
Supporting Information

Well-defined, persistent, chiral phthalocyanine nanoclusters via G-quadruplex assembly

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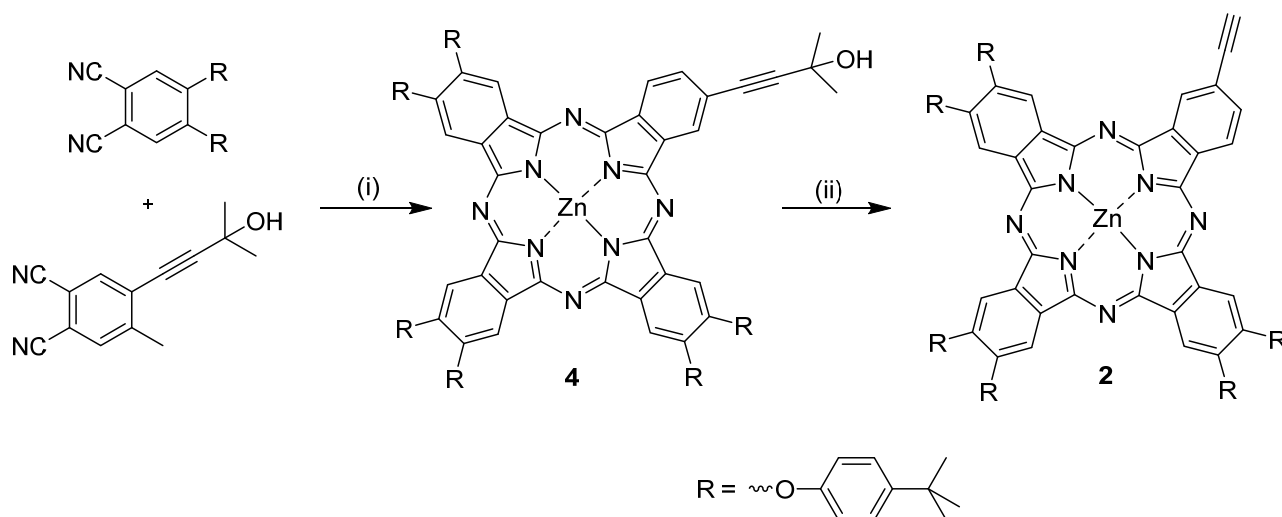
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1. GENERAL METHODS

MALDI-TOF-MS spectra were obtained from a *BRUKER ULTRAFLEX III* instrument equipped with a nitrogen laser operating at 337 nm. **NMR** spectra were recorded with a *BRUKER AC-300* (300 MHz) and *BRUKER DRX* 500 MHz instrument. The temperature was actively controlled at 298 K. Chemical shifts are measured in ppm using the signals of the deuterated solvent as the internal standard. **Column chromatography** was carried out on silica gel Merck-60 (230–400 mesh, 60 Å), and TLC on aluminum sheets precoated with silica gel 60 F254 (E. Merck). **UV/Vis spectra** were recorded with a *JASCO V-660*. **Emission spectra** were obtained with a *JASCO-V8600*. **CD spectra** were recorded with a *JASCO V-815* equipment. In all these instruments the temperature was controlled using a *JASCO Peltier* temperature controller with a range of 263–383 K, adjustable temperature slope and accuracy of ± 0.1 K.

2. SYNTHESIS AND CHARACTERIZATION

Chemicals were purchased from commercial suppliers and used without further purification. Solid, hygroscopic reagents were dried in a vacuum oven before use. Reaction solvents were thoroughly dried before use using standard methods. The synthesis and characterization of bromoguanosine **3** from commercial guanosine has been reported before by us.^[1] The synthesis of ethynyl-Pc **2** (Scheme S1) is described below.



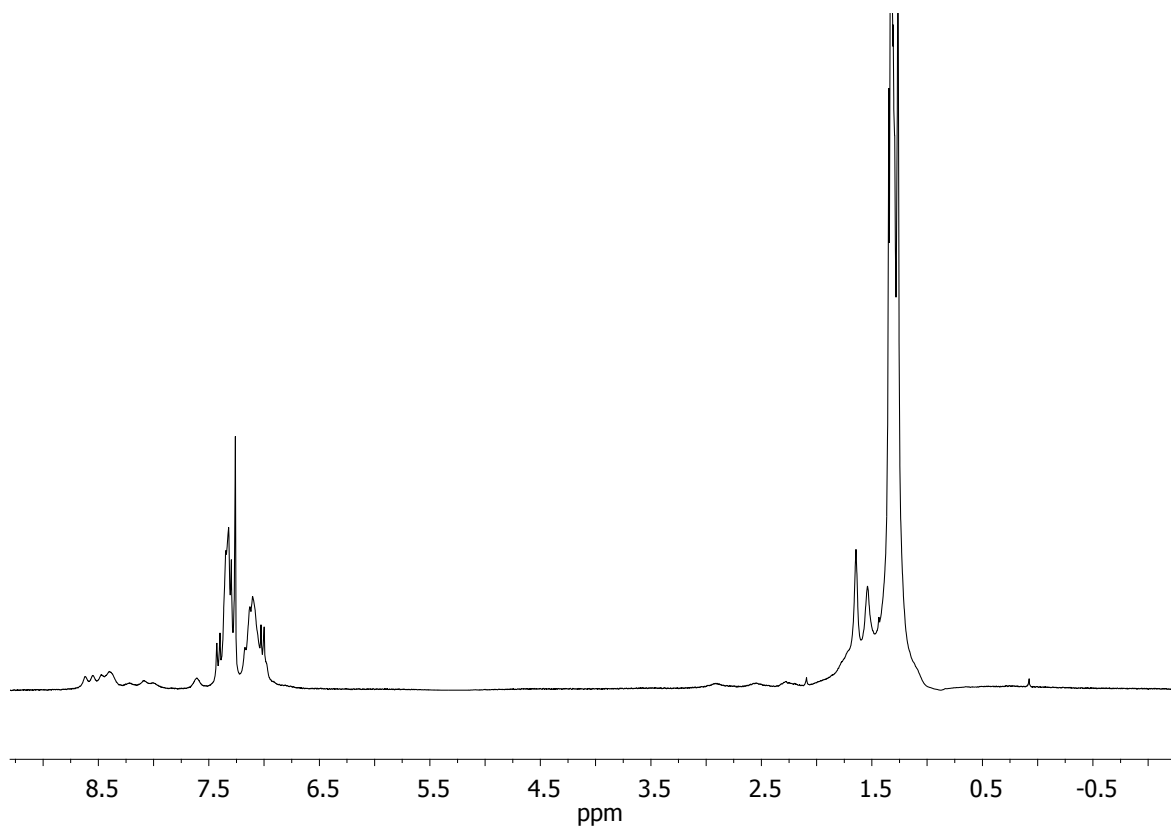
Scheme S1. Synthetic route to **2**: (i) $ZnCl_2$, DMAE, reflux, 16h, 14%. (ii) NaOH, toluene, reflux, 6h, 71%.

