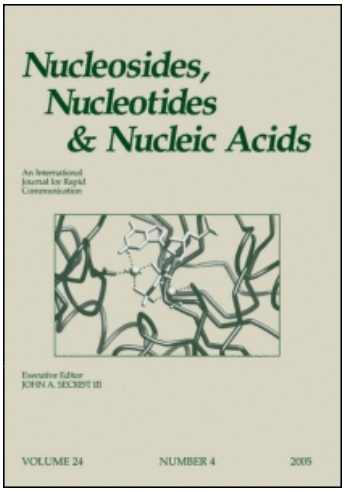

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DNA BASE PAIR BINDING SPECIFICITY OF CC-1065

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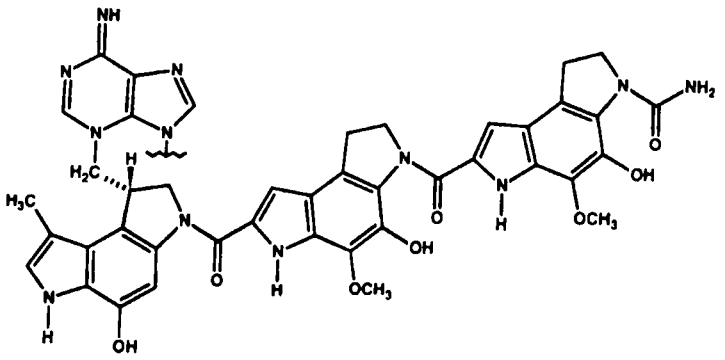
Abstract: Oligomer duplexes were prepared by a solid-phase phosphoramidite triester coupling approach in order to study the DNA base pair binding specificity of the antitumor antibiotic CC-1065 by CD spectroscopy.

CC-1065 is a very potent cytotoxic antitumor antibiotic isolated from *Streptomyces zelensis*.[1] In its interaction with DNA, it binds preferentially in the minor groove of a B-form double-stranded DNA at AT rich regions[2,3] by at least two mechanisms which produce large induced CD curves. It can bind irreversibly which results in a covalent bond between N-3 of adenine and the methylene carbon of the cyclopropyl group of CC-1065 as shown below.[4] The arrangement is such that CC-1065 extends in the 5'-direction from the site of attachment. The other mechanism results in a reversibly bound species which converts to the irreversibly bound species with varying kinetics depending on the DNA sequence.[2,5] CC-1065 has shown a sequence specificity towards 5'PuNTTA and 5'AAAAA according to Maxam and Gilbert sequencing studies.[6] As a result of its binding to DNA, CC-1065 produces remarkable helix stabilization and is a potent inhibitor of DNA synthesis.[3,7]



In order to study more extensively the DNA base pair binding specificity of CC-1065 at a molecular level with CD spectroscopy, several oligodeoxyribonucleotide sequences were designed and synthesized. These had a minimum number of adenine residues in order to limit the binding to only one CC-1065 molecule per sequence. The binding region was flanked by GC base pairs in order to stabilize duplex formation.

The oligonucleotides, varying in length from a 7-mer to a 14-mer, were synthesized using the solid-phase phosphoramidite triester coupling approach developed by Beaucage and Caruthers.[8] The yields based on the absorbance at 504 nm after detritylation with 1.5% TCA/CH_2Cl_2 ranged from 25-100%. The oligomers were fully deprotected as previously described[9,10] and purified by either polyacrylamide gel electrophoresis[9,10] or anion-exchange high pressure liquid chromatography (HPLC). The HPLC purification was carried out successfully using two different sets of conditions on a Nucleogen DEAE 60-7 column[11] as shown in Figure 1. While a good separation was achieved in both cases, the volatility of the triethylammonium bicarbonate buffer favored its use over the sodium acetate-LiCl system which required desalting on a Waters Sep-Pak C-18 cartridge.

The primary and complementary strands were annealed in 10 mM Tris-HCl (pH 7.8), 75 mM NaCl by incubation at 65°C for 10 min followed by a slow cool down to room temperature over a 6 hour period and freezing. All the duplexes synthesized exhibited a normal B-form spectrum by CD spectroscopy. CC-1065 was added to a final concentration of one CC-1065 molecule per duplex and the CC-1065-DNA interaction followed by CD. Table 1 summarizes the results of our findings.

