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Synthesis and Utility of Novel *C-meso*-Glycosylated Metalloporphyrins

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Abstract—Novel hybrid porphyrins bearing two and four suitably protected glycosidic units appended at the *meso* positions of the central macrocycle through robust carbon–carbon bonds have been constructed and characterized. Metallation of these constructs with certain bivalent metal ions then produced a series of porphyrinato entities which had all the sugar protecting groups removed to arrive at the corresponding water soluble porphyrin–sugar hybrid species. It is noteworthy that two palladium derivatives, compounds **6** and **10**, proved to be efficient reagents for the selective cleavage of double strand DNA into form II nicked circular DNA upon exposure to visible light at room temperature in aqueous media.

Introduction

The identification of materials that are able to achieve non-random DNA strand scission proves to be an invaluable resource in the design of potential anticancer or antiviral drugs.[1] In particular, compounds conjugating a reactive chromophore to proper functional vectors such as peptide,[2] oligonucleotide,[3] and carbohydrate[4] segments have attracted special interest, these hybrids being constructed to behave simultaneously as nucleic acid binders and degradative chemical substances.[5] Advantages of this approach can be found in compounds which are not toxic in the dark but can be rendered active by light.[6] Such is the case with certain photochemotherapeutic agents that are currently under evaluation in the treatment of solid tumors,[7] as well as the emerging field of viral photoinactivation.[8]

The aim of the present work was to construct porphyrin–sugar conjugates[9] and their metallo species by anchoring a carbohydrate or a nucleoside motif to the *meso* positions of the macrocycle through robust carbon–carbon bonds and to evaluate their ability to cleave nucleic acids upon exposure to visible light. The rationale behind these conjugates is that the water solubilizing carbohydrate portion close to the chromophore can potentiate the recognizing capability and specificity of the entire molecule for nucleic acids via noncovalent associations, bringing the photoactive porphyrin core into the vicinity of the target and inducing selective DNA strand breakage when irradiated. We report herein the viability of this project and the resultant new class of potential DNA photocleavers.

Synthesis and Characterization

Our choice was to assemble two hybrid porphyrin prototypes, **5** and **9**—and the corresponding deprotected free bases **7** and **14**—bearing two and four peripheral furanose components, respectively, and to prepare certain neutral, water soluble bivalent metal complexes, namely, palladium porphyrins **6** and **10**, as well as copper, zinc, and nickel species **11**, **12**, and **13**.

The synthesis of ligand **5** (Scheme 1), possessing alternate uridine and *p*-fluorophenyl groups at the 5, 10, 15, 20 *meso* positions, was planned and executed as indicated, by coupling of protected dipyrryluridine **3** to *p*-fluorobenzaldehyde (**4**) followed by oxidation by DDQ. To assemble the key dipyrryl nucleoside **3**, condensation between readily available aldehydo uridine **2**[10] and pyrrole (**1**) was attained by using $SnCl_4$ as the Lewis acid promoter. The reaction proceeded smoothly, giving the expected adduct **3** as the sole component in 33% yield. Exposure of a 1:1 molar mixture of **3** and *p*-fluorobenzaldehyde (**4**) to BF_3 etherate (0.5 mol equiv.) in CH_2Cl_2 at room temperature, followed by DDQ oxidative treatment, according to a MacDonald-type [2+2] condensation procedure,[11] allowed the assembly

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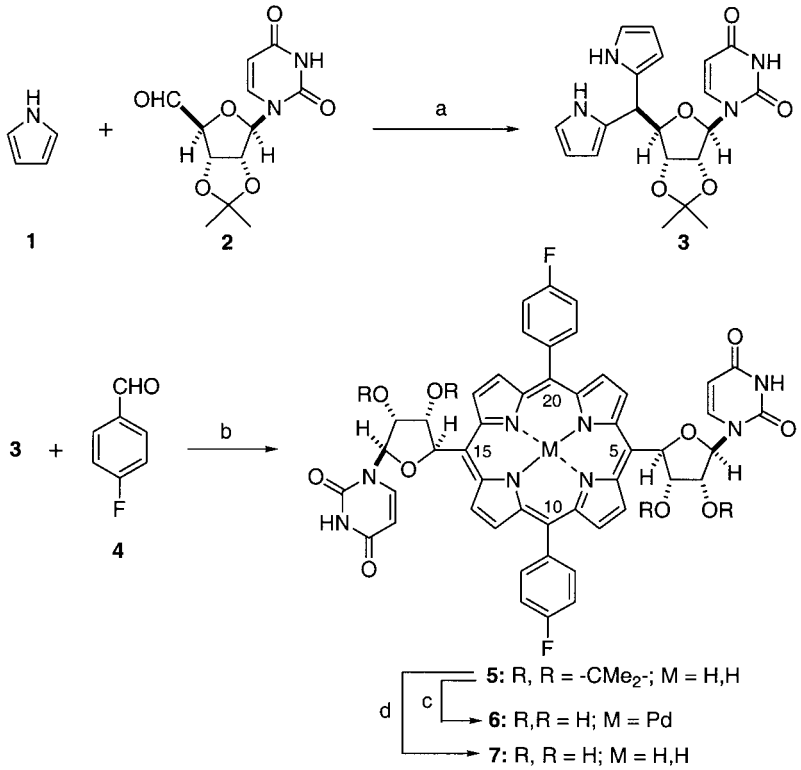
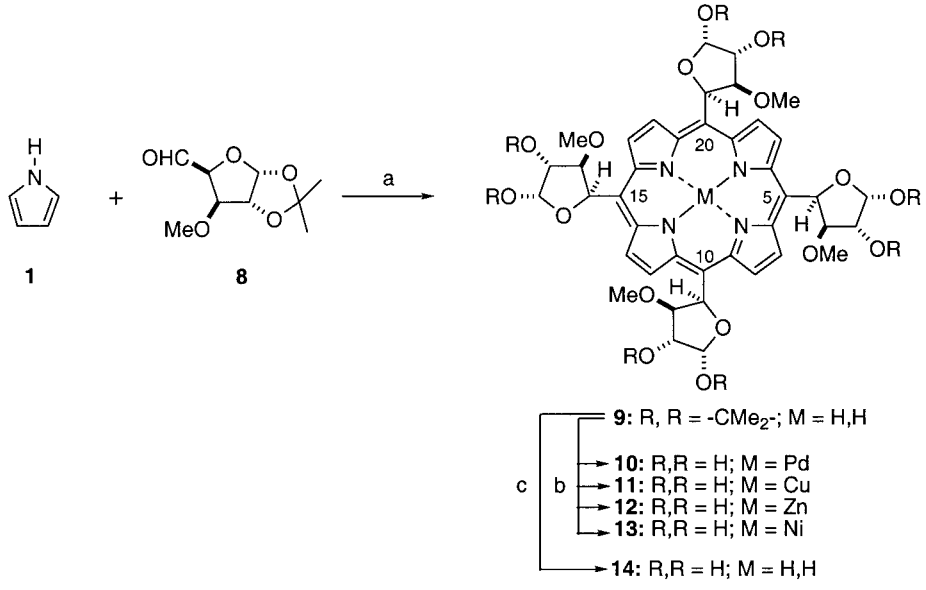


