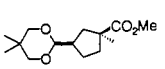
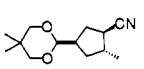
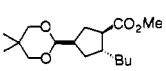
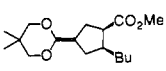
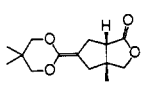
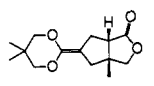
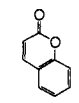
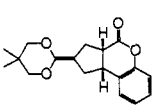
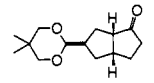
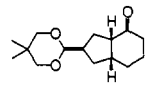
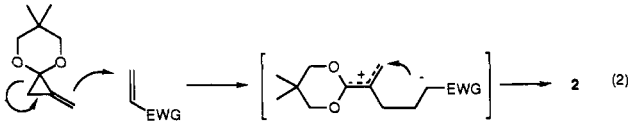
Table I. Cycloaddition of Methylenecyclopropanone Ketal **1** with Electron-Deficient Olefins[a]

entry	olefin	(equiv)	temp (°C)	time (h)	product	% yield[b]
1		(1.1)	80	18		91
2		(1.1)	80	39		85[c]
3		(1.1)	70	26		89
4		(1.1)	70	46		86[d]
5		(0.85)	80	90		84
6		(0.85)	70[e]	16		87
7		(1.1)	80	20		95
8		(1.1)	80	28		88
9		(2.0)	80	28		85

[a] The reaction was carried out in CH_3CN (2.5 mL/mmol except in entries 3 and 4, 0.5 mL/mmol, and in entries 7 and 8, 1.0 mL/mmol). The cycloadduct consisted of a single product (except entries 2 and 4) as determined by capillary GLC and by ^{13}C NMR for the equivalent reaction carried out in CD_3CN. [b] Isolated yield of the ester **3** obtained after hydrolysis of the ketene acetal **2** (Amberlyst 15 in aqueous acetonitrile at room temperature for 30 min). The yields are based on **1** except in entries 5 and 6 wherein they are based on the butenolide. [c] Both the starting olefin and the product were a 88:12 mixture of *E* and *Z* isomer. [d] The starting olefin was 100% *Z*, and the product was 98% *Z*. [e] The reaction was carried out under high pressure (13 kbar) in CH_2Cl_2.

suggests that the major pathway of the present reaction involves a concerted cycloaddition of a TMM intermediate (e.g., **4**).[9] At the present time, however, other possibilities including a step-wise mechanism (eq 2) cannot be rigorously eliminated. Mechanistic studies as well as the synthetic exploration of this new reaction is under active investigation.



(2)

1

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Supplementary Material Available: Spectral data (IR, ^1H NMR, and ^{13}C NMR) for the new compounds (6 pages). Ordering information is given on any current masthead page.

(8) Curiously, however, there has been reported, to our knowledge, only a single case of *thermal* [3 + 2] cycloaddition of a methylenecyclopropane to olefins (i.e., 2,2-diphenyl derivative added to tetracyanoethylene and related highly reactive olefins, ref 2c).

(9) For the reactivities and the orbital properties of **4**, see: Siemionko, R.; Shaw, A.; O'Connell, G.; Little, R. D.; Carpenter, B. K.; Shen, L.; Berson, J. A. *Tetrahedron Lett.* **1978**, 3529.

Cooperative Site Specific Binding of Oligonucleotides to Duplex DNA

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Cooperative interactions between DNA binding ligands are critical to their specificity, affinity, and biological activity.[1-4] Triple helix formation by oligonucleotides is the most powerful *chemical* approach to date for the sequence-specific recognition of double helical DNA.[5-9] Hoogsteen hydrogen bonded base triplets, TAT and C+GC, result from pyrimidine oligonucleotides binding site specifically to purine duplex sequences. In the triple helical model, a binding site size of 18 purine base pairs affords 36 discrete sequence-specific hydrogen bonds for recognition of DNA in the major groove. As a possible mechanism for improving the specificity of triple helix formation, we tested whether oligonucleotides could cooperatively bind to a double-stranded DNA template.

