# Facile Preparation of Well-Defined $AB_2$ Y-Shaped Miktoarm Star Polypeptide Copolymer via the Combination of Ring-Opening Polymerization and Click Chemistry

Jingyi Rao, Yanfeng Zhang, Jingyan Zhang, and Shiyong Liu*

*Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, Hefei National Laboratory for Physical Sciences at the Microscale, University of Science and Technology of China, Hefei, Anhui 230026, China*

*Received April 28, 2008; Revised Manuscript Received May 27, 2008*

Well-defined $AB_2$ Y-shaped miktoarm star polypeptide copolymer, PZLL-*b*-$(PBLG)_2$, was synthesized via a combination of ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydride (NCA) and click chemistry, where PZLL is poly(ϵ-benzyloxycarbonyl-L-lysine) and PBLG is poly(γ-benzyl-L-glutamate). First, two types of primary-amine-containing initiators, *N*-aminoethyl 3,5-bis(propargyloxyl)-benzamide and 3-azidopropylamine, were synthesized and employed for the ROP of NCA, leading to the formation of dialkynyl-terminated PZLL and azide-terminated PBLG, *dialkynyl*-PZLL and PBLG-$N_3$, respectively. The subsequent copper(I)-catalyzed cycloaddition reaction between *dialkynyl*-PZLL and slightly excess PBLG-$N_3$ led to facile preparation of PZLL-*b*-$(PBLG)_2$ Y-shaped miktoarm star polypeptide copolymer. The excess PBLG-$N_3$ was scavenged off by reacting with alkynyl-functionalized Wang resin. The obtained Y-shaped miktoarm star polypeptide copolymer was characterized by gel permeation chromatograph (GPC), Fourier transform-infrared spectroscopy (FT-IR), and $^1$H NMR. Moreover, after the hydrolysis of protecting benzyl and benzyloxycarbonyl groups of PZLL-*b*-$(PBLG)_2$, water-soluble pH-responsive Y-shaped miktoarm star polypeptide copolymer, PLL-*b*-$(PLGA)_2$, was obtained, where PLL is poly(L-lysine) and PLGA is poly(L-glutamic acid). It can self-assemble into PLGA-core micelles at acidic pH and PLL-core micelles at alkaline pH, accompanied with the coil-to-helix transition of PLGA and PLL sequences, respectively. The spontaneous pH-responsive supramolecular assembly of PLL-*b*-$(PLGA)_2$ miktoarm star polypeptide copolymer has been investigated via a combination of $^1$H NMR, laser light scattering (LLS), transmission electron microscopy (TEM), and circular dichroism (CD) spectroscopy.

## Introduction

Compared to linear block copolymers, miktoarm star copolymers exhibit unique phase-separation behavior either in bulk or in solution due to that more than two building blocks have been linked to a single junction point.[1–17] This has provoked considerable interest in the preparation of a variety of miktoarm star copolymers with varying arm numbers, chemical composition, and chain topology. Their self-assembly in selective solvents can create novel nanostructures with potential applications in diverse fields such as drug nanocarriers, diagnose assays, nanopatterns, and photonics.[7–10,18–22]

On the other hand, polypeptide hybrid miktoarm star copolymers consisting of polypeptide and conventional polymer building blocks can provide extra opportunity for hierarchical self-assembly due to the formation of secondary structures from the polypeptide sequence, which is unobtainable with conventional miktoarm star copolymers.[23–25] Kim et al.[23] synthesized 3-miktoarm star copolymers consisting of polystyrene (PS) and poly(γ-benzyl-L-glutamate) (PBLG) via the combination of atom transfer radical polymerization (ATRP) and ring-opening polymerization (ROP) of *N*-carboxyanhydride (NCA) and investigated their supramolecular assembly and phase-transition behavior in the bulk state. Recently, Lecommandoux et al.[24] synthesized linear polystyrene-*b*-poly(L-glutamic acid), PS-*b*-PLGA, and 3-miktoarm star PS-*b*-$(PLGA)_2$ copolymers, and compared their self-assembly behavior. Irrespective of the PLGA contents the miktoarm star copolymer exhibited a stacked microstructure, which was drastically different from that of linear PS-*b*-PLGA copolymer.

It is expected that miktoarm star copolymers consisting of purely synthetic polypeptide sequences would be quite intriguing due to its nonlinear topology and the formation of complex secondary structures associated with different types of polypeptide. It should be noted that a series of polypeptide diblock copolymers have already been reported, which were typically synthesized via the sequential ROP of corresponding α-amino acid NCAs or the solid phase peptide synthesis technique.[26–39] Lecommandoux et al.[26] reported that in aqueous solution, poly(L-glutamic acid)-*b*-poly(L-lysine) (PLGA-*b*-PLL) can self-assemble into two types of unilamellar vesicles at pH 3 and 12, respectively, resulting from the formation of secondary structures and block composition asymmetry. Deming et al.[27] prepared nonionic block copolypeptides of L-leucine and ethylene glycol-modified L-lysine residues, which adopted a rod-like conformation and self-assembled into complex nanostructures, such as large vesicles, sheet-like membranes, and irregular aggregates in aqueous solution.

To the best of our knowledge, the synthesis and characterization of nonlinear-shaped purely synthetic polypeptide-based miktoarm star copolymers have not been reported yet, partially due to that considerable challenges exist during their preparation. This is quite different from that of linear polypeptide-containing diblock copolymer, in which the ROP of the corresponding NCA using primary amine-terminated polypeptide or synthetic polymer can lead to the facile preparation.[40–44]

* To whom correspondence should be addressed. E-mail: sliu@ustc.edu.cn.

10.1021/bm800462q CCC: $40.75 
Published on Web 07/09/2008

More recently, a synthetic coupling strategy to polymers with complex structures based on "click chemistry" has been successfully developed.[45–52] The term "click chemistry" was coined by Sharpless and co-workers,[48] who renovated the Huisgen's 1,3-dipolar cycloadditions between azides and alkynyls (or nitriles) using copper salts as catalysts. Recently, Lecommandoux et al.[53] reported the novel syntheses of well-defined block copolymers composed of a PBLG sequence and a poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) block by "click" coupling alkynyl- and azide-terminated PBLG and PDMAEMA precursors. This approach can successfully avoid the ROP initiating capability of tertiary amine residues in PDMAEMA block.

Herein, we report for the first time the synthesis of well-defined nonlinear-shaped PZLL-*b*-$(PBLG)_2$ miktoarm star polypeptide copolymer via a combination of ROP and click chemistry, where PZLL is poly($\epsilon$-benzyloxycarbonyl-L-lysine) and PBLG is poly($\gamma$-benzyl-L-glutamate). Primary amine-containing initiators, *N*-aminoethyl 3,5-bis(propargyloxyl)benzamide and 3-azidopropylamine were synthesized and employed for the preparation of dialkynyl-terminated PZLL (*dialkynyl*-PZLL) and azide-terminated PBLG (PBLG-$N_3$). The subsequent copper(I)-catalyzed cycloaddition reaction between *dialkynyl*-PZLL and PBLG-$N_3$ led to the facile preparation of Y-shaped miktoarm star polypeptide copolymer, PZLL-*b*-$(PBLG)_2$. Moreover, after removing protecting groups, water-soluble double hydrophilic miktoarm star polypeptide copolymer, PLL-*b*-$(PLGA)_2$, was obtained. Its pH-responsive self-assembly behavior in aqueous solution was then thoroughly investigated via a combination of $^1$H NMR, laser light scattering (LLS), transmission electron microscopy (TEM), and circular dichroism (CD) spectroscopy.

