

Synthesis, Characterization and Phase Behaviors of Polypeptides Bearing Biphenyl Mesogens and Oligo-Ethylene-Glycol Tails^{*}

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Abstract A series of polypeptides bearing biphenyl mesogenic side-chains and oligo-ethylene-glycol (OEG) tails (PPLG_n-g-BPOEG_m, $n = 26$ and 63 , $m = 2, 3$, and 7) has been synthesized via a 1,3-dipolar cycloaddition with quantitative grafting density. FTIR results revealed that the polypeptides adopted highly stable α -helix in the temperature range of 25–200 °C. DSC, POM and WAXS analysis revealed that PPLG_n-g-BPOEG_m ($m \leq 3$) samples with short OEG tail length showed two main phase transitions including crystal to liquid crystalline (smectic C, SmC) phase transition and the melting transition of crystalline E-phase, while PPLG_n-g-BPOEG₇ with longer OEG tail length ($m = 7$) exhibited the melting transitions without the formation of liquid crystalline phase.

Keywords: Liquid-crystalline polymers; Polypeptides; Click chemistry; Smectic phases.

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INTRODUCTION

Synthetic polypeptides are mimics of natural peptides with many attractive properties, such as biocompatibility, relatively stable conformation (*e.g.*, α -helix and β -sheet), and highly ordered self-assemble structures in solution and solid state^[1–3]. Homopolypeptides and copolypeptides with various topological structures^[4–13] (*e.g.*, block copolymers^[14–17] and molecular bottle-brushes^[18, 19]) can be readily prepared by controlled ring-opening polymerization^[20], which is possible for large-scale production. Some polypeptides with short main-chain length can undergo unique conformationally coupled phase transition in the solid state^[21, 22]. Therefore, polypeptide materials have gained significant attention for potential applications that range from nanotechnology to biomedical applications^[23–25].

Polypeptides with mesogenic side-chains, namely side-chain liquid crystalline (SCLC) polypeptides can form hierarchical supermolecular ordering due to a biaxial orientation of rigid polypeptide main-chain and mesogenic side-chains^[26, 27]. SCLC polypeptides have two mesogenic elements, *i.e.*, the rigid helical main-chain and the mesogenic side-chains which are likely oriented perpendicular to the main-chain. The competition of the ordering principles of main-chains and side-chains plays an important role in the formation of thermotropic liquid crystalline phases. This concept was firstly demonstrated by Watanabe and Tominaga^[26]. They prepared

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poly(glutamates) bearing biphenyl mesogens and alkyl tails by transesterification. These samples exhibited two transitions and formed three distinct phases, including the crystalline, the smectic A and the cholesteric phases. Later, Deming and coworkers reported a series of thermotropic polylysines bearing variable lengths of flexible spacers between polypeptide main-chain and side-on mesogens^[27]. These samples formed an unusual mesophase where both mesogenic side-chain and polypeptide main-chain were ordered and coexist in a nematic-hexagonal structure. Recently, we have demonstrated that liquid crystalline polypeptides bearing biphenyl mesogens and alkyl tails (*i.e.*, PPLG_{*n*}-g-BPC_{*m*}) can be synthesized by a copper-mediated 1,3-dipolar cycloaddition with quantitative grafting densities^[28]. The polypeptides underwent a reversible crystalline to liquid crystalline phase (smectic C or smectic E phases) transition depending on the main-/side-chain lengths. Yet, there are still many questions with regard to the structure-property relation of side-chain LC polypeptides to be addressed. For example, the effect of the nature of the flexible side-chain tails on the molecular packing and phase transitions are still unrevealed.

In this contribution, we report the synthesis and solid-state phase behaviors of polypeptides bearing biphenyl mesogens and oligo-ethylene-glycol (OEG) tails (*i.e.*, PPLG-g-BPOEGs). The molecular structures of the resulting polypeptides were studied by ¹H-NMR, FTIR, and GPC. The liquid crystalline structures and phase transitions were strongly depending on their main-chain/OEG tail lengths, which were investigated by a combination of characteristic techniques including DSC, POM and WAXS.

EXPERIMENTAL

Materials

Triethylene glycol monomethylether (98%) was purchased from TCI-EP. Polyethylene glycol monomethylether ($M_w = 350$) and *p*-toluenesulfonyl chloride were purchased from Alfa-Aesar. Diethylene glycol monomethylether (99%), 4,4'-dihydroxydiphenyl (99%), 3-propargyl bromide (99%), *N,N,N',N'',N'''*-penta-methyldiethylenetriamine (PMDETA, 99%), copper bromide (CuBr, 99%), and all other chemicals were purchased from Aladdin and used as received. Poly(γ -azidopropyl-L-glutamates) (PAPLGs) were synthesized by a reported procedure^[29].

Instruments and Methods

¹H-NMR spectra were recorded on a BRUKER ARX 400 MHz spectrometer. Chemical shifts (δ) were referenced to the protio impurities in deuterated solvents. Gel permeation chromatography (GPC) measurements were performed using a system equipped with an isocratic pump (Agilent 1100), four Phenomenex 5 μ m, 300 mm $\times$ 7.8 mm columns (10 nm, 50 nm, 10² nm, and 10³ nm), a DAWN HELEOS multiangle light scattering (MALS) detector ($\lambda = 658$ nm), and a Wyatt OptilabREX differential refractive index (DRI) detector. DMF containing 0.1 mol·L⁻¹ LiBr was used as the eluent at a flow rate of 1.0 mL·min⁻¹. The column temperature was 60 °C and the detector temperature was 25 °C for both MALS and DRI detectors. The measured dn/dc of PAPLG in DMF/0.1 mol·L⁻¹ LiBr at 25 °C and 658 nm wavelength is 0.076 mL·g⁻¹. Fourier transform infrared (FTIR) spectra of samples at room temperature (25 °C) were recorded on a Thermo Scientific Nicolet 6700 FTIR spectrometer. Variable-temperature FTIR spectra were recorded on a PE-Spectrum Oen FTIR spectrometer. The MALDI-TOF mass spectra (MS) were recorded on a Bruker autoflexTOF/TOF. Thermogravimetric analysis (TGA) was carried out on a STA 449C instrument at a heating rate of 10 K·min⁻¹ under nitrogen (flow rate = 60 mL·min⁻¹). Differential scanning calorimetry (DSC) experiments were carried out on a TA DSC Q100 calorimeter with a programed heating and cooling procedure under nitrogen at a heating/cooling rate of 10 K·min⁻¹ in the range from -70 °C to 200 °C. The temperature and heat flow were calibrated with benzoic acid and indium. The samples were encapsulated in hermetically sealed aluminum pans with a typical sample weight of ~4 mg. The crystalline and liquid crystalline textures of the samples were studied under a polarized optical microscope (POM, Leica DM-LM-P) coupled with a Mettler-Toledo hot stage (FP82HT). The films for POM test were prepared by drop casting from polymer/DMF solutions onto glass slides and dried at room temperature inside a drybox. One-dimensional wide-angle X-ray scattering (1D WAXS) measurements were performed on a Bruker D8 Advance diffractometer equipped with an SSD160' celerator detector in the reflection mode. The