2,3,9,10,16,17-*tert*-butylphenoxy-23-(3-hydroxy-3-methyl-1-butynyl)-5,28:14,19-diimino-7,12:21,26-dinitrilotetrabenzo[*c,h,m,r*][1,6,11,16]tetraazacicloecosinato-(2)-N²⁹,N³⁰,N³¹,N³²zinc(II) (4)

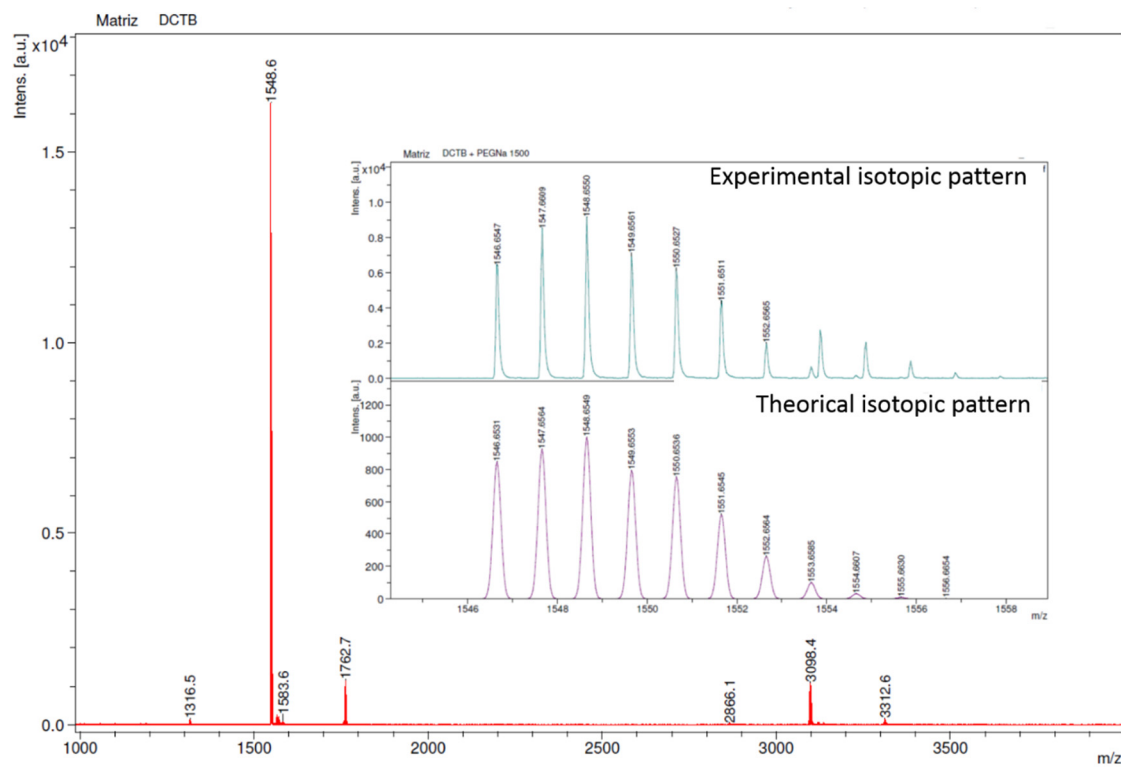
A dimethylaminoethanol (DMAE) (2 mL) solution of, 4,5-*tert*-butylphenoxyphthalonitrile^[iii] (708 mg, 1.67 mmol), 4-(3-hydroxy-3-methyl-1-butynyl)phthalonitrile^[iii] (117 mg, 0.55 mmol) and ZnCl₂ (75 mg, 0.55 mmol) was stirred at reflux under argon atmosphere for 16 h. The reaction was allowed to cool to room temperature and the crude was precipitated and triturated in methanol / water (1:1) mixture. The resulting green solid was then purified by column chromatography on silica gel using as a mobile phase hexane/dioxane (4:1). The fractions containing the desired product were then evaporated yielding phthalocyanine (**1**) as a green solid in 14% yield.

M.p.: > 250 °C. **¹H NMR** (400 MHz, CDCl₃, 25 °C, TMS), δ = 8.8-7.8 (m, 8H), 7.73-7.52 (m, 1H), 7.75-7.2 (m, 12H), 7.2-6.9 (m, 12H), 1.65-1.1 (s, 60H) ppm. **FT-IR** (KBr): 2933, 2929, 2855, 1721, 1603, 1507, 1450, 1394, 1394, 1363, 1266, 1214, 1172, 1088, 1013, 891, 827, 743, 732, 545, 439 cm⁻¹. **UV/Vis** (THF): λ_{max} (log ϵ) = 671 (5.1), 617 (4.6), 601 (4.4), 357 (4.8), 293 (4.6) nm. **MS** (MALDI-TOF, dithranol: *m/z* (%) 1546.65-1552.65 (100) [M]⁺. **HR MALDI-TOF MS**, dithranol: *m/z* 1546.6547 [M]⁺, calcd for C₉₇H₉₄N₈O₇Zn: 1546.6531.

