The Solution Conformation of a Carbocyclic Analog of the Dickerson-Drew Dodecamer: Comparison with its own X-ray Structure and that of the NMR Structure of the Native Counterpart

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Summary

The NMR conformation of a carbocyclic analog of the Dickerson-Drew dodecamer (dCCCGAATT*CGCGG)\textsubscript{2} containing 6'-\alpha-Me carbocyclic thymidines (T*) has been determined and compared with that of its X-ray structure. The solution structure of the 6'-\alpha-Me carbocyclic thymidine modified duplex has also been compared with the solution structure of the corresponding unmodified Dickerson-Drew duplex solved by us under the same experimental conditions. The NMR structures have been based on 24 experimental distance and torsion constraints per residue for (dCCCGAATT*CGCGG)\textsubscript{2} (1) and on 21 constraints per residue for the natural counterpart. In general, both final NMR structures are more close to the B-type DNA. The cyclopentane moieties of the carbocyclic thymidine residues adopt C1'-exo B-DNA type puckers (the phase angles \(\Phi = 136-139^\circ\) and the puckering amplitudes \(\Psi = 36-37^\circ\)) that are close to their previously published crystal C1'-exo or C2'-endo puckers. The main differences between the two NMR structures are for \(\beta(T^*)\) and \(\xi(T^*)\) backbone torsions (27-50\(^\circ\)), for basepair twist for the 7-8 and 8-9 basepair steps (5-6\(^\circ\)), tilt for the 8-9 step (7\(^\circ\)), roll for the 7-8 step (7\(^\circ\)), shift for the 7-8 step (0.9\(\AA\)) and slide for the 9-10 step (0.6\(\AA\)). The relatively small deviations of helical structure parameters lead to structural isomorphism of these duplexes in aqueous solutions (atomic RMSD = 1.0\(\AA\)). The difference of the minor groove widths (less than 0.7\(\AA\)) in the core part of the modified duplex in comparison with the native one is much smaller than the difference between the X-ray structures of these duplexes. A detailed comparison of NMR and X-ray structure parameters showed significant monotonic differences (0.9-2.5\(\AA\)) for all basepair slides in both duplexes. Deviations between NMR and X-ray structure parameters for the modified duplex were also found for basepair tilt of the 4-5 step (13\(^\circ\)), rolls for the 8-9 and 10-11 steps (16\(^\circ\) and 19\(^\circ\)), twist of the 3-4 step (8\(^\circ\)) and shift of the 9-10 step (0.9\(\AA\)).

Introduction

Owing to the insufficient stability and cellular penetration properties of natural oligo-DNA and -RNA, the chemically modified oligonucleotides (1,2) (and references therein) have played an important role in the design of the antisense and anti-gene therapy (3). Three important features in these chemically modified oligonucleotides are that they should bind strongly to the opposite strand, show resistance to the nucleolytic degradation and, ideally, should penetrate into the cell more easily than the natural counterpart. Although many modified oligonucleotides have so far been prepared and their binding data to the opposite DNA or RNA strand have been reported, very little information (4-6) is however available regarding how the structure of these covalently modified oligonucleotides changes in aqueous solution vis-a-vis their natural counterpart, or what are the quantitative structural differences between the solution and the solid state structure of these modified duplexes.

Although the introduction of 6'-\alpha-methyl-2'-deoxy-carbocyclic or 6'-\alpha-hydroxy-
sequence-dependent manner. Since both the X-ray crystal structure of two antisense DNA duplexes containing 6'-α-methyl- and 6'-α-hydroxy carbocyclic thymines, duplex (I) \(5'(\text{C}^{\text{G}}\text{C}\text{G}\text{A}^6\text{T}^\text{Me},^8\text{T}^\text{Me},^3\text{G}^{11}\text{C}^{12}\text{G})_2^3\) and duplex (III) \(5'(\text{C}^{\text{G}}\text{C}\text{G}^6\text{A}^6\text{T}^\text{OH},^8\text{T}^\text{OH},^3\text{G}^{11}\text{C}^{12}\text{G})_2^3\), as well as the NMR structure of the duplex (I) are now known, we argued that we have a good case in our hand to study if there is any correlation of anyone of the above properties of the carbocyclic-modified oligo-DNs with their structure and the hydration pattern. We also argued that this study is additionally interesting because of the absence of O4'-oxygen in the carbocyclic moieties which makes them lipophilic as well as devoid of stereoelectronic anomic and gauche effects (22) making them more flexible than the furanose counterpart, and thereby allowing the modified oligo-DNA to adjust smoothly to the geometrical constraints exerted by the phosphate backbones to mimic the structure of the B-form DNA of the natural counterpart.

