Supramolecular Inclusion Complexes of a Star Polymer Containing Cholesterol End-Capped Poly(ε-caprolactone) Arms with β-Cyclodextrin

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ABSTRACT: A novel hexa-armed and star-shaped polymer containing cholesterol end-capped poly(ε-caprolactone) arms emanating from a phosphazene core (N3P3-(PCL-Chol)6) was synthesized by a combination of ring-opening polymerization and “click” chemistry techniques. For this purpose, the terminal −OH groups of the synthesized precursor (N3P3-(PCL-OH)6) were converted into −Chol through a series of reaction. Both N3P3-(PCL-OH)6 and N3P3-(PCL-Chol)6 were then employed in the preparation of supramolecular inclusion complexes (ICs) with β-cyclodextrin (β-CD). The latter formed ICs with β-CD in higher yield. The host–guest stoichiometry (ε-CL:β-CD, mol:mol) in the ICs of N3P3-(PCL-Chol)6 was found to be 1.2. The formation of supramolecular ICs of N3P3-(PCL-Chol)6 with β-CD was confirmed by using Fourier transform infrared (FTIR) and 1H nuclear magnetic resonance (NMR) spectroscopic methods, wide-angle X-ray diffraction (WAXD), and thermal analysis techniques. WAXD data showed that the obtained ICs with N3P3-(PCL-Chol)6 had a channel-type crystalline structure, indicating the suppression of the original crystallization of N3P3-(PCL-Chol)6 in β-CD cavities. Moreover, the thermal stabilities of ICs were found to be higher than those of the free star polymer and β-CD. Furthermore, the surface properties of N3P3-(PCL-Chol)6 and its ICs with β-CD were investigated by static contact angle measurements. The obtained results proved that the wettability of N3P3-(PCL-Chol)6 successfully increased with the formation of its ICs with β-CD.

KEYWORDS: cholesterol; click chemistry; β-cyclodextrin; ε-caprolactone; inclusion chemistry; inclusion complex; star polymers; supramolecular structures

INTRODUCTION Supramolecular inclusion complexes (ICs) formed between cyclodextrins (CDs) and polymers have attracted much attention because of their potential biomedical applications in drug delivery systems and tissue engineering.1–5 CDs are water-soluble macrocyclic oligosaccharides, which are composed of six (α-CD), seven (β-CD), eight (γ-CD) glucose units connected by α-1,4 bondings.6–10 The peculiar geometric structure of these compounds resembles a hollow truncated cone and the presence of hydroxyl groups only on the outer surface of CD molecules provide a hydrophobic internal cavity and hydrophilic outer surface.2,11,12 Their geometries give a hydrophobic cavity having a depth of about 8.0 Å, and an internal diameter of about 4.5 Å for α-, about 7.0 Å for β-, and about 8.5 Å for γ-CD.6,13,14 CDs have the feature of forming ICs with various hydrophobic guest molecules with suitable polarity and dimension (e.g., adamantane, cholesterol) in their inner cavities and thus, enhance the solubility of these molecules in aqueous media.15 Therefore, they can be employed in pharmaceutical industry to enhance solubility, chemical stability, and bioavailability of poorly soluble drugs, to reduce toxicity and to control the rate of release.5,16–18 Besides, these macrocycles have been used in analytical sciences,13 material science,19 separation technology,20 catalysis,21 cosmetic,22 textile,23 food,9,24 and packaging industry.2,25

Complexation studies of CDs with polymers started with the work by Harada and Kamachi and they reported that α-CD gave stoichiometric ICs with poly(ethylene glycol) (PEG) of various molecular weights.26 Since CDs are biocompatible, biodegradable, water-soluble, and environmentally safe compounds, their ICs with biodegradable polyesters have extensively been investigated by many research groups. Harada and coworkers investigated ICs of CDs with linear aliphatic polyesters, such as poly(ε-caprolactone) (PCL), poly(ethylene adipate) (PEA), poly(trimethyleneadipate) (PTA), and poly(1,4-butylene adipate) (PBA).27–29 Tonelli and coworkers reported that the formation of CD-ICs reduced phase separation in immiscible polymer blends, like poly(ε-caprolactone)/...
and fluidity. This ability of cholesterol arises from the affinity and its ability to change the membrane’s permeability.

Cholesterol is one of the most important sterols in the membrane of eukaryotic cells because of its high thermodynamic affinity and its ability to change the membrane’s permeability and fluidity. This ability of cholesterol arises from the rigidity of the steroid ring system and plays an important role in self-association of molecules in biological systems. Even though the contents of cholesterol in polymers are very low, it has a strong tendency for self-association. As these properties are important for cell attachment and proliferation, the cholesterol end-capped oligomers/polymers are expected to have promising applications in drug delivery systems and tissue engineering scaffolds.

Recently, cholesterol end-capped star polymers were synthesized to form supramolecular structures via complexation with CDs forming ICs. In addition, cholesterol has unique features such as chirality, amphiphilicity, liquid crystallinity, and biocompatibility. Cholesterol has the ability to form ICs with CDs. Thus, cholesterol end-capped oligomers/polymers have been studied to form supramolecular structures via complexation with CDs forming ICs.

