Our results establish that 14-electron, d^0, mixed-ring bent-metallocene complexes of general type $(Cp^*)(C_2B_9H_{11})M(R)$ are accessible by reaction of group 4 alkyls with $C_2B_9H_{13}$ and are structurally and electronically similar to d^0 $(C_5R_5)_2M(R)$ and $(C_5R_5)_2M(R)^+$ species.[1-4] The observation of strong ligand binding by **1** and **2** and agostic Zr···H-C and Zr···H-B interactions in **3** and **7** indicates that these complexes are highly electrophilic. This feature is reflected in the high olefin and acetylene insertion reactivity and in the facile reactions leading to μ-CH_2 complexes **7** and **8**.

Recent studies establish that 14-electron cationic $Cp_2Zr(R)^+$ species exhibit enhanced insertion reactivity vs 16-electron neutral $Cp_2Zr(R)(X)$ complexes.[3] The high insertion reactivity reported here for neutral **1**, **2**, and **5** and the high reactivity of neutral d^0 $(C_5R_5)_2M(R)$ complexes[1,2] suggest that this difference results largely from the increased unsaturation of $Cp_2Zr(R)^+$ rather than the charge.

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Supplementary Material Available: Synthetic procedures and characterization data for new compounds and details of the X-ray structure determinations of **3** and **7** (34 pages); listing of observed and calculated structure factors for **3** and **7** (24 pages). Ordering information is given on any current masthead page.

Sequence-Specific Bifunctional DNA Ligands Based on Triple-Helix-Forming Oligonucleotides Inhibit Restriction Enzyme Cleavage under Physiological Conditions

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Intermolecular triplex formation has been demonstrated to inhibit DNA-protein interactions by the observation that selective binding of a third strand of DNA at the recognition site of restriction/modification enzymes prevents cleavage or methylation of the duplex.[1-3] Triplex formation has the potential to precisely modulate gene expression if targeted at protein binding sites involved in the regulation of a specific gene.

Intermolecular triplex formation occurs by binding of a homopyrimidine oligonucleotide to the major groove of a hompurine-homopyrimidine stretch of DNA, parallel to the purine strand. Sequence specificity results from Hoogsteen pairing between thymine and protonated cytosine in the third pyrimidine strand and the Watson-Crick A·T and G·C pairs of the duplex, respectively.[4-8]

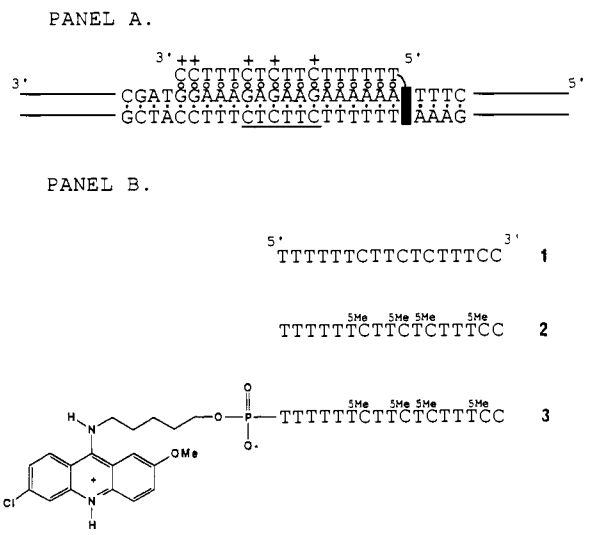

Figure 1. Panel A: Structure and sequence of the triple helix with the covalently attached acridine intercalated at the 5′ triplex-duplex junction, showing the recognition sequence (underlined) of *Ksp*632 I. Filled dots and open circles between bases indicate Watson-Crick and Hoogsteen pairing, respectively. Protonated cytosines are indicated by the + symbol. Panel B: Structures of the oligonucleotides used for triple-helix formation. The unmodified oligonucleotide (**1**) and the oligonucleotide containing 5-methylcytosine (**2**) were synthesized by using phosphoramidite chemistry on a Pharmacia automated DNA synthesizer. The oligonucleotide with an acridine covalently attached to the 5′ end (**3**) was prepared by solid-phase synthesis using the phosphoramidite derivative of 2-methoxy-6-chloro-9-[(ω-hydroxypentyl)amino]acridine as described.[12] Bases which are 5-methylcytosine are indicated 5Me. All oligonucleotides are parallel to the purine strand of the duplex.

