applications. He has published 88 papers in refereed scientific journals.

Marinos Pitsikalis (far left) received his B.Sc. (1989) and Ph.D. (1994, advisor, Professor Nikos Hadjichristidis) from the University of Athens, Greece. His postdoctoral research was done at the University of Alabama at Birmingham, with Professor J. W. Mays (1995−1996). He joined the Department of Chemistry of the University of Athens as Lecturer (1998), followed by promotions to Assistant Professor (2002) and Associate Professor (2009). He has been a Visiting Scientist at the University of Milan (March 1991), University of Alabama at Birmingham (September−October 1993), Max Plank Institute for Polymer Science (August 1994), National Institute for Standards and Technology (December 1995), IBM Almaden Research Center (February 1995), University of Wisconsin (September 2002), and University of Tennessee at Knoxville (August 2003, August 2005). His research interests include the synthesis and characterization of complex macromolecular architectures by anionic, coordination, controlled/living radical, and ring-opening polymerization techniques, the self-assembly behavior of copolymers in selective solvents, etc. He has published 88 papers in refereed scientific journals.

Georgios Sakellariou (second from left) received his B.Sc. (1999) and Ph.D. (2003, advisor, Professor Nikos Hadjichristidis) from the University of Athens. He undertook a postdoctoral fellowship with Professor Jimmy W. Mays (2004−2006), at the University of Tennessee at Knoxville, and then returned to the University of Athens as a Research Associate (2006−2009). In 2009, he was elected as Lecturer in the Department of Chemistry, University of Athens. He has been a Visiting Scientist at the University of Alabama at Birmingham (February−April 2001 and January−March 2002) and University of Tennessee at Knoxville (November−December 2007). His research interests include the synthesis and characterization of complex macromolecular architectures by anionic, controlled/living radical, and ring-opening polymerization techniques and surface polymerization from carbon nanotubes and gold and silicon substrates. He has published 22 papers in refereed scientific journals.

Scheme 1. Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides (NCAs)



tory. Here below, we discuss the most accepted mechanisms and side reactions reported up to 1997, when Deming's seminal work rejuvenated reasearch on synthetic polypeptides by the ROP of NCAs. Efforts after 1997 will also be presented.

2.1. Earlier Studies before 1997. Mechanistic Considerations

Most authors agreed that the most likely pathways for the ROP of NCAs are the "normal amine" (NA) and the "activated monomer" (AM) mechanisms. The first is attributed to the polymerization with primary amines, stronger nucleophiles than basic initiators, and the second to the metal alkoxide or tertiary amines, stronger basic than nucleophile initiators. The coexistence of both mechanisms was proposed when secondary amines, weak nucleophile/basic initiators, were the active species. Other initiating systems, such as metal salts, have been developed but will not be discussed here due to their limited use.

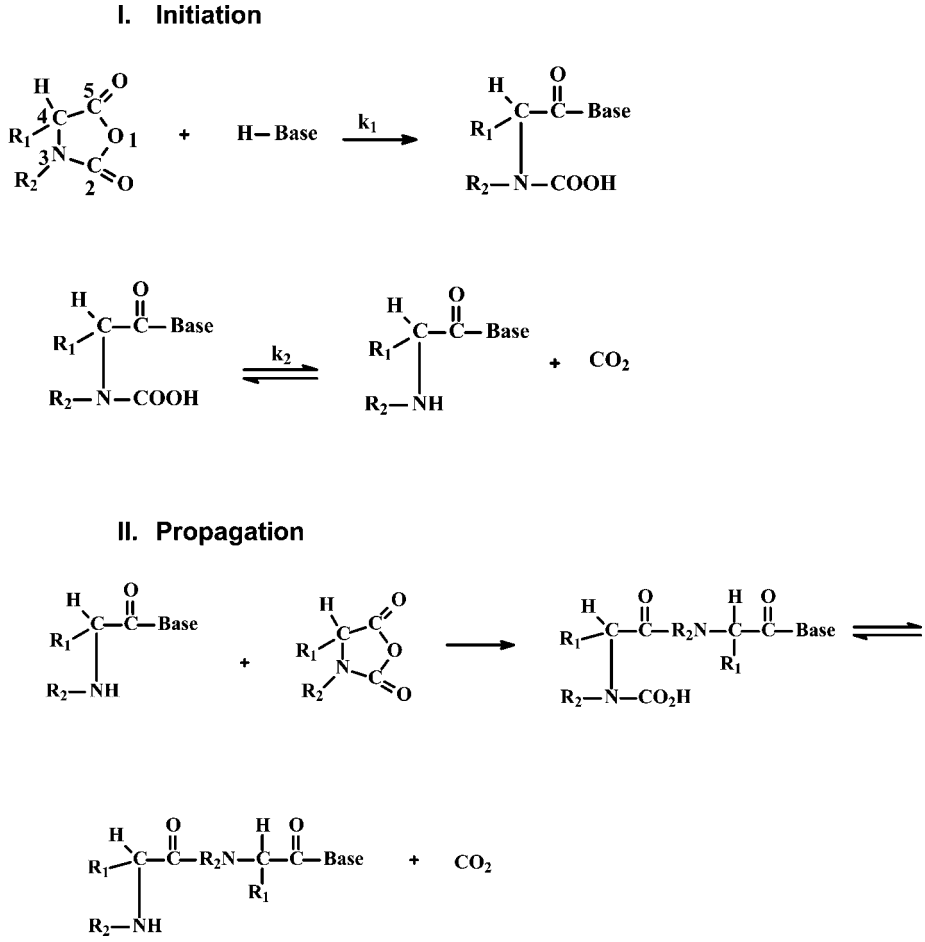
2.1.1. Normal Amine Mechanism (NAM)

This mechanism is generally applied for the polymerization of NCAs initiated by nonionic initiators having at least one mobile hydrogen atom (base-H), such as primary and secondary amines, alcohols, and water. The general reactions involved in the initiation and propagation steps are given in Scheme 2.

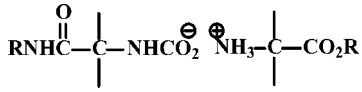
This mechanism applies to the N-unsubstituted NCAs ($R_2 = H$) as well as to N-substituted NCAs ($R_2 \neq H$).

The initiation is based on the nucleophilic attack of the base, which carries the labile hydrogen. The intermediate unstable carbamic acid decarboxylates to give a new free amino group, which promotes the polymerization. Primary amine initiators gave the best results concerning the agreement between the experimentally observed and the stoichiometric molecular weights. Since primary amines are more nucleophilic than the ω-amino groups of the propagating chains, the initiation rate is faster than propagation, leading to polypeptides with low polydispersity indices (PDI = M_w/M_n). A proof of this mechanism was demonstrated independently by Peggion et al.[8] and Goodman et al.,[9] by confirming the incorporation of the initiator fragment in the

Scheme 2. Initiation and Propagation Steps According to NAM



Scheme 3. Salt between the Carbamic Acid and the End Amino Groups of Two Polypeptide Chains



final polymer. The incorporation ratio varies with the structure of the initiator, from 100% for primary amines to only 10% for secondary amines, such as diisopropylamine, which mainly follows the AM pathway.[9,10] This is due to the higher basicity and steric hindrance of diisopropylamine compared to *n*-hexylamine.[10−12]

Apart from the desired reaction pathway obtained with primary amines, such as *n*-hexylamine, deviations plaguing the living character of the ROP of NCAs as well as the synthesis of high molecular weight polypeptides were identified by several groups and are described below. Factors causing deviations from the living nature of ROP include the following: carbamic acid−CO_2 equilibrium, solvent, and the presence of water and other impurities.

The intermediate carbamic acid plays a critical role in NAM. Ballard et al.[13,14] presented the peculiar kinetic features of the polymerization of sarcosine NCA (Sar-NCA) in nitrobenzene. They pointed out that the carbamic acid forms a salt with the amino groups of the propagating chains (Scheme 3), which catalyzes the propagation of the polymerization, and thus, higher than the normal first order kinetics were observed. The effect disappeared when the polymerization occurred in dimethylformamide (DMF).[15] This phenomenon was attributed to the higher acidity of DMF than that of nitrobenzene, which reduces the basicity of the amine active site, thereby preventing the salt formation.

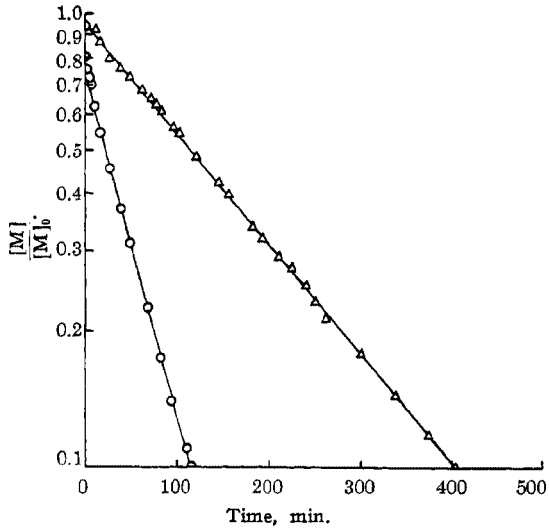
Another factor which prevents the reaction from following the desired first order kinetics is the pressure under which the polymerization is conducted. Under constant pressure (continuous removal of CO_2 from the solvent), carbamic acid is unstable[14] and first order kinetics are observed, whereas when the reaction takes place without CO_2 removal and carbamic acid salt formation is favored, different kinetics are observed.[15−17]

It should be noted that the stability of carbamic acid depends on the electronegativity of the substituents on the nitrogen atom. Thus, the higher the electronic density of nitrogen, the greater the stability of the carbamic acid. For example, the carbamic acid of sarcosine is more stable than that of glycine and consequently influences the ROP with primary amines to a greater extent.

Another key issue in the ROP is the purity of the NCAs, as pointed out by many authors and in particular by Ballard et al. When these authors conducted the ROP of highly purified γ-benzyl-L-glutamate NCA (BLG-NCA), first order kinetics was observed (Scheme 4) throughout the entire polymerization. In contrast, when less rigorously purified BLG-NCA was used, initially first order kinetics were observed, followed by a faster polymerization at later stages.[18]

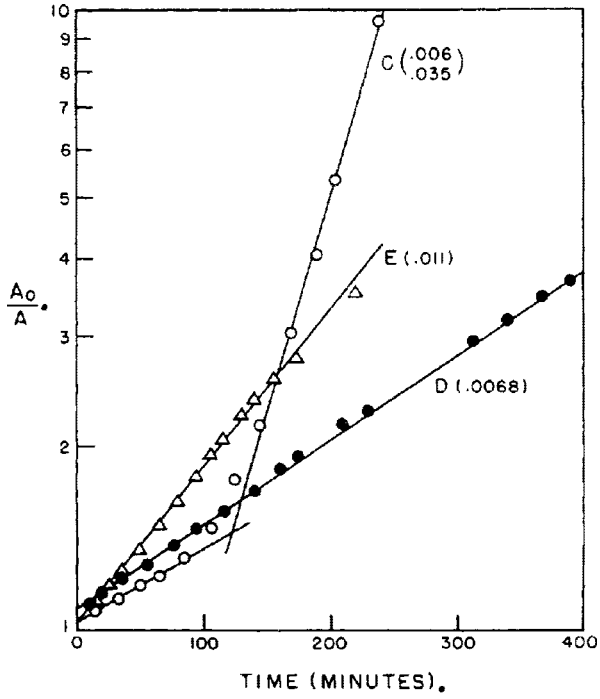
Doty et al.[19] questioned these results, showing that high purity PLG-NCA gives dual kinetics, whereas low purity (addition of 0.67 mol %, of *n*-hexylamine HCl salt) BLG-NCA could also give linear kinetics throughout the entire polymerization (Scheme 5). This particular salt was added

Scheme 4. Kinetics of Highly Purified γ-Benzyl−L-Glutamate NCA Initiated by *N*-Hexylamine in Dioxane at 25°C[a]



[a] Initial anhydride concentration: $[M]_0 = 0.151$ mol L^{-1}. *n*-Hexylamine concentrations: ○, 3×10^{-2} mol L^{-1}; Δ, 1×10^{-2} mol L^{-1}. Reprinted with permission from ref 18. Copyright 1957 American Chemical Society.

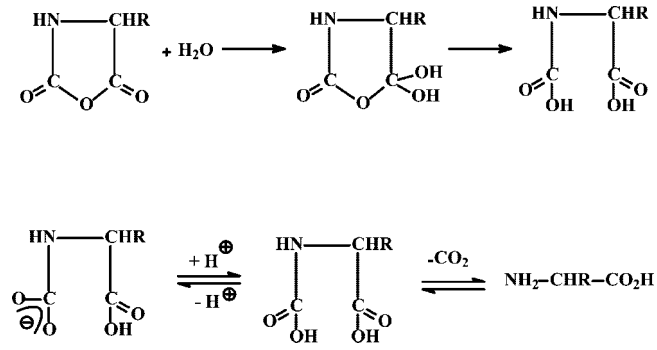
Scheme 5. Kinetics of the γ-Benzyl−L-Glutamate NCA Initiated by *n*-Hexylamine ([Anhydride]/[Initiator] = 20) in Dioxane at the Concentration 4 g/100 cc at 25°C[a]



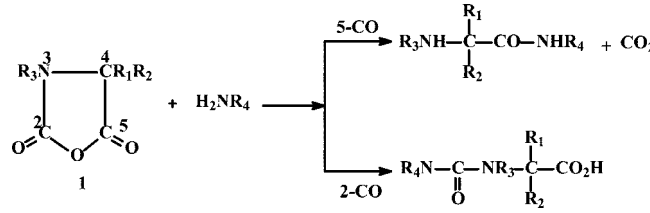
[a] C is a highly purified anhydride (HCl < 0.1 mol %); D and E correspond to the addition of *n*-hexylamine hydrochloride to the extent of 0.67 and 5.0 mol %, respectively. *A* is absorbance (a.u.). In parenthesis are indicated the slopes of the curves. Reprinted with permission from ref 19. Copyright 1957 American Chemical Society.

since HCl, a common impurity generated during the synthesis of NCAs, can react with the amino active sites to give the HCl salts. We can conclude from the results above that the purity of the NCAs, as well as of the reaction system plays a critical role on the kinetics of the polymerization initiated by primary amines. This is the reason there are contradictory and confusing results in the literature.

Scheme 6. Hydrolytic Mechanisms of NCAs



Scheme 7. Reaction of the Primary Amine Initiator with 5-C or 2-C



Water is a common impurity, which can influence the ROP of NCAs. The reaction of NCAs with water was carefully investigated by several authors.[1,20−26] They reported that the reaction of NCAs resulted in the formation of polypeptides when the NCA/H_2O ratio was higher than 10, whereas complete hydrolysis occurred when this ratio was lower than 10^{-3}. Intermediate ratios favor the formation of oligopeptides. The temperature of the system is also critical for the hydrolysis of NCAs, since at low temperatures it proceeds slower. Additional parameters that influence the initiation of the polymerization of NCAs with water include the pH and the bulkiness of the N-substituent of the NCA. According to Bartlett et al.,[25,26] the hydrolytic mechanism is the one shown in (Scheme 6).

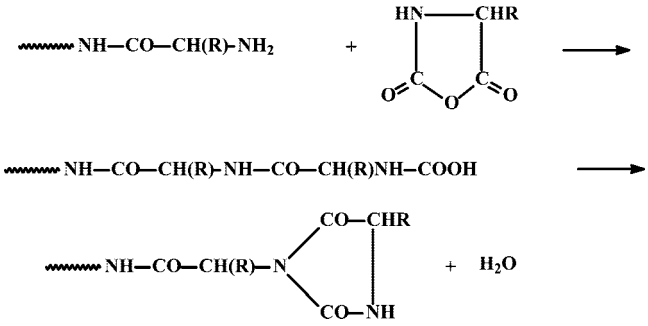
On exposure to moisture, even solid crystalline anhydrides undergo an autoaccelerated polymerization with evolution of carbon dioxide. This solid-state reaction proceeds without any observable change in the crystals, as confirmed by X-ray spectroscopy.[27]

Secondary reactions can also occur from the attack of the amino group to the 2-CO instead of the desired 5-CO, leading to the formation of the ureido acid chain end, mainly during the initiation step (Scheme 7). The greater the nucleophilicity of the amino initiator used, the lower is the percentage of the attack at the 2-CO group. For example, in the *n*-hexylamine-initiated polymerization of ^{14}C-labeled BLG-NCA, Goodman et al.[9,17] found this 2-CO deviation to be very limited (<0.15% of the initial active sites). Similar results were obtained by Katchalski et al.[28]

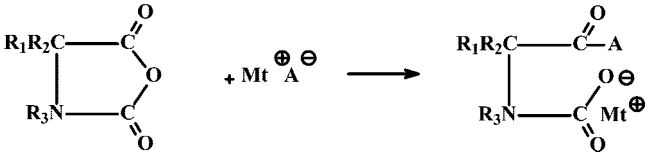
Another undesired termination reaction was reported in the water-initiated polymerization of glycine and alanine NCAs at elevated temperatures, leading to the formation of terminal hydantoin rings (Scheme 8).[29]

At this point we would like to include the "Blout mechanism",[30] an ionic version of the NAM, proposed in the case of BLG-NCA polymerization in dioxane with sodium methoxide as initiator. The initiation step involves the nucleophilic attack of the anionic moiety of the basic salt, followed by the ring-opening. No decarboxylation takes place during this step (Scheme 9).

Scheme 8. Termination Reaction of Primary Amine Initiating Polymerization at High Temperatures, Leading to the Formation of Hydantoin Rings



Scheme 9. Initiation of Polymerization of NCAs According to "Blout's Mechanism"



In the propagation step, the resulting carbamate anion behaves like the methoxide anion, followed by the release of carbon dioxide (Scheme 10).

This mechanism does not require a proton from the initiator and can be applied to both N-unsubstituted and N-substituted NCAs. As in the case of NAM, it implies the incorporation of the initiator moiety in the resulting polymer chain. Although there is evidence[9,17] that "Blout's mechanism" works under certain conditions, it is generally accepted that the polymerization of NCAs with strong bases, such as sodium methoxide, proceeds via the activated monomer mechanism.

2.1.2. Activated Monomer Mechanism (AMM)

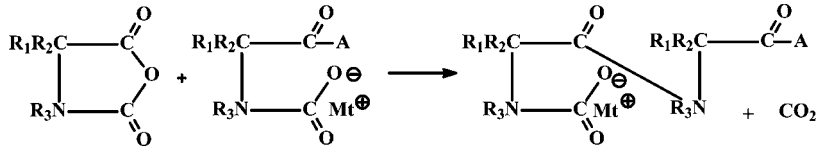
This mechanism was first proposed by Ballard et al.[31] to explain the ROP of DL-phenylalanine NCA, initiated by a tertiary amine.[32,33] Later, Szwarc[34] proposed that the AMM was also valid for the basic salt-initiated polymerization of N-unsubstituted NCAs. In the cases of tertiary and secondary amine, as well as the alkali halide-initiated polymerizations, it is believed that AMM and NAM coexist. Following are given the different steps of AMM.

2.1.2.1. Preinitiation Reaction. Here, the initiator does not act as a nucleophile, as in the NAM, but rather as a base, subtracting the proton of the 3-N of NCA, resulting in the corresponding anion. Therefore, in this mechanism the "initiator" acts as a catalyst and not as a chain initiating compound. It is obvious that this mechanism can only be applied to N-unsubstituted NCAs (Scheme 11), bearing a proton at the 3-N position of the NCA.

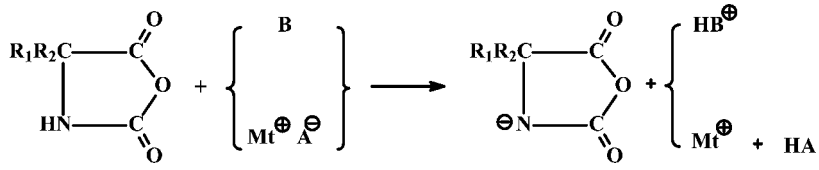
2.1.2.2. Initiation. The actual initiator is the resulting NCA anion, which attacks the 5-CO to give a tadpole dimer, followed by reaction with another NCA, creating a new anion with the simultaneous release of carbon dioxide (Scheme 12).

2.1.2.3. Propagation. The tadpole dimer is attacked by a new anion, to give a tadpole trimer, and so on, followed by the creation of NCA anion at each reaction step (Scheme 13).

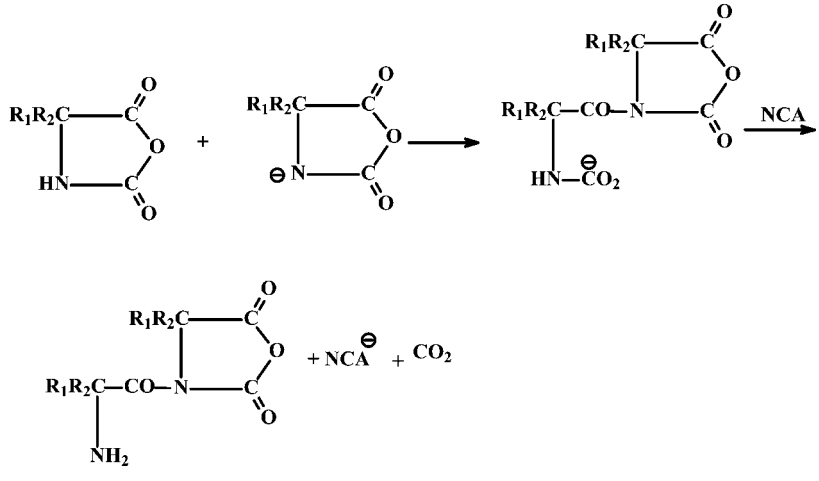
Scheme 10. Propagation of Polymerization of NCAs According to "Blout's Mechanism"



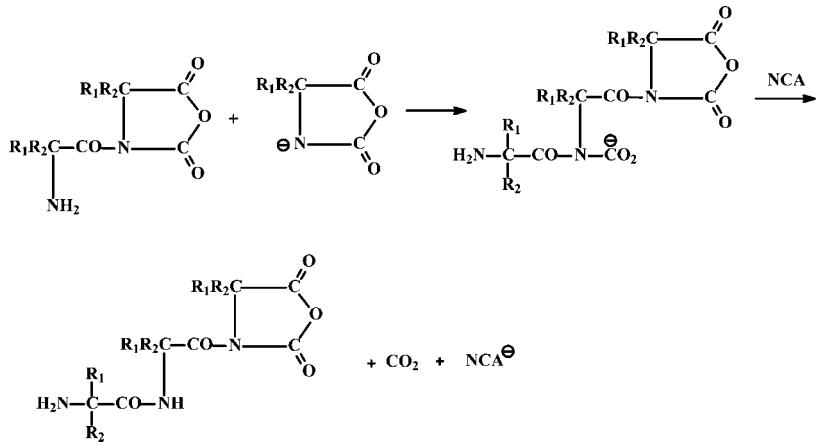
Scheme 11. Preinitiation Reaction of the Activated Monomer Mechanism



Scheme 12. Initiation Reaction of the Activated Monomer Mechanism



Scheme 13. Propagation Reaction of the Activated Monomer Mechanism



Bamford's group found that Sar-NCA was almost unreactive when diisopropylamine was used as initiator, while the polymerization of γ-ethyl-L-glutamate NCA is even faster under these conditions,[33,35] compared to the *n*-hexylamine-initiated polymerization. Steric hindrance inhibits the NAM initiation with diisopropylamine, and at the same time the system cannot follow the AMM, since the Sar-NCA lacks the necessary proton. However, the high basicity of diisopropylamine may result in the proton subtraction from the 3-N of γ-ethyl-L-glutamate NCA (AMM).

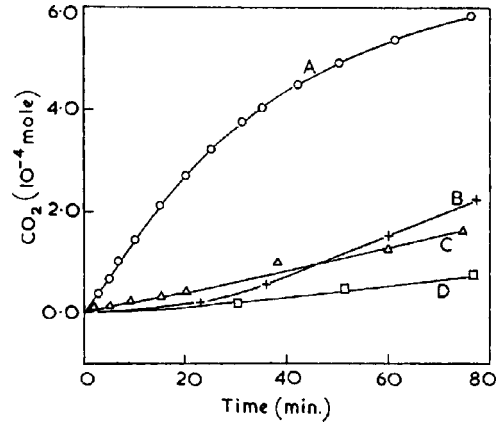
An even more convincing argument for the AM mechanism was provided by Bamford et al.[36] Tri-*n*-butylamine in dimethylformamide induced only slow polymerization of L-proline NCA (absence of 3-N hydrogen), whereas the polymerization of γ-ethyl-L-glutamate NCA was very fast under similar conditions, even when the concentration of the base was reduced by a factor of 10. This provides a very strong indication of the occurrence of AMM, since proline NCA is an extremely reactive monomer and polymerizes rapidly with primary amines.

Many authors polymerized N-substituted NCAs, such as sarcosine, proline, and (*S*)-thiazolidine-4-carboxylic acid NCAs,[9,14,36−39] using tertiary amines or pyridine derivatives. Surprisingly, these polymerizations proceeded, in spite of the absence of a hydrogen either in the initiator (NAM) or on the 3-N (AMM). Bamford's group[14,36,40] attributed these polymerizations to protic impurities or to the relatively acidic proton 4-C of the NCA monomer. The protons originating from such impurities or from 4-C react with the basic compounds to form NAM initiators. In order to prove this hypothesis, Bamford and co-workers[36] added a small amount of 3-methyl hydantoin to the until then unreactive Sar-NCA/basic compound solution. The same is valid for L-proline NCA. Rapid polymerization was immediately observed (Scheme 14). This rapid polymerization was attributed to the ionization of 3-methylhydantoin and to its addition to the 5-CO of the NCA monomer, resulting in a heterodimer, bearing a secondary amino end group, followed by NAM propagation (Scheme 15).

The monomer and solvent purity is very critical for the AMM polymerization. An example is given by Ballard et al.,[36] who reported that water accelerated the polymerization of proline NCA (tributyl amine in DMF), while, in a dry system, the kinetics were very slow, as expected from the AMM.

Even the presence of traces of salts can affect the normal pathway of AMM polymerization. Bamford et al.[32] found

Scheme 14. Conversion−Time Curves for Reaction of Sarcosine *N*-Carboxyanhydride with Tributylamine in DMF at 25 °C[a]



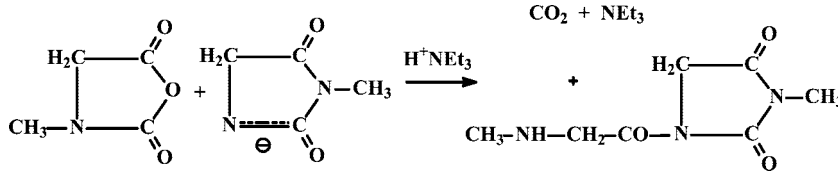
[a] Concentrations in mol/L. Sarcosine *N*-carboxy anhydride: A, 0.332; B, 0.317; D, 0.289. 3-Methylhydantoin: A, 0.178; B, 0.185; D, 0. Tributylamine: A, 0.042; B, 0; D, 0.262. Curve C is for L-proline NCA (0.291) and tributylamine (0.129). Complete conversion for 0.332 mol/L of sarcosine NCA yields 10^{-3} mol of CO_2. Reprinted with permission from ref 36. Copyright 1961 Royal Society of Chemistry.

that LiCl is an efficient initiator for the polymerization of NCAs in dimethylformamide. Initially, they suggested that Li^+ was the base that subtracted the H from the N of the NCA. The activated NCA then reacts with a nonactivated monomer, generating the initiator to give a dimeric carbamic acid. However, it was observed that lithium perchlorate in DMF does not initiate the polymerization in systems for which lithium chloride is an efficient initiator. This proves that the reaction involves the negative ion, i.e. Cl^- or ClO_4^-, and not the common Li^+ ion. The polymerization was attributed to the Cl^- anions, which are stronger bases than perchlorate. Later, the concept of the proton transfer to the base was accepted, and the AMM mechanism was extended not only to aprotic bases, but also to secondary amines.

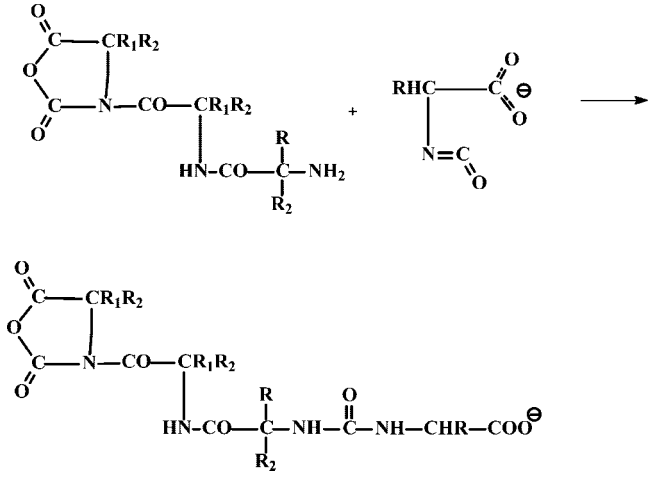
NCA anions are well-known to rearrange to α-isocyanatocarboxylates, and therefore, a termination process arises from the reaction with the amine at the end of the polymeric chain (Scheme 16).[41]

The intramolecular coupling of the terminal amine with the NCA-cyclic end of the same polypeptide is another termination process,[42−47] resulting in the formation of cyclic polypeptides. The rate of this reaction depends on the molecular weight of the polymer, since the higher the

Scheme 15. Reaction of Sarcosine NCA with 3-Methylhydantoin, Resulting in a Heterodimer Bearing a Secondary Amino End Group



Scheme 16. Reaction of α-Isocyanatocarboxylates with the Amine at the End of the Polymeric Chain



molecular weight, the lower the probability of ring closure. This feature may account for the high polydispersity of those polypeptides produced from aprotic bases.

Another probable mode of termination of the propagating chains may result from the unwanted addition of an activated monomer to the NCA-cyclic chain end (Scheme 17).

Lastly, it should be noted that since the AMM proceeds via anions, it is expected to proceed with a higher propagation rate than that of the NAM leading to higher molecular weights. On the other hand, since the initiation in AMM is slower than the propagation, polypeptides with high polydispersity indices are obtained.[48]

2.2. Studies after 1997. Living ROP of α-Amino Acid NCAs

2.2.1. Transition Metal Complexes Based on Co and Ni

Until 1997, efforts to synthesize high molecular weight homo- and copolypeptides with controlled molecular characteristics and low polydispersity were unsuccessful, due to the unwanted side reactions previously discussed. Deming[49−51] discovered a new class of NCA-initiators based on organonickel compounds, which were able to overcome termination reactions. By using the zero valent nickel complex bipyNi(COD) (bipy = 2,2′-bipyridyl, COD = 1,5-cyclooctadiene), Deming was able to synthesize homo- and block copolypeptides with predictable molecular characteristics and low PDIs. Later, he found that cobalt initiators of the $(PMe_3)_4Co$ type were also efficient.[51]

Both these metals react with NCA monomers to afford, by oxidative addition to the anhydride bonds of NCA, metallacyclic complexes, which react with a second NCA monomer to give a 6-membered amido-alkyl metallacycle (Scheme 18).

The amido-alkyl metallacycles react with another NCA and, simultaneously eliminating CO_2, result in a large metallacycle and, via migration of an amide proton to the metal-bound carbon, produce a 5-membered amido-amidate complex, which is the active polymerization intermediate (Scheme 19).[52]

The propagation proceeds through this 5-membered amido-amidate complex by attack of the nucleophilic amido group on the electrophilic 5-C carbonyl of NCA to regenerate the active species upon elimination of CO_2 and proton migration. Therefore, the metal migrates to the newly added NCA, while being held by a robust chelate at the active end (Scheme 20).

The formation of the chelating metallacyclic intermediate is a general requirement for living NCA polymerizations with transition metal initiators. These organometal initiators are capable of giving homo- and copolypeptides with narrow molecular weight distributions ($M_w/M_n < 1.3$), controlled molecular weights ($5.0\times 10^2 < M_n < 5.0 \times 10^5$),[53] with a wide range of NCAs, preserving the original chirality of the NCA monomers.[54,55] Moreover, from kinetic analysis performed with γ-benzyl-L-glutamate NCA in DMF, it was concluded that the polymerization rate is rather high, since the observed rate constant was 2.7×10^{-4} s^{-1} at 298 K.[49]

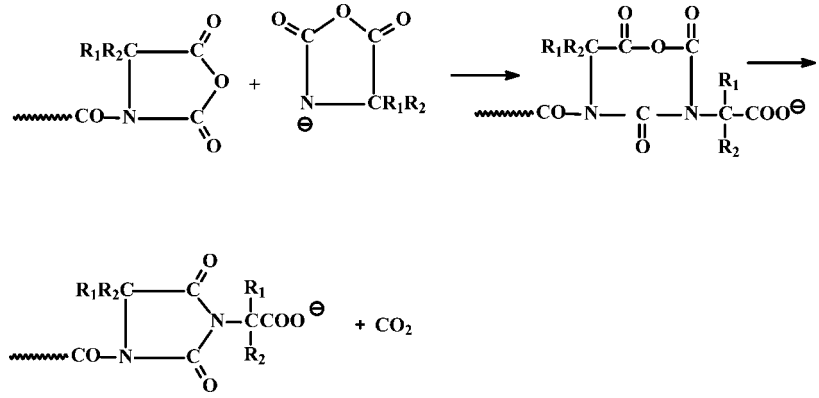
One minor drawback of this mechanism, which can be easily remedied, is the presence of the metals in the final polypeptides. Moreover, the method requires a proton at the 3-N of the NCA and thus cannot be applied to N-substituted NCAs such as proline NCA.[52] As Deming indicated, in the case of the $(PPh_3)_2Ni(COD)$ for the ROP of L-proline NCA, which lacks a proton at the 3-N, the expected 6-membered amido-alkylmetallacycle intermediate formed, but unfortunately, very low yields were obtained (Scheme 21).[52]

When L-proline or Sar-NCA was reacted with bpyNi(COD) or $(PMe_3)_4Co$ in THF or DMF, very little polypeptide was also formed (∼2−5%).

