Conformational analysis of r(CGCGCG) in aqueous solution: an A-type double helical conformation studied by two-dimensional nuclear Overhauser effect spectroscopy

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ABSTRACT

The conformation of the hexanucleoside pentaphosphate r(CGCGCG) in aqueous solution was studied by circular dichroism, $^1\text{H}$- and $^{31}\text{P}$-NMR spectroscopy. The base-, H1'- and H2'-proton resonances were assigned by means of 2D-NOE spectroscopy. The base- and H1'-proton chemical shifts were studied as a function of temperature. Proton-proton distances are computed in A- and A'-RNA as well as in A-, B- and Z-DNA. A qualitative interpretation of the observed 2D-NOE intensities shows that r(CGCGCG) adopts a regular A-type double helical conformation under our experimental conditions. The CD- and $^{31}\text{P}$-NMR experiments described in this paper are in agreement with this structure both under low- and high-salt conditions.

INTRODUCTION

X-ray diffraction studies of DNA-fibres and DNA-crystals have shown that DNA conformations can be divided in three major classes, viz. A-, B- and Z-DNA. Apart from morphological differences, A- and B-DNA are right-handed double helical structures which differ at the atomic level mainly in the pucker of the sugar rings (N-type (C3'-endo) for A-DNA; S-type (C2'-endo) for B-DNA) and in the glycosidic torsion angle ($\chi$). The third conformation, Z-DNA, was discovered by Wang et al. in a X-ray diffraction study of a single crystal of d(CGCGCG) grown in the presence of spermine. Later, this form was also observed in fibres of poly-d(GC)•poly-d(GC), poly-d(GT)•poly-d(AC) and poly-d(AsT)•poly-d(AsT). Features of this Z-type conformation can be summarized as follows: [1] the structure seems to be restricted to alternating purine-pyrimidine base sequences; [2] the glycosidic torsion angle is syn for the purine bases and anti for the pyrimidine bases; [3] the sugar rings alternately take up an N-type (for the purine moieties) and S-type (for the pyrimidine moieties) conformation and [4] the phosphodiester backbone torsion angles $\alpha$ and $\zeta$ adopt a ($g^+, g^+$) conformation in the pyrimidine-purine (py-pu) dimeric units and a ($g^-, t$) conformation in the purine-pyrimidine (pu-py) dimeric units of Z-DNA.
Before Z-DNA was discovered, Pohl and Jovin found a striking change in the CD spectrum of poly-d(GC)*poly-d(GC) upon addition of salt (NaCl, LiCl, CsCl or MgCl₂) or ethanol: a positive Cotton effect around 290 nm was observed for poly-d(GC)*poly d(GC) under low salt conditions whereas a negative Cotton effect around 290 nm was found under high-salt (4M NaCl) conditions. The CD spectra of d(CGCGCG) displayed a similar salt dependent change and it was shown by Thamann et al. that a B- to Z-DNA conformational transition was responsible for this inversion of the Cotton effect around 290 nm in the CD spectra of d(CGCGCG) and poly-d(GC)*poly-d(GC). Detailed CD and NMR studies showed that this B- to Z transition is inhibited by the addition of certain intercalating drugs. On the other hand, oligo- or poly-cationic organic molecules such as spermine or poly(arginine) have been reported to stabilize the Z-type conformation of poly-d(GC)*poly-d(GC) and related polynucleotides.

As of to date X-ray studies of RNA duplexes have come up only with structures belonging to the A-type conformation of double helices. However, when poly-r(GC)*poly-r(GC) was found to display a negative CD effect in the region of 290 nm even under low salt conditions, Uesugi et al. suggested that this RNA duplex adopts a Z-like conformation. This conclusion, which was solely based on this negative CD effect, was questioned and, moreover, a recent calculation of the CD spectrum using revised monomer transition parameters predicted a negative long wavelength Cotton effect for r(CG)-duplexes in a normal A-type conformation. A recent CD and NMR study of r(CG)-oligonucleotides settled this controversy by showing that these molecules indeed adopt an A-type double helical conformation.

In order to elucidate the intimate conformational aspects of CG-sequences in RNA we synthesized the self-complementary hexamer r(CGCGCG) and studied it using CD and NMR techniques. 2D-NOE ³¹P-NMR enabled the assignment of all the base-, H1'- and H2'-proton resonances of the hexamer and showed that r(CGCGCG) adopts an A-type conformation in solution at low salt concentrations. CD spectra measured at several salt concentrations (NaCl as well as MgCl₂) and ³¹P-NMR spectra recorded in the presence of NaCl and methanol gave no indications for an A-to-Z transition under these conditions.

