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

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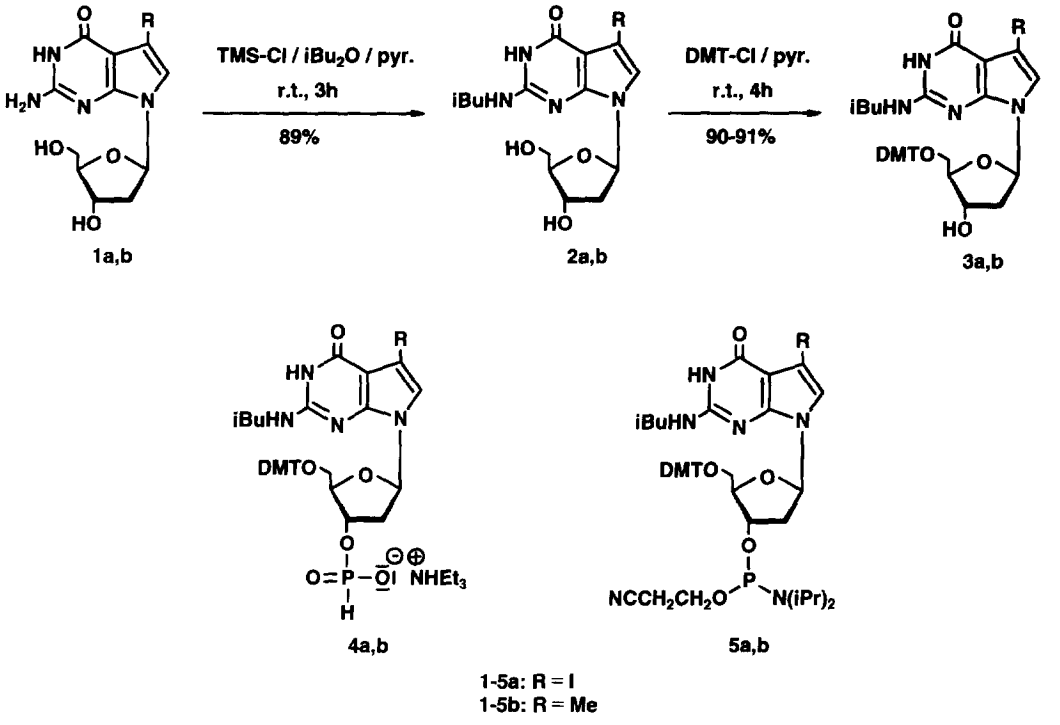
Abstract. Oligonucleotides containing 7-substituted 7-deazaguanine residues (7-methyl, 7-iodo) have been synthesized. The self-complementary octamer d(I^7c^7G-C)$_4$ containing 7-iodo-7-deaza-2'-deoxyguanosine forms a stabilized duplex compared to the parent oligomer d(G-C)$_4$ (ΔT_m = +10° C). Also the complex between the oligodeoxynucleotide d($I^7c^7G_5$-G) and poly(C) is stabilized (ΔT_m = +10°) over that of d(c^7G_5-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 analysis[6]). 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 isobutyryl residue was introduced as amino protecting group [11] (**2a,b**; 89 % yield). The protecting group half-lifes (25% aq. NH_3, 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** (PCl_3/N-methylmorpholine/1H-1,2,4-triazole) and the cyanoethyl phosphoramidites **5a,b**. All compounds were characterized by ^{1}H-, ^{31}P-, and ^{13}C-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 μM. [b] 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1 M NaCl.

The replacement of the 7-hydrogen by a 7-methyl group [d(Me^7c^7G-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(I^7c^7G-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 $Me^7c^7G_d$ or $I^7c^7G_d$ 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, $Me^7c^7G_d$, and $I^7c^7G_d$ show that the latter exhibits a significantly higher positive charge at H-N(1) ($Q_{H\text{-}N(1)}$) compared with the other 7-deaza-2'-deoxyguanosines (c^7G_d = +0.243; $Me^7c^7G_d$ = +0.243; $I^7c^7G_d$ = +0.264). This is underlined by the pK_a-values of deprotonation (c^7G_d : 10.3; $I^7c^7G_d$: 10.0).