## Experimental Section

**Materials.** $\gamma$-Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) and $\epsilon$-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (ZLL-NCA) were synthesized according to literature procedures.[54,55] Wang resin (1.47 mmol/g), copper(I) bromide (CuBr, 99.99%), 18-crown-6 (98%), and propargyl bromide (80% solution in toluene) were purchased from Aldrich and used as received. Sodium hydride (NaH, 57−63% suspension in oil) and hydrobromic acid in glacial acetic acid (HBr/AcOH, 45% w/v) were purchased form Alfa Aesar and used without further purification. Methyl 3,5-dihydroxybenzoate and 3-azidopropylamine were prepared according to literature procedures.[56,57] Tetrahydrofuran (THF), petroleum ether, acetone, and *N*,*N*-dimethylformamide (DMF) were dried by refluxing over sodium/benzophenone and distilled just prior to use. Trifluoroacetic acid (TFA), 1,2-diaminoethane, and all other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received.

**Sample Preparation.** General approaches to the preparation of *N*-aminoethyl 3,5-bis(propargyloxyl)benzamide (**1**), PBLG-$N_3$ and *dialkynyl*-PZLL homopolymers, and Y-shaped miktoarm star polypeptide copolymers, PZLL-*b*-$(PBLG)_2$ and PLL-*b*-$(PLGA)_2$, were shown in Scheme 1.

*Synthesis of Methyl 3,5-Bis(propargyloxyl)benzoate.*[57] Methyl 3,5-dihydroxybenzoate (16.8 g, 100 mmol) and propargyl bromide (26.2 g, 220 mmol) were dissolved in 300 mL of acetone, followed by the addition of potassium carbonate (15.1 g, 109 mmol) and 18-crown-6 (0.1 g, 0.4 mmol). The reaction mixture was heated at reflux under $N_2$ protection for 24 h. After filtration, the filtrate was concentrated under reduced pressure. The crude product was then crystallized in methanol to give methyl 3,5-bis(propargyloxyl)- benzoate as pale yellow crystals (20.4 g, 82.9%). $^1$H NMR in $CDCl_3$, $\delta$ (ppm): 2.55 (2H, *CCH*), 3.91 (3H, $CH_3O$), 4.73 (4H, $CH_2CCH$), 6.82 (1H, aromatic), 7.29 (2H, aromatic) (Figure 1a).

*Synthesis of N-Aminoethyl 3,5-Bis(propargyloxyl)benzamide (1).*[58] A solution of methyl 3,5-bis(propargyloxyl)benzoate (6.25 g, 25.6 mmol) in 30 mL of methanol was added dropwise into a stirred solution of 1,2-diaminoethane (73.9 g, 1.23 mol) in 120 mL of methanol at 0 °C. After the addition was completed within ~1 h, the mixture was allowed to warm to room temperature and stirred for 96 h. The solvent was removed under reduced pressure using a rotary evaporator and the temperature remained below 40 °C during this process. Subsequently, the excess 1,2-diaminoethane was removed using an azeotropic mixture of toluene and methanol (9:1 v/v). After drying in a vacuum oven for 24 h, the compound **1** was obtained as a slightly yellowish oil with a yield of 96%. $^1$H NMR in $CDCl_3$, $\delta$ (ppm): 1.61 (2H, $CH_2NH_2$), 2.55 (2H, *CCH*), 2.95 (2H, $CH_2NH_2$), 3.49 (2H, CONH$CH_2$), 4.73 (4H, $CH_2CCH$), 6.68 (1H, CO*NH*), 6.76 (1H, aromatic), 7.05 (2H, aromatic; Figure 1b).

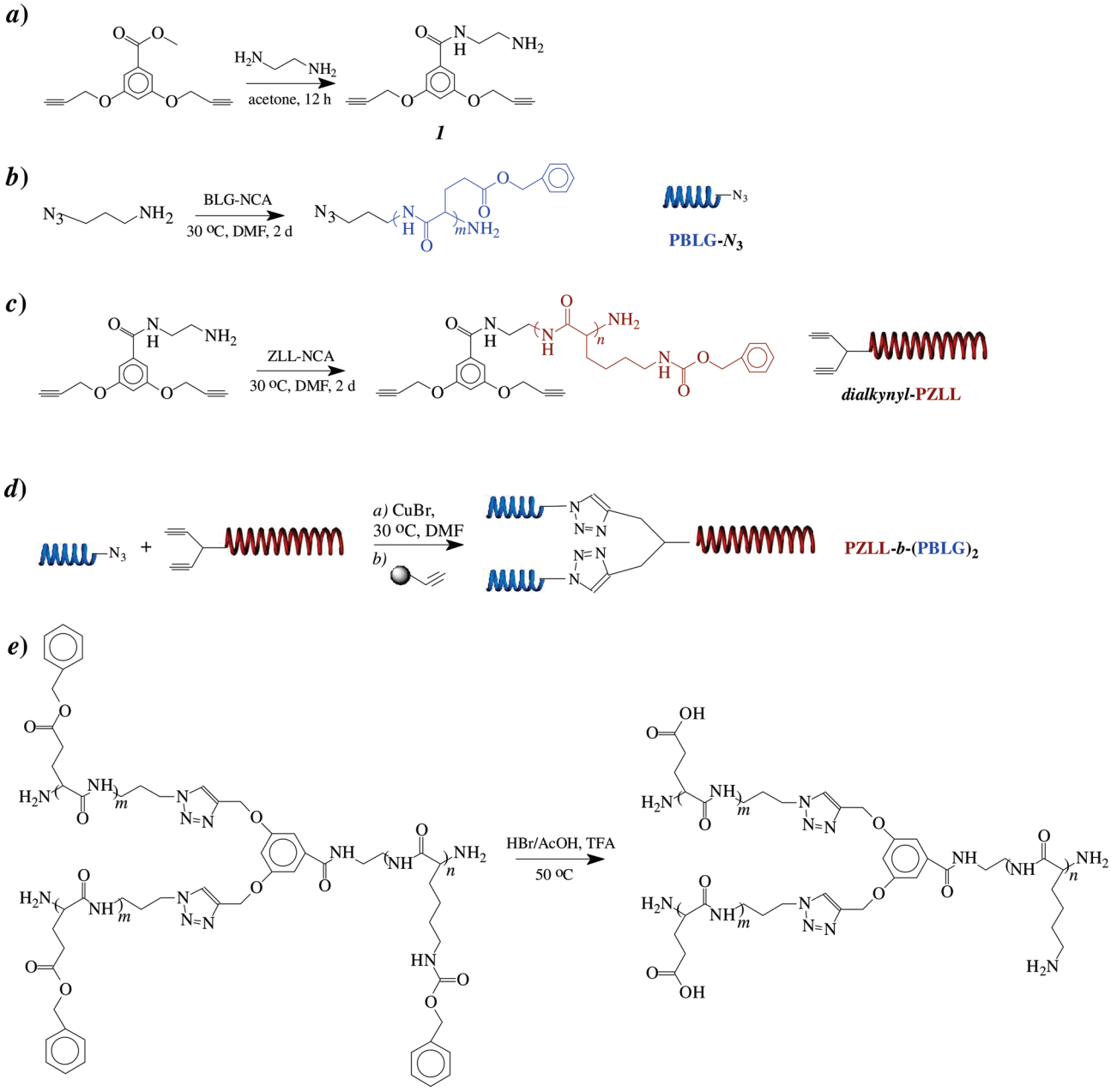
*Synthesis of PBLG$_{15}$-$N_3$.* A typical procedure for the synthesis of $PBLG_{15}$-$N_3$ was as follows (Scheme 1b). 3-Azidopropylamine (0.1 g, 1.0 mmol) was dissolved in 30 mL of anhydrous DMF in a 250 mL baked flask. Freshly prepared BLG-NCA (5.24 g, 20 mmol) was dissolved in 25 mL of anhydrous DMF in a separate 100 mL flask. The mixture was then cannulated into the 3-azidopropylamine/DMF solution via a double-tipped stainless needle. The reaction mixture was allowed to stir for 3 days at room temperature under a dry $N_2$ atmosphere. After partially removing the solvent, the reaction mixture was precipitated into an excess of anhydrous diethyl ether. This purification cycle was repeated three times. The obtained white solids (4.2 g, 80% yield) were dried in a vacuum oven overnight at room temperature. The molecular weight and molecular weight distribution of $PBLG_{15}$-$N_3$ were determined by GPC using DMF as the eluent: $M_n$ = 9,600, $M_w/M_n$ = 1.17 (Figure 2). The actual degree of polymerization (DP) of PBLG homopolymer was determined to be 15 by $^1$H NMR analysis in $CDCl_3$ (containing ~10 v/v % TFA). The obtained azide-terminated polymer was denoted as $PBLG_{15}$-$N_3$ (Figure 3).

