DNA Interactions with Porphyrins Bearing Ammonium Side Chains[1]

Hans-Jörg Schneider* and Manxue Wang

Fachrichtung Organische Chemie, Universität des Saarlandes, D66041 Saarbrücken, Germany

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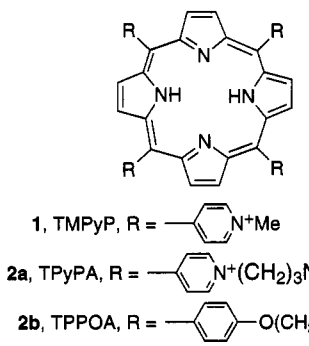
Eleven new porphyrins bearing either tertiary amine or ammonium groups with different spacers in the meso position have been synthesized. Five of them were water-soluble enough for DNA binding studies. UV/vis measurements of some derivatives as a function of pH quantifies the porphyrin core protonation postulated recently by Marzilli et al. with an uptake of two protons and pK values of 6.0 or 6.4, respectively. The porphyrins as well as their Cu(II) or Zn(II) derivatives decrease the viscosity of ds(CT) DNA slightly and increase the melting point of DNA by only up to 1.7 °C at DNA base pair/ligand ratios of 40. DNA causes either a red shift (≤6 nm) or a blue shift (≥−7 nm) of the Soret bands. These results as well as the observed small hypochromicities show that all new porphyrins interact with DNA without intercalation. However, their affinity in comparison with other macrocycles bearing four positive charges is, based on ethidium bromide assays with C_{50} values of 7×10^{-8} to 2×10^{-7} M relatively high and points to groove binding contributions by other than electrostatic forces.

Introduction

The binding of cationic porphyrins to DNA is of considerable interest in view of their potential uses as antitumor or antiviral agents; their metal complexes may also serve for the selective cleavage of nucleic acids.[2] Further development will rely upon improved understanding of the underlying binding mechanisms, to which the work particularly of Fiel,[2e] Mansuy,[3] and Marzilli[4] and their groups have greatly contributed. Until now most of the porphyrins which have shown intercalation bear positive charges close to the macrocycle, such as in the commercially available TMPyP **1** (Scheme 1).

In view of the strong stacking interactions of porphyrins with aromatic ligands which we were able to quantify for the first time as well as in the absence of additional ionic contributions, and which by NMR were characterized by face-to-face orientations,[5] we wanted to explore the possibility of intercalating porphyrins bearing positively charged ammonium units at a greater distance from the macrocycle. The combination of extended electroneutral aromatic systems with such aminoalkyl chains has been shown to lead to intercalation into double stranded DNA. In particular, we wanted to address the question to which degree stacking interactions of the

Scheme 1



electroneutral porphyrin skeleton—which, e.g. for naphthalene-like ligands alone including heterocycles was shown to reach in water about 15 kJ mol^{-1}[5]—would overcome the energy necessary to separate DNA base pairs upon intercalation. We also wanted to factorize the ionic contributions to groove binding, if possible, with the same salt bridge increments which have been used before for the description of polyamine binding.[6,7] While our work was in progress, Marzilli et al.[4] published evidence that depending on the electron donating or accepting properties of the linker unit R in **2a** (TPyPA) or **2b** (TPPOA), intercalation (**2a**) or outside (groove) binding (**2b**) prevails. They also found that for **2b** protonation inside the porphyrin occurs even at neutral pH in the presence of DNA. The present paper extends these studies and provides new and independent insight into protonation and binding properties of such substituted porphyrins.

Results

Compounds. The porphyrins **2b**, **2d**, **3**, **4**, and **5** in Schemes 2–4 were synthesized on the basis of the *meso*-phenyl-substituted macrocycles which were modified to include one or two (**3**) or four ammonium groups (**2b**, **2d**,

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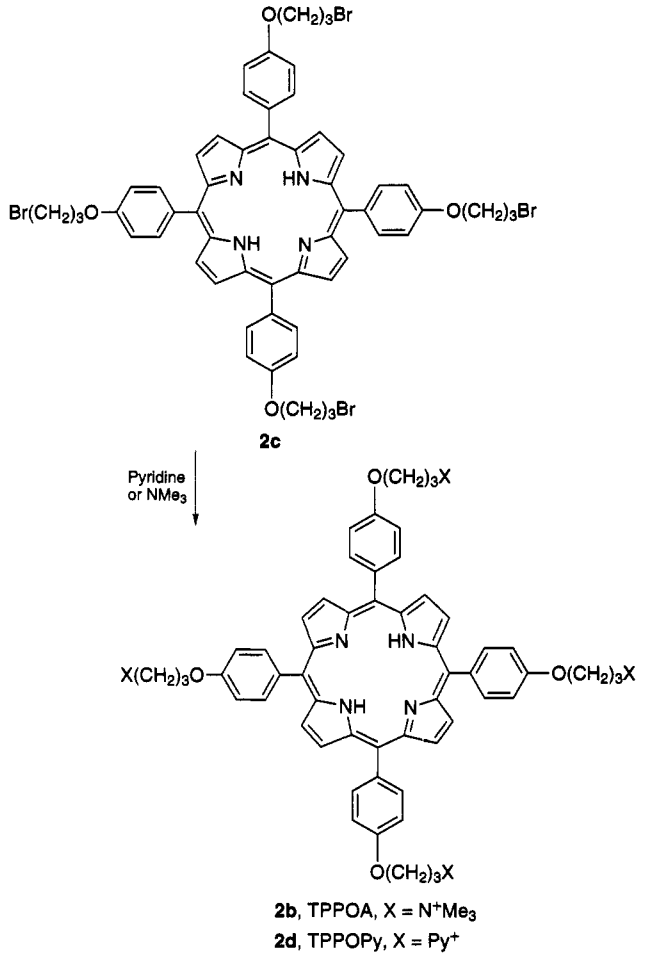
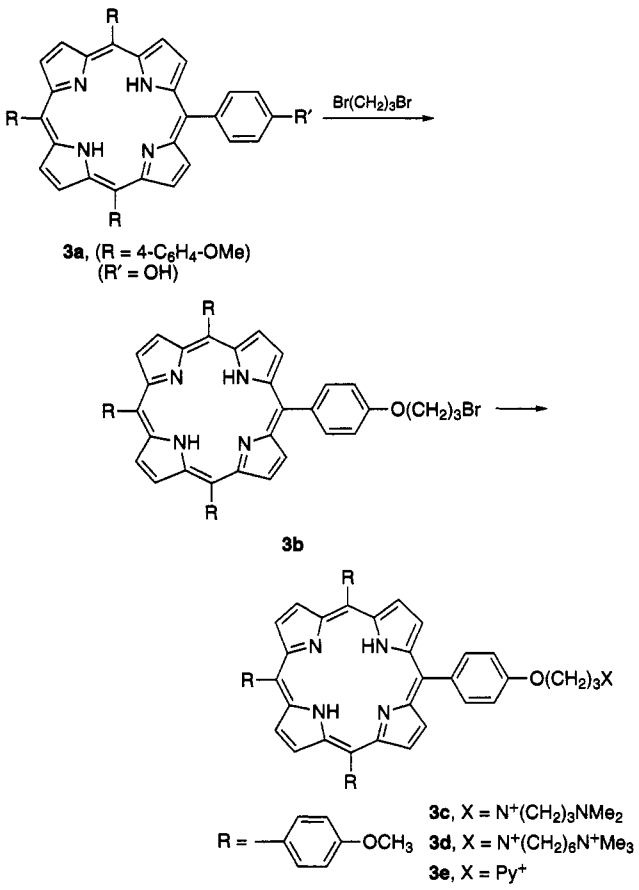
Scheme 2