MATERIALS AND METHODS

The compounds r(CGCGCG) and d(CGCGCG) were synthesized via an improved phosphotriester method. After purification, the compounds were treated with Dowex 50W cation-exchange resin to yield the sodium salts, followed by desalination on Sephadex G10.
CD samples were dissolved in a 10 mM sodium phosphate buffer, pH = 7.0; salt (NaCl or MgCl₂) was added to obtain the appropriate salt concentrations. Nucleotide concentrations were determined spectrophotometrically; extinction coefficients at room temperature for r(CGCGCG) (ε₂₅₄ = 48,800) and d(CGCGCG) (ε₂₅₄ = 49,200) were inferred from snake venom phosphodiesterase digestion experiments using extinction coefficients of the constituent nucleotides and nucleosides.

CD spectra were measured on a CNRS Roussel-Jouan III Dichrograph (Jobin Yvon, France) interfaced with an Apple II computer. The spectra were digitized, stored on floppy disks and transferred to an Amdahl V7B computer. The data were baseline corrected, smoothed, plotted and integrated by a modified version of the program CDSTAK.

NMR-samples were lyophilized three times from D₂O (99.75%) and finally dissolved in D₂O (99.95%). The pH was adjusted to 7.0 (meter reading) and TMA (0.5 mM) was added as an internal reference. The samples were degassed and sealed under nitrogen gas in a 5 mm NMR-tube. Oligonucleotide concentrations were determined spectrophotometrically.

¹H-NMR spectra were measured on a Bruker WM-500 spectrometer interfaced to an ASPECT-2000 computer and a real time pulser board. Suppression of the residual HDO-peak in the 1D spectra was achieved by applying the DASWEFT pulse scheme. Chemical shifts are reported relative to TMA; they may be converted to the more familiar DSS (or TMS) scale by adding 3.18 ppm to the quoted values. Exact sample temperatures were derived from the chemical shift of the residual HDO-peak.

The 2D-NOE spectrum was recorded using the standard pulse scheme:

\[(90° - t₁ - 90° - τₘ - 90° - t₂)\]

with a mixing period of 0.6 sec. In order to reduce the solvent signal a selective, continuous irradiation of the water resonance was applied at all times except during the observation period \(t₂\).

Phosphorus-31 NMR spectra were acquired on a Bruker WM-300 spectrometer operating at 121.53 MHz. Heteronuclear broadband proton decoupling was used throughout; \(^{31}\)P chemical shifts are reported relative to external phosphoric acid.

RESULTS AND DISCUSSION

Analysis of the spatial structure of r(CGCGCG) by means of 2D-nuclear Overhauser enhancement spectroscopy.

Two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) is
a powerful and efficient method for studying oligonucleotides in aqueous solution. With a single instrument setting the NOESY experiment provides a complete set of proton-proton Overhauser effects in the molecule at issue and thus map all interproton distances of, say, less than 5 Å. It has been shown that these NOE "connectivities" can be used for the assignment of the individual proton resonances in the NMR spectrum. In the present paper we will demonstrate that the NOESY-data also allow discrimination between A-, B- and Z-type conformations of double helical oligonucleotides.

The 2D-NOE spectrum of r(CGCGCG) recorded at 297 K is presented as an
Fig. 2: Expansions of the NOESY contourplot of r(CGCGCG) given in Fig. 1, showing (A) the cross peaks between the base- and H1'-protons, (B) the cross peaks between the H1'- and the remaining ribose protons, (C) the cross peaks between the base protons and the H2'/H3'/H4'/H5'/H5'' protons.