powder samples were placed in tinfoil slots (size: 12 mm $\times$ 8 mm) with a typical sample weight of 70–80 mg. Two-dimensional wide-angle X-ray scattering (2D WAXS) experiments were conducted on a Bruker D8Discover diffractometer with a 2D detector of GADDS in the transmission mode. The X-ray sources (Cu K α , λ = 0.154 nm) were provided by 3 kW ceramic tubes, and the peak positions were calibrated with silicon powder ($2\theta > 15^\circ$) and silver behenate ($2\theta < 10^\circ$). The background scattering was recorded and subtracted from the sample patterns. The oriented samples used in 2D WAXS were prepared by mechanical shearing at respective liquid crystalline phase. The point-focused X-ray beam was aligned perpendicular to the shear direction.

Synthesis of 4-Toluenesulfonyl-terminated Oligo-ethylene-glycols (Ts-OEGs, 2a–2c)

In an ice-water bath, the THF solution (7.0 mL) of diethylene glycol monomethylether (**1a**, 3.1 mL, 27 mmol) was mixed with the NaOH aqueous solution (4.8 mol·L⁻¹, 8.2 mL) in a round-bottomed flask (50 mL), followed by slow addition of the THF solution (7.0 mL) of *p*-toluenesulfonylchloride (5.00 g, 26 mmol). The solution was stirred at 0 °C for 4 h, and the THF was evaporated under vacuum to yield a liquid residue that was extracted with diethyl ether (7.0 mL $\times$ 3). The organic layers were combined and washed with brine until pH = 7. Drying with anhydrous sodium sulfate, filtration and removing the solvent under vacuum afforded the final product as a pale yellow liquid (**2a**, 3.80 g, 53% yield). ¹H-NMR (CDCl₃, δ): 7.79 (d, 2H, CH₃Ar–), 7.33 (d, 2H, –ArSO₂), 3.69–4.16 (m, 4H, –CH₂CH₂OSO₂), 3.47–3.57 (m, 4H, –OCH₂CH₂O–), 3.34 (t, 3H, –OCH₃), 2.44 (s, 3H, CH₃Ar–). Ts-OEG₃ (**2b**, 75% yield) was synthesized using the same method as Ts-OEG₂ (**2a**). ¹H-NMR (CDCl₃, δ): 7.79 (d, 2H, CH₃Ar–), 7.33 (d, 2H, –ArSO₂), 3.68–4.16 (m, 4H, –CH₂CH₂OSO₂), 3.52–3.58 (m, 8H, –OCH₂CH₂O–), 3.36 (t, 3H, –OCH₃), 2.44 (s, 3H, CH₃Ar–). Ts-OEG₇ (**2c**, 69% yield) was synthesized using the same method as Ts-OEG₂ (**2a**). ¹H-NMR (CDCl₃, δ): 7.79 (d, 2H, CH₃Ar–), 7.33 (d, 2H, –ArSO₂), 3.67–4.15 (m, 4H, –CH₂CH₂OSO₂), 3.54–3.64 (m, 24H, –OCH₂CH₂O–), 3.37 (t, 3H, –OCH₃), 2.44 (s, 3H, CH₃Ar–).

Synthesis of 4'-Prop-2-ynyloxy-biphenyl-terminated Oligo-ethylene-glycols (Pr-BPOEGs, 4a–4c)

A round-bottom flask (100 mL) was charged with 4,4'-dihydroxydiphenyl (1.00 g, 5.37 mmol), potassium hydroxide (1.11 g, 19.82 mmol) and dimethyl sulfoxide (DMSO, 50.0 mL) under nitrogen. The mixture was stirred at 60 °C for 6 h. Ts-OEG₂ (**2a**, 1.81 g, 6.60 mmol) was slowly added and stirred at 60 °C for 24 h. Deionized water (12.5 mL) was added to the above mixture, followed by extracting with diethyl ether (30.0 mL $\times$ 3). The aqueous solution was collected and acidified by HCl aqueous solution (1.0 mL, 12 mol·L⁻¹). Then, the aqueous solution was extracted with chloroform (30.0 mL $\times$ 3). The organic layers were combined and washed with brine (50.0 mL $\times$ 3). Drying with anhydrous sodium sulfate, filtration and removing the solvent under vacuum afforded the intermediate (**3a**) as an orange-red solid (obtained 0.92 g).

3a (0.92 g, 3.19 mmol), potassium carbonate (1.32 g, 0.095 mol) and acetone (40 mL) were mixed in a round-bottomed flask (100 mL). The mixture was heated to the reflux temperature (56 °C) and stirred for 3 h, followed by slow addition of 3-propargyl bromide (0.75 mL, 9.60 mmol). The mixture was stirred at the reflux temperature for 24 h. Filtration and removing the solvent afforded an orange solid that was purified by silica gel chromatography using ethyl acetate/methanol/petroleum ether (volume ratio = 19:1:5, R_f = 0.93) as the eluent. Crystalline product (Pr-BPOEG₂, **4a**, 0.40 g, 22% yield) was obtained after recrystallization from methanol. ¹H-NMR (CDCl₃, δ): 7.47 (d, 4H, biphenyl), 7.02 (d, 2H, biphenyl), 6.96 (d, 2H, biphenyl), 4.73 (s, 2H, –OCH₂C $\equiv$ CH), 3.59–4.18 (m, 8H, –OCH₂CH₂–), 3.40 (s, 3H, –OCH₃), 2.54 (s, 1H, –OCH₂C $\equiv$ CH). MALDI-TOF-MS (m/z) [M]⁺ calculated for C₂₀H₂₂O₄, 326.152; found 326.187. Pr-BPOEG₃ (**4b**) was synthesized using the same method as **4a** (R_f = 0.84, obtained 0.53 g, 34% yield). ¹H-NMR (CDCl₃, δ): 7.47 (d, 4H, biphenyl), 7.02 (d, 2H, biphenyl), 6.96 (d, 2H, biphenyl), 4.73 (s, 2H, –OCH₂C $\equiv$ CH), 3.38–4.16 (m, 12H, –OCH₂CH₂–), 3.38 (s, 3H, –OCH₃), 2.54 (s, 1H, HC $\equiv$). MALDI-TOF-MS (m/z) [M]⁺ calculated for C₂₂H₂₆O₅, 370.178; found 370.231. Pr-BPOEG₇ (**4c**) was synthesized using the same method as **4a** (R_f = 0.57–0.86, obtained 1.64 g, 54% yield). ¹H-NMR (CDCl₃, δ): 7.47 (d, 4H, biphenyl), 7.02 (d, 2H, biphenyl), 6.96 (d, 2H, biphenyl), 4.73 (s, 2H, –OCH₂C $\equiv$ CH), 3.54–4.16 (t, 28H, –OCH₂CH₂–), 3.37 (s, 3H, –OCH₃), 2.54 (s, 1H, HC $\equiv$). MALDI-TOF-MS (m/z) [M]⁺ calculated for C₃₀H₄₂O₉, 546.283; found 546.337.

