Matrix,[14] but calculations at the UHF/6-31G* level do not reveal a stable gas-phase structure for this species. The short C_2–H distance in **3** suggests that the 1,3-shift should be better seen as two consecutive 1,2-shifts. Although 1,2-hydrogen shifts barriers are close to the dissociation limit in most radical systems,[15-17] the involvement of the double bond in this case facilitates the migration.

The above results not only suggest that suprafacial 1,3-hydrogen shifts in olefin radical cations should be facile processes but also hint at a general willingness of radical cations to undergo sigmatropic rearrangements that would be difficult in the neutral parent molecules.

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Registry No. 1, 34504-10-4.

Supplementary Material Available: Archive entries for MP2/6-31G*//6-31G* calculations on **1–4** (2 pages). Ordering information is given on any current masthead page.

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Metalloregulation in the Sequence Specific Binding of Synthetic Molecules to DNA

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Specific metal cations exert remarkable effects on the transcription of several prokaryotic and eukaryotic genes.[1] Recently, the DNA-binding eukaryotic transcription factor TFIIIA has been characterized as a series of peptide fingers connected by zinc-binding domains.[1a] These observations raise the issue of whether the binding of synthetic molecules to DNA could in some way be regulated by the addition of specific metal ions.[2]

Bis(netropsin)-3,6,9,12,15-pentaoxaheptadecanediamide (Figure 1)[3] was synthesized to test the possibility of *metalloregulation* in the sequence specific binding of a small molecule to DNA. **1** is a dimer of netropsin analogues[4] connected by a tetraethylene glycol tether, a multidentate acyclic neutral ligand (podand) for metal cations. The tether's terminal glycolamide groups provide additional oxygen donors and might allow **1** to wrap up as a pseudomacrocycle which would mimic the hole size of 18-crown-6.[5] For simultaneous binding of the dipeptide subunits,[6] one might anticipate that the energetic benefit of filling the pseudomacrocycle with a specific metal cation would be important. Attachment of EDTA to one terminus of the molecule allows use of the affinity cleaving method[7] to determine the DNA-binding efficiency, sequence specificity, and binding site size of **1**·Fe(II), in the presence and absence of various alkali, alkaline earth and transition-metal cations.[8,9]

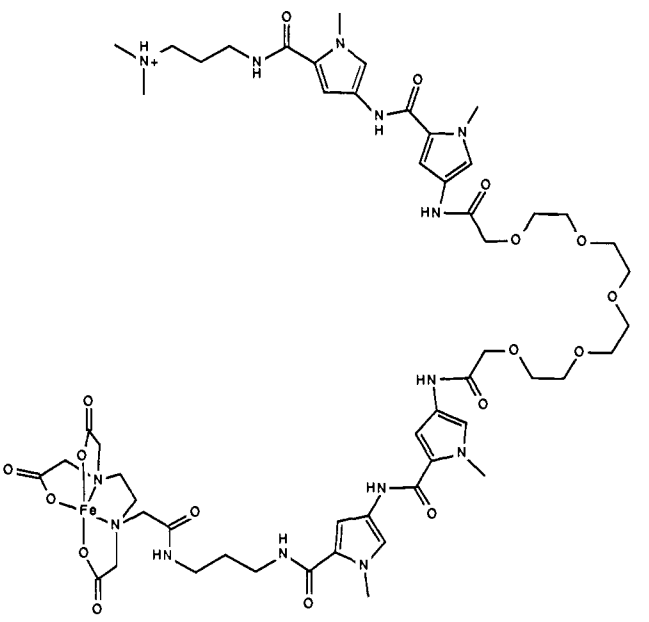

Figure 1. Bis(netropsin)-3,6,9,12,15-pentaoxaheptadecanediamide-EDTA·Fe (**1**·Fe).

