

## **Single-molecule visualisation of DNA G-quadruplex formation in live cells.**

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#### **Supplementary Reference**

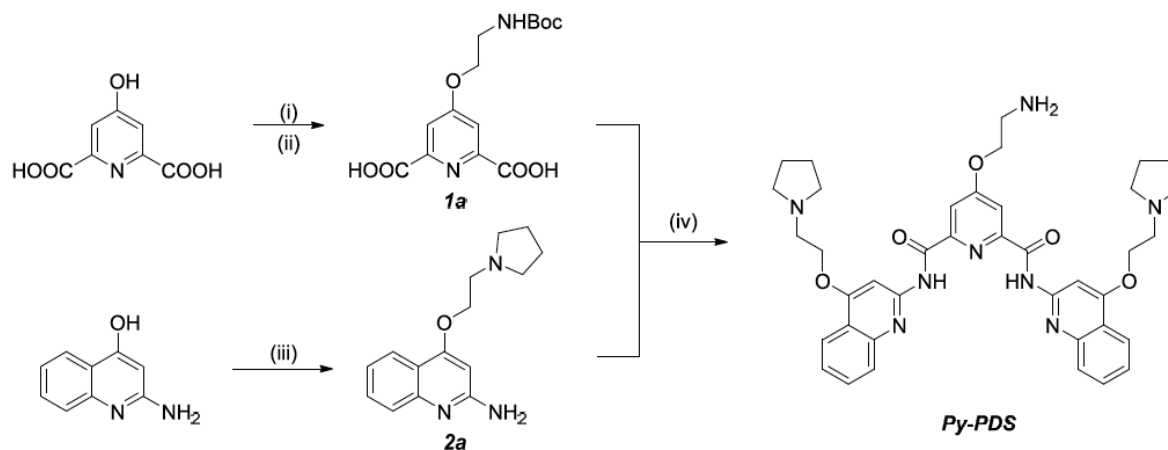
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## Synthesis of SiR-PyPDS analogues

All solvents and reagents were purified by standard techniques reported in Armarego, W.L.F., Chai, C.L.L., Purification of Laboratory Chemicals, 5th edition, Elsevier, 2003; or used as supplied from commercial sources (Sigma-Aldrich Corporation® unless stated otherwise). NMR spectra were acquired on Bruker® DRX-400, Bruker® DPX-400 and DRX-500 instruments using deuterated solvents as detailed and at ambient probe temperature (300 K). Chemical shifts ( $\delta$ ) are given in ppm and coupling constants in Hz.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra are referenced to the deuterated solvent residual peaks, namely  $\text{CDCl}_3$  (7.260 ppm; 77.16 ppm),  $\text{CD}_3\text{OD}$  (3.310 ppm and 4.780 ppm; 49.15 ppm). Notation for the  $^1\text{H}$ -NMR spectral splitting patterns includes: singlet (*s*), doublet (*d*), triplet (*t*), broad (*br*) and multiplet/overlapping peaks (*m*). Signals are quoted as  $\delta$  values in ppm, coupling constants (*J*), are quoted in Hertz and approximated to the nearest 0.5. For the assignments of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR DQF-COSY, HSQC and HMBC experiments were also performed. Data analysis for the nuclear magnetic resonance (NMR) spectra was performed using MestReNova® software, version 11.0.4. Mass spectra were recorded on a Micromass® Q-ToF (ESI) spectrometer. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 plates, and spots were visualized under UV light. Flash chromatography (FC) was performed using Merck Kieselgel 60 at rt under a positive pressure of nitrogen using previously distilled solvents. High performance liquid chromatography (HPLC) purification was carried out on all final compounds by using a Varian Pursuit C18, 5  $\mu$  column (250  $\times$  21.2 mm) and a gradient elution with  $\text{H}_2\text{O}/\text{MeCN}$  containing 0.1% TFA at a flow rate of 12.0 mL/min. Values were recorded on a Perkin Elmer® 241 polarimeter. All final compounds had a purity of  $\geq 95\%$  as assessed by LCMS.

### Synthesis of Py-PDS, as reported in supporting reference 1.

Unlike PDS, pyrrolidine-based side chains on the quinoline rings of the scaffold were employed in the synthesis of SiR-PyPDS and SiR-iPyPDS. This chemical modification ensured chemo-selective SiR-labelling of the central pyridine over the two quinolines of the scaffold.



**(i) Chelidamic acid dimethyl ester.** Chelidamic acid hydrate (10.0 g, 50.8 mmol) was dissolved in 100 ml  $\text{SOCl}_2$  and refluxed overnight. The solvent was removed *in vacuo* and a yellow solid was obtained, which was quenched with 100 mL of freshly distilled MeOH at 0 °C. The excess solvent was then removed *in vacuo*. The crude product was dissolved in 200 mL of a sat. aq.  $\text{NaHCO}_3$  solution and washed with 100 mL EtOAc. The aqueous layer was then recovered, acidified with conc. HCl to pH 4 and extracted with EtOAc. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and the solvent removed *in vacuo* to obtain the product as a white powder (3.4 g, 16.1 mmol, 29%).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta\text{H}$  7.39 (2H, br s), 3.92 (6H, s). The spectroscopic data were in agreement with the literature (2).