Recently, cholesterol end-capped star polymers were synthesized to form supramolecular structures via complexation with CDs. Setijadi et al. prepared ICs of cholesterol end-capped three-armed star-shaped poly(polyethylene glycol) acrylate (polyPEG-A) with β-CD. In another study, star shaped PEG with cholesterol end-groups and star-shaped PEG with β-cyclodextrin end-groups (β-CD) are prepared in order to obtain hydrogels via the ICs of cholesterol/β-CD. Nevertheless, there is no publication in the literature which reports ICs formed between β-CDs and cholesterol end-capped PCL star-like architectures.

Phosphazenes, having [N=PR2]- repeating units, are cyclic, linear short-chain or high molecular weight polymer compounds and constitute a very important compound family in heteroatom chemistry. The halogen “R” groups can easily be replaced by alkoy, aryloxy, and amino groups via nucleophilic reactions. Depending on the nature of the substituent moieties, phosphazene compounds can be tailored to possess a wide variety of physical and chemical properties, such as flame retardancy, super hydrophobicity, ion conductivity, biocompatibility, and so on. Besides, they exhibit high thermal and chemical stabilities. Therefore, cyclophosphazenes with proper functional groups were employed as initiators or starting compounds in the preparation of dendrimers, star, and cyclomatrix polymers.

With these backgrounds, we report the synthesis of a novel hexa-armed and cholesterol (Chol) end-capped star-shaped PCL with phosphazene core (N3P3-(PCL-Chol)6) (P4) by a combination of ring-opening polymerization (ROP) and “click” chemistry techniques with the motivation of investigating the effect of cholesterol end groups on the inclusion complexation behavior of the star polymer having six PCL arms (P4) with β-CD (Scheme 1). To the best of our knowledge, this is the first report on the synthesis of polypepsidoturaxanes composed of a star polymer having cholesterol end-capped PCL arms with β-CD.

The synthesis of linear PCL having cholesterol end groups at both ends (Chol-PCL-Chol) and cholesterol end-capped phosphazene compound (N3P3-Chol) (5) without PCL arms is also reported herein to investigate their complexation behaviors with β-CD (Schemes 2 and 3, respectively). The modified polymers, their ICs with β-CD, and the intermediates at various stages of synthesis were characterized using Fourier transform infrared (FTIR), 1H NMR, 31P NMR, and gel permeation chromatography (GPC) as well as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Wide-angle X-ray diffraction (WAXD). The surface properties of β-CD formed complexes with the cholesterol end capped structures (P4, L4, and 5) were investigated by static contact angle measurements.

Materials

β-Cyclodextrin (β-CD, Merck, ≥98%) was used without further purification. ε-caprolactone (ε-CL, Aldrich, 97%) was dried over calcium hydride (CaH2, Sigma-Aldrich, 95%), distilled at 97 °C under 10 mmHg and stored over dry 4 Å molecular sieves. Cholesteryl chloroformate (C26H42ClO2, Aldrich, 98%) was used as supplied. Sodium hydride (NaH, Merck, 60% in mineral oil) was washed with dry hexane just prior to use. Triethylamine (TEA, ≥99.5%) was obtained from Fluka, dried over CaH2 and stored over 3 Å molecular sieves. Dichloromethane (DCM, Merck, 99.8%) was dried over phosphorus pentoxide. Dimethylformamide (DMF, Merck, 99.8%) was both dried and stored over activated 4 Å molecular sieves. Ethanediol (Fluka, ≥99.5%), tin(II) 2-ethylhexanoate (Sn(Oct)2, Aldrich, 95%), 2-bromo-2-methylpropanoyl bromide (C6H13Br2O, Aldrich, 98%), sodium azide (NaN3, Sigma-Aldrich, 99.5%), propargyl alcohol (C3H5O, Merck), N,N,N′,N′-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%), copper(I) bromide (CuBr; Sigma-Aldrich, 98%), methanol (Merck, 99.9%), methane iodide (CH3I, Sigma-Aldrich, 99%), and 1-bromonaphthalene (Br-
Naphthalene, Sigma-Aldrich, 97%) were used as purchased without further purification. Azido functional Merrifield resin was prepared according to the literature. Measurements

$^1$H and $^{31}$P NMR spectra were obtained on a Varian INOVA 500 MHz (202 MHz for $^{31}$P) spectrometer at 25 °C using CDCl$_3$ and $d_6$-DMSO as the deuterated solvents. TMS was the internal reference for $^1$H NMR, while 85% H$_3$PO$_4$ was the internal reference for $^{31}$P NMR. Mass spectra measurements were obtained by Bruker MicroTOF LC-MS (electron spray ionization) and Bruker microflex LTMAIDI-TOF MS (nitrogen UV-laser ionization at 337 nm) spectrometers. Fourier-transform IR spectra were recorded on Perkin-Elmer Paragon 1000 spectrometer equipped with PIKE MIRacle Diamond ATR and the results were uncorrected. Average molecular weights and molecular weight distributions of the polymers were estimated on an Agilent GPC Instrument (Model 1100) consisting of a pump, a refractive index detector, and two Waters Styragel column (HR 5E) and using THF as eluent at a flow rate of 0.5 mL/min at 23 °C. Melting points ($T_m$) and crystallization temperatures ($T_c$) of the polymers were measured on Mettler Toledo DSC 822 calorimeter under nitrogen flow (10 mL/min). All the samples were first

