Oligo(β-peptoid)s with Backbone Chirality from Aspartic Acid Derivatives: Synthesis and Property Investigation

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ABSTRACT: Poly(β-peptoid)s (N-substituted poly-β-alanines) are an intriguing class of pseudopeptidic materials for biomedical applications, but the polymers prepared by solution polymerization have restricted diversity and functionality due to synthetic difficulty. Synthesis of structurally diverse poly(β-peptoid)s is highly desirable yet challenging. Herein, we report a new approach to synthesize skeletal chiral β-peptoid polymers from readily available aspartic acid derivatives. Two types of N-substituted β-homoalanine monomers, i.e., N-(methyl propionate)-Asp-OMe (N-MeP-Asp-OMe) and N-(tert-butyl propionate)-Asp-OMe (NtBuP-Asp-OMe), were synthesized in high yield via anaza-Michael addition reaction between L-aspartic acid-1-methyl ester (L-Asp-OMe) and acrylate species. Both N-substituted β-homoalanines can be readily converted into polymerizable N-substituted β-homoalanine N-carboxyanhydrides (β-NNCAs). Subsequent ring-opening polymerization (ROP) of these β-NNCA monomers provides access to oligo(β-peptoid)s and mPEG-poly(β-peptoid) diblocks with backbone chirality. Their conformations were preliminarily studied by circular dichroism (CD) spectra and Fourier transform infrared spectroscopy (FT-IR). The synthetic strategy would significantly facilitate the development of novel poly(β-peptoid)s with well-defined and diverse structures.

1. INTRODUCTION

Synthetic peptide analogues have been long sought after for their promising applications in biotechnology. Poly(β-peptoid)s (N-substituted poly-β-alanines) are particularly intriguing class of pseudopeptidic polymers, which can formally be gained by the addition of a methylene unit in the backbone of polypeptoids or the N-substitution of poly(β-peptide). Tertiary amides in a β-peptoid backbone offer higher proteolytic resistance and less polar as compared to typical peptide amide bonds. Benefiting from a combination of biocompatibility, degradability, metabolic stability, and processability, β-peptoids have vast potential for therapeutic applications. Moreover, the extra C–C bond in the β-peptoid backbone would greatly expand the potential diversity of the peptoid shape as compared to α-peptides. In this regard, a great deal of effort has been made to develop poly(β-peptoid) materials with interesting structures for property investigation and biological applications in recent years.

Generally, β-peptoids are conformationally flexible and do not form stable folding structures due to missing amide protons and chiral centers on their backbones. Therefore, approaches to restricting the structural flexibility of β-peptoids are attractive and significant. It has been demonstrated that β-peptoids with stable helical structures can be achieved by introducing chiral N-substituents. However, the requirement of submonomers with bulky and chiral substituents in this strategy impedes the utility for the synthesis of chemically diverse β-peptoids with well-defined folded structures. In addition to β-peptoids with chiral N-substituents, β-peptoids with backbone chirality have been shown to form defined secondary structures as well. Specifically, Lim and co-workers reported a series of N-alkylated β-homoalanine oligomers (α-ABpeptoids) via solid-phase synthesis. These α-ABpeptoid oligomers with different side chains showed distinctive circular dichroism (CD) characteristics, indicative of ordered folding conformations. Besides, β-ABpeptoids with chiral groups at β-positions rather than α-positions were prepared via a submonomer solid-phase strategy. Very recently, Morimoto and co-workers reported that the introduction of bulky substituents at β-carbon would strongly promote the folding of β-peptoids. All of the abovementioned β-peptoids are synthesized by the monomer or unique “submonomer” method, where the β-peptoid chain is extended in an iterative and stepwise manner. These strategies provide facile access to unique structural control and diversity in a synthetic polymer. However, their multistep synthesis and tedious purification greatly restrict the large-scale synthesis and wide application of β-peptoids.
Ring-opening polymerization (ROP) of α-N-carboxyanhydrides (α-NCAs) represents an efficient and expedient pathway to obtain poly(α-peptide)s and poly(α-peptoid)-s with controlled structures and compositions. In contrast to poly(α-peptide)s and poly(α-peptoid)-s, studies on the ROP of β-NCAs were rarely reported. In 1954, Birkof and co-workers described the successful synthesis and polymerization of N-p-tolyl-β-alanine-NCa by both thermal and p-toluidine initiation. Later on, they reported the polymerization of N-phenyl-β-alanine-NCa, initiated with water or by heating above the melting point. Zilkha and co-workers demonstrated the synthesis of poly(N-benzyl-β-alanine) from ROP of corresponding β-NCAs. A long time later, Luxenhofer and co-workers demonstrated the living character of β-NCA polymerization from kinetic studies. Homo and block copolymers with Poisson distribution were successfully obtained via azamichael addition. However, the poor solubility limits further investigation. Recently, an alternative route for the synthesis of β-peptoids was reported through copolymerization of N-alkylaziridines with carbon monoxide using a metal-mediated ROP approach. Although a few β-peptoid materials have been prepared via different synthetic routes based on ROP, synthetic versatility of functional monomers and polymers is still challenging. Especially, β-peptoids with backbone chirality have never been prepared by the ROP strategy in the literature to the best of our knowledge. There is still a significant lack in our understanding of the synthesis and physicochemical properties of β-peptoids. Hence, it is particularly intriguing and highly desirable to develop a novel and effective strategy to obtain structurally diverse β-peptoid materials.

