Association of Star-Shaped Poly(D,L-lactide)s Containing Nucleobase Multiple Hydrogen Bonding

Afia S. Karikari, Brian D. Mather, and Timothy E. Long*

Department of Chemistry, Macromolecules and Interfaces Institute, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

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A new family of associating polymers based on four-arm, star-shaped poly(D,L-lactide) (PDLLA) containing peripheral complementary hydrogen-bonding sites is described. Hydroxy-terminated, four-arm, star-shaped PDLLAs of controlled molar mass were functionalized with complementary DNA base pairs, adenine (A) and thymine (T), to obtain PDLLA−A and PDLLA−T, respectively. ^{1}H NMR spectroscopy confirmed quantitative functionalization and the subsequent formation of PDLLA−A and PDLLA−T hydrogen-bonded complexes. Job's analysis revealed a 1:1 optimal stoichiometry for the hydrogen-bonded complexes, and the association constant (K_a) that was determined using the ^{1}H NMR-based Benesi−Hildebrand treatment was 84 M^{-1} for the low molar mass complementary polymers. Furthermore, the PDLLA-based hydrogen-bonded complexes exhibited higher solution viscosities compared to the corresponding non-hydrogen-bonded precursors, which further confirmed strong complementary multiple-hydrogen-bonding associations between the star-shaped polymers with terminal adenine and thymine groups. Moreover, variable-temperature ^{1}H NMR studies demonstrated the thermoreversibility of the hydrogen-bonded PDLLA-based complexes in solution.

Introduction

The design of supramolecular polymers utilizing well-defined hydrogen-bonding interactions has received renewed attention. Multiple hydrogen bonding combines several hydrogen bonds in a single functional unit, and as a consequence, enhanced thermal stability compared to that of single-hydrogen-bonding systems is observed.[1] Multiple-hydrogen-bonding interactions, which are moderately strong and highly directional, lead to the synthesis of polymers with desirable properties such as thermoreversibility and responsiveness to external stimuli including pH, solvent polarity, temperature, and concentration.[1−3] Multiple-hydrogen-bonding scaffolds are typically classified as either self-complementary when hydrogen bonds form between identical hydrogen-bonding units or complementary when hydrogen bonds form between dissimilar yet complementary donor and acceptor units.[4]

Meijer and co-workers[5−8] and many other researchers[9−14] have demonstrated the versatility of multiple-hydrogen-bonding interactions in the preparation of polymers incorporating both self-complementary hydrogen-bonding and complementary hydrogen-bonding groups. Our research group has previously reported the synthesis of self-complementary and complementary multiple-hydrogen-bonding polymers based on polystyrene,[4,15−17] poly(alkyl acrylate)s,[18] poly(alkyl methacrylate) copolymers,[19−22] and star-shaped poly(isoprene)s[23] for various applications including electrospinning and surface modification. Our recent efforts include the introduction of multiple-hydrogen-bonding units to biodegradable polymers for use in biomedical technologies. Synthetic biopolymers with molecular recognition abilities offer intriguing possibilities due to potential interactions with diverse biological environments in a controlled manner.[24] Biomimetic polymers offer applications in molecular imprinting and patterning, biosensing, tissue engineering, and targeted drug/protein delivery. For example, Liu et al.[25] reported the synthesis of a temperature-sensitive poly(*N*-isopropylacrylamide-*co*-methacrylic acid) gel that imprinted L-pyroglutamic acid through multiple-hydrogen-bonding interactions.

Biocompatible and biodegradable polymers have received significant interest in applications such as surgical fixation devices, controlled drug delivery, and tissue engineering scaffolds.[26−28] However, reports on synthetic biocompatible and biodegradable polymers that contain nucleobase molecular recognition sites are more limited.[29−32] The utility of biocompatible and biodegradable polymers as supramolecular structures with molecular recognition ability offers the potential for advanced biopolymers with biomimetic properties. Biocompatible and biodegradable polymers containing multiple-hydrogen-bonding units are expected to exhibit high strength, toughness, and elasticity, low-temperature processability, favorable degradation, and biocompatibility.[33] Recently, van Hest and co-workers reported on the synthesis of adenine- and thymine-containing diblock copolymers using ATRP from PEG macroinitiators.[32] Meijer et al.[33] recently reported the synthesis of oligomeric poly(ϵ-caprolactone)s (PCLs) containing self-complementary quadruple-hydrogen-bonding 2-ureido-4[1*H*]-pyrimidinone (UPy) units. Mixing of the UPy-functionalized PCL with UPy-functionalized peptides resulted in bioactive supramolecular structures that exhibited strong and specific cell binding properties. In addition, Guan et al.[30] reported the synthesis of a modular polymer based on a double-closed-loop (DCL) peptidomimetic β-sheet module containing quadruple-hydrogen-bonding units. The DCL-based modular polymer showed more uniform sawtooth patterns than the UPy system[31] due to sequential unfolding of the modular polymer upon stretching.

In biological systems, complementary multiple-hydrogen-bonding interactions are paramount and the basic concept of molecular recognition occurs in adenine−thymine (A−T), adenine−uracil (A−U), and guanine−cytosine (G−C) base pairs

* To whom correspondence should be addressed. Phone: (540) 231-2480. Fax: (540) 231-8517. E-mail: telong@vt.edu.

