pH-Independent Recognition of the dG·dC Base Pair in Triplex DNA: 9-Deazaguanine N^7-(2′-Deoxyribonucleoside) and Halogenated Derivatives Replacing Protonated dC

by **Frank Seela***, **Khalil I. Shaikh**, and **Thomas Wiglenda**

Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastrasse 7, D-49069 Osnabrück, and Laboratory of Bioorganic and Biophysical Chemistry, Center for Nanotechnology (CeNTech), Gievenbecker Weg 11, D-48149 Münster
(phone: +49 541 969 2791; fax: +49 541 969 2370; e-mail: Frank.Seela@uni-osnabrueck.de)

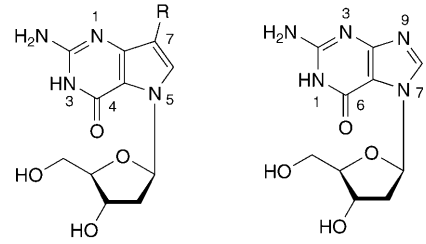
Triplex-forming oligonucleotides (TFOs) containing 9-deazaguanine N^7-(2′-deoxyribonucleoside) **1a** and halogenated derivatives **1b**,**c** were synthesized employing solid-phase oligonucleotide synthesis. For that purpose, the phosphoramidite building blocks **5a**–**c** and **8a**–**c** were synthesized. Multiple incorporations of **1a**–**c** in place of dC were performed within TFOs, which involved the sequence of five consecutive **1a**–**c**·dG·dC triplets as well as of three alternating **1a**–**c**·dG·dC and dT·dA·dT triplets. These TFOs were designed to bind in a parallel orientation to the target duplex. Triplex forming properties of these oligonucleotides containing **1a**–**c** in the presence of Na^+ and Mg^{2+} were studied by UV/melting-curve analysis and confirmed by circular-dichroism (CD) spectroscopy. The oligonucleotides containing **1a** in the place of dC formed stable triplexes at physiological pH in the case of sequence of five consecutive **1a**·dG·dC triplets as well as three alternating **1a**–**c**·dG·dC and dT·dA·dT triplets. The replacement of **1a** by 9-halogenated derivatives **1b**,**c** further enhanced the stability of DNA triplexes. Nucleosides **1a**–**c** also stabilized duplex DNA.

Introduction. – Since the discovery of triple-helical DNA [1], triplex-forming oligonucleotides (TFOs) have attracted attention due to their application in the sequence-specific recognition of duplex DNA thereby blocking gene expression ('antigene strategy') [2–4]. Biologically active TFOs were shown to induce targeted mutations [5][6]. Conjugates of oligonucleotides with psoralen and alkylating agents have been used to demonstrate the accessibility of a particular DNA target sequence in cell nuclei [7][8]. TFOs have found application in oligonucleotide diagnostics [9][10].

Essentially two families of DNA triple helices have been characterized *i*) those containing the pyrimidine·purine·pyrimidine motif, and *ii*) others forming a purine·purine·pyrimidine scheme. These two families differ in their third-strand composition and orientation. In the more commonly described pyrimidine·purine·pyrimidine motif, the third pyrimidine strand has been shown to bind within the major groove of duplex DNA in a parallel orientation with respect to the purine strand to form the triple helix. In this motif, the third-strand recognition occurs by the formation of dT·dA·dT and dCH^+·dG·dC base triades through *Hoogsteen* pairing [2][3]. The necessary protonation of cytosine limits the use of this method. Much effort has been devoted to increase the stability of triplexes under neutral conditions. The replacement of cytosine by 5-methylcytosine increases the stability of triplexes but does not alleviate the pH dependence [11–14]. Oligonucleotides containing N^7-glycosylated purine nucleosides,

acyclic derivatives, or peptide nucleic acid (PNA) analogs have been studied for the same purpose [15–19]. Also base-modified oligonucleotides incorporating pyrazolo[4,3-*d*]pyrimidines [20][21], 5-halogenated pyrimidines [11], or 6-oxocytidines [22] were investigated. In particular, the guanine N^7-(2′-deoxyribonucleoside) **2**, a non-natural purine analogue of a 2′-deoxycytidine which displays the H-bonding pattern of a protonated cytidine, shows a high third-strand-binding affinity being specific to the dG·dC base pair, and the corresponding triplexes are stable under physiological pH [15–18].

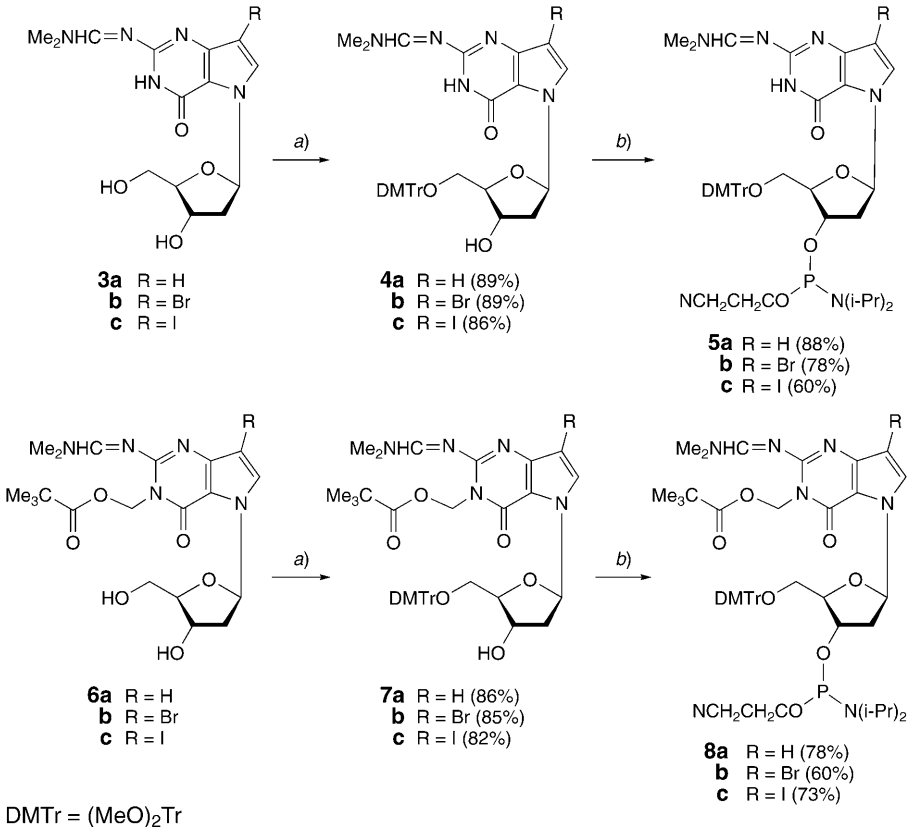
Earlier experiments with halogenated derivatives of 7-deazapurine nucleosides [23–26] or 8-aza-7-deazapurine nucleosides have shown that 7-halogeno substituents stabilize the DNA duplex structure significantly [27][28]. The incorporation of 7-chloro-7-deaza-2′-deoxyguanosine into TFOs in place of 2′-deoxyguanosine leads to a triplex stabilization [29]. This phenomenon was studied on nucleosides related to the purine constituents of the third strand of triplexes but not on those mimicking the protonated dC residues. We are now investigating the effect of bulky substituents introduced in the third strand of triplex-forming oligonucleotides. For this, we chose the 9-deazapurine (=pyrrolo[3,2-*d*]pyrimidine) system as a purine surrogate which can be glycosylated regioselectively at the 7-position of the base (purine numbering is used throughout the discussion section) and can be substituted at the 9-position with any substituent of choice. This report describes on the synthesis of the phosphoramidite building blocks containing N^7-glycosylated 9-deazaguanine 2′-deoxyribonucleosides **1a**–**c**, which are remarkably stable structural analogs of the purine nucleoside **2**. As the base triad **2**·dG·dC is structurally related to that of dCH^+·dG·dC, oligonucleotides were synthesized for the investigation of their third-strand binding. Preliminary results of a part of this work have been reported in a short communication [30][31].



