Synthesis and G-quadruplex binding studies of new 4-*N*-methylpyridinium porphyrins

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A series of cationic porphyrins carrying 1–3 *meso*-*N*-pyridinium groups has been synthesised, and their binding to G-quadruplex DNA has been explored by surface plasmon resonance (SPR) and circular dichroism spectroscopy. Two *trans* substituents appear to be sufficient for tight binding; preferential binding to the anti-parallel intramolecular human telomeric DNA was observed for the A_2 *trans* and A_3 porphyrins. The A_2 *trans* is able to induce the formation of an anti-parallel G-quadruplex in a K^+ free solution, mimicking the effect of a molecular chaperone.

Introduction

Intramolecular G-quadruplexes are four stranded DNA secondary structures formed by G-rich DNA sequences containing four tracks of at least two consecutive guanines.[1] In the presence of monovalent cations (preferentially K^+), the tetrads of hydrogen bonded guanines are held together by $C{=}O\cdots M^+$ and π–π or hydrophobic interactions. During the past decade, there has been growing interest in the structure, recognition and function of intramolecular G-quadruplexes. The best studied example is the human telomeric DNA quadruplex that leads to inhibition of telomere extension by telomerase, an enzyme active in most cancer cells.[2–4] Recently, a large number of putative quadruplex forming sequences have also been identified throughout the human genome.[5] A few examples of quadruplexes located in the promoter regions of oncogenes have been postulated to act as regulator elements controlling the level of expression of these genes.[6–9]

There is now considerable interest in designing new quadruplex binding ligands as effective therapeutic agents. Most of the molecules reported to date contain extended aromatic surfaces with flat ring systems and are believed to interact with the external tetrad of the quadruplex.[10,11] Tetra-*N*-methylpyridiniumporphyrin, H_2-TMPyP4, was previously reported to bind to the intramolecular human telomeric quadruplex with low micromolar affinity.[12,13] More recently it was also shown to bind to other quadruplexes with similar affinities.[14] A number of tetra-substituted porphyrins have been designed to target quadruplex DNA.[15,16] Herein, we describe the synthesis and quadruplex binding affinities of new mono-, bi-, and tri-*meso* substituted porphyrins in an attempt to define the requisite structural features.

Results and discussion

In order to determine what structural changes to the substitution pattern of H_2-TMPyP4 can be tolerated, four porphyrins (A_1, A_2 *cis*/*trans* and A_3. Fig. 1) lacking *meso* substituents were prepared. These porphyrins systematically vary the number and position of *N*-methylpyridinium groups. The synthesis of A_2 *trans* was previously reported by R. K. Wall *et al.* in very low yields,[17] but A_1, A_2 *cis* and A_3 have not been previously reported. All porphyrins were synthesised by rational methods, mainly by the MacDonald-type of dipyrromethane coupling,[18] as outlined in Scheme 1. The trifluoroacetic acid (TFA) catalysed condensation of **1** with 2,2′-dipyrromethane **2** in THF yielded 4-pyridylporphyrin **3** (7–10% after purification by chromatography). In order to obtain the 5,15-di(4-pyridyl)porphyrin **7**, 5-(4-pyridyl)dipyrromethane **5** was reacted with trimethyl orthoformate **6** in the presence of catalytic amount of trichloroacetic acid (TCA) under a nitrogen atmosphere for 4 h, followed by the addition of pyridine and further stirring for 12 h. Purification was carried out by column chromatography giving 9% of porphyrin. The 5,10-di-(4-dyridyl)porphyrin **11** was obtained by the condensation

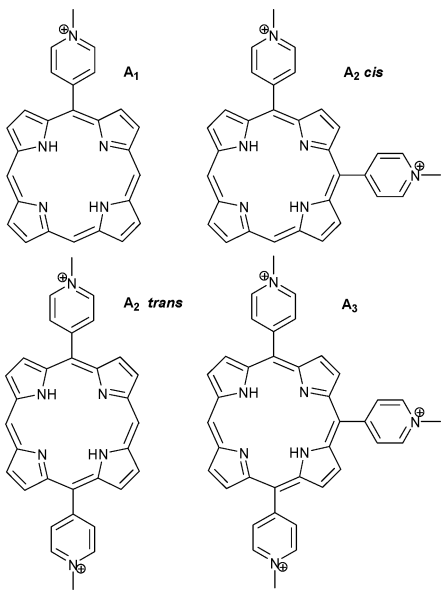

Fig. 1 Structure of the four *N*-methylpyridinium porphyrins used in this study (A_1, A_2 *cis*, A_2 *trans*, A_3).

