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^{1}H-NMR Studies Of The Interactions Of Two Distamycin Analogues With The Dodecamer d(CGCGAATTCGCG)$_2$.

Maria Rosaria Conte, Ernesto Fattorusso*, Luigi Gomez Paloma and Luciano Mayol

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II,
via Domenico Montesano 49, I-80131 Napoli, Italy

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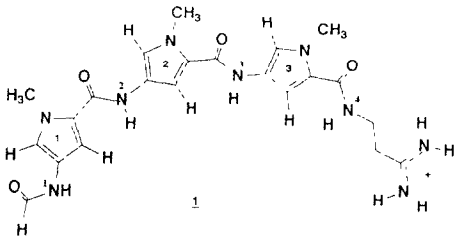
Abstract

High resolution proton NMR techniques have been used to study the interaction between the self complementary Dickerson dodecamer d(CGCGAATTCGCG)$_2$ and two distamycin analogues containing a retroinverted amide bond. The results indicated that both analogues, although binding the Dickerson dodecamer less strongly than distamycin, span the central AATT segment in the minor groove in a similar fashion.

Investigation of the binding of small ligands to DNA is an important topic in physical as well as in medicinal chemistry. In fact, due to the central role of DNA in the regulation of biochemical processes, compounds capable of interacting with it exhibit a wide spectrum of antiviral and antitumoral activity and the comprehension of the parameters governing the binding is a significant step in a rational approach to design new drugs which perform their action at the gene level. Many biophysical studies of the interaction of small molecules with synthetic oligonucleotides are currently carried out both in the crystalline state and in solution, where Nuclear Magnetic Resonance has resulted to be the most powerful tool for the analysis of the structure and dynamics of drug-nucleic acid complexes[1]. In this context the natural oligopeptide antibiotic distamycin (1) has received particular attention as a model of compounds which bind preferentially to the AT rich region in the minor groove of the right-handed B-DNA double helix. It has been shown that the molecule adopts a planar crescent-shaped conformation that enables it to fit snugly in the narrow minor groove forming close Van der Waals contacts between the hydrogens of the pyrrole rings with C2H protons of adenine moieties, as well as hydrogen bonds between the amide hydrogen atoms and the N-3 of adenines and O-2 of thymines. The AT binding specificity is explained by the presence of the guanine NH_2 groups lying in the minor groove of GC rich regions which sterically inhibits the penetration of the molecule[2].

In order to give a further insight in the mode of interaction of small non-intercalating ligands to DNA and, particularly, to get information about the relative weight of the various factors which are responsible for the binding, we have undertaken a project dealing with the study of the complexes formed between some distamycin analogues and synthetic fragments of DNA. In this frame we have been investigating two compounds which differ from distamycin in possessing a retroinversion at the level of the first (2) or the third (3) amide bond and we present here ^{1}H-NMR data[3] on their interaction with the Dickerson dodecamer d(CGCGAATTCGCG)$_2$.

The two distamycin analogues 2 and 3 have been synthesized as described previously[4]. The self complementary dodecamer d(CGCGAATTCGCG)$_2$ was prepared using a Beckman 200A synthesizer, purified by ion exchange HPLC on a Partisil 10 SAX column using linear gradient of KH_2PO_4 20% CH_3CN, pH=7 from 10 mM to 0.35 M and desalted by gel filtration on a Biogel P2 column. Double strand DNA concentration used in the various NMR experiments ranged from 1 to 4 mM, in buffer consisting of 10 mM sodium phosphate (pH 7.00) and 10 mM sodium chloride. Both drugs and DNA concentration were determined spectrophotometrically using the appropriate extinction coefficients.



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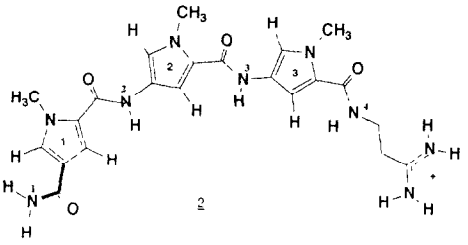

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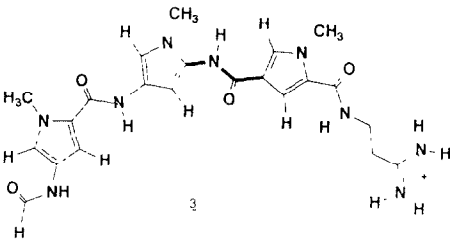

