Oligonucleotides containing 7- or 8-methyl-7-deazaguanine: steric requirements of major groove substituents on the DNA structure

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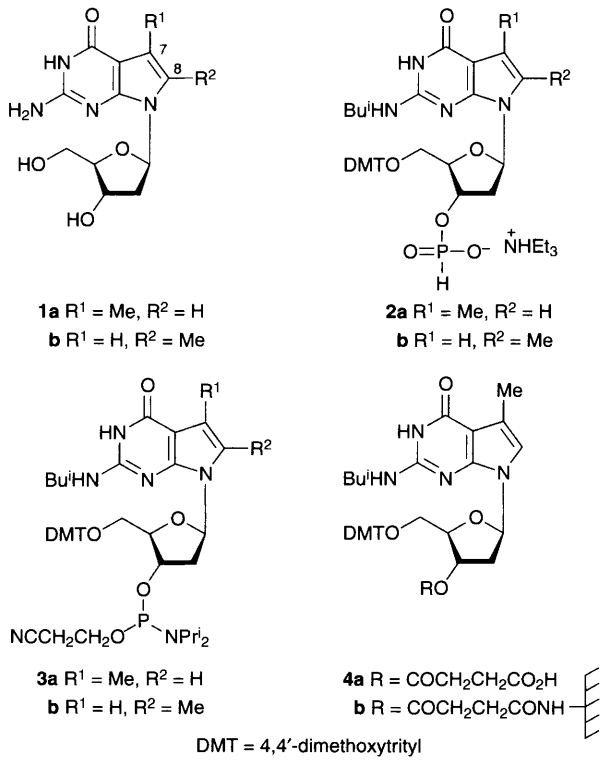
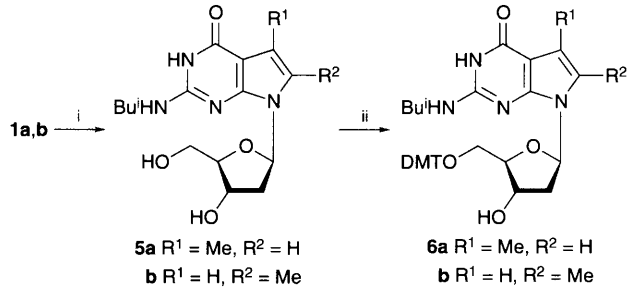
Methyl groups in alternating octanucleotides containing 7-methyl-7-deazaguanine and cytosine have steric freedom and maintain the B-DNA structure, whereas the isomeric 8-methyl-7-deazaguanine causes a B–Z transition.

DNA can exist in various conformations, *e.g.* A-, B- and Z-DNA. Model building shows that small 8-methyl groups located in the major groove of B-DNA can interfere with the sugar phosphate backbone of the B-DNA molecule. This causes steric strain in 8-methylated G-C-rich oligonucleotides, which is reduced by a transition from B- to Z-DNA.[1] Furthermore, bulky 8-substituents can change the purine base from *anti* to *syn*, the latter is adapted by Z-DNA.[2] Up to now it has not been known whether these steric restrictions are also valid for 7-methyl groups or whether the generation of a positive charge as introduced by the 7-methylation of guanine plays the main role in the B–Z transition of oligonucleotides with alternating dC-dG. In order to investigate this matter the 7-deazaguanine skeleton was chosen as a substitute for the guanine base. The guanine and 7-deazaguanine heterocycles are isosteric and the latter stays uncharged if methylated at position 7, whereas the 7-methylguanine moiety carries a charge. The parent nucleoside, 7-deaza-2′-deoxyguanosine (c^7G_d), as well as the 7-methyl derivative **1a**, have been synthesised previously.[3,4] The 8-methyl derivative of c^7G_d is also available by convergent nucleoside synthesis.[5] Studies on the structure of such oligonucleotides allow differentiation between steric effects of the 7-methyl *vs.* the 8-methyl group in the absence of a charged purine moiety.