The X-ray structures of antisense duplex (I) and (III) (20) show the following characteristic features: (i) They are isomorphous to the native Drew-Dickerson dodecamer (3), and they only have relatively small geometrical deviations. (ii) Nevertheless, the hydration patterns of the minor grooves of the two carbocyclic oligonucleotides are significantly different from their natural counterpart as a result of changing polarity in the minor grooves of duplexes (I) and (III). (iii) A comparison of the duplex (I) with the native counterpart shows that two shell water molecules (w33 and w35) are replaced by the four methyl groups in the former (Figure 2). This leads to the isolation of the first shell water molecule (w67) from the spine of hydration (20). It has been shown that this water molecule still has the hydrogen bonds with oxygens O2 of T and O9T residues. (iv) In the duplex (III), there are hydrogen bonds between the 6'-α-hydroxyl group of the carbocyclic thymines and N3 or O2 atoms of 3'-adjacent A or T, respectively, in the central portion of the minor groove. Interestingly, the polar 6'-α-hydroxyl substituent in the duplex (III) distorts the minor groove hydration more severely than the nonpolar 7'-α-methyl group in the duplex (I). However, this is compensated by the 6'-hydroxyl group acting as the hydrogen-bonding acceptor for additional water molecules at the groove periphery. This additional hydrogen bond has been used to explain the higher thermodynamic stability of the duplex (III) than duplex (I).

![Diagram](image1)

**Figure 2:** Schematic representation of the minor groove and its hydration in the natural Dickerson-Drew dodecamer in (A) and duplex (I), \(5'(\text{C}^{\text{G}}\text{C}\text{G}^6\text{A}^6\text{T}^\text{OH},^8\text{T}^\text{OH},^3\text{G}^{11}\text{C}^{12}\text{G})_2^3\) in (B). The numbering of water molecules has been used in a way similar to the one reported by (20) in their X-ray study.
In this work, we provide evidence through the use of the carbocyclic analogue of Dickerson-Drew dodecamer (I) that the favourable hydrogen-bonding interactions found in the first shell of hydration in the natural counterpart, d(GCG-GAATTCCGGC)₂ (3,20) are one of the main factors that are responsible for longer residence time of the bound water (∼0.6 ns at 10°C) (23). This work also shows that the residence time of the bound water molecule near the 7'-α-methyl (Tₘ) carbocyclic thymines (Figure 1A) in the minor groove of duplex (I) is less than 0.36 ns because of the hydrophobicity of Tₘ moieties (the oil effect), whereas in relatively more polar duplex (II), d(F16G25C34G43A60G63T82T106C110G111C125G)₂, containing polar 6'-α-hydroxyl (Tₙ) and Aₙ moieties (Figures 1B and 1C), the correlation time of this hydroxyl group is much longer than 0.36 ns, which is very much similar to the native 12-mer DNA duplex (7,10) as well as to those found near H₁' in an oligo-RNA: r(CDCAAAUUGGCG)₂ (19).