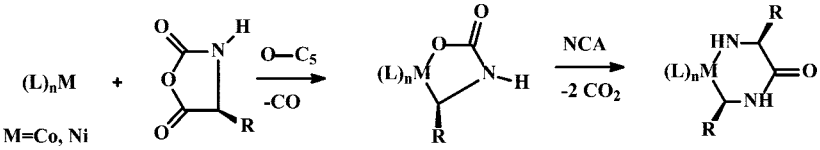
2.2.2. Primary Amine Hydrochlorides

Another novel approach to control the amine initiated NCA polymerization was reported by Schlaad et al. in 2003.[56] In order to avoid the AMM mechanism, this group used primary amine hydrochloride salts as macroinitiators. The addition of protons to the system prevents the formation of the NCA anions, provided that reprotonation of the NCA anions is faster than the nucleophilic attack to another NCA monomer. The reactivity of primary amine hydrochlorides toward NCAs was first investigated by Knobler et al.,[57,58] in the 1960s. These authors found that only one NCA molecule reacts with such salts, without propagation, since the hydrochloric salt of the primary amine formed is less nucleophilic than the parent amine. The dormant amine hydrochloride species is favored in this equilibrium, and therefore, the free amines are reactive for only a very short time and cannot propagate. From Knobler's work,[57,58] it was well-known that the concentration of the free amine species increases, as does the exchange rate between free amine and

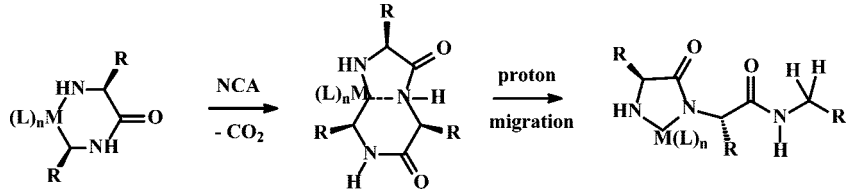
Scheme 17. Unwanted Addition of an Activated Monomer to the NCA-Cyclic Chain End



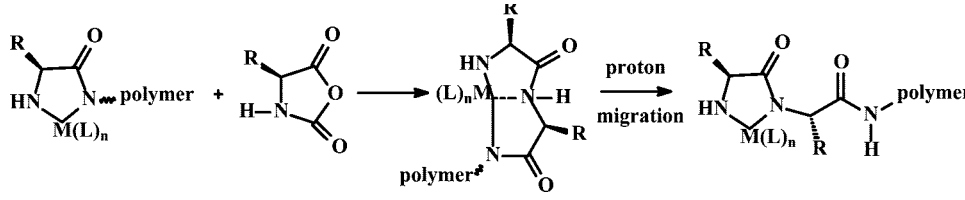
Scheme 18. Initiation Reactions of the Mechanism for the Polymerization of NCAs with bipyNi(COD) (bipy = 2,2′-Bipyridyl, COD = 1,5-Cyclooctadiene) or $(PMe_3)_4Co$ Complexes Proposed by Deming et al.



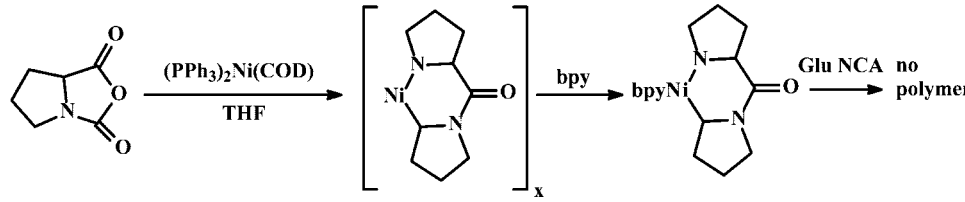
Scheme 19. Initiation Reactions of the Mechanism for the Polymerization of NCAs with bipyNi(COD) (bipy = 2,2′-bipyridyl, COD = 1,5-Cyclooctadiene) or $(PMe_3)_4Co$ Complexes Proposed by Deming et al.



Scheme 20. Propagation Reactions of the Mechanism for the Polymerization of NCAs with bipyNi(COD) (bipy = 2,2′-Bipyridyl, COD = 1,5-Cyclooctadiene) or $(PMe_3)_4Co$ Complexes Proposed by Deming et al.



Scheme 21. Polymerization of L-Proline NCA Using $(PPh_3)_2Ni(COD)$ (COD = 1,5-Cyclooctadiene) Initiator, Where Low Yield Was Obtained

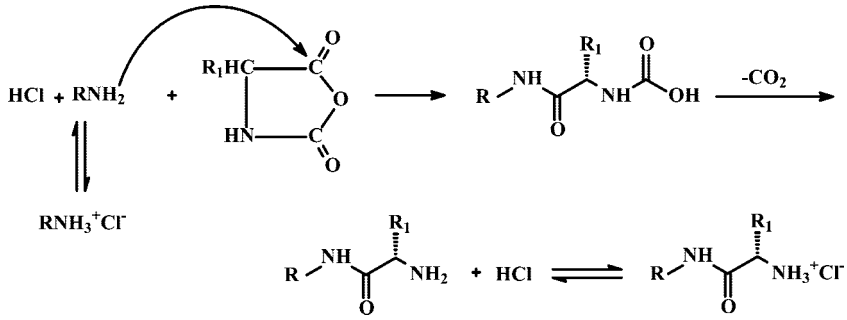


amine hydrochloride, by increasing the temperature. Schlaad's group, based on this work and the fact that the newly formed free amine end group would be immediately protonated (dormant), envisioned that the AMM pathway would be avoided. An example is provided by the synthesis of polystyrene-*b*-polypeptide hybrid copolymers, using primary amine hydrochloride end-capped polystyrene as the macroinitiator to polymerize *Z*-L-lysine-NCA in DMF (Scheme 22).

In this case, the polymerization lasted 3 days, at temperatures between 40 and 80 °C, with yields ranging from 64 to 84%, with the higher being obtained at higher temperatures. Even though the molecular weight distributions of the copolymers were as low as 1.03, the molecular weights of the polypeptides were found to be 20−30% higher than the expected ones. This was attributed to partial termination of the initiating species by traces of impurities accompanying the NCA monomer, although the presence of unreacted macroinitiator species was not taken into account.

The incomplete polymerization of the monomer in Schlaad's approach is due to the low reactivity of the active sites, which are in equilibrium with the dormant amine hydrochloride species. Therefore, in order to synthesize block copolypeptides, it is necessary to remove the ureacted monomer before the addition of the second monomer. Moreover, it is very difficult to synthesize copolypeptides with complex macro-

Scheme 22. Reaction of a Hydrochloric Salt of a Primary Amine with a NCA



molecular architectures such as multiblock multicomponent and branched copolypeptides, when linking agents are required.

2.2.3. Primary Amines and High Vacuum Techniques

In 2004, Aliferis, Iatrou, and Hadjichristidis[59] reported the first living polymerization of NCAs using neat primary amines as initiators and using high vacuum techniques (HVT). The Athens Group is very experienced in the use of HVT for the living anionic polymerization of conventional monomers, such as styrene, dienes, etc.[60] The necessity of such techniques addresses the sensitivity of initiators (e.g *sec*-BuLi) and macroanions to trace amounts of water, carbon dioxide, oxygen, and other reactive impurites. The use of such techniques is also effective in cases where the reaction duration is rather long (e.g., a few days to a few weeks). This is a small price one has to pay in order to achieve model complex macromolecular architectures.[61] The alternative approach, inert atmosphere techniques, is not efficient to obtain the necessary purity of the system and to maintain it for long periods of time.

Based on the previous work on the polymerization of NCAs, it was concluded that NAM has certain weak points. It is sensitive to impurities, such as HCl salts and acyl chlorides of α-amino acids (coming from the synthesis of NCAs), that lead to termination reactions, as well as to other initiating species (water and amines), leading to multimodal polymeric materials. In addition, the presence of CO_2 slows the kinetics and leads to incomplete polymerization.

Since NCAs are just as sensitive to impurities as are the macroanions, the Athens Group decided to apply HVT for their polymerization.[60]

By applying the HV techniques, the low boiling point species, i.e. the solvent (DMF) and the initiators (*n*-hexylamine or 1,6-diaminohexane), were purified rigorously. Since DMF decomposes when distilled at elevated temperatures (bp = 153 °C), distillation under HV is needed (35−40 °C). In addition, the DMF should pass through a NCO-column in order to eliminate the last traces of dimethylamine. The purification of the monomer is very critical. With the exception of L-proline-NCA, which can be sublimed and thus can be easily purified, most of the NCAs are solid and require very careful repeated crystallizations under vacuum (to avoid contamination from air humidity) for their purification. Before crystallizations, these NCAs must be treated according to a recent method which eliminates the chloroimpurities coming from the NCA synthesis.[62] The Athens Group[59] recently found that when the monomer crystallization was carried out in a drybox, the levels of impurities were not low enough to allow living polymerization. In contrast, when the recrystallizations were conducted using HVT, the polymerization successfully led to well-defined polypeptides. The proof of the system's purity under HVT is the indefinite maintenance of a DMF-NCA solution without polymerization or decomposition.[63,64]

A key parameter for the successful living ROP of NCAs is the elimination of the CO_2 produced during the propagation. As reported previously, the propagation of NAM involves two steps (Scheme 2), the ROP of NCA and the decarboxylation of the formed carbamic acid. Although the solubility of CO_2 in DMF is low compared to those of other solvents used in the ROP of NCAs,[65] its reactivity toward bases is much higher in DMF than in other solvents such as nitrobenzene.[14,66] Consequently, the decarboxylation of the carbamic acid does not occur rapidly, and some active sites may remain as is or form salts with the active amines. In both cases, the formed species are inactive and lead to incomplete polymerization and/or termination reactions.

Incomplete polymerization was observed under HVT when the reactors used had smaller volumes than that of the CO_2 evolved during the ROP. Using large reactors connected to the vacuum line and periodically degassing the system, living polymerization of NCAs was achieved. The high vacuum continuously removes the CO_2 produced during the reaction from the solvent.[59]

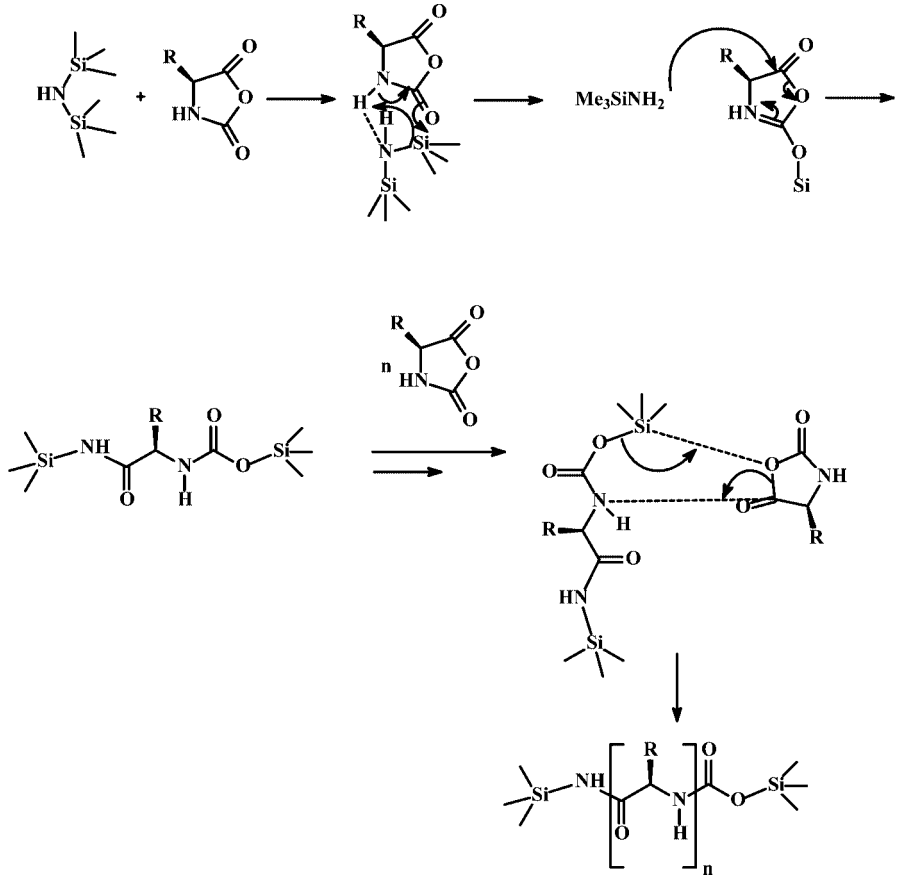
With these methods and techniques, the Athens Group revealed the living nature of the ROP of a wide variety of NCAs to produce homo- and block copolypeptides with high degrees of molecular and compositional homogeneity.[59] The kinetic study of the polymerization of γ-benzyl-L-glutamate NCA in DMF gave a rather high rate constant of 5.12×10^{-3} s^{-1}. This technique is general, since it can be used to polymerize both substituted and unsubstituted NCAs. The HVTs were also utilized to synthesize well-defined homo- and copolypeptides with complex macromolecular architectures, such as multiblock linear as well as star and star-block copolypeptides.[67]

HVTs were also applied successfully for the synthesis of polypeptide hybrids with complex macromolecular architectures.[68] It is clear that HV techniques have a great potential for the synthesis of other well-defined materials with complex macromolecular architectures, such as multiblock, cyclic, super-H, and miktoarm stars.

2.2.4. Primary Amines and Low Temperatures

In 2004, Vayaboury et al.[69] studied the polymerization of ε-trifluoroacetyl-L-lysine NCA (TFA-Lys NCA) in DMF with *n*-hexylamine initiator, as a function of temperature, using

Scheme 23. Polymerization Mechanism of NCAs Using Hexamethyldisilazane Initiators



Shlenk techniques under nitrogen. After complete consumption of NCA monomer, the crude polymerization mixtures were characterized by SEC and nonaqueous capillary electrophoresis (NACE), capable of separating and quantifying polymeric chains with different chain ends. These authors observed that, by lowering the temperature from 20 to 0 °C, the amount of living chains increased from 22 to 99%, respectively. Also, by adding more NCA at 0 °C, the molecular weight was increased without an increase of the terminated chain ends. They concluded that, at 0 °C, the activation energy barrier for chain propagation becomes lower than that of the side reactions and thus dominates kinetically.

The decrease in termination reactions upon lowering the temperature has also been observed in other types of polymerizations, such as anionic and cationic. The termination reactions under high temperatures observed by Vayaboury et al. could be accounted for by the low purity of the system. This is supported by the fact that, even at 0 °C where termination is very limited, bimodal polypeptide chains were obtained, probably due to the existence of two different initiating species.

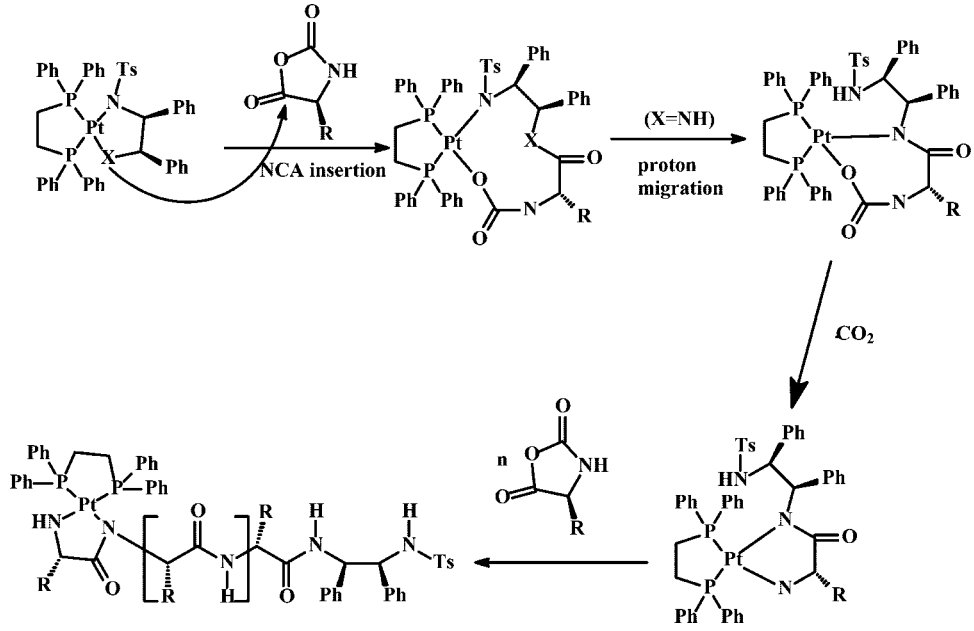
2.2.5. Silazane Derivatives

In 2007, Lu et al.[70] used hexamethyldisilazane (HMDS) for the controlled polymerization of the BLG-NCA. They found that the polypeptides obtained with this initiator exhibited low polydisperisty indices (1.19−1.26), the molecular weights obtained were the expected ones, and the yield was almost 100%. As a secondary amine, HMDS is expected to act either as a nucleophile to open the NCA ring at 5-C (NAM) or as a base to deprotonate the 3-N position (AMM), like other secondary amines with bulky alkyl groups. Indeed, according to the mechanism proposed, the HMDS deprotonates the 3-N in the first step to form Me_3SiNH_2 (TMS amine), while the other TMS group is attached at 2-C (Scheme 23). The generated TMS amine attacks the 5-C to form a TMS carbamate (TMS-CBM). Then, the polypeptide chains propagate through the transfer of the TMS group from the terminal TMS-CBM to the incoming monomer to form a new TMS-CBM terminal propagating group.

These HMDS-mediated NCA polymerizations are similar to the group transfer polymerizations (GTP) of acrylic monomers initiated by similar organosilicon compounds. Although GTPs require Lewis acid activators or nucleophilic catalysts to facilitate polymerizations, HMDS-mediated NCA polymerizations do not require any additional catalyst as activators. The authors reported that it is unclear whether the TMS group transfers through an anionic process as GTP or through a proposed concerted process. Block copolypeptides, such as poly(γ-benzyl-L-glutamate)-*b*-poly(ε-carbobenzyloxy-L-lysine) (PBLG-*b*-PZLL) were synthesized by the sequential addition of the corresponding NCAs, with low polydispersity indices and the expected composition.

In a later work this group showed that other N-TMS amines, such as benzylamine, morpholine, propargylamine, *N*-(aminoethylene)-5-norbornene-endo-2,3-dicarboximide, and $mPEG_{2000}$ amine, were also efficient initiators.[71] These initiators contain functional groups that can be used for further reactions such as "click" chemistry and ring-opening metathesis polymerization.

Scheme 24. Polymerization Mechanism of NCAs Using [Bis(diphenylphosphino)ethane][*N*-((1*S*,2*R*)-2-amido-1,2-diphenylethyl)-4-methylbenzenesulfonamidato]platinum Complex Initiators



According to the proposed mechanism, the weak point of this approach is that a proton is required on the 3-N of the NCA in order to initiate the polymerization. Thus, the TMS-amine initiator's approach is not general, since it cannot be used for the polymerization of N-unsubstituted NCAs, such as proline and sarcosine.

2.2.6. Transition Metal Complex Based on Pt

Finally, in 2008, Peng et al.[72] reported a novel platinum complex {[bis(diphenylphosphino)ethane][*N*-((1*S*,2*R*)-2-amido-1,2-diphenylethyl)-4-methylbenzenesulfonamidato]-platinum [(dppe)Pt(MBS-NH)]} as an efficient initiator for the ring-opening polymerization of Z-Lys-NCA. They also synthesized an alternative complex, {[bis(diphenylphosphino)ethane][*N*-((1*S*,2*R*)-2-hydroxo-1,2-diphenylethyl)-4-methylbenzenesulfonamidato]platinum(II) [(dppe)Pt(MBS-O)]} in order to explain the reactivity of the former initiator. Initially, they conducted a polymerization of Z-Lys-NCA with the complex (dppe)Pt(MBS-O) at room temperature and with a monomer/initiator ratio of 20. No reaction was observed. When the same system was heated at 60 °C, the yield of polymerization was only 9.3%. The same system at 60 °C, without the initiator, gave the same polymerization characteristics, implying that the complex had no effect on the polymerization or the system was not pure enough. When the first complex, (dppe)Pt(MBS-NH), was used, a well-defined polypeptide was obtained with the expected molecular weight and a low polydispersity index. The molecular weight of the polymer was increased by adding another amount of monomer, thereby showing the living nature of the polymerization. They reported that the system follows the same mechanism proposed for the amido-amidate metal complexes of Ru and Ni proposed by Deming et al. (Scheme 24). The probable first step of the reaction is the insertion of NCA into the complex molecule, followed by a proton migration from X (when X = NH) to the 3-N. In the case where X = O, the polymerization does not proceed due to the absence of an H atom, as observed for the (dppe)Pt(MBS-NH) complex. Decarbonylation generates a new amido-amidate platinum complex, which will restart a new NCA insertion and so on.

As in the case of Deming et al., the method does not apply to N-substituted NCAs and an extra step is necessary to remove the metal.

3. Polypeptide-Based Macromolecular Architectures

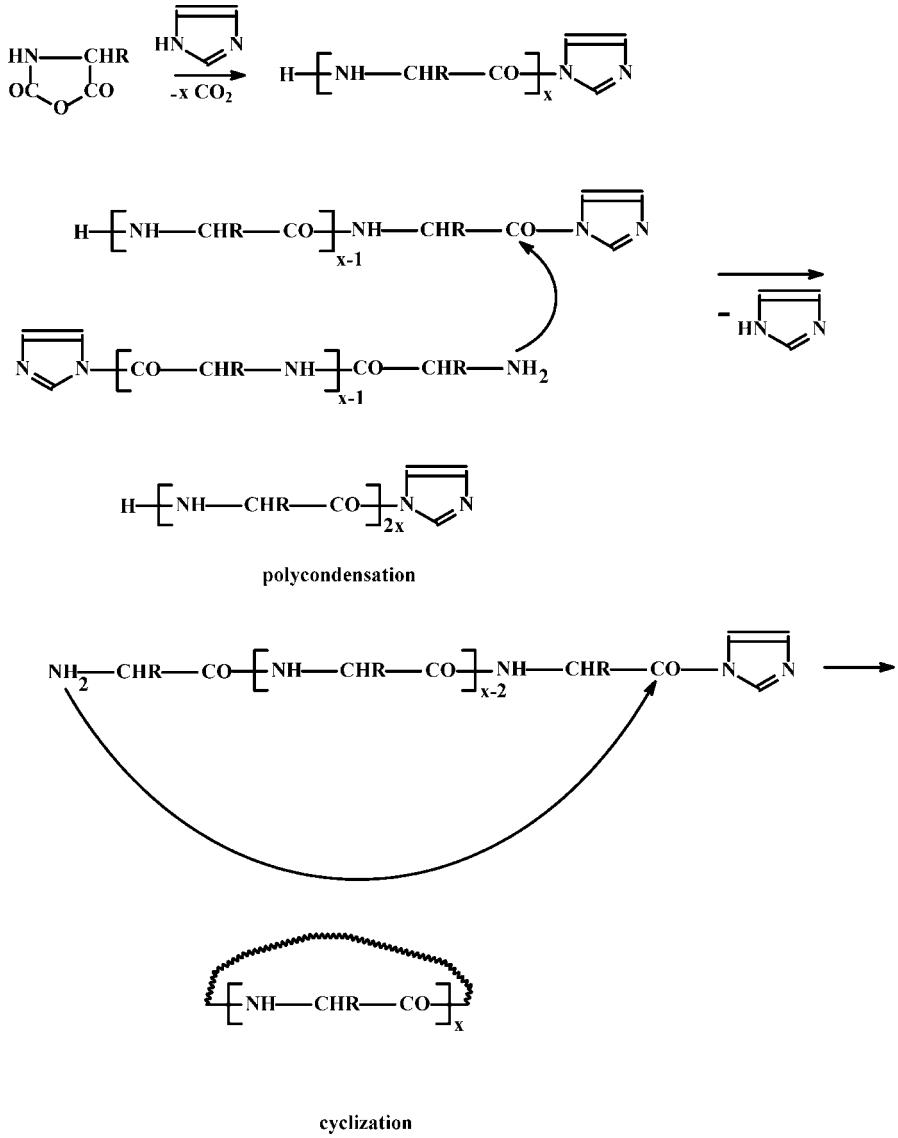
A large variety of linear homopolypeptides has already been reported in the literature (e.g refs 4c, 49, and 59) and thus will not be discussed here. Early studies led to the synthesis of rather ill-defined polypeptide-based polymers. However, the recent advances in the living NCA-ROP have allowed the synthesis of well-defined materials with different architectures. This chapter is dedicated to the synthesis of cyclic homopolypeptides, random and block copolypeptides, hybrid block copolymers, star structures, and more complex architectures, such as graft and highly branched materials. Emphasis will be given to rather well-defined structures.

3.1. Cyclic Polypeptides

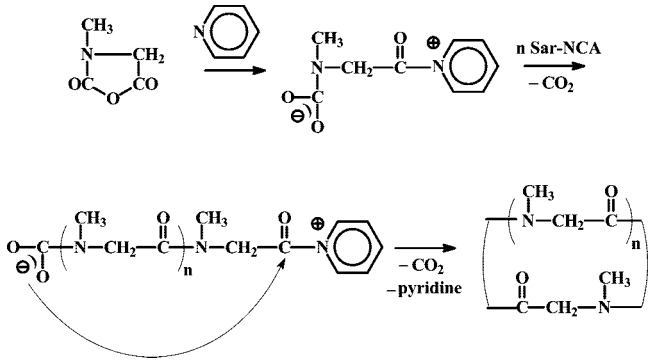
The use of protic nucleophiles, such as primary amines (e.g., *n*-hexylamine) or alcohols leads almost exclusively to linear polymers. However, secondary or tertiary amines may promote the polymerization of NCAs to cyclic polypeptides, depending on the amine basicity, nucleophilicity and steric hindrance effects, through pathways different from those discussed above.

It was proposed that certain protic nucleophiles can act as initiators to generate end groups, which are reactive enough to enable end-to-end cyclization of the growing peptide chains. In these cases, the ROP kinetics is transformed to a kinetically controlled polycondensation. Sar-NCA, D,L-Leu-NCA, and D,L-Phe-NCA were polymerized by Kricheldorf et al.[73] in dioxane at 60 °C using imidazole as initiator, leading to cyclic polymers as the main product (Scheme 25). In contrast, the cyclization reaction was hindered in the case of L-Ala-NCA, due to the formation

Scheme 25. Cyclic Polymers from Imidazole Initiated Polymerization



Scheme 26. Zwitterionic Polycondensation Mechanism for the Polymerization of Sar-NCA to Cyclic Products

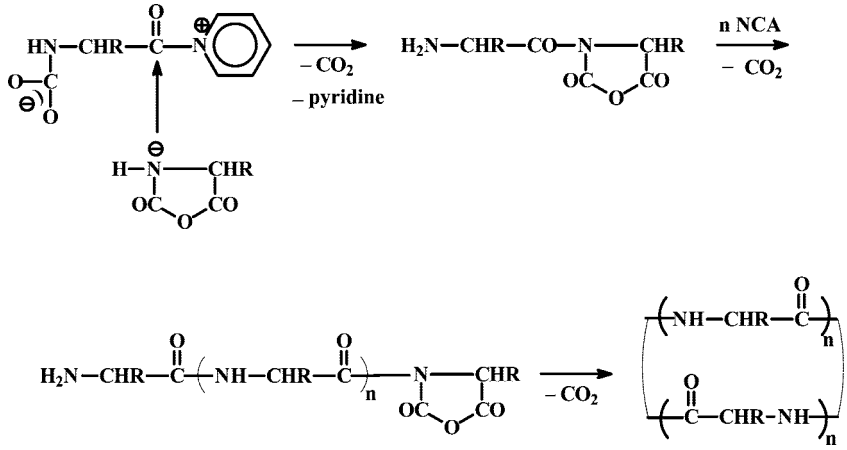


and rapid precipitation of β-sheet crystals for low molecular weight polymers (DP $\leq$ 10) and α-helices for higher molecular weight polymers (DP > 10).

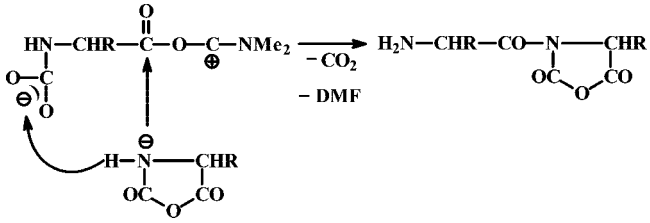
An alternative route for the synthesis of cyclic polypeptides was reported by Kricheldorf et al.[64] using pyridine as initiator. At least in the case of Sar-NCA, a zwitterionic polycondensation mechanism was involved, as shown in Scheme 26. In the case of N-unsubstituted NCAs, the zwitterion formation may be followed by reaction with another NCA to provide an N-aminoacyl-NCA (Scheme 27). It was also reported that spontaneous or solvent-induced polymerizations may take place. Solvents with high nucleophilicity and electron donor ability may catalyze the zwitterionic polymerization of NCAs. The reactivity of certain solvents for the polymerization of D,L-Phe-NCA was found to decrease in the order *N*-methylpyrrolidone, NMP > dimethyl sulfoxide, DMSO > *N*,*N*-dimethyl formamide, DMF > tetramethylurea, TMU. According to the proposed mechanism, the solvent acted as a nucleophile and electron donor generating zwitterionic species as active intermediates. MALDI-TOF spectra unambiguously supported the formation of cyclic products, but the proposed mechanistic procedures were not based on experimental data and the catalytic effect of the solvent was not explained in detail. It is possible that the presence of amine or alcohol impurities in the solvents was responsible for this behavior. The molecular characteristics of these samples were not reported.

Sterically hindered trialkylamines are not strong enough nucleophiles to promote the zwitterionic polymerization of NCAs. However, they can polymerize N-unsubstituted NCAs in dioxane to afford cyclic polypeptides. In this case, the polymerization proceeds through the deprotonation of the NCAs, followed by a nucleophilic attack of the resulted NCA

Scheme 27. Zwitterionic Mechanism for the Polymerization of N-Unsubstituted NCAs to Cyclic Products



Scheme 28. Nucleophilic Attack of Deprotonated NCA onto Another Monomer



anion onto the 5-CO of another NCA, as shown in Scheme 28. To support this assumption, the polymerization of Sar-NCA was attempted using *N*-ethyldiisopropylamine as initiator.[64] The polymerization yield was very low, and only linear products were prepared. In all other cases using N-unsubstituted NCAs, cyclic polypeptides were mainly observed.

The thermal polymerization of NCAs to low molecular weight products was first reported by Leuchs, who mentioned that NCAs decompose when heated at temperatures around or above their melting temperatures. In a recent study, Kricheldorf et al.[74] revealed, by NMR and MALDI-TOF characterization, that the thermal polymerization is accompanied by cyclization reactions. The thermal polymerization of Sar-NCA at 120 °C led mainly to the synthesis of cyclic products. It was proposed that the polymerization proceeds through a zwitterionic mechanism considering either a chain-growth or a step-growth process. Experimental evidence supporting this mechanism was not provided, and furthermore, the molecular characteristics of the produced polymers were not given.

Cyclic products were also observed from the thermal polymerization of N-unsubstituted NCAs, such as L-Ala-NCA, D,L-Leu-NCA, D,L-Val-NCA, and D,L-Phe-NCA.[75] The situation was very complex, since it was found that oligomers coexist with higher molecular weight products. Detailed mechanistic studies and molecular characterization of the products were not reported.

A different approach was also developed by Kricheldorf et al.[76] for the synthesis of cyclic polySar. Difunctional primary amines (1,12-diaminododecane, DAD, or 1,13-diamino-4,7,10-trioxatridecane, DATT) were employed for the synthesis of linear well-defined telechelic polymers bearing amino end groups. Subsequent reaction of the telechelic polymers with 4,4′-diisocyanatodiphenylmethane led to chain extension and finally exclusively to cyclic polymers. For higher molecular weight precursors it was not possible to identify the extent of cyclization. Using either hydroxysuccinimide sebacate or sebasic acid bisimidazolide as linking agents, it was found that at least 50% of cyclic products were obtained in addition to linear polycondensates. Due to the step-growth nature of the linking reaction, the molecular weight distribution of the final products was broad (PDI ≥ 2). Moreover, the cyclic products were not isolated from the mixture with the linear chains.

3.2. Copolypeptides and Hybrids

3.2.1. Random Copolypeptides

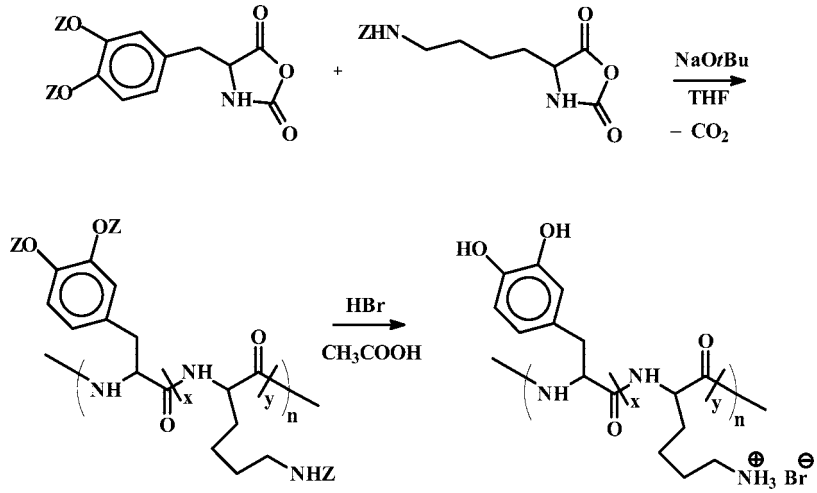
Polypeptides are valuable tools for the study of their complex relatives, the proteins. However, it is well-known that proteins are composed of up to 20 different α-amino acids. Therefore, random or statistical copolypeptides, which architecturally approach those structures found in nature, are definitely better models than homopolypeptides.

Random copolypeptides can be prepared by two different methods. The first involves the simultaneous copolymerization of two or more different NCAs, whereas the second involves the partial deprotection of the side protective groups of various homopolypeptides. The (co)polymerization can be effected by ring-opening procedures, using amine initiators or suitable transition metal complexes.

