Efficient Photocleavage of DNA by Cationic Porphyrin–Acridine Hybrids with the Effective Length of Diamino Alkyl Linkage

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Positively charged porphyrins bearing an acridine with various lengths of diamino alkyl linkage, 5-[4-[(6-chloro-2-methoxy-9-acridyl)aminoalkylaminocarbonyl]phenyl]-10,15,20-tris(4-*N*-methylpyridiniumyl)porphine triiodide, alkyl=ethyl, butyl, hexyl, or octyl, were synthesized. They exhibited more enhanced photocleavage activity of pUC18 plasmid DNA than TMPyP, *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphine, which is well known to bind to DNA tightly and to cleave DNA effectively; the hybrid linked with the hexamethylene chain showed particularly high activity. An equilibrium dialysis experiment demonstrated that the binding ability of the hybrids to calf thymus (CT) DNA correlated quantitatively with the photocleavage activity. The lack of the substantial red-shift of the Soret maxima of the hybrids through the titration with CTDNA denied the intercalative binding of the porphyrin part. In their circular dichroism (CD) spectral change on binding to CTDNA, two negative peaks appeared at 275 nm and at 285—290 nm in the UV range. The latter negative peak was observed for hybrids, but not for TMPyP, and thus we assigned it to induced CD (ICD) derived from intercalation of acridine chromophore. In the visible range, the hybrids showed only a positive peak around their Soret maxima, and this feature suggested the porphyrin moiety lay in the DNA groove. In addition, the length of the linker markedly influenced the ellipticity of their visible ICD, suggesting that the proximity of the porphyrin moiety to DNA was greatly affected by the linker.

Key words porphyrin; DNA; acridine; circular dichroism; photocleavage; equilibrium dialysis

The development of compounds which modify nucleic acid structure is a current research area and can make a significant contribution to anti-tumor and -viral chemotherapy.[1,2] For example, drugs like bleomycin (BLM)[3—5] capable of binding to and cleaving nucleic acids are expected to suppress the proliferation of malignant cells by blocking the flow of genetic information. From this point of view, cationic *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphine (TMPyP) has been carefully studied and noted for its bifunctionality: i) the tight interaction with DNA through the three types of binding mode including intercalation, outside binding in the groove, and outside binding with self-stacking along the DNA surface,[6,7] and ii) the marked photochemical nuclease activity.[8—10] Such a positively charged photosensitizer[11,12] would also be useful in the photodynamic therapy of cancer.[13,14]

Some hybrid molecules composed of a non-charged porphyrin and acridines have been synthesized as a functional BLM model.[15—17] Acridine is known as an intercalator,[18,19] and hence the metal complexes of the hybrids bind to DNA *via* the acridine moiety, and then cause strand breaks by generating reactive oxygen species in the presence of reducing or oxidizing agents.

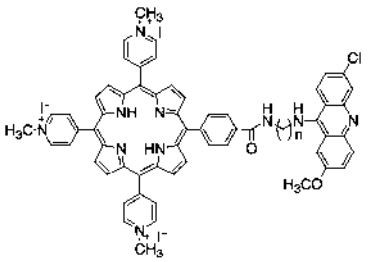
To achieve higher and more efficient chemical modification of negatively charged DNA, we have for the first time synthesized positively charged porphyrin–acridine hybrids, **1**—**4**, which are linked with various lengths of a diamino alkyl chain. In this paper we report their photo-nuclease activity and interaction with DNA. Our data show that they exhibit more enhanced photo-induced nuclease activity than TMPyP, and suggest that acridine and porphyrin moiety in the hybrids interact with DNA by the intercalative and outside groove binding, respectively.

Results and Discussion

Synthesis of the Cationic Porphyrin–Acridine Hybrids

The synthetic procedures for the positively charged porphyrin–acridine hybrids **1**—**4** are illustrated in Chart 1. One equivalent of terephthalaldehydic acid methyl ester, 3 eq of pyridine-4-aldehyde, and 4 eq of pyrrole were reacted in propionic acid for 1.5 h. The resultant mixture including six porphyrin isomers was separated on silica gel. The fifth eluted isomer, 5-(4-methoxycarbonylphenyl)-10,15,20-tris(4-pyridyl)-porphine, **9**, obtained in 5.9% yield was then hydrolyzed with lithium hydroxide to give 5-(4-carboxyphenyl)-10,15,20-tris(4-pyridyl)porphine, **10**, almost quantitatively. Condensation of 1 eq of **10** with 5—7 eq of acridine-diaminoalkane derivatives,[20] **5**—**8**, using 5—7 eq of carbonyldiimidazole (CDI) in an absolute 50/50 (v/v) DMF–DMSO solution afforded corresponding porphyrin–acridine hybrids, **11**—**14**, in 22—46% yield. The products were then methylated with excess methyl iodide in DMF for 3 h to give the final products, **1**—**4**, quantitatively. Characterization of the compounds was accomplished using ^{1}H-NMR spectroscopy, matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and elemental analysis.

Photocleavage of DNA To estimate the potency of these novel hybrids, the photo-nuclease activity of **1**—**4** was exam-



1: n = 2
2: n = 4
3: n = 6
4: n = 8

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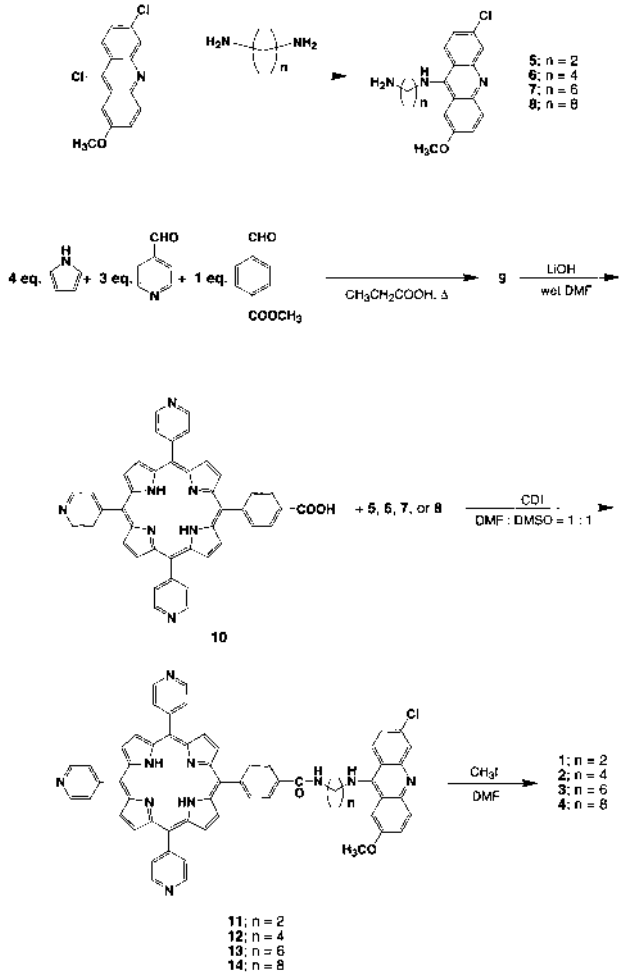


