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Tandem Catalysis for the Preparation of Cylindrical Polypeptide Brushes

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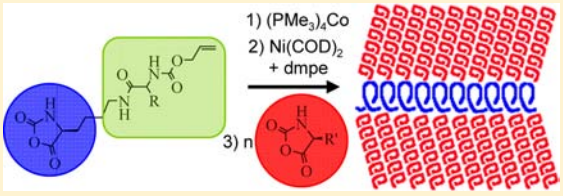
Supporting Information

ABSTRACT: Here, we report a method for synthesis of cylindrical copolypeptide brushes via *N*-carboxyanhydride (NCA) polymerization utilizing a new tandem catalysis approach that allows preparation of brushes with controlled segment lengths in a straightforward, one-pot procedure requiring no intermediate isolation or purification steps. To obtain high-density brush copolypeptides, we used a "grafting from" approach where alloc-α-aminoamide groups were installed onto the side chains of NCAs to serve as masked initiators. These groups were inert during cobalt-initiated NCA polymerization and gave allyloxycarbonyl-α-aminoamide-substituted polypeptide main chains. The alloc-α-aminoamide groups were then activated *in situ* using nickel to generate initiators for growth of side-chain brush segments. This use of stepwise tandem cobalt and nickel catalysis was found to be an efficient method for preparation of high-chain-density, cylindrical copolypeptide brushes, where both the main chains and side chains can be prepared with controlled segment lengths.



INTRODUCTION

Branched chain copolypeptides have intrigued scientists for many years. Sela performed pioneering studies on what he termed "multi-chain polypeptides" that were evaluated for their immunostimulating properties.[1] More recently, hyperbranched and dendritic polypeptides, with their abundance of functional groups and three-dimensional globule-like presentation of functionality, have been found valuable for multiple presentation of antigens in vaccines, as imaging agents, and for drug and oligonucleotide delivery.[2−7] Although the properties of branched polypeptides show great promise, controlled synthesis of these materials remains challenging. Stepwise peptide dendrimer synthesis provides excellent control over polypeptide branching, but is a tedious process that can be difficult to scale. An alternative is the preparation of hyperbranched or dendrigraft polypeptides via polymerization of α-amino acid *N*-carboxyanhydrides (NCAs).[8−12] These materials have been prepared via a stepwise sequence of polymerization, side-chain deprotection, and polymerization,[8−11] as well as by simultaneous polymerization and deprotection.[12] Although these methods are more efficient and scalable compared to dendrimer preparation, they tend to give limited control over branch architecture, or provide only low branch density in polymer brushes.[8−12] High-density cylindrical copolypeptide brushes are desirable synthetic targets since the potential to control their three-dimensional shape makes them intriguing as components in block copolymers, which can then be used for preparation of self-assembled materials with complex morphologies.[13−15] Although there have been many successes in controlled synthesis of cylindrical hybrid−polypeptide copolymer brushes,[16−20] the preparation of entirely polypeptide-based cylindrical copolymer brushes has not been achieved. Here, we report a new method for synthesis of cylindrical copolypeptide brushes via NCA polymerization utilizing a tandem catalysis approach that allows preparation of brushes with controlled segment lengths in a straightforward, one-pot procedure that requires no intermediate isolation or purification steps.

The synthesis of branched polypeptides via NCA polymerization is challenging since protecting groups typically need to be removed from side-chain functionalities (e.g., primary amines of lysine) to generate initiators for branch points.[8−12] Although this can be done *in situ* via hydrogenation to generate hyperbranched polypeptides,[12] this approach is difficult to control, and more regular branched structures are typically obtained only when polymers with deprotected side chains are isolated and purified before resuming polymerization.[8−11] To avoid this need for intermediate purification steps and to obtain high-density brush copolypeptides, we pursued a "grafting from" approach where alloc-α-aminoamide groups (alloc = allyloxycarbonyl) were installed onto the side chains of NCAs to serve as masked initiators. These groups were envisioned to be inert during NCA polymerization to give alloc-α-aminoamide-substituted polypeptide main chains, but then would be activated *in situ* to generate NCA polymerization initiators for side-chain brush growth (Figure 1). We have shown previously that alloc-α-aminoamides react quantitatively with $L_2Ni(COD)$ (L = donor ligand, COD = cyclooctadiene) in dimethylformamide (DMF) to generate amido-amidate nickelacycles, which are efficient initiators for living polymerization of NCAs.[21] We