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A prerequisite for the study of the interaction drug-DNA is the assignment of the signals of both components in the complex. In the case of the complex formed between distamycin and the Dickerson dodecamer, this was accomplished by transferring the information gained on the free oligonucleotide by sequence specific assignment to the complex through 2D exchange and 1D NOE (in H_2O) NMR experiments[5]. The situation was quite different for the complexes between the same dodecamer and the two analogues 2 and 3. In fact, both compounds are less active than distamycin and, accordingly, they show higher rate of dissociation with DNA[6] thus forming kinetically instable complexes on the NMR time scale. Therefore, when a less than equimolecular amount of drug is present in the solution, no apparent doubling of DNA or drug proton resonances is observed except for a broadening of some signals in the complex formed between 2 and the dodecamer (at 290°K), which turn in sharper peaks at higher temperature (>310°K). The signals of most of the aromatic protons in the complexes have been consequently assigned by inspection of spectra obtained at several points in a titration of the $d(CGCGAATTCGCG)_2$ duplex with 2 or 3.

Fig. 1 shows the aromatic region of the ^1H spectra of the mixture 1:1 of $d(CGCGAATTCGCG)_2$ with 3 (a) and the dodecamer alone (b) in D_2O solution whereas in table 1 are reported the variations of chemical shifts for aromatic, H1', H2' and H2" protons of the same dodecamer induced by formation of the complex with 2 in comparison with the corresponding data for protons which have been positively identified in the complex with distamycin[5].

From these data it is apparent that the binding of the dodecamer with 2 and 3, as in the case of distamycin, results in the largest shift differences for the central four -AATT- base pairs. Similarly, the C2H resonances of A5 and A6 are drifted downfield while those of the other more affected protons, namely C6H of T7 and T8, are shifted upfield.

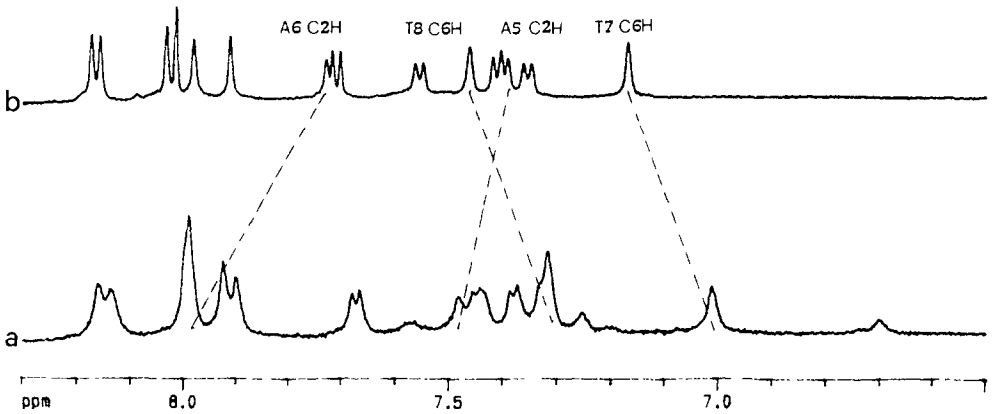

Fig 1. Aromatic region of 500 MHz ^{1}H-NMR spectrum of the dodecamer d(CGCGAATTCGCG)$_2$ alone (b) and in presence of an equimolecular amount of the analogue 3 (a).

Table 1: variations of chemical shifts (ppm) of DNA resonances caused by the presence of equimolecular amounts of distamycin (1) ($\Delta\delta$ 1)[a] and of the analogue 2 ($\Delta\delta$ 2)[b].