Chart 1

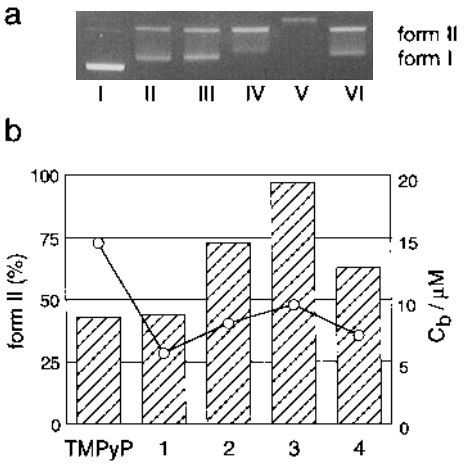


Fig. 1. Agarose Gel (0.8%) Electrophoresis of Plasmid DNA Treated with TMPyP and **1**—**4** in the Presence of Light

Cleavage conditions: 57.9 μM pUC18 plasmid DNA, 10 mM sodium phosphate buffer (pH 7.0), irradiation at 526 nm for 30 min at 25 °C. a) Lane I, DNA alone; lane II to VI, 1.0 μM TMPyP, **1**, **2**, **3**, and **4**. Form I and form II denote the supercoiled and nicked circular forms of plasmid DNA, respectively; b) a bar graph shows the densitometric data for form II (%) of each lane, and a line graph shows the amount of TMPyP and **1**—**4** bound to CTDNA. The dialysis results were obtained after equilibration for 24 h, using 1.0 μM TMPyP and **1**—**4** in each dialysate solution and 58.0 μM CTDNA in the dialysis tube.

ined using supercoiled double-stranded pUC18 plasmid DNA. A mixture of the hybrid (1.0 μM) and the plasmid DNA (57.9 μM) in a buffer (10 m M sodium phosphate, pH 7.0) was irradiated at 526 nm of the Q band region for 30 min at 25 °C. Acridine moiety in the hybrids is not excited at this wavelength. After irradiation, the conversion of the supercoiled DNA (form I) to nicked circular DNA (form II) was visualized by agarose gel electrophoresis with ethidium bromide staining. Figure 1 shows the results of the gel electrophoresis (a), and the densitometric data of the form II distribution of each lane as a bar graph (b). In this reaction condition, **1**, having an acridine with dimethylene linkage, and TMPyP, having no acridine, showed almost the same degrees (44 and 43%, respectively) of the conversion from form I to form II. However, the hybrids **2** (73%), **3** (97%) and **4** (63%) with longer linkage exhibited higher degrees of the conversion than TMPyP. Based on these results, it is clear that the positively charged porphyrin–acridine hybrid **3** with hexamethylene linkage has the most efficient photo-nuclease activity of all the porphyrins tested, and that the effective length of the diamino alkyl linkage in the hybrids is responsible for this high nuclease activity.

Equilibrium Dialysis An equilibrium dialysis experiment to know how many hybrids can bind to DNA was performed. Five-tenths milliliters of calf thymus DNA (CTDNA) solution (58.0 μM) was dialyzed against 100 ml of a dialysate buffer solution (10 mM sodium phosphate, pH 7.0) containing a hybrid (1.0 μM). After equilibration at 25 °C for 24 h, the amount of the hybrid bound to CTDNA (C_b) was evaluated by a visible absorbance measurement.[21] Results are summarized in Fig. 1b as a line graph. Among the hybrids, **3** (C_b=9.6 μM) was bound in the highest amount, and the amount of other hybrids bound to CTDNA decreased in the order of **2** (8.1 μM), **4** (7.1 μM), and **1** (5.7 μM). These equilibrium dialysis results of the hybrids closely correlated with their photo-nuclease activity, in that their quantitative relation for the amount of the hybrid bound to CTDNA corresponded to their relative capability of photochemical modification of the plasmid DNA.[22]

Absorption Spectra Complexation between a ligand molecule and DNA leads to absorption spectral changes that can be used to monitor the binding process. All hybrid solutions (5.0 μM) were titrated with a stock solution of CTDNA in a buffer (100 mM NaCl and 10 mM sodium phosphate, pH 7.0). The intensity of the Soret bands for all hybrids decreased at the initial stage, and then increased with further DNA additions. The spectral change for titration of **1** with CTDNA is shown in Fig. 2 as a representative of all hybrids, and Fig. 3 shows its absorbance change at the Soret maximum. The spectral behavior demonstrated that at least two binding steps exist. While the large decrease in absorbance (hypochromicity) was observed in common among **1**, **2**, **3**, and **4** (33, 38, 38, and 34%, respectively), the substantial red-shift of the Soret bands was not observed through the titration. The absence of the red-shift indicates that there is no sign of intercalation of TMPyP moiety in the hybrids.[23]

At the initial step below the DNA concentration of 6—8 μM the intensity of the Soret maximum decreased monotonously for each hybrid. Since isosbestic points were observed for this step, as shown in Fig. 4 for **1**, the optical contributions should come from two distinct species. We thus performed Scatchard analysis for the initial binding

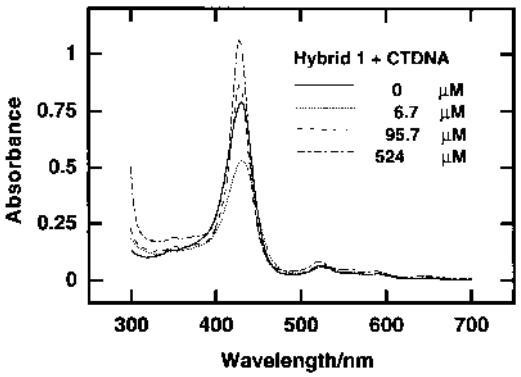


Fig. 2. Absorption Spectra of **1** (5.0 μM) with Addition of Various CTDNA Concentrations in 10 mM Sodium Phosphate, 0.1 M NaCl (pH 7.0)

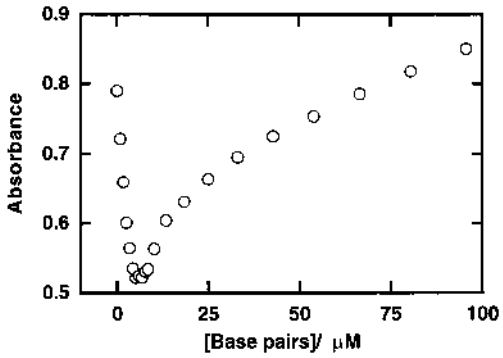


Fig. 3. Absorbance Change of **1** at 429 nm with Addition of CTDNA

equilibrium.[24] The Scatchard plot for the binding of **1** to CTDNA is shown in Fig. 5, and the values of K, the binding constant, and n, the number of base pairs occupied by the bound hybrid, are summarized in Table 1. The values of n evaluated for all hybrids suggest that one hybrid molecule occupies one base pair site of DNA at the initial binding step, though it is not clear whether the orientation of hybrids is ordered (*e.g.*, outside stacking along the DNA surface) or not. Since the values of K and n were obtained at R (input ratio of [hybrid]/[base pairs])$>$0.6, care should be taken that these are not associated with other experiments performed at $R \leq 0.1$.