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in DNA and RNA.[34] A wide variety of synthetic polymers were functionalized earlier with the complementary nucleobases adenine and thymine;[4,15,35,36] however, few reports describe the incorporation of adenine and thymine in biocompatible polymers.[37,38] Our research laboratories demonstrated earlier the synthesis of well-defined, four-arm, star-shaped poly(D,L-lactide) (PDLLA) oligomers of controlled molar mass and narrow molar mass distribution for use as potential biological adhesives.[39] Recently, we reported the synthesis of well-defined, four-arm, star-shaped PDLLAs as potential biological adhesives.[39] In this study, end group modification of the four-arm star PDLLA with cross-linkable methacrylic groups with or without urethane groups and the influence of hydrogen bonding on the mechanical properties of photo-cross-linked PDLLA networks were reported. Herein, we describe the first report of complementary multiple-hydrogen-bonding association of star-shaped PDLLA using DNA nucleobases. Heterocyclic base pairs were introduced to the termini of star-shaped PDLLA using a Michael addition strategy to obtain adenine- and thymine-functionalized star PDLLA (PDLLA−A and PDLLA−T, respectively).[4] Complex formation and structures were examined using ^{1}H NMR spectroscopy. The continuous variation method (Job's method),[40] which is widely utilized in determining the stoichiometry of associations, established the stoichiometry of the hydrogen-bonded PDLLA−A and PDLLA−T complexes in $CHCl_3$. The association constant, K_a, was determined using ^{1}H NMR titration, and solution rheological studies revealed supramolecular association through systematic changes in specific viscosity.

Experimental Section

Materials. Tetrahydrofuran (99.98%, EMD Chemicals) was distilled from sodium and benzophenone (99%, Aldrich) immediately prior to use. Acryolyl chloride (95%, Aldrich) was distilled under nitrogen immediately prior to use. Triethylamine (99.5%, Aldrich) was distilled over calcium hydride prior to use. Adenine (99%, Aldrich) and thymine (99%, Aldrich) were dried for 24 h under reduced pressure at 60 °C prior to use. Dimethyl sulfoxide (DMSO; >99.9+%, anhydrous, Aldrich) and potassium *tert*-butoxide (95%, Aldrich) were used without further purification.

Synthesis of Acrylated, Four-Arm, Star-Shaped PDLLA. The synthesis and characterization of hydroxyl-terminated, four-arm, star-shaped PDLLA was conducted in a fashion similar to that reported in our earlier paper.[39] Star-shaped PDLLA (20 g, 0.0019 mol) was weighed under nitrogen into a 500 mL flame-dried, septum-sealed, two-neck, round-bottomed flask with a magnetic stir bar. The flask was equipped with an addition funnel and placed in a water bath. Tetrahydrofuran (THF) (100 mL) was added under nitrogen to prepare an 18 wt % polymer solution. Triethylamine (TEA) (15-fold excess, 16 mL) in 100 mL of THF was added by syringe into the reaction mixture under nitrogen, and the mixture was cooled to 0 °C. Acryloyl chloride (15-fold excess, 9.2 mL), which was dissolved in 200 mL of THF and syringed into the addition funnel, was added dropwise to the reaction mixture under a continuous nitrogen flow. The reaction was allowed to proceed for 24 h at 23 °C. The mixture was filtered to remove triethylamine hydrochloride salt. THF was removed using rotary evaporation, and the product was redissolved in chloroform, precipitated repeatedly into a hexane/methanol (90:10, v/v) mixture, and dried under reduced pressure at 23 °C for 24 h. Quantitative functionalization was confirmed using ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.8−6.6 (3H, −OCC*H*═CH_2,), 5.1−5.3 (poly(D,L-lactide), 1H per repeating unit, −C*H*(CH_3)−), 4.1−4.4 (2H, −OCH_2CH_2O(PDLLA)), 3.5−3.8 (16H, ethylene oxide units, −OCH_2CH_2−), 3.4 (2H, CCH_2O−), 1.3−1.8 (poly(D,L-lactide), 3H per repeating unit, −CH(CH_3)−).