Results and Discussion. – 1. *Synthesis of Monomers.* The synthesis of the 9-deazaguanine N^7-(2′-deoxyribonucleoside) **1a** was already reported [32]. Recently, an improved synthesis protocol for **1a** and also for the 9-halogenated derivatives **1b**,**c** was published [33]. The partially deprotected intermediates **3a**–**c** and **6a**–**c** obtained during nucleoside synthesis [33] were used as precursors to prepare phosphoramidite building blocks for solid-phase oligonucleotide synthesis. The two series of starting materials differ in their protecting design. In the series **3a**–**c**, the lactam moiety of

the 9-deazaguanine residue is unprotected, while this moiety is protected by a (pivaloyloxy)methyl (= (2,2-dimethyl-1-oxopropoxy)methyl) residue in the series **6a**–**c**. At first, compounds **3a**–**c** or **6a**–**c** were converted to the 4,4′-dimethoxytrityl derivatives **4a**–**c** and **7a**–**c**, respectively, with $(MeO)_2TrCl$ in pyridine (*Scheme*). Subsequently, the treatment of **4a**–**c** and **7a**–**c** with 2-cyanoethyl diisopropylphosphoramidochloridite yielded the phosphoramidites **5a**–**c** and **8a**–**c**, respectively, which were further used in solid-phase oligonucleotide synthesis.

Scheme



a) $(MeO)_2TrCl$, pyridine; 5 h, r.t. *b*) $(i\text{-}Pr)_2NP(Cl)(OCH_2CH_2CN)$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 30 min.

To assure the stability and applicability of the amino-protecting groups during oligonucleotide syntheses, the deprotection of the formamidine protecting group of compounds **3a**–**c** was studied UV-spectrophotometrically in 25% aqueous ammonia solution (40°). The half-life value for the deprotection of the 9-unsubstituted compound **3a** measured spectrophotometrically at 260 nm (25% aq. ammonia, 40°) was 12.3 min, while the 9-substituted derivatives **3b**,**c** show higher half-life values (**3b** 22.5 min, **3c** 40 min). This demonstrates that halogen substituents increase the stability of the amino-protecting groups in the pyrrolo[3,2-*d*]pyrimidine system.

All synthetic intermediates were characterized by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectroscopy as well as by elemental analyses (*Table 1* and *Exper. Part*). According to *Table 1*, the C(9) signals of nucleobases show the most dramatic changes upon halogen substitution. In all cases, the C(9) signal shows an upfield shift of *ca.* 12 ppm when a Br-substituent is introduced, while the I-substituent causes a larger shift of *ca.* 42 ppm compared to the 9-unsubstituted compounds.

Table 1. *^{13}C-NMR Chemical Shifts of Nucleosides and Derivatives*[a])

	4a	**4b**	**4c**	**7a**	**7b**	**7c**
C(2)[b]), C(2)[c])	154.5[d])	154.7[d])	154.7[d])	153.7[d])	153.9[d])	153.7[d])
C(4)[b]), C(6)[c])	155.1[d])	155.1[d])	154.8[d])	154.3[d])	154.2[d])	154.0[d])
C(4a)[b]), C(5)[c])	113.9	113.9	114.1	112.8	112.7	112.9
C(6)[b]), C(8)[c])	127.8	129.6	130.3	127.9	129.8	131.6
C(7)[b]), C(9)[c])	102.6	90.1	59.2	102.9	90.3	59.3
C(7a)[b]), C(4)[c])	144.9	144.8	144.8	145.0	144.9	144.8
Me(Piv)	–	–	–	26.9	26.8	26.7
MeN	34.4	34.4	34.5	34.6	34.6	34.6
N=CH	157.0	157.0	156.9	157.0	157.0	156.9
C(1′)	85.4[d])	85.5[d])	85.5[d])	85.2[d])	85.6[d])	85.7[d])
C(2′)	[e])	[e])	[e])	39.9	39.9	39.9
C(3′)	70.5	70.3	70.4	70.5	70.4	70.5
C(4′)	85.1[d])	85.4[d])	85.0[d])	85.4[d])	85.6[d])	85.6[d])
C(5′)	64.1	63.9	64.0	64.1	64.0	64.0
OCH_2	–	–	–	65.5	65.5	65.5
C=O	–	–	–	176.5	176.8	176.8

[a]) Measured in (D_6)DMSO. [b]) Systematic numbering. [c]) Purine numbering. [d]) Tentative. [e]) Superimposed by the DMSO signal.

2. *Synthesis and Characterization of Oligonucleotides.* Oligonucleotides **9**–**28** (see *Tables 2* and *3* for *Formulae*) were synthesized in an automated *ABI 392-08* DNA synthesizer with the phosphoramidites **5a**–**c** and **8a**–**c**. The oligonucleotides were purified by reversed-phase HPLC (see *Exp. Part*). The composition of the oligonucleotides was determined by HPLC (*RP-18*) after tandem enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1M Tris·HCl buffer (pH 8.3) at 37°. The HPLC profiles of the reaction products obtained after enzymatic digestion clearly demonstrate that the 9-bromo- and 9-iodo-substituted compounds **1b**,**c** are much more hydrophobic (*Fig. 1*,*b* and *c*) compared to the unsubstituted nucleoside **1a** (*Fig. 1*,*a*). MALDI-TOF mass spectra were measured for the modified oligonucleotides, correct masses were found in all cases (see *Exper. Part*).

3. *Duplexes Containing Base Pairs of the 9-Deazaguanine* N^7*-(2′-Deoxyribonucleoside)* **1a** *and the 9-Halogenated Derivatives* **1b**,**c** *with* m^5iC_d. Before investigating the stability of triplexes, the effect of compounds **1a**–**c** on the duplex stability was studied. Previously, our laboratory had reported on the incorporation of N^7-glycosylated 2′-deoxyadenosine and N^7-glycosylated 2′-deoxyguanosine **2** in oligonucleotide duplexes [34][35]. Compound **2** forms stable base pairs with 2′-deoxy-5-methylisocytidine (=m^5iC_d) (duplex **11**·**12**, *Table 2*) [36]. Now, we incorporated the 9-deazaguanine

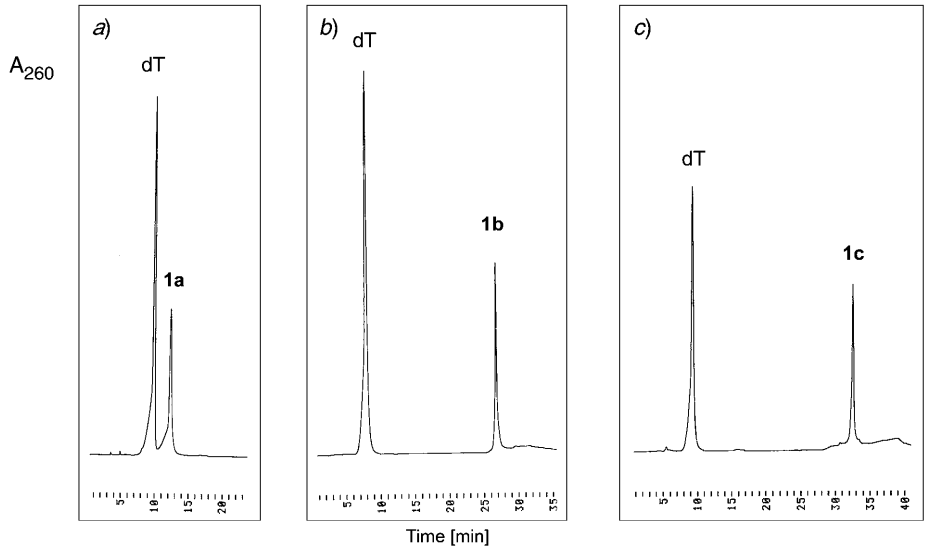


Fig. 1. *Reversed-phase HPLC profiles after the enzymatic hydrolysis of the oligonucleotides* a) **25** *containing* **1a**, b) **26** *containing* **1b**, *and* c) **27** *containing* **1c**, *with snake-venom phosphodiesterase and alkaline phosphatase in 0.1*M Tris·*HCl buffer* (pH 8.3). Gradient: 20 min *A*, 20–60 min 50% *B* in *A*, flow rate 0.7 ml/min (*A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5, *B* = MeCN).