**SCHEME 1** Synthesis of the star polymer containing cholesterol end-capped PCL arms and a phosphazene core (P4).
heated from $-25 \, ^\circ C$ to $100 \, ^\circ C$ with a heating rate of $10 \, ^\circ C$/min and kept isothermal at $100 \, ^\circ C$ for 5 min to erase the thermal history, then cooled to $-25 \, ^\circ C$ at $10 \, ^\circ C$/min, and finally heated to $100 \, ^\circ C$ at $10 \, ^\circ C$/min. TGA was performed on a Mettler Toledo TGA/SDTA 851 thermogravimetric analyzer from room temperature to $700 \, ^\circ C$ with a heating rate of $10 \, ^\circ C$/min under inert nitrogen atmosphere. X-ray diffraction (XRD) patterns of powder samples were obtained at room temperature on a Rigaku D-MAX 2200 X-ray diffractometer using Cu Kα radiation with a wavelength of 1.54 Å over a 2θ range from 2° to 40° at a speed of 0.1° min$^{-1}$. The voltage and the current were set to 40 kV and 40 mA, respectively. KSV-CAM 200 contact angle meter apparatus was used to measure the static contact angles of the liquids (water, methylene iodide, and 1-bromonaphthalene) under air at room temperature. Equilibrium ($\theta_e$) contact angles of

SCHEME 2 Synthesis of $\alpha,\omega$-cholesterol end-capped linear PCL (L4).
these liquids were measured by using 3 μL droplet volumes to neglect the gravity flattening effect. Contact angle measurements were taken over three different areas for each polymer sample. Average and standard deviation of θ values were calculated as less than ±2.

**Synthesis of Cholesteryl 2-Propyn-1-yl Carbonate (1)**

Cholesteryl 2-propyn-1-yl carbonate (1) was synthesized according to the literature method with minor modifications.68 Prop-2-yn-1-ol (0.30 g, 5.35 mmol) was dissolved in dry DCM (20 mL) in a three-necked flask equipped with a pressure-equalized dropping funnel under inert argon atmosphere. After the addition of TEA (0.90 g, 8.89 mmol), the reaction mixture was cooled to −15 °C by means of ice–NaCl mixture. Then, cholesterylchloroformate (2.0 g, 4.45 mmol) was dissolved in dry DCM (10 mL) and added dropwise to the mixture within 30 min. The reaction mixture was stirred for additional 48 h at room temperature. It was then diluted with DCM and washed with saturated NaHCO3 solution (2 × 25 mL) and deionized water (2 × 25 mL). After the organic layers were combined and dried with MgSO4, the solvent was removed via rotary evaporator. The crude product was purified by column chromatography over silica using dichloromethane as the eluent. Yield: 1.64 g (79%). m.p.: 72–75 °C. MS (m/z): calcd for C31H48O3, 468.71; found, 468.31 [M+K]+. FTIR (ATR, cm−1): 3299 and 3259 (B–C–A–H); 2131 (A–C–B–C–A); 2936 and 2876 (C–A–H), 1742, 1471, 1367, 1294, 1240, 1162, 1047, 959, 840, 749, 733. 1H NMR (500 MHz, CDCl3, δ, ppm): 0.60–2.35 (overlapping multiplets, 43H),
2.41 (s, 1H, −C≡CH), 4.44 (m, 1H, −COOC<), 4.65 (s, 2H, −CH₂O–), 5.32 (d, 1H, >C=CH—).

Synthesis of Hexa-Armed Star Polymer Containing Cholesterol End-Capped PCL Arms (N₃P₃-(PCL-Chol)₆) (P₄)

P₁, P₂, and P₃ were synthesized according to the literature method.⁶₅ P₃ (0.30 g, 0.02 mmol) and cholesteryl 2-propyn-1-yl carbonate (1) (0.12 g, 0.26 mmol) were dissolved in degassed DMF (10 mL) under inert argon atmosphere. PMDETA (0.06 g, 0.35 mmol) was added and the solution was gently purged with argon for 10 min. Then, copper (I) bromide (0.05 g, 0.35 mmol) was added to the reaction mixture, and it was again degassed by purging argon for additional 5 min. The reaction mixture was stirred at room temperature for 48 h. After the solution was diluted to 150 mL with DCM, it was washed successively with brine (2 × 50 mL) and water (50 mL). Subsequent to collecting and drying of organic phases with MgSO₄, the solvent was concentrated to 5 mL by rotary evaporator. Then, P₄ was isolated as a white product by precipitating from cold hexane. Yield: 0.34 g (95%).