^1H NMR of 4 (CDCl_3)



MALDI-TOF spectra with (inset) isotopic distribution pattern HR-MALDI-TOF spectrum of 4

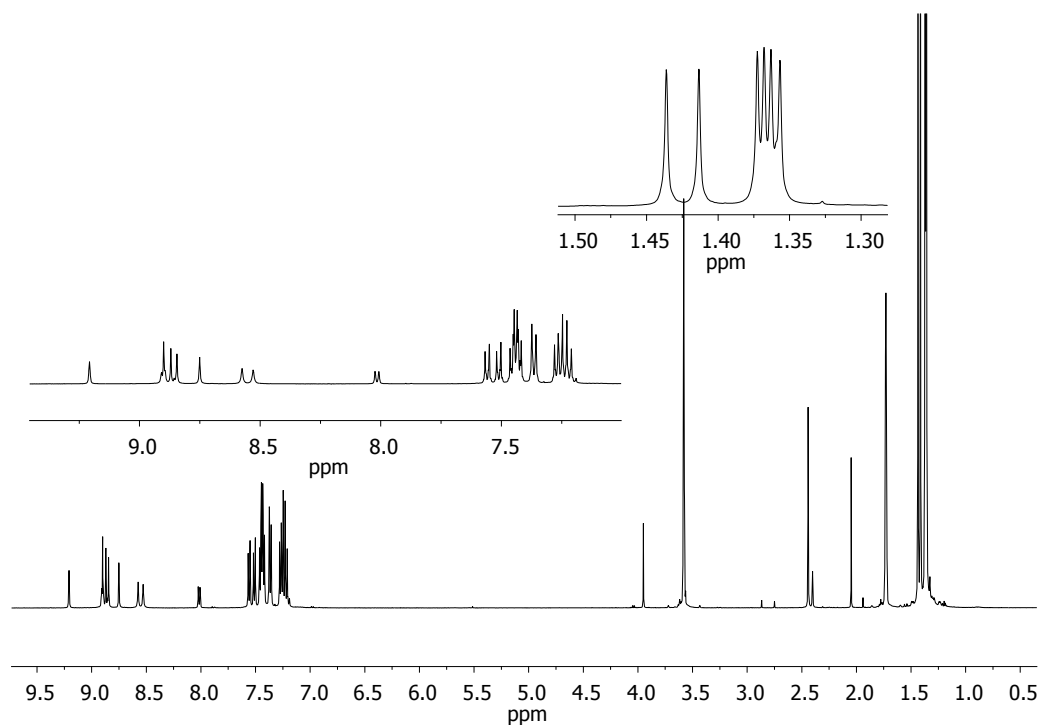


2,3,9,10,16,17-*tert*-butylphenoxy-23-ethynyl-5,28:14,19-diimino-7,12:21,26-dinitrilotetrabenzo[*c,h,m,r*][1,6,11,16]tetraazacicloecosinato-(2)-N²⁹,N³⁰,N³¹,N³²zinc(II) (2)

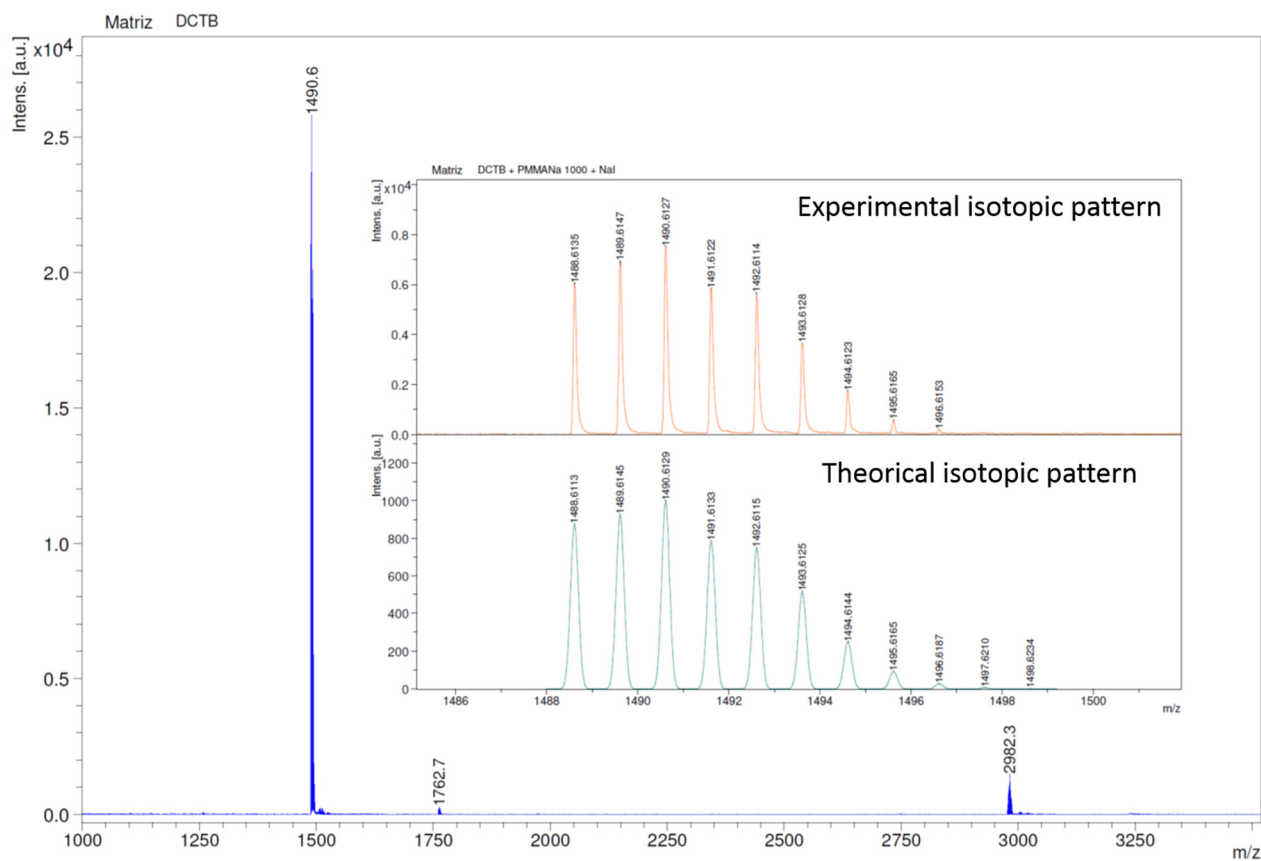
A dry toluene solution (2 mL) of phthalocyanine **4** (108 mg, 0.07 mmol) and dry NaOH (3 mg, 0.075 mmol) was stirred in a 10 mL flask under reflux in argon atmosphere for six hours. The solvent was removed at the rotary evaporator and the solid residue was extracted with CH₂Cl₂ and washed with water. The organic phase was dried over MgSO₄ and the solvent evaporated. The resulting crude was purified by column chromatography on silica gel using heane/ dioxane (5:1) as eluent. Phthalocyanine **2** was obtained as a green solid in a 71% yield.

