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Adenine-Containing Block Copolymers via Ring-Opening Metathesis Polymerization: Synthesis and Self-Assembly into Rod Morphologies

Hassan S. Bazzi and Hanadi F. Sleiman*

Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, Quebec, H3A 2K6 Canada

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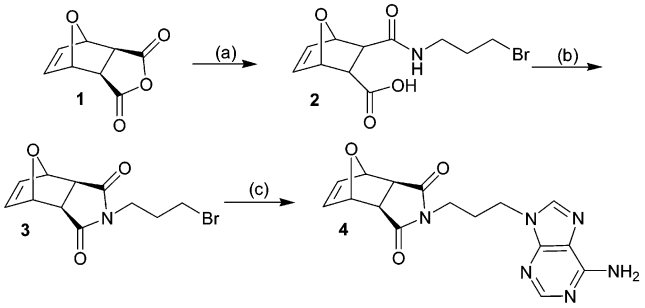
The control of strand−strand association in synthetic polymers has been the subject of intense investigation in recent years.[1] For this, a number of noncovalent forces, such as van der Waals, electrostatic, and single-point hydrogen-bonding interactions, have been used.[1] Biological macromolecules (e.g., nucleic acids), on the other hand, derive their remarkable ability for cooperative and selective binding from molecular recognition or the association of complementary partners via multiple hydrogen bonds.[2] The addition of molecular recognition to the manifold of polymer interactions can thus endow synthetic polymers with the ability to select and bind their complementary partners as well as interface efficiently with biological systems.[3−7] In the past few years, a number of important contributions have described the synthesis of polymers containing molecular recognition units and their ability to selectively bind complementary molecules, polymers, and colloidal gold particles.[3−6] In most of these previous systems, molecular recognition units were incorporated into polymers generated by conventional free radical polymerization or statistically attached to a block copolymer backbone.[3,4] Atom transfer radical polymerization has been used to synthesize homopolymers containing uridine and adenosine monomers.[6]

We have recently initiated a research program to examine the use of the ring-opening metathesis polymerization[8,9] to construct "DNA-mimetic" polymers and block copolymers containing regioregular polymer backbones, which are substituted with DNA bases or analogues.[10] These polymers are expected to show high binding affinity and selectivity to their complementary partners, as well as to natural nucleic acids, with potential applications as biomolecule sensors and DNA-delivery agents. We here report the first synthesis of adenine-containing homopolymers and block copolymers via controlled ring-opening metathesis polymerization and the ability of these hydrogen-bonding and self-complementary block copolymers to self-assemble into novel nanoscale rod morphologies.

Scheme 1. Synthesis of Monomer 4: (a) $BrCH_2CH_2CH_2NH_2 \cdot HBr$, $NaHCO_3$, 63%; (b) Acetic Anhydride, NaOAc, 90 °C, 70 %; (c) Adenine, NaH, DMF, 60 °C, 48%



The synthesis of adenine-containing monomer **4** was performed using the furan−maleic anhydride *exo*-adduct **1**[11] as starting material[12] (Scheme 1). Treatment of this adduct with 3-bromopropylamine hydrobromide resulted in ring-opening of the anhydride to yield the amide/acid product **2**. Condensation to the imide in a NaOAc/acetic anhydride mixture yielded the bromo-derivative **3**. Reaction of adenine with NaH in hot DMF followed by addition of **3** gave adenine-substituted monomer **4**.[13]

We have recently used living ring-opening metathesis polymerization (ROMP) with the ruthenium alkylidene (Grubbs catalyst) **5**[9] (Chart 1) to construct polymers and

* Corresponding author: e-mail hanadi.sleiman@mcgill.ca.

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Scheme 2. (A) ROMP Reaction of Noncovalently Protected Monomer 4; (B) Synthesis of Copolymers Incorporating Blocks of Poly(4)

