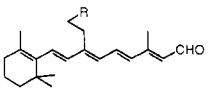
Table I. Absorbance Maxima of Pigments and H^+ Pumping Ability



	R	pigment[a] (λ_{max} nm)	H^+ pump[b] (%)
12	-OH	560	40
19	-OCOH	452	*c*
20	$-OCOCH_3$	452	
21	$-OCOCHN_2$	452	12
22	-OCO-Pr	452	12
23	$-OCO(CH_2)_8CH{=}CH_2$	452	10
24	adamantanecarbonyl-O-	452	7
25	retinoyl-O-	450	
26	$-(CH_2)_{13}CH_3$	452	
8	$-OCO(CH_2)_9OH$	452	7
14	$-OCO(CH_2)_9OSO_3K$	475	12

[a] Pigment with bleached bR-opsin, 10 mM HEPES buffer pH 7.0, room temperature, dark. [b] H^+ pumping ability of pigments, relative to *all-trans*-retinal (100%), generated with JW2N cell vesicles, irradiation >435 nm; >530 nm for pigment **12**. [c] Not tested.

sulfate **15** yielded a pigment only after 6 h; in contrast, retinal sulfates **16–18** did not reconstitute pigments after 16–24 h, thus suggesting that the chain length in retinal sulfate **15** is the shortest necessary for the chromophore to reach the binding site.[17] This result, together with the fact that the direction of proton translocation in these vesicles is normal (pH decreases upon irradiation, Table I, below) shows that the *substituents at C-9, including 9-methyl in native bR, face the extracellular side of the membrane*. Since the Stokes or hydrodynamic radius of the sulfate group is around 2.30 Å[18] and the C–C bond length in an alkyl chain (zigzag) is 1.25 Å,[19] the distance between C-9 of *all-trans*-retinal and the sulfate oxygen, or *the depth of C-9 from the extracellular side of the membrane surface, can then be estimated to be ca. 15 Å*.

The pigment from hydroxyethyl analogue **12** absorbs at 560 nm and is similar to native bR, while other pigments reconstituted from **7–11**/**19–26** (Table I), *all* absorb around 450 nm, including C_{16}–OH analogue **7**, hydroxyethyl formate **19** and -O-CO-adamantyl analogue **24**. This trend indicates that interaction between the C-9 hydroxyethyl (**12**) and surrounding bR α-helices is similar to that of native bR, while substituents extending beyond ca. 4 Å, independent of the nature of the neutral group beyond the ester bond, lead to similar perturbations in the interactions between the chromophore and binding site.[20] Retinal sulfates **13–15** gave 23-nm red-shifted pigments compared to corresponding alcohols **7–9**, presumably because electrostatic interaction of the sulfate group with the membrane surface gives rise to chromophoric dislocation.

Proton pumping abilities of pigments reconstituted from retinal analogues **8**, **12**, **14**, **21**, **22**, **23**, and **24** with white membrane cell vesicles were measured (Table I). All 9-alkylretinal pigments tested showed pH decreases resulting from proton extrusion, which were completely blocked by the uncoupler nigericin (data not shown); however, the efficiency of proton translocation was lower than the *all-trans*-retinal pigment. The fact that functional bR analogues can be reconstituted efficiently from retinal analogues with long C-9 alkyl chains indicate that such analogues can be used for affinity labeling studies of bR and related photoreceptors; the latter aspect is under investigation.[21,22]

Acknowledgment. We are grateful to Dr. Fadila Derguini for suggestions and discussions. This work has been supported by NSF CHE 18263.

(16) When pigments **7–11**, **13**, and **14** were incubated with *all-trans*-retinal in the dark, 25 °C, pH 7.0, the 9-alkyl chromophore was replaced; after >24 h the λ_{max} was shifted to 562 nm. All pigments were not stable when treated with 0.1 M NH_2OH and 450-nm irradiation; $t_{1/2}$ 5–10 min.

(17) Reconstitution experiment with JW2N white membrane cell vesicles showed the same results.

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(22) Experimental and other details of this communication will be submitted shortly for publication.