M.p.: > 250 °C. **¹H NMR** (500 MHz, THF-*d*₈, 25 °C, TMS), δ 9.19 (s, 1H), 9.0 – 8.9 (m, 2H), 8.87 (s, 1H), 8.85 (s, 1H), 8.75 (s, 1H), 8.57 (s, 1H), 8.53 (s, 1H), 8.02-8.01 (m, 1H), 7.61–7.53 (m, 2H), 7.53–7.48 (m, 2H), 7.48–7.40 (m, 8H), 7.40 – 7.32 (m, 4H), 7.33 – 7.15 (m, 8H), 7.93 (s, 1H), 1.43 (s, 9H), 1.41(s, 9H), 1.37 (m, 36H) ppm. **¹³C NMR** (126 MHz, THF-*d*₈, 25 °C, TMS), δ 157.49, 157.42, 157.29, 157.22, 156.81, 156.45, 153.69, 153.58, 153.51, 153.11, 153.07, 152.57, 152.55, 152.16, 152.13, 151.65, 151.42, 150.95, 150.50, 147.28, 147.00, 146.64, 146.54, 146.44, 146.42, 139.03, 138.56, 136.04, 135.95, 135.57, 135.42, 134.81, 133.27, 127.74, 127.70, 127.52, 127.49, 127.45, 127.39, 124.07, 123.61, 119.50, 119.09, 118.49, 118.35, 118.24, 118.20, 116.46, 116.37, 116.22, 116.06, 115.13, 114.04.85.56, 80.74, 35.30, 35.26, 35.18, 35.16, 32.20, 32.16 ppm. **FT-IR** (KBr): 2959, 2905, 2867, 2106, 1602, 1507, 1452, 1404, 1363, 1267, 1214, 1178, 1088, 1029, 892, 828, 744, 723, 547 cm⁻¹. **UV/Vis** (THF): λ_{max} (log ε) = 686 (5.2), 631 (4.4), 601 (4.2), 356 (4.7) nm. **MS** (MALDI-TOF, dithranol: m/z (%) 1488.6-1496.6 (100) [M]⁺. **HR MALDI-TOF MS**, dithranol: m/z 1488.6135 [M]⁺, calcd for C₉₄H₈₈N₈O₆Zn: 1488.6113.

^1H NMR of 2 (THF- d_6)



MALDI-TOF spectra with (inset) isotopic distribution pattern HR-MALDI-TOF spectrum (b) of 2



Zn(II)Pc-G (1)

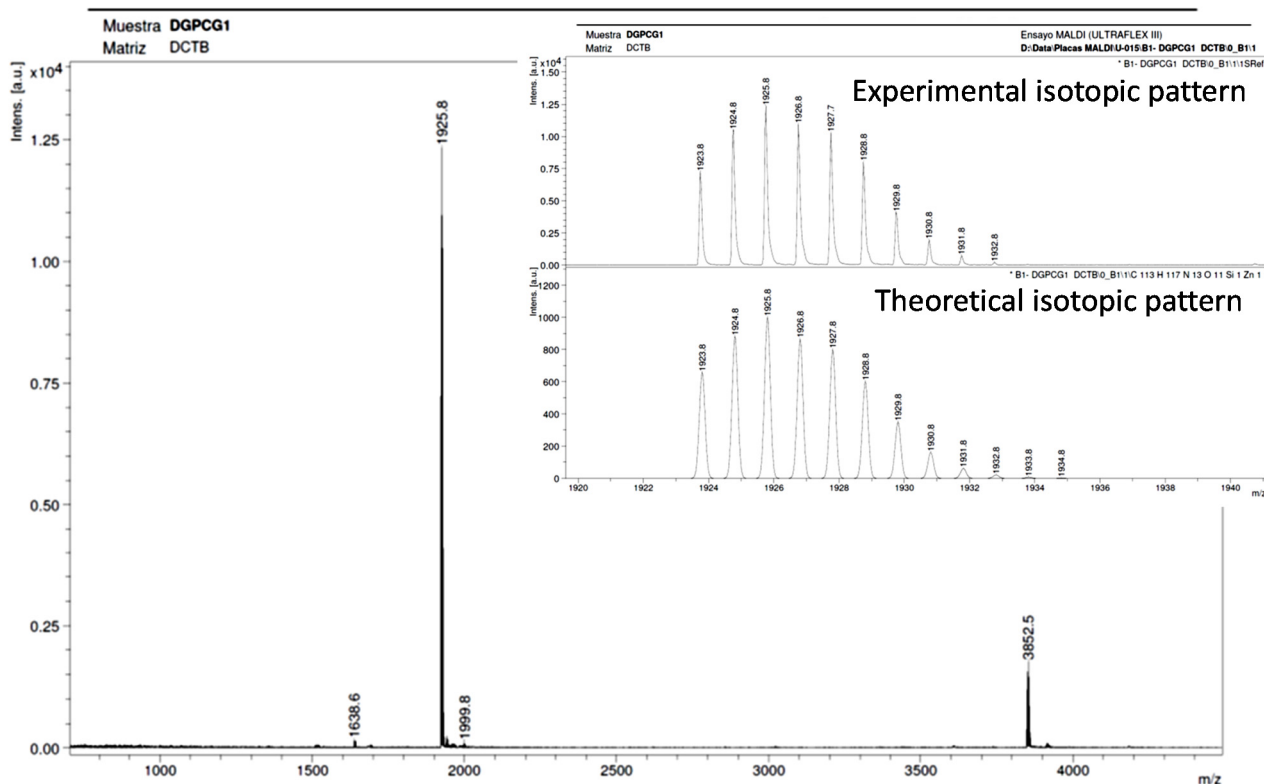
A dry a Toluene/THF/NEt(*i*Pr)₂ (3:1:1) mixture (2 mL) was subjected to deoxygenation by three freeze-pump-thaw cycles with argon and poured over a mixture of ethynyl-Pc **2** (236 mg, 0.16 mmol), bromoguanosine **3** (68 mg, 0.13 mmol), Pd(PPh₃)₄ (2.3 mg, 0.002 mmol), and CuI (0.2 mg, 0.001 mmol). The mixture was stirred under argon for 16 h at 60 °C. Once the ethynyl derivative is consumed, monitored by TLC, the mixture was filtered over a celite plug and the solvent was evaporated under reduced pressure. The product was purified by two consecutive column chromatographies on silica gel using first hexane/1,4-dioxane (1:1) and then CHCl₃/MeOH (50:1) as eluents, affording **1** as a green solid (30 mg, 12 %).

