Effect of Precursor's Chemical Composition on the Formation and Stability of G-Quadruplex Core Supramolecular Star

Polymers

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General Methods and Materials

Guanosine, succinic anhydride, (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI.HCl), 1-Hydroxybenzotriazole (HOBt) were purchased from commercial sources. Hydroxy terminated polybutadienes were purched from Polymer source Inc. NMR spectra were recorded on a Bruker AV300 MHz and AV500 MHz spectrometers, using CDCl₃, as the solvent and tetramethyl silane (TMS) as internal standard and used as such without further drying.

For mixed solvent study 1.25mM solution of octamer was prepared in 0.5 mL of $CDCl_3$ and gradually added DMSO- d_6 , the percent of DMSO- d_6 used was (v/v). Mass spectrometry analyses were performed using an electron ionization (EI) MS spectrometer (Micromass AutoSpec-Ultima). UV-visible spectroscopic analysis was performed on JASCO V-670 UV-Vis-NIR spectrophotometer. Circular dichroism spectroscopic analysis was performed on JASCO J-715 spectrometer using 1.0 mm path length and 0.3 mL quartz cuvette.

Synthesis

General esterification procedure: A mixture of guanosine acid (0.575 g, 1.36 mmol), EDCI.HCl (0.374 g, 1.81 mmol), HOBt (0.245 g, 1.81 mmol), DMAP (0.03 g, 0.27 mmol), and hydroxy terminated polybutadiene ($M_n = 1000$) (10. g, 0.90 mmol) in pyridine (12 mL) was stirred at 25 °C for 48 h. Pyridine was removed under reduced pressure and the crude mixture was purified by column chromatography (DCM/MeOH, 95:5) using KMnO₄ as TLC stain to yield 0.82 g of polymer 1 (Yield = 59%). ¹H-NMR (δ, ppm, 500 MHz, CDCl3): 12.01 (s, 1H, NH), 7.60 (s, 1H), 6.14 (br s, 2H), 5.94 (s, 1H), 5.57 - 5.25 (br m, 50H), 4.98 (m, 8H), 4.62 (m, 1H), 4.42 (m, 1H), 4.21 (m, 1H), 4.12 – 3.99 (m, 2H), 3.60 – 3.36 (m, 1H), 2.62 (s, 4H), 2.08 – 1.18 (br m, 116H), 0.86 (m, 8H); ¹³C-NMR (δ, ppm, 125 MHz, CDCl₃): 172.59, 172.54, 172.37, 159.35, 153.71, 151.26, 142.93, 142.88, 141.71, 141.64, 136.98, 131.45, 131.10, 130.78, 130.72, 130.64, 130.33, 130.22, 129.83, 129.65, 129.46, 129.18, 129.01, 128.70, 128.55, 128.27, 128.09, 118.18, 115.97, 115.83, 115.39, 115.27, 114.65, 114.54, 114.45, 114.15, 113.82, 90.69, 85.04, 84.64, 81.87, 71.01, 69.69, 68.69, 64.66, 64.27, 64.01, 63.52, 43.95, 43.84, 43.73, 41.80, 41.63, 41.57, 40.83, 40.74, 39.92, 39.29, 38.39, 35.32, 35.26, 35.10, 35.05, 34.48, 34.42, 34.33, 34.18, 33.75, 33.65, 32.89, 30.27, 29.43, 29.22, 27.68, 27.40, 25.64, 25.19, 25.09, 25.02,

23.79, 19.99, 19.36, 19.25, 18.90, 11.80, 11.70,11.61; UV/Vis (chloroform) $\lambda_{max} = 256$ nm.

Polymers 2, 3 and 4 were synthesized in a similar fashion.

Polymer 2: (Yield = 41%) ¹H-NMR (δ, ppm, 500 MHz, CDCl3): 12.03 (s, 1H, NH), 7.60 (s, 1H), 6.19 (br s, 2H), 5.95 (s, 1H), 5.55 – 5.26 (br m, 60H), 4.96 (m, 9H), 4.62 (m, 1H), 4.42 (m, 1H), 4.21 (m, 1H), 4.06 (m, 2H), 3.60 – 3.43 (m, 1H), 2.63 (s, 4H), 2.08 – 1.11 (br m, 131H), 0.84 (m, 8H); ¹³C-NMR (δ, ppm, 125 MHz, CDCl₃): 172.57, 172.52, 172.36, 159.35, 153.68, 151.27, 142.92, 142.86, 141.70, 141.62, 136.96, 131.46, 131.08, 130.78, 130.72, 130.64, 130.31, 130.04, 129.82, 129.64, 129.46, 129.18, 129.01, 128.70, 128.55, 128.27, 128.09, 118.15, 115.94, 115.80, 115.24, 114.64, 114.52, 114.43, 90.71, 85.05, 84.64, 81.87, 71.00, 69.68, 68.67, 64.65, 64.26, 64.00, 63.51, 53.62, 43.93, 43.83, 43.70, 40.80, 40.72, 39.91, 39.28, 38.41, 35.31, 35.24, 35.09, 35.03, 34.82, 34.74, 34.47, 34.40, 34.32, 34.16, 33.74, 33.64, 32.92, 30.40, 30.33, 30.29, 29.91, 29.41, 29.24, 27.73, 27.60, 27.39, 25.62, 25.18, 25.08, 25.00, 23.73, 19.97, 19.34, 19.23, 18.88, 11.77, 11.67, 11.59; UV/Vis (chloroform) λ_{max} = 256 nm.