Scheme 3



4). The entry to the different systems **3c–e** with one or two charges was straightforward starting from the 5-(4-hydroxyphenyl)-10,15,20-tris(4-methoxyphenyl)porphyrin (**3a**). Unfortunately, the porphyrins **3c–e** were not water-soluble enough for reliable measurements with DNA. This was not the case with most of the systems containing four charges. The derivatives **2b** and **2d** containing phenoxy ether linkers were prepared from the tetraphenolic porphyrin **2c**, whereas the corresponding ester and amide derivatives **4** and **5** were accessible from the known 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin. These compounds were water-soluble enough for binding studies with DNA. In spite of several attempts, however, the ester derivatives **4a** and **4b** hydrolyzed partially during the necessary column chromatography.

Methods for Binding Studies. Affinities to ds-calf thymus (CT-) DNA were evaluated by a fluorimetric assay with ethidium bromide (**EB**) as described earlier.[7] Melting points of the CT-DNA–ligand **L** complexes with ratios of DNA base pair to **L** of 40 and corresponding viscosity changes at variable ratios from 20 to 100 were measured by known procedures,[7b] as were UV hypochromicities H of the DNA complexes (H%) and Soret band changes ($\Delta\lambda$) (Results, see Table 1). Due to solubility and aggregation problems of the porphyrins, NMR could not be used for the detection of binding modes to DNA in water.

Protonation State of the Porphyrins. Until recently[4] it was assumed that porphyrins at around pH 7 are not substantially protonated at the center of the macrocycle. There are few potentiometric and spectroscopic studies supporting this; they were based, however, on porphyrins bearing sulfonic acid groups in not well defined numbers and positions.[8] With the porphyrins **4e** and **4f** we observe in the UV/vis spectrum the appearance of new bands at 443 nm upon lowering the pH to 5. The absorbance of the new band first increases and then decreases upon addition of CT-DNA. With porphyrin **5b**, the new band appears also after the addition of DNA. In agreement with Marzilli et al.,[4] we attribute this to core protonation of the porphyrin, which for the first time we have secured also by following the spectra as a function of pH (Figures 1, 2). With **5b** protonation is fast as observed usually;[4] however for **4e** and **4f** a constant spectrum is reached in the dilute solutions ([**P**] = 10^{-6} M^{-1}) only after 18 to 24 h. Similar observations have been reported earlier.[4b] The slow processes might also be due to slow dissociation of self-aggregated porphyrins. The latter was checked by controlling the extinctions as a function of concentration. Porphyrins **2b** and **2d** aggregate in water solution even in the concentration range of 1.91×10^{-6} to 2.69×10^{-5} M. A linear Lambert–Beer correlation was found with [**P**] $< 7 \times 10^{-5}$ for compounds **4e**, **4f**, and **5b**, which, however, does not rule out either very strong or very weak self-associations. Nevertheless, the observed consumption of about two protons in the acid–base titration points in the case of the porphyrins **4e**, **4f**, and **5b** to *double* protonation. Due to the close-lying pK values (see Figure 2) it was not possible to determine the constants for the different protonation steps separately,[8a] nor could they be mea-