*Synthesis of dialkynyl-PZLL$_{24}$.* According to similar procedures employed for the preparation of $PBLG_{15}$-$N_3$, *dialkynyl*-PZLL was synthesized using **1** (0.16 g, 0.6 mmol) as the initiator for the ROP of ZLL-NCA (5.19 g, 17 mmol) in anhydrous DMF (Scheme 1c). The obtained white solids (4.7 g, 87% yield) were dried in a vacuum oven overnight at room temperature. The molecular weight and molecular weight distribution of *dialkynyl*-PZLL were determined by GPC using DMF as the eluent: $M_n$ = 18500, $M_w/M_n$ = 1.12 (Figure 2). The actual degree of polymerization (DP) of PZLL homopolymer was determined to be 24 by $^1$H NMR analysis in $CDCl_3$ (containing ~10 v/v% TFA; Figure 3). The obtained alkynyl end functional polymer was denoted as *dialkynyl*-$PZLL_{24}$.

*Alkynyl-Functionalized Wang Resin.* Alkynyl-functionalized Wang resin was synthesized as follows. Commercial Wang resin (5 g, 7.35 mmol) was dispersed in 40 mL of dry DMF under stirring. After cooling to 0 °C, NaH (467 mg, 11.7 mmol) was added into the reaction flask. The reaction mixture was stirred at 0 °C for 50 min followed by the addition of propargyl bromide (1.6 g, 13.4 mmol) under a $N_2$ atmosphere. After 12 h, the suspension was filtrated and thoroughly washed with THF and methanol. After drying in a vacuum oven, alkynyl-functionalized Wang resin was obtained with quantitative yield. FT-IR analysis revealed the presence of characteristic alkynyl signals at ~2100 cm$^{-1}$.

*Synthesis of Miktoarm Star Copolymer PZLL$_{24}$-b-(PBLG$_{15}$)$_2$ by Click Chemistry.* The synthesis of PZLL-*b*-$(PBLG)_2$ miktoarm star polypeptide copolymer was accomplished by the click coupling between *dialkynyl*-PZLL and PBLG-$N_3$ using CuBr as the catalyst (Scheme 1d). The mixed solution of $PBLG_{15}$-$N_3$ (1.67 g, 0.5 mmol) and *dialkynyl*-$PZLL_{24}$ (1.0 g, 0.15 mmol) in 10 mL DMF was degassed by two freeze−pump−thaw cycles. CuBr (11 mg, 0.075 mmol) was then introduced into the glass ampule under a $N_2$ atmosphere. After stirring for 20 h at 60 °C, an aliquot was taken for GPC analysis and alkynyl-

**Scheme 1.** Preparation of (a) *N*-Aminoethyl 3,5-Bis(propargyloxyl)benzamide, (b) PBLG-$N_3$, (c) *dialkynyl*-PZLL, (d) PZLL-*b*-$(PBLG)_2$, and (e) PLL-*b*-$(PLGA)_2$ Y-Shaped Miktoarm Star Polypeptide Copolymers




functionalized Wang resin (0.43 g, 0.6 mmol) was then added. The suspension was kept stirring for a further 4 h. After suction filtration, the filtrate was concentrated on a rotary evaporator and then precipitated to an excess of anhydrous diethyl ether. After dissolving in DMF, the product was further purified by passing through a neutral alumina column to remove copper catalyst. After precipitating into an excess of anhydrous diethyl ether, the obtained product was dried overnight in a vacuum oven at room temperature (1.7 g, 85% yield). The molecular weight and molecular weight distribution of PZLL-*b*-$(PBLG)_2$ were determined by GPC using DMF as the eluent: $M_n$ = 26700, $M_w/M_n$ = 1.13. The obtained diblock copolymer was denoted as $PZLL_{24}$-*b*-$(PBLG_{15})_2$.

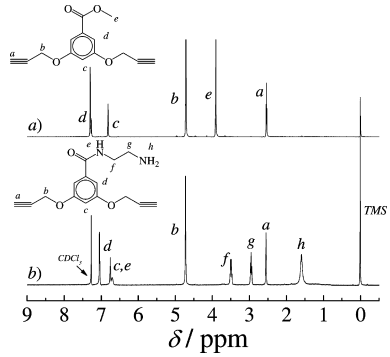



**Figure 1.** $^1H$ NMR spectra of (a) methyl 3,5-bis(propargyloxyl)benzoate and (b) *N*-aminoethyl 3,5-bis(propargyloxyl)benzamide in $CDCl_3$.

*Hydrolysis of $PZLL_{24}$-b-$(PBLG_{15})_2$.* The removal of protecting benzyl and benzyloxycarbonyl groups of $PZLL_{24}$-*b*-$(PBLG_{15})_2$ was conducted as follows (Scheme 1e).[59–61] $PZLL_{24}$-*b*-$(PBLG_{15})_2$ (0.7 g, 0.07 mmol) was dissolved in 25 mL of TFA under stirring. HBr/AcOH (3.8 mL, 30 mmol HBr) was then dropwise introduced into the reaction vessel. After stirring for 18 h at room temperature, the solvent was removed under reduced pressure. The obtained solids were redispersed in water and the solution pH was adjusted to 7 with the addition of NaOH. The aqueous solution was then dialyzed (cutoff molar mass of 3500 g/mol)

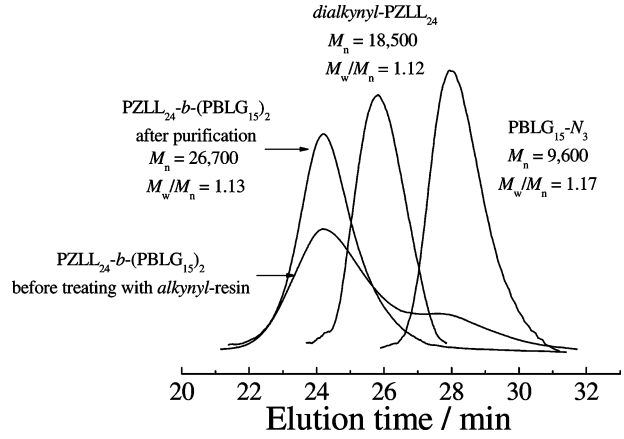



**Figure 2.** DMF GPC traces of (a) $PBLG_{15}$-$N_3$, (b) *dialkynyl*-$PZLL_{24}$, and the target Y-shaped miktoarm star polypeptide copolymer, $PZLL_{24}$-*b*-$(PBLG_{15})_2$, before (c) and after (d) treating with alkynyl-functionalized Wang resin.