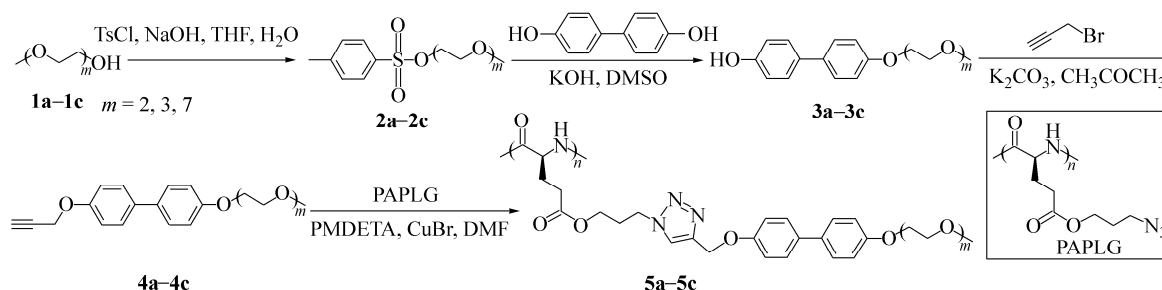
Synthesis of Poly(γ -propyl-L-glutamate)-graft-(biphenyl-terminated oligo-ethylene-glycols) (PPLG-g-BPOEGs, 5a–5c)

Poly(γ -3-azidopropyl-L-glutamate) (PAPLG, 10 mg, 0.047 mmol of azido groups), **4a** (16 mg, 0.050 mmol), CuBr (8 mg, 0.050 mmol) and PMDETA (12.1 μ L, 0.050 mmol) were mixed in DMF (3 mL) under nitrogen. The click reaction was stirred at room temperature. The conversion of azido groups was monitored by FTIR spectroscopy. The signal of azido groups at around 2094 cm^{-1} disappeared within an hour. Nevertheless, the reaction was stirred at room temperature for 24 h before purification to ensure quantitative grafting density. The product was purified by dialysis against DI-H₂O (HCl_{aq} was added to remove the copper ion at pH = 5–7) in a dialysis bag with a cutoff molecular weight of 3000 $\text{g}\cdot\text{mol}^{-1}$ for 4 days. The product (PPLG-g-BPOEG₂, **5a**) was collected by evaporating the solvent under vacuum. **5a** (18.2 mg, 70% yield) was further purified by dissolving in chloroform and precipitation from diethyl ether triple times. ¹H-NMR (CDCl₃, δ): 7.69 (s, 1H, triazole), 6.88–7.35 (m, 8H, biphenyl), 5.05 (s, 2H, $-\text{CH}_2\text{OAr}-$), 4.38 (t, 2H, $-\text{ArOCH}_2\text{CH}_2\text{O}-$), 4.07 (br s, 5H, $-\text{COCHCH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ and $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.81 (t, 2H, $-\text{ArOCH}_2\text{CH}_2\text{O}-$), 3.68 (t, 2H, $-\text{OCH}_2\text{CH}_2\text{OCH}_3$), 3.55 (t, 2H, $-\text{OCH}_2\text{CH}_2\text{OCH}_3$), 3.36 (t, 3H, $-\text{OCH}_3$), 2.67 (br s, 2H, $-\text{COCHCH}_2\text{CH}_2-$), 2.36 (t, 2H, $-\text{COCHCH}_2\text{CH}_2-$), 2.15 (m, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$). PPLG-g-BPOEG₃ (**5b**) was synthesized using the same method as **5a** (66% yield). ¹H-NMR (CDCl₃, δ): 7.72 (s, 1H, triazole), 6.89–7.35 (m, 8H, biphenyl), 5.09 (s, 2H, $-\text{CH}_2\text{OAr}-$), 4.39 (t, 2H, $-\text{ArOCH}_2\text{CH}_2\text{O}-$), 4.06 (br s, 5H, $-\text{COCHCH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ and $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.81 (t, 2H, $-\text{ArOCH}_2\text{CH}_2\text{O}-$), 3.55–3.64 (m, 8H, $-\text{OCH}_2\text{CH}_2-$), 3.34 (t, 3H, $-\text{OCH}_3$), 2.66 (br s, 2H, $-\text{COCHCH}_2\text{CH}_2-$), 2.43 (t, 2H, $-\text{COCHCH}_2\text{CH}_2-$), 2.15 (m, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$). PPLG-g-BPOEG₇ (**5c**) was synthesized using the same method as **5a** (74% yield). ¹H-NMR (CDCl₃, δ): 7.71 (s, 1H, triazole), 6.89–7.35 (m, 8H, biphenyl), 5.03 (s, 2H, $-\text{CH}_2\text{OAr}-$), 4.39 (t, 2H, $-\text{ArOCH}_2\text{CH}_2\text{O}-$), 4.06 (br s, 5H, $-\text{COCHCH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ and $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.80 (t, 2H, $-\text{ArOCH}_2\text{CH}_2\text{O}-$), 3.52–3.62 (m, 24H, $-\text{OCH}_2\text{CH}_2-$), 3.35 (t, 3H, $-\text{OCH}_3$), 2.68 (br s, 2H, $-\text{COCHCH}_2\text{CH}_2-$), 2.43 (t, 2H, $-\text{COCHCH}_2\text{CH}_2-$), 2.17 (m, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$).

RESULTS AND DISCUSSION

Synthesis

Polypeptides bearing biphenyl mesogens and oligo-ethylene-glycol tails (*i.e.*, PPLG-g-BPOEGs, **5**) were synthesized by click chemistry (*i.e.*, copper-mediated alkyne-azide [2 + 3] 1,3-dipolar cycloaddition) between 4'-prop-2-ynyloxy-biphenyl-terminated oligo-ethylene-glycols (Pr-BPOEGs) and poly(γ -3-azidopropyl-L-glutamate) (PAPLG) with quantitative grafting density (Scheme 1). Pr-BPOEGs having biphenyl mesogens and oligo-ethylene-glycol (OEG) tails were synthesized in three steps. Specifically, Pr-BPOEGs (**4**) with variable OEG tail length ($m = 2, 3$ and 7) were synthesized *via* the esterification of **1** with *p*-toluenesulfonyl chloride in the presence of sodium hydroxide to afford **2**, followed by the mono-etherification of 4,4'-dihydroxydiphenyl in the presence of potassium hydroxide to afford **3** which was further etherified with 3-propargyl bromide in K₂CO₃/acetone. Purification of **4** was achieved by silica gel chromatography and recrystallization in methanol.