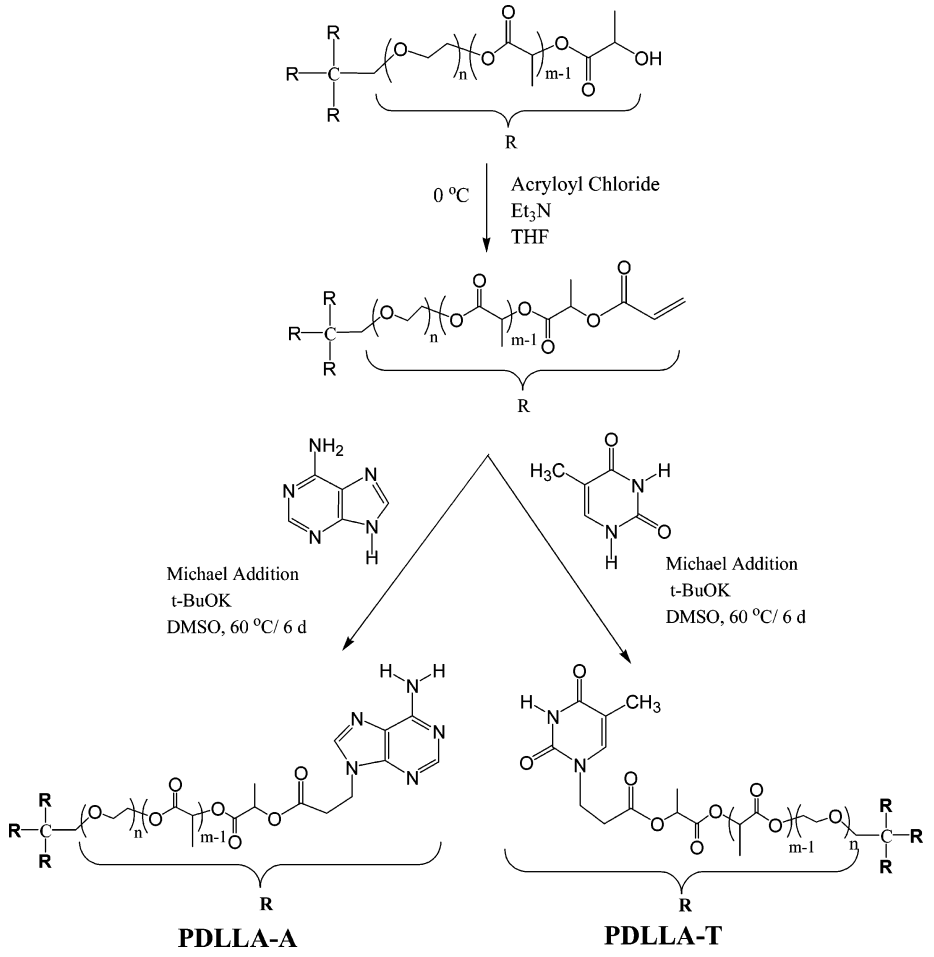
Synthesis of Adenine-Terminated, Four-Arm, Star-Shaped PDLLA (PDLLA−A). The acrylated star PDLLA (5.0 g) was added under nitrogen to a flame-dried, 50 mL round-bottomed flask containing a magnetic stir bar. Potassium *tert*-butoxide (10 mg) was added as a catalyst under nitrogen, and the flask was sealed with a rubber septum. Due to the insolubility of adenine in DMSO at 60 °C, 5.6 g (22-fold molar excess) was dissolved separately in 60 mL of DMSO and the resulting solution heated to 120 °C. The adenine solution was added by syringe into the reaction mixture under nitrogen, and the round-bottomed flask was immersed in a 60 °C oil bath. The reaction was allowed to proceed for 6 d, and during this time, the adenine precipitated partially. The mixture was filtered, and the DMSO was removed at 50 °C and 100 mTorr. The product was redissolved in chloroform, precipitated several times into a hexane/methanol (90:10, v/v) mixture, and dried under reduced pressure at 50 °C for 24 h. Quantitative functionalization was confirmed using ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.3 (1H, −N═C*H*N−), 8.19 (1H, ═NC*H*═N−), 5.1−5.3 (poly(D,L-lactide), 1H per repeating unit, −C*H*(CH_3)−, and 2H, −NH_2), 4.59 (2H, −NCH_2CH_2), 4.1−4.4 (2H, −CH_2CH_2O-(PDLLA)), 3.5−3.8 (16H, ethylene oxide units, −OCH_2CH_2−), 3.4 (2H, CCH_2O−), 3.04 (2H, −OCOCH_2CH_2N−), 1.3−1.8 (poly(D,L-(lactide), 3H per repeating unit, −CH(CH_3)). M_n was calculated from the relative ^{1}H NMR integration ratio of DLLA methine protons (−C*H*(CH_3)) at 5.1−5.3 ppm compared to the β-methylene protons of the ethylene oxide unit adjacent to the DLLA repeat units (2H, −OCH_2CH_2−; 1H, −N═C*H*N−; and 1H, ═NC*H*═N) at 4.1−4.4, 8.3, and 8.19 ppm, respectively.

Synthesis of Thymine-Terminated Four-Arm, Star-Shaped PDLLA (PDLLA−T). The acrylated star PDLLA (5.0 g) was added under nitrogen to a flame-dried, 50 mL round-bottomed flask containing a magnetic stir bar. Thymine (5.7 g, 24-fold excess) and 10 mg of the catalyst, potassium *tert*-butoxide, were added under nitrogen. The flask was sealed with a rubber septum, and 60 mL of DMSO was added by syringe into the reaction mixture under nitrogen. The round-bottomed flask was immersed in a 60 °C oil bath, and the reaction was allowed to proceed for 6 d. The mixture was filtered to remove unreacted thymine, and the DMSO was removed at 50 °C and 100 mTorr. The product was redissolved in chloroform, precipitated several times into a hexane/methanol (90:10, v/v) mixture, and dried under reduced pressure at 50 °C for 24 h. Quantitative functionalization was confirmed using ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.17 (1H, −CON*H*CO−), 7.2 (1H, −C(CH_3)═C*H*−), 5.1−5.3 (poly(D,L-lactide), 1H per repeating unit, −C*H*(CH_3)−), 4.1−4.4 (2H, −CH_2CH_2O-(PDLLA)), 3.99 (2H, −NCH_2CH_2), 3.5−3.8 (16H, ethylene oxide units, −OCH_2CH_2−), 3.4 (2H, CCH_2O−), 2.85 (2H, OCOCH_2CH_2N−), 1.89 (3H, −C(CH_3)═CH−), 1.3−1.8 (poly(lactide backbone), 3H per repeating unit, −CH(CH_3)−). M_n was calculated from the relative ^{1}H NMR integration ratio of DLLA methine protons (−C*H*(CH_3) at 5.1−5.3 ppm compared to the β-methylene protons of the ethylene oxide unit adjacent to the DLLA repeat units (2H, −OCH_2CH_2−; 1H, −CON*H*CO−; and 1H, −C(CH_3)═C*H*−) at 4.1−4.4, 8.17, and 7.2 ppm, respectively.

