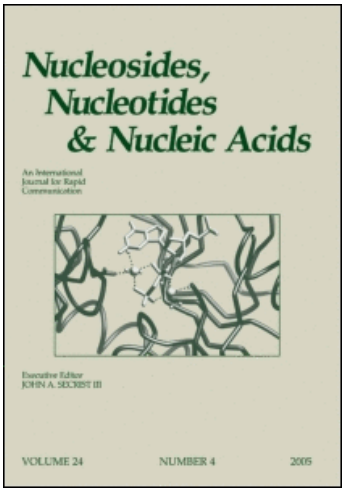

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Parallel-Stranded Duplex DNA and Self-Assembled Quartet Structures Formed by Isoguanine and Related Bases

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PARALLEL-STRANDED DUPLEX DNA AND SELF-ASSEMBLED QUARTET STRUCTURES FORMED BY ISOGUANINE AND RELATED BASES

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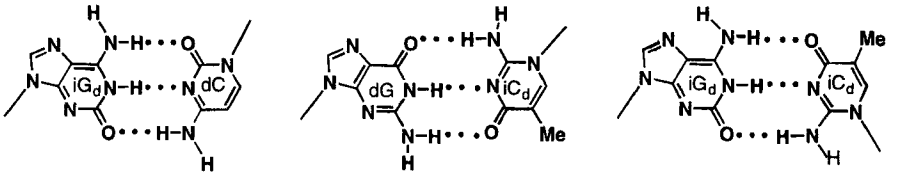
ABSTRACT: Parallel-stranded (ps) oligonucleotide duplexes are described containing isoguanine-cytosine and/or 5-methylisocytosine-guanine base pairs. A parallel hybrid is also formed when 5-aza-7-deazaguanine base pairs with guanine while the base pair with isoguanine results in an antiparallel duplex. Oligomers such as d(T_4isoG_4T_4) form self-assembled tetraplexes which show a cation selectivity different from that of the G-quartet.

INTRODUCTION

Parallel-stranded DNA structures (ps-DNA) can be formed by oligonucleotide duplexes, triplexes and in tetrameric aggregates. A high dA-dT content and a special sequence design is a prerequisite for a parallel-stranded arrangement of a DNA duplex structure.[1] Recently, a new type of parallel-stranded DNA duplexes has been described with oligonucleotides containing isoguanine-cytosine or isocytosine-guanine base pairs (motifes **I** and **II**).[2-4] As these modified base pairs are remarkably stable they dictate the strand polarity even in the presence of a high dA-dT content. It was also reported that parallel duplexes can be formed using other modified bases e.g. utilizing the base pair of 5-aza-7-deazaguanine and guanine.[5] Apart from the ability of modified bases to induce parallel duplex structures their self-assembly to higher aggregates is of interest. A tetrameric assembly of isoguanine-rich oligonucleotides was recently established.[6] It was shown that the hydrogen bonding pattern of this quartet is different from that of the guanine tetrad.[7]

This manuscript reports on oligonucleotide duplexes containing isoguanine or/and 5-methylisocytosine. When these oligonucleotides pair with a second strand

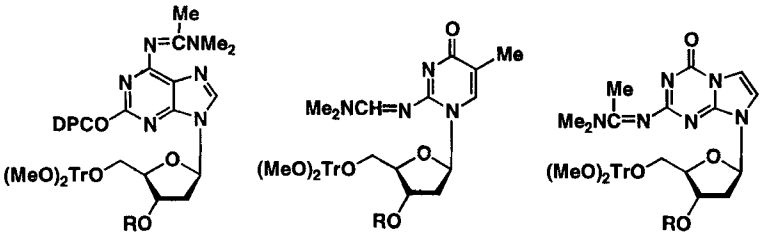
which contains cytosine or guanine parallel duplexes are formed while the base pairing between isoguanine and 5-methylisocytosine results in an antiparallel strand orientation (motif **III**).[8] As 5-aza-7-deazaguanine has the same donor-acceptor pattern as isocytosine its base pairing capability with guanine or isoguanine will also be investigated. Apart from the investigation on isoguanine duplex structures the ion-dependent stability of the isoG-quartet will be examined.