Scheme 1. Synthesis of *C-meso*-linked aryl-uridinyl porphyrins **5**–**7**. (a) $SnCl_4$, $CHCl_3$, rt, 1 h (33%); (b) $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 3 h, then DDQ, sonication, 30 min (15%); (c) $Pd(OAc)_2$, $CH_3OH/CHCl_3$ (1:1, v/v), sonication, 2 h, then 75% aq TFA, CH_2Cl_2, rt, 3 h (78%); (d) 75% aq TFA, CH_2Cl_2, sonication, 3 h (90%).

of *C-meso*-linked aryl–uridinyl porphyrin hybrid construct **5** in an acceptable 15% yield over two steps.

In order for the water-soluble palladium porphyrin **6** to be prepared, a two-step protocol was efficiently carried out (78% yield), consisting of metallation of free ligand **5** [$Pd(OAc)_2$, $CH_3OH/CHCl_3$, sonication] and subsequent acidic full deacetonidation (75% aq TFA). In this context, water-soluble ligand **7** was also synthesized, by simply exposing **5** to 75% aq TFA under ultrasonic irradiation (90% yield).

The design of tetra-*C*-glycosylated porphyrin **9** entails two-component macrocyclization involving commercially available dialdose **8** and pyrrole (**1**) (1:1 molar ratio), by following essentially the Lindsey protocol (Scheme 2).[12]



Scheme 2. Synthesis of tetra-*C*-glycosylated porphyrins **9**–**14**. (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 3 h, then DDQ, Et_3N, rt, 18 h (6%); (b) $M(OAc)_2$, $CH_3OH/CHCl_3$ (1:1, v/v), sonication, then 75% aq TFA, CH_2Cl_2, rt, 3 h (63–90%); (c) 75% aq TFA, CH_2Cl_2, sonication, 3 h (90%).

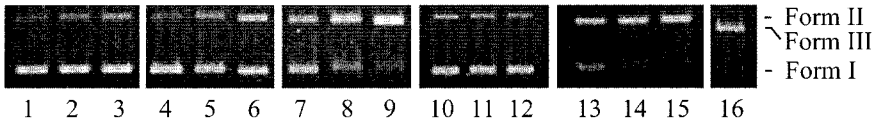


Figure 1. Agarose gel electrophoresis pattern for the porphyrin-triggered photocleavage of ds pUC18 DNA. Final porphyrin concentration, 25.7 μM; irradiation times, 3, 6, and 9 h at 27±0.5°C. pUC18 control experiment after 3, 6, and 9 h irradiation (lanes 1–3); pUC18 in the presence of **7** (lanes 4–6); **6** (lanes 7–9); **14** (lanes 10–12); **10** (lanes 13–15); linearized pUC18 (form III) (lane 16).

Indeed, direct condensation between **8** and **1** under BF_3 etherate guidance followed by DDQ oxidation afforded sugar–porphyrin assemblage **9** in 6% overall yield. As previously described, metallation/deprotection of **9** to water-soluble palladium porphyrin **10** was executed [$Pd(OAc)_2$, $CH_3OH/CHCl_3$, sonication; 75% aq TFA, 90% yield], while similar treatment of **9** with copper(II), zinc(II), and nickel(II) acetates followed by acidic deacetonidation allowed the porphyrin series **11**, **12**, and **13** to be prepared in 87, 80, and 63% yields, respectively. In addition, fully deprotected ligand **14** resulted from simple deprotection of the parent porphyrin **9**, as indicated (90% yield).