Herein, we report an efficient way to make N-substituted β3-homoalanines using an azamichael addition reaction between commercially available L-aspartic acid 1-methyl ester (L-Asp-OMe) and acrylate species (Scheme 1). Two types of optically active N-substituted β3-homoalanine monomers, i.e., N-(methyl propionate)-Asp-OMe (NMeP-Asp-OMe) and N-(tert-butyl propionate)-Asp-OMe (NtBuP-Asp-OMe), were synthesized in high yield. Both of the N-substituted β3-homoalanines can be readily converted into polymerizable N-substituted β3-homoalanine N-carboxyanhydrides (β-NCNAs). Subsequent ROP of these β-NCNA monomers provides access to oligo(β-peptoid)s and mPEG-poly(β-peptoid)s diblocks. The key point of our design is the introduction of the chiral backbone from aspartic acid derivatives using the efficient azamichael addition reaction. Aspartic acid, the only naturally occurring proteinogenic β-amino acid, is readily available and shares many excellent properties of natural amino acids. Aza-michael addition can proceed efficiently under benign conditions and has high tolerance of functional groups. The synthetic strategy represents an efficient methodology to prepare poly(β-peptoid)s with well-defined and diverse structures.

2. RESULTS AND DISCUSSION

2.1. Synthesis of N-Substituted L-Asp-OMe. Scheme 1 shows the synthetic procedures of N-substituted l-aspartic acid derivatives. Generally, L-aspartic acid 1-methyl ester (L-Asp-OMe) reacted with acrylate species (methyl acrylate and tert-butyl acrylate) via the azamichael addition reaction to give β-peptoid monomers. The azamichael conjugate addition was conducted in methanol under weak basic conditions to avoid hydrolysis of the ester bond. The reactions proceeded efficiently at ambient temperature to give clean products of N-(methyl propionate)-Asp-OMe (NMeP-Asp-OMe) and N-(tert-butyl propionate)-Asp-OMe (NtBuP-Asp-OMe) as white powders in excellent yields, which can be easily purified by washing. The structures of NMeP-Asp-OMe and NtBuP-Asp-OMe were characterized by 1H and 13C NMR spectra (Figure S1a–d). All of the peaks were well assigned, indicative of the successful N-substitution of L-Asp-OMe. Furthermore, electrospray ionization tandem mass spectrometry (ESI-MS) results confirmed the successful preparation of NMeP-Asp-OMe and NtBuP-Asp-OMe (Figure S2). Note that there were no substituted byproducts, possibly due to the large steric hindrance, relatively low activity of the α-amino group, and mild reaction conditions. Aspartic acid, the only naturally occurring proteinogenic β-amino acid, is readily available and shares many excellent properties of natural amino acids. The synthetic protocols are facile and readily scalable in high yield, significantly facilitating the synthesis of optically active β-peptoid monomers and relevant conformational study of β-peptoid polymers.