absolute value contour plot in Fig. 1. At this temperature the molecule is in its dimeric form as will be shown in a following section. The contours (= peaks) along the diagonal arise from the protons that did not cross-relax with other protons during the mixing time $t_m$ and therefore display a gross correspondence with the normal 1D-spectrum (see Fig. 1, top trace). The off-diagonal contours (= cross peaks), which appear symmetrically with respect to the diagonal and characterized by e.g. the coordinates $(\delta_A, \delta_B)$ and $(\delta_B, \delta_A)$, manifest NOEs between the protons resonating at $(\delta_A, \delta_A)$ and $(\delta_B, \delta_B)$, respectively.
The cross peaks between the aromatic base proton region (around 4.5 ppm) and the H1'-region (around 2.5 ppm) are presented on an expanded scale in Fig. 2A. The NOE cross peaks interconnecting the doublet resonances ($^3J = 7$ Hz) in the aromatic region (typical for C-H6 protons) to the doublets in the H1'-region (typical for C-H5 protons), cf. Fig. 2A - dashed lines, are readily identified as the intra-base NOEs between the H5- and H6-protons of the three cytosine moieties in the r(CGCGCG) molecule. The remaining cross peaks all arise from NOEs between aromatic base protons on the one hand and H1' sugar protons on the other.

In order to rationalize the latter NOE cross peaks an analysis of the inter-proton distances occurring in the three families (A, B and Z) of double helices is mandatory. Table I summarizes the relevant proton-proton distances which were calculated by means of the computer program BUILDER on basis of the published X-ray coordinates for the aforementioned double helical structures. Perusal of these interproton distances shows that in the righthanded A-form as well as in the righthanded B-form the purine H8 or pyrimidine H6 base proton is not only close to its intranucleotide H1'-proton (3.6 - 3.9 Å), but also to the H1'-proton of its 5'-adjacent nucleotide (2.8 - 4.6 Å). It is therefore expected that in a 2D-NOE experiment each of the purine H8 and pyrimidine H6 base protons (except those at the 5' termini) will be connected via NOE cross peaks to two H1'-protons.

Inspection of the corresponding distances in the lefthanded Z-type double helix indicates that the NOE cross peak pattern expected for an oligonucleotide duplex in a Z-type conformation will be quite different. The cytidine H6 base proton is again roughly equidistant to both its intranucleotide and its 5'-adjacent internucleotide H1'-proton (ca. 3.5 Å), but the guanosine residues display a relatively short intranucleotide G-H8/G-H1' distance of ca. 2.6 Å in combination with a large 5'-adjacent internucleotide G-H8/C-H1' distance of ca. 6.5 Å. As the latter distance surpasses the limit that can be detected by NOEs, one expects the guanosine H8-base protons in Z-DNA to be connected only to its intranucleotide H1'-proton via a NOE cross peak (with an intensity comparable to the NOE cross peaks connecting the intranucleotide C-H5/C-H6 protons).

Turning to Fig. 2A it is seen that all base protons but one display NOE cross peaks with two H1'-protons, thus ruling out a lefthanded Z-like conformation for r(CGCGCG), but in agreement with a righthanded double helical conformation (A or B).

Armed with the knowledge that the double helical structure of the r(CGCGCG) duplex is righthanded, the base- and H1'-proton resonances can be assigned using the procedure described by (amongst others) Haasnoot et al. 29.
Table 1.
(a) Calculated intranucleotide proton-proton distances.

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<td>2.9 Å</td>
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<tr>
<td>G-H8/G-H3'</td>
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(b) Calculated internucleotide proton-proton distances for the base protons of nucleotide (n) to the ribose protons of the 5'-adjacent ribose ring (n-1).

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<tr>
<td>G-H8/G-H3'</td>
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1Distances calculated on basis of the torsion angles given in ref. 33. Note that some of the interproton distances are unrealistically small (<2.0 Å) due to the assumption of a rigid, uniform geometry and the omission of taking hydrogen atoms into account in the derivation of the A- and B-models. In reality these distances will certainly increase to values in the range of 2.0 - 2.3 Å.

2Distances calculated on basis of the torsion angles given in refs. 6 and 35.

In Fig. 2A it is seen that there is one base-proton (at 4.85 ppm) which displays only one cross peak to the H1'-protons. This must be the H6 base proton of the rC(1)-residue, because this residue has no 5'-neighbour so only one (intranucleotide) NOE cross peak is to be expected. This particular assignment can be verified by taking a closer look at Fig. 1: the intranucleotide NOE cross peaks that link the 5'-terminal H5'/H5"'-protons (which always resonate at the highest field if a 5'-phosphate is absent) to their corresponding rC(1)-H6 base proton (cf. drawn lines in Fig. 1), also assigns the H6-proton at 4.85 ppm to the rC(1)-residue [C(1)-H6]. Fig. 2A shows that the H1'-proton of this residue [C(1)-H1'] resonates at 2.33 ppm, moreover, the resonance at 2.81 ppm is now readily assigned to the C(1)-H5 proton (dashed lines).