Proton	$\Delta\delta$ 1	$\Delta\delta$ 2	Proton	$\Delta\delta$ 1	$\Delta\delta$ 2
C1 C6H	0.00	0.01	T7 C6H	0.16	0.09
C1 C5H	-	0.02	T7 C1'H	0.22	0.23
C1 C1'H	-	0.00		0.45	
C1 C2'H	-	0.00	T7 C2'H	-	0.18
C1 C2"H	-	0.00	T7 C2"H	-	0.14
G2 C8H	0.00	0.01	T8 C6H	0.23	0.18
G2 C1'H	-	0.01		0.31	
G2 C2'H	-	-0 03	T8 C1'H	0.45	0.37
G2 C2"H	-	-0.02		0.60	
C3 C6H	0.00	0.01	T8 C2'H	-	0.22
C3 C5H	-	-0.02	T8 C2"H	-	0.14
C3 C1'H	-	0.01	C9 C6H	0.07	0.12
C3 C2'H	-	0.01		0.35	
C3 C2"H	-	-0.06	C9 C5H	0.00	0.09
G4 C8H	0.06	0.04		0.13	
G4 C1'H	0.28	-0.07	C9 C1'H	0.21	0.24
G4 C2'H	-	0.00	C9 C2'H	-	0.13
G4 C2"H	-	-0.01	C9 C2"H	-	0.16
A5 C8H	-0.05	-0.02	G10 C8H	0.07	0.03
A5 C2H	-0.10	-0.10	G10 C1'H	0.07	0.02
	-0.20		G10 C2'H	-	-0.03
A5 C1'H	0.42	0.35	G10 C2"H	-	-0.01
A5 C2'H	-	-0.05	C11 C6H	0.00	0.01
A5 C2"H	-	0.10	C11 C5H	0.04	0.00
A6 C8H	0.00	-0.07	C11 C1'H	-	0.01
	-0.14		C11 C2'H	-	-0.03
A6 C2H	-0.51	-0.43	C11 C2"H	-	-0.01
	-0.59		G12 C8H	0.00	-0.01
A6 C1H	0.58	0.15	G12 C1'H	-	-0.01
A6 C2'H	-	0.04	G12 C2'H	-	-0.03
A6 C2"H	-	0.12	G12 C2"H	-	-0.02

a. The loss of the symmetry of the DNA duplex caused by binding of distamycin and the slow rate of dissociation of the complex lead to a doubling of many DNA resonances over the spectrum.

b. The resonances for H1', H2' and H2" of the DNA in the complex have been assigned by a combination of NOESY and COSY spectra.

These data indicated that both analogues, although binding the Dickerson dodecamer less strongly than distamycin, also span the central -AATT- segment in a similar way. This interaction could be confirmed in the case of the complex with compound 2 by observing the Nuclear Overhauser Effect (NOE) between proximal protons from both drug and DNA[7]. Preliminarly, it was necessary to assign resonances for the protons on the concave side of the curved molecule of compound 2 in the complex. This was accomplished as follows.

In the free drug the six aromatic protons resonate as broad singlets in the region from δ 7.6 to 6.8 (D_2O solution). The distinction between α- (δ 7.59, 7.24, 7.24) and β- (δ 7.30, 6.98, 6.90) pyrrole protons was straightforward, based on the NOE effect observed for H-5 signals by irradiation at the methyl groups' resonances. Then, the mutually long range coupled signals at δ 7.59 and 7 30 could be confidently assigned to H-5 and H-3 of the pyrrole ring 1 on the basis of their higher chemical shifts. The latter resonance in the complex was identified as the singlet at δ 7.08, partially overlapped to the T7 C6H signal, by following the variation of chemical shift during a titration of the drug with the dodecamer and was the starting datum to assign the other important signals for the drug protons, namely H-3 of ring 2 and 3 and the two central amide protons, through a NOESY experiment in H_2O (fig. 2). In this solvent not all the exchangeable protons could be observed; among the signals present in the spectrum, the singlet at δ 10.54 showed two cross peaks with the signals at δ 7.08 (H-3 of ring 1) and δ 7.03, whereas the proton resonating at δ 9.67 was correlated with protons resonating at δ 7 03 and δ 6.77. Consequently, the signals at δ 10.54 and 9.67 were identified as the resonances of the amide protons NH-2 and NH-3 respectively, and the signals at δ 7.03 and 6.77 could be assigned to H-3 protons of ring 2 and 3, respectively.

