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# **Supporting Information**

# Visible-light photoswitching of ligand binding mode suggests Gquadruplex DNA as a target for photopharmacology

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# Contents

1	Exp	erimental details	3		
	1.1	FRET melting assays	3		
	1.2	Circular dichroism titrations	5		
	1.3	UV-visible spectroscopy	5		
	1.4	NMR spectroscopy of G-quadruplexes	5		
	1.5	Photoirradiation experiments	6		
	1.6	Notes on procedure – avoiding unwanted photoisomerization	6		
	1.7	Determination of apparent dissociation constants	7		
	1.8	Molecular dynamics simulations	8		
	1.9	Cytotoxicity studies	9		
2	Sup	plementary tables	10		
	Tab	le S1: FRET stabilisation values for compound <b>1o</b>	10		
	Tab	le S2: FRET stabilisation values for compound <b>1c</b>	10		
3	Sup	Supplementary figures12			
4	Ger	General synthetic route to ligand <b>10</b>			
5	Syn	Synthetic procedures and compound characterisation			
6	Ref	References3			

### 1 Experimental details

#### 1.1 FRET melting assays

Fluorescence resonance energy transfer (FRET) melting assays were performed according to the procedure reported by De Cian and co-workers<sup>[1]</sup> on Roche LightCycler 480 qPCR instrument. In these assays, the oligonucleotides of interest were obtained labelled at the 5' and 3' ends with FAM (a fluorescence donor) and TAMRA (a fluorescence quencher) respectively. In the folded state, proximity of the donor and quencher mean that FAM fluorescence is not observed since energy is transferred non-radiatively to TAMRA by FRET. As the temperature is raised and the secondary structure denatures, the fluorophores move further apart and hence the fluorescence signal increases. From the resulting curve, the characteristic melting temperature ( $T_{1/2}$ ) is defined as that at which the normalised fluorescence signal equals 0.5. The change in melting temperature ( $\Delta T_m$ ) induced by a small molecule ligand compared to that of the oligonucleotide in the absence of ligand provides an indication of the ligand's ability to stabilise the G4 structure. The assay is shown in schematic form below:



All oligonucleotides used were purchased from Eurogentec (Belgium), purified by HPLC and delivered dry. Oligonucleotide concentrations were determined by UV-absorbance using a NanoDrop 2000 Spectrophotometer from Thermo Scientific. The oligonucleotides used were:

DNA model	Sequence		
F21T (human telomeric G4)	5'-FAM-GGGTTAGGGTTAGGGTTAGGG-TAMRA-3'		
F10T (duplex)	5'-FAM-TATAGCTATA-HEG-TATAGCTATA-TAMRA-3'		
ds26			

FAM = 6-carboxyfluorescein; TAMRA = 6-carboxy-tetramethylrhodamine; HEG = [(-CH<sub>2</sub>CH<sub>2</sub>O-)<sub>6</sub>]

wavelength (Figure S0 below).

All sequences were annealed before use by heating for 2 minutes at 90°C and then placed immediately into ice. The final concentration of oligonucleotide was 200 nM in all cases. The buffer used depended on the sequence in question, for F21T in Na<sup>+</sup> conditions, the final buffer contained 100mm NaCl, and 10 mm Li cacodylate. F21T in K<sup>+</sup> conditions and F10T, 10 mM KCl, 90 mM LiCl and 10 mM Li cacodylate were used. Ligand concentrations were either 1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M or 10  $\mu$ M. Each sample was tested in duplicate on the same plate, and each plate was repeated in at least duplicate to assess the reproducibility of all results. Oligonucleotide denaturation was detected by monitoring FAM fluorescence ( $\lambda_{ex}$  = 494 nm,  $\lambda_{em}$  = 518 nm). Control experiments ensured that the compounds **10/1c** did not display fluorescence upon excitation at this

Appropriate control experiments were also carried out for each sample set. Data processing was carried out using Origin 9, with  $\Delta T_{1/2}$  used to represent  $\Delta T_m$ .



Figure S0: Emission spectra of (a) **1o** and (b) **1c** excited at different wavelengths. Neither isomer is emissive when irradiated at the wavelength of the FAM dye (495 nm). The ligand concentration was 50  $\mu$ M in 100 mM potassium phosphate buffer, pH 7.4.

