on the olation reaction. The two H_3O_2 bridges in this dimer hold the two metal atoms in a mutual orientation suitable for the olation reaction. This reaction may proceed by a water molecule elimination from each of the two H_3O_2 bridges, leaving two μ-hydroxo bridges between the two metal atoms. This mechanism may also operate in the olation of aquo ions such as $Cr(H_2O)_5OH^{2+}$ in which the "Chromic Dimer"[7] $[(H_2O)_4Cr(OH)_2Cr(H_2O)_4]^{4+}$ is formed. In some cases, the formation of these species may be followed by the rupture of one bridge to form a singly bridged species[8] as in eq 3.

$$[Cr(OH)_2Cr]^{4+} + H^+ \rightarrow [Cr(OH)Cr]^{5+} + H_2O \quad (3)$$

Work is in progress on the isolation of other crystalline compounds with μ-H_3O_2 bridges. The existence of bridged species of these complex ions in solution will be investigated.

Acknowledgment. A.B. is a "Bat-Sheva" fellow. We are grateful to the Bat-Sheva de Rothschild Foundation for financial support.

Registry No. $[Cr(bpy)_2(H_2O)_2]^{3+}$, 36513-26-5; $[(bpy)_2Cr(H_3O_2)_2Cr(bpy)_2]I_4 \cdot 2H_2O$, 87764-12-3.

Supplementary Material Available: Tables of atomic positional and thermal parameters (2 pages). Ordering information is given on any current masthead page.

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Sequence-Specific Double-Strand Cleavage of DNA by Bis(EDTA-distamycin·Fe^{II}) and EDTA-Bis(distamycin)·Fe^{II}

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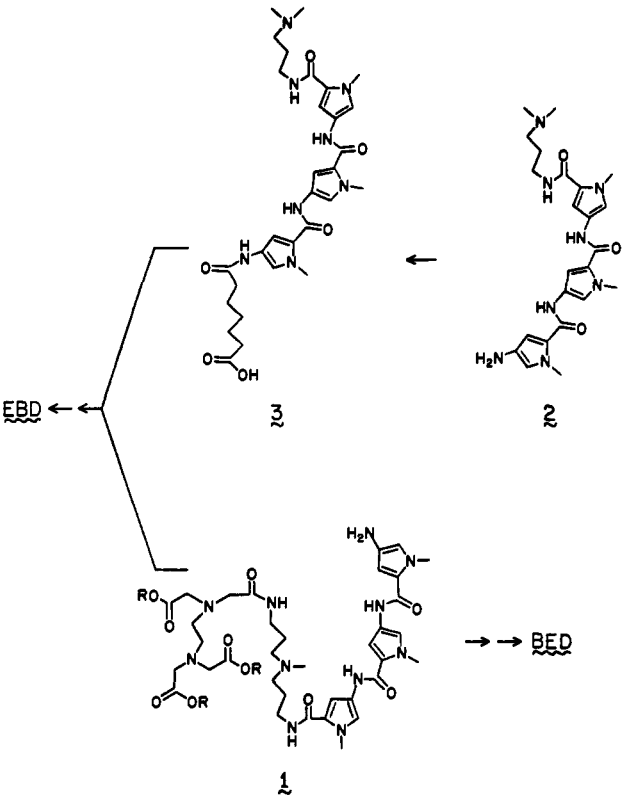
Restriction enzymes (type II) cleave double-helical DNA on opposite strands at or close to a defined recognition site four-six base pairs in size.[3] The ability of these enzymes to cleave DNA into unique fragments is useful for DNA sequencing, chromosome analyses, gene isolation, and recombinant DNA manipulations. Attachment of EDTA·Fe^{II} to a DNA binding molecule creates a DNA cleaving molecule.[4] Distamycin-EDTA·Fe^{II} (DE·Fe^{II}) and EDTA-distamycin·Fe^{II} (ED·Fe^{II}), which contain EDTA tethered to the amino or carboxy terminus of an *N*-methylpyrrole tripeptide DNA binding unit, single strand cleave DNA adjacent to five base pair A + T recognition sites.[5] The pentapeptide penta-*N*-methylpyrrolecarboxamide-EDTA·Fe^{II} (P5E·Fe^{II}) achieves double-strand cleavage of DNA adjacent to a six-seven base pair A + T recognition site in a catalytic reaction.[6] One general approach for designing double-strand DNA-cleaving molecules with defined target sequences and binding-site sizes would be to couple sequence-specific DNA binding molecules of similar (or diverse) base pair specificities and attach one (or more) DNA cleaving moieties such as EDTA·Fe^{II}.

