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DESIGNED DOUBLE-STRAND DNA CLEAVAGE WITH CHELATE-APPENDED PORPHYRINS

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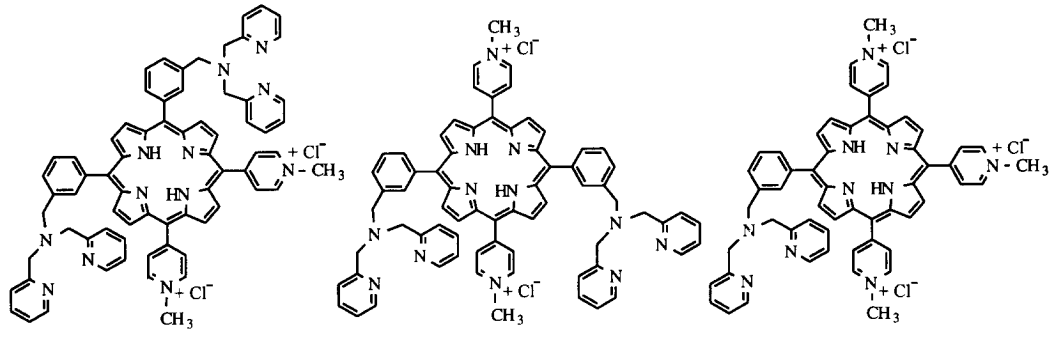
Abstract. The first example of DNA double-strand cleavage mediated by a synthetic porphyrin is described. Two new porphyrins, each with two metal complexing appendages, were shown to mediate the oxidative double-strand cleavage of pBR322 plasmid DNA. By contrast, a porphyrin with one appendage was found to mediate only single-strand breaks.

The sequence-selective cleavage of DNA by the antitumor-antibiotic bleomycin and synthetic analogs continues to be of considerable interest for chemotherapeutic, diagnostic, and analytical applications.[1] Tetra-4-(N-methyl)pyridylporphyrin (TMPyP) is widely known to bind specifically to tumor cells and to bind DNA.[2] Selective intercalation into poly d(GC)-d(GC) and binding in the groove of poly d(AT)-d(AT) have been demonstrated. We have previously shown that derivatives of TMPyP with a metal-binding aminopyridyl appendage would bind to plasmid DNA and induce single-strand breaks under copper-dependent, oxidative conditions.[3a] To improve the efficacy of such compounds, we have sought to incorporate two, high turn-over, iron binding sites[3b,c] on the periphery of the porphyrin core.

We describe here two new porphyrins, (5,10-bis[3-[N,N-bis-(2-pyridylmethyl)aminomethyl] phenyl]-15,20-bis-(1-methyl-4-pyridyl)porphine (**1**) and 5,15-bis-[3-[N,N-bis-(2-pyridylmethyl)aminomethyl]-phenyl]-10,20-bis-(1-methyl-4-pyridyl)porphine (**2**)), each with this lobster claw motif, and show that these porphyrins mediate the efficient double-strand cleavage of pBR322 plasmid DNA. By contrast, a porphyrin with only one metal chelating arm, (5-[3-[N,N-bis-(2-pyridylmethyl)-aminomethyl]phenyl]-10,15,20-tri-(1-methyl-4-pyridyl)-porphine (**3**)), was found to mediate only single strand breaks.