### 1.2 Circular dichroism titrations

Circular Dichroism (CD) titrations were recorded using a Jasco J-810 spectrometer fitted with a Peltier temperature controller. Measurements were taken in a quartz cuvette with a path length of 5 mm, at 20 °C, at a 100 nm / min scanning speed at 1 nm intervals, with a 1 nm bandwidth. The CD spectra were recorded between 800 and 200 nm, and baseline corrected for the buffer used. The oligonucleotide sequences used were: telo23: (hybrid model, 5'-TAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3') and telo22 (antiparallel model, 5'-AGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3'). The oligonucleotides were purchased from Eurogentec (Belgium), purified by HPLC and delivered dry. Oligonucleotide concentrations were determined by UV-absorbance using a NanoDrop 2000 Spectrophotometer from Thermo Scientific. The oligonucleotide was annealed before use by heating for 2 minutes at 90°C and then placed immediately into ice. The oligonucleotide was at a concentration of 4.2  $\mu$ M which gave an OD of 1 and the buffer used was either sodium (telo22) or potassium (telo23) phosphate (100 mM, pH 7.4). The ligand was added by aliquot from a 1mM stock solution in the appropriate buffer (containing 10% DMSO to ensure solubility). The reported spectrum for each sample represents the average of 3 scans. Data processing was carried out using Prism 7 with an 8-point second order smoothing polynomial applied to all spectra. Observed ellipticities were converted to mean residue ellipticity ( $\theta$ ) = deg cm<sup>2</sup> dmol<sup>-1</sup> (molar ellipticity).

#### 1.3 UV-visible spectroscopy

UV spectra were recorded on a Thermo Scientific BIOMATE 3S UV-vis Visible Spectrophotometer at ambient temperature. Measurements were taken in a 3 mL quartz cuvette with a path length of 10 mm. The UV-visible spectra were recorded between 800 nm and 200 nm and baseline corrected for the buffer used.

### 1.4 NMR spectroscopy of G-quadruplexes

<sup>1</sup>H NMR spectra of G-quadruplex sequences were recorded at 298 K using a 600 MHz Varian VNMRS spectrometer equipped with a triple resonance cryogenically cooled probe head. The oligonucleotide sequences used were: telo23: (hybrid model, 5'-TAGGGTTAGGGTTAGGGTTAGGG-3') and telo22 (antiparallel model, 5'-AGGGTTAGGGTTAGGGTTAGGGTTAGGG-3'). Samples of oligonucleotide were dissolved in 90% H<sub>2</sub>O/10% D<sub>2</sub>O containing either 25 mM sodium phosphate (pH = 7.0) and 70 mM sodium chloride (for telo22) or 20 mM potassium phosphate (pH = 7.0) and 70mM potassium chloride (for telo23). All experiments employed sculpted excitation water suppression. The final NMR samples contained 600  $\mu$ L of 185  $\mu$ M oligonucleotide. Samples were annealed before use by heating for 2 minutes at 90°C and then placed immediately into ice.

Aliquots of ligand (10 mM in DMSO- $d_6$ ) were added to the appropriate yield titration points, the sample was mixed thoroughly and NMR spectra were recorded immediately after the addition of ligand. Data were processed using MestReNova software (version 11.0.2). Resonances were assigned from data provided in the literature by Wang and Patel (telo22)<sup>[2]</sup> and by Patel *et al.*<sup>[3]</sup> Photoirradiation of NMR samples was conducted using the protocol described in Section 1.5.

### 1.5 Photoirradiation experiments

Samples of **1o** and **1c** (50  $\mu$ M in 100 mM sodium phosphate buffer, pH 7.4) were irradiated with monochromatic light at 450 nm (for photocyclization) and 635 nm (for photocycloreversion). The irradiation was performed in a 3 mL quartz cuvette containing a magnetic stirrer and a total volume of 2 mL solution. The irradiation sources were collimated laser diode modules (ThorLabs, CPS450 and CPS635, 4.5 mW, elliptical beam). The photoreaction was followed by recording the UV-visible spectra at appropriate time points (see Section 1.3).

For the kinetic experiments (Figure 1c), samples of **1o** (10  $\mu$ M) and the appropriate DNA sequence (30  $\mu$ M by strand) were exposed to ambient room light under otherwise identical conditions. The irradiation was performed in a 3 mL quartz cuvette containing a total volume of 1.5 mL solution. The buffer used was either sodium (telo22) or potassium (telo23 and ds26) phosphate (100 mM, pH 7.4).

For photoirradiation experiments conducted in the presence of oligonucleotides and followed by NMR spectroscopy (see Section 1.4) to ensure even irradiation of the sample, the solution (600  $\mu$ L) was transferred to a 1 mL quartz cuvette containing a magnetic stirrer and irradiated at the appropriate wavelength for the time specified, then transferred back to the NMR tube for analysis.

### 1.6 Notes on procedure – avoiding unwanted photoisomerization

Since it is impractical to avoid working under conditions of ambient light, significant care was taken with sample handling to avoiding the unwanted photoisomerisation between **1o/1c** in experiments where the effect of the individual isomers was considered.