Preparation of PDLLA−A and PDLLA−T Blends (PDLLA(A−T)). A typical procedure for the preparation of the PDLLA(A−T) complexes for ^{1}H NMR spectroscopy and solution rheological characterization involved mixing PDLLA−A and PDLLA−T in $CDCl_3$ at room temperature. The PDLLA(A−T) solutions for solution rheological studies consisted of a 1:1 (w/w) mixture of PDLLA−A and PDLLA−T, and blend compositions of varied stoichiometry were used for association constant analysis.

Stoichiometry and Association Constant Determinations. Job's method of continuous variation[40−44] was utilized for stoichiometric analysis. A series of PDLLA(A−T) solutions comprising various mole fractions of PDLLA−A and PDLLA−T (0:1 to 1:0) were prepared in $CDCl_3$. The mole fractions of PDLLA−A and PDLLA−T were calculated on the basis of SEC M_n of the precursor polymer. The total concentration was maintained at 1.5 wt % (4.0, 2.0, and 0.66 mM at M_n = 5490, 10600, and 33300 g/mol, respectively). During variable-

Scheme 1. Synthetic Methodology for the Functionalization of Four-Arm, Star-Shaped PDLLA To Produce PDLLA−A and PDLLA−T



temperature ^{1}H NMR experiments, the temperature was allowed to equilibrate for 10 min before collection of each spectrum.

Characterization. The polymer composition, number average molar mass, and percent functionalization were determined using ^{1}H NMR spectroscopy. The ^{1}H NMR spectra were obtained using a Varian UNITY spectrometer operating at 400 MHz using $CDCl_3$ as the solvent. The molar mass and molar mass distribution were determined at 40 °C in THF (ACS grade) at a flow rate of 1 mL/min using a Waters 717 autosampler equipped with a Waters 2410 refractive index detector, a Wyatt Technology MiniDAWN MALLS detector, and a Viscotek 270 viscosity detector. Reported molar masses are based on absolute measurements using the MALLS detector. Solution rheological analysis was performed using a VOR Bohlin strain-controlled solution rheometer at 25 ± 0.2 °C equipped with a concentric cylinder geometry.

Results and Discussion

The preparation of nucleobase-terminated PDLLA star polymers proceeded via a four-step synthesis as shown in Scheme 1. Hydroxyl-terminated star-shaped PDLLAs with molar masses ranging from 5490 to 33300 g/mol were first synthesized with narrow molar mass distributions (M_w/M_n = 1.07−1.37). In the second step, acryloyl chloride was reacted with the terminal hydroxyl groups of PDLLA in solution to introduce acrylate end groups. ^{1}H NMR spectroscopy confirmed quantitative end group functionalization (Figure 1) on the basis of the disappearance of the PDLLA hydroxyl resonance (Figure 1A, peak f) at 2.73 ppm and the concurrent appearance of resonances h, i, and j at 6.49, 6.18, and 5.89 ppm, respectively, which were assigned to the olefinic protons of the acrylated star PDLLA (Figure 1B). Michael addition of the acrylated star PDLLA with heterocyclic bases, such as adenine (A) or thymine (T), resulted in four-arm, star-shaped polymers with complementary multiple-hydrogen-bonding (CMHB) end groups.

Successful incorporation of the heterocyclic base pairs was confirmed using ^{1}H NMR spectroscopy, and star-shaped PDLLA−A and PDLLA−T precursors were obtained. The olefinic protons at 5.8−6.5 ppm disappeared, confirming quantitative Michael addition of the heterocycles to the acrylated PDLLA. The Michael addition of thymine to acrylated PDLLA occurred regiospecifically at N1, as observed previously.[4] PDLLA−A offers four new resonances that are consistent with adenine at 8.31 ppm (−N═C*H*N−), 8.19 ppm (═NC*H*═N−), 4.59 ppm (−NCH_2CH$_2$−), and 3.03 ppm (−OCOCH_2CH$_2$N). Amine NH_2 resonances that are associated with adenine overlapped with the methine protons in the PDLLA repeat unit at 5.1−5.3 ppm. Five new resonances characteristic of thymine were observed in the ^{1}H NMR spectrum of PDLLA−T at 8.18 ppm (−CON*H*CO−), 7.20 ppm (−C(CH$_3$)═C*H*−), 3.99 ppm (−NCH_2CH$_2$−), 2.85 ppm (−OCOCH_2CH$_2$N), and 1.89 ppm (−C(CH_3)═CH−).[4]

The formation of complementary multiple hydrogen bonds was probed via analysis of a 1:1 (w/w) mixture of 5.5K PDLLA−A and PDLLA−T (PDLLA(A−T)) at 1.5 wt % (4.0 mM). The ^{1}H NMR resonances of the PDLLA(A−T) mixture were compared with the resonances of the PDLLA−A and PDLLA−T precursors. All of the other monitored resonances of the mixture remained unshifted from their original chemical shifts in the corresponding PDLLA−A and PDLLA−T precursors except for the NH and NH_2 protons. A downfield shift was observed for the NH resonance of PDLLA−T from 8.18 to 8.83