Circular Dichroism (CD) Spectra CD is a powerful spectroscopic technique that has been widely used to detect conformational changes of DNA and to study the effects of interaction with external ligands.[25,26] The CD spectra generally consist of positive and/or negative bands and are therefore more sensitive and informative than absorption spectra.[27] In particular, the CD spectra in the UV range is sensitive to the conformational change of the duplex, and hence CTDNA was titrated with a stock solution of TMPyP or hybrids in a buffer (100 mM NaCl and 10 mM sodium phosphate, pH 7.0). Figure 6 shows the CD spectral changes on addition of TMPyP (top), **1** (middle), and **3** (bottom) to CTDNA. In the case of TMPyP, the decrease of positive CD at 270 nm was observed through the titration.[28] The appearance of this negative peak is thought to reflect the conformational perturbation of DNA caused by interaction with TMPyP. On the other hand, in the case of hybrids another negative peak at 285—290 nm appeared together with a negative peak at around 275 nm. This large negative peak at

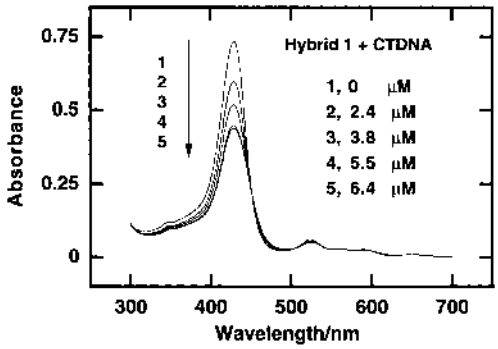


Fig. 4. Absorption Spectra of **1** (5.0 μM) with Addition of CTDNA at $R>0.75$ in 10 mM Sodium Phosphate, 0.1 M NaCl (pH 7.0)

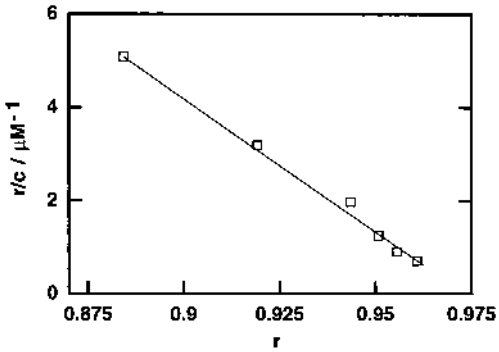


Fig. 5. Scatchard Plot for the Binding of **1** to CTDNA

Table 1. Binding Parameters of Hybrids to CTDNA at $R>0.6$

	1	**2**	**3**	**4**
$K/\mu M^{-1}$	58	23	8.9	8.1
n	0.97	0.94	0.96	0.98

285—290 nm was observed for all hybrids, but not for TMPyP, and thus we assign it to induced CD derived from intercalation of acridine chromophore in the hybrids.

Induced CD (ICD) Spectra ICD is very helpful for analysis of the interaction of chiral DNA with achiral porphyrin.[6,7,23] The ICD spectra can clarify the binding mode of the porphyrin chromophore. Figure 7 shows the ICD spectra for TMPyP and hybrids bound to CTDNA in the 350—500 nm region at $R=0.10$ (top), 0.043 (middle), and 0.010 (bottom) in a buffer (100 mM NaCl and 10 mM sodium phosphate, pH 7.0). For TMPyP both positive and negative peaks showing almost equivalent ellipticity were observed in the Soret region. However, the ICD for all the hybrids showed only large positive peaks and decrease of the ellipticity in the order of **1**, **2**, **3**, and **4**.

To examine the influence of ionic strength on the binding to CTDNA, the ICD were recorded at various salt (NaCl) concentrations. Figure 8 shows the ICD spectra at $R=0.017$ in a buffer containing 100 mM (top), 20 mM (middle), or 0 mM (bottom) of NaCl. The positive peak of TMPyP decreased with decrease in the salt concentration. The ICD spectral change would indicate alteration of the binding mode. On the other hand, for hybrids the presence of the positive peaks and the order of their ellipticity remained unchanged. These spectral data under the conditions employed at various R or

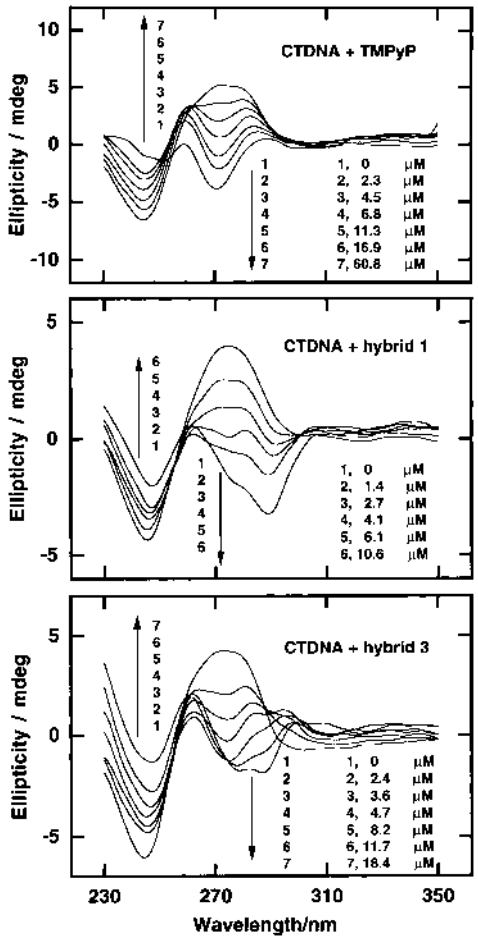


Fig. 6. CD Spectral Changes of CTDNA with Addition of TMPyP (Top), **1** (Middle), and **3** (Bottom) in 10 mM Sodium Phosphate, 0.1 M NaCl (pH 7.0)

The CTDNA concentrations were as follows: top, 31.4 μM; middle, 31.0 μM; bottom, 28.6 μM.