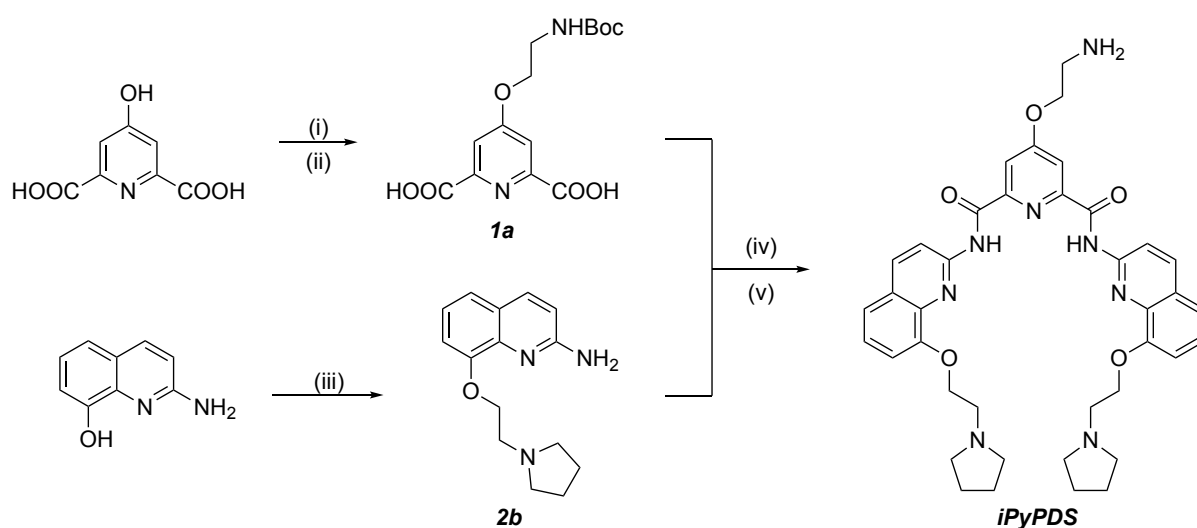
**(ii) 4-(2-((tert-butoxycarbonyl)amino)ethoxy)pyridine-2,6-dicarboxylate (1a).** Chelidamic acid dimethyl ester (1.5 g, 7.1 mmol), N-Boc-ethanolamine (1.7 g, 10.5 mmol) and triphenylphosphine (3.7 g, 14.1 mmol) were dissolved in 50 mL freshly distilled THF and cooled to 0 °C. DIAD (1.9 mL, 9.9 mmol) was added dropwise under argon. The mixture was allowed to warm to rt and stirred for 3 d. The solvent was removed *in vacuo* and the product purified by column chromatography (50% EtOAc, 50% petroleum ether) to obtain the dimethyl ester of the title compound as a white powder. This compound was dissolved in 50 mL MeOH and deprotected by slowly adding a solution of NaOH (0.4 g, 10.6 mmol) in 50 mL  $\text{H}_2\text{O}$ . The solvent was evaporated *in vacuo* and the remaining solid dissolved in  $\text{H}_2\text{O}$ . The solution was acidified with 5%  $\text{HCOOH}_{(\text{aq})}$  and extracted with EtOAc. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and the solvent removed *in vacuo* to obtain the title compound as a white powder (1.5 g, 4.6 mmol, 64%).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta\text{H}$  7.86 (2H, s), 4.26 (2H, t,  $J$  5.0 Hz), 3.49 (2H, t,  $J$  5.0 Hz), 1.42 (9H, s);  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta\text{C}$  169.4, 167.0, 158.4, 150.3, 115.2, 80.5, 69.6, 40.7, 28.6; HRMS (ES) calculated for  $\text{C}_{14}\text{H}_{17}\text{N}_2\text{O}_7$  ( $[\text{M}+\text{H}]^+$ )  $m/z$ : 325.1041, found 325.1046. The spectroscopic data were in agreement with the literature (2).

**(iii) 4-(2-(pyrrolidin-1-yl)ethoxy)quinolin-2-amine (2a).** 2-Amino-quinolinone (0.68 g, 4.2 mmol), N-(2-hydroxyethyl)-pyrrolidine (1.2 mL, 10.6 mmol, 2.5 equiv.) and triphenylphosphine (2.8 g, 10.6 mmol) were dissolved in 100 mL of freshly distilled THF and cooled to 0 °C. DIAD (2.1 mL, 10.6 mmol) was added dropwise under argon. The mixture was allowed to warm to rt and stirred for 4 d. The reaction was worked up by extracting three times with  $\text{HCl}_{(\text{aq})}$  0.1M, then the aqueous fraction was neutralized with  $\text{NaOH}_{(\text{aq})}$  and extracted again with DCM. The solvent was removed *in vacuo* and the product purified by column chromatography (89.5% EtOAc, 10% MeOH, 0.5% TEA) and left for 3 d under high vacuum to remove residues of alcohol and to obtain the title compound as a white powder (219 mg, 0.9 mmol, 21%).  $^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta\text{H}$  8.00 (1H, dd,  $J$  8.0, 1.0 Hz), 7.53 (1H, dd,  $J$  8.0, 1.5 Hz), 7.48 (1H, ddd,  $J$  8.0, 6.5, 1.0 Hz), 7.20 (1H, ddd,  $J$  8.0, 6.5, 1.5 Hz), 6.28 (1H, s), 4.33 (2H, t,  $J$  5.5 Hz), 3.10 (2H, t,  $J$  5.5 Hz), 2.81-2.73 (4H, m), 1.94-1.82 (4H, m);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta\text{C}$  163.0, 160.3, 148.0, 130.2, 124.1, 121.9, 121.6, 110.8, 90.4, 67.6,

54.8, 54.6, 23.3; HRMS (ES) calculated for  $C_{15}H_{20}N_3O$  ( $[M+H]^+$ )  $m/z$ : 258.1606, found 258.1611. The spectroscopic data were in agreement with the literature (2).

**(iv) 4-(2-aminoethoxy)-N2,N6-bis(4-(2-(pyrrolidin-1-yl)ethoxy)quinolin-2-yl)pyridine- 2,6-dicarboxamide (Py-PDS).** The pyridine dicarboxylate **1a** (56 mg, 0.17 mmol) was dissolved in DCM ( $\approx 0.5$  M) at 0 °C and 1-chloro-N,N,2-trimethylpropenylamine (47.6  $\mu$ L, 0.36 mmol, 2.2 equiv.) was slowly added. The reaction was allowed to stir at rt for 1.5 h checking the completion by LC-MS. The solution was then cooled to 0 °C and triethylamine (50.2  $\mu$ L, 0.36 mmol, 2.2 equiv.) was carefully added dropwise. The reaction was then allowed to warm to rt and it was kept stirring for another 1 h. The quinoline **2a** (92 mg, 0.36 mmol, 2.2equiv.) was added to the mixture as a DCM solution ( $\approx 1.0$  M) and stirred at rt overnight. The intermediate was nearly purified by flash column (94.5% DCM, 5% MeOH, 0.5% TEA) to obtain a crude that was treated overnight with a 1:10 mixture of TFA in DCM. The final compound was purified using HPLC (gradient: 10% MeCN/90%  $H_2O$ , 0.1% TFA to 100% MeCN, 0.1% TFA over 30 min,  $R_t$ =12.0-13.5 min) to afford the TFA salt of the title compound as a white powder (Py-PDS- $3CF_3CO_2H$ , 27.8 mg, 0.027 mmol, 16%).  $^1H$ -NMR (400 MHz,  $CD_3OD$ )  $\delta$  8.48 (2H, br d,  $J$  8.0 Hz), 8.21 (2H, s), 8.17 (2H, s), 8.12 (2H, br d,  $J$  8.0 Hz), 8.01 (2H, ddd,  $J$  8.0, 6.5, 1.0 Hz), 7.75 (2H, ddd,  $J$  8.0, 6.5, 1.0 Hz), 4.98-4.93 (4H, m), 4.69-4.63 (2H, m), 4.07-3.99 (4H, m), 3.97-3.85 (4H, m), 3.61-3.53 (2H, m), 3.48-3.34 (4H, m), 2.36-2.08 (8H, m);  $^{13}C$ -NMR (100 MHz,  $CD_3OD$ )  $\delta$  168.2, 167.2, 163.7, 151.0, 149.7 139.5, 134.4, 127.6, 123.5, 121.3, 118.8, 113.9, 94.7, 66.4, 65.7, 55.2, 53.6, 39.1, 23.1; HRMS (ES) calculated for  $C_{39}H_{45}N_8O_5$  ( $[M+H]^+$ )  $m/z$ : 705.3501, found 705.3510. The spectroscopic data were in agreement with the literature (1).