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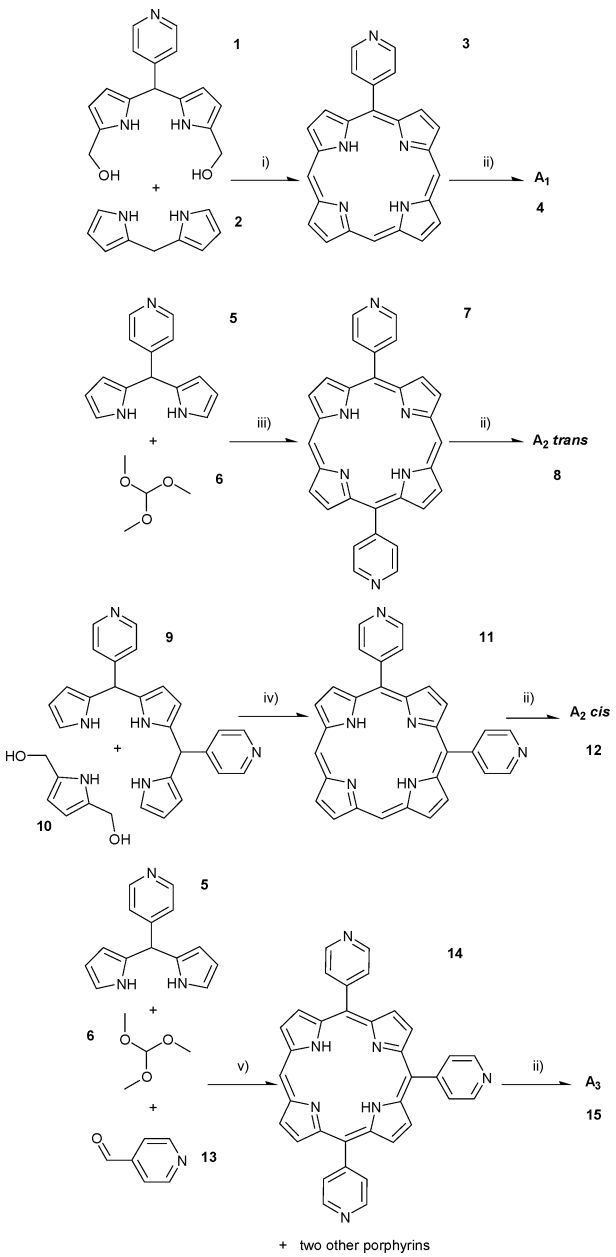

Scheme 1 Synthetic pathways for the synthesis of the A_1, A_2 *cis*/*trans* and A_3 porphyrins. i) THF, N_2(g) and TFA. After 1 h of reaction DDQ was added. ii) CH_3I in DMF. iii) DCM, TCA, N_2(g) and after 4 h pyridine was added. iv) DCM, TFA under N_2(g). v) DCM, TCA under N_2(g). After 4 h pyridine was added, followed by DDQ after 3 h.

of tripyrromethane **9** with 2,5-bis(hydroxymethyl)pyrrole **10** in DCM, under nitrogen atmosphere catalysed by TFA and purified by column chromatography (3%). The condensation of 5-(4-pyridyl)dipyrromethane **5** with trimethyl orthoformate **6** and with pyridine-3-carboxaldehyde **13**, was carried out at room temperature under nitrogen atmosphere and catalysed by TCA, giving after column chromatography purification three porphyrins: 5,15-di(4-pyridyl)porphyrin **7**, tetrapyridylporphyrin, and the desired 5,10,15-tri(4-pyridyl)porphyrin **15** with a 0.8% yield.

