Electronic Supplementary Information

Non-natural G-quadruplex in a non-natural environment

*Shinaj K. Rajagopal and Mahesh Hariharan**

School of Chemistry, Indian Institute of Science Education and Research Thiruvananthapuram, CET Campus, Sreekaryam, Thiruvananthapuram, Kerala, INDIA 695016

E-mail: mahesh@iisertvm.ac.in or mahesh.hrn@gmail.com

Materials and methods

All chemicals were obtained from commercial suppliers and used as received without further purification. All reactions were carried out in glassware oven-dried prior to use and wherever necessary, were performed under dry nitrogen in dried, anhydrous solvents using standard gastight syringes, cannulae, and septa. Solvents were dried and distilled by standard procedures. TLC analyses were performed on precoated aluminium plates of silica gel 60 F254 plates (0.25 mm, Merck) and developed TLC plates were visualized under short and long wavelength UV lamps. Flash column chromatography was performed using silica gel of 200-400 mesh employing a solvent polarity correlated with the TLC mobility observed for the substance of interest. Yields refer to chromatographically and spectroscopically homogenous substances. Melting points were obtained using a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IRPrestige-21 FT-IR spectrometer as KBr pellets. ^{1}H and ^{13}C NMR spectra were measured on a 500 MHz Bruker advanced DPX spectrometer. Internal standard used for ^{1}H and ^{13}C NMR is 1,1,1,1-tetramethyl silane (TMS).

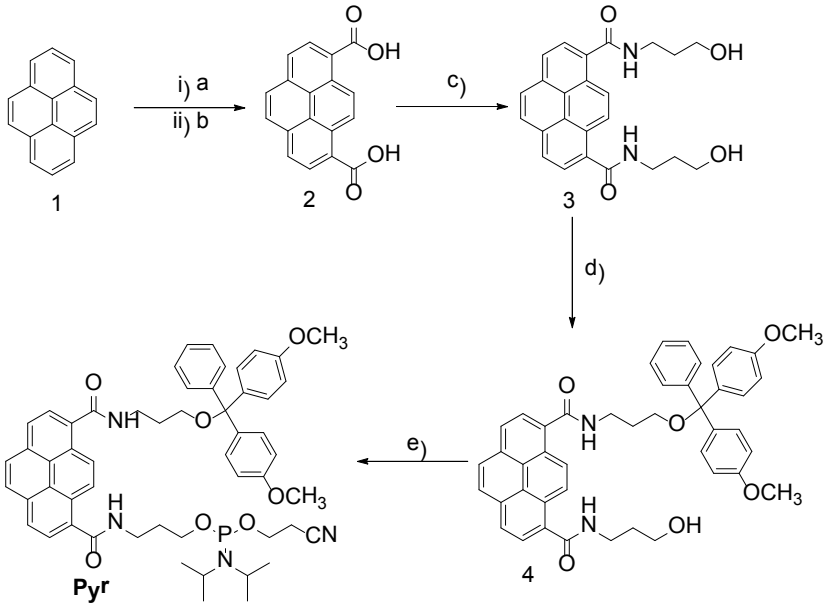
Oligonucleotides were synthesized on a K&A Laborgeraete DNA synthesizer. Oligonucleotides were synthesized on 1 μmol scale with appropriate controlled pore glass (CPG) beads used as 3' solid support using conventional phosphoramidite chemistry, following the procedure of Letsinger and Wu.[1] Oligonucleotides thus synthesized were isolated as trityl-on derivatives and purified using Reverse Phase High Performance Liquid Chromatography performed using a Shimadzu Prominence Liquid Chromatography with a Phenomenex column (Luna 5u C18(2) 100A, 250 x 10 mm) with a

gradient of 20 mM ammonium acetate buffer and acetonitrile with a flow rate of 1 ml/min. Molecular masses of the oligonucleotides were determined following desalting by means of MALDI-TOF mass spectroscopy on a Bruker Apex III MALDI spectrometer. Absorption, emission and circular dichroism spectra were recorded on Shimadzu UV-3600 UV-VIS-NIR, Horiba Jobin Yvon Fluorolog and Jasco J-815 spectrometers respectively. Water content of the DES is measured using the Karl Fischer Titrator, model: $\mu AquaCal_{100}$ from Analab Scientific Instruments Pvt. Ltd.