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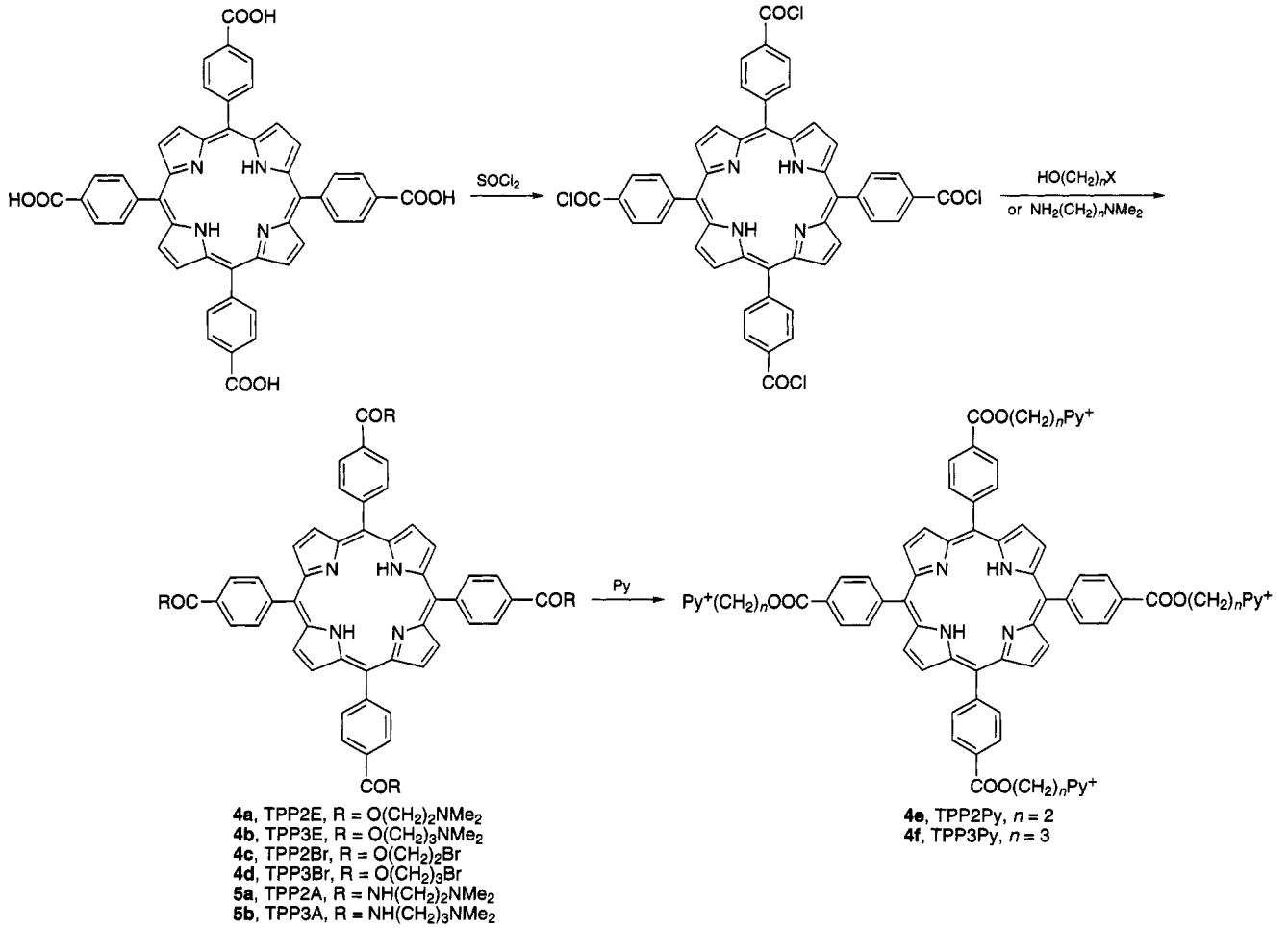
Scheme 4



4a, TPP2E, R = $O(CH_2)_2NMe_2$
4b, TPP3E, R = $O(CH_2)_3NMe_2$
4c, TPP2Br, R = $O(CH_2)_2Br$
4d, TPP3Br, R = $O(CH_2)_3Br$
5a, TPP2A, R = $NH(CH_2)_2NMe_2$
5b, TPP3A, R = $NH(CH_2)_3NMe_2$

4e, TPP2Py, $n = 2$
4f, TPP3Py, $n = 3$

Table 1. Interaction Results of Porphyrins P and DNA[a]

P	C_{50} ($\times 10^{-7}$ M)[b]	H (%)[c]	$\Delta\lambda$ (nm)[c]	ΔT (°C)[d]	ΔT (°C)[e]
TPP3Py	1.15	35	−1	3.9 [21.1]	1.0
TPP2Py	1.60	4	5	3.5 [20.9]	0.7
TPPOA	1.06	52	6	2.5 [32.0]	0.7
TPP3A	2.22	22	−4	0 [47.7]	0.5
TPPOPy	0.783	43	6	3.5 [15.2]	1.7
CuTPP3Py	0.702	18	−7		0
CuTPP2Py	1.40	30	1		
CuTPPOA	0.846	41	3	3.5 [13.6]	1.0
CuTPPOPy	0.738	38	5	4.5 [16.1]	1.0
ZnTPP3A	1.89	45	6		1.1

[a] Metalloporphyrins (CuTTOPy, CuTPPOA, CuTPP2Py, CuTPP3Py, and ZnTPP3A) are derived from the free base porphyrins shown in Schemes 2–4 by replacing the two inner pyrrole protons by a metal ion. The initial positions of the Soret peak (before CT DNA addition) are 415 (TPP3Py), 415 (TPP2Py), 418 (TPPOA), 415 (TPP3A), 418 (TPPOPy), 416 (CuTPP3Py), 410 (CuTPP2Py), 416 (CuTPPOA), 417 (CuTPPOPy), and 422 (ZnTPP3A). All measurements were performed in SHE buffer at pH 7. [b] Titration experiments were performed in duplicate. C_{50} values provided figures with approximately 1% error associated with them. [c] Final [porphyrin]:[DNA base pair] ratio $R_f = 0.04$. [d] Data in brackets are the ratios of DNA base pair to porphyrin. [e] The ratio of DNA base pair to porphyrin is 40.

sured by NMR for the above-mentioned reasons. The observed stoichiometry and the peak at longer wavelength (compared to the Soret band) indicate that the band at 443 nm is due to the uptake of two protons. In accordance with Marzilli et al.[4b] we conclude that the presence of electron-withdrawing linker units in the para position of *meso*-phenylporphyrins leads to negligible protonation. It should be borne in mind that association of the porphyrins to the DNA phosphates may well increase the basicity of the macrocycle by several units.[9] From Figure 2, the plots of the absorbances at the Soret band, and the new band of **4e** and **4f** vs pH, one derives a midpoint pH of 3.0 and an apparent pK_a of 6.0 ($pK_3 + pK_4$), for **5b** (midpoint pH 3.2) an apparent pK_a of 6.4.

Binding Affinities and Modes. The binding of porphyrins to ds DNA is usually[2f–h] grouped into several categories: (i) Intercalation with *meso* pyridinium derivatives as long as bulky groups or those in positions leading to nonplanar arrangements of the *meso*-aryl unit do not interfere. Intercalation is characterized by Soret band changes of ≥15 nm, of hypochromicities H differences higher than 35%, by negative CD signals, by viscosity, and by melting point increases. All these indicators were investigated with the derivatives **2b**, **2d**, **4e**, **4f**, and **5b** and their Cu(II) or Zn(II) complexes, and conclusively *all* showed the absence of intercalation (Table 1). This leaves as binding modes (ii) outside binding stablized by self-stacking of the porphyrin, which would apply only to the *meso*-tetra(4-aniliniumyl)-porphyrin. The observed *de*creasing viscosity (Figure 3) and small increase of melting points (Figure 4), however, speak for the third alternative, which is (iii) outside random binding, typical for *meso*-tetrakis(2-

