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OFFICE OF NAVAL RESEARCH

Grant N00014-92-J1052

R&T Code 4135018

Technical Report #6

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**"Specific Recognition of CG Base Pairs by 2-Deoxynebularine
within the Purine•Purine•Pyrimidine Triple-Helix Motif"**

by
H. U. Stilz and P. B. Dervan

**California Institute of Technology
Division of Chemistry and Chemical Engineering
Pasadena, CA**

January 11, 1993

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Specific Recognition of CG Base Pairs by 2-Deoxynebularine within the Purine•Purine•Pyrimidine Triple-Helix Motif⁺

Hans Ulrich Stilz and Peter B. Dervan*

Beckman Institute
California Institute of Technology
Pasadena, California 91125

RUNNING TITLE: Recognition of CG-base pairs

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* To whom correspondence should be addressed

ABSTRACT

The sequence-specific recognition of double-helical DNA by purine-rich oligodeoxyribonucleotide-directed triple-helix formation is limited to purine tracts. Within the geometric constraints of the phosphate-deoxyribose position of a purine•purine•pyrimidine triple helical structure model building studies suggested that the deoxyribonucleoside 2'-deoxynebularine (dN) might form one specific hydrogen bond with cytosine (C) or adenine (A) of Watson-Crick cytosine-guanine (CG) or adenine-thymine (AT) base pairs. 2-Deoxynebularine (dN) was incorporated by automated methods into purine-rich oligodeoxyribonucleotides. From affinity cleavage analysis, the stabilities of base triplets within a purine•purine•pyrimidine (pu•pu•py) triple helix were found to decrease in order N•CG~N•AT >>N•GC~N•TA (pH 7.4, 37 °C). Oligodeoxyribonucleotides containing two N residues were shown to bind specifically within plasmid DNA a single 15 base pair site of the human immunodeficiency virus genome containing two CG base pairs within a purine tract. This binding event occurs under physiologically relevant pH and temperature (pH 7.4, 37 °C) and demonstrates the utility of the new base. Quantitative affinity cleavage titration reveals that, in the particular sequence studied, an N•CG base triplet interaction results in a stabilization of the local triple helical structure by 1 kcal•mol⁻¹ (10 mM NaCl, 1 mM spermine tetrahydrochloride, 50 mM Tris-acetate, pH 7.4, 4 °C) compared to an A•CG base triplet mismatch.

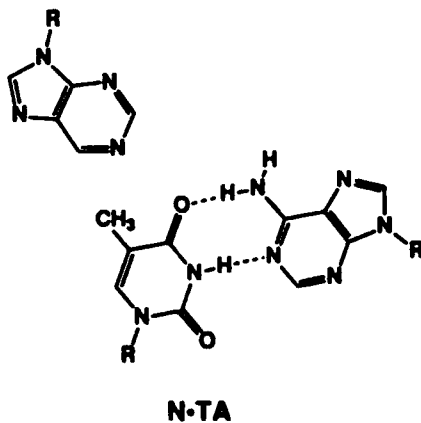
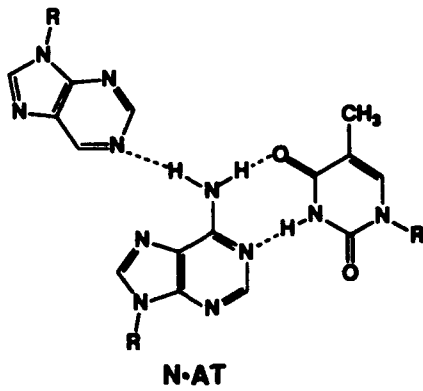
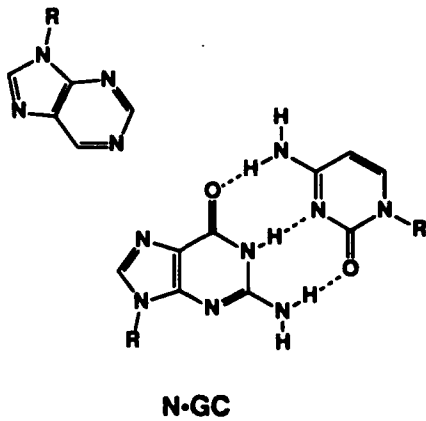
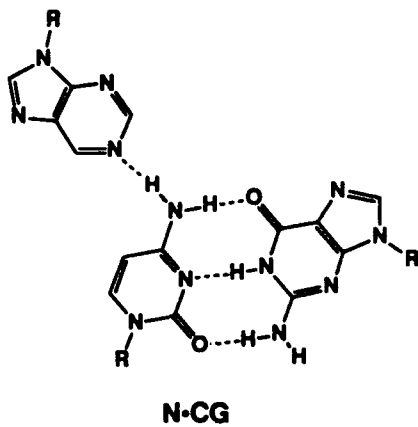