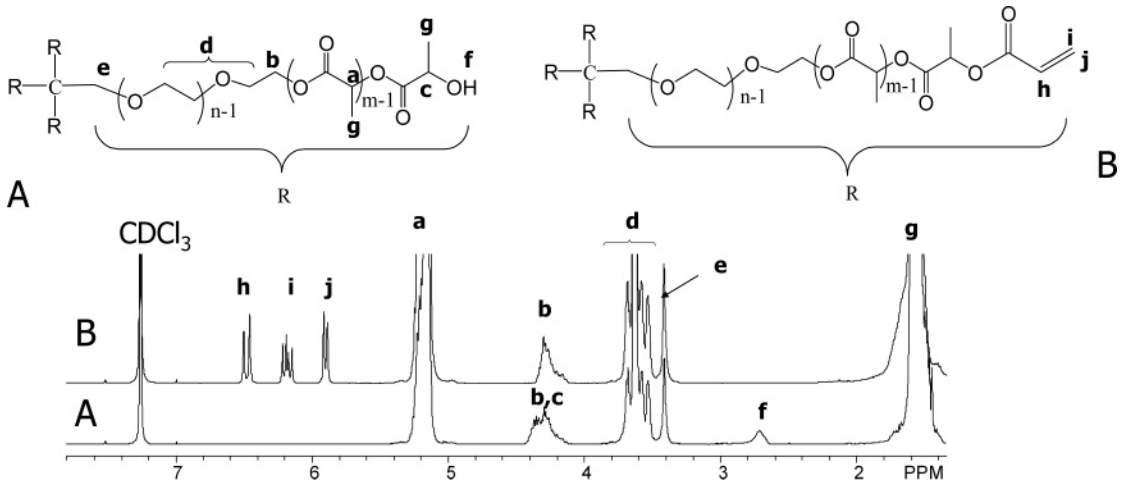


Figure 1. Stacked ^{1}H NMR spectra of (A) hydroxyl-terminated and (B) acrylate-terminated, four-arm star-shaped PDLLA (M_n = 5490 g/mol, M_w/M_n = 1.07).

Table 1. Molar Mass and CMHB End Group Content (mol %) for Four-Arm, Star-Shaped PDLLA−A and PDLLA−T

sample	backbone	precursor M_n[a] (g/mol)	precursor M_n[b] (g/mol)	M_w/M_n	M_n[c] (g/mol)	CMHB[c] end group content (mol %)
1	PDLLA−A	6420	5490	1.07	6730	4.8
2	PDLLA−T	6420	5490	1.07	6910	4.7
3	PDLLA−A	11000	10600	1.11	13100	2.3
4	PDLLA−T	11000	10600	1.11	14900	2.0
5	PDLLA−A	42900	33300	1.37	46000	0.6
6	PDLLA−T	42900	33300	1.37	44500	0.7

[a] Determined using ^{1}H NMR in $CDCl_3$. [b] Determined using SEC MALLS. [c] M_n after functionalization as determined using ^{1}H NMR spectroscopy.

ppm upon solution blending, while the NH_2 resonance of PDLLA−A, which previously overlapped with the methine proton in the PDLLA repeat unit at 5.1−5.3 ppm, shifted downfield to 6.2 ppm. These downfield shifts were consistent with previous literature[41] and suggested the formation of intermolecular hydrogen bonds between the heterocyclic-modified, star-shaped PDLLA. On the other hand, a slight upfield shift was observed for the PDLLA−A adenine ring proton at 8.1 ppm after solution blending, while the more upfield adenine ring proton chemical shift remained unchanged. This change was attributed to hydrogen-bonding interactions between the adenine and thymine groups. The complementary A−T hydrogen bonds are located closer to the adenine ring proton which exhibited the change in chemical shift.[45]

A series of four-arm, star-shaped PDLLA−A and PDLLA−T precursors were prepared over a molar mass range of 5490−33300 g/mol to evaluate the effect of molar mass on hydrogen-bonding associations. Three four-arm, star-shaped PDLLA precursors (5490, 10600, and 33300 g/mol) were divided into two portions and modified with either adenine or thymine to obtain PDLLA−A and PDLLA−T samples with equivalent molar concentration of CMHB end groups, molar mass, and molar mass distribution (Table 1). Solutions of equivalent molar mass PDLLA−A and PDLLA−T (PDLLA(A−T)) (1:1, w/w, ratio) were prepared in chloroform-*d* (2.6 wt %). Three complexes, PDLLA(A−T)5.5K, PDLLA(A−T)10.6K, and PDLLA(A−T)33.3K, were obtained, and these corresponded to total molar solution concentrations of 7.04, 3.65, and 1.16 mM, respectively. Complexation in the solutions was observed by monitoring their respective thymine NH chemical shifts.

The PDLLA(A−T) NH resonance shifted upfield from 9.38 to 7.58 ppm as the molar mass increased from 5490 to 33300 g/mol (Figure 2). The significant upfield shift observed with increasing molar mass occurred due to the decreased concentration of CMHB end groups. The molar concentration of CMHB end groups was calculated via comparison of the methine protons of the PDLLA repeat unit and characteristic resonances assigned to the heterocyclic end groups and decreased as the molar mass was increased from 5490 to 33300 g/mol. This decreased CMHB concentration shifted the equilibrium (A + T ⇌ A−T) toward a less associated state. The low molar mass PDLLA(A−T)5.5K complex had 4.8 mol % CMHB end groups compared with 2.2 and 0.7 mol % for the PDLLA(A−T)10.6K and PDLLA(A−T)3.3K complexes, respectively (Table 1). Hence, the relatively high 9.38 ppm NH chemical shift observed in the PDLLA(A−T)5.5K complex was a result of the presence of a higher concentration of CMHB end groups and higher total solution concentration.