### Synthesis of iPyPDS



**(iii) 8-(2-(pyrrolidin-1-yl)ethoxy)quinolin-2-amine (2b).** 2-Amino-8-quinolinol (2.1 g, 13 mmol), N-(2-hydroxyethyl)-pyrrolidine (3.8 mL, 32 mmol, 2.5 equiv.) and

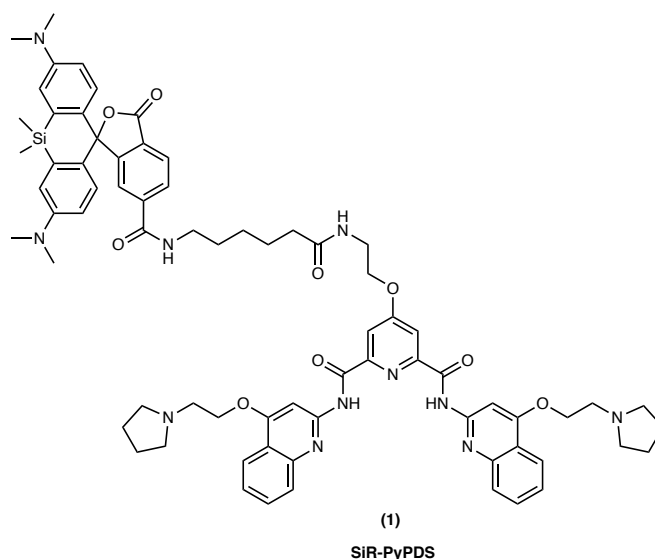
triphenylphosphine (8.5 g, 32 mmol) were dissolved in 100 mL of freshly distilled THF and cooled to 0 °C. DIAD (6.4 mL, 32 mmol) was then added dropwise under argon. The mixture was allowed to warm to rt and stirred for 4 d. The reaction was worked up by extracting three times with 0.1 M HCl<sub>(aq)</sub>, then the aqueous fraction was neutralized with NaOH<sub>(aq)</sub> and extracted again with DCM. The solvent was removed *in vacuo* and the product purified by column chromatography (89.5% DCM, 10% MeOH, 0.5% TEA) and left for 1 d under high vacuum to remove residues of alcohol and to obtain the title compound as a white powder (1.45 g, 5.7 mmol, 44%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 7.73 (d, *J* = 8.9 Hz, 1H), 7.15 (d, *J* = 7.3 Hz, 1H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 7.3 Hz, 1H), 6.68 (d, *J* = 8.9 Hz, 1H), 4.28 (t, *J* = 5.5 Hz, 2H), 3.12 (t, *J* = 5.4 Hz, 2H), 2.82 – 2.74 (m, 4H), 1.93 – 1.85 (m, 4H); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>) δ 158.0, 152.1, 137.9, 137.9, 123.9, 121.4, 120.1, 112.9, 109.8, 66.5, 55.0, 54.1, 23.6; HRMS (ES) calculated for C<sub>15</sub>H<sub>20</sub>N<sub>3</sub>O ([M+H]<sup>+</sup>) *m/z*: 258.1601, found 258.1594.

(iv) **4-(2-aminoethoxy)-N2,N6-bis(8-(2-(pyrrolidin-1-yl)ethoxy)quinolin-2-yl)pyridine-2,6-dicarboxamide (iPyPDS)**. The pyridine dicarboxylate 1b (50 mg, 0.15 mmol) was dissolved in DCM (≈ 0.5 M) at 0 °C and 1-chloro-N,N,2-trimethylpropenylamine (42.3 μL, 0.32 mmol, 2.2equiv.) was added slowly. The reaction was allowed to stir at rt for 1.5 h checking the completion by LC-MS. The solution was then cooled to 0 °C again and triethylamine (44.6 μL, 0.32 mmol, 2.2equiv.) was carefully added dropwise inside the solution and it was allowed to warm to rt and stirred for another 1 h. The quinoline 2b (82 mg, 0.32 mmol, 2.2equiv.) was added to the mixture as DCM solution (≈ 1.0 M) and stirred at rt overnight. The intermediate was nearly purified by flash column (79.5% DCM, 20% MeOH, 0.5% TEA) to obtain a crude that was treated overnight with a 1:10 mixture of TFA in DCM. The final compound was purified using HPLC (gradient: 10% MeCN/90% H<sub>2</sub>O, 0.1% TFA to 100% MeCN, 0.1% TFA over 30 min, Rt=12.0-13.5 min) to afford the TFA salt of the title compound as a white powder (iPyPDS·3CF<sub>3</sub>CO<sub>2</sub>H, 18.9 mg, 0.018 mmol, 12%). <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD) δ 8.58 (d, *J* = 8.9 Hz, 2H), 8.43 (d, *J* = 9.0 Hz, 2H), 8.17 (s, 2H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.59 (t, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 7.3 Hz, 2H), 4.62 – 4.57 (m, 6H), 3.70 – 3.62 (m, 4H), 3.57 – 3.49 (m, 4H), 3.31 – 3.28 (m, 4H), 3.09 – 2.09 (m, 2H), 1.93 – 1.73 (m, 8H); <sup>13</sup>C-NMR (126 MHz, CD<sub>3</sub>OD) δ 168.9, 163.9, 162.5, 162.2, 152.7, 151.8, 151.1, 141.7, 141.5, 128.9, 127.7, 122.2, 117.3, 113.6, 112.8, 66.6, 65.2, 55.1, 54.8, 39.7, 23.6; HRMS (ES) calculated for C<sub>39</sub>H<sub>45</sub>N<sub>8</sub>O<sub>5</sub> ([M+H]<sup>+</sup>) *m/z*: 705.3501, found 705.3487.