We report the synthesis of two sequence-specific double-strand DNA cleaving molecules, bis(EDTA-distamycin·Fe^{II}) (BED·Fe^{II}) and EDTA-bis(distamycin)·Fe^{II} (EBD·Fe^{II}) (Chart I). These molecules contain two *N*-methylpyrrole tripeptide units[7] coupled at the amino termini via a flexible tether with EDTA attached to one or both carboxy termini. In the presence of O_2 and dithiothreitol (DTT), *nanomolar* concentrations of BED·Fe^{II} and EBD·Fe^{II} cleave DNA (25 °C, pH 7.9). BED·Fe^{II} and EBD·Fe^{II} cleave pBR 322 plasmid DNA (4362 base pairs) on opposite strands to afford *discrete* DNA fragments. High-resolution gel electrophoresis of an end-labeled restriction fragment containing a major binding site reveals cleavage contiguous to an *eight* base pair sequence 5′-TTTTTATA-3′.

Reaction of 2 equiv of amine **1** with the di-*N*-hydroxysuccinimide ester of heptanedioic acid followed by hydrolysis (0.25 M, LiOH), acidification, and chromatography (silica gel, NH_3/MeOH) afforded BED. Condensation of diamine **2** with an excess of the monoimidazolide of heptanedioic acid afforded the amino acid **3**. Reaction of the imidazolide of **3** with amine **1** followed by hydrolysis, acidification, and chromatography (silica gel, NH_3/MeOH) afforded EBD. BED and EBD were rendered



metal free by supporting each on Amberlite XAD-2 and washing with 5% aqueous Na_2EDTA and deionized water and eluting with methanol.[8]

The DNA cleavage efficiency of BED·Fe^{II} and EBD·Fe^{II} was followed by monitoring the conversion of supercoiled pBR 322 plasmid DNA (form I) to open circular (form II) and linear forms (form III).[9,10] One single-strand scission converts form I to form

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(10) The correction factor for the decreased stainability of form I pBR-322 DNA is 1.22.[4]

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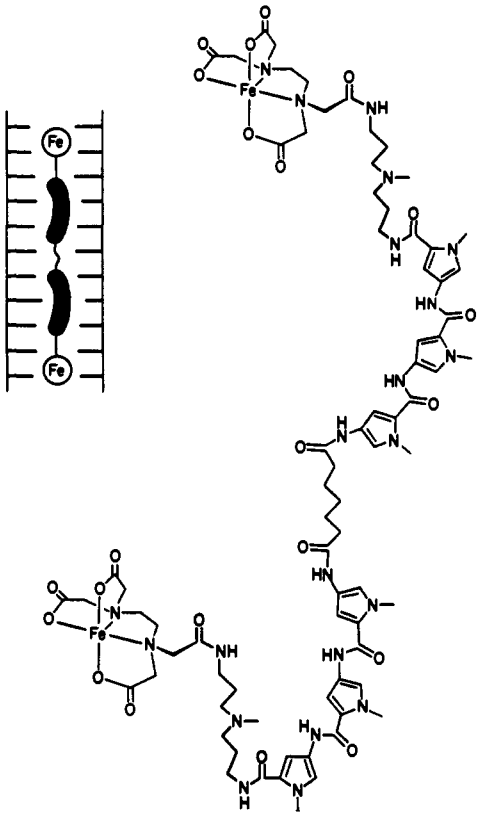
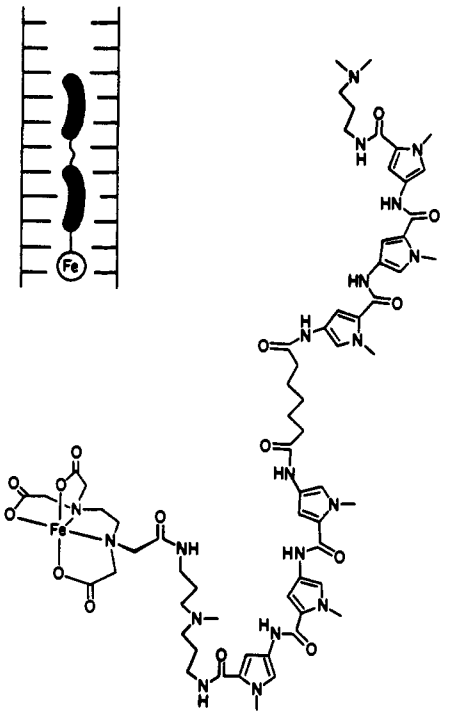
Chart I