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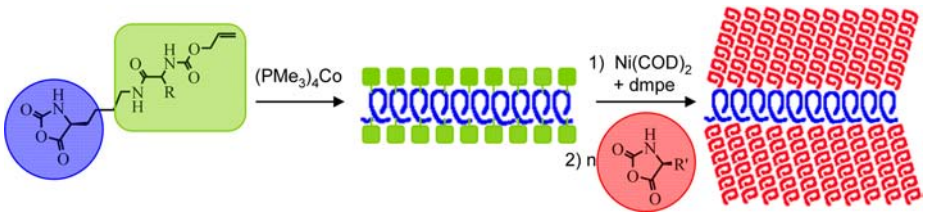


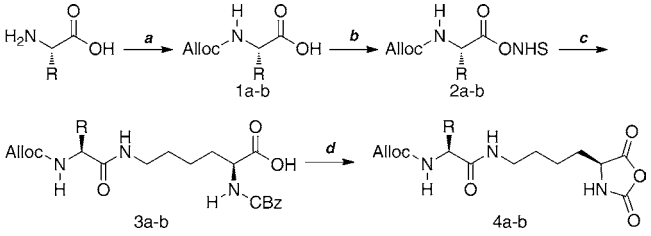
Figure 1. Schematic diagram showing two-stage, one-pot synthesis of cylindrical brush copolypeptides. The *N*-carboxyanhydride (NCA) component (blue) of N_ε-(alloc-L-methionyl)-L-lysine NCA is first polymerized using $(PMe_3)_4Co$ initiator to give a linear polypeptide that bears pendant initiator precursors (green). Side-chain initiators are then activated using dmpeNi(COD), followed by addition of a second NCA monomer (red) to give the brush copolymers.

have also found (*vide infra*) that this reaction is selective for Ni(0), since Co(0) complexes (i.e., $(PMe_3)_4Co$) do not react with alloc-α-aminoamides under similar conditions. Since $(PMe_3)_4Co$ reacts directly with NCAs to generate amido-amidate cobaltacycles, which also initiate the living polymerization of NCAs,[22] use of stepwise tandem cobalt and nickel catalysis should enable the facile synthesis of brush copolypeptides from alloc-α-aminoamide-substituted NCAs (Figure 1). A key feature of this process is the envisioned use of different initiator formation mechanisms for main-chain and side-chain growth,[23] so that these polymerizations can be sequentially controlled in a single-pot procedure.

RESULTS AND DISCUSSION

For the synthesis of alloc-α-aminoamide-substituted NCAs, we chose to use L-lysine as the main-chain-forming NCA, as substituted lysine NCAs are readily polymerized and the side-chain amine group is easily functionalized.[24−26] We used the hydrophobic amino acids L-isoleucine and L-methionine for construction of the alloc-α-aminoamide side-chain groups (Scheme 1) since these required no protecting groups and

Scheme 1. Synthesis of Allyloxycarbonyl-Aminoamide-Containing NCA Monomers[a]



[a]Reagents and conditions: (a) allylchloroformate, Na_2CO_3, β-cyclodextrin, H_2O, 3.5 h (88% yield). (b) DCC, NHS, THF, 0−21 °C, 1 h (69% yield). (c) N_α-Cbz-L-Lys-OH, Na_2CO_3, 1:1 THF:H_2O, 21 °C, 48 h (72% yield). (d) DCMME, DCM, 40 °C, 36 h (70% yield). **4a** = N_ε-(allyloxycarbonyl-L-methionyl)-L-lysine-*N*-carboxyanhydride (R = $-CH_2CH_2SCH_3$), **4b** = N_ε-(allyloxycarbonyl-L-isoleucyl)-L-lysine-*N*-carboxyanhydride (R = $-CH(CH_3)CH_2CH_3$).

similar residues had been found to form good initiators in earlier work.[20] Methionine was also chosen since it provides a means for chemoselective, post-polymerization cleavage of side-chain segments by reaction with CNBr.[27] The methionine- and isoleucine-derivatized lysine NCA monomers were prepared using standard methods (Scheme 1) and were obtained in reasonable yields after purification using flash column chromatography.[28] Although both monomers were found to be efficiently polymerized using $(PMe_3)_4Co$, we have focused the studies here on the methionine-based monomer, N_ε-(alloc-L-methionyl)-L-lysine-*N*-carboxyanhydride (K^{AM} NCA), to take advantage of the side-chain segment cleavability at this residue.

