7-Deazapurine Oligodeoxyribonucleotides: The Effects of 7-Deaza-8-methylguanine on DNA Structure and Stability

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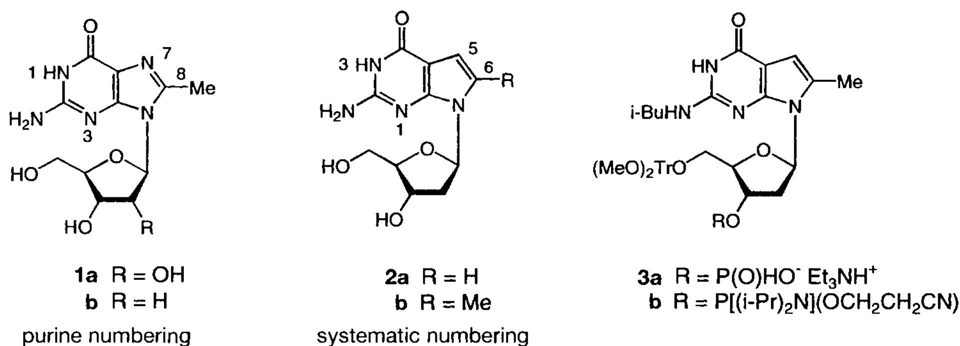
Oligodeoxyribonucleotides containing 7-deaza-2′-deoxy-8-methylguanosine ($m^8c^7G_d$; **2b**) were prepared. For this purpose, the phosphonate **3a** and the phosphoramidite **3b** were synthesized and employed in solid-phase oligodeoxyribonucleotide synthesis. The structures and the thermodynamic data of duplex formation of oligodeoxyribonucleotides containing **2b** were investigated by temperature-dependent CD and UV spectra and compared with those containing 7-deaza-2′-deoxy-7-methylguanosine ($m^7c^7G_d$) or 7-deaza-2′-deoxyguanosine (c_7G_d; **2a**). In general, compound **2b** reduces the duplex stability. In case of the sequence d(m^8c^7G-C)$_4$ (**18**), the B → Z transition was facilitated by the incorporation of **2b**. Moreover, a single 7-deaza-8-methylguanine residue present in an oligodeoxyribonucleotide tract of guanine residues destabilizes the dG quadruplex significantly. This destabilization is more pronounced than in the case of 7-deazaguanine or 7-deaza-7-methylguanine.

Introduction. – The purine residues bearing alkyl or halogen substituents at C(8) have a strong influence on the nucleoside conformation and ultimately on the structure of DNA [1–8]. Single-crystal X-ray analysis has shown that the orientation around the *N*-glycosylic bond is often biased towards *syn* in the case of 8-substituted guanine ribonucleosides [1–4]. This is the result of steric repulsion between the 8-substituents and the sugar moiety. In the solid state, these nucleosides show a C(2′)-*endo* pucker ($^2T_3$) and an intramolecular O(5′)–H···N(3) H-bond [1–4]. The 8-methylguanosine (**1a**) and 2′-deoxy-8-methylguanosine (**1b**) have been incorporated into oligonucleotides. These residues markedly stabilize the Z-conformation of the oligonucleotide duplex, even at low salt concentration [5–8]. In such a Z-DNA structure, the guanine residues adopt the *syn*/C(3′)-*endo* conformation. The 8-substituents are located in the periphery of the left-handed helix and exposed to the solvent [5][6].

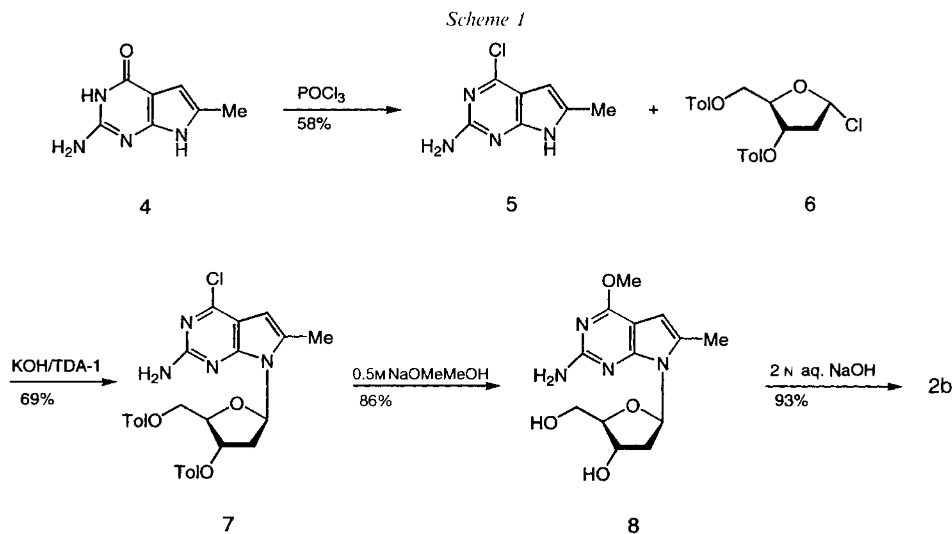
Earlier, the role of 7-substituted 7-deazapurine nucleosides on the DNA structure and stability was studied [9–12]. It was found that substituents at the 7-position have steric freedom within the major groove of B-DNA, thereby stabilizing the duplex. Now, the 8-substituted 7-deazapurine derivative, 7-deaza-2′-deoxy-8-methylguanosine ($m^8c^7G_d$; **2b**), is incorporated [13] into oligonucleotides. For this molecule, a single-crystal X-ray structure has been reported which is the first solid-state structure of an 8-substituted purine 2′-deoxyribonucleoside related to 2′-deoxyguanosine. This structure shows a peculiarity. The glycosylic-bond orientation is *anti* and not *syn* as observed for 8-substituted guanine ribonucleosides [14]. This manuscript reports on the structure and stability of oligonucleotides containing compound **2b** within various sequence patterns which were obtained by solid-phase synthesis using phosphonate **3a** or phosphoramidite **3b**. The structural changes of single-stranded oligonucleotide chains, duplexes, and

quadruplex structures will be investigated and compared with oligonucleotides bearing the Me group at the 7-position.



Results and Discussion. – *Synthesis and Properties of 7-Deaza-2'-deoxy-8-methylguanosine* (**2b**). First, 7-deaza-8-methylguanine (**4**) was prepared as described in [15]. Chlorination of **4** was then performed with $POCl_3$ yielding the 6-chloro derivative **5** in 58% yield (*Scheme 1*). Nucleobase-anion glycosylation with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**6**) [16] in the presence of KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) in MeCN afforded the β-D-nucleoside **7** (69% yield). Compound **7** was treated with 0.5M NaOMe in MeOH to give the methoxy nucleoside **8** (86% yield). The latter was converted into nucleoside **2b** using 2N aq. NaOH (93% yield). The overall yield of the reaction sequence was 32% based on compound **4** as starting material.