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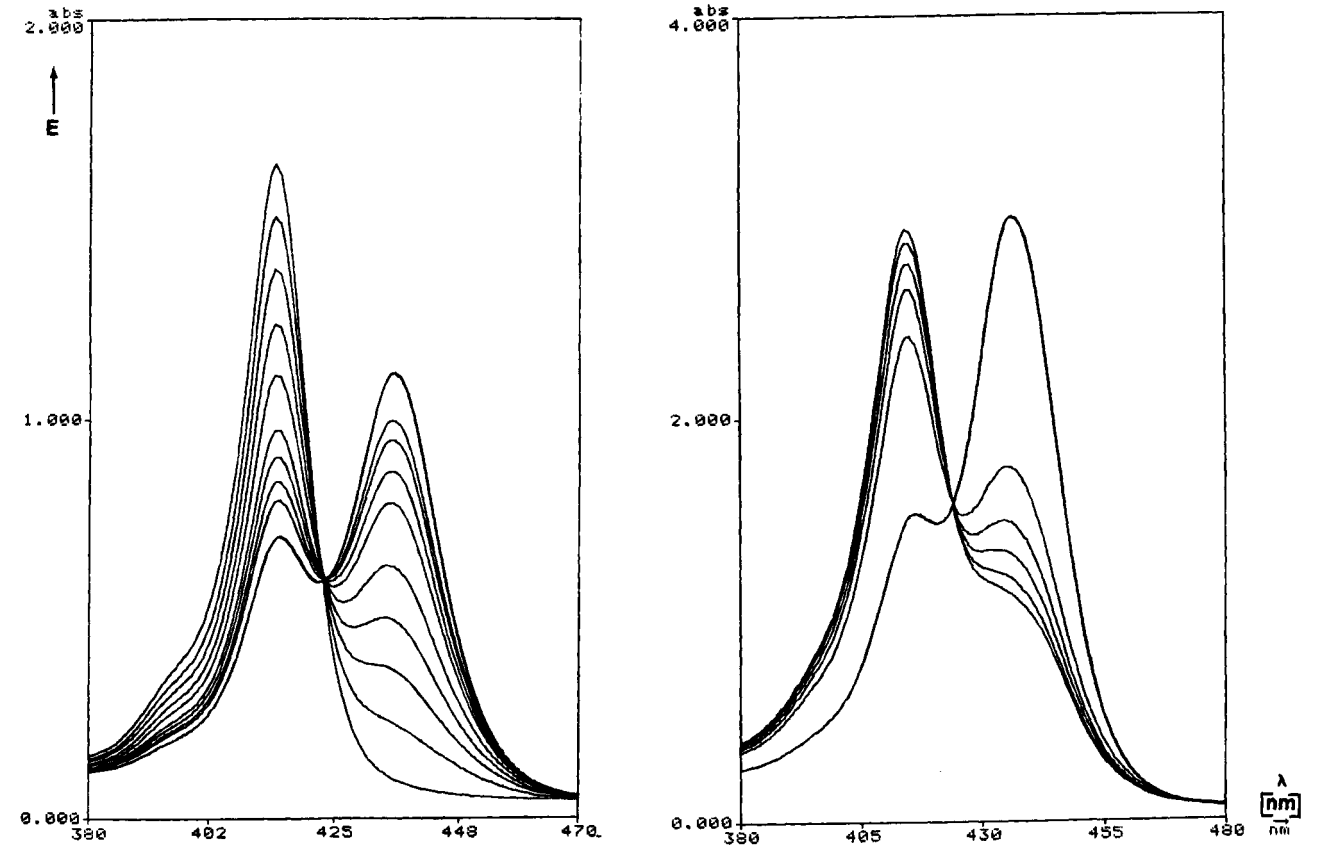

Figure 1. UV/vis spectra of TPP3A (**5b** , left) and TPP2Py (**4e**, right) at different pH.

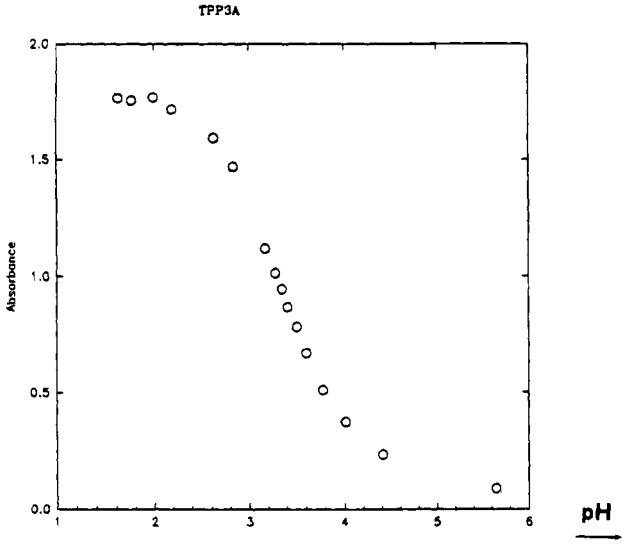

Figure 2. UV/vis extinction of TPP3a (**5b**) at 443 nm as a function of pH.

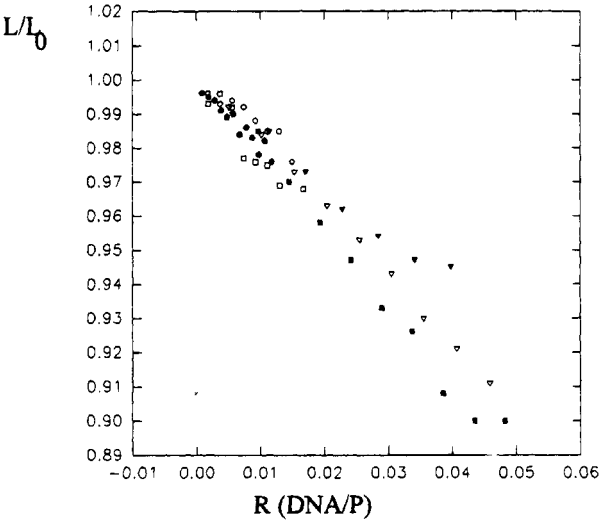

Figure 3. Plot of relative viscosity vs r, the ratio of added porphyrin to DNA in base pairs. TPP2Py, ○; TPPOPy, ▽; TPP3Py, □; TPPOA, ●; CuTPPOA, ▼; CuTPPOPy, ■.

methylpyridiniumyl)porphyrin, etc., and shows the characteristic features of purely electrostatic groove association such as strong affinity dependence on ionic strength.

