Electronic Supplementary Material (ESI) for RSC Advances

A Pyridyl Carboxamide Molecule Selectively Stabilizes G-quadruplex and Regulates Duplex-Quadruplex Competition

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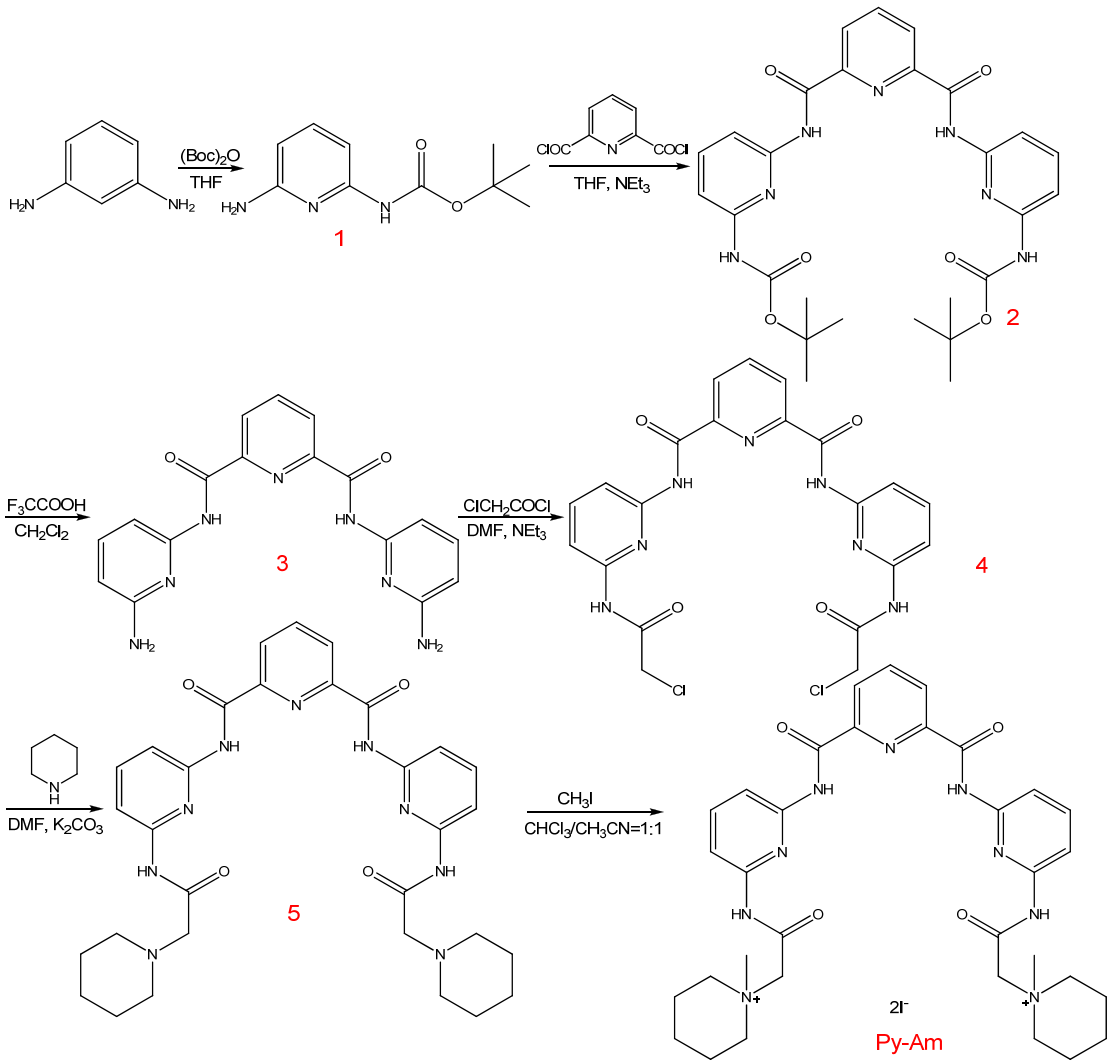
Supplementary Information

Synthesis of Py-Am.