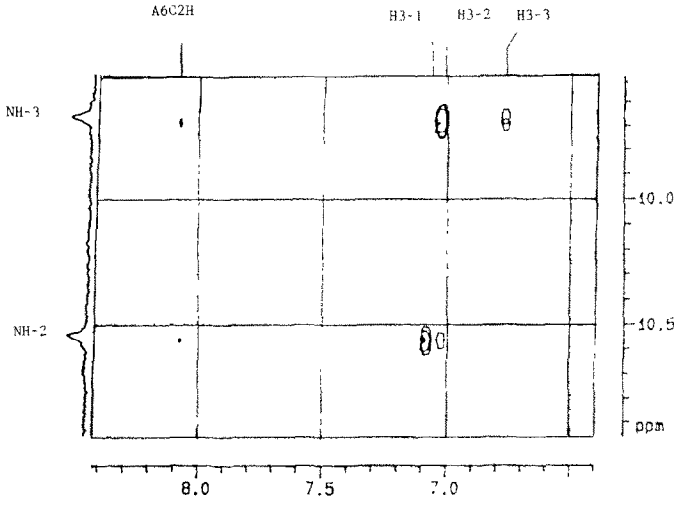

Fig. 2. Part of 500 MHz NOESY (τm 300 ms) spectrum in H_2O of the complex of the analogue 2 with the dodecamer d(CGCGAATTCGCG)$_2$ showing the intramolecular and intermolecular NOE contacts of the amide protons NH-2 and NH-3 of the drug.

Once identified most of the drug proton resonances in the spectrum of the complex, the binding configuration was established from the intermolecular NOEs observed between compound 2 and the DNA through a series of NOESY spectra in D_2O performed at different mixing times (100, 150, 300, 500 ms). These spectra contain both intermolecular NOEs used to assign H1', H2' and H2" DNA resonances following the sequencial connectivities[8], and intermolecular NOEs to identify drug-DNA contact points (fig. 3). The above cited H_2O NOESY also showed two intermolecular contacts between both the two central amide protons and the C2H proton of adenine 6. No other cross peak involving exchangeable resonances could be noticed in this spectrum.

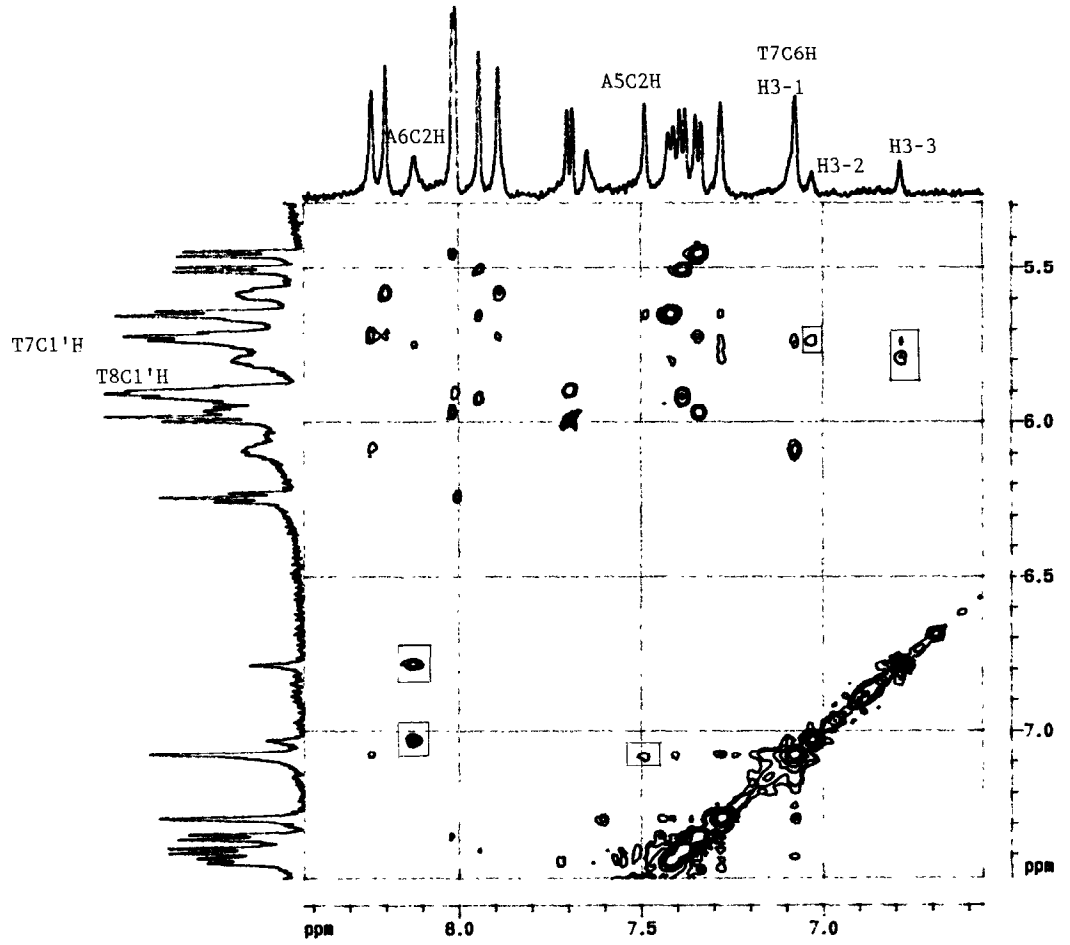

Fig. 3. Part of 500 MHz NOESY (τm 300 ms) spectrum in D_2O of the complex of the analogue 2 with the Dickerson dodecamer showing the DNA intramolecular base-H1′ cross peaks and the intermolecular NOE contacts (in boxes) involving the β protons of the pyrrole rings in the drug.