However, the affinities of all porphyrins, as given by the **EB** assay, are with C_{50} around 10^{-7} M (Table 1) higher than to be expected on the basis of pure electrostatic binding. Thus, the typical C_{50} value for open chain or cyclic tetramines is around 10^{-6} M, in accordance with the dependence on the number of salt bridges.[7] Only for the porphyrins **2b** and **2d** bearing electron donating linkers and thus additional positive charges by core protonation are the C_{50} values in line with pure electrostatic interactions. For the other porphyrins, including the metal derivatives (see Table 1), additional van der Waals forces obviously enhance the groove binding. The apparent insensitivity of these interactions against electron-donating or -accepting substituents is in line with similar findings with stacking complexes of porphyrins and a variety of different guest ligands.[5] The relatively large hypochromicities observed with TPPOPy, CuTPPOA, TPPOA, and TPP3Py may be due to the self-aggregation induced by the formation of DNA complexes.

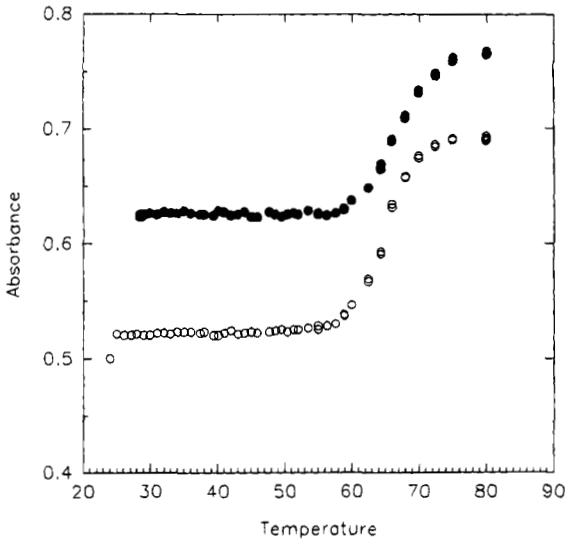

Figure 4. (○): melting curve of DNA. (●): melting curve of DNA with TPPOPy (DNA/P = 40).

Experimental Part

All chemicals were reagent grade and purchased from Aldrich, Fluka, or Merck. Dried CH_2Cl_2 was refluxed with P_2O_5, distilled over P_2O_5, and kept over 4-Å molecular sieves. NEt_3 was distilled over KOH. Pyrrole was freshly distilled before use.

NMR spectra were recorded on a Bruker AM400 spectrometer at 400 MHz with a digital resolution of 0.3 Hz. Assignments of porphyrin structures by ^1H NMR were based on chemical shifts and coupling constants.

Fast atom bombardment mass spectra (FAB-MS) were recorded on a Varian-Mat 311 mass spectrometer, using mixtures of either glycol and HCOOH or glycol, MeOH, and HCOOH as matrix. The molecular ions of all the porphyrins appeared as the base peaks in FAB-MS unless noted. Elemental analysis was performed on a Carlo Erba 1106 instrument. UV/vis spectral and melting point measurement of DNA were carried out on KONTRON UVIKON 860 double beam recording spectrophotometer with a thermostated cell compartment. Fluorescence studies were performed on a Hitachi F 3000 fluorescence spectrometer. Viscosity titrations were carried out with a Schott viscosimeter (type 53610/I) with a 0.4 mm microcapillary.

All porphyrins appeared as purple or brown red crystals or powders.

5,10,15,20-Tetrakis[4-(3-pyridiniumpropoxy)phenyl]-porphyrin Tetrabromide (TPPOPy) (2d). 5,10,15,20-Tetrakis[4-(3-bromopropoxy)phenyl]porphyrin[10] (**2c**) (50 mg) in pyridine (5 mL) was refluxed for about 1 h. After cooling, the precipitate was collected by filtration, washed with $CHCl_3$ thoroughly, and dried. The yield is quantitative. ^1H NMR (DMSO-d_6, TMS): δ −2.89 (s, 2H), 2.68 (m,8H, J = 6.0 Hz), 4.46 (t, 8H, J = 5.3 Hz), 5.03 (t, 8H, J = 6.7 Hz), 7.30 (d, 8H, J = 8.5 Hz), 8.14 (d, 8H, J = 8.4 Hz), 8.31 (t, 8H, J = 7.1 Hz), 8.75 (t, 4H, J = 7.8 Hz), 8.87 (s, 8H), 9.35 (d, 8H, J = 5.5 Hz). UV/vis (H_2O): 418 (4.58×10^4), 522 (1.39×10^4), 561(1.21×10^4), 589 (shoulder), 649 (5.25×10^3). FAB-MS m/z 1173.3 (M^+, calcd 1174.656 for $C_{52}H_{54}N_8O_4Br_4$). Anal. Calcd for TPPOPy·$2H_2O$: C, 51.59; H, 4.83; N, 9.26. Found: C, 51.33; H, 5.14; N, 8.97.

5,10,15,20-Tetrakis{4-[3-(*N,N,N*-trimethylammonium)-propoxy]phenyl}porphyrin Tetrabromide (TPPOA) (2b). Trimethylamine (1.1 g) was bubbled into the DMF (20 mL) solution of porphyrin **2c**[10] (53 mg, 46 mol) overnight. A little precipitate was obtained by filtration. After the ether was added to the mother liquor, more product was obtained. The porphyrin was recrystallized in a mixture of methanol and acetone to yield 26 mg (41%) of the product. ^1H NMR (DMSO-d_6, TMS): δ −2.89 (s, 2H), 2.39 (m, 8H), 3.22 (s, 36H), 3.68 (t, 8H, J = 8.25 Hz), 4.38 (t, 8H); 7.42 (8H, d, J = 8.17 Hz), 8.15 (d, 8H, J = 8.42 Hz), 8.86 (s, 8H). UV/vis (H_2O): 418, 522, 561, 590 (shoulder), 641 nm. FAB-MS m/z 1399.3 (M^+, calcd 1399.104 for $C_{68}H_{86}N_8O_4Br_4$).