M.p.: > 250 °C. **¹H NMR** (300 MHz, THF-*d*₈- DMSO-*d*₆ (2:1), 25 °C, TMS), δ = 10.81 (s(b), 1H), 9.49 (s, 1H), 9.25 (d, 1H, *J*_o = 6.5 Hz), 9.06 (s, 1H), 9.0 – 8.8 (m, 5H), 8.23 (d, 1H, *J*_o = 6.5 Hz), 7.5 – 7.0 (m, 24H), 6.50 (s(b), 2H), 6.37 (s, 1H), 5.59 (d, 1H, *J* = 6.7 Hz), 5.22 (m, 1H), 4.16 (m, 1H), 3.77 (d, 2H, *J* = 6.8 Hz), 1.54 (s, 3H), 1.33 (s, 3H), 1.3-1.1 (m, 54H), 0.76 (s, 9H), -0.11 (s, 3H), -0.13 (s, 3H). **UV/Vis** (THF): λ_{max} (log ε) = 687 (5.2), 615 (4.4), 356 (4.8) nm. **MS** (MALDI-TOF, dithranol): *m/z* (%) 1923.8-1932.8 (100) [M]⁺, 3847.5-3860.5 (20) [2M]⁺. **HR MALDI-TOF MS**, dithranol: *m/z* 1923.8056 [M]⁺, calcd for C₁₁₃H₁₁₇N₁₃O₁₁SiZn: 1923.8056.

¹H NMR of **1** (THF-*d*₈- DMSO-*d*₆ (2:1))

