Supporting Information

A G-quadruplex ligand with a 10000-fold selectivity over duplex DNA.

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Synthetic procedure

All commercially available compounds were purchased from Aldrich. Neutral alumina was obtained from Merck (ref 101077). 5,10,15,20-tetra(*p*-aminophenyl)porphyrin was prepared by reduction of 5,10,15,20-tetra(*p*-nitrophenyl)porphyrin with stannous chloride.[1] DMF was dried over 4 Å molecular sieves. ^{1}H-NMR spectra were recorded on a Bruker Avance 300 spectrometer using the residual solvent peak as internal calibration. Mass spectra were recorded either on a Perkin-Elmer SCIEX API 365 (electrospray) or on a Nermag R1010 apparatus (chemical ionization). UV-vis spectra were recorded on a Hewlett Packard 8452A spectrophotometer.

Synthesis of the free-base precursor[2]

3-Pyridinepropionic acid (0.63 g, 4.15 mmol, 2 eq/NH_2, 8 eq/porph), 1-hydroxybenzotriazole (HOBT, 0.56 g, 2 eq/NH_2, 8 eq/porph) and O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU, 1.42 g, 1.8 eq/NH_2, 7.2 eq/porph) were dried under vacuum for 2 h in a 250-ml two-necked flask. Under argon, dry DMF (30 mL) and diisopropylethylamine (1.6 mL, 4.4 eq/NH_2, 17.6 eq/porph) were added with a syringe. After 15 min stirring under argon, 5,10,15,20-tetra(*p*-aminophenyl)porphyrin was added (0.35 g, 1 eq) and the mixture was stirred for 2h, under argon and in the dark. The reaction was quenched by the slow addition of water (100 mL) and the organic compounds were extracted in CH_2Cl_2. The chlorinated layer was evaporated and the solid was triturated with MeOH. Purification was achieved by filtration on neutral Al_2O_3 (L19,5, Ø 2.8 cm) with a $CHCl_3$ / EtOH / NEt_3 mixture (from 95:5:0 to 80:20:1). Finally, a $CHCl_3$ / EtOH / pentane precipitation (1:0.5:10) at 4°C overnight gave a solid which was filtered off, washed with water to remove traces of colourless salts and dried under vacuum. Yield 0.19 g (30%, *i.e.* 74% per coupling).

^{1}H-NMR (300 MHz, $CDCl_3$+20% CD_3OD), δ (ppm) : 8.81 (broad s, 8H, pyrroles), 8.52 (d, 4H, H_2, ^4J = 1.5 Hz), 8.41 (dd, H_6, ^3J = 5 Hz, ^4J = 1.5 Hz), 8.11 (d, 8H, H_o, ^3J = 8.4 Hz), 7.91 (d, 8H, H_m, ^3J = 8.4 Hz), 7.76 (m, 4H, H_4), 7.33 (dd, 4H, H_5, ^3J = 7.3 Hz, ^3J = 5 Hz), 3.16 (t, 8H, $COCH_2$, ^3J = 7.5 Hz), 2.84 (t, 8H, CH_2py, ^3J = 7.5 Hz).

CI-MS : $[M+H]^+$ = 1207.

Quaternization of the pyridyl nitrogens

5,10,15,20-tetra(PhNHCOCH$_2$CH$_2$(3-pyridyl)) porphyrin (0.1 g, 0.083 mmol) was dissolved in $CHCl_3$/MeOH (1:1, 10 mL). Methyl iodide (1.1 mL, 50 eq/py, 200 eq/porph) was added and the mixture was heated at 50°C in the dark for 24 h. After evaporation, the solid was dried under vacuum. Yield 0.16 g (quantitative).

