130. Duplex Stability of 7-Deazapurine DNA: Oligonucleotides Containing 7-Bromo- or 7-Iodo-7-deazaguanine

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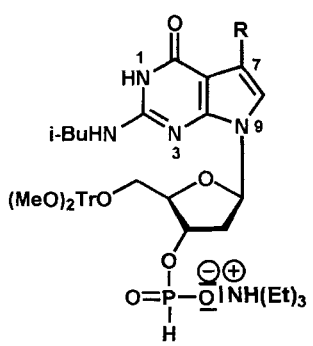
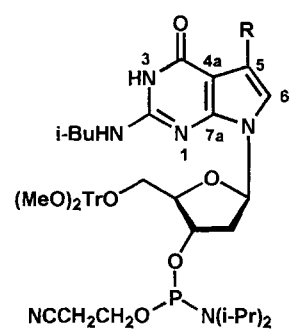
(14.V.96)

The oligonucleotide building blocks, the phosphonates **1a,b** and the phosphoramidites **2a,b** derived from 7-iodo- and 7-bromo-7-deaza-2′-deoxyguanosines **3a,b** were prepared. They were employed in solid-phase oligonucleotide synthesis of the alternating octamers d(Br^{7}c^{7}G-C)$_4$ (**8**) and d(I^{7}c^{7}G-C)$_4$ (**9**) as well as the homo-oligonucleotides d[(Br^{7}c^{7}G)$_5$-G] (**11**) and d[(I^{7}c^{7}G)$_5$-G] (**12**). The melting profiles and CD spectra of oligonucleotide duplexes were measured. The T_m values as well as the thermodynamic data were determined and correlated to the major-groove modification of this DNA. The self-complementary octamers **8** and **9** form more stable duplexes compared to the parent oligomer d(G-C)$_4$. The heteroduplex of d[(I^{7}c^{7}G)$_5$-G] (**12**) with d(C$_6$) is slightly destabilized ($\Delta T_m = -12°$) over that of d[(c^{7}G)$_5$-G] with d(C$_6$). However, the complex of **12** with poly(C) is more stable than that of d[(c^{7}G$_5$-G)] with poly(C).

The presence of alkyl, alkynyl, as well as halogeno substituents in position 7 of 7-deazaadenine-containing oligonucleotides leads to duplex stabilization with retention of the particular DNA structure [1] [2]. This stabilization was observed for alternating, consecutive, or randomly distributed 7-substituted 7-deazaadenine residues. The increased stability of the duplex structures can be explained by hydrophobization of the modified DNA, by stronger H-bonding, and/or more favorable stacking interactions. The findings are similar to those observed with oligonucleotides containing dT or 5-halogenated 2′-deoxyuridines, when compared with dU [3] [4]. The situation is different for substituents located in the 8-position of purines or 7-deazapurines [5–7]. In these cases, a destabilization of oligonucleotide duplexes occurs because of steric interference with the



1 a R = I
b R = Br
purine numbering

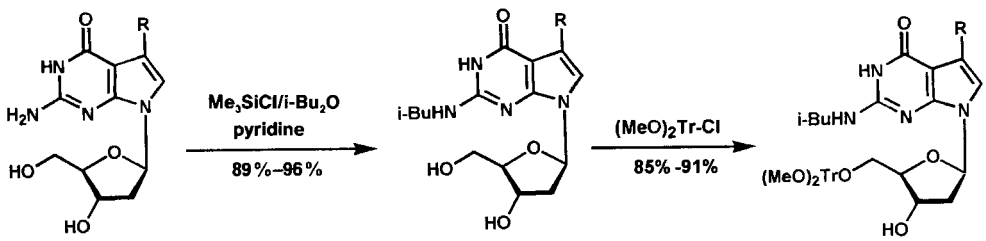


2 a R = I
b R = Br
systematic numbering

sugar-phosphate backbone. As a consequence, the DNA evades from a B-type structure into other DNA structures with less steric stress.

The duplex stabilization observed for oligonucleotides containing 7-substituted 7-deazaadenines was also expected for oligonucleotides with 7-substituted 7-deazaguanine residues. Recently, the synthesis of 7-chloro-, 7-bromo-, and 7-iodo-7-deaza-2′-deoxyguanosine was described [8]. The 7-bromo- (**3b**) and 7-iodo derivatives (**3a**) have now been converted into building blocks **1a,b** and **2a,b** for solid-phase synthesis and have been incorporated into oligonucleotides containing the modified bases. The modified bases are present in two sequence patterns. They either alternate with dC or are part of homooligomer tracts. The secondary structure of single strands and self-complementary duplexes is studied by CD spectroscopy, and the thermodynamic stability of duplexes is determined from T_m measurements.