2.2. Preparation and Characterization of β-NCNAs. Monomers of NMeP-Asp-OMe and NtBuP-Asp-OMe were then converted into the corresponding β-NCNAs by adopting a modified procedures, as outlined in Scheme 1. Note that anhydrous tetrahydrofuran (THF) was applied as a solvent for the preparation of Boc-protected precursors due to the hydrolysis of the ester bond in an alkaline aqueous solution. Subsequently, Boc-protected precursors underwent ring closure into β-NCNAs using PCl3 in anhydrous dichloromethane (DCM) under N2 purge. The obtained two β-NCNAs were generally viscous oils at ambient temperature, which were then fractionally precipitated in hexane from a THF solution (THF/hexane = 1:3) to yield samples as colorless oils in 64 and 59% yields, respectively. Both β-NCNA monomers were readily soluble in common solvents such as ethyl ether, toluene, THF, ethyl acetate, DCM, and dimethylformamide (DMF), except hexane. Their structures were unambiguously characterized by 1H and 13C NMR (Figure S3a–d). All of the chemical shifts in NMeP-Asp-OMe and NtBuP-Asp-OMe NCAs agreed well with the designed
structures, indicating the successful cyclization of NMeP-Asp-OMe and NβBuP-Asp-OMe monomers. Furthermore, Fourier transform infrared spectroscopy (FT-IR) spectra confirmed the successful preparation of two types of β-NNCA monomers with the appearance of a new peak at about 1805 cm\(^{-1}\) (Figures S4 and S5).

2.3. Ring-Opening Polymerization of β-NNCAs. It was reported that the N-substituted β-alanine NCAs can undergo living nucleophilic polymerization.\(^{17}\) We then focus on the ROP of the N-substituted L-Asp-OMe NCA. We have previously prepared several N-substituted α-amino acids and corresponding NCAs via Schiff base and reductive amination reactions.\(^{34,35}\) Unfortunately, previous studies showed that the ROP of NCAs was not successful due to the steric hindrance from the double substitution at the nitrogen atom as well as the C3 atom. For the case of β-NNCAs, which have slightly large ring size and reduced steric hindrance, we hence expected that double substituted β-NNCAs might undergo feasible ROP under appropriate conditions to prepare poly(β-peptoid)s as well as copoly(β-peptoid)s with backbone chirality.

We first studied the effects of the solvent on the ROP of N-substituted L-aspartic acid NCAs with benzylamine as an initiator. All of the reactions were conducted at 50 °C under reduced pressure to remove the generated carbon dioxide. FT-IR was used to monitor the progress of the reaction.\(^{17}\) H NMR and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were used to confirm the chemical structures and the degree of polymerization (DP) of the polymers. Considering the solubility of the polymers, polymerization in polar solvents such as DMF, N-methyl-2-pyrrolidone (NMP), and THF was first studied. At predetermined time intervals, a small portion of the reaction solution was taken out for FT-IR measurement. FT-IR spectra suggest the (nearly) complete conversion of β-NNCA monomers within 3 days in DMF and NMP. Also, the monomers in THF underwent complete consumption within 2 days. Surprisingly, only oligopolymers with DP around 3 were obtained regardless of solution concentrations (20–100 mg mL\(^{-1}\)) and the initial monomer-to-benzylamine ratio ([M]\(_0\)/[BnNH\(_2\)]\(_0\) = 50:1 or 100:1) (Figure 1a and Table S1). Note that extension of the reaction time did not result in polymers with higher DPs, indicating the absence or limited extent of the interchain coupling reaction. Also, polymerization in toluene (TOL), which is a typical nonpolar solvent for the ROP of NCAs, has been investigated.\(^{36,37}\) During the polymerization process, precipitates in the form of paste appeared and accumulated on the wall of the Schlenk tube while maintaining the reaction solution in a clear state. The monomer was consumed within 1.5 days with the disappearance of an absorbance peak at about 1805 cm\(^{-1}\) and the appearance of a new peak at 1645 cm\(^{-1}\) (Figure S4) and the products were precipitated using THF/ether to afford oligo(β-peptoid)s with a DP of around 7. In addition, the mixture solvents of THF and TOL (v/v = 1:1) were also attempted, giving oligo(β-peptoid)s with DP around 5 within 2 days. Apparently, TOL appeared to be the best solvent to produce oligomers in a shorter time among these solvents. The possible reason was that β-peptoid precipitated during polymerization due to its poor solubility in TOL, which confined active chain ends to react with monomers.\(^{38}\) The solvent dependence of the polymerization behavior has been reported previously by other groups.\(^{39,40}\) Furthermore, polymerization of NMeP-Asp-OMe NCA in the absence of any solvent did not work well to form P(NMeP-Asp-OMe),\(_7\) (Table S1). We also attempted the polymerization in TOL using other initiator systems such as BnNH\(_2\)/DBU, BnOH/TU/TBD, n-hexylamine, and hexamethyldisiloxane (HMDS). Unfortunately, only oligomers with DP < 10 were obtained (Table S1).