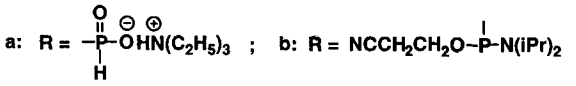
I (ps) **II** (ps) **III** (aps)

RESULTS AND DISCUSSION

Synthesis of Oligonucleotides. The oligodeoxyribonucleotides **4-20** were synthesized according to standard protocols of phosphoramidite or phosphonate chemistry.[3,9] The building blocks of $isoG_d$ (**1a,b**), $^{Me}iC_d$ (**2a,b**) and $c^7z^5G_d$ (**3a,b**) were prepared according to published procedures.[3,10,11]



1a, b **2a, b** **3a, b**



a: R = $-\overset{\overset{O}{\|}}{\underset{\underset{H}{|}}{P}}-O^{\ominus}H\overset{\oplus}{N}(C_2H_5)_3$; **b:** R = $NCCH_2CH_2O-\overset{|}{P}-N(iPr)_2$

The solid-phase oligonucleotide synthesis was performed in a 1 μmol scale on an Applied Biosystems ABI 392-08 synthesizer. The work-up followed the standard protocol. The oligonucleotides were purified on OPC™ cartridges (Applied Biosystems, USA). The purity of the oligonucleotides was proven by ion exchange HPLC on a

NucleoPac PA 100 column.[6] The nucleoside composition was determined by enzymatic hydrolysis using snake-venom phosphodiesterase followed by alkaline phosphatase and analyzed by RP-HPLC.[3]

Oligonucleotide Duplexes with Isoguanine-Cytosine and/or 5-Methylisocytosine-Guanine Base Pairs. The aps-duplex **5•7** was used as a standard for the investigation of various base pairing pattern within parallel-stranded or antiparallel-stranded oligonucleotide duplexes. The duplex **5•7** shows a T_m-value of 50.5°C (Table).

When one of the strands (**5** or **7**) contains $isoG_d$ instead of dG and 5-methyl-$isoC_d$ instead of dC and the sequence is arranged in a parallel orientation (oligomers **4** and **6**) hybrids are formed with T_m-values being 6°C (**6•7**) or 12°C (**4•5**) lower than that of the antiparallel duplex **5•7** (Table). The antiparallel duplex (**4•6**) containing $isoG_d$- $^{Me}iC_d$ base pairs exhibits a higher T_m-value than that of the antiparallel construct (**5•7**). A similar observation as observed on these duplexes has been made on hybrids with alternating bases such as **8•9** compared to **9•10**. From these results one could reason that duplexes containing isoguanine-cytosine base pairs are always less stable than their guanine-cytosine counterparts. It is demonstrated that this is not the case with the duplexes **11•12** in comparison to **12•13** as well as with **14•15** compared to **16•16**. In both cases the T_m-values of the parallel constructs are higher than those of their antiparallel counterparts (Table). A special characteristic of these oligonucleotides is their consecutive arrangement of the isoguanine residues.

CD Spectra. In order to investigate the conformational properties of ps-DNA in solution, the CD spectra of ps-**4•5**, ps-**6•7**, aps-**4•6**, and aps-**5•7** were examined in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM Na-cacodylate buffer (pH 7.0). The result is shown in Fig. 1.

The ps-oligonucleotides show negative lobes around 250 nm as it is observed for the aps-duplex **5•7**. However, compared to the CD spectrum of aps-**5•7** (B-DNA) the ps-duplexes **4•5** and **6•7** exhibit only weak positive bands near 275 nm. The CD spectra of $isoG_d$- and $^{Me}iC_d$-containing ps-DNA (ps-**4•5** and ps-**6•7**) are very similar to that of $isoG_d$- and $^{Me}iC_d$-containing aps-DNA (aps-**4•6**) but apparently different from that of unmodified B-DNA (aps-**5•7**). These differences are not only due to the change of the chain orientation but result also from the altered spectroscopic properties of the modified bases.