Early work in the field was devoted to the determination of the reactivity ratios of the different NCA monomers and the determination of the amino acid sequence distribution, mainly by ^{1}H-, ^{15}N-, and ^{13}C-CP/MAS NMR techniques. Among the structures prepared were the following: random copolymers of γ-benzyl L-glutamate and L-methionine,[77] γ-benzyl L-glutamate and L-valine,[78,79] glycine and alanine,[80] D,L-leucine and D,L-valine,[81,82] N^{ε}-carbobenzoxy L-lysine and β-benzyl L-aspartate,[83] *O*-acetyl L-tyrosine with L-valine and glycine,[84] L-alanine and L-valine,[85] L-alanine and sarcosine,[86] N^{ε}-carbobenzoxy L-lysine and L-valine,[87] and random terpolymers, such as of glycine, L-leucine, and L-valine.[88]

A more recent work by Wamsley et al.[89] examined the terpolymerization of L-Leu, L-Val, and BLA (β-benzyl L-aspartate) in dioxane with triethylamine as initiator. Binary copolymers of L-Leu with BLA, BLA with L-Val, and L-Leu with L-Val were also prepared, and their reactivity ratios were obtained through the Fineman−Ross and Kelen−Tüdos graphical methods and the nonlinear least-squares curve-fitting method. On the basis of these reactivity ratios, the Alfrey−Goldfinger equations were used to estimate the terpolymer compositions. No statistical difference between the calculated and the actual terpolymer compositions was

Scheme 29. Synthesis of Random Copolymers of N^{ε}-Carbobenzoxy L-Lysine and *O,O′*-Dicarbobenzoxy L-Dihydroxyphenylalanine



found. These results indicate that random copolymers and terpolymers were predominately formed.

Recent studies were focused on the synthesis of random copolypeptides for specific applications or for the study of their solution properties. For example, Yu et al.[90] synthesized random copolymers of N^{ε}-carbobenzoxy L-lysine and *O,O′*-dicarbobenzoxy L-dihydroxyphenylalanine by ROP of the corresponding NCAs in THF with sodium *tert*-butoxide as initiator. Subsequent treatment of the copolymers with HBr in acetic acid resulted in the partial cleavage of the carbobenzoxy-protective groups (Scheme 29). The molecular weights determined by SEC analysis revealed the presence of high molecular weight copolymers with rather broad PDIs. It was found that aqueous solutions of these copolymers, when mixed with suitable oxidizing agents, can form cross-linked networks that may be suitable as moisture-resistant adhesives for a variety of substrates.

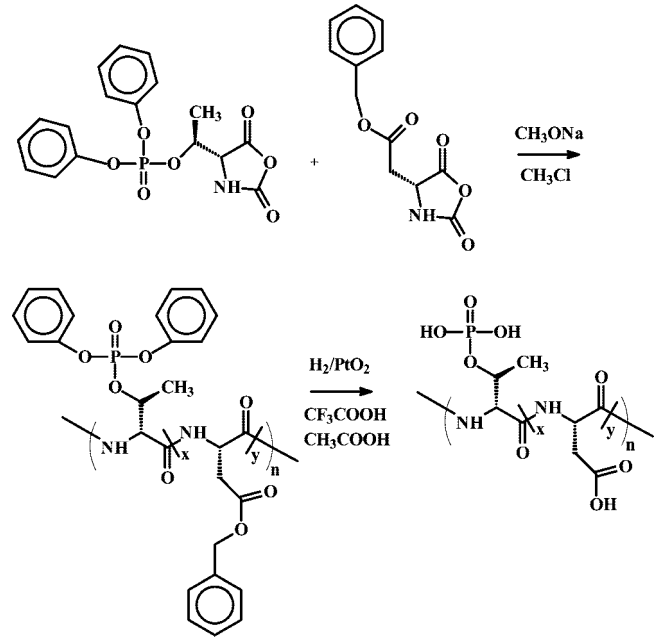
Hayashi et al.[91] prepared random copolypeptides of *O*-phospho-L-threonine and L-aspartic acid by the corresponding phenyl- or benzyl-protected NCAs using sodium methoxide as initiator. The deprotection was performed by catalytic hydrogenolysis over PtO_2 in high but not quantitative yield (up to 81%) (Scheme 30). The molecular weights were low, as estimated by viscometry. The PDIs were not reported in this work. The effect of these copolypeptides on the crystallization of $CaCO_3$ was examined.

Amphiphilic random copolypeptides composed of combinations of the hydrophilic amino acid L-Lys and one of the hydrophobic amino acids L-Leu, L-PheAla, L-Isoleu, L-Val, or L-Ala were prepared using the Deming initiator $Co(PMe_3)_4$.[92] Products with different molecular weights and varied compositions were obtained. SEC analysis revealed that the samples were characterized by relatively narrow molecular weight distributions. These copolymers may mimic the cationic and amphiphilic nature of many natural antimicrobial peptides.

The NCAs of *δ*-*N*-benzyloxycarbonyl-L-ornithine and *δ*-coumaryloxyacetyl-L-ornithine (5−10% mol) were copolymerized in DMF using triethylamine as initiator.[93] The products were characterized by dilute solution viscometry. Subsequent irradiation induced intermolecular dimerization reactions between coumarin moieties and slowly led to the formation of transparent hydrogels.

The copolymerization of BLG-NCA and *ε*-carbobenzoxy L-lysine NCA (ZLL-NCA) with lysine NCAs carrying labile protective groups such as N^{ε}-trifluoroacetyl- (TFA), N^{ε}-(*tert*-butoxycarbonyl)- (Boc), N^{ε}-(9-fluorenylmethoxycarbonyl)- (Fmoc), and N^{ε}-(6-nitroveratryloxycarbonyl)- (Nvoc) was conducted in DMF with *n*-hexylamine by Klok and Hernádez[94] (Scheme 31). Low molecular weight copolypeptides were obtained and analyzed by NMR and MALDI-TOF. These species contain different side groups that can be selectively removed and thus can be used as scaffolds for the preparation of synthetic antigens or protein mimetics.

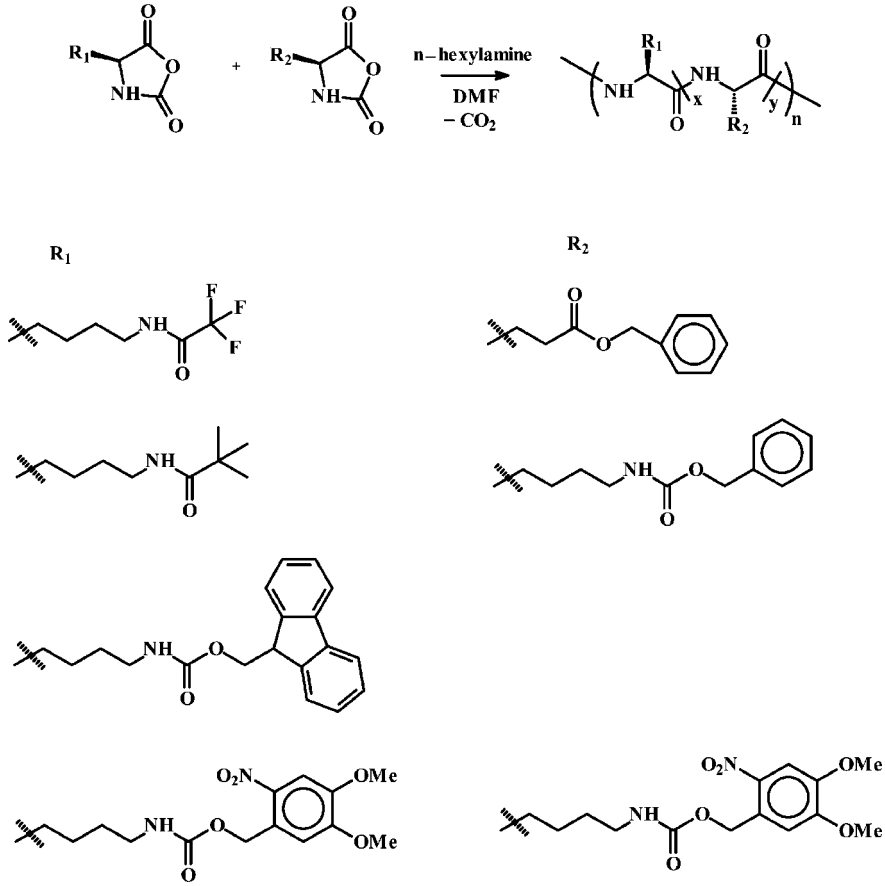
Scheme 30. Synthesis of Random Copolypeptides of *O*-Phospho-L-threonine and L-Aspartic Acid



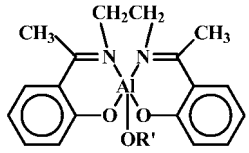
Random copolypeptides of Pro and Ala were synthesized by Kakinoki et al.[95] using *n*-hexylamine as initiator. The yields were rather low (up to 60%), but the molecular weight distributions were relatively narrow. The solution properties of these copolypeptides and the effect of irradiation with *γ*-rays were also examined.

Aluminum Schiff's base complexes, initially used for the ring-opening polymerization of lactides,[96] were employed as initiators for the copolymerization of Leu and *γ*-methyl-L-glutamate NCAs (MLG-NCA), to give the corresponding

Scheme 31. Copolymerization of γ-Benzyl-L-glutamate NCA and N^{ε}-Carbobenzoxy L-Lysine NCA with Lysine NCAs Carrying Labile Protective Groups



Scheme 32. Aluminum Schiff's Base Complex Initiators for the Random Copolymerization of Leu and γ-Methyl-L-glutamate NCAs



HAPENAlOMe R'=OMe

HAPENAlO^{i}Pr R'=O^{i}Pr

random copolypeptides (Scheme 32).[97] The high values of both reactivity ratios, calculated by the Fineman−Ross and Kelen−Tüdos graphical methods, revealed a multiblock structure. NMR analysis showed that slightly longer MLG block sequences existed. The molecular weights were estimated by dilute solution viscometry, whereas the molecular weight distributions were not reported.

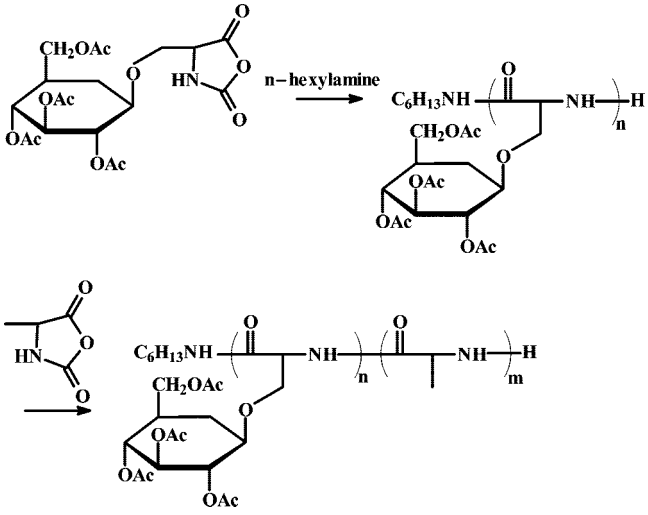
The second method for the synthesis of random copolypeptides, involving the homopolymerization of NCA bearing protected side groups and followed by the partial deprotection, was adopted by Higuchi et al.[98,99] for the synthesis of poly[(γ-methyl-L-glutamate)-*co*-(L-glutamic acid)] (poly(MLG)-*co*-(LGA)). The γ-methyl-L-glutamate-NCA was polymerized in 1,2-dichloroethane employing *n*-hexylamine. The final homopolymer was dissolved in a mixture of methanol and isopropanol and treated with aqueous NaOH for 10 h, followed by treatment with trifluoroacetic acid to afford the random copolypeptide. NMR analysis revealed that 30% of the monomer units had been transformed to glutamic acid.

3.2.2. Block Copolypeptides and Polypeptide-Hybrids

Block copolymers containing polypeptide blocks can be divided into two different categories. The first includes copolypeptide structures, where both blocks are polypeptides, while the second category involves structures where polypeptide blocks are combined with other nonpeptide polymer chains (hybrids). The copolypeptides are usually prepared by sequential addition of different NCAs to either amine or transition metal complex initiators. On the other hand, the hybrid copolymers can be prepared by combining several polymerization techniques for the synthesis of the nonpolypeptide block with the ROP of the NCAs. All these polypeptide-based materials present very interesting properties both in solution and in bulk, since they combine the conformational features of the polymeric and polypeptide components. A major issue is raised by the difficulty to find a common good solvent for both blocks in the copolypeptides or the hybrid copolymers. The direct consequences of this event are that the polymerization becomes heterogeneous during the reaction and the copolymers tend to form micellar structures, thus hindering the detailed molecular characterization by techniques such as SEC. In the following sections, selected examples will be presented describing the synthesis of block copolypeptides and hybrid copolymers.

3.2.2.1. Block Copolypeptides. Several polypeptide block combinations have been described, including both natural and synthetically prepared amino acids, leading to a variety

Scheme 33. Synthesis of Block Copolymers of *O*-(Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-serine with Ala



of structures. Most efforts have been devoted to the synthesis of diblock copolypeptides. However, triblock copolypeptides of the ABA type and other more complex linear copolypeptides have been prepared. In almost all cases, the synthesis was achieved by sequential addition of monomers, assuming complete conversion of the previous monomer.

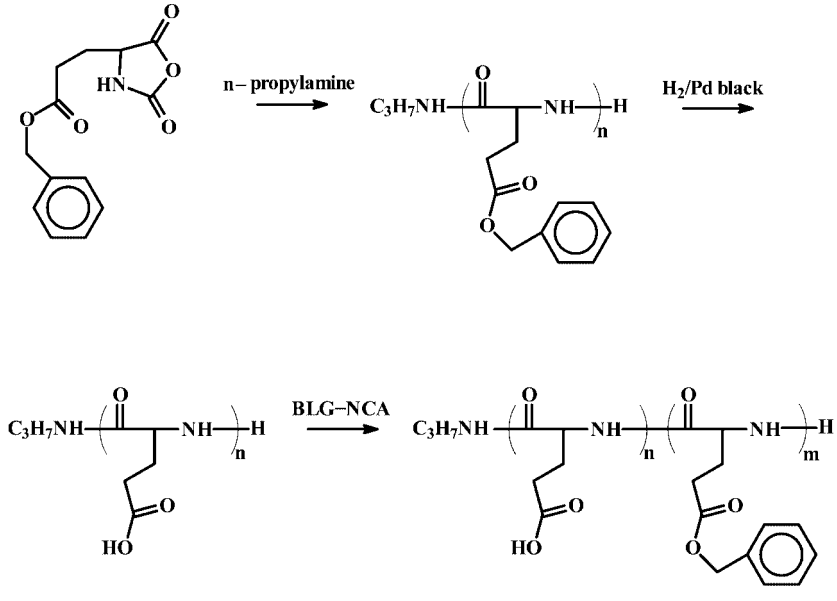
O-(Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-serine-NCA was polymerized by Aoi et al.[100] using *n*-hexylamine as initiator followed by the addition of Ala-NCA to afford the corresponding block copolymer. SEC analysis confirmed the complete consumption of the first block. Both blocks had low molecular weights (^{1}H NMR), whereas the molecular weight distributions (SEC) were very narrow. In addition, the block copolymer composition was almost identical with the feed molar ratio of the two monomers. Deacetylation of the glycopeptides was achieved by treatment with hydrazine monohydrate in methanol at 25 °C for 6 h (Scheme 33).

The amphiphilic block copolymers poly[(BLG)-*b*-(LGA)] were prepared by Higashi et al.[101] according to the reactions in Scheme 34. Initially, BLG-NCA was polymerized by *n*-propylamine. The polymer was precipitated in diethyl ether followed by catalytic hydrogenolysis (Pd/H_2) to remove the benzyl protective groups and afford PLGA. The amine end group of the polymer served to initiate the BLG-NCA polymerization. NMR spectroscopy was used to monitor the synthesis and to characterize the final product. This polymerization led to low molecular weight copolymers. Unfortunately, the molecular weight distributions were not reported.

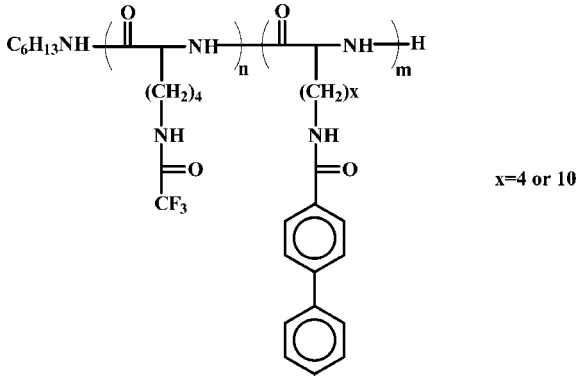
Using a similar methodology, Guillermain et al.[102,103] synthesized block copolypeptides of poly(N^ε-trifluoroacetyl-L-lysine) and another poly(L-lysine) block carrying liquid crystalline side groups. These liquid crystalline blocks include poly[11-(biphenyl-4-carboxamido)undecanamido-L-lysine] and poly(N^ε-4-phenylbenzamido-L-lysine) (Scheme 35). The first block was not polymerized quantitatively. It was therefore precipitated and purified before proceeding with the polymerization of the second monomer. Due to solubility problems, it was not possible to provide the molecular weight distributions by SEC analysis. The amide functions of the poly(N^ε-trifluoroacetyl-L-lysine) could be selectively hydrolyzed by NaOH, leading to the synthesis of amphiphilic block copolymers.

In a series of papers, the high vacuum technique (HVT) was applied by Aliferis et al.[59,104,105] for the sequential polymerization of different NCAs using primary amines as initiators to afford various well-defined block copolypeptides. It is widely accepted that HVT, more than any other techniques, ensures that all the reagents and the level of impurities of the reaction environment are sufficiently low in all the reaction steps. Therefore, under these experimental conditions, the living nature of a polymerization method can be promoted. The complete consumption of the monomer, the linear relationship between the molecular weight of the produced polypeptides and the conversion, the excellent agreement between the stoichiometric and the experimentally measured molecular weights, the narrow molecular weight distributions of the polymers, and the ability to synthesize samples of very high molecular weight confirm that the polymerization proceeds in a living fashion. Consequently, the preparation of well-defined block copolypeptides is feasible with this technique, as proven by the synthesis of the following copolypeptides: PBLG-PZLL, PBLG-PTYR, PBLG-PGLY, PZLL-PBLG, and PBLG-PLEU. The syntheses above were monitored by SEC, and the molecular weights

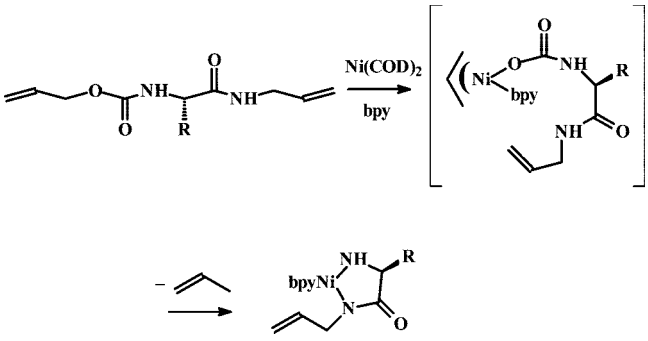
Scheme 34. Synthesis of Poly[(*γ*-benzyl-L-glutamate)-*b*-(L-glutamic acid)]



Scheme 35. Block Copolypeptides of Poly(N^{ε}-trifluoroacetyl-L-lysine) and Either Poly[11-(biphenyl-4-carboxamido)undecanamido-L-lysine] or Poly(N^{ε}-4-phenylbenzamido-L-lysine)



Scheme 36. Synthesis of Amido Amidate Metallacycle Propagating Species



were measured by membrane osmometry. It was found that the yields were quantitative, the molecular weight distribution very narrow, and the molecular weights in very close agreement with the stoichiometric values for both blocks. These results confirm that this technique maintains the level of impurities sufficiently low, thus avoiding termination reactions and promoting the living polymerization of the NCAs. Only for samples of high molecular weights were the distributions somewhat broader. In this case the initiator concentration is very low and becomes comparable to that of the system's impurities.

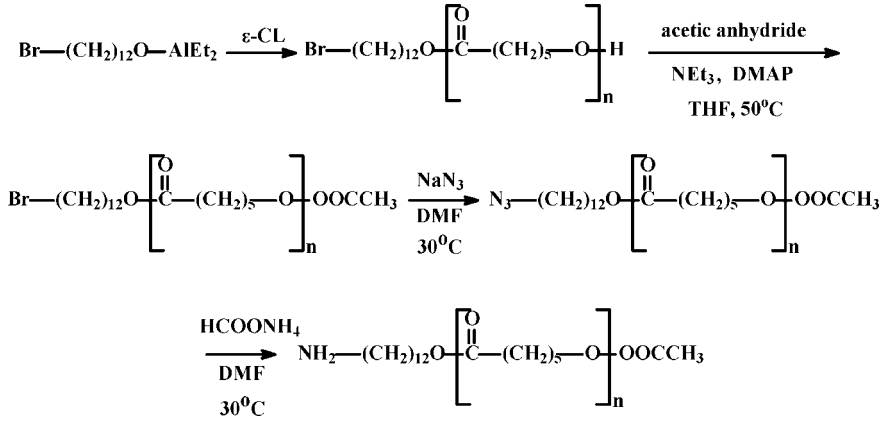
Polypeptides have been efficiently prepared through the use of zero valent transition metal complexes. The best results were obtained with nickel and cobalt complexes [i.e., bpyNi(COD) and $Co(PMe_3)_4$], leading to polymers with controlled molecular weights and narrow molecular weight distributions ($M_w/M_n < 1.2$) in almost quantitative yields. Efficient initiators for the polymerization of NCAs were found to be zero (or generally low) valent metals, to be capable of undergoing 2-electron oxidative-addition reaction, to have strong electron donating ligands, and to demonstrate stability of the metal complex toward the amino acids functionalities. Extensive mechanistic and kinetic studies revealed that the homopolymerization of NCAs is living, therefore allowing for the efficient synthesis of block copolypeptides through the sequential addition of monomers. However, mechanistic restrictions do not permit the polymerization of N-substituted amino acids, such as sarcosine. Employing this methodology, the following copolypeptides have been prepared:[49,51,106−113] Lys-BLG, BLG-Lys, Lys-Leu, BLG-Pro, BLG-Leu, Leu-Val, Lys-Ala, Lys-Gly, Asp-(PheAla-*co*-Leu), poly(N^{ε}-2[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine)-*b*-poly(L-aspartic acid sodium salt).

A drawback of the method is that the active propagating species are generated *in situ* and therefore do not permit for controlled functionalization of the polypeptide chain end. To overcome this problem, amido amidate metallacycle propagating species were synthesized, as shown in Scheme 36.[106] Using N^{α}-allyloxycarbonyl-amino acid allyl amides as substrates and zero valent nickel complexes, the desired amido-amide nickelacycle was formed in good yield. These initiators were efficiently used to produce block copolypeptides with controlled sequences and compositions and relatively low molecular weight distribution. The products were received in high but not quantitative yields.

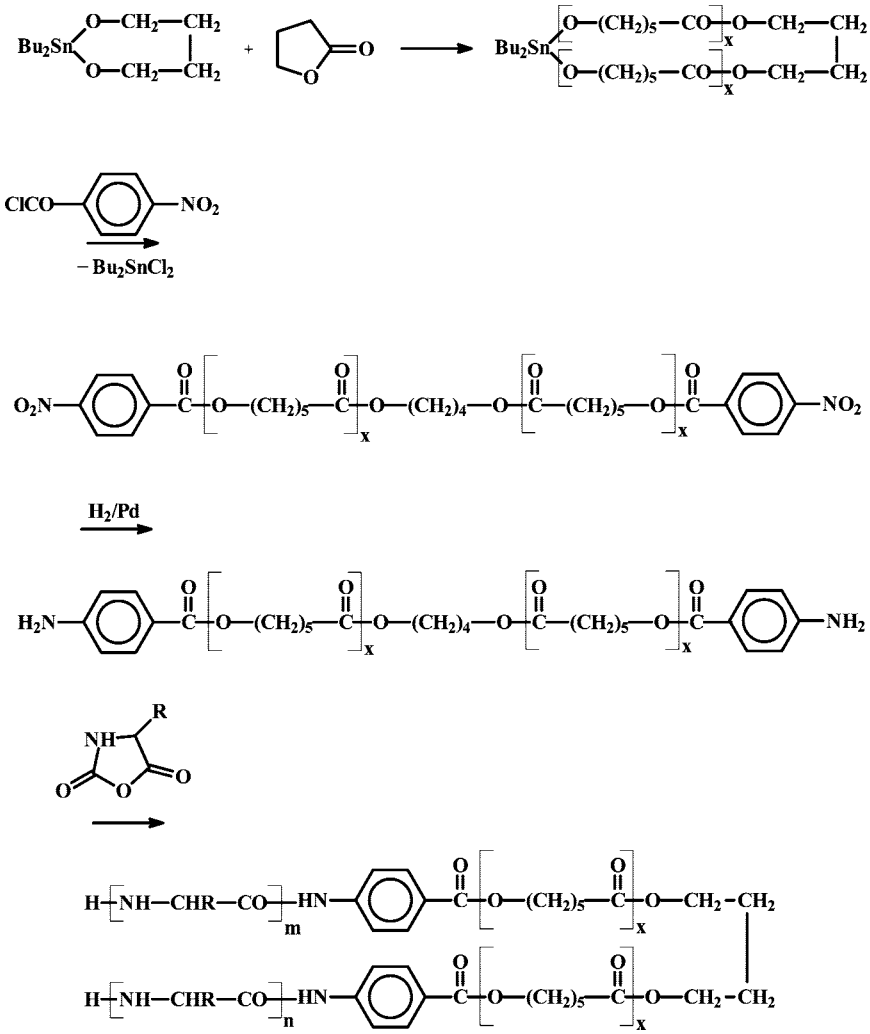
Schiff's base aluminum alkoxide complexes (Scheme 32) were also employed by Goury et al.[97] for the synthesis of poly[(γ-methyl-L-glutamate)-*b*-(L-leucine)] block copolypeptides. NMR analysis revealed that the desired copolypeptides have been successfully prepared. However, the molecular characteristics and details concerning the efficiency of the crossover reaction from the first block to the second one were not reported.

Triblock copolypeptides of the ABA type have also been prepared using similar methods to those reported above. Difunctional amine initiators have been efficiently used for the synthesis of symmetric triblock copolypeptides by a two-step sequential addition of NCAs. The most commonly employed initiator was 1,6-hexamethylenediamine. Early reports failed to give pure triblocks, since the products were contaminated with both A and B homopolypeptides. Using this method, poly[(BLG)-*b*-(L-leucine)-*b*-(γ-BLG)] and the corresponding poly[(LGA)-*b*-(L-leucine)-*b*-(LGA)] triblock copolypeptides were syn-

Scheme 37. Synthesis of α-Acetyl-ω-NH_2-PCL Macroinitiator



Scheme 38. Synthesis of ABA Triblock Copolymers, Where B Is PCL and A Is PGly, PAla, PPhe, or PBLG



thesized by Minoura et al.[114,115] This result can be attributed to the incomplete purification of the reagents (solvent, monomers, etc.). It is obvious that amine impurities may exist, leading to undesired homopolymerization of both NCAs. This was further confirmed by the synthesis of poly[(ZLL)-*b*-(BLG)-*b*-(ZLL)] copolypeptides using the HVT and performing extensive purification procedures.[59,116] Under these conditions, products with controlled molecular weights, with narrow molecular weight distributions, and free of homopolymer or diblock copolymer impurities were obtained. In principle, primary amines can also be employed as initiators for the synthesis of asymmetric triblock copolypeptides or even terpolypeptides.

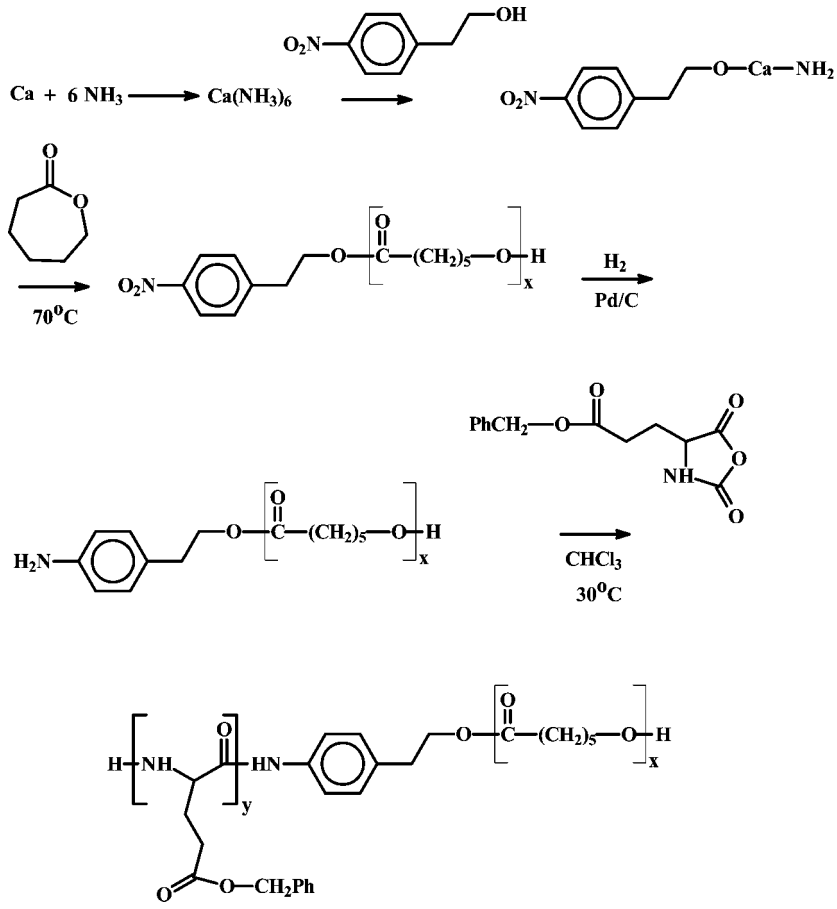
Transition metal mediated polymerization of NCAs was also used for the synthesis of triblock copolypeptides. In this case, only asymmetric species can be prepared by a three-step polymerization procedure. Only by a careful control of molecular weights can symmetric structures be obtained. Using $Co(PMe_3)_4$ as initiator, the copolypeptide poly[(N^{ε}-carbobenzoxy L-lysine)-*b*-(L-leucine)-*b*-(N^{ε}-carbobenzoxy L-lysine)] was prepared.[109,117] Complete consumption of each monomer and relatively narrow molecular weight distributions were reported. Theoretically, symmetric triblock copolymers can be prepared using suitable difunctional amido amidate metallacycle propagating species. Special care should be given in this case to the purity of the products, since the polymerization yields are not quantitative.

3.2.2.2. Polypeptide-Hybrids. Research on the synthesis of polypeptide hybrid block copolymers began in the mid 1970s.[118] Advances in polymer chemistry have allowed for the preparation of a wide variety of hybrids by a combination of different polymerization techniques. The structures are described below according to the initiators used for the ROP of the NCAs: (a) amines, (b) transition metal complexes, and (c) amine salts.

3.2.2.2.1. Primary Amine Macroinitiators. End-functionalized polymers carrying amines on one or both chain ends can be used as macroinitiators for the polymerization of NCAs. This remains the most widely applied method for the synthesis of a plethora of hybrid block copolymers. The major advantage of this method is the fact that several living/controlled polymerization techniques are able to prepare a variety of well-defined polymers with a high degree of amine functionalization. When combined with the recently developed ROP of NCAs under high vacuum, these methods lead to well-defined poplypeptide hybrids.

The vast majority of these hybrid copolymers involve the use of semi- and telechelic poly(ethylene oxide), PEO, carrying amine end group(s). This interest can be attributed to the fact that the amino-PEOs are commercially available in a variety of molecular weights with narrow molecular weight distributions, are water-soluble, and are biocompatible. Furthermore, these PEO polypeptide hybrids can form amphiphilic, double hydrophilic, and rod−coil block co-

Scheme 39. Synthesis of PCL-*b*-PBLG Hybrids



polymers, leading to materials with very interesting properties in solution and in bulk. Characteristic examples include diblock copolymers of PEO with poly(L-2-anthraquinonylalanine),[119] PBLG,[120,121] and poly[(DL-Val)-*co*-(DL-Leu)].[122] The molecular characterization in most cases was incomplete, since SEC traces were not provided and details concerning the efficiency of the macroinitiator were not given. In many cases, the presence of free homoblock was detected, implying a lack of control over the block copolymerization reaction. α,ω-Diamino-PEOs were also employed as difunctional macroinitiators for the synthesis of ABA-type triblock copolymers, where B is PEO and A is the polypeptide. Employing this technique, PBLG-*b*-PEO-*b*-PBLG,[123,124] PZLL-*b*-PEO-*b*-PZLL,[125,126] and poly[(DL-Val)-*co*-(DL-Leu)]-*b*-PEO-*b*-poly[(DL-Val)-*co*-(DL-Leu)][122] have been prepared. Earlier studies resulted in poorly defined products, since diblocks and homopolymers were present in the final reaction products.

However, recent reports revealed that a higher level of structural control was achieved, most possibly through a better purification of the reagents used. Thus, using a PEO-NH_2 macroinitiator and employing high vacuum techniques, the synthesis of the PEO-*b*-PZLL-*b*-PBLG triblock terpolymers was reported by Karatzas et al.[127] using sequential polymerization. SEC analysis revealed that the macroinitiator was quantitatively consumed and that the final products displayed monomodal peaks of relatively narrow molecular weight distribution. In addition, the molecular weights were in very close agreement with the stoichiometric values, indicating that under the experimental conditions used in this work the polymerization was free of termination reactions.