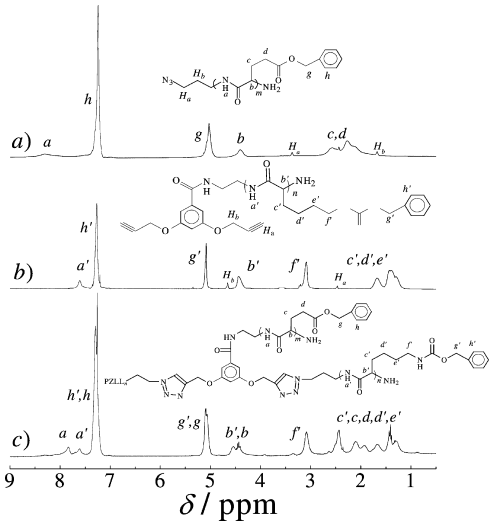



**Figure 3.** $^1$H NMR spectra of (a) $PBLG_{15}$-$N_3$, (b) *dialkynyl*-$PZLL_{24}$, and (c) $PZLL_{24}$-*b*-$(PBLG_{15})_2$ in $CDCl_3$/TFA mixture.

against deionized water for 12 h with frequent replacement of fresh water. The obtained $PLL_{24}$-*b*-$(PLGA_{15})_2$ was dried in a vacuum oven overnight at room temperature (0.34 g, 71% yield). The complete removal of benzyl and benzyloxycarbonyl protecting groups was further confirmed by $^1$H NMR and FT-IR analysis. Following similar procedures, *dialkynyl*-$PZLL_{24}$ and $PBLG_{15}$-$N_3$ were also hydrolyzed into $PLL_{24}$ and $PLGA_{15}$.

**Characterization.** Molecular weight distributions were determined by GPC using a series of two linear Styragel columns HT3 and HT4 and a column temperature of 35 °C. Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30 °C) was used. The eluent was DMF at a flow rate of 1.0 mL/min. A series of six low polydispersity polystyrene standards with molecular weights ranging from 800 to 400000 g/mol were used for the GPC calibration. All $^1$H NMR spectra were recorded at 25 °C on a Bruker AV300 NMR spectrometer (resonance frequency of 300 MHz for $^1$H) operated in the Fourier transform mode. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker VECTOR-22 IR spectrometer. The spectra were collected over 64 scans with a spectral resolution of 4 $cm^{-1}$. Circular dichroism (CD) spectra were recorded at 25 °C with a Jasco J-720 (Tokyo, Japan) spectrophotometer.

*Potentiometric Titrations.* The aqueous solutions (0.1 g/L) of $PLL_{24}$, $PLGA_{15}$, and $PLL_{24}$-*b*-$(PLGA_{15})_2$ were titrated by the dropwise addition of HCl or NaOH solutions. The solution pH was monitored with a Corning Check-Mite pH meter (precalibrated with pH 4, 7, and 10 buffer solutions).

*Laser Light Scattering (LLS).* A commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multitau digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He−Ne laser ($\lambda_0$ = 632 nm) as the light source was employed for dynamic laser light scattering (LLS) measurements. Scattered light was collected at a fixed angle of 90° for duration of ~10 min. Distribution averages and particle size distributions were computed using cumulants analysis and CONTIN routines. All data were averaged over three measurements.

*Transmission Electron Microscopy (TEM).* TEM observations were conducted on a Philips CM 120 electron microscope at an acceleration voltage of 100 kV. Samples for TEM observations were prepared by placing 10 $\mu$L of micelle solutions at a concentration of 0.1 g/L on copper grids, which were successively coated with thin films of Formvar and carbon. No staining was required.

## Results and Discussion

**Syntheses of $PZLL_{24}$-*b*-$(PBLG_{15})_2$ and $PLL_{24}$-*b*-$(PLGA_{15})_2$ Miktoarm Star Polypeptide Copolymers.** General approaches employed for the preparation of Y-shaped miktoarm star polypeptide copolymers, PZLL-*b*-$(PBLG)_2$ and PLL-*b*-$(PLGA)_2$, were shown in Scheme 1. The trifunctional initiator **1** bearing two alkynyl and one primary amine groups was synthesized via aminolysis of methyl 3,5-bis(propargyloxyl)benzoate with an excess of 1,2-diaminoethane. $^1$H NMR spectrum of methyl 3,5-bis(propargyloxyl) benzoate and the corresponding peak assignments were shown in Figure 1a. After aminolysis, the characteristic signal at $\delta$ = 3.91 ppm for methyl protons completely disappeared, new resonance signals at $\delta$ = 6.68, 3.49, 2.95, and 1.61 ppm can be ascribed to protons of amide group (*e*), two methylene groups (*f*, *g*), and primary amine (*h*), respectively (Figure 1b).

*Synthesis of $PBLG_{15}$-$N_3$ and dialkynyl-$PZLL_{24}$.* It has been well-established that primary amine residues can be employed for the ROP of α-amino acid NCA, resulting in the formation of polypeptide polymers.[37,38,40–44,62,63] In the current study, 3-azidopropylamine and **1** were used as initiators for the ROP of ZLL-NCA and BLG-NCA, leading to the formation of PBLG-$N_3$ and *dialkynyl*-PZLL (Scheme 1).[62] In both cases, the polymerization proceeded homogeneously in dry DMF. GPC analysis in DMF revealed monomodal and symmetric peaks for PBLG-$N_3$ and *dialkynyl*-PZLL (Figure 2). The number-average molecular weights, $M_n$, were determined to be 9600 and 18500, with polydispersity, $M_w/M_n$, being 1.17 and 1.12 for PBLG-$N_3$ and *dialkynyl*-PZLL, respectively.

Figure 3 showed the $^1$H NMR spectra of PBLG-$N_3$ and *dialkynyl*-PZLL in $CDCl_3$ containing ~10 v/v % TFA. For PBLG-$N_3$, the resonance signals of protons of amide group (*a*), phenyl group (*h*), methylene group of benzyl (*g*), α-methine group (*b*), and β- and γ-methylene groups (*c*, *d*) occurred at $\delta$ = 8.0−8.5, 7.3, 5.0, 4.4, and 1.8−2.8 ppm, respectively (Figure 3a). Signals of methylene protons ($H_a$, $H_b$) adjacent to the azide group in PBLG-$N_3$ were observed at $\delta$ = 3.37 and 1.68 ppm, respectively. Based on the integral ratio of peaks $H_a$ and *g*, the degree of polymerization, DP, of PBLG homopolymer was calculated to be 15. Thus, azide-functionalized PBLG homopolymer was denoted as $PBLG_{15}$-$N_3$.