Figure 1

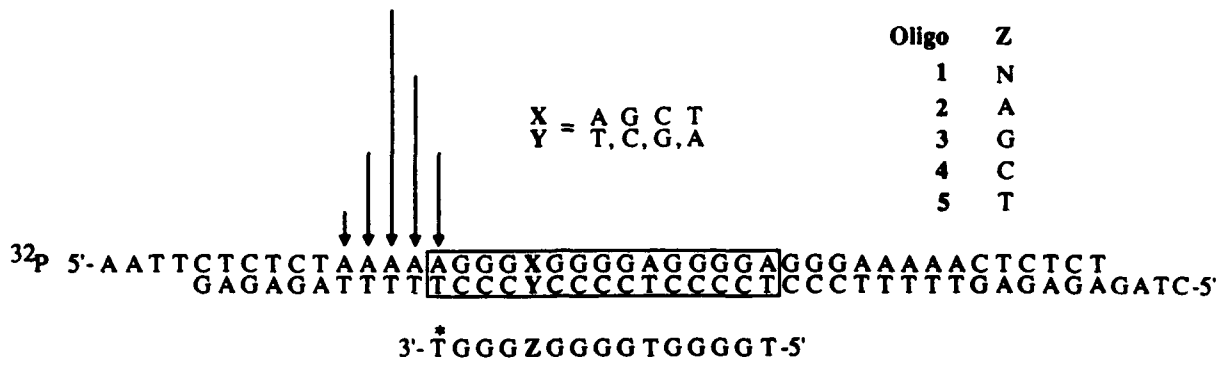
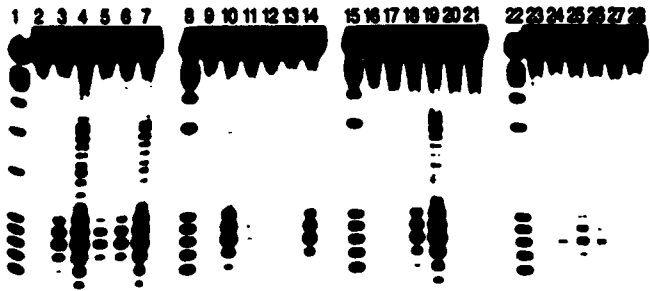