Results and Discussion. – 1. *Monomers.* It has been shown that the *N*-isobutyryl (i-Bu) protecting group of 7-deaza-2′-deoxyguanosine shows almost the same half-life for the NH_2-group deprotection as 2′-deoxyguanosine (c^7G_d: *t*/2 109 min; dG: *t*/2 112 min; 25% aqueous NH_3 solution, 40°) [9]. Therefore, the same group was selected for the halogeno derivatives **3a** and **3b** (*Scheme*). Nevertheless, it is expected that the electron-withdrawing properties of the 7-halogeno substituents in compounds **3a,b** alter the electron density of the pyrrolo[2,3-*d*]pyrimidine moiety compared to 7-deaza-2′-deoxyguanosine (**3c**). This is documented by the change of the pK_a values. The pK_a values were determined UV-spectrophotometrically. The non-substituted nucleoside **3c** showed a pK_a of 1.1; the 7-halogeno derivatives were more difficult to protonate: pK_a = 0.7 and 0.6 for **3a** and **3b**, respectively. The pK_a values of deprotonation were found to be 10.3 for **3c** and 10.0 for **3a** and **3b**. The position of protonation is N(3) for **3c** [10] and is expected to be the same for **3a** and **3b**.



3a R = I
b R = Br
c R = H

4a R = I
b R = Br
c R = H

5a R = I
b R = Br
c R = H

The i-Bu protecting groups were introduced using the protocol of transient protection [11]. As expected, the half-lives of **4a,b** are significantly shorter (*t*/2 64 ± 5 min) than those of **4c**. The intermediates **4a,b** were converted into the 4,4′-dimethoxy-trityl ((MeO)$_2$Tr) derivatives **5a,b** under standard conditions in yields of 85–91%. From the (MeO)$_2$Tr derivatives, the phosphonates **1a,b** were prepared using PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole. Phosphitylation of **5a,b** with chloro(2-cyanoethyl)diisopropylamino)phosphine in CH_2Cl_2 in the presence of (i-Pr)$_2$EtN furnished the phosphor-

amidites **2a,b**. Structural proof of all compounds was achieved by ^{1}H-, ^{31}P-, and ^{13}C-NMR spectra (*Table 1* and *Exper. Part*) as well as by elemental analyses. According to *Table 1*, the 7-iodo substituent changes the chemical shift of C(7) dramatically. This is due to a positive mesomeric effect of the I-substituent. A similar effect is found for the 7-bromosubstituted compounds. However, the electronic effects of the 7-substituents are vague as no linear correlation can be observed between the ^{13}C-NMR chemical shift of C(7) and either the *Hammett* constants or different types of electronegativity values of the substituents.

Table 1. *^{13}C-NMR Chemical Shifts of Pyrrolo[2,3-d]pyrimidine Nucleosides*[a])

	C(2)[b]) C(2)[c])	C(6)[b]) C(4)[c])	C(5)[b]) C(4a)[c])	C(7)[b]) C(5)[c])	C(8)[b]) C(6)[c])	C(4)[b]) C(7a)[c])	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
3a	152.7	158.0	99.8	54.9	121.6	150.5	82.2	[d])	70.9	87.1	61.8
b	153.0	157.7	90.4	98.2	116.5	150.2	82.3	[d])	71.0	87.2	61.9
c	152.5	158.5	100.0	102.1	116.7	150.5	82.2	[d])	70.8	86.9	61.9
4a	147.5	156.1	103.9	55.5	124.3	147.0	82.6	[d])	70.8	87.3	61.7
b	147.3	155.6	90.7	102.0	119.1	147.0	82.6	[d])	70.7	87.3	61.7
5a	147.6	156.1	104.2	55.0	124.1	147.1	82.7	[d])	70.6	85.5	64.1
b	147.4	155.6	90.8	102.2	119.0	147.1	82.6	[d])	70.5	85.5	64.1
1a	147.7	156.1	104.1	55.0	124.1	147.2	82.8	[d])	72.9	85.6	63.8
b	147.5	155.6	90.9	102.2	119.0	147.2	82.6	[d])	72.8	85.5	63.9

[a]) Measured in (D_6)DMSO at 25°. [b]) Purine numbering. [c]) Systematic numbering. [d]) Superimposed by DMSO.

2. *Oligonucleotides*. 2.1. *Synthesis*. DNA can adopt several conformations depending on the compositions, the sequence, or the environmetal conditions [12]. The transformation between different secondary structures appears to be intimately related both to ionic interactions and the state of hydration of the nucleic acid. Thus, for G/C-rich DNA, the availability or lack of excess H_2O will dictate the DNA conformation: under high H_2O activity, the major groove will be filled, and the phosphates will form more extensive hydration complexes; under low H_2O activity, the major groove will collapse, and the phosphate O-atoms will become buried. The 7-deaza-2′-deoxyguanosine is already more hydrophobic than 2′-deoxyguanosine and shows a number of unusual properties. In alternating d(C-c^{7}G)$_3$ the duplex does not undergo a B → Z transition [13]. The c^{7}G$_d$ molecule destroys dG quartets in oligonucleotides with consecutive dG residues. It is also observed that the c^{7}G$_d$·dC *Watson-Crick* (*WC*) base pair is less stable than that of the parent dG·dC [13].