Both NMR dispersion studies with ²H and ¹⁷O (23-25) as well as combination of NOESY and ROESY (26) experiments have been developed to assess the residence times of water in protein (27-31) and DNA or RNA (7,9,10,23,24). One essential assumption for the NMR dispersion (NMRED) study is the averaging of the correlation time (τ₁) of long-lived water molecules (23). To extract a single correlation time, a water molecule is displaced by chemical modification or mutation, and the correlation time is a function of the difference in the dispersion profiles of the parent and the modified compound (23-25). The information on residence time of bound water molecule from both the NMRED and the combination of NOESY and ROESY studies have been found to complement each other. In this communication, we have used a combination of NOESY and ROESY experiments as our initial attempt to study the residence time of water molecules in duplexes (I) and (II) because of the following reasons: (i) The use of 7'-α-methyl groups for the measurement (see Material and Method) of the relative residence time of the bound water in duplex (I) allows us to disperse the water-DNA cross-peak at the chemical shift of water to estimate the individual correlation times based on both methyl groups as well as on H2A's. (ii) The 7'-α-methyl groups are pointed to the minor groove in duplex (I) just as the H2 proton of an adenine (A) moiety, and hence provide an independent marker. (iii) The 7'-α-methyl groups in duplex (I) are far away (>5.2Å) from the neighbouring labile exchangeable protons, hence they can be more confidently used for assessment of straight dipole-dipole interaction between bound-water and DNA-proton compared to NOEs from exchange relayed NOEs with labile DNA protons. We have also measured the residence times of the bound-water molecules in the minor grooves of both duplexes (I) and (II) by using H2A proton for the sake of comparison, although they are shown to be affected (32) by the two-step relay process via the neighbouring exchangeable protons under certain conditions, and hence relatively less reliable.

In analogy with the hydration pattern found in the X-ray structure of duplex (III), we herein conclude that the longer residence time of the water molecule in the minor groove in the duplex (II) suggests that they are potentially capable of forming the hydrogen bonds with the neighbouring N3 or O2 atoms, and can contribute in the thermodynamic stabilisation of the duplex II more than the duplex I.

The above conclusions are based on several unexpected interesting features that have been observed for the cross-peaks at the water chemical shift in the NOESY and ROESY spectra of duplexes (I) and (II) (Figure 3,4).

Results and Discussion

The NMR conformation of the carbocyclic analog of the Dickerson-Drew dodecamer [d(CCGGAAT*T*CGCG)]₂ containing 6'-α-Me carbocyclic thymines (T*) has been determined by us (enclosed manuscript) and compared with that of
its X-ray structure. The solution structure of the 6'-α-Me carbocyclic thymidine modified duplex has also been compared with the solution structure of the corresponding unmodified Dickerson-Drew duplex solved by us under the same experimental conditions.

The assignment of all protons for duplex (I) has been also performed in a conventional manner, using NOESY and COSY-DQF experiments, and the data will be published elsewhere together with its full structural analysis.

(1) Water-7'-α-methyl Contact of T<sub>Me</sub> in Duplex (I)

In both NOESY and ROESY spectra of duplex (I) at 10°C [Figures 3(Bii) and 3(Biv)] the cross-peaks between bound water molecule and 7'-α-methyl of T<sub>Me</sub> are negative with respect to the diagonal peaks, indicating that σ<sub>Me</sub>/σ<sub>O</sub> > 0 (see Figure 5 for the phase distortion problem, and Material and Methods). This unambiguously indicates that the residence time of the bound water molecule located near 7'-α-methyl of T<sub>Me</sub> groups is less than 0.36 ns (27,28,31). It is noteworthy that this is a well known behaviour (7b,10,33) for the water located in the major groove of native DNA in proximity with methyl groups of thymidine moieties (Me-T). Indeed, the cross-peaks corresponding to water-(Me-T) are negative for both duplexes (I) and (II) [Figure 3(Aii), 3(Aiv) and 3(Bii), 3(Biv)].

(2) Water-H2A Contact

(i) Duplex (I)

For duplex (I) the cross-peaks between water and H2A protons have vanished to zero in the NOESY spectrum, and they are negative in the ROESY spectrum for all temperatures between 5 and 20°C (Figures 3 and 4). These cross-peaks are interpreted as direct intramolecular nOe or Oe (see below) with a short residence time of ~ 0.36 ns for the bound water. However, a more detailed survey of the NOESY/ROESY spectra at 20°C of the water-2H<sub>A</sub> and water-2H<sub>A</sub> cross-peaks [Figure 4(Bi) and 4(Bii)] shows some minor differences: the intensity of the former cross-peak has slightly increased with increasing temperature and has a negative sign, showing that the hydration pattern is indeed different for 5A and 6A.