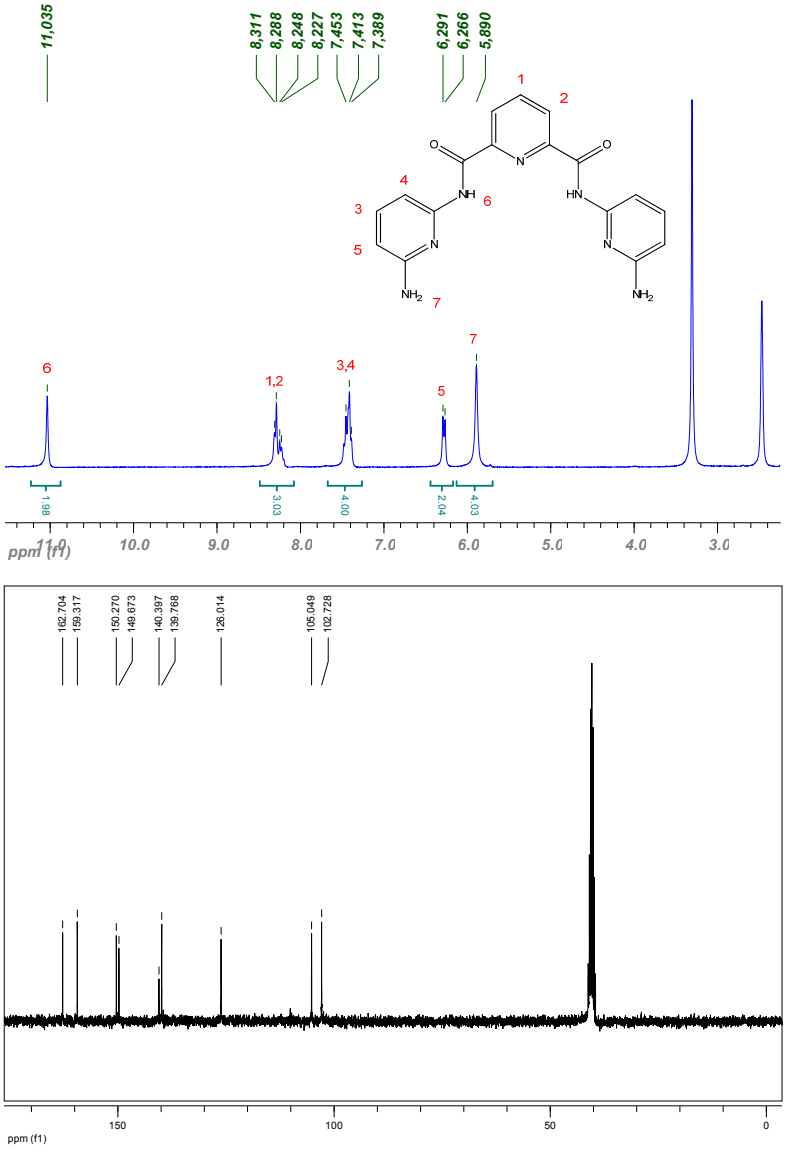


Compounds 1 and 2 were synthesized with the reported methods.[1]