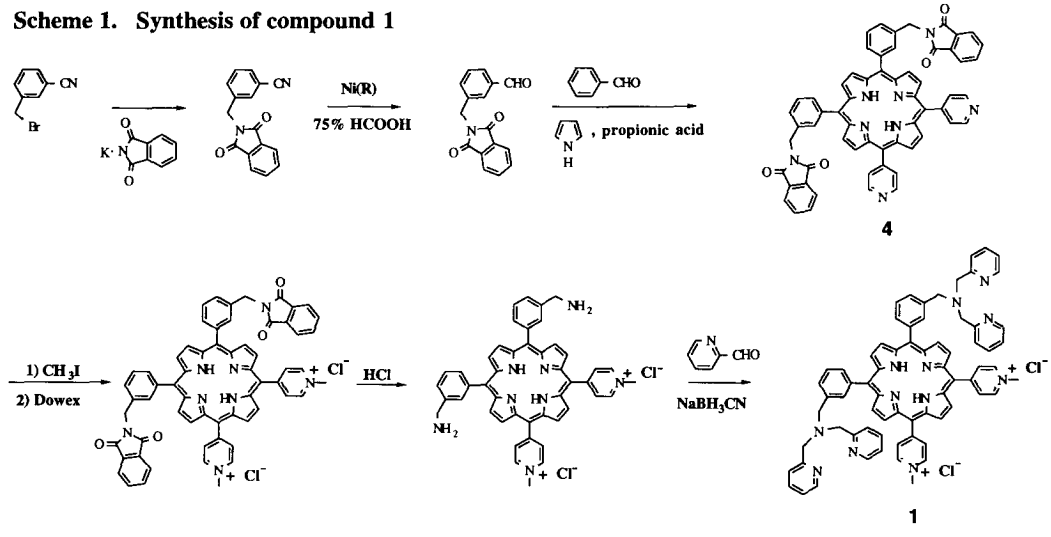
1 **2** **3**

DNA strand cleavage by these porphyrins required Fe^{2+} and oxygen and was stimulated by DTT, indicating that the strand cleavage involved typical oxidation of the DNA. Significantly, **1** and **2** produced double-strand breaks only when more than one equiv of Fe^{2+} was used. Thus, the binding of **1** or **2** to DNA, and the subsequent binding of two equiv of iron, must produce two reactive centers in the ternary DNA-porphyrin-metal complex. These two sites must each react to cut opposite strands of duplex DNA before the porphyrin diffuses from the scissile site.

Synthesis. The syntheses of **1–3** were conducted as shown in Scheme 1. Pyrrole, 3-(phthalimidomethyl)-benzcarboxyaldehyde and 4-pyridinecarboxyaldehyde were gently refluxed in propionic acid. 5,10-Bis-[3-(phthalimidomethyl-phenyl]-15,20-bis-(4-pyridyl)porphine (**4**), (5,15-bis-[3-(phthalimidomethyl)phenyl]-10,20-bis-(4-pyridyl)porphine (**5**) and 5-[3-(phthalimidomethyl)-phenyl]-10,15,20-tris-(4-pyridyl)porphine (**6**) (not shown in Scheme 1) were separated from the reaction mixture by column chromatography (silica gel; $CHCl_3$-MeOH). Compound **4–6** were methylated with CH_3I in CH_2Cl_2.

The methylated compounds were applied to Dowex 1X8-200 in methanol to exchange the counter anion from I^- to Cl^- after which treatment with HCl to afforded the benzylic amines. The metal chelate appendages were assembled by reductive amination with 2-pyridylcarboxyaldehyde to give **1** (81%), **2**, and **3**. Compounds **1–3** were characterized by their UV and NMR spectra.[4]

Scheme 1. Synthesis of compound 1



Binding to DNA. The nature of DNA binding of compounds **1–3** was revealed by observing changes of their UV spectra upon titration with d(GC)-d(GC) or d(AT)-d(AT) co-polymers. Intercalation of TMPyP into poly d(GC) induces a 20 nm red shift and a 40% hypochromism of the Soret band while groove binding into d(AT)-d(AT) causes a 7 nm red shift and a 7% hypochromism.[2] For compound **1**, a 4.4 nm of red shift (14.0% hypochromism) with poly d(GC)-d(GC) and a 3.6 nm red shift (14.5% hypochromism) with poly d(AT)-d(AT) was observed, indicating that **1** was bound in the groove of DNA. For compound **2**, an 18.0 nm red shift (22.2% hypochromism) and a 9.4 nm red shift (14.0 % hypochromism) were observed when it bound to poly

d(GC)-d(GC) or poly d(AT)-d(AT), respectively, showing that **2** displayed intermediate character between intercalation and groove binding to DNA. By contrast, **3**, which has only one appendage behaved similarly to TMPyP.