Irreversible binding is characterized by a shift to shorter wavelength of the induced CD band (392→371 nm), reflecting the reaction between the cyclopropyl group of CC-1065 and the N-3 of adenine. This binding mode was observed with most of the oligonucleotides, although with varying kinetics depending on the DNA sequence. A purine or a pyrimidine at position 5 (or 4) from the site of attachment did not seem to effect the binding. Equivalent induced molar ellipticities (~180,000) were observed for both 14-mer sequences --5'GAGTTA and --5'GTGTTA differing only at position 5; the same held true for position 4 (--5'GCTTA and --5'GGTTA). We also found that the longer the oligomer, the more rigidly bound the CC-1065, the greater the

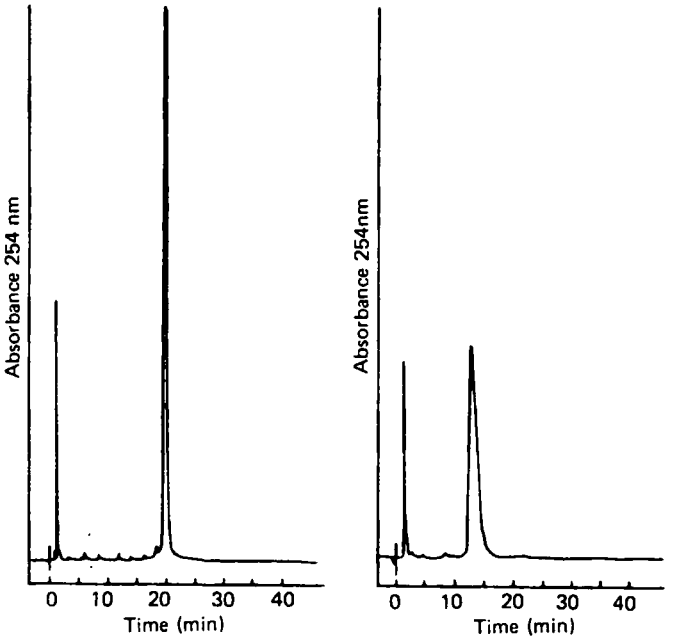


FIG. 1: HPLC chromatograms of 5'CCCTAATC$_3$' reaction mixture.

TABLE 1

STUDIES OF CC-1065 BINDING TO DNA OLIGOMERS BY CD

Oligomers (duplex) (5' → 3')	Induced molar ellipticity	λ_{max} (nm)		Complete	Type of binding
		1 hr	1 day		
CGGAGTTAGGGGCG	186,000	371	371	1 hr	irreversible
CGGTGTTAGGGGCG	180,000	372	371	1 hr	irreversible
GTTAGGG	54,000	390	376	1-8 days	rev. → irrev.
GATTAGGG	100,000	390	371	1 day	rev. → irrev.
GGATTAGG	140,000	375	370	1 day	irreversible
CGATTAGC	132,000	374	370	3 hr	irreversible
CGCTTAAGCG	140,000	374	371	1 hr	irreversible
CCGGTTAACCGG	160,000	371	370	1 hr	irreversible
CGCGAATTCGCG	190,000	392	392	>>28 days	reversible
CGCGATATCGCG	134,000	391	389	6-15 days	rev. → irrev. (slow)
CGCGTATACGCG	96,000	387	374	5-14 days	rev. → irrev. (slow)
CGCGAAAACGCG	158,000	386	371	1 day	rev. → irrev.
GCGAAGAACGCG	135,000	391	391	>>15 days	reversible
CGCAGAAACGCG	165,000	372	371	1 day	irreversible

induced CD (10-mer (140,000)<12-mer (160,000)<14-mer (186,000)). CPK models of the adduct predict that the drug molecule should cover about 5 base pairs.[12] We found that, as the number of bases to the 5'-end of the 5'TTA binding sequence increased, a greater induced CD was observed (5'GTTA*GGG (54,000)<5'GATTA*GGG (100,000)<5'GGATTA*GG (140,000)) and the rate of conversion from reversibly to irreversibly bound species also increased. The replacement of recognition sequence 5'TTA by 5'ATAT and 5'TATA resulted in a lower induced CD and a slower conversion to an irreversibly bound species occurring over a 5-15 days period. The sequences 5'GAAAA and 5'AGAAA did bind CC-1065 irreversibly at a normal rate of species conversion. However, a G at position 3 from the site of attachment (5'AAGAA) hindered the formation of the irreversibly bound species. Another sequence, 5'GAATT, which also has a G at position 3, only bound CC-1065 reversibly. That a G at position 3 would hinder binding was predicted by molecular modeling studies.[5] The results for 5'AAGAA indicates that CC-1065 can bind "around" this base pair, but only in a position that results in a very slow conversion of the reversibly bound species to the irreversibly bound species. In general, the data of Table 1 indicate that the best irreversible binding of CC-1065 occurs for sequences which have dA-dT base pairs in positions 1,2 and 3, as long as these bases do not have an alternating arrangement.

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