N^7-(2′-deoxyribonucleosides) **1a**–**c** in duplex DNA and studied their base pairing with m^5iC_d (*Table 2*). According to *Table 2*, compounds **1a**–**c** form base pairs with m^5iC_d (which was deduced from sigmoidal melting profiles). Incorporation of nucleoside **1a** (duplex **13**·**14**) results in a T_m value of 40° (*Table 2*) which is identical to that of the duplex **11**·**12** containing the N^7-glycosylated guanine **2** but lower than the duplex **9**·**10** containing the two canonical base pairs at the corresponding positions (*Fig. 2*, motif **III**). The replacement of **1a** by the bulkier 9-functionalized nucleosides **1b**,**c** increases the T_m value by 1° per modification (see duplex **15**·**14** and **16**·**14**). This indicates that the bulky 9-substituents of N^7-glycosylated 9-deazapurines are well accommodated in duplex DNA. These results are in line with observations reported for other halogenated 7-deazapurine and 8-aza-7-deazapurine 2′-deoxyribonucleosides [23–28]. However, the stabilization by the halogeno substituents in a base pair of 7-functionalized 7-deazapurine nucleosides with regular glycosylation sites is slightly higher than those of the uncommonly glycosylated analogs [26]. Regarding the base-pair motifs formed between **1a**–**c** and m^5iC_d, we suggest a similar motif as it was proposed for the purine analog **2** (motif **II**) [35]. In both cases, tridentate base pairs are formed similarly to the case of the canonical dG·dC base pair (motif **I**). The lower stability of the base pairs shown in the motifs **II** and **III** might result from the differences in overlap of the surface areas of the heterocycles (stacking) as well as from weaker H-bonding (*Fig. 2*).

4. *Triplexes Containing the 9-Deazaguanine* N^7*-(2′-Deoxyribonucleoside)* **1a** *and 9-Halogenated Derivatives* **1b**,**c**. Protonated cytosine is required to form two H-bonds in the triplex motif **IV** (*Fig. 3*). The pK_a value of the monomeric 2′-deoxycytidine is 4.3.

Table 2. T_m *Values of Oligonucleotide Duplexes Containing* **1a**–**c** *and* **2**[a])

Duplexes	T_m [°]	ΔH° [kcal/mol]	ΔS° [kcal/mol]	ΔG°_{310} [kcal/mol]
5′-d(TAG GTC AAT ACT)-3′ (**9**)	47	−89	−253	−10.9
3′-d(ATC CAG TTA TGA)-5′ (**10**)				
5′-d(TA**iC** **iC**TC AAT ACT)-3′ (**11**)	40	−57	−156	−8.4
3′-d(AT**2** **2**AG TTA TGA)-5′ (**12**)				
5′-d(TA**1a** **1a**TC AAT ACT)-3′ (**13**)	40	−74.9	−212.6	−8.9
3′-d(AT**iC** **iC**AG TTA TGA)-5′ (**14**)				
5′-d(TA**1b** **1b**TC AAT ACT)-3′ (**15**)	42	−64.9	−181.1	−9.7
3′-d(AT**iC** **iC**AG TTA TGA)-5′ (**14**)				
5′-d(TA**1c** **1c**TC AAT ACT)-3′ (**16**)	42	−75.8	−212.8	−9.8
3′-d(AT**iC** **iC**AG TTA TGA)-5′ (**14**)				

[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 μM of single-strand concentration. **iC** = 2′-deoxy-5-methylisocytidine.

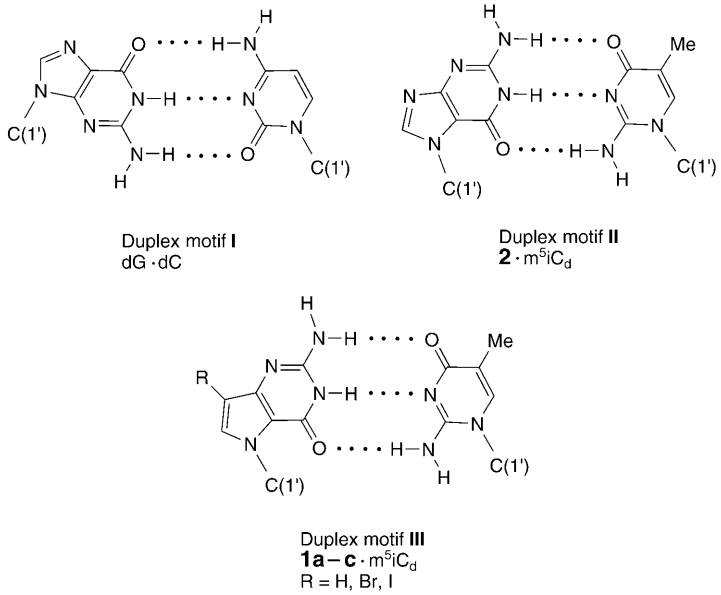


Fig. 2. *Base pairs formed by* **1a**–**c** *and* **2** *with* m^5iC_d

When incorporated into oligonucleotides, the pK_a will be higher but will be still below pH 7.0. This limits the use of triplex motif **IV** *in vivo* as an optimal triplex stability occurs only under acidic conditions; the intracellular pH is around 7.3. The first solution to provide more stable $dCH^+ \cdot dG \cdot dC$ triplexes was the use of 2′-deoxy-5-methylcytidine instead of dC [11–14]. This stabilizes the triplex significantly although its pK_a value is only 0.2 units higher than that of dC. Thus stabilization cannot be traced back to pK_a changes. Apparently the hydrophobic Me group has a favorable influence on entropic changes. Various base modifications were performed to overcome this prob-

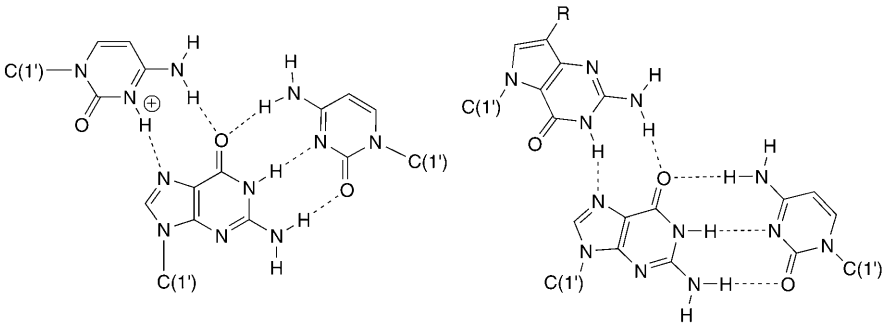


Fig. 3. *Triplex Motifs of Protonated 2′-Deoxycytidine with the dG·dC* (motif **IV**) *and of* **1a**–**c** *with the dG·dC Base Pair* (motif **V**)

lem[11][15][19–22]. Earlier, the guanine N^7-(2′-deoxyribonucleoside) **2** [15][35] has been incorporated into the TFOs. It was shown that the nucleoside **2** resembles the recognition site of a protonated 2′-deoxycytidine. A high third-strand-binding affinity specific to the dG·dC base pair was observed at neutral pH [15–18]. Also related pyrazolo[4,3-*d*]pyrimidine nucleosides (P_1 and P_2) were investigated [20][21]. Pyrrolo[3,2-*d*]-pyrimidines (=9-deazapurines) glycosylated at the 7-position mimic a protonated dC within a triplex structure and can be functionalized with various residues including any kind of reporter groups at the position 9. Therefore, we incorporated the N^7-glycosylated 9-deazapurine 2′-deoxyribonucleoside **1a** and its 9-halogen derivatives **1b**,**c** into the TFOs. Its recognition site mimics that of protonated dC. We then examined the formation of triplexes with oligonucleotides containing 15 base pairs with multiple incorporations of the modified nucleosides, both involving either a 'consecutive' run of non-canonical residues or those following an 'alternating' scheme. The target duplexes **17**·**18** and **19**·**20** shown in *Table 3* follow the same sequence which has been already used by *McLaughlin* and co-workers [37]. The third strands containing the $dCH^+ \cdot dG \cdot dC$ motif is expected to bind in parallel orientation to the target duplex by *Hoogsteen* base pairing. The triplexes differ from each other by the number of $dCH^+ \cdot dG \cdot dC$ base triads. In one series, the pyrimidine-motif-forming triplex is constructed from consecutive dCH^+ residues, while in the other, the dCH^+ residues are alternating with dT thereby diluting the number of dCH^+ residues within the particular DNA fragment from five to three.