DNA Cleavage Reactions. DNA cleavage was detected by observing changes in the relative amounts of the three forms of plasmid pBR322 measured from densitometric scans of photographs of 1% agarose gels stained with ethidium bromide. DNA cleavage reactions required Fe^{2+} and the reaction was accelerated by DTT, ascorbic acid, or hydrogen peroxide, similar to results we have described for this ligand attached to a complementary oligonucleotide.[3b,c] The reaction was inhibited by preincubation with EDTA or catalase, demonstrating that iron and hydrogen peroxide were required for these cleavage reactions. Iron was by far the most active metal.

Double-Strand DNA Cleavage by Compound 1. A significant difference was observed in the time course of the appearance of forms II and III plasmid when compounds **1** and **3** were compared under oxidative cleavage conditions. The number of single strand cuts per plasmid[5] over time was calculated for samples taken every 5-10 min over 1 h by densitometric integration. The results of three experiments (a,b,c) are superimposed in Figure 1. In the case of experiment (a) (**1** (1 μM), Fe^{2+} (10 μM), line 1-3), a steady decrease in the relative amount of form I (line 1) was observed, while form II increased quickly, reaching a steady state and then declining. Most significantly, form III could be detected early in the reaction profile, and well before the complete reaction of form I.

Figure 1

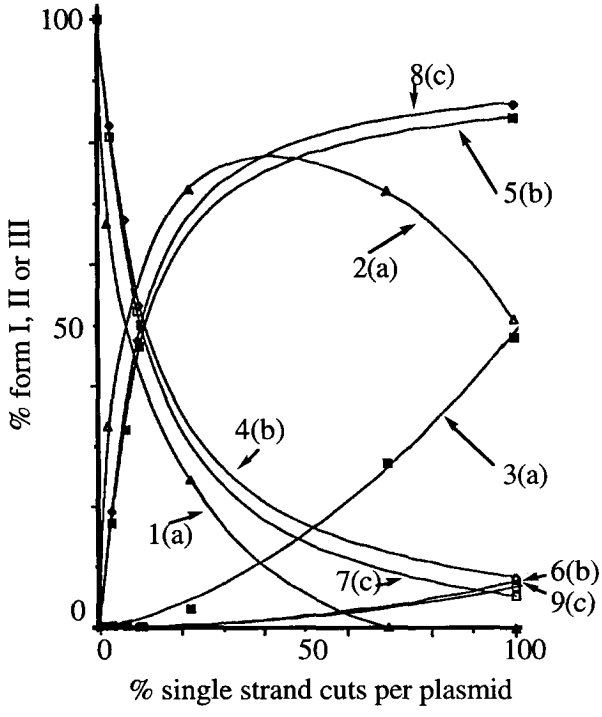


Figure 1. DNA cleavage reactions (a)-(c) were conducted at 25 °C for 1 h. (a) contained **1** (1 μM), Fe^{2+} (10 μM), DTT (100 μM), (b) contained **1** (1 μM), Fe^{2+} (1 μM), DTT (100 μM), exp(c) contained **3** (1 μM), Fe^{2+} (1 μM), DTT (100 μM), all in 10 mM Tris-HCl (pH 7.4). Total volume was 10 mL. Three forms of plasmid DNA were separated by 1% agarose gel electrophoresis. Data presented are for triplicate runs. Lines 1–3, 4–6, and 7–9 are the results obtained under conditions (a), (b), and (c) respectively. Lines 1, 4, and 7 represent the time course for decrease of form I. Lines 2, 5, and 8 represent changes in the amounts of form II, while lines 3, 6, and 9 represent changes in form III. Negligible cleavage occurred under these conditions in the absence of **1** (7-8% after 1 h), cf. Figure 2, scan 6, and reference 3b.

By contrast, in experiment (b) (**1** (1 μM), Fe^{2+} (1 μM), lines 4–6), a decrease in the relative amount of form I (line 4) and an increase of form II (line 5) were observed. Under these conditions, form III (line 6) appeared only after all of form I had reacted as expected for serial, single-strand breaks. Compound **3**, with a single ligating appendage, gave results similar to that of compound **1** with limited iron (experiment (c) (**3** (1 μM), Fe^{2+} (1 μM), line 7-9)). Higher iron concentrations did not affect the results with compound **3**. These results show that double-strand DNA cleavage was effected by **1**-Fe_2 whereas **1**-Fe was a single-strand cutter. Significantly, no scenario of isolated single-strand cuts can lead to the outcome observed here for **1**-Fe_2.