A 517 base pair restriction fragment (Eco RI/Rsa I) from plasmid pBR322 DNA, labeled with ^{32}P on the 5′ end, was incubated with **1**·Fe(II) (20 μM) in the presence of various cations (1 mM)[8] for 2 h at 37 °C (pH 7.6). Dithiothreitol (1 mM) was added to initiate the cleavage reactions which were allowed to proceed for 1.5 h at 37 °C. The DNA cleavage products were separated on a DNA sequencing gel and visualized by autoradiography (Figure 2). **1**·Fe(II) (20 μM) in the absence of metal cation or in the presence of 1 mM concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, Ag^+, Ni^{2+}, Cd^{2+}, or Hg^{2+} produces little DNA cleavage.[8,9] However, addition of Sr^{2+} or Ba^{2+} results in strong

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(8) The cations chosen have appreciable affinity for 18-crown-6 in water. Log K (H_2O) values (M^{-1}) are Cd^{2+} (5.4); Ba^2 (3.9); Sr^{2+} (2.8); Hg (2.4); K^+ (2.1); Ag^+ (1.6); NH_4^+ (1.2); Na^+ (0.8); Ca^{2+} (0.5). Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, D. *Chem. Rev.* **1985**, *85*, 271–339.
(9) In a formal sense, metalloregulation of the sequence specific cleavage can be due to metal-ion specific *inhibition* of the cleavage reaction (by competitive binding to the EDTA moiety) or binding event (by competitive binding to the DNA) rather than metal-ion specific *enhancement* of the sequence-specific binding perhaps due to a conformational change in ligand **1**. To avoid the complication of metal-ion-specific inhibition, *controls* show that the cations (1 mM) used here do *not* diminish the sequence specific DNA cleaving efficiency of distamycin-EDTA·Fe(II).[7]

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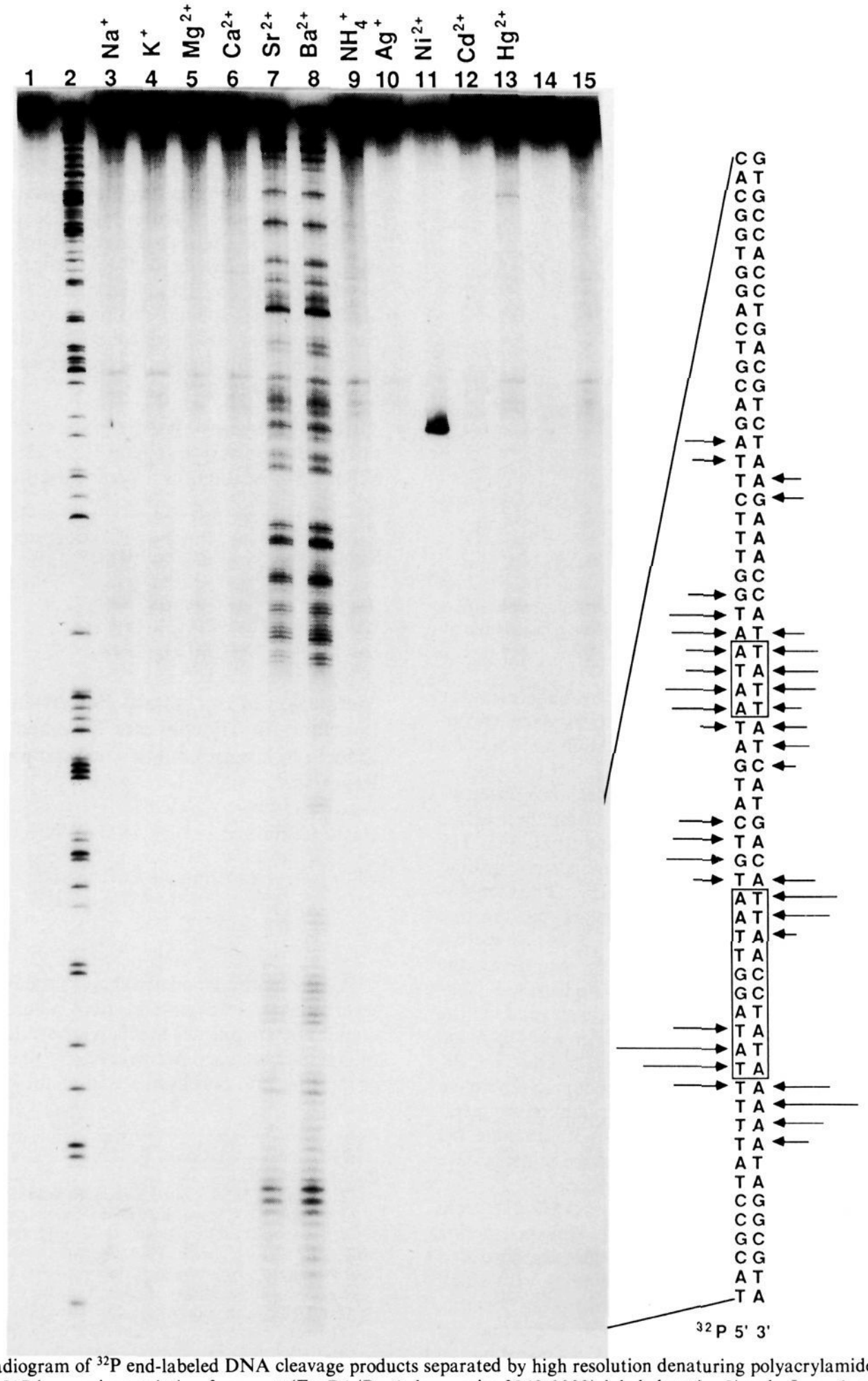