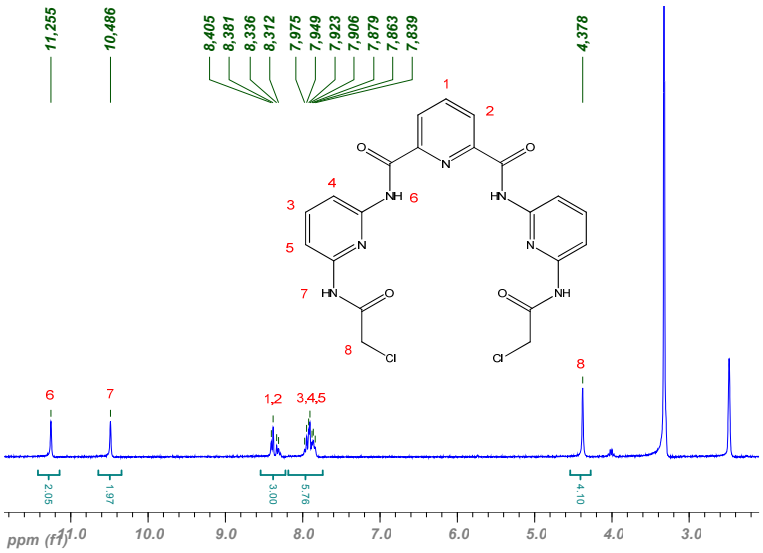
Synthesis of compound 3(2,6-Bis-(6-aminopyridin-2-ylcarbamoyl)pyridine). Compound 2 (1 g, 1.82 mmol) was dissolved into 20 mL CH_2Cl_2 under the ice bath. After the addition of 2 mL F_3CCOOH, the mixture was further stirred at r. t. for 12 h. The reaction mixture was concentrated under reduced pressure and then treated with 20 mL 2 mol/L NaOH. The precipitate was filtrated, washed with water, and finally dried to afford white powder compound 3 (620 mg, 97.5%).

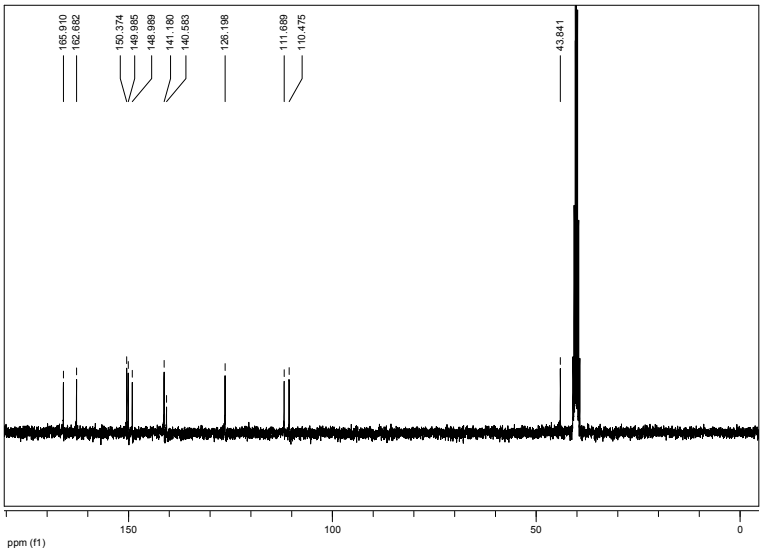
^{1}H NMR (300 MHz, [D6] DMSO) δ = 11.04 (s, 2H), 8.23-8.31 (m, 3H), 7.39-7.45 (m, 4H), 6.28 (d, *J* = 7.5 Hz, 2H), 5.89 (br, 4H) ppm.

^{13}C NMR (75 MHz, [D6] DMSO) δ = 162.7, 159.3, 150.3, 149.7, 140.4, 139.8, 126.0, 105.1, 120.7 ppm.
HRMS(ESI): m/z: calculated for $C_{17}H_{15}N_7O_2$: 350.1360 ($M+H^+$); found: 350.1365 ($M+H^+$).