## CONJUGATION with SiR-C<sub>n</sub>

In a typical reaction, 30-50 equivalents of DIEA and 1.1 equivalents of a 100 mM solution of TSTU in DMSO are added to a DMSO solution of SiR-C<sub>n</sub>-COOH (Spirochrome ®) 1 equivalent. The colourless mixture is kept at rt for 5 min under shaking followed by addition of a solution of PDS analogue (1.1 equivalents of a 50 mM DMSO solution). The mixture is kept shaking for 4-18 hours until completion, as assessed by LC-MS. Afterwards, a double volume of milliQ water is added to the mixture and directly injected on HPLC (gradient: 10% MeCN/90% H<sub>2</sub>O, 0.1% TFA to 100% MeCN, 0.1% TFA over 30 min, Rt=11.0-14.5 min). The collected peaks are dried and dissolved in MeOD for spectroscopic characterization.

### SiR-PyPDS (1)

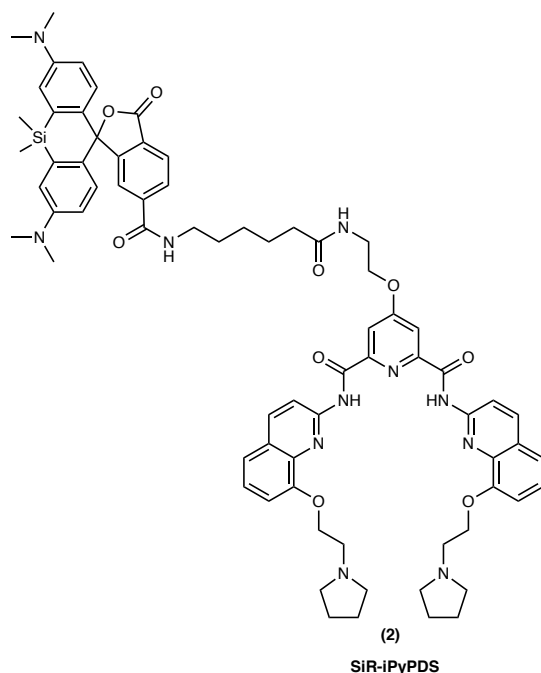


SiR-C6-COOH (183  $\mu$ L, 13.5 mM, 1.1 equiv.), DIEA (10  $\mu$ L, 30 eq), TSTU (25  $\mu$ L, 100 mM, 1.1 eq) and Py-PDS (45  $\mu$ L, 50 mM, 1 eq) were left react together for 12 hours following the general method above. The final compound was dried to afford the TFA salt of the title compound as an intense blue powder (SiR-C6-PyPDS $\cdot$ 2CF<sub>3</sub>CO<sub>2</sub>H, 1.94 mg, 1.29  $\mu$ mol, 58%). <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  8.40 (dd,  $J$  = 8.3, 0.8 Hz, 2H), 8.20 (s, 2H), 8.15 (d,  $J$  = 8.2 Hz, 1H), 8.06 (s, 2H), 8.05 – 8.00 (m, 3H), 7.90 (td,  $J$  = 8.4, 7.0, 1.4 Hz, 2H), 7.82 (d,  $J$  = 1.4 Hz, 1H), 7.66 (td,  $J$  = 8.1, 6.9, 1.1 Hz, 2H), 7.18 (d,  $J$  = 2.8 Hz, 2H), 6.89 (d,  $J$  = 9.4 Hz, 2H), 6.66 (dd,  $J$  = 9.4, 2.9 Hz, 2H), 4.83 (t,  $J$  = 4.6 Hz, 4H), 4.31 (t,  $J$  = 5.5 Hz, 2H), 3.97 (t,  $J$  = 4.6 Hz, 4H), 3.64 (t,  $J$  = 5.3 Hz, 2H), 3.37 (t,  $J$  = 6.9 Hz, 2H), 3.16 (s, 12H), 2.27 (t,  $J$  = 7.1 Hz, 2H), 2.19 (s, 8H), 1.75 – 1.66 (m, 2H), 1.66 – 1.59 (m, 2H), 1.46 – 1.36 (m, 2H), 0.68 (s, 3H), 0.52 (s, 3H). <sup>13</sup>C-NMR (126 MHz, CD<sub>3</sub>OD)  $\delta$  176.5, 169.7, 168.2, 165.1, 164.0, 162.3, 162.0, 152.8, 151.5, 146.1, 139.7, 133.2, 130.3, 128.8, 127.1, 126.4, 123.5, 120.5, 120.4, 114.9, 113.8, 95.7, 68.9, 66.1, 56.2, 54.8, 41.0, 40.8, 39.4, 36.7, 31.7, 30.0, 29.1, 27.4, 26.4, 24.0, 22.4, 13.3, -0.3, -1.9. HRMS (ES) calculated for C<sub>72</sub>H<sub>82</sub>N<sub>11</sub>O<sub>9</sub>Si<sup>+</sup> ([M+H]<sup>2+</sup>)  $m/z$ : 636.8067, found 636.8047.

The solid was dissolved in 129.2  $\mu$ L of DMSO (10 mM solution) and kept in the freezer

at -20 °C.

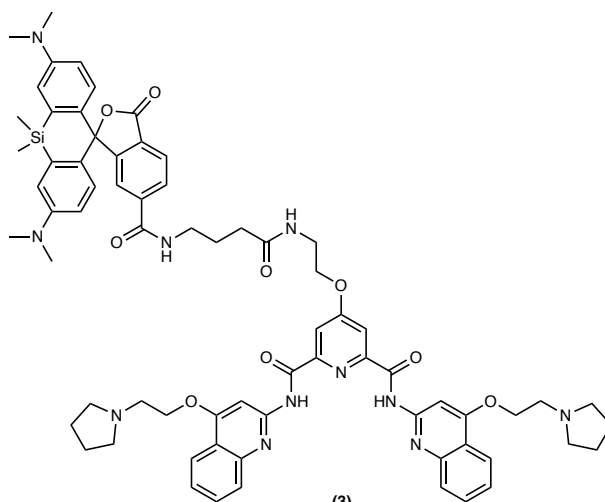
## SiR-iPyPDS (2)