BED • Fe (II)



EBD • Fe (II)

II. In the presence of O_2 and DTT, nanomolar concentrations of BED·Fe^{II} and EBD·Fe^{II} cleave DNA (10 μM base pair), almost 3 orders of magnitude lower concentration than required for efficient ED·Fe^{II} cleavage (Table I). BED·Fe^{II} affords approximately twice as much form III linear DNA as EBD·Fe^{II} suggesting that at some binding sites BED·Fe^{II} may cleave both DNA strands in a single binding event.

Sequence-specific double-strand cleavage of DNA by BED·Fe^{II} and EBD·Fe^{II} was examined on linear pBR 322 plasmid DNA (4362 bp).[11] BED·Fe^{II} or EBD·Fe^{II} (0.25 or 0.06 μM) was equilibrated with linear pBR 322 DNA (50 μM bp) for 30 min (37 °C) followed by addition of DTT (5 mM). After 1 h, the reaction was quenched and analyzed by agarose gel electrophoresis (Figure 1). Both BED·Fe^{II} and EBD·Fe^{II} (0.06 μM) cleave linear pBR 322 into discrete fragments. Restriction mapping indicates major cleavage sites at approximately 3.3 and 4.2 kbases,[12] regions of pBR 322 with high poly(dA)·poly(dT) content. Cleavage specificity diminishes at higher concentrations of BED·Fe^{II} and EBD·Fe^{II}, presumably due to cleavage at sites of lower affinity.

The sequence and size of BED·Fe^{II}/EBD·Fe^{II} recognition sites can be resolved by analysis of DNA cleavage patterns using ^{32}P end-labeled restriction fragments and high-resolution denaturing polyacrylamide gel electrophoresis.[13,14] A 517 base pair *Rsa I*/*Eco RI* restriction fragment (3848–4362 bp) from pBR 322[15,16] was labeled separately with ^{32}P (*Eco RI* site) on the 5′ and 3′ ends.[5b,6] The resulting DNA fragments were allowed to react with BED·Fe^{II} or EBD·Fe^{II} at dimer/bp ratios of 0.01 in the presence of DTT (1 mM) for 1 h[17] and analyzed by gel electrophoresis. A histogram of the DNA cleavage patterns obtained from densitometric analysis of the autoradiograms reveals a major cleavage site contiguous to the eight base pair sequence 5′-TTTTTATA-3′

Table I. Cleavage of pBR 322 Plasmid[a]

reagent	concn, μM	form, % I	II	III
ED·Fe^{II}	10.0	0	80	20
ED·Fe^{II}	1.0	10	85	5
EBD·Fe^{II}	0.10	0	66	34
EBD·Fe^{II}	0.010	0	82	18
EBD·Fe^{II}	0.001	43	48	9
BED·Fe^{II}	0.10	0	17	53 (30)[b]
BED·Fe^{II}	0.010	9	55	36
BED·Fe^{II}	0.001	48	42	10

[a] Form I pBR 322 (10 μM bp), reagent, buffer (40 mM Tris base, 5 mM NaOAc, pH 7.9), and DTT (5 mM) were allowed to react at 25 °C for 1 h and quenched. In all cases reactions were carried to completion. Forms I, II, and III were analyzed by agarose gel electrophoresis and quantitated by densitometry after ethidium bromide staining. [b] Lower molecular weight linear DNA.

(11) Linear pBR-322 was obtained by digestion of supercoiled pBR 322 with *Eco RI* and *Sal I* restriction enzymes, respectively.

(12) The cleavage sites were located by initially linearizing pBR-322 with *Eco RI*, *Sal I*, *Nde I*, *Ava I*, *Eco RI* + *Sal I*, *Hind II* + *Ava I*, *Taq I*, or *Rsa I* restriction enzymes, followed by BED·Fe^{II} or EBD·Fe^{II} cleavage. Changes in the lengths of the resulting DNA fragments could be correlated with cleavage sites.