(ii) Duplex (II)

A completely different picture has been observed for duplex (II) with 6'-α-hydrox-
yl groups compared to duplex (I) with 7′-α-methyl substituents. The cross-peaks on the water line from 2H²A and 2H⁴A are very intense and have positive signs in the NOESY (τ_m = 200 ms) [Figure 3(Aii), 4(Aii)] and negative signs in the ROESY (τ_m = 100 ms) spectrum [Figures 3(Aiii), and 4(Aiii)]. Their intensities are almost identical, which is a sign of long correlation times (27,28,31) as found for covalently bound non-exchangeable protons in DNA. The recent X-ray structure of duplex (III) has shown that the 6′-α-hydroxyl group of T⁸OH is hydrogen-bonded to O2 of T⁹OH and 6′-α-hydroxyl group of T⁹OH is hydrogen-bonded to N3 of ²⁶OH in the "first hydration sphere", and that the T⁸OH and ⁸T⁸OH also act as hydrogen-bonding acceptors for an additional water molecule, bridging the 6′-α-hydroxyl group of ²⁰T⁸OH and ¹⁹T⁸OH in the opposite strand. In our duplex (II), we have different environments for ²H²A and ²H⁴A with respect to the opposite strand. Indeed for ²H²A, the 6′-α-hydroxyl group of ²T⁹OH has the potential to bridge with the 6′-α-hydroxyl group ²⁰T⁸OH through a water molecule in the same manner as for duplex (III). On the other hand, a 6′-α-hydroxyl group of ⁶A⁹OH is present around ²H²A, but there is no 6′-α-hydroxyl group-containing nucleotide on the opposite strand or in close proximity. Despite this different water environment in the second shell of hydration, we find that relative intensities of NOESY and ROESY cross-peaks on the water line of ²H²A and ²H⁴A to be almost identical (see Figures 3 and 4). This makes it tempting to suggest that the cross-peaks from ²H²A and ²H⁴A on the water line are perhaps owing to the dipole-dipole interaction between covalently bound 6′-α-hydroxyl group and H2As, which is considered by Portmann et al as the model of the covalently bound water in the minor groove. We cannot however rule out the contribution of the exchange mechanism in the above process (30). The fact that we see the cross-peaks from ²H²A and ²H⁴A on the water line allows us to put an upper limit for the residence time for any non-covalently bound water in the minor groove of any DNA duplex, which should be less than the correlation time of 6′-α-hydroxyl groups of that of a non-exchangeable DNA protons (~6 ns). Hence, the ratio of intensities in NOESY and ROESY cross-peaks on the water line of ²H²A and ²H⁴A in duplex (II) have been used as a qualitative reference point for the estimation of the correlation time of the non-covalently bound water molecule in duplex (I) and in the natural counterpart.

The experimental data obtained in this work has shown that for duplex (I), the intensities of the water and 7′-α-methyl cross-peaks (Figures 3 and 4) are very similar. This indeed supports the previous X-ray study that the bound water molecule is located between four α-methyl groups involving ²⁷T⁴Me, ⁸T⁴Me, ¹⁹T⁴Me and ²⁰T⁴Me residues of both strands. The estimated distances [both from NMR (enclosed MS) and X-ray structure (20)], between water-oxygen (w67 in Figure 2) and carbon of

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**Figure 4:** Comparison (at 20°C) of the cross-sections through the NOESY [panels: A(i), A(ii), B(i) and B(iii)] and ROESY [panels: A(iii), A(iv), B(iii) and B(iv)] spectra of duplex (II) in (A), and duplex (I) in (B). See Figure 2 for comparison and abbreviations at 10°C.
the 7'-α-methyl group of 7'T, are ~3.6 Å. According to the X-ray data, the next water (w41) and the neighbouring 7'-α-methyl of 7'T, are more than 9 Å away. This means that there is most probably one bound water molecule with a residence time of <0.36 ns in the proximity of the 7'-α-methyl of 7'T, which is much shorter than the residence times of water (>0.6 ns) (10°C) around 6T and 19T, and 5T in the natural dodecamer (10,23,25). For duplex (I), the residence time between water and the 7'-α-methyl of 7'T, (0.36 ns) correlates well with those based on water-H2(6A) results (0.3-0.5 ns). It is noteworthy that this residence time of water near H2(6A) in duplex (I) is shorter than 0.6 ns for the same residue in the natural counterpart.