Based on the results of our photoisomerisation studies (Figure 1b) the red-light promoted cycloreversion of **1c** to **1o** was not considered to be the source of potential artefacts. In fact, it was actually the photocylisation of **1o** to **1c** under the blue component of ambient room light which was of more concern (see further discussion below). Regarding the cycloreversion of **1c** to **1o**, direct irradiation close to the absorbance

maximum of **1c** (635 nm) in an otherwise dark fumehood was required for several hours to achieve significant cycloreversion of **1c** to **1o**. Indeed, over 4 hours of irradiation with red light were required to achieve almost complete cycloreversion of **1c**, versus less than 10 min irradiation with visible blue light (450 nm) to achieve complete cyclisation of **1o** (see Figure 1b). Nonetheless, samples of **1c** were kept in the dark and exposed to ambient light only for a few seconds in order to take aliquots for addition to titrations and other experiments. We are confident that photochemical back-isomerisation was negligible under the experimental conditions.

Regarding the more concerning propensity of 10 to cyclise under ambient light (owing to the presence of the blue component in fluorescent room lights) we took great care to keep the samples in the dark and minimise exposure to ambient light when transferring aliquots. Indeed, we performed NMR and UV-vis titrations in dim conditions. Even partial unwanted cyclisation of **10** to **1c** could be detected by eye (since the solutions turned green) but the absence of **1c** could also be confirmed by UV-vis. By employing careful handling technique, no unwanted cyclisation of **10** to **1c** was observed during the experiments. Indeed, examination of the raw data from the absorbance titrations (see Figures S9 and S18) reveals no **1c** is generated during the experimental procedure, since no appearance of the visible absorbance band ( $\lambda_{max} = 670$  nm) is detected.

### 1.7 Determination of apparent dissociation constants

Apparent dissociation constants for **10 and 1c** were determined through UV-visible spectroscopy titration experiments. The raw spectra were recorded as described in Section **1.3**. The concentration of ligand was fixed at 10  $\mu$ M in a constant volume of **1.5** mL buffer. The buffer used was either sodium (telo22) or potassium (telo23) phosphate (100 mM, pH 7.4). During the titration, aliquots of sample were removed and replaced with aliquots of oligonucleotide to give the required titration points (from a 100  $\mu$ M stock solution in appropriate buffer containing also 10  $\mu$ M ligand to maintain constant ligand concentration). NB: the oligonucleotide solution was annealed by heating to 90 °C for 2 minutes and then cooling on ice **prior** to the addition of ligand (to avoid annealing in the presence of ligand). Following addition, the solution was mixed thoroughly and the UV-visible spectrum was acquired immediately. Data were fitted to an independent-and-equivalent-sites binding model (Equation 1) using Prism 7 software, a full derivation of which is provided by (amongst others) Thordarson,<sup>[4]</sup> adapted to an independent and equivalent sites model by (amongst others) Buurma and Gade.<sup>[5]</sup> The stoichiometry of the complex (*N*) was chosen as the lowest integer value that provided a satisfactory fit, *R*<sup>2</sup> > 0.97 (*N* = 2 for **10** and **1c**) which was also inferred from the NMR titration experiment of **1c** with telo22.

$$\Delta A = \varepsilon_{\Delta complex}([\text{complex}])$$

where:

 $[\text{complex}] = \frac{1 + N \cdot K_a \cdot [\text{DNA}]_{tot} + K_a \cdot [\text{ligand}]_{tot}}{2 \cdot K_a} - \sqrt{(1 + N \cdot K_a \cdot [\text{DNA}]_{tot} + K_a \cdot [\text{ligand}]_{tot})^2 - 4 \cdot N \cdot K_a^2 \cdot [\text{DNA}]_{tot} \cdot [\text{ligand}]_{tot}}$ 

 $\Delta A$  = absorbance change at each titration point relative to free ligand (observed parameter)

 $\varepsilon_{\Delta complex}$  = change in the molar extinction coefficient between free ligand and DNA/ligand complex (fitted parameter)

N = the binding stoichiometery of ligand to DNA (selected parameter)

 $K_{\rm a} = the apparent association constant$  (fitted parameter),  $K_{\rm d} = \frac{1}{K_{\rm c}}$ 

 $[DNA]_{tot} = the concentration of added DNA (known parameter)$ 

 $[ligand]_{tot} = the total ligand concentration (fixed parameter)$ 

### 1.8 Molecular dynamics simulations

We performed docking calculation of ligands **1o** and **1c** to telo22 G4 DNA (PDB code: 143D)<sup>[2]</sup> and telo24 G4 DNA (PDB code: 2GKU)<sup>[3]</sup> in order to predict all the available high-affinity binding modes. Note: telo24 is a modified form of telo23 selected to provide a single solution confirmation to facilitate structure determination. For more information see the discussion by Patel *et al.*<sup>[3]</sup> The ligand molecule was optimised using PerkinElmer Chem3D software using the MM2 forcefield. Docking calculations were performed using AutoDock Vina<sup>[6]</sup> with the DNA structure kept fixed in its original solution conformation throughout the docking procedures. The highest affinity binding pose was then submitted to microsecond molecular dynamics simulations in order to test the stability of the binding pose.