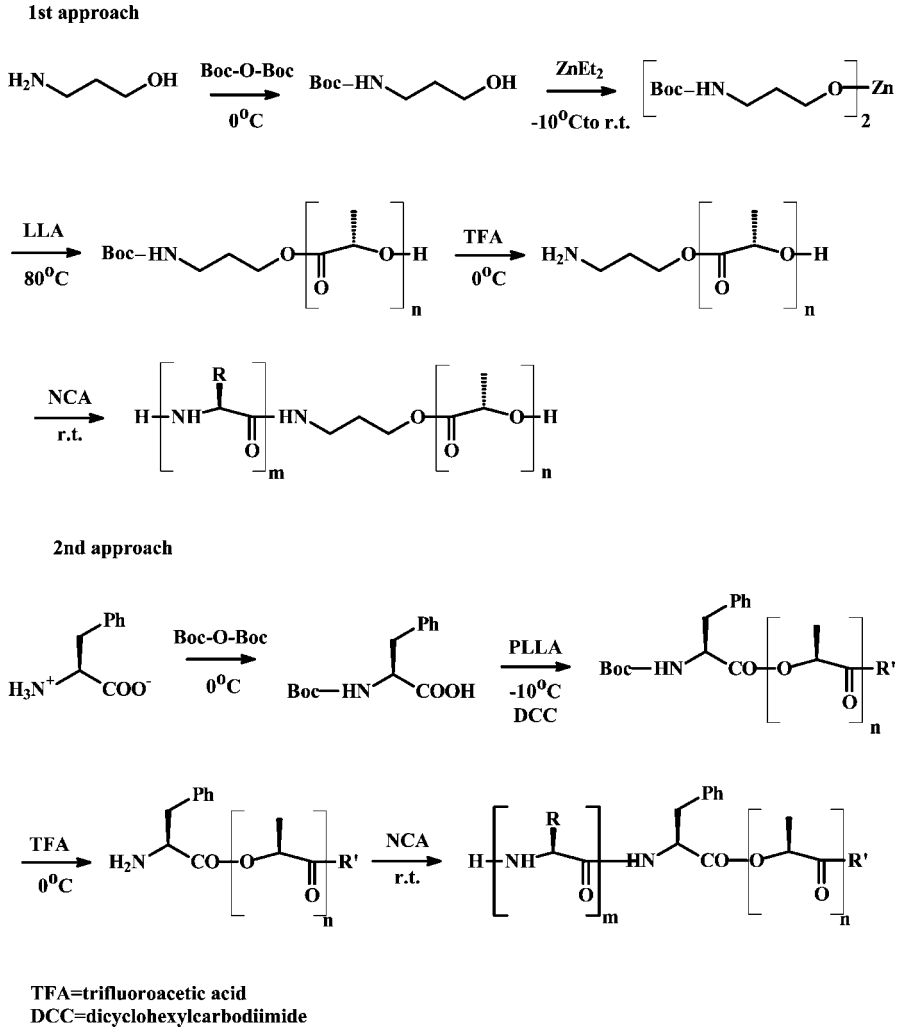
Hybrid block copolymers with aliphatic polyesters, such as poly(ε-caprolactone), PCL, and polylactide, PLA, represent a very interesting class of polymeric materials. Due to the low immunogenicity, biocompatibility, biodegradability and excellent mechanical properties of aliphatic polyesters, these hybrids have found applications in tissue engineering and drug delivery.[128] Moreover, the presence of the polyesters modifies the stability of the hybrids, since it reduces the enzymatic degradation of the polypeptides. Finally, the formation of crystalline polyester domains greatly influences the self-assembly behavior of the hybrid structures.

The general synthetic scheme for the polyester hybrids starts with the synthesis of the mono- or diamino polyester (A) macroinitiators, followed by the ROP of the desired NCA to give the corresponding AB and BAB hybrids, respectively, where B are the polypeptide chains.

A diethyl aluminum alkoxide bearing an alkylbromide functional group was employed by Degée et al.[129] for the polymerization of CL. The hydroxyl end group was protected by reaction with acetic anhydride. The bromo end group was subsequently converted to the corresponding azido group, which was then reduced to a primary amine group (Scheme 37). The resulting α-acetyl-ω-NH_2-PCL initiated the polymerization of the BLG-NCA. The molecular weight (NMR) was in close agreement with the stoichiometric value, revealing that the macroinitiator was quantitatively consumed. However, detailed molecular characterization was not provided.

The seven-membered cyclic initiator 2,2-dibutyl-2-stanna-1,3-dioxepane was used by Kricheldorf et al.[130] to produce macrocyclic PCL (Scheme 38). Subsequent reaction of the

Scheme 40. Synthesis of Block of Hybrids PLA with PAla, PPhe, PLeu, PBLG, and P(β-benzyl-L-aspartate)

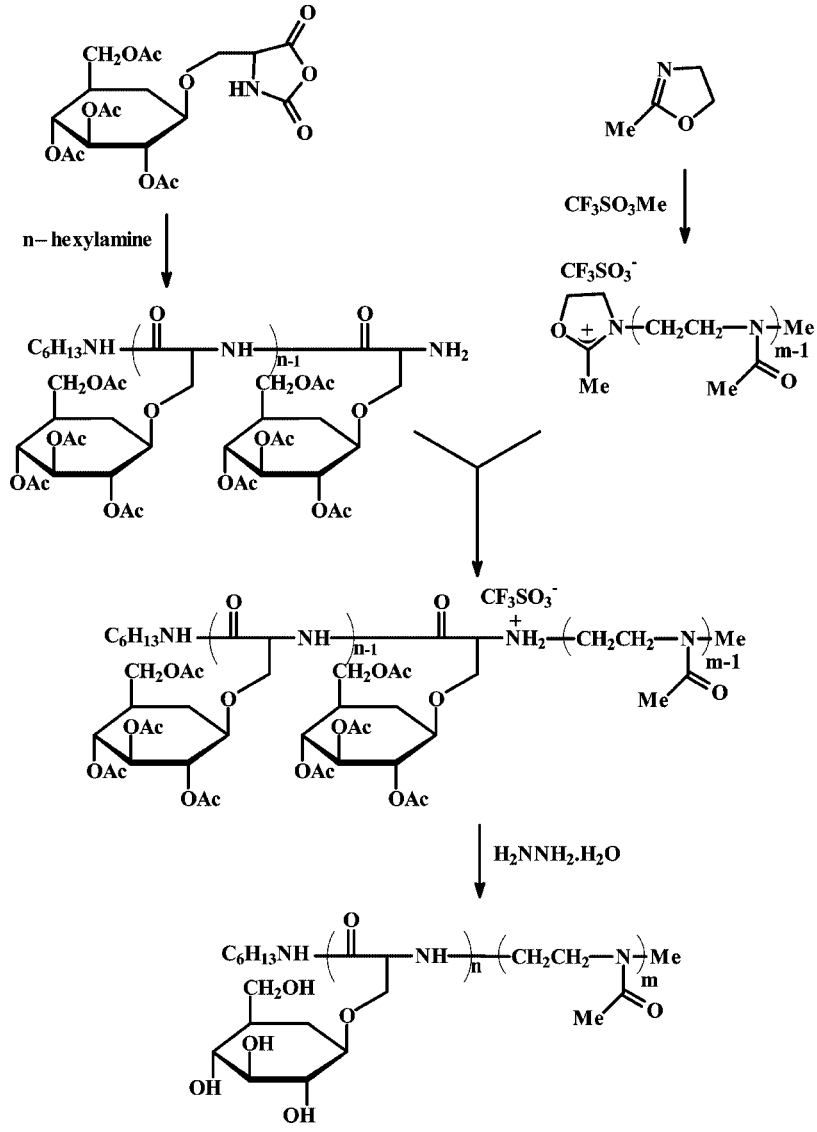


two OH's with 4-nitrobenzoyl chloride yielded the corresponding α,ω-dinitrobenzoyl PCL, which was then converted to the α,ω-diamine PCL by catalytic hydrogenation. These polymers were then employed as macroinitiators for the polymerization of the Gly-, Ala-, Phe-, and BLG-NCAs to afford the respective triblock hybrids.[131] The low nucleophilicity of the 4-aminobenzoyl end groups prevented the aminolytic cleavage of the PCL chains during synthesis and storage but also raised questions regarding their reactivity as initiators for the polymerization of NCAs. The samples were characterized via viscometry, IR and NMR spectroscopy. The yields of the NCA polymerization were high but not quantitative (between 60 and 80%). The poor solubility of the polypeptides in organic solvents prevented the determination of the molecular weight distribution of the copolymers by SEC. However, there was evidence (NMR) that, during the BLG-NCA polymerization, a mixture of di- and triblocks was obtained, due to the low initiation rate compared to that of propagation for this specific monomer.

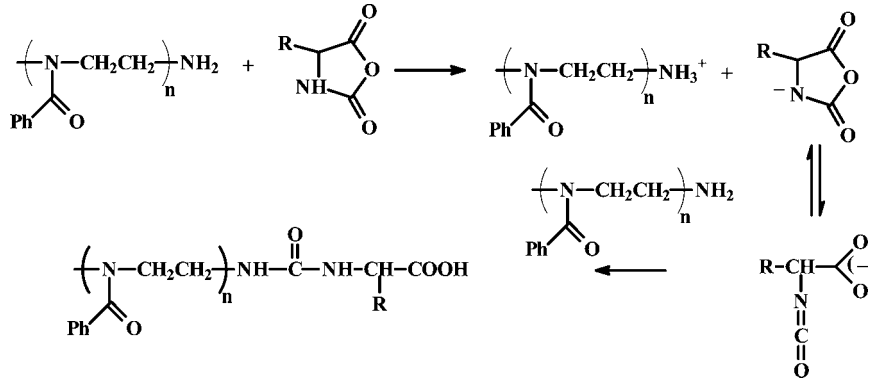
Calcium 4-nitrophenethoxide was used by Rong et al.[132] to polymerize CL, leading to polymers bearing nitrophenyl end groups. These end groups were subsequently transformed to aminophenyl moieties through catalytic hydrogenation. The aromatic amine groups served as initiating sites for the polymerization of the BLG-NCA to afford PCL-*b*-PBLG (Scheme 39). Due to the association of the aromatic amino groups of the initiator, the molecular weights were higher than expected. In addition, SEC analysis could not be carried out.

Hybrids have also been prepared using PLA macroinitiators. Two different approaches have been developed by Gotsche et al.[133] for the synthesis of these macroinitiators (Scheme 40). The first involved the use of *tert*-butoxycarbonyl-protected 1-amino-propanol, a heterofunctional initiator bearing both an OH and a protected amine group. Reaction with Et_2Zn yields the corresponding zinc alcoholate, followed by polymerization of LA. The amino deprotection was subsequently performed under anhydrous conditions with trifluoroacetic acid at 0 °C, leading to the synthesis of the desired PLA macroinitiator. Alternatively, the PLA macroinitiator was prepared by end-capping the hydroxyl end-functionalized PLA, prepared by classic ROP, with *tert*-butoxycarbonyl-protected phenylalanine. Using either of these methods, block hybrids of PLA with PAla, PPhe, PLeu, PBLG, or P(β-benzyl-L-aspartate) have been prepared.[133−135] Using the end-capping method, the triblock terpolymer PEG-*b*-PLA-*b*-PBLG has been synthesized.[136] Characterization of the products revealed that the macroinitiators were efficiently prepared and were quantitatively consumed during the polymerization reactions. Self-assembly phenomena observed for most of these structures prevented the use of SEC for the determination of the molecular weight distribution.

Scheme 41. Synthesis of Poly(2-methyl-2-oxazoline)-*b*-poly[*O*-(tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-serine]



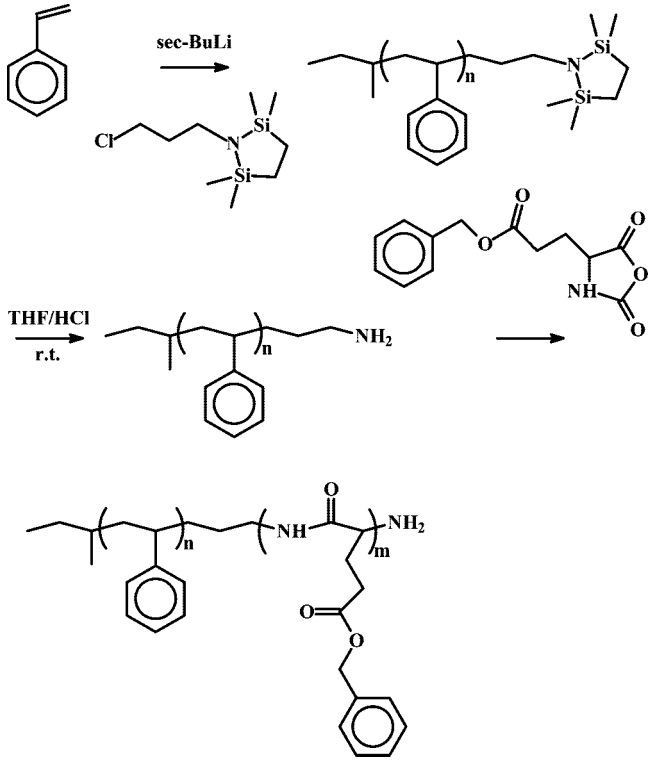
Scheme 42. Production of Inactive Poly(2-phenyl-2-oxazoline) Bearing a Hydantoic Acid *ω*-End



Block hybrids with polyoxazolines have also been prepared, leading to the formation of amphiphilic copolymers with interesting solution properties. The synthesis of poly(2-methyl-2-oxazoline)-*b*-poly[*O*-(tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-serine] has been conducted by Tsutsumichi et al.[137] according to the reactions given in Scheme 41. The glucosylated-NCA was polymerized with *n*-hexylamine, whereas the oxazoline was polymerized by cationic polymerization using methyl trifluoromethanesulfonate as initiator. Equimolar quantities of the macromolecular reagents were mutually terminated to give the desired block copolymer, with a conversion of 87% after 120 h of reaction. The contamination with the homopolymeric chains cannot be avoided, and therefore, extensive purification is required, leading to low overall yield (45%). The block copolymer was deacetylated by hydrazine under mild conditions to yield the corresponding poly(glucopeptides)−polyoxazoline block copolymers.

Alternatively, polyoxazoline and polypeptide block copolymers have been prepared using *ω*-amino-terminated polyoxazoline macroinitiators, prepared by the cationic ring-

Scheme 43. Synthesis of Oligostyrene-*b*-PBLG Hybrids

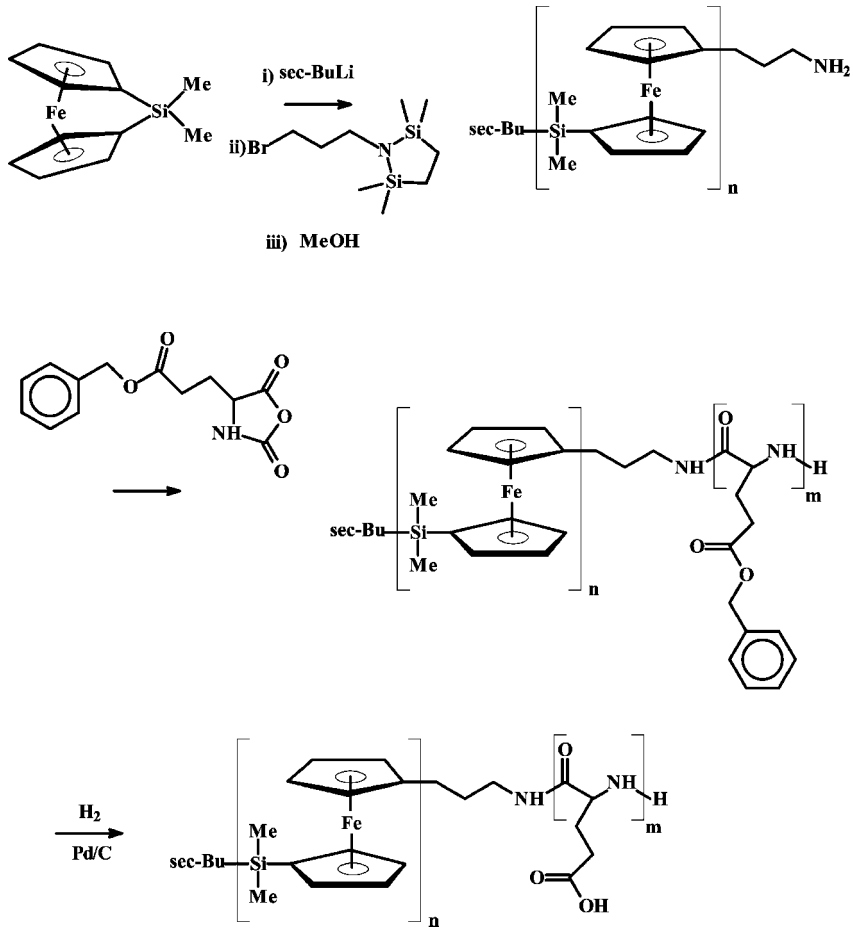


opening polymerization of 2-oxazolines with methyl *p*-toluenesulfonate as initiator and followed by termination with ammonia. Using this approach, the following hybrids have been efficiently prepared by Tsutsumichi et al.:[138,139] poly(2-methyl-2-oxazoline)-*b*-poly(γ-benzyl-L-glutamate), poly(2-methyl-2-oxazoline)-*b*-poly(phenylalanine), poly(2-methyl-2-oxazoline)-*b*-poly[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine], and poly(2-phenyl-2-oxazoline)-*b*-poly[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine]. SEC analysis confirmed the quantitative consumption of the macroinitiator, leading to copolymers with molecular weights close to the stoichiometric values and relatively narrow molecular weight distributions ($1.14 \leq M_w/M_n \leq 1.18$). On the contrary, the synthesis of poly(2-phenyl-2-oxazoline)-*b*-poly(γ-benzyl-L-glutamate) led to a mixture of the desired copolymer and inactive poly(2-phenyl-2-oxazoline) bearing a hydantoic acid ω-end (Scheme 42).

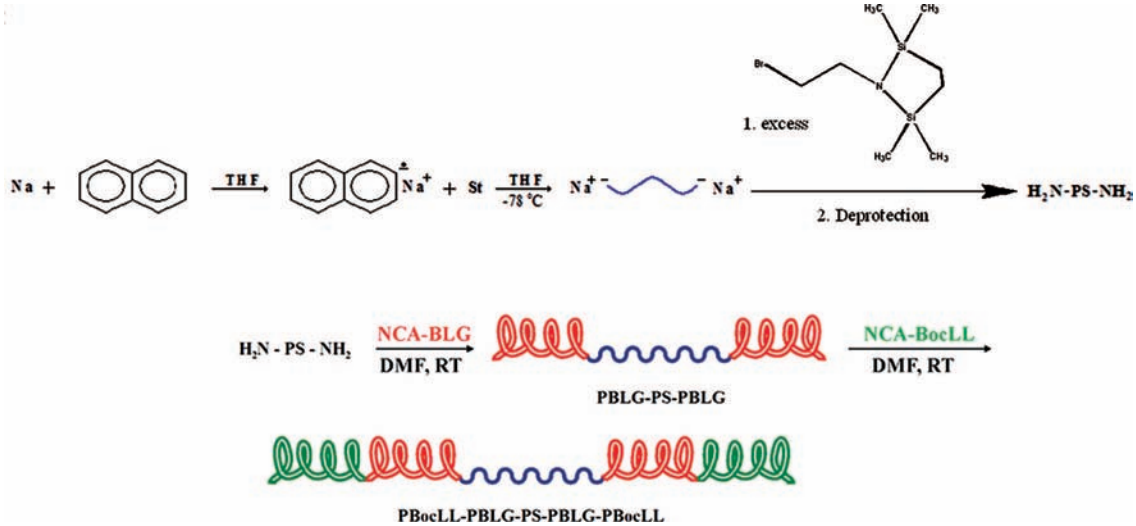
Living or even controlled polymerizations offer the advantage of quantitative introduction of amine groups at one or both chain ends, with the best results being obtained from anionic polymerization. In this respect, an ω-amino-functionalized styrene oligomer was synthesized by Klok et al.[140] after termination of the living oligomer with 1-(3-chloropropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane, followed by acidolysis of the protective group (yield: 85%). The unreacted styrene oligomer was removed by flash chromatography. The amino-functionalized oligomers were subsequently used to initiate the polymerization of BLG-NCA, leading to well-defined copolymers that were characterized by SEC and NMR techniques (Scheme 43).

A similar approach was adopted by Kim et al.[141] for the synthesis of the poly(ferrocenyldimethylsilane)-*b*-poly(γ-benzyl-L-glutamate) (PFS-*b*-PBLG) (Scheme 44). Dimethyl[1]-silaferrocenophane was polymerized anionically at room

Scheme 44. Synthesis of Poly(ferrocenyldimethylsilane)-*b*-PBLG Hybrids



Scheme 45. Synthesis of PBocLL-*b*-PBLG-*b*-PS-*b*-PBLG-*b*-PBocLL Pentablock Terpolymers



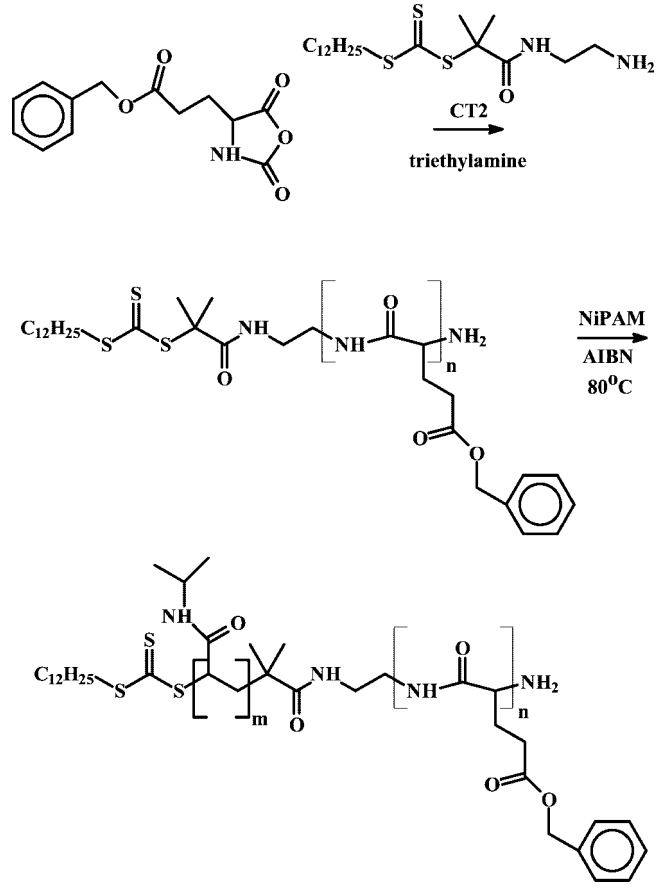
temperature followed by quenching with 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane at −76 °C and deprotection by precipitation in methanol. The unfunctionalized polymer was removed by column chromatography. The PFS-NH_2 macroinitiators were then used to polymerize the BLG-NCA, leading to the desired hybrid. Monomodal peaks of narrow molecular weight distributions were revealed by SEC. Catalytic hydrogenation of the PBLG blocks afforded the corresponding poly(ferrocenyldimethylsilane)-*b*-poly(LGA) block copolymer.

Linear pentablock terpolymers PBLL-*b*-PBLG-*b*-PS-*b*-PBLG-*b*-PBLL (BLL is *tert*-butyloxycarbonyl-L-lysine) were recently synthesized by Karatzas et al.[68] using α,ω-diamino-PS. This polymer was prepared by anionic polymerization using sodium/naphthalene as a difunctional initiator and terminated with 1-(3-bromopropyl)-2,2,5,5-tetramethylaza-2,5-disilacyclopentane, followed by deprotection using successive precipitations of the polymer in methanol. The PS difunctional macroinitiator was subsequently used for the sequential polymerization of BLG-NCA and BLL-NCA under high vacuum (Scheme 45). The molecular weights were in very close agreement with the stoichiometric values, and the molecular weight distribution is very narrow, indicating that well-defined samples were prepared with this methodology.

Conventional radical polymerization was employed by Cheonet et al.[142] for the synthesis of amine-terminated poly(N-isopropylacrylamide) in the presence of 2-aminoethanthiol hydrochloride as the chain transfer agent. These amino-macroinitiators were subsequently employed for the synthesis of block copolymers with PBLG. The samples were not characterized in detail.

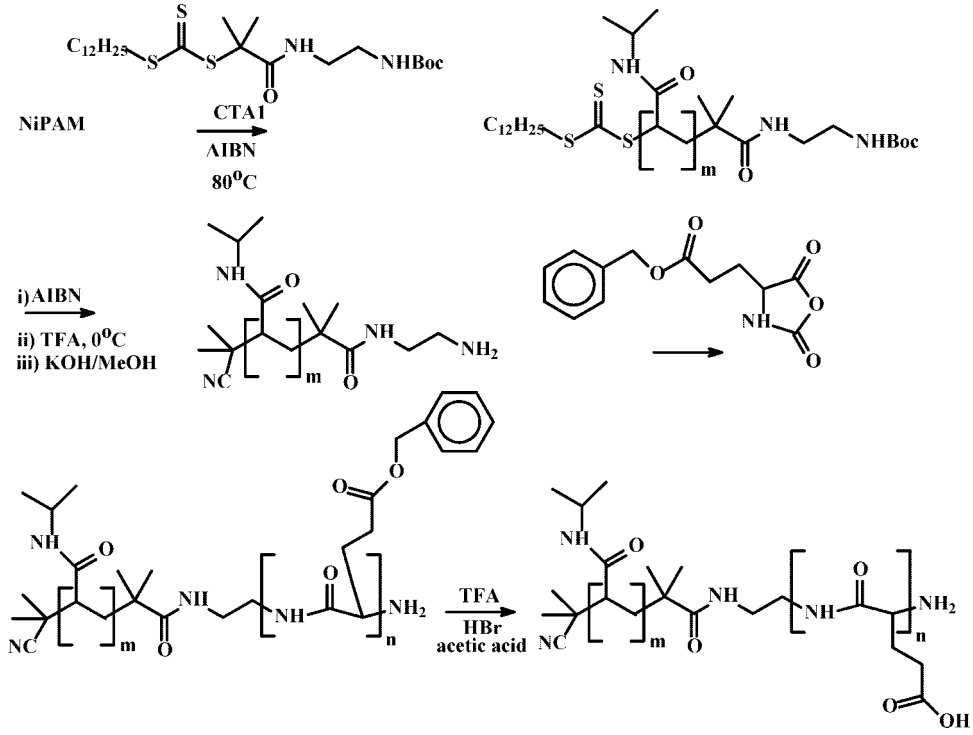
A better control for the synthesis of the same product was achieved with the RAFT polymerization. Two different approaches were employed by Zhang et al.[143] The first (Scheme 46) is based on the synthesis of a suitable macromolecular chain transfer agent (CTA), while the second is based on the synthesis of a macroinitiator (Scheme 47).[144] CTA-1 was synthesized and transformed to CTA-2 after removal of the Boc protective group. CTA-2 was employed for the polymerization of BLG-NCA through its free amino group to yield the macromolecular PBLG-CTA, followed by the RAFT polymerization of *N*-isopropylacrylamide. The polymerization yields were not quantitative, and the molecular weight

Scheme 46. Synthesis of PNiPAM-*b*-PBLG Copolymers through the Macromolecular Chain Transfer Agent Route

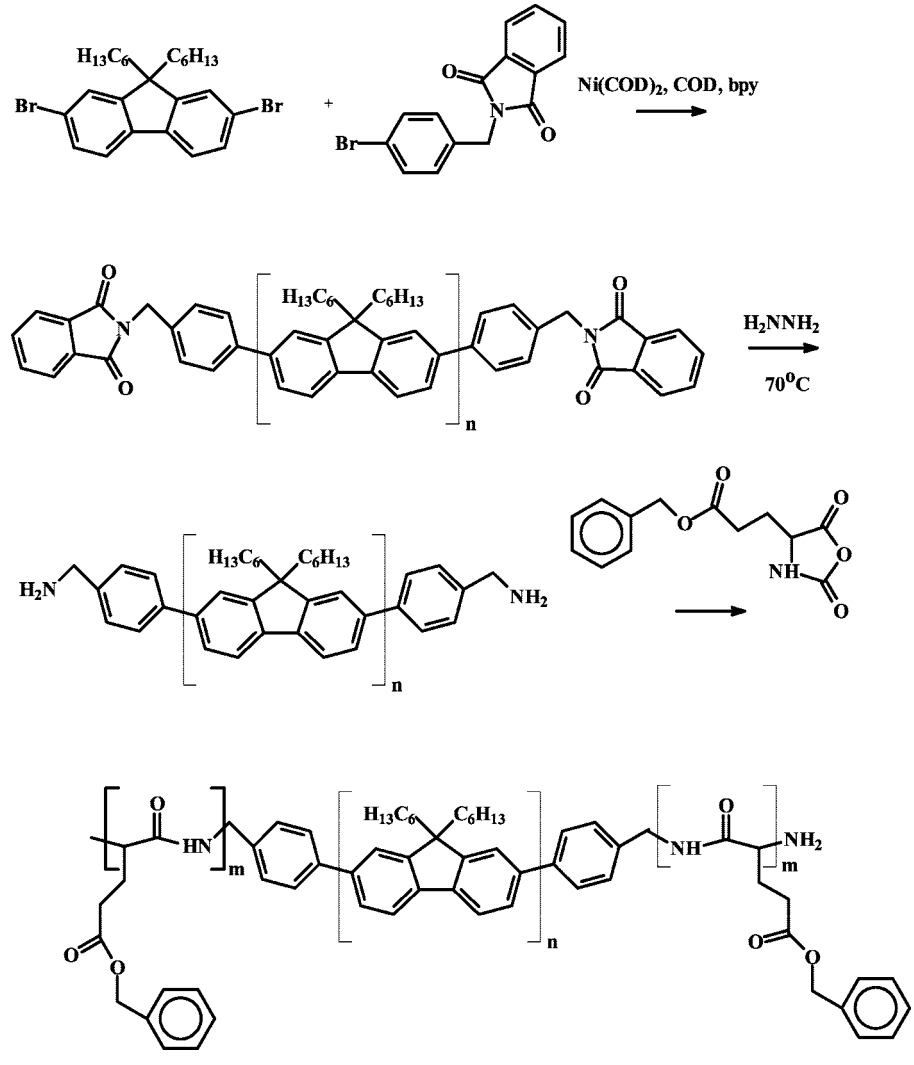


distributions were rather broad (up to 1.65). Aminolysis of the trithiocarbonate group by the free amino end group from the polypeptide block was considered the main side reaction, leading to relatively broad distributions. According to the second route, *N*-isopropylacrylamide was polymerized first using CTA-1 followed by the deprotection of the amino end group, to afford the macroinitiator, which was then utilized to polymerize the BLG-NCA. The second method led to monomodal SE chromatograms for most samples with relatively narrow molecular weight distributions ($1.19 \leq M_w/M_n \leq 1.40$).

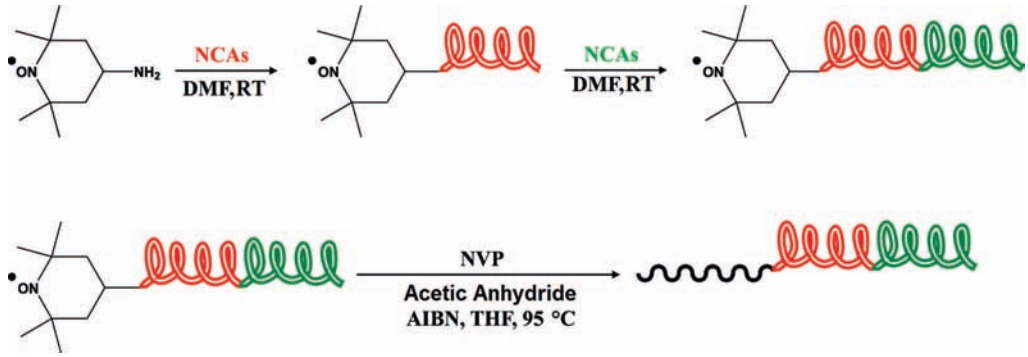
Scheme 47. Synthesis of PNiPAM-*b*-PBLG Copolymers through the Macroinitiator Route



Scheme 48. Synthesis of ABA Block Copolymers, Where B Is Poly[2,7-(9,9-dihexylfluorene)] and A Is PBLG



Scheme 49. Synthesis of PNVP-*b*-PBLG-*b*-PZLL Triblock Terpolymers



Step-growth polymerization is another technique for the synthesis of telechelic amine macroinitiators. The Yamamoto coupling polymerization of 2,7-dibromo-9,9-dihexylfluorene was performed by Kong et al.[145] followed by the end-capping reaction with *N*-(*p*-bromobenzyl)phthalimide. Deprotection with hydrazine afforded the telechelic amine macroinitiators, which were subsequently used for the polymerization of BLG-NCA. Due to the nature of the step-growth polymerization, the molecular weight distribution of the triblock copolymers was very broad (M_w/M_n values higher than 2.0) (Scheme 48).

An amine-functionalized TEMPO radical was employed by Karatzas et al.[127] for the synthesis of poly(*N*-vinylpyrrolidone), PNVP, copolymers with PBLG and PZLL blocks, i.e. PNVP-*b*-PBLG, PNVP-*b*-PZLL, and PNVP-*b*-PBLG-*b*-PZLL (Scheme 49). The polypeptide blocks were initially prepared through the amino group. The TEMPO moiety did not interfere with the polymerization and allowed the synthesis of polypeptides with very narrow molecular weight distributions. The final step involved the nitroxide-mediated polymerization of NVP, leading to the synthesis of copolymers with relatively low polydispersity and controlled molecular weights.

A novel approach, based on "click" chemistry, was applied by Lecommandoux et al.[146] for the synthesis of glycoprotein analogues in which a polysaccharide block is chemically linked to a polypeptide chain (Scheme 50). Reductive amination of dextran was performed using propargylamine in acetate buffer (pH = 5.0) in the presence of sodium cyanoborohydride. In another reactor, 1-azido-3-aminopropane was employed as a functional initiator for the polymerization of BLG-NCA leading to a polypeptide chain with azide end groups. The polysaccharide and polypeptide blocks were then coupled in DMSO, a good solvent for both blocks, in the presence of CuBr and the ligand pentamethyldiethylenetriamine, PMDETA, at room temperature. An excess of dextran was employed for the quantitative reaction of the functionalized PBLG chain. The excess dextran was removed by dialysis against water. The synthesis was confirmed by NMR and IR spectroscopy.

