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Phosgene-free Synthesis of Polypeptides using Activated Urethane Derivatives of α-Amino Acids: An Efficient Synthetic Approach to Hydrophilic Polypeptides

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ABSTRACT

A series of polypeptides have been synthesized from the corresponding *N*-phenoxycarbonyl derivatives of hydroxyl- or amino-functionalized α-amino acids such as L-serine, L-cysteine, L-threonine, L-tyrosine, L-glutamine, and L-asparagine. These derivatives are prepared by *N*-carbamylation of tetrabutylammonium salts of α-amino acids with diphenyl carbonate (DPC). The synthesis of polypeptides can be achieved by heating these *N*-phenoxycarbonyl derivatives at 60 ^{o}C in *N*,*N*-dimethylacetamide (DMAc) in the presence of *n*-butylamine through the polycondensation of the derivatives with accompanying the elimination of phenol and CO_2. The NMR and MALDI-TOF mass analyses of the resulting polypeptides revealed that *n*-butylamine was successfully incorporated into the chain end of polypeptides. Molecular weights of the polypeptides were controlled by varying feed ratio of the urethane derivatives to *n*-butylamine. The employment of an amine-terminated poly(ethylene glycol) in place of *n*-butylamine permitted the successful synthesis of the corresponding diblock copolymers composed of polyether and polypeptide segments.

INTRODUCTION

Polypeptides have been widely employed to prepare a wide variety of the functional biomaterials for the use of drug delivery system (DDS) and tissue engineering, because of their excellent biocompatibility and biodegradability.[1] One of the most efficient routes for the synthesis of polypeptides is the ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs).[2] The polymerization gives polypeptides with well-defined structures involving molecular weights and terminal structures and thus widely used as a highly reliable method for the precision synthesis of polypeptide-containing materials. However, the synthesis of NCAs usually requires highly toxic phosgene and its derivatives.[3] In addition, sensitive nature of NCAs to moisture and heat has prevented the production and the utilization of NCAs in an industrial scale.

We have developed a phosgene-free synthesis of NCA using diphenyl carbonate (DPC) as an alternative of phosgene.[4] The synthesis has been achieved by the selective intramolecular cyclization of *N*-phenoxycarbonyl α-amino acids that can be easily synthesized by *N*-carbamylation of onium salts of α-amino acid with DPC. These urethane derivatives can be used not only as precursors of NCAs but also as useful monomers for a more straightforward synthesis of polypeptides, where heating these urethane derivatives in the presence of amines gives the corresponding polypeptides through the *in situ* formation of NCAs and their polycondensation along with the elimination of phenol and CO_2.[5] The advantages of this

synthetic approach are 1) the successful incorporation of amine residue in the chain end, 2) the control of molecular weight by varying feed ratio between the urethane derivative and amine, and 3) the easy handling and simple procedure without the use and formation of any toxic compounds. In the previous study, we have synthesized poly(Z-L-lysine) and hydrophilic polypeptides such as poly(L-alanine), poly(L-valine), poly(L-methionine) and poly(L-tryptophan), by utilizing polycondensation of the corresponding urethane derivative.[5]

Herein, we report the applicability of the polycondensation system to the synthesis of polypeptides from α-amino acids with functional groups such as L-serine, L-cysteine, L-threonine, L-tyrosine, L-glutamine, and L-asparagine. In addition to the resulting well-defined synthesis of those polypeptides, a facile synthesis of diblock copolymers composed of both polyether and polypeptide segment is also reported.