Scheme 1



The structure of compound **2b** was confirmed by single-crystal X-ray analysis (*Fig. 1*) [14]. The glycosylic torsion angle ($\chi = -115°$) is in the *anti*-range, and the sugar ring has a C(2')-*endo* (^2E) pucker. The conformation around the C(4')–C(5') bond is *trans*. This

is different from most of the 8-substituted guanine ribonucleosides which normally show a *syn*-conformation, a C(2')-*endo* pucker, and a (+) *gauche* conformation (+ *sc*) around the C(4')–(5') bond, being able to form an intramolecular O(5')–H···N(3) H-bond [1–4]. Even the unsubstituted 2'-deoxyguanosine crystallizes in the *syn*-conformation showing a 2'-*endo* ($^2T_3$) sugar pucker [17].

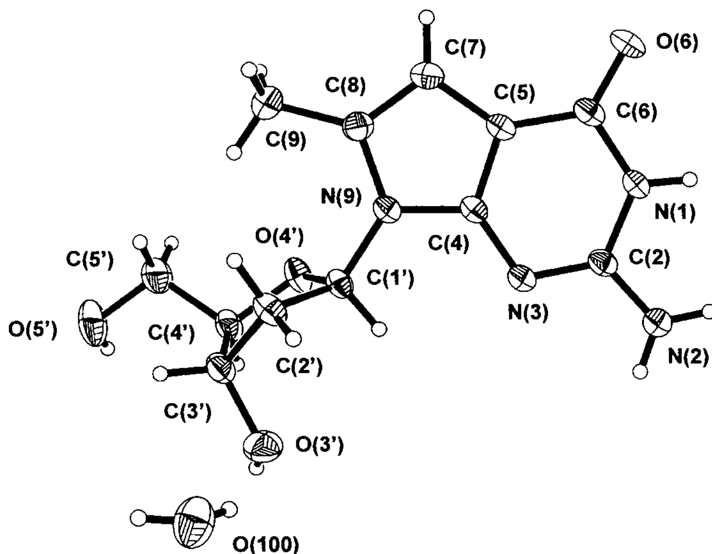


Fig. 1. *Molecular structure of 7-deaza-2'-deoxy-8-methylguanosine* (**2b**) *in the solid state and atom numbering.* Anisotropic displacement ellipsoids representing the 50% probability density of the corresponding atoms are shown; H-atoms are shown as spheres with arbitrary radius.

The sugar puckering was also investigated in solution on the basis of vicinal $^1H,^1H$-coupling constants using the PSEUROT program (version 6.2) [18]. From these data, the N/S ratio was found to be 18:82 for compound **2b**. In comparison, the conformer ratio N/S of 2'-deoxyguanosine is 29:71 and that of 7-deaza-2'-deoxyguanosine (**2a**) 28:72 [18]. In solution, the conformation of **2b** around the *N*-glycosylic bond was difficult to determine. Due to the 8-methyl group, the NOE data could not be used to quantify the ratio of *syn*/*anti* conformers. However, a strong NOE value of H–C(1') (10.6%) upon saturation of the 8-methyl protons indicated a significant population of the *anti*-form.

All monomeric compounds were characterized by ^{1}H- and ^{13}C-NMR spectra as well as by elemental analyses. Generally, gated-decoupled spectra were used for the unambiguous assignment of the ^{13}C-NMR chemical shifts. *Table 1* summarizes the ^{13}C-NMR data of the various monomers. A 10-ppm downfield shift of C(8) was observed for the methyl compound **2b** compared to **2a**. A similar shift was observed for **5**, **7**, and **8** when compared to the non-methylated compounds [19].

Synthesis of the Oligonucleotides. For the solid-phase oligonucleotide synthesis, the phosphonate **3a** and the phosphoramidite **3b** were prepared. For this purpose, compound **2b** was blocked at the 2-amino group with an isobutyryl (i-Bu) residue using the

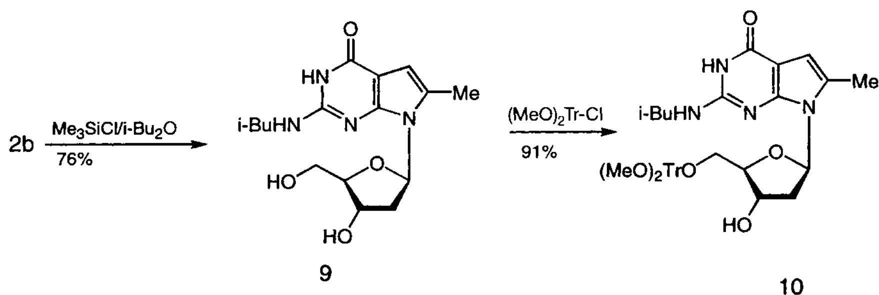
Table 1. *^{13}C-NMR Chemical Shifts* [ppm] *of Pyrrolo[2,3-*d*]pyrimidine 2'-Deoxyribonucleosides*[a])

	C(2)[b]) C(2)[c])	C(4)[b]) C(6)[c])	C(4a)[b]) C(5)[c])	C(5)[b]) C(7)[c])	C(6)[b]) C(8)[c])	C(7a)[b]) C(4)[c])	Me	CO
2a	152.5	158.5	100.0	102.1	116.7	150.5		
2b	151.7	158.1	99.3	100.9	128.1	150.9	13.5	
4	151.9	158.6	99.9	98.7	126.4	151.2	13.0	
5	149.0[d])	158.8[d])	109.4	95.7	133.7	154.9	13.0	
7	149.9[d])	158.7[d])	108.6	98.4	135.2	154.6	13.7	
8	158.6	162.2	96.8	97.7	131.0	154.8	13.7	
9	145.9	156.2	103.4	102.0	131.5	148.2	13.9	180.0
10	145.6	156.1	103.5	101.7	131.2	147.8	13.5	179.7
3a	145.5	156.4	103.5	101.5	131.7	147.5	13.2	179.8
	C(1')	C(2')	C(3')	C(4')	C(5')	MeO		
2a	82.2	–	70.8	86.9	61.9			
2b	82.7	38.2	70.7	86.7	61.9			
7	83.3	34.8	75.0	80.8	64.1			
8	83.1	38.1	71.1	87.0	62.1	52.9		
9	82.6	38.1	70.5	86.7	61.6			
10	82.1	38.2	70.5	85.1	64.1	54.9		
3a	82.7	36.6	71.7	85.1	62.5	55.0		

[a]) Measured in (D_6)DMSO. [b]) Systematic numbering. [c]) Purine numbering. [d]) Tentative.

protocol of transient protection [20] which yielded the crystalline derivative **9** (76%) (*Scheme 2*). The stability of the protecting group of **9** was determined UV-spectrophotometrically by hydrolysis in 25% aqueous NH_3 solution at 40°. The half-life of the deprotection was found to be 91 min for **9** compared to 109 min for isobutyrylated **2a** [11]. Next, the 4,4'-dimethoxytriphenylmethyl ($(MeO)_2Tr$) group was introduced at the 5'-position using the standard protocol which furnished compound **10** in 91 % yield. This derivative was converted into phosphonate **3a** (PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole; 90% yield) as well as into the phosphoramidite **3b** (chloro(2-cyanoethoxy)-(diisopropylamino)phosphane (= 2-cyanoethyl diisopropylphosphoramidochloridite; 73% yield). The structures of **9**, **10**, and **3** were established by their spectroscopic data (see *Exper. Part* and *Table 1*).