To study the effect of 7-halogeno substituents on the *WC*-duplex stability, the phosphonates **1a,b** as well as that of c^{7}G$_d$ were employed in automated oligodeoxynucleotide synthesis. The synthesis followed a protocol of phosphonate chemistry which was published earlier [1] [14]. The oligonucleotides **6–13** were prepared, recovered from the polymer support, deprotected, and purified using oligonucleotide purification cartridges. The composition of the oligonucleotides was established by MALDI-TOF-MS (matrix-assisted laser-desorption-ionization time-of-flight mass spectroscopy) which gave the expected molecular weights (*Table 2*).

2.2. *Self-Complementary Duplexes*. It was observed earlier that 7-halogenated 7-deazaadenines increase the stability of self-complementary dodecanucleotide duplexes.

Table 2. *Molecular Weights Determined by MALDI-TOF Mass Spectra and Yield of Oligonucleotides*

	M^+ (found)	M^+ (calc.)	A_{260} units
d(G-C)$_4$ (**6**)	[a])	2411.6	11.4
d(c^{7}G-C)$_4$ (**7**)	[a])	2407.6	17.0
d(Br^{7}c^{7}G-C)$_4$ (**8**)	2725.5	2723.2	12.6
d(I^{7}c^{7}G-C)$_4$ (**9**)	2911.5	2911.5	12.0
d[(c^{7}G)$_5$-G] (**10**)	1912.7	1908.3	5.7
d[(Br^{7}c^{7}G)$_5$-G] (**11**)	2304.3	2302.9	5.4
d[(I^{7}c^{7}G)$_5$-G] (**12**)	2538.9	2537.8	13.5
d(C$_6$) (**13**)	[a])		14.2

[a]) Not measured.

The dodecanucleotides d(Cl^{7}c^{7}A-T)$_6$, d(Br^{7}c^{7}A-T)$_6$, or d(I^{7}c^{7}A-T)$_6$ show much higher T_m values than the parent d(A-T)$_6$ and compared to that of d(c^{7}A-T)$_6$ [1] [2]. A similar stabilization was now observed for the octanucleotides **8** and **9** containing 7-iodo- or 7-bromo-7-deazaguanine residues in comparison to oligomers containing 7-deazaguanine or guanine. The T_m values of the octanucleotides **8** and **9** were taken from temperature-dependent UV spectra as described in [15] and compared with the parent d(c^{7}G-C)$_4$ (**7**) as well as d(G-C) (**6**) (see *Table 3*). As reported earlier for other duplexes, the octamer duplex **7**·**7** containing 7-deaza-2′-deoxyguanosine is significantly less stable than the duplex of the parent oligonucleotide. The alternating octamers **8** and **9** with 7-bromo- or 7-iodo-7-deaza-2′-deoxyguanosine, however, exhibit significantly higher T_m values than those containing guanine (*Fig. 1*). The stabilizing effect of the 7-halogeno substituents is opposite to the influence of 8-substituents in guanine which strongly destabilize the duplex structure [7].

To shed more light onto the structural influences caused by the 7-substituents of 7-deazaguanine-containing oligonucleotides, CD spectra were measured. Earlier, the CD spectra of alternating d(C-c^{7}G)$_3$ and d(C-G)$_3$ were compared [13]. According to their very similar shapes it was concluded that both hexamers form B-like DNA-duplex structures. The CD spectra of the halogenated duplexes **8**·**8** and **9**·**9** do not differ significantly from these CD spectra which shows that **8** and **9** form a B-like duplex as well. As the 7-substituents are located in the major groove of the B-DNA, they have considerable

Table 3. T_m *Values and Thermodynamic Data of Oligonucleotide Duplexes*[a])

	T_m [°C][b])	ΔH [kcal/mol]	ΔS [cal/mol·K]
d(G-C)$_4$·d(G-C)$_4$ (**6**·**6**)	60	−81	−244
d(c^{7}G-C)$_4$·d(c^{7}G-C)$_4$ (**7**·**7**)	53	−62	−190
d(Br^{7}c^{7}G-C)$_4$·d(Br^{7}c^{7}G-C)$_4$ (**8**·**8**)	67	−72	−212
d(I^{7}c^{7}G-C)$_4$·d(I^{7}c^{7}G-C)$_4$ (**9**·**9**)	70	−91	−266
d[(c^{7}G)$_5$-G]·d(C$_6$) (**10**·**13**)	27	−48	−157
d[(Br^{7}c^{7}G)$_5$-G]·d(C$_6$) (**11**·**13**)	23	−44	−145
d[(I^{7}c^{7}G)$_5$-G]·d(C$_6$) (**12**·**13**)	15	−41	−138

[a]) Oligomer concentration, 7.5 μM of single strands.