The triplex **17**·**18**·**24** containing alternating $dCH^+ \cdot dG \cdot dC$ and $dT \cdot dA \cdot dT$ base triads in the third strand (*Hoogsteen* strand) is composed of unmodified nucleoside residues (5′-d(TTT CTT TTC TCT CTT)-3′ (**24**)). It forms a stable triplex under acidic conditions (pH value 6.5) in the presence of spermine and shows a T_m value of 45° for the *Hoogsteen*-strand melting (triplex melting) (*Table 3*). A decrease in the third-strand melting from 45° to 17° is observed at pH 8.0. This results from the deprotonation of the 2′-deoxycytosine residue being necessary for triplex formation. No changes in the higher T_m value (duplex melting) at 72° are observed at pH 6.5 and 8.0. In the case

Table 3. T_m *Values of Oligonucleotide Triplexes Containing 9-Deazaguanine* N^7*-(2′-Deoxyribonucleoside)* **1a** *and Its 9-Halogenated Derivatives* **1b**,**c** *in Alternating and Consecutive Sequences*[a])[b])

Triplex (alternating)											T_m [°]	
											pH 6.5	pH 8.0
21	5′-d(TTT	**1a**	TT	TT	**1a**	T	**1a**	T	**1a**	TT)-3′		
17	5′-d(GCGCGAAA	**G**	AA	AA	**G**	A	**G**	A	**G**	AACCCGG)-3′	45/72	45/72
18	3′-d(CGCGCTTT	**C**	TT	TT	**C**	T	**C**	T	**C**	TTGGGCC)-5′		
22	5′-d(TTT	**1b**	TT	TT	**1b**	T	**1b**	T	**1b**	TT)-3′		
17	5′-d(GCGCGAAA	**G**	AA	AA	**G**	A	**G**	A	**G**	AACCCGG)-3′	48/72	47/72[c])
18	3′-d(CGCGCTTT	**C**	TT	TT	**C**	T	**C**	T	**C**	TTGGGCC)-5′		
23	5′-d(TTT	**1c**	TT	TT	**1c**	T	**1c**	T	**1c**	TT)-3′		
17	5′-d(GCGCGAAA	**G**	AA	AA	**G**	A	**G**	A	**G**	AACCCGG)-3′	48/72[c])	48/72[c])
18	3′-d(CGCGCTTT	**C**	TT	TT	**C**	T	**C**	T	**C**	TTGGGCC)-5′		
24	5′-d(TTT	**C**	TT	TT	**C**	T	**C**	T	**C**	TT)-3′		
17	5′-d(GCGCGAAA	**G**	AA	AA	**G**	A	**G**	A	**G**	AACCCGG)-3′	45/72	17/72
18	3′-d(CGCGCTTT	**C**	TT	TT	**C**	T	**C**	T	**C**	TTGGGCC)-5′		
Triplex (consecutive)												
25	5′-d(TTT	**1a**	TT	TT	**1a**	**1a**	**1a**	**1a**	**1a**	TT)-3′		
19	5′-d(GCGCGAAA	**G**	AA	AA	**G**	**G**	**G**	**G**	**G**	AACCCGG)-3′	53/74	52/74
20	3′-d(CGCGCTTT	**C**	TT	TT	**C**	**C**	**C**	**C**	**C**	TTGGGCC)-5′		
26	5′-d(TTT	**1b**	TT	TT	**1b**	**1b**	**1b**	**1b**	**1b**	TT)-3′		
19	5′-d(GCGCGAAA	**G**	AA	AA	**G**	**G**	**G**	**G**	**G**	AACCCGG)-3′	57/74	56/74[c])
20	3′-d(CGCGCTTT	**C**	TT	TT	**C**	**C**	**C**	**C**	**C**	TTGGGCC)-5′		
27	5′-d(TTT	**1c**	TT	TT	**1c**	**1c**	**1c**	**1c**	**1c**	TT)-3′		
19	5′-d(GCGCGAAA	**G**	AA	AA	**G**	**G**	**G**	**G**	**G**	AACCCGG)-3′	57/74	57/74[c])
20	3′-d(CGCGCTTT	**C**	TT	TT	**C**	**C**	**C**	**C**	**C**	TTGGGCC)-5′		
28	5′-d(TTT	**C**	TT	TT	**C**	**C**	**C**	**C**	**C**	TT)-3′		
19	5′-d(GCGCGAAA	**G**	AA	AA	**G**	**G**	**G**	**G**	**G**	AACCCGG)-3′	24/74	n.t.[d])/74
20	3′-d(CGCGCTTT	**C**	TT	TT	**C**	**C**	**C**	**C**	**C**	TTGGGCC)-5′		

[a]) Measured at 260 nm in 10.5 mM HEPES (=4-(2-hydroxyethyl)-1-piperazineethansulfonic acid) with 50 mM NaCl, 10 mM $MgCl_2$ and 0.5 mM spermine. [b]) The concentration of single strand was 1 μM. [c]) Observed with poor hypochromicity. [d]) No triplex formed.

of the triplex **19**·**20**·**28**, in which a third strand contains five adjacent dC residues (5′-d(TTT CTT TTC CCC CTT)-3′ (**28**)), the stability of the triplex at pH 6.5 decreases to 24°, and no triplex is formed at pH 8.0. Thus the formation of triplexes is restricted to pH 6.5, while duplex melting stays almost unchanged (T_m 74°) (*Table 3*). Apparently, the presence of adjacent charged dCH^+ residues leads to a charge repulsion resulting in a destabilization of the triplex structure [16].

The 9-deazaguanine N^7-(2′-deoxyribonucleoside) **1a** can form a bidentate *Hoogsteen* pair with dG·dC without protonation thereby resembling the dCH^+·dG·dC base-pairing motif (see *Fig. 3*, motif **IV** and **V**). For this purpose, oligonucleotides **21**–**23** and **25**–**27** were studied next. Typical first derivatives of the melting profiles are shown in *Fig. 4*.

The replacement of dC by nucleoside **1a**, as shown in *Table 3* results in a stable triplex structure **17**·**18**·**21** showing T_m values for duplex and triplex melting similar to

those incorporating dCH^+ residues, *i.e.*, **17·18·24**. The most significant difference between the triplex **17·18·24** containing dCH^+ the triplex and **17·18·21** containing **1a** lies in the finding that a stable triplex is formed at pH 8.0 with the latter one (45°) (*Fig. 4*,*a*). At pH 8.0, triplex **17·18·24** showed a rather low stability (17°). This demonstrates that triplex formation takes place pH-independently when dC is replaced by compound **1a**. In particular, replacements of dC residues by **1a** with five consecutive incorporations in the triplex **19·20·25** (*Table 3*) forms a much more stable triplex (T_m 53° at pH 6.5 and 52° at pH 8.0). The stabilizing effect of **1a** is much higher in the consecutive sequence motif as compared to the alternating arrangement of bases. We propose that this stabilization is due to an improved base stacking between adjacent N^7-glycosylated guanine residues, the absence of charges, and an increased surface area compared to the protonated dC residues. Thus the destabilization of triplexes caused by adjacent charged dCH^+ residues can be circumvented when dC is replaced by nucleoside **1a**, and the stable triplexes can be formed under neutral conditions.