Figure 2A



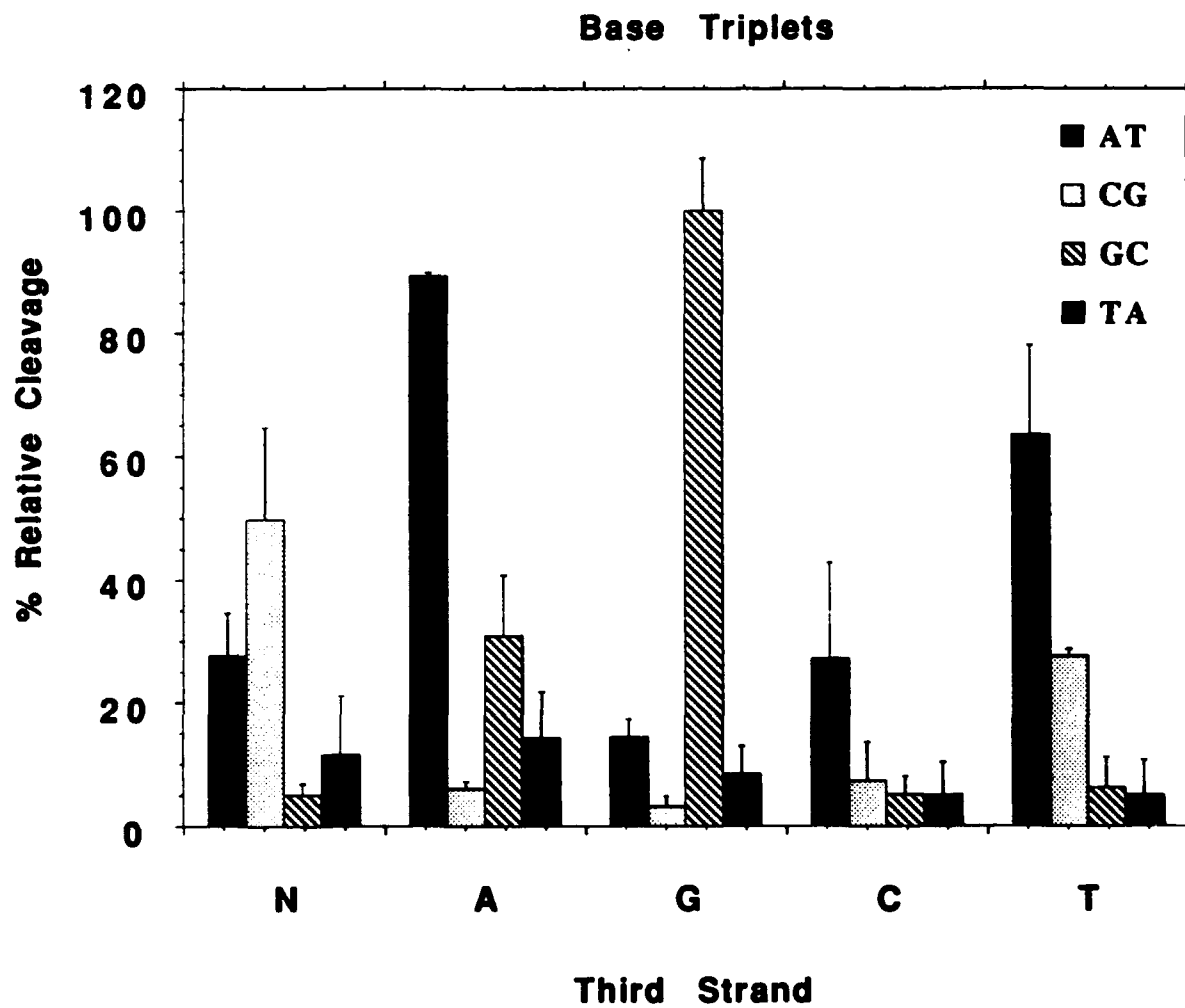


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