Double-Strand DNA Cleavage by Compound 2. Behavior similar to that observed for compound **1** was also obtained with compound **2** when the reaction was followed over 60 min as described above. Integrated densitometer scans are depicted in Figure 2. Again, form II plasmid was observed to increase quickly and significant amounts of form III appeared before the complete reaction of form I. Thus, as was the case with compound **1**, compound **2** mediated a significant number of double strand breaks.

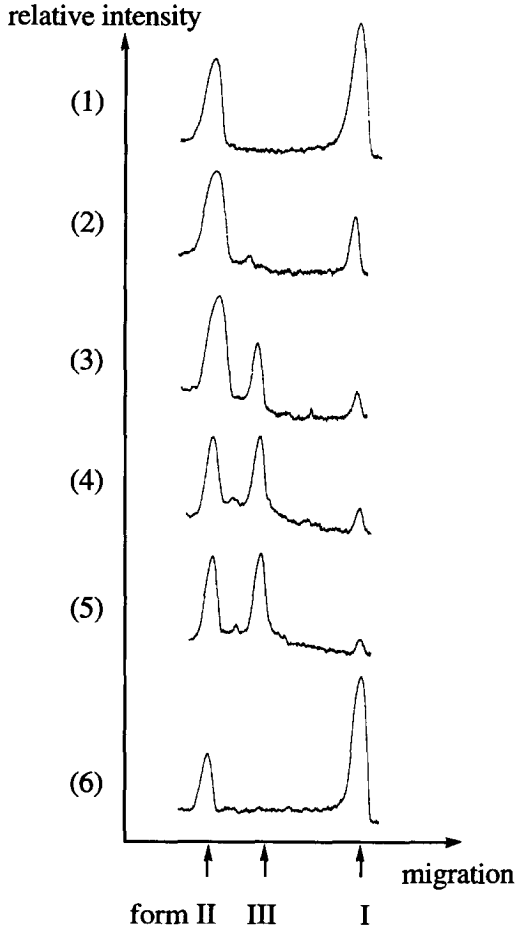


Figure 2. Densitometric determinations of DNA cleavage reactions mediated by porphyrin **2** (1 μM) in the presence of Fe^{2+} (10 μM), DTT (100 μM) in Tris-HCl buffer (pH 7.4) at 37 °C. Reactions were quenched after (1) 5 min; (2) 10 min; (3) 20 min; (4) 40 min; and (5) 60 min. Scan (6) is a control reaction after 20 min in the absence of **2**. Negligible cleavage (<5% form II) occurred under these conditions in the presence of Fe^{2+} (10 μM), DTT (100 μM), and the ligand, N-benzyl-N,N-di-{(2-pyridyl) methyl amine (10 μM), cf. reference 3b. The horizontal axis is electrophoretic migration on 1% agarose; the vertical axis is densitometric intensity.

Comparison between 1 and Bleomycin in the DNA Cutting Efficiency. As can be seen in Table 1, the strand cutting activity of **1**-Fe_2 was found to be comparable to that observed under similar conditions with bleomycin. Bleomycin is notable both for its high cutting efficiency and for inducing double-strand breaks.[1g,6] Thus, at 2 μM **2**, 10 μM Fe^{2+} and no added DTT, compound **1** showed significant cleavage as compared to a control. Both compound **1** and bleomycin showed a large stimulation of activity with DTT.

Table 1. DNA Cleavage by Compound 1 and Bleomycin

	conc (μM)	DTT (μM)	form I	form II	form III
Compound **1**	1.0	0	95.3	4.7	0.0
	2.0	0	83.3	16.7	0.0
Bleomycin	0.5	0	91.1	8.9	0.0
	1.0	0	45.3	47.6	7.1
Compound **1**	1.0	100	2.1	84.0	13.9
	2.0	100	0.0	76.6	23.4
Bleomycin	0.5	100	17.6	53.5	28.9
	1.0	100	0.0	28.3	71.7
Control	0	0	94.8	5.2	0

DNA 80 μM (bp), $FeSO_4$: 10 μM (included in every experiment except control), bleomycin: Blenoxane, buffer: 10 mM Tris-HCl (pH 7.5), reaction: 22 °C, 1 h.