Scheme 2



Solid-phase synthesis of the oligonucleotides was performed on an automated synthesizer using standard protocols of H-phosphonate or phosphoramidite chemistry [21][22]. The oligonucleotides were purified on an oligonucleotide-purification cartridge (see *Exper. Part*). *Tables 2–5* contain all oligonucleotides **11–35** which were synthesized and summarize the physicochemical properties. The base composition of the oligonucleotides was confirmed by enzymatic hydrolysis using snake-venom phosphodiesterase followed by alkaline phosphatase. For some oligonucleotides, MALDI-TOF mass spectra were taken (see *Exper. Part*).

Single-Stranded Oligonucleotides. Due to stacking of the bases, the single-stranded helices do form well-organized structures. These structures have been studied in a number of cases [23][24]. Nevertheless, single-stranded oligonucleotides containing at least three to four consecutive deoxyguanosine residues form G quadruplets. Therefore, they cannot be studied in the form of single strands. Oligonucleotides containing 7-deazapurine nucleosides cannot form such aggregates [25][26] which enables one to study their single strands, even in the case of consecutive 7-deazaguanine residues, *e.g.*, by CD spectroscopy.

The CD spectrum of the 8-methylated oligomer d[$(m^8c^7G)_5$–G] (**14**) shows small CD signals compared to either the non-methylated oligonucleotide d[$(c^7G)_5$–G] (**11**) or the 7-methylated oligonucleotide d[$(m^7c^7G)_5$–G] (**13**) (*Fig. 2, b*). The CD spectra of the monomeric nucleosides $m^8c^7G_d$ (**2b**), c^7G_d (**2a**), and $m^7c^7G_d$ are shown for comparison (*Fig. 2, a*). According to earlier results, the oligonucleotides **11** and **13** containing either 7-deazaguanine or 7-deaza-7-methylguanine show sigmoidal melting profiles indicating highly ordered single strands [12]. According to the CD spectrum of **14**, this oligonucleotide apparently shows more conformational flexibility than d[$(c^7G)_5$–G]. In other words, the preorganization of a helix is very weak in the case of oligonucleotide **14** containing the 8-substituted 7-deazaguanine.

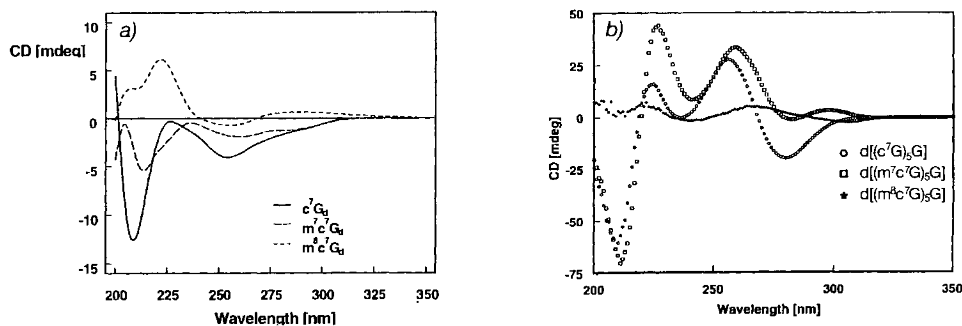


Fig. 2. a) *CD Spectra of nucleosides* c^7G_d (**2a**), $m^7c^7G_d$, *and* $m^8c^7G_d$ (**2b**) (nucleoside concentration 100 μM in 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1M NaCl, pH 7.0 at 25°); b) *CD spectra of oligonucleotides d[*$(c^7G)_5$*–G]* (**11**), *d[*$(m^7c^7G)_5$*–G]* (**13**), *and d[*$(m^8c^7G)_5$*–G]* (**14**) (oligonucleotide concentration 25 μM in 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1M NaCl, pH 7.0, at 5°)

Oligonucleotide Duplexes. It was reported that the stabilities of duplexes increase in the order $d(c^7G–C)_4$ (**16**) < $d(m^7c^7G–C)_4$ (**17**) < $d(G–C)_4$ (**15**); data of compounds **15a** and **15b** were shown for comparison [13]. Thermodynamic parameters were determined using the program MeltWin (see *Exper. Part*). Some earlier data which used

another program are now revised [10][12][13]. According to *Table 2*, the duplex d(m^8c^7G–C)$_4$ (**18**; T_m 47°) is enthalpically destabilized compared to d(c^7G–C)$_4$ (**16**; T_m 52°) and also to the 7-methylated d(m^7c^7G–C)$_4$ (**17**). Due to this, stacking and/or H-bonding of the 8-substituted oligonucleotide must be reduced compared to the 7-methylated derivative. According to the CD spectrum of oligomer **18**, a Z-DNA is formed [13]. Similarly to the 8-substituted purine oligoribonucleotides, the bulky 8-methyl groups force the oligonucleotide from the B- to the Z-DNA structure [5][6]. This conformational change is neither observed in the case of the 7-substituted 7-deazaguanine oligonucleotides nor in the case of oligonucleotides containing 7-deazaguanine.

Table 2. T_m *Values, Thermodynamic Data, and DNA Structure of Self-complementary Oligonucleotides*[a])

	T_m [°]	ΔH [kcal/mol]	ΔS [cal/K · mol]	ΔG [kcal/mol]	DNA Structure
d(G–C)$_4$ (**15**)	59	–67	–179	–11.9	B-DNA
d(c^7G–C)$_4$ (**16**)	52	–65	–178	–10.2	B-DNA
d(m^7c^7G–C)$_4$ (**17**)	55	–67	–182	–11.0	B-DNA
d(m^8c^7G–C)$_4$ (**18**)	47	–62	–171	–9.0	Z-DNA
d(C–G)$_4$ (**15a**)	59	–67	–178	–11.7	B/Z-DNA
d(C-m^7c^7G)$_4$ (**15b**)	55	–57	–149	–10.5	B-DNA

[a]) Single-strand concentration is 10 μM measured in 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1M NaCl, pH 7.0.