As discussed above, we became also interested in studying the influence of bulky 9-halogeno substituents incorporated in the same triplexes. For that purpose compound **1a** was replaced by its 9-bromo and 9-iodo derivatives **1b**,**c** (*Table 3*). According to *Table 3*, triplexes containing **1b**,**c** exhibit significantly higher T_m values than those containing the 9-unsubstituted nucleoside **1a**. These results are in line with our expectation derived from earlier experiments [11]. *Table 3* also demonstrates that the effect of stabilization of triplexes by 9-bromo and 9-iodo nucleosides **1b** and **1c**, respectively, is similar. The presence of **1b** and **1c** in the 'alternating' triplexes **17·18·22** and **17·18·23** leads to T_m values around 48° at both pH 6.5 and 8.0 (*Fig. 4*,*b*). This is also much higher when compared with that of nucleoside **1a**. The effect of stabilization by **1b**,**c** is also similar in the case of consecutive incorporations; the triplexes **19·20·26** and **19·20·27**) containing five adjacent incorporations of **1b**,**c** show a T_m value of 57° at pH 6.5 and 8.0 (*Fig. 4*,*c*). This clearly reveals that the 9-halogeno substituents of 9-deazapurine nucleoside **1a** stabilize DNA triplexes, and the 9-substituents are well accommodated in triplex DNA. This stabilizing effect can be attributed to the enhanced stacking interactions of the 9-halogenated 9-deazapurine residues within the triplex structure. Furthermore, the halogeno substituents make the triplex structure more hydrophobic leading to favorable entropic changes. These results clearly demonstrate that the destabilization of triplexes in case of consecutive incorporations of dCH^+ caused by charge–charge repulsion of consecutive incorporations can be alleviated when dC is replaced by **1a**–**c**.

Next, the CD spectra of the triplexes formed between the duplex target site and modified oligonucleotides in the case of alternating as well as of consecutive sequences were measured (*Fig. 5*). The appearance of an intense negative short wavelength band (210–220 nm) in the CD spectra strongly supports the formation of triple-stranded complexes [38–43]. The absorption at 210–220 nm was observed for both the 'alternating' modified triplexes **17·18·21**, **17·18·22,** and **17·18·23** (*Fig. 5*,*a*) as well as for the triplexes **19·20·25**, **19·20·26**, and **19·20·27** with consecutive incorporations (*Fig. 5*,*b*) but not for the duplexes **17·18** and **19·20**.

Conclusions. – Triplex DNA containing the $dCH^+ \cdot dG \cdot dC$ motif is susceptible to pH changes as only the protonated dC or its 5-methyl derivative are able to form

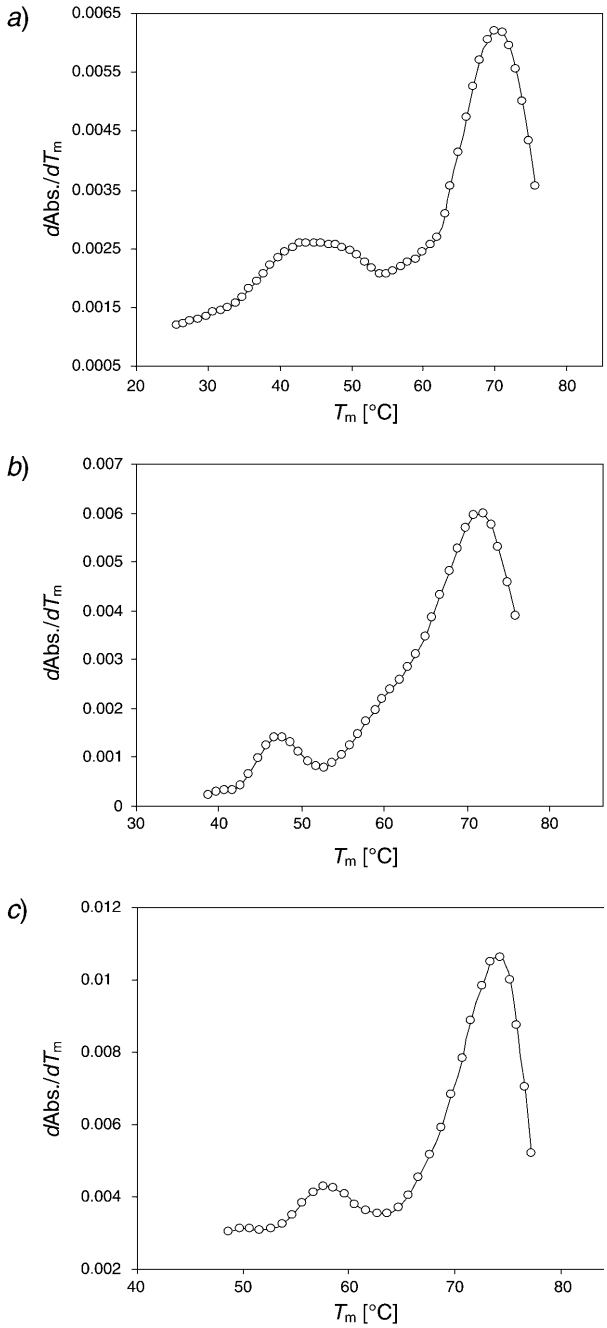


Fig. 4. *First derivatives of the UV/melting curves of the triplexes* a) **17**·**18**·**21** (containing **1a**), b) **17**·**18**·**22** (containing **1b**) *at pH 6.5, and* c) **19**·**20**·**27** (containing **1c**) *at pH 8.0.* Measured at 260 nm in 10.5 mM HEPES, 50 mM NaCl, 10 mM $MgCl_2$, and 0.5 mM spermine.

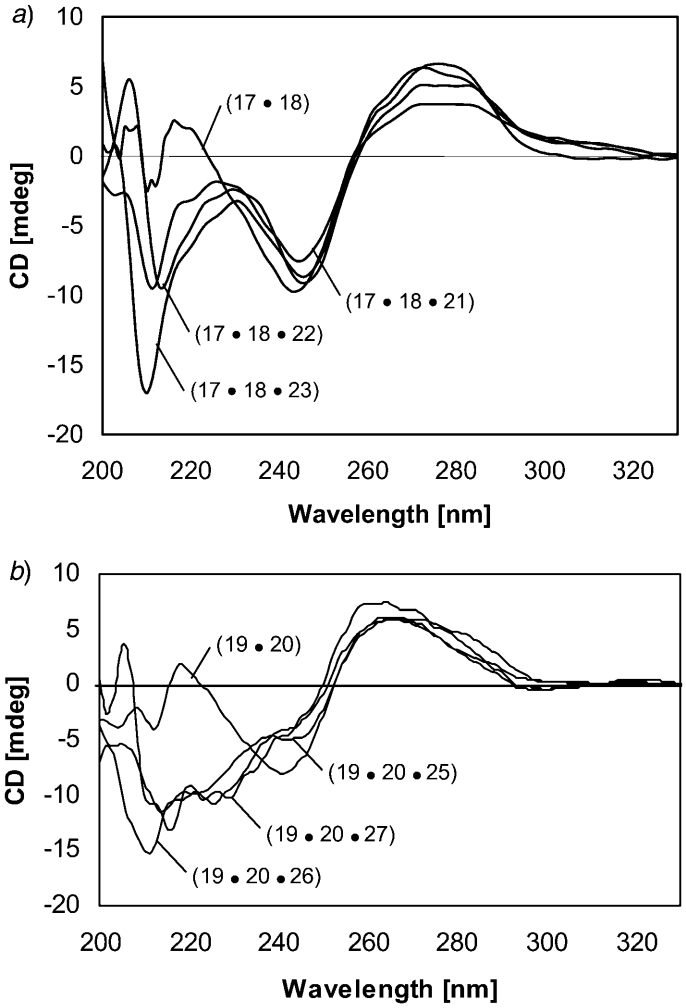


Fig. 5. *CD Spectra of DNA triplexes* a) *containing alternating* **1a**–**c**·*dG*·*dC and dT*·*dA*·*dT base triplets* (triplexes **17**·**18**·**21**, **17**·**18**·**22**, and **17**·**18**·**23**) *and* b) *containing consecutive* **1a**–**c**·*dG*·*dC base triplets* (triplexes **19**·**20**·**25**, **19**·**20**·**26**, and **19**·**20**·**27**). Measured in 10.5 mM HEPES, 50 mM NaCl, 10 mM $MgCl_2$, and 0.5 mM spermine at pH 6.5.

Hoogsteen base pairs. This behavior is a drawback for the triplex application in DNA diagnostics or antigene therapy. The replacement of dC by the uncommonly linked 9-deazapurine N^7-deoxyribofuranoside **1a** within TFOs results in stable triplex formation under neutral conditions. Thus the 9-deazaguanine base mimics the recognition site of a protonated cytosine. The stability of these triplexes is further enhanced when the nucleoside **1a** is substituted at the 9-position by bromo or iodo substituents (see **1b**,**c**). The effect of triplex stabilization is particularly pronounced in the case of consecutive incorporations of **1a**–**c** as compared to alternating **1a**–**c**·dT sequence.