Figure 2. (left) Autoradiogram of ^{32}P end-labeled DNA cleavage products separated by high resolution denaturing polyacrylamide gel electrophoresis. DNA substrate was a 517 base pair restriction fragment (EcoR1/Rsa1, base pairs 3848-0002) labeled at the 5′ end. Lane 1, control, intact DNA; lane 2, Maxam–Gilbert chemical sequencing G reaction; lanes 3–13, 15 **1**·Fe(II) at 20 μM with the indicated cation (NaOAc, KOAc, $Mg(OAc)_2$, $Ca(OAc)_2$, $Sr(NO_3)_2$, $Ba(OAc)_2$, NH_4OAc, AgOAc, $Ni(OAc)_2$, $Cd(OAc)_2$, $Hg(OAc)_2$) at 1 mM; lane 14, control, Ba^{2+} at 1 mM without **1**·Fe(II); lane 15, control, **1**·Fe(II) in the absence of added cation. (right) Sequence from bottom half of the sequencing gel. Arrows indicate positions of major cleavage. Boxes are binding locations based on affinity cleaving model[7] and footprinting controls on **1**·In^{3+}.[10]

specific patterns of DNA cleavage. Densitometric analysis of the cleavage patterns in the lower half of the sequencing gel reveals two well-resolved binding sites, 5′-TATAGGTTAA-3′ and 5′-AATA-3′[10,11] (Figure 2).

The dependence of DNA cleavage efficiency by **1**·Fe(II) upon Ba^{2+} concentration was examined. **1**·Fe(II) produces sequence-specific cleavage in the presence of stoichiometric concentrations of Ba^{2+} (20 μM) which increases with Ba^{2+} concentration up to 10 mM. At higher concentrations of Ba^{2+} the amount of cleavage decreases. A plot of the relative amounts of cleavage produced at the 5′-TATAGGTTAA-3′ site by **1**·Fe(II) vs. Ba^{2+} concen-

(10) Assignment of the binding sites was made on the basis of the cleavage patterns from the reaction of **1**·Fe(II) with DNA labeled with ^{32}P at the 5′ (Figure 2) and 3′ (data not shown) ends and interpreted according to the affinity cleaving model described in ref 7. The assignment of binding sites was confirmed independently by MPE·Fe(II) footprinting[7b] (cleavage inhibition at those sites) with **1**·In(III) which is competent to bind but not cleave DNA.

(11) Incubation of **1**·Fe(II) with DNA in the absence of any added cation gives very weak cleavage (by a factor of 10 less) flanking sites of pure A,T DNA and not identical with the sites seen in the presence of Ba^{2+} and Sr^{2+}.