All the simulations were performed using the Gromacs-5.0 software package.<sup>[7]</sup> The recently introduced parm-BSC1 force field was used for the DNA parameterization. For the ligand, the General Amber Force Field (GAFF) were used to generate parameters.<sup>[8,9]</sup> The charges were calculated using the restrained electrostatic potential (RESP) fitting procedure.<sup>[10]</sup> The RESP fit was performed onto a grid of electrostatic potential points calculated at the HF/6-31G(d) level as recommended by the force-field designers and recent literature.<sup>[9,10]</sup>

To start the simulations, each docked ligand/DNA complex was solvated in a cubic box with the dimension of 74 x 74 x 74 Å<sup>3</sup> along with 12982 TIP3P explicit water molecules.<sup>[11]</sup> An extra 19 Na+ (telo22) and 21 K<sup>+</sup>

ions (telo24) were added to neutralize the system.<sup>[12]</sup> The complexes were minimized prior to the equilibration and production run as follows: the minimization of the solute hydrogen atoms on the DNA and the ligand was followed by the minimization of the counterions and the water molecules in the box. In the next step, the DNA backbone along with the all the heavy atoms on the ligand were kept frozen, and the solvent molecules with counterions were allowed to move during a 50 ps MD run, to relax the density of the whole system. In the next step the nucleobases were relaxed in several minimization runs with decreasing force constants applied to the DNA backbone atoms, however, a few phosphate atoms were kept restrained with a force constant of 2.39 kcal.mol<sup>-1</sup>. Å<sup>-2</sup>. After the full relaxation, the system was slowly heated to the room temperature to 300K using V-rescale thermostat with a coupling constant of 0.5 ps employing an NVT (constant-temperature, constant-volume) ensemble.<sup>[13,14]</sup> As the system reached the temperature of interest, the equilibration simulation was performed for 10000 ps (10 ns) using an NPT ensemble with Berendsen thermostat and Berendsen barostat, and 0.5 ps was used again as the coupling constant for both temperature and pressure, respectively.<sup>[15]</sup> Finally, the production run was set for 1000000000 ps (1µs) using Nose-Hoover thermostat<sup>[16,17]</sup> and Parrinello-Rahman barostat<sup>[18]</sup> with the same coupling constant as previously taken in the equilibration simulation in the NPT ensemble. All the simulations were carried out under the periodic boundary conditions (PBC). The particle-mesh Ewald (PME) method was used to calculate the electrostatic interactions with in a cut-off of 10 Å.<sup>[19]</sup> The same cut-off was used for Lennard-Jones (LJ) interactions. All simulations were performed with a 1.0 fs time step.

### 1.9 Cytotoxicity studies

All cell culture reagents and media were purchased from Invitrogen, Life Technologies (Thermo-Fisher). HeLa cells were maintained at 37 °C and 5 % CO<sub>2</sub> in high glucose DMEM (4.5 g/L) supplemented with 10% hiFBS, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine and non-essential amino acids (1 X).

Cytotoxicity was measured through the alamarBlue assay (ThermoFisher scientific). Briefly, 1 x 10<sup>5</sup> HeLa cells were seeded in 96-wells plates (100 uL/well) in the presence of increasing concentrations of ligands. Dosing was carried out in a 2-fold serial dilution from a starting concentration of 100 uL ligand, and each experiment was performed in triplicate. After 72 hrs of incubation at 37 °C, cells were washed three times with PBS before addition of alamarBlue solution (5% v/v) in FBS-free media (100 uL) were added to each well and cells were reincubated for 1 hr at 37 °C. Cell viablility was then measured by alamarBlue fluorescence ( $\lambda_{ex} = 555$  nm,  $\lambda_{em} = 590$  nm) using a CLARIOstar plate reader. Dose response (inhibition) curves were plotted in

GraphPad Prism using non-linear regression to determine EC<sub>50</sub> values. The values reported are the average of three independent experiments, with error reported as standard deviation.