Scheme 1 The synthetic route to poly(γ -propyl-L-glutamate)-graft-(biphenyl-terminated oligo-ethylene-glycols) (PPLG-g-BPOEGs)

The molecular structures of **4** were verified by $^1\text{H-NMR}$ (Fig. 1a, Figs. S1–S2) and FTIR spectroscopy (Fig. 2). The integrations and chemical shifts of $^1\text{H-NMR}$ resonances are consistent with their chemical structures. In the FTIR spectra, the characteristic bands at 2128, 1498, and 1100 cm^{-1} correspond to ν_{alkyne} , ν_{biphenyl} , and $\nu_{\text{C-O-C}}$, respectively. A series of PAPLGs with various degree of polymerization ($\text{DP} = 26$ and 63 , Fig. S3) was prepared by a reported procedure^[29].

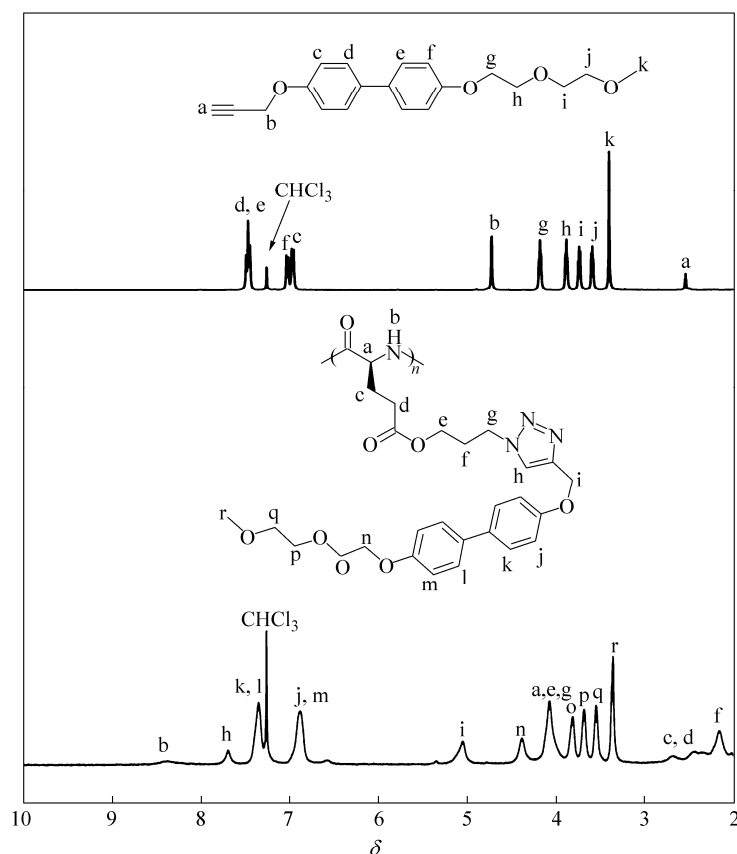


Fig. 1 $^1\text{H-NMR}$ spectra of (a) Pr-BPOEG₂ and (b) PPLG₂₆-g-BPOEG₂ in CDCl_3

The click chemistry between PAPLGs and Pr-BPOEGs was catalyzed by CuBr in the presence of *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA) at room temperature under nitrogen. Purification of poly(γ -propyl-L-glutamate)-graft-(biphenyl-terminated oligo-ethylene-glycols) (PPLG-*g*-BPOEGs, **5**) involved dialysis against deionized water at $\text{pH} = 5\text{--}7$ to remove the copper ions and repeat dissolution-precipitation in chloroform-diethyl ether to remove any excess **4**. The molecular structures of PPLG_{*n*}-*g*-BPOEG_{*m*} samples with variable main-chain ($n = 26$ and 63) and OEG tail length ($m = 2, 3$, and 7 , Scheme 1) were verified by $^1\text{H-NMR}$ (Fig. 1b, Figs. S4–S5), FTIR (Fig. 2b) and GPC (Fig. S3). For example, the appearance of $^1\text{H-NMR}$ resonance at $\delta = 7.70$ in the $^1\text{H-NMR}$ spectrum (Fig. 1b) and characteristic peaks at 1498 and 1100 cm^{-1} in the FTIR spectrum (Fig. 2b) suggested the formation of triazole groups and covalent assembly of the polypeptide backbones and biphenyl and OEG tails. The 1:2 integration ratio of H_h and H_n in the $^1\text{H-NMR}$ spectrum (Fig. 1b) and the absence of $\nu_{\text{N=N=N}}$ in the FTIR spectrum (Fig. 2b) suggested a quantitative grafting density. The successful grafting of Pr-BPOEGs to the polypeptide main-chains was also verified by GPC (Fig. S3), which showed a noticeable peak shift by comparing PPLG₂₆-*g*-BPOEG₇ sample with PAPLG₂₆ and Pr-BPOEG₇ samples. FTIR has been used to investigate the secondary structures of PPLG-*g*-BPOEGs (**5**) in the solid-state (Fig. 2b). Typically, polypeptides exhibit amide I bands at around 1630, 1656, and 1693 cm^{-1} that are characteristic of β -sheet, α -helix and random-coil conformations^[30, 31]. PPLG_{*n*}-*g*-BPOEG_{*m*} samples showed exclusive α -helical

conformation in temperature range of 25–200 °C (Fig. S6). This is consistent with previous reports that only polypeptides with short main-chain lengths (*e.g.*, DP < 18) exhibited a mixture of lamellar assembly of β -sheets and columnar hexagonal arrays of α -helices^[21, 32].