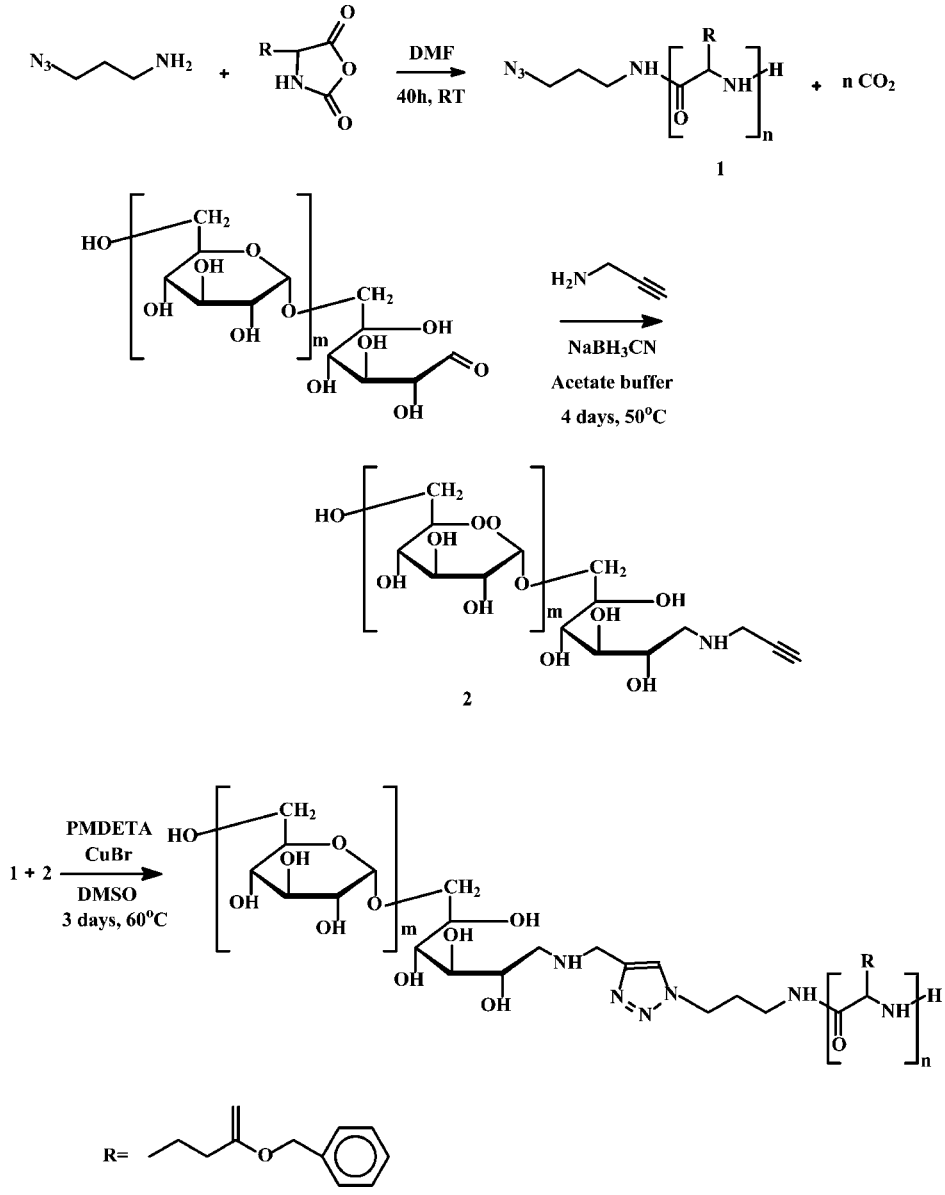
3.2.2.2.2. Transition Metal Complex Macroinitiators. Zero valent metal complexes cannot be used directly for the synthesis of polypeptide hybrid block copolymers. However, N^{α}-allyloxycarbonyl-amino acid allyl amides can be used as universal precursors for the amido-amidate nickelacycle initiators (Scheme 51). As shown in the scheme, the N^{α}-allyloxycarbonyl-amino acid derivatives may undergo tandem oxidative additions to nickel(0) giving the nickelacycle initiators. This method was initially employed for the synthesis of block copolypeptides and was then expanded by Deming and co-workers to a variety of hybrid structures.

This methodology was applied for the synthesis of PBLG-*b*-polyoctenamer-*b*-PBLG and PBLG-*b*-polyethylene-*b*-PBLG triblock copolymers[147] using the reaction sequence given in Scheme 52. Acyclic diene metathesis, ADMET, polymerization was employed for the synthesis of α,ω-diamino-functionalized polyoctenamer using Grubbs' catalyst $RuCl_2$-(=CHPh)$(PCy_3)_2$. This product was converted to the bisallylformate-L-leucine-terminated polyoctenamer, followed by reaction with [1,2-bis(diethylphosphino)ethane]Ni(COD), depeNi(COD), to produce the active sites for the subsequent polymerization of BLG-NCA. The Wilkinson catalyst was finally employed for the hydrogenation of the polyoctenamer block to afford the corresponding polyethylene block. Since the polyoctenamer precursor was insoluble in DMF, where the polymerization of BLG-NCA took place, unreacted polymer was filtered prior to the polymerization of the NCA. Due to the nature of ADMET polymerization and to the heterogeneity of the NCA polymerization, the produced triblock copolymers were polydisperse even though SEC analysis and degradation studies confirmed the quantitative consumption of the purified polyoctenamer precursor. This work was also applied for the synthesis of triblock copolymers where the middle block was either poly(ethylene glycol), PEG, or poly(dimethyl siloxane), PDMS.[148]

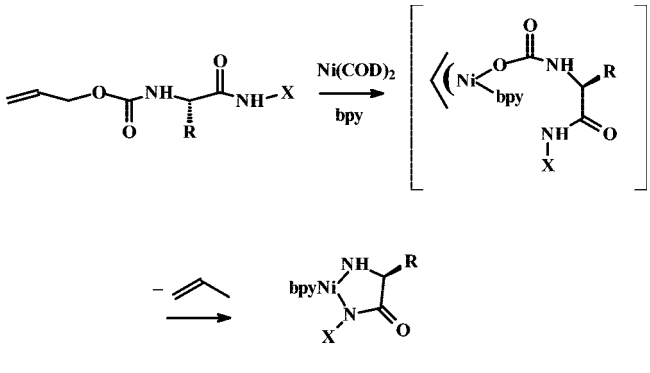
The same approach was also efficiently employed for the synthesis of poly(methyl acrylate)-*b*-PBLG hybrids[149] (Scheme 53). ATRP was successfully used to produce amino end-functionalized poly(methyl acrylate), which was then transformed to Ni-macroinitiators for the polymerization of BLG-NCA, leading to well-defined copolymers.

It is well-known that amido-metallacycle end groups are the active species for the synthesis of polypeptides by transition metal complexes. Therefore, electrophilic reagents, such as isocyanates, can react with these end groups through formation of stable urea linkages. Using this idea, isocyanate end-capped PEG was reacted in excess with living PBLG to produce PBLG-*b*-PEG[148] (Scheme 54). SEC and NMR measurements revealed that the coupling reaction was near quantitative. This work was further expanded to the synthesis of CABAC pentablock terpolymers, where C was PEG, A was PBLG, and B was polyoctenamer, PEG or PDMS. The synthesis was achieved through the coupling reaction of the living ABA triblock copolymers with isocyanate end-capped PEG. Repeated precipitation from THF solutions into methanol was employed for the purification of the pentablocks. SEC analysis confirmed that monomodal traces of relatively narrow molecular weight distribution were obtained.

Scheme 50. Synthesis of Block Copolymers of Dextran and PBLG



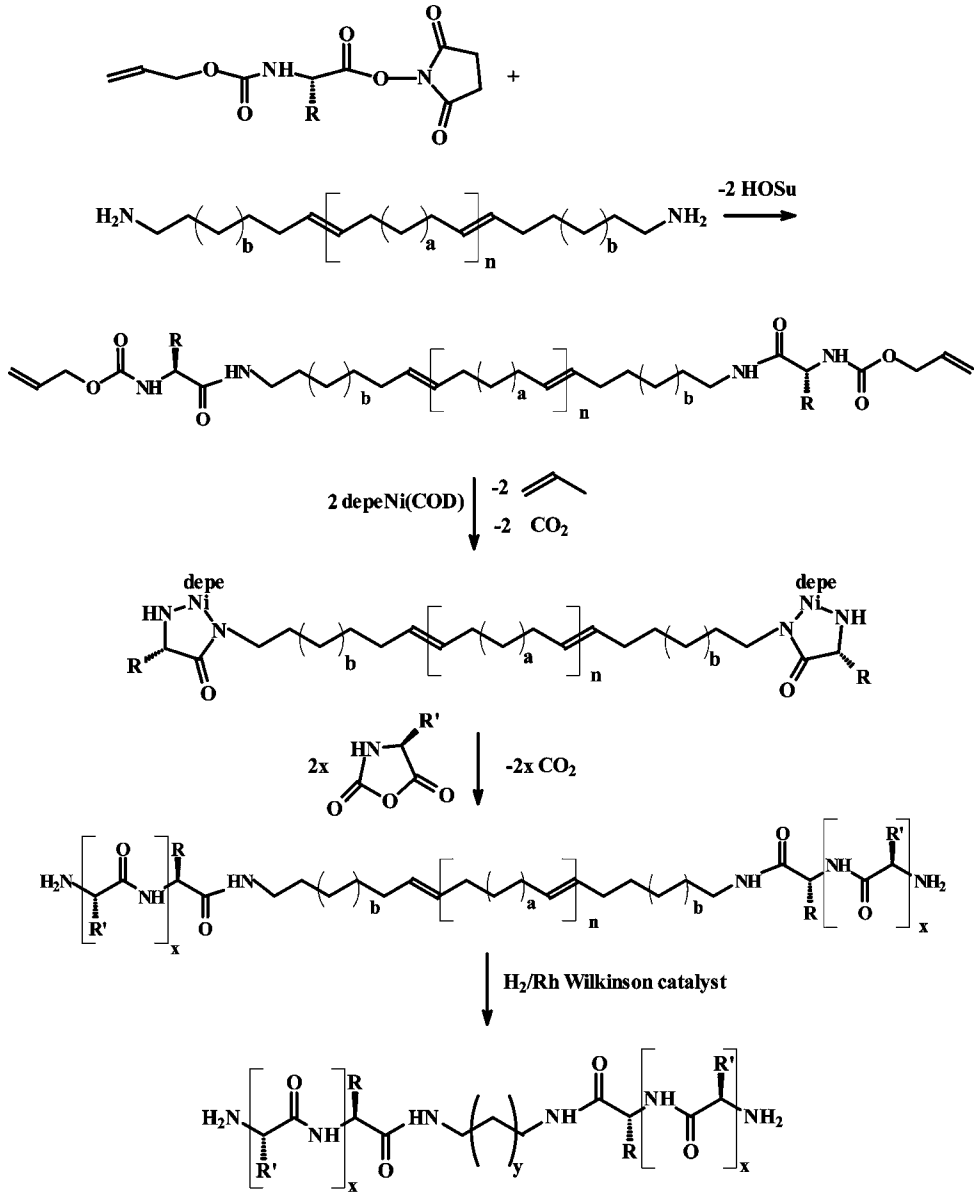
Scheme 51. Nickelacycle Macroinitiators for the Synthesis of Hybrid Copolymers



Rod−rod hybrid block copolymers of PBLG and either poly((*S*)-(−)-α-methylbenzyl isocyanide) or poly(L-isocyanoalanyl-L-alanine methyl ester) were prepared by Kros et al.[150] using Ni(COD)(bpy) and sequential addition of monomers, starting from BLG-NCA. Since the amidonickelacycle end groups are known to react with electrophiles, it was assumed that the isocyanide monomer interacts with the nickel center through the formation of a temporary five-coordinate complex to yield a carbine-like initiator complex, which can promote the polymerization of isocyanides (Scheme 55). The final products were contaminated by homopolymers and were found to display relatively broad molecular weight distributions (M_w/M_n values up to 1.47).

3.2.2.2.3. Primary Amine Hydrochloride Macroinitiators. Primary amine hydrochlorides can be used as initiators for the polymerization of NCAs, as discused above. In this respect, Schlaad et al.[56,151] anionically prepared amino end-functionalized PS, served as macroinitiators for the synthesis of PS-*b*-PZLL hybrid block copolymers. The final products were purified by extraction with cyclohexane to remove unreacted PS precursor. The purified copolymers were characterized by very narrow molecular weight distributions. However, the efficiency of the initiator was not quantitative (around 80% at most) and the molecular weight of the

Scheme 52. Synthesis of PBLG-*b*-polyoctenamer-*b*-PBLG and PBLG-*b*-polyethylene-*b*-PBLG Triblock Copolymers



HOSu=N-hydroxysuccinimide
depe= 1,2-bis(diethylphosphino)ethane

polypeptide block was very low. It was observed that, by increasing the molecular weight of the polypeptide, the molecular weight distribution was substantially broadened.

The same methodology was also adopted by Lutz et al.[152] for the synthesis of PEO-*b*-PBLG and PEO-*b*-poly(β-benzyl-L-aspartate) using amine hydrochloride-functionalized PEO macrioinitiators. The hybrids obtained had very narrow molecular weight distributions, but the degree of polymerization for the peptide block was very low (up to 10). A small amount of homopolypeptides was also present in the crude product and was eliminated by selective precipitation.

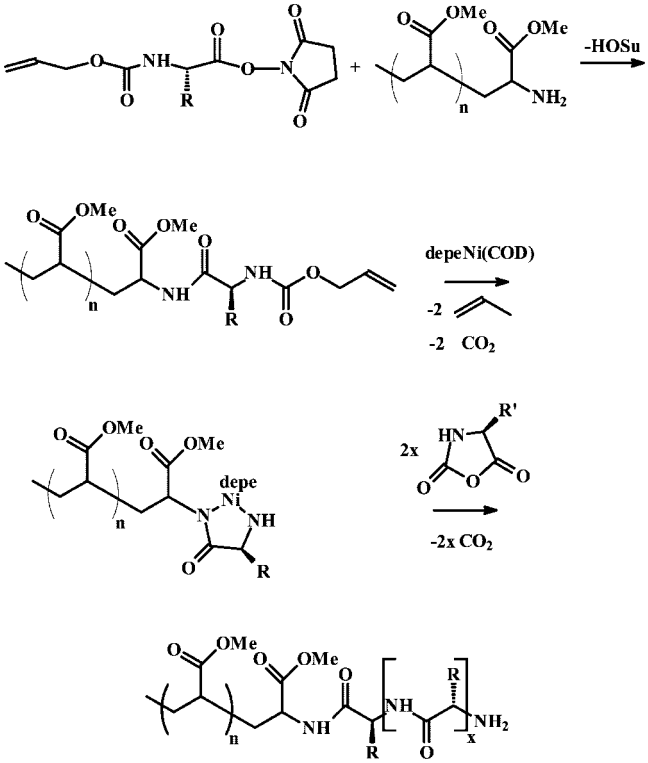
3.3. Star-Shaped Architectures

Star polymers are branched polymers consisting of several linear chains linked to a central core. Among the general synthetic routes[153] that have been developed for the synthesis of star polymers, two have preferentially been used for the synthesis of structures bearing polypeptides arms, i.e. the use of multifunctional initiators and multifunctional linking agents.

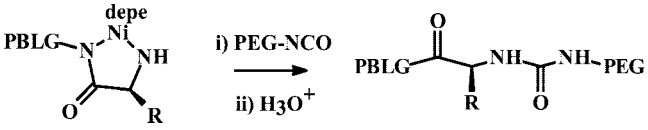
3.3.1. Multifunctional Initiators

This method is referred to as the "core-first" or "arm-out" or divergent approach. According to this procedure, multifunctional compounds capable of simultaneously initiating the polymerization of several arms are used. There are several requirements a multifunctional initiator has to fulfill in order to produce star polymers with controllable molecular weights, uniform arm lengths, and low molecular weight distribution. All initiating sites must be equally reactive and the initiation rate must be higher than the propagation rate. The characterization of the star polymers produced by this method is difficult, since the molecular weight of the arms cannot be measured directly. The number of arms can be defined

Scheme 53. Synthesis of Poly(methyl acrylate)-*b*-PBLG Hybrids



Scheme 54. Reaction of Isocyanate End-Capped PEG with Living PBLG To Produce PBLG-*b*-PEG Copolymers



indirectly by several methods, such as end-group analysis or determination of the branching parameters, which are the ratios of either the mean square radius of gyration, the intrinsic viscosity, or the hydrodynamic radius of the star to the corresponding linear with the same molecular weight. Finally, the determination of the functionality can be achieved by isolation of the arms after cleavage (i.e., hydrolysis), if possible, and subsequent analysis. This approach is the most widely used for the synthesis of polypeptide stars.

3.3.2. Multifunctional Linking Agents

This method is referred to as the "arm-first" or "arm-in" or convergent approach. It involves the synthesis of living macromolecular chains and their subsequent reaction with a multifunctional linking agent. It is probably the most efficient way to synthesize well-defined star polymers because of the absolute control that can be achieved in all synthetic steps. The functionality of the linking agent determines the number of the branches of the star polymer, provided that the linking reaction is quantitative. The living arms can be isolated before linking and characterized independently along with the final star. Consequently, the functionality of the star can be measured directly and with accuracy. Disadvantages of the method can be considered to be the long time required for the linking reaction in most cases and the need to perform fractionation in order to obtain the pure star polymer, since a small excess of the living arm is needed to ensure complete linking.

Using these methodologies and a combination of different polymerization techniques, several polypeptide-based star polymers have been prepared. Perylene derivatives functionalized with four primary amine groups were employed by Klok et al.[154] as tetrafunctional initiators to provide 4-arm PBLG and PZLL stars (Scheme 56). The polymerization yields were not very high, especially for the PZLL stars. It was found that the products had relatively broad molecular weight distributions and that for longer polypeptides (DP > 400) the molecular weights substantially deviated from the stoichiometric values, due to extended termination reactions.

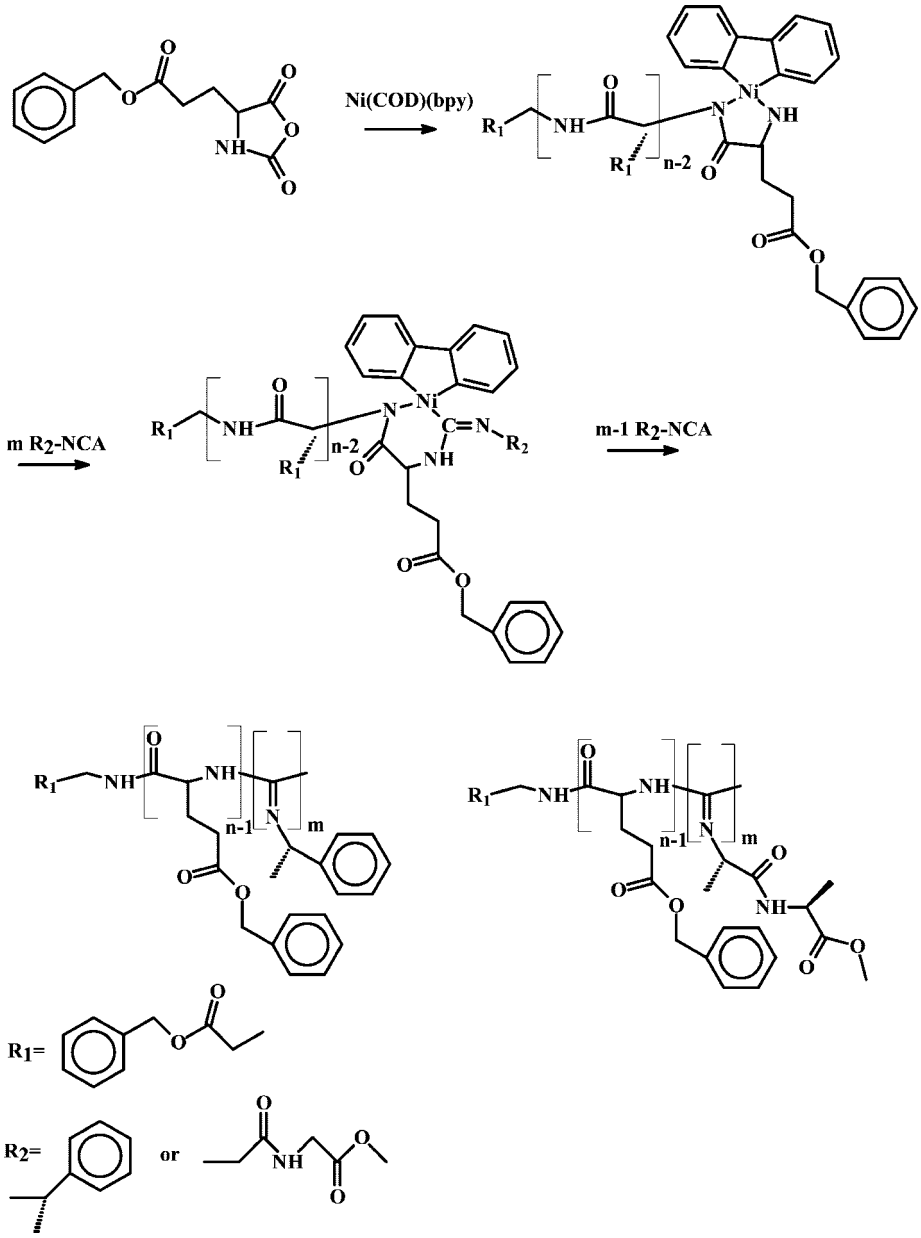
Hexakis(4-benzylamino-1-oxy)- and hexakis(4-aminophenoxy) cyclotriphosphazene were employed by Inoue et al.[155,156] as hexafunctional initiators for the synthesis of 6-arm PBLG star polymers (Scheme 57). The first multifunctional initiator carrying primary amines was successfully employed for the synthesis of stars with the desired functionality. However, for structures with higher arm molecular weight (DP > 33), a second SEC trace was observed and the products possessed broader molecular weight distributions. On the other hand, the second multifunctional initiator carrying aromatic amines was found to produce stars with lower functionalities depending on the molar ratio of the monomer to the initiator.

Dendrimers functionalized with a specific number of primary amine groups at their periphery were used as multifunctional initiators for the polymerization of NCAs. For example, poly(amido amine) dendrimers of the third to fifth generations were employed for the polymerization of NCAs bearing sugars as side groups.[157] In addition, poly(trimethyleneimine) dendrimers produced 64-arm stars of polysarcosine,[158] whereas poly(propylene imine) dendrimers gave 4- and 8-arm P(BLG-*co*-DL-Val) stars.[159] The functional amine groups quantitatively served as initiating sites, leading to narrow molecular weight distribution stars.

In a more recent study, 2-(aminomethyl)-2-methyl-1,3-propanediamine (Scheme 58) was employed by Aliferis et al.[160] as a trifunctional initiator for the synthesis of P(BLL-*b*-BLG)$_3$ 3-arm star-block copolypeptides. BLG-NCA was polymerized first followed by the addition of BLL-NCA. Monomodal peaks of very narrow molecular weight distributions were obtained by SEC. Furthermore, the composition and the molecular weights were in agreement with the expected values, indicating that well-defined structures were synthesized.

The use of polypropylenimine tetraamine dendrimer and amidoethylethanolamine dendrimer (PAMAM) as tetrafunctional initiator for the preparation of P(BLG-*b*-BLL)$_4$ 4-arm star-block copolypeptides failed to give monomodal SEC traces. A second peak was observed, attributed to linear polypeptides, which resulted from the tertiary amines of the multifunctional initiators. This problem was not encountered in previous studies with dendrimers. The high purity of the system and the low generation of the dendrimers, which allows for the easy approach of the monomer to the tertiary amine groups, may account for the formation of linear polypeptides. The pure star-block copolymers were obtained by fractionation.

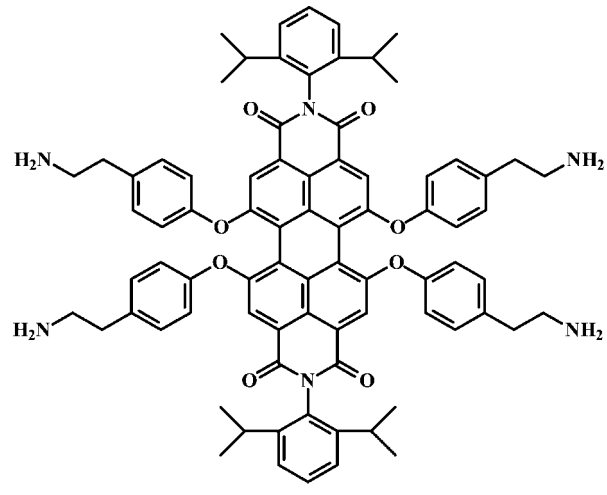
Scheme 55. Synthesis of a Hybrid Block of Copolymers PBLG and Either Poly((*S*)-(−)-α-methylbenzyl isocyanide) or Poly(L-isocyanoalanyl-L-alanine methyl ester)



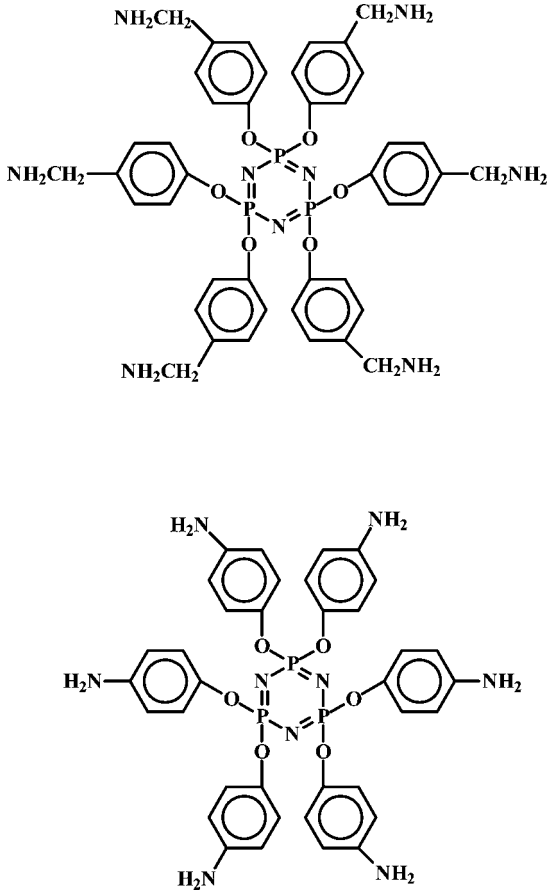
Four-arm PEO stars end-functionalized with primary amine groups were efficiently used by Karatzas et al.[127] for the polymerization of BLG-NCA leading to the synthesis of (PEO-*b*-PBLG)$_4$ star-block copolymers (Scheme 59). The macroinitiator was quantitatively consumed, leading to a product of relatively narrow molecular weight distribution and molecular weight very close to the stoichiometric value.

A combination of ATRP and ROP was performed by Abraham et al.[161] for the synthesis of (PS-*b*-PBLG)$_3$ three-arm star-block hybrid copolymers. ATRP techniques were initially adopted for the synthesis of a three-arm PS star using a suitable cynurate-based initiator. The end-bromine groups were transformed to amines by a three-step azidation route followed by conversion to nickel−amine complex macroinitiators suitable for the polymerization of BLG-NCA (Scheme 60). The functionality of the precursor PS star was confirmed by the measurement of the molecular weights of the stars and the corresponding arms, which were isolated

Scheme 56. Perylene Derivatives Functionalized with Four Primary Amine Groups



Scheme 57. Hexakis(4-benzylamino-1-oxy)- and Hexakis(4-aminophenoxy) Cyclotriphosphazene Hexafunctional Initiators



through hydrolysis of the ester groups connecting the initiator and the arms. The final products were obtained in moderate yields and possessed relatively narrow molecular weight distributions ($1.1 < M_w/M_n < 1.4$).

The second methodology involving suitable linking chemistry was adopted by Aliferis et al.[67] for the synthesis of $(PBLG)_3$, $(PZLL)_3$ 3-arm star homopolymers and (PBLG-*b*-PZLL)$_3$ and (PZLL-*b*-PBLG)$_3$ 3-arm star-block copolypeptides. The living homo- and block copolypeptides carrying amine end groups were reacted with tris(4-isocyanatophenyl)methane (Scheme 61). For the efficient linking reaction, an excess of the living arms was used. The pure stars were obtained by performing the salting-out technique. SEC and membrane osmometry measurements were conducted on both the arms and the stars, revealing that well-defined three-arm stars of very narrow molecular weight distribution were synthesized.

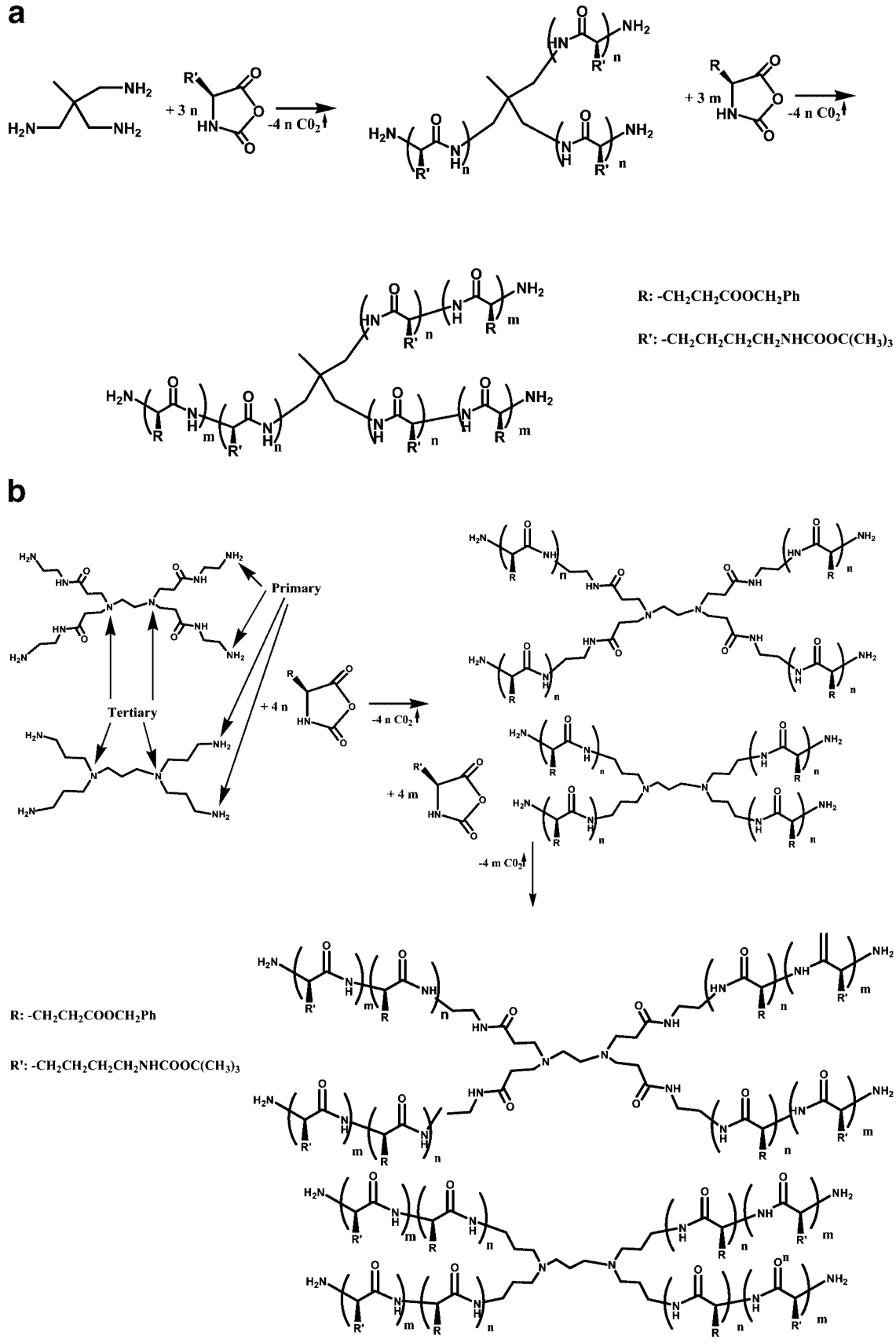
The recent development of several living or controlled polymerization techniques has allowed the synthesis of miktoarm star polymers, i.e. stars consisting of chemically different arms, including polypeptide chains. A combination of ATRP and ROP was employed by Babin et al.[162,163] for the synthesis of $PS(PBLG)_2$ miktoarm star copolymers, as outlined in Scheme 62. Styrene was initially polymerized by ATRP followed by reaction with a large excess of 1-aminotriethylenetriamine for the nucleophilic substitution of the end-bromine group, leading to the synthesis of PS chains bearing two amine end groups. This reaction was not quantitative. The nonfunctionalized PS chains were efficiently removed by selective precipitation in hexane after treatment of the $PS(NH_2)_2$ chains with aqueous HCl. The end-amine groups were subsequently used for the polymerization of BLG-NCA, to afford the desired structures. Hydrolysis using an excess of hydrogen bromide in trifluoroacetic acid led to the cleavage of the benzyloxycarbonyl protective groups and the synthesis of the corresponding amphiphilic miktoarm stars carrying PLGA arms. SEC analysis revealed that the $PS(NH_2)_2$ macroinitiators were quantitatively consumed and that the molecular weight distributions of the final products were fairly narrow ($1.09 < M_w/M_n < 1.22$).

$(PLLA)_2$PBLG mikto arm stars were prepared by Sun et al.[164] using the trifunctional initiator 2-benzyloxycarbonylamino-1,3-propanediol (Scheme 63). The two available hydroxyl groups were employed for the polymerization of L-lactide in the presence of $Sn(Oct)_2$. Subsequent cleavage of the benzyloxycarbonyl protective group, using hydrogen bromide in acetic acid, afforded PLLA chains with a central amine group, $(PLLA)_2NH_2$, which served as macroinitiator for the polymerization of BLG-NCA to give the desired product. NMR analysis revealed that the $(PLLA)_2NH_2$ macroinitiator was efficiently prepared. SEC experiments confirmed the complete consumption of the macroinitiator and the absence of any homopolymer trace. However, the molecular weight distributions of the final products were not measured due to the self-assembly of the miktoarm star copolymers in the solvent used for SEC.