Subsequently, polymerization of NMeP-Asp-OMe NCA in toluene was investigated with benzylamine at various reaction temperatures. Figure 1b shows the DP and polymerization time (the time approaching ∼100% monomer consumption) versus temperature plots for NMeP-Asp-OMe NCA. Apparently, the DP slightly increased from 4 to 10 as the temperature increased from 25 to 80 °C. Meanwhile, the polymerization time gradually decreased, indicating accelerated monomer consumption at elevated temperatures. Further increasing the temperature from 80 to 120 °C, however, leads to oligomers with almost no further increased DPs. Representative MALDI-TOF analysis of P(NMeP-Asp-OMe),\(_7\) is shown in Figure S6, confirming the chemical structure of oligo(β-peptoid) (mass of repeat unit, Δm = 215.1 Da, [C\(_9\)H\(_{13}\)NO\(_5\)]\(^+\) = 215.1 g mol\(^{-1}\); DP\(_n\) = ~7; residual mass, r.m. = 106.1 (BnNH = C,H,N)). As for NβBuP-Asp-OMe NCA, only oligomers with 3–4 repeat units were obtained under all conditions studied. The characteristics of the prepared oligomers are summarized in Table S1. We assumed that three possible reasons led to low DPs. In contrast to α-NCAs and α-NNCAs, the less ring strain in β-NNCAs makes them less active to undergo ROP.\(^{17}\) Moreover, the steric hindrance of the propagating species limits the further chain propagation of the disubstituted β-peptoids. Besides, spontaneous polymerizations induced by solvents, water, or other nucleophilic

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Figure 1. Influence of (a) solvents (at 50 °C) and (b) reaction temperatures (in TOL) on the degree of polymerization (DP) and polymerization time for NMeP-Asp-OMe NCA ([M]\(_0\)/[BnNH\(_2\)]\(_0\) = 50:1). All samples were prepared at 100 mg mL\(^{-1}\).
Impurities could also result in uncontrollable polymerizations with low DPs and yields (Figure S7).\textsuperscript{41,42}

2.4. Diblock Copoly(β-peptoid) Synthesis. To obtain polymers with high molecular weights, we synthesized diblock copolymers of mPEG-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) and mPEG-\(b\)-P(\(n\)BuP-Asp-OMe)\(_n\) via ROP of β-NNCAs using mPEG-NH\(_2\) as a macromolecular initiator. Note that the copolymerization in THF and TOL was also investigated, giving similar results to the homopolymerization reactions. Thus, the copolymerizations were all conducted in TOL. The corresponding diblocks were characterized using gel permeation chromatography (GPC) and \(^1\)H NMR (Figures 2, S8, and S9). The molecular characteristics of obtained polymers are listed in Table S2. Figure 2 gives the typical GPC traces of mPEG45-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) and mPEG113-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) polymerized in TOL.

![Figure 2. GPC traces of mPEG45-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) (red line) and mPEG113-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) (blue line) polymerized in TOL.](https://dx.doi.org/10.1021/acsomega.0c04726)

Butyl groups in α-C side chains can offer strong hydrophobicity.

2.6. Conformational Studies. It is known that the introduction of backbone chirality can offer ordered folding structures for β-peptoids.\textsuperscript{14,15,19,43} Thus, circular dichroism (CD) spectroscopy was utilized to investigate the potential folding structures of the samples. The CD spectra of oligo(β-peptoids) were obtained in H\(_2\)O at 25 °C in the varying length (190–280 nm). As shown in Figure 3a, P(\(n\)MeP-Asp-OMe)\(_n\) displayed characteristic CD signals with intense minima around 208 nm and shallow minima around 230 nm at pH ~ 7, which were identical in shape to those of oligo(β-peptoids) prepared by solid-phase synthesis.\textsuperscript{15,16} The diblock copolypeptoids of mPEG45-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) and mPEG113-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) showed nearly identical CD signals to P(\(n\)MeP-Asp-OMe)\(_n\) indicating the similarly ordered structure (Figure S11b,c). Also, mPEG45-\(b\)-P(\(n\)BuP-Asp-OMe)\(_n\) and mPEG113-\(b\)-P(\(n\)BuP-Asp-OMe)\(_n\) presented ordered structures with intense minima around 195 nm and shallow minima around 228 nm (Figure S11d,e).