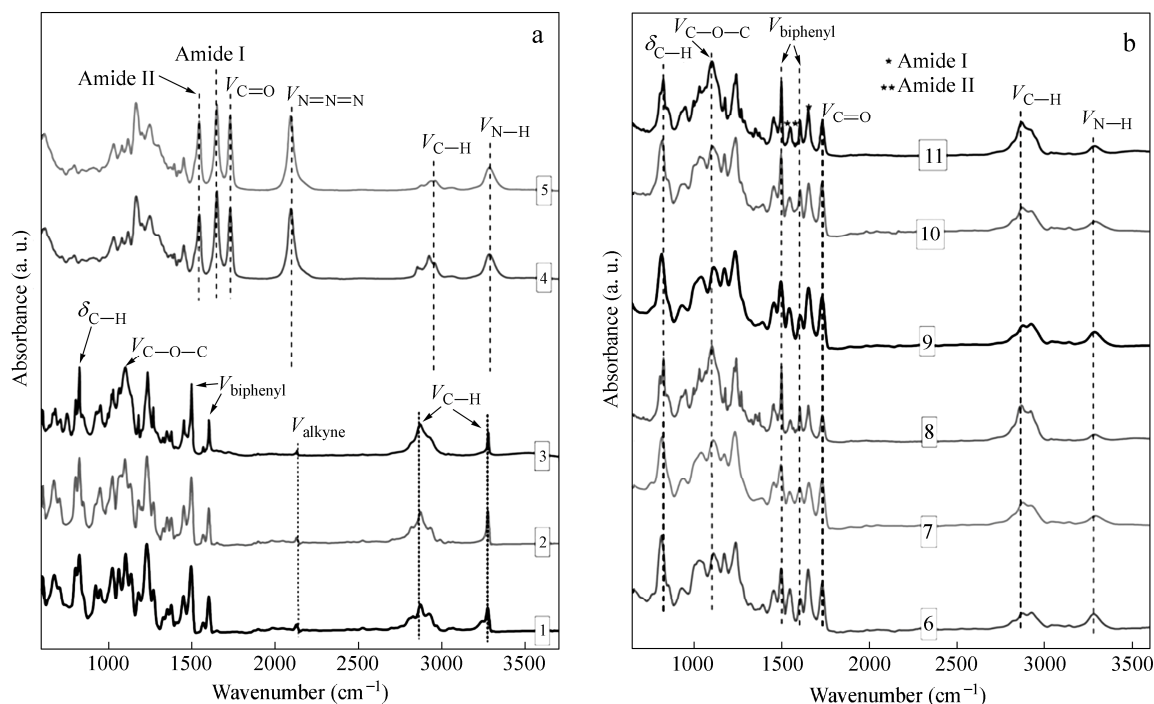


Fig. 2 FTIR spectra of (a) Pr-BPOEG_m ($m = 2, 3$ and 7 , curves 1–3), PAPLG_n ($n = 26$ and 63 , curves 4–5) and (b) PPLG₂₆-g-BPOEG_m ($m = 2, 3$ and 7 , curves 6–8), PPLG₆₃-g-BPOEG_m ($m = 2, 3$ and 7 , curves 9–11)

Phase Behaviors

Differential scanning calorimetry (DSC, Fig. 3) was used to study the thermal properties of the mesogenic side-chains (*i.e.*, Pr-BPOEGs) and PPLG-g-BPOEGs. Pr-BPOEG samples showed melting transition temperatures (T_m s) at 93.2, 85.7, and 47.3 °C (Table S1), respectively, as shown in the second heating cycles (Fig. 3, curves 1, 4, and 7). The T_m s decreased as the OEG tail length increased due to an increase of side-chain flexibility. Pr-BPOEG samples exhibited two crystallization transition temperatures (T_c s), which is likely due to either a polymorphism of respective crystallites or different crystal perfection during crystallization process. Their heat of melting (ΔH_m) and total heat of crystallization (ΔH_c) were tantamount, suggesting a reversible melting/crystallization behavior (Table S1). The phase transitions of Pr-BPOEG were further studied by polarized optical microscopy (POM, Fig. 4). All Pr-BPOEG samples showed birefringence at the temperatures below respective T_m s and exhibited isotropic phases above the T_m s, suggesting the melting transitions (Fig. S7).

Prior to the DSC test, thermogravimetric analysis (TGA) was used to investigate the thermal stabilities of PPLG-g-BPOEGs. All samples showed initial decomposition temperatures above 250 °C (Fig. S8). In the DSC thermograms (Fig. 3), PPLG-g-BPOEGs exhibited glass transitions and melting/crystallization transitions in the heating/cooling cycles, suggesting the coexistence of amorphous domains and crystalline domains. The glass transition temperature (T_g) was determined directly by DSC and marked by a downward pointing arrow (Fig. 3). Meanwhile, the melting transition temperature (T_m) was determined by the combination of DSC and variable-temperature wide-angle X-ray scattering (WAXS) which indicated the transitions of crystal E-phases in the solid-state. For example, PPLG₂₆-g-BPOEG₃ showed a weak endothermic peak at 105.9 °C where the E-phase started to disappear as confirmed by the variable-temperature WAXS result, indicating a melting transition temperature of crystal E-phase at 105.9 °C. In the heating cycle, the glass transition temperature (T_g) and T_m increased as increasing the main-chain length due to the increase of van der Waals interaction between the

adjacent polymers. For example, PPLG₂₆-g-BPOEG₂ showed a T_g at 131.1 °C (Table 1, entry 1) while PPLG₆₃-g-BPOEG₂ showed a T_g at 136.9 °C (Table 1, entry 4). PPLG₂₆-g-BPOEG₃ exhibited a T_m at 105.9 °C (Table 1, entry 2) while PPLG₆₃-g-BPOEG₃ exhibited a T_m at 114.4 °C (Table 1, entry 5). The T_g and T_m significantly decreased as increasing the side-chain length due to the increase of side-chain flexibility. For instance, the T_g of PPLG₆₃-g-BPOEG₂ was 47.3 K higher than that of PPLG₆₃-g-BPOEG₃ (Table 1, entry 4 and 5). The T_m of PPLG₆₃-g-BPOEG₃ was 27.9 K higher than that of PPLG₆₃-g-BPOEG₇ (Table 1, entry 5 and 6). In the cooling cycle, we observed crystallization transitions and glass transition temperatures, indicating a reversible thermal behavior.