RESULTS AND DISCUSSION

In this study, six functionalized amino acids, L-serine bearing OH group, L-cysteine bearing SH group, L-threonine bearing OH group, L-tyrosine bearing phenolic OH group, L-glutamine bearing amide NH_2, and L-asparagine bearing amide NH_2, were used as the starting materials. These amino acids were derived into their protected forms **1** by the established methods for *O*-alkylation, *S*-alkylation, and tritylation of amide group.[6-9] Scheme 1 shows the process of the derivation of the protected amino acids **1** into the corresponding urethanes **2**, which was

conducted according to the reported procedure for the synthesis of the urethane derivatives of *O*-benzyl-L-serine, *O*-benzyl-L-cysteine, *O*-benzyl-L-threonine, *O*-benzyl-L-tyrosine, N_{δ}-trityl-L-glutamine, and N_{γ}-trityl-L-asparagine.[5] This synthetic route to urethane derivatives **2** consists of two step reactions: In the first step, the amino acids were treated with tetrabutylammonium hydroxide to convert them into the corresponding ammonium salts. In the second step, to the ammonium salts diphenyl carbonate (DPC) was added to achieve *N*-carbamoylation. The resulting urethane derivatives were isolated by column chromatography and/or recrystallization. Their molecular structures were confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, elemental analysis, and high-resolution mass spectrometry. These urethane derivatives are storable for at least two months under ambient conditions without special caution to air and moisture. Previously, we have reported that analogous urethane derivatives of various α-amino acids were efficiently converted into the corresponding polypeptides by heating them in *N*,*N*-dimethylacetamide (DMAc) at 60 ^{o}C in the presence of amines.[5] The addition of primary amine to this system gave the corresponding polypeptide with primary amine-incorporated terminal end.

With following the procedure, we investigated the polymerization of **2** (Scheme 2). First, that of L-serine-derivative **2a** was performed by heating it in DMAc (0.5 mol/L) in the presence of *n*-$BuNH_2$ (2 to 20 mol%). The progress of polycondensation was tracked by monitoring the amount of phenol eliminated from the urethane derivative. The urethane

derivative was completely consumed within 24h. At that time, white precipitates were observed, suggesting the formation of polypeptide insoluble in DMAc. The resulting polypeptides were isolated as ether-insoluble fractions. The FT-IR spectrum of this product displays that the signal of urethane linkage (1716 cm^{-1}) was completely disappeared, while absorbance at 1628 cm^{-1} and 1506 cm^{-1}, which are the characteristic signals from the vibration of the peptide linkage (Figure 1), was observed. The spectrum indicates also that the **P2a** maintains the β-sheet form in the solid state.

In order to confirm the structure of **P2a**, it was analyzed with ^{1}H NMR. As shown in Figure 2, ^{1}H NMR spectrum of **P2a** in deuterated TFA indicated the singles assignable to the terminal *n*-$BuNH_2$ residue at δ = 0.80, 1.08-1.46, 2.85-3.27 ppm. In addition, degree of polymerization (DP) of **P2a** (Table 1, Entry 2) was determined to be about 10 from the integrated intensity of peak between the terminal *n*-$BuNH_2$ residue and methine proton at around δ = 4.83 ppm. This DP is close to the predicted one based on the feed ratio. The resulting polypeptides **P2a** were insoluble in acetone, CH_2Cl_2, THF, EtOAc and toluene, but soluble in TFA. It was partially soluble in DMF. SEC analysis of the only soluble part in DMF containing 10 mM LiBr was performed to estimate the molecular weight of **P2a**. We found that the molecular weight of the soluble part increased as the feed ratio of **2a** to *n*-$BuNH_2$ increased in the range from 5 to 20 (Table 1, Entries 1-3), while the polydispersity index remained relatively narrow (1.12-1.17). However, in all case, polycondensation of **2a** gave a

lower molecular weight than theoretical one estimated with the feed ratio, due to the precipitation of **P2a** from the reaction mixture.

A MALDI-TOF mass spectrum of **P2a** exhibited one prominent series of mass signals with *Δ*177, which is good agreement with the monomer unit of **P2a** and the respective peaks is assigned to the polymer structure possessing *n*-$BuNH_2$-incorporated initiating end and amino group at the propagating end (Figure 3). The detected signals are distributed in the range of DP from 7 to 16. From these result, we have confirmed that *n*-$BuNH_2$ serves as an initiator at the initial stage of the polycondensation system to give the well-defined terminal structure.