FTIR (ATR, cm⁻¹): 2946 and 2866 (C–H); 1722 (O=C); 1470 (C–H); 1239 ((C≡O)=O); 1294 and 1178 (P=N); 1046 (C–O–C); 959 (P–O–C). ¹H NMR (500 MHz, CDCl₃, δ ppm): 7.79 (d, C₂H₃N₃ (triazole ring); 7.18, 7.16, and 6.91, 6.90 (AA'BB' pattern, C₆H₄); 5.38 (d, 1H, >C=CH— in cholesterol); 5.28 (s, 2H, −CH₂O–); 5.05 (s, C₆H₄CH₂O); 4.37 (m, 1H, −COOC< in cholesterol); 4.13 (m, terminal CH₂O(C=O)CH₂); 4.05 (m, CH₂O(C=O) in PCL); 2.28 (m, O(C=O)CH₂ in PCL); 1.93 (m, terminal O(C=O)CH₂); 1.65 (m, CH₂O(C=O)CH₂); 1.4 (m, CH₂O(C=O) in PCL); 1.38 (m, O(C=O)CH₂).

The linear PCL with cholesterol groups at both ends (Chol-PCL-Chol) (L₄) and cholesterol end-functional phosphazene compound (N₃P₃-(Chol)₆) (5) were synthesized via “click chemistry” technique as shown in Schemes 2 and 3, respectively. They were also employed in the preparation of supramolecular ICs with β-CD (see the Supporting Information for experimental details).

Preparation of Inclusion Complexes of N₃P₃-(PCL-Chol)₆ with β-CD (P₄-β-CD)

P₄ (0.25 g, 0.01 mmol) was dissolved in 20 mL of acetone and β-CD (2.12 g, 1.87 mmol) was dissolved in 15 mL of distilled water. Then, P₄ solution was added dropwise to the
\( \beta \)-CD solution at 60 °C with vigorous stirring. After stirring at 60 °C for 3 h, the heat bath was removed and the mixture was left stirring at room temperature overnight. The precipitates were collected by filtration, washed with acetone (2 × 15 mL) and distilled water (2 × 15 mL) to remove free polymers and uncomplexed \( \beta \)-CD, respectively. The obtained white powder product was dried in vacuo at 45 °C until a constant weight. The structures of \( \beta \)-CD formed complexes with P4 are depicted in Scheme 4. Yield: 1.59 g (63.5%). ¹H NMR (500 MHz, DMSO-\( d_6 \), \( d \), ppm): 5.74 (d, 8H, O(7)H of \( \beta \)-CD); 5.68 (d, 8H, O(8)H of \( \beta \)-CD); 4.83 (d, 8H, C(1)H of \( \beta \)-CD); 4.48 (t, 8H, O(9)H of \( \beta \)-CD); 3.98 (m, C(1)H of \( \beta \)-CD); 3.74–3.26 (m, 48H, C(5)H, C(6)H, C(3)H, C(2)H and C(4)H of \( \beta \)-CD); 2.27 (m, O(C(=O)CH_{2}CH_{2}CH_{2}CH_{2}O(C=O) in PCL); 0.86 (m, O(C(=O)CH_{2}CH_{2}CH_{2}CH_{2}O(C=O) in PCL).

The same procedure was employed for the preparation of ICs of N3P3-(PCL-OH)6 with \( \beta \)-CD (P1-\( \beta \)-CD). Yield: 1.52 g (56.7%). ¹H NMR (500 MHz, DMSO-\( d_6 \), \( d \), ppm): 5.72 (d, 8H, O(7)H of \( \beta \)-CD); 5.67 (d, 8H, O(8)H of \( \beta \)-CD); 4.82 (d, 8H, C(1)H of \( \beta \)-CD); 4.47 (t, 8H, O(9)H of \( \beta \)-CD); 3.98 (m, C(1)H of \( \beta \)-CD); 3.74–3.26 (m, 48H, C(5)H, C(6)H, C(3)H, C(2)H and C(4)H of \( \beta \)-CD); 2.27 (m, O(C(=O)CH_{2}CH_{2}CH_{2}CH_{2}O(C=O) in PCL; 0.86 (m, O(C(=O)CH_{2}CH_{2}CH_{2}CH_{2}O(C=O) in PCL.