The quaternisation of the pyridyl groups was achieved by methylation with iodomethane in DMF at 40 °C yielding quantitatively the final A_1, A_2 *cis*/*trans* and A_3 porphyrins without need for further purification.

To improve the solubility of porphyrins A_1 and A_2 *cis*/*trans* in water, an anion exchange from iodide to chloride was carried out. While this approach was successful for A_2 *cis*/*trans*, the water solubility of A_1 as either an iodide or chloride salt was too poor to allow DNA binding studies.

The binding of the four porphyrin derivatives A_2 *cis*, A_2 *trans*, A_3 and H_2-TMPyP4 (control porphyrin) to duplex (5-biotin-[GGCATAGTGCGTGGGCGTTAGC]-3 hybridised with its complementary sequence) and quadruplex DNA (5-biotin-[GTTA(GGGTTA)$_4$GG]-3) was investigated using surface plasmon resonance (SPR), allowing binding events to be monitored in real time, without the use of labels (example shown in Fig. 2).[19] The results are summarised in Table 1.

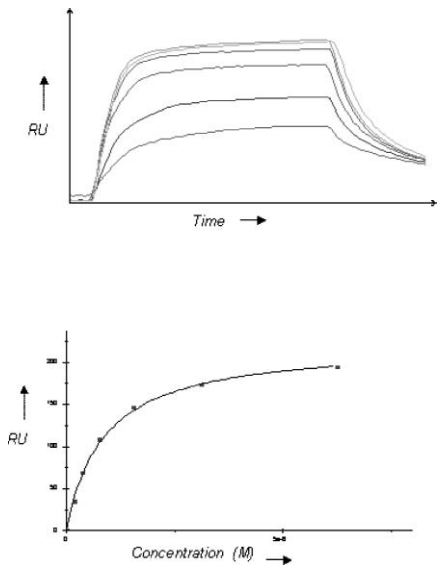

Fig. 2 Sensorgram overlay obtained for 6 different concentrations of the A_2 *trans* porphyrin (6.25, 3.12, 1.56, 0.78, 0.39, 0.20 μM, top to bottom) binding to the human telomeric quadruplex and the corresponding binding curve obtained using the BIAeval software (BIAcore).

As shown previously with tetrasubstituted porphyrins,[19] all new porphyrins we tested have similar affinities for duplex (data not shown) and quadruplex DNA. However the number of peripheral *meso* pyridinium groups and their location proved to be important for good binding. The A_2 *trans* porphyrin presents the lowest dissociation constant ($K_D = 0.83 \pm 0.10$ μM), comparable to that obtained for H_2-TMPyP4 ($K_D = 0.63 \pm 0.08$ μM). The porphyrins A_2 *cis* and A_3 show significantly weaker binding (K_D values of 18.9 ± 2.80 μM and 5.88 ± 0.90 μM respectively) (Table 1). These

Table 1 Dissociation constants (K_D) of H_2-TMPyP4 (control porphyrin), A_2 *cis*, A_2 *trans* and A_3 porphyrins to quadruplex DNA. The SPR experiments were carried out in 50 mM Tris·HCl pH 7.4, 100 mM KCl using a streptavidin functionalised chip on a Biacore 2000 SPR biosensor

Porphyrins	K_D/(μM)
H_2-TMPyP4	0.63 ± 0.08
A_2 *cis*	18.90 ± 2.80
A_2 *trans*	0.83 ± 0.10
A_3	5.88 ± 0.90

results indicate that two pyridinium substituents can be sufficient for quadruplex recognition as long as they are *trans* orientated to each other. Any other combination of less than four pyridiniums seems to significantly reduce quadruplex binding. This *trans* substitution pattern is comparable in geometry to other reported quadruplex binding platforms (*e.g.* 3,6-disubstituted acridines) that simultaneously target the external guanine and two opposite grooves.[20]

It is noteworthy that all these porphyrins seem to bind to the quadruplex structure with a high stoichiometry. Similar results have been observed by others using SPR and other techniques.[12,16]