Our data has shown that the relative intensities in NOESY and ROESY for the water-H2(6A) and water-H2(5A) cross-peaks in duplexes (I) and (II) are indeed quite different. For duplex (II), they are similar to those observed in natural Dickerson-Drew duplex (10), where water molecules “live” longer than 0.36 ns. But for the duplex (I), they are more similar to the hydration pattern of the dodecamer with a central (TTAA)2 position (7a) with a residence time of the water molecule of ~0.36 ns.

The occurrence of long-lived water molecules is believed (7a) to depend on the presence of a narrow minor groove. Our comparative NMR structural elucidation of duplex (I) and the natural Dickerson-Drew dodecamer duplex shows that their minor groove widths are similar, with an r.m.s.d of <0.63 Å. Additionally, it has been recently proposed (18) that the hydration kinetics in the rCGCCAAAUU-UGCG2 duplex may be dictated by hydrogen-bonding of water with 2'-OH groups than by the groove width. Moreover, the hydrogen bridging of bound water molecule with O2 atoms of 7'T, and 19T, residues are found to be similar (20) (Figure 2) for duplex (I) and the natural Dickerson-Drew dodecamer duplex based on X-ray data. Additionally, in the X-ray structure of duplex (I), there are at least two water molecules present adjacent (~3.5 Å) to H2(6A) or H2(19A) (in Figure 2B, see

Figure 5: Comparison of the cross-sections through the NOESY spectra of duplex (I), containing 7'-α-methyl (T,2) carbocyclic thymidines, taken along F2 at the F1 frequency of the water signal, showing no projection of cross-peaks between methyl and 7'-α-methyl protons of the DNA and water at different mixing times (marked at the left side of the panel) at 20°C. The assignments are shown at the top part of the panel.
w67 and w53 for 2H®A and w67 and w41 for 2H®15A). In the neighbourhood of H2(6A) / H2(17A), there are however four water molecules w53, w55 and w41, w84 (20). This hydration pattern is also quite similar to the natural Dickerson-Drew (3,20) dodecamer structure (Figure 2A). This similarity of hydration patterns in the first shell of hydration found in the X-ray structure is actually in conflict with the much reduced residence time of water molecules found in the minor grooves of duplex (I) compared to the natural counterpart based on NMR.

There are two possible explanations: (i) Since the NMR structure suggests that both the carbocyclic analogue and the natural 12-mer have almost the same minor groove width (r.m.s.d is < 0.63 Å), the replacement of the second shell of hydration is caused by the hydrophobic methyl groups of the carbocyclic nucleotide moieties, which, in turn, influence the life-time of the bound water molecule in the first shell of hydration, or, (ii) the water molecule in the proximity of the 6'-methyl of 7Me has lost the extra hydrogen-bonded stabilisation with O4' (34) in the sugar moiety because of its replacement by the hydrophobic 6'-methyleneclospentane moiety. It is also possible that both (i) and (ii) might contribute synergistically to the reduced residence time of water molecule in the minor groove in duplex (I). These suggestions are also supported by X-ray data (20) that the second shell of hydration in the centre of the modified duplex (I) is disorganised by the hydrophobic methyl groups compared with the natural counterpart (3). Moreover there was no hydrogen bond found between water molecule W67 and O4' in duplex (I) compared with the natural counterpart (2,34). It appears unlikely that the bound water molecule can distinguish between structures with conformational variation of the minor groove smaller than 0.63 Å as found between duplex (I) and its native counterpart.

The residence times of the water molecules located in the first shell of hydration around H2(6A) / H2(17A) and H2(6A) / H2(17A) protons are slightly longer (~0.36 ns) than for the water molecule located in the proximity of the 6'-methyl group of 7°Me (< 0.36 ns), which is consistent with X-ray data that the hydration pattern is restored (20) after 6A or 15A residues in duplex (I). It is however not possible to rule out the absence of any relay effect contributing partially to the enhancement of crosspeaks of H2A's at the chemical shift of water, which could be one of the reasons also for the overestimation of the residence times of water molecule around H2A protons.