In contrast to d(G-C)$_4$ (**6**) which forms a left-handed Z-DNA duplex structure in aqueous 4 M NaCl solution[13] neither d(c^7G-C)$_4$ (**7**) nor d(I^7c^7G-C)$_4$ (**9**) or other c^7G_d-containing oligonucleotides[14] show this B→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 concentation, the typical shape of a B-DNA CD spectrum is retained in case of the oligonucleotides **7**-**9** (positive B_{2u} transition at 283 nm; negative B_{1u} transition at 258 nm).

Furthermore, we measured the complex formation of the homooligomer **10** [d($I^7c^7G_5$-G)] with poly(C) (identical base concentrations) and compared the T_m-value with that of **11** · poly(C) [**11**: d(c^7G_5-G)] . The latter was used as reference because oligonucleotides containing c^7G_d clusters do not form high-molecular weight aggregates (e.g. tetrades) like those with several consecutive dG residues. The heteroduplex of poly(C) with d($I^7c^7G_5$-G) exhibits a T_m value of 52°C which is 10° higher than that of poly(C) and d(c^7G_5-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'→5' exonuclease) as well as calf spleen phosphodiesterase (CS-PDE, 5'→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'→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(I^{7}c^{7}G-C)$_4$ **9**	d(I^{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 µg; CS-PDE: 20 µg; [c] room temperature.

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REFERENCES AND NOTES

1. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
2. Sanghvi, Y. S.; Cook, P. D. In *Carbohydrate Modifications in Antisense Research*; American Chemical Society: Washington DC, ACS Symposium Series, 1994; Vol 580.
3. Froehler, B. C.; Wadwani, S.; Terhorst, J. J.; Gerrad, S. K. *Tetrahedron Lett.* **1992**, *33*, 5307.
4. Möller, A.; Nordheim, A.; Kozlowski, S. A.; Patel, D. J.; Rich, A. *Biochemistry* **1984**, *23*, 54.
5. Kanaya, E. N.; Howard, F. B.; Frazier, J.; Miles, H. T. *Biochemistry* **1987**, *26*, 7159.
6. Seela, F.; Zulauf, M.; Reuter, H. **1995**, unpublished data.
7. Seela, F.; Thomas, H. *Helv. Chim. Acta* **1995**, *78*, 98.
8. 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.
9. Winkeler, H.-D.; Seela, F. *Liebigs Ann. Chem.* **1984**, 708.
10. Ramzaeva, N.; Seela, F. *Helv. Chim. Acta* **1995**, *78*, 1083.
11. Ti, G.S.; Gaffney, B.L.; Jones, R.A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.
12. Compd. **4a** (systematic numbering): ^{1}H-NMR (d$_6$-DMSO): δ, 1.15 (m, 5 CH$_3$), 2.36, 2.37 (m, H-2'), 2.76 (m, CH), 2.98 (m, 3 CH$_2$), 3.20 (m, 2H-5'), 3.75 (s, 2 CH$_3$O), 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). ^{31}P-NMR (d$_6$-DMSO) δ, 1.05 (^{1}J(P,H) = 587 Hz; ^{3}J(P,H) = 8.3 Hz).
    Compd. **4b**: ^{1}H-NMR (d$_6$-DMSO): δ, 1.16 (m, 5 CH$_3$), 2.19 (s, CH$_3$), 2.30 (m, H-2'), 2.74 (m, CH), 3.00 (m, 3 CH$_2$), 3.13, 3.18 (2m, 2H-5'), 3.75 (s , 2 CH$_3$O), 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). ^{31}P-NMR (d$_6$-DMSO): δ, 0.95 (^{1} J(P,H) = 584 Hz; ^{3}J(P,H) = 8.1 Hz).
13. Saenger, W. *Principles of Nucleic Acid Structure*, Springer Verlag Inc., New York, 1984; p. 283.
14. Seela, F.; Driller, H. *Nucleic Acids Res.* **1989**, *17*, 901.

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