# 2 Supplementary tables

Δ <i>T</i> <sub>m</sub> / °C	1 µM	2 μM	5 μΜ	10 µM
F21T (K <sup>+</sup> )	3 ± 0.2	5 ± 0.8	8 ± 0.1	10 ± 0.2
F21T (Na <sup>+</sup> )	0 ± 0.2	1 ± 0.3	$1 \pm 0.4$	4 ± 0.5
F10T (K <sup>+</sup> )	0 ± 0.1	0 ± 0.1	0 ± 0.1	1 ± 0.1

### Table S1: FRET stabilisation values for compound 10

### Table S2: FRET stabilisation values for compound 1c

Δ <i>T</i> <sub>m</sub> / °C	1 μM	2 μM	5 μΜ	10 µM
F21T (K <sup>+</sup> )	3 ± 0.2	6 ± 0.2	9 ± 0.2	13 ± 0.5
F21T (Na <sup>+</sup> )	0 ± 0.2	0 ± 0.3	2 ± 0.4	4 ± 0.3
F10T (K <sup>+</sup> )	0 ± 0.1	0 ± 0.1	1 ± 0.1	1 ± 0.3



Figure S1: Isomerisation of **1o** to **1c** upon exposure to UV light followed by <sup>1</sup>H NMR in  $D_2O$  [10/1c]= 1 mM.



Figure S2: Example FRET melting curves for F21T (K<sup>+</sup>) G4 alone (blue trace) and in the presence of 10  $\mu$ M **1o** (black trace) and 10  $\mu$ M **1c** (green trace).



Figure S3: Thermal instability of ligand **1c** in potassium FRET buffer at 70 °C. [**1c**] + [**1o**] = 10  $\mu$ M. The cycloreversion is clearly visible by the attenuation of the **1c** red absorption band (670 nm).



Figure S4: assignment of imino and aromatic resonances of telo22 (antiparallel G4) in sodium NMR buffer (a) cartoon representation of the G4 topology and colour-coded labelling of proton numbering (b) imino region, i.e. guanosine H1 resonances (green) and (c) aromatic region of the <sup>1</sup>H NMR spectrum (see Section 1.4 for further experimental details). Assignments were made in accordance with the literature structure provided by Wang and Patel.<sup>[2]</sup> Aromatic assignments are for adenonsine H8 and guanosine H8 resonances (blue), adenosine H2 resonances (red) and thymidine H6 resonances (orange). Overlapping signals in the bracketed region could not be unambiguously assigned. The visible A1H2 and A7H2 resonances are overlapped at 7.83 ppm and could not be unambiguously assigned.



Figure S5: stacked NMR spectra (imino/aromatic region) of telo22 (antiparallel G4) titrated with ligand **1c**. See Section 1.4 for further experimental details.



Figure S6: stacked NMR spectra (carbohydrate/aliphatic region) of telo22 (antiparallel G4) titrated with ligand **1c**. See Section 1.4 for further experimental details.



Figure S7: stacked NMR spectra (imino/aromatic region) of telo22 (antiparallel G4) titrated with ligand **10**. See Section 1.4 for further experimental details.



Figure S8: stacked NMR spectra (carbohydrate/aliphatic region) of telo22 (antiparallel G4) titrated with ligand **10**. See Section 1.4 for further experimental details.



Figure S9: binding poses of ligand (a) **10** and (b) **1b** calculated from molecular dynamic simulations. See Section 1.8 for full experimental details.



Figure S10: Example UV-visible titration and determination of apparent binding constant of telo22 (antiparallel G4) and ligand 1**o**. [**1o**] = 10  $\mu$ M, buffer: 100 mM sodium phosphate, pH 7.4. (a) Raw UV spectra, (b) 380 nm binding isotherm and data fitting parameters. See Section 1.7 for full experimental details. The values quoted in the main manuscript are the averages from two independent experiments.



Figure S11: UV-visible titration and determination of apparent binding constant of telo22 (antiparallel G4) and ligand **1c**. [**1c**] = 10  $\mu$ M, buffer: 100 mM sodium phosphate, pH 7.4. (a) Raw UV spectra, (b) 670 nm binding isotherm and data fitting parameters. See Section 1.7 for full experimental details. The values quoted in the main manuscript are the averages from two independent experiments.



Figure S12: reversible switching of ligand  $1c \leftrightarrow 1o$  demonstrating change switch in ligand binding mode and affinity (a) imino NMR specta showing reversible shift of G8 (purple dot) and G4 (orange dot), (b) chemical shift switches of G8, (c) chemical shift switches of G4. See Section 1.5 for full experimental details.



Figure S13: assignment of imino resonances of telo23 (hybrid G4) in sodium NMR buffer (a) cartoon representation of the G4 topology and colour-coded labelling of proton numbering (b) imino region, i.e. guanosine H1 resonances (green). Assignments were made in accordance with the literature structure provided by Patel *et al.*<sup>[3]</sup> Under the experimental conditions, a second (minor) topology is observed in the absence of ligand, resonances are indicated (**x**).



