7-Deazaisoguanine quartets: self-assembled oligonucleotides lacking the Hoogsteen motif

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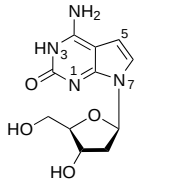
Oligonucleotides containing consecutive 7-deazaisoguanine residues form self-assembled quartets, indicating that the purine nitrogen-7 of isoguanine is not participating in the hydrogen bonding pattern.

Oligodeoxyribonucleotides with consecutive isoguanine residues, *e.g.* d(T_4isoG$_4T_4$),† form self-assembled structures.[1,2] Such aggregates have also been observed in the case of the homopolyribonucleotide poly(isoguanylic acid)[3] and the monomeric ribonucleoside isoguanosine.[4] Like the G-quartet the aggregate of isoguanine has properties of an ionophore.[5] The stability of the metal complexes depend on the size of the particular ion.[6] Ion-exchange HPLC experiments showed that the aggregate of d(T_4isoG$_4T_4$) has the same number of negative phosphate charges as that of d($T_4G_4T_4$). Due to this observation a tetrameric structure was also proposed for d(T_4isoG$_4T_4$).[2] The stoichiometry of this complex was established using isoguanine-containing oligonucleotides with different dT-tails at the 3′- and 5′-termini.[7] So far, the isoguanine-quartet shows behaviour similar to that of guanine. Although the tetrameric assembly of isoguanine has been verified, several structural motifs have been proposed for the quartet structure.[2–4]

Base-modified nucleosides are useful tools to probe the structure of such high-molecular assemblies, in particular those which are held together by hydrogen bonds. Regarding this it has already been shown that the base pairing pattern can be restricted to the Watson–Crick motif when 7-deazaguanine is replacing guanine.[8] As a result, Hoogsteen quartets cannot be formed and the oligonucleotides are unable to form aggregates.[9] Based on this principle, 7-deaza-2′-deoxyisoguanosine **1** (c^7iG_d) has been employed to probe the oligonucleotide quartet structure formed by 2′-deoxyisoguanosine **2** (isoG$_d$). New oligonucleotide quartets should be accessible when the purine nitrogen-7 does not participate in the hydrogen bonding pattern. For this purpose the oligonucleotide building blocks **3** and **4** were synthesised. Oligonucleotides with consecutive 7-deaza-isoguanine residues were prepared and their ability to form self-assembled aggregates was studied.



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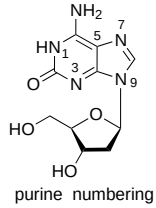

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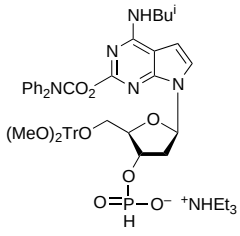

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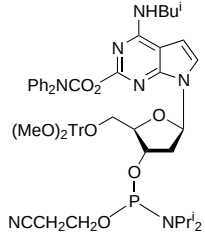

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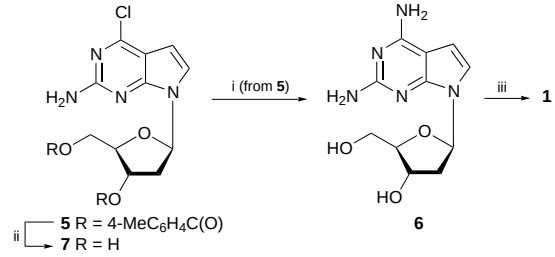


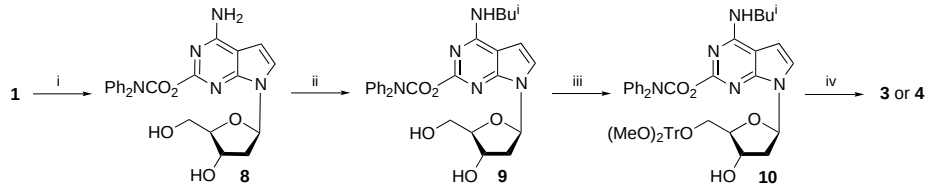
Scheme 1 *Reagents and conditions*: i, 25% NH_3–dioxane (1:1 v/v), 120 °C, 3 d, 88%; ii, 25% NH_3–dioxane (1:1 v/v), 60 °C, 3 d, 80%; iii, $NaNO_2$, 20% aq. AcOH, 67%