DNA Cleavage by a Metal Chelating Tricationic Porphyrin

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The demonstrated chemotherapeutic effects of porphyrins[1] have stimulated recent investigations into the nature of porphyrin-cell interactions. That *meso*-tetra(4-*N*-methylpyridyl)porphine (TMPyP) intercalates the base pairs of DNA selectively at G-C rich regions has been well-established.[2] ^1H and ^{31}P NMR experiments with oligonucleotides have indicated that the preferred sequence for intercalation of TMPyP is CpG.[2h] The axially ligated metalloderivatives of TMPyP {Fe(III), Mn(III), and Co(III)} have also been determined to bind to DNA; however, these metalloporphyrins were observed to bind A-T rich regions in the minor groove.[2d,e] In the presence of high intensity visible light and oxygen, various water-soluble porphyrins, including TMPyP, were found to induce single-strand scissions in DNA.[3] DNA cleavage was also observed with the metalloderivatives of TMPyP {Fe(III), Mn(III), and Co(III)}, but these porphyrins generally required reduced forms of oxygen for activity.[4,5] The broad spectrum reactivity of related iron hemes has been utilized in the design of functional bleomycin models[6] and heme–oligonucleotide adducts which are capable of cleaving complementary single-stranded DNA.[7]

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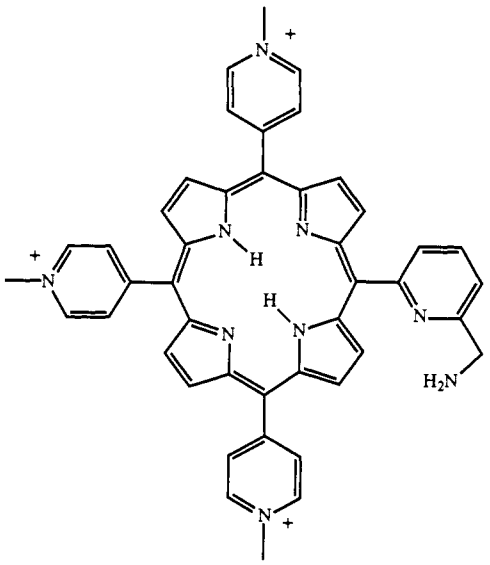
Table I. DNA Cleavage Results[f]

components	% ccc[a]	% oc	% linear	no. single strand breaks (ssb)[b]
pBR32 alone	80	20	0	
Cu(II)	80 (37)[d] [4][e]	20 (67) [96]	0 (0) [0]	0 (0.77) [3.2]
1	62	38	0	0.26
1 + Cu(II)	29 (0) [0]	71 (57) [55]	0 (43) [45]	1.02 (16.6) [17.3]
1 + Zn(II) + Cu(II)[c]	64	36	0	0.22
1 + neocuproine	76	24	0	0.052
1 + EDTA	79	21	0	0.016
1 + catalase	77	23	0	0.041
1 + boiled (Δ) catalase	62	38	0	0.26
1 + Cu(II) + EDTA	70	30	0	0.14
1 + Cu(II) + catalase	74	26	0	0.081
1 + Cu(II) + Δ catalase	27	73	0	1.09
TMPyP + Cu(II)	80 (37)	20 (67)	0 (0)	0 (0.77)
AMP + Cu(II)	80	20	0	0
1 + Cu(II) + SOD	27	73	0	1.09
1 + Cu(II) + NaO_2CH	29	71	0	1.02
1 + Cu(II) + EB	65	35	0	0.21

[a] The reduced stainability of cccDNA was corrected for by using a factor of 1.22.[19] [b] The number of single strand breaks were calculated as previously described.[10,11a,20] [c] Zn(II) was added prior to Cu(II). [d] Results appearing in parentheses refer to experiments carried out in 1 mM DTT for 3 h. [e] Results appearing in brackets refer to experiments carried out in 1 mM DTT/1 mM hydrogen peroxide for 0.5 h. [f] Reaction components were present at the following concentrations: pBR322 (100 μM); **1**, TMPyP and 2-(aminomethyl)pyridine (AMP) (7 μM); metals (48 μM); neocuproine (48 μM); EDTA (2 mM); catalase and superoxide dismutase (SOD) (60 μg/mL); sodium formate (20 mM); and ethidium bromide, EB, (35 μM). Reactions were conducted as described in Figure 2.