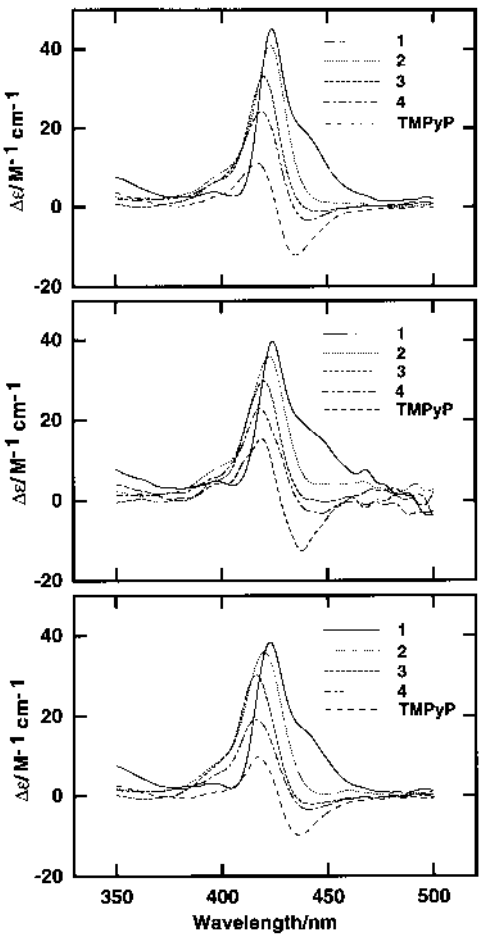
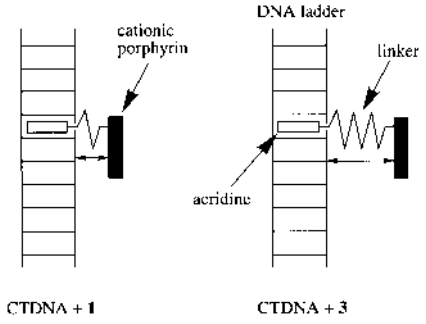


Fig. 7. ICD Spectra of Hybrids and TMPyP in the Presence of CTDNA in 10 mM Sodium Phosphate, 0.1 M NaCl (pH 7.0)

The concentrations of the porphyrins and CTDNA were as follows: top, 5.0 μM and 50 μM; middle, 5.0 μM and 116 μM; bottom, 4.9 μM and 500 μM.

salt concentrations suggest that the porphyrin part in all the hybrids always lay in the DNA groove. Furthermore, it was deduced that the interaction of the porphyrin chromophore in the hybrid with DNA reduced by degrees, as the diamino alkyl linkage became longer. It was concluded that the introduction of an acridine intercalator to the lead compound TMPyP could result in outside groove binding selectivity to the cationic porphyrin.



Conclusion We have synthesized new DNA-interactive, cationic porphyrin–acridine hybrids linked with various lengths of a diamino alkyl chain. They exhibited more efficient photo-nuclease activity than TMPyP. The introduction of acridine intercalator to cationic porphyrin chromophore brought about outside groove binding selectivity to the porphyrin, and the various lengths of their diamino alkyl linkage markedly influenced the ellipticity of their visible ICD. Since the photo-nuclease activity of hybrids varied depending on the length of their diamino alkyl linkage, a definitive factor for the activity must be a suitable distance between the outside groove surface of DNA and the positively charged porphyrin moiety in the hybrids. These hybrids are the first cationic porphyrins showing more excellent photocleavage of DNA than TMPyP.

Experimental

Instrumentation The ^{1}H-NMR spectra were recorded on a JEOL GX-400 or JNM-A-500 spectrometer. The MALDI TOF mass spectra were measured on a Bruker REFLEX^{TM}. The UV–visible absorption measurement was performed on a Beckman DU650 spectrophotometer. The CD spectra were recorded on a JASCO J-720 spectropolarimeter.

Materials and Methods CTDNA was purchased from Sigma Chemical Co. CTDNA solutions were quantitated spectrophotometrically using ε_{260}= 13,200 M (base pairs)$^{-1}$ cm^{-1}. The tosylate salt of TMPyP was purchased from Dojin Chemical Co.

9-(2-Aminoethyl)amino-6-chloro-2-methoxyacridine (5) 6,9-Dichloro-2-methoxyacridine (1.20 g, 4.30 mmol) was dissolved in ethylenediamine (20 ml) and stirred at 70 °C for 1 h. After filtration, water (100 ml) was added to the filtrate. The yellow powder was separated, collected on a filter, and dried. Yield 1.0 g (81%).

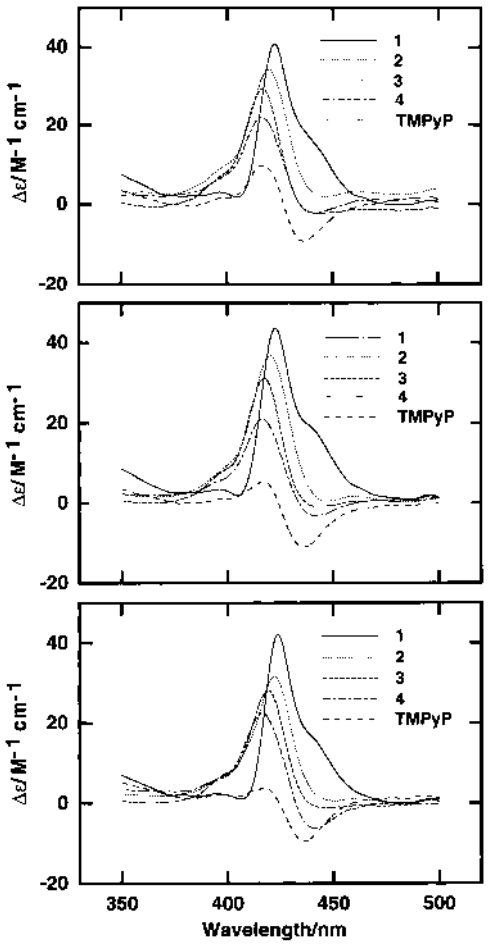


Fig. 8. ICD Spectra of Hybrids and TMPyP in the Presence of CTDNA in 10 mM Sodium Phosphate and Various Amounts of NaCl (pH 7.0)

The concentrations of the porphyrins, CTDNA, and NaCl were as follows: top, 4.9 μM, 298 μM, and 0.1 M; middle, 5.0 μM, 298 μM, and 0.02 M; bottom, 5.0 μM, 298 μM, and 0 M.