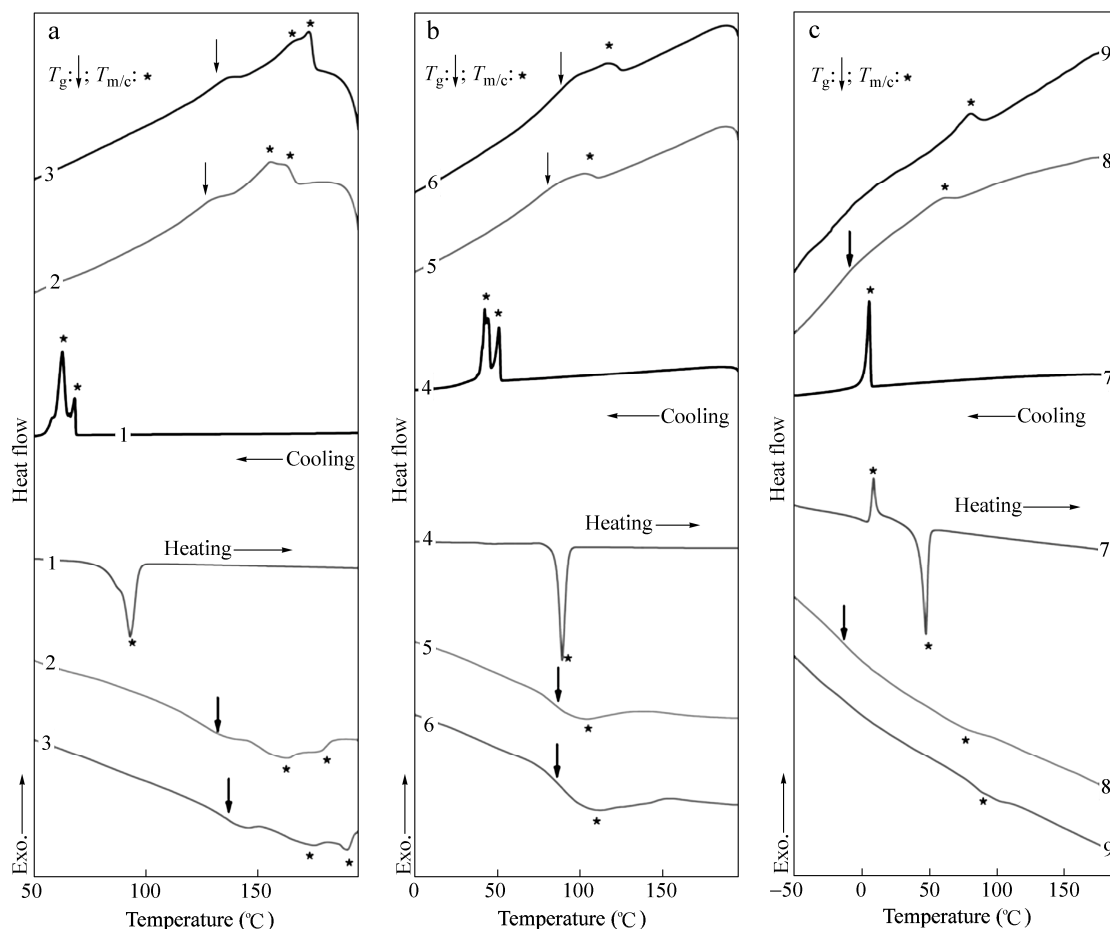


Fig. 3 DSC thermograms of (a) Pr-BPOEG₂ (curve 1), PPLG_n-g-BPOEG₂ ($n = 26$ and 63 , curves 2–3), (b) Pr-BPOEG₃ (curve 4), PPLG_n-g-BPOEG₃ ($n = 26$ and 63 , curves 5–6), and (c) Pr-BPOEG₇ (curve 7), PPLG_n-g-BPOEG₇ ($n = 26$ and 63 , curves 8–9)

The phase transition temperatures and phase behaviors of PPLG_n-g-BPOEG_m samples were determined by the combination of DSC (Fig. 3), POM (Fig. 4) and WAXS (Fig. 5). For example, all samples showed birefringence under POM at the temperatures below respective T_g s or T_m s and they showed ordered structures as evidenced by the WAXS results, suggesting crystalline phase below respective T_g s or T_m s. As increasing the temperature, some samples (*e.g.*, PPLG_{26/63}-g-BPOEG₇) showed extinction under POM and disappearance of sharp scattering peaks in WAXS, suggesting a transition to isotropic phases. Above respective T_g s, some samples (*e.g.*, PPLG_{26/63}-g-BPOEG_{2/3}) showed liquid crystalline phase (*i.e.*, SmC) and crystal E-phase as determined from WAXS. The E-phase transition was also determined by WAXS and the transition temperature was obtained from DSC.

Table 1. Compositional parameters and DSC results of PPLG-*g*-BPOEG samples

Entry	PPLG _{<i>n</i>} - <i>g</i> -BPOEG _{<i>m</i>}				Second heating		First cooling	
	<i>M_n</i> ^a	<i>M_w</i> / <i>M_n</i> ^b	<i>n</i> ^c	<i>m</i> ^d	<i>T_g</i> ^e (°C)	<i>T_m</i> ^f (°C)	<i>T_g</i> ^e (°C)	<i>T_c</i> ^g (°C)
1	14000	1.20	26	2	131.1	160.5, 178.1	125.8	155.0, 167.9
2	15100	1.20	26	3	82.2	105.9	74.9	100.8
3	19700	1.20	26	7	−15.6	73.5	−14.6	58.6
4	33900	1.28	63	2	136.9	172.5, 190.3	129.6	152.3, 173.2
5	36700	1.28	63	3	89.6	114.4	89.0	116.2
6	47800	1.28	63	7	—	86.5	—	80.1

^a Number-average molecular weights were calculated from the molecular weight of repeating units $\times n$; ^b Molecular weight distribution of PAPLG was determined from GPC; ^c Degree of polymerization of PAPLG was determined by GPC; ^d Degree of polymerization of OEG tails; ^e Glass transition temperatures; ^f Melting transition temperature; ^g Crystallization transition temperature

Specifically, PPLG_{*n*}-*g*-BPOEG₇ samples with long OEG tails ($m = 7$) exhibited crystalline textures at temperatures below respective T_m s and became isotropic above the T_m s (Fig. S9), suggesting a crystal to isotropic transition (Table 2, entry 3 and 6). In the cooling cycle, the crystalline texture (spherulite) appeared, indicating a reversible crystalline behavior, which was consistent with the DSC results. PPLG_{*n*}-*g*-BPOEG_{*m*} samples with short OEG tails (*i.e.*, $m \leq 3$) exhibited stable birefringence below respective decomposition temperatures (Fig. S10).