We report that two different pyrimidine oligonucleotides, which are nine bases in length, *cooperatively* bind to an 18 base-pair homopurine site in bacteriophage λ genomic DNA by triple helix formation. The purine target sequence 5′-$A_4GA_6GA_4GA$-3′ occurs once in λ DNA[10] (48.5 kilobase pairs) and can be considered as two contiguous unique half-sites, 5′-A_4GA_4-3′ and 5′-A_2GA_4GA-3′.

† Howard Hughes Medical Institute Doctoral Fellow.

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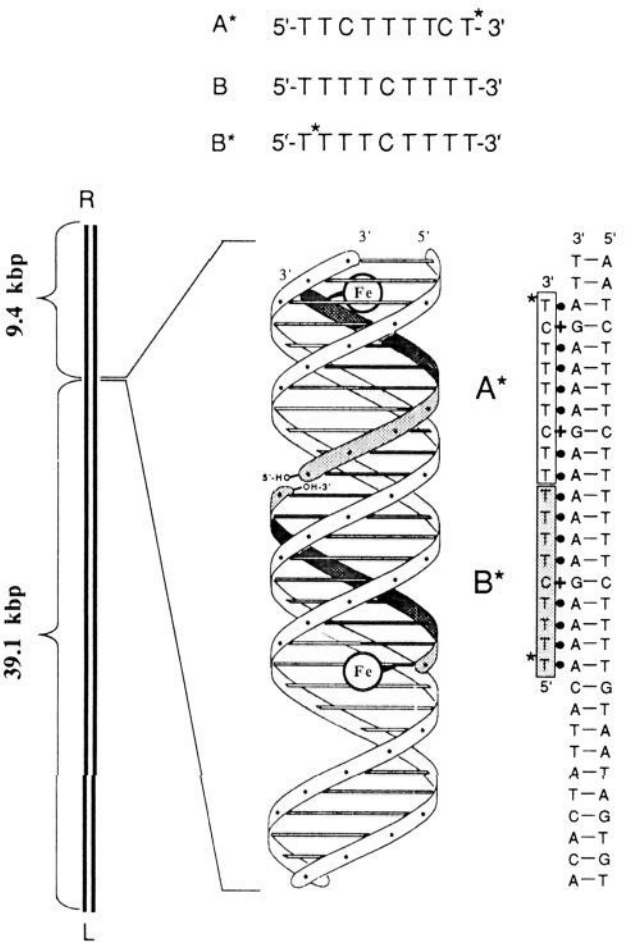

Figure 1. Upper: pyrimidine oligonucleotides designed to bind adjacent half-sites of the target sequence. A* and B* contain an EDTA cleaving function at their 3′ and 5′ termini, respectively. Lower: double strand cleavage of λ DNA affords two fragments, 39.1 and 9.4 kbp in size. Simplified models of the triple helix complex between adjacent Watson–Crick homopurine–homopyrimidine half-sites and the Hoogsteen pyrimidine oligonucleotides A* and B* bound head-to-tail.

Two pyrimidine oligonucleotides, specific for the adjacent half sites, were tested for cooperative binding interaction by the affinity cleaving method.[5,11,12]

Oligonucleotides A*, B, and B* were synthesized by automated methods (Figure 1). A modified nucleoside for cleaving DNA, thymidine-EDTA (T*),[12] was placed at the 3′ end of 5′-$T_2CT_4CT^*$-3′, (A*).[13] Oligonucleotides 5′-T_4CT_4-3′ were synthesized with and without a T* moiety at the 5′ end (B* and B, respectively). λ DNA[12] was labeled with ^{32}P at the right end (R) with AMV reverse transcriptase.[5b] Specific double strand oxidative cleavage at the target site by an oligonucleotide-EDTA·Fe affords a 9.4 kbp fragment (Figure 2).[5b] The intensity of the 9.4 kbp fragment indicates the extent of oligonucleotide binding.