Compound **2b** was incorporated also into another oligonucleotide with alternating dG–dT. Oligonucleotides with this sequence normally do not form a Z-DNA structure. As it can be seen from *Table 3*, the non-self-complimentary duplex d(m^8c^7G–T)$_4$ · d(A–C)$_4$ (**23** · **20**; T_m 13°) is strongly destabilized compared to its unmethylated d(c^7G–T)$_4$ · d(A–C)$_4$ (**21** · **20**; T_m 37°). A similar result was found for the duplex d[T_4(m^8c^7G)$_4T_4$] · d($A_4C_4A_4$) (**28** · **25**) containing four consecutive 8-methylated 7-deazaguanine residues (T_m 25°); the parent duplex d[T_4(c^7G)$_4T_4$] · d($A_4C_4A_4$) (**26** · **25**) shows a T_m value of 45°. In these cases, the destabilization is caused by an unfavorable enthalpy, which is partially compensated by a more favorable entropy.

Table 3. T_m *Values, Thermodynamic Data, and DNA Structure of Non-self-complementary Oligonucleotides*[a])

	T_m [°]	ΔH [kcal/mol]	ΔS [cal/K · mol]	ΔG [kcal/mol]
d(G–T)$_4$ · d(A–C)$_4$ (**19** · **20**)	38	–69	–195	–8.0
d(c^7G–T)$_4$ · d(A–C)$_4$ (**21** · **20**)	37	–72	–205	–7.8
d(m^7c^7G–T)$_4$ · d(A–C)$_4$ (**22** · **20**)	40	–61	–169	–8.4
d(m^8c^7G–T)$_4$ · d(A–C)$_4$ (**23** · **20**)	13	–47	–139	–3.8
d($T_4G_4T_4$) · d($A_4C_4A_4$) (**24** · **25**)	49	–86	–242	–11.1
d[T_4(c^7G)$_4T_4$] · d($A_4C_4A_4$) (**26** · **25**)	45	–78	–219	–9.8
d[T_4(m^7c^7G)$_4T_4$] · d($A_4C_4A_4$) (**27** · **25**)	45	–92	–264	–10.5
d[T_4(m^8c^7G)$_4T_4$] · d($A_4C_4A_4$) (**28** · **25**)	25	–72	–217	–4.9

[a]) Single-strand concentration is 10 μM measured in 10 mM Na-cacodylate, 10 mM $MgCl_2$, 0.1M NaCl, pH 7.0.

From the CD spectra, it can be seen that the duplexes $d(m^8c^7G-T)_4 \cdot d(A-C)_4$ (**23** · **20**) and $d[T_4(m^8c^7G)_4T_4] \cdot d(A_4C_4A_4)$ (**28** · **25**) still retain the B-form of DNA with an *anti*-orientation of 8-methylated 7-deazaguanine residues. However, this B-form requires some extra energy to be formed compared to duplexes with 7-substituents. This is reflected by the lower T_m values. In the case of $d(m^8c^7G-C)_4$ (**18**; *Table 2*), the T_m is not as strongly reduced as the 8-methyl groups are located on the periphery of the Z-helix having more steric freedom than within the B-DNA duplex structure.

It has been reported that the 'homooligomers' containing 7-deazaguanine or 7-methylated 7-deazaguanine form duplexes with $d(C_6)$ (see **11** · **12** and **13** · **12**, resp., in *Table 4*) as well with poly(C). In all cases, the duplexes are destabilized when a methyl group is present. However, the destabilization is almost negligible in the case of the 7-methylated duplexes (containing **13**) whereas the 8-substituted derivatives (containing **14**) suffer severe destabilization. According to the CD spectra (data not shown), these duplexes have an A-DNA or A-like structure.

Table 4. T_m *Values, Thermodynamic Data, and Duplex Structures*[a])

	T_m [°]	ΔH [kcal/mol]	ΔS [cal/K · mol]	ΔG [kcal/mol]	Structure
$d[(c^7G)_5-G] \cdot d(C_6)$ (**11** · **12**)[b])	25	−53	−152	−5.5	A-DNA
$d[(m^7c^7G)_5-G] \cdot d(C_6)$ (**13** · **12**)[b])	24	−51	−147	−5.5	A-DNA
$d[(m^8c^7G)_5-G] \cdot d(C_6)$ (**14** · **12**)[b])	< 10				A-DNA
$d[(c^7G)_5-G] \cdot$ polyC[c])	42				A-Type
$d[(m^7c^7G)_5-G] \cdot$ polyC[c])	41				A-Type
$d[(m^8c^7G)_5-G] \cdot$ polyC[c])	25				A-Type

[a]) Measured in 10 mM Na-cacodylate, 10 mM $MgCl_2$, 0.1M NaCl, pH 7.0. [b]) Single-strand oligonucleotide concentration is 10 μM. [c]) Identical base composition.

Oligonucleotide Quadruplexes. Next, the behavior of methylated 7-deazaguanine residues was studied with regard to quadruplex destabilization. For this purpose, the oligonucleotide $d(T_4G_8T_4)$ (**29**) was synthesized, and a number of guanine residues were replaced stepwise by 7- or 8-methylated 7-deazaguanine residues (oligonucleotides **29**–**35**, *Table 5*). The replacement of dG by c^7G_d was also undertaken for comparison. From the latter, it was already known that it destroys the quadruplex structure when fully replacing dG [25][26]. However, it was unknown to what extent the replacement is necessary to be effective in disaggregation and how substituents in the 7- or 8-position show an additional effect on the quadruplex stability. As reported earlier, oligonucleotide aggregate formation can be monitored by ion-exchange HPLC. This method is able to separate the single-stranded chains from the tetrameric aggregates (quadruplexes). The fast-migrating peaks obtained by this method represent the single strands, whereas the slower ones are due to quadruplex formation. To examine the aggregation properties of compounds **29**–**35**, the oligomers were dissolved in 1M NaCl, kept in the refrigerator (−18°) overnight, brought to room temperature, and then applied to an ion-exchange HPLC. The chromatographic profiles (for a selection, see *Fig. 3*) show a peak composition which depends on the structure and the amount of the residues being incorporated. *Table 5* summarizes the peak areas of the single strands and quadruplexes at different temperatures and correlates them also with the number of residues.

Table 5. *Relative Peak Areas of Oligonucleotide Single Strands* (SS) *and Qudruplexes* (QP) *Obtained from Ion-exchange HPLC* [a])

	25°		50°		90°	
	SS	QP	SS	QP	SS	QP
$d(T_4G_8T_4)$ (**29**)	–	99	2	97	3	96
$d(T_4GGG$**c^7**$GGGGGT_4)$ (**30**)	36	61	70	26	83	12
$d(T_4GGG$**m^{7}c^7**$GGGGGT_4)$ (**31**)	7	91	14	80	40	40
$d(T_4GGG$**m^{8}c^7**$GGGGGT_4)$ (**32**)	4	96	17	82	71	12
$d(T_4GGG$**c^7**G**c^7**$GGGGT_4)$ (**33**)	23	75	74	21	94	–
$d(T_4GGG$**m^{7}c^7**G**m^{7}c^7**$GGGGT_4)$ (**34**)	46	35	66	22	83	–
$d(T_4GGG$**m^{8}c^7**G**m^{8}c^7**$GGGGT_4)$ (**35**)	94	4	98	–		

[a]) Data were determined spectrophotometrically at 260 nm. Experimental procedure, see [29].