[b]) Measured in 0.1M NaCl containing 10 mM Na-cacodylate, and 10 mM $MgCl_2$, pH 7.0.

steric freedom which is not observed for bulky substituents located in position 8. In this case, the substituents interfere sterically with the sugar-phosphate backbone.

To exclude hairpin formation of the self-complementary oligonucleotides, the concentration dependence of the T_m values was measured (data not shown) which confirmed duplex formation in all cases. From these data, thermodynamic parameters of helix → coil transition were determined. The ΔH and ΔS values calculated from the concentration dependence of T_m and those evaluated from the fitting of the truncated melting curves were identical within the limits of error. According to these thermodynamic data (*Table 3*) the 7-halogeno substituents stabilize the duplex structure enthalpically but lead to an entropic destabilization. This is opposite to the data observed for alternating oligonucleotides with halogenated 7-deazaadenine residues [1].

2.3. *Homooligonucleotide Duplexes.* For the study of homooligonucleotide duplexes, the oligomers **11** and **12** were hybridized with $d(C_6)$ (**13**) (*Figs. 2* and *3*), and their T_m values were compared with that of $d[(c^7G)_5\text{-}G]\cdot d(C_6)$ (**10**·**13**). The latter was used as a reference, because oligonucleotides containing c^7G_d tracts do not form G quartets as observed in the case of dG. Surprisingly, the heteroduplex of $d(C_6)$ (**13**) with $d[(Br^7c^7G)_5\text{-}G]$ (**11**; T_m 23°) is slightly less stable than that with the non-substituted $d[(c^7G)_5\text{-}G]$ (**10**; T_m 27°; see *Table 3*). The T_m value of $d[(I^7c^7G)_5\text{-}G]$ (**12**) with $d(C_6)$ (**13**) is even 12° lower than that of the parent duplex. Apparently, homooligonucleotide duplexes containing 7-substituted c^7G_d tracts are more sensitive to steric repulsion of the consecutively arranged 7-halogeno substituents than homooligonucleotides with 7-substituted 7-deazaadenines. There is also a critical limit of the size of the 7-substituents in the duplexes described above. This phenomenon has been already observed in the case of 5-substituted pyrimidines, *e.g.*, by the introduction of an i-Pr residue into synthetic poly(dA-dT), which led to a decrease of the T_m value and a transition into a new secondary structure designated as X-DNA [16]. The introduction of the consecutively arranged bulky and lipophilic substituents can also mediate the interaction of DNA with the aqueous environment leading to the transformation of the secondary structure. However, the destabilization of the 7-substituents in $d(c^7G)_6\cdot d(C)_6$ is much smaller than in the case of 8-substituted 7-deazaguanine or guanine-containing oligonucleotides. In the case of 7-substituted 7-deazaadenine-containing oligonucleotides, generally more stable duplexes are formed – either in sequences with alternating or consecutive modified bases [1].

The conservation of the helix geometry of the heteroduplexes **10**·**13**, **11**·**13**, and **12**·**13** (*Fig. 4, a*) and the conformation of the single-stranded oligodeoxynucleotides **10**–**12** (*Fig. 4, b*) can be derived from their CD spectra. The spectrum of **12**·**13** exhibits a weak negative band at 290 nm and a very strong positive band at 268 nm which is similar to the CD spectra of RNA or A-DNA but different from typical conservative B-DNA spectra [12]. On this basis, these duplexes are tentatively assigned to the A form. The similarity of the CD spectra of the single-stranded oligomers **10** and **11** as well as of their heteroduplexes with $d(C_6)$ in sign, frequency, and band shape (see *Fig. 4*) suggests that the Br-substituent does not significantly alter the predominant single-strand conformation within this series. It is evident, however, that in the duplex **12**·**13**, the 7-iodo substituent must significantly affect the conformation, leading to a noticeable destabilization of the duplex. The inspection of the CD spectrum of the single-stranded **12** (*Fig. 4, b*) shows a strong negative band at 290 nm which shares some aspects of the left-handed Z-confor-