In contrary to duplex (I) with hydrophobic 7°-methyl group of 7°Me, the correlation time of the 6'-hydroxyl groups in the minor groove in the duplex (II), with polar 6'-hydroxyl groups of AOH or T0H pointed to the minor groove, has been found to be much longer than 0.36 ns. Both the duplex (II) [T<sub>m</sub> = 48.3°C at 1μM] and the natural counterpart [T<sub>m</sub> = 48.4°C at 1μM] have longer residence times of water molecules located near H2A, owing to their stabilisation by the hydrogen bonding with the 6'-hydroxyl group of AOH or T0H in duplex or with N3 and O2, which, in turn, culminate into their stronger thermodynamic stabilisation than duplex I [T<sub>m</sub> = 44.1°C at 1μM] with 7°Me groups.

The work is now in progress to elucidate the hydration pattern in an analogue of duplex (I) containing 2'-deoxyaristeromycin (Figure 1E), which will enable us to dissect and quantify the stereoelectronic (22) versus steric effects, and will also allow us to understand the importance of the hydrophobic effect in the design of antisense oligonucleotides.

**Conclusion**

The ability to chemically modify oligonucleotides provides a powerful tool in the design of the antisense and antigen therapy by controlling important properties such as their stability to cellular nucleases, stronger bonding to the target DNA or RNA as well as cellular penetration. Despite the fact that many modified oligonucleotides

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**The Residence Time of the Bound Water in the Minor Groove**

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have so far been prepared and their binding data to the opposite DNA or RNA strand have been reported, very little information is available regarding how the structure of these covalently modified duplexes change in aqueous solution vis-a-vis their natural counterpart, or what are the quantitative structural differences between the solution and the solid state structure of these modified duplexes. Our findings on the hydration of the carbocyclic modified oligonucleotides are as follows:

(i) The residence time of the bound water molecule near the 7′-α-methyl (T<sub>M</sub>) carbocyclic thymidines in the minor groove of the self-complementary hydrophobic antisense oligodeoxyribonucleotides, i.e. duplex (I), is less than 0.36 ns because of the hydrophobic repulsion by T<sub>M</sub> groups (the oil effect) which induces the replacement of the second shell of hydration and the loss of the extra hydrogen-bonded water molecule with O4′ in the sugar moiety caused by the hydrophobic methyl groups of the carbocyclic nucleotide.

(ii) In relatively more polar duplex (II), containing polar 6′-α-hydroxyl (T<sub>OH</sub> and A<sub>OH</sub>) blocks, the correlation time of this hydroxyl group is much longer than 0.36 ns because these hydroxyl groups are potentially capable of forming the hydrogen bonds with the neighbouring N3 or O2 atoms, and can contribute in the thermodynamic stabilisation of the duplex (II) more than the duplex (I).

Materials and Methods

(A) NMR Sample Preparation

The oligomers d<sup>5′</sup>(C<sub>c</sub>G<sub>c</sub>C<sub>c</sub>G<sub>c</sub>A<sub>c</sub>A)<sub>T<sub>M</sub>8A<sub>T<sub>M</sub>8T<sub>M</sub>8G<sub>c</sub>G<sub>c</sub>11C<sub>c</sub>G<sub>c</sub>)<sub>2</sub> (duplex I) and d<sup>5′</sup>(C<sub>c</sub>G<sub>c</sub>C<sub>c</sub>G<sub>c</sub>A<sub>c</sub>A)<sub>OH</sub>4A<sub>OH</sub>8T<sub>OH</sub>8T<sub>OH</sub>8C<sub>c</sub>G<sub>c</sub>11C<sub>c</sub>G<sub>c</sub>)<sub>2</sub> (duplex II) were prepared as described before (20). Purified samples (~160 nM units) were dissolved in 0.6 ml of the following buffer for NMR measurements: 100 mM NaCl, 10 mM Na<sub>2</sub>PO<sub>4</sub>, 10 µM EDTA, pH 7.0 in 90% H<sub>2</sub>O:10% D<sub>2</sub>O.