Preparation of Physical Blend of N3P3-(PCL-Chol)6 with \( \beta \)-CD (P4/\( \beta \)-CD)

The physical blend of N3P3-(PCL-Chol)6 with \( \beta \)-CD (P4/\( \beta \)-CD), were prepared by using the same weight ratio as related to ICs. P4 was mixed with \( \beta \)-CD and pulverized in a

\[ M_n, \text{NMR} \] was determined by ¹H NMR spectroscopy.
\[ M_n, \text{GPC} \] and \( M_w/M_n \) were determined by GPC analysis with polystyrene standards. THF was used as eluent (RI detector).

\[ T = 115 \degree \text{C}; \text{bulk}; 49/\text{[SnOct}_2] = 300; \text{polymerization time} = 24 \text{h}. \]

\[ M_{\text{NMR}} \] was determined by \( M_{\text{NMR}} = \text{M} \cdot 115 \degree \text{C}. \]

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The chemical structure of the cholesterol end-functional star PCL polymer (P4) was verified by FTIR and $^1$H NMR spectroscopic techniques andGPC analysis. Figure 1 shows the FTIR spectra of azide and cholesterol end-capped star-shaped PCL polymers (P3 and P4, respectively). Upon azidification of the precursor bromide functional star polymer (P2), the peak emerged at 2110 cm$^{-1}$ indicates the presence of azide functional groups in the structure of P3 [Fig. 1(a)]. The disappearance of azide peak in the FTIR spectrum of P4 clearly confirms the completeness of the click reaction [Fig. 1(b)]. Also, the strong and sharp peak at 1723 cm$^{-1}$ in FTIR spectra of both P3 and P4 is attributed to the carbonyl groups in the repeating units of the PCL chains.

In the $^1$H NMR characterization, the comparison of the $^1$H NMR spectrum of P3 [Fig. 2(a)] with that of P4 [Fig. 2(b)] also supports the complete attachment of cholesterol groups after “click” reaction. The peak (H$_i$) depicted at 1.47 ppm in the $^1$H NMR spectrum of P3 [Fig. 2(a)] shifted to the lower field by 0.46 ppm on Cu(I) catalyzed click reaction [Fig. 2(b)]. The methyl protons of the terminal 2-methylpropanoyl group was deshielded by the triazole and cholesterol groups and therefore shifted to the lower magnetic fields. Beside this, some new peaks also appeared in the $^1$H NMR spectrum of P4. The methylene protons next to the triazole ring (5.27 ppm, H$_i$), methine proton in the triazole ring (7.68 ppm, H$_m$), and finally cholesterol protons (5.44 ppm (6-H, d), 4.37 ppm (3z-H, m), and 0.65–2.46 ppm) came out as a result of the click reaction. Moreover, the experimental and theoretical integral ratios between the proton signal of the methylene group adjacent to the benzene ring (H$_i$) and the cholesterol proton (H$_m$) are very close to each other (H$_i$/H$_m$, experimental = 1.94, the theoretical value should be 2.0), indicating the complete and successful end functionalization of PCL arms with cholesterol moiety.

The number- and weight-average molecular weights of the cholesterol end-functional linear and star-shaped PCLs as well as its polydispersity index values were determined by GPC and related data are summarized in Table 1. The $^1$H NMR spectra of the polymers enabled us to calculate the composition and $M_{n,NMR}$ values as well. The average number of $\varepsilon$-CL units per branch of P4 was calculated to be 20, using the ratio of the integral value of methylene protons in PCL chains (—CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$O(C=O)—) to that in the initiator (—C$_6$H$_5$CH$_2$O—). Thus, the number-average molecular weight of P4 was determined using the following formula:

$$M_{n,NMR} = a \times 20 (\text{number of } \varepsilon-\text{CL in PCL chains}) \times 114.14 + M_w \text{ of the initiator} + a \times M_w \text{ of chain end group, where } a = 6 (a = 2 \text{ for L4). The discrepancies between } M_{n,GPC} \text{ and } M_{n,NMR} \text{ values are due to hydrodynamic volume differences between the synthesized PCL polymers and polystyrene standards with the same molecular weight.}$

Inclusion Complexes of P4 with $\beta$-CD (P4-$\beta$-CD)

The key parameters affecting the stability of ICs are intermolecular interactions and size compatibility between host and guest molecules. Internal diameter of the $\beta$-CD molecules do not closely fit external diameter of PCL chains as in the case of $\varepsilon$-CDs, resulting in weaker interactions between $\beta$-CD molecules and PCL chains. This was reflected by the complexation ratios, in that, $\varepsilon$-CL:$\beta$-CD ratio reported in the literature$^{27}$ was around 1.5, considerably higher than $\varepsilon$-CL:$\varepsilon$-CD$^{77,28}$ (close to 1.0). In fact, these ratios were observed notably higher for star-shaped polymers due to steric effects induced by increasing number of arms around core unit.$^{36,39}$

RESULTS AND DISCUSSION

Synthesis of Hexa-Armed Star Polymer Containing Cholesterol End-Capped PCL Arms (P4)