This reveals charge repulsion of protonated dC residues which does not occur in triplexes containing **1a**–**c**. All 9-deazapurine nucleosides are almost completely stable against N–glycosyl bond hydrolysis, and the bulky 9-halogeno substituents are well accommodated in the DNA triplexes.

We thank Dr. *H. Rosemeyer* and Dr. *Y. He* for the NMR spectra. We also thank *M. Dubiel*, *E. Michalek*, and *S. Budow* for their kind help. Financial support by *Roche Diagnostics GmbH* (Penzberg, Germany) is gratefully acknowledged.

Experimental Part

General. All chemicals were purchased from *Aldrich*, *Sigma*, or *Fluka* (*Sigma-Aldrich Chemie GmbH*, Deisenhofen, Germany). Solvents were of laboratory grade. Thin layer chromatography (TLC): aluminium sheets, silica gel *60* F_{254}, 0.2 mm layer (*VWR*, Germany). Column flash chromatography (FC): silica gel *60* (*VWR*, Germany) at 0.4 bar; sample collection with an *UltroRac II* fractions collector (*LKB Instruments*, Sweden). UV Spectra: *U-3200* spectrometer (*Hitachi*, Tokyo, Japan); λ_{max} (ε) in nm. CD Spectra: *Jasco 600* (*Jasco*, Japan) spectropolarimeter with thermostatically (*Lauda-RCS-6* bath) controlled 1-cm cuvettes. NMR Spectra: *Avance-250* or *AMX-500* spectrometers (*Bruker*, Karlsruhe, Germany), at 250.13 MHz for ^{1}H and ^{13}C; δ in ppm rel. to Me_4Si as internal standard or external 85% H_3PO_4 for ^{31}P; *J* values in Hz. Elemental analyses were performed by *Mikroanalytisches Laboratorium Beller* (Göttingen, Germany).

UV/Melting Curves. The melting temp. were measured with a *Cary-1/3* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller. The temperature was measured continuously in the reference cell with a *Pt-100* resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program [44].

Oligonucleotides. The oligonucleotide syntheses were carried out in an *ABI 392-08* DNA synthesizer (*Applied Biosystems*, Weiterstadt, Germany) at a 1-μmol scale with the phosphoramidites **5a**–**c** and **8a**–**c** following the synthesis protocol for 3′-(2-cyanoethyl phosphoramidites) (user manual for the *392* DNA synthesizer, *Applied Biosystems*, Weiterstadt, Germany). The coupling efficiency was always higher than 97%. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aq. NH_3 soln. for 14–16 h at 60° [45].

Purification of the 5′-dimethoxytrityl oligomers was performed by reversed-phase HPLC (*RP-18*; gradient system (*A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5, *B* = MeCN): 3 min 20% *B* in *A*, 12 min 20–50% *B* in *A*, and 25 min 20% *B* in *A*; flow rate 1.0 ml/min). The soln. was dried and treated with 2.5% $CHCl_2COOH$ in CH_2Cl_2 for 5 min at 0° to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC (gradient: 0–20 min 0–20% *B* in *A*; flow rate 1.0 ml/min). The oligomers were desalted (*RP-18*, silica gel) and lyophilized on a *Speed-Vac* evaporator to yield colorless solids which were frozen at −24°. For UV/melting analysis of the triplexes, the oligonucleotides (single-strand concentration 1 μmol at 260 nm) were mixed in the buffer soln. containing 10.5 mM HEPES (= 2-[4-(2-hydroxyethyl)piperazine]-1-ethansulfonic acid), 50 mM NaCl, 10 mM $MgCl_2$, and 0.5 mM spermine, and heated to 85° for 5 min, allowed to cool to r.t. followed by overnight storage at 4°. The temp. was measured continuously by heating with the rate of 0.5°/min from 20° to 90°.

The enzymatic hydrolysis of the oligonucleotides was performed as described by *Seela* and *Becher* [46] with snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamenteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli* from *Roche Diagnostics GmbH*, Germany) in 0.1M *Tris*·HCl buffer (pH 8.3) at 37°. The reaction products were subjected to reversed-phase HPLC (*RP-18*, 200 × 10 mm column; gradient: 20 min *A*, 20–60 min 50% *B* in *A*). Quantification of the constituents were made on the basis of the peak areas, which were divided by the extinction coefficients ε_{260} of the nucleosides: dT 8800, dC 7300, dA 15400, **1a** 5500, **1b** 4000, and **1c** 4100.

The molecular masses of the oligonucleotides were determined by MALDI-TOF-MS with a *Biflex-III* instrument (*Bruker Saxonia*, Leipzig, Germany) and 3-hydroxypicolinic acid (3-HPA) as a matrix (*Table 4*).

Table 4. *Molecular Masses* ($[M+H]^+$) *of the Oligonucleotides Measured by MALDI-TOF Mass Spectrometry*

	$[M+H]^+$ (calc.)	$[M+H]^+$ (found)
5′-d(TA**1a 1a**TC AAT ACT)-3′ (**13**)	3641.6	3641.1
5′-d(TA**1b 1b**TC AAT ACT)-3′ (**15**)	3799.2	3801.1
5′-d(TA**1c 1c**TC AAT ACT)-3′ (**16**)	3895.2	3895.7
5′-d(TTT **1a**TT TT**1a** T**1a**T **1a**TT)-3′ (**21**)	4598.0	4598.3
5′-d(TTT **1a**TT TT**1a 1a1a1a 1a**TT)-3′ (**25**)	4647.1	4647.5
5′-d(TTT **1b**TT TT**1b** T**1b**T **1b**TT)-3′ (**22**)	4913.6	4915.1
5′-d(TTT **1b**TT TT**1b 1b1b1b 1b**TT)-3′ (**26**)	5119.5	5120.5
5′-d(TTT **1c**TT TT**1c** T**1c**T **1c**TT)-3′ (**23**)	5101.9	5101.2
5′-d(TTT **1c**TT TT**1c 1c1c1c 1c**TT)-3′ (**27**)	5401.5	5401.6

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]amino}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one* (**4a**). Compound **3a** [33] (375 mg, 1.16 mmol) was dried by repeated co-evaporation with anh. pyridine (2×3 ml) and dissolved in anh. pyridine (5 ml). Then, 4,4′-dimethoxytrityl chloride (550 mg, 1.62 mmol) was added at r.t. while stirring, and stirring was continued for another 5 h. The mixture was poured into a 5% aq. $NaHCO_3$ soln. (30 ml) and extracted with CH_2Cl_2 (2×30 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the resulting residue subjected to FC (silica gel, column 3×12 cm, CH_2Cl_2/Me_2CO 9:1 (300 ml), then CH_2Cl_2/MeOH 95:5): **4a** (650 mg, 89%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.3. UV (MeOH): 232 (34700), 252 (29600), 293 (21300). ^{1}H-NMR ((D$_6$)DMSO): 11.18 (*s*, NH); 8.54 (*s*, N=CH); 7.40 (*d*, *J* = 2.85, H–C(6)); 7.40–7.23 (*m*, arom. H); 6.94–6.84 (*m*, arom. H, H–C(1′)); 6.13 (br. *s*, H–C(7)); 5.31 (*m*, OH–C(3′)); 4.29 (*m*, H–C(3′)); 3.90 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.10 (*m*, MeN, CH_2(5′)); 2.98 (*s*, MeN); 2.36–2.27 (*m*, CH_2(2′)). Anal. calc. for $C_{35}H_{37}N_5O_6$ (623.70): C 67.40, H 5.98, N 11.23; found: C 66.49, H 5.88, N 10.92.