(B) NMR Experiment

<sup>1</sup>H NMR spectra were recorded on a Bruker DRX 500 NMR spectrometer (1H at 500 MHz). Phase-sensitive NOESY experiments with water suppression were achieved by the use of two short spinlock pulses, SL<sub>94</sub> and SL<sub>108</sub>, as described by (26) using the following parameters: mixing times (τ<sub>m</sub>) were varied between 0.03 and 0.200 s to observe the spatial contact of the non-exchangeable protons with water; 4K complex data points in τ<sub>y</sub>, 512 complex data points in τ<sub>t</sub>, the relaxation delay between pulse sequence was 2.0 s. SL<sub>94</sub> and SL<sub>108</sub> are equal to 0.5 ms and 3 ms, respectively, the delay between spinlock pulses τ is equal to 167 μs, the carrier was set at the water frequency, 32 scans/FID were used for quadrature detection in F<sub>t</sub> - dimension with time proportional phase incrementation (TPPI). 2D data sets for ROESY spectra with the water suppression are achieved with one short spinlock pulse, SL<sub>94</sub> (26). During the mixing time sequence of n(π/6) pulses with length 3.4 μs separated by delay, Δ (34.5 μs) provides a similar effect as spin-lock SL<sub>94</sub> of the NOESY experiment, so that the spectra were recorded with spinlock duration between 0.03 and 0.10 s using 6.25 kHz rf field for all pulses and a recycle delay of 2 s. Typically 4K data points were collected for each τ<sub>i</sub> 512 values during experiments. A 3 ms saturation pulse is applied after data acquisition. The spectral excitation profile in these experiment is proportional to sin(Ωτ) where Ω is the angular frequency relative to the carrier and τ = 167 μs. The nonuniform spectral excitation in F<sub>2</sub>-dimension were corrected by multiplying with 1/sin(Ωτ) function. The assignment of all protons of duplexes (I) and (II) were done in a conventional manner, using NOESY and COSY DQF experiments. They will be published elsewhere together with structural analysis.

The troubling difference between water-[7′-α-methyl of (T<sub>M</sub>) and water-(Me-T) cross-peaks is the phase behaviour in the NOESY spectra (Figure 5) at shorter mix-
The ratio of NOESY and ROESY (ρ) cross-peak intensities was used to elucidate the correlation time, $\tau_{r}$, (18,23,38). If the bound water molecule undergoes rapid or large amplitude motion, the spectral density function becomes complex, and depends on the order parameter in the spectral density function (38). The theoretical evaluation of the transformation of correlation time from ratio/ or sign of $\sigma_{w1}$ to $\sigma_{w2}$ is model-dependent (8,23). However, it has been shown (23) for the Dickerson-Drew dodecamer, using NMR dispersion (NMRD) and NOESY/ROESY methods, that there is a high degree of orientation order with little local motion during the residence time of water molecule in the minor groove. However, in major groove the motion of water can be unrestricted. With this in mind, we have interpreted the ratio/ or sign of $\sigma_{w1}$ to $\sigma_{w2}$ of water-DNA cross-peaks and correlation time in this work assuming the approximation of rigid binding for water in the minor groove for all three dodecamers (I), (II) and Dickerson-Drew, because of the width of the minor groove in the NMR spectra and the first-shell water position in the X-ray structure (duplex I) have remained unchanged. Hence, all through our evaluation of signs of crosspeaks of water-H2A or 7'-α-methyl (Tm) of DNA in the NOESY and ROESY experiments, the following criteria were applied: The change of positive to negative NOE sign corresponds to the correlation time of ~0.36 ns (at 500 MHz) and the positive/negative NOE sign are less/more than 0.36 ns, respectively. Note, no comparison has however been used amongst the positive signs or the negative signs.

(C) Melting measurements. UV melting profiles were obtained by scanning $A_{260}$ absorbency versus time at heating rate of 1°C/min and temperature gradient 20-80°C (60 min). The $T_m$s were calculated from the maximum points of the first derivatives of the melting curves. For thermodynamic calculations, an average of five $T_m$ values were used at each concentration of 8, 12, 16, 20 and 24 μM. All measurements were carried out in 200 mM Na2HPO4/NaH2PO4, 1M NaCl buffer at pH 7.3. Before each melting experiment denaturation and renaturation of the samples were carried out by heating solutions to 80°C for 15 min followed by slow cooling to the RT and keeping it standing at RT overnight.

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References and Footnotes