7-Deaza-2′-deoxyisoguanosine **1** has already been prepared (i) by convergent nucleoside synthesis[10] or (ii) by photosubstitution on 2-chloro-7-deaza-2′-deoxyadenosine.[11] Now, a more efficient route has been employed using glycosylation of the 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine anion[12] with 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride. The resulting nucleoside **5** was treated with conc. NH_3–dioxane (autoclave, 120 °C, 3 d) to give 2-amino-7-deaza-2′-deoxyadenosine **6**.[13] The deprotected compound **7** was also obtained (60 °C, conc. NH_3–dioxane, 3 d). Selective deamination of **6** ($NaNO_2$ in aq. AcOH) furnished the nucleoside **1** (Scheme 1). It is stable in acidic solution whereas 2′-deoxyisoguanosine **2** is an extremely labile nucleoside.[1]

For the preparation of **3** and **4**, compound **1** was blocked on the 2-oxo function with a $Ph_2NC(O)$ group (Ph_2NCOCl, Pr^i_2NEt–pyridine, room temp.) to give derivative **8**. The isobutyryl residue was then introduced to protect the amino group, yielding compound **9**. Standard conditions were employed to block the 5′-hydroxy group with a DMT residue† (**9** → **10**).‡ The phosphonate **3** as well as the phosphoramidite **4** were prepared from **10** according to known procedures (Scheme 2).

As discussed above, DNA quartets have been constructed from oligonucleotides containing short runs of isoguanine, *e.g.* d(T_4isoG$_4T_4$).[1,2] These self-assembled structures were identified by HPLC ion exchange chromatography [Fig. 1(*a*)].[2] Their chromatographic behaviour was almost identical to that observed for d($T_4G_4T_4$). The chromatogram shows two peaks, a fast migrating peak for the monomeric oligonucleotide and a slow migrating peak for the tetramer (Fig. 1). The same experiment was then performed with oligonucleotides containing consecutive 7-deazaisoguanine residues.§ For this purpose, the oligomers 5′-d($T_4c^7iG_4T_4$) **11** and 5′-d($T_2c^7iG_6T_4$) **12** were prepared by solid-phase synthesis.

The oligomer 5′-d($T_4c^7iG_4T_4$) **11** gives only one peak when chromatographed on an ion exchange HPLC column under the conditions described for d(T_4isoG$_4T_4$) [Fig. 1(*b*)].[2] This fast migrating peak contains only the single-stranded oligomer. However, when the number of consecutive c^7iG_d-residues is increased from four to six by replacing another two dT-residues as in the oligomer d($T_2c^7iG_6T_4$) **12**, a slow migrating zone



Scheme 2 *Reagents and conditions*: i, Ph_2NCOCl, 80%; ii, Me_3SiCl, Bu^iCl, 80%; iii, DMTCl, 51%; iv (for **3**) PCl_3, *N*-methylmorpholine, 1*H*-1,2,4-triazole, 93%; (for **4**) $(NCCH_2CH_2O)(Pr^i_2N)PCl$, $EtNPr^i_2$, 85%

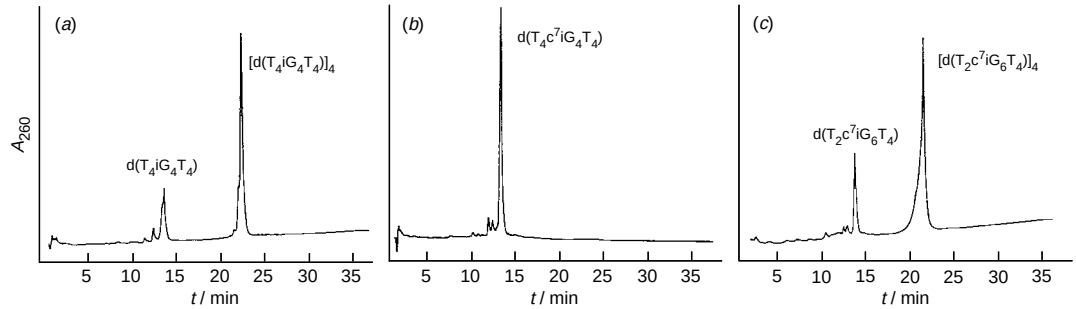


Fig. 1 Ion exchange HPLC profiles of (*a*) 5′-d(T_4isoG_4T_4), (*b*) 5′-d($T_4c^7iG_4T_4$) and (*c*) 5′-d($T_2c^7iG_6T_4$) at 25 °C. Solvent systems: A = 25 mM Tris-HCl (containing 1 mM EDTA buffer, pH 8.0)–MeCN, 90 : 10; B = A containing 1.0 M NaCl. Gradient: t = 0–30 min, 20 → 80% B in A; t = 30–35 min, 80% B in A.