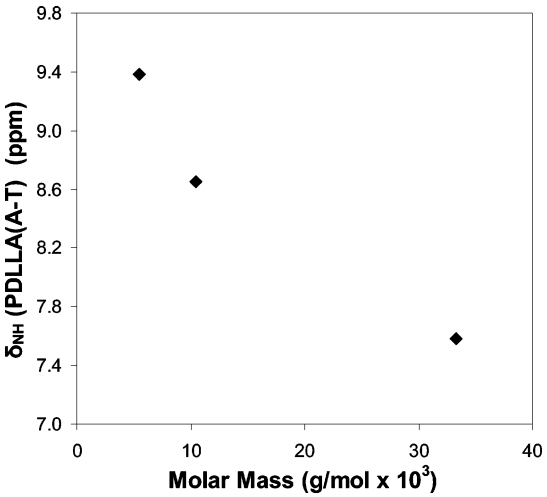


Figure 2. Relationship between the ^{1}H NMR NH chemical shift and the PDLLA(A−T) molar mass ($CDCl_3$, 27 °C, 2.6 wt %).

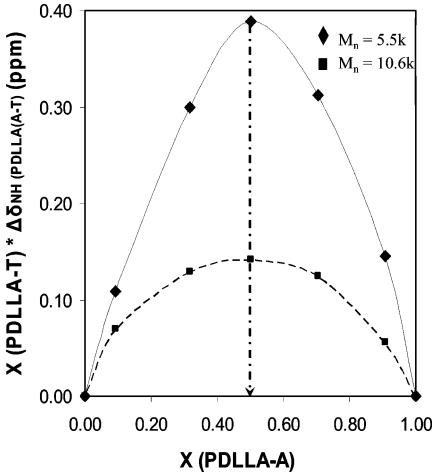


Figure 3. Job's plot to determine the stoichiometry of PDLLA(A−T)5.5K and PDLLA(A−T)10.6K complexes in solution (chloroform-*d*, 27 °C, 1.5 wt %).

The continuous variation method,[42,44] also known as Job's method,[46] was employed to investigate the stoichiometry of the PDLLA(A−T) complexes in solution. Determination of the PDLLA(A−T) complex stoichiometry using ^{1}H NMR spectroscopy afforded an understanding of the molar ratio of PDLLA−A and PDLLA−T in the complex.

To determine the stoichiometry of the hydrogen-bonding complex, solutions containing PDLLA−A and PDLLA−T were prepared in chloroform-*d*. The molar ratios of the two components were varied, while the total solution concentration was maintained at 1.5 wt %. The chemical shift of the thymine NH resonance was recorded at different mole fractions, and the change in chemical shift ($\Delta\delta$) of the PDLLA(A−T) complex relative to a nonassociated PDLLA−T solution was calculated. Stoichiometric determinations were not conducted for the PDLLA(A−T)33.3K complex due to the difficulty of monitoring the NH protons at low end group concentrations. Job's plots generated for the PDLLA(A−T)5.5K and PDLLA(A−T)10.6 K complexes are shown in Figure 3. The *x*-axis values at the parabolic curve maxima determined the stoichiometry of the complex. Both plots were highly symmetric with a maximum at 0.5 mole fraction, indicating 1:1 base pairing. Thus, when various fractions of star-shaped PDLLA−A and PDLLA−T were mixed in solution, a maximum concentration of the hydrogen-bonded PDLLA(A−T) complex was formed at a 1:1 PDLLA−A/PDLLA−T equimolar concentration.[47]

To further confirm the structure of the hydrogen-bonded complexes in solution, 12 solutions of PDLLA(A−T) complexes were prepared where the PDLLA−T concentration was maintained at 1.5 wt % (4.0 and 2.0 mM for the PDLLA(A−T)5.5K and PDLLA(A−T)10.6.K complexes, respectively). The PDLLA−A concentration, however, was systematically increased from 0 to 6.5 wt % (0−10 mM for PDLLA(A−T)5.5K and 0−20 mM for PDLLA(A−T)10.6.K). The change in the chemical shift of the thymine NH resonance in the PDLLA(A−T) complex was observed with increasing PDLLA−A solution concentration. The change in the chemical shift with complexation was due to the fast exchange between associated and dissociated A−T complexes, which led to an averaged NH resonance, rather than two separate NH resonances.[4,42] Plots of induced change in chemical shift versus PDLLA−A concentration were obtained, and a nonlinear correlation was revealed (Figure 4). This type of curve is typically observed during the NMR titration experiment, and the fit corresponded to the

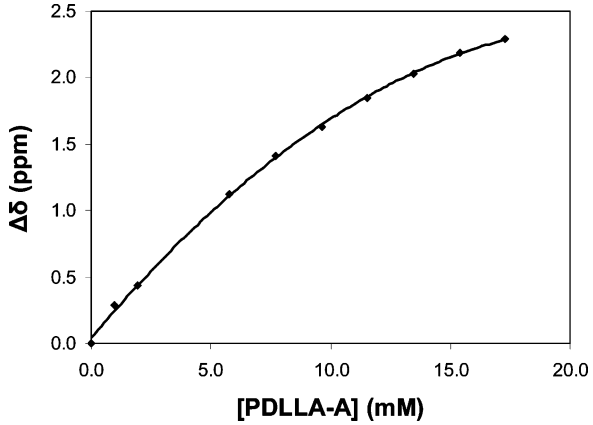


Figure 4. Nonlinear relationship between the induced change in the thymine NH chemical shift and the solution concentration (M_n = 5490 g/mol, [PDLLA−T] = 4.0 mM, 27 °C).