Likewise, other polypeptides (**P2b-P2f**) were also obtained under same reaction condition and their results are summarized in Table 1. The heating of **2b-2f** /DMAc solution in the presence of *n*-$BuNH_2$ successfully afforded the corresponding polypeptides in the excellent yield (>88%). In the case of poly(*O*-benzyl-L-cysteine) **P2b** and poly(*O*-benzyl-L-tyrosine) **P2d**, their polypeptides were immediately precipitated from the reaction mixture due to quite low solubility in DMAc solution. Therefore, higher molecular weight polypeptides (M_n = >2000) are not obtained at all. On the other hand, poly(*O*-benzyl-L-threonine) **P2c** showed a good solubility in DMAc solution**,** leading to the homogeneous system without any precipitations during polycondensation. The molecular weight increased up to M_n = 6500, when the feed ratio ($[\mathbf{2c}]_0/[n\text{-}BuNH_2]_0$) was varied in the range of 5 to 50. As shown by the SEC trace in Figure 4, that of **P2c** (Table 1, Entries 10 and 11) are unimodal and the PDI

remained relatively low. However, the PDI became gradually broad for the synthesis of higher molecular weight of **P2c** ($[\mathbf{2c}]_0/[n\text{-BuNH}_2]_0$ = 50), suggesting that small amount of the side reactions might be present. It is noteworthy the polycondensation of **P2c** required much longer reaction time than those of other urethane derivatives to reach the high conversion. This difference in the reaction time is likely due to the difference in the rate of NCA formation of **P2c** that depends on steric hindrance of the substituent. The prolonged reaction time might increase probability of undesired side reaction.

We next investigated the polypeptide synthesis of poly(*N*-trityl-L-glutamine) **P2e** and poly(*N*-trityl-L-asparagine) **P2f** through polycondensation of urethane derivative. In all case, the urethane derivatives of **2e** and **2f** were completely consumed within 24 h at 60 ^{o}C in DMAc solution to give the corresponding polypeptide, even though the bulky trityl group is introduced as protecting group on the side chain. For synthesis of **P2e** with low molecular weight, the obtained polypeptides possessed a good solubility in DMAc solution. All reactions were homogeneous and gave polypeptides with well-controlled molecular weight and narrow PDI (1.12-1.16). However, for the synthesis of polypeptide with higher molecular weight ($[\mathbf{2e}]_0/[n\text{-BuNH}_2]_0$ = 50), precipitation was observed in DMAc solution, and this polycondensation showed the formation of polypeptide with a high PDI. Polycondensation of **2f** was also investigated. For polycondensation of **P2f** with low molecular weight, polypeptide with well-controlled manner was observed ($[\mathbf{2f}]_0/[n\text{-BuNH}_2]_0$ = 5 and 10), but higher

molecular weight (M_n = >2300) was not obtained, because of restricted chain extension caused by steric hindrance of bulky trityl group close to main chain. Their polycondensation gave the formation of oligopeptides as main product. Additionally, each molecular weight (**P2e** and **P2f**) estimated from SEC analysis was much lower than that predicted from the feed ratio monomer to initiator. This might be due to the aggregations of polypeptides containing the bulky trityl group in DMF with 10 mM LiBr when compared with polystyrene standards. The MALDI-TOF mass analysis of all polypeptides indicated that the incorporation of *n*-BuNH_2 residue at the terminal end of polypeptides and therefore it acts as an initiator during the polycondensation system (for the spectra of all polypeptides see the Supporting Information).