Polymerization of K^{AM} NCA using $(PMe_3)_4Co$ in tetrahydrofuran (THF) proceeded readily at ambient temperature to give poly(N_ε-(alloc-L-methionyl)-L-lysine), poly(K^{AM}), with complete monomer conversion and no reaction at the side-chain alloc groups. To determine chain lengths, K^{AM} NCA was polymerized at different monomer-to-initiator ratios, and after complete monomer consumption, active chains were end-capped with isocyanate-terminated poly(ethylene glycol) (PEG, M_n = 2000 Da).[29] Compositional analysis of purified, end-capped polymers by ^1H NMR gave number-average poly(K^{AM}) chain lengths that increased linearly with stoichiometry (Figure 2). Chain length distributions of these poly(K^{AM}) samples were

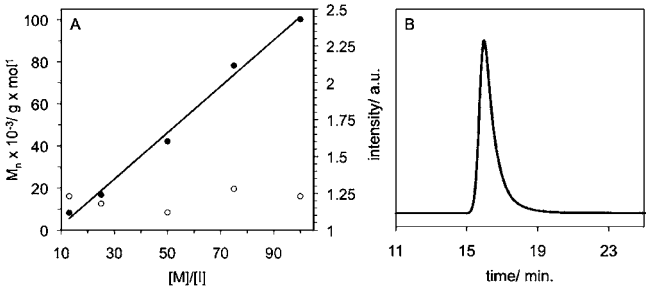


Figure 2. (a) Molecular weight (M_n, filled circles) and polydispersity index (M_w/M_n, open circles) of poly(K^{AM}) as a function of monomer-to-initiator ratio ([M]:[I]) after 100% monomer conversion. M_n and M_w/M_n were determined by ^1H NMR and gel permeation chromatography (GPC/LS). (b) GPC chromatogram (normalized LS intensity in arbitrary units versus elution time) of a poly(K^{AM}) sample (Table S1, entry 2).

obtained by gel permeation chromatography (GPC)/LS analysis and were found to possess low polydispersity indices (M_w/M_n) between 1.12 and 1.28, indicating the formation of well-defined polypeptides (Figure 2). Poly(K^{AM}) was prepared in high yield with precisely controlled chain lengths up to nearly 300 residues long, and could also be prepared as diblock copolymers with other amino acids (Table 1). Overall, these data show that K^{AM} NCA, similar to other NCAs, is able to undergo living polymerization when initiated with $(PMe_3)_4Co$. Circular dichroism spectroscopy of poly(K^{AM}) in THF revealed that this polymer, similar to other poly(L-lysine) derivatives,[24−26] is predominantly α-helical (see Supporting Information (SI), Figure S1), which imparts poly(K^{AM}) with good solubility in organic solvents and may provide an exposed presentation of the side-chain alloc-α-aminoamide groups.[19]

The key feature of poly(K^{AM}) is the reactivity of its side-chain alloc-L-methionyl groups that will be utilized for

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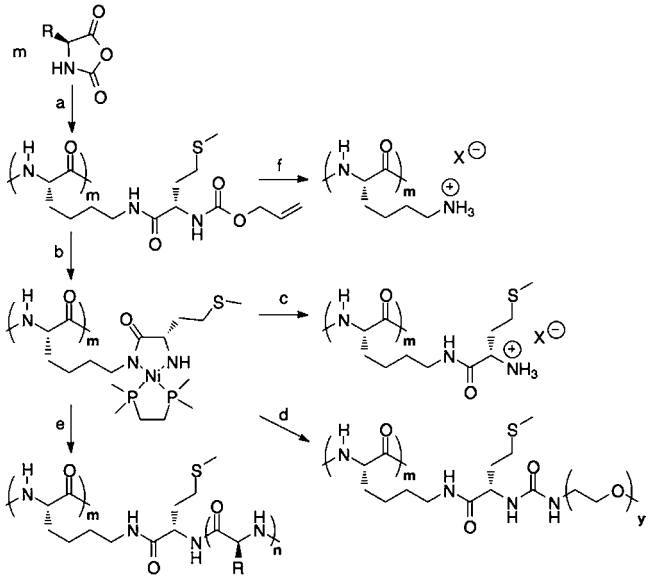
Table 1. Synthesis of Diblock Copolypeptides Using $(PMe_3)_4Co$ in THF at 21 °C