Fully protected porphyrins **5** and **9**, the corresponding water soluble deprotected counterparts **7** and **14**, and the various novel metallated species **6**, **10**, **11**, **12**, and **13** were fully characterized by ^{1}H NMR analysis, high resolution mass spectrometry, and, in some instances, elemental analyses. Furthermore, UV–visible and circular dichroism spectroscopies provided immediate proof of the existence of the porphyrin ring chromophore. As an example, uridinyl porphyrin **5** had an exact mass of 1003.3216 *m/z*, as expected, corresponding to a molecular formula $[M+H]^+$ $C_{54}H_{45}F_2N_8O_{10}$ (calculated 1003.3226 *m/z*). The UV–vis spectrum showed an intense Soret band centered at 414 nm, accompanied by four less intense Q bands at 514, 549, 590, and 644 nm. The ^{1}H NMR spectrum of **5** at 400 MHz indicated a D_2 symmetry of the whole molecule under the experimental conditions (25°C in $CDCl_3$), displaying two doublets each integrating to four protons for the β-pyrrolic resonances and only one resonance system for the two appended nucleoside chiral fragments.

As expected, tetrasubstituted sugar porphyrin **9** (*m/z* 999.4247; $C_{52}H_{63}N_4O_{16}$) showed a rather simple ^{1}H NMR profile with a single broad resonance at δ=9.73 integrating to eight protons. Clearly, this pattern reflects a high level of molecular symmetry (D_4), and this arrangement is corroborated by a single set of signals for the *meso*-furanose moieties. A very intense Soret band at 419 nm in the UV–vis spectrum ($CHCl_3$) was indicative of some level of deviation from planarity of the macrocycle, though this diminished symmetry was not evident in its ^{1}H NMR spectrum.

All the metallated porphyrins of this study invariably showed exact masses in line with the corresponding postulated molecular formulas, with precisions within the usual confidence level. Clear, fully detailed ^{1}H NMR spectra were measured for the important palladium porphyrins **6** and **10**, showing diagnostic resonances for all the constitutional fragments, the β-pyrrole system, the aromatic frames, and the sugar units. For bis-uridinyl palladium porphyrin **6** (*m/z* 1027.1481; $C_{48}H_{35}F_2N_8O_{10}Pd$), the D_2 symmetry of the parent ligand **5**, as manifested in its ^{1}H NMR spectrum, was completely lost, possibly due to a ring distortion induced by palladium ion complexation. By contrast, the highly substituted palladium porphyrin **10** retained the D_4 symmetry of its free ligand precursor **9** as indicated by its ^{1}H NMR spectrum which reveals a single resonance at δ=9.65 for all the eight pyrrole protons.

DNA Photocleavage Experiments

The DNA-cleaving activities of porphyrin bases **7** and **14**, as well as the metallo derivatives **6**, **10**, **11**, **12**, and **13**, were tested under visible light irradiation (100 W tungsten lamp) by monitoring the conversion of the supercoiled pUC18 (form I), a well characterized plasmid from *E. coli* (2686 base pairs), into nicked circular and linear DNA (forms II and III).

The effect of light was quantified by also taking into account the amount of nicked DNA spontaneously produced by the plasmid without added porphyrins. Under dark conditions none of the products showed any DNA cleaving ability. The DNA photocleavage results are reported in Fig. 1. Upon irradiation at [porphyrin]=25.7 μM, free aryl–uridinyl porphyrin **7** and ligand **14**, which bears four pendant furanose moieties, behaved similarly, showing moderate cleaving activity with ca. 15% nicked, circular DNA (form II) produced within 9 h of light exposure. Strongly enhanced activity was displayed by the palladium complexes of the same ligands. The porphyrinato derivative **6** exhibited 19, 47, and 62% conversion to form II DNA within 3, 6, and 9 h of irradiation, respectively, whereas the porphyrinato counterpart **10** produced about 60% of form II DNA at 3 h irradiation, suggesting an even greater photodynamic activity.

Copper porphyrin **11** produced only 16% of form II after 9 h of irradiation, showing a photodynamic activity similar to the free porphyrin bases. In contrast, under the same conditions, zinc and nickel complexes **12** and **13** had no DNA-cleaving activity (<5%). None of the considered porphyrins were able to produce the linear form III under the tested experimental conditions.

Conclusions

An easy preparation of *C-meso*-glycosylated porphyrin ligands has been described, by using either a dipyrrylmethane/aldehyde [2+2] cyclocondensation protocol, or a direct pyrrole/aldehyde macrocyclization. When complexed

with a palladium ion, and fully liberated from the various protecting groups, bis-uridinyl porphyrin **5** and tetra-glycosylated porphyrin **9** gave rise to water soluble metallated species, **6** and **10**, which proved to be efficient DNA cleavers upon exposure to visible light. These experiments enlighten the central role played by the water-solubilizing carbohydrate attached to the lipophilic porphyrin during cleavage of the DNA target. Further studies aimed at a better understanding of the basic mechanisms of recognition and binding involving the DNA-based constructs and these promising hybrid structures are in progress.