Figure S14: NMR titration (imino and aromatic region) of telo23 (hybrid G4) with ligand **1c**. (a) stacked NMR spectra of each titration point, (b) overlaid imino region. See Section 1.4 for further experimental details.



Figure S15: stacked NMR spectra (carbohydrate/aliphatic region) of telo23 (hybrid G4) titrated with ligand **1c**. See Section 1.4 for further experimental details.



Figure S16: circular dichroism titration of telo23 (hybrid G4) with (a) ligand **1c** and (b) ligand **1o**. See Section 4.2 for full experimental details.



Figure S17: Figure S13: NMR titration (imino and aromatic region) of telo23 (hybrid G4) with ligand **1o**. (a) stacked NMR spectra of each titration point, (b) imino region. See Section 1.4 for further experimental details.



Figure S18: stacked NMR spectra (carbohydrate/aliphatic region) of telo23 (hybrid G4) titrated with ligand **10**. See Section 1.4 for further experimental details.



Figure S19: UV-visible titration and determination of apparent binding constant of telo23 (hybrid G4) and ligand **10**. [**10**] = 10  $\mu$ M, buffer: 100 mM potassium phosphate, pH 7.4. (a) Raw UV spectra, (b) 380 nm binding isotherm and data fitting parameters. See Section 1.7 for full experimental details. The values quoted in the main manuscript are the averages from two independent experiments.



Figure S20: UV-visible titration and determination of apparent binding constant of telo23 (hybrid G4) and ligand **1c**. [**1c**] = 10  $\mu$ M, buffer: 100 mM potassium phosphate, pH 7.4. (a) Raw UV spectra, (b) 670 nm binding isotherm and data fitting parameters. See Section 1.7 for full experimental details. The values quoted in the main manuscript are the averages from two independent experiments.



Figure S21: switching of G-tetrad structure of telo23 with alternate irradiation with UV and visible light. The shaded envelope shows the area integrated to quantify the degree of G-tetrad perturbation, as shown in the main manuscript, Figure 4e.

# 4 General synthetic route to ligand **10**



Scheme S1: Synthetic route to compound **1o**. The low yields of **8** result from the difficulty of the removing triphenylphosphine oxide requiring multiple purification steps. Full details are provided in Section 5.

## 5 Synthetic procedures and compound characterisation

### **General experimental**

Chemicals were purchased and used without further purification. Dry solvents were obtained by distillation using standard procedures, or by passage through a column of anhydrous alumina using equipment from Anhydrous Engineering (University of Bristol) based on the Grubbs' design.<sup>[20]</sup> Reactions requiring anhydrous conditions were performed under N<sub>2</sub>; glassware and needles were either flame dried immediately prior to use, or placed in an oven (150 °C) for at least 2 h and allowed to cool in a desiccator or under reduced pressure. Liquid reagents, solutions or solvents were added via syringe through rubber septa; solid reagents were added via Schlenk type adapters. Reactions were monitored by TLC on Kieselgel 60F<sub>254</sub> (Merck), with UV light (254 nm) detection and by staining with basic potassium permanganate solution. Flash column chromatography was performed according to Still and co-workers<sup>[21]</sup> using silica gel [Merck, 230–400 mesh (40–63 µm)]. Solvents for flash column chromatography (FCC) and thin layer chromatography (TLC) are listed in volume:volume percentages. Extracts were concentrated in vacuo using both a Heidolph HeiVAP Advantage rotary evaporator (bath temperatures up to 50 °C) at a pressure of 15 mmHg (diaphragm pump) or 0.1 mmHg (oil pump), as appropriate, and a high vacuum line at room temperature. Water soluble compounds were freeze dried on a Lytotrap Plus (LTE Scientific LTD). <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were measured at 25°C in the solvent specified with Varian or Bruker spectrometers operating at field strengths listed. Chemical shifts are quoted in parts per million with spectra referenced to the residual solvent peaks. Multiplicities are abbreviated as: br (broad), s (singlet), d (doublet), t (triplet), g (guartet), p (pentet), m (multiplet) and app. (apparent) or combinations thereof. Assignments of <sup>1</sup>H NMR and <sup>13</sup>C NMR signals were made where possible, using COSY, HSQC and HMBC experiments. Mass spectra were obtained by the University of Bristol mass spectrometry service by electrospray ionisation (ESI) or matrix assisted laser desorption ionisation (MALDI) modes. Infra-red spectra were recorded in the range 4000-400 cm<sup>-1</sup> on a Perkin Elmer Spectrum either as neat films or solids compressed onto a diamond window.