It follows from the data summarized in Table 1 that C(1)-H1' should also
Fig. 3: Average proton-proton distances between the H1'-proton and the H2'-, H3'- and H4'-protons in a N-type (C3'-endo) ribose ring.

display a NOE cross peak to G(2)-H8. Following the arrowed line in Fig. 2A, starting at the cross peak connecting C(1)-H6 and C(1)-H1', we indeed find this connectivity thereby leading to the conclusion that G(2)-H8 resonates at 4.64 ppm. The second NOE cross peak found for this G(2)-H8 resonance identifies the G(2)-H1' resonance (at 2.52 ppm). Persisting this line of reasoning, all the base- and H1'-protons are readily assigned (cf. the arrowed lines in Fig. 2A); the result of this assignment procedure is summarized in Fig. 2A (top- and right-trace).

The assignment of the 1H-NMR spectrum of r(CGCGCG) is easily extended to the H2' protons of this molecule. Figure 3 gives the intranucleotide proton-proton distances between the H1'-proton on the one hand and the H2', H3' and H4'-protons on the other hand. These distances were calculated for a ribose ring having an N-type (C3'-endo) conformation (which is the dominating conformer under the conditions employed, vide infra). The shortest distance calculated is the H1'/H2' distance (ca. 2.8 Å), from which it follows that the most intense NOEs that are found in the region containing the cross peaks that link the H1'-protons to the H2', H3', H4', H5' and H5''-protons, originate from NOEs between H1' and H2' protons belonging to the same sugar residue. In Figure 2B it is seen that for each H1'-proton indeed one cross peak occurs which is much stronger than the remaining cross peaks observed for that particular H1' proton. Using these specific connectivities in combination with the assignment of the H1'-protons (vide supra) one is able to assign all the H2'-resonances in the r(CGCGCG) molecule (dashed lines in Fig. 2B); Fig. 2B (top trace) summarizes the results for this part of the assignment.

Inspection of the interproton distances listed in Table I shows that there are some characteristic distances which may allow discrimination between A- and B-like double helical structures in nucleic acids. For instance, in A- and A'-RNA the distance between the base proton (C-H6 or G-H8) and the H2' of its
"own" sugar ring is relatively large (ca. 4.0 Å) compared to the distance between that base-proton and the H2' of its 5'-neighbouring sugar ring (ca. 1.8 Å). In B-DNA it is just the other way around: the intranucleotide base proton/H2' distance is small (ca. 2.0 Å) in comparison with the internucleotide base proton/H2' (of the 5'-adjacent nucleotide) distance (ca. 3.9 Å).

In Fig. 2C the region of the 2D-NOE spectrum is shown in which the cross peaks occur between the base- and H2'-protons. Using the assignments described above (Figs. 2A and 2B), it is found that all base-protons, except of course that of the rC(1')-residue, display larger NOEs (i.e. larger intensities for the NOE cross peaks) to the H2'-proton of their respective 5'-neighbouring sugar ring than to their "own" (intranucleotide) H2'-proton. This is illustrated in Fig. 2C for the C(3')-H6 and the G(6')-H8 base protons (drawn lines to their "own" H2'-protons and dashed lines to the H2'-protons of the 5'-neighbouring sugar rings). Since NOE cross peak intensities vary, ceteris paribus, with the inverse sixth power of the distance between interacting protons, this observation indicates that the internucleotide base proton/H2' distance is smaller than the intranucleotide base proton/H2' distance, hence it is concluded that the r(CGCGCG) duplex adopts an A-type double helical conformation in aqueous solution under low salt conditions.

One-dimensional 1H-NMR of r(CGCGCG).

X-ray diffraction studies have shown that in general the sugar rings in A-type helices take up an N-type conformation, while the sugar rings in B-type helices adopt an S-type conformation, but exceptions to this rule have been reported. On basis of a generalized Karplus equation, Altona reported that an N-type sugar ring is characterized by a small coupling constant $J_{1'-2'}$ of ~1 Hz while an S-type sugar ring features a $J_{1'-2'}$ of ~8 Hz. All H1'-protons but one appear in the resolution enhanced 500 MHz NMR spectrum (not shown) as singlets (i.e. $J_{1'-2'} \leq 1$ Hz), from which it is concluded that all sugar rings but one adopt pure N-type conformations. In other words, r(CGCGCG) adopts a regular A-type double helical conformation. The exception is formed by G(6) of which the H1' resonance displays a coupling constant $J_{1'-2'}$ of ca. 2.5 Hz, thereby indicating that the ribose ring positioned at the terminus of the double helix retains some conformational freedom. The origins of this phenomenon have not been established but it is noted that similar "end effects" have been observed in other double helical RNA fragments by Petersheim and Turner as well as in a double helical DNA fragment by Sanderson et al.