Table. T_m-Values of Oligonucleotides Modified by iG_d and/or $^{Me}iC_d$ [a]

ps-duplexes[b]	T_m(°C)	aps-duplexes	T_m(°C)
5'-d(ATiCiCAiGTTATiGA) (**4**) • • * * • * • • • • * • 5'-d(TAG GT C AATA CT) (**5**)	38	3'-d(ATCCAGTTATGA) (**7**) • • • • • • • • • • • • • 5'-d(TAGGTCAATACT) (**5**)	50.5
5'-d(TiCATAAiCTiGiGAT) (**6**) • * • • • • * • * • • • 5'-d(AGTAT TGA C CTA) (**7**)	44	5'-d(ATiCiCAiGTTATiGA)(**4**) • • * * • • * • • • • • * • 3'-d(TAiGiGTiCAATAiCT)(**6**)	58.5
5'-d(iGTiGTiGTiGTiGTiGT) (**8**) * • • • * • • * • * • * 5'-d(CA CA CA CA CA CA) (**9**)	47	3'-d(GTGTGTGTGTGT) (**10**) • • • • • • • • • • • • 5'-d(CACACACACACA) (**9**)	59
5'-d(iGiGiGAAA) (**11**) * * * • • • 5'-d(C C CTTT) (**12**)	29	5'-d(GGGAAA) (**13**) • • • • • • 3'-d(CCCTTT) (**12**)	<12
5'-d(iGiGiG C C C) (**14**) * * * * * * 5'-d(C C CiGiGiG) (**15**)	47	5'-d(GGGCCC) (**16**) • • • • • • 3'-d(CCCGGG) (**16**)	36

a) Measured in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.0;[3] the oligomer concentration is 5 μM. b) d(iC) = $^{Me}iC_d$ = 2'-deoxy-5-methylisocytidine.

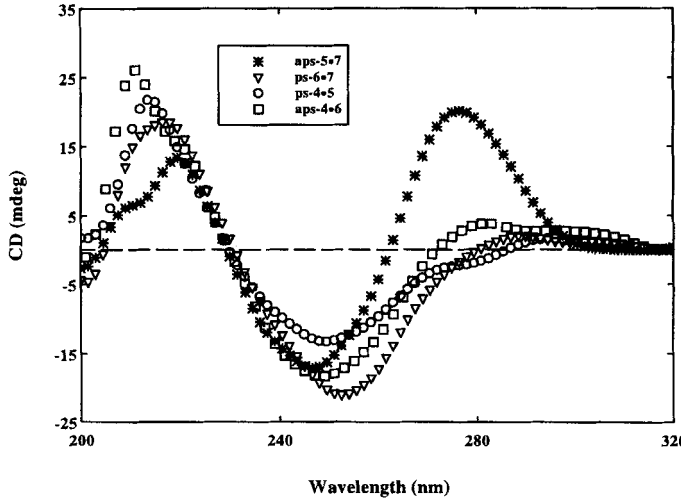


FIG. 1. CD spectra of ps-**4•5**, ps-**6•7**, aps-**5•7** and aps-**4•6** measured in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM Na-cacodylate buffer (pH 7.0) at 5°C. Oligomer concentration is 5 μM.