5-(4-Hydroxyphenyl)-10,15,20-tris(4-methoxyphenyl)-porphyrin[11] (3a). The propionic acid solution (400 mL) of pyrrole (5.6 mL, 80 mmol), 4-methoxybenzaldehyde (7.3 mL, 60 mmol), and 4-hydroxybenzaldehyde (3.7 g, 30 mmol) was refluxed for 1 h and cooled overnight; no precipitate was observed from the mixture as described in the literature. The solvent was removed. DMF was added to dissolve the residue. Acetone was used to allow the purple mixed porphyrin crystals to precipitate. The mixed porphyrins were collected by filtration through sintered glass filter, washed by MeOH and hot water, and purified on a dry neutral alumina column (eluent: $CHCl_3$). The first band of the column was *meso*-tetrakis(4-methoxyphenyl)porphyrin as byproduct. The second band (porphyrin **3a**) was rechromatographed on silica gel using $CHCl_3/CH_3OH/CH_3COOC_2H_5$ (40:1:1) as eluent. The third main band was collected to give porphyrin **3a** (yield 7%). ^1H NMR ($CDCl_3$, TMS): δ 4.10 (9H, s), 7.21 (d, 2H, J = 8.4 Hz), 7.29 (6H, d, J = 8.6 Hz), 8.07 (d, 2H, J = 8.4 Hz), 8.12 (d, 6H, J = 8.5 Hz), 8.86 (s, 8H).

5-[4-(3-Bromopropoxy)phenyl]-10,15,20-tris(4-methoxyphenyl)porphyrin[10b] (3b). This porphyrin was prepared from 200 mg (280 mol) of porphyrin **3a** to yield 170 mg (72%) of the product. ^1H NMR ($CDCl_3$, TMS): δ −2.74 (s, 2H), 2.55 (m, 2H), 3.79 (t, 2H), 4.40 (t, 2H), 4.10 (s, 9H), 7.26 (m 8H), 8.12 (m, 8H), 8.85 (s, 8H). UV/vis ($CHCl_3$): 421, 517, 554, 590, 647 nm.

5-{4-[3-[[3-(*N',N'*-Dimethylamino)propyl]ammonium]-propoxy]phenyl}-10,15,20-tris(4-methoxyphenyl)porphyrin Bromide (3c). Porphyrin **3b** (100 mg, 0.12 mmol) and 1,3-(*N,N,N',N'*-tetramethyldiamino)propane (1 mL, 6 mmol) in DMF (20 mL) were heated to 90 °C. The reaction was followed by analytical TLC. After 4 h, the solution was poured into 50 mL of ethyl acetate. The precipitate was filtered and washed by $CHCl_3$ (3 times) and ether to give 70 mg (61%) of the new product. Further purification was carried out by reprecipitation by adding $CHCl_3$ to the DMF solution of the porphyrin. ^1H NMR (DMSO-d_6, TMS): δ −2.88 (s, 2H), 1.97 (m, 2H), 2.20 (s, 6H), 2.35 (m, 4H), 3.62 (m, 2H), 3.17 (s, 6H), 4.40 (t, 4H), 7.40 (m, 8H,), 8.13 (m, 8H), 8.87 (s, 8H). UV/vis (MeOH): 416.5, 514.5, 550.5, 590, 645.5 nm. FAB-MS m/z 975.7 (M + 3, 15.04), 976.4 (M + 4, 16.22), 977.3 (M + 5, 21.49), 978.0 (M + 6, 16.26) (calcd 972.041 for $C_{57}H_{59}N_6O_4Br$). Solubilities: soluble in DMF and DMSO; slightly soluble in $CHCl_3$ and MeOH; insoluble in H_2O, Et_2O, and CH_3CO_2Et.

6-(*N',N'*-Dimethylamino)-1-(*N,N,N*-trimethylammonium)hexane Iodide. Methyl iodide (1.2 mL, 20 mmol) was added in 10 min to 40 mL of an ice-cooled $CHCl_3$ solution of *N,N,N',N'*-tetramethyl-1,6-diaminohexane (10 mL, 46 mmol) with stirring. A white precipitate formed immediately, which was filtered. ^1H NMR proved that it was the byproduct of the dimethylated ammonium salt. Ether was added to the filtrate to precipitate the monomethylated product. The pure compound was obtained by recrystallization from a mixture of acetone and water to give a white hygroscopic powder 4.7 g (75%). ^1H NMR (D_2O, TSP): δ 1.40 (m, 6H), 1.75 (m, 2H), 2.17 (s, 6H), 2.32 (t, 2H), 3.07 (s, 9H), 3.27 (t, 2H).

5-{4-[3-[[6-(*N',N',N'*-Trimethylammonium)hexyl]-*N,N*-dimethylammonium]propoxy]phenyl}-10,15,20-tris(4-methoxyphenyl)porphyrin (3d). Porphyrin **3b** (100 mg, 0.12 mmol) and 1-(*N,N,N*-trimethylammonium)-3-(*N',N'*-dimethylamino)hexane (189 mg, 0.6 mmol) were heated in 60 mL of DMF to 90 °C for 3 h. TLC was used to follow the reaction. After the reaction, the solvent was removed under reduced pressure and the residue was extracted by $CHCl_3$ thoroughly to remove the unreacted ammonium salt, yielding 76 mg (55%) of the product. ^1H NMR (DMSO-d_6, TMS): δ

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−2.88 (s, 2H), 1.41 (m, 2H), 1.74 (m, 2H), 1.82 (m, 2H), 2.38 (m, 2H), 3.18 (s, 6H), 3.31 (m, 4H), 3.64 (t, 4H), 4.06 (s, 9H), 4.40 (t, 2H), 7.41 (m, 8Hl), 8.13 (m, 8H), 8.87 (s, 8H). UV/vis (MeOH): 418, 517, 554, 593, 650 nm. FAB-MS m/z 1155.1 (M^+, calcd 1156.061 for $C_{61}H_{68}N_6O_4BrI$). Solubilities: very soluble in DMF and DMSO; moderately soluble in MeOH; insoluble in $CHCl_3$, ether, and H_2O.