Synthesis of compound 4 (2,6-Bis-(6-chloracetylaminopyridin-2-ylcarbamoyl)pyridine). Compound 3 (349 mg, 1mmol) was dissolved into the mixture of dry DMF (20 mL) and Et_3N (1 g). $ClCH_2COCl$ (600 mg) was then slowly added into the stirring mixture at r. t. The mixture was further stirred for 2 h, and then concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel ($CH_2Cl/CH_3COOCH_2CH_3$ = 3⁄1-2⁄1) to afford compound 4 as white powder (328 mg, 65.6%).
^{1}H NMR (300 MHz, [D6] DMSO) δ = 11.26 (s, 2H), 10.49 (s, 2H), 8.31-8.41 (m, 3H), 7.84-7.98 (m, 6H), 4.38 (s, 4H) ppm.
^{13}C NMR (75 MHz, [D6] DMSO) δ = 165.9, 162.7, 150.4, 150.0, 149.0, 141.2, 140.6, 126.2, 111.7, 110.5, 43.8 ppm.
HRMS(ESI): m/z: calculated for $C_{21}H_{17}N_7O_4Cl_2$: 502.0792 ($M+H^+$); found: 502.0800 ($M+H^+$).



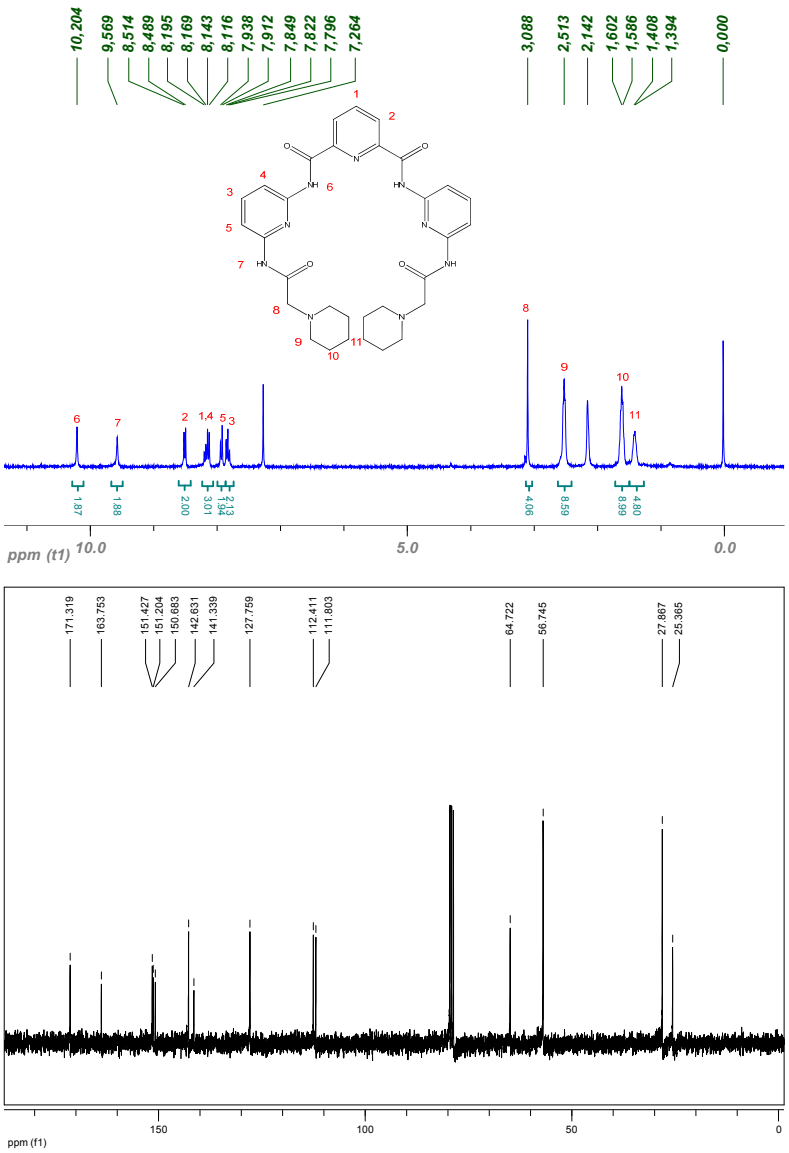


Synthesis of compound 5 (2,6-Bis-(6-piperidyl-*N*-acetylaminopyridin-2-ylcarbamoyl)pyridine). Excess piperidine (2 mL) was added into the mixture of compound 4 (251 mg, 0.5 mmol), DMF (20 mL) and K_2CO_3 (1 g). The mixture was stirred at 90 °C for 12 h, then filtered and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel (CH_2Cl_2/petroleum ether = 2/1) to afford compound 5 as white powder (140 mg, 46.7%).