SiR-C6-COOH (183  $\mu$ L, 13.5 mM, 1.1 equiv.), DIEA (10  $\mu$ L, 30 eq), TSTU (25  $\mu$ L, 100 mM, 1.1 eq) and iPyPDS (45  $\mu$ L, 50 mM, 1 eq) were left react together for 12 hours following the general method above. The final compound was dried to afford the TFA salt of the title compound as an intense blue powder (SiR-C6-iPyPDS-2CF<sub>3</sub>CO<sub>2</sub>H, 0.76 mg, 0.51  $\mu$ mol, 23%). <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  8.55 (d, *J* = 9.0 Hz, 2H), 8.43 (d, *J* = 8.9 Hz, 2H), 8.20 (d, *J* = 8.2 Hz, 1H), 8.10 (s, 2H), 8.06 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.75 (d, *J* = 1.8 Hz, 1H), 7.68 (dd, *J* = 8.3, 1.1 Hz, 2H), 7.60 (t, *J* = 8.0 Hz, 2H), 7.39 (dd, *J* = 7.9, 1.2 Hz, 2H), 7.29 (d, *J* = 2.9 Hz, 2H), 6.94 (d, *J* = 9.5 Hz, 2H), 6.73 (dd, *J* = 9.5, 2.8 Hz, 2H), 4.57 (t, *J* = 5.0 Hz, 4H), 4.38 (t, *J* = 5.4 Hz, 2H), 3.66 (dt, *J* = 5.4, 4.9 Hz, 6H), 3.38 (t, *J* = 7.2 Hz, 2H), 3.26 (s, 12H), 2.26 (t, *J* = 7.3 Hz, 2H), 1.83 (m, 8H), 1.65 (dq, *J* = 22.2, 7.6 Hz, 5H), 1.41 (p, *J* = 8.2 Hz, 2H), 0.85 (s, 1H), 0.65 (s, 3H), 0.58 (s, 3H). <sup>13</sup>C-NMR (126 MHz, CD<sub>3</sub>OD)  $\delta$  176.5, 169.7, 168.2, 165.1, 164.0, 162.3, 162.0, 152.8, 151.5, 146.1, 139.7, 133.2, 130.3, 128.8, 127.1, 126.4, 123.5, 120.5, 120.4, 114.9, 113.8, 95.7, 68.9, 66.1, 56.2, 54.8, 41.00, 40.8, 39.4, 36.7, 30.0, 27.4, 26.4, 24.0, -0.3, -1.9. HRMS (ES) calculated for C<sub>72</sub>H<sub>82</sub>N<sub>11</sub>O<sub>9</sub>Si<sup>+</sup>([M+H]<sup>+</sup>) *m/z*: 1272.6066, found 1272.6047.

The solid was dissolved in 50.6  $\mu$ L of DMSO (10 mM solution) and kept in the freezer at -20 °C.

### SiR-C4-PyPDS (3)



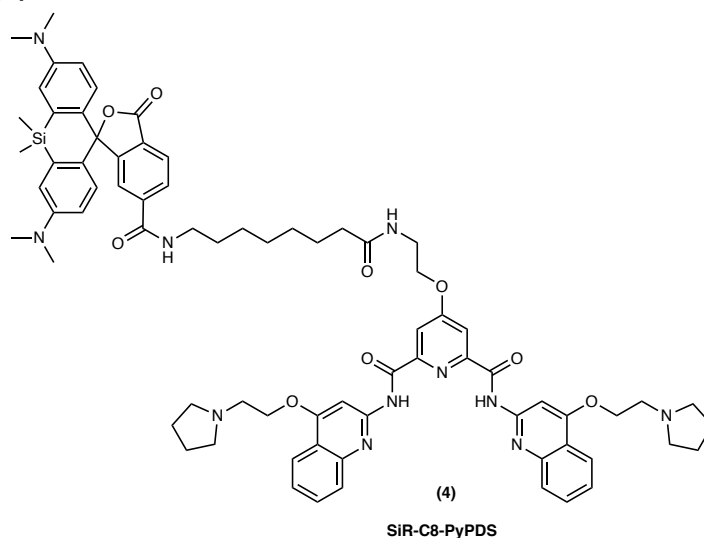
SiR-C4-PyPDS

SiR-C4-COOH (74  $\mu$ L, 13.5 mM, 1 equiv.), DIEA (5  $\mu$ L, 30 eq), TSTU (12  $\mu$ L, 100 mM, 1.1 eq) and Py-PDS (25  $\mu$ L, 50 mM, 1.1 eq) were left react together for 4 hours following the general method described above. The final compound was dried to afford the TFA salt of the title compound as an intense blue powder (SiR-C4-PyPDS $\cdot$ 2CF<sub>3</sub>CO<sub>2</sub>H, 1.06 mg, 0.72  $\mu$ mol, 72%). <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  8.43 (dd,  $J$  = 8.4, 0.8 Hz, 2H), 8.27 – 8.18 (m, 3H), 8.14 – 8.05 (m, 5H), 7.94 (ddd,  $J$  = 8.4, 7.0, 1.5 Hz, 2H), 7.81 (d,  $J$  = 1.6 Hz, 1H), 7.69 (ddd,  $J$  = 8.2, 6.9, 1.1 Hz, 2H), 7.23 (d,  $J$  = 2.9 Hz, 2H), 6.93 (d,  $J$  = 9.4 Hz, 2H), 6.70 (dd,  $J$  = 9.5, 2.9 Hz, 2H), 4.33 (t,  $J$  = 5.3 Hz, 2H), 4.04 – 3.95 (m, 4H), 3.65 (t,  $J$  = 5.3 Hz, 2H), 3.45 (t,  $J$  = 6.9 Hz, 2H), 3.20 (s, 12H), 2.36 (t,  $J$  = 7.0 Hz, 2H), 2.20 (s, 8H), 1.95 (p,  $J$  = 6.9 Hz, 2H), 0.66 (s, 3H), 0.55 (s, 3H). <sup>13</sup>C-NMR (126 MHz, CD<sub>3</sub>OD)  $\delta$  176.0, 169.8, 168.1, 165.7, 164.2, 161.7, 161.5, 152.7, 151.3, 144.9, 133.6, 129.0, 127.4, 125.6, 123.7, 120.7, 120.3, 115.0, 114.0, 95.7, 69.1, 66.4, 56.2, 54.7, 40.8, 39.7, 34.3, 31.7, 29.1, 26.0, 24.0, 22.4, 13.3, -0.4, -1.9. HRMS (ES) calculated for C<sub>70</sub>H<sub>78</sub>N<sub>11</sub>O<sub>9</sub>Si<sup>+</sup> ([M+H]<sup>+</sup>)  $m/z$ : 1244.5753, found 1244.5739.

The solid was dissolved in 71.9  $\mu$ L of DMSO (10 mM solution) and kept in the freezer at -20  $^{\circ}$ C.