G-quadruplex sample preparation: Solution of oligonucleotides (5-7 μM) was prepared in either 10 mM cacodylate buffer (pH 7.2) containing 100 mM KCl or deep eutectic solvent (DES) medium containing 100 m*m* KCl. Prior to the analysis, G-quadruplex DNA was equilibrated by heating the oligonucleotide in the respective solvent for 5 minutes at 90 °C and then slowly cooling to room temperature. In the case of G-quadruplex in DES medium, the samples were subjected to vacuum centrifuge until a constant mass was reached (12 hrs).

Abbreviations used in the manuscript: m*m* is an abbreviation for millimolal while mM is an abbreviation for millimolar.

Experimental section



a) chloroacetyl chloride, $AlCl_3$, b) NaOH/Pyridine, 90 °C, 24 hrs c) $SOCl_2$/ C_6H_6, aminopropanol, THF d) DMT-Cl, pyridine, 70 °C e) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, diisopropylethylamine, dry DCM, room temperature.

Scheme S1 Shows the synthesis of pyrene phosphoramidite **Pyr**.

Preparation of Pyrene-1, 8-dicarboxylic acid [2]: Pyrene (**1**) (15 g, 0.07 mol) dissolved in CS_2 (yellow solution) was cooled to 0 °C (ice bath) and subsequently anhydrous $AlCl_3$ was added. Chloroacetyl chloride was then syringed slowly to the suspension and the ice bath was removed. Hydrogen chloride was liberated upon warming to room temperature. After 3 hrs, the mixture was added slowly to a vigorously stirred mixture of ice and concentrated HCl. The resulting suspension was filtered and vacuum dried (mixture of 1,1'-(pyrene-1,6-diyl)bis(2-chloroethanone) and 1,1'-(pyrene-1,8-diyl)bis(2-chloroethanone)). Acetic acid was added to this crude product and the mixture was refluxed for 1 hr and filtered. Filtration was repeated thrice. The filtrate was cooled to 0 °C and the precipitate yielded 1,1'-(pyrene-1,8-diyl)bis(2-chloroethanone). 1,1'-(pyrene-1,8-diyl)bis(2-chloroethanone) was then mixed with pyridine and was stirred for 3 hrs at 90 °C, cooled to room temperature and stirred overnight. The suspension was then filtered after cooling to 0 °C and the solid was dried. Then 14% NaOH was added and the suspension was heated to 90 °C and stirred for 3 hrs till it became red. After cooling to room temperature poured into ice water maintain pH 2 and dried to yield pyrene-1, 8 -dicarboxylic acid. mp >300°C; ^{1}H NMR [500 MHz, DMSO (D_6), δ] 9.29 (s, 2H),

8.66 (d, J= 8.1, 2H), 8.43 (d, J= 7.8, 2H), 8.35 (s, 2H). ^{13}C NMR [125MHz, DMSO (D_6), δ] 168.89, 133.01, 129.16, 129.01, 128.65, 126.19, 125.78, 125.60, 123.64. IR (KBr, cm^{-1}): 3012.81, 1674.21, 1271.09, 839.03. m/z 290.0584; calculated [$C_{18}H_{10}O_4^+$; 290.0581].

Preparation of N^1, N^8-bis(3-hydroxypropyl)pyrene-1,8-dicarboxamide [3]: A suspension of **2** in benzene, dimethylformamide and thionyl chloride was added and refluxed overnight. Removal of solvent at reduced pressure affords the corresponding acid chloride as a yellow residue. Tetrahydrofuran was added and the resulting slurry was slowly poured into a well stirred solution of aminopropanol and triethylamine in methanol at 0 °C. After warming to room temperature, the mixture was concentrated to 20 ml and poured into water. The solid was collected and washed successively with methanol to give the yellow product (yield = 63%). mp 152 °C. ^{1}HNMR [500 MHz, DMSO (D_6), δ]: 8.72 (t, J= 5.5, 2H), 8.54 (s, 2H), 8.38 (d, J= 7.8, 2H), 8.29 (s, 2H), 8.16 (d, J= 7.8, 2H), 4.60 (t, J= 5.1, 2H), 3.61 (q, J= 5.5, 4H), 3.51 (q, J= 6.5, 4H), 1.84 (m, 4H). ^{13}C NMR [125 MHz, DMSO (D_6), δ] 168.70, 132.66, 131.55, 128.04, 127.29, 125.45, 125.33, 125.10, 123.70, 58.69, 36.73, 32.45. IR (KBr, cm^{-1}): 3390.86, 3296.35, 2941.44, 2877.79, 1637.56, 1616.35, 1533.41, 1053.13. m/z 404.1743; calculated [$C_{24}H_{24}N_2O_4^+$; 404.1741].