We describe here DNA strand scission mediated by a tricationic porphyrin with a metal binding appendage. The 6′-(aminomethyl)pyridylporphyrin **1** (structure **1**) was synthesized via cross



1

condensation of pyrrole, 4-pyridinecarboxaldehyde, and 6-(hydroxymethyl)-2-pyridinecarboxaldehyde followed by subsequent manipulations of the side chain.[8] Spectrophotometric titrations of **1** with various nucleic acids indicated that **1** was bound by intercalation into poly d(G-C)-d(G-C), groove bound to poly d(A-T)-d(A-T) and bound by a combination of these modes to calf thymus DNA. This behavior is analogous to that of TMPyP.[2d,e,j] Upon stepwise addition of poly d(G-C)-d(G-C) to a solution containing **1**, the Soret band (420 nm, $\epsilon_{420} = 2.3 \times 10^5$ M^{-1} cm^{-1}) was shifted to 440 nm with a reduced absorbance ($\epsilon_{440} = 1.3 \times 10^5$ M^{-1} cm^{-1}) indicative of intercalation. On the basis of the results of this titration, the apparent binding constant of **1** for poly d(G-C)-d(G-C) was calculated to be 7.0×10^5 M^{-1} ([Na^+] = 0.2 M, 25 °C), similar to that of TMPyP.[2d,e,j,9]

Porphyrin **1** mediated strand scission as determined by the partial conversion of the covalently closed circular plasmid (cccpBR322) to open circular DNA (ocDNA), a process which requires only one nick (Figure 1, Table I). Inhibition (80–90%) by catalase (60 μg/mL) or EDTA (2 mM) suggests that hydrogen peroxide and adventitious metal ions participated in the cleavage reaction. The presence of 48 μM Cu(II) stimulated DNA strand scission; however, the other metals assayed {Zn(II), Co(II), Ni(II), Hg(II), Pb(II), Mn(II), Fe(III), and Cd(II)} were unable to elicit the same effect. These results and inhibition of cleavage by neocuproine (48 μM) imply that copper was the adventitious metal required for reactivity. The Cu(II)–**1** system produced extensive DNA cleavage (full length linear DNA (LDNA) and smaller linear fragments were observed) in 1 mM DTT and 1 mM DTT/1 mM hydrogen peroxide.[10,11] That hydrogen peroxide was generated in situ from molecular oxygen was demonstrated by the observation that a solution containing Cu(II)–**1** (1 mM DTT), which had been deoxygenated by several freeze–pump–thaw cycles, induced only 10% of the single strand breaks generated by a control solution which had not been deoxygenated.[12]

Evidence from control experiments suggests that the pendant aminomethylpyridyl moiety of **1** was intimately involved in the chemistry. The visible spectrum observed after incubation of **1** with poly d(G-C)-d(G-C), poly d(A-T)-d(A-T), and calf thymus DNA, under reaction conditions analogous to those described in Figure 1, lane 3, was characteristic of that of the free-base **1** rather than a copper porphyrin.[13,14] Furthermore, when Zn(II) (48 μM) had been preequilibrated with DNA-bound **1**, added Cu(II) did not accelerate the cleavage reaction. In addition, solutions con-

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(12) The final concentrations of the reagents were pBR322 (7 μM in base pairs), Cu(II) (3.4 μM), **1** (0.44 μM), and DTT (1 mM).

(13) Free Porphyrin was released to the medium upon increasing the ionic strength to 2 M.