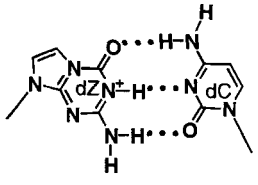
Oligonucleotide Duplexes with 5-Aza-7-Deazaguanine-Guanine or 5-Aza-7-Deazaguanine-Isoguanine Base Pairs. It has been reported that 5-aza-7-deaza-2'-deoxyguanosine ($c^7z^5G_d \equiv dZ$) is able to form stable base pairs with dC at low pH (motif **IV**), while they are destabilized under neutral conditions.[11] In this case the chain orientation is antiparallel. A parallel chain orientation occurs when 5-aza-7-deazaguanine pairs with guanine (ps-**17•18**, motif **V**).[5] As isocytosine has the same donor-acceptor pattern as 5-aza-7-deazaguanine, similar to the antiparallel isoC-isoG base pair (motif **III**), an aps-duplex formation can be anticipated between $c^7z^5G_d$ and $isoG_d$ (motif **VI**). This was proven with the oligonucleotides 5'-d(GGGZZZ) (**17**) and 5'-d(iGiGiGCCC) (**19**). They were hybridized and a stable duplex with a T_m-value of 41°C was measured (1 M NaCl, 0.1 M $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.0). According to the sequence of the oligonucleotides this duplex has an antiparallel chain orientation.

5'-d(G G G Z Z Z) (**17**)
* * * * * *
5'-d(Z Z Z G G G) (**18**)

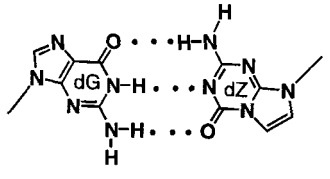
ps-**17•18**: T_m = 50°C

5'-d(G G G Z Z Z) (**17**)
* * * * * *
3'-d(C C C iG iG iG) (**19**)

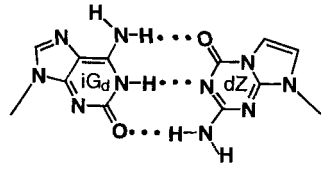
aps-**17•19**: T_m = 41°C



IV (aps)



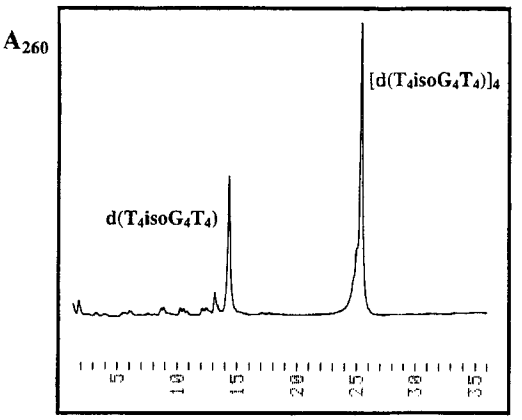
V (ps)

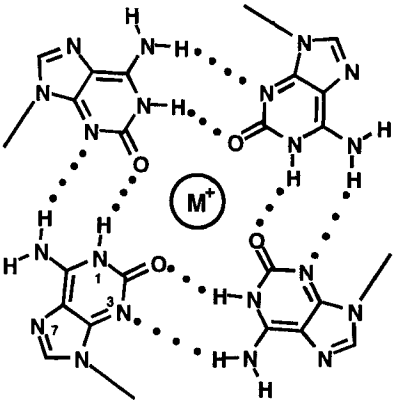


VI (aps)

Tetrameric DNA Formed by Isoguanine-Quartets. Oligodeoxyribonucleotides containing short runs of guanines - such as $d(TG_4T)$, $d(T_4G_4)$ or $d(T_4G_4T)$ - form tetrads.[7] The assembly is built by Hoogsteen base pairing and the strands show parallel orientation. Earlier, the aggregation of poly(isoguanylic acid)[12] and monomeric isoguanine nucleoside[13] has been demonstrated. A quartet structure was also established for isoguanine-rich oligonucleotides.[3,6]

The oligonucleotide $d(T_4isoG_4T_4)$ (**20**) forms a tetramer by the self-assembly of the isoguanine-residues.[3,6] The ion exchange chromatography profile of $d(T_4isoG_4T_4)$ (**20**)





VII

FIG. 2. Ion-Exchange HPLC Profiles of 5'-d(T_4isoG$_4T_4$).