#### 1,5-bis(5-chloro-2-methylthiophen-3-yl)pentane-1,5-dione, 4



2-Chloro-5-methylthiophene (**3**, 3.1 g, 24 mmol) and glutaryl chloride (**2**, 2.0 g, 12 mmol) were dissolved in dichloromethane (150 mL) and anhydrous aluminium chloride (4.6 g, 34 mmol) was added. The solution was stirred for at rt for 18 h, then poured onto ice water (200 mL). The layers were separated and the aqueous back extracted with dichloromethane (2 x 100 mL). The combined organic extractions were dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo* to afford the title compound **4** as a pale brown solid (3.6 g, 97%). v<sub>max</sub> / cm<sup>-1</sup> (compressed solid) 3093 (w), 2961 (w), 2923 (w), 2896 (w), 1673 (s), 1524 (m), 1455 (s), 1409 (m), 1374 (m), 1243 (m), 1203 (s), 1156 (m), 1130 (m), 1006 (m), 970 (m), 822 (m), 784 (m), 729 (m), 483 (s); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.18 (2H, s, 4-CH), 2.85 (4H, t, *J* = 7.0 Hz, 7-CH<sub>2</sub>), 2.66 (6H, s, 9-CH<sub>3</sub>), 2.05 (2H, app. p, *J* = 7.0 Hz, 8-CH<sub>2</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  195.0 (6-CO), 147.9 (ArC), 135.0 (ArC), 127.0 (4-CH), 125.4 (ArC), 40.6 (7-CH<sub>2</sub>), 18.3 (8-CH<sub>2</sub>), 16.2 (9-CH<sub>3</sub>); ESI-LRMS for C<sub>15</sub>H<sub>14</sub>Cl<sub>2</sub>O<sub>2</sub>S<sub>2</sub>Na<sup>+</sup> [MNa]<sup>+</sup> calcd: 382.9, found: 383.0. Proton and carbon NMR were consistent with literature data.<sup>[22]</sup>

### 1,2-bis(5-chloro-2-methylthiophen-3-yl)cyclopent-1-ene, 5



To a stirred vigorously suspension of zinc powder (2.1 g, 31.67 mmol) in anhydrous THF (250 mL), TiCl<sub>4</sub> (2.9 mL, 27 mmol) was added dropwise. The solution was heated to reflux for 1 h. The mixture was then cooled to 0 °C and thiophene **4** (3.8 g, 10.6 mmol) was added to the suspension. The mixture was heated to reflux for 4 h, then quenched with sat. aq. Na<sub>2</sub>CO<sub>3</sub> (40 mL) causing the evolution of a white precipitate. The solution was filtered through Celite, washing with EtOAc. The filtrate was dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was purified by flash silica chromatography, eluting with hexane, to afford the title compound **10** as a white solid (2.8 g, 81%).  $v_{max}$  / cm<sup>-1</sup> (compressed solid) 2950 (m), 2919 (m), 2844 (m), 1553

(w), 1538 (w), 1457 (s), 1439 (s), 1378 (w), 1310 (w), 1291 (w), 1202 (m), 1159 (m), 1144 (m), 1055 (w), 1014 (m), 922 (s), 964 (w), 827 (s), 748 (w), 667 (w), 651 (w), 482 (s); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.58 (2H, s, 4-*CH*), 2.72 (4H, t, *J* = 7.5 Hz, 7-*CH*<sub>2</sub>), 2.02 (2H, p, *J* = 7.5 Hz, 8-*CH*<sub>2</sub>), 1.89 (6H, s, 9-*CH*<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  135.0 (Ar*C*), 134.6 (5-*C*), 133.4 (Ar*C*), 126.8 (4-*C*H), 125.3 (Ar*C*), 38.5 (7-*C*H<sub>2</sub>), 23.0 (8-*C*H<sub>2</sub>), 14.3 (9-*C*H<sub>3</sub>). Proton and carbon NMR were consistent with literature data.<sup>[22]</sup>