In the case of *dialkynyl*-PZLL, the signals at $\delta$ = 7.5−7.7, 7.3, 5.1, 4.4, 3.1, and 1.2−1.8 ppm were ascribed to protons of amide group (*a*′), phenyl group (*h*′), methylene group of benzyl (*g*′), α-methine group (*b*′), ϵ-methylene group (*f*′), and three methylene groups (*c*′, *d*′,*e*′). Signals at $\delta$ = 4.7 and 2.5 ppm were ascribed to protons of methylene ($H_b$) and alkynyl ($H_a$) groups. The actual DP of *dialkynyl*-PZLL was determined to be 24 by comparing the integration areas of peaks $H_b$ and *g*′.

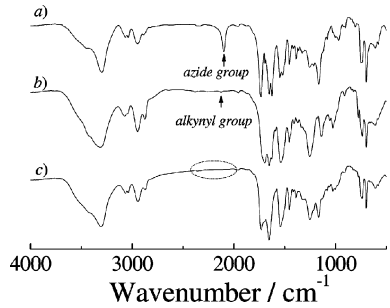



**Figure 4.** FT-IR spectra of (a) $PBLG_{15}$-$N_3$, (b) *dialkynyl*-$PZLL_{24}$, and (c) $PZLL_{24}$-*b*- $(PBLG_{15})_2$.

The product was denoted as *dialkynyl*-$PZLL_{24}$. Moreover, FT-IR spectra of PBLG-$N_3$ and *dialkynyl*-PZLL homopolymer clearly revealed the presence of absorbance peaks at 2097 and 2127 $cm^{-1}$, which were characteristic of azide and alkynyl moieties, respectively (Figure 4).

*Synthesis of Miktoarm Star Polypeptide Copolymer by Click Chemistry.* The first example of the synthesis of $AB_2$ miktoarm star copolymers using ATRP and click chemistry was reported by Monteiro and co-workers.[52] The click reaction of azide-terminated polymer (A) with excess tripropargylamine led to the formation of dialkynyl-terminated precursor, and its subsequent click coupling with azide-terminated polymer (B) resulted in $AB_2$ miktoarm star copolymer. In the current study, the click reaction between *dialkynyl*-$PZLL_{24}$ and $PBLG_{15}$-$N_3$ afforded the corresponding miktoarm star polypeptide copolymer, PZLL-*b*-$PBLG_2$ (Scheme 1d). It should be noted that the click reaction was conducted in DMF under ligand-free conditions. It has been established previously that the click reaction can be quite efficient even in the absence of ligand if the solvent can facilitate sufficient solubility of copper catalyst.[64] During click reaction, excess $PBLG_{15}$-$N_3$ was used to ensure the complete consumption of *dialkynyl*-PZLL. Fortunately, the excess $PBLG_{15}$-$N_3$ can be facilely removed by clicking with alkynyl-functionalized Wang resin and the subsequent simple filtration procedure.[65]

The formation of well-defined Y-shaped miktoarm star polypeptide copolymer was confirmed by GPC and $^1H$ NMR analysis. GPC traces of crude and purified products were shown in Figure 2. The crude product exhibited a bimodal GPC trace, clearly revealing a shoulder peak corresponding to the excess PBLG-$N_3$. However, after treating with alkynyl-functionalized Wang resin, a monomodal peak can be observed, suggesting the complete removal of excess $PBLG_{15}$-$N_3$. The elution trace of the purified $PZLL_{24}$-*b*-$(PBLG_{15})_2$ was quite symmetric, with no tailing at the lower molecular weight side. Compared to that of *dialkynyl*-$PZLL_{24}$ and $PBLG_{15}$-$N_3$, we can clearly observe that the elution peak of $PZLL_{24}$-*b*-$(PBLG_{15})_2$ shifted to higher molecular weight side, giving an $M_n$ of 26700 and an $M_w/M_n$ of 1.13.

$^1H$ NMR spectrum of $PZLL_{24}$-*b*-$(PBLG_{15})_2$ was shown in Figures 3c, which revealed all the characteristic signals of PZLL and PBLG. Most importantly, the composition ratio between PBLG and PZLL components derived from signal integral ratios of peak *f′* to peaks *g′* and *g* agreed quite well with the desired structure of Y-shaped miktoarm star copolymer. Figure 4c shows the FT-IR spectrum of the purified Y-shaped miktoarm star polypeptide copolymer, $PZLL_{24}$-*b*-$(PBLG_{15})_2$, after treating with alkynyl-functionalized Wang resin. The complete disappearance of characteristic azide and alkynyl absorbance peaks at ~2100 $cm^{-1}$ further confirmed the complete click reaction.

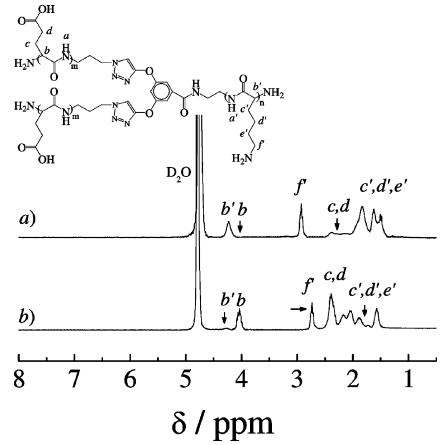



**Figure 5.** $^1H$ NMR spectra of $PZLL_{24}$-*b*-$(PBLG_{15})_2$ in $D_2O$ at (a) pH 2 and (b) pH 12, respectively.

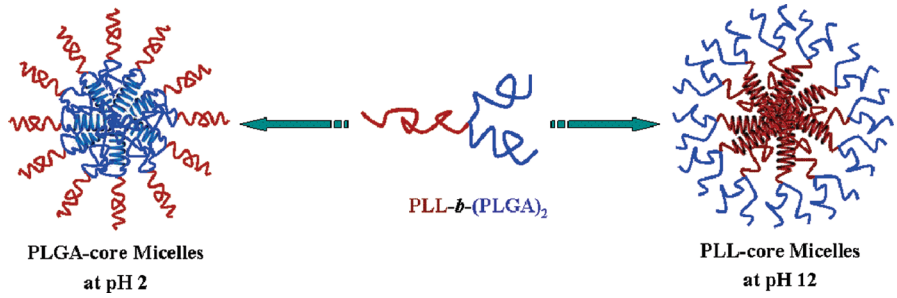
On the basis of above GPC, $^1H$ NMR, and FT-IR results, we can conclude that Y-shaped $AB_2$ 3-miktoarm star polypeptide copolymer, $PZLL_{24}$-*b*-$(PBLG_{15})_2$, was successfully synthesized. The combination of ROP and subsequent click reactions can thus provide a suitable platform for the synthesis of nonlinear shaped miktoarm star polymers consisting of purely synthetic peptides.

*Synthesis of PLL-b-(PLGA)$_2$.* The obtained $PZLL_{24}$-*b*-$(PBLG_{15})_2$ was then subjected to hydrolysis to remove benzyl and benzyloxycarbonyl protecting groups. A mixture of TFA and 45% w/v HBr/AcOH was employed for the hydrolysis reaction according to previous literature procedures.[59–61] Figure 5 shows the $^1H$ NMR spectrum of hydrolysis product, $PLL_{24}$-*b*-$(PLGA_{15})_2$, in $D_2O$. Two conditions, pH 2 and 12, were chosen to ensure the water-solubility of PLL and PLGA blocks, respectively. As compared to that of the precursors (Figure 3), we can clearly see that signals characteristic of benzyl and benzyloxycarbonyl groups of PBLG and PZLL building blocks at 7.3 and 5.0 ppm totally disappeared, indicating the complete removal of benzyl and benzyloxycarbonyl groups.

