

OLIGONUCLEOTIDE DUPLEX STABILITY CONTROLLED BY THE 7-SUBSTITUENTS OF 7-DEAZAGUANINE BASES

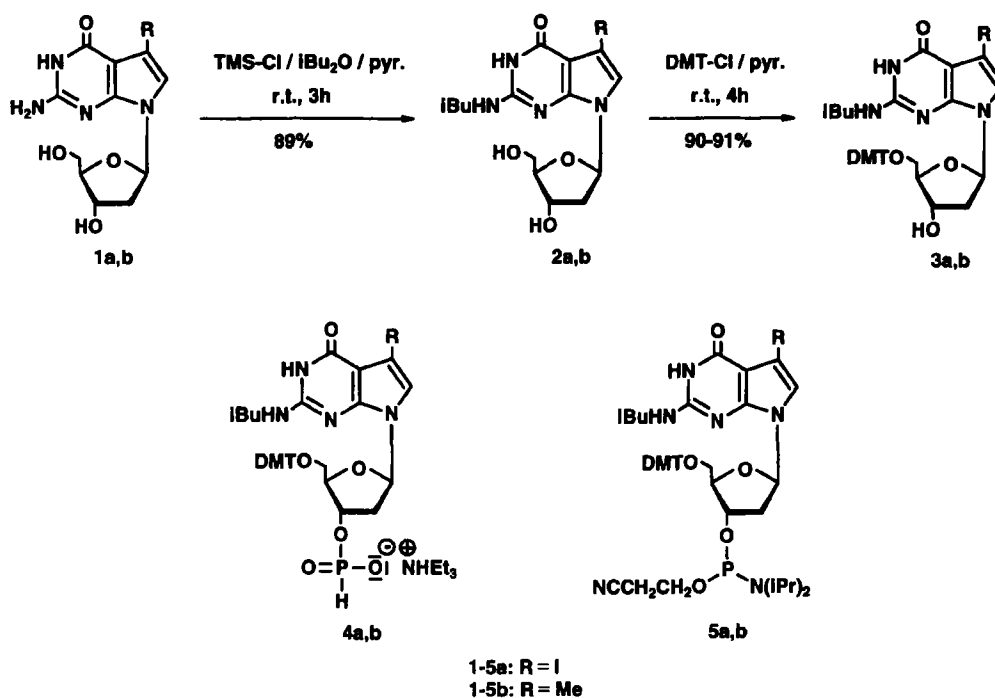
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Abstract. Oligonucleotides containing 7-substituted 7-deazaguanine residues (7-methyl, 7-iodo) have been synthesized. The self-complementary octamer d(I⁷c⁷G-C)₄ containing 7-iodo-7-deaza-2'-deoxyguanosine forms a stabilized duplex compared to the parent oligomer d(G-C)₄ ($\Delta T_m = +10^\circ \text{C}$). Also the complex between the oligodeoxynucleotide d(I⁷c⁷G₅-G) and poly(C) is stabilized ($\Delta T_m = +10^\circ$) over that of d(c⁷G₅-G) with poly(C).

Synthetic oligodeoxyribonucleotides targeted to certain segments of m-RNA (antisense oligonucleotides) have concerned the center of oligonucleotide research within the last few years.^{1,2} The low duplex stability, unfavorable delivery, and low biostability of naturally-occurring oligonucleotides have been improved by phosphodiester backbone- or base-modification. In this respect it was shown that the 5-substituents of pyrimidines which are located in the major groove of B-DNA can stabilize the duplexes structure.³ On the contrary, the 8-substituents of purines which are also located in the major groove lead to destabilization. This is a result of steric repulsion which drives the molecule into the syn-conformation.^{4,5} On the other hand, an anti-conformation, as observed for the common DNA constituents, was established for 7-substituted 7-deazapurine nucleosides, e.g. for 2'-deoxy-7-iodotubercidin (X-ray analysis⁶). Furthermore, the 7-substituents do not interfere sterically with the sugar-phosphate backbone and should fit exactly into the major groove of the B-DNA.⁷ The corresponding 7-deazapurine 2',3'-dideoxyribonucleoside triphosphates labelled with bulky fluorescent reporter groups are already in use as chain terminators in the Sanger dideoxy sequencing.⁸

Recently, the 7-methyl- and 7-iodo-7-deaza-2'-deoxyguanosine were synthesized.^{9,10} These compounds have now been incorporated into oligonucleotides. For this purpose standard conditions were chosen and the isobutryl residue was introduced as amino protecting group¹¹ (**2a,b**; 89 % yield). The protecting group half-lives (25% aq. NH₃, 40 °C) showed significant differences (64 min for **2a**; 93 min for **2b**). Subsequently, the 4,4'-dimethoxytriphenylmethyl (DMT) group was introduced (**3a,b**; 90% yield). They have been converted into the phosphonates **4a,b** (PCl₃/N-methylmorpholine/1H-1,2,4-triazole) and the cyanoethyl phosphoramidites **5a,b**. All compounds were characterized by ¹H-, ³¹P-, and ¹³C-NMR spectra as well as elemental analyses.¹²