### 1,2-bis(2-methyl-5-(pyridin-4-yl)thiophen-3-yl)cyclopent-1-ene, 8



Thiophene 5 (250 mg, 0.74 mmol) was dissolved in anhydrous THF (5 mL) and the solution cooled to 0 °C. n-BuLi (2.5 M in hexanes, 1.6 mmol) was added dropwise, and the solution stirred for 30 min. tris-n-butylborate (460 µL, 2.2 mmol) was added and the resulting solution warmed to rt and stirred for 1 h. Separately, Pd(PPh<sub>3</sub>)<sub>4</sub> (86 mg, 0.074 mmol) and 4-bromopyridine hydrochloride (7, 320 mg, 1.6 mmol) were dissolved in 2.5 M aq. K<sub>2</sub>CO<sub>3</sub> (5 mL) and the solution was degassed by evacuation under vacuum and backfilling with nitrogen three times. The boronic ester solution was then added to this mixture and the resulting solution heated to 70 °C and stirred for 18 h. The mixture was cooled and diluted with dichloromethane (50 mL) and washed with water (50 mL). The aqueous was back extracted with dichloromethane (2 x 50 mL) and the combined organic extractions dried (MgSO<sub>4</sub>) and filtered. The filtrate was concentrated in vacuo and the residue purified by flash silica chromatography, eluting with 2:1 hexane:EtOAc, to yield a pale violet solid. This was further purified by trituration in 1:1 Et<sub>2</sub>O:pentane to afford the title compound **8** as a pale violet solid (81 mg, 26%). v<sub>max</sub> / cm<sup>-1</sup> (compressed solid) 3065 (w), 3028 (w), 2952 (w), 2920 (w), 2946 (w), 1596 (s), 1540 (w), 1498 (m), 1461 (m), 1437 (m), 1411 (m), 1378 (w), 1310 (w), 1294 (w), 1220 (m), 1171 (w), 1120 (w), 992 (m), 909 (w), 857 (w), 814 (s), 729 (m), 601 (m), 542 (m), 4723 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.53 (4H, d, J = 5.2 Hz, 12-CH), 7.34 (4H, d, J = 5.2 Hz, 11-CH), 7.21 (2H, s, 4-CH), 2.85 (4H, t, J = 7.5 Hz, 7-CH<sub>2</sub>), 2.11  $(2H, p, J = 7.5 Hz, 8-CH_2)$ , 2.02 (6H, s, 9-CH<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  150.4 (12-CH), 141.4 (10-C), 137.4 (ArC), 137.2 (ArC), 136.8 (ArC), 135.0 (6-C), 126.4 (4-CH), 119.4 (11-CH), 38.6 (7-CH<sub>2</sub>), 23.1 (8-CH<sub>2</sub>), 14.8 (9CH<sub>3</sub>); ESI-LRMS for  $C_{25}H_{23}N_2S_2^+$  [MH]<sup>+</sup> calcd: 415.1, found: 415.1. Proton and carbon NMR were consistent with literature data.<sup>[22]</sup>

### 4,4'-(cyclopent-1-ene-1,2-diylbis(5-methylthiophene-4,2-diyl))bis(1-methylpyridin-1-ium) iodide, 1o



Compound **8** (40 mg, 0.96 mmol) was dissolved in dichloromethane (1 mL). Methyl iodide (13  $\mu$ L, 0.21 mmol) was added and the solution stirred at rt for 3 h, then concentrated *in vacuo*. The residue was suspended in 1:1 hexane:EtOAc (5 mL) and the precipitate filtered and dried under vacuum to afford the title compound **1o** as a green powder (52 mg, 78%). v<sub>max</sub> / cm<sup>-1</sup> (compressed solid) 3026 (w), 2946 (w), 2922 (w), 2841 (w), 1634, (s), 1595 (m), 1533 (m), 1511 (s), 1471 (m), 1435 (m), 1416 (m), 1372 (w), 1342 (w), 1313 (w), 1293 (w), 1260 (w), 1220 (m), 1190 (s), 1181 (s), 1150 (m), 1069 (w), 1047 (w), 1028 (w), 993 (w), 920 (w), 893 (w), 830 (s), 756 (w), 720 (w), 541 (m), 471 (s); <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  8.51 (4H, d, *J* = 6.7 Hz, 12-CH), 7.97 (4H, dd, *J* = 6.7 Hz, 11-CH), 7.84 (2H, s, 4-CH), 4.24 (6H, s, 13-CH<sub>3</sub>), 2.89 (7H, t, *J* = 7.5 Hz, 7-CH<sub>2</sub>), 2.14 (8H, p, *J* = 7.5 Hz, 8-CH<sub>2</sub>), 2.06 (6H, s, 9-CH<sub>3</sub>); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  148.7 (10-C), 144.9 (ArC), 144.4 (12-CH), 138.8 (ArC), 135.3 (3-C), 132.9 (5-C), 132.5 (ArC), 121.5 (11-CH), 46.8 (13-CH<sub>3</sub>), 37.9 (7-CH<sub>2</sub>), 22.7 (8-CH<sub>2</sub>), 14.1 (9-CH<sub>3</sub>); ESI-LRMS for C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>S<sub>2</sub><sup>2+</sup> [M]<sup>2+</sup> calcd: 222.1, found: 222.1. Proton and carbon NMR were consistent with literature data.<sup>[22]</sup>

### 6 References

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