^{1}H-NMR (CD_3OD) δ: 3.01 (2H, t, J=6.6 Hz), 3.85 (2H, t, J=6.6 Hz), 3.98 (3H, s), 7.31 (1H, dd, J=9.2, 2.2 Hz), 7.42 (1H, dd, J=9.5, 2.6 Hz), 7.53 (1H, d, J=2.6 Hz), 7.83 (1H, d, J=9.5 Hz), 7.86 (1H, d, J=2.2 Hz), 8.27 (1H, d, J=9.2 Hz). UV λ_{max} (DMSO) nm (ε): 420 (6420), 362 (2900), 345 (2270), 329 (1300), 285 (39800). EI-MS *m/z*: 301 (M^+, Calcd for $C_{16}H_{16}ClN_3O$: 301.78). *Anal*. Calcd for $C_{16}H_{16}ClN_3O \cdot H_2O$: C, 60.09; H, 5.67; N, 13.14. Found: C, 60.09; H, 5.68; N, 13.19.

9-(2-Aminobutyl)amino-6-chloro-2-methoxyacridine (6) 6,9-Dichloro-2-methoxyacridine (0.64 g, 2.31 mmol) was dissolved in 1,4-diaminobutane (8 ml) and stirred at 70 °C for 1 h. After filtration, water (100 ml) was added to the filtrate. The yellow powder was separated, collected on a filter, and dried. Yield 472 mg (58%).

^{1}H-NMR (CD_3OD) δ: 1.52 (2H, m), 1.79 (2H, m), 2.62 (2H, t, J=7.3 Hz), 3.79 (2H, t, J=7.3 Hz), 3.95 (3H, s), 7.25 (1H, dd, J=9.4, 2.0 Hz), 7.39 (1H, dd, J=9.5, 2.6 Hz), 7.47 (1H, d, J=2.6 Hz), 7.80 (1H, d, J=9.5 Hz), 7.83 (1H, d, J=2.2 Hz), 8.21 (1H, d, J=9.4 Hz). UV λ_{max} (DMSO) nm (ε): 421 (7740), 363 (3330), 345 (2740), 329 (1620), 285 (44700). EI-MS *m/z*: 329 (M^+, Calcd for $C_{18}H_{20}ClN_3O$: 329.83). *Anal*. Calcd for $C_{18}H_{20}ClN_3O \cdot H_2O$: C, 62.15; H, 6.38; N, 12.08. Found: C, 62.11; H, 6.05; N, 11.76.

9-(2-Aminohexyl)amino-6-chloro-2-methoxyacridine (7) 6,9-Dichloro-2-methoxyacridine (0.63 g, 2.28 mmol) was dissolved in 1,6-diaminohexane (8 ml) and stirred at 80 °C for 1 h. After filtration, water (200 ml) was added to the filtrate. The yellow powder was separated, collected on a filter, and dried. Yield 611 mg (74 %).

^{1}H-NMR (CD_3OD) δ: 1.38 (6H, m), 1.76 (2H, m), 2.55 (2H, t, J=7.3 Hz), 3.79 (2H, t, J=7.3 Hz), 3.95 (3H, s), 7.24 (1H, dd, J=9.2, 2.2 Hz), 7.38 (1H, dd, J=9.5, 2.6 Hz), 7.47 (1H, d, J=2.6 Hz), 7.80 (1H, d, J=9.5 Hz), 7.84 (1H, d, J=2.2 Hz), 8.20 (1H, d, J=9.2 Hz). UV λ_{max} (DMSO) nm (ε): 420 (7070), 362 (3070), 345 (2500), 328 (1530), 285 (42700). EI-MS *m/z*: 358 (M^+, Calcd for $C_{20}H_{24}N_3O_1Cl_1$: 357.89). *Anal*. Calcd for $C_{20}H_{24}N_3O_1Cl_1 \cdot 1$ (H_2O): C, 63.91; H, 6.97; N, 11.18. Found: C, 63.84; H, 6.59; N, 10.91.

9-(2-Aminooctyl)amino-6-chloro-2-methoxyacridine (8) To 1,8-diaminooctane (7.7 g) melted at 80 °C, 6,9-dichloro-2-methoxyacridine (0.77 g, 2.82 mmol) was added and stirred for 1 h. Water (300 ml) was added to the reaction mixture, and then it was cooled in a refrigerator. The oily product was dissolved in hydrochloric acid (15 ml) and water (100 ml) by warming. After filtration, orange powder precipitated, then was filtered off, washed with tetrahydrofuran (THF) and dried. Yield 854 mg (62%).

^{1}H-NMR (CD_3OD) δ: 1.41 (8H, m), 1.66 (2H, m), 2.00 (2H, m), 2.91 (2H, t, J=7.7 Hz), 4.01 (3H, s), 4.14 (2H, t, J=7.7 Hz), 7.48 (1H, dd, J=9.2, and 1.8 Hz), 7.63 (1H, dd, J=9.5, 2.2 Hz), 7.75 (1H, d, J=9.5 Hz), 7.79 (1H, d, J=2.2 Hz), 7.83 (1H, d, J=1.8 Hz), 8.45 (1H, d, J=9.2 Hz). UV λ_{max} (DMSO) nm (ε): 425 (8170), 345 (3310), 329 (2970), 284 (44900). EI-MS *m/z*: 386 (M^+, Calcd for $C_{22}H_{28}N_3O_1Cl_1$: 385.94). *Anal*. Calcd for $C_{22}H_{28}ClN_3O \cdot 2HCl \cdot 1.5H_2O$: C, 54.38; H, 6.85; N, 8.65. Found: C, 54.41; H, 6.53; N, 8.29.

5-(4-Methoxycarbonylphenyl)-10,15,20-tris(4-pyridyl)porphine (9) A mixture of terephthalaldehydic acid methyl ester (1.6 g, 0.01 mol) and pyridine-4-aldehyde (2.7 ml, 0.03 mol) was dissolved in propionic acid (160 ml), and was refluxed with stirring. To this solution was slowly added pyrrole (2.6 ml, 0.04 mol). The mixture was successively refluxed for 1.5 h. After propionic acid was removed under reduced pressure, chloroform (100 ml) was added to the residue, which was then neutralized with saturated $NaHCO_3$ solution. The organic phase was dried with anhydrous Na_2SO_4, and then the crude material including six porphyrin isomers was chromatographed on silica gel (2% methanol/$CHCl_3$). Addition of heptane to the eluent and slow evaporation of the solvent afforded **9** as purple crystals. Yield: 400 mg (5.9%)

^{1}H-NMR ($CDCl_3$) δ: −2.89 (2H, s), 4.12 (3H, s), 8.16 (6H, m), 8.30 (2H, d, J=8.1 Hz), 8.47 (2H, d, J=8.1 Hz), 8.86 (8H, m), 9.06 (6H, m). UV λ_{max} (DMSO) nm (ε): 642 (2010), 587 (3420), 546 (3720), 513 (10100), 418 (191000). FAB-MS *m/z*: 676 (M^+, Calcd for $C_{43}H_{29}N_7O_2$: 675.76). *Anal*. Calcd for $C_{43}H_{29}N_7O_2 \cdot 0.5CHCl_3$: C, 71.04; H, 4.04; N, 13.33. Found: C, 70.80; H, 4.04; N, 13.72.