The oligomer **29** shows one main peak of the quadruple assembly formed by the 8 dG quartets, and only a trace of the monomer peak is visible (*Fig. 3,a*; 90°). Replacement of one dG residue in position 4 (always counted from the 5′-end of the dG-tract)

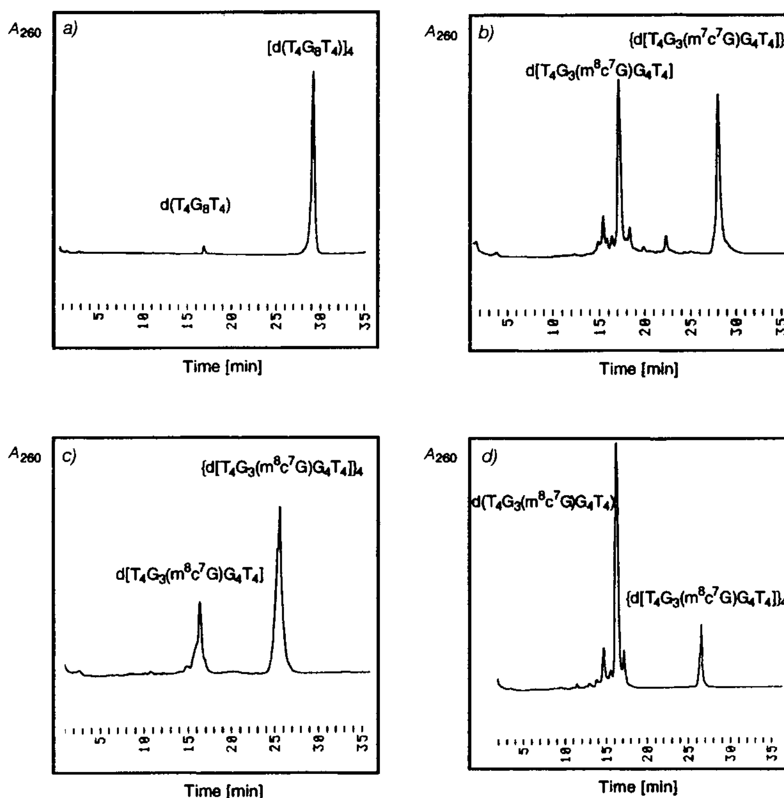


Fig. 3. *Ion-exchange HPLC profiles of* a) $d(T_4G_8T_4)$ (**29**) *at 90°*, b) $d[T_4G_3(m^7c^7G)G_4T_4]$ (**31**) *at 90°*, c) $d[T_4G_3(m^8c^7G)G_4T_4]$ (**32**) *at 50°, and* d) $d[T_4G_3(m^8c^7G)G_4T_4]$ (**32**) *at 90°*

by c^7G_d (see oligomer **30**) reduces the area of the quadruplex to 12% (90°) and 61% at 25° (*Table 5*). However, when the 7-methyl derivative $m^7c^7G_d$ or the 8-methyl derivative $m^8c^7G_d$ (**2b**) were incorporated instead of c^7G_d (oligomers **31** and **32**, same position) the tetrameric aggregate becomes stabilized. This is valid for temperatures of 25 and 50°. At 90°, the aggregate containing c^7G_d (see **30**), and also that containing the 8-methyl derivative (see **32**), becomes less stable, while the 7-methyl derivative (see **31**) still has a reasonable stability (*Fig. 3, b–d*). However, the replacement of two dG residues in the oligonucleotide **29** by c^7G_d, $m^7c^7G_d$, or $m^8c^7G_d$ (see **33**–**35**) results in a strong destabilization of the aggregate which is most pronounced in the case of the 8-methylated oligomer **35** (*Table 5*). From these experiments, it can be concluded that $m^8c^7G_d$ (**2b**) destabilizes the quadruplex structure more than $m^7c^7G_d$ or c^7G_d (**2a**). This observation is in line with the finding that guanine residues in oligodeoxynucleotide quadruplexes are in the *anti*-conformation around the *N*-glycosylic bond [27][28]. Therefore, the 8-methyl derivative **2b** which has the tendency to adopt the *syn*-conformation might distort the oligonucleotide backbone more than 7-deazaguanine or its 7-methyl derivative. According to *Table 5*, it is obvious that tracts of guanine residues cannot form stable quadruplexes when the runs of guanines contain less than 3 consecutive dG residues, *e.g.*, by separating the tracts by one 7-deazapurine moiety.

The CD spectrum of $d(T_4G_8T_4)$ (**29**) at 25° is typical for a parallel-stranded quadruplex structure (*Fig. 4,a*) [30][31]. After replacement of one dG residue by $m^7c^7G_d$ or $m^8c^7G_d$ (position 4, oligomers **31** and **32**), the band around 250 nm is reduced compared to the parent oligomer **29**. The incorporation of c^7G_d (same position) leads to a further decrease. These findings are in agreement with the HPLC experiments. They showed that a single replacement of dG by c^7G resulted in stronger disaggregation than in the case of the replacement by methylated derivatives. The CD spectrum of oligomer **35** (*Fig. 4,b*) with two 7-deaza-8-methylguanine residues does not display the typical shape of the tetramer (see **29**). A shoulder at 275 nm and a maximum at 250 nm are observed. The features observed in the CD spectra are also in agreement with those found by ion-exchange HPLC.

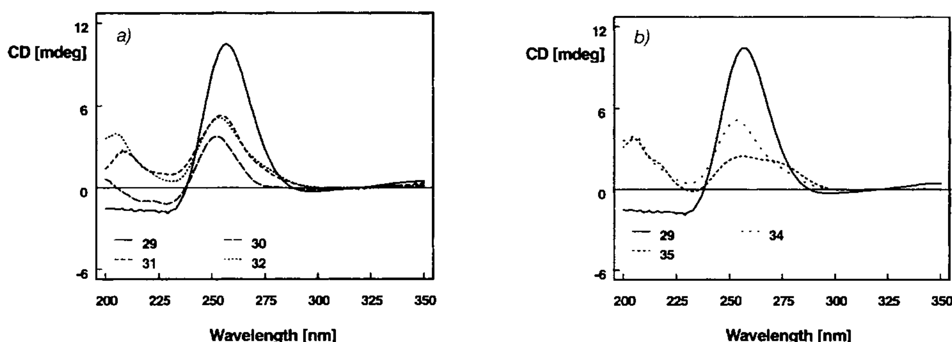


Fig. 4. *CD Spectra of* a) *oligonucleotides* **29**–**32** *and* b) *oligonucleotides* **29**, **34**, *and* **35** *at 25°*. In 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1M NaCl, pH 7.0.