The results of these experiments are summarized in table 2. Among the most intense peaks there are those correlating the three H-3 protons of the pyrrole rings with the C2H hydrogens of the adenine moieties, thus clearly indicating that these protons are the points of closest contacts between the drug and DNA. The above data provide direct evidence that the drug molecule indeed binds the central-AATT-sequence of the Dickerson dodecamer as in the case of distamycin. Quantitative NOE measurements are currently in progress to get a better determination of the complex in solution with the aid of computer methods.

A significant feature of the interaction of distamycin and other minor groove binders with symmetrical DNA fragments pertains to the presence of two equivalent and exchanging ligand binding sites on the

Table 2. Summary of intermolecular NOEs for the analogue 2 -DNA complex.

Drug proton	DNA proton	Drug proton	DNA proton
H3-1	A5 C2H	H3-3	T7 C1'H
H3-1	A6 C1'H	H3-3	T8 C1'H
H3-2	A6 C2H	NH-2	A6 C2H
H3-2	T7 C1'H	NH-3	A6 C2H
H3-3	A6 C2H		

oligonucleotide sequence. When the kinetics of binding is slow on the NMR time scale, this process is supported by the presence of exchange cross peaks connecting two DNA resonances in the complex that were originally derived from a symmetry related pair (the binding of the asymmetric drug molecule to the oligomer destroys the symmetry of the duplex)[5]. The high rate of dissociation of the complexes formed by compound 2 and 3 with the dodecamer and the consequent absence of doubling of any resonance in the ^{1}H- NMR spectrum prevented us from observing such an exchange of the ligands between the two binding sites of the DNA sequence.

This study along with the investigation of complexes formed by 2 and 3 or other distamycin analogues with related DNA sequences will allow to better understand the force responsible for the interaction of these minor groove binding molecules with DNA. The preliminary results described here seem to emphasize the role played by the hydrogen bonds involving NH-1 and NH-3 in the binding of the natural antibiotic distamycin to DNA. At the moment, the lack of these interactions appears to be the sole cause why the Dickerson dodecamer embodies compound 2 and 3 in the minor groove less efficiently than distamycin. In fact, molecular modeling of compounds 2 and 3 indicated that both molecules can assume a crescent shape which should match the curvature of the minor groove as well as distamycin, thus allowing Van der Waals interactions between the aromatic pyrroles of the drug and adenine rings to occur. This conclusion is supported, in the case of the complex of 2 with the Dickerson dodecamer, by the intense NOE intermolecular cross peaks involving the protons belonging to the above cited moieties.

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References

1. Blackburn, G.M.; Gait, M.J. *Nucleic Acids in Chemistry and Biology. Oxford University Press* **1990**.
2. Pelton, J.G.; Wemmer, D.E. *J. Am. Chem. Soc.* **1990**, *112*, 1393-99 and references cited therein.
3. NMR experiments were performed on a Bruker AMX 500 spectrometer. Two dimensional spectra were obtained in the phase sensitive mode.
4. Animati, F.; Arcamone, F.; Lombardi, P.; Rossi, C. *The Total Synthesis of Novel "Retrouniverso" Distamycin Analogues. 12th International Symposium "Synthesis in Organic Chemistry"* Cambridge, 23-25 July **1991**.
5. Klevit, R.E.; Wemmer, D.E.. Reid, B.R. *Biochemistry* **1986**, *25*, 3296-3303.
6. Feigon, J.; Denny, W.A.; Kearns, D.R. *J. Med. Chem.* **1984**, *29*, 450-456.
7. The higher instability of 3 in aqueous solutions prevented us from performing any NMR experiment requiring relatively long acquisition time
8. Wüthrich, K. *NMR of Proteins and Nucleic Acids. John Wiley & Sons, New York* **1986**.