5-(4-Carboxyphenyl)-10,15,20-tris(4-pyridyl)porphine (10) To a solution of **9** (400 mg, 0.592 mmol) in 40 ml of DMF was added lithium hydroxide monohydrate (1.20 g, 28.6 mmol) and water (5 ml), and the mixture was stirred at room temperature for 10 h. The pH of the reaction mixture was adjusted to 4 with 1 M HCl, and the product was extracted from the aqueous layer with chloroform. The organic layer was washed with water twice and dried with anhydrous Na_2SO_4. Addition of heptane and slow evaporation gave purple crystals. Yield: 340 mg (84%).

^{1}H-NMR ($CDCl_3$) δ: −3.02 (2H, s), 8.27 (6H, m), 8.34 (2H, d, J=8.1 Hz), 8.40 (2H, d, J=8.1 Hz), 8.90 (8H, m), 9.05 (6H, m). UV λ_{max} (DMSO) nm (ε): 642 (1790), 587 (3390), 546 (3610), 513 (11300), 418 (232000). FAB-MS *m/z*: 662 (M^+, Calcd for $C_{42}H_{27}N_7O_2$: 661.73). *Anal*. Calcd for $C_{42}H_{27}N_7O_2 \cdot H_2O$: C, 74.21; H, 4.30; N, 14.42. Found: C, 74.02; H, 3.99; N, 14.18.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminoethylaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphine (11) Compound **10** (48 mg, 73 μmol) was dissolved in 20 ml of an absolute 50/50 (v/v) DMF–DMSO solution under argon at 0 °C. To this solution 8 ml of an absolute 50/50 (v/v) DMF–DMSO solution of CDI (35 mg, 0.22 mmol) was slowly added, and the reaction mixture was stirred for 6 h at 0 °C. To this reaction mixture was added 10 ml of an absolute 50/50 (v/v) DMF–DMSO solution of **5** (109 mg, 0.36 mmol) in 20 min at 0 °C. The solution was stirred 20 h at room temperature. Chloroform (100 ml) was poured into the solution, which was then washed with saturated $NaHCO_3$ solution three times and water once, and dried with anhydrous Na_2SO_4. The solvent was removed and hybrid **11** was purified on a silica gel chromatograph (5 to 20% methanol/$CHCl_3$). Addition of heptane to the eluent and slow evaporation gave a brown powder, which was collected by centrifugation and dried. Yield: 15 mg (22%).

^{1}H-NMR (DMSO-d_6) δ: 3.92 (2H, q, br), 4.02 (3H, s), 4.36 (2H, m, br), 7.48 (1H, d, J=8.8 Hz), 7.65 (1H, d, J=9.2 Hz), 7.89 (1H, d, J=9.2 Hz), 7.98 (1H, s), 8.09 (1H, s), 8.25 (4H, s, br), 8.68 (6H, d, J=5.7 Hz), 8.70 (1H, d, J=8.8 Hz), 9.01 (8H, m), 9.24 (6H, d, J=5.7 Hz). UV λ_{max} (DMSO) nm (ε): 642 (2870), 587 (5480), 546 (5750), 512 (17600), 418 (371000), 285 (70500). MALDI-TOF-MS *m/z*: 946.19 (M^+, Calcd for $C_{58}H_{41}ClN_{10}O_2$: 945.49). *Anal*. Calcd for $C_{58}H_{41}N_{10}O_2Cl_1 \cdot 0.5CHCl_3 \cdot 1.5H_2O$: C, 68.07; H, 4.35; N, 13.57. Found: C, 67.82; H, 4.37; N, 13.38.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminobutylaminocarbonyl]phenyl]-

10,15,20-tris(4-pyridyl)porphine (12) Compound **10** (65 mg, 98 μmol) was dissolved in 2 ml of an absolute 50/50 (v/v) DMF–DMSO solution under argon at 0 °C. To this solution 2 ml of an absolute 50/50 (v/v) DMF–DMSO solution of CDI (80 mg, 0.49 mmol) was slowly added, and the reaction mixture was stirred for 6 h at 0 °C. To this reaction mixture was slowly added 2 ml of an absolute 50/50 (v/v) DMF–DMSO solution of **6** (239 mg, 0.687 mmol) at 0 °C. The solution was stirred 37 h at room temperature. Chloroform (70 ml) was poured into the solution, which was then washed with saturated $NaHCO_3$ solution three times and water once, and dried with anhydrous Na_2SO_4. The solvent was removed and hybrid **12** was purified on a silica gel chromatograph (5 to 20% methanol/$CHCl_3$). Addition of heptane to the eluent and slow evaporation gave a brown powder, which was collected by centrifugation and dried. Yield: 39 mg (38%).

^{1}H-NMR (DMSO-d_6) δ: −3.03 (2H, s), 1.75 (2H, m, br), 1.93 (2H, m, br), 3.45 (2H, q, br), 3.98 (3H, s), 4.00 (2H, m, br), 7.40 (1H, d, J=8.4 Hz), 7.50 (1H, d, J=9.0 Hz), 7.77 (1H, s), 7.81 (1H, d, J=9.0 Hz), 7.86 (1H, s), 8.24 (2H, d, J=8.4 Hz), 8.27 (6H, d, J=5.7 Hz), 8.29 (2H, d, J=8.4 Hz), 8.44 (1H, d, J=8.4 Hz), 8.88 (1H, t, br), 8.90 (8H, s, br), 9.05 (6H, d, J=5.7 Hz). UV λ_{max} (DMSO) nm (ε): 642 (3430), 587 (6680), 546 (7070), 513 (22500), 418 (601000), 285 (75900). MALDI-TOF-MS *m/z*: 974.13 (M^+, Calcd for $C_{60}H_{45}ClN_{10}O_2$: 973.55). *Anal*. Calcd for $C_{60}H_{45}N_{10}O_2Cl_1 \cdot CH_3OH \cdot 2H_2O$: C, 70.34; H, 5.13; N, 13.45. Found: C, 70.36; H, 4.96; N, 13.05.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminohexylaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphine (13) Compound **10** (89 mg, 0.134 mmol) was dissolved in 10 ml of an absolute 50/50 (v/v) DMF–DMSO solution under argon at 0 °C. To this solution CDI (153 mg, 0.941 mmol) was added, and the reaction mixture was stirred for 2 h at 0 °C. To this reaction mixture was added **7** (341 mg, 0.941 mmol) at 0 °C. The solution was stirred 47 h at room temperature. Chloroform (100 ml) was poured into the solution, which was then washed with diluted HCl solution three times in order to remove unreacted **7**, and with water three times, and dried with anhydrous Na_2SO_4. The solvent was removed and hybrid **13** was purified on a silica gel chromatograph (5 to 20% methanol/$CHCl_3$). The brown powder was precipitated by addition of diethyl ether, collected on a filter and dried. Yield: 64 mg (46%).