**pH-Responsive Micellization of PLL-*b*-(PLGA)$_2$.** It is well-known that PLGA and PLL will adopt a random coil conformation in aqueous solution at alkaline and acidic pH conditions, respectively, due to the ionization of side carboxyl groups and protonation of primary amine residues.[66–68] The water-solubility for PLGA and PLL will considerably decrease in acidic and alkaline media, respectively, due to the pH-induced coil-to-helix transitions. Lecommandoux et al.[26] reported that PLGA-*b*-PLL diblock copolymer can spontaneously form two types of vesicles at pH 3 and 12, respectively. For Y-shaped $AB_2$ miktoarm star copolymer, $PLL_{24}$-*b*-$(PLGA_{15})_2$, we expect that they will exhibit similar pH-responsive supramolecular self-assembly behavior in aqueous solution. Most importantly, this process will be accompanied with coil-to-helix or helix-to-coil transitions for PLL and PLGA sequences (Scheme 2).

The titration results of $PLGA_{15}$ and $PLL_{24}$ homopolymers were shown in Figure 6a,b. From the insets, we can see that the degree of protonation ($\alpha$) of $PLGA_{15}$ and $PLL_{24}$ increase from 0 to 1 as the solution pH decreases from 6.78 to 3.51 and 11.02 to 8.43, revealing p$K_a$ values of 4.36 and 9.29, respectively. These results were in general agreement with literature reports.[69,70] During titration, we can observe that $PLGA_{15}$ homopolymer remained water-soluble above pH 4.2, but exhibited phase separation from the solution below pH 4.2. On the other hand, $PLL_{24}$ became water-insoluble above pH 9.6.

**Scheme 2.** pH-Responsive Micellization of $PLL_{24}$-*b*-$(PLGA_{15})_2$ Associated with Coil-to-Helix Transitions




Considering the zwitterionic nature of $PLL_{24}$-*b*-$(PLGA_{15})_2$, an isoelectric point (IEP) was expected and calculated to be at ~pH 5.[71,72]

Figure 6c shows the titration curve of $PLL_{24}$-*b*-$(PLGA_{15})_2$ in aqueous solution. We can observe the formation of stable dispersion with characteristic bluish tinge at pH above 6.2 and below pH 4.6, indicating the formation of colloidal aggregates. In the pH range of 4.6−6.2, macroscopic phase separation can be observed. This should be ascribed to the presence of IEP, and charge neutralization lead to the formation of aggregates lacking sufficient stabilization from surface charges. However, the precipitates can be easily redispersed by the addition of base or acid.

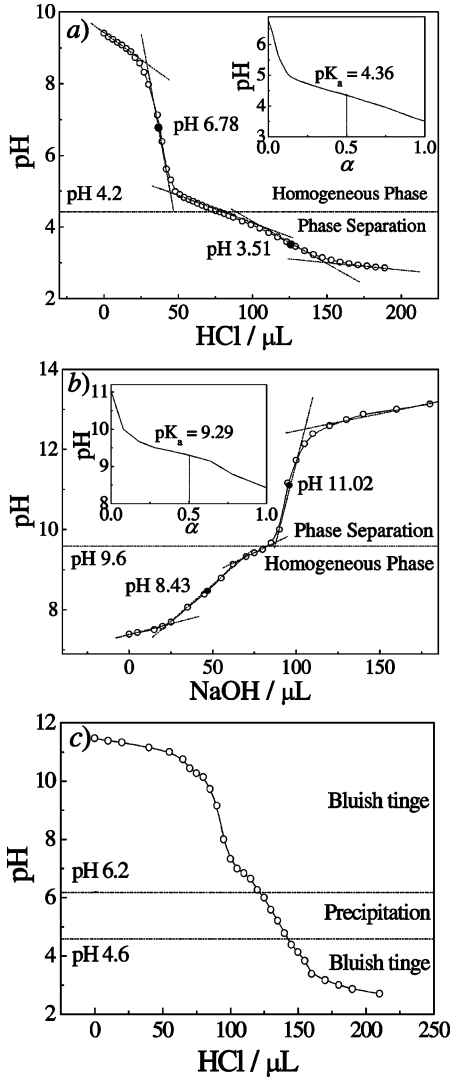



**Figure 6.** Titration curves of 1.0 g/L (a) $PLGA_{15}$, (b) $PLL_{24}$ homopolymer, and (c) $PLL_{24}$-*b*-$(PLGA_{15})_2$ Y-shaped miktoarm star copolymer. The insets show same titration curves with the *x*-axis expressed as the degree of protonation α.

A reexamination of $^1$H NMR results of $PLL_{24}$-*b*-$(PLGA_{15})_2$ shown in Figure 5 further revealed the pH-responsive supramolecular assembly behavior. At acidic pH, the PLGA block is neutralized and the formation of compact α-helical structure leads to decreased solubility. Thus, NMR resonance signals characteristic of PLGA block at 4.0 and 2.0−2.5 ppm almost completely disappeared at pH 2, indicating that PLGA block gets insoluble; on the other hand, resonance signals characteristic of PLL segments are still clearly evident (Figure 5a). These results suggest the formation micelles consisting of PLGA cores stabilized by PLL coronas (Scheme 2).

According to the titration results (Figure 6), PLL block is fully deprotonated and PLGA block remains completely ionized at pH 12. $^1$H NMR spectrum clearly reveals a signal shift from 2.9 to 2.6 ppm for ϵ-methylene proton (*f*′) in PLL segment upon changing from pH 2 to 12. Moreover, the signals at 1.4−2.0 ppm corresponding to protons of three methylene groups (*c*′, *d*′, *e*′) of PLL segment considerably decreases in intensity (Figure 5b), which is in line with the results previously reported for coil-to-helix transition of PLL upon pH increase.[26,32,73] This supports the formation of PLL-core micelles stabilized by ionized PLGA coronas at pH 12.

The pH-induced formation of PLGA-core and PLL-core micelles were further characterized by dynamic LLS. Figure 7 showed the hydrodynamic radius distributions, $f(R_h)$, for aqueous solutions of $PLL_{24}$-*b*-$(PLGA_{15})_2$ at pH 2 and 12, respectively. The average hydrodynamic radii, $<R_h>$, were determined to be 77 and 85 nm for PLGA-core and PLL-core micelles, respectively. Both types of micelles were relatively narrow-disperse with the polydispersities ($\mu_2/\Gamma^2$) being 0.1 and 0.08, respectively.

The actual morphology of the two types of micelles has been be obtained by TEM observation (Figure 8), which clearly reveals the presence of spherical micelles with average diameters

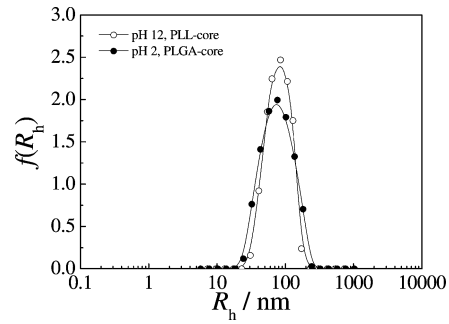



**Figure 7.** The hydrodynamic radius distributions, $f(R_h)$, obtained for 1.0 g/L aqueous solution of $PLL_{24}$-*b*-$(PLGA_{15})_2$ at 25 °C at pH 2 (○) and 12 (•), respectively.