Polymer 3: (Yield = 40%) ¹H-NMR (δ, ppm, 500 MHz, CDCl3): 12.08 (s, 1H, NH), 7.61 (s, 1H), 5.98 (br s, 2H), 5.95 (s, 1H), 5.56 – 5.26 (br m, 200H), 4.97 (m, 20H), 4.64 (m, 1H), 4.42 (m, 1H), 4.19 (m, 1H), 4.11 – 4.03 (m, 2H), 3.61 – 3.40 (m, 1H), 2.63 (s, 4H), 2.16 – 1.12 (br m, 500H), 0.86 (m, 12H); ¹³C-NMR (δ, ppm, 125 MHz, CDCl₃): 172.52, 172.50, 172.37, 159.26, 153.81, 151.17, 142.91, 142.85, 141.71, 141.62, 136.93, 131.45, 131.12, 130.78, 130.80, 130.70, 130.66, 130.32, 130.18, 129.81, 129.60, 129.44, 129.18, 129.03, 128.70, 128.53, 128.29, 128.12, 118.20, 115.97, 115.80, 115.37, 115.29, 114.66, 114.56, 114.48, 114.18, 113.80, 90.70, 85.06, 84.60, 81.88, 71.03, 69.69, 68.66, 64.65, 64.28, 64.03, 63.55, 43.98, 43.85, 43.74, 41.82, 41.64, 41.58, 40.85, 40.75, 39.93, 39.30, 38.40, 35.34, 35.28, 35.12, 35.05, 34.49, 34.44, 34.34, 34.20, 33.75, 33.65, 32.90, 30.28, 29.44, 29.24, 27.69, 27.42, 25.65, 25.19, 25.09, 25.03, 23.78, 19.99, 19.38, 19.25, 18.92, 11.82, 11.72,11.61; UV/Vis (chloroform) λ_{max} = 256 nm.

Polymer 4: (Yield = 25%) ¹H-NMR (δ , ppm, 500 MHz, CDCl3): 12.06 (s, 1H, NH), 7.61 (s, 1H), 5.94 (s, 1H), 5.76 (br s, 1H), 5.56 – 5.25 (br m, 380H), 4.97 (m, 40H), 4.65 (m, 1H), 4.42 (m, 1H), 4.18 (m, 1H), 4.11 – 4.03 (m, 2H), 2.63 (s, 4H), 2.14 – 1.13 (br m, 1109H), 0.86 (m, 12H); UV/Vis (chloroform) $\lambda_{max} = 256$ nm.

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General self-assembly procedure: All the polymeric building blocks (1, 2, 3 and 4) were thoroughly washed with Millipore water to remove any metal-cation impurity that may be present in the material (these metal-cation impurities can come from the multistep synthetic protocols necessary for the preparation of the building blocks). All the building blocks were then dried under high vacuum conditions and stored at (-30) °C.

A stock solution of KI was prepared in acetone. Required amount of the KI solution was placed in a glass vial and the acetone was removed completely under reduced pressure. Then, known amount of the desired polymer building block (1, 2, 3 and 4) was added to this vial. Chloroform was then added and the mixture was stirred overnight. Removal of chloroform gave access to the octameric assemblies, 1_8 .K⁺, 2_8 .K⁺, 3_8 .K⁺, 4_8 .K⁺ in the semi-solid form. These samples were the dried and used for further studies. However, it is difficult to say if trace amounts of water was present or absent from the systems due to the complexity in the 1-2 ppm region of the ¹H-NMR spectra. Concentrations of the arm and the octamer were based on their respective molecular weights.



Figure S1. ¹H-NMR spectra of **2** (bottom), with 1/8 eq KI (middle), and 1/2 eq KI (top) in CDCl₃. TMS is used as an internal standard.



Figure S2. ¹H-NMR spectra of **3** (bottom), with 1/8 eq KI (middle), and 1/2 eq KI (top) in CDCl₃. TMS is used as an internal standard.



Figure S3. ¹H-NMR spectra of **4** (bottom), with 1/8 eq KI (middle), and 1/2 eq KI (top) in CDCl₃. TMS is used as an internal standard.



Figure S4. Variable-temperature ¹H-NMR spectra of 2_8 .K⁺ in CDCl₃. TMS is used as an internal standard.



Figure S5. Variable-temperature ¹H-NMR spectra of $\mathbf{3}_{8}$.K⁺ in CDCl₃. TMS is used as an internal standard.



Figure S6. UV-Vis absorption spectra of **2** (a), **3** (b), and **4** (c) (0.0703 mM) as solid line and $\mathbf{2}_8$.K⁺ (a), $\mathbf{3}_8$.K⁺ (b), and $\mathbf{4}_8$.K⁺ (c) (0.0703 mM) as dashed line, in chloroform.



Figure S7. Variable-concentration ¹H-NMR spectra of $\mathbf{1}_{8}$.K⁺ in CDCl₃. TMS is used as an internal standard.



Figure S8. Variable-concentration ¹H-NMR spectra of 2_8 .K⁺ in CDCl₃. TMS is used as an internal standard.



Figure S9. Variable-concentration ¹H-NMR spectra of $\mathbf{3}_{8}$.K⁺ in CDCl₃. TMS is used as an internal standard.



Figure S10. Variable-concentration ¹H-NMR spectra of 4_8 .K⁺ in CDCl₃.



Figure S11. ¹H-NMR spectra of 2_8 .K⁺ upon addition of DMSO-d₆ in CDCl₃. TMS is used as an internal standard.



Figure S12. ¹H-NMR spectra of $\mathbf{3}_{8}$.K⁺ upon addition of DMSO-d₆ in CDCl₃. TMS is used as an internal standard.



Figure S13. MALDI-TOF mass spectrum of 1 (a), 2 (b), 3 (c), and 4 (d).