Preparation of N^1-(3-bis(4-methoxyphenyl)(phenyl)methoxy)propyl-N^8-(3-hydroxypropyl) pyrene-1, 8-dicarboxamide [4]: To a well stirred solution of **3** in dry pyridine, 4, 4'-dimethoxytrityl chloride in pyridine was added and refluxed overnight and the resulting mixture was quenched with $NaHCO_3$, and washed thrice with water and purified by flash chromatography (yield= 52%). mp 182 °C . ^{1}HNMR [500 MHz, CD_2Cl_2, δ] 8.09 (t, J= 5.5, 2H), 7.21 (m, 21H), 3.76 (t, J= 5.5, 2H), 3.65 (m, 4H), 3.54 (s, 6H), 3.25 (t, J= 6, 2H), 1.98 (m, 2H), 1.84 (m, 2H). ^{13}C NMR [125 MHz, CD_2Cl_2, δ] 170.06, 169.33, 144.47, 135.94, 130.64, 130.50, 130.48, 130.22, 129.82, 129.31, 127.48, 127.25, 127.12, 126.30, 126.13, 124.09, 123.75, 123.44, 123.0, 122.43, 122.21, 112.48, 85.77, 61.60, 58.61, 38.17, 35.92, 32.06, 28.79. IR (KBr, cm^{-1}): 3398.57, 3257.77, 2927.94, 2870.08, 1631.78, 1537.27, 1508.33, 1031.92. m/z 706.8215; calculated [$C_{45}H_{42}N_2O_4^+$; 706.8213].

Preparation of 3-(((8-((3-bis(4-methoxyphenyl)(phenyl)methoxy)propylcarbamoyl)pyren-1-yl)methyl)amino)propyl (2-cyanoethyl) diiopropylphosphoramidite [Pyr]: 1 equivalent of **4** was dissolved in dry CH_2Cl_2 and 3 equivalent diisopropylethylamine. Then 1 equivalent of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite under N_2 atmosphere was added and stirred at room temperature for 1 hr. The reaction mixture was directly purified through column chromatography (silica gel CH_2Cl_2:MeOH (98:2) + 2% Et_3N) to give the phosphoramidite **Pyr** (yield= 22%). ^{1}HNMR [500 MHz, CD_2Cl_2, δ] 7. 01 (m, 21H), 3.58 (s, 6H), 3-3.3 (m, 22H), 2.35 (m, 2H), 2.03 (m, 2H), 1.04 (d, J= 6.8, 4H), 1.03 (d, J= 6.8, 4H). ^{13}C NMR [125 MHz, CD_2Cl_2, δ] 169.66, 169.61, 158.52, 145.28, 136.37, 131.23, 131.20, 131.0, 129.96, 128.96, 128.12, 127.84, 127.64, 126.70, 124.54, 123.0, 118.05, 117.22, 113.07, 86.15, 62.19, 61.85, 58.51, 55.12, 45.23, 43.14, 38.21, 37.73, 31.06, 29.74, 22.65, 20.39, 19.93. ^{31}P NMR [202MHz, CD_2Cl_2, δ] 149.89.

Table S1 m/z values of G-rich oligonucleotide **Pyr1-Pyr3** and the model oligonucleotide (**G_3T_3**) determined by MALDI-TOF mass spectroscopy.