^{1}H-NMR (300 MHz, CD_3OD, 45°C), δ (ppm) : 9.05 (s, 4H, H_2), 8.88 (s, 8H, pyrroles), 8.83 (d, 4H, H_6, ^3J = 6.3 Hz), 8.67 (d, 4H, H_4, ^3J = 8.4 Hz), 8.52 (d, 8H, H_o, ^3J = 8.0 Hz), 8.26 (d,

8H, H_m, ^3J = 8.1 Hz), 8.12 (dd, 4H, H_5, ^3J = 6.2 Hz, ^3J = 8.1 Hz), 4.50 (s, 12 H, CH_3), 3.42 (t, 8H, CH_2, ^3J = 7 Hz), 3.15 (t, 8H, CH_2, ^3J = 7.2 Hz).
ES^+-MS : $[M\text{-}I^-]^+$ = 1648, $[M\text{-}2I^-]^{2+}$ = 760.5.

Metallation with manganese(III)

A solution of the quaternized porphyrin (50 mg, 0.028 mmol) in DMF (5 mL) and 2,4,6-collidine (0.5 mL) was heated to 110°C in the dark. A first batch of manganese(II) acetate tetrahydrate was added as a solution in 0.5 mL H_2O (55 mg, 8 eq) and the mixture was heated at 110°C in the dark for 3 h. A second batch of manganese(II) acetate tetrahydrate was added as a solution in 0.5 mL H_2O (55 mg, 8 eq), followed by some more collidine (0.5 mL) and the mixture was heated at 110°C in the dark for a further 3 h. A UV-Vis control showed the presence of both free-base and metallated porphyrin. Therefore a third batch of manganese(II) acetate tetrahydrate was added (110 mg, 16 eq) as well as some more collidine (1 mL) and heating was maintained overnight. After evaporation of the water, the porphyrins were precipitated by the addition of Et_2O (15 V), collected, dissolved in MeOH and precipitated with Et_2O (15 V) at 4°C over the weekend. The precipitate was filtered on a fritted glass (porosity 4), the pale yellow filtrate was discarded. The precipitate was washed with Et_2O and dissolved in MeOH, which leaves most of the excess of manganese salts behind (brown solid remaining on the filter while the filtrate is green). Some DOWEX 1x8-200 resin (chloride form) was added to the porphyrin solution in 4.5 mL MeOH and the mixture was gently stirred over the weekend. The resin was filtered and washed with MeOH, the filtrate was evaporated and dried under vacuum. Yield 39 mg (93%).
ES^+-MS : $[M\text{-}5Cl^-\text{-}3C_9H_{11}NO]^+$ = 874.50, $[M\text{-}5Cl^-\text{-}C_9H_{11}NO]^{2+}$ = 585.05, $[M\text{-}5Cl^-\text{-}2C_9H_{11}NO+2H]^{2+}$ = 511.25, $[M\text{-}5Cl^-]^{3+}$ = 439.75, $[M\text{-}5Cl^-\text{-}C_9H_{11}NO]^{3+}$ = 390.35, $[M\text{-}5Cl^-]^{4+}$ = 330.15, $[M\text{-}5Cl^-\text{-}C_9H_{11}NO+H]^{4+}$ = 292.85.
UV-Vis (H_2O) λ_{max}/nm (ε/mol^{-1} L cm^{-1}) : 382 (37300), 402 (37600), 422 (32300), 470 (59300), 562 (8000), 602 (8100).

Surface plasmon resonance (BIACORE) analysis.

Principle.

Binding events between two molecules are monitored in real time, without the use of any label using an optical phenomenon called Surface Plasmon Resonance (SPR). Biomolecular binding events cause changes in the refractive index close to the surface layer of a chip, which are detected as changes in the SPR signal. During a binding analysis SPR changes occur as a solution is passed over the surface of a sensorchip. To perform an analysis, one interactant (ligand) is immobilized over a carboxymethylated dextran matrix of a sensorchip. The sensor surface forms one wall of a flow cell. Sample containing the other interactant (analyte) is injected over this surface in a precisely controlled flow. The progress of an interaction is monitored as a sensorgram that expresses Resonance Units (RU) as a function of time. The analyte binds to the surface-attached ligand during sample injection, resulting in an increase in signal. At the end of the injection, the sample is replaced by a continuous flow of buffer and the decrease in signal reflects dissociation of interactant from the surface-bound complex.