^{1}H-NMR (DMSO-d_6) δ: −3.03 (2H, s), 1.46 (4H, m, br), 1.64 (2H, m, br), 1.85 (2H, m, br), 3.64 (2H, q, br), 3.89 (2H, m, br), 3.96 (3H, s), 7.40 (1H, d, J=9.2 Hz), 7.49 (1H, d, J=8.7 Hz), 7.75 (1H, s), 7.80 (1H, d, J=8.7 Hz), 7.85 (1H, s), 8.26 (6H, d, J=5.9 Hz), 8.27 (2H, d, J=8.4 Hz), 8.30 (2H, d, J=8.4 Hz), 8.40 (1H, d, J=9.2 Hz), 8.82 (1H, t, br), 8.90 (8H, m, br), 9.04 (6H, d, J=5.9 Hz). UV λ_{max} (DMSO) nm (ε): 642 (3540), 587 (6770), 546 (7140), 513 (22100), 418 (486000), 284 (66700). MALDI-TOF-MS *m/z*: 1001.7 (M^+, Calcd for $C_{62}H_{49}ClN_{10}O_2$: 1001.60). *Anal*. Calcd for $C_{62}H_{49}N_{10}O_2Cl_1 \cdot 2H_2O$: C, 71.77; H, 5.15; N, 13.50. Found: C, 71.55; H, 4.87; N, 13.53.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminooctylaminocarbonyl]phenyl]-10,15,20-tris(4-pyridyl)porphine (14) Compound **10** (88 mg, 0.133 mmol) was dissolved in 10 ml of an absolute 50/50 (v/v) DMF–DMSO solution under argon at 0 °C. To this solution CDI (151 mg, 0.931 mmol) was added, and the reaction mixture was stirred for 2 h at 0 °C. To this reaction mixture was added **8** (453 mg, 0.931 mmol) and 4-ethylmorpholine (355 μl, 2.79 mmol) at 0 °C. The solution was stirred 40 h at room temperature. Chloroform (100 ml) was poured into the solution, which was then washed with diluted HCl solution three times and water three times, and dried with anhydrous Na_2SO_4. The solvent was removed and hybrid **14** was purified on a silica gel chromatograph (5 to 20% methanol/$CHCl_3$). The brown powder was precipitated by addition of diethyl ether, collected by centrifugation and dried. Yield: 33 mg (24%).

^{1}H-NMR (DMSO-d_6) δ: −3.02 (2H, s), 1.33 (8H, m, br), 1.61 (2H, m, br), 1.78 (2H, m, br), 3.39 (2H, q, br), 3.81 (2H, m, br), 3.93 (3H, s), 7.35 (1H, d, J=9.2 Hz), 7.43 (1H, d, J=9.2 Hz), 7.68 (1H, s), 7.78 (1H, d, J=9.2 Hz), 7.83 (1H, s), 8.26 (6H, d, J=5.9 Hz), 8.27 (2H, d, J=8.6 Hz), 8.30 (2H, d, 8.6 Hz), 8.35 (1H, d, J=9.2 Hz), 8.81 (1H, t, br), 8.90 (8H, m, br), 9.04 (6H, d, J=5.9 Hz). UV λ_{max} (DMSO) nm (ε): 642 (3730), 587 (7170), 546 (7580), 513 (23600), 418 (519000), 285 (67800). MALDI-TOF-MS *m/z*: 1029.8 (M^+, Calcd for $C_{64}H_{53}ClN_{10}O_2$: 1029.65). *Anal*. Calcd for $C_{64}H_{53}ClN_{10}O_2 \cdot 1.5H_2O$: C, 72.75; H, 5.34; N, 13.26. Found: C, 72.83; H, 5.13; N, 13.25.