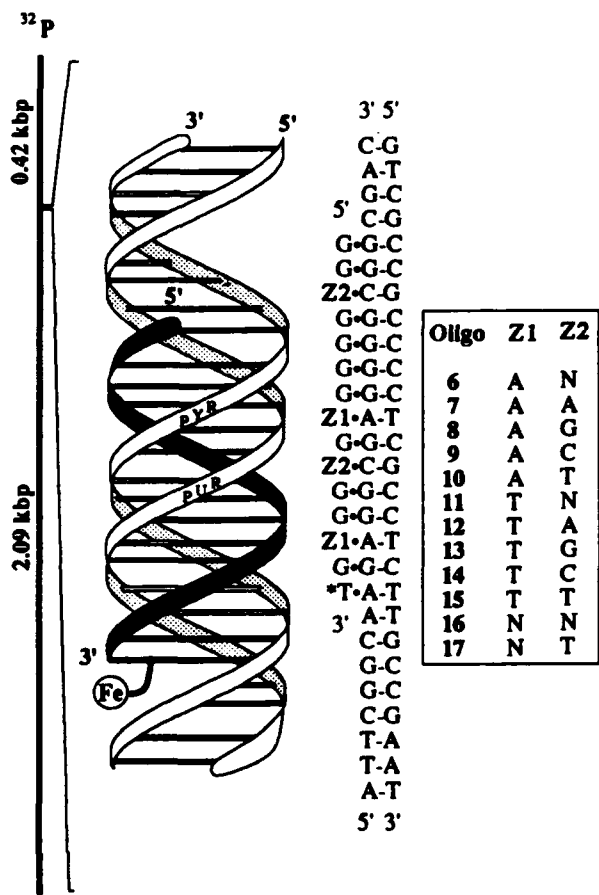
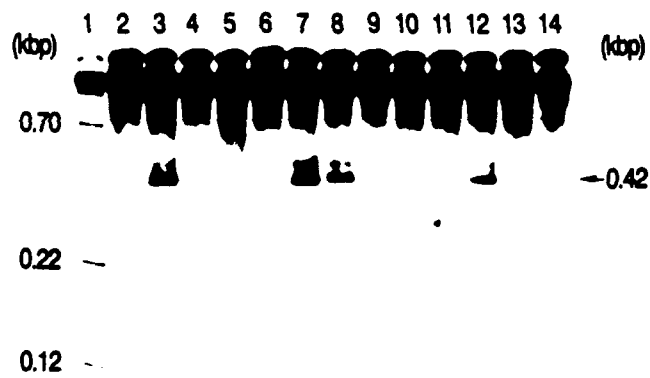
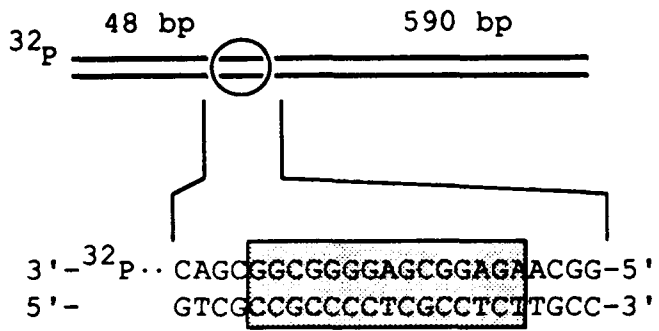