Table I. Concentrations of Oligonucleotides Required for 50% Inhibition of Restriction Endonuclease Cleavage by *Ksp*632 I via Triple-Helix Formation[a]

oligonucleotide	concn required for 50% inhibn of cleavage, μM		T_m of triplex-to-duplex transition at
	pH 7.0	pH 7.7	pH 7.0, °C
1, native	4.4	>50	15 ± 0.5
2, 5-methylcytosine	0.85	36	22 ± 3
3, 5-methylcytosine/acridine	0.15	7.5	35 ± 1

[a] T_m measurements were performed as previously described[11] in 50 mM sodium cacodylate buffer (pH 7.0) by using a Kontron Uvikon 820 spectrophotometer.

The concentrations of the third strand required for specific inhibition of restriction enzyme cleavage via triple-helix formation are in the micromolar range,[1,2] and triplex formation and hence inhibition are dependent on low pH.[1,2] In order to use intermolecular triplex formation as a tool to modulate gene expression in vivo, conditions must be established whereby triplex formation is stable under physiological conditions of pH and at a concentration of the third strand that can be realistically attained in the nucleus of living cells.

In this study we have attempted to optimize the concentrations of oligodeoxynucleotides required for site-specific inhibition of protein binding to DNA by the replacement of cytosine with 5-methylcytosine and by attachment of an acridine residue to the

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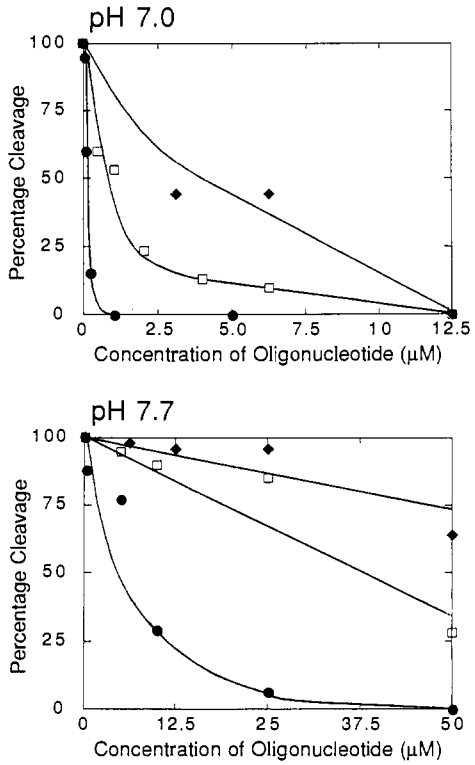

Figure 2. Specific inhibition of *Ksp*632 I cleavage by intermolecular triplex formation: plots of percentage cleavage of the DNA fragment versus the concentration of oligonucleotide in the reaction mixture at pH 7.0 and pH 7.7. The symbols on the plot indicate the oligonucleotides with 5-methylcytosine and an acridine (**3**) (●), 5-methylcytosine only (**2**) (□), and the natural 17-mer (**1**) (◆). Assays were performed by using a *Not*I–*Hin*dIII restriction fragment of pDCl,[13] labeled at one end by ^{32}P, which contains a single *Ksp*632 I cleavage site within the 17-bp recognition site for triple-helix formation. The triple helix was formed in restriction buffer A (33 mM Tris acetate, 33 mM potassium acetate, 0.5 dithiothreitol, 10 mM $MgCl_2$, and 100 μM spermine, at pH 7.0 or 7.7) by incubation for >1 h at 30 °C in order to avoid kinetic effects. Three units of *Ksp*632 I (Boehringer Mannheim) was then added and the mixture incubated for a further 20 min before the reaction was stopped with the addition of EDTA to 50 mM. Cleavage was analyzed by PAGE and quantified by using an LKB laser densitometer, using exposures within the linear range of the film.