^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.20 (s, 2H), 9.57 (s, 2H), 8.12-8.20 (m, 3H), 7.92 (d, *J* = 7.8 Hz, 2H), 7.82 (t, J = 7.8 Hz, 2H), 3.09 (s, 4H), 2.51 (br, 8H), 1.60 (br, 8H), 1.39 (br, 4H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.3, 163.8, 151.4, 151.2, 150.7, 142.6, 141.3, 127.8, 112.4, 111.8, 64.7, 56.7, 27.9, 25.4 ppm.

HRMS(ESI): m/z: calculated for $C_{31}H_{37}N_9O_4$: 600.3041 ($M+H^+$); found: 600.3037 ($M+H^+$).

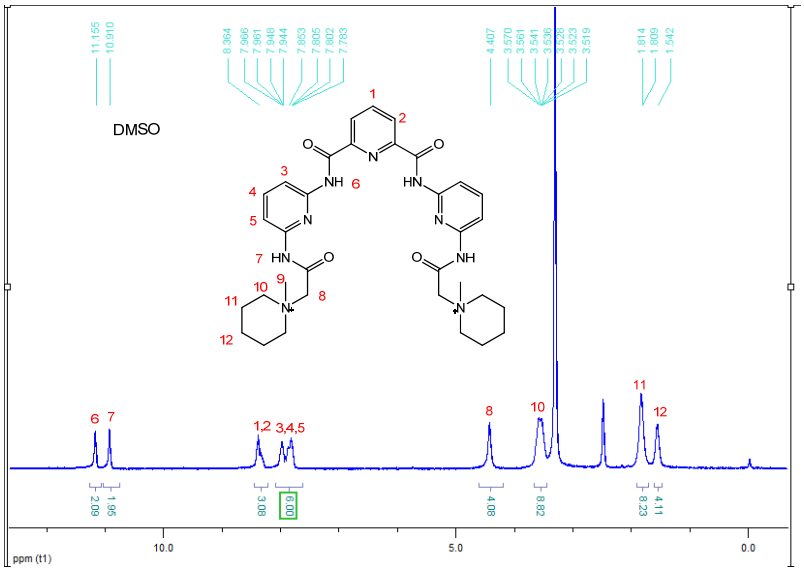


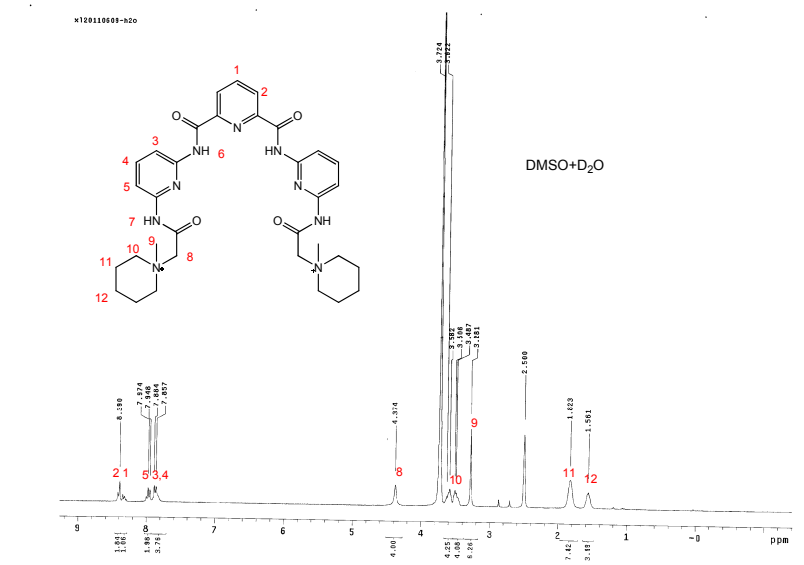
Synthesis of Py-Am (2,6-Bis-(6-piperidyl-*N*-methyl-*N*-acetylaminopyridin-2-ylcarbamoyl)pyridine iodide). Compound 5 (30 mg, 0.05 mmol) was dissolved into the solution of 10 mL $CHCl_3$/CH_3CN (1/1). CH_3I (1 mL) was added into the mixture under ice bath. The reaction mixture was then stirred at 20 °C for 18 h. The precipitate was filtrated under reduced pressure, washed with $CHCl_3$, and finally dried to afford white powder Py-Am (40 mg, 87.1%).