shows two well separated peaks with greatly different retention times (Fig. 2). Both peaks contain the same oligonucleotide **20**. The fast migrating zone contains the single-stranded oligomer **20** while the slow migrating peak represents an aggregate. By using oligonucleotides with 7-deazaisoguanine instead of isoguanine it was shown that the isoG quartet is formed by an aggregate lacking the Hoogsteen motif (motif **VII**).[14]

It is known from previous studies that monovalent cations stabilize the G-quartet structure. The ion-dependent stability of the guanine-quartet has been determined by gel electrophoresis under non-denaturating conditions,[7] circular dichroism[15] and free energy perturbation studies.[16] The stability decreases in the order $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$. The cation dependence of G-quartet is now studied by ion-exchange chromatography. For this purpose samples of the oligonucleotide d($T_4G_4T_4$) were heated to 90°C for 2 min in the presence of various monovalent cations (M^+ in motif **VII**) followed by a 7-days storage at -20°C to form aggregates. The samples were then applied to ion-exchange HPLC on a NucleoPac PA-100 column.[6] The relative amounts of single stranded and tetrameric oligonucleotides were estimated as a function of the ion radii. The stabilities were determined at two different temperatures on the basis of the peak areas (Fig. 3, left). In this case the cation dependence was the same as it was determined earlier by other techniques.

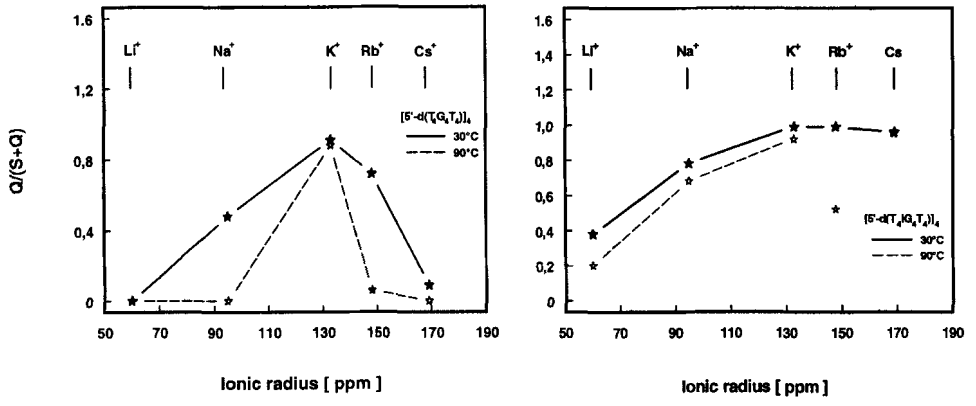


FIG. 3. Relative stabilities of $[d(T_4G_4T_4)]_4$ (*left*) and $[d(T_4isoG_4T_4)]_4$ (*right*) as a function of alkali ion radii. Data are taken from peak areas of ion-exchange chromatography (30°C and 90°C). *S* and *Q* correspond two areas of the monomeric and tetrameric species in the HPLC profiles.

The same experiment as performed with $d(T_4G_4T_4)$ was also carried out with the isoguanine-containing oligomer $d(T_4isoG_4T_4)$ (**20**). The correlation between ionic radii and quartet stability is shown in Fig. 3 (right). From these measurement it is concluded that the isoG quartet is generally more stable than the G-quartet.[17] Similar, as in case of the guanine-tetrad the most stable isoG-tetrad is formed in the presence of K^+ as the central ion. However, different from the G-tetrad also other cations form stable complexes. According to Fig. 3 (right) the tetrad with Rb^+ and Cs^+ are very stable and the complex with Na^+ seems to be also more stable as in the case of the self-assembled G4-structure. It is not clear whether Li^+ can also form such a complex or not, as a residual content of other cations cannot be excluded which may obscure this measurement. A very stable isoG-quartet with Cs^+ as the central ion has been found for the monomeric isoguanosine.[18] Due to these observations oligonucleotides with stretches of isoguanine are useful components for nanostructure devices which can act as ionophores with selective cation binding ability.

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