Based on the above results, we have attempted a facile synthesis of diblock copolymer using poly(ethylene glycol) containing amino group at the terminal (PEG-NH_2) as a source of polyether segment (Scheme 3). According to the procedure described above, **2a** and PEG-NH_2 (M_n =2100 and PDI =1.05) were dissolved in DMAc solution ([**2a**]$_0$/[amine]$_0$ = 10) and then the mixture was stirred at 60 ^oC for 12h. After the reaction for 12h, ^1H NMR analysis showed that **2a** was converted into polypeptide through polycondensation without any precipitation from the reaction mixture. Pouring the reaction mixture into diethyl ether gave white powder in a high yield (89%). The formation of polypeptide was verified by ^1H NMR analysis (see Supporting Information) and the SEC profile in DMF appeared a unimodal and narrow peak,

which is clearly shifted to a higher molecular weight region from that of the original PEG-NH_2. Compared with a original poly(*O*-benzyl-L-serine), the solubility of diblock copolymer was improved by the conjugation of PEG chain possessing high solubility in common organic solvent and therefore it is soluble in DMF, DMAc, acetone, THF, and $CHCl_3$.These results clearly indicated the successful conjugation of poly(*O*-benzyl-L-serine) segment into PEG chain. Also, we further investigated the synthesis of diblock copolymer by using other urethane derivative such as **2b** and **2f** to demonstrate the versatility of our polycondensation system and the results are summarized in Table 2. Polycondensation of **2b** with PEG-NH_2 successfully gave the desired diblock copolymer in high yield (91%). Using a polypeptide having high solubility; poly(*N*-trityl-L-glutamine), we could find the adjustment of polypeptide chain length by varying the feed ratio between **2e** and amine in PEG ($[\mathbf{2e}]_0/[\text{amine}]_0$ = 10 and 20), while the PDI remains narrow even after polycondensation (Figure 5). With the utilization of other functional amines, synthesis of polypeptide-based material with functional group on the side chain and their application to biomedical engineering is now on progress.

CONCLUSION

Facile and phosgene-free approach to the synthesis of polypeptides with functional groups such as L-serine, L-cysteine, L-threonine, L-tyrosine, L-glutamine, and L-asparagine has been developed by polycondensation of their urethane derivative with the elimination of phenol and CO_2. The polycondensation smoothly take place by heating urethane derivatives at 60 ^oC *N*,*N*-dimethylacetamide (DMAc) in the presence of *n*-$BuNH_2$ to give the corresponding polypeptides in high yield. The detailed analysis with NMR and MALDI-TOF mass spectrometry appeared that *n*-$BuNH_2$ was successfully incorporated into the chain end of polypeptide during the polycondensation system. In addition, chain length of polypeptide was adjusted by feed ratio between urethane derivative and amine. Based on successful synthesis of polypeptides in the presence of amine, we could prove the synthesis of a diblock copolymer by utilizing amine-terminated poly(ethylene glycol) as a source of the polyether segment.

EXPERIMENTAL SECTION

Materials and Instruments.

Polyethylene glycol containing amino group at terminal (SUNBRIGHT MEPA-20H, (MeO-PEG-$(CH_2)_3$-NH_2), M_n =2100, PDI = 1.05) was purchased from NOF Corporation. All other chemicals and solvents were purchased from Tokyo Chemical Industry and Watanabe

Chemical Industry, and used without further purification unless otherwise denoted below. A *n*-butylamine (*n*-$BuNH_2$) and *N*,*N*-dimethylacetamide (DMAc) were purified by the distillation over calcium hydride (CaH_2), prior to use. For the protection of hydroxyl group of L-serine, we have conducted benzyl protection for hydroxyl group using benzyl bromide (see Supporting Information). The introduction of the protecting group for other α-amino acids was carried out in accordance with the published procedures to give the corresponding **1a-1f** (*S*-benzyl-L-cysteine,[6] *O*-benzyl-L-threonine,[7] *O*-benzyl-L-tyrosine,[8] N_δ-trityl-L-glutamine,[9] and N_γ-trityl-L-asparagine [9]). Analytical TLC was performed on commercial Merck plates coated with silica gel (TLC Silica gel 60 F_{254}). Column chromatography was carried out by using Kanto Silica Gel 60N (spherical, neutral, 63-210 μm). ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a JEOL ECS-400 spectrometer, and chemical shifts were recorded in ppm units using tetramethylsilane (TMS) as an internal standard. Fourier transform infrared spectroscopy (FT-IR) analysis was recorded on Thermo Scientific Nicolet iS10 over a range from 600 to 4000 cm^{-1}. Size exclusion chromatography (SEC) was carriedout on TOSOH HLC-8220 system equipped with three consecutive polystyrene gel columns [TSK-gels (bead size, exclusion limited molecular weight); super-AW4000 (6 μm, > 4×10^5), super-AW3000 (4 μm, > 6×10^4) and super-AW2500 (4 μm, > 2×10^3)] and refractive index and ultraviolet detectors at 40 ^oC. The system was operated using 10 mM LiBr in DMF as eluent at a flow rate of 0.5 mL/min. Polystyrene standards were employed for