## SiR-C8-PyPDS (4)



SiR-C8-COOH (50  $\mu$ L, 18 mM, 1 equiv.), DIEA (8  $\mu$ L, 50 eq), TSTU (11  $\mu$ L, 100 mM, 1.2 eq) and Py-PDS (27  $\mu$ L, 50 mM, 1.5 eq) were left react together for 18 hours following the general method above. The final compound was dried to afford the TFA salt of the title compound as an intense blue powder (SiR-C8-PyPDS $\cdot$ 2CF<sub>3</sub>CO<sub>2</sub>H, 0.99 mg, 0.65  $\mu$ mol, 72%). <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  8.40 (dd, *J* = 8.3, 0.8 Hz, 2H), 8.23 – 8.17 (m, 3H), 8.08 (s, 2H), 8.06 – 8.01 (m, 3H), 7.91 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 2H), 7.80 (d, *J* = 1.7 Hz, 1H), 7.66 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 2H), 7.20 (d, *J* = 2.8 Hz, 2H), 6.89 (d, *J* = 9.5 Hz, 2H), 6.66 (dd, *J* = 9.5, 2.9 Hz, 2H), 4.38 (t, *J* = 5.3 Hz, 2H), 4.00 – 3.94 (m, 4H), 3.69 (t, *J* = 5.3 Hz, 2H), 3.38 (t, *J* = 7.0 Hz, 2H), 3.20 (s, 12H), 2.37 – 2.03 (m, 10H), 1.44 – 1.20 (m, 10H), 0.69 (s, 3H), 0.52 (s, 3H). <sup>13</sup>C-NMR (126 MHz, CD<sub>3</sub>OD)  $\delta$  176.7, 169.7, 168.2, 165.3, 164.0, 161.7, 161.4, 154.4, 152.7, 151.4, 145.8, 133.3, 128.8, 127.2, 126.2, 123.6, 120.9, 120.3, 114.9, 113.8, 95.6, 69.0, 66.2, 56.2, 54.8, 41.2, 40.8, 39.4, 36.9, 31.8, 31.7, 31.5, 30.3, 30.1, 30.0, 29.4, 29.3, 29.1, 29.0, 28.8, 27.9, 26.8, 24.0, 22.7, 22.6, 22.4, 22.3, 22.1, 13.6, 13.4, 13.3, 13.1, 13.0, -0.4, -1.9. HRMS (ES) calculated for C<sub>74</sub>H<sub>86</sub>N<sub>11</sub>O<sub>9</sub>Si<sup>+</sup> ([M+H]<sup>+</sup>) *m/z*: 1300.6379, found 1300.6338.

The solid was dissolved in 64.7  $\mu$ L of DMSO (10 mM solution) and kept in the freezer at -20  $^{\circ}$ C.

## Supplementary methods

### Oligonucleotides used

All oligonucleotides were HPLC purified and used as supplied. Stock solutions of 100  $\mu$ M were prepared in MiliQ purified water. Unless otherwise stated all fluorescence measurements were taken at 20 °C on a Varian Cary Eclipse Fluorescence Spectrometer fitted with a Varian temperature control unit. The sequences used for the various experiments described in the manuscript are reported in Table 1.

Name	Supplier	Sequence (5' to 3')
FRET H-telo	Biomers	FAM-GGGTTAGGGTTAGGGTTAGGG-TAMRA
FRET c-Kit1	Biomers	FAM-GGG AGG GCG CTG GGA GGA GGG-TAMRA
H-telo complementary	Sigma	CCC TAA CCC TAA CCC TAA CCC
c-Kit1 complementary	Sigma	CCC TCC TCC CAG CGC CCT CCC
FRET2 H-telo	IDT	Cy5-AGGGTTAGGGTTAGGGTTAGGGAGAGGTAAAAG GATAATGG CCACGGTGC GGACGGC-Biotin
FRET2 Myc	IDT	Cy5-TGGGTGGGTAGGGTGGGAGAGGTAAAAGGATAAT GGCCACGGTGC GGACGGC-Biotin
FRET2 c-Kit1	IDT	Cy5-AGGGAGGGCGCTGGGAGGAGGGAGAGGTAAAAG GATAATGGCCACGGTGC GGACGGC-Biotin
FRET2 comp	IDT	GCCGTCCGCACCGTGGCCATTATCCTTT-Cy3-TACCTCT
c-Kit1	Sigma	AGG GAG GGC GCT GGG AGG AGG G
Myc	Sigma	TGG GTG GGT AGG GTG GGT AA
h-Telo	Invitrogen	A GGG TTA GGG TTA GGG TTA GGG T
dsDNA	Invitrogen	CAA TCG GAT CGA ATT CGA TCC GAT TG
MYC-mut	Invitrogen	T TGA GTG TGT GTA GTG TGT GTA AA
MYC for sm-binding	Invitrogen	Biotin-TGA GGG TGG GTA GGG TGG GTA A-3'-Alexa488
MYC-mut for sm-binding	Invitrogen	Biotin-TGA GTG TGT GTA GTG TGT GTA A-3'-Alexa488

**Table 1:** Oligonucleotide sequences used in this study.

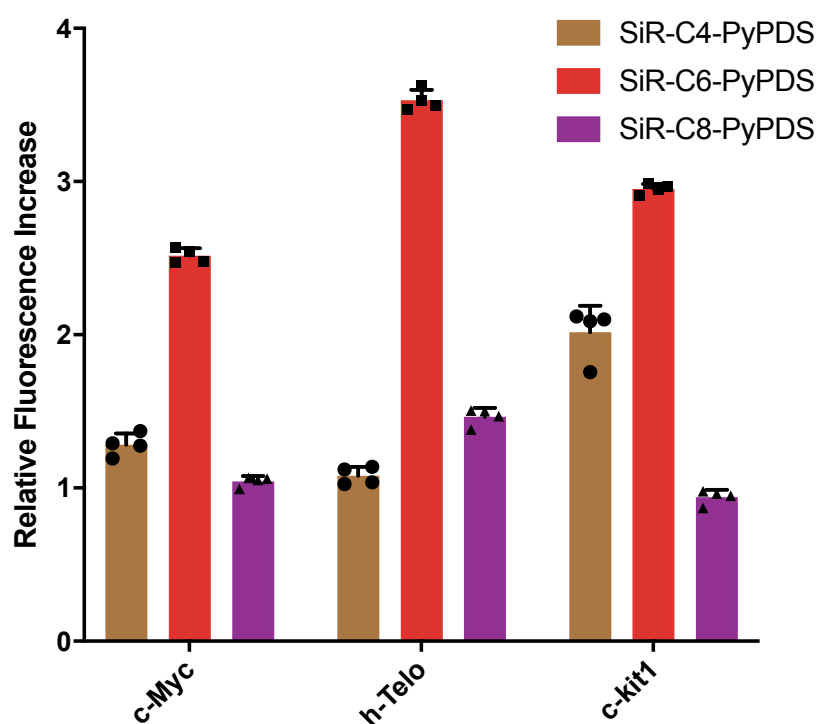
All oligonucleotides were HPLC purified and used as supplied. Stock solutions of 100  $\mu$ M were prepared in MiliQ purified water. Unless otherwise stated all fluorescence measurements were taken at 20 °C on a Varian Cary Eclipse Fluorescence Spectrometer fitted with a Varian temperature control unit.