Figure 4 shows the chemical shift versus temperature profiles for the C-H6 and G-H8 base protons (A), the C-H5 base protons (B) and the H1' sugar pro-
Fig. 4: Chemical shifts versus temperature profiles recorded for the base- and H1'-protons of r(CGCGCG), 5 mM in D2O, 0.1 M NaCl, pH = 7.4; (A) G-H8 and C-H6 base protons, (B) C-H5 base protons, (C) H1' protons. It is seen that at high temperatures, where r(CGCGCG) is in a random coil conformation, the resonances belonging to the various groups of protons (e.g. the C-H6 protons) all attain very similar chemical shifts. This phenomenon, together with the steep sigmoidal melting curves, especially those of the G(4)-H8, C(5)-H6, G(6)-H8, C(3)-H5, C(5)-H5, C(3)-H1' and C(5)-H1' protons, indicates that these chemical shift profiles monitor the double helix to random coil transition with a melting temperature (Tm) of ca. 338 K.

Another aspect of these melting curves we want to draw attention to is the
temperature dependence of the chemical shifts of the G(6)-H8, C(1)-H6 and C(1)-H1' protons. In contrast to the shifts of the other protons, the former display significant chemical shift changes below 315 K. Since the protons involved are located at the termini of the double helix this phenomenon is clearly the expression of an (other?) "end effect". Judging from the profile displayed by especially the C(1)-H1' proton the gradual chemical shift changes below 315 K cannot or only partially be explained by fraying phenomena occurring at the end of the duplex. Instead, we ascribe these chemical shift changes at lower temperatures to intermolecular association of double helical fragments. It is tempting to relate such a duplex association to the intermolecular stacking arrangements found \(^{36,41}\) for A-type helices in the solid state where the flat end base pair of one duplex is in close proximity to the sugar phosphate backbone in the shallow minor groove of another duplex, but the data at hand do not give rise to such an interpretation although they neither dismiss this possibility. However, the B-type duplex formed by d(GGC*C*GGCC) displays \(^{40}\) similar chemical shift "end effects" as found in the present case of r(CGCGCG). These effects were ascribed \(^{40}\) to fraying of the terminal base pair but since the observed chemical shift changes at lower temperatures are of the same order of magnitude and have the same sign as the chemical shift changes induced by the random coil to duplex transition, intermolecular duplex association for d(GGC*C*GGCC) is a more likely explanation. This being the case, the NMR data obtained for r(CGCGCG) and d(GGC*C*GGCC) are to be interpreted in terms of head-to-head and/or head-to-tail types of duplex association since intermolecular stacking as described above for A-type helices are supposed \(^{41}\) not to take place in duplexes having a B-type conformation.

\(^{31}\)P-NMR spectroscopy.

Phosphorus-31 NMR chemical shifts can provide \(^{42}\) conformational information about the phosphodiester backbone torsion angles \(\alpha\) and \(\zeta\). The two phosphodiester conformations present in Z-DNA (\(g^+, g^+\) and \(g^-, t\)) should give rise to a token difference in chemical shifts for the corresponding phosphorus resonances, and, indeed, such a difference (\(-1.5\) ppm) has been reported by e.g. Patel et al. \(^{43}\) for poly-d(GC*)•poly-d(GC*) in a Z-DNA conformation. Figure 5 presents the broadband proton decoupled \(^{31}\)P-NMR spectra of r(CGCGCG) measured at 298 K in D\(_2\)O containing methanol and NaCl in several concentrations, viz. 0.1 M NaCl (bottom), 0.1 M NaCl + 37.5% methanol (middle) and 3.5 M NaCl (top). It is seen that the dispersion of the five \(^{31}\)P-resonances spans a region of less than 0.6 ppm under these conditions. Hence, the phosphodiester torsion angles \(\alpha\) and \(\zeta\) in r(CGCGCG) are all very similar as is expected for a regular
A- (or B-) type double helix (all phosphodiesters characterized by \(g^-, g^-\)). The \(^{31}\)P-NMR spectra therefore exclude a Z-like RNA structure in r(CGCGCG) under low salt conditions as well as at high methanol or NaCl concentrations.