The concentrations of porphyrin required to run SPR experiments are below those leading to self aggregation (<2 μM). This value was obtained by running Beer–Lambert aggregation experiments (data not shown). Hence, self aggregation of the porphyrins appears to occur *after* binding to G-quadruplex DNA. This aggregation phenomenon has also been described for H_2-TMPyP4 complexes with G-quadruplex; again it was reported that this porphyrin exists as a monomer in water even in the presence of concentrated inorganic salts.[21–23]

The human telomeric DNA can exist as a mixture of the parallel and anti-parallel G-quadruplex conformations when in a K^+ solution.[24–26] In order to investigate if the porphyrins were selective for any particular G-quadruplex conformation, circular dichroism (CD) experiments were performed. Those experiments were carried out on the 5-GGATTGGGATTGGGATTGGGATTGGG-3 (*Htelo*) DNA sequence, which was previously annealed in a 50 mM Tris·HCl pH 7.4, 150 mM KCl buffer. The CD spectra of 10 μM of *Htelo* quadruplex showed significant changes when in the presence of 15 equivalents of the A_2 *trans* and the A_3 porphyrin (Fig. 3). It was observed that those two porphyrins prefer the anti-parallel conformation which is indicated by the characteristic positive peak near 295 nm.[24] On the other hand, the A_2 *cis* porphyrin did not seem to alter the CD spectra of the G-quadruplex significantly, suggesting no specific preference for any of the two conformations, supporting the SPR results.

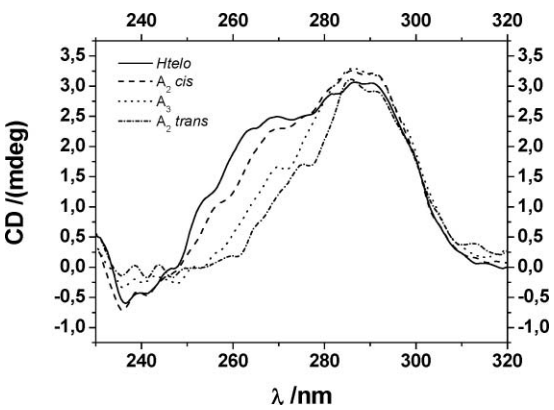

Fig. 3 CD spectra of 10 μM of *Htelo* quadruplex (solid line) in the presence of 15 equiv. of: A_2 *trans* (short dash dotted line); A_3 (dotted line); and A_2 *cis* (dashed line). Measurements were carried out at 20 °C in a 50 mM Tris·HCl pH 7.4, 150 mM KCl buffer.

It was also of interest to investigate if the porphyrins could induce the formation of a G-quadruplex in a K^+ free buffer with non-annealed *Htelo* DNA. The A_2 *trans* was the only porphyrin that clearly could achieve this: CD spectra show the suppression of the 255 nm positive peak of the non-annealed *Htelo* (solid line) in favour of the positive peak around 290 nm of the anti-parallel G-quadruplex conformation (dotted line) (Fig. 4). A clear isoelliptic point at around 270 nm suggests a transition between two conformation states upon addition of the porphyrin.

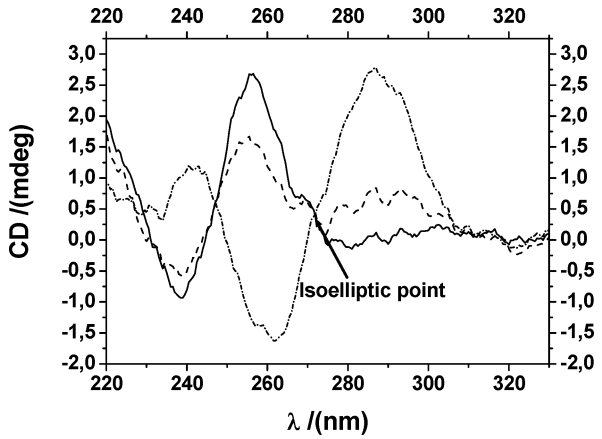

Fig. 4 CD spectra of 10 μM of non-annealed *Htelo* in a 50 mM Tris·HCl pH 7.4 buffer (solid line) in the presence of 2.2 equiv. (dashed line) and 12 equiv. (dotted line) of A_2 *trans*.