Sequence	m/z calculated	m/z found
G_3T_3	6615.2	6617.3
Pyr1	6163.9	6159.7
Pyr2	5721.9	5723.3
Pyr3	5582.9	5583.4

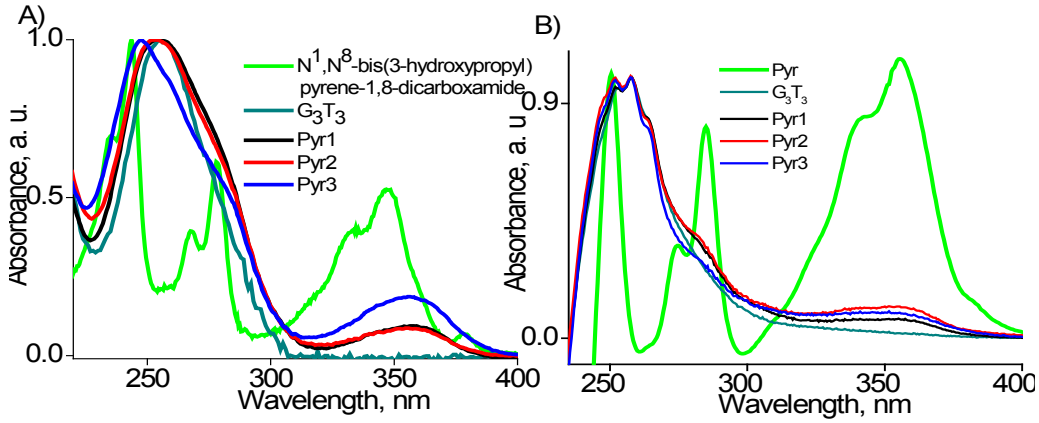


Fig. S1 Normalized absorption spectra of **N^1, N^8-bis(3-hydroxypropyl)pyrene-1,8-dicarboxamide**, **G_3T_3**, **Pyr1**, **Pyr2** and **Pyr3** in A) 10 mM sodium cacodylate buffer (pH 7.2) containing 100 mM KCl and B) Normalized absorption spectra of **Pyr**, **G_3T_3**, **Pyr1**, **Pyr2** and **Pyr3** in DES containing 100 m*m* KCl.

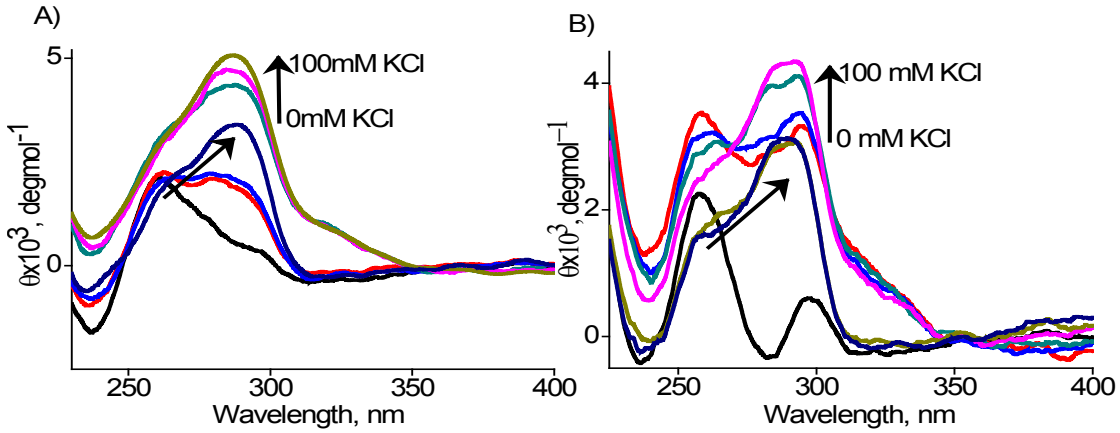


Fig. S2 Representative circular dichroism spectra of A) **Pyr2** and B) **Pyr3** in 10 mM sodium cacodylate buffer (pH 7.2) with increase in concentration of KCl.

References

1. R. L. Letsinger and T. Wu, Use of a stilbenedicarboxamide bridge in stabilizing, monitoring, and photochemically altering folded conformations of oligonucleotides, *J. Am. Chem. Soc.*, 1995, **117**, 7323-7328.