calibration. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was performed at room temperature on a Shimadzu Biotech AXIMA Confidence using α-Cyano-4-hydroxycinnamic acid (CHCA) and sodium trifluoroacetate, as a matrix material and a cationization agent, respectively. In order to prepare the sample for MALDI-TOF mass analysis, the obtained polypeptide was dissolved in TFA (2 mg/mL), and then the solution was mixed with CHCA/THF solution (5 mg/mL) and sodium trifluoroacetate/THF solution (1 mg/mL) at a ratio of 1:2:1. The final mixture was deposited onto sample plate and allowed to air dry. The resulting sample were irradiated with 337 nm of nitrogen laser and detected on positive mode at 20 kV. Melting point was measured on Bibby Stuart scientific melting point apparatus SMP3. Elemental analysis was performed on LECO CHNS-932 analyzer. High-resolution mass spectrometry was conducted using a JEOL JMS-700 mass spectrometer.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-*O*-benzyl-L-serine (2a)

To a stirred suspension of *O*-benzyl-L-serine (2.4g, 12.5 mmol) in methanol 15 mL, tetrabutylammonium hydroxide (37% in methanol) (8.8 g, 12.5 mmol) was slowly added at room temperature. After stirring for 1 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved into acetonitrile (15 mL), and then the resulting solution added dropwise to a stirred solution of diphenyl carbonate (DPC) (2.7 g, 12.5 mmol)

in acetonitrile (15 mL) at room temperature. After stirring the solution for 1h, the distilled water (100 mL) was added into the resulting mixture. The resulting mixture was acidified to pH 2-3 with 1M HCl, and then extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. The crude products were purified with by column chromatography (eluting with a gradient from 30-70% ethyl acetate in *n*-hexane), and then recrystallization from ethyl acetate/*n*-hexane. Yield: 3.1 g (78%) of **2a** as white powder, mp: 96.5-97.7 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.76-3.85 (m, 1H), 3.95-4.02 (m, 1H), 4.54-4.63 (m, 3H), 5.94 (d, 1H, J = 8.4 Hz), 7.02-7.15 (m, 2H), 7.16-7.22 (m, 1H), 7.26-7.38 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 54.44, 69.46, 73.67, 121.66, 125.69, 127.90, 128.18, 128.67, 129.45, 137.19, 150.88, 154.62, 174.99. IR (neat, cm^{-1}): 3326, 1716, 1659, 1528, 1490, 1202, 1140, 1107, 1063, 744, 698. Anal. calcd for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.43; N, 4.44. Found: C, 64.68; H, 5.39; N, 4.40.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-*S*-benzyl-L-cysteine (2b)

According to the procedure described above, the urethane derivative was synthesized from *S*-benzyl-L-cysteine and **2b** was obtained as white powder in 70% yield, mp: 111.5-113.0 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.87-3.03 (m, 2H), 3.76 (s, 2H), 4.65 (m, 1H), 5.80 (d, 1H, J = 8.0 Hz), 7.08-7.15 (m, 2H), 7.17-7.38 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):

33.35, 36.86, 53.48, 121.64, 125.78, 127.53, 128.83, 129.09, 129.50, 137.51, 150.80, 154.45, 174.92. IR (neat, cm^{-1}): 3348, 3028, 1721, 1705, 1665, 1520, 1489, 1388, 1254, 1218, 1187, 767, 698, 685. Anal. calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.23. Found: C, 61.51; H, 5.04; N, 4.18.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-*O*-benzyl-L-threonine (2c)