Experimental

General. Flash chromatography was performed on 40–60 μm silica gel (Merck) or Florisil (100–200 mesh, Carlo Erba), using the indicated solvent mixtures. Analytical thin-layer chromatography was performed on Merck silica gel 60 F_{254} plates (0.25 mm). The compounds were visualized by dipping the plates in a solution of cerium(IV) sulfate/ammonium molybdate. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AMX-400, Bruker AC-300, or Bruker AC-100 spectrometer, and are reported in parts per million (δ) relative to tetramethylsilane (0.0 ppm) as an internal reference, with coupling constants in Hertz (Hz). Optical rotations were measured on a Rudolph Research Autopol III polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Circular dichroism spectra were obtained using a Jasco J715 spectropolarimeter apparatus and molar elipticity [θ] is reported in deg cm^2 dmol^{-1}. Infra-red spectra were recorded on a Jasco FT/IR-300E spectrometer. Ultraviolet–visible spectra were recorded on a Kontron Uvicon 860 spectrophotometer. Elemental analyses were performed by the Microanalytical Laboratory of University of Parma. Low-resolution mass spectra were obtained on a Finnigan 1060 6c mass spectrometer, and high-resolution spectra were obtained on a Kratos MS08RFA mass spectrometer using the chemical ionization method (CH_4). All the solvents were distilled before use according to standard methods.

Pyrrole (**1**), *p*-fluorobenzaldehyde (**4**), and 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylopentodialdofuranose-(1,4) (**8**) were obtained from commercial suppliers (Fluka) and used without further purification.

Aldehyde **2**. To a stirring solution of uridine (Aldrich) (0.55 g, 2.25 mmol) in dry acetone (45 mL), a catalytic amount of H_2SO_4 was added dropwise, under argon atmosphere at room temperature. After being stirred for 1 h, the reaction mixture was quenched with $BaCO_3$ until neutralization. The heterogeneous mixture was filtered on a celite pad, and the filtrates were evaporated under reduced pressure, to give a crude white solid which was subjected to flash chromatographic purification on silica gel (90:10 CH_2Cl_2/acetone). A pure protected uridine intermediate was recovered (510 mg, 80% yield) as a white solid.

To a stirring solution of CH_2Cl_2 (8 mL) and DMF (2 mL) under argon were sequentially added pyridine (1.16 mL, 14.4 mmol) and CrO_3 (720 mg, 7.2 mmol), and the resulting mixture was allowed to stir at room temperature for 20 min. To this reaction mixture was added the above obtained protected uridine (510 mg, 1.8 mmol) dissolved in 8 mL of a 4:1 CH_2Cl_2/DMF solution, and the resulting mixture was treated with acetic anhydride (0.68 mL, 7.2 mmol). After 10 min, the reaction was quenched with EtOH (0.9 mL) and poured into ethyl acetate (90 mL). The resulting mixture was filtered with gentle suction through a sintered glass funnel packed, as a column, with silica topped with a layer of anhydrous Na_2SO_4. After elution with ethyl acetate, the solvent was evaporated under reduced pressure, affording 406 mg (80% yield) of aldehyde **2**, which was used as such in the subsequent coupling step. Compound **2**: ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.45 (s, 1H), 7.25 (d, J=7.9 Hz, 1H), 5.78 (d, J=7.9 Hz, 1H), 5.49 (s, 1H), 5.22 (dd, J=6.3, 1.6 Hz, 1H), 5.11 (d, J=6.3 Hz, 1H), 4.56 (d, J=1.6 Hz, 1H), 1.54 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=199.4, 163.6, 151.0, 144.3, 112.0, 103.0, 100.2, 94.2, 85.0, 83.8, 26.6, 25.0. $C_{12}H_{14}N_2O_6$ (282.26): calcd. C 51.07, H 5.00, N 9.92; found C 50.96, H 5.13, N 9.84.

Dipyrryluridine **3**. To a stirring solution of pyrrole (**1**) (0.20 mL, 2.88 mmol) and aldehyde **2** (406 mg, 1.44 mmol) in $CHCl_3$ (60 mL), $SnCl_4$ (80 μL, 0.72 mmol) was added dropwise at room temperature under argon atmosphere. After being stirred for 1 h, the pale-violet reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ and solid NH_4Cl. The resulting slurry was extracted with CH_2Cl_2 (3×25 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated under vacuum. The crude material was purified by flash chromatography (75:25 CH_2Cl_2/acetone) to furnish 190 mg (33% yield) of pure **3** as a colorless oil. $[\alpha]_D^{20}$=−208.0 (c 0.024, $CHCl_3$). UV–vis ($CHCl_3$) λ_{max} 258 nm (ϵ=3440 cm^{-1} M^{-1}). ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.38 (bs, 1H), 8.37 (bs, 1H), 8.28 (bs, 1H), 6.72 (m, 1H), 6.69 (m, 1H), 6.34 (d, J=8.1 Hz, 1H), 6.18 (m, 2H), 6.13 (m, 2H), 5.90 (d, J=2.9 Hz, 1H), 5.61 (d, J=8.1 Hz, 1H), 4.77 (dd, J=6.8, 4.6 Hz, 1H), 4.65 (dd, J=6.8, 2.9 Hz, 1H), 4.58 (dd, J=4.6, 4.4 Hz, 1H), 4.56 (d, J=4.4 Hz, 1H), 1.32 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (25 MHz, $CDCl_3$): δ=163.4, 162.6, 150.6, 141.9, 129.2, 129.0, 117.4, 117.3, 114.7, 108.3, 108.1, 106.7, 106.6, 102.9, 92.2, 87.8, 83.3, 81.1, 27.1, 25.3. $C_{20}H_{22}N_4O_5$ (398.42): calcd. C 60.29, H 5.57, N 14.06; found C 60.40, H 5.65, N 14.00.