We then studied the concentration dependence of the CD signals of β-peptoids. The spectral shapes and intensities of P(\(n\)MeP-Asp-OMe)\(_n\), mPEG45-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\), mPEG113-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\), mPEG45-\(b\)-P(\(n\)BuP-Asp-OMe)\(_n\), and mPEG113-\(b\)-P(\(n\)BuP-Asp-OMe)\(_n\) remained almost unchanged in water under various concentrations, i.e., 0.1, 0.2, 0.5, and 1 mg mL\(^{-1}\) (Figure S11a–e). Moreover, P(\(n\)MeP-Asp-OMe)\(_n\) and mPEG45-\(b\)-P(\(n\)P-Asp-OH)\(_n\) showed identical spectral signals in methanol under various concentrations (Figure S12). These results indicate that the spectral shape is not due to the aggregation of the peptoids.\textsuperscript{14} Note that the CD spectra of P(\(n\)BuP-Asp-OMe)\(_n\) was not given due to the poor solubility in water. Next, we investigated the stability of the peptoids under different pH values. Typically, the CD spectrum of P(\(n\)BuP-Asp-OMe)\(_n\) was almost unchanged in shape as the pH increased from 2 to 7 (Figure 3a). However, distinct features were observed for P(\(n\)MeP-Asp-OMe)\(_n\) as the pH value was further increased from 7 to 10, where the minima moved from 208 to 220 nm and the shallow minima around 230 nm almost disappeared. The changes possibly arise from the increased deprotonation of the amino groups at the chain end of the polymer, which influence intramolecular hydrogen bonds.\textsuperscript{14} Also, similar pH dependence was observed for the diblock copolymer of mPEG45-\(b\)-P(\(n\)MeP-Asp-OMe)\(_n\) (Figure S13b). CD spectra of N-terminal capped P(\(n\)MeP-Asp-OMe)\(_n\) were almost unchanged as the pH increased from 3.7 to 6.9 and to 10.4, confirming the effect of terminal amino groups on folding propensities (Figure S14).

To investigate the charge state on folding structures, P(\(n\)MeP-Asp-OMe)\(_n\) was hydrolyzed using LiOH to obtain the completely deprotected oligomer of P(\(n\)Asp-OMe)\(_n\) (Scheme 2). The chemical structures of P(\(n\)P-Asp-OH)\(_n\), mPEG45-\(b\)-P(\(n\)P-Asp-OH)\(_n\), and mPEG113-\(b\)-P(\(n\)P-Asp-OH)\(_n\) were characterized by \(^1\)H NMR (Figure S10a–c). After hydrolysis, P(\(n\)P-Asp-OH)\(_n\) showed decreased solubility in organic solvents, while increased solubility in water. The folding propensity of P(\(n\)P-Asp-OH)\(_n\) was explored in aqueous solutions under diverse pH values. As revealed by CD (Figure 3b), the deprotected P(\(n\)P-Asp-OH)\(_n\) displayed a maximum near 195 nm and a minimum at about 215 nm at pH ~ 7, which were distinct from unhydrolyzed P(\(n\)MeP-Asp-OMe)\(_n\). As the pH was raised from 3 to 11, the intensities of
both maximum and minimum bands gradually increased with the maxima and minima shifting from 193 to 197 nm and from 207 to 216 nm, respectively. It should be noted that there was a sharp increase as the pH value increased from 6 to 7, possibly due to the increased deprotonation degree of $-\text{COOH}$ moieties. The structure changes were also investigated by FT-IR spectra (Figure 3c), in which the carbonyl at 1721 cm$^{-1}$ gradually reduced as the pH increased from 3 to 7 and almost disappeared for samples at pH values of 8, 9, and 10. Further, the carbonyl at 1633 cm$^{-1}$ exhibited a red shift as the pH increased from 3 to 10. The deprotected diblock copolypeptoid of mPEG$_{45}$-b-P($^\text{N}^\text{P}$-Asp-OMe)$_{13}$ showed identical pH-dependent transitions in aqueous solutions of P($^\text{N}^\text{P}$-Asp-OH)$_{10}$ (Figure 3d). Moreover, all of the deprotected samples show almost unchanged spectral shapes with concentration (Figure S15a–c).