			first segment[b]			diblock copolymer[c]			
entry	1st monomer[a]	2nd monomer[a]	M_n	M_w/M_n	DP	M_n	M_w/M_n	DP	yield (%)[d]
1	17 K NCA	17 K^{AM} NCA	18 700	1.18	71	43 000	1.12	142	99
2	17 K NCA	6 K^{AM} NCA	18 700	1.18	71	29 300	1.29	102	97
3	50 K^{AM} NCA	50 K NCA	31 200	1.11	91	55 000	1.12	184	100
4	50 K^{AM} NCA	25 K NCA	31 200	1.11	91	45 300	1.25	145	100

[a]First and second monomers added stepwise to the initiator; number indicates equivalents of monomer per $(PMe_3)_4Co$. K NCA = N_ε-Cbz-L-lysine-*N*-carboxyanhydride. K^{AM} NCA = N_ε-(alloc-L-methionyl)-L-lysine-*N*-carboxyanhydride. [b]Molecular weight and polydispersity index after polymerization of the first monomer (determined by GPC/LS for poly(K); determined by GPC/LS and ^{1}H NMR for poly(K^{AM})). [c]Molecular weight and polydispersity index after polymerization of the second monomer (as determined by GPC/LS and ^{1}H NMR). [d]Total isolated yield of diblock copolypeptide. DP = degree of polymerization.

cylindrical polypeptide brush growth. To evaluate this chemistry, the side chains in freshly prepared, unpurified poly(K^{AM}) (Scheme 2a) were reacted with stoichiometric

Scheme 2. Synthesis and Reactivity of Poly(K^{AM})[a]



[a]Reagents and conditions: (a) $(PMe_3)_4Co$, THF, 21 °C, 1 h. (b) dmpeNi(COD), DMF, 80 °C, 16 h. (c) 4.0 M HCl, 21 °C, 2 h. (d) α-Methoxy-ω-isocyanoethyl-poly(ethylene glycol), PEG-NCO (M_w = 350 Da), DMF, 21 °C, 16 h. (e) Bn-Glu NCA, DMF, 21 °C, 16 h. (f) 0.25 M cyanogen bromide in 70% formic acid in water, 4 h.

$Ni(COD)_2$ and bis(dimethylphosphino)ethane (dmpe) at 80 °C to generate amido-amidate nickelacycle initiating groups (Scheme 2b). This reaction is known to proceed quantitatively on small molecules,[21] yet we needed to confirm that active nickelacycle initiators were also formed in high yield on the poly(K^{AM}) side chains. As a first step, to determine if all alloc-L-methionyl groups react with Ni(0), the product of this reaction was quenched by addition of 4.0 M HCl. While alloc-L-methionyl groups are stable to these conditions, the activated nickel complexes are hydrolyzed to give poly(N_ε-(L-methionyl)-L-lysine) (Scheme 2c). Analysis of the polymer product by ^{1}H NMR showed that at least 91% of the alloc groups had reacted when stoichiometric Ni(0) was used (Figure 3). It is likely that higher conversion of alloc groups could be obtained by using excess Ni(0), but this was not pursued since free Ni(0) will also react with NCAs and would need to be removed in a subsequent purification step.[23] To show that the alloc-L-methionyl groups not only react with Ni(0) but also form