Conclusions

A series of porphyrins systematically lacking *meso* pyridinium substituents has been synthesised and their binding to telomeric G-quadruplex was assessed by SPR and CD spectroscopy. According to the SPR results two *meso* substituents in a *trans* orientation proved to be sufficient for tight quadruplex binding. Both A_2 *trans* and A_3 porphyrins showed preferential binding to the anti-parallel conformation of the intramolecular human telomeric quadruplex, and the A_2 *trans* is capable of acting as a molecular chaperone by inducing the formation of the anti-parallel G-quadruplex in a K^+ free buffer.

Experimental

^{1}H NMR spectra were recorded on Bruker DPX 400 and DRX 500 instruments, whilst ^{13}C spectra were collected on a Bruker DRX 500, equipped with a Cryopobe. Chemical shifts are quoted in parts per million; *J*-values are in Hz. Exact mass spectra were recorded on a Waters-LCT Premier-time of flight mass spectrometer. UV-Vis spectra were recorded on a HP 8452A Diode Array UV-Vis spectrophotometer. Circular dichroism experiments were performed on a JASCO model J-810 circular dichroism spectrapolarimeter equipped with a Peltier temperature controller. Preparative thin layer chromatography were carried out on Kieselgel 60 F_{254} (Merck) 0.2 mm plates. The porphyrin precursors were visualised by UV-visible absorption (245–365 nm). Flash column chromatography was carried out using Kieselgel 60 (Merk) 230–400 mesh and distilled solvents. Pyrrole was distilled before use and all other chemicals were purchased as reagent grade, being used without any further purification.

1,9-Diformyl-5-(4-pyridyl)dipyrromethane

Vilsmeier reagent was prepared by adding $POCl_3$ (32 mmol, 3.0 mL) drop wise under N_2 to DMF (20 mL) at 0 °C. 5-(4-pyridyl)dipyrromethane **5**[27](1.0 g, 4.5 mmol) was dissolved in DMF (15 mL) under N_2 and the solution was cooled to 0 °C. To this stirred solution was added drop wise the Vilsmeier reagent (7.7 mL, 10.8 mmol) and the mixture was stirred at 0 °C for 1.5 h. Saturated aqueous sodium acetate solution (50 mL) was carefully added and the mixture was stirred for 4 h at room temperature. The solution was extracted three times with ethyl acetate, the extracts were washed with brine and water, dried over Na_2SO_4 and evaporated *in vacuo* to give a brown oil. This was purified by flash chromatography (silica: eluted initially with chloroform, then gradually increasing to chloroform–methanol 10 : 1) to give after evaporation of the solvent *in vacuo*, 58% (729 mg) of diformylated dipyrromethane. ^{1}H NMR (500 MHz, $CDCl_3$), $\delta = 11.10$ (br s, 2H), 9.16 (s, 2H), 8.51 (d, $J = 6.1$ Hz, 2H), 7.20 (d, $J = 6.1$ Hz, 2H), 6.85–6.86 (m, 2H), 6.03–6.04 (m, 2H) and 5.58 (s, 1H) ppm. ^{13}C NMR (500 MHz Cryo, $CDCl_3$), $\delta = 179.3$, 150.1, 148.4, 139.9, 133.0, 123.6, 122.4, 111.9 and 43.8 ppm. Exact mass: calculated: 280.1086; found: 280.1088 ($M + H^+$).