SEE FIGURE S1 BELOW

MALDI-TOF spectra with (inset) isotopic distribution pattern of **1**



3. FIGURES S1-S5.

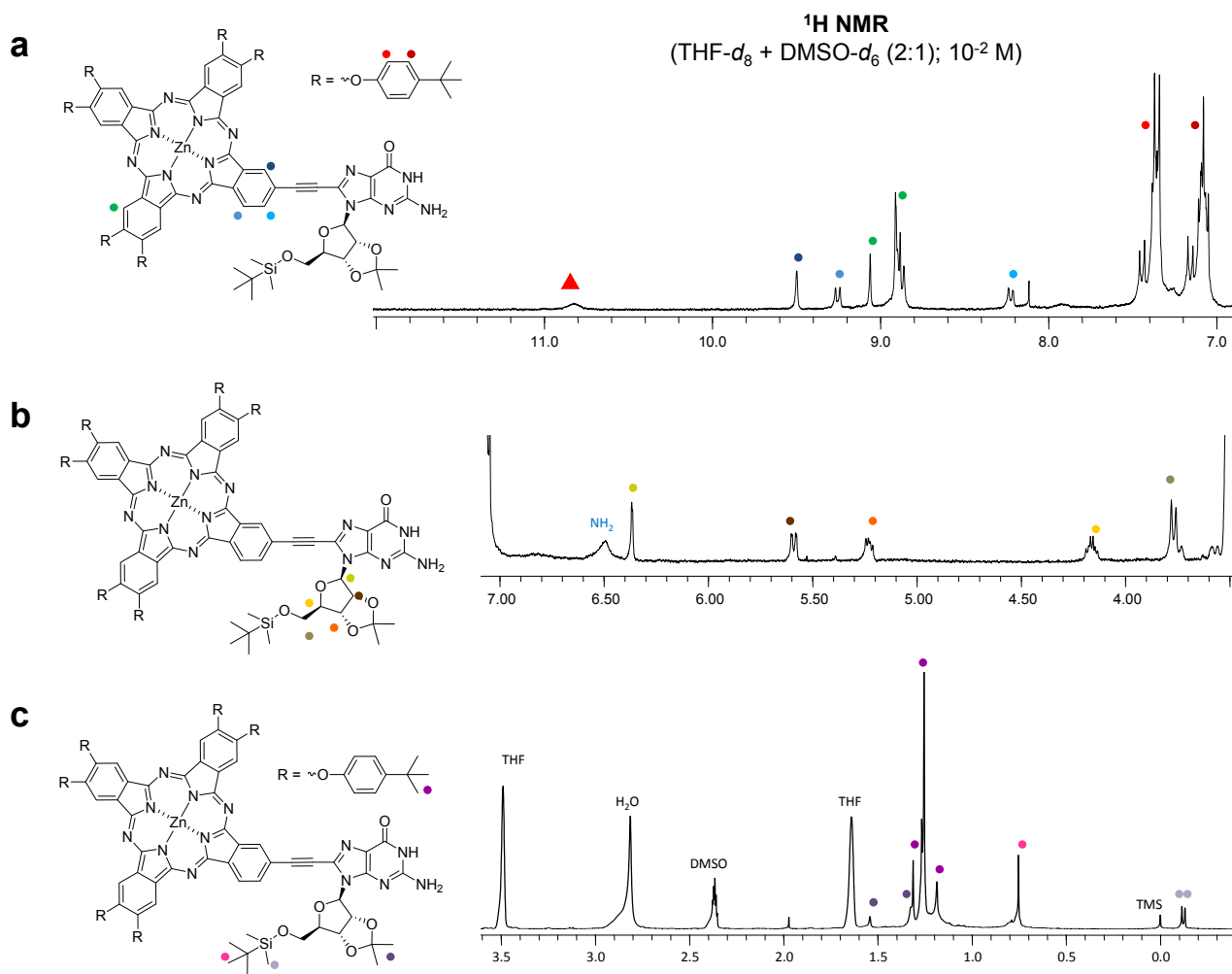


Figure S1. Three regions of the ^1H NMR spectrum of Pc-G **1** in a 2:1 mixture of THF- d_8 and DMSO- d_6 where aggregation at a 10^{-2} M concentration is prevented.

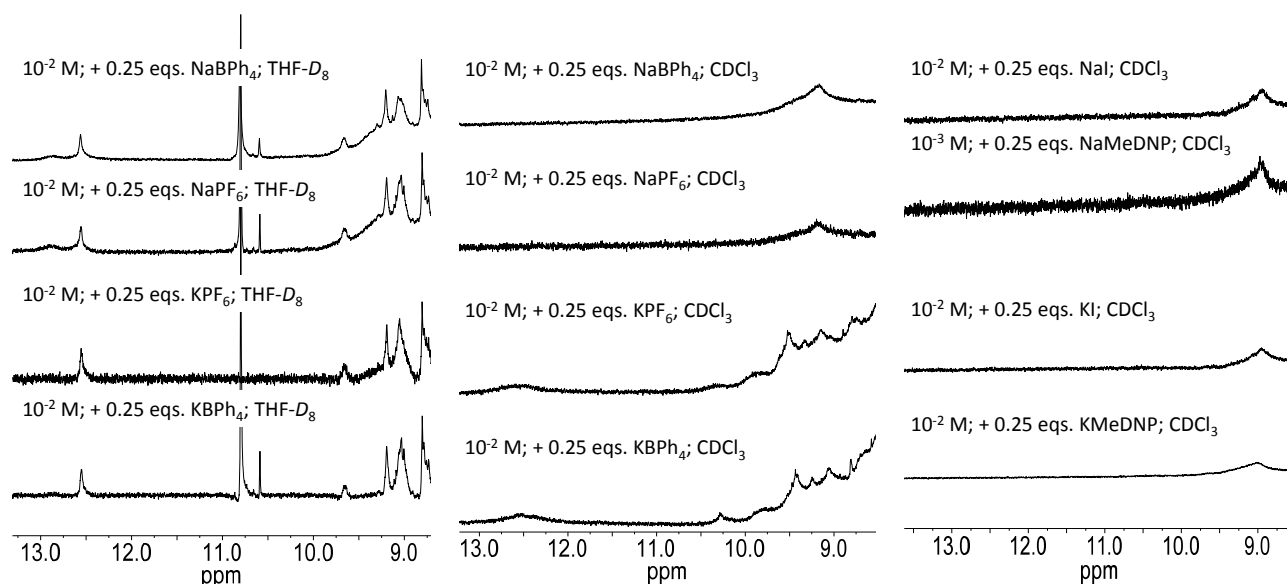


Figure S2. Low-field region of the ^1H NMR spectrum of Pc-G **1** at 25 °C in different solvents in the presence of K^+ or Na^+ salts. Only in THF- d_8 well-resolved signals could be obtained that helped us assign quadruplex structure. The Pc-G **1** octamer can be assembled in this solvent at NMR concentrations in the presence of K^+ or Na^+ salts bearing non-coordinating anions like BPh_4^- or PF_6^- . However, the complexes with Na^+ salts are weaker and showed only partial formation, revealing a mixture of the Pc-G **1** octamer (sharp signal at 12.5 ppm) and Pc-G **1** monomer (broad signal at 12.8 ppm) in slow exchange. In CDCl_3 and toluene- d_8 , however, very broad signals were obtained that precluded any assignment. We believe the G-quadruplexes may further aggregate in these solvents at the high NMR concentrations. In acetone- d_6 the monomer and quadruplexes are not soluble enough.