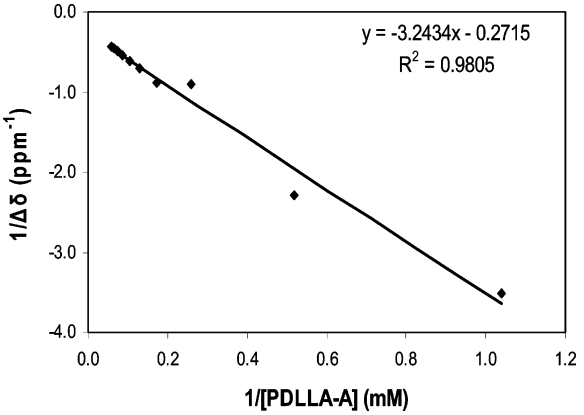


Figure 5. Benesi−Hildebrand plots of PDLLA−A/PDLLA−T association in $CDCl_3$ (M_n = 5490 g/mol, [PDLLA−T] = 4.0 mM, 27 °C).

Benesi−Hildebrand model, which further confirmed the 1:1 stoichiometry.

The Benesi−Hildebrand model, which is a mathematical method of determining the association constant (K_a) from NMR titration data, was used to fit the nonlinear chemical shift data as expected for a dimeric hydrogen bond association.[42,48] The model assumes a 1:1 stoichiometry for the complex. The fitting of the induced change in chemical shift with concentration provided further evidence for a 1:1 complex stoichiometry. A double reciprocal plot of the association of PDLLA−A and PDLLA−T (M_n = 5490 g/mol) revealed a linear relationship as shown in Figure 5 and further confirmed the prevalence of a 1:1 PDLLA−A/PDLLA−T complex in solution.[50]

The Benesi−Hildebrand analysis was performed to calculate the association constant, K_a, using the equation

$$1/\Delta\delta = 1/(K_a\Delta\delta_{max}[\text{PDLLA}-\text{A}]) + 1/\Delta\delta_{max}$$

where $\Delta\delta_{max}$ is the maximum change of the chemical shift of the thymine NH protons corresponding to complete formation of the associated complex. The slope of the double reciprocal plot is $1/K_a\Delta\delta_{max}$, and the intercept is $1/\Delta\delta_{max}$. The K_a that was calculated from the slope of the plot was 84 M^{-1}, which is consistent with K_a values reported earlier for adenine−thymine base pair recognition (ca. 10−100 M^{-1} in $CDCl_3$).[47,51] In addition to complementary hydrogen-bonding interactions, self-association of the adenine and thymine groups, although relatively insignificant, occurred and may have contributed to the calculated association constants. However, the reported K_a

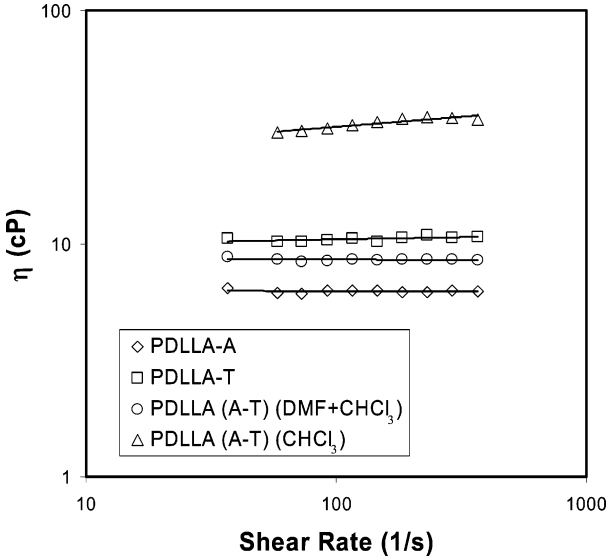


Figure 6. Solution viscosities of PDLLA−A, PDLLA−T, and PDLLA-(A−T)33.3K (M_n = 33300 g/mol) in $CHCl_3$ and $CHCl_3$/DMF (50:50) at 25 °C and 10 wt % concentration.

values for adenine and thymine self-association are relatively low ($K_{a(A-A)}$ = 2.4 M^{-1}, $K_{a(T-T)}$ = 3.5 M^{-1} in $CDCl_3$).[52,53]

