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PARALLEL-STRANDED DUPLEX DNA FORMED BY A NEW BASE PAIR BETWEEN GUANINE AND 5-AZA-7-DEAZAGUANINE

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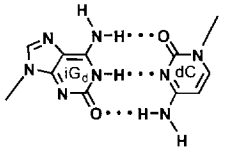
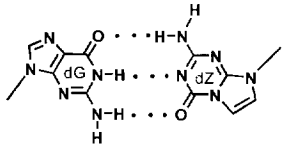
Abstract. Oligonucleotides containing 5-aza-7-deazaguanine residues have been synthesized. A DNA duplex with parallel strand orientation is assembled by a new base pair between guanine and 5-aza-7-deazaguanine. © 1997 Elsevier Science Ltd.

Naturally-occurring DNA forms double helices in which two strands are arranged in antiparallel orientation. However, also a parallel strand orientation is feasible in which both strands show in the 5'→3'-direction. Parallel strands (ps) are realized in triplexes[1,2] and tetraplex structures.[3] A predominant A, T-content is required for a parallel strand arrangement of DNA containing the four natural bases (ps-DNA).[4] Recently, our laboratory has reported on oligonucleotide duplexes which form parallel chains, exclusively.[5,6] They contain isoguanine-cytosine base pairs which dictate the strand orientation (motif **I**). These studies were performed on duplexes with purine-pyrimidine base pairs. A parallel duplex should also be accessible in the case of a "purine-purine" base pair.[7,8] These studies are now undertaken on a potential base pair formed by 5-aza-7-deazaguanine (purine numbering) and guanine (motif **II**).



I (ps)



II (ps)

For this purpose, the phosphoramidite **3** was synthesized from 5-aza-7-deaza-2'-deoxyguanosine (**1**, $c^7z^5G_d$ or dZ).[9] This nucleoside is related to 2'-deoxyguanosine but lacks the hydrogen at position N-1. As a result this position is now available as a proton acceptor site, while the other atoms are capable to form hydrogen bonding according to the Watson-Crick mode. For the solid-phase oligonucleotide synthesis compound **1** was protected with an amidine residue at the base and a DMT group at the OH-5'-position. The intermediate **2** was converted into **3** (Scheme). All new compounds were characterized by NMR spectra.[11]

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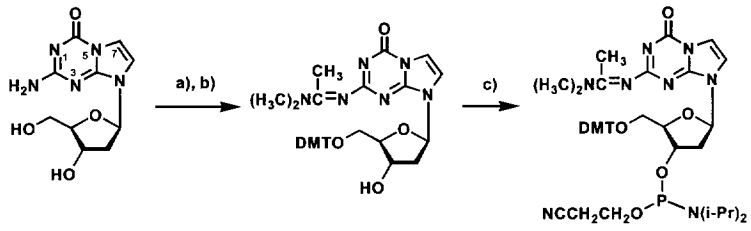


1 2 3

Scheme. *a) DMTrCl, pyridine, r.t., 3.5h, 57%. b) $(CH_3)_2NC(CH_3)(OCH_3)_2$, CH_3OH, 50°C, 2h, 90%. c) $EtN(iPr)_2$, $(iPr_2N)(NCCH_2CH_2O)PCl$, CH_2Cl_2, r.t., 30 min, 58%.*

The phosporamidite **3** was employed in solid-phase synthesis to yield the oligonucleotides 5'-d(G-G-G-Z-Z-Z) (**4**), 5'-d(Z-Z-Z-G-G-G) (**5**) and 5'-d(Z-Z-Z-Z-Z-Z) (**6**). The synthesis was performed on a 1 μM scale with an Applied Biosystems ABI 392-08 synthesizer. The work-up followed the standard protocol.[12] The oligonucleotides were purified on an OPC™ cartridge (Perkin Elmer Applied Biosystems, USA)[13] and, if necessary, by reversed-phase HPLC. The purity of the oligonucleotides was proven by ion exchange HPLC on a NucleoPac PA 100 column.[14] The nucleoside composition was determined by enzymatic hydrolysis using snake-venom phosphodiesterase followed by alkaline phosphatase and analyzed by reversed-phase HPLC.[5,6]

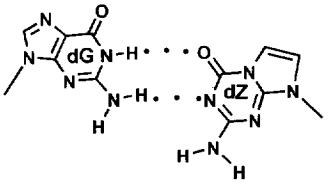
Both oligomers **4** and **5** contain three consecutive 5-aza-7-deaza-2'-deoxyguanosine and three 2'-deoxyguanosine residues. The only difference is their location either at the 5'- or the 3'-termini. In principle, both individual oligomers can form self-complementary duplexes with parallel or antiparallel chain orientation (ps-**4•4** or aps-**4•4**). The parallel duplex could contain three base pairs with *three* hydrogen bonds each between 5-aza-7-deazaguanine and guanine (motif **II**), while the antiparallel duplex is formed by six base pairs with only *two* hydrogen bonds between the bases (motif **III**). The same is expected for the duplex of **5•5**. Both duplexes (**4•4** and **5•5**) are expected to be quite labile. Nevertheless, an equimolar mixture of **4** and **5** should form a much more stable duplex when the strands adopt parallel chain orientation.