A commercially available PEO bearing two central- and two end-amine groups was employed by Cho et al.[165] as a macroinitiator for the polymerization of BLG-NCA, leading to the synthesis of (PEO-*b*-$PBLG)_2(PBLG)_2$ miktoarm star copolymers (Scheme 64). The molecular weights were determined by NMR analysis. However, details concerning the efficiency of this synthetic approach and the characterization of the products were not provided.

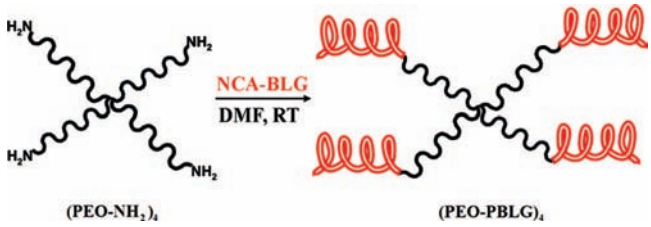
Taking advantage of the unique features of anionic polymerization and the possibilities offered for the synthesis of end- or in-chain amino-functionalized polymers as well as of the living nature of the ROP of NCAs under high vacuum conditions, a variety of well-defined miktoarm star hybrids (macromolecular *chimeras)* was synthesized and fully characterized by Karatzas et al.[68] These samples include the following structures: $(PS)_2(PBLG)$, $(PS)_2(PBLL)$, (PS)(PI)(PBLG), (PS)(PI)(PBLL), $(PS)_2[P(\alpha\text{-MeS})](PBLG)$, $(PS)_2[P(\alpha\text{-MeS})](PBLL)$, $(PS)_2(PBLG)_2$, and $(PS)_2(PBLL)_2$ (Scheme 65). The synthetic strategy involved the preparation of diphenyl ethylene, DPE, functionalized polymers [DPE-chain-end-functionalized PI (PI-D), DPE-in-chain-functionalized polystyrene (PS-D-PS), and DPE-in-chain-difunctionalized polystyrene], shown in Scheme 66. These structures were subsequently activated by reaction with a living polymer chain or *s*-BuLi followed by reaction with 1-(3-bromopropyl)-2,2,5,5-tetramethyl-aza-2,5-disilacyclopentane. The silyl-protected group was cleaved by treatment with HCL or *p*-toluenesulfonic acid, leading to the synthesis of the corresponding amine-functionalized polymers. These products were employed as macroinitiators for the polymerization of BLG- and BLL-NCAs to afford the desired miktoarm stars. Combined characterization by NMR, SEC, and light scattering measurements confirmed the efficiency of this synthetic scheme and the homogeneity of prepared products. The only restriction of this procedure was the steric hindrance of the in-chain functionalized macroinitiators. This became obvious from the fact that

Scheme 58. (a) General Reactions Used for the Synthesis of 3-Arms Star Copolypeptides Using the 2-(Aminomethyl)-2-methyl-1,3-propanediamine Initiator. (b) General Reactions Used for the Synthesis of 4-Arm Star Copolypeptides Using the Amidoethylethanolamine Dendrimer, the 1,4-Diaminobutane Core, Generation 0.0 (PAMAM) and Polypropylenimine Tetraamine Dendrimers, and Generation 1.0 (DAB) Initiators, Respectively



$(PS)_2(NH_2)_2$ macroinitiators led to miktoarm stars with broader molecular weight distributions, whereas the more sterically hindered $(PS)_4(NH_2)_2$ macroinitiators afforded multimodal distributions.

Scheme 59. Synthesis of (PEO-*b*-PBLG)$_4$ Star-Block Copolymers



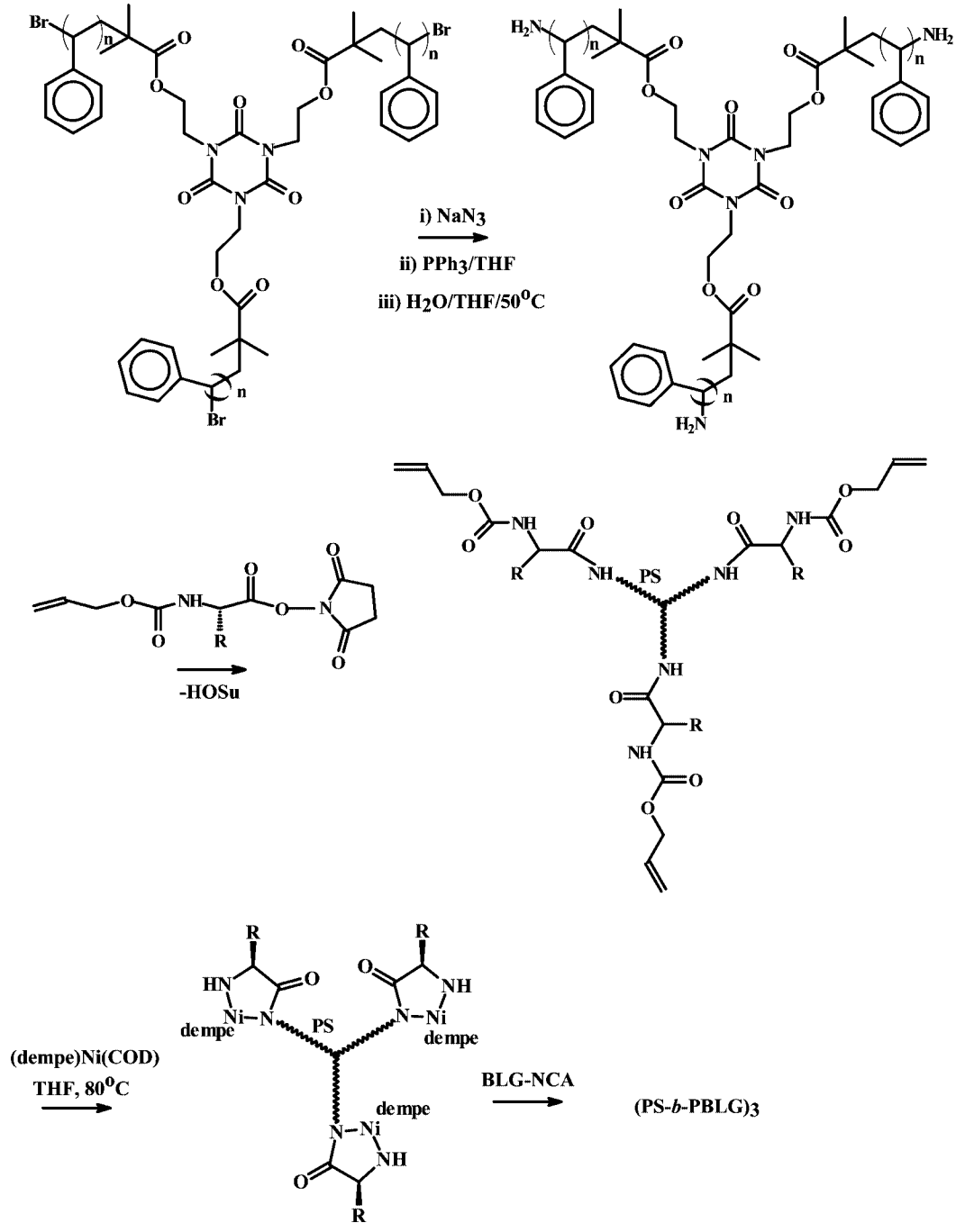
3.4. Complex Architectures

Recent advances in synthetic methodologies have allowed for the synthesis of novel macromolecular structures with excellent control over the topology, microstructure, and composition.[166] The application of organic chemistry reactions for the transformation of the macromolecular chains and for the synthesis of new initiators and linking agents along with the combination of different polymerization methods are the main tools for the synthesis of complex macromolecular architectures. However, the limitations in achieving true living polymerization conditions of NCAs have, in the past, rendered difficult the synthesis of more complex structures containing polypeptide chains. The most common structures, other than those reported above, include graft copolymers.

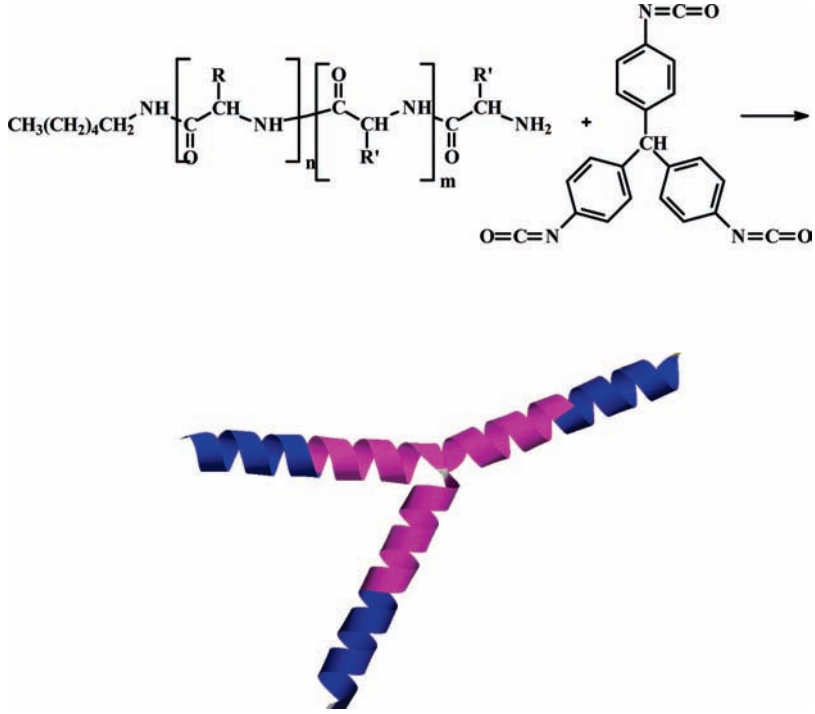
Three general methods have been developed for the synthesis of randomly branched graft copolymers: (a) the "grafting onto", (b) the "grafting from", and (c) the macromonomer method (or "grafting through" method).

The "grafting onto" method involves the use of a backbone chain containing functional groups randomly distributed

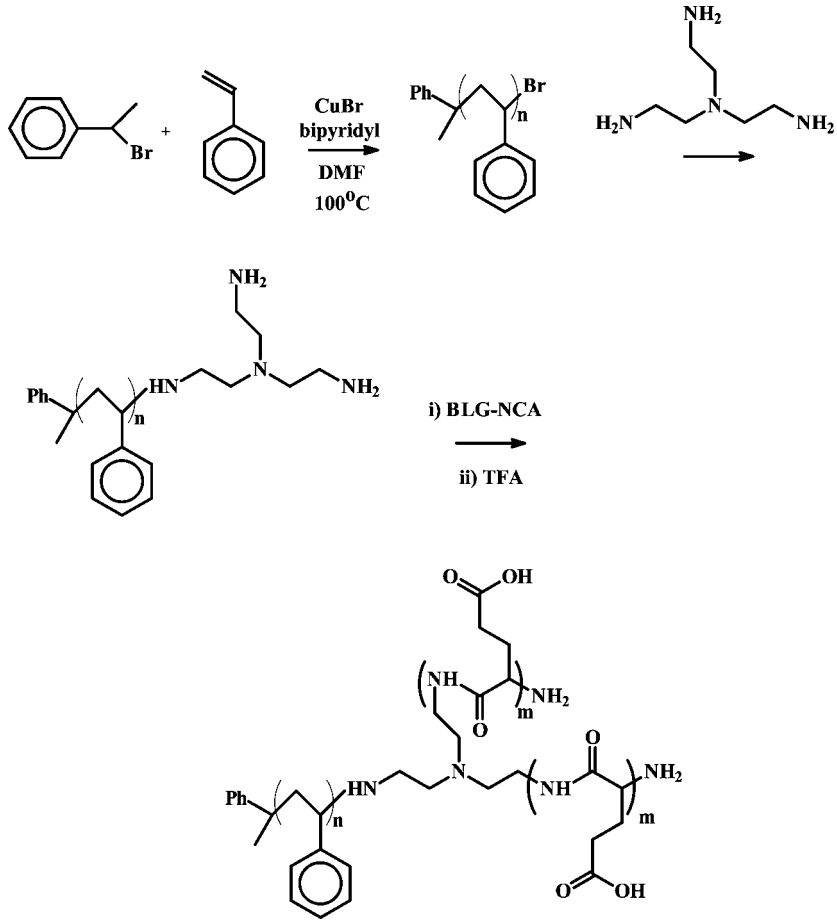
Scheme 60. Synthesis of (PS-*b*-PBLG)$_3$ Star-Block Hybrid Copolymers



Scheme 61. Synthesis of (PBLG-*b*-PZLL)$_3$ Star-Block Copolymers through Suitable Linking Chemistry

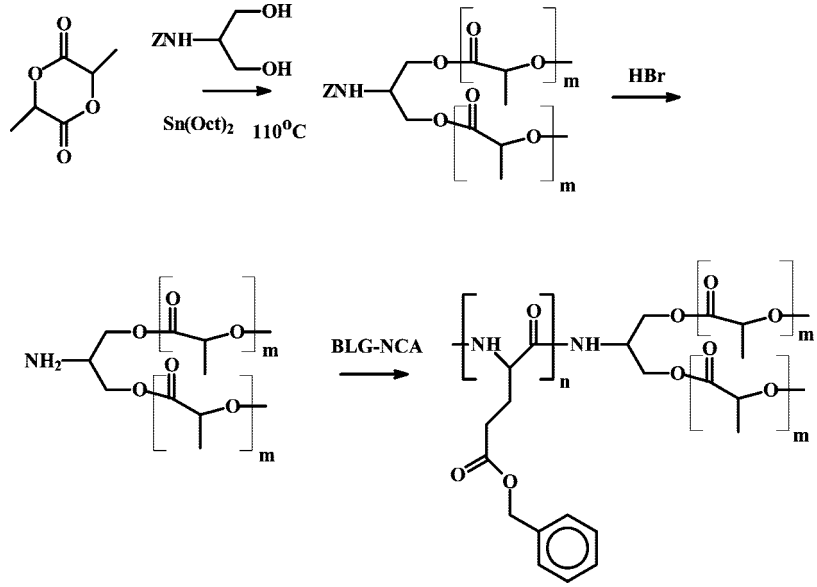


Scheme 62. Synthesis of PS(PBLG)$_2$ Miktoarm Star Copolymers

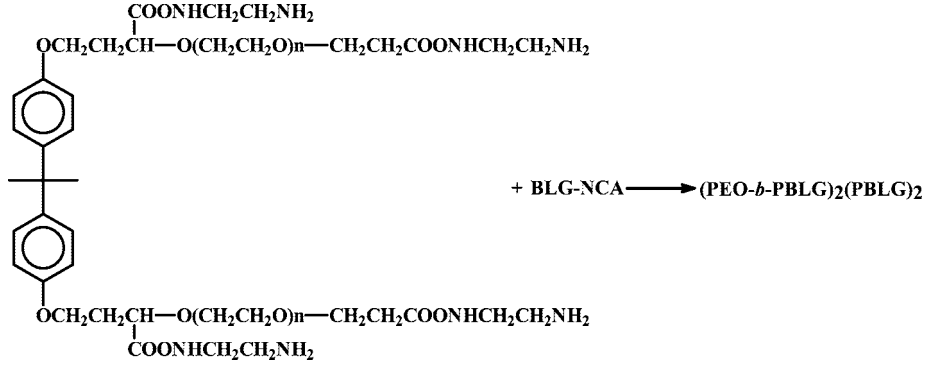


along the chain and branches having reactive chain ends. The coupling reaction between the functional backbone and the end-reactive branches leads to the formation of graft copolymers. There are very few reports using this technique. As an example, the synthesis of poly(*N*-hydroxyethyl L-glutamine)-*g*-poly(tryptophan) was reported by Sugimoto et al.[167] (Scheme 67). PBLG was prepared and partially saponified. The resulting free carboxyl groups were linked with the end-amine groups of linear chains of polytryptophan in the presence of 1,3-dicyclohexylcarbodiimide, DCC, and

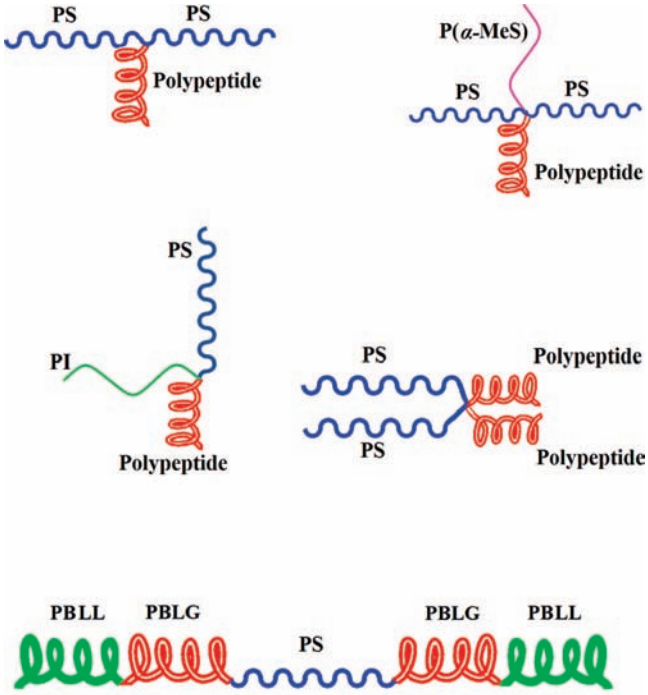
Scheme 63. Synthesis of (PLLA)$_2$PBLG Miktoarm Stars



Scheme 64. Synthesis of (PEO-*b*-PBLG)$_2$(PBLG)$_2$ Miktoarm Star Copolymers



Scheme 65. Macromolecular Chimeras Based on Polypeptides

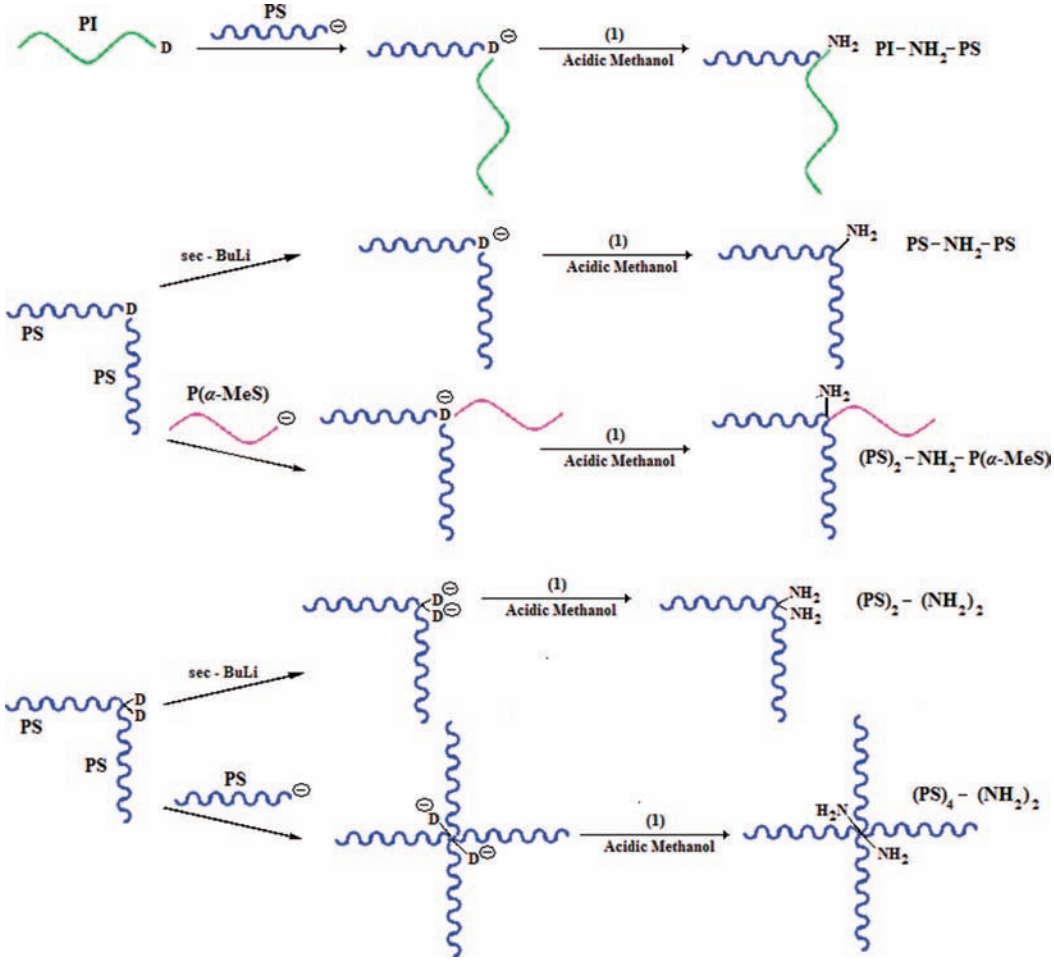


1-hydroxybenzotriazole. Subsequent aminolysis with 2-amino-1-ethanol was performed, leading to the target structures. Unfortunately, the molecular characteristics of the graft copolymers were not reported.

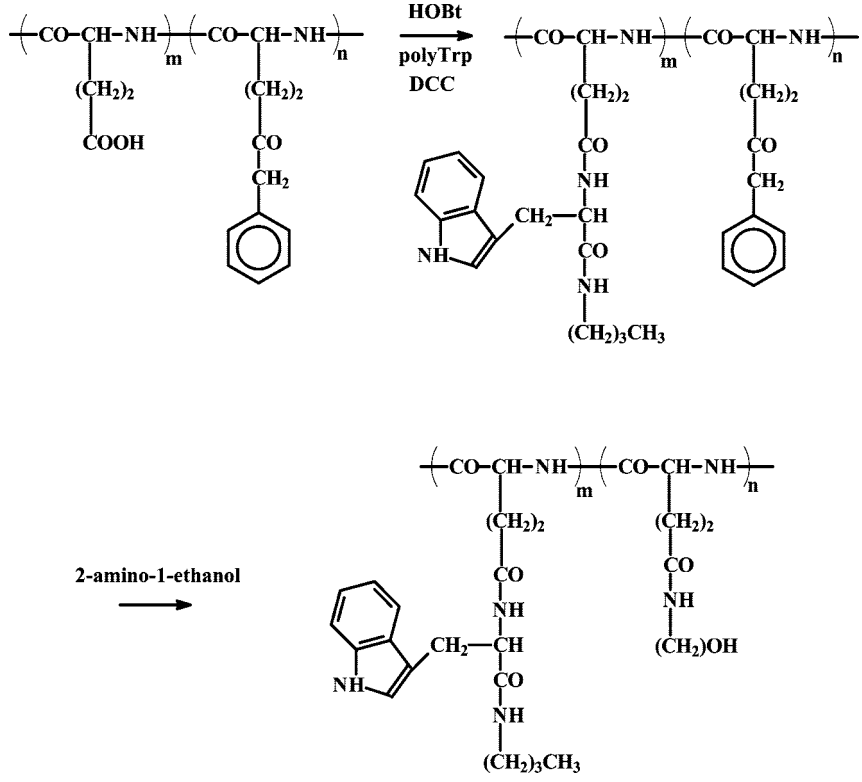
In the "grafting from" method, active sites are generated randomly along the backbone. These sites are capable of initiating the polymerization of a second monomer, leading to the synthesis of graft copolymers. This method was widely used for the synthesis of graft hybrids, especially with polypeptide side chains. Primary amine groups were generated along the backbone and then served as initiation sites for the polymerization of NCAs. Many of the early reports were based on this synthetic strategy. For example, *N*-benzylacrylamide was copolymerized with *N*-(3-aminopropyl)methacrylamide hydrochloride by conventional radical polymerization. The copolymer was then dehydrochlorinated to afford free primary amine groups, subsequently functioning as initiation sites for the polymerization of Ala-NCA[168] (Scheme 68). In another study, *N*-methyl-*N*-(4-vinylphenethyl)ethylenediamine was copolymerized with 2-hydroxyethyl methacrylate. The side amine groups were subsequently used to copolymerize the NCAs of β-benzyl L-aspartate and β-(4-phenylazobenzyl) L-aspartate to give the graft copolymer hybrid[169,170] (Scheme 69). These structures were not characterized thoroughly in terms of the molecular and structural characteristics.

The "grafting from" approach was also employed in more recent studies. *N*-*tert*-Butoxycarbonyl-*N*′-(2-methacryloyl)-1,3-diaminopropane was prepared by Zhang et al.[171] and polymerized by free radical polymerization. The

Scheme 66. Synthetic Strategy Involved for the Synthesis of Macromolecular Chimeras

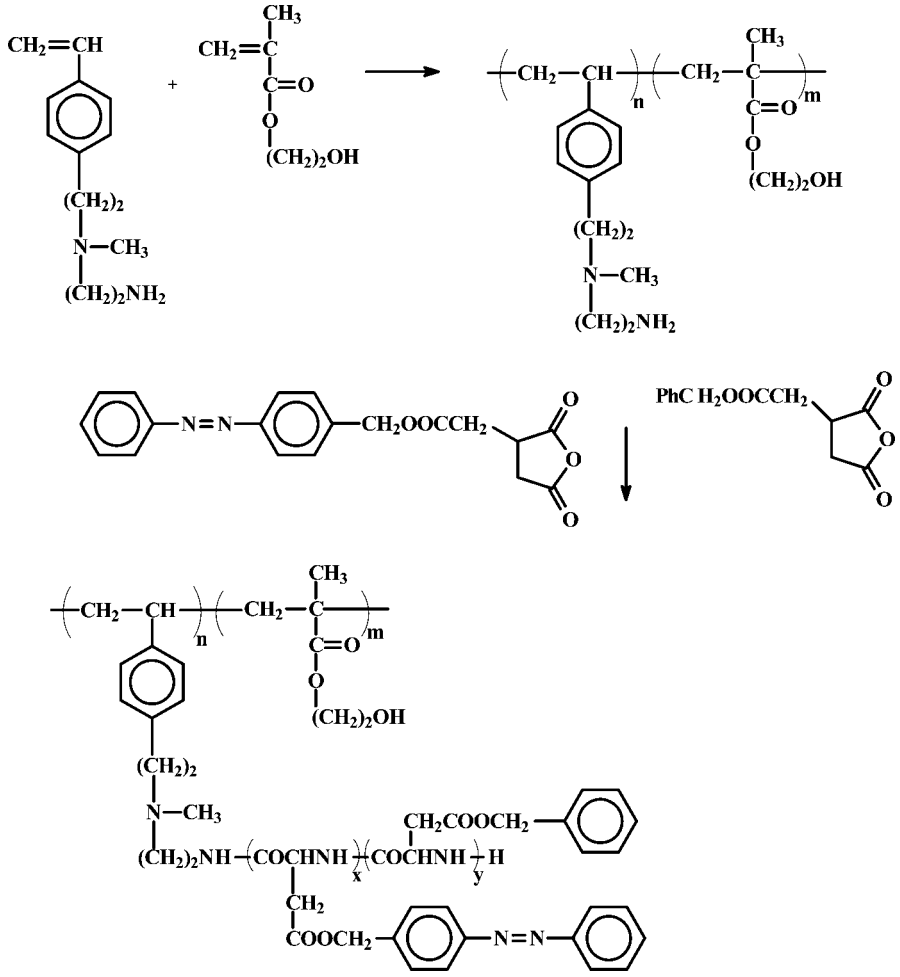


Scheme 67. Synthesis of Poly(*N*-hydroxyethyl L-glutamine)-*g*-poly(tryptophan)



quantitative deprotection of the amine group was performed in a mixture of dichloromethane and trifluoroacetic acid, followed by oligomerization of the BLG- or ZLL-NCAs, affording the corresponding molecular brushes (Scheme

Scheme 68. Synthesis of Poly(*N*-benzylacrylamide)-*g*-polyalanine Graft Copolymers



70). The samples were characterized by NMR and AFM techniques.

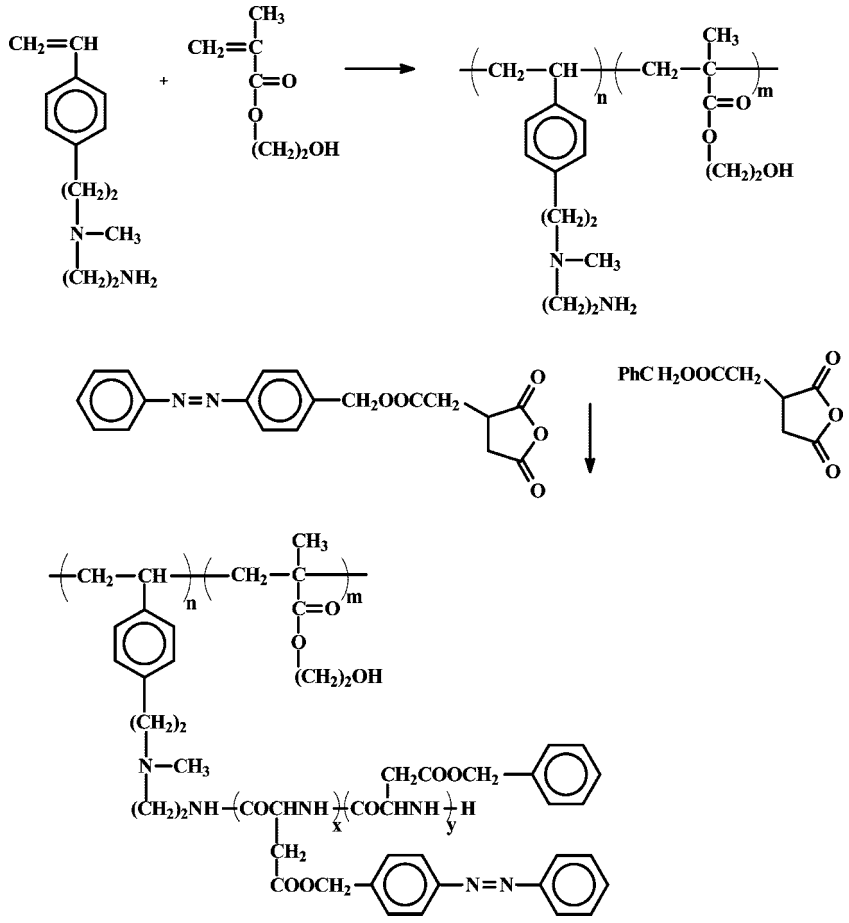
A similar methodology was applied by Xiang et al.[172] for the synthesis of chitosan-*g*-poly(L-tryptophan) graft copolymers (Scheme 71). Deacetylated chitosan (deacetylation degree of amino groups 75%) was employed as a multifunctional macroinitiator for the polymerization of the L-tryptophan-NCA in ethyl acetate solutions, leading to the final graft copolymers. The products were analyzed by NMR and IR spectroscopy.

The last method for the synthesis of graft copolymers is the macromonomer method. A macromonomer is an oligomeric or polymeric chain bearing a polymerizable end group. Macromonomers having two polymerizable end groups have also been reported. Copolymerization of preformed macromonomers with another monomer yields graft copolymers. In an early report, *m*,*p*-vinylbenzylamine was used as initiator for the oligomerization of DL-phenylalanine-NCA, PPhe-NCA, leading to a narrow molecular weight distribution of macromonomers of low molecular weight (Scheme 72). These macromonomers were then copolymerized with MMA and S, to give PMMA-*g*-PPhe and PS-*g*-PPhe graft copolymers, respectively.[173]

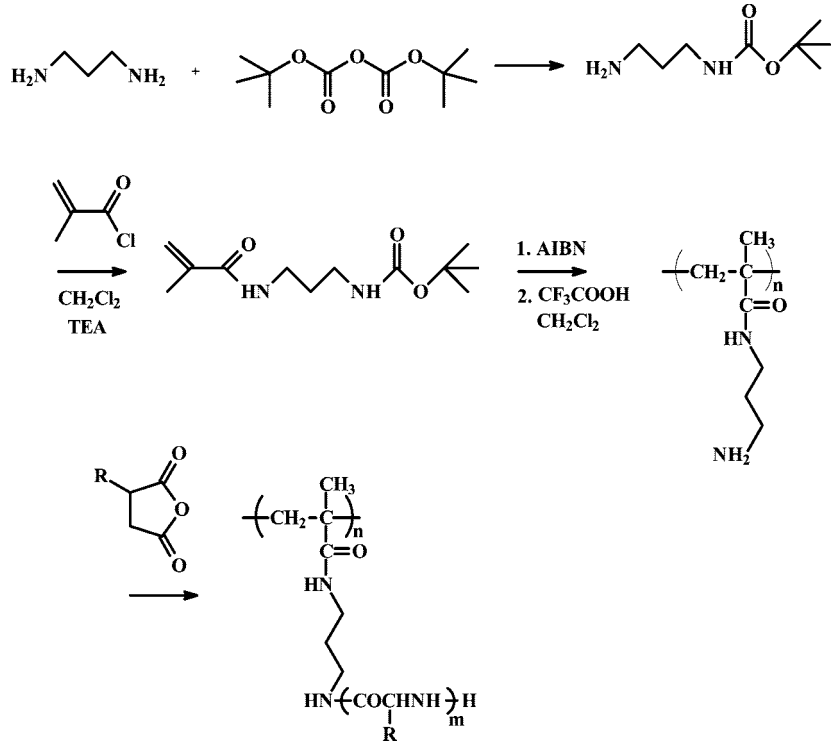
Highly branched and dendritic-like polylysines have also been prepared. The synthetic strategy involves the selective deprotection of the polylysine side amine groups and their subsequent use as initiating sites for the polymerization of new L-lysine monomers. The synthesis of dendritic-graft polylysine by Klok and Hernádez[174] is outlined in Scheme 73. The methodology involved the use of two orthogonally N^{ε}-protected L-lysine NCAs. One of the monomers was masked with a temporary protecting group, which could be removed under mild conditions. The second monomer contained a permanent protecting group, which could not be removed under experimental conditions suitable for the cleavage of the temporary protective group. The Boc (*tert*-butoxycarbonyl-) group was selected as the temporary protective group, since it can be removed under moderately acidic conditions, whereas the Z (benzyloxycarbonyl-) group was the permanent one, since it can be removed only in HBr/acetic acid solutions or by hydrogenation. Both monomers were polymerized using a primary amine initiator. The Boc group was selectively cleaved to afford a number of primary amine groups, which can act as initiators for the grafting of the first generation of polypeptide arms onto the core. Repetition of this procedure yields the dendritic-graft polymer. The samples were rather polydisperse and structurally not uniform. However, the employed procedure is rather simple and avoids extensive isolation and purification processes.