Solid-phase oligonucleotide synthesis furnished the oligonucleotides **6** - **11** (Table 1 and 2). They have been purified by HPLC and their base composition was confirmed by enzymatic hydrolysis. Table 1 summarizes T_m -values as well as the thermodynamic data of duplex formation of the self-complementary octanucleotides **6** - **9**. As can be seen, 7-deaza-2'-deoxyguanosine [$d(c^7G-C)_4$, **7**] destabilizes the duplex structure significantly compared to the parent oligonucleotide containing 2'-deoxyguanosine [$d(G-C)_4$, **6**].

Table 1. T_m -Values and Thermodynamic Data of Duplex Formation of Oligonucleotides^{a,b}

Oligonucleotides	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/K·mol]
$d(G-C)_4$ (6)	60	-81 ± 2	-244 ± 5
$d(c^7G-C)_4$ (7)	53	-62 ± 2	-190 ± 5
$d(Me^7c^7G-C)_4$ (8)	58	-81 ± 2	-245 ± 5
$d(I^7c^7G-C)_4$ (9)	70	-91 ± 3	-266 ± 5

^a Oligonucleotide conc. 10 μ M. ^b 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1 M NaCl.

The replacement of the 7-hydrogen by a 7-methyl group [$d(\text{Me}^7\text{c}^7\text{G-C})_4$, **8**] increases the duplex stability being close to that of the parent oligonucleotide **6**. An extraordinary stable duplex is formed when a 7-iodo-substituent was introduced [$d(\text{I}^7\text{c}^7\text{G-C})_4$, **9**]. From this finding it is concluded that the duplex stability can be tuned by the appropriate selection of a 7-substituent within the 7-deazaguanine-cytosine base pair. The thermodynamic data show that the high T_m -value of the oligomer **9** is caused by a high reaction enthalpy compensating a slightly unfavorable entropy.

The stabilization of the duplex structure by incorporation of $\text{Me}^7\text{c}^7\text{G}_d$ or $\text{I}^7\text{c}^7\text{G}_d$ instead of c^7G_d can be traced back to (i) a hydrophobization of the major groove, (ii) increased stacking interactions of the modified bases, and (iii) better proton donor properties of H-N(1) causing stronger hydrogen bonding within the base pair. Charge density calculations (Alchemy III, Tripos Ass. Inc.) on c^7G_d , $\text{Me}^7\text{c}^7\text{G}_d$, and $\text{I}^7\text{c}^7\text{G}_d$ show that the latter exhibits a significantly higher positive charge at H-N(1) ($Q_{\text{H-N(1)}}$) compared with the other 7-deaza-2'-deoxyguanosines ($\text{c}^7\text{G}_d = +0.243$; $\text{Me}^7\text{c}^7\text{G}_d = +0.243$; $\text{I}^7\text{c}^7\text{G}_d = +0.264$). This is underlined by the pK_a -values of deprotonation ($\text{c}^7\text{G}_d : 10.3$; $\text{I}^7\text{c}^7\text{G}_d : 10.0$).

In contrast to $d(\text{G-C})_4$ (**6**) which forms a left-handed Z-DNA duplex structure in aqueous 4 M NaCl solution¹³ neither $d(\text{c}^7\text{G-C})_4$ (**7**) nor $d(\text{I}^7\text{c}^7\text{G-C})_4$ (**9**) or other c^7G_d -containing oligonucleotides¹⁴ show this B \rightarrow Z transition. This was proved by CD spectroscopy of **6-9** in neutral buffer solutions containing three different NaCl concentrations (100 mM, 1 M, or 4 M). While the parent oligomer **6** exhibits the expected inversion of the CD bands under high-salt concentration, the typical shape of a B-DNA CD spectrum is retained in case of the oligonucleotides **7-9** (positive B_{283} transition at 283 nm; negative B_{188} transition at 258 nm).

Furthermore, we measured the complex formation of the homooligomer **10** [$d(\text{I}^7\text{c}^7\text{G}_5\text{-G})$] with poly(C) (identical base concentrations) and compared the T_m -value with that of **11** · poly(C) [**11**: $d(\text{c}^7\text{G}_5\text{-G})$]. The latter was used as reference because oligonucleotides containing c^7G_d clusters do not form high-molecular weight aggregates (e.g. tetradecanates) like those with several consecutive dG residues. The heteroduplex of poly(C) with $d(\text{I}^7\text{c}^7\text{G}_5\text{-G})$ exhibits a T_m value of 52°C which is 10° higher than that of poly(C) and $d(\text{c}^7\text{G}_5\text{-G})$ (42°C). The high duplex stability of such 7-substituted 7-deazapurine oligonucleotides make these compounds interesting for antisense investigation.