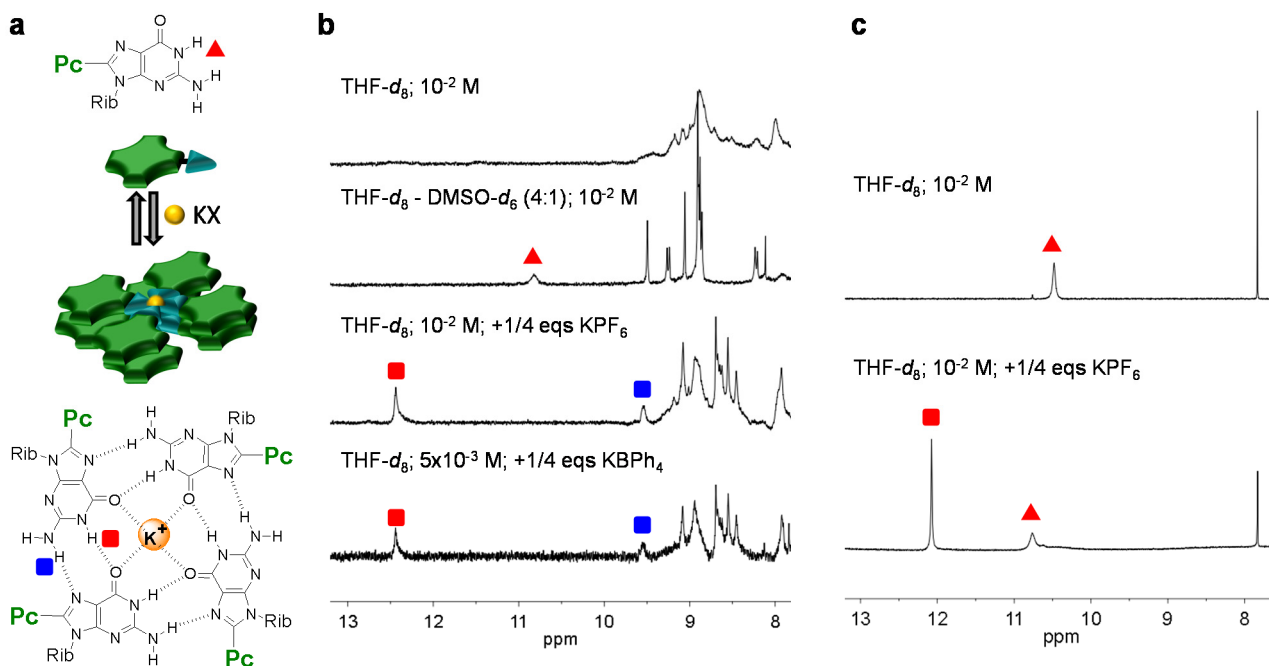


Figure S3. (a) Pc-G **1** octamer formation in the presence of K⁺ salts. The most characteristic amide and amine protons are shown at the monomer and G-quartet states. (b) Low-field region of the ¹H NMR spectrum of Pc-G **1** in different solvents and concentrations and in the absence or presence of K⁺ salts. (c) Low-field region of the ¹H NMR spectrum of G **3** in THF-*d*₈ at a 10⁻² M concentration in the absence or presence of KPF₆ salts.

In the presence of KPF₆, a slow equilibrium between monomer **3** (red triangle) and the (**3**)₈·K⁺ complex (red square) was observed in THF-*d*₈, as reported previously by us (see refs. 5g and 7c in the text). In contrast, in the same conditions, no signal for monomer **1** was detected (see 3rd spectrum in (b)), suggesting an additional stabilization of the quadruplex caused by the presence of the Pc macrocycle. This stabilization effect is further supported by the observation of the H-bound G-amine proton at around 9.5 ppm (blue square), which is typically found as a broad signal at room temperature for **3** and other G derivatives (see also Figure S3 and refs. 5g and 7c in the text).

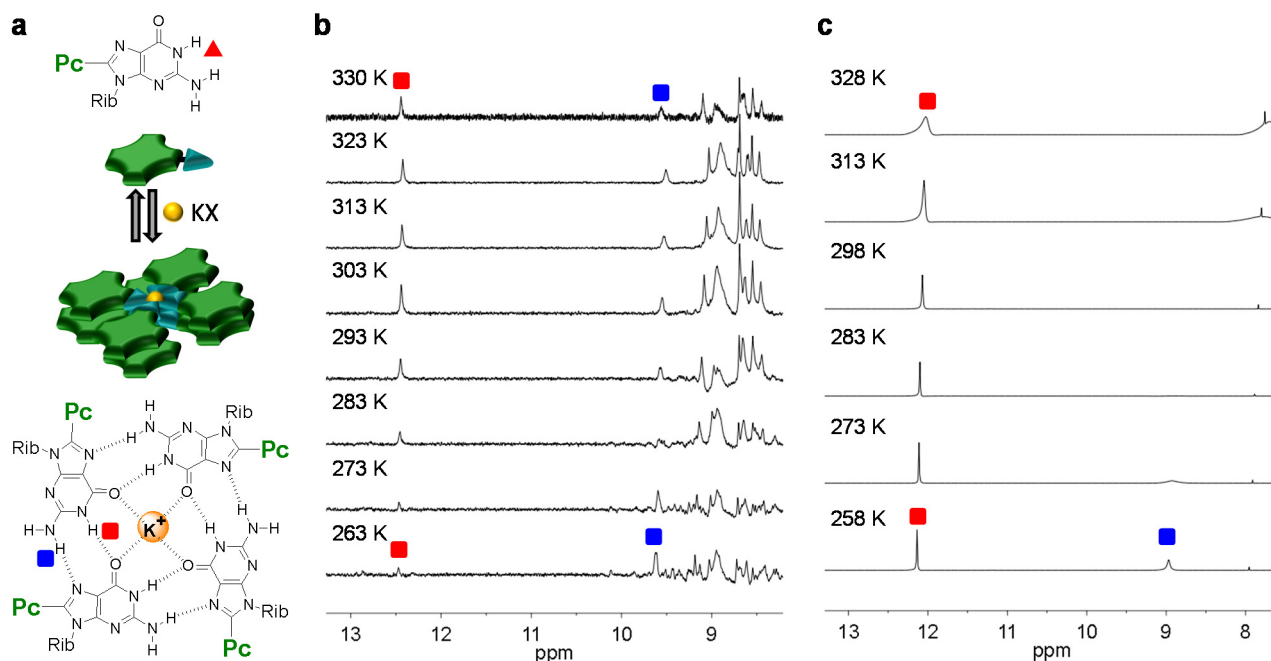


Figure S4. (a) Pc-G **1** octamer formation in the presence of K⁺ salts. The most characteristic amide and amine protons are shown at the monomer and G-quartet states. (b,c) Low-field region of the ¹H NMR spectrum of (b) Pc-G **1** at a 10⁻² M concentration and (c) G **3** in THF-*d*₈ at a 10⁻¹ M concentration in the presence of KPF₆ salts and as a function of sample temperature.

The additional quadruplex stabilization brought by the presence of the Pc cores (please, compare Figures S3b and c) is manifested by the persistence of the H-bound G-amine proton at around 9.5 ppm (blue square) in the whole range of temperatures (for the the (**3**)₈·K⁺ complex it is only detected at low temperatures), and the constant shape and position of the G-amide proton signal in (**1**)₈·K⁺ (red square).