### Growth inhibition

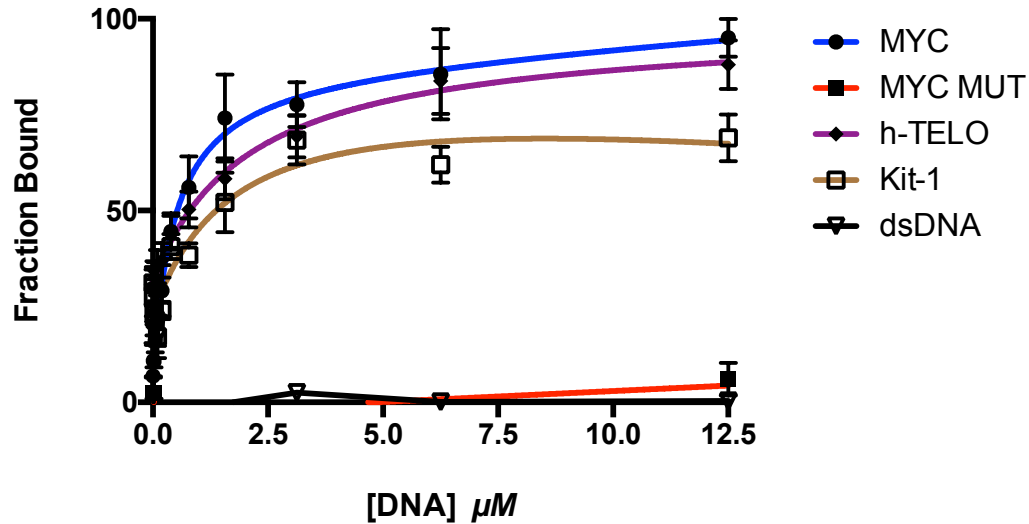
SiR-PDS and SiR-iPDS were stocked at 10mM concentration, dissolved in DMSO (Thermofisher Scientific, cat# 20688). U2OS in a density of 40,000 cells/ml were seeded one night before they were treated with serial dilutions of SiR-PDS and SiR-iPDS (the maximum concentration for both drugs were 50 $\mu$ M) for 24 h. Cell viability

was then determined via a CellTiter-Glo One Solution assay (Promega, cat # G8461) according to manufacturer's protocol. Each serial dilution has 4 replications. Curves were plotted averaging the 4 replicates in Prism (GraphPad v6) using a non-linear regression model, "dose-response – inhibition" equation [log(inhibitor) vs. normalised response - variable slope] and GI<sub>50</sub> values calculated.

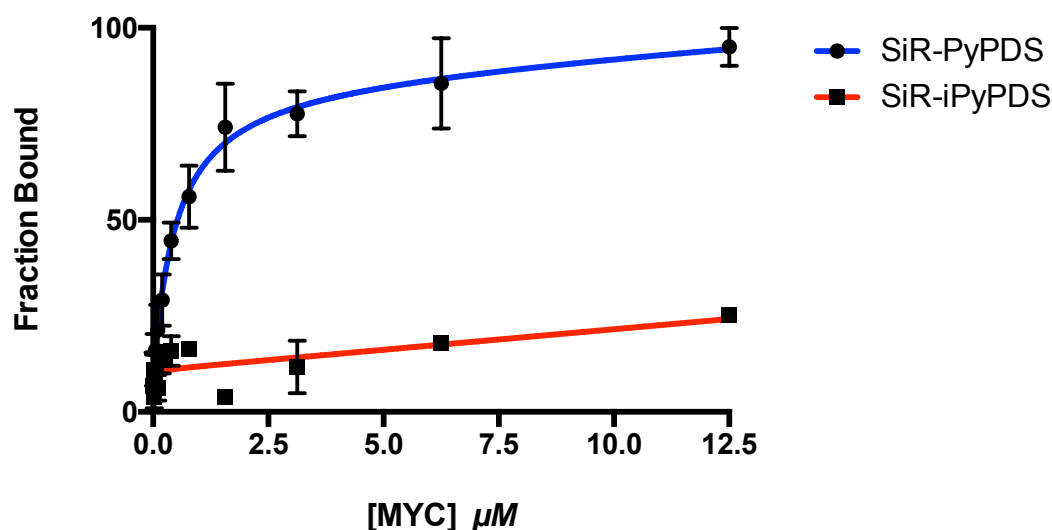
## Supplementary Figures



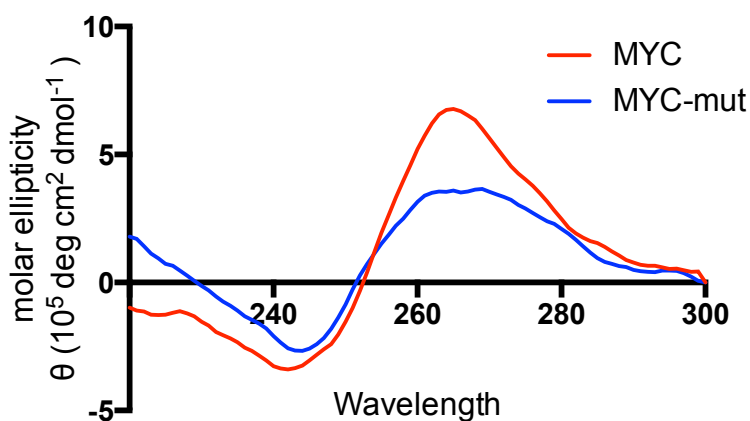
**Supplementary Figure 1: Optimization of the linker length between PyPDS and SiR.** Fluorescence light-up measurements obtained with 100 nM solutions of SiR-C4-PyPDS, SiR-C6-PyPDS and SiR-C8-PyPDS in the presence of different oligonucleotides (10 mM), as described in the methods section: "SiR-PyPDS analogues G4-binding comparison". Varying the linker length between SiR and the PyPDS scaffold revealed that the highest fluorescence light-up response was observed for the C6 linker in the presence of G4-folding oligonucleotides. The data are plotted as the ratio of the fluorescence intensity emission of each SiR-compound (100 nM) measured at 633 nm in the presence of 10 mM G4-oligos (c-Myc, hTelo and c-kit1), over the fluorescent emission measured at 633 nm for the same SiR-analogue (100 nM) in buffer only. Data are plotted as the average of 4 independent replicates. Error bars indicate mean  $\pm$  sd.