The phosphodiester hydrolysis of the oligonucleotide duplexes **6-9** as well as of the oligonucleotide single strands **10** and **11** has been tested using snake venom phosphodiesterase (SV-PDE, 3' \rightarrow 5' exonuclease) as well as calf spleen phosphodiesterase (CS-PDE, 5' \rightarrow 3' exonuclease). Table 2 shows that all oligonucleotides containing 7-substituted 7-deaza-2'-deoxyguanosines are hydrolyzed significantly slower by both exonucleases, in particular by the 5' \rightarrow 3' calf spleen phosphodiesterase.

Table 2. Half-life Values (τ) of Enzymatic Phosphodiester Hydrolyses of the Oligomers 6-11 Towards Snake Venom (SV-PDE) and Calf Spleen Phosphodiesterase (CS-PDE)^{a,b,c}

Exonuclease	τ [min]					
	d(G-C) ₄ 6	d(c ⁷ G-C) ₄ 7	d(Me ⁷ c ⁷ G-C) ₄ 8	d(1 ⁷ c ⁷ G-C) ₄ 9	d(1 ⁷ c ⁷ G ₅ -G) 10	d(c ⁷ G ₅ -G) 11
SV-PDE	2.5	1.3	16	17	26	2.5
CS-PDE	82	~1000	>1200	>1200	>1200	>1200

^a 0.1 M TRIS-HCl, pH 8.3; ^b SV-PDE: 4 μ g; CS-PDE: 20 μ g; ^c room temperature.

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REFERENCES AND NOTES

- Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
- Sanghvi, Y. S.; Cook, P. D. In *Carbohydrate Modifications in Antisense Research*; American Chemical Society: Washington DC, ACS Symposium Series, 1994; Vol 580.
- Froehler, B. C.; Wadwani, S.; Terhorst, J. J.; Gerrad, S. K. *Tetrahedron Lett.* **1992**, *33*, 5307.
- Möller, A.; Nordheim, A.; Kozlowski, S. A.; Patel, D. J.; Rich, A. *Biochemistry* **1984**, *23*, 54.
- Kanaya, E. N.; Howard, F. B.; Frazier, J.; Miles, H. T. *Biochemistry* **1987**, *26*, 7159.
- Seela, F.; Zulauf, M.; Reuter, H. **1995**, unpublished data.
- Seela, F.; Thomas, H. *Helv. Chim. Acta* **1995**, *78*, 98.
- Prober, J.M.; Trainor, G.L.; Dam, R.J.; Hobbs, F.W.; Robertson, C.W.; Zagursky, R.J.; Cocuzza, A.J.; Jensen, M.A.; Baumeister, K. *Science* **1987**, *238*, 336.
- Winkeler, H.-D.; Seela, F. *Liebigs Ann. Chem.* **1984**, 708.
- Ramzaeva, N.; Seela, F. *Helv. Chim. Acta* **1995**, *78*, 1083.
- Ti, G.S.; Gaffney, B.L.; Jones, R.A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.
- Compd. **4a** (systematic numbering): ¹H-NMR (d₆-DMSO): δ , 1.15 (m, 5 CH₃), 2.36, 2.37 (m, H-2'), 2.76 (m, CH), 2.98 (m, 3 CH₂), 3.20 (m, 2H-5'), 3.75 (s, 2 CH₃O), 4.11 (m, H-4'), 4.80 (m, H-3'), 6.44 (t, H-1'), 6.09 (s, PH), 6.87-7.39 (m, aromatic H + H-6), 11.79 (br., 2 NH). ³¹P-NMR (d₆-DMSO) δ , 1.05 (¹J(P,H) = 587 Hz; ³J(P,H) = 8.3 Hz).
Compd. **4b**: ¹H-NMR (d₆-DMSO): δ , 1.16 (m, 5 CH₃), 2.19 (s, CH₃), 2.30 (m, H-2'), 2.74 (m, CH), 3.00 (m, 3 CH₂), 3.13, 3.18 (2m, 2H-5'), 3.75 (s, 2 CH₃O), 4.01 (m, H-4'), 4.77 (m, H-3'), 6.43 (d, J(P,H) = 346 Hz, PH), 6.45 (t, H-1'), 6.8-7.4 (m, aromatic H + H-6), 11.67, 11.69 (2s, 2 NH). ³¹P-NMR (d₆-DMSO): δ , 0.95 (¹J(P,H) = 584 Hz; ³J(P,H) = 8.1 Hz).
- Saenger, W. *Principles of Nucleic Acid Structure*, Springer Verlag Inc., New York, 1984; p. 283.
- Seela, F.; Driller, H. *Nucleic Acids Res.* **1989**, *17*, 901.

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