4-Pyridylporphyrin 3

To a stirred solution of 1,9-diformyl-5-(4-pyridyl)dipyrromethane (165 mg, 0.59 mmol) in THF–methanol (10 : 1, 30 mL) was added, under N_2, $NaBH_4$ (0.46 g, 11.8 mmol) in small portions (every 2 min). After 40 min at room temperature, the reaction mixture was poured into a mixture of saturated aqueous ammonium chloride and chloroform (1 : 1, 50 mL). The organic phase was isolated, washed with water (2 times) and dried. The solvent was evaporated *in vacuo*. The residue was immediately dissolved in acetonitrile (56 mL), 2,2′-dipyrromethane **2**[28] (86.2 mg, 0.59 mmol) was added. The resulting mixture was stirred for 5 min at room temperature, then TFA (0.54 mL, 7.1 mmol) was added, and the mixture was stirred for one hour. DDQ (0.4 g, 1.8 mmol) in toluene (10 mL) was added. After mixing for 1 h at room temperature, triethylamine (1 mL, 7.1 mmol) was added. The crude mixture was evaporated to dryness, dissolved in chloroform and purified by preparatory TLC (eluted by chloroform–methanol 10 : 0.5). The fraction containing the porphyrin (as assessed by UV-visible) was collected and extracted from the silica. Evaporation of the solvent *in vacuo* afforded 7 to 10% of red product (16.0 to 22.8 mg). $\lambda_{max}(CH_2Cl_2)$/nm 400 (log ε/dm^3 mol^{-1} cm^{-1} 4.53), 494 (3.12), 530 (1.67) and 566 (1.49). ^{1}H NMR (500 MHz, acetone-d_6), $\delta = 10.50$ (s, 2H), 10.49 (s, 1H), 9.64–9.66 (m, 4H), 9.63 (d, $J = 4.6$ Hz, 2H), 9.25 (d, $J = 6.5$ Hz, 2H), 9.17 (d, $J = 4.6$ Hz, 2H), 8.81 (d, $J = 6.5$ Hz, 2H) and −3.59 (br s, 2H, NH) ppm. ^{13}C NMR (500 MHz Cryo, acetone-d_6), $\delta = 158.3$, 150.2, 150.1, 149.6, 147.7, 145.0, 132.9, 132.7, 132.4, 132.1, 130.7, 113.1, 106.0 and 105.9 ppm. Exact mass: calculated: 388.1562; found: 388.1558 ($M + H^+$).

5,15-Di(4-pyridyl)porphyrin 7

To a stirred solution of 5-(4-pyridyl)dipyrromethane **5** (500 mg, 2.24 mmol) and trimethyl orthoformate **6** (18 mL, 0.17 mol) in dry DCM (630 mL) previously degassed by bubbling with argon, was added drop wise, over 15 min, a solution of TCA (8.83 g, 54 mmol) in dry DCM (227 mL). After the addition was complete, the solution was stirred in the dark and at room temperature, for 4 h, before being quenched with pyridine (15.6 mL) and stirred, again in the dark for further 17 h. The solution was the purged with air for 10 min and stirred, under ambient lighting conditions, for 4 h. Solvent was evaporated *in vacuo*, first using water aspiration, and then vacuum overnight. The resulting black solid was preadsorbed onto silica and loaded onto the top of a flash chromatography column. The crude product was eluted from the column using a mixture of chloroform–methanol (10 : 1). Evaporation of the eluent afforded 9% of a purple solid (91 mg). ^{1}H NMR (500 MHz, $CDCl_3$), $\delta = 10.36$ (s, 1H), 9.43 (d, $J = 4.6$ Hz, 4H), 9.05 (d, $J = 5.4$ Hz, 4H), 9.03 (d, $J = 4.6$ Hz, 4H), 8.19 (d, $J = 5.8$ Hz, 4H) and −3.19 (br s, 2H, NH) ppm.