(t_R = 22 min) appeared along with the fast migrating peak (t_R = 14 min) [Fig. 1(*c*)]. The latter contains the single-stranded oligomer, while the slow migrating peak represents the aggregate. Consequently, the aggregate of d($T_2c^7iG_6T_4$) **12** reflects a self-assembled structure. The almost identical mobilities of compounds d(T_4isoG_4T_4) and d($T_2c^7iG_6T_4$) on ion-exchange HPLC indicate that both aggregates are formed by assembly of four strands. This is the result of an identical number of negative phosphate charges, which are responsible for the chromatographic mobility.

From model building it is obvious that the structure of the isoguanine quartet has to be different from that of guanine. The structure of the two bases differs with regard to the position of the purine substituents, thereby changing their donor–acceptor pattern. Various aggregate motifs have been suggested for the polyribonucleotide poly(isoG),[3] for the monomeric isoguanosine,[4] and also for oligodeoxyribonucleotides.[2,14] Some structures do not use the purine nitrogen-7 to form the supramolecular assembly. Among them the most probable structure for the quartet of **12** is **A** (Fig. 2). It follows a motif which has been proposed for a monomeric isoguanosine derivative.[4]

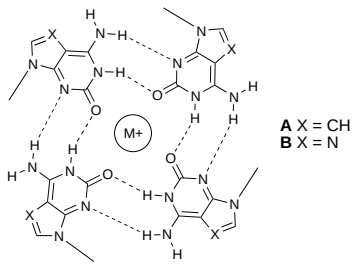


Fig. 2

The experiments described suggest that oligonucleotides containing either 7-deazaisoguanine or isoguanine form similar supramolecular structures with an identical hydrogen bonding pattern (**A** or **B**). Nevertheless, due to the structural changes of the heterocyclic base—a purine system is replaced by a pyrrolo[2,3-*d*]pyrimidine heterocycle—the strength of the hydrogen bonding array results in somewhat lower stability for the 7-deazaisoguanine quartet **A** compared to that formed by isoguanine (**B**). Unlike 2′-deoxyisoguanosine, which is very sensitive to acidic conditions (glycosylic bond hydrolysis), 7-deaza-2′-deoxyisoguanosine is very stable. This makes it a good candidate to form ionophors with good chemical stability.

Footnotes and References

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† *Abbreviations*: $isoG_d$ or d(isoG), 2′-deoxyisoguanosine; c^7iG_d or d(c^7iG), 7-deaza-2′-deoxyisoguanosine; DMT or $(MeO)_2Tr$, 4,4′-dimethoxytriphenylmethyl.

‡ *Selected data* for **10** (systematic numbering): λ_{max}(MeOH)/nm (ε) 274 (9800), 294 (7600); δ_H ([$^2H_6$]DMSO) 1.13, 1.14 (2 s, 2 CH_3), 2.30 [m, H-C(2′)], 2.88 (m, CH), 3.16 [m, 2 H-C(5′)], 3.72 (s, 2 CH_3O), 3.95 [m, H-C(4′)], 4.38 [m, H-C(3′)], 5.37 [br s, HO-C(3′)], 6.58 [m, H-C(1′)], 6.83 [d, *J* 7.6, H-C(5)], 7.22–7.46 [m, H-C(6) and 23 arom. H]; calc. for $C_{49}H_{47}N_5O_8$ (833.95): C, 70.57; H, 5.68; N, 8.40; found: C, 70.29; H, 5.65; N, 8.33%.

§ *General procedure*. The samples for ion exchange HPLC were prepared by preheating to 90 °C for 2 min, cooling to room temperature for 10 min, and storing at −20 °C for 30 min. The sample was then brought to room temperature and injected onto a NucleoPac PA-100 column. Elution was performed with Tris-HCl buffer, pH 8.0, by increasing the NaCl concentration from 0.2 to 0.8 M (ref. 2).

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