In order to perform solid-phase oligonucleotide synthesis the building blocks **2a,b** and **3a,b**, as well as silica-bound **4b**, were prepared. Compounds **1a,b** were blocked on the 2-amino group with an isobutyryl residue using the protocol of transient protection. The derivatives **5a,b** were obtained crystalline (**5a**: 89%; **5b**: 85%). Next, the 4,4′-dimethoxytriphenylmethyl (DMT) group was introduced at the 5′-hydroxy position using the standard conditions which furnished compounds **6a,b** (**6a**: 90%, **6b**: 88%). These derivatives were converted into the phosphonates **2a,b** (PCl_3–*N*-methylmorpholine–1*H*-1,2,4-triazole) and the phosphoramidites **3a,b** [chloro(2-cyanoethoxy)-(diisopropylamino)phosphane]. Succinylation of **6a** yielded the derivative **4a** (80%), which was activated to the 4-nitrophenyl ester and linked to amino-functionalised Fractosil, forming the solid support **4b**. The ligand concentration was 70 μmol g^{-1} silica. Solid-phase oligonucleotide synthesis was performed on an automated synthesiser. The octanucleotides shown in Table 1 were synthesised and then they were purified by OPC cartridges, and their base composition was confirmed by enzymatic hydrolysis.

From the melting curves of the oligonucleotides, the T_m values were determined and the thermodynamic parameters ΔH and ΔS calculated using shape analysis of the melting curve.[7]





Scheme 1 *Reagents*: i, Me_3SiCl, Bu^i_2O, 89%(**5a**) or 85% (**5b**); ii, 4,4′-dimethoxytrityl chloride, 90% (**6a**) or 88% (**6b**)

Table 1 T_m Values, thermodynamic data and DNA structure of self-complementary oligonucleotides

Oligonucleotides	T_m/°C[a]	ΔH/ kcal mol^{-1}	ΔS/ cal K^{-1} mol^{-1}	DNA structure 0.1 and 4 M NaCl
d(G-C)$_4$ **7**	61	−82	−247	B-DNA
d(c^{7}G-C)$_4$ **8**[b]	53	−62	−190	B-DNA
d(m^{7}c^{7}G-C)$_4$ **9**[b]	58	−82	−250	B-DNA
d(m^{8}c^{7}G-C)$_4$ **10**	46	−70	−218	Z-DNA
d(C-G)$_4$ **11**	59	−84	−251	B/Z-DNA
d(C-m^{7}c^{7}G)$_4$ **12**	56	−81	−247	B-DNA

[a] Oligonucleotide conc. is 10 μM. Measurements were performed in 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1 M NaCl, pH 7.0. [b] Ref. 6.

As it can be seen, the duplex d(c^7G-C)$_4$ **8** is less stable than that of d(G-C)$_4$ **7**. This destabilisation was already recognised on shorter oligonucleotides[8] as well as on polynucleotide duplexes having the same composition.[9] The 7-methylated octanucleotide d(m^7c^7G-C)$_4$ **9** shows a significantly increased duplex stability over that of d(c^7G-C)$_4$ **8**, coming close to that of the parent purine oligonucleotide **7**. The same was found for d(C-m^7c^7G)$_4$ **12** compared to d(C- G)$_4$ **11**. On the other hand, the duplex d(m^8c^7G-C)$_4$ **10** with an 8-methyl group is labile, even in comparison with d(c^7G-C)$_4$ **8**. From these results it can be concluded that a methyl group located at the 8-position of 7-deazaguanine destabilises the DNA duplex, in a similar fashion to the 8-methyl group of guanine.[1] A methyl group located at the 7-position of 7-deazaguanine stabilises the duplex. As it can be seen from Table 1 the comparably low duplex stability of the oligonucleotides **8** and **10** is caused by an unfavourable enthalpy resulting from weaker H-bonding and/or stacking interactions. The 7-methylated oligomers **9** and **12** show about the same enthalpy and entropy changes as the corresponding purine oligonucleotides **7** and **11**.