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(17) The cleavage reactions were run with >600 cpm of ^{32}P end-labeled restriction fragments made up to a total DNA concentration of 100 μM (bp) with sonicated calf thymus DNA. The reactions were run at 25 °C for 1 h and terminated by freezing followed by lyophilization and suspension in 4 μL of a pH 8.3 100 mM Tris–borate, 50% formamide solution. These solutions were heat denatured and loaded on a 0.4 mm thick, 40 cm long, 8% polyacrylamide, 1:20 cross-linked, 50% urea gel and electrophoresed at 1500 V. Autoradiography of the gels was carried out at −50 °C on Kodak, X-Omat AR film, and the autoradiograms scanned at 485 nm. The relative peak area for each site was equated to the relative cleavage efficiency.

(18) The DNA cleavage products are consistent with oxidative cleavage of the deoxyribose ring affording a 5′-phosphate DNA terminus and approximately equal proportions of 3′-phosphate and 3′-phosphoglycolic acid termini.[19]

(19) Hertzberg, R.; Dervan, P. B., unpublished results.

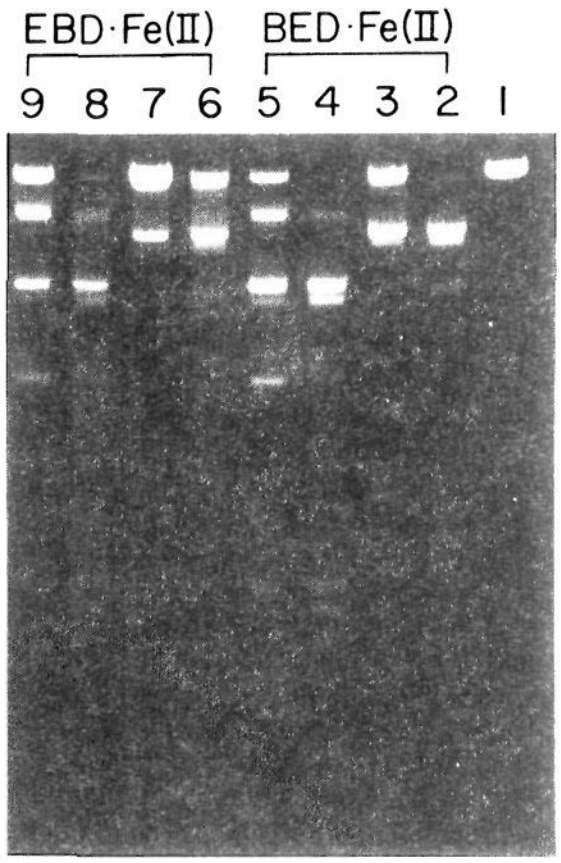

Figure 1. 1% agarose gel/ethidium bromide staining. Lane 1, intact linear pBR-322 DNA (50 μM in base pairs). Lanes 2, 3, 6, and 7 are linearized by *Eco RI* digestion. Lanes 4, 5, 8, and 9 are linearized by *Sal I* digestion. *Lanes* 2 and 4 BED·Fe^{II} at 0.25 μM; lanes 3 and 5 BED·Fe^{II} at 0.06 μM; lanes 6 and 8 EBD·Fe^{II} at 0.25 μM; lanes 7 and 9 EBD·Fe^{II} at 0.06 μM.

Figure 2. Histogram of BED·Fe^{II} and EBD·Fe^{II} cleavage patterns from high-resolution denaturing gels. BED·Fe^{II} (1.0 μM) cleavage reactions on 5′ and 3′ end-labeled 517 bp DNA fragment (100 μM bp). EBD·Fe^{II} (1.0 μM) cleavage reactions on 5′ and 3′ end-labeled 517 bp DNA fragment (100 μM bp). Arrows represent amount of cleavage resulting in removal of indicated base. Solid boxes are the binding site. Assignment is based on the model described in ref 5b,c.

and a minor site contiguous to the five base pair sequence 5′-AATAA-3′ (Figure 2).

The multiple asymmetric cleavage patterns on opposite DNA strands presumably result from a diffusible oxidizing species, such as hydroxyl radical, generated in the minor groove of a right-handed DNA double helix.[5b,c,6,18] Like the tripeptides DE and ED, the dimer EBD can apparently assume two orientations on the DNA.[5,6] The eight base pair (5′-TTTTTATA-3′) and the five base pair (5′-AATAA-3′) binding sites suggest that the hydrocarbon tether allows both dimeric *and* monomeric binding modes. Changes in linker length and/or flexibility might lead to exclusive dimeric binding.