Combinations of oligonucleotides A*, B, and B* at 5 μM concentrations were incubated at 24 °C with $Fe(NH_4)_2(SO_4)_2$, spermine (1.0 mM), λ DNA (approximately 4 μM in base pairs), 100 mM NaCl, and 25 mM tris-acetate at pH 6.6. Dithiothreitol (4 mM) was added after 0.5 h to initiate strand cleavage. The

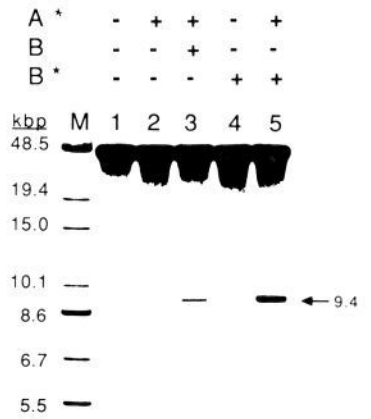

Figure 2. Site-specific double strand cleavage of bacteriophage λ DNA. Autoradiogram of ^{32}P right end-labeled λ DNA, 48.5 kbp in size, on an 0.5% agarose gel. Lane M, DNA size markers obtained by restriction enzyme digestion of left end-labeled DNA with BamH1, Apa I, and Sma I; digestion of right end-labeled DNA with BamH1, Sma I, and Xho I: 48.5 (undigested DNA), 19.4, 15.0, 10.1, 8.6, 6.7, and 5.5 kbp. Lane 1, right end-labeled intact λ DNA control. Lane 2, right end-labeled λ DNA with 5.0 μM A* and 5 μM Fe(II). Lane 3, λ DNA with 5.0 μM A*, 5.0 μM B, and 5.0 μM Fe(II). Lane 4, λ DNA with 5.0 μM B* and 5.0 μM Fe(II). Lane 5, λ DNA with 5.0 μM A*, 5.0 μM B*, and 10 μM Fe(II). Arrow on the right indicates 9.4 kbp cleavage fragment.

reactions proceeded for 6 h (24 °C) and were stopped by ethanol precipitation. Double strand cleavage products were separated by agarose gel electrophoresis and visualized by autoradiography (Figure 2). Cleavage efficiency was quantitated by scintillation counting of the individual bands.

A* incubated in the absence of B generated a marginally detectable level of cleavage, 1.3 (±0.2)% (Figure 2, lane 2). A similar result 1.7 (±0.2)% was observed for B* incubated alone, (lane 4). This suggests that these nine base pyrimidine oligonucleotides have low binding affinity for their target sites under the conditions chosen (24 °C, pH 6.6), consistent with previous observations.[5a] In contrast, when A* was incubated in the presence of B, the cleavage efficiency improved to 4.4 (±0.5)% (lane 3). This 3.5-fold improvement in double strand cleavage indicates a positive binding interaction between contiguous oligonucleotides A* and B, aligned head-to-tail in the major groove of duplex DNA. Double strand cleavage can be further increased to 10.5 (±1.1)% when both oligonucleotides were equipped with EDTA (A* in the presence of B*, lane 5).

Cooperative binding could arise from two different interactions: base stacking of the terminal thymine bases of the Hoogsteen strand in the major groove and/or an induced conformational change at contiguous DNA sites. There is literature precedent for both types of interactions, although in significantly different systems.[2-4] If base stacking between the terminal bases of contiguous oligonucleotides in the triple helical complex is important for cooperative binding,[13] then disruption of this interaction could be utilized to make sequence-specific recognition highly sensitive to single base mismatches. Design of structural motifs that enhance the cooperative interaction of adjacent sequence-specific binding oligonucleotides on duplex DNA is in progress.

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