Oligonucleotides containing alternating d(C-G) undergo a B–Z transition. This transition produces a dramatic change of the CD spectrum.[10] As the shape of the CD spectra is diagnostic of a particular DNA structure, the CD spectra of the compounds **7–12** were measured. At low salt concentration the CD spectra of the oligomers **7–9** and **11–12** are similar. As those of **7** and **8** are in accordance with a B-like DNA structure, a structural change to Z-DNA is not observed when the 7-deazaguanine moiety carries a 7-methyl group. The oligonucleotide d(C-G)$_4$ **11** has a Z-DNA structure in 4 M NaCl similar to poly[d-(C-m^7G)] under physiological conditions.[11] The oligomer **12** containing 7-methyl-7-deazaguanine maintains the B-DNA duplex form even at high salt concentration. As the charged 7-methylated guanine base facilitates the transition from B- to Z-DNA while the 7-methyl- 7-deazaguanine does not, it is suggested that the transition is caused by the charge and not by the spatial effect of the methyl group. The CD spectra of the oligomer **10** containing 8-methyl-7-deazaguanine residues are different from that of the d(c^7G-C)$_4$. Similar to the parent purine oligonucleotides, the 8-methyl substituent of 7-deazagaunine also forces the oligonucleotide from the B- to the Z-form (Fig. 1).

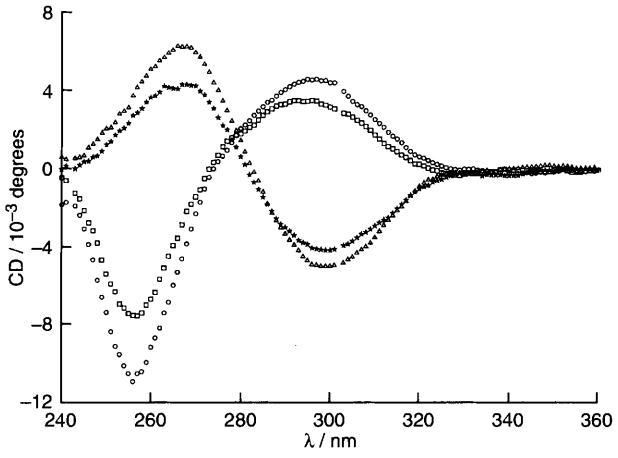


Fig. 1 CD Spectra of the oligonucleotide duplexes **9** and **10** (10 μM) in Na-cacodylate (10 mM), $MgCl_2$ (10 mM) and NaCl (0.1 or 4 M), measured at 20 °C; (□) d(m^7c^7G-C)$_4$ in 0.1 M NaCl, (○) d(m^7c^7G-C)$_4$ in 4 M NaCl, (★) d(m^8c^7G-C)$_4$ in 0.1 M NaCl, (△) d(m^8c^7G-C)$_4$ in 4 M NaCl

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References

1 H. Sugiyama, K. Kawai, A. Matsunaga, K. Fujimoto, I. Saito, H. Robinson and A. H. J. Wang, *Nucleic Acids Res.*, 1996, **24**, 1272.
2 A. H. J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Grawford, J. H. van Boom, G. van der Marel and A. Rich, *Nature (London)*, 1979, **282**, 680.
3 H. D. Winkeler and F. Seela, *J. Org. Chem.*, 1983, **48**, 3119.
4 H. D. Winkeler and F. Seela, *Liebigs Ann. Chem.*, 1984, 708.
5 F. Seela and Y. Chen, unpublished data.
6 F. Seela, N. Ramzaeva and Y. Chen, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 3049.
7 L. A. Marky and K. J. Breslauer, *Biopolymers*, 1987, **26**, 1601.
8 F. Seela and H. Driller, *Nucleic Acids Res.*, 1989, **17**, 901.
9 L. J. P. Latimer and J. S. Lee, *J. Biol. Chem.*, 1991, **266**, 13849.
10 F. M. Pohl and T. M. Jovin, *J. Mol. Biol.*, 1972, **67**, 375.
11 A. Möller, A. Nordheim, S. R. Nichols and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 4777.

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