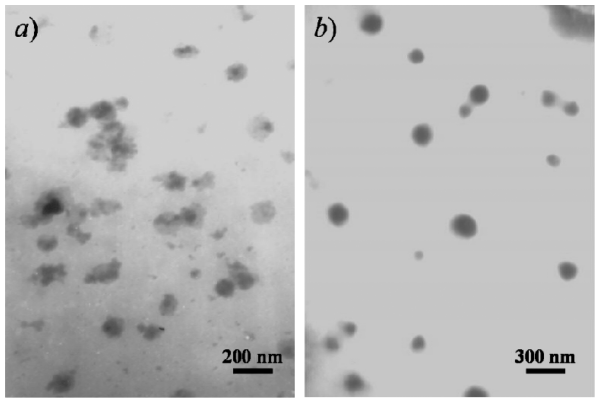



**Figure 8.** TEM images of PLGA-core and PLL-core micelles formed from $PLL_{24}$-*b*-$(PLGA_{15})_2$ Y-shaped miktoarm star polypeptide copolymer at (a) pH 2 and (b) pH 12, respectively.

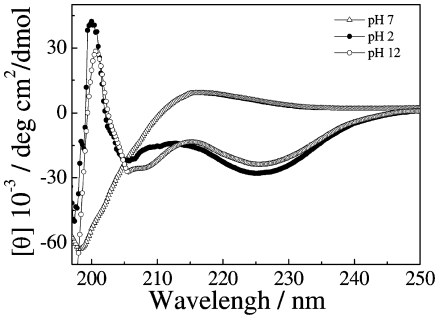



**Figure 9.** Circular dichroism spectra obtained for 0.2 g/L aqueous solution of $PLL_{24}$-*b*-$(PLGA_{15})_2$ at different pH.

of ~90 and 110 nm for PLGA-core (pH 2) and PLL-core micelles (pH 12), respectively. The particle sizes estimated from TEM were systematically smaller than those obtained by dynamic LLS, which is reasonable considering that the former reflects conformations in the dry state. It should be noted that Lecommandoux et al. reported that polypeptide-based block copolymer, $PLGA_{15}$-*b*-$PLL_{15}$, can self-assemble into two types of vesicles with invertible microstructures at pH 3 and 12.[26] In the current study, miktoarm polypeptide $PLL_{24}$-*b*-$(PLGA_{15})_2$ copolymer formed spherical PLGA-core and PLL-core micelles at low and high pH conditions, respectively. This might be due to differences in chain topology.

To further probe the coil-to-helix and helix-to-coil transitions associated with the pH-responsive micellization behavior, CD measurements were conducted and results were shown in Figure 9. At pH 7, CD spectrum shows a typical inflected curve with a gradual positive maximum at 215 nm and a minimum at 198 nm, which is characteristic of a random coil conformation. Upon changing to pH 12 or 2, two negative minima at 221 and 208 nm could be apparently detected, suggesting the formation of α-helical conformation from PLL and PLGA sequences, respectively. Thus, we can conclude that the pH-responsive micellization of the novel Y-shaped miktoarm star polymer, $PLL_{24}$-*b*-$(PLGA_{15})_2$, was also accompanied with transition between α-helix and coil conformations (Scheme 2).

## Conclusion

In summary, well-defined PZLL-*b*-$(PBLG)_2$ Y-shaped miktoarm star polypeptide copolymer was synthesized for the first time via a combination of ring-opening polymerization (ROP) and click chemistry, where PZLL is poly(ε-benzyloxycarbonyl-L- lysine) and PBLG is poly(γ-benzyl-L-glutamate). On the basis of gel permeation chromatograph (GPC), Fourier transform-infrared spectroscopy (FT-IR), and $^1$H NMR results, the click reaction has led to the quantitative preparation of nonlinear-shaped miktoarm star copolymer consisting of purely synthetic polypeptide. Moreover, after the hydrolysis of protecting groups of PZLL-*b*-$(PBLG)_2$, water-soluble Y-shaped miktoarm star polypeptide copolymer, PLL-*b*-$(PLGA)_2$, was obtained, where PLL is poly(L-lysine) and PLGA is poly(L-glutamic acid). It can self-assemble into PLGA-core micelles at acidic pH and PLL-core micelles at alkaline pH, accompanied with the coil-to-helix transition of PLGA and PLL sequences, respectively. It can be expected that the synthetic strategy of Y-shaped miktoarm star polypeptide copolymer and the pH-responsive self-assembly behavior might represent a promising new direction in the field of macromolecular design and bionanotechnology within the context of both academic studies and practical applications.

**Acknowledgment.** The financial supports of National Natural Scientific Foundation of China (NNSFC) Projects (20534020, 20674079, and 50425310), Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP), and the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT) are gratefully acknowledged.

## References and Notes

(1) Birshtein, T. M.; Polotsky, A. A.; Abetz, V. *Macromol. Theory Simul.* **2004**, *13*, 512–519.
(2) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Pispas, S.; Avgeropoulos, A. *Prog. Polym. Sci.* **2005**, *30*, 725–782.
(3) Hadjichristidis, N.; Pitsikalis, M.; Pispas, S.; Iatrou, H. *Chem. Rev.* **2001**, *101*, 3747–3792.
(4) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200–1205.
(5) Huckstadt, H.; Gopfert, A.; Abetz, V. *Macromol. Chem. Phys.* **2000**, *201*, 296–307.
(6) Iatrou, H.; Hadjichristidis, N. *Macromolecules* **1992**, *25*, 4649–4651.
(7) Lohse, D. J.; Hadjichristidis, N. *Curr. Opin. Colloid Interface Sci.* **1997**, *2*, 171–176.
(8) Milner, S. T. *Macromolecules* **1994**, *27*, 2333–2335.
(9) Pispas, S.; Hadjichristidis, N.; Potemkin, I.; Khokhlov, A. *Macromolecules* **2000**, *33*, 1741–1746.
(10) Pispas, S.; Poulos, Y.; Hadjichristidis, N. *Macromolecules* **1998**, *31*, 4177–4181.
(11) Pochan, D. J.; Gido, S. P.; Pispas, S.; Mays, J. W. *Macromolecules* **1996**, *29*, 5099–5105.
(12) Shi, P. H.; Li, Y. G.; Pan, C. Y. *Eur. Polym. J.* **2004**, *40*, 1283–1290.
(13) Sioula, S.; Hadjichristidis, N.; Thomas, E. L. *Macromolecules* **1998**, *31*, 8429–8432.
(14) Sotiriou, K.; Nannou, A.; Velis, G.; Pispas, S. *Macromolecules* **2002**, *35*, 4106–4112.
(15) Tselikas, Y.; Iatrou, H.; Hadjichristidis, N.; Liang, K. S.; Mohanty, K.; Lohse, D. J. *J. Chem. Phys.* **1996**, *105*, 2456–2462.
(16) Yang, L. Z.; Gido, S. P.; Mays, J. W.; Pispas, S.; Hadjichristidis, N. *Macromolecules* **2001**, *34*, 4235–4243.
(17) Tang, P.; Qiu, F.; Zhang, H. D.; Yang, Y. L. *J. Phys. Chem. B* **2004**, *108*, 8434–8438.
(18) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967–973.
(19) Maldovan, M.; Urbas, A. M.; Yufa, N.; Carter, W. C.; Thomas, E. L. *Phys. Rev. B: Condens. Matter Mater. Phys.* **2002**, *65*, 165123.
(20) Park, M.; Harrison, C.; Chaikin, P. M.; Register, R. A.; Adamson, D. H. *Science* **1997**, *276*, 1401–1404.
(21) Pitsikalis, M.; Pispas, S.; Mays, J. W.; Hadjichristidis, N. *Adv. Polym. Sci.* **1998**, *135*, 1–137.
(22) Thurn-Albrecht, T.; Schotter, J.; Kastle, C. A.; Emley, N.; Shibauchi, T.; Krusin-Elbaum, L.; Guarini, K.; Black, C. T.; Tuominen, M. T.; Russell, T. P. *Science* **2000**, *290*, 2126–2129.
(23) Abraham, S.; Ha, C. S.; Kim, I. *J. Polym. Sci., Part A: Polym. Chem* **2006**, *44*, 2774–2783.
(24) Babin, J.; Taton, D.; Brinkmann, M.; Lecommandoux, S. *Macromolecules* **2008**, *41*, 1384–1392.
(25) Dimitrov, I. V.; Berlinova, I. V.; Vladimirov, N. G. *Macromolecules* **2006**, *39*, 2423–2426.