Materials.

All binding studies based on SPR phenomenon were performed on a four-channel BIACORE 3000 optical biosensor instrument (BIAcore AB, Uppsala, Sweden). All experiments were performed on sensorchips SA (sensorchips with streptavidin covalently immobilized on a carboxymethylated dextran matrix) obtained from Biacore AB, Uppsala, Sweden.

Immobilization of biotinylated DNA probes.

Both flow cells of an SA streptavidin sensor chip were coated with biotinylated probes. Three 5'-biotin-labeled oligonucleotide sequences (Eurogentec, Belgium) were used in these experiments. Two hairpin duplexes were chosen from previous published work,[3] referred to as

the 22-mer [$(CG)_4$], (5'-TTCGCGCGCGTTTTCGCGCGCG sequence) (1000 RU immobilized on flow cell 2) and the 20-mer [AATT] (5'-CGAATTCGTCTCCGAATTCG sequence) (1000 RU immobilized on flow cell 3) and the human telomeric quadruplex DNA, referred to as the 22-mer [G_4], corresponding to the 5'-AGGGTTAGGGTTAGGGTTAGGG sequence (1000 or 300 RU immobilized on flow cell 4 for Figures 2 and 3, respectively). No target oligonucleotide was captured on flow cell 1 so that it could be used as a reference surface. All immobilization steps were performed at a final DNA concentration of 10 nM and at a flow rate of 2 μL/min. Injections were stopped when sufficient RU levels were obtained.

BIA analysis.

Binding analyses were performed with multiple injections of different compound concentrations over the immobilized DNA surface at 25°C. All samples were diluted in HBS-EP/KCl buffer and were injected over the sensor surface for 5 minutes at a flow rate of 20 µL/min. All diluted samples were injected at the same time over the four channels (flow cells). Flow cell 1 was used to obtain control sensorgrams showing nonspecific binding to the streptavidin-coated surface as well as refractive index changes resulting from changes in the bulk properties of each solution. Control sensorgrams were substracted from sensorgrams obtained with immobilized DNAs to yield true binding responses. Kinetics constants were calculated using BIAevaluation 4.0.1 software and apparent association constants (K_a) were calculated as the ratio of $k_{on}/k_{off.}$

For each molecule, we calculated k_{on}, k_{off} and K_a constants using both 1-site (Langmuir) and 2-site algorithms and we selected the best fit corresponding to the lower Chi2 parameter value (not shown).

Sensorgrams (resonance units, RU, versus time) for the concentration-dependent binding of manganese porphyrin **1** on duplex and quadruplex DNA. The sensorgrams were recorded at 1.5, 3, 6.25, 12.5, 25, 50, 100, 200, 400 nM concentrations of **1** for duplex and quadruplex DNA in Figure 2. In Figure 3 the sensorgrams were recorded at 0.3, 0.75, 1.5, 3, 6.25, 12, 25, 50 nM concentrations of **1**.

Scatchard Analysis.

For all the molecules tested, data obtained from sensorgrams were used for Scatchard analysis using the equation $R_{eq}/C = K_a\ (R_{max} - R_{eq})$ where R_{eq} is the response at equilibrium in Resonance Units (RU), C is the concentration of analyte in solution (nM) and R_{max} is the theoretical maximum response (proportional to the amount of immobilized ligand). R_{eq} was calculated by BIAevaluation 4.0.1 software. As R_{max} remains constant, a plot of R_{eq}/C versus R_{eq} has a slope of $-K_a$ in the case of 1-site model of interaction. From Scatchard plots was determined the 1-site or non-equivalent 2-site model of interaction for quadruplex DNA.