*7-Bromo-5-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)-methylidene]amino}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one* (**4b**). As described for **4a**, with **3b** [33] (400 mg, 1.00 mmol), 4,4′-dimethoxytrityl chloride (440 mg, 1.30 mmol), and pyridine (5 ml): **4b** (625 mg, 89%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.3. UV (MeOH): 231 (27300), 260 (21800), 297 (16600). ^{1}H-NMR ((D$_6$)DMSO): 11.39 (*s*, NH); 8.50 (*s*, N=CH); 7.62 (*s*, H–C(6)); 7.39–7.23 (*m*, arom. H); 6.87–6.84 (*m*, arom. H, H–C(1′)); 5.30 (*d*, *J* = 4.2, OH–C(3′)); 4.30 (*m*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.13–3.08 (*m*, MeN, CH_2(5′)); 3.00 (*s*, MeN); 2.39 (*m*, H_α–C(2′)); 2.28 (*m*, H_β–C(2′)). Anal. calc. for $C_{35}H_{36}BrN_5O_6$ (702.59): C 59.83, H 5.16, N 9.97; found: C 59.82, H 5.17, N 9.83.

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]amino}-3,5-dihydro-7-iodo-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one* (**4c**). As described for **4a**, with **3c** [33] (250 mg, 0.58 mmol), 4,4′-dimethoxytrityl chloride (271 mg, 0.80 mmol), and pyridine (4 ml): **4c** (359 mg, 86%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.27. UV (MeOH): 231 (25600), 262 (20200), 298 (15600). ^{1}H-NMR ((D$_6$)DMSO): 11.33 (*s*, NH); 8.49 (*s*, N=CH); 7.62 (*s*, H–C(6)); 7.39–7.20 (*m*, arom. H); 6.90–6.86 (*m*, arom. H, H–C(1′)); 5.29 (*d*, *J* = 4.1, OH–C(3′)); 4.30 (*m*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.73 (*s*, 2 MeO); 3.19–3.09 (*m*, MeN, CH_2(5′)); 3.00 (*s*, MeN); 2.37 (*m*, H_α–C(2′)); 2.27 (*m*, H_β–C(2′)). Anal. calc. for $C_{35}H_{36}IN_5O_6$ (749.59): C 56.08, H 4.84, N 9.34; found: C 56.13, H 4.77, N 9.40.

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]amino}-3-[(2,2-dimethyl-1-oxopropoxy)methyl]-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one* (**7a**). As described for **4a**, with **6a** [33] (375 mg, 0.86 mmol), 4,4′-dimethoxytrityl chloride (339 mg, 1.0 mmol), and pyridine (5 ml): **7a** (550 mg, 86%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.36. UV (MeOH): 236 (23600), 257 (25200), 298 (18600). ^{1}H-NMR ((D$_6$)DMSO): 8.56 (*s*, N=CH); 7.72 (*s*, H–C(6)); 7.38–7.20 (*m*, arom. H); 6.87–6.84 (*m*, arom. H, H–C(1′)); 6.16 (*s*, CH_2O); 5.37 (*d*,

J=4.3, OH–C(3′)); 4.33 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.15–3.10 (*m*, MeN, CH_2(5′)); 2.98 (*s*, MeN); 2.43 (*m*, H_β–C(2′)); 2.38 (*m*, H_α–C(2′)); 1.08 (*s*, 3 Me). Anal. calc. for $C_{41}H_{47}N_5O_8$ (737.84): C 66.74, H 6.42, N 9.49; found: C 66.53, H 6.31, N 9.54.

*7-Bromo-5-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)-methylidene]amino}-3-[(2,2-dimethyl-1-oxopropoxy)methyl]-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one* (**7b**). As described for **4a**, with **6b** [33] (262 mg, 0.509 mmol), 4,4′-dimethoxytrityl chloride (300 mg, 0.88 mmol), and pyridine (5 ml): **7b** (355 mg, 85%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.3. UV (MeOH): 237 (24000), 265 (25900), 312 (19300). ^{1}H-NMR ((D$_6$)DMSO): 8.52 (*s*, N=CH); 7.71 (*s*, H–C(6)); 7.38–7.20 (*m*, arom. H); 6.87–6.84 (*m*, arom. H, H–C(1′)); 6.16 (*s*, CH_2O); 5.37 (*d*, J=4.3, OH–C(3′)); 4.33 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.15–3.10 (*m*, MeN, CH_2(5′)); 2.98 (*s*, MeN); 2.40 (*m*, H_β–C(2′)); 2.32 (*m*, H_α–C(2′)); 1.08 (*s*, 3 Me). Anal. calc. for $C_{41}H_{46}BrN_5O_8$ (816.74): C 60.29, H 5.68, N 8.57; found: C 60.22, H 5.78, N 8.45.

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]amino}-3-[(2,2-dimethyl-1-oxopropoxy)methyl]-3,5-dihydro-7-iodo–4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one* (**7c**). As described for **4a**, with **6c** [33] (150 mg, 0.26 mmol), 4,4′-dimethoxytrityl chloride (170 mg, 0.50 mmol), and pyridine (4 ml): **7c** (176 mg, 82%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.3. UV (MeOH): 237 (21500), 267 (25000), 313 (18800). ^{1}H-NMR ((D$_6$)DMSO): 8.51 (*s*, N=CH); 7.70 (*s*, H–C(6)); 7.39–7.22 (*m*, arom. H, H); 6.88–6.86 (*m*, H–C(1′)); 6.17 (*s*, CH_2O); 5.36 (*d*, J=4.3, Hz, OH–C(3′)); 4.33 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.73 (*s*, 2 MeO); 3.19–3.11 (*m*, MeN, CH_2(5′)); 2.99 (*s*, MeN); 2.41 (*m*, H_β–C(2′)); 2.31 (*m*, H_α–C(2′)); 1.09 (*s*, 3 Me). Anal. calc. for $C_{41}H_{46}IN_5O_8$ (863.74): C 57.01, H 5.37, N 8.11; found: C 57.10, H 5.48, N 7.98.

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]-amino}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5a**). To a soln. of **4a** (300 mg, 0.50 mmol) in anh. CH_2Cl_2 (8 ml) were added ^{i}Pr$_2$NEt (210 µl, 1.17 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (166 µl, 0.75 mmol) while stirring under Ar at r.t. Stirring was continued for another 30 min, and then CH_2Cl_2 (30 ml) was added. The soln. was then washed with 5% aq. $NaHCO_3$ soln. (30 ml), the aq. layer extracted with CH_2Cl_2 (2×30 ml), the combined org. layer dried (Na_2SO_4) and evaporated, the resulting residue subjected to FC (silica gel, column 6×1.5 cm, CH_2Cl_2/Me_2CO 8:2): **5a** (352 mg, 88%). Colorless foam. TLC (CH_2Cl_2/Me_2CO 8:2): R_f 0.7. ^{31}P-NMR ($CDCl_3$): 150.00; 149.41.

*7-Bromo-5-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)-methylidene]amino}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5b**). As described for **5a**, with **4b** (200 mg, 0.28 mmol), ^{i}Pr$_2$NEt (79 µl, 0.46 mmol), 2-cyanoethyl diisopropylphosphoramidochloridite (72 µl, 0.33 mmol), and CH_2Cl_2 (4 ml): **5b** (200 mg, 78%). Colorless foam. TLC (CH_2Cl_2/Me_2CO 8:2): R_f 0.64. ^{31}P-NMR ($CDCl_3$): 150.08, 149.53.

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]-amino}-3,5-dihydro-7-iodo-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5c**). As described for **5a**, with **4c** (300 mg, 0.40 mmol), ^{i}Pr$_2$NEt (100 µl, 0.58 mmol), 2-cyanoetyl diisopropylphosphoramidochloridite (101 µl, 0.46 mmol), and CH_2Cl_2 (5 ml): **5c** (228 mg, 60%). Colorless foam. TLC (CH_2Cl_2/Me_2CO 8:2): R_f 0.60. ^{31}P-NMR ($CDCl_3$): 150.05; 149.52.

*5-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]amino}-3-[(2,2-dimethyl-1-oxopropoxy)methyl]-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**8a**). As described for **5a**, with **7a** (200 mg, 0.28 mmol), ^{i}Pr$_2$NEt (79 µl, 0.46 mmol), 2-cyanoethyl diisopropylphosphoramidochloridite (72 µl, 0.33 mmol), and CH_2Cl_2 (4 ml): **8a** (200 mg, 78%). Colorless foam. TLC (CH_2Cl_2/Me_2CO 9:1): R_f 0.71. ^{31}P-NMR ($CDCl_3$): 150.00; 149.41.