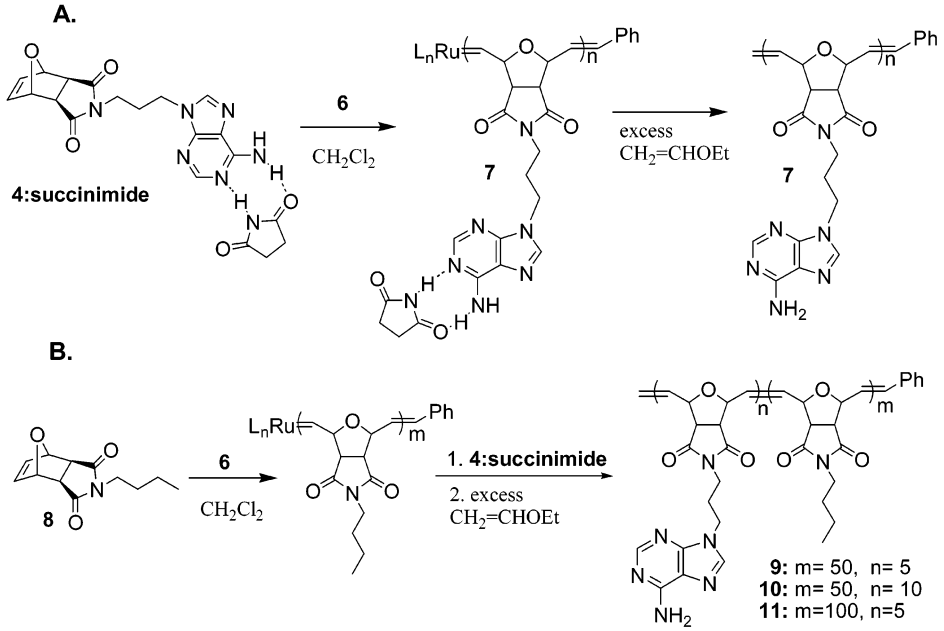
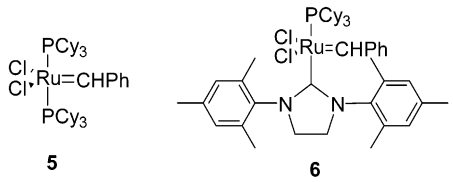


Chart 1



block copolymers containing thymine analogues in their repeat units.[10] We thus first studied the ROMP reaction of monomer **4** with catalyst **5** in dichloromethane. No polymerization was observed even after 2 days at room temperature. This can be due to deactivation of catalyst **5** upon coordination of the ruthenium to the adenine moiety in **4**. Consistent with this, ^{31}P NMR of the reaction mixture showed the disappearance of the resonance corresponding to the phosphine in **5** at 36.61 ppm[9] and the appearance of two new resonances for free and bound phosphine at 25.34 and 51.05, respectively. The new generation catalyst **6** has been shown to display higher activity in ROMP reactions than **5**.[9] When monomer **4** (10 equiv) was allowed to react with catalyst **6** in dichloromethane for 12 h at room temperature, adenine homopolymer **7** was generated as a sparingly soluble solid in 70% yield. However, higher monomer conversions could not be achieved, likely due to the insolubility of polymer **7**, which possesses self-complementary adenine units.[2]

We reasoned that the addition of a complementary molecule, such as succinimide, to monomer **4** would result in hydrogen-bond-mediated association of this molecule to its adenine moiety (Scheme 2A). This could provide a novel noncovalent method to "protect" monomer **4** during its polymerization and increase the solubility of the resulting polymer, thus preventing its precipitation before the reaction is complete. Addition of 2 equiv of succinimide to **4** resulted in a downfield shift of both the adenine amino and the succinimide NH protons by ^{1}H NMR (0.6 and 0.8 ppm, respectively, in $CDCl_3$), consistent with the formation of a succinimide:adenine complex. When the monomer **4**/succinimide mixture was reacted with catalyst **6** in CH_2Cl_2, the monomer conversion increased to 90%, and the solubility of the resulting polymer was significantly increased. This suggests that hydrogen bonding of a complementary molecule to a ROMP monomer can potentially be used as a new method to enhance the efficiency of the ROMP reaction. Quenching with ethyl vinyl ether, followed by precipitation from methanol yielded adenine polymer **7** in 80% yield. ^{1}H NMR of **7** in d_6-DMSO showed the characteristic downfield shift of the olefin signals upon ring-opening, and a polymer double bond cis-to-trans ratio of 52:48. MALDI−TOF analysis of the polymer showed a repeat unit consistent with the molecular weight of the monomer.[13,14]