The temperature-dependent CD spectra of the oligomer quadruplexes containing one or two residues of compound $m^8c^7G_d$ (**2b**) or $m^7c^7G_d$ are shown in *Fig. 5*. It is apparent that the intensities of the CD bands are lowered with increasing temperature. However,

the incorporation of two residues (oligomers **34** and **35**) leads to a change of the spectra reflecting the formation of single strands. Again, the decay of the quadruplex is strongest in the case of compound **35**, a finding which is in agreement with ion-exchange HPLC data.

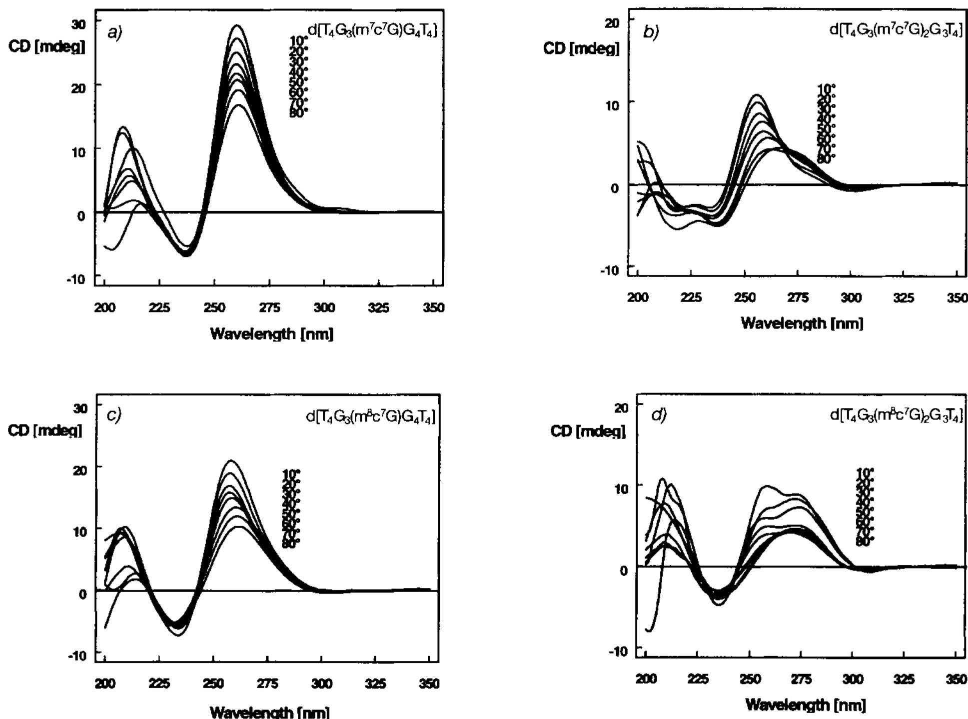


Fig. 5. *Temperature-dependent CD spectra of* a) *d[$T_4G_3(m^7c^7G)G_4T_4$]* (**31**) b) *d[$T_4G_3(m^7c^7G)_2G_3T_4$]* (**34**), c) *d[$T_4G_3(m^8c^7G)G_4T_4$]* (**32**), *and* d) *d[$T_4G_3(m^8c^7G)_2G_3T_4$]* (**35**). 5 µM Oligonucleotide in 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1 M NaCl, pH 7.0.

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Experimental Part

General. TLC: Aluminum sheets coated with a 0.2-mm layer of silica gel *60 F_{254}* (*Merck*, Germany). Flash chromatography (FC): at 0.5 bar (silica gel *60 H* (*Merck*, Germany)). A *Uvicord S* (*LKB Instruments*, Sweden) was used for detection. Solvent systems for TLC and FC: cyclohexane/AcOEt 2:1 (*A*), CH_2Cl_2/MeOH 95:5 (*B*), CH_2Cl_2/MeOH 9:1 (*C*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*D*), CH_2Cl_2/AcOEt/Et_3N 47.5:47.5:5 (*E*). UV Spectra: *Hitachi-150-20* spectrometer (*Hitachi*, Japan). M.p.: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland). NMR Spectra: *Bruker-AC-250* and *-AMX-500* spectrometer; δ values in ppm rel. to Me_4Si as internal standard (^1H and ^{13}C) or to external phosphoric acid (^{31}P). Elemental analyses were performed by *Mikroanalytisches Laboratorium Beller*, Göttingen, Germany.

Oligonucleotides. Oligonucleotide synthesis based on phosphonate chemistry was carried out on an automated DNA synthesizer, model *380B* (*Applied Biosystems*, Germany). The phosphonates of dA, dG, dT, and dC were prepared by Ms *E. Feiling*. For phosphoramidite chemistry, the synthesis of oligonucleotides was carried out on

an automated DNA synthesizer, model *ABI 392-08* (*Applied Biosystems*, Germany). The phosphoramidites of dA, dG, dT, dC, and the controlled-pore-glass (CPG) columns were purchased from *PerSeptive Biosystems GmbH*, Germany. The phosphoramidites of 7-deaza-2'-deoxyguanosine and 7-deaza-2'-deoxy-7-methylguanosine were synthesized previously [12][32]. The coupling yields obtained with the phosphonates of modified nucleosides were *ca.* 92%, compared with 95% of regular phosphonates. The coupling yields in phosphoramidite chemistry were 95% for the modified building blocks and 98% for non-modified compounds. The oligonucleotides were purified by means of oligonucleotide-purification cartridges (OPC; *Applied Biosystems*). The yields of the isolated and purified oligonucleotides were between 15 and 25%. Oligonucleotides with consecutive dG residues were difficult to purify (→ $(dG)_4$ aggregates) and contained in some cases trace amounts (< 5%) of impurities. Reversed-phase HPLC: 4 × 250 mm *Rp-18* (10 μm) *LiChrosorb* column (*Merck*) with a *Merck-Hitachi* HPLC pump (model *655 A-12*) connected with a variable-wavelength monitor (model *655-A*), a controller (model *L-5000*), and an integrator (model *D-2000*). Snake-venom phosphodiesterase (EC 3.1.4.1) and alkaline phosphatase (EC 3.1.3.1) were gifts from *Boehringer Mannheim GmbH*, Germany. CD Spectra: *Jasco-600* spectropolarimeter; thermostatically controlled 1-cm cuvettes connected to a *Lauda-RCS-6* bath (*Lauda*, Germany). T_m Values were determined with a *Cary-1E* UV/VIS spectrophotometer (*Varian*, Melbourne, Australia) [33]. The temperature was increased by 60°/h. The T_m values were determined using the software package '2hDNA' (Dr. *Apel*, *Varian*, Darmstadt, Germany). Thermodynamic data were calculated from the melting profiles with MeltWin [34].