*7-Bromo-5-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-2-{[(dimethylamino)-methylidene]amino}-3-[(2,2-dimethyl-1-oxopropoxy)methyl]-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**8b**). As described for **5a**, with **7b** (200 mg, 0.24 mmol), ^{i}Pr$_2$NEt (67 µl, 0.39 mmol), 2-cyanoethyl diisopropylphosphoramidochloridite (66 µl, 0.30 mmol), and CH_2Cl_2 (4 ml): **8b** (150 mg, 60%). Colorless foam. TLC (CH_2Cl_2/Me_2CO 9:1): R_f 0.70. ^{31}P-NMR ($CDCl_3$): 150.02; 149.60.

5-[2-Deoxy-5-O-*(4,4′-dimethoxytrityl)-β*-D-erythro-*pentofuranosyl]-2-{[(dimethylamino)methylidene]amino}-3-[(2,2-dimethyl-1-oxopropoxy)methyl]-3,5-dihydro-7-iodo-4*H-*pyrrolo[3,2*-d*]pyrimidin-4-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**8c**). As described for **5a**, with **7c** (100 mg, 0.11 mmol), $^{i}Pr_2NEt$ (33 µl, 0.19 mmol), 2-cyanoethyl diisopropylphosphoramidochloridite (33 µl, 0.15 mmol), and CH_2Cl_2 (2 ml): **8c**. (90 mg, 73%). Colorless foam. TLC (CH_2Cl_2/Me_2CO 9:1): R_f 0.68. ^{31}P-NMR ($CDCl_3$): 149.98; 149.57.

REFERENCES

[1] G. Felsenfeld, D. R. Davies, A. Rich, *J. Am. Chem. Soc.* **1957**, *79*, 2023.
[2] H. E. Moser, P. B. Dervan, *Science (Washington, D.C.)* **1987**, *238*, 645.
[3] T. Le Doan, L. Perrouault, D. Praseuth, N. Habhoub, J.-L. Decout, N. T. Thuong, J. Lhomme, C. Hélène, *Nucleic Acids Res.* **1987**, *15*, 7749.
[4] C. Hélène, *Anticancer Drug Des.* **1991**, *6*, 569.
[5] A. F. Faruqi, S. H. Krawczyk, M. D. Matteucci, P. M. Glazer, *Nucleic Acids Res.* **1997**, *25*, 633.
[6] A. Majumdar, A. Khorlin, N. Dyatkina, F.-L. M. Lin, J. Powell, J. Liu, Z. Fei, Y. Khripine, K. A. Watanabe, J. George, P. M. Glazer, M. M. Seidman, *Nature Genet.* **1998**, *20*, 212.
[7] C. Giovannangeli, S. Diviacco, V. Labrousse, S. Gryaznov, P. Charneau, C. Hélène, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 79.
[8] E. S. Belousov, I. A. Afonina, I. V. Kutyavin, A. A. Gall, M. W. Reed, H. B. Gamper, R. M. Wydro, R. B. Meyer, *Nucleic Acids Res.* **1998**, *26*, 1324.
[9] C. Hélène, in '*Triplex Forming Oligonucleotides*', Eds. C. Malvy, A. Harel-Bellan, L. L. Pritchard, and M. A. Norwell, Kluwer Academic, 1999.
[10] V. N. Soyfer, V. N. Potaman, 'Triple-Helical Nucleic Acids', Springer, New York, 1995.
[11] T. J. Povsic, P. B. Dervan, *J. Am. Chem. Soc.* **1989**, *111*, 3059.
[12] L. E. Xodo, G. Manzini, F. Quadrifoglio, G. A. van der Marel, J. H. van Boom, *Nucleic Acids Res.* **1991**, *19*, 5625.
[13] S. F. Singleton, P. B. Dervan, *Biochemistry* **1992**, *31*, 10995.
[14] G. E. Plum, Y.-W. Park, S. F. Singleton, P. B. Dervan, K. J. Breslauer, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9436.
[15] J. Hunziker, E. S. Priestley, H. Brunar, P. B. Dervan, *J. Am. Chem. Soc.* **1995**, *117*, 2661.
[16] H. Brunar, P. B. Dervan, *Nucleic Acids Res.* **1996**, *24*, 1987.
[17] A. St. Clair, G. Xiang, L. W. McLaughlin, *Nucleosides Nucleotides* **1998**, *17*, 925.
[18] M. D'Costa, V. A. Kumar, K. N. Ganesh, *J. Org. Chem.* **2003**, *68*, 4439.
[19] J. Marfurt, C. Leumann, *Angew. Chem.* **1998**, *110*, 184.
[20] J. S. Koh, P. B. Dervan, *J. Am. Chem. Soc.* **1992**, *114*, 1470.
[21] I. Radhakrishnan, D. J. Patel, E. S. Priestley, H. M. Nash, P. B. Dervan, *Biochemistry* **1993**, *32*, 11228.
[22] U. Parsch, J. W. Engels, *Chem.–Eur. J.* **2000**, *6*, 2409.
[23] F. Seela, H. Thomas, *Helv. Chim. Acta* **1995**, *78*, 94.
[24] F. Seela, M. Zulauf, *Chem.–Eur. J.* **1998**, *4*, 1781.
[25] N. Ramzaeva, F. Seela, *Helv. Chim. Acta* **1996**, *79*, 1549.
[26] N. Ramzaeva, C. Mittelbach, F. Seela, *Helv. Chim. Acta* **1997**, *80*, 1809.
[27] F. Seela, M. Zulauf, *J. Chem. Soc., Perkin Trans. 1* **1999**, 479.
[28] F. Seela, G. Becher, *Helv. Chim. Acta* **1999**, *82*, 1640.
[29] Y. Aubert, L. Perrouault, C. Hélène, C. Giovannangeli, U. Asseline, *Bioorg. Med. Chem.* **2001**, *9*, 1617.
[30] P. Leonard, T. Wiglenda, F. Seela, *Nucleosides, Nucleotides, Nucleic Acids* **2001**, *20*, 1279.
[31] T. Wiglenda, Diplomarbeit, Universität Osnabrück, Osnabrück, Germany, 2001.
[32] N. S. Girgis, H. B. Cottam, S. B. Larson, R. K. Robins, *J. Heterocycl. Chem.* **1987**, *24*, 821.
[33] F. Seela, K. I. Shaikh, T. Wiglenda, P. Leonard, *Helv. Chim. Acta* **2004**, *87*, 2507.
[34] F. Seela, H. Winter, '10th International Round Table', Park City, Utah, USA. 1992.
[35] F. Seela, P. Leonard, *Helv. Chim. Acta* **1996**, *79*, 477.

[36] P. Leonard, Ph. D. Thesis, University of Osnabrück, Osnabrück, Germany, 1998.
[37] G. Xiang, R. Bogacki, L. W. McLaughlin, *Nucleic Acids Res.* **1996**, *24*, 1963.
[38] D. M. Gray, A. R. Morgan, R. L. Ratliff, *Nucleic Acids Res.* **1978**, *5*, 3679.
[39] D. M. Gray, S.-H. Hung, K. H. Johnson, *Methods Enzymol.* **1995**, *246*, 19.
[40] K. Liu, H. T. Miles, J. Frazier, V. Sasisekharan, *Biochemistry* **1993**, *32*, 11802.
[41] N. Sugimoto, P. Wu, H. Hara, Y. Kawamoto, *Biochemistry* **2001**, *40*, 9396.
[42] A. Aviñó, M. Frieden, J. C. Morales, B. G. de la Torre, R. G. García, F. Azorín, J. L. Gelpí, M. Orozco, C. González, R. Eritja, *Nucleic Acids Res.* **2002**, *30*, 2609.
[43] S. D. Osborne, V. E. C. Powers, D. A. Rusling, O. Lack, K. R. Fox, T. Brown, *Nucleic Acids Res.* **2004**, *32*, 4439.
[44] M. Petersheim, D. H. Turner, *Biochemistry* **1983**, *22*, 256.
[45] 'Users Manual of the DNA Synthesizer', Applied Biosystems, Weiterstadt, Germany.
[46] F. Seela, G. Becher, *Nucleic Acids Res.* **2001**, *29*, 2069.

Received November 17, 2005