Several interesting features of the DNA binding and cutting by compounds **1–3** can be discerned from the data. First, the mobility of the porphyrin derivatives from site to site along the DNA must be slower or of the same order as the progress of strand nicking (minutes). Otherwise, only single-strand cuts would have been observed. Secondly, the single-armed derivative **3** apparently cannot make a second cut on the opposite strand efficiently, even though such a metal complex could be catalytic. Accordingly, these porphyrins must be relatively fixed at their initial reaction site. If one assumes random binding along the DNA, the cleavage pattern should be a typical Poison distribution. Thus, at low concentrations of **1** on the plasmid, one can assume that form II of the plasmid derived only from single strand cuts (**S**) while form III arose from double-strand cuts (**D**).[7] With a molar ratio of **1**:DNA of 80, the ratio **S/D** could be estimated to be 23, or 4% double-strand cuts. The theoretical ratio of **S/D** should be 129 from the equation of Freifelder and Trumbo.[8] So the double-strand cleavage observed was five times greater than expected for random, independent single cuts within 16 base pairs.

This is the first observation of double-strand DNA cleavage mediated by a designed porphyrin derivative. Compounds with this property may produce DNA lesions that are difficult to repair, affording a new strategy for the design of more effective anti-cancer drugs.

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4. Spectral data for compounds **1**, **2**, and **3**.

1: NMR (DMSO-d_6) δ: -3.01 (2H, s, NH), 3.97 (8H, s, $4CH_2$), 4,71 (6H, s, $2CH_3$), 7.20 (4H, d-d, arom.), 7.62 (4H, d, arom.), 7.64 (4H, d-d, arom.), 8.42 (4H, d, arom.), 7.83 (2H, d, arom.), 8.08 (2H, d, arom.), 8.22 (2H, s, arom.), 8.40 (4H, d, arom.), 8.42 (4H, d, arom.), 8.84 (4H, d, arom,), 8.88 (4H. d, arom.), 9.48 (4H, d, arom.), Vis (Tris-HCl, pH 7.4) 420 (1.4×10^5), 518 (1.3×10^4), 555 (7.0×10^3), 590 (5.0×10^3).

2: NMR (DMSO-d_6) δ: -2.99 (2H, s, NH), 3.94 (8H, s, $4CH_2$), 3.98 (4H, s, $2CH_2$), 4.73 (6H, s, $2CH_3$), 7.23 (4H, d-d, arom.), 7.61 (4H, d, arom.), 7.70 (4H, d-d, arom.), 7.80 (2H, d-d, arom. J = 7, J = 7), 7.90 (2H, d, arom. J=7), 8.09 (2H, d, arom. J=7), 8.22 (2H, s, arom.) 8.46 (4H, d, arom. J = 4), 8.90 (4H, d, arom. J = 5), 8.96 (4H, d, arom. J = 5), (9.00 (4H, d, arom. J = 5), 9.55 (4H, d, arom. J = 4), 9.55 (4H, d, arom. J = 4), Vis (10 mM Tris-HCl, pH 7.4) 419 nm (2.3×10^5), 518 (1.3×10^4), 558 (6.9×10^3), 585 (6.1×10^3).

3: NMR (DMSO-d_6) δ: -3.04 (2H, s, NH), 3.94 (4H, s, CH_2), 3.96 (2H, s, CH_2), (9H, s, $3CH_3$), 7.25 (2H, d-d, arom.), 7.64 (2H, d, CH_2), 7.72 (2H, d, CH_2), 7.72 (2H, d-d, arom.), 7.82 (1H, d-d, arom.), 7.93 (1H, d, arom.), 8.10 (1H, d, arom.), 8.23 (1H, s, arom.), 8.47 (2H, d, arom.), 8.8-9.2 (14H, m, arom.), 9.4-9.6 (6H, m, arom.), Vis (10 mM Tris-HCl, pH 7.4), 420 nm (2.0×10^5), 518 (1.2×10^4), 554 (6.3×10^3), 581 (5.8×10^3).

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