Porphyrin **5**. To a solution of dipyrryl uridine **3** (170 mg, 0.43 mmol) in dry CH_2Cl_2 (60 mL) were added sequentially 4-fluorobenzaldehyde (**4**) (46 μL, 0.43 mmol) and $BF_3{\cdot}OEt_2$ (26 μL, 0.22 mmol), while a stream of pure argon was passing. The dark-red solution was carefully shielded from light and stirring was continued for 3 h. Then, dichlorodicyanobenzoquinone (DDQ) (159 mg, 0.7 mmol) was added and the resulting dark reaction mixture was kept under ultrasonic irradiation for 30 min. The solvent was evaporated under vacuum and the product was purified by flash chromatography on Florisil (75:25 CH_2Cl_2/acetone) to furnish 32 mg of porphyrin **5** (15% yield) as a red-brown glassy solid. $[\alpha]_D^{20}$=−675.0 (c 0.03, $CHCl_3$). FT-IR (KBr) 3350 (N–H), 1681 (C=O), 1600, 1360, 1040, 770 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 414 nm (ϵ=1.15×10^5 cm^{-1} M^{-1}), 514 (ϵ=5900), 549 (ϵ=2810), 590 (ϵ=2190), 644

(ϵ=1350). CD (7.5×10^{-4} M, $CHCl_3$) $[\theta]_{300}$=−815 deg cm^2 dmol^{-1}, $[\theta]_{439}$=−940, $[\theta]_{540}$=+500. CD (6.2×10^{-5} M, $CHCl_3$) $[\theta]_{371}$=+2470 deg cm^2 dmol^{-1}, $[\theta]_{404}$=+6930, $[\theta]_{420}$=+5140, $[\theta]_{429}$=+5260, $[\theta]_{602}$=−2470. ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.70 (d, J=5.0 Hz, 4H), 8.87 (d, J=5.0 Hz, 4H), 8.80 (bs, 2H), 8.13 (m, 4H), 7.45 (m, 6H), 7.18 (d, J=8.1 Hz, 2H), 5.76 (d, J=8.1 Hz, 2H), 5.50 (bs, 2H), 5.16 (d, J=6.0 Hz, 2H), 4.98 (d, J=6.0 Hz, 2H), 2.63 (s, 6H), 2.17 (s, 6H), −2.67 (bs, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.5 (2C), 162.5 (2C), 162.1 (2C), 161.4 (2C), 149.9 (2C), 143.1 (2C), 138.3 (2C), 135.6 (4C), 132.4 (4C), 128.5 (4C), 127.2, 119.2, 116.2 (2C), 113.7 (2C), 113.4 (2C), 103.5 (2C), 102.7 (2C), 95.7 (3C), 93.4 (2C), 88.8 (3C), 87.6 (2C), 82.5 (2C), 29.3 (2C), 29.2, 27.6. HRMS (CI, CH_4) m/z: 1003.3216 $[M+H]^+$ (calcd 1003.3226 for $C_{54}H_{45}F_2N_8O_{10}$). $C_{54}H_{44}F_2N_8O_{10}$ (1003.00): calcd C 64.67, H 4.42, N 11.17; found C 64.51, H 4.59, N 10.99.

Porphyrin **6**. To a solution of protected porphyrin **5** (22 mg, 0.02 mmol) in 50 mL of methanol/chloroform (1:1, v/v), $Pd(OAc)_2$ (6.7 mg, 0.03 mmol) was added under stirring and the resulting reaction mixture was kept under ultrasonic irradiation until complete disappearance of the starting porphyrin as judged by TLC analysis (2 h). The mixture was filtered and the solvent was removed under reduced pressure, furnishing a protected palladium porphyrin intermediate (24 mg, 98%) as a brown glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.2 (s, 2H), 9.88 (bs, 1H), 9.72 (s, 2H), 9.40 (s, 2H), 9.35 (bs, 1H), 8.6-9.0 (m, 2H), 7.5–8.3 (m, 12H), 6.05 (d, J=7.1 Hz, 2H), 5.52 (d, J=1.0 Hz, 2H), 5.29 (d, J=3.0 Hz, 2H), 5.08 (d, J=3.0 Hz, 2H), 3.5–3.7 (4s, 12H). This intermediate (24 mg, 0.019 mmol) was dissolved in 15 mL of CH_2Cl_2 and treated with 8 mL of a 75% aqueous trifluoroacetic acid solution at room temperature. After stirring for 3 h, the organic phase discolored completely, while the aqueous phase assumed a pink-brown color. The aqueous layer was separated, washed with ether, and neutralized by addition of a 2 M ammonia solution. Evaporation of the water solvent under reduced pressure gave crude palladium porphyrin **6**, that was subjected to flash chromatographic purification on silica gel (80:20 CH_2Cl_2/acetone). Porphyrin **6** was obtained (16 mg, 78% yield from **5**) as a red-brown glassy solid. $[\alpha]_D^{20}$=−3.9 (c 0.07, $CHCl_3$). FT-IR (KBr) 3330 (broad), 1680 (C=O), 1600, 1363, 1275, 1152, 1038, 840, 770 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 410 nm (ϵ=9110 cm^{-1} M^{-1}), 522 (ϵ=845), 556 (ϵ=270), 586 (ϵ=110). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=10.00 (d, J=5.1 Hz, 2H), 9.5–10.1 (m, 6H), 8.80 (d, J=5.1 Hz, 2H), 8.20 (m, 4H), 7.98 (d, J=8.1 Hz, 2H), 7.80 (d, J=8.1 Hz, 1H), 7.63 (d, J=7.1 Hz, 1H), 7.55 (m, 4H), 5.90 (m, 2H), 5.3–5.7 (m, 6H), 2.90 (bs, 4H). HRMS (CI, CH_4) m/z: 1027.1481 $[M+H]^+$ (calcd 1027.1473 for $C_{48}H_{35}F_2N_8O_{10}Pd$).