According to the procedure described above, the urethane derivative was synthesized from *O*-benzyl-L-threonine. After purifying the crude product with column chromatography, **2c** was obtained as colorless oil in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.23-1.40 (m, 3H), 4.19-4.28 (m, 1H), 4.38-4.49 (m, 2H), 4.55-4.63 (m, 1H), 5.87 (d, 1H, J = 9.2 Hz), 7.03-7.20 (m, 3H), 7.21-7.40 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 16.41, 58.74, 71.32, 74.25, 121.61, 125.62, 127.87, 128.07, 128.55, 129.41, 137.49, 150.94, 155.28, 175.58. IR (neat, cm^{-1}): 2978, 1715, 1514, 1484, 1453, 1380, 1309, 1201, 1155, 1089, 1051. HRMS (FAB, *m/z*): calcd for $C_{18}H_{19}NO_5$, 329.1263; found, 330.1346 $(M + H)^+$.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-*O*-benzyl-L-tyrosine (2d)

According to the procedure described above, the urethane derivative was synthesized from *O*-benzyl-L-tyrosine and **2d** was obtained as white powder in 41% yield, mp: 115.3-117.0 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.93-3.27 (m, 2H), 4.63-4.72 (m, 1H), 5.01 (s, 2H), 5.50

(d, 1H, J = 7.8 Hz), 6.87-6.96 (m, 2H), 7.04-7.20 (m, 5H), 7.26-7.43 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 36.97, 55.00, 70.16, 115.24, 121.63, 125.69, 127.62, 128.11, 128.70, 129.45, 130.45, 136.99, 150.81, 154.34, 158.23, 176.01. IR (neat, cm^{-1}): 3324, 3030, 1723, 1690, 1530, 1509, 1487, 1384, 1239, 1005, 807, 742, 693. Anal. calcd for $C_{23}H_{21}NO_5$: C, 70.58; H, 5.41; N, 3.58. Found: C, 70.88; H, 5.43; N, 3.73.

Synthesis of Urethane Derivative: N_α-(phenoxycarbonyl)-N_δ-trityl-L-glutamine (2e)

According to the procedure described above, the urethane derivative was synthesized from N_δ-trityl-L-glutamine and **2e** was obtained as white powder in 81% yield, mp: 121.2-122.6 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.70-1.83 (m, 1H), 1.93-2.05 (m, 1H), 2.35-2.52 (m, 2H), 3.95-4.05 (m, 1H), 7.09 (d, 2H, J = 7.4 Hz), 7.14-7.29 (m, 16H), 7.34-7.40 (m, 2H), 8.03 (d, 1H, J = 8.0 Hz), 8.64 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 26.78, 32.50, 53.56, 69.24, 121.57, 124.99, 126.32, 127.45, 128.51, 129.28, 144.87, 150.98, 154.39, 171.13, 173.37. IR (neat, cm^{-1}): 1746, 1715, 1636, 1535, 1488, 1205, 749, 699. Anal. calcd for $C_{31}H_{28}N_2O_5$: C, 73.21; H, 5.55; N, 5.51. Found: C, 73.51; H, 5.83; N, 5.27.

Synthesis of Urethane Derivative: N_α-(phenoxycarbonyl)-N_γ-trityl-L-asparagine (2f)

According to the procedure described above, the urethane derivative was synthesized from N_γ-trityl-L-asparagine and **2f** was obtained as white powder in 56% yield, mp: 181.4-183.0 ^{o}C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.67-2.79 (m, 2H), 4.28-4.39 (m, 1H), 7.05-7.10 (m, 2H), 7.12-7.30 (m, 16H), 7.35-7.42 (m, 2H), 8.05 (d, 1H, J = 8.3 Hz), 8.68 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 37.92, 51.16, 69.46, 121.46, 125.01, 126.38, 127.44, 128.56, 129.31, 144.71, 150.92, 153.97, 168.66, 172.87. IR (neat, cm^{-1}): 3030, 1702, 1640, 1520, 1490, 1445, 1293, 1214, 1028, 750, 698. Anal. calcd for $C_{30}H_{26}N_2O_5$: C, 72.86; H, 5.30; N, 5.66. Found: C, 72.61; H, 5.37; N, 5.58.