5,10-Di(4-pyridyl)porphyrin 11

5,10-Di(4-pyridyl)tripyrromethane[27] (0.45 g, 1.0 mmol) was added to the stirred solvent of dry DCM (200 mL) previously degassed by bubbling with argon at room temperature protected from light. A solution of 2,5-bis(hydroxymethyl)pyrrole **10**[29] (0.127 g, 1.0 mmol) in methanol (5 mL) was added to the stirred solution. After 10 min, TFA (15.6 μL, 0.2 mmol) was added with a micro syringe, and then the reaction mixture was stirred for 1.5 h. DDQ (0.22 g, 1.0 mmol) was then added, followed by triethylamine (0.03 mL, 0.2 mmol). The crude mixture was evaporated to dryness, dissolved in chloroform and purified by preparative TLC (eluted by chloroform–methanol 10 : 1). The fraction containing the porphyrin (as assessed by UV-visible) was collected and extracted from the silica. Evaporation of the solvent *in vacuo* afforded 3% purple product (14 mg). $\lambda_{max}(CH_2Cl_2)$/nm 406 (log ε/dm^3 mol^{-1} cm^{-1} 5.18), 500 (4.01), 578 (3.62), 660 (3.20) and 698 (3.16). ^{1}H NMR (500 MHz, $CDCl_3$), $\delta = 10.28$ (s, 2H), 9.46 (s, 2H), 9.39 (d, $J = 4.6$ Hz, 2H), 9.04 (d, $J = 5.8$ Hz, 4H), 8.97 (d, $J = 4.6$ Hz, 2H), 8.90 (s, 2H), 8.77 (d, $J = 5.8$ Hz, 4H) and −3.45 (br s, 2H, NH) ppm. ^{13}C NMR (500 MHz Cryo, $CDCl_3$), $\delta = 150.2$, 148.3, 130.0–133.0, 129.5, 116.6 and 105.2 ppm. Exact mass: calculated: 465.1828, found 465.1818 ($M + H^+$).

5,10,15-Tri(4-pyridyl)porphyrin 14

To a stirred solution of 5-(4-pyridyl)dipyrromethane **5** (500 mg, 2.24 mmol), 4-pyridinecarboxaldehyde **13** (0.4 mL, 4.1 mmol) and trimethyl orthoformate **6** (9 mL, 82.5 mmol) in dry DCM (630 mL) previously degassed by bubbling with argon, was added drop wise, over 15 min, a solution of TCA (8.83 g, 54 mmol) in dry DCM (227 mL). After the addition was complete, the solution was stirred in the dark and at room temperature, for 4 h, before being quenched with pyridine (15.6 mL) stirred, again in the dark for further 2 h and then DDQ was added (0.3 g, 1.3 mmol). Solvent was evaporated *in vacuo*, first using water aspiration, and then vacuum overnight. The resulting black solid was preadsorbed onto silica and loaded onto the top of a flash chromatography column. The crude product was eluted from the column using a mixture of chloroform–methanol (10 : 1); the second porphyrinic fraction corresponded to the expected product. Evaporation of the eluent afforded 0.8% of a purple solid (5.1 mg). $\lambda_{max}(CH_2Cl_2)$/nm 414 (log ε/dm^3 mol^{-1} cm^{-1} 4.97), 508 (3.86), 580 (3.38) and 636 (2.64). ^{1}H NMR (500 MHz, $CDCl_3$), $\delta = 10.40$ (s, 1H), 9.44 (d, $J = 4.6$ Hz, 2H), 9.07 (br s), 9.02 (d, $J = 4.8$ Hz, 2H), 9.00 (d, $J = 4.6$ Hz, 2H),

8.92 (d, $J = 4.8$ Hz, 2H), 8.75 (d, $J = 5.6$ Hz, 2H), 8.18 (d, $J = 5.6$ Hz, 4H), 7.65 (dd, $J = 6.6$, 1.5 Hz, 2H) and −3.06 (br s, 2H, NH) ppm. ^{13}C NMR (500 MHz Cryo, $CDCl_3$), $\delta = 150.9$, 149.2, 148.5,147.0, 132.8, 131.6, 131.1, 130.1, 129.4, 123.5, 117.5, 114.8 and 107.6 ppm. Exact mass: calculated: 542.2093; found: 542.2079 ($M + H^+$).

General procedure for the methylation of the porphyrins pyridyl groups

The quaternisation of the pyridyl groups was achieved by methylation with a large excess of iodomethane in DMF, at 40 °C for 3 to 7 h, in quantitative yields. The anion-exchange was performed with Dowex 1 × 2–200 in the chloride form, which was shaken together with the previously dissolved porphyrins in 20% acetone 80% water for 2 h. The resin was then filtered off and washed with water, giving the final porphyrins without any need of further purification.