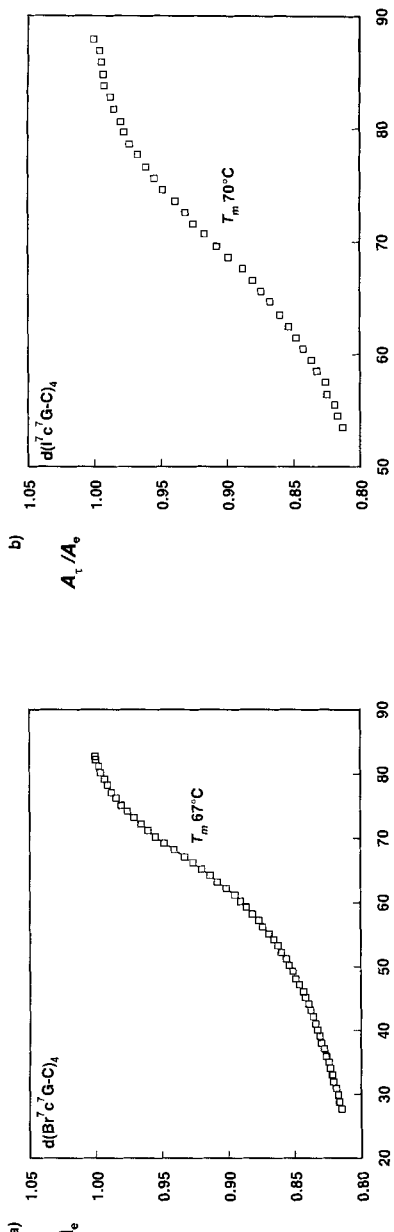


Fig. 1. *Normalized melting profiles of alternating duplexes* a) *d(Br*^{7}*c*^{7}*G-C)*$_4$ (**8**) *and* b) *d(7c*^{7}*G-C)*$_4$ (**9**). The melting curves were measured at 280 nm in 10 mM Na-cacodylate, 0.1 M NaCl, and 10 mM $MgCl_2$ at pH 7.0; the oligomer concentration was 7.5 μM of single strands.

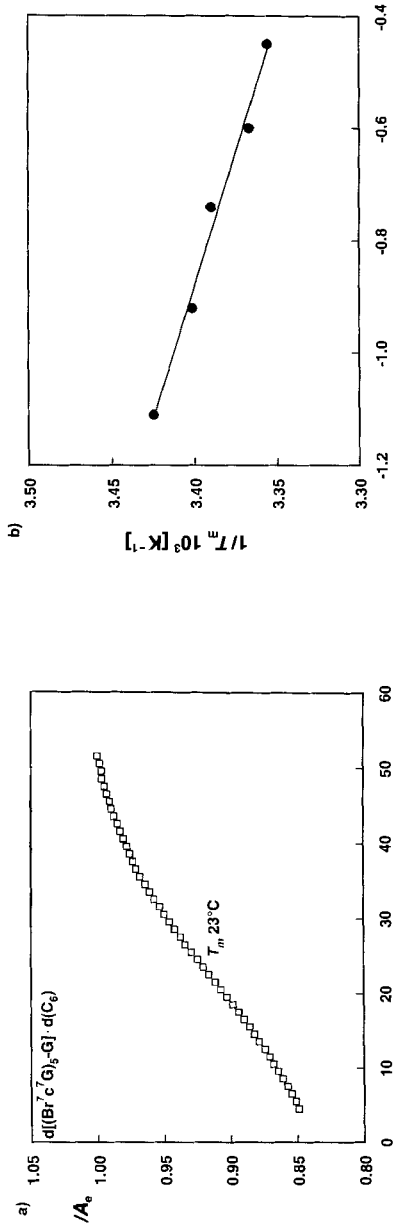


Fig. 2 a) *Normalized melting profile of heteroduplex d[(Br*^{7}*c*^{7}*G)*$_5$*-G]·d(C*$_6$*)* (**11**·**13**) *and* b) $1/T_m$ vs. *log* ($c_T/4$) *of* **11**·**13**. Conditions, see *Fig. 1*.

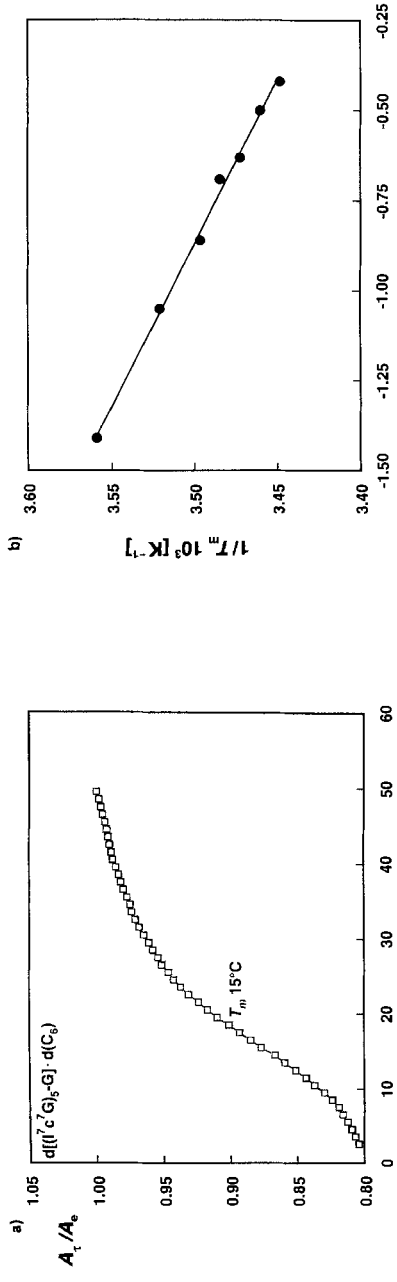


Fig. 3 a) *Normalized melting profile of heteroduplex d[(T⁷c⁷G)₅-G]·d(C₆)* (**12·13**) *and* b) $1/T_m$ *vs. log* ($c_T/4$) *of* **12·13**. Conditions, see *Fig. 1*.