While adenine-containing homopolymers have been previously synthesized using free radical polymerization methods,[4,6] there are no known examples of block copolymers containing adenine units. Considering the molecular recognition ability of these units, as well as their hydrogen-bond self-complementarity,[2] we were interested in using the above method to generate block copolymers containing **4**. The ROMP reaction is well-known to be a powerful tool for the synthesis of block polymers through the sequential addition of monomers. As a first step, *exo*-*N*-butyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide (**8**)[15] (50 equiv) was reacted with initiator **6** in CH_2Cl_2 at room temperature for 30 min (Scheme 2B). An aliquot of this solution was removed, and its ^{1}H NMR revealed the complete conversion of monomer **8**. To the remaining solution of poly(**8**), a dichloromethane solution of monomer **4** (5 equiv) and succinimide (10 equiv) was added. ^{1}H NMR showed more than 95% conversion of monomer **4** after 12 h at room temperature. After quenching and precipitation in hexanes, block copolymer **9** was obtained in 85% yield. Other block copolymers (**10**, **11**) with 50:10 and 100:5 ratios of **8** to **4** were also generated following the same procedure.[13] These copolymers were characterized by ^{1}H and ^{13}C NMR, FT-IR, UV/vis, and gel permeation chromatography. All spectroscopic methods showed the presence of the two polymer blocks (poly(**4**) and poly(**8**)). GPC analysis of the copolymers showed monomodal

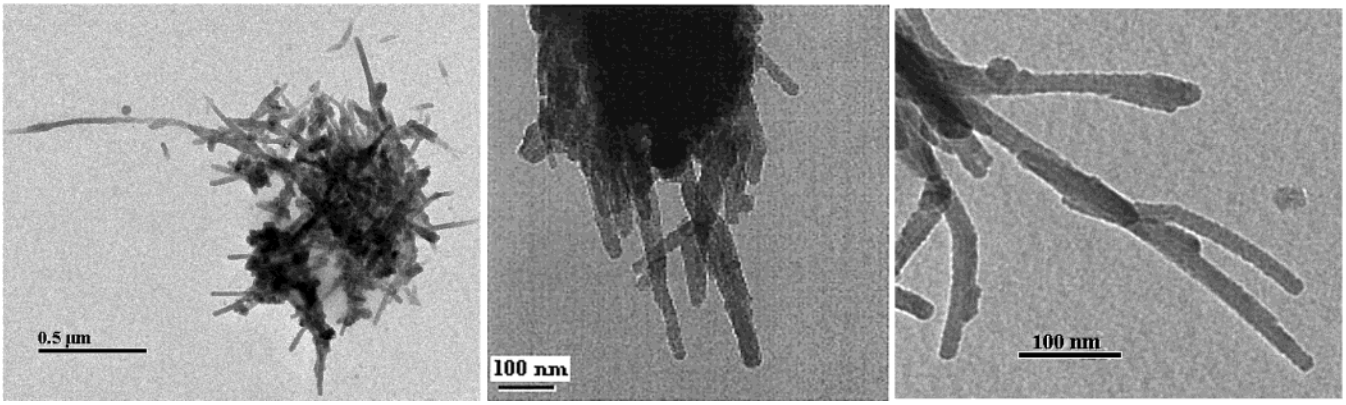


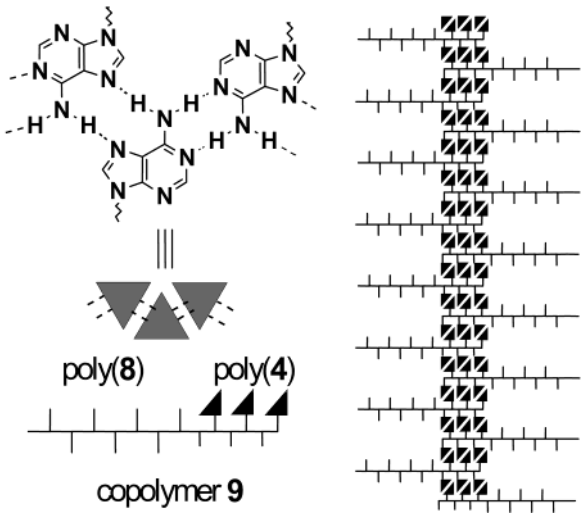
Figure 1. Transmission electron micrographs of copolymer **9** from a THF solution.

distributions, as well as the disappearance of the homopolymer trace, and the appearance of peaks at higher molecular weight than the homopolymer poly(**8**).[13] The polydispersities for the block copolymers ranged from 1.20 to 1.60. These somewhat broader distributions than those normally obtained for catalyst **5**[9] are most likely due to the use of catalyst **6**, which typically displays faster ROMP propagation rates, compared to initiation.[9]