Highly branched poly(L-lysine) was also prepared by a repetitive sequence of NCA polymerization and end-functionalization/deprotection reactions.[175] Z-Lys-NCA or ε-trifluoroacetyl-L-lysine-NCA was initially polymerized with *n*-hexylamine. The polymer was then end-functionalized by reaction with N^{α},N^{ε}-diFmoc L-lysine (Fmoc: 9-fluorenylmethoxycarbonyl) under standard peptide coupling conditions. Selective cleavage of the Fmoc group (20% v/v

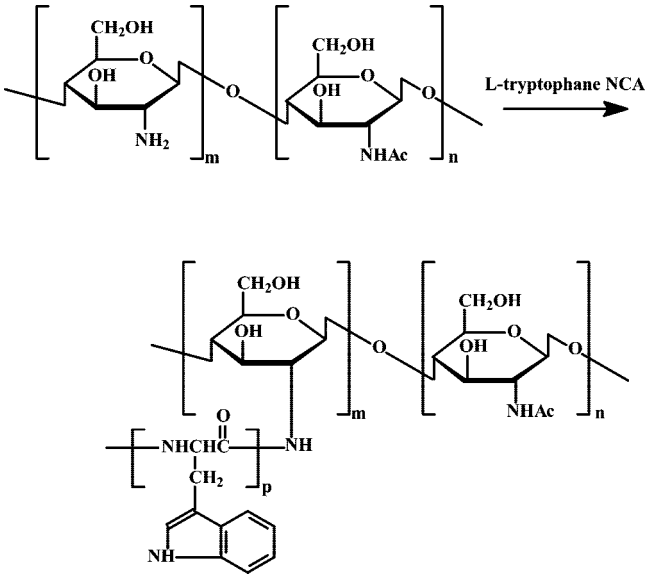
Scheme 69. Synthesis of Poly[*N*-methyl-*N*-(4-vinylphenethyl)ethylenediamine]-*co*-2-hydroxyethyl methacrylate-*g*-poly[*β*-benzyl L-aspartate-*co*-(*β*-(4-phenylazobenzyl) L-aspartate)] Graft Copolymers



Scheme 70. Synthesis of Molecular Brushes



Scheme 71. Synthesis of Chitosan-*g*-poly(L-tryptophan) Graft Copolymers



solution of piperidine in DMF) resulted in a linear PLL bearing two free primary amine end groups. These amine groups served as initiators for the growth of the first generation of branches. One repetition of this procedure afforded a highly branched poly(L-lysine) (Scheme 74). The SEC traces of the first generation were monomodal and of moderate molecular weight distribution. In the second generation polymers, a shoulder was observed at the lower molecular weights, indicating that incomplete end-functionalization and/or deprotection of the precursor took place. This result indicates that the method cannot be applied for the synthesis of higher than second generation structures, due to the lack of control of the polymerization steps. Moreover, the same authors prepared α,ω-branched polymers using the same methodology and 1,4-diaminobutane.

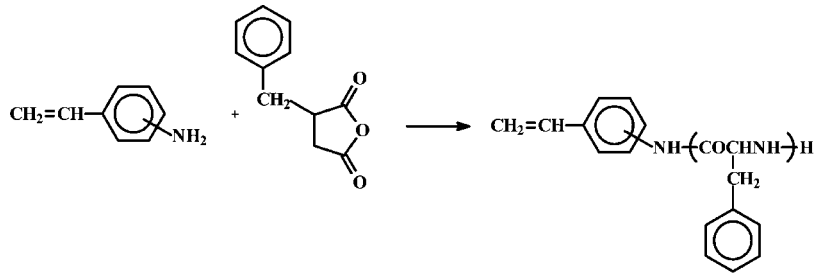
Polystyrenes dendronized with L-lysine dendrons of different generations were prepared by the procedure given in Scheme 75.[176] 4-Vinylbenzylamine was linked with the appropriate protected L-lysine dendrons under standard peptide coupling conditions, affording the corresponding dendronized macromonomers. The macromonomers were then subjected to conventional radical polymerization to give products with broad molecular weight distributions. Monomer conversion and polymer molecular weight were found to decrease with increasing dendron size.

A polyamidoamine dendron (PAMAM) carrying a Boc-protected amine group was also synthesized by Harada et al.[177,178] The protective group was then removed, and the primary amine group was employed as initiator for the polymerization of ZLL-NCA, leading to a linear dendritic block copolymer (Scheme 76).

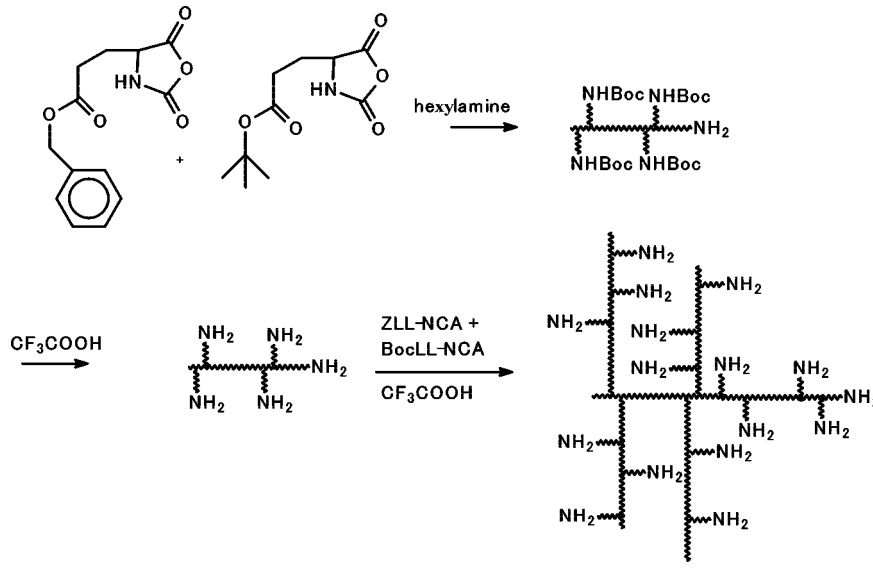
4. Surface-Bound Polypeptides

This section reviews the recent work on the synthesis of surface-bound polypeptides. Polypeptide chains can be immobilized onto a surface through physical interactions or covalent bonds. Physical interactions are relatively weak

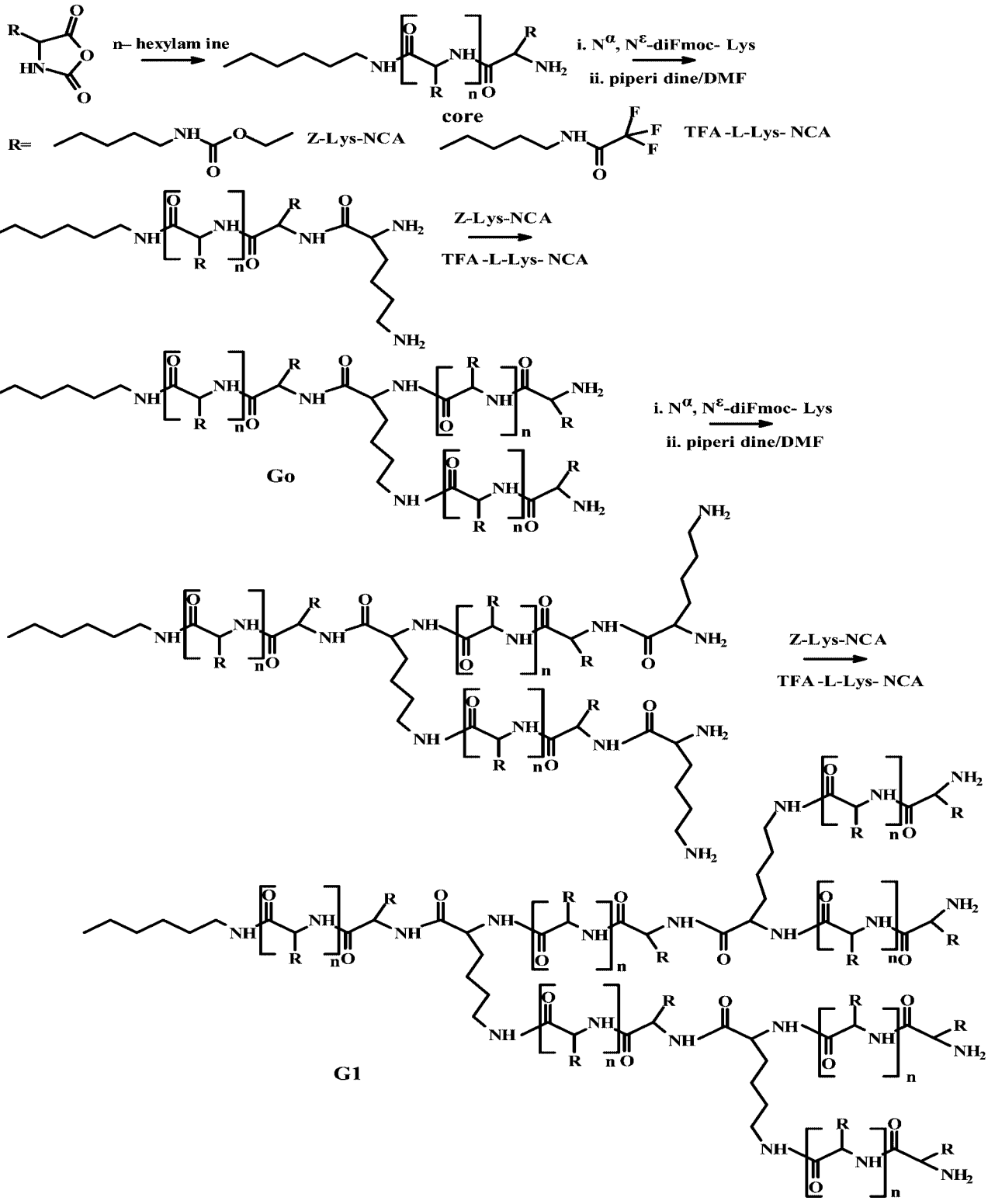
Scheme 72. Synthesis of Poly(DL-phenylalanine) Macromonomers



Scheme 73. Synthesis of Highly Branched Polylysine



Scheme 74. Synthesis of Highly Branched Polylysine

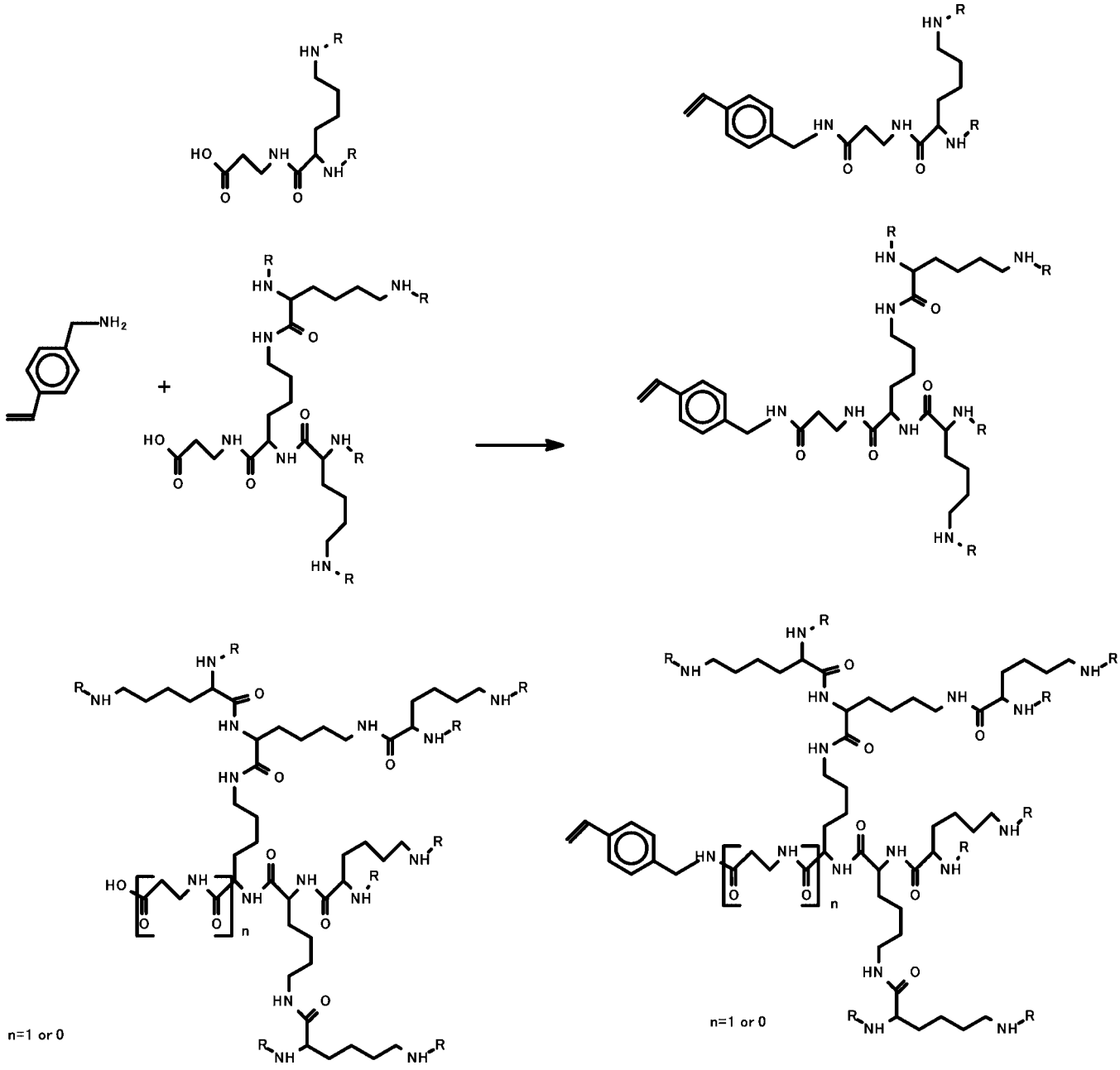


affinity forces, whereas covalent chemical bonds can stably immobilize the polymer chains. Polypeptide chains that are attached by one end to the surface and oriented along the direction normal to the grafting surface are referred to as polypeptide brushes. Polypeptide brushes are of paramount practical and scientific importance, since they can self-assemble into well-ordered secondary structures (α-helices, β-sheets) on surfaces. This ability plays a central role in the fabrication of artificial biocompatible surfaces.

4.1. Surface-Bound Polypeptides via Physical Interactions

General methods for the preparation of physically adsorbed polypeptide thin films on surfaces of solid substrates include the Langmuir−Blodgett (LB) process and the $\pi-\pi$ stacking interaction process. The LB technique has been the dominantly preferred method for noncovalently attached polypeptide thin films on flat surfaces. On the other hand, the $\pi-\pi$ stacking interaction

Scheme 75. Synthesis of Styrene Macromonomers Carrying L-Lysine Dendrons



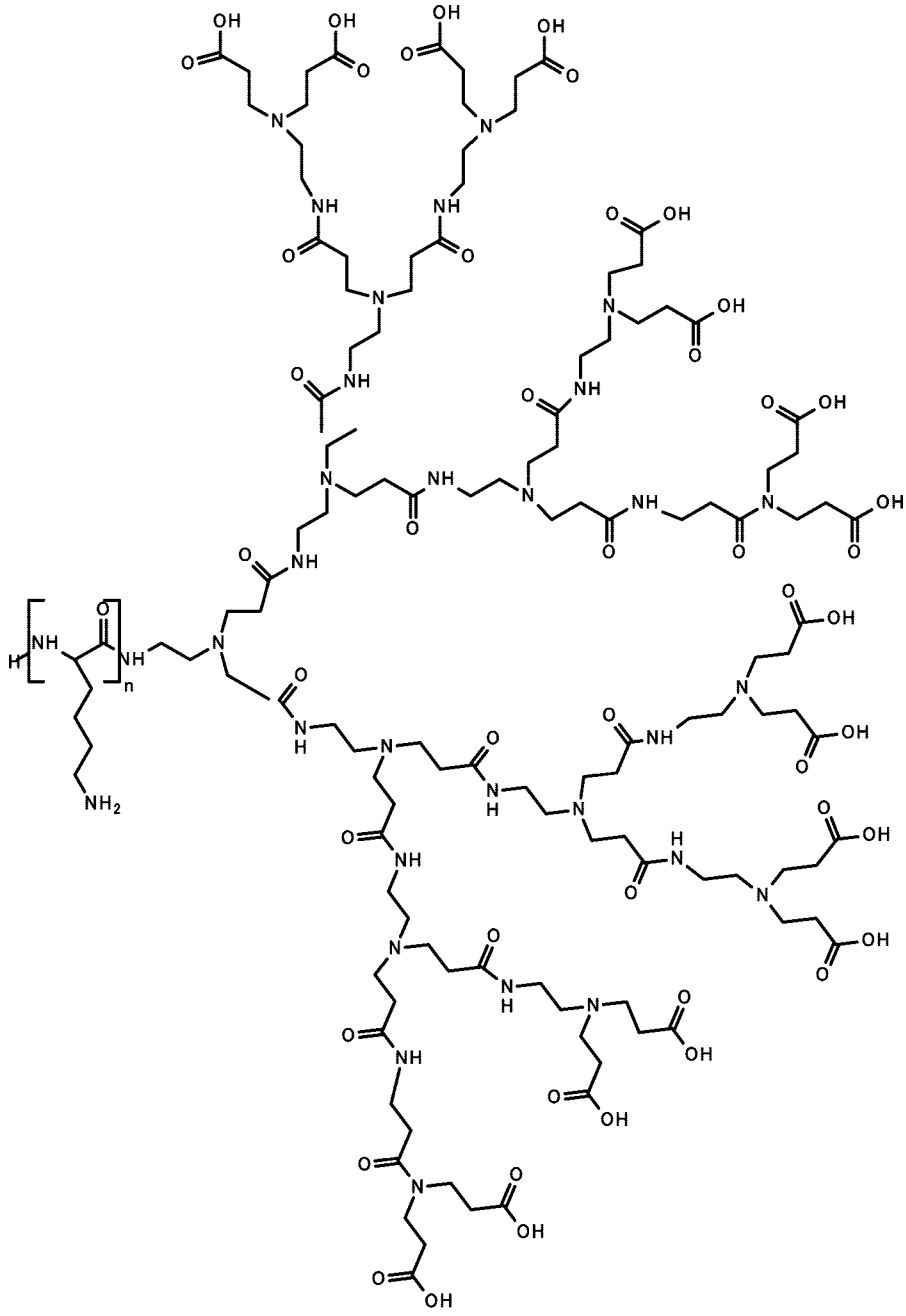
has been used widely in wrapping polypeptide chains around carbon nanotubes (CNTs) to enhance the biocompatibility and solubility of CNTs.

4.1.1. The Langmuir−Blodgett Process

The LB technique involves the formation of densely packed monolayers at the air−liquid interface followed by transfer of the monolayer to the surface, by dipping and lifting the substrate, under a constant surface pressure. If desired, the dipping and lifting process can be repeated to obtain multilayer LB films. In one case in the paper by Shibata et al.,[179] a monolayer of γ-dodecyl L-glutamate (DLG) *N*-carboxy anhydride (NCA) monomer was spread at the air−water interface from a dichloromethane solution, followed by polymerization immediately after spreading, as confirmed by the surface pressure−area isotherms. The polymerized monolayer was continuously transferred by the LB technique on a calcium fluoride plate until a thickness of 20 layers was achieved. Polarized infrared spectra of the resulting polypeptide LB films indicated that the ratio of α-helix to β-sheet conformations in the PDLG LB films depends on the surface pressure.

Menzel et al.[180−182] demonstrated the preparation of a "hairy rod" type LB film originating from graft copolymer consisting of a rigid rodlike PBLG backbone and flexible side chains (**1** and **2**, Scheme 77). These graft copolymers were spread from a chloroform solution onto ultrapure water. After a period of at least 15 min, the monolayer was compressed to the intended surface pressure. When the surface area had stabilized at this pressure, the monolayer was transferred to a silicon wafer or a quartz slide surface. The resulting LB film of PBLG can be represented as a deformed hairy rod arranged in bilayers. In these anisotropic films, the rodlike backbones are preferentially oriented in the dipping direction and, in each layer, are arranged antiparallel in order to cancel out the macrodipole moment.

Scheme 76. Linear Dendritic Block Copolymers of PZLL and PAMAM Dendrons

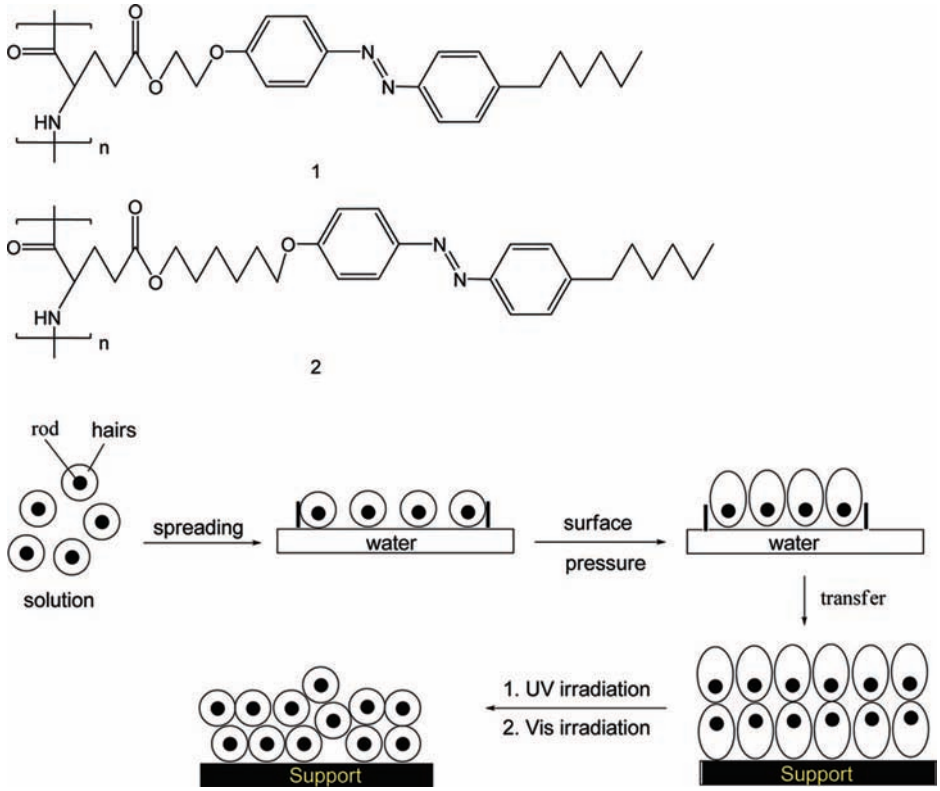


After UV/Vis irradiation the asymmetric bilayered structure is lost and the concomitant relaxation of the hairy rod polymer results a new arrangement of the side groups. For clarification, see Scheme 77.

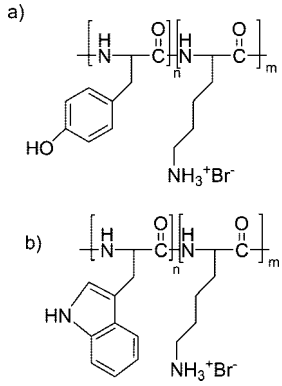
4.1.2. The $\pi-\pi$ Stacking Interaction Process

A number of research groups have investigated the feasibility of enhancing biocompatibility and altering the wetting properties of carbon nanotubes (CNTs) by immobilizing polypeptide chains onto the sidewalls of CNTs through $\pi-\pi$ interactions. Dai et al.[183] presented a method for directly measuring the physical interaction between polypeptides and CNTs using atomic force microscopy (AFM). The method involves coating the silicon nitride AFM probe with polylysine or polytryptophan and calculating the adhesion force between the polypeptide and an individual CNT. The results suggest that there was almost no interaction between the polylysine-modified tips and pristine CNTs over the entire pH range. However, the adhesion between the above modified tips and carboxylated (oxidized) CNTs decreased with increasing pH. The variation of adhesion as a function of pH may result from the protonation/deprotonation of carboxyl groups on the carboxylated CNTs and the $-NH_2$ groups of polylysine. At low pH values, the protonated $-NH_3^+$ groups of polylysine show a relatively strong adhesive force with the $-$COOH groups on the oxidized CNTs. At high pH, the $-NH_3^+$ and $-$COOH are deprotonated into $-NH_2$ and $-$COONa moieties to significantly reduce the adhesion force between them. In the case of polytryptophan modified tips, the adhesion forces with the oxidized CNTs are stronger than those between the polylysine and oxidized CNTs under the same conditions. This effect is due to the dominant role of the $\pi-\pi$ stacking interaction (indol moiety/CNT), especially at low carboxylic

Scheme 77. Chemical Structure of the "Hairy Rod" Polymers and the Langmuir−Blodgett Process



Scheme 78. Chemical Structures of the Random Copolymers (a) Poly-L-(Tyr,Lys-HBr) and (b) Poly-L-(Trp,Lys-HBr)



content, as well as to the hydrogen bonding between the amine on the polytryptophan chain and the carboxylic groups of CNTs.

Salzmann et al.[184] tested random copolymers of poly(L-Tyr-*co*-L-Lys-HBr) and poly(L-Trp-*co*-L-Lys-HBr) (Scheme 78) as dispersing agents for single-walled carbon nanotubes (SWCNTs). The π-stacking of the aromatic amino acids tyrosine and tryptophan with the extended π-system of the CNTs was the most important force for the noncovalent adsorption. The hydrophilic properties of lysine hydrobromide rendered the CNTs/polypeptide conjugate water-soluble, as proved by optical absorbance and fluorescence spectroscopy. In another study,[185] the same group synthesized polylysine, with pyrene side groups randomly distributed along the chain. These pyrene groups bound the otherwise not absorbable polylysine on the surface of pristine SWCNTs, and maximal dispersion could be thus achieved. Saito et al.[186] reported another method of wrapping a polypeptide backbone on a SWCNT through $\pi-\pi$ interactions between the porphyrin side groups and the extended π-electron system on the SWCNT surface. This technique was used to separate SWCNTs of different sizes.

4.2. Surface-Bound Polypeptides via Covalent Bonding

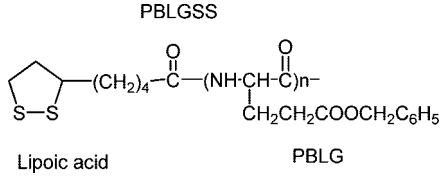
Polypeptide brushes are surface end-grafted polypeptides, oriented normal to the grafting surface. Covalently bound polypeptide brushes can be prepared by the "grafting to" and "grafting from" strategies. In the "grafting to" approach, preformed polypeptide chains are end-grafted onto a surface from solution through chemical reaction. The "grafting to" methods are limited by relatively low grafting density due to increased steric hindrance as grafting density increases. The "grafting from" approaches are more promising for constructing polypeptide brushes with relatively high grafting density. The "grafting from" strategies can be achieved by grafting initiating sites onto a surface followed by *in situ* polymerization of the appropriate monomers.

4.2.1. The "Grafting to" Strategies for Polypeptide Brushes

"Grafting to" strategies involve the reaction of preformed end-functionalized polypeptides with suitable functionalized surfaces under appropriate conditions. The chains, in their equilibrium state, point away from the plane of the grafting surface, preferably perpendicular to the grafting surface. One advantage of the "grafting to" strategy is the use of preformed polymers with known characteristics, in contrast to the "grafting from" methods.

The most expedient process for synthesis of well-defined polypeptides is currently the ROP of NCAs with primary amines. End-functional groups can be selectively placed at either the α-end (C-terminus) or the ω-end (N-terminus). For example, C-terminus-functionalization can be achieved

Scheme 79. Chemical Structure of PBLG-SS



by using functional initiators, while N-terminus-functionalization can be accomplished by postpolymerization end-group modification. As mentioned earlier, NCA polymerization by the amine mechanism is often plagued with premature termination. These unwanted termination steps are responsible not only for uncontrollable molecular weight and broad molecular weight distribution but also lower functionality at the N-terminus (ω-ends).

Enriquez et al.[187,188] investigated the reaction of a ω-disulfide PBLG (PBLG-SS), where a disulfide moiety is attached at the N-terminus of PBLG, with a gold surface. PBLG-SS was synthesized by condensation of PBLG with lipoic acid (Scheme 79). The chemisorption film of PBLG-SS on gold was compared with (i) physically adsorbed unfunctionalized PBLG on gold and (ii) Langmuir−Blodgett (LB) deposited PBLG monolayers. It was concluded that the PBLG-SS self-assembled (SA) on the gold surface through the disulfide groups. The self-assembled PBLG chains retained their α-helical conformation with their axes tilted with respect to the surface. In the cases of the physically adsorbed unfunctionalized PBLG film and the LB monolayers, the PBLG helices lie in the plane of the gold surface and the grafting efficiency is lower. Worley et al.[189] studied the influence of the electric field on the alignment of these PBLG-SS on a gold surface. By applying an appropriate voltage between two gold electrodes combined with an optimized electrolyte concentration, thicker self-assembled (SA) films were produced as compared to PBLG-SS SA films without voltage. The observed film thickness indicated that these PBLG-SS are preferably deposited on the negative electrode rather than at the positive electrode or on an unbiased control substrate. This observation supports the theory that helical polypeptides have a macroscopic dipole moment from the N-terminus to the C-terminus.

Niwa et al.[190] investigated the reversible formation of single- and double-layered helical PBLG assemblies on a gold surface due to helix−macrodipole interactions. It is well-known that helical polypeptide chains display a macroscopic dipole moment, that is, in this case, the electrostatic potential from the N-terminus to the C-terminus. This strong intermolecular dipole interaction leads to the formation of an antiparallel pair of PBLG helical rodlike structures (Scheme 80). The adsorbed PBLG segment bearing two alkyl chains with disulfide end groups (**1**) in $CHCl_3$ (helicogenic solvent) retained the α-helical conformation (94%), leading to double-layered structures. In contrast, when DMSO (nonhelicogenic solvent) was used, **1** formed monolayers with lower helix content (62%) due to the decreased helix−macrodipole interaction.

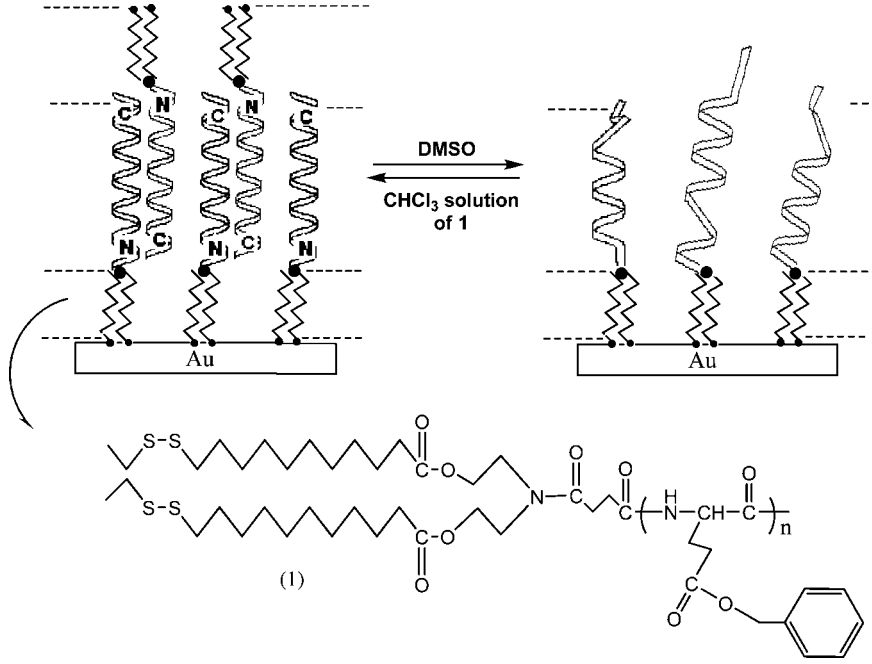
Williams et al.[191,192] reported the synthesis and characterization of C-terminus disulfide-functionalized PBLG (SS-PBLG) with low polydispersity ($M_w/M_n \leq 1.2$). The SS-PBLG was prepared by ROP of BLG-NCA with an ω-amine disulfide initiator (*N*-lipoyl-1,3-diaminopropane) in anhydrous chloroform (Scheme 81). The merit of this method lies in the fact that no second step for the polypeptide coupling with sulfur-containing molecules is required. In addition, this method provides a route to polypeptide brushes having the macrodipole orientation toward the surface, in contrast to the coupling strategy. Surface plasmon resonance revealed that self-assembly of high molecular weight polypeptides on gold substrates proceeded rapidly. The effect of the film thickness on the properties of the polypeptide brush was also investigated. An increase in the length of the polypeptide brush causes an increase in the average tilt angle of the helix axis with a simultaneous increase in the dipole moment. In addition, the time allowed for assembly significantly influences the surface coverage as well as the tilt angle. Longer times led to a decrease in surface coverage and an increase in tilt angle, which was more pronounced for the shorter polypeptide brushes.

In addition to the organosulfur compounds on gold, alkylsilanes on silicon oxide are another efficient chemisorption system, which leads to densely packed self-assembled monolayers (SAMs).