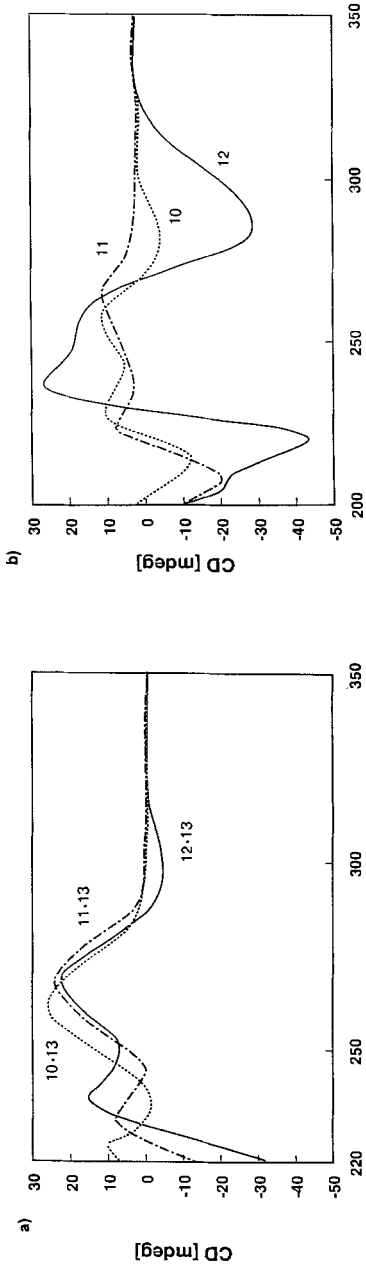


Fig. 4. a) *CD Spectra of heteroduplexes d[(c⁷G)₅-G]·d(C₆)* (**10·13**), *d[(Br⁷c⁷G)₅-G]·d(C₆)* (**11·13**), *and d[(T⁷c⁷G)₅-G·d(C₆)* (**12·13**), *measured at 5° at 5 μM single-strand concentration and* b) *CD spectra of single-stranded homooligonucleotides d[(c⁷G)₅-G]* (**10**), *d[(Br⁷c⁷G)₅-G]* (**11**), *and d[(T⁷c⁷G)₅-G]* (**12**) *at 20° at 10 μM oligomer concentration*. Conditions otherwise as in *Fig. 1*.

mation, since such a negative *Cotton* effect is common in CD spectra of left-handed Z-DNA [17]. None of the monomers, neither $I^7c^7G_d$ nor $Br^7c^7G_d$, exhibit any CD effect between 240 and 350 nm, neither at room temperature nor at 5°. This comparison indicates that the structural requirements for double-helix formation are better met by the conformation of the single-stranded **10** and **11** than by the single-stranded **12** which exhibits a preorganization of the single strand – a phenomenon which affects the thermodynamics of duplex formation.

The A-DNA which is less stretched compared to B-DNA gives more space to bulky substituents. When poly(C) was used as pairing partner, the heteroduplex of d[$(I^7c^7G)_5$-G] (**12**) with poly(C) exhibits a T_m value of 52° which is 10° higher than that of d[$(c^7G)_5$-G] (**10**) and poly(C) (42°) [18]. This significant increase of the T_m of the double-helical complex formed with RNA makes this modification ($I^7c^7G_d$) interesting for the antisense purposes which are currently studied.

We thank Mrs. *E. Feiling* and Mrs. *A. Lohmann* for excellent technical assistance. Financial support by the *Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie* is greatfully acknowledged.