*4-Chloro-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine* (**5**). A suspension of **4** [12] (10.0 g, 6.1 mmol) in $POCl_3$ (100 ml) containing two drops of *N,N*-dimethylaniline was heated under reflux for 3 h under stirring. The mixture was slowly poured into crashed ice (700 ml), and stirring was continued for 1 h. The mixture was adjusted to pH 4–5 with conc. aq. ammonia. The precipitate was crystallized from AcOEt: light yellow solid (6.5 g, 58%). TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.5. UV (MeOH): 320 (5730), 260 (4520) 233 (24800). ^{1}H-NMR ((D_6)DMSO): 2.24 (*s*, Me); 5.90 (*s*, H–C(5)); 6.35 (*s*, NH_2); 11.30 (*s*, NH). Anal. calc. for $C_7H_7ClN_4$ (182.6): C 46.04, H 3.86, Cl 19.41, N 30.68; found: C 46.04, H 3.97, Cl 19.28, N 30.74.

*4-Chloro-7-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine* (**7**). To a suspension of **5** (1.0 g, 5.4 mmol) in anh. MeCN (80 ml), powdered KOH (1.2 g, 17.8 mmol) and TDA-1 (200 μl, 0.62 mmol) were added under stirring at r.t. After stirring for 5 min, 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl chloride (**6**) [16] (2.3 g, 6.0 mmol) was added, and stirring was continued for another 15 min. Insoluble material was filtered off and washed with MeCN, and the filtrate was evaporated. The residue was purified by FC (column 8 × 3 cm, cyclohexane/AcOEt 2:1). The content of the main zone was crystallized from MeOH: colorless crystals (2.0 g, 69%). M.p. 150–151°. TLC (silica gel, cyclohexane/AcOEt 2:1): R_f 0.5. ^{1}H-NMR ((D_6)DMSO): 2.35 (*s*, Me); 2.38 (*s*, 2 Me); 3.49 (*m*, 1 H–C(2')); 4.54 (*m*, 2 H–C(5')); 4.70 (*m*, H–C(4')); 5.81 (*m*, H–C(3')); 6.09 (*s*, H–C(5)); 6.41 (*t*, *J* = 7.2, H–C(1')); 6.62 (*s*, NH_2); 7.3–7.9 (*m*, arom. H). Anal. calc. for $C_{28}H_{27}ClN_4O_5$ (535.0): C 62.86, H 5.09, N 10.47; found: C 63.01, H 5.12, N 10.51.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-4-methoxy-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine* (**8**). A soln. of **7** (1.0 g, 1.87 mmol) in 0.5M NaOMe/MeOH (40 ml) was heated under reflux for 4 h. The soln. was cooled and adsorbed on silica gel, and the mixture was evaporated. The silica gel was loaded on the top of a column (9 × 4 cm) and chromatographed (CH_2Cl_2/MeOH 95:5). The substance of the main zone was crystallized from MeCN: colorless crystals (0.47 g, 86%). M.p. 192–194°. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.4. UV (MeOH): 289 (7300), 262 (10900), 226 (21900). ^{1}H-NMR ((D_6)DMSO): 2.00, 2.81 (2*m*, 2 H–C(2')); 2.32 (*s*, Me); 3.59 (*m*, 2 H–C(5')); 3.76 (*m*, H–C(4')); 3.88 (*s*, MeO); 4.33 (*m*, H–C(3')); 5.20 (*d*, *J* = 5.1, OH–C(3')); 5.27 (*t*, *J* = 6.0, OH–C(5')); 5.99 (*s*, H–C(5), NH_2); 6.30 (*t*, *J* = 6.5, H–C(1')). Anal. calc. for $C_{13}H_{18}N_4O_4$ (294.3): C 53.05, H 6.16, N 19.04; found: C 52.64, H 6.02, N 18.87.

*2-Amino-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-6-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**2b**). A suspension of **8** (1.0 g, 3.4 mmol) in 2N aq. NaOH (100 ml) was heated under reflux for 15 h. Upon neutralization with 20% aq. HCl soln., **2b** crystallized: colorless crystals (0.84 g, 88%). M.p. 230° (dec.). TLC (silica gel, CH_2Cl_2/MeOH 4:1): R_f 0.5. UV (H_2O): 262 (12900), 290 (sh, 6520). ^{1}H-NMR ((D_6)DMSO): 1.99, 2.71, (2 *m*, 2 H–C(2')); 2.29 (*s*, Me); 3.54, 3.60 (2 *m*, 2 H–C(5')); 3.74 (*m*, H–C(4')); 4.31 (*m*, H–C(3')); 5.02, 5.18 (2*m*, OH–C(3'), OH–C(5')); 6.00 (*s*, H–C(5)); 6.07 (*s*, NH_2); 6.31 (*t*, *J* = 6.6, H–C(1')); 10.37 (br., NH). Anal. calc. for $C_{12}H_{16}N_4O_4$ (280.3): C 51.42, H 5.75, N 19.99; found: C 51.32, H 5.76, N 20.09.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-2-(isobutyrylamino)-6-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**9**). Compound **2b** (450 mg, 1.6 mmol) was dried by co-evaporation of dry pyridine (3 × 15 ml) and then suspended in dry pyridine (8 ml). Chlorotrimethylsilane (1.0 ml, 8.0 mmol) was added at r.t. After stirring for 15 min, the soln. was treated with isobutyric anhyride (1.3 ml, 8.0 mmol) and maintained at r.t. for 3 h. The mixture was then cooled in an ice bath, and H_2O (1.6 ml) was added. After 5 min, 25% aq. ammonia (1.6 ml) was added and stirring continued for 15 min. The mixture was then evaporated to near dryness and the residue

crystallized from H_2O: colorless powder (430 mg, 77%). M.p. 212–214°. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.4. UV (MeOH): 302 (13000), 273 (13300). ^{1}H-NMR ((D_6)DMSO): 1.10 (*d*, *J* = 6.5, *Me*$_2$CH); 2.00, 2.71 (2*m*, 2 H–C(2'), Me_2C*H*); 2.37 (*s*, Me); 3.55 (*m*, 2 H–C(5')); 3.72 (*m*, H–C(4')); 4.31 (*m*, H–C(3')); 4.83 (*m*, OH–C(5')); 5.23 (*m*, OH–C(3')); 6.20 (*s*, H–C(5)); 6.46 (*t*, *J* = 6.7, H–C(1')); 11.38, 11.76 (2*s*, 2 NH). Anal. calc. for $C_{16}H_{22}N_4O_5$ (350.4): C 54.85, H 6.33, N 15.99; found: C 54.75, H 6.36, N 16.01.