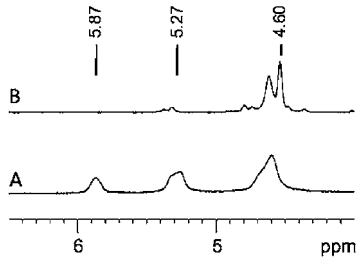


Figure 3. Activation and quenching of alloc side chains in poly(K^{AM}). (a) Partial ^{1}H NMR spectrum in TFA-*d* showing alloc proton resonances of poly(K^{AM}). Resonances: $-CH_2CH{=}CH_2$ (5.87 ppm), $-CH_2CH{=}CH_2$ (5.27 ppm), and $-CH_2CH{=}CH_2$ and α-carbon protons (4.60 ppm). (b) Partial ^{1}H NMR spectrum in TFA-*d* of poly(K^{AM}) after activation with dmpeNi(COD) followed by quenching with 4.0 M HCl to remove nickel complexes. By NMR integrations, 91− 100% of alloc side chains were activated (see SI).

active nucleophilic initiators, we reacted them with different lengths of isocyanate-terminated PEG (M_n = 350 or 1000 Da), which have been shown to react quantitatively with active nickelacycle chain ends (Scheme 2d).[29] Analysis of these products (see SI, Table S2) revealed that the Ni-activated side chains of poly(K^{AM}) were capped by PEG chains with 91−100% efficiency, showing that the formation of active nickelacycle initiators at each side chain in poly(K^{AM}) proceeds with high efficiency.

Having verified that the side-chain groups of poly(K^{AM}) can be converted *in situ* to active nickelacycle initiators, we next explored the grafting of cylindrical polypeptide brushes from these activated polymers (Scheme 2e). To obtain high-chain-density cylindrical brushes, the initiation efficiency for side-chain initiating groups needs to be very high to ensure that polypeptides grow from each side chain. We had previously found that initiation efficiency for NCA polymerization using small-molecule nickelacycle initiators was low in THF, due to nickelacycle aggregation.[23] However, initiation efficiency was quantitative in DMF, which better solubilizes these complexes.[23] Here, we also found that growth of side-chain brush segments was optimal in DMF, and that nickelacycle aggregation on activated poly(K^{AM}) was further suppressed by use of 2 equiv of dmpe per nickel center. For the purpose of being able to adequately characterize side-chain segment growth, we used a block copolymer main chain, poly(N_ε-Cbz-L-lysine)-*block*-poly(K^{AM}), poly(K)-*b*-poly(K^{AM}), where the poly(K) segment served as a high-molecular-weight end-group for determination of average side-chain segment lengths by ^{1}H NMR analysis (see SI, Table S3). For initial proof of concept, γ-benzyl-L-glutamate NCA (E NCA) was used for

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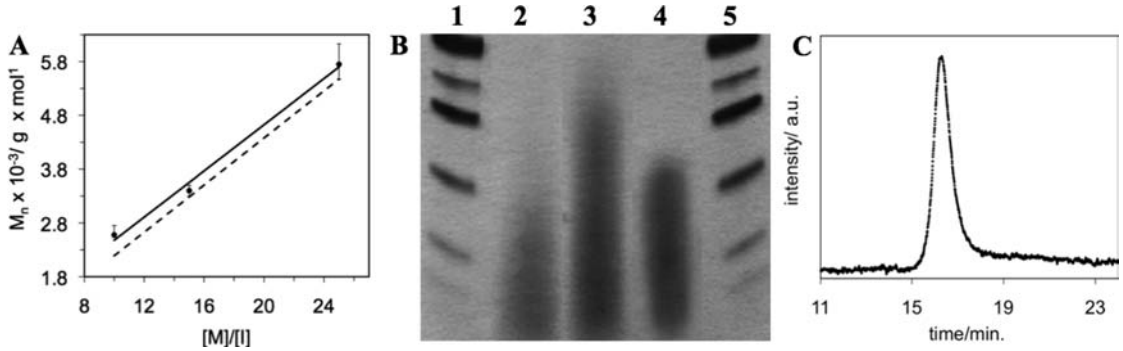