Experimental Part

General. See [1]. Oligonucleotide syntheses were carried out on an automated synthesizer, model *380 B* (*Applied Biosystems*, Weiterstadt, Germany). Purification of oligonucleotides was performed using *Oligonucleotide Purification Cartridges* (*Applied Biosystems & Inc.*). Flash chromatography (FC): at 0.5 bar with silica gel *60 H* (*Merck*, Germany); solvent systems for FC and TLC: CH_2Cl_2/MeOH 90:10 (*A*), CH_2Cl_2/acetone 85:15 (*B*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*C*), CH_2Cl_2/AcOEt/Et_3N 69:30:1 (*D*). CD Spectra: *Jasco-600* spectropolarimeter, thermostatical controlled 1-cm cuvettes with a *Lauda-RCS-6* bath. Melting curves: *Cary-1E-UV/VIS* spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller; the actual temp. was measured in the reference cell with a Pt-100 resistor. NMR Spectra: *AMX-500* and *AC-250* spectrometers (*Bruker*, Germany); δ in ppm rel. to internal $SiMe_4$ (^1H,^{13}C) and to external 85% H_3PO_4 soln. (^{31}P). MALDI-TOF-MS were measured by Mrs. *Stephanie Hahner* at the Institute of Medical Physics and Biophysics (Director Prof. Dr. *Hillenkamp*) of the Westfälische Wilhelms Universität Münster on a home built apparatus.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-5-iodo-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**4a**). Compound **3a** (300 mg, 0.76 mmol) was dried by co-evaporation with pyridine (3 × 5 ml). The residue was suspended in pyridine (4 ml), and Me_3SiCl (0.48 ml, 3.75 mmol) was added at r.t. After 15 min stirring, the soln. was treated with isobutyric anhydride (0.62 ml, 3.78 mmol) and maintained at r.t. for 3 h. The mixture was cooled in an ice bath, H_2O (1 ml) and subsequently (5 min later) 25% aq. NH_3 soln. (1 ml) were added, and stirring was continued for 15 min. The soln. was evaporated to near dryness and the residue crystallized from H_2O: colorless crystals (312 mg, 89%). M.p. 188°. TLC (silica gel, *A*): R_f 0.4. ^{1}H-NMR ((D$_6$)DMSO): 1.01 (2 Me); 2.12 (*m*, H_α–C(2′)); 2.37 (*m*, H_β–C(2′)); 2.73 (*m*, CH); 3.50 (*m*, 2 H–C(5′)); 3.78 (*m*, H–C(4′)); 4.30 (*m*, H–C(3′)); 4.89 (*t*, OH–C(5′)); 5.20 (*d*, OH–C(3′)); 6.35 (*t*, H–C(1′)); 7.43 (*s*, H–C(6)); 11.49, 11.76 (2*s*, 2 NH). Anal. calc. for $C_{15}H_{19}IN_4O_5$ (462.2): C 38.98, H 4.14, N 12.12; found: C 39.11, H 4.37, N 11.96.

*5-Bromo-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**4b**). As described for **4a**, with **3b** (345 mg, 1.0 mmol): colorless crystals (400 mg, 96%) from H_2O. M.p. 182°. TLC (silica gel, *A*): R_f 0.4. ^{1}H-NMR ((D$_6$)DMSO): 1.10 (*d*, J = 6.6, 2 Me); 2.12 (*m*, H_β–C(2′)); 2.42 (*m*, H_α–C(2′)); 2.73 (*m*, CH); 3.50 (*m*, 2 H–C(5′)); 3.78 (*m*, H–C(4′)); 4.30 (*m*, H–C(3′)); 4.93 (*t*, OH–C(5′)); 5.24 (*d*, OH–C(3′)); 6.38 (*t*, J = 6.0, H–C(1′)); 7.42 (*s*, H–C(6)); 11.56, 11.81 (2*s*, 2 NH). Anal. calc. for $C_{15}H_{19}BrN_4O_5$ (415.2): C 43.39, H 4.61, N 13.49; found: C 43.31, H 4.64, N 13.36.

*7-[2-Deoxy-5-*O*-(4,4-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-iodo-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**5a**). Compound **4a** (400 mg, 0.87 mmol) was dried by repeated co-evaporation from anh. pyridine and then dissolved in pyridine (5 ml). At r.t., 4,4′-dimethoxytrityl chloride (328 mg, 0.95 mmol) was added and the soln. stirred overnight. MeOH (3 ml) and 5% aq. $NaHCO_3$ soln. (30 ml) were added. The aq. layer was extracted 3 times with CH_2Cl_2, the org. layer dried (Na_2SO_4) and evaporated, and the residue applied to FC (column 10 × 5 cm, *B*): colorless, amorphous solid (600 mg, 91%). TLC (silica gel, *B*): R_f

0.5. ^{1}H-NMR ((D$_6$)DMSO): 1.13 (*m*, 4 Me); 2.24 (*m*, H–C(2′)); 2.77 (*m*, CH); 3.12 (*m*, 2 H–C(5′)); 3.75 (*s*, 2 MeO); 3.93 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.30 (*d*, OH–C(3′)); 6.39 (*t*, H–C(1′)); 6.86–7.39 (*m*, arom. H, H–C(6)); 11.54, 11.82 (2*s*, 2 NH). Anal. calc. for $C_{36}H_{37}IN_4O_7$ (764.6): C 56.55, H 4.88, N 7.33; found: C 56.42, H 4.82, N 7.30.