The hexa-armed star polymer containing cholesterol end-capped PCL arms (P4) was synthesized following four-step synthetic procedures using “core-first” methodology as depicted in Scheme 1. In the first step, the hydroxyl end-groups blend. This was succeeded by taking the advantage of the Cu(I) salt catalyzed 1,3-dipolar cycloaddition reaction between azide groups of the polymers and ethyne group of the precursor bromide functional star polymer (P2). The synthetic importance of the Cu(I) salt catalyzed “click chemistry” stems from its versatility, reliability, and specificity. Moreover, this method hardly demands functional group protection and has an exceptional selectivity for 1,4-triazole formation.$^{69–72}$
It is known from the literature that cholesterol molecules can be threaded by 2 or 3 β-CD; whereas, monomer units of PCL have a tendency to form 1:1 complex with β-CD. Therefore, this motivated us to prepare cholesterol end-capped star-shaped PCL polymer (P4) and to investigate to what extent cholesterol end-groups would hold together β-CD molecules threaded on the PCL arms and thus would decrease e-CL:β-CD ratio in IC of the star polymer with β-CD. The star polymer with OH end-functional groups (P1) was also used in the preparation of supramolecular ICs with β-CD in order to investigate the effect of cholesterol-end group on the complexation yield. The complexation behavior of the synthesized cholesterol end-capped polymers with β-CD was thoroughly characterized in detail by means of 1H NMR, FTIR, DSC, TGA, and WAXD. ICs of P4 with β-CD were successfully prepared by adding dropwise polymer solutions in acetone into aqueous β-CD solution, followed by rigorous stirring.

FTIR is a very useful tool to obtain the evidence for the inclusion complexation. It can give information about the formation of ICs and confirm the existence of both host and guest components in ICs. Figure 3 shows the FTIR spectra of P1-β-CD and P4-β-CD together with pure β-CD. The broad signal at 3311 cm\(^{-1}\) in the spectrum of β-CD [Fig. 3(a)] is due to symmetric and asymmetric OH stretching bands. The other notable signals are observed at 1155 cm\(^{-1}\) (C\(\equiv\)O\(\equiv\)C), 1078 cm\(^{-1}\) (C\(\equiv\)C) and 1025 cm\(^{-1}\) (C\(\equiv\)O). Comparing the FTIR spectrum of P4-β-CD with the spectra of its components (the physical blend of P4 and β-CD), almost all bands for P4-β-CD can be related to its individual components with slight up-shifting and decrease in the intensity of carboxyl.
group; no additional bands are observed [Fig. 3(b)]. It reflects that β-CD molecules were threaded onto the branch chains of P4 in the ICs, as shown in Scheme 4. Additionally, the shift of the bands in P4-β-CD IC could be connected to hydrogen bonds formation, which mainly took place between the β-CD hydroxyl groups and the carbonyl groups in the guest PCL polymers, as well as between β-CD hydroxyl groups threaded onto neighboring chains of the PCL polymers.34,39 Moreover, the intensity of carbonyl signal in the FTIR spectra of P4-β-CD IC [Fig. 3(c)] is more suppressed than that in the FTIR spectra of P1-β-CD IC, indicating that complexation yield is higher in the former.

Further evidence for the inclusion complexations of P1 and P4 with β-CD was carried out by 1H NMR measurement. 1H NMR spectra of P1-β-CD and P4-β-CD is given in Figure 4(a,b), respectively. The host–guest stoichiometry of P1-β-CD was determined by integral ratio of CH proton (H1) signal of β-CD to that of methylene protons (Hg) signals in the ε-CL repeating units of P1. The stoichiometric ratio of ε-CL to β-CD for P1-β-CD was 1.5. Due to the steric hindrance, a few ε-CL units close to the phosphazene core might not have been included by β-CD molecules, resulting in increased ε-CL:β-CD ratio as reported in the literature.26 As for the β-CD ICs of cholesterol-containing star-shaped polymers (P4-β-CD),

### TABLE 2 Type of ICs of the PCLs with CD

<table>
<thead>
<tr>
<th>Polymer</th>
<th>CD</th>
<th>Yield (wt %)</th>
<th>ε-CL:β-CD (mol:mol)</th>
<th>Mₙ,GPC</th>
<th>References</th>
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<td>Chol-(CL)ₐ</td>
<td>α-CD</td>
<td>70</td>
<td>1.49</td>
<td>3,040</td>
<td>Guo et al.⁵²</td>
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<td>≈1.0</td>
<td>3,040</td>
<td>Guo et al.⁵³</td>
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<td>1</td>
<td>830</td>
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<td>530</td>
<td>Kawaguchi et al.²⁷</td>
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<td>1.58</td>
<td>1,200</td>
<td>Dai et al.⁶⁶</td>
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<tr>
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<td>1.02</td>
<td>1,200</td>
<td>Chan et al.⁶⁶</td>
</tr>
<tr>
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<td>γ-CD</td>
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<td>2.2</td>
<td>7,800</td>
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* The number-average molecular weights (Mₙ) of a single arm were characterized by ¹H NMR spectroscopic functional groups analysis.