Asymmetric block copolymers are known to undergo self-assembly into nanoscale aggregates in selective solvents.[1] In copolymer **9**, the *N*-butyl-containing block poly(**8**) is soluble in most organic solvents, while the adenine containing block poly(**4**) is soluble in DMSO, slightly soluble in $CHCl_3$, and insoluble in THF. Thus, these block copolymers are expected to show aggregation behavior in THF and $CHCl_3$. The self-assembly of copolymer **9** (PDI = 1.60) was investigated in THF solution. Dynamic light scattering measurements of **9** in THF (1% w/v) showed the presence of large, nonspherical aggregates (diameters ranging from 869 ± 24 to 1238 ± 10 nm, depending on the scattering angle). Transmission electron microscopy analysis of a sample of **9**, prepared by evaporation of a THF solution (1% w/v), revealed the presence of large aggregates of well-defined cylindrical rods[13] (Figure 1). The diameter of these rods (30 ± 2 nm) corresponds to the approximate length of two partially stretched chains of copolymer **9** (Scheme 3). The adenine block poly(**4**) is expected to reside in the core of these cylindrical strutures due to its significantly low solubility in THF, and the *N*-butyl block poly(**8**) constitutes the corona. Indeed, while the ^{1}H NMR of copolymer **9** in d_6-DMSO showed peaks corresponding to both blocks of poly(**8**) and poly(**4**), no adenine peaks were visible when the ^{1}H NMR was taken in d_8-THF, consistent with lower mobility and decreased solvation for this block.[16] On the other hand, the *N*-butyl block signals were still strong, indicating that the poly(**8**) block constitutes the corona of these micelles, and the adenine poly(**4**) block is located in their core. Therefore, the adenine-containing block most likely resides in a relatively THF-free microenvironment, thus allowing the adenine units to associate via hydrogen bonding (vide infra).

The observation of rod morphologies in copolymer **9** is unexpected, considering the polymer composition (core:corona ratio 1:10). Coil−coil asymmetric copolymers are known to aggregate into cylindrical structures only when the core block is significantly larger than the corona and form starlike spherical micelles at low core: corona ratios (such as in **9**).[1] Recently, the crystalline

Scheme 3. Self-Complementarity of Adenine and Association of the Adenine Units in Copolymer 9



nature of the core block has been shown to play an important role in the formation of cylindrical micelles.[17] Interestingly, DSC runs on the adenine homopolymer **7** showed a small amount of crystallinity in this polymer (T_m = 130 °C). Wide-angle X-ray scattering (WAXS) measurements[13] also revealed some crystallinity in homopolymer **7** and to a lesser extent in copolymer **9** (*d* spacing of 4.8 Å was observed in both systems). While we are currently investigating this phenomenon further, we suggest that the ability of this system to form rod morphologies stems from the hydrogen-bonding self-complementarity of adenine,[2] in addition to its propensity for aromatic π-stacking.[18,19] This can result in noncovalent cross-linking of the micellar core, thus providing rigidity, crystallinity, and resistance toward interfacial curvature (Scheme 3).

In summary, we have shown the first synthesis of adenine-containing polymers and block copolymers using the ring-opening metathesis polymerization of noncovalently protected monomers. Studies of the self-assembly of copolymer **9** reveal an unexpected cylindrical morphology, which can be interpreted as arising from the hydrogen-bonding self-complementarity of the adenine units in the micellar core. Further studies are currently being conducted to fully characterize this morphogenesis mechanism as well as to study the effect of hydrogen-bonding guests on the polymer morphologies.

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Supporting Information Available: Full experimental details of the synthesis and characterization of monomer **4** and the polymers, UV−vis and WAXS spectra, and additional transmission electron micrographs. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) See Supporting Information.

(14) Quenching the polymerization with ethyl vinyl ether results in decomplexation of the succinimide from polymer **7**. The resulting polymer was too insoluble to obtain a GPC analysis; however, it was characterized by ^{1}H and ^{13}C NMR, FTIR, and UV/vis as well as MALDI-TOF MS (see Supporting Information).

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(18) Because the polymer backbone in **7** and **9** contains a nearly equal number of cis and trans double bonds (52:48), its contribution to crystallinity is expected to be negligible.

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