5′ end of the oligonucleotide via a pentamethylene linker (Figure 1, panel B). Methylation of cytosines increases the affinity of the third strand for the duplex,[2,10] as a result of a small p*K* change and the formation of a helical spine of hydrophobic methyl groups in the major groove, and an acridine covalently attached to the 5′ end of the oligomer is able to intercalate site-specifically into the DNA at the triplex–duplex junction (Figure 1, panel A), increasing the affinity of the third strand for the target duplex.[11]

We tested the effect of these modifications on inhibition of protein binding to DNA by determining the relative concentrations required for 50% inhibition of cleavage of DNA by the restriction endonuclease *Ksp*632 I. The results of these experiments are shown in Figure 2 and summarized in Table I. Both of these modifications can substantially reduce the concentration required to inhibit cleavage (Figure 2). At both pH 7.0 and pH 7.7, substitution of 5-methylcytosine for cytosine produces an approximately 5-fold increase in inhibition of cleavage, and the presence in addition of an acridine residue covalently attached to the 5′ end of the oligomer produces an overall 30-fold increase in inhibition of cleavage over the unmodified oligomer (Table I). These results reflect the increase in the melting temperature of the triplex-to-duplex transition (Table I) resulting from these modifications. The increased concentrations of all oligodeoxynucleotides required for inhibition of *Ksp*632 I cleavage at pH 7.7 compared to pH 7.0 reflect the inherent dependence of intermolecular triplex formation on protonation of cytosines. The oligoacridine was able to give 100% inhibition of *Ksp*632 I cleavage at only 1 μM at neutral pH. This is the first demonstration of *complete inhibition* of endonuclease cleavage *at neutral pH* by intermolecular triplex formation.

We have shown that modification of oligonucleotides can be effectively used to strongly increase the competitive inhibition of protein binding to DNA via intermolecular triplex formation. This will allow the design of effective inhibitors of gene transcription or DNA replication and, thus, the production of potential therapeutic agents or tools for investigating the biological function of specific segments of DNA in a highly selective manner.

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Synthesis and Crystal Structure of a Four-Metal Ketenylidene: $[PPN]_2[Fe_3CuI(CO)_9(CCO)]$

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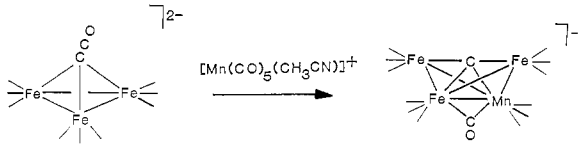
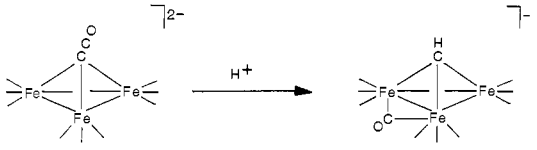
The reactions of the coordinated ketenylidene ligand, CCO, have received considerable attention in part because of its interesting and varied chemistry.[1-4] This chemistry includes both metal cluster building reactions and a variety of transformations of the CCO ligand. The majority of ketenylidene complexes are trimetallic carbonyl clusters and cluster building reactions on these lead to carbide clusters resulting from the cleavage of the C–CO bond, eq 1.



(1)

The addition of H^+ to $[PPN]_2[Fe_3(CO)_9(CCO)]$ (I) also results in the cleavage of the α-C–β-CO bond and formation of the methylidyne, eq 2.[2] By contrast, protonation of the ruthenium



analogue, $[PPN]_2[Ru_3(\mu\text{-}CO)_3(CO)_6(CCO)]$, occurs on the metal framework with retention of the ketenylidene ligand.[3b]

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