Figure 4. Lengths and chain length distributions of poly(E) segments grown from activated poly(K^{AM}) side chains. (a) Number-average molecular weight after 100% monomer conversion of poly(E) segments as a function of monomer-to-activated K^{AM} initiator ratio ([M]:[I]) in poly(K)-*b*-poly(K^{AM}) block copolymers. Values were determined using ^{1}H NMR integrations (filled circles), and each data point represents the average of four repeat experiments (error bars show the range of data obtained). Dotted line represents the expected calculated values. (b) Visualization of PGA chain length distributions using SDS−PAGE. Lanes 1 and 5: protein molecular weight standards (from top: 25, 20, 15, 10, 5, and 2 kDa). Lanes 2 and 3: synthetic PGA standards (lane 2, M_n = 5120, M_w/M_n = 1.05; lane 3, M_n = 18 900, M_w/M_n = 1.13). Lane 4: PGA sample (M_n = 3820) cleaved from a brush copolymer using CNBr. (c) GPC chromatogram (normalized LS intensity in arbitrary units versus elution time) of a PGA sample (M_n = 3820) cleaved from a brush copolymer using CNBr (same as in lane 4 of panel b) that was re-benzylated and found to have M_w/M_n = 1.13.

side-chain segment growth since it forms soluble, α-helical chains that can be readily distinguished from the lysine-based main chain. For copolypeptide brush preparation, poly(K)-*b*-poly(K^{AM}) copolymers of different segment lengths (Table S3) were prepared in THF using $(PMe_3)_4Co$ as described above. Brush copolymers could also be prepared using poly(K^{AM}) homopolymers, if desired. After concentration of the crude reaction mixtures under vacuum followed by dilution with DMF, the K^{AM} side chains in the copolymers were activated by reaction with $Ni(COD)_2$ and dmpe at 80 °C to give macroinitiators that were used directly with no further isolation or purification steps.

Different amounts of E NCA in DMF were added directly to the block copolymer macroinitiator solutions, resulting in growth of the brush segments. The polymerizations of E NCA were found to go to completion and the copolypeptide brushes were obtained in high yields (Table S3). Compositional analysis of the copolypeptide brushes by ^{1}H NMR showed that average poly(γ-benzyl-L-glutamate), poly(E), segment lengths increased linearly with E NCA monomer-to-activated K^{AM} initiator ratios and were close to values expected for 100% brush initiation efficiency, indicating controlled polymerization of the side-chain segments (Figure 4a). Poly(E) segments were grown to degrees of polymerization of <25 for ease of characterization relative to the main chains. However, on the basis of previous results using similar initiators,[21,29] we expect longer side-chain segments could be grown. To measure chain length distributions of the poly(E) segments, we utilized the methionine linker to cleave the brush segments from the main chain. A model reaction was performed by mixing N_ε-(alloc-L-methionyl)-N_α-Cbz-L-lysine with CNBr, resulting in complete cleavage of the alloc-L-methionyl group from the lysine residue, which confirmed the utility of this reaction for chemoselective peptide cleavage (see SI). A similar reaction on poly(K^{AM}) showed that CNBr cleavage at the methionine linkers works equally as well on this polypeptide, and gave the expected alloc-homoserine lactone byproduct (Scheme 2f). To perform this reaction on the copolypeptide brushes, the poly(E) segments were first deprotected to poly(L-glutamic acid), PGA, to improve their solubility in polar media, and the brushes were then incubated with CNBr in aqueous formic acid (see SI). The resulting cleaved polypeptide segments were then analyzed using SDS−PAGE and compared to narrow molecular weight distribution PGA standards (M_w/M_n = 1.05−1.13) (Figure 4b). We found that the cleaved PGA segments gave bands similar in width and location to the standards, indicating narrow chain length distributions and molecular weights close to expected values. These results were further supported by rebenzylation of a PGA sample to give a poly(E) for analysis using GPC/LS (Figure 4c) that was also found to possess a narrow molecular weight distribution (M_w/M_n = 1.13), which confirmed the growth of a high density of uniform brush segments.

■ CONCLUSION

The use of tandem cobalt and nickel catalysis has been shown to be an efficient method for preparation of high chain density, cylindrical copolypeptide brushes, where both the main chains and side chains can be prepared with controlled segment lengths. This new methodology avoids the need for intermediate deprotection and purification steps, yields well-defined copolymers, and should be valuable for the straightforward preparation of new copolypeptide architectures. We plan to use this chemistry to create three-dimensional copolymers, with block segments either along the main chain or in the side chains, which should give rise to new self-assemblies that can incorporate the useful properties of branched polypeptides.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data for all new compounds; polymerization data; M_n vs [M]/[I] plots; and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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