Synthesis of Polypeptides by Polycondensation of Urethane Derivatives

Typical procedure: Urethane derivatives of *O*-benzyl-L-serine **2a** (315 mg, 1 mmol) was dissolved in anhydrous DMAc (2 mL), and the solution was added into a Schlenk tube. After the addition of *n*-BuNH_2/DMAc solution (25 μl, 2 ×10^{-3} mmol/μL), the resulting mixture was stirred at 60 ^{o}C for 24 h under argon atmosphere. The reaction mixture was cooled to room temperature, and poured into diethyl ether (100 mL). Precipitates were isolated by the filtration and then dried under vacuum to yield 138 mg (78 %) of **P2a** as white powder. ^{1}H NMR (400 MHz, TFA-*d*, δ, ppm): 3.50-4.03 (br, 2H), 4.28-4.62 (br, 2H), 4.74-4.96 (br, 1H), 6.93-7.34 (br, 5H). IR (neat, cm^{-1}): 3281, 2860, 1628, 1506, 1452, 1100, 732, 693.

Other polypeptides (**P2b** and **P2d**) were obtained by similar procedure as white powder. For the polycondensation of **2c**, **2e**, and **2f**, the reaction mixture was poured into water/methanol

mixture (vol. 8:2) with stirring, and white precipitates were collected by filtration. The physical data is listed below.

P2b: ^{1}H NMR (400 MHz, TFA-*d*, δ, ppm): 2.59-2.99 (br, 2H), 3.65 (s, 2H), 4.35-4.63 (br, 1H), 7.02-7.15 (br, 5H). IR (neat, cm^{-1}): 3269, 3026, 2914, 1623, 1513, 1492, 1229, 694. **P2c:** ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.70-1.35 (br, 3H), 3.61-4.20 (br, 1H), 4.25-4.86 (br, 3H), 7.05-7.35 (br, 5H). IR (neat, cm^{-1}): 3286, 2975, 2868, 1633, 1494, 1452, 1379, 1345, 1208, 1091, 733, 694. **P2d:** ^{1}H NMR (400 MHz, TFA-*d*, δ, ppm): 2.71-3.10 (br, 2H), 4.62-5.11 (m, 3H), 6.70-7.40 (m, 9H). IR (neat, cm^{-1}): 3267, 2923, 1624, 1508, 1232, 1174, 1020, 733, 692. **P2e:** ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.55-1.98 (br, 2H), 2.27 (br, 2H), 4.19 (br, 1H), 7.05-7.36 (br 15H), 7.50-7.83 (br, 1H), 8.15-8.40 (br, 1H). IR (neat, cm^{-1}): 3306, 3055, 1649, 1488, 1445, 1186, 750, 696. **P2f:** ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.58-2.83 (br, 2H), 3.96-4.83 (br, 1H), 6.90-7.34 (br, 15H), 7.41 (s, 1H), 7.90-8.82 (br, 1H). IR (neat, cm^{-1}): 3313, 3055, 1667, 1489, 749, 698, 633.

Synthesis of Diblock Copolymer by Polycondensation of Urethane Derivatives in the Presence of PEG-NH_2

Typical procedure: urethane derivative **2a** (315 mg, 1 mmol) and PEG-NH_2 (M_n =2100, 200 mg, 0.1 mmol) were dissolved in anhydrous DMAc (2 mL) and and the solution was added into a Schlenk tube. The resulting mixture was stirred at 60 ^oC for 12 h under argon

atmosphere. The reaction mixture was cooled to room temperature, and poured into diethyl ether (100 mL). Precipitates were isolated by the filtration and then dried under vacuum to give 335 mg (89%) of diblock copolymer: PEG-*b*-poly(*O*-benzyl-L-serine), as white powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.42-3.66 (br, 20H), 4.33-4.51 (br, 2H), 4.61-4.75 (br, 1H), 7.11-7.33 (br, 5H), 8.13-8.41 (br, 1H). IR (neat, cm^{-1}): 3271, 2862, 1626, 1525, 1341, 1097, 961, 841, 733, 694. From ^{1}H NMR spectrum (see Supporting Information), degree of polymerization for poly(*O*-benzyl-L-serine) in diblock copolymer is estimated to be 9.