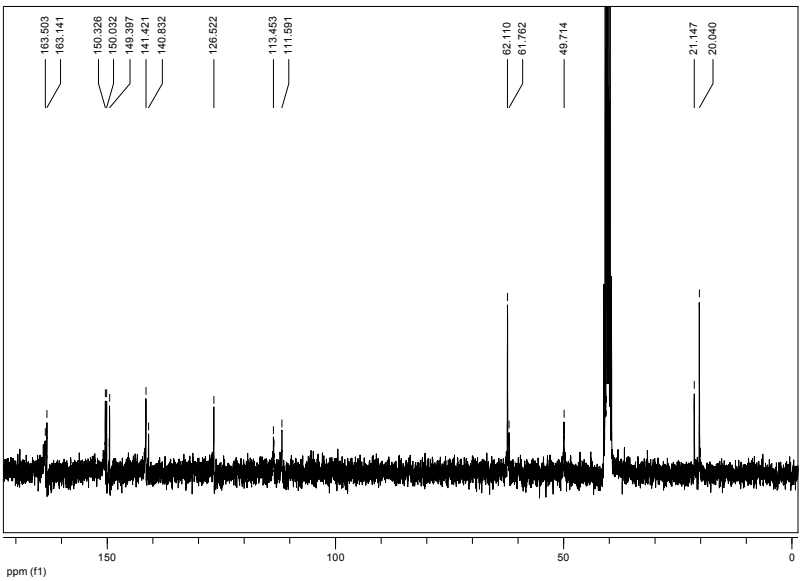
^{1}H NMR (300 MHz, [D6] DMSO) δ = 11.15 (s, 2H), 10.91 (s, 2H), 8.30-8.36 (m, 3H), 7.78-7.99 (m, 6H), 4.41 (s, 4H), 3.52-3.57 (m, 8H), 3.28 (s, 6H) from DMSO+D_2O spectrum, 1.81 (br, 8H), 1.54 (br, 4H) ppm.

^{13}C NMR (75 MHz, [D6] DMSO) δ = 163.5, 163.1, 150.3, 150.0, 149.4, 141.4, 140.8, 126.5, 113.4, 111.6, 62.1, 61.7, 49.7, 21.1, 20.0 ppm.

HRMS(ESI): m/z: calculated for $C_{33}H_{43}N_9O_4I_2$: 314.6714 ([M-2I$^-$]/2); found: 314.6715 ([M-2I$^-$]/2).







Figures.