Circular Dichroism of r(CGCGCG) and d(CGCGCG).

In Figure 6 the CD spectra of r(CGCGCG) are shown at several NaCl and MgCl\(_2\) concentrations (top), together with the CD spectra of d(CGCGCG) recorded under corresponding conditions (bottom). In the right upper corner of each spectrum the band intensities at different wavelengths are plotted as a function of the salt concentration.

The CD spectrum of d(CGCGCG) shows the same salt concentration dependence as the CD spectra of poly-d(GC)*poly-d(GC)\(^8\) and d(C*GC*GC*G)\(^44\) which was ascribed\(^10\) to a B-to-Z transition. In r(CGCGCG) such a salt concentration dependence is absent (Fig. 6) which indicates the absence of a major conformational change, as for instance an A-to-Z transition, at the higher salt concentrations.

Ethidium bromide is known to reverse the B-to-Z transition of DNA at high salt concentrations\(^12,13\), and as a consequence the negative Cotton effect around 290 nm displayed by poly-d(GC)*poly-d(GC) at high salt concentrations...
Fig. 6: CD spectra of r(CGCGCG), top, and d(CGCGCG), bottom, at different salt concentrations: 1.0 M NaCl (1); 3.0 M NaCl (2); 5.0 M NaCl (3); 10 mM phosphate buffer (4); 0.5 M MgCl$_2$ (5) and 1.0 M MgCl$_2$ (6). Single strand concentration ~0.02 mM, T = 280 K. The insets show the integrated band intensities as a function of salt concentration: 260-270 nm (squares), 280-290 nm (circles) and 285-295 nm (triangles).

Fig. 7: CD-spectra recorded for 0.02 mM r(CGCGCG) in water, 0.1M NaCl, T = 275 K, before (-----) and after (------) addition of 0.01 mM ethidium bromide.
disappears when ethidium bromide is added to the sample. When ethidium bromide was added to a CD sample of r(CGCGCG), the negative effect around 290 nm did not disappear (Fig. 7), again indicating that this negative CD effect does not originate from a Z-like structure of r(CGCGCG) but arises as a consequence of the C-G base sequence in a normal A-type conformation.

CONCLUSIONS

The $^1$H-NMR chemical shift vs. temperature profiles recorded for the base- and H1'-protons showed that below 315 K r(CGCGCG) adopts a double helical structure. From the 2D-NOESY experiment it was inferred that the double helix is of the A-type. The H1'-H2' vicinal coupling constants indicate that all sugars attain a pure N-type conformation except for the terminal base pairs where some conformational freedom retains. Linebroadening and precipitation of the hexamer prohibited the recording of $^1$H-NMR spectra at high salt conditions, but from the $^{31}$P-NMR and CD spectra it is inferred that the A-type double helical conformation is retained when salt or methanol is added.

The CD experiments with salt and ethidium bromide make a Z-like structure of r(CGCGCG) unlikely, even at high salt concentrations, in spite of the negative Cotton effect around 290 nm.

ACKNOWLEDGEMENTS

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*To whom correspondence should be addressed

NOTES AND REFERENCES

1. Abbreviations used:

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<th>Abbreviation</th>
<th>Definition</th>
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<td>r(CGCGCG)</td>
<td>cytidylyl (3'-5') gyanylyl (3'-5') cytidylyl (3'-5') guanylyl (3'-5') cytidylyl (3'-5') guanosine.</td>
</tr>
<tr>
<td>d(CGCGCG)</td>
<td>2'-deoxycytidylyl (3'-5') 2'-deoxyguanylyl (3'-5') 2'-deoxycytidylyl (3'-5') 2'-deoxyguanylyl (3'-5') 2'-deoxycytidylyl (3'-5') 2'-deoxyguanosine.</td>
</tr>
<tr>
<td>C*</td>
<td>5-methyl-2'-deoxyctydine.</td>
</tr>
<tr>
<td>CD</td>
<td>Circulair Dichroism.</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance.</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser Enhancement.</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million.</td>
</tr>
</tbody>
</table>
TMA = tetramethylammonium (nitrate).
DSS = 2,2-dimethyl-2-silapentane-5-sulphate (sodium form).
TMS = tetramethylsilane.