Other diblock copolymers were also prepared by same procedure using urethane derivative **2b** or **2e**. All polymers were collected as white powder. The physical data is listed below.

PEG-*b*-poly(*S*-benzyl-L-cysteine): ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.48-2.85 (br, 2H), 3.49 (s, 18H), 3.64-3.78 (br, 2H), 4.55-4.75 (br, 1H), 7.13-7.33 (br, 5H), 8.26-8.60 (br 1H). IR (neat, cm^{-1}): 3268, 2876, 1624, 1520, 1341, 1100, 961, 841, 697. **PEG-*b*-poly(*N*-trityl-L-glutamine):** ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.43-1.97 (br, 2H), 2.10-2.43 (br, 2H), 3.49 (s, 18H), 4.05-4.34 (br, 1H), 7.02-7.43 (br, 15H), 7.72-8.10 (br, 1H), 8.39-8.74 (br, 1H). IR (neat, cm^{-1}): 3307, 2865, 1651, 1489, 1446, 1095, 752, 697.

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REFERENCES

1. (a) T. J. Deming, *Adv. Drug Delivery Rev.* **2002**, *54*, 1145-1155. (b) Y. Bae, S. Fukushima, A. Harada, K. Kataoka, *Angew. Chem. Int. Ed.* **2002**, *42*, 4640-4630. (c) T. Nakanishi, S. Fukushima, K. Okamoto, M. Suzuki, Y. Matsumura, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka *J. Controlled release* **2001**, *74*, 295-302. (d) L. Griffith, G. Naughton, *Science* **2002**, *295*, 1009-1010. (e) R. A. McMillan, V. P. Conticello, *Macromolecules* **2000**, *33*, 4809-4821.

2. (a) T. J. Deming, *J. Polym. Sci. PartA: Polym. Chem.* 2000, *38*, 3011-3018. (b) H. R. Kricheldorf, *Angew. Chem. Int. Ed.* **2006**, *45*, 5752-5784.

3. (a) A. C. Farthing, *J. Chem. Soc*, **1950**, 3213-3217. (b) D. Coleman, A.C. Farthing, *J. Chem. Soc*, **1950**, 3218-3222. (c) R. Wilder, S. Mobashery, *J. Org. Chem*. **1992**, *57*, 2755-2756.

4. K. Koga, A. Sudo T. Endo, *J. Polym. Sci. Part A: Polym. Chem*. **2010**, *48*, 4351-4355.

5. (a) S. Yamada, K. Koga, T. Endo, *J. Polym. Sci. Part A: Polym. Chem*. **2012**, *50*, 2527-2532. (b) S. Yamada, K. Koga, A. Sudo, M. Goto, T. Endo, *J. Polym. Sci. Part A: Polym. Chem*. **2013**, *51*, 3726-3731. (c) S. Yamada, A. Sudo, M. Goto, T. Endo, *J. Polym. Sci. Part A: Polym. Chem*. **2013**, *51*, 4565-4571.

6. M. Frankel, D. Gertner, H. Jacobson, A. Zilkha, *J. Chem. Soc.* **1960**, 1390-1393.

7. T. Mizoguchi, G. Levin, D. W. Woolley, J. M. Stewart, *J. Org. Chem.* **1968**, *33*, 903-904.

8. E. Wünsch, G. Fries, A. Zwick, *Chem. Ber.* **1958**, *91*, 542-547.

P. Sieber, B. Riniker, *Tetrahedron Lett.* **1991**, *32*, 739-742.