5-[4-(3-Pyridinium)propoxy)phenyl]-10,15,20-tris(4-methoxyphenyl)porphyrin (3e). Porphyrin **3b** (100 mg, 0.12 mmol) in pyridine (5 mL) was refluxed for 1 h. After cooling, the precipitate was collected, washed thoroughly with $CHCl_3$, and dried to give the product **3e** with a yield of 91 mg (84%). ^1H NMR ($CDCl_3$, TMS): δ −2.78 (s, 2H), 2.78 (m, 2H), 4.42 (t, 2H, J = 5.4 Hz), 4.10 (s, 9H), 5.25 (t, 2H, J = 7.1 Hz), 7.13 (d, 2H, J = 8.4 Hz), 7.33 (d, 6H), 8.13 (m, 10H), 8.55 (m, 2H), 8.87 (m, 7H), 9.36 (d, 2H, J = 5.9 Hz). UV/vis (MeOH): 416.5, 513.5, 550, 588, 644.5 nm. FAB-MS m/z 931.1 (M^+, calcd 920.908 for $C_{55}H_{46}N_5O_4Br$). Solubilities: very soluble in DMF, DMSO, and pyridine; moderately soluble in $CHCl_3$; slightly soluble in MeOH; insoluble in H_2O.

***meso*-Tetrakis[4-[(3-bromopropoxy)carbonyl]phenyl]-porphyrin[12] (TPP3Br) (4d).** 5,10,15,20-Tetrakis(4-carboxyphenyl)porphyrin[13] (0.5 g, 0.6 mmol) was refluxed with $SOCl_2$ (10 mL) for 2 h. Excess reagent was removed by evaporation under reduced pressure to dryness. The porphyrin acid chloride was dissolved in dry CH_2Cl_2 (50 mL) and refluxed with 3-bromopropanol (0.6 mL, 7 mmol) and dry NEt_3 (1 mL) overnight. The solvent was removed, and water was used to wash the solid several times. The solid was dried and chromatographed on silica gel (eluent: $CHCl_3$). The main purple fraction was collected to yield 490 mg of the product (61%). The analytical sample was rechromatographed using $CHCl_3/CH_3OH$ (20:1) as eluent. ^1H NMR ($CDCl_3$, TMS): δ −2.78 (s, 2H), 2.46 (m, 8H, J = 6.3 Hz), 3.68 (t, 8H, J = 6.5 Hz), 4.66 (t, 8H, J = 6.0 Hz), 8.32 (d, 8H, J = 8.2 Hz), 8.46 (d, 8H, J = 8.2 Hz), 8.83 (s, 8H). UV/vis ($CHCl_3$): 419, 514, 549, 587, 643 nm. FAB-MS m/z 1275.1 (M^+, calcd 1274.696 for $C_{60}H_{50}N_4O_8Br_4$).

***meso*-Tetrakis[4-[(2-bromoethoxy)carbonyl]phenyl]-porphyrin (TPP2Br) (4b).** This porphyrin was prepared according to the same procedure as that of TPP3Br (**4d**) from TCP (0.5 g, 0.6 mmol) and 2-bromoethanol (7 mmol). Eluent: $CHCl_3$. The first band was collected. Yield 476 mg (62%). ^1H NMR ($CDCl_3$, TMS): δ −2.81 (s, 2H), 3.81 (t, 8H, J = 6.0 Hz), 4.84 (t, 8H, J = 6.0 Hz), 8.32 (d, 8H, J = 8.2 Hz), 8.49 (d, 8H, J = 8.2 Hz), 8.83 (s, 8H). UV/vis ($CHCl_3$): 420, 514, 548, 587, 643 nm. FAB-MS m/z 1218.9 (M^+, calcd 1218.588 for $C_{56}H_{42}N_4O_8Br_4$). Anal. Calcd for TPP2Br·2H_2O: C, 54.98; H, 4.15; N, 4.27. Found C, 55.18; H, 4.03; N, 4.23.

***meso*-Tetrakis[4-[(2-pyridiniumethoxy)carbonyl]-phenyl]porphyrin Tetrabromide (TPP2Py) (4e).** TPP2Br (**4c**) (100 mg, 82 mol) in pyridine (5 mL) was refluxed for 1 h. The precipitated pyridinium salt was filtered off and purified by usual workup (as for TPPOA) to yield quantitative product. ^1H NMR (DMSO-d_6, TMS): δ −2.95 (s, 2H), 4.99 (t, 8H), 5.22 (t, 8H), 8.31 (m, 8H), 8.38 (m, 16H), 8.73 (m, 4H), 8.84 (s, 8H), 9.36 (d, 8H, 2.6). UV/vis (H_2O): 415 (2.75 × 10^5), 443 (8.18 × 10^4), 521 (1.61 × 10^4), 558 (8.95 × 10^3), 592 (6.25 × 10^3), 649 (4.26 × 10^3). FAB-MS m/z 1535.4 (M^+, calcd 1535.04 for $C_{76}H_{62}N_8O_8Br_4$). Anal. Calcd for TPP2Py: C, 59.47; H, 4.07; N, 7.30. Found: C, 59.42; H, 4.06; N, 6.81.

***meso*-Tetrakis[4-[(3-pyridiniumpropoxy)carbonyl]-phenyl]porphyrin Tetrabromide (TPP3Py) (4f).** TPP3Py (**4f**) was synthesized using the same procedure as that of TPP2Py from 100 mg (78 mol) of TPP3Br (**4d**). The yield was quantitative. ^1H NMR (DMSO-d_6, TMS): δ −2.88 (s, 2H), 2.68 (m, 8H), 4.46 (t, 8H), 5.03 (t, 8H), 7.30 (d, 8H), 8.14 (d, 8H), 8.31 (d, 8H), 8.75 (m, 4H), 8.87 (s, 8H), 9.35 (d, 8H, 2,6). ^1H NMR (MeOH-d_4): δ 2.69 (m, 8H), 4.67 (t, 8H, J = 5.4 Hz), 5.00 (t, 8H, J = 7.02 Hz), 8.21 (t, 8H, J = 6.64 Hz, J = 6.71 Hz), 8.31 (d, 8H, J = 8.0 Hz), 8.41 (d, 8H, J = 7.87 Hz), 8.65 (t, 4H), 8.85 (s, 8H), 9.21 (d, 8H, J = 5.87 Hz). UV/vis (H_2O): 415 (3.96 × 10^5), 519 (1.19 × 10^4), 557 (6.90 × 10^3), 585 (4.35 × 10^3), 646 (2.81 × 10^3). FAB-MS m/z 1591.2 (M^+, calcd 1591.104 for $C_{80}H_{70}N_8O_8Br_4$). Anal. Calcd for TPP3Py·5H_2O: C, 57.16; H, 4.80; N, 6.67. Found: C, 56.93; H, 4.99; N, 6.34.