Porphyrin **7**. To a solution of protected porphyrin **5** (10 mg, 0.001 mmol) in CH_2Cl_2 (5 mL) a 75% aqueous trifluoroacetic acid solution (1 mL) was added under stirring at room temperature. After 10 min, the mixture was subjected to ultrasonic irradiation and was allowed stirring for 3 h. The organic phase discolored completely, while the aqueous phase assumed a green color indicative of the formation of a porphyrin dication. The aqueous layer was separated, washed with ether, and neutralized by addition of a 2 M aqueous ammonia solution. Evaporation of the water solvent under vacuum gave crude compound **7**, that was purified by a short flash chromatographic column on silica gel (60:40 acetone/$CHCl_3$), furnishing 8 mg (90% yield) of pure free base **7** as a red-brown glassy solid. $[\alpha]_D^{20}$=+7.1 (c 0.06, MeOH). $[\alpha]_{546}^{20}$=+15.5 (c 0.06, $CHCl_3$). FT-IR (KBr) 3330 (broad), 1682 (C=O), 1600, 1360, 1040, 770 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 409 nm (ϵ=1.6×10^4 cm^{-1} M^{-1}), 511 (ϵ=1550), 545 (ϵ=670), 587 (ϵ=550), 642 (ϵ=280). UV–vis (MeOH) λ_{max} 416 nm (ϵ=1260 cm^{-1} M^{-1}), 545 (ϵ=130), 589 (ϵ=90). CD (2.3×10^{-3} M, MeOH) $[\theta]_{413}$=+96 deg cm^2 dmol^{-1}, $[\theta]_{553}$=−46. ^{1}H NMR (400 MHz, [D$_6$]acetone/CD_3OD): δ=9.99 (bs, 4H), 9.55 (bs, 4H), 8.35 (m, 4H), 7.93 (m, 4H), 7.30 (m, 2H), 7.22 (d, J=8.0 Hz, 2H), 6.51 (d, J=8.0 Hz, 2H), 6.40 (bs, 2H), 5.51 (d, J=3.5 Hz, 2H), 5.25 (m, 2H). HRMS (CI, CH_4) m/z: 923.2595 $[M+H]^+$ (calcd 923.2600 for $C_{48}H_{37}F_2N_8O_{10}$). $C_{48}H_{36}F_2N_8O_{10}$ (922.87): calcd C 62.47, H 3.93, N 12.14; found C 62.55, H 3.81, N 11.96.

Porphyrin **9**. To a stirring solution of pyrrole (**1**) (170 μL, 2.5 mmol) and dialdose **8** (500 mg, 2.5 mmol) in anhydrous CH_2Cl_2 (200 mL), freshly distilled BF_3 etherate (30 μL, 0.25 mmol) was added under argon atmosphere at room temperature. The reaction vessel was carefully shielded from light, and stirring was continued for 3 h. Triethylamine (70 μL, 0.50 mmol) and DDQ (610 mg, 2.70 mmol) were added, and the dark-violet reaction mixture was stirred at room temperature for an additional 18 h. The solvent was evaporated under vacuum and the resulting solid was dissolved in CH_2Cl_2/ethyl acetate (93:7 ratio, 5 mL). Florisil (1 g) was added and the solvent was evaporated to give a powder which was charged at the top of a short silica gel column. Elution with CH_2Cl_2/ethyl acetate (90:10) solvent mixture furnished pure glycosylated porphyrin **9** (37 mg, 6% yield) as a dark-violet powder. $[\alpha]_D^{20}$=+135.4 (c 0.01, $CHCl_3$). FT-IR (KBr) 3320 (N–H), 1640, 1370, 1057, 745 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 419 nm (ϵ=1.4×10^5 cm^{-1} M^{-1}), 523 (ϵ=8945), 560 (ϵ=1840), 595 (ϵ=3100), 652 (ϵ=2130). CD ($CHCl_3$) $[\theta]_{414}$=−57745 deg cm^2 dmol^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 9.73 (bs, 8H), 7.87 (d, J=3.0 Hz, 4H), 6.69 (d, J=4.0 Hz, 4H), 5.16 (d, J=4.0 Hz, 4H), 4.61 (d, J=3.0 Hz, 4H), 2.48 (s, 12H), 1.90 (s, 12H), 1.60 (s, 12H), −2.62 (bs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=145.4 (8C), 129.9 (8C), 112.0 (4C), 109.8 (4C), 104.9 (4C), 90.0 (4C), 88.4 (8 C), 58.8 (4C), 27.4 (4C), 26.6 (4C). HRMS (CI, CH_4) m/z: 999.4247 $[M+H]^+$ (calcd 999.4239 for $C_{52}H_{63}N_4O_{16}$). $C_{52}H_{62}N_4O_{16}$ (999.09): calcd C 62.51, H 6.26, N 5.61; found C 62.63, H 6.23, N 5.52.