General Procedure for Methylation of Hybrids Hybrids **11**—**14** were methylated in 5 ml of DMF with methyl iodide (0.8 ml) for 3 h at room temperature. The solvent and methyl iodide were removed under vacuum. The residue was taken up in DMF and precipitated with diethyl ether. The brown powder was collected by centrifugation, washed with diethyl ether and dried. This reaction is quantitative.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminoethylaminocarbonyl]phenyl]-10,15,20-tris(4-*N*-methylpyridiniumyl)porphine Triiodide (1): ^{1}H-NMR (DMSO-d_6) δ: −3.03 (2H, s), 3.97 (2H, q, br), 4.09 (3H, s), 4.38 (2H, m, br), 4.72 (6H, s), 4.73 (3H, s), 7.55 (1H, d, J=9.2 Hz), 7.68 (1H, d, J=9.9 Hz), 7.86 (1H, d, J=9.9 Hz), 7.91 (1H, s), 7.95 (1H, s), 8.24 (2H, d, J=8.3 Hz), 8.31 (2H, d, J=8.3 Hz), 8.68 (1H, d, J=9.2 Hz), 8.97 (6H, d, J=6.6 Hz), 9.17 (8H, m), 9.43 (6H, d, J=6.6 Hz). UV λ_{max} (DMSO) nm (ε): 644 (2320), 589 (6020), 551 (6150), 516 (16900), 423 (261000), 286 (65900). MALDI-TOF-MS *m/z*: 990.33 (M^+, Calcd for $C_{61}H_{50}ClN_{10}O_2$: 990.60). *Anal*. Calcd for $C_{61}H_{50}ClN_{10}O_2I_3 \cdot 4H_2O \cdot CH_3I$: C, 46.97; H, 3.88; N, 8.84. Found: C, 47.07; H, 3.88; N, 9.10.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminobutylaminocarbonyl]phenyl]-10,15,20-tris(4-*N*-methylpyridiniumyl)porphine Triiodide (2): ^{1}H-NMR (DMSO-d_6) δ: −3.03 (2H, s), 1.82 (2H, m, br), 2.08 (2H, m, br), 3.50 (2H, q, J=6.1 Hz), 4.02 (3H, s), 4.25 (2H, m, br), 4.72 (6H, s), 4.73 (3H, s), 7.60 (1H, d, J=9.2 Hz), 7.75 (1H, d, J=11.7 Hz), 7.84 (1H, d, J=11.7 Hz), 7.86 (1H, s), 7.95 (1H, s), 8.32 (4H, s, br), 8.62 (1H, d, J=9.5 Hz), 9.01 (6H, d, J=6.6 Hz), 9.10 (8H, m), 9.49 (6H, d, J=6.6 Hz). UV λ_{max} (DMSO) nm (ε): 644 (2730), 589 (7510), 551 (7590), 517 (21600), 425 (313000), 286 (67500). MALDI-TOF-MS *m/z*: 1018.82 (M^+, Calcd for $C_{63}H_{54}ClN_{10}O_2$: 1018.65). *Anal*. Calcd for $C_{63}H_{54}ClN_{10}O_2I_3 \cdot 8H_2O \cdot CH_3I \cdot DMF$: C, 45.76; H, 4.59; N, 8.76. Found: C, 45.85; H, 4.27; N, 8.84.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminohexylaminocarbonyl]phenyl]-10,15,20-tris(4-*N*-methylpyridiniumyl)porphine Triiodide (3): ^{1}H-NMR (DMSO-d_6) δ: −3.02 (2H, s), 1.53 (4H, m, br), 1.70 (2H, m, br), 1.99 (2H, m, br), 3.45 (2H, q, J=6.2 Hz), 4.01 (3H, s), 4.14 (2H, m, br), 4.73 (6H, s), 4.74 (3H, s), 7.60 (1H, d, J=9.2 Hz), 7.73 (1H, d, J=11.4 Hz), 7.84 (1H, d, J=11.4 Hz), 7.85 (1H, s), 7.95 (1H, s), 8.33 (4H, s, br), 8.57 (1H, d, J=9.2 Hz), 9.01 (6H, d, J=6.6 Hz), 9.19 (8H, m), 9.49 (6H, d, J=6.6 Hz) UV λ_{max} (DMSO) nm (ε): 644 (2480), 589 (6710), 551 (6810), 517 (18900), 425 (298000), 285 (60800). MALDI-TOF-MS *m/z*: 1046.60 (M^+, Calcd for $C_{65}H_{58}N_{10}O_2Cl_1$: 1046.71). *Anal*. Calcd for $C_{65}H_{58}ClN_{10}O_2I_3 \cdot 8H_2O$: C, 49.68; H, 4.75; N, 8.91. Found: C, 49.79; H, 4.47; N, 9.13.

5-[4-[(6-Chloro-2-methoxy-9-acridyl)aminooctylaminocarbonyl]phenyl]-10,15,20-tris(4-*N*-methylpyridiniumyl)porphine Triiodide (4): ^{1}H-NMR (DMSO-d_6) δ: −3.02 (2H, s), 1.41 (8H, m, br), 1.67 (2H, m, br), 1.93 (2H, m, br), 3.43 (2H, q, J=6.6 Hz), 3.99 (3H, s), 4.12 (2H, m, br), 4.73 (6H, s), 4.74 (3H, s), 7.58 (1H, d, J=9.2 Hz), 7.72 (1H, d, J=9.2 Hz), 7.82 (1H, d, J=9.5 Hz), 7.84 (1H, s), 7.93 (1H, s), 8.34 (4H, s, br), 8.54 (1H, d, J=9.2 Hz), 9.01 (6H, d, J=6.6 Hz), 9.09 (8H, m), 9.25 (6H, d, J=6.6 Hz). UV λ_{max} (DMSO) nm (ε): 644 (2450), 589 (6580), 551 (6700), 517 (18800), 425 (287000), 285 (62600). MALDI-TOF-MS *m/z*: 1074.19 (M^+, Calcd for $C_{67}H_{62}ClN_{10}O_2$: 1074.76). *Anal*. Calcd for $C_{67}H_{62}ClN_{10}O_2I_3 \cdot 7H_2O$: C, 50.88; H, 4.84; N, 8.86. Found: C, 50.88; H, 4.99; N, 9.08.

DNA Photocleavage Assay Photoirradiation was performed at 25 °C using a Hitachi 650—660 fluorescence spectrophotometer. A sample containing supercoiled pUC18 plasmid DNA (57.9 μM) and a hybrid (1.0 μM) was irradiated in a buffer (10 μM sodium phosphate, pH 7.0) at 526 nm for 30 min. After irradiation, DNA was analyzed by 0.8% agarose gel electrophoresis at 100 V for 45 min. The gel was incubated in a solution of ethidium bromide for 5 min and the DNA bands were detected by fluorescence under a UV lamp. The densitometric data of the bands were obtained with an ATTO Densitograph version 4.0 for Macintosh.

Equilibrium Dialysis Assay A volume of 100 ml of the dialysate buffer solution (10 mM sodium phosphate, pH 7.0) containing 1.0 μM hybrid was placed in a beaker. Extinction coefficient of the Soret band of the hybrid was then spectrophotometrically determined. A 0.5 ml buffer solution (10 mM sodium phosphate, pH 7.0) containing CTDNA (58.0 μM) was pipetted into a 0.5 ml Spectro/Por DispoDialyzer tube, which was then soaked in the dialysate buffer solution. The beaker was covered with Parafilm and wrapped in foil, and its content was allowed to equilibrate with stirring for 24 h at 25 °C. After the completion of equilibration, the DNA sample was transferred to a tube, and was taken to a final concentration of 1% sodium dodecyl sulfate by the addition of its 10% stock solution. The total concentration of the hybrid (C_t) within the dialysis tube was determined spectrophotometrically using wavelength and extinction coefficient of the hybrid. The free hybrid concentration (C_f) was determined spectrophotometrically using an aliquot of the dialysate solution. The amount of bound hybrid (C_b) was determined by the difference, $C_b = C_t - C_f$.

Spectral Measurements Aliquots of a CTDNA solution were added to the solution of a hybrid (5.0 μM). The spectral measurement was performed at 25 °C in a buffer containing 10 mM sodium phosphate and 100 mM sodium chloride (pH 7.0). All spectral data were corrected for the dilution effect.

The binding constant (K) and the number of base pairs occupied by the bound drug (n) were estimated from the absorbance change of the Soret maximum, using the Scatchard equation:

$$r/c = K(n-r)$$

where r is the binding ratio and c is the concentration of unbound hybrid. Values for r and c were calculated according to the treatment of Peacocke and Skerrett.[29] The linear fitting of r *vs*. r/c was carried out using a KaleidaGraph version 3.0. To a buffered aqueous solution (10 mM sodium phosphate and 100 mM sodium chloride, pH 7.0) of CTDNA (about 30 μM) was added aliquots of the TMPyP or hybrid solution, and CD spectra in the UV range were recorded.

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