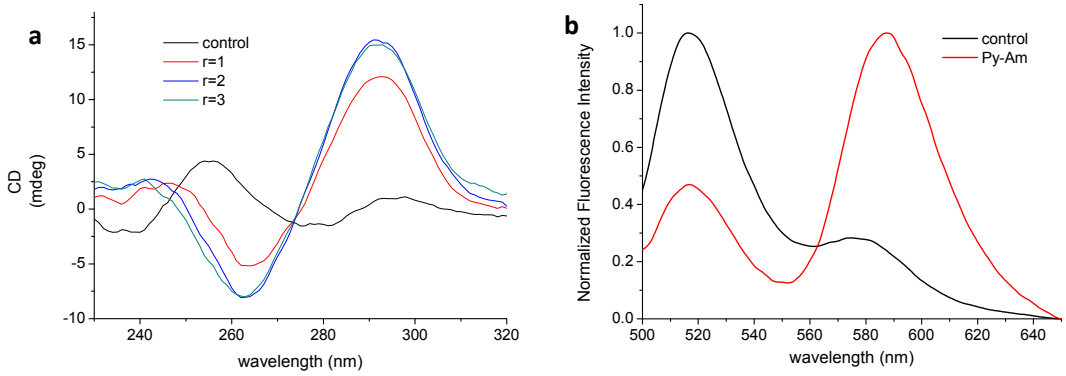


Fig. S1. G-quadruplex formation of the telomere DNA induced by Py-Am. a, CD spectra of G4A. Conditions: 20 μM G4A, 10mM Tris-HCl. r: mol ratio of Py-Am to G4A. b, FRET induced by Py-Am. Blue curve is the spectrum of the control sample containing 50 nM F21T, 10 mM Tris-HCl; red curve is the spectrum of the sample added 500 nM Py-Am.

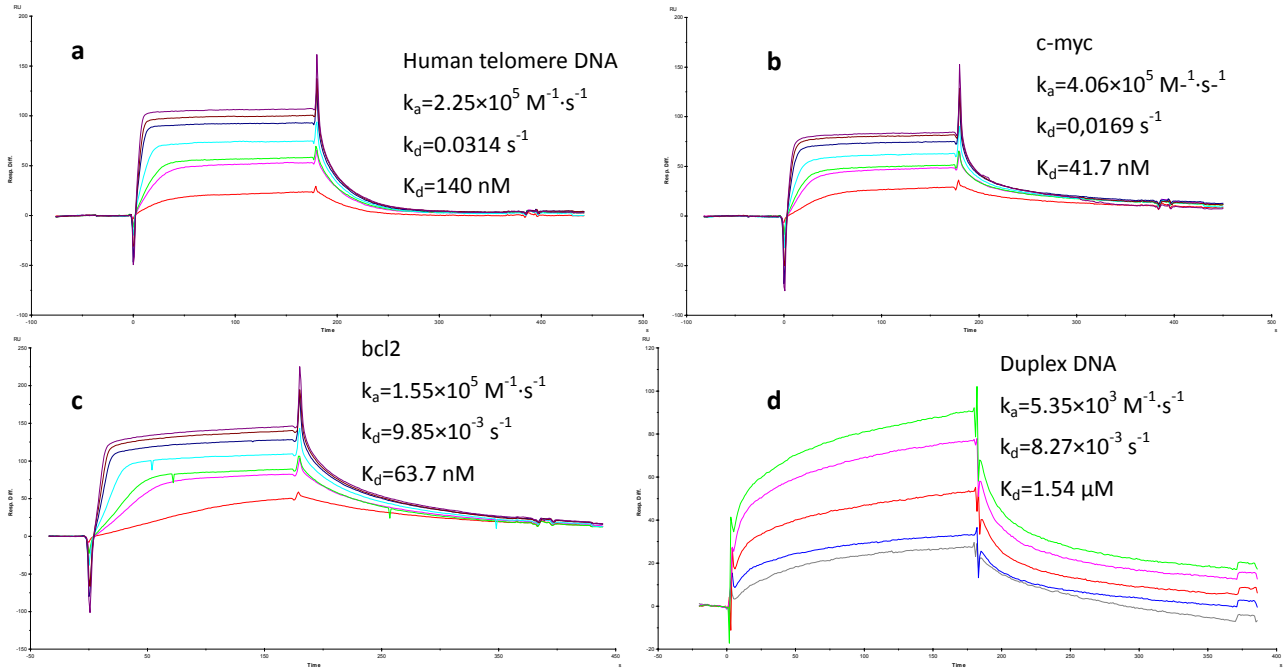


Fig. S2. SPR sensorgram overlay for binding of Py-Am to G-quadruplex (a, b and c) and duplex (d) at 25 °C. The unbound ligand concentrations in the flow solution were 50, 100, 150, 200, 400, 500, 600 nM from the lowest curve to the top curve for G-quadruplex, and the unbound ligand concentrations in the flow solution were 200, 300, 60, 800, 1200 nM from the lowest curve to the top curve for duplex.