Porphyrin **10**. The title compound was obtained from protected porphyrin **9** (25 mg, 0.025 mmol) and $Pd(OAc)_2$ (45 mg, 0.2 mmol) following the two-step procedure described for **6**. After the metallation reaction (24 h), a protected palladium porphyrin intermediate was obtained (26 mg, 94%) as a blue-magenta glassy solid. UV–vis ($CHCl_3$) λ_{max} 419 nm (ϵ=3.0×10^4 cm^{-1} M^{-1}), 534 (ϵ=2200). ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.67 (s, 8H), 7.73 (d, J=3.0 Hz, 4H), 6.65 (d, J=4.0 Hz, 4H), 5.12 (d, J=4.0 Hz, 4H), 4.54 (d, J=3.0 Hz, 4H), 2.44 (s, 12H), 1.87 (s, 12H), 1.58 (s, 12H). HRMS (CI, CH_4) m/z: 1103.0470

$[M+H]^+$ (calcd 1103.3111 for $C_{52}H_{61}N_4O_{16}Pd$). This intermediate (26 mg, 0.024 mmol) was subjected to TFA-promoted deprotection treatment as described for compound **6**. After flash chromatographic purification (85:15 CH_2Cl_2/ethyl acetate), pure deprotected palladium porphyrin **10** was obtained (21 mg, 90% yield from **9**) as a red-brown glassy solid. $[\alpha]_D^{20}=+7.6$ (*c* 0.02, $CHCl_3$). FT-IR (KBr) 3330 (broad), 1608, 1371, 1275, 1152, 1055, 839, 745 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 411 nm (ϵ=9900 $cm^{-1}\,M^{-1}$), 527 (ϵ=1000), 563 (ϵ=610). ^1H NMR (300 MHz, [D_6]-acetone/CD_3OD): δ =9.65 (bs, 8H), 7.69 (d, *J*=3.0 Hz, 4H), 6.62 (d, *J*=4.0 Hz, 4H), 5.10 (d, *J*=4.0 Hz, 4H), 4.51 (d, *J*=3.0 Hz, 4H), 2.41 (s, 12H). HRMS (CI, CH_4) *m/z*: 943.1850 $[M+H]^+$ (calcd 943.1859 for $C_{40}H_{45}N_4O_{16}Pd$).

Porphyrin **11**. The copper porphyrin **11** was obtained from protected porphyrin **9** (10 mg, 0.01 mmol) and $Cu(OAc)_2$ (2.0 mg, 0.01 mmol) following the two-step procedure described for **6**. After the metallation reaction (1 h), a protected copper(II) porphyrin intermediate was obtained (10 mg, 95%) as a blue-magenta glassy solid. UV–vis ($CHCl_3$) λ_{max} 419 nm (ϵ=9.5×10^4 $cm^{-1}\,M^{-1}$), 550 (ϵ=4300), 591 (ϵ=1830). ^1H NMR (300 MHz, $CDCl_3$): δ=10.2–9.3 (m, 8H), 8.2-7.9 (m, 4H), 7.0-6.9 (m, 4H), 6.6-6.2 (m, 4H), 5.3-4.9 (m, 4H), 3.1-2.8 (2s, 12H), 2.5-1.5 (2s, 24H). HRMS (CI, CH_4) *m/z*: 1060.3390 $[M+H]^+$ (calcd 1060.3378 for $C_{52}H_{61}N_4O_{16}Cu$). This intermediate (10 mg, 0.009 mmol) was subjected to TFA-promoted deprotection treatment, as described for compound **6**. After a short-column flash chromatographic purification (80:20 CH_2Cl_2/ethyl acetate), pure deprotected copper(II) porphyrin **11** was obtained (8 mg, 87% yield from **9**) as a dark red solid. FT-IR (KBr) 3330 (broad), 1602, 1370, 1271, 1148, 1058, 850, 750 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 414 nm (ϵ=6500 $cm^{-1}\,M^{-1}$), 547 (ϵ=305). ^1H NMR (300 MHz, [D_6]acetone/CD_3OD): δ=9.8–9.2 (m, 8H), 8.1–7.8 (m, 4H), 7.0–6.8 (m, 4H), 6.5–6.2 (m, 4H), 5.0–4.7 (m, 4H), 3.1–2.8 (2s, 12H). HRMS (CI, CH_4) *m/z*: 900.2132 $[M+H]^+$ (calcd 900.2126 for $C_{40}H_{45}N_4O_{16}Cu$).

Porphyrin **12**. The zinc porphyrin **12** was obtained from **9** (10 mg, 0.01 mmol) and $Zn(OAc)_2$ (2.2 mg, 0.01 mmol) following the two-step procedure described for **6**. After the metallation reaction (30 h), a protected zinc porphyrin intermediate was obtained (9 mg, 85%) as a red-brown glassy solid. UV–vis ($CHCl_3$) λ_{max} 425 nm (ϵ=1.4×10^5 $cm^{-1}\,M^{-1}$), 561 (ϵ=6500), 600 (ϵ=2740), 620 (ϵ=1830). ^1H NMR (300 MHz, $CDCl_3$): δ=9.67 (s, 8H), 7.85 (d, *J*=3.0 Hz, 4H), 6.62 (d, *J*=4.0 Hz, 4H), 5.14 (d, *J*=4.0 Hz, 4H), 4.50 (d, *J*=3.0 Hz, 4H), 2.40 (s, 12H), 1.80 (s, 12H), 1.50 (s, 12H). HRMS (CI, CH_4) *m/z*: 1061.3382 $[M+H]^+$ (calcd 1061.3374 for $C_{52}H_{61}N_4O_{16}Zn$). This intermediate (9 mg, 0.0085 mmol) was subjected to TFA-promoted deprotection treatment as described for compound **6**. After flash chromatographic purification (85:15 CH_2Cl_2/ethyl acetate), pure deprotected zinc porphyrin **12** was recovered (7 mg, 80% yield from **9**) as a dark red glass. FT-IR (KBr) 3330 (broad), 1370, 1268, 1147, 1057, 838, 748 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 416 nm (ϵ=1.4×10^4 $cm^{-1}\,M^{-1}$), 556 (ϵ=1470), 590 (ϵ=380). ^1H NMR (300 MHz, [D_6]acetone/CD_3OD): δ=9.66 (bs, 8H), 7.85 (d, *J*=3.0 Hz, 4H), 6.59 (d, *J*=4.0 Hz, 4H), 5.13 (d, *J*=4.0 Hz, 4H), 4.48 (d, *J*=3.0 Hz, 4H), 2.37 (s, 12H). HRMS (CI, CH_4) *m/z*: 901.2130 $[M+H]^+$ (calcd 901.2122 for $C_{40}H_{45}N_4O_{16}Zn$).