In conclusion, we have found that dimerization of EDTA–distamycin results in molecules capable of double-strand cleavage of DNA (25 °C, pH 7.9) at the eight base pair A + T recognition level and that function effectively at nanomolar concentrations. This work illustrates one approach for the design of double-strand DNA cleaving molecules with defined target sequences and binding site sizes.

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Registry No. 1, 87802-66-2; **2**, 78486-18-7; **3**, 87802-67-3; BED, 87802-68-4; BED·Fe(II), 87802-64-0; EBD, 87802-70-8; EBD·Fe(II), 87802-65-1; heptanedioic acid di-*N*-hydroxysuccinimide ester, 74648-14-9; heptanedioic acid monoimidazolide ester, 87802-69-5; Fe, 7439-89-6.

Supplementary Material Available: Autoradiograms used for results in Figure 2 (1 page). Ordering information is given on any current masthead page.

Syntheses and X-ray Crystal Structures of Two New Transition-Metal Complexes Having Diphosphene Ligands in Novel Bonding Modes†

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A number of recent publications have shown that diphosphenes, RP=PR, can bind in a variety of ways to transition metals. For example, the structures of four complexes in which the diphosphene behaves as a side-on ligand have been published.[1] More recently, the use of large substituents at phosphorus has permitted the isolation of the first end-on bonded complex [*trans*-{[Fe$(CO)_4]_2$[$PCH(SiMe_3)_2]_2$}] (**1**), where each phosphorus atom behaves as a simple two-electron donor.[2]

We now report the syntheses and X-ray crystal structures of two new complexes having diphosphene ligands in previously unobserved bonding modes.[3] The compounds are [$Cr(CO)_5$-$[PCH(SiMe_3)_2]_2$] (**2**), in which only one of the phosphorus atoms behaves as a two-electron donor to a chromium atom, and [Fe$(CO)_4$[μ-$Fe(CO)_4$][P(2,4,6-*t*-$Bu_3C_6H_2O$)$]_2$] (**3**), where the diphosphene behaves both as a side-on and end-on bonded ligand to two iron atoms. Both **2** and **3** were synthesized by the reaction of the appropriate carbonylmetalate dianion with PCl_2[CH-$(SiMe_3)_2$] or PCl_2(2,4,6-*t*-$Bu_3C_6H_2O$) in ether solution, and the workup was as previously described.[2] The yields (based on phosphorus) were 45% for **2**, orange-yellow crystals, mp 109–114 °C, and 40% for **3**, orange-red crystals, mp 190–191 °C.

The structures of both **2**[4a] and **3**[4b] were determined by single-crystal X-ray diffraction. For the chromium complex, Figure 1, it can be seen that the diphosphene ligand, $(Me_3Si)_2CHP$=$PCH(SiMe_3)_2$, is bound to chromium through one phosphorus atom only. This is in sharp contrast to the structure of **1** in which each phosphorus atom behaves as a donor to an $Fe(CO)_4$ moiety. We attribute this difference primarily to an increase in crowding, the $Cr(CO)_5$ fragment being larger than $Fe(CO)_4$. The result of the increased steric requirements of $Cr(CO)_5$ can be seen in Figure 1 where both Me_3Si groups on C(6) are oriented away from the $Cr(CO)_5$ moiety whereas in **1** the Me_3Si groups orient toward the smaller $Fe(CO)_4$. This conformational change effectively prevents coordination to a second $Cr(CO)_5$, and the unique structure observed is the result. The P(1)–P(2) distance, 2.027

† No reprints available.

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(4) (a) Mo Kα radiation (λ = 0.71069 Å), T = 293 K; triclinic $P\bar{1}$, a = 9.139 (2) Å, b = 10.851 (1) Å, c = 18.059 (3) Å, α = 76.83 (1)°, β = 80.36 (2)°, γ = 65.01 (1)°; Z = 2; μ = 6.3 cm^{-1}; 2932 unique data, 324 parameters, R = 0.051. (b) Mo Kα radiation (λ = 0.71069 Å), T = 140 K; monoclinic $P2_1/c$, a = 11.243 Å, (3) Å, b = 29.250 (6) Å, c = 15.300 (3) Å, β = 110.67 (1)°; Z = 4; μ = 7.3 cm^{-1}; 6144 unique data, 319 parameters, R = 0.060.

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