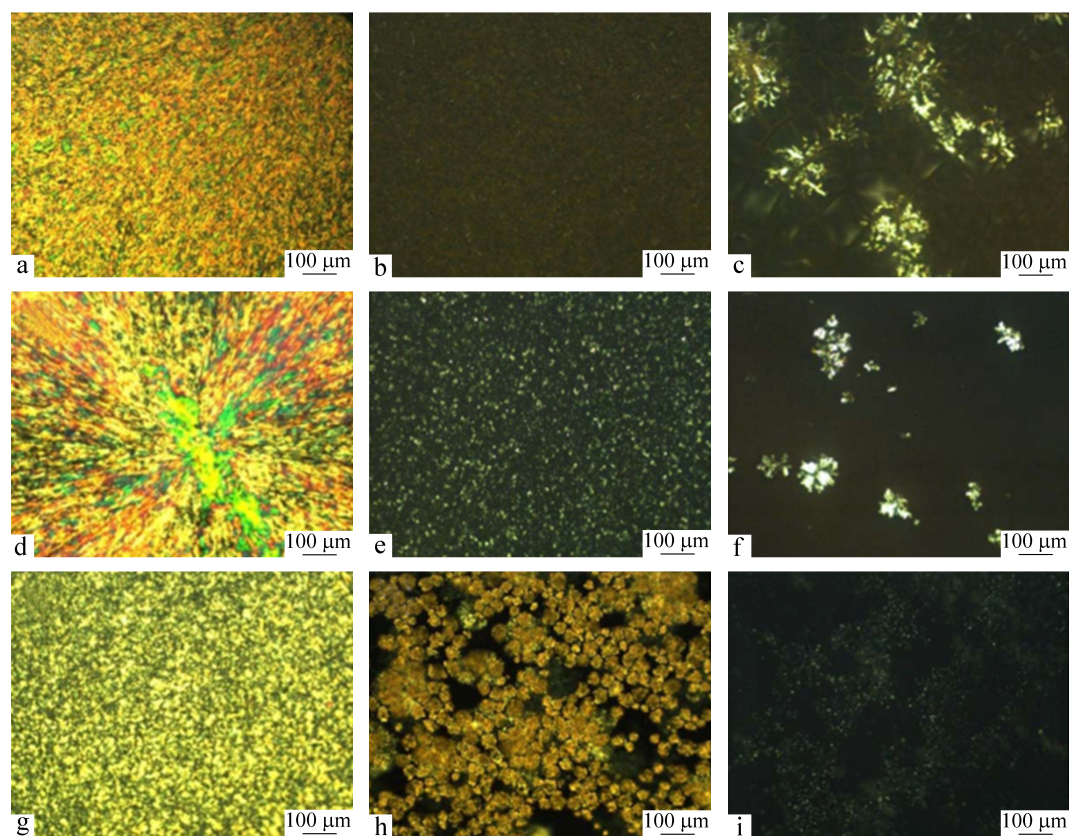


Fig. 4 POM images of Pr-BPOEG_{*m*} ($m = 2, 3$, and 7 , images a, d, and g), PPLG_{*n*}-*g*-BPOEG₂ ($n = 26$ and 63 , images b, c), PPLG_{*n*}-*g*-BPOEG₃ ($n = 26$ and 63 , images e–f), and PPLG_{*n*}-*g*-BPOEG₇ ($n = 26$ and 63 , images h–i) at 25°C

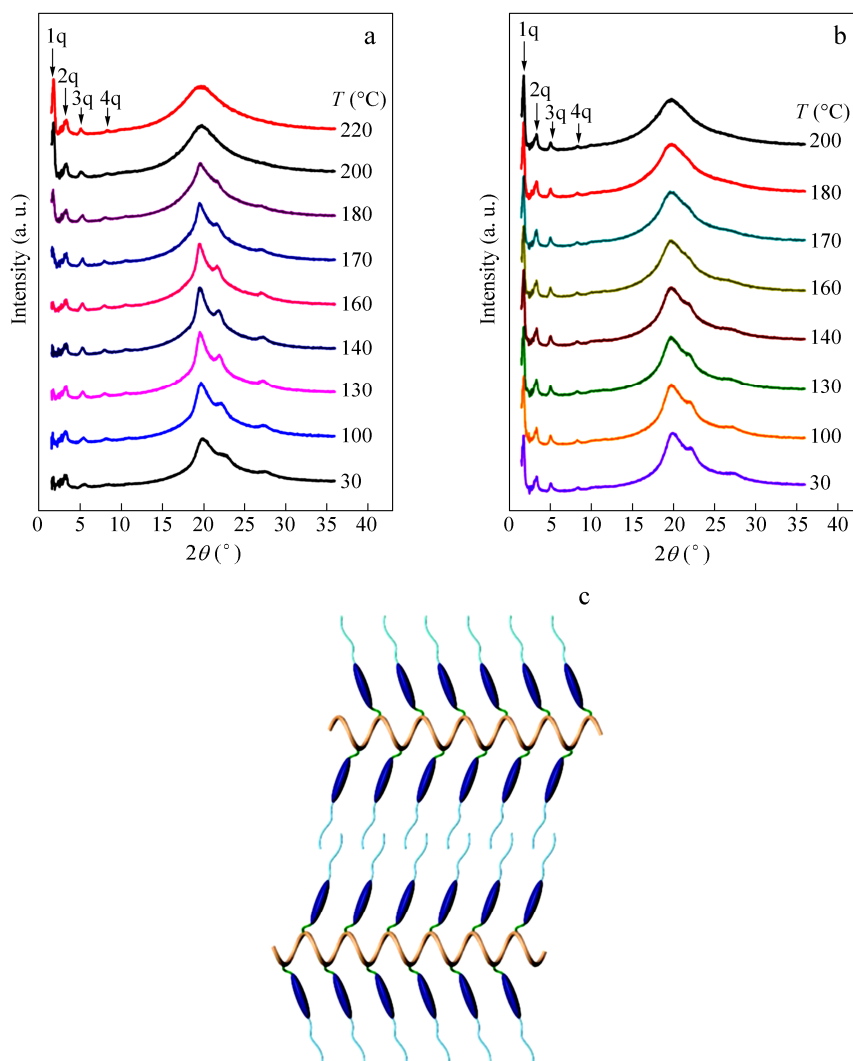


Fig. 5 Variable-temperature 1D WAXS data of PPLG₂₆-g-BPOEG₂ in the (a) heating and (b) cooling cycles; (c) proposed molecular packing of PPLG₂₆-g-BPOEG₂ and PPLG₂₆-g-BPOEG₃ (The helix and ellipsoid signify the α -helix and biphenyl mesogen, respectively.)

Table 2. WAXS data and phase transitions of PPLG-g-BPOEG samples

Entry ^a	Smectic C ^b		Phase transition ^d (°C)	
	d_{layer} (nm)	Tilt angle (°)		
1	5.07	NA ^c	Crystal $\xleftrightarrow[125.8]{131.1}$ SmC + E phase $\xleftrightarrow[167.9]{178.1}$ SmC	
2	5.32	53.2	Crystal $\xleftrightarrow[74.9]{82.2}$ SmC + E phase $\xleftrightarrow[100.8]{105.9}$ SmC	
3	—	—	Crystal $\xleftrightarrow[58.6]{73.5}$ Isotropic	
4	5.32	NA ^c	Crystal $\xleftrightarrow[129.6]{136.9}$ SmC + E phase $\xleftrightarrow[173.2]{190.3}$ SmC	
5	5.37	58.7	Crystal $\xleftrightarrow[89.0]{89.6}$ SmC + E phase $\xleftrightarrow[116.2]{114.4}$ SmC	
6	—	—	Crystal $\xleftrightarrow[80.1]{86.5}$ Isotropic	

^a The entry number is the same as in Table 1; ^b Smectic C phase was determined by 1D/2D WAXS experiments;

^c The tilt angle was not available due to poor flow ability of the samples above respective T_{mS} ; ^d Phase transition of each sample was determined by combination of DSC, POM and WAXS results.