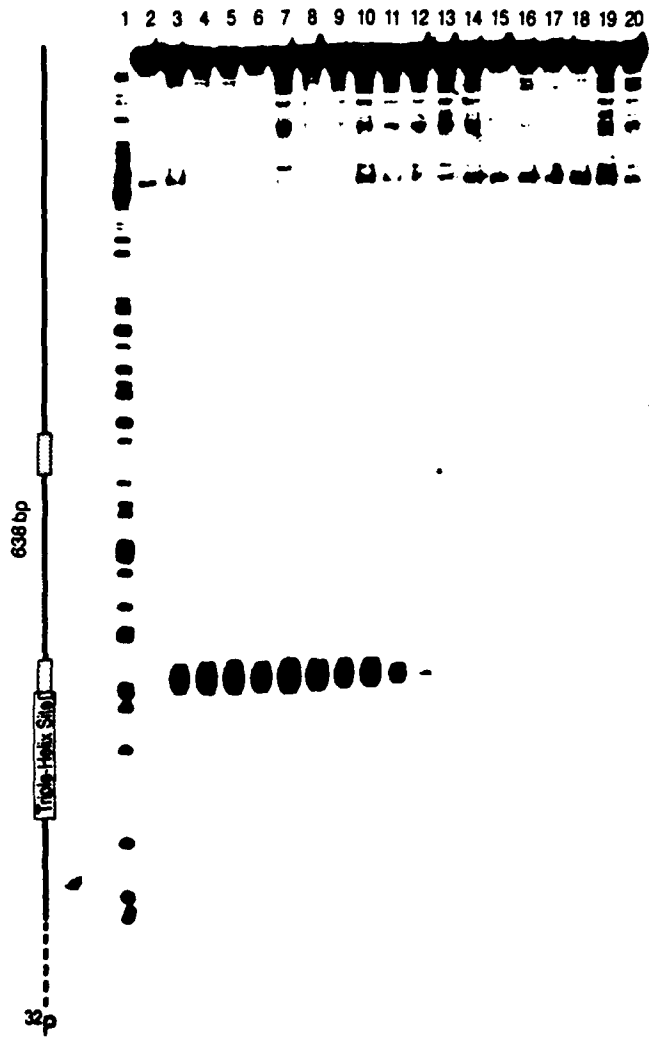
Figure 4A





- 6 5' - GGNGGGGAGNGGAGT*
- 7 5' - GGAGGGGAGAGGAGT*
- 10 5' - GGTGGGGAGTGGAGT*
- 11 5' - GGNGGGGTGNGGTGT*
- 17 5' - GGTGGGGNGTGGNGT*