*7-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-6-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one* (**10**). Compound **9** (330 mg, 0.94 mmol) was dried by repeated co-evaporation with dry pyridine (3 × 20 ml) and then dissolved in dry pyridine (8 ml). 4,4'-Dimethoxytrityl chloride (397 mg, 1.2 mmol) was introduced at r.t. while stirring, and stirring was continued for 6 h. Then MeOH (5 ml) and 5% aq. $NaHCO_3$ soln. (80 ml) were added. The aq. layer was extracted with CH_2Cl_2 (3 × 50 ml), the combined org. layer dried ($NaSO_4$) and evaporated and the residue submitted to FC (silica gel, column 4 × 8 cm, CH_2Cl_2/MeOH 95:5 containing traces of Et_3N): colorless powder (560 mg, 91%). TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.4. ^{1}H-NMR ((D_6)DMSO): 1.10 (*d*, *J* = 6.7, *Me*$_2$CH); 2.16 (*s*, Me); 2.20, 2.40 (2*m*, 2 H–C(2')); 2.74 (*q*, *J* = 6.8, Me_2C*H*); 3.12 (*m*, 2 H–C(5')); 3.72 (*s*, 2 MeO); 3.89 (*m*, H–C(4')); 4.34 (*m*, H–C(3')); 5.30 (*d*, *J* = 3.7, OH–C(3')); 6.38 (*t*, *J* = 6.7, H–C(1')); 6.7–7.4 (*m*, arom. H, H–C(6)); 11.46, 11.71 (2*s*, 2 NH). Anal. calc. for $C_{37}H_{40}N_4O_7$ (652.8): C 68.08, H 6.18, N 8.58; found: C 68.88, H 6.16, N 8.56.

*7-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-6-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one 3'-(Triethylammonium Phosphonate)* (**3a**). To a soln. of PCl_3 (200 µl, 2.26 mmol) and *N*-methylmorpholine (2.5 ml) in CH_2Cl_2 (14 ml), 1*H*-1,2,4-triazole (523 mg, 7.30 mmol) was added at r.t. After 30 min, the mixture was cooled to 0° and a soln. of **10** (300 mg, 0.46 mmol) in CH_2Cl_2 (14 ml) added slowly. After stirring for 30 min at r.t., the mixture was poured into 1M (Et_3NH)HCO_3 (40 ml) and shaken. The aq. layer was extracted with CH_2Cl_2 (3 × 40 ml), the combined org. extract dried (anh. Na_2SO_4) and evaporated, and the residue submitted to FC (silica gel, column 3 × 7 cm, CH_2Cl_2/MeOH/Et_3N 88:10:2). The fraction of the main zone was evaporated, the residue dissolved in CH_2Cl_2, and the soln. washed with 0.1M (Et_3NH)HCO_3 (5 × 20 ml), dried (Na_2SO_4), and evaporated: colorless foam (340 mg, 90%). TLC (silica gel, CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f 0.5. ^{1}H-NMR ((D_6)DMSO): 1.11 (*t*, *J* = 7.2, 5 Me); 2.26 (*m*, 1 H–C(2')); 2.34 (*s*, Me); 2.70 (*q*, *J* = 6.8, Me_2C*H*); 2.91 (*q*, *J* = 7.2, 3 CH_2); 3.05, 3.15 (2*m*, 2 H–C(5')); 3.70 (*s*, MeO); 3.95 (*m*, H–C(4')); 5.49 (*m*, H–C(3')); 6.43 (*d*, *J*(P,H) = 346, PH); 6.28 (*t*, *J* = 6.7, H–C(1')); 6.8–7.2 (*m*, arom. H); 11.62, 12.03 (2*s*, 2 NH). ^{31}P-NMR ((D_6)DMSO): 1.44 (^1J(P,H) = 591, ^3J(P,H–C(3')) = 9.9). Anal. calc. for $C_{43}H_{56}N_5O_9P$ (817.9): C 63.14, H 6.90, N 8.56; found: C 63.13, H 6.98, N 8.61.

*7-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-6-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**3b**). To a soln. of **10** (100 mg, 0.15 mmol) in anh. CH_2Cl_2 (6 ml) (i-Pr)$_2$EtN (0.086 ml, 0.49 mmol) and 2-cyanoethyl diisopropyl phosphoramidochloridite (0.06 ml, 0.27 mmol) were added at r.t. After stirring for 30 min, the soln. was diluted with CH_2Cl_2 (4 ml), and 5% aq. $NaHCO_3$soln. (5 ml) was added. The mixture was extracted with CH_2Cl_2 (3 × 15 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, column 5 × 2 cm, CH_2Cl_2/AcOEt/Et_3N 16:4:1): colorless foam (95 mg, 73%). TLC (silica gel, CH_2Cl_2/AcOEt 3:1): R_f 0.7. ^{31}P-NMR ((D_6)DMSO): 148.2, 148.5.

Analysis of Oligonucleotides. a) *Enzymatic Hydrolysis*. The oligonucleotides (0.2 A_{260} units) were dissolved in 0.1M *Tris* · HCl buffer (pH 8.3, 200 µl) and treated with snake-venom phosphodiesterase (6 µg) at 37° for 45 min and alkaline phosphatase (2 µg) at 37° for 30 min. The mixture was analyzed by reversed-phase HPLC (*RP-18*, 20 min 100% 0.1M (Et_3NH)OAc (pH 7)/MeCN 95:5, flow rate 0.6 ml/min). Quantification was made at 260 nm on the basis of the peak areas which were divided by the extinction coefficients of the nucleoside constituent (ε_{260}: dA 15400, dT 8800, dG 11400, dC 7300, c^7G_d 13500, $m^7c^7G_d$ 11000, $m^8c^7G_d$ 12900).

b) *Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry*. See *Table 6*.

Ion-Exchange HPLC. The ion-exchange chromatography was performed on a 4 × 50 mm *NucleoPac-PA-100* column (*Dionex*, P/N 043018, USA) using a *Merck-Hitachi* HPLC apparatus with one pump (model *655 A-12*) and a controller (model *L-5000*) which was connected with an integrator (model *D-2000*). A column oven (model *L-7350*, *Merck*, Germany) was used to control the temp. of the ion-exchange column. The oligonucleotide samples were prepared as follow: A sample of 0.1 A_{260} unit was dissolved in 1M NaCl buffer (1M NaCl, 10 mM *Tris* · HCl, 100 µl), heated to 95° for 2 min, and kept overnight in the refrigerator (–18°) to became frozen. The samples were warmed up to r.t. and injected onto the ion-exchange column which was already preheated to the required temp. The following eluent system was used: 25 mM *Tris* · HCl containing 1 mM EDTA buffer (pH 8)/MeCN 90:10 (*A*); 25 mM *Tris* · HCl, 1.0M NaCl containing 1 mM EDTA buffer (pH 8)/MeCN 90:10 (*B*); gradient: 0–30 min 20–80% *B* in *A*, 5 min 80% *B* in *A*; flow rate 0.75 ml/min.

Table 6. *MALDI-TOF MS Data of Oligonucleotides*

	m/z			m/z	
	calc.	found		calc.	found
11	1909	1909	**13**	1980	1978
14	1980	1979	**18**	2465	2465
21	2469	2470	**22**	2525	2523
23	2525	2524	**24**	3690	3689
26	3686	3688	**28**	3742	3742
29	5005	5004	**30**	5004	5004
31	5019	5019	**32**	5019	5019
33	5003	5004	**34**	5032	5035
35	5032	5032			

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