To investigate the $-\text{COOH}$ content on the folding properties, mPEG$_{45}$-b-P($^{t}\text{Bu}$P-Asp-OMe)$_{9}$ was partially deprotected by CF$_3$COOH to remove $t$-butyl groups on the side chains but reserve the methyl ester bonds (Scheme 2). The partially deprotected product mPEG$_{45}$-b-P($^{t}\text{Bu}$P-Asp-OMe)$_{9}$ was characterized by $^1$H NMR (Figure S10d). The CD spectrum of mPEG$_{45}$-b-P($^{t}\text{Bu}$P-Asp-OMe)$_{9}$ presented intense minima around 195 nm and shallow minima around 228 nm at pH ~ 7 (Figure S15c), which are identical to undeprotected mPEG$_{45}$-b-P($^{t}\text{Bu}$P-Asp-OMe)$_{9}$. We assumed that partial deprotection cannot change the folding structure. Further, CD measurements were conducted at different pH values. Obviously, the CD spectrum of mPEG$_{45}$-b-P($^{t}\text{Bu}$P-Asp-OMe)$_{9}$ was almost unchanged in shape as the pH increased from 2 to 7 (Figure S13c). As the pH increased from 7 to 10, however, the minima moved from 195 to 217 nm and the shallow minima around
228 nm almost disappeared, mostly due to the increased deprotonation degree of −COOH groups at the side chains.

3. CONCLUSIONS

In summary, we have demonstrated a facile approach to synthesize backbone chiral β-peptoid polymers from readily available aspartic acid derivatives via an aza-Michael addition reaction. Two types of N-substituted β3-homoalanine monomers, i.e., N-(methyl propionate)-Asp-Ome (MeP-Asp-Ome) and N-(tert-butyl propionate)-Asp-Ome (BuP-Asp-Ome), were synthesized in high yield. Successful cyclization and polymerization of these N-substituted β3-homoalanines provide access to oligo(β-peptoid) and mPEG-poly(β-peptoid) diblocks with backbone chirality. It was found that both oligo(β-peptoid)s and diblocks have good solubility in common organic solvents but behave differently in water due to their structural differences. Their conformations were preliminarily studied by CD and FT-IR. De

4. EXPERIMENTAL SECTION

4.1. Materials and Instruments. Hexane, tetrahydrofuran (THF), dichloromethane (DCM), and toluene (TOL) were first dried with calcium hydride and purified by passing through activated alumina columns prior to use. i-Aspartic acid 1-methyl ester was purchased from GL Biochem (Shanghai) Ltd. Methyl acrylate, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) were purchased from Energy Chemical. Methoxypolyethylene glycol (5000 Da) was purchased from Jenkem Technology Co, Ltd. 1-Methyl ester was purchased from GL Biochem (Shanghai) Ltd. 1-Methyl ester was purchased from GL Biochem (Shanghai) Ltd. 5000 Da) was purchased from Jenkem Technology Co, Ltd.

4.2. General Procedure for the Synthesis of N-Substituted l-Aspartic Acid 1-Methyl Ester (MeP-Asp-Ome or BuP-Asp-Ome). A typical example is described below. Methyl acrylate (10.9 mL, 122.0 mmol) was added dropwise into a mixture of l-aspartic acid-1-methyl ester (15 g, 102.0 mmol), methanol (200 mL), and triethylamine (15.6 mL). After stirring at room temperature for 12–18 h, the solution changed from turbid to clear, suggesting completion of the reaction. After solvent evaporation, the crude product was washed twice with 100 mL of diethyl ether. A white powder was obtained via filtration and dried under vacuum to constant weight (23.2 g, 97.6% yield).1H NMR (400 MHz, D2O) δ 4.20 (t, 1H), 3.74 (s, 3H), 3.65 (s, 3H), 3.35 (t, 2H), 2.81 (m, 4H).13C NMR (101 MHz, CD3OD) δ 48.24, 48.03, 47.82, 47.60, 47.39, 47.18, 46.97.