The effect of hydrogen-bonding associations on solution viscosity was investigated using 10 wt % solutions of the four-arm star-shaped PDLLA−A and PDLLA−T as well as the PDLLA(A−T) complexes (1:1, w/w, PDLLA−A/PDLLA−T mixture) in $CHCl_3$ at ambient temperature. The solubilities of the 5490 and 10600 g/mol samples were limited at 10 wt % due to enhanced hydrogen-bonding associations and subsequent aggregation as a result of the high concentration of CMHB end groups. Consequently, only the 33300 g/mol polymers were evaluated. The zero shear viscosities (η_o) of the 33300 g/mol samples were determined from extrapolation to the *y*-axis (Figure 6). The PDLLA(A−T)33.3K solution yielded a near-Newtonian fluid with an η_o of approximately 33 cP, which was significantly higher than those of the individual PDLLA−A and PDLLA−T solutions of the same molar mass (6.2 and 10 cP, respectively). This increase in viscosity was attributed to the formation of intermolecular, complementary hydrogen bonds for the PDLLA(A−T)33.3K 1:1 complex. The large increase in the solution viscosity of the PDLLA(A−T)33.3K complex relative to the PDLLA−A and PDLLA−T solutions was attributed to the formation of a supramolecular structure of CMHB-terminated star polymers in solution. The slightly higher η_o of the PDLLA−T solution relative to the PDLLA−A solution was consistent with the more favorable self-association of thymine ($K_{a(T-T)}$ = 3.5 M^{-1}, $K_{a(A-A)}$ = 2.4 M^{-1}).[52,53] It is well-known that *N*,*N*-dimethylformamide (DMF) disrupts hydrogen bonding.[54,55] We observed a significant decrease in the solution viscosity of the PDLLA(A−T)33.3K 1:1 complex in a 1:1 (v/v) mixture of $CHCl_3$ and DMF, and this was attributed to a disruption of the hydrogen-bonding interactions between complementary PDLLA−A and PDLLA−T.

The complexes consisting of an equimolar mixture of the nucleobase-functionalized star PDLLA were analyzed using variable-temperature ^{1}H NMR spectroscopy in toluene-d_8 to investigate the effect of temperature on the extent of hydrogen bonding. Thus, toluene-d_8 (boiling point 110 °C) permitted analysis at typical temperatures for dissociation.[16,18,19] The temperature dependence of the NH proton chemical shift of the PDLLA(A−T)10.6K complex (1:1) at 4.4 wt % (3.8 mM) in toluene-d_8 is shown in Figure 7. The NH resonance shifted

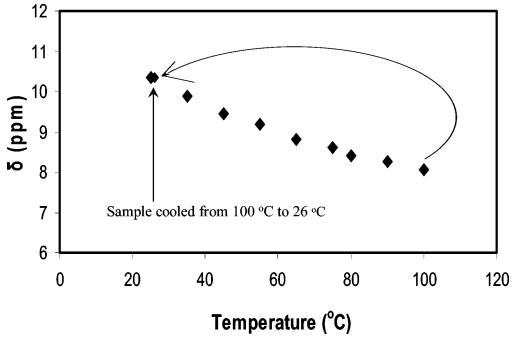


Figure 7. ^{1}H NMR NH chemical shift of the PDLLA(A−T)10.6K complex (1:1) as a function of temperature (3.8 mM (4.4 wt %) in toluene-d_8). Samples were allowed to equilibrate for 10 min at each temperature.

upfield systematically from 10.3 to 8.0 ppm as the temperature was raised from 25 to 100 °C. The gradual decrease in the NH resonance with increasing temperature was attributed to dissociation of the complementary hydrogen bonds. However, once the sample was cooled from 100 to 26 °C, the NH resonance returned to its original position at 10.3 ppm. This observation suggested that the PDLLA-based hydrogen-bonded supramolecular complexes were thermoreversible in toluene-d_8.

The hydrogen bonding was thermoreversible since the NH resonance reproducibly shifted upfield and downfield as the temperature was increased to 100 °C and cooled to 27 °C. Our earlier efforts also described hydrogen-bonded complexes based on linear polystyrenes that were modified with adenine and thymine end groups, and high levels of dissociation occurred at 95 °C.[4] We observed that the NH resonance of the star-shaped PDLLA complex continued to shift upfield from 95 to 105 °C. This implied that the four-arm, star-shaped PDLLA-based complexes remained relatively more associated at 100 °C, which may suggest increased hydrogen-bonding interactions for the star architecture.

Conclusions

Well-defined hydroxyl-terminated four-arm, star-shaped PDLLAs of controlled molar mass ranging from 5490 to 33300 g/mol were synthesized and successfully functionalized to achieve peripheral complementary multiple-hydrogen-bonding DNA base pair units. Mixing of star-shaped PDLLA−A and PDLLA−T in solution resulted in the formation of supramolecular structures through hydrogen-bonding interactions. Job's plots and Benesi−Hildebrand analysis revealed a 1:1 complexation between the star-shaped PDLLA−A and PDLLA−T. A K_a of 84 M^{-1} was observed for the PDLLA(A−T)5.5K complex based on lower molar mass polymers. Additionally, a PDLLA(A−T) hydrogen-bonded complex exhibited higher solution viscosity compared to the corresponding non-hydrogen-bonded PDLLA−A and PDLLA−T samples, confirming strong hydrogen-bonding associations between the PDLLA−A and PDLLA−T star-shaped polymers. Moreover, variable-temperature ^{1}H NMR studies also demonstrated the reversibility of the hydrogen-bonded PDLLA-based supramolecular structures.

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Supporting Information Available. Stacked ^{1}H NMR spectra of four-arm star-shaped PDLLA−A, PDLLA−T, and PDLLA(A−T). This material is available free of charge via the Internet at http://pubs.acs.org.

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