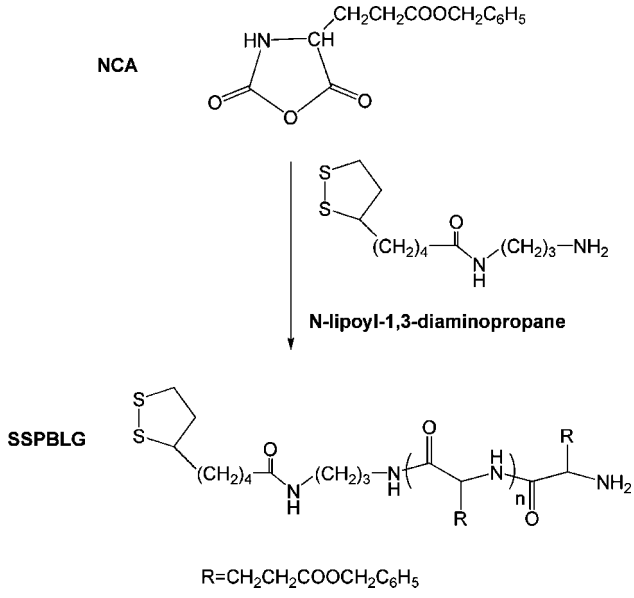
Chang and Frank[193] compared three strategies for preparing chemically grafted PBLG brushes on flat silicon surfaces. In the first method, a coupling compound, 3-(trichlorosilyl-)propyl chloroformate (CFPS), was first attached on the surface through reaction of chlorosilane groups and the OH of the surface. The SAM was used to immobilize preformed PBLG (DP: 100) at the N-terminus via reaction of the remaining chloroformate group with the amino group of PBLG. In the second method, a clean silicon wafer was immersed in a solution of PBLG-APS (DP: 40) (APS: 3-(aminopropyl)triethoxysilane), while, in the third method, a SAM of APS was formed on the silicon wafer surface and the remaining amino group was then used to initiate the polymerization of BLG-NCA. The first method leads to a pure α-helical conformation aligned parallel to the surface, resulting in low grafting density. The similar hydrophobicity of the CFPS-modified silicon surface (contact angle 71°) and the PBLG film (contact angle 74°) may cause a strong association and might be responsible for the parallel orientation of the PBLG helices on the surface. In contrast, a film with relatively higher density can be obtained by the second method. This can be attributed to the fact that the triethoxysilane end groups of PBLG react not only with the silicon substrate but also to cross-link intermolecularly before attachment to the surface. The cross-linked PBLG, through siloxane bonds, attach to the surface in bundles, leading to a tightly packed film. However, a higher portion of the β-sheet conformation was found in the film prepared by the second method due to the lower degree of polymerization of PBLG. The surface itself does not induce a transition in the secondary structure, likely caused by the high mobility of the β-sheet during the adsorption. Surprisingly, the third method was not successful in grafting. The authors reasoned that the surface-initiated NCA polymerization was terminated in the early stage, before the polypeptide chains were long enough to form α-helices, since the initiating sites in the SAM were too close to each other.

A "grafting to" method for attaching PLGA on the surface of microporous membranes was developed by Hollman and Bhattacharyya.[194] The microporous membranes were made from bacterial cellulose fibers, which contain a good amount of surface aldehyde groups. The grafting of PLGA was achieved by the reaction of the primary amine terminal groups with the aldehyde groups of the membrane. These functionalized membranes allow for the controlled transport of both water and charged solutes in dilute systems. The level

Scheme 80. Schematic Illustration of the Single- and Double-Layered Polypeptide Assemblies on a Gold Substrate and the Chemical Structure of the PBLG Segment Bearing Two Alkyl Chains with Disulfide End Groups



Scheme 81. Synthesis of SS-PBLG Using *N*-Lipoylamine as Initiator



of tenability regarding water permeation and ion exclusion is due to the conformational transitions of the attached polypeptide from a helical to random-coil motif.

Although single covalent bonding methods have most frequently been used to prepare polypeptide brushes on surfaces, the multicovalent bonding methods can also be applied for the attachments of multibound polypeptide chains on surfaces. For example, Zhang et al.[195] reported a method where PLL is attached on an oxidized SWCNT through reaction of the primary amine side groups on PLL and the carboxylic acid groups in the presence of DCC. Their analytical results showed that all the SWCNT carboxylic acid groups completely reacted, while a large quantity of residual free amino groups remained, which can be useful in promoting cell adhesion on the PLL surface.

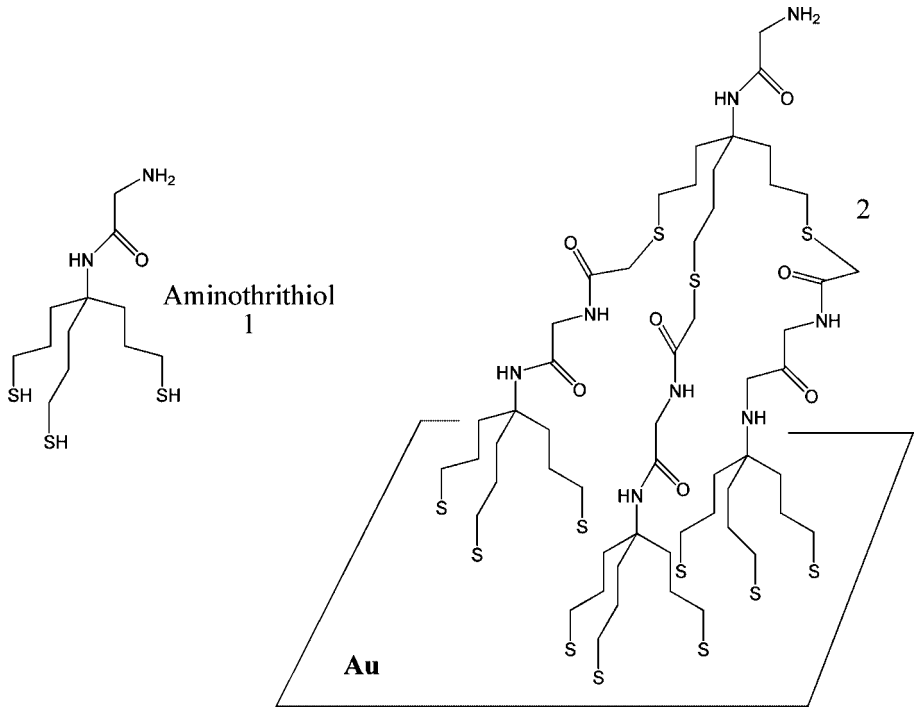
4.2.2. The "Grafting from" Strategies for Polypeptide Brushes

The "grafting from" approach is more promising for the preparation of high density polymer brushes. High density polymer brushes are expected to possess special properties due to the significant interaction between adjacent chains. Growth of polypeptide chains from a surface can be achieved by attaching the initiating sites onto a surface followed by in situ ROP of the NCA monomers. Methods of immobilizing initiation sites on surfaces include the following: (a) self-assembly monolayer (SAM) process (on gold and silicon oxide surfaces), (b) glow discharging in the presence of a gas (on polymer membrane surfaces), and (c) chemical modification of surface functional groups (on CNT and PS particle surfaces). Primary amines are the most widely used initiators for NCA polymerization. A drawback of the "grafting from" approach is the relatively broad molecular weight distribution obtained due to the increased difficulty in controlling both the initiation and propagation steps, compared with the case of the nonimmobilized initiators.

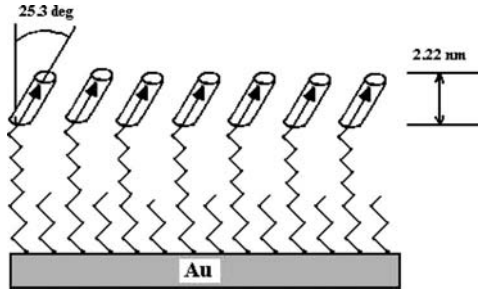
4.2.2.1. Methods of Immobilizing Initiator on Surfaces. It is essential that the initiation sites are attached on the surface at controlled intervals so that the grafted peptide chains can have the necessary space to adopt the desired secondary structure or to switch from one higher ordered structure to another. The SAM process using chemisorption systems, such as organosulfide compounds on gold or alkylsilanes on silicon oxide, often leads to closely packed monolayers in which the initiation sites occupy a smaller space than the cross-sectional area of the helical structure of a polypeptide chain. Several research groups have thus developed methods to control the spacing of the immobilized initiation sites on surfaces.

Whitesell et al.[196] reported the use of a specially designed amino-trithiol (**1**) to immobilize primary amine groups on flat gold surfaces (Scheme 82). Since this compound occupies a space smaller than that required by helical polyphenylalanine, attempts to grow polyphenylanine were not suc-

Scheme 82. Chemical Structures of the Amino-Trithiol 1 and the Amino-Nonathiol 2



Scheme 83. Structural Model of a Poly(L-Leuc) Layer on a C11N/C4 Mixed SAM

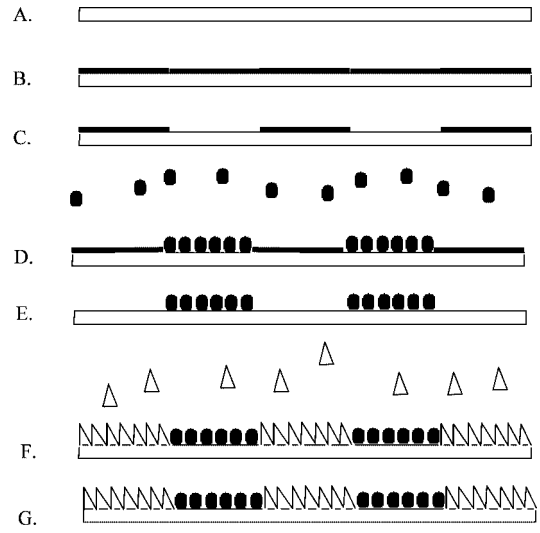


cessful. In order to overcome this problem, the authors prepared and attached the amino-nonathiol (**2**). Subsequent polymerization of phenylanine NCA was successful.

Higuchi et al.[197] reported another method for the preparation of a SAM containing a primary amine at designated intervals on a gold surface. The SAM consists of a mixture of 11-amino-1-undecanethiol (C11N) and butyldisulfide (C4) at an appropriate molar ratio. Stepwise polymerization of leucine NCA initiated by this SAM composition was successful, while the same process containing only C11N SAM gave no polymer (Scheme 83). The same authors[198] have also prepared a mixed SAM containing C11N and C4 on a gold nanoparticle surface. Polymerization of γ-methyl L-glutamate (MLG) NCA initiated by this SAM was successful. Heise et al.[199] explored the fabrication of mixed SAMs of $Br(CH_2)_{11}SiCl_3$ and $(CH_3(CH_2)_{10}SiCl_3$ on flat silicon surfaces. The bromo groups were converted into primary amines *in situ* and used for the polymerization of NCAs.

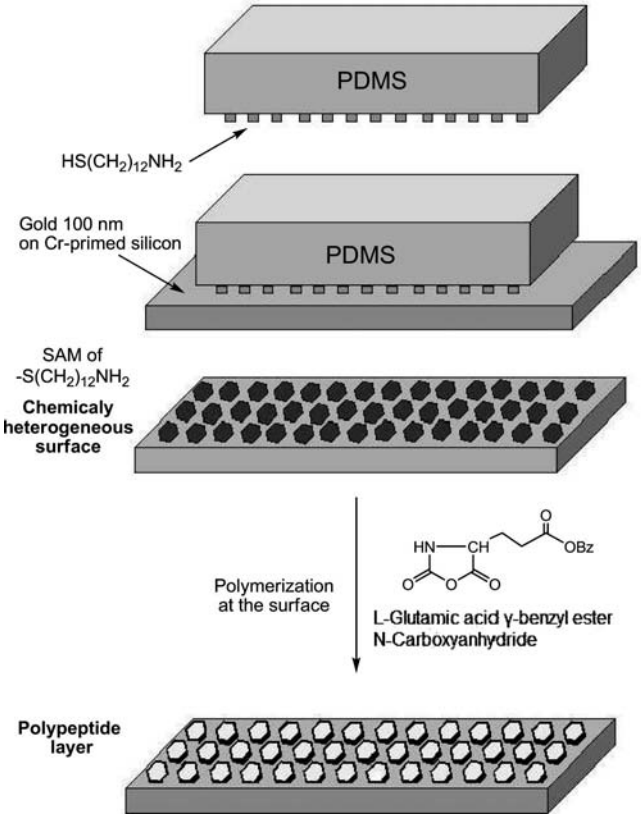
Another example of the controlled immobilization of initiation sites involves patterned SAMs. Many potential applications of surface-bound polypeptides require the ability to pattern the position of the grafted polypeptide chains. Patterned polymer brushes can be obtained by surface-initiated NCA polymerization from patterned SAMs containing initiation sites. The patterned SAMs can be prepared by traditional photolithography or microcontact printing (μCP) methods.

Scheme 84. Micropatterned Surface Containing Regions Derivatized with Methylsilane (Close Circles) and Aminosilane Functional Groups (Open Triangles)



Britland et al.[200] described a method for micropatterning surface-initiated polypeptide brushes by the traditional photolithography method (Scheme 84). On a clean glass or fused silica slide (A), positive photoresist was spun onto the surface and then baked at 90 °C for half an hour (B). The resist was then illuminated by light transmitted through a photolithographic mask containing a relief image of the desired pattern. Exposed resist was removed, and the surface was rinsed thoroughly (C). The substrate was then immersed in a solution of dimethyltrichlorosilane followed by rinsing with the solvent (D). Next, the unexposed resist was removed (E). The surface was immersed in a solution of 3-[(2-aminoethyl)amino]propyltrimethoxysilane (F). Finally, the surface was rinsed and baked at 115 °C for 10 min to yield a micropatterned surface containing regions derivatized with methyl and amino functional groups (G).

Scheme 85. Preparation of Thin Patterned Polypeptide Layers by Microprinting and Surface-Initiated Polymerization



Kratzmüller et al.[201] developed an approach to prepare a patterned primary amine-containing SAM by μCP for the construction of PBLG brushes on a flat gold surface. In this method, a polydimethylsiloxane (PDMS) stamp was treated with a solution of 12-mercaptododecylamine [$HS(CH_2)_{11}NH_2$] in ethanol. After the solvent was evaporated under nitrogen, the stamp was brought into contact with the gold surface. A microstructured patterned SAM containing primary amine groups was thus obtained (Scheme 85).

Wang et al.[202] reported a method of preparing patterned two-level polypeptide brushes. Here, a clean silicon oxide substrate was masked with a patterned photoresisting layer by conventional photolithography. A SAM of 3-aminopropyltriethoxysilane (APS) formed on the uncovered silicon oxide surface. After removing the photoresisting layer, poly(γ-methyl-L-glutamate) (PMLG) was grown from the SAM of APS. To pattern the second-level polypeptide brushes, the patterned photoresisting layer was deposited rotated 90° with respect to the previous pattern. Then PBLG was grown from the exposed PMLG chain ends. A covalently bound, multilevel pattern consisting of grafted PMLG and PMLG-*b*-PBLG was thus obtained.

Methods of immobilizing primary amine functional groups for surface-initiated NCA polymerization on the side walls of CNTs have also been reported. Ramanathan et al.[203] reported two different approaches of chemically modifying SWCNT with primary amine groups. The first approach involves a direct coupling of ethylenediamine with the carboxylic acid groups on the SWCNT surface to introduce primary amine groups through amide formation. The other approach converts the surface carboxylic acid groups into hydroxymethylene groups, followed by transformation of these hydroxymethylene groups into aminomethylene groups (Scheme 86). Detailed characterization was performed by FTIR and XPS.

Yao et al.[204] reported another amide functionalization process for surface initiated PBLG on SWCNTs. Raw multiwalled carbon nanotubes (MWCNTs) were first oxidized in concentrated strong acids to create carboxylic acid groups at the side walls (MWCNT-COOH). These carboxylic groups were converted into acyl chloride groups and subsequently reacted with excess ethylenediamine to generate primary amine decorated MWCNTs. Thermogravimetric analysis (TGA) indicated that the resulting surface-functionalized CNT contained 2.1 mmol of $-NH_2$ per gram of carbon.

In another study by Li et al.,[205] the acyl chloride MWCNTs were treated with excess 1,6-diaminohexane to produce primary amine-grafted MWCNTs. TGA showed that there was approximately 0.75 mmol of $-NH_2$ per gram of carbon.

Surface-initiated NCA polymerization from polymer membranes can be realized by introducing primary amine groups onto the surface, followed by NCA polymerization. The primary amine groups were introduced by the so-called "glow-discharge treatment" or "plasma treatment". In the "glow-discharge treatment" reported by Ito et al.,[206] polymer membranes of polytetrafluoroethylene (PTFE)/polyethylene (PE) copolymer were maintained at a pressure of 0.05 Torr in the presence of ammonia gas and were glow-discharged using a high-frequency modulator (400 W). The number of $-NH_2$ thus produced at the polymer membrane surface was found to be increased with increasing power and time of the glow-discharge treatment.

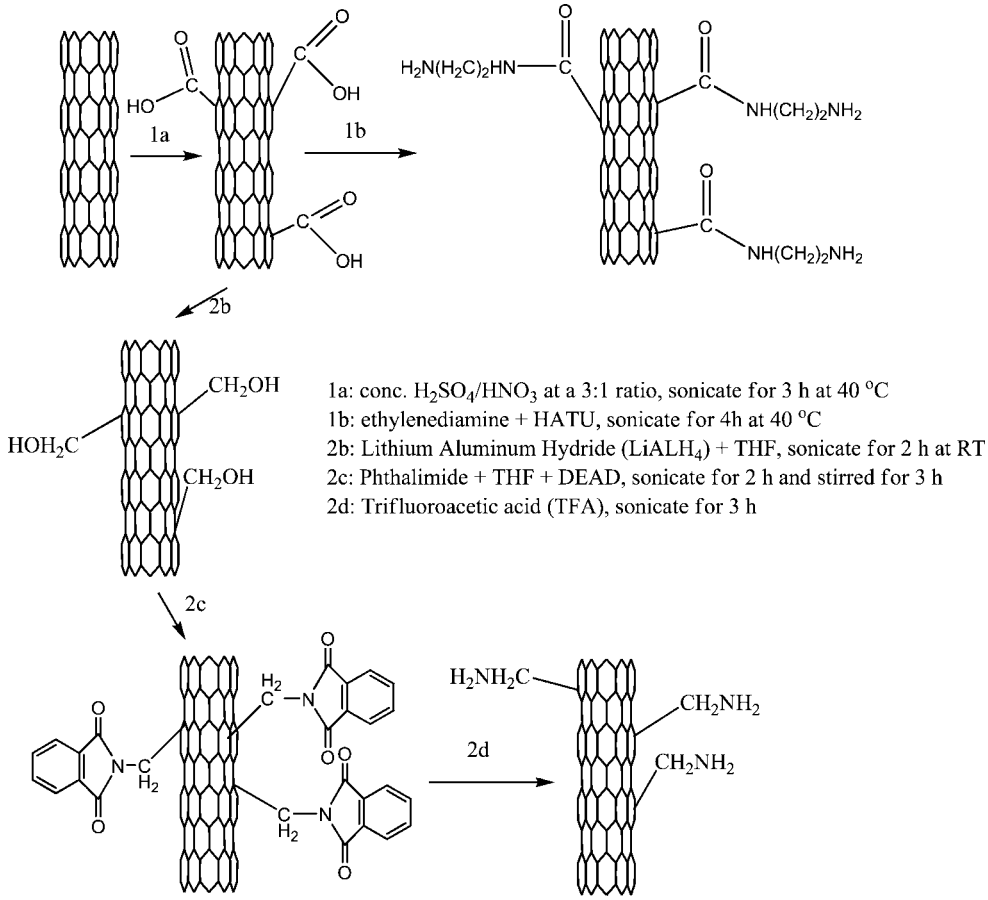
Liu et al.[207] attached primary amine groups on the surface of microporous polypropylene (PP) membranes by a similar approach using a plasma reactor. The PP membrane was exposed to ammonia plasma, which was generated at a given ammonia pressure, for a predetermined period of time. The amount of the $-NH_2$ groups on the treated polymer membrane was determined by the ninhydrin method[208] and calibrated using *n*-octylamine.

4.2.2.2. Surface-Initiated NCA Polymerization. Surface-initiated NCA polymerizations have been conducted by conventional ring-opening NCA polymerization with amines as well as transition metal-mediated initiators.

4.2.2.2.1. NCA Polymerization with Amino Initiators. Conventional ring-opening NCA polymerizations have been performed in solution or in bulk. Heise et al.[199] performed the polymerization of BLG-NCA from a primary amine end-functionalized SAM on a silicon wafer, in dry dioxane under argon at room temperature for 14 days. The initiating SAM was prepared by silanization with mixed functionalized and nonfunctionalized trichlorosilane compounds. It was found that the thickness and roughness of the resulting polypeptide thin film varied with the concentration of the initiation sites. A minimum concentration is necessary to obtain a certain thickness and minimum roughness. Beyond a certain concentration, the thickness of the polypeptide thin film remained constant while its roughness increased.

The degree of polymerization is very limited when carried out in solution, due to extensive chain-transfer or termination side reactions. Wieringa and Shouten[209] investigated the polymerizations in bulk and concluded that solid state NCA polymerization gave better and reproducible brushes than those conducted in solutions. The solid-phase polymerization was carried out by spin-coating a thin film of monomer onto silicon wafers, pretreated with a primary amine containing a silanization agent, followed by heating the samples above

Scheme 86. Covalent Grafting of Primary Amine on SWCNTs



the melting point of the monomer. The polypeptide brushes thus produced appear to adopt a pure α-helical conformation. In addition, the total grafted amount can be controlled by varying the spinning rate. Higher spinning rates resulted in smaller amounts of grafted polymer. However, approximately 80% of the polymerized material was actually nongrafted on the substrate. This free polymer was thermally initiated and had both α-helical and β-sheet conformations.

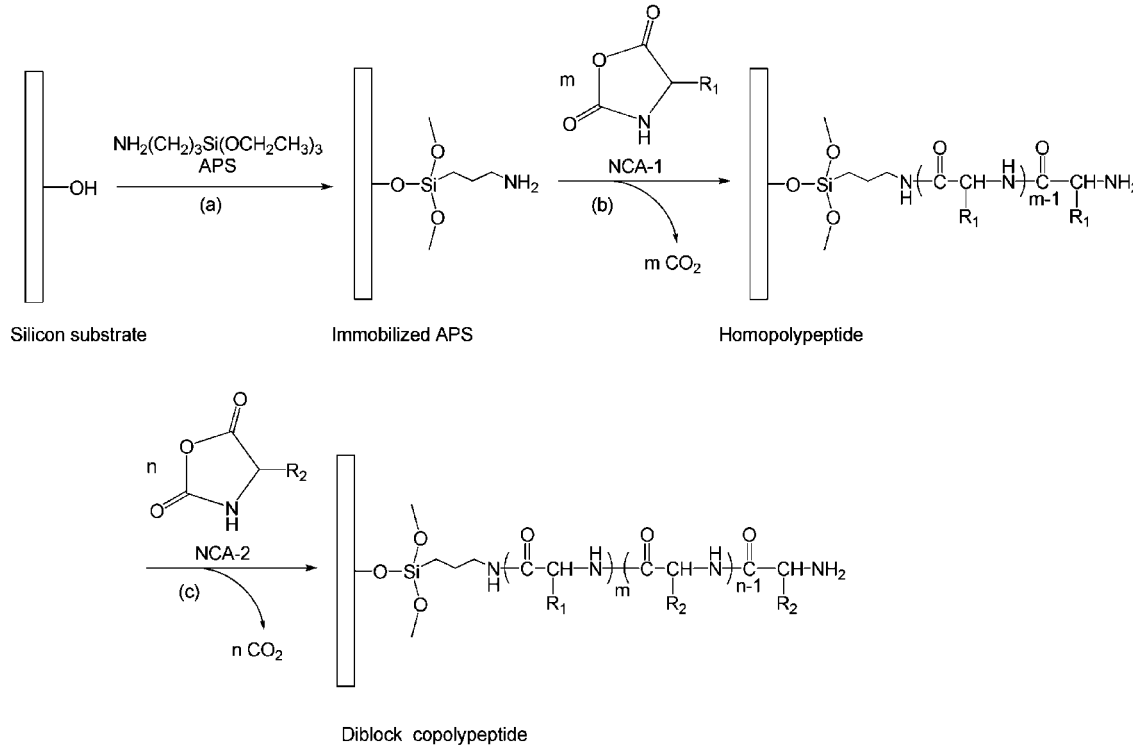
Luijten et al.[210] reported a surface-initiated polymerization of β-phenethyl-L-aspartate NCA from an APS-treated surface of silicon or quartz. The polymerization was performed in dry chloroform at 40 °C under inert atmosphere. As the surface-initiated polymerization started, the solution became slightly cloudy, and after 24 h, precipitation occurred. The obtained layer thickness was limited because the growing chain ends were blocked by the precipitated, nongrafted polymer chains formed as byproduct. Although the polymerization was plagued with side reactions, the grafted polyaspartate brushes were long enough to adopt an α-helical secondary conformation. It is interesting to note that this α-helical conformation can be reversibly inverted to other helical structures such as left-handed ω-helices and π-helices upon heating at 140−150 °C. This conformational variation is caused by the fact that the peptide bonds in polyaspartates cannot form perfectly stable H-bonding along the main chain, due to competitive H-bonding between the NH groups in the backbone and C═O groups in the side chain. In certain polyaspartates and copolyaspartates, the helical sense in solution and in the solid state varies with temperature. Thus, these brushes exhibit a thermally induced α−ω and α−π transition.

Wieringa et al.[211−213] studied the surface-initiated NCA polymerization of γ-benzyl L-glutamate (BLG) and γ-methyl L-glutamate (MLG) in anhydrous DMF at 40 °C. The monomer solution was added to the silanized silicon wafer surface, prepared by treating the wafer with refluxing APS vapor. It was reported that most of the polymer growth takes place during the first 5 h. The role of the monomer concentration was also reported. Higher monomer concentration led to a thicker polypeptide thin film. The orientation of the helices was closer to perpendicular with respect to the grafting surface with higher monomer concentrations and shorter reaction times. The differences in tilt angles with the substrate of PBLG and PMLG can be explained by changes in grafting density, caused mainly by the size of the monomer.

The synthesis of surface-grafted block copolypeptides of PBLG as the first block and PMLG as the second block was also described. The primary amine-pretreated silicon wafers initiated the surface polymerization of the first monomer. After removal of the excess monomer solution, the amine end groups of the formed PBLG blocks acted as initiators for the second monomer. This method provides the possibility of making layered structures of surface-grafted block copolymers with tuned properties.

Chang et al.[214,215] introduced a novel dry process for surface-initiated NCA polymerization, i.e. vapor deposition polymerization on a surface. In contrast to the case for melt polymerization, where local monomer concentration may vary dramatically, the vapor deposition method offers better control of the monomer distribution over the whole surface area. This should lead to a narrower molecular weight distribution of the grafted brushes. The chemistry of the growing of homo- and block

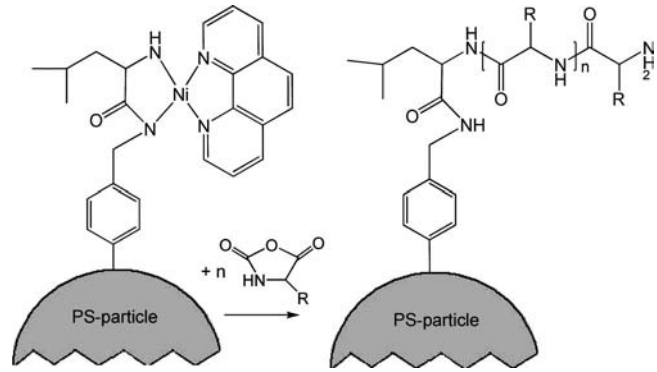
Scheme 87. Surface-Initiated Homo- and Block Copolymerization on a Silicon Surface



copolypeptides from the APS modified silicon surface is provided in Scheme 87. In this method, the reaction rate can be controlled by changing the temperature, substrate−monomer distance, pressure, and reaction time to obtain the desired film properties. PBLG films with thicknesses from 4 to 40 nm have been reproducibly prepared. All of the PBLG films above 4 nm thick adopt a pure α-helical conformation with a 45° tilt angle and have homogeneous surface structure with surface roughness, rms, within 3.5 nm.

Niwa et al.[216] reported the surface-initiated polymerization of BLG-NCA monomer in the presence of helical PBLG immobilized on a gold surface (for a better understanding, see also Scheme 81). In this study, PBLG-N-SS or PBLG-C-SS was grafted in advance onto the gold surface. A mixed SAM composed of an amine-terminated disulfide compound and an amine-free disulfide compound was then prepared on the uncovered surface. The ratio of these two compounds was used so as to satisfy spatial requirements. The surface-initiated polymerization of BLG was controlled by the direction of the macrodipole of the pregrafted helical rods (PBLG-N-SS or PBLG-C-SS). The rate of polymerization in the presence of PBLG-N-SS was accelerated by a factor of about 10 compared to that in the absence of PBLG matrix. In contrast, the presence of PBLG-C-SS suppressed the BLG-NCA polymerization. These effects of helical PBLG matrices on polymerization could be a result of their macrodipole interaction. In the case of PBLG-N-SS, the helix-macrodipole moment direction is antiparallel to that of the polymerized PBLG chain, and therefore, the growth of the PBLG chain by polymerization between PBLG-N-SS matrices (DP: 95) would be much more favorable than that without PBLG matrix (DP: 73). Conversely, the presence of PBLG-C-SS, with a macrodipole moment direction parallel to the growing chains, resulted in an energetic disadvantage for the PBLG chain growth (DP: 15).

Scheme 88. Ni-Mediated NCA Polymerization on a PS-Particle Surface



Surface-initiated polymerization from the side walls of CNTs is a heterogeneous process. Li et al.[205] prepared poly-L-lysine grafted MWCNT (MWCNT-*g*-PLL) by first suspending the amine-functionalized CNTs (CNT-NH_2) in anhydrous tetrahydrofuran (THF). After the mixture was sonicated for 30 min, the monomer was added and the polymerization was allowed to proceed for 48 h under inert atmosphere at 30 °C. The final reaction mixture was diluted with THF and sonicated followed by filtration through a 0.2 μm PTFE membrane. The solid was collected and washed with a large quantity of THF to remove free polypeptides.

4.2.2.2.2. NCA Polymerization with Transition Metal Initiators. The feasibility of living NCA polymerization grafting from surfaces using nickel-complex initiators was explored by Witte et al.[217] The polymerization was carried out on the spherical surfaces of commercially available polystyrene (PS) resins (Scheme 88). Two approaches have been investigated, namely the block copolymerization approach and the alloc-amide approach. The block copolymerization approach includes four steps: (1) immobilization

of one unit of the NCA monomer onto the surface of the PS beads; (2) reaction of the bound NCA monomers with an excess amount of Ni-complex initiator to create living chain ends; (3) removal of the excess nonbound Ni-complex initiator; and (4) addition of NCA monomer and ROP. The bound polypeptide chains, cleaved from the PS resins by photolysis, were found to have polydispersity indices of 1.52 and almost Gaussian molecular weight distributions. Unfortunately, the unbound polypeptide was approximately 82%. In order to overcome this drawback, the authors proposed the alloc-amide approach, which gives well-defined initiators at the surface and substantially reduces the formation of free polymer in solution (45−50%). In this approach, the initiating Ni(amido-amidate) complex is created directly on the surface. These grafted initiation sites are activated with excess $Ni(COD)_2$ and phenanthroline as ligand.

5. Concluding Remarks

It is clear that the new developments in the ROP of NCAs hold tremendous promise that well-defined polypeptides with controllable molecular weight, sequence, composition, and molecular weight distribution can be synthesized. Although much development is still required, the synthetic polypeptide-based materials begin to rival the natural counterparts in terms of complexity and accuracy. Such well-defined polypeptide-based materials will greatly assist in the development of new biomedical and pharmaceutical materials with a wide range of tunable properties. On the other hand, studies on these materials will shed light on the natural phenomena associated with proteins.

6. Acknowledgments

The Research Committee of the University of Athens and the General Secretariat of Research and Technology of Greece are acknowledged for financial support.

7. List of Symbols and Abbreviations

AFM	atomic force microscopy
AMM	activated monomer mechanism
APS	3-(aminopropyl)triethoxysilane
ATRP	atom transfer radical polymerization
BLL	*tert*-butyloxycarbonyl-L-lysine
BLG	γ-benzyl-L-glutamate
COD	1,5-cyclooctadiene
CTA	chain transfer agent
CNT	carbon nanotube
CFP	3-(trichlorosilyl)propyl chloroformate
μCP	microcontact printing
DAD	1,12-diaminododecane
DATT	1,13-diamino-4,7,10-trioxatridecane
DCC	dicyclohexylcarbodiimide
DMF	*N*,*N*′-dimethylformamide
DMSO	dimethylsulfoxide
DP	degree of polymerization
dppe	[bis(diphenylphosphino)ethane]
HMDS	hexamethyldisilazane
HVT	high vacuum techniques
LB	Langmuir−Blodgett
LGA	L-glutamic acid
α-MeS	α-methylstyrene
MWCNTs	multiwalled carbon nanotubes
NACE	nonaqueous capillary electrophoresis
NAM	normal amine mechanism
NCA	*N*-carboxyanhydride
NMP	*N*-methylpyrrolidone
PBLG	poly(γ-benzyl-L-glutamate)
PBLL	poly(*tert*-butyloxycarbonyl-L-lysine)
PCL	poly(ε-caprolactone)
PDMS	polydimethylsiloxane
PDI	polydispersity index (M_w/M_n)
PEO	polyethyleneoxide
PFS	poly(ferrocenyldimethylsilane)
PGLY	polyglycine
PI	polyisoprene
PLA	polylactide
PLEU	polyleucine
PLGA	poly(L-glutamic acid)
PLL	poly(L-lysine)
PMLG	poly(methyl-L-glutamate)
PNVP	poly(*N*-vinylpyrollidone)
PP	polypropylene
PS	polystyrene
PTFE	polytetrafluoroethylene
PTYR	polytyrosine
PZLL	poly(ε-carbobenzyloxy-L-lysine)
ROP	ring opening polymerization
SAM	self-assembled monolayer
Sar	sarcosine
SEC	size exclusion chromatography
SWCNTs	single-walled carbon nanotubes
TEM	transmition electron microscopy
TFA	ε-trifluoroacetyl-L-lysine
TGA	thermogravimetric analysis
TMS	trimethylsilyl amine
TMU	tetramethylurea
Z	carbobenzyloxy

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