(26) Rodriguez-Hernandez, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026–2027.
(27) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244–248.
(28) Breedveld, V.; Nowak, A. P.; Sato, J.; Deming, T. J.; Pine, D. J. *Macromolecules* **2004**, *37*, 3943–3953.
(29) Constancis, A.; Meyrueix, R.; Bryson, N.; Huille, S.; Grosselin, J. M.; Gulik-Krzywicki, T.; Soula, G. *J. Colloid Interface Sci.* **1999**, *217*, 357–368.
(30) Doi, T.; Kinoshita, T.; Kamiya, H.; Tsujita, Y.; Yoshimizu, H. *Chem. Lett.* **2000**, 262–263.
(31) Doi, T.; Kinoshita, T.; Kamiya, H.; Washizu, S.; Tsujita, Y.; Yoshimizu, H. *Polym. J.* **2001**, *33*, 160–164.
(32) Harada, A.; Cammas, S.; Kataoka, K. *Macromolecules* **1996**, *29*, 6183–6188.
(33) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature* **2002**, *417*, 424–428.
(34) Pochan, D. J.; Pakstis, L.; Ozbas, B.; Nowak, A. P.; Deming, T. J. *Macromolecules* **2002**, *35*, 5358–5360.
(35) Deming, T. J. *Adv. Polym. Sci.* **2006**, *202*, 1–18.
(36) Schlaad, H. *Adv. Polym. Sci.* **2006**, *202*, 53–73.
(37) Klok, H. A.; Lecommandoux, S. *Adv. Polym. Sci.* **2006**, *202*, 75–111.
(38) Aliferis, T.; Iatrou, H.; Hadjichristidis, N. *Biomacromolecules* **2004**, *5*, 1653–1656.
(39) Iatrou, H.; Frielinghaus, H.; Hanski, S.; Ferderigos, N.; Ruokolainen, J.; Ikkala, O.; Richter, D.; Mays, J.; Hadjichristidis, N. *Biomacromolecules* **2007**, *8*, 2173–2181.
(40) Cardinaux, F.; Howard, J. C.; Taylor, G. T.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 2005–2028.
(41) Howard, J. C.; Cardinaux, F.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 2029–2051.
(42) Klok, H. A.; Langenwalter, J. F.; Lecommandoux, S. *Macromolecules* **2000**, *33*, 7819–7826.
(43) Webster, O. W. *Science* **1991**, *251*, 887–893.
(44) Woodward, R. B.; Schramm, C. H. *J. Am. Chem. Soc.* **1947**, *69*, 1551–1552.
(45) Altintas, O.; Hizal, G.; Tunca, U. *J. Polym. Sci., Part A: Polym. Chem* **2006**, *44*, 5699–5707.
(46) Altintas, O.; Yankul, B.; Hizal, G.; Tunca, U. *J. Polym. Sci., Part A: Polym. Chem* **2006**, *44*, 6458–6465.
(47) Durmaz, H.; Dag, A.; Altintas, O.; Erdogan, T.; Hizal, G.; Tunca, U. *Macromolecules* **2007**, *40*, 191–198.
(48) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
(49) Opsteen, J. A.; van Hest, J. C. M. *Chem. Commun.* **2005**, 57–59.
(50) Quemener, D.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Chem. Commun.* **2006**, 5051–5053.
(51) Reynhout, I. C.; Cornelissen, J. J. L. M.; Nolte, R. J. M. *J. Am. Chem. Soc.* **2007**, *129*, 2327–2332.
(52) Whittaker, M. R.; Urbani, C. N.; Monteiro, M. J. *J. Am. Chem. Soc.* **2006**, *128*, 11360–11361.
(53) Agut, W.; Taton, D.; Lecommandoux, S. *Macromolecules* **2007**, *40*, 5653–5661.
(54) Daly, W. H.; Poche, D. *Tetrahedron Lett.* **1988**, *29*, 5859–5862.
(55) Wang, L. Y.; Wang, S. G.; Bei, J. Z. *Polym. Adv. Technol.* **2004**, *15*, 617–621.
(56) Lewis, W. G.; Magallon, F. G.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 9152–9153.
(57) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Frechet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed* **2004**, *43*, 3928–3932.
(58) Lee, J. W.; Kim, B. K.; Kim, H. J.; Han, S. C.; Shin, W. S.; Jin, S. H. *Macromolecules* **2006**, *39*, 2418–2422.
(59) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley-Interscience: New York, 1999.
(60) Hiskey, R. G.; Adams, J. B. *J. Am. Chem. Soc.* **1965**, *87*, 3969–3973.
(61) Aboderin, A. A.; Delpierre, G. R.; Fruton, J. S. *J. Am. Chem. Soc.* **1965**, *87*, 5469–5472.
(62) Sanda, F.; Gao, G. Z.; Masuda, T. *Macromol. Biosci.* **2004**, *4*, 570–574.
(63) Lowik, D. W. P. M.; Ayres, L.; Smeenk, J. M.; Van Hest, J. C. M. *Adv. Polym. Sci.* **2006**, *202*, 19–52.
(64) Sumerlin, B. S.; Tsarevsky, N. V.; Louche, G.; Lee, R. Y.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 7540–7545.
(65) Chen, G. J.; Tao, L.; Mantovani, G.; Ladmiral, V.; Burt, D. P.; Macpherson, J. V.; Haddleton, D. M. *Soft Matter* **2007**, *3*, 732–739.
(66) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222–245.
(67) Greenfield, N. J.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108–4116.
(68) Johnson, W. C.; Tinoco, I. *J. Am. Chem. Soc.* **1972**, *94*, 4389–4390.
(69) Kukula, H.; Schlaad, H.; Antonietti, M.; Forster, S. *J. Am. Chem. Soc.* **2002**, *124*, 1658–1663.
(70) Kruijff, B. D.; Rietveld, A.; Telders, N.; Vaandrager, B. *Biochim. Biophys. Acta* **1985**, *820*, 295–304.
(71) Patrickios, C. S.; Hertler, W. R.; Abbott, N. L.; Hatton, T. A. *Macromolecules* **1994**, *27*, 930–937.
(72) Patrickios, C. S.; Sharma, L. R.; Armes, S. P.; Billingham, N. C. *Langmuir* **1999**, *15*, 1613–1620.
(73) Bradbury, E. M.; Crane-Robinson, C.; Goldman, H.; Rattle, H. W. E. *Biopolymers* **1968**, *6*, 851–862.

BM800462Q