*5-Bromo-7-[2-deoxy-5-*O*-(4,4-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one* (**5b**). As described for **5a**, with **4b** (300 mg, 0.72 mmol) and $(MeO)_2TrCl$ (276 mg, 0.8 mmol). FC yielded a colorless foam (440 mg, 85%). TLC (silica gel, *B*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 1.13 (*d*, *J* = 6.4, 2 Me); 2.24 (*m*, H–C(2′)); 2.77 (*m*, CH); 3.12 (*m*, 2 H–C(5′)); 3.74 (*s*, 2 MeO); 3.93 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.31 (*d*, OH–C(3′)); 6.41 (*t*, *J* = 6.6, H–C(1′)); 6.85–7.38 (*m*, arom. H, H–C(6)); 11.56, 11.85 (2*s*, 2 NH). Anal. calc. for $C_{36}H_{37}BrN_4O_7$ (717.6): C 60.25, H 5.20, N 7.81; found: C 60.29, H 5.11, N 7.83.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl-3,7-dihydro-5-iodo-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-(Triethylammonium Phosphonate)* (**1a**). To a soln. of PCl_3 (180 µl, 2 mmol) and *N*-methylmorpholine (2.2 ml) in CH_2Cl_2 (12 ml) was added 1*H*-1,2,4-triazole (480 mg, 6.8 mmol). The soln. was cooled to 0°, and **5a** (306 mg, 0.4 mmol) in CH_2Cl_2 (12 ml) was added slowly. Stirring was continued for 30 min at 0°, then the mixture was poured into 1M $(Et_3NH)HCO_3$ (**TBK**, pH 8.0, 25 ml), shaken, and separated. The aq. layer was extracted with CH_2Cl_2 (3 × 30 ml) and the combined org. extract dried (Na_2SO_4) and evaporated. FC (column 10 × 5 cm, CH_2Cl_2/Et_3N 98:2, then *C*) furnished a main zone which was evaporated. The residue was dissolved CH_2Cl_2, extracted with aq. 0.1M TBK (8 × 20 ml), dried with Na_2SO_4, and evaporated: colorless foam (320 mg, 86%). TLC (silica gel, *C*): R_f 0.7. ^{1}H-NMR ((D$_6$)DMSO): 1.15 (*m*, 5 Me); 2.36, 2.37 (*m*, 2 H–C(2′)); 2.76 (*m*, CH); 2.98 (*m*, 3 CH_2); 3.20 (*m*, 2 H–C(5′)); 3.75 (*s*, 2 MeO); 4.11 (*m*, H–C(4′)); 4.80 (*m*, H–C(3′)); 6.44 (*t*, H–C(1′)); 6.09 (*s*, PH); 6.87–7.39 (*m*, arom. H, H–C(6)); 11.79 (br., 2 NH). ^{31}P-NMR ((D$_6$)DMSO): 1.05 (^1J(P,H) = 587, ^3J(P,H) = 8.3). Anal. calc. for $C_{42}H_{53}IN_5O_9P$ (929.8): C 54.26, H 5.75, N 7.53; found: C 54.39, H 5.91, N 7.58.

*5-Bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-(Triethylammonium Phosphonate)* (**1b**). As described for **1a**, with **5b** (237 mg, 0.33 mmol), PCl_3 (150 µl, 1.7 mmol), *N*-methylmorpholine (1.8 ml), and 1*H*-1,2,4-triazole (400 mg, 5.6 mmol): colorless foam (250 mg, 86%). TLC (silica gel, *C*): R_f 0.7. ^{1}H-NMR ((D$_6$)DMSO): 1.15 (*m*, 5 Me); 2.33 (*m*, H–C(2′)); 2.72 (*m*, CH); 2.98 (*m*, 3 CH_2); 3.18 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 4.08 (*m*, H–C(4′)); 4.73 (*m*, H–C(3′)); 6.45 (*t*, H–C(1′)); 6.64 (*d*, *J* = 585, PH); 6.83–7.62 (*m*, arom. H, H–C(6)); 11.79, 11.85 (2*s*, 2 NH). ^{31}P-NMR ((D$_6$)DMSO): 0.98 (^1J(P,H) = 585.3, ^3J(P,H) = 8.97). Anal. calc. for $C_{42}H_{53}BrN_5O_9P$ (882.8): C 57.14, H 6.05, N 7.93; found: C 57.30, H 6.16, N 7.92.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-iodo-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite]* (**2a**). A soln. of **5a** (230 mg, 0.3 mmol) in anh. CH_2Cl_2 (5 ml) was pre-flushed with Ar. Then (i-Pr)$_2$EtN (200 µl, 1.2 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphine (100 µl, 0.44 mmol) were added under Ar. After stirring for 0.5 h, 5% aq. $NaHCO_3$ soln. (8 ml) was added and the mixture extracted with CH_2Cl_2 (2 × 20 ml). The org. layer was dried (Na_2SO_4), filtered, and evaporated. FC (silica gel, column 5 × 10 cm, *D*) gave a mixture od diastereoisomers **2a** (125 mg, 52%). TLC (silica gel, *D*): R_f 0.8. ^{31}P-NMR ($CDCl_3$): 148.05, 148.75.

*5-Bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite]* (**2b**). As described for **2a**, with **5b** (216 mg, 0.3 mmol), (i-Pr)$_2$EtN (200 µl, 1.2 mmol), and chloro(2-cyanoethoxy)(diisopropylamino)phosphine (100 µl, 0.44 mmol): colorless foam (140 mg, 50%). TLC (silica gel, *D*): R_f 0.8. ^{31}P-NMR ($CDCl_3$): 148.1, 148.8.

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