Porphyrin **13**. The nickel porphyrin **13** was obtained from **9** (10 mg, 0.01 mmol) and excess $Ni(OAc)_2{\cdot}4H_2O$ (100 mg, 0.4 mmol) following the two-step procedure described for **6**. After the metallation reaction (10 days), a protected nickel porphyrin intermediate was obtained (7.4 mg, 70%) as a brown-red glass. UV–vis ($CHCl_3$) λ_{max} 421 nm (ϵ=1.0×10^4 $cm^{-1}\,M^{-1}$), 547 (ϵ=590), 589 (ϵ=210). ^1H NMR (300 MHz, $CDCl_3$): δ=9.36 (s, 8H), 7.09 (d, *J*=3.0 Hz, 4H), 6.48 (d, *J*=4.0 Hz, 4H), 4.98 (d, *J*=4.0 Hz, 4H), 4.33 (d, *J*=3.0 Hz, 4H), 2.52 (s, 12H), 1.75 (s, 12H), 1.51 (s, 12H). HRMS (CI, CH_4) *m/z*: 1054.9804 $[M+H]^+$ (calcd 1055.3436 for $C_{52}H_{61}N_4O_{16}Ni$). This intermediate (7.4 mg, 0.007 mmol) was subjected to TFA-promoted deprotection treatment, as described for compound **6**. After flash chromatographic purification (80:20 CH_2Cl_2/ethyl acetate), pure deprotected nickel porphyrin **13** was recovered (5.6 mg, 63% yield from **9**) as a brown-red glass. FT-IR (KBr) 3330 (broad), 1618, 1372, 1272, 1147, 1054, 837, 754 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 418 nm (ϵ=4550 $cm^{-1}\,M^{-1}$). ^1H NMR (300 MHz, [D_6]acetone/CD_3OD): δ=9.35 (bs, 8H), 7.05 (d, *J*=3.0 Hz, 4H), 6.45 (d, *J*=4.0 Hz, 4H), 5.00 (d, *J*=4.0 Hz, 4H), 4.30 (d, *J*=3.0 Hz, 4H), 2.50 (s, 12H). HRMS (CI, CH_4) *m/z*: 895.2191 $[M+H]^+$ (calcd 895.2184 for $C_{40}H_{45}N_4O_{16}Ni$).

Porphyrin **14**. Deprotected free base **14** was obtained from **9** (10 mg, 0.01 mmol) following the procedure described for **7** (reaction time 3 h). After flash chromatographic purification on silica gel (90:10 CH_2Cl_2/MeOH), pure free base **14** was recovered (7.5 mg, 90%) as a brown-red glassy solid. FT-IR (KBr) 3320 (broad), 1640, 1370, 1057, 745 cm^{-1}. UV–vis ($CHCl_3$) λ_{max} 421 nm (ϵ=4750 $cm^{-1}\,M^{-1}$), 523 (ϵ=220), 600 (ϵ=100), 652 (ϵ=60). ^1H NMR (300 MHz, CD_3OD): δ=10.0–9.6 (m, 8H), 7.7–7.4 (m, 4H), 6.4–5.6 (m, 4H), 4.65 (m, 4H), 4.43 (m, 4H), 2.55 (s, 12H). HRMS (CI, CH_4) *m/z*: 839.2996 $[M+H]^+$ (calcd 839.2987 for $C_{40}H_{47}N_4O_{16}$).

Gel electrophoretic experiments. Plasmid pUC18 (300 ng), dissolved in 10 mM MES (2-morpholinoethanesulfonic acid) at pH 6.0 ([pUC18]=8.6 nM), was irradiated for 3, 6, and 9 h at 27.0±0.5°C with a 100 W tungsten lamp in the absence or in the presence of ethanolic 25.7 μM solutions of porphyrins **7**, **6**, **14**, and **10**. Linearized pUC18 was obtained by digestion of 50 ng of pUC18 at 37.0±0.5°C for 2 h with 5U of EcoRI restriction enzyme. Samples were loaded on a 0.8% agarose gel in a 40 mM tris-acetate pH 7.7/1 mM EDTA buffer, submitted to electrophoresis (run at constant 100 V), and then stained with ethidium bromide. The DNA bands were revealed by UV light and a polaroid photograph taken; the amounts of DNA forms were quantified by scan densitometer with software CREAM (KEN EN TEC), Copenhagen.

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