***meso*-Tetrakis[4-[[3-(*N,N*-dimethylamino]propoxy]carbonyl]phenyl]porphyrin (TPP3E) (4b)** was prepared from 0.5 g (0.63 mmol) of *meso*-(4-hydroxyphenyl)porphyrin and 0.9 mL (8 mmol) of $HO(CH_2)_3NH_2$ using a similar method as for TPP3Br (**4d**). After reaction the solvent was removed and water was used to wash the residue. The solid was stirred with NaOH (pH 13 ~ 14) to deprotonate the tertiary amines. The solid was filtered, dried and purified on silica gel two times. The first eluent was: $CHCl_3$/MeOH/NH_3·H_2O (25%) (10:10:1), the second $CHCl_3/CH_3OH/NH_3$·H_2O (25%) (3:3:1). Yield 235 mg (33%). ^1H NMR ($CDCl_3$): δ −2.80 (s, 2H), 2.14 (m, 8H, J_1 = 7.6 Hz, J_2 = 7.0 Hz), 2.39 (s, 24H), 2.65 (t, 8H, J = 7.5 Hz), 4.58 (t, 8H, J = 6.4 Hz), 8.30 (d, 8H, J = 8.1 Hz), 8.45 (d, 8H, J = 8.1 Hz), 8.82 (s, 8H). UV/vis (MeOH): 415, 511, 544, 588, 643 nm. FAB-MS m/z 1130.6 (M^+, calcd 1131.388 for $C_{68}H_{74}N_8O_8$). Anal. Calcd for TPP3E: C, 69.96; H, 6.73; N, 9.60. Found: C, 69.72; H, 7.01; N 9.20.

***meso*-Tetrakis[4-[[2-(*N,N*-dimethylamino)ethoxy]carbonyl]phenyl]porphyrin (TPP2E) (4a)** from 0.5 g (0.6 mmol) of *meso*-(4-carboxyphenyl)porphyrin and 0.8 mL (8 mmol) of $HO(CH_2)_2NH_2$ following the same method as for TPP3E (**4b**). Eluent: CH_2Cl_2:MeOH:NH_3·H_2O(25%) = 5:5:1. Yield 194 mg (29%). ^1H NMR ($CDCl_3$, TMS): δ −2.81 (s, 2H), 2.45 (s, 24H), 2.87 (t, 8H, J = 5.8 Hz), 4.63 (t, 8H, J = 5.8 Hz), 8.29 (d, 8H, J = 8.2 Hz), 8.46 (d, 8H, J = 8.2 Hz), 8.82 (s, 8H). UV/vis (MeOH): 414, 511, 543, 587, 641 nm. FAB-MS m/z 1075.7 (M^+, calcd 1075.28 for $C_{64}H_{66}N_8O_8$). Anal. Calcd for TPP2E.3CH_2Cl_2·H_2O: C, 60.50; H, 5.46; N, 8.42. Found C, 60.22; H, 5.87; N 8.32.

***meso*-Tetrakis[4-[[[2-(*N,N*-dimethylamino)ethyl]amino]-carbonyl]phenyl]porphyrin (TPP2A) (5a).** The porphyrin acid chloride (from 0.5 g of porphyrin acid and 10 mL of $SOCl_2$) was prepared using the same method as for **4b**. The porphyrin acid chloride was dissolved in CH_2Cl_2 (50 mL) and then treated with *N,N*-dimethylethylenediamine (0.9 mL, 8 mmol) in the presence of NEt_3 (1 mL) at room temperature overnight. After the solvent was removed under reduced pressure, the residue was stirred with 2 N NaOH to deprotonate the tertiary amine. The pure product was obtained by separation on silica gel, using as eluent $CHCl_3$/MeOH/NH_3·H_2O (25%) (5:5:1). The second band was recrystallized in a mixture of MeOH and water to yield 360 mg (53%) of the porphyrin amide. ^1H NMR ($CDCl_3$, TMS): δ −2.86 (s, 2H), 2.38 (s, 24H), 2.67 (t, 8H, J = 5.8 Hz), 3.72 (q, 8H, J_1 = 5.4 Hz, J_2 = 5.8 Hz), 7.17 (br s, 4H), 8.20 (d, 8H, J = 8.0 Hz), 8.27 (d, 8H, J = 7.8 Hz), 8.82 (s, 8H). UV/vis ($CHCl_3$): 419, 514, 549, 588, 643 nm. FAB-MS m/z 1071.2 (M^+, calcd 1071.344 for $C_{64}H_{70}N_{12}O_4$). Anal. Calcd for TPP2A: C, 59.74; H, 6.59, N, 15.69. Found: C, 59.54; H, 6.59; N, 15.31.

***meso*-Tetrakis[4-[[[3-(*N,N*-dimethylamino)propyl]amino]carbonyl]phenyl]porphyrin (TPP3A) (5b).** This porphyrin amide was prepared from 0.5 g (0.6 mmol) of porphyrin acid and 0.9 mL (7 mmol) of $NH_2(CH_2)_3NMe_2$ using the same method as for TPP2A. Eluent: CH_2Cl_2/MeOH/NH_3·H_2O (25%) (5:5:1). Yield: 333 mg (47%). ^1H NMR ($CDCl_3$, TMS): δ −2.93 (s, 2H), 1.94 (m, 8H), 2.45 (s, 24H), 2.68 (t, 8H, J = 5.5 Hz), 3.77 (q, 8H, J = 5.4 Hz), 8.19 (d, 8H, J = 8.0 Hz), 8.27 (d, 8H, J = 8.0 Hz), 8.83 (s, 8H), 8.86 (s, 4H). UV/vis (MeOH): 414, 511, 545, 587, 642 nm. UV/vis (SHE buffer, pH = 7.0): 415 (2.37 × 10^5), 522 (9.09 × 10^3), 562 (6.59 × 10^3), 654 (2.87 × 10^3). FAB-MS m/z 1127.5 (M^+, calcd 1127.452 for $C_{68}H_{78}N_{12}O_4$). Anal. Calcd for TPP3A·3H_2O: C, 69.13; H, 7.17; N, 14.23. Found: C, 68.85; H, 6.59; N 14.61.

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