Figure 5



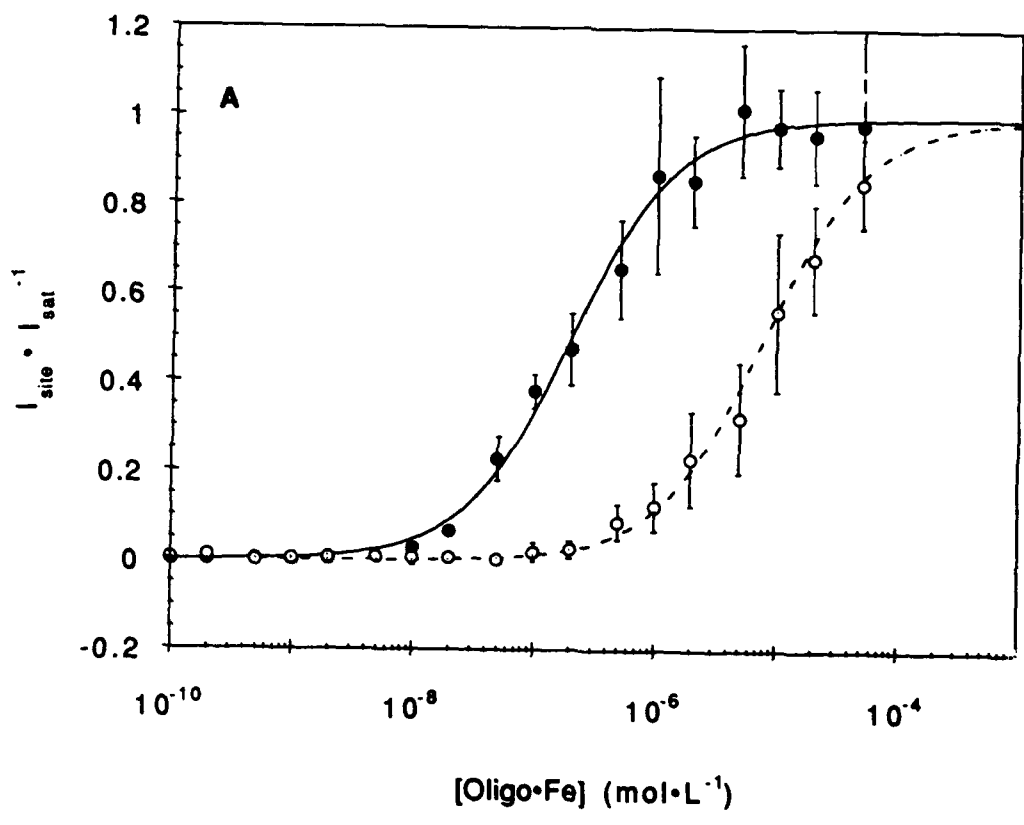


Figure 7A

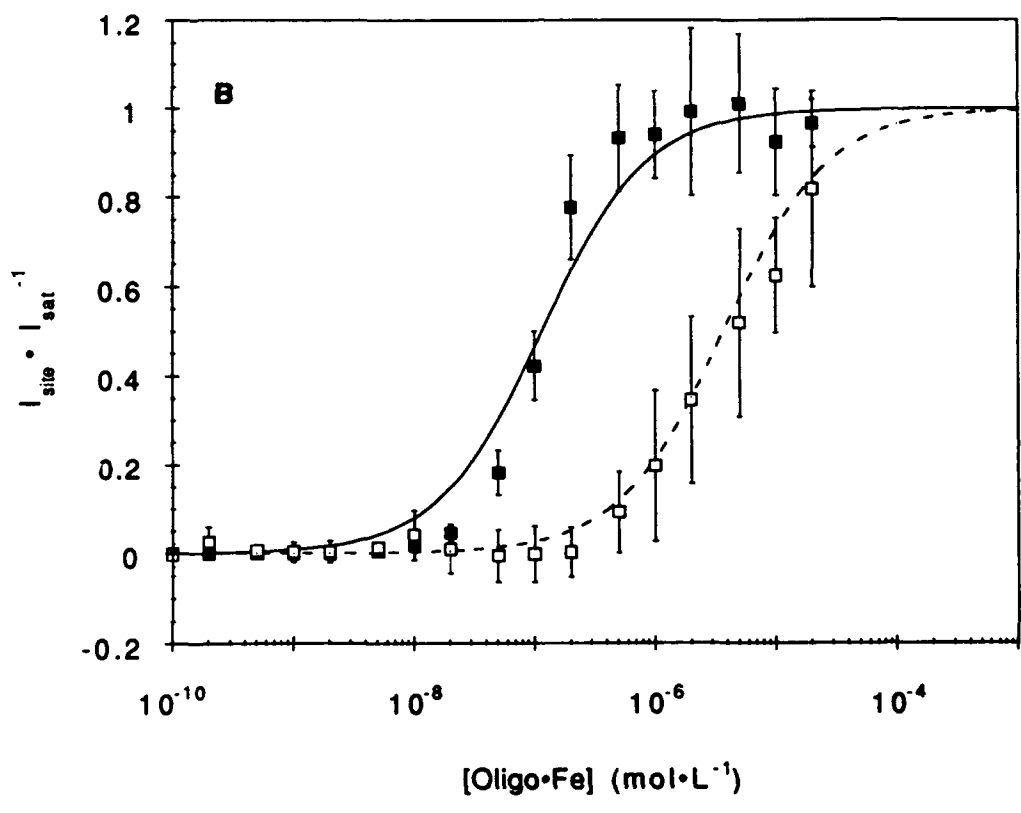


Figure 7B

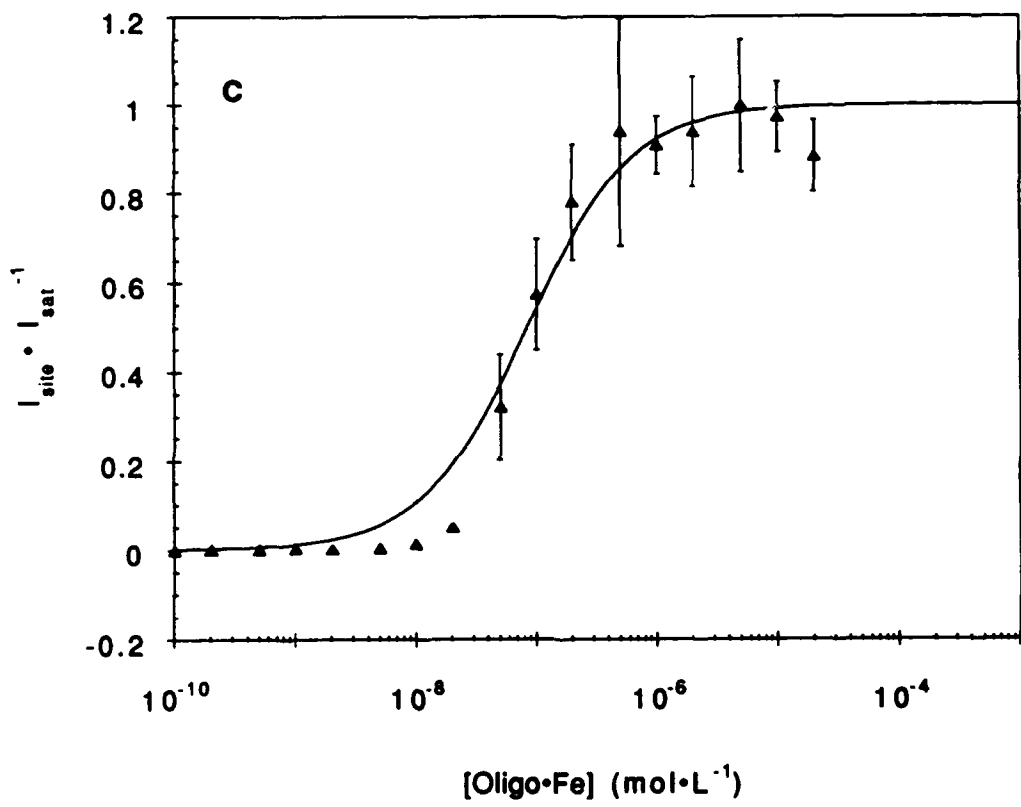
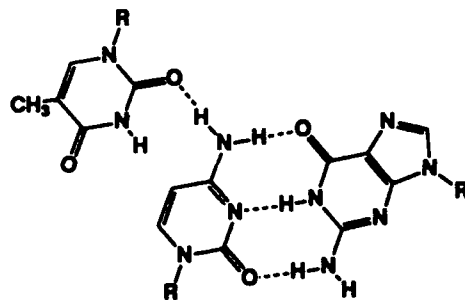
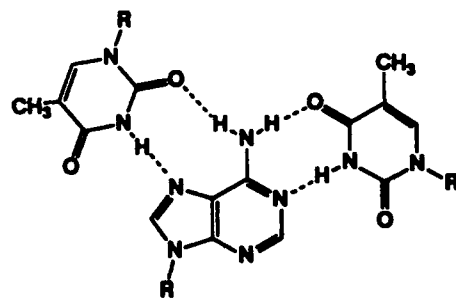


Figure 7c



T-CG



T-AT

Figure 8

Table I: Association Constants at pH = 7.4 and 4 °C.^a

Oligo	Base Triplets (n) ^b	K_T (M ⁻¹)	ΔG_T (kcal·mol ⁻¹)
6	G•GC (10), A•AT (2), N•CG (2)	$5.0 (\pm 1.8) \times 10^7$	- 9.8 (± 0.3)
7	G•GC (10), A•AT (2), A•CG (2)	$1.3 (\pm 0.5) \times 10^6$	- 7.8 (± 0.3)
10	G•GC (10), A•AT (2), T•CG (2)	$1.2 (\pm 0.3) \times 10^8$	- 10.2 (± 0.3)
11	G•GC (10), T•AT (2), N•CG (2)	$8.6 (\pm 2.0) \times 10^7$	- 10.1 (± 0.2)
17	G•GC (10), N•AT (2), T•CG (2)	$2.7 (\pm 2.0) \times 10^6$	- 8.2 (± 0.8)

^aValues listed are mean values measured from affinity cleavage titration experiments performed in Association buffer (10 mM NaCl, 1 mM spermine tetrahydrochloride, 0.1 mM-bp calf thymus DNA, 50 mM Tris-acetate, pH 7.4).

^bn = number of triplets within each triple helical complex.