![FIGURE 5 3¹P NMR spectra of (a) P4 and (b) P4-β-CD.](image-url)
CD), $\epsilon$-CL:β-CD ratio was found to be 1.2. This is less than the $\epsilon$-CL:CD values reported in the literature for the CD ICs of 8-, 6-, and even 4-armed star-shaped polymers with the close molecular weight. The decreased branch chain density can be induced by the increasing polymer molecular weight or the increasing branch arm length. It is known from the literature that, the internal diameter of α-CDs fits well to the cross-sectional diameter of PCLs, whereas, the

<table>
<thead>
<tr>
<th>Entry</th>
<th>Yield (wt %)</th>
<th>$\epsilon$-CL:β-CD (mol/mol)</th>
<th>$T_{d,free}$ (°C)$^b$</th>
<th>$T_{d,ICs}$ (°C)$^c$</th>
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<td>β-CD</td>
<td>PCL Polymers</td>
</tr>
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<td>1.5</td>
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<td>371.9</td>
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<tr>
<td>P4-β-CD</td>
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<td>1.2</td>
<td>307.4</td>
<td>289.7</td>
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<tr>
<td>L1-β-CD</td>
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<td>1.3</td>
<td>307.4</td>
<td>278.4</td>
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<tr>
<td>L4-β-CD</td>
<td>59</td>
<td>≈1</td>
<td>307.4</td>
<td>294.3</td>
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</table>

$^a$ The stoichiometry ($\epsilon$-CL:β-CD, mol/mol) of each monomeric repeating unit PCL/β-CD was determined by $^1$H NMR.

$^b$ $T_{d,free}$ denotes the initial decomposition temperature of free β-CD and cholesterol end-capped PCL polymers, respectively.

$^c$ $T_{d,ICs}$ denotes the initial decomposition temperature of the host β-CD and the guest cholesterol end-capped PCL polymers included in the ICs, respectively.

FIGURE 6 DSC thermograms of β-CD, L4, L4-β-CD, L4/β-CD physical blend, P4, P4-β-CD, and P4/β-CD physical blend in (a) the first heating run, (b) cooling run, and (c) second heating run. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
larger diameter of β-CDs brings about lower affinity and the twisting of PCL polymer to fill the internal cavity of β-CDs. Therefore, in general, less β-CD molecules can be threaded onto PCL chains than α-CDs do. In the present study, the bulky cholesterol end-units held together the β-CDs, which were threaded onto the PCL chains like the string of beads, due to close fitting of their outer radius to the internal diameters of β-CDs. Besides, each of the cholesterol end-groups can be encapsulated by one or two β-CD units, this phenomenon also makes contribution to low ε-CL:β-CD values in the corresponding ICs. It can be also said that these ratios approximately vary as the incorporated cholesteryl moiety can form stable inclusion complex with β-CD. The internal diameter of γ-CDs is the largest of all. Since γ-CDs can accommodate more than one PCL chain, they were kept out of the scope of this comparison.

The signals belonging to β-CD and cholesterol end-capped PCL star polymer can be clearly seen in 1H NMR spectrum in Figure 4(b). These results are in accordance with the literature findings on the CD ICs of PCLs, which are summarized in Table 2.

Moreover, the structure of the phosphazene core of P4 was determined by means of 31P NMR [Fig. 5(a)]. The appearance of only one signal at 8.30 ppm in the 31P NMR is interpreted as a reliable indication for P4 having phosphazene core.

Additionally, the absence of any signal in 31P NMR of P4-β-CD is a clear evidence for suppression of phosphorous signal after complexation process between P4 and β-CD [Fig. 5(b)]. In this study, the ICs of β-CD with hydroxyl and cholesterol end-capped linear PCL polymers (L1 and L4, respectively) together with cholesterol end-capped phosphazene derivative (5) were also prepared in order to compare their complexation behaviors with β-CD to that of P4-β-CD and to investigate the effect of cholesterol end units on complexation behavior (see the Supporting Information for experimental and characterization details of L1, L4, 5, and their ICs with β-CD). The host–guest stoichiometries (ε-CL:β-CD) in L1-β-CD and L4-β-CD ICs were determined using the same method as in the case of P4-β-CD IC. Since steric hinderence effects seen in IC of the star polymers (P1-β-CD and P4-β-CD) were not effective to that of linear polymers (L1-β-CD and L4-β-CD), the host–guest stoichiometries in the complexes of linear polymers were found to be lower than those of star polymers (see Table 3). Besides, ε-CL:β-CD ratio was considerably lower in the IC of cholesterol end-capped linear PCL polymer (L4-β-CD) than that of hydroxyl end capped linear polymer (L1-β-CD). DSC was used to determine the melting and crystallization behaviors of cholesterol functional P4, L4, and their ICs; the related thermograms are presented in Figure 6. The DSC curves of β-CD and the polymer/β-CD physical blends are also given for comparison. The melting peaks of P4 and L4 were observed at 52.5 and 53.2 °C, respectively, in the first heating run; on the contrary, the DSC plots of β-CD and the ICs did not exhibit any observable melting peaks [Fig. 6(a)]. In a similar way, in the cooling [Fig. 6(b)] and in the second heating runs [Fig. 6(c)], both the crystallization and the second melting peaks of P4 and L4 disappeared upon complexation with β-CD. These observations agreed with the literature and showed that the crystallization of the cholesterol end-capped star and linear polymers threaded by β-CD were totally inhibited. Besides, DSC thermograms of the physical blends were nearly identical to those of P4 and L4.