<u>Telomeric repeat amplification protocol, TRAP assay.</u>

Exponentially growing HeLa cell cultures were trypsinized, washed in PBS and S-100 extracts obtained as described.[4] Stock protein concentration was adjusted to 5 µg mm^{-3}, flash-frozen and stored at -80°C. To assess telomerase activity, compounds were serially diluted in lysis buffer (range, 50 µM to 25 nM) and mixed 1:1 with 1.25 µg of HeLa cell extract (final volume, 5 mm^3). Extension and amplification reactions and electrophoresis were carried out as described.[4] For quantification, autoradiographs were scanned in a Storm 860 scanner and signal intensity of telomerase ladder and PCR internal control (ITAS) measured using ImageQuant v1.2 software. Dose-dependent PCR inhibition was observed (diminished or absent internal control (ITAS) signal at concentrations >25 µM); these datapoints were therefore excluded from analysis. Regression curves and IC_{50} values were calculated using GraphPad Prism software, and values expressed as % of activity of an equal amount of nontreated HeLa cell extract. Negative controls were included in all assays by preincubating HeLa extracts with RNAse for 10 min at 30°C prior to the extension reaction. Compound **1**

was assayed in three separate TRAP assays (the concentration of **1** is given in μM on the figure). Regression curves were highly reproducible.

Figures and Tables.

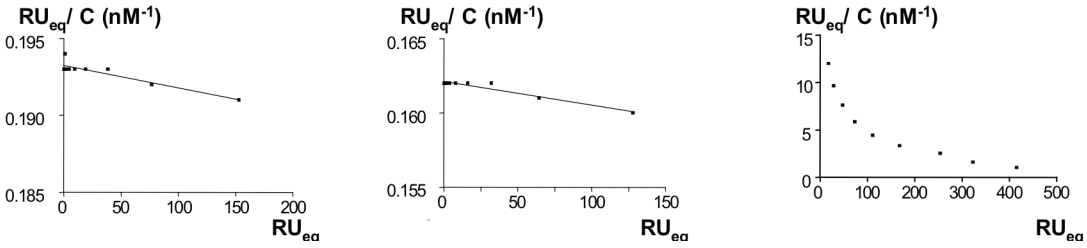

Figure S1. Scatchard plots for the concentration-dependent binding of manganese porphyrin **1** on duplex and quadruplex DNA at high chip loading. Data from Figure 2.

Table S1. Kinetic and equilibrium constants for the interaction of **1** with duplex and quadruplex DNA. In italics, values corresponding to a site of lower affinity. Data from Fig. 2.

	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_a (M^{-1})
GC Duplex	0.7×10^{2}	3.0×10^{-3}	2.5×10^{4}
AT Duplex	0.6×10^{2}	5.1×10^{-3}	1.2×10^{4}
Quadruplex*	3.3×10^{2}	1.7×10^{-6}	1.9×10^{8}
	3.9×10^{4}	8.8×10^{-3}	4.5×10^{6}

* The data for quadruplex DNA is biased by the high chip loading (1000 RU) due to the very strong binding of **1**. See Figure 3 for optimized experimental conditions.

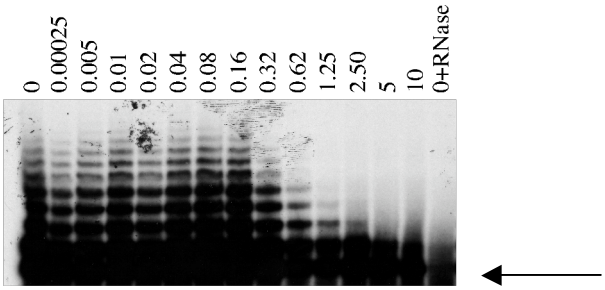

Figure S2. TRAP assay gel for **1** showing the ladders produced by PCR amplification of the oligonucleotides generated by the activity of telomerase. Arrow : internal control.

References.

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