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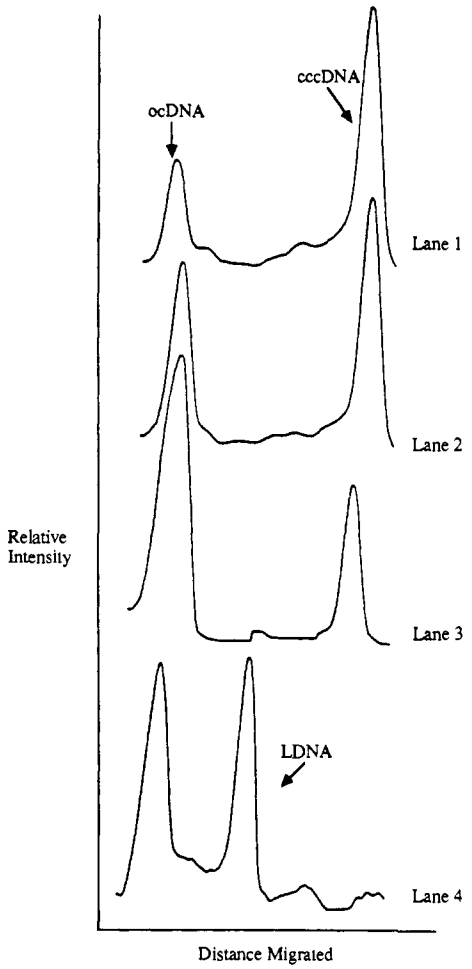

Figure 1. Cleavage of pBR 322 by **1**. All reactions were carried out in 89 mM trizma, 89 mM boric acid buffer (pH = 8.3) in 13 μL total volume at 37 °C in the dark. The concentration of pBR322 was constant at 100 μM in base pairs: lane 1, pBR322 alone, 6 h; lane 2, **1** (7 μM), 6 h; lane 3, **1** (7 μM) and $Cu(OAc)_2$ (48 μM), 6 h; lane 4, **1** (7 μM); $Cu(OAc)_2$ (48 μM), and DTT (1 mM), 3 h. After separation of the DNA by agarose gel electrophoresis, the ethidium bromide stained gel was photographed with a Polaroid MP-4 Land camera equipped with Tiffen (23A) filter and Polaroid Type 665 positive/negative film. The DNA bands were quantitated by densitometric analysis of the negative using a Biorad 620 video densitiometer.

taining hydrogen peroxide (10 μM) and cupric acetate (48 μM) or TMPyP (7 μM), cupric acetate (48 μM) and DTT (1 mM) did not promote DNA cleavage beyond what was observed in the controls, results which confirm the requirement for the aminomethylpyridyl group on **1**. Substantial inhibition (80%) by ethidium bromide (35 μM, 5 equiv based on **1**), a known intercalator, suggests that intercalation of the porphyrin is the binding mode most favorable for the reaction of Cu(II)–**1** with DNA. The inability of the copper complex of 2-(aminomethyl)pyridine to cleave DNA demonstrates that the porphyrin must direct the chemistry of the attached chelator.

With regard to the nature of the cleavage chemistry, the Cu(II)–**1** system may be compared to other known Cu(II) dependent cleaving agents such as bleomycin[15] and 1,10-phenanthroline.[16] Complexed with 1,10-phenanthroline, chelated Cu(I) (generated in situ) is believed to react with hydrogen peroxide (produced via Cu(I) reduction of dioxygen) to form reactive copper species which may then cause site-selective DNA cleavage. The involvement of hydrogen peroxide manifested in the Cu(II)–**1** system suggests a similar redox mechanism.[17,18] Preliminary sequencing studies have indicated that, like copper-bleomycin,[15b] Cu(I)–**1** produced DNA cleavage at discrete sites.[15c] Accordingly, the reactive species responsible for DNA strand scission by **1** is suggested to be a copper species formed upon reaction of hydrogen peroxide with Cu(I)–**1** rather than hydroxyl radical.

These results demonstrate the first example of a DNA cleaving agent which utilizes a porphyrin solely as a DNA recognizing element. With straightforward synthetic modifications, analogues of **1** have been developed which show intriguing variations in cleavage chemistry depending on the nature of the pendant chelator. Clarification of these observations and the possible application of this class of molecules to cancer chemotherapy are currently under investigation.

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Surfactant Structure and Aggregate Morphology. The Urge for Aggregate Stability

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Surfactant aggregation in water is an intricate process. Above a critical concentration, cooperative association sets in and the Gibbs energy of the system is minimized through a compromise of a variety of often opposing forces.[1] These forces depend both on the molecular architecture of the surfactant and on the peculiar solvent properties of water. The urge for optimum aggregate stability is reflected in the rich variety of possible aggregate morphologies, each with its particular mode of alkyl chain packing and headgroup arrangement.[2-6]

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