5' - d(G G G Z Z Z)
5' - d(G G G Z Z Z)

ps-**4•4**

5' - d(G G G Z Z Z)
3' - d(Z Z Z G G G)

aps-**4•4**



III (aps)

5' - d(G G G Z Z Z)
5' - d(Z Z Z G G G)

ps-**4•5**

In order to prove the duplex stability, temperature-dependent UV-measurements were performed. Both oligonucleotides **4•4** and **5•5** show sigmoidal melting profiles with low T_m-values (Table). This is the result of

self-pairing. The concentration dependence of the T_m-values (data not shown) indicates duplex melting and no destacking of single-stranded chains. According to the number of hydrogen bonds a duplex with antiparallel chain orientation is favored (aps-**4•4** or aps-**5•5**; motif **III**). However, the mixture of the oligomers **4** and **5** gives rise to a much more stable duplex showing a T_m-value of 50°C (Table, Figure). According to these findings the duplex **4•5** must adopt parallel strand orientation following the base pair motif **II**.

Table. T_m-Values of oligonucleotide duplexes[a]

Oligomers[b]		Duplexes	T_m [°C]
5'-d(G-G-G-Z-Z-Z)	**(4)**	**4•4**	20
5'-d(Z-Z-Z-G-G-G)	**(5)**	**5•5**	28
		4•5	50
5'-d(Z-Z-Z-Z-Z-Z)	**(6)**		
5'-d(c^7G)$_6$ **(7)**		**6•7**	42

[a] UV-measurements (260 nm) were performed in 1 M NaCl, 0.1 M $MgCl_2$ and 60 mM Na-cacodylate buffer, pH 7.0. Single strand conc. is 8 µM.
[b] dZ = $c^7z^5G_d$ = 5-aza-7-deaza-2'-deoxyguanosine; d(c^7G) = c^7G_d = 7-deaza-2'-deoxyguanosine.

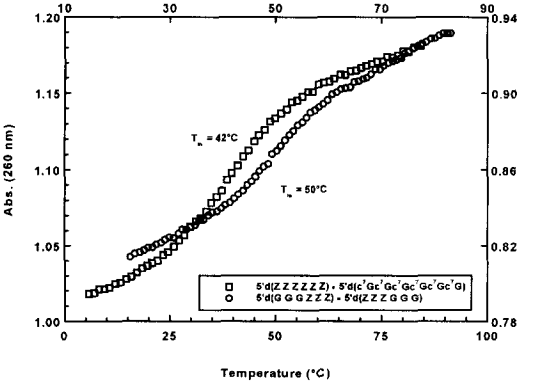
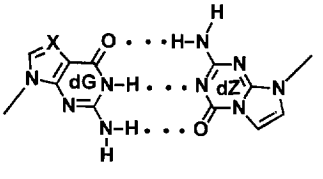
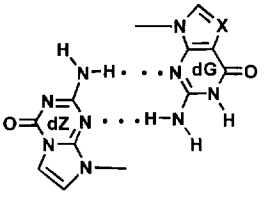


***Figure**: UV-Melting profiles of the duplexes* **4•5** *(o) (left and bottom scale) and* **6•7** *(□) (right and top scale); conditions see Table.*

Furthermore, the duplex formation between the homooligonucleotides 5'-d(Z-Z-Z-Z-Z-Z) **(6)** and 5'-d($c^7Gc^7Gc^7Gc^7Gc^7G$) **(7)** was studied. A stable duplex with a T_m-value of 42°C (Figure) was observed in this case (Table). As the oligomers **6** and **7** contain 7-deazapurine bases (7-deazaguanine and 5-aza-7-deazaguanine) lacking nitrogen-7 as acceptor site a base pair motif following the *Hoogsteen* mode can be excluded. However, two different motifs (**IV** and **VI**) still have to be considered. Both are in accordance with a parallel duplex structure, e.g. **6•7**, with the sugar residues in anti-conformation. The base pair **IV** is held together by *three* hydrogen bonds whereas only *two* hydrogen bonds contribute to the base pair **VI**. Consequently, the motif **IV** is the most likely one forming the highly stable duplex **6•7**. If one transfers these findings towards the hybrid of **4•5** the base pair motif **II** is a most likely pattern for the parallel alignment of the duplex structure.



II (ps, X = N)
IV (ps, X = CH)



V (ps, X = N)
VI (ps, X = CH)

In conclusion, 5-aza-7-deazaguanine forms a base pair with guanine resulting in stable oligonucleotide duplexes. These "purine-purine" base pairs direct the oligonucleotide strands into a parallel chain orientation thereby expanding the pairing modes of synthetic DNA.

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11. Compd. **2**: ^{1}H-NMR (d_6-DMSO): δ, 2.10 (s, 3 CH_3), 2.42 (m, H_α-2'), 2.56 (m, H_β-2'), 3.02 (2 CH_3), 3.15 (m, 2H-5'), 3.72 (s, 2 CH_3O), 3.91 (m, H-4'), 4.32 (m, H-3'), 5.4 0 (m, 3'-OH), 6.25 (t, H-1'), 6.73 (m, aromatic H), 7.19-7.45 (m, aromatic H + H-7 + H-8).
    Compd. **3**: ^{31}P-NMR ($CDCl_3$): δ, 149.45, 149.38.
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