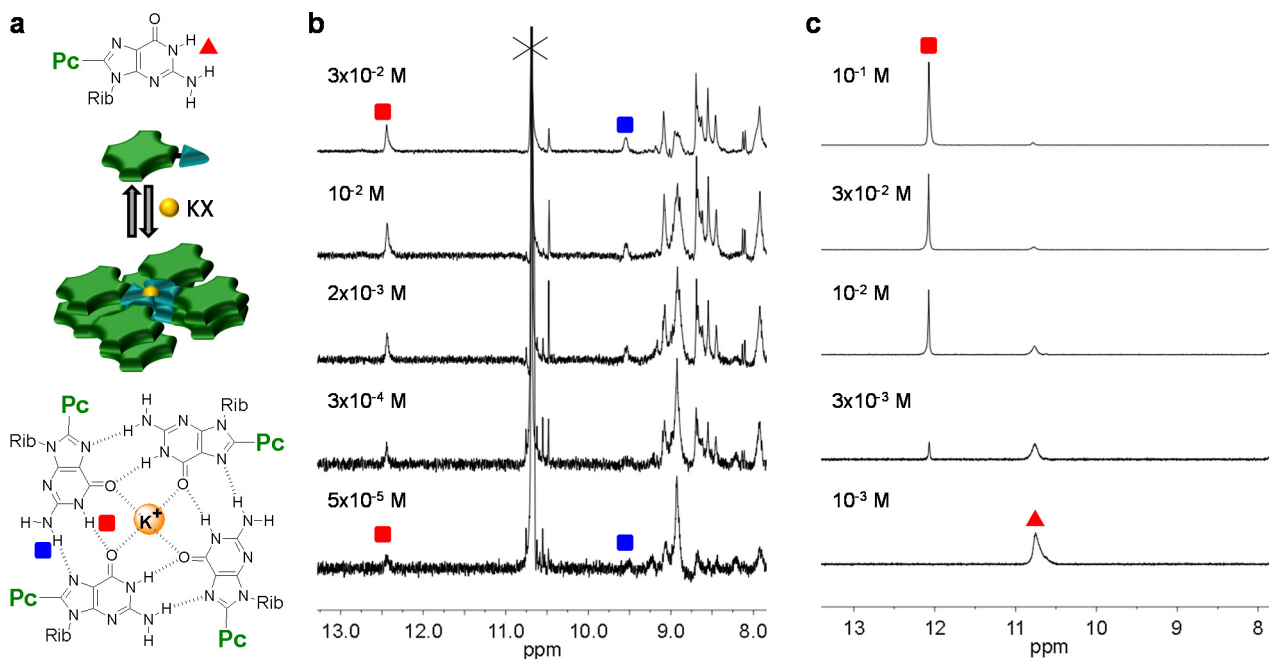


Figure S5. (a) Pc-G **1** octamer formation in the presence of K⁺ salts. The most characteristic amide and amine protons are shown at the monomer and G-quartet states. (b,c) Low-field region of the ¹H NMR spectrum of (b) Pc-G **1** and (c) G **3** in THF-*d*₈ at room temperature in the presence of KPF₆ salts and as a function of sample concentration.

The additional quadruplex stabilization brought by the presence of the Pc cores (please, compare Figures S4b and c) is manifested again by the persistence of the H-bound G-amine proton at around 9.5 ppm (blue square) in the whole range of concentrations, and the constant shape and position of the G-amide proton signal (red square).

Also note that the (3)₈·K⁺ complex dissociates upon dilution and, at 10⁻³ M, no signal of the octamer is detected anymore. In marked contrast, the (1)₈·K⁺ complex H-bound signals persist until the NMR detection limits.

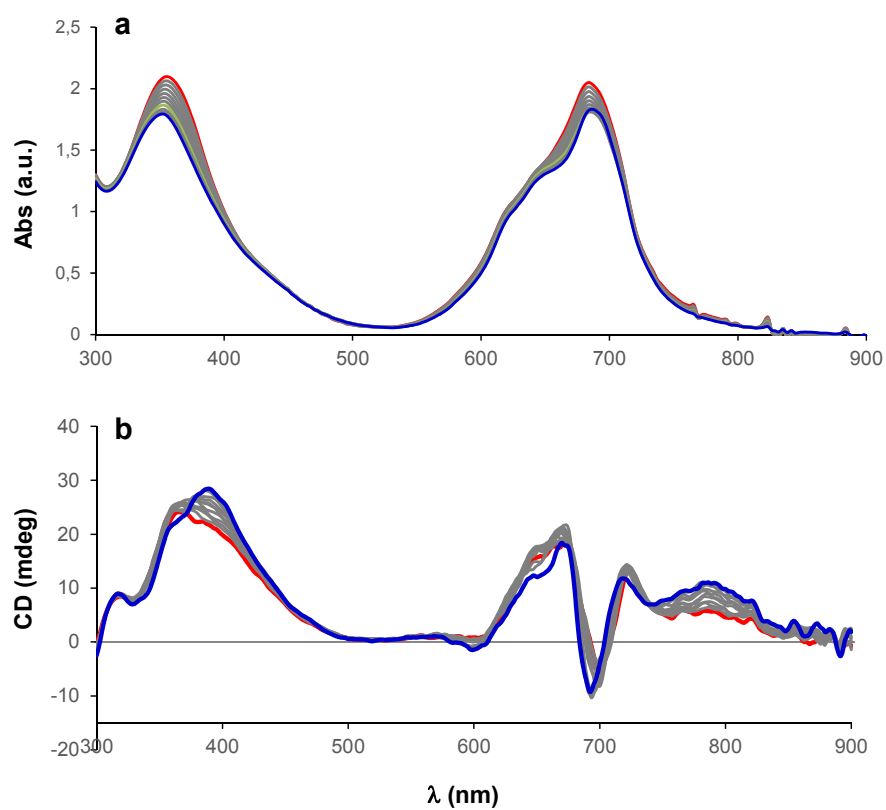


Figure S6. (a) Absorption and (b) CD spectra of Pc-G **1** in toluene at a 4×10^{-6} M concentration at different temperatures, from 268 K (blue spectra) to 368 K (red spectra).

The characteristic absorption and CD features of the (**1**)₈·K⁺ complex are maintained along the whole temperature range, suggestion no significant dissociation in toluene, even at low concentrations.

References (S.I.)

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- (iii) M. S. Marcuccio, I. Polina, S. Greenberg, A.B.P. Lever, C.C. Leznoff, B. Tomer, *Can. J. Chem.* 1985, **63**, 3057.