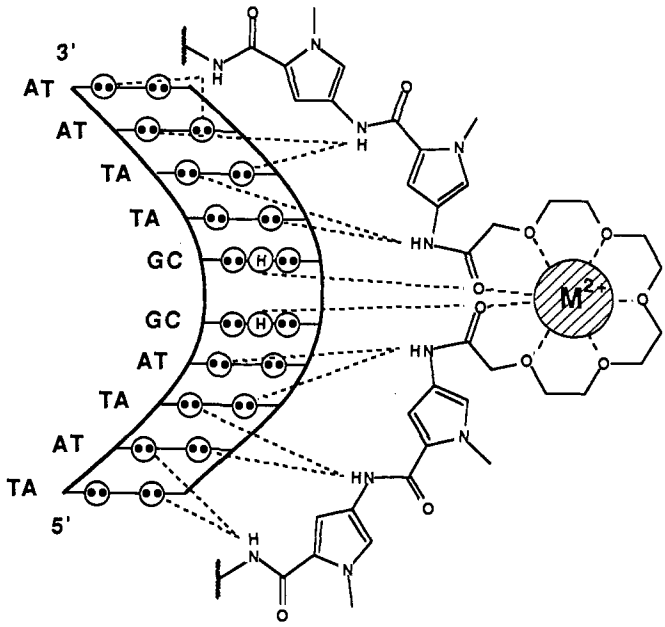

Figure 3. Hypothetical model for binding of **1** in the presence of a specific metal ion (M = Ba^{2+} or Sr^{2+}) to a ten base pair sequence 5'-TATAGGTTAA-3'.

tration indicates saturation binding. The decrease in cleavage efficiency above 10 mM Ba^{2+} may be due to displacement of **1**·Fe(II) from the DNA polyanion by the dication and/or competition for the EDTA moiety by Ba^{2+}.

For recognition of DNA by *N*-methylpyrrolecarboxamide we would expect that each dipeptide subunit of **1**, having three amide NHs, should bind to four contiguous A,T base pairs.[12,13] The observed binding site, 5'-AATA-3' reveals that monomeric binding can be facilitated by the presence of Ba^{2+} or Sr^{2+}. The fact that monomeric binding is metalloregulated indicates that the free ligand **1** may be in a conformational state that is less competent to bind unless a specific metal ion is present. Moreover, the observed ten base pair binding site, 5'-TATAGGTTAA-3', suggests that the dipeptides of **1**·Fe^{II} bind in a *dimeric mode* in the presence of Sr^{2+} or Ba^{2+} and produce an *additional* DNA-ion binding subunit specific for the central sequence, 5'-GG-3'. CPK models indicate that for a heptacoordinate pseudomacrocycle the lone pair electrons on the carboxamide oxygens anti to the metal coordination site may form hydrogen bonds with guanine N2 amino groups which protrude from the floor of the minor groove in right-handed double helical DNA[14] (Figure 3).

In conclusion, addition of Sr^{2+} or Ba^{2+} converts **1**·Fe(II) from a species which produces little DNA cleavage to a sequence specific DNA binding/cleaving molecule. We have thus demonstrated metalloregulation in the sequence specific binding of a small synthetic molecule to DNA. The effect is metal-ion specific, occurring with the heavier alkaline earth cations. We cannot yet dissect the relative contributions of cation size, coordination number and "hardness" to the metallospecificity.[15] Ba^{2+} and Sr^{2+} are known to bind more strongly to 18-crown-6 in water than either the alkali metals or the lighter alkaline earth metals.[8] Ba^{2+} is more effective than Sr^{2+} in producing sequence specific cleavage which is consistent with the greater affinity of Ba^{2+} for 18-crown-6 in water.[8] Why Ba^{2+} and Sr^{2+} are more effective than Cd^{2+} is not understood but may be due to the "harder" cations having higher affinity for the phosphate backbone of DNA.[16] Perhaps bound **1** in the minor groove of DNA creates a "cavity" for Sr^{2+} or Ba^{2+}, consisting of a neutral heptaoxamacrocycle capped on top and bottom by the phosphate oxygen anions on the neighboring DNA backbone. The observation of specific metal-ion dependent binding may be interpreted in terms of allosteric models in which complexation of Sr^{2+} or Ba^{2+} by **1** induces a change which allows monomeric binding and, at least at one DNA site, simultaneous binding of two subunits to form a crescent-shaped molecule complementary to the minor groove of DNA with dipeptides specific for $(A,T)_4$ flanking a podand/cation complex specific for $(G,C)_2$ (Figure 3).

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Metalation of Surfactant Porphyrins at Anionic Interfaces in Micelles and Reversed Micelles: Dramatic Effects of Chain Length and Atropisomer Structure on Reactivity

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Porphyrin metalation in solution has been well-studied; however, several mechanistic questions remain unresolved.[1-12] Metalation in microheterogeneous media has been less investigated,[13-20] although its biological occurrence,[21-23] the amphiphilic character of most natural porphyrins, and metal ion-porphyrin solubility

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