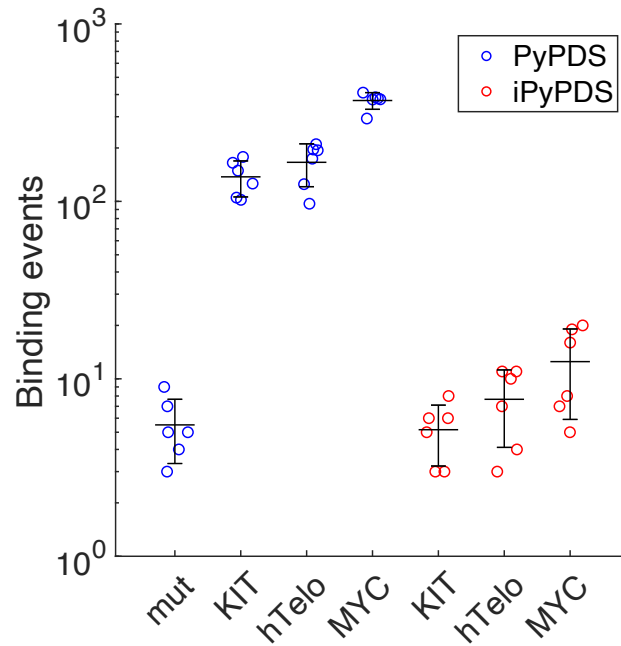
**Supplementary Figure 2: SiR-PyPDS fluorescence light-up response upon G4-binding.** Fluorescence titration of SiR-PyPDS in presence of different oligonucleotides, as described in the methods section: “Fluorescence titrations”. SiR-PyPDS displayed a selective light-up response upon titration with G4-folding oligonucleotides, whereas negligible fluorescence increase was measured upon titration with ssDNA and dsDNA. Data are plotted as the ratio of the SiR fluorescence emission at 633 nm for every titration point over the emission measured in buffer alone and normalised to the highest fluorescence emission measured (100%). The data are plotted as an average of 3 independent replicates. Error bars indicate mean  $\pm$  sd. SiR-PyPDS measured apparent  $K_d$  are: **MYC**  $0.63 \pm 0.08 \mu\text{M}$ ; **Kit-1**  $1.0 \pm 0.1 \mu\text{M}$ ; **h-Telo**  $2.0 \pm 0.8 \mu\text{M}$ ; Non-ambiguous values cannot be calculated for dsDNA and MYC-mutant.



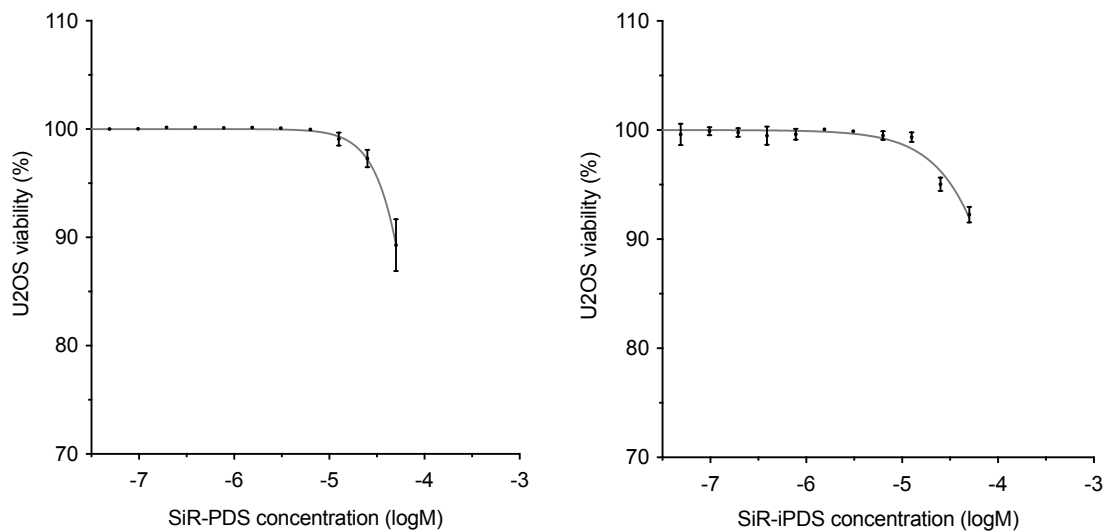
**Supplementary Figure 3: Differential G4-binding of SiR-PyPDS and SiR-iPyPDS.** Fluorescence titrations obtained exciting at 633nm a solution of either SiR-PyPDS or SiR-iPyPDS in the presence of increasing concentrations of a G4-folded oligonucleotide (MYC), as described in the methods section: “Fluorescence titrations”. SiR-PyPDS displayed a selective light-up response upon G4-binding whereas SiR-iPyPDS showed negligible fluorescence emission variation upon titration with either oligonucleotide. Data are plotted as average of 3 independent replicates and using the same normalisation processes of Supplementary Figure 2. Error bars indicate mean  $\pm$  sd.



**Supplementary Figure 4: Circular Dichroism analysis of MYC oligonucleotides used for *in vitro* experiments:** CD spectra trace confirming folded state of the G4-MYC oligonucleotide used for surface binding experiments and unfolded state of the mutant version MYC-mut both used at 10  $\mu\text{M}$  concentration and annealed in 100 mM  $\text{K}^+$  buffer pH = 7.4. This experiment has been repeated 3 times with consistent outcomes.



**Figure 5: Binding of SiR-PyPDS to different quadruplexes in vitro.** The number of binding events observed for SiR-PyPDS and SiR-iPyPDS binding to G4s in vitro varies for different G4 sequences (250 pM concentration of both probes in all cases except for MYC where it was 25 pM). Error bars indicate mean  $\pm$  sd.  $n = 6$  measurements for each condition.



**Figure 6: Growth inhibition curves obtained with SiR-PyPDS (left) and SiR-iPyPDS (right) in U2OS cells.** Growth inhibition studies indicate that no significant cellular toxicity is elicited by treatment of either SiR-PyPDS or SiR-iPyPDS over a 24 h treatment for doses up to 10  $\mu$ M. Error bars indicate mean  $\pm$  sd.  $n = 4$  measurements taken from 4 independent replicates of each treatment condition.

## Supporting References.

- (1) *Organic & Biomolecular Chemistry*, 10, 6537–6546, 2012.
- (2) *Dalton Transactions*, 6, 862–871, 2004.