The crystalline and liquid crystalline structures were studied by variable-temperature WAXS. PPLG_{*n*}-g-BPOEG₂ samples with short side-chain length (*i.e.*, *m* = 2) exhibited two sets of scattering peaks (Fig. 5 and S11). For example, in the low-angle region (< 5°), the scattering peaks of PPLG₂₆-g-BPOEG₂ were found to possess a periodicity of 1, 2, 3 and 4 ($q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ is the X-ray wavelength), indicating a lamellar structure with a *d*-spacing ($d = 2\pi/q$) of 5.07 nm (Fig. 5c). The scattering intensity increased and the *d*-spacing decreased as increasing the temperature, indicating a promoted ordering. In the high-angle region (> 15°), the scattering peaks showed the scattering peaks at $2\theta = 19.48^\circ$, 21.82° and 27.18° ascribing to the crystal E phase^[33, 34]. When the temperature was above the *T_g* (131.1 °C for PPLG₂₆-g-BPOEG₂ and 136.9 °C for PPLG₆₃-g-BPOEG₂) and below the *T_m* (178.1 °C for PPLG₂₆-g-BPOEG₂ and 190.3 °C for PPLG₆₃-g-BPOEG₂), the scattering peaks at low-/high-angle regions were retained, indicating heterogeneous phases comprising of liquid crystalline Smectic C (SmC) phase^[35, 36] and crystal E phase (Table 2, entry 1 and 4). The phase transitions of PPLG_{*n*}-g-BPOEG₂ sample exhibited no isotropic phase below respective decomposition temperatures. The full width at half-height (fwhh) was used to investigate the phase transition of the crystal E phase of PPLG₆₃-g-BPOEG₂ (Fig. S12). The fwhh of scattering peak at $2\theta = 19.48^\circ$ decreased from 2.85° to 2.00° in the temperature range of 30–160 °C, indicating a promoted side-chain ordering due to the increase of molecular mobility above *T_g* (136.9 °C). The fwhh started to increase at ~170 °C corresponding to the melting transition temperature observed by DSC, suggesting the melting transition of the crystal E-phase. The fwhh continued to increase until at 190–200 °C corresponding to the second melting transition temperature of the crystal E-phase (Table 2, entry 1 and 4). This was consistent with the DSC results that PPLG_{*n*}-g-BPOEG₂ with short OEG tail length (*m* = 2) likely processed crystalline polymorphism of E-phase.

PPLG_{*n*}-g-BPOEG₃ samples with medium OEG tail length (*m* = 3) showed molecular packing (Fig. S13 and S14) and phase transitions (Table 2, entry 2 and 5) similar to those of PPLG_{*n*}-g-BPOEG₂. Yet, the higher ordered scattering peak in the low-angle region is fewer and the scattering intensity in the high-angle region is weaker, indicating a less ordered molecular packing due to the increased flexibility of OEG tails and decreased rigidity of the polypeptides. Nevertheless, PPLG_{*n*}-g-BPOEG₇ samples with longer OEG tail length (*m* = 7) exhibited ill-defined scattering peaks in the high-angle region corresponding to a spherulite crystal (Fig. S15). The absence of liquid crystalline phase of these samples suggested disordered molecular packing by increasing the OEG tail length. The WAXS diffractogram of PPLG_{*n*}-g-BPOEG₇ became featureless at high temperatures (*i.e.*, 73.5 °C for PPLG₂₆-g-BPOEG₇ and 86.5 °C for PPLG₆₃-g-BPOEG₇) due to the melting transition of the spherulite crystal (Table 2, entry 3 and 6). These results suggest that long main-chain length and short OEG tail length are essential to form liquid crystalline phase of polypeptides.

Two dimensional wide-angle X-ray scattering (2D WAXS) was used to further identify the phase structures of PPLG-g-BPOEGs. The samples were mechanically sheared at a proper temperature above the *T_m*s and then quenched to room temperature. Specifically, the shearing temperatures for PPLG₂₆-g-BPOEG₃ and PPLG₆₃-g-BPOEG₃ are 170 and 180 °C, respectively. The samples with OEG₂ tails showed poor flow ability above respective *T_m*s and were not able to be sheared. In the 2D WAXS patterns (Figs. S16 and S17), the shearing direction was along the meridian and normal to the incident X-ray beam. The scattering arcs were ill-defined because that the polymers were too rigid to flow well above respective *T_m*s, which led to poor orientation. Yet, the azimuthal integrity of the scattering halos at $2\theta = 3.29^\circ$ – 3.41° and 21.86° – 21.87° revealed the orientation of the main-chain and mesogenic side-chains. Two scattering arcs in the low-angle region were concentrated on the meridian ($\chi = 90^\circ$ and 270°) while four scattering arcs in the high-angle region are mainly located at $\chi = 36.8^\circ$, 139.2° , 210.4° and 331.1° for PPLG₂₆-g-BPOEG₃ (Fig. S16c) and at $\chi = 31.3^\circ$, 141.6° , 208.7° , and 329.3° for PPLG₆₃-g-BPOEG₃ (Fig. S17c), suggesting the smectic C phases of PPLG₂₆-g-BPOEG₃ and PPLG₆₃-g-BPOEG₃ with tilt angles^[35] of 53.2° and 58.7° (Table 2, entry 2 and 5), respectively.

CONCLUSIONS

We have demonstrated that polypeptides bearing biphenyl mesogens and oligo-ethylene-glycol tails (*i.e.*, PPLG_{*n*}-g-BPOEG_{*m*}s, *n* = 26 and 63, *m* = 2, 3, and 7) can be facily synthesized by a 1,3-dipolar cycloaddition with quantitative grafting density. FTIR analysis revealed that the polymers adopted only α -helical conformation in the temperature range of 25–200 °C. DSC, POM and WAXS results indicated that the phase behaviors of PPLG_{*n*}-g-BPOEG_{*m*} samples strongly depended on the main-chain and OEG tail lengths. Samples with short OEG tail length (*m* ≤ 3) exhibited a phase transition sequence of crystal ↔ smectic C (SmC) + E-phase ↔ SmC, while samples with longer OEG tail length (*m* = 7) possessed a phase transition of crystal ↔ isotropic. The phase behaviors of the resulting polypeptides will shed some light on future molecular design of crystalline and liquid crystalline polymers.

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¹H-NMR spectra of Pr-BPOEG_{3/7}, PPLG₂₆-g-BPOEG_{3/7} (Figs. S1–S2, S4–S5), GPC data (Fig. S3), variable-temperature FTIR spectra of PPLG₂₆-g-BPOEG₇ (Fig. S6), POM images of Pr-BPOEG_{*m*} (Fig. S7), PPLG₂₆-g-BPOEG₃ (Fig. S10), and PPLG₂₆-g-BPOEG₇ (Fig. S9) at different temperatures, TGA thermograms of PPLG₂₆-g-BPOEG_{*m*} (Fig. S8), variable-temperature 1D WAXS data of PPLG₂₆-g-BPOEG_{3/7} (Figs. S13 and S15), PPLG₆₃-g-BPOEG_{2/3} (Figs. S11 and S14), temperature dependence of d-spacing and fwhh for PPLG₆₃-g-BPOEG₂ (Fig. S12), 2D WAXS patterns and azimuthal scan data of PPLG_{26/63}-g-BPOEG₃ (Figs. S16 and S17). DSC results of Pr-BPOEG samples (Table S1).

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