4.3. General Procedure for the Synthesis of NNCAs (MeP-Asp-Ome and BuP-Asp-Ome NCAs). A typical example of a NNCAs in THF (20 mL) was dissolved in anhydrous CH2Cl2 (250 mL) under nitrogen. PCl3 (5.4 mL) was added dropwise to the reaction solution at 0 °C, and then the solution was stirred for 0.5 h in an ice bath and for another 3 h at RT. After the solvent was removed under vacuum, the solution was extracted twice with CH2Cl2 (2 × 20 mL) and filtered. The filtrate was evaporated to give a colorless oil. After three times dissolving/precipitating with THF/hexane in a glovebox, the solution of 5MeP-Asp-Ome NCA was obtained (7.5 g, 64.4% yield). 1H NMR (400 MHz, CDCl3) δ 4.85 (s, 1H), 3.98–3.86 (m, 1H), 3.81 (s, 3H), 3.70 (s, 3H), 3.56–3.46 (m, 1H), 3.20–2.98 (m, 2H), 2.98–2.86 (m, 1H), 2.74–2.59 (m, 1H).

4.4. General Procedure for the Synthesis of Oligo(β-peptoid)s [P(5MeP-Asp-Ome) and P(5BuP-Asp-Ome)]. The ROP of NNCAs was performed in toluene (TOL) using benzylamine as an initiator. A benzylamine/TOL (0.1 M) solution was injected into NNCAs/TOL (100 mg mL−1) under nitrogen. The solution was then stirred at a designed temperature under vacuum. The progress of the reaction was monitored by FT-IR. The reaction system was then precipitated in cold diethyl ether. The polymer was isolated by centrifugation and drying. The yield was about 20%, and the oligomer was characterized using MALDI-TOF and 1H NMR.

4.5. General Procedure for the Synthesis of Diblock Copolymers [mPEG-b-P(5MeP-Asp-Ome) and mPEG-b-P(5BuP-Asp-Ome)]. Typically, mPEG45-NH2 (77.5 mg, 0.0386 mmol) was dissolved in THF/hexane (2 mL) was added dropwise to the reaction solution at 0 °C, and then the solution was stirred for 0.5 h in an ice bath and for another 3 h at RT. After the solvent was removed under vacuum, the solution was extracted twice with CH2Cl2 (2 × 20 mL) and filtered. The filtrate was evaporated to give a colorless oil. After three times dissolving/precipitating with THF/hexane in a glovebox, the solution of 5MeP-Asp-Ome NCA was obtained (7.5 g, 64.4% yield). 1H NMR (400 MHz, CDCl3) δ 4.85 (s, 1H), 3.98–3.86 (m, 1H), 3.81 (s, 3H), 3.70 (s, 3H), 3.56–3.46 (m, 1H), 3.20–2.98 (m, 2H), 2.98–2.86 (m, 1H), 2.74–2.59 (m, 1H).
tube, and the solution was then stirred at 50 °C for 48 h under vacuum. The reaction mixture was precipitated in cold diethyl ether, centrifuged, and dried. A white solid was obtained with a 35% yield, and the copolymers were characterized using GPC and 1H NMR.

4.6. General Procedure for Hydrolysis of Methyl Ester Groups. Typically, 0.18 g of P(5MeP-Asp-OME)9, LiOH-H₂O (0.18 g, 4.3 mmol), and 10 mL of deionized water were added in a flask, and the mixture was stirred at RT for 4 h. Then, the solution mixture was put into a suitable dialysis bag and dialyzed in distilled water for 2 days with changing water eight times. Dialyzed solutions were lyophilized to obtain P(5P-Asp-OH)₁₀ as a white solid.

4.7. General Procedure for Hydrolysis of tert-Butyl Ester Groups. Typically, 0.15 g of mPEG₄₅-b-(4BuP-Asp-OME)₉ was dissolved in 15 mL of CF₃COOH (TFA) at 0 °C, and the mixture was stirred at RT for 2 h. The solution was divided into a 20 mL of water and loaded into a dialysis bag, and the mixture was stirred at RT for 4 h. The solution was added in a flask, and the mixture was stirred at RT for 2 h. A solution was added to 15–20 mL of water and loaded into a dialysis bag, and then the solution was dialyzed in distilled water for 2 days with water changing eight times. Dialyzed solutions were lyophilized to obtain mPEG₄₅-b-(4P-Asp-OME)₉ as a white solid.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04726.

Detailed additional 1H NMR spectra; GPC results; FT-IR spectra; and CD measurements (PDF)

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