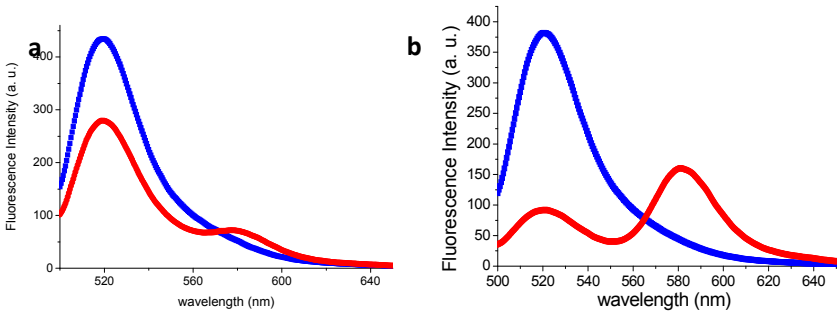


Fig. S3. G-quadruplex formation in the presence of Py-Am. a, fluorescence spectra of F-ckit-T in the absence (blue) and presence (red) of Py-Am. Measurements were carried out under these conditions: 50 nM F-ckit-T, 55 nM ckit-c, 100mM KCl and 10mM Tris-HCl buffer. Herein, the concentration of c-kit-c was a little more than that of F-ckit-T in order to make the F-ckit-T can totally form duplexes. 1μM Py-Am was added to study its transformation ability. The samples were all heated at 95 °C for 5 min and then slowly cooled down to the room temperature before measurement. b, fluorescence spectra of F21T in the absence (blue) and presence (red) of Py-Am. Measurements were carried out under these conditions: 50 nM F21T, 55 nM 21G-c, 100mM KCl and 10mM Tris-HCl buffer. Samples were treated with the same procedure as the former ones.

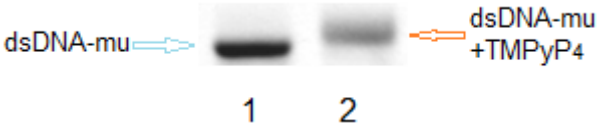


Fig. S4. Gel analysis of TMPyP4 with duplex DNA. The long dsDNA without G-quadruplex forming region (dsDNA-mu) was selected in this gel analysis experiment. Each sample included 1μM FAM labeled DNA. Lane 1: dsDNA-mu alone; Lane 2: dsDNA-mu treated with TMPyP4 (50 μM). Both the two samples were treated with heat denaturation and renaturation before loaded onto the gel. We could easily find that even if no G-quadruplex could form within this long duplex DNA, TMPyP4 could still interact with the duplex structure to make the duplex migrate a little slower. This result demonstrated that the slightly slower migration of the c-myc long dsDNA treated with TMPyP4 (see the text, Figure 5, Lane 7) was not because of the formation of G-qaudruplex but due to the interaction between TMPyP4 and the duplex structure.

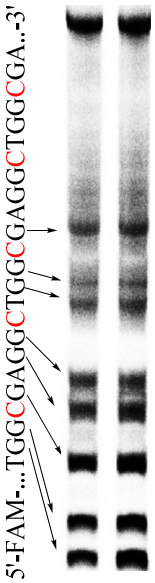


Fig. S5. DMS foot printing for dsDNA-mu. The left lane was the sample of the long dsDNA without Py-Am; the right lane was the sample treated with Py-Am. It was clearly that no difference between the samples in the absence and presence of Py-Am could be observed.

Ref:

1. E. Kolomiets, V. Berl, J. M. Lehn *Chem. Eur. J.* 2007, **13**, 5466–5479.