5-(*N*-Methylpyridinium-4-yl)porphyrin 4. λ_{max}(MeCN)/nm 398 (log ε/dm^3 mol^{-1} cm^{-1} 4.84), 496 (3.89), 538 (3.69) and 580 (3.73). ^{1}H NMR (500 MHz, CD_3NO_2), $\delta = 10.59$ (s, 2H), 10.54 (s, 1H), 9.83 (d, $J = 4.6$ Hz, 2H), 9.66 (s, 4H), 9.23 (d, $J = 6.5$ Hz, 2H), 9.18 (d, $J = 4.6$ Hz, 2H), 8.98 (d, $J = 6.5$ Hz, 2H), 4.86 (s, 3H) and −3.77 (br s, 2H, NH) ppm. ^{13}C NMR (500 MHz Cryo, CD_3NO_2), $\delta = 160.3$, 144.5, 134.4, 133.3, 133.6, 130.6, 112.2, 107.4 and 49.2 ppm. Exact mass: calculated: 402.1719; found: 402.1711 (M).

5,15-Di(*N*-methylpyridinium-4-yl)porphyrin 8. ^{1}H NMR (500 MHz, DMSO), $\delta = 10.85$ (s, 2H), 9.86 (d, $J = 4.7$ Hz, 4H), 9.53 (d, $J = 6.6$ Hz, 4H), 9.25 (d, $J = 4.7$ Hz, 4H), 9.08 (d, $J = 6.6$ Hz, 4H), 4.74 (s, 6H) and −3.34 (br s, 2H, NH) ppm.

5,10-Di(*N*-methylpyridinium-4-yl)porphyrin 12. λ_{max}(MeCN)/nm 412 (log ε/dm^3 mol^{-1} cm^{-1} 5.04), 504 (4.01), 574 (3.52) and 632 (2.95). ^{1}H NMR (500 MHz, CD_3CN (75%) and CD_3OD (25%)), $\delta = 10.61$ (s, 2H), 9.66 (br s, 4H), 9.18 (d, $J = 4.5$ Hz, 4H), 9.07 (br s, 4H), 8.88 (d, $J = 4.5$ Hz, 4H) and 4.7 (s, 6H) ppm. ^{13}C NMR (500 MHz Cryo, CD_3CN (75%) and CD_3OD (25%)), $\delta = 159.1$, 145.0, 134.1, 114.2, 108.3 and 49.4 ppm. Exact mass: calculated: 247.1104; found: 247.1070 (M).

5,10,15-Tri(*N*-methylpyridinium-4-yl)porphyrin 15. λ_{max}(MeCN)/nm 420 (log ε/dm^3 mol^{-1} cm^{-1} 5.04), 510 (4.21), 548 (4.08) and 580 (4.07). ^{1}H NMR (500 MHz, CD_3NO_2), $\delta = 10.73$ (s, 1H), 9.72 (d, $J = 4.7$ Hz, 2H), 9.28 (d, $J = 6.5$ Hz, 4H), 9.26 (d, $J = 6.7$ Hz, 2H), 9.18 (d, $J = 4.7$ Hz, 2H), 9.16 (d, $J = 4.9$ Hz, 2H), 9.11 (d, $J = 4.9$ Hz, 2H), 8.97 (d, $J = 6.5$ Hz, 4H), 8.94 (d, $J = 6.7$ Hz, 2H) and 4.86 (s, 9H) ppm. ^{13}C NMR (500 MHz Cryo, CD_3NO_2), $\delta = 160.0$, 159.2, 144.9, 144.8, 134.1, 133.9, 117.0, 115.4, 109.6 and 49.4 ppm. Exact mass: calculated: 195.4234; found: 195.4224 (M).

SPR studies

Surface plasmon resonance was performed on a Biacore 2000 instrument, using degassed Tris·HCl running buffer (50 mM Tris·HCl, 100 mM KCl, pH 7.4). Sensor chips (Type SA, Biacore) were loaded with approximately 600 RU of *Htelo* (and duplex DNA as a control). Six serial dilutions of compound were injected at a flow rate of 20 μL min^{-1} and the equilibrium response determined relative to the baseline (blank flow cell). The maximum compound concentrations were 6.25 μM for A_2 *trans* and H_2-$TMPyP_4$ and 25 μM for A_2 *cis* and A_3. Between injections the sensor surface was refreshed with injections of 1 M KCl and buffer. All experiments were carried out in duplicate.

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