FIGURE 7 XRD patterns of (a) β-CD, (b) L4, (c) L4-β-CD, (d) L4/β-CD, (e) P4, (f) P4-β-CD, and (g) P4/β-CD physical blend. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 8 TGA thermograms of (a) β-CD, (b) L4, (c) L4-β-CD, (d) P4, and (e) P4-β-CD. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
The X-ray diffraction (XRD) is a strong method to clarify the crystalline structure of ICs in the solid state. Figure 7 showed XRD patterns of the uncomplexed polymers with significant peaks at $21.4^\circ$ and $23.7^\circ$ for P4 and at $21.5^\circ$ and $23.8^\circ$ for L4, which were consistent with those of linear PCL crystals located at $21.4^\circ$ and $23.8^\circ$. This indicates that cholesterol end-capped PCL polymers have crystalline structure similar to that of linear PCL in the solid state. For the ICs, major diffraction peaks were observed at $7.5^\circ$ and $38.4^\circ$ for P4-β-CD and at $6.9^\circ$, $39.9^\circ$, and $28.0^\circ$ for L4-β-CD, while the main crystalline peaks for the pure PCL polymers disappeared. The diffraction patterns of the ICs were quite different from those of their constituents and polymer/β-CD physical blends, pointing to the existence of different packing pattern and the formation of ICs between cholesterol end-capped PCL polymers and β-CD. Also, due to long chain nature of the cholesterol end-capped PCL polymer guests, their ICs with β-CD were thought to have a channel-type crystalline structure, which is a typical structure of ICs formed by the linear stack of β-CD rings on a polymer chain.

Thermal properties of P4, L4, and 5 and their ICs were evaluated by TGA technique from room temperature up to $700^\circ$C at a rate of $10^\circ$C min$^{-1}$ under nitrogen atmosphere and the related thermograms are depicted in Figure 8. As seen from the figure, thermal degradation of the ICs occurred in two steps, which were assigned to the decompositions of β-CD host molecules and threaded polymer chains, respectively, which is very similar to those of other CD-PCL ICs reported in the literature.40,52,53 Table 3 summarizes the preparation and the initial decomposition temperatures of all ICs, the free PCL polymers, and β-CD. According to these data, the host and guest constituents of ICs started to decompose at higher temperatures than their free states, which indicates that formation of ICs between free PCL polymers and β-CD enhanced the thermal stability of both the guest PCL polymers and the host β-CD and that the ICs were more thermally stable.

**Contact Angle**

The contact angle measurements of P4, L4, and 5 and their ICs with β-CD were conducted in the solid state using MeI$_2$ and BrNaphthalene as the test liquids and the static $\theta_s$ values are given in Figure 9. Also, the water contact angles were measured for P4, L4, and 5 as $104^\circ$, $89^\circ$, and $105^\circ$, respectively. But the water contact angles for both the cholesterol-functionalized phosphazene derivative (5) and PCL polymers (P4 and L4) ICs could not be measured.

**FIGURE 9** Equilibrium contact angle results of test liquids on free PCL polymers (P4 and L4), 5 and their ICs.
because of the rapid spreading of water on these surfaces. The films of the samples on glass slides were prepared using the sample solutions of 15 mg/mL and dried under vacuum at 45 °C. Equilibrium (θe) contact angles of MeL2 and BrNaphthalene were determined by static contact angle measurements using 3 μL droplet volumes. β-CD is a very hydrophilic material due to its OH groups. Therefore, the formation of the ICs of the cholesterol end-capped macromolecules with β-CD was expected to increase their polarity and hydrophilicity. As expected, complexation of the macromolecules with β-CD induced a significant reduction in θe values of MeL2 and BrNaphthalene as compared with their uncompressed states.

CONCLUSION

A novel star polymer containing cholesterol end-capped PCL arms emanating from a phosphazene core (P4) were synthesized via core-first approach and the complete attachment of bulky cholesterol groups at the arm-termini was achieved via Cu(I) catalyzed “click” reactions. P4 was then utilized to form supramolecular structures with β-CD. The linear PCL with cholesterol groups at both ends (L4) and cholesterol end-capped phosphazene compound (S) without PCL arms were also synthesized to compare their complexation behaviors with β-CD to that of P4. The stochiometry (c:CL: β-CD, mol:mol) of the ICs formed between cholesterol end-capped polymers with β-CD was found to be lower than that of polymers having no cholesterol functional end groups. The structures of the polymers were confirmed using FTIR, 1H NMR, 31P NMR, GPC, and WAXD. The hydrophilicity of the cholesterol functionalized polymers was improved via forming ICs with β-CD while decreasing the crystallinity of the PCL backbone. The melting and crystallization temperatures of P4 and L4 in the ICs couldn’t be observed in the DSC thermograms due to the presence of β-CD molecules threaded onto the branches